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Daniel C. Capaldi^a; Douglas L. Cole^a; Vasulinga T. Ravikumar^a

^a Isis Pharmaceuticals, Carlsbad, CA

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SYNTHESIS OF DEOXYRIBONUCLEOSIDE PHOSPHORODITHIOATE DIMERS BY A TRIESTER METHOD

Daniel C. Capaldi, Douglas L. Cole & Vasulinga T. Ravikumar*

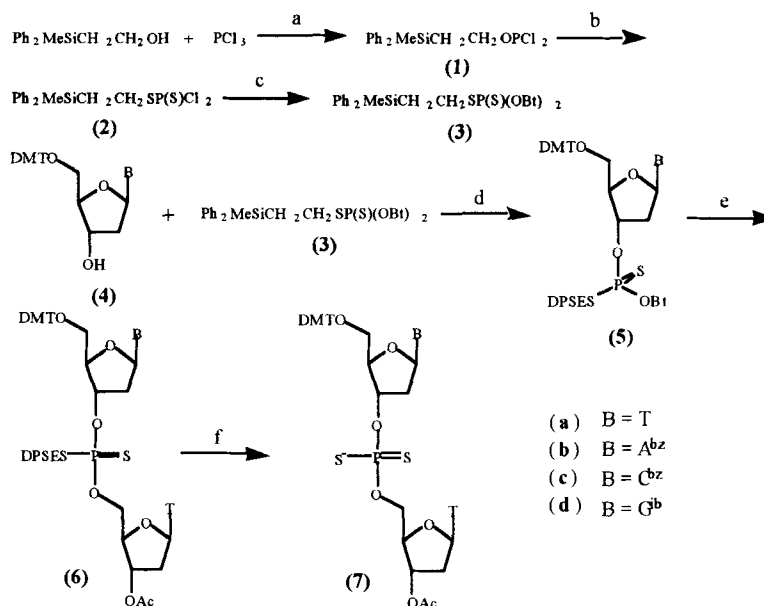
Isis Pharmaceuticals, 2290 Faraday Avenue, Carlsbad, CA 92008

Modified oligodeoxynucleotides have recently received much attention due to their therapeutic applications. Among the more promising are phosphorodithioates where both nonbridging oxygen atoms in the phosphate diesters are replaced by sulfur. Deoxynucleoside phosphorodithioate dimers have been prepared in several ways, using H-phosphonate, phosphordiamidite, phosphoramidite, and thiophosphoramidite methods. Reports have also appeared on the synthesis of oligonucleotides with alternating phosphodiester and dithiophosphodiester linkages, as well as one on ribonucleoside dimers. Of the above methods, the thiophosphoramidite method has been applied successfully for the preparation of mixed base oligonucleotides containing contiguous phosphorodithioate linkages. However, this method gives products which contain varying amounts of phosphorothioate linkages (2 - 10%) due to factors associated with the involvement of trivalent thiophosphorus compounds. In addition, the thiophosphoramidite synthons are difficult to purify on silica gel column, and have a tendency to dismutate in presence of acidic catalysts such as tetrazole. The thiophosphite intermediate which is formed is also unstable to tetrazole. Similarly in the thio- and dithio-H-phosphonate method, the primary coupling products are unstable to catalysts, pivaloyl chloride and iodine. Recently, Dahl *et al* reported¹⁻² synthesis of dimers and oligomers upto octamer which also leads to formation of small amounts of phosphorothioate linkages. In addition, about 1.2% per phosphorodithioate linkage of the oligomer is cleaved during

deprotection. In this paper, we report the preparation of deoxynucleoside phosphorodithioate dimers by a triester method using a new dithiophosphorylating reagent, RSP(S)Cl_2 where $\text{R} = 2$ -diphenylmethyl silylethyl (DPSE). This protecting group has been utilized in the synthesis of oligonucleotides via phosphoramidite approach. The strategy used is outlined in Scheme 1.

2-Diphenylmethylsilylethyl dithiophosphorodichloridate (**2**) was prepared by reacting the alcohol with phosphorus trichloride to give phosphorodichloridite (**1**) (δ 175.5 ppm) which on refluxing with thiophosphoryl chloride, sulfur and charcoal afforded (**2**) (δ 71.45 ppm).^{3,4} Treatment with 2 equivalents of 1-hydroxy-6-trifluoromethyl benzotriazole gave *O,O*-bis(benzotriazole-1-yl) S-2-diphenylmethylsilyl-ethyldithiophosphorotriester (**3**) (δ 114.1 ppm) which gave the protected dinucleoside dithiophosphorotriesters (**6a-d**) (δ 99.28, 98.32 ppm) with protected nucleosides in 65–72% yields. Dahl *et al* reported that S-4-chlorobenzyl dithiophosphorodichloridate underwent rapid S-dealkylation with 1-hydroxybenzotriazole and 1-hydroxy-6-trifluoromethylbenzotriazole to give triethylammonium *O,O*-bis(benzotriazole-1-yl) phosphorodithioate as the isolated product. However, we did not observe such reactions with the DPSE protecting group. Deprotection of the S-silylethyl group (δ 121.63 ppm) was achieved by treatment with *tetra*-butylammonium fluoride (1 M, THF) at room temperature in <5 min. ³¹P NMR and HPLC analyses showed no trace of formation of phosphorothioate or phosphodiester linkages. In addition no cleavage of the internucleotide bond was observed.

In a typical experiment, 2-diphenylmethylsilylethyl dithiophosphorodichloridate (5.9 g, 15 mmol) was coevaporated with dry dioxane (10 ml), then redissolved in dioxane (40 ml) and dry pyridine (2.4 ml, 30 mmol). 1-Hydroxy-6-trifluoromethylbenzotriazole (6.1 g, 30 mmol) was added and stirred at ambient temperature for 1 h. The pyridinium hydrochloride salt was filtered under argon and used as a stock solution. The above bis-phosphorylating reagent (10 ml, ca. 3 mmol) was added to a stirred solution of 5'-*O*-4,4'-dimethoxytritylthymidine (1.09 g, 2 mmol) in dry dioxane (5 ml). The mixture was allowed to stir for 2 h at room temperature, then 3'-*O*-acetylthymidine (0.57 g, 2 mmol) and 1-methylimidazole (0.8 ml, 10 mmol) were added. The mixture was allowed to stir overnight, then partitioned between ethyl acetate (100 ml) and saturated aqueous sodium hydrogen carbonate (50 ml). The organic layer was dried (Na_2SO_4) and evaporated under



Scheme 1: a. ether, 0°C, 2 h; b. P(S)Cl₃, sulfur, charcoal, reflux, 8 h; c. pyridine, 1-hydroxy-6-trifluoromethylbenzotriazole, dioxane, rt, 1 h; d. pyridine, dioxane, rt, 2 h; e. 3'-O-acetylthymidine, NMI, rt, 18 h; f. TBAF (1M, THF)

reduced pressure. The crude product was purified by silica gel chromatography (ethyl acetate-hexane) (4:1, v/v) and the fractions containing (6a) combined and concentrated to afford the product as colorless glass (1.5 g; 65%).

In conclusion, we have shown that protected dinucleoside phosphorodithioates can be obtained in fairly good yields by a phosphotriester method using the new phosphorylating agent (2), thus circumventing tervalent thiophosphorus reagents which are prone to decomposition and also avoids use of thiolate which has unpleasant odor.

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4. The mechanism of this sulfur transfer reaction is currently under investigation.