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SYNTHESIS OF URACIL AND THYMINE NUCLEOSIDES OF UNSATURATED 5-(AMINOACYL)AMINOPENTOFURANOSES

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ABSTRACT

Improved syntheses of 1-(2,3-dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-uracil (**5a**) and -thymine (**5b**) have been achieved *via* treatment of the corresponding 3′,5′-oxetane with sodium hydroxide in hexamethylphosphoric triamide. The 2′,3′-unsaturated nucleosides (**5a** and **5b**) were converted into 1-(5-amino-2,3,5-trideoxy-β-D-*glycero*-pent-2-enofuranosyl)-uracil (**8a**) and -thymine (**8b**), respectively. A new type of aminoacyl nucleoside, the 1-[5-(aminoacyl)amino-2,3,5-trideoxy-β-D-*glycero*-pent-2-enofuranosyl)-uracils and -thymines, has been obtained by condensation of **8a** and **8b** with the active esters of several amino acid derivatives followed by deprotection. These nucleosides were examined for *in vivo* antitumor activity against Sarcoma 180 (solid tumor). However, none of the compounds exhibited significant antitumor activity.

INTRODUCTION

Such nucleoside antibiotics as puromycin[1], gougerotin[2], polyoxins[3], and blasticidin S (ref. 4) contain amino sugar residues in their molecules, and the amino group is linked to at least one amino acid by a peptide bond. Among these antibiotics, the unique structure of blasticidin S, which has an endocyclic double bond at C-2 and C-3 of the sugar residue, is of interest in connection with the significant biological roles of 2,3-unsaturated sugars in metabolic pathways. As it has been reported[5] that these antibiotics exhibit antitumor, antibacterial, or other important biological activities, considerable effort has been devoted to the synthesis of various aminonucleosides[6–25]. Some of these synthetic aminonucleosides exhibit significant biological activities. 5′-Amino-5′-deoxythymidine[8] is of especially great interest, because it has been shown to possess strong antiviral potency and activity *vs.* the thymidine kinases from Walker 256 carcinoma and mouse ascites Sarcoma 180. As

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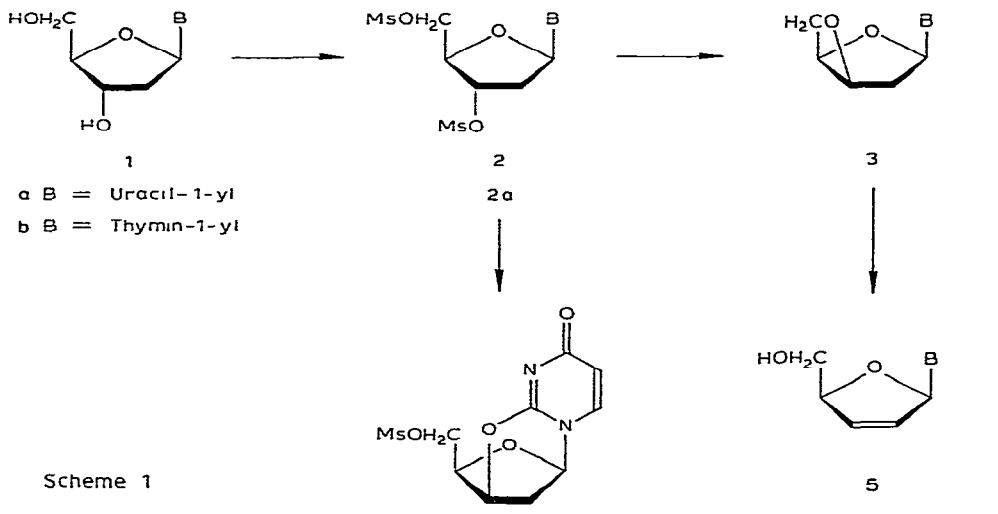
part of a program involving the design of nucleoside analogs having chemotherapeutic activities, it seemed feasible that the hybrid molecule consisting of a residue of blasticidin S and a residue of a pyrimidine nucleoside of a 5-amino-2,5-dideoxy sugar should provide a new class of biologically active compounds.

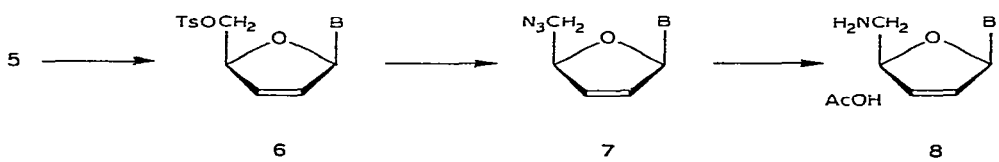
RESULTS AND DISCUSSION

We now describe the synthesis of representatives of a new type of unsaturated aminoacyl nucleoside, namely, 1-[5-(aminoacyl)amino-2,3,5-trideoxy-β-D-*glycero*-pent-2-enofuranosyl]-uracils (**12a–d**) and -thymines (**13a–d**), by condensation of 5′-amino-2′,3′-unsaturated nucleosides (**8a** and **8b**) with several amino acid derivatives (**9a–d**).

For the synthesis of **8a** and **8b**, the 2′,3′-unsaturated nucleosides **5a** and **5b** were chosen as intermediates (see Schemes 1 and 2). A few synthetic routes leading to **5a** and **5b** have been described[26–28]. Our initial study of the preparation of **5a** and **5b** was conducted according to the method of Horwitz and co-workers[26], with



Scheme 1



a B = Uracil-1-yl
b B = Thymin-1-yl

Scheme 2

certain modifications. The method involves two base-catalyzed reactions: conversion of the 3′,5′-dimesylate **2** into **3** by treatment with aqueous sodium hydroxide, and reaction of **3** with potassium *tert*-butoxide in dimethyl sulfoxide to give **5**. Although this set of reactions provides a convenient method for small-scale preparations, simplification and improvement of the method were desirable for large-scale preparations.

It was found that **3a** could be readily obtained in good yield *via* direct treatment of the crude reaction-mixture containing **2a** with aqueous sodium hydroxide at pH 12. The pH of the reaction mixture seemed to be critical for formation of the oxetane. Thus, treatment at pH 9.0 afforded 2,3′-anhydro-[2′-deoxy-5′-*O*-(methylsulfonyl)-uridine] (**4**), a compound proposed by Horwitz *et al.*[26] as an intermediate in the formation of **3a**. The last step of the reactions occasionally led to a complication: decomposition of the product during evaporation of the solvent (dimethyl sulfoxide). Therefore, isolation of **5a** was achieved by chromatography on a column of silica gel, and the yield decreased to 40–60%.

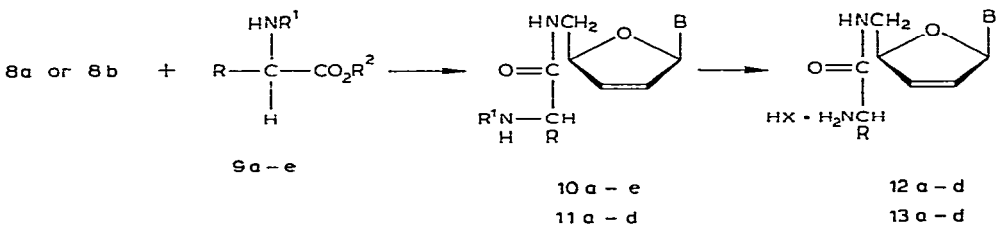
It is well known that hexamethylphosphoric triamide serves as an excellent solvent for nucleophilic substitution-reactions, and that it may be readily removed from an aqueous solution by extraction with chloroform (due to formation of a complex with chloroform). Accordingly, it seemed possible that hexamethylphosphoric triamide might serve as a substitute for dimethyl sulfoxide. Preliminary reactions were attempted in hexamethylphosphoric triamide by treating **3a** with various bases, such as sodium hydroxide, potassium hydroxide, sodium hydride, sodamide, and 1,8-diazabicyclo[5.4.0]-7-undecene (DBU), and it was found that, except for DBU, each of the bases promoted the reaction in this solvent. Thus, **5** could be prepared in high yield by the reaction of **3** with sodium hydroxide (3 molar equiv.) in hexamethylphosphoramide for 3 h at 70–80° without chromatographic purification.

Tosylation of **5a** was performed with *p*-toluenesulfonyl chloride in pyridine at 5°, to give 1-(2,3-dideoxy-5-*O*-*p* tolylsulfonyl-β-D-*glycero*-pent-2-enofuranosyl)uracil (**6a**) in 85% yield. Subsequent treatment of **6a** with sodium azide in *N*,*N*-dimethylformamide for 3 h at 70–80° afforded the 5′-azide **7a** in 84% yield. Its i.r. spectrum showed a characteristic azide band at 2100 cm^{-1}. The corresponding thymidine derivatives could be obtained analogously. Selective reduction of the azides **7a** and **7b** was first attempted by using sodium borohydride; however, the amines **8a** and **8b** were obtained in rather low yield by chromatographic purification on a column of charcoal. The selective reduction of **7a** and **7b** to the corresponding amines **8a** and **8b** was achieved in high yield by use of hydrogen sulfide in aqueous pyridine[29].

Next, introduction of aminoacyl groups onto the 5′-amino group of the nucleoside was studied. This involved two important problems: choice of (*a*) a method of coupling between the 5′-amino group and the *N*-protected amino acid, and (*b*) the *N*-protecting group of the amino acids. Either the active-ester or the *N*,*N*′-dicyclohexylcarbodiimide (DCC) method has usually been employed for the preparation of nucleoside peptides. The *tert*-butoxycarbonyl (Boc) or the benzyloxycarbonyl (Z) group has been widely used as an *N*-protecting group of amino acids. However, it

seemed probable that the benzyloxycarbonyl group would be unsuitable for *N*-protection in the present work, because the products (**8**) have an olefinic bond which would presumably be saturated upon later hydrogenolysis of the Z group. In addition, the lability of the glycosyl bond to strong acid precluded the use of protection by the Z group. Therefore, Boc protection was first tested in the synthesis of **12a**. Coupling of **8a** with the *p*-nitrophenyl ester of *N*-(*tert*-butoxycarbonyl)-L-phenylalanine (**9e**) was attempted in *N*,*N*-dimethylformamide at room temperature in the presence of triethylamine, to give 1-{5-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]amino-2,3,5-trideoxy-β-D-*glycero*-pent-2-enofuranosyl}uracil (**10e**) in 82% yield. The structure was determined by elemental analysis and by n.m.r. spectroscopy. The n.m.r. spectrum showed characteristic signals for both the aminoacyl group and the nucleoside residue (see Experimental section). Deprotection of **10e** was attempted by using various acids (hydrogen chloride, hydrogen fluoride, formic acid, and trifluoroacetic acid), but 3M hydrochloric acid in aqueous 1,4-dioxane or methanol seemed the most appropriate for the purpose. Treatment of **10e** with 3M hydrochloric acid in 1:1 water–1,4-dioxane at room temperature, followed by neutralization with Amberlite IR-45 anion-exchange resin, gave the desired nucleoside, namely, 1-[2,3,5-trideoxy-5-(L-phenylalanyl)amino-β-D-*glycero*-pent-2-enofuranosyl]uracil (**12b**) as an amorphous powder contaminated with small proportions of impurities. Attempts at purification by chromatography and reprecipitation were unsuccessful.

Therefore, protection by the (*o*-nitrophenyl)sulfenyl (Nps) group was attempted as an alternative. Coupling of **8a** with the *N*-hydroxysuccinimide ester of *N*-(*o*-nitrophenyl)sulfenyl-L-phenylalanine (**9b**) in *N*,*N*-dimethylformamide at room temper-



9–13	*R*	R^1	R^2
a	H	Nps[a]	Su[b]
b	$PhCH_2$	Nps	Su
c	$MeS(CH_2)_2$	Nps	Np[c]
d	$NpsNH(CH_2)_4$	Nps	Su
e	$PhCH_2$	Boc[d]	Np

a Nps = (*o*-Nitrophenyl)sulfenyl
b Su = Succinimido.
c Np = *p*-Nitrophenyl
d Boc = *tert*-Butoxycarbonyl

Scheme 3

TABLE I

YIELD AND COMPOSITION OF (AMINOACYL)AMINONUCLEOSIDES (**12a–d** AND **13a–d**)

Compound	*B*[a]	*R*	*Formula*	*Yield (%)*	*Analysis*					
					Calc			*Found*		
					C	*H*	*N*	*C*	*H*	*N*
12a	U	H	$C_{11}H_{11}N_4O_4 \cdot AcOH \cdot 2/3\ H_2O$	84	46.15	5.75	16.56	45.88	5.89	16.21
12b	U	$PhCH_2$	$C_{18}H_{20}N_4O_4 \cdot H_2O$	91	57.74	5.92	14.97	58.00	5.69	14.48[b]
12c	U	$MeS(CH_2)_2$	$C_{14}H_{20}N_4O_4S \cdot AcOH \cdot 1/2\ H_2O$	77	46.92	6.15	13.68	46.96	6.04	13.78
12d	U	$H_2N(CH_2)_4$	$C_{15}H_{23}N_5O_4 \cdot 2\ AcOH \cdot H_2O$	83	47.99	6.99	14.72	47.81	7.51[b]	13.94[b]
13a	T	H	$C_{12}H_{16}N_4O_4 \cdot HCl \cdot H_2O$	59	43.04	5.83	16.74	42.93	5.68	16.45
13b	T	$PhCH_2$	$C_{19}H_{21}N_4O_4 \cdot HCl \cdot 3/2\ H_2O$	77	52.71	5.82	12.94	52.33	5.72	12.62
13c	T	$MeS(CH_2)_2$	$C_{15}H_{22}N_4O_4S \cdot HCl \cdot 1/2\ H_2O$	63	44.93	6.28	13.97	44.76	6.09	13.69
13d	T	$H_2N(CH_2)_4$	$C_{16}H_{25}N_5O_4 \cdot 2\ HCl \cdot H_2O$	50	43.44	6.61	15.83	43.36	6.52	15.53

[a]U = uracil-1-yl; T = thymin-1-yl. [b]A very hygroscopic compound for which better analyses could not be obtained.

TABLE II

PHYSICAL CONSTANTS OF COMPOUNDS **12a–d** AND **13a–d**

Compound	*M.p. (degrees)*	$\lambda_{max}^{H_2O}$ *in nm* (ε_{mM})	$[\alpha]_D^{25}$ *(degrees)*[a]
12a	147–149	261 5 (10.65)	−86
12b	64–85	261.5 (8.46)	−71
12c	—	262 (9 76)	−17
12d	—	261.5 (9 00)	− 7 5
13a	194	266 (9.47)	−19.5
13b	160–163	267 (11 075)	+16.5
13c	115–140	266 (9.59)	+20 5
13d	181–183	266 (8 85)	−52 5

[a]At c = 1, in water.

ature afforded 1-{2,3,5-trideoxy-5-[(*N*-*o*-nitrophenylsulfenyl)-L-phenylalanyl]amino-β-D-*glycero*-pent-2-enofuranosyl}uracil (**10b**) in high yield. Analogous reactions of **8a**, with *N*-hydroxysuccinimide esters of (*o*-nitrophenylsulfenyl)glycine (**9a**) and N^1,N^6-di-(*o*-nitrophenylsulfenyl)-L-lysine (**9d**), respectively, gave the corresponding nucleosides in fairly good yields. In the case of L-methionine, the *p*-nitrophenyl ester of (*o*-nitrophenylsulfenyl)-L-methionine (**9c**) was employed as the active ester. Except for the glycine derivative (**10a**), the products were obtained as yellow, amorphous solids that had no sharp melting-point. However, the structures could be convincingly determined by n.m.r. spectroscopy. Treatment of **10b** with two molar equiv. of 2-mercaptobenzothiazole (MBT) in 2:1 ethanol–acetic acid for 1.5 h at room temperature gave, after chromatography on silica gel, the desired product **12b** in 91% yield as an amorphous material. Structural elucidation of the deprotected nucleosides **12a–d** was achieved by n.m.r. spectroscopy and elemental analysis (see Tables I–III).

In the synthesis of the thymine series, the foregoing set of reactions was applied. Thus, condensation of **8b** with each of the active esters **9a–d** gave the corresponding derivatives **11a–d** in fairly good yield. Subsequent deprotection of **11a–d** was conducted with MBT in 2:1 ethanol–acetic acid at room temperature, to afford the corresponding aminoacyl nucleosides **13a–d** as acetates that were very hygroscopic, amorphous materials. The homogeneity of each was indicated by thin-layer chromatography; however, correct analytical data could not be obtained. Accordingly, the acetic salts were converted into the corresponding hydrochloric salts by treatment with Diaion SA-11B (Cl^-) ion-exchange resin. These salts, also, did not crystallize, but were obtained as chromatographically pure foams by lyophilization; their hygroscopicity was generally less than that of the acetic salts, and acceptable values of elemental analyses were obtained. The structures were confirmed by the n.m.r. spectra of these salts.

The nucleoside derivatives (**12a–d** and **13a–d**) were examined *in vivo* for anti-

TABLE III

N.M.R.-SPECTRAL DATA FOR COMPOUNDS **12a–d** AND **13a–d**

Compound	*Solvent*	*Chemical shifts*[a]					
		H-1'	*H-2'*	*H-3'*	*H-4'*	*H-6*	*Others*
12a	Me_2SO-d_6	6 85(m)	6.48(m)	6.0(m)	4.88(m)	7.47(d)	1.89 (s, Ac), 3.22 (s, CH_2CO) 3.42 (m, H-5'), 5.7 (d, *J* 7.8 Hz, H-5), 8.15 (m, NHCO)
12b	D_2O	6 75(m)	6.25(m)	5.85(m)		7 42(d)	3.0 (d, *J* 6.6 Hz, CH_2Ph), 3.32 (d, *J* 4 8 Hz, H-5'), 3 8 (m, CH), 5.76 (d, *J* 7.8 Hz, H-5), 7.27 (s, Ph)
12c	Me_2SO-d_6	6 81(m)	6.42(m)	5.98(m)	4.85(m)	7 43(d)	1.91 (s, Ac), 2.05 (s, MeS), 3 35 (m, H-5' +CH), 5.68 (d, *J* 7 8 Hz, H-5), 8.12 (m, NHCO)
12d	Me_2SO-d_6	6 8(m)	6 45(m)	6 0(m)		7 45(d)	1.45 [m, $(CH_2)_3$], 1.82 (s, Ac), 3 35 (m, H-5' +CH), 5.67 (d, *J* 7.8 Hz, H-5), 8.2 (m, NHCO)
13a	Me_2SO-d_6	6 78(m)	6.48(m)	5.95(m)	4 8(m)	7.28(s)	1.82 (s, Me-5), 3.4 (m, H-5') 3.51 (s, CH_2), 8 77 (*m*, NHCO)
13b	Me_2SO-d_6	6 78(m)	6.38(m)	5.82(m)	4.71(m)	7.22(s)	1.78 (s, Me-5), 4.05 (m, CH) 7.27 (s, Ph), 8.46 (m, N^+H_3), 8.95 (m, NHCO), 11.35 (s, H-3)
13c	Me_2SO-d_6–D_2O	6 85(m)	6.52(m)	6.02(m)	4.9(m)	7.34(br s)	1.89 (s, Me-5), 2.1 (s, MeS) 3.48 (m, H-5'), 3.88 (m, CH)
13d	Me_2SO-d_6–D_2O	6 88(m)	6.6(m)	6.1(m)	5 0(m)	7.45(br s)	1.65 [m, $(CH_2)_3$], 1.95 (s, Me-5), 2.98 (m, CH_2N), 3.55 (m, H-5'), 3.9 (m, CH)

[a]In p.p.m. from an internal standard of tetramethylsilane.

tumor activity against Sarcoma 180 (solid tumor) in ICR female mice. Test compounds in normal saline containing 0.5% of (carboxymethyl)cellulose were administered intraperitoneally (100 mg/kg/day for 5 days). The results indicated that none of the compounds exhibited significant antitumor activity against this tumor.

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, and 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, and i.r. spectra, with a Shimadzu IR-27G spectrometer. Optical rotations were measured with a JASCO DIP-4 automatic polarimeter.

2,3'-Anhydro-[2'-deoxy-5'-O-(methylsulfonyl)uridine] (**4**). — Reaction of **1a** (2.5 g, 11 mmol) with methanesulfonyl chloride (3.78 g, 33 mmol) in pyridine was conducted according to the method of Michelson and Todd[30]. The resulting mixture was poured into ice–water (300 mL), and the pH was adjusted to 9.0 by adding M sodium hydroxide. The mixture was then boiled under reflux for 2 h, cooled, brought to pH 6.0 with acetic acid, and evaporated *in vacuo*. The residue was crystallized from water (10 mL) to give 1.7 g (54%) of **4**, m.p. 180–182° (dec.). Recrystallization from 2:1 ethanol–water gave an analytically pure sample having the same melting point; $\lambda_{max}^{pH\ 6\ 8}$ 230, 245, 267 (sh) nm; n.m.r. data (Me_2SO-d_6): δ 3.2 (s, Me), 3.3 (s, H-5'), 4.1–4.7 (m, H-2',4'), 5.39 (m, H-3'), 5.78 (d, $J_{5,6}$ 8 Hz, H-5), 5.98 (br s, H-1'), and 7.68 (d, $J_{5,6}$ 8 Hz, H-6).

Anal. Calc. for $C_{10}H_{12}N_2O_6S$ (288.3): C, 41.66; H, 4.20; N, 9.72; S, 11.12. Found: C, 41.51; H, 4.32; N, 9.70; S, 11.12.

*1-(2,3-Dideoxy-β-*D*-glycero-pent-2-enofuranosyl)uracil* (**5a**). — To a solution of **3a** (6.94 g, 33 mmol) in hexamethylphosphoric triamide (69 mL) was added pulverized sodium hydroxide (3.96 g, 99 mmol) at room temperature, and the mixture was heated for 3.5 h at 70–80°. The solution was cooled, poured into water (500 mL), and the mixture extracted with chloroform (3 × 150 mL) to remove hexamethylphosphoric triamide. The pH of the aqueous layer was then adjusted to 6 with 5% hydrochloric acid, and the solution was evaporated to dryness *in vacuo*. The residue was extracted with hot acetone (3 × 500 mL), and the extract was evaporated, to give 6.2 g (89%) of **5a**, m.p. 160–162°, which was identical with an authentic sample[26].

*1-(2,3-Dideoxy-β-*D*-glycero-pent-2-enofuranosyl)thymine* (**5b**). — Similar treatment of **3b** (22.84 g, 102 mmol) with sodium hydroxide (12.24 g, 306 mmol) in hexamethylphosphoric triamide (228 mL) and processing as for **5a**, gave 19.3 g (84%) of **5b**, m.p. 168–170°, identical with an authentic sample[26].

*1-(2,3-Dideoxy-5-O-*p*-tolylsulfonyl-β-*D*-glycero-pent-2-enofuranosyl)uracil* (**6a**). — To a solution of **5a** (10.8 g, 51.4 mmol) in pyridine (108 mL) was added, in portions, *p*-toluenesulfonyl chloride (19.58 g, 103 mmol) at 5°, and the mixture was kept overnight at the same temperature. The resulting solution was then poured into ice–

water (1 L). The crystals that separated were collected by filtration, washed with 2-propanol, and dried, to give 15.82 g (85%) of **6a**, m.p. 168–169°; λ_{max}^{EtOH} 261.5 (ε_{mM} 9.76) and 224 nm (14.9); ν_{max}^{Nujol} 3180, 3050, 1700, 1630, and 1595 cm^{-1}; n.m.r. data (Me_2SO-d_6): δ 2.42 (s, Me), 4.21 (m, H-5′), 4.99 (m, H-4′), 5.46 (d, $J_{5',6}$ 8 Hz, H-5), 6.02 (m, H-3′), 6.38 (m, H-2′), 6.78 (m, H-1′), 7.27 (d, $J_{5,6}$ 8 Hz, H-6), 7.42 and 7.75 (d, J 8 Hz, arom. H), and 11.47 (br s, NH).

Anal. Calc. for $C_{16}H_{16}N_2O_6S$: C, 52.75; H, 4.43; N, 7.69. Found: C, 52 51; H, 4.57; N, 7.73.

*1-(2,3-Dideoxy-5-*O*-*p*-tolylsulfonyl-β-*D*-*glycero*-pent-2-enofuranosyl)thymine* (**6b**). — Analogous reaction of **5b** (31.6 g, 141 mmol) with *p*-toluenesulfonyl chloride (53.7 g, 282 mmol) in pyridine (316 mL) gave, after processing and recrystallization from ethanol, 48.5 g (90%) of **6b**, m.p. 138° (dec.) [lit.[31] m.p. 111–113° (dec.)]; λ_{max}^{EtOH} 264 and 271 (sh) nm; n.m.r. data (Me_2SO-d_6): δ 1.78 (s, Me-5), 2.42 (s, Me-Ph), 4.22 (d, $J_{4',5'}$ 3 Hz, H-5′), 5.0 (m, H-4′), 6.05 (m, H-3′), 6.38 (m, H-2′), 6.82 (m, H-1′), 7.22 (br s, H-6), 7.45 and 7.77 (d, J 8 Hz, arom. H), and 11.38 (br s, NH).

Anal. Calc. for $C_{17}H_{18}N_2O_6S$: C, 53.97; H, 4.80, N, 7.41; S, 8.48. Found: C, 53.49; H, 4.83; N, 7.23; S, 8.31.

*1-(5-Azido-2,3,5-trideoxy-β-*D*-glycero-pent-2-enofuranosyl)uracil* (**7a**). — A mixture of **6a** (5.1 g, 14 mmol) and sodium azide (1.28 g, 19.7 mmol) in *N,N*-dimethylformamide (225 mL) was stirred for 3 h at 70–80°, cooled, and evaporated to dryness *in vacuo*. The residue was extracted with hot acetone (400 mL), the extract was evaporated to dryness, and the residue was crystallized from 1:1 2-propanol–diisopropyl ether, to afford 2.9 g (88%) of **7a**, m.p. 132–133°. Recrystallization from ethanol gave an analytical sample, m.p. 136–137°; λ_{max}^{EtOH} 260 nm (ε_{mM} 9.42); ν_{max}^{Nujol} 3150–3050, 2100, 1706, and 1685 cm^{-1}; n.m r. data ($CDCl_3$, Me_2SO-d_6): δ 3.6 (m, H-5′), 5.0 (m, H-4′), 5.66 (d, $J_{5,6}$ 8 Hz, H-5), 5.95 (m, H-3′), 6.37 (m, H-2′), 6.98 (m, H-1′), 7.51 (d, $J_{5,6}$ 8 Hz, H-6), and 8.0–9 0 (br, NH).

Anal. Calc. for $C_9H_9N_5O_3$: C, 45.96; H, 3.86; N, 29.78. Found: C, 45.99; H, 3.96; N, 29.69.

*1-(5-Azido-2,3,5-trideoxy-β-*D*-glycero-pent-2-enofuranosyl)thymine* (**7b**). — A mixture of **6b** (45 g, 119 mmol) and sodium azide (10.9 g, 168 mmol) in *N,N*-dimethylformamide (225 mL) was stirred for 3 h at 70–80°, and evaporated *in vacuo*. The residue was slowly poured into water (1.5 L), and the resulting precipitate was collected by filtration, washed with water, and dried, to give 24.2 g (82%) of **7b**, m.p. 167–168° (dec.). Recrystallization from methanol gave an analytical sample, m.p. 169–170° (dec.); λ_{max}^{MeOH} 265 nm (ε_{mM} 8.72); n.m.r. data (Me_2SO-d_6): δ 1.78 (s, Me-5), 3.59 (d, $J_{4',5'}$ 3 Hz, H-5′), 4.95 (m, H-4′), 6.02 (m, H-3′), 6.4 (m, H-2′), 6.82 (m, H-1′), 7.35 (br s, H-6), and 11 33 (br. NH).

Anal. Calc. for $C_{10}H_{11}N_5O_3$: C, 48.19; H, 4.45; N, 28.10. Found: C, 48.24; H, 4.50; N, 28.09.

*Synthesis of 1-(5-amino-2,3,5-trideoxy-β-*D*-glycero-pent-2-enofuranosyl)-uracil* (**8a**) *and -thymine* (**8b**). — Selective reduction of the unsaturated nucleoside azides (**7a,b**) to the corresponding amines (**8a,b**) was conducted as previously reported[29].

1-{5-[N-(tert-Butoxycarbonyl)-L-phenylalanyl]amino-2,3,5-trideoxy-β-D-glycero-pent-2-enofuranosyl}uracil (**10e**). — To a solution of **8a** (404 mg, 1.5 mmol) and triethylamine (152 mg, 1.5 mmol) in *N,N*-dimethylformamide (3 mL) was added **9e** (638 mg, 1.65 mmol) at 5°, and the mixture was stirred for 4 h at the same temperature. The solution was then poured into ethyl acetate (50 mL), and the ethyl acetate solution was successively washed with 1% hydrochloric acid and a 4% aqueous solution of sodium hydrogencarbonate, dried (magnesium sulfate), and concentrated to ~5 mL, giving 565 mg (82%) of **10e**, m.p. 182–184° (dec.). Recrystallization from ethyl acetate afforded colorless prisms having the same melting point: ν_{max}^{Nujol} 3390, 3175, 1715, 1705 (sh), 1700, 1685, 1650, and 1550 cm^{-1}; n.m.r. data (Me_2SO-d_6): δ 1.32 (s, Me_3C), 2.9 (m, H-5'), 3.33 (m, CH_2Ph), 4.15 (m, CH-NH), 4.75 (m, H-4'), 5.65 (d, $J_{5,6}$ 8 Hz, H-5), 5.96 (m, H-3'), 6.28 (m, H-2'), 6.8 (m, H-1' + Boc-NH), 7.27 (s, Ph), 7.37 (d, $J_{5,6}$ 8 Hz, H-6), 8.1 (m, CH_2NH), and 11.36 (br s, H-3).

Anal. Calc. for $C_{23}H_{28}N_4O_6$: C, 60.51; H, 6.18; N, 12.27. Found: C, 60.41; H, 6.22; N, 12.03.

p-*Nitrophenyl ester of* N-(o-*nitrophenylsulfenyl*)-L-*methionine* (**9c**). — To a solution of *N*-(*o*-nitrophenylsulfenyl)-L-methionine (1.74 g, 5.8 mmol) and *p*-nitrophenol (0.81 g, 5.8 mmol) in ethyl acetate (40 mL) was added *N,N'*-dicyclohexylcarbodiimide (1.2 g, 5.8 mmol) at ~5°, and the mixture was stirred overnight at room temperature. The resulting precipitate was removed by filtration, and the filtrate was successively washed with a 4% aqueous solution of sodium hydrogencarbonate, water, and saline, dried, and evaporated *in vacuo*; the residue was triturated with ethanol–diisopropyl ether, to provide 1.77 g (72%) of **9c** as yellow crystals, m.p. 80–82°. Recrystallization from ethyl acetate–petroleum ether afforded an analytical sample, m.p. 82–84°: $[\alpha]_D^{25}$ −122° (*c* 1.0, *N,N*-dimethylformamide); ν_{max}^{Nujol} 3340, 1765, 1758, 1611, 1587, 1563, 1525, 1342, and 1328 cm^{-1}; n.m.r. data ($CDCl_3$): δ 2.2 (s, Me), 2.38 (m, $CHCH_2$), 2.85 (m, SCH_2), 3.53 (deformed d, NH), 4.02 (m, CH), and 7.25–8.5 (m, arom. H).

Anal. Calc. for $C_{17}H_{17}N_3O_6S_2$: C, 48.23; H, 4.05; N, 9.93; S, 15.14. Found: C, 48.04; H, 4.11; N, 9.77; S, 15.02.

N-*Hydroxysuccinimide ester of* N^1,N^6-*di*-(o-*nitrophenylsulfenyl*)-L-*lysine* (**9d**). — To a solution of N^1,N^6-di-(*o*-nitrophenylsulfenyl)-L-lysine (2.71 g, 6 mmol) and *N*-hydroxysuccinimide (0.81 g, 7 mmol) in tetrahydrofuran (20 mL) was added *N,N'*-dicyclohexylcarbodiimide (1.44 g, 7 mmol) at ~5°, and the mixture was stirred for 4 h. The solution was then evaporated *in vacuo* below 25°, and ethyl acetate (20 mL) was added to the residue. The insoluble substances were removed by filtration. The filtrate was successively washed with a 4% aqueous solution of sodium hydrogencarbonate and water, dried (sodium sulfate), and evaporated, to give 3.38 g of **9d** as an orange paste. Its i.r. spectrum showed absorption bands at 1815, 1785, and 1741 cm^{-1}, indicative of the carbonyl bonds of an active ester. This compound was used for the next step without further purification.

General procedure for the preparation of the protected aminoacylamino nucleosides (**10a–d** *and* **11a–d**). — To a suspension of 5 mmol of the amino nucleoside **8**

TABLE IV

PROTECTED AMINOACYLAMINO NUCLEOSIDES (**10a–d** AND **11a–d**)

Compound	*Method of purification*	*M p (degrees)*	*Yield (%)*
10a	A[a]	179–180	81
10b	B[b]	100–110	80
10c	B	80–82	78
10d	B	70–90	70
11a	C[c]	161–162	74
11b	B	135–140	67
11c	B	190–193	67
11d	B	105–115	80

[a]A, Recrystallization from methanol–*N*,*N*-dimethylformamide [b]B, Column chromatography on silica gel. [c]C, Recrystallization from ethyl acetate–*N*,*N*-dimethylformamide

in *N*,*N*-dimethylformamide (10 mL) was added the active ester **9** at ~5°, and the mixture was stirred overnight at room temperature, diluted with ethyl acetate (50 mL), successively washed with a 4% aqueous solution of 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 with 9:1 chloroform–methanol (see Table IV). All of the compounds (**10a–d** and **11a–d**) were chromatographically homogeneous; however, except for **10a**, elemental analyses did not give acceptable results.

General procedure for the preparation of the deprotected aminoacylamino nucleosides (**12a–d** and **13a–d**). — A mixture of 4 mmol of the protected aminoacylamino nucleoside (**10** or **11**) and 8 mmol of 2-mercaptobenzothiazole in 3:1 ethanol–acetic acid (48 mL) was stirred for 2–6 h at room temperature. The resulting precipitate was removed by filtration, and a portion was purified by chromatography on a column of silica gel, to give di-(*o*-nitrophenyl) disulfide and di-(2-benzothiazolyl) disulfide in the ratio of ~1:1.

The filtrate was evaporated *in vacuo* below 35°, followed by repeated co-evaporation with ethanol. To the residue was added water (20 mL), the insoluble material (MBT) was removed by filtration, and the filtrate was washed with benzene to remove MBT completely. The aqueous layer was treated with Norit, and lyophilized, to give the desired product (**12a–d**) (see Tables I–III). In the thymine series, the acetic salts thus obtained were converted into the corresponding hydrochloric salts by use of SA-11B (Cl^-) ion-exchange resin (20 mL), and the eluate was lyophilized, to give **13a–d** (see Tables I–III).

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