Application of the Michaelis–Arbusov Reaction to the Synthesis of Internucleoside 3'-*S*-Phosphorothiolate Linkages

Xiang Li,[a] Gerard K. Scott,[a] Anthony D. Baxter,[b] Roger J. Taylor,[b] Joseph S. Vyle[a] and Richard Cosstick*,[a]

[a] *Robert Robinson Laboratories, Department of Chemistry, University of Liverpool, PO Box 147, Liverpool L69 3BX, UK*

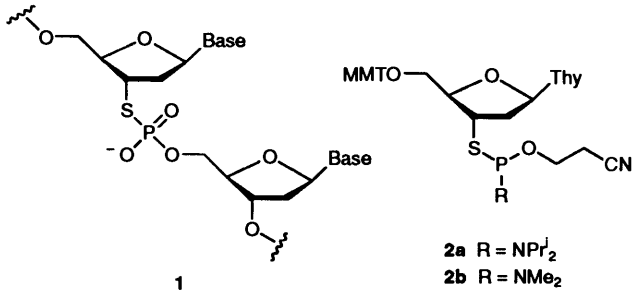
[b] *Ciba Central Research, Ciba-Geigy PLC, Hulley Road, Macclesfield SK10 2NX, UK*

The 5'-*O*-monomethoxytrityl-3'-*S*-(aryldisulfanyl)-3'-deoxythymidines **7** and **8** have been prepared by the reaction of 5'-*O*-monomethoxytrityl-3'-thiothymidine with the appropriate arenesulfenyl chloride. These disulfides undergo a Michaelis–Arbusov reaction with simple trialkyl phosphites to yield 5'-*O*-monomethoxytrityl-3'-thiothymidin-3'-yl *O,O*-dialkyl phosphorothiolates. More interestingly, 3'-deoxy-3'-*S*-(2,4-dinitrophenylsulfanyl)-5'-*O*-monomethoxytritylthymidine **8** reacts with a variety of thymidin-5'-yl dialkyl phosphites to give dithymidine phosphorothiolate triesters with the phosphorothiolate group protected with either a methyl or a 2-cyanoethyl group.

3'-*O*-(*tert*-Butyldimethylsilyl)thymidin-5'-yl triethylammoniumphosphonate **17** is converted into the corresponding bis-(*O*-trimethylsilyl) phosphite by treatment with bis(trimethylsilyl)trifluoroacetamide. *in situ* Reaction of this phosphite with disulfide **8** gives, after work-up, the dithymidine phosphorothiolate diester directly. Methylation of compound **17** with methyl chloromethanoate, followed by silylation and subsequent reaction with disulfide **8**, gives the methyl-protected dithymidine phosphorothiolate triester.

Oligonucleotides containing a 3'-*S*-phosphorothiolate linkage **1** have attracted increasing interest as probes for studying the interaction of nucleic acids and their processing enzymes. In particular these analogues have been used to investigate cleavage processes catalysed by the restriction endonuclease *Eco* RV,[1] the *Tetrahymena* ribozyme[2] and the DNA repair enzyme T4 endonuclease 4V.[3] In view of the potential utility of these compounds we have sought to develop efficient and convenient procedures for their preparation which are potentially suited to automation.

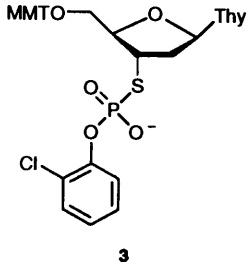
We initially investigated the possibility of using 3'-*S*-thiophosphoramidites **2** which are relatively stable and easy to prepare. The first examined, the *N,N*-diisopropylthiophosphoramidite **2a**, was shown to be surprisingly unreactive (about two orders of magnitude less reactive than the corresponding phosphoramidite) and it was necessary to use 5-(4-nitrophenyl)-tetrazole as an activating agent which is more acidic than the conventionally used tetrazole.[4–6] Unfortunately, under these acidic conditions the alkylsulfanyl group is particularly susceptible to displacement from the phosphorus centre and coupling reactions with compound **2a** are accompanied by the formation of many side-products and hence give low and very variable yields (generally 30–60%). Smaller dialkylamino groups [dimethylamino (**2b**) and pyrrolidino (**2c**)] show increased reactivity and give consistently higher yields (75–80%),[7] although the yields are still significantly below the level necessary to sustain repetitive rounds of automated synthesis. In another approach the phosphorothiolate diester **3** has been shown to couple cleanly and efficiently to yield the fully protected dinucleoside monophosphate.[8] However, the oximate-mediated removal of the aryl protecting group, which proceeds *via* nucleophilic attack at phosphorus, results in 1–10% cleavage of the phosphorus–sulfur bond, depending on the exact deprotection conditions.



1

2a R = NPr^i_2
2b R = NMe_2

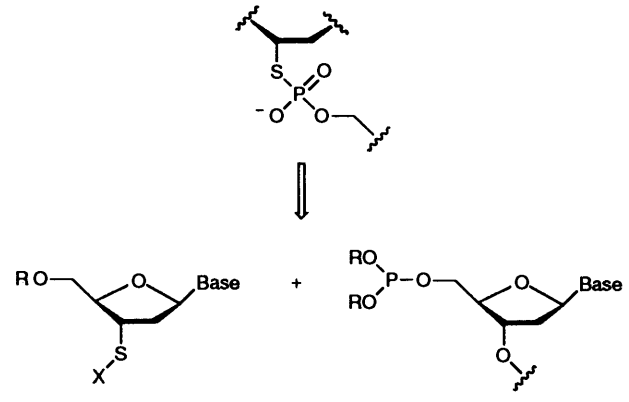
2b R = N



3

Thy = Thymin-1-yl
MMT = monomethoxytrityl

These difficulties encouraged us to investigate an alternative approach that was not available for the preparation of natural phosphodiester linkages and involved disconnection across the phosphorus–sulfur bond to an electrophilic thiol derivative **4** and a nucleoside phosphite **5** (Scheme 1). It is well established that a Michaelis–Arbusov-type reaction between disulfides and phosphite triesters provides an efficient route to phosphorothiolates under relatively mild conditions.[9–11] In a preliminary publication we have described the preparation of dinucleoside 3'-*S*-phosphorothiolates using a simple nucleoside 5'-*O*-(dimethyl phosphite).[8] The present study is a much more extensive exploration of the scope and potential of this type of Michaelis–Arbusov reaction and in particular we wish to draw attention to the versatility of nucleoside silyl phosphites in the synthesis of internucleoside phosphorothiolate linkages.

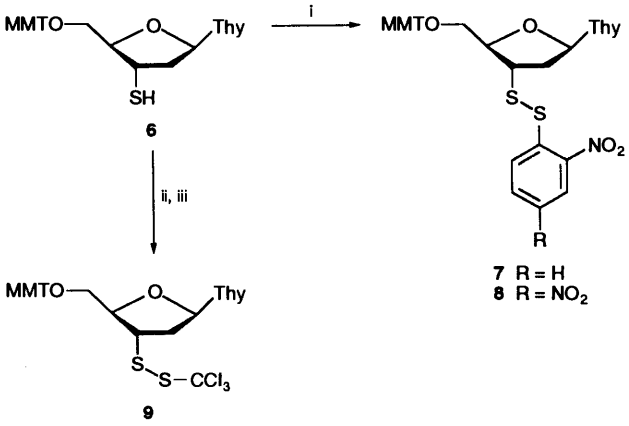


Scheme 1

Results and Discussion

Synthesis of Electrophilic Thiol Derivatives.—Fundamental studies by Harvey *et al.* on the reaction of phosphite triesters with disulfides established that the more polarised unsymmetrical disulfides are more reactive than symmetrical disulfides; an observation that is consistent with an ionic rather than a radical mechanism.[9] Additionally, the cleavage of unsymmetrical disulfides was shown to proceed to eliminate the more stable thiolate anion.

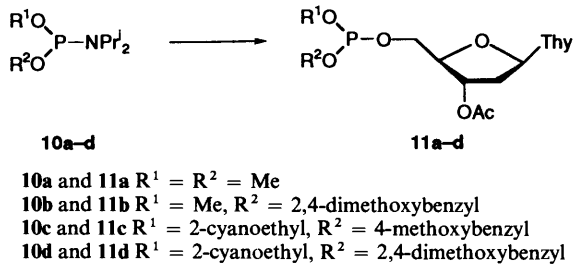
These results led us to select compounds **7**–**9** (Scheme 2) as potentially reactive disulfides which we hoped would be readily accessible from the thionucleoside **6**. The aryl disulfides **7** and **8**



Scheme 2 *Reagents:* i, ArSCl, THF; ii, $AgNO_3$, Et_3N, EtOH; iii, CCl_3SCl, THF

were both prepared in yields in excess of 70% by reaction of the thionucleoside **6** with 1.5 mole equivalents of the appropriate arenesulfenyl chloride in dry tetrahydrofuran (THF) at 0 °C. Although triethylamine was initially included in the reaction mixture to prevent detritylation it was noted that the presence of this base promoted the unwanted sulfenylation of the thymine heterocycle. However, contrary to our expectation, detritylation does not appear to be a significant problem in the absence of a base although some protection against this eventuality was afforded by drying of the thiol **6** by evaporation of pyridine. The trichloromethyl disulfide **9** could not be prepared by the same procedure, but was obtained in 70% yield by reaction of trichloromethanesulfenyl chloride with the silver salt derived from thiol **6** (Scheme 2).

Preparation of Nucleoside Phosphites.—In designing nucleoside 5′-phosphites that were likely to be most suitable for the Michaelis–Arbusov-type reaction it was necessary to consider the fact that the alkyl group retained in the reaction was required to be a suitable protecting group for the resulting phosphorothiolate triester. We have previously shown that the cyanoethyl group is ideal as a blocking group for internucleotide phosphorothiolate linkages;[4–6] additionally we believed that the methyl group, which is removed by nucleophilic attack at the methyl carbon atom, would also be suitable for this purpose. Phosphites **11a** and **11b** (Scheme 3)



10a–d **11a–d**

10a and **11a** $R^1 = R^2 = Me$
10b and **11b** $R^1 = Me$, R^2 = 2,4-dimethoxybenzyl
10c and **11c** R^1 = 2-cyanoethyl, R^2 = 4-methoxybenzyl
10d and **11d** R^1 = 2-cyanoethyl, R^2 = 2,4-dimethoxybenzyl

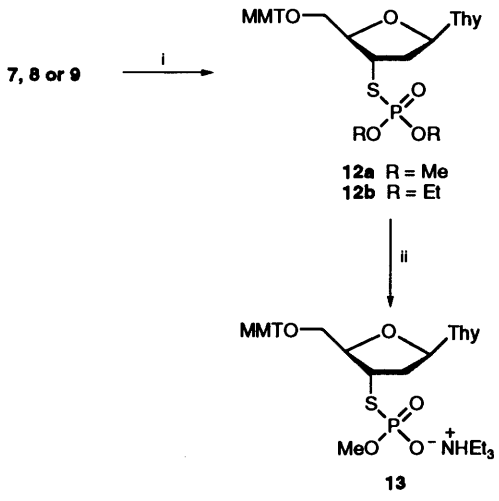
Scheme 3 *Reagents:* 3-*O*-acetylthymidine, tetrazole, DMAP, MeCN

were selected as Michaelis–Arbusov substrates that would introduce a methyl-protecting group into the product, whilst phosphites **11c** and **11d** were chosen to produce a cyanoethyl-protected phosphorothiolate. The methoxybenzyl substituents in phosphites **11b**–**11d** were incorporated to increase the nucleophilicity of the phosphorus atom which was thought to be particularly desirable in the case of compounds **11c** and **11d** in order to compensate for the electron-withdrawing cyanoethyl group.

The precursor phosphoramidites **10b**–**10d** were prepared by reaction of the appropriate chloro(alkoxy)-*N*,*N*-diisopropylaminophosphine with the appropriate benzyl alcohol in diethyl ether in the presence of *N*,*N*-diisopropylethylamine and the products were obtained in very good yield (75–90%) after purification by column chromatography.[12] Diisopropylamino-(dimethoxy)phosphane **10a** was prepared as previously described by Mag and Engels.[13]

There have been several reports on the use of nucleoside phosphite triesters as intermediates in the synthesis of modified nucleotides. In particular, simple nucleoside 3′-*O*-(dialkyl phosphites) have been used to prepare dinucleoside monophosphate analogues containing 5′-*N*-phosphoramidite linkages by a Staudinger reaction followed by a Michaelis–Arbusov-type transformation.[13,14] In both these reports the nucleoside phosphites were used *in situ* and no attempts were made to isolate these intermediates. However, for a systematic investigation of the utility of the nucleoside phosphites we believed that it would be advantageous to isolate and characterise these compounds. The phosphites **11a**–**11d** (Scheme 3) were prepared by reaction of 3′-*O*-acetylthymidine with the appropriate phosphoramidite in acetonitrile in the presence of tetrazole. After work-up the nucleoside phosphites were isolated in good yield (78% or greater) by flash chromatography on a silica gel column with an eluent containing 10% triethylamine. The inclusion of triethylamine in the eluent was found to be critically important for efficient recovery of the nucleoside 5′-phosphites from the column and in the absence of this base the isolated yields were very poor. It should be noted that Honjo *et al.* have previously found it necessary to use alumina for the chromatographic purification of a nucleoside 5′-(diethyl phosphite) although in that case the isolated yield was less than 50%.[15]

Michaelis–Arbusov-type Reactions.—In model studies the electrophilic thiol derivatives **7**–**9** were treated with triethyl and/or trimethyl phosphite in toluene at room temperature (Scheme 4). Although compounds **7**–**9** all reacted over a period of 1–2 days with these simple phosphites to give the 3′-*S*-



Scheme 4 *Reagents:* i, $(RO)_3P$, THF; ii, PhSH, Et_3N, 1,4-dioxane

Table 1 Reaction of nucleosidyl phosphites with the dinitrophenyl disulfide **8**

Nucleosidyl phosphite	Reaction time (*t*/h)	Reaction temperature (*T*/°C)	Yield (product) (%)
11a	16	ambient	89 (**14**)
11b	2	ambient	94 (**14**)
11c	96	ambient	74 (**15**)
11c	24	45	80 (**15**)
11d	24	ambient	94 (**15**)
11d	10	45	84 (**15**)

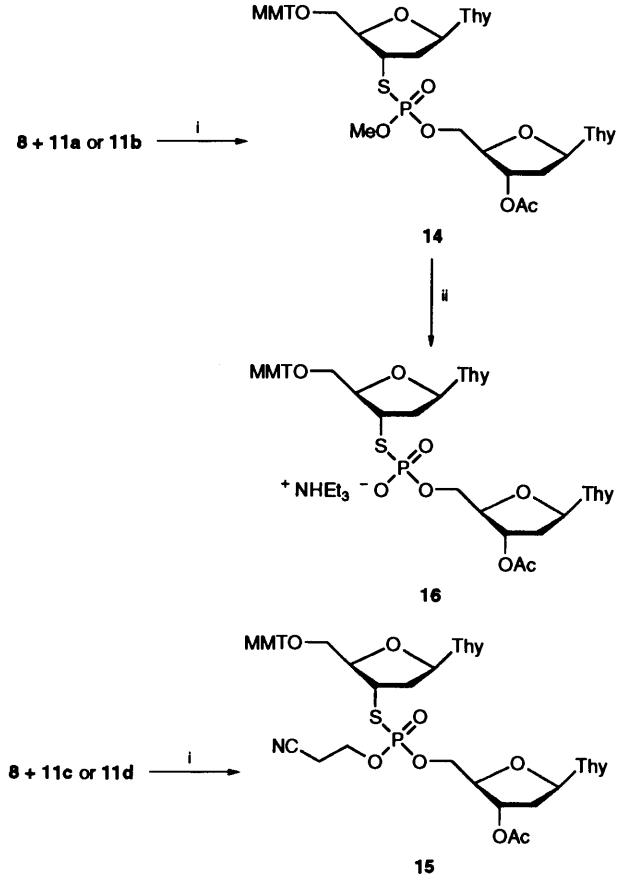
phosphorothiolates **12**, the aryl disulfides gave the cleanest products and highest yields. To establish the compatibility of the methyl protecting group with the phosphorothiolate linkage, demethylation of compound **12a** was studied by ^{31}P NMR spectroscopy; treatment with benzenethiol–triethylamine–1,4-dioxane (1:2:4) for 1 h at room temperature resulted in quantitative deprotection and the methyl phosphorothiolate diester **13** was isolated in 82% yield, after chromatography.

The synthesis of the dinucleoside 3′-*S*-phosphorothiolates was initially investigated by reaction of the dinitrophenyl disulfide **8** and the nucleoside dimethyl phosphite **11a** (Scheme 5 and Table 1). Use of 3 mole equivalents of the phosphite in dry toluene at room temperature led to a smooth reaction over a period of *ca.* 16 h to give the methyl-protected dinucleotide **14** in 89% yield. Under the same conditions the more nucleophilic dimethoxybenzyl phosphite **11b** reacted more rapidly to give compound **14** in greater than 90% yield within 1 h.

As expected the cyanoethyl phosphites reacted with compound **8** much more slowly; although compound **15** (Scheme 5) could be obtained from either phosphite **11c** or **11d** in a yield of 80% or greater by performing the reaction at 45 °C. It was noted that in all of these Michaelis–Arbusov-type transformations high yields were dependent on the use of pure samples of the dinitrophenyl disulfide **8**. The other electrophilic thiol derivatives (compounds **7** and **9**) generally gave poorer yield in the Michaelis–Arbusov-type reactions and their use was not investigated systematically.

Quantitative demethylation of compound **14** was achieved by treatment with benzenethiol–triethylamine–1,4-dioxane (1:2:4) for 2 h at room temperature and the phosphate-deprotected dinucleoside **16** was isolated in 82% yield after chromatography. ^{31}P NMR studies indicated that there was no measurable cleavage of the internucleoside linkage under these conditions.

Michaelis–Arbusov-type Reactions with Nucleoside Silyl Phosphites.—It is known that the introduction of a silyl group

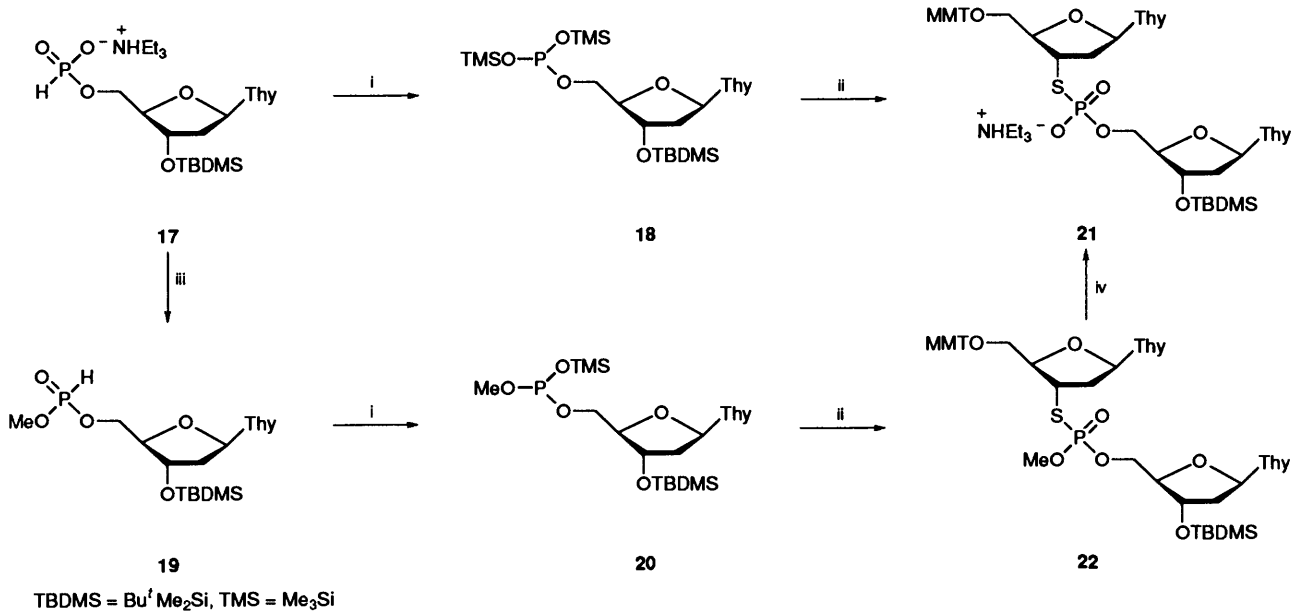


Scheme 5 *Reagents and conditions:* i, toluene; ii, PhSH, Et_3N, 1,4-dioxane

in place of an alkyl group in the phosphite increases the nucleophilicity of the tricoordinate phosphorus centre and thereby increases their reactivity in the Michaelis–Arbusov reaction. Additionally, when the reaction was performed with mixed alkyl silyl phosphites it was shown to proceed by desilylation and not dealkylation.[16] It appeared, therefore, that nucleoside silyl phosphites would most closely meet our requirements for reactive Michaelis–Arbusov substrates potentially suited to automated solid-phase synthesis.

Although the synthesis and utilisation of a nucleoside silyl phosphite was first described by Hata and Skeine[17] almost 20 years ago their use has not been widely reported in the literature. For our purposes the nucleosidyl bis(trimethylsilyl) phosphite **18** and mixed alkyl silyl phosphite **20** appeared to be suitable Michaelis–Arbusov substrates that would be readily available from the nucleoside 5′-*H*-phosphonate **17** (Scheme 6).

The nucleoside 5′-*H*-phosphonate **17** was obtained in 89% yield by reaction of 3′-*O*-(*tert*-butyldimethylsilyl)thymidine with tris(1,2,4-triazolyl) phosphite[18] and subsequent hydrolysis. Conversion into the desired bis(trimethylsilyl) phosphite **18** was initially achieved using trimethylsilyl chloride and triethylamine in pyridine according to the procedure described by Hata and Sekine.[17] It was subsequently found that a cleaner product, uncontaminated with triethylammonium hydrochloride, was obtained when the silylation was performed using 1.5 mole equivalents of *N*,*N*-bis(trimethylsilyl)trifluoroacetamide in CH_2Cl_2. For both procedures ^{31}P NMR spectroscopy established that the bis(trimethylsilyl) phosphite **18**, which was identified by a single resonance at δ_P 116, was generated almost quantitatively. The *in situ* reaction of this intermediate with the disulfide **8** was followed by ^{31}P NMR spectroscopy and was shown to proceed cleanly and rapidly to give the desired



Scheme 6 *Reagents:* i, *N*,*N*-bis(trifluoromethyl)trifluoroacetamide, CH_2Cl_2; ii, **8**, CH_2Cl_2; iii, $ClCO_2Me$, pyridine; iv, PhSH, Et_3N, 1,4-dioxane

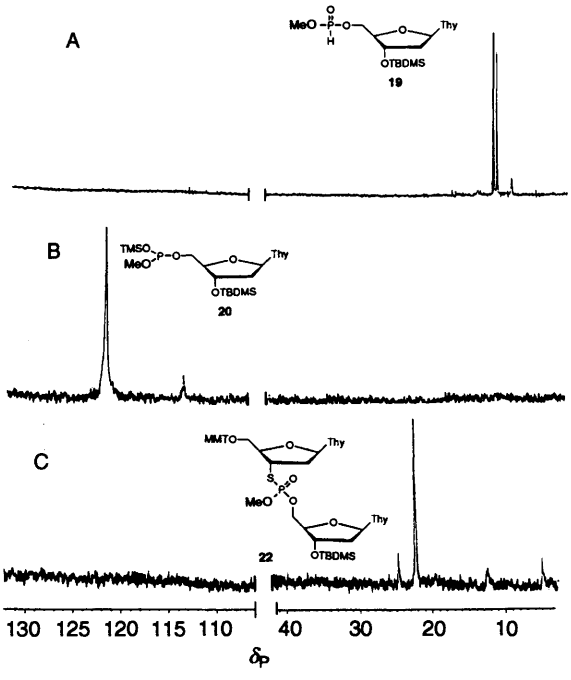


Fig. 1 ^{31}P NMR spectra of the intermediates in the sequential conversion of the phosphonate **17** into the dithymidine phosphorothiolate triester **22**. Panel A; spectrum of the crude methyl phosphonate **19**, obtained after treatment of compound **17** with methyl chloromethanoate, pyridine and work-up. Panel B; spectrum of the crude trimethylsilyl phosphite **20**, obtained after silylation of phosphonate **19** with *N*,*N*-bis(trimethylsilyl)trifluoroacetamide. Panel C; spectrum of the crude dithymidine phosphorothiolate triester **22**, obtained after addition of disulfide **8**.

dinucleoside phosphorothiolate. After aqueous work-up, which removes the trimethylsilyl group from the phosphorothiolate triester, and column chromatography, the product diester **21** was obtained in 65% yield.

The phosphite **20** was prepared by initial methylation of the *H*-phosphonate with methyl chloromethanoate[19] in CH_2Cl_2–pyridine to give the *H*-phosphonate diester **19** in almost quantitative yield. Although this compound could be isolated and characterised it was generally silylated *in situ* to give compound **20** and this then reacted directly with the disulfide **8** to effect a one-pot conversion of compound **17** into the phosphorothiolate triester **22**. This series of reactions was conveniently monitored by ^{31}P NMR spectroscopy and the overall efficiency of the process can be appreciated from the set of NMR spectra presented in Fig. 1. Removal of the methyl protecting group from compound **22** proceeded efficiently using benzenethiol and triethylamine under the standard conditions.

Conclusions.—Our studies show that a Michaelis–Arbusov reaction between a nucleosidyl 5′-phosphite and a nucleosidyl 3′-*S*-disulfide is a versatile, efficient and clean method for the synthesis of internucleotide 3′-*S*-phosphorothiolate linkages. In particular, the phosphite component can be chosen to produce a phosphorothiolate group protected with either a 2-cyanoethyl or a methyl group, whilst reaction with a nucleosidyl 5′-bis-(trimethylsilyl) phosphite yields the deprotected linkage directly. Reactions between the silyl phosphites and the dinitrophenyl disulfide **8** were shown to be exceptionally rapid and may prove applicable to a solid-phase-based procedure. The use of the silyl phosphites in an intramolecular version of this Michaelis–Arbusov reaction[20] to construct analogues of 3′,5′-cyclic nucleotides is currently being explored.

Experimental

Fast-atom bombardment (FAB) mass spectra were recorded on a VG Analytical 7070E mass spectrometer operating with a PDP 11/250 data system and an Ion Tech FAB ion gun working at 8 kV. High-resolution FAB mass spectra were obtained on a VG ZAB/E spectrometer at the SERC Mass Spectrometry Service Centre (Swansea, UK) and reported masses are accurate to ± 5 ppm. 3-Nitrobenzyl alcohol was used as a matrix unless stated otherwise. ^1H NMR spectra were measured on either a Bruker AMX400 or a Bruker AC200 spectrometer, chemical shifts are given in ppm downfield from tetramethylsilane as internal standard, and *J* values are given in Hz. Peaks displaying obvious diastereoisomeric splitting are denoted with an asterisk. ^{31}P NMR spectra are referenced to 85% phosphoric acid. Nucleosides were visualised as a black spot by spraying with a solution of 5% (v/v) sulfuric acid and 3% (w/v) phenol in ethanol and charring at 120 °C. Light petroleum refers to the fraction with distillation range 60–80 °C.

General Method for the Preparation of 3′-(*Aryldisulfanyl*) 3′-*Deoxy*-5′-O-(*monomethoxytrityl*)*thymidines* **7** *and* **8**.—The

thionucleoside **6** (1.0 g, 1.9 mmol), dried by coevaporation with pyridine to yield a thick oil, was dissolved in dry THF (20 cm^3) and the solution was added dropwise to a stirred and cooled (ice-bath) solution of the appropriate arenesulfenyl chloride (3.8 mmol) in THF (40 cm^3). After a further 30 min at this temperature the mixture was allowed to warm to room temperature and was then stirred for 1 h. The reaction mixture was then partitioned between CH_2Cl_2 and saturated aq. $NaHCO_3$, and the organic phase was dried ($MgSO_4$) and then evaporated. Silica gel chromatography (CH_2Cl_2 containing 1–3% MeOH) gave the *disulfide* as a yellow solid.

3'-*Deoxy*-5'-O-*monomethoxytrityl*-3'-(2-*nitrophenyldisulfanyl*)*thymidine* **7**. 74% Yield (Found: C, 63.0; H, 4.9; N, 6.1. $C_{36}H_{33}N_3O_7S_2$ requires C, 63.2; H, 4.87; N, 6.15%); δ_H(200 MHz; $CDCl_3$) 9.20 (1 H, s, NH), 8.28 (2 H, d, *J* 8.1, ArH), 8.15 (2 H, d, *J* 8.1, ArH), 7.62 (1 H, s, 6-H), 7.20–7.40 (12 H, m, ArH), 6.82 (2 H, d, *J* 8.8, C_6H_4OMe), 6.20 (1 H, t, *J* 5.1, 1'-H), 4.09 (1 H, m, 4'-H), 3.79 (3 H, s, OMe), 3.75 (1 H, m, 3'-H), 3.60 (1 H, m, 5'-H), 3.38 (1 H, m, 5'-H), 2.50 (2 H, m, 2'-H_2) and 1.43 (3 H, s, 5-Me); *m/z* (FAB^+) 648 ($M + H^+$).

3'-*Deoxy*-3'-(2,4-*dinitrophenyldisulfanyl*)-5'-O-(*monomethoxytrityl*)*thymidine* **8**. 72% Yield (Found: C, 59.6; H, 4.5; N, 7.6. $C_{36}H_{32}N_4O_9S_2$ requires C, 59.3; H, 4.42; N, 7.69%); δ_H(200 MHz; $CDCl_3$) 9.24 (1 H, s, NH), 9.09 (1 H, s, ArH), 8.34 (2 H, s, ArH), 7.61 (1 H, s, 6-H), 7.20–7.48 (12 H, m, ArH), 6.81 (2 H, d, *J* 8.8, C_6H_4OMe), 6.21 (1 H t, *J* 5.2, 1'-H), 4.09 (1 H, m, 4'-H), 3.80 (3 H, s, OMe), 3.75 (1 H, m, 3'-H), 3.65 (1 H, m, 5'-H), 3.39 (1 H, m, 5'-H), 2.53 (2 H, m, 2'-H_2) and 1.47 (3 H, s, 5-Me); *m/z* (FAB^+) 729 ($M + H^+$).

3'-*Deoxy*-5'-O-*monomethoxytrityl*-3'-(*trichloromethyldisulfanyl*)*thymidine* **9**.—To a stirred solution of the thionucleoside **6** (870 mg, 1.64 mmol) in ethanol (70 cm^3) was added silver nitrate (418 mg, 2.46 mmol). After stirring of the mixture for 1 h, triethylamine (0.5 cm^3) was added, the reaction mixture concentrated to 20 cm^3, and the precipitate was collected by filtration. The precipitate was washed sequentially with ethyl acetate, ethanol and water and dried *in vacuo* to give the silver salt of compound **6** (0.96 g, 92%) as a yellow solid. (This material was used directly without characterisation or further purification.)

Trichloromethanesulfenyl chloride (180 mm^3, 0.73 mmol) was added, by syringe, to a stirred suspension of the silver salt (155 mg, 0.24 mmol) in dry THF (5 cm^3) at 0 °C. After the mixture had been stirred for 10 min the ice-bath was removed and the reaction was allowed to continue for a further 10 min at room temperature. The reaction mixture was diluted with CH_2Cl_2, washed with aq. $NaHCO_3$, dried ($MgSO_4$) and evaporated. Silica gel chromatography (CH_2Cl_2 containing 1–3% MeOH) gave the disulfide **9** (70% yield from the silver salt) [Found: $(M + H)^+$, 679.0662. $C_{31}H_{30}{}^{35}Cl_3N_2O_5S_2$ requires $(M + H)^+$, 679.0662]; δ_H(200 MHz; $CDCl_3$) 9.10 (1 H, s, NH), 7.72 (1 H, s, 6-H), 7.27–7.46 (12 H, m, ArH), 6.84 (2 H, d, *J* 8.7, C_6H_4OMe), 6.31 (1 H, t, *J* 5.2, 1'-H), 4.34 (1 H, m, 4'-H), 4.22 (1 H, m, 3'-H), 3.80 (3 H, s, OMe), 3.65 (1 H, m, 5'-H), 3.35 (1 H, m, 5'-H'), 2.53 (2 H, m, 2'-H_2) and 1.44 (3 H, s, 5-Me).

General Method for the Preparation of the Nucleoside 5'-*Phosphites* **11a–11d**.—To a solution of 3'-*O*-acetylthymidine (300 mg, 1.05 mmol), tetrazole (298 mg, 4.2 mmol) and 4-(dimethylamino)pyridine (DMAP) (128 mg, 1.05 mmol) in dry acetonitrile (5 cm^3) at room temperature was added a solution of a dialkoxy(diisopropylamino)phosphane **10a–d** (2.5 mmol) in dry acetonitrile by syringe. After 80 min the precipitated material was removed by filtration and washed with ethyl acetate (2 × 10 cm^3). The combined filtrate was washed with aq. $NaHCO_3$, dried ($MgSO_4$) and evaporated. Silica gel chromatography [CH_2Cl_2–ethyl acetate–NEt_3 (5:4:1)] gave the nucleoside phosphite (78–90% yield). Phosphites **11b–d** were isolated as a mixture of diastereoisomers.

3'-O-*Acetylthymidin*-5'-*yl dimethyl phosphite* **11a**. δ_H(200 MHz; $CDCl_3$) 9.16 (1 H, s, NH), 7.64 (1 H, s, 6-H), 6.43 (1 H, dd, *J* 8.4 and 6.0, 1'-H), 5.31 (1 H, m, 3'-H), 4.17 (1 H, m, 4'-H), 4.08 (2 H, m, 5'-H_2), 3.59 (6 H, d, J_{PH} 9.7, POMe), 2.38 (1 H, m, 2'-H), 2.19 (1 H, m, 2'-H'), 2.12 (3 H, s, Ac) and 1.95 (3 H, s, 5-Me); $\delta(^{31}P)$ (81 MHz; $CDCl_3$) 143.0; *m/z* (FAB^+) 377 $(M + H)^+$.

3'-O-*Acetylthymidin*-5'-*yl* 2,4-*dimethoxybenzylmethyl phosphite* **11b**. δ_H(200 MHz; $CDCl_3$) 9.01 (1 H, s, NH), 7.66 (1 H, s, 6-H), 7.22* (1 H, d, *J* 8.1, ArH), 6.44 (3 H, m, ArH and 1'-H), 5.25 (1 H, m, 3'-H), 4.87* (2 H, d, J_{PH} 8.5, CH_2Ar), 4.15 (1 H, m, 4'-H), 4.05 (2 H, m, 5'-H_2), 3.80* (6 H, m, OMe), 3.52* (3 H, d, J_{PH} 10.3, POMe), 2.31 (1 H, m, 2'-H), 2.16 (1 H, m, 2'-H'), 2.10 (3 H, s, Ac) and 1.90 (3 H, s, 5-Me); $\delta(^{31}P)$ (81 MHz; $CDCl_3$) 143.2 and 143.0; *m/z* (FAB^+) 513 $(M + H)^+$.

3'-O-*Acetylthymidin*-5'-*yl* 2-*cyanoethyl* 4-*methoxybenzyl phosphite* **11c**. δ_H(200 MHz; $CDCl_3$) 8.72 (1 H, s, NH), 7.49 (1 H, s, 6-H), 7.26 (2 H, m, ArH), 6.88 (2 H, d, *J* 8.6, ArH), 6.44 (2 H, m, ArH), 6.37 (1 H, m, 1'-H), 5.25 (1 H, m, 3'-H), 4.88* (2 H, d, J_{PH} 9.2, CH_2Ar), 4.14–3.94 (5 H, m, OCH_2, 4'-H and 5'-H_2), 3.81 (3 H, s, OMe), 2.60 (2 H, t, *J* 6.0, CH_2CN), 2.34 (1 H, m, 2'-H), 2.15 (1 H, m, 2'-H'), 2.11 (3 H, s, Ac) and 1.90 (3 H, s, 5-Me); $\delta(^{31}P)$ (81 MHz; $CDCl_3$) 142.1 and 141.9; *m/z* (FAB^+) 522 $(M + H)^+$.

3'-O-*Acetylthymidin*-5'-*yl* 2-*cyanoethyl* 2,4-*dimethoxybenzyl phosphite* **11d**. δ_H(200 MHz; $CDCl_3$) 7.55 (1 H, s, 6-H), 7.22 (1 H, m, ArH), 6.46 (2 H, m, ArH), 6.39 (1 H, m, 1'-H), 5.26 (1 H, m, 3'-H), 4.90* (2 H, d, J_{PH} 9.0, CH_2Ar), 4.15–3.96 (5 H, m, OCH_2, 4'-H and 5'-H_2), 3.81 (3 H, s, OMe), 2.59* (2 H, t, *J* 6.1, CH_2CN), 2.34 (1 H, m, 2'-H'), 2.17 (1 H, m, 2'-H), 2.11 (3 H, s, Ac) and 1.90 (3 H, s, 5-Me); $\delta(^{31}P)$ (81 MHz; $CDCl_3$) 142.4 and 142.1; *m/z* (FAB^+) 552 $(M + H)^+$.

General Procedure for the Preparation of O,O-*Dialkyl* 5'-O-*Monomethoxytrityl*-3'-*thiothymidin*-3'-*yl Phosphorothiolates* **12a–b**.—To a solution of the disulfide **8** (135 mg, 0.186 mmol) in dry THF (3 cm^3) was added the appropriate trialkyl phosphite (1.86 mmol) and the reaction mixture was stirred at room temperature for *ca.* 20 h before being partitioned between CH_2Cl_2 (20 cm^3) and brine (20 cm^3). After drying ($MgSO_4$), the organic phase was evaporated and the residue was purified by silica gel chromatography with CH_2Cl_2 containing 2–3% methanol as the eluent. The pure fractions were evaporated to dryness, redissolved in CH_2Cl_2, and precipitated into light petroleum.

O,O-*Dimethyl* 5'-O-*monomethoxytrityl*-3'-*thiothymidin*-3'-*yl phosphorothiolate* **12a**. 83% Yield (Found: C, 59.9; H, 5.6; N, 4.3. $C_{32}H_{35}N_2O_8PS$ requires C, 60.18; H, 5.52; N, 4.39%); δ_H(200 MHz; $CDCl_3$) 8.39 (1 H, s, NH), 7.62 (1 H, s, 6-H), 7.45–7.23 (12 H, m, ArH), 6.84 (2 H, d, *J* 8.7, C_6H_4OMe), 6.24 (1 H, t, *J* 5.0, 1'-H), 4.02 (2 H, m, 4'- and 3'-H), 3.80 (3 H, s, OMe), 3.72 (3 H, d, J_{PH} 12.8, POMe), 3.69 (3 H, d, J_{PH} 12.8, POMe), 3.58–3.46 (2 H, m, 5'-H_2), 2.65 (2 H, m, 2'-H_2) and 1.41 (3 H, s, 5-Me); $\delta(^{31}P)$ (81 MHz; $CDCl_3$) 29.4; *m/z* (FAB^-) 637 $(M - H)^-$ and 623 $(M - CH_3)^-$.

O,O-*Diethyl* 5'-O-*monomethoxytrityl*-3'-*thiothymidin*-3'-*yl phosphorothiolate* **12b**. 75% Yield (Found: C, 60.9; H, 6.0; N, 4.1. $C_{34}H_{39}N_2O_8PS$ requires: C, 61.25; H, 5.90; N, 4.20%); δ_H(200 MHz; $CDCl_3$), 8.65 (1 H, s, NH), 7.60 (1 H, s, 6-H), 7.29–7.43 (12 H, m, ArH), 6.86 (2 H, d, *J* 8.9, C_6H_4OMe), 6.25 (1 H, t, *J* 4.9, 1'-H), 4.07 (6 H, m, 2 × OCH_2, 4'- and 3'-H), 3.79 (3 H, s, OMe), 3.61–3.39 (2 H, m, 5'-H_2), 2.66 (2 H, m, 2'-H_2), 1.42 (3 H, s, 5-Me), 1.28 (6 H, 2 × t, *J* 7.0, 2 × Me); $\delta(^{31}P)$ (81 MHz; $CDCl_3$) 25.7; *m/z* (FAB^-) 665 $(M - H)^-$ and 637 $(M - Et)^-$.

General Procedure for Michaelis–Arbusov Reactions using Nucleoside 5'-*Phosphites*.—To the disulfide **8** (105 mg, 0.143

mmol, dried by coevaporation of dry pyridine and dry toluene) a solution of a nucleoside phosphite **11** (3 mol equiv.) in dry toluene (2 cm^3) was added. The reaction mixture was stirred at the requisite temperature until the reaction was judged to be complete (Table 1), and was then evaporated, and the product was isolated, as a mixture of diastereoisomers, by silica gel chromatography (CH_2Cl_2 containing 3–5% MeOH). After evaporation of the solvents the pure fractions were dissolved in CH_2Cl_2 and precipitated into light petroleum (see Table 1 for yields).

Methyl-protected dinucleoside 3′-S-*phosphorothiolate triester* **14**. (Found: FAB *m/z* $(M + H)^+$, 891.2676. $C_{43}H_{48}N_4O_{13}PS$ requires (M + H), 891.2676); δ_H(200 MHz; $CDCl_3$) 9.67* (1 H, s), 9.62* (1 H, s), 7.59* (1 H, s), 7.43–7.24 (13 H, m), 6.84 (2 H, d, *J* 8.8), 6.33 (2 H, m), 5.26 (1 H, m), 4.36–4.11 (4 H, m), 3.89 (1 H, m), 3.79 (3 H, s), 3.69* (3 H, d, J_{PH} 12.8), 3.60 (1 H, m), 3.43 (1 H, m), 2.68 (2 H, m), 2.37 (1 H, m), 2.17 (1 H, m), 2.10 (3 H, s), 1.90* (3 H, s) and 1.45* (3 H, s); $\delta(^{31}P)$ (81 MHz; $CDCl_3$) 29.2 and 29.0.

2-Cyanoethyl-protected dinucleoside 3′-S-*phosphorothiolate triester* **15**. [Found: FAB *m/z* $(M + H)^+$, 930.2785. $C_{45}H_{49}N_5O_{13}PS$ requires (M + H), 930.2785]; δ_H(200 MHz; $CDCl_3$) 9.40* (2 H, s), 7.58* (1 H, s), 7.57–7.20 (13 H, m), 6.84 (2 H, d, *J* 8.9), 6.27 (2 H, m), 5.28 (1 H, m), 4.40–4.04 (7 H, m), 3.79* (3 H, s), 3.56 (1 H, m), 3.43 (1 H, m), 2.78–2.64 (4 H, m), 2.37 (1 H, m), 2.23 (1 H, m), 2.10 (3 H, s), 1.90 (3 H, s) and 1.45* (3 H, s); $\delta(^{31}P)$ (81 MHz; $CDCl_3$) 26.0 and 26.4.

General Procedure for the Demethylation of Nucleoside 3′-S-*Phosphorothiolate Triesters*.—To the triester [*e.g.*, **12a** (104 mg, 0.162 mmol)] was added a mixture of 1,4-dioxane–triethylamine–benzenethiol (5:4:1, v/v/v; 2.5 cm^3) and the solution was stirred for 1–2 h at room temperature. Once the reaction was complete the solvents were evaporated off under reduced pressure, the residue was dissolved in CH_2Cl_2, the solution was washed with saturated brine, dried (Na_2SO_4), and evaporated, and the residue was purified by silica gel chromatography with CH_2Cl_2 containing 2% NEt_3 and 4–6% methanol as the eluent. The products were isolated in ~80% yield as their triethylammonium salts after precipitation.

Methyl 5′-O-*monomethoxytrityl*-3′-*thiothymidin*-3′-*yl hydrogen phosphorothiolate, triethylammonium salt* **13**. δ_H(200 MHz; $CDCl_3$) 7.55 (1 H, s, 6-H), 7.40–7.11 (12 H, m, ArH), 6.75 (2 H, d, *J* 8.9, C_6H_4OMe), 6.12 (1 H, t, *J* 5.9, 1′-H), 4.02 (1 H, m, 4′-H), 3.77 (1 H, m, 3′-H), 3.71 (3 H, s, OMe), 3.44 (2 H, m, 5′-H_2), 3.43 (3 H, d, J_{PH} 12.3, POMe), 2.97 [6 H, q, *J* 7.3, $N(CH_2Me)_3$], 2.62 (2 H, m, 2′-H_2), 1.33 (3 H, s, 5-Me) and 1.22 [9 H, t, *J* 7.3, $N(CH_2Me)_3$]; $\delta(^{31}P)$ (81 MHz; $CDCl_3$) 16.3; *m/z* (FAB^-) 623 $(M - H)^-$.

5′-O-*Monomethoxytrityl*-3′-*thiothymidylyl*-(3′-5′)-(3′-O-*acetylthymidine*), *triethylammonium salt* **16**. δ_H(200 MHz; $CDCl_3$) 9.10 (2 H, br s), 7.70 (1 H, s), 7.53 (1 H, s), 7.41–7.18 (12 H, m), 6.84 (2 H, d, *J* 8.8), 6.38 (1 H, t, *J* 5.8), 6.22 (1 H, t, *J* 5.9), 5.32 (1 H, m), 4.17–3.77 (5 H, m), 3.80 (3 H, s), 3.48 (2 H, m), 3.06 (6 H, q, *J* 8.8), 2.71 (2 H, m), 2.28 (2 H, m), 2.06 (3 H, s), 1.89 (3 H, s), 1.43 (3 H, s) and 1.29 (9 H, t, *J* 8.8); $\delta(^{31}P)$ (81 MHz; $CDCl_3$) 16.7; *m/z* (FAB^-) 875 $(M - H)^-$.

5′-O-*Monomethoxytrityl*-3′-*thiothymidylyl*-(3′-5′)-[3′-O-(tert-*butyldimethylsilyl*)*thymidine*] **22**. δ_H(200 MHz; $CDCl_3$) 7.65 (1 H, s), 7.60 (1 H, s), 7.37–7.19 (12 H, m), 6.78 (2 H, d, *J* 8.7), 6.26 (1 H, t, *J* 5.7), 6.16 (1 H, t, *J* 5.8), 4.38 (1 H, m), 4.12 (1 H, m), 3.96 (2 H, m), 3.86 (2 H, m), 3.73 (3 H, s), 3.43 (2 H, m), 3.01 (6 H, q, *J* 7.7), 2.67 (2 H, m), 2.10 (2 H, m), 1.84 (3 H, s), 1.43 (3 H, s), 1.30 (9 H, t, *J* 8.8), 0.83 (9 H, s) and 0.1 (6 H, s); $\delta(^{31}P)$ (81 MHz; $CDCl_3$) 14.1; *m/z* (FAB^-) 947 $(M - H)^-$.

3′-O-(tert-*Butyldimethylsilyl*)*thymidin*-5′-*yl Phosphonate, Triethylammonium Salt* **17**.—To a stirred solution of phosphorus trichloride (1.2 cm^3, 14 mmol) and *N*-methylmorpholine (15.7 cm^3, 140 mmol) in anhydrous CH_2Cl_2 (14 cm^3) was added 1,2,4-triazole (3.2 g, 47 mmol). After 30 min the reaction mixture was cooled to 0 °C. A solution of 3′-*O*-(*tert*-butyldimethylsilyl)thymidine (1.0 g, 2.8 mmol, previously dried by coevaporation with anhydrous acetonitrile) in dry CH_2Cl_2 (38 cm^3) was added dropwise over a period of 20 min and the mixture was stirred for a further 10 min. The reaction mixture was poured into aq. triethylammonium hydrogen carbonate (TEAB) (1.0 mol dm^{-3}, pH 8.5; 110 cm^3) and the organic phase was separated. The aqueous phase was reextracted with CH_2Cl_2 (200 cm^3) and the combined organic phases were dried (Na_2SO_4) and evaporated to give a viscous oil. Silica gel chromatography (CH_2Cl_2 containing 2% NEt_3 and 3–5% MeOH) and evaporation of the pooled fractions yielded the title compound **17** as the triethylammonium salt (1.29 g, 89%). In some preparations it was necessary to remove small amounts of contaminating 1,2,4-triazole by extracting the pooled fractions with TEAB (0.1 mol dm^{-3}). The product had δ_H(400 MHz; $CDCl_3$) 9.18 (1 H, br s, NH), 7.74 (1 H, s, 6-H), 6.84 (1 H, d, J_{PH} 617, HP), 6.34 (1 H, t, *J* 7.2, 1′-H), 4.49 (1 H, m, 4′-H), 4.08 (1 H, m, 3′-H), 4.02 (2 H, m, 5′-H_2), 3.10 (6 H, q, *J* 7.2, 3 × CH_2), 2.18 (2 H, m, 2′-H_2), 1.94 (3 H, s, 5-Me), 1.32 (9 H, t, *J* 7.2, 3 × Me), 0.88 (9 H, s, Bu^t) and 0.08 (6 H, s, 2 × SiMe); $\delta(^{31}P)$ (81 MHz; $CDCl_3$) 6.3; *m/z* (FAB^-) 419 $(M - Et_3HN)^-$.

3′-O-(tert-*Butyldimethylsilyl*)*thymidin*-5′-*yl Methyl Phosphonate* **19**.—To a solution of compound **17** (1 g, 1.92 mmol) and methyl chloromethanoate (0.15 cm^3, 1.92 mmol) in anhydrous CH_2Cl_2 (7.5 cm^3) was added pyridine (0.16 cm^3, 1.92 mmol) dropwise. After 30 min the reaction mixture was partitioned between CH_2Cl_2 and saturated aq. $NaHCO_3$. The organic phase was dried ($MgSO_4$) and evaporated to give an oil. Silica gel chromatography (CH_2Cl_2 containing 2–6% MeOH) and evaporation of the pooled fractions yielded the title compound (728 mg, 87%); δ_H(200 MHz; $CDCl_3$) 8.75 (1 H, br s, NH), 7.31 (1 H, s, 6-H), 6.79 (1 H, d, J_{PH} 704, HP), 6.20 (1 H, t, *J* 7.2, 1′-H), 4.31 (1 H, m, 4′-H), 4.16 (2 H, m, 5′-H_2), 3.90 (1 H, m, 3′-H), 3.72 (3 H, d, J_{PH} 11.8, POMe), 2.14 (2 H, m, 2′-H_2), 1.85 (3 H, s, 5-Me), 0.80 (9 H, s, Bu^t) and 0.02 (6 H, s, 2 × SiMe); $\delta(^{31}P)$ (81 MHz; $CDCl_3$) 12.0 and 11.1; *m/z* (FAB^+) 435 $(M + H)^+$.

General Procedure for the Preparation of Nucleoside Silyl Phosphites.—To a solution of the H-phosphonate (1.0 mmol, dried by coevaporation with anhydrous acetonitrile) in dry CH_2Cl_2 (2 cm^3) was added bis(trimethylsilyl)trifluoroacetamide (0.4 cm^3, 1.47 mmol). ^{31}P NMR spectroscopy showed that the silyl phosphites were formed in almost quantitative yield within 15 min, and these products were used directly in the Michaelis–Arbusov reaction without isolation.

3′-O-(tert-*Butyldimethylsilyl*)*thymidin*-5′-*yl bis*(*trimethylsilyl*) *phosphite* **18**. $\delta(^{31}P)$ (81 MHz; $CDCl_3$) 116.0.

3′-O-(tert-*Butyldimethylsilyl*)*thymidin*-5′-*yl methyl trimethylsilyl phosphite* **20**. δ(31P) (81 MHz; $CDCl_3$) 122.0.

5′-O-*Monomethoxytrityl*-3′-*thiothymidylyl*-(3′-5′)-[3′-O-(tert-*butyldimethylsilyl*)*thymidine*] *Triethylammonium Salt* **21**.—To a solution of the bis(trimethylsilyl) phosphite **18** (0.96 mmol) in anhydrous CH_2Cl_2 (2 cm^3) was added the disulfide **8** (698 mg, 0.96 mmol) in the above solvent (1 cm^3). An intense red colour was formed and after 30 min ^{31}P NMR spectroscopy indicated the complete conversion of starting material into a new product. The reaction was then quenched with water (0.1 cm^3) and subsequently partitioned between CH_2Cl_2 and saturated aq. $NaHCO_3$. The organic phase was dried (anhydrous $MgSO_4$) and reduced to give an oil. Silica gel chromatography (2% Et_3N–5% MeOH–CH_2Cl_2) gave the title

compound **21** (681 mg, 68%); δ_H(200 MHz; $CDCl_3$) 7.65 (1 H, s), 7.61 (1 H, s), 7.19–7.37 (12 H, m), 6.78 (2 H, d, *J* 8.1), 6.26 (1 H, t), 6.16 (1 H, t), 4.38 (1 H, m), 4.12 (1 H, m), 3.96 (2 H, m), 3.86 (2 H, m), 3.73 (3 H, s), 3.43 (2 H, m), 3.0 (6 H, q), 2.67 (2 H, m), 2.10 (2 H, m), 1.84 (3 H, s), 1.36 (3 H, s, 5-Me), 1.27 (9 H, t), 0.83 (9 H, s) and 0.01 (6 H, s); $\delta(^{31}P)$ (81 MHz; $CDCl_3$), 14.1; *m/z* (FAB^-) 947 (M^-).

Methyl-protected Dinucleoside 3'-S-Phosphorothiolate Triester **22**.—A solution of the disulfide **8** (828 mg, 1.15 mmol) in anhydrous CH_2Cl_2 (1 cm^3) was added to a solution of the trimethylsilyl phosphite **20** (1.15 mmol) in CH_2Cl_2 (2 cm^3). An intense red colour was formed and after 30 min ^{31}P NMR spectroscopy indicated the complete conversion of starting material into a new product. The reaction mixture was then quenched with water (0.1 cm^3) and partitioned between CH_2Cl_2 and saturated aq. $NaHCO_3$. The organic phase was dried ($MgSO_4$) and reduced to give an oil. Silica gel chromatography (3% MeOH in CH_2Cl_2) gave the *title compound* **22** (774 mg, 70%) [Found: *m/z* (FAB^+), 985.3255. (M + Na), $C_{47}H_{59}N_4NaO_{12}PSSi$ requires *m/z*, 985.3255]; δ_H(400 MHz; $CDCl_3$) 8.78* (2 H, s), 7.52* (1 H, s), 7.27 (13 H, m), 6.84 (2 H, d, *J* 8.1), 6.23 (2 H, m), 4.40 (1 H, m), 4.32 (1 H, m), 4.13 (1 H, m), 3.95 (2 H, m), 3.78 (3 H, s, OMe), 3.71* (3 H, d, J_{PH} 12.1), 3.30–3.65 (2 H, m), 2.60 (2 H, m), 2.13 (2 H, m), 2.08 (1 H, m), 1.87* (3 H, s), 1.44* (3 H, s), 0.88 (9 H, s) and 0.07 (6 H, s); $\delta(^{31}P)$ (81 MHz; $CDCl_3$) 27.0 and 26.7.

Acknowledgements

We are grateful to the Science and Engineering Research Council (X. L., G. K. S. and J. S. V.) and Ciba Central Research (G. K. S.) for financial support. We also thank Mr. A. Mills (Liverpool) and the SERC service at Swansea for obtaining FAB mass spectra.

References

1 J. S. Vyle, D. Kemp, R. Cosstick and B. A. Connolly, *Biochemistry*, 1992, **31**, 3012.
2 J. Piccirilli, J. S. Vyle, M. H. Caruthers and T. R. Cech, *Nature*, 1993, **361**, 85.
3 T. Murata, S. Iwai and E. Ohtsuka, *Heterocycles*, 1992, **33**, 529.
4 R. Cosstick and J. S. Vyle, *Tetrahedron Lett.*, 1989, **30**, 4693.
5 R. Cosstick and J. S. Vyle, *Nucleic Acids Res.*, 1990, **18**, 829.
6 X. Li, D. M. Andrews and R. Cosstick, *Tetrahedron*, 1992, **48**, 2729.
7 J. S. Vyle and R. Cosstick, unpublished results.
8 J. S. Vyle, X. Li and R. Cosstick, *Tetrahedron Lett.*, 1992, **33**, 3017.
9 R. G. Harvey, H. I. Jacobson and E. V. Jensen, *J. Am. Chem. Soc.*, 1963, **85**, 1618.
10 D. Cooper, S. Trippet and C. White, *J. Chem. Res.*, 1983, (*S*), 234.
11 C. E. Muller and H. J. Roth, *Tetrahedron Lett.*, 1990, **31**, 501.
12 The phosphoramidites **10b–d** were prepared according to the method of S. L. Beaucage and M. H. Caruthers, *Tetrahedron Lett.*, 1981, **22**, 1859.
13 M. Mag and J. W. Engels, *Nucleic Acids Res.*, 1989, **17**, 5973.
14 R. L. Letsinger and G. A. Heavner, *Tetrahedron Lett.*, 1975, 147.
15 M. Honjo, Y. Adachi and T. Maruyama, *J. Org. Chem.*, 1988, **53**, 4552.
16 J. Chojnowski, M. Cypryk and J. Michalski, *J. Organomet. Chem.*, 1981, **215**, 355.
17 T. Hata and M. Sekine, *J. Am. Chem. Soc.*, 1974, **96**, 7363.
18 B. C. Froehler, P. G. Ng and M. D. Matteucci, *Nucleic Acids Res.*, 1986, **14**, 5399.
19 D. G. Hewitt, *Aust. J. Chem.*, 1979, **32**, 463.
20 X. Li and R. Cosstick, *J. Chem. Soc., Perkin Trans. 1*, 1993, 1091.

Paper 4/01160K
Received 25*th February* 1994
Accepted 10*th March* 1994