Target-Directed Enediynes: Designed Estramycins

Graham B. Jones,*,† George Hynd,† Justin M. Wright,† Ajay Purohit,† Gary W. Plourde II,† Robert S. Huber,‡ Jude E. Mathews,‡ Aiwen Li,‡ Michael W. Kilgore,‡ Glenn J. Bubley,§ Molly Yancisin,|| and Myles A. Brown||

Department of Chemistry, Northeastern University, Boston, Massachusetts 02115, GHS-CU Biomedical Cooperative, Clemson University, Clemson, South Carolina, 29634, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02215, and Dana-Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts 02115

grjones@lynx.neu.edu

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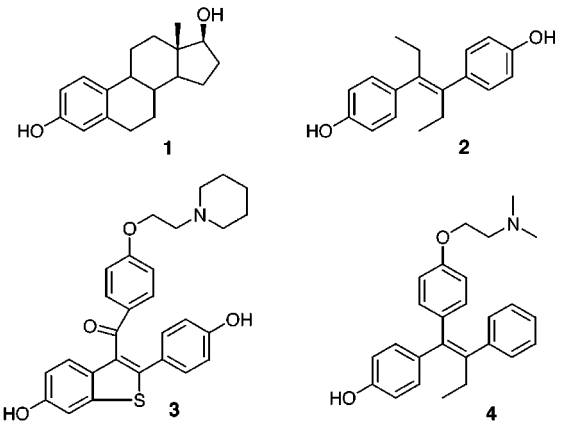
The goal of selective targeting of enediyne cytotoxins has been investigated using estrogenic delivery vehicles. A series of estrogen−enediyne conjugates were assembled, and affinity for human estrogen receptor [hERα] was determined. The most promising candidate induced receptor degradation following Bergman cycloaromatization and caused inhibition of estrogen-induced transcription in T47-D human breast cancer cells.

Introduction

Estrogens regulate cellular events through specific interactions with intracellular receptors (designated ER α and *β*) that function as ligand-inducible transcription factors.[1] In its inactive form, ER is bound to one or more heat shock proteins,[2] which are believed to mediate folding of the protein during translation and aid in the stability of the receptor. After hormone binding to the estrogen receptor, a series of events follow that include the dissociation of heat shock proteins,[3] dimerization,[4] recruitment of coactivators,[5] and finally recognition and binding to specific DNA sequences known as estrogen response elements. Although the exact sequence of these events is the subject of a great deal of investigation, all these events precede the interaction of the complex with the basic transcriptional machinery.[5]

Modulation of estrogen receptor function is of paramount importance for a variety of diseases including breast cancer and osteoporosis. Indeed, it is well-recognized that chemotherapeutic management of breast cancer is most successful when the tumor, which may contain up to 20 000 ERs per cell, is in the estrogen-responsive phase. The development of competitive inhibitors as antiestrogens has been influenced by QSAR analysis of the endogenous agonist estradiol **1** and synthetic estrogens including diethylstilbestrol **2** and its dihydro derivative, hexestrol.[6] It has been noted that an intraphenolic distance of ∼10−11 Å confers potent estrogenic activity. However, introduction of specific (basic) functionality to these frameworks can confer antiestrogenic character, as exemplified by raloxifene **3** and even the tamoxifen metabolite **4**, which act through through a combination of competitive binding and inducing changes in the receptor topology.[7] The recent disclosure of the X-ray crystal structures of **1** and **3** bound to the hormone binding domain of ERα suggested that subtle differences in binding interactions may translate to profound differences in ER mediated physiological effects.[8]



A potential strategy for therapeutic management of breast cancer involves use of cytotoxic antiestrogens, where a chemically reactive functional group is attached

† Northeastern University.
‡ Clemson University.
§ Beth Israel Deaconess Medical Center, Harvard Medical School.
|| Dana-Farber Cancer Institute, Harvard Medical School.

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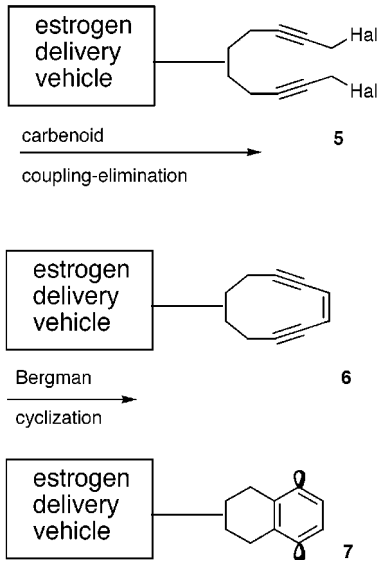
to a carrier substrate that has affinity for ER and can thus potentially accumulate the cytotoxin in an ER-rich cell.[9] In most cases reported to date, the principal target of these agents is the DNA of ER-responsive tumor cells.[9,10] Though clinical candidates have emerged, generally this has been an unsuccessful strategy, largely because of independent cytotoxicity and poor accumulation of the drugs in the desired target cells. These problems are often a consequence of the structural features of the cytotoxic group, which may reduce the overall binding affinity of the hybrid for ER. Subtle changes in lipophilicity often adversely affect ER binding affinity, and there are few chemically reactive functional groups that will not have a pronounced effect on this parameter.[6,9] Guided by these constraints, we sought to incorporate carbocyclic *enediynes* into cytotoxic estrogen hybrids.

Enediynes are one of the most recently discovered classes of antitumor agents and have garnered a great deal of interest over the past decade due to their exceptional cytotoxicity, a consequence of their ability to generate diyl radicals on cycloaromatization of the enediyne core.[11] The presumed target of these diyl radicals is DNA, resulting in single- and double-stranded lesions. However, protein targets have also been identified for specific enediynes, resulting in proteolysis,[12] protein agglomeration,[13] and protein dimerization,[14] and at the molecular level it has been demonstrated that amino acyl radicals are generated when amino acids are exposed to diyl radicals.[15] Harnessing the proteolytic/DNA cleaving capacity of a designed enediyne toward the transcriptional machinery of an ER-rich tumor cell therefore constituted an attractive proposition.[16] A number of tumors have been shown to possess high concentrations of ER including breast cancer, prostatic carcinoma, melanoma, ovarian adenocarcinoma, colon adenocarcinoma, hypernephroma, and endometrial carcinoma. The half-lives of unstrained C-10 monocyclic enediynes is approximately 12−18 h at physiological temperature, making them ideal as thermally generated cytotoxins.[17] Our aim was to assemble conjugates **6** using a method for enediyne synthesis pioneered in this laboratory, which involves an in situ coupling elimination from bis propargylic halides **5** (Scheme 1).[18] Incubation of substrates **6** with ER-rich cells then offers the opportunity to gauge the effects of the 1,4-diyl cycloaromatization products **7** on the transcriptional machinery.[19]

Scheme 1. Design for Enediyne Hybrids



Results and Discussion

Since a common feature of many molecules with affinity for ER is a phenolic group (viz. **1**−**4**), we elected to initially study coupling of the enediyne moiety to the carrier molecule via a phenolic ether linkage. We recently developed a late-stage coupling method for the synthesis of linear and cyclic enediynes[18] and, to avoid thermal decomposition during synthetic manipulation, elected to attach the cyclic enediyne *precursors* to the desired phenol. Using phenol itself as a model study, readily available diyne **8** was converted to free alcohol **9**, coupled under Mitsonobu conditions, and then transformed into propargyl bromide **10** (Scheme 2). Direct conversion to the enediyne **12** was effected using the metallohalocarbenoid coupling−elimination; however, to enable storage of the enediyne, conversion to the corresponding dicobalt hexacarbonyl complex **11** was effected. Liberation of the unmasked enediyne **12** was effected using TBAF,[20] and in the presence of excess 1,4-cyclohexadiene, cycloaromatization to yield **14** took place, presumably via intermediate diyl **13**. The identity of **14** was confirmed by independent synthesis involving Mitsonobu coupling of phenol with 1,2,3,4-tetrahydro-2-naphthalene methanol. Kinetics of the Bergman cycloaromatization were investigated, and it was determined that the $t_{1/2}$ for enediyne **12** is 18 h at 37 °C.

With the model study in place, we turned our efforts toward bona fide estrogen conjugates and selected the readily available bis-phenols hexestrol and diethylstilbestrol (DES). Since our coupling protocol involves mask-

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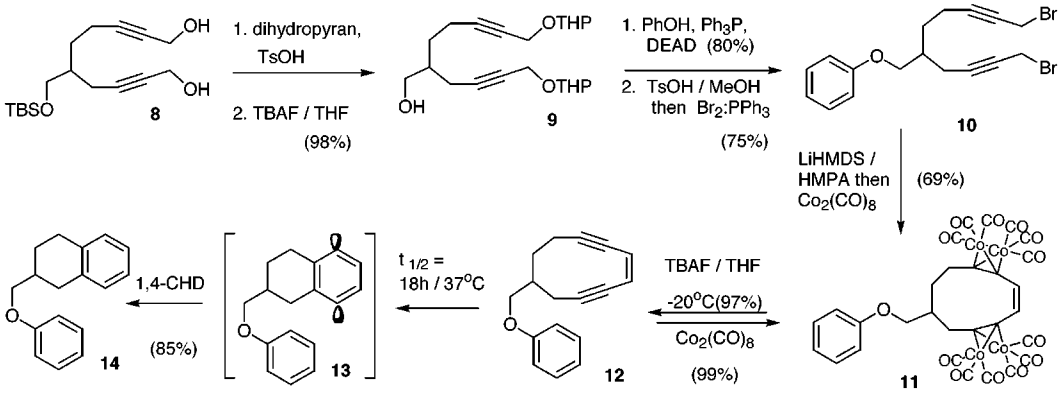
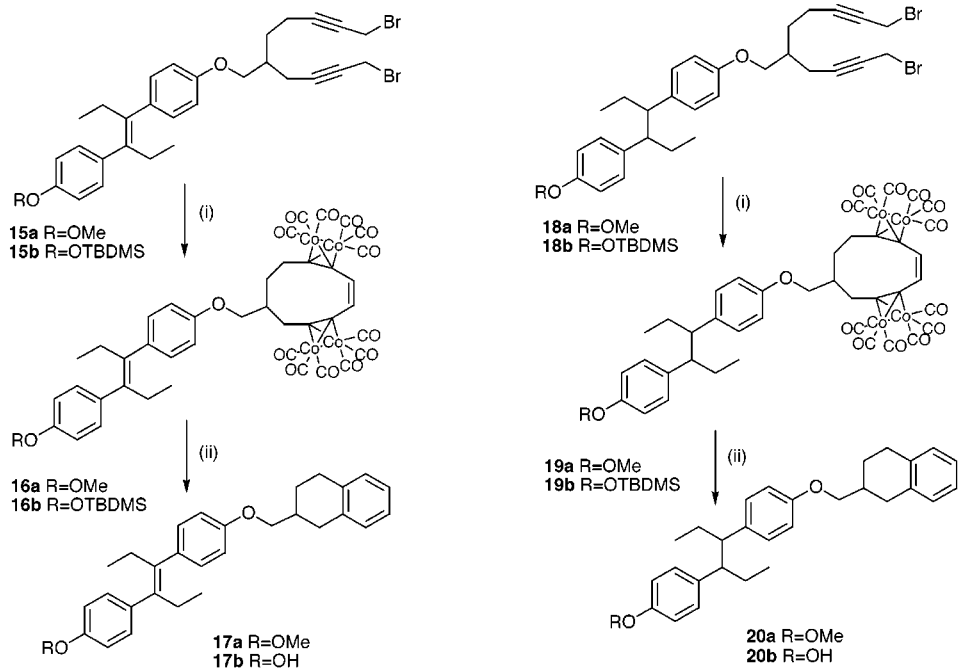
Scheme 2. Model Studies for Phenol-Linked Enediynes



Scheme 3. Preparation and Cycloaromatization of DES and Hexestrol Conjugates[a]



[a] Legend: (i) LiHMDS/HMPA then $Co_2(CO)_8$ (16−81%); (ii) TBAF/THF −20 °C then 1,4-CHD, 37 °C/48 h (84−89%).

ing of one of the two primary binding sites of these estrogens, we also wished to prepare suitable reference compounds for use as control elements in subsequent bioassay. Accordingly, coupling DES with diyne **9** followed by phenolic protection (methyl or TBDMS ether) and subsequent propargylic bromination gave enediyne precursors **15** (Scheme 3). Carbenoid coupling followed by immediate protection of the enediyne core gave **16**, which when unmasked under controlled conditions and the enediyne subjected to cycloaromatization in the presence of 1,4-cyclohexadiene gave adducts **17**. In the case of **16b**, the method of deprotection used facilitated in situ desilation, to give the free phenol **17b**. Similarly, using hexestrol as the phenolic component, bromides **18** were prepared, which gave arenes **20** on cycloaromatization of the derived enediynes (Scheme 3).

Finally, an estradiol conjugate was prepared. This required coupling of dialkyne **9** with commercially available estrone, giving the steroidal diyne **21** following reduction of the 17 keto group (Scheme 4). Protection of the 17β alcohol, liberation of the propargyl alcohols, and subsequent bromination then gave carbenoid precursor **22**. In situ coupling−elimination gave the corresponding enediyne in good yield, isolated as the dicobalt hexacarbonyl complex **23**. Finally, one-pot decomplexation−desilation gave the thermally unstable enediyne, which underwent cycloaromatization and trapping to give arene **24**.

Receptor Binding. To assess the ability of the compounds to recognize and bind to the presumed target, ERα, a competitive binding assay was employed. The assay requires incubation with ligand over extended periods, during which time the enediyne would undergo cycloaromatization. Accordingly, we elected to assay the cycloaromatized products rather than the enediynes themselves. However, since the Bergman cycloaromatization proceeds via a late-stage transition state, the diyl radical intermediates are "product-like", suggesting the arene products are appropriate structural mimics of the diyls. Accordingly, arenes **17**, **20**, and **24** were incubated with recombinant ER in the presence of ^{3}H estradiol, and affinity for ER was calculated using Scatchard analysis.[21] Under the conditions employed (Table 1) affinity for all compounds was disappointingly low, underscoring the

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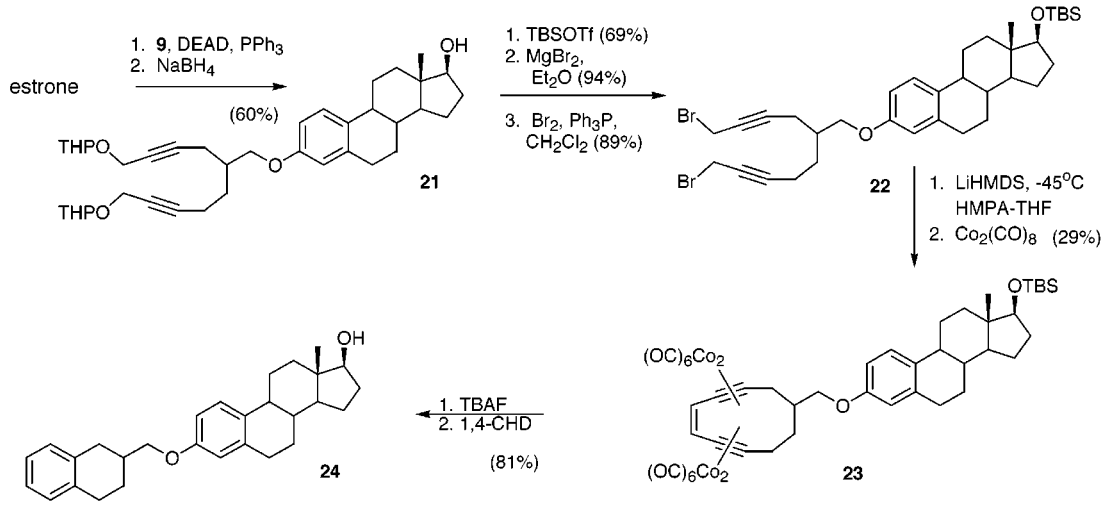
Scheme 4. Preparation of 3-Hydroxy Estradiol Conjugate



Scheme 5. Attempted Route to 17α−Alkynyl Estradiol Conjugate

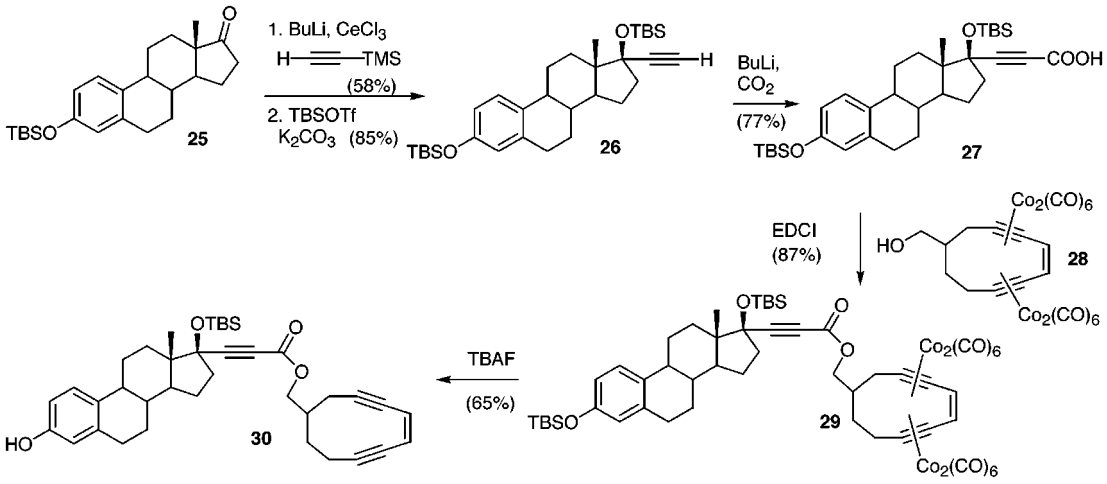


Table 1. Binding Affinity for ERα[a]

compd	RBA/M
1	1.0×10^{-9}
17a	4.3×10^{-4}
17b	2.6×10^{-5}
20a	6.4×10^{-4}
20b	3.1×10^{-5}
24	7.7×10^{-5}
35	5.1×10^{-7}

[a] Candidates/controls + ^{3}H **1** (2.5 nM) incubated with cytosol at 4 °C. Unbound agents removed (DCC) and bound ^{3}H **1** measured by scintillation counter.[21] RBA corresponds to concentration required to reduce ER-bound ^{3}H **1** by 50%.

importance of not only having one but two hydroxy functions available for productive binding interactions with the receptor.

Refinement. Though a multitude of substituted steroid templates are available via direct synthesis, we were particularly interested in commencing from a commercially available building block. Surveying numerous options, it was decided to utilize estrone, introducing the enediyne functionality at the 17α position. Through QSAR analysis it has been shown that 17α *alkynyl* derivatives of estradiol show comparable ER affinity to estradiol,[22] and we decided to construct a hybrid with an alkynyl linker between the enediyne and the steroidal template. Accordingly, the TBDMS ether of estrone **25** was prepared and a 17α alkyne moiety introduced (Scheme 5). A one-pot protection of the 17*β* hydroxy group and deprotection of the alkynyl silyl group was achieved, giving alkyne **26**. To introduce convergence to the synthesis, for the enediyne component, we elected to use a preformed C-10 enediyne, available from our library of existing structures.[18] We opted to couple the steroid and enediyne via an ester linkage, and conversion of **26** to carboxylate **27** was thus conducted. Direct coupling of **27** with the shelf-stable masked enediyne complex **28** was effected, giving the ester hybrid **29** in excellent yield. It was anticipated that global deprotection using TBAF would give the free steroidal enediyne (**33**). However, despite exhaustive efforts, **30** was the only product isolated. Indeed, a variety of alternate methods for deprotection at this (17*β*) position were attempted without success, confirming earlier reports of the problems encountered in deprotection at this position when a 17α substituent is in place.[16a]

Commencing from commercially available ethynyl estradiol **31**, monoprotection followed by carboxylation of the dianion gave alkynoic acid **32** directly (Scheme 6). The triethylsilyl derivative was chosen over the *tert*-butyldimethylsilyl to permit more rapid deprotection.

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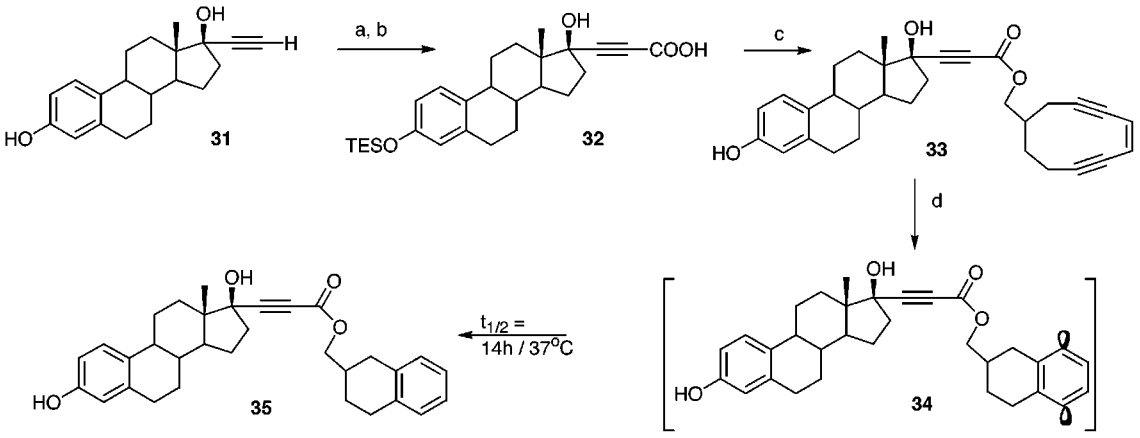
Scheme 6. Modified Route to 17α−Alkynyl Estradiol Candidate[a]



[a] Legend: (a) TESCl (94%); (b) BuLi, CO_2 (69%); (c) EDCI, **28** then TBAF (78%); (d) 1,4-CHD (69%).

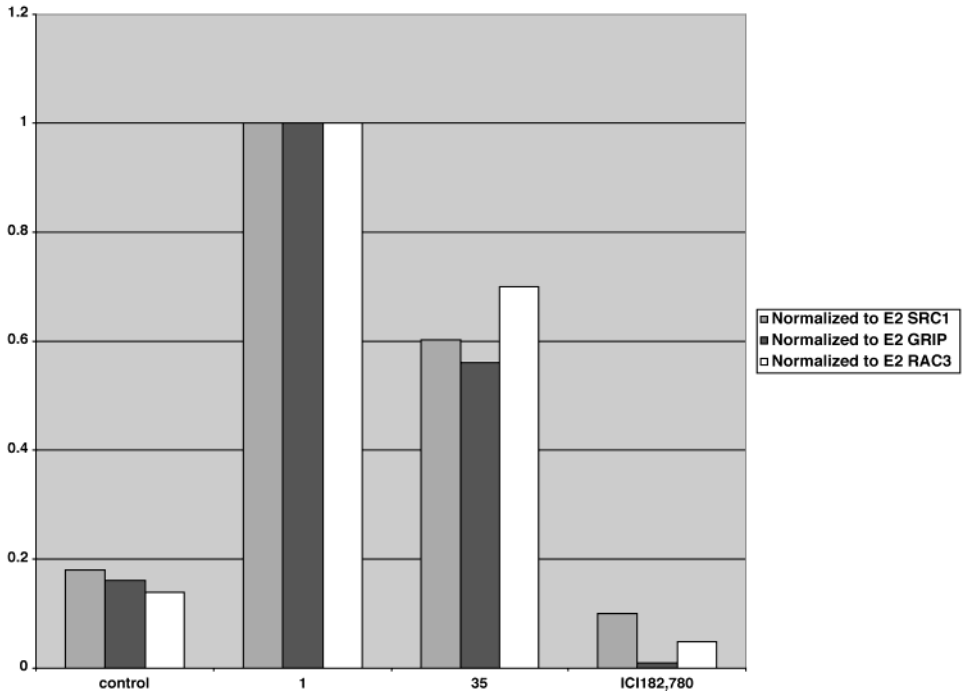


Figure 1. Ability of glutathione-bound ERα to recruit SRC1, GRIP, and RAC3 coactivators in the presence of 10^{-6} M **1**, **35**, or ICI 182,780.

Esterification with cobalt complex **28** followed by mild deprotection gave desired enediyne **33** in good yield, which underwent cycloaromatization to arene **35** $t_{1/2}$ = 14 h/37 °C. Gratifyingly, the binding affinity of arene **35** was in the sub-micromolar range, finally allowing biological evaluation of a relevant estrogen−enediyne conjugate.

Co-Activator Recruitment Assays. To determine the nature of the interaction of **35** (and therefore **33**) with ERα, a recruitment assay was conducted, which assessed the ability of candidate compounds to allow the hormone binding domain of hERα to bind key co-activators. The assay revealed that **35**, like β-estradiol, is capable of promoting recruitment of the SRC1, GRIP1, and RAC3 estrogen receptor co-activator proteins, which are important for formation of estrogenic complexes competent for transcription (Figure 1). This suggests that **35** has estrogen-like properties in contrast to antiestrogens (e.g., ICI 182,780), which inhibit co-activator recruitment.

Receptor Degradation. Our initial assumption was that **33**, when bound to the hERα, may induce proteolysis of either the receptor or its ternary complexes, following Bergman cycloaromatization to **34**. To address this possibility, a freshly prepared sample of **33** was incubated with ^{35}S-labeled full-length hERα at various concentrations for 2 half-lives (36 h) at either 4 or 37 °C, then the protein was separated using SDS−PAGE and visualized using fluorography. The results indicate that the enediyne induces degradation of the receptor (Figure 2, lanes 13−14) and that the process has concentration and temperature-dependent components (lanes 6−7). Control reactions with either estradiol, the antiestrogens 4-hydroxytamoxifen or ICI 182,780,[23] or arene **35** indicate the enediyne−estrogen conjugate is responsible for the degradation, which may imply a proteolytic mechanism. The

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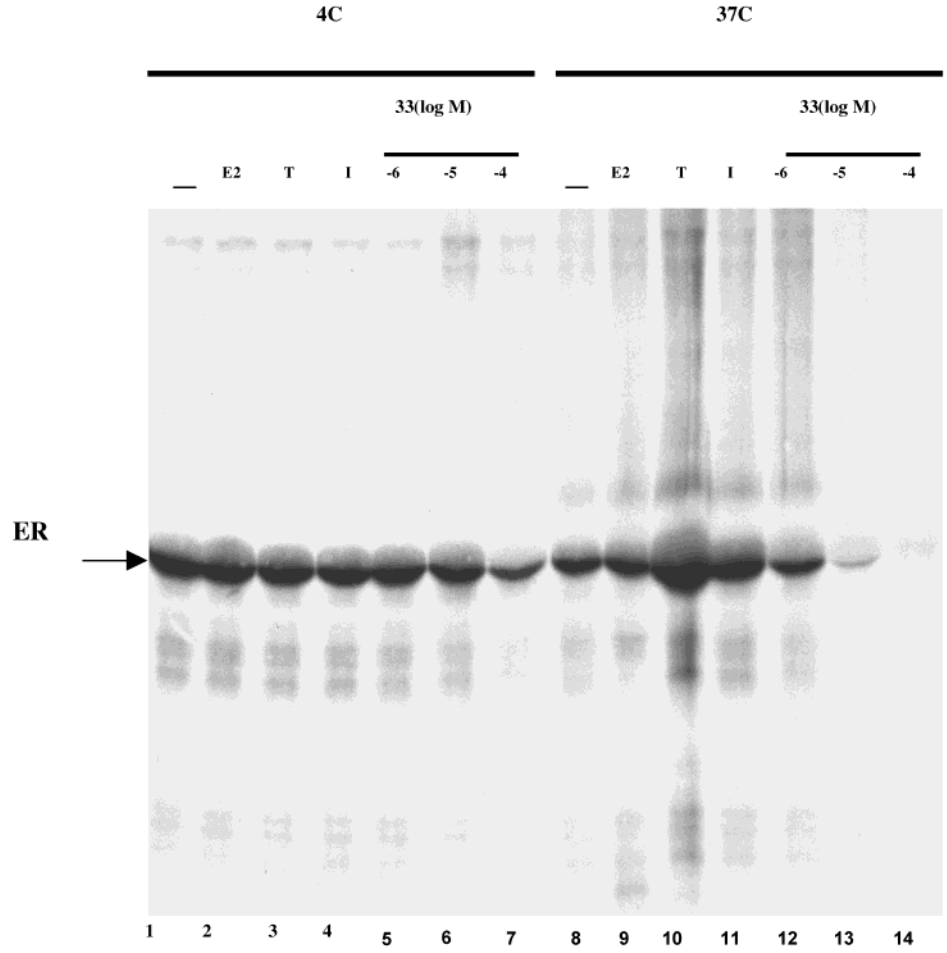

Figure 2. ERα degradation mediated by enediyne **33**·35*S*-methionine-labeled full-length hERα incubated with either ethanol alone (lanes 1 and 8), estradiol (lanes 2 and 9, 10 μM), 4-OH-tamoxifen (lanes 3 and 10, 10 μM), ICI182,780 (lanes 4 and 11, 10 μM) or **33** (lanes 5−7 and 12−14 at concentrations indicated) at either 37 or 4 °C for 36 h.

observation of receptor degradation at 10 μM **33** is encouraging since the binding affinity of **35** for hERα was only 0.51 μM. The efficiency of atom transfer from enediyne-derived 1,4-diyls (e.g., **34**) is often low, typically requiring multiple (>30) equivalents of donor species. Thus, within the context of receptor affinity, the observed degradation is relatively efficient and may encourage other applications of this strategy to be developed. Though no specific fragments were isolated, it is entirely possible that improved analogues can be developed that approach the binding affinity levels observed for natural ligands, including β estradiol, which in turn may improve both the specificity and selectivity of the degradation events. Incubation of the enediyne derived from **16a** (deprotection with TBAF) showed no receptor degradation at 10^{-5} M. Since the RBA of its cycloaromatization product (**17a**) is lower than that of **35** (Table 1), this implies a tentative correlation between receptor affinity and cleavage potential.[24] Intriguingly, receptor degradation induced by **33** was partially attenuated when conducted in the presence of estradiol, though the mechanism of this inhibition is unresolved.[25]

Transcription Inhibition. Since the degradation assay revealed partial decomposition of receptor at micromolar levels, we opted to study the effect of the diyl radical on receptor mediated transcriptional activation. Indeed, this is logical given the fact that the diyl, when generated, is presumably positioned several angstroms remote from the primary binding interactions in the receptor−steroid complex. We thus probed estrogenic activity under the influence of enediyne **33** and the endogenous ligand for hER, estradiol, by employing a luminometric transcriptional activation assay.[26] Specifically, **33** was analyzed for its ability to limit estrogen-induced transcription in T47-D breast cancer cells using an assay procedure that relies on an estrogen response element (ERE), coupled with a luciferase gene, to allow luminometric quantitation of transcription. Controls were employed using cells lacking the response element. In preliminary studies, transfected cells were pretreated for 16 h with enediyne **33** (10^{-7} M) or media only and then treated for 6 h with estradiol (E_2, 10^{-9} M). These analyses (Figure 3) demonstrate that in just 6 h estradiol clearly activates transcription of its target sequence between 2- and 3-fold. Significantly, *pretreating for 16 h with enediyne **33** results in a marked and statistically significant*

(24) Similar results were obtained with the enediyne derived from **19a**.

(25) The degradation of hER using **33** (10^{-5} M, 37 °C) is partially suppressed when assays are conducted in the presence of estradiol (up to 10^{-5} M). At estradiol concentrations of 10^{-6} M, this apparent inhibitory effect is negligible, and no effect is observed at lower concentrations, suggesting the observation is not directly related to displacement. Intriguingly, a similar observation was found with phenol (10^{-4} M), tempting speculation that an electron-transfer process involving phenoxy radicals may be involved.

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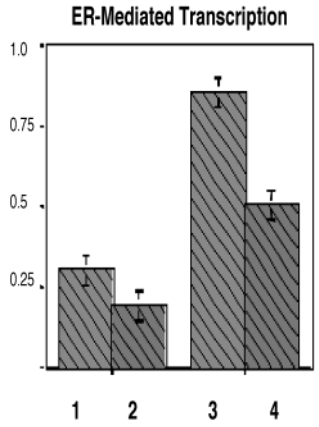

Figure 3. ER transcription in T47-D cells. Data reported as fold induction over transfected control cells (no steroid treatment): 1 = control cells, 16 h; 2 = control + 10^{-7} M enediyne/16 h; 3 = as 1 then + 10^{-9} M **1** /6 h; 4 = as 2 then + 10^{-9} M **1**/6 h. Responses calculated as raw luminometric units (RLU).

Table 2. Growth Inhibition of 33[a]

cells	IC_{50} (μM)
MCF-7	31
MDB-231	48
LNCaP	33
HEK-293	10

[a] Cells split, grown to 50% confluence, then treated with **33** (triplicate, 10^{-3}−10^{-9} M) and ^{3}H thymidine.[28]

reduction in the ability of the cells to respond to subsequent estrogen treatment to initiate transcription. This result indicates that alteration in the ability of the receptor to bind estrogen, recognize and bind response elements on the DNA, or interact with the transcriptional machinery has been significantly impaired by enediyne treatment. While these data do not allow us to interpret the molecular mechanism of this effect, it clearly indicates that the cells ability to transduce the estrogen signal has been altered by preincubation with **33**. Thus, while it is possible that the diyl **34** degrades the actual receptor via a proteolytic mechanism (Figure 2), it may instead impair the fidelity of the transcriptionally active complex via a more subtle mechanism.

Growth Inhibition. The antiproliferative effect of enediyne **33** was assessed using a hERα-rich cell line (MCF-7 human breast cancer), a hERα-deficient cell line (MDB-231), an androgen receptor (AR)-positive cell line (LNCaP), and an AR-negative cell line which is sensitive to cytotoxins (HEK-293).[27] A standard thymidine uptake assay was employed,[28] with a 56 h incubation time, corresponding to 4 half-lives of **33**. Though the IC_{50} values show elevated growth inhibition toward the ER and AR positive cells relative to the ER negative (Table 2), the marked inhibition of the HEK-293 cells suggests an independent mechanism for cytotoxicity. Complicating the issue is the fact that the cycloaromatization product (**35**) is a demonstrated estrogen (Figure 1), which could be expected to stimulate growth of the ER rich cells. Indeed, addition of estradiol (10^{-6} M) followed by **33** (10^{-6} M) resulted in net growth of MCF-7 cells, suggesting that either the estradiol displaces the enediyne from the receptor or interacts with the enediyne to diminish its potency.[25]

Conclusions

The goal of selective targeting of enediyne cytotoxins has been applied to estrogenic delivery vehicles. A general strategy for the synthesis of 17α substituted enediyne derivatives of estradiol has been developed. Binding affinity, transcription inhibition, and estrogen receptor degradation show some correlations, suggesting the possibility of a mode of action involving the transcriptional machinery. On the basis of these preliminary findings, it is now of interest to perform in-depth biological screens and to develop optimized candidates with nanomolar level affinity. The commercial availability of steroid **31** and easy access to cyclic enediynes including **28** will expedite this quest. Refinement of these hybrids may pave the way for more specific cytotoxins, including photoactivated enediyne cores, which may function as photodynamic therapies,[29] or enzyme inhibitors.[30] Perhaps the greatest potential of such enediyne−hormone conjugates is as dynamic probes of transcription factor assembly, an application that presumably could be extended to other members of the nuclear receptor superfamily.

Experimental Procedures[18]

3-Triethylsilyloxy-17-α-ethynyl-*β*-estradiol. Et_3SiCl (1.68 g, 11.13 mmol, 1.87 mL) and imidazole (1.03 g, 15.2 mmol) were added to a solution of *β*-ethynyl estradiol **31** (3.0 g, 10.2 mmol) in anhydrous DMF (25 mL). The resulting solution was stirred for 3 h at 25 °C and then diluted with EtOAc (100 mL) and poured onto cold HCl (1%, 100 mL). The phases were separated and the aqueous layer further extracted with EtOAc (3 × 50 mL). The combined organic extracts were washed with water (3 × 100 mL) followed by saturated $NaHCO_3$ (2 × 100 mL) and saturated aqueous NaCl (1 × 100 mL), dried over Na_2SO_4, and concentrated in vacuo. Chromatography (90:10 hexanes/EtOAc) of the residual oil afforded the title compound (3.9 g, 94%) as a colorless oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.13 (d, J = 8.7 Hz, 1H), 6.63 (dd, J = 8.1, 2.4 Hz, 1H), 6.57 (d, J = 3.0 Hz, 1H) 2.80 (m, 2H), 2.60 (s, 1H), 2.36 (m, 2H), 2.22 (m, 1H), 1.86 (m, 5H), 1.44 (m, 3H), 1.10 (t, J = 7.9 Hz, 9H), 0.99 (s, 3H), 0.74 (m, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.2, 137.8, 132.8, 126.1, 119.8. 117.0, 87.5, 79.9, 74.0, 49.5, 47.1, 43.5, 39.3, 38.9, 32.7, 29.6, 27.2, 26.3, 22.8, 12.7, 6.8, 5.0. Anal. Calcd for $C_{26}H_{38}O_2Si$: C, 76.04; H, 9.33. Found: C, 75.71; H, 9.21.

3-Triethylsilyloxy-17-α-(2-propynoic)-*β*-estradiol (32). *n*-BuLi (19.5 mmol, 7.8 mL of 2.5 M in hexanes) was added dropwise over 10 min at −15 °C to a solution of 3-*O*-triethylsilyloxy-17-α-ethynyl-*β*-estradiol (1.60 g, 3.9 mmol) in THF (20 mL). The mixture was stirred at −15 °C for 0.5 h, and then anhydrous CO_2 (dried by passage through 4 Å molecular sieves and then concentrated H_2SO_4) was bubbled through the solution for 1 h. The mixture was warmed to 25 °C, and CO_2 bubbling continued until the reaction mixture reached dryness. The residue was dissolved in EtOAc (50 mL) and the resulting solution poured onto HCl (1%, 30 mL). The layers were separated, and the aqueous layer was extracted

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with EtOAc (3 × 30 mL). The combined organic extracts were washed with HCl (1%, 2 × 50 mL) and saturated aqueous NaCl, (2 × 50 mL), dried over Na_2SO_4, and concentrated in vacuo. The residual oil was purified via chromatography (30:70−100:0 EtOAc/hexanes) to yield **32** (1.22 g, 69%) as a thick oil: ^{1}H NMR (300 MHz, CD_3OD) δ 7.10 (d, J = 8.1 Hz, 1H), 6.57 (dd, J = 8.1, 2.4 Hz, 1H), 6.51 (d, J = 2.4 Hz, 1H) 2.75 (m, 2H), 2.26 (m, 3H), 1.86 (m, 8H), 1.39 (m, 4H), 0.99 (t, J = 8.1 Hz, 9H), 0.86 (s, 3H), 0.71 (m, 6H); ^{13}C NMR (75 MHz, CD_3OD) δ 156.2, 152.2, 137.5, 133.1, 125.8, 118.9. 118.1, 87.8, 80.8, 74.2, 50.1, 48.2, 44.3, 38.4, 38.2, 32.8, 30.1, 28.2, 27.5, 23.9, 13.7, 7.0, 4.9. Anal. Calcd for $C_{27}H_{38}O_4Si$: C, 71.32; H, 8.42. Found: C, 71.49; H, 8.56.

17α-([5-Cyclodecen-3,7-diynylmethyl]-2-propynoate)-β-estradiol (33). A solution of **32** (0.409 g, 0.899 mmol), EDCI (0.172 g, 0.899 mmol), **28** (0.692 g, 0.899 mmol),[18] and DMAP (0.012 g, 0.0899 mmol) in DMF (10 mL) was stirred at 0 °C for 5 h. The mixture was then poured onto a biphasic mixture of water (15 mL) and EtOAc (15 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (3 × 25 mL). The combined organic extracts were washed with HCl (2%, 2 × 30 mL) and saturated aqueous NaCl (2 × 30 mL), dried over Na_2SO_4, and concentrated in vacuo. The residual oil was immediately dissolved in THF (3 mL) and the solution cooled to 0 °C. Bu_4NF (8.99 mmol, 8.99 mL of 1.0 M in THF) was added dropwise over 10 min, and the mixture was stirred at 0 °C for an additional 1 h and then poured onto a saturated solution of NH_4Cl (3.0 mL). The layers were separated, and the aqueous layer was extracted with Et_2O (3 × 5 mL). The combined organic extracts were washed with HCl (1%, 2 × 5 mL), $NaHCO_3$ (2 × 5 mL), and saturated aqueous NaCl (1 × 5 mL) dried over $MgSO_4$, and concentrated in vacuo. The residual oil was purified via radial chromatography (10:90−50:50 EtOAc/hexanes) to yield **33** (0.339 g, 78%) as a yellow oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.12 (d, J = 8.4 Hz, 1H), 6.62 (dd, J = 8.4, 2.7 Hz, 1H), 6.55 (d, J = 2.4 Hz, 1H), 5.82 (s, 2H), 4.11 (m, 2H), 2.79 (m, 3H), 2.19 (m, 7H), 1.46 (m, 13H), 0.93 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 153.8, 153.5, 138.0, 132.0, 126.4, 123.3, 123.0, 115.1, 112.6, 103.3, 101.4, 91.6, 83.1, 83.0, 79.9, 69.4, 49.7, 47.8, 43.1, 40.6, 39.2, 38.5, 32.9, 32.5, 31.5, 29.5, 27.0, 26.1, 24.7, 22.9, 22.6, 12.6.

17α-[(1,2,3,4-Tetrahydro-2-naphthalenenylmethyl)-2-propynoate]-β-estradiol (35). A solution of enediyne **33** (0.097 g, 0.2 mmol) in 1,4-cyclohexadiene (5 mL) was degassed and then sealed in a 10 mm NMR tube and incubated for 48 h at 37 °C in a constant temperature heating bath. On cooling, the solution was filtered through a silica gel plug and then condensed in vacuo, and the residual oil was purified via radial chromatography (10:90−50:50 EtOAc/hexanes) to yield **35** (0.067 g, 69%) as a thick foam: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.10 (m, 5H), 6.63 (dd, J = 8.1, 2.4 Hz, 1H), 6.56 (d, J = 3.0 Hz, 1H), 4.20 (dd, J = 6.6, 1.2, 2H), 2.86 (m, 4H), 2.31 (m, 6H), 1.57 (m, 13H), 0.93 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.0, 153.4, 138.1, 136.2, 135.1, 132.3, 129.1, 128.9, 126.5, 125.8, 125.7, 115.2, 112.6, 91.0, 80.0, 78.0, 70.2, 55.1, 54.9, 49.8, 47.9, 43.2, 39.3, 33.6, 32.3, 29.5, 28.4, 27.0, 26.2, 25.9, 22.9, 12.7. Anal. Calcd for $C_{32}H_{36}O_4$: C, 79.31, H, 7.49. Found: C, 79.89, H, 7.56. Authenticity of the material was confirmed by independent synthesis, coupling **32** with 1,2,3,4-tetrahydro-2-naphthalenylmethanol followed by deprotection (HF/Py).[34]

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Supporting Information Available: Synthetic procedures and spectroscopic data for the preparation of **8**−**30** and procedures used for bioassay of **33**. This material is available free of charge via the Internet at http://pubs.acs.org.

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