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SYNTHESES AND CONFIGURATIONAL ASSIGNMENTS OF THE DIASTEREOMERS OF THE 4-NITROPHENYL ESTERS OF THYMIDINE 3'-(N-PHENYL PHOSPHORAMIDATE) AND THYMIDINE 5'-(N-PHENYL PHOSPHORAMIDATE)

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Summary: The separated diastereomers of 3'-(4-nitrophenyl N-phenyl phosphoramidate)-5'-monomethoxytrityl thymidine and 3'-monomethoxytrityl-5'-(4-nitrophenyl N-phenyl phosphoramidate) thymidine have been prepared and their absolute configurations at phosphorus have been determined.

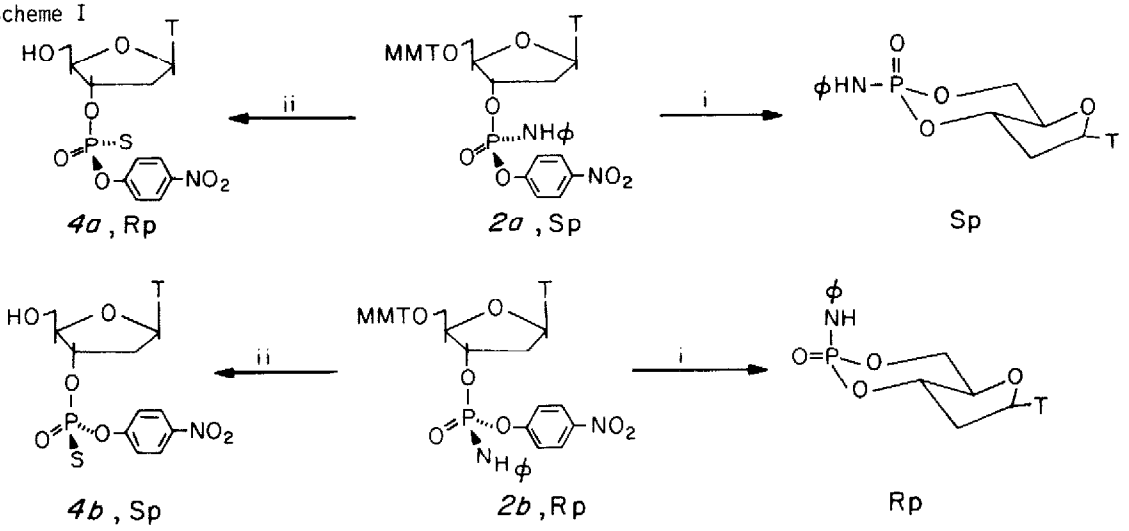
Recent work in this laboratory (1) and that of W. J. Stec (2) has shown that the sodium salts of N-phenyl phosphoramidate diesters react with [^{18}O]-carbonyl compounds (carbon dioxide (1) or benzaldehyde (2)) to yield oxygen chiral phosphodiesters; this reaction proceeds with retention of configuration at phosphorus. Because this reaction appears to be general, the synthesis of a wide variety of oxygen chiral phosphodiesters is now possible. Such materials will be useful in determining the stereochemical course of both enzymatic and nonenzymatic reactions involving phosphoryl group transfer (3). Since we are interested in the mechanisms of the reactions catalyzed by nucleases and, in general, phosphodiesterases, we plan to synthesize thymidine 3'- and 5'-(4-nitrophenyl phosphates) which are chiral by virtue of oxygen isotopes; these chromophoric phosphodiesters are substrates for a number of deoxyribonucleases. In this communication we report the syntheses and configurational assignments of all the necessary N-phenyl phosphoramidate precursors for the desired oxygen chiral phosphodiesters.

4-Nitrophenyl N-phenyl phosphoramidic chloride (1) was prepared by the reaction of 4-nitrophenyl phosphorodichloridate with two equivalents of aniline in refluxing benzene (4). After extraction of a chloroform solution of the product with water and removal of the solvent, the product was crystallized from benzene as pale yellow crystals, m.p. 126.8-128.0°; ^{31}P NMR +1.62 ppm ($CDCl_3$, downfield relative to external 85% H_3PO_4).

Reaction of 5'-monomethoxytrityl thymidine (5) with a 10% excess of 1 in dry pyridine gave a diastereomeric mixture of 3'-(4-nitrophenyl N-phenyl phosphoramidate)-5'-monomethoxytrityl thymidines (2a and 2b, see Scheme I for structures) as a pale yellow foam in about 75% yield. In $CDCl_3$ the ^{31}P NMR spectrum of this mixture showed two resonances of equal intensities at -5.52 and -5.76 ppm; on silica gel with CH_2Cl_2:2-propanol (25:1) as eluent, two spots were observed with R_f 0.45 (2a) and 0.37 (2b). The diastereomers could be separated by short column

chromatography on Merck silica gel 60H. 2a had a ^{31}P NMR chemical shift of -5.91 ppm and 2b had a chemical shift of -5.49 ppm.

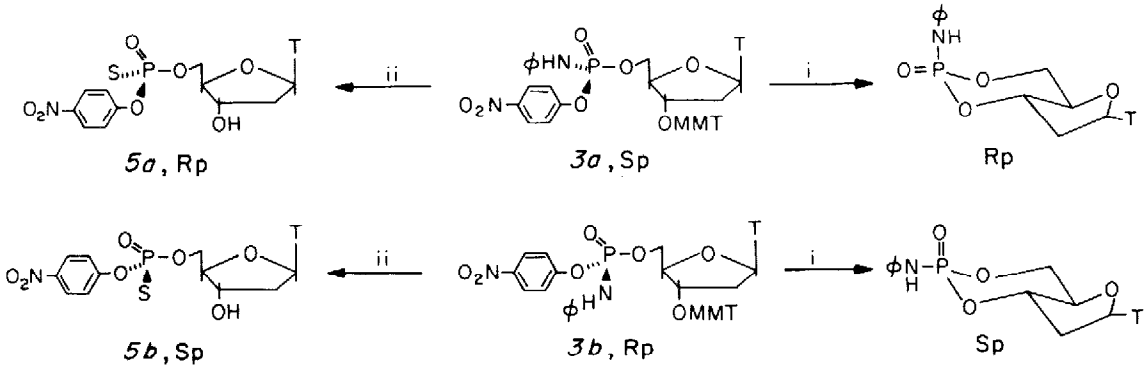
Scheme I



i = 80% acetic acid and t-butoxide in DMF; ii = NaH/CS_2 and 80% acetic acid

Reaction of 3'-monomethoxytrityl thymidine (7) with a 10% excess of 1 in dry pyridine gave a diastereomeric mixture of the 3'-monomethoxytrityl-5'-(4-nitrophenyl N-phenyl phosphoramidate) thymidines (3a and 3b, see Scheme II for structures) in about 75% yield. In $CDCl_3$ the ^{31}P NMR spectrum of this mixture showed two resonances at -4.18 and -4.50 ppm, in an intensity ratio of 100:81; on silica gel a single spot was observed with R_f 0.45. The diastereomers could be separated on a 2 x 90 cm column of silica gel 60H. The first component to elute from the column (3a) had a ^{31}P NMR chemical shift of -4.67 ppm ($CDCl_3$) and the second component had a chemical shift of -5.01 ppm ($CDCl_3$).

Scheme II



i = 80% acetic acid and t-butoxide in DMF; ii = NaH/CS_2 and 80% acetic acid

2a, 2b, 3a, and 3b were separately dissolved in dry pyridine and treated with a three-fold excess of sodium hydride. After thirty minutes, excess carbon disulfide was added to each reaction mixture, and the mixtures were heated at 50° for two hours. After evaporation of the solvent, the phosphorothioates were treated with 80% acetic acid in water to remove the monomethoxytrityl groups. The products were purified by chromatography on DEAE-Sephadex A-25, using a linear gradient of triethylammonium bicarbonate as eluent. The fractions containing the (4-nitrophenyl phosphorothioate) products were combined, concentrated, converted to the corresponding sodium salt by ion-exchange chromatography, and lyophilized to yield the diastereomeric phosphorothioates in about 70% overall yield. Both thymidine 3'-(4-nitrophenyl phosphorothioates) (4a and 4b, obtained from 2a and 2b respectively) had ^{31}P NMR chemical shifts of 50.64 ppm in D_2O, as judged by a spectrum recorded on the mixture of diastereomers. The thymidine 5'-(4-nitrophenyl phosphorothioates) (5a and 5b, obtained from 3a and 3b respectively) had different ^{31}P NMR chemical shifts, with that of 5a being 51.87 ppm and that of 5b being 52.09, as judged by a spectrum recorded on the mixture of diastereomers. The ^{1}H NMR spectra of 4a and 4b were virtually identical, as were the ^{1}H NMR spectra of 5a and 5b. 4a, 4b, 5a, and 5b were pure by tlc.

2a, 2b, 3a, and 3b were separately treated with 80% acetic acid in water to remove the monomethoxytrityl groups. Each N-phenyl phosphoramidate was dissolved in dry DMF and treated with a ten-fold excess of potassium t-butoxide; after forty five minutes at room temperature, the reactions were stopped by addition of an excess of ammonium chloride dissolved in water and evaporated to dryness. The cyclic N-phenyl phosphoramidates of thymidine 3',5'-cyclic phosphate were dissolved in CH_3OD, and ^{31}P NMR spectra were recorded on each sample. By this procedure, 2a and 3b were converted to the equatorial N-phenyl phosphoramidate of the cyclic phosphate, as judged by the chemical shift of the product (1.62 ppm); this cyclic N-phenyl phosphoramidate has the S_P configuration (8). 2b and 3a were converted to the axial N-phenyl phosphoramidate of the cyclic phosphate, as judged by the chemical shift of the product (-1.39 ppm); this cyclic N-phenyl phosphoramidate has the R_P configuration. Each cyclization reaction was observed to proceed with complete stereospecificity.

Although it would be predicted that the t-butoxide catalyzed cyclization reaction should proceed with inversion of configuration at phosphorus, thereby allowing the configurations of 2a, 2b, 3a, and 3b to be assigned, it is essential to obtain independent proof for this prediction if these acyclic N-phenyl phosphoramidates are to be used to prepare oxygen chiral phosphodiesters for stereochemical studies. Such information can be obtained by comparing the rates of hydrolysis of 5a and 5b in the presence of snake venom phosphodiesterase, which is known to be stereospecific for the R_P diastereomer of chiral nucleoside phosphorothioates (9). The rates of hydrolysis of 5a, 5b, and thymidine 5'-(4-nitrophenyl phosphate) (NPpT) were measured at pH 8.9 in the presence of 20 mM $MgCl_2$; measurements were made at several concentrations of each diester so that values for V_{max} and K_m could be determined. The values obtained are collected in the Table. Since 5a was hydrolyzed faster than 5b, 5a has the R_P configuration and 5b has the S_P configuration (the configurational assignments are summarized in the Schemes). Since the reaction of a sodium salt

of an N-phenyl phosphoramidate with carbon disulfide proceeds with retention of configuration at phosphorus (10), 3a has the S_p configuration and 3b has the R_p configuration. This assignment demonstrates that the t-butoxide catalyzed cyclization reaction does, in fact, proceed with inversion of configuration at phosphorus. Since it is highly probable that the stereochemical course of the ring closure reactions for the 3'- and 5'-N-phenyl phosphoramidates would be identical, 2a can be assigned the S_p configuration and 2b the R_p configuration; therefore, 4a can be assigned the R_p configuration and 4b the S_p configuration. Stec's group has also synthesized the diastereomers of thymidine 3'-(4-nitrophenyl phosphorothioate) by an analogous procedure but did not assign their absolute configurations at phosphorus (11).

Table. Kinetic Parameters for the Hydrolysis of NPpT, 5a and 5b by Snake Venom Enzyme

Substrate	V_{max} (μmoles/(min mg))	K_m (mM)	V_{max}/K_m
NPpT	23.5	0.17	138
5a	6.0	0.038	157
5b	1.0	0.59	1.7

The configurational assignments reported in this communication permit the confident synthesis of thymidine 3'- and 5'-(4-nitrophenyl phosphates) which are chiral by virtue of oxygen isotopes, and these syntheses are currently underway in this laboratory.

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