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Protein-Degrading Enediynes: Library Screening of Bergman Cycloaromatization Products

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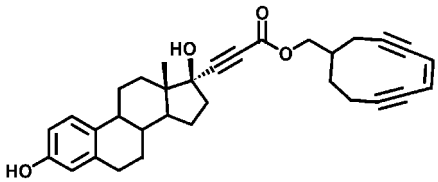
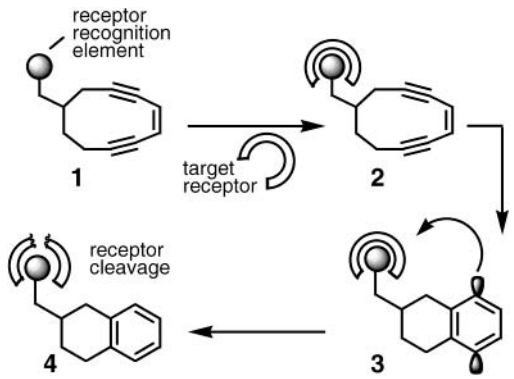
ABSTRACT



A screening method based on Bergman cycloaromatization products was applied to a compact library of estrogenic-enediyne hybrids. An enediyne candidate identified from the screen was subsequently synthesized, and it induced temperature- and concentration-dependent degradation of human estrogen receptor α upon cycloaromatization.

The enediyne antitumor antibiotics are capable of inducing a wide range of biological events, including DNA strand scission, RNA cleavage, protein agglomeration, and apoptosis.[1] Derivatives of both calicheamicin and neocarzinostatin are currently undergoing clinical evaluation, and the challenge of designing synthetic enediyne hybrids continues to attract interest.[2] While the in vitro and in vivo effectiveness of enediynes against certain cancers is unquestioned, the exact mechanism(s) of biological activity remains to be clarified. On the basis of the reported protein-modulating ability of synthetic enediynes,[3] and the recent confirmation that amino acid radicals can be generated from enediynes,[4] we became interested in the possibility of designing enediyne hybrids **1** capable of interacting with specific receptor targets to form **2** (Scheme 1). Such systems could have vast

Scheme 1. General Strategy for Enediyne "Affinity Cleavage Agents"



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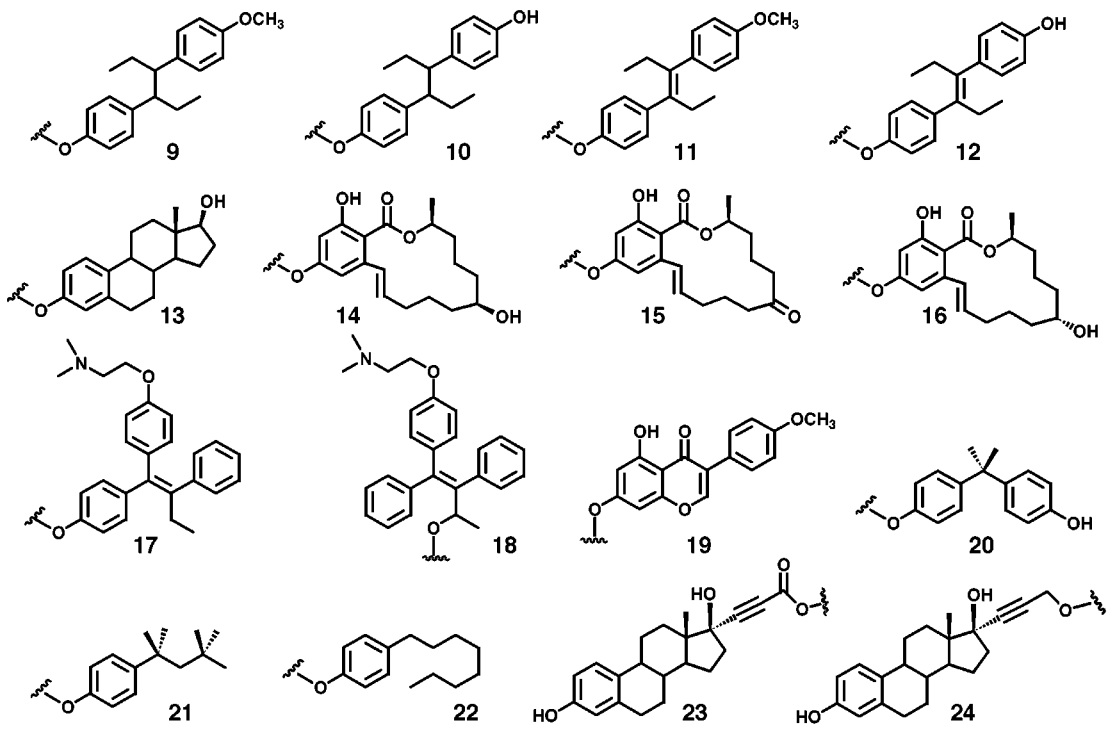
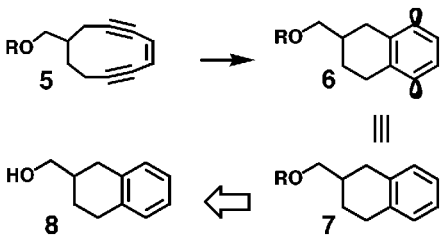


Figure 1. Library of estrogenic probes synthesized **7**, R = **9**−**24**.

potential, interaction of the cycloaromatized diyls with the protein target (**3**) serving either as dynamic probes of protein architecture or as irreversible inhibitors of protein function. Should proteolysis result (**4**), the enediyne would constitute an affinity cleavage system, complementing established methods.[5] An enediyne-based system could offer several advantages in this regard, most importantly that the entire probe is hydrophobic, ideal for receptors whose endogenous ligands are lipophilic, including the nuclear receptor superfamily.[6]

Due to the thermal instability and often sensitive chemistry involved, the synthesis and manipulation of enediynes requires special attention. Accordingly, rather than assembling an *enediyne* library, we wished to outline a rapid screening method for the *identification* of promising candidate compounds, based on their presumed affinity for a target receptor. Since the cycloaromatization of C-10 carbocyclic enediynes **5** (Scheme 2) takes place via a late-stage transition state, the active diyl radicals **6** bear a close structural resemblance to the arene products **7**. A viable strategy could therefore be to couple readily available alcohol **8**[7] with structures having affinity to the receptor of interest and screen for receptor affinity, thus identifying the most promising enediyne candidates for subsequent synthesis. Due to its importance in endocrinological pathways related to cancer, we focused our attention on the human estrogen receptor α(hERα).[8] High affinity ligands identified for this receptor include a wide variety of phenols which mimic the endogenous agonist, *β*-estradiol.[9] Accordingly, **8** was coupled to a library of known ligands, using Mitsonobu coupling methods with the appropriate phenols and alcohols (DEAD, PPh_3, DMF, 12 h/0 °C). Mimics prepared (Figure 1) include the tetrahydronaphthyl ethers of hexestrols and stilbestrols **9**−**12**, *β*-estradiol **13**, zeranols **14**−**16**, hydroxylated tamoxifen derivatives **17** and **18**, biochanin A **19**, and the alkyl phenols **20**−**22**. Analysis of affinity for the ligand-binding

Scheme 2. Post-Bergman Cycloaromatization Products as Diyl Isosteres



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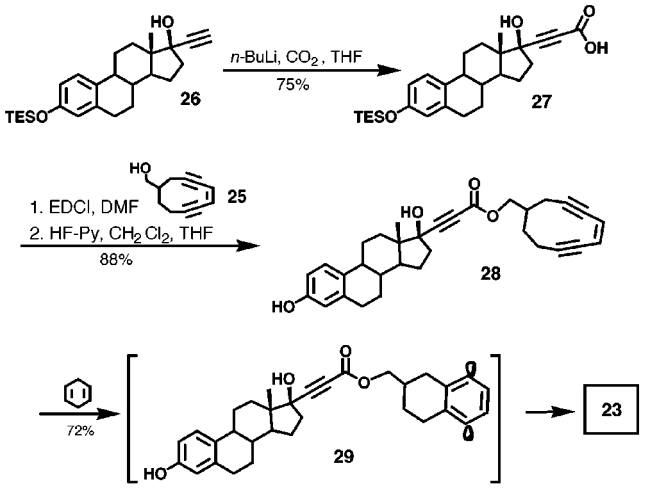
domain of hERα was conducted using a competitive displacement assay,[10] in most cases indicating relatively poor affinity for the receptor. This is presumably due to the reduction in hydrogen bonding capacity relative to the native ligands, two such interactions between β-estradiol and the receptor having been identified.[8] Our attention therefore turned to accessible ligands which possess an enediyne-tethering site *in addition* to diol functionality. It is known that certain 17α-alkynyl steroid derivatives possess comparable binding to the parent steroidal nucleus;[9] thus analogues **23** and **24** were prepared by coupling **8**, with either the alkynyl carboxylate or propargyl alcohol, both of which are readily prepared from commercially available 17α-ethynyl estradiol. Ester **23** and ether **24** both showed sub-micromolar affinity to hERα;[10] however, in the case of **24**, the compound proved unstable, decomposing to a mixture of products on standing for extended periods. Subsequent investigations revealed that **23**, like β-estradiol, is capable of recruiting the AIB1, GRIP1, and RAC3 estrogen receptor coactivator proteins, which are important for formation of estrogenic complexes competent for transcription.[11] For these reasons, the enediyne-estrogen derived from **23** became the candidate for synthesis.[12] The thermally labile enediyne core **25** was prepared in eight steps from commercially available methyl hexynoate, the key enediyne closure utilizing an intramolecular carbenoid coupling reaction.[13] Alkyne **26**, prepared from ethynyl estradiol, was converted to alkynyl carboxylate **27** and immediately coupled with freshly prepared **25** (Scheme 3). Subsequent desilylation gave key enediyne-

Scheme 3. Preparation of Chemically Reactive Estrogen-Enediyne Probe



estrogen **28** in good yield. As expected, in the presence of a hydrogen donor, this enediyne underwent Bergman type cycloaromatization to yield adduct **23** (half-life approximately 15 h at 37 °C), identical in all respects to the authentic material, and underscoring our design philosophy (Scheme 2). We were now able to study the key interaction of diyl **29** with the receptor target. Accordingly, a freshly prepared sample of **28** was incubated with ^{35}S-labeled full length hERα at various concentrations for two half-lives (36 h), and 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

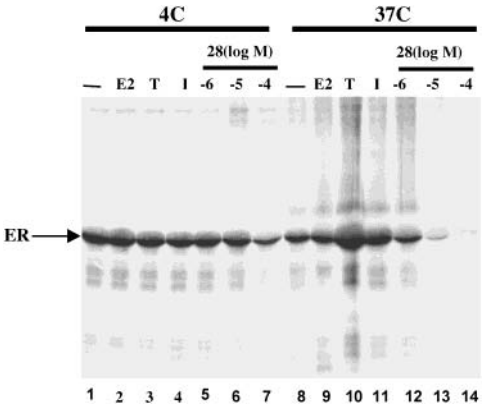


Figure 2. ERα degradation mediated by enediyne **28**· ^{35}S-Methionine-labeled full length hERα incubated with either ethanol alone (lanes 1 and 8), estradiol (lanes 2 and 9, 10 μM), 4-OH-tamoxifen (lanes 3 and 10, 10 μM), ICI182,780 (lanes 4 and 11, 10 μM), and **28** (lanes 5−7 and 12−14 at concentrations indicated) at either 37 °C or 4 °C for 36 h. The samples were resolved (10% SDS-PAGE), fixed, enhanced, dried, and visualized using fluorography. Incubation with **23** or **25** (1 mM) produced no change relative to control (data not shown).

13 and14), and that the process has concentration and temperature-dependent components (lanes 6 and 7). This finding constitutes the first example of targeted protein degradation using a designed enediyne. Control reactions either with estradiol, the antiestrogens 4-hydroxytamoxifen or ICI 182,780,[14] arene **23**, or enediyne **25** indicate the enediyne-estrogen conjugate is responsible for the degradation, which implies a proteolytic mechanism involving diyl **29**.[3,4] *The observation of receptor degradation at micromolar concentration is especially encouraging given the fact that the affinity of **23** for hERα was only in the low micromolar range.* It is thus possible that improved analogues can be found which approach the nanomolar affinity levels observed for natural ligands, including β-estradiol, which in turn may improve both the specificity and selectivity of the degradation event.

(10) Relative binding affinities (RBA's) determined by displacement of ^{3}H estradiol from ligand binding domain of hERα using increasing concentrations (nM through mM) of candidate compounds at 4 °C/37 °C. RBA's of compounds **9**−**22** were all >1 μM, **23** (0.5 μM), and **24** (0.1 μM) relative to estradiol (1 nM). Specific details of the entire screen will be published in a full account of this work.

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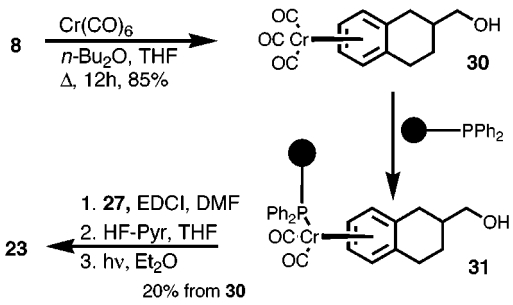
Scheme 4. Polymer-Supported Route to Library



Having demonstrated a screening method based on post-Bergman activated enediynes, it will now be of interest to perform refined screens to find optimized candidates, targeted toward this and other nuclear receptors. In pursuit of this goal, it may be desirable to employ solid-phase synthesis methods, and we elected to investigate the utility of the newly discovered "traceless linker" method.[15] Accordingly, alcohol **8** was converted to tricarbonyl chromium complex **30** and subjected to photolytic ligand exchange with polymer-supported triphenylphosphine to yield adduct **31** (Scheme 4). Esterification with **27** followed by deprotection were successful on the polymer-supported analogue, decomplexation giving a sample of **23**, identical with that prepared using the solution-phase method. We envisage this technology will now permit the rapid assembly of diverse libraries of enediyne mimics. Due to the promising activity of the estrogen enediyne hybrid **28** (Figure 2), we anticipate many applications will be forthcoming.

In summary, a convenient library screening method identified an estrogenic enediyne candidate. Following synthesis, the enediyne-induced temperature- and concentration-dependent degradation of the human estrogen receptor α occurred at micromolar levels,[16] providing the first proof-of-concept for designed protein-targeted enediynes.[17]

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(16) At micromolar levels, degradation of other proteins might also be expected to compete. In a preliminary survey, trace degradation of lysozyme and bovine serum albumin is observed, whereas moderate degradation of the transcription factor FKHR is induced. The scope and selectivity of degradation induced by **28** and refined analogues will be reported in a full account of this work.

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