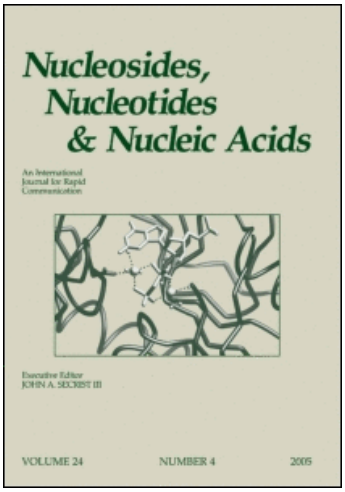

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Preparation and ^{17}O NMR Spectra of ^{17}O-Labeled Thymidine 5'-Phosphate Triesters, Alkylphosphonates, Dialkylphosphinates, and Phosphoramidates

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PREPARATION AND ^{17}O NMR SPECTRA OF ^{17}O-LABELED THYMIDINE 5'-PHOSPHATE TRIESTERS, ALKYLPHOSPHONATES, DIALKYLPHOSPHINATES, AND PHOSPHORAMIDATES

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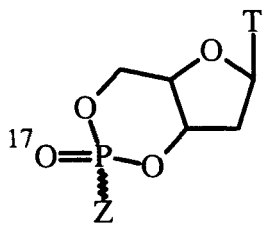
Abstract: The ^{17}O chemical shifts of the title compounds cover a range of values and appear to be useful for the assignment of structure. However, the individual diastereomers of derivatives containing a stereogenic phosphorus center did not display discernably different ^{17}O chemical shifts.

^{17}O NMR spectroscopy is a valuable technique for the study of molecular structure.[1-5] For example, recent studies have demonstrated that ^{17}O chemical shifts for diverse functional groups are quantitatively related to torsion angle changes.[3] The ^{17}O NMR spectral studies of a number of phosphorus derivatives have been reported, including cyclic and bicyclic phosphites, phosphates, and thiophosphates.[6] Recently we reported the successful use of ^{17}O chemical shifts to assign configuration at phosphorus to diastereomeric thymidine cyclic 3',5'-monophosphate esters, methyl phosphonates and N,N-dimethyl phosphoramidates:[7]



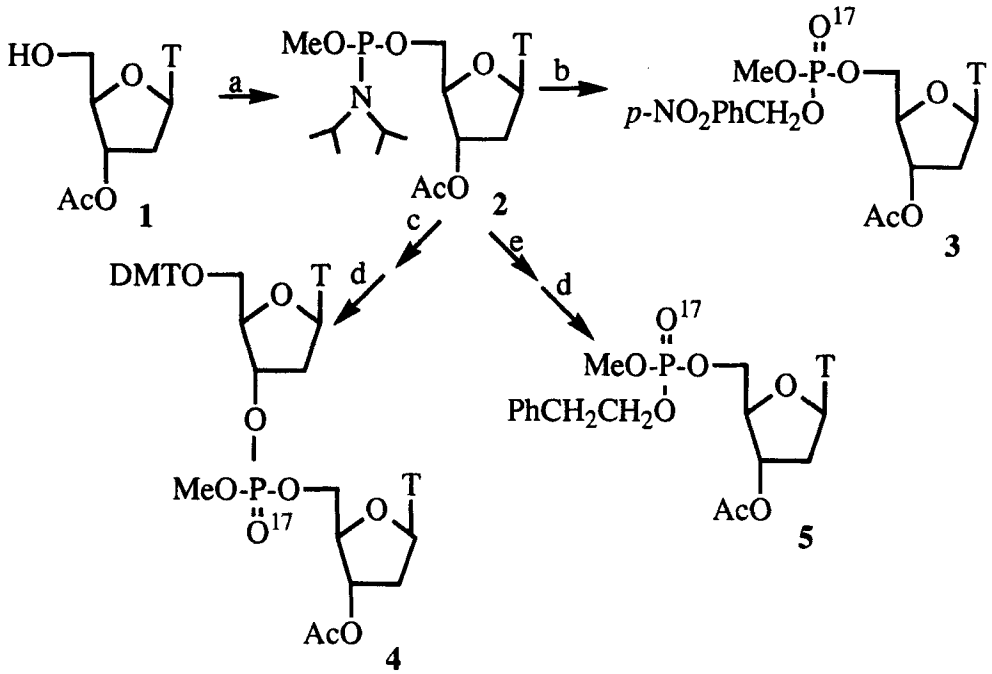
Z = Me_2N, Me, MeO

Thus, it seemed reasonable to expect that the individual diastereomers of neutral acyclic phosphorus derivatives of thymidine featuring a stereogenic (chiral) phosphorus, such as phosphates **3-5**, methylphosphonates **7-9**, dialkylphosphinates **10** and **11** and phosphoramidate **13**, might have well-differentiated ^{17}O chemical shifts.

In continuation of our studies of applications of ^{17}O NMR to nucleotides, we report the synthesis of the above derivatives of thymidine, ^{17}O labeled at phosphoryl oxygen, along with their measured ^{17}O chemical shifts and one-bond coupling constants, $^{1}J_{OP}$. An overall 33 ppm range of chemical shifts is observed for these derivatives. However, the chemical shifts of the diastereomers of individual compounds are identical within the ^{17}O resolution limits. The ^{31}P chemical shifts for the same molecules cover more than 70 ppm. Comparison of the ^{17}O and ^{31}P NMR chemical shifts of the thymidine derivatives studied suggests that chemical shift variations of the two nuclei may complement one another in distinguishing different phosphorus functionalites.

Labeled phosphate **4** was prepared by standard phosphoramidite coupling techniques.[17] Thus, reaction of 3'-*O*-acetylthymidine[8] with methyl *N,N*-diisopropylaminophosphorochloridite, MeO(*i*-Pr_2N)PCl,[9] in dichloromethane in the presence of (*i*-$Pr_2N)_2$NEt afforded the corresponding phosphoramidite, **2** (Scheme 1). Condensation of intermediate **2** with 5'-*O*-dimethoxytritylthymidine yielded the dinucleoside phosphite (not shown, 85% yield) which was oxidized[10] with iodine/$H_2{}^{17}O$ to give the desired known phosphotriester, **4**,[15] with ^{17}O-labeled phosphoryl oxygen. Product **4** displayed one set of equal-intensity signals in its ^{31}P NMR spectrum at δ - 0.41 and - 0.78, assigned to the individual diastereomers of the $P{=}O^{16}$ material. Somewhat smaller peaks, also of equal intensity, were seen at δ -0.45 and -0.82. The latter signals are attributed to the presence of $P{=}O^{18}$, arising from $H_2{}^{18}O$ in the $H_2{}^{16}O/H_2{}^{17}O/H_2{}^{18}O$ solution used in the oxidation, which shifts the ^{31}P resonances upfield.[18] The ^{31}P resonance from the $P{=}O^{17}$ phosphate is not seen in such cases because of phosphorus-oxygen coupling and severe quadrupolar line broadening.[18b,19] The structure of **4** was confirmed by comparison of its ^{1}H NMR spectrum to the published parameters[15] of the fully deblocked diester.

Similarly, phosphoramidite **2** was condensed with *p*-nitrobenzyl alcohol and 2-phenylethyl alcohol to form the requisite phosphites that were oxidized to the corresponding oxygen-labeled phosphate triesters **3** and **5** (Scheme 1). These products, as expected, also showed four ^{31}P NMR signals, in two sets. The structures of **3** and **5** were confirmed by ^{1}H NMR spectroscopy and mass spectrometry (Experimental

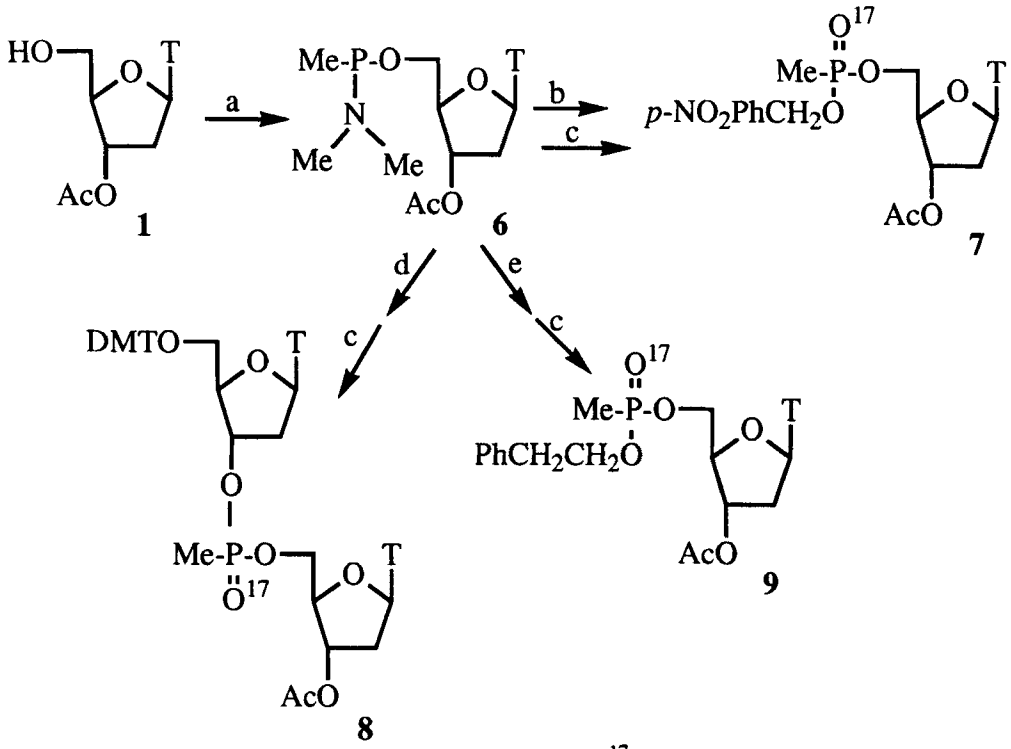


(a) *i*-$Pr_2NP(OMe)Cl$/*i*-Pr_2NEt. (b) *p*-$NO_2C_6H_4CH_2OH$/1H-tetrazole (c) 1*H*-tetrazole/5'-*O*-Dimethoxytritylthymidine (d) $I_2/H_2{}^{17}O$ (e) 1*H*-tetrazole/$PhCH_2CH_2OH$

Scheme 1

Section). The ^{1}H MeO resonances of the individual diastereomers of **3-5** were well-separated.

Methylphosphonate derivatives **7-9** were prepared by the procedure of Jager and Engels,[11] (Scheme 2). Thus 3'-*O*-acetylthymidine on reaction with bis-(*N*,*N*-dimethylamino)methylphosphine gave the corresponding phosphonamidite, **6**, in 78% yield as two diastereomers (~50/50 ratio, ^{31}P chemical shifts, δ 143.2, 141.6), used without further purification. On condensation with 5'-*O*-dimethoxytritylthymidine in acetonitrile in the presence of benzotriazole, compound **6** gave a phosphonite intermediate, which was oxidized without further purification to **8** using a mixture of iodine, pyridine and ^{17}O labeled water in tetrahydrofuran. Known product **8**,[11] (82% yield) showed the requisite two sets of ^{31}P NMR signals at δ 32.94, 33.45 (^{16}O) and



(a) $(Me_2N)_2PMe$ (b) p-$NO_2C_6H_4CH_2OH$ (c) $H_2{}^{17}O/I_2$
(d) 5'-Dimethoxytritylthymidine/1*H*-tetrazole
(e) $PhCH_2CH_2OH$/1*H*-tetrazole

Scheme 2

32.87, 33.41 (^{18}O). The structure of this compound was confirmed by ^1H NMR spectroscopy and by comparison of the ^{31}P chemical shifts to the literature values.[12]

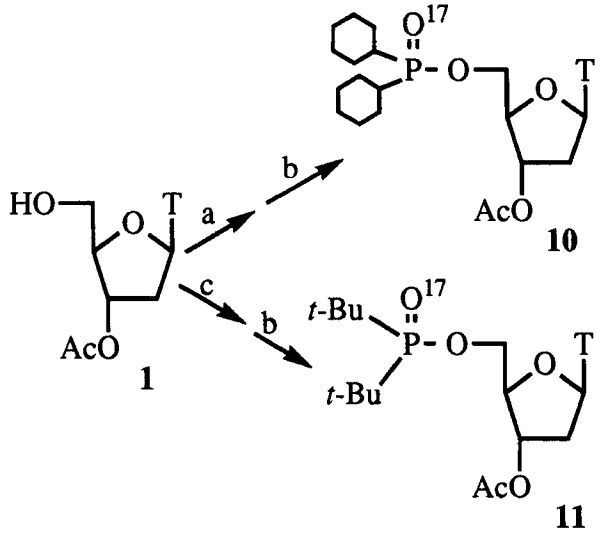
Similarly, condensation of **6** either with *p*-nitrobenzyl alcohol or 2-phenylethyl alcohol, followed by oxidation and chromatographic purification, gave phosphonates **7** and **9** in 79% and 48% yields, respectively (Scheme 2). Two sets of ^{31}P resonances also were observed for **7** and **9** (Experimental Section). Unlike the MeO ^1H resonances of **3-5**, the doublets of the MeP(O) functionality of the individual diastereomers of **7-9** had indistinguishable ^1H chemical shifts. The structures of **7** and **9** were confirmed by ^1H NMR spectroscopy and mass spectrometry (Experimental Section). For those

molecules with a stereogenic phosphorus center, for which ^1H NMR data are recorded in the Experimental Section (**3**, **5**, **7**, **9**, and **13**), the NH (thymin-1-yl ring), H6 or H1' resonance was doubled because of the presence of two diastereomers.

Dialkylphosphinates **10** and **11** were prepared by treatment of 3'-*O*-acetylthymidine either with dicyclohexyl- or with di-*tert*-butylchlorophosphine followed by $H_2{}^{17}O/I_2$ oxidation (Scheme 3). These products were isolated by HPLC purification on silica, both in 41% yield. Their structures were confirmed by ^1H NMR spectroscopy and mass spectrometry. No doubling of the base ring proton ^1H NMR resonances of the type noted for those molecules with chiral phosphorus was seen for **10** and **11**, as is consistent with the absence of a stereogenic phosphorus center. However, the diastereotopic *t*-butyl groups of **11** appeared as two separate doublets, $^3J_{HP}$ = 15 Hz.

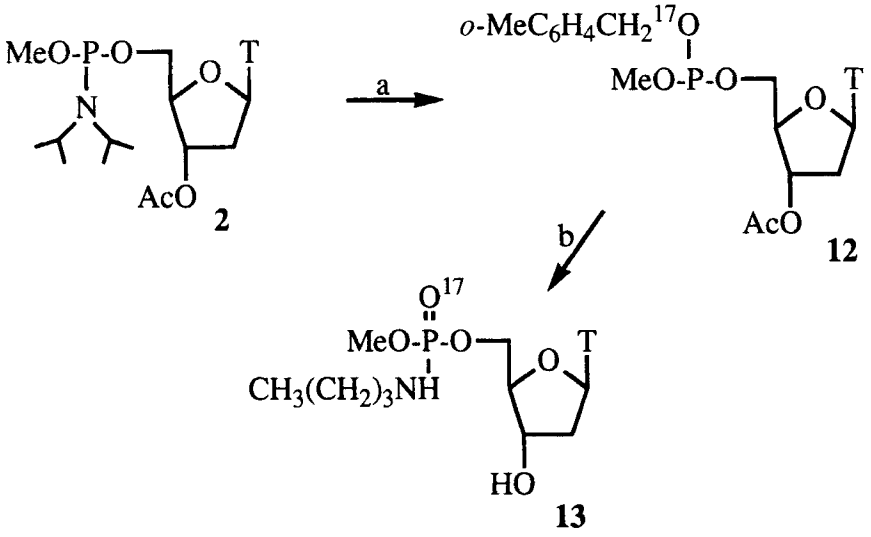
The synthesis of phosphoramidate **13** (Scheme 4) was based on knowledge that the *o*-methylbenzyl substituent, used as a protecting group in nucleoside phosphite triester chemistry, is readily lost on I_2/H_2O oxidation of phosphites to afford the phosphate diester.[13] A related protecting group, the 2-cyano-1,1,-dimethyl substituent behaves analogously with I_2/H_2O. However, when the phosphite containing the latter substituent is treated with I_2 under *anhydrous* conditions, but in the presence of primary amine, the protecting group is eliminated, and a phosphoramidate analogous to **13** is generated.[14] When phosphoramidite **2** was reacted with ^{17}O-labeled *o*-methylbenyzyl alcohol, phosphite **12** was formed. This product was used without further purification in reaction with I_2 in the presence of *n*-butylamine to form phosphoramidate **13** with ^{17}O-labeled phosphoryl oxygen, isolated in pure form by HPLC in 42% yield. Its ^{31}P NMR spectrum showed the presence of two diastereomers in essentially equal amounts. Interestingly, the formation of **13** was accompanied by the loss of the 3'-acetyl group. Structural identification was based on ^1H NMR and mass spectrometry. Unlike the phosphates and methylphosphonates, phosphinates **10** and **11** and phosphoramidate **13** did not display perceptible ^{18}O-induced shifts in their ^{31}P resonances.

In the Table are recorded the ^{17}O chemical shifts and one-bond phosphorus oxygen coupling constants ($^1J_{OP}$) for the above molecules along with the ^{31}P chemical shifts for their $P{=}O^{16}$ counterparts. Unlike the ^{31}P chemical shifts that readily



(a) (cyclo $C_6H_{11})_2PCl/i\text{-}Pr_2NEt$ (b) $I_2/H_2{}^{17}O$ (c) $t\text{-}Bu_2PCl$

Scheme 3



(a) $o\text{-}MeC_6H_4CH_2{}^{17}OH/1H\text{-}$tetrazole (b) $I_2/n\text{-}BuNH_2$

Scheme 4

TABLE. ^{17}O and ^{31}P NMR Parameters

Cmpd.	$\delta^{17}O^{e}$	$\delta^{31}P^{a}$	Cmpd.	$\delta^{17}O^{e}$	$\delta^{31}P^{a}$
3	81.1	0.72	**9**	106.0	31.9
	(160)[b]	1.01		(161)[b]	32.3
4	82.9	-0.41	**10**	76.6	61.5[d]
	(c)	-0.78		(159)	
5	80.7	0.40	**11**	83.9	71.2[d]
	(156)	0.56		(176)	
7	110.6	32.9	**13**	82.1	12.1
	(161)	33.4		(139)	12.2
8	111.8	32.9			
	(c)	33.5			

[a] ^{31}P Chemical shifts for two diasteromers ($CDCl_3$) of P=O^{16}-containing compounds. [b] $^{1}J_{OP}$ in Hz. [c] Not resolved. [d] Not diasteromeric at phosphorus. [e] CD_3CN at 75 °C.

differentiate R_P and S_P diastereoisomers, the individual diastereomers of a particular thymidine derivative do not show different chemical shifts, even though the ^{17}O chemical shift range covers 34 ppm. In addition, on comparison of ^{17}O and ^{31}P chemical shift data in the Table, one notes a parallel inability of either method to greatly differentiate chemical shifts for the phosphate triester series **3-5** ($\Delta\delta^{17}O$ = 2.2; $\Delta\delta^{31}P$ = 1.1, 1.9 two diastereomers). Interestingly, the dicyclohexyl- and di-*t*-butylphosphinate functionalities of **10** and **11** feature a large chemical shifts difference within *both* their ^{17}O and ^{31}P NMR spectra. By contrast, while the average ^{31}P chemical shifts for the methylphosphonates **7-9** cover a range of only δ 31.9-33.4, the ^{17}O chemical shifts are more sensitive to change in structure and range from δ106.0 to 111.8. In particular $\Delta\delta^{17}O$ for methylphosphonates **7** and **9** is 4.6 ppm, even though their structural difference is rather subtle. These results suggest that for nucleoside derivatives containing uncharged, four-coordinate phosphorus ^{17}O and ^{31}P chemical shifts may work in parallel fashion in some instances and in other cases in a complementary way to provide structural information.

A future direction of this work will be to use other solvents, lanthanide and other shift reagents, (including optically active ones such as *t*-BuPhP(S)OH[20]) and

optically active solvents to attempt to differentiate the ^{17}O chemical shifts of individual diasteromers. The ability of $Eu(FOD)_3$ to differential diastereotopic oxygens of sulfones that have accidentally isochronous ^{17}O resonances has been demonstrated.[16] Also, other nucleobases may better differentiate the ^{17}O chemical shifts of diastereomeric phosphoryl groups than does the thymin-1-yl substitutent. Although ^{31}P NMR is effective in distinguishing between derivatives that are diastereomeric at phosphorus, the ultimate potential of ^{17}O NMR is in the observation of a single ^{17}O-enriched, and thereby labeled phosphate, phosphonate, or phosphoramidate moiety in an oligonucleotide sequence containing more than one such monomer unit.

Experimental

General.

Proton NMR spectra were obtained on Varian XL-300 or VXR-500 spectrometers. ^{31}P NMR spectra were taken on a Varian XL-300 spectrometer at 121 MHz under proton decoupling conditions. ^{31}P chemical shifts in $CDCl_3$ are reported in δ ppm downfield (+) or upfield (-) from external 85% H_3PO_4. ^{17}O NMR spectra were run in CD_3CN at 75 °C on a Varian XL-400 (54.2 MHz) or VXR-500 (67.6 MHz) NMR spectrometer. Chemical shifts are relative to H_2O as external standard and are all downfield (+) of the internal standard. $H_2{}^{17}O$ (20% ^{17}O), purchased from Cambridge Isotope Laboratories was sometimes diluted with $H_2{}^{16}O$ before use. Mass spectra were obtained on VG 7050E double-focussing, high resolution and Hewlett Packard 5971A MSD mass spectrometers.

Preparation of ^{17}O-Labeled *o*-Methylbenzyl Alcohol.

To a solution of *o*-methylbenzyl chloride (500 mg) and water (120 uL $H_2{}^{17}O$, 100 uL $H_2{}^{16}O$) in DMF (3 mL) was added sodium carbonate (20 mg). The solution was heated at 150°C for 2 h, then allowed to cool to room temperature and partitioned between CH_2Cl_2 (30 mL) and water (10 mL). The organic layer was separated, dried (sodium sulphate), and the solvent was removed. The residue obtained was dissolved in methylene chloride (3 mL) and added to hexane (100 mL). After about 2 h of stirring, the hexane layer was separated and concentrated under reduced pressure. This solution was purified by column chromatography over silica gel using dichloromethane as the eluent to give ^{17}O-labeled product alcohol that was in all respects identical to an authentic, unlabeled sample (TLC, ^1H NMR, HPLC). 220 mg (51%).

3'-*O*-Acetylthymidine (1).

3'-*O*-Acetylthymidine was prepared after the procedure of Michelson and Todd[8] except that the 5'-hydroxyl function was protected with dimethoxytrityl instead of the trityl group. Reaction of thymidine (5 g, 20.6 mmol) in pyridine (80 mL) with 4,4'-dimethoxytrityl chloride (8.08 g, 41.2 mmol, 1.2 eq.) afforded the 5-dimethoxytrityl derivative which after treatment with acetic anhydride and removal of the dimethoxytrityl group left a residue that was triturated with hexane. The resulting solid was crystallized from acetone/*n*-hexane to yield 3.75 g (64%) of the product.

3'-*O*-Acetylthymidin-5'-yl Methyl N,N-Diisopropylphosphoramidite (2).

3'-*O*-Acetylthymidine (0.25 g, 0.88 mmol) was dried by coevaporation with anhydrous pyridine (10 mL). The residue was dried overnight under high vacuum. To it was added *i*-Pr_2NEt (0.766 mL, 3.51 mmol) and CH_2Cl_2 (5 mL). After the nucleoside had partially dissolved, *i*-$Pr_2NP(OMe)Cl$ (0.40 mL, 2.1 mmol) was added dropwise, and the reaction mixture was stirred at room temperature for 1 h. MeOH (0.02 mL) was added, and after a few minutes the reaction mixture was diluted with approximately 30 mL of a 5% triethylamine-ethyl acetate solution. The organic phase was washed with saturated sodium bicarbonate solution and dried (sodium sulphate), and the solvent was removed under reduced pressure. The residue was precipitated from hexane at -20° (306 mg, 84% yield). ^{31}P NMR ($CDCl_3$) δ 149.7, 149.8 (ratios of peak areas ca. 50/50).

5'-*O*-Dimethoxytritylthymidin-3'-yl 3'-*O*-Acetylthymidin-5'-yl Methyl Phosphate (4).

The above phosphoramidite, **2** (104 mg, 0.25 mmol), 5'-*O*-dimethoxytritylthymidine (136 mg, 0.25 mmol), and 1*H*-tetrazole (70.1 mg, 1.0 mmol) were dissolved in freshly distilled acetonitrile (3 mL) under an argon atmosphere. The solution was stirred at room temperature for 20 min, diluted with 5% triethylamine-ethyl acetate and washed with saturated sodium bicarbonate solution. The aqueous layer was re-extracted with ethyl acetate. The organic layer was dried (sodium sulphate), and the solvent was removed under reduced pressure to give 182 mg (85%) of the product as a colorless glass. ^{31}P NMR ($CDCl_3$) δ 140.0, 140.9 (approximately equal peak areas).

The latter was dissolved in THF-pyridine (5 mL, 4:1), and the solution was cooled to -45°. To it was added, dropwise, a solution of iodine in THF-$H_2{}^{17}O$ (iodine, 63 mg; $H_2{}^{17}O$, 100 μL; THF, 1 mL) over a period of 20 min. The reaction solution was diluted with about 10 mL of water, mixed with 3 mL of a 5% sodium bisulphite solution, and extracted several times with ethyl acetate. The organic layer was dried (sodium sulphate), and the solvent was removed under reduced pressure to give 160 mg (86%) of product **4** as a colorless glass, shown to be pure by ^{31}P and ^1H NMR spectroscopy and by TLC on silica gel (5% MeOH/CH_2Cl_2). Its ^1H NMR parameters agreed with published values.[15] ^{31}P NMR ($CDCl_3$) δ -0.41, -0.78 (approximately equal peak areas); ^{18}O shifted peaks at δ -0.45, -0.82. Lit.[15] for fully deblocked methyl ester -0.60, -0.69 (DMSO-d_6, added 8-hydroxy quinoline).

3'-*O*-Acetylthymidin-5'-yl *p*-Nitrobenzyl Methyl Phosphate (3).

To a solution of phosphoramidite **2** (250 mg, 0.603 mmol) and p-nitrobenzyl alcohol (92 mg, 0.60 mmol) in 3 mL of CH_3CN was added 1*H*-tetrazole (84 mg, 1.2 mmol). After this solution was stirred at room temperature for 15 min, a solution of iodine in THF-pyridine-water (iodine, 165 mg; THF, 0.4 mL; pyridine, 0.4 mL; $H_2{}^{17}O$, 0.1 mL) was added dropwise until the color of iodine persisted. The reaction mixture was diluted with ethyl acetate and washed with water containing a small amount of sodium bisulphite. The organic layer was dried (sodium sulphate) and removed under reduced pressure. The residue was purified by HPLC on SiO_2 (eluent: 0-5% MeOH-CH_2Cl_2) to yield 70 mg (23%) of pure product **3**. ^{31}P NMR ($CDCl_3$) δ 0.72, 1.07; ^{18}O-shifted peaks, δ 0.68, 0.97. ^1H NMR ($CDCl_3$) δ 9.20 (br s, NH), 9.24 (br s, NH), 7.44 (q, H6), 7.45 (q, H6), 7.55, 7.58 (2 d, *o*-C_6H_4), 8.23, 8.26 (2 d, *m*-C_6H_4), 6.37, 6.40 (m, H1'), 5.18-5.26 (m, H3, OCH_2Ar), 4.28-4.37 (m, H5'), 4.13-4.19 (m, H4'), 3.81 (d, J_{HP} = 11.4 Hz, OMe), 3.82 (d, J_{HP} = 11.1 Hz, OMe), 2.05-2.25 (m, H2'), 2.12 (s, $OCOCH_3$), 1.92 (br s, CH_3). FAB mass spectrum: 514 (M + 1).

3'-*O*-Acetylthymidin-5'-yl 2-Phenylethyl Methyl Phosphate (5).

The reaction was carried out in exactly the manner described for the preparation of **3**. The product was purified by column chromatography on silica gel (5% Et_3N/CH_2Cl_2) to yield 66 mg (24%) of pure **6**. ^{31}P NMR ($CDCl_3$) δ 0.40, 0.56; ^{18}O-shifted peaks 0.36, 0.52. ^1H NMR ($CDCl_3$) δ 9.03 (br s, NH), 9.02 (br s, NH), 7.45 (q, H6), 7.50 (q, H6), 6.55-6.65 (m, Ph), 6.39 (dd, H1'), 5.22-5.24 (m, H3'),

4.10-4.36 (m, H4', H5', OCH_2CH_2Ph), 3.72 (d, J_{HP} = 11.4 Hz, OMe), 3.71 (d, J_{HP} = 11.1 Hz, OMe), 2.92-3.03 (m, OCH_2CH_2Ph), 2.00-2.42 (m, H2'), 2.11 (s, $COCH_3$), 1.92 (d, 5-CH_3). FAB mass spectrum: 483 (M + 1).

3'-*O*-Acetylthymidin-5'-yl Methyl *N,N*-Diisopropylphosphonamidite (6).

To a suspension of 3'-*O*-acetylthymidine (800 mg, 2.81 mmol) in dichloromethane (10 mL) under argon was added methylbis(dimethylamino)phosphine, $(Me_2N)_2PMe$ (755 mg, 5.62 mmol). The reaction mixture was stirred at room temperature for 15 h, and the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (1.5 mL), and the solution was added dropwise to stirred *n*-pentane (40 mL). After 2 h, the supernatant was decanted, and the residue was dissolved in dichloromethane and washed with water. The organic layer was dried (sodium sulphate) and evaporated to give 820 mg (78%) of product **6** as a colorless foam of high purity (^{31}P NMR). ^{31}P NMR ($CDCl_3$) δ 141.46, 143.18.

5'-*O*-Dimethoxytritylthymidin-3'-yl 3'*O*-Thymidin-5'-yl Methylphosphonate (8).

Following the procedure of Jager and Engels,[11] to a solution of **6** (75 mg, 0.20 mmol) and 5'-*O*-dimethoxytritylthymidine (109 mg, 0.20 mmol) in dry acetonitrile (1 mL) was added benzotriazole (95 mg, 0.80 mmol) at room temperature. After 2 min 0.3 mL of an iodine solution (iodine, 165 mg; THF, 0.4 mL; pyridine, 0.4 mL; $H_2{}^{17}O$, 100 μL) was added, and 15 min later the reaction mixture was diluted with ethyl acetate (50 mL). The organic phase was washed with water containing a small amount of sodium bisulphite. The aqueous layer was re-extracted with ethyl acetate, dried (sodium sulphate), evaporated under reduced pressure, and azeotroped consecutively with toluene, ethanol and dichloromethane to yield 153 mg (82%) of product **8** as a colorless glass. ^{31}P NMR ($CDCl_3$) δ 32.91, 33.46, near-equal intensities, (lit.[12] 31.7, 32.2, CD_3CN); ^{18}O-shifted peaks at δ 32.87, 33.41

3'-*O*-Acetylthymidin-5'-yl 2-Phenylethyl Methylphosphonate (9).

Similarly, benzotriazole (95 mg, 0.80 mmol) and 2-phenylethyl alcohol (23.9 μL, 0.20 mmol) were dissolved in acetonitrile (1 mL) and added to the solid methylphosphonite **6** (74.77 mg, 0.20 mmol) under an argon atmosphere. After 75 s at room temperature, an iodine solution (iodine, 170 mg; THF, 0.4 mL; pyridine, 0.4 mL, $H_2{}^{17}O$ 100 μL) was added until the yellow color persisted. The reaction mixture was diluted with ethyl acetate (20 mL) and washed with water containing a small amount

of sodium bisulphite. The aqueous layer was re-extracted, and the combined organic layer was dried (sodium sulphate). The solvent was evaporated under reduced pressure, and the product was azeotroped consecutively with toluene, ethanol, dichloromethane, and finally purified by HPLC on SiO_2 (0-5% MeOH-CH_2Cl_2) to yield 45 mg (48%) of **9** as a colorless glass. ^{31}P NMR ($CDCl_3$) δ 31.91, 32.31, near-equal intensities; ^{18}O-shifted peaks at 31.86, 32.26. ^1H NMR ($CDCl_3$) δ 9.48 (s, NH), 7.56 (q, H6) 7.46 (q, H6), 7.05-7.20 (m, Ph), 6.29, 6.35 (m, H1'), 5.18-5.38 (m, H3'), 3.90-4.44 (m, H4', H5', O*C*H_2CH_2), 2.94-3.02 (m, OCH_2C*H*$_2$Ph), 2.32-2.47 (m, H2'), 2.11 (s, COCH_3), 1.91 (d, 5-Me), 1.43 (d, J_{HP} = 17.4 Hz, P-CH_3). HRMS (CI), mass calculated for $C_{21}H_{28}N_2O_8P$: 467.15695. Found: 467.15832.

3'-*O*-Acetylthymidin-5'-yl *p*-Nitrobenzyl Methylphosphonate (7).

Analogously, a solution of benzotriazole (319 mg, 2.68 mmol) and p-nitrobenzyl alcohol (103 mg, 0.670 mmol) in dry acetonitrile (2 mL) was added to the solid methylphosphonite **6**. After 90 s a solution of iodine (See preparation of **9**.) was added dropwise at room temperature until the yellow color persisted. The reaction mixture was diluted with ethyl acetate and washed with water containing a small amount of sodium bisulphite. The organic layer was dried and removed under reduced pressure. The residue was azeotroped consecutively with toluene, ethanol, and dichloromethane. The residue was chromatographed on a silica gel filtration column using 0-5% methanol in 2% Et_3N-methylene chloride as the eluent. The appropriate fractions (TLC) yielded 263 mg (79%) of pure **10**. ^{31}P NMR ($CDCl_3$) δ 32.86, 33.34; ^{18}O-shifted peaks 32.81, 33.33. ^1H NMR ($CDCl_3$) δ 9.60 (s, NH), 9.5 (s, NH), 7.54, 7.57 (2 d, *o*-C_6H_4), 8.22, 8.24 (2 d, *m*-C_6H_4) 7.48 (q, H6), 7.43 (q, H-6), 6.24-6.40 (m, H1'), 5.12-5.39 (m, H3', O*C*H_2Ph-NO_2-*p*), 3.90-4.41 (m, H4', H5'), 2.30-2.49 (m, H2'), 2.11 (s, COCH_3), 1.93 (d, 5-CH_3), 1.61 (d, J_{HP} = 17.4 Hz, P-CH_3). FAB mass spectrum: 498 (M + 1).

3'-*O*-Acetylthymidin-5'-yl Dicyclohexylphosphinate (10).

To a suspension of 3'-*O*-acetylthymidine, **1** (250 mg, 0.879 mmol), and diisopropylethylamine (0.77 mL, 3.52 mmol) in anhydrous dichloromethane (4 mL) was added dicyclohexylchlorophosphine (307 mg, 1.31 mmol). The reaction mixture was stirred at room temperature for 23 h. A solution of iodine (iodine, 170 mg; pyridine, 0.1 mL; $H_2{}^{17}O$ 0.4 mL; THF, 0.4 mL) was added dropwise until the yellow

color of the iodine solution persisted. After 5 min the reaction mixture was diluted with methylene chloride and washed with water containing a small amount of sodium bisulphite. The organic layer was dried (sodium sulphate) and evaporated under reduced pressure. The product was purified by HPLC (0-5% MeOH-CH_2Cl_2) to afford 180 mg (41%) of pure product. ^{31}P NMR ($CDCl_3$) δ 61.45. ^{1}H NMR ($CDCl_3$) δ 10.06 (br s, NH), 7.52 (q, H6), 6.33 (dd, H1'), 5.22 (d, H3'), 4.18-4.36 (m, H4', H5'), 2.35-2.50 (m, H2'), 2.11 (s, $COCH_3$), 1.96 (d, 5-CH_3), 1.00-1.90 (m, cyclohexyl). HRMS (CI): mass calculated for $C_{21}H_{27}N_2O_9P$: 497.24165. Found: 497.24086.

3'-*O*-Acetylthymidin-5'-yl Di-*t*-butylphosphonate (11).

Phosphonate **11** was prepared in analogous fashion with molar ratios the same as those used for **10**. The product was isolated by HPLC on SiO_2 (0-5% MeOH-CH_2Cl_2). Yield, 160 mg (41%). ^{31}P NMR δ 71.20. ^{1}H NMR ($CDCl_3$) δ 8.30 (q, H6), 6.25 (d, H1'), 5.36 (m, H3'), 4.17 (dd, H5'), 3.89-4.10 (m, H4'), 2.54 (ddd, H2'), 2.33 (ddd, H2"), 2.07 (s, $COCH_3$), 2.04 (d, 5-CH_3), 1.42 (d, J_{HP} = 15.3 Hz, $C(CH_3)_3$), 1.37 (d, J_{HP} = 15.3 Hz, $C(CH_3)_3$). Mass spectrum (EI): 446 (M). Anal. Calcd for $C_{20}H_{33}N_2O_7P{\cdot}H_2O$: C, 51.94; H, 7.63; N, 6.06. Found: C, 52.19; H, 7.56; N, 6.13.

Thymidin-5'-yl Methyl *N*-Butylphosphoramidate (13).

To a solution of **2** (104 mg, 0.250 mmol) and ^{17}O-labeled 2-methylbenzyl alcohol (31 mg, 0.25 mmol) in dry acetonitrile (4 mL) was added 1*H*-tetrazole (35 mg, 0.25 mmol). The reaction mixture was stirred at room temperature for 15 min and was then diluted with 5% triethylamine-methylene chloride, washed with saturated sodium bicarbonate solution and dried (sodium sulphate). The solvent was evaporated under reduced pressure. The residue obtained was dried for about 2 h at room temperature under high vacuum and then dissolved in a solution of *n*-butylamine (5 mL) and anhydrous THF (0.5 mL) to which a solution of iodine (50.0 mg, 0.20 mmol) in anhydrous THF (0.5 mL) was added dropwise until the color of iodine persisted. The reaction mixture was concentrated under reduced pressure and then purified by HPLC on SiO_2 with 0-10% MeOH-CH_2Cl_2 as eluent. Yield, 41 mg (42%). ^{31}P NMR ($CDCl_3$) δ 12.05, 12.12. ^{1}H NMR ($CDCl_3$): δ 9.9 (br s, NH), δ 7.53 (q, H6), 7.45 (q, H6), 6.25-6.35 (m, H1'), 4.52- 4.65 (m, H3'), 4.05-4.30 (m, H4', H5'), 3.75 (d, J_{HP} = 11.4 Hz, OMe), 3.74 (d, J_{HP} = 11.4 Hz, OMe), 3.45-3.60 (m, $NHCH_2CH_2CH_2CH_3$),

2.80-2.90 (m, $NHCH_2CH_2CH_2CH_3$), 2.15-2.50 (m, H2'), 1.90 (d, 5-Me), 1.20-1.60 (m, $NHCH_2CH_2CH_2CH_3$), 0.85-0.95 (m, $NH(CH_2)_3CH_3$). FAB mass spectrum: 392 (M + 1).

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