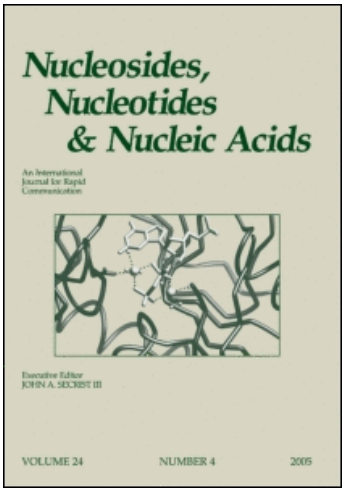

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Hybridization of Alternating Cationic/Anionic Oligonucleotides to RNA Segments

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To cite this Article Jung, Paul M. , Histand, Gary and Letsinger, Robert L.(1994) 'Hybridization of Alternating Cationic/Anionic Oligonucleotides to RNA Segments', Nucleosides, Nucleotides and Nucleic Acids, 13: 6, 1597 — 1605
To link to this Article: DOI: 10.1080/15257779408012174
URL: http://dx.doi.org/10.1080/15257779408012174

NUCLEOSIDES & NUCLEOTIDES, 13(6&7), 1597-1605 (1994)

HYBRIDIZATION OF ALTERNATING CATIONIC/ANIONIC OLIGONUCLEOTIDES TO RNA SEGMENTS#

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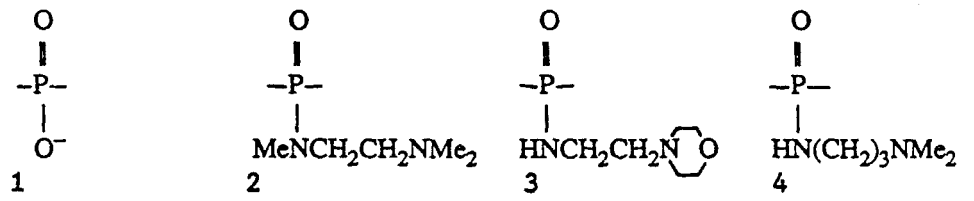
ABSTRACT It is shown that oligonucleotide analogues containing alternating phosphodiester groups and cationic groups linked to phosphorus through an amidate bond hybridize to complementary RNA segments. An improved synthetic procedure for the alternating compounds is also described.

INTRODUCTION

In earlier papers we have described the synthesis and properties of "cationic" oligonucleotides.[1,2] Sites for positive charges were introduced along the backbone of the polymers during synthesis by replacing phosphodiester groups (1) by aminoalkylamino-phosphoramidate groups (2). In aqueous solution at appropriate pH the terminal amino



1 2 3 4

groups in the pendant amidates acquire a positive charge. The net charge on the oligomers in this system can be varied either by altering the phosphodiester/aminoalkyl-phosphoramidate ratio during synthesis or by changing the pH of a solution of a given

This paper is dedicated to Professor Morio Ikehara in recognition of his seminal contributions to nucleic acids chemistry.

oligomer. When the net charge on the oligomer is positive, the stability of hybrids formed with complementary DNA segments varies inversely with the ionic strength of the solution; when the positive sites balance the negative charges, the stability of the hybrids is independent of the ionic strength.[1-3] In either case, in solutions of low ionic strength (e.g. < 0.1 M NaCl) the duplex hybrids formed by the cationic derivatives proved to be more stable than the corresponding DNA duplexes. These properties raise interesting possibilities for applications as tools in biochemical systems. However, in contrast to the case for DNA targets, thermal dissociation studies indicated that a fully substituted cationic oligo(dT) derivative did not react significantly with poly(rA)[1]. Apparently the (cationic probe)/poly(rA) complex is more sensitive to distortions in structure induced by the pendant phosphoramidate substituents than is the (cationic probe)/poly(dA) complex.

On the assumption that the unfavorable distortions might be relieved by alternating the phosphoramidate links with natural type phosphodiester links in the probe oligomer, we have investigated interactions of RNA targets with several probes possessing alternating charges along the backbone. In the present paper we (a) report that zwitterionic type oligomers containing alternating phosphoramidate and phosphodiester internucleoside links indeed bind to RNA targets, and (b) describe an improved method for synthesizing alternating cationic/anionic oligonucleotide derivatives. These experiments were carried out with dimethyaminopropyl phosphoramidate derivatives of type **4** since exploratory experiments[4] have shown these derivatives to be superior to those of type **2** as probes for DNA, and, in contrast to derivatives of type **3**, they hybridized well to DNA strands in solutions in the pH 7 range. The new results make the alternating cationic phosphoramidate derivatives promising candidates for use in studies involving hybridization to specific RNA sequences.

RESULTS AND DISCUSSION

The initial experiments were carried out with a homothymidylate derivative, pentadecamer I. This compound was prepared by extending a stepwise procedure previously described for synthesis of shorter oligomers.[1,2] In accord with the proposed

I. T-T+T-T+T-T+T-T+T-T+T-T+T-T+T, + = internucleoside link 4

structure it exhibited two peaks of about equal intensity in the ^{31}P NMR spectrum, one

at 10.57 ppm upfield (phosphoramidate) and the other 0.81 ppm downfield (phosphodiester) relative to H_3PO_4. Melting curves for complexes of I with poly(dA) and poly(rA) are shown in Figure 1. Sigmoidal curves were obtained in both cases, clearly demonstrating that *compound I forms a relatively stable complex with poly(rA)* as well as with poly(dA). As generally observed for complexes of oligo(dT) and its analogues, the Tm value for the complex with poly(rA) is somewhat lower than that for the complex with poly(dA). The absence of significant net charge on oligomer I would be expected to faciliate formation of a triple stranded complex, even in solutions at low ionic strength. Mixing curve experiments with varying percentages of compound I and poly(dA) showed, however, that only a double stranded complex was formed. Similarly, attempts to observe binding between compound I and double stranded poly(dA)/poly(dT) were negative. Therefore, with respect to triple strand formation, unfavorable steric effects apparently more than compensate for favorable electrostatic effects.

A disadvantage of the synthetic procedure used in preparing I is that each hydrogen phosphonate intermediate has to be converted to the corresponding phosphoramidate before proceeding with the next phosphoramidite coupling and iodine oxidation, since the iodine reagent oxidizes hydrogen phosphonate groups as well as phosphite triesters. Each amidation with the amine/CCl_4 mixture required at least 15 minutes; so the overall time for synthesis of a 15-mer became quite lengthy. Our observation that the hydrogen phosphonate intermediates are relatively stable to t-butyl hydroperoxide, an effective reagent for converting phosphite triesters to phosphodiesters,[5] opened the way to a more rapid synthetic protocol. In this scheme, each intermediate phosphotriester generated from an amidite coupling was oxidized with t-butyl hydroperoxide rather than iodine. The hydrogen phosphonate intermediates were left intact until all nucleotide units had been added to the chain, then all of the phosphonates were converted to amidates in a single oxidative step. It was found that cyanoethyl phosphoramidate reagents serve quite satisfactorily in these couplings, so they were employed in place of methyl phosphoramidite reagents in subsequent work. A direct comparison of the old and new method was made by synthesizing d(T-T+T-T+T) (+ represents amidate link 4). The cycle time for introducing each phosphoramidate link was reduced from 20 min. for the old procedure to 5 min for the new. Average coupling yields judged by the DMT color test were good in both cases (97-98%); however, the new method afforded a cleaner

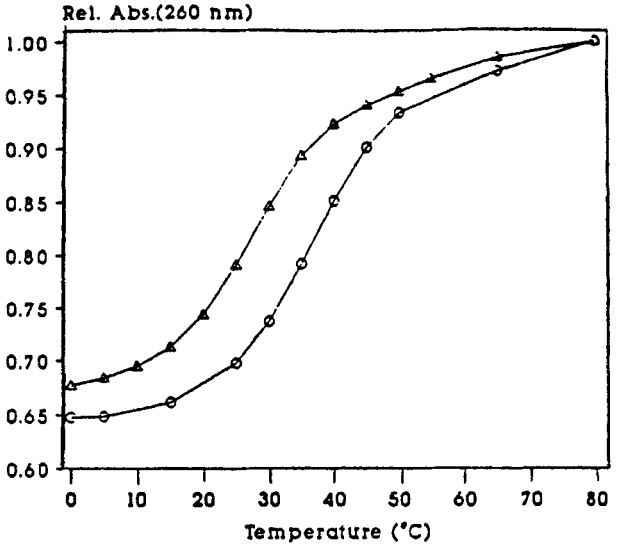


Figure 1. Absorbance profile for dissociation of complexes at pH 7 in 10 mM Tris.HCl buffer, 0.1 M NaCl: d[(T-T+),T], cpd.I, with Poly(dA),-O-; with Poly(rA),-△-.

product (88% of the total nucleotide product obtained on RP HPLC was the desired oligomer, as compared to 75% for the product obtained by the earlier route).

To explore the hybridization properties of mixed-base cationic oligonucleotide derivatives compounds II-VI were prepared. The structures and some properties of these compounds are listed in Table 1. Compound II has alternating phosphodiester and dimethylaminopropyl phosphoramidate links. Compounds III and IV have the same sequence as II, but III has only cationic internucleoside links and IV has only phosphodiester links. Oligos V and VI are the complementary DNA and RNA targets.

Compound II was isolated in 25% overall yield by RP HPLC. Like compound I it exhibited two peaks in the ^{31}P NMR spectrum (a broad band near +10.6 ppm attributable to the phosphoramidate groups and a sharper band near -2 ppm, attributable to the phosphodiesters; Figure 2). The fully modified oligomer (III) showed a single major band, broadened as a consequence of the heterogeneity of environment of the

Table 1. Properties of Oligonucleotides

	Compound	RP HPLC,min	TLC[a],Rf	PAGE[b],RM	Tm[c]
II	d(C+T-G+A-A+A-A+T-G+G)	16.8	0.5	+0.02	35
III	d(C+T+G+A+A+A+A+T+G+G)	19.8	0.3	+1.7	31
IV	d(C-T-G-A-A-A-A-T-G-G)	12.2	0.3	-1.3	31
V	d(C-C-A-T-T-T-T-C-A-G)	13.4	0.3	-1.3	
VI	r(C-C-A-U-U-U-U-C-A-G)	11.5	0.3	-1.2	

a. Brinkmann silica gel 60, mobile phase nPrOH:H_2O:NH_4OH (55:35:10, v/v/v. b.Mobility relative to methylene blue: "+" denotes movement toward the cathode; "-" denotes movement toward the anode. c. The conditions were the same as in Figure 4.

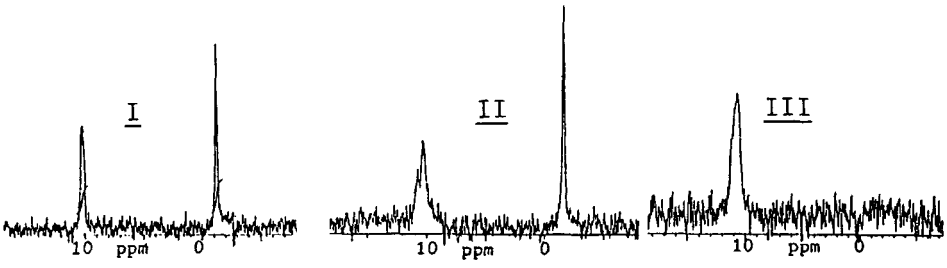


Figure 2. ^{31}P NMR spectra of compounds I, II, and III in D_2O.

phosphoramidate links (Figure 2,III). In contrast to the phosphodiester oligomers, which were efficiently hydrolyzed to the corresponding nucleosides by snake venom phosphodiesterase and alkaline phosphatase within 2 hours at 37 °C, compound II was resistant to attack by these nucleases (see Figure 3). The Tm data (Table 1) for the complexes formed with the deoxyribonucleotide target are consistant with results obtained previously for other cationic derivatives and serve to further characterize these compounds.

Data on the interactions of the oligomers with a complementary RNA strand, VI, are presented in Table 2, and some representative dissociation curves are given in Figure 4. Several interesting features may be noted. The melting curves provide good evidence that the *alternating oligomer containing mixed bases (compound II), like the homooligomer (I), hybridizes with a complementary RNA oligonucleotide*. In 0.1 M NaCl the affinity is

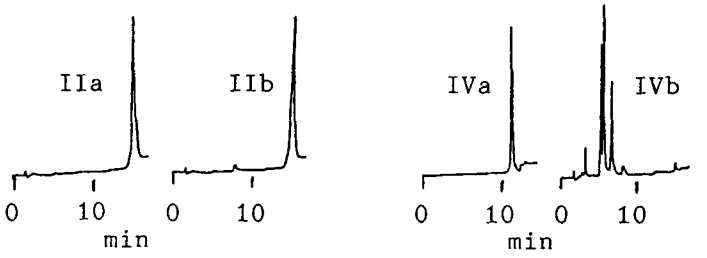


Figure 3. RP HPLC profiles for enzymatic hydrolysis of compounds II and IV: (a) before treatment with enzyme; (b) after treatment with enzyme. The major peaks in the profile for IV are, respectively, dC, dI[8], dG, and dT.

Table 2. Estimated Tm values for complexes with CCAUUUUCAG[a]

Compound		Concentration of NaCl		
		23 mM	0.1 M	1.0 M
II	C+T-G+A-A+A-A+T-G+G	12	12	10
III	C+T+G+A+A+A+A+T+G+G	b	c	c
IV	C-T-G-A-A-A-A-T-G-G	<5	13	21

(a) The values are approximate since the lower base lines for the melting curves are not clearly defined. (b) A precipitate formed. (c) No precipitate; no evidence for hybridization from the melting curve data.

comparable to that for the corresponding phosphodiester probe (Figure 4). Experiments at different salt concentration show that Tm for formation of the complex involving the zwitterionic probe is little affected by changes in the ionic strength of the solution. This behavior resembles that for complexes derived from zwitterionic probes and oligodeoxyribonucleotide targets. In contrast, the complex formed from IV and VI behaves normally; stability increases with increasing salt concentration (Table 2). It is noteworthy that in the low salt system (23 mM NaCl) the complex derived from the RNA oligonucleotide and the zwitterionic oligomer is more stable than that formed from IV+VI. The fully modified cationic oligonucleotide (III) formed a precipitate when mixed with the RNA target (VI) in the low salt medium. This precipitate appears to be simply a manifestation of non-specific polyanion-polycation interaction.

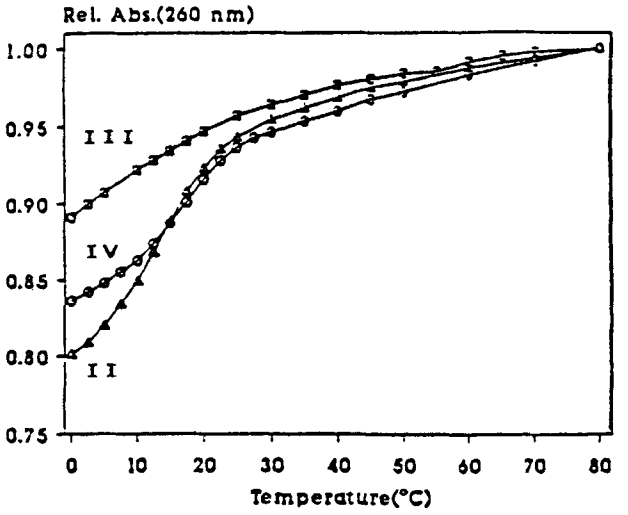


Figure 4. Melting curves for interaction of oligomer II (Δ), oligomer III (□), and oligomer IV (○) with CCAUUUUCAG (oligomer VI); in 0.1 M NaCl, pH 7.0.

SUMMARY

Previous studies have revealed unique features for hybridization of cationic probes with DNA targets. The new results open the way for applications of the alternating cationic/anionic oligomers as probes and inhibitors of processes involving RNA targets as well.

EXPERIMENTAL SECTION

General Methods. ^{31}P NMR spectra were determined on a Varian XLA-400 NMR spectrometer. High performance liquid chromatography (HPLC) was carried out with a C-18 reversed-phase column (5-μm Hypersil ODS; 100 x 2.1 mm) on a Hewlett-Packard 1090 chromatograph with aqueous 30 mM triethylammonium acetate (pH 7.0), using a linear acetonitrile gradient (1%/min starting at 0%, flow rate 1.0 ml/min). Polyacrylamide gel electrophoresis (PAGE) was carried out with a 20% gel (19:1 acrylamide/bis-acrylamide in 89 mM Tris.borate (pH 8.3) at a constant 200 V in

commercial equipment (R. Schadel, Inc.). Enzymatic hydrolyses were carried out as previously described.[6] Thermal dissociation curves were obtained by following changes in absorbance at 260 nm as samples were warmed from 0°C to 80°C in 2.5 or 5°C increments, allowing 3 or 5 min, respectively, for equilibration at each step.

Oligonucleotide Syntheses. The alternating thymidylate analogue, I, was prepared by successively alternating hydrogen phosphate and phosphoramidate synthetic chemistry as described previously,[1] but with use of 3-dimethylaminopropylamine as the amine component in the oxidative-coupling step. Fully substituted cationic oligomer III was prepared by use of conventional hydrogen phosphonate chemistry with a final oxidative step with CCl_4 and dimethylaminopropylamine (see method in ref 1). The new procedure for synthesizing the zwitterionic oligomers is described below.

Reactions were carried out manually by the syringe technique using commercially available CPG supports loaded with a 1-1.5 μmol of nucleoside. Conventional washing procedures were employed. Hydrogen phosphonate couplings were effected by successively drawing in 0.5 mL of pyridine/CH_3CN 20 mM in DMTr-nucleoside-H-phosphonate and 0.5 mL of a pyridine/CH_3CN solution containing trimethylacetyl chloride (100 mM). After 3 min the mixture was expelled from the syringe. Amidite couplings were carried out with 1 mL of 25 mM DMT-protected nucleoside-ß-cyanoethylphosphor-amidite and 0.5 mL of 570 mM 1-H-tetrazole in CH_3CN. After 2 min the support was washed with 4:1 CH_3CN/pyridine, CH_3CN, and CH_2Cl_2 (2x1 mL each), and the intermediate phosphites were oxidized by a 2 min treatment with a 1.1 M solution of t-BuOOH in CH_2Cl_2. At the end of the synthetic sequence, oxidative amidation of the H-phosphonate groups was accomplished by a 20 min treatment with a solution of 3-dimethylaminopropylamine/pyridine/CCl_4 (1/5/5 v/v/v). The DMT group was then removed and the oligonucleotides were cleaved from the support with concd. NH_4OH (6.5 h, 55 °C). The products were purified by RP HPLC, and the purity was confirmed by analytical HPLC; 32-36 A_{260} units (~25 % yield) of oligomer was obtained in each case.

The RNA oligomer was synthesized by the procedure of Wu et al.[8]

ACKNOWLEDGMENT

This work was supported by the National Institute of General Medical Sciences (Grant GM 10265) and by the National Cooperative Drug Discovery Group for the

treatment of AIDS of the National Cancer Institute and the National Institute of Allergy and Infectious Diseases (Grant UOI AI24846).

REFERENCES AND NOTES

1. Letsinger, R.L., Singman, C.N., Histand, G., and Salunkhe, M. *J. Am. Chem. Soc.* **1988**, *110*, 4470-4471.

2. Letsinger, R.L., Clare, S.E., Histand, C.D., Salunkhe, M., Sarin, P.S., Singman, C.N., Tanaka, K., and Zhang, "Solid Phase Synthesis of Novel Oligonucleotide Analogues," in *Innovations and Perspectives in Solid Phase Synthesis*, Editor, Roger Epton, SPCC (UK) Ltd. **1990**, 183-195.

3. Zwitzer and coworkers have recently described a related family of zwitterionic oligonucleotide derivatives in which cationic charges are introduced *via* 5-aminoalkyl- 2'-deoxyuridine substituents: (a) Hashimoto, H., Nelson, M.G., and Switzer, C. *J. Org. Chem.* **1993**, *58*, 4194-4195; *J. Am. Chem. Soc.* **1993**, *115*, 7128-7134.

4. K. Tanaka and R.L. Letsinger, unpublished work.

5. Hayakawa, Y., Wakabayashi, S., Kato, H., and Noyori, R. *J. Am. Chem. Soc.* **1990**, *112*, 1691-1696.

6. Miller, P.S., Dreon, N., Pulford, S.M., and McParland, K.B. *J. Biol. Chem.*, **1980**, *255*, 9659-9665.

7. dA is converted to dI by adenosine deaminase in the enzyme mixture.

8. Wu, T., Ogilvie, K.K., and Pon, R.T. *Nucleic Acids Res.* **1989**, 3501-3517.

Received 12/21/93
Accepted 2/7/94