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A Novel Solid Support for Synthesis of Oligonucleotide 3′-Phosphorothioate Monoesters

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Abstract—A new reagent immobilized on solid support allowing for solid-phase synthesis of oligonucleotides with a 3′-terminal phosphorothioate monoester is described. The support is compatible with phosphoramidite chemistry for automated oligonucleotide synthesis. Final deprotection with ammonia under standard conditions leads to oligonucleotide 3′-terminal phosphorothioate.

There is increasing interest in synthesis of oligonucleotides with ligands such as amino, carboxyl, thiol or phosphate groups attached at the 3′- or 5′-termini as these are widely used research tools for genetic analysis, gene functionalization, and elucidation of mechanisms of action in molecular biology.[1–3] Synthesis of 3′-phosphorylated oligonucleotides is also of interest because it permits for oligonucleotide circularization by template-derived chemical ligation,[4,5] modifications at internucleotide linkages[6] and conjugation of reporter groups at the 3′-terminus.[7–11] 3′-Terminal phosphorothioate oligonucleotide (TPT) has also been identified as a minor process related impurity in synthesis of phosphorothioate oligodeoxyribonucleotides with purines at the 3′ terminus.

A number of methods have been reported for selective introduction of phosphate/phosphorothioate groups at the 3′-terminus of oligonucleotides. Dithioethanol and disulfide linkers allow for standard synthesis of oligonucleotides on solid support and liberate the oligomer 3′-phosphate by cleavage of a disulfide bond during final deprotection with dithiothreitol.[12–14] Another method involves condensation of cyanoethyl phosphoramidite to amino alkyl-CPG followed by cleavage of 3′-terminal phosphoramidate diester under prolonged treatment with 80% aqueous acetic acid.[15] A universal allyl linker for solid phase oligonucleotide synthesis was also reported, in which the allyl group is cleaved under Pd(0) conditions and is orthogonal to other protecting groups used during oligonucleotide synthesis.[16] Recently, Guzaev et al. reported a novel solid support for synthesis of 3′-phosphorylated oligonucleotides.[17] The methods reported earlier require a two-step final deprotection procedure or require drastic conditions or unpleasant reagents to liberate the 3′-thiophosphate/phosphate oligonucleotide. Herein, we report a novel solid support useful for synthesis of oligonucleotide 3′-phosphorothioate/3′-phosphate monoesters. The solid support is compatible with standard oligomerization conditions and the desired compound is generated during the final ammonia deprotection step.

The design of a quasi phosphate protecting group was based on the 'trimethyl lock' concept shown earlier to significantly facilitate lactonization of 2-hydroxybenzenepropionic acids.[18,19] The 3′-(2′-hydroxy-4′,6′-dimethyl)-2,2-dimethylpropionic acid moiety has been used as a prodrug of amine-containing compounds. A redox sensitive linker for solid phase peptide synthesis where the trimethyl lock system in a substituted hydroquinone moiety facilitates cyclic ether formation with the release of carboxylate compound was also reported recently.[20] We hypothesized that 3′-(2′-hydroxy-4′,6′-dimethyl)-2,2-dimethylpropionic acid would undergo similar cyclization to form a cyclic ether and liberate the 3′-phosphorothioate oligonucleotide under basic deprotection conditions.

Preparation of the solid support is shown in Scheme 1. Thus, the primary hydroxyl group of compound **1** (whose synthesis has been reported in the literature[21])

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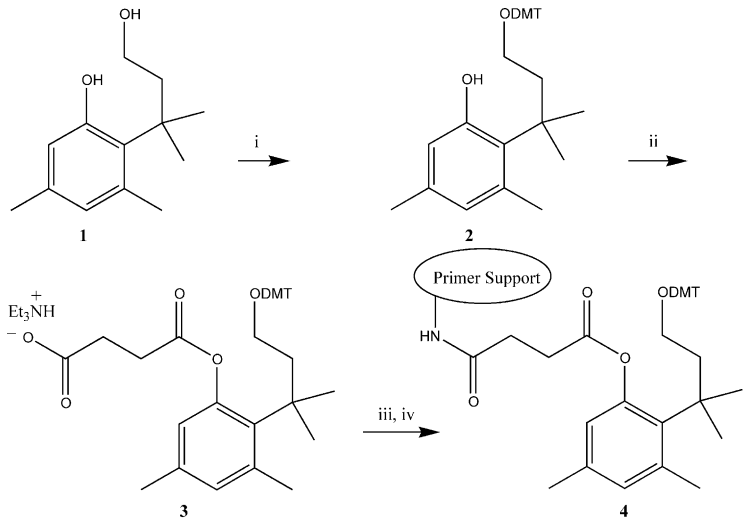


Scheme 1. (i) DMT-Cl/Py; (ii) succinic anhydride/DMAP/Py; (ii) Pharmacia HL-30; (iv) Ac_2O/*N*-methylimidazole/Py/THF.

was selectively dimethoxytritylated (82%) by treatment with 4′,4′-dimethoxytrityl chloride in pyridine to give **2**. Succinylation of the phenolic hydroxyl of **2** by treatment with succinic anhydride in the presence of DMAP and pyridine yielded compound **3** as the triethylammonium salt in 85% yield. Immobilization of succinate **3** on Pharmacia HL30 primer using HBTU as condensing agent afforded the desired solid support **4** (loading 92 μmol/g).

Suitability of support **4** for synthesis of 3′-phosphorothioate oligonucleotide was demonstrated by synthesizing (GCCCAAGCTGGCATCCGTCp), a TPT analogue of ISIS-2302 (*n*−1)-mer. Synthesis was performed using a Pharmacia OligoPilot II DNA/RNA synthesizer at 180–190 μmol scale using standard β-cyanoethyl phosphoramidites (0.2 M solution in acetonitrile). Phenylacetyl disulfide (PADS) (0.2 M solution in 3-picoline/acetonitrile, 1:1, v/v) was used for sulfurization of phosphite triester linkages.[22] After chain assembly, the oligonucleotide was deprotected with ammonia at 55 °C for 16 h. Reversed phase purification followed by final detritylation afforded the desired oligonucleotide, whose structure was established by LC–MS, ^{31}P NMR (Fig. 1) and capillary gel electrophoresis (Fig. 2). TPT analogues of multiple phosphorothioate oligodeoxyribonucleotides were synthesized to show the versatility of this chemistry.

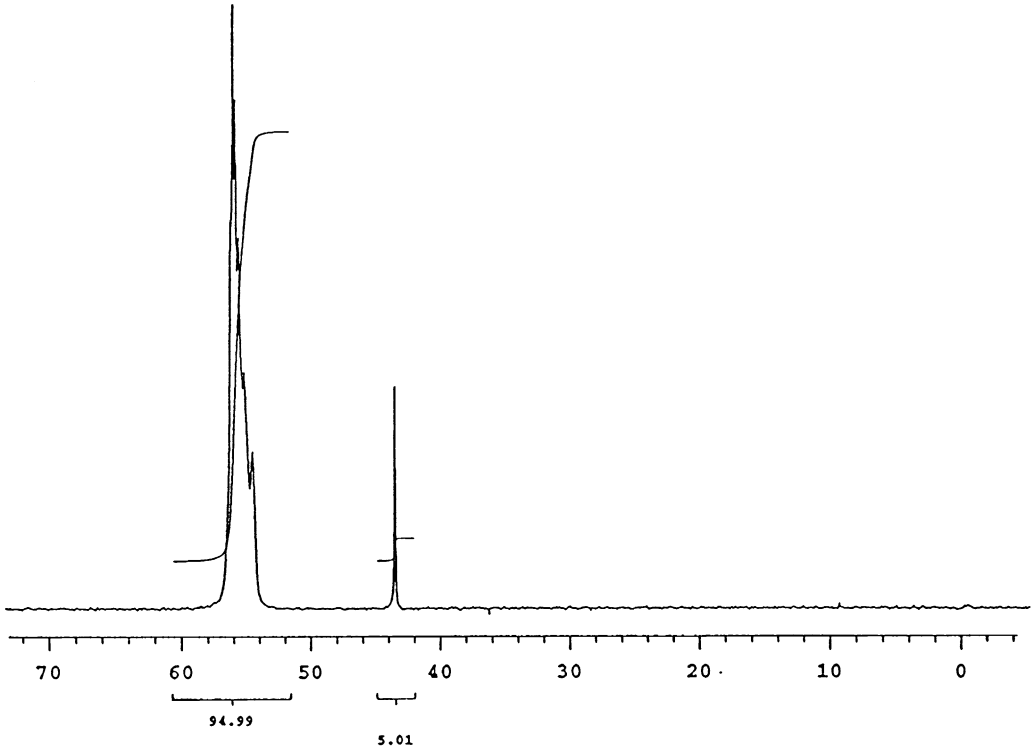


Figure 1. $^{31}P(D_2O)$ NMR of TPT oligonucleotide synthesized.

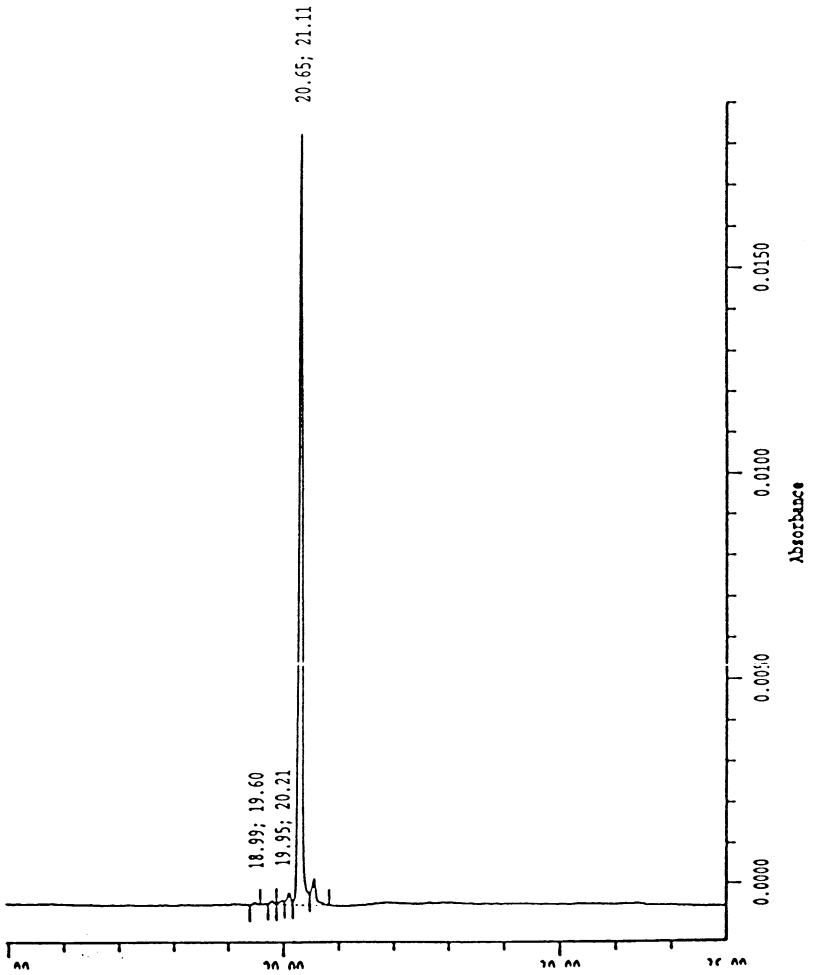
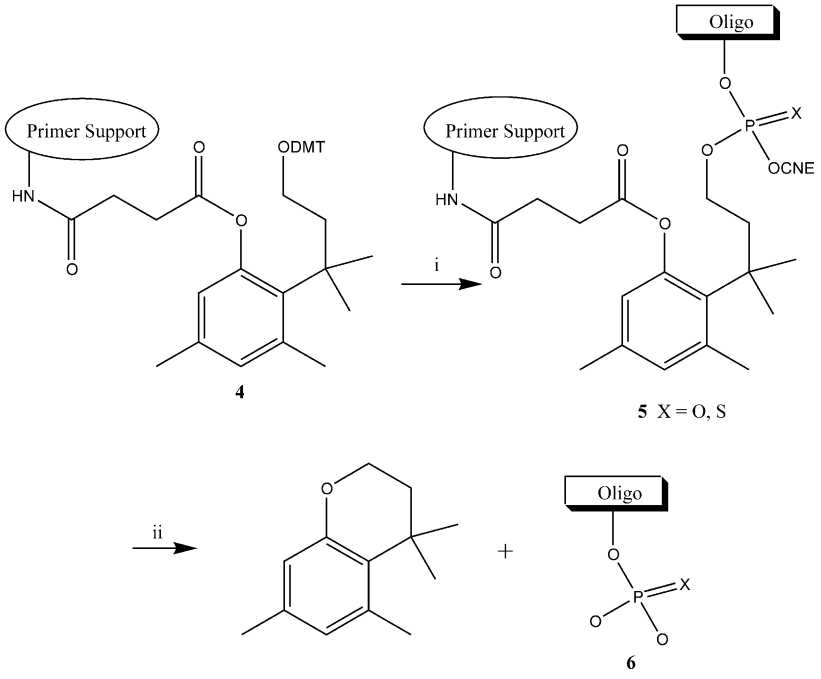


Figure 2. CGE analysis of TPT oligonucleotide synthesized.



Scheme 2. (i). Standard oligonucleotide synthesis; (ii) ammonia, 55 °C, 16 h.

In conclusion, we have demonstrated that 3′-terminal phosphorothioate oligonucleotides may be efficiently synthesized using 3′-(2′-hydroxy-4′,6′-dimethyl)-2,2-dimethylpropionic acid moiety under standard oligomerization conditions (Scheme 2). In order to synthesize the 3′-phosphate analogue, sulfurization step needs to be replaced with an oxidation step and *tert*-butyl hydroperoxide could be employed as the oxidation reagent. The 3′-(2′-hydroxy-4′,6′-dimethyl)-2,2-dimethylpropionic acid moiety in which the aromatic hydroxyl group is protected as an ester can potentially be used as a phosphate protecting group in oligonucleotide synthesis via the phosphoramidite chemistry and also as exocyclic amino protecting group and is under investigation in our laboratory.

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