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New Solid Phase Synthesis of Oligodeoxythymidine Phosphorodithioates by a Modified HObt-Method

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Abstract: Two new dithiophosphorylating reagents **2a,b** have been prepared and shown to give nucleoside monomers **3a,b** with reactivities suitable for solid phase synthesis. An octamer and a nonamer deoxythymidine phosphorodithioate were prepared on a TentaGel support with good coupling efficiencies. The products after deblocking are free from phosphorothioate contaminations (detection limit 0.5%).

Oligodeoxynucleoside phosphorodithioates, which are of interest as inhibitors of viral gene expression,[1] have been prepared by various methods.[2-4] We have recently published a triester method (the HODhbt-method) for the preparation of phosphorodithioates using solution phase chemistry.[4] It is suitable for large scale synthesis of phosphorodithioates free from phosphorothioate impurities but the coupling rates are too slow to be convenient for solid phase synthesis. Stec *et al.* have published a solid phase triester method involving the use of dithiaphospholane nucleoside derivatives, but this method is only suitable for pyrimidines and produces *ca.* 1% phosphorothioate.[2]

We now wish to report the use of a modified HObt-method (HObt is 1-hydroxybenzotriazole) for solid phase synthesis of oligodeoxythymidine phosphorodithioates. The strategy used is a variation of van Boom's HObt-method.[5] The optimised coupling conditions were developed using solution phase chemistry as outlined in **Figure 1**.

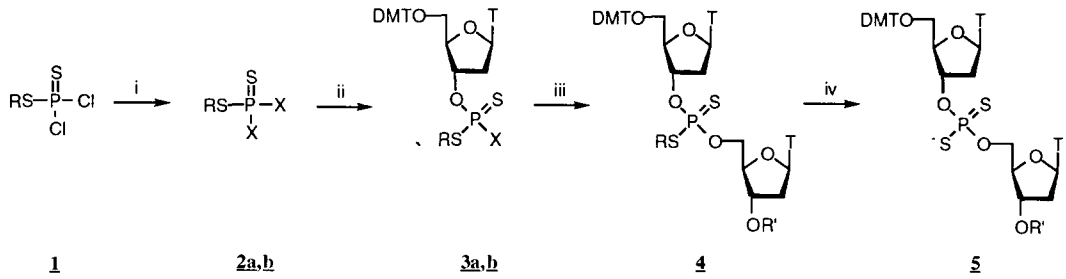

Figure 1: Synthesis of the dinucleoside phosphorodithioate **5** (1 mmol scale). R is 2,4-dichlorobenzyl and R' is phenoxyacetyl. For **2a** and **3a** X is HObt: i) HObt (2.0 mmol) and imidazole (2.0 mmol) in 10 ml diethyl ether, 15 min. at r.t., followed by filtration, evaporation *in vacuo* and redissolvation in acetonitrile (2.5 ml). ii) 5'-*O*-Dimethoxytrityldeoxythymidine (1.0 mmol) and pyridine (4.0 mmol), 5 min. at r.t.. iii) 3'-*O*-Phenoxyacetyl-deoxythymidine[4] (1.4 mmol) and *N*-methylimidazole (10 mmol), 15 min. at r.t.. For **2b** and **3b** X is HOnbt (6-nitro-1-hydroxybenzotriazole[6]): i) HOnbt (2.0 mmol) and imidazole (2.0 mmol) in dioxane (10 ml), 15 min. at r.t., followed by filtration. ii) 5'-*O*-Dimethoxytrityldeoxythymidine (1.0 mmol) and pyridine (4.0 mmol), 15 min. at r.t., followed by evaporation *in vacuo* and redissolvation in acetonitrile (2.5 ml). iii) 3'-*O*-Phenoxyacetyldeoxythymidine (1.4 mmol) and *N*-methylimidazole (10 mmol), 3 min. at r.t.. iv) Thiophenol :pyridine:triethylamine (1:1:1, v/v/v), overnight at r.t..

In order to obtain the *O,O*-bis(benzotriazol-1-yl) *S*-2,4-dichlorobenzyl dithiophosphortriester **2a** or the HOnbt derivative **2b** it is crucial to use a relatively nonpolar solvent such as diethyl ether or dioxane in combination with a base which forms an insoluble hydrochloride.[7] Preparation of **3a** and **3b** were performed under slightly different conditions due to the low solubility of HOnbt in diethyl ether and the higher reactivity of **2b**. **2a** was prepared in diethyl ether, the ethereal solution of **2a** was filtered under nitrogen from the hydrochloride and evaporated *in vacuo*. The activated dithiophosphortriester **2a** was more than 98% pure according to ^{31}P n.m.r: δp (diethyl ether) 111.4 ppm. The activated nucleoside dithiophosphortriester **3a** was prepared by redissolving **2a** in acetonitrile and transferring the solution to 5'-*O*-dimethoxytrityldeoxythymidine and pyridine. After 5 min.[8] the reaction mixture was evaporated *in vacuo*. The activated nucleoside dithiophosphortriester **3a** was 94% pure according to ^{31}P n.m.r: δp (acetonitrile) 103.7+104.0 ppm.[9] **2b** was prepared as **2a** but in dioxane, δp (dioxane) 115.9 ppm. The conversion of **2b** to **3b** was performed in dioxane but otherwise analogous to the preparation of **3a**. The reaction was complete in less than 15 min: δp (dioxane) 105.6 + 105.5 ppm. The resulting foams of **3a** and **3b** could be stored for at least six weeks at -20°C under nitrogen without detectable decomposition. Optimal coupling conditions for the formation of the fully protected dimer **4** were found by solution phase chemistry using the least reactive activated monomer **3a** as reference (**Table 1**). NMI and ethyldiisopropylamine were tried as they have been shown to have a dramatic effect on the rate of coupling in the classical HObt-method [10], 3-nitro-1,2,4-triazole and 5-(2-nitrophenyl)-tetrazole have been shown to be nucleofilic catalysts in the classical phosphortriester method [11], and 1-hydroxy-7-azabenzotriazole have been shown to speed up peptide coupling rates.[12]

Table 1: Coupling conditions and coupling times for the reaction of **3a,b** with 3'-*O*-phenoxyacetyldeoxythymidine. **3a,b** (1.0 mmol) + 3'-*O*-phenoxyacetyldeoxythymidine (1.4 mmol) in acetonitrile (2.5 ml) were mixed with the catalyst, and the progress of the reaction was followed by ^{31}P n.m.r. at 27°C.

Activated monomer	Coupling conditions	Coupling time in min. [a]
3b	NMI (10 eq.)	<3
3a	NMI (50 eq.) [b]	8
3a	NMI (10 eq.) + 1-hydroxy-7-azabenzotriazole (2 eq.)	10
3a	NMI (10 eq.)	15
3a	NMI (10 eq.) + ethyldiisopropylamine (10 eq.)	15
3a	NMI (10 eq.) + 3- nitro-1,2,4-triazole (3 eq.)	30
3a	NMI (10 eq.) + 5-(2-nitrophenyl)-tetrazole (3 eq.)	30
3a	NMI (4 eq.)	36
3a	NMI (10 eq.) + DBU (10 eq.)	- [c]

a) Time for >98% conversion. b) No acetonitrile added. c) Complete decomposition of **3a** occurred in 5 min.

We have examined two of the common solid supports for DNA synthesis, LCAA-CPG and TentaGel, and found that TentaGel gave the highest coupling yields.[13] In a typical coupling, solid **3a** or **3b** (90 μmol) was dissolved in NMI (100 μl) and acetonitrile (310 μl) and injected onto the TentaGel-T support using a polypropylene syringe.[14] The coupling efficiency was monitored by measuring the release of the DMT cation by UV-spectroscopy at 499 nm, and averaged 98.7% for **3a** over seven couplings and 95.1% for **3b** over six couplings. A complete cycle is shown in **Table 2.**

The dithioate dT_8-mer and dT_9-mer were dealkylated at sulphur with thiophenol:triethylamine:pyridine (1:1:1 v/v/v).[15] The oligomers were cleaved from the support with conc. aqueous ammonia for 2 hours at r.t.. The crude oligomers, with DMT-on, was purified using a Hamilton PRP-1 HPLC column and the homogeneity of the products checked by PAGE (see **Figure 2** and **Figure 3**).

Table 2: Cycle used for solid phase synthesis of oligodeoxythymidine phosphorodithioates using **3a** or **3b**.

Steps	Solvents and reagents	Time (min.)
1	3% Trichloroacetic acid in dichloromethane	1
2	Acetonitrile	1
3	Coupling	**3a**: 140 or **3b**: 25
4	Acetonitrile	1
5	Capping (NMI/THF + Ac_2O/THF)	0.5
6	Acetonitrile	1

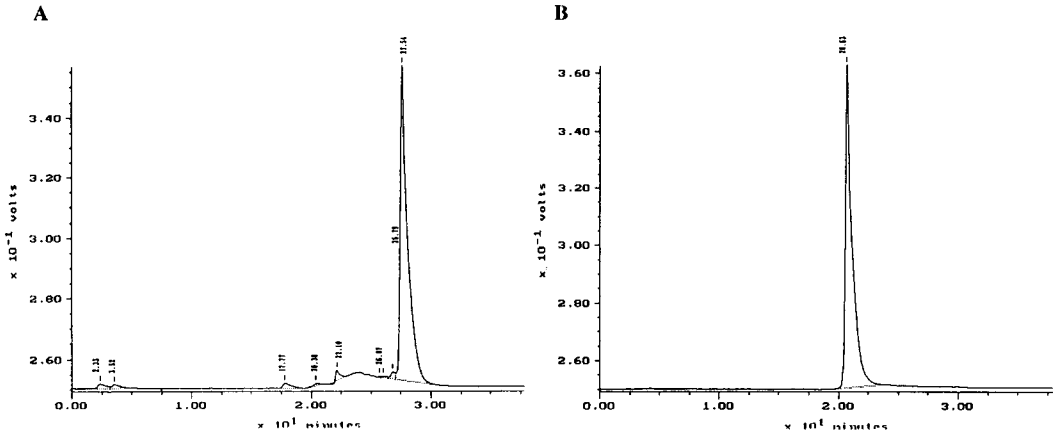

Figure 2 Purification of the dithioate dT8-mer, prepared using **3b** as the phosphorylating reagent (similar data was obtained using **3a**). **A** and **B**: HPLC chromatograms (Hamilton PRP-1 column, 50°C, eluted with buffer a: 5% acetonitrile in aqueous buffer b: 80% acetonitrile in 0.1M aqueous ammonium hydrogencarbonate, pH=9.0; 100% a for 5 min, 0-100% b for 40 min, 100% b for 5 min). **A**: Crude dithioate dT8-mer with DMT-on, **B**: HPLC-purified and fully deprotected dithioate dT8-mer.

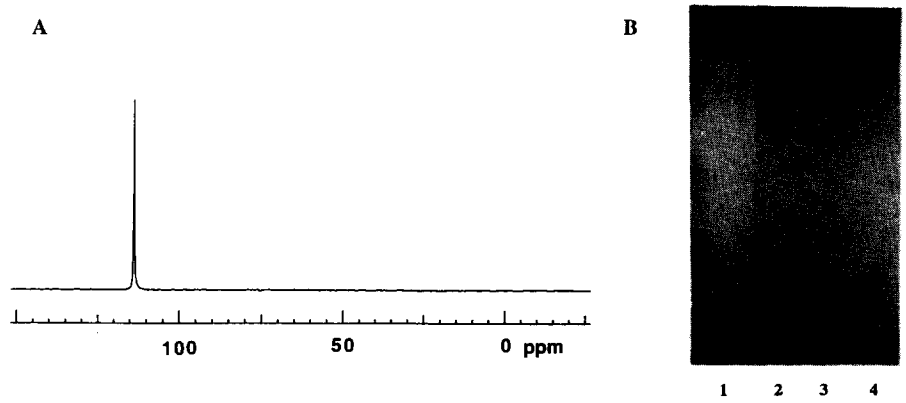

Figure 3 Characterisation of the dithioate dT8-mer. **A**: ^{31}P n.m.r. (D_2O/H_2O) of the HPLC purified dithioate dT8-mer. **B**: PAGE of the fully deprotected dithioate dT8-mer and dT9-mer (20% acrylamide/7 M urea gel, buffer 90 mM Tris-borate, 2.5 mM Na_2EDTA, pH=8.0, samples (0.25 OD) dissolved in 20 µl 7 M urea/10xbuffer (9:1) and heated to 90°C for 0.5 h, run at 500V, visualised by UV shadowing). Lane 1: Bromophenol blue; lane 2: A ladder of oligothymidines (T_{14}, T_{12}, T_{10} and T_8); lane 3: Dithioate dT8-mer and lane 4: Dithioate dT9-mer.

In conclusion we have developed a triester solid phase method for the preparation of oligodeoxythymidine phosphorodithioates. The coupling times are acceptable, the yields are high and the products are not contaminated with phosphorothioates, as is the case for other solid phase synthesis methods.[2,3] Work is in progress to use the method for the synthesis of mixed-base sequences and to fully automate the method.

Acknowledgements

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References and notes

1. Marshall, W. S.; Caruthers M. H., *Science* **1993**, 259, 1564-1570.
2. Okyuszek, A. ; Sierzchata, A.; Sochacki, M., Stec, J. W., *Tetrahedron Lett.* **1992**, 33, 7585-7588.
3. Wiesler, W. T.; Marshall, W. S.; Caruthers M. H., In *Methods in Molecular Biology, Vol. 20: Protocols for Oligonucleotides and Analogs, Synthesis and Properties.* Edited by Agrawal, S.: Humana Press inc., Totowa **1993**, p. 191.
4. Eldrup, A. B.; Bjergårde K.; Felding J. ; Kehler, J.; Dahl, O., *Nucleic Acids Research* **1994**, 22, 1797-1804.
5. Marugg, J. E.; Tromp, M.; Jhurani, P.; Hoyng, C. F.; van der Marel, G. A.; van Boom, J. H., *Tetrahedron* **1984**, 40, 73-78.
6. 6-nitro-1-hydroxybenzotriazole was prepared by a modified literature procedure[17] as follows: A solution of hydrazine hydrate (40 ml, 0.83 mol) in absolute ethanol (100 ml) was added dropwise to a solution of 2,4-dinitroiodobenzene (29.4 g; 0.1 mol) in absolute ethanol (250 ml) under nitrogen. The resulting solution was refluxed for 3h, cooled to 0°C and the crystalline precipitate collected as yellow crystals. The yellow crystals were redissolved in water (150 ml) and acidified with 4M hydrochloric acid (34 ml). 6-nitro-1-hydroxybenzotriazole was collected after washing with 4M hydrochloric acid (2x80 ml), dried over P_2O_5, and recrystallised from acetonitrile (570 ml). Yield 9.32 g (52%), m.p. 203-207°C dec., (lit.[17] 190-192°C dec.).
7. Under the reaction conditions outlined in Figure 1 more than 99% of imidazole hydrochloride precipitates in dry diethyl ether, 99% in dioxane and 80% in THF. The use of pyridine instead of imidazole leads to extensive dealkylation of **2a** (see ref. 16). This is probably due to the higher solubility of pyridine hydrochloride in these solvents.
8. The reaction rate is strongly influenced by the solvent. In acetonitrile the reaction is complete in 5 min. against 30 min. in dichloromethane and 60 min. in THF. DMF causes side reactions as shown by a signal at 132.9 ppm.
9. The ^{31}P-spectrum showed, beside some hydrolysis, a 3% impurity at 132.9 ppm. The amount of produced sideproduct at 132.9 ppm was strongly dependent on the solvent: In dichloromethane it was 3%, in THF 12% and in DMF 32%.
10. Marugg, J. E.; McLaughlin, L. W.; Piel, N.; Tromp, M.; van der Marel, G. A.; van Boom, J. H., *Tetrahedron Lett.* **1983**, 24, 3989-3992.
11. Reese, C. B. and Pei-Zhuo, Z., *J. Chem. Soc. Perkin Trans. I*, **1993**, 2291-2301.
12. Carpino, L. A., *J. Am. Chem. Soc.*, **1993**, 115, 4397-4398.
13. Coupling on the dT-CPG support (Cruachem, loading 32 μmol/g) gave significant lower coupling yields (*ca.* 50% DMT yield). This was also the case when a CPG-bound oligodeoxythymidine sequence (5- or 10-mer) was elongated with **3a** (42-47% DMT yield).
14. TentaGel-T support (Rapp Polymere, Tübingen, Germany, capacity 0.219 mmol/g,): 1.3 μmol scale. The solution was injected in two portions of *ca.* 0.2 ml with 20 min. (**3a**) or 5 min. (**3b**) intervals followed by the last *ca.* 0.2 ml which was left in contact with the support for 100 min. (**3a**) or 15 min. (**3b**).
15. It is essential that the *S*-protection groups are completely removed before treating the solid support with conc. aqueous ammonia to avoid phosphorothioate contamination. Therefore the treatment with thiophenolate was performed overnight before deblocking with conc. aqueous ammonia.
16. Dahl, H. B.; Bjergårde, K.; Nielsen, J.; Dahl, O., *Tetrahedron Lett.* **1990**, 31, 3489-3492.
17. Curtius, T., *J. Prakt. Chemie* **1907**, 76, 374.

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