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DEOXYNUCLEOSIDE PHOSPHORODITHIOATES. PREPARATION BY A TRIESTER METHOD

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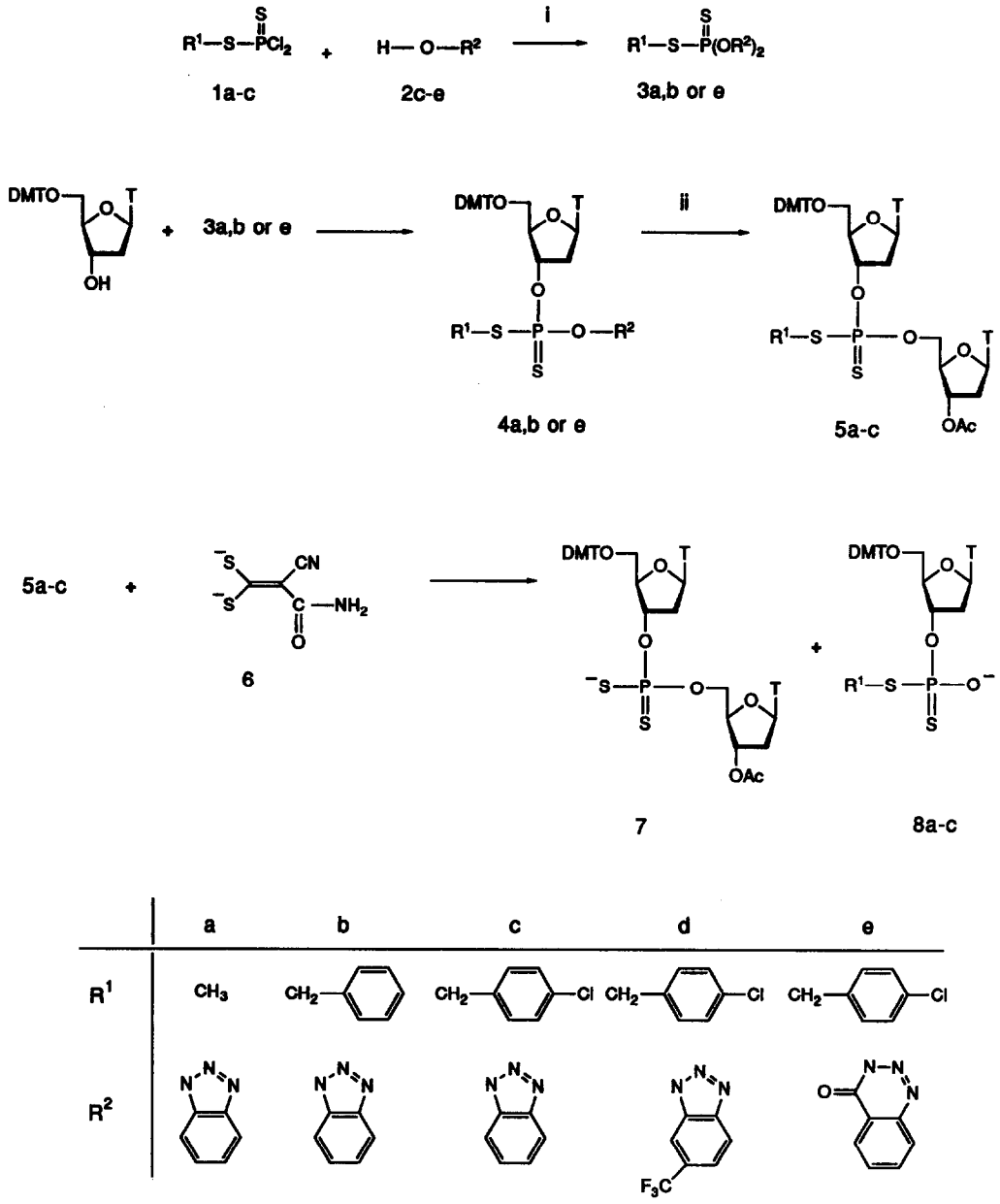
A series of active esters of dithiophosphoric acid (3) have been prepared and their properties as potential precursors for dideoxynucleoside phosphorodithioates evaluated.

Modified oligodeoxynucleotides have recently received much attention due to their therapeutic possibilities.[1,2,3] Among the more promising are deoxynucleoside phosphorodithioates where both nonbridging oxygen atoms in the phosphate diesters are substituted with sulphur. Deoxynucleoside phosphorodithioate dimers have recently been prepared by several groups, using phosphorodiamidite,[4] phosphoramidite[5-7] or H-phosphonate methods.[8-10] Reports have also appeared on the preparation of oligodeoxynucleoside phosphorodithioates from thiophosphoramidites[11,12] and of oligodeoxynucleotides with alternating phosphordiester and dithiophosphordiester linkages,[13] as well as one recently on ribonucleoside dimers.[14]

Of the preparative methods mentioned above, the one using nucleoside thiophosphoramidites has been the most successful so far, but an inherent weakness of this method is the reactivity of the thiophosphoramidites and the thiophosphites which are the primary products. Thus thiophosphoramidites, which are reactive enough to couple efficiently in a few minutes, cannot be purified on silica columns, and have a tendency to dismutate in the presence of an acidic catalyst such as tetrazole.[7] The thiophosphite primary product is likewise unstable in the presence of tetrazole.[7] Thio- and dithio-H-phosphonate monomers are stable but the primary coupling products are unstable in the presence of the catalysts, pivaloyl chloride[8] or iodine.[10]

In this paper we report the preparation of deoxynucleoside phosphorodithioate dimers by a triester method, where no unstable tervalent thiophosphorus intermediates are involved. The strategy used, a variation of van Booms HOBT method,[15] is outlined in the Scheme.

The S-alkyl dithiophosphorodichloridates (**1a,b**) are known compounds.[16] Treatment with 2 equivalents of 1-hydroxybenzotriazole (**2c**) gave the O,O-bis(benzotriazol-1-yl) S-alkyl dithiophosphortriesters (**3a,b**).[17] These gave the protected dinucleoside dithiophosphortriesters (**5a,b**)[18] with protected nucleosides. However, S-dealkylation of the protected dimers (**5a,b**) with 2-carbamoyl-2-cyanoethylene-1,1-dithiolate (**6**)[19] in N,N-dimethylformamide (DMF) gave, besides **7**, significant cleavage of the dimers.[20] This was also the case when thiophenol was used.[20] We therefore decided to use a more labile alkyl group, 4-chlorobenzyl[6,13], for the S-protection.



Scheme: Strategy to make dinucleoside phosphorodithioates by a triester method, i) Pyridine and ii) 3′-acetylthymidine.

When S-4-chlorobenzyl dithiophosphorodichloridate (1c)[22] was treated with 2 equivalents of 2c in the same way as described for 3a,b,[17] S-dealkylation occurred rapidly and only triethylammonium O,O-bis(benzotriazole-1-yl) phosphorodithioate could be isolated.[23] When we used 1-hydroxy-6-trifluoromethyl-benzotriazole (2d) instead of 2c an analogous dealkylated product was formed. The less nucleophilic 3,4-dihydro-3-hydroxy-4-oxobenzotriazin (2e), however, with 1c gave O,O-bis(3,4-dihydro-4-oxobenzotriazin-3-yl) S-4-chlorobenzyl dithiophosphortriester (3e) without significant S-dealkylation.[24] This reagent was used successfully to obtain 5c as described below.

In a typical experiment 2e (680 mg, 4.2 mmol) in anhydrous dioxane (8 ml) and pyridine (0.55 ml) was mixed with 1c (520 mg, 2.0 mmol) in dioxane (2 ml). After 45 min. at r.t. the reaction to 3e was complete (^{31}P n.m.r.),[24] and 5′-dimethoxytritylthymidine (1.00 g, 1.8 mmol) was added. The formation of 4e was complete after 20 min. at r.t. (^{31}P n.m.r.),[25] at which time 3′-acetylthymidine (250 mg, 2.5 mmol) in pyridine (2 ml) was added. After 45 min. at r.t. the formation of 5c was complete (^{31}P n.m.r.), and the reaction mixture was evaporated to dryness, the residue dissolved in dichloromethane (50 ml), washed with 5% aq. sodium hydrogen carbonate (3x50 ml), dried (magnesium sulphate), and evaporated to dryness. The crude product was purified by silica gel chromatography (eluent chloroform/pyridine, 99/1 v/v containing 1-10% v/v of methanol) and the fractions containing 5c (R_f 0.31 in chloroform/methanol/pyridine 90/9/1 v/v) combined and evaporated to dryness. Precipitation in petroleum ether from ethylacetate/triethylamine (99.5/0.5 v/v) gave pure 5c (1.35 g, 79%).[26]

Depotection of 5c with an excess of 6 (1.7 M in DMF, ca. 26°C, $t_{1/2}$ ca. 3 min) gave 7 (δp 115.2) accompanied by only minor cleavage of the dimer to 8c (δp 69.7+68.8, 4% according to ^{31}P n.m.r. after 30 min). Alternatively, thiophenol/triethylamine/dioxane (1:1:2 v/v, ca. 26°C, $t_{1/2}$ ca. 25 min) gave 7 together with 3% cleavage (^{31}P n.m.r. after 5h). The completely deblocked dimer phosphorodithioate was obtained by standard treatment with aq. ammonia followed by 80% aq. acetic acid and was identical (^{1}H and ^{31}P n.m.r.) to the compound described earlier.[7]

In conclusion we have shown that protected dinucleoside phosphorodithioates can be obtained in good yields by a phosphortriester method using the phosphorylating agent 3e, thus circumventing tervalent thiophosphorus reagents which are prone to decompositions. The method seems useful for preparation of large amounts of shorter oligonucleoside phosphorodithioates by solution chemistry, but a better S-protecting group than 4-chlorobenzyl is probably needed before the method can be used for polymer supported synthesis of longer oligomers.

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17) Preparation of **3a,b**: **1a** or **1b** (1 mmol) in dioxane (1 ml) was mixed with **2c** (2.1 mmol) in dioxane (2 ml) and anhydrous pyridine (2.2 mmol). After 1 h the reaction mixture was filtered, and the filtrate evaporated to an oil, which was dissolved in dry acetonitrile. This solution, which was stable for at least a week at -20°C, was used for reactions with 5′-dimethoxytritylthymidine. $\delta_P(CH_3CN)$: 116.1 ppm (**3a**), 90% pure according to ^{31}P n.m.r.: 113.7 ppm (**3b**), 70% pure.
18) Preparation of protected dinucleoside dithiophosphortriesters (**5a,b**): To 5′-dimethoxytritylthymidine was added O,O-bis(benzotriazol-1-yl) S-alkyl dithiophosphortriester (**3a,b**) (1.05 eq) in acetonitrile. When the reaction was finished (TLC/^{31}P n.m.r.), 3′-acetylthymidine (1.5 eq) in pyridine was added, and the reaction mixture was stirred for approximately 16 h at r.t. and then evaporated to dryness. The product was purified on a silica column (eluated with chloroform/pyridine (99/1, v/v) containing 1,2...10% methanol) followed by precipitation in petroleumether from dichloromethane. **5a**: Yield 76%, $\delta_P(CDCl_3)$; 93.5+93.3 ppm. $\delta_H(CDCl_3)$; 9.6 (s, 2x1H, NH), 7.6-7.2 and 6.9-6.8 (15H, arom. and 2xH-6), 6.6-6.3 (2H, H-1′), 5.7-5.2 (2H, H-3′), 4.5-4.1 (4H, H-4′ and H-5′), 3.8 (s, 6H, OCH_3), 3.5 (2H, H-5′), 2.9-2.2 (7H, H-2′ and CH_3), 2.1 (s, 3H, $C(O)CH_3$), 2.0 (s, 3H, CH_3-5) and 1.5 (s, 3H, CH_3-5). **5b**: Yield 72%, $\delta_P(CDCl_3)$; 98.0+96.4 ppm, $\delta_H(CDCl_3)$; 9.7-9.1 (2xH, NH), 7.7-7.1 and 6.9-6.7 (20H, 2xH-6 and arom.), 6.6-6.3 (2H, H-1′), 5.5-5.2 (2H, H-1′), 5.2-5.0 (1H, H-3′), 4.7-4.5 (1H, H-3′), 4.4-3.9 (4H, H-5′and SCH_2), 3.7 (s, 6H, OCH_3), 3.6-3.3 (2H, H-5′), 2.6-2.0 (4H, H-2′), 2.1 (s, 3H, $C(O)CH_3$), 1.9 (s, 3H, CH_3-5) and 1.5 (s, 3H, CH_3-5).
19) B. H. Dahl, K. Bjergårde, L. Henriksen and O. Dahl, Acta Chem. Scand., 44 (1989), in press.
20) The dealkylations were followed by ^{31}P n.m.r. With an excess of **6** (1.7 M in DMF, ca. 26°C), the dimer disappeared with a $t_{½}$ of 40 min (**5a**) or 8 min (**5b**). With thiophenol/triethylamine/dioxane (1:1:2 v/v) **5a** disappeared with $t_{½}$ of 2h. In all cases the product **7** (δ_P 115.1 ppm in DMF) was formed together with a byproduct, identified as **8a** or **8b**.[21] The final amount of **8a** was 35% using **6** and 45% using thiophenol; 20% of **8b** was formed using **6**.
21) The byproduct from **5a** had δ_P 71.8+71.2 ppm in the DMF reaction mixture. Authentic **8a** was prepared in the same way as **5a**,[18] using water instead of 3′-acetylthymidine. The product was purified by extraction of a dichloromethane solution with 5% aq. sodium hydrogencarbonate and precipitation in pretroleumether. Yield 93%, δ_P(DMF) 69.0+68.4 ppm. Addition of this compound to the dealkylation reaction mixture intensified the byproduct signals at 71.8+71.2 ppm. Similar results were obtained for **8b** (δ_P 71.1+69.9 ppm in the DMF reaction mixture).
22) S-4-Chlorobenzyl dithiophosphorodichloridate (**1c**) was prepared in an analogous way to **1b**,[16] but purified by recrystallization from hexane at -20°C instead of distillation. Yield 88%, $\delta_P(CDCl_3)$ 67.8 ppm, 96% purity according to ^{31}P n.m.r.
23) When **1c** was treated with **2c** as described[17] the main product (δ_P 132.5 ppm, 94%) could be purified on a silica gel column (eluted with chloroform/methanol/triethylamine 45:45:10 v/v) to give pure triethylammonium O,O-bis(benzotriazol-1-yl) phosphorodithioate. $\delta_P(CDCl_3)$ 132.5; $\delta_H(CDCl_3)$ 8.7 (s, 1H, NH), 8.0 (d, J=7.9 Hz, 2H) and 7.4 (m, J=7.9 Hz, 6H, arom.), 3.2 (q, J=7.3 Hz, 6H, CH_2) and 1.2 (t, J=7.3 Hz, 9H, CH_3). $\delta_C(CDCl_3)$ 142.2, 128.4, 127.3, 124.2, 118.6 and 110.6 (arom.), 46.2 (CH_2) and 8.3 (CH_3).
24) **3c**: δ_P(dioxane) 112.5 ppm, 97% pure according to ^{31}P n.m.r., A 3% impurity (δ_P 131.7) is presumably the S-dealkylated product; the amount of this increased to 11% after one week at -20°C.
25) **4c**: δ_P(dioxane) 106.0+104.7 ppm, 97% pure according to ^{31}P n.m.r.
26) **5c**: $\delta_P(CDCl_3)$ 97.6+96.1 ppm, (Litt.[6] $\delta_P(CDCl_3)$ 96.44 ppm) more than 99% pure according to ^{31}P n.m.r., $\delta_H(CDCl_3)$; 9.6-8.5 (2H, NH), 7.6-7.1 and 7.0-6.7 (19H, 2xH-6 and arom.), 6.5-6.1 (2H, H-1′), 5.6-5.2 (2H, H-1′), 5.1-4.9 (1H, H-3′), 4.6-4.5 (1H, H-3′), 4.3-3.8 (4H, H-5′ and SCH_2), 3.8 (s, 6H, OCH_3), 3.6-3.3 (2H, H-5′), 2.7-2.0 (7H, H-2′ and $C(O)CH_3$), 1.9 (s, 3H, CH_3-5) and 1.5 (s, 3H, CH_3-5).

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