SYNTHESIS OF STEROIDAL CYCLOPHOSPHAMIDES*

Emerson L. Foster and Robert T. Blickenstaff [1]

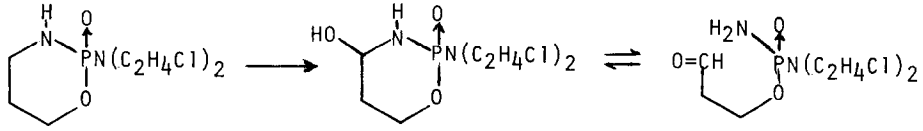
Veterans Administration Hospital, and Biochemistry Department, Indiana University School of Medicine, Indianapolis, Indiana 46202

Received: 9/29/75

ABSTRACT

The Reformatsky product of estrone methyl ether and ethyl bromoacetate was transformed by two separate routes to 21-amino-3-methoxy-17α-pregna-1,3,5(10)-trien-17β-ol (9). Cyclization with bis-(2-chloroethyl)phosphoramide dichloride produced the steroidal cyclophosphamide 10. Analogous syntheses transformed androstenolone into steroidal cyclophosphamide 20 and androstenedione into steroidal cyclophosphamide 28.

Cyclophosphamide (1) is in extensive use clinically as an antitumor agent [2]. It is activated in the liver to 4-hydroxycyclophosphamide (2) [3], which is in equilibrium with the open-chain form 3 [4]. Both hydroxy compound 2 and the corresponding 4-hydroperoxy derivative have been synthesized recently, and both exhibit high in vivo and in vitro cytotoxic activity [5].



1 2 3

Despite only spotty successes from previous attempts [6,7], the concept of linking some form of a N-mustard to steroids persists [8,9], primarily for the selective delivery of the active moiety to target sites and to reduce toxicity. The present work describes the synthesis of potential antitumor steroidal cyclophosphamide derivatives.

*Published in preliminary form: Steroids, 24, 737 (1974)

Ethyl 17β-hydroxy-3-methoxy-17α-pregna-1,3,5(10)-trien-21-oate (5) [10], from the Reformatsky reaction of estrone methyl ether (4), was hydrolyzed to the corresponding acid; the action of methyl chloroformate on the triethylammonium salt 6 gave the mixed anhydride 7. Without purification the latter was treated with ammonia to produce the amide 8, which by reduction with diborane [11] afforded the amine 9. Cyclization with bis-(2-chloroethyl)phosphoramide dichloride [12] in the presence of triethylamine [13] produced the spiro steroid cyclophosphamide 10. Its structure is supported by the absorption bands for NH (3150 cm^{-1}) and PO (1212 cm^{-1}) in the ir spectrum; furthermore, all the resonances in the nmr spectrum of cyclophosphamide (1) are observed except the missing methylene protons adjacent to oxygen.

A second route to the amino alcohol 9 required more isolated intermediates, but proceeded in higher overall yield. The ester 5 was reduced to the diol 11 with lithium aluminum hydride. Selective tosylation produced monotosylate 12, which was quantitatively converted to the azide 13, and reduction of the latter produced the amine 9. This route was used in subsequent syntheses; intermediates and final products are listed in Table 1. 3β-Hydroxy-5-androsten-17-one tetrahydropyranyl ether [14] underwent Reformatsky condensation to give the ester 14, which was reduced with lithium aluminum hydride to diol 15. Selective tosylation gave the tosylate 16, which was converted to the azide 17. Reduction [15] of the latter gave amine 18, which was cyclized as before to the steroid cyclophosphamide 19. Under conditions used previously to hydrolyze steroidal ketals [16], the protective group was removed giving the hydroxy compound 20. Attempts to oxidize Δ^5-3β-ol 20 to a Δ^4-3-keto steroid have led to intractible mixtures.

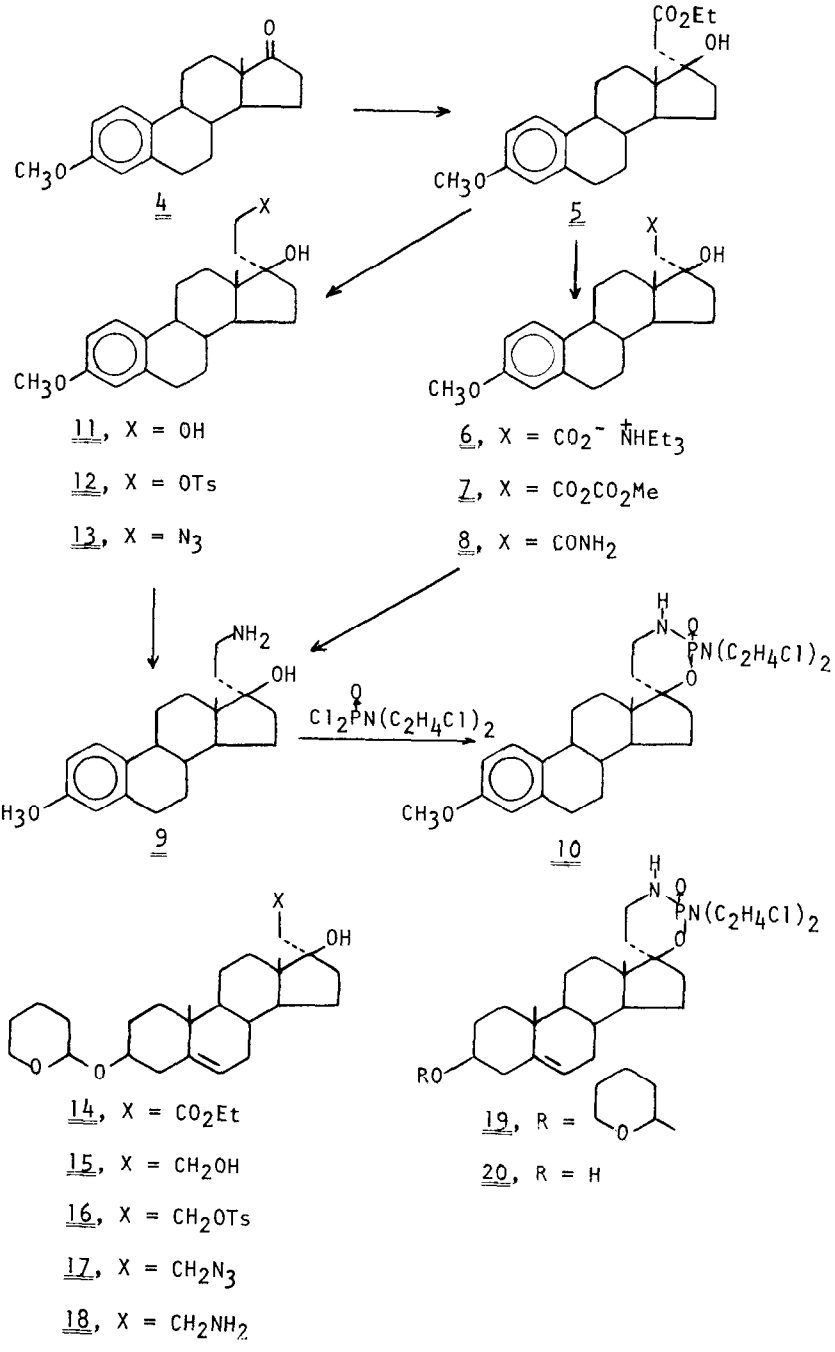
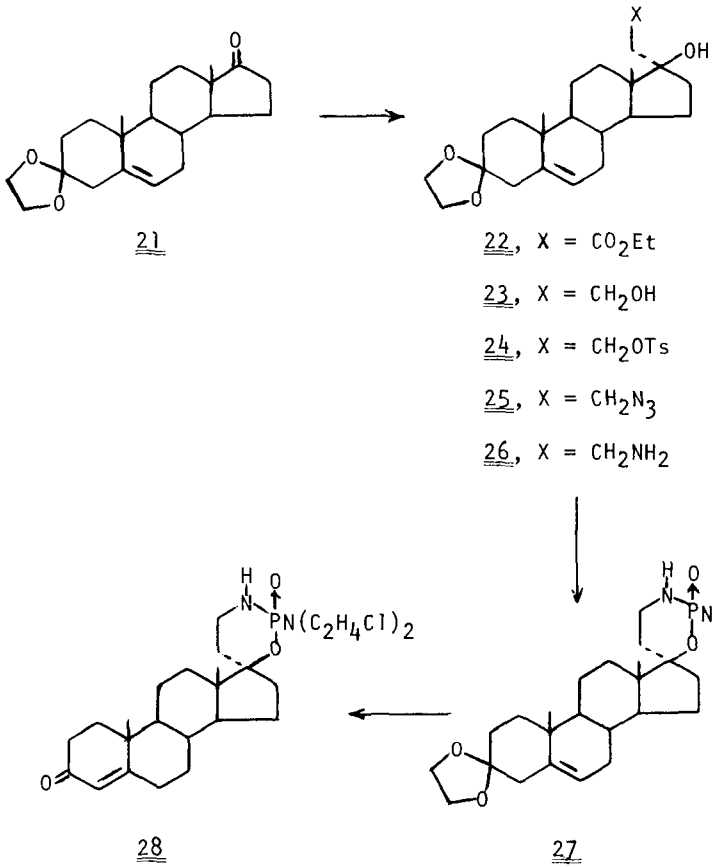

O
CH3O
4
CO2Et
OH
5
X
OH
11, X = OH
12, X = OTs
13, X = N3
6, X = CO2⁻ ⁺NHEt3
7, X = CO2CO2Me
8, X = CONH2
NH2
Cl2P(O)N(C2H4Cl)2
9
10
H
N
P(O)N(C2H4Cl)2
14, X = CO2Et
15, X = CH2OH
16, X = CH2OTs
17, X = CH2N3
18, X = CH2NH2
RO
19, R =
20, R = H

Table 1. The Synthesis of Steroidal Cyclophosphamides

Compd.	m.p. (°C)	Yield (%)	IR (cm^{-1})	Elemental Analyses C	H	X
				(found)		
14	113-114[a]	59	3470 (OH), 1700 (C=O), 1020	73.35 (73.32)	9.29 (9.80)	Calcd. Fnd.
15	202-205[b]	80	3300-3250 (OH), 1027	74.59 (74.50)	10.11 (10.10)	
16	138-139[b]	65	1592, 1170 (SO_3)	69.19 (69.28)	8.45 (8.56)	5.59 (S) (5.59)
17	152-154[a]	91	3350 (OH), 2112 (N_3)	70.39 (70.08)	9.32 (9.30)	9.47 (N) (9.44)
18	204-206[a]	75	3300-3100 (OH and NH), 715	74.77 (74.14)	10.38 (10.32)	3.25 (N) (3.22)
19	182-187[b]	27	3325-3200 (NH), 1200-1180 (PO), 1030-1020, 980-968, 712	59.69 (59.68)	8.18 (8.33)	4.64 (N) (4.47) 5.13 (P) (4.99) 11.74 (Cl) (11.63)
20	228-230[b]	77	3300-3200 (OH and NH), 1216 (PO), 978, 962	57.80 (57.58)	7.96 (7.87)	5.39 (N) (5.53) 5.96 (P) (5.93) 13.65 (Cl) (13.47)
22	135-136[a]	41	3500 (OH), 1706 (C=O), 1180, 1110, 1034, 1015	71.74 (71.39)	9.15 (9.40)	
23	225-228[a]	77	3280 (OH), 1108, 1032	73.37 (73.66)	9.64 (9.94)	
25	154-155[a]	60	3535 (OH), 2100 (N_3), 1100	68.79 (68.63)	8.79 (8.65)	10.46 (N) (10.39)
26	217-219[a,c]	74	3325 (OH), 3230 (NH), 1114	73.55 (73.42)	9.93 (9.62)	3.72 (N) (3.41)
27	192-193[b,d]	40	3200 (NH), 1192 (PO), 1100, 975	57.75 (57.40)	7.72 (7.90)	4.99 (N) (4.91) 5.51 (P) (5.62) 12.62 (Cl) (12.90)
28	172-176[d,c]	75	3152 (NH), 1675 (C=O), 1620 (C=C) 1216 (PO), 1130, 1086, 985, 975, 956	58.02 (59.04)	7.59 (7.73)	5.41 (N) (5.51) 5.99 (P) (6.28) 13.70 (Cl) (14.06)

a) Recrystallized from MeOH; b) from acetone; c) chromatographed on basic Al_2O_3; d) chromatographed on Florisil; e) recrystallized from EtOAc.

The Δ^4-3-keto cyclophsophamide (28) was synthesized instead by a sequence beginning with the 3-ethyleneketal (21) of 4-androsten-3,17-dione [19]. Its Reformatsky product 22 was reduced, the 21-tosylate 24 without purification was converted to the azide 25, and reduction gave the hydroxy amine 26, which cyclized to the cyclophosphamide 27. Hydrolysis of intermediate 27 in acetic acid - water - methanol at room temperature for 3 days gave the keto product 28.



21

22, X = CO_2Et

23, X = CH_2OH

24, X = CH_2OTs

25, X = CH_2N_3

26, X = CH_2NH_2

28

27

EXPERIMENTAL SECTION [17]

Ethyl 17β-hydroxy-3-methoxy-17α-pregna-1,3,5(10)-trien-21-oate (5) Estrone methyl ether (4, 6.0 g) was treated with 6.0 ml of ethyl bromoacetate, 4 g of activated Zn, one drop of methyl iodide and one crystal of I_2 in benzene at reflux to give 4.63 g of crude 5. Chromatography on Al_2O_3 gave 352 mg of starting material (eluted by benzene) and 2.03 g (25.8%) of 5 (eluted by Et_2O), m.p. 104-106° (lit. [10] m.p. 104-106°).

17β-Hydroxy-3-methoxy-17α-pregna-1,3,5(10)-trien-21-amide (8). Alkaline hydrolysis of 5 gave the corresponding acid, which was isolated as the triethylammonium salt 6. Methyl chloroformate reacted with 1.36 g of 6 to form the corresponding mixed anhydride 7. Without purification the anhydride was treated in hot THF with ammonia; filtering and evaporating gave 800 mg of 8. Recrystallization from methanol-acetone gave the analytical sample, m.p.; 198-199°; ir 3375-3325 (OH), 3150 (NH), 1672 (C=O), 1608, 720 cm^{-1}.

Anal. Calcd for $C_{21}H_{29}NO_3$: C, 73.43; H, 8.51; N, 4.07. Found: C, 73.47; H, 8.39; N, 3.94.

21-Amino-3-methoxy-17α-pregna-1,3,5(10)-trien-17β-ol (9). To a solution of 6 ml of diborane in THF under nitrogen, cooled by an ice bath, was added slowly 476 mg of 8 in 10 ml of THF. The mixture was heated at reflux 1 hour and cooled. A solution of 1.20 ml of conc. HCl made up to 2 ml was cooled and added to the cold reaction mixture. Sodium hydroxide pellets were added to precipitate the product (400 mg), m.p. 134-135°. Recrystallization from methanol raised the m.p. to 139-140°, and chromatography on basic Al_2O_3 gave a hydrate of 9 (eluted by NH_4OH-MeOH 1:4), 88%, m.p. 141-142.5°; ir 3350-3250 (OH and NH), 1035, 720 cm^{-1}.

Anal. Calcd for $C_{21}H_{31}NO_2 \cdot 1\ 1/2\ H_2O$: C, 70.75; H, 9.61: N, 3.93 Found: C, 70.85, 70.78; H, 8.97, 9.18; N, 3.60, 3.97.

The N-acetyl derivative, small plates out of MeOH-H_2O, m.p. 177-178°, ir 3350 (OH), 3295 (NH), 1675 (C=O), 1624, 1612, 1298, 1255 cm^{-1}.

Anal. Calcd for $C_{23}H_{33}NO_3$; C, 74.36; H, 8.95; N, 3.77. Found: C, 74.12; H, 9.08; N, 3.89.

17α-Pregna-1,3,5(10)-triene-3,17β,21-triol, 3-methyl ether (11). A solution of 16.789 g (45.07 mM) of 5 in 200 ml of benzene was concentrated to 180 ml for drying; it was then added dropwise to a suspension of 15 g of $LiAlH_4$ in 500 ml of THF stirred at reflux. The mixture was stirred at reflux 3 hr, cooled to room temperature, treated with aq NaOH until inorg salts clumped [18]. The organic layer was diluted with Et_2O, washed with brine, dried (Na_2SO_4) and evaporated: 14.5 g. Recrystallization from MeOH gave 11, m.p. 160-164° (84.6%), suitable for tosylation. The analytical sample crystallized from acetone-petroleum ether, m.p. 164-165°, ir 3300-3250 (OH), 1606 (arom), 1240, 1034 cm^{-1}.

Anal. Calcd for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 75.77; H, 9.10.

17α-Pregna-1,3,5(10)-triene-3,17β,21-triol,3-methyl ether, 21-tosylate (12). A solution of 13.26 g (40.12 mM) of 11 and 13.26 g of tosyl chloride in 95 ml of dry pyridine stood in the refrigerator overnight, then was poured on ice. The oil that separated was triturated in cold, dilute HCl until it solidified, then was washed thoroughly

and dried to give 17.86 g (91.8%) of crude 12, m.p. 149-153°, suitable for reaction with sodium azide. Recrystallization from acetone-petroleum ether and from benzene-petroleum ether gave the analytical sample, m.p. 155-157°, ir 3525 (wk, OH), 1602 (arom), 1172 (SO_3), 896 cm^{-1}.

Anal. Calcd for $C_{28}H_{36}O_5S$: C, 69.39; H, 7.49; S, 6.62. Found: C, 69.42; H, 7.54; S, 6.55.

21-Azido-17α-pregna-1,3,5(10)-triene-3,17β-diol, 3-methyl ether (13). A mixture of 17.863 g (36.86mM) of 12 and 8.93 g of NaN_3 in 375 ml of DMF was kept at 88° for 4 hr, cooled to room temperature, diluted with H_2O to turbidity, and refrigerated overnight. The crystalline product was filtered, washed thoroughly with H_2O and vacuum dried: 13.18 g (100%), m.p. 160-162°; ir 3550 (OH), 2070 (N_3), 1600 (arom), 1235, 1040 cm^{-1}, suitable for reduction to the amine. Recrystallization in MeOH gave the analytical sample, needles, m.p. 160-162°.

Anal. Calcd for $C_{21}H_{29}N_3O_2$: C, 70.95; H, 8.22; N, 11.82. Found: C, 71.02; H, 8.35; N, 11.80.

21-Amino-17α-pregna-1,3,5(10)-triene-3,17β-diol, 3-methyl ether (9). A mixture of 13.00 g (36.57 mM) of 13 and 6.00 g of $NaBH_4$ in 400 ml of i-PrOH was stirred and heated at reflux for 24 hr. Tlc indicated a trace of starting material remaining, so 10 ml of hydrazine hydrate (85%) and a scoop of Raney Ni were added; after an additional 5 min of reflux, MeOH was added, the mixture filtered hot, and the filtrate evaporated. The white solid residue was thoroughly washed with H_2O, filtered and vacuum dried. The crude product, 12.145 g (100%), was recrystallized in MeOH-H_2O to give 9, identical in m.p. and ir to that prepared from the amide 8.

3-Methoxy-1,3,5(10)-estratriene-17(R)-spiro-6'-2'-[bis-(2-chloroethyl)amino]-2'-oxo-1'-oxa-3'-aza-2'-phosphorane (10). A solution of 9 (1.46 g) in 40 ml of THF and 1.3 ml of triethylamine was added dropwise to 1.1 g of bis-(2-chloroethyl)phosphoramide dichloride in 40 ml of THF at room temp. with stirring. After 5 hours the mixture was filtered and the filtrate concentrated to dryness using a rotary evaporator to give an oily residue. Crystallization from ethyl acetate gave 372 mg (16.3%) of 10, m.p. 190-191°; ir 3150 (NH), 1606, 1240, 1212 (PO), 1084, 1034, 982, 902 cm^{-1}; nmr $CDCl_3$ δ 7.2, 6.74 and 6.63 (m, arom) 3H, 3.77 (s, CH_3O) 3H, 3.7-3.0 (m) 10H, 2.6 (broad s, NH) 1H, 2.9-1.2 (m) 17H, 0.94 (s, 18-CH_3) 3H.

Anal. Calcd for $C_{25}H_{37}Cl_2N_2O_3P$: C, 58.25; H, 7.24; Cl, 13.75; N, 5.43; P, 6.01. Found: C, 58.71, 57.67; H, 7.38, 7.07; Cl, 13.90; N, 5.38; P, 6.08.

For cyclophosphamide (1), nmr δ 4.6-4.0 (m, C-6 methylene) 2H, 3.8-2.9 (m, C-4 methylene and side chain methylenes) 10H, 2.37 (s, NH) 1H, 2.1-1.7 (m, C-5 methylene) 2H.

Acknowledgements. We are grateful to Mr. Dominique Breaux for expert technical assistance, to Dr. Gordon C. Wolf for helpful discussions, and to Dr. Douglas Dorman (Eli Lilly and Company) for the nmr spectra and their interpretation. This work was supported by Veterans Administration project no. 0997-05.

References

1. To whom inquiries should be addressed.
2. Camerman, N., and Camerman, A., J. Amer. Chem. Soc., 95, 5038 (1973).
3. van der Steen, J., Timmer, E. C., Westra, J. G., and Benckhuysen, C., J. Amer. Chem. Soc., 95, 7335 (1973).
4. Hohorst, H. J., Draeger, U., Voelker, G., and Brock, N., Abstr. 11th Int. Cancer Congr., Florence, Oct. 20-26, 1964, p. 729.
5. Takamizawa, A., Matsumoto, S., Iwata, T., Tochino, Y., Katagiri, K., Yamaguchi, K., and Shiratori, O., J. Med. Chem., 18, 376 (1975).
6. Jones, J. B., Adam, D. J., and Leman, J. D., J. Med. Chem., 14, 827 (1971) and references therein.
7. Masnyk, I. B., Steroids, 19, 771 (1972).
8. Ansfield, F. J., Carter, A. C., Goldenberg, I. S., and Segaloff, A., Cancer Chemother. Rep., 55, 259 (1971).
9. Everson, R. B., Hall, T. C., and Wittliff, J. L., Cancer Chemother. Rep., 57, 353 (1973).
10. M. Mousseron-Canet and Y. Beziat, Bull. Soc. Chim. France, 581 (1965).
11. Brown, H. C., and Heim, P., J. Amer. Chem Soc., 86, 3566 (1964).
12. Romania Ministry of Petroleum and Chemical Industry, Brit. Patent 1,030,836 (1966); Chem. Abstr., 65, 5366h (1966).
13. Asta-Werke Akt-Ges. Chemische Farbrik, Brit. Patent 812,651 (1959); Chem. Abstr., 54, 5472t (1960). Arnold, H., and Baurseaux, F., Angew. Chem., 70, 539 (1958).
14. Ott, A. C., Murray, M. F., and Pederson, R. L., J. Amer. Chem. Soc., 74, 1239 (1952). Bowers, A., and Becerra, R., J. Amer. Chem. Soc., 82, 4007 (1960).
15. Foster, E. L., and Blickenstaff, R. T., J. Org. Chem., 31, 1500 (1966). Smith, P. A. S., Hall, J. H., and Kau, R. O., J. Amer. Chem. Soc., 84, 685 (1962).
16. Lewbart, M. L., J. Org. Chem., 37, 1233 (1972).
17. Melting points were taken with a Thomas-Hoover capillary melting point apparatus and are corrected. Infrared spectra were obtained on mineral oil mulls with a perkin-Elmer 247 Spectrophotometer. Nuclear magnetic spectra were obtained in deuterochloroform on a Varian HA-100 Spectrophotometer. Elemental analyses were performed by Galbraith Laboratories, Knoxville, Tennessee.
18. Fieser, L. F., and Fieser, M., "Reagents for Organic Synthesis," John Wiley and Sons, New York, 1967, p. 584.
19. Dauben, H. J., Jr., Loken, B., and Ringold, H. J., J. Am. Chem. Soc., 76, 1359 (1954).