A mixture of the alcohol, acetic anhydride, and pyridine in a 3:5:1 ratio was placed in a flask fitted with a reflux condenser and a drying tube. After the flask was heated on a steam bath overnight, the reaction mixture was washed successively with approximately five times its volume of each of the following solutions: (1) saturated NaCl–$NaHCO_3$ solution, (2) 10% HCl in saturated NaCl solution, and (3) saturated NaCl–$NaHCO_3$ solution. The ester was then dried over molecular sieves for several days and an IR was taken to confirm the conversion of the alcohol to ester.

Pyrolysis Procedure. The ester was dropped onto an electrically heated column packed with Pyrex helecies at a rate of about 1–2 drops every 5 s. The column was heated to 450 ± 5 °C and the column was flushed with N_2 at a rate estimated to be 0.5 ml/s. The receiver at the bottom of the column was kept in a dry ice–acetone bath.

Analysis of the Pyrolysis Products. The pyrolysis products were analyzed on a Varian Aerograph Model 700 (commonly known as an Autoprep) equipped with a thermal conductivity detector. To separate the isomers, a 15 or 30 ft × 0.25 in. i.d. column packed with a saturated $AgNO_3$–ethylene glycol solution on Chromosorb W (40–60 mesh) was used. The ratio of absorbent to $AgNO_3$ solution was 2:1. The column temperature was about 35 °C. A flow rate of approximately 60 ml/min of He was generally used. The column decomposed rapidly (1 week) when left at room temperature, and was kept in a freezer when not in use.

The peaks were identified by simultaneous injection into the VPC of a mixture of the pyrolysis product mixture and a sample of the olefin in question. To determine the relative amounts of the isomeric olefins in the mixture, the VPC trace of the product mixture was Xeroxed and the peaks were cut and weighed.

Registry No.—1-Pentene, 109-67-1; *trans*-2-pentene, 646-04-8; *cis*-2-pentene, 627-20-3; 3-methyl-1-pentene, 760-20-3; *trans*-3-methyl-2-pentene, 616-12-6; *cis*-3-methyl-2-pentene, 922-62-3; 4,4-dimethyl-1-pentene, 762-62-9; *trans*-4,4-dimethyl-2-pentene, 690-08-4; *cis*-4,4-dimethyl-2-pentene, 762-63-0; 2-methyl-1-pentene, 763-29-1; 2-methyl-2-pentene, 625-27-4; 2,4-dimethyl-1-pentene, 2213-32-3; 2,4-dimethyl-2-pentene, 625-65-0; 2,4,4-trimethyl-1-pentene, 107-39-1; 2,4,4-trimethyl-2-pentene, 107-40-4.

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Carbamate Analogues of Oligonucleotides

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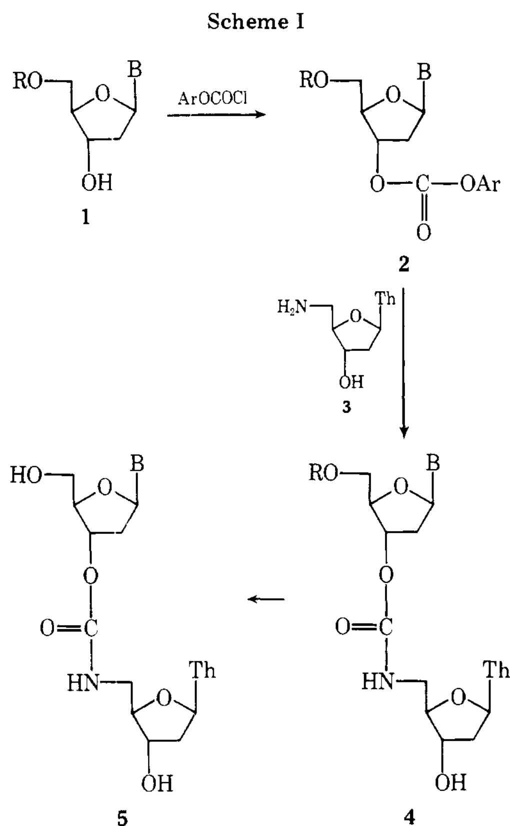
Some dinucleoside phosphate and trinucleoside diphosphate analogues that possess internucleoside carbamate bonds (–OCONH–) are described. These compounds are prepared in good yields under mild conditions by reaction of a nucleoside 3′-*O*-*p*-nitrophenyl carbonate intermediate with a 5′-aminonucleoside. The internucleoside carbamate linkage is stable and does not hydrolyze in acidic or basic solution or in solutions containing snake venom or spleen phosphodiesterase.

We describe in this paper the synthesis and chemical properties of oligonucleotide analogues containing 3′–5′ carbamate linkges between nucleoside units.[1] These compounds were prepared as models to explore the synthesis and stability of oligonucleotides with such linkages. Our interest in this class of compounds was stimulated by the prospect that the stepwise chemical synthesis of such analogues might be readily achieved and that these unique analogues might have important biochemical properties such as template activity, resistance to enzyme-catalyzed degradation, interferon induction, or phosphodiesterase inhibition. The carbamate linked analogues were particularly interesting since Baker and co-workers, working with isolated enzyme systems, found evidence for the simulation of phosphate by the *O*-carbamate in a derivative of 6-mercaptopurine.[2] However, these same workers were unable to detect such simulation with several other nucleoside carbamates.[3] Various oligonucleotide analogues containing such linkages as phosphonate,[4] thiophosphate,[5] phosphoramidate,[6] carboxymethyl,[7] and carbonate[8,9] have been reported in the literature and several of these have been found to have interesting biochemical activity.

The general synthetic approach for the formation of the carbamate linkage was modeled after the active ester method of polypeptide synthesis and Baker's method of nucleoside

carbamate synthesis.[3] Appropriately protected deoxynucleosides were converted into the 3′-O-p-nitrophenyl carbonate derivatives by the method of Letsinger and Ogilvie.[10] It was found that if dioxane was used as the solvent, instead of pyridine, this reaction proceeded at a much faster rate and that the 3′-O-carbonates (**2**) could be obtained in better than 80% yields by precipitation from hexane. Further purification was generally not required. The 3′-O-p-nitrophenyl carbonates (**2a** and **2b**) reacted smoothly with 5′-amino-5′-deoxythymidine[11] (**3**) in pyridine at room temperature to yield the protected dinucleoside carbamates **4a** and **4b**. A blocking

Scheme I



1a, 2a, and 4a, R = trityl; B = thymine
1b, 2b, and 4b, R = monomethoxytrityl; B = N-benzoylcytosine
5a, B = thymine
5b, B = cytosine

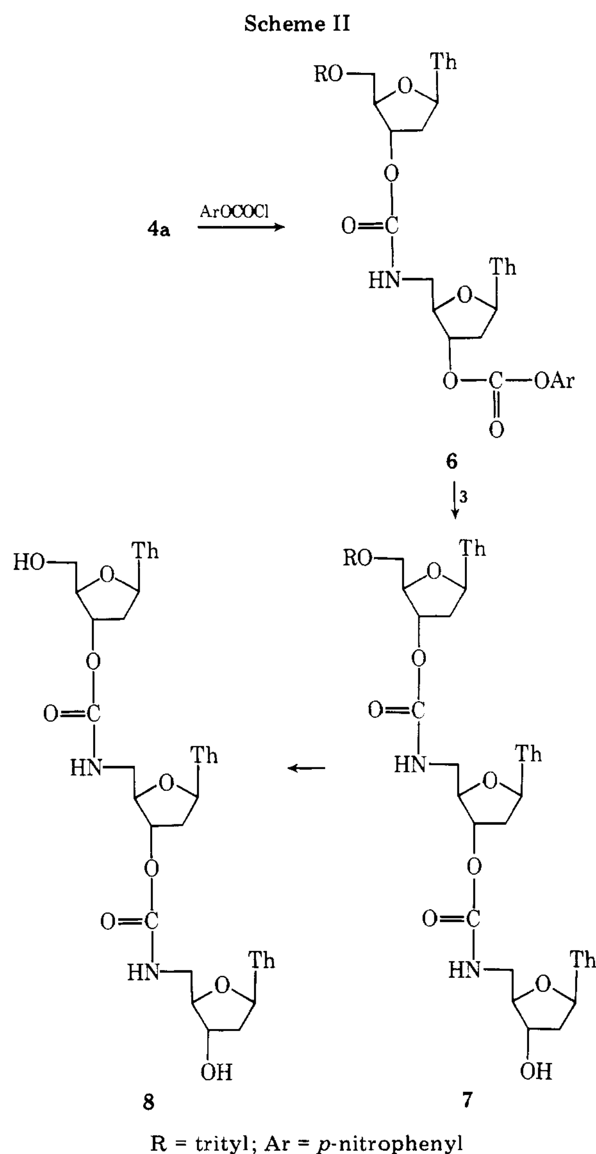
group at the 3′ position of the aminonucleoside is not required to achieve a selective reaction to form the 3′–5′ linkage. Isolated yields of better than 70% of crystalline product **4a** were typical. Reaction of the 3′-O-phenyl carbonate of **2a** with **3** was very sluggish under these conditions, and acceptable yields were obtained only upon prolonged reaction in N,N-dimethylformamide at 50 °C.[12]

In agreement with expectations, the internucleoside carbamate linkages were very stable to hydrolytic conditions. The 5′-O-trityl group was selectively removed from **4a** by treatment with aqueous acetic acid at 100 °C without detectable degradation of the carbamate linkage, and the product (**5a**) was isolated in over 80% yield. Elemental and spectroscopic analyses confirmed the structure of the product. Compound **5a** was found to be stable at room temperature in 0.1 M NaOH or in 0.1 M HCl solutions for periods of at least 24 h. When treated with snake venom phosphodiesterase or spleen phosphodiesterase under conditions where complete hydrolysis of thymidylyl-(3′-5′)-thymidine rapidly occurred, compound **5a** was found to be completely resistant to degradation. Heating **5a** in 2 M NaOH at 100 °C for 2 h resulted in partial hydrolysis of the carbamate linkage. Thymidine and **3** were identified by paper chromatography as the hydrolysis products.

The preparation of compound **4b** demonstrates the utility of this synthetic sequence for the preparation of a dinucleoside carbamate which requires a protecting group on the base. The N-benzoyl and monomethoxytrityl protecting groups were removed by the usual treatment first with ammonium hydroxide and then with acetic acid to yield **5b**. Spectroscopic analysis of the product showed the expected shift of λ_{max} to higher wavelength on going from a neutral to an acidic solution. This is characteristic of cytosine derivatives.

To test this synthetic procedure in the preparation of oligonucleotide analogues containing more than two nucleoside units, compounds **7** and **8** were prepared. Treatment of compound **4a** with a tenfold excess of p-nitrophenyl chloroformate in dioxane–pyridine (8:1) for 30 min gave a quantitative yield of the 3′-O-p-nitrophenyl carbonate derivative (**6**). Attempts

Scheme II



R = trityl; Ar = p-nitrophenyl

to prepare this derivative in pyridine had resulted in slow reactions and poor yields owing to the decomposition of the carbonate in this solvent. Compound **6** reacted rapidly (less than 1 h) with the aminonucleoside to give compound **7** in a 61% isolated yield. This compound was purified by recrystallization from tetrahydrofuran, and the elemental analysis and spectroscopic data were consistent with the assigned structure. The blocking group was selectively removed from

7 by treatment with acetic acid and the trinucleoside dicarbamate, **8**, was isolated in 70% yield after purification by preparative thin layer chromatography.

This work demonstrates that carbamate analogues of oligonucleotides can be synthesized in good yields under mild conditions with short reaction times. The carbamate linkage has been shown to be one of the most stable linkages that has been developed for oligonucleotide analogues. Further analysis of these model compounds as enzyme substrates is in progress.

Experimental Section

Infrared spectra were recorded on Perkin-Elmer Model 621 or 137 spectrophotometers and ultraviolet spectra were recorded on a Cary 14 spectrophotometer. Melting points were determined with a Fisher-Johns apparatus and are not corrected. Elemental analyses were made by Galbraith Laboratories, Knoxville, Tenn.

Reagent grade pyridine and 2,6-lutidine were each distilled from *p*-toluenesulfonyl chloride, redistilled from calcium hydride, and stored over Linde 4A molecular sieves. Reagent grade 1,4-dioxane was distilled from lithium aluminum hydride and stored over molecular sieves. Reagent grade triethylamine was distilled from *p*-toluenesulfonyl chloride and redistilled from calcium hydride. *p*-Nitrophenyl chloroformate was purified by sublimation at reduced pressure.

For analytical TLC, Eastman 6060 silica gel plates were used. Preparative TLC was done using plates with a 1-mm silica layer obtained from Quantum Industries (PQ1F). Nucleosides and their derivatives were located under UV light. In addition, trityl containing materials were detected by spraying the chromatogram with 10% $HClO_4$ and drying under a stream of hot air, and compounds containing the *p*-nitrophenyl carbonate moiety were detected by exposing the chromatogram to ammonia fumes. Column chromatography was performed using 60–200 mesh chromatographic grade silica gel from Sargent-Welch Co. Paper chromatography was carried out on Whatman 3MM paper using the descending technique with a solvent system of isopropyl alcohol–concentrated ammonium hydroxide–water (7:1:2 v/v/v).

Reactions were generally run in septum-sealed flasks or test tubes with stirring provided by a Teflon-coated magnet. Solvents were removed under reduced pressure with a bath temperature less than 35 °C. Phosphodiesterase catalyzed rections were carried out by the procedures previously described.[6] Hydrolytic analyses were monitored by paper chromatography.

5′-*O*-Tritylthymidylyl-(3′-5′ carbamoyl)-5′-amino-5′-deoxythymidine (4a). *p*-Nitrophenyl 5′-tritylthymidine 3′-carbonate[10] (1.00 g, 1.54 mmol) was added to 5′-amino-5′-deoxythymidine[11] (0.37 g, 1.54 mmol) in 30 ml of pyridine. The solution turned yellow immediately owing to the formation of the *p*-nitrophenoxide anion, and after 30 min triethylamine (0.16 g, 1.5 mmol) was added. TLC analysis (ethyl acetate) showed that the reaction was complete after 1 h. The reaction mixture was stirred overnight without further change in the TLC analysis. Then 60 ml of ethyl acetate was added, the solution was concentrated under reduced pressure, and 100 ml of ether–ethyl acetate (1:1) was added. The resulting precipitate was isolated by filtration and dried under vacuum at 50 °C. The yield was 0.83 g (71%) of compound **4a** which was homogeneous on TLC with ethyl acetate (R_f 0.07) and with acetone (R_f 0.35). This compound was recrystallized from methanol–tetrahydrofuran: mp 230–232 °C; λ_{max} 262 nm (ϵ 2.4 $\times 10^4$ in dioxane); λ_{min} 242 nm.

Anal. Calcd for $C_{40}H_{41}N_5O_{10}$: C, 63.90; H, 5.50; N, 9.31. Found: C, 64.00; H, 5.76; N, 9.21.

Thymidylyl-(3′-5′-carbamoyl)-5′-amino-5′-deoxythymidine (5a). Compound **4a** (3.77 mg, 0.05 mmol) was dissolved in 3 ml of acetic acid–water (4:1 v/v) and heated on the steam bath for 40 min. The solvent was then evaporated under reduced pressure and the last traces of acid were removed by coevaporation with ethanol. The resulting solid residue was dissolved in solvent grade tetrahydrofuran and compound **5a** was isolated in an 83% yield after the addition of hexane: mp 219–221 °C; λ_{max} 262 nm (ϵ 2.0 $\times 10^4$ in dioxane), λ_{min} 234 nm; homogeneous on paper chromatography (R_f 0.52) and on TLC in tetrahydrofuran (R_f 0.39).

Anal. Calcd for $C_{21}H_{27}N_5O_{10}\cdot\frac{1}{2}H_2O$: C, 48.64; H, 5.44; N, 13.50. Found: C, 48.74; H, 5.52; N, 13.27.

Preparation of 4a from Phenyl 5′-*O*-Tritylthymidine 3′-Carbonate.[12] Phenyl 5′-*O*-tritylthymidine 3′-carbonate[13] (88 mg, 0.15 mmol) and 5′-amino-5′-deoxythymidine (35 mg, 0.15 mmol) were dissolved in 10 ml of *N,N*-dimethylformamide. Reaction progress, which was monitored by TLC, was very slow at room temperature so the reaction was placed in a bath at 50 °C. After 36 h the product was isolated (81 mg, 72%) as described above. This material was identical with compound **4a**, prepared from the *p*-nitrophenyl carbonate derivative, on TLC analysis, melting point, and infrared analysis.

***p*-Nitrophenyl 5′-*O*-Monomethoxytrityl-*N*-benzoyl-2′-deoxycytidine 3′-Carbonate (2b).** 5′-*O*-Monomethoxytrityl-*N*-benzoyl-2′-deoxycytidine[14] (69.9 mg, 0.12 mmol) was dried by the evaporation of two 4-ml portions of dioxane under reduced pressure. The resulting solid was dissolved in 4 ml of dioxane. The solution was cooled to 10 °C, treated with 2,6-lutidine (0.1 ml, 0.9 mmol) and *p*-nitrophenyl chloroformate (35.7 mg, 0.18 mmol), and stirred at room temperature for 2 h. A second portion of the chloroformate (24 mg, 0.12 mmol) was then added and stirring was continued for 18 h. Chloroform (50 ml) was added to the reaction mixture and this solution was washed with two 50-ml portions of an aqueous solution buffered at pH 5, dried over sodium sulfate, and concentrated under reduced pressure. The resulting residue was dissolved in a minimum of tetrahydrofuran, and this solution was added to an excess of hexane. The precipitate which formed was isolated by filtration and dried to give 79 mg (86%) of the *p*-nitrophenyl carbonate **2b**, which on TLC analysis in ethyl acetate (R_f 0.62) was shown to contain both the monomethoxytrityl and the *p*-nitrophenoxy groups by the appropriate tests. This compound was used in the preparation of **4b** without further purification.

5′-*O*-Monomethoxytrityl-*N*-benzoyldeoxycytidylyl-(3′-5′-carbamoyl)-5′-amino-5′-deoxythymidine (4b). Compound **2b** (78 mg, 0.10 mmol) and 5′-amino-5′-deoxythymidine (38 mg, 0.16 mmol) were dissolved in 5 ml of pyridine at 0 °C. TLC analysis showed that the reaction had gone to completion in 30 min. After the solvent was removed under reduced pressure, the residue was dissolved in tetrahydrofuran–ethyl acetate (1:1 v/v) and washed with two portions of salt water. The organic layer was dried over sodium sulfate, concentrated under reduced pressure, and applied to a silica gel column (2.5 $\times$ 40 cm). The column was first eluted with ethyl acetate and then the product was obtained by elution with tetrahydrofuran. Compound **4b** was isolated as a dihydrate by precipitation from hexane in a 47% yield (41 mg): mp 147–154 °C; UV max (CH_3OH) 261 nm (ϵ 3.1 $\times 10^4$) and 303 (9.8 $\times 10^3$); homogeneous on TLC in ethyl acetate–tetrahydrofuran (1:1 v/v, R_f 0.23).

Anal. Calcd for $C_{47}H_{46}N_6O_{11}\cdot 2H_2O$: C, 62.51; H, 5.35; N, 9.31. Found: C, 62.02; H, 5.37; N, 9.51.

For removal of the blocking groups, 6 mg of **4b** was dissolved in 0.25 ml of pyridine and 0.25 ml of concentrated ammonium hydroxide was added. After standing overnight at room temperature, this solution was concentrated under a stream of nitrogen and the resulting residue was dissolved in 0.25 ml of acetic acid. The solution was heated on the steam bath for 15 min and again concentrated under a stream of nitrogen. The residue was chromatographed on paper and the only nucleoside product, **5b** (R_f 0.49), was eluted from the paper and analyzed by UV spectroscopy: λ_{max} 266 nm (H_2O, pH 7), and λ_{max} 271 (H_2O, pH 1). The product was homogeneous on TLC in methanol (R_f 0.58).

***p*-Nitrophenyl 5′-*O*-Tritylthymidylyl-(3′-5′-carbamoyl)-5′-amino-5′-deoxythymidine 3′-Carbonate (6).** The protected dinucleoside carbamate, **4a** (520 mg, 0.70 mmol), was dried by the evaporation of two 10-ml portions of pyridine, dissolved in a mixture of 0.5 ml of pyridine and 4 ml of dioxane, and treated with *p*-nitrophenyl chloroformate (1.40 g, 7.0 mmol) at 0 °C. Then the reaction mixture was removed from the ice bath, and after 30 min at room temperature, TLC analysis showed no unreacted dinucleoside. Anhydrous ethanol (2 ml) was added to the reaction mixture and solvents were evaporated under reduced pressure. The residue was dissolved in ethanol and concentrated to a gum to remove pyridine. After dissolving the residue in tetrahydrofuran–ethyl acetate (1:1 v/v) and washing with two portions of aqueous pH 5 buffered solution, the organic layer was dried over sodium sulfate, concentrated, and added to a 20-fold volume of hexane. The resulting precipitate was collected by filtration and dried under reduced pressure: yield 0.80 g, mp 148–155 °C, TLC analysis in acetone–benzene (1:1, R_f 0.11) showed only one major component which gave positive tests for the trityl and the *p*-nitrophenyl groups.

5′-*O*-Tritylthymidylyl-(3′-5′-carbamoyl)-5′-amino-5′-deoxythymidylyl-(3′-5′-carbamoyl)-5′-amino-5′-deoxythymidine (7). Compound **6** (15.3 mg, 0.016 mmol) and 5′-amino-5′-deoxythymidine (7.8 mg, 0.032 mmol) were dissolved in 1 ml of pyridine. After 1 h, TLC analysis showed that the reaction had gone to completion and the solvent was evaporated under reduced pressure. The residue was dissolved in tetrahydrofuran–ethyl acetate and the solution was washed with a saturated sodium bicarbonate solution. The organic layer was dried over sodium sulfate and concentrated. Compound **7**

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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(12) Compound **4a** has been reported to have been prepared from **2a** (Ar = phenyl) previously; see ref 1a.

(13) This compound, **2a** (Ar = phenyl), was prepared by the method of Letsinger and Ogilvie (ref 10) in a 90% yield and is considerably more stable than the *p*-nitrophenyl carbonate, **2a** (Ar = *p*-nitrophenyl): mp 96–100 °C, homogeneous on TLC in ethyl ether (R_f 0.34) and ethyl acetate (R_f 0.50); NMR and IR consistent with structure.

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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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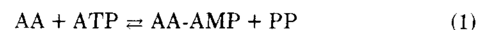
The synthesis of the title compounds XIIa and XIIb is described. 2′,3′-*O*-Isopropylidene-1,2-di(adenosin-N^6-yl)ethane (I) on reaction with 4-methoxytrityl chloride in pyridine gave ditrityl derivative III accompanied by trityl compound IV. The formation of IV was suppressed by blocking of the remaining 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′ ⇄ 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.

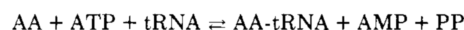
Scheme I



$$\text{AA} + \text{ATP} \rightleftarrows \text{AA-AMP} + \text{PP} \qquad (1)$$

$$\text{AA-AMP} + \text{tRNA} \rightleftarrows \text{AA-tRNA} + \text{AMP} \qquad (2)$$

Scheme II



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

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).