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Template-Directed Organic Synthesis. A Model for the Peptidyl Transfer Reaction of Protein Biosynthesis[1]

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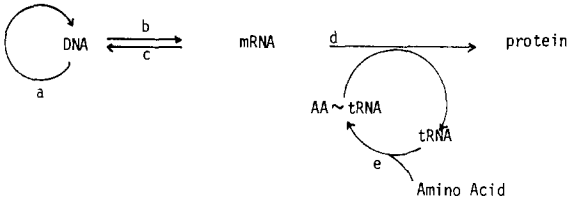
The effects of polynucleotide templates on the self-condensations in water and in pyridine of 2′ (and 3′)-*O*-glycyladenosine, 2′ (and 3′)-*O*-glycyluridine, and 5′-glycyladenylate were studied. Poly(U) template was found to have a favorable effect on the self-condensations of glycine esters of adenosine and adenylate in both media.

INTRODUCTION AND BACKGROUND

The genetic information of a living system, recorded in deoxyribonucleic acid (DNA), is first transcribed into messenger RNA (mRNA). The nucleotide sequence of mRNA is then translated into the amino acid sequences of proteins on ribosomes. Each group of three consecutive nucleotides (codon) in the mRNA is read as the signal for incorporation of a particular amino acid. To achieve this, amino acids are first activated by ATP and then are attached to cognate transfer RNA (tRNA). The nucleotide sequence of a codon in the mRNA is recognized by the tRNA by interaction with a nucleotide sequence, the anti-codon. The interactions between codon and anti-codon arise by base pairing between the complementary nucleotides, namely, adenylic (AMP) and uridylic acid (UMP), and guanylic (GMP) and cytidylic acid (CMP) (*1*, *2*).



(a) Replication (DNA polymerase)
(b) transcription (RNA polymerase)
(c) transcription (reverse transcriptase)
(d) translation (peptidyl transferase)
(e) (aminoacyl-tRNA ligase)

SCHEME 1. The central dogma of molecular biology.

Cognate transfer RNAs have chain lengths of approximately 80 nucleotides (MW ~ 25,000), and they are folded into semi-rigid L-shaped molecules. In addition to the

[1] Dedicated to Professor William S. Johnson on the occasion of his 65th birthday and in recognition of his pioneering contributions to the art and science of synthetic organic chemistry.

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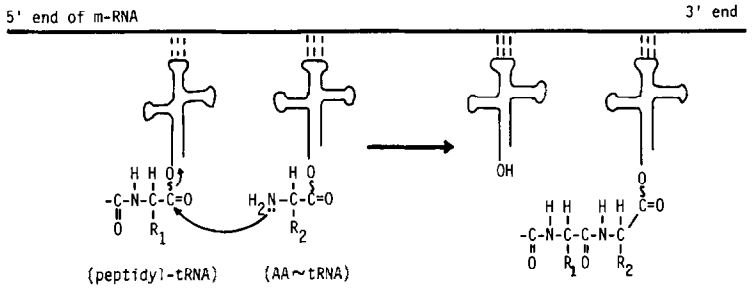
common nucleotides (A, C, G, U), they also contain rare nucleotides. Despite the fact that the nucleotide sequence of the tRNA clearly determines its specificity, some nucleotide sequences are common to all tRNAs. For example, the sequence of –CCA is always found at the 3′-end of tRNAs (*3*, *4*). Amino acids are attached through their carboxylic groups to the 3′-terminal adenosine of their cognate tRNAs. The aminoacyl ester in the aminoacyl–tRNA(AA ~ tRNA) is relatively labile because of the presence of the α-hydroxyl group (*5–7*).

After activation, the AA ~ tRNA molecules diffuse to the ribosomes, which are spherical particles on which the peptide bonds are formed. Ribosomes (MW ~3 × 10^6) are constructed from a large (50S) and a small (30S) subunit. Messenger RNA attaches to ribosomes and moves across them to bring successive codons into position to select the correct AA ~ tRNA precursors. The peptide chain grows stepwise, beginning at the amino terminal end. Attachment of the nascent chain to the ribosome occurs through binding of the chain's terminal tRNA moiety to a site (P-site) in the ribosomal surface. Precursor AA ~ tRNA molecules enter another site (A-site), allowing the subsequent formation of a peptide bond to the growing chain held in the P-site.



SCHEME 2. Peptidyl transfer reaction.

Formation of the peptide bond presumably involves nucleophilic attack of the amino group of the AA ~ tRNA in the A-site at the labile ester moiety of peptidyl-tRNA in the P-site (*8*), the reactivity of 2′(or 3′)-aminoacyl-tRNA and model esters being well documented (*5*). The specificity of the interactions between mRNAs and tRNAs is such that once an amino acid is loaded onto its cognate tRNA the recognition depends only on the specificity of the tRNA, *not* on the structure of the amino acid being transported. Thus, when α-hydroxyaminoacyl-tRNA was incubated with ribosomal preparations, polyester was formed instead of polypeptide (*9*).

Base pairing interactions between complementary poly–poly nucleotides, poly–oligo nucleotides, poly–mono nucleotides, and mono–mono nucleotides have been extensively studied by physical methods (*10–13*), and more recently by examining the template-directed syntheses of nucleotides (*14–16*). Organized helical structures were reportedly formed when polyuridylic acid [poly(U)] was mixed with adenosine and its derivatives (*17*). These helical structures are believed to be held together by hydrogen bonds between the uracil and adenine rings. Poly(C) was also found to form similar helical structures with guanosine and its derivatives. Stacking interaction between the purine rings presumably plays an important role in stabilizing the helical structure, since poly(A) [or poly(G)] does not form similar helical structures with monomers of the complementary pyrimidine nucleoside.

The first reported template-directed organic synthesis was the condensation of two hexathymidylic acid residues to give dodeca-T by a water-soluble carbodiimide in the presence of a poly(A) template (*14*). Subsequently, the condensation of adenylic acid with adenosine (or adenylic acid) was reported to be greatly enhanced in the presence of poly(U) template (*18*). It is to be noted that the condensation of AMP with adenosine (or AMP) on a poly(U) template yields a product which is predominantly 2′–5′ linked, while the enzymic reactions of RNA polymerase yields exclusively the 3′–5′ isomers. Although there has been significant progress in the area of template-directed organic synthesis, this approach has been limited largely to nucleotide synthesis.

It appeared to us that it should be possible, at least in principle, to utilize base-pairing interactions between complementary nucleosides (or nucleotides) in organic syntheses other than nucleotide synthesis. Based on the known and presumed mechanisms of protein synthesis on the ribosome, it might become possible to induce condensations between the reactive units attached to the 2′(or 3′) hydroxyl groups of tRNAs (or their functional equivalents) in the presence of a nucleotide template of defined length and sequence. This kind of synthetic approach would be exceptionally attractive in the synthetic operations that depend on the repetitive condensations of both identical and non-identical units, for example, terpenoids and polyketides. Once the appropriate conditions are met, it should also be possible to control the *number* of condensing units as well as the *sequence* of the condensations. In the present account, we describe the initial experiments with aminoacyl nucleosides in the presence of polynucleotides.

RESULTS AND DISCUSSION

The aminoacyl-nucleosides, 2′(and 3′)-*O*-cbz-glycyladenosine, 2′(and 3′)-*O*-cbz-glycyluridine, 2′(and 3′)-*O*-cbz-(L)-phenylalanyluridine, and 3′-*O*-cbz-(L)-phenylalanylthymidine, were prepared essentially by the literature procedures (*19*, *20*). As expected (*5*), the aminoacyl esters on the ribosyl moiety of adenosine and uridine reacted readily with nucleophiles such as methanol (*20*) and amino acids to yield methyl esters of the amino acids and dipeptides, respectively. Next, to examine the degree of interactions between nucleosides, the reactions of 2′(and 3′)-*O*-(cbz-glycyl)-adenosine with 3′-*O*-(L)-phenylalanyl-5′-*O*-trityl-2′-deoxyadenosine (for the interaction between A and A), and with 3′-*O*-(L)-phenylalanyl-5′-*O*-trityl-thymidine (for the interaction between A and T) were studied in dry pyridine at room temperature. It was observed that the amino group of phenylalanine on A interacts readily with the amino acid ester of A to give dipeptidyladenosine, while the amino group of phenylalanine on T does not readily interact with the same aminoacyl ester of A. These results can be best explained in terms of a strong interaction between A and T in such a manner that the amino nucleophile does not come into reacting distance with the active ester moiety, namely, an anti-catalysis because of a better interaction between A and T.

After confirming the reactivity of 2′(and 3′)-aminoacyl esters on the ribosyl group and the favorable interaction between the complementary nucleosides, e.g., A and T in pyridine, we have examined the effect of templates on the self-condensation of the glycine esters of 2′(and 3′)-adenosine, -uridine, and of 5′-AMP. The results are shown in the Tables 1 to 3 (Scheme 3). The first template examined was 1,1′-dimethylenebisuracil (*21*) (U–C_2–U) (Table 1, runs 10 and 11). The result of the

TABLE 1

SELF-CONDENSATION OF 2'(AND 3')-*O*-GLYCYL-ADENOSINE ON POLY(U) TEMPLATE

Run	Substrate (m*M*)	Template (m*M*)	Conditions	Identification of products
1	40	40	0.1 *M* KH_2PO_4 (pH 7.5), pyr. suspension, rt, 4 days	Gly, $(gly)_2$, $(gly)_3$, $(gly)_4$—trace
2	20	20	0.5 *M* NaCl, 0.05 *M* $MgCl_2$ pyr. suspension, rt, 12 days	Gly, $(gly)_2$, $(gly)_3$
3	10	10	0.5 *M* NaCl, 0.05 *M* $MgCl_2$, pyr. suspension, rt, 17 days	Gly, $(gly)_2$—trace
4	10	10	0.1 *M* NaCl, 0.01 *M* $MgCl_2$, pyr. suspension, rt, 17 days	Gly
5	10	—	Pyridine solution, rt, 17 days	Gly
6	20	20	0.5 *M* NaCl, 0.05 *M* $MgCl_2$, H_2O, −13°C, approx. rt, cycle 12 days	Gly, $(gly)_2$—trace
7	10	10	0.5 *M* NaCl, 0.05 *M* $MgCl_2$, H_2O, rt, 17 days	Gly, $(gly)_2$
8	10	10	0.1 *M* NaCl, 0.01 *M* $MgCl_2$, H_2O, rt, 17 days	Gly, $(gly)_2$
9	10	—	0.5 *M* NaCl, 0.05 *M* $MgCl_2$, H_2O, rt, 17 days	Gly
10	40	U–C_2–U[a]	Pyridine, rt, 6 days	Gly, $(gly)_2$
11	40	—	Pyridine, rt, 6 days	Gly, $(gly)^2$

[a] See the text.

experiment with U–C_2–U was not significantly different from that of of the control experiment, both yielding glycine and glycine dimer. However, the lack of the template effect by U–C_2–U could have been due to its poor solubility in the reaction medium. After many trials, conditions were found (Table 1 runs 1 and 2; Table 2, run 3) for the

TABLE 2

SELF-CONDENSATION OF 5'-GLYCYL-AMP ON POLY(U) TEMPLATE

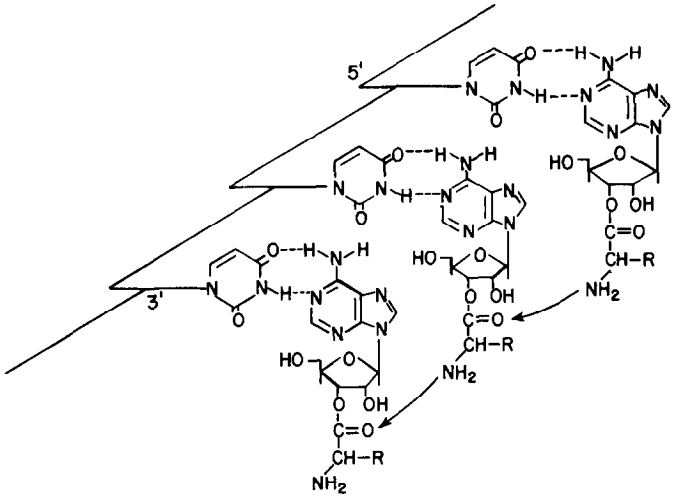
Run	Substrate (m*M*)	Template (m*M*)	Conditions	Identification of products
1	20	20	0.5 *M* NaCl, 0.05 *M* $MgCl_2$, Pyridine suspension, rt, 8 days	Gly, $(gly)_2$
2	20	—	Pyridine solution, rt, 8 days	Gly, $(gly)_2$
3	20	20	0.5 *M* NaCl, 0.05 *M* $MgCl_2$, H_2O, rt, 2 days	Gly, $(gly)_2$, $(gly)_3$
4	20	—	0.5 *M* NaCl, 0.05 *M* $MgCl_2$, H_2O, rt, 2 days	Gly, $(gly)_2$—trace
5	20	—	H_2O, rt, 2 days	Gly, $(gly)_2$—trace

TABLE 3

SELF-CONDENSATION OF 2′ (AND 3′)-*O*-GLYCYL-URIDINE ON POLY(A) TEMPLATE

Run	Substrate (m*M*)	Template (m*M*)	Conditions	Identification of products
1	20	20	0.1 *M* KH_2PO_4(pH 7.5), pyr. suspension, rt, 16 days	Gly, $(gly)_2$—trace
2	20	20	0.5 *M* NaCl, 0.05 *M* $MgCl_2$, pyr. suspension, rt, 16 days	Gly, $(gly)_2$—trace
3	20	—	Pyridine solution, rt, 16 days	Gly, $(gly)_2$—trace
4	20	20	0.5 *M* NaCl, 0.05 *M* $MgCl_2$, H_2O, rt, 16 days	Gly

formation of trimers and tetramers[2] of glycine mediated by the presence of the poly(U) template.



SCHEME 3. 3′-Aminoacyladenosines on poly(U) template.

The results of runs 1 through 5 (Table 1) suggest that in dry pyridine, where no competing hydrolytic reaction is available, higher concentration, higher salt concentration, and the presence of poly(U) template seem to have favorable effects on the self-condensation of the reactive ester of adenosine. The results of runs 6 through 9 again indicate that even in the water, in which hydrolysis is competing with the self-condensation, the presence of the template has a favorable, although small, effect.

With the more reactive ester 5′-glycyl-AMP, prepared according to the literature method (*22*, *23*), a similarly favorable effect of the complementary template is apparent (Table 2). In contrast, the experiments in Table 3 clearly indicate that poly(A) does not have any significant template effect on the self-condensation of 2′ (and 3′)-*O*-glycyl-uridine in either water or pyridine. The data on hand (Table 1) do not indicate whether

[2] The presence of higher oligomers of glycine cannot be excluded on the basis of the analytical protocol (see Experimental).

the freeze–thaw cycle has any advantage over the room temperature reaction. We are currently conducting the T_m measurements of various aminoacyladenosines on poly(U) template to gain further information on this point. If the longer peptidyl chain derivatives of adenosine on poly(U) have higher T_m values than the shorter ones, oscillating the reaction temperature will favor formation of oligomers. The ionic strengths used thus far are not much higher than those of the enzymatic system. Perhaps it may be necessary to use a much higher salt concentration to get a tighter binding between the template and the monomers. The work currently in progress involves a systematic study of the effects of pH, ionic strength, and temperature on the condensations of various amino acid derivatives of adenosine on the poly(U) template, and a possible use of the interaction between dinucleotides and the polymeric template.

Summary and prognosis. It has been shown that oligopeptide synthesis (gly_4) can be induced by weak interactions (e.g., hydrogen bonding) of the complementary base pairs of genetic material. The obvious extension of this work to programmed organic synthesis involving more sophisticated templates (e.g., —U–U–A–U–U–A—) and building blocks (e.g., acyl, malonyl, propionyl esters of A, A–A, A–A–U, etc.) can now be approached with a view to achieving fidelity of sequencing not only oligopeptide synthesis, but also the formation of "mixed" oligoketides related to fatty acid, phenolic, and macrolide biosynthesis, based on singlet, doublet or, if necessary, triplet complementarity in template and 2′(or 3′)-acylated substrate.

EXPERIMENTAL

Nuclear magnetic resonance spectra were recorded on Varian Associates EM 360, and Perkin Elmer R 32 spectrometers. Unless otherwise stated, deuterochloroform was used as solvent with chemical shifts reported as δ values in parts per million relative to tetramethylsilane. Silica gel 60 (EM) and cellulose F (EM) plates were used for thin-layer chromatography (tlc). Spots were detected by uv light or I_2 vapor. Electrophoretic analyses were performed on Whatman No. 1 paper, using a Savant Flat Plate Electrophoresis system (voltage gradient length, 60 cm). Visualization of the electrophoretogram was done by Fluram in acetone spray (Roche Diagnostics). All of the amino acids, peptide standards, nucleosides, and polynucleotides were purchased from Sigma Chemical Co.

2′(and 3′)-O-Carbobenzyloxyglycyladenosine was prepared by a procedure similar to literature methods (*19–20*). A mixture of adenosine (2.67 g, 10 mmol) and trityl chloride (5.56 g, 20 mmol) in dry pyridine (40 ml) was heated at 60°C for 9 hr to yield 5′-*O*-tritylladenosine. This mixture was treated with carbobenzyloxy(cbz)-glycine (2.09 g, 10 mmol) and dicyclohexylcarbodiimide (DCC) (3.10 g, 15 mmol) over 15 hr at RT. An extractive work-up with chloroform followed by silica gel column chromatography (10% ethyl acetate in benzene) gave the condensation product (3.0 g). nmr; 7.90, 7.96 (each, S, 1H), 5.82 (br d, 1H, 1′H), 5.45 (br, 1H, 2′ and 3′ H), 5.05 (S, 2H, cbz), 4.25 (br S, 1H, 2′ and 3′ H), 4.08 (br S, 1H, 4′H), 3.98 (br d, 2H, $COCH_2$–N), 3.75 (br S, 2H, 5′Hs).

Removal of 5′-*O*-trityl group was carried out in refluxing 80% aqueous acetic acid

over 15 min. nmr; 8.16, 8.26 (each, S, 1H), 5.82 (br d, 1H, 1'H), 5.45 (br, 1H, 2' and 3' H), 5.04 (S, 2H, cbz), 4.20 (br, 1H, 2' and 3'H), 4.03 (br S, 2H, 5'Hs), 4.00 (br S, 1H, 4'H), 3.80 (br S, 2H, CO–CH_2–N).

The carbobenzyloxy (cbz) group was removed immediately before use by hydrogenolysis in acetic acid over $Pd/BaSO_4$ at 0°C.

2'(and 3')-O-cbz-glycyluridine was prepared similarly. nmr; 7.88, 7.96 (each S, 1H), 5.95 (br d, 1H, 1'H), 5.65 (br, 1H, 2' and 3' H), 5.08 (S, 2H, cbz), 4.50 (br, 1H, 2' and 3'H), 4.17 (br, 1H, 4'H), 4.05 (d, 2H, $COCH_2NH$), 3.80 (br S, 2H, 5'Hs). nmr of 5'-*O*-trityl derivative; 7.65, 7.22 (each S, 1H), 5.95 (br, 2H, 1'H and amide H), 5.32 (br, 1H, 2' and 3'H), 5.06 (S, 2H, cbz), 4.57 (br, 1H, 2' and 3'H), 4.08 (br S, 1H, 4'H), 4.02 (br S, 2H, $COCH_2NH$), 3.45 (br S, 2H, 5'Hs).

2'(and 3')-O-cbz-(L)*-phenylalanyluridine.* This nucleoside was prepared similarly. nmr; 7.88 and 7.96 (each S, 1H), 5.95 (br d, 1H, 1'H), 5.30 (br S, 1H, 2' and 3'H), 5.02 (S, 2H, cbz), 4.55 (br, 2H, 2' and 3'H and CO–CH–NH), 4.0 (br S, 1H, 4'H), 3.75 (br S, 2H, 5'Hs), 3.08 (br, 2H, benzylic). nmr of 5'-*O*-trityl derivative; 7.65, 7.73 (each, S, 1H), 6.0 (br, 2H, 1'H and amide), 5.2–5.4 (br, 1H, 2' and 3'H), 5.0 (S, 2H, cbz), 4.5–4.7 (br, 2H, 2' and 3'H and COCHN), 4.05 (br S, 1H, 4'H), 3.40 (br, 2H, 5'Hs), 3.08 (br, 2H, benzylic).

5'-O-Trityl-3'-O-cbz-(L)*-phenylalanylthymidine* was prepared similarly. nmr; 9.1 (S, 1H, Th), 6.25 (br t, 1H, 1'H), 5.35 (br, 1H, 3'H), 5.05 (S, 2H, cbz), 4.6 (br, 1H, COCHN), 3.75 (br S, 1H, 4'H), 3.35 (br d, 2H, 5'Hs), 3.0 (b, 2H, benzylic), 2.6 (br, 2H, 2'Hs), 1.38 (S, 3H, CH_3).

5'-O-Trityl-3'-O-[cbz-glycyl-(L)*-phenylalanyl]-thymidine* was prepared in the usual manner from 5'-*O*-trityl-3'-*O*-(L)-phenylalanylthymidine, cbz-glycine, and DCC. nmr; 9.34 (S, 1H, Th), 6.25 (br t, 1H, 1'H), 5.35 (br, 1H, 3'H), 5.09 (S, 2H, cbz), 3.85 (br, 3H, 4'H and $COCH_2N$), 3.4 (br S, 2H, 5'Hs), 3.05 (br S, 2H, benzylic), 2.35 (br, 2H, 2'Hs), 1.40 (S, 3H, CH_3).

5'-O-Trityl-3'-O-[cbz-(L)*-phenylalanyl]-2'-deoxyadenosine.* A solution of 2'-deoxyadenosine monohydrate (2.5 g, 10 mmol) in diethylformamide dimethylacetal (5 ml) was kept overnight in a flask protected from moisture. Solvents were removed under high vacuum at below 60°C, and the mixture was redissolved in chloroform (12 ml). Dilution with petroleum ether (200 ml) resulted in the formation of a precipitate, which was quickly filtered and dried under vacuum. The crude product was treated with trityl chloride and pyridine overnight and poured into water. An extractive work-up with chloroform (250 ml) gave a crude product. This product was successively treated with cbz-(L)-phenylalanine (1.5 g, 5 mmol) and DCC (1.5 g, 7.5 mmol) in dry pyridine (10 ml) overnight, and with acetic acid (10 ml) in chloroform (15 ml) and 95% ethanol (25 ml) for 5 hr at RT. Evaporation of the solvents followed by chromatography on a silica gel column (50% ethyl acetate in benzene) gave the desired product (3 g). nmr; 7.92 and 8.20 (each, S, 1H, Ad), 6.47 (br S, 1H, 1'H), 5.45 (br, 1H, 3'H), 5.09 (S, 2H, cbz), 4.65 (br, 1H, COCHN), 4.0 (br, 1H, 4'H), 3.36 (br d, 2H, 5'Hs), 3.07 (br d, 2H, benzylic), 2.50 (br, 2H, 2'Hs).

5'-O-Trityl-3'-O-[cbz-glycyl-(L)*-phenylalanyl]-2'-deoxyadenosine.* This compound was prepared in the usual way from 5'-*O*-trityl-3'-*O*-(L)-phenylalanyl-2'-deoxyadenosine, cbz-glycine, and DCC. nmr; 7.92, 8.16 (each, S, 1H, Ad), 6.25 (br t, 1H, 1'H), 5.4 (br S, 1H, 3'H), 5.11 (S, 2H, cbz), 4.80 (br, 1H, COCHN), 4.05 (br S,

1H, 4'H), 3.86 (d, $J = 7$ Hz, 2H, $COCH_2N$), 3.35 (br d, 2H, 5'Hs), 3.07 (d, $J = 7$ Hz, 2H, benzylic), 2.3–2.6 (br, 2H, 2'Hs).

Reactivity of 2'(and 3')-O-cbz-(L)*-phenylalanyluridine.* A solution of the substrate (52.6 mg, 0.1 mmol), glycine ethyl ester hydrochloride (139 mg, 1 mmol), and a trace of triethylamine in chloroform (5 ml) were kept at RT for several days. By tlc analysis, a spot identical in R_f to that of authentic cbz-(L)-phenylalanylglycine ethyl ester (from cbz-L-phenylalanine and glycine ethyl ester) was detected. After a month, an extractive work-up gave the same compound in good yield. nmr; 1.20 (t, $J = 7$ Hz, 3H), 3.06 (d,d, $J = 9$ and 4 Hz, 2H, benzylic), 3.90 (d, $J = 7$ Hz, 2H, $NHCH_2CO$), 4.15 (q, $J = 7$ Hz, 2H), 4.52 (q, $J = 7$ Hz, 1H, N–CH–CO), 5.01 (S, 2H, cbz), 5.65 (d, $J = 9$ Hz, 1H, NH), 6.73 (br t, 1H, NH).

Reactivity of 2'(and 3')-O-cbz-glycyladenosine. The substrate reacted with (L)-phenylalanine ethyl ester in chloroform containing a catalytic amount of triethylamine to give the ethyl ester of cbz-glycyl-(L)-phenylalanine. nmr; 1.10 (t, $J = 7$ Hz, 3H), 3.0 (d, $J = 7$ Hz, 2H, benzylic), 3.75 (d, $J = 7$ Hz, 2H, NCH_2CO), 4.05 (q, $J = 7$ Hz, 2H), 4.80 (q, $J = 7$ Hz, 1H, NCHCO), 5.01 (S, 2H, cbz), 5.75 (t, $J = 7$ Hz, NH), 6.83 (d, $J = 9$ Hz, NH).

With glycine ethyl ester, the substrate similarly reacted to give the ethyl ester of cbz-glycylglycine. nmr; 1.24 (t, $J = 7$ Hz, 3H), 3.94 (d,d, $J = 9$, 4 Hz, 4H, $NHCH_2CO$), 4.17 (q, $J = 7$ Hz, 2H), 5.09 (S, 2H, cbz), 5.76 (br t, 1H, NH), 6.90 (br, 1H, NH).

Interactions of 2'(and 3')-O-(cbz-glycyl)-adenosine with 3'-O-[(L)-phenylalanyl)-5'-O-trityl-2'-deoxyadenosine and with 3'-O-(L)-phenylalanyl-5'-O-tritylthymidine. A solution of 3'-*O*-(L)-phenylalanyl-5'-*O*-trityl-2'-deoxyadenosine (50 mg), prepared from its cbz-protected derivative by hydrogenolysis, and 2'(and 3')-*O*-(cbz-glycyl)-adenosine (13.5 mg) in dry pyridine (0.5 ml) containing a trace of triethylamine, was kept at RT for 1 week. A spot identical in its R_f to that of the authentic 3'-*O*-[cbz-glycyl-(L)-phenylalanyl]-5'-*O*-trityl-2'-deoxyadenosine was clearly identified by tlc analysis (silica gel, 20% methanol in chloroform).

A similar reaction with 3'-*O*-(L)-phenylalanyl-5'-*O*-tritylthymidine over 2 months failed to show any sign of 3'-*O*-[cbz-glycyl-(L)-phenylalanyl]-5'-*O*-tritylthymidine.

5'-O-(cbz-glycyl)-Adenylate. 5'-*O*-(cbz-glycyl)-Adenylate was prepared essentially according to the literature procedure (*22*). To a solution of cbz-glycine (630 mg, 3 mmol) and tri-(*n*-butyl) amine in dry dioxane (4 ml) at 0°C was added ethyl chloroformate (0.33 ml) in dry dioxane (1 ml). The solution was stirred for 0.5 hr at 0°C before a solution of tri-(*n*-octyl) amine salt of adenylic acid (720 mg, 1 mmol) in dry DMF (1.5 ml) and dry dioxane (5 ml) were added. The solution was stirred for 2 hr at 0°C, overnight at RT, before it was poured into ether (100 ml). The precipitate filtered was essentially pure 5'-*O*-(cbz-glycyl)-adenylate: R_f on cellulose (t-amyl alcohol/formic acid/water, 3/2/1) was 0.66.

5'-O-Glycyladenylate. This compound was prepared by hydrogenating the cbz-derivative in 80% aqueous acid over $Pd/BaSO_4$ at 0°C.

General procedure of condensation experiments. The stock solutions of the substrate were prepared by dissolving the freshly prepared substrate in the indicated solution at 0°C. The stock solutions of the templates were similarly prepared. The substrate and the template solutions were mixed at 0°C to give the indicated concentration. The formation of organized structures was usually evident by the appearance of cloudiness

when the substrate and template solutions were mixed. In the pyridine suspension experiments, the aqueous solution of the mixture was quickly frozen at −78°C and lyophilized to yield a powder. The powdery mixture was suspended in pyridine for the indicated periods. For the aqueous experiments, the combined mixture of substrate and template was either kept at a fixed temperature or was subjected to several freeze–thaw cycles. The progress of the reactions could be monitored by following the disappearance of the starting material by tlc analysis. The experiments were stopped by treating the reaction mixture with 1 *N* NaOH solution over 0.5 hr at RT. Neutralization with 1 *N* HCl followed by lyophilization gave the total product, which was dissolved in a fixed amount of water to give the analytical sample. The total product was analyzed by paper electrophoreses at two different pHs, borate buffer (pH 9.2) and formate buffer (pH 1.5) on Whatman No. 1 paper. Identification of the products was made by direct comparison of their mobilities with those of standards. Compounds containing amino groups were visualized with Fluram spray. The relative mobilities of glycine-containing compounds are as follows (*24*):

Compound	Distance from the origin (cm)	
	pH 1.5[3]	pH 9.2[4]
Glycine	20.1	0.8
$(Gly)_2$	18.1	14.5
$(Gly)_3$	15.6	11.0
$(Gly)_4$	14.0	6.7
$(Gly)_5$	12.1	3.5
$(Gly)_6$	11.9	—

[3] Developed for 80 min at a voltage gradient of 2000 V/60 cm.
[4] Developed for 110 min at a voltage gradient of 2000 V/60 cm.

ACKNOWLEDGMENT

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