Synthesis of Deoxydinucleoside Phosphates Containing 4-Thio Substituted Pyrimidine Nucleobases

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The preparations, by the hydrogenphosphonate approach in solution, of the five pyrimidine deoxydinucleoside monophosphates **14a**, **14b**, **16a**, **16b** and **17** containing a 4-thiosubstituted pyrimidine nucleobase at either their 3'- or 5'-end are described. These syntheses were conveniently accomplished using 4-thionucleoside derivatives having their sulfur function protected by *S*-pivaloyloxymethylation. The behaviour of unprotected 4-thionucleoside derivatives was also investigated and they were shown to give comparable overall yields.

Modified oligodeoxynucleotides are currently attracting considerable attention. So far most of the known modifications have been introduced either on the phosphate backbone or in the sugar moieties. Another domain of interest is the case of base-modified oligonucleotides.[1]

As part of our ongoing research in this series, we have needed substantial amounts of dinucleoside phosphates incorporating a sulfur-substituted pyrimidine such as 4-thiouracil (s^4U) or 4-thiothymine (s^4T) which are structurally closely related to the natural constituents of RNA and DNA, respectively. These nucleobase analogues which exhibit specific photochemical properties have been proposed as intrinsic photoaffinity probes for nucleic acid structure and interactions.[2] The photochemistry of s^4U is thought to occur *via* its triplet state and model studies with a number of its derivatives have disclosed two types of photochemical reactions, *i.e.* [2 + 2] cycloadditions with olefinic compounds and coupling reactions with hydrogen donors *via* hydrogen abstraction and radical recombination.[2,3] More recently, this photochemical behaviour has been observed in the dinucleotide series leading to the very efficient formation of (6–4) pyrimidine pyrimidinone photoproducts as well as new types of photoproducts.[4]

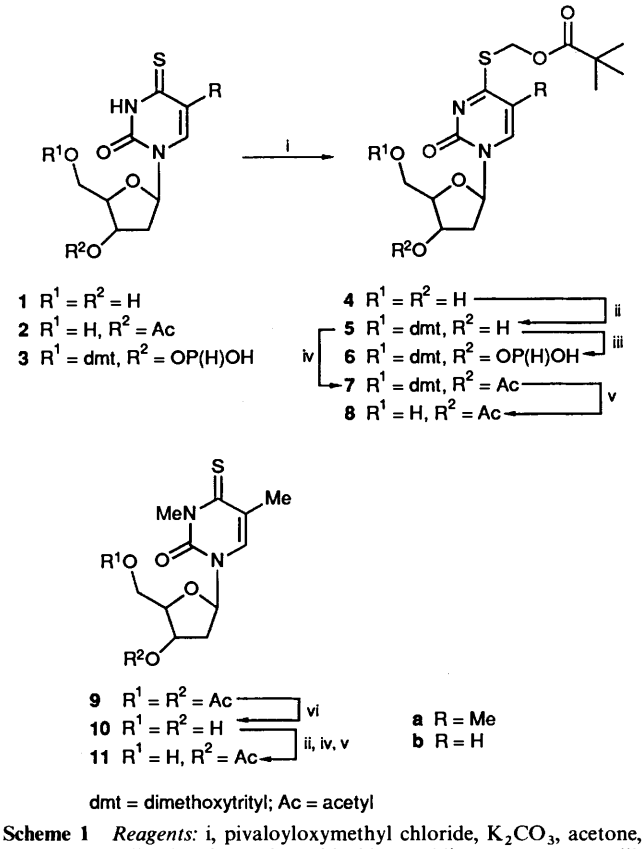
Herein we wish to report on the synthesis of the four dimers **14a**, **14b**, **16a** and **16b** incorporating s^4U and s^4T at either 5' or 3' position[5] and of the 3-*N*-methyl derivative **17**.

Results and Discussion

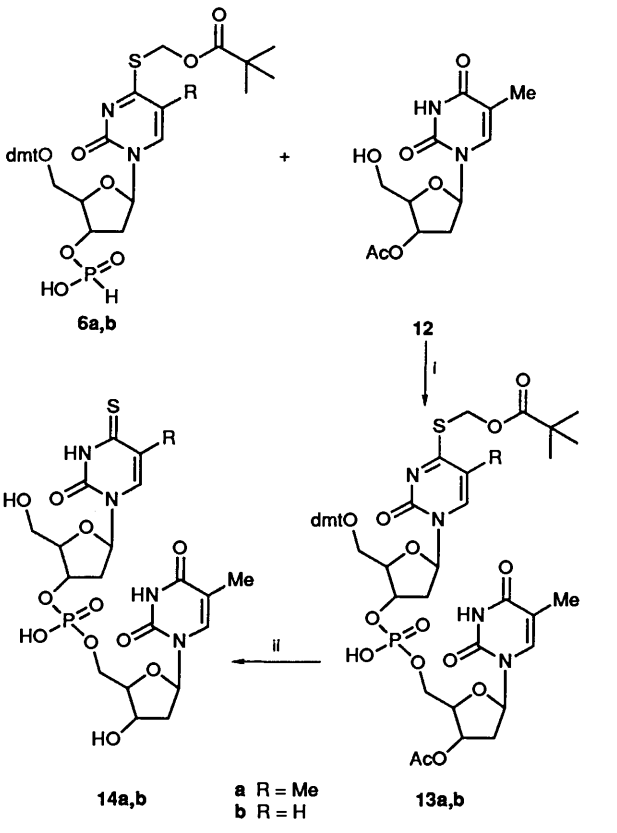
A number of strategies can be used for the thiolation of a pyrimidine base at the dinucleotide level. In a first instance,[4b] we have made use of the reactivity of 4-triazolylpyrimidinone which was developed by Sung.[6] The phosphoramidite of such a derivatized pyrimidine nucleoside can be prepared and used for oligonucleotide synthesis.[7] The last step of the synthesis consists of the displacement of the triazolyl group by the hydrosulfide anion.[4b] A recent report has introduced the use of thioacetate as a nucleophile for the substitution of the triazole residue.[8] In another version of this methodology, thioacetate displacement of a 4-thiophenyl or 4-*p*-nitrothiophenyl group, instead of 4-triazolyl, was proposed.[9]

For the sake of simplification, we searched to establish a route starting from an appropriately sulfur-protected 4-thionucleoside. Although phosphorylation reactions have been accomplished with nucleosides having unprotected nucleobases,[10] it is generally recommended to block all the potential nucleophilic positions of these moieties. Moreover, in the case of 4-thiouracil (or 4-thiothymine), careful attention should be paid not only to the nucleophilicity but also to the oxidative character of the mercapto function when using P^{III} phosphoramidite[11] or hydrogenphosphonate[12] chemistries whose protocols require iodine oxidation steps. Accordingly, sulfur protection by means of *S*-methylsulfonylation[13] and *S*-cyanoethylation[14] have recently been proposed to fulfil the requirements of the phosphoramidite methodology. In this laboratory, after several unfavourable results using various acyl groups to protect the sulfur function of these bases, we have found *S*-pivaloyloxymethylation to be the best choice,[5] showing perfect compatibility with the two P^{III}-based phosphorylation strategies. In our hands, it gave particularly good results for the semi-preparative synthesis (0.5 mmol scale) of dinucleoside phosphates containing 4-thiouracil (or 4-thiothymine) at either the 5' or the 3' end by the hydrogenphosphonate approach in solution. However, a recent paper has suggested that hydrogenphosphonylation of 5'-*O*-(4,4-dimethoxy)trityl-4-thiothymidine using the phosphorus trichloride–imidazole combination and condensation of the resulting hydrogenphosphonate **3a** with a 5'-*O*-unprotected nucleoside attached to a solid support was practicable to obtain 4-thiothymidine-containing oligonucleotides.[15] This prompted us to use 3'-acetyl-4-thiothymidine **2a** and 3'-acetyl-2'-deoxy-4-thiouridine **2b** in combination with a deoxynucleoside hydrogenphosphonate derivative of thymidine to prepare the corresponding dimers **16a** and **16b** in solution. We have found the overall yields to be comparable by the protected and the unprotected approach.

The phosphonate **6a** (or **6b**) needed for the preparation of the dimer **14a** (or **14b**) having a mercaptopyrimidine at the 5' end was synthesized by the following sequence (Scheme 1). Treatment of an acetone solution of 4-thiothymidine **1a** (or 2'-deoxy-4-thiouridine **1b**) with pivaloyloxymethyl chloride in the presence of potassium carbonate at room temperature afforded the corresponding 4-*S*-(pivaloyloxymethylthio)pyrimidin-2-one deoxyribofuranoside **4a** or **4b** in over 65% yields. Subsequent reaction of both derivatives with dimethoxytrityl chloride provided the 5'-protected nucleosides **5a** and **5b**, respectively. Their corresponding 3'-hydrogenphosphonates **6** were prepared by using two published procedures, either the phosphorus trichloride–imidazole system[16] or by activation of phosphorus acid with pivaloyl chloride.[17] In both cases, the expected hydrogenphosphonates **6a** and **6b** were obtained in comparable satisfactory yields. These compounds exhibited ^1H and ^{31}P NMR spectroscopic data in agreement with their proposed structures. Condensation of phosphonates **6a** or **6b** with 3'-*O*-acetylthymidine **12** (Scheme 2) was accomplished by means of bis(2-oxo-3-oxazolidinyl)phosphinic chloride[18] in a mixture of



Scheme 1 *Reagents:* i, pivaloyloxymethyl chloride, K_2CO_3, acetone, room temp.; ii, dimethoxytrityl chloride, pyridine, room temp.; iii, phosphorus trichloride, imidazole[16] or phosphorus acid, pivaloyl chloride;[17] iv, acetic anhydride, pyridine, room temp.; v, 3% trifluoroacetic acetic acid in CH_2Cl_2; vi, MeOH–H_2O–$N(Et)_3$, (8:1:1), room temp., 16 h



Scheme 2 *Reagents:* i (*a*), bis(2-oxo-3-oxazolidinyl)phosphinic chloride, acetonitrile–pyridine (1:1); (*b*), iodine, water–pyridine (1:9); ii (*a*), NH_4OH–pyridine (1:1); (*b*), 80% acetic acid; (*c*) RP 18 column chromatography, water–acetonitrile gradient

acetonitrile–pyridine (1:1). *In situ* oxidation of the resulting hydrogenphosphonate diester with iodine in water–pyridine (1:9) solution provided the expected protected dinucleoside phosphate **13a** or **13b**. Complete deprotection was carried out in two steps: overnight ammonia treatment [conc. ammonium hydroxide–pyridine (1:1)] at room temperature to eliminate simultaneously the pivaloyloxymethyl and acetyl groups followed 80% acetic acid treatment to eliminate the remaining 5′-*O*-dimethoxytrityl group. The crude dimer **14a** (or **14b**) was fully purified over a Lichroprep RP 18 (Merck, Art. 13900) reversed-phase column using a water–acetonitrile gradient as eluent.

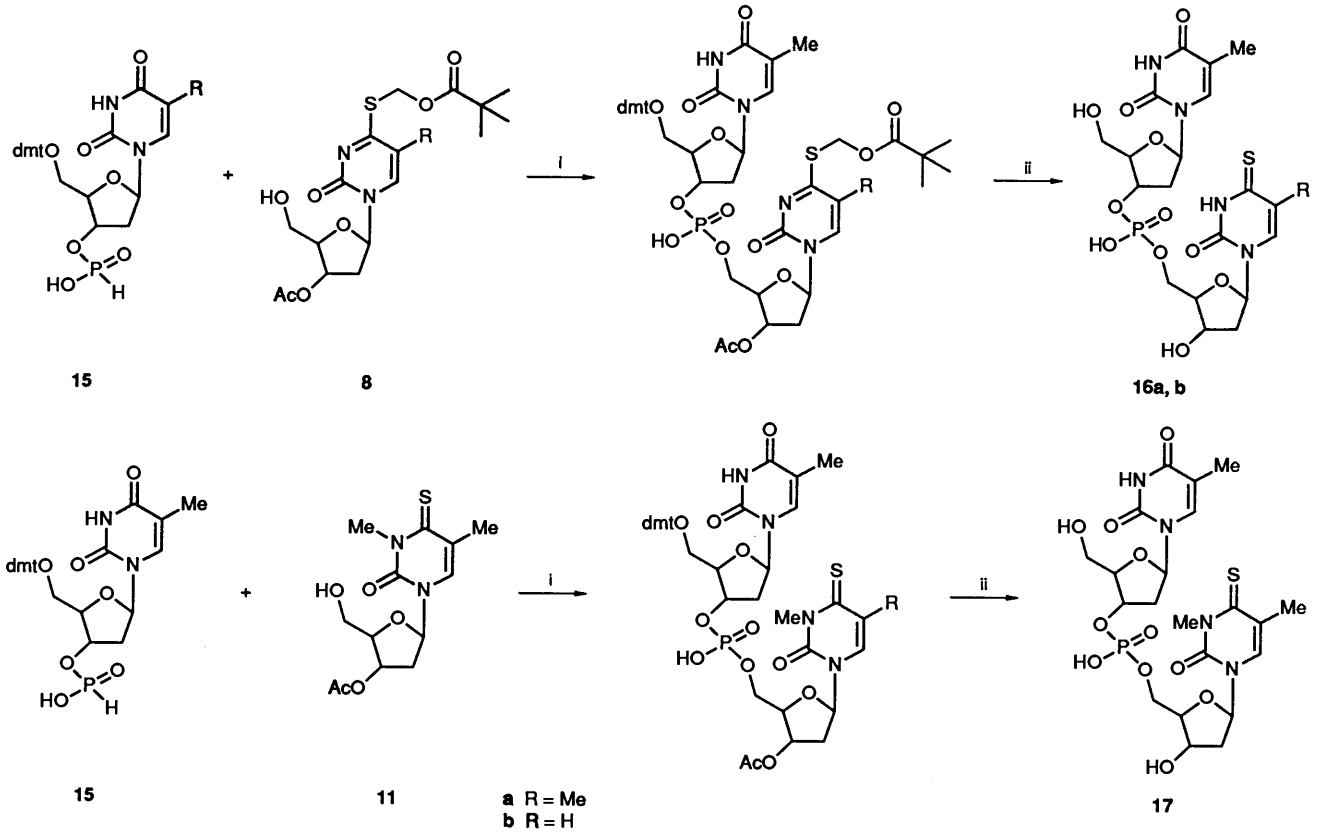
The introduction of a sulfur-modified residue at the 3′-end of a dimer requires the preparation of the corresponding 5′-OH free and 3′-*O*-acetylated nucleoside. The suitable intermediates **8a** or **8b** were obtained from 4-*S*-pivaloyloxymethylthionucleosides **4a** or **4b** in two fashions. In one case, the 5′-*O*-dimethoxytritylated derivative **5b** was acetylated and the resulting compound deprotected by acidic treatment to give intermediate **8b**. Alternatively, **8a** was prepared in two steps: one pot tritylation and acetylation of **4a** followed by detritylation.

Similarly, we have synthesized compound **11** which to our knowledge is unknown in the literature. It was prepared starting from 3′,5′-di-*O*-acetylthymidine which after treatment with methyl iodide in the presence of K_2CO_3 in acetonitrile afforded 3′,5′-di-*O*-acetyl-N^3-methylthymidine in 95% yield. Thiation of the latter using phosphorus pentasulfide in dioxane gave the corresponding 3′,5′-di-*O*-acetyl-N^3-methyl-4-thiothymidine **9** in moderate yield (45%). This is probably due to the fragile character of the glycosidic bond as manifested by the isolation of N^3-methyl-4-thiothymine. Subsequently, compound **9** was smoothly deacetylated using a mixture of methanol–triethylamine–water (8:1:1) to give **10** in 92% yield. It served to prepare 3′-*O*-acetyl-N^3-methyl-4-thiothymidine **11** by following the same route as above for **8**.

These 3′-*O*-acetyl derivatives **8a**, **8b** and **11** were employed in a phosphorylation reaction by the hydrogenphosphonate approach using (5′-*O*-dimethoxytrityl)thymidin-3′-yl hydrogenphosphonate **15** as described in the previous examples. However, in the case of **17**, the deacetylation step was carried out in a mixture of methanol–triethylamine–water (8:1:1) overnight at room temperature (Scheme 3).

Finally, we examined the possibility of preparing the two dimers **16a** and **16b** using derivatives having no protection at the sulfur position. Thus, **15** was condensed either with 3′-*O*-acetyl-4-thiothymidine **2a** or 3′-*O*-acetyl-2′-deoxy-4-thiouridine **2b**. These two nucleosides were prepared from the known 5′-*O*-dimethoxytrityl-4-thiothymidine[19] and 5′-*O*-dimethoxytrityl-2′-deoxy-4-thiouridine[19] in two steps (acetylation, detritylation) without purification of the intermediates. The overall yields of fully purified dimers after successive condensation, oxidation, deprotection and reverse phase chromatography were in the range of 56–58% which compare well with those obtained by the above described sequence including the sulfur protection step.

In conclusion, dinucleoside phosphates containing either 4-thiouracil or 4-thiothymine have been prepared in satisfactory yields by the hydrogenphosphonate approach in solution. The pivaloyloxymethyl group has been found suitable for the protection of the sulfur function of the modified nucleic acid bases. However, in the case of simple dimers, this protection, that we recommend for the multistep solid phase synthesis of oligonucleotides,[20] can be omitted since the overall yield of the complete sequence remains within an acceptable range.



Scheme 3 *Reagents:* i (*a*), bis(2-oxo-3-oxazolidinyl)phosphinic chloride, acetonitrile–pyridine (1:1); (*b*), iodine, water–pyridine (1:9); ii (*a*), NH_4OH–pyridine (1:1); (*b*), 80% acetic acid; (*c*), RP 18 column chromatography, water–acetonitrile gradient

Experimental

^1H, ^{13}C and ^{31}P NMR spectra were recorded on Bruker AC 250 or AM 400, Bruker AC 250 and Bruker AC 300-P spectrometers, respectively. ^1H chemical shifts (δ_H) are reported either relative to tetramethylsilane (TMS) in deuteriochloroform ($CDCl_3$) or to 3-(trimethylsilyl)propionic acid sodium salt (TSP) in deuterium oxide (D_2O), *J* values are given in Hz. ^{13}C chemical shifts (δ_C) are reported relative to $CDCl_3$ (77.7 ppm) in $CDCl_3$ and to dioxane (67.8 ppm) in D_2O. ^{31}P chemical shifts (δ_P) are reported relative to an external capillary standard of 85% phosphoric acid.

Mass spectroscopy was carried out using a A.E.I. M.S. 50 instrument for electron impact spectra or a Kratos M.S. 80 machine for high resolution measurements (HRMS) or fast atom bombardment spectra (FAB) using thioglycerol as the matrix.

M.p.s were determined with a Reichert apparatus and are uncorrected.

Pyridine and acetonitrile (HLPC grade, $H_2O < 0.05\%$) were dried by heating, under reflux, with calcium hydride and phosphorus pentoxide (P_2O_5), respectively. Phosphorus trichloride and pivaloyl chloride were freshly distilled prior to use. Imidazole was sublimed before use.

5′-*O*-Dimethoxytritylthymidine 3′-hydrogenphosphonate triethylammonium salt **15** was synthesized according to published procedures.[16,17] Phosphonates and acetates were dried at room temp. in a dessicator over P_2O_5 under vacuum one night before condensation.

All reactions were carried out at room temperature unless otherwise stated.

Chromatography was performed on Merck 7729 silica gel. Thin layer chromatography (TLC) was run on pre-coated silica gel sheets with luminescer (254 nm) manufactured by Schleicher and Schuell (ref 394 732). Lichroprep RP 18 (Merck 13 900) was employed for reversed-phase chromatography.

3′,5′-*Di*-O-*acetyl*-N^3-*methylthymidine*.—To a stirred solution of 3′,5′-di-*O*-acetylthymidine (4.0 g, 12.3 mmol) in acetonitrile (60 cm^3) was added K_2CO_3 (4.0 g, 2.4 equiv.) followed by iodomethane (4 cm^3, 5.2 equiv.) After 3 h, the reaction was filtered on Celite and washed with brine. The organic phase was collected, dried over sodium sulfate and concentrated *in vacuo*. The crude product was filtered on a silica gel column with dichloromethane (CH_2Cl_2) as eluent leading to 3′,5′-di-*O*-acetyl-N^3-methylthymidine (3.98 g, 95%) (HRMS Found: M^+, 340.1270. $C_{15}H_{20}N_2O_7$ requires *M*, 340.1270); $\delta_H(CDCl_3)$, 1.95 (3 H, s), 2.14 (6 H, s), 2.26 (1 H, dt, *J* 14.5, 6.9), 2.50 (1 H, br dd, *J* 14.5, 6.1), 3.31 (3 H, s), 4.28 (1 H, br s), 4.36 (2 H, br s), 5.26 (1 H, dd, *J* 6.1, 2.3), 6.35 (1 H, t, *J* 6.1) and 7.36 (1 H, s); $\delta_C(CDCl_3)$ 13.4, 20.9, 27.9, 37.6, 64.0, 74.3, 82.2, 85.8, 110.3, 132.9, 151.0, 163.4, 170.3 and 170.4.

3′,5′-*Di*-O-*acetyl*-N^3-*methyl*-4-*thiothymidine* **9**.—To a stirred solution of 3′,5′-di-*O*-acetyl-N^3-methylthymidine (3.98 g, 11.7 mmol) in dioxane (75 cm^3) at 80 °C was added P_2S_5 (2.6 g). After 90 min, the reaction was filtered on Celite, concentrated *in vacuo* and purified by flash chromatography using heptane–ethyl acetate (9:1) as eluent to give **13** (1.89 g, 45%) (HRMS Found: M^+, 356.1038. $C_{15}H_{20}N_2O_6S$ requires *M*, 356.1042); $\delta_H(CDCl_3)$ 2.11 (6 H, s), 2.16 (3 H, s, 1 H, m), 2.58 (1 H, ddd, *J* 14.5, 6.1, 2.3), 3.79 (3 H, s), 4.30 (1 H, m), 4.38 (2 H, m), 5.22 (1 H, dt, *J* 6.1, 2.3), 6.28 (1 H, dd, *J* 8.4, 6.1) and 7.37 (1 H, s); $\delta_C(CDCl_3)$ 19.9, 21.5, 36.1, 38.7, 64.3, 74.7, 83.2, 87.1, 120.5, 128.2, 149.5, 170.8, 171.0 and 191.9.

N^3-*Methyl*-4-*thiothymidine* **10**.—A solution of 3′,5′-di-*O*-acetyl-N^3-methyl-4-thiothymidine **9** (680 mg, 1.91 mmol) in MeOH–TEA–H_2O (8:1:1) (10 cm^3) was stirred at room temperature for 5 h. The solution was then concentrated *in vacuo* and the residue flash chromatographed using CH_2Cl_2–

MeOH (98:2) as eluent to give **10** (478 mg, 92%), m.p. 114–117 °C (from ethyl acetate) (Found: C, 48.55; H, 5.6; N, 10.35; S, 11.85. $C_{11}H_{16}N_2O_4S{\cdot}H_2O$ requires C, 48.52; H, 5.92; N, 10.29; S, 11.70%); $\delta_H(CDCl_3)$ 2.16 (3 H, s), 2.42 (2 H, t, *J* 6.9), 3.80 (3 H, s), 3.87 (1 H, dd, *J* 11.8, 3.0), 3.97 (1 H, dd, *J* 11.8, 3.8), 4.06 (1 H, q, *J* 3.8), 4.61 (1 H, q, *J* 4.6), 6.18 (1 H, t, *J* 6.9) and 7.51 (1 H, s); $\delta_C(D_2O)$ 19.6, 36.6, 40.6, 62.2, 71.4, 88.2, 121.3, 132.2, 151.0 and 192.4.

4-S-*Pivaloyloxymethyl*-4-*thiothymidine* **4a**.—To a stirred solution of 4-thiothymidine **1a** (4.32 g, 16.7 mmol) in acetone (100 cm^3), was added K_2CO_3 (5.8 g, 2.5 equiv.). After 15 min, pivaloyloxymethylchloride (5.5 cm^3, 2.3 equiv.) was added dropwise and the reaction stirred 18 h. The mixture was then filtered on Celite, the filtrate concentrated under reduced pressure, diluted with CH_2Cl_2, washed with brine, and dried over sodium sulfate. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel, eluting with CH_2Cl_2–CH_3OH (95:5) to give the title compound **4a** (4.25 g, 68.5%). Crystallization of the latter material from H_2O gave colourless crystals; m.p. 128–130 °C (Found: C, 51.6; H, 6.4; N, 7.35; S, 8.8. $C_{16}H_{24}N_2O_6S$ requires C, 51.61; H, 6.45; N, 7.53; S, 8.60%); $\delta_H(CDCl_3)$ 1.14 (9 H, s), 1.99 (3 H, s), 2.24 (1 H, m), 2.54 (1 H, m), 3.88 (2 H, br s), 4.06 (1 H, br s), 4.53 (3 H, br s), 5.76 (2 H, s), 6.15 (1 H, t, *J* 6.1) and 8.00 (1 H, s); $\delta_C(CDCl_3)$ 14.7, 27.5, 39.4, 41.7, 61.5, 62.3, 71.0, 88.3, 88.5, 113.2, 140.4, 154.6, 176.1 and 179.0.

4-S-*Pivaloyloxymethyl*-2′-*deoxy*-4-*thiouridine* **4b**.—Compound **1b** (2.4 g, 9.8 mmol) was treated as described for the preparation of **4a** to give **4b** in 66.5% yield (HRMS Found: M^+, 358.1191. $C_{15}H_{22}N_2O_6S$ requires *M*, 358.1199); $\delta_H(CDCl_3)$ 1.17 (9 H, s), 2.26 (1 H, m), 2.60 (1 H, m), 3.89 (2 H, br s), 4.11 (3 H, br s), 4.53 (1 H, br s), 5.74 (2 H, m), 6.16 (1 H, t, *J* 5.3), 6.35 (1 H, d, *J* 6.9) and 8.25 (1 H, d, *J* 6.9); $\delta_C(CDCl_3)$ 27.5, 39.4, 41.9, 61.8, 62.2, 71.1, 88.5, 104.5, 142.7, 154.8, 176.0 and 179.0.

5′-O-*Dimethoxytrityl*-4-S-*pivaloyloxymethyl*-4-*thiothymidine* **5a**.—To a solution of 4-*S*-pivaloyloxymethyl-4-thiothymidine **4a** (280 mg, 0.75 mmol) in pyridine (5 cm^3) was added dimethoxytrityl chloride (300 mg, 0.88 mmol, 1.17 equiv.). The solution was stirred overnight at room temperature. The solvent was then removed under reduced pressure and the residue diluted with CH_2Cl_2, washed with sodium hydrogen carbonate and twice with brine. The organic phase was dried over sodium sulfate and concentrated *in vacuo*. The crude product was purified by flash chromatography using heptane–ethyl acetate (25:75) to give **5a** (280 mg, 55.4%); FAB (thioglycerol + LiCl) *m/z* 681 $(M + Li^+)^+$; $\delta_H(CDCl_3)$ 1.18 (9 H, s), 1.56 (3 H, s), 2.30 (1 H, m), 2.79 (1 H, m), 3.43 (2 H, m), 3.78 (6 H, s), 4.20 (1 H, br s), 4.60 (1 H, br s), 5.82 (2 H, m), 6.37 (1 H, t, *J* 6.5), 6.82 (4 H, d, *J* 9.1), 7.13–7.52 (9 H, m) and 7.96 (1 H, s); $\delta_C(CDCl_3)$ 14.1, 27.5, 39.3, 42.9, 55.9, 61.3, 63.9, 72.3, 87.3, 87.7, 112.8, 113.9, 127.7, 128.6, 130.7, 136.1, 139.3, 145.0, 154.3, 159.3, 175.8 and 179.1

5′-O-*Dimethoxytrityl*-4-S-*pivaloyloxymethyl*-2′-*deoxy*-4-*thiouridine* **5b**.—Compound **4b** (1.0 g, 2.8 mmol) was tritylated as described for the preparation of **5a** in 75.5% yield; FAB (thioglycerol + LiCl) *m/z* 667 $(M + Li^+)^+$; $\delta_H(CDCl_3)$ 1.18 (9 H, s), 2.28 (1 H, m), 2.71 (1 H, m), 3.47 (2 H, m), 3.79 (6 H, s), 4.14 (1 H, br s), 4.56 (1 H, m), 5.78 (2 H, m), 5.90 (1 H, d, *J* 6.9), 6.24 (1 H, t, *J* 5.3), 6.83 (4 H, d, *J* 8.4), 7.13–7.47 (9 H, m) and 8.13 (1 H, d, *J* 6.9); $\delta_C(CDCl_3)$ 27.5, 39.4, 42.6, 55.9, 61.5, 63.1, 71.3, 87.0, 87.7, 103.8, 113.9, 127.8, 128.7, 130.7, 136.0, 141.8, 144.9, 154.4, 159.3, 175.2 and 179.0.

5′-O-*Dimethoxytrityl*-4-(S-*pivaloyloxymethyl*)-4-*thiothymidin*-3′-*yl Hydrogenphosphonate* (*Triethylammonium Salt*) **7a**.—*Method A*.[16] To a stirred solution of imidazole (1.5 g, 22 mmol) in CH_2Cl_2 (10 cm^3) was added diisopropylethylamine (4.5 cm^3, 26 mmol) followed by slow introduction of PCl_3 (250 cm^3, 2.86 mmol). After 30 min, the solution was chilled to 0 °C and **6a** (280 mg, 0.415 mmol) in CH_2Cl_2 (2.5 cm^3) was added. One hour later, the reaction was diluted with CH_2Cl_2, washed twice with a 1 mol dm^{-3} triethylammonium hydrogen carbonate solution, dried over sodium sulfate and concentrated *in vacuo*. The crude product was purified by flash chromatography using a gradient of MeOH in ethyl acetate (from 0 to 30%) to give the expected hydrogenphosphonate (268 mg, 77%).

Method B.[17] This compound was also synthesized accord' 'g to Stawinski's method using pivaloyl chloride on a 0.49 mmol scale in 52.5% yield; FAB *m/z* 737 $(M - H^+)^-$; $\delta_P(CDCl_3)$ −3.79 (J_{PH} 622.1; $^3J_{PH}$ 8.5); $\delta_H(CDCl_3)$ 1.20 (9 H, s), 1.34 (9 H, t, *J* 7.3), 1.50 (3 H, s), 2.37 (1 H, m) and 2.83 (1 H, m), 3.06 (6 H, m), 3.38 (1 H, dd, *J* 10.7, 3.0), 3.50 (1 H, dd, *J* 10.7, 2.6), 3.78 (6 H, s), 4.30 (1 H, m), 4.97 (1 H, m), 5.85 (2 H, s), 6.35 (1 H, t, *J* 6.2), 6.82 (4 H, d, *J* 8.8), 6.86 (1 H, d, *J* 622.1), 7.13–7.45 (9 H, m) and 7.88 (1 H, s).

5′-O-*Dimethoxytrityl*-4-(S-*pivaloyloxymethyl*)-2′-*deoxy*-4-*thiouridin*-3′-*yl Hydrogenphosphonate* (*Triethylammonium Salt*) **6b**.—This compound was prepared following either the above procedure A on 0.73 mmol in 67% yield or procedure B on a 0.5 mmol scale in 58.2% yield; FAB *m/z* 723 $(M - H^+)^-$; $\delta_P(CDCl_3)$ −3.61 (J_{PH} 621.2); $\delta_H(CDCl_3)$ 1.20 (9 H, s), 1.32 (9 H, t, *J* 6.4), 2.40 (1 H, m), 2.83 (1 H, m), 3.06 (6 H, m), 3.46 (2 H, m), 3.79 (6 H, s), 4.29 (1 H, m), 4.97 (1 H, m), 5.79 (2 H, s), 5.82 (1 H, d, *J* 7.3), 6.23 (1 H, m), 6.82 (4 H, d, *J* 8.8), 6.88 (1 H, d, *J* 621.2), 7.08–7.50 (9 H, m) and 8.10 (1 H, d, *J* 7.3).

3′-O-*Acetyl*-5′-O-*dimethoxytrityl*-4-S-*pivaloyloxymethyl*-4-*thiothymidine* **7a**.—A solution of 4-*S*-pivaloyloxymethyl-4-thiothymidine **4a** (4.25 g, 11.4 mmol) and dimethoxytritylchloride (4.6 g, 1.2 equiv.) in pyridine (35 cm^3) was stirred at room temperature for 5 h. Then, acetic anhydride (20 cm^3) was added and the solution stirred 90 min. Methanol (30 cm^3) was added and the solution stirred another 30 min. After concentration of the solution *in vacuo*, dilution with CH_2Cl_2, washing with a saturated sodium hydrogen carbonate solution, brine, drying over sodium sulfate and concentration under reduced pressure, the crude product was filtered on a short silica gel column [eluent: heptane–ethyl acetate (1:1)] to give **7a** (7.57 g, 92.8%); $\delta_H(CDCl_3)$ 1.18 (9 H, s), 1.53 (3 H, s), 2.06 (3 H, s), 2.33 (1 H, dt, *J* 14.5, 6, 9), 2.73 (1 H, br dd, *J* 14.5, 6.1), 3.44 (2 H, m), 3.76 (6 H, s), 4.19 (1 H, br s), 5.39 (1 H, m), 5.83 (2 H, s), 6.38 (1 H, dd, *J* 6.9, 6.1), 6.81 (4 H, d, *J* 9.1), 7.10–7.40 (9 H, m) and 7.85 (1 H, s); $\delta_C(CDCl_3)$ 14.0, 21.6, 27.5, 39.3, 39.9, 55.8, 61.2, 64.0, 75.6, 85.2, 87.2, 87.7, 112.6, 113.9, 127.8, 128.6, 130.6, 135.8, 138.9, 144.8, 153.9, 159.4, 171.0, 176.0 and 179.0.

3′-O-*Acetyl*-5′-O-*dimethoxytrityl*-N^3-*methyl*-4-*thiothymidine*.—This compound (400 mg, 1.47 mmol) was prepared according to the procedure described for preparation of **7a** (Yield 76.7%); $\delta_H(CDCl_3)$ 1.66 (3 H, s), 2.08 (3 H, s), 2.43 (1 H, ddd, *J* 14.5, 8.4, 6.1), 2.56 (1 H, m), 3.47 (2 H, m), 3.78 (6 H, s), 3.80 (3 H, s), 4.18 (1 H, br s), 5.43 (1 H, br d, *J* 5.3), 6.43 (1 H, dd, *J* 8.4, 5.3), 6.83 (4 H, d, *J* 9.1), 7.20–7.45 (9 H, m) and 7.69 (1 H, s); $\delta_C(CDCl_3)$ 19.0, 21.6, 36.2, 39.1, 55.8, 64.1, 75.7, 84.9, 86.5, 87.8, 113.9, 120.6, 127.8, 128.6, 129.0, 130.6, 135.8, 144.8, 149.7, 159.4, 170.9 and 192.0.

3′-O-*Acetyl*-4-S-*pivaloyloxymethyl*-4-*thiothymidine* **8a**.—3′-*O*-Acetyl-5′-*O*-dimethoxytrityl-4-*S*-pivaloyloxymethyl-4-thiothymidine **7a** (7.57 g, 10.57 mmol) was dissolved in CH_2Cl_2 containing 3% of trifluoroacetic acid (50 cm^3). The solution was stirred 3 h at room temperature and methanol was added until discolouration. After concentration under reduced

pressure, the crude product was purified by column chromatography on silica gel [eluent: heptane–ethyl acetate (1:1)] to give **8a** (2.5 g, 58%), m.p. 154–155 °C (from water) (Found: C, 52.35; H, 6.2; N, 6.5; S, 7.85. $C_{18}H_{26}N_2O_7S$ requires C, 52.17; H, 6.28; N, 6.76; S, 7.73%); $\delta_H(CDCl_3)$ 1.13 (9 H, s), 1.98 (3 H, s), 2.06 (3 H, s), 2.32 (1 H, dt, *J* 14.5, 6.9), 2.57 (1 H, ddd, *J* 14.5, 6.1, 2.3), 3.92 (2 H, m), 4.14 (1 H, br s), 5.33 (1 H, m), 5.78 (2 H, s), 6.23 (1 H, dd, *J* 6.9, 6.1) and 7.97 (1 H, s); $\delta_C(CDCl_3)$ 14.6, 21.5, 27.4, 39.2, 61.2, 62.7, 75.3 (CH), 86.5, 88.2, 112.8, 139.9, 154.2, 171.2, 176.1 and 179.0.

3′-O-*Acetyl*-N^3-*methyl*-4-*thiothymidine* **11**.—3′-*O*-acetyl-5′-*O*-dimethoxytrityl-N^3-methyl-4-thiothymidine (170 mg, 0.286 mmol) was treated as described for the preparation of **10** (Yield 69%) (Found: C, 49.65; H, 5.8; N, 8.7; S, 10.0. $C_{13}H_{18}N_2O_5S$ requires C, 49.68; H, 5.77; N, 8.91; S, 10.18%) (HRMS Found: M^+, 314.0915. $C_{13}H_{18}N_2O_5S$ requires *M*, 314.0936); $\delta_H(CDCl_3)$ 2.10 (3 H, s), 2.14 (3 H, s), 2.43 (2 H, m), 3.78 (3 H, s), 3.92 (2 H, m), 4.12 (1 H, q, *J* 2.3), 5.35 (1 H, dt, *J* 5.3, 2.3), 6.24 (1 H, dd, *J* 8.0, 6.10) and 7.64 (1 H, s); $\delta_C(CDCl_3)$ 19.9, 21.6, 36.2, 38.4, 63.1, 75.2, 86.1, 88.0, 120.5, 129.9, 149.7, 171.3 and 192.0.

3′-O-*Acetyl*-4-(S-*pivaloyloxymethyl*)-2′-*deoxy*-4-*thiouridine* **8b**.—To a solution of 5′-*O*-dimethoxytrityl-4-(*S*-pivaloyloxymethyl)-2′-deoxy-4-thiouridine **5b** (930 mg, 1.41 mmol) in pyridine (5 cm^3) was added acetic anhydride (7 cm^3). The solution was stirred overnight, then methanol (10 cm^3) was added. The reaction mixture was concentrated under reduced pressure, diluted with CH_2Cl_2, washed with sodium hydrogen carbonate, twice with brine, dried over sodium sulfate and evaporated. The residue (920 mg) was treated with CH_2Cl_2 containing 3% of trifluoroacetic acid (7.5 cm^3) at room temperature during 30 min. Then, methanol (1 cm^3) was added and the reaction mixture was diluted with CH_2Cl_2, washed with sodium hydrogen carbonate, brine, dried over sodium sulfate and concentrated under reduced pressure. The crude product was purified by flash chromatography using heptane–ethyl acetate (1:1) to give **6b** (410 mg, 72.7%) (HRMS Found: M^+, 400.1302. $C_{17}H_{24}N_2O_7S$ requires *M*, 400.1305); $\delta_H(CDCl_3)$ 1.17 (9 H, s), 2.09 (3 H, s), 2.33 (1 H, m), 2.66 (1 H, ddd, *J* 14.5, 6.1, 3.0), 2.97 (1 H, br s), 3.93 (2 H, m), 4.17 (1 H, br s), 5.34 (1 H, m), 5.77 (2 H, s), 6.25 (3 H, m) and 8.17 (1 H, d, *J* 6.9); $\delta_C(CDCl_3)$ 21.6, 27.5, 39.4, 61.4, 62.9, 75.2, 86.6, 88.5, 104.3, 142.2, 154.3, 171.4, 175.7 and 179.0.

5′-O-*Thymidylyl*-4-*thiothymidine* (Tps^{4}T) **16a**.—*Condensation*. To a mixture of dried hydrogen phosphonate **15** (300 mg, 0.42 mmol, 1.1 equiv.) and acetate **8a** (160 mg, 0.38 mmol, 1 equiv.) in anhydrous acetonitrile–pyridine (1:1) (5 cm^3) was added bis(2-oxo-3-oxazolidinyl)phosphinic chloride (250 mg, 0.98 mmol, 2.58 equiv.). The reaction was stirred 40 min at room temperature. CH_2Cl_2 was added and the solution washed twice with brine and concentrated under reduced pressure.

Oxidation.—To the residue diluted with pyridine (2 cm^3) and water (one drop) was added iodine (140 mg). After 1 h, a saturated sodium thiosulfate solution was added to the reaction mixture until it became colourless, then the solution was diluted with CH_2Cl_2. The organic phase was washed with saturated solutions of sodium hydrogen carbonate and sodium chloride and concentrated under reduced pressure.

Removal of the pivaloyloxymethyl- and acetyl protecting groups. The residue was dissolved in a mixture of NH_4OH–pyridine (1:1) (4 cm^3) and kept overnight at room temperature. The solution was concentrated under reduced pressure.

Detritylation. The residue was taken in 80% acetic acid (4 cm^3). After 30 min of stirring, the mixture was concentrated *in vacuo* the residue dissolved in water. The aqueous solution was washed twice with CH_2Cl_2 and concentrated. The crude product **2a** was purified on a short column (9.5 × 2 cm i.d.) of reversed-phase silica gel RP 18 with water as eluent (50 cm^3) then water–acetonitrile 2% (50 cm^3), 4% (50 cm^3), 8% (50 cm^3). Fractions (4 cm^3) were collected. Those containing **16a** were concentrated then lyophylized leading to 98 mg (0.17 mmol) of a yellow foam (46%, 4 steps); FAB *m/z* 561 $(M - H^+)^-$, 583 $(M - 2H^+ + Na^+)^-$; $\delta_P(D_2O)$ 0.45; $\delta_H(D_2O)$ 1.89 (3 H, s), 2.07 (3 H, s), 2.32 (1 H, m), 2.43 (2 H, t, *J* 6.6), 2.55 (1 H, m), 3.80 (2 H, m), 4.09 (1 H, m), 4.16 (2 H, m), 4.20 (1 H, m), 4.61 (1 H, m), 4.8, 6.18 (1 H, dd, *J* 7.1, 6.3), 6.28 (1 H, t, *J* 6.6), 7.65 (1 H, s) and 7.73 (1 H, s); $\delta_C(D_2O)$ 12.7, 17.7, 38.9, 40.3, 62.4, 66.0, 71.5, 76.4, 86.5, 86.9, 112.8, 121.4, 135.3, 138.6, 150.3, 152.7, 167.5 and 192.3.

5′-O-*Thymidylyl*-3-*methyl*-4-*thiothymidine* (TpMe^{3}s^{4}T) **3**.—Compound **15** (150 mg, 0.21 mmol, 1.1 equiv.) and **11** (60 mg, 0.19 mmol, 1 equiv.) were treated as described for the synthesis of **16a** except that deacetylation was performed in a mixture of CH_3OH–TEA–H_2O (8:1:1) overnight at room temperature to give in 58.7% overall yield; FAB *m/z* 577 $(M + H^+)^+$, 599 $(M + Na^+)^+$; $\delta_P(D_2O)$ 0.40; $\delta_H(D_2O)$ 1.85 (3 H, s), 2.10 (3 H, s), 2.29 (1 H, m), 2.43 (2 H, m), 2.52 (1 H, m), 3.69 (3 H, s), 3.78 (2 H, m), 4.09 (1 H, m), 4.17 (3 H, m), 4.59 (1 H, m), 4.80, 6.12 (1 H, dd, *J* 7.1, 6.0), 6.29 (1 H, t, *J* 6.3), 7.63 (1 H, s) and 7.74 (1 H, s); $\delta_C(D_2O)$ 13.1, 19.8, 36.8, 39.1, 40.6, 6.32, 65.9, 71.3, 76.4, 86.0, 86.4, 86.7, 87.9, 112.6, 121.2, 131.9, 138.5, 150.9, 152.5, 167.3 and 192.2.

2′-*Deoxy*-5′-O-*thymidylyl*-4-*thiouridine* (Tps^{4}U) **16b**.—A mixture of compounds **15** (205 mg, 0.33 mmol, 1.1 equiv.) and **8b** (120 mg, 0.3 mmol, 1 equiv.) was treated as described for the synthesis of **16a** to give the dinucleoside phosphate **16b** in 43% overall yield; FAB *m/z* 547 $(M - H^+)^-$, 569 $(M - 2H^+ + Na^+)^-$; $\delta_P(D_2O)$ 0.33; $\delta_H(D_2O)$ 1.85 (3 H, s), 2.39 (4 H, m), 3.75 (2 H, m), 4.01 (1 H, m), 4.15 (3 H, m), 4.34 (1 H, m), 4.73 (1 H, m), 6.19 (2 H, m), 6.49 (1 H, d, *J* 7.4), 7.60 (1 H, s) and 7.68 (1 H, d, *J* 7.4); $\delta_C(D_2O)$ 12.9, 38.9, 40.3, 62.5, 66.2, 71.6, 76.5, 86.5, 86.7, 87.0, 87.3, 112.8, 114.8, 138.2, 138.7, 150.3, 152.8, 167.6 and 191.9.

5′-O-(4-*Thiothymidylyl*)*thymidine* (s^{4}TpT) **14a**.—A mixture of compounds **6a** (130 mg, 0.15 mmol, 1 equiv.) and **12** (48 mg, 0.169 mmol, 1.09 equiv.) was treated as described for the synthesis of **16a** to give the dinucleoside phosphate **14a** in 53.4% overall yield; FAB *m/z* 561 $(M - H^+)^-$; $\delta_P(D_2O)$ 0.39; $\delta_H(D_2O)$ 1.80 (3 H, s), 1.97 (3 H, s), 2.29 (2 H, t, *J* 6.4), 2.33 (1 H, m), 2.55 (1 H, dt, *J* 13.8, 5.1), 3.72 (1 H, dd, *J* 12.7, 4.2), 3.79 (1 H, dd, *J* 12.7, 3.2), 4.04 (3 H, m), 4.13 (1 H, q, *J* 3.7), 4.51 (1 H, q, *J* 5.0), 4.69 (1 H, m), 6.07 (1 H, t, *J* 6.4), 6.23 (1 H, t, *J* 6.6), 7.63 (1 H, s) and 7.70 (1 H, s); $\delta_C(D_2O)$ 12.9, 17.6, 39.4, 40.1, 62.2, 66.1, 71.7, 76.0, 86.3, 87.2, 112.7, 121.4, 135.3, 138.6, 150.2, 152.8, 167.3 and 192.4.

5′-O-(2-*Deoxy*-4-*thiouridylyl*)*thymidine* (s^{4}UpT) **14b**.—A mixture of compound **6b** (82 mg, 0.1 mmol, 1 equiv.) and **12** (28 mg, 0.1 mmol, 1 equiv.) was treated as described for the synthesis of **16a** to give the dinucleoside phosphate **14b** in 58.7% overall yield; FAB *m/z* 547 $(M - H^+)^-$, 569 $(M - 2H^+ + Na^+)^-$; $\delta_P(D_2O)$ 0.47; $\delta_H(D_2O)$ see ref. 21; $\delta_C(D_2O)$ 13.0, 39.5, 40.1, 62.3, 66.2, 71.8, 76.3, 86.3, 86.5, 87.6, 112.8, 114.8, 138.0, 138.7, 150.1, 152.9, 167.4 and 192.0.

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Paper 2/02110B
Received 24*th April* 1992
Accepted 10*th June* 1992