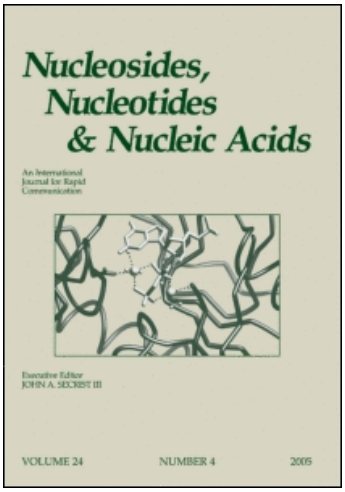

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Oligonucleotide Phosphonates—Solid Phase Synthesis and Biochemical Characterization

D. Bankaitis-Davis[a]; L. Huang[a]; T. Koga[a]; G. Beaton[a]
[a] Amgen Boulder Inc., St. Boulder, CO

To cite this Article Bankaitis-Davis, D. , Huang, L. , Koga, T. and Beaton, G.(1997) 'Oligonucleotide Phosphonates—Solid Phase Synthesis and Biochemical Characterization', Nucleosides, Nucleotides and Nucleic Acids, 16: 7, 945 — 949
To link to this Article: DOI: 10.1080/07328319708006112
URL: http://dx.doi.org/10.1080/07328319708006112

NUCLEOSIDES & NUCLEOTIDES, 16(7-9), 945-949 (1997)

OLIGONUCLEOTIDE PHOSPHONATES - SOLID PHASE SYNTHESIS AND BIOCHEMICAL CHARACTERIZATION

D. Bankaitis-Davis, L. Huang, T. Koga and G. Beaton*

Amgen Boulder Inc., 3200 Walnut St., Boulder, CO. 80301.

ABSTRACT: A novel method for the automated synthesis of oligonucleotide-3'-phosphonates is described. Oligonucleotide analogs synthesized by this method exhibited appreciable stability to exonuclease digestion.

Some nucleic acid analogs, particularly phosphorothioates, have recently been shown to mediate some of their biological effects through non antisense mechanisms[1]. We have been interested in oligonucleotide 3'-hydroxymethylene phosphonates (**fig. 1**) and their derivatives as alternative analogs for antisense experiments. Recently we developed an approach to the solid-phase synthesis of oligonucleotide 3'-hydroxymethylene phosphonates[2] that provides efficient access to analogs of potential biological interest as well as a scaffold for the synthesis of other 3'-phosphonate analogs through modification of the 3'-hydroxyl group.

A basic set of monomers **1-4** (**fig. 2**) was constructed that allows for the synthesis of phosphonate linkages in homopolymers or chimeric oligonucleotides. 3'-C-diphenylimidazolidino-5'-O-methylphosphite **1** functions as a monomer for chain extension of the phosphonate backbone. This is derived from the nucleoside **2**, which may be used to introduce a 3'-formyl equivalent to solid support. Alternatively, this may be accomplished by attachment of the phosphoramidites **3** to universal resins[3]. The 5'-O-methyl phosphites **4** provide a method for introducing a 3'-hydroxyl function to the growing end of the oligonucleotide chain.

Homopolymer oligonucleotide phosphonates were constructed from solid supports loaded with 3'-C-diphenylimidazolidino nucleosides (**fig. 3**). Acidic deprotection of the 3'-diphenylimidazolidino group with trichloroacetic acid followed by coupling of the primary monomer **1** yielded the 3'-hydroxymethylene phosphonate linkage. Chain extension was realized with repetition of the acid deprotection and coupling to the desired

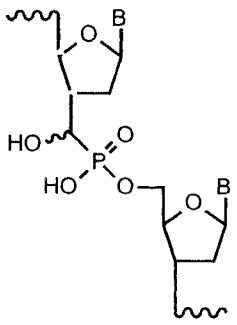

FIG. 1

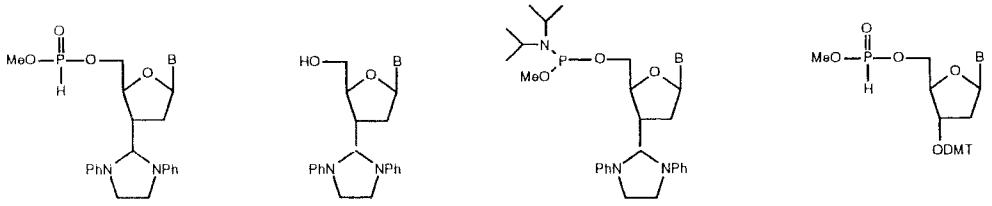

FIG. 2

oligomer length. The oligonucleotide chain was terminated by a final coupling with the methyl phosphite **4**. Deprotection with 2-carbamoyl-2-cyanoethylene-1,1-dithiolate or thiophenol followed by cleavage from the support with ammonium hydroxide gave the free oligonucleotide. Chimeric oligonucleotides containing both 3'-hydroxymethylene phosphonate and phosphodiester linkages were also constructed using monomers **3** and **4** interchangeably to introduce either the phosphonate or phosphodiester linkages as required. Trityl cation assay and ^{31}P NMR indicated coupling efficiencies of 95-97% for oligonucleotides prepared by this method.

The stability of a heptamer thymidylate uniformly modified with the 3'-hydroxymethylenephosphonate linkage to nuclease digestion was qualitatively compared to its phosphodiester counterpart. For digestion by snake venom phosphodiesterase (SVPDE) the oligomers were labeled with ^{32}P and digested with a series of enzyme concentrations ([E], **fig. 4**). Cleavage products were analyzed by gel electrophoresis.

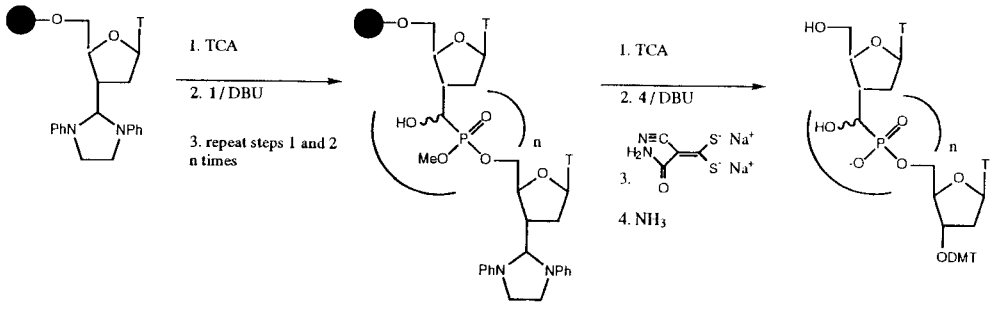

FIG. 3

SVPDE Digestion of Oligonucleotide 3'-hydroxymethylene phosphonates

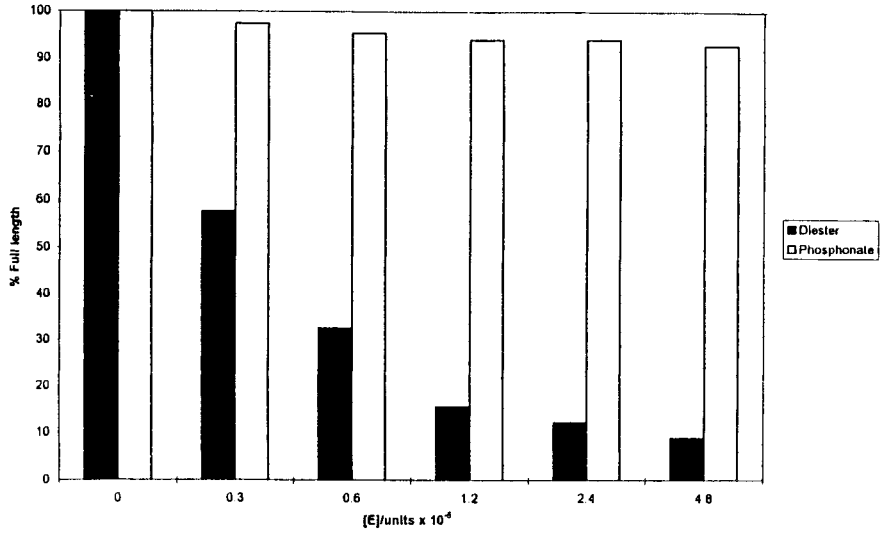

FIG. 4

HPLC Analysis of Calf Spleen Phosphodiesterase Digestion

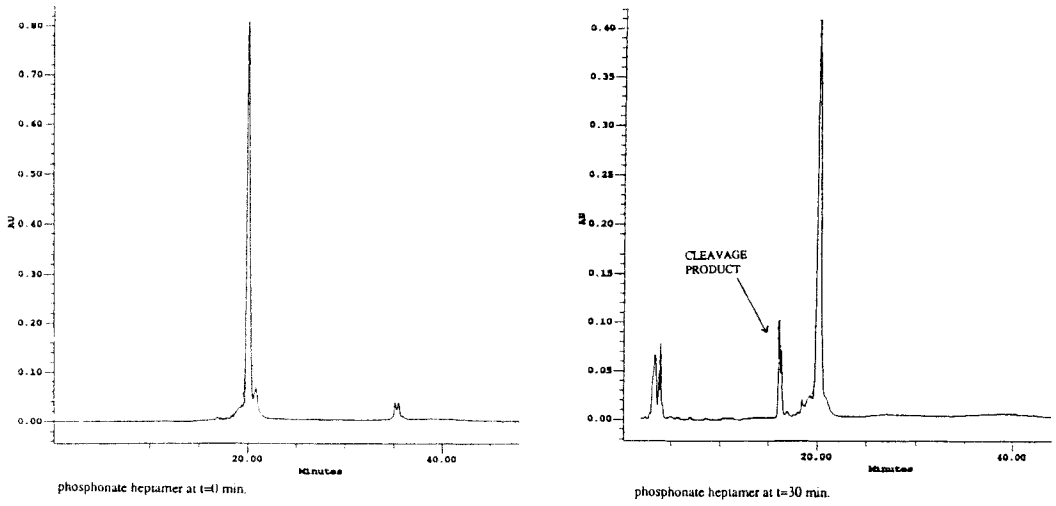

FIG. 5

Fig. 4 indicates the amount of full length oligomer remaining following a 30 minute incubation of the test oligonucleotide with enzyme at 37°C. Preliminary results indicated that for a series of concentrations, the phosphodiester was rapidly degraded whereas the analog showed no detectable degradation. Hplc analysis of the same oligonucleotides for their susceptibility to calf spleen phosphodiesterase (0.25 units) is depicted in **fig. 5**. While the phosphodiester was 80% degraded by the enzyme after 30 minutes, only 16% of a cleavage product, presumably the thymidine 3'-hydroxymethylene phosphonate monoester was observed for the analog (**fig. 5**, void volume peaks were attributable to the enzyme). These results were interesting compared to previous reports for the enzymatic stability of oligonucleotide methylene phosphonates[4].

An efficient method for the rapid automated synthesis of oligonucleotide 3'-hydroxymethylenephosphonates has been described. Current approaches may allow further access to a series of other 3'-phosphonate analogs of biochemical interest via modification of the hydroxymethylene group. Analogs of this type clearly show resistance to degradation by enzymes. A more detailed analysis of the biochemical properties of these analogs is in progress.

REFERENCES

1a) Stein, C. A.; Cheng, Y.-C. *Science* **1993**, 261, 1004. (b) Stein, C. A. *Leukemia* **1992**, 16, 967.

2) Bankaitis-Davis, D. M.; Huang, L.; Koga, T. M.; Beaton, G., (*manuscript in preparation*)

3) Scott, S.; Hardy, P.; Sheppard, R. C.; McLean, M. J. *Innovations and Perspectives in Solid Phase Synthesis* Epton, R. ed. Mayflower, Birmingham, 1994, 115.

4) Breaker, R. R.; Gough, G. R.; Gilliam, P. T. *Nucleic Acids Res.* **1990** 18, 3085.