New Approach to the Synthesis of Nucleoside Phosphorothioates

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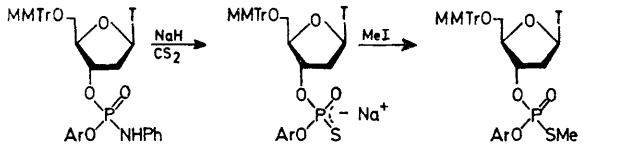
Summary Phosphorylation of 5′-monomethoxytritylthymidine (II) with aryl-*N*-phenylphosphoramidochloridate gave the diastereoisomers (IV) and (V), which are enantiomeric at the P atom; removal of the *P*-anilido group of (IV) and (V) by treatment with NaH, followed by CS_2 gave the corresponding 5′-monomethoxytritylthymidine-3′-*O*-arylphosphorothioates, (VI) and (VII), in good yield which were converted into their 5-methyl esters.

IN view of the increasing interest in the biological properties of nucleoside phosphorothioates[1] we report a convenient new route for the synthesis of this important class of compounds. Recently we demonstrated the phosphorylating ability of *O*-phenyl-*N*-phenylphosphoramidochloridate (I) by its reaction with 5′-*O*-monomethoxytritylthymidine (II) to give 5′-*O*-monomethoxytritylthymidine-3′-phenyl-phosphoranilidate (IV).[2]

As expected, the two P-enantiomeric diastereoisomers (IVa) and (IVb) were formed, as shown by t.l.c. and ^{31}P n.m.r. spectroscopy [(IVa), R_f 0·38; (IVb), R_f 0·49; δ (IVa, IVb) +2·5 p.p.m. ($CDCl_3$)].† However, treatment of (II) with *o*-chlorophenyl-*N*-phenylphosphoramidochlor-

† Analytical t.l.c. was carried out on pre-coated plates, silica gel $60F_{254}$ (Merck). Preparative t.l.c. was performed on plates covered with silica gel HF_{254} (Merck).

idate (III) (50% molar excess), m.p. 91–95 °C, $\delta(^{31}P)$ −2·50 p.p.m. ($CDCl_3$), in dry pyridine solution at room temperature gave the diastereoisomeric anilidates (V) (70% yield) in the ratio 62:38; (Va) $[\alpha]_D^{20} = +18{\cdot}33°$ (*c* 1·6, MeOH), $\delta(^{31}P)$ +2·75 p.p.m., (*M* + 1) ion by field-desorption mass-spectrometry 780 a.m.u.; (Vb) $[\alpha]_D^{20} = +16{\cdot}74°$ (*c* 0·9, MeOH), $\delta(^{31}P)$ +2·25 p.p.m., (*M* + 1) ion 780 a.m.u. To our knowledge this is the first example of an asymmetric synthesis during phosphorylation of a nucleoside. Diastereoisomers (Va) and (Vb) could be easily separated by preparative t.l.c.† The procedure previously reported for the stereospecific conversion of *P*-anilidates into *P*-thiolates,[3] allowed the ready synthesis of the thioate (VI) and the diastereoisomeric thioates (VII). These compounds were best characterized by conversion into *S*-methylthioates (VIII), and (IXa) and (IXb) (see Scheme) by reaction with methyl iodide.



(IV) Ar = Ph
(V) Ar = *o*-ClC_6H_4
(VI) Ar = Ph
(VII) Ar = *o*-ClC_6H_4
(VIII) Ar = Ph
(IX) Ar = *o*-ClC_6H_4

MMTr = *p*-MeO·$C_6H_4Ph_2C$; T = thymin-1-yl

SCHEME

As an example, treatment of (IV) with an excess of NaH in dioxan followed by addition of CS_2[4] and heating to 60 °C gave (VI a and b) in nearly quantitative yield.† (VI) in dry benzene was treated with methyl iodide at room temperature for 12 h. The product (VIII) consisted of an equimolar mixture of the P-enantiomeric diastereoisomers as shown by their ^1H n.m.r. spectra ($CDCl_3$) [two doublets at 2·21 and 2·30 p.p.m. ^3J (P–H) 16·5 Hz, intensity 1:1, characteristic for the P(O)SMe group]. The conversions (Va)→(VIIa)→(IXa) and (Vb)→(VIIb)→(IXb) were carried out in the same way (see Scheme).‡

TABLE

	(Va)	(Vb)	(VIIa)	(VIIb)	(IXa)[a]	IXb)[a]
R_f value (t.l.c.)	0·26[d]	0·34[d]	0·28[e]	0·38[e]	0·64[d]	0·64[d]
$\delta(^{31}P)$[b]	+2·75	+2·25	−52·94[c]	−51·94[c]	−26·00	−25·62

[a] The presence of an SMe group was demonstrated by ^1H n.m.r. spectroscopy; δ (IXb) 2·31 (d), ^3J (P–H) 16·6 Hz, δ (IXa) 2·33 (d), ^3J (P–H) 16·6 Hz ($CDCl_3$, Me_4Si as internal reference.) [b] in $CDCl_3$, ext. H_3PO_4, low-field negative shift. [c] D_2O–MeOH solution, 2:1. [d] Solvent: $CHCl_3$–Me_2CO (10:3). [e] Solvent: BuOH–$CHCl_3$ (1:1).

It should be emphasized that both conversions (Va, b)→(IXa, b) are completely stereospecific[3] as demonstrated spectroscopically.

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‡ Both isomers of (IX) were identified by mass spectrometry.

[1] F. Eckstein, *Angew. Chem. Internat. Edn.*, 1975, **14**, 160.
[2] W. S. Zielinski and Z. Leśnikowski, *Synthesis*, 1976, (3), 185.
[3] W. J. Stec, A. Okruszek, K. Lesiak, B. Uznański, and J. Michalski, *J. Org. Chem.*, 1976, **41**, 227.
[4] Treatment of (V a and b) with NaH followed by reaction of the resulting salt with CO_2 gave the corresponding sodium 5′-*O*-monomethoxytritylthymidine-3′-*o*-chlorophenylphosphate in 80% yield. This new deblocking technique complements that of M. Ikehara, S. Uesugi, and T. Fukui, *Chem. Pharm. Bull.*, 1967, **15**, 440.