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CYCLOPHOSPHAMIDE STEREOCHEMISTRY. I. SYNTHESIS AND CONFIGURATIONAL STABILITY OF DIASTEREOMERIC CYCLOPHOSPHAMIDE DERIVATIVES

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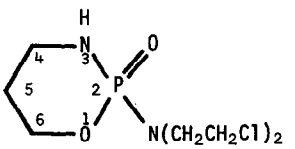
Cyclophosphamide (1) is a clinically useful anticancer drug which is believed to require enzymatic C_4-hydroxylation before exhibiting its chemotherapeutic effects.[1] Our interest in elucidating the stereospecificity of this metabolic activation process toward (R)- and (S)-1 has led to preliminary evaluation of *in vitro* configurational stability at the chiral phosphorus center in 1 by studies utilizing suitable diastereomeric derivatives, which we now wish to report.



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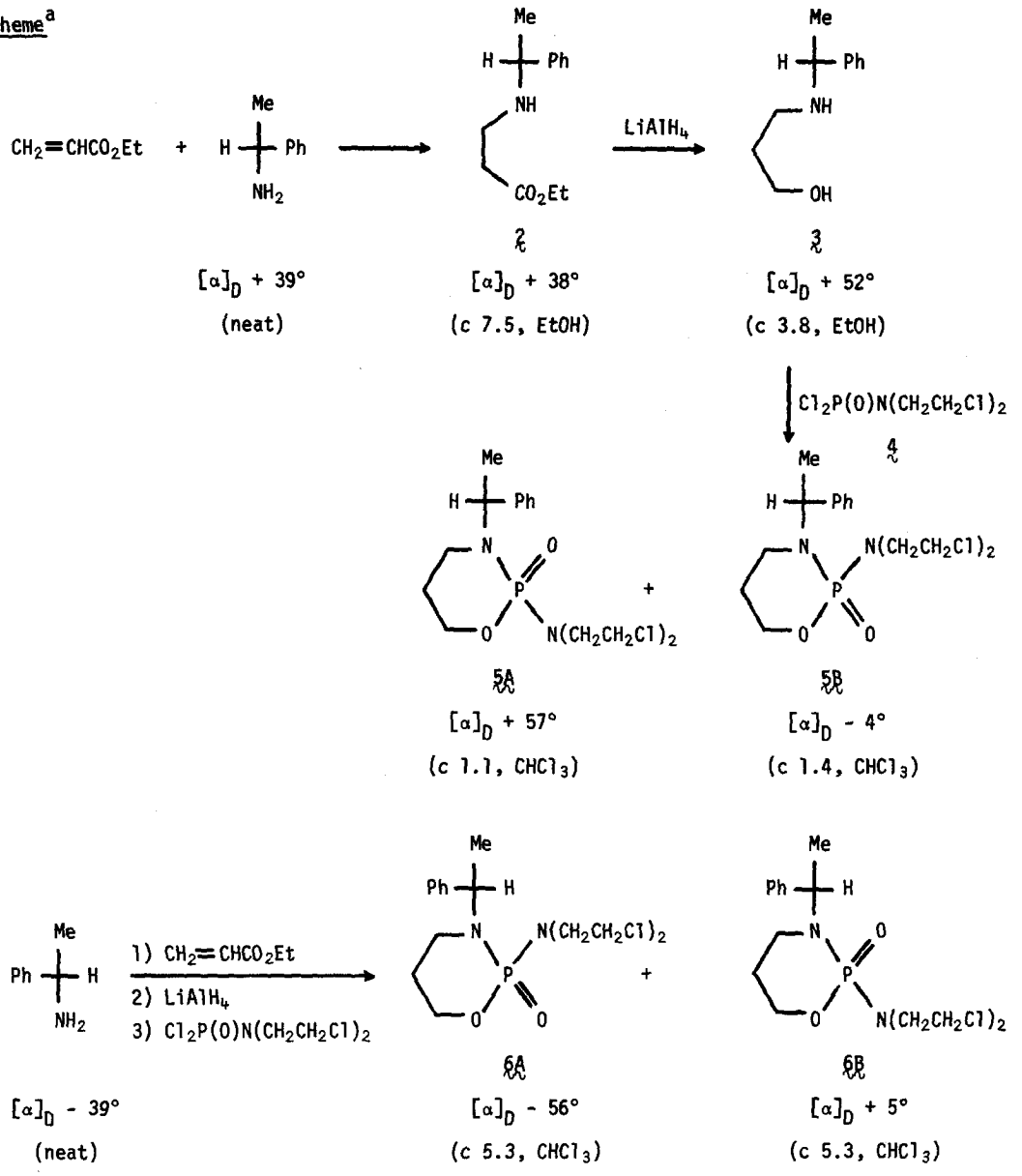
Addition of optically pure (+)-(R)-α-methylbenzylamine to ethyl acrylate in refluxing absolute EtOH afforded 2,[2] which was reduced with $LiAlH_4$ to 3 in 59% overall isolated yield (see Scheme). Treatment (4 days, 25°) of this presumably optically pure amino alcohol with 4[3] in EtOAc containing 2 equiv. Et_3N yielded (63%), upon careful chromatography (silica gel; Et_2O : $CHCl_3$ = 1 : 1), near equal amounts of the desired cyclophosphamide diastereomers, 5A (R_f = 0.74) and 5B (R_f = 0.57). Use of optically pure (-)-(S)-α-methylbenzylamine in the synthetic Scheme led to isolation of the respective enantiomers of 5, 6A and 6B. To our knowledge, this stereoisomer set represents the first example of cyclophosphamide derivatives which can be used as a model system for probing chemotherapeutic consequences of configurational homogeniety at phosphorus in 1.[4]

Spectroscopic assessment of diastereomeric purity of 5A and 5B was readily accomplished by monitoring their individual methyl group ^1H nmr (60 MHz, $CDCl_3$) doublets (J = 7 Hz) at δ 1.55 and 1.68, respectively. At higher magnetic field strength (220 MHz), configurationally pure (≥ 99%, tlc) samples of 5 failed to show additional methyl group proton signals at 20°. This evidence and the broad band decoupled high resolution ^{13}C nmr spectrum of 1 (68.9 MHz, $CDCl_3$),[5]

Scheme[a]



[a] Rotational differences between enantiomers of 5 and 6 are ascribed to contamination by solvent and H_2O in these viscous hygroscopic oils.

which exhibits only one set of absorption peaks at 40°, together with reported[6] conformational analyses of analogous ring systems, are consistent with our assumption that the six-membered ring in 5 is conformationally mobile at room temperature.

Rapid interconversion of (R)- and (S)-1 under physiological conditions can, in principle, occur by reversible coordination of water to initiate formation of a symmetrical phosphorane, or by attack at phosphorus of some other nucleophilic species with subsequent intramolecular ligand exchange in phosphorane intermediates.[7] The likelihood of such events, which if operative would preclude differential antitumor activity between enantiomers of 1, was gauged indirectly via 5.

Initial ^{1}H nmr experiments were performed with 5 (0.34 M) in D_2O having 75 v/v% DMSO-d_6 as co-solvent. Mildly acidic (pH ~ 5.6) or basic (pH ~ 8.4) samples of pure 5A or pure 5B showed no detectable ($\lesssim$ 5%) diastereomer crossover after 15 hr at 50°, as did diastereomer samples containing 0.5 - 1.0 equiv. of either NaCl, NaOAc, or $MgCl_2$.[8] More significantly, it was found that neither diastereomer of 5 underwent stereomutation ($\leq$ 1%, tlc) while dissolved in human blood plasma (1 mg/ml) at 37° for a period of 14 hr.

The presently reported configurational stability at phosphorus in model substrate 5 suggests that, in the absence of special in vivo effects, enantiomerically pure cyclophosphamide should not suffer racemization during its relatively rapid transport ($\lesssim$ 15 min[9]) to the hepatic enzyme system which effectuates C_4-hydroxylation. Consequently, the conceivable therapeutic advantages of administering enantiomerically pure 1 remain as viable research goals. A similar conclusion extends to isophosphamide,[10] which is a chiral antitumor agent structurally related to 1. Studies concerning the resolution of 1 will be reported in the future.

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