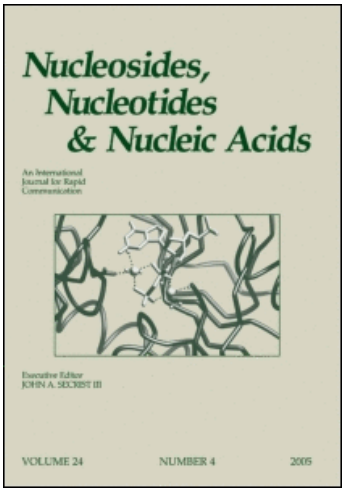

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Studies on Nucleoside H-Phosphonothioates as Synthons in the Synthesis of Oligonucleotide Analogues

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STUDIES ON NUCLEOSIDE H-PHOSPHONOTHIOATES AS SYNTHONS IN THE SYNTHESIS OF OLIGONUCLEOTIDE ANALOGUES

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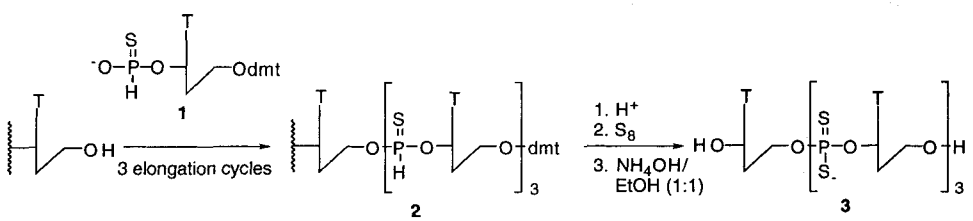
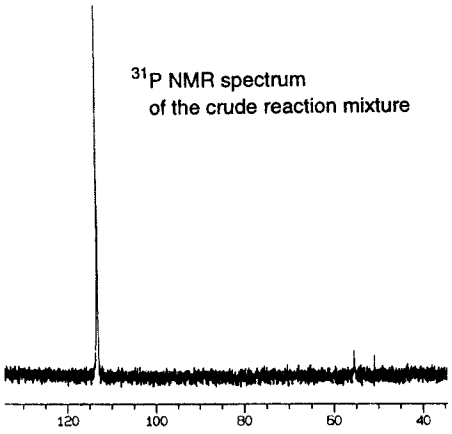
Recently, we have reported that H-phosphonothioate diesters[1] are versatile synthetic intermediates for the preparation of phosphorothioates, phosphorodithioates, or other phosphodiester analogues. The most suitable coupling agents to produce H-phosphonothioate diesters were found to be various chlorophosphates[1] [*e.g.*, 2-chloro-5,5-dimethyl-2-oxo-2λ^5-1,3,2-dioxaphosphinane (NEP), diphenyl phosphorochloridate]. These reagents secure the required chemoselectivity during the condensation and are unreactive towards the P-H function in H-phosphonothioate diesters[1,2].

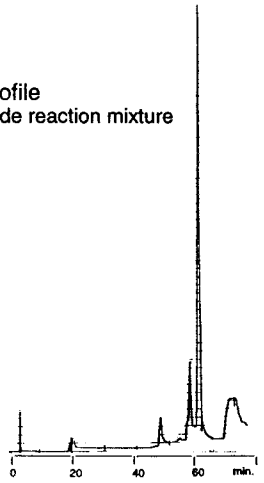
To evaluate the possibility of synthesizing oligonucleotide analogues *via* H-phosphonothioate intermediates on solid support, we carried out preliminary studies on the preparation of oligonucleoside phosphorodithioates, as it is shown below.



To this end we synthesised on solid support tetrathymidine triphosphorodithioate **3** *via* the NEP promoted condensation of nucleoside H-phosphonothioate **1**[3] to produce H-phosphonothioate **2**, followed by its sulfurization as the last step. The reactions were carried out in a Hamilton syringe using 30 mg of solid support (CPG 500 Å) loaded with 5'-O-(dimethoxytrityl)-3'-O-succinylthymidine. The synthetic protocol was as follows

(cycle name, reagent, time): (i) detritylation, 2% dichloroacetic acid in dichloroethane (4 x 1 mL), 4 min; (ii) washing, dichloroethane (3 x 1 mL), pyridine (3 x 1 mL), 6 min; (iii) coupling, **1** (33 mM in pyridine, 600 μL) and NEP (100 mM in pyridine, 600 μL), 5 min; (iv) washing, pyridine (3 x 1 mL), dichloroethane (3 x 1 mL), 6 min. In these experiments, the capping step was omitted. The coupling yield per step was ~94% as judged from the trityl assay. On completion of the cycles, the support-bound oligomer **2** containing H-phosphonothioate internucleosidic bonds was sulfurised with elemental sulfur in pyridine for 30 min and then washed successively with pyridine (3 x 1 mL) and dichloroethane (3 x 1 mL). Product **3** was cleaved from the support by treatment with 33% aqueous ammonia - ethanol (1:1, v/v) and the crude reaction mixture was subjected to HPLC [C-18 column, gradient of CH_3CN in 25 mM CH_3COONH_4 (0 to 20%)] and ^{31}P NMR analyses.





The HPLC profile (see above) is consistent with the observed coupling efficiency (the major peak from **3** and some truncated sequences) and the ^{31}P NMR spectrum confirms the presence of phosphorodithioate linkages in the major product (δ_P ~114 ppm). The origin of traces of phosphoromonothioates (signals at ~55 ppm) is unknown. Optimization of the synthetic protocol is in progress.

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