Facile Synthesis of a Phosphotriester Intermediates for Solution-Phase Preparation of Oligonucleotide Phosphorothioates

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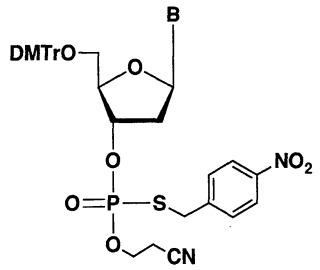
Fully protected 2'-deoxynucleoside phosphorothioate triester derivatives, which are key starting materials for the large-scale solution-phase preparation of phosphorothioate oligonucleotides, were easily synthesized by a new method starting from common, fully protected 2'-deoxynucleosides.

Phosphorothioate oligonucleotides are the most intensively investigated nuclease-resistant antisense analogues. They have been noted as therapeutic agents for several diseases and a number of clinical trials are being undertaken.[1-3] For clinical evaluation, large-scale phosphorothioate oligonucleotides are needed. The synthesis of a phosphorothioate oligomer is usually accomplished by an automated solid-phase phosphoramidite method[4,5] which, however, gives only a small quantity of the product compared to the solution-phase method. During the past decade, some attempts to prepare the oligomers on a large scale by the solution-phase phosphotriester method have been investigated.[6-10] For example, Barber et al.[10] demonstrated the feasibility of this strategy through the synthesis of several phosphorothioate oligomers by the condensation of "phosphorothioate blockmer" to reduce the reaction steps and by-products. The key starting material they utilized in this procedure is compound **1**, which was prepared by the reaction of the fully



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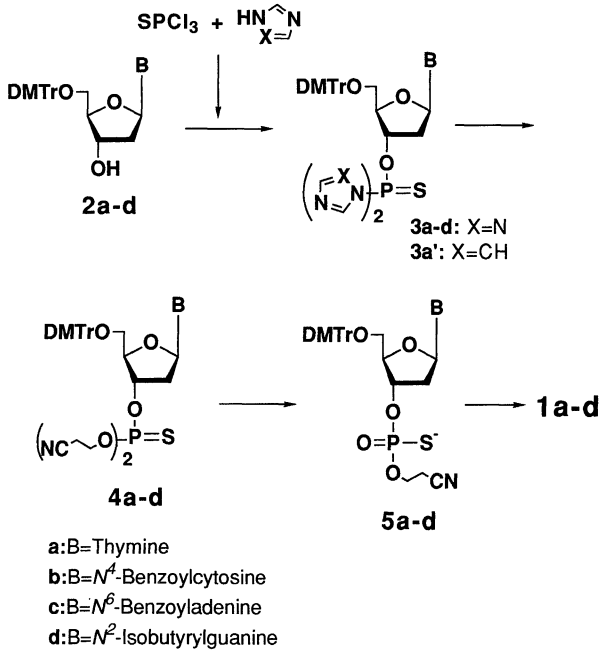
protected nucleoside 3'-hydrogen phosphonate with elemental sulfur and subsequent protection of the sulfur.[10] The method appeared to be efficient, although the hydrogen phosphonate is rather expensive. Other attempts to prepare the analogous compound of **1** from the corresponding fully protected 3'-*O*-phosphoramidite derivatives with an appropriate thiol derivative and subsequent oxidation seem to give a substantial amount of side products.[6,7] Thus, a convenient synthetic method of compound **1** starting from easily accessible and cheap starting material is highly desirable for easier supply of compound **1** in terms of the large-scale synthesis of the phosphorothioate oligomer. Here we wish to report a new facile synthesis of **1** from a common nucleoside derivative, namely, 5'-*O*-DMTr protected 2'-deoxynucleoside.

As the source of sulfur, we utilized thiophosphoryl chloride because of its high reactivity. Thus, 5'-*O*-DMTr protected 2'-deoxythymidine (**2a**) (4.36 g, 8.0 mmol) was allowed to react with a slight excess of thiophosphoryl tris(1,2,4-triazolide) or thiophosphoryl tris(imidazolide), prepared from thiophosphoryl chloride (1.0 ml, 10 mmol) and either 1,2,4-triazole or imidazole (2.1 g, 30 mmol) in a mixed solvent of THF/pyridine (3:2, 50 ml), at room temperature to give bistriazolide intermediate **3a** or bisimidazolide intermediate **3a'**, respectively and



Scheme 1.

quantitatively.[11,12] The reactivity of the bisimidazolide intermediate **3a'** was, however, found to be low in the following step compared to the bistriazolide counterpart **3a**. Therefore, we decided to concentrate our effort on compound **3a** in the following steps. To the above reaction mixture containing **3a** was added ethylenecyanohydrine (1.4 ml, 20 mmol) to generate biscyanoethyl phosphorothioate triester **4a**. It should be noted that compound **4a** is unstable in the presence of a trace amount of water and attempts to isolate the pure compound were unsuccessful. Subsequent brief treatment of **4a** with triethylamine (2.8 ml, 20 mmol) and water (5 ml) without isolation of the compound resulted in removal of one of the cyanoethyl protecting groups on the phosphate moiety to give phosphorothioate diester (**5a**).[13] Compound **5a** was easily isolated from the mixture by simple extraction with $CHCl_3$ and retained enough purity to be used in the next step without further

purification. Thus, one-pot synthesis of diester compound **5a** from 5'-*O*-DMTr-thymidine (**2a**) was successfully attained. Since elemental sulfur was not used as the source of sulfur in this procedure, the reactions were easily worked up and purification of the intermediates by column chromatography was not required.[10] After workup of diester **5a**, the thiol moiety was protected with a *p*-nitrobenzyl group using 4-nitrobenzyl bromide[14] to give the desired phosphorothioate triester (**1a**). The protecting group was once mentioned as an appropriate S-protecting group in earlier work.[10] After the protection, **1a** was purified by silica gel column chromatography.[15] The overall yield of **1a** after the purification from the protected nucleoside **2a** was quite satisfactory (4.8 g, yield 73 % from **2a**, see Table 1).

Table 1. Overall yields of the phosphorothioate triesters

Compound	Yield/%
1a	73
1b	37
1c	37
1d	54

The method was also applicable to other naturally occurring deoxynucleoside derivatives. Thus, the phosphorothioate triesters of N^4-benzoyl-2'-deoxycytidine (**1b**), N^6-benzoyl-2'-deoxyadenosine (**1c**), and N^2-isobutyryl-2'-deoxyguanosine (**1d**) were prepared from the corresponding base-protected 5'-*O*-DMTr nucleosides (**2b-c**) essentially in the same manner as described for **1a**.[16] The yields of the triester derivatives (**1a-d**) obtained by the current method are given in Table 1. In Table 1, the yields of **1b** and **1c** were somewhat lower than those of the other analogs. At this moment, we presume that this is due to the instability of the diester derivatives (**5b** and **5c**) during the hydrolysis. However, the yields listed in Table 1 are the overall yields after 4-step reactions starting from the base-protected 5'-*O*-DMTr nucleosides and, therefore, these are still satisfactory. Compound **1a-d** obtained by this new synthetic method reported here could be utilized in phosphorothioate oligomer synthesis by the already known solution-phase and blockmer condensation method.[10]

In conclusion, the phosphorothioate triester derivatives (**1a-d**) which are key intermediates for the large-scale solution-phase synthesis of phosphorothioate oligomer were easily obtained from the corresponding base-protected 5'-*O*-DMTr-nucleosides by a facile method. The method reduces the synthetic and purification steps of the triester derivatives compared to the preceding methods and, therefore, provides an easy supply of **1**.

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13 Data for **5a** (Ref.10): ^{31}P NMR (CD_3CN) δ 59.62, 59.49: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.19 (s, br, 1H, NH), 7.61-6.82 (m, 14H, H6 and DMTr-H), 6.39 (m, 1H, H1'), 5.30 (m, 1H, H3'), 4.37 (m, 1H, H4'), 4.28-3.90 (m, 2H, P-OCH_2), 3.79 (s, 6H, OCH_3), 3.55-3.37 (m, 2H, H5' and H5"), 3.07 (q, Et_3N), 2.68-2.13 (m, 4H, CH_2CN, H2' and H2"), 1.40 and 1.36 [d, 3H, CH_3 (thymine)], 1.32 (t, Et_3N). FAB$^-$MS 692 $(M-H)^-$, 690 $(M-3H)^-$.
14 To a stirred solution of phosphorothioate diester **5a** in CH_3CN(100 ml) were added 2,6-lutidine (1.9 ml, 16 mmol) and 4-nitrobenzyl bromide (3.5 g, 16 mmol). The reaction mixture was stirred at room temperature for 4 h.
15 Data for **1a** (Ref.10): ^{31}P NMR (CD_3CN) δ 27.55, 27.48: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.44 (s, br, 1H, NH), 8.16 [m,2H, H meta of *p*-nitrobenzyl], 7.50 [m, 3H, H ortho of *p*-nitrobenzyl and H6], 7.36-6.83 (m, 13H, DMTr-H), 6.37 (m, 1H, H1'), 5.20 (m, 1H, H3'), 4.23-4.09 (m, 5H, P-OCH_2, $POSCH_2$ and H4'), 3.79 (s, 6H, OCH_3), 3.48 and 3.31 (m, 2H, H5' and H5"), 2.73-2.38 (m, 4H, CH_2CN, H2' and H2"), 1.44 [s, 3H, CH_3 (thymine)]. FAB$^+$MS 828 $(M+H)^+$, 827 (M^+).
16 Data for **5b**: ^{31}P NMR (CD_3CN) δ 61.15: FAB$^-$MS 781 $(M-H)^+$. Data for **5c**: ^{31}P NMR (CD_3CN) δ 58.62, 58.38: FAB$^-$MS 805 $(M-H)^+$. Data for **5d**: ^{31}P NMR (CD_3CN) δ 58.06: FAB$^-$MS 787 $(M-H)^+$. Data for **1b**: ^{31}P NMR (CD_3CN) δ 28.06, 27.99: FAB$^+$MS 918 $(M+H)^+$, 917 (M^+). Data for **1c**: ^{31}P NMR (CD_3CN) δ 29.03, 28.29: FAB$^+$MS 942 $(M+H)^+$. Data for **1d**: ^{31}P NMR (CD_3CN) δ 29.33, 29.22: FAB$^+$MS 924 $(M+H)^+$.