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FACILE PREPARATION OF THE INDIVIDUAL DIASTEREOMERS OF THYMIDINE 3',5'-CYCLIC PHOSPHOROTHIOATE (cTMPS)

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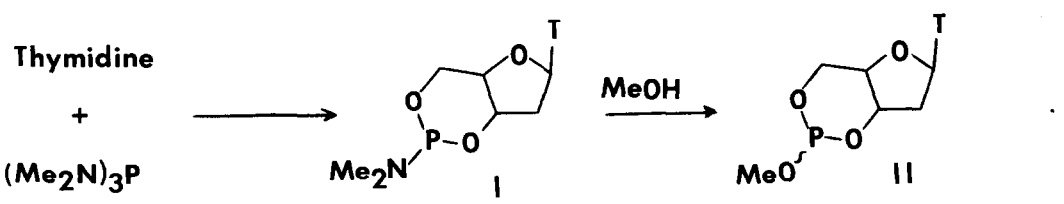
Summary: A facile synthesis of the individual S_p and R_p 3,5-cyclic thymidine phosphorothioates is reported. The key intermediate is a methyl phosphite triester which is readily converted under free-radical conditions to the diastereomeric thiophosphates which are separated on SiO_2 and then cleanly dealkylated by t-$BuNH_2$ to the amine salts of title compounds.

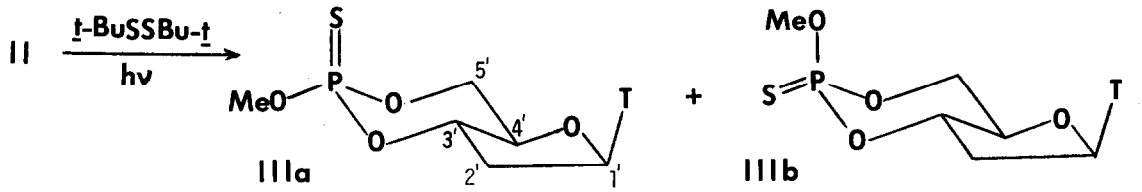
Nucleoside phosphorothioates of known configuration at phosphorus are of considerable current interest for studies of the overall stereochemistries of enzymatic processes[1,2] and as stereochemical probes of receptor-site substrate binding requirements.[1,3] The two individual diastereomers of adenosine 3,5-cyclic phosphorothioate (cAMPS) have recently been prepared[4] and the stereochemistry of hydrolysis of the S_p diastereomer by beef heart phosphodiesterase determined.[5]

The recent confirmation of the presence of cytidine 3',5'-cyclic monophosphate (cCMP) in rat liver and L1210 leukemia cells[6] as well as the discovery of a phosphodiesterase selective for cCMP[7] focuses special attention on the importance of pyrimidine-derived 3',5'-cyclic phosphorothioates. We report here the ready synthesis of the diastereomerically pure thymidine 3',5'-cyclic phosphorothiates (cTMPS) in four straightforward steps (intermediates I-IV) beginning with thymidine. Only one other method of preparation of cTMPS diastereomers has been published.[8] Our method is one step step shorter and utilizes the readily prepared cyclic phosphite II, whose isolation was reported previously.[9] Indeed, starting from II, the overall yield of the diastereomers of IV normally is greater than 80%. From thymidine itself, one can readily prepare 100-400 mg amounts of each dia-

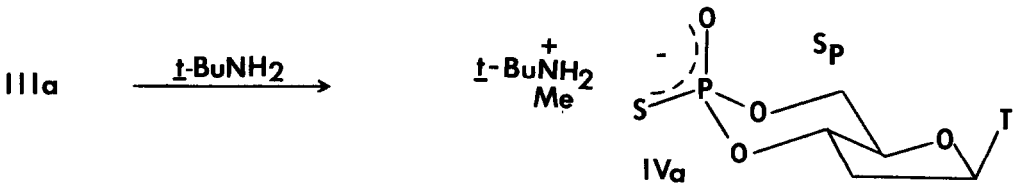


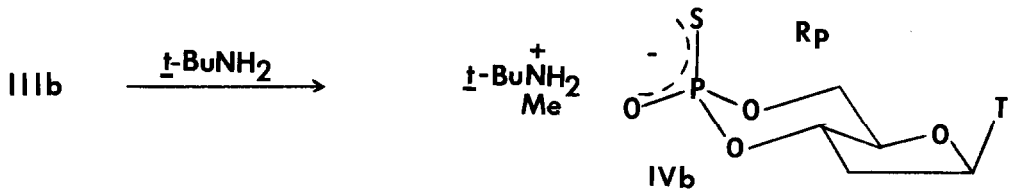
stereomer of cTMPS.[10]

For example, irradiation through Pyrex (medium pressure Hanovia 450 W lamp) of a solution of 500 mg (1.7 mmol) of II and 1.35 g (7.6 mmol) of t-BuSSBu-t dissolved in 25 ml of 70/30 benzene/acetone gave, after single-pass medium pressure liquid chromatographic (MPLC) purification on a 1000 X 15 mm SiO_2 (97/3 $CHCl_3$/MeOH eluant), 460 mg (1.4 mmol) of diastereomeric III (IIIa/IIIb about 50/50 by ^{31}P NMR).[11] The individual isomers of III were isolated by MPLC on the above column



(80/20 CCl_4/i-PrOH). Thus, 400 mg of the above mixture, chromatographed in four batches, gave 183 mg of IIIa (δ ^{31}P = +67.7, $CDCl_3$) and 180 mg of IIIb (δ ^{31}P = +65.8, $CDCl_3$);[12] overall isolated yield based on starting II, 82%. The desired diastereomers of cTMPS, crystalline salts IVa and IVb, are obtained virtually quantitatively from solutions of IIIa and IIIb, respectively, in t-$BuNH_2$ after 15 hours of reflux.[13] Diastereomeric purity was >95% as judged by ^{31}P NMR (D_2O).





Configurational assignments to IVa and IVb were made by comparisons of their ^{31}P chemical shifts (D_2O at +54.9 (IVa) and +53.1 (IVb) to those at +54.7 and 52.1 reported for the corresponding ammonium salts.[8] Further confirmation of these assignments comes from our results. The known correlation [14] of higher field ^{31}P chemical shifts for thiophosphates with RO axial fixes the con-

figurational assignments of IIIa and IIIb. In addition, the relative $C_{3'}$ and $C_{5'}$ ^{13}C chemical shifts for IIIa and IIIb (vs. TMS) are consistent with certain ^{13}C chemical shift relationships recently noted in such ring systems.[15] Thus, the MeO-cis isomer (IIIb) has its $C_{3'}$ peak at δ 7.76. Similarly, the $C_{5'}$ resonances for IIIb and IIIa are at 70.2 and 69.2, respectively. Demethylation of III is stereospecific and proceeds stereochemically as shown.

The above reaction sequence further demonstrates the versatility of the isolated cyclic phosphite II[9] for the introduction of various functionality at phosphorus in P-derivatized nucleoside 3',5'-cyclic monophosphates (1,3,2-dioxaphosphorinanes) using chemistry well-established in non-nucleoside systems. In principle this route also should be applicable to ribonucleosides via their $O_{2'}$-protected derivatives. The photo-initiated free radical sulfurization step (II → III) provides an alternative to the usual S_8 method which in our case gave large amounts of side product. It may find application as well to other phosphorothioate syntheses using phosphite triester intermediates.[16]

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10. This compares with an overall yield (based on 5'-O-monomethoxytritylthymidine) for the previous method of about 30%.[8] Although the comparatively low yield of starting II (presently ∿20%) results in overall yields of IV based on thymidine somewhat lower than those of Stec et al.,[8] the ready availability of thymidine and comparative simplicity of our approach recommend its use. Improved methods for preparing I (near-quantitatively converted to II[9]), which will be applicable as well to deoxyribonucleosides less readily available than thymidine, are under investigation in this laboratory.

11. RS• oxidations of six-membered ring cyclic phosphites are known to be stereospecific with retention of phosphorus configuration (W.G. Bentrude, J.H. Hargis, N.A. Johnson, T.B. Min, P.E. Rusek, Jr., H.W. Tan, and R.A. Wielesek, J. Am. Chem. Soc., 98, 5348 (1976)).

12. Positive ^{31}P chemical shifts in ppm downfield from external 85% H_3PO_4.

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