dence to predict good cyclic analogues of linear peptides.

The conformational study of linear tuftsin suggested the presence of a type IV β-turn at the Lys-Pro position. This information combined with previous experimental/synthetic studies led us to propose four cyclic analogues of tuftsin. Quenched dynamical searches of the conformational space of the four proposed cyclic tuftsin analogues revealed the following: direct cyclization of tuftsin through the N and C termini, as in $ctuf_1$, resulted in a complete loss of the conformational characteristics (type IV β-turn) of the linear tuftsin. Also, addition of Lys at position 5 prior to cyclization, as in $ctuf_3$, resulted in a different conformation relative to the linear tuftsin. However, addition of either Gly or Asp at position 5 ($ctuf_2$ and $ctuf_4$, respectively) resulted in a similar backbone conformation (type IV β-turn) at Lys-Pro. Also, side-chain conformations of $ctuf_2$ and $ctuf_4$ displayed most of the characteristics of the linear analogue. Thus, using the strategy outlined here, we conclude that $ctuf_2$ and $ctuf_4$ are the most reasonable candidates for synthesis and biological testing (K. Nishioka, work in progress). These studies will provide some further insight on the validity of these particular theoretical methods.

Acknowledgment. We would like to thank Prof. V. J. Hruby and Prof. K. Nishioka for numerous interesting discussions and the Robert A. Welch Foundation and the National Institutes of Health for partial support.

Note Added in Proof: K. Nishioka has performed experiments which indicate that $ctuf_2$ is 50 times more potent than tuftsin in a phagocytosis assay.

Registry No. Tyr-Gly-Gly-Phe-Met, 58569-55-4; Thr-Lys-Pro-Arg, 9063-57-4; cyclo(Thr-Lys-Pro-Arg), 83797-39-1; cyclo-(Thr-Lys-Pro-Arg-Gly), 140175-51-5; cyclo(Thr-Lys-Pro-Arg-Lys), 140175-52-6; cyclo(Thr-Lys-Pro-Arg-Asp), 140175-53-7; (S)-$CH_3CHBrCH_2CH_3$, 5787-32-6.

Nucleosides and Nucleotides. 112. 2-(1-Hexyn-1-yl)adenosine-5′-uronamides: A New Entry of Selective A_2 Adenosine Receptor Agonists with Potent Antihypertensive Activity[1]

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Chemical modifications of the potent A_2 adenosine receptor agonist 2-(1-hexyn-1-yl)adenosine (**7**, 2-HA) at the 5′-position have been carried out to find more potent and selective A_2 agonists. These analogues were evaluated for adenosine A_1 and A_2 receptor binding affinity in rat brain tissues and antihypertensive effects in spontaneously hypertensive rats (SHR). Among the series of compounds, 2-(1-hexyn-1-yl)adenosine-5′-N-cyclopropyluronamide (**16d**) had the most potent affinity to the A_2 receptor with a K_i of 2.6 nM, which is essentially the same as that of the parent agonist, 2-HA. However, the most selective agonist for the A_2 receptor was 2-(1-hexyn-1-yl)-adenosine-5′-N-methyluronamide (**16b**) with a K_i of 11 nM and a 162-fold selectivity. The N-alkyl substituents of 5′-uronamide derivatives did not seem to potentiate the A_2 binding affinity but drastically reduced the A_1 affinity compared with the parent 2-HA. Therefore, the A_1/A_2 selectivity was consequently increased. Other 5′-deoxy-5′-substituted derivatives of 2-HA such as the chloro (**20**), carboxamide (**27**, **28**), sulfonamide (**29**), urea (**30**), and thiourea (**22**) analogues were also prepared. Among these nucleosides, no active compounds with potent or selective affinities to both receptors were found except **20**. Although glycosyl conformations and sugar-puckering of these nucleosides were studied by ^1H NMR spectroscopy, there were no positive correlations between active and inactive agonists. 2-(1-Hexyn-1-yl)adenosine-5′-uronamide (**16a**) and **16d** had a potent hypotensive effect at ED_{30} values of 0.18 and 0.17 μg/kg, respectively, upon iv administration to anesthetized SHR.

There is considerable evidence to indicate that adenosine derivatives specifically modulate coronary vasodilation via one of the cell surface adenosine receptors, termed A_2, which mediates stimulation of intracellular cAMP accumulation.[2] Selective A_2 adenosine receptor agonists elicit antihypertensive activity in rats while analogues having preferential binding affinity for the A_1 receptor produce depression in heart rate and cardiac contractility, which is considered to be a side effect for antihypertensive agents.[3] Therefore, selective A_2 receptor agonists have a potential for the treatment of cardiovascular diseases with minimized toxic effects.

Recently, several A_2 selective agonists, such as N^6-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl]adenosine (**3**, DPMA),[4] 2-[[4-(2-carboxyethyl)phenethyl]amino]-adenosine-5′-N-ethyluronamide (**4**, CGS 21680),[5] 2-[2-(4-methylphenyl)ethoxy]adenosine (**5**, MPEA),[6] and 2-[(2-cyclohexylethyl)amino]adenosine (**6**, CGS 22492),[7] which are shown in Chart I along with classical standards, have been reported. We have also reported that 2-alkynyl-

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(1) Part 111: Nishio, H.; Ono, A.; Matsuda, A.; Ueda, T. Thermal stability of oligodeoxyribonucleotide duplexes containing N^6-hydroxyladenine in substitution for adenine. *Chem. Pharm. Bull.* 1992, *40*, 1355–1357.

(2) Haleen, S. J.; Evans, D. B. Selective effects of adenosine receptor agonists upon coronary resistance and heart rate in isolated working rabbit hearts. *Life Sci.* 1985, *36*, 127–137.
(3) Hutchison, A. J.; Webb, R. L.; Oei, H. H.; Ghai, G. R.; Zimmerman, M. B.; Williams, M. CGS 21680C, an A_2 selective adenosine receptor agonists with preferential hypotensive activity. *J. Pharmacol. Exp. Ther.* 1989, *251*, 47–55.
(4) Bridges, A. J.; Bruns, R. F.; Ortwine, D. F.; Priebe, S. R.; Szotek, D. L.; Trivedi, B. K. N^6-[2-(3,5-Dimethoxyphenyl)-2-(2-methylphenyl)ethyl]adenosine and its uronamide derivatives. Novel adenosine agonists with both high affinity and high selectivity for the adenosine A_2 receptor. *J. Med. Chem.* 1988, *31*, 1282–1285.
(5) Hutchison, A. J.; Williams, M.; de Jesus, R.; Yokoyama, R.; Oei, H. H.; Ghai, G. R.; Webb, R. L.; Zoganas, H. C.; Stone, G. A.; Jarvis, M. F. 2-(Arylalkylamino)adenosine-5′-uronamides: A new class of highly selective adenosine A_2 receptor ligands. *J. Med. Chem.* 1990, *33*, 1919–1924.

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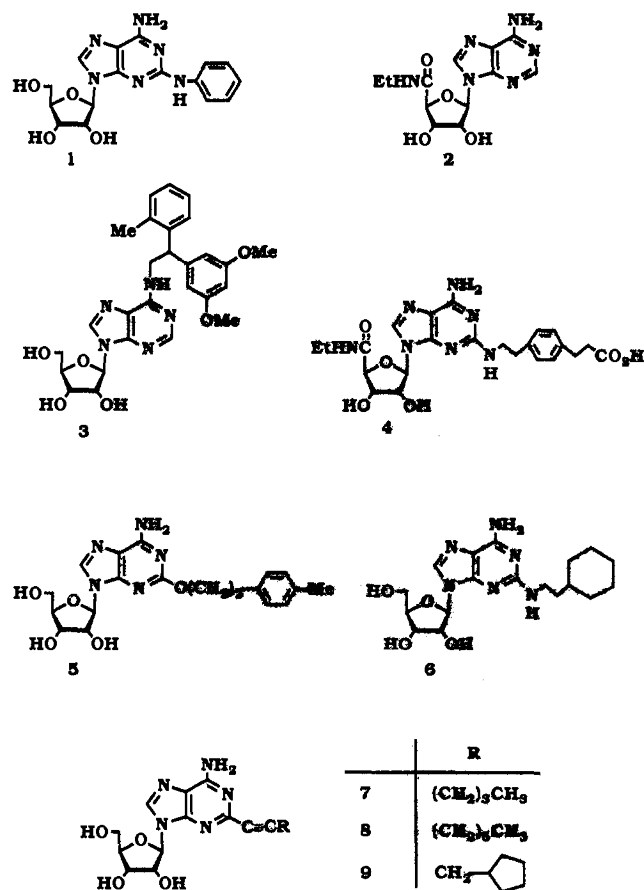
Chart I



adenosines have a profound A_2 adenosine receptor selectivity in rat brain.[8,9] From studies of the structure–activity relationships, an acetylenic functional group at the C-2 position of adenosine was essential for having affinities to both receptors, and the length of the alkyl side chain attached to the ethynyl group influenced either binding affinity or receptor selectivity. Of a series of 2-alkynyladenosines, 2-(1-hexyn-1-yl)adenosine (**7**) showed the most potent A_2 adenosine receptor affinity with a K_i of 2.8 nM and an A_1/A_2 selectivity ratio of about 40-fold.[8] This compound also had a blood pressure (BP) lowering activity, with 0.11 μg/kg as an ED_{30} upon iv administration to anesthetized spontaneously hypertensive rats (SHR), and showed a markedly potent and long-lasting antihypertensive activity upon oral administration to conscious SHR.[9] We also described cardiovascular effects of 2-(1-octyn-1-yl)adenosine (**8**, YT-146) in various mammalian preparations in comparison with adenosine as well as 2-chloroadenosine, and it was shown to be a potent, orally active, and long-lasting hypotensive agent having less cardiac depressant activity.[10] In addition, we also found that 2-(3-cyclopentylpropyn-1-yl)adenosine (**9**)[11] was a more selective A_2 agonist (70-fold) than **7** and **8**. However, 2-alkynyladenosines having 3-hydroxy-3-substituted-propyn-1-yl substituents showed a superior affinity compared with those of **7** and **9** but were not selective for the A_2 receptor.[8,11] These compounds caused a profound decrease in heart rate (HR) at lower doses than that inducing hypotensive effects in SHR.

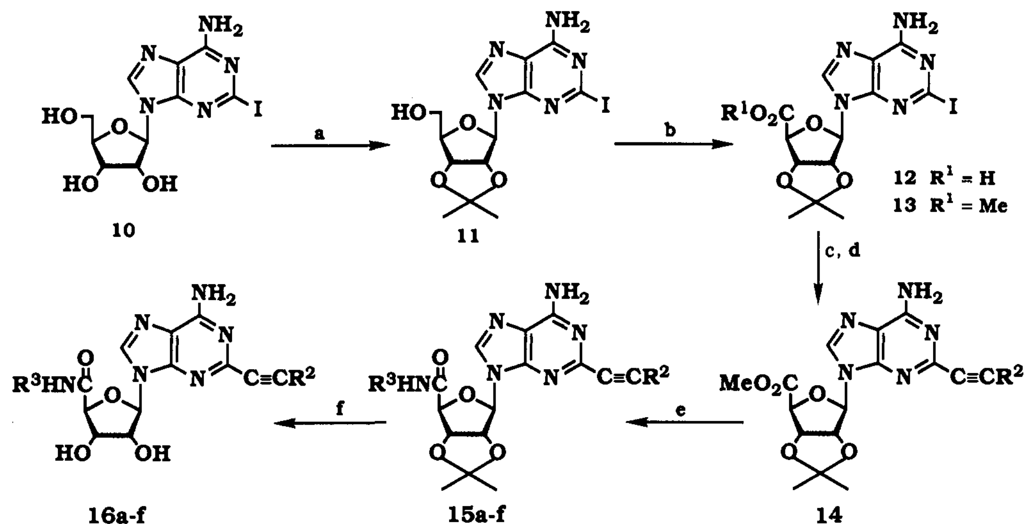
Though 1-(6-amino-9*H*-purin-9-yl)-1-deoxy-*N*-ethyl-β-D-ribofuranuronamide (**2**, NECA) is a highly potent A_2 agonist but not very selective for the A_2 receptor, a combination of the structural features of **2** and CV 1808 (**1**), classical A_2 selective standards, in one molecule has recently been reported to increase selectivity for the A_2 receptor. One such derivative is 2-[[2-(4-chlorophenyl)-ethyl]amino]adenosine-5'-*N*-ethyluronamide that has as much as 200-fold selectivity for the A_2 receptor in rat brain.[5] Other modifications at both N^6 and 5' or N^6 and C-2 positions of adenosine had no or slight enhancement of the A_2 selectivity, although great enhancement of the A_1 affinity was observed.[4,12,13] In these cases, the N^6 or C-2 substituents seemed to be the primary determinant at the A_1 or A_2 receptor. Therefore, whether a combination of both structure features of 2-alkynyladenosines and **2** (C-2-alkynyl and 5'-(*N*-alkylcarboxamide) groups) into one molecule shows additive effects to the A_2 receptor binding affinity or not, it is of interest to get more information on binding regions of the A_2 receptor. In this report, we fixed a C-2 substituent of adenosine as a 1-hexyn-1-yl group and synthesized its 5'-(*N*-alkyluronamide) derivatives, **16a–f**. For further comparison of structural requirements for the binding affinity we prepared 5'-(acylamino)-5'-deoxy and 5'-deoxy-5'-ureido analogues of 2-HA **27–31**, which have reversed relationships of the substituent from **2** (4'-CONHR to 5'-NHCOR). Additionally, binding regions near the 5' position of adenosine are explored using 5'-chloro-5'-deoxy derivative **20**. Moreover, we examined BP-lowering activities and effects on HR in SHRs of these nucleosides.

Results and Discussion

Chemistry. In Scheme I, the synthetic route of 2-(1-hexyn-1-yl)adenosine-5'-uronamides **16a–f** is depicted. The acetonide **11** of 2-iodoadenosine was prepared in 96% yield from 2-iodoadenosine (**10**)[8,14] by reaction with acetone with 70% $HClO_4$ as acid catalyst. Oxidation of the 5'-hydroxyl group in **11** with RuO_4 (prepared from RuO_2 with $NaIO_4$ in a mixture of CH_3CN, $CHCl_3$, and H_2O) afforded

(6) Ueeda, A.; Thompson, R. D.; Arroyo, L. H.; Olsson, R. A. 2-Aralkoxyadenosines: potent and selective agonists at the coronary artery A_2 adenosine receptor. *J. Med. Chem.* **1991**, *34*, 1340–1344.
(7) Francis, J. E.; Webb, R. L.; Ghai, G. R.; Hutchison, A. J.; Moskal, M. A.; deJesus, R.; Yokoyama, R.; Rovinski, S. L.; Contardo, N.; Dotson, R.; Barclay, B.; Stone, G. A.; Jarvis, M. F. Highly selective adenosine A_2 receptor agonists in a series of N-alkylated 2-aminoadenosines. *J. Med. Chem.* **1991**, *34*, 2570–2579.
(8) Matsuda, A.; Shinozaki, M.; Yamaguchi, T.; Homma, H.; Nomoto, R.; Miyasaka, T.; Watanabe, Y.; Abiru, T. Nucleosides and Nucleotides. 103. 2-Alkynyladenosines: a novel class of selective adenosine A_2 receptor agonists with potent antihypertensive effects. *J. Med. Chem.* **1992**, *35*, 241–252.
(9) Abiru, T.; Yamaguchi, T.; Watanabe, Y.; Kogi, K.; Aihara, K.; Matsuda, A. The antihypertensive effect of 2-alkynyladenosines and their selective affinity for adenosine A_2 receptors. *Eur. J. Pharmacol.* **1991**, *196*, 69–76.
(10) Kogi, K.; Uchibori, T.; Aihara, K.; Yamaguchi, T.; Abiru, T. Pharmacological profile of the 2-alkynyladenosine derivative 2-octynyladenosine (YT-146) in the cardiovascular system. *Jpn. J. Pharmacol.* **1991**, *57*, 153–165.
(11) Abiru, T.; Miyashita, T.; Matanabe, Y.; Yamaguchi, T.; Matsuda, A. Nucleosides and Nucleotides. 107. 2-(Cycloalkylalkynyl)adenosines: Adenosine A_2 receptor agonists with potent antihypertensive effects. *J. Med. Chem.*, in press.
(12) Olsson, R. A.; Kusachi, S.; Thompson, R. D.; Ukena, D.; Padgett, W.; Daly, J. W. N^6-Substituted *N*-alkyladenosine-5'-uronamides: Bifunctional ligands having recognition groups for A_1 and A_2 adenosine receptors. *J. Med. Chem.* **1986**, *29*, 1683–1689.
(13) Trivedi, B. K.; Bridges, A. J.; Bruns, R. F. In *Adenosine and Adenosine Receptors*; Williams, M., Ed.; The Humana Press Inc.: Clifton, NJ, 1990; pp 57–103.
(14) Nair, V.; Young, D. A. A new synthesis of isoguanosine. *J. Org. Chem.* **1985**, *50*, 406–408.

Scheme I[a]



$R^2 = (CH_2)_3CH_3$

R^3 : a series, H; b series, Me; c series, Et;

d series, cyclopropyl; e series, Pr; f series, Bu.

[a] Reaction conditions: (a) acetone, 70% $HClO_4$; (b) RuO_2, $NaIO_4$ in CH_3CN, $CHCl_3$, and H_2O (1:1:2); (c) $SOCl_2$ in MeOH; (d) HC≡C(C-$H_2)_3CH_3$, $(Ph_3P)_2PdCl_2$, CuI, Et_3N in DMF; (e) R^3NH_2 in MeOH; (f) 80% aqueous trifluoroacetic acid.

crude carboxylic acid 12 in 68% yield with contamination by some metals. In this oxidation, the iodo group at the C-2 position was not found to be oxidized. However, oxidation of 11 with $KMnO_4$ in aqueous alkaline solution, even under high-dilution conditions, gave a complex mixture due to oxidation of the 2-iodo group. The crude 12 was then converted into its methyl ester 13 with thionyl chloride in MeOH in 78% yield as a colorless foam. The palladium-catalyzed cross-coupling reaction of 12 with 1-hexyne was done as described previously with bis(triphenylphosphine)palladium dichloride in the presence of CuI in DMF containing Et_3N to give 2-(1-hexyn-1-yl) derivative 14 in almost quantitative yield.[8,15]

Compound 14 was heated in a MeOH solution of NH_3, methylamine, ethylamine, cyclopropylamine, *n*-propylamine, or *n*-butylamine in a sealed tube which gave the corresponding 5'-uronamides 15a–f in moderate to high isolated yields. Cleavage of the acetonides of 15a–f with aqueous 80% trifluoroacetic acid at room temperature furnished *N*-alkyl-2-(1-hexyn-1-yl)adenosine-5'-uronamides 16a–f in excellent yields.

A solid-state conformation of NECA (2) was recently disclosed in syn-conformation around its glycosyl linkage in which a hydrogen bond is observed between the N^3 position of the adenine ring and the NH of the carboxamide group.[16] In solution, 2 prefers a *syn*-glycosyl conformation as determined from its nuclear Overhouser effect (NOE) of the ^1H NMR spectra.[17] If the substituents change from 4'-CONHR into 5'-NHCOR, in which the amido proton could have a similar pK_a value to that of 2, comparable effects on the conformation around the glycosyl linkage can be achieved. Since the 5' binding region of the adenosine A_2 receptor has been reported to accommodate only sizes as small as an ethyl and a cyclopropyl,[17] we introduced carboxamides, sulfonamides, ureas, and thioureas, with small substituents, at the 5' position. We also prepared the 5'-chloro-5'-deoxy derivative of 7.

Compound 11 was initially converted into 2-(1-hexyn-1-yl) derivative 17 as described above, and then the 5'-hydroxyl group was transformed to other functional groups as shown in Scheme II. On treatment of 17 with triphenylphosphine and diethyl azodicarboxylate in the presence of diphenyl phosphorazidate in THF,[18] the desired 5'-azido-5'-deoxy derivative 18 was obtained in 83% yield as a foam. Reduction of the azide group in 18 was done by a reaction with triphenylphosphine in aqueous pyridine to give amino derivative 21 as a crude foam, which was further treated with acetic formic anhydride or acetic anhydride in pyridine, affording the corresponding formamide and acetamide 22 and 23 in good yields. Methylsulfonylation of 21 gave raise to the methanesulfonamide 24. Reaction of 21 with methyl isocyanate or methyl isothiocyanate in pyridine at room temperature furnished the methylureido and methylthioureido derivatives 25 and 26 in good yields, respectively. The 5'-chloro derivative 19 was also prepared from 17 with triphenylphosphine in CCl_4 in 81% yield. Deprotection of the isopropylidene group of the nucleosides 19 and 22–26 was done similarly as described above to furnish 20 and 27–31 in high isolated yields.

As described above, NECA (2) prefers the syn-conformation around the glycosyl linkage in both solution and

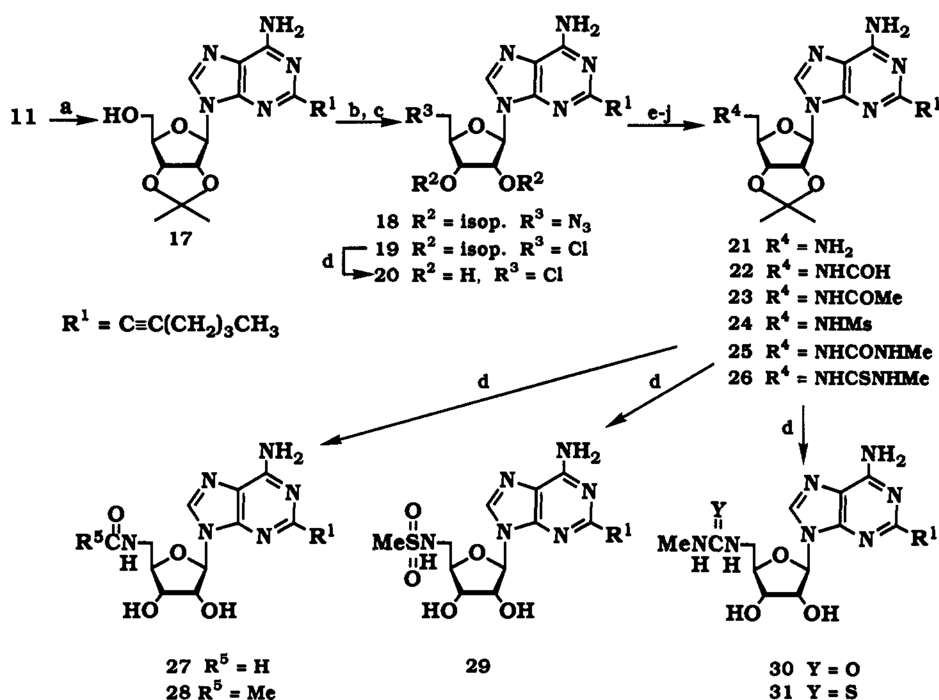
(15) Matsuda, A.; Shinozaki, M.; Miyasaka, T.; Machida, H.; Abiru, T. Palladium-catalyzed cross-coupling of 2-iodoadenosine with terminal alkynes: synthesis and biological activities of 2-alkynyladenosines. *Chem. Pharm. Bull.* 1985, *33*, 1766–1769.

(16) Moos, W. H.; Hamilton, H. W.; Ortwine, D. F.; Taylor, M. D.; McPhail, A. T. Adenosine receptor agonists. X-ray crystal structure of NECA 1-(6-amino-9*H*-purin-9-yl)-1-deoxy-*N*-ethyl-β-D-ribofuranuronamide. *Nucleosides Nucleotides* 1989, *8*, 449–461, and references cited therein.

(17) Prasad, R. N.; Bariana, D. S.; Fung, A.; Savic, M.; Tietje, K. 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.

(18) Matsuda, A.; Yasuoka, J.; Sasaki, T.; Ueda, T. Nucleosides and Nucleotides. 95. Improved synthesis of 1-(2-azido-2-deoxy-β-D-arabinofuranosyl)cytosine (Cytarazid) and -thymine. Inhibitory spectrum of cytarazid on the growth of various human tumor cells in vitro. *J. Med. Chem.* 1991, *34*, 999–1002.

Scheme II[a]



[a] Reaction conditions: (a) HC≡C$(CH_2)_3CH_3$, $(Ph_3P)_2PdCl_2$, CuI, Et_3N in DMF; (b) (17→18), diphenylphosphoryl azide, Ph_3P, DEAD in THF; (c) (17→19), CCl_4, Ph_3P; (d) 80% aqueous trifluoroacetic acid; (e) (18→21), Ph_3P in aqueous pyridine; (f) (21→22), acetic formic anhydride in pyridine; (g) (21→23), Ac_2O in pyridine; (h) (21→24), MsCl, Et_3N in CH_2Cl_2; (i) (21→25), methyl isocyanate in pyridine, (j) (21→26), methyl isothiocyanate in pyridine.

Table I. Conformational Analysis of 2-(1-Hexyn-1-yl)adenosine Derivatives and NECA (2)

compd:	16c		20		27		7 (2-HA)		2 (NECA)	
$J_{1',2'}$ (Hz)	7.7		5.9		6.6		6.1		7.8	
$J_{3',4'}$ (Hz)	1.1		3.4		*a*		3.3		1.5	
2'-end %[b]	88		63		*a*		65		84	
irrd proton:	[8]	[1']	[8]	[1']	[8]	[1']	[8]	[1']	[8]	[1']
NOE (%)[c] 1'	12.4	*d*	5.9	*d*	8.5	*d*	6.6	*d*	11.8	*d*
2'	3.2	2.4	5.7	3.1	4.0	1.7	4.1	2.7	3.2	2.2
3'	*d*	*d*	0.9	*d*	1.3	*d*	0.8	*d*	*d*	*d*
4'	*d*	2.4	*d*	2.5	*d*	1.7	*d*	2.2	*d*	2.7
8	*d*	12.9	*d*	5.2	*d*	9.1	*d*	5.9	*d*	9.7

[a] H-3' and H-4' protons were overlapped. [b] Calculated as described in ref 19. [c] When indicated proton is saturated, enhancements (%) are described below. [d] Not observed.

solid state,[16,17] the characters of which in NECA were expected to increase affinities to the A_2 receptor.[16] We therefore analyzed sugar-puckering and glycosyl conformations of **2**, **7**, **16c**, **20**, and **27** using ^{1}H NMR spectroscopy. From vicinal coupling constants between H-1' and H-2', and between H-3' and H-4',[19] **2** and **16c** showed a preferential equilibrium for C-2'-endo forms (84% and 88%, respectively), while **7** and **20** had a similar tendency toward C-2'-endo equilibrium (63% and 65%, respectively) but to a lesser extent than 5'-carboxamide derivatives. On **27**, as both H-3' and H-4' appeared at 4.00 ppm as a broad singlet, the $J_{3',4'}$ value could not be measured. The value of $J_{1',2'}$ (6.6 Hz) suggested that **27** would also have a equilibrium preferential to the C-2'-endo form. These data together with nuclear Overhauser effects (NOE) of the compounds are listed in Table I. When H-8 of **2** was irradiated, enhancements of the H-1' (11.8%) and the H-2' (3.2%) resonances were observed, and NOEs were also observed at H-8 (9.7%), H-2' (2.2%), and H-4' (2.7%) signals when H-1' of **2** was saturated. The marked enhancements of H-8 and H-1' confirm the close proximity

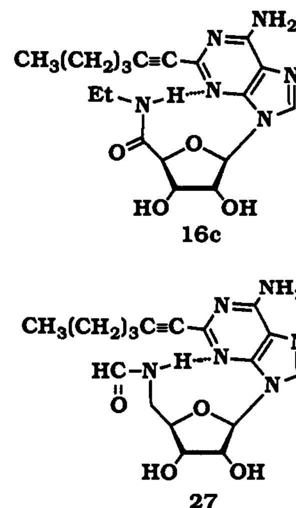
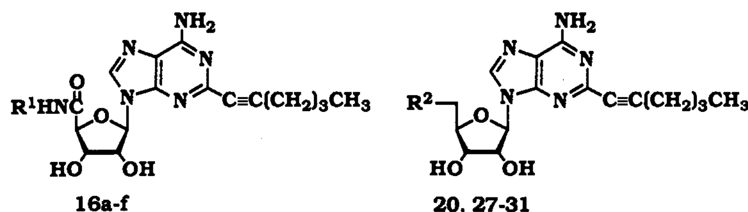


Figure 1. Syn conformation of **16c** and **27**.

(19) Altona, C.; Sundaralingam, M. Conformational analysis of the sugar ring in nucleosides and nucleotides. Improved method for the interpretation of proton magnetic resonance coupling constants. *J. Am. Chem. Soc.* **1973**, *95*, 2333–2344.

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



compd	R^1 or R^2	K_i,[a] nM ± SEM A_1	K_i,[a] nM ± SEM A_2	selectivity: A_1/A_2	BP ED_{30}[b] ± SEM (μg/kg)	HR ED_{10}[c] ± SEM (μg/kg)
16a	R^1 = H	1270 ± 215	19 ± 6.7	67	0.18 ± 0.02	>100
16b	R^1 = Me	1785 ± 264	11 ± 1.6	162	0.51 ± 0.13	>100
16c	R^1 = Et	276 ± 25	4.8 ± 1.3	58	0.30 ± 0.07	>100
16d	R^1 = c-Pr	136 ± 17	2.6 ± 0.6	52	0.17 ± 0.02	40.7 ± 2.4
16e	R^1 = Pr	1807 ± 192	44 ± 6.3	41	2.88 ± 0.19	>100
16f	R^1 = Bu	1932 ± 286	169 ± 40	11	26.4 ± 3.1	>100
20	R^2 = Cl	217 ± 21	5.2 ± 0.9	42	>3	>3
27	R^2 = NHCHO	>472	>181	-	>100	>100
28	R^2 = NHCOMe	>472	>181	-	>100	>100
29	R^2 = $NHSO_2Me$	>472	>181	-	>100	>100
30	R^2 = NHCONHMe	>472	>181	-	>100	>100
31	R^2 = NHCSNHMe	>472	>181	-	54.5 ± 9.2	>100
1 (CV1808)		529 ± 32	83 ± 7.3	6	6.10 ± 1.95	>100
2 (NECA)		8.3 ± 1.6	10 ± 2.5	0.8	0.23 ± 0.05	0.35 ± 0.02
3 (DPMA)		64 ± 5.1	4.6 ± 1.3	14	12.3 ± 1.6	>100
4 (CGS21680)		1138 ± 173	10 ± 1.4	114	0.83 ± 0.20	>100
7 (2-HA)		103 ± 21	2.4 ± 0.3	43	0.11 ± 0.03	70.4 ± 14.3
8 (2-OA)		117 ± 17	6.1 ± 0.9	19	0.35 ± 0.02	>100
CPA		0.76 ± 0.08	336 ± 6.4	0.002	0.53 ± 0.05	0.17 ± 0.04

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

between both protons as required in the syn-conformation.[20] Compound **16c** has almost the same glycosyl conformation as that of **2** (Figure 1). Although **27** preferred a syn-conformation, but to a lesser extent than **2** and **16c** in syn population, it might also be due to hydrogen-bonding between N^3 of the adenine ring and NH of the 5′-carboxamide group. Both **7** and **20** exist in about a 1:1 anti-syn conformational ratio. Thus, the 2-hexynyl substituent does not interfere with rotation around the glycosyl linkage.

Adenosine Receptor Binding Affinity

The A_1 and A_2 adenosine receptor binding affinities for the analogues **16a–f**, **20**, and **27–31** with relevant standards were measured in the presence of adenosine deaminase by standard radioligand binding methods and are summarized in Table II. Adenosine A_1 receptor binding assays were done with rat brain (without using cerebellum and brain stem) homogenates by [^{3}H]cyclohexyladenosine (CHA) binding by the procedure described previously.[8,22] Adenosine A_2 receptor binding assays were done with rat striatum and [^{3}H]NECA by the previous methods.[8,22]

As shown in Table II, in a series of 2-(1-hexyn-1-yl)-adenosine-5′-uronamides **16a–f**, the *N*-cyclopropyl derivative **16d** showed the highest binding activity for both A_1 and A_2 receptors with K_is of 136 and 2.6 nM, respectively. A decrease in the carbon number of the *N*-alkyl substituent at the 5′-carboxamide gradually reduced the A_2 affinity (**16a**/**16d** = 7). However, increases in the carbon number reduced the A_2 binding affinity rather drastically (**16f**/**16d** = 65). For the A_1 receptor, similar tendencies in the binding affinity were also observed, but the ratio was not very different (**16a**/**16d** = 9 and **16f**/**16d** = 14). Since the potency of the binding affinity to both receptors of the parent nucleoside, 2-HA (**7**), was quite similar to those of **16d**, an introduction of the carboxamide group at the 5′-position of **7** seems not to be advantageous for the potency of the A_2 receptor binding affinity. In a series of *N*-alkyl adenosine-5′-uronamides, the order of effects of the alkyl substituent on the potency at A_2 receptor was reported to be Et (K_i = 10.3 nM) ≥ cyclopropyl (13.4 nM) > Me (67 nM) > H (120 nM).[13] The order of A_2 potency for **16a–d** is cyclopropyl (K_i = 2.6 nM) > Et (4.8 nM) > Me (11 nM) > H (19 nM) > Pr (44 nM) > Bu (169 nM). Therefore, *N*-alkyl substituents can only regulate the A_2 binding potency. However, the affinity to the A_1 receptor was substantially reduced: a 17-fold reduction to **16b** from 2-HA was observed. Consequently, the A_1/A_2 ratio is increased 43-fold for 2-HA to 162-fold for **16b**. On the other hand, for the other parent nucleoside, NECA (**2**), the binding potency is increased about 2 times for **16c** to the A_2 receptor and is decreased 33 times to the A_1 receptor. Therefore, the C-2 hexynyl group seems to be a primary determinant at the A_2 receptor. The carboxamide group at the 5′-position can diminish the affinity to the A_1 re-

(20) Rosemeyer, H.; Toth, G.; Golankiewicz, B.; Kazimierczuk, Z.; Bourgeois, W.; Kretschmer, U.; Muth, H-P.; Seela, F. Syn-anti conformational analysis of regular and modified nucleosides by 1D ^{1}H NOE difference spectroscopy: A simple graphical method based on conformationally rigid molecules. *J. Org. Chem.* 1990, *55*, 5784–5790.

(21) 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-Schmiedebergs Arch. Pharmacol.* 1986, *332*, 179–183.

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

ceptor. Subsequently, the A_1/A_2 selectivity ratio should be considerably increased. While doubly modified adenosines such as (*N*-alkyl-2-amino)adenosine-5′-*N*-ethyluronamides often showed a profound selectivity to A_2 receptor, this also would be because of reduction to A_1 binding affinity, although there are some exceptions.[5]

Among other 5′-substituted derivatives, the 5′-chloro-5′-deoxy derivative **20** was almost equipotent to those of **16c** at both A_1 and A_2 receptors. However, 5′-chloro-5′-deoxyadenosine itself was reported as a rather A_1 selective agonist with K_i values of 19.5 and 151 nM for A_1 and A_2 receptors, respectively.[13] Thus, **20** shows 11-fold reduction for the A_1 and 29-fold enhancement for the A_2 receptor over 5′-chloro-5′-deoxyadenosine. However, 5′-deoxy-5′-carboxamides **27** and **28**, sulfonamido **29**, ureido **30**, and thioureido **31** showed loss of affinity up to 472 and 181 nM to A_1 and A_2 receptors, respectively.

As described above, the *syn*-glycosyl conformation of **2** was thought to be one of the important factors for receptor binding activity.[16] However, in this study, we found that the glycosyl conformation and the sugar-puckering between the active nucleosides **7**, **16c**, and **20**, and the inactive derivative **27** did not seem to show positive correlations to the binding affinity. Since these nucleosides are not fixed in certain conformations, unlike a cyclonucleoside, which is fixed in a certain conformation by bridges between the sugar and nucleobase moieties, they could be rather flexible molecules while **2** and **16c** prefer *syn*-glycosyl conformations via the intramolecular hydrogen bonding. If these active agonists bind to the receptors, their glycosyl conformations as well as the sugar-puckering would be fixed in a certain manner at the binding sites by at least several hydrophobic and hydrogen-binding interactions. Therefore, one of the major determinants for the affinity would be bulkiness of substituents attached at the 5′ position of the adenosine derivatives or the distance between distal substituents at C-2 and 5′ positions besides the nature of the C-2 substituents.

Antihypertensive Activity

The potency of test compounds to decrease BP and HR were compared upon iv administration in the spontaneously hypertensive rat model. The relative potency to decrease BP was estimated on the basis of the ED_{30} value, the mean dose that produced a 30% decrease in BP. Similarly, the relative potency to decrease HR was estimated on the basis of the ED_{10} value, the mean dose that produced a 10% decrease in HR. These data are shown in Figure 2 and listed in Table II.

Thirteen newly synthesized nucleosides and appropriate standards were tested intravenously. 5′-Carboxamide derivatives **16a–f** of **7**, showed significant hypotensive effects at doses of 0.2 μg/kg and up to 30 μg/kg. These compounds had dose-related BP-lowering activity when administered as multiple doses, which is shown in Figure 2 (upper). From the data in Table II, **16d** had one of the most potent hypotensive activities with an ED_{30} of 0.17 μg/kg. This effectiveness is about 5 times greater than that of **4**. This activity is comparable to those of **2** and **7** but slightly less than that of **9**, which had an ED_{30} of 0.05 μg/kg in our previous report.[11] Although it is difficult to compare in vitro receptor binding affinities directly with in vivo pharmacological activities, the BP-lowering potency seems proportional to the binding affinity to the A_2 receptor in this study, except those of **16a** and **20**. The A_2 binding affinity ratios of **16d** vs **16c**, **16b**, **16e**, and **16f** were 1.8, 4.2, 17, and 65, which correlate well with the ED_{30} of the hypotensive activity ratio (1.8, 3, 17, and 155, respectively). For compound **7a**, although the A_2 affinity is 7-fold less

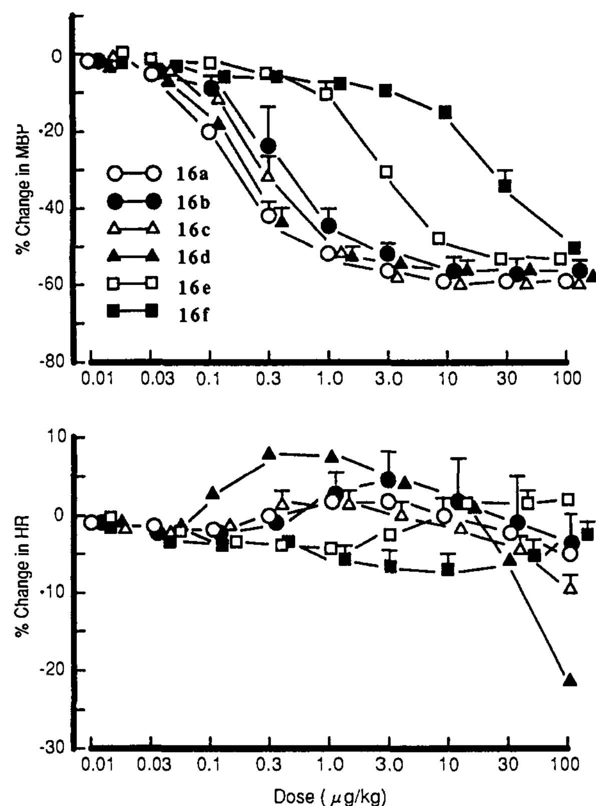


Figure 2. Effects of **16a–f** on blood pressure in anesthetized male SHR. Each compound was administered iv in a cumulative manner at 5-min intervals. Each value represents the mean ± SE of four animals.

than that of **16d**, the hypotensive potency is almost same as that of **16d**. While the A_2 binding affinity of **20** is comparable to that of **16c**, the BP-lowering activity was less than 10-fold although we could not measure an exact EC_{30} value for **20** due to its insolubility. The reason for these differences is not clear in this study, but there might be mechanisms in the BP-lowering activity of **16a** and **20** other than the A_2 receptor activation. Compound **16b** showed a 162-fold A_2 selectivity, making it the most selective A_2 agonist in this series, together with the 2-alkynyladenosines and 2-(cycloalkylalkynyl)adenosines we described previously.[8,11] However, **16d** caused a significant tachycardia at a dose range between 0.1 and 10 μg/kg, but at higher doses (an ED_{10} of 40 μg/kg) bradycardia was observed (Figure 2, lower). Since bradycardia has been described to be mediated via the A_1 receptor, a low selective adenosine agonist would cause a decrease of HR. These examples are **2**, **7**, and N^6-cyclopentyladenosine (CPA) in Table II, although the values are not proportional to the A_1 binding affinity. Relatively selective agonists **16a–d** also showed a slight increase and decrease of HR at rather higher doses but not a significant one.

In summary, we have synthesized several 5′-modified 2-(1-hexyn-1-yl)adenosine analogues. Modification at the 5′-position slightly modulated the affinity at the A_2 receptor but greatly reduced the affinity at the A_1 receptor compared with the parent agonist, 2-HA (**7**). Consequently A_1/A_2 selectivity was increased 162-fold in **16b**. Conformational analyses of these active and inactive nucleosides did not seem to have positive correlations to the affinity. In this study, we found that the C-2 hexynyl group would be the primary determinant, and bulkiness at the 5′-position was not favorable for the A_2 receptor. Antihypertensive activity of these active agonists upon iv administration to SHR was proportionally related to the A_2

binding affinity, except for **16a** and **20**.

Experimental Section

Melting points were measured on a Yanagimoto MP-3 micromelting point apparatus and are uncorrected. The ^{1}H NMR spectra were recorded on a JEOL JNM-FX 100 (100 MHz) or JEOL JNM-GX 270 (270 MHz) spectrometer with tetramethylsilane as internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad). All exchangeable protons were detected by addition of D_2O. The NOE measurements were done according to ref 20 using JEOL JNM-GX 270, and the solutions (ca. 0.05 M in DMSO-d_6) were degassed by bubbling N_2 through them followed by ultrasound sonication. In the experiments using 5′-deoxy-N^3,5′-anhydroisoguanosine, we observed similar enhancements as described previously.[20] UV absorption spectra were recorded with a Shimadzu UV-240 spectrophotometer. Mass spectra (MS) were measured on a JEOL JMX-DX303 spectrometer. IR spectra was recorded with 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).

2′,3′-*O*-Isopropylidene-2-iodoadenosine (11). 70% Perchloric acid (2 mL) was added to a suspension of 2-iodoadenosine (10) (5.0 g, 12.7 mmol) in acetone (150 mL) at 0 °C. The mixture was stirred for 4 h at room temperature, and the solution was neutralized by concentrated NH_4OH. The solvent was removed by evaporation in vacuo, and the residue was crystallized from hot EtOH to afford 11 (5.3 g, 96%): mp 178–181 °C; MS *m/z* 433 (M^+); NMR (100 MHz, $CDCl_3$) 7.74 (1 H, s, H-8), 6.10 (2 H, br s, 6-NH_2), 5.80 (1 H, d, H-1′, $J_{1',2'}$ = 4.4 Hz), 5.30–5.10 (3 H, m, H-2′, 3′, 5′-OH), 4.45 (1 H, br s, H-4′), 4.02 (1 H, br dd, H-5′a, $J_{5'a,4'}$ = 1.4, $J_{5'a,b}$ = 12.7 Hz), 3.80 (1 H, br d, H-5′b), 1.64, 1.41 (each 3 H, s, isoprop). Anal. ($C_{13}H_{16}IN_5O_4$) C, H, N.

2′,3′-*O*-Isopropylidene-2-iodoadenosine-5′-uronic Acid (12). RuO_2 (200 mg) was added to a solution of 11 (4.33 g, 10 mmol) and $NaIO_4$ (3.45 g, 15 mmol) in a mixture of CH_3CN (60 mL), $CHCl_3$ (60 mL, EtOH free), and H_2O (90 mL). The mixture was vigorously stirred for 5 h with a mechanical stirrer at room temperature. Further amounts of $NaIO_4$ (3.45 g, 15 mmol) were added to the reaction mixture, and the whole was stirred for 2 h more. 2-Propanol (10 mL) was added to the mixture, which was filtered through a Celite pad. The filtrate was concentrated to about one-half volume, which was acidified to pH 2–3 by 2 N HCl at 0 °C. The resulting colored precipitates were collected by filtration and washed with Et_2O to give crude 12 (3.1 g, 68%, as a foam): MS *m/z* 433 (M^+ + H-Me); NMR (DMSO-d_6) 8.16 (1 H, s, H-8), 7.70 (2 H, br s, 6-NH_2), 6.27 (1 H, s, H-1′), 5.49 (1 H, dd, H-3′, $J_{3',2'}$ = 5.9, $J_{3',4'}$ = 1.7 Hz), 5.39 (1 H, d, H-2′, $J_{2',3'}$ = 5.9 Hz), 4.68 (1 H, d, H-4′, $J_{4',3'}$ = 1.7 Hz), 1.52, 1.36 (each 3 H, s, isoprop).

Methyl 2′,3′-*O*-Isopropylidene-2-iodoadenosine-5′-uronate (13). Thionyl chloride (130 μL, 1.8 mmol) was added to a suspension of 12 (400 mg, 0.9 mmol) in MeOH (50 mL) at 0 °C. The mixture was stirred for 2 h at room temperature and was concentrated to dryness in vacuo. The residue was neutralized with saturated aqueous $NaHCO_3$, the whole was extracted several times with $CHCl_3$, and the organic phase was washed with saturated aqueous $NaHCO_3$ and NaCl solutions. The separated organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was purified by column chromatography on silica gel (2.2 × 12 cm) with 4% MeOH in $CHCl_3$, giving 13 (320 mg, 78%, as a foam): MS *m/z* 461 (M^+); NMR (100 MHz, $CDCl_3$) 7.82 (1 H, s, H-8), 6.13 (1 H, s, H-1′), 5.90 (2 H, br s, 6-NH_2), 5.68 (1 H, dd, H-3′, $J_{3',2'}$ = 5.9, $J_{3',4'}$ = 1.7 Hz), 5.41 (1 H, d, H-2′, $J_{2',3'}$ = 5.9 Hz), 4.83 (1 H, d, H-4′, $J_{4',3'}$ = 1.7 Hz), 3.65 (3 H, s, CO_2Me), 1.60, 1.44 (each 3 H, s, isoprop).

Methyl 2′,3′-*O*-Isopropylidene-2-(1-hexyn-1-yl)-adenosine-5′-uronate (14). A mixture of 13 (267 mg, 0.58 mmol), bis(triphenylphosphine)palladium dichloride (41 mg, 10 mol%), 1-hexyne (137 μL, 1.2 mmol), CuI (40 mg), and Et_3N (97 μL, 0.7 mmol) in DMF (10 mL) was stirred at 70 °C for 2 h under argon atmosphere. The solvent was removed in vacuo, and the residue was dissolved in $CHCl_3$. H_2S gas was introduced to the solution for 30 s, and then N_2 gas was purged. Insoluble materials were removed by filtration through a Celite pad, and the filtrate was washed with saturated aqueous $NaHCO_3$, then dried (Na_2SO_4), and concentrated to dryness. The residue was purified by a silica gel column (2.3 × 10 cm) with 4% EtOH in $CHCl_3$ to afford 14 (239 mg, 99%, as a foam): MS *m/z* 415 (M^+); IR (Nujol) ν C≡C 2240 cm^{-1}; NMR (100 MHz, $CDCl_3$) 7.91 (1 H, s, H-8), 6.17 (1 H, s, H-1′), 5.79–5.65 (3 H, m, 6-NH_2, H-3′), 5.43 (1 H, d, H-2′, $J_{2',3'}$ = 6.1 Hz), 4.81 (1 H, s, H-4′), 3.56 (3 H, s, OMe), 2.52–2.38 (2 H, m, ≡$CCH_2(CH_2)_2CH_3$), 1.70–1.37 (4H, m, ≡$CCH_2(CH_2)_2CH_3$), 1.60, 1.43 (each 3 H, s, isoprop), 1.03–0.88 (3 H, m, ≡$CCH_2(CH_2)_2CH_3$).

General Method for the Synthesis of 15a–f. A mixture of 14 in a MeOH solution of NH_3 (saturated at 0 °C) or amines in a sealed bottle was heated for an appropriate time. The solvent was removed in vacuo, and the residue was purified by a silica gel column to afford **15a–f**.

2′,3′-*O*-Isopropylidene-2-(1-hexyn-1-yl)adenosine-5′-uronamide (15a). From 14 (250 mg, 0.6 mmol) in NH_3/MeOH (20 mL, saturated at 0 °C) at 80 °C for 3 h was obtained **15a** (193 mg, 80%, as a foam) by silica gel column chromatography (2.2 × 5 cm, with 10% MeOH in $CHCl_3$): MS *m/z* 400 (M^+); NMR (100 MHz, $CDCl_3$) 8.10 (1 H, br s $CONH_2$), 7.84 (1 H, s, H-8), 6.16 (2 H, br s, 6-NH_2), 6.03 (1 H, d, H-1′, $J_{1',2'}$ = 3.0 Hz), 5.92 (1 H, br s, $CONH_2$), 5.29–5.27 (2 H, m, H-2′,3′), 4.71 (1 H, d, H-4′, $J_{4',3'}$ = 1.5 Hz), 2.50–2.30 (2 H, m, ≡$CCH_2(CH_2)_2CH_3$), 1.64 (3 H, s, isoprop), 1.60–1.40 (4 H, m, ≡$CCH_2(CH_2)_2CH_3$), 1.39 (3 H, s, isoprop), 1.00–0.88 (3 H, m, ≡$CCH_2(CH_2)_2CH_3$).

2′,3′-*O*-Isopropylidene-2-(1-hexyn-1-yl)adenosine-5′-*N*-methyluronamide (15b). From 14 (415 mg, 1 mmol) in CH_3NH_2/MeOH (20 mL, saturated at 0 °C) at 80 °C for 3 h was obtained **15b** (267 mg, 65%, as a solid) by silica gel column chromatography (2.8 × 6 cm, with EtOAc): MS *m/z* 416 (M^+); NMR (100 MHz, $CDCl_3$) 7.90–7.80 (1 H, br s CONH), 7.83 (1 H, s, H-8), 5.94 (1 H, d, H-1′, $J_{1',2'}$ = 4.2 Hz), 5.76 (2 H, br s, 6-NH_2), 5.28 (1 H, dd, H-2′, $J_{2',1'}$ = 4.2, $J_{2',3'}$ = 6.1 Hz), 5.14 (1 H, dd, H-3′, $J_{3',2'}$ = 6.1, $J_{3',4'}$ = 1.7 Hz), 4.74 (1 H, d, H-4′, $J_{4',3'}$ = 1.7 Hz), 2.91 (3 H, d, NMe), 2.50–2.38 (2 H, m, ≡$CCH_2(CH_2)_2CH_3$), 1.71 (3 H, s, isoprop), 1.60–1.40 (4 H, m, ≡$CCH_2(CH_2)_2CH_3$), 1.37 (3 H, s, isoprop), 1.00–0.94 (3 H, m, ≡$CCH_2(CH_2)_2CH_3$).

2′,3′-*O*-Isopropylidene-2-(1-hexyn-1-yl)adenosine-5′-*N*-ethyluronamide (15c). From 14 (100 mg, 0.24 mmol) in aqueous 70% $EtNH_2$ (1 mL) and MeOH (2 mL) at 80 °C for 4 h was obtained **15c** (73 mg, 71%, as a foam) by silica gel column chromatography (2.2 × 6 cm, with hexane/EtOAc = 1/4): MS *m/z* 429 (M^+ + H); NMR (100 MHz, $CDCl_3$) 7.83 (1 H, s, H-8), 7.60–7.36 (1 H, br s, CONH), 6.00 (1 H, d, H-1′, $J_{1',2'}$ = 3.7 Hz), 5.67 (2 H, br s, 6-NH_2), 5.35–5.15 (2 H, m, H-2′, 3′), 4.69 (1 H, d, H-4′, $J_{4',3'}$ = 1.7 Hz), 3.49–3.22 (2 H, m, NCH_2CH_3), 2.50–2.37 (2 H, m, ≡$CCH_2(CH_2)_2CH_3$), 1.62 (3 H, s, isoprop), 1.86–1.26 (4 H, m, ≡$CCH_2(CH_2)_2CH_3$), 1.38 (3 H, s, isoprop), 1.04–0.87 (6 H, m, NCH_2CH_3, ≡$CCH_2(CH_2)_2CH_3$).

2′,3′-*O*-Isopropylidene-2-(1-hexyn-1-yl)adenosine-5′-*N*-cyclopropyluronamide (15d). From 14 (100 mg, 0.24 mmol) in 98% cyclopropylamine (1 mL) and MeOH (2 mL) at 80 °C for 6 h was obtained **15d** (100 mg, 95%, as a foam) was obtained by silica gel column chromatography (1.8 × 2 cm, with EtOAc): MS *m/z* 441 (M^+ + H); NMR (100 MHz, $CDCl_3$) 7.80 (1 H, s, H-8), 6.82–6.78 (1 H, br s, CONH), 6.03 (1 H, d, H-1′, $J_{1',2'}$ = 2.4 Hz), 5.64 (2 H, br s, 6-NH_2), 5.42–5.33 (2 H, m, H-2′, 3′), 4.64 (1 H, d, H-4′, $J_{4',3'}$ = 1.7 Hz), 2.66–2.38 (3 H, m, *N*-cyclopropyl, ≡$CCH_2(CH_2)_2CH_3$), 1.61 (3 H, s, isoprop), 1.38 (3 H, s, isoprop), 1.70–1.26 (4 H, m, ≡$CCH_2(CH_2)_2CH_3$), 1.01–0.82 (3 H, m, ≡$CCH_2(CH_2)_2CH_3$), 0.77–0.60 (2 H, m, *N*-cyclopropyl), 0.46–0.34 (2 H, m, *N*-cyclopropyl).

2′,3′-*O*-Isopropylidene-2-(1-hexyn-1-yl)adenosine-5′-*N*-propyluronamide (15e). From 14 (416 mg, 1 mmol) in *n*-propylamine (1 mL) and MeOH (10 mL) at 70 °C for 12 h was obtained **15e** (293 mg, 66%, as a foam) was obtained by silica gel column chromatography (2.8 × 6 cm, with hexane/EtOAc = 1/2): MS *m/z* 442 (M^+); NMR (100 MHz, $CDCl_3$) 7.83 (1 H, s, H-8), 7.30 (1 H, br t, CONH), 6.00 (1 H, d, H-1′, $J_{1',2'}$ = 3.4 Hz), 5.88 (2 H, br s, 6-NH_2), 5.36–5.18 (2 H, m, H-2′, 3′), 4.69 (1 H, d, H-4′, $J_{4',3'}$ = 2.0 Hz), 3.50–3.00 (2 H, m, *N*-propyl), 2.44 (2 H, t, ≡$CCH_2(CH_2)_2CH_3$), 1.63 (3 H, s, isoprop), 1.38 (3 H, s, isoprop), 1.86–1.31 (6 H, m, *N*-propyl, ≡$CCH_2(CH_2)_2CH_3$), 1.01–0.72 (6 H, m, *N*-propyl, ≡$CCH_2(CH_2)_2CH_3$).

2′,3′-*O*-Isopropylidene-2-(1-hexyn-1-yl)adenosine-5′-*N*-butyluronamide (15f). From 14 (416 mg, 1 mmol) in *n*-bu-

tylamine (1 mL) and MeOH (10 mL) at 70 °C for 6 h was obtained **15f** (366 mg, 80%, as a foam) was obtained by silica gel column chromatography (2.2 × 6 cm, with hexane/EtOAc = 1/2): MS m/z 456 (M^+); NMR (100 MHz, $CDCl_3$) 7.83 (1 H, s, H-8), 7.23 (1 H, br t, CONH), 6.02 (1 H, d, H-1', $J_{1',2'}$ = 3.7 Hz), 5.92 (2 H, br s, 6-NH_2), 5.31 (1 H, dd, H-2', $J_{2',1'}$ = 3.7, $J_{2',3'}$ = 6.2 Hz), 5.24 (1 H, dd, H-3', $J_{3,2'}$ = 6.2, $J_{3',4'}$ = 2.2 Hz), 4.69 (1 H, d, H-4', $J_{4',3'}$ = 2.2 Hz), 3.50–3.32 (1 H, m, N-butyl), 3.18–3.05 (1 H, m, N-butyl), 2.50–2.42 (2 H, m, $\equiv CCH_2(CH_2)_2CH_3$), 1.63 (3 H, s, isoprop), 1.38 (3 H, s, isoprop), 1.70–1.12 (8 H, m, N-butyl, $\equiv CCH_2(CH_2)_2CH_3$), 0.99–0.92 (3 H, m, $\equiv CCH_2(CH_2)_2CH_3$), 0.87–0.82 (3 H, m, N-butyl).

2',3'-O-Isopropylidene-2-(1-hexyn-1-yl)adenosine (17). A mixture of 11 (2.0 g, 4.62 mmol), 1-hexyne (0.7 mL, 6.1 mmol), bis(triphenylphosphine)palladium dichloride (324 mg), and CuI (300 mg) in DMF (20 mL) containing Et_3N (0.77 mL, 5.54 mmol) was heated at 70 °C for 2 h under argon atmosphere. The solvent was removed in vacuo, and the residue was dissolved in $CHCl_3$. H_2S gas was introduced into the solution for 30 s, and then N_2 gas was purged. Insoluble materials were removed by filtration through a Celite pad, and the filtrate was washed with saturated aqueous $NaHCO_3$, then dried (Na_2SO_4), and concentrated to dryness. The residue was purified by a silica gel column (4.5 × 10 cm) with 4% EtOH in $CHCl_3$ to afford 17 (1.50 g, 84%, as a foam): MS m/z 387 (M^+); IR ν C≡C 2240 cm^{-1}; NMR (270 MHz, $CDCl_3$) 7.83 (1 H, s, H-8), 6.12 (1 H, dd, 5'-OH), 5.85 (2 H, br s, 6-NH_2), 5.82 (1 H, d, H-1', $J_{1',2'}$ = 5.1 Hz), 5.21 (1 H, dd, H-2', $J_{2',1'}$ = 5.1, $J_{2',3'}$ = 5.9 Hz), 5.13 (1 H, dd, H-3', $J_{3',2'}$ = 5.9, $J_{3',4'}$ = 0.7 Hz), 4.53 (1 H, br s, H-4'), 3.99 (1 H, ddd, H-5'b, $J_{5'b,4'}$ = 1.5, $J_{a,b}$ = 13.2, $J_{5'b,OH}$ = 2.2 Hz), 3.80 (1 H, ddd, H-5'a, $J_{5'a,4'}$ = 1.5, $J_{a,b}$ = 13.2, $J_{5'a,OH}$ = 11.7 Hz), 2.45 (2 H, t, $\equiv CCH_2(CH_2)_2CH_3$), 1.76–1.44 (4 H, m, $\equiv CCH_2(CH_2)_2CH_3$), 1.64, 1.37 (each 3 H, s, isoprop), 0.94 (3 H, m, $\equiv CCH_2(CH_2)_2CH_3$). Anal. ($C_{19}H_{25}N_5O_4$) C, H, N.

5'-Azido-5'-deoxy-2',3'-O-isopropylidene-2-(1-hexyn-1-yl)adenosine (18). A mixture of diphenyl phosphorazidate (0.83 mL, 3.87 mmol) and diethyl azodicarboxylate (0.62 mL, 3.87 mmol) in THF (20 mL) was added dropwise over 10 min to a solution of 17 (1.0 g, 2.58 mmol) and triphenylphosphine (1.02 g, 3.87 mmol) in THF (30 mL) with stirring at 0 °C. Then, the mixture was stirred for 4 h further at room temperature and was concentrated to dryness in vacuo. The residue was purified by a silica gel column (3.3 × 11 cm) with 4% MeOH in $CHCl_3$ to afford 18 (882 mg, 83%, as a foam): MS m/z 412 (M^+); IR 2240 (C≡C), 2100 (N_3) cm^{-1}; NMR (100 MHz, $CDCl_3$) 7.91 (1 H, s, H-8), 6.12 (1 H, d, H-1', $J_{1',2'}$ = 2.2 Hz), 5.88 (2 H, br s, 6-NH_2), 5.35 (1 H, dd, H-2', $J_{2',1'}$ = 2.2, $J_{2',3'}$ = 6.4 Hz), 5.09 (1 H, dd, H-3', $J_{3',2'}$ = 6.4, $J_{3',4'}$ = 3.4 Hz), 4.43–4.28 (1 H, m, H-4'), 3.67–3.61 (2 H, m, H-5'a,b), 2.46 (2 H, t, $\equiv CCH_2(CH_2)_2CH_3$), 1.89–1.25 (4 H, m, $\equiv CCH_2(CH_2)_2CH_3$), 1.62, 1.40 (each 3 H, s, isoprop), 0.95 (3 H, m, $\equiv CCH_2(CH_2)_2CH_3$).

5'-Chloro-5'-deoxy-2',3'-O-isopropylidene-2-(1-hexyn-1-yl)adenosine (19). A suspension of 17 (100 mg, 0.26 mmol) and triphenylphosphine (102 mg, 0.39 mmol) in CCl_4 (10 mL) was heated under reflux for 24 h. The solvent was removed in vacuo, and the residue was purified by a silica gel column (1.8 × 4 cm) with hexane/EtOAc (1:2) to afford 19 (85 mg, 81%, as a foam): MS m/z 405 (M^+); IR 2240 (C≡C) cm^{-1}; NMR (270 MHz, $CDCl_3$) 7.91 (1 H, s, H-8), 6.13 (1 H, d, H-1', $J_{1',2'}$ = 2.4 Hz), 5.86 (2 H, br s, 6-NH_2), 5.35 (1 H, dd, H-2', $J_{2',1'}$ = 2.4, $J_{2',3'}$ = 6.4 Hz), 5.19 (1 H, dd, H-3', $J_{3',2'}$ = 6.4, $J_{3',4'}$ = 3.4 Hz), 4.47 (1 H, ddd, H-4', $J_{4',3'}$ = 3.4, $J_{4',5'a}$ = 7.3, $J_{4',5'b}$ = 5.4 Hz), 3.90 (1 H, dd, H-5'a, $J_{5'a,4'}$ = 7.3, $J_{5'a,b}$ = 11.2 Hz), 3.67 (1 H, dd, H-5'b, $J_{5'b,4'}$ = 5.4, $J_{5'a,b}$ = 11.2 Hz), 2.46 (2 H, t, $\equiv CCH_2(CH_2)_2CH_3$), 1.68–1.25 (4 H, m, $\equiv CCH_2(CH_2)_2CH_3$), 1.63, 1.41 (each 3 H, s, isoprop), 0.96 (3 H, m, $\equiv CCH_2(CH_2)_2CH_3$).

5'-Amino-5'-deoxy-2',3'-O-isopropylidene-2-(1-hexyn-1-yl)adenosine (21). A solution of triphenylphosphine (190 mg, 0.72 mmol) in a mixture of H_2O (2 mL) and pyridine (5 mL) was added dropwise over 25 min to a mixture of 18 (200 mg, 0.49 mmol) in pyridine (10 mL) with stirring at 0 °C. The mixture was stirred for a further 1 h at 0 °C and was concentrated to dryness in vacuo. The residue was coevaporated several times with toluene and was purified by a silica gel column (2.2 × 7 cm) with 8% MeOH in $CHCl_3$ to afford 21 (103 mg, 55%, as a solid): MS m/z 387 (M^+ + H); NMR (100 MHz, DMSO-d_6) 8.41 (1 H, s, H-8), 7.42 (2 H, br s, 6-NH_2), 6.04 (1 H, d, H-1', $J_{1',2'}$ = 3.2 Hz), 5.36 (1 H, dd, H-2', $J_{2',1'}$ = 3.2, $J_{2',3'}$ = 6.1 Hz), 4.98 (1 H, dd, H-3', $J_{3',2'}$ = 6.1, $J_{3',4'}$ = 2.7 Hz), 4.20–4.00 (1 H, m, H-4'), 3.00 (2 H, br s, 5'-NH_2), 2.80–2.60 (2 H, m, H-5'a,b), 2.42 (2 H, t, $\equiv CCH_2(CH_2)_2CH_3$), 1.60–1.20 (4 H, m, $\equiv CCH_2(CH_2)_2CH_3$), 1.54, 1.34 (each 3 H, s, isoprop), 0.99 (3 H, m, $\equiv CCH_2(CH_2)_2CH_3$).

5'-Deoxy-5'-formamido-2',3'-O-isopropylidene-2-(1-hexyn-1-yl)adenosine (22). Acetic formic anhydride (85 μL, 1.34 mmol) was added to a solution of 21 (250 mg, 0.67 mmol) in pyridine (10 mL). The mixture was stirred for 1 h at room temperature and was concentrated to dryness in vacuo. The residue was coevaporated several times with toluene and was partitioned between $CHCl_3$ and saturated $NaHCO_3$. The separated organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was purified by a silica gel column (1.8 × 6 cm) with 4% MeOH in $CHCl_3$ to afford 22 (205 mg, 77%, as a foam): MS m/z 414 (M^+); NMR (100 MHz, $CDCl_3$) 9.01–8.90 (1 H, m, CONH), 8.60 (1 H, s, CHO), 7.84 (1 H, s, H-8), 5.92 (2 H, br s, 6-NH_2), 5.24 (1 H, d, H-1', $J_{1',2'}$ = 4.9 Hz), 5.24 (1 H, dd, H-2', $J_{2',1'}$ = 4.9, $J_{2',3'}$ = 6.1 Hz), 4.83 (1 H, dd, H-3', $J_{3',2'}$ = 6.1, $J_{3',4'}$ = 2.2 Hz), 4.55–4.53 (1 H, m, H-4'), 4.45–4.20 (1 H, m, H-5'a), 3.30–3.15 (1 H, m, H-5'b), 2.50–2.40 (2 H, m, $\equiv CCH_2(CH_2)_2CH_3$), 1.80–1.30 (4 H, m, $\equiv CCH_2(CH_2)_2CH_3$), 1.63, 1.35 (each 3 H, s, isoprop), 0.95 (3 H, t, $\equiv CCH_2(CH_2)_2CH_3$).

5'-Acetamido-5'-deoxy-2',3'-O-isopropylidene-2-(1-hexyn-1-yl)adenosine (23). Acetic anhydride (31 μL, 0.32 mmol) was added to a solution of 21 (63 mg, 0.16 mmol) in pyridine (5 mL). The mixture was stirred for 2 h at room temperature and was concentrated to dryness in vacuo. The residue was coevaporated several times with toluene and was partitioned between $CHCl_3$ and saturated $NaHCO_3$. The separated organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was purified by a silica gel column (1.8 × 3 cm) with 8% MeOH in $CHCl_3$ to afford 23 (59 mg, 85%, as a foam): MS m/z 428 (M^+); NMR (100 MHz, $CDCl_3$) 7.82 (1 H, s, H-8), 7.78 (1 H, d, CONH, J = 8.5 Hz), 5.83 (2 H, br s, 6-NH_2), 5.80 (1 H, d, H-1', $J_{1',2'}$ = 4.6 Hz), 5.16 (1 H, dd, H-2', $J_{2',1'}$ = 4.6, $J_{2',3'}$ = 6.1 Hz), 4.83 (1 H, dd, H-3', $J_{3',2'}$ = 6.1, $J_{3',4'}$ = 2.7 Hz), 4.46 (1 H, dd, H-4', $J_{4',3'}$ = 2.7, $J_{4',5'}$ = 5.4 Hz), 4.15 (1 H, ddd, H-5'a, $J_{5'a,NH}$ = 8.5, $J_{a,b}$ = 14.7, $J_{5'a,4'}$ = 5.4 Hz), 3.40–3.23 (1 H, m, H-5'b), 2.42 (2 H, t, $\equiv CCH_2(CH_2)_2CH_3$), 2.19 (3 H, s, Ac), 1.60–1.20 (4 H, m, $\equiv CCH_2(CH_2)_2CH_3$), 1.62, 1.36 (each 3 H, s, isoprop), 0.94 (3 H, t, $\equiv CCH_2(CH_2)_2CH_3$).

5'-Deoxy-5'-(methanesulfonamido)-2',3'-O-isopropylidene-2-(1-hexyn-1-yl)adenosine (24). Methanesulfonyl chloride (90 μL, 1.16 mmol) and Et_3N (0.18 mL, 1.32 mmol) were added to a solution of 21 (300 mg, 0.78 mmol) in CH_2Cl_2 (10 mL). The mixture was stirred for 1 h at room temperature and was washed with saturated $NaHCO_3$ and saturated NaCl. The separated organic phase was dried (Na_2SO_4) and concentrated to dryness. The residue was purified by a silica gel column (2.2 × 8 cm) with 2% MeOH in $CHCl_3$ to afford 24 (154 mg, 43%, as a foam): MS m/z 464 (M^+); NMR (100 MHz, $CDCl_3$) 7.80 (1 H, s, H-8), 7.60–7.45 (1 H, m, NH), 5.78 (1 H, d, H-1', $J_{1',2'}$ = 3.4 Hz), 5.70 (2 H, br s, 6-NH_2), 5.38–5.24 (1 H, m, H-2'), 5.06 (1 H, dd, H-3', $J_{3',2'}$ = 5.8, $J_{3',4'}$ = 2.0 Hz), 4.53 (1 H, br s, H-4'), 3.80–3.40 (2 H, m, H-5'a, H-5'b), 3.01 (3 H, s, Ms), 2.46 (2 H, t, $\equiv CCH_2(CH_2)_2CH_3$), 1.80–1.20 (4 H, m, $\equiv CCH_2(CH_2)_2CH_3$), 1.62, 1.38 (each 3 H, s, isoprop), 0.96 (3 H, t, $\equiv CCH_2(CH_2)_2CH_3$).

5'-Deoxy-5'-(3-methylureido)-2',3'-O-isopropylidene-2-(1-hexyn-1-yl)adenosine (25). Methyl isocyanate (78 μL, 1.32 mmol) was added to a solution of 21 (254 mg, 0.66 mmol) in pyridine (10 mL) at 0 °C. The mixture was stirred for 1 h at room temperature and was concentrated to dryness. The residue was purified by a silica gel column (2.8 × 6 cm) with 8% MeOH in $CHCl_3$ to afford 25 (252 mg, 86%, as a foam): MS m/z 444 (M^+); NMR (100 MHz, $CDCl_3$) 7.82 (1 H, s, H-8), 7.10–7.02 (1 H, m, NH), 5.97 (2 H, br s, 6-NH_2), 5.72 (1 H, d, H-1', $J_{1',2'}$ = 5.4 Hz), 5.40–5.28 (1 H, m, NH), 5.15 (1 H, dd, H-2', $J_{2',1'}$ = 5.4, $J_{2',3'}$ = 5.9 Hz), 4.86 (1 H, dd, H-3', $J_{3',2'}$ = 5.9, $J_{3',4'}$ = 1.5 Hz), 4.48 (1 H, br s, H-4'), 4.18 (1 H, ddd, H-5'a, $J_{5'a,4'}$ = 2.1, $J_{a,b}$ = 14.7, $J_{5'a,NH}$ = 9.9 Hz), 3.27–3.13 (1 H, m, H-5'b), 2.84 (3 H, d, Me), 2.45 (2 H, t, $\equiv CCH_2(CH_2)_2CH_3$), 1.73–1.23 (4 H, m, $\equiv CCH_2(CH_2)_2CH_3$), 1.62, 1.35 (each 3 H, s, isoprop), 0.94 (3 H, t, $\equiv CCH_2(CH_2)_2CH_3$).

5'-Deoxy-5'-(3-methylthioureido)-2',3'-O-isopropylidene-2-(1-hexyn-1-yl)adenosine (26). Methyl isothiocyanate (22 μL, 0.31 mmol) was added to a solution of 21 (100 mg, 0.26 mmol)

in pyridine (5 mL) at 0 °C. The mixture was stirred for 2 h at room temperature and was concentrated to dryness. The residue was purified by a silica gel column (1.8 × 6 cm) with 4% MeOH in $CHCl_3$ to afford **26** (105 mg, 88%, as a foam): MS *m/z* 459 (M^+); NMR (270 MHz, $CDCl_3$) 8.30–8.20 (1 H, m, NH), 7.82 (1 H, s, H-8), 6.68–6.66 (1 H, m, NH), 5.92 (2 H, br s, 6-NH_2), 5.74 (1 H, d, H-1', $J_{1',2'}$ = 5.5 Hz), 5.11 (1 H, dd, H-2', $J_{2',1'}$ = 5.5, $J_{2',3'}$ = 1.7 Hz), 5.02–4.98 (1 H, m, H-3'), 4.94–4.90 (1 H, m, H-5'a), 4.55–4.54 (1 H, m, H-4'), 3.52–3.47 (1 H, m, H-5'b), 3.13 (3 H, d, Me, *J* = 4.4 Hz), 2.44 (2 H, t, ≡$CCH_2(CH_2)_2CH_3$), 1.80–1.26 (4 H, m, ≡$CCH_2(CH_2)_2CH_3$), 1.62 1.35 (each 3 H, s, isoprop), 0.94 (3 H, t, ≡$CCH_2(CH_2)_2CH_3$).

General Procedure for Deblocking of 15a–f, 19, and 22–26. Compound **15a–f**, **19**, or **22–26** was treated with 80% aqueous trifluoroacetic acid (TFA) for several hours at room temperature. The solvent was removed in vacuo, and the residue was coevaporated several times with EtOH. The residue was dissolved in MeOH, and the whole was mixed with silica gel which was evaporated to dryness in vacuo. The residue was placed on a silica gel column which was eluted with an appropriate mixture of EtOH and $CHCl_3$.

2-(1-Hexyn-1-yl)adenosine-5'-uronamide (16a). Treatment of **15a** (60 mg, 0.15 mmol) with TFA (5 mL) for 2 h gave **16a** (52 mg, 96%, crystallized from EtOH): mp 176–178 °C; MS *m/z* 360 (M^+); IR ν C≡C 2240 cm^{-1}; NMR (270 MHz, DMSO-d_6) 8.46 (1 H, s, H-8), 8.27 (1 H, br s, $CONH_2$), 7.53 (1 H, br s, $CONH_2$), 7.49 (2 H, br s, 6-NH_2), 5.95 (1 H, d, H-1', $J_{1',2'}$ = 7.7 Hz), 5.70 (1 H, br d, 2'-OH), 5.56 (1 H, br d, 3'-OH), 4.54 (1 H, dd, H-2', $J_{2',1'}$ = 7.7, $J_{2',3'}$ = 4.4 Hz), 4.28 (1 H, d, H-4', $J_{4',3'}$ = 1.7 Hz), 4.15 (1 H, dd, H-3', $J_{3',2'}$ = 4.4, $J_{3',4'}$ = 1.7 Hz), 2.45 (2 H, t, ≡CCH_2-$(CH_2)_2CH_3$), 1.59–1.48 (2 H, m, ≡$CCH_2(CH_2)_2CH_3$), 1.46–1.35 (2 H, m, ≡$CCH_2(CH_2)_2CH_3$), 0.91 (3 H, t, ≡$CCH_2(CH_2)_2CH_3$). Anal. ($C_{16}H_{20}N_6O_4$·$^1/_3H_2O$) C, H, N.

2-(1-Hexyn-1-yl)adenosine-5'-*N*-methyluronamide (16b). Treatment of **15b** (243 mg, 0.59 mmol) with TFA (7 mL) for 2 h gave **16b** (201 mg, 92%, crystallized from EtOH): mp 187–189 °C; MS *m/z* 374 (M^+); IR ν C≡C 2240 cm^{-1}; NMR (270 MHz, DMSO-d_6) 8.80–8.70 (1 H, m, CONH), 8.41 (1 H, s, H-8), 7.55 (2 H, br s, 6-NH_2), 5.92 (1 H, d, H-1', $J_{1',2'}$ = 7.7 Hz), 5.76 (1 H, d, 3'-OH, *J* = 4.0 Hz), 5.54 (1 H, d, 2'-OH, *J* = 6.2 Hz), 4.54 (1 H, ddd, H-2', $J_{2',1'}$ = 7.7, $J_{2',OH}$ = 6.2, $J_{2',3'}$ = 4.8 Hz), 4.31 (1 H, d, H-4', $J_{4',3'}$ = 1.7 Hz), 4.12 (1 H, ddd, H-3', $J_{3',2'}$ = 4.8, $J_{3',OH}$ = 4.0, $J_{3',4'}$ = 1.7 Hz), 2.79 (3 H, d, NMe), 2.42 (2 H, t, ≡CCH_2-$(CH_2)_2CH_3$), 1.55–1.35 (4 H, m, ≡$CCH_2(CH_2)_2CH_3$), 0.90 (3 H, t, ≡$CCH_2(CH_2)_2CH_3$). Anal. ($C_{17}H_{22}N_6O_4$·$^1/_2H_2O$) C, H, N.

2-(1-Hexyn-1-yl)adenosine-5'-*N*-ethyluronamide (16c). Treatment of **15c** (69 mg, 0.16 mmol) with TFA (7 mL) for 2 h gave **16c** (54 mg, 86%, crystallized from EtOH): mp 147–150 °C; MS *m/z* 389 (M^+ + H); IR ν C≡C 2240 cm^{-1}; NMR (270 MHz, DMSO-d_6) 8.79 (1 H, t, CONH), 8.41 (1 H, s, H-8), 7.55 (2 H, br s, 6-NH_2), 5.92 (1 H, d, H-1', $J_{1',2'}$ = 7.7 Hz), 5.77 (1 H, d, 3'-OH, *J* = 4.0 Hz), 5.57 (1 H, d, 2'-OH, *J* = 6.6 Hz), 4.57 (1 H, ddd, H-2', $J_{2',1'}$ = 7.7, $J_{2',OH}$ = 6.6, $J_{2',3'}$ = 4.8 Hz), 4.30 (1 H, d, H-4', $J_{4',3'}$ = 1.1 Hz), 4.11 (1 H, ddd, H-3', $J_{3',2'}$ = 4.8, $J_{3',OH}$ = 4.0, $J_{3',4'}$ = 1.1 Hz), 3.36–3.25 (2 H, m, NCH_2CH_3), 2.41 (2 H, t, ≡CCH_2-$(CH_2)_2CH_3$), 1.56–1.35 (4 H, m, ≡$CCH_2(CH_2)_2CH_3$), 1.08 (3 H, t, NCH_2CH_3), 0.91 (3 H, t, ≡$CCH_2(CH_2)_2CH_3$). Anal. ($C_{18}H_{24}N_6O_4$·H_2O) C, H, N.

2-(1-Hexyn-1-yl)adenosine-5'-*N*-cyclopropyluronamide (16d). Treatment of **15d** (100 mg, 0.23 mmol) with TFA (5 mL) for 2 h gave **16d** (67 mg, 73%, crystallized from EtOH): mp 133–138 °C; MS *m/z* 400 (M^+); IR ν C≡C 2240 cm^{-1}; NMR (270 MHz, DMSO-d_6) 8.52 (1 H, s, H-8), 8.25 (1 H, d, CONH, *J* = 3.9 Hz), 7.46 (2 H, br s, 6-NH_2), 5.94 (1 H, d, H-1', $J_{1',2'}$ = 6.6 Hz), 5.66 (1 H, d, 3'-OH, *J* = 5.0 Hz), 5.59 (1 H, d, 2'-OH, *J* = 6.0 Hz), 4.61 (1 H, ddd, H-2', $J_{2',1'}$ = 6.6, $J_{2',OH}$ = 6.0, $J_{2',3'}$ = 4.4 Hz), 4.27 (1 H, d, H-4', $J_{4',3'}$ = 2.2 Hz), 4.17 (1 H, ddd, H-3', $J_{3',2'}$ = 4.4, $J_{3',OH}$ = 5.0, $J_{3',4'}$ = 2.2 Hz), 2.72–2.65 (1 H, m, *N*-cyclopropyl), 2.41 (2 H, t, ≡$CCH_2(CH_2)_2CH_3$), 1.58–1.36 (4 H, m, ≡$CCH_2(CH_2)_2CH_3$), 0.91 (3 H, t, ≡$CCH_2(CH_2)_2CH_3$), 0.69–0.61 (2 H, m, *N*-cyclopropyl), 0.50–0.43 (2 H, m, *N*-cyclopropyl). Anal. ($C_{19}H_{24}N_6O_4$·$^4/_3H_2O$) C, H, N.

2-(1-Hexyn-1-yl)adenosine-5'-*N*-propyluronamide (16e). Treatment of **15e** (276 mg, 0.63 mmol) with TFA (10 mL) for 2 h gave **16e** (200 mg, 80%, crystallized from EtOH/hexane): mp 142–144 °C; MS *m/z* 402 (M^+); IR ν C≡C 2240 cm^{-1}; NMR (270 MHz, DMSO-d_6) 8.69 (1 H, t, CONH), 8.42 (1 H, s, H-8), 7.54 (2 H, br s, 6-NH_2), 5.93 (1 H, d, H-1', $J_{1',2'}$ = 7.8 Hz), 5.76 (1 H, d, 2'-OH, *J* = 3.9 Hz), 5.57 (1 H, d, 3'-OH, *J* = 6.4 Hz), 4.59 (1 H, ddd, H-2', $J_{2',1'}$ = 7.8, $J_{2',OH}$ = 3.9, $J_{2',3'}$ = 4.4 Hz), 4.32 (1 H, s, H-4'), 4.12 (1 H, dd, H-3', $J_{3',2'}$ = 4.4, $J_{3',OH}$ = 6.4 Hz), 3.36–3.31 (2 H, m, *N*-propyl), 2.42 (2 H, t, ≡$CCH_2(CH_2)_2CH_3$), 1.57–1.23 (6 H, m, *N*-propyl, ≡$CCH_2(CH_2)_2CH_3$), 0.91 (3 H, t, ≡CCH_2-$(CH_2)_2CH_3$), 0.83 (3 H, t, *N*-propyl). Anal. ($C_{19}H_{26}N_6O_4$·H_2O) C, H, N.

2-(1-Hexyn-1-yl)adenosine-5'-*N*-butyluronamide (16f). Treatment of **15f** (350 mg, 0.77 mmol) with TFA (10 mL) for 2 h gave **16f** (310 mg, 97%, crystallized from EtOH): mp 130–133 °C; MS *m/z* 416 (M^+); IR ν C≡C 2240 cm^{-1}; NMR (270 MHz, DMSO-d_6) 8.64 (1 H, t, CONH), 8.43 (1 H, s, H-8), 7.54 (2 H, br s, 6-NH_2), 5.93 (1 H, d, H-1', $J_{1',2'}$ = 7.3 Hz), 5.72–5.56 (2 H, m, 2'-OH, 3'-OH), 4.58 (1 H, ddd, H-2', $J_{2',1'}$ = 7.3, $J_{2',3'}$ = 4.4 Hz), 4.31 (1 H, d, H-4', $J_{4',3'}$ = 1.5 Hz), 4.11 (1 H, dd, H-3', $J_{3',2'}$ = 4.4, $J_{3',4'}$ = 1.5 Hz), 3.44–3.16 (2 H, m, *N*-butyl), 2.41 (2 H, t, ≡$CCH_2(CH_2)_2CH_3$), 1.56–1.21 (8 H, m, *N*-butyl, ≡$CCH_2(CH_2)_2CH_3$), 0.93–0.84 (6 H, m, *N*-butyl, ≡$CCH_2(CH_2)_2CH_3$). Anal. ($C_{20}H_{28}N_6O_4$·$^1/_2H_2O$) C, H, N.

5'-Chloro-5'-deoxy-2-(1-hexyn-1-yl)adenosine (20). Treatment of **19** (70 mg, 0.17 mmol) with 80% TFA (5 mL) for 2 h gave **20** (50 mg, 79%, crystallized from EtOAc): mp 151–154 °C; MS *m/z* 365 (M^+); IR ν C≡C 2240 cm^{-1}; NMR (270 MHz, DMSO-d_6) 8.37 (1 H, s, H-8), 7.42 (2 H, br s, 6-NH_2), 5.89 (1 H, d, H-1', $J_{1',2'}$ = 5.9 Hz), 5.65–5.40 (2 H, m, 2'-OH, 3'-OH), 4.67 (1 H, dd, H-2', $J_{2',1'}$ = 5.9, $J_{2',3'}$ = 5.4 Hz), 4.18 (1 H, dd, H-3', $J_{3',2'}$ = 5.4, $J_{3',4'}$ = 3.4 Hz), 4.09 (1 H, ddd, H-4', $J_{4',3'}$ = 3.4, $J_{4',5'a}$ = 4.9, $J_{4',5'b}$ = 6.4 Hz), 3.94 (1 H, dd, H-5'a, $J_{5'a,4'}$ = 4.9, $J_{5'a,b}$ = 11.7 Hz), 3.84 (1 H, dd, H-5'b, $J_{5'b,4'}$ = 6.4, $J_{5'a,b}$ = 11.7 Hz), 2.40 (2 H, t, ≡$CCH_2(CH_2)_2CH_3$), 1.55–1.35 (4 H, m, ≡$CCH_2(CH_2)_2CH_3$), 0.90 (3 H, t, ≡$CCH_2(CH_2)_2CH_3$). Anal. ($C_{16}H_{20}ClN_5O_3$) C, H, N.

5'-Deoxy-5'-formamido-2-(1-hexyn-1-yl)adenosine (27). Treatment of **22** (272 mg, 0.67 mmol) with TFA (10 mL) for 1 h gave **27** (226 mg, 92%, crystallized from EtOH): mp 135–137 °C; MS *m/z* 373 (M^+); IR ν C≡C 2240 cm^{-1}; NMR (270 MHz, DMSO-d_6) 8.52 (1 H, br s, NH), 8.39 (1 H, s, H-8), 8.15 (1 H, s, CHO), 7.47 (2 H, br s, 6-NH_2), 5.84 (1 H, d, H-1', $J_{1',2'}$ = 6.6 Hz), 5.5–5.3 (2 H, br s, 2'-, 3'-OH), 4.70–4.58 (1 H, m, H-2'), 4.10–3.90 (2 H, m, H-3', 4'), 3.60–3.30 (2 H, m, H-5'a,b), 2.41 (2 H, t, ≡$CCH_2(CH_2)_2CH_3$), 1.60–1.35 (4 H, m, ≡$CCH_2(CH_2)_2CH_3$), 0.91 (3 H, t, ≡$CCH_2(CH_2)_2CH_3$). Anal. ($C_{17}H_{21}N_6O_4$·$^2/_3H_2O$) C, H, N.

5'-Acetamido-5'-deoxy-2-(1-hexyn-1-yl)adenosine (28). Treatment of **23** (152 mg, 0.35 mmol) with TFA (10 mL) for 4 h at 0 °C gave **28** (123 mg, 89%, crystallized from EtOH): mp 198–203 °C; MS *m/z* 388 (M^+); IR ν C≡C 2240 cm^{-1}; NMR (270 MHz, DMSO-d_6) 8.38 (1 H, s, H-8), 8.13 (1 H, t, NH), 7.43 (2 H, br s, 6-NH_2), 5.82 (1 H, d, H-1', $J_{1',2'}$ = 6.1 Hz), 5.46 (1 H, d, 2'-OH, $J_{2',OH}$ = 5.0 Hz), 5.26 (1 H, br s, 3'-OH), 4.56 (1 H, br s, H-2'), 4.02 (1 H, br s, H-3'), 3.93 (1 H, br s, H-4'), 3.46–3.37 (2 H, m, H-5'a,b), 2.41 (2 H, t, ≡$CCH_2(CH_2)_2CH_3$), 1.87 (3 H, s, Ac), 1.58–1.38 (4 H, m, ≡$CCH_2(CH_2)_2CH_3$), 0.91 (3 H, t, ≡CCH_2-$(CH_2)_2CH_3$). Anal. ($C_{18}H_{24}N_6O_4$·$^1/_2H_2O$) C, H, N.

5'-Deoxy-5'-(methanesulfonamido)-2-(1-hexyn-1-yl)adenosine (29). Treatment of **24** (150 mg, 0.32 mmol) with TFA (10 mL) for 4 h at 0 °C gave **29** (126 mg, 92%, crystallized from EtOH): mp 122–126 °C; MS *m/z* 424 (M^+); IR ν C≡C 2240 cm^{-1}; NMR (270 MHz, DMSO-d_6) 8.39 (1 H, s, H-8), 7.41 (2 H, br s, 6-NH_2), 7.32 (1 H, t, NH), 5.85 (1 H, d, H-1', $J_{1',2'}$ = 6.6 Hz), 5.51 (1 H, d, 2'-OH, $J_{2',OH}$ = 6.0 Hz), 5.30 (1 H, d, 3'-OH, $J_{3',OH}$ = 4.4 Hz), 4.68–4.60 (1 H, m, H-2'), 4.12 (1 H, br s, H-3'), 4.00–3.95 (1 H, m, H-4'), 3.36–3.16 (2 H, m, H-5'a,b), 2.91 (3 H, s, Ms), 2.41 (2 H, t, ≡$CCH_2(CH_2)_2CH_3$), 1.60–1.35 (4 H, m, ≡CCH_2-$(CH_2)_2CH_3$), 0.91 (3 H, t, ≡$CCH_2(CH_2)_2CH_3$). Anal. ($C_{17}H_{24}N_6O_5S$·H_2O) C, H, N.

5'-Deoxy-5'-(3-methylureido)-2-(1-hexyn-1-yl)adenosine (30). Treatment of **25** (240 mg, 0.54 mmol) with TFA (10 mL) for 3 h at −20 °C gave **30** (180 mg, 82%, crystallized from EtOH): mp 146–148 °C; MS *m/z* 403 (M^+); IR ν C≡C 2240 cm^{-1}; NMR (270 MHz, DMSO-d_6) 8.39 (1 H, s, H-8), 7.44 (2 H, br s, 6-NH_2), 6.17 (1 H, t, NH), 5.83 (1 H, d, H-1', $J_{1',2'}$ = 6.6 Hz), 5.74 (1 H, br s, NH), 5.60–5.00 (2 H, m, 2'-, 3'-OH), 4.55 (1 H, dd, H-2', $J_{2',1'}$ = 6.6, $J_{2',3'}$ = 5.0 Hz), 4.02 (1 H, dd, H-3', $J_{3',2'}$ = 5.0, $J_{3',4'}$ = 3.3 Hz), 3.95–3.90 (1 H, m, H-4'), 3.80–3.50 (1 H, m, H-5'a), 3.40–3.25

(1 H, m, H-5′b), 2.56 (3 H, s, Me), 2.41 (2 H, t, ≡CCH_2(CH_2)$_2$$CH_3$), 1.60–1.35 (4 H, m, ≡$CH_2$(C$H_2$)$_2$$CH_3$), 0.91 (3 H, t, ≡$CH_2$($CH_2$)$_2CH_3$). Anal. ($C_{18}H_{25}N_7O_4$·$H_2O$) C, H, N.

5′-Deoxy-5′-(3-methylthioureido)-2-(1-hexyn-1-yl)-adenosine (31). Treatment of 26 (200 mg, 0.44 mmol) with TFA (10 mL) for 6 h at −20 °C gave 31 (96 mg, 53%, crystallized from EtOH): mp 131–135 °C; MS m/z 419 (M^+); IR ν C≡C 2240 cm^{-1}; NMR (270 MHz, DMSO-d_6) 8.39 (1 H, s, H-8), 7.61 (1 H, br s, NH), 7.41 (3 H, br s, NH, 6-NH_2), 5.83 (1 H, d, H-1′, $J_{1',2'}$ = 6.6 Hz), 5.47 (1 H, br s, 2′-OH), 5.28 (1 H, br s, 3′-OH), 4.59 (1 H, br s, H-2′), 4.15–4.00 (2 H, m, H-3′, 4′), 3.90–3.60 (2 H, m, H-5′a,b), 2.81 (3 H, s, Me), 2.41 (2 H, t, ≡CH_2(CH_2)$_2$$CH_3$), 1.60–1.36 (4 H, m, ≡$CH_2$(C$H_2$)$_2$$CH_3$), 0.91 (3 h, t, ≡$CH_2$($CH_2$)$_2CH_3$). Anal. ($C_{18}H_{25}N_7O_3S$·$H_2O$) C, H, N.

A_1 and A_2 Binding Assay. The A_1 and A_2 receptor binding affinities for the test compounds were measured in the presence of adenosine deaminase (type VI, Sigma) in triplicate by previously described methods.[8] A_1 receptor binding was measured in adenosine deaminase (ADA) pretreated rat brain (without cerebellum and brain stem) homogenates with 2.5 nM [^{3}H]CHA as a radioligand, and binding to the A2 receptor was measured in ADA-pretreated rat striatal membranes using 5 nM [^{3}H]NECA in the presence of 50 nM CPA. IC_{50} values were calculated by a computerized nonlinear, least-squares analysis program (SP-1.2.3, programmed by Dr. Ono, University of Tokyo, Tokyo, Japan).

Cardiovascular Effects. Effects of the compound on BP and HR after iv administration were measured in anesthetized male SHR as described previously.[8] Briefly, each test compound (0.03–100 μg/mL) was administered iv at 5-min intervals in a cumulative manner. The relative potency to decrease BP was estimated on the basis of the ED_{30} value, the mean dose that produced a 30% decrease in BP of SHR. Similarly, the relative potency to decrease HR was estimated on the basis of the ED_{10} value, the mean dose that produced a 10% decrease in HR of SHR.

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Anti-HIV-I Activity of Linked Lexitropsins

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Five groups of lexitropsin oligopeptides have been synthesized that are structurally related to the natural antiviral agents netropsin and distamycin and bearing two such moieties joined by flexible or rigid linkers. Inhibitory activity of these types of agents against murine leukemia retrovirus (MuLV) led to an evaluation of their inhibition of HIV-I in cell culture. The antiretroviral activity of the five different classes of lexitropsins is discussed in terms of their structural differences.

Introduction

World Health Organization (WHO) data suggest that some 1.2 million cases of AIDS have occurred worldwide in the past 10 years and that 10 million people could be infected.[1] This last figure may rise to 30–40 million by the end of the century.[1] Although great strides have been made in understanding the molecular biology of the generally accepted causative agent, the human immunodeficiency viruses, HIV,[2,3] and some palliative agents are available clinically,[4,5] AIDS is still an incurable disease.

Wellcome's Zidovudine (AZT)[6] is the principal agent being used clinically for treating HIV infection,[4,5,7] although related nucleoside structures are anticipated to obtain U.S. Food and Drug Administration (FDA) approval soon. Experience with AZT has led to improved protocols such as lower doses—from daily doses of 1–2 g per day in early 1990, to about 0.5 g per day at the present time.[5] There has also been a trend to the earlier use of AZT to treat AIDS and ARC (AIDS related complex), although this may allow a reservoir of virus to build up in the population.[4,5,7] The challenge, fueled by the increasing resistance of HIV to AZT, is to develop alternative chemotherapeutic agents.

The majority of drugs being developed are structurally related to AZT, i.e. nucleoside derivatives that are reverse transcriptase (RT) inhibitors. Such agents, including dideoxyinosine (ddI), dideoxycytidine (ddC), and 3′-azidodideoxyuridine (AzdU) are likely to exhibit similar toxicity (pancreatitis and nerve damage) and resistance problems as AZT.[5] Therefore there is an urgent demand not only for alternative structures effective against HIV but also for agents that operate by different mechanisms. Tetrahydroimidazobenzodiazepinone (TIBO) is one such agent that has been uncovered by systematic screening procedures in Belgium.[5]

We describe the design and synthesis of certain extended oligopeptide lexitropsins that, based on their inhibition of

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(1) Grmek, M. D. In *History of Aids. Emergence and Origin of a Modern Pandemic*; Princeton University Press: Princeton, 1990.
(2) Broder, S.; Gallo, R. C. *N. Engl. J. Med.* **1984**, *311*, 1292–1297.
(3) Popovic, M.; Sarngadharan, M. G.; Read, E.; Gallo, R. C. *Science* **1984**, *224*, 497–500.
(4) Mitsuya, H.; Broder, S. *Nature* **1987**, *325*, 773–778.
(5) Nelson, S. *Chem. Br.* **1991**, 294–296.
(6) Horwitz, J. P.; Chua, J.; Noel, M. *J. Org. Chem.* **1964**, *29*, 2076–2078.
(7) Reference 1, pp 183–186.

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