N-methyls), and 2.44 ppm (six-line m, 2, CH_2D, $J_{allylic}$ = 0.8, $J_{H,D}$ = 2.3 Hz); uv λ_{max}^{EtOH} 281, 241 mμ (sh).

5-(2-Propynyloxy)uridine (9, R = H).—Propargyl bromide (19.04 g 0.16 mol) was added to a solution of 5-hydroxyuridine[20] (20.8 g 0.08 mol) in 50% aqueous methanol (500 ml) containing sodium hydroxide (3.2 g 0.08 mol). The mixture was stirred at room temperature for 10 hr, by which time most of the starting material had reacted (tlc, CH_2Cl_2–MeOH, 5:1; $FeCl_3$ spray). Removal of solvent and crystallization of the syrupy residue from methanol afforded 14.7 g (61%) of product, mp 152–153°. A single recrystallization gave pure material: mp 155–156°; uv (pH 1) λ_{max} 276, λ_{min} 242 mμ; (pH 12) λ_{max} 275, λ_{min} 252 mμ; nmr (DMSO-d_6–D_2O) δ 7.80 (s, 1, H-6), 5.81 (m, 1, H-1′), 4.67 (d, 2, $-OCH_2-$, J = 2.3 Hz), ~4.27–3.55 (m, HOD + H-2′, 3′, 4′, 5′a, 5′b), and 3.49 ppm (t, 1, CCH_3).

Anal. Calcd for $C_{12}H_{14}N_2O_7$: C, 48.32; H, 4.73; N, 9.39. Found: C, 48.16; H, 4.80; N, 9.46.

Tri-*O*-acetyl-5-(2-propynyloxy)uridine (9, R = $COCH_3$).—Acetylation of **9** (R = H) (1.5 g) in acetic anhydride (5 ml)–pyridine (20 ml) for 1 hr at room temperature, and isolation of the product by the standard chloroform extraction and washing procedure, afforded the tri-*O*-acetate as a chromatographically pure amorphous foam (2.1 g, 89%): nmr ($CDCl_3$) δ 7.43 (s, 1, H-6), 6.20 (m, 1, H-1′), 5.37 (m, 2, H-2′ and H-3′), 4.72 d (1, $-OCH_2-$, J = 2.2 Hz), 4.37 (broad s, 3, H-4′ and H-5′a,b), 2.66 (t, 1, CH), and 2.20, 2.12, and 2.10 (three singlets, 9 protons, acetyls). This compound contained considerable amounts of entrapped chloroform which was not removed on storage at 40° under vacuum for 24 hr. At higher temperatures, darkening and partial rearrangement to **11** took place. The nmr spectrum showed more $CHCl_3$ than did a $CDCl_3$ blank run at the same amplitude; the presence of $CHCl_3$ was confirmed by elemental analysis.

Anal. Calcd for $C_{18}H_{20}N_2O_{10}\cdot 0.13\ CHCl_3$: C, 49.50; H, 4.61; N, 6.37; Cl, 3.29. Found: C, 49.52; H, 4.55; N, 6.13; Cl, 3.29.

1-(β-D-Ribofuranosyl)-6-methylfuro[3,2-*d*]pyrimidine-2,4-[1*H*,3*H*]-dione (10, R = H). Method A.—A solution of **9** (R = H) (1 g) in DMSO (20 ml) was heated at 135° for 1.5 hr. Concentration of the solution to a semisolid (bath 55–60°) and recrystallization from hot water furnished 835 mg (83.5%) of **10** (R = H): mp 246–248°; uv (pH 1) λ_{max} 248, 280, λ_{min} 233, 257; (pH 12) λ_{max} 242 sh, 282; λ_{min} 259 mμ; nmr (DMSO-d_6) δ 10.45 (s 1, NH), 6.95 (broadened s, 1, H-7 allylic coupling not resolved), 5.93 (d, 1, H-1′, $J_{1',2'}$ = 6 Hz), ~5.33–4.90 (m, 3, hydroxyls), ~4.50–4.00 (m, 2, H-2′, H-3′), ~4.00–3.50 (m, 3, H-4′, H-5′a,b), and 2.45 ppm (broadened s, 3, 6-CH_3).

Anal. Calcd for $C_{12}H_{14}N_2O_7$: C, 48.32; H, 4.73; N, 9.39. Found: C, 48.10; H, 4.79; N, 9.19.

Method B.—A solution of **9a** (3 g) in water (75 ml) was refluxed for 3.5 hr (tlc, CH_2Cl_2–MeOH, 5:1). The concentrated solution deposited 2.66 g (88%) of **10** (R = H), mp 246–247°.[21]

1-(Tri-*O*-acetyl-β-D-ribofuranosyl)-6*H*-pyrano[3,2-*d*]pyrimidine-2,4-[1*H*,3*H*]-dione (11).—A solution of **9** (R = $COCH_3$) (400 mg) in toluene (20 ml) was refluxed for 3.5 hr, at which time all the starting material had rearranged as shown by the nmr spectrum of an evaporated sample of the reaction mixture. Removal of solvent afforded **11** as an amorphous, yellow solid. Thick layer chromatography (EtOAc–benzene, 4:1; zone eluted with $CHCl_3$) afforded the analytical sample as a rigid foam: uv (pH ~1) λ_{max}^{EtOH} 341, λ_{min} 276; (pH ~ 12) λ_{max}^{EtOH} 343, λ_{min} 294 mμ; nmr ($CDCl_3$) δ 6.55 (m, 1, H-8, $J_{7,8}$ = 10 Hz, $J_{6,8}$ not fully resolved), 6.10 (double triplet, 1, H-7, $J_{6,7}$ = 4 Hz), 5.87–5.25 (m, 3, H-1′, 2′, 3′), 4.68 (dd, 2, H-6a,b, $J_{6,8}$ ~1 Hz), ~4.50–4.08 (m, 3, H-4′,5′a,b), and 2.09 ppm (s, 9, acetyls). Chloroform entrapped in this compound was not removed during 24 hr at 40° under vacuum. Partial decomposition took place at higher temperatures as reflected by darkening and appearance of a peak at ~450 mμ in the uv spectrum at pH 12. Nmr and elemental analysis indicated chloroform.

Anal. Calcd for $C_{18}H_{20}N_2O_{10}\cdot 0.17CHCl_3$: C, 49.08; H, 4.57; N, 6.30; Cl, 4.06. Found: C, 48.81; H, 4.86; N, 5.90; Cl, 3.93.

Registry No.—**2**, 35042-03-6; **3**, 35042-04-7; **4**, 35042-05-8; **5**, 35042-06-9; **9** (R = H), 35042-07-0; **9** (R = $COCH_3$), 35042-08-1; **10** (R = H), 35042-09-2; **11**, 35042-10-5.

(20) D. W. Visser in "Synthetic Methods in Nucleic Acid Chemistry," Vol. 1, W. W. Zorbach and R. S. Tipson, Ed., Interscience, New York, N. Y., 1968, p 428.

(21) Boiling water is also the solvent of choice for the Claisen rearrangement of 5-allyloxyuridine to 6-allyl-5-hydroxyuridine (>90% yield). The rearrangement in boiling DMF (79% yield) has been reported[2] previously.

Aminoacyl Derivatives of Nucleosides, Nucleotides, and Polynucleotides. XIV. A General Synthesis of Adenosine 2′(3′)-*O*-Peptidyl Derivatives[1]

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Reaction of the adenosine 2′(3′)-*O*-L-phenylalanyl, -L-leucyl, and -L-alanyl derivatives (**1a–c**) with 5-chloro-8-hydroxyquinoline esters of protected amino acids or dipeptide (**2b–e**) affords the protected adenosine 2′(3′)-*O*-peptidyl derivatives (**3b, 3c, 3g, 3e** and **3f**) in good yields. Removal of protecting groups gives 2′(3′)-*O*-L-phenylalanylphenylalanyl, -L-lysylphenylalanyl, -L-phenylalanylleucyl, -L-leucylalanyl, and -L-serylphenylalanylphenylalanyl adenosines (**3i–m**) in excellent yields. Similarly, 5-chloro-8-hydroxyquinoline acetate (**2a**) acylates 2′(3′)-*O*-L-phenylalanyl adenosine (**1a**) and 2′(3′)-*O*-L-leucyladenosine (**1b**) to give 2′(3′)-*O*-(*N*-acetyl-L-phenylalanyl)-adenosine (**3a**) and 2′(3′)-*O*-(*N*-acetyl-L-leucyl)adenosine (**3d**) in high yields. The usefulness of the described acylation reaction for the synthesis of peptidyl or *N*-acylaminoacyl oligoribonucleotides is discussed.

The 2′(3′)-*O*-aminoacyl derivatives of nucleosides and oligonucleotides may be used as suitable substrates in investigation of the mechanism of the transpeptidation process in ribosomal systems.[3,4] The adenosine 2′,3′-*O*-bisaminoacyl derivatives and 2′(3′)-*O*-peptidyl derivatives represent potential substrates for detailed investigations of the formation of the peptide bond on ribosomes.[5]

In the present paper, we report a general synthesis of adenosine 2′(3′)-*O*-peptidyl derivatives starting from 2′(3′)-*O*-aminoacyladenosines[6] as key intermediates. An earlier paper of this series[7] described the preparation of adenosine 2′(3′)-*O*-peptidyl derivatives con-

(1) Part XIII: I. Rychlík, J. Černá, S. Chládek, P. Pulkrábek, and J. Žemlička, *Eur. J. Biochem.*, **16**, 136 (1970).

(2) Present address where correspondence should be sent: Detroit Institute of Cancer Research, Division of the Michigan Cancer Foundation, 4811 John R Street, Detroit, Michigan 48201.

(3) I. Rychlík, S. Chládek, and J. Žemlička, *Biochim. Biophys. Acta*, **138**, 640 (1967).

(4) I. Rychlík, J. Černá, S. Chládek, J. Žemlička, and Z. Haladová, *J. Mol. Biol.*, **43**, 13 (1969).

(5) J. Černá, S. Chládek, I. Rychlík, and J. Žemlička, *Biochim. Biophys. Acta*, **199**, 291 (1970).

(6) S. Chládek, P. Pulkrábek, J. Sonnenbichler, J. Žemlička, and I. Rychlík, *Collect. Czech. Chem. Commun.*, **35**, 2296 (1970).

(7) S. Chládek and J. Žemlička, *ibid.*, **33**, 4299 (1968).

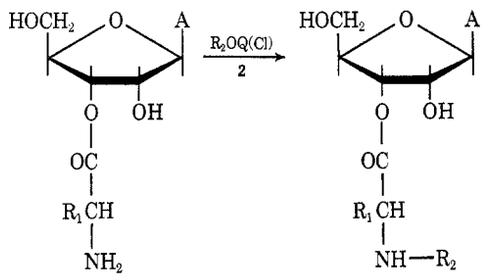
taining glycine as the C-terminal unit with the use of a selective acylation of the ortho ester type intermediates by the action of active esters of protected amino acids.

Later on,[8,9] this method was also applied to the synthesis of analogous dinucleoside phosphate derivatives, and the esters of 5-chloro-8-hydroxyquinoline[10] (chloroxine) were used as selective acylating agents (for a detailed discussion, see ref 8).

The use of chloroxine esters **2** has been now extended to the acylation of various adenosine 2′(3′)-*O*-aminoacyl derivatives **1**. As the starting material, adenosine esters of the amino acids L-phenylalanine, L-leucine, and L-alanine[11] (**1a-c**) were used.

The latter were condensed (see Scheme I) in di-

SCHEME I



1 **3**

A = adenine
Q(Cl) = 5-chloroquinolin-8-yl

1a, $R_1 = C_6H_5CH_2$ **2a**, $R_2 = CH_3CO$
b, $R_1 = (CH_3)_2CHCH_2$ **b**, R_2 = ZPhe
c, $R_1 = CH_3$ **c**, R_2 = ZLys (Z)
d, R_2 = ZLeu
e, R_2 = ZSer(*t*-Bu)Phe

3a, $R_1 = C_6H_5CH_2$; $R_2 = CH_3CO$
b, $R_1 = C_6H_5CH_2$; R_2 = ZPhe
c, $R_1 = C_6H_5CH_2$; R_2 = ZLys (Z)
d, $R_1 = (CH_3)_2CHCH_2$; $R_2 = CH_3CO$
e, $R_1 = (CH_3)_2CHCH_2$; R_2 = ZPhe
f, $R_1 = CH_3$; R_2 = ZLeu
g, $R_1 = C_6H_5CH_2$; R_2 = ZSer(*t*-Bu)Phe
h, $R_1 = C_6H_5CH_2$; R_2 = ZSerPhe
i, $R_1 = C_6H_5CH_2$; R_2 = Phe
j, $R_1 = C_6H_5CH_2$; R_2 = Lys
k, $R_1 = (CH_3)_2CHCH_2$; R_2 = Phe
l, $R_1 = CH_3$; R_2 = Leu
m, $R_1 = C_6H_5CH_2$; R_2 = SerPhe

methylformamide with chloroxine esters of *N*-benzyloxycarbonylamino acids **2b–d** or with chloroxine acetate (**2a**). The reaction mixture was separated by preparative thin layer chromatography on silica gel. The yields of peptidyl and *N*-acetylaminoacyl derivatives **3** are shown in Table I. The following compounds were detected as by-products: the unreacted aminoacyl derivative **1** (or a trace of adenosine due to its decomposition) and small amounts of *N*-acetylaminoacyl

TABLE I
REACTION OF ACTIVE ESTERS **2** WITH ADENOSINE 2′(3′)-*O*-AMINOACYL DERIVATIVES **1**

Acyl-adenosine	Active ester	Product	Yield,[a] %
A-Phe (**1a**)	Ac-OQ(Cl) (**2a**)	A(Ac-Phe) (**3a**)	78
A-Phe (**1a**)	Z-Phe-OQ(Cl) (**2b**)	A(Z-Phe-Phe) (**3b**)	89
A-Phe (**1a**)	Z-Lys(Z)-OQ(Cl) (**2c**)	A(Z-Lys(Z)-Phe) (**3c**)	94
A-Phe (**1a**)	Z-Ser(*t*-Bu)Phe-OQ(Cl) (**2e**)	A(Z-Ser(*t*-Bu)Phe-Phe) (**3g**)	42
A-Leu (**1b**)	Ac-OQ(Cl) (**2a**)	A(Ac-Leu) (**3d**)	70
A-Leu (**1b**)	Z-Phe-OQ(Cl) (**2b**)	A(Z-Phe-Leu) (**3e**)	77
A-Ala (**1c**)	Z-Leu-OQ(Cl) (**2d**)	A(Z-Leu-Ala) (**3f**)	66

[a] The yields were determined by weight.

derivatives (*e.g.*, **3a** accompanying the reaction of compound **1a** with the ester **2b**). Formation of the by-products (*e.g.*, **3a**) may be explained by an exchange reaction[12] between the active esters **2b–e** and the acetate ions (their presence in the reaction mixture is due to dissociation of acetates of bases **1** which represent the virtual starting material; *cf.* ref 6). The extent of this side reaction is negligible with chloroxine esters under conditions used.

By the action of hydrogen bromide in acetic acid, the chloroxine ester of *N*-benzyloxycarbonyl-L-phenylalanine was converted to L-phenylalanine chloroxine ester dihydrobromide.[10] Condensation of the latter with *N*-benzyloxycarbonyl-*O*-*tert*-butyl-L-serine, according to the anhydride method,[13] afforded the required dipeptide ester. In the synthesis of the derivative **3g**, the protected active ester of the dipeptide **2e** was condensed with the aminoacyl derivative **1a**. After the usual isolation, the yield of the protected tripeptide ester was 42%. The above procedure precludes racemization of the phenylalanine unit in the resulting tripeptide ester during aminolysis of the active ester **2e** (*cf.* ref 14).

Lapidot and coworkers[15,16] used *N*-hydroxysuccinimide esters of protected oligopeptides in a recent synthesis of a tRNA oligopeptidyl derivative. Esterification of *N*-hydroxysuccinimide was effected with *N*,*N*′-dicyclohexylcarbodiimide. This procedure, however, does not guarantee the optical purity of the product, since the carboxylic function of the peptide is activated by the action of *N*,*N*′-dicyclohexylcarbodiimide.[14] Similarly, Gottikh, *et al.*,[17,18] have prepared imidazoyl derivatives of protected peptides by the esterification used with *N*,*N*′-carbonyldiimidazol. The activated esters of peptides were used for the acylation of 5′ nucleotides, but again the optical purity of resulting compounds [2′(3′)-*O*-peptidyl-5′ nucleotides] is subject to question.[14]

(8) S. Chládek and J. Žemlička, *Chem. Commun.*, **35**, 89 (1970).
(9) V. Gut, S. Chládek, and J. Žemlička, *ibid.*, **35**, 2398 (1970).
(10) H. D. Jakubke and A. Voigt, *Chem. Ber.*, **99**, 2944 (1966).
(11) All amino acids and their derivatives were of the L configuration. For abbreviations, see ref 6 and 7. The symbol -OQ(Cl) designates an ester of 5-chloro-8-hydroxyquinoline.
(12) J. Žemlička and S. Chládek, *Biochim. Biophys. Acta*, **246**, 487 (1971).
(13) J. R. Vaughn, Jr., *J. Amer. Chem. Soc.*, **73**, 3547 (1951).
(14) F. Weygand, A. Prox, and W. König, *Chem. Ber.*, **99**, 1451 (1966).
(15) Y. Lapidot and N. de Groot, *Biochim. Biophys. Acta*, **179**, 521 (1969).
(16) Y. Lapidot, N. de Groot, and S. Rappoport, *ibid.*, **182**, 105 (1969).
(17) B. P. Gottikh, A. A. Krayevsky, and P. P. Purygin, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 1104 (1970).
(18) P. P. Purygin, A. A. Krayevsky, and B. P. Gottikh, *ibid.*, 1369 (1970).

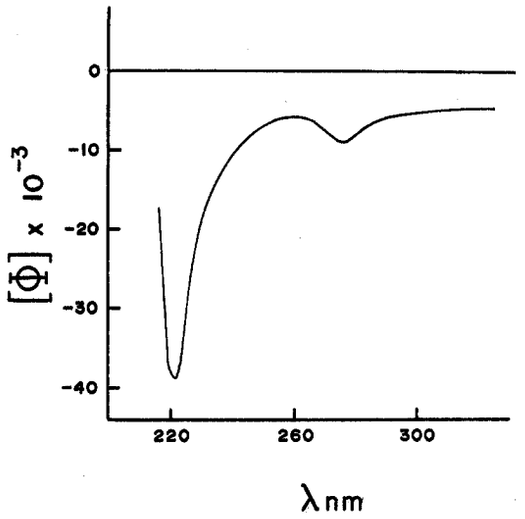


Figure 1.—Optical rotatory dispersion curve of a mixture of products obtained by dissolving compound **3i** in dimethylformamide (methanol).

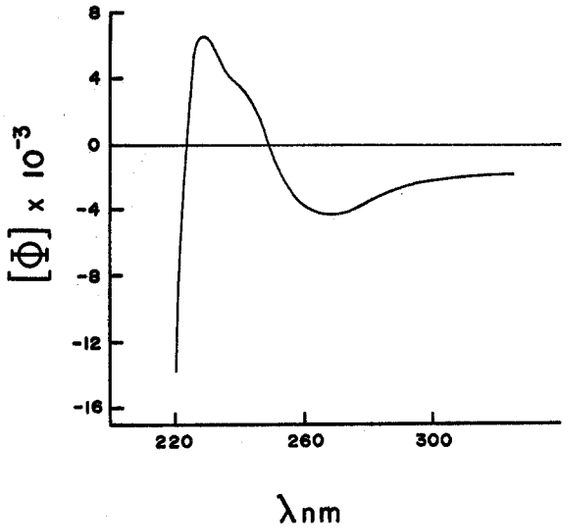


Figure 2.—Optical rotatory dispersion curve of compound **3i** (0.1 *M* HCl).

The protected peptides and the *N*-acetylaminoacyl derivatives **3a–g** were characterized by thin layer chromatography on silica gel and paper chromatography as well as by ultraviolet and infrared spectra when a sufficient amount of material was available. In the case of derivatives **3b**, **3c**, **3e**, and **3f**, the protecting *N*-benzyloxycarbonyl group was removed by the usual procedure of hydrogenolysis over a palladium catalyst in acetic acid as the solvent.[6] In the synthesis of the tripeptidyl derivative **3m**, the *O-tert*-butyl group in the serine unit of compound **3g** was quantitatively removed by the action of trifluoroacetic acid prior to hydrogenolysis. The final products were characterized by paper chromatography, electrophoresis at pH 3.4, alkaline hydrolysis to adenosine, and the corresponding peptide derivative, as well as by ratio of amino acids to adenosine (see Table II).

TABLE II

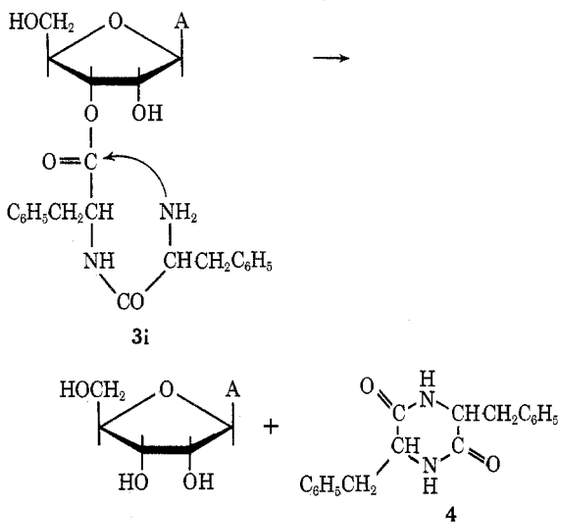
ADENOSINE 2′(3′)-*O*-PEPTIDYL DERIVATIVES **3**

Compd	Yield, %	Ratio of components[a]
A(Phe-Phe) (**3i**)	74	Phe:A = 1.90:1
A(Lys-Phe) (**3j**)	53	Lys:Phe:A = 0.96:1.09:1
A(Phe-Leu) (**3k**)	61	Phe:Leu:A = 0.63:0.87:1
A(Leu-Ala) (**3l**)	88	Leu:Ala:A = 0.90:0.95:1
A(Ser-Phe-Phe) (**3m**)	50	Ser:Phe:A = 1.00:2.36:1

[a] See ref 7.

2′(3′)-*O*-L-Phenylalanyl-L-phenylalanyladenosine (**3i**) rapidly decomposes when dissolved in dimethylformamide even in the absence of a base, in contrast to the 2′(3′)-*O*-aminoacyl derivatives **1** which are relatively stable in dimethylformamide. The dipeptide ester[19] **3i** is readily cyclized to the corresponding 2,5-piperazinedione **4** with the simultaneous removal of adenosine (Scheme II). The optical rotatory dispersion spectrum of the reaction mixture (Figure 1) differs considerably from that of the starting compound (Figure 2). A distinct Cotton effect in the 220-nm region strongly favors the presence of 2,5-piperazinedione **4** in the reaction mixture. A similar Cotton effect of an unusually high amplitude has been observed[20] with 2,5-piperazinedione derived from L-phenylalanine. This observation is in accordance with a report of Lapidot and coworkers,[15,16] who recorded the formation of cyclopeptides during hydrolysis of several tRNA peptidyl derivatives.

SCHEME II



In our opinion, the present procedure is applicable to the synthesis of various oligonucleotide peptidyl derivatives. In this respect, the chloroxine esters fulfill all fundamental requirements; namely, they are selective, do not react with other functions present in the molecule of aminoacyl oligonucleotides (especially with the phospho diester linkage[8,9]), and do not acylate the cytidine amino group in contrast to other active esters.[9] Consequently, the chloroxine esters are highly advantageous from the preparative point of view.

Experimental Section

General Procedures.—All evaporations were carried out *in vacuo* at <35° bath temperature. Some general methods used

(19) It has been well established that aminoacyl and peptidyl derivatives of nucleosides, oligonucleotides, or tRNA behave as active esters; *e.g.*, see H. G. Zachau and H. Feldmann, *Progr. Nucleic Acid Res. Mol. Biol.*, **4**, 217 (1965).

(20) K. Bláha and I. Frič, *Collect. Czech. Chem. Commun.*, **35**, 619 (1970).

in this study were described in an earlier paper.[6] Adenosine aminoacyl derivatives 1a–c were prepared according to ref 6. The active esters 2a–d were prepared by known procedures.[9,10,21] Protected amino acids and peptides were prepared in the Peptide Department of this institute. Descending chromatography was performed on Whatman No. 1 paper in the solvent systems S_1, 2-propanol–concentrated ammonium hydroxide–water (7:1:2); S_2, 1-butanol–acetic acid–water (5:2:3), and S_3, 1-butanol–water–pyridine–acetic acid (90:72:60:18). Thin layer chromatography was performed with aluminum foils precoated with silica gel and starch as binder (Silufol, Kavalier Glassworks, Votice, Czechoslovakia) in the solvent systems S_4, methylene chloride–methanol (95:5), and S_5, methylene chloride–methanol (9:1). Preparative thin layer chromatography employed the method described previously.[6] Spots were detected under ultraviolet light and, whenever possible, by the ninhydrin spray. For R_f values, see Table III. Electrophoresis was performed on Whatman No. 1 paper in 0.05 *M* sodium hydrogen citrate (pH 3.4). For electrophoretic mobilities, see Table IV. Melting points were taken on a heated microscope stage (Kofler block). Infrared spectra (Table V) were measured on a UR-10 spectrophotometer in chloroform at a concentration of 5% or, in the region of hydrogen bonds, of 3×10^{-3} *M*. ORD spectra were recorded on a Jasco ORD/UV apparatus without temperation in 1 mm cells at a concentration of 1 μmol per 1 ml.

TABLE III

R_f VALUES IN PAPER AND THIN LAYER CHROMATOGRAPHY OF STARTING COMPOUNDS, PRODUCTS, AND AUTHENTIC SPECIMENS

Compd	S_1	S_2	S_3	S_4	S_5
A	0.51	0.56	0.55	start	0.08
Phe	0.59	0.70	0.53		
A-Phe (1a)	*a*	0.73			0.09
A(Z-Phe-Phe) (3b)	*a*	0.90		0.26	0.57
A(Ac-Phe) (3a)	*a*	0.80		0.05	0.32
Ac-Phe	0.74				
A(Phe-Phe) (3i)	*a*	0.85			
Phe-Phe	0.79	0.89			
A(Z-Lys(Z)-Phe) (3c)	*a*	0.94			0.65
A(Lys-Phe) (3j)	*a*	0.55	0.49		
Lys-Phe	0.61	0.57	0.39		
Lys	0.32	0.29	0.11		
A(Z-Ser(*t*-Bu)-Phe-Phe) (3g)	*a*	0.92		0.08	0.47
A(Z-Ser-Phe-Phe) (3h)	*a*	0.90		0.03	0.35
A(Ser-Phe-Phe) (3m)	*a*	0.78	0.80		
Ser	0.38	0.29	0.17		
Ser-Phe-Phe	0.72	0.89	0.70		
A-Leu (1b)	*a*	0.68			0.06
Leu	0.62	0.70	0.54		
A(Ac-Leu) (3d)	*a*	0.85			0.35
A(Z-Phe-Leu) (3e)	*a*	0.95			0.20
A(Phe-Leu) (3k)	*a*	0.82			
Phe-Leu	0.82	0.88			
A-Ala (1c)	*a*	0.44			0.04
Ala	0.47	0.41	0.21		
A(Z-Leu-Ala) (3f)	*a*	0.90		0.10	0.20
A(Leu-Ala) (3l)	*a*	0.64			
Leu-Ala	0.67	0.72	0.63		

[a] Decomposition.

Authentic Samples.—L-Phenylalanyl-L-phenylalanine was prepared by removal of the benzyloxycarbonyl group from *N*-benzyloxycarbonyl-L-phenylalanyl-L-phenylalanine[22] with hydrogen bromide in acetic acid. L-Phenylalanyl-L-leucine was obtained according to ref 23. L-Leucyl-L-alanine was prepared by condensation of L-alanine benzyl ester *p*-toluenesulfonate with *N*-benzyloxycarbonyl-L-leucine by the action of *N*,*N*′-dicyclohexylcarbodiimide and the subsequent hydrogenolysis of protecting groups.[7] L-Lysyl-L-phenylalanine was obtained analogously by condensation of N^{α},N^{ϵ}-bisbenzyloxycarbonyl-L-lysine with L-phenylalanine benzyl ester *p*-toluenesulfonate[24] and the subsequent hydrogenolysis. L-Seryl-L-phenylalanyl-L-phenylalanine was obtained by hydrogenolysis of *N*-benzyloxy-L-seryl-L-phenylalanyl-L-phenylalanine (*vide infra*). Amino acid analysis: LysPhe,Lys, Phe = 1.02; SerPhePhe,Ser, Phe = 0.445.

TABLE IV

RELATIVE MOBILITY OF PRODUCTS IN THE PAPER ELECTROPHORESIS (pH 3.4; ADENOSINE MOBILITY, 1.00)

Compd	Mobility	Compd	Mobility
A-Phe (1a)	2.1	Leu	0.71
Phe	0.47	Phe-Leu	1.29
A(Phe-Phe) (3i)	1.30	A-Ala (1c)	2.40
Phe-Phe	0.83	Ala	0.74
A(Lys-Phe) (3j)	3.00	A(Leu-Ala) (3l)	1.80
Lys	4.00	Leu-Ala	1.02
Lys-Phe	2.2	A(Ser-Phe-Phe) (3m)	1.50
A-Leu (1b)	2.1	Ser	0.72
A(Phe-Leu) (3k)	1.37	Ser-Phe-Phe	0.54

N-Benzyloxycarbonyl-*O*-*tert*-butyl-L-seryl-L-phenylalanine 5-Chloro-8-hydroxyquinoline Ester (2e).—A mixture of *N*-benzyloxycarbonyl-*O*-*tert*-butyl-L-serine (3.1 mmol, 0.92 g), *N*-ethyl piperidine (3.1 mmol, 0.43 ml), and chloroform (10 ml) was cooled to −20°, treated with *sec*-butyl chloroformate (3 mmol, 0.4 ml), and then held at 5° for 10 min. The solution was cooled again to −20° and treated with L-phenylalanine 5-chloro-8-hydroxyquinoline ester dihydrobromide[10] (3.1 mmol, 1.52 g), and the resulting mixture was stirred for 1 hr at −20° and for an additional 2 hr at room temperature. The solvent was evaporated under diminished pressure and the residue was dissolved in chloroform (50 ml). The solution was washed successively with water, two portions of 20% aqueous citric acid, water, 5% aqueous sodium hydrogen carbonate, and water again. The dried (Na_2SO_4) solution was evaporated to dryness under diminished pressure. The residue solidified on trituration with a mixture of nitromethane and water. Recrystallization from aqueous 2-propanol afforded 0.83 g (44%) of compound **2e**, mp 151–153°.

Anal. Calcd for $C_{33}H_{34}ClN_3O_6$: C, 65.61; H, 5.67; N, 6.95; Cl, 5.87. Found: C, 65.90; H, 5.76; N, 7.09; Cl, 6.03.

N-Benzyloxycarbonyl-L-seryl-L-phenylalanyl-L-phenylalanine Benzyl Ester.—A solution of the active ester **2e** (0.3 g, 0.5 mmol) in dimethylformamide (5 ml) was added to a 1 *M* solution (0.5 ml) of L-phenylalanine benzyl ester (liberated from the *p*-toluenesulfonate by the action of triethylamine[24]) in ethyl acetate. The resulting mixture was kept at room temperature for 60 hr and then diluted with ethyl acetate (40 ml). The solution was washed successively with water and five 20-ml portions of 0.5 *M* H_2SO_4, and then once more with water. The dried (Na_2SO_4) ethyl acetate solution was evaporated under diminished pressure. The residual oil was dissolved in ethyl acetate and a solid was precipitated with petroleum ether (bp 30–60°). The gelatinous precipitate was collected and dissolved in trifluoroacetic acid (2 ml), the solution was allowed to stand at room temperature for 2 hr and evaporated to dryness under diminished pressure, and the residue was coevaporated twice with dioxane to remove the traces of trifluoroacetic acid. Crystallization from ethyl acetate–petroleum ether afforded 128 mg of a crude product, mp 143–145°, which was recrystallized twice from ethyl acetate to afford 50 mg of the title compound, mp 161–163°.

Anal. Calcd for $C_{36}H_{37}N_3O_7$: C, 69.33; H, 5.97; N, 6.74. Found: C, 69.08; H, 6.12; N, 6.89.

Reaction of 2′(3′)-*O*-Aminoacyl Derivatives of Adenosine 1 with the Active Esters 2.—Compound 1 (50 μmol obtained from a stock solution in 80% aqueous acetic acid by lyophilization, dried at 10^{-3} Torr, and washed with two portions of ether) in dimethylformamide (0.5 ml) was treated with a solution of the active ester 2 (100 μmol) in dimethylformamide (0.5 ml), and the entire mixture was allowed to stand at room temperature for

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TABLE V

INFRARED SPECTRA (cm^{-1}) AND ULTRAVIOLET SPECTRA (nm) OF PRODUCTS

Compd[a]	ν(CO) Ester	ν(CO) Urethane	ν(CO) Amide	Adenine	ν(NH_2)	ν(NH)	λ_{max}	Log ε
3a	1750, 1729[b]		1664	1632	3526, 3412	3438	260[c]	3.97
3b	1745	1718	1679	1634	3526	3413[d]	259[c]	4.14
3d	1743, 1730[b]		1658	1643[e]	3521, 3415	3442	260[f]	
3g[g]	1746	1726	1676, 1510	1639[e]	3526, 3414	3431[d]	260[c]	
3e	1750	1715	1680		3524, 3414	3431[d]	260[c]	

[a] ν(OH) 3200 cm^{-1} (hydrogen bonding) for all compounds. [b] Hydrogen bonding. [c] In ethanol. [d] Together with $\nu(NH_2)_{sym}$, shoulder. [e] Together with ν(CO) amide bonded. [f] 0.01 *M* HCl. [g] A weak band at 3357 cm^{-1}, probably NH bonded; *cf.* the spectra of adenosine 2′(3′)-*O*-bisaminoacyl derivatives.[6]

20–24 hr and evaporated to dryness under diminished pressure. The residue was dissolved in the solvent system S_5 and chromatographed on a thin layer (loose, 3 mm thick, 20 × 50 cm) of silica gel in the same solvent system. Detection showed a band of the unreacted starting compound **1**, a very weak band of the *N*-acetyl derivative of compound **1**, and the principal band of the main product **3**. The latter band was eluted with the above solvent system and the eluate was evaporated under diminished pressure (when traces of chloroxine were present, the product was rechromatographed). Powdered products were obtained by lyophilization of dioxane solutions. For yields, see Table I. The products were homogeneous as shown by paper and thin layer chromatography; some of them were characterized by infrared spectra.

2′(3′)-*O*-(*N*-Benzyloxycarbonyl-L-seryl-L-phenylalanyl-L-phenylalanyl)adenosine (3h).—Compound **3g** (50 μmol) was dissolved in trifluoroacetic acid[6] (1 ml), and the solution was allowed to stand at room temperature for 45 min and evaporated to dryness under diminished pressure. The residue was repeatedly lyophilized with five portions of dioxane until colorless. The resulting powder was chromatographically homogeneous in the solvent systems S_2 and S_5 and different from the starting derivative **3g**.

Adenosine 2′(3′)-*O*-Peptidyl Derivatives (3i–3m).—The hydrogenolysis of the *N*-benzyloxycarbonyl group was performed over 5% palladium oxide on barium sulfate catalyst in 80% aqueous acetic acid, as reported earlier.[6] Yields of the peptidyl derivatives **3i–3m** were determined spectrophotometrically with the use of aliquots of stock solutions in 80% acetic acid diluted with 0.01 *M* HCl. For yields of products and the corresponding amino acid analyses, see Table II. The products were also characterized by paper chromatography in the solvent systems S_2 and S_3 (Table III), electrophoresis at pH 3.4 (Table IV), and alkaline hydrolysis in the solvent system S_1 or in 0.2 *M* NaOH (30 min at 20°) to adenosine and the parent peptide. Products of hydrolysis were compared with authentic specimens in the solvent systems S_1, S_2, and S_3 as well as in electrophoresis at pH 3.4.

Cleavage of Compound 3i in Dimethylformamide.—Compound **3i** (1.09 μmol) was dissolved in dimethylformamide (0.2 ml). After 4 hr at room temperature the reaction mixture was analyzed by chromatography in the solvent systems S_1 and S_2. Adenosine was determined as the single reaction product (detection under ultraviolet light and with ninhydrin). The solution was evaporated to dryness under diminished pressure, and the residue was repeatedly lyophilized with water and dried at 10^{-4} Torr. The residue was finally dissolved in hot methanol and the small amount of insoluble material was removed by filtration. The ORD measurements (Figures 1 and 2) were performed with the filtrate as well as with the starting compound **3i**.

Registry No.—**1a**, 2′-isomer, 25164-30-1; **1a** 3′-isomer, 5956-81-0; **1b** 2′-isomer, 34996-43-5; **1b** 3′-isomer, 5957-19-7; **1c** 2′-isomer, 4217-73-6; **1c** 3′-isomer, 4217-74-7; **2a**, 10173-02-1; **2b**, 7797-44-6; **2c**, 27785-02-0; **2d**, 7797-39-9; **2e**, 34996-31-1; **3a** 2′-isomer, 44996-45-7; **3a** 3′-isomer, 34996-32-2; **3b** 2′-isomer, 34996-46-8; **3b** 3′-isomer, 34969-19-2; **3c** 2′-isomer, 34996-47-9; **3c** 3′-isomer, 34996-33-3; **3d** 2′-isomer, 34996-48-0; **3d** 3′-isomer, 34996-34-4; **3e** 2′-isomer, 34996-49-1; **3e** 3′-isomer, 35000-88-5; **3f** 2′-isomer, 35000-98-7; **3f** 3′-isomer, 35000-89-6; **3g** 2′-isomer, 35000-99-8; **3g** 3′-isomer, 35000-90-9; **3h** 2′-isomer, 35001-00-4; **3h** 3′-isomer, 35000-91-0; **3i** 2′-isomer, 35001-01-5; **3i** 3′-isomer, 35000-92-1; **3j** 2′-isomer, 35001-02-6; **3j** 3′-isomer, 35000-93-2; **3k** 2′-isomer, 35001-03-7; **3k** 3′-isomer, 35000-94-3; **3l** 2′-isomer, 35001-04-8; **3l** 3′-isomer, 35000-95-4; **3m** 2′-isomer, 35001-05-9; **3m** 3′-isomer, 35000-96-5; *N*-benzyloxycarbonyl-L-seryl-L-phenylalanyl-L-phenylalanine benzyl ester, 35000-97-6.

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