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SYNTHESIS AND ANTITUMOR ACTIVITY OF CYTOSINE AND ADENINE NUCLEOSIDES OF UNSATURATED 5-(AMINOACYL)AMINOPENTOFURANOSES

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ABSTRACT

Direct synthesis of the 1- and 9-(5-azido-2,3,5-trideoxy-β-D-*glycero*-pent-2-enofuranosyl) derivatives (**3a** and **3b**) of cytosine and adenine, respectively, has been accomplished *via* treatment of the corresponding 2′,3′-unsaturated nucleosides (**1a** and **1b**) with triphenylphosphine and carbon tetrabromide in the presence of lithium azide. Members of a new type of (aminoacyl)amino nucleoside, the 1- and 9-[5-(aminoacyl)amino-2,3,5-trideoxy-β-D-*glycero*-pent-2-enofuranosyl] derivatives of cytosine and adenine, respectively, have been obtained by condensation of the corresponding, unsaturated amino nucleosides with the active esters of several amino acid derivatives, followed by deprotection. These nucleosides were examined for *in vivo* antitumor activity against leukemia L-1210 and Sarcoma 180 (solid tumor) in mice; none of them exhibited antitumor activity against L-1210 in mice, but compounds **1a**, **3a**, and 1-[2,3,5-trideoxy-5-(L-methionyl)amino-β-D-*glycero*-pent-2-enofuranosyl]cytosine exhibited weak activity against Sarcoma 180 (solid tumor).

INTRODUCTION

In recent years, special attention has been directed to the biological activities of amino nucleoside derivatives in relation to such nucleoside antibiotics as puromycin[1], gougerotin[2], the polyoxins[3], and blasticidin S (ref. 4). The unique structure and biological activities of blasticidin S, which contains an endocyclic double-bond at C-2,C-3 of the pyranosyl residue, prompted the synthesis, and exploration of the biological activity, of (aminoacyl)amino unsaturated nucleosides structurally related to blasticidin S. Our interest in such amino sugar nucleosides was also roused by the recent observation that 5′-amino-5′-deoxythymidine[5] is a potent inhibitor of mammalian thymidine kinase, an enzyme that shows activity in many tumor and virus-infected systems.

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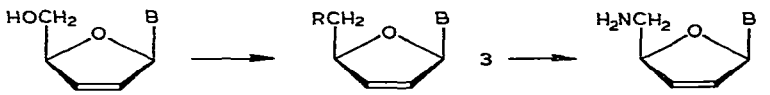
We have previously reported[6] the synthesis of members of a new type of (aminoacyl)amino unsaturated nucleoside, the 1-[5-(aminoacyl)amino-2,3,5-trideoxy-β-D-*glycero*-pent-2-enofuranosyl]-uracils and -thymines. These compounds were tested for biological activity against Gram-positive and -negative bacterial organisms, and Sarcoma 180 (solid tumor) in mice, but none of them exhibited inhibitory activity. In pursuit of therapeutically useful agents among the 5′-amino unsaturated nucleosides, it was therefore of interest to investigate the cytosine and adenine counterparts, and we now report the synthesis of some [5-(aminoacyl)-amino-2,3,5-trideoxy-β-D-*glycero*-pent-2-enofuranosyl]-cytosines (**8a–d**) and -adenines (**9a–f**), and their antitumor activity against Sarcoma 180 (solid tumor) in mice and murine leukemia L-1210.

RESULTS AND DISCUSSION

The synthesis of a variety of 5′-(aminoacyl)amino unsaturated nucleosides is outlined in Schemes 1 and 2.



1 a,b

a B = Cytosin-1-yl

b B = Adenin-9-yl

2a R = TsO

3a R = N_3

3b R = N_3

4

Scheme 1

The starting materials, **1a** and **1b**, were conveniently prepared from the corresponding 2-*O*-acetyl-3-bromo-3-deoxy-β-D-xylofuranosyl nucleosides *via* an electrochemical method[7] developed in our laboratory, followed by deacetylation with methanolic ammonia. Tosylation of **1a** with tosyl chloride in dry pyridine gave tosylate **2a** in rather low yield (57%) compared to those in the uracil and thymine series[6]. The tosylated site was confirmed by the appearance of an NH_2 resonance at 7.22 p.p.m. in its n.m.r. spectrum and an absorption maximum at 270 nm in its u.v. spectrum. Treatment of **2a** with sodium azide in *N*,*N*-dimethylformamide at 70–80° gave, in 70% yield, crystalline azide **3a**, the i.r. spectrum of which exhibited a sharp absorption band at 2080 cm^{-1} characteristic of an azide group.

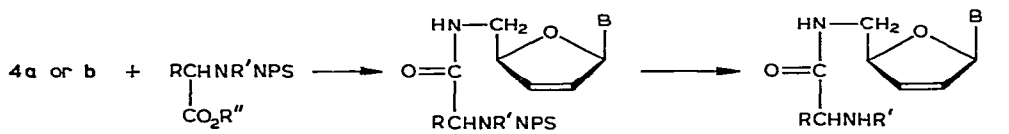
In the adenine series, some difficulties were anticipated in the tosylation and subsequent azide formation, as Jahn reported[8] that the 5′-*O*-tosyl derivative of (N^6-unprotected) adenosine tends to undergo intramolecular cyclization to afford the N^3,5′-anhydronucleoside; we therefore applied an alternative route for the preparation of azide **3b**.

Recently, several methods for direct replacement of hydroxyl groups by halogens or alkylthio groups, using such trivalent phosphorus reagents as triphenylphosphine, tributylphosphine, triphenyl phosphite, and hexamethylphosphoric triamide, have been developed[9]. Hata and co-workers successfully extended this method to the

direct introduction of an azide group onto C-5′ of nucleosides[10], and we applied this method to the preparation of **3b**. The reactions of **1b** with several combinations of reagents, namely, Ph_3P–CBr_4, Ph_3P–CCl_4, Ph_3P–*N*-bromosuccinimide (NBS), $(PhO)_3P$–CBr_4, and $(PhO)_3P$–NBS in the presence of LiN_3, were examined under a variety of conditions. Azide **3b** was obtained in the best yield (63%) by the reaction of **1b** with 1.5 molar equiv. each of triphenylphosphine and carbon tetrabromide and 5 molar equiv. of lithium azide in *N*,*N*-dimethylformamide at room temperature. Although slight cleavage of the glycosyl bond under these conditions was detected by t.l.c., the N^3,5′-intramolecular cyclization was not observed. The structure of **3b** was fully characterized by elemental analysis and spectroscopic data. The i.r. spectrum showed a characteristic azide band at 2075 cm^{-1}, and the n.m.r. spectrum exhibited the amino protons (broad singlet) at 7.26 p.p.m. This method could also be applied successfully to **1a**, to give **3a** in 64.6% yield. Transformation of **3a** and **3b** into the corresponding amines, **4a** and **4b**, was attained in high yield by use of hydrogen sulfide in aqueous pyridine[11].

In studies on the structure–activity relationship of puromycin and gougerotin analogs, it has been noted that the nature of the aminoacyl group affects the biological activity of the parent amino nucleoside[1,12]. We therefore attempted preparation of the aminoacyl derivatives **8a–d** and **9a–f** by a method usual for peptide synthesis.

The reactions of **4a** with the active esters, **5a–d**, of the *N*-(*o*-nitrophenyl)sulfenyl amino acids were conducted in *N*,*N*-dimethylformamide at room temperature, and the desired products, **6a–d**, were obtained in good yields. Treatment of **4a** with the glycine derivative **5e** showed a complicated feature, and the desired product could



5a–f 6a–d or 7a–f 8a–d or 9a–f

NPS = (*o*-Nitrophenyl) sulfenyl

6,8 B = Cytosin-1-yl

7,9 B = Adenin-9-yl

5-9	*R*	*R′*	*R″*
a[a]	$PhCH_2$	H	Su[b]
b[a]	*p*-$MeOPhCH_2$	H	Su
c[a]	$MeS(CH_2)_2$	H	Np[c]
d[d]	Ph	H	Su
e	H	H	Su
f	H	Me	Su

a The L Isomer was employed.

b Su = Succinimido.

c Np = *p*-Nitrophenyl.

d The D Isomer was employed.

Scheme 2

TABLE I

COMPOSITIONS AND YIELDS OF (AMINOACYL)AMINO NUCLEOSIDES (**8a–d** AND **9a–f**)

Compound	*B*[a]	*R*	*R'*	*Formula*	*Yield (%)*	*Analysis Calc. (Found)*			
						C	*H*	*N*	*S*
8a	C	$PhCH_2$	H	$C_{18}H_{21}N_5O_3 \cdot (CO_2H)_2 \cdot H_2O$	68	51.83 (52.17	5.44 5.12	15.11 14.99)	
8b	C	*p*-$MeOPhCH_2$	H	$C_{19}H_{23}N_5O_4 \cdot (CO_2H)_2 \cdot 1.5\ H_2O$	72	50.20 (50.17	5.62 5.28	13.94 13.68)	
8c	C	$MeS(CH_2)_2$	H	$C_{14}H_{21}N_5O_3S \cdot 0.5\ (CO_2H)_2 \cdot H_2O$	67	44.77 (45.36	6.01 5.60	17.40 16.97	7.97 7.48)[b]
8d	C	Ph	H	$C_{17}H_{19}N_5O_3 \cdot (CO_2H)_2 \cdot 0.5\ H_2O$	69	51.82 (51.76	5.04 4.79	15.90 15.49)	
9a	A	$PhCH_2$	H	$C_{19}H_{21}N_7O_2 \cdot (CO_2H)_2$	57	53.73 (54.00	4.94 5.24	20.89 20.99)	
9b	A	*p*-$MeOPhCH_2$	H	$C_{20}H_{23}N_7O_3 \cdot AcOH$	76	56.28 (56.16	5.80 5.80	20.89 21.12)	
9c	A	$MeS(CH_2)_2$	H	$C_{15}H_{21}N_7O_2S \cdot 0.5\ (CO_2H)_2 \cdot H_2O$	77	45.06 (45.28	5.67 5.67	22.99 23.06	7.52 7.51)
9d	A	Ph	H	$C_{18}H_{19}N_7O_2 \cdot 0.5\ AcOH$	88	57.71 (57.83	5.61 5.45	24.80 24.46)	
9e	A	H	H	$C_{12}H_{15}N_7O_2 \cdot 0.5\ H_2O$	67	48.31 (48.40	5.41 5.50	32.87 32.91)	
9f	A	H	Me	$C_{13}H_{17}N_7O_2 \cdot Me_2CHOH$	72	52.88 (52.64	6.93 6.86	26.98 27.21)	

[a]C = cytosin-1-yl; A = adenin-9-yl.
[b]Better analyses could not be obtained, because of hygroscopicity of the compound.

TABLE II

PHYSICAL CONSTANTS OF COMPOUNDS **8a–d** AND **9a–f**

Compound	*M.p. (degrees)*	$\lambda_{max}^{H_2O}$ *in nm* (ε_{mM})	$[\alpha]_D^{25}$ *(degrees) (c, in H_2O)*
8a	130–135	271.5 (8.68)	−12.4 (0.725)
8b	100–125	272.5 (10.19)	−14.1 (1.35)
8c	132–140	271 (7.67)	−4.5 (1.07)
8d	141–145	271 (6.98)	−57.8 (0.675)
9a	125–132	261 (13.98)	+1.1 (0.91)
9b	153–156	261.5 (14.57)	−25.8 (1.55)
9c	81–110	261 (13.48)	+15.2 (1.12)
9d	88–94	261 (13.59)	−23.4 (0.94)
9e	129–131	261 (11.73)	−59.5 (1.26)
9f	96–100	260.5 (14.48)	−42.5 (0.54)

not be obtained, whereas, in the adenine series, the corresponding product, **7e**, was obtained from **4b** in 87% yield.

The coupling reactions of **4b** with the active esters **5a–f** were conducted under conditions similar to those used for the cytosine series, to give the corresponding protected aminoacyl nucleosides **7a–f** in fairly good yields. After purification by chromatography on a column of silica gel, all of the products, except for the D-2-phenylglycine and glycine derivatives (**7d** and **7e**), were obtained as yellow, homogeneous (in t.l.c.) foams that did not show sharp melting-points. The structures were confirmed by n.m.r. spectroscopy and elemental analysis.

Deprotection of **6a–d** was conducted in 10:1 ethanol–acetic acid containing 2 equiv. of 2-mercaptobenzothiazole, to give the desired products, **8a–d**, in high yields. However, in the adenine series, treatment of the protected nucleosides **7a–f** under the same conditions led to extensive glycosylic cleavage, to afford adenine in 80% yield, due to the acetic acid present. After several experiments, the deprotection was found to proceed effectively in 10:10:1 ethanol–pyridine–acetic acid; cleavage of the glycosyl bond was not observed to an appreciable extent, but the reaction rate was much lower than that in ethanol–acetic acid solution. Thus, treatment of the adenine analogs **7a–f** with two equiv. of 2-mercaptobenzothiazole under the foregoing conditions gave the expected products, **9a–f**, in good yields. The unprotected (aminoacyl)amino nucleosides (**8a–d** and **9a–f**) were obtained as extremely hygroscopic solids, and, therefore, most of them were converted into the oxalates for analysis (see Table I). The structures of these products (see Table II) were confirmed by elemental analysis and n.m.r. spectroscopy. The n.m.r. spectra of **8a–d** and **9a–f** showed characteristic signals of H-1′, -2′, -3′, and -4′ due to the 2′,3′-unsaturated sugar residues, and also, the presence of the respective amino acid residues (see Tables III and IV).

TABLE III

N.M.R.-SPECTRAL DATA FOR THE CYTIDINE ANALOGS **8a–d**

Compound	*Solvent*	*Chemical shifts*[a]					
		H-1′	*H-2′*	*H-3′*	*H-5*	*H-6*	*Others*
8a	Me_2SO-d_6–D_2O	6.85(m)	6.3(m)	6.0(m)	5.82(d) $J_{5,6}$ 7.8 Hz	7.4(d)	2.9 (m, PhCH_2), 3.4 (m, H-5′ + CH_2CH), 4.8 (m, H-4′), 7.3 (s, Ph)
8b	D_2O		6.41(m)	6.15(m)	6.05(d) $J_{5,6}$ 7.2 Hz	7.6(d)	3.1 (m, PhCH_2), 3.78 (s, Me), 3.2 (m, H-5′ + CH_2CH), 7.07 (m, H-1′ + arom. H)
8c	D_2O	6.8(m)	6.45(m)	6.05(m)	6.04(d) $J_{5,6}$ 7.8 Hz	7.6(d)	2.15 (m, CH_2CH), 2.03 (s, Me), 2.55 (m, CH_2S), 3.45 (d, $J_{4',5'}$ 5.4 Hz, H-5′), 4.06 (t, J 6 Hz, CH)
8d	D_2O	6.65(m)	6.4(m)	5.95(m)	6.0(d) $J_{5,6}$ 7.2 Hz	7.58(d)	3.45 (deformed d, H-5′), 5.05 (s, CH), 7.42 (s, Ph)

[a]In p.p.m. from an internal standard of Me_4Si or sodium 2,2-dimethyl-2-silapentane-5-sulfonate (in D_2O).

TABLE IV

N.M.R.-SPECTRAL DATA FOR THE ADENOSINE ANALOGS **9a–f**

Compound	*Chemical shifts*[a]							
	H-1′	*H-2′*	*H-3′*	*H-4′*	*H-5′*	*H-2* or *H-8*		*Others*
9a	6.95(m)	6.3(m)	6.1(m)	4.85(m)	3.35(m)	8.07(s)	8.19(s)	3.0 (m, PhCH_2), 3.75 (m, CH), 7.21 (s, Ph)
9b	7.0(m)	6.4(m)	6.2(m)	4.9(m)		8.13(s)	8.35(s)	1.95 (s, Ac), 2.85 (m, PhCH_2), 3.45 (m, H-5′ + CH), 3.75 (s, MeO), 7.0 (m, arom. H)
9c	7.0(m)	6.55(m)	6.25(m)	5.05(m)	3.45(m)	8.22(s)	8.26(s)	1.95 (m, CH_2CH), 1.99 (s, Me), 3.75 (m, CH)
9d	6.95(m)	6.45(m)	6.2(m)	4.95(m)	3.4(m)	8.17(s)	8.2(s)	1.88 (s, Ac), 4.53 (m, CH), 7.3 (m, Ph)
9e	6.98(m)	6.49(m)	6.25(m)	5.0(m)	3.4(m)	8.16(s)	8.23(s)	3.16 (s, CH_2)
9f	6.92(m)	6.5(m)	6.18(m)	4.96(m)	3.4(m)	8.09(s)	8.18(s)	1.04 (d, J 6 Hz, Me_2CH), 2.2 (s, MeN), 3.04 (s, CHN), 3.75 (m, Me_2CH)

[a]In p.p.m. from an internal standard of Me_4Si (in Me_2SO-d_6–D_2O).

Antitumor activity. — The 5′-(aminoacyl)amino unsaturated nucleosides **8a–d** and **9a–f**, and some of the intermediates (**1a**, **1b**, **3a**, **3b**, **4a**, and **4b**), were examined for antitumor activity against Sarcoma 180 (solid tumor) and leukemia L-1210 in mice, following the general protocols outlined in the Experimental section. None of the compounds exhibited inhibitory activity against leukemia L-1210 at 100 mg/kg/day × 5, i.p.; however, against Sarcoma 180 (solid tumor), compounds **1a**, **3a**, and **8c** exhibited weak activity (29.2, 32.9, and 36.5% inhibition, respectively) at 100 mg/kg/day × 7, i.p.

EXPERIMENTAL

General. — Melting points were determined on a Yamato melting-point apparatus and are uncorrected. N.m.r. spectra were recorded with a Hitachi Perkin–Elmer R-20A spectrometer; chemical shifts are reported in p.p.m. downfield from an internal standard of tetramethylsilane. U.v. spectra were recorded with a Hitachi EPS-3T spectrometer. Optical rotations were measured with a JASCO DIP-4 automatic polarimeter.

*1-(2,3-Dideoxy-5-*O*-*p*-tolylsulfonyl-β-*D*-glycero-pent-2-enofuranosyl)cytosine* (**2a**). — To a solution of **1a** (0.21 g, 1 mmol) in pyridine (5 mL) was added *p*-toluenesulfonyl chloride (0.29 g, 1.5 mmol) at −10°, and the mixture was stirred overnight at room temperature. The mixture was then poured into ice–water (50 mL), and the crystals that separated were collected by filtration, and washed with 2-propanol, to give 0.21 g (57%) of **2a**, m.p. 163–165°. An analytical sample of **2a**, recrystallized from ethanol, had m.p. 167–168°; λ_{max}^{MeOH} 226 (ε_{mM} 21.98) and 270 nm (8.27); n.m.r. (Me_2SO-d_6): δ 2.42 (s, Me), 4.18 (d, $J_{4',5'}$ 3 Hz, H-5′), 4.75–5.15 (m, H-4′), 5.62 (d, $J_{5,6}$ 7.2 Hz, H-5), 5.85–6.1 (m, H-3′), 6.16–6.4 (m, H-2′), 6.76–6.94 (m, H-1′), 7.22 (br s, NH_2), 7.26 (d, *J* 7.2 Hz, H-6), 7.39 and 7.77 (d, *J* 8 Hz, arom. H).

Anal. Calc. for $C_{16}H_{17}N_3O_5S$: C, 52.89; H, 4.72; N, 11.57; S, 8.85. Found: C, 52.93; H, 4.74; N, 11.50; S, 8.91.

*1-(5-Azido-2,3,5-trideoxy-β-*D*-glycero-pent-2-enofuranosyl)cytosine* (**3a**). — *A.* A mixture of **2a** (0.36 g, 1 mmol) and sodium azide (0.13 g, 2 mmol) in *N*,*N*-dimethylformamide (5 mL) was stirred for 3 h at 70–80°, and evaporated *in vacuo*. Water (10 mL) was added to the residue, and the aqueous solution was extracted with chloroform (5 × 10 mL). The extracts were combined, dried (magnesium sulfate), and evaporated *in vacuo*, to give 0.16 g (70%) of **3a**, m.p. 173–175°. An analytical sample (recrystallized from 2-propanol) had m.p. 176–177° (dec.), $[\alpha]_D^{27}$ +50.6° (*c* 0.08, *N*,*N*-dimethylformamide); λ_{max}^{MeOH} 240 (ε_{mM} 7.68) and 270.5 nm (7.68); ν_{max}^{Nujol} 2080 cm^{-1} (azide); n.m.r. (Me_2SO-d_6): δ 3.56 (d, $J_{4',5'}$ 4.2 Hz, H-5′), 4.7–5.1 (m, H-4′), 5.76 (d, $J_{5,6}$ 7.8 Hz, H-5), 5.85–6.15 (m, H-3′), 6.22–6.5 (m, H-2′), 6.8–7.05 (m, H-1′), 7.26 (br s, NH_2), and 7.46 (d, *J* 7.8 Hz, H-6).

Anal. Calc. for $C_9H_{10}N_6O_2$: C, 46.15; H, 4.30; N, 35.88. Found: C, 46.35; H, 4.59; N, 36.00.

B. A mixture of **1a** (1.46 g, 7 mmol), triphenylphosphine (2.75 g, 10.5 mmol),

and lithium azide (1.71 g, 35 mmol) in *N,N*-dimethylformamide (21 mL) was heated until a clear solution was obtained. The solution was then cooled to ~5°, a solution of carbon tetrabromide (3.48 g, 10.5 mmol) in *N,N*-dimethylformamide (7 mL) was added, under nitrogen, during 40 min, and the mixture was stirred overnight at room temperature. After addition of methanol (6 mL), the mixture was evaporated *in vacuc* below 45°, and the residue was dissolved in chloroform (50 mL). The solution was extracted with 5% hydrochloric acid (3 × 30 mL), and the extract was made neutral with sodium hydrogencarbonate, and evaporated to dryness. The residue was extracted with hot ethanol, the extract was evaporated to dryness, and the residue was purified by chromatography on silica gel (40 g), using 17:3 chloroform–methanol, to give 1.03 g (63%) of **3a**, m.p. 176–177°, identical in every way with the sample obtained by method A.

*9-(5-Azido-2,3,5-trideoxy-β-*D-glycero-*pent-2-enofuranosyl)adenine* (**3b**). — A mixture of **1b** (11.66 g, 50 mmol), triphenylphosphine (19.67 g, 75 mmol), and lithium azide (12.24 g, 250 mmol) in *N,N*-dimethylformamide (120 mL) was treated with carbon tetrabromide (24.88 g, 75 mmol) in *N,N*-dimethylformamide (40 mL) in a manner similar to that described for **3a**. After addition of methanol (20 mL), the solution was evaporated *in vacuo*, the residue was partitioned between water (100 mL) and chloroform (200 mL), and the aqueous layer was further extracted with chloroform (3 × 200 mL). The organic layer was dried (magnesium sulfate), and evaporated, and the residue was crystallized from 2-propanol (60 mL), to give 5.01 g of **3b**, m.p. 143–145° (dec.). The mother liquor was evaporated to dryness, and the residue was purified by chromatography on silica gel (200 g), using 19:1 chloroform–methanol, to give triphenylphosphine oxide (19.24 g, 92%); subsequent elution with 17:3 chloroform–methanol gave an additional 3.33 g of **3b**; total yield of **3b**, 8.34 g (65%). An analytical sample, recrystallized from ethanol, had m.p. 146° (dec.), $[\alpha]_D^{27}$ +46.7° (*c* 1.07, methanol); λ_{max}^{MeOH} 260 nm (ε_{mM} 13.53); ν_{max}^{Nujol} 2075 cm^{-1} (azide); n.m.r. (Me_2SO-d_6): δ 3.61 (d, $J_{4',5'}$ 4.8 Hz, H-5'), 4.9–5.3 (m, H-4'), 6.2–6.42 (m, H-3'), 6.42–6.65 (m, H-2'), 6.95–7.1 (m, H-1'), 7.32 (br s, NH_2), 8.15 (s, H-2 or H-8), and 8.24 (s, H-2 or H-8).

Anal. Calc. for $C_{10}H_{10}N_8O_2$: C, 46.51; H, 3.90; N, 43.39. Found: C, 46.24; H, 3.99; N, 43.14.

*Synthesis of 1-(5-amino-2,3,5-trideoxy-β-*D-glycero-*pent-2-enofuranosyl)cytosine* (**4a**) *and 9-(5-amino-2,3,5-trideoxy-β-*D-glycero-*pent-2-enofuranosyl)adenine* (**4b**). — Selective reduction of the unsaturated nucleoside azides (**3a** and **3b**) to the corresponding amines (**4a** and **4b**) was achieved according to a published method[11].

N-*Hydroxysuccinimide ester of* O-*methyl*-N-(o-*nitrophenylsulfenyl*)-L-*tyrosine* (**5b**). — After removal of dicyclohexylamine from the dicyclohexylamine salt (14.83 g, 28 mmol) of *O*-methyl-*N*-(*o*-nitrophenylsulfenyl)-L-tyrosine *via* treatment with 0.5M sulfuric acid in the usual way, the resulting acid was dissolved in oxolane (84 mL), the solution was cooled to ~5°, *N*-hydroxysuccinimide (3.22 g, 28 mmol) and dicyclohexylcarbodiimide (5.78 g, 28 mmol) were added successively, and the mixture was stirred overnight at room temperature, and evaporated. The residue was suspended in

ethyl acetate (20 mL), and dicyclohexylurea was removed by filtration. The filtrate was successively washed with water and saline, dried (magnesium sulfate), concentrated to ~10 mL, and diluted with petroleum ether, to give 10.3 g (83%) of **5b**, m.p. 65–75° (dec.), as a yellow powder. Crystallization from ethyl acetate–petroleum ether gave an analytical sample, m.p. 148–149°, $[\alpha]_D^{27}$ −10.7° (*c* 0.52, chloroform); ν_{max}^{Nujol} 3325, 1810, 1785, and 1738 cm^{-1}; n.m.r. (Me_2SO-d_6): δ 2.9 (s, CH_2CH_2), ~2.9–3.6 (m, CH_2CH), 3.82 (s, Me), ~3.8–4.28 (m, CH), 5.68 (d, *J* 10.5 Hz, NH), and 6.7–7.7 and 7.95–8.4 (m, arom. H).

Anal. Calc. for $C_{20}H_{19}N_3O_7S$: C, 53.93; H, 4.30; N, 9.43; S, 7.20. Found: C, 53.67; H, 4.49; N, 9.21; S, 7.02.

N-Hydroxysuccinimide ester of N-(o-*nitrophenylsulfenyl*)*sarcosine* (**5f**). — A mixture of *N*-(*o*-nitrophenylsulfenyl)sarcosine [obtained from its dicyclohexylammonium salt (8.47 g, 20 mmol)], *N*-hydroxysuccinimide (2.3 g, 20 mmol), and dicyclohexylcarbodiimide (4.13 g, 20 mmol) was treated as described for **5b**. The resulting, yellow precipitate (containing **5f** and dicyclohexylurea) was collected by filtration, and extracted with hot *N,N*-dimethylformamide to remove the dicyclohexylurea. The extract was concentrated to ~20 mL, and the resulting precipitate (of dicyclohexylurea) was removed by filtration. Dilution of the filtrate with ether gave 4.81 g (71%) of **5f**, m.p. 189–191°, as yellow crystals. Recrystallization from *N,N*-dimethylformamide–diethyl ether gave an analytically pure sample, m.p. 200° (dec.); ν_{max}^{Nujol} 1839, 1788, and 1738 cm^{-1}; n.m.r. (Me_2SO-d_6): δ 2.88 (s, CH_2CH_2), 3.01 (s, Me), 4.44 (s, CH_2), and 7.26–8.42 (m, arom. H).

Anal. Calc. for $C_{13}H_{13}N_3O_6S$: C, 46.02; H, 3.86; N, 12.38; S, 9.45. Found: C, 45.99; H, 4.06; N, 12.42; S, 9.35.

General procedure for preparation of the protected (*aminoacyl*)*amino nucleosides* (**6a–d** and **7a–f**). — To a suspension of the aminonucleoside **4** (5 mmol) in *N,N*-dimethylformamide (15 mL) was added an equimolar amount of the active ester **5** at ~5°, and the mixture was stirred overnight at room temperature. It was then diluted with ethyl acetate or chloroform (150 mL), and the solution was washed successively with 4% aqueous sodium hydrogencarbonate, water, and saline, dried (magnesium sulfate), and evaporated to dryness *in vacuo*. The residue was purified by recrystallization, or by chromatography on silica gel using appropriate solvents (see Table V).

General procedure for preparation of the unprotected (*aminoacyl*)*amino nucleosides* (**8a–d**) (*cytosine series*). — A mixture of the protected (aminoacyl)amino nucleoside **6** (2 mmol) and 2-mercaptobenzothiazole (4 mmol) in 10:1 ethanol–acetic acid (11 mL) was stirred overnight at room temperature. The resulting precipitate was removed by filtration, and the filtrate was concentrated *in vacuo* below 30°, followed by coevaporation with ethanol. Water (15 mL) was added to the residue, and the insoluble material was filtered off. The filtrate was washed with benzene (3 × 20 mL), and lyophilized, to give a very hygroscopic foam. This was dissolved in ethanol (20 mL), and oxalic acid (~1.8 mmol) was added to the solution, to give **8a–d** as a white powder. The yields and physical constants are given in Tables I–III.

TABLE V

PROTECTED (AMINOACYL)AMINO NUCLEOSIDES (6a–d AND 7a–f)

Compound	*M.p. (degrees)*	*Yield (%)*	*Formula*	*Analysis Calc. (Found)*			
				C	*H*	*N*	*S*
6a	130–140	84	$C_{24}H_{24}N_6O_5S \cdot 0.5\ H_2O$	55.69	4.87	16.24	6.20
				(55.71	4.78	16.15	6.34)
6b	140–150	75	$C_{25}H_{26}N_6O_6S \cdot 0.5\ H_2O$	54.83	4.97	15.35	5.86
				(54.78	4.80	15.06	5.92)
6c	>116[a]	76	$C_{20}H_{24}N_6O_5S_2 \cdot 0.5\ H_2O$	47.89	5.02	16.76	12.79
				(48.23	4.87	16.77	12.82)
6d	143–150	68	$C_{23}H_{22}N_6O_5S \cdot 0.5\ H_2O$	54.86	4.60	16.69	6.38
				(55.11	4.61	16.46	6.62)
7a	110–120	89	$C_{25}H_{24}N_8O_4S$	56.38	4.54	21.04	6.02
				(56.39	4.59	20.69	5.96)
7b	126–135	78	$C_{26}H_{26}N_8O_5S \cdot 0.5\ H_2O$	54.63	4.76	19.60	5.61
				(54.80	4.61	19.38	5.74)
7c	88–110	86	$C_{21}H_{24}N_8O_4S_2$	48.82	4.68	21.69	12.41
				(48.79	4.88	21.30	12.14)
7d	180–181	82	$C_{24}H_{22}N_8O_4S$	55.59	4.28	21.61	6.18
				(55.46	4.52	21.32	6.02)
7e	163–164	87	$C_{18}H_{18}N_8O_4S$	48.86	4.10	25.33	7.25
				(48.72	4.05	25.65	7.29)
7f	113–118	77	$C_{19}H_{20}N_8O_4S \cdot 0.5\ H_2O$	49.02	4.55	24.08	6.89
				(49.19	4.57	23.80	6.96)

[a]Gradually decomposes.

General procedure for preparation of the (aminoacyl)amino nucleosides (**9a–f**) *(adenine series).* — A mixture of **7** (5 mmol) and 2-mercaptobenzothiazole (10 mmol) in 10:10:1 ethanol–pyridine–acetic acid (21 mL) was stirred overnight at room temperature. The resulting precipitate was filtered off, and the filtrate was concentrated *in vacuo* below 30°. The residue was purified by chromatography on a column of silica gel, using 17:3 chloroform–methanol, to give a homogeneous glass which was converted into the oxalate if necessary. In the purification of **9e** and **9f** by chromatography, elution was performed with 3:1 chloroform–methanol. The results are given in Tables I, II, and IV.

Biological test systems. — *Anti-L-1210 activity.* BDF_1 mice were inoculated intraperitoneally (i.p.) with 1×10^5 L-1210 cells. A solution of the test substance in 0.9% aqueous sodium chloride was administered i.p., treatment being started 24 h after the leukemic-cell inoculation and continued daily for 5 days. The daily dose employed in i.p. administration was 100 mg/kg. Antitumor activity was calculated by comparing the mean survival-time of the treated animals to that of control animals.

Anti-Sarcoma 180 (solid tumor) activity. ICR strain mice were inoculated subcutaneously with 2×10^6 tumor cells. A solution of the test substance in 0.9% aqueous sodium chloride was administered intraperitoneally. Treatment was started 24 h after inoculation with the Sarcoma 180 cells and continued for 7 days in test groups of 5 animals. The daily dose employed was 100 mg/kg. On the 10th day after tumor inoculation, the average weight of the tumors was compared with that of those in a control group.

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