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, 137569-72-3; 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<sup> $\dagger,1$ </sup>

Akira Matsuda,<sup>\*,†</sup> Misao Shinozaki,<sup>§</sup> Toyofumi Yamaguchi,<sup>∥</sup> Hiroshi Homma,<sup>‡</sup> Rie Nomoto,<sup>§</sup> Tadashi Miyasaka,<sup>§</sup> Yohko Watanabe,<sup>∥</sup> and Toichi Abiru<sup>∥</sup>

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- $\beta$ -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,<sup>2</sup> inhibition of neurotransmitter release,<sup>3</sup> hypothermia,<sup>4</sup> negative inotropic and chronotropic action,<sup>5</sup> inhibition of lipolysis,<sup>6</sup> inhibition of renin release,<sup>7</sup> inhibition of platelet aggregation,<sup>8</sup> and peripheral and cerebral vasodilation.<sup>9</sup> 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,<sup>10</sup> deamination to inosine by adenosine deaminase,<sup>11</sup> 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,



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

<sup>\*</sup> Author to whom correspondence should be addressed.

<sup>&</sup>lt;sup>†</sup>This paper is dedicated to the memory of Professor Tohru

Ueda, deceased Sept 19, 1990.

<sup>&</sup>lt;sup>‡</sup>Hokkaido University.

Showa University.

Yamasa Shoyu Co. Ltd.

Scheme I<sup>a</sup>



<sup>a</sup>(a) CuI, I<sub>2</sub>, CH<sub>2</sub>I<sub>2</sub>, isoamyl nitrite in THF, 80 °C; (b) NH<sub>3</sub>/MeOH; (c) HC=C(CH<sub>2</sub>)<sub>n</sub>CH<sub>3</sub>, (PPh<sub>3</sub>)<sub>2</sub>PdCl<sub>2</sub>, CuI, Et<sub>3</sub>N 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<sub>1</sub> agonists.<sup>12</sup> However, only a few potent and/or

- 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'-Calkynyl-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<sub>1</sub> and A<sub>2</sub> 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.

selective A<sub>2</sub> adenosine receptor agonists have been reported. 2-(Phenylamino)adenosine (CV-1808) was originally reported as a potent coronary vasodilator<sup>13</sup> and confirmed as a selective A<sub>2</sub> agonist.<sup>14</sup> 1-(6-Amino-9H-purin-9-yl)-1-deoxy-N-ethyl- $\beta$ -D-ribofuranuronamide (NECA) was also found to be a coronary vasodilator<sup>15</sup> and a highly potent A<sub>2</sub> agonist but not selective to the A<sub>2</sub> receptor.<sup>14</sup> Recently, more selective adenosine A<sub>2</sub> agonists such as DPMA,<sup>16</sup> CGS 21680,<sup>17</sup> and MPEA<sup>18</sup> shown in Chart I have been synthesized and have shown certain pharmacological activities.

- (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<sup>6</sup>-[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<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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)<sup>α</sup>

	additives			reaction	% isolated yield		
solvent	$\overline{\mathrm{CH}_{2}\mathrm{I}_{2}}$	$I_2$	CuI	time, min	2	3a or 3c <sup>b</sup>	
CH <sub>3</sub> CN	+	-	-	15	57	27	_
CH <sub>3</sub> CN	-	+	-	15	39	42	
CH <sub>3</sub> CN	-	-	+	15	42	с	
CH <sub>3</sub> CN	+	-	+	15	66	с	
CH <sub>3</sub> CN	-	+	+	15	74	с	
CH <sub>3</sub> CN	+	+	+	15	82 (76)	5	
THF	+	-	-	60	64	21	
THF	-	+	-	30	57	с	
THF	-	+	+	45	80 (77)	11	
THF	+	-	+	45	81 (82)	с	
THF	+	+	+	45	89 (85)	(2.7)	

<sup>a</sup> The reaction of 2 (2 mmol) with isoamyl nitrile (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 ( $CH_{2}I_{2}$ , 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. <sup>b</sup>When using acetonitrile, the byproduct is 3a, and 3c was obtained from the reaction in THF. <sup>c</sup>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<sup>19</sup> and had cardiovascular effects in normotensive rats.<sup>20</sup> Our recent report has shown that 2-(1-hexyn-1yl)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.<sup>21</sup> Thus, synthesis of other 2-AA derivatives and investigation of their pharmacological activities are necessary to develop a more selective A<sub>2</sub> 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  $1-\beta$ -D-ribofuranosyl-6-(1-octyn-1-yl)purine (12) and 8-(1-octyn-1vl)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 6chloro-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<sub>2</sub>I<sub>2</sub>) in small quantities.<sup>23</sup> 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)-2acetamido-6-chloropurine (3a) was obtained as a byproduct. This compound (3a) could be obtained by the reaction of a purin-2-yl radical,<sup>23</sup> 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 6chloropurine 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,<sup>24</sup> 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-iodo-adenosine  $(4a)^{25}$  as crystals from water in 90% yield.

<sup>(19)</sup> 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.

<sup>(20)</sup> Matsuda, A.; Ueda, T. The synthesis, mutagenic and pharmacological activities of 2-carbon-substituted adenosines. Nucleosides Nucleotides 1987, 6, 85–94.

<sup>(21)</sup> 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<sub>2</sub> receptors. Eur. J. Pharmacol. 1991, 196, 69-76.

<sup>(22)</sup> 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.

<sup>(23)</sup> Nair, V.; Richardson, S. G. Modification of nucleic acid gases via radical intermediates: Synthesis of dihalogenated purine nucleosides. Synthesis 1982, 670–673.

<sup>(24)</sup> Nair, V.; Chamberlain, S. D. Reductive deamination of aminopurine nucleosides. Synthesis 1984, 401-403.

<sup>(25)</sup> Nair, V.; Young, D. A. A new synthesis of isoguanosine. J. Org. Chem. 1985, 50, 406-408.





<sup>a</sup> (a) 1 N NaOH, MeOH, then Ac<sub>2</sub>O, pyridine; (b)  $O_2$ , Cu(OAc)<sub>2</sub>, pyridine; (c) NH<sub>3</sub>/MeOH; (d) compound 4b, (PPh<sub>3</sub>)<sub>2</sub>PdCl<sub>2</sub>, CuI, Et<sub>3</sub>N in DMF, 80 °C.

For the palladium-catalyzed cross-coupling reaction of 4a with terminal alkynes, a modification by Sonogashira<sup>26</sup> of a method originally developed by Heck<sup>27</sup> 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, <sup>1</sup>H 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.<sup>28,29</sup> The pattern of the <sup>1</sup>H 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 ( $\delta$  8.39 in DMSO- $d_6$ ) of **5b** showed a broad singlet with a half-width of  $\sim 15$  Hz, presumably due to the chelation of metals between 6-amino and N7-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<sub>2</sub>S, 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.

- (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 8cyanoadenosines 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.





<sup>a</sup> (a) HC=C(CH<sub>2</sub>)<sub>5</sub>CH<sub>3</sub>, (PPh<sub>3</sub>)<sub>2</sub>PdCl<sub>2</sub>, Et<sub>3</sub>N in DMF, 70 °C; (b) NH<sub>3</sub>/MeOH.

Although both the <sup>1</sup>H NMR spectral patterns were quite similar, except for the absence of the acetylenic proton in 8, the UV spectrum of 8 ( $\lambda_{max}$  at 331 and 263 nm) is different from that of 7 ( $\lambda_{max}$  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.<sup>30</sup> Compound 10 was prepared from a similar cross-coupling reaction of tri-Oacetyl-2-iodoadenosine 4b with 7. Comparison of the UV spectra of both derivatives identifies 8 as 1,4-bis(2,3,5tri-O-acetyladenosin-2-yl)buta-1,3-diyne. These also suggest that the cross-coupled nucleosides without treatment with H<sub>2</sub>S 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

<sup>(30)</sup> 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

	Ki,ª	nM	selectivity	BP ED <sub>20</sub> , <sup>b</sup>	HR ED	
no.	A <sub>1</sub>	$A_2$	$A_1/A_2$	$\mu g/kg$	$\mu g/kg$	
5a	$186 \pm 9.7$	$21.2 \pm 2.3$	8.8	$0.48 \pm 0.13$	$18.4 \pm 8.4$	
5b	$154 \pm 17$	$7.5 \pm 2.1$	20.5	$0.18 \pm 0.02$	$41.5 \pm 1.5$	
5c	$126 \pm 8.8$	$2.8 \pm 0.3$	45.0	$0.11 \pm 0.03$	$66.9 \pm 6.8$	
5đ	$170 \pm 21$	$5.4 \pm 1.7$	31.5	$0.14 \pm 0.01$	>100	
5e	$202 \pm 20$	$12.1 \pm 1.3$	16.7	$0.34 \pm 0.02$	>100	
5 <b>f</b>	$257 \pm 15$	$33.7 \pm 5.6$	7.6	$0.53 \pm 0.09$	>100	
5g	$232 \pm 23$	$32.8 \pm 6.1$	7.1	$0.46 \pm 0.05$	>100	
5 <b>h</b>	$267 \pm 14$	$44.6 \pm 5.7$	6.0	$2.05 \pm 0.25$	>100	
51	$10.1 \pm 1.4$	$20.5 \pm 2.3$	0.49	$0.95 \pm 0.14$	$1.19 \pm 0.1$	
$5\mathbf{m}$	$81.4 \pm 11$	$40.1 \pm 6.4$	2.0	$2.53 \pm 0.18$	$15.3 \pm 2.7$	
5 <b>n</b>	$38.1 \pm 5.1$	$88.1 \pm 9.8$	0.43	$23.8 \pm 2.9$	$44.2 \pm 3.6$	
50	$18.5 \pm 1.9$	$10.6 \pm 1.4$	1.8	$1.06 \pm 0.02$	$9.59 \pm 0.1$	
5p	$128 \pm 15$	$10.2 \pm 1.8$	12.6	$1.10 \pm 0.09$	$79.9 \pm 6.4$	
5q	$1498 \pm 189$	$307 \pm 53$	4.9	$9.16 \pm 1.50$	>100	
5 <b>r</b>	$244 \pm 17$	$12.8 \pm 2.1$	19.1	$1.42 \pm 0.05$	>100	
6	$69.8 \pm 7.8$	$630 \pm 91$	0.11	$1.15 \pm 0.01$	>100	
9	$272 \pm 30$	$37.6 \pm 4.8$	7.3	$5.91 \pm 1.10$	$5.76 \pm 0.8$	
12	>567	>567		>100	>100	
14	>567	>567		>100	>100	
17	>567	>567		>100	>100	
18	>567	>567		>100	>100	
19	>567	$150 \pm 20$		$5.00 \pm 1.10$	>100	
21	>567	>567		$46.7 \pm 2.4$	$50.3 \pm 5.1$	
22	>567	>567		>100	>100	
23	>567	>567		>100	>100	
CPA	$1.21 \pm 0.1$	$421 \pm 68$	0.003	$0.50 \pm 0.05$	$0.14 \pm 0.04$	
NECA	$13.3 \pm 2.1$	$12.4 \pm 2.3$	1.1	$0.19 \pm 0.05$	$0.35 \pm 0.02$	
CV1808	$780 \pm 72$	$107 \pm 8.2$	7.3	$5.82 \pm 1.8$	>100	
CG21680	$1232 \pm 95$	$8.8 \pm 1.2$	140	$0.83 \pm 0.2$	>100	
DPMA	$103 \pm 10$	6.9 ± 1.3	14.9	$12.1 \pm 1.6$	>100	

<sup>a</sup> Inhibition constant for  $A_1$  (rat brain membranes, [<sup>3</sup>H]CHA) or  $A_2$  (rat striatal membranes, [<sup>3</sup>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 [<sup>3</sup>H]CHA and [<sup>3</sup>H]NECA were 2.18 ± 0.05 and 3.60 ± 0.90 nM, respectively. <sup>b</sup>Dose of compound which produced a 30% decrease in blood pressure of anesthetized SHRs. <sup>c</sup>Dose of compound which produced a 10% decrease in heart rate of anesthetized SHRs. ED<sub>30</sub> and ED<sub>10</sub> values were the mean of four animals.

Scheme IV<sup>a</sup>



(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- $\beta$ -Dribofuranosyl-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 <sup>1</sup>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).<sup>29</sup> 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-[(pchlorophenyl)thio]vinyl]adenosine (20) in 97% yield. The Z geometry of these nucleosides was identified by the

<sup>(31)</sup> 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.

<sup>(32)</sup> 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<sup>a</sup>



<sup>a</sup> (a) H<sub>2</sub>, Pd/C, EtOH; (b) (i) Ac<sub>2</sub>O, Et<sub>3</sub>N, DMAP in CH<sub>3</sub>CN, (ii) H<sub>2</sub>, Lindlar catalyst, quinoline, in EtOAc and MeOH, (iii) NH<sub>3</sub>/MeOH; (c) NaSPh-p-Cl, MeOH; (d) Hg(OAc)<sub>2</sub>, aqueous AcOH, then H<sub>2</sub>S; (e) 0.2 N HCl in dioxane, 100 °C; (f) NaNO<sub>2</sub>, 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.<sup>33</sup> Adenosine  $A_2$  receptor binding assays were done in rat striatum with tritiated NECA by the previous methods.<sup>34</sup> 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<sup>17</sup> and DPMA<sup>16</sup> 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

<sup>(33)</sup> 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.

<sup>(34)</sup> Bruns, R. F.; Lu, G. H.; Pugsley, T. A. Characterization of the A<sub>2</sub> adenosine receptor labeled by [<sup>3</sup>H]NECA in rat striatal membranes. *Mol. Pharmacol.* 1986, 29, 331-346.

atoms (hexynyl), while the  $A_1/A_2$  affinity ratios of 2-(1butyn-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 1hexyn-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<sup>18,35,36</sup> 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-1yl]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 (51-r). 1-Hydroxypropyn-1-yl derivative 51, 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 50 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 50, they indicated greatly reduced affinities at the  $A_1$  receptor compared to 50 and similar  $A_2$  selectivities to 5e. However, 4-(octyloxy)butyn-1-yl derivative 5g 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<sub>1</sub> 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<sup>17,18,35,36</sup> constitute a more precise model of the A<sub>2</sub> add nosine receptors around the C-2 pocket. Addenotions having -NH-, -O-, -S-, and -C=C- groups at the C-2 position show A<sub>2</sub> addenosine 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<sup>37</sup> and 3deazaadenosines<sup>38</sup> 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,<sup>18,35</sup> 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<sup>18,35</sup> 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.<sup>20</sup> 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.<sup>21</sup> It has been described how a selective adenosine  $A_2$  agonist showed preferential vasodilator activity.<sup>18,35,39</sup> We also indicated that hypotensive effects reflect A2 receptor activation and bradycardia reflects A<sub>1</sub> receptor activation.<sup>21</sup> 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  $ED_{30} = 0.11 \ \mu g/kg$ , 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.<sup>21</sup>

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

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

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

<sup>(37)</sup> 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.

<sup>(38)</sup> 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.

<sup>(39)</sup> 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.<sup>32</sup> 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 <sup>1</sup>H 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 ( $\delta$ ), 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<sub>2</sub>O. 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-2iodopurine (2). (a). Isoamyl nitrite (5 mL, 37 mmol) was added to a mixture of 9-(2,3,5-tri-O-acetyl-1-\$-D-ribofuranosyl)-2mino-6-chloropurine<sup>22</sup> (1, 5.12 g, 12 mmol), I<sub>2</sub> (3.04 g, 12 mmol), CH<sub>2</sub>I<sub>2</sub> (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<sub>3</sub> until the iodine color disappeared, then eluted with 1% EtOH in CHCl<sub>3</sub>. 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<sup>+</sup>). Continuous elution of the column with 2% EtOH in CHCl<sub>3</sub> 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<sup>+</sup>); NMR (CDCl<sub>3</sub>)  $\delta$  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<sub>3</sub> until removal of the color of iodine and then eluted with 1% EtOH in CHCl<sub>3</sub>. Compound 2 was obtained (877 mg, 82%, crystallized from EtOH). Further elution of the column with 4% EtOH in CHCl<sub>3</sub> gave 3a (47 mg, 5%, as a foam): MS m/z 469 (M<sup>+</sup>); NMR (CDCl<sub>3</sub>)  $\delta$  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  $\text{CH}_{2}I_{2}$  (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<sub>3</sub> to give 2 (673 mg, 63%, crystallized from EtOH).

Further elution of the column with 4% EtOH in CHCl<sub>3</sub> afforded **3b** (62 mg, 6%, as a foam): MS m/z 485 (M<sup>+</sup>); NMR (CDCl<sub>3</sub>)  $\delta$  0.94 (3 H, d, CHMe<sub>2</sub>), 1.28 (3 H, d, CHMe<sub>2</sub>), 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<sub>2</sub>), 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.<sup>25</sup> mp 142-144 °C); MS m/z 393 (M<sup>+</sup>); UV (H<sub>2</sub>O)  $\lambda_{max}$  267 nm ( $\epsilon$  13 200); NMR (DMSO- $d_6$  + D<sub>2</sub>O)  $\delta$  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<sub>10</sub>H<sub>12</sub>IN<sub>5</sub>O<sub>4</sub>) 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<sub>2</sub>O (1.5 mL) in CH<sub>3</sub>CN (30 mL).<sup>40</sup> 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<sub>2</sub>O. The separated organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>) 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<sup>+</sup>); NMR (CDCl<sub>3</sub>)  $\delta$  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<sub>2</sub>), 6.13 (1 H, d, 1'-H,  $J_{1',2'}$  = 4.9 Hz), 7.84 (1 H, s, 8-H). Anal. (C<sub>16</sub>H<sub>18</sub>IN<sub>5</sub>O<sub>7</sub>) 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<sub>3</sub>/EtOH = 10:1, v/v), the reaction mixture was concentrated to dryness under reduced pressure. The residue was dissolved in CHCl<sub>3</sub>, and H<sub>2</sub>S gas (30 s) and then N<sub>2</sub> gas were introduced to the solution. The suspension was filtered through a Celite pad and washed with CHCl<sub>3</sub>. 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<sub>3</sub> 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<sub>2</sub>O): mp 129–132 °C; MS m/z 333 (M<sup>+</sup>), 201 (B<sup>+</sup> + 1); IR (KBr)  $\nu$ C=C 2230 cm<sup>-1</sup>; UV  $\lambda_{max}$  (H<sub>2</sub>O) 287 (sh) ( $\epsilon$  9300), 270 nm ( $\epsilon$  13 600); UV  $\lambda_{max}$  (0.5 N HCl) 290 (sh) ( $\epsilon$  12 700), 272 nm ( $\epsilon$  18 600); NMR (DMSO- $d_6$  + D<sub>2</sub>O)  $\delta$  1.01 (3 H, t, Me), 1.58 (2 H, m, CH<sub>2</sub>CH<sub>3</sub>), 2.39 (2 H, m, C=CCH<sub>2</sub>), 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<sub>15</sub>H<sub>19</sub>N<sub>5</sub>O<sub>4</sub>·<sup>1</sup>/<sub>2</sub>H<sub>2</sub>O) C, H, N.

**2-(1-Hexyn-1-y1)adenosine (5c).** The reaction of **4a** with 1-hexyne for 1 h gave 5c (302 mg as a hydrate, 85%, crystallized from MeOH/H<sub>2</sub>O): mp 121-125 °C; MS m/z 347 (M<sup>+</sup>), 215 (B<sup>+</sup> + 1); IR (KBr)  $\mu$ C=C 2230 cm<sup>-1</sup>; UV  $\lambda_{max}$  (H<sub>2</sub>O) 286 (sh) ( $\epsilon$  12000), 270 nm ( $\epsilon$  17000); UV  $\lambda_{max}$  (0.5 N HCl) 286 (sh) ( $\epsilon$  14000), 270 nm ( $\epsilon$  19600); NMR (DMSO- $d_6$  + D<sub>2</sub>O)  $\delta$  0.92 (3 H, t, Me), 1.51 (4 H, m, CH<sub>2</sub>CH<sub>2</sub>CH<sub>3</sub>), 2.42 (2 H, m, C=CCH<sub>2</sub>), 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<sub>16</sub>H<sub>21</sub>N<sub>5</sub>-O<sub>4</sub>·H<sub>2</sub>O) 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<sub>2</sub>O): mp 113-115 °C; MS m/z 361 (M<sup>+</sup>), 229 (B<sup>+</sup> + 1); IR (KBr)  $\delta C \equiv C$  2230 cm<sup>-1</sup>; UV  $\lambda_{max}$  (H<sub>2</sub>O) 286 (sh) ( $\epsilon$  9800), 270 nm ( $\epsilon$  14 500); UV  $\lambda_{max}$  (0.5 N HCl) 288 (sh) ( $\epsilon$  13 200), 272 nm ( $\epsilon$  19 300); NMR (DMSO- $d_6$  + D<sub>2</sub>O)  $\delta$  0.89 (3 H, t, Me), 1.42 (6 H, m, (CH<sub>2</sub>)<sub>3</sub>), 2.52 (2 H, m, C = CH<sub>2</sub>), 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<sub>17</sub>H<sub>23</sub>N<sub>5</sub>O<sub>4</sub>·<sup>1</sup>/<sub>2</sub>H<sub>2</sub>O) 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<sub>2</sub>O): mp 101-103 °C; MS m/z 375 (M<sup>+</sup>), 243 (B<sup>+</sup>

<sup>(40)</sup> Matsuda, A.; Shinozaki, M.; Suzuki, M.; Watanabe, K.; Miyasaka, T. A convenient method for the selective acylation of guanine nucleosides. *Synthesis* 1986, 385-386.

<sup>(41)</sup> Cheng, Y.; Prusoff, W. H. Relationship between the inhibition constant (Ki) and the concentration of the inhibitor which causes 50 percent inhibition (IC<sub>50</sub>) of an enzynic reaction. Biochem. Pharmacol. 1973, 22, 3099-3108.
(42) Fleming, W. W.; Westfall, D. P.; De La Lande, I. S.; Jellett, L.

<sup>(42)</sup> Fleming, W. W.; Westfall, D. P.; De La Lande, I. S.; Jellett, L. B. Log-normal distribution of equieffective doses of nor-epinephrine and acetylcholine in several tissues. J. Pharmacol. Exp. Ther. 1972, 181, 339-345.

+ 1); IR (KBr)  $\nu C \equiv C 2230 \text{ cm}^{-1}$ ; UV  $\lambda_{max}$  (H<sub>2</sub>O) 287 (sh) ( $\epsilon 10200$ ), 269 nm ( $\epsilon 15300$ ); UV  $\lambda_{max}$  (0.5 N HCl) 294 ( $\epsilon 9900$ ), 271 nm ( $\epsilon 14800$ ); NMR (DMSO- $d_6$  + D<sub>2</sub>O)  $\delta 0.88$  (3 H, t, Me), 1.34 (8 H, m, (CH<sub>2</sub>)<sub>4</sub>), 2.48 (2 H, m, C \equiv CCH<sub>2</sub>), 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<sub>18</sub>H<sub>25</sub>N<sub>5</sub>O<sub>4</sub>·H<sub>2</sub>O) 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<sub>2</sub>O): mp 89–94 °C dec; IR (KBr)  $\nu$ C=C 2230 cm<sup>-1</sup>; UV  $\lambda_{max}$  (H<sub>2</sub>O) 284 (sh) ( $\epsilon$  13 000), 270 nm ( $\epsilon$  17 400); UV  $\lambda_{max}$  (0.5 N HCl) 292 ( $\epsilon$  14 000), 271 nm ( $\epsilon$  18 800); NMR (DMSO- $d_6$  + D<sub>2</sub>O)  $\delta$  0.87 (3 H, t Me), 1.28–1.56 (10 H, m, (CH<sub>2</sub>)<sub>6</sub>), 2.40 (2 H, m, C=CH<sub>2</sub>), 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<sub>19</sub>H<sub>27</sub>N<sub>5</sub>O<sub>4</sub><sup>-1</sup>/<sub>2</sub>H<sub>2</sub>O) 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<sub>2</sub>O): mp 123 °C; MS m/z 403 (M<sup>+</sup>), 272 (B<sup>+</sup> + 1); IR (KBr)  $\nu$ C=C 2230 cm<sup>-1</sup>; UV  $\lambda_{max}$  (MeOH) 287 (sh) ( $\epsilon$  11000), 269 nm ( $\epsilon$  15 900); NMR (DMSO- $d_6$  + D<sub>2</sub>O)  $\delta$  0.86 (3 H, t, Me), 1.28 (12 H, m, (CH<sub>2</sub>)<sub>6</sub>), 2.41 (2 H, m, C=CCH<sub>2</sub>), 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<sub>20</sub>H<sub>29</sub>H<sub>5</sub>-O<sub>4</sub>·H<sub>2</sub>O) 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<sup>+</sup>), 300 (B<sup>+</sup> + 2); IR (KBr)  $\nu$ C=C 2220 cm<sup>-1</sup>; UV  $\lambda_{max}$  (MeOH) 287 (sh) ( $\epsilon$  11 000), 268 nm ( $\epsilon$  15 700); NMR (DMSO- $d_6$  + D<sub>2</sub>O)  $\delta$  0.84 (3 H, t, Me), 1.25 (16 H, m, (CH<sub>2</sub>)<sub>8</sub>), 2.40 (2 H, m, C=CCH<sub>2</sub>), 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<sub>22</sub>H<sub>33</sub>N<sub>5</sub>O<sub>4</sub>·H<sub>2</sub>O) 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<sup>+</sup>), 328 (B<sup>+</sup> + 1); IR (KBr)  $\nu$ C=C 2230 cm<sup>-1</sup>; UV  $\lambda_{max}$  (MeOH) 285 (sh) ( $\epsilon$  11 000), 269 nm ( $\epsilon$  15 900); NMR (DMSO- $d_6$  + D<sub>2</sub>O)  $\delta$  0.86 (3 H, t, Me), 1.28 (20 H, m, (CH<sub>2</sub>)<sub>10</sub>), 2.41 (2 H, m, C=CCH<sub>2</sub>), 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<sub>24</sub>-H<sub>37</sub>N<sub>5</sub>O<sub>4</sub>) 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<sup>+</sup>), 356 (B<sup>+</sup> + 2); IR (KBr  $\nu$ C=C 2220 cm<sup>-1</sup>; UV  $\lambda_{max}$  (MeOH) 284 (sh) ( $\epsilon$  11 000), 269 nm ( $\epsilon$  15 800); NMR (DMSO- $d_6$  + D<sub>2</sub>O)  $\delta$  0.84 (3 H, t, Me), 1.25 (24 H, m, (CH<sub>2</sub>)<sub>12</sub>), 2.40 (2 H, m, C=CCH<sub>2</sub>), 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<sub>26</sub>H<sub>41</sub>N<sub>5</sub>O<sub>4</sub>-1/<sub>2</sub>H<sub>2</sub>O) 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<sup>+</sup>), 384 (B<sup>+</sup> + 2); IR (KBr)  $\nu$ C=C 2220 cm<sup>-1</sup>; UV  $\lambda_{max}$  (MeOH) 287 (sh) ( $\epsilon$  11000), 270 nm ( $\epsilon$  15800); NMR (DMSO-d<sub>6</sub> + D<sub>2</sub>O)  $\delta$  0.86 (3 H, t, Me), 1.27 (28 H, m, (CH<sub>2</sub>)<sub>14</sub>), 2.39 (2 H, m, C=CCH<sub>2</sub>), 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<sub>28</sub>H<sub>45</sub>N<sub>5</sub>O<sub>4</sub>·<sup>1</sup>/<sub>2</sub>H<sub>2</sub>O) C, H, N.

2-(3-Hydroxy-1-propyn-1-yl)adenosine (51). Compound 51 (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<sup>+</sup>); UV  $\lambda_{max}$  (H<sub>2</sub>O) 289 ( $\epsilon$  8900), 270 ( $\epsilon$  13 300), 267 (sh) nm ( $\epsilon$  13 000); UV  $\lambda_{max}$  (0.5 N HCl) 294 ( $\epsilon$  11 900), 271 ( $\epsilon$  17 800), 282 (sh) nm ( $\epsilon$  17 300); NMR (DMSO- $d_6$  + D<sub>2</sub>O)  $\delta$  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<sub>2</sub>OH), 4.55 (1 H, t, 2'-H), 5.88 (1 H, d, 1'-H), 8.42 (1 H, s, H-8). Anal. (C<sub>13</sub>H<sub>15</sub>N<sub>5</sub>O<sub>5</sub>) 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<sup>+</sup>); IR (KBr)  $\nu$ C==C 2230 cm<sup>-1</sup>; UV  $\lambda_{max}$  (H<sub>2</sub>O) 286 (sh) ( $\epsilon$  10 100), 270 nm ( $\epsilon$  14 800); UV  $\lambda_{max}$  (0.5 N HCl) 291 (sh) ( $\epsilon$  13 300), 271 nm ( $\epsilon$  19 600); NMR (DMSO- $d_6$  + D<sub>2</sub>O)  $\delta$  3.59 (2 H, m, CH<sub>2</sub>CH<sub>2</sub>OH), 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<sub>5</sub>O<sub>5</sub>·H<sub>2</sub>O) 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  $\lambda_{max}$  (H<sub>2</sub>O) 289 (sh), 270 ( $\epsilon$  13 200), 264 (sh) nm; UV  $\lambda_{max}$  (0.5 N HCl) 294 ( $\epsilon$  9600), 271 ( $\epsilon$  14 100), 264 (sh) nm; NMR (DMSO- $d_6$  + D<sub>2</sub>O)  $\delta$  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<sub>2</sub>), 4.55 (1 H, t, 2'-H), 5.87 (1 H, d, 1'-H), 8.43 (1 H, s, H-8). Anal. (C<sub>14</sub>H<sub>17</sub>N<sub>5</sub>O<sub>5</sub>-1/<sub>2</sub>H<sub>2</sub>O) C, H, N.

**2-(3-Butoxy-1-propyn-1-yl)adenosine (50).** Compound **50** (198 mg, 51%, crystallized from EtOH) was obtained from **4a** with 3-butoxy-1-propyne for 1 h: mp 105–110 °C; UV  $\lambda_{max}$  (H<sub>2</sub>O) 289 ( $\epsilon$  8900), 270 nm ( $\epsilon$  13000); UV  $\lambda_{max}$  (0.5 N HCl) 290 ( $\epsilon$  9800), 270 nm ( $\epsilon$  13000); UV  $\lambda_{max}$  (0.5 N HCl) 290 ( $\epsilon$  9800), 270 nm ( $\epsilon$  14000); NMR (DMSO- $d_6$  + D<sub>2</sub>O)  $\delta$  0.90 (3 H, t, Me), 1.34, 1.52 (each 2 H, m, CH<sub>2</sub>CH<sub>2</sub>), 3.51 (2 H, t, CH<sub>2</sub>O), 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<sub>2</sub>), 4.54 (1 H, t, 2'-H), 5.86 (1 H, d, 1'-H), 8.43 (1 H, s, H-8). Anal. (C<sub>17</sub>H<sub>23</sub>N<sub>5</sub>O<sub>5</sub>·<sup>2</sup>/<sub>3</sub>H<sub>2</sub>O) C, H, N.

**2-(4-Propoxy-1-butyn-1-yl)adenosine (5p).** Compound **5p** (162 mg, 42%, as a powder from CHCl<sub>3</sub>/EtOAc) was obtained from **4a** with 4-propoxy-1-butyne for 1 h: IR (KBr)  $\nu$ C=C 2230 cm<sup>-1</sup>; UV  $\lambda_{max}$  (H<sub>2</sub>O) 284 (sh), 270 nm ( $\epsilon$  14 000); UV  $\lambda_{max}$  (0.5 N HCl) 293 ( $\epsilon$  10 600), 270 nm ( $\epsilon$  15 200); NMR (DMSO- $d_6$  + D<sub>2</sub>O)  $\delta$  0.87 (3 H, t, Me), 1.53 (2 H, m, CH<sub>2</sub>CH<sub>3</sub>), 2.65 (2 H, t, C=CCH<sub>2</sub>), 3.38–3.65 (6 H, m, 5'-Ha,b, CH<sub>2</sub>OCH<sub>2</sub>), 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<sub>17</sub>H<sub>23</sub>N<sub>5</sub>O<sub>5</sub>·1/<sub>2</sub>H<sub>2</sub>O) C, H, N.

**2-[4-(Octyloxy)-1-butyn-1-yl]adenosine (5q).** Compound **5q** (36 mg, 8%, as a powder from MeOH/H<sub>2</sub>O) was obtained from **4a** with 4-(octyloxy)-1-butyne for 1 h: IR (KBr)  $\nu$ C=C 2230 cm<sup>-1</sup>; UV  $\lambda_{max}$  (MeOH) 290 (sh), 270 nm ( $\epsilon$  14 200); NMR (DMSO-d<sub>6</sub> + D<sub>2</sub>O)  $\delta$  0.84 (3 H, t, Me), 1.23–1.47 (12 H, m, (CH<sub>2</sub>)<sub>6</sub>), 2.63 (2 H, t, C=CCH<sub>2</sub>), 3.42 (2 H, t, CH<sub>2</sub>O), 3.53–3.69 (4 H, m, 5'-Ha,b, CH<sub>2</sub>O), 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<sub>22</sub>H<sub>33</sub>N<sub>5</sub>O<sub>5</sub>·<sup>1</sup>/<sub>2</sub>H<sub>2</sub>O) C, H, N.

**2-(5-Ethoxy-1-pentyn-1-yl)adenosine (5r).** Compound **5r** (143 mg, 38%, as a powder from CHCl<sub>3</sub>/Et<sub>2</sub>O) was obtained from **4a** with 5-ethoxy-1-pentyne for 1 h: IR (KBr)  $\nu$ C=C 2230 cm<sup>-1</sup>; UV  $\lambda_{max}$  (H<sub>2</sub>O) 285 (sh), 270 nm ( $\epsilon$  13 200); UV  $\lambda_{max}$  (0.5 N HCl) 293 ( $\epsilon$  10 000), 270 nm ( $\epsilon$  14 400); NMR (DMSO- $d_6$  + D<sub>2</sub>O)  $\delta$  1.12 (3 H, t, Me), 1.76 (2 H, t, OCH<sub>2</sub>CH<sub>2</sub>), 2.44 (2 H, t, C=CCH<sub>2</sub>), 3.42-3.70 (6 H, m, 5'-Ha,b, CH<sub>2</sub>OCH<sub>2</sub>), 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<sub>16</sub>H<sub>18</sub>N<sub>5</sub>O<sub>6</sub>·<sup>1</sup>/<sub>2</sub>H<sub>2</sub>O) C, H, N.

**2-[2-(Trimethylsilyl)-1-ethyn-1-yl]adenosine (6).** Compound 6 (352 mg, 97%, crystallized from MeOH/H<sub>2</sub>O) was obtained from 4a with (trimethylsilyl)acetylene for 1 h: mp 168-170 °C; MS m/z 363 (M<sup>+</sup>), 231 (B<sup>+</sup> + 1); IR (KBr)  $\nu$ C=C 2160 cm<sup>-1</sup>; UV  $\lambda_{max}$  (H<sub>2</sub>O) 293 ( $\epsilon$  11 400), 271 nm ( $\epsilon$  16 200); NMR (DMSO- $d_6$  + D<sub>2</sub>O)  $\delta$  0.23 (9 H, s, SiMe<sub>3</sub>), 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<sub>15</sub>H<sub>21</sub>N<sub>5</sub>O<sub>4</sub>Si) 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  $(3.3 \times 12 \text{ cm})$ , which was washed with 1-4% EtOH in CHCl<sub>3</sub> then eluted with 8% EtOH in CHCl<sub>3</sub>. 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<sup>+</sup>), 188 (B<sup>+</sup> + 1); IR (KBr)  $\nu$ C=C 2220 cm<sup>-1</sup>; UV  $\lambda_{max}$  (H<sub>2</sub>O) 288 (sh) ( $\epsilon$  12000), 270 nm ( $\epsilon$  17000); NMR (DMSO- $d_6$  + D<sub>2</sub>O)  $\delta$  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<sub>2</sub>S, 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<sub>2</sub>O (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<sub>3</sub>. From this fraction, 7 (2.6 g, 77%, crystallized from MeOH) was obtained: mp 174-176 °C; IR (KBr)  $\nu$ C=C 2110 cm<sup>-1</sup>; UV  $\lambda_{max}$  (MeOH) 290 ( $\epsilon$  8700), 267 nm (ε 14 100); NMR (CDCl<sub>3</sub>) 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<sub>2</sub>), 8.46 (1 H, s, 8-H). Anal.  $(C_{18}N_{19}N_5O_7^{-1}/_2H_2O)$  C, H, N. Continuous elution of the column with 8% EtOH in CHCl<sub>3</sub> afforded 8 (90 mg, 3%, crystallized from EtOH): mp 239-242 °C; IR (KBr)  $\nu$ C=C 2130 cm<sup>-1</sup>; UV  $\lambda_{max}$ (MeOH) 331 ( $\epsilon$  27 100), 263 nm ( $\epsilon$  29 400); UV  $\lambda_{min}$  285 ( $\epsilon$ , 15 400), 258 nm (ε 28 900); NMR (DMSO-d<sub>6</sub>) 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<sub>2</sub>), 8.49 (2 H, s, 8-H). Anal. (C<sub>36</sub>H<sub>36</sub>N<sub>10</sub>O<sub>14</sub>) C, H, N.

Synthesis of 8 from 7. Oxygen was introduced to a solution of 7 (50 mg, 0.12 mmol) and Cu(OAc)<sub>2</sub>·H<sub>2</sub>O (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<sub>3</sub>. 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<sub>3</sub>N (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<sub>3</sub>. H<sub>2</sub>S 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<sub>3</sub>. From this fraction, a hygroscopic reddish crystalline 10 (342 mg, 83%, crystallized from hot EtOH) was obtained: mp 149-155 °C; UV  $\lambda_{max}$  (MeOH) 313 ( $\epsilon$  19300), 266 nm ( $\epsilon$  25000): UV  $\lambda_{min}$ (MeOH) 285 (ε 13800), 257 nm (ε 23000); NMR (DMSO-d<sub>6</sub>) 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<sub>2</sub>), 8.53 (2 H, s, 8-H). Anal.  $(\tilde{C}_{34}H_{36}N_{10}O_{14}H_2O)$  C, H, N.

**2-(1-Ethyn-1-yl)adenosine (9).** Compound 6 (1.39 g, 3.8 mmol) in NH<sub>3</sub>/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<sup>+</sup>), 159 (B<sup>+</sup> + 1); IR (KBr)  $\nu$ C=C 2110 cm<sup>-1</sup>; UV  $\lambda_{max}$  (H<sub>2</sub>O) 286 (sh) ( $\epsilon$  9200), 268 ( $\epsilon$  15 000), 263 (sh) nm ( $\epsilon$  14 500); UV  $\lambda_{min}$  (H<sub>2</sub>O) 242 nm ( $\epsilon$  7300); NMR (DMSO- $d_6$  + D<sub>2</sub>O)  $\delta$  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<sub>12</sub>H<sub>13</sub>N<sub>5</sub>O<sub>4</sub>) 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<sub>3</sub>N (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<sub>3</sub>. 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<sup>+</sup>); UV  $\lambda_{max}$  (MeOH) 266 nm ( $\epsilon$  14 300); UV  $\lambda_{min}$  (MeOH) 239 nm ( $\epsilon$ 6300); NMR (CDCl<sub>3</sub>)  $\delta$  1.28 (3 H, t, CH<sub>2</sub>CH<sub>3</sub>), 2.74 (2 H, d,  $\begin{array}{l} {\rm CH_2CO_2Et},\,J=4.9\,{\rm Hz}),\,3.76\,(1\,{\rm H},\,{\rm dd},\,{\rm H-5'a},\,J_{4',5'a}=2.9,\,J_{5'a,5'b}\\ =\,12.5\,{\rm Hz}),\,3.95\,(1\,{\rm H},\,{\rm dd},\,{\rm H-5'b},\,J_{4',5'b}=1.0\,{\rm Hz}),\,4.19\,(2\,{\rm H},\,{\rm q},\\ {\rm CH_2CH_3}),\,4.43\,(1\,{\rm H},\,{\rm br}\,{\rm s},\,{\rm H-4'}),\,5.13\,(1\,{\rm H},\,{\rm dd},\,{\rm H-3'},\,J_{2',3'}=6.4,\\ J_{3',4'}=2.4\,{\rm Hz}),\,5.27\,(1\,{\rm H},\,{\rm dd},\,{\rm H-2'},\,J_{1',2'}=3.4,\,J_{2',3'}=6.4\,{\rm Hz}),\,5.78\\ (1\,{\rm H},\,{\rm t},\,{\rm end}\,\,{\rm H},\,J=4.9\,{\rm Hz}),\,5.89\,(1\,{\rm H},\,{\rm d},\,{\rm H-1'},\,J_{1',2'}=3.4\,{\rm Hz}),\\ 6.52\,(2\,{\rm H},\,{\rm br}\,{\rm s},\,6-{\rm NH}_2),\,7.76\,(1\,{\rm H},\,{\rm s},\,{\rm H-8}).\,\,{\rm Anal.}\,\,({\rm C}_{15}{\rm H_{18}}{\rm IN}_5{\rm O_8})\\ {\rm C},\,{\rm H},\,{\rm N}.\,\,{\rm Data}\,\,{\rm for}\,\,16:\,\,{\rm mp}\,108-110\,\,^{\circ}{\rm C};\,{\rm MS}\,\,m/z\,\,491\,\,({\rm M^+});\,{\rm UV}\\ \lambda_{\rm max}\,\,({\rm MeOH})\,\,266\,\,{\rm nm}\,\,(\epsilon\,14\,000);\,{\rm UV}\,\lambda_{\rm min}\,\,({\rm MeOH})\,\,239\,\,{\rm nm}\,\,(\epsilon\,6300);\\ {\rm NMR}\,\,({\rm CDCl}_3)\,\,1.30\,\,(3\,{\rm H},\,{\rm t},\,{\rm CH}_2{\rm CH}_3),\,2.88\,\,(2\,{\rm H},\,{\rm d},\,{\rm CCO}_2{\rm Et},\\ J=5.4\,{\rm Hz}),\,3.78\,\,(1\,{\rm H},\,{\rm d},\,{\rm H-5'a},\,J_{4',5'a}=2.0,\,J_{5'a,5'b}=12.7\,{\rm Hz}),\\ 3.99\,\,(1\,{\rm H},\,{\rm dd},\,{\rm H-5'b},\,J_{4',5'b}=1.5\,{\rm Hz}),\,4.21\,\,(2\,{\rm H},\,{\rm q},\,{\rm CH}_2{\rm O}_2{\rm Hz}),\\ (1\,{\rm H},\,{\rm br}\,\,{\rm s},\,{\rm H-4'}),\,5.05\,\,(1\,{\rm H},\,{\rm dd},\,{\rm H-3'},\,J_{2',3'}=5.9\,\,J_{3',4'}=1.0\,{\rm Hz}),\\ 5.21\,\,(1\,{\rm H},\,{\rm dd},\,{\rm H-2'},\,J_{1',2'}=4.9,\,J_{2',3'}=5.9\,\,{\rm Hz}),\,5.51\,\,(1\,{\rm H},\,{\rm t},\,{\rm exo}\\{\rm H},\,J=5.4\,{\rm Hz}),\,5.86\,\,(1\,{\rm H},\,{\rm d},\,{\rm H-1'},\,J_{1',2'}=4.9\,\,{\rm Hz}),\,5.51\,\,(1\,{\rm H},\,{\rm t},\,{\rm exo}\\{\rm H},\,J=5.4\,{\rm Hz}),\,5.86\,\,(1\,{\rm H},\,{\rm d},\,{\rm H-1'},\,J_{1',2'}=4.9\,\,{\rm Hz}),\,5.51\,\,(1\,{\rm H},\,{\rm t},\,{\rm exo}\\{\rm H},\,J=5.4\,\,{\rm Hz}),\,5.86\,\,(1\,{\rm H},\,{\rm d},\,{\rm H-1'},\,J_{1',2'}=4.9\,\,{\rm Hz}),\,5.51\,\,(1\,{\rm H},\,{\rm t},\,{\rm exo}\\{\rm H},\,J=5.4\,\,{\rm Hz}),\,5.86\,\,(1\,{\rm H},\,{\rm d},\,{\rm H-1'},\,J_{1',2'}=4.9\,\,{\rm Hz}),\,6.25\,\,(2\,{\rm H},\,{\rm br}\\{\rm s},\,6-{\rm NH}_2),\,7.75\,\,(1\,{\rm H},\,{\rm s},\,{\rm H-8}).\,\,{\rm Anal.}\,\,({\rm C}_{15}{\rm H_{18}}{\rm IN}_5{\rm O_6}\,\,{\rm C},\,{\rm H},\,{\rm N}.\,\,{\rm Anal.}\,\,({\rm C}_{15}{\rm H_{18}}{\rm IN}_5{\rm O_6}\,\,{\rm C},\,{\rm H},\,{\rm N}.\,\,{\rm Anal.}\,\,({\rm Ch}_{15}{\rm H_{10}}\,{\rm H}),\,{\rm Ho}\,{\rm H},$ 

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<sub>2</sub>O to give 17 (260 mg, 88%): mp 116–118 °C. Synthesis of this compound was reported by Marumoto et al.,<sup>31</sup> but the melting point was not given.

2-Acetyladenosine (21). Hg(OAc)<sub>2</sub> (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<sub>2</sub>S 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<sub>2</sub>O to give 21 (148 mg, 68%): mp 232–233 °C; IR (KBr)  $\nu$ C==O 1705 cm<sup>-1</sup>; UV  $\lambda_{max}$  (H<sub>2</sub>O) 312 nm ( $\epsilon$  5800), 265 (sh) ( $\epsilon$  10 300), 261 nm ( $\epsilon$  10 400); UV  $\lambda_{min}$  (H<sub>2</sub>O) 281 ( $\epsilon$  4100), 249 nm ( $\epsilon$  7900); NMR (DMSO-d<sub>6</sub> + D<sub>2</sub>O)  $\delta$  2.66 (3 H, s, COCH<sub>3</sub>), 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<sub>1',2'</sub> = 5.9 Hz), 8.53 (1 H, s, H-8). Anal. (C<sub>12</sub>H<sub>15</sub>N<sub>5</sub>O<sub>5</sub>) 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<sub>3</sub>CN (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<sub>2</sub>O. 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<sup>+</sup>); IR (KBr)  $\nu$ C=C 2230 cm<sup>-1</sup>; UV  $\lambda_{max}$  (MeOH) 287 (sh) ( $\epsilon$  9800), 268 nm ( $\epsilon$  14700): UV  $\lambda_{min}$  (MeOH) 244 nm (ε 6800); NMR (CDCl<sub>3</sub>) δ 0.89 (3 H, t, Me), 1.31 (4 H, m, CH<sub>2</sub>), 1.66 (2 H, m, CH<sub>2</sub>), 2.07 (3 H, s, Ac), 2.15 (6 H, s, Ac), 2.16  $(3 \text{ H}, \text{ s}, \text{ Ac}), 2.45 (2 \text{ H}, \text{ t}, \text{C}=CCH_2), 4.39-4.42 (3 \text{ H}, \text{ m}, 4', 5'-\text{H}),$ 5.56 (1 H, dd, 3'-H), 5.77 (1 H, dd, 2'-H), 5.81 (2 H, br s, 6-NH<sub>2</sub>), 6.32 (1 H, d, 1'-H,  $J_{1',2'}$  = 5.9 Hz), 8.00 (1 H, s, 8-H). Anal. (C<sub>24</sub>H<sub>31</sub>N<sub>5</sub>O<sub>7</sub><sup>-1</sup>/<sub>5</sub>H<sub>2</sub>O) 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<sub>3</sub>/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<sub>3</sub> to afford 19 (1.34 g, 89%, crystallized from aqueous MeOH): mp 125-126 °C; UV  $\lambda_{max}$  (H<sub>2</sub>O) 268 nm ( $\epsilon$ 13800); UV  $\lambda_{min}$  (H<sub>2</sub>O) 251 nm ( $\epsilon$  9000); UV  $\lambda_{max}$  (0.5 N HCl) 273 nm ( $\epsilon$  14100); UV  $\lambda_{min}$  (0.5 N HCl) 252 nm ( $\epsilon$  8600); NMR  $(DMSO-d_6) \delta 0.85 (3 H, t, Me), 1.24-1.45 (8 H, m, CH_2 \times 4), 2.83$ (2 H, m, CH=CHCH<sub>2</sub>), 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-CH=CH, J = 12.2 Hz), 7.16 (2 H, br s, 6-NH<sub>2</sub>), 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<sub>2</sub> atmosphere. The catalyst was removed by filtration and the

filtrate was concentrated to dryness. The residue was treated with NH<sub>3</sub>/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<sub>3</sub> to afford 18 (710 mg, 90%, as a foam): UV  $\lambda_{max}$  (H<sub>2</sub>O) 263 nm ( $\epsilon$  13700); UV  $\lambda_{min}$  (H<sub>2</sub>O) 229 nm ( $\epsilon$  3300); UV  $\lambda_{max}$  (0.5 N HCl) 258 nm ( $\epsilon$  14000); UV  $\lambda_{min}$  (0.5 N HCl) 232 nm ( $\epsilon$  4000); NMR (DMSO-d<sub>6</sub>)  $\delta$  0.85 (3 H, t, Me), 1.24-1.76 (12 H, m, CH<sub>2</sub> × 6), 2.61 (2 H, t, 2-C=CH<sub>2</sub>), 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<sub>2</sub>), 8.22 (1 H, s, H-8). Anal. (C<sub>18</sub>H<sub>29</sub>N<sub>5</sub>O<sub>4</sub>·<sup>1</sup>/<sub>2</sub>H<sub>2</sub>O) C, H, N. **2-(1-Octyn-1-yl)inosine** (22). NaNO<sub>2</sub> (2.0 g, 29 mmol) was

2-(1-Octyn-1-yl)inosine (22). NaNO<sub>2</sub> (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<sub>2</sub>O (100 mL) was extracted with a mixture of CHCl<sub>3</sub>/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<sub>3</sub> to afford 22 (1.25 g, 66.5%, as a powder from EtOAc/Et<sub>2</sub>O): IR (KBr)  $\nu$ C=C 2230 cm<sup>-1</sup>; UV  $\lambda_{max}$  (H<sub>2</sub>O) 291 nm ( $\epsilon$  12 200); UV  $\lambda_{max}$  (0.5 N NaOH) 264 nm ( $\epsilon$  12 000); NMR (DMSO-d<sub>6</sub>)  $\delta$  0.89 (3 H, t, Me), 1.31 (4 H, m, CH<sub>2</sub>) × 4), 1.44 (2 H, m, CH<sub>2</sub>), 1.57 (2 H, m, CH<sub>2</sub>), 2.49 (2 H, t, 2-C=CCH<sub>2</sub>), 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<sub>1',2'</sub> = 5.5 Hz), 8.35 (1 H, s, H-8). Anal. (C<sub>18</sub>H<sub>24</sub>N<sub>4</sub>O<sub>5</sub>·1/<sub>2</sub>H<sub>2</sub>O) 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)  $\nu C \equiv C 2230 \text{ cm}^{-1}$ ; UV  $\lambda_{max}$  (H<sub>2</sub>O) 285 (sh), 270 nm ( $\epsilon$  13 000); UV  $\lambda_{min}$  (H<sub>2</sub>O) 246 nm ( $\epsilon$  5700); UV  $\lambda_{max}$  (0.5 N HCl) 258 (sh), 270 nm ( $\epsilon$  16 900); UV  $\lambda_{min}$  (0.5 N HCl) 250 nm ( $\epsilon$  7800); UV  $\lambda_{max}$  (0.5 N NaOH) 280 nm ( $\epsilon$  14 300); UV  $\lambda_{min}$  (0.5 N NaOH) 254 nm ( $\epsilon$  5600); NMR (DMSO-d<sub>6</sub>)  $\delta$  0.89 (3 H, t, Me), 1.28-1.56 (8 H, m, CH<sub>2</sub> × 4), 2.39 (2 H, t, 2-C =CCH<sub>2</sub>), 7.20 (2 H, br s, 6-NH<sub>2</sub>), 8.13 (1 H, s, H-8). Anal. (C<sub>13</sub>H<sub>17</sub>N<sub>5</sub>) 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<sub>2</sub>O to give 20 (427 mg, 97%): mp 146-148 °C eff; UV  $\lambda_{max}$  (MeOH) 322 ( $\epsilon$  24 800), 267 nm ( $\epsilon$  14 400); UV  $\lambda_{min}$ (MeOH) 282 ( $\epsilon$  10 200), 248 nm ( $\epsilon$  6900); NMR (DMSO-d<sub>6</sub> + D<sub>2</sub>O)  $\delta$  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<sub>1',2'</sub> = 5.9 Hz), 6.51 (1 H, d, vinyl, J<sub>a,b</sub> = 10.3 Hz), 7.08 (1 H, d, vinyl, J<sub>a,b</sub> = 10.3 Hz), 7.41-7.63 (5 H, m, Ph), 8.35 (1 H, s, H-8). Anal. (C<sub>18</sub>H<sub>18</sub>ClN<sub>5</sub>O<sub>4</sub>S) 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<sub>3</sub>N (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<sub>3</sub> to give 9-(2,3,5-tri-Oacetyl- $\beta$ -D-ribofuranosyl)-6-octynylpurine (549 mg, 57%, as a foam). A solution of the above compound (486 mg, 1 mmol) was treated with NH<sub>3</sub>/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<sup>+</sup>); UV  $\lambda_{max}$  (MeOH) 288 nm ( $\epsilon$  16 600); UV  $\lambda_{min}$  (MeOH) 249 nm ( $\epsilon$  5000); NMR (DMSO- $d_6$  + D<sub>2</sub>O)  $\delta$  0.89 (3 H, t, Me), 1.26–1.62 (8 H, m, CH<sub>2</sub>-(CH<sub>2</sub>)<sub>4</sub>Me), 2.62 (2 H, m, CH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>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<sub>3</sub>N (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<sub>3</sub>. H<sub>2</sub>S 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<sub>3</sub> 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<sup>+</sup>); IR (KBr)  $\nu$ C=C 2230 cm<sup>-1</sup>; NMR (CDCl<sub>3</sub>)  $\delta$ 0.91 (3 H, t, Me), 1.26-1.74 (8 H, m, CH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>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<sub>2</sub>-(CH<sub>2</sub>)<sub>4</sub>Me), 4.28–4.58 (3 H, m, H-4', 5'a,b), 5.97 (3 H, m, H-3', 6-NH<sub>2</sub>), 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<sup>+</sup>); IR (KBr)  $\nu$ C=C 2230 cm<sup>-1</sup>; UV  $\lambda_{max}$  (MeOH) 301 (sh) ( $\epsilon$  14 800), 293 ( $\epsilon$  20 600), 287 (sh) nm ( $\epsilon$ 19000); UV  $\lambda_{\min}$  (MeOH) 248 nm ( $\epsilon$  3300); NMR (DMSO- $d_{\epsilon}$ )  $\delta$ 0.88 (3 H, t, Me), 1.13-1.76 (8 H, m, CH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>Me), 2.57 (2 H, m, CH<sub>2</sub>(CH<sub>2</sub>)<sub>4</sub>Me), 3.63 (2 H, m, H-5'a,b), 3.99 (1 H, m, H-4'), 4.19  $(1 \text{ H}, \text{ m}, \text{H-3'}), 4.97 (1 \text{ H}, \text{q}, \text{H-2'}), 5.94 (1 \text{ H}, \text{d}, \text{H-1'}, J_{1',2'} = 6.4$ Hz), 7.55 (2 H, br s, 6-NH<sub>2</sub>), 8.14 (1 H, s, H-2). Anal. ( $C_{18}H_{25}N_5O_4$ ) C, H, N.

Adenosine Receptor Binding Assay. The A<sub>1</sub> receptor binding assay was done by previously described method,<sup>38</sup> 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  $\mu$ L containing 2.5 nM [<sup>3</sup>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  $\mu$ M 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  $\mu$ g/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,<sup>34</sup> 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<sub>2</sub> and 5 nM [<sup>3</sup>H]NECA in place of 2.5 nM [<sup>3</sup>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  $\mu$ M.

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.<sup>41</sup>

Blood Pressure and Heart Rate. Male SHR anesthetized by an iv injection of urethane (500 mg/kg) and  $\alpha$ -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  $\mu$ g/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<sub>30</sub> 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<sub>10</sub> value, the mean dose that produced a 10% decrease in HR of SHR. ED<sub>30</sub> and ED<sub>10</sub> values were calculated by the method of Fleming et al.<sup>42</sup>

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  $1 \times 10^{-4}$  M of the compounds (5a–e) and 15  $\mu$ g of the enzyme were incubated at 25 °C for 26 h. The absorbances at  $\lambda_{max}$  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, 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,<sup>†</sup> Philippe L. Durette,<sup>†</sup> Thomas Rollins,<sup>‡</sup> Salvatore Siciliano,<sup>‡</sup> Dana N. Cianciarulo,<sup>‡</sup> Sumire V. Kobayashi,<sup>‡</sup> Charles G. Caldwell,<sup>†</sup> Martin S. Springer,<sup>‡</sup> and William K. Hagmann<sup>\*,†</sup>

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<sub>50</sub>'s = 3.3 and 12  $\mu$ g/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.<sup>1-4</sup> 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.<sup>5-7</sup> 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<sup>8</sup> and porcine<sup>9</sup>

- Hugli, T. E. The Structural Basis for Anaphylatoxin and Chemotactic Functions of C3a, C4a, and C5a. CRC Crit. Rev. Immunol. 1981, 1, 321-366.
- 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.

<sup>\*</sup> To whom correspondence and reprint requests should be sent.

<sup>&</sup>lt;sup>†</sup>Department of Medicinal Chemistry Research.

<sup>&</sup>lt;sup>‡</sup>Department of Immunology Research.