Design, Synthesis, and Biological Evaluation of Ellipticine−Estradiol Conjugates

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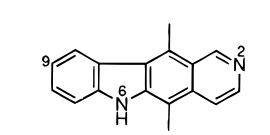
Three ellipticine−estradiol conjugates were synthesized in an effort to target the cytotoxicity of ellipticine to estrogen-receptor positive cells. The three conjugates were prepared with linker chains extending from the 17α position of the estradiol to N-2 (compound **3**), N-6 (compound **4**), and C-9 (compound **5**) positions of ellipticine. The ellipticine−estradiol conjugates were evaluated for their abilities to bind to estrogen receptors, to inhibit topoisomerase II, and for their cytotoxicities in human cancer cell lines. Conjugates **3** and **5** displayed weak binding affinities of 0.132 and 0.303 for the estrogen receptor (relative to estradiol = 100), while conjugate **4** did not show any detectable binding to the estrogen receptor. Compound **3** was a moderate inhibitor of topoisomerase II (IC_{50} 24.1 μM), while **4** and **5** were inactive. Conjugate **3** was consistently more cytotoxic (GI_{50} values 1−10 μM) than compounds **4** and **5** (GI_{50} values 10−100 μM) in a variety of human cancer cell lines. None of the compounds displayed any selectivity for estrogen-receptor positive cell lines, which probably reflects their weak affinities for estrogen receptors.

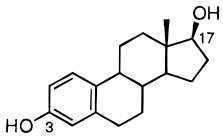
Introduction

The apparent nonselectivity of many antitumor agents and hence their acute toxicity toward rapidly proliferating tissues have long been the major deterrent in the use of these agents for the treatment of human cancer. One approach toward ameliorating this nonselectivity is to couple them to carriers which have shown selectivity toward the tumors themselves[1,2] or the tissues from which these tumors are derived.[3−5] This might enable the selective delivery of the cytotoxic agent to the cells or tissues bearing a high concentration of binding sites for the carrier. Thus, an estrogen can be used, in principle, to target breast cancers, since a large number of breast cancers have been shown to be estrogen-receptor (ER) positive and markedly concentrate estrogens within the cell nucleus.[6] Both the growth of the endometrium and the growth of breast tissue are significantly dependent on estrogens, and these cells are rich in ERs.[6] The ER belongs to a class of gene regulatory proteins referred to as the nuclear receptor superfamily, which includes receptors for steroids, thyroid hormones, and retinoids.[7,8] A typical target tissue cell contains about 5000−20 000 receptor molecules.[9] Although there are many theories regarding the precise mechanism of action of both estrogens and antiestrogens, it is now generally accepted that the steroid hormones bind to a receptor dimer−DNA complex within the nucleus, which then alters the rate of transcription by an as yet unknown mechanism.[9] Estrogens should thus be able to serve as effective carriers of DNA-directed cytotoxic agents into the nuclei of ER-rich cells. Several attempts have been made to utilize this approach by conjugating estradiol with alkylating moieties such as nitrogen mustards,[10] nitrosoureas,[11] aziridines,[12] dichloroplatinum,[13] and intercalators such as ellipticine.[14] In order for this approach to be successful, the conjugates should exhibit antitumor activity and also possess sufficient binding to the ER, which would allow the selective accumulation of the conjugates in ER-rich cells. Katzenellenbogen and Katzenellenbogen[15] have suggested that the relative binding affinity (RBA) value of such a conjugate should be at least 1% of that of estradiol (RBA = 100%), based on the number of receptors per cell and the possible drug concentration.

Ellipticine (**1**) is a naturally occurring indole alkaloid which exhibits cytotoxicity due to both inhibition of topoisomerase II and intercalation into DNA.[16,17] It was



1, Ellipticine



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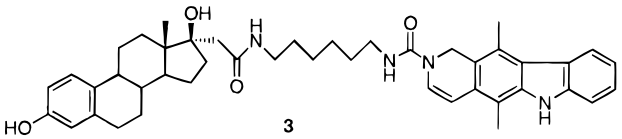

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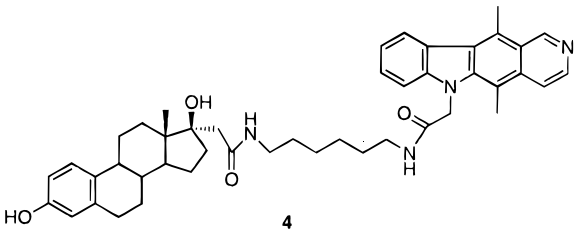

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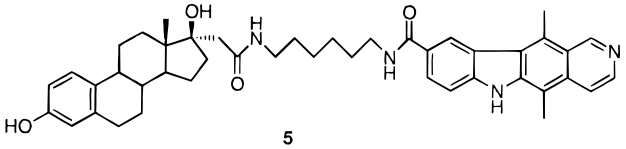

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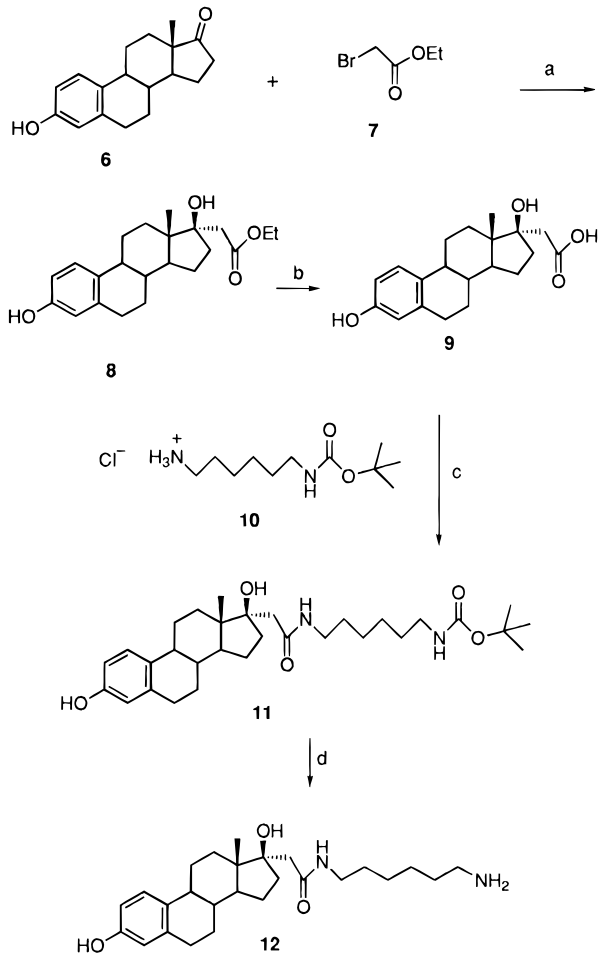
Scheme 1[a]



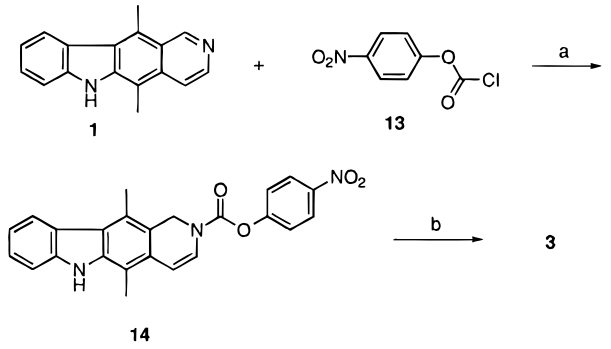
[a] Reagents: (a) activated Zn, THF, ultrasound, 23 °C (7 h), 50 °C (2 h); (b) EtOH, 10% aqueous NaOH, 23 °C (48 h); (c) Et_3N, EDCI, HOBt, DMF, −15 °C (4 h), 23 °C (4 h); (d) TFA, CH_2Cl_2, 0−5 °C (6 h).

thus of interest to link ellipticine to a potent estrogen such as estradiol (**2**) to provide conjugates that can potentially bind to the ER and exhibit antitumor activity. Structure−activity relationships have indicated that the most crucial units required for binding of estradiol to ER are the free 3-OH and the 17β-OH groups.[18,19] The initial ellipticine−estradiol conjugate that was reported by Delbarre *et al.*[14] utilized a short linker to attach the 17α-position of estradiol to N-2 of ellipticine. Our study was aimed at synthesizing ellipticine−estradiol conjugates with a long linker chain which would potentially position the ellipticine nucleus away from the 17β-hydroxyl group, which is critical for binding to the ER. In addition, we were also interested in investigating the point of attachment of the linker chain to different regions of the ellipticine ring system. Initially, three conjugates, **3**−**5**, were designed in which the linker chain extended from a fixed 17α-position of estradiol to three different regions of the ellipticine nucleus: the 2-position (**3**), the 6-position (**4**), and the 9-position (**5**) of ellipticine. The present report discusses the synthesis of the three ellipticine−estradiol conjugates **3**−**5**, their binding affinity for ER, their topoisomerase II inhibitory activity, and their *in vitro* cytotoxicity against a variety of human cancer cell lines.

Chemistry

The synthesis of the estradiol-linker **12** is outlined in Scheme 1, which was then attached to the different

Scheme 2[a]



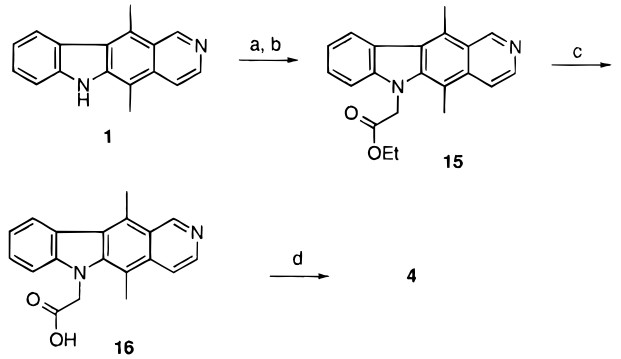
[a] Reagents: (a) THF, −70 °C (5 min), than 1.0 M $NaCNBH_3$ in THF, −70 °C (10 min); (b) **12**, DMF, 23 °C (64 h).

regions of the ellipticine ring system. The Reformatsky reaction of estrone (**6**) with ethyl bromoacetate (**7**) in the presence of activated zinc afforded only a 26% yield of the desired product **8**.[14] Ultrasonication has been used to accelerate the rate and increase yields of the Reformatsky reaction.[20] The reaction was therefore carried out in the presence of ultrasound and afforded the desired product in improved (56%) yield. Saponification of the ester **8** with 10% aqueous sodium hydroxide in aqueous ethanol gave the desired acid **9** in 80% yield. Activation of the acid **9** at low temperature with EDCI/HOBt using triethylamine as the base and reaction with *N*-[(*tert*-butyloxycarbonyl)amino]-1,6-hexanediamine hydrochloride (**10**) provided the coupled product **11** in 73% yield. Cleavage of the BOC group was achieved with trifluoroacetic acid in methylene chloride at 0−5 °C to afford the estradiol-linked amine **12** in good yield (90%). Deprotection of **11** at room temperature resulted in significantly lower yields due to formation of side products.

The attachment of the estradiol-linker **12** to the 2-position of ellipticine is shown in Scheme 2. Utilizing the procedure we have developed for the facile synthesis of 2-acyl-1,2-dihydroellipticines,[21] the acylation of ellipticine (**1**) with *p*-nitrophenyl chloroformate (**13**) at −70 °C in tetrahydrofuran, followed by the *in situ* reduction of the acylated intermediate with sodium cyanoborohydride, afforded the desired 2-acyl-1,2-dihydroellipticine **14** in almost quantitative yield. The desired conjugate **3** was obtained by the nucleophilic displacement of *p*-nitrophenol from **14** with the amine **12** in *N*,*N*-dimethylformamide.

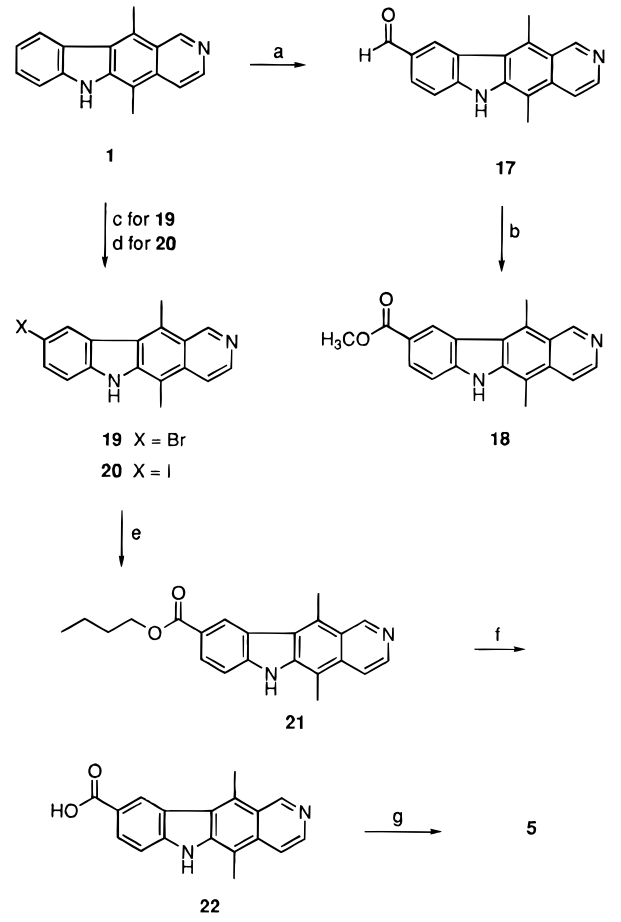
The synthesis of the conjugate **4**, in which the linker chain is attached to the 6-position of ellipticine, is shown in Scheme 3. Deprotonation of the 6-NH of ellipticine with sodium hydride in anhydrous *N*,*N*-dimethylformamide at 0 °C, followed by addition of ethyl bromoacetate, afforded the desired 6-functionalized derivative **15** in 49% yield along with a significant amount of a highly colored polar side-product which was not identified. Hydrolysis of the ester was achieved with 10% aqueous sodium hydroxide in a mixture of ethanol and tetrahydrofuran to afford the desired acid **16** in 90% yield. Initial attempts to couple the acid **16** with the steroidal amine **12** were unsuccessful due to the poor solubility of the acid **16** in conventional organic solvents. However, the triethylammonium salt of **16**, formed by the reaction of the acid with triethylamine in anhydrous *N*,*N*-dimethylformamide at 75 °C, had sufficient solubility in this solvent to effect reaction. Activation of the

Scheme 3[a]



[a] Reagents: (a) NaH, DMF, 0 °C (10 min); (b) ethyl bromoacetate, 0 °C (1 h); (c) THF, EtOH, 10% aqueous NaOH, reflux (4 h); (d) Et_3N, DMF, 75 °C (30 min), then EDCI, HOBt, **12**, 23 °C (48 h).

Scheme 4[a]



[a] Reagents: (a) hexamethylenetetramine, TFA, reflux (20 min); (b) NaCN, MnO_2, MeOH, reflux (96 h); (c) pyridinium bromide perbromide, AcOH, 60 °C (12 h); (d) I_2, $Hg(OAc)_2$, AcOH, 60 °C (12 h); (e) $Pd(PPh_3)_4$, nBuOH, nBu_3N, CO gas at 1 atm, DMF, 80 °C (15 h); (f) THF, EtOH, H_2O, LiOH, 60 °C (14 h); (g) EDCI, HOBt, Et_3N, **12**, DMF, 23 °C (24 h).

acid with EDCI/HOBt and reaction with the amine **12** afforded the desired conjugate **4** in 39% yield.

The synthesis conjugate **5**, in which the linker chain is attached to C-9 of ellipticine, is shown in Scheme 4. Formylation at the reactive 9-position of ellipticine was carried out as reported[22] with hexamethylenetetramine in refluxing trifluoroacetic acid to yield 9-formylellipticine (**17**). However, several attempts to oxidize the 9-aldehyde group present in **17** to the ester, **18**, or the acid, **22**, failed to provide the required oxidized product(s) in usable yield. Oxidation of **17** with manganese dioxide and sodium cyanide[23] in refluxing methanol was the best method and afforded the desired methyl ester **18** in very low yield (15%). Other methods were then explored in order to synthesize the 9-substituted ellipticine at the right oxidation level. Bromination of ellipticine was carried out with pyridinium bromide perbromide[24] in acetic acid at 60 °C to afford the required 9-bromoellipticine (**19**) in 60% yield. In a similar manner, iodination of ellipticine with iodine and mercuric acetate[25] in hot acetic acid furnished 9-iodoellipticine (**20**) in moderate yield (55%). Palladium-catalyzed carbonylation[26] of **20** with tetrakis(triphenylphosphine)palladium(0), *n*-butanol, and *n*-butylamine in DMF at 80 °C with constant bubbling of CO gas at 1 atm for 15 h afforded the desired 9-butyl ester **21** in 59% yield. A similar palladium-catalyzed carbonylation of 9-bromoellipticine (**19**) at 80 °C for 24 h also yielded **21** (47%). Hydrolysis of the butyl ester was achieved with aqueous lithium hydroxide in a mixture of tetrahydrofuran and ethanol at 60 °C to give the acid **22** (Scheme 4). Reaction of the triethylammonium salt of the acid **22** in anhydrous *N*,*N*-dimethylformamide with EDCI/HOBt and steroidal amine **12** gave the final target conjugate **5** in 45% yield.

Table 1. Relative Estrogen Receptor Binding Affinities and Topoisomerase II Inhibitory Activities of Ellipticine−Estradiol Conjugates **3**−**5**

compd	RBA[a]	IC_{50} in μM[b]
3	0.132 (±0.028)	24.1 (±8.3)
4	*c*	>200
5	0.303 (±0.030)	>200
8	3.66 (±1.00)	−
9	0.039 (±0.005)	−
11	0.056 (±0.010)	−
12	0.041 (±0.002)	−

[a] Relative binding affinity for the estrogen receptor determined in a competitive radiometric assay at 0 °C (estradiol = 100). [b] IC_{50} values for topoisomerase II inhibition determined in a catenation inhibition assay. The IC_{50} value for ellipticine (**1**) = 1.9 μM. [c] RBA value below detectable limit.

Biological Results and Discussion

The three ellipticine−estradiol conjugates **3**−**5** were evaluated for their ability to bind to the estrogen receptor[27] and their ability to inhibit topoisomerase II.[28] The effects of linker chain attachment to the estradiol moiety on affinity for the estrogen receptor were also investigated using the synthetic intermediates **8**, **9**, **11**, and **12**. In addition, conjugates **3**−**5** were submitted for cytotoxicity testing in the National Cancer Institutes *in vitro* drug discovery screen.[29−31] This screen has been designed to identify compounds with selective cytotoxicities for various types of human cancer cells.

The relative binding affinities (RBAs) of the conjugates **3**−**5** and modified estradiols **8**, **9**, **11**, and **12** for the estrogen receptor were determined using competitive radiometric binding assays,[27] and their values are listed in Table 1 as compared to estradiol (**2**) (RBA = 100, K_d = 0.3 nM). Conjugates **3** and **5**, which have the estradiol-linker attached to the 2- and 9-position, respectively, bound weakly to the estrogen receptor relative to estradiol, but their affinities were not negligible. In contrast, conjugate **4**, with the linker attached to the 6-position of ellipticine, displayed negligible affinity for the estrogen receptor. The low affinities of conjugates **3**, **4**, and **5** cannot be ascribed to the presence of a

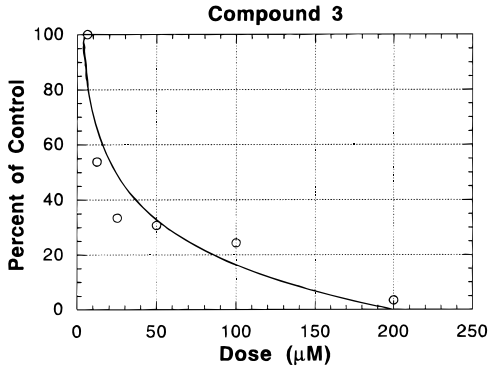


Figure 1. Inhibition of topoisomerase II by ellipticine−estradiol conjugate **3** as determined in a catenation inhibition assay.[21,28] The sample was tested at concentrations ranging from 3.12 to 200 μM, and the IC_{50} value was determined to be 24.1 μM. Ellipticine (**1**) displayed an IC_{50} of 1.9 μM in this system.

quaternary carbon at C-17 of the estradiol moiety per se, because intermediate **8** still maintained a relatively high binding affinity for the estrogen receptor. The conversion of the ester **8** to the acid **9** resulted in a drop in affinity by about 2 orders of magnitude, and this loss was not recovered in the amide derivatives **11** and **12**. However, the attachment of **12** to C-9 of ellipticine through an amide linkage in **5** actually resulted in a 7-fold increase in affinity for the estrogen receptor. In contrast, its attachment to ellipticine in **3** and **4** did not change the affinity appreciably. These results with **3**, **4**, and **5** indicate that the point of attachment to the "distant" ellipticine nucleus also plays a role in binding to the estrogen receptor.

The conjugates **3**−**5** were tested as inhibitors of topoisomerase II (Table 1) in a decatenation assay.[28] The decatenation inhibitory activity results are also predictive of the ability to inhibit the cleavable complex, with both assays being indicative of the intrinsic potency of the compounds against the topoisomerase II enzyme. Only compound **3** showed moderate inhibition of topoisomerase II with an IC_{50} of 24.1 μM (Figure 1). Both of the other conjugates **4** and **5** were inactive in this assay. Ellipticine itself inhibited topoisomerase II with an IC_{50} of 1.9 μM. These results suggest that the point of attachment of the estradiol-linker to the ellipticine nucleus is an important determinant in the activity of these agents against topoisomerase II. The lower topoisomerase II inhibitory potency of the conjugate **3** is not related to its 2-acyl-1,2-dihydroellipticine structure, since we have observed that other compounds with a similar functionality have shown good inhibitory potency against topoisomerase II.[19] The lower activity is probably related to the presence of a bulky steroid at the end of the linker which could potentially hamper binding to the covalent enzyme−nucleic acid complex. It is therefore evident that the linkage of estradiol to ellipticine, at least using the strategies reported here, results in a significant attenuation of both the estrogen receptor binding affinity of the estradiol moiety as well as the topoisomerase II inhibitory activity of the ellipticine fragment.

In spite of the negligible to moderate activity shown by the conjugates against topoisomerase II, it was still of interest to evaluate these compounds for their cytotoxic activity since ellipticine exerts its antitumor effects via multiple modes including inhibition of topoisomerase II, intercalation into DNA, and oxidative conversion into more cytotoxic metabolites.[14,15] The 3 conjugates were tested in approximately 55 five cell lines, representing human leukemia, nonsmall cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostrate cancer, and breast cancer cell lines. The results from representative cell lines for each of these nine different types of cancer are listed in Table 2. Within the breast cancer cell lines, the two MCF-7 lines are estrogen receptor positive, and the GI_{50} values for these are shown along with two nonestrogen receptor positive cell lines.

Table 2. Cytotoxicities of Ellipticine **(1)** and Ellipticine−Estradiol Conjugates **3**−**5** (GI_{50} Values in μM)[a]

panel/cell line	**1**	**3**	**4**	**5**
leukemia				
MOLT-4	0.38	2.9	46.3	38.3
nonsmall cell lung cancer				
NCI-H322M	0.31	2.68	71.4	28.0
colon cancer				
HCT-116	0.29	3.55	39.9	31.2
CNS cancer				
U251	0.93	7.64	72.4	55.9
melanoma				
LOX IMVI	0.56	7.97	50.9	51.4
ovarian cancer				
IGROV1	0.86	8.53	>100	>100
renal cancer				
SN12C	0.73	9.56	>100	>100
prostrate cancer				
PC-3	−	3.51	57.1	74.0
breast cancer				
MCF-7	0.71	3.39	>100	−
MCF-7/ADR-RES	0.74	9.96	>100	>100
MDA-MB-231/ATCC	−	19.7	>100	34.7
HS 578T	−	16.6	46.8	>100

[a] The cytotoxicity GI_{50} values are the concentrations corresponding to 50% growth inhibition.

The cytotoxicity data indicate that the 2-position conjugate **3** is more potent (GI_{50} ~1−10 μM) against the tumor cell panel compared to conjugates **4** and **5** (GI_{50} ~10−100 μM). However, conjugate **3** did not show any selectivity for the estrogen receptor positive cell lines. This lack of selectivity can be attributed to the low affinity of conjugate **3** for the estrogen receptor (RBA = 0.132).

Although the present effort can be categorized as another failed attempt to increase the selectivity of a cytotoxic agent for cells rich in estrogen receptors by conjugating it to estradiol, the reported chemistry opens the way for the preparation of additional conjugates that might display the desired selectivity. Further modifications can be pursued in order to increase the estrogen receptor binding affinity of the 2-acyldihydroellipticine−estradiol series (represented by **3**) by changing the functional groups present in the linker chain and by exploring attachments of the linker to the 11β- and 7α-positions of the estradiol molecule.[18,19] In addition, the antitumor potency of this conjugate **3** can be potentially improved by the substitution of a 9-methoxy or a 9-hydroxy group on the ellipticine nucleus; such substitutions are known to increase the antitumor activity and topoisomerase inhibitory activity of several ellipticine analogs.[16,17] Additional work will be required in order to successfully conjugate a cytotoxic agent to a carrier with affinity for the estrogen receptor without significantly compromising the activity of either fragment.

Experimental Section

General. Melting points were determined in capillary tubes on a Thomas−Hoover Unimelt apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Bruker ARX-300 and Varian VXR-500 spectrometers. IR spectra were recorded on a Perkin-Elmer 1600 FT-IR spectrophotometer. Low-resolution chemical ionization mass spectra (CIMS) were determined on a Finnigan 4000 spectrometer using 2-methylpropane as the reagent gas. High-resolution CIMS, low-resolution fast atom bombardment mass spectra (FABMS), and peak match FABMS were obtained on a Kratos MS50 spectrometer. Analytical thin-layer chromatography was done on Whatman silica 60 K6F glass-coated plates with fluorescent indicator. Column chromatography was performed using Merck silica gel (230−400 mesh). Solvents were dried over 4 Å molecular sieves before using. Microanalyses were performed at the Purdue Microanalysis Laboratory, and all values are within ±0.4% of the calculated compositions.

Ethyl 3,17*β*-Dihydroxy-1,3,5-estratriene-17α-acetate (8). A solution of ethyl bromoacetate (**7**) (10.81 g, 64.8 mmol) in anhydrous toluene (15 mL) was added dropwise over a period of 30 min to a mixture of activated zinc (4.0 g, 61.1 mmol) and estrone (**6**) (5 g, 18.5 mmol) in anhydrous THF (60 mL), and the resulting mixture was stirred at room temperature for 1 h. The mixture was ultrasonicated for a period of 6 h and then warmed to 50 °C for a period of 2 h. After cooling to room temperature, the reaction mixture was treated with 1% sulfuric acid (150 mL) and then ethyl ether (100 mL) and stirred for 20 min. The organic layer was separated, and the aqueous layer was extracted with ethyl ether (2 × 100 mL). The combined organic layers were washed with 10% $NaHCO_3$ solution (100 mL), and brine (100 mL), dried (Na_2SO_4), and filtered. The filtrate was evaporated under reduced pressure to dryness, and the residue was flash chromatographed on silica gel, eluting with 2:1, hexanes/ethyl ether. The fractions showing a single spot corresponding to $R_f = 0.32$ were pooled and evaporated under reduced pressure to dryness to afford **8** (3.68 g, 56%) as a thick oil which slowly solidified on storing at 5 °C to afford a white colored solid: mp 66−68 °C; TLC (1:1 ethyl ether/hexanes) $R_f = 0.32$; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.14 (d, 1 H, $J = 9$ Hz), 6.62 (dd, 1 H, $J = 9.0$ Hz, 3.0 Hz), 6.56 (d, 1 H, $J = 3$ Hz), 4.96 (br s, 1 H), 4.39 (br s, 1 H), 4.21 (q, 2 H, $J = 6$ Hz), 2.80−2.85 (m, 2 H), 2.67 (d, 1 H, $J = 18$ Hz), 2.52 (d, 1 H, $J = 18$ Hz), 2.24−2.31 (m, 1 H), 2.05−2.13 (m, 1 H), 1.60−1.98 (m, 5 H), 1.26−1.58 (m, 6 H), 1.31 (t, 3 H, $J = 6$ Hz), 0.94 (s, 3 H); low-resolution CIMS m/z (%) 359 (MH^+, 15), 341 (100); high-resolution CIMS calculated MH^+ 359.2222, found 359.2207.

3,17*β*-Dihydroxy-1,3,5-estratriene-17α-acetic Acid (9). A 10% aqueous solution of NaOH (20 mL) in ethanol (80 mL) was added to a solution of **8** (3.13 g, 8.74 mmol), and the resulting solution was stirred for 48 h at room temperature. Most of the ethanol was removed under reduced pressure, and the reaction mixture was diluted with water (150 mL). The aqueous layer was extracted with ether (2 × 50 mL), and this organic layer was discarded. The aqueous layer was then acidified to pH 1 with 1 N HCl and reextracted with ether (3 × 100 mL). The combined organic layers were washed with brine (100 mL), dried (Na_2SO_4), and filtered. The filtrate was subjected to flash column chromatography on silica gel, eluting first with EtOAc (250 mL) and then with 1% MeOH in EtOAc to afford fractions showing a single spot on silica gel at $R_f = 0.25$ ($CHCl_3$/MeOH, 9:1). These fractions were pooled and evaporated to dryness under reduced pressure and then coevaporated 3 times from a hexanes/ether mixture. This residue was dried over $CaSO_4$ under vacuum to afford **9** (2.31 g, 80%) as a white solid: mp 142−144 °C (lit.[17] mp 142 °C); TLC $R_f = 0.25$ $CHCl_3$/MeOH (9:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.13 (d, 1 H, $J = 9$ Hz), 6.63 (dd, 1 H, $J = 9$ Hz, 3 Hz), 6.57 (d, 1 H, $J = 3$ Hz), 2.80−2.83 (m, 2 H), 2.76 (d, 1 H, $J = 15$ Hz), 2.59 (d, 1 H, $J = 15$ Hz), 2.28−2.32 (m, 1 H), 2.04−2.10 (m, 1 H), 1.27−1.94 (m, 11 H), 0.95 (s, 3 H); low-resolution CIMS m/z (relative intensity) 331 (MH^+, 15), 313 (100); high-resolution CIMS calculated MH^+ 331.1909, found 331.1899.

***N*-[6-[(*tert*-Butyloxycarbonyl)amino]hexyl]-3,17*β*-dihydroxy-1,3,5-estratriene-17α-acetamide (11).** Triethylamine (3.4 mL, 24 mmol), HOBt (1.22 g, 9 mmol), and *N*-[(*tert*-butyloxycarbonyl)amino]-1,6-hexanediamine hydrochloride (**10**) (1.90 g, 7.5 mmol) were added to a solution of **9** (1.98 g, 6 mmol) in dry DMF (60 mL). The reaction mixture was cooled to −15 °C, and EDCI·HCl (1.61 g, 8.4 mmol) was added in portions as a solid. The resulting mixture was stirred at −15 °C for a period of 4 h and slowly allowed to warm to room temperature. After a period of 48 h, the reaction mixture was evaporated under reduced pressure to dryness (oil pump). The residue was dissolved in CH_2Cl_2 (250 mL) and washed with a saturated solution of sodium bicarbonate (2 × 100 mL), water (100 mL), and brine (100 mL), dried ($MgSO_4$), and filtered. The filtrate was flash chromatographed on silica gel, eluting with a gradient of 25−40% EtOAc in hexanes to afford fractions showing a single spot on TLC. These fractions were pooled and evaporated under reduced pressure to dryness to afford **11** (2.3 g, 73%) as a white solid: mp 105−107 °C; TLC $R_f =$ 0.44 EtOAc/hexanes (3:1); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.10 (d, 1 H, $J = 9$ Hz), 6.70 (br s, 1 H), 6.64 (dd, 1 H, $J = 9$ Hz, 3 Hz), 6.58 (d, 1 H, $J = 3$ Hz), 4.57 (br s, 1 H), 3.24−3.37 (m, 2 H), 3.13 (m, 2 H), 2.77 (m, 2 H), 2.55 (d, 1 H, $J = 15$ Hz), 2.34 (d, 1 H, $J = 15$ Hz), 1.81−1.86 (br t, 4 H), 1.10−1.70 (m, 17 H), 1.44 (s, 9 H), 0.90 (s, 3 H); low-resolution FABMS m/z 529 (MH^+); high-resolution FABMS calculated MH^+ 529.3641, found 529.3652. Anal. ($C_{31}H_{48}N_2O_5 \cdot 0.5H_2O$) C, H, N.

***N*-(6-Aminohexyl)-3,17*β*-hydroxy-1,3,5-estratriene-17α-acetamide (12).** A solution of **11** (2.26 g, 4.28 mmol) in CH_2Cl_2 (100 mL) was cooled in an ice bath to 0−5 °C. Trifluoroacetic acid (15 mL) was added, and the deep yellow solution was stirred at 0−5 °C for 6 h. Water (200 mL) was added carefully to the reaction mixture and the organic layer removed. The aqueous layer was basified to pH 8.5 with a saturated solution of $NaHCO_3$ and extracted with 10% MeOH in CH_2Cl_2 (3 × 80 mL). The combined organic layers were washed with brine (50 mL), dried (K_2CO_3), and filtered. The filtrate was evaporated under reduced pressure to dryness and flash chromatographed on silica gel, eluting first with 10% MeOH in $CHCl_3$ (250 mL), followed by 15% MeOH in $CHCl_3$, to afford fractions showing a single spot on TLC. These fractions were pooled and evaporated under reduced pressure to dryness to afford a brown oil. On drying this oil under vacuum, a shiny brown hygroscopic solid formed which was collected to afford **12** (1.65 g, 90%): mp softens and shrinks at 85 °C; TLC $R_f = 0.15$ $CHCl_3$/MeOH/NH_4OH (40:10:0.5); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 8.11 (t, 1 H, $J = 6$ Hz), 7.03 (d, 1 H, $J = 9$ Hz), 6.49 (dd, 1 H, $J = 9$ Hz, 2 Hz), 6.42 (d, 1 H, $J = 2$ Hz), 3.05 (br apparent q, 2 H, $J = 6$ Hz), 2.67−2.71 (m, 2 H), 2.56 (t, 2 H, $J = 6$ Hz), 2.22−2.27 (m, 3 H), 2.03 (m, 1 H), 1.50−1.85 (m, 5 H), 1.10−1.45 (m, 15 H), 0.80 (s, 3 H); low-resolution CIMS m/z (relative intensity) 429 (MH^+, 10), 411 (20), 271 (40), 159 (100); high-resolution CIMS calculated MH^+ 429.3117, found 429.3112. Anal. ($C_{26}H_{40}N_2O_3 \cdot 0.5CF_3COOH$) C, H, N.

2-[(*p*-Nitrophenoxy)carbonyl]-1,2-dihydroellipticine (14). A suspension of ellipticine **(1)** (0.246 g, 1 mmol) in anhydrous THF (11 mL) was cooled to −70 °C in a dry ice/acetone bath, and a solution of *p*-nitrophenyl chloroformate (**13**) (0.242 g, 1.2 mmol) in THF (5 mL) was added slowly to this suspension. There was immediate formation of a red precipitate. After stirring for 5 min, a 1.0 M solution of $NaCNBH_3$ in THF (2.5 mL, 2.5 mmol) was added slowly via a syringe drive and the mixture stirred for 10 min at −70 °C. The mixture was poured into ice/water (100 mL) and extracted with $CHCl_3$ (2 × 80 mL). The combined $CHCl_3$ extracts were washed with 1 N HCl (80 mL) and cold water (80 mL), dried ($MgSO_4$), and filtered. The filtrate was evaporated under reduced pressure to afford **14** (0.41 g, 99%) as an orange-colored solid: mp 233−234 °C (dec.); TLC $R_f = 0.62$ EtOAc/hexanes (1:1); ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.19 (s, 1 H), 8.33 (d, 2 H, $J = 9$ Hz), 8.14 (dd, 1 H, $J = 8, 9$ Hz), 7.63 and 7.58 (2 d, 2 H, $J = 9$ Hz), 7.51 (d, 1 H, $J = 9$ Hz), 7.37 (dd, 1 H, $J = 7, 8$ Hz), 7.15 (dd, 1 H, $J = 7, 8$ Hz), 6.97 and 6.94 (2 d, 1 H, $J = 9$ Hz), 6.36 (d, 1 H, $J = 9$ Hz), 5.22 and 5.02 (2 s, 2 H), 2.71 (s, 3 H), 2.51 (s, 3 H); low-resolution CIMS m/z (relative intensity) 414 (MH^+, 100), 247 (40), 140 (20); high-resolution CIMS calculated MH^+ 414.1454, found 414.1441.

2-[[[*N*-(3,17*β*-Dihydroxy-1,3,5-estratrien-17α-yl)acetamido]-*N*-hexyl]carbamoyl]-1,2-dihydroellipticine (3). A mixture of 2-[(*p*-nitrophenoxy)carbonyl]-1,2-dihydroellipticine (**14**) (0.070 g, 0.17 mmol) and the steroidal amine **12** (0.086 g, 0.2 mmol) was stirred in anhydrous DMF (2 mL) at room temperature under argon. After 16 h, more amine (0.043 mg, 0.1 mmol) was added and the reaction continued for a period of 48 h. The reaction mixture was then evaporated to dryness under reduced pressure, and the residue was dissolved in MeOH (30 mL). Silica gel (500 mg) was added to this solution and the suspension evaporated to dryness. This plug was poured on top of a silica gel column (1.8 × 15 cm, 230−400 mesh, flash chromatography grade) and flash chromatographed with $CHCl_3$ and then with 100 mL portions of 10, 20, 30, 40, and 50% EtOAc in $CHCl_3$. Fractions showing two spots on silica gel (EtOAc) at $R_f = 0.55$ and $R_f = 0.20$ were pooled and evaporated to a small volume. The organic layers were extracted with 1 N HCl (3 × 50 mL), then washed with water (50 mL) and a saturated solution of $NaHCO_3$ (75 mL), dried (Na_2SO_4), and filtered. The organic layer now showed that the spot at $R_f = 0.20$, corresponding to ellipticine, had disappeared due to the acidic extraction. The filtrate was evaporated under reduced pressure to dryness and dried under vacuum/$CaSO_4$ for 24 h to afford **3** (0.054 g, 45%) as an orange-colored solid: mp 175−178 °C (dec.); TLC $R_f = 0.55$ EtOAc; IR (nujol) 3677, 1641, 1594 cm^{-1}; ^1H NMR (DMSO-d_6, 300 MHz) δ 11.04 (s, 1 H), 8.98 (s, 1 H), 8.12 (overlapping br t and d, 2 H, $J = 8$ Hz), 7.47 (d, 1 H, $J = 8$ Hz), 7.32 (dd, 1 H, $J = 7.5$, 8 Hz), 7.17 (br t, 1 H, $J = 6$ Hz), 7.11 (dd, 1 H, $J = 8$, 7.5 Hz), 7.03 (d, 2 H, $J = 8$ Hz), 6.50 (dd, 1 H, $J = 8$, 2.5 Hz), 6.43 (d, 1 H, $J = 2.5$ Hz), 5.96 (d, 1 H, $J = 8$ Hz), 5.59 (s, 1 H), 4.88 (s, 2 H), 3.06−3.20 (m, 4 H), 2.71 (br s, 5 H), 2.45 (s, 3 H), 2.29 (s, 2 H), 2.24 (br m, 1 H), 2.0−2.10 (m, 1 H), 1.15 −1.85 (m, 19 H), 0.80 (s, 3 H); low-resolution FABMS *m*/*z* 702 (M^+); HPLC retention time = 9.06 min (Vydac C-18 column, 75−100% MeOH in H_2O in 30 min, flow rate 1.0 mL/min). Anal. ($C_{44}H_{54}N_4O_4 \cdot 1.70H_2O$) C, H, N.

Ellipticine-6-acetic Acid Ethyl Ester (15). Sodium hydride (0.036 g, 1.5 mmol, 95%) was added to a cooled (0 °C) suspension of ellipticine (**1**) (0.246 g, 1 mmol) in anhydrous DMF (10 mL) under argon. The mixture was stirred for 10 min at this temperature, and then a solution of ethyl bromoacetate (**7**) (0.267 g, 1.6 mmol) in anhydrous DMF (2 mL) was added slowly over a period of 15 min. The red solution was stirred for 1 h at 0 °C under an argon atmosphere, and the DMF was distilled off under reduced pressure. The resulting residue was stirred in water (50 mL) and filtered. The residue was washed well with water (20 mL), air-dried, dissolved in MeOH, and evaporated on silica gel. This material was poured on top of a silica gel column and eluted with 100% ether followed by 10% THF in ether and then finally with 20% THF in ether. Fractions showing a single spot were pooled and evaporated to afford a residue which was crystallized from ethyl ether to afford **15** (0.162 g, 49%) as an orange-colored solid: mp 206−208 °C (dec.); TLC (silica gel, THF) $R_f = 0.63$; ^1H NMR (300 MHz, DMSO-d_6) δ 9.73 (s, 1 H), 8.47 (d, 1 H, $J = 6$ Hz), 8.42 (d, 1 H, $J = 8$ Hz), 8.02 (d, 1 H, $J = 6$ Hz), 7.66 (d, 1 H, $J = 8$ Hz), 7.58 (dd, 1 H, $J = 7$, 8 Hz), 7.34 (dd, 1 H, $J = 8$, 7 Hz), 5.54 (s, 2 H), 4.21 (q, 2 H, $J = 7$ Hz), 3.26 (s, 3 H), 2.88 (s, 3 H), 1.22 (t, 3 H, $J = 7$ Hz); low-resolution FABMS *m*/*z* 333 (MH^+); high-resolution FABMS calculated MH^+ 333.1603, found 333.1585. Anal. ($C_{21}H_{20}N_2O_2 \cdot 0.25H_2O$) C, H, N.

Ellipticine-6-acetic Acid (16). A 10% aqueous NaOH solution (2 mL) was added to a solution of **15** (0.12 g, 0.36 mmol) in a mixture of ethanol and THF (2:1, 15 mL), and the resulting mixture was heated at reflux for a period of 4 h. The solvents were removed under reduced pressure, and the residue was stirred in water (10 mL). The pH of the mixture was adjusted to 6.5 with dropwise addition of 1 N HCl, and the resulting suspension was stored at 5 °C for 24 h and filtered. The residue was washed with cold water (5 mL) and air-dried. The residue was further washed with ether and dried over P_2O_5/vacuum at 78 °C to afford 0.099 g (90%) of **16** as an orange solid: TLC $R_f = 0.13$ $CHCl_3$/MeOH (3:1); ^1H NMR (300 MHz, DMSO-d_6) δ 9.74 (s, 1 H), 8.41−8.48 (2 overlapping d, 2 H), 8.03 (d, 1 H, $J = 6$ Hz), 7.65 (d, 1 H, $J = 8$ Hz), 7.58 (dd, 1 H, $J = 7$, 7.5 Hz), 7.34 (dd, 1 H, $J = 7.5$, 7 Hz), 5.44 (s, 2 H), 3.27 (s, 3 H), 2.92 (s, 3 H); low-resolution FABMS *m*/*z* 305 (MH^+); high-resolution FABMS calculated MH^+ 305.1290, found 305.1278.

6-[[[[*N*-(3,17*β*-Dihydroxy-1,3,5-estratrien-17α-yl)acetamido]-*N*-hexyl]carbamoyl]methyl]ellipticine (4). To a suspension of the acid **16** (0.023 g, 0.075 mmol) in anhydrous DMF (4 mL) was added triethylamine (71.3 μL, 0.5 mmol), and the reaction mixture was stirred at 75 °C for 30 min under argon. The reaction mixture was then cooled to room temperature and HOBt (0.027 g, 0.2 mmol) was added followed by the steroidal amine **12** (0.064 g, 0.15 mmol). To this mixture was added EDCI·HCl (0.038 g, 0.2 mmol) and the reaction continued for 48 h at room temperature under argon. The mixture was evaporated to dryness under reduced pressure and the residue stirred in water for 2 h. The suspension was cooled to 5 °C and filtered. The residue was washed with water and air-dried. The residue was dissolved in a mixture of MeOH/$CHCl_3$, and silica gel (500 mg) was added. The suspension was evaporated to dryness under reduced pressure. This silica gel material was poured on top of a dry silica gel column (1.8 × 15 cm, 230−400 mesh, flash chromatography grade) and flash chromatographed, eluting sequentially with 100 mL portions of 10%, 20% to 60% acetone in $CHCl_3$. Fractions showing the desired product spot along with some trailing impurity on silica gel were pooled and evaporated to dryness. The residue was dissolved in absolute ethanol containing several drops of concentrated HCl, and the solution was evaporated to dryness under reduced pressure. The residue was dissolved in a minimum amount of ethanol and diluted with ethyl ether. This solution was left at 0 °C for 48 h. The precipitated solid was collected by filtration and dried at once over $CaSO_4$ under vacuum to afford **4** (0.021 g, 39%) as a yellow solid: mp 176−180 °C (dec.); TLC (silica gel, 1:1 $CHCl_3$/acetone) $R_f = 0.20$; IR (nujol) 3677, 1660, 1591 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 9.74 (s, 1 H), 8.98 (s, 1 H), 8.41−8.46 (2 overlapping d, 2 H, $J = 6.4$, 8 Hz), 8.34 (t, 1 H, $J = 5.4$ Hz), 8.02−8.07 (overlapping t and d, 2 H, $J = 6.4$, 7.5 Hz), 7.59 (d, 1 H, $J = 8$ Hz), 7.53 (dd, 1 H, $J = 6.5$, 7.8 Hz), 7.33 (dd, 1 H, $J = 7.5$ Hz, 6.8 Hz), 7.02 (d, 1 H, $J = 8.4$ Hz), 6.49 (d, 1 H, $J = 8.1$ Hz), 6.43 (s, 1 H), 5.58 (s, 1 H), 5.22 (s, 2 H), 3.27 (s, 3 H), 3.02−3.16 (2 overlapping m, 4 H), 2.91 (s, 3 H), 2.69 (m, 2 H), 2.15−2.26 (m, 3 H), 1.95−2.05 (m, 1 H), 1.20−1.80 (m, 19 H), 0.79 (s, 3 H); low-resolution FABMS *m*/*z* 715 (MH^+); HPLC retention time = 4.99 min (Vydac C-18 column, 75−100% MeOH in H_2O in 30 min, flow rate 1.0 mL/min). Anal. ($C_{45}H_{54}N_4O_4 \cdot HCl \cdot H_2O$) C, H, N.

9-Formylellipticine (17). A mixture of ellipticine (**1**) (0.246 g, 1 mmol) and hexamethylenetetramine (1.55 g, 11 mmol) in trifluoroacetic acid (45 mL) was heated at reflux for 20 min. The reaction mixture was evaporated to one-fourth of its original volume under reduced pressure and diluted with water (20 mL). The solution was cooled in an ice bath and carefully neutralized by addition of solid sodium bicarbonate. This suspension was extracted with 10% MeOH in $CHCl_3$ (4 × 100 mL). The combined organic layers were washed with a saturated solution of $NaHCO_3$ (100 mL) and brine (100 mL), dried (Na_2SO_4), and filtered. The filtrate was evaporated to dryness under reduced pressure to afford **17** (0.246 g, 90%) as a yellow-colored solid which was sufficiently pure for use in further synthetic transformations: mp >300 °C (dec.) (lit.[20] mp >350 °C dec.); TLC (9:1 $CHCl_3$/MeOH) $R_f = 0.24$; low-resolution FABMS *m*/*z* 275 (MH^+).

Ellipticine-9-carboxylic Acid Methyl Ester (18). A mixture of 9-formylellipticine (**17**) (0.040 g, 0.144 mmol), manganese oxide (200 mg), and sodium cyanide (200 mg) in MeOH (50 mL) was brought to reflux and stirred for a period of 96 h. The TLC on silica gel ($CHCl_3$/MeOH/NH_4OH, 90:9:1) showed the disappearance of the starting material at $R_f = 0.77$ and formation of a major spot at $R_f = 0.81$. The reaction mixture was cooled to room temperature and filtered through Celite. The catalyst was washed with MeOH (20 mL). The combined filtrates were evaporated under reduced pressure to dryness. This residue was stirred in water (12 mL). The pH of this suspension was brought to 8 with dropwise addition of acetic acid. This suspension was stored at 5 °C for 18 h and filtered. The residue was washed well with water, air-

dried, and subjected to flash chromatography on silica gel, eluting with a gradient of 2−8% MeOH in $CHCl_3$. Appropriate fractions were pooled and evaporated to afford a residue which was dissolved in glacial acetic acid and filtered. This solution was evaporated to dryness under reduced pressure, and the residue was dissolved in hot EtOAc and left at 0 °C for 72 h. The deposited solid was filtered and dried to afford **18** (0.006 g, 15%) as a yellow solid: mp 272−274 °C (dec.); TLC (9:1 benzene/EtOH) $R_f = 0.24$; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.82 (s, 1 H), 9.72 (s, 1 H), 8.91 (s, 1 H), 8.46 (br d, 1 H, J = 6 Hz), 8.15 (d, 1 H, J = 8.2 Hz), 7.94 (br d, 1 H, J = 6 Hz), 7.61 (d, 1 H, J = 8.2 Hz), 3.91 (s, 3 H), 3.52 (s, 3 H), 2.79 (s, 3 H); low-resolution FABMS m/z 305 (MH^+); high-resolution FABMS calculated MH^+ 305.1290, found 305.1278. Anal. ($C_{19}H_{16}N_2O_2 \cdot 1.5CH_3COOH$) C, H, N.

9-Bromoellipticine (19). Pyridinium bromide perbromide (0.24 g, 0.75 mmol) was added to a solution of ellipticine (**1**) (0.12 g, 0.5 mmol) in glacial acetic acid (4 mL). The temperature of the reaction mixture was raised to 60 °C and the suspension stirred for a period of 12 h. The reaction mixture was then cooled to room temperature and diluted with water (20 mL). The pH was adjusted to 8 with the dropwise addition of concentrated NH_4OH. This mixture was extracted with 5% MeOH in EtOAc (2 × 30 mL). The combined organic layer was washed with water (30 mL) and brine (30 mL), dried ($MgSO_4$), and filtered. The filtrate was evaporated under reduced pressure to dryness, and the residue was subjected to flash column chromatography on silica gel, eluting with 1−10% EtOH in benzene. Fractions showing the desired spot were pooled and evaporated to afford a residue, which was dissolved in glacial acetic acid, filtered, and reevaporated to dryness under reduced pressure. This residue was dissolved in boiling EtOAc and left at 0 °C to deposit a solid which was filtered and dried to give **19** (0.096 g, 60%) as an orange solid: mp >250 °C (dec.); TLC (9:1 benzene/EtOH) R_f = 0.24; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.53 (s, 1 H), 9.69 (s, 1 H), 8.43 (overlapping peaks, 2 H), 7.91 (d, 1 H, J = 6 Hz), 7.66 (dd, 1 H, J = 1.5, 8.5 Hz), 7.50 (d, 1 H, J = 8.5 Hz), 3.20 (s, 3 H), 2.76 (s, 3 H); low-resolution FABMS m/z 327 (MH^++2), 325 (MH^+); high-resolution FABMS calculated MH^+ 325.0340, found 325.0347. Anal. ($C_{17}H_{13}N_2Br \cdot 0.5CH_3COOH$) C, H, N, Br.

9-Iodoellipticine (20). A solution of $Hg(OAc)_2$ (0.24 g, 0.75 mmol) in glacial AcOH (3 mL) was added under argon to a solution of ellipticine (**1**) (0.12 g, 0.5 mmol) in glacial AcOH (2 mL) maintained at 60 °C. A precipitate formed within 5 min. After 15 min, a solution of I_2 (0.19 g, 0.75 mmol) in glacial AcOH (7 mL) was added to the suspension and the mixture stirred for 12 h at 60 °C. The reaction mixture was cooled to room temperature and poured into water (150 mL). The pH of the solution was adjusted to 8 with dropwise addition of concentrated NH_4OH. The suspension was extracted with EtOAc (2 × 150 mL). The combined organic layer was washed with brine (150 mL), dried (Na_2SO_4, K_2CO_3), and filtered. Silica gel (0.8 g) was added to the filtrate, and the suspension was evaporated to dryness under reduced pressure. This plug was poured on top of a dry silica gel column and eluted with 1−12% EtOH in benzene. Fractions showing the desired spot were pooled and the solvent was evaporated to afford a residue which was dissolved in glacial acetic acid and filtered. This solution was evaporated again under reduced pressure to dryness. The residue was dissolved in boiling EtOAc and left at 0 °C to deposit a solid which was filtered and dried to afford **20** (0.102 g, 55%) as a yellowish brown solid: mp >250 °C (dec.); TLC (9:1 benzene/EtOH) R_f = 0.26; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.53 (s, 1 H), 9.71 (s, 1 H), 8.61 (app d, 1 H), 8.44 (d, 1 H, J = 6 Hz), 7.93 (d, 1 H, J = 6 Hz), 7.81 (dd, 1 H, J = 1.5, 8.5 Hz), 7.42 (d, 1 H, J = 8.5 Hz), 3.22 (s, 3 H), 2.78 (s, 3 H); low-resolution FABMS m/z 373 (MH^+); high-resolution FABMS calculated MH^+ 373.0202, found 373.0210. Anal. ($C_{17}H_{13}N_2I \cdot 0.5CH_3COOH$) C, H, N, I.

Ellipticine-9-carboxylic Acid Butyl Ester (21). Tri-*n*-butylamine (0.056 g, 0.3 mmol) was added to a solution of 9-iodoellipticine (**20**) (0.056 g, 0.15 mmol) in anhydrous DMF (4 mL) and *n*-BuOH (2 mL). The reaction mixture was heated to 80 °C, and $Pd[P(Ph_3)]_4$ (0.017 g, 10 mol%) was added to this solution. Carbon monoxide gas at 1 atm was constantly bubbled into this hot solution for a period of 15 h (*note: this reaction must be performed in a hood in order to avoid exposure to carbon monoxide gas*). The reaction mixture was then cooled to room temperature and filtered through a pad of Celite. The Celite was washed with DMF (2 × 5 mL), and the filtrate was evaporated to dryness under reduced pressure. The residue was dissolved in 5% MeOH in $CHCl_3$ (50 mL) and washed with water (25 mL) and brine (25 mL), dried ($MgSO_4$), and filtered. Silica gel (0.5 g) was added to the filtrate and it was evaporated to dryness. This silica gel plug was loaded on top of a dry SiO_2 column and flash chromatographed with 1−10% EtOH in benzene as the eluant. Fractions showing the desired spot were pooled, and the solvent was evaporated to afford a residue which was dissolved in glacial acetic acid, filtered, and reevaporated to dryness. The residue was dissolved in boiling EtOAc and left at 0 °C for 72 h. The solid that had deposited was filtered and dried to afford **21** (0.031 g, 59%) as a yellow solid: mp 162−166 °C (dec.); TLC (9:1 benzene/EtOH) R_f = 0.30; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.80 (s, 1 H), 9.71 (s, 1 H), 8.88 (s, 1 H), 8.44 (br d, 1 H, J = 6 Hz), 8.13 (d, 1 H, J = 8.5 Hz), 7.94 (br d, 1 H, J = 6 Hz), 7.61 (d, 1 H, J = 8.5 Hz), 4.34 (t, 2 H, J = 6.5 Hz), 3.24 (s, 3 H), 2.77 (s, 3 H), 1.72−1.81 (m, 2 H), 1.46−1.58 (m, 2 H), 1.00 (t, 3 H, J = 7 Hz); low-resolution FABMS m/z 347 (MH^+); high-resolution FABMS calculated MH^+ 347.1760, found 347.1750. Anal. ($C_{22}H_{22}N_2O_2 \cdot CH_3COOH$) C, H, N.

In a similar manner, the reaction of 9-bromoellipticine (**19**) (0.05 g, 0.15 mmol) with CO and Pd(0) at 80 °C for 24 h also afforded 0.024 g (47%) of compound **21**.

Ellipticine-9-carboxylic Acid (22). A solution of $LiOH \cdot H_2O$ (0.063 g, 1.5 mmol) in H_2O (1 mL) was added to a suspension of **21** (0.052 g, 0.15 mmol) in THF/EtOH/H_2O (4:1:1, 10 mL). The reaction mixture was then warmed to 60 °C for 14 h. The solvent was removed under reduced pressure and the residue stirred in H_2O. The pH of the solution was adjusted to 6 with dropwise addition of AcOH. The resulting suspension was left at 5 °C for 6 h and filtered. The residue was washed well with water, acetone (5 mL), and ether. The residue was then dried over P_2O_5/vacuum to afford 0.041 g (94%) of **22** as a brown solid which was carried forward directly to the next step: TLC (3:1 $CHCl_3$/MeOH) R_f = 0.25; low-resolution FABMS m/z 291 (MH^+).

9-[[[*N*-(3,17*β*-Dihydroxy-1,3,5-estratrien-17α-yl)acetamido]-*N*-hexyl]carbamoyl]ellipticine (5). Ellipticine-9-carboxylic acid (**22**) (0.04 g, 0.14 mmol) was dissolved in a mixture of anhydrous DMF (3 mL) and triethylamine (0.5 mL). HOBt (0.038 g, 0.28 mmol) was added to this solution, followed 5 min later by the steroidal amine **12** (0.09 g, 0.21 mmol). A solution of EDCI·HCl (0.054 g, 0.28 mmol) in anhydrous DMF (1 mL) was then added, and the reaction mixture was stirred for 24 h at room temperature. The solvent was evaporated under reduced pressure and the residue stirred in H_2O. The pH was adjusted to 8 with dropwise addition of dilute NH_4OH, and the suspension was stored at 5 °C for 6 h. The suspension was filtered and the residue washed well with water and air-dried. The residue was dissolved in $CHCl_3$/MeOH (3:1, 50 mL), and SiO_2 (0.5 g) was added to the solution. The suspension was evaporated to dryness. The resulting material was loaded on top of a dry SiO_2 column (1.8 × 15 cm, 230−400 mesh, flash chromatography grade) and flash chromatographed with 1−10% EtOH in $CHCl_3$ containing 0.1% triethylamine. The fractions showing a single spot were pooled and evaporated to dryness under reduced pressure. The residue was stirred with anhydrous ether, filtered, and dried to afford **5** (0.043 g, 45%) as a yellow solid: mp 210−214 °C (dec.); TLC R_f = 0.71 $CHCl_3$/MeOH (3:1); IR (nujol) 3753, 1636, 1595 cm^{-1}; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 11.65 (s, 1 H), 9.74 (s, 1 H), 8.99 (s, 1 H), 8.86 (s, 1 H), 8.60 (t, 1 H, J = 5.4 Hz), 8.46 (d, 1 H, J = 6 Hz), 8.13 (t, 1 H, J = 5.2 Hz), 8.07 (d, 1 H, J = 8.5 Hz), 7.95 (d, 1 H, J = 6 Hz), 7.57 (d, 1 H, J = 8.5 Hz), 7.01 (d, 1 H, J = 8.4 Hz), 6.49 (dd, 1 H, J = 2, 8.4 Hz), 6.42 (d, 1 H, J = 2 Hz), 5.59 (s, 1 H), 3.33 (s, 3 H), 3.11 (m, 2 H), 2.81−2.88 (overlapping s and m, 5 H), 2.68 (br m, 2 H), 2.28 (s, 2 H), 2.21 (br m, 1 H), 2.02 (br m, 1 H), 1.10−1.80 (m, 19 H), 0.79 (s, 3 H); low-resolution FABMS m/z 701 (MH^+); HPLC retention time = 3.67 min (Vydac C-18 column, 75−

100% MeOH in H_2O in 30 min, flow rate 1.0 mL/min). Anal. ($C_{44}H_{52}N_4O_4 \cdot EtOH \cdot 2.5H_2O$) C, H, N.

Measurement of Estrogen Receptor Relative Binding Affinities (RBAs). Estrogen receptor RBAs were determined using competitive radiometric binding assays using a tritium-labeled estrogen as the tracer, and immature rat uterine cytosol as the source of receptor, according to the methods described previously.[27] By definition, estradiol is given an RBA value of 100. These assays were conducted in duplicate at 0 °C (18 h). The coefficient of variation in replicate experiments is typically less than 30%.

Cytotoxicity Screening Results. Screening was performed using the previously described protocols and reporting procedures.[32] DMSO solutions of the compounds were routinely diluted by a factor of 500 with aqueous media. Aliquots of the resulting aqueous solutions or suspensions were tested in the cell cultures for 48 h. The cells were exposed to the compound for the entire 48 h of the assay. Cell growth was monitored colorimetrically using sulforhodamine B, a bright pink anionic dye that binds electrostatically to the basic amino acid residues of TCA-fixed cells.[30]

Decatenation Assay.[21,28] Each reaction mixture containing 1.8 μg/reaction of KDNA (TopoGEN, Inc., Columbus, OH), 67 mM Tris-HCl (pH 8), 160 mM KCl, 13 mM $MgCl_2$, 0.7 mM ATP, 0.7 mM dithiothreitol, 0.06 μg of bovine serum albumin (BSA), 17 μL of test compound, and 1 μL of human placenta Topo II enzyme (2 units) was incubated at 37 °C for 5 min. Reactions were stopped with 3 μL of stop buffer (5% Sarkosyl, 0.0025% bromophenol blue, and 25% glycerol). Samples were electrophoresed in 0.6% TBE−agarose gels at a constant 30 mA. Ethidium bromide-stained gels (0.5 μg/mL) were photographed using Poloroid 6700 High Speed film (Poloroid Corp., Cambridge, MA) using UV fluorescence at 300 nm. The decatenated KDNA was quantitated by densitometric analysis (Collage image-analysis software; Fotodyne, Inc., New Berlin, WI).

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References

(1) Upeslacis, J.; Hinman, L. Chemical Modification of Antibodies for Cancer Chemotherapy. *Annu. Rep. Med. Chem.* **1988**, *23*, 151−160.
(2) Blatter, W. A.; Lambert, J. M.; Goldmacher, V. S. Realising the Full Potential of Immunotoxins. *Cancer Cells* **1989**, *1*, 50−55.
(3) Krohn, K.; Kulikowski, K.; Leclercq, G. Diethylstilbesterol-Linked Cytotoxic Agents: Synthesis and Binding Affinity for Estrogen Receptors. *J. Med. Chem.* **1989**, *32*, 1532−1538.
(4) Schmidt, B. F.; Hernandez, L.; Routzer, C.; Czerwinski, G.; Chmurny, G.; Michejda, C. J. Peptide-Linked 1,3-Dialkyl-3-acyltriazenes: Gastrin Receptor Directed Antineoplastic Alkylating Agents. *J. Med. Chem.* **1994**, *37*, 3812−3818.
(5) Varga, J. M.; Asato, N.; Lande, S.; Lerner, A. B. Melanotropin−Daunomycin Conjugate Shows Receptor-Mediated Cytotoxicity in Cultured Murine Melanoma Cells. *Nature (London)* **1977**, *267*, 56−558.
(6) Epstein, R. J. The Clinical Biology of Hormone-Responsive Breast-Cancer. *Cancer Treat. Rev.* **1988**, *15*, 33−51.
(7) Evans, R. M. The Steroid and Thyroid Hormone Receptor Superfamily. *Science* **1988**, *240*, 889−895.
(8) Beato, M. Gene Regulation by Steroid Hormones. *Cell* **1989**, *56*, 335−344.
(9) Katzenellenbogen, J. A. Probes for Steroid Receptors−Studies at the Molecular, Cellular, and Whole Organism Levels. In *Proceedings of the Robert A. Welch Foundation Conference on Chemical Research XXXV Chemistry at the Frontiers of Medicine*; The Robert A. Welch Foundation: Houston, TX, 1991; pp 229−257.
(10) Hamacher, H.; Bormann, B.; Christ, E. N-Mustard Groups Linked to Estrogens: Problems of Synthesis and Receptor Affinity. In *Cytotoxic Estrogens in Hormone Receptive Tumors*; Raus, J., Martens, H., Leclercq, G., Eds.; Academic Press: London, 1980; pp 107−122.
(11) Lam, H.-Y. P.; Begleiter, A.; Goldenberg, G. J.; Wong, C.-M. Synthesis of Steroidal Nitrosoureas with Antitumor Activity. *J. Med. Chem.* **1979**, *22*, 200−202.
(12) Schönemann, K. H.; van Vliet, N. P.; Zeelen, F. J. Potential Antitumour Agents: 11β-(1-Aziridinylmethyl) Derivatives of Estradiol and Estrone. *Eur. J. Med. Chem.-Chim. Ther.* **1980**, *15*, 333−335.
(13) Chesne, C.; Leclercq, G.; Pointeau, P.; Patin, H. Synthesis and Biological Studies of Aminoestradiol−Platinum(II) Conjugates. *Eur. J. Med. Chem.-Chim. Ther.* **1986**, *21*, 321−327.
(14) Delbarre, A.; Oberlin, R.; Roques, B. P.; Borgna, J.-L.; Rochefort, H.; Le Pecq, J.-P.; Jacquemin-Sablon, A. Ellipticine Derivatives with an Affinity to the Estrogen Receptor, An Approach to Develop Intercalating Drugs with a Specific Effect on the Hormone-Dependent Breast Cancer. *J. Med. Chem.* **1985**, *28*, 752−761.
(15) Katzenellenbogen, J. A.; Katzenellenbogen, B. S. Considerations in the Design and Evaluation of Cytotoxic Estrogens. *Breast Cancer Res. Treat.* **1982**, *2*, 347−353.
(16) Gribble, G. W. Synthesis and Antitumor Activity of Ellipticine Alkaloids and Related Compounds. In *The Alkaloids*; Brossi, A., Ed.; Academic Press, Inc.: San Diego, 1990; Vol. 39; pp 239−352.
(17) Sainsbury, M. Ellipticines. In *Chemistry of Antitumor Agents*; Wilman, D. E. V., Ed.; Chapman and Hall: New York, 1990; pp 410−435.
(18) Bucourt, R.; Vignau, M.; Torelli, V.; Richard-Foy, H.; Geynet, C.; Secco-Millet, C.; Redeuilh, G.; Baulieu, E. E. New Biospecific Adsorbents for the Purification of Estradiol Receptor. *J. Biol. Chem.* **1978**, *253*, 8221−8228.
(19) Zeelen, F. J.; Bergink, E. W. Structure−Activity Relationships of Steroid Estrogens. In *Cytotoxic Estrogens in Hormone Receptive Tumors*; Raus, J., Martens, H., Leclercq, G., Eds.; Academic Press: London, 1980; pp 39−48.
(20) Han, B.-H.; Boudjouk, P. Organic Sonochemistry. Sonic Acceleration of the Reformatsky Reaction. *J. Org. Chem.* **1982**, *47*, 5030−5032.
(21) Devraj, R.; Fernandez, J. A.; Barrett, J.; Cushman, M. Synthesis of a Series of Cytotoxic 2-Acyl-1,2-Dihydroellipticines which Inhibit Topoisomerase II. *Anti-Cancer Drug Design*, in press.
(22) Plug, J. P. M.; Koomen, G.-J.; Pandit, U. K. An Expedient Synthesis of 9-Hydroxyellipticine. *Synthesis* **1992**, 1221−1222.
(23) Corey, E. J.; Gilman, N. W.; Ganem, B. E. New Methods for the Oxidation of Aldehydes to Carboxylic Acids and Esters. *J. Am. Chem. Soc.* **1968**, *90*, 5616−5617.
(24) Piers, K.; Meimaroglou, C.; Jardine, R. V.; Brown, R. K. The Preparation of 3-Bromoindole. *Can. J. Chem.* **1963**, *41*, 2399−2401.
(25) He, H.-M.; Fanwick, P. E.; Wood, K.; Cushman, M. A Novel 1,3 O-C Silyl Shift and Deacylation Reaction Mediated by Tetra-*n*-butylammonium Fluoride in an Aromatic System. *J. Org. Chem.* **1995**, *60*, 5905−5909.
(26) Narayana, C.; Periasamy, M. Organic Synthesis via Carbonylation of Organometallic Reagents with Carbon Monoxide. *Synthesis* **1985**, 253−268.
(27) Katzenellenbogen, J. A.; Johnson, H. J., Jr.; Myers, H. N. Photoaffinity Labels for Estrogen Binding Proteins of Rat Uterus. *Biochemistry* **1973**, *12*, 4085−4092.
(28) Muller, M. T.; Helal, K.; Spitzner, J. R. A Rapid and Quantitative Microtiter Assay for Eukaryotic Topoisomerase II. *Nucleic Acids Res.* **1989**, *17*, 9499−9501.
(29) Boyd, M. R. Status of the NCI Preclinical Antitumor Drug Discovery Screen. *Principles and Practices of Oncology* **1989**, *3*, 1−12.
(30) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. R. Feasibility of a High-Flux Anticancer Screen Using a Diverse Panel of Cultured Human Tumor Lines. *J. Natl. Cancer. Inst.* **1991**, *83*, 757−766.
(31) Paull, K. D.; Shoemaker, R. H.; Hodes, L.; Monks, A.; Scudiero, D. A.; Rubinstein, L.; Plowman, J.; Boyd, M. R. Development of Mean Graph and COMPARE Algorithm. *J. Natl. Cancer Inst.* **1989**, *81*, 1088−1092.
(32) Boyd, M. R.; Paull, K. D. Some Practical Considerations and Applications of the National Cancer Institute In Vitro Anticancer Drug Discovery Screen. *Drug Dev. Res.* **1995**, *34*, 91−109.
(33) Skehan, P.; Storeng, R.; Scuderio, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, F. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. New Colorimetric Assay for Anticancer-Drug Screening. *J. Natl. Cancer Inst.* **1990**, *82*, 1107−1112.

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