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Synthesis of Dicytidylyl-(3′–5′)-1,2-di(adenosin-N^6-yl)ethane and Dicytidylyl-(3′–5′)-1,4-di(adenosin-N^6-yl)butane: Covalently Joined Terminals of Two Transfer Ribonucleic Acids and Their Behavior toward Snake Venom Phosphodiesterase†

Jiří Žemlička

ABSTRACT: The chemical synthesis of the title bridged trinucleoside diphosphates **3e** and **3f** along with the corresponding dinucleoside phosphates **3c** and **3d** is described. Bridged nucleosides **3a** and **3b** gave on treatment with triethyl orthoformate in the presence of *p*-toluenesulfonic acid in dimethylformamide the cyclic orthoesters **2a** and **2b**. Condensation of **2a** and **2b** with *N*,2′,5′-*O*-triacetylcytidine 3′-phosphate (**1**) using dicyclohexylcarbodiimide in pyridine afforded after deblocking and chromatographic separation products **3c–f**. The latter were readily degraded with pancreatic RNase, but **3c** and **3e** were completely resistant toward snake venom phosphodiesterase whereas **3d** and **3f** were digested to the extent of 65 and 43%, respectively. The major product of degradation of **3f** with phosphodiesterase was compound **3d** resulting from the combined action of phosphodiesterase and contaminating phosphomonoesterase. The results are explained in terms of stacking of terminal bridged nucleoside units in **3c–f**. The implications of these findings for the function of snake venom phosphodiesterase are discussed.

Previous studies have established that suitably functionalized bridged adenosines are valuable spacer probes for ribosomal peptidyltransferase from *Escherichia coli* (Li et al., 1978). The results of those investigations led us to propose a possible "transition state" for the reaction of peptidyl- and aminoacyl-tRNA[1] catalyzed by peptidyltransferase which includes an intercalation of both 3′ C-A terminals (Li et al., 1978). In order to examine this possibility, it was necessary to prepare the appropriate oligonucleotides derived from the 3′ terminal of tRNA and linked covalently between their purine moieties. Such models are of additional interest as substrates for nucleolytic enzymes involved also in the metabolism of tRNA. The present report describes chemical synthesis of the requisite models which incidentally constitute the first oligonucleotides

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[1] Abbreviations used: TLC, thin-layer chromatography; DEAE, diethylaminoethyl; NMR, nuclear magnetic resonance; RNase, ribonuclease; OD, optical density; UV, ultraviolet; DCC, dicyclohexylcarbodiimide; DMF, dimethylformamide; *N*-AcPhe-tRNA, *N*-acetyl-L-phenylalanyl-tRNA; CD, circular dichroism; CPK models, Corey–Pauling–Koltun models; TosOH, *p*-toluenesulfonic acid; Et, ethyl; Ac, acetyl; other abbreviations conform with the recommendations of the IUPAC–IUB Commission on Biochemical Nomenclature (1971).

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derived from bridged nucleosides and their biochemical examination.

Materials and Methods

General Methods [*See Li & Žemlička (1977)*]. TLC, including preparative TLC, was performed as described by Žemlička & Owens (1977) in solvent S_1, dichloromethane–methanol (9:1). Paper chromatography was conducted on Whatman No. 1 paper in solvent S_2, 2-propanol–NH_4OH–H_2O (7:1:2), and S_3, 1-butanol–acetic acid–H_2O (5:2:3). Paper electrophoresis was performed by using a flat plate apparatus (Savant Instruments, Hicksville, NY) which was cooled with tap water or a circulator bath maintained at 15 °C in phosphate (0.02 M Na_2HPO_4, pH 7) and borate (0.02 M sodium borate, pH 9) buffer. For preparative chromatography and electrophoresis paper Whatman No. 3 MM was used; elution of materials from paper or cellulose was carried out by centrifugation. Column chromatography was conducted with DEAE-cellulose (Cellex D, Sigma Chemical Co., St. Louis, MO) and DEAE-Sephadex (A-25, Pharmacia, Uppsala, Sweden). The conversion of DEAE-cellulose from Cl^- to HCO_3^- form was performed as described (Zadražil, 1973) whereas the HCO_3^- form of DEAE-Sephadex was obtained as follows. A 50-g portion was swelled in 1 M ammonium carbonate (1 L), the supernatant was decanted, and the Sephadex was rinsed with 0.01 M triethylammonium hydrogen carbonate (3 × 300 mL). After the column was packed, it was washed with the same buffer (1 L). NMR spectra were obtained by using a Varian A-60 A instrument using sodium 2,2-dimethyl-2-silapentane-5-sulfonate as the internal reference.

Yields of the products were determined spectrophotometrically by using the following $\epsilon_{260}^{pH^2} \times 10^{-3}$ values from the literature: 6.8 (Cyd-3′-P; Calbiochem-Behring Corp., 1978); 25.6 (**3a**); 35.9 (**3b**; Žemlička & Owens, 1977). The corresponding values for oligonucleotides were calculated as the sum of the $\epsilon_{260}^{pH^2} \times 10^{-3}$ for Cyd-3′-P and appropriate bridged nucleoside **3a** or **3b**, disregarding a possible hypochromism due to the interaction of cytosine and purine moieties: 32.4 (**3c**); 39.2 (**3d**); 42.7 (**3e**); 49.5 (**3f**).

Starting Materials. *N*,2′,5′-*O*-Triacetylcytidine 3′-phosphate (**1**) was prepared by a modification of the described procedure (Lohrmann & Khorana, 1964) using *N*,5′-*O*-diacetylcytidine 3′-phosphate (Chládek & Žemlička, 1967). NMR (D_2O) showed three distinct acetyl groups at δ 2.20, 2.15, and 2.12. Nucleosides **3a** and **3b** were obtained as described (Žemlička & Owens, 1977).

Enzymes. Pancreatic RNase, A, Type III-A, was the product of Sigma Chemical Co., St. Louis, MO. Phosphodiesterase from Russel's viper venom (B grade) containing 0.24% of phosphomonoesterase activity was purchased from Calbiochem., San Diego, CA.

1,2-Bis[2′,3′-O-(ethoxymethylene)adenosin-N⁶-yl]ethane (**2a**). Compound **3a** (dihydrate, 0.3 g, 0.5 mmol) dried by evaporation with DMF (15 mL) at 0.1 mmHg and room temperature was dissolved in DMF (15 mL), and triethyl orthoformate (1 mL) and TosOH (monohydrate, 0.4 g, 2.1 mmol) were added. The solution was stirred for 20 h at room temperature. Triethylamine (0.5 mL) was then added, and the mixture was evaporated at 0.1 mmHg and room temperature. The residue was dissolved in chloroform (40 mL), and the solution was washed with saturated aqueous $NaHCO_3$ (2 × 20 mL) and with water (20 mL). The dried ($MgSO_4$) organic phase was evaporated, and the residue was chromatographed on a 2-mm thick loose layer of silica gel (35 × 15 cm) in S_1 containing 0.1% triethylamine. The major UV absorbing band was eluted with the same solvent, the eluate was evaporated, and the residue was precipitated from chloroform (5 mL) by the addition of petroleum ether (100 mL) to give 0.13 g (38%) of **2a**; TLC (S_1) showed a trace of a slower moving contaminant. This product was sufficiently pure to be used in the preparation of **3c** and **3e**. An analytical sample was obtained by chromatography of an 80-mg portion on a 2-mm thick 20 × 20 cm silica gel GF 254 layer in S_1 containing 0.1% triethylamine (developed twice), and the major UV absorbing band was worked up as described above to give 52 mg of **2a**: TLC (S_1) homogeneous; UV max (ethanol) 272 nm (ϵ 31 900); NMR (CD_3SOCD_3) δ 8.43 and 8.39 (2s, 2, H_8), 8.29 (s, 2, H_2), 8.03 (br s, disappeared on addition of D_2O, 2, NH), 6.29 and 6.21 (2s overlapped with 2d, 4, $H_{1'}$ and CH of EtOCH), 5.50 and 5.13 (2m, 4, H_2 and H_3), 3.66 (q, overlapped with ribose protons, CH_2 of EtO), 1.27 (m, 6, CH_3 of EtO). Anal. Calcd for $C_{28}H_{36}N_{10}O_{10}\cdot H_2O$: C, 48.69; H, 5.55; N, 20.28. Found: C, 48.68; H, 5.40; N, 20.55.

1,4-Bis[2′,3′-O-(ethoxymethylene)adenosin-N⁶-yl]butane (**2b**). Compound **3b** (0.25 g, 0.4 mmol) dried by evaporation with DMF (5 mL) at 0.1 mmHg and room temperature was dissolved in DMF (10 mL), and triethyl orthoformate (0.8 mL, 4.8 mmol) and TosOH (monohydrate, 0.23 g, 1.2 mmol) were added. The solution was stirred for 22 h at room temperature. It was worked up in the same fashion as given for compound **2a**. The crude product was chromatographed twice on a 2-mm thick 20 × 20 cm silica gel GF 254 layer in S_1 containing 0.1% triethylamine. The main UV absorbing band was worked up as described for compound **2a** to give **2b** (0.21 g, 75%): TLC (S_1) homogeneous; UV max (0.01 N HCl) 265 nm (ϵ 35 300); NMR (CD_3SOCD_3 and D_2O) δ 8.45 and 8.41 (3s, 4, H_8 and H_2), 6.28 and 6.20 (2s, 4, CH of CHOEt overlapped with $H_{1'}$), 5.49 and 5.16 (2m, 4, $H_{2'}$ and $H_{3'}$), 1.74 (br m, 4, $-C-(CH_2)_2C-$), 1.22 (m, 6, CH_3 of EtO). Anal. Calcd for $C_{30}H_{40}N_{10}O_{10}\cdot H_2O$: C, 50.13; H, 5.89; N, 19.49. Found: C, 49.85; H, 5.61; N, 19.24.

Cytidylyl-(3′–5′)-1,2-di(adenosin-N⁶-yl)ethane (**3c**) *and Dicytidylyl-(3′–5′)-1,2-di(adenosin-N⁶-yl)ethane* (**3e**). Ethoxymethylene derivative **2a** (67 mg, 0.1 mmol) and *N*,2′,5′-*O*-triacetylcytidine 3′-phosphate (**1**, 224 mg, 0.4 mmol) were coevaporated 5 times with pyridine (5-mL portions) at 0.2 mmHg and room temperature. The residue was dissolved in pyridine (2 mL), and DCC (1 g, 4.8 mmol) was added. Immediately, a syrup precipitated and the solution turned red. The mixture was shaken for 7 days at room temperature. Water (1 mL) was added, the mixture was kept for 1 h at room temperature, and the insoluble solid (dicyclohexylurea) was filtered off and washed with 10% aqueous pyridine (10 mL) and pyridine (5 mL). The filtrate was extracted with petroleum ether (3 × 10 mL) and evaporated at 0.2 mmHg and room temperature. The residue was lyophilized from water (2 × 10 mL) to remove pyridine. It was then dissolved in cold 80% HCOOH (4 mL) and the solution was kept at 0 °C for 20 min. Water (10 mL) was added and the mixture was lyophilized. The residue was lyophilized from water (5 × 10 mL) and 10% pyridine (20 mL) to remove HCOOH. It was dissolved in methanol saturated with ammonia at 0 °C (10 mL), and the solution was kept for 16 h at room temperature and then evaporated. The residue was dissolved in water (20 mL), the insoluble portion was filtered off, and the filtrate was put on the top of the DEAE-cellulose column (HCO_3^-; 47 × 3 cm). The column was washed with water to the disappearance of UV absorption and then eluted with a linear gradient of triethylammonium bicarbonate: 0.25 M (2 L) in

Table I: Identification of Products of Condensation of *N*,2′,5′-*O*-Triacetylcytidine 3′-Phosphate (**1**) with 1,2-Bis[2′,3′-*O*-(ethoxymethylene)adenosin-N^6-yl]ethane (**2a**) on a DEAE-cellulose Column (for Details See Materials and Methods)

peak	OD_{260}^{pH2} units	fractions pooled	identification[a]
A	175	61–65	cytidine-2′(3′)-methyl phosphate[b]
B	385	68–72	Cyd-2′:3′-P
C	600	75–83	**3c**
D	1230	98–119	Cyd-3′-P plus **3e**

[a] Peaks were identified by paper chromatography (S_2 and S_3), electrophoresis (phosphate), and UV spectrophotometry at pH 2 (0.01 N HCl). Peaks A and B were degraded (A partially) with pancreatic RNase. [b] Tentative.

Table II: R_f Values and Electrophoretic Mobilities of **3c–f** (for Details See Materials and Methods)

compd	$R_f(S_2)$[a]	$R_f(S_3)$[a]	mobility phosphate[a]	mobility borate[b]
3c	1.23	0.62	0.20	1.00
3d	1.23	0.58	0.15	
3e	0.88	0.10	0.44	1.17
3f	1.10	0.11	0.36	1.07
Cyd-2′:3′-P	1.44	1.00	0.64	

[a] Relative to R_f of Cyd-(2′)3′-P = 1.00. [b] Relative to the mobility of **3c** = 1.00.

Table III: UV Spectral Data of Compounds **3c–f** in 0.01 N HCl (pH 2)

compd	max (nm)	min (nm)	A_{250}/A_{260}	A_{280}/A_{260}	A_{290}/A_{260}
3c	275,[a] 265	235	0.82	1.30	0.72
3d	275,[a] 267	236	0.69	0.84	0.38
3e	277	237	0.63	1.15	0.70
3f	270	235	0.60	0.91	0.46

[a] Shoulder.

the reservoir; 2 L of water in the mixing vessel; flow rate 0.7 mL/min; 21-mL fractions taken. For fraction pooling and identification of peaks, see Table I. Peaks A–D were evaporated in vacuo and the corresponding residues were coevaporated 3 times with methanol (see Table I for details).

Peak C was chromatographed on one 2-mm thick 20 × 20 cm layer of Avicel in solvent S_2, the main UV absorbing band close to the origin was eluted with water, and the eluate was lyophilized to give **3c** (390 OD_{260}^{pH2} units, 12 μmol, 12%), uniform on paper chromatography (S_2 and S_3) and electrophoresis (phosphate and borate, Table II) (for UV data see Table III).

Peak D was chromatographed as in the case of peak C, and the main band from the origin was worked up as described above to give **3e** (218 OD_{260}^{pH2} units, 5.5 μmol, 5.5%), uniform on paper chromatography (S_2 and S_3) and electrophoresis (phosphate and borate, Table II) (for UV data see Table III).

Cytidylyl-(3′–5′)-1,4-di(adenosin-N^6-yl)butane (**3d**) *and Dicytidylyl-(3′–5′)-1,4-di(adenosin-N^6-yl)butane* (**3f**). The experiment was performed as described in the case of **3c** and **3e** on the 0.05-mmol scale of **2b**. After the usual workup, paper electrophoresis (phosphate) of the crude product showed the presence of five components: nucleoside **3b**, **3d**, **3f**, Cyd-2′:3′-P, and Cyd-3′-P. This mixture was chromatographed on the DEAE-Sephadex column (HCO_3^-; 55 × 2.5 cm) which was first washed with 0.01 M triethylammonium hydrogen carbonate until the disappearance of UV absorption and then

Table IV: Identification of Products of Condensation of *N*,2′,5′-*O*-Triacetylcytidine 3′-Phosphate (**1**) with 1,4-Bis[2′,3′-*O*-(ethoxymethylene)adenosin-N^6-yl]butane (**2b**) on a DEAE-Sephadex Column (for Details Cf. Materials and Methods)

peak	OD_{260}^{pH2} units	fractions pooled	identification[a]
A	20	71–76	unidentified
B	76	78–83	cytidine-2′(3′)-methyl phosphate[b]
C	24	88–93	unidentified
D	588	97–107	Cyd-2′:3′-P plus **3d**
E	285	156–161	Cyd-3′-P
F	795	164–173	**3f**

[a] Peaks were identified by paper electrophoresis (phosphate), by UV spectrophotometry at pH 2, and by comparison with similar products obtained from the experiment with **2a**, Table I. [b] Tentative.

eluted with a linear gradient of the same buffer: 0.4 M (2 L) in the reservoir; 2 L of water in the mixing vessel; flow rate 1.05 mL/min; 21-mL fractions taken. Peaks A–F were pooled and worked up as given in the preceding experiment (see Table IV for details).

Peak D was subjected to preparative electrophoresis on a 27-cm wide strip of Whatman 3 MM paper using 0.03 M triethylammonium hydrogen carbonate for 4 h at 20 V/cm. The band of **3d** was eluted with water, and the eluate was lyophilized to give 80 OD_{260}^{pH2} units (1.9 μmol, 4%) of **3d**. Peak F afforded 795 OD_{260}^{pH2} units (16 μmol, 32%) of **3f**. For a further characterization of **3d** and **3f** see Tables II and III.

Degradation of **3c–f** *with Pancreatic RNase.* Compounds **3c–f** (0.6 μmol of each) were incubated with pancreatic RNase (0.2 mg) in 0.1 M Tris-HCl (pH 7.5), total volume 0.2 mL, for 22–24 h at 37 °C. The mixtures were applied on Whatman 3 MM paper and subjected to electrophoresis in phosphate buffer. The corresponding UV absorbing spots were eluted with 0.01 N HCl (5 mL each), and the UV spectrum of each eluate was determined by using an appropriate blank solution. The amount of undegraded (2′–5′) material was 1–1.5% in the case of **3c** and **3e**, and the ratio Cyd-3′-P/**3a** was 1.1 for **3c** and 1.8 for **3e**. Compound **3d** was degraded from 96%, and Cyd-3′-P/**3b** was 1.2 whereas **3f** gave an almost quantitative cleavage to Cyd-3′-P and **3b** in the ratio of 1.8. In the latter case, only traces of undegraded material (2′–5′ isomer of **3d**) were detected.

In separate experiments, the incubation mixtures of **3c** and **3e** were chromatographed on Whatman 3 MM paper in solvent S_2, the spots of nucleoside **3a** were eluted with water, and the eluates were lyophilized and subjected to paper electrophoresis in borate buffer. The presence of a single spot corresponding to that of **3a** was ascertained in each case.

Degradation of **3c–f** *with Snake Venom Phosphodiesterase.* Compounds **3c–f** (0.3 μmol of each) were incubated with Russell's viper venom phosphodiesterase (20 units) in water (0.1 mL), 0.1 M Tris–acetate (pH 8.8; 0.125 mL), and 0.3 M magnesium acetate (0.025 mL) for 22 h at 37 °C. The mixtures were subjected to paper electrophoresis, and the resulting spots were analyzed by UV spectrophotometry as described for pancreatic RNase. No degradation of compounds **3c** and **3e** was observed. Dinucleoside phosphate **3d** was cleaved from 65%, giving Cyd and bridged derivatives **3b** and **4**. The ratio of Cyd/(**3b** plus **4**) was 1.3, and that of **4**/**3b** was 5.5. The structure of **4** followed from the UV spectrum which was identical with that of **3b** and from the electrophoretic mobility [phosphate, 0.47 of Cyd-2′(3′)-P]. Trinucleoside diphosphate **3f** was degraded from 43%. For the

Scheme I

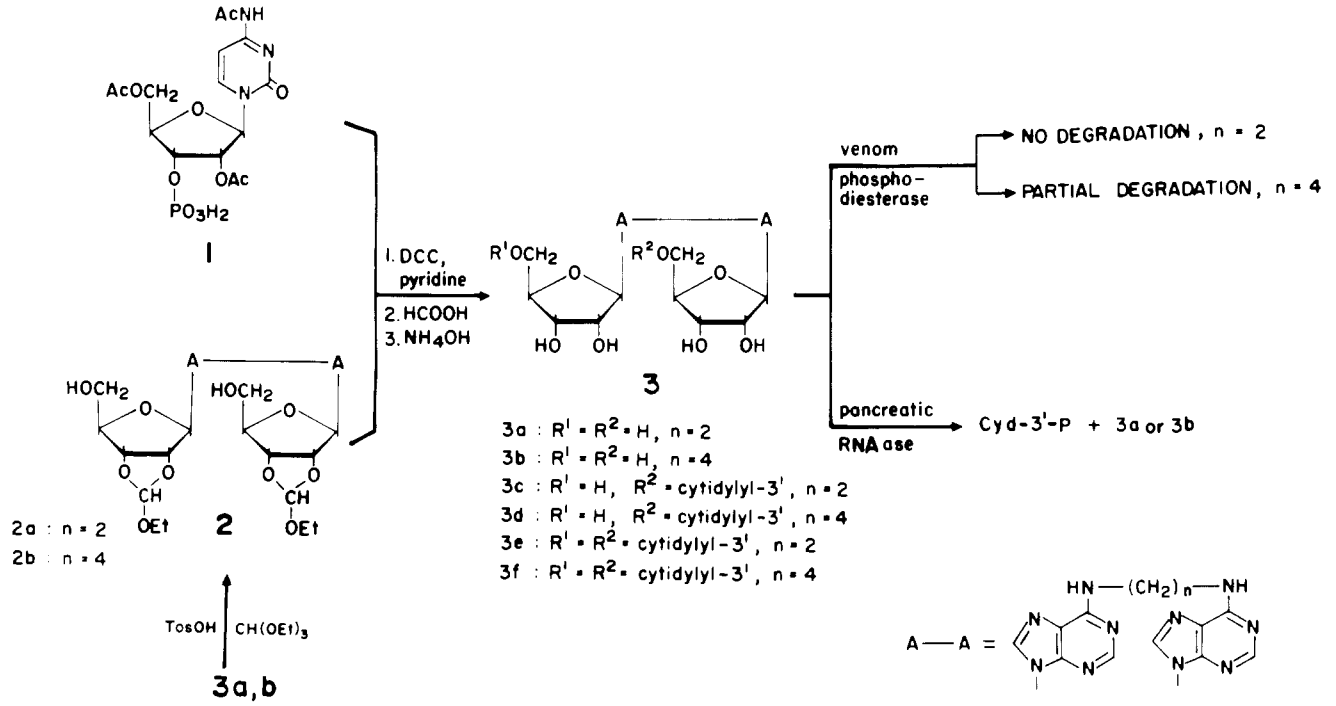


Table V: Degradation of Trinucleoside Diphosphate **3f** (0.3 μmol) with Snake Venom Phosphodiesterase (for Details See Materials and Methods)

product	$OD_{260}^{pH^2}$ units	% OD[a]	yield (μmol)	yield (%)
3d	3.7	27.4	0.087	28.9
3f	7.6	57.1	0.154	51.2
3b[b]	0.85	6.4	0.024	7.9
Cyd[c]	1.2	9.0	0.177	29.4

[a] Based on $OD_{260}^{pH^2}$ recovered (90%). [b] Electrophoretic mobility (phosphate): −0.09 of Cyd-2'(3')-P. [c] Electrophoretic mobility (phosphate): −0.16 of Cyd-2'(3')-P.

product composition see Table V. In a control experiment, U-A was quantitatively digested to Urd and Ado-5'-P within 6 h.

Results and Discussion

Synthesis. For the preparation of model compounds **3c–f** a general method of condensation of a suitably protected 3'-nucleotide with a nucleoside cyclic 2',3'-orthoformate was employed (Žemlička et al., 1966). The starting components **2a** and **2b** resulted from an orthoester exchange reaction of the appropriate bridged nucleosides **3a** and **3b** with triethyl orthoformate in DMF catalyzed with TosOH (Scheme I). The yields of **2a** and **2b** were 38 and 75%, respectively. NMR spectra, which confirmed the structure of both intermediates, were rather complex, and splitting of signals indicated the presence of a diastereoisomeric mixture resulting from the introduction of two new asymmetric carbon atoms of cyclic 2',3'-orthoformate into the molecule. In view of the existence of a plane of symmetry in **3a** and **3b**, three stereoisomers of **2a** and **2b**, i.e., exo/exo, exo/endo, and endo/endo, are possible. *N*,2',5'-*O*-Triacetylcytidine 3'-phosphate (**1**; Lohrmann & Khorana, 1964) was used as a nucleotide component. The condensation of **1** with nucleosides **2a** and **2b** was effected with DCC for 7 days at room temperature.

The strategy of deblocking was of considerable importance for obtaining products with a minimum of isomerization of 3'–5' internucleotide bonds. Thus, the orthoester grouping was first hydrolyzed with cold 80% HCOOH. This treatment presumably left the "critical" 2'-*O*-acetyl groups in the products intact. All acyl functions were subsequently removed by using methanolic ammonia. The resultant mixture of products was then chromatographed on the DEAE-cellulose column (DEAE-Sephadex in the case of **3d** and **3f**), and the materials obtained thereby were further purified by either preparative TLC (**3c** and **3e**) or electrophoresis (**3d**). It is of interest to note that overall yields were higher with the more "extended" bridged nucleoside **2b** (**3d** plus **3f**, 36%) compared to **2a** (**3c** plus **3e**, 17.5%). Similarly, the reaction at both 5'- and 5''-hydroxy groups was favored in the case of **2b** where a 32% yield of trinucleoside diphosphate **3f** and only 4% of dinucleoside phosphate **3d** were obtained. In contrast, nucleoside **2a** afforded trinucleoside diphosphate **3e** in 5.5% yield together with dinucleoside phosphate **3c** (12%). All products were characterized by usual criteria: UV spectroscopy (Table III), paper chromatography, electrophoresis (Table II), and degradation with pancreatic RNase. The latter confirmed the correct nucleotide/nucleoside ratio: 1 in the case of **3c** and **3d** and 2 for **3e** and **3f**. The amount of unnatural isomers with the 2'–5' internucleotide bond was 1–1.5% for **3c** and **3e** and <1–4% for **3d** and **3f**.

Biochemical Results. (*A*) *Protein Synthesis.* Compounds **3e** and **3f** did not inhibit the puromycin reaction in the *N*-AcPhe-tRNA–*E. coli* 70S ribosomes–poly(U) system at concentrations of 8×10^{-4} M (P. Bhuta, unpublished results).

(*B*) *Snake Venom Phosphodiesterase Studies.* Compounds **3c** and **3e** are completely resistant toward venom phosphodiesterase from Russel's viper. In contrast, derivative **3d** was digested to the extent of 65%, giving phosphate **4** and cytidine as the major products along with some bridged nucleoside **3b** (Scheme II). The occurrence of **3b** in the mixture is not surprising considering the presence of a minute amount of phosphomonoesterase in the enzyme preparation and also rather forcing reaction conditions—excess enzyme and a prolonged incubation period (22 h). The stoichiometry of Cyd formed corresponded to that of **3b** plus **4**. Compound **3f** was degraded from 43%, giving **3d** as the major product. Again, the occurrence of **3d** in the reaction mixture is readily ac-

Scheme II

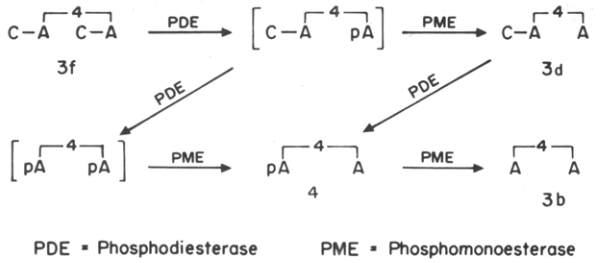
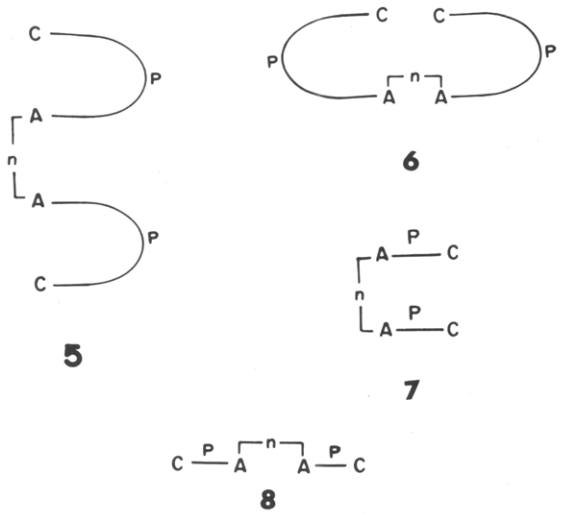


Chart I[a]



[a] P = phosphodiester bridge; C = cytosine; A = purine (N^6-alkyladenine). Series **a**, $n = 2$; series **b**, $n = 4$. For a similar notation cf. Bangerter & Chan (1969). Stacked base residues are arranged vertically; unstacked are arranged horizontally. For CPK models of **5a** and **5b**, see Figure 1.

counted for by the presence of phosphomonoesterase (Scheme II and Table V). It is somewhat surprising that nucleotide **4** was not found in the mixture whereas a small amount of **3b** was present. Thus, partial degradation of **3f** can be explained in terms of a series of reactions catalyzed by phosphodiesterase and phosphomonoesterase (Scheme II). As already mentioned, the chemical synthesis afforded compound **3d** only in 4% yield, whereas a 32% yield of **3f** was obtained. Therefore, some improvement can be attained by a preparative use of phosphodiesterase degradation of **3f** to **3d** in 9% overall yield.

The difference in substrate activities essentially reflects lengthening of the aliphatic chain in **3d** and **3f** relative to **3c** and **3e**. A set of conformations can be considered for all these oligonucleotides. Thus, fully stacked structures **5a** and **5b** (Chart I and Figure 1) can be derived from the fact that in bridged nucleosides **3a** and **3b** the purine residues are stacked (Žemlička & Owens, 1977) and from an assumption that a similar type of interaction could also take place between cytosine and purine moieties as found in the case of C-A (Bangerter & Chan, 1969; Warshaw & Tinoco, 1966). Thus, structures **5a** and **5b** (Chart I and Figure 1) may adequately represent the arrangement of base residues in **3e** and **3f**. A similar stacking pattern can also be considered for compounds **3c** and **3d**. However, occurrence of other stacked conformations cannot be excluded in the present time. Extended forms with a partially destacked bridged nucleoside portion (Chart I, formula **6**), a cytosine moiety (formula **7**), or a completely "unwound" structure **8** (the corresponding CPK models are not shown) are less likely.

In a functional model of venom phosphodiesterase (Wigler, 1963), a pocket for the base residue together with binding sites

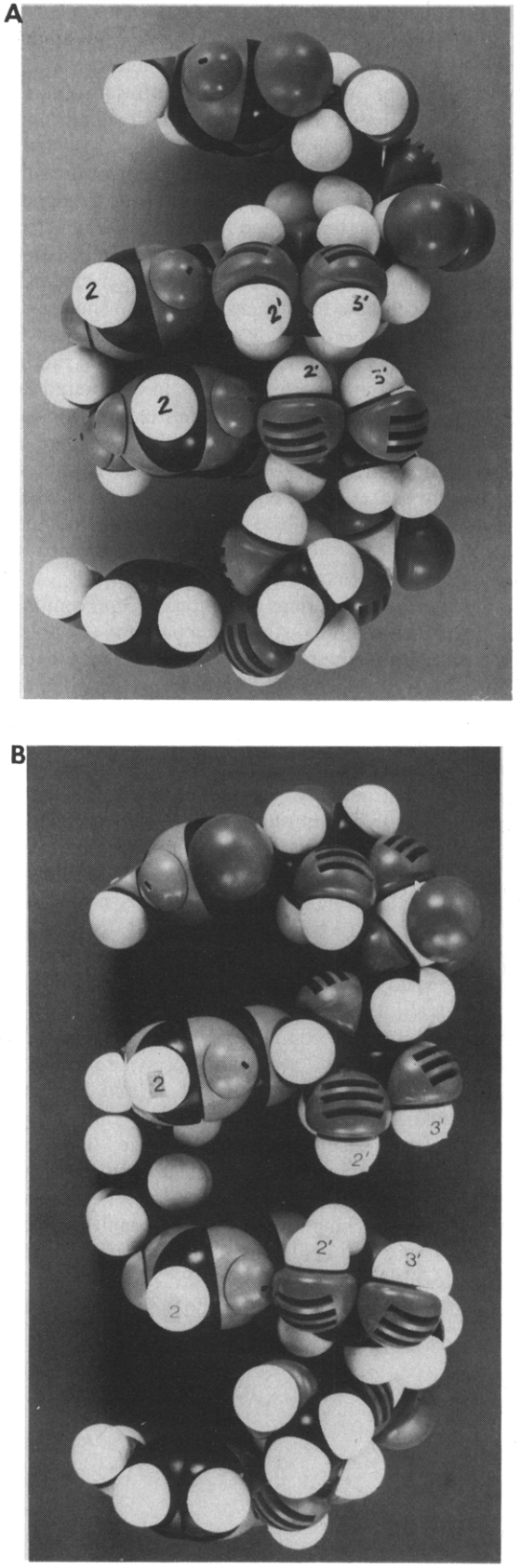


FIGURE 1: (A) CPK model of bridged oligonucleotide **3e** in a stacked conformation. For an abbreviated notation see Chart I, formula **5a**. Note the closeness of both 2′,3′-vicinal glycol functions of the bridged ribonucleoside moiety. All base residues are in anti conformation; $C_{4'}$–$C_{5'}$ bond is in *g,g* form. Number 2 denotes the appropriate hydrogen atoms of the bridged purine moiety. (B) CPK model of bridged oligonucleotide **3f** in a stacked conformation. See also Chart I, formula **5b**. Note that both purine and 2′,3′-vicinal glycol portions are more distal than in **3e**.

for the phosphodiester grouping and a water molecule has been suggested. According to the proposal there is no binding site or pocket for the ribose portion. It is therefore likely that extended forms of **3e** and **3f** represented by formulas **6** and **8** should be amenable to degradation with venom phosphodiesterase. It can be argued that the presence of the N^6–N^6 aliphatic chain may interfere with the binding of the substrate to the enzyme surface. However, there is little to suggest that an N^6-alkylation of the adenine moiety seriously affects the substrate activity (Schweizer et al., 1971). In addition, it is difficult to explain why the presence of a longer chain (**3d** and **3f**) is more acceptable for the enzyme than a shorter one (**3c** and **3e**). On the other hand, it is possible to account for the observed differences by invoking fully stacked structures **5a** and **5b** (Chart I and Figure 1). Thus, in compound **3e** (formula **5a**, Figure 1A) both phosphodiester functions are accessible but purine and ribose moieties are close together. It is likely that tightly stacked base residues cannot fit the corresponding pocket on the enzyme surface. Such a situation is somewhat reminiscent of the covalently linked photodimers of DNA which are known to be resistant toward venom phosphodiesterase (Setlow et al., 1964).

In contrast, the purine rings of compound **3f** in conformation **5b** (Figure 1B) are more distal and apparently are able to fit, at least partly, into the pocket. However, it may be argued that the observed differences can be caused by one or both bridged bases of **3c–f** in or close to a syn conformation. Recently, it has been shown that a nucleoside phosphodiester having a syn conformation of the 3′-terminal base residue is only partially degraded by venom phosphodiesterase (Ogilvie & Hruska, 1976). The question of anti–syn conformation of bridged nucleosides **3a** and **3b** remains unsettled. The CD spectra (Žemlička & Owens, 1977) seem to rule out the possibility of both purine residues being in a syn conformation. In addition, some 2′(3′)-*O*-aminoacyl derivatives of **3a** were found to be excellent substrates for ribosomal peptidyltransferase catalyzed peptide bond formation (Bhuta et al., 1977; Li et al., 1978), a process which exhibits a distinct requirement for an anti conformation (Žemlička et al., 1975). However, an alternative occurrence of syn rotamers in the overall conformational population of **3a** and **3b** cannot be entirely excluded. It is also recognized that association (stacking) of two base residues can "freeze" one particular type of rotamer by preventing rotation around the *N*-glycosyl linkage (Saenger, 1973). Thus, changes in anti–syn population of rotamers and stacking of bases can be coupled. Therefore, such effects may also contribute to the observed differences in substrate activity of **3c–f**.

It is concluded that stacking phenomena, coupled possibly with changes in anti–syn rotameric population, can satisfactorily account for the behavior of oligonucleotides **3c–f** toward venom phosphodiesterase. Such an explanation is in accord with the proposed mechanism of venom phosphodiesterase action (Wigler, 1963). Additional models are needed to examine whether a further extension of aliphatic chain can lead to a full restoration of substrate activity.

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