due to the presence of both α- and β-isomers.] IR ($CHCl_3$) ν_{max} 3340 (NH, OH), 1730 (OCO), 1710 ($COCH_3$), 1650 (γ-pyrone), 1610, and 1585 cm^{-1} (Ar); MS (FAB), m/z (relative intensity) 731 (0.2, MH^+), 730 (0.1, M^+) 391 (0.1, O-sugar), 356 (0.8, MH^+ - sugar), 355 (0.3, M^+ - sugar), 340 [0.8, MH^+ - (O-sugar)], 339 [1.8, M^+ - (O-sugar)], 338 (0.9, 339 - H), 321 (1.2, 338 - OH), 320 (1.9, 338 - H_2O), 279 [5.1 (391 - $NHCOCF_3$)], 225 (2.8, 391 - $C_7H_4NO_4$), 122 (8.3, $C_6H_4NO_2$), 121 (100, 122 - H), 104 (15.3, C_7H_4O).

Mild Base Deprotection of 11 Affording the Glycosides 13 and 14. Compound 11 (85 mg) was dissolved in 15 mL of acetone, the solution was treated with 0.1 N NaOH (9 mL), and the reaction mixture was stirred for 30 min under a nitrogen atmosphere. The pH of the solution was adjusted to 8 by adding a few drops of 5% HCl and the solution was then extracted with ethyl acetate (5 × 25 mL). The ethyl acetate extract was washed with water and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was dissolved in acetone and applied to preparative TLC plates and eluted with $CHCl_3$–MeOH (4:1). Three bands appeared on the plates which were removed separately and the silica was extracted with acetone and stirred for 30 min and filtered. Removal of the solvent from band I gave **13**, which was purified by recrystallization from acetone to give the pure partially deprotected compound **13**: 14 mg (yield 18%); mp 270–285 dec; ^1H NMR (Me_2SO-d_6, $CDCl_3$ 1:1) δ 1.3 (t, 3 H, C'_5CH_3), 1.70 (m, 2 H), 2.10 (m, 1 H), 2.30 (d, 3 H, $COCH_3$), 2.55 (m, 2 H), 3.2 (m, 2 H), 3.60 (d, 1 H), 4.1 and 4.4 (q, both signals integrate for 1 H), 4.25 (m, 1 H), 4.7 and 4.85 (d, both signals integrate for 1 H, C'_4OH, exch), 5.2 and 5.4 (bs, 1 H, C_7H_e, $J_{1/2}$ = 3 Hz), 5.3 and 5.5 (d, 1 H), 7.45 (t, 1 H, Ar), 7.62 (dd, 1 H, Ar), 7.80 (m, 1 H, Ar), 8.25 (m, 2 H, one proton is exch), 8.8 (bs, 1 H, exch), 12.20 (s, 1 H, $C_{11}OH$, exch); IR ($CHCl_3$) ν_{max} 3300 (NH, OH), 1710 and 1695 ($COCH_3$), 1645 (amide), 1625 (γ-pyrone), 1605, and 1590 cm^{-1} (Ar); MS (FAB, glycerol), m/z (relative intensity) 566 (0.2, MH^+), 324 [1.4, MH^+ - (O-sugar)], 323 [4.2, M^+ - (O-sugar)], 322 (1.1, 323 - H), 279 (6.5, 322 - $COCH_3$), 278 [1.8, 322 - ($COCH_3$ + H)], 242 (0.6, O-sugar), 226 (0.9, 242 - O). The third TLC band upon workup gave compound 14 which was further purified by recrystallization from acetone–ether (1:1): 7 mg (yield 10%); mp 200–205 °C dec; IR (KBr) ν_{max} 3400 (NH_2, OH), 1700 ($COCH_3$), 1650 and 1625 (γ-pyrone), 1610, and 1590 cm^{-1} (Ar); MS (FAB, glycerol), m/z (relative intensity) 470 (1.9, MH^+), 469 (0.2, M^+), 335 (0.4, M^+ - sugar), 319 [0.5, M^+ - (O-sugar)], 318 (0.2, 319 - H), 303 (0.4, 318 - CH_3), 275 (0.7, 318 - $COCH_3$), 134 (0.5, sugar), 133 (1.4, 134 - H), 115 (18.7, 133 - H_2O), 99 (3.5, 115 - NH_2).

Mild Base Deprotection of 12 Leading to the Glycoside 15. When a procedure similar to that for the deprotection of 11 was used, compound **12** was deprotected and purified by TLC (on preparative silica plates) and eluted with ether to give **15** which was further purified by recrystallization from THF–ether (1:1) (yield 25%): mp 185 and 210 °C dec; ^1H NMR (Me_2SO-d_6) δ 1.1 (q, 1 H, C'_5CH_3), 1.50 (m, 1 H), 2.20 (d + m, 6 H, $COCH_3$ + 3 H), 2.90 (m, 2 H), 3.50 (m, 2 H, one of the proton is exch), 4.02 (m, 1 H), 4.18 (q, 1 H), 4.95 (q, <1 H, exch), 5.10 (t, <1 H), 5.25 (m, 1 H), 5.40 (t, <1 H), 5.50 (d, <1 H, exch), 7.50 (t, 1 H, Ar), 7.65 (m, 1 H, Ar), 7.95 (m, 1 H, Ar), 8.20 (d, 1 H, Ar), 9.12 (t, 1 H, NH, exch), 9.40 (d, 1 H, C_6OH, exch), 12.16 (d, 1 H, COH, exch); IR ($CHCl_3$) ν_{max} 3400 (NH, OH), 1725, 1705 ($COCH_3$), 1650 (amide), 1625 (γ-pyrone), 1610, and 1590 cm^{-1} (Ar); MS (FAB, glycerol–sulfolane), m/z (relative intensity) 582 (0.5, MH^+), 581 (0.2, M^+), 356 (2.1, MH^+ - sugar), 355 (0.7, M^+ - sugar), 340 [1.6, MH^+ - (O-sugar)], 339 [3.5, M^+ - (O-sugar)], 338 [1.7, M^+ - (O-sugar)], 323 (1.0, 338 - CH_3), 321 (2.9, 338 - OH), 320 (4.2, 338 - H_2O), 295 (5.4, 323 - CO), 294 (2.3), 295 - H), 277 (39, 294 - OH), 242 (1.9, O-sugar), 241 (11.0, 242 - H), 226 (5.5, sugar), 225 (1.8, 226 - H), 104 (9.6, C_7H_4O).

Acknowledgment. This investigation was supported by grants to J.W.L. from the National Cancer Institute of Canada and the Natural Sciences and Engineering Research Council of Canada and by a contract with the National Foundation for Cancer Research. We thank Dr. John H. Peters and Dr. G. Ross Gordon of SRI Internationsl, Menlo Park, CA, for the augmentation of hepatic microsome oxygen consumption data and Dr. John Stezowski and E. Eckle, of the Institut fur Organische Chemie, Biochemie and Isotopenforschung der Universitat Stuttgart, for the X-ray determination. We also thank Dr. Robert Newman of the M. D. Anderson Tumor Institute, Houston, and Dr. Miles Hacker of the Vermont Regional Cancer Center, Burlington, for the antileukemia cytotoxicity data, Dr. Tom Nakashima and his associates for the high-field NMR measurements, and Dr. Alan Hogg and his colleagues for the high-resolution mass spectra.

Supplementary Material Available: X-ray crystallographic data (16 pages). Ordering information is given on any current masthead page.

Expedient Chemical Synthesis of Sequence-Specific 2′,5′-Oligonucleotides

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Received July 13, 1984

A rapid chemical approach to the preparation of sequence-specific 2′,5′-oligonucleotides and analogues of 2-5A is described. For instance, reaction of the 5′-phosphoromorpholidate of adenosine (MopA, **14**) with the 5′-phosphoroimidazolidate of inosine (ImpI, **17**), under conditions of lead ion catalysis, gave MopA2′p5′I (**19a**) in 21% yield. Acid hydrolysis of **19a** gave pA2′p5′I, which then was converted to the corresponding 5′-phosphoroimidazolidate Imp5′A2′p5′I (**19a**) through redox condensation with triphenylphosphine, imidazole, and 2,2-dipyridyl disulfide. Lead ion catalyzed condensation of **19c** with Mop5′A (**14**) gave Mop5′a2′p5′A2′p5′I (**30**) in 17% yield. By acid hydrolysis, **30** could be converted to the corresponding 5′-monophosphate, or, by reaction with pyrophosphate in DMF, to the corresponding 2-5A analogue, ppp5′A2′p5′A2′p5′I (**5b**). The following oligonucleotides were prepared by using similar methodology: ppp5′A2′p5′A2′p5′A (**1b**), ppp5′A2′p5′I2′p5′A (**4b**), ppp5′I2′p5′A2′p5′A (**3b**), and ppp5′A2′p5′A2′p5′ (2′dA) (**2b**).

The natural occurrence of the 2′,5′-phosphodiester bond in 5′-triphosphoryladenylyl(2′→5′)adenylyl(2′→5′)-adenosine (2-5A or 2′,5′-oligo A), a mediator of interferon action,[2-4] and in pre-tRNA,[5] has led to increased interest in the synthesis of such unusually linked oligonucleotides.[6,7] For studies on the relationship of oligonucleotide structure

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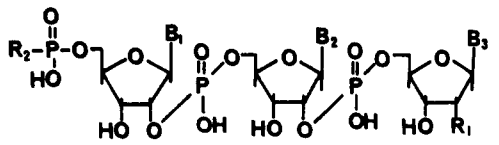
Scheme I



1a,b $R_1 = OH$, $B_1 = ade$, $B_2 = ade$, $B_3 = ade$

2a,b $R_1 = H$, $B_1 = ade$, $B_2 = ade$, $B_3 = ade$

3a,b $R_1 = OH$, $B_1 = hyp$, $B_2 = ade$, $B_3 = ade$

4a,b $R_1 = OH$, $B_1 = ade$, $B_2 = hyp$, $B_3 = ade$

5a,b $R_1 = OH$, $B_1 = ade$, $B_2 = ade$, $B_3 = hyp$

a, $R_2 = OH$; b, $R_2 = O\text{-}P(O)(OH)\text{-}O\text{-}P(O)(OH)\text{-}OH$

to the activation of the 2-5A-dependent endonuclease, 2-5A itself and numerous analogues have been prepared by a variety of methodologies including the phosphotriester strategy[8-16] and the lead ion-catalyzed polymerization approach.[17,18]

Thus far, 2′,5′-oligoadenylate analogues prepared have been of the N_pN_pN or N_pN_pX[19-24] type where X may be introduced during a stepwise synthesis[19-21] in a phosphotriester approach or via chemical modification[22] of the A_pA_pA structure. Increasingly, however, it has become important to explore the biological activity of 2′,5′-oligonucleotides in which the first, second, or third nucleotide residue has been altered in order to determine the relative contribution of each residue to the binding and enzyme activation processes.[25] For instance, the dinucleotide, ppp5′A2′p5′A, does not bind well to or activate the 2-5A-dependent endonuclease whereas the trinucleotide ppp5′A2′p5′A2′p5A does,[1] thereby suggesting the importance of the third or 2′-terminal nucleotide residue in the binding and activation process. On the other hand, the trinucleotide, p5′A2′p5′A2′p5′A, will bind well to but not activate the endonuclease of mouse L cells whereas the corresponding 5′-di- or 5′-triphosphate, (p)-pp5′A2′p5′A2′p5′A,[26,27] will activate the enzyme, thereby assigning some importance to the 5′-terminus of the molecule in enzyme activation.

In this context, it is clear that a rapid chemical approach to sequence-specific 2′,5′-linked oligonucleotides would be of considerable value. Herein we describe such an approach and illustrate its use by the synthesis of native 2-5A (**1b**) itself, a 2′-terminal deoxy analogue, pppA2′p5′A2′p5′(2′dA) (**2b**), and three heterobase analogues; namely, ppp5′I2′p5′A2′p5′A (**3b**) ppp5′A2′p5′I2′p5′A (**4b**), and ppp5′A2′p5′A2′p5′I (**5b**) (Scheme I).

Results

To date, the most facile and useful method for the synthesis of 2′,5′-oligoadenylates has been the lead ion-catalyzed polymerization of adenosine 5′-phosphoroimidazolidate (ImpA).[17,28-30] Without the use of any sugar or base-protecting groups, this method gives a high yield of various 2′,5′-oligoadenylates of increasing chain length together with a small percentage of 3′,5′-linkage isomers which can be removed readily by digestion with P_1 nuclease or by HPLC.[18,25] Because (i) radioactive adenosine added to the reaction mixture during the lead ion-induced polymerization of adenosine 5′-phosphoroimidazolidate gave a series of 5′-radioactive adenosine terminated oligomers and (ii) because oligomers such as $(pA)_4$ could not be elongated by addition of adenosine 5′-phosphoroimidazolidate under conditions of lead ion catalysis, Lohrmann and Orgel[31,32] suggested that polymerization proceeded in the 2′→5′ direction rather than the 5′→2′ direction.

In concert with these latter findings, when we repeated the same reaction of adenosine (**6**, 2 mol) with ImpA (**7**, 1 mol), A2′p5′A (**8**) was generated but so was an equal amount of A3′p5′A for a total product yield of 55% based on input ImpA. Similarly, the reaction of adenosine (**6**, 2 mol) with ImpA2′p5′A (**11c**, 1 mol) gave a mixture of A2′p5′A2′p5′A (**12**, 25%) and A3′p5′A2′p5′A (**13**, 21%) together with the hydrolysis product pA2′p5′A (31.5%). It is likely that the loss of selectivity for 2′,5′-linked product in the polymerization reaction may be due to the deletion of the 5′-monophosphate moiety which may serve to influence the relative reactivity of the 2′- and 3′-hydroxyl groups. However, as may be predicted from Lohrmann and Orgel's results, the reaction of AMP (**10**) with ImpA (**7**) in the presence of lead ion at pH 7.5 gave no oligomerization: instead only hydrolysis of ImpA to pA occurred.

The above considerations suggested that a suitable 5′-phosphate blocking group might be able to serve as a

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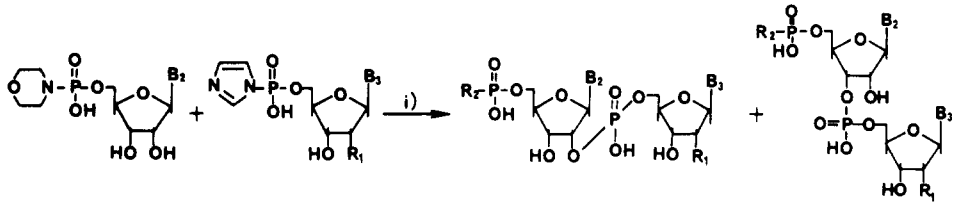
Scheme II



14, B_2 = ade; 15, B_2 = hyp

7, B_3 = ade; R_1 = OH; 16, B_3 = ade; R_1 = H; 17, B_3 = hyp; R_1 = OH

11a,b,c B_2 = B_3 = ade; R_1 = OH; 18a,b,c B_2 = B_3 = ade; R_1 = H; 19a,b,c B_2 = ade; B_3 = hyp; R_1 = OH; 20a,b,c B_2 = hyp; B_3 = ade; R_1 = OH

21a,b B_2 = B_3 = ade; R_1 = H

a, R_2 = Mo; b, R_2 = OH; c, R_2 = Im

i) Pb^{+2}, pH 7.0–7.5, imidazole buffer, 4°

Table I. Lead-Catalyzed Coupling of Nucleoside Phosphoromorpholidates and Dinucleotide Phosphoroimidazolidates

morpholidate[a]		imidazolidate[a]		reaction condition[b]		purification[e] by chromatography[f]			main product			byproduct		
						column size, cm × cm	eluting concn (M) for							
compd	amt, μmol	compd	amt, μmol	buffer,[c] mL	catalyst,[d] μL		main product	byproduct	compd	amt, μmol	yield,[g] %	compd	amt, μmol	yield,[g,h] %
6	80	**11c**	40	2.5	150	1.6 × 16	0.11	0.13	**12**	10.0	25	**13**	8.4	21
24	124	**11c**	62.0	1.24	124	1.6 × 16	0.20	0.22	**24**	11.2	18	**25**	5.4	8.8
14	173	**18c**	86.6	1.73	173	1.6 × 25	0.19	0.22	**26**	19.9	23	**27**	9.6	11
15	160	**11c**	80.0	2.00	200	1.6 × 25	0.21	0.24	**28**	14.4	18	**29**	7.0	8.7
14	136	**19c**	68.0	1.36	136	1.6 × 25	0.22	0.26	**30**	11.6	17	**31**	5.2	7.7
14	120	**20c**	60.0	1.20	120	1.6 × 24	0.22	0.24	**32**	11.4	19	**33**	5.0	8.3

[a] Sodium salts. [b] Temperature, 4 °C. [c] 0.2 M imidazolium nitrate (pH 6.75). [d] 0.25 M lead nitrate. [e] Reaction mixture was treated with Chelex 100 resin (ammonium form 2–3 mL bed volume) before application to the Sephadex column. [f] DEAE Sephadex A-25 (HCO_3^- form). Elution was with a linear gradient of 0.05–0.30 M TEAB (pH 7.6). (Total volume was 1000 mL). [g] Yields were calculated from absorbance reading and ε values determined in the way described in the experimental section. [h] In addition, 25–35% yields of the hydrolysis products, dinucleotide 5′-monophosphates, were obtained.

compromise between the nonreactive phosphate moiety and the lack of selectivity of the free 5′-hydroxyl terminus. To this end, the morpholine group was chosen for the following reasons: (i) Nucleoside 5′-phosphoromorpholidates are readily accessible via dicyclocarbodiimide coupling[33-35] or through redox condensation.[36]

(ii) The phosphoromorpholidate is relatively stable at the pH (6.5–7.5) where the lead ion-catalyzed coupling is conducted (vide infra).

(iii) The morpholine moiety undergoes facile removal by incubation at pH 4 at 37 °C for 5 min, and no internucleotide hydrolysis occurs under these conditions (vide infra).

(iv) Finally, the morpholidate group has been well established as an excellent leaving group for the preparation of di- and triphosphates by reaction with inorganic phosphate or pyrophosphate, respectively.[33-35]

Thus (Scheme II), reaction of adenosine 5′-phosphoromorpholidate (MopA, **14**, 1.5 equiv) with ImpA (≥1.0 equiv) in 0.2 M imidazole buffer (pH 7.0) in the presence of Pb^{2+} (0.5 equiv) for 24 h gave Mop5′A2′p5′A (**11a**, isolated yield 23%) together with a minor amount of oligoadenylates such as 2′,5′-$(pA)_2$ and 2′,5′-$(pA)_3$ and a 4% yield of the linkage isomer MopA3′p5′A (not isolated). The structure assigned to **11a** (Scheme II) was supported by the ^1H NMR (Table III) which revealed characteristic morpholine methylene protons at 3.15 and 2.41 ppm, together with two anomeric (5.99 and 5.67 ppm) protons and four adenine ring protons, and by the fact that mild acid hydrolysis of **11a** gave a product identical with authentic pA2′p5′A (**11b**).

To determine the potential scope of this reaction and to clarify the relative importance of the 2′- and 3′-hydroxyl groups of the ribose ring in the Pb^{2+}-catalyzed polymerization, both 3′-deoxyadenosine 5′-phosphoroimidazolidate (**22**) and 2′-deoxyadenosine 5′-phosphoroimidazolidate (**16**) were reacted separately with MopA (**14**) (Scheme II). Except for imidazolidate hydrolysis, no reaction proceeded between the 3′-deoxyadenosine derivative (**22**) and (**14**), whereas two products (**18a** and **21a**) were generated in the reaction of the 2′-deoxyadenosine derivative (**16**) with MopA (**14**). When 3′-deoxyadenosine 5′-phosphoromorpholidate (**23**) was subjected to the usual Pb^{2+}-catalysis conditions with ImpA (**7**), no coupling occurred even after 2 days incubation at 4 °C. The assigned structures of **18a** and **21a** (Scheme II) were based on, in addition to PMR spectra, the observations that (i) when treated with nuclease P_1, only **21b** was digested to a 1:1 mixture of the 5′-monophosphates of adenosine and 2′-deoxyadenosine whereas **18a** was completely resistant to degradation (Table V). (ii) Treatment of **18a** and **21a** with snake venom phosphodiesterase gave, in both cases, a 1:1 mixture of 5′-monophosphates of adenosine and 2′-deoxyadenosine (Table V).

These results show that while the 2′-hydroxyl group is dispensable, the 3′-hydroxyl group is essential for the Pb^{2+}-catalyzed oligomerization to occur. It is possible that a chelate structure may form between the Pb^{2+}, the phosphate residue and the 3′-hydroxyl group that structure

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Table II. Retention Times (R_T) and Extinction Coefficients (ϵ) of Mono- and Oligonucleotides

compd	R_T column 1,[a] program 1[b]	R_T column 3,[c] program 2[d]	ϵ[f]
A	26.0	2.6	15.4
pI	4.6	6.6	12.2
pA	8.4	6.4	15.4
p2'dA	15.5	6.5	15.3
MopI	22.5	3.6	12.2
MopA	26.1	3.6	15.4
A2'p5'A	27.1	3.7	25.8
A3'p5'A	31.6	3.7	26.3
MopA2'p5A	21.7	4.6	25.8
ImpA2'p5A	21.0	6.8	25.8
MopA2'p5(2'dA)	24.6	4.7	25.8
ImpA2'p5'(2'dA)	24.2	6.8	25.8
MopA2'p5'I	20.9	4.9	24.1
ImpA2'p5'I	20.4	6.9	24.1
MopI2'p5'A	22.1	4.9	23.4
ImpI2'p5'A	21.6	6.7	23.4
pA2'p5'A	13.7	12.0	25.8
pA2'p5(2'dA)	17.3	11.5	25.8
pA3'p5'(2'dA)	23.2	13.9	26.3
pA2'p5'I	10.2	12.5	24.1
pI2'p5'A	13.0	11.5	23.4
A2'p5'A2'p5'A	27.3	7.7	34.5
A3'p5'A2'p5'A	27.7	8.6	
MopA2'p5'A2'p5'A	21.3	10.5	34.5
MopA3'p5'A2'p5'A	25.3	12.7	
MopA2'p5'A2'p5'(2'dA)	23.6	10.3	34.5
MopA3'p5'A2'p5'(2'dA)	27.7	12.8	
MopI2'p5'A2'p5'A	23.2	10.7	31.8
MopI3'p5'A2'p5'A	24.0	12.3	
MopA2'p5'I2'p5'A	22.7	11.5	31.7
MopA3'p5'I2'p5'A	25.7	13.5	
MopA2'p5'A2'p5'I	20.0	11.1	30.3
MopA3'p5'A2'p5'I	24.7	13.5	
pA2'p5'A2'p5'A	16.4	19.2	34.5
pA2'p5'A2'p5'(2'dA)	18.5	18.4	34.5
pI2'p5A2'p5'A	16.9	17.9	31.8
pA2'p5I2'p5'A	16.3	21.0	31.7
pA2'p5A2'p5'I	13.9	20.0	30.3
pppA2'p5'A2'p5'A	13.0[g]		34.5
pppA2'p5'A2'p5'(2'dA)	16.3[g]		34.5
pppI2'p5'A2'p5'A	13.5[g]		31.8
pppA2'p5'I2'p5A	13.1[g]		31.7
pppA2'p5'A2'p5'I	9.1[g]		30.3

[a] Column 1: ultrasphere ODS (4.1 × 250 mm). [b] Program 1: solvent A, 50 mM ammonium phosphate (pH 7.0); solvent B, MeOH/H_2O = 1:1; 0–50% B (in 25 min), 50–100% B (in 5 min), flow rate 1 mL/min. [c] Column 3: synchropak AX-300. [d] Program 2: solvent A, 10 mM sodium phosphate, monobasic (pH 5.4), 20% MeOH (v/v); solvent B, 10 mM sodium phosphate, monobasic (pH 5.9), 500 mM ammonium sulfate, 20% MeOH (v/v), 0–20% B (in 20 min) flowrate 1.0 mL/min. [f] ϵ values were determined in the manner detailed in the Experimental Section. [g] Retention times were determined with column 2 (μ-Bondapak C18) eluted in program 1.

may be necessary for the coupling reaction to proceed.

Next to be examined was the condensation of nucleotides of different heterocyclic bases (Scheme II). The reaction of MopA (**14**) with the 5'-phosphoroimidazolidate of inosine (ImpI, **17**) gave MopA2'p5'I (**19a**) as a major product in 21% yield, whereas reaction of the morpholidate of 5'IMP (MopI, **15**) with ImpA (**7**) gave MopI2'p5'A (**20a**) in 19% yield. The dinucleotides p5'I2'p5'A (**20b**) and p5'A2'p5'I (**19b**), obtained by acid hydrolysis of **20a** and **19a**, respectively, were completely resistant to degradation by either nuclease P_1 or RNase T_2, but were completely digested with snake venom phosphodiesterase give p5'I and p5'A in a 1:1 ratio (Table V). Sequential digestion of **19b** with bacterial alkaline phosphatase followed by snake venom phosphodiesterase gave Ado and 5'ImP in a 1:1 ratio (Table V). Similarly, the same sequential digestion of **20b** gave Ino and 5'AMP in a 1:1 ratio. These observations, together with PMR spectra (Table III), corroborated the assigned structure.

To continue the extension of the oligonucleotide chain (Scheme III), the dimer 5'-monophosphates (**11b**, **18b–20b**) were converted to the corresponding 5'-phosphoroimidazolidates (**11c**, **18c–20c**) and then subjected to Pb^{2+}-catalyzed coupling with monomer morpholidate (**14** or **15**) (Scheme III). Optimum conditions for this coupling required a lower pH (6.75) than for the previous monomer–monomer couplings. At more basic pH (7.5–8.0), extensive exchange of morpholidate with the starting dimer occurred to give MopA2'p5'A (**11a**) and little desired trimeric product was formed. Experimentation with various aspects of the reaction, including ratio of starting materials, Pb^{2+} concentration, and the nature and pH of the buffer, revealed that only the latter two parameters could influence the ratio of morpholidate exchange to trinucleotide generation. For instance, when *N*-methylimidazole (pH 7.5) was employed as reaction buffer, no trimer species was formed and the exchange product, **11a**, was found in even greater yield. Furthermore, while slightly alkaline pH favored the morpholidate exchange, pH values much less than 6.75 showed increasing hydrolysis of the starting dimeric phosphoroimidazolidate (e.g., **11c**) to p5'A2'p5'A (**11b**) and led to a further decrease in the yield of desired trinucleotide. Thus, the optimum reaction conditions for the Pb^{2+}-catalyzed coupling included the use of imidazole buffer at pH of 6.75 (see Table I).

Coupling of MopA (**14**) with Imp5'A2'p5'A (**11c**) thus produced Mop5'A2'p5'A2'p5'A (**24**, 18%) and Mop5'A3'p5'A2'p5'A (**25**, 8.8%) after DEAE-Sephadex chromatography. The structure of **24** was confirmed from its proton NMR spectrum (Table III) and by the fact that mild acid hydrolysis produced a product indistinguishable from authentic p5'A2'p5'A2'p5'A (**1a**). The identity of compound **25** was established from its proton NMR (Table III), its digestion with nuclease P_1 to give a 1:1 ratio of 5'AMP and p5'A2'p5'A (Table V), and by the fact that mild acid hydrolysis of **25** yielded a product that was identical with an authentic sample of p5'A3'p5'A2'p5'A.

Lead ion catalyzed coupling was performed between MopA (**14**) and ImpA2'p5'(2'dA) (**18c**), between MopI (**15**) and ImpApA (**17c**), between MopA (**14**) and ImpI2'p5'A (**19c**) and between **14** and ImpA2'p5'I (**20c**) in a similar manner to that described for the above coupling of MopA and ImpApA (Scheme III, Table I). Yields of the desired all 2',5'-linked isomers (**26**, **28**, **30**, **32**) were in the range of 17–22% based on starting imidazolidate with accompanying quantities of the 3',5'-linkage isomers (**27**, **29**, **31**, **33**) in 8–11% yield.

In support of the assigned structures, the acid hydrolysis products of **26**, **28**, **30**, and **32**, i.e., compounds **2a**, **3a**, **4a**, and **5a**, were all resistant to the action of nuclease P_1 but were degraded by snake venom phosphodiesterase (Table V). Although the base sequence isomers, p5'I2'p5'A2'p5'A (**3a**), p5'A2'p5'I2'p5'A (**4a**), and p5'A2'p5'A2'p5'I (**5a**) all gave the same degradation products (5'AMP and 5'IMP) in the same 2:1 ratio upon digestion with snake venom phosphodiesterase, p5'I2'p5'A2'p5'A (**3a**) could be distinguished from the other two isomers, **4a** and **5a**, if the oligomers were sequentially digested first with bacterial alkaline phosphatase and then with snake venom. In this case, **3a** gave free Ino and 5'AMP in a 1:2 ratio whereas the other two isomers, **4a** and **5a**, gave Ado, 5'AMP, and 5'IMP in a 1:1:1 ratio. Additional confirmation of the

Table III. Characteristic Proton NMR Signals of Oligonucleotides[a]

oligonucleotide	anomeric protons (C1)	purine ring protons (C2 + C8)	morpholine ring proteins	
			CH_2NCH_2	CH_2OCH_2
11a, MopA2′p5′A	5.99 (1 H, s), 5.67 (1 H, d, J = 3.2)	8.01 (1 H, s), 7.90 (1 H, s), 7.86 (1 H, s), 7.75 (1 H, s)	3.15 (4 H, m)	2.41 (4 H, m)
11b, pA2′p5′A	6.01 (1 H, d, J = 4.1), 5.68 (1 H, s)	8.15 (1 H, s), 7.99 (1 H, s), 7.86 (1 H, s), 7.80 (1 H, s)		
18a, MopA2′p5′(2′dA)	6.03 (1 H, t, J = 6.5), 5.94 (1 H, s)	8.00 (1 H, s), 7.94 (1 H, s), 7.88 (1 H, s), 7.74 (1 H, s)	3.14 (4 H, m)	2.40 (4 H, m)
18b, pA2′p5′(2′dA)	6.04 (1 H, t, J = 6.1), 5.98 (1 H, d, J = 4.2)	8.16 (1 H, s), 7.98 (1 H, s), 7.90 (1 H, s), 7.77 (1 H, s)		
21a, MopA3′p5′(2′dA)	6.20 (1 H, t, J = 6.2), 5.70 (1 H, d, J = 4.5)	8.14 (1 H, s), 8.12 (1 H, s), 9.93 (1 H, s), 7.92 (1 H, s)	3.36 (4 H, m)	2.74 (4 H, m)
21b, pA3′p5′(2′dA)	6.19 (1 H, t, J = 5.1), 5.73 (1 H, d, J = 4.1)	8.26 (1 H, s), 8.14 (1 H, s), 7.92 (1 H, s), 7.85 (1 H, s)		
20a, MopI2′p5′A	5.98 (1 H, d, J = 2.2), 5.74 (1 H, d, J = 3.0)	8.02 (1 H, s), 8.00 (1 H, s), 7.99 (1 H, s), 7.75 (1 H, s)	3.23 (4 H, t, J = 4.5)	2.52 (4 H, m)
20b, pI2′p5′A	6.01 (1 H, d, J = 3.8), 5.74 (1 H, d, J = 3.9)	8.12 (1 H, s), 8.01 (1 H, s), 7.96 (1 H, s), 7.76 (1 H, s)		
19a, MopA2′p5′I	6.01 (1 H, d, J = 4.5), 5.64 (1 H, d, J = 3.8)	8.04 (1 H, s), 7.92 (1 H, s), 7.90 (1 H, s), 7.84 (1 H, s)	3.16 (4 H, m)	2.45 (4 H, m)
19b, pA2′p5′I	6.01 (1 H, d, J = 4.5), 5.64 (1 H, d, J = 3.9)	8.23 (1 H, s), 7.95 (1 H, s), 7.86 (1 H, s), 7.83 (1 H, s)		
24, MopA2′p5′A2′p5′A	5.87 (1 H, s), 5.77 (1 H, d, J = 3.5), 5.62 (1 H, d, J = 4.6)	7.99 (1 H, s), 7.90 (1 H, s), 7.83 (1 H, s), 7.75 (1 H, s), 7.70 (1 H, s), 7.64 (1 H, s)	3.10 (4 H, m)	2.33 (4 H, m)
1a, pA2′p5′A2′p5′A	5.89 (1 H, d, J = 3.0), 5.75 (1 H, d, J = 3.7), 5.64 (1 H, d, J = 4.0)	7.98 (1 H, s), 7.97 (1 H, s), 7.80 (1 H, s), 7.76 (1 H, s), 7.73 (1 H, s), 7.70 (1 H, s)		
1b, pppA2′p5′A2′p5′A	5.88 (1 H, s), 5.76 (1 H, d, J = 3.0), 5.64 (1 H, d, J = 3.8)	7.99 (1 H, s), 7.97 (1 H, s), 7.82 (1 H, s), 7.74 (1 H, s), 7.70 (1 H, s), 7.68 (1 H, s)		
25, MopA3′p5′A2′p5′A	5.99 (1 H, d, J = 2.6), 5.67 (1 H, d, J = 3.6), 5.57 (1 H, d, J = 5.6)	8.04 (1 H, s), 8.03 (1 H, s), 7.97 (1 H, s), 7.96 (1 H, s), 7.85 (1 H, s), 7.69 (1 H, s)	3.35 (4 H, t, J = 4.4)	2.70 (4 H, m)
26, MopA2′p5′A2′p5′(2′dA)	6.00 (1 H, t, J = 5.6), 5.86 (1 H, s), 5.73 (1 H, d, J = 3.3)	7.95 (1 H, s), 7.94 (1 H, s), 7.91 (1 H, s), 7.84 (1 H, s), 7.79 (1 H, s), 7.67 (1 H, s)	3.07 (4 H, m)	2.30 (4 H, m)
2a, pA2′p5′A2′p5′(2′dA)	6.03 (1 H, t, J = 6.3), 5.90 (1 H, d, J = 2.7), 5.72 (1 H, d, J = 3.5)	7.97 (2 H, s), 7.81 (2 H, s), 7.70 (1 H, s), 7.67 (1 H, s)		
26, pppA2′p5′A2′p5(2′dA)	6.03 (1 H, t, J = 6.7), 5.89 (1 H, d, J = 1.8), 5.73 (1 H, d, J = 3.8)	7.98 (1 H, s), 7.97 (1 H, s), 7.82 (1 H, s), 7.80 (1 H, s), 7.69 (1 H, s), 7.64 (1 H, s)		
27, MopA3′p5′A2′p5(2′dA)	6.03 (1 H, t, J = 5.9), 5.94 (1 H, d, J = 3, 1 H), 5.58 (1 H, d, J = 5.5)	8.04 (1 H, s), 8.02 (1 H, s), 7.91 (1 H, s), 7.87 (1 H, s), 7.68 (1 H, s)	3.32 (4 H, m)	2.69 (4 H, m)
28, MopI2′p5′A2′p5A	5.84 (1 H, s), 5.83 (1 H, d, J = 3.1), 5.63 (1 H, d, J = 4.0)	7.97 (1 H, s), 7.93 (1 H, s), 7.91 (1 H, s), 7.78 (1 H, s), 7.70 (1 H, s), 7.69 (1 H, s)	3.22 (4 H, t)	2.52 (4 H, m)
3a, pI2′p5′A2′p5A	5.84 (2 H, m), 5.65 (1 H, d, J = 3.9)	8.01 (1 H, s), 7.98 (1 H, s), 7.89 (1 H, s), 7.80 (1 H, s), 7.71 (1 H, s)		
3b, pppI2′p5′A2′p5′A	5.83 (2 H, br s), 5.65 (H, d, J = 3.7)	7.98 (1 H, s), 7.95 (1 H, s), 7.89 (1 H, s), 7.78 (1 H, s), 7.68 (1 H, s)		
29, MopI3′p5′A2′p5′A	5.98 (1 H, d, J = 1.8), 5.63 (1 H, d, J = 3.3), 5.53 (1 H, d, J = 6.2)	8.02 (1 H, s), 8.00 (1 H, s), 7.93 (1 H, s), 7.84 (1 H, s), 7.69 (1 H, s), 7.67 (1 H, s)	3.35 (4 H, m)	2.69 (4 H, m)
30, MopA2′p5′I2′p5′A	5.93 (1 H, d, J = 1.4), 5.73 (1 H, d, J = 2.5), 5.71 (1 H, d, J = 4.9)	8.01 (1 H, s), 7.94 (1 H, s), 7.90 (1 H, s), 7.81 (2 H, s), 7.64 (1 H, s)	3.13 (4 H, m)	2.39 (4 H, m)
4a, pA2′p5′I2′p5′A	5.95 (1 H, d, J = 3.3), 5.73 (1 H, d, J = 2.6), 5.73 (1 H, s)	8.11 (1 H, s), 8.01 (1 H, s), 7.92 (1 H, s), 7.84 (1 H, s), 7.79 (1 H, s), 7.68 (1 H, s)		
4b, pppA2′p5′I2′p5′A	5.94 (1 H, d, J = 2.5), 5.73 (1 H, d, J = 3.7), 5.72 (1 H, s)	8.02 (2 H, s), 7.90 (1 H, s), 7.81 (1 H, s), 7.78 (1 H, s), 7.67 (1 H, s)		
31, MopA3′p5′I2′p5′A	5.98 (1 H, d, J = 2.8), 5.71 (1 H, d, J = 2.5), 5.69 (1 H, d, J = 3.0)	8.13 (1 H, s), 8.01 (1 H, s), 7.97 (1 H, s), 7.96 (1 H, s), 7.92 (1 H, s), 7.70 (1 H, s)	3.34 (4 H, m)	2.71 (4 H, m)

Table III (Continued)

oligonucleotide	anomeric protons (C1)	purine ring protons (C2 + C8)	morpholine ring proteins	
			CH_2NCH_2	CH_2OCH_2
32, MopA2′p5′A2′p5′I	5.88 (1 H, s), 5.79 (1 H, d, J = 3.8), 5.61 (1 H, d, J = 4.5)	7.95 (1 H, s), 7.92 (1 H, s), 7.86 (1 H, s), 7.76 (1 H, s), 7.72 (1 H, s), 7.71 (1 H, s)	3.10 (4 H, m)	2.33 (4 H, m)
5a, pA2′p5′A2′p5′I	5.90 (1 H, d, J = 2.8), 5.77 (1 H, d, J = 3.9), 5.64 (1 H, d, J = 4.3)	8.03 (1 H, s), 7.95 (1 H, s), 7.83 (1 H, s), 7.79 (1 H, s), 7.78 (1 H, s), 7.74 (1 H, s)		
5b, pppA2′p5′A2′p5′I	5.88 (1 H, s), 5.78 (1 H, d, J = 3.7), 5.63 (1 H, d, J = 4.3)	7.97 (1 H, s), 7.95 (1 H, s), 7.83 (1 H, s), 7.77 (1 H, s), 7.76 (1 H, s), 7.72 (1 H, s)		
33, MopA3′p5′A2′p5′I	5.98 (1 H, d, J = 2.6), 5.61 (1 H, d, J = 3.1), 5.55 (1 H, d, J = 5.6)	8.03 (1 H, s), 8.01 (1 H, s), 7.95 (1 H, s), 7.91 (1 H, s), 7.82 (1 H, s), 7.73 (1 H, s)	3.33 (4 H, m)	2.68 (4 H, m)

[a] Chemical shifts were determined in D_2O with acetone (δ 2.05) as an internal standard. s, singlet; d, doublet; t, triplet; m, multiplet. Coupling constants are given in parentheses (Hz).

Table IV. Comparison of ^{31}P NMR Chemical Shifts of 2′,5′-Linked Oligonucleotides[a]

oligonucleotide	internucleotide phosphates	5′-Phosphates		
		α	β	γ
pppA2′p5′A2′p5A	−1.06 (1 P, s), −1.38 (1 P, s)	−11.38 (1 P, d)	−22.52 (1 P, t)	−6.84 (1 P, d)
pppA2′p5′A2′p5(2′dA)	−0.96 (1 P, s), −1.35 (1 P, s)	−11.31 (1 P, d)	−22.38 (1 P, t)	−8.39 (1 P, d)
pppI2′p5′A2′p5′A	−0.85 (1 P, s), −1.09 (1 P, s)	−11.02 (1 P, d)	−21.27 (1 P, t)	−6.60 (1 P, d)
pppA2′p5I2′p5′A	−0.96 (2 P, s)	−11.00 (1 P, d)	−21.78 (1 P, t)	−6.90 (1 P, d)
pppA2′p5′A2′p5′I	−0.82 (1 P, s), −1.14 (1 P, s)	−10.98 (1 P, d)	−21.73 (1 P, t)	−6.74 (1 P, d)
pA2′p5A2′p5′A	−0.96 (1 P, s), −1.19 (1 P, s)	0.96 (1 P, s)		
pA2′p5′A2′p5′(2′dA)	−0.99 (1 P, s), −1.36 (1 P, s)	1.60 (1 P, s)		
pI2′p5A2′pA	−0.91 (1 P, s), −1.12 (1 P, s)	1.60 (1 P, s)		
pA2′p5I2′p5A	−1.22 (1 P, s)	0.55 (1 P, s)		
pA2′p5′A2′p5′I	−0.88 (1 P, s), −1.15 (1 P, s)	1.00 (1 P, s)		
pA2′p5′A	−1.19 (1 P, s)	0.82 (1 P, s)		
pA2′p5′(2′dA)	−1.07 (1 P, s)	0.83 (1 P, s)		
pI2′p5′A	−1.23 (1 P, s)	1.10 (1 P, s)		
pA2′p5′I	−1.02 (1 P, s)	1.60 (1 P, s)		

[a] Chemical shifts were determined in D_2O with 0.85% H_3PO_4 as an external standard. d = doublet; t = triplet; J_{P-O-P} = ~19 Hz. Spectra were measured in a D_2O solution of EDTA (6 mM).

Table V. Enzymatic Digestion Analysis[a] of 2′,5′-Linked Oligonucleotides

oligonucleotide substrate	nuclease P_1[b]	SVP[c]	(1) BAP,[d] (2) SVP[e]
pA2′p5′A	N.R.	pA	A + pA (1:1)
pA2′p5′(2′dA)	N.R.	pA + p(2′dA) (1:1)	A + p(2′dA) (1:1)
pA3′p5′(2′dA)	pA + p(2′dA) (1:1)	pA + p(2′dA) (1:1)	A + p(2′dA) (1:1)
pA2′p5′I	N.R.	pA + pI (1:1)	A + pI (1:1)
pI2′p5′A	N.R.	pA + pI (1:1)	I + pA (1:1)
pA2′p5′A2′p5′A	N.R.	pA	A + pA (1:2)
pA2′p5′A2′p5′(2′dA)	N.R.	pA + p(2′dA) (2:1)	A + pA + p(2′dA) (1:1:1)
pI2′p5′A2′p5′A	N.R.	pI + pA (1:2)	I + pA (1:2)
pA2′p5I2′p5′A	N.R.	pI + pA (1:2)	A + pI + pA (1:1:1)
pA2′p5′A2′p5′I	N.R.	pI + pA (1:2)	A + pA + pI (1:1:1)

[a] Digestion products were analyzed either by TLD (System A) or HPLC (column 1, program 1). Retention times of products are listed in Table II. [b] Conditions: ~1.0 $OD_{\lambda max}$ substrate and 1 μg enzyme in 50 μL of 10 mM NH Ac (pH 5.75) at 37 °C for 2.5 h. N.R. indicates no reaction. [c] Conditions: ~1.0 $OD_{\lambda max}$ substrate and 0.1 unit snake venom phosphodiesterase (SVP) in 50 μL of 10 mM Tris–acetate (pH 8.8), 10 mM $MgCl_2$ at 37 °C for 2.5 h. [d] Conditions: ~5.0 $OD_{\lambda max}$ substrate and 0.05 unit bacterial alkaline phosphatase (BAP) in 50 μL of Tris–acetate (pH 8.5), 1.0 mM $MgCl_2$ at 37 °C for 2.5 h. [e] After alkaline phosphatase digestion, the solution was heated at 100 °C for 5 min and then centrifuged. The supernatant was lyophilized and the residue was taken up in methanol. The methanol solution was concentrated to dryness. The residue was dissolved in the buffer for snake venom phosphodiesterase digestion.

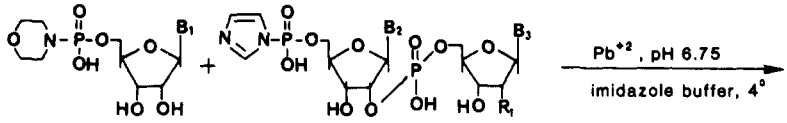
assigned structures was obtained from the proton NMR spectra (Table III). It was noteworthy that the chemical shifts (δ) of the morpholine methylene protons of the 3′-5′, 2′-5′ linked isomers (**25**, **27**, **29**, **31**, and **33**) showed a significantly higher value (0.2–0.3 ppm) than the corresponding all 2′,5′-linked isomers (**24**, **26**, **28**, **30**, and **32**).

Finally, each of the synthesized trimer morpholidates, **24**, **26**, **28**, **30**, and **32**, were converted readily to the corresponding trimer triphosphates, **1b**, **2b**, **3b**, **4b**, and **5b**, respectively (Scheme I), by treatment with tri-*n*-butylammonium pyrophosphate in DMF. Yields ranged from 74–91% and each product's assigned structure was confirmed by proton (Table III) and phosphorus NMR (Table IV).

Discussion

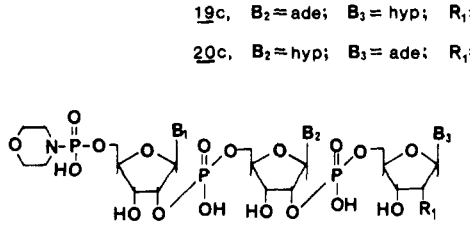
Lead ion catalyzed condensation of unprotected mono- and dinucleotide phosphoroamidates has been found to be a convenient method for the preparation of various 2′-5′ linked oligonucleotides. Several advantages of the methodology are descirbed in this paper: (a) no protection is necessary for the base amino or sugar hydroxyl groups of nucleosides or nucleotides; (b) unlike some conventional phosphotriester syntheses, this coupling reaction requires

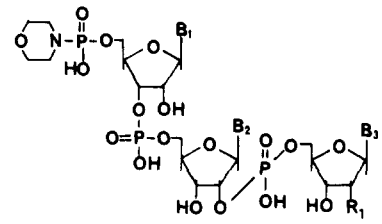
Scheme III



14, B_1 = ade
15, B_1 = hyp

11c, $B_2 = B_3$ = ade; R_1 = OH
18c, $B_2 = B_3$ = ade; R_1 = H
19c, B_2 = ade; B_3 = hyp; R_1 = OH
20c, B_2 = hyp; B_3 = ade; R_1 = OH





24, $B_1 = B_2 = B_3$ = ade; R_1 = OH
26, $B_1 = B_2 = B_3$ = ade; R_1 = H
28, B_1 = hyp; $B_2 = B_3$ = ade; R_1 = OH
30, $B_1 = B_3$ = ade; B_2 = hyp; R_1 = OH
32, $B_1 = B_2$ = ade; B_3 = hyp; R_1 = OH

25, $B_1 = B_2 = B_3$ = ade; R_1 = OH
27, $B_1 = B_2 = B_3$ = ade; R_1 = H
29, B_1 = hyp; $B_2 = B_3$ = ade; R_1 = OH
31, $B_1 = B_3$ = ade; B_2 = hyp; R_1 = OH
33, $B_1 = B_2$ = ade; B_3 = hyp; R_1 = OH

no extreme conditions such as anhydrous solvents or low reaction temperature since the reaction proceeds smoothly in buffered aqueous solution at 4 °C; (c) progress of the reaction can be monitored either by reverse-phase or ion-exchange HPLC, and the desired coupling products are easily purified from the reaction mixture by DEAE Sephadex chromatography; (d) both 5′-mono- and triphosphates of desired oligonucleotides can be prepared simply by treating the corresponding phosphoromorpholidate with mild acid or pyrophosphate, respectively. The utility and efficiency of Pb^{2+}-catalyzed condensation of nucleotide phosphoroamidates has been demonstrated by its application to the synthesis of 2-5a (**1a**, **1b**), as well as 2′-terminal deoxy analogues (**2a**, **2b**) and sequence-specific inosine/adenosine analogues (**3a**, **3b**, **4a**, **4b**, **5a**, and **5b**). Biological activities of these 2-5A analogues will be reported in a separate publication.[37]

Experimental Section

Materials and Methods. Reagents. Adenosine 5′-monophosphate (free acid), 2′-deoxyadenosine 5′-monophosphate (free acid), 2,2-dipyridyl disulfide (Aldrithiol-2), triphenylphosphine, morpholine, triethylamine, dimethyl sulfoxide, dimethylformamide, lead nitrate, 1-methylimidazole, and deuterium oxide were purchased from Aldrich Chemical Co. (Milwaukee, WI). Inosine 5′-monophosphate (IMP, free acid), cordycepin 5′-monophosphate (free acid), and sodium iodate were obtained from Sigma Chemical Co. (St. Louis, MO); imidazole and sodium pyrophosphate came from J. T. Baker Chemical Co. (Phillipsburg, NJ); HPLC-grade ammonium phosphate, ammonium acetate, ammonium sulfate, and sodium phosphate (monobasic) were products of Fisher Scientific Co. (Fair Lawn, NJ).

Ion-Exchange Resins. Dowex 50X8, DEAE-Sephadex and Chelex 100 were from J. T. Baker, Pharmacia Fine Chemicals (Piscataway, NJ) and Bio-rad Laboratories (Richmond, CA), respectively.

Enzymes. Nuclease P_1 was from Calbiochem. (LaJolla, CA); bacterial alkaline phosphatase was from Sigma Chemical Co. (St. Louis, MO); and snake venom phosphodiesterase was from P. L. Biochemicals (Piscataway, NJ).

TLC Analysis. Analytical TLC was done on precoated PEI-Cellulose F plates (E. Merck, Darmstadt, West Germany) with solvent systems A (0.1 M HN_4HCO_3) or B (0.25 M NH_4HCO_3).

Spectral Measurements. Proton NMR spectra were recorded with a Varian HR220 instrument operating at 220 MHz. The solvent was D_2O with acetone (2.05 ppm from Me_4Si) as an internal standard. Chemical shifts (δ) are reported in ppm. Multiplicity is abbreviated as s (singlet), d (doublet), t (triplet), or m (multiplet), and coupling constants are expressed in hertz (Hz). Each sample (5–7 μmol) was dissolved in D_2O and evaporated under vacuum. The residue was redissolved in D_2O (~700 μL) and the solution was transferred into an NMR tube. Phosphorus NMR spectra were obtained with a JEOL instrument FX100 at 100 MHz. The solvent was D_2O and chemical shifts are reported in ppm using 0.85% H_3PO_4 as external reference. ^{31}P spectra were measured in the presence of EDTA (5–7 μmol). UV spectra were recorded with a Hewlett-Packard 8450A UV/vis spectrophotometer.

High-Performance Liquid Chromatography Instrumentation. (a) HPLC system 1 consisted of two Model 110A solvent delivery systems and a Model 421 controller from Beckman Instrument (Berkeley, CA). The detector was an ISCO (Lincoln, NE) Model UA-J absorbance/fluorescence monitor. The area integration of the chromatogram was determined with an Altex (Berkeley, CA) Model C-RIA integrator.

(b) HPLC System 2 consisted of two Model 112 solvent delivery systems, a Model 420 controller, and a Model 153 analytical UV detector from Beckman Instruments, and a Hewlett-Packard 3390A integrator.

Columns and elution conditions were as follows: (a) Ultrasphere ODS reverse phase column (4.6 × 250 mm) (Altex Scientific, Inc., Berkeley, CA) eluted with program 1: solvent A, 50 mM ammonium phosphate (pH 7.0); solvent B, methanol/H_2O = 1:1, flow rate, 1.0 mL/min; 0–50% B (25 min), 50–100% B (5 min).

(b) μ-Bondapak C_{18} reverse phase (3.9 × 300 mm) (Waters Associates, Milford, MS).

(c) Synchropak AX-300 anion exchange (4.1 × 250 mm) (Sychrom, Inc., Leonden, IN) with elution in program 2: solvent A, 10 mM sodium phosphate, monobasic (pH 5.9) and 20% methanol (v/v); solvent B, 10 mM sodium phosphate monobasic, 500 mM ammonium sulfate (pH 5.9) and 20% methanol (v/v); flow rate 1.0 mL/min 0–20% B (20 min).

Enzymatic digestion of the various oligomers was carried out with the following enzymes under the indicated conditions.

(1) P_1 nuclease: substrate ~1.0 $OD_{\lambda max}$, enzyme 1 μg, buffer 0.1 M ammonium acetate (pH 5.75), total volume 50 μL, incubation temperature 37 °C, time 2.5 h.

(37) Imai, J.; Lesiak, K.; Torrence, P. F. *J. Biol. Chem.* **1985**, *260*, 1390–1393.

(2) Bacterial alkaline phosphatase: substrate ~1.0 $OD_{\lambda max}$, enzyme 0.01 unit, buffer 0.1 M Tris–acetate (pH 8.5), 0.001 M $MgCl_2$, total volume 50 μL, incubation temperature 37 °C, time 2.5 h.

(3) Snake venom phosphodiesterase: substrate ~1.0 $OD_{\lambda max}$, enzyme 0.1 unit, buffer 0.01 M Tris–acetate (pH 8.8), 0.01 $MgCl_2$, total volume 50 μL, incubation temperature 37 °C, time 25 h.

Determination of Extinction Coefficients of Oligonucleotides. (1) Determination of Calibration Curves for Standard Mononucleotides. One millimolar solutions of AMP, 2'dAMP, and IMP were prepared and 1–10 μL of each solution was injected into the HPLC (column 1, program 1). The resulting area integral values were poltted against the injected amounts of each solution and calibration curves were constructed from them. For the determination of extinction coefficients, 2'dAMP and IMP solution prepared above also were used as internal calibration standards: 2'IMP for pA2'p5'I, pI2'p5'dA, pI2'p5'A2'p5'A, pA2'p5'I2'p5A, and pA2'p5'A2'p5'I, and 2'dAMP for pA2'p5'A, pA2'p5'(2'dA), pA3'p5'(2'dA), pA2'p5'A2'p5'A, and pA2'p5'A2'p5'(2'dA).

(2) Snake Venom Digestion. An accurately determined amount (~ A_{260} unit) of oligonucleotide and 20 μL of one of the above calibration standards were dissolved in the buffer (vide infra) for snake venom digestion. The solution was divided into two equal parts; and then snake venom phosphodiesterase (0.1 unit, 1 μL) was added to one of these solutions and it was incubated at 37 °C for 2 h. At the conclusion of the digestion, 10 μL of each mixture was injected separately into the HPLC using a column 1 and program 1. The concentrations of each of the products of each digestion was calculated from the observed normalized peak integral and the predetermined calibration curve. The extinction coefficient then could be calculated from the A_{260} value input to the digestion and the μmoles of nucleotide as determined by HPLC.

Preparation of Nucleoside 5'-Phosphoromorpholidates (13 and 14) and Nucleoside Phosphoroimidazolidates (7, 15, and 16). Adenosine 5'-Phosphoromorpholidate (14). Adenosine 5'-monophosphate (**10**, free acid, 694 mg, 2.0 mmol) was dissolved in dry Me_2SO (4.0 mL). Pulverized triphenylphosphine (1.31 g, 5.0 mmol), morpholine (871 μL, 10 mmol), and 2,2-dipyridyl disulfide (1.10 g, 5.0 mmol) were added successively to this solution, and the resulting yellow-colored reaction mixture was stirred at room temperature for 40 min. After the completion of the reaction had been assured by TLC (solvent system A), the whole mixture was poured into 0.1 M sodium iodide solution in acetone (90 mL) with stirring. The white precipitate thus deposited was allowed to settle, and a large portion of the supernatant was decanted. The precipitate was collected either by filtration or centrifugation and washed several times with fresh acetone until the yellow color was gone. After drying over P_2O_5 at room temperature for 1 h under vacuum, the sodium salt of MopA (**14**, 8.60 g, 1.96 mmol, yield 98.0%) was stored in a tightly sealed desiccator. No decomposition was observed for a few months as long as it was kept in a freezer (−20 °C). Other nucleoside 5'-phosphoromorpholidates (**15**, **23**) and 5'-phosphoroimidazolidates (**7**, **16**, and **17**) were synthesized in a similar manner in yields of 95–100%.

Lead Ion Catalyzed Coupling of Nucleoside 5'-Phosphoromorpholidates and Nucleoside 5'-Phosphoroimidazolidates. Preparation of MopA2'p5'A (11a). $Pb(NO_3)_2$ (0.25 M, 2 mL) was added to a mixture of MopA (**14**, 1.5 mmol as sodium salt), and ImpA (**7**, 1.0 mmol as sodium salt) dissolved in imidazole nitrate buffer (20 mL, 0.2 M, pH 7.0) at 4 °C. After 24 h of stirring at 4 °C, Chelex 100 (NH_4^+ form, 5 mL) was added to the turbid reaction mixture which gradually became clear. The Chelex was removed by filtration, and the filtrate was diluted with water to 100 mL and applied to a DEAE-Sephadex A-25 column (1.6 × 20 cm), preequilibrated with water. Elution was with a linear gradient of 0.0 M (500 mL) to 0.2 M (500 mL) triethylammonium bicarbonate (pH 7.6). Appropriate fractions were combined and evaporated to dryness in vacuo. Water was added and removed by evaporation from the residue in order to remove all triethylammonium bicarbonate. The product MopA2'p5'A (**11a**) was isolated as the triethylammonium salt in 23.5% yield based on starting ImpA. Other dinucleotide 5'-phosphoromorpholidates (**18a**, **19a**, and **20a**) were prepared in a similar manner. The yields were as follows: **18a**, 24%; **19a**, 21%; **20a**, 19%.

Acid Hydrolysis of Dinucleotide Phosphoromorpholidates. Preparation of pA2'p5'A (11b). MopA2'p5'A (**11a**, 5930 A_{258} units, 230 μmol triethylammonium salt) was dissolved in water (5 mL) and the pH of the solution was adjusted to 4.0 with 10% acetic acid. The reaction mixture was incubated at 37 °C for 5 h. After the completion of the hydrolysis was assured by TLC (solvent system A) and HPLC (column a, program 1), the solution was neutralized with triethylamine and subsequently evaporated to dryness. The residue was redissolved in methanol (2 mL) and ether (5 mL) was added to it. The resulting precipitate was centrifuged down and the supernatant was removed. After washing with ether a few more times, the precipitate was dried in vacuo. The product pA2'p5'A (**11b**) was thus isolated as a triethylammonium salt (5730 A_{258} units, 222 μmol) in 97% yield.

Other dinucleotide 5'phosphates (**18b**, **19b**, and **20b**) were prepared in the same fashion from their respective 5'-phosphoromorpholidates (**18a**, **19a**, and **20a**).

Preparation of Dinucleotide 5'-Phosphoroimidazolidates (17c, 18c, 19c, and 20c). Preparation of ImpA2'p5'A (11c). Dipyridyl disulfide (244 mg, 1.11 mmol) was added to a mixture of pA2'p5'A (**11b**, 222 μmol, 5730 A_{258} units, triethylammonium salt), triphenylphosphine (290 mg, 1.11 mmol) and imidazole (142 mg, 2.22 mmol) in DMF (3.45 mL) and Me_2SO (0.69 mL), and the resulting yellow solution was stirred for 40 min at ambient temperature. When completion of the reaction had been confirmed by TLC (solvent system A), the entire mixture was poured into a solution of NaI in acetone (0.1 M, 60 mL). The white precipitate that formed was collected by filtration, washed several times with acetone, and then dried over P_2O_5 at room temperature for 1 h in vacuo. The sodium salt of ImpA2'p5'A (**11c**, 5550 A_{258} units, 215 μmol) was obtained in a yield of 93% and was used directly in the next step of the synthesis without further purification.

Other dinucleotide 5'-phosphoroimidazolidates (**18c**, **19c**, and **20c**) were prepared from the corresponding 5'-monophosphates in an analogous manner.

Lead Ion Catalyzed Coupling of Nucleoside 5'-Phosphoromorpholidates (14, 15) and Dinucleotide 5'-Phosphoroimidazolidate (11c, 18c, 19c, and 20c). Preparation of MopA2'p5'A2'p5'A (24). Lead nitrate solution (0.25 M, 124 μL) was added to a solution of MopA (**14**, 1910 A_{258} units, 124 μmol as sodium salt) and ImpA2p5'A (**11c**, 1600 A_{258} units, 62 μmol as sodium salt) in imidazole nitrate buffer (0.2 M, 1.24 mL, pH 6.75). Progress of the reaction was monitored by removing a 20-μL aliquot, diluting 5 times with H_2O and treating with Chelex 100 resin (20 μL) for 10 min with occasional vortexing of the solution. After the Chelex was centrifuged down, 10 μL of the sample was analyzed by HPLC using system c. When all of the ImpA2'p5'A (**13c**, retention time 18.5 min) had disappeared, the entire reaction mixture was diluted with H_2O (10 mL) and treated with Chelex 100 resin (3.0 mL) for 30 min. After the Chelex was removed by filtration, the filtrate was further diluted with H_2O to a final volume of 30 mL and applied to a DEAE Sephadex A-25 column (1.6 × 16 cm, preequilibrated with triethylammonium bicarbonate, pH 7.6). Elution was with a linear gradient of 0.0–0.3 M triethylammonium bicarbonate (pH 7.5, 500 and 500 mL). A total of 162 fractions were collected. Fractions 87–107 were pooled and concentrated in vacuo, and water was added to and evaporated from the resulting residue to remove residual triethylammonium bicarbonate. The desired product, MopA2'p5'A2'p5'A (**24**), was isolated in 18% yield as the triethylammonium salt (372 A_{258} units, 10.8 μmol). Fractions 110–128 and 130–171 contained the 3',5'-linked isomer MopA3'p5'A2'p5'A (**25**, 190 A_{258} units, 5.4 μmol, 8.8% yield) and hydrolyzed pA2'p5'A (**11b**, 550 A_{258} units, 21 μmol, 34.4% yield), respectively. HPLC retention times of these compounds are given in Table II.

Again, the remaining 5'-phosphoromorpholidates of the trinucleotides (**26**, **28**, **30**, and **32**) were prepared in a similar manner. The specifics of their preparation and HPLC characteristics are given in Tables I and II, respectively.

Hydrolysis of 2',5'-Linked Trinucleotide 5'-Phosphoromorpholidates. Preparation of pA2'p5'A2'p5'A. The pH of an aqueous solution (2 mL) of MopA2'p5'A2'p5'A (**24**, 207 A_{258}

units, 6 μmol) was adjusted to 4.0 with 10% HOAc. The solution was incubated at 37 °C for 5 h, neutralized with 5% NH_4OH, and then applied to a column (1 × 20 cm) of DEAE-Sephadex A-25 which was eluted with a linear gradient of 0.1–0.4 M triethylammonium bicarbonate buffer (pH 7.6, 250 and 250 mL). From a total of 82 fractions, numbers 47–58 were pooled and evaporated to dryness in vacuo. After several additions and evaporations of water, the residue was dissolved in CH_3OH (200 μL) and poured into a solution of sodium iodide in acetone (0.1 M, 4 mL). The resulting white precipitating was centrifuged down and washed several times with acetone. After in vacuo drying, pA2′p5′A2′p5′A (**1a**, 203 A_{258} units, 5.88 μmol) was obtained as the sodium salt in a yield of 98%. By HPLC (program 1), the purity of **1a** was 99.9%.

Other trinucleotide 5′-monophosphates were generated in an analogous fashion; thus, hydrolysis of MopA2′p5′A2′p5′(2′dA) (**26**) gave p5′A2′p5′A2′p5′(2′dA) (**28**, 136 A_{258} units, 3.94 μmol, 97% yield), MopI2′p5′A2′p5′A gave p5′I2′p5′A2′p5′A (**3a**, 194 A_{258} units, 98% yield). MopA2′p5′I2′p5′A (**30**) gave p5′A2′p5′I2′p5′A (**4a**, 152 A_{258} units, 98% yield) and MopA2′p5′A2′p5′I (**32**) gave p5′A2′p5′A2′p5′I (**5a**, 128 A_{258} units, 98% yield). HPLC retention times and UV ε values are given in Table II whereas proton and ^{31}P NMR spectra of these products are listed in Tables IV and V.

Preparation of Trinucleotide 5′-Triphosphates. Preparation of ppp5′A2′p5′A2′p5′A (2-5A, 1b). Compound **24** (MopA2′p5′A2′p5′A, triethylammonium salt, 207 A_{258} units, 6 μmol) was dissolved in a solution (200 μL) of tri-*n*-butylammonium pyrophosphate in DMF (0.5 M), and the reaction mixture was kept at room temperature for 3 days. After dilution with H_2O (1 mL), the solution was applied to a DEAE Sephadex A25 column (HCO_3^- form, 1 × 20 cm) which then was eluted with a linear gradient of 0.2–0.56 M triethylammonium bicarbonate (pH 7.6, 250 and 250 mL). From the 82 fractions collected, fractions 53–63 were pooled and reduced to dryness in vacuo. After several additions and evaporations of water, the residue was dissolved in CH_3OH (200 μL) and treated with a solution of sodium iodide in methanol (0.1 M, 4 mL). The resultant white precipitate was centrifuged down, washed with acetone, and dried in vacuo over P_2O_5 for 1 h. In this way, 2-5A (pppA2′p5′A2′p5′A, **1b**, 152 A_{258} units, 4.48 μmol) was obtained in 75% yield. The product had identical TLC and HPLC properties with authentic material and its UV, ^1H, and ^{31}P spectra were also identical with authentic 2-5A.

The other trinucleotide 5′-triphosphates (**2b**, **3b**, **4b**, and **5b**) were synthesized in the same manner from their respective morpholidates. Tables II–IV give pertinent chromatographic and spectral characteristics.

Registry No. **1a** (Na salt), 95314-17-3; **1b**, 65954-93-0; **2a**, 95314-26-4; **2b**, 82137-97-1; **3a**, 95314-27-5; **3b**, 95313-96-5; **4a**, 95314-28-6; **4b**, 95313-97-6; **5a**, 95314-29-7; **5b**, 95313-98-7; **6**, 58-61-7; **7** (Na salt), 60031-83-6; **8**, 2273-76-9; **10** (free acid), 61-19-8; **11a** (triethylammonium salt), 95314-02-6; **11b** (triethylammonium salt), 95314-03-7; **11c** (Na salt), 95313-99-8; **12**, 70062-83-8; **13**, 75074-06-5; **14** (Na salt), 95314-00-4; **15**, 27908-35-6; **16**, 26568-05-8; **17**, 66536-80-9; **18a**, 95314-04-8; **18b**, 95314-10-6; **18c**, 95314-11-7; **19a**, 95314-06-0; **19b**, 95314-09-3; **19c**, 95314-12-8; **20a**, 95314-07-1; **20b**, 95314-08-2; **20c**, 95314-13-9; **21a**, 95314-05-9; **23**, 95314-30-0; **24** (trimethylammonium salt), 95314-15-1; **25**, 95314-16-2; **26**, 95314-18-4; **27**, 95314-22-0; **28**, 95314-19-5; **29**, 95314-23-1; **30**, 95314-20-8; **31**, 95314-24-2; **32**, 95314-21-9; **33**, 95314-25-3; A3′p5′A, 2391-46-0; p5′A3′p5′A2′p5′A, 78983-51-4.

Photochemistry of Phthalimides with Olefins. Solvent-Incorporated Addition vs. Cycloaddition to Imide C(=O)—N Bond Accompanying Ring Enlargement

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Received September 5, 1984

The photoreactions of phthalimides **1a–c** and a variety of olefins (**2a–g**) have been investigated. Irradiation of methanol solutions of **1a** in the presence of electron-rich olefins **2a–f** leads to formation of methanol-incorporated adducts **3a** + **4a**, **9a,b**, **19**, **29a,b**, **30a,b**, and **31**. Irradiation of acetonitrile solutions of **1a** in the presence of relatively electron-poor aliphatic olefins **2f,g** gives ring-enlarged cycloaddition products **23**, **32**, and **34**, probably by a mechanism which involves collapse of an exciplex. Photolyses of **1a** and **2a** in less polar alcohols afford the two types of products, simultaneously. With decrease of the solvent polarity, the yields of the solvent-incorporated adducts decrease and that of the ring-enlarged cycloaddition products increase. Irradiation of **1a** and **2c** leads to formation of the other types of products: in methanol **20** is obtained together with **19**, and in acetonitrile **22** and **24** are formed together with **23**. The formation of **24** is rationalized by a mechanism in which degradation of an oxetane (**27**) is involved. The products **20** and **22** appear to be derived through electron transfer from **2c** to the excited state of **1a**. Phenanthrene (electron-transfer) sensitization of the reaction **1a** + **2c** gives **20** and **23** in methanol and **22** in acetonitrile, selectively. These results and ΔG values associated with the electron transfer support an electron-transfer mechanism for the solvent-incorporated adduct formation.

The photochemistry of imides has been the subject of intensive investigations over several years.[1] Concerning the photochemistry of imides with olefins, we found that oxetane formation was the most typical process in the photolyses of alicyclic imides with olefins (eq 1), illustrating its normal $n\pi^*$ carbonyl photoreactivity.[2] On the other hand, photoreactions of phthalimides with olefins are quite different from those of alicyclic imides. On the intramolecular photoreactions of *N*-alkenylphthalimides, the re-

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