On the Formation of Homo-azasteroidal Esters of *N,N*-Bis(2-chloroethyl)aminobenzoic Acid Isomers and their Antitumor Activity

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The *N,N*-bis(2-chloroethyl)aminobenzoate isomers and the 4-methyl-3-*N,N*-bis(2-chloroethyl)aminobenzoate of 3β-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam, 3α-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam, 3β-hydroxy-13α-amino-13,17-seco-5-androsten-17-oic-13,17-lactam and 17β-hydroxy-3-aza-A-homo-4α-androsten-4-one, have been prepared and their biological activity evaluated against P388 leukemia *in vivo* and Ehrlich Ascites tumor (EAT), P388 and L1210 leukemias and Baby Hamster cells (BHK) *in vitro*. The esters in which the alkylating congener is linked to the lactam alcohol in the axial position are inactive *in vivo* in P388 leukemia, while compounds **1**, **4**, **6**, **13**, **14** and the alkylating congeners **17**, **18** and **20** are active. The effect of the homo-azasteroidal of *N,N*-bis(2-chloroethyl)aminobenzoic acid isomers and of 4-methyl-3-*N,N*-bis(2-chloroethyl)aminobenzoic acid on the incorporation of the radioactive precursor into the DNA of L1210, P388 leukemias, Ehrlich ascites tumor and, baby Hamster kidney cells was investigated. Higher inhibitory effects on the incorporation of the radioactive precursor was obtained with the *ortho* derivatives, yielding >70% inhibition of thymidine incorporation in all tumor lines tested.

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The concept of designing and developing hybrid anticancer compounds, agents which combine in one molecule two such as steroidal lactam esters of carboxylic derivatives of *N,N*-bis(2-chloroethyl)aniline, arose from the following reasons.

The steroidal lactam molecule characterized by the -NHCO group, may be structurally specific and therefore more prolonged as a result of the multiple interactions of such a group with similar groups that exist in proteins and nucleic acids.

The reduction of the amide group to the amine (-NHCO- → -$NHCH_2$-) diminishes the activity in Ehrlich Ascites Tumor (EAT), Lewis Lung Carcinoma (LLC) and P388, L1210 leukemias [1]. The amide group of a lactam molecule may be essential for activity. The lactam is probably transformed by a metabolic process to the active species which could attack the cancer cells.

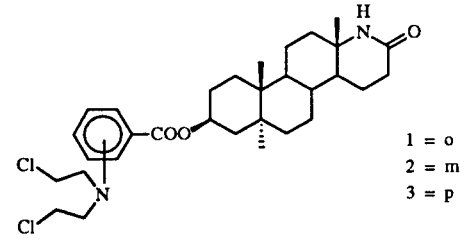
Most steroidal alkylating agents have proved inactive in L1210 leukemia [2], but most homo-azasteroidal esters gave satisfactory results in early and advanced P388, L1210 leukemias [3-5] and solid tumors [6-8], with substitution to date in either the D-ring or the A-ring by an easily cleaved ester bond.

The study of the electronic effect in compounds containing aromatic ring is especially recommended since the inductive effect is readily transmitted through such conjugated systems.

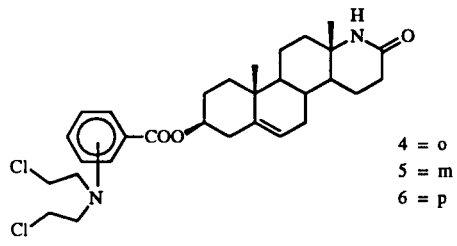
The homo-azasteroidal esters of benzoic acid mustard isomers have been selected for evaluation of their antitumor activity because of the presence of a conjugation system suitable for the study of steric and electronic effect on antitumor activity.

It is expected that the *ortho*-substituted nitrogen mustard as compared to the *meta*- and *para*-isomers will be more active, due to steric inhibition of resonance because twisting the nitrogen out of the ring plane prevents π-orbital overlap.

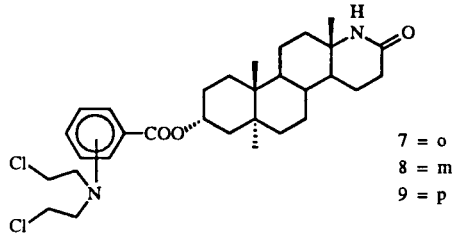
The biological basis of the benzoic acid mustard isomers [9] and the 4-methyl derivatives [10] used were the steroidal lactams, namely, 3β-hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam [11], the isomeric 3α-hydroxy-13a-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam, the 3β-hydroxy-13α-amino-13,17-seco-5-androsten-17-oic-13,17-lactam [12] and 17β-hydroxy-3-aza-A-homo-4α-androsten-4-one [13].



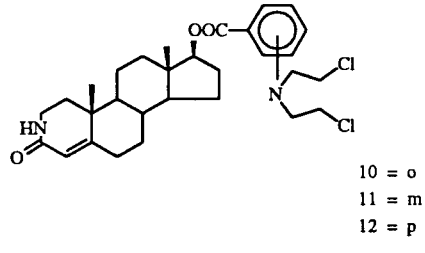
Figures 1-3. 3—Hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam (*o-*, *m-*, *p-*)-*N,N*-(2-chloroethyl)aminobenzoate.



Figures 4-6. 3-—Hydroxy-13α-amino-13,17-seco-5-androsten-17-oic-13,17-lactam (*o*-, *m*-, *p*-)-*N,N*-bis(2-chloroethyl)aminobenzoate.



Figures 7-9. 3α-Hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam (*o*-, *m*-, *p*-)-*N,N*-bis(2-chloroethyl)aminobenzoate.



Figures 10-12. 17β-Hydroxy-3-aza-A-homo-4α-androsten-4-one (*o*-, *m*-, *p*-)-*N,N*-bis(2-chloroethyl)aminobenzoate.

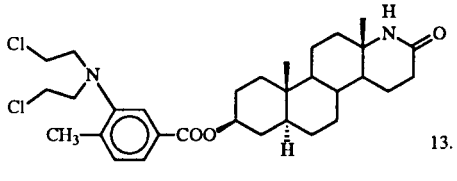


Figure 13. 3β-Hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam 4-methyl-3-*N,N*-bis(2-chloroethyl)aminobenzoate.

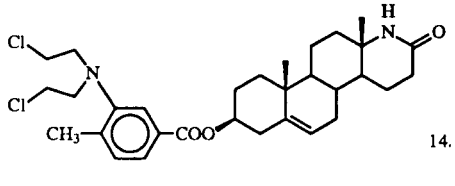


Figure 14. 3β-Hydroxy-13α-amino-13,17-seco-5-androsten-17-oic-13,17-lactam 4-methyl-3-*N,N*-bis(2-chloroethyl)aminobenzoate.

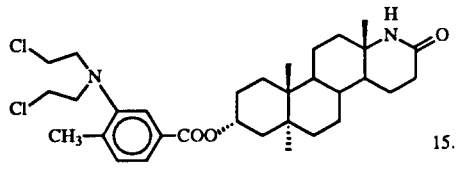


Figure 15. 3α-Hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam 4-methyl-3-*N,N*-bis(2-chloroethyl)aminobenzoate.

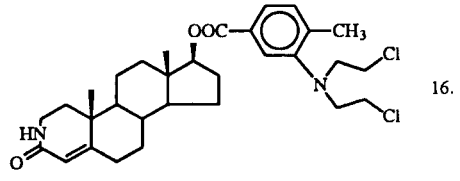


Figure 16. 17-Hydroxy-3-aza-A-homo-4α-androsten-4-one 4-methyl-*N,N*-bis(2-chloroethyl)aminobenzoate.

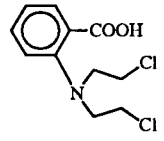


Figure 17. *o*-*N,N*-Bis(2-chloroethyl)aminobenzoic acid (*o*-ABA).

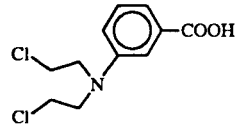


Figure 18. *m*-*N,N*-Bis(2-chloroethyl)aminobenzoic acid (*m*-ABA).

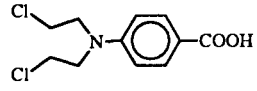


Figure 19. *p*-*N,N*-Bis(2-chloroethyl)aminobenzoic acid (*p*-ABA).

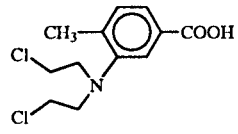


Figure 20. 4-Methyl-3-*N,N*-bis(2-chloroethyl)aminobenzoic acid (MABA).

Chemistry.

General Considerations for the Synthesis of Homo-azasteroidal Esters of *N,N*-Bis(2-chloroethyl)aminobenzoic

Acid Isomers (*o*-, *m*-, *p*- ABA) and 4-methyl-3-*N,N*-bis(2-chloroethyl)aminobenzoic Acid (4-MABA).

The alcoholysis of an acid chloride is a general method which has been widely used for the esterification of homo-azasteroidal alcohols with various carboxylic derivatives of *N,N*-bis(2-chloroethyl)aniline.

In order to synthesize the esters of various homo-azasteroidal alcohols with 4-methyl-3-*N,N*-bis(2-chloroethyl)aminobenzoic acid and *N,N*-bis(2-chloroethyl)-aminobenzoic acid isomers, we first applied the acid chloride method.

Due to the low solubility of the above acids in benzene, the preparation method of the acid chlorides by treatment of the corresponding acids with thionyl chloride at room temperature could not be utilized. Therefore, we applied the method described by Niculescu *et al* [9] which was used for the preparation of 3-*N,N*-bis(2-chloroethyl)-aminobenzoyl chloride. The carboxylic acids were refluxed with excess thionyl chloride in benzene. 3-*N,N*-Bis(2-chloroethyl)aminobenzoyl chloride and 4-methyl-3-*N,N*-bis(2-chloroethyl)aminobenzoyl chloride were prepared. In contrast the 2- and 4-benzoic acid isomers were not converted into the corresponding chlorides. An alternative method for the preparation of the latter compounds using cyanuric chloride as the chlorinating agent, failed also to give these acid chlorides.

Attempts to synthesize the homo-azasteroidal esters of 3-*N,N*-bis(2-chloroethyl)aminobenzoic acid (*m*-ABA) and 4-methyl-3-*N,N*-bis(2-chloroethyl)aminobenzoic acid (4-MABA) by the reaction of the corresponding acid chlorides with homo-azasteroidal alcohols in dry benzene led, in all cases, in two main derivatives: a. Amide derivatives, acylated on the nitrogen atom of the A- or D-lactam moiety, having the 17- or the 3-hydroxyl group free; b. derivatives acylated both on the hydroxyl group and on the nitrogen atom of the lactam moiety.

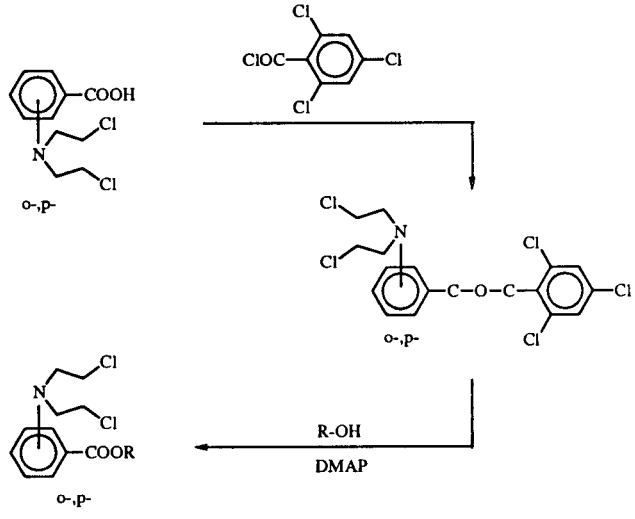
The desired ester derivatives were isolated in very low yields.

Due to the problems described above, we experimented with an alternative way to synthesize the ester derivatives. The reaction of the symmetrical anhydrides of the above acids, (which were prepared by the reaction of the acid with its corresponding acid chloride in the presence of pyridine), with homo-azasteroidal alcohols alone, or in the presence of 4-dimethylaminopyridine, gave the desired esters. The presence of 4-dimethylaminopyridine raised the yield about 10% and reduced the reaction time to one half.

As we have mentioned above, it was not possible to prepare the 2- and 4-*N,N*-bis(2-chloroethyl)aminobenzoyl chlorides by the thionyl or cyanuric chloride methods in sufficient amounts for further reaction. Therefore the preparation of the symmetrical anhydrides of these acids as described for the 3-*N,N*-bis(2-chloroethyl)amino- and 4-methyl-*N,N*-bis(2-chloroethyl)amino- derivatives was not possible and consequently, the symmetrical anhydride method for the synthesis of the esters of these acids could not be applied.

Having however the experience that the symmetrical acid anhydride method worked well for the synthesis of the homo-azasteroidal esters of 3-*N,N*-bis(2-chloroethyl)-amino- and 4-methyl-3-*N,N*-bis(2-chloroethyl)amino-benzoic acids, we considered using mixed anhydrides to esterify the 2- and 4-*N,N*-bis(2-chloroethyl)aminobenzoic acids (Scheme 1). The mixed anhydrides of the above acids were prepared using 2,4,6-trichlorobenzoyl chloride as the condensing agent and triethylamine as an auxiliary base. As a component of the mixed anhydrides, 2,4,6-trichlorobenzoyl chloride proved to be a good example, because the combination of the steric hindrance and electron density concerning its carbonyl group, as shown from esters, favours nucleophilic attack on the carbonyl group of the 2- and 4-benzoic acid mustard isomers. The alcoholysis step was carried out in benzene and in the presence of 4-dimethylaminopyridine. The esters were isolated in good yields, whereas the corresponding 2,4,6-trichlorobenzoic acid esters, were isolated as by-products (3-6%).

Scheme 1. General Approach for the Synthesis of Homo-azasteroidal Esters



R = Modified Steroidal Skeleton

Biological Data.

In vivo Experiments.

Materials and Methods.

Drugs.

Stock solutions of all drugs used in this study were

made immediately before use. They were suspended in corn oil in the desired concentration, following dissolution in 10% of DMSO, and a volume of 0.6 ml/mouse was injected.

Mice.

BALB/C, DBA/2 and DBF_1 mice of both sexes were used for toxicity studies and antitumor testing. Mice, obtained from the experimental section of Theagenion Cancer Hospital, were under conditions of constant temperature and humidity, in sterile cages, with water and food.

Toxicity.

The acute toxicity of the compounds was determined following a single i.p. injection into BALB/C mice in groups, of 10 mice/dose at three different dosages. The mice were observed for 30 days and the Lethal 1 Dose (LD) 50 and LD10 doses were evaluated graphically.

Lymphocytic leukemia P388 was obtained from Professor K. Harrap, Section of Drug Development of the Institute of Cancer Research, U.K., maintained in ascitic form in DBA/2 mice, by injection of 10^6 cells 0.1 ml at 7-day intervals into the peritoneal cavity.

Life Extension Assay.

The evaluation of the antitumor activity of all compounds in P388 leukemia was performed as follows:

Mice were implanted with 10^6 P388 leukemic cells (i.p.) under 0.1 ml per mouse. One day later the animals were divided into groups. One group of 8 animals was used as a control (treated with corn oil only), whereas the other groups were treated i.p., 0.1 ml per mouse, with the drugs intermittently on days 1, 5 and 9 [14]. The efficacy of the given drugs was determined by T/C % (mean survival time of treated mice/mean survival time of control mice x 100).

Cell Cultures.

The L1210 and P388 leukemic cells were obtained from Professor K. Harrap, Section of Drug Development, Institute of Cancer Research, U.K., and were grown in RPMI-1640.

The Ehrlich Ascites tumor cells (EAT) and Baby Hamster Kidney cells (BHK) were obtained from the Research Department of Theagenion Cancer Institute and were grown in medium 199 and MEM, respectively. All the media were supplemented with 10% calf serum, 100 units/ml penicillin, 100 µg/ml streptomycin and 42 m*M* HEPES, in an atmosphere of 5% carbon dioxide and air at 37°.

Treatment with the Drugs.

The experiments were carried out with 10^6 cells/ml at 37°. The compounds were dissolved in DMSO and added to the culture medium containing the tumor cells. The final concentration of DMSO, no more than 0.5%, had no cytotoxic effect in our testing system. The incubation time was 30 minutes at concentration of 25 µg/ml.

Measurement of [Methyl-^{3}H]thymidine Incorporation Values.

DNA synthesis was determined after 30 minutes incubation of 10^6 cells and 1 µCi of the radioactive precursor ^{3}H-Thymidine in 1 ml final volume of the media. At the end of the labelling period cells were washed twice in Phosphate-Buffered Saline (PBS) and resuspended into 50 µl of the same solution. The cell suspension was placed on Whatman filters No. 41 and the wet filters were soaked in 5% trichloroacetic acid cold (TCA) for 10 minutes. The filters were further washed twice in 5% trichloroacetic acid (TCA), twice in 96% alcohol, once in ether:ethanol 1:1, and once in ether. After drying, the filters were placed in scintillation liquid and the radioactivity was determined using a Packard Scintillation Counter. The samples of each experiment was triplicate and the inhibitory effect of the compounds was represented by the % inhibition of the incorporation of [methyl-^{3}Thymidine into DNA of treated cells versus untreated.

Table I

Toxicity of the Homo-azasteroidal Esters of *o*-, *m*-, *p*-*N*,*N*-Bis(2-chloroethyl)aminobenzoic Acid, 4-Methyl-3-*N*,*N*-bis(2-chloroethyl)aminobenzoic Acid and Their Alkylating Congeners

Compound Number	LD_{50} [a] mg/kg	LD_{10} mg/kg
1	600	450
2	350	200
3	280	80
4	300	170
5	200	100
6	320	100
7	280	170
8	550	200
9	300	180
10	300	150
11	300	125
12	500	280
13	280	140
14	570	250
15	600	300
16	200	90
17, *o*-ABA	25	10
18, *m*-ABA	58	40
19, *p*-ABA	80	45
20, 4-MABA	18	10

[a] LD_{50} values were estimated graphically, where the percentage of deaths due to the toxicity of each dose is shown in the ordinate, while the administered doses are indicated on the abscissae on semi-logarithmic paper.

Results.

The toxicity of homo-azasteroidal esters and the mustards of benzoic acid isomers are reported in Table I.

The alkylating congeners *o*-, *m*-, *p*-*N,N*-bis(2-chloroethyl)aminobenzoic acids (*o*-, *m*-, *p*-ABA) (**17**, **18**, **19**) and the 4-methyl-3-*N,N*-bis(2-chloroethyl)aminobenzoic acid (4-MABA) (**20**) are more toxic than the corresponding esters.

The esters and the alkylating congeners, have been tested against P388 leukemia *in vivo*.

The esters in which the alkylating congeners is linked to the lactam alcohol in the axial position, **7**, **8**, **9**, **15** are inactive in the above experimental leukemia system. Similar results have been obtained with homo-azasteroidal esters of cinnamic acid isomers [15]. Probably, this is due to the rate of hydrolysis. The results of activity are reported in Table II.

Table II

In vivo Antitumor Activity of theHomo-azasteroidal Ester of *N,N*-Bis(2-chloroethyl)aminobenzoic Acid Isomers, 4-Methyl-3-*N,N*-bis(2-chloroethyl)aminobenzoic Acid and their Alkylating Congeners in P388 Lymphocytic Leukemia

Compound Number	Dosage [1] mg/kg/day	MST [2] Non-treated animals days	MST Treated nnimals days	T/C [3] %
1	225	10.6	15.5	146
2	100	13.3	14.7	110
3	40	11.0	12.0	109
4	85	10.1	15.0	148
5	40	10.1	11.0	109
6	50	6.0	8.8	146
7	85	10.1	11.0	109
8	200	10.6	11.75	110
9	75	9.8	11.2	114
10	62.5	10.1	11.1	109
11	75	9.0	10.5	117
12	100	11.0	13.0	118
13	70	13.3	30.1	226
14	125	9.8	19.0	193
15	50	9.0	10.5	117
16	45	9.0	10.6	118
17, *o*-ABA	7.5	10.1	18.6	184
18, *m*-ABA	20	6.0	12.3	205
19, *p*-ABA	25	13.3	13.3	100
20, 4-MABA	5.0	13.3	19.1	144

The most active compounds were **1**, **4**, **6**, **13** and **14**. Out of the congeners, *o*-, *m*-*N,N*-bis(2-chloroethyl)aminobenzoic acid and 4-methyl-3-*N,N*-bis(2-chloroethyl)-aminobenzoic acid showed the best activity of *m*-ABA with T/C 205% at a dose of 20 mg/kg. *In vivo* incorporation studies demonstrated higher inhibitory effect on the incorporation of the radioactive precursor for compounds **1**, **4**, **7**, **8**, **13** and **16**. The *in vitro* results are reported in Table III.

Table III

Inhibition of DNA Synthesis of the Homo-azasteroidal Esters of *N,N*-Bis(2-chloroethyl)aminobenzoic Acid Isomers, 4-Methyl-3-*N*,-bis(2-chloroethyl)aminobenzoic Acid and their Alkylating Congeners on EAT, P388, L1210 and BHK Culture Lines

Compound Number	% Inhibition of [^{3}H-methyl]thymidine incorporation [a] EAT	P388	L1210	BHK
1	80	86	86	76
2	52	49	55	40
3	87	73	88	69
4	78	85	85	74
5	49	55	51	31
6	78	68	76	54
7	87	84	88	76
8	84	84	87	76
9	67	69	63	40
10	77	62	73	62
11	68	54	70	69
12	70	66	78	60
13	80	79	92	71
14	79	54	77	71
15	74	74	83	70
16	74	85	87	74
17, *o*-ABA50	55	49	42	
18, *m*-ABA	77	73	81	20
19, *p*-ABA33	27	28	15	
20, 4-MABA	51	48	39	30

[a] Each % of inhihition is the mean value of three determinations.

Discussion.

The present results show that the esters of 4-methyl-3-*N,N*-bis(2-chloroethyl)aminobenzoic acid of D-steroidal lactams (Figures 13, 14) are active in P388 leukemia. The *ortho* alkylating congener produces esters of D-lactam-alcohols with satisfactory activity in the above leukemia (Figures 1, 4) as well.

Our previous studies with homo-azasteroidal esters of *p*-*N,N*-bis(2-chloroethylaminophenylacetic acid [3], *p*-*N,N*-bis(2-chloroethyl)aminophenylbutyric acid [4] and *p*-*N,N*-bis(2-chloroethyl)aminophenoxyacetic acid [5] showed good activity in L1210 and P388 leukemias, even in advanced L1210 leukemia [5]. Probably, the direct conjugation system diminishes the activity, due to the rate of hydrolysis.

Table IV

Physical Properties of Homo-azasteroidal Esters of *N,N*-Bis(2-chloroethyl)aminobenzoic Acid Isomers and of 4-Methyl-3-*N,N*-bis(2-chloroethyl)aminobenzoic Acid

Compound No.	Formula	Yield%	Mp C°	Recrystalization Solvent	Carbon Calcd.	Carbon Found	Hydrogen Calcd.	Hydrogen Found	Nitrogen Calcd.	Nitrogen Found	$[\alpha]_D$ ($CHCl_3$, 18°C)
1	$C_{30}H_{42}N_2O_3Cl_2$	70	151-153	$CH_3COOC_2H_5$	65.57	65.80	7.65	7.69	5.10	4.88	+6α [a]
2	$C_{30}H_{42}N_2O_3Cl_2$	80	223-225	$CH_3COOC_2H_5$	65.57	65.80	7.65	7.36	5.10	5.02	+10α [a]
3	$C_{30}H_{42}N_2O_3Cl_2$	80	225-227	$CH_3COOC_2H_5$	65.57	65.41	7.65	7.80	5.10	5.31	+22α [a]
4	$C_{30}H_{40}N_2O_3Cl_2$	68	173-174	$CH_3COOC_2H_5$	65.81	66.01	7.31	7.20	5.12	4.99	-30 [a]
5	$C_{30}H_{40}N_2O_3Cl_2$	75	263-265	$CH_3COOC_2H_5$	65.81	65.80	7.31	7.36	5.12	5.02	-30α [a]
6	$C_{30}H_{40}N_2O_3Cl_2$	78	148-150	$CH_3COOC_2H_5$	65.81	65.68	7.31	7.56	5.12	5.14	+2α [a]
7	$C_{30}H_{42}N_2O_3Cl_2$	60	142-145	$CH_3COOC_2H_5$/ *n*-hexan	65.57	65.69	7.65	7.71	5.10	5.31	+5α [a]
8	$C_{30}H_{42}N_2O_3Cl_2$	72	137-140	$CH_3COO_2H_5$	65.57	65.71	7.65	7.68	5.10	5.24	+6α [a]
9	$C_{30}H_{42}N_2O_3Cl_5$	56	148-150	$CH_3COOC_2H_5$/ *n*-hexan	65.5	65.83	7.65	7.77	5.10	5.09	+3α [a]
10	$C_{30}H_{40}H_2O_3Cl_2$	60	133-135	$CH_3COOC_2H_5$/ *n*-hexan	65.81	65.71	7.48	7.48	5.12	5.08	+42α [a]
11	$C_{30}H_{40}N_2O_3Cl_2$	75	233-236	$CH_3COOC_2H_5$	65.81	66.01	7.31	7.21	5.12	5.28	+68α [a]
12	$C_{30}H_{40}N_2O_3Cl_2$	63	200-203	$CH_3COOC_2H_5$	65.81	65.71	7.31	7.33	5.12	5.01	+117β [b]
13	$C_{31}H_{44}N_2O_3Cl_2$	72	220-223	$CH_3COOC_2H_5$	66.07	65.90	7.82	7.84	4.97	4.88	+10α [a]
14	$C_{31}H_{42}N_2O_3Cl_2$	68	215-217	$CH_3COOC_2H_5$	66.31	66.40	7.49	7.50	4.99	4.89	-20α [a]
15	$C_{31}H_{44}N_2O_3Cl_2$	63	223-226	$CH_3COOC_2H_5$	66.07	66.00	7.82	7.99	4.97	4.69	-5β [b]
16	$C_{31}H_{42}N_2O_3Cl_2$	41	243-244	$CH_3COOC_2H_5$/ $CHCl_3$	66-31	66-11	7.49	7-38	4.99	4-80	+74α [a]

[a] C (concentration) = 1 g/100 ml. [b] C = 0.046 g/100 ml .

Table VI

Nuclear Magnetic Resonance Spectral Data of the Homo-azasteroidal Esters of 4-Methyl-3-*N,N*-bis(2-chloroethyl)aminobenzoic Acid and *N,N*-Bis(2-chloroethyl)aminobenzoic Acid Isomers

Compound Number	δ (ppm) Ar-H	NH	H-6	H-4	H-3	H-17	$N\text{-}CH_2CH_2Cl$	$ArCH_3$	$19\text{-}CH_3$	$18\text{-}CH_3$
1	7.88-7.07	6.42	-	-	4.93	-	3.52	-	1.17	0.96
2	7.43-6.85	6.27	-	-	4.92	-	3.72	-	1.15	0.86
3	7.52-6.65	6.47	-	-	4.89	-	3.73	-	1.15	0.85
4	7.65-7.08	6.26	5.45	-	4.83	-	3.53	-	1.18	1.04
5	7.44-6.86	6.73	5.43	-	4.84	-	3.70	-	1.18	1.06
6	7.93, 6 67	6.38	5.42	-	4.80	-	3.73	-	1.18	1.05
7	7.97-7.13	6.66	-	-	5.27	-	3.56	-	1.17	0.82
8	7.65-6.87	6.10	-	-	5.29	-	3.72	-	1.16	0.83
9	7.96, 6 70	6.37	-	-	5.24	-	3.73	-	1.16	0.82
10	7.66-7.05	6.62	-	5.75	-	4.82	3.52	-	1.16	0.90
11	7.44-6.86	6.62	-	5.74	-	4.81	3.71	-	1.16	0.94
12	7.93, 6.65	6.23	-	5.75	-	4.80	3.73	-	1.15	0.85
13	7.80-7.25	6.99	-	-	4.93	-	3.47	2.39	1.15	0.87
14	7.81-7.26	6.01	5.43	-	4.82	-	3.46	2.39	1.19	1.07
15	7.87-7.27	5.89	-	-	5.27	-	3.47	2.39	1.16	0.83
16	7.83-7.26	6.54	-	5.75	-	4.82	3.45	2.39	1.17	0.95

Table V

Infrared Spectral Data of the Homo-azasteroidal Esters of 4-Methyl-3-*N,N*-bis(2-chloroethyl)aminobenzoic Acid and *N,N*-bis(2-chloroethyl)aminobenzoic Acid Isomers

Compound Number	-NH-	-CO- stretching	-NHCO-	aromatic abs.
		cm^{-1}		cm^{-1}
1	3160, 3060	1715	1680	760, 725
2	3175, 3040	1715	1675	750
3	3180, 3050	1700	1660	770, 730
4	3160, 3060	1715	1680	760, 735
5	3175, 3060	1710	1660	750
6	3200, 3130	1690	1648	765, 730
7	3200, 3060	1710	1675	760,720
8	3210, 3080	1720	1660	750
9	3200, 3060	1700	1660	770, 730
10	3180, 3050	1720	1665	760, 730
11	3170	1715	1660	750
12	3180	1700	1660	765,730
13	3190, 3060	1715	1660	760, 735
14	3180, 3050	1710	1655	760, 730
15	3170, 3060	1710	1660	760, 730
16	3130, 3090	1710	1660	760, 730

EXPERIMENTAL

Melting points were obtained on a Fisher-Johns apparatus and are uncorrected. The ir spectra were obtained on a Perkin-Elmer 298 spectrophotometer with polystyrene as the reference peak. The nmr spectra were obtained on a Bruker WC250 spectrometer (250 Hz) in deuteriochloroform with tetramethylsilane as an internal standard.

3α-Hydroxy-13α-amino-13,17-seco-5α-androstan-17-oic-13,17-lactam was prepared by the procedure described by Regan and Hayes [11] to produce lactam in 65% yield mp >300° methanol.

Anal. Calcd. for $C_{19}H_{31}NO_2$: C, 74.75; H, 10.16; N, 4.59. Found: C, 73.98; H, 10.20; N, 4.80.

General Procedure for the Synthesis of Homo-azasteroidal Esters with Symmetrical Carboxylic Aniline Mustard Anhydrides. (The esters **2, 5, 8, 11, 13-16** were prepared by this method).

Method A.

A solution of 3-*N,N*-bis(2-chloroethyl)aminobenzoic acid (5.7 mmoles) in dry benzene (30 ml) was treated with freshly distilled thionyl chloride (28.5 mmoles) and refluxed for one hour. The excess thionyl chloride was destroyed by the addition of a few drops of pure formic acid to the solution, and the solvent was removed under reduced pressure. The remaining acid chloride was dissolved in dry benzene (30 ml), and 5.7 mmoles of acid and dry pyridine (11.4 mmoles) were added. The mixture was refluxed for 2 hours. Pyridine hydrochloride was removed by filtration, and the excess pyridine and solvent was removed under reduced pressure. The remaining symmetrical anhydride was dissolved in dry benzene (150 ml) and homo-azasteroidal alcohol (5.7 mmoles) and 4-dimethylaminopyridine (5.7 mmoles) were added. The reaction mixture was refluxed for 48 hours. The solvent was evaporated, and the residue was dissolved in chloroform, washed with 3% aqueous hydrochloride acid, water, aqueous sodium becarbonate and water, dried over sodium sulfate and filtered. The solvent was evaporated under reduced pressure and the residue was chromatographed on silica gel. Elution with chloroform gave the desired ester. The compounds prepared are reported in Table IV.

The structures of the esters were identified by means of ir and nmr spectra (Tables V and VI).

Method B.

General Procedure for the Synthesis of Homo-azasteroidal Esters with Mixed Carboxylic Aniline Mustard Anhydrides. (The esters **1, 3, 4, 6, 7, 9, 10, 12** were prepared by this method).

A solution of 4-*N,N*-bis(2-chloroethyl)aminobenzoic acid (5.7 mmoles) was added to 30 ml of dry benzene. The mixture was treated with 2,4,6-trichlorobenzoyl chloride (5.7 mmoles) and triethylamine (5.7 mmoles), and refluxed for 2 hours. Triethylamine hydrochloride was removed by filtration and the solvent was removed under reduced pressure. The residue was then dissolved in toluene and the solvent was removed under reduced pressure. The remaining mixed carboxylic anhydride was dissolved in 150 ml of dry benzene, and homo-azasteroidal alcohol (5.7 mmoles) and 4-dimethylaminopyridine (5.7 mmoles) were added. The reaction mixture was refluxed for 48 hours. The solvent was evaporated, the residue was dissolved in chloroform, washed successively with 3% aqueous hydrochloric acid, water, aqueous sodium bicarbonate and water, and dried over sodium sulfate. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel. Elution with chloroform gave the desired compounds. The prepared compounds are given in Table IV.

The structures of the esters were identified by means of ir and nmr spectra (Table V and VI).

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