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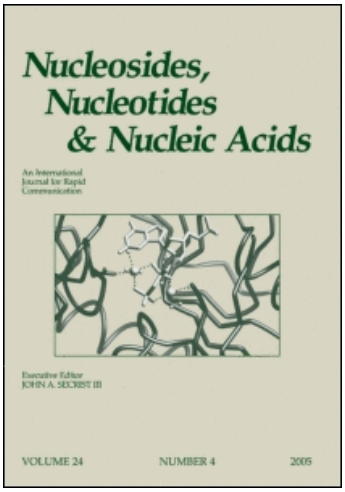

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THE SYNTHESIS, MUTAGENIC AND PHARMACOLOGICAL ACTIVITIES OF 2-CARBON-SUBSTITUTED ADENOSINES†

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Abstract: 2'-Deoxy-2-(p-nitrophenyl)-adenosine (15) was found to be the most potent mutagen when tested with S. typhimurium TA 98 and TA 100 in the series of compounds, whose activity towards TA 98 is one order of magnitude greater than that of 4-nitroquinoline N-oxide.

In another series of reactions, synthesis of 2-alkynyl-adenosines (18) was achieved through the palladium catalyzed cross-coupling of terminal alkynes with 2-iodoadenosine (17). Compounds bearing the 2-C≡CCH(R)OH side-chain exhibited potent antiallergic activity in rats. These compounds, however, possessed hypotensive activity accompanied by a decrease in heart rate whereas with a longer alkyl side-chain at the 2-position, compounds showed selective hypotensive activity.

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Adenosine plays many important roles in initiating numerous metabolic actions in a variety of cells.[2] These include many pharmacological activities and certain adenosine analogs show antiviral and anticancer activities. However, adenosine itself and its analogs have been known to be metabolically unstable due to their deamination by adenosine deaminase and phosphorylation by adenosine kinase. In order to circumvent these disadvantages, numerous analogs of adenosine bearing a substituent at the C-2 position have been synthesized. In contrast to the development of methods for the introduction of hetero-atoms into the C-2 position of adenosine, only few studies have been reported on carbon-carbon bond formation reactions. These so far involve the

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classical condensation between pre-formed 2-substituted adenines and sugars,[3] the cyclization of appropriately-substituted imidazole nucleosides,[4] homolytic methylation[5] and nucleophilic substitution of 2-methanesulfonyladenosine with cyanide.[6]

Recently, we have found a convenient procedure for the introduction of phenyl and heteroaryl groups at the 2-position of 2-amino-6-chloropurine riboside[7] and a versatile method involving a cross-coupling of 2-iodoadenosine with terminal alkynes.[8] During the course of these synthetic studies we have also found a mutagenic activity in a crude preparation of 2-phenyladenosine and we report some pharmacological activities including antiallergic and hypotensive activities shown by 2-alkynyladenosines.

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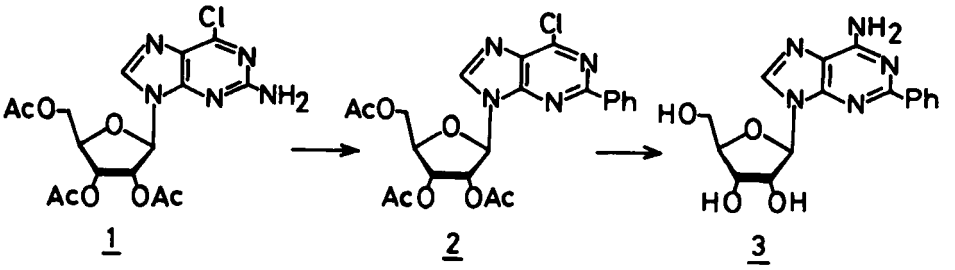
1. SYNTHESIS AND MUTAGENIC ACTIVITIES OF 2-(NITROPHENYL)-ADENINES

When 2-amino-6-chloropurine riboside (1) was heated in benzene with an excess of isopentyl nitrite in the presence of copper(I) oxide, 6-chloro-2-phenylpurine nucleoside (2) was obtained.[7] The crude 2 was then heated with NH_3/MeOH at 70°C to give crude 2-phenyladenosine (3). A series of nucleoside derivatives including the crude 2-phenyladenosine has been tested for mutagenesis in the Ames' Salmonella system[8](Salmonella typhimurium TA 98 which is a frameshift-mutagen detector, and TA 100 which is a base-pair change-mutagen detector). Interestingly, the crude preparation of 3 was found to be mutagenic. However, 2-phenyladenosine (3) purified by HPLC did not show any mutagenic activity. Instead, the mutagenic activity toward both TA 98 and TA 100 (without metabolic activation) was almost entirely concentrated in one of the minor peaks which seemed to consist of at least two compounds. From ca. 200 mg of crude 3, 0.8 mg of a mixture of the minor compounds was isolated by using a semi-preparative reverse phase column. From mass (M^+,m/z 388) and ^{1}NMR spectroscopic data, this material was shown to be a mixture of isomeric 2-(nitrophenyl)-adenosines (5). In



scheme 1

order to determine the position of the nitro group in the phenyl ring, we synthesized 2-(o-,m- and p-nitrophenyl)-adenosines (5o,m,p) in yields of 20, 51 and 71%, respectively, in an unambiguous manner from 4-cyano-5-aminoimidazole nucleoside (4) by treatment with o-, m- or p-nitrobenzonitriles in NH_3/MeOH[4] (scheme 2). The 2-(m- and p-nitrophenyl)-adenosines (5m,p) had the almost same retention time (13.7 and 14.0 min, respectively) as the minor peak (13-17 min) and both had mutagenic activity to TA 98 and TA 100. On the other hand, the o-nitro derivative (5o), whose retention time is 4.7 min, did not show any activity at all.

It is of interest to study whether the mutagenicity of these compounds (5m,p) arises from the nucleosidic character or whether they are behaving as aromatic nitro compounds. To clarify the structure-activity relationships of these compounds, several nucleoside and base analogs were synthesized (scheme 3). Compound 5p was deaminated with HNO_2 to afford the inosine analog 6. The p-anilino nucleoside 7 was obtained by catalytic reduction of 5p. Compounds 5p and 6 were treated with 2N HCl to give the base analogs 8 and 9, respectively. 2'-Deoxy-2-(p-nitrophenyl)-adenosine (15) was also synthesized by the following sequence: selective protection of the the 3',5'-hydroxyls of 10 with TIPDS, Barton deoxygenation of the thiocarbonyl derivative of 12, the synthesis of the nitrophenylpyrimidine portion (13) and the deblocking of 14 to give 15 (scheme 4).

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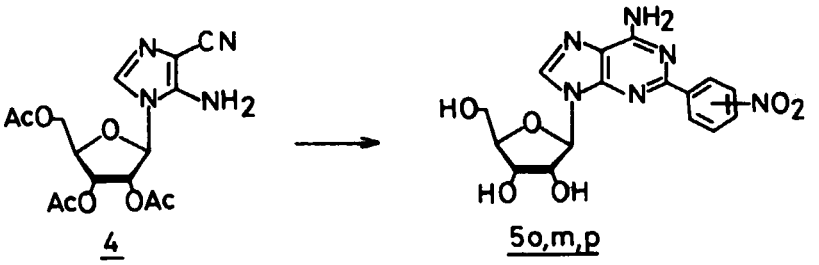
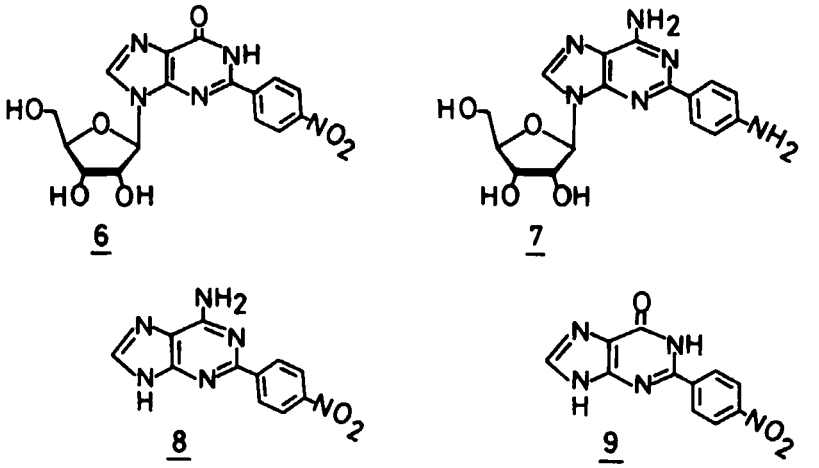
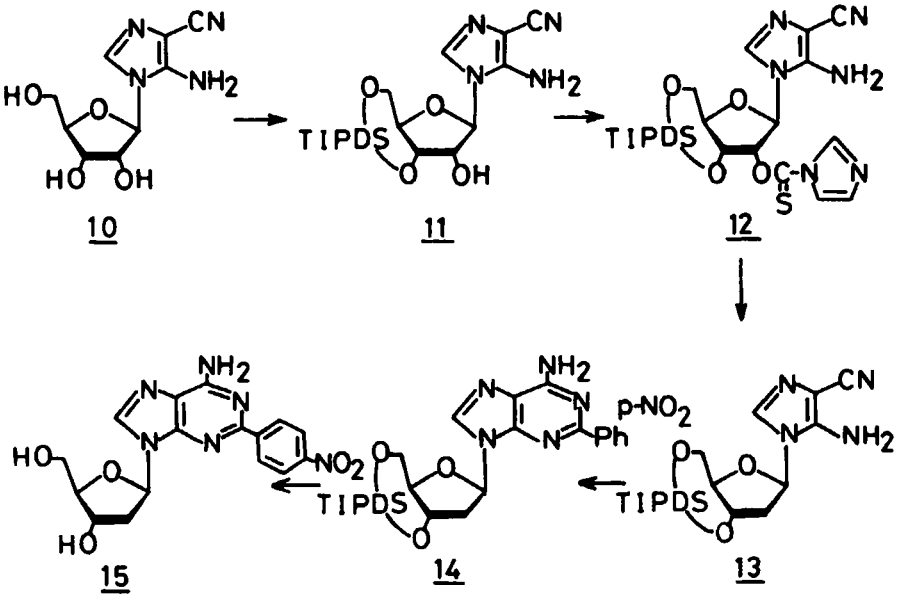


scheme 2



scheme 3



scheme 4

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The deaminated nucleoside (6) was less mutagenic than 5p, whereas the diamino compound (7) did not show any activity with either TA 98 or TA 100. This means that the mutagenic activity is based upon the nitro function. The activity of the adenine derivative (8) was found to be much greater than that of 5p. The hypoxanthine derivative (9) was also mutagenic, but it was again much less potent than 8. Surprisingly, 2'-deoxy-2-(p-nitrophenyl)-adenosine (15) was four times more potent than 8 in the reversion of TA 98 (12,000 revertants per μg) and also exhibited significant mutagenicity to the TA 100 strain (3,700 revertants per μg). The activity of 15 towards TA 98 is one order of magnitude greater than that of 4-nitroquinoline N-oxide which is known to be as a powerful mutagen and carcinogen. The mutagenic mechanism of action of both compounds (8,15) remains the subject of further study. However, it was shown that they required nitroreductase activation to exhibit the mutagenicity since they show no or markedly less activity with the nitroreductase-deficient mutants, TA 100/1,8-DNP and TA 100NR.

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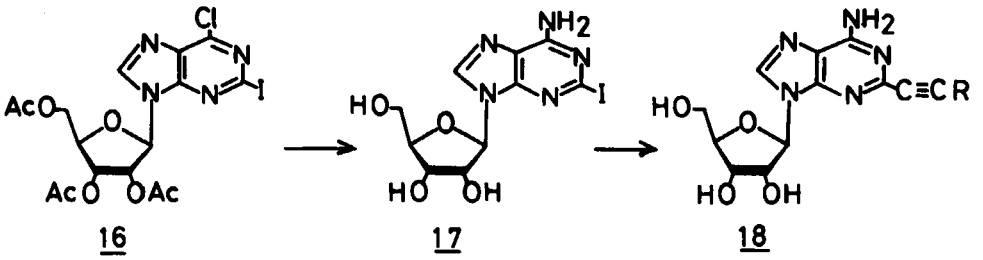
2. SYNTHESIS AND PHARMACOLOGICAL ACTIVITIES OF 2-ALKYNYL-ADENOSINES

In order to find a more general method for the synthesis of 2-carbon-substituted adenosines, we tried the cross-coupling reaction of 2-iodoadenosine (17) with terminal alkynes. Compound 17 was easily prepared from 6-chloro-2-iodopurine riboside (16) by treatment with NH_3/MeOH. The synthesis of 16, originally prepared on a small scale by Nair and Richardson,[10] with the diazotization-substitution reaction of 1 in CH_2I_2, could be achieved on a large scale by using CH_3CN or THF as a cosolvent in the presence of CH_2I_2, I_2 and CuI. When 17 was treated with a slight molar excess of trimethylsilylacetylene in the presence of catalytic amounts of $(PPh_3)_2PdCl_2$ and CuI in Et_3N/DMF at 80°C for 1 h, 2-(2-trimethylsilylethynyl)-adenosine (18a) was ob-



16 17 18

scheme 5

tained in crystalline form after treatment with H_2S.[8] Several of the terminal alkynes reacted in a similar manner with compound 17 to give 2-alkynyladenosines (18a-l) in high yields.

Among the series of 2-alkynyladenosines (18), only 2-ethynyl- and 2-(2-trimethylsilylethynyl)-adenosines (18b,a) had a moderate growth inhibitory activity (ID_{50}; 1.6 and 3.2 μg/ml, respectively) against murine leukemic L5178Y cells in culture. The inhibitory activity of 18 on the 48 hr-passive cutaneous anaphylaxis (PCA) reaction was also examined by the i.v. route in rats sensitized with rat anti-dinitrophenylated-ascaris extract antiserum (Table 1). Compounds bearing -C≡CCH(R)OH side-chains at the C-2 position were found to be the most potent inhibitors of the series and were more active than disodium cromoglycate (DSCG), the widely used antiallergic drug. An increase or decrease in the number of carbon atoms in the R substituent resulted in a reduction or the loss of the PCA-inhibitory activity. Furthermore, increasing the size of the R-substituent also reduced or eliminated the inhibitory activity.

In order to elucidate the structure-activity relationships further, we synthesized several analogs including 2-alkyl and alkenyl derivatives derived from 18d, positional isomers of hydroxy-alkynyl side-chains other than at the C-2 position and sugar analogs of 18d. When 18d was reduced catalytically with 10% Pd/C, 2-(3-hydroxypropyl)-adenosine

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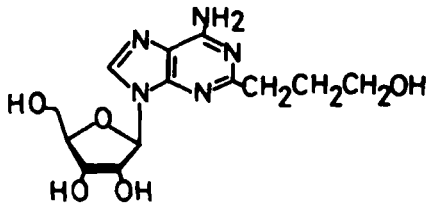
Table 1. Pharmacological activities of 2-alkynyladenosines (18a-l)

	R	% inhibition of 48 h-PCA (mg/kg, iv) 0.12	0.4	1.2	4.0	cardiovascular responses (10μg/kg, iv) BP(%)	HR(%)	duration
a	$SiMe_3$	20	48	76	75	-8	-3	0.5 min
b	H	0	10	50	100	—	—	—
c	Ph	0	0	0	0	-8	0	0.5
d	CH_2OH	0	100	100	100	-44	-33	20
e	CH_2CH_2OH	0	0	100	100	-37	-14	20
f	$CH(OH)CH_3$	0	100	100	100	-53	-46	25
g	CH(OH)Ph	0	100	100	100	-43	-59	90
h	$(CH_2)_2CH_3$	0	30	100	100	-29	-3	—
i	$(CH_2)_3CH_3$	0	0	0	0	-45	-8	15
j	$(CH_2)_4CH_3$	0	0	0	0	-41	0	15
k	$(CH_2)_5CH_3$	0	0	0	0	-44	0	25
l	$(CH_2)_7CH_3$	0	0	0	0	-21	0	45



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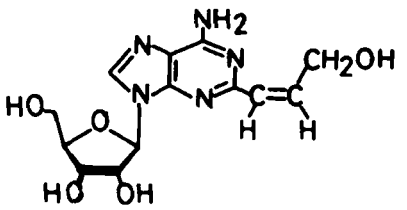

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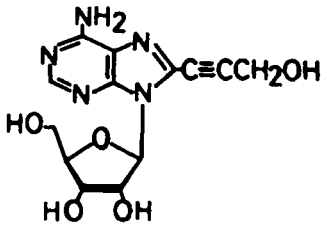

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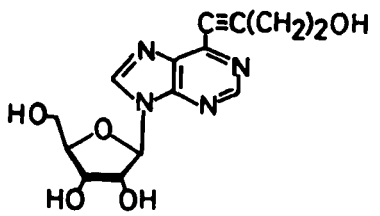

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scheme 6

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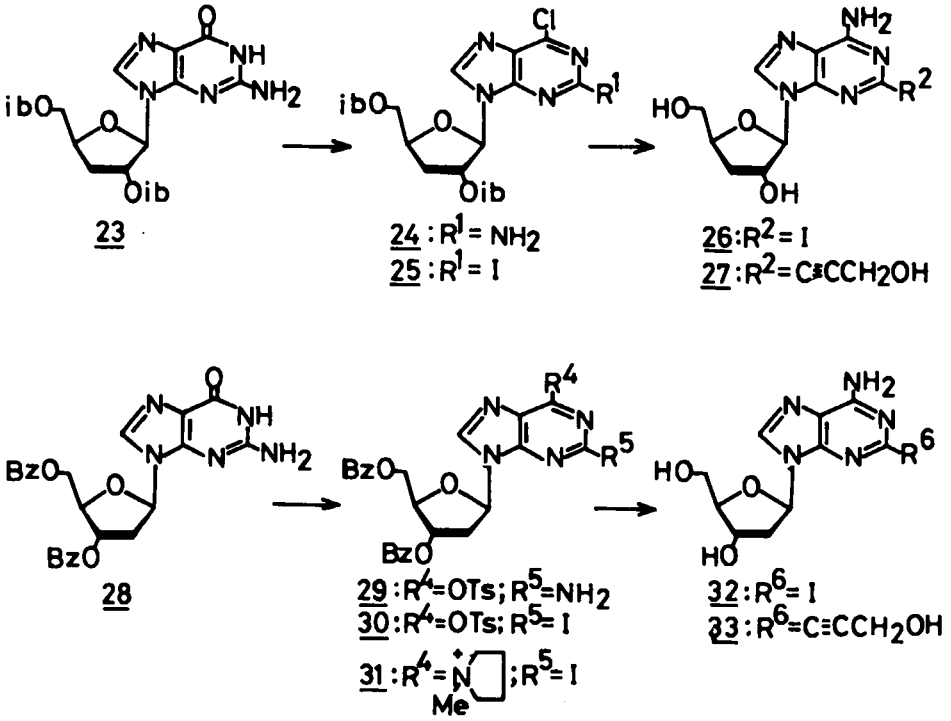

scheme 7

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(19) was obtained, whereas 2-(Z)-(3-hydroxypropenyl)-adenosine (20) was obtained using the Lindlar catalyst and quinoline. 8-(3-Hydroxy-1-propynyl)-adenosine (21) and 6-(4-hydroxy-1-butynyl)-purine riboside (22) were synthesized from respectively 8-bromoadenosine and 6-chloropurine riboside via the organopalladium intermediates. 2'-Deoxy- and 3'-deoxy-2-(3-hydroxy-1-propynyl)-adenosines (27,33) were also synthesized (scheme 7).

The inhibitory activity in the 48 hr-PCA reaction was markedly reduced in compounds 21 and 22. Compound 19 had one third of the activity of 18d whereas 20 was not active even at a ten-times higher concentration. Furthermore, both the 2'-deoxy and 3'-deoxy analogs (27,33) did not exhibit any significant activity. These results show that in the adenosine system, substituents at C-2 containing 3-4 carbon atoms

with a terminal triple bond and a sugar moiety containing the 2'-, 3'-cis-diol system with a ribo-configuration are required for inhibitory activity in the PCA reaction in rats. During the course of this study, we have found that the antiallergic activity of 18 was accompanied by a hypotensive activity and a decrease in heart rate following intravenous administration in normal rats. Interestingly, some of the 2-alkynyladenosines having longer alkyl side-chains showed potent hypotensive activity without an antiallergic effect and a heart rate decrease. It should be noted that these compounds still have a hypotensive activity in the spontaneously hypertensive rat (SHR).

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REFERENCES

1. This paper constitutes Nucleosides and Nucleotides Part 71: Part 70; H.Inoue, N.Saito and T.Ueda, Chem. Pharm. Bull., 34, in press (1986).
2. For recent reviews, see J.R.S.Arch and E.A.Newsholme, Essays Biochem., 14,82 (1978); I.H.Fox and W.N.Kelley, Ann. Rev. Biochem., 47, 655 (1978).
3. G.Gough and M.H.Maguire, J. Med. Chem., 8,866 (1965).
4. R.Marumoto, Y.Yoshioka, O.Miyashita, S.Shima, K.Imai, K.Kawazoe, and M.Honjo, Chem. Pharm. Bull., 23,759 (1975); A.Yamazaki, I.Kumashiro and T.Takenishi, J. Org. Chem., 32,3258 (1967); T.Murakami, M.Otsuka, S.Kobayashi and M.Ohno, Heterocycles, 16, 1315 (1981).
5. M.Maeda, K.Nushi and Y.Kawazoe, Tetrahedron, 30, 2677 (1974).

6. A.Matsuda, Y.Nomoto and T.Ueda, Chem. Pharm. Bull., 27, 183 (1979).
7. A.Matsuda, K.Satoh, H.Tanaka and T.Miyasaka, Synthesis, 1984, 963.
8. A.Matsuda, M.Shinozaki, T.Miyasaka, H.Machida and T.Abiru, Chem. Pharm. Bull., 33, 1766 (1985).
9. B.N.Ames, J.McCann and E.Yamasaki, Mutation Res., 31, 347 (1975).
10. V.Nair and S.G.Richardson, Synthesis, 1982, 670.

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