was isolated as a precipitate from hexane and dried to give 10.2 mg (61%) of material which was homogeneous on TLC in ethyl acetate–tetrahydrofuran (1:1, R_f 0.22) and in tetrahydrofuran (R_f 0.54). This material was purified for analysis by recrystallization from tetrahydrofuran and isolated as a bis solvate: mp 168–170 °C (loss of solvent at 152–155 °C); λ_{max} (CH_3OH) 267 nm (ϵ 2.8 × 10^4), λ_{min} 234 nm.

Anal. Calcd for $C_{51}H_{54}N_8O_{15}\cdot 2C_4H_8O$: C, 60.91; H, 6.06; N, 9.63. Found: C, 60.75; H, 6.07; N, 9.34.

The trityl blocking group was removed from compound **7** (30 mg) by the usual treatment with hot 80% acetic acid. After preparative TLC on a 1 mm thick silica plate with dioxane, compound **8** was isolated in a 70% yield: R_f 0.51 in tetrahydrofuran; λ_{max} 267 nm; paper chromatography R_f 0.38.

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Registry No.—**1b,** 60920-99-2; **2a** (Ar = *p*-nitrophenyl), 10270-35-6; **2a** (Ar = phenyl), 34311-55-2; **2b** (Ar = *p*-nitrophenyl), 60921-00-8; **3,** 25152-20-9; **4a,** 54666-95-4; **4b,** 60921-01-9; **5a,** 54667-52-6; **5b,** 60921-02-0; **6,** 60921-03-1; **7,** 60921-04-2; **8,** 60921-05-3; *p*-nitrophenyl chloroformate, 7693-46-1.

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Simple Models of Nucleic Acid Interactions. 2. Aminoacyl Derivatives of "Bridged" Nucleosides: Synthesis of 2′(3′)-*O*-L-Phenylalanyl- and 2′(3′)-*O*-L-Leucyl-1,2-di(adenosin-N^6-yl)ethane[1]

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The synthesis of the title compounds XIIa and XIIb is described. 2′,3′-*O*-Isopropylidene-1,2-di(adenosin-N^6-yl)ethane (I) on reaction with 4-methoxytrityl chloride in pyridine gave ditrityl derivative III accompanied by tritrityl compound IV. The formation of IV was suppressed by blocking of the remaining cis-diol group in I with 2′,3′-*O*-dimethylaminomethylene function (intermediate VII). Acetylation of I gave the corresponding tetraacetyl derivative II, whereas III and IV afforded di- and monoacetyl derivatives V and VI, respectively. Condensation of III with ZPheOH or ZLeuOH using dicyclohexylcarbodiimide in pyridine led to the phenylalanyl or leucyl derivative VIIIa and VIIIb. Deblocking of VIIIa and VIIIb with 80% acetic acid afforded intermediates Xa and Xb which after treatment with 90% trifluoroacetic acid or Dowex 50 (H^+) gave the *N*-benzyloxycarbonylaminoacyl derivatives XIa and XIb as the mixtures of 2′ and 3′ isomers. Hydrogenolysis of XIa and XIb using PdO–$BaSO_4$ in cold 80% acetic acid as catalyst led to the phenylalanyl and leucyl derivatives XIIa and XIIb. Equilibration of 2′ and 3′ isomers of compounds XIa and XIb is also described.

According to the current views[2] protein biosynthesis takes place in two distinct steps: (a) formation of aminoacyl transfer ribonucleic acids (AA-tRNA) catalyzed by aminoacyl–tRNA synthetases and (b) formation of the peptide bond between peptidyl and aminoacyl tRNA which is catalyzed by ribosomes. The process (a) involves activation of an amino acid (AA) by reaction with adenosine 5′-triphosphate (ATP) to give aminoacyl adenylate (AA-AMP) and inorganic pyrophosphate (PP, Scheme I, eq 1) followed by a transfer of aminoacyl residue of AA-AMP to the 2′ or 3′ hydroxy group of tRNA's terminal adenosine unit[3a] (Scheme I, eq 2). An alternate concerted mechanism has also been proposed where ATP, AA, and tRNA react simultaneously (Scheme II) to afford AA-tRNA, AMP, and PP.[3a] Although a considerable body of information has been gathered on the substrate requirements of the process,[3a,b] the mutual orientation (topochemistry) of AA-AMP and tRNA in the last step of the transformation (Scheme I, eq 2) remains an intriguing problem of molecular biology.

Scheme I

$$\text{AA} + \text{ATP} \rightleftharpoons \text{AA-AMP} + \text{PP} \quad (1)$$

$$\text{AA-AMP} + \text{tRNA} \rightleftharpoons \text{AA-tRNA} + \text{AMP} \quad (2)$$

Scheme II

$$\text{AA} + \text{ATP} + \text{tRNA} \rightleftharpoons \text{AA-tRNA} + \text{AMP} + \text{PP}$$

It is conceivable that in the process of AA-tRNA formation the adenosine moiety of AA-AMP and that of the 3′ terminal of tRNA are stacked. Thus, a space-filling (CPK) model can be constructed for such a situation (adenine–adenine stacking) in which the aminoacyl residue of AA-AMP would be in a suitable position to attack the 2′ or 3′ hydroxy group of the adenosine terminal unit of tRNA (Figure 1).

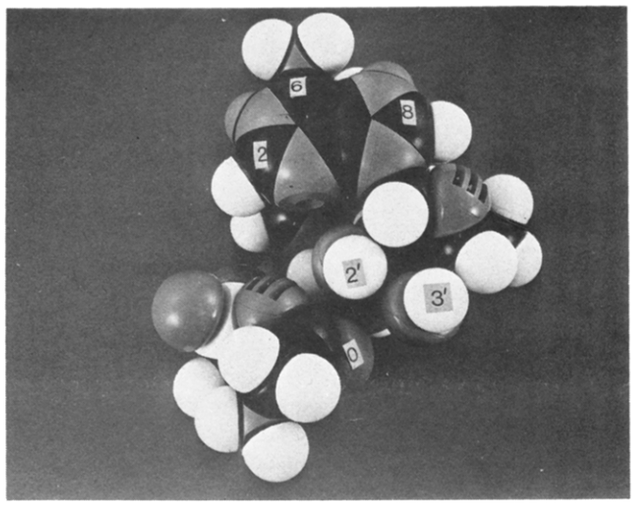


Figure 1. Possible CPK model of transfer of an aminoacyl (glycyl) residue from AA-AMP to tRNA. For simplicity only terminal adenosine of tRNA is shown. Conformations of both adenine residues and $C_{4'}$–$C_{5'}$ are arbitrary anti-g,g. Note the stacking of adenine rings and the closeness of glycyl carbonyl group (O) to the 2′ and 3′ hydroxy groups of terminal adenosine. Adenine moiety of adenosine is on top of that of AA-AMP. Also note the possibility of bridging the N-6 positions of both bases. Numbering is in accord with the current nomenclature of purine ribonucleosides.

It was, therefore, of interest to study simple adenosine derivatives which would combine the structural features of aminoacyl adenylates, AMP, and AA-tRNA, including the stacking of the adenine residues (transition state analogues). A "bridged" nucleoside in which two adenosine units were covalently joined through an aliphatic chain of the N-6 positions to ensure base stacking and containing an amino acid residue could, therefore, be useful. The synthesis and stacking properties of some N^6–N^6 bridged adenosine nucleosides have been the subject of a previous report.[1a] In this communication we describe the introduction of one amino acid residue into a bridged adenine nucleoside leading to compounds XIIa and XIIb.

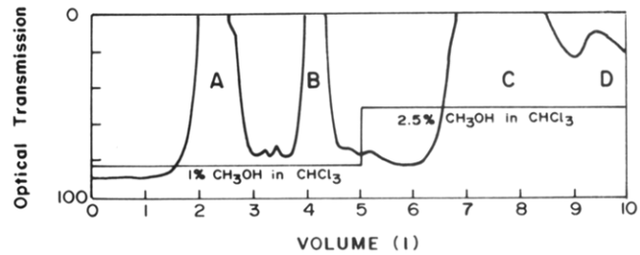


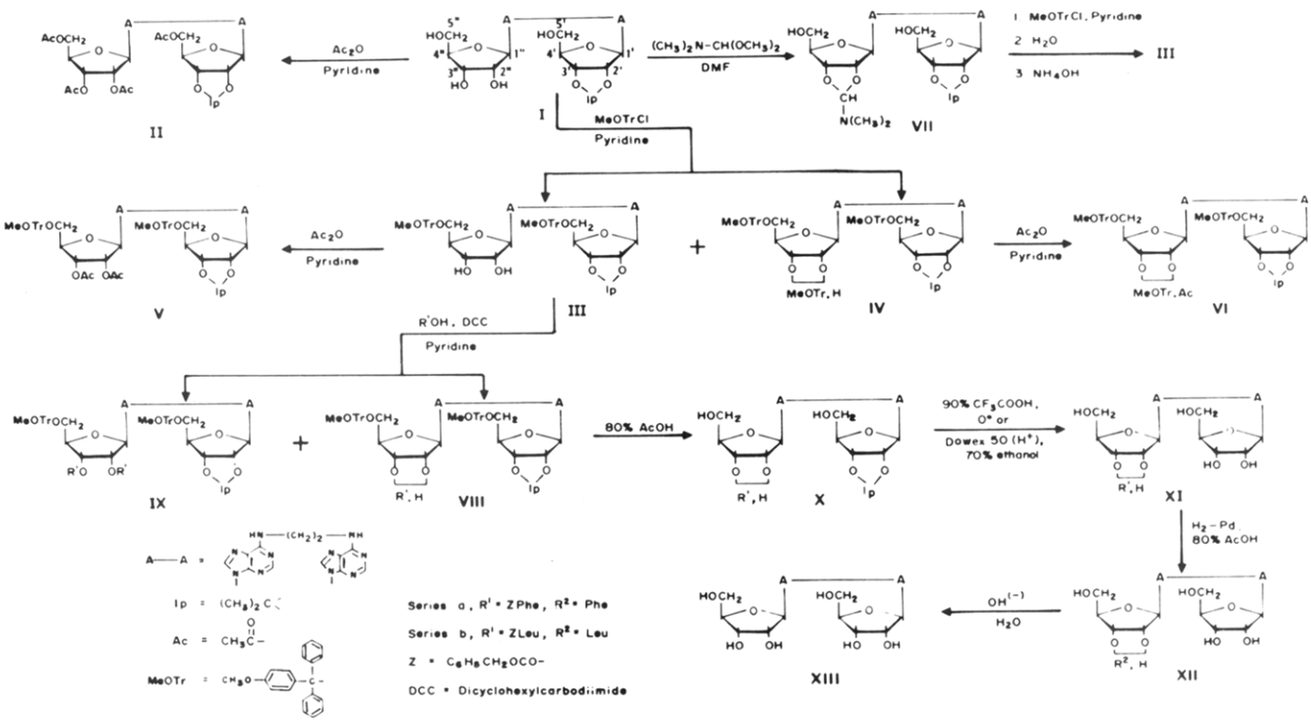
Figure 2. Chromatography of the products from the reaction of 4-methoxytrityl chloride with compound I in pyridine on 45 × 5 cm silica gel column (see Experimental Section). Identification of peaks: A, 4-methoxytriphenylcarbinol; B, tritrityl derivative IV; C, dititryl derivative III; D, monotrityl derivative(s). Tops of the main peaks were off scale.

The selective substitution of one ribofuranose residue in a bridged nucleoside by an amino acid requires a specific strategy not dissimilar from that employed in the synthesis of oligonucleotides.[4] However, the considerable lability of an aminoacyl ester group at the 2′ or 3′ position of a ribonucleoside severely restricts the choice of appropriate protecting groups for the successful course of the synthesis.

The 2′,3′-*O*-isopropylidene-1,2-di(adenosin-N^6-yl)ethane (I) provided a convenient starting material in the present approach. The latter, which was prepared in 70% yield by an improved coupling procedure[1a] from 6-chloro-9-(β-D-2,3-*O*-isopropylideneribofuranosyl)purine with N^6-(2-aminoethyl)adenosine, has both ribofuranose residues (vicinal glycol groups) functionally differentiated. In addition, the 2′,3′-*O*-isopropylidene group can be easily removed with trifluoroacetic acid[5] using conditions under which the aminoacyl ester bond is essentially stable.[6]

Compound I was tritylated using 2 equiv of 4-methoxytrityl chloride in pyridine to give two major products, the desired dititryl derivative III (55%) and tritrityl derivative IV (12%), which were isolated by column chromatography (Figure 2). The formation of IV could be suppressed with a stoichiometric amount of 4-methoxytrityl chloride (2 mol) but the reaction mixture contained a considerable amount of monotrityl derivatives as indicated by TLC (Scheme III).

Scheme III



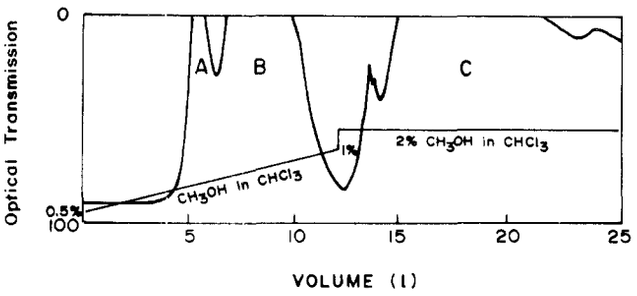


Figure 3. Chromatography of the products from the condensation of ZLeuOH with compound III using DCC in pyridine on 50 × 5 cm silica gel column (see Experimental Section). Identification of peaks: A, dileucyl derivative IXb; B, monoleucyl derivative VIIIb; C, starting material III. Tops of the main peaks were off scale.

The structures of III and IV followed from UV and NMR spectra. Thus, the UV spectra (the longer wavelength absorption bands) were very similar to the parent nucleoside XIII which indicated the absence of N-tritylation.[7] NMR spectra of III and IV (integration of aromatic protons) were in agreement with the presence of two and three trityl groups, respectively. Interestingly, the aromatic methoxy groups were distinctly different in III whereas in compound IV only a single signal for all three methoxy groups was observed. It is recognized that the conformation of ribofuranose and 2′,3′-*O*-isopropylideneribofuranose moieties is different.[8] This difference may be reflected in magnetic nonequivalency of the two methoxy groups. It is, however, more difficult to explain the presence of only one signal for the methoxy group in the tritrityl derivative IV. One can argue that introduction of a second trityl group into the ribofuranose moiety of III may lead to a conformational change similar to that caused by 2′,3′-*O*-isopropylidene group. However, a simple comparison of $J_{1',2'}$ for 2′,5′- and 3′,5′-di-*O*-trityluridine (7.5 and 5 Hz, respectively)[9] with that of 2′,3′-*O*-isopropylideneuridine (2.4 Hz)[8a] is not compatible with such an argument.[10] It appears then that more subtle conformational changes resulting in the different shielding of 4-methoxytrityl groups in III and IV are operational.

The structures of III and IV were further confirmed by oxidation with periodate (only III is oxidized) and by acetylation studies. Acetylation of I using acetic anhydride in pyridine gave only the tetraacetyl derivative II whose UV spectrum was very similar to that of XIII. Thus, the exocyclic (N-6) imino groups are not acetylated under the conditions used. An NMR spectrum of II showed the acetoxy methyl groups as two singlets which integrated for 3 and 9 protons, respectively. As expected, acetylation of III and IV led to the formation of di- and monoacetyl derivatives V and VI whose UV and NMR spectra were in accord with the proposed structures and with the absence of any N-tritylation or N-acetylation. The methoxy group in the NMR spectrum of V showed as two singlets (cf. compound III) whereas the acetoxy signals (methyl) appeared as a lone singlet. On the other hand, all three methoxy groups in VI were nonequivalent which is in sharp contrast to the findings for compound IV. It was possible to separate 2′- and 3′-*O*-acetyl isomers of compound VI by preparative TLC. The acetoxy (methyl) signal in the faster moving (presumably 3′-*O*-acetyl isomer) was slightly more shielded than that of the 2′ isomer. The fact that both isomers were identified is consistent with the possibility that compound IV (starting material for VI) is also a mixture of isomeric 2′ and 3′ tritylated derivatives.

Undesired 2′(3′)-*O*-tritylation could be suppressed if compound I first was reacted with dimethylformamide dimethyl acetal in dimethylformamide (DMF).[11] The resultant 2′,3′-*O*-isopropylidene-2″,3″-*O*-dimethylaminomethylene derivative VII, obtained as a syrup, was then tritylated as above to give III in 85% yield after hydrolysis of the 2″,3″-*O*-dimethylaminomethylene group in NH_4OH (Scheme III).

Protected compound III with a free vicinal glycol grouping was condensed with *N*-benzyloxycarbonyl-L-phenylalanine (ZPheOH) or *N*-benzyloxycarbonyl-L-leucine (ZLeuOH) using dicyclohexylcarbodiimide (DCC) in pyridine.[12] The resultant mixture, consisting of the starting material III, monoaminoacyl derivative VIIIa or VIIIb, and diaminoacyl compound IXa or IXb, was separated by preparative TLC (series a) or column chromatography (series b, Figure 3). The products were characterized by their UV and NMR spectra. The integrated NMR signals in VIIIa and VIIIb were in accord with the presence of only one aminoacyl residue (Phe or Leu) in the molecule. As in compound III, the methoxy groups in both VIIIa and VIIIb showed as two well-separated singlets. The attempted condensation of tritrityl derivative IV with ZPheOH using DCC in pyridine was not successful, presumably owing to the steric hindrance of the 2′ or 3′ hydroxy groups by a neighboring bulky 4-methoxytrityl function. By contrast, acetylation of IV was achieved without difficulty (cf. compound VI).

The removal of 4-methoxytrityl and isopropylidene groups was performed separately. Thus, for reasons which are not apparent the complete deprotection of VIIIa in a single step using 90% trifluoroacetic acid afforded XIa in a low yield after extensive purification by TLC. Therefore, the 4-methoxytrityl group was selectively removed in 80% acetic acid to give the isopropylidene derivatives Xa and Xb which were purified by preparative TLC. Compounds Xa and Xb were then treated with 90% trifluoroacetic acid for 20 min at 0 °C to give the corresponding *N*-benzyloxycarbonyl derivatives XIa and XIb in 83 and 85% overall yield, respectively. Alternatively, the isopropylidene group of Xa and Xb could be removed by treatment with Dowex 50 (H^+) in 70% ethanol for 1 h at room temperature. The latter procedure is very mild and accordingly should find a wider application in nucleic acid chemistry.

The structures XIa and XIb were confirmed by spectral (UV and NMR) data. Although two sets of $H_{1'}$ signals corresponding to two ribofuranose moieties are clearly discernible in the NMR spectra of XIa and XIb, it was impossible to determine the ratio of 2′ and 3′ aminoacyl isomers, primarily because of insufficient resolution of signals. However, direct evidence for the presence of both isomers was provided by TLC in solvent S_6 where compounds XIa and XIb moved as two distinct spots in the ratio of 7:3; the faster moving component was more abundant. It seemed conceivable that in both cases the 2′ and 3′ aminoacyl isomers were resolved. Thus, the ratio 7:3 corresponded as well to the isomeric composition of related 2′(3′)-*O*-(*N*-benzyloxycarbonyl)aminoacyl ribonucleosides.[13] In addition, the prevalence of the faster moving component indicated that it was probably the 3′ isomer.[14] In a micropreparative (μmol scale) experiment the separated bands of both isomers were equilibrated at room temperature in solvent S_5. A 7:3 mixture of isomers was again obtained from both bands and in both cases (XIa and XIb) after TLC in solvent S_6.

In the final step, the *N*-benzyloxycarbonyl group of XIa and XIb was removed by hydrogenolysis using PdO–$BaSO_4$ as catalyst in 80% acetic acid at 0 °C for 2 h in the usual fashion.[12] The resultant phenylalanyl and leucyl derivatives XIIa and XIIb, obtained in 81 and 85% yield, respectively, were characterized by TLC, paper chromatography, electrophoresis, and UV spectra. In addition, alkaline hydrolysis afforded the parent nucleoside XIII and amino acid (PheOH or LeuOH) in a manner similar to other 2′(3′)-*O*-aminoacyl nucleosides.[12]

The biological testing of XIIa and XIIb along with that of some precursors will be reported elsewhere.

Experimental Section

General Procedures. See ref 1a. Samples for analysis were dried at room temperature for 15–20 h at 10^{-2} mm over P_2O_5 and paraffin oil. Descending paper chromatography was performed on Whatman No. 1 paper in the following solvents: S_1, 2-propanol–concentrated NH_4OH–water (7:1:2), and S_2, 1-butanol–acetic acid–water (5:2:3). Thin layer chromatography (TLC) including preparative TLC was conducted as described[1a] in solvents: S_3, chloroform–methanol (95:5); S_4, chloroform–methanol (9:1); S_5, chloroform–methanol (4:1); and S_6, chloroform–methanol–acetic acid (8:1:1). For paper electrophoresis 0.02 M $Na_2B_4O_7$ (pH 9.0) and 1 M acetic acid were used as buffers at 40 V/cm and 1–2 h. NMR spectra were determined using a Varian A-60A instrument. Tetramethylsilane was used as an internal reference in $CDCl_3$ whereas in CD_3SOCD_3 the internal reference was sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). Detection of cis-diol groups was performed on TLC plates by spraying with 0.5% aqueous $NaIO_4$ and, after several minutes, with 0.5% benzidine in acetic acid–ethanol (1:4) mixture. A positive reaction showed a white spot on a dark blue background.[15] Because benzidine is carcinogenic the use of a hood for the spraying operation is imperative. 4-Methoxytrityl groups were detected by spraying with 10% $HClO_4$.[16]

Column Chromatography. The short column chromatographic technique[17] was modified as follows. Silica gel GF 254 (type 60, for TLC, Merck, Darmstadt, Germany) and glass columns (Glenco Scientific, Inc., Houston, Texas, 60 × 5 cm) were used. Silica gel was mixed in a blender with chloroform or dichloromethane (3.5 ml/l g) and the slurry was poured into the column with the tap open. A slight pressure of nitrogen was then applied until the descendant liquid caught up with the silica gel surface; 100 g of silica gel settles to ca. 200 ml. The solution of the mixture to be separated was allowed to soak into the column without applying any pressure, the space above the silica gel was filled with the solvent, and FMI laboratory pump Model RPP (Fluid Metering, Inc., Oyster Bay, N.Y.) was attached. The use of an adjustable plunger (Glenco Scientific Co., Houston, Texas) eliminates the dead volume above the silica gel which is of particular importance when a gradient elution technique is employed. It is imperative to remove all air from the solvents used by evacuation (aspirator). All air bubbles from the whole system should be removed prior to the elution by opening the vent in the top screw of the column. The chromatography was monitored continuously by UV absorption (transmission) at 260 nm using LKB Uvicord II (LKB, Bromma, Sweden) and the fractions were collected using a linear fraction collector LKB Ultrorac. The flow rate was maintained at 3 ml/min. The ratio of the mixtures of compounds to silica gel was 1/40 (w/w) for easy separations (as judged from TLC) and 1/100 for difficult ones.

Starting Materials and Reagents. Pyridine (reagent grade) was distilled twice (from *p*-toluenesulfonyl chloride and KOH) and it was stored over Linde molecular sieves, 4A. Trifluoroacetic acid was distilled before use. 4-Methoxytrityl chloride (MeOTrCl), dimethylformamide dimethyl acetal, and dicyclohexylcarbodiimide (DCC) were products of Aldrich Chemical Co., Milwaukee, Wis. *N*-Benzyloxycarbonyl-L-phenylalanine (ZPheOH) was purchased from Sigma Chemical Co., St. Louis, Mo., and *N*-benzyloxycarbonyl-L-leucine (ZLeuOH) was the product of Tridom Fluka Chemical Co., Hauppauge, N.Y.

2',3'-*O*-Isopropylidene-1,2-di(adenosin-N^6-yl)ethane (I). The previously described[1a] procedure was modified as follows. The solution of 6-chloro-2',3'-*O*-isopropylidene(9-β-D-ribofuranosyl)purine (6.2 g, 20 mmol), N^6-(2-aminoethyl)adenosine[18] (6.55 g, 20 mmol), and triethylamine (14.05 ml, 100 mmol) in DMF (100 ml) was stirred for 4 days at room temperature. Crystalline triethylamine hydrochloride was filtered off and the filtrate was evaporated at 0.04 mm and room temperature. The residue was dissolved in 50% aqueous ethanol (100 ml) and the solution was passed through a Dowex 1 (X2, 200–400 mesh) column (150 ml, HCO_3^- form). The column was eluted with the same solvent (1.7 l.). Evaporation of the eluate afforded a white solid (12 g) which was washed twice with acetone (50 and 20 ml). The resultant solid was dissolved in 50% aqueous pyridine (80 ml) and the solution was passed through a Dowex 50 (X2, 100–200 mesh) column (350 ml, pyridinium form) and the column was eluted with the same solvent (2 l.). Evaporation of the eluate gave a white solid which was washed with acetone–ether to afford 8.4 g (70%) of I, mp 154–156 °C, lit.[1a] 154–155 °C, homogeneous on TLC (S_5) and paper electrophoresis ($Na_2B_4O_7$); UV and NMR spectra were identical with those of an authentic sample.[1a]

5',5''-*O*-Di(4-methoxy)trityl-2',3'-*O*-isopropylidene-1,2-di(adenosin-N^6-yl)ethane (III) and 2''(3''),5',5''-*O*-tri(4-methoxy)trityl-2',3'-*O*-isopropylidene-1,2-di(adenosin-N^6-yl)ethane (IV). A. Direct Tritylation of I. A solution of compound I (6.0 g, 10 mmol) and 4-methoxytrityl chloride (12.36 g, 40 mmol) in pyridine (50 ml) was stirred for 10 h at room temperature. The reaction mixture was then added dropwise into cold water (1 l.) with stirring, and the precipitate was collected by filtration, washed with water, and dried at 0.04 mm at room temperature. The crude product was dissolved in a minimum amount of chloroform and the solution was added dropwise into an excess of petroleum ether. The resultant solid was collected by filtration and further purified by column chromatography on 400 g of silica gel (Figure 2). Peak B afforded after evaporation compound IV (1.7 g, 12%), homogeneous on TLC (S_3), UV max (ethanol) 274 nm (ε 34 800), min 249 (24 200); NMR ($CDCl_3$) δ 8.43 and 8.17 (2 s, 2, H_8), 7.82 and 7.66 (2 s, 2, H_2, the signal at 7.66 is partially overlapped with phenyl), 7.59–6.70 (m, 42, 4-methoxytrityl), 6.07 (broad s, 2, $H_{1'}$), 3.56 (s, 9, CH_3O), 1.62 and 1.42 (2 s, 6, CH_3 of isopropylidene).

Anal. Calcd for $C_{85}H_{80}N_{10}O_{11}$: C, 72.01; H, 5.69; N, 9.88. Found: C, 71.76; H, 5.77; N, 9.60.

Evaporation of peak C afforded ditrityl derivative III (6.3 g, 55%): homogeneous on TLC (S_3); UV max (ethanol) 274 nm (ε 33 500), min 248 (20 200); NMR ($CDCl_3$) δ 8.18 and 8.10 (2 s, 2, H_8), 7.97 and 7.55 (2 s, 2, the signal at 7.55 is partially overlapped with phenyl), 7.55–6.70 (m, 28, 4-methoxytrityl), 6.30 and 6.08 (2 broad s, 2, $H_{1'}$), 3.64 and 3.49 (2 s, 6, CH_3O), 1.65 and 1.46 (2 s, 6, CH_3 of isopropylidene).

Anal. Calcd for $C_{65}H_{64}N_{10}O_{10}$: C, 68.16; H, 5.63; N, 12.23. Found: C, 68.31; H, 5.69; N, 12.00.

B. Tritylation via Intermediate VII. Compound I (6.0 g, 10 mmol) was dried by evaporation with DMF at 0.05 mm and room temperature, the residue was dissolved in DMF (50 ml), dimethylformamide dimethyl acetal (13.4 ml, 100 mmol) was added, and the solution was kept for 17 h at room temperature. Evaporation at 0.05 mm and room temperature afforded a syrup VII which was coevaporated with DMF (50 ml) and dried at 0.05 mm and room temperature overnight. The residue was dissolved in pyridine (50 ml), 4-methoxytrityl chloride (18.5 g, 60 mmol) was added, and the solution was stirred at room temperature for 20 h. The reaction mixture was then poured on ice (ca. 700 g) and extracted with chloroform (3 × 400 ml) and the combined organic layers were dried ($MgSO_4$). Evaporation afforded a syrup which was coevaporated with ethanol (500 ml) and dissolved in a mixture of dioxane (100 ml) and concentrated NH_4OH (10 ml). The solution was kept overnight at room temperature and then it was evaporated. The residue was partitioned between chloroform and water, and the organic layer was dried ($MgSO_4$) and evaporated to give III as a TLC (S_3) homogeneous foam. Purification by column chromatography (see method A and Figure 2) afforded 9.7 g (85%) of III, UV and NMR identical with those of the sample obtained by method A.

Aminoacylation of Ditrityl Derivative III. A. Preparation of Phenylalanyl Derivatives VIIIa and IXa. A mixture of compound III (0.8 g, 0.7 mmol) and ZPheOH (0.22 g, 0.77 mmol) was dried by evaporation with pyridine (20 ml) at 0.05 mm and room temperature. The residue was dissolved in cold pyridine (7 ml) and the cooled solution of DCC (0.16 g, 0.77 mmol) in pyridine (3 ml) was added. The reaction mixture was stirred for 1 h at 0 °C and 24 h at room temperature. A piece of ice was then added, and dicyclohexylurea was filtered off and washed with pyridine (5 ml). The filtrate was evaporated at 0.05 mm and room temperature to a syrup which was lyophilized from dioxane (50 ml). The resultant white solid was partitioned between chloroform and saturated aqueous $NaHCO_3$, and the combined organic layers were washed with water (2 × 100 ml), dried ($MgSO_4$), and evaporated. The residue was chromatographed on 4 mm thick loose layer of silica gel (35 × 15 cm) in solvent S_3. The three major UV absorbing bands were obtained which were eluted with the same solvent and the eluates evaporated. The fastest band afforded diaminoacyl derivative IXa: 36 mg (3%); TLC (S_3) homogeneous; UV max (ethanol) 274 nm (ε 34 900), min 248 (22 100); NMR ($CDCl_3$) δ 8.26 and 8.16 (2 s, 2, H_8), 7.80 and 7.63 (2 s, 2, H_2), 7.40–6.86 (m, 48, 4-methoxytrityl + phenyl), 6.18–6.07 (poorly resolved m, 2, $H_{1'}$), 4.96 (s, 4, CH_2 of benzyloxycarbonyl), 3.71 and 3.61 (2 s, partially overlapped, 6, CH_3O), 1.63 and 1.40 (2 s, 6, CH_3 of isopropylidene).

Anal. Calcd for $C_{99}H_{94}N_{12}O_{16}\cdot 3H_2O$: C, 67.29; H, 5.99; N, 9.51. Found: C, 67.14; H, 5.93; N, 9.34.

The next (slower) band afforded compound VIIIa: 0.28 g (28%); TLC (S_3) homogeneous; UV max (ethanol) 274 nm (ε 34 300), min 248 (21 200); NMR ($CDCl_3$) δ 8.10 and 8.05 (2 s, 2, H_8), 7.65 and 7.48 (2 s partially overlapped with phenyl, 2, H_2), 7.20–6.71 (m, 38, 4-methoxytrityl + phenyl), 6.00 and 5.90 (2 d, 2, $H_{1'}$), 5.01 (s, 2, CH_2 of benzyloxycarbonyl), 3.55 and 3.50 (2 s, 6, CH_3O), 1.62 and 1.41 (2 s, 6, CH_3 of isopropylidene).

Anal. Calcd for $C_{82}H_{79}N_{11}O_{13}$: C, 69.04; H, 5.58; N, 10.80. Found: C, 68.90; H, 5.56; N, 10.79.

The third (slowest) band afforded after evaporation 0.35 g (43%) of starting material III.

B. Preparation of Leucyl Derivatives VIIIb and IXb. Method A was followed except that a 8.4-mmol (9.6 g) of III scale and ZLeuOH instead of ZPheOH were used. After the workup the crude product was purified by column chromatography on 550 g of silica gel (Figure 3). Peak A afforded diaminoacyl derivative IXb: 1 g (7%); TLC (S_3) homogeneous; UV max (ethanol) 274 nm (ϵ 34 300), min 248 (21 500); NMR ($CDCl_3$) δ 8.28 and 8.17 (2 s, 2, H_8), 7.98 and 7.81 (2 s partially overlapped with phenyl, 2, H_2), 7.69–6.68 (m, 38, 4-methoxytrityl + phenyl), 6.31 and 6.08 (2 d, 2, $H_{1'}$), 5.04 (s, 4, CH_2 of benzyloxycarbonyl), 3.63 and 3.53 (2 s, 6, CH_3O), 1.64 and 1.42 (the former signal is overlapped with CH_2 of Leu, 2 s, 6, CH_3), 0.92 (m, 12, CH_3 of ZLeu).

Anal. Calcd for $C_{93}H_{98}N_{12}O_{16}$: C, 68.11; H, 6.02; N, 10.25. Found: C, 67.83; H, 6.16; N, 10.27.

Peak B afforded compound VIIIb: 5.54 g (50%); TLC (S_3) homogeneous; UV max (ethanol) 273 nm (ϵ 34 500), min 247 (20,900); NMR ($CDCl_3$) δ 8.30 and 8.23 (2 s partially overlapped, 2, H_8), 7.80 and 7.64 (2 s partially overlapped with phenyl, 2, H_2), 7.39–6.68 (m, 33, 4-methoxytrityl + phenyl), 6.13 and 6.03 (2 d, 2, $H_{1'}$), 5.18 (s, 2, CH_2 of benzyloxycarbonyl), 3.67 (s, 6, CH_3O), 1.69 and 1.46 (overlapped with CH_2 of Leu, 2 s, 6, CH_3), 1.03 (m, 6, CH_3 of ZLeu).

Anal. Calcd for $C_{79}H_{81}N_{11}O_{13}$: C, 68.13; H, 5.86; N, 11.06. Found: C, 67.91; H, 5.95; N, 11.01.

Peak C afforded after evaporation 2.85 g (31%) of the starting material III.

2′(3′)-*O*-(*N*-Benzyloxycarbonyl)-L-phenylalanyl-1,2-di(adenosin-N^6-yl)ethane (XIa). A. Using 80% CH_3COOH and 90% CF_3COOH. A solution of compound VIIIa (0.28 g, 0.2 mmol) in 80% acetic acid (20 ml) was kept at room temperature for 6 h; the detritylation was complete as judged from TLC (S_4). The reaction mixture was evaporated at 0.04 mm, the residue was coevaporated with ethanol, and crude Xa was chromatographed on 4 mm thick loose silica gel layer 35 × 15 cm in solvent S_4. The major UV absorbing band of Xa was eluted with the solvent, the eluate was evaporated, and the residue dissolved in cold 90% CF_3COOH (3 ml). The solution was stirred for 20 min at 0 °C and then evaporated at 0.04 mm. The resultant syrup was stirred with Dowex 1 (acetate form, 20 ml) in 70% ethanol (40 ml) for 30 min at room temperature. The resin was filtered off, and washed with 70% ethanol and the filtrate was evaporated. The crude XIa was chromatographed on 4 mm thick loose layer of silica gel in solvent S_4. The major UV absorbing band was worked up as above to give phenylalanyl derivative XIa, 0.14 g (82%), homogeneous on TLC in S_4, in solvent S_6 two spots were obtained (vide supra): UV max (ethanol) 273 nm (ϵ 32 800), min 223 (5000);[19] NMR (CD_3SOCD_3) δ 8.38 and 8.28 (2 s partially overlapped, 4, H_8 and H_2), 7.35 (s, 10, phenyl), 6.03 and 5.93 (2 s, 2, $H_{1'}$), 5.07 (s, 2, CH_2 of benzyloxycarbonyl).

Anal. Calcd for $C_{39}H_{43}N_{11}O_{11}\cdot H_2O$: C, 54.47; H, 5.28; N, 17.92. Found: C, 54.54; H, 5.47; N, 17.69.

B. Using 80% CH_3COOH and Dowex 50 (H^+). Intermediate Xa (0.13 g, 0.15 mmol) obtained by method A (deblocking in 80% CH_3COOH) was stirred in 70% ethanol with Dowex 50 (H^+, 3 ml) for 1 h at room temperature. The resin was filtered off and washed with 50% pyridine (60 ml), the filtrate was evaporated, and the residue lyophilized from dioxane (30 ml). The crude product was chromatographed on one 4 mm thick 15 × 35 cm loose layer of silica gel in solvent S_4. The major UV absorbing band was eluted with the same solvent and the eluate evaporated to give XIa (0.07 g, 80%), homogeneous on TLC (S_4) and identical (UV, NMR) with the sample obtained by method A.

2′(3′)-*O*-(*N*-Benzyloxycarbonyl)-L-leucyl-1,2-di(adenosin-N^6-yl)ethane (XIb). Method A for preparation of compound XIa was followed on a 1-mmol scale via intermediate Xb to give 0.68 g (85%) of XIb: TLC (S_4) homogeneous; in solvent S_6 two spots were observed (vide supra); UV max (ethanol) 274 nm (ϵ 32 200), min 222 (5000);[19] NMR (CD_3SOCD_3) δ 8.47 and 8.36 (2 s partially overlapped, 4, H_8 and H_2), 7.4 (s, 5, phenyl), 6.04 and 5.95 (2 s, 2, $H_{1'}$), 5.14 (s, 2, CH_2 of benzyloxycarbonyl), 1.67 (m, 2, CH_2 of Leu), 0.97 and 0.90 (2 s partially overlapped, CH_3 of ZLeu).

Anal. Calcd for $C_{36}H_{45}N_{11}O_{11}\cdot 1\frac{1}{4}H_2O$: C, 52.06; H, 5.79; N, 18.55. Found: 52.26; H, 5.96; N, 18.22.

Using method B compound XIb was prepared in 81% yield.

2′(3′)-*O*-L-Phenylalanyl-1,2-di(adenosin-N^6-yl)ethane (XIIa). A slow stream of hydrogen was bubbled through a solution of compound XIa (10–20 μmol) in 80% acetic acid (2 ml) containing 5% PdO–$BaSO_4$ (20–30 mg) at 0 °C with stirring for 2 h. The catalyst was filtered off through a thin Celite bed and it was washed with cold 80% acetic acid (4 ml). The volume of the clear filtrate was adjusted to 10 ml (volumetric flask), the aliquots were pipetted for UV spectra, TLC, paper chromatography, and electrophoresis, and the bulk was lyophilized. UV (0.01 N HCl) corresponded to that of the parent nucleoside XIII: max 263 nm, min 232, shoulder ca. 276. TLC (S_4) showed the complete absence of the starting material XIa; compound XIIa was homogeneous (R_f 0.65) on paper chromatography in S_2 and electrophoresis in 1 M acetic acid (mobility 1.93 of PheOH). It was nynhydrin positive and in S_1 it was hydrolyzed to nucleoside XIII and PheOH. Yield (determined spectrophotometrically at 263 nm using ϵ_{263} 26 300)[1a] was 81%.

2′(3′)-*O*-L-Leucyl-1,2-di(adenosin-N^6-yl)ethane (XIIb) was prepared and characterized as stated above for compound XIIa, yield 85%, R_f 0.70 (S_2), electrophoretic mobility in 1 M acetic acid 2.2 of PheOH. In solvent S_1 compound XIIb was hydrolyzed to nucleoside XIII and LeuOH.

Acetyl Derivatives II, V, and VI. Compounds I, III, and IV were acetylated as follows. The solution of the compound (0.5 mmol) in a mixture of pyridine (2 ml) and acetic anhydride (1 ml) was kept overnight at room temperature. The reaction mixture was evaporated at 0.05 mm and room temperature, the residue was dissolved in dioxane (10 ml), and the solution was lyophilized to a white solid which was further purified by precipitation from chloroform solution using petroleum ether. After drying at 0.04 mm and room temperature an essentially quantitative yield of the corresponding acetyl derivative was obtained.

Tetraacetyl Derivative II. Homogeneous on TLC (S_3); UV max (ethanol) 270 nm (ϵ 32 300), min 229 (5100);[19] NMR ($CDCl_3$) δ 8.29 (s, 2, H_8), 7.85 and 7.77 (2 s, 2, H_2), 6.02 (m, 2, $H_{1'}$), 3.96 (broad s, 4, dimethylene bridge), 2.1 (3 s partially overlapped, 9) and 1.96 (s, 3, CH_3 of acetyl), 1.62 and 1.44 (2 s, 6, CH_3 of isopropylidene).

Anal. Calcd for $C_{33}H_{40}N_{10}O_{12}\cdot 3H_2O$: C, 48.17; H, 5.51; N, 17.02. Found: C, 48.34; H, 5.14; N, 17.02.

Diacetyl Derivative V. Homogeneous on TLC (S_3); UV max (ethanol) 274 nm (ϵ 34 200), min 248 (21 800); NMR ($CDCl_3$) δ 8.16 and 7.79 (2 s, 4, $H_8 + H_2$), 7.17–6.7 (m, 28, 4-methoxytrityl), 6.12 and 5.98 (2 m, 2, $H_{1'}$), 3.62 and 3.58 (2 s, 6, CH_3O), 2.02 (s, 6, CH_3 of acetyl), 1.62 and 1.40 (2 s, 6, CH_3 of isopropylidene).

Anal. Calcd for $C_{69}H_{68}N_{10}O_{12}$: C, 66.45; H, 5.66; N, 11.23. Found: C, 66.73; H, 5.63; N, 10.94.

Monoacetyl Derivative VI. Two partially overlapped spots on TLC (S_3)—2′ and 3′ isomers; UV max (ethanol) 274 nm (ϵ 34 900), min 248 (24 600). The isomers were separated on a loose layer of silica gel in solvent S_3. The faster moving compound (presumably 3′-*O*-acetyl derivative) was obtained in 60% yield: NMR ($CDCl_3$) δ 8.25 and 8.00 (2 s, 2, H_8), 7.64 and 7.54 (2 s, 2, H_2), 7.15–6.7 (m, 42, 4-methoxytrityl), 5.97 (s, 2, $H_{1'}$), 3.67, 3.64, and 3.62 (3 s partially overlapped, 9, CH_3O), 2.05 (s, 3, CH_3 of acetyl), 1.60 and 1.40 (2 s, 6, CH_3 of isopropylidene). The slower moving component (presumably 2′-*O*-acetyl derivative) was obtained in 40% yield: NMR ($CDCl_3$) δ 1.92 (s, 3, CH_3 of acetyl), the rest of the signals were identical with those of the faster moving isomer.

Anal. (mixture of isomers). Calcd for $C_{87}H_{82}N_{10}O_{12}$: C, 71.54; H, 5.73; N, 9.57. Found: C, 71.46; H, 5.97; N, 9.76.

Equilibration of 2′ and 3′ Isomers of Phenylalanyl and Leucyl Derivative XIa and XIb. Compound XIa (2 mg, 2.5 μmol) was chromatographed on a precoated silica gel aluminum foil (10 × 5 cm) in solvent S_6. Two well-separated UV absorbing bands were eluted with solvent S_5 and the eluates were evaporated. UV spectra of both bands in 0.01 N HCl were identical (max 263, shoulder 276 nm) and corresponded to nucleoside XIII. The ratio of faster/slower moving component as determined from UV was 7/3. Both bands were dissolved in solvent S_5 and the solution was kept at room temperature overnight. Subsequent TLC in S_6 showed the presence of both isomer in each sample in the same ratio as above.

The same result was obtained with leucyl derivative XIb.

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Registry No.—I, 60996-27-2; II, 60967-39-7; III, 60967-40-0; IV 3′-*O*-MeOTr isomer, 60967-41-1; IV 2′-*O*-MeOTr isomer, 60996-28-3; V, 60967-42-2; VI 3′-*O*-acetyl isomer, 60967-43-3; VI 2′-*O*-acetyl isomer, 60996-29-4; VII, 60967-44-4; VIIIa 3′-*O*-ZPhe isomer, 60996-30-7; VIIIa 2′-*O*-ZPhe isomer, 60996-31-8; VIIIb 3′-*O*-ZLeu isomer, 60967-45-5; VIIIb 2′-*O*-ZLeu isomer, 60967-46-6; IXa, 60967-47-7; IXb, 60967-48-8; Xa 3′-*O*-ZPhe isomer, 60967-49-9; Xa, 2′-*O*-ZPhe isomer, 60967-50-2; Xb 3′-*O*-ZLeu isomer, 60967-51-3; Xb

2′-O-ZLeu isomer, 60967-52-3; XIa 3′-O-ZPhe isomer, 60967-53-5; XIa 2′-O-ZPhe isomer, 60967-54-6; XIb 3′-O-ZLeu isomer, 60967-55-7; XIb 2′-O-ZLeu isomer, 60967-56-8; XIIa 3′-O-Phe isomer, 60967-57-9; XIIa 2′-O-Phe isomer, 60967-58-0; XIIb 3′-O-Leu isomer, 60967-59-1; XIIb 2′-O-Leu isomer, 60967-60-4; XIII, 60687-64-1; 4-methoxytrityl chloride, 14470-28-1; dimethylformamide dimethyl acetal, 4637-24-5; ZPheOH, 1161-13-3; ZLeuOH, 2018-66-8.

References and Notes

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Nucleosides. 104. Synthesis of 4-Amino-5-(D-ribofuranosyl)pyrimidine *C*-Nucleosides from 2-(2,3-*O*-Isopropylidene-5-*O*-trityl-D-ribofuranosyl)acetonitrile[1]

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2,4-Diamino-5-(β-D-ribofuranosyl)pyrimidine and 5-(β-D-ribofuranosyl)-2-thiocytosine (2-thiopseudocytidine) were synthesized along with their α isomers in four steps from 2-(2,3-O-isopropylidene-5-O-trityl-D-ribofuranosyl)-acetonitrile.

As a part of our program in search of anticancer agents, we have recently developed[2] a novel method for general synthesis of pseudouridine (**1**) and its analogues, e.g., pseudoisocytidine (**2**) and 2-thiopseudouridine (**3**), from a common intermediate, ethyl 2-(2,3-*O*-isopropylidene-5-*O*-trityl-D-ribofuranosyl)acetate (**4**). Pseudoisocytidine (**2**) was found[3] to be as active chemotherapeutically as the naturally occurring antibiotic 5-azacytidine,[4] against various mouse leukemias, and more importantly, **2** was effective against arabinofuranosylcytosine (ara-C)-resistant mouse leukemia cell lines.[3] Further biochemical and preclinical toxicological studies are currently underway with **2** in our institute in preparation for clinical trials.

In our previous communication,[5] we have briefly described the use of 2-(2,3-*O*-isopropylidene-5-*O*-trityl-D-ribofuranosyl)acetonitrile (**5**) for the synthesis of 3-(2,3-*O*-isopropylidene-5-*O*-trityl-D-ribofuranosyl)-4-thio-6-oxopyrazolo[1,5-*a*]-1,3,5-triazine (**7**) via the 3-aminopyrazole derivative (**6**).

In this report we describe the synthesis of pyrimidine C-5 nucleosides bearing a 4-amino function from the versatile intermediate **5** which was prepared previously by Ohrui et al.[6] Formylation of **5** with ethyl formate and sodium hydride in a mixture of ether and ethanol gave crude sodium enolate **8** which, without further purification, was treated with methyl iodide in dimethylformamide. Two products (**9** and **10**) in a ratio of ~4:1 were detected on a thin layer chromatogram and separated by silica gel column chromatography. ^1H NMR analyses showed that these products were the β (**9**) and α (**10**)