Design, Synthesis, and Biological Evaluation of Doxorubicin−Formaldehyde Conjugates Targeted to Breast Cancer Cells

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The anthracycline antitumor drug doxorubicin (DOX) has been utilized for decades as a broad-spectrum chemotherapeutic. Recent literature evidence documents the role of formaldehyde in the cytotoxic mechanism, and anthracycline−formaldehyde conjugates possess substantially enhanced activity in vitro and in vivo. Targeting a doxorubicin−formaldehyde conjugate specifically to cancer cells may provide a more efficacious chemotherapeutic. The design and 11-step synthesis of doxorubicin−formaldehyde conjugates targeted to the estrogen receptor, which is commonly overexpressed in breast cancer cells, are reported. The formaldehyde is incorporated in a masked form as an N-Mannich linkage between doxorubicin and salicylamide. The salicylamide triggering molecule, previously developed to release the doxorubicin−formaldehyde active metabolite, is tethered via derivatized ethylene glycols to an *E* and *Z* mixture of 4-hydroxytamoxifen. The targeting group, *E*/*Z*-4-hydroxytamoxifen, was selected for its ability to tightly bind the estrogen receptor and antiestrogen binding sites. The targeted doxorubicin−formaldehyde conjugates' estrogen receptor binding and in vitro growth inhibition were evaluated as a function of tether length. The lead compound, DOX-TEG-TAM, bearing a triethylene glycol tether, binds the estrogen receptor with a binding affinity of 2.5% relative to *E*/*Z*-4-hydroxytamoxifen and inhibits the growth of four breast cancer cell lines with 4-fold up to 140-fold enhanced activity relative to doxorubicin.

Introduction

Metastatic breast cancer is the second leading cause of tumor-related death in women behind only lung cancer.[1] The anthracycline antitumor antibiotic doxorubicin (DOX) is one of the most effective chemotherapeutics employed in the treatment of breast cancer. Increased survival, particularly in the context of drug-resistant breast cancer, is likely to result from the development of targeted, highly specific treatments.

Doxorubicin, a natural product discovered more than 30 years ago, exhibits a wide range of antineoplastic activity and, consequently, has stimulated an immense research effort to understand its mode of action and to develop superior derivatives. While the cytotoxic mechanism of doxorubicin remains somewhat controversial, there is substantial evidence to suggest that the following events play a role: (1) intercalation and alkylation of DNA, (2) induction of topo-II mediated strand breaks, (3) interference with DNA unwinding and helicase activity, (4) lipid peroxidation, and (5) direct membrane effects.[2,3]

While there remains substantial debate as to the cytotoxic mechanism, DNA has clearly emerged as a target, with the bulk of the intracellular drug residing in the nucleus, a portion of which intercalates DNA.[4] Recent literature reports suggest that DOX chelates iron to catalytically produce formaldehyde for use in DNA alkylation.[5,6] The proposed mechanism is as follows. Doxorubicin sequesters iron from iron storage proteins and induces oxidative stress. The oxidative stress product(s) oxidizes a carbon source to produce formaldehyde, and DOX then traps a portion of the formaldehyde at its amino-alcohol functionality, intercalates, and then alkylates DNA.[7,8] The most stable covalent linkage occurs at 5′-NGC-3′ sites in DNA between the 3′-amino substituent of the drug and the exocyclic amine of the guanine base. The structure of the linkage, and the presence of the bridging methylene, has been established by X-ray crystallography, NMR, mass spectrometry, and chemical trapping.[9−13] The term "*virtual cross-link*" has been invoked to describe the combination of intercalation, alkylation, and hydrogen-bonding interactions[11] as shown in Figure 1. The *virtual cross-link*, in rapidly dividing cells, leads to cell death, and in some cells, apoptosis.[14,15] Furthermore, formaldehyde-releasing prodrugs have been shown to act synergistically with doxorubicin.[16,17]

One of the limitations of chemotherapeutic protocols is the development of dose-limiting acute and chronic side effects. In the case of the anthracyclines, the induction of oxidative stress culminating in the production of formaldehyde gives rise to many drug side effects, the most serious of which is chronic cardiotoxicity.[18] Furthermore, chemotherapy is often rendered ineffective by the ability of cancer cells to develop resistance in response to a cytotoxic assault. Two common resistance mechanisms observed in the multidrug resistance (MDR) response include the overexpression of the p-170 glycoprotein drug efflux pump and the overexpression of enzymes that neutralize oxidative stress,[5,19] necessary for anthracycline activation.

Recently a doxorubicin−formaldehyde conjugate, doxoform, was developed in an attempt to circumvent these resistance mechanisms.[20] The formaldehyde conjugate,

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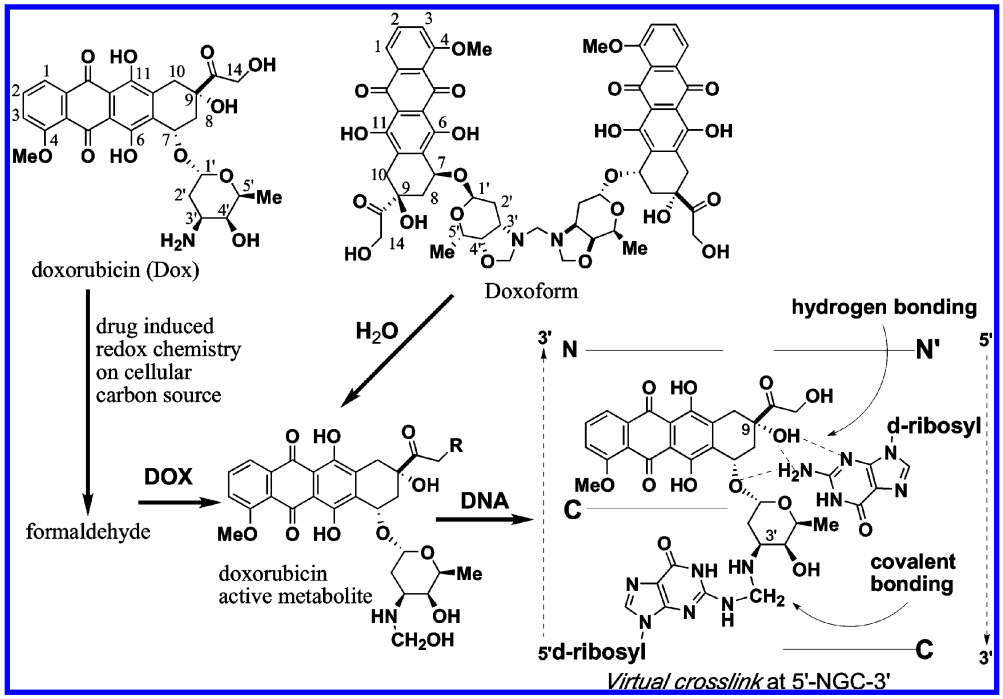


Figure 1. Doxorubicin and doxoform formation of the active metabolite and the subsequent DOX-DNA lesion.

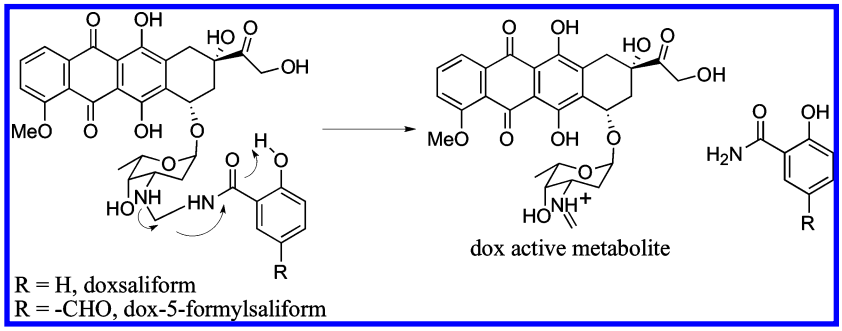


Figure 2. Structure of second generation of doxorubicin−formaldehyde conjugates.

shown in Figure 1, was designed to circumvent the induction of oxidative stress (to produce formaldehyde), which would overcome the overexpression of enzymes that neutralize oxidative stress. Doxoform was posited as a prodrug that would hydrolyze to the doxorubicin active metabolite and subsequently form DNA *virtual cross-links*. Indeed, doxoform was found to be substantially more toxic than doxorubicin to MCF-7 sensitive (100-fold) and doxorubicin-resistant (MDR expressing) MCF-7/Adr (10000-fold) breast cancer cells.[20] Fluorescence microscopy reveals doxoform localizes to the cell nucleus; flow cytometry shows higher drug levels and longer retention times in MCF-7 and MCF-7/Adr cells when treated with doxoform relative to doxorubicin.[8] Although doxoform exhibits greater cytotoxicity than doxorubicin, there are some insurmountable problems associated with it as a potential chemotherapeutic. Doxoform possesses very poor solubility characteristics because of its symmetry, has a very short lifetime in the vascular system, hydrolyzes too rapidly (half-life of ~10 min under physiological conditions), and exhibits higher mouse toxicity than doxorubicin.

In an attempt to address these issues, a second generation of doxorubicin−formaldehyde conjugates was developed to deliver the doxorubicin active metabolite in the form of a more hydrolytically robust N-Mannich base.[21,22] The lead compound from that study, doxsaliform, is shown in Figure 2. Doxsaliform has a half-life for hydrolysis to the doxorubicin active metabolite of 57 min under physiological conditions. Furthermore, the prodrug was more cytotoxic than doxorubicin in MCF-7 sensitive (4-fold) and MCF-7/Adr resistant (10-fold) breast cancer cells.[21,23] The aromatic moiety is amenable to functionalization, such as in the case of the formyl group in DOX-5-formylsaliform (Figure 2), permitting conjugation to a targeting molecule.

Targeting has the potential of selective delivery of a cytotoxic assault on the cancerous target cells while sparing indiscriminant damage to peripheral tissue. This could serve to lower the required dose and reduce unacceptable side effects, enhancing the therapeutic index of a drug. Although targeted cytotoxins have not yet found widespread application in the clinic, several interesting new strategies have recently been investigated such as androgen and estrogen receptor targeted geldanamycin,[24,25] cytotoxic analogues of hypothalamic peptides,[26] peptidyl targeted anthracyclines,[27,28] doxorubicin targeted to tumor vasculature,[29] folic acid conjugates,[30] and targeting matrix metalloproteinases,[31] to name a few. In an attempt to deliver selectively a doxorubicin−formaldehyde conjugate, we have designed, synthesized, and evaluated a new class of conjugates nominally targeted to estrogen receptor (ER) positive breast cancer cells.

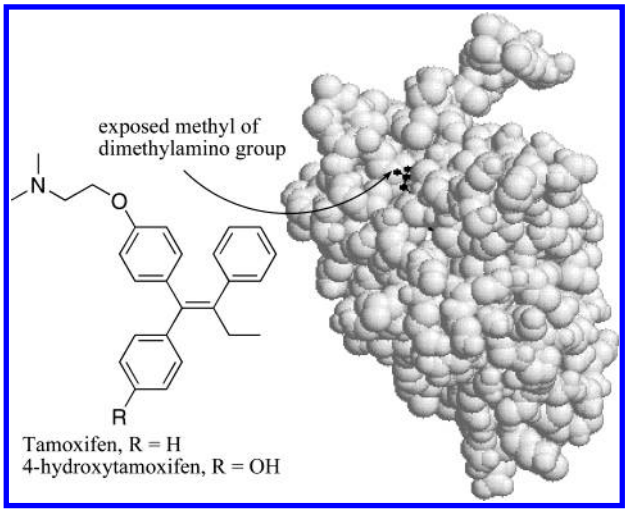


Figure 3. Cocrystal structure of 4-OHT bound to the ligand binding domain of ERα. The ligand is shown in "ball and stick" form (from Shiau and co-workers, PDB code: 3ERT).

Estrogen receptors, which are commonly overexpressed in breast tumor cells, have long been exploited as therapeutic targets. Tamoxifen, a nonsteroidal antiestrogen, has been used over the past 3 decades in the treatment of hormone responsive breast cancers.[32] The estrogen receptor (now referred to as ERα) resides primarily in the nucleus; the binding of an agonist, such as estradiol, triggers the expression of multiple genes, ultimately leading to cell proliferation. Upon binding estradiol, ER undergoes a conformational change, dissociates from heat shock proteins, homodimerizes, and binds estrogen response elements leading to transcription and cell proliferation.[32,33] More recently, a new ER subtype, ERβ, has been identified.[34,35] While ERα has been extensively studied, the physiological role of ERβ, particularly with respect to breast pathobiology, remains unclear.[36]

We now describe the design, synthesis, and preliminary evaluation of a third generation of doxorubicin−formaldehyde conjugate that bears the doxsaliform moiety tethered to hydroxytamoxifen as a targeting group. The lead compound, which we call DOX-TEG-TAM, consists of doxsaliform tethered to hydroxytamoxifen (TAM) via a triethylene glycol unit (TEG).

Design

Several classes of molecules bind with high affinity to ER from which a targeting strategy could be developed. Perhaps the most obvious choice would be to tether the doxorubicin−formaldehyde conjugate (DOX-5-formylsaliform, Figure 2) to estradiol, the native ER ligand. However, we hypothesize that the presence of a growth-stimulating hormone with a cytotoxin may not result in the most potent growth inhibitory conjugate. Alternatively, conjugation to an antiestrogen, such as tamoxifen, would deliver the cytotoxin to ER-overexpressing breast cancer cells without the concomitant growth stimulation.

In 1999, Shiau and co-workers published a cocrystal structure of 4-hydroxytamoxifen (4-OHT), the active metabolite of tamoxifen, bound to the ligand binding domain of ERα.[37] Figure 3 shows a space-filling model of this cocrystal structure created in RasMol from the coordinates available in the Protein Data Bank (PDB code: 3ERT). The cocrystal structure reveals that one methyl group of the dimethylamino function of 4-OHT is exposed at the surface, perhaps providing an ideal location to tether a cytotoxic moiety. A further advantage to a targeting strategy based on tamoxifen, or 4-hydroxytamoxifen, is the binding interaction that triarylbutene antiestrogens have with antiestrogen binding sites (AEBS). Antiestrogen binding sites are cytosolic, membrane-bound protein complexes that tightly bind tamoxifen and 4-OHT but exhibit virtually no affinity for estradiol.[38,39] The structure and natural function of AEBS remain poorly understood; however, there is some evidence to suggest that AEBS overexpression plays a role in tamoxifen resistance.[40] Additionally, AEBS are commonly expressed in hormone refractory, ER-negative breast cancer cell lines.[41,42] Therefore, a targeting strategy that utilizes a ligand, such as 4-hydroxytamoxifen, that has high affinity to both ER and AEBS could serve to deliver a cytotoxin to a broader range of breast cancer cell types.

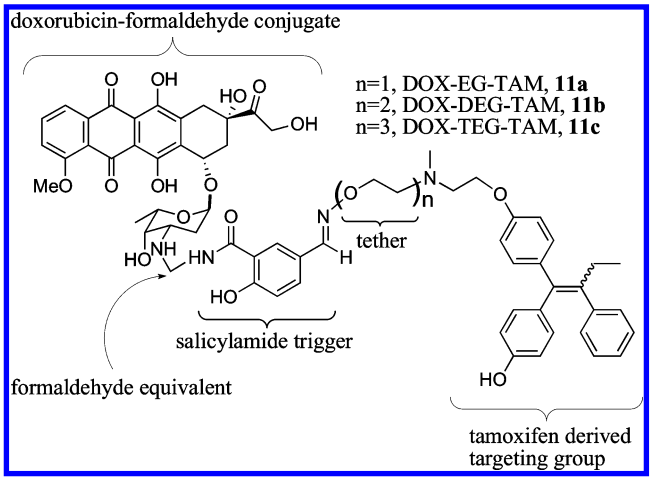


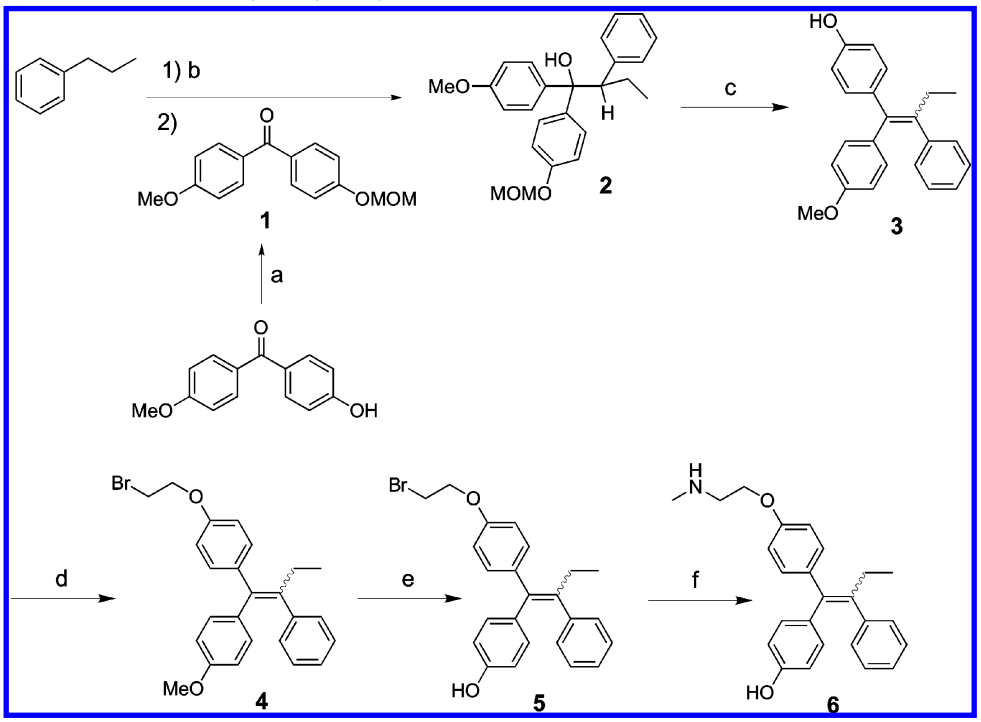
Figure 4. ER-targeted doxorubicin−formaldehyde conjugate.

The design of the ER-targeted doxorubicin−formaldehyde conjugates is shown in Figure 4. The formaldehyde was incorporated in the form of an N-Mannich base between the amide function of the salicylamide moiety and the amine of doxorubicin. A functionalized salicylamide was used as a trigger moiety to release the doxorubicin active metabolite.[21] The trigger was tethered to the targeting group with derivatized ethylene glycol units to confer enhanced water solubility. The tamoxifen active metabolite 4-hydroxytamoxifen was utilized as a targeting group based on the favorable attributes described above. An equimolar mixture of *E* and *Z* geometric isomers of the targeting group was utilized because previous work has demonstrated that para-hydroxy-substituted triarylbutenes isomerize under cell culture conditions, compromising the interpretation of the activity of pure isomers.[43] Furthermore, both geometric isomers of tamoxifen have been found to bind AEBS comparably.[39]

Chemistry

The overall synthetic strategy for **11a**−**c** required the synthesis of desmethyl-4-hydroxytamoxifen **6**, which could then be joined to various protected tethers, followed ultimately by oximation with DOX-5-formylsaliform (Figure 2). The synthesis of desmethyl-4-hydroxytamoxifen was accomplished as shown in Scheme

Scheme 1. Synthesis of *E*/*Z*-Desmethyl-4-hydroxytamoxifen[a]



[a] Reagents and conditions: (a) NaH, MOM−Cl (95%); (b) (1) n-BuLi, KOtBu, TMEDA, (2) **1,** −78 °C to room temp (97%); (c) 6 M HCl (93%); (d) $(n\text{-Bu})_4HSO_4$, NaOH, 1,2-dibromoethane (90%); (e) BBr_3 (57%); (f) $MeNH_2$, 60 °C, sealed tube (91%).

1. The phenolic function in 4-hydroxy-4′-methoxybenzophenone was first protected as the methoxymethyl (MOM) ether under standard conditions, providing benzophenone **1** in good yield. The other commercially available starting material, *n*-propylbenzene, was metalated at the α position using Schlosser's base[44] and then combined with **1** to provide carbinol **2** in 97% yield. Carbinol **2** was then dehydrated and MOM-deprotected in one step under strongly acidic conditions to yield triarylbutene **3** in good overall yield. The phenolic function of triarylbutene **3** was bromoethylated under phase-transfer conditions to achieve a 90% yield of **4**. Triarylbutene **4** was then demethylated with boron tribromide to provide free phenol **5** in serviceable yield (57%). Early attempts at the demethylation resulted in the facile loss of the bromoethyl group as well as the methyl ether, providing the triarylbutene bisphenol as the unwanted major product. Running the reaction at higher dilution and closely following the reaction by HPLC circumvented this problem; once the reaction had proceeded to the point at which roughly 60% of the starting material was demethylated, the reaction was quenched. The starting material **4** was then recycled to improve the overall yield from 57% to 75%. Finally, the primary bromide **5** was aminated with methylamine in 91% yield to complete the synthesis of the targeting group, *E*/*Z*-desmethyl-4-hydroxytamoxifen **6**.

The targeting/tether intermediates, **9a**−**c**, were synthesized as illustrated in Scheme 2. Commercially available *N*-hydroxynorbornyldicarboximide, utilized as protected hydroxylamine, was O-alkylated under mildly basic conditions with bis-halo derivatives of ethylene glycols to provide protected tethers **7a**−**c** in 66−72% yield. The protected tethers, **7a**−**c**, were joined to the targeting group (**6**) in the presence of Hunig's base to achieve the protected targeting/tether intermediates **8a**−**c** in serviceable yields. Finally, the norbornyl protecting group was removed via hydrazinolysis, exposing the hydroxylamine ether functionality (**9a**−**c**) in 67−74% yield.

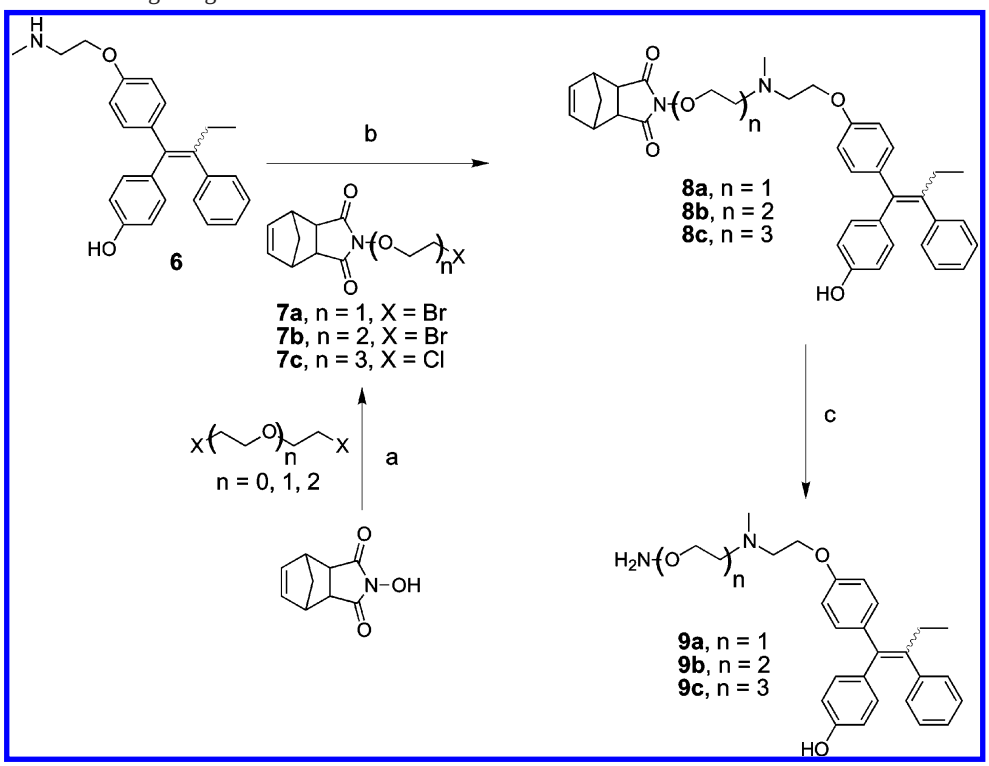
The synthetic work was completed as shown in Scheme 3. First, the hydroxylamine ether targeting/tether intermediates **9a**−**c** were joined with the triggering molecule 5-formylsalicylamide[45] to provide **10a**−**c** in 72−88% yield. HPLC indicated the formation of one isomer about the oxime double bond, which presumably is the less sterically demanding anti product. The trigger/targeting molecules termed SAL-EG-TAM (**10a**), SAL-DEG-TAM (**10b**), and SAL-TEG-TAM (**10c**) were synthesized to evaluate the presence (**11a**−**c**) and absence (**10a**−**c**) of doxorubicin on the ER relative binding affinity of the derivatized hydroxytamoxifen targeting group.

Second, the complete drug was prepared by joining, via oximation, DOX-5-formylsalicylamide to **9a**−**c**. The reaction was performed in a 1:1 mixture of 95% ethanol and 0.5% aqueous trifluoroacetic acid to stabilize the base-labile N-Mannich linkage.[22] The targeted formaldehyde conjugates, **11a**−**c**, were isolated by preparative HPLC in 50% yield. The targeted formaldehyde conjugates, termed DOX-EG-TAM (**11a**), DOX-DEG-TAM (**11b**), and DOX-TEG-TAM (**11c**) to denote the length of the tether in ethylene glycol units, were fully characterized by COSY, HSQC 2D-NMR, and ESI-HRMS.

Results and Discussion

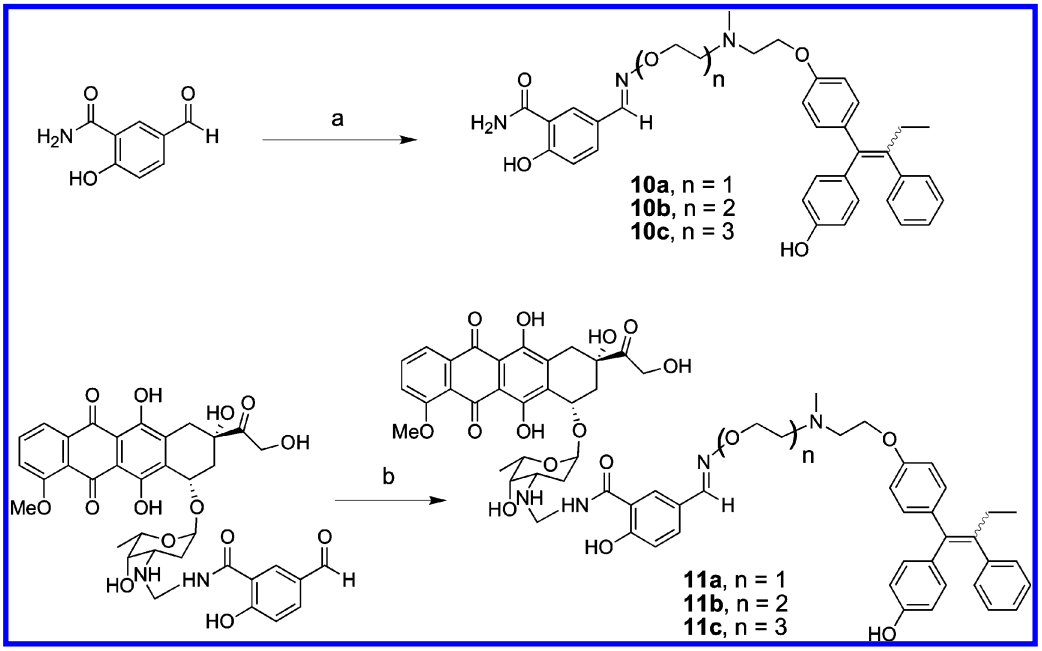
Hydrolysis and Stability. A standard solution of DOX-TEG-TAM (**11c**) in dimethyl sulfoxide (DMSO) containing 1% v/v acetic acid (AcOH) was diluted 1:100 in two different buffers: lysis buffer (pH 7.4, 10 mM Tris, 1.5 mM EDTA, 10 mM Na_2MoO_4) used for binding experiments or TE buffer (pH 7.6, 10 mM Tris, 1 mM EDTA). Samples incubated at 37 and 4 °C were moni-

Scheme 2. Synthesis of Targeting/Tether Intermediates[a]



[a] Reagents and conditions: (a) triethylamine, DMF (**7a**, 69%; **7b**, 72%; **7c**, 66%); (b) DIPEA, THF, sealed tube, 60 °C (+NaI, **7c**) (**8a**, 68%; **8b**, 55%; **8c**, 61%); (c) hydrazine, EtOH, 60 °C (**9a**, 71%; **9b**, 67%; **9c**, 74%).

Scheme 3. Oximation with 5-Formylsalicylamide and DOX-5-formylsaliform[a]



[a] Reagents and conditions: (a) **9a**−**c**, EtOH (**10a**, 81%; 1**0b**, 72%; **10c**, 88%); (b) **9a**−**c**, trifluoroacetic acid, EtOH, H_2O (**11a**−**c**, ∼50%).

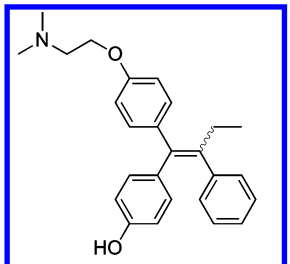
tored by HPLC to detect the loss of intact targeted drug and the formation of doxorubicin. The hydrolysis data were fit using first-order reaction kinetics to provide first-order rate constants; the hydrolysis half-life was then calculated using $t_{1/2} = (\ln 2)/k$. The half-life for hydrolysis of **11c** was found to be 76 min (pH 7.4) and 58 min (pH 7.6) at 37 °C, while at 4 °C the half-life was 180 h (pH 7.4) and 119 h (pH 7.6).

Estrogen Receptor Binding. The estrogen receptor source for the competitive binding experiments was an MCF-7 cell lysate. The crude cell lysate was utilized as a binding medium to account for other specific protein−ligand interactions that may occur under physiological conditions as well. The lysate was co-incubated with 1 nM tritium-labeled estradiol (^{3}H-E2) and various radioinert competitors at various concentrations for 18 h at 4 °C. Following incubation, free and unbound steroids were stripped from solution with a 1% dextran-coated charcoal (DCC) buffered suspension; receptor-bound ^{3}H-E2 in solution was then quantified via

scintillation counting. Nonspecific ^{3}H-E2 binding was determined with 2000-fold diethylstilbestrol, an ER-competitive ligand, which competes off all ER-bound ^{3}H-E2. All assays were performed at least in duplicate, and scintillation counting was performed in triplicate to ensure reproducibility. Complete results for all competitors are shown in Table 1. The IC_{50} for each competitor is defined as the concentration required to inhibit 50% of the ^{3}H-E2 binding. The relative binding affinity (RBA) is, by definition, the ratio (as a percentage) of the molar concentrations of a reference competitive compound and a test compound required to decrease the proportion of specifically bound ^{3}H-E2 by 50%. The RBA^{OHT} is the binding affinity of a competitor relative to *E*/*Z*-4-OHT.

To ensure that the developed assay would provide meaningful data, a control experiment in which cold E2 was used as the competitor was performed in duplicate. In both cases, 1.5 nM cold E2 was found to reduce the bound ^{3}H-E2 by 50%, indicating that the developed method is a valid measure of competitive binding. As a further control, tamoxifen, which weakly binds ER, was utilized as a competitor. As expected, tamoxifen exhibited a weak interaction with ER with an IC_{50} of 2000 nM.

First, the effect of tethering to 4-hydroxytamoxifen on ER binding was measured. A 1:1 mixture of *E* and *Z* geometric isomers of 4-hydroxytamoxifen, **12**, gave an



E/Z-4-hydroxytamoxifen, **12**

IC_{50} of 5 nM, which was assigned an RBA^{OHT} of 100. In the absence of the sterically demanding doxorubicin moiety (**10a**−**c**), the shortest tether, derived from ethylene glycol (**10a**), exhibits an RBA^{OHT} of 1.7, while **10b** and **10c** possess similar RBA^{OHT} values of 3.3. While in the presence of the doxorubicin moiety, the formaldehyde conjugates **11a**−**c** were found to have IC_{50} values from 200 to 300 nM. The formaldehyde conjugate with the longest tether, DOX-TEG-TAM (**11c**), was found to possess slightly better binding characteristics (RBA^{OHT} = 2.5) relative to **11a** and **11b** (RBA^{OHT} = 1.7).

It is interesting to note that in the case of the triethylene glycol derived tether, for example, the addition of doxorubicin only decreases the RBA^{OHT} from 3.3 (**10c**) to 2.5 (**11c**). This suggests that it is the presence of the (poly)ethylene glycol tether unit or the triggering salicylamide moiety that is dominating the inhibition of the native antiestrogen binding interaction. However, the data indicate, albeit to a lesser extent, that ER binding is enhanced as the tether length increases. While the binding affinity of *E*/*Z*-4-OHT is clearly compromised by the steric demands of the tether/trigger/DOX moiety, the observed binding affinities of **11a**−**c** may be sufficient to elicit a targeting response. It is encouraging that all three targeted conjugates possess substantially better binding characteristics than tamoxifen.

Table 1. Relative Binding Affinities of ER Ligands

competitor	IC_{50} (nM)[a]	RBA^{OHT}
E2	1.5 ± 0.04	330
E/*Z*-4-OHT, **12**	5 ± 0.3	100
DOX-EG-TAM, **11a**	300 ± 42	1.7
DOX-DEG-TAM, **11b**	300 ± 27	1.7
DOX-TEG-TAM, **11c**	200 ± 20	2.5
SAL-EG-TAM, **10a**	300 ± 33	1.7
SAL-DEG-TAM, **10b**	150 ± 20	3.3
SAL-TEG-TAM, **10c**	150 ± 17	3.3
tamoxifen	2000 ± 140	0.3

[a] The concentration of competitor that inhibited 50% of ^{3}H-E2 binding was determined using a competitive binding assay in which an MCF-7 cell lysate was the ER source. Lysates were incubated with ^{3}H-E2 and various concentrations of competitors for 18 h at 4 °C as described in the Experimental Section.

Breast Cancer Cell Growth Inhibition. The ER-targeted DOX−formaldehyde conjugates **11a**−**c** were evaluated against four breast cancer cell lines that differ in terms of estrogen receptor and multidrug resistance expression. MCF-7 cells are human breast adenocarcinoma cells that express estrogen receptor at a level of 195 000 sites per cell.[46] MCF-7/Adr cells are an ER-negative, doxorubicin-resistant variant of the parent MCF-7 that express the multidrug resistance (MDR) phenotype.[19] MDA-MB-231 and MDA-MB-435 are ER-negative human breast adenocarcinoma and ductal carcinoma cells, respectively. All cytotoxins were formulated as 100× solutions in DMSO/1% AcOH and delivered to cells as 1% DMSO (0.01% AcOH) in cell culture medium. In all experiments, cell treatment lasted 4 h. Representative values for the IC_{50}, the concentration inhibiting 50% of cell growth, are shown in Table 2.

In all four cell lines the targeted formaldehyde conjugates, **11a**−**c**, were more cytotoxic than doxorubicin. In the case of doxorubicin-sensitive MCF-7 cells, the most active targeted conjugates, **11b** and **11c**, were 6- to 10-fold more cytotoxic than doxorubicin. In the case of ER-negative, multidrug-resistant MCF-7/Adr cells, the targeted formaldehyde conjugates were 40-fold (**11a**) and 140-fold (**11b** and **11c**) more active than doxorubicin. In the case of ER-negative, drug-sensitive breast cancer cells, MDA-MB-231 and MDA-MB-435, all three targeted formaldehyde conjugates (**11a**−**c**) were 8- to 10-fold and 2- to 4-fold more cytotoxic, respectively.

There are several relevant controls necessary to interpret the growth inhibition data. When a 1:1 mixture of *E* and *Z* isomers of 4-hydroxytamoxifen (**12**) was administered to the cells, we found that only ER-overexpressing MCF-7 cell growth was appreciably inhibited with an IC_{50} of 300 nM. In the ER-negative cell lines (MCF-7/Adr, MDA-MB-231, and MDA-MB-435), the IC_{50} was 20000−40000 nM, likely the result of nonspecific toxicity. Interestingly, when DOX and *E*/*Z*-4-OHT were coadministered as an equimolar mixture, a synergistic effect was observed with a 2- to 5-fold increase in cytotoxicity relative to doxorubicin for all four cell lines. This synergism is not without precedent as Volm and co-workers have reported the sensitization of L1210/DOX multidrug-resistant leukemia cells when cotreated with doxorubicin and tamoxifen.[47] De Vincenzo and co-workers have reported a modest

Table 2. Growth Inhibition for Various Breast Cancer Cell Lines[a]

	IC_{50} (nM)			
	MCF-7	MCF-7/Adr	MDA-MB-231	MDA-MB-435
DOX	200 ± 26	10000 ± 1300	300 ± 33	150 ± 14
E/*Z*-4-OHT, **12**	300 ± 33	40000 ± 5600	30000 ± 7000	20000 ± 4000
DOX and *E*/*Z*-4-OHT co-treat	70 ± 11	2000 ± 260	100 ± 11	100 ± 21
doxsaliform	70 ± 5	2000 ± 320	80 ± 9	50 ± 9
DOX-EG-TAM, **11a**	80 ± 6	250 ± 35	40 ± 3	60 ± 7
DOX-DEG-TAM, **11b**	20 ± 3	70 ± 8	30 ± 4	40 ± 6
DOX-TEG-TAM, **11c**	30 ± 5	60 ± 9	30 ± 5	40 ± 6

[a] The IC_{50} was determined as described in the Experimental Section. All determinations were done at least in duplicate, with representive data shown above. Error bars represent one standard deviation about the mean for the six wells per lane measured for each drug concentration.

decrease in the IC_{50} for doxorubicin when MCF-7/Adr cells were cotreated with tamoxifen.[48] Nevertheless, it is intriguing that the synergism would be observed in all four cell lines regardless of ER or MDR expression.

Perhaps the most relevant control is the comparison of the targeted formaldehyde conjugates (**11a**−**c**) with the untargeted doxorubicin−formaldehyde equivalent, doxsaliform. Doxsaliform was prepared as the N-Mannich base as previously described.[21] Doxsaliform was found to be equally cytotoxic (MDA-MB-435) or slightly (2- to 3-fold) less cytotoxic (MCF-7 and MDA-MB-231) than **11a**−**c** in three of the four breast cancer cell lines tested. However, an interesting result is observed in the case of the multidrug-resistant MCF-7/Adr cells; the targeted formaldehyde conjugates are 8-fold (**11a**) and 28-fold (**11b**, **11c**) more cytotoxic than untargeted doxsaliform. This result is even more interesting in the context of the fact that MCF-7/Adr cells are ER-negative. One possible explanation for this observation is that the p-170 glycoprotein drug-efflux pump, which is overexpressed as part of the multidrug-resistance phenotype, is rapidly pumping out doxorubicin and untargeted doxsaliform, while the targeted formaldehyde conjugates (**11a**−**c**) are entering the cell and experiencing a binding interaction with AEBS that serves to sequester the molecule, preventing drug-efflux pump-mediated excretion. Indeed, increased lipophilicity, endowed by the presence of the triarylbutene moiety, should make the targeted formaldehyde conjugates poor substrates for the p-170 glycoprotein.[49] In summary, the targeted formaldehyde conjugates **11a**−**c** were more cytotoxic relative to doxorubicin and untargeted doxsaliform in both ER-positive (MCF-7) and ER-negative (MCF-7/Adr, MDA-MB-231) breast cancer cell lines.

With the ER binding affinity (Table 1) and in vitro growth inhibition data (Table 2) serving as a guide, we have moved ahead with **11c** as the lead compound. This doxorubicin−formaldehyde conjugate, derived from the longest tether, is characterized by the most favorable in vitro data. While the in vitro data look encouraging, we hypothesize that ultimately the best indication of efficacy of this targeting strategy will come from an in vivo experiment. To that end, **11c** is currently under evaluation in a pilot mouse experiment. Preliminary mouse formulation experiments demonstrate that the acute toxicity of **11c** is substantially less than that of doxoform; complete results from the mouse experiments will be published in due course. Further investigation into tethers that accommodate enhanced ER binding, as well as the study of these doxorubicin−formaldehyde conjugates' interaction with AEBS, is currently ongoing.

We have synthesized a new class of targeted doxorubicin−formaldehyde conjugates that exhibit favorable in vitro characteristics in the treatment of sensitive and resistant breast cancer relative to both the clinical drug doxorubicin and an untargeted doxorubicin−formaldehyde conjugate doxsaliform. We have also demonstrated that tethering from the *N*-methyl group of 4-hydroxytamoxifen does not eliminate the native ER binding affinity of the antiestrogen. Future work will be directed toward investigating the targeting mechanism and the observed enhanced activity of the targeted doxorubicin−formaldehyde conjugates.

Experimental Section

1. General Remarks. Thin-layer chromatography (TLC) was performed on precoated aluminum sheets of silica gel 60 F_{254} from EM Science (Gibbstown, NJ). Flash column chromatography was performed on Sorbent Technologies silica gel with particle size 32−63 μm and 60 Å pore size. Analytical HPLC was performed on a Hewlett-Packard 1050/1100 chromatograph equipped with a diode array UV−vis detector interfaced to an Agilent ChemStation data system. Analytical HPLC injections were performed on a Hewlett-Packard 5 μm reverse-phase octadecylsilyl (ODS) microbore column, 2.1 mm i.d. × 100 mm, eluting at 0.5 mL/min while being monitored at 256 and 310 nm. Analytical separation was achieved using method 1 parameters: flow rate, 0.5 mL/min; eluents A = CH_3CN and B = 20 mM triethylammonium acetate, pH 7.4; gradient, 5:95 A/B at 0 min to 50:50 A/B at 7 min to 85:15 A/B at 12 min, isocratic to 17 min, back to 5:95 A/B at 20 min. Preparative HPLC was performed on a hybrid chromatograph consisting of a Rainin Rabbit HP gradient pumping system and a Hewlett-Packard 1040 diode array UV−vis detector interfaced to an Agilent ChemStation data system. Preparative HPLC purification of the targeted doxorubicin−formaldehyde conjugates was performed on an Agilent Zorbax Rx-C8 9.4 mm × 25 cm semipreparative column. Preparative purification was achieved using method 2 parameters: flow rate, 2.5 mL/min; eluents A = CH_3CN and B = 0.1% trifluoroacetic acid in purified water; gradient, 2:98 A/B at 0 min to 62:38 A/B at 22 min, isocratic to 28 min, back to 2:98 A/B at 30 min. As a consequence of the hydrolytic instability of the targeted compounds, the degree of purity was established by two independent HPLC methods. The first system (method 2) was the HPLC configuration described above for the preparative purification of **11a**−**c**. The second HPLC method for establishing purity, denoted method 3, was performed on the Hewlett-Packard 1040A system described above using an Agilent Zorbax Rx-C8 4.6 mm × 15 cm analytical column. Method 3 parameters were as follows: flow rate, 1.0 mL/min; eluents A = CH_3CN and B = 0.1% trifluoroacetic acid in purified water; gradient, 2:98 A/B at 0 min to 70:30 A/B at 24 min, isocratic to 28 min, back to 2:98 A/B at 30 min. In all cases, the *E* and *Z* isomers were reported as two peaks, typically overlapping at half peak height. The HPLC method for purification of DOX-5-formylsaliform, denoted method 4, was performed on the hybrid chromatograph described above with a Rainin Dynamax Microsorb 10 mm C8-guard column. Method 4

parameters were as follows: flow rate, 2.0 mL/min; eluents A = CH_3CN and B = 0.1% trifluoroacetic acid in purified water; gradient, 2:98 at 0 min to 35:65 at 16 min, 35:65 at 16 min to 85:15 at 20 min, back to 2:98 at 22 min. Molecular ions for all intermediates and final structures were determined using ESI-MS performed with a 3 T Finnigan FTMS-2000 Fourier transform mass spectrometer at The Ohio State University (Prof. Christopher Hadad). ^{1}H NMR spectra were acquired with a Varian Unity INOVA 500 MHz spectrometer, with the exception of the doxorubicin−formaldehyde conjugates that were analyzed on a Varian INOVA 400 MHz spectrometer with a 3 mm microprobe. NMR assignments for the protons in the tamoxifen, salicylamide, and doxorubicin portions of the final structures, **10a**−**c** and **11a**−**c**, are denoted "TAM", "SAL", and "DOX." Absolute ethanol was from AAPEK Alcohol and Chemical Company (Shelbyville, KY). All other reagents and solvents were from Aldrich (Milwaukee, WI) and Fisher Scientific (Fair Lawn, NJ), respectively, and were used without further purification.

The concentrations of test compounds were determined UV/vis spectrophotometrically with a Hewlett-Packard 8452A diode array spectrophotometer interfaced to an Agilent ChemStation data system as described for each biological assay. ^{3}H-estradiol was obtained from Amersham Pharmacia Biotech (Buckinghamshire, England). Cell lysis was performed with a Misonix sonicator ultrasonic processor fitted with a microtip. Ultracentrifugation of cell lysates was accomplished with a Beckman TL-100 ultracentrifuge. Tamoxifen, cold estradiol, and diethylstilbestrol were all obtained from Sigma (St. Louis, MO). Complete Mini protease inhibitor cocktail tablets were obtained from Boehringer Mannheim (Indianapolis, IN). Cell lysate total protein was measured with a Micro Protein Determination kit from Sigma Diagnostics (Dorset, England). Liquid scintillation counting was performed using a Packard (Downers Grove, IL) Tri-Carb 1600 TR liquid scintillation analyzer. Econo-Safe biodegradable liquid scintillation cocktail was from Research Products International Corporation (Mount Prospect, IL).

All tissue culture materials were obtained from Gibco Life Technologies (Grand Island, NY) unless otherwise stated. MCF-7 and MDA-MB-231 cells were obtained from American Type Culture Collection (Rockville, MD). MCF-7/Adr doxorubicin resistant cells were a gift from W. W. Wells (Michigan State University, East Lansing, MI). MDA-MB-435 cells were generously provided by Dr. Renata Pasqualini (MD Anderson Cancer Center, Houston, TX). MCF-7, MCF-7/Adr, and MDA-MB-231 cells were maintained in vitro by serial culture in RPMI 1640 medium supplemented with 10% fetal bovine serum (Gemini Bioproducts, Calbassas, CA), L-glutamine (2 mM), HEPES buffer (10 mM), penicillin (100 units/mL), and streptomycin (100 μg/mL). MDA-MB-435 cells were maintained in vitro by serial culture in DMEM medium supplemented with 5% fetal bovine serum, L-glutamine (2 mM), sodium pyruvate (1 mM), and nonessential amino acids and vitamins for minimum essential media. Phenol-red-free media supplemented with L-glutamine and dextran-coated charcoal stripped fetal bovine serum were obtained from Sigma (St. Louis, MO). Cells were maintained at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air.

2. Synthesis. 4-(Methoxymethoxy)-4′-methoxybenzophenone (1). Sodium hydride (352 mg, 8.8 mmol) as a 60% dispersion in mineral oil was dissolved in 7 mL of dry dimethylformamide (DMF) and cooled to 0 °C under argon (Ar). In a separate vial 4-hydroxy-4′-methoxybenzophenone (1 g, 4.4 mmol) was dissolved in 4 mL of dry DMF and added to the reaction flask containing the NaH/DMF mixture. The reaction mixture was stirred at 0 °C under Ar for 1 h. Chloromethyl methyl ether (0.67 mL, 8.8 mmol) was added, and the reaction mixture allowed to warm to room temperature. After 2 h, TLC revealed complete consumption of the benzophenone starting material. The reaction mixture was diluted with 120 mL of methylene chloride (CH_2Cl_2), washed 2× with 50 mL of 1 M sodium carbonate, dried (Na_2SO_4), and concentrated in vacuo to give the crude product as an oil. The product was flash-chromatographed on silica gel (4:1 hexanes/ethyl acetate to 3:2 hexanes/ethyl acetate) to yield 1.14 g (95%) of desired product as a clear, oily residue. TLC (SiO_2, 3:2 hexanes/ethyl acetate): R_f = 0.55. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.49 (3H, s, MOM−OMe), 3.87 (3H, s, −OMe), 5.24 (2H, s, MOM−CH_2−), 6.95 (2H, d, J = 8.9 Hz, 2′), 7.09 (2H, d, J = 8.7 Hz, 2), 7.76 (2H, d, J = 8.9 Hz, 3′), and 7.79 (2H, d, J = 8.7 Hz, 3). HRMS $[M + Na]^+$ calculated 295.0941, found 295.0941.

1-(4-Methoxymethoxyphenyl)-1-(4-methoxyphenyl)-2-phenylbutan-1-ol (2). To a solution of 23 mL of dry hexanes in an oven-dried three-neck, 500 mL round-bottom flask was added 25.1 mL of potassium *tert*-butoxide (25.1 mmol) as a 1.0 M solution in tetrahydrofuran (THF) and *n*-propylbenzene (3.5 mL, 25.1 mmol). The reaction mixture was stirred at room temperature under Ar. By use of an oven-dried, Ar-purged syringe, *n*-butyllithium (15.7 mL, 25.1 mmol) as a 1.6 M solution in hexanes was added, followed by tetramethylethylenediamine (7.6 mL, 50.2 mmol). The reaction mixture was stirred at room temperature under Ar for 30 min, at which point it was cooled to −78 °C. In a separate flask, electrophile **1** (1.14 g, 4.19 mmol) was dissolved in 50 mL of dry THF and added dropwise over 30 min. Following the addition of the electrophile, the reaction mixture was allowed to warm to room temperature over 4 h, at which time TLC revealed complete consumption of the electrophile **1**. The reaction was quenched through the addition of 50 mL of saturated ammonium chloride followed by 100 mL of distilled water. The aqueous phase was extracted 3× with 100 mL of CH_2Cl_2. The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo, providing a colorless, oily residue. The crude product was purified via flash chromatography on silica gel (4:1 hexanes/ethyl acetate), providing 1.6 g (97%) of carbinol **2** as an equimolar mixture of diastereomers. TLC (SiO_2, 3:1 hexanes/ethyl acetate): R_f = 0.38. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.76 (3H, t, J = 7.5 Hz, 4), 1.83 (2H, m, J = 9.8, 7.5, 4.7 Hz, 3), 3.42 (1.5H, s, MOM−OMe diast 1), 3.51 (1.5H, s, MOM−OMe diast 2), 3.56 (1H, dd, J = 9.8, 4.7 Hz, 3), 3.70 (1.5H, s, −OMe diast. 2), 3.82 (1.5H, s, −OMe diast. 1), 5.08 (1H, s, MOM−CH_2− diast. 1), 5.20 (1H, s, MOM−CH_2− diast. 1), 6.66 (1H, d, J = 8.8 Hz, MeO−Ph 2 diast. 1), 6.80 (1H, d, J = 8.8 Hz, MeO−Ph 2 diast. 2), 6.91 (1H, d, J = 8.6 Hz, MOM−Ph 2 diast. 1), 7.04 (1H, d, J = 8.8 Hz, MOM−Ph 2 diast. 2), 7.10 (2H, m, phenyl−ortho), 7.14 (1H, d, J = 8.8 Hz, MeO−Ph 3 diast. 1), 7.15 (1H, d, J = 8.8 Hz, MeO−Ph 3 diast. 2), 7.15 (3H, m, phenyl−meta, para), 7.46 (1H, d, J = 8.6 Hz, MOM−Ph 3 diast. 1), 7.48 (1H, d, J = 8.8 Hz, MOM−Ph 3 diast. 2). HRMS $[M + Na]^+$ calculated 415.1880, found 415.1886.

***E/Z*-1-(4-Hydroxyphenyl)-1-(4-methoxyphenyl)-2-phenylbutene (3).** To a solution of carbinol **2** (989 mg, 2.5 mmol) in 19 mL of CH_2Cl_2 was added 19 mL of 95% ethanol and 19 mL of 6 M hydrochloric acid. The reaction mixture was stirred vigorously and heated under reflux overnight. After 18−24 h, 120 mL of sodium carbonate was added and the aqueous layer was extracted 4× with 100 mL of CH_2Cl_2. The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo, providing the crude reaction product. The crude product was purified via flash chromatography on silica gel (85% hexanes/15% ethyl acetate), providing 772 mg (93%) of triarylbutene **3** as an equimolar mixture of *E* and *Z* stereoisomers as a light-yellow oil. TLC (SiO_2, 3:1 hexanes/ethyl acetate): R_f = 0.27. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.94 (1.5H, t, J = 7.5 Hz, 4 diast. 1), 0.95 (1.5H, t, J = 7.5 Hz, 4 diast. 2), 2.50 (2H, q, J = 7.5 Hz, 3), 3.70 (1.5H, s, −OMe diast. 1), 3.85 (1.5H, s, −OMe diast. 2), 6.48 (1H, d, J = 8.8 Hz, HO−Ph 3 diast. 2), 6.57 (1H, d, J = 8.8 Hz, HO−Ph 3 diast. 1), 6.75 (1H, d, J = 8.8 Hz, MeO−Ph 3 diast. 2), 6.79 (1H, d, J = 9.0 Hz, HO−Ph 2 diast. 2), 6.82 (1H, d, J = 8.6 Hz, MeO−Ph 3 diast. 1), 6.90 (1H, d, J = 8.8 Hz, HO−Ph 2 diast. 1), 7.12 (4H, d, J = 8.6 Hz, MeO−Ph 2 and phenyl−ortho), 7.18 (3H, d, J = 8.6 Hz, phenyl−meta, para). HRMS $[M + Na]^+$ calculated 353.1512, found 353.1505.

***E/Z*-1-[4-(2-Bromoethoxy)phenyl]-1-(4-methoxyphenyl)-2-phenylbut-1-ene (4).** To a stirred solution of **3** (676 mg, 2.05 mmol) dissolved in 16.7 mL of 1,2-dibromoethane was

added 18.9 mL of 1 M sodium hydroxide and tetrabutylammonium hydrogen sulfate (646 mg, 1.85 mmol). The biphasic reaction mixture was stirred vigorously at room temperature overnight. After 18 h, TLC revealed complete consumption of the starting material **3**. The reaction was worked up via the addition of 100 mL of CH_2Cl_2 and 100 mL of sodium bicarbonate. The aqueous layer was washed 1× with 100 mL of CH_2Cl_2; the combined organic layers were dried with Na_2SO_4 and concentrated in vacuo to yield the crude product as a light-yellow oil. The material was purified via flash chromatography on silica gel (85% hexanes/15% ethyl acetate), providing 807 mg (90%) of triarylbutene **4** as an equimolar mixture of diastereomers. TLC (SiO_2, 4:1 hexanes/ethyl acetate): R_f = 0.53. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.98 (3H, dt, J = 7.5 Hz, 4), 2.53 (2H, dq, J = 7.5 Hz, 3), 3.57 (1H, t, J = 6.4 Hz, Br−CH_2− diast. 1), 3.68 (1H, t, J = 6.4 Hz, Br−CH_2− diast. 2), 3.71 (1.5H, s, −OMe diast. 1), 3.86 (1.5H, s, −OMe diast. 2), 4.17 (1H, t, J = 6.4 Hz, −O−CH_2− diast. 2), 4.34 (1H, t, J = 6.4 Hz, −O−CH_2− diast. 1), 6.59 (2H, d, J = 8.8 Hz, Br−EtO−Ph 3 both diast), 6.82 (2H, dd, J = 8.8, 9.0 Hz, MeO−Ph 3 both diast), 6.93 (2H, dd, J = 8.8 Hz, Br−EtO−Ph 2 both diast), 7.15 (3H, m, phenyl−meta, para), 7.20 (4H, m, J = 8.8 Hz, MeO−Ph 2 and phenyl−ortho). HRMS $[M + Na]^+$ calculated 459.0930, found 459.0935.

***E/Z*-1-[4-(2-Bromoethoxy)phenyl]-1-(4-hydroxyphenyl)-2-phenylbutene (5).** To a stirred solution of triarylbutene **4** (689 mg, 1.58 mmol) in 130 mL of CH_2Cl_2 was added boron tribromide (1.58 mmol) as a 1 M solution in CH_2Cl_2. The reaction mixture was stirred under an inert atmosphere of Ar. The reaction was quenched at 4.5 h when analytical HPLC revealed about 60% formation of desired demethylated product; further reaction resulted in loss of the bromoethyl functional group. The reaction was quenched via the addition of 200 mL of 2 M NaCl. The aqueous phase was extracted 2× with 100 mL of CH_2Cl_2; the combined organic layers were washed 1× with 100 mL of distilled water. The organic phase was concentrated in vacuo to yield the crude product as a light-yellow oil. The material was purified via flash chromatography on silica gel (95% hexanes/5% ethyl acetate to 80% hexanes/20% ethyl acetate), providing 383 mg (57%) of phenol **5** as an equimolar mixture of diastereomers. The remaining starting material was then recycled to achieve a two-reaction yield of 75%. TLC (SiO_2, 4:1 hexanes/ethyl acetate): R_f = 0.23. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.97 (3H, dt, J = 7.5 Hz, 4), 2.52 (2H, dq, J = 7.5 Hz, 3), 3.57 (1H, t, J = 6.4 Hz, Br−CH_2− diast. 1), 3.68 (1H, t, J = 6.4 Hz, Br−CH_2− diast. 2), 4.17 (1H, t, J = 6.4 Hz, −O−CH_2− diast. 2), 4.34 (1H, t, J = 6.4 Hz, −O−CH_2− diast. 1), 6.49 (1H, d, J = 8.6 Hz, HO−Ph 3 diast. 2), 6.59 (1H, d, J = 9.0 Hz, HO−Ph 3 diast. 1), 6.76 (1H, d, J = 8.8 Hz, RO−Ph 3 diast. 2), 6.83 (2H, dd, J = 8.8, 9.0 Hz, HO−Ph 2 both diast.), 6.93 (1H, d, J = 8.8 Hz, RO−Ph 3 diast. 1), 7.14 (4H, m, RO−Ph 2 and phenyl−ortho), 7.20 (3H, m, phenyl−meta, para). HRMS $[M + Na]^+$ calculated 445.0774, found 445.0760.

***E/Z*-1-(4-Hydroxyphenyl)-1-[4-(2-methylaminoethoxy)phenyl]-2-phenylbutene (6).** Bromide **5** (344 mg, 0.81 mmol) was dissolved in 8.1 mL of a 2 M solution of methylamine (16.2 mmol) in THF. The reaction mixture was stirred in a sealed tube for 48 h at 60 °C. After 48 h, analytical HPLC revealed complete consumption of starting material. Reaction workup was accomplished via the addition of 100 mL of CH_2Cl_2. The organic phase was washed 1× with 50 mL of a pH ∼10 Na_2CO_3/$NaHCO_3$ aqueous buffer. The aqueous phase was then extracted 4× with 50 mL of CH_2Cl_2. The combined organics were then dried (Na_2SO_4) and concentrated in vacuo to yield a yellow, oily reaction product. The material was purified via flash chromatography on silica gel (90% chloroform/10% methanol), providing 276 mg (91%) of *E*/*Z*-desmethylhydroxytamoxifen **6** as an equimolar mixture of diastereomers. TLC (SiO_2, 4:1 chloroform/methanol): R_f = 0.21. ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.93 (3H, dt, J = 7.5 Hz, 4), 2.49 (1.5H, s, N−Me diast. 1), 2.51 (2H, dq, J = 7.5 Hz, 3), 2.55 (1.5H, s, N−Me diast. 2), 2.94 (1H, t, J = 5.1 Hz, −N−CH_2− diast. 1), 3.04 (1H, t, J = 5.1 Hz, −N−CH_2− diast. 2), 3.95 (1H, t, J = 5.1 Hz, −O−CH_2− diast. 1), 4.11 (1H, t, J = 5.1 Hz, −O−CH_2− diast. 2), 6.43 (1H, d, J = 8.6 Hz, HO−Ph 3 diast. 2), 6.49 (1H, d, J = 8.8 Hz, HO−Ph 3 diast. 1), 6.67 (1H, d, J = 8.6 Hz, RO−Ph 3 diast. 2), 6.75 (2H, dd, J = 8.4, 8.8 Hz, HO−Ph 2 both diast), 6.83 (1H, d, J = 8.6 Hz, RO−Ph 3 diast. 1), 7.03 (1H, d, J = 8.6 Hz, RO−Ph 2 diast. 2), 7.11 (3H, m, RO−Ph 2 diast. 1 and phenyl−ortho), 7.16 (3H, m, phenyl−meta, para). HRMS $[M + H]^+$ calculated 374.2115, found 374.2113.

4-(2-Bromoethoxy)-4-azatricyclo[5.2.1.0^{2,6}]dec-8-ene-3,5-dione (7a). To a solution of *N*-hydroxynorbornyl dicarboximide (1 g, 5.6 mmol) in 6.8 mL of dry DMF was added triethylamine (2 mL, 14.0 mmol) and 1,2-dibromoethane (2.4 mL, 28.0 mmol). The reaction mixture was stirred at room temperature overnight. At 18 h, TLC revealed consumption of starting material. The reaction workup was achieved via the addition of 200 mL of CH_2Cl_2 and 150 mL of 1 M $NaHCO_3$; the phases were separated, and the aqueous phase was extracted 2× with 100 mL of CH_2Cl_2. The combined organic layers were washed 1× with 100 mL of 3 M NaCl, dried (Na_2SO_4), and concentrated in vacuo. The material was purified via flash chromatography on silica gel (60% hexanes/40% ethyl acetate), providing 1.105 g (69%) of **7a** as a white solid. TLC (SiO_2, 4:1 ethyl acetate/hexanes): R_f = 0.61. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.50 (1H, dbs, 10b), 1.76 (1H, dt, J = 1.8 Hz, 10a), 3.20 (2H, m, 1, 7), 3.42 (2H, m, 2, 6), 3.47 (2H, m, Br−CH_2−), 4.20 (2H, m, −O−CH_2−), 6.15 (2H, t, J = 1.9 Hz, 8, 9). HRMS $[M + Na]^+$ calculated 307.9893, found 307.9890.

4-[2-(2-Bromoethoxy)ethoxy]-4-azatricyclo[5.2.1.0^{2,6}]-dec-8-ene-3,5-dione (7b). To a solution of *N*-hydroxynorbornyl dicarboximide (0.5 g, 2.8 mmol) in 2.8 mL of dry DMF was added triethylamine (1 mL, 7.0 mmol) and 2-bromoethyl ether (1.8 mL, 14.0 mmol). The reaction and workup were performed exactly as described for **7a** above. The material was purified via flash chromatography on silica gel (60% hexanes/40% ethyl acetate), providing 669 mg (72%) of **7b** as a clear, colorless oil. TLC (SiO_2, ethyl acetate): R_f = 0.63. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.48 (1H, dbs, 10b), 1.73 (1H, dt, J = 1.8 Hz, 10a), 3.18 (2H, m, 1, 7), 3.39 (2H, m, 2, 6), 3.45 (2H, m, Br−CH_2−), 3.73 (2H, m, −N−O−CH_2−CH_2−), 3.80 (2H, m, −N−O−CH_2−CH_2−), 4.10 (2H, m, −O−CH_2−CH_2−Br), 6.12 (2H, t, J = 1.9 Hz, 8, 9). HRMS $[M + Na]^+$ calculated 352.0155, found 352.0163.

4-{2-[2-(2-Chloroethoxy)ethoxy]ethoxy}-4-azatricyclo-[5.2.1.0^{2,6}]dec-8-ene-3,5-dione (7c). To a solution of *N*-hydroxynorbornyl dicarboximide (0.5 g, 2.8 mmol) in 2.4 mL of dry DMF was added triethylamine (1 mL, 7.0 mmol) and 1,2-bis(2-chloroethoxy)ethane (2.2 mL, 14.0 mmol). The reaction mixture was stirred at 60 °C overnight. At 18 h, TLC revealed consumption of starting material. The reaction workup was performed exactly as described for **7a** above. The material was purified via flash chromatography on silica gel (50% hexanes/50% ethyl acetate), providing 614 mg (66%) of **7c** as a clear, colorless oil. TLC (SiO_2, ethyl acetate): R_f = 0.59. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.47 (1H, dbs, 10b), 1.71 (1H, dt, J = 1.8 Hz, 10a), 3.16 (2H, m, 1, 7), 3.37 (2H, m, 2, 6), 3.61 (6H, m, −O−CH_2−CH_2−O−CH_2−CH_2−Cl), 3.70 (4H, m, −N−O−CH_2−CH_2−), 4.08 (2H, m, −O−CH_2−CH_2−Cl), 6.10 (2H, t, J = 1.9 Hz, 8, 9). HRMS $[M + Na]^+$ calculated 352.0922, found 352.0924.

***E/Z*-4-{2-[(2-{4-[1-(4-Hydroxyphenyl)-2-phenylbut-1-enyl]phenoxy}ethyl)methylamino]ethoxy}-4-azatricyclo-[5.2.1.0^{2,6}]dec-8-ene-3,5-dione (8a).** A solution of **7a** (99.3 mg, 0.35 mmol) in dry THF (1.5 mL) was added to **6** (86.3 mg, 0.23 mmol). Diisopropylethylamine (60 μL, 0.35 mmol) was added, and the reaction mixture was transferred to a sealed tube and heated to 60 °C for 24 h. Analytical HPLC revealed that the reaction had reached equilibrium with over 90% of the starting material consumed. The reaction workup consisted of dilution into 50 mL of ethyl acetate followed by washing of the organic phase 1× with 50 mL of a pH ∼10 Na_2CO_3/$NaHCO_3$ aqueous buffer. The aqueous phase was extracted 3× with 25 mL of ethyl acetate; the combined organic layers were dried (Na_2SO_4) and concentrated in vacuo. The crude material was purified via flash chromatography on silica gel (90% ethyl acetate/10%

hexanes), providing 90.4 mg (68%) of **8a** as a clear, colorless oil. TLC (SiO_2, 4:1 ethyl acetate/hexanes): $R_f = 0.55$. ^1H NMR (500 MHz, $CDCl_3$): δ 0.92 (3H, dt, $J = 7.3$ Hz, TAM 4), 1.48 (1H, m, 10b), 1.74 (1H, m, 10a), 2.46 (5H, m, TAM 3, N−Me), 2.89 (3H, m, $-CH_2-N-CH_2-CH_2-O-Ar$, $-CH_2-N-CH_2-CH_2-O-Ar$ diast. 1), 2.99 (1H, t, $J = 5.3$ Hz, $-CH_2-N-CH_2-CH_2-O-Ar$ diast. 2), 3.16 (2H, m, 1, 7), 3.40 (2H, m, 2, 6), 3.94 (1H, t, $J = 5.5$ Hz, $-N-O-CH_2-$ diast. 2), 4.11 (3H, m, $Ar-O-CH_2-$, $-N-O-CH_2-$ diast. 1), 6.11 (2H, dt, $J = 2.0$ Hz, 8, 9), 6.45 (2H, dd, $J = 8.8$, 8.6 Hz, RO−Ph 2), 6.71 (2H, dd, $J = 8.8$ Hz, HO−Ph 3), 6.80 (2H, dd, $J = 8.8$, 8.6 Hz, RO−Ph 3), 7.10 (7H, m, HO−Ph 2, phenyl). HRMS $[M + Na]^+$ calculated 601.2673, found 601.2692.

***E/Z*-4-{2-{2-[(2-{4-[1-(4-Hydroxyphenyl)-2-phenylbut-1-enyl]phenoxy}ethyl)methylamino]ethoxy}ethoxy)-4-azatricyclo[5.2.1.0^{2,6}]dec-8-ene-3,5-dione (8b).** A solution of **7b** (223 mg, 0.6 mmol) in dry THF (4.0 mL) was added to **6** (297 mg, 0.9 mmol). Diisopropylethylamine (157 μL, 0.9 mmol) was added. The reaction and workup were performed exactly as described for **8a** above. The crude material was purified via flash chromatography on silica gel (98:2 to 100:0 ethyl acetate/hexanes), providing 205 mg (55%) of **8b** as a light-yellow oil. TLC (SiO_2, 4:1 chloroform/methanol): $R_f = 0.54$. ^1H NMR (500 MHz, $CDCl_3$): δ 0.92 (3H, dt, $J = 7.3$ Hz, TAM 4), 1.45 (1H, m, 10b), 1.73 (1H, m, 10a), 2.46 (5H, m, TAM 3, N−Me), 2.78 (1H, t, $J = 5.6$ Hz, $-CH_2-N-CH_2-CH_2-O-Ar$ diast. 1), 2.85 (1H, t, $J = 5.6$ Hz, $-CH_2-N-CH_2-CH_2-O-Ar$ diast. 2), 2.90 (1H, t, $J = 5.6$ Hz, $-CH_2-N-CH_2-CH_2-O-Ar$ diast. 1), 3.00 (1H, t, $J = 5.6$ Hz, $-CH_2-N-CH_2-CH_2-O-Ar$ diast. 2), 3.15 (2H, m, 1, 7), 3.38 (2H, m, 2, 6), 3.65 (4H, m, $-N-O-CH_2-CH_2-O-CH_2-CH_2-N-$), 3.95 (1H, t, $J = 5.6$ Hz, $-N-O-CH_2-$ diast. 2), 4.08 (3H, m, $Ar-O-CH_2-$, $-N-O-CH_2-$ diast. 1), 6.12 (2H, m, 8. 9), 6.42 (1H, d, $J = 8.7$ Hz, RO−Ph 2 diast. 2), 6.46 (1H, d, $J = 8.7$ Hz, RO−Ph 2 diast. 1), 6.67 (1H, d, $J = 8.7$ Hz, HO−Ph 3 diast. 1), 6.72 (1H, d, $J = 8.9$ Hz, HO−Ph 3 diast. 2), 6.78 (2H, m, RO−Ph 3), 7.10 (7H, m, HO−Ph 2, phenyl). HRMS $[M + H]^+$ calculated 623.3116, found 623.3133.

***E/Z*-4-[2-(2-{2-[(2-{4-[1-(4-Hydroxyphenyl)-2-phenylbut-1-enyl]phenoxy}ethyl)methylamino]ethoxy}ethoxy)-ethoxy]-4-azatricyclo[5.2.1.0^{2,6}]dec-8-ene-3,5-dione (8c).** A solution of **7c** (190 mg, 0.57 mmol) in dry THF (2.5 mL) was added to **6** (143 mg, 0.38 mmol). Diisopropylethylamine (100 μL, 0.57 mmol) and sodium iodide (114 mg, 0.76 mmol) were added, and the reaction mixture was transferred to a sealed tube and heated to 60 °C for 24 h. Analytical HPLC revealed that the reaction had proceeded to 90% consumption of starting material. The reaction workup was performed exactly as describe above for **8a**. The crude material was purified via flash chromatography on silica gel (98% ethyl acetate/2% methanol to 92% ethyl acetate/8% methanol), providing 154 mg (61%) of **8c** as a light-yellow oil. TLC (SiO_2, 4:1 ethyl acetate/methanol): $R_f = 0.14$. ^1H NMR (500 MHz, $CDCl_3$): δ 0.91 (3H, dt, $J = 7.3$ Hz, TAM 4), 1.47 (1H, m, 10b), 1.73 (1H, m, 10a), 2.42 (3H, ds, N−Me), 2.47 (2H, q, $J = 7.3$ Hz, TAM 3), 2.75 (1H, t, $J = 5.7$ Hz, $-CH_2-N-CH_2-CH_2-O-Ar$ diast. 1), 2.81 (1H, t, $J = 5.7$ Hz, $-CH_2-N-CH_2-CH_2-O-Ar$ diast. 2), 2.86 (1H, t, $J = 5.5$ Hz, $-CH_2-N-CH_2-CH_2-O-Ar$ diast. 1), 2.96 (1H, t, $J = 5.5$ Hz, $-CH_2-N-CH_2-CH_2-O-Ar$ diast. 2), 3.15 (2H, m, 1, 7), 3.39 (2H, m, 2, 6), 3.63 (8H, m, $-N-O-CH_2-CH_2-O-CH_2-CH_2-O-CH_2-CH_2-N-$), 3.94 (1H, t, $J = 5.7$ Hz, $-N-O-CH_2-$ diast. 2), 4.10 (3H, m, $Ar-O-CH_2-$, $-N-O-CH_2-$ diast. 1), 6.12 (2H, m, 8, 9), 6.41 (1H, d, $J = 9.0$ Hz, RO−Ph 2 diast. 2), 6.47 (1H, d, $J = 8.8$ Hz, RO−Ph diast. 1), 6.68 (2H, dd, $J = 8.8$, 9.0 Hz, HO−Ph 3), 6.79 (2H, dd, $J = 8.8$, 8.6 Hz, RO−Ph 3), 7.10 (7H, m, HO−Ph 2, phenyl). HRMS $[M + Na]^+$ calculated 689.3197, found 689.3203.

***E/Z*-1-(4-{2-[(2-Aminooxyethyl)methylamino]ethoxy}-phenyl)-1-(4-hydroxyphenyl)-2-phenylbutene (9a).** To a solution **8a** (22.7 mg, 39 μmol) dissolved in 0.5 mL of 95% ethanol was added hydrazine monohydrate (7 μL, 195 μmol). The reaction mixture was transferred to a sealed tube and heated to 60 °C. After 2 h, TLC revealed complete consumption of starting material. The reaction mixture was transferred to a round-bottom flask and concentrated in vacuo. The crude product was purified via flash chromatography on silica gel (90% chloroform/10% methanol), providing 16.9 mg (71%) of **9a** as a clear, colorless oil. TLC (SiO_2, 9:1 chloroform/methanol): $R_f = 0.17$. ^1H NMR (500 MHz, CD_3CN): δ 0.87 (3H, dt, $J = 7.3$ Hz, 4), 2.25 (1.5H, s, N−Me diast. 1), 2.32 (1.5H, s, N−Me diast. 2), 2.42 (2H, dq, $J = 7.3$ Hz, 3), 2.59 (1H, t, $J = 5.7$ Hz, $H_2N-O-CH_2-CH_2-N-CH_2-$ diast. 1), 2.65 (1H, t, $J = 5.7$ Hz, $H_2N-O-CH_2-CH_2-N-CH_2-$ diast. 2), 2.69 (1H, t, $J = 5.9$ Hz, $H_2N-O-CH_2-CH_2-N-CH_2-$ diast. 1), 2.79 (1H, t, $J = 5.9$ Hz, $H_2N-O-CH_2-CH_2-N-CH_2-$ diast. 2), 3.64 (1H, t, $J = 5.7$ Hz, $H_2N-O-CH_2-$ diast. 1), 3.69 (1H, t, $J = 5.7$ Hz, $H_2N-O-CH_2-$ diast. 2), 3.90 (1H, t, $J = 5.9$ Hz, $Ar-O-CH_2-$ diast. 1), 4.06 (1H, t, $J = 5.9$ Hz, $Ar-O-CH_2-$ diast. 2), 6.45 (1H, d, $J = 8.6$ Hz, HO−Ph 3 diast. 2), 6.54 (1H, d, $J = 9.0$ Hz, HO−Ph 3 diast. 1), 6.70 (1H, d, $J = 8.6$ Hz, RO−Ph 3 diast. 2), 6.77 (1H, d, $J = 8.8$ Hz, RO−Ph 3 diast. 1), 6.79 (1H, d, $J = 8.6$ Hz, HO−Ph 2 diast. 2), 6.88 (1H, d, $J = 8.8$ Hz, HO−Ph 2 diast. 1), 7.05 (1H, d, $J = 8.6$ Hz, RO−Ph 2 diast. 2), 7.13 (6H, m, RO−Ph 2 diast. 1, phenyl). HRMS $[M + Na]^+$ calculated 455.2305, found 455.2275.

***E/Z*-1-[4-(2-{[2-(2-Aminooxyethoxy)ethyl]methylamino}-ethoxy)phenyl]-1-(4-hydroxyphenyl)-2-phenylbutene (9b).** To a solution of **8b** (45.7 mg, 73.4 μmol) dissolved in 1.0 mL of 95% ethanol was added hydrazine hydrate (11.5 μL, 367 μmol). The reaction was performed exactly as described for **9a** above. The crude product was purified via flash chromatography on silica gel (98:2 to 92:8 chloroform/methanol), providing 23.5 mg (67%) of **9b** as a clear, colorless oil. TLC (SiO_2, 9:1 chloroform/methanol): $R_f = 0.12$. ^1H NMR (500 MHz, CD_3CN): δ 0.87 (3H, dt, $J = 7.3$ Hz, TAM 4), 2.26 (1.5H, s, N−Me diast. 1), 2.33 (1.5H, s, N−Me diast. 2), 2.42 (2H, dq, $J = 7.3$ Hz, TAM 3), 2.58 (1H, t, $J = 5.8$ Hz, $Ar-O-CH_2-CH_2-N-CH_2-$ diast. 1), 2.64 (1H, t, $J = 5.8$ Hz, $Ar-O-CH_2-CH_2-N-CH_2-$ diast. 2), 2.71 (1H, t, $J = 5.8$ Hz, $Ar-O-CH_2-CH_2-N-CH_2-$ diast. 1), 2.81 (1H, t, $J = 5.8$ Hz, $Ar-O-CH_2-CH_2-N-CH_2-$ diast. 2), 3.52 (4H, m, $-N-O-CH_2-CH_2-O-CH_2-$), 3.66 (2H, m, $-N-O-CH_2-$), 3.90 (1H, t, $J = 5.6$ Hz, $Ar-O-CH_2-$ diast. 2), 4.06 (1H, t, $J = 5.6$ Hz, $Ar-O-CH_2-$ diast. 1), 6.45 (1H, d, $J = 8.5$ Hz, HO−Ph 3 diast. 2), 6.53 (1H, d, $J = 8.7$ Hz, HO−Ph 3 diast. 1), 6.70 (1H, d, $J = 8.5$ Hz, RO−Ph 3 diast. 2), 6.77 (1H, d, $J = 8.7$ Hz, RO−Ph 3 diast. 1), 6.79 (1H, d, $J = 8.5$ Hz, HO−Ph 2 diast. 2), 6.88 (1H, d, $J = 8.7$ Hz, HO−Ph 2 diast. 1), 7.05 (1H, d, $J = 8.5$ Hz, RO−Ph 2 diast. 2), 7.14 (6H, m, RO−Ph 2 diast. 1, phenyl). HRMS $[M + Na]^+$ calculated 499.2567, found 499.2545.

***E/Z*-1-{4-[2-({2-[2-(2-Aminooxyethoxy)ethoxy]ethyl}-methylamino)ethoxy]phenyl}-1-(4-hydroxyphenyl)-2-phenylbutene (9c).** To a solution of **8c** (135 mg, 203 μmol) dissolved in 2.6 mL of 95% ethanol was added hydrazine hydrate (31.8 μL, 1.02 mmol). The reaction was performed exactly as described for **9a** above. The crude product was purified via flash chromatography on silica gel (98:2 to 90:10 chloroform/methanol), providing 78.4 mg (74%) of **9c** as a clear, colorless oil. TLC (SiO_2, 4:1 chloroform/methanol): $R_f = 0.16$. ^1H NMR (500 MHz, CD_3CN): δ 0.87 (3H, dt, $J = 7.3$ Hz, 4), 2.25 (1.5H, s, N−Me diast. 1), 2.32 (1.5 H, s, N−Me diast. 2), 2.42 (2H, dq, $J = 7.3$ Hz, 3), 2.57 (1H, t, $J = 5.7$ Hz, $-O-CH_2-CH_2-N-CH_2-CH_2-O-Ar$ diast. 1), 2.64 (1H, t, $J = 5.9$ Hz, $-O-CH_2-CH_2-N-CH_2-CH_2-O-Ar$ diast. 2), 2.70 (1H, t, $J = 5.9$ Hz, $-O-CH_2-CH_2-N-CH_2-CH_2-O-Ar$ diast. 1), 2.80 (1H, t, $J = 5.7$ Hz, $-O-CH_2-CH_2-N-CH_2-CH_2-O-Ar$ diast. 2), 3.52 (8H, m, $H_2N-O-CH_2-CH_2-O-CH_2-CH_2-O-CH_2-CH_2-N-$), 3.67 (2H, m, $H_2N-O-CH_2-$), 3.89 (1H, t, $J = 5.9$ Hz, $Ar-O-CH_2-$ diast. 1), 4.05 (1H, t, $J = 5.7$ Hz, $Ar-O-CH_2-$ diast. 2), 6.45 (1H, d, $J = 8.8$ Hz, HO−Ph 3 diast. 2), 6.53 (1H, d, $J = 8.8$ Hz, HO−Ph 3 diast. 1), 6.69 (1H, d, $J = 8.8$ Hz, RO−Ph 3 diast. 2), 6.76 (1H, d, $J = 8.8$ Hz, RO−Ph 3 diast. 2), 6.79 (1H, d, $J = 8.6$ Hz, HO−Ph 2 diast. 2), 6.87 (1H, d, $J = 8.6$ Hz, HO−Ph diast. 1), 7.04 (1H, d, $J = 8.6$ Hz, RO−Ph 2 diast. 1), 7.14 (6H, m, RO−Ph 2 diast. 2, phenyl). HRMS $[M + Na]^+$ calculated 543.2829, found 543.2796.

***E/Z*-2-Hydroxy-5-({2-[(2-{4-[1-(4-hydroxyphenyl)-2-phenylbut-1-enyl]phenoxy}ethyl)methylamino]ethoxyimino}-

methyl)benzamide (10a). To a solution of **9a** (33 mg, 76.3 μmol) in 7.6 mL of 95% ethanol was added 5-formylsalicylamide (10 mg, 68.7 μmol). The reaction mixture was stirred vigorously overnight. Analytical HPLC revealed complete consumption of starting material after 18 h. The material was concentrated in vacuo and purified via flash chromatography on silica gel (100:0 to 95:5 chloroform/methanol), providing 32.2 mg (81%) of **10a** as a clear, colorless oil. TLC (SiO_2, 9:1 chloroform/methanol): $R_f = 0.23$. ^{1}H NMR (500 MHz, CD_3CN): δ 0.86 (3H, dt, $J = 7.5$ Hz, TAM 4), 2.30 (1.5H, s, N−Me diast. 1), 2.39 (3.5H, m, N−Me diast. 2, TAM 3), 2.74 (2H, m, =N−O−CH_2−CH_2−N−), 2.82 (2H, m, Ar−O−CH_2−CH_2−N−), 3.90 (1H, t, $J = 5.9$ Hz, =N−O−CH_2− diast. 1), 4.06 (1H, t, $J = 5.9$ Hz, =N−O−CH_2− diast. 2), 4.17 (1H, t, $J = 5.7$ Hz, Ar−O−CH_2− diast. 1), 4.22 (1H, t, $J = 5.7$ Hz, Ar−O−CH_2− diast. 2), 6.44 (1H, d, $J = 8.6$ Hz, HO−Ph 3 diast. 2), 6.52 (1H, d, $J = 8.8$ Hz, HO−Ph 3 diast. 1), 6.68 (1H, d, $J = 8.8$ Hz, RO−Ph 2 diast. 2), 6.73 (1H, d, $J = 8.8$ Hz, RO−Ph 2 diast. 1), 6.79 (1H, d, $J = 8.6$ Hz, HO−Ph 2 diast. 2), 6.86 (1H, d, $J = 8.8$ Hz, HO−Ph 2 diast. 1), 6.92 (1H, dd, $J = 8.6$, 2.2 Hz, SAL 3), 7.10 (7H, m, RO−Ph 3, phenyl), 7.66 (1H, m, SAL 4), 7.81 (1H, dd, $J = 7.3$, 2.0 Hz, SAL 6), 8.00 (0.5H, s, oxime diast. 1), 8.02 (0.5H, s, oxime diast. 2). HRMS $[M + H]^+$ calculated 580.2806, found 580.2837. Degree of purity: HPLC method 2, retention times of 26.1 and 26.5 min, 97.3%; method 3, retention times of 21.4 and 21.7 min, 95.4%.

***E*/*Z*-2-Hydroxy-5-[(2-{2-[(2-{4-[1-(4-hydroxyphenyl)-2-phenylbut-1-enyl]phenoxy}ethyl)methylamino]ethoxy}-ethoxyimino)methyl]benzamide (10b).** To a solution of **9b** (35 mg, 73 μmol) in 7.3 mL of 95% ethanol was added 5-formylsalicylamide (10.9 mg, 66 μmol). The reaction was performed exactly as described for **10a** above. The crude product was purified via flash chromatography on silica gel (100:0 to 90:10 chloroform/methanol), providing 33.1 mg (72%) of **10b** as a clear, colorless oil. TLC (SiO_2, 9:1 chloroform/methanol): $R_f = 0.16$. ^{1}H NMR (500 MHz, CD_3CN): δ 0.86 (3H, dt, $J = 7.5$ Hz, TAM 4), 2.26 (1.5H, s, N−Me diast. 1), 2.33 (1.5H, s, N−Me diast. 2), 2.40 (2H, dq, $J = 7.5$ Hz, TAM 3), 2.59 (1H, t, $J = 5.9$ Hz, Ar−O−CH_2−CH_2−N−CH_2− diast. 1), 2.65 (1H, t, $J = 5.7$ Hz, Ar−O−CH_2−CH_2−N−CH_2− diast. 2), 2.71 (1H, t, $J = 5.7$ Hz, Ar−O−CH_2−CH_2−N−CH_2− diast. 1), 2.81 (1H, t, $J = 5.9$ Hz, Ar−O−CH_2−CH_2−N−CH_2− diast. 2), 3.52 (1H, t, $J = 5.9$ Hz, =N−O−CH_2−CH_2−O−CH_2− diast. 2), 3.58 (1H, t, $J = 5.7$ Hz, =N−O−CH_2−CH_2−O−CH_2− diast. 1), 3.68 (2H, m, =N−O−CH_2−CH_2−O-), 3.89 (1H, t, $J = 5.7$ Hz, Ar−O−CH_2− diast. 1), 4.05 (1H, t, $J = 5.9$ Hz, Ar−O−CH_2− diast. 2), 4.20 (2H, m, =N−O−CH_2−CH_2−O-), 6.44 (1H, d, $J = 8.6$ Hz, HO−Ph 3 diast. 2), 6.52 (1H, d, $J = 8.8$ Hz, HO−Ph 3 diast. 1), 6.68 (1H, d, $J = 8.6$ Hz, RO−Ph 2 diast. 2), 6.74 (1H, d, $J = 8.8$ Hz, RO−Ph 2 diast. 1), 6.79 (1H, d, $J = 8.6$ Hz, HO−Ph 2 diast. 2), 6.86 (1H, d, $J = 8.8$ Hz, HO−Ph 2 diast. 1), 6.92 (1H, d, $J = 8.8$ Hz, SAL 3), 7.03 (1H, d, $J = 8.8$ Hz, RO−Ph 3 diast. 1), 7.12 (6H, m, RO−Ph 3 diast, 2, phenyl), 7.66 (1H, m, SAL 4), 7.83 (1H, m, SAL 6), 8.02 (0.5H, s, oxime diast. 1), 8.04 (0.5H, s, oxime diast. 2). HRMS $[M + H]^+$ calculated 624.3068, found 624.3023. Degree of purity: HPLC method 2, retention times of 26.8 and 27.2 min, 99.7%; method 3, retention times of 21.7 and 22.0 min, >99.7%.

***E*/*Z*-2-Hydroxy-5-{[2-(2-{2-[(2-{4-[1-(4-hydroxyphenyl)-2-phenylbut-1-enyl]phenoxy}ethyl)methylamino]ethoxy}-ethoxy)ethoxyimino]methyl}benzamide (10c).** To a solution of **9c** (33 mg, 63 μmol) in 6.3 mL of 95% ethanol was added 5-formylsalicylamide (9.4 mg, 57 μmol). The reaction was performed exactly as described for **10a** above. The crude product was purified via flash chromatography on silica gel (100:0 to 95:5 chloroform/methanol), providing 33.5 mg (88%) of **10c** as a clear, colorless oil. TLC (SiO_2, 9:1 chloroform/methanol): $R_f = 0.17$. ^{1}H NMR (500 MHz, CD_3CN): δ 0.89 (3H, t, $J = 7.5$ Hz, TAM 4), 2.28 (1.5H, s, N−Me diast. 1), 2.35 (1.5H, s, N−Me diast. 2), 2.43 (2H, dq, $J = 7.5$ Hz, TAM 3), 2.60 (1H, t, $J = 5.9$ Hz, Ar−O−CH_2−CH_2−N−CH_2− diast. 1), 2.66 (1H, t, $J = 5.7$ Hz, Ar−O−CH_2−CH_2−N−CH_2− diast. 2), 2.73 (1H, t, $J = 5.7$ Hz, Ar−O−CH_2−CH_2−N−CH_2− diast. 1), 2.83 (1H, t, $J = 5.7$ Hz, Ar−O−CH_2−CH_2−N−CH_2− diast. 2), 3.56 (6H, m, =N−O−CH_2−CH_2−O−CH_2−CH_2−O−CH_2−CH_2−N−), 3.72 (2H, m, =N−O−CH_2−CH_2−), 3.92 (1H, t, $J = 5.7$ Hz, Ar−O−CH_2− diast. 1), 4.08 (1H, t, $J = 5.7$ Hz, Ar−O−CH_2− diast. 2), 4.23 (2H, m, =N−O−CH_2−), 6.47 (1H, d, $J = 8.6$ Hz, HO−Ph 3 diast. 2), 6.56 (1H, d, $J = 8.8$ Hz, HO−Ph 3 diast. 1), 6.71 (1H, d, $J = 8.6$ Hz, RO−Ph 2 diast. 2), 6.79 (1H, d, $J = 8.8$ Hz, RO−Ph 2 diast. 1), 6.82 (1H, d, $J = 8.6$ Hz, HO−Ph 2 diast. 2), 6.91 (1H, d, $J = 8.8$ Hz, HO−Ph 2 diast. 1), 6.95 (1H, d, $J = 8.6$ Hz, SAL 3), 7.07 (1H, d, $J = 8.6$ Hz, RO−Ph 3 diast. 1), 7.16 (6H, m, RO−Ph 3 diast. 2, phenyl), 7.70 (1H, m, SAL 4), 7.87 (1H, m, SAL 6), 8.06 (0.5H, s, oxime diast. 1), 8.07 (0.5H, s, oxime diast. 2). HRMS $[M + Na]^+$ calculated 690.3150, found 690.3096. Degree of purity: HPLC method 2, retention times of 27.1 and 27.4 min, 99.4%; method 3, retention times of 21.7 and 22.0 min, >99.7%.

***E*/*Z*-*N*-[2-Hydroxy-5-({2-[(2-{4-[1-(4-hydroxyphenyl)-2-phenylbut-1-enyl]phenoxy}ethyl)methylamino]-ethoxyimino}methyl)benzamide]doxorubicin (11a).** DOX-5-formylsaliform was synthesized on the basis of the procedure previously described for doxsaliform.[21,23] To a solution of 23 mg of 5-formylsalicylamide[45] dissolved in 2 mL of DMF was added 20.8 μL of formalin and 20 mg of doxorubicin hydrochloride. The reaction mixture was stirred at 55 °C for 45 min. Following reaction, the solvent was concentrated in vacuo and the material was purified by preparative HPLC using method 4 and carried forward without further characterization. A solution of DOX-5-formylsaliform acetate salt (4.4 mg, 5.8 μmol) in a mixture of 3.0 mL of 0.5% trifluoroacetic acid in water and 1.5 mL of 95% ethanol was measured for DOX chromophore concentration spectrophotometrically at 480 nm (ϵ = 11 500 L/(mol·cm)). Targeting/tether group **9a** (3.0 mg, 7.0 μmol) was dissolved in 1.5 mL of 95% ethanol and added to the reaction mixture. The reaction mixture was stirred at room temperature for 2 h. The reaction mixture was then filtered with a 4 mm, 0.45 μm HPLC syringe filter (Alltech Associates, Inc., Deerfield, IL) and purified via preparative HPLC. Following each injection, the desired product peaks (both *E* and *Z* isomers) at $t_R = 24.5$ and 24.8 min were collected into a round-bottom flask and 200 μL of glacial acetic acid was added. Following peak collections, the material was concentrated in vacuo, providing 3.4 mg of the acetate salt of **11a** (50%) as a red solid. ^{1}H NMR (400 MHz, CD_3OD): δ 0.86 (3H, dt, $J = 7.4$ Hz, TAM 4), 1.32 (3H, d, $J = 5.5$ Hz, DOX 5′-Me), 2.17 (3H, m, DOX 8 and DOX 2′), 2.41 (3H, m, DOX 2′ and TAM 3), 2.98 (1.5H, s, TAM N−Me), 3.02 (2H, ab, DOX 10), 3.06 (1.5H, s, TAM N−Me), 3.66 (6H, bm, DOX 3′ and −CH_2−N−CH_2−), 3.98 (3H, s, DOX 4-OMe), 4.21 (1H, t, $J = 4.9$ Hz, =N−O−CH_2− diast. 1), 4.30 (1H, bm, DOX 5′), 4.37 (1H, t, $J = 4.9$ Hz, =N−O−CH_2− diast. 2), 4.42 (1H, t, $J = 4.7$ Hz, Ar−O−CH_2− diast. 1), 4.47 (1H, bt, Ar−O−CH_2− diast. 2), 4.70 (4H, m, DOX 14 and −N−CH_2−N−), 4.87 (1H, under CD_3OH, DOX 4′), 5.13 (1H, bs, DOX 7), 5.47 (1H, bs, DOX 1′), 6.37 (1H, d, $J = 8.7$ Hz, TAM HO−Ph 3 diast. 2), 6.57 (3H, m, SAL 3 and TAM HO−Ph 3 diast. 1, TAM RO−Ph 2 diast. 2), 6.73 (2H, m, TAM RO−Ph 2 diast. 1 and TAM HO−Ph 2 diast. 2), 6.94 (3H, m, TAM HO−Ph 2 diast. 1 and TAM RO−Ph 3), 7.08 (5H, m, TAM phenyl), 7.33 (1H, m, SAL 4), 7.52 (1H, d, $J = 8.5$ Hz, DOX 3), 7.85 (4H, m, oxime, SAL 6, DOX 1 and DOX 2). HRMS $[M + H]^+$ calculated 1135.4547, found 1135.4564. Degree of purity: HPLC method 2, retention times of 24.5 and 24.8 min, 98.3%; method 3, retention times of 20.5 and 20.8 min, 98.5%.

***E*/*Z*-*N*-(2-Hydroxy-5-[(2-{2-[(2-{4-[1-(4-hydroxyphenyl)-2-phenylbut-1-enyl]phenoxy}ethyl)methylamino]ethoxy}-ethoxyimino)methyl]benzamide)doxorubicin (11b).** The reaction and purification as described above for **11a** were utilized only substituting targeting/tether group **9b**. Purification provided the acetate salt of **11b** (50%) as a red solid. ^{1}H NMR (400 MHz, CD_3OD): δ 0.86 (3H, dt, $J = 7.3$ Hz, TAM 4), 1.33 (3H, d, $J = 6.4$ Hz, DOX 5′-Me), 2.16 (3H, bm, DOX 8 and DOX 2′), 2.42 (3H, m, TAM 3 and DOX 2′), 2.94 (1.5H, s, TAM N−Me diast. 1), 3.01 (1.5H, s, TAM N−Me diast. 2), 3.04 (2H, ab, DOX 10), 3.50 (4H, bm, −CH_2−N−CH_2−), 3.80 (5H, bm, =N−O−CH_2−CH_2−O−CH_2−CH_2−N− and DOX 3′), 3.97

(3H, s, DOX 4′-OMe), 4.18 (2H, m, =N−O−CH_2−), 4.23 (1H, bt, TAM Ar−O−CH_2− diast. 1), 4.31 (1H, bq, DOX 5′), 4.34 (1H, bt, TAM Ar−O−CH_2− diast. 2), 4.70 (4H, ds, DOX 14 and −N−CH_2−N−), 4.94 (1H, under CD_3OH, DOX 4′), 5.13 (1H, bs, DOX 7), 5.48 (1H, bs, DOX 1′), 6.36 (1H, d, J = 8.4 Hz, TAM HO−Ph 3 diast. 2), 6.57 (3H, m, SAL 3 and TAM HO−Ph 3 diast. 1, TAM RO−Ph 2 diast. 2), 6.73 (2H, m, TAM RO−Ph 2 diast. 1 and TAM HO−Ph 2 diast. 2), 6.95 (3H, m, TAM HO−Ph 2 diast. 1 and TAM RO−Ph 3), 7.07 (5H, m, TAM phenyl), 7.29 (1H, m, SAL 4), 7.52 (1H, d, J = 8.5 Hz, DOX 3), 7.70 (1H, ds, oxime), 7.81 (2H, m, DOX 2 and SAL 6), 7.91 (1H, d, J = 7.6 Hz, DOX 1). HRMS $[M + H]^+$ calculated 1179.4809, found 1179.4709. Degree of purity: HPLC method 2, retention times of 24.9 and 25.2 min, >99.5%; method 3, retention times of 20.4 and 20.6 min, 97.8%.

***E/Z-N*-(2-Hydroxy-5-{[2-(2-{2-[(2-{4-[1-(4-hydroxyphenyl)-2-phenylbut-1-enyl]phenoxy}ethyl)methylamino]ethoxy}ethoxy)ethoxyimino]methyl}benzamide)doxorubicin (11c).** The reaction and purification as described above for **11a** were utilized by only substituting targeting/tether group **9c**. Purification provided the acetate salt of **11c** (50%) as a red solid. ^{1}H NMR (400 MHz, CD_3OD): δ 0.86 (3H, t, J = 7.4 Hz, TAM 4), 1.34 (3H, d, J = 6.5 Hz, DOX 5′-Me), 2.14 (2H, m, DOX 8), 2.30 (1H, m, DOX 2′), 2.41 (3H, m, DOX 2′ and TAM 3), 2.94 (1.5H, s, N−Me diast. 1), 3.0 (2H, ab, DOX 10), 3.02 (1.5H, s, N−Me diast. 2), 3.64 (9H, m, =N−O−CH_2−CH_2−O−CH_2−CH_2−O−CH_2−CH_2−N−CH_2−CH_2−O−Ar and DOX 3′), 3.82 (4H, m, =N−O−CH_2−CH_2−O−CH_2−), 3.94 (3H, s, DOX 4′-OMe), 4.13 (2H, bm, =N−O−CH_2−), 4.19 (1H, t, J = 5.0 Hz, Ar−O−CH_2− diast. 1), 4.32 (1H, m, DOX 5′), 4.36 (1H, t, J = 4.9 Hz, Ar−O−CH_2− diast. 2), 4.72 (4H, ds, DOX 14 and −N−CH_2−N−), 4.86 (1H, under CD_3OH, DOX 4′), 5.09 (1H, bs, DOX 7), 5.45 (1H, bs, DOX 1′), 6.37 (1H, d, J = 8.6 Hz, TAM HO−Ph 3 diast. 2), 6.45 (1H, bm, SAL 3), 6.59 (2H, m, TAM HO−Ph 3 diast. 1 and TAM RO−Ph 2 diast. 2), 6.74 (TAM RO−Ph 2 diast. 1 and TAM HO−Ph 2 diast. 2), 6.95 (2H, m, TAM HO−Ph 2 diast. 1 and TAM RO−Ph 3 diast. 2), 7.07 (6H, m, TAM RO−Ph 3 diast. 1 and TAM phenyl), 7.23 (1H, bm, SAL 4), 7.47 (1H, d, J = 8.3 Hz, DOX 3), 7.57 (1H, ds, oxime), 7.66 (1H, bs, SAL 6), 7.70 (1H, bm, DOX 2), 7.84 (1H, bd, DOX 1). HRMS $[M + Na]^+$ calculated 1245.4890, found 1245.4966. Degree of purity: HPLC method 2, retention times of 24.8 and 25.0 min, >99.7%; method 3, retention times of 20.2 and 20.5 min, 98.8%.

***E/Z*-4-Hydroxytamoxifen (12).** Bromide **5** (112 mg, 0.26 mmol) was dissolved in 2.6 mL of a THF solution containing a 2 M concentration of dimethylamine (5.2 mmol). The mixture was transferred to a sealed tube and heated to 60 °C. After 43 h, TLC revealed consumption of starting material **5**. Reaction workup was accomplished via the addition of 30 mL of CH_2Cl_2. The organic phase was extracted 1× with 50 mL of a pH ∼10 Na_2CO_3/$NaHCO_3$ aqueous buffer. The aqueous phase was washed 4× with 15 mL of CH_2Cl_2. The combined organics were then dried (Na_2SO_4) and concentrated in vacuo to yield a yellow, oily reaction product. The material was purified via chromatography on silica gel (95:5 to 90:10 chloroform/methanol), providing 31 mg (31%) of *E*/*Z*-4-hydroxytamoxifen **12**. ^{1}H NMR (500 MHz, $CDCl_3$) established the structure as previously described[44] and indicates >99% purity.

3. Biological Evaluation. Hydrolysis and Stability. The half-life for hydrolysis was determined for the lead compound, DOX-TEG-TAM (**11c**), at 4 and 37 °C. The concentration of a stock solution of DOX-TEG-TAM in DMSO/1% AcOH was found to be 1.2 mM by vis absorption at 480 nm (ϵ = 11 500 L/(mol·cm)). The DOX-TEG-TAM was diluted 1:100 in either pH 7.6 TE buffer (10 mM Tris, 1 mM EDTA buffer) or pH 7.4 lysis buffer (10% v/v glycerol, 10 mM Tris, 1.5 mM EDTA, 10 mM Na_2MoO_4). A sample of each buffer was kept at 37 and 4 °C and monitored by HPLC using method 2 (described above) to track the loss of DOX-TEG-TAM and the subsequent formation of doxorubicin. The area-under-the-curve (AUC) was used to calculate the percentage of intact material versus time. The hydrolysis data were fit to first-order kinetics using the Regression (Blackwell Scientific Publishing, London) software. The reaction rate constants were the following: 0.012 ± 0.0007 min^{-1} (pH 7.6) and 0.0092 ± 0.0003 min^{-1} (pH 7.4) at 37 °C; 0.0058 ± 0.0005 h^{-1} (pH 7.6) and 0.0038 ± 0.0003 h^{-1} (pH 7.5) at 4 °C. The half-life for hydrolysis was then calculated from the rate constants using $t_{1/2}$ = (ln 2)/k.

Estrogen Receptor Binding Assay. The relative binding affinity of each test compound was measured through competition assay with tritiated estradiol (^{3}H-E2) through a procedure adapted from several sources.[50−53] MCF-7 cells were utilized as the ERα source. Cells were cultured in six T-175 flasks to 80% confluence, at which time the full RPMI media was replaced with phenol-red-free RPMI media supplemented with 10% dextran-coated charcoal (DCC) stripped fetal calf serum (henceforth referred to as "stripped media"); the cells were cultured for an additional 24 h. Four hours prior to harvesting, the growth medium was replaced with fresh stripped media. To harvest, cells were washed with 10 mL of Hank's balanced salt solution and dissociated from the flasks with 2 mL of trypsin. Trypsinization was quenched with 10 mL of fresh stripped media; cells from six T-175 flasks were combined and pelleted by centrifugation at 300g for 5 min at 25 °C. The supernatant was decanted, the cells were resuspended in 50 mL of stripped media and enumerated with a hemacytometer. The cells were then pelleted again by centrifugation. The supernatant was decanted, and the cells were suspended in pH 7.4 lysis buffer (10% v/v glycerol, 10 mM Tris, 1.5 mM EDTA, 0.5 mM dithiothreitol, 10 mM Na_2MoO_4, 1.0 mM phenylmethylsulfonyl fluoride, supplemented with Complete-Mini protease inhibitors) at 4 °C such that the cell density was 25 million cells per milliliter of lysis buffer. Cells were lysed at 0 °C via sonication with a microtip set at maximum power for 10 cycles of 6 s on followed by 24 s off. The ER-enriched lysate was obtained by ultracentrifugation of the homogenate at 225000g for 45 min at 4 °C. The supernatant was dispensed into 100 μL aliquots and stored at −70 °C. The lysate protein density was measured with a Sigma Diagnostics Total Protein kit; for all experiments the protein density was between 3.1 and 4.0 mg/mL.

Competitive ligands were prepared as 120× stock solutions in DMSO containing 1% acetic acid. Competitor concentrations were determined for ligands containing the DOX chromophore by optical density at 480 nm (ϵ = 11 500 L/(mol·cm)), while ligands containing only the salicylamide/triarylbutene chromophore were measured at 280 nm (ϵ = 29 500 L/(mol·cm)). Typically four different concentrations of competitor were prepared as 120× solutions in DMSO containing 1% acetic acid. Tritiated estradiol was prepared as a 120 nM stock solution (120×) in DMSO containing 1% acetic acid. The 120× solutions were then diluted 1:10 in pH 7.6 TE (10 mM Tris, 1 mM EDTA) buffer to provide 12× solutions of ^{3}H-E2 and competitor. Aliquots of cell lysate (100 μL) were thawed at 4 °C; 10 μL of 12× competitor was added, followed by 10 μL of 12× ^{3}H-E2. Total binding was measured by addition of vehicle in the absence of competitor, while nonspecific binding was determined by incubation of ^{3}H-E2 in the presence of 2000× diethylstilbestrol. Reaction lysates were vortexed vigorously and stored at 4 °C for 18 h. Following incubation, unbound steroids were stripped from the lysate through the addition of 280 μL of DCC as a 1% w/v suspension in pH 7.6 TE buffer (10 mM Tris, 1.0 mM EDTA). Following the addition of the DCC, the reaction lysates were vortexed and stored on ice for 15 min, with vortexing every 5 min. DCC was pelleted by centrifugation at 3000g for 10 min at 4 °C; 300 μL of lysate supernatant was transferred to scintillation vials containing 4 mL of Econosafe biodegradable scintillation cocktail. The vials were then vortexed vigorously, and each sample was counted for five repetitions of 3 min counts. This counting protocol was then repeated to ensure reproducibility. Scintillation counting background was subtracted from all measurements. The relative binding affinity (RBA) for each test compound was calculated from the ratio of the molar concentrations of *E*/*Z*-4-OHT and the test compound required to decrease the proportion of specifically bound ^{3}H-E2 by 50%. Scintillation counting was performed in triplicate, and each

competitor was assayed at least in duplicate. Error bars for each determination represent one standard deviation about the mean for scintillation counting statistics.

In Vitro Cellular Growth Inhibition Experiments. The IC_{50} for the targeted formaldehyde conjugates and all control compounds were performed as previously described[21] with minor modifications. All compounds were solubilized in dimethyl sulfoxide containing 1% v/v acetic acid. The concentrations of all 100× DMSO/1% AcOH drug solutions were determined spectrophotometrically by absorbance at 480 nm (ϵ = 11 500 L/(mol·cm)). Drug treatment lasted 4 h, and cells were cultured until the control wells had achieved 80% confluence (typically 4−5 days). For every experiment, each drug level and the controls were performed in hexuplicate; each experiment was performed at least in duplicate. Error bars represent one standard deviation about the mean for the six wells per lane measured for each drug concentration.

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Supporting Information Available: ^{1}H NMR spectra for all isolated intermediates and final products used in the biological evaluation; HPLC data for establishing degree of purity. This material is available free of charge via the Internet at http://pubs.acs.org.

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