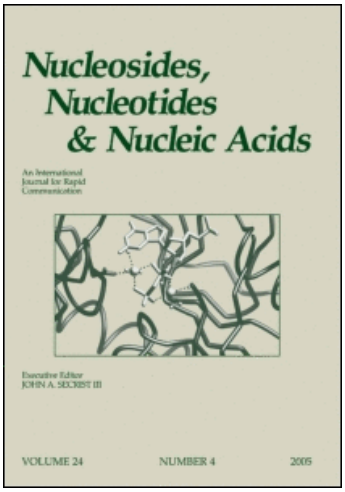


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# Solid Phase Synthesis of 5'-Methylenephosphonate DNA

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# SOLID PHASE SYNTHESIS OF 5'-METHYLENEPHOSPHONATE DNA

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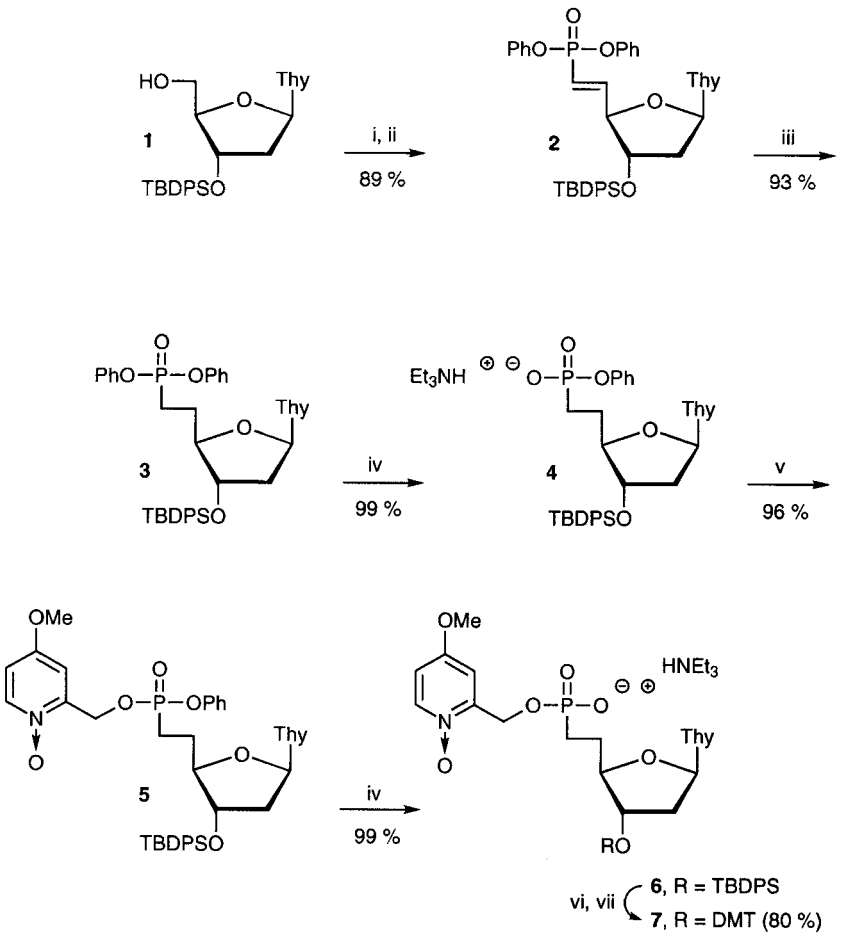
**Abstract.** A new approach to the solid phase synthesis of 5'-methylenephosphonate DNA is described. It makes use of intramolecular catalysis which ensures rapid and high-yielding condensations and thus provides a convenient entry to ionic, achiral analogs of thymidylic acids up to 20 nucleotides in length.



In order for the antisense oligonucleotide strategy to be effective, it is assumed that the natural phosphodiester internucleotidic linkage has to be replaced with a chemical entity which retains or strengthens the properties of the phosphate diester.[1] The most well-studied phosphate analogs of DNA are those having the methylphosphonate[2] or the phosphorothioate[3] internucleotidic linkages. Normally, chemical syntheses of these analogs leads to a pool of diastereoisomeric products, some of which may display physicochemical properties, e.g., hybridization to a target sequence, significantly different from other diastereomers present in the product mixture. This stereochemical problem can be avoided by employing achiral phosphate analogs as potential antisense oligonucleotides. Phosphorodithioate DNA represents a major candidate in this class of analogs.[4] Several other possible[5] achiral phosphate analogs deserve attention as potential antisense compounds and this necessitates studies directed towards their chemical preparation.

We have chosen the 5'-methylenephosphonate analog[6] of DNA as a target modification for several reasons. Firstly, a synthetic route for the introduction of the requisite phosphonate function on nucleoside level was at hand,[7] and secondly, preliminary studies[8] indicated clean and rapid condensations when a phosphonate




(i) DCC, DMSO; (ii) $Ph_3P{=}CHP(O)(OPh)_2$; (iii) $H_2$, Pd/C; (iv) 2-pyridinealdoxime, TMG; (v) 4-methoxy-1-oxido-2-pyridinemethanol, 2-chloro-5,5-dimethyl-2-oxo-1,3,2-dioxaphosphinane; (vi) $Bu_4NF$; (vii) DMT-Cl, DMAP

SCHEME 1

protecting group enabling intramolecular catalysis was employed. Synthesis of the thymidine monomer **7** required for solid phase synthesis of 5'-methylenephosphonate analogs of oligothymidylic acid is outlined in Scheme 1.

Oxidation of 3'-*O*-*tert*-butyldiphenylsilylthymidine **1** with DCC/DMSO and treatment of the resulting aldehyde with the mixed phosphorane-phosphonate Wittig reagent, diphenyl triphenylphosphoranylidenemethylphosphonate[9], afforded the

TABLE 1. Protocol for the manual solid phase synthesis of 5'-methylenephosphonate DNA.

| | Description | Volume | Time |
|---|---|---|---|
| Elongation cycle | | | |
| 1. | DCE wash | 5 × 1 mL | |
| 2. | Detritylation[a] | 5 × 1 mL | 5 × 1 min |
| 3. | DCE wash | 5 × 1 mL | |
| 4. | Pyridine wash | 5 × 1 mL | |
| 5. | Coupling mixture[b] | | 5 min |
| 6. | Pyridine wash | 5 × 1 mL | |
| 7. | Repeat steps 1-6 | | |
| End cycle | | | |
| 8. | DCE wash | 5 × 1 mL | |
| 9. | Detritylation[a] | 5 × 1 mL | 5 × 1 min |
| 10. | Dioxane wash | 5 × 1 mL | |
| 11. | Dealkylation[c] | 1 mL | 60 min |
| 12. | Methanol wash | 5 × 1 mL | |
| 13. | Ether wash | 5 × 1 mL | |
| 14. | Cleavage from support[d] | 1 mL | overnight |

a) 1 % TFA in DCE. b) 2,4,6-Triisopropylbenzenesulfonyl chloride (50 μmol) was added to a solution of **7** (30 μmol) in pyridine (500 μL) and the resulting mixture drawn into the syringe. c) Thiophenol-triethylamine-dioxane, 1:1:2. d) conc. $NH_3$-ethanol, 3:1



vinylphosphonate **2**. Catalytic hydrogenation at atmospheric pressure gave *C*-phosphonate **3**. Introduction of the catalytic 2-picolyl derivative was very efficient and involved: (i) selective deprotection of one phenyl group from **3** to produce **4**; (ii) coupling of **4** with 4-methoxy-1-oxido-2-pyridinemethanol[10] followed by (iii) removal of the phenyl group from **5** to produce **6**. Finally, the silyl protecting group of **6** was removed and replaced by the dimethoxytrityl group. The resulting building block **7** was obtained in 62 % overall yield from 3'-*O*-*tert*-butyldiphenylsilylthymidine. Clearly, the chemistry outlined in Scheme 1 constitutes a very efficient route to the required monomer.

Solid phase synthesis of 5'-methylenephosphonate DNA was performed manually with a Hamilton syringe charged with the CPG-bound nucleoside. 3'-*O*-Dimethoxy-

tritylthymidine was anchored to CPG *via* its 5'-*O*-succinate ester and the oligomers were synthesized according to the protocol given in Table 1.

Several oligomers up to 20-mers were prepared on a 1 μmol scale according to this protocol (coupling yields as estimated by the trityl assays were in the range 95-98 %), lyophilized and the crude products analyzed by reversed phase HPLC. In all cases the desired oligomer was the major reaction product clearly separated from small amounts of truncated sequences. This demonstrates the usefulness of the approach for the preparation of 5'-methylenephosphonate DNA, a potential antisense oligonucleotide analog. We are currently extending this approach to include all four common nucleosides, thus adding a new member to the class of achiral phosphate analogs of DNA.

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