Notes

Functionalized Congeners of Adenosine: Preparation of Analogues with High Affinity for A_1-Adenosine Receptors

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A series of functionalized congeners of adenosine based on N^6-phenyladenosine, a potent A_1-adenosine receptor agonist, was synthesized. Derivatives of the various congeners should be useful as receptor and histochemical probes and for the preparation of radioligands and affinity columns or as targeted drugs. N^6-[4-(Carboxymethyl)phenyl]adenosine served as the starting point for synthesis of the methyl ester, the methyl amide, the ethyl glycinate, and various substituted anilides. One of the latter, N^6-[4-[[[4-(carbomethoxymethyl)anilino]carbonyl]methyl]phenyl]adenosine, served as the starting point for the synthesis of another series of congeners including the methyl amide, the hydrazide, and the aminoethyl amide. The terminal amino function of the last congener was acylated to provide further analogues. The various congeners were potent competitive antagonists of binding of N^6-[^{3}H]cyclohexyladenosine to A_1-adenosine receptors in rat cerebral cortical membranes. The affinity of the congener for the A_1 receptor was highly dependent on the nature of the spacer group and the terminal moiety with K_i values ranging 1–100 nM. A biotinylated analogue had a K_i value of 11 nM. A conjugate derived from the Bolton–Hunter reagent had a K_i value of 4.5 nM. The most potent congener contained a terminal [(aminoethyl)amino]carbonyl function and had a K_i value of less than 1 nM.

A "congener approach" to drug design was recently described by Goodman and co-workers.[1-5] In this approach, a functionalized chain is incorporated into the primary drug structure (pharmacophore) at a point that does not perturb biological activity. The resulting active congener is then covalently joined through the functional group on this chain to various organic moieties, e.g., amines and peptides.[3-5] One goal is to attach the functionalized congeners to directed carriers for drug targeting. The biological activity of the final conjugates often is influenced markedly by changes in the structures of the attached moieties, even though such changes may be physically remote from the primary pharmacophore. In a series of analogues of isoproterenol both the potency and duration of action in vivo could be modulated dramatically by attachment of the pharmacophore through a long chain to various amines or oligopeptides.[1-4]

This approach has now been extended to the development of a series of functionalized congeners of adenosine. There are at least two classes of adenosine receptors that have apparent important physiological functions in the cardiovasculature, central nervous system, endocrine glands, gastrointestinal tract, platelet, kidney, and immune systems.[6] The A_1-adenosine receptor appears to be involved in cardiac and central depressant and antilipolytic functions of adenosine, while an A_2-adenosine receptor appears to be involved in endocrine, vasodilation, spasmolytic, and antithrombotic functions. The A_1 receptors in fat cells, brain, and heart are inhibitory to adenylate cyclase, while A_2 receptors in many cells are stimulatory. Although adenosine analogues at present are not used in a therapeutic capacity, functionalized adenosine congeners with high selectivity and/or potency at A_1 and A_2 receptors would have potential for drug development and would be useful as receptor probes (e.g., radioactive, fluorescent[7], or irreversible ligands), in preparation of affinity columns, and for histochemical localization and study of adenosine receptors.

A functionalized derivative of N^6-phenyladenosine was the starting point for the preparation of congeners, and since N^6-substituted adenosines exhibit selectivity for A_1-adenosine receptors,[6,8,9] the initial biological testing consisted of determining the competitive antagonism of binding of N^6-[^{3}H]cyclohexyladenosine to an A_1-adenosine receptor site in brain membranes.[10] While this binding site may represent a receptor linked in an inhibitory manner to brain adenylate cyclase, this is not certain. The A_1-receptor designation is used in the present paper to avoid the explicit implications of inhibitory and activating links to adenylate cyclase of the alternate R_i and R_a terminology introduced by Londos et al.[9]

Results and Discussion

The N^6-amino group was selected as the point of attachment of the functionalized chain, since at that position structural requirements for high affinity at A_1-adenosine

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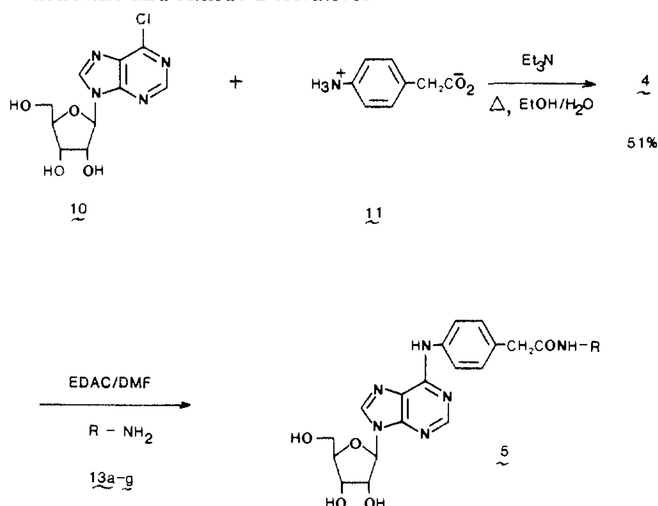
Scheme I. Synthesis of a Carboxylic Acid Congener of Adenosine and Amide Derivatives



receptors are broad.[6] N[6]-substituted adenosines including N^6-phenyladenosine appear to be full agonists at A_1-adenosine receptors[8,9] while having only partial agonist activity at A_2 receptors.[11] The N^6-phenyl derivative of adenosine (**1**) is very potent with a K_i value in competitive binding assay against N^6-[^{3}H]cyclohexyladenosine on rat cerebral cortex membranes of 3.2 nM (Table I). Various para substitutions on the phenyl ring (e.g., compounds **2** and **3**) did not appear to be detrimental to biological receptor binding.[11] Therefore, N^6-[4-(carboxymethyl)-phenyl]adenosine (**4**) was synthesized with the view of using the carboxyl group as the functional site in elaboration of a series of congeners and related analogues. The potency of these analogues was then assessed by measuring inhibition of binding of N^6-[^{3}H]cyclohexyladenosine to A_1 receptors in rat cerebral cortical membranes (Table I).

The carboxylic acid **4** and the methyl amide derivative **5a** both proved to be potent (Table I). Seven other simple amides were synthesized, six of which contained an additional aryl ring. Earlier results with functionalized congeners of isoproterenol indicated that distal aryl groups could be important determinants of biological activity.[1,2] The activity of two of the aromatic amides (**5e**,**f**) suggested that another series of functionalized congeners could be based on further extension of the chain from CH_2CO_2H to $CH_2CONHC_6H_4CH_2CO_2H$. The ester (**6**), amide (compounds **7a**, **8**, **9a–c**), and hydrazide (**7b**) derivatives of this carboxylic acid were synthesized and proved very potent.

The synthetic route to the carboxylic acid congener **4** and its amide derivatives is shown in Scheme I. The initial displacement on 6-chloropurine ribonucleoside (**10**) by 4-aminophenylacetic acid, is similar to methods used previously[12,13] and provided a facile entry into this series. The carboxylic acid congener **4** was coupled in variable yields to amines through activation by a water-soluble carbodiimide in DMF. Often the product precipitated in a chromatographically pure state upon addition of water to the reaction mixture. To extend the chain further, methyl 4-aminophenylacetate reacted in relatively good yield with **1** with use of carbodiimide/hydroxybenzotriazole activation to give the methyl ester **6** (Scheme II). Among amides synthesized from **4** were two conjugates containing *p*-amino-L-phenylalanine. This choice was based on the experience with isoproterenol congeners when

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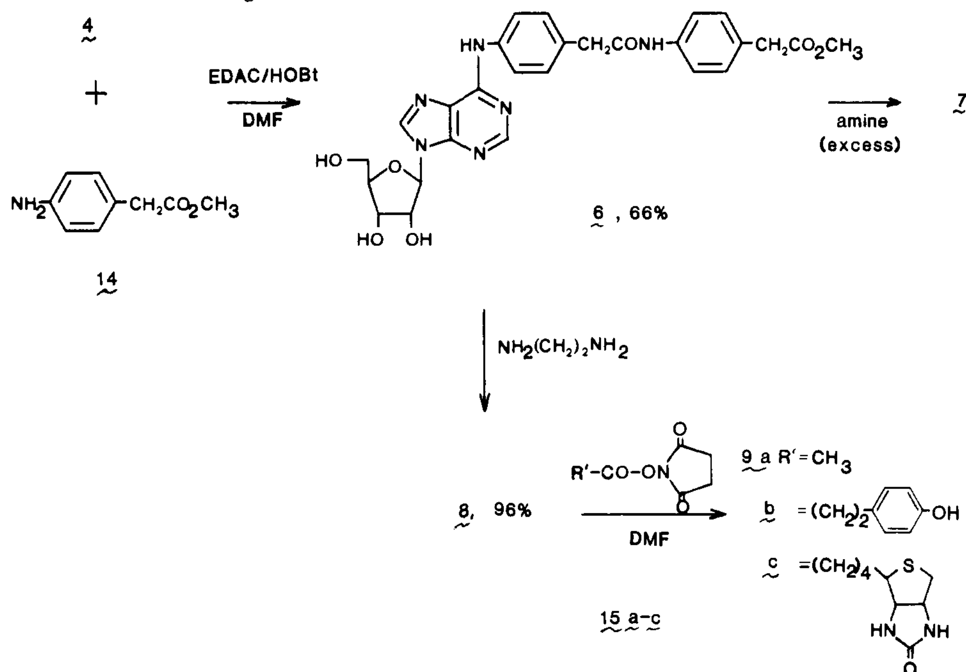
Table I. Effect of Functionalization on the Activity of N^6-Phenyladenosine at A_1-Adenosine Receptors

compd	A_1 receptor:[a] K_i, nM
(general structure: N^6-(4-R-phenyl)adenosine)	
1, R = H	3.2 ± 0.5
2, R = CH_3	2.5 ± 0.05
3, R = OCH_3	2.3 ± 0.8
R = $CH_2C(O)R'$	
4, R′ = OH	210 ± 57
5a, R′ = $NHCH_3$	16 ± 10
5b, R′ = $NHCH_2CO_2C_2H_5$	15 ± 8
5c, R′ = NH–C_6H_4–CH_3 (ortho)	6.8 ± 3
5d, R′ = NH–C_6H_4–CH_3 (meta)	2.0 ± 0.1
5e, R′ = NH–C_6H_4–CH_3	1.7 ± 0.2
5f, R′ = NH–C_6H_4–CF_3	3.3 ± 0.4
5g, R′ = NH–C_6H_4–$CH_2CH(NHCO_2C(CH_3)_3)CONHCH_2CONHCH_3$	13 ± 0
5h, R′ = NH–C_6H_4–$CH_2CH(NHCOCF_3)CO_2CH_3$	18 ± 1.4
R = $CH_2C(O)NH$–C_6H_4–$CH_2C(O)R''$	
6, R″ = OCH_3	2.5 ± 0.7
7a, R″ = $NHCH_3$	6.7 ± 0.7
7b, R″ = $NHNH_2$	4.5 ± 0.7
8, R″ = $NHCH_2CH_2NH_2$	0.85 ± 0.35
9a, R″ = $NHCH_2CH_2NHCOCH_3$	9.3 ± 1.7
9b, R″ = $NHCH_2CH_2NHCO(CH_2)_2$–C_6H_4–OH	4.5 ± 0.04
9c, R″ = $NHCH_2CH_2NHCO(CH_2)_4$–(biotinyl bicyclic ring: S, HN, NH, O)	11.4 ± 0.4

[a] IC_{50} values for A_1 receptors were obtained from antagonism of binding of 1 nM [^{3}H]cyclohexyladenosine to cerebral cortical membranes. $K_i = IC_{50}/1$ + concentration [^{3}H]cyclohexyladenosine/K_a for cyclohexyladenosine (the K_a value is equal to ≈ 1 nM). Values are means ± SEM for two to four experiments, each of which included triplicate determinations for each point of the curve.

it was demonstrated that monodisperse oligopeptides, attached to the congener through *p*-aminophenylalanine,[2-5] could serve as drug carriers. It was also shown in the isoproterenol system that drug carriers may alter favorably

Scheme II. Synthesis of an Amino Congener of Adenosine and Various Amide Derivatives



the pharmacological properties.[4]

Amides were prepared from 6 by aminolysis of the methyl ester. When an excess of ethylenediamine was used, the amino congener 8 resulted. Compound 8 could be acylated selectively on the primary amine with use of active esters of *N*-hydroxysuccinimide to give compounds **9a–c** (Scheme II).

The conjugate of 8 with *d*-biotin (compound **12c**) was synthesized as an analogue that is expected to form a very tight complex with the glycoprotein avidin while the A_1-receptor binding characteristics are maintained. The avidin complex, in turn, may be coupled to probes, other proteins, or insoluble polymers, following the approach described by Bayer and Wilchek.[14]

Biological Testing

N^6-Phenyladenosine (1) has high potency at A_1-adenosine receptors while being relatively inactive compared to adenosine at A_2 receptors.[6] Substituents at the para position (2, 3) had little effect on the A_1-receptor affinity as antagonists of binding of N^6-[^{3}H]cyclohexyladenosine in rat cerebral cortical membranes (Table I). The presence of a reactive *p*-CH_2CO_2H moiety (compound 4) decreased potency considerably, suggesting that an anionic substituent is not well tolerated near the N^6-aryl binding region of the A_1 receptor. It is interesting to note in relation to applying the functionalized congener approach to new receptor systems that this observation parallels results with catecholamine series in which a carboxylic acid congener was considerably less potent than derivatives blocked as amides, particularly aryl amides.[1] Conversion of acid 4 to the methyl amide (**5a**) afforded a relatively potent congener with a K_i value of 16 nM. The amide of ethyl glycinate **5b** was also relatively potent with a K_i value of 8.5 nM. The most strongly active compounds of this series of eight congeners were those which were simple anilides (**5c–f**). These had K_i values of 2–7 nM and thus were comparable in receptor affinity to the simple N^6-phenyladenosines. The position of the electron-density methyl substituent in the *o*- (**5c**), *m*- (**5d**), and *p*-toluidides (**5e**) had little effect on potency nor did replacement of the *p*-methyl substituent with a highly electron-withdrawing substituent, the trifluoromethyl group (compound **5f**). The high affinity of these anilides suggests the presence of a distal aryl binding site in the subregion of the receptor that binds N^6-substituents. The remaining two anilides (**5g,h**) are conjugates derived from *p*-amino-L-phenylalanine. Both were several-fold less potent than the simple anilides, suggesting that steric bulk even tethered to the primary pharmacophore through a functionalized chain may not be tolerated at the A_1 binding site.

A second series of functionalized congeners was synthesized wherein the side chain of 4 was further extended by introduction of the (4-aminophenyl)acetyl moiety. In this series (Table I), the neutral ester and amides (compounds **7a**, **8**, **9a-c**) afforded very potent congeners. The hydrazide **7b** was also quite potent and is potentially useful in coupling to aldehydes and carbohydrates. The most potent congener (8) contained the terminal $NHCH_2CH_2NH_2$ grouping and had a K_i value of less than 1 nM (Table I). This analogue thus appears to have considerable potential for the preparation of affinity columns designed to isolate A_1-adenosine receptors.

Although the terminal amine of compound 8 is removed from the adenosine pharmacophore by a through-bond distance of 17 atoms, conversion to an acetyl derivative (**9a**) resulted in about a tenfold reduction in receptor affinity. A similar increase in potency dependent on a distal amino group was observed in a series of functionalized xanthines acting as adenosine antagonists.[18] Of course, contributions to the potency by folded conformations in which the amine resides close to the primary pharmacophore cannot be ruled out. Such indications that minor structural changes on the attached carrier may alter the potency even in vitro parallels earlier results with conjugates of isoproterenol.[1-4]

The final two acyl derivatives of compound 8 were somewhat more potent than **9a**. The (4-hydroxyphenyl)propionyl derivative **9b** was designed to be radioiodinated. Indeed, the phenolic moiety of compound **9b** is derived from the Bolton–Hunter reagent,[15] which has

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Table II. Analytic Data

compd	method	% yield	mp, °C	formula	anal.	MS[f]
4	*a*	51	186–188	$C_{18}H_{19}N_5O_6\cdot{}^1/_2H_2O$	C, H, N	402, 358, 270, 226
5a	*b*	49[e]	215–218	$C_{19}H_{22}N_6O_5$	*i*	415, 283
5b	*b*	10	195–198	$C_{22}H_{26}N_6O_7\cdot 3H_2O$	C, H, N	487, 355
5c	*b*	55	248–250	$C_{25}H_{26}N_6O_5\cdot{}^1/_2H_2$	C, H, N	491, 359
5d	*b*	72	242–244	$C_{25}H_{26}N_6O_5\cdot{}^1/_2DMF\cdot{}^1/_2H_2O$	C, H, N	491, 359
5e	*b*	45	246–249	$C_{25}H_{26}N_6O_5\cdot{}^1/_2H_2O$	C, H, N	491, 359
5f	*b*	37	231–234	$C_{25}H_{23}N_6O_5F_3\cdot{}^1/_2DMF\cdot H_2O$	C, H, N	545, 413
5g	*b*	36	194–199	$C_{35}H_{43}N_9O_9\cdot DMF\cdot H_2O$	C, H, N	734[g]
5h	*b*	55	233–236	$C_{30}H_{30}N_7O_8F_3\cdot{}^3/_2H_2O$	C, H, N	674[g]
6	*a*	66	235–237	$C_{27}H_{28}N_6O_7\cdot H_2O$	C, H, N	549[g]
7a	c	78	227–229	$C_{27}H_{29}N_7O_6\cdot{}^3/_2H_2O$	C, H, N	548[g]
7b	*c*	68	227–230 dec	$C_{26}H_{28}N_8O_6$	*i*	549[g]
8	*c*	96	221–223	$C_{28}H_{32}N_8O_6\cdot DMF\cdot H_2O$	C, H, N	577[g]
9a	*d*	81	243–246	$C_{30}H_{34}N_8O_7\cdot DMF\cdot H_2O$	C, H, N	619[g]
9b	*d*	63	195–198	$C_{37}H_{40}N_8O_8$	C, H, N	725[g]
9c	*d*	50	199–202	$C_{38}H_{46}N_{10}O_8S\cdot 3H_2O$	C, H, N	803[g]
12	*a*	89	100.5–101	$C_{12}H_{11}N_2O_5F_3$	C, H, N	338
13g	*a*	86	111–111.5	$C_{12}H_{13}N_2O_3F_3$	H, N; C[j]	308, 291
14	*a*	75	197–199	$C_9H_{12}NO_2Cl$	C, H, N	166
15a	*a*	90	128–130	$C_6H_7NO_4$	C, H, N	158[h]

[a] Refer to Experimental Section. [b] Carbodiimide coupling, as in procedure for **5d**. [c] Aminolysis as in procedure for 8. [d] Acylation by *N*-hydroxysuccinimide ester as in procedure for **9c**. [e] Isolated by preparative TLC. [f] Chemical ionization using ammonia gas, unless noted. [g] Californium plasma desorption mass spectrometry,[19] positive ions. [h] Methane gas ionization. [i] Noncrystalline solid, could not be recrystallized. [j] C: calcd, 49.65; found, 49.15.

been widely used for radioiodination of proteins. While radioiodinated analogues of adenosine previously have been reported,[16] compound **9b** illustrates a new general approach to radiolabeled analogues derived from functionalized congeners. Thus, if a functionalized chain is incorporated in a drug molecule at a position that accomodates steric changes, the resulting compound (for example, 4 or 8) may be connected to a *variety* of groups[17] that either contain a radioisotope or are specifically designed to accept an isotope. In such an approach it is also possible to introduce bifunctional prosthetic groups currently used for protein radiolabeling.[17]

We have used the functionalized congener approach to enhance water solubility of selective adenosine receptor antagonists.[18] In the present series of adenosine analogues, the water solubility varied, but for most of the derivatives was not enhanced. The solubility of the carboxylic acid congener 4 was 5.5 mM in 0.01 M phosphate buffer at pH 7.2, roughly a threefold increase over N^6-phenyladenosine. The solubility of the amino congener 8 was 0.37 mM, i.e., diminished but still suitable for in vivo testing.

Conclusions

The congener approach to analogue development has been extended to adenosine. The functionalized congeners and related analogues of N^6-phenyladenosine proved potent in antagonizing binding of N^6-[^{3}H]cyclohexyladenosine to A_1-adenosine receptors in brain membranes. The potency at the receptor is dependent on groups distal from the pharmacophore. Certain congeners appear potentially useful for making conjugates as receptor probes.[14] A parallel study has been carried out with xanthines as A_1-adenosine receptor antagonists.[18]

Further evaluation of these adenosine analogues as activators of adenylate cyclases (A_2 receptors), as vasodilators (A_2 receptors), as stimulants of endocrine glands (A_2 receptors), as inhibitors of adenylate cyclase (A_1 receptors), as cardiac and central depressants (A_1 receptors), and as antilipolytic agents (A_1 receptors) will be required in order to evaluate possible development as therapeutic agents. The biotinyl conjugate **9c** is being investigated as a ligand for histochemical localization of A_1-adenosine receptors.[14]

Experimental Section

Chemical Methods and Materials. Thin-layer chromatography was carried out with use of Analtech silica gel GF plates with mixtures of chloroform/methanol/acetic acid (v/v/v; A, 50/50/5; B, 85/10/5). Reagent grade dimethylformamide (DMF, Aldrich gold label) was stored over 3-Å molecular sieves. 1-Ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDAC) was purchased from Sigma. 4-Aminophenylacetic acid and succinimido 3-(4-hydroxyphenyl)propionate were purchased from Fluka.

The adenosine derivatives were characterized by proton NMR with a Varian 220-MHz spectrophotometer in the Fourier transform mode. Some of the adenosine derivatives (Table II) gave parent ion (M + 1) peaks by chemical-ionization (NH_3) mass spectrometry on a Finnigan 10150 instrument. In a complete study of this series by Pannell and Fales of NHLBI, molecular weights of adenosine analogues were determined by californium plasma desorption mass spectroscopy.[19] Elemental analyses were carried out by the Microanalytical and Instrumentation Section of the NIADDK-LC at the National Institutes of Health, under the direction of Dr. David Johnson.

N^6-[4-(Carboxymethyl)phenyl]adenosine[23] (4). 4-Aminophenylacetic acid (11; 2.80 g, 18.5 mmol) and 6-chloropurine

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(23) IUPAC names for adenosine derivatives described in synthetic procedures: [*p*-[(9-β-D-ribofuranosyl-9*H*-purin-6-yl)amino]phenyl]acetic acid (4), 2-[*p*-[(9-β-D-ribofuranosyl-9*H*-purin-6-yl)amino]phenyl]-*p*-acetotoluidide (**5d**), methyl 2-[*p*-[2-[*p*-[(9-β-D-ribofuranosyl-9*H*-purin-6-yl)amino]phenyl]acetamido]phenyl]acetate (**6b**), α-(methylcarbamoyl)-2-[*p*-[(9-β-D-ribofuranosyl-9*H*-purin-6-yl)amino]phenyl]-*p*-acetotoluidide (**7a**), α-[(2-aminoethyl)carbamoyl]-2-[*p*-[(9-β-D-ribofuranosyl-9*H*-purin-6-yl)amino]phenyl]-*p*-acetotoluidide (8).

Table III. NMR Data[a] for Adenosine Derivatives

A B C D R

N^6—C_6H_4—CH_2—CO—NH—C_6H_4—R

compd	A[c]	B[c]	C[b]	D	R
4	7.96	7.28	3.60		
5a	7.80	7.19	3.60		2.58 (3 H)
5c	7.89	7.33	3.65	7.1–7.3 (4 H)	2.19 (s, 3 H)
5	7.84	7.28	3.60	7.1–7.4 (4 H)	2.27 (s, 3 H)
5e	7.86	7.30	3.60	7.49,[c] 7.10[c]	2.25 (s, 3 H)
5f	7.87	7.30	3.66	7.67,[c] 7.82[c]	
5g	7.85	7.28	3.59	7.49,[c] 7.15[c]	4.09 (1 H, C_αPhe), 3.66 (2 H, CH_2Gly), 2.92 and 2.73 (2 H, C_βPhe), 3.58 (3 H, CH_3), 1.32 (s, 9 H, *t*-Bu)
5h	7.86	7.29	3.60	7.52,[c] 7.17[c]	4.58 (1 H, C_αPhe), 3.68 (s, 3 H, CH_3), 3.13 and 2.96 (2 H, C_βPhe)
6	7.87	7.30	3.61[d]	7.53,[c] 7.21[c]	3.61 (s, 3 H, CH_3, overlapping), 2.56 (3 H)
7a	7.87	7.30	3.60[d]	7.52,[c] 7.17[c]	
7b	7.86	7.29	3.61[d]	7.54,[c] 7.18[c]	
8	7.90	7.29	3.60[d]	7.50,[c] 7.18[c]	3.06 (2 H, CH_2NHCO), 2.64 (2 H, CH_2)
9a	7.85	7.28	3.59[d]	7.51,[c] 7.11[c]	3.1 (4 H, CH_2), 1.78 (s, 3 H, CH_3)
9b	7.84	7.28	3.59[d]	7.51,[c] 7.14[c]	6.94,[c] 6.64,[c] 3.05 (4 H, CH_2), 2.67 (t, 2 H, CH_2), 2.26 (t, 2 H, CH_2)
9c	7.85	7.29	3.60[d]	7.51,[c] 7.17[c]	6.43[e] and 6.36[e] (each s, 1 H, NH), 4.3[e] and 4.1[e] (each m, 1 H, CHN), 3.1[e] (1 H, CHS), 3.08 (4 H, CH_2NH), 2.8[e] (q, 1 H, CH_2S), 2.6[e] (d, 1 H, CH_2S), 2.05 (t, 2 H, CH_2CO), 1,3–1.6[e] (l H)

[a] In Me_2SO-d_6, δ from Me_4Si. Signals from the adenosine moiety are not included. Typical values are as follows (for compound **5e**): 8.54 and 8.39 (each s, 1 H, adenine), 5.96 (d, 1 H, ribose C_1, J = 6 Hz), 5.48 (d, 1 H, OH, J = 6 Hz), 5.29 (t, 1 H, OH), 5.21 (d, 1 H, OH, J = 4 Hz), 4.64, 4.13, and 3.99 (each 1 H, ribose CHO), 3.63 and 3.69 (m, 2 H, CH_2OH). [b] Singlet, 2 H, unless noted. [c] Doublet, 2 H, aromatic, J = 8–9 Hz. [d] Singlet, 4 H, includes two protons from Ar CH_2CO of R group. [e] Biotin moiety.

ribonucleoside (**10**; Aldrich, 5.30 g, 8.5 mmol) were added to a mixture of ethanol, water, and triethylamine in the ratio of volumes of 2:5:5 (24 mL). The suspension was heated in an oil bath at 80 °C for 3 days under argon. The resulting yellow solution was evaporated to near dryness. The residue was dissolved in 150 mL of ethanol, and this solution was treated with 50 mL of ether. The resulting precipitate was filtered to give 1.3 g of impure product, which was not characterized further. Evaporation of the mother liquor followed by crystallization of the residue from ethanol/ether/petroleum ether provided 3.80 g of the pure product: $[\alpha]^{25}D$ −25.5° (DMF, *c* 0.4); UV peak (95% ethanol) at 303 nm (log ϵ 4.47).

Compounds **5a–h** were synthesized according to the representative coupling procedure given below. Methylamine (**13a**) and glycine ethyl ester (**13b**) were obtained commercially as the hydrochloride salts and were first neutralized with 1 equiv of *N*-ethylmorpholine. (*tert*-Butyloxycarbonyl)-*p*-amino-L-phenylalanylglycine *N*-methylamide (**13g**) was prepared as in Jacobson et al.[5]

***N^6*-[4-[(4-Toluidinocarbonyl)methyl]phenyl]adenosine (5e).** Compound 4 (112 mg, 0.28 mmol), *p*-toluidine (**13e**; 30 mg, 0.28 mmol), and EDAC (64 mg, 0.34 mmol) were dissolved in 5 mL of DMF, and the solution was stirred overnight. The product precipitated slowly after addition of water (40 mL). The solid was collected on a fine sintered glass filter and dried in vacuo at 80 °C to give 62 mg of white solid.

***N^6*-[4-[[[4-(Carbomethoxymethyl)anilino]carbonyl]methyl]phenyl]adenosine[23] (6).** Compound 4 (2.00 g, 50 mmol) was dissolved ion DMF (40 mL) and, after cooling in an ice bath, was treated successively with 1-hydroxybenzotriazole (0.67 g) and EDAC (0.96 g, 60 mmol). After several minutes methyl *p*-aminophenylacetate hydrochloride (**14**; 1.11 g, 55 mmol) and *N*-ethylmorpholine (0.70 mL) were added, and stirring was continued at room temperature for 2 h. An equal volume of water was added. The precipitate (1.80 g) was collected, washed with methanol and ether, and dried. The product could be recrystallized from DMF/ethyl acetate to give a pure white solid: $[\alpha]^{25}_D$ −43.5° (DMF, *c* 0.8); UV (95% ethanol) 303 nM, (log ϵ 4.51), 250 (log ϵ 4.31), 241 (log ϵ 4.30).

***N^6*-[4-[[[4-[[(Methylamino)carbonyl]methyl]anilino]carbonyl]methyl]phenyl]adenosine[23] (7a).** Compound 6 (26 mg, 0.048 mmol) was dissolved in 0.5 mL of DMF and the solution treated with a 40% aqueous solution of methylamine (1.0 mL, Kodak). After 1 day the solvent was evaporated, leaving a white solid (20 mg), homogeneous by TLC (solvent B).

***N^6*-[4-[[[4-[[[(2-Aminoethyl)amino]carbonyl]methyl]anilino]carbonyl]methyl]phenyl]adenosine[23] (8).** Compound 6 (40.7 mg, 0.074 mmol) was dissolved in 0.4 mL of DMF and treated with 0.6 mL of ethylenediamine. After the mixture was allowed to stand overnight, the volume was reduced under a stream of nitrogen. Trituration of the resulting oil with methanol produced a solid, which was collected, washed with ether, and dried in vacuo at 80 °C: yield 41 mg; $[\alpha]^{25}_D$ −39.7° (DMF, *c* 0.7); UV spectrum showed peaks at 303, 251, and 241 nm.

(3a*S*,4*S*,6a*R*)-Hexahydro-2-oxo-*N*-[2-[2-[*p*-[2-[*p*-[(9-β-D-ribofuranosyl-9*H*-purin-6-yl)amino]phenyl]acetamido]phenyl]acetamido]ethyl]-1*H*-thieno[3,4-*d*]imidazole-4-valeramide (9c). Compound 8 (57 mg, 0.10 mmol) was suspended in 2 mL of DMF and the solution treated with *N*-succinimidyl-*d*-biotin (Sigma, 50 mg, 0.15 mmol). After the mixture was stirred for 2 days, methanol and ether (2 mL each) were added. The precipitate was collected, washed with ether, and dried. The product, 76 mg, was homogeneous by TLC (solvent B), using UV detection and a biotin-specific spray reagent[20] consisting of 0.1% 4-(dimethylamino)cinnamaldehyde in 1% sulfuric acid.

(Trifluoroacetyl)-*p*-nitro-L-phenylalanine Methyl Ester (12). *p*-Nitro-L-phenylalanine methyl ester hydrochloride[5] (0.73 g, 2.8 mmol) was suspended in ethyl acetate (20 mL) and cooled in an ice bath. *N*-Ethylmorpholine (0.36 mL, 2.8 mmol) and trifluoroacetic anhydride (0.40 mL, 2.8 mmol) were added. After stirring for 1 h, the solution was filtered, extracted with water and dilute HCl, and evaporated. The product was recrystallized from ethyl acetate/petroleum ether to give 0.79 g: $[\alpha]^{25}_D$ −15.4° (EtOH, c 05).

(Trifluoroacetyl)-*p*-amino-L-phenylalanine Methyl Ester (13h). Compound 12 (0.33 g, 1.03 mmol) was dissolved in methanol (10 mL) and hydrogenated over 5% palladium on charcoal in a Parr shaker. After 1 h the mixture was filtered, evaporated, and chromatographed on a silica TLC plate with ethyl acetate/petroleum ether (1:1). The band of product was extracted with ethyl acetate to give a yield of 257 mg: NMR (ppm, $CDCl_3$) 6.83 (d, 2 H, Ar, 8 Hz), 6.60 (d, 2 H, Ar), 4.81 (q, 1 H, C_αPhe), 3.78 (s, 3 H, CH_3), 3.09 (d, 2 H, C_βPhe, J = 6 Hz).

Methyl *p*-Aminophenylacetate Hydrochloride (14). *p*-Aminophenylacetic acid (6.27 g, 41.5 mmol) was esterified by bubbling dry HCl gas through a cold, stirred methanolic solution for 4 h. Ether was added, and the crystals were collected to give 6.27 g: NMR (ppm, Me_2SO-d_6) 7.39 (s, 4 H, Ar), 3.75 (s, 2 H, CH_2), 3.64 (s, 3 H, CH_3).

Succinimido Acetate (15a). *N*-Hydroxysuccinimide (17.33 g, 0.15 mol) was suspended in 120 mL of THF and cooled in an ice bath. Acetic anhydride (16 mL, 0.17 mol) and then triethylamine (22 ml, 0.16 mol) were added slowly. The solid dissolved, and then gradually a white precipitate appeared. After

0.5 h an equal volume of petroleum ether was added, and the crystalline product was collected to give 21.22 g (mp 128–130 °C, lit.[21] mp 131–132 °C).

Biochemical Assay. Inhibition of binding of 1 nM N^6-[^{3}H]cyclohexyladenosine was assayed as described.[22] Inhibition of binding by a range of concentrations of each adenosine analogue was assessed in triplicate for at least two separate experiments, and the IC_{50} values were estimated graphically for each experiment.

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Registry No. 1, 23589-16-4; 2, 29204-54-4; 3, 29204-77-1; 4, 96760-57-5; 5a, 96760-58-6; 5b, 96760-59-7; 5c, 96760-60-0; 5d, 96760-61-1; 5e, 96760-62-2; 5f, 96760-63-3; 5g, 96760-64-4; 5h, 96760-65-5; 6, 96760-66-6; 7a, 96760-67-7; 7b, 96760-68-8; 8, 96760-69-9; 9a, 96760-70-2; 9b, 96760-71-3; 9c, 96760-72-4; 10, 5399-87-1; 11, 1197-55-3; 12, 96760-73-5; 13a, 74-89-5; 13b, 459-73-4; 13c, 95-53-4; 13d, 108-44-1; 13e, 106-49-0; 13f, 455-14-1; 13g, 89545-73-3; 13h, 96760-74-6; 14, 83528-16-9; 15a, 14464-29-0; ethylenediamine, 107-15-3; N-succinimidyl-d-biotin, 35013-72-0; p-nitro-L-phenylalanine methyl ester hydrochloride, 17193-40-7; trifluoroacetic anhydride, 407-25-0; N-hydroxysuccinimide, 6066-82-6; acetic anhydride, 108-24-7.

New Cysteamine (2-Chloroethyl)nitrosoureas. Synthesis and Preliminary Antitumor Results

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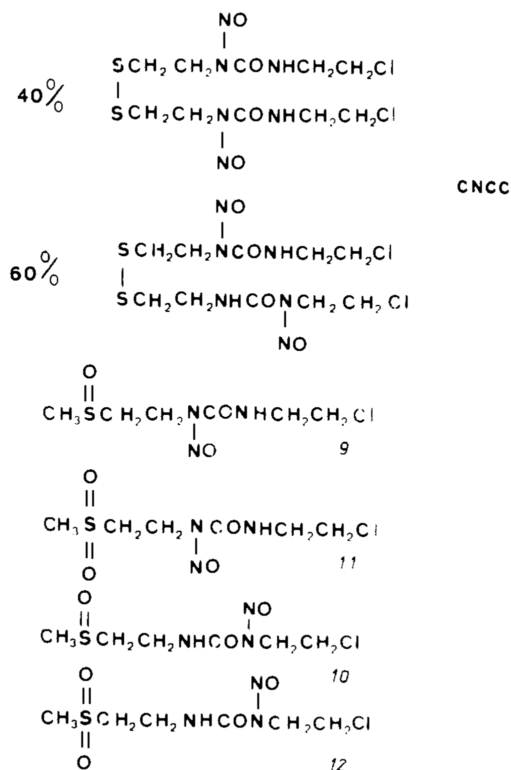
Three chemical pathways were used for the synthesis of four new N′-(2-chloroethyl)-N-[2-(methylsulfinyl)ethyl]- and N′-(2-chloroethyl)-N-[2-(methylsulfonyl)ethyl]-N- or -N′-nitrosoureas. These compounds are plasma metabolites of CNCC, a promising antineoplastic (2-chloroethyl)nitrosourea. Preliminary antitumor evaluation was performed against L1210 leukemia implanted intraperitoneally in mice. Among these compounds, two of them exhibited a greater antitumor activity compared to that of the parent mixture.

(2-Chloroethyl)nitrosoureas, a class of very active antitumor agents, are of interest for the treatment of glioblastoma, Hodgkin's disease, non-Hodgkin's lymphoma, bronchial carcinoma, and gastric cancers.[1–5] To date the most widely used in cancer chemotherapy are BCNU, CCNU, and MeCCNU. Among the several compounds of this important series, the more effective agents chlorozotocin, ACNU, MeCCNU, and RFCNU have undergone phase I–II clinical trials.[6] They were selected on the basis of their wide spectrum of activity. CNCC (Chart I), a recently developed (2-chloroethyl)nitrosourea,[6–10] shows a very promising antineoplastic activity in several experimental animal tumor models.[6,7,10]

In vivo metabolic studies of CNCC[11,12] allowed us to identify active plasma metabolites in different animal species (mouse, rat, rabbit) and in man. These biotransformation products originate in the breakdown of the disulfur bridge with subsequent methylation and oxidation of the sulfur atom. Structure analysis of the four isolated metabolites listed in Chart I shows that they could be antitumor agents. To test this hypothesis we have synthesized **9–12** (Chart I) and evaluated their antitumor activity. This paper describes the synthesis and the preliminary biological results of antitumor testing against L_{1210} in mice.[13]

Chemistry. The four compounds were prepared by several routes. Scheme I leads to the mixture of sulfoxides and sulfones. The urea **1** is easily prepared by reacting 2-chloroethyl isocyanate with 2-(methylthio)ethylamine in diethyl ether. Conventional nitrosation of **1** gives dominance to the formation of the N-nitroso-N′-(2-chloroethyl) compound **2**. Oxidation with H_2O_2 in acetone or formic acid yields a mixture of nitroso sulfoxides and sulfones.

Chart I



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