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SYNTHESIS OF DINUCLEOSIDE AND DINUCLEOTIDE PHOSPHORODITHIOATES VIA A PHOSPHOTRIESTER APPROACH

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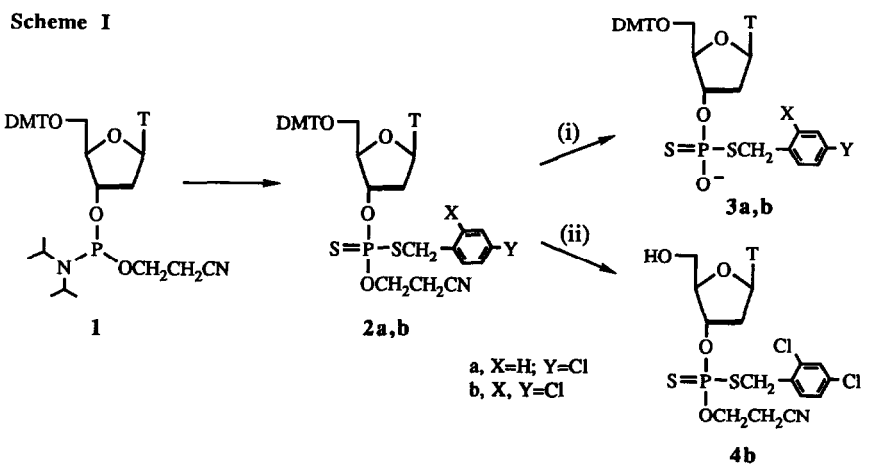
5′-O-Dimethoxytrityl and S-protected thymidine-3′-phosphorodithioate, 3′-O-acetylthymidine or thymidine-3′-O-[β-cyanoethyl-(S-2,4-dichlorobenzyl)]-phosphorodithioate, 1-methylimidazole, and 2,4,6-triisopropylbenzenesulfonylchloride react to give excellent yields of dinucleoside phosphorodithioates.

Oligonucleotides bearing phosphorodithioate internucleotide linkages have recently shown potential as antiviral agents and as reagents useful for a large number of biological and biochemical applications.[1] Although these analogs have been synthesized via several different methods,[2-10] the procedures, which are primarily adaptable to polymer supports, are designed for rapidly synthesizing small quantities of DNA (1 μmole or less). For certain applications such as therapeutic or biophysical (NMR and X-ray crystallography) studies, considerably larger quantities of phosphorodithioate DNA will be needed. Here we report our results on adapting the phosphate triester approach[11-14] toward this objective.

Fully protected thymidine phosphorodithioate triesters (**2a** and **2b**) were prepared from commercially available 5′-O-dimethoxytritylthymidine-3′-[(β-cyanoethyl)-N,N-diisopropyl]-phosphoramidite (**1**) by treatment first with 4-chloro or 2,4-dichlorobenzylmercaptan and tetrazole in CH_3CN for 40 min and then, without isolation, a saturated sulfur solution in toluene:2,6-lutidine (19:1; v:v). The resulting mixture was stirred at r.t. for 1 h and the product isolated by precipitation from pentane. As these reactions were quantitative, further purification by silica gel column chromatography was unnecessary.[15-17]

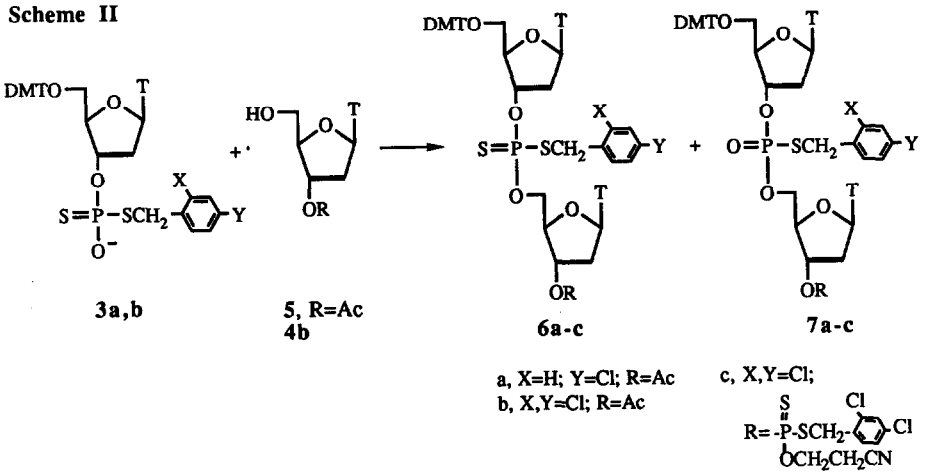
In order to synthesize phosphorodithioate internucleotide linkages from these monomers, the dimethoxytrityl and β-cyanoethyl protecting groups were selectively removed using methods developed for synthesizing unmodified DNA via the phosphotriester approach. The β-cyanoethyl group was eliminated by stirring **2a** and **2b** (0.14 M) with Et_3N:CH_3CN (1:1, v:v) at r.t. for 2-3 h, and the resulting products purified by

Scheme I



Reagents: (i) Et_3N:CH_3CN (1:1, v:v); (ii) 2% *p*-toluenesulfonic acid or 3% trichloroacetic acid.

Scheme II



silica gel column chromatography to yield **3a** and **3b** as their triethylammonium salts[18,19] (72% and 93% overall yields, respectively, from **1**). Acid hydrolysis of the 5′-dimethoxytrityl group from **2b** with either 2% *p*-toluenesulfonic acid in CH_2Cl_2:CH_3OH (75:25; v:v) or 3% trichloroacetic acid in CH_2Cl_2 at 0°C for 10-30 min yields **4b**[20] in 77-80% overall yield from **1**.

A major challenge in this approach was to identify proper conditions for the chemoselective activation of the oxygen atom at the phosphorus center of **3b** to yield **6c**. In order to determine these conditions, **3a** or **3b** was reacted with 3′-O-acetylthymidine (**5**) using various condensing agents in different solvents (Table 1). Mesitylenesulfonyl-3-nitrotriazolide (MSNT, entry 1) gave the desired dinucleoside phosphorodithioate **6a** in 75% yield (^{31}P NMR δ 95.0 and 94.4) and the undesired phosphorothioate **7a** in 25% yield (^{31}P NMR δ 26.9 and 26.7).[21] With diphenyl chlorophosphate (entry 2), the products were 3′-O-acetylthymidine-5′-O-diphenylphosphate (^{31}P NMR δ -12.1) and starting material **3a**. Since **3a** and diphenyl chlorophosphate were observed to initially form an asymmetric pyrophosphate intermediate (^{31}P NMR δ 83.4 and 82.4), the 5′-hydroxyl of **5** must, in the second step, preferentially attack the phosphate center rather than the phosphorodithioate in this pyrophosphate. When triisopropylbenzenesulfonylchloride (TPSCl, 3 eq) and 1-methylimidazole (7.7 eq) were used (entry 3), the coupling reaction of **3a** and **5** was complete within 2 h with a good ratio of **6a**:**7a** (97:3). Similar results were obtained when **3b** (0.18 mmole) replaces **3a** (entry 4). In this reaction, product **6b**,[22] contaminated with only 1% of **7b** (^{31}P NMR), was isolated in 91% yield after silica gel column purification. Mesitylenesulfonylchloride and 1-methylimidazole in pyridine or CH_3CN (entries 5,6) also gave the desired dimer **6b**; however, the ratios of **6b**:**7b** (96:4 and 95:5, respectively) were not as acceptable as with TPSCl. Pivaloylchloride (entry 7) failed to give the desired product. Of these various condensing agents, TPSCl was clearly superior. We then examined different solvents (entries 8-14) by condensing **3b** with **5** (1.2 eq)in the presence of TPSCl (3 eq) and 1-methylimidazole (5 eq). With CH_3CN or pyridine:CH_3CN (1:1, v:v), results were similar to those found with pyridine. In addition to the product peaks (^{31}P NMR δ 95 and 94), a small peak (3-7% intensity relative to product peaks) at 100 ppm was also observed in pyridine, CH_3CN, and pyridine:CH_3CN. The compound corresponding to this peak could be the chlorophosphorodithioate derivative of **3** as the same peak arose as the major ^{31}P NMR signal when **3b**, TPSCl and

Table 1. Synthesis of Dinucleotide Phosphorodithioates.

Entry	Reactants	Condensing agents*	Solvent	Reaction# Time	Products & Ratios† 6	7	Yield (%)
1	**3a**, **5** (1 eq)	MSNT (1.5 eq)	pyridine	1 day	75	25	
2	**3a**, **5** (2.5 eq)	$ClP(O)(OPh)_2$ (2 eq)‡	pyridine	NP	—	—	
3	**3a**, **5** (1.2 eq)	TPSCl	pyridine	2 h	97	3	
4	**3b**, **5** (1.35 eq)	TPSCl (3.4 eq)	pyridine	2 h	98	2	91
5	**3b** (1.2 eq), **5**	MSCl	pyridine	—¶	96	4	
6	**3b** (1.2 eq), **5**	MSCl	CH_3CN	—¶	95	5	
7	**3b**, **5**	pivaloyl chloride (2eq)	pyridine	NP	—	—	
8	**3b**, **5**	TPSCl	—**	1.5 h	98	2	
9	**3b**, **5**	TPSCl	CH_3CN	2 h	98	2	
10	**3b**, **5**	TPSCl	DMF	NP	—	—	
11	**3b**, **5**	TPSCl	dioxane	>1 day	94	4	
12	**3b**, **5**	TPSCl	CH_2Cl_2	1.5 h	99	1	
13	**3b**, **5**	TPSCl	THF◊	1.5 h	99	1	
14	**3b**, **5**	TPSCl♦	THF	2 h	99	1	
15	**3b** (1.15 eq), **4b**	TPSCl	THF	1.5 h	99	1	95

*Unless otherwise noted, the reactions were carried out by adding the condensing agents (3 eq) as one portion to the solutions of 0.07 M of **3** (1 eq) and **5** (1.2 eq) at r.t. under an inert atmosphere. For TPSCl and MSCl, 1-methylimidazole (5-7.7 eq) was added as a nucleophilic catalyst. #Completion was determined by the disappearance of the ^{31}P NMR signals of **3**, pyrophosphate intermediates (^{31}P NMR δ 83 and 82), and methylimidazolide intermediates (^{31}P NMR δ 90); NP - no product. †The ratio of **6**:**7** was determined by the integration ratio of their ^{31}P NMR signals. ‡Compound **3a** was allowed to react with $ClP(O)(OPh)_2$ for 10 min prior to the addition of **5**. ¶The reaction time was not determined; however, it was estimated to be less than 2 h. **Pyridine:CH_3CN (1:1, v:v). ◊Salt precipitation was observed in this solvent. ♦The reaction was carried out initially at 0°C. After the addition of an ice-cold solution of TPSCl in THF and 1-methylimidazole, the reaction was warmed to r.t.

1-methylimidazole were mixed in pyridine. This compound also decomposed during silica gel column chromatography. Certain other solvents such as DMF or dioxane gave either no desired product (DMF) or results less satisfactory than with pyridine (dioxane). The best results were with CH_2Cl_2 or THF as coupling reactions in both solvents were complete within 1.5 h, gave ratios of **6b**:**7b** as high as 99:1, and did not generate a peak at 100 ppm. An attempt to further improve the ratio of **6b**:**7b** by initially lowering the reaction temperature to 0°C during the slow addition of TPSCl in THF (entry 14) did not alter the results. Finally, **3b** (1.15 eq) was allowed to react with **4b** (0.3 mmol) in a manner similar to that described in entry 13. Again, a ratio of 99:1 for **6c**:**7c** was observed and pure **6c** (free of **7c**) was isolated in 95% yield[23] by silica gel filtering-column chromatography[24] (column prewashed with CH_2Cl_2:pyridine, 98:2 and then eluted with CH_2Cl_2:MeOH, 96:4).

This chemistry, in a manner similar to the phosphate triester approach for synthesizing natural DNA, should be adaptable to the synthesis of oligonucleotide dithioates in large quantities. The results show that dinucleotide phosphorodithioate synthons can be produced in high overall yields in a chemoselective manner while using only small excesses of the mononucleotide diester. All these features are quite attractive for large scale synthesis.

These results suggest the following general strategy for research involving dithioate DNA. For the rapid synthesis of a large number of dithioate containing oligonucleotides, the preferred approach will be to use deoxynucleoside 3′-phosphorothioamidites as synthons on polymeric supports.[5] Once a particular sequence has been shown to be interesting for a unique application requiring large quantities of material, then synthesis in solution via a phosphate triester approach will be preferred.

Acknowledgements

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15. ^{1}H and ^{31}P NMR were recorded in $CDCl_3$ unless otherwise specified with tetramethylsilane and 85% H_3PO_4 as reference standards, respectively. The matrix for FAB mass spectra was magic bullet.
16. **2a**. FAB$^+$ mass spectrum, 834 (MH)$^+$; FAB$^-$ mass spectrum 779 (M - CH_2CH_2CN)$^-$; ^{31}P NMR δ 95.3 and 95.0.
17. **2b**. FAB$^-$ mass spectrum, 813 (M - CH_2CH_2CN)$^-$. 708 (M - dichlorobenzyl)$^-$; ^{31}P NMR δ 95.8 and 95.5.
18. **3a**. FAB$^-$ mass spectrum, 779 (M)$^-$; ^{31}P NMR δ 74.6 and 72.6; ^{1}H NMR δ 9.80 (br s, NH), 7.63, 7.62 (2s, H_6), 7.40-6.80 (m, ArH), 6.45 (m, H_1'), 5.30 (m, H_3'), 4.20 (m, H_4'), 3.95 (m, SCH_2), 3.78, 3.76 (2s, OCH_3), 3.29 (m, $H5'$), 2.98 (q, Et_3N), 2.40 (m, H_2'), 1.44-1.20 (m, CH_3, Et_3N).
19. **3b**. FAB$^-$ mass spectrum, 813 (M)$^-$; ^{31}P NMR δ 73.9 and 72.3; ^{1}H NMR δ 7.63, 7.60 (2s, H_6), 7.53-6.81 (m, ArH), 6.46 (m, H_1'), 5.33 (m, H_3'), 4.28-3.90 (m, H_4', SCH_2), 3.78, 3.77 (2s, OCH_3). 3.38 (m, $H5'$), 2.98 (q, Et_3N), 2.42 (m, H_2'), 1.38-1.22 (m, CH_3, Et_3N).
20. **4b**. FAB$^+$ mass spectrum, 566 (MH)$^+$; FAB$^-$ mass spectrum, 511 (m - CH_2CH_2CN)$^-$; ^{31}P NMR δ 95.0 and 94.7; ^{1}H NMR δ 9.76, 9.73 (2 br s, NH), 7.50-7.24 (m, H_6, ArH), 6.19 (m, H_1'), 5.31 (m, H_3'), 4.38-4.09 (m, OCH_2, SCH_2), 3.88 (m, $H5'$), 3.26 (m, H_4'), 2.79 (two sets of t, J = 6 Hz, CH_2CN), 2.47 (m, H_2'), 1.90 (s, CH_3).
21. The assignment of **7a** was based on the reported ^{31}P chemical shift value of 26 ppm for a similar phosphorothioate triester: R. R. Hodges, N. G. Conway, and L. W. McLaughlin, *Biochemistry* **28**, 261 (1989) and references cited therein.
22. **6b**. FAB$^+$ mass spectrum, 1081 (MH)$^+$; FAB$^-$ mass spectrum, 1079 (M - 1)$^-$, 921 (M - dichlorobenzyl)$^-$; ^{31}P NMR δ 97.8 and 96.4. The ^{1}H NMR data was consistent with the results reported previously for the same compound (ref. 2).
23. **6c**. FAB$^-$ mass spectrum, 1307 (M - CH_2CH_2CN)$^-$, 1202 (M - dichlorobenzyl)$^-$. ^{31}P NMR δ 97.9, 96.6, 96.4, 95.8, 95.6, 95.4, and 95.2; ^{31}P NMR (THF, ext. lock) δ 94.6, 94.1, 93.4, and 93.3; ^{1}H NMR δ 9.50, 9.42 (2 br s, NH), 7.60-6.83 (m, H_6, ArH), 6.47, 6.30 (2m, H_1'), 5.46, 5.21 (2m, H_3'), 4.37-4.08 (m, OCH_2, SCH_2, $H4'$, H_5'), 3.79, 3.78 (2s, OCH_3), 3.44 (m, H_5'), 2.74 (m, CH_2CN), 2.42 (m, H_2'), 1.90 (s, CH_3), 1.44, 1.43 (2s, CH_3).
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