Synthesis of Steroidal Cyclophosphamide, 2-Bis (2-chloroethyl)amino-2-oxo-6-(5α-cholestan) 1, 3,2-oxazaphosphorinane

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Cyclophosphamide and its analogues are important clinical agents in the treatment of cancer. We have prepared steroidal cyclophosphamides (1a and 1b). The approach used for the synthesis of 1a and 1b is outlined in Scheme 1. Treatment of cholestanone (2) with n-butyllithium and acetonitrile gave a 72.5% yield of β-hydroxyiminonitrile derivative 3, which was subsequently reacted with LiAlH4 to give aminoethylnol derivative 4.2 Cyclization of 4 with bis(2-chloroethyl)phosphoramic dichloride (5) in the presence of 2 equiv. of Et3N afforded crude mixtures of 1a and 1b, which were chromatographed on silica gel with EtOAc:CH2Cl2:hexane = 2:2:1 to give analytically pure crystals of the faster (mp, 192-194°C) and slower (mp, 178-180°C) eluting diastereomers of 1a and 1b in 58% yield. Assignment of cyclophosphamide structures to the faster and slower eluting diastereomeric cyclization products has been suggested by the IR; 1H-NMR; 13P-NMR; and 31P-NMR.

Our measurements of 1a and 1b indicated the 1H-NMR chemical-shift difference between the NH resonances at 2.73 and 2.50 ppm for the faster and slower eluting diastereomers of 1a and 1b, respectively. The substantial deshielding (0.23 ppm) of N-H proton thus exhibited by the faster moving compound 1a, suggests more efficient intramolecular H-bonding to the adjacent P=O functionality. This difference in H-bonding was also founded in 31P-NMR by the deshielding of chemical shift[41.9 ppm (-NH-CH2)] in the proposed 1a, as opposed by the shielding of chemical shift [36.0 ppm (-NH-CH2)] in the proposed 1b. These compounds may have a greater impact as anticancer agents by their lipophilicity. Compounds 1a and 1b were found no activity against Hepatoma cells.

Experimental

3-Cyanomethyl-5α-cholestan-3-ol (3). To a stirred solution of 1.6 M n-butyllithium in 9.5 ml (15 mmol) hexane, at -80°C under nitrogen, was rapidly added a solution of 0.82 ml (15 mmol) of acetonitrile in 30 ml of anhydrous THF. After stirring for 1 hr, the resulting white suspension was treated with a solution of 3.0 g (75 mmol) 2 in 10 ml of THF. The cold-ice bath was removed and stirred for additional 10 min before it was poured into ice-water hydrochloric acid. The aqueous layer was extracted with three 50 ml portions of EtO. The combined ether extracts were dried(MgSO4) and evaporated in vacuo, and the residual crude product was chromatographed on silica gel with CH2Cl2 as an eluent, and obtained 2.4 g (73% yield) of white solids, mp 158-159°C; 1H-NMR (CDCl3) δ 2.6 (s, 2H, -CH2CN), 0.6-2.0 (m, 1H, steroid); IR (KBr) 3480 (-OH), 2930, 2255 (-CN), 1460, 1370, 1080, 1050 cm-1.

3β-Aminoethylene-5α-cholestan-3-ol (4). To a stirred solution of 1.7 g (3.9 mmol) of 3 in 150 ml of anhydrous THF was added in small portions, 0.75 g (19.5 mmol) of lithium aluminum hydride. The mixture was refluxed with stirring for 17 hrs. After decomposing excess lithium aluminum hydride with 0.75 ml water and 2.3 ml of 20% NaOH, the mixture was filtered and filtrate was evaporated in vacuo to obtain yellow oily residues (45% yield). All attempts
to obtain crystallization was unsuccessful. IR (KBr) 3330 (NH2), 1560 (NH), 1470, 1380, 1150, 1020 cm⁻¹.

2-Bis(2-chloroethyl)amino-2-oxo-6-(5α-cholestanyl)-1,3,2-oxazaphosphorinane (1a and 1b). A crude 1.7 g (3.9 mmol) of 4 and 1.0 g (3.9 mmol) of bis(2-chloroethyl)phosphoramic dichloride (5) was dissolved in 160 ml of anhydrous THF, and added 0.79 ml (7.8 mmol) of anhydrous Et3N. The reaction mixture was vigorously stirred for 24 hrs. and the B2O3 • HCl formed was filtered. The filtrate was evaporated in vacuo and the residue was chromatographed on silica gel using EtOAc: CH2Cl2: Hexane (2: 2: 1) to give fractions containing faster eluting 1a and slower eluting 1b (1a: 1b = 1: 1.2; 1.2 g; 58% yield). For 1a: mp. 192-194°C; ¹H-NMR (CDCl₃) 6 3.61 (t. J = 7.40, 4H, 2 x -NCH₂CH₂Cl), 3.25-3.50 (m, 4H, 2 x -NCH₂CH₂Cl), 3.19 (m, 2H, -NCH₂CH₂CH₂), 2.73 (br s, 1H, NH), 2.12 (m, 2H, -NCH₂CH₂CH₂), 0.6-2.0 (m, H steroid); ¹³C-NMR (CDCl₃) 8 84.9 (d, JCF = 7.8, spiro carbon). 493 (d, JCF = 3.0, 2 x -NCH₂CH₂Cl), 425 (2 x -NCH₂CH₂Cl), 419 (d, JCF = 8.5, -NCH₂CH₂Cl), 36.0 (N-NH₂CH₂Cl), 12.0, 12.1, 18.7, 21.3, 22.5, 23.8, 24.2, 28.0, 28.2, 31.9, 32.0, 35.5, 35.8, 36.0, 36.2, 36.5, 40.0, 42.5, 42.6, 43.7, 45.4, 56.3, and 56.5 (steroid carbons); ³¹P-NMR (CDCl₃) 8 10.25; Mass (FAB) (m/z) 618 (M++1); Anal. Calcd. for C₃₂H₃₆N₄O₆PCl₄: C, 64.17; H, 6.62; N, 4.43. Found: C, 64.09; H, 6.4; N, 4.4. For 1b: mp. 178-180°C; ¹H-NMR (CDCl₃) 8 3.59 (t, J = 6.9, 4H, 2 x -NCH₂CH₂Cl), 3.28-3.54 (m, 4H, 2 x -NCH₂CH₂Cl), 3.19 (m, 2H, -NCH₂CH₂Cl), 2.50 (br d, 1H, NH), 2.12 (m, 2H, -NCH₂CH₂Cl), 0.8-4.20 (m, H steroid); ¹³C-NMR (CDCl₃) 8 84.7 (d, JCF = 7.8, spiro carbon). 494 (d, JCF = 3.0, 2 x -NCH₂CH₂Cl), 425 (2 x -NCH₂CH₂Cl), 419 (d, JCF = 8.5, -NCH₂CH₂Cl), 36.0 (N-NH₂CH₂Cl), 12.0, 12.1, 18.7, 21.3, 22.5, 23.8, 24.2, 28.0, 28.2, 31.9, 32.0, 35.5, 35.8, 36.0, 36.2, 36.5, 40.0, 42.5, 42.6, 43.7, 45.4, 56.3, and 56.5 (steroid carbons); ³¹P-NMR (CDCl₃) 8 10.48; Mass (FAB) (m/z) 618 (M++1); Anal. Calcd. for C₃₂H₃₆N₄O₆P₃: C, 64.17; H, 6.4; N, 4.3. Found: C, 64.32; H, 9.98; N, 4.49.

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References

Transformation Mechanism of Bicyclic Ketal Compound to 1,5-Diketone

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The chemistry of bicyclic ketals in the 6,8-dioxabicyclo[3.2.1]octane series are very unique and interesting. Our initial success in the preparation of 1,5-diketone from bicyclic ketal expanded the utilities of this bicyclic ketal system to the direct synthesis of 2,6-disubstituted pyridines, 2,3,6-trisubstituted pyridines, cyclohexene derivatives, and cyclopentanediol derivatives. The 1,5-diketone is thought to be an active intermediate for these transformation reactions. We proposed two possible mechanisms for the formation of 1,5-diketone from bicyclic ketal using aluminium chloride-sodium iodide in methylene dichloride. The mechanism "a" in Scheme 1 involves O(6)-C(5) bond cleavage followed by 1,2-hydride shift via an epoxide intermediate, whereas the alternative mechanism "b" involves O(8)-C(5) bond cleavage followed by proton abstraction.

Scheme 1.

Scheme 2.