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Modification of the 5′ Position of Purine Nucleosides. 1. Synthesis and Biological Properties of Alkyl Adenosine-5′-carboxylates

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A series of esters of adenosine-5′-carboxylic acid has been prepared. Most of the compounds were nontoxic, causing prolonged increases in coronary sinus PO_2 when administered to anesthetized dogs; the ethyl ester was most active. Nitrosation and oxidation of the ethyl ester **12** gave respectively inactive inosine ethyl ester **30** and the fairly active N^1-oxide ethyl ester **29**.

The overall pharmacological profile of ethyl adenosine-5′-carboxylate (**12**) has been reported.[2-5] Evaluation of **12** in animals shows a profile of activity which suggests possible antianginal properties in man. Thus, **12** represents a new type of relatively nontoxic potent coronary vasodilator,[5] orally active in the dog. Currently, **12** is undergoing extensive clinical evaluation as a potential antianginal agent.

Studies aimed at delineating the structure–activity relationships of esters of adenosine-5′-carboxylic acid (**8**) are reported in this paper.

Chemistry. The synthetic steps leading to the formation of the esters (**4–7**, Table I) of 2′,3′-*O*-isopropylideneadenosine-5′-carboxylic acid[6,7,11] are outlined in Scheme I. Similarly, the esters (**11–25**, Table II) of adenosine-5′-carboxylic acid (**8**)[6-11] were prepared by the reaction of appropriate alcohols with an uncharacterized acid chloride **9** (obtained from **8** and $SOCl_2$) or alternatively by other methods summarized in Scheme II.

Since a wide variety of esters were desired for pharmacological screening, an extensive study of esterification methods was made. Four different methods were required for the preparation of the esters listed in Tables I and II.

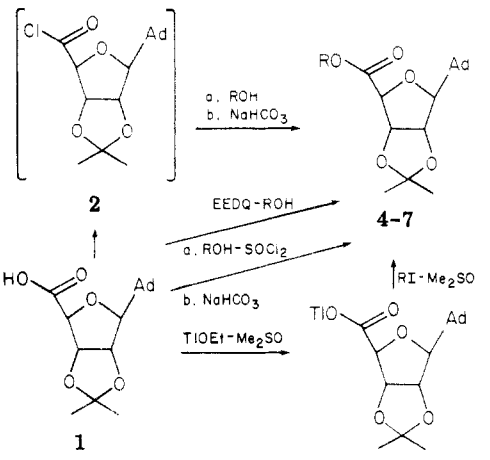
The thallous ethoxide method, which was used first, was found to be inconvenient due to the poor solubility of the intermediate thallous salts obtained from **1** and **8**.

Esterification of **1** and **8** with alcohols in the presence of $SOCl_2$ proceeded normally in most cases. However, in some cases, as in the esterification of **1** with 2-propanol and 1-butanol, the isopropylidene group was cleaved to the extent of 30–40%.

The carboxylic acids (**1** and **8**) on treatment with $SOCl_2$ gave the uncharacterized acid chlorides **2** and **9**. Reaction of **2** and **9** with the appropriate alcohol followed by $NaHCO_3$ was the next method of choice. However, in this case, traces of water in the alcohol caused the formation of varying amounts of the acids (**1** and **8**) as by-products.

The attempted synthesis of the 5′-*N*-alkylamide of **1** using an aliphatic amine and EEDQ in alcohol as a solvent gave a mixture of amide and ester (corresponding to the alcohol used). This observation led to the use of EEDQ as an esterification catalyst, as in the preparation of the *sec*-butyl ester **7**.

Scheme I



Ad = 9-adenyl

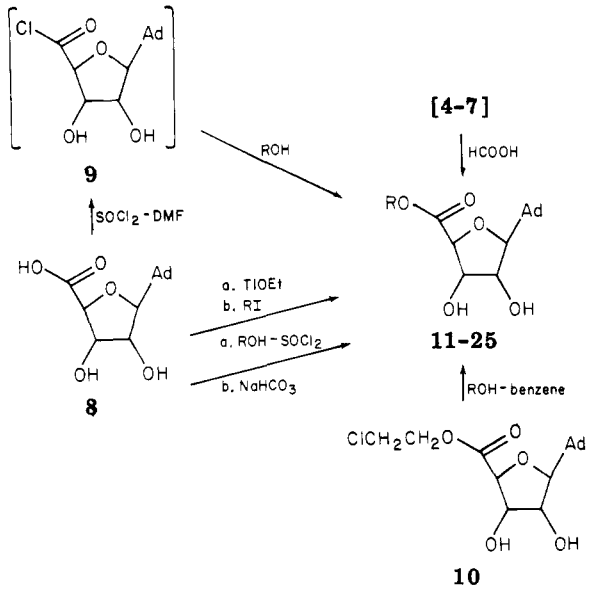
It was also observed that boiling ethanol or methanol in the presence of a small amount of benzene converted the β-chloroethyl ester **10** into the ethyl (**13**) or the methyl (**11**) ester in less than 30 min. The methyl ester **11** itself was unaffected even after a 24-h reflux period in ethanol. Use of a β-chloroethyl ester for such a mild ester interchange does not appear to have been reported before.

The 6-NH_2 group of **12** was smoothly deaminated by nitrosation in dilute acetic acid to give ethyl inosine-5′-carboxylate (**30**, Table III) in high yield. Another route for the preparation of **30** was by the esterification of inosine-5′-carboxylic acid.[12,13]

Oxidation of **1** by H_2O_2–AcOH gave adenosine-5′-carboxylic acid N^1-oxide (**28**) in good yield. The latter, on esterification, gave **29** (Table III), the desired N^1-oxide of **13**. Details of the methods are described in the Experimental Section.

Nuclear Overhauser studies[18] of the most active ester, **12**, show that there is 11.4% enhancement of H-8 reso-

Scheme II



nance when its H-1′ proton was irradiated. No such enhancement was noticed on the irradiation of the H-2′ proton. In cycloguanosine, locked in the syn conformation, there is 39% enhancement of H-8 proton resonance showing interaction between H-1′ and H-8 protons.[19] The data may simply be accounted for by a model in which the ester **12** has a conformation intermediate between syn and anti.

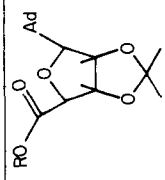
Results and Discussion

All compounds reported herein were evaluated for PO_2 activity in open-chest anesthetized dogs. It was assumed that with coronary arterial blood of relatively constant PO_2 (partial pressure of O_2 in mmHg) supplying the normal heart muscle, any compound causing an increase in coronary sinus blood PO_2 without concomitant increase in work load or changes in blood pressure (BP) would be of value to ischemic heart tissue. The method used has been described by Shoepke et al.[15] Representative effects are reproduced in Table III. Compounds which caused minimal effects on PO_2 (less than 40% increase for less than 10 min) were tested at 10 mg/kg, intravenously, in one animal only; those possessing greater activity were retested in different animals. The variability occurring with this experimental preparation is illustrated with **12**.

Esters **10**, **12**, **14**–**16**, **18**, **19**, and **23** raised the PO_2 value at a dose of 1 mg/kg or lower. Of these, compounds **10**, **12**, **15**, **18**, and **19** have very little or no effect on BP and HR. The methyl ester **11** was only weakly active, whereas its next higher homologue exerted a potent and long-lasting effect on PO_2.[5] The ethyl ester **12** was of the order of 100 times as active in the PO_2 test as the methyl (**11**) or propyl (**18**) esters. However, as the ester chain length was increased (Table III) gradually to *n*-butyl, the potency fell off sharply.

It appears that the greatest activity is retained in the two-carbon chain ester $R'CH_2CH_2OC(=O)-$, Figure 1, the ethyl ester (**12**, R′ = H) being the most active. The chloroethyl ester (**10**, R′ = Cl) was active at the 0.3 mg/kg dose. However, since the $ClCH_2CH_2-$ group of **10** is readily cleaved or replaced by other groups, it was not investigated further. The β-hydroxy ethyl ester (**14**, R′ = OH) retained a good deal of activity of the parent compound (**12**) but was obviously lower in potency. Replacement of R′ by phenyl (**20**), OEt (**16**), or cyclopentyl (**25**) groups resulted

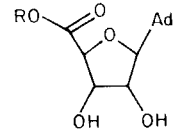
Table I

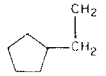


Compd no.	R	Mp, °C	Recrystn solvent[a]	Method (yield, %)[b]	Specific notation, deg	Optical rotation °C	Concn (*c*)	Solvent[c]	R_f[d]	Formula	Analyses
4	CH_3[e]	241–243[f]	M	A (91)	−11 ± 0.23	26	4	HCl		$C_{14}H_{17}N_5O_5$	C, H, N
				B (40)							
5	CH_3CH_2[e]	171–172[g]	E + B	A (42)	−14 ± 1.7	28	0.57	EtOH	0.73 (Y)	$C_{15}H_{19}N_5O_5$	C, H, N, O
				B (65)							
				C (90)							
				G (75)							
6	$(CH_3)_2CH$[e]	105–110[h]	Et	H[i] (30)	−20 ± 1	26	1	HCl	0.68 (X)	$C_{16}H_{21}N_5O_5$	C, H, N, O
7	$CH_3CH_2\underset{\displaystyle CH_3}{\underset{|}{CH}}$	93–95	Et	A (50)					0.77 (X)	$C_{17}H_{23}N_5O_5 \cdot 0.5H_2O$	C, H, N
				B (60)							
				C (80)							

[a] B, C_6H_6; E, EtOH; Et, Et_2O; M, MeOH. [b] Yields are those of the crude solids, under each method. [c] Acid used for solution was 1 N HCl. [d] Solvent systems used for TLC are indicated in parentheses and described in the Experimental Section. [e] When this work was finished, the preparation of some of these compounds appeared[12] in the literature. [f] Reported mp are 244,[12] 239–240,[14] 245–248 °C.[11] [g] Reported mp is 168–169 °C.[12] [h] Reported mp is 114 °C.[12] [i] After the addition of $SOCl_2$ (2 h), the reaction mixture was heated to 70–75° and this temperature was maintained for 2 h and then left at the room temperature for 24 h. Only a part of the 2′,3′-isopropylidene group was cleaved to give **19**.

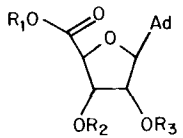
Table II



Compd no.	R	Mp, °C	Recrystn solvent[a]	Method (yield, %)[b]	Specific notation, deg	Optical rotation °C	Optical rotation Concn (c)	Solvent[c]	R_f[d]	Formula	Analyses
11	CH_3[e]	220-222[f] dec	M	G (86) H (63) I (100)	−23 ± 0.43	26	2.34	HCl	0.60 (X)	$C_{11}H_{13}N_5O_5$	C, H, N, O
12	CH_3CH_2	207-209	E	G (97) F (88)	−22 ± 0.55	26	1.8	H_2O		$C_{12}H_{15}N_5O_5 \cdot HCl$	C, H, Cl, N
13	CH_3CH_2[e]	210-211[g]	E + B	F (63) E (66) I (83) D[h] (50)[i]	−29 ± 0.6	27	1.0	HCl	0.56 (X)	$C_{12}H_{15}N_5O_5$	N
10	$ClCH_2CH_2$	208-210	A	G (76)	−35 ± 2.2	26	0.96	HCl	0.44 (Y)	$C_{12}H_{14}ClN_5O_5$	C, H, Cl, N, O
14	$HOCH_2CH_2$	206-207	E	H (47)	−16 ± 0.6	26	1.5	HCl	0.82 (X)	$C_{12}H_{15}N_5O_6$	C, H, N
15	$CH_2=CHCH_2$	210-211	A	G (70)	−25 ± 1.5	26	1.4	HCl	0.50 (Y)	$C_{13}H_{14}N_5O_5$	C, H, N, O
16	$EtOCH_2CH_2$	150-152	A	G (15)	−33 ± 1.8	26	1.8	HCl	0.33 (Y)	$C_{14}H_{19}N_5O_6$	C, H, N, O
17	$N_3CH_2CH_2$	151-153	A	G (29)	−77 ± 1.5	26	1.3	HCl	0.43 (Y)	$C_{12}H_{14}N_8O_5$	C, H, O
18	$CH_3CH_2CH_2$	198-200	Ea + C	H (33)	−25 ± 1	27	1	HCl	0.51 (X)	$C_{13}H_{17}N_5O_5$	C, H, N, O
19	$(CH_3)_2CH$[e]	222-223[j]	A	J (45)	−26 ± 1.3	26	1.34	HCl	0.55 (Y)	$C_{13}H_{17}N_5O_5 \cdot H_2O$	C, H, N, O
20	$C_6H_5CH_2CH_2$	170-172	A	G (38)	−30 ± 1.5	26	2	HCl		$C_{18}H_{19}N_5O_5$	C, H, N, O
21	$CH_3(CH_2)_2CH_2$[e]	145-148[k]	M	H (40)	−20 ± 2	27	0.5	HCl	0.54 (X)	$C_{14}H_{19}N_5O_5 \cdot 0.5H_2O$	C, H, O
22	$CH{\equiv}CCH_2$	197-198	A	F (85)	−30 ± 1	26	2.2	HCl		$C_{13}H_{13}N_5O_5$	C, H, N
23	c-C_3H_5-CH_2	222-223 dec	D + Et	G (40)						$C_{14}H_{17}N_5O_5$	C, H, N
24	c-C_5H_9	228-232 dec	E	F (20)						$C_{15}H_{19}N_5O_5$	C, H, N
25		143-145	E	F (32)					0.71 (X)	$C_{17}H_{23}N_5O_5$	C, H, N

[a] A, $(CH_3)_2CO$; B, C_6H_6; C, $CHCl_3$; D, dioxane; E, EtOH; Ea, CH_3COOEt; Et, Et_2O; M, MeOH. [b] Yields given are those of crude solids, under each method. [c] The acid used for solution was 1 N HCl. [d] Solvent systems used for TLC are indicated in parentheses and described in the Experimental Section. [e] When this work was finished, the preparation of some of these compounds by different methods was reported.[4,12,14] [f] Reported mp are 217,[12] 215-217 °C.[14] [g] Reported mp are 196-199,[4] 218,[12] 204-205 °C.[14] [h] Heated for 5 min at 90 °C. [i] Yield calculated from 1. [j] Reported mp 208 °C.[12] [k] Reported mp 166-167 °C.[14]

Table III



Compd no.	R_1	R_2	R_3	Dose,[a] mg/kg	PO_2[b] %	PO_2[b] Duration[c]	BP[d] mmHg	BP[d] Duration[c]	HR[e] %	HR[e] Duration[c]	
1	H	Isopropylidene		1	0	0	0	0	−4	9	
4	CH_3	Isopropylidene		10	7	3	−6	8	0	0	
5	CH_3CH_2	Isopropylidene		1	0	0	0	0	−2	21	
	Na	Isopropylidene		10	0	0	0	0	−3	5	
26	Na	H	H	10	22	7	0	0	0	0	
8	H	H	H	1	59	2	0	0	0	0	
11	CH_3	H	H	1	13	30	−8	30	0	0	
12	CH_3CH_2	H	H	0.05	106[f] ± 28	42 ± 6	−2 ± 2	17 ± 8	21 ± 6	43 ± 2	
				0.10	177[g] ± 34	48 ± 11	9 ± 4	33 ± 3	7 ± 8	58 ± 11	
				0.1 (id)	67	89	−2	60	2	36	
10	$ClCH_2CH_2$	H	H	0.1	26	9	0	0	3	0	
				0.3	103	31	1	40	5	40	
14	$HOCH_2CH_2$	H	H	0.05	94	6	7	7	0	0	
				0.1	100	19	−30	27	−15	10	
15	$CH_2=CHCH_2$	H	H	1	28	60	0	0	−5	6	
16	$EtOCH_2CH_2$	H	H	1	42	60	−10	60	10	60	
17	$N_3CH_2CH_2$	H	H	1	4	30	−4	30	−12	17	
18	$CH_3CH_2CH_2$	H	H	1	47	60	0	0	3	5	
19	$(CH_3)_2CH$	H	H	1	29	60	−6	60	2	60	
20	$C_6H_5CH_2CH_2$	H	H	10	9	18	−8	9	19	11	
21	$CH_3(CH_2)_2CH_2$	H	H	1	8	20	0	0	0	0	
22	$CH{\equiv}CCH_2$	H	H	1	−5	14	−7	14	9	15	
23	c-C_3H_5-CH_2	H	H	1	86	28	−7	40	30	45	
24	c-C_5H_9	H	H	10	39	40	−7	50	8	50	
25	c-C_5H_9-CH_2CH_2	H	H	0.1	11	10	0	0	0	0	
27	$(CH_3)_3N^+CH_2CH_2OH$[h]	H	H	10	29	11	36	7	−14	6	
	Thioethyl ester[h]	H	H	1	9	60	0	0	−6	12	
30	CH_3CH_2[h]	H	H	1	0	0	0	0	0	0	Inosine ethyl ester
29	CH_3CH_2[h]	H	H	0.1	94	40	−3	17	25	25	N^1-Oxide of 13
	Adenosine			2	150	4	−50	2	−40	3	
				15 (id)	3	15	0	0	−2	15	

[a] Unless otherwise specified, the drugs were administered by the intravenous route; id = intraduodenal. [b] PO_2 = partial pressure of oxygen, change from control; initial values ranged from 15 to 25 mmHg. [c] Duration is the total time (in minutes) elapsed from the initiation of the change until return to the pretest value. [d] BP = mean aortic blood pressure, change from control. [e] HR = heart rate, beats/min. [f] Mean ± SEM; $N = 3$. [g] Mean ± SEM; $N = 4$. [h] Physical properties are described in the Experimental Section.

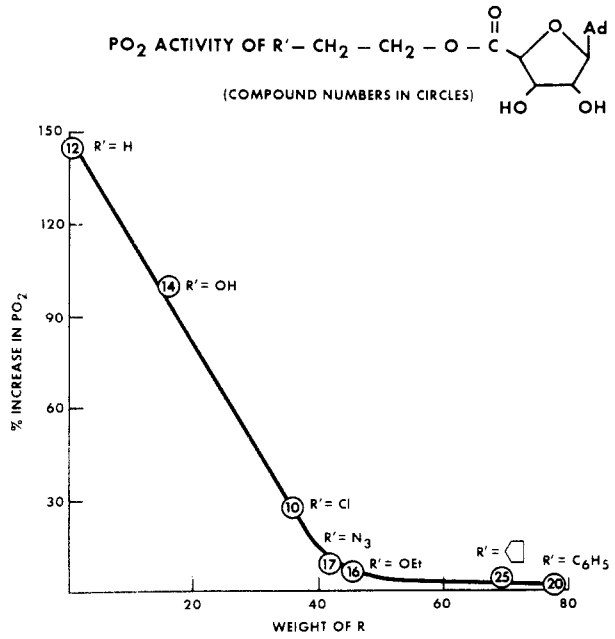


Figure 1.

in decrease or complete loss of PO_2 activity. Thus, roughly speaking, in the two-carbon chain esters, the activity decreases with increase in molecular weight.

Of the three-carbon chain esters, the activity is in the order: *n*-propyl (**18**) > isopropyl (**19**) > allyl (**15**) > propargyl (**22**). Thus, in a three-carbon chain, increasing the degree of unsaturation results in progressively decreasing activity. The cyclopropyl methyl ester **23** is relatively more active than the *n*-butyl ester **21**.

The N^1-oxide of **13** (**29**) showed only marginally lower activity than the parent compound.

Adenosine-5′-carboxylic acid (**8**) which is insoluble in water and common organic solvents was only slightly active at 10 mg/kg. The water-soluble sodium salt of **8** (compound **26**) was inactive. Choline reacts with **8** to form a crystalline salt **27** which also was inactive. Evidently, the activity of the ethyl ester is a property of the ester molecule as a whole. These esters (Table III) may be considered as modified nucleosides which are capable of penetrating cell membranes. A rapid onset of PO_2 increases of the order of 100% is seen in dogs, lasting for approximately 30 min, when **12** is administered intravenously at 50 μg/kg. Intraduodenal administration of **12** at 0.10 mg/kg causes greater than 60% increases in PO_2 lasting for approximately 90 min. These results indicate that **12** is well absorbed and not hydrolyzed rapidly to the very weakly active acid **8**. Studies by Merits and Anderson[16] indicate that **8** is a major metabolite eliminated in the urine when [8-^{14}C]-**12** is administered either orally or intravenously in the rat, mouse, and dog.

Ethyl 2′,3′-*O*-isopropylideneadenosine-5′-carboxylate (**5**) showed essentially no cardiovascular activity at ten times the dose of unsubstituted **12**. Quite likely, the two hydroxyl groups may be involved in bonding, anchoring the molecule at the receptor. Adenosine deaminase does not play a major role in the metabolism of **12**, since only a small amount of inosine-5′-carboxylic acid is eliminated from the urine in all three species.[16] This was predictable from enzyme studies[4] which showed that **12** was not a ready substrate for adenosine deaminase in vitro nor was it an inhibitor of deaminase activity. The inosine ester **30**, probably the precursor of the inactive inosine-5′-carboxylic acid, is also inactive. One may be led to agree with Bloch et al.[17] that the removal or alteration of the 4′-CH_2OH (e.g., to 4′-COOEt in **12**) leads to a loss of substrate specificity for adenosine deaminase.

Though the mechanism of the vasoactive action of **12** and other esters in the series is unknown, it appears that the activity does not involve adenosine deaminase but that it is dependent upon the 2′,3′-hydroxyl and the N^6-amino groups.

Experimental Section

Chemical Methods and Materials. Thin-layer chromatography was performed using Eastman 6060 silica gel chromagram sheet with fluorescent indicator with solvent systems: X, *n*-BuOH–H_2O (47:3); Y, *n*-BuOH–NH_4OH–H_2O (86:5:14). Physical properties of these compounds were determined with the following instruments: Thomas-Hoover apparatus (melting point, uncorrected); Unicam SP-800A UV spectrometer (uv spectra); Beckman IR-8 spectrometer (ir spectra, KBr); Hilger and Watts Standard (MK-III) polarimeter (optical rotation); Varian HA-100 spectrometer (NMR spectra, Me_4Si). Mass spectra were determined on a AE1, MS-902 spectrometer. The fragmentation of these nucleosides in the mass spectrometer, in general, gave the major ions consisting of the intact base plus certain portions of the sugar skeleton. Thus, in every case, the characteristic $M^+ - (OR)$ and $M^+ - (COOR)$ peaks for the loss of the alkoxy and the alkoxycarbonyl radicals were clearly evident.[20]

Elemental analyses were performed by the Microanalytical Services of Abbott Laboratories, North Chicago, Ill. When analyses are indicated by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

The general preparative procedures for compounds listed in Tables I and II are illustrated by the following examples.

2′,3′-*O*-Isopropylideneadenosine-5′-carboxylic Acid Methyl Ester (4).[11,12,14] **Method A.** Powdered **1** (9.4 g, 0.03 mol) was added (in 1 min) to gently stirred $SOCl_2$ (30 ml) at 0–10°. The cooling bath was removed and DMF (10 drops) was added to the cloudy mixture. The reaction mixture was stirred (0.5 h) at 50° and the clear solution was evaporated under reduced pressure. The residue was diluted with ether and evaporated again. This process was repeated thrice. Finally the residue was triturated with ether and filtered to give **2** (quantitative), mp 195–200° dec.

The same product was obtained without the use of DMF. However, in that case, the initial reaction was run at 5–10° and then stirred at the room temperature for 2 h and finally the solution was poured onto a large volume of dry ether to give **2**. After drying in vacuo over P_2O_5 for 24 h, the melting point was 205–210° dec.

The dried **2** (3.2 g) was added to absolute methanol (50 ml) at room temperature. The mixture was stirred (15 h) at room temperature and evaporated under reduced pressure at 35°. The residue was stirred with 10% aqueous $NaHCO_3$ solution at 10° to give **4** (3 g, 91%) melting at 230–242°. Recrystallization from a large volume of methanol gave the pure methyl ester **4** as colorless crystals.

2′,3′-*O*-Isopropylideneadenosine-5′-carboxylic Acid Ethyl Ester (5).[12] **Method B.** A solution of **1** (6.4 g, 0.02 mol) in warm dry Me_2SO (75 ml) was cooled (5 °C) and thallous ethoxide (10.0 g, 0.04 mol) was added. The cloudy mixture was stirred at 20° (1 h), diluted with dry ether (40 ml), and filtered to give 9.0 g (90%) of the thallous salt **3**. This salt (**3**) could also be obtained in 80% yield using a large volume of DMF as the solvent.

A cloudy solution of **3** (2.3 g, 0.0044 mol) in hot Me_2SO (150 ml) was mixed with ethyl iodide (2.0 g, 0.012 mol) and heated (90–100°). After 15 min an orange-yellow solid (1.45 g) was filtered off and washed (Me_2SO, 10 ml; $CHCl_3$, 50 ml). The combined filtrate was diluted with water (500 ml) and extracted with 5 × 80 ml of $CHCl_3$. The extract was washed successively with 2 × 50 ml of $Na_2S_2O_7$ (10% solution), water (2 × 50 ml), and saturated NaCl, dried, and distilled. Recrystallization of the residue gave the desired ester: ir 1740 cm^{-1} (ester).

2′,3′-*O*-Isopropylideneadenosine-5′-carboxylic Acid *sec*-Butyl Ester (7). Method C. A mixture of **1** (9.4 g, 0.03 mol) and EEDQ (*N*-ethoxycarbonyl-2-dihydroquinoline, 8.1 g, 0.033 mol) in *sec*-butyl alcohol (450 ml) was stirred at 95° (3 h). The

cloudy mixture was then evaporated to dryness under reduced pressure. The residue was triturated with ether (350 ml) and filtered from undissolved starting acid (2.5 g). The ether filtrate was evaporated; the residue was stirred with 10% $NaHCO_3$ solution (50 ml), filtered, and washed with water to give 3.5 g of **7**.

This product was identical with the *sec*-butyl ester (mp 93–95°) obtained by methods A and B.

Adenosine-5'-carboxylic Acid Ethyl Ester (13).[12] **Method D.** The cleavage of the 2',3'-*O*-isopropylidene group of **5** was affected by the use of 50% formic acid at the temperature and duration indicated in Table II to give **13**. Two recrystallizations from methanol gave the analytical sample.

Adenosine-5'-carboxylic Acid Ethyl Ester Monohydrochloride (12). Method E. The acid **8** was converted into its thallous salt (83% yield) which gave the ethyl ester **13**, in essentially the same way as described for the preparation of **5**, method B, and subsequently converted to the hydrochloride.

Adenosine-5'-carboxylic Acid Cyclopentyl Ester (24). Method F. Dried **8** (2.81 g, 0.01 mol) was added to $SOCl_2$ (25 ml) at 10–15°. The cloudy mixture was allowed to warm to the room temperature. DMF (8 drops) was added and then the reaction mixture was stirred at 50° (30 min). The mixture was then evaporated under reduced pressure at 30°. The residue was triturated with ether (50 ml) and evaporation continued. This process was repeated thrice. Finally, the residue was stirred vigorously with dry ether and filtered to give **9** as a white solid. The product, after drying in a vacuum oven at 80–85° (over P_2O_5), melted at 180–185° dec (yield quantitative).

Dried **9** (6.0 g) was stirred (20 h) in cyclopentanol (50 ml) at room temperature, diluted with ether, and then filtered. The residue was (sometimes purified as the HCl salt as in the case of **12** or) converted into the free base **24** by stirring with cold $NaHCO_3$ solution. Recrystallization from ethanol gave the pure cyclopentyl ester **24**.

Adenosine-5'-carboxylic Acid Allyl Ester (15). Method G. $SOCl_2$ (2 ml) was added dropwise to a suspension of **8** (1.0 g, 0.0036 mol) in allyl alcohol (35 ml) at 0–5°. The mixture was stirred (18–48 h; in some cases, e.g., for phenethyl ester, 5–6 h of stirring completed the reaction) at room temperature, then cooled to −10°, diluted with ether, and filtered. The residue was washed with ether. (In the case of compound **12**, this residue was recrystallized from boiling absolute ethanol to give the ethyl ester as a monohydrochloride salt, **12**.)

The residue was dissolved in cold water and basified with aqueous $NaHCO_3$. The free base **15** which separated as white solid (mp 209–210°) was recrystallized from acetone.

Adenosine-5'-carboxylic Acid *n*-Butyl Ester (21).[14] **Method H.** Thionyl chloride (3.5 ml) was added to a stirred suspension of **1** (3.21 g, 0.01 mol) in 1-butanol (50 ml) at 5°. After the addition was completed, the cloudy mixture was stirred at the room temperature (sometimes the reaction mixture was heated for a period indicated in the Table) for 24 h. The clear solution was poured onto 100 ml of ice, basified (solid $NaHCO_3$), and filtered to give 1.5 g (40%) of **21**. Two recrystallizations from methanol gave the analytical sample.

Adenosine-5'-carboxylic Acid Methyl Ester (11).[12] **Method I.** A solution of the 2-chloroethyl ester of adenosine-5'-carboxylic acid (1.0 g) in methanol (30 ml) was boiled (15–20 min) with an excess of benzene (50 ml) until the crystallization point. The solution, on standing at the room temperature, gave **11** identical in every respect with the methyl ester prepared by method G.

Adenosine-5'-carboxylic Acid Isopropyl Ester (19).[12] **Method J.** This ester was prepared by the reaction of adenosine-5'-carboxylic acid chloride (**9**) with 2-propanol (freshly distilled) in the manner described under method G, with the exception that the mixture of **9** and 2-propanol was kept at 65–70° for 3.5 h. The reaction mixture was cooled to the room temperature and ether was added. The hydrochloride of the isopropyl ester, which precipitated out, was filtered, washed with ether, and converted (in the usual way) to its free base.

Adenosine-5'-thiocarboxylic Acid Ethyl Ester [4'-C(=O)SEt]. Dried **2** (2.0 g, 0.006 mol) was added, portionwise, to a mixture of ethanethiol (4.2 g, 0.06 mol) and pyridine (4.8 g, 0.06 mol) at 10–15° and then stirred at room temperature (24 h). The mixture was then evaporated under reduced pressure at 30°. The residue was triturated with ether (100 ml) and filtered. The ether filtrate was evaporated and the residue was triturated with petroleum ether (bp 30–60°) to give 1.2 g (80%) of 2',3'-*O*-isopropylideneadenosine-5'-thiocarboxylic acid ethyl ester (mp 97–100°). A part (0.8 g) of this crude material was mixed with formic acid (35 ml of 20%) and allowed to stand for 14 days at room temperature. The clear solution was evaporated to dryness under reduced pressure. The residue was stirred with cold 10% aqueous $NaHCO_3$ (40 ml), filtered, washed with cold water, and recrystallized from $CHCl_3$ to give pure adenosine-5'-thiocarboxylic acid ethyl ester: yield 30%; mp 178–180° (from chloroform); $[\alpha]^{26}D$ −10 ± 2° (*c* 0.5, 0.5 N HCl); R_f 0.6 (A). Anal. ($C_{12}H_{15}N_5O_4S \cdot 0.5H_2O$) C, H, N, O, S.

Salts of 1 and 8. The acid was neutralized with aqueous $NaHCO_3$ solution, filtered (Norite), and evaporated to near dryness. The residue was triturated with absolute ethanol, filtered, and recrystallized from ethanol.

Sodium 2',3'-*O*-isopropylideneadenosinecarboxylate: mp 280 °C. Anal. ($C_{13}H_{14}N_5O_5Na$) C, H, N.

Sodium adenosine-5'-carboxylate dihydrate (26): mp 280 °C. Anal. ($C_{10}H_{10}N_5O_5Na \cdot 2H_2O$) N.

Choline Adenosine-5'-carboxylate (27). The acid **8** (2.8 g, 0.01 mol) was stirred with 25 ml of a 50% solution of choline in methanol for 1 h, diluted with methanol (100 ml), refluxed (0.5 h), and filtered. The filtrate was concentrated to 50 ml and poured onto well-stirred ether (1 l.). The mixture was cooled (overnight), filtered (3.4 g), and recrystallized from ethanol–benzene to give **27** as shining flakes melting at 149–157° (foams): $[\alpha]^{25}D$ −10.3 ● 1.0° (*c* 0.97, H_2O). Anal. ($C_{15}H_{24}N_6O_6$) C, H.

Adenosine-5'-carboxylic Acid N^1-Oxide (28). A suspension of **1** (9.6 g, 0.03 mol) in acetic acid (750 ml) and H_2O_2 (100 ml of 30% aqueous solution) was stirred for 7 days at room temperature. At the end of this period, the mixture was filtered to remove a small amount (0.2 g) of suspended material, the filtrate was cooled (10–15°), and the excess H_2O_2 was destroyed by 5% Pd/C (6 g). The dark mixture was filtered; the filtrate was concentrated to 75 ml under reduced pressure and poured onto stirred ether (200 ml). The grey solid was filtered and recrystallized (H_2O) to give 3.6 g (dried at 120°/6 h in vacuo) of **28** (mp 220° dec) contaminated with traces of the N^1-oxide of **1**. This material was suitable for esterification. The pure acid **28** was obtained by incubating the crude material (0.84 g) in 50% HCOOH (35 ml) at room temperature for 3 days. The clear solution was evaporated under reduced pressure. The residue was triturated several times with methanol, filtered (0.5 g), and recrystallized from water to give analytically pure **28**, mp 220° dec. Anal. ($C_{10}H_{11}N_5O_6$) C, H, N, O.

Ethyl Adenosine-5'-carboxylate N^1-Oxide (29). Thionyl chloride (2 ml) was added dropwise to a suspension of **28** (1.8 g, 0.006 mol) in EtOH (100 ml) at 5–10°. After 30 min at 5–10°, the clear solution was stirred (16 h) at room temperature. The mixture was then kept at 50° for 0.5 h, concentrated to 20 ml under reduced pressure, and poured onto stirred ether (150 ml). The precipitate was filtered; the residue was stirred with aqueous $NaHCO_3$ solution (20 ml) at 10–15° and filtered to give 0.8 g (45%) of **29** (mp 197–200°). Recrystallization (EtOH) gave **29** as a semihydrate: mp 204–206°; $[\alpha]^{26}D$ −22 ± 2.0° (*c* 0.65, 1 N HCl); characteristic ir and NMR spectra. Anal. ($C_{12}H_{15}N_5O_6 \cdot 0.5H_2O$) C, H, N.

Ethyl Inosine-5'-carboxylate (30). A solution of **12** (7.0 g, 0.02 mol) in water (150 ml) containing acetic acid (12.0 g) was cooled to 0 to −10° and mixed with an aqueous solution of sodium nitrite (16.0 g, 0.23 mol in 50 ml of water). The reaction mixture was gently stirred for 4 h at 10° and then at room temperature for 4 days. At the end of this period, the mixture was cooled, filtered, and washed successively with plenty of cold water, acetone, and ether to give 4.5 g of inosine-5'-carboxylic acid ethyl ester. Recrystallization (EtOH) gave the pure **30** (mp 228° dec[13]): ir 1745, 1735 cm^{-1}. The compound had the characteristic NMR and mass spectra.

Pharmacological Studies. The compounds were evaluated in a cardiovascular test in which blood pressure, heart rate, and coronary sinus PO_2 were monitored continuously on a Grass Model 7 polygraph. Dogs of either sex were anesthetized with 250 mg/kg of barbital sodium, intravenously, after sedation with 3 mg/kg, subcutaneously, of morphine sulfate. Under artificial respiration,

an incision was made in the fourth right intercostal space and the pericardium was incised. A Beckman polarographic PO_2 microelectrode (in a Riley needle) was placed in the coronary sinus via the superior vena cava. Compound, dissolved in isotonic saline, was administered as a bolus either intravenously (jugular) or intraduodenally through an indwelling catheter.

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Analogues of 8-Azaguanosine

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Two routes for the synthesis of 6-substituted 8-azaguanosine analogues are described. 2,5,6-Triamino-4(3*H*)-pyrimidinethione (**1**) was converted by methylation, nitrosation, and acetylation to *N*-acetyl-7-(methylthio)-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-5-amine (**5**). The reaction of **5** with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride gave a mixture of the 7-, 8-, and 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-8-azapurines **4a–c** which was converted to 8-azaguanosine (**7c**) and the corresponding 7- and 8-substituted isomers **7a** and **7b**. **4a–c** were also converted with NaOMe to 6-*O*-methyl-8-azaguanosine (**8c**) and to the corresponding 7- and 8-substituted isomers **8a** and **8b**. The preferred route, however, to 6-substituted 8-azaguanosine analogues is an unambiguous synthesis through N^2-acetyl-6-(benzylthio)-N^4-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-5-nitro-2,4-pyrimidinediamine (**13**), prepared from the reaction of the chloropyrimidine **10** with the aminoribose **11**. Catalytic hydrogenation of **13** gave the aminopyrimidine **14**, which was converted with nitrous acid to the nucleoside β-**20**. Treatment of β-**20** with dilute acid gave 7-(benzylthio)-3-β-D-ribofuranosyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-5-amine (**19**). Replacement of the benzylthio group of **19** with various nucleophilic reagents gave 8-aza-6-thioguanosine **17** and analogues **8c**, **15**, and **16**. The thione **17** rearranges in aqueous solution to the thiadiazolopyrimidine **21**. The parent [1,2,3]thiadiazolo[5,4-*d*]pyrimidine-5,7-diamine (**24a**) was prepared by nitrosation of the triaminopyrimidine (**23a**). Rearrangement of **24a** in the presence of base gave a high yield of the thione **25a** which could be rearranged with heat to **24a**. Compounds **8a–c**, **15–19**, **24a**, and **25a** were evaluated in the H.Ep.-2 cell culture screen and compounds **8c**, **15–19**, **24a**, and **25a** in the L1210 mouse leukemia screen. Only one compound, **8c**, showed high cytotoxicity and borderline L1210 activity resulting from its enzymatic conversion to 8-azaguanosine.

The anticancer activity of 8-azaadenosine (3-β-D-ribofuranosyl-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-amine)[1] and 8-azainosine (3,6-dihydro-3-β-D-ribofuranosyl-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one)[1,2] in experimental animals systems coupled with the clinical efficacy of the 6-thiopurines against human leukemias[3,4] led us to prepare alkoxy- and sulfur-containing congeners of these nucleosides. These compounds proved to be substrates for adenosine kinase, to be cytotoxic to cell lines possessing this enzyme but not to lines deficient in it, and to show some activity against murine leukemias.[1,5] These initially encouraging results and the knowledge of the anticancer activity of 6-thioguanine[3] and 8-azaguanine (5-amino-3,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-one)[6] suggested the preparation and reevaluation of the known 8-aza-6-thioguanine (5-amino-3,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidine-7-thione) (**25a**)[7] and the synthesis of its ribonucleoside **17** since, although 6-thioguanosine is rapidly phosphorylyzed to 6-thioguanine,[3] 8-azapurine ribonucleosides such as 8-azainosine are not and show activity not possessed by the parent heterocycles.[1,2]

The successful synthesis of 7-(methylthio)-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-3*H*-1,2,3-triazolo[4,5-*d*]-pyrimidine[8] by the molecular sieve catalyzed reaction of 7-(methylthio)-3*H*-1,2,3-triazolo[4,5-*d*]pyrimidine with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl chloride and the reactivity of the methylthio group of this nucleoside to nucleophilic displacement reactions[8,9] suggested a similar sequence for the preparation of 8-aza-6-thioguanosine (5-amino-3,6-dihydro-3-β-D-ribofuranosyl-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidine-7-thione, **17**).

2,5,6-Triamino-4(3*H*)-pyrimidinethione[10] (**1**) was alkylated with methyl iodide to give a 72% yield of 6-(methylthio)-2,4,5-pyrimidinetriamine (**2**). This route to