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SYNTHESIS OF DINUCLEOSIDE PHOSPHORODITHIOATES VIA THIOAMIDITES

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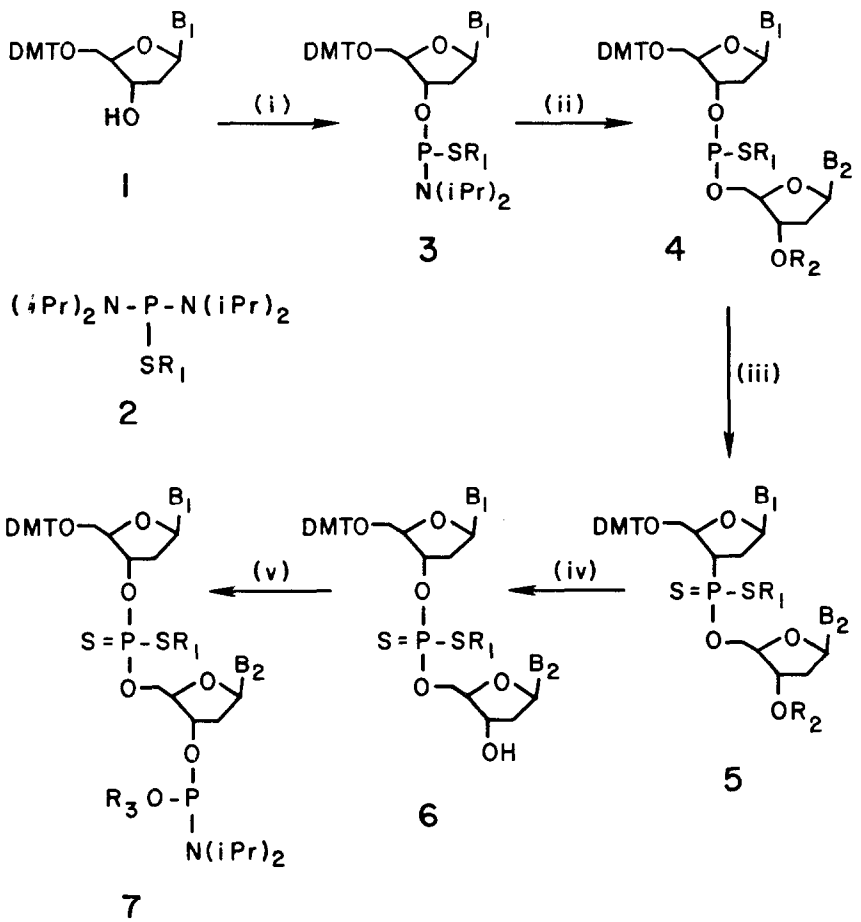
Deoxynucleosides and S-(4-chlorobenzyl)-phosphorothiodiamidites react via phosphorothioamidites to form thiophosphite triesters. Sulfur oxidation yields dinucleoside phosphorodithioate triesters which can be used to selectively introduce dithioate linkages into DNA.

Phosphorus modified polynucleotides are receiving increasing attention primarily due to their potential as antiviral agents.[1,2,3] Of the various possible derivatives, the internucleotide phosphorodithioate linkage would appear to be an excellent candidate because it is isosteric with phosphodiesters, is nuclease resistant, and should have other biochemical and biophysical properties similar to natural DNA.

Recently a pathway for introducing phosphorodithioate moieties as dinucleotide synthons into DNA was developed[4] and used successfully to synthesize several *lac* operators (unpublished results). However, this approach is not yet adaptable to the preparation of DNA containing exclusively phosphorodithioate linkages nor is it easily used to prepare these analogs from mononucleotide synthons which are superior for automation.[5] In order to achieve this versatility, we have recently been focusing our attention on a pathway analogous to the phosphite triester method for synthesizing DNA whereby S-alkylphosphorothioamidites are used as mononucleotide synthons. Here we report the preparation and application of these compounds to the synthesis of dinucleotides containing phosphorodithioate internucleotide linkages.

The deoxynucleoside phosphorothioamidites (**3**) are obtained selectively through the condensation of S-(4-chlorobenzyl)-N,N,N',N'-tetraisopropylphosphorothiodiamidite[6] (**2**) (470 mg, 1.2 mmol) with 5'-O-dimethoxytrityl and base protected deoxynucleosides (1 mmol each) in anhydrous acetonitrile (10 ml) using tetrazole (168 mg, 2.4 mmol) as catalyst. Reaction mixtures are stirred for 8 h at r.t., worked up by a standard procedure,[8] and fractionated by flash column chromatography (B_1 = T, C^{Bz}, A^{Bz}, ethylacetate:chloroform:triethylamine, 45:45:10, v/v/v; B_1 = G^{Ib}, ethylacetate:chloroform:triethylamine:acetonitrile, 41:41:9:9, v/v/v/v) to yield homogeneous, amorphous powders after precipitation from pentane (55-65%).[9-12] A key decision was selection of the 4-chlorobenzyl protecting group as it reflects a compromise between reasonable reactivity of the phosphorothioamidite and facil deprotection at the end of a deoxyoligonucleotide synthesis ($t_{1/2}$ = 16 min in dioxane:triethylamine:thiophenol, 2:1:1, v/v/v).

The next step leading to **5**, a dinucleoside phosphorodithioate, is condensation of **3** with a 3'-protected deoxynucleoside in the presence of pyridinium tetrafluoroborate[13] to yield the dinucleoside thiophosphite (**4**). Thus in a typical reaction, 3'-O-acetylthymidine (142 mg, 0.5 mmol) is allowed to react with **3** (B_1 = T, 833 mg, 1 mmol) in the presence of pyridinium tetrafluoroborate (334 mg, 2 mmol) in dry acetonitrile (5 ml). After ten minutes the reaction mixture is quenched by addition of 20 atomic equivalents of sulfur (640 mg) in pyridine (2 ml), concentrated *in vacuo* to a gum, redissolved in ethylacetate (50 ml), and the excess sulfur



Synthesis of Dinucleotide Phosphorodithioates. (i) tetrazole; (ii) 3'-O- and base protected deoxynucleoside + pyridinium tetrafluoroborate; (iii) sulfur; (iv) *t*-butylamine:methanol when R_2 is acetyl, imidazole when R_2 is 4-chlorophenylcarbonyl; (v) N,N,N',N'-tetraisopropyl-β-cyanoethylphosphorodiamidite + tetrazole. Abbreviations: DMT, 4,4'-dimethoxytrityl; iPr, isopropyl; B_1 and B_2, thymine (T), N4-benzoylcytosine (C^{Bz}), N6-benzoyladenine (A^{Bz}), and N2-isobutyryl-guanine (G^{Ib}); R_1, 4-chlorobenzyl; R_2, acetyl or 4-chlorophenylcarbonyl; R_3, β-cyanoethyl.

removed by filtration. Following a standard aqueous work-up[8] and flash column chromatography (CH_3CCl_3:CH_3OH, 95:5, v/v), 5 was isolated by precipitation into pentane (60% yield).[14] Other deoxydinucleoside phosphorodithioates (5, B_1 = T, B_2 = G^{Ib}; 5, B_1 = C^{Bz}, B_2 = A^{Bz})[15,16] in comparable yields have been synthesized analogously.

During our work with phosphorothioamidites, we have observed that manipulation of these compounds, in contradiction to handling of normal O-alkylphosphoramidites,[5] should be done strictly under an inert atmosphere. Handling in air leads to the formation of various amounts of the corresponding oxides.[17] Also compounds tentatively assigned as the 4-chlorobenzylphos-

phonothioamidates are formed when phosphorothioamidites are reacted with acidic catalysts.[18] These reactions, however, do not necessarily interfere with coupling as complete conversion of the 3'-protected deoxynucleoside to **4** can be achieved by using an excess of **3** and high concentrations of both deoxynucleoside derivatives. Preliminary investigations have also revealed that the resulting thiophosphite triesters (^{31}P NMR (acetonitrile): $B_1 = B_2 = T$, δ 191.7; $B_1 = C^{Bz}$, $B_2 = A^{Bz}$, δ 191.4) are stable toward nonnucleophilic base and undergo rapid acid catalyzed hydrolysis to hydrogen phosphonates. They are susceptible to rapid oxidation by air or *t*-butylhydroperoxide to yield phosphorothioates and by sulfur to the phosphorodithioate triester. Further investigations of the properties and reactivity of thiophosphite triesters are currently in progress.

The dinucleoside phosphorodithioates (**5**) can be further converted to synthons useful for DNA synthesis by a two step process involving first removal of R_2 to yield **6** and then conversion to the dinucleotide 3'-phosphoramidite (**7**). For thymidine containing dinucleotides ($B_1 = B_2 = T$), the acetyl group is satisfactory for 3'-protection as it can be removed using *t*-butylamine in methanol.[4] However, this procedure cannot be extended to other bases as partial deprotection of the exocyclic amines, especially of cytosine containing dimers, is observed. As a consequence, the 4-chlorophenoxycarbonyl group has been tested and found to be completely satisfactory. It is stable to reagents used for detritylation and internucleotide bond formation but is readily removed with imidazole (1 M in acetonitrile:water, 99:1, v/v, 5 h). Thus synthesis of **7** from **5** proceeds by first removing R_2 with either *t*-butylamine in methanol or imidazole in aqueous acetonitrile. The dinucleotide (**6**) is recovered following aqueous extraction and precipitation into pentane. Synthesis of **7** then involves condensing **6** (80 mg, 0.08 mmol) with bis-(diisopropylamino)-2-cyanoethoxyphosphine (30 mg, 0.1 mmol) in dry acetonitrile (10 ml) using tetrazole (6 mg, 0.08 mmol) as catalyst (2 h at r.t.).[19] These dimers are now being used in combination with unmodified deoxynucleoside 3'-phosphoramidites in order to synthesize various DNA fragments.

Generally, this synthetic route whereby internucleotide dithioate linkages can be prepared from mononucleotide synthons is found to be more attractive for introducing phosphorodithioate linkages into DNA than the pathway described previously.[4] This is because the phosphorothioamidites, in contrast to most diamidites, are reasonably stable to work up conditions and can be isolated, stored and used whenever necessary. Chemically the pathway is also more desirable as the intermediate thiophosphite triesters are readily oxidized to the phosphorodithioates in a protected form which makes further synthesis possible with a minimum of manipulations following each condensation.[20] We therefore anticipate that a pathway as outlined here will be readily adaptable to polymer supported synthesis of DNA containing internucleotide phosphorodithioate linkages.

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6. Synthesis of S-(4-chlorobenzyl)-N,N,N',N'-tetraisopropylphosphorothiodiamidite. 4-Chlorobenzylmercaptan (7.93 g, 50 mmol) and NaH (2.4 g of a 50% NaH suspension in oil, 50 mmol) were mixed into dry ethyl ether (300 ml) and stirred for 4 h at room temperature until evolution of H_2 ceased. N,N,N',N'-Tetraisopropylaminochlorophosphine (13.34 g, 50 mmol)[7] was added and the reaction mixture stirred for an additional 2 h. After removal of the resulting salt by filtration, the clear solution was concentrated *in vacuo* to yield a white, amorphous solid which was recrystallized from acetonitrile (70%). ^{31}P NMR ($CDCl_3$) δ 93.45; ^{1}H NMR δ 7.35 (aromatic), 3.4-3.9 (CH_2 and H of iPr), 1.22 (CH_3 of iPr); FAB^+ mass spectrum, 389 (M^+); FAB^- mass spectrum 387 (M - 1).
7. Houben Weyl Methoden der Organischen Chemie Bd. E1, Organische Phosphorverbindungen, H. G. Theime Verlag, Stuttgart, 1982, pg. 389.
8. Reaction mixtures are diluted with deacidified ethylacetate (50 ml) and extracted with sodium bicarbonate (2 x) and brine. The organic layer is dried over magnesium sulfate, filtered, and concentrated to a glass *in vacuo*. Products are purified by column chromatography on 85 g of silica 60 (0.06-0.063 mm/230-400 mesh ASTM Machery Nagel).
9. 5'-O-Dimethoxytritylthymidine 3'-O-(S-4-chlorobenzyl)diisopropylaminophosphorothioamidite (**3**). FAB^+ mass spectrum, 830 (M - 2)$^-$; ^{31}P NMR ($CDCl_3$) δ 161.3, 159.97.
10. 5'-O-Dimethoxytrityl-N4-benzoyldeoxycytidine 3'-O-(S-4-chlorobenzyl)diisopropylaminophosphorothioamidite (**3**). FAB^+ mass spectrum, 922 $(M-H)^+$; ^{31}P NMR ($CDCl_3$) δ 164.7, 163.7.
11. 5'-O-Dimethoxytrityl-N6-benzoyldeoxyadenosine 3'-O-(S-4-chlorobenzyl)diisopropylaminophosphorothioamidite (**3**). FAB^+ mass spectrum, 944 $(M + H)^+$; ^{31}P NMR ($CDCl_3$) δ 162.3, 161.5.
12. 5'-O-Dimethoxytrityl-N2-isobutyryldeoxyguanosine 3'-O-(S-4-chlorobenzyl)diisopropylaminophosphorothioamidite (**3**). FAB^- mass spectrum, 926 $(M - H)^-$; ^{31}P NMR ($CDCl_3$) δ 162.6, 160.9.
13. Anhydrous pyridinium tetrafluoroborate. A solution of dry pyridine (791 mg, 10 mmol) in dry ethylether (50 ml) was prepared and HBF_4 (10 mmol, 1.9 g of a diethyletherate, Aldrich) in dry dichloromethane (5 ml) was added with stirring. After 2 h the salt was removed by filtration, washed with dry ether, and dried in a dessicator over P_2O_5 (92%).
14. 5'-O-Dimethoxytritylthymidine S-(4-chlorobenzyl)-3'-O-(5'-O-thymidylyl)-phosphorodithioate. FAB^+ mass spectrum, 1005 (M^+), 847 (M - 4-chlorobenzylmercaptyl), 703 (M - DMT + H)$^+$, 455 (M - DMT-4-chlorobenzylmercaptyl + H)$^+$; FAB^- mass spectrum, 879 (M - 4-chlorobenzyl)$^-$, 779 (M - 5'-thymidylyl)$^-$, 477 (thymidine-S-4-chlorobenzylphosphorodithioate), 355 (thymidine 5'-phosphorodithioate); ^{31}P NMR ($CDCl_3$) δ 96.44 UV (EtOH) λmax 228, 268 nm.
15. 5'-O-Dimethoxytritylthymidine S-(4-chlorobenzyl)-3'-O-(5'-O-N2-isobutyryldeoxyguanosinyl)-phosphorodithioate. FAB^+ mass spectrum, 1277 (M - Na)$^+$, 952 (M - DMTr)$^+$; ^{31}P-NMR ($CDCl_3$) δ 95.8, 96.14; UV (EtOH) λmax 262 nm.
16. 5'-O-Dimethoxytrityl-N6-benzoyldeoxyadenosine S-(4-chlorobenzyl)-3'-O-(5'-O-N4-benzoyldeoxycytidine)-phosphorodithioate. ^{31}P NMR ($CDCl_3$) δ 93.89, 93.31.
17. Oxidized products can be observed with all four amidites. ^{31}P NMR ($CDCl_3$) δ 32.3 (B_1 = T), 31.4 (B_1 = C^{Bz}), 31.3 (B_1 = A^{Bz}), 32.3 (B_1 = G^{Ib}). Similar products are obtained when phosphorothioamidites are oxidized with *t*-butylhydroperoxide.
18. A complex decomposition of the phosphorothioamidites is observed with time upon treatment with acids in the absence of nucleoside nucleophiles. Further studies are in progress to reveal these mechanisms.
19. 5'-O-Dimethoxytritylthymidylyl S-(4-chlorobenzyl)-3'-O-(5'-O-thymidylyl-3'-O[N,N-diisopropylamino-β-cyanoethoxyphosphinyl])-phosphorodithioate. ^{31}P NMR ($CDCl_3$) δ 148.9 (amidite), 97.15, 95.7 (dithioate); UV (EtOH) λmax 229, 268 nm.
20. Recently we have observed that dinucleoside phosphoramidites react selectively with mercaptans to form thiophosphite triesters which similarly can be oxidized with sulfur to yield the corresponding dinucleotide phosphorodithioate.

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