Synthesis of 4-Nitrophenyl Esters of Thymidine 3′-Phosphate and 3′-Phosphorothioate using a New Phosphorylating Agent

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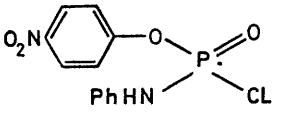
Summary 2′-Deoxynucleoside-3′-*O*-(4-nitrophenyl) phosphoranilidates (*e g* , **2**), prepared by the action of *O*-(4-nitrophenyl) *N*-phenylphosphoramidochloridate (**1**) on alcohols, are converted into the corresponding 4-nitrophenyl phosphate esters (*e g* , **3**) or P-chiral 4-nitrophenyl phosphorothioate esters (*e g* , **4**) by treatment with NaH–CX_2 (X = O or S)

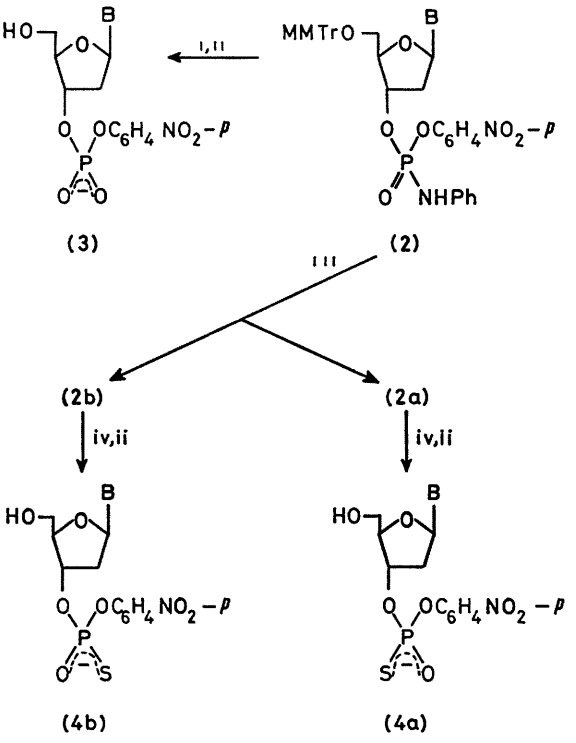
A SUCCESSFUL application of an unsymmetrical phosphorylating agent in the phosphotriester approach to the chemical synthesis of oligonucleotides has recently been demonstrated [1] The key step in the preparation of nucleotide building blocks for this purpose involves the selective removal of the one of the protecting groups



(1)

Recently, we have found that *O*-(4-nitrophenyl) *N*-phenylphosphoramidochloridate (**1**) can be successfully used for phosphorylation of deoxynucleosides Besides the simple preparation of (**1**), the yields for conversion into the appropriate 2′-deoxynucleoside-3′-O-(4-nitrophenyl) phosphoranilidates (**2**) are in the range 70—90% The anilidodiesters (**2**) are easily converted by means of NaH–CX_2 (X = O or S) into the corresponding 2′-deoxynucleoside-3′-*O*-(4-nitrophenyl) phosphates (**3**) or phosphorothioates (**4**) The former may serve as a substrates for oligonucleotide synthesis [2]

For our requirements an essential property of (**2**) is its intrinsic diastereomeric character Furthermore, the separation of the diastereomers (**2a**) and (**2b**) by a simple short column chromatography technique[3] and their subsequent stereospecific conversion[4] into the corresponding (**4a**) and (**4b**) diastereomers is possible (see Scheme) Thus, we obtained the P-chiral substrates (**4a**) and (**4b**) for use in



SCHEME B = thymin-1-yl, MMTr = *p*-$MeOC_6H_4Ph_2C$- I, $NaH–CO_2$ in dry pyridine, II, 80% AcOH, III, separation of diastereomers, IV, $NaH–CS_2$ in dry pyridine

stereochemical studies of enzyme–substrate interactions[5] in the family of exolytic enzymes Essential in our procedure is the presence of a chromophoric 4-nitrophenyl substituent in (**3**) and (**4**) which facilitates the spectroscopic assay of the lytic reaction progress

The following example illustrates the advantage and simplicity of our procedure † *O*-(4-Nitrophenyl) phosphorodichloridate[6] (0 1 mol) reacts with aniline (0 2 mol) in

† The procedures described here are general for the preparation of 2′-deoxynucleoside-3′-*O* (4 nitrophenyl) phosphates, (**3**, B = Ade, Gua, Cyt) and -3′-*O*-(4-nitrophenyl) phosphorothioates (**4**, B = Ade, Gua, Cyt)

benzene solution (100 ml) to give (**1**) in 83% yield, m.p. 126—128 °C (benzene); $\delta(^{31}P)$ −1·61 p.p.m. (Me_2CO); *m/z* 312 (M^+, 100%).‡

5′-Monomethoxytritylthymidine (7 mmol) in dry pyridine (20 ml), when treated with (**1**) (10·5 mmol), gives (**2**) in 90% yield. This mixture can be separated into diastereomers by use of short column chromatography§ to give (**2a**): R_f 0·33 (A); $\delta(^{31}P)$ +3·37 p.p.m. ($CDCl_3$); *m/z* 789 (M^+-1) and 517 (M^+-MMTrH) and (**2b**): R_f 0·24 (A); $\delta(^{31}P)$ −3·17 p.p.m.; *m/z* 789 (M^+-1) and 517 (M^+-MMTrH).¶ Each of the diastereomers is converted, under the conditions described in our previous report[4] ($NaH-CS_2$) followed by treatment with 80% AcOH and purification by paper chromatography and BioGel P-2 filtration, into the thymidine 3′-*O*-(4-nitrophenyl) phosphorothioates, isolated as their ammonium salts, (**4a**): yield 69%; R_f 0·69 (B); $\delta(^{31}P)$ −50·68 p.p.m. (pyridine); *m/z* 459 (M^+-NH_3); $\epsilon(H_2O$; 267 nm) 14,200; (**4b**): yield 75%; R_f 0·71 (B); $\delta(^{31}P)$ −50·76 p.p.m. (pyridine); *m/z* 459 (M^+-NH_3); $\epsilon(H_2O$; 267 nm) 13,600.

The diastereomeric mixture of (**2**) (0·5 mmol) in dry pyridine, on treatment with NaH (50% molar excess) followed by dry CO_2 (gas), is converted, in high yield (80%), into P-achiral thymidine 3′-*O*-(4-nitrophenyl) phosphate (**3**): R_f 0·65 (B); $\delta(^{31}P)$ +6·28 p.p.m. (pyridine); *m/z* 443 (M^+-NH_3); $\epsilon(H_2O$; 267 nm) 15,600.

This preparative method of (**3**) is simple, efficient, and may be considered an alternative to those reported by Turner and Khorana[7] and Glinski *et al.*[8]

Assays of spleen phosphodiesterase EC 3.1.4.18 with (**3**), (**4a**), and (**4b**) will be published elsewhere.

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‡ External H_3PO_4 as reference; low-field shifts are negative.

§ On silica gel MN (200—300 mesh); chloroform–acetone (1—2·5%) solvent mixtures.

¶ T.l.c. on pre-coated plates, silica gel $60F_{254}$ (Merck) in solvent system (A), chloroform–acetone (10 : 3), or cellulose F (Merck) in solvent system (B), n-butanol–acetic acid–water (5 : 1 : 3). Field desorption mass spectral data are given for (**2**)—(**4**).

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