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# OXIDATIVE AND NONOXIDATIVE FORMATION OF INTERNUCLEOTIDE LINKAGES

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Activation of nucleoside 3′-hydrogenphosphonodithioates with iodine in the presence of nucleosides and amines yields dinucleoside phosphorodithioates and nucleoside phosphorodithioamidates, respectively. Similar activation of nucleoside 3′-methylphosphonothioates yields dinucleoside methylphosphonothioates.

Oligonucleotides bearing phosphate modifications have recently shown potential as antiviral agents[1] and as probes for many biochemical studies.[2] These results have led us[3-6] and more recently others[7,8] to develop methods for synthesizing oligonucleotides with sulfur bonded to phosphorus at the nonbridging valencies. This previously unknown analog having phosphorodithioate internucleotide linkages may be particularly useful for many biochemical, diagnostic, and therapeutic applications because it is achiral and isoelectronic with normal DNA, nuclease resistant,[3,5] and a very potent inhibitor of Avian and Human Immune Deficiency Virus reverse transcriptases.[9] These results have prompted us to continue investigations on the synthesis of phosphorodithioate DNA. Here we report that deoxynucleoside hydrogenphosphonodithioates can be used as synthons for preparing several DNA analogs, including the phosphorodithioate derivative.

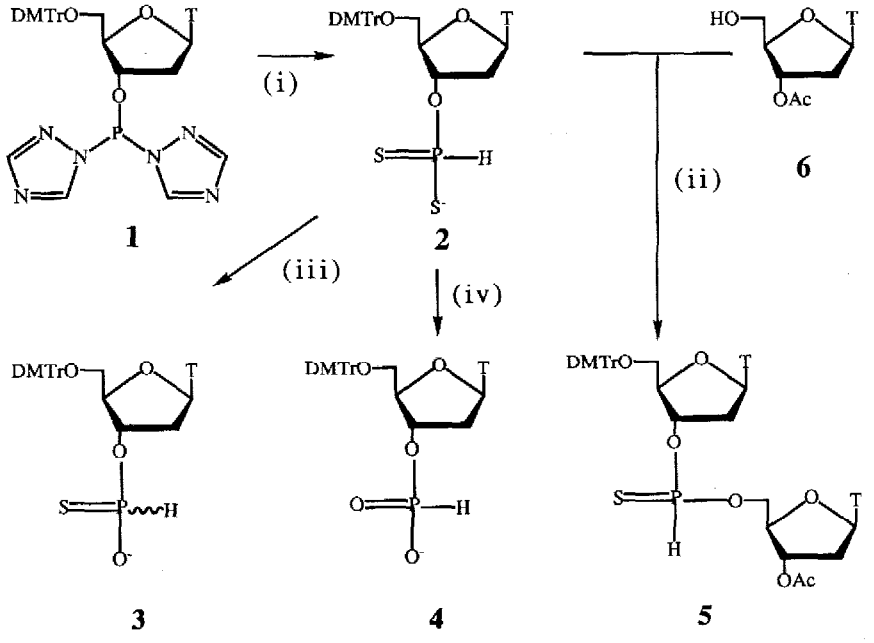


Figure 1. Nonoxidative Condensation Reactions on Phosphorus. (i) $H_2S$ + N-methylmorpholine; (ii) N-methyl-2-chloropyridinium iodide; (iii) N-methyl-2-chloropyridinium iodide + 1 eq. water in pyridine or dicyclohexylcarbodiimide (DCC) + 1 eq water in pyridine; (iv) 1,3-(dimethylaminopropyl)-3-ethylcarbodiimide + excess water. Abbreviations: DMTr, dimethoxytrityl; T, thymine; Ac, acetyl.

5′-O-Dimethoxytritylthymidine 3′-hydrogenphosphonodithioate **2** was prepared by passage of $H_2S$ through a reaction mixture of the 3′-phosphorobistriazolide **1**[10] for five minutes (Figure 1). After purging with argon to remove excess $H_2S$ and concentration to dryness, the product was isolated as the triethylammonium salt by aqueous extraction, silica gel flash column chromatography (ethylacetate:dichloromethane:methanol: triethylamine, 60:30:5:5,v:v:v:v), and precipitation from pentane (65% yield based on protected deoxynucleoside).[11,12]

Deoxynucleoside hydrogenphosphonodithioates were found to undergo desulfurization and other nonoxidative reactions. Thus when **2** was allowed to react with one equivalent each of water and DCC or N-methyl-2-chloropyridinium iodide in pyridine for 30 minutes, the hydrogen phosphonothioate **3** forms in essentially quantitative yield.[13] Exhaustive desulfurization to the hydrogenphosphonate **4**[14] was achieved (30 min) with 4 equivalents of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide in a two phase system (dichloromethane:water).[15] Formation of dithymidine hydrogen phosphonothioate **5**, which can be further converted to dinucleoside phosphorothioates, phosphorodithioates, phosphorothioamidates, and alkylphosphonothioates,[3] was completed by treatment of **2** (0.1 mmol, 74 mg) with 3'-O-acetylthymidine **6** (0.1 mmol, 28 mg) and N-methyl-2-chloropyridinium iodide (0.15 mmol, 38 mg) in pyridine. After 15 min, **5** was isolated in 45% yield by column chromatography on silica gel ($CH_3CCl_3$:$CH_3OH$: $(CH_3CH_2)_3N$, 90:9.5:0.5, v:v:v) and precipitation from n-pentane. Detectable side-products were unreacted starting material as well as hydrolysis products, mainly **3**.

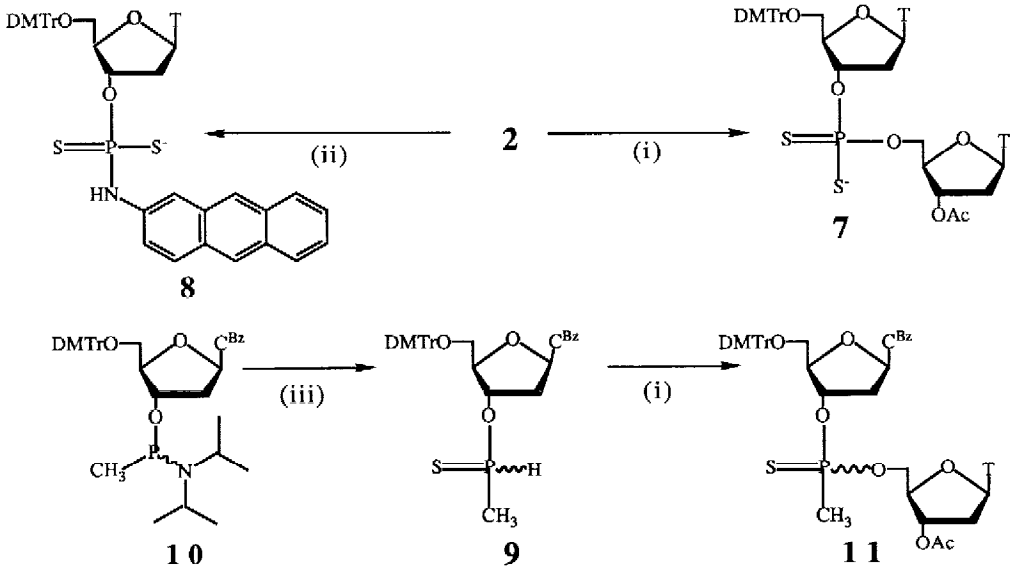


Figure 2. Iodine Mediated Condensation Reactions. (i) 1 eq. iodine in pyridine + 3′-O-acetylthymidine; (ii) 1 eq. iodine + 2-aminoanthracene; (iii) $H_2S$ + 5 eq tetrazole in acetonitrile. Abbreviations: $C^{Bz}$, N4-benzoylcytosine.

Dinucleoside phosphorodithioates were synthesized successfully from **2** by exploiting their oxidizability (Figure 2). When **2** (0.1 mmol, 74 mg) and **6** (0.1 mmol, 28 mg) in pyridine were treated with iodine (0.1 mmol of a 0.1 M solution in pyridine), the dinucleoside phosphorodithioate **7**[3] was the only detectable product.[16] After

addition of $NaHSO_3$ to reduce any possible excess iodine and filtration to remove salts, compound **7** can be isolated as its triethylammonium salt by silica gel flash column chromatography ($CH_3CCl_3$:$CH_3OH$:$(CH_3CH_2)_3N$, 84.5:15:0.5, v:v:v) and precipitation from 250 ml n-pentane (57%, 63 mg). An especially attractive feature of this activation process is that the progress of the reaction can be monitored by decolorization of the iodine solution. This is especially useful as the persistence of a light brown color in the presence of excess iodine indicates when coupling is complete. When excess **2** and iodine are used, an additional $^{31}P$ NMR signal (116.7 ppm in pyridine), which may be due to "overoxidized" compounds,[17] is observed. This signal disappears when the brown iodine solution is decolorized by adding solid $NaHSO_3$ and water. Using a similar approach, compound **8** was synthesized by treating a pyridine solution (0.9 ml) of **2** (0.1 mmol, 74 mg) and 2-aminoanthracene (0.12 mmol, 23 mg) with iodine (0.1 mmol of a 0.1 M pyridine solution). A side product (10% by $^{31}P$ NMR, 105.4 ppm in pyridine) that formed during this coupling reaction could not be transformed to the desired product by treatment with $NaHSO_3$ and water but was removed by silica gel column chromatography ($CH_3CCl_3$: $CH_3OH$:$(CH_3CH_2)_3N$, 85:14.5:0.5, v:v:v:). Compound **8** was isolated in 60% yield (44 mg)[18] by precipitation from pentane.

Extension of the iodine mediated coupling procedure to the synthesis of dinucleoside methylphosphonothioates [19,20] was only moderately successful. Thus, when **9** (0.1 mmol, 71 mg) [21,22] and **6** (0.1 mmol, 28 mg) were treated with one equivalent iodine, the reaction mixture contained **11**, the desired product ($^{31}P$ NMR (pyridine) δ 98.1 and 97.2), and three side products ($^{31}P$ NMR (pyridine) δ 86.7, 86.1, and 85.2).[23] Purification of crude **11** was done by silica gel column chromatography. Side products were first removed as one fraction with $CH_3CCl_3$: $CH_3OH$ (9:1, v:v). Compound **11** was then eluted using $CH_3CCl_3$:$CH_3OH$ (4:1,v:v) and isolated in 47% yield (47 mg) by precipitation from n-pentane.[24]

These results outline a method whereby deoxynucleoside hydrogenphosphonodithioates can be used as synthons for preparing phosphorodithioate and H-phosphonothioate linked DNA as well as nucleoside phosphorodithioamidates. The synthons are easily prepared via readily scaleable procedures and appear to be stable toward normal laboratory conditions. A particularly useful feature as well is the observation that activation with iodine directly yields the dinucleoside phosphorodithioate as the only detectable product. This procedure is also scaleable and should be compatible with nonpolymer support methods for synthesizing deoxyoligonucleotides in the large quantities needed for various therapeutic testing programs. Finally, the method appears to be relatively free of reactions that lead to significant quantities of phosphorothioates as side products. Presumably this is because contaminating oxygen does not interfere with the activation process and elemental sulfur, which is difficult to solubilize and is a rather poor oxidant of P(III) compounds, is not part of the sulfurization reactions.

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10. B. C. Froehler and M. D. Matteucci, *Tetrahedron Lett.* **27**, 469 (1986).
11. $^{1}H$ and $^{31}P$ NMR were recorded in deuterated chloroform unless otherwise specified with tetramethylsilane and 85% $H_3PO_4$ as reference standards, respectively. The matrix for FAB MS was magic bullet (dithiothreitol and dithioerythritol) unless otherwise stated.
12. Compound **2**. FAB+ mass spectrum, 832 (M + matrix + K), 655 (M + O -1), 368 (M - DMTr + S); FAB- mass spectrum (thioglycerol), 639 (M - 1), 671 (M - 1 + S), 745 (M - 1 + matrix); $^{31}P$ NMR $\delta$ 83.5. $^{1}H$ NMR $\delta$ 8.59 (d, $J_{HP}$ = 591 Hz, P-H), 8.37 (broad s, NH), 7.56 (s, $H_6$ of thymine), 7.16-7.40 (m, 9, phenyl, C2-H and C6-H of anisyl) 6.81 (2d, $J_{HH}$ = 8.9 Hz, 4, C3-H and C5-H of anisyl), 6.44 (m, 1, $H_{1'}$), 5.35 (m, 1, $H_{3'}$), 4.35 (m, 1, $H_{4'}$), 3.76 (s, 6, $CH_3$ of anisyl), 3.36-3.50 (m, 2, $H_{5'}$), 3.19 (q, $J_{HH}$ = 7.3 Hz, 6, $CH_2$ of triethylamine), 1.32-1.35 (m, t($J_{HH}$ = 7.3 Hz, 9, $CH_3$ of triethylamine), 3, $CH_3$ of thymine).
13. Compound **3**. $^{31}P$ NMR $\delta$ 52.7 and 52.2, $J_{PH}$ = 577 Hz (2 diastereomers).
14. Compound **4**. $^{31}P$ NMR $\delta$ 2.7, $J_{HP}$= 623.77 Hz, $J_{HH}$ = 8.5 Hz.
15. One mg Aliquat 336 was added to reduce emulsification (Aldrich).
16. Compound **7**. $^{31}P$ NMR (pyridine) $\delta$ 115.5; $^{31}P$ NMR ($CDCl_3$) $\delta$ 113.0.
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18. Compound **8**. FAB+ mass spectrum, 736 (M + 2H), 455 (DMTr + matrix); FAB- mass spectrum, 1135 (M + 2 x matrix), 829 (M - 1), 527 (M - DMTr), 458 (DMTr + matrix); $^{31}P$ NMR $\delta$ 95.5. $^{1}H$ NMR $\delta$ 8.52 (broad s, NH), 8.22, 8.14 and 7.83-7.9 (m, 5, anthracene), 7.57 (s, 1, $H_6$ of thymine), 7.11-7.14 (m, 13, phenyl, C2-H and C6-H of anisyl, 4H of anthracene), 6.69 (2d, $J_{HH}$ = 9 Hz,4, C3-H and C5-H of anisyl), 6.48 (m, 1, $H_{1'}$), 5.69 (d, $J_{HP}$ = 11 Hz, 1, NH of 2-anthracene amide), 5.33-5.42 (m, 1, $H_{3'}$), 4.32 (m, 1, $H_{4'}$),3.72-3.77 (m, 2, $H_{5'}$), 3.69 (d, 6, $CH_3$ of anisyl), 3.18 (q, $J_{HH}$ = 7.3 Hz, 8, $CH_2$ of triethylammonium), 2.73-2.81 (m, 1, $H_{2'}$), 2.25-2.34 (m, 1, $H_{2'}$), 1.40 and 1.39 (2s, 3, $CH_3$ of thymine), 1.34 (t, 18, $CH_3$ of triethylammonium); UV (ethanol) $\lambda_{max}$ (nm) 240 (I = 1), 258 (shoulder, I = 1.26), 264 (I = 1.43).
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21. Compound **9** was synthesized by sulfhydrolysis of **10**[19] using $H_2S$ in the presence of tetrazole (5 eq.) in dry acetonitrile. The product was isolated in 94% yield (335 mg) from an 0.5 mmol preparation after aqueous work-up and precipitation into n-pentane.
22. Compound **9**. FAB+ mass spectrum, 712 ($M^+$); FAB- mass spectrum, 710 (M - H), 726 (M - H + O), 742 (M - H + S); $^{31}P$ NMR $\delta$ 70.7 and 70.5, $J_{HP}$ = 529 Hz (2 diastereomers); $^{1}H$ NMR $\delta$ 8.85 (broad s, NH), 8.15 and 8.14 (2s, $J_{HH}$ = 7.5 Hz, 1, $H_6$ of cytosine, 2 diastereomers), 7.86 (s, $J_{HH}$ = 8.3 Hz, 2, C2-H and C6-H of benzoyl), 7.85 (m, $J_{HP}$ = 543 Hz) and 7.80 (m, $J_{HP}$ = 520 Hz) (1, P-H), 7.13-7.63 (m, 13, $H_5$ of cytosine, (C3-H, C4-H and C5-H of benzoyl), phenyl, C2-H and C5-H of anisyl), 6.32 (q (apparent), 1, $H_{1'}$), 5.29-5.32 (m, 1, $H_{3'}$), 4.26 and 4.38 (2 m, 1, $H_{4'}$ (2 diastereomers)), 3.76 (2s, 6, $CH_3$ of anisyl), 3.38-3.66 (m, 2, $H_{5'}$), 2.77-2.92 (m, 1, $H_{2'}$), 2.22-2.45 (m, 1, $H_{2'}$), 1.86 (m, 3, P-$CH_3$ (2 diastereomers)).
23. When the reaction was carried out in dichloromethane, essentially no dimer forms. Instead several compounds having $^{31}P$ NMR signals 85.3-93.4 ppm were synthesized.
24. Compound **11**. FAB+ mass spectrum, 1016 (M - 1 + Na), 616 (5′-dimethoxytrityl-O2,3′-anhydro-N4-benzoylcytidine), 455 (DMTr + matrix); FAB- mass spectrum, 991 (M - 3), 376 (3′-O-acetylthymidine-5′-methylphosphonothioate); $^{31}P$ NMR $\delta$ 98.2, 97.4; $^{1}H$ NMR $\delta$ 8.76 (broad s, NH), 8.13 and 8.17 (2d, $J_{HH}$ = 8 Hz, 1, $H_6$ of cytosine (2 diastereomers)), 7.88 (2d, $J_{HH}$ = 8.5 Hz, 2, C2-H and C6-H of benzoyl), 7.46-7.63 (m, 4(C3-H, C4-H, and C5-H of benzoyl), $H_6$ of thymine), 7.18-7.39 (m, 10, $H_5$ of cytosine, phenyl, C2-H and C6-H of anisyl), 6.84 (2d, $J_{HH}$ = 8.8 Hz, 4, C3-H and C5-H of anisyl), 6.22-6.31 (m, 2, $H_{1'}$), 5.41 (m, 1, $H_{3'}$), 5.13 and 5.24 (2m, 1, $H_{3'}$ (2 diastereomers)), 4.06-4.31 (m, 4, $H_{5'}$ and $H_{4'}$), 3.78 and 3.77 (2s, 6, $CH_3$ of anisyl), 3.44 (m, 2, $H_{5'}$), 2.79-2.86 (m, 1, $H_{2'}$), 2.16-2.43 (m, 3, $H_{2'}$), 2.07 and 2.08 (2s, 3, $CH_3$ of acetyl), 1.90 and 1.92 (2s, 3, $CH_3$ of thymine), 1.87 (d, $J_{HP}$ = 15.4 Hz) and 1.82 (d, $J_{HP}$ = 15.2 Hz) (3, P-$CH_3$ (2 diastereomers)).


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