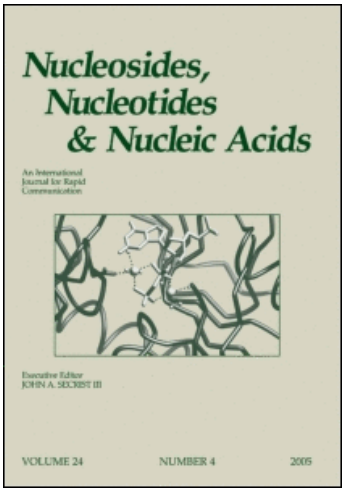

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Synthesis and Properties of Oligonucleotide (2-Aminoethyl)Phosphonates

Alan F. Cook[a]; Reza Fathi[a]; Qing Huang[a]; George Coppola[a]; William Delaney[a]; Jia-Lin Syi[a]
[a] PharmaGenics, Inc., 4 Pearl Court, Allendale, New Jersey, USA

To cite this Article Cook, Alan F. , Fathi, Reza , Huang, Qing , Coppola, George , Delaney, William and Syi, Jia-Lin(1995) 'Synthesis and Properties of Oligonucleotide (2-Aminoethyl)Phosphonates', Nucleosides, Nucleotides and Nucleic Acids, 14: 3, 1005 — 1008
To link to this Article: DOI: 10.1080/15257779508012521
URL: http://dx.doi.org/10.1080/15257779508012521

NUCLEOSIDES & NUCLEOTIDES, 14(3-5), 1005-1008 (1995)

SYNTHESIS AND PROPERTIES OF OLIGONUCLEOTIDE (2-AMINOETHYL)PHOSPHONATES

Alan F. Cook*, Reza Fathi, Qing Huang, George Coppola, William Delaney, and Jia-Lin Syi

PharmaGenics, Inc., 4 Pearl Court, Allendale
New Jersey, USA 07401

ABSTRACT

Oligonucleotides with novel, cationic backbone substituents have been prepared. Dinucleotide aminoethylphosphonates have been synthesized and the isomers were separated and used to prepare oligonucleotides with alternating positive and negative charges. The properties of these oligonucleotides have been examined.

Although a large number of modifications to the phosphodiester backbone of oligonucleotides have been reported in the search for analogs with improved properties[1], most modifications have been either neutral or negatively charged. In contrast, relatively few reports of oligonucleotides with cationic backbone substituents have been described[2-4]. In this study we have developed methods for the synthesis of oligonucleotides with cationic (2-aminoethyl)phosphonate backbone groups, and the chemical, biophysical and biological properties of analogs of this type have been determined.

Oligonucleotide synthesis. Phthalimido or halogenated phthalimido derivatives of (2-aminoethyl)phosphonic acid were prepared and condensed with 5'-dimethoxytrityl-thymidine to give the protected mononucleotides **1a-c** (Figure 1). These monomers were coupled with thymidine to produce the dimers **2a-c** as mixtures of isomers which could be separated by column chromatography. The stereochemical assignments for the dimers were made primarily on the basis of 2D NMR experiments. Studies on the deprotection of

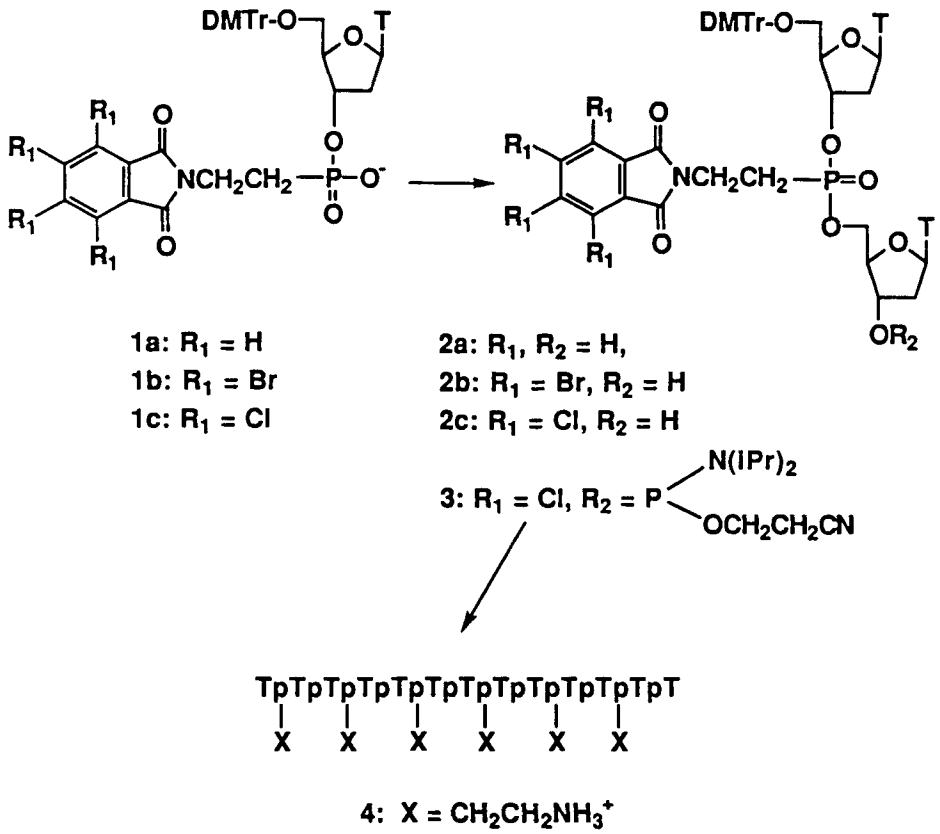

FIGURE 1

the phthalimido substituents showed that removal of the unsubstituted phthalimido group from **2a** required relatively harsh conditions, whereas the tetrahalophthalimido groups could be cleanly removed from either **2b** and **2c** using ethylenediamine. The tetrachlorophthalimido group was subsequently selected as the protecting group of choice for oligonucleotide synthesis. The dimer **2c** was converted to its phosphoramidite derivative **3** and the latter was coupled in a DNA synthesizer to produce single isomer, net neutral, alternating backbone, T_{13} oligonucleotides of structure **4**.

Hybridization properties. The hybridization properties of the single isomer, alternating backbone oligonucleotides were compared with

TABLE 1

HYBRIDIZATION OF ALTERNATING BACKBONE T_{13} OLIGONUCLEOTIDES TO DNA AND RNA

BACKBONE TYPE	ISOMER	MELTING TEMP (°C) DNA TARGET[a]	RNA TARGET[a]
$P\text{-}O^-$	-	35	29.5
$P\text{-}CH_2NH_3^+/P\text{-}O^-$	Sp	<10	-
$P\text{-}CH_2CH_2NH_3^+/P\text{-}O^-$	Sp	12	-
$P\text{-}CH_2NH_3^+/P\text{-}O^-$	Rp	45	34
$P\text{-}CH_2CH_2NH_3^+/P\text{-}O^-$	Rp	51	34.5
$P\text{-}CH_3/P\text{-}O^-$	MIXED	32	32
$P\text{-}S^-/P\text{-}O^-$	MIXED	27	27

[a]DNA: d-A(pA)$_{12}$, RNA: r-A(pA)$_{12}$, in 160 mM salt.

phosphodiester controls and with the corresponding aminomethyl derivatives[5] (Table 1).

One of these single isomer, net neutral oligonucleotides (**4**, Rp isomer) formed more stable hybrids with either DNA or RNA targets than their corresponding natural counterparts, whereas the other (Sp) isomer did not form stable hybrids. Interestingly, the duplex of **4** (Rp isomer) with its DNA complement displayed a higher dissociation temperature than the corresponding aminomethyl derivative, even though the former possessed more steric bulk. The effect of salt concentration on dissociation temperature was less marked than for the natural duplex, which is to be expected for hybrids in which the charge-charge repulsions are reduced. Dissociation temperatures were strongly pH-dependent, with the more stable hybrids being formed under conditions which favored protonation of the aminoalkyl groups.

Hydrolytic stability. Since previous work had demonstrated that oligonucleotide (aminomethyl)phosphonates were hydrolyzed in aqueous solution[5], it was of interest to examine the hydrolytic stability of the aminoethyl

series. A dinucleotide (aminomethyl)phosphonate in aqueous solution at 37° at pH 7 was hydrolyzed with a $t_{1/2}$ of 45 hours, whereas the corresponding (2-aminoethyl)phosphonate was much more stable with a $t_{1/2}$ of more than 30 days. This difference in stability might be caused by a reduction in the electropositivity of the phosphorus atom due to the additional intervening methylene group.

Biological studies. Oligonucleotides **4** (Rp and Sp isomers) were virtually undegraded after digestion for 24 h with S1 nuclease, whereas a natural sequence was rapidly degraded ($t_{1/2}$ = 4 min) under the same conditions. Oligomer **4** (Rp isomer) did not induce RNase-H-mediated cleavage of a complementary RNA strand under conditions which were suitable for the cleavage of a natural duplex. Stability in serum-containing media was evaluated for an alternating backbone sequence possessing a ^{32}P label at the 5'-position; minimal degradation was observed over 24 hours.

REFERENCES

1. Uhlmann, E.; Peyman, A. *Chem. Rev.* (1990) **90**, 543-584.
2. Letsinger, R. L.; Singman, C. N.; Histand, G.; Salunkhe, M. *J. Amer. Chem. Soc.* (1988) **110**, 4470-4471.
3. Froehler, B. C. *Tetrahedron Lett.*. (1986) **27**, 5575-5578.
4. Jung, P. M.; Histand, G.; Letsinger, R. L. *Nucleosides & Nucleotides* (1994) **13**, 1597-1604.
5. Fathi, R.; Huang, Q.; Syi, J.-L.; Delaney, W.; Cook, A. F. *Bioconjugate Chem.* (1994) **5**, 47-57.