Selective Delivery of Cytotoxic Compounds to Cells by the LDL Pathway[1,2]

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Cancer cells need cholesterol to make new membrane. They get it either by de novo synthesis or from low-density lipoprotein (LDL), or both. Some types of cancer have very high LDL requirements. LDL particles, which circulate in the blood, contain a cholesteryl ester core surrounded by a phospholipid coat containing apoproteins that are recognized by LDL receptors on cell surfaces. After attachment to cells, LDL is endocytosed into lysosomes, where the core is exposed and hydrolyzed. A technique is known whereby LDL can be isolated, its core removed and replaced by a compatible lipophilic substance, and then reconstituted into intact LDL particles that are recognized and internalized by cells in the normal manner. A series of cytotoxic compounds has been synthesized, designed to be compatible with reconstituted LDL, and directed against cancers that copiously internalize LDL. They were evaluated by measuring the toxicity of reconstituted LDL toward test cells bearing LDL receptors. Selectivity was determined by comparison, either with mutant cells with few LDL receptors or with reconstituted methylated LDL (which is not recognized by LDL receptors) on normal cells. Two compounds, **19** and **25**, were found that reconstitute well, kill or arrest the test cells at reasonably low concentrations, and are completely selective, suggesting that they are delivered to cells exclusively via the LDL pathway.

Background. There is a constant search for differences between malignant and normal cells that can be exploited in chemotherapy. One such difference that has not yet been utilized is in the rate of metabolism of low-density lipoprotein (LDL).[3] Body cells obtain cholesterol either by de novo synthesis or from LDL, or both. Cancer cells, like all dividing ones, need large amounts of cholesterol because they are making new membrane. Thus it is logical to expect that at least some types of cancer cells will have higher LDL requirements than any normal cell type, and to date the following types are reported to do so: acute myeloid leukemia,[4] cervical epidermoid carcinoma EC-50,[5] and several other gynecological malignancies.[6] Most types of cancer have yet to be surveyed in this regard.

LDL, the principal cholesterol transport lipoprotein in human plasma, consists of spherical particles 220 Å in diameter. Each LDL particle contains a nonpolar core composed of approximately 1500 cholesterol molecules esterified to long-chain fatty acids. The core is surrounded by a polar coat of phospholipid, unesterified cholesterol, and apoprotein that is recognized by cell surface receptors. After binding to receptors, which are grouped together in coated pits on the surfaces of cells, LDL particles are endocytosed and taken into lysosomes, where the cholesteryl esters are hydrolyzed and the cholesterol made available to the rest of the cell. The mechanism of the LDL system was elucidated by Brown, Goldstein, and co-workers.[7-9]

It is possible to extract the cholesterol esters out of the core of LDL and reconstitute the core with other hydrophobic compounds.[10] In many cases, such reconstituted LDL (r-LDL) is essentially identical with native LDL in its ability to bind to LDL receptors, be internalized by cells, and be hydrolyzed in lysosomes. Thus LDL can serve to deliver a broad range of compounds into mammalian cells. For example, when LDL is reconstituted with the toxic compound 25-hydroxycholesterol oleate, cells that express the LDL receptor pathway are killed when they are incubated in the presence of this reconstituted LDL. It may therefore be possible to prepare reconstituted LDLs that can efficiently kill cancer cells that express high levels of LDL receptors.[11-13]

The successful reconstitution of LDL is facilitated when the hydrophobic compound to be reconstituted contains certain groups that render it compatible with LDL's phospholipid coat and lipophilic core.[14] Among the groups that act in this way are oleyl, retinyl, and cholesteryl. We refer to them as LDL anchors.

Since LDL is taken first to lysosomes after entering cells, attachment of an LDL anchor seemed a worthwhile application of lysosomotropic detergents. These are long-chain alkylamines of intermediate basicity such that they are substantially protonated only within the acidic milieu of lysosomes, where they are changed into powerful detergents the rupture the lysosomal membrane. The ensuing release of lysosomal enzymes into the cytoplasm kills the cell.[2,15,16] Lysosomotropic detergents are thus toxic

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(1) On the part of the Merck group this paper is regarded as no. 6 in the series Lysosomotropic Agents.
(2) Paper 5: Miller, D. K.; Griffiths, E.; Lenard, J.; Firestone, R. A. *J. Cell. Biol.* **1983**, *97*, 1841.
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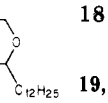
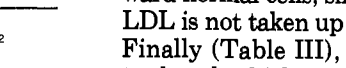
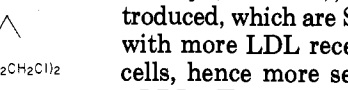
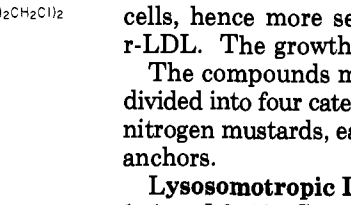
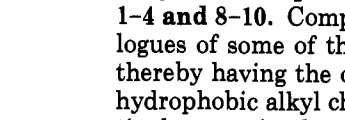
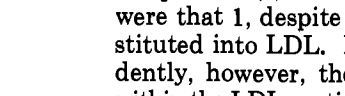
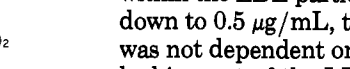
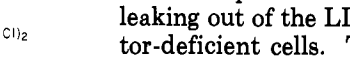
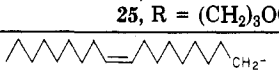
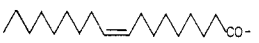
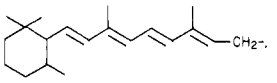
Table I. Compounds[a]

1, *N*-oleylimidazole
2, *N*-oleylmorpholine
3,
4, *N*-retinylmorpholine[21]
5, $C_{12}H_{25}NHCH_2CF_3$[15]
6, $C_{12}H_{25}NHCH_2CHF_2$
7,
8, oleyl-OCON-$(C_{12}H_{25})CH_2CF_3$
9, oleyl-OCON-$(C_{12}H_{25})_2$-CHF_2
10, 
11, $HN(CH_2CH_2Cl)_2$
12, oleyl-OCON-$(CH_2CH_2Cl)_2$
17, $C_8F_{17}CH_2CH_2$-NH_2[24]
22, 
24, 

13-16, 18-21, 23, 25
13, 
14, R = CONH-oleyl
15 R = $CH_2OCONHC_{12}H_{25}$
16, R = $CH_2OCONHCH_2CH_2C_8F_{17}$
18, 
19, R = $CH_2OCON(CH_2CH_2Cl)_2$
20, 
21, 
23, R = $CONMeOCON(CH_2CH_2Cl)_2$
25, R = $(CH_2)_3OCON(CH_2CH_2Cl)_2$

[a] Oleyl ≡ 
oleoyl ≡ 
retinyl ≡ 

to all lysosome-bearing cells but do not normally distinguish between normal and malignant cells, because they enter cells readily by diffusion through the plasma membrane.[2] By restricting the entry of detergent amines to the LDL pathway we hoped to achieve selectivity.

There are four criteria that must be met for the successful use of the LDL system to deliver anticancer compounds: (1) the compounds must be capable of reconstitution with LDL, (2) the reconstituted LDL (r-LDL) must be toxic to cells, (3) it must also be selective, i.e., enter cells only via the LDL pathway, (4) the quantity of cytotoxic compound that can be delivered in LDL must be sufficient to kill all, or almost all, of the target cells.

Results and Discussion

During the course of the study, the test system was modified twice as improvements were introduced. Initially (Table II), the toxicity of r-LDL was measured by its ability to inhibit the growth of normal human fibroblasts or Chinese hamster ovary (CHO) cells,[17] which have normal LDL receptor activity. Growth was measured on a scale of 0–6, with 6 representing normal growth as exhibited by cells that received no r-LDL. Selectivity was measured by comparing the effects on normal cell growth with those on the growth of mutant human fibroblasts (receptor-negative form of homozygous familial hypercholesterolemia) or V-79 CHO cells,[10,18] strains of cells that express few LDL receptors (2–20% of normal). Subsequently (Table II), an improved index of selectivity was substituted, in which methylated r-LDL was compared with normal r-LDL toward normal cells, since it had been found that methylated LDL is not taken up at all by functional LDL receptors.[8,19] Finally (Table III), the new test cells SV-589[20] were introduced, which are SV-40 transformed human fibroblasts with more LDL receptors than the previously used test cells, hence more sensitive to a given concentration of r-LDL. The growth scale here was 0–4.

The compounds made and tested in this study can be divided into four categories: lysosomotropic detergents and nitrogen mustards, each with first one, and then two LDL anchors.

Lysosomotropic Detergents with One LDL Anchor, 1–4 and 8–10. Compounds 1–3 are simply the oleyl analogues of some of the more powerful detergent amines, thereby having the oleyl group serve in the dual role of hydrophobic alkyl chain and LDL anchor. Since the retinyl group is also compatible with LDL,[14] *N*-retinylmorpholine (4)[21] was also tested. The results (Table II) were that 1, despite its oleyl group, could not be reconstituted into LDL. Fortunately, the others could. Evidently, however, they were not bound tightly enough within the LDL particles, because although they were toxic down to 0.5 μg/mL, they were not selective; that is, killing was not dependent on uptake via LDL receptors. Only by leaking out of the LDL could they have killed the receptor-deficient cells. The extra methyl groups in 3 were designed to improve containment within the core of LDL but did not work. *N*-Retinylmorpholine (4) was indeed acting as a lysosomotropic detergent because retinol was nontoxic at 10 μg/mL.

Detergent amines such as 1–4 enter cells freely by permeation[2] and need no activation other than protonation to exert their effect. Therefore a stable, deactivating, polar link that impeded entry by diffusion but could be hydrolyzed intracellularly was indicated. Since carbamates have been previously used in antineoplastics,[22] oleyl alcohol was linked to the detergent amines 5–7 as carbamates 8–10. These substances reconstituted well but were not toxic. The free amines 5 and 6 (without LDL) were toxic at 10 and 1 μg/mL respectively, and 7 is known to be even more potent (unpublished data), so it seemed likely that either the carbamates were not efficiently hydrolyzed by the cells or else LDL cannot carry enough drug into the cells to kill them.

Nitrogen Mustard with One LDL Anchor, 11. The dose–response of lysosomotropic detergents is not smooth but sigmoidal; i.e., below a threshold concentration their activity is low, and above it, high.[15,16] Thus carbamates 8–10 might have given rise to the free amines 5–7 but not enough for killing. Nitrogen mustard 11 is a cytotoxic compound that is not a detergent and ought not to exhibit a sigmoidal dose–response. Therefore its oleyl carbamate 12 was prepared. It reconstituted well, and at 10 μg/mL it was highly toxic to both normal and mutant cells, showing that it too leaks out of LDL. However, at 2 μg/mL there remained considerable toxicity and for the first time significant, though not complete, selectivity. This showed, at least for nitrogen mustard, that the LDL pathway has sufficient capacity to kill cells.

Lysosomotropic Detergents with Two LDL Anchors, 13–16, 18. If a single LDL anchor was insufficient

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Table II. Test Results with Compounds 1–24

compd	type[a]	reconst	concn,[b] μg/mL	growth of cells[c]: normal + r-LDL	mutant + r-LDL	normal + Me-r-LDL	toxic	specific
1	det	no						
2	det	yes	0.5	0	1		yes	no
3, 4	det	yes	0.5	0	0		yes	no
8–10	det	yes	10	6	6		no	
12	NM	yes	10	0	0			
			2	2	5		yes	slightly
13, 15	det	yes	10	6	6		no	
14	det	yes	20	6	6		no	
22	NM	yes	20	6	6		no	
16	det	no						
18	det	yes	10	6	6		no	
19	NM	yes	10	0[d]	6		yes	yes
20, 21	NM	yes	80	6		6	no	
23	NM	yes	40	5		6	slightly	yes
24	NM	yes	40	6		6	no	

[a] NM = nitrogen mustard, det = detergent. [b] Measured as concentration of LDL protein, approximately equals concentration of compound. [c] Control cells (no r-LDL) always had growth = 6. [d] Growth 98% arrested, about half the cells dead.

Table III. Test Results Using SV-589 Cells and 20, 21, and 25

compd	type[a]	reconst	concn,[b] μg/mL	growth of cells[c]: + r-LDL	+ Me-r-LDL	toxic	specific
20, 21	NM	yes	80	4	4	no	
25	NM	yes	1	4	4		
			3	2	4		
			10, 20, 40	0	4	yes	yes

[a,b] Same as above. [c] Only normal cells used. Growth of control cells = 4.

to prevent leakage, then two anchors might do it. We chose the cholesterol nucleus, esterified at the 3β-OH with oleic or ricinoleic acid and with the cytotoxic moiety attached either on the D-ring side chain or on the ricinoleic acid's alcohol group.

The amides **13** and **14** were easy to prepare and reconstitute but inactive, presumably owing to the resistance of simple amides to hydrolysis, so that once again we turned to carbamates. Compound **15**, designed to release the highly toxic dodecylamine within the cells, also reconstituted well but was inactive. In addition to the previous explanations (inefficient hydrolysis, insufficient delivery), a third possibility was that the free amine, a strong base, upon liberation within lysosomes raised the lysosomal pH,[2,23] stopping further hydrolysis. Compound **16**, which carries the less basic but very toxic amine **17**,[24] failed to reconstitute, apparently because the long perfluorinated chain is incompatible with LDL despite its great hydrophobicity. Compound **18**, bearing the weakly basic but also highly toxic **7**, reconstituted but was inactive.

Nitrogen Mustards with Two LDL Anchors, 19–25. The steroid mustard carbamate **19** was our first good compound. It reconstituted well, had fairly good toxicity, and was completely selective, showing that the double anchor worked well. However, although 10 μg/mL of **19** arrested growth of 98% of the normal cells, only about half of these were actually killed, and the toxic effects were not increased at higher doses, reflecting the saturability of the LDL pathway. If intracellular hydrolysis of the carbamate in **19** was complete, then the maximum amount of nitrogen mustard **11** that could be delivered in LDL was almost, but not quite, enough. However, if hydrolysis was incomplete, improvements were still possible.

With the chlorambucil derivatives **20** and **21** and phenesterine **22**, no hydrolysis is required since the link to the steroid does not deactivate the mustard. Unfortunately, although they reconstituted well, they were inactive.

Better hydrolysis might be attainable with a more labile link. An obvious choice was a hydroxamic acid ester,[25] expected to hydrolyze much faster than a carbamate. Indeed, **23** was so unstable that it was difficult to handle. Nevertheless, although it reconstituted well and was selective, it was much less potent than **19**. Evidently this link is resistant to lysosomal hydrolysis despite its lability under nonenzymatic conditions.

Steric effects were next considered. In the cholesteryl ricinoleate derivative **24**, the mustard carbamate has been moved from a rather congested site on the D ring down onto the oleyl chain. However, this compound, while it reconstituted well, was inactive.

Another way to alter the steric environment of the mustard carbamate was to move it into a less crowded locale by extending the C-17 side chain, as in **25**. This compound met our expectations (Table III). It reconstituted well, was completely selective, and killed 100% of the test cells at about 5 μg/mL and above.

Conclusion. We have succeeded in developing a cytotoxic compound, **25**, capable of reconstitution with LDL and thus being delivered to cells exclusively via the LDL pathway in amounts capable of killing 100% of cells bearing a normal number of LDL receptors. If this level of toxicity to the target cells holds in vivo, then efficacious dose levels might be attainable, even when the medicated LDL is diluted by native LDL already present in the patient's blood. Furthermore, this dilution can be reduced by prior removal of native LDL,[26] which would also give

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the benefit of opposing the down regulation of LDL receptors on the cells that is produced by high levels of LDL in the plasma.[27] LDL-binding activity can also be increased by suppressing cholesterol biosynthesis with inhibitors.[28] Thus, by a combination of these methods it may be possible to obtain good anticancer activity at reasonably small dose levels.

Synthesis

The compounds in Table I were synthesized by the routes shown in Scheme I. During the preparation of **7**, the dodecyl chain had such a great deactivating effect on all reactions that forcing conditions were almost always required. The nitrogen was protected by the relatively hard-to-remove tosyl because BOC protection failed, owing to involvement of its carbonyl during attempted cyclization. The lengthy sequence to **7** was needed because the standard cyclization conditions (H_2SO_4, 170 °C), which give 2-methylmorpholine from $HOCH_2CH_2NHCH_2CHMeOH$[29] (41% in our hands), failed completely with the dodecyl analogue.

Reaction of 3β-hydroxy-22,23-bisnorcholenic acid with oleoyl chloride produced the 3-oleate cleanly and quantitatively without protection of the carboxylic acid group. We believe this occurred via the mixed anhydride which transferred only the oleoyl group to the 3-OH because the bisnorcholenic acid's carbonyl is so hindered. In contrast, direct oleoylation of 3β-hydroxycholenic acid gave a messy polymeric product mixture, which we attribute to unselective acylation of the 3-OH by both acyl moieties of the mixed anhydride, since the cholenic acid's carbonyl is relatively unhindered. That is why 3β-cholenic acid was esterified before oleoylation. The LiI demethylation was somewhat capricious, giving variable ratios of free acid to unreacted methyl ester. The latter is easily removed after the next step and recycled. An alternative protecting group is the *p*-bromophenacyl ester, prepared from 3β-hydroxycholenic acid and α,*p*-dibromoacetophenone (Me_2SO–THF, Et_3N, room temperature, 18 h), and removed with Zn (AcOH–THF, room temperature, 18 h) from the 3-oleate.

All other reactions were uneventful.

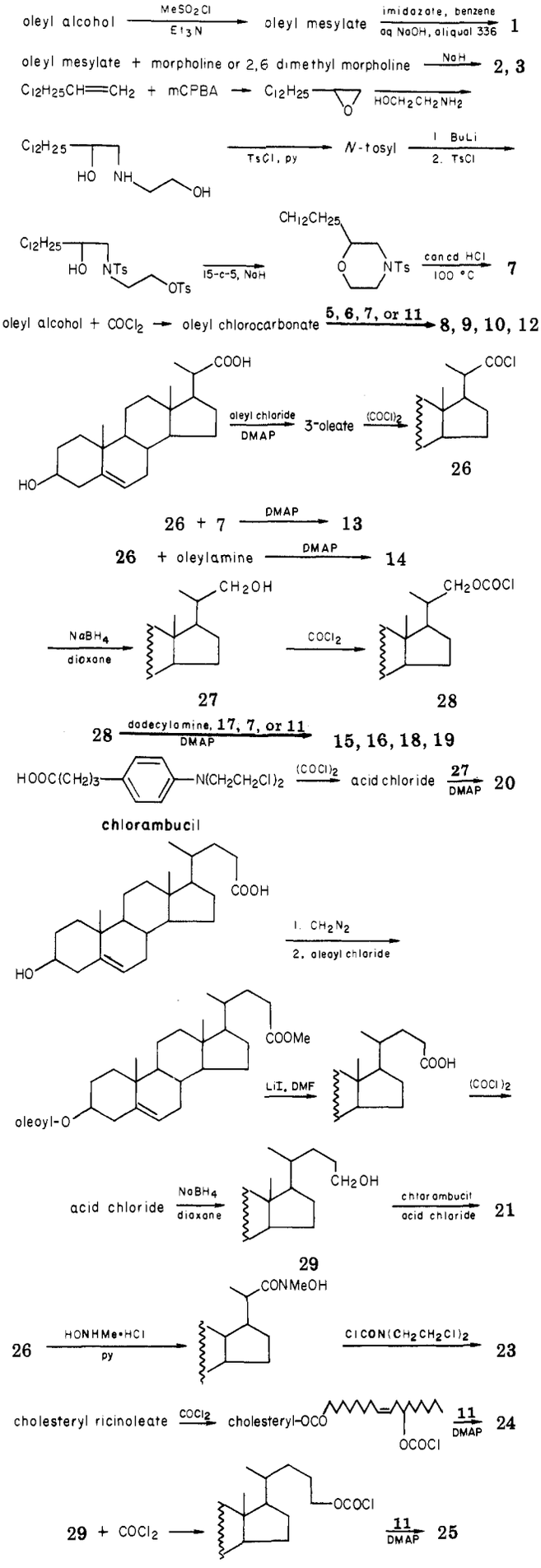
Experimental Section

All reactions done under nitrogen employed a Firestone Valve (Ace Glass Co., catalog no. 8766-12). NMR spectra were run on Varian T-60 and SC-300 instruments and are reported in ppm from Me_4Si in $CDCl_3$. Phenesterine was from Upjohn. TLC and PLC used Analtech silica gel plates. Column chromatograms used E. Merck silica gel 60 (40–63 μm, 400–230 mesh; E. Merck No. 9385).

The procedure for reconstitution of LDL (or methyl-LDL) has been published.[10] Cells were grown as described,[11] plated 20 000/well in 24-well Linbro plates, and incubated 4 h. The reconstituted LDL was then added and incubation continued. On day 3, the medium was removed and the dish was washed with phosphate-buffered saline (PBS). This removed dead cells. The live cells that adhered to the dish were fixed with 15% formalin for 15 min, then stained with crystal violet. Excess stain was washed off with water, and the dish with stained and fixed cells was dried and evaluated visually.

Reconstituted LDL typically contains approximately equal weights of core and LDL–protein.[10] In the present study, this ratio was measured for r-(**22**)LDL and r-(**24**)LDL. To an aliquot

Scheme I. Synthesis of Compounds 1–25



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(29) Berlin, A. Y.; Synchera, J. P. *Zh. Obshch. Khim.* **1950**, *20*, 640.

of r-LDL solution containing 10 μg of r-LDL protein was added 10 μg of stigmasterol in ethanol, 0.200 mL of PBS (pH 7.4), and 4 mL of 2:1 $CHCl_3$–MeOH. The mixture was vortexed, aged 30 min, treated with 0.8 mL of water, vortexed, centrifuged 10 min at 2000 rpm, and separated. The organic phase was evaporated, treated with 0.100 mL of heptane and 0.4 mL of 0.625 N ethanolic KOH, heated 45 min at 80 °C, diluted with 1 mL water, and extracted with 2:1 $CHCl_3$–MeOH. The organic extract was washed with water, dried, and analyzed by GLC for cholesterol (4 ft 3% OV-17, 250 °C, flow 20 mL/min, flame ionization), using stigmasterol as internal standard. The content of **22** and **24** in r-LDL was found to be 1.01 and 1.14 mg, respectively, per milligram of LDL protein. Values for methyl r-LDL were found to be comparable. Samples of r-LDL are stored at 4 °C for up to 1 week. During this period no change in properties has ever been seen.

***N*-Oleylimidazole (1).** Oleyl alcohol was converted to its mesylate by overnight stirring at 0 °C under N_2 of 7.48 g (28 mmol) of oleyl alcohol, 3.4 mL of redistilled methanesulfonyl chloride, and 3.7 mL of triethylamine in 100 mL of CH_2Cl_2. Dilution with hexane, filtration, and evaporation of solvents afforded 6.83 g (71%) of oleyl mesylate: TLC R_f 0.8, 10:1 $CHCl_3$–EtOAc; NMR δ 3.00 (s, CH_3S), 4.21 (t, J = 7 Hz, (CH_2OMs)), 3.6 (trace of CH_2OH). This compound (6.83 g, 19.8 mmol), 2.67 g (39 mmol) of imidazole, 50 mL of 1 N aqueous NaOH, 50 mL of benzene, and 1.96 mg of aliquat 336 (0.0049 mmol) (methyltrioctylammonium chloride; General Mills Chemicals Inc.) were refluxed with stirring overnight. The benzene layer was washed with brine, dried with K_2CO_3, filtered, and evaporated, leaving 3.39 g of crude 1. Of this, 242 mg was chromatographed on a 20 × 20 cm plate, 2 mm, 1:1 $CHCl_3$–EtOAc, R_f 0.4, providing 160 mg of pure 1 (25%): NMR δ 7.50 (br s), 7.10 (br s), and 6.94 (br s) (each 1 H, imidazole CH's), 3.96 (t, J = 7 Hz, CH_2N; mass spectrum, m/e 318.

***N*-Oleylmorpholine (2).** Oleyl mesylate (2.5 g, 6.7 mmol) was refluxed overnight in 50 mL of acetonitrile with 161 mg of oil-free NaH (6.7 mmol) and 1.4 mL (16.2 mmol) of morpholine. The reaction mixture was diluted with hexane, washed with water, aqueous K_2HPO_4, and brine, dried over K_2CO_3, filtered, and stripped, leaving 1.62 g of crude **2**. Chromatography with 1:1 petroleum ether:ether afforded 1.5 g (65%) of pure **2**: R_f 0.3 on TLC; NMR δ 3.75–3.95 (m, 4 H, CH_2O), 2.4–2.6 (m, 4 H, ring CH_2N), 2.1 (br m, 6 H, chain CH_2N and CH_2CH=); mass spectrum, m/e 337.

***N*-Oleyl-2,6-dimethylmorpholine (3).** Oleyl mesylate (1.40 g, 4.04 mmol) was refluxed 40 h in 40 mL of acetonitrile with 92 mg of oil-free NaH (3.8 mmol) and 4.6 g (40 mmol) of 2,6-dimethylmorpholine. After evaporation in vacuo of the solvent, the residue was taken up in hexane, washed twice with water, dried over K_2CO_3, filtered, evaporated, and chromatographed, 1:1 $CHCl_3$–EtOAc, TLC R_f 0.5, affording 476 mg (32%) of pure **3**: NMR δ 3.5–4.0 (m, 2 H, CH_3CHO), 1.8–2.9 (m, 10 H, CH_2N and CH_2CH=); mass spectrum, m/e 365.

1-Tetradecene Oxide. 1-Tetradecene (78.4 g, 0.40 mol), *m*-chloroperbenzoic acid (85%, 83.1 g, 0.41 mol), and 400 mL of CH_2Cl_2 were mixed at 0 °C and stirred at 25 °C 48 h. The solution was filtered, washed with saturated aqueous $NaHCO_3$, water, and brine, dried over $MgSO_4$, filtered, stripped to give 87.9 g of tetradecene oxide: NMR δ 2.5–3.1 (m, 3 H, CH_2CHO), no vinyl or allylic H.

1-Dodecyl-2-[(2-hydroxyethyl)amino]ethanol. Tetradecene oxide (13 g, 6.1 mmol) and 44 g (722 mmol) of ethanolamine in 200 ml of ethanol were stirred at 25 °C overnight. Volatiles were pumped off at 0.1 torr, and the semisolid residue was washed with water and dried, affording 10.2 g (61%) of product: NMR δ 3.75 (m, 3 H, CHO, CH_2O), 2.8 (m, 7 H O*H*, CH_2NH, which falls to 3 H with D_2O added to the $CDCl_3$); mass spectrum (silylated), m/e 418.

***N*-Tosyl-1-dodecyl-2-[(2-hydroxyethyl)amino]ethanol.** The previous product (273 mg, 1 mmol) and 191 mg (1 mmol) of toluenesulfonyl chloride in 1 mL of pyridine were stirred overnight under N_2 at 25 °C. Volatiles were stripped at 0.1 torr and the residue treated a few minutes with a few drops water. Ethyl acetate was added, and the solution was washed twice with dilute aqueous HCl (no emulsions), water, aqueous K_2HPO_4, and brine, dried with $MgSO_4$, filtered, and evaporated, leaving 141 mg of product (33%): single spot on TLC (4:1 $CHCl_3$–EtOAc, R_f 0.25); NMR δ 7.7 (d), 7.4 (d) (4 H, J = 9 Hz, C_6H_4), 4.5 (br s, 2 H, OH), 3.8 (br m, 3 H, CHO, CH_2O), 3.25 (br m, 4 H, CH_2N), 2.45 (s, 3 H, Ar CH_3).

***N*-Tosyl-2-dodecylmorpholine.** To 141 mg (0.33 mmol) of the previous product in 2 mL of THF under N_2 at −78 °C was added 0.194 mL of 1.7 M *n*-BuLi (0.33 mmol). After 5 min 63 mg (0.33 mmol) of tosyl chloride in 1 mL of THF was added. The mixture was stirred 30 min at −78 °C and allowed to warm to 25 °C. NaH (12 mg, 0.5 mmol), oil-free, and 4 μL of 15-crown-5 in 1 mL of THF were added. The mixture was stirred overnight under N_2 at 25 °C. The mixture was stripped, taken into CH_2Cl_2, washed with water, aqueous K_2HPO_4, and brine, dried with K_2CO_3, filtered, evaporated, and chromatographed on a 20 × 20 cm plate (2 mm) with 50:1 $CHCl_3$–EtOAc, giving 52 mg of product, R_f 0.6 (two spots on TLC), and 21 mg (15%) of recovered starting material. The product was rechromatographed on a 1-mm plate with 100:1 $CHCl_3$–EtOAc, providing 34 mg (25%) of pure compound: NMR δ 3.2–4.0 (br m, CH_2N, CHO, CH_2O, 5 H), 1.8–2.4 (m, 2 H, CH_2N); mass spectrum, m/e 409. An alternative to repeated chromatography of crude samples, which contained *O*-tosylates by NMR, was overnight stirring with 50% aqueous NaOH.

2-Dodecylmorpholine (7). The cyclization of the previous example was performed on 5.0 g (11.7 mmol) of starting material, producing 5.93 g of crude cyclized *N*-tosyl compound, which was cleaned up by stirring overnight at 25 °C with 150 mL of 50% aqueous NaOH. The mixture was diluted with water and extracted with EtOAc, which was dried over K_2CO_3, filtered, and stripped, giving 4.0 g of pure cyclic *N*-tosyl compound. This was stirred vigorously 16.5 h at 100 °C with concentrated HCl in a sealed tube, poured into 150 mL of 10% NaOH, extracted with EtOAc, dried with K_2CO_3, filtered, and stripped, leaving 1.6 g of 7 (54%): NMR δ 3.2–4.1 (br m, 3 H, CHO and CH_2O), 2.4–3.1 (br m, 4 H, CH_2N, 2.1 (br s, 1 H, NH; mass spectrum, m/e 255; TLC, 50:1 $CHCl_3$–EtOAc, one spot, R_f 0.5. Anal. ($C_{16}H_{33}NO$) N; C: calcd, 75.29; found, 73.16. H: calcd, 12.94; found, 12.09.

Oleyl Chlorocarbonate. A solution of 3.16 g (10 mmol) of oleyl alcohol in 20 mL ether was added over 15 min at room temperature with stirring to 25 mL of 1.1 M phosgene in benzene (27.5 mmol). Evaporation of solvent left 3.79 g of product: IR 5.60 μm; NMR δ 4.31 (t, J = 6 Hz, CH_2OCOCl), 5.37 (m, CH=CH).

***N*-[(Oleyloxy)carbonyl]-*N*-(2,2,2-trifluoroethyl)dodecylamine (8).** To 134 mg (0.5 mmol) of *N*-(2,2,2-trifluoroethyl)dodecylamine (**5**)[15] and 122 mg (1 mmol) of 4-(dimethylamino)pyridine (DMAP) in 20 mL of ether was added dropwise with stirring under N_2 190 mg of oleyl chlorocarbonate in 1 mL of ether (0.5 mmol). After the mixture was stirred for 24 h, 1 mL of water was added dropwise. The mixture was washed with water, 3 M aqueous H_3PO_4, water, aqueous K_2HPO_4, and brine, dried with $MgSO_4$, filtered, and evaporated, leaving 130 mg of crude product. This was purified by PLC on two 20 × 20 cm plates, 2 mm, $CHCl_3$–EtOAc (50:1), R_f 0.7, affoding 11 mg (17%) of **8**: IR (film) 5.81 μm; NMR δ 3.34 (t, J = 6 Hz, $NCH_2C_{11}H_{23}$), 3.89 (q, J = 9 Hz, CH_2CF_3), 4.12 (t, J = 6 Hz, CH_2O); mass spectrum, m/e 561.

***N*-[(Oleyloxy)carbonyl]-*N*-(2,2-difluoroethyl)dodecylamine (9).** This was prepared in the same manner as **8** by using 129 mg (0.5 mmol) of *N*-(2,2-difluoroethyl)dodecylamine (**6**),[15] 61 mg (0.5 mmol) of DMAP, and 190 mg of oleyl chlorocarbonate, giving 235 mg (87%) of pure **7**: R_f 0.6, using $CHCl_3$ for the PLC; IR (film) 5.85 μm; NMR δ 3.35 (t, J = 7 Hz, $NCH_2C_{11}H_{23}$), 3.58 (d of t, J = 6, 14 Hz, NCH_2CHF_2), 4.16 (t, J = 6 Hz, CH_2O); mass spectrum, m/e 543.

***N*-[(Oleyloxy)carbonyl]-2-*n*-dodecylmorpholine (10).** This was prepared in the same way as **8**, using 400 mg of **7** (1.57 mmol), 211 mg of DMAP (1.73 mmol), and 648 mg of oleyl chlorocarbonate (1.71 mmol), reaction time 1 h. The crude product was chromatographed on 50 g of silica gel, eluting with $CHCl_3$–EtOAc 50:1, TLC R_f 0.6, providing 814 mg of pure **10** (78%).

***N*-[(Oleyloxy)carbonyl]-*N*,*N*-bis(2-chloroethyl)amine (12).** To 142 mg (1 mmol) of *N*,*N*-bis(2-chloroethyl)amine free base (prepared from the hydrochloride as in ref 24) and 122 mg (1 mmol) of DMAP in 100 mL of ether under N_2 with stirring was added over 2 h 379 mg (1 mmol) of oleyl chlorocarbonate in 5 mL of ether. The workup and purification were the same as for **8**, providing 375 mg (86%) of pure **12**: R_f 0.5, 50:1 $CHCl_3$–EtOAc; IR (film) 5.86 μm; NMR δ 3.69 (s, 8 H, O=$CNCH_2CH_2Cl$);

mass spectrum, m/e 435. Anal. ($C_{23}H_{43}NO_2Cl_2$) C, H, N.

3β-(Oleoyloxy)-22,23-bisnorcholenic Acid. To 173 mg (0.5 mmol) of 3β-hydroxy-22,23-bisnorcholenic acid (Steraloids) and 122 mg (1 mmol) of DMAP in 2 mL of pyridine was added under N_2 dropwise 150 mg (0.5 mmol) of oleoyl chloride (Nu-Chek), rinsing in with a little pyridine. The reaction was stirred 2 h at room temperature and then 15 min on the steam bath, cooled, and stripped at 0.1 torr. Water was added dropwise over 5 min and then excess 1 M aqueous H_3PO_4 and $CHCl_3$ were added and the mixture was stirred. The $CHCl_3$ layer was separated, washed with brine, dried with $MgSO_4$, filtered, and evaporated at 0.1 torr, leaving 333 mg of product (ca. 100%): IR (film) 5.75, 5.85 μm, with the latter disappearing in morpholine, showing that it is a free COOH and the other an ester; NMR δ 4.6 (m, 1 H, 3α-H; comes at 3.58 with unesterified 3β-ol); mass spectrum (FD) of methyl ester (with CH_2N_2), m/e 635, 342; no 280 (methyl oleate), no 688 (steroid–COO–steroid–COOMe), trace 360 (steroid methyl ester).

3β-(Oleoyloxy)-22,23-bisnorcholenic Acid Chloride (26). The previous product (243 mg, 0.398 mmol), in 1.5 mL of CH_2Cl_2 was treated with 0.040 mL of degassed oxalyl chloride[30] (0.469 mmol) (no reaction) and then 0.8 μL of dimethyl formamide (DMF). Effervescence started immediately, continuing for 1 h. After a total of 1.8 h under N_2, the solvent was pumped off, 5 mL of benzene was added, and it was pumped off at 0.1 torr, leaving 224 mg of **26**: IR (film) 5.56, 5.75 μm.

3β-(Oleoyloxy)-22,23-bisnorcholenic 2-*n*-Dodecylmorpholide (13). Compound **26** (224 mg, 0.356 mmol) in 1 mL of benzene, was added dropwise over 3 min to a mixture of 91 mg of **7**, 45.5 mg of DMAP (0.356 mmol), and 1 mL of pyridine with stirring. The mixture was stirred an additional 15 min and kept overnight in the refrigerator. Solvents were stripped at 0.1 torr. Ether and water were added, and then (after 1 min) dilute aqueous H_3PO_4 was added. The ether layer was separated, washed with more aqueous H_3PO_4, water, aqueous K_2HPO_4, and brine, dried with $MgSO_4$, filtered, and evaporated, leaving 293 mg of crude **13**. This was chromatographed on two 20 × 20 cm plates, 2 mm silica gel, using $CHCl_3$–EtOAc (25:1), affording 150 mg (50%) of pure **13**: R_f 0.4; IR (film) 5.75, 6.1 μm; NMR δ 5.4 (m, 3 H, vinyl H's), 4.6 (m, 1 H, 3α-H), 3.1–4.5 (m, 7 H, morpholine ring H's; CH_2N's shifted from 2.8 m); mass spectrum, m/e 848.

***N*-Oleyl-3β-(oleoyloxy)-22,23-bisnorcholenamide (14).** To **26**, made as described above from 100 mg (0.15 mmol) of acid, was added 4 mL of THF, and at 0 °C, 0.040 mL of pyridine and 52 mg of oleylamine (85% pure, 0.165 mmol) were added. After overnight stirring at 25 °C, the product was diluted with water, extracted with EtOAc, and chromatographed, eluting with 2:1 ether–hexane, R_f 0.45, affording 88 mg of pure **14**: IR (film) 5.75, 6.08 μm; NMR δ 3.04–3.33 (m, 2 H, $NHCH_2$), 4.38–4.72 (m, 1 H, 3α-H), 5.18–5.46 (m, 5 H, olefins).

2-[3β-(Oleoyloxy)androst-5-en-17β-yl]propan-1-ol (27). The (oleoyloxy)bisnorcholenic acid (2.00 g, 3.29 mmol) was converted to **26** as described above. This was taken up in 5 mL of dioxane and added dropwise under N_2 over 7 min at room temperature with stirring to 152 mg of $NaBH_4$ (4 mmol) in 5 mL of dioxane. The $NaBH_4$ gradually dissolved. After another 5 min the mixture was heated for 10 min on the steam bath, cooled to 0 °C, and treated with water dropwise (ca. 4 mL) until effervescence ceased, ca. 10 min. Volatiles were pumped off at 0.1 torr. The residue was taken up in CH_2Cl_2 and water, treated with aqueous HCl (no reaction), and neutralized with $NaHCO_3$ and then aqueous K_2HPO_4. The layers were separated, and the aqueous portion was extracted twice more with CH_2Cl_2. The combined organic layers were washed with brine, dried with $MgSO_4$, filtered, and evaporated, leaving 1.949 g of crude product. From this, 0.436 g was chromatographed on two 20 × 20 cm plates, 2 mm, $CHCl_3$–EtOAc (50:1), R_f 0.4, providing 230 mg (53%) of pure **27**: NMR δ 5.4 (m, 3 H, vinyl H's), 4.6 (m, 1 H), 3.2–3.8 (m, 2 H, CH_2H); mass spectrum, m/e 596.

***N*-[[[2-[3β-(Oleoyloxy)androst-5-en-17β-yl]propyl]oxy]carbonyl]-*N*-dodecylamine (15).** To 176 mg (0.295 mmol) of **27**, dried by flushing twice with benzene, was added 5 mL of ether and 1.70 mL of 1.74 M $COCl_2$ in benzene (2.96 mmol; the excess is needed). After 1 h at room temperature the solvents were evaporated, and more benzene was added and pumped off at 0.1 torr: IR (film) 5.63, 5.77 μm, absorbance ratio 1.36. The crude chlorocarbonate **28** was treated with 53 mg (0.295 mmol) of *n*-dodecylamine and 36 mg (0.295 mmol) of DMAP in 10 mL of ether, stirring overnight. The reaction mixture was washed with water, aqueous H_3PO_4 twice, water, aqueous K_2HPO_4 twice, and brine, dried with $MgSO_4$, filtered, and evaporated, leaving 174 mg of crude **15**. This was chromatographed on two 20 × 20 cm plates, 1 mm, $CHCl_3$–EtOAc (50:1), R_f 0.6, providing 98 mg (41%) of pure **15**: NMR δ 3.6–4.2 (m, 2 H, $CH_2OC{=}O$), 3.1 (m, 2 H, CH_2N).

***N*-[[[2-[3β-(Oleoyloxy)androst-5-en-17β-yl]propyl]oxy]carbonyl]-*N*-[2-(*n*-perfluorooctyl)ethyl]amine (16).** An ether solution of 2-(*n*-perfluorooctyl)ethylamine (**17**)[24] was prepared from 330 mg (0.66 mmol) of its hydrochloride by stirring with 20 mL of ether, 20 mL of 50% aqueous NaOH, and 5 mL of water for 30 min, saturaing the aqueous layer with solid K_2CO_3, separating and drying the ether layer with K_2CO_3. To this solution was added **28**, made from 390 mg (0.66 mmol) of **27** and 81 mg (0.66 mmol) of DMAP. After 3 days of stirring under N_2, the reaction mixture was washed with water, aqueous H_3PO_4, water, aqueous K_2HPO_4, and brine, dried with $MgSO_4$, filtered, and chromatographed on a 20 × 20 cm plate, 1.5 mm, $CHCl_3$–EtOAc (50:1), affording 315 mg (44%) of **16**: R_f 0.6, single spot in several TLC systems; NMR δ 3.5 (br m, $C_8F_{17}CH_2CH_2$), 3.7–4.2 (m, $CH_2OC{=}O$); mass spectrum, m/e 1086, 804 (M^+ – oleoyl). Anal. ($C_{51}H_{72}O_4NF_{17}$) H, N; C: calcd, 56.40; found, 54.98. F: calcd, 29.74; found, 31.21.

4-[[[2-[3β-(Oleoyloxy)androst-5-en-17β-yl]propyl]oxy]carbonyl]-2-*n*-dodecylmorpholine (18). To **28**, made from 178 mg (0.28 mmol) of **278** was added 72 mg (0.28 mmol) of **7** and 34 mg (0.28 mmol) of DMAP in 5 mL of ether, stirring overnight under N_2. The workup was the same as for **15** and **16**. The crude product (255 mg) was chromatographed on a 20 × 20 cm plate, 2 mm, $CHCl_3$–EtOAc (50:1), R_f 0.5, yielding 149 mg (61%) of pure **18**: NMR δ 5.4 (m), 4.6 (m), 3.1–4.5 (m, morpholine ring H's), 3.7–4.2 (m); mass spectrum, m/e 878.

***N*-[[[2-[3β-(Oleoyloxy)androst-5-en-17β-yl]propyl]oxy]carbonyl]-*N*,*N*-bis(2-chloroethyl)amine (19).** To **28** in 10 mL of benzene, made from 450 mg (0.755 mmol) of **27**, were added 0.4 mL of nitrogen mustard free base and 180 mg of DMAP (1.47 mmol). The mixture was stirred 17.5 h at room temperature, stirred 5 min with a few drops of water, diluted with EtOAc, washed with aqueous H_3PO_4, water, aqueous K_2HPO_4, and brine, dried with $MgSO_4$, filtered, and stripped, giving 576 mg of crude **19**. This was chromatographed on 40 g of silica gel with $CHCl_3$, TLC R_f 0.4, giving 410 mg (71%) of pure **19**: NMR δ 5.4 (m, 3 H), 4.6 (m, 1 H), 3.8–4.2 (m, 2 H), 3.68 (s, 8 H, $O{=}CNCH_2CH_2Cl$); mass spectrum, m/e 764 (Cl_2), 481 (Cl_2, M^+ – oleoyloxy). Anal. ($C_{45}H_{75}NO_4Cl_2$) C, H, N.

2-[3β-(Oleoyloxy)androst-5-en-17β-yl]propanol Chlorambucil Ester (20). Chlorambucil (Sigma; 91 mg, 0.29 mmol) was converted into its acid chloride by treatment for 40 min with 0.25 mL of oxalyl chloride and 2 μL of DMF in 2 mL of CH_2Cl_2 and then stripping in vacuo. This was added to 0.29 mmol of **27** and 40 mg (0.33 mmol) of DMAP in 1.5 mL of benzene, stirring under N_2 20 h. The mixture was diluted with ether, stirred 1 min with a few drops of H_2O, washed with pH 5.5 aqueous phosphate (two times), aqueous K_2HPO_4, and brine, dried with $MgSO_4$, filtered, evaporated (252 mg crude), and chromatographed on two 20 × 20 cm plates (1 mm), using 10:1 hexane–EtOAc, R_f 0.5, providing 152 mg (59%) of pure **20**: NMR δ 7.03 (d), 6.64 (d, J = 9 Hz, 4 H, aromatic CH), 5.2–5.5 (m, 3 H, olefinic CH), 4.6 (m, 3α-H), 3.6–4.2 (m, 2 H, $CH_2OC{=}O$), 3.65 (s), 8 H, $O{=}CNCH_2CH_2Cl$); mass spectrum, m/e 881 (Cl_2).

Methyl 3β-Hydroxy-5-cholenate. 3β-Hydroxycholenic acid (749 mg, 2 mmol; Steraloids) was treated in 25 mL of ether and 15 mL of $CHCl_3$ for 2.5 h with diazomethane made from 0.6 g of nitrosomethylurea (>4 mmol), 1.2 mL of 40% aqueous KOH, 10 mL of ether (0 °C, 30 min, then excess K_2CO_3). The reaction mixture was filtered and stripped, leaving 602 mg (77%) of ester: NMR δ 3.60 (s, 3 H, $COOCH_3$), 3.5 (m, 1 H, 3α-H); mass spectrum, m/e 388; TLC R_f 0.35, 15:1 CH_2Cl_2–EtOAc.

Methyl 3β-(Oleoyloxy)-5-cholenate. To the previous compound (598 mg, 1.54 mmol), in 10 mL CH_2Cl_2, was added 198 mg

(30) Degassed by three freeze–pump–thaw cycles to remove HCl.

of DMAP (1.62 mmol) and 464 mg of oleoyl chloride (1.54 mmol) (NuChek) in 5 mL of CH_2Cl_2. After 17 h of stirring at 25 °C, the mixture was stirred with water for 5 min, washed with aqueous 1 M H_3PO_4, water, and brine, dried with Na_2CO_3, filtered, and evaporated, giving 997 mg of product: IR 5.73 μm, no OH, COOH or COCl; NMR δ 4.6 (m, 1 H), 3.67 (s, 3 H); mass spectrum, *m/e* 652; TLC R_f 0.7, 50:1 CH_2Cl_2–EtOAc.

3β-(Oleoyloxy)-5-cholenic Acid. The previous compound (128 mg, 0.197 mmol) was dried by benzene flush and heated under N_2 with 497 mg of LiI (3.70 mmol) in 28 mL of DMF, distilling 25 mL of DMF at 1 atm over 4 h, and refluxing 2 h more. The reaction mixture was washed with 1 N aqueous HCl, H_2O (three times), and brine, dried with $MgSO_4$, filtered, and stripped, affording 108 mg of product, which was 9:1 free acid–unreacted methyl ester: NMR δ 10.5 (br s, 1 H, COOH, 5.25–5.48 (m, 3 H, vinyl H's), 4.6 (br s, 1 H, C*H*O oleoyl), 3.66 (s, ca. $^1/_3$ H, $COOCH_3$); mass spectrum, *m/e* 356 (M^+ – oleoyloxy), 370 (w, Me ester – oleoyloxy).

4-[3β-(Oleoyloxy)androst-5-en-17β-yl]pentanol (29). To the previous compound (108 mg, 0.17 mmol; flushed twice with benzene) was added 1 mL of CH_2Cl_2, of 50 μL degassed[30] oxalyl chloride, and 1 μL of DMF. The mixture was stirred 1 h at 25 °C under N_2, stripped, and flushed with benzene at 0.1 torr: IR 5.54, 5.77 μm (acid Cl, ester). The acid chloride, together with 1.5 mL of dioxane and 37 mg (1 mmol) of $NaBH_4$ was stirred under N_2 for 5 min at 25 °C and 10 min at 100 °C. At 0 °C the excess $NaBH_4$ was decomposed with H_2O and then dilute HCl. After neutralizing with K_2HPO_4, the mixture was extracted twice with CH_2Cl_2, which was washed with brine, dried with $MgSO_4$, filtered, evaporated (96 mg crude), and chromatographed on a 20 × 20 cm plate (1 mm), using 50:1 $CHCl_3$–EtOAc. A 52-mg sample of product (49%) was obtained: R_f 0.5; NMR δ 3.5–3.8 (m, 2 H, CH_2OH); mass spectrum, *m/e* 624.

4-[3β-(Oleoyloxy)androst-5-en-17β-yl]pentanol Chlorambucil Ester (21). To chlorambucil acid chloride, made as described above from 35 mg (0.115 mmol) of chlorambucil, was added 62 mg (0.1 mmol) of **29** in 1 mL of 1:1 benzene–CH_2Cl_2. The mixture was stirred overnight under N_2, diluted with ether, washed with aqueous pH 5.7 phosphate, aqueous K_2HPO_4, and brine, dried with K_2CO_3, filtered, evaporated (88 mg), and chromatographed on a 20 × 20 cm plate (1 mm), using 10:1 hexane–EtOAc. Product **21** (66 mg, 73%), R_f 0.4, was obtained: NMR δ 3.9–4.2 (m, 2 H, $CH_2OC{=}O$), otherwise like **20**; mass spectrum, *m/e* 909 Cl_2.

***N*-Methyl-*N*-[[[bis(2-chloroethyl)amino]carbonyl]oxy]-3β-(oleoyloxy)-22,23-bisnor-5-cholenamide (23).** 3β-(Oleoyloxy)-22,23-bisnorcholenic acid (1.284 g, 2.11 mmol) was converted to **26** and then in 10 mL of benzene was added dropwise to a solution of 194 mg (2.32 mmol) of *N*-methylhydroxylamine hydrochloride in 10 mL of pyridine over 35 min under N_2. The mixture was stirred overnight, stripped in vacuo, taken up in 50 mL of benzene, washed with water, aqueous 1 M H_3PO_4 (two times), and brine, dried with $MgSO_4$, filtered, stripped, and rapidly chromatographed[31] on 73 g of silica gel (E. Merck, 70–230 mesh), eluting with 1:1 hexane–EtOAc at about 2.5 psi, TLC R_f 0.3. The steroid hydroxamic acid was obtained: 197 mg, 14.6%; NMR δ 8.6 (br s, 1 H, OH), 3.4 (2 s, 3 H, NCH_3); mass spectrum, *m/e* 640. This sample (0.307 mmol) was flushed with benzene and taken up in 2 mL of CH_2Cl_2. DMAP (38 mg, 0.307 mmol) and 63 mg (0.307 mmol) of bis(2-chloroethyl)amine chlorocarbonate[32] were added in 1 mL of CH_2Cl_2, and the mixture was stirred overnight at 25 °C under N_2. The mixture was diluted with ether and stirred 10 min with water. The organic layer was washed with dilute aqueous pH 2 phosphate, water, and brine, dried with $MgSO_4$, filtered, stripped (216 mg crude), and rapidly chromatographed[31] on 20.8 g of silica gel with 2.5:1 hexane–EtOAc as before, TLC R_f 0.35. There was obtained 60 mg of pure **23** (24%): NMR δ 5.22–5.48 (m, 3 H, vinyl H's), 4.6 (br s, 3α-H), 3.71 (s, 8 H, $O{=}CNCH_2CH_2Cl$), 3.32 (s, 3 H, NCH_3); mass spectrum, *m/e* 807 (Cl_2). These compounds could not be chromatographed by PLC or HPLC.

12-[[[[Bis(2-chloroethyl)amino]carbonyl]oxy]oleoyl]-cholesterol (24). Cholesteryl ricinoleate (Nu-Chek; 156 mg, 0.234 mmol) was flushed with benzene at 0.1 torr and dissolved in 5 mL of ether. Phosgene (1.74 M in benzene; 2.00 mL, 3.48 mmol) was added. After 24 h at 25 °C (2 h was greatly insufficient), solvents were evaporated at 0.1 torr, leaving 167 mg of crude chlorocarbonate: IR 5.62, 5.77 μm. To this in 5 mL of ether were added 36 mg (0.258 mmol) of bis(2-chloroethyl)amine and 31 mg (0.258 mmol) of DMAP in 5 mL of ether. The mixture was stirred 42 h under N_2, diluted with 40 mL of EtOAc, stirred 10 min with water, washed with dilute aqueous H_3PO_4, water, aqueous K_2HPO_4, and brine, dried with $MgSO_4$, filtered, stripped (184 mg crude), and chromatographed on two 20 × 20 cm plates (1 mm) with 12:1 hexane–EtOAc (R_f 0.5) affording 160 mg of pure **24** (83%): NMR δ 5.22–5.48 (m, 3 H, vinyl H's), 4.83 (m, 1 H, C*H*OCON), 4.6 (m, 1 H, 3β-H), 3.61 (br s, 8 H, $O{=}CNCH_2CH_2Cl$); mass spectrum, *m/e* 833–836.

***N*-[[[4-[3β-(Oleoyloxy)androst-5-en-17β-yl]pentyl]oxy]carbonyl]-*N*,*N*-bis(2-chloroethyl)amine (25).** Compound **29** (82 mg, 0.131 mmol) was flushed twice with benzene at 0.1 torr. Phosgene (1.74 M in benzene, 1.5 mL, 2.6 mmol) was added. After 2 h at 25 °C, solvents were removed in vacuo: IR 5.63, 5.76 μm. Benzene (2 mL) was added, followed by 0.2 g of bis(2-chloroethyl)amine (1.4 mmol) and 32 mg (0.26 mmol) of DMAP. The mixture was stirred under N_2 at 25 °C for 17 h, treated with a few drops of water for 2 min, diluted with EtOAc, washed with aqueous H_3PO_4, water, aqueous K_2HPO_4, and brine, dried with $MgSO_4$, filtered, stripped (103 mg crude), and chromatographed on a 1-mm plate, 20 × 20 cm, with 50:1 $CHCl_3$–EtOAc, R_f 0.45, providing 78 mg of pure **25**: 75%; NMR δ 4.0–4.3 (m, 2 H, CH_2OCON), 3.67 (s, 8 H, $O{=}CNCH_2CH_2Cl$); mass spectrum, *m/e* 792 Cl_2.

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