Aminoacyl Derivatives of Nucleosides, Nucleotides, and Polynucleotides. XVIII. Synthesis of 2′(3′)-*O*-Aminoacyl Derivatives of Dinucleoside Phosphates[1]

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The synthesis of C-A-Phe (**5a**), C-U-Phe (**5b**), U-A-Phe (**5c**), U-U-Phe (**5d**), C-dA-Phe (**5e**), and C-dA-Gly (**5f**), potential substrates for ribosomal peptidyl transferase, is described. 2′,5′-Di-*O*-(1-ethoxyethyl)uridine 3′-phosphate (**1a**) and *N*-dimethylaminomethylene-2′,5′-di-*O*-(1-ethoxyethyl)cytidine 3′-phosphate (**1b**) were condensed with A(Z-Phe) (**2a**), U(Z-Phe) (**2b**), dA(Z-Phe) (**2c**), and dA(Z-Gly) (**2d**) in the presence of DCC in pyridine to afford intermediates **3a–d**. The latter were hydrolyzed in dilute acetic acid to C-A(Z-Phe) (**4a**), C-U(Z-Phe) (**4b**), U-A(Z-Phe) (**4c**), U-U(Z-Phe) (**4d**), C-dA(Z-Phe) (**4e**), and C-dA(Z-Gly) (**4f**), which were isolated by preparative tlc on microcrystalline cellulose. Hydrogenolysis (Pd-$BaSO_4$) of **4a–f** in 80% acetic acid at 0° gave C-A-Phe (**5a**), C-U-Phe (**5b**), U-A-Phe (**5c**), U-U-Phe (**5d**), C-dA-Phe (**5e**), and C-dA-Gly (**5f**). An improved method for the preparation of compound **1a** is also described.

The major stimulus for the synthesis of 2′(3′)-*O*-aminoacyl ribooligonucleotides lies in the fact that such compounds have found considerable use in the investigation of the mechanism of protein biosynthesis, particularly its peptide bond formation step.[2] The need for a simple general synthesis of the title compounds is further stressed by the fact that enzymic methods (degradation of the aminoacyl or peptidyl-tRNA with T_1-RNase[3]) suffer from an inherent drawback: they do not provide materials other than those derived from the natural 3′-terminal sequence of tRNA. The first syntheses of 2′(3′)-*O*-aminoacyl ribooligonucleotides, 2′(3′)-*O*-glycyl derivatives, made use of the orthoester exchange of the *N*-benzyloxycarbonylglycine orthoester with unprotected ribodinucleoside phosphates[4] or condensation of a nucleotide or nucleoside component carrying a cyclic glycine orthoester grouping with a suitably protected counterpart.[5] The cyclic orthoester intermediate was then hydrolyzed and the *N*-benzyloxycarbonyl group was removed to give the 2′(3′)-*O*-glycyl derivatives of a number of dinucleoside phosphates.[4-6] This simple, efficient, and highly selective method can in principle be extended to the preparation of 2′(3′)-*O*-glycyl derivatives of longer ribooligonucleotides. However, this method is not general, since orthoesters of optically active amino acids have not been described to date.[7]

Recently, a report of the preparation of the 2′(3′)-*O*-aminoacyl derivatives of C-A and C-C-A appeared describing the aminoacylation of protected ribodi- or trinucleoside phosphates with *N*-*tert*-butoxycarbonylamino acids and DCC in pyridine.[10] Although this reaction afforded the requisite products, the yields of the starting ribooligonucleotides as well as those of the aminoacylation were very low. Moreover, as can be expected from an analogy with simple ribonucleoside derivatives[11] the course of aminoacylation is not uniform and 2′,3′-*O*-bisaminoacylation always occurs. In addition, aminoacylation of unblocked cytosine amino groups also takes place, although the authors claim that the *N*-*tert*-butoxycarbonylaminoacyl groups are removed in the subsequent deprotecting step. The severe conditions that must be employed for the removal of the *N*-*tert*-butoxycarbonyl group (concentrated CF_3COOH) may have led to degradation or isomerization of the oligonucleotide moiety and could account for the low yields of the final products.[10]

Our present work describes a different approach to the synthesis of 2′(3′)-*O*-aminoacyl ribooligonucleotides and involves the condensation of protected ribonucleoside 3′-phosphates with 2′(3′)-*O*-(*N*-benzyloxycarbonyl)aminoacyl ribonucleosides and subsequent deblocking in dilute CH_3COOH followed by hydrogenolysis. Using this method, we have prepared four 2′(3′)-*O*-(L-phenylalanyl) ribodinucleoside phosphates. Our work also includes the synthesis of two 3′-*O*-aminoacyl derivatives of dinucleoside phosphates containing a 3′-terminal 2′-deoxyribonucleoside.

Results and Discussion

2′(3′)-*O*-Aminoacyl ribooligonucleotides contain two extremely sensitive functions, a fact which must be taken into account before any serious attempt of their synthesis is to be undertaken. The aminoacyl ester bond is relatively stable in acid but highly labile in alkaline or neutral media, and the phosphodiester linkage is reasonably stable in alkali but isomerizes in acidic media. Thus, the different requirements of these sensitive functions make the judicious choice of the requisite protecting groups very difficult. In our approach we have chosen protecting groups for the nucleotide components that can be removed by treatment with dilute CH_3COOH. Such conditions are mild enough to minimize the isomerization of the phosphodiester linkage while ensuring the integrity of the aminoacyl residue.

As nucleotide components we have used *N*-dimethylaminomethylene-2′,5′-di-*O*-(1-ethoxyethyl)cytidine 3′-phosphate (**1b**)[12] and 2′,5′-di-*O*-(1-ethoxyethyl)uridine 3′-phosphate (**1a**)[13] (Scheme I). A new simple, and convenient procedure has been found for the preparation of **1a** which avoids the use of an external acid catalyst (HCl or CF_3COOH) and isolation of the product by selective precipitation of Ca, Li, or Ag salts or by anion exchange chromatography.[15] Thus, uridine 3′-phosphate (pyridinium salt) on treatment with ethyl vinyl ether in dimethylformamide at room temperature gave an 80% yield of **1a** (isolated as the triethylammonium salt).

2′(3′)-*O*-(*N*-Benzyloxycarbonyl-L-phenylalanyl) derivatives **2a**, **2b**, and **2c** were prepared as described earlier,[16] with some modifications in the synthesis of the corresponding intermediates. Compound **2d** was prepared analogously to **2c** from 2′-deoxy-5′-*O*-(4-methoxy)trityladenosine and *N*-benzyloxycarbonylglycine using DCC in pyridine. The isomeric composition of ribonucleoside derivatives **2a** and **2b** was determined from their nmr spectra (anomeric protons).[17] The well-resolved spectrum of U(Z-Phe) (**2b**) made it possible to estimate the ratio of 2′ and 3′ isomers not only from their anomeric protons, but also from the H_5 proton[18] and the CH_2 signal of the benzyloxycarbonyl group (Table I). The condensation of protected

Table I
Determination of Isomeric Composition of 2b from Different Signals in the Nmr Spectrum

Signal[a]	3′ isomer, %	2′ isomer, %
$H_{1'}$	71	29
$H_{1'}$[b]	72	28
H_5[b]	74	26
CH_2 of benzyl[b]	73	27

[a] Composition determined from the height of the signals. The intermediary 5′-*O*-(4-methoxytrityl) derivative had virtually the same isomeric composition as determined from the $H_{1'}$ signal: 71.5% of the 3′ isomer and 28.5% of the 2′ isomer, respectively. [b] Determined after addition of D_2O.

Table II
2′(3′)-*O*-(*N*-Benzyloxycarbonyl)aminoacyl Dinucleoside Phosphates 4a–f

Compd	Yield, %	Uv spectrum (0.01 *N* HCl) max	250/260	280/260	290/260
C-A(Z-Phe) **4a**	22	265	0.74	0.72	0.47
C-U(Z-Phe) **4b**	25	267	0.61	0.91	0.60
C-dA(Z-Phe) **4e**	21	265	0.76	0.74	0.48
C-dA(Z-Gly) **4f**	39	266	0.73	0.72	0.45
U-A(Z-Phe) **4c**	25	257	0.91	0.33	0.11
U-U(Z-Phe) **4d**	21	260[a]	0.76	0.34	0.05

[a] 95% ethanol.

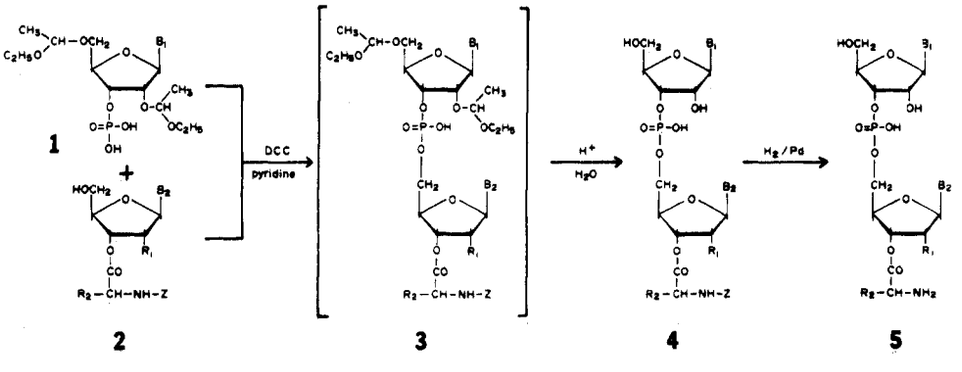
nucleotides **1a** and **1b** with nucleoside components **2a–d** was effected by DCC in pyridine. The intermediates **3** (not isolated) were deblocked in 80% acetic acid to remove the 1-ethoxyethyl groups and (in the case of cytidine derivatives) in solvent S_2 to remove the *N*-dimethylaminomethylene group. The resulting products **4a–f** (yields 17–25%, see Table II) were isolated by preparative tlc on microcrystalline cellulose. The use of TPS instead of DCC in the condensation step did not improve the yields and led to extensive isomerization of **4** (up to 30% of the pancreatic RNase resistant 2′–5′ isomer). In control experiments we have confirmed that nucleotides **1a** and **1b** are unstable under the reaction conditions. Thus, in the presence of TPS in pyridine, extensive removal of the 1-ethoxyethyl group takes place.[19]

The amino group of the adenine moiety of nucleosides **2a**, **2c**, and **2d** was not protected in view of the fact that it is not easily phosphorylated, unlike the cytosine amino group.[20] This was further ascertained in the case of C-A(Z-Phe) (**4a**), which after reaction with dimethylformamide dineopentyl acetal in DMF afforded quantitatively the corresponding bis-*N*-dimethylaminomethylene derivative as shown by its uv spectrum. Dimethylformamide acetals are known to substitute the amino functions of base residues to give the corresponding *N*-dimethylaminomethylene derivatives. The quantitative reaction of **4a** with dimethylformamide dineopentyl acetal has therefore verified the absence of phosphorylation at the adenine amino group.

Nucleoside components **2a** and **2b** have only one hydroxy group protected by an aminoacyl residue. We anticipated that the relatively bulky[21] *N*-benzyloxycarbonylaminoacyl group will provide protection for the neighboring hydroxyl group by steric hindrance.[22] Several examples from the literature have lent support to our assumption. 2′-*O*-Tetrahydropyranyl ribonucleosides are phosphorylated at the 5′ hydroxy group,[23] and diphenylmethyl esters of ribonucleoside 3′-phosphates were employed for the synthesis of ribodinucleotides without protection of the 2′ hydroxy group.[24] It has also been claimed that a variety of puromycin analogs are not phosphorylated at the 2′ hydroxy group by either $POCl_3$ or protected ribonucleoside 3′-phosphates.[25] However, in neither case has a systematic search for possible contaminating 2′- or 3′-phosphorylated isomers been undertaken.

The extent of phosphorylation at the 2′ or 3′ hydroxy groups of **2a** and **2b** was determined by degradation of products **4** or **5** with snake venom phosphodiesterase. The latter is specific for esters of 5′ nucleotides, and thus 3′–2′ and 3′–3′ isomers will not be degraded. In all cases the above isomers were essentially absent (Table III). The results obtained with phosphodiesterase were generally in agreement with those found by degradation with methanolic ammonia.[26] The latter method is based on preferential degradation of 3′–3′ and 3′–2′ ribooligonucleotides relative to the more stable[27] 3′–5′ or 2′–5′ isomers (Scheme II). It should be emphasized, however, that the above method, which is a part of an efficient synthetic procedure for preparation of 3′–5′ ribooligonucleotides from unprotected ribonucleosides, has not been used as an analytical tool to date. We have found that some 3′–5′ oligonucleotides (*e.g.*, C-A and C-dA) are also degraded to some extent (*ca.* 5%).[28]

Scheme I



Z = $C_6H_5CH_2OCO$
DCC = dicyclohexylcarbodiimide
1a: B_1 = uracil
1b: B_1 = N^6-dimethylaminomethylenecytosine
1c: B_1 = cytosine
2a: B_2 = adenine, R_1 = OH, R_2 = $C_6H_5CH_2$
2b: B_2 = uracil, R_1 = OH, R_2 = $C_6H_5CH_2$
2c: B_2 = adenine, R_1 = H, R_2 = $C_6H_5CH_2$
2d: B_2 = adenine, R_1 = R_2 = H
4a, 5a: B_1 = cytosine, B_2 = adenine, R_1 = OH, R_2 = $C_6H_5CH_2$
4b, 5b: B_1 = cytosine, B_2 = uracil, R_1 = OH, R_2 = $C_6H_5CH_2$
4c, 5c: B_1 = uracil, B_2 = adenine, R_1 = OH, R_2 = C_6H_5
4d, 5d: B_1 = B_2 = uracil, R_1 = OH, R_2 = $C_6H_5CH_2$
4e, 5e: B_1 = cytosine, B_2 = adenine, R_1 = H, R_2 = $C_6H_5CH_2$
4f, 5f: B_1 = cytosine, B_2 = adenine, R_1 = R_2 = H

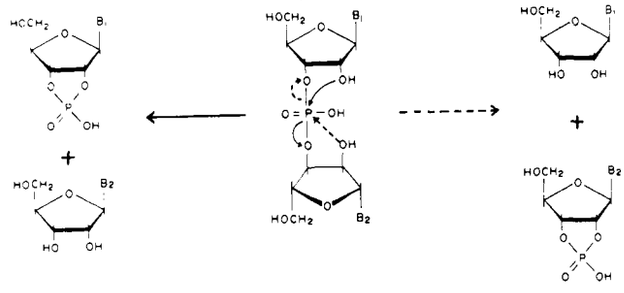
Compounds 2a, 2b, 4a, 5a, 4b, 5b, 4c, 5c, 4d, and 5d are mixtures of 2′ and 3′-aminoacyl derivatives

Table III
2′(3′)-*O*-Aminoacyl Dinucleoside Phosphates

Compd	Yield, %	Uv spectrum (0.01 *N* HCl) max	250/260	280/260	290/260	2′–5′,[a] %	N^1/N^2 [a]	3′–3′ [b] or 3′–2′, %
C-A-Phe **5a**	80	265	0.78	0.68	0.40	4	0.81	0
C-U-Phe **5b**	82	267	0.69	0.79	0.47	3.4	0.85	0
U-A-Phe **5c**	22[c]	257	0.91	0.23		2.2	0.95	0
U-U-Phe **5d**	60[c]	262	0.76	0.46	0.06	3.5	0.95	0
C-dA-Phe **5e**	84	265	0.77	0.71	0.43	6.5	0.97	
C-dA-Gly **5f**	84	265	0.80	0.70	0.49	4.9	0.84	

[a] Determined by pancreatic RNase digestion. [b] Determined by snake venom phosphodiesterase digestion. [c] Purified by preparative tlc in solvent S_6.

Scheme II



The final products, 2′(3′)-*O*-L-phenylalanyl derivatives **5a–f**, obtained by hydrogenolysis[4,5] of **4a–f**, were also characterized by the more common criteria: paper chromatography and electrophoresis (Tables IV and V), tlc, uv spectra, and degradation with pancreatic RNase, which showed the fidelity of the 3′–5′ interribonucleotide linkages (Table III), and by alkaline hydrolysis to the parent dinucleoside phosphate and amino acid.

Experimental Section

General Methods. Chromatography and Electrophoresis. Paper chromatography was performed by the descending technique on Whatman No. 1 or 3 MM paper in the following solvent systems: S_1, 2-propanol–concentrated ammonium hydroxide–water (7:1:2); S_2, 1-butanol–acetic acid–water (5:2:3); S_3, ethanol–1 *M* ammonium acetate (5:2). R_f values are given in Table IV. Thin layer chromatography (tlc) was performed on silica gel coated aluminum foils (Merck, Darmstadt, Germany) in solvents S_4, $CHCl_3$–CH_3OH (95:5), and S_5, $CHCl_3$–CH_3OH (9:1), and on glass plates coated with Avicel microcrystalline cellulose[29] in solvents S_1 and S_2. Preparative tlc was performed on 5-mm thick nonadhering (loose) layers of silica gel, 70–325 mesh ASTM (Merck, Darmstadt, Germany), containing 1% of a fluorescent indicator (Leuchtpigment ZS Super, Riedel-deHaën, Hannover, Germany, or Lumilux Green ZS, American Hoechst Corp., Somerville, N. J.). Microcrystalline cellulose Avicel TG-101 (FMC Corp., American Viscose Division, Newark, Del.) with 1% of the above indicator was used for preparative layers (2 mm thick, 20 × 20 cm) on glass plates, using 1-butanol saturated with 10% acetic acid (S_6) as the developer. These layers were prepared as follows. A thick suspension of Avicel (200 g) in 95% ethanol (600 ml) was stirred for 10 min in a blender. The suspension was then applied directly or by means of a spreader on five to six glass plates and the layers were made smooth, while still wet, by shaking with the hand. Paper electrophoresis was conducted on a Savant flat plate, using 1 *M* CH_3COOH as a buffer or in 0.02 *M* Na_2HPO_4 (pH 7.0) on Whatman No. 1 paper at 40 V/cm 1–2 hr. Electrophoretic mobilities are given in Table V. Uv-absorbing compounds were detected using a Mineralight lamp; ninhydrin-positive substances were detected by spraying with 0.1% ninhydrin in ethanol.

Spectra. Uv spectra were obtained using a Cary Model 11 recording spectrophotometer or a Beckman Model DB-GT grating spectrophotometer. Yields of oligonucleotides were determined spectrophotometrically at pH 2 (0.1 *N* HCl) using the following extinction coefficients: C-A, ϵ_{260} 21,200; C-dA, ϵ_{260} 20,900; C-U, ϵ_{260} 16,750; U-A, ϵ_{260} 24,200; U-U, ϵ_{260} 20,000. Nmr spectra were obtained using a Varian A-60A spectrophotometer. $(CH_3)_4Si$ was used as an internal standard with $CDCl_3$ and CD_3COCD_3 and sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) with CD_3SOCD_3.

Pancreatic Ribonuclease Degradation. Pancreatic ribonuclease degradation was performed with 1–2 μmol of compound **5e,f** in a mixture of Tris-HCl buffer (0.1 *N*, pH 7.8, 0.2 ml) and 0.2 mg of ribonuclease A (Sigma Chemical Co., St. Louis, Mo.). The solution was incubated for 4–6 hr at 37°, applied to Whatman 3 MM

Table IV
Paper Chromatography of Starting Compounds, Products, and Authentic Specimens

Compd	R_f S_1	S_2
A	0.54	0.59
dA	0.64	
U	0.50	0.59
Cp	0.12	0.26
Up	0.11	
Ccp	0.43	
Ucp	0.42	
Gly	0.36[a]	
Phe	0.66[a]	0.70[a]
$(EtOEt)_2$Cp **1c**	0.49	
$(EtOEt)_2$Up **1a**	0.58[b]	
C-A	0.26	
C-U	0.20	
C-dA	0.34	
U-U	0.21	
U-A	0.30	
C-A(Z-Phe) **4a**	*c*	0.71
C-U(Z-Phe) **4b**	*c*	0.68
C-dA(Z-Phe) **4e**	*d*	0.80
C-dA(Z-Gly) **4f**	*d*	0.65
U-U(Z-Phe) **4d**	*c*	0.60
U-A(Z-Phe) **4c**	*c*	0.60
C-A-Phe **5a**	*c*	0.45[a]
C-U-Phe **5b**	*c*	0.38[a]
C-dA-Phe **5e**	*d*	0.56[a]
C-dA-Gly **5f**	*d*	0.18[a]
U-U-Phe **5d**	*c*	0.24[a]
U-A-Phe **5c**	*c*	0.24[a]

[a] Color reaction with ninhydrin. [b] S_3, R_f 0.62. [c] Hydrolysis to parent dinucleoside phosphate and amino acid. [d] Partial hydrolysis to parent dinucleoside phosphate and amino acid.

Table V
Paper Electrophoretic Mobilities of Products (1 *M* Acetic Acid, pH 3.4)

Compd	Mobility[a]
Gly	−3.0
Phe	−7.0
C-A-Phe **5a**	−22.0
C-U-Phe **5b**	−14.0
C-dA-Phe **5e**	−22.6
C-dA-Gly **5f**	−22.2
U-U-Phe **5d**	−0.16[b]
U-A-Phe **5c**	−0.63[b]

[a] Relative to cytidine 3′-phosphate mobility = 1.00, unless stated otherwise. [b] Relative to uridine 3′-phosphate mobility = 1.00.

paper, and developed in system S_1. For deoxy derivatives **5e** and **5f**, the solution was lyophilized after incubation and the residue was dissolved in methanolic ammonia (0.5 ml). After 2 hr at room temperature, the solution was evaporated and applied to the chromatogram. The spots of the products of enzymic degradation were eluted with 0.01 *N* HCl and the optical density of the eluates was measured. Compound **5c** was not completely soluble under the conditions used. Therefore, it was hydrolyzed during paper chromatography in solvent S_1, and the spot of U-A was eluted with water, the eluate was lyophilized, and the residue was incubated as above.

Russell's Viper Venom Phosphodiesterase Degradation. The compound (1 μmol) was dissolved in Tris-acetate buffer (pH 8.8, 0.1 *M*, 0.25 ml) and magnesium acetate (0.3 *M*, 0.05 ml) and incubated with the enzyme (20 units in 0.1 ml of water, Calbiochem, Los Angeles, Calif., grade B) for 4–6 hr at 37°. The estimation of degradation products was performed as was described for pancreatic ribonuclease. For compound **5c**, a procedure similar to that given for the corresponding ribonuclease degradation was followed. Simultaneously, a control experiment (without enzyme) was run under the same conditions to ascertain complete stability of the phosphodiester linkage(s) at pH 8.8 and during chromatography in solvent S_1.

Starting Materials. Cytidine 3′-phosphate (free acid) and uridine 3′-phosphate (ammonium salt) were prepared from the corresponding 2′,3′-cyclic phosphates by known methods.[30] Uridine 3′-phosphate (pyridinium salt) was prepared according to the procedure described for 5′-*O*-acetyluridine 3′-phosphate.[15c] The solution of uridine 3′-phosphate prepared from uridine 2′(3′)-phosphate (10 g, 30.8 mmol) obtained after pancreatic ribonuclease degradation of the corresponding 2′,3′-cyclic phosphate was passed through the Dowex 50 (X4, 200–400 mesh, pyridinium form) column (34 × 5 cm) equilibrated with 1% pyridinium acetate (pH 6.0) and the column was eluted with the same buffer. The eluate (*ca.* 3 l.) was lyophilized and the product which solidified after addition of ethanol (200 ml) was filtered and dried over KOH at 0.01 mm, 8.41 g (69%). The uv spectrum in 0.01 *N* HCl indicated the presence of pyridine (λ 255 nm, shoulder at 260), and the nmr spectrum showed the presence of pyridine (0.8 equiv as determined from the integration curve of the signals at δ 8.33 and 8.92) and DMF (0.15 equiv, δ 3.32, d) and the absence of the 2′ isomer as judged[18] from the signals for H_6, H_5, and H_1'. Attempts to remove both solvents by repeated lyophilization from water or prolonged drying over P_2O_5 or concentrated H_2SO_4 at room temperature were not successful and therefore this material was used in the subsequent step as such. The purity of nucleotides **1a** and **1b** was checked by paper chromatography (S_1) and electrophoresis (pH 7.0) and uv and nmr spectroscopy. The latter showed the absence of the 2′ isomer.[18] 2′(3′)-*O*-(*N*-Benzyloxycarbonyl-L-phenylalanyl)adenosine was prepared as described.[11b] Dimethylformamide (DMF) and pyridine were dried with Linde Molecular Sieves, 4A. Dimethylformamide dineopentyl and dimethyl acetal were products of Aldrich Chemical Co., Milwaukee, Wis. Petroleum ether was of 30–60° boiling range. PdO (5%) on $BaSO_4$ was a product of Engelhard, Newark, N. J.

3′-*O*-(*N*-Benzyloxycarbonyl-L-phenylalanyl)-2′-deoxyadenosine (2c). 2′-Deoxy-5′-*O*-(4-methoxytrityl)adenosine (1.05 g, 2 mmol) was dissolved in DMF (5 ml), dimethylformamide dimethyl acetal (0.5 ml) was added, and the mixture was kept at ambient temperature for 16 hr. The solution was evaporated to dryness *in vacuo*, and the uv spectrum of the residue in ethanol showed quantitative formation of the *N*-dimethylaminomethylene derivative[31a] (max 310 nm, min 264). The residue was evaporated several times with anhydrous pyridine and then dissolved in pyridine (10 ml); *N*-benzyloxycarbonyl-L-phenylalanine (1.2 g, 4 mmol) was added, and the reaction mixture was cooled to 0°. A cold solution of DCC (1.3 g, 6 mmol) in pyridine (3 ml) was added and the mixture was kept at 0° for 20 min and then for 1 week at room temperature. Tlc (S_4) showed nearly quantitative reaction. Ice (10 ml) and petroleum ether (20 ml) were added, the dicyclohexylurea was filtered off, washed with 50% pyridine, and the filtrate was evaporated *in vacuo* to dryness. The residue was coevaporated with toluene (2 × 25 ml) and extracted with petroleum ether and the residue was dissolved in 80% CH_3COOH (25 ml). After 10 hr at room temperature the reaction mixture was diluted with 1-butanol (50 ml) and water (15 ml); the solution was kept overnight at room temperature and evaporated to dryness *in vacuo*, and the residue was dissolved in dioxane and lyophilized. Tlc (S_5) indicated that **2c** was the major product. The residue was applied to two loose layers (15 × 35 cm) of silica gel that were developed in S_5. The main band was eluted (S_5) and the eluate was evaporated *in vacuo*. Compound **2c** solidified after treatment with $CHCl_3$ and petroleum ether. The yield was 0.43 g (40%) of tlc-homogeneous (S_5) product **2c** which was identical (ir, uv) with the compound prepared earlier:[16] nmr (CD_3COCD_3–D_2O) δ 8.31 (s, 1, H_8), 8.25 (s, 1, H_2), 7.33 (s, 10, C_6H_5), 6.37 (q, 1, $H_{1'}$), 5.11 (s, 2, CH_2-benzyl).

3′-*O*-(*N*-Benzyloxycarbonylglycyl)-2′-deoxyadenosine (2d). The title compound was prepared by the same method as described for **2c**: yield 35%; tlc (S_5) homogeneous; uv (95% ethanol) max 260 nm (ε 14,500); ir ($CHCl_3$) 3180 (OH bonded), 3451 (NH), 3524, 3412 (NH_2), 1745 (CO ester), 1721 (CO urethane), 1632 (adenine, ring stretch), 1591 (phenyl), 1514 cm^{-1} (CONH, amide II); nmr (CD_3COCD_3–D_2O) δ 7.90 (s, 1, H_8), 7.83 (s, 1, H_2), 7.02 (s, 5, C_6H_5), 6.12 (q, 1, $H_{1'}$), 4.91 (s, 2, CH_2-benzyl).

Anal. Calcd for $C_{20}H_{22}N_6O_6 \cdot H_2O$: C, 52.17; H, 5.25; N, 18.25. Found: C, 52.68; H, 4.90; N, 17.98.

***N*-Dimethylaminomethyleneadenosine.** *N*-Dimethylaminomethyleneadenosine was prepared as described:[31a] mp 201–204° (lit.[31a] mp 205–207°); the uv spectrum was identical with that reported;[31a] nmr (CD_3SOCD_3) δ 9.00 [s, 1, CH of $(CH_3)_2NCH$=], 8.57 (s, 1, H_8), 8.50 (s, 1, H_2), 6.02 (d, 1, $H_{1'}$, $J_{1',2'}$ = 5.5 Hz), *ca.* 5.28 (broad m which disappeared on addition of D_2O, 2, OH), 3.20 [d, 6, $(CH_3)_2N$].

5′-*O*-(4-Methoxytrityl)adenosine. A stirred suspension of *N*-dimethylaminomethyleneadenosine (22.8 g, 0.071 mol) in pyridine (350 ml) was treated with 4-methoxytrityl chloride (26.3 g, 0.085 mol) at room temperature. Stirring was continued for 67 hr, whereupon the reaction mixture was poured onto ice (*ca.* 2500 g) and the precipitated syrup was extracted with $CHCl_3$ (4 × 1000 ml); the combined extracts were dried ($MgSO_4$) and evaporated *in vacuo*. The syrupy residue was dissolved in 20% NH_3 in methanol (500 ml) and the solution was kept for 48 hr at room temperature.[32] Evaporation *in vacuo* afforded almost white 5′-*O*-(4-methoxytrityl)adenosine, which was washed with ether (500 ml) and petroleum ether (500 ml): yield 33.3 g (87%); mp 180–182° dec (lit.[30] mp 189–191° dec); uv (95% ethanol) max 233 nm (ε 13,800), 259 (12,500), min 245 (10,000) (lit.[30] max 233 and 260 nm); nmr (CD_3SOCD_3) δ 8.26 (s, 1, H_8), 8.12 (s, 1, H_2), 7.33 and 6.84 (m, 14, phenyl and 4-methoxyphenyl), 5.97 (d, 1, $H_{1'}$, $J_{1',2'}$ = 4 Hz), 5.51 (d, 1, OH, *J* = 5 Hz), 5.18 (d, 1, OH, *J* = 5 Hz), 3.75 (s, 3, CH_3O).

5′-*O*-(4-Methoxytrityl)uridine. The described procedure[16] was modified as follows. Uridine (4.88 g, 20 mmol) dried by evaporation with pyridine at 0.1 mm and <50° (bath temperature) was dissolved in pyridine (80 ml), 4-methoxytrityl chloride (6.8 g, 22 mmol) was added, and the solution was kept for 3 days at room temperature. The reaction mixture was then poured onto ice and water (*ca.* 400 ml) with stirring, the syrupy precipitate was extracted with $CHCl_3$ (2 × 200 ml), and the combined extracts were dried ($MgSO_4$). Evaporation *in vacuo* afforded a syrup that was dissolved in benzene (70 ml). Addition of cyclohexane (325 ml) precipitated 5′-*O*-(4-methoxytrityl)uridine, yield 10.28 g (90%); tlc (S_4) showed traces of two faster moving components, presumably 2′,5′- and 3′,5′-di-*O*-(4-methoxytrityl) derivatives. The crude product was dissolved in benzene (150 ml) and the solution was applied to a silica gel column (13 × 6.5 cm). The column was eluted with benzene (400 ml), $CHCl_3$ (1200 ml), 1% CH_3OH in $CHCl_3$ (400 ml), and 3% CH_3OH in $CHCl_3$ (1200 ml). Elution with S_4 (2000 ml) afforded 5′-*O*-(4-methoxytrityl)uridine as an amorphous powder after precipitation from benzene with petroleum ether: yield 10.1 g (88%); tlc (S_4) homogeneous; uv (95% ethanol) max 231 nm (ε 15,600), 264 (9800), min 250 (8100) [lit.[33] max 232 nm (ε 18,000), 263 (10,400)]; nmr (CD_3COCD_3) δ 7.83 (d, 1, H_6, $J_{6,5}$ = 8 Hz), 7.33 and 6.91 (m, 14, phenyl and 4-methoxyphenyl, the latter forms an A_2B_2 system the lower field half of which is overlapped with phenyl), 5.92 (d, 1, $H_{1'}$, $J_{1',2'}$ = 3 Hz), 5.31 (d, 1, H_5, $J_{5,6}$ = 8 Hz), 3.77 (s, 3, CH_3O), *ca.* 4.37 (poorly resolved m, 3, CH of ribose), 3.46 (d, 2, $H_{5'}$), 2.75 (s, disappears on addition of D_2O, 2, OH).

2′(3′)-*O*-(*N*-Benzyloxycarbonyl)-L-phenylalanyluridine (2b). The procedure described earlier[16] was modified as follows. A mixture of 5′-*O*-(4-methoxytrityl)uridine (4.54 g, 8.8 mmol) and *N*-benzyloxycarbonyl-L-phenylalanine (2.9 g, 9.7 mmol) was dried by evaporation with pyridine at 0.02 mm. The residue was dissolved in ice-cold pyridine (20 ml) and a cooled solution of DCC (1.86 g, 9 mmol) in pyridine (10 ml) was added. The reaction mixture was stirred for 1 hr at 0° and then for 23 hr at room temperature. Water (10 ml) was added, dicyclohexylurea was filtered off, washed with 50% pyridine (10 ml) and pyridine (10 ml); the filtrate was evaporated at 0.04 mm, the residue was dissolved in dioxane (100 ml), and the filtered solution was lyophilized. The

residue was dissolved in $CHCl_3$ and put onto a silica gel column (330 g, 85 × 3 cm). The column was eluted (flow rate 6 ml/min, 500-ml fractions taken) with $CHCl_3$ (500 ml), 1% CH_3OH in $CHCl_3$ (3000 ml), and 3% CH_3OH in $CHCl_3$ (4000 ml). Fraction 8 gave after evaporation syrupy 2′,3′-*O*-bis(*N*-benzyloxycarbonyl)-L-phenylalanyl-5′-*O*-(4-methoxytrityl)uridine, which was dissolved in CH_2Cl_2 and precipitated with petroleum ether to give an amorphous powder, 1.2 g (13%), tlc (S_4) homogeneous. Fractions 9–11 gave after evaporation a monoaminoacyl derivative contaminated as shown by tlc (S_4) with small amounts of the bis derivative and 5′-*O*-(4-methoxytrityl)uridine. A portion of this product was chromatographed on a loose layer of silica gel in S_1 to give after elution of the main band with the same solvent and evaporation of the eluate tlc (S_4) homogeneous 2′(3′)-*O*-(*N*-benzyloxycarbonyl)-L-phenylalanyl-5′-*O*-(4-methoxytrityl)uridine: nmr (CD_3COCD_3) δ 7.74 (d, 1, H_6, $J_{6,5}$ = 7.5 Hz), 7.23 and 7.04 (m, 24, phenyl and 4-methoxyphenyl partially overlapped with phenyl), 6.10 (d, $H_{1'}$ of 2′ isomer, $J_{1',2'}$ = 4.0 Hz), 5.98 (d, $H_{1'}$ of 3′ isomer, $J_{1',2'}$ = 5.0 Hz), 5.38 (d, 1, H_5, $J_{5,6}$ = 7.5 Hz), 3.73 (s, 3, CH_3O). The crude product was dissolved in 80% acetic acid (60 ml) and the solution was kept for 23 hr at room temperature and then lyophilized. The residue was dissolved in CH_2Cl_2 and precipitated with petroleum ether to give an amorphous powder which was chromatographed on two 4-mm thick layers of silica gel (35 × 15 cm) in S_2. The main band (**2b**) was eluted with the same solvent, the eluate was evaporated, and the residue was dissolved in CH_2Cl_2 and precipitated with petroleum ether to give a white, amorphous product that was homogeneous on tlc (S_5): yield 1.635 g (35%); nmr (CD_3COCD_3) δ 7.92 (d, 1, H_6, $J_{6,5}$ = 8 Hz), 7.27 (two poorly resolved s, 10, phenyl), 6.12 (d, $H_{1'}$ of 2′ isomer, $J_{1',2'}$ = 5 Hz), 5.97 (d, $H_{1'}$ of 3′ isomer, $J_{1',2'}$ = 6 Hz), 5.63 (d, 1, H_5, $J_{5,6}$ = 8 Hz), 5.06 (s, CH_2 of benzyl of 3′ isomer), 5.03 (s, CH_2 of benzyl of 2′ isomer). On addition of D_2O, the H_5 signals of the 2′ and 3′ isomers were resolved.

Further elution of the column with S_4 (3000 ml) afforded after evaporation of fractions 12–21 and precipitation of the residue from CH_2Cl_2 with petroleum ether starting 5′-*O*-(4-methoxytrityl)uridine, 1.255 g (28%).

Attempted Reaction of 2b with DCC in Pyridine. Compound **2b** (60 mg, 0.12 mmol) was dissolved in pyridine-d_5 (1 ml) dried with Linde molecular sieves (4A). DCC (0.125 g, 0.64 mmol) was added and the nmr spectrum of the clear, colorless solution was recorded in intervals of 15 min, 5 hr, 24 hr, and 1 week. No change in chemical shifts or coupling constants of the $H_{1'}$ and heteroaromatic protons was observed over a period of 7 days. No change was evident after heating the sample for 3 hr at 100°. In a blank experiment the nmr spectrum of the solution of DCC and compound **2b**, respectively, in pyridine-d_5 were scanned in the same intervals.

2′,5′-Di-*O*-(1-ethoxyethyl)uridine 3′-Phosphate (1a). A. A stirred suspension of uridine 3′-phosphate (NH_4 salt, 1.5 g, 4.5 mmol) in DMF (50 ml) containing ethyl vinyl ether (10 ml) was cooled to −30° and 4.47 *N* HCl in DMF (2 ml, 9 mmol) was added. The starting material gradually dissolved and a fine precipitate (NH_4Cl) appeared. The reaction mixture was stirred for 30 min at −30°, 30 min at −10°, and 45 min at 0°. Tlc (S_1) showed that the reaction was about 90% complete. Additional ethyl vinyl ether was added and stirring at room temperature was continued for 1.5 hr. As the reaction mixture darkened on standing at room temperature, the reaction mixture was stirred for an additional 1 hr at 0°. Concentrated NH_4OH (2 ml) was added, the white precipitate (NH_4Cl) was filtered off and washed with DMF, and the filtrate was evaporated at 0.1 mm and 35° (bath temperature). The residue was dissolved in 50% methanol and put on the top of the DEAE cellulose column (capacity 0.9 mequiv/ml, Sigma Chemical Co., St. Louis, Mo., HCO_3^- form, 85 × 2.5 cm). The chromatography was performed with a linear gradient of 0.2 *M* triethylammonium bicarbonate in 50% methanol (2 l.) in the reservoir and 50% methanol (2 l.) in the mixing vessel. The single nucleotide peak gave, after evaporation *in vacuo*, compound **1b** containing according to tlc (S_1) *ca.* 5% of a uv-absorbing contaminant of a lower mobility, presumably 5′-*O*-(1-ethoxyethyl)uridine 3′-phosphate. Crude **1a** (4.05 mmol) was chromatographed on two 5-mm thick loose layers (15 × 35 cm) of microcrystalline cellulose (Avirin, FMC Corp., American Viscose Division, Newark, Del.) in 1-butanol saturated with concentrated NH_4OH. The band of product **1a** was eluted with 1% triethylamine in methanol and the eluate was evaporated. The resultant residue was dried at room temperature (0.04 mm) to give a solid which, on washing with ether and further drying at room temperature (10^{-3} mm), gave 0.86 g (37%) of the triethylammonium salt of **1a** which was homogeneous according to paper chromatography (S_1, S_3) and paper electrophoresis: uv (0.01 *N* HCl) max 261 nm (ϵ 10,200), min 231 (2300).

Anal. Calcd for $C_{23}H_{44}N_3O_{11}P$: C, 48.50; H, 7.79; N, 7.38; P, 5.44. Found: C, 48.24; H, 8.06; N, 7.59; P, 5.43.

In another experiment performed as above with 1 g (3 mmol) of uridine 3′-phosphate (NH_4 salt), the filtrate obtained after treating the reaction mixture with NH_4OH and containing an excess of triethylamine was evaporated to *ca.* 20 ml. The residue was poured with stirring into the solution of $CaCl_2$ (2.5 g, 22.5 mmol) in ethanol (500 ml). On standing for 4 days at −20°, the Ca salt of **1a** was deposited as a solid (1 g, 48%) containing 5% of partially deblocked nucleotide **1a** according to paper chromatography (S_3); it was identical by paper chromatography and electrophoresis with the analytical sample: mol wt calcd for a decahydrate 688.6; found (spectrophotometrically) 696.

B. Uridine 3′-phosphate (pyridinium salt, 1 g, 2.5 mmol) was dried by evaporation with DMF (30 ml). The foamy residue was dissolved in DMF (25 ml) under N_2 and ethyl vinyl ether (5 ml) was added. The almost colorless solution was stirred for 24 hr at room temperature. To the resultant light brown solution triethylamine (5 ml) was added with stirring and the mixture was evaporated first using a rotary evaporator (35° bath temperature) and then at room temperature at 0.1 mm. The syrupy residue was dissolved in methanol (20 ml, 0.1% triethylamine) and the solution was added dropwise into vigorously stirred dry ether (500 ml, 0.1% triethylamine). The solution was kept at −20° overnight, a small amount of brown semisolid was filtered, and the filtrate was treated with an equal volume of petroleum ether (bp 30–60°) to give a syrup which solidified after standing overnight at 0°; 0.8 g (56%) of **1a** (triethylammonium salt) was obtained after washing with ether containing 0.1% triethylamine. The mother liquors were evaporated to dryness *in vacuo* to give 0.36 g (25%) of the same product. The product was identical (uv, paper chromatography in S_1 and S_3) with authentic specimens described above. It contained a trace of a uv-absorbing impurity (R_f 0.9).

Condensation of 2′,5′-Di-*O*-(1-ethoxyethyl) Nucleotides 1 with 2′(3′)-*O*-(*N*-Benzyloxycarbonyl)aminoacyl Nucleosides 2. Compound **1a** or **1b** (0.1 mmol) was dissolved in cold 5% pyridine (2–3 ml), the solution was passed through a 5-ml column of Dowex 50 (pyridinium) at 4°, and the column was eluted with 50% pyridine (15–20 ml). The eluate was evaporated *in vacuo* at room temperature and the residue was dried by coevaporation with anhydrous pyridine *in vacuo*. Nucleoside component **2** (0.2 mmol) was then added and the mixture was again coevaporated with anhydrous pyridine. Atmospheric pressure was always restored by dry nitrogen. The residue was dissolved in pyridine (1 ml), dicyclohexylcarbodiimide (DCC, 0.25 g, 1.2 mmol) was added, and the solution was kept in a well-stoppered flask for 4–6 days at ambient temperature. Ice and petroleum ether (10 ml) were added, the solution was diluted with 50% pyridine (*ca.* 10 ml) and extracted with petroleum ether (3 × 10 ml), and the precipitated dicyclohexylurea was filtered. The solution was evaporated *in vacuo* and the residue was lyophilized three times from dioxane, then dissolved in system S_2 (15 ml)[34] and kept overnight at room temperature.[31b] The solution was evaporated to dryness *in vacuo*, and the residue was dissolved in 80% acetic acid (15 ml) and kept for 4 hr in the case of compounds **4a**, **4b**, **4e**, and **4f** and 1 hr in the case of compounds **4c** and **4d**. The solution was lyophilized and the residue was dissolved in a minimum volume of ethanol–water or methanol containing a trace of acetic acid (*ca.* 1–2 ml) and applied to two cellulose layers (20 × 20 cm). The layers were developed in system S_6. Usually three bands were detected (in order of increasing R_f values): nucleoside 3′-phosphate, protected aminoacyl dinucleoside phosphate (**4**), and nucleoside (**2**). The band of product **4** was eluted with the system S_2 in a chromatographic column or by centrifugation and the eluate was evaporated *in vacuo* at room temperature. The residue was lyophilized from water (twice), dissolved in ethanol–water–CH_3COOH or methanol containing a trace of CH_3COOH,[35] and filtered through a small cotton filter. After the volume was adjusted with ethanol or methanol, the yield was measured spectrophotometrically. The products **4a–f** were chromatographically uniform in system S_2; ammonolysis (in S_1) led to the parent dinucleoside phosphates. For yields, uv spectra, and degradation with pancreatic ribonuclease, see Table IV.

2′(3′)-*O*-Aminoacyl Dinucleoside Phosphates (5). *N*-Benzyloxycarbonyl derivative **4** (10–20 μmol) was dissolved in 80% acetic acid (5 ml), 5% PdO–$BaSO_4$ (100–200 mg) was added, and a gentle stream of hydrogen was bubbled through the stirred solution at 0°. After 1.5 hr the catalyst was filtered through a Celite

bed and the filtrate was lyophilized. The residue was dissolved in 80% acetic acid (10 ml) and the yield was determined spectrophotometrically after appropriate dilution with 0.01 *N* HCl (Table V). Aliquots of the stock solution were pipetted for enzymatic degradations, alkaline hydrolysis (which gives the parent amino acid and dinucleoside phosphate), tlc, paper chromatography, and electrophoresis. In the case of cytidine derivatives **5a–f,** no further purification was necessary. Compounds **5c** and **5d** were purified by preparative tlc (S_6). The major band was eluted with solvent S_2 and the eluate was evaporated at room temperature and 0.1 mm. Lower yields reflect this purification and volatility of the products (fluffy powders) during high-vacuum evaporation.

Reaction of Compound 4a with Dimethylformamide Dineopentyl Acetal. Compound **4a** (0.43 μmol) was repeatedly coevaporated with DMF, dissolved in the same solvent (0.5 ml); dimethylformamide dineopentyl acetal (0.1 ml) was added and the solution was kept at room temperature for 16 hr. After evaporation *in vacuo* the uv spectrum of the residue (95% ethanol) showed max 312 nm and min 266 nm, which corresponds to bis-*N*-dimethylaminomethylene derivative of C-A.

Reaction of Nucleotides 1a and 1b with TPS. The pyridinium salt of nucleotide **1a** or **1b** (0.1 mmol) was repeatedly evaporated with pyridine and dissolved in pyridine (2 ml) and TPS) (150 mg, 0.5 mmol) was added. After 3 days at room temperature ice was added, the solution was evaporated, the residue was dissolved in water, and the solution was lyophilized. Paper chromatography of the crude product (S_1) revealed several spots, including the nucleoside 2′,3′-cyclic phosphate, the nucleoside 2′(3′)-phosphate, starting material **1a** or **1b,** and symmetrical pyrophosphate derived from **1a** or **1b.**

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Registry No.—**1a** triethylammonium salt, 51599-88-3; **2b** 2′ isomer, 51599-89-4; **2b** 3′ isomer, 27552-68-7; **2c,** 27552-73-4; **2d,** 51599-90-7; **4a** 2′ isomer, 51599-91-8; **4a** 3′ isomer, 51599-92-9; **4b** 2′ isomer, 51599-93-0; **4b** 3′ isomer, 51599-94-1; **4c** 2′ isomer, 51599-95-2; **4c** 3′ isomer, 51599-96-3; **4d** 2′ isomer, 51599-97-4; **4d** 3′ isomer, 51599-98-5; **4e,** 51599-99-6; **4f,** 51635-70-2; **5a** 2′ isomer, 51600-00-1; **5a** 3′ isomer, 51600-01-2; **5b** 2′ isomer, 51600-02-3; **5b** 3′ isomer, 51600-03-4; **5c** 2′ isomer, 51600-04-5; **5c** 3′ isomer, 51600-05-6; **5d** 2′ isomer, 51600-06-7; **5d** 3′ isomer, 51600-07-8; **5e,** 51600-08-9; **5f,** 51600-09-0; 2′-deoxy-5′-*O*-(4-methoxytrityl)adenosine, 51600-10-3; *N*-benzyloxycarbonyl-L-phenylalanine, 1161-13-3; *N*-dimethylaminomethyleneadenosine, 17331-15-6; 5′-*O*-(4-methoxytrityl)adenosine, 51600-11-4; 5′-*O*-(4-methoxytrityl)uridine, 51600-12-5; 2′-*O*-(*N*-benzyloxycarbonyl)-L-phenylalanyl-5′-*O*-(4-methoxytrityl)uridine, 51635-71-3; 3′-*O*-(*N*-benzyloxycarbonyl)-L-phenylalanyl-5′-*O*-(4-methoxytrityl)uridine, 51635-72-4; uridine 3′-phosphate NH_4 salt, 51600-13-6.

References and Notes

(1) (a) For a preceding report of this series (XVII), see S. Chládek, D. Ringer, and J. Žemlička, *Biochemistry,* **12,** 5135 (1973). (b) Presented in part at the 6th Regional Meeting of the American Chemical Society, Houghton, Mich., June 1972, Abstracts, p 23. (c) This investigation was supported in part by U. S. Public Health Service Research Grant GM-19111 from the Division of General Medical Sciences. (d) For abbreviations used, see *Biochim. Biophys. Acta,* **247,** 1 (1971), and ref 1a. Other abbreviations: tlc, thin layer chromatography; DCC, dicyclohexylcarbodiimide; TPS, 2,4,6-triisopropylbenzenesulfonyl chloride; DSS, sodium 2,2-dimethyl-2-silapentane-5-sulfonate; DMF, dimethylformamide; EtOEt, 1-ethoxyethyl; C-A-Phe, cytidylyl-(3′-5′)-2′(3′)-*O*-L-phenylalanyladenosine; C-U-Phe, cytidylyl-(3′-5′)-2′(3′)-*O*-L-phenylalanyluridine; U-A-Phe, uridylyl-(3′-5′)-2′(3′)-*O*-L-phenylalanyladenosine; U-U-Phe, uridylyl-(3′-5′)-2′(3′)-*O*-L-phenylalanyluridine; C-dA-Phe, cytidylyl-(3′-5′)-2′-deoxy-3′-*O*-L-phenylalanyladenosine; C-dA-Gly, cytidylyl-(3′-5′)-2′-deoxy-3′-*O*-glycyladenosine; C-A(Z-Phe), cytidylyl-(3′-5′)-2′(3′)-*O*-(*N*-benzyloxycarbonyl-L-phenylalanyl)adenosine; C-U(Z-Phe), cytidylyl-(3′-5′)-2′(3′)-*O*-(*N*-benzyloxycarbonyl-L-phenylalanyl)uridine; U-A(Z-Phe), uridylyl-(3′-5′)-2′(3′)-*O*-(*N*-benzyloxycarbonyl-L-phenylalanyl)adenosine; U-U(Z-Phe), uridylyl-(3′-5′)-2′(3′)-*O*-(*N*-benzyloxycarbonyl-L-phenylalanyl)uridine; C-dA(Z-Phe), cytidylyl-(3′-5′)-2′-deoxy-3′-*O*-(*N*-benzyloxycarbonyl-L-phenylalanyl)adenosine; C-dA(Z-Gly), cytidylyl-(3′-5′)-2′-deoxy-3′-*O*-(*N*-benzyloxycarbonylglycyl)adenosine.

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(21) It should be kept in mind that the effectiveness of protection may vary with the nature of the N-blocking group and amino acid itself.

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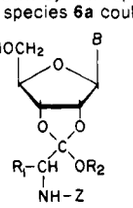

6

6a R_1 = alkyl or aralkyl, R_2 = H

6b R_1 = alkyl or aralkyl, R_2 = R_3–O–P(=O)(OH)–O–

where R_3 is a ribonucleoside 3′-residue

tent for the protection of 2′ or 3′ hydroxy groups in **2a** and **2b.** However, a possible interaction of **6a** with the nucleotide component under the reaction conditions to give a mixed anhydride derivative **6b** *cannot be rigorously excluded.* In such a case, because of an excess of nucleoside component **2a** or **2b** present, **6b** could in principle phosphorylate both itself and the "uncyclized" **2a** or **2b.** Because of bulkiness of the "leaving group" (anion of **6a**) interme-

diate **6b** might also contribute to the observed high selectivity of phosphorylation.

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Total Synthesis of Steroids. V.[1] Synthesis of *rac*-3-Methoxy-14α-hydroxy-8α-estra-1,3,5(10)-triene-11,17-dione and Its Derivatives

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The cyclization of 8,14-seco-3-methoxy-11ξ-hydroxyestra-1,3,5,8-tetraene-14,17-dione (**2a**) in the presence of Meerwein reagents led to the formation of tetracyclic products **6a** and **6b**. The stereochemistry of the latter compounds was established by spectral evidence and by their conversion to *rac*-13-isoestrone. Further transformations of **6a** and **6b** led to the new 11-keto compound **19** epimeric at C-14.

In our first paper on the total synthesis of steroids[2] we reported on the peracid oxidation of Torgov's secodione **1** and now we wish to describe the complete synthesis of the title compound from the same starting material. The secodione **1** was converted as previously described[2] into the mixture of **2a** and **2b**, which was submitted to the action of different acidic reagents in order to produce tetracyclic compounds. The resulting products are presented in Scheme I and listed in Table I. The trione **3**, which did not react further, was obtained in all instances except from the reaction with acetic acid–boron trifluoride. Surprisingly, in three cases 14-dehydroequilenin methyl ether was obtained in substantial amounts. We suggest that this is produced by dehydration of **2a**, leading to an intermediate A, which undergoes an isomerization to the naphthalene derivative B (Scheme II). The latter can cyclize easily to yield **4**, as is known from the literature[3] and as was proved in our previous paper.[4]

We reported previously[2] on the synthesis of the tetracyclic ketones **6a,b**[5] from **2a** in very low yield by the action of boron trifluoride etherate. We have now found that the yield of these required compounds can be increased tenfold by use of Meerwein reagents, *e.g.*, $Me_3O^+BF_4^-$ or $Et_3O^+BF_4^-$ (Scheme III). We did not investigate the course of the reaction of Meerwein reagents with **2a,b** in detail, but we observed on tlc plates the formation of ketal **2c**, which could be isolated and converted in a separate experiment into the triketone **3a,b**. The latter compound is almost always the minor product in the cyclization reaction. Another product formed initially under the influence of $Et_3O^+BF_4^-$ has a polarity similar to that of

Table I

No.	Acid	Product (yield, %)
1	$SnCl_4$	**3a,b** (95) + traces of other products
2	$CH_3CO_2H + BF_3 \cdot Et_2O$	**3a,b** (50) + **4** (24)
3	$CH_3CO_2H + BF_3 \cdot CH_3CO_2H$	**4** (40) + **5** (41)
4	CF_3CO_2H	**3a,b** (50) + (50)
5	$BF_3 \cdot Et_2O$	**3a,b** (33) + **6a,b** (6.5)
6	Meerwein reagents	**3a,b** (25) + **6a,b** (75)
7	CH_3CO_2H and chlorinated acetic acids	No effect

Scheme I