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Chemical synthesis of hormone receptor probes: high affinity photoactivated enediyne-estrogens

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Abstract—A family of enediyne-estrogens has been prepared, and evaluated for affinity to hER. The optimal ligand has nM affinity, and undergoes photoBergman cycloaromatization.

Steroid hormone receptors are members of a superfamily of nuclear transcription factors which modulate gene activity by both hormone dependent and hormone independent mechanisms.[1] Hormonal response is mediated by the interaction of the ligand with its receptor, which then initiates the binding of the complex to specific regulatory sequences at target genes, referred to as hormone response elements (HRE's). In the case of the human estrogen receptor (hER α/β) distinct subdomains have been characterized including those for transactivation (A, B) DNA binding (C) and hormone binding (E) (Fig. 1), which recognizes the endogenous ligand β-estradiol with nanomolar affinity.[2] Estrogen-mediated pathways are of paramount importance in endocrinology and have a strong correlation with the progression of mammary tumors, and continue to be the focus of numerous studies.[3] On the basis of QSAR analysis, selective antagonists of β-estradiol have been developed including tamoxifen and raloxifene, both of which are competitive antagonists and show efficacy against human breast cancer.[4] A complimentary strategy is the disruption of estrogen biosynthesis, as exemplified by the ketosteroid isomerase inhibitor **1**

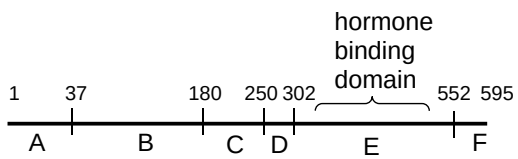
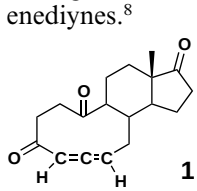


Figure 1. Human ER binding domains.

pioneered by Covey and Robinson.[5] Our own efforts have been directed towards agents with the ability to selectively interfere with the transcriptional activation process, and we recently developed a class of enediyne-estrogens exemplified by the thermally labile ligand **2**.[6] Enediynes and related ene–yne–allenes are thermally activated to produce diyl radicals by a processes referred to as the Bergman or Myers-Saito cyclizations, respectively (Scheme 1).[7] These high energy species are capable of abstracting hydrogen atoms from DNA (causing strand scission) and from proteins (leading to degradation and/or agglomeration). In the case of **2**, though this molecule was effective in inducing temperature dependent degradation of hERα, and of modulating transcription, its affinity for hER was only 10^{-6} M presumably due to the hydrophilic bridging group linking the enediyne to the alkynyl-steroid.[6] Encouraged by this observation, we have been actively pursuing systems with the nM affinity of the endogenous estradiol, and that are thermally stable but can be *activated on demand*. Our strategy was to use affinity [hERα] driven synthesis of either 17α substituted steroidal ene–yne–allenes or enediynes to determine the minimal linker unit coupling the warhead and steroid, avoiding hydrophilic groups which are known to reduce binding affinity. A central feature was to incorporate enediyne building blocks which have been made available through our carbenoid route to linear and cyclic enediynes.[8]



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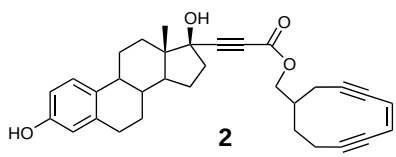

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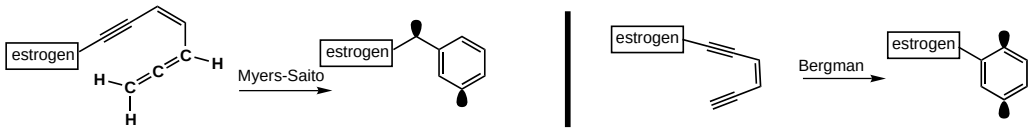


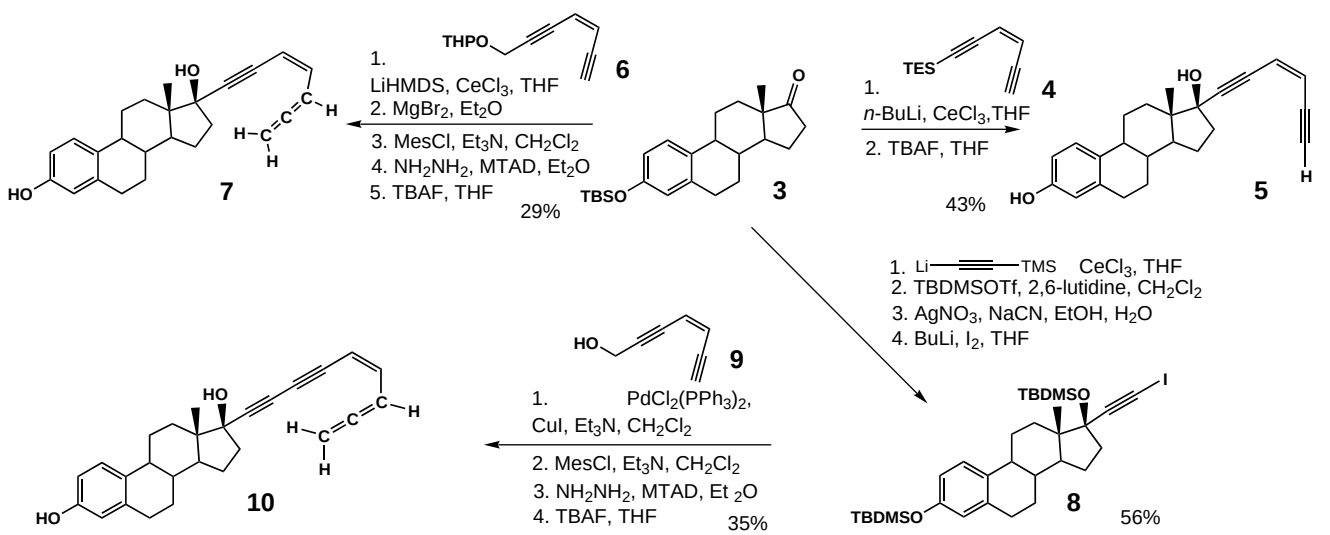
Scheme 1. Possible pathways for generation of proteolytic diradical-estrogen conjugates.

Our first target was enediyne **5**, which was available by coupling the acetylide of monoprotected linear enediyne **4**[8] with estrone derivative **3**, followed by desilylation (Scheme 2). Though compound **5** had enhanced affinity for the estrogen receptor (Table 1), it failed to undergo thermal Bergman cycloaromatization even at elevated temperatures ($\sim$100°C). Prolonged thermolysis resulted in isomerization to the corresponding *E* enediyne, prompting us to search for an alternate diyl progenitor. Accordingly, the ene–yne–allene **7** was pursued. Estrone **3** was coupled with THP ether **6** via its organocerium derivative,[8] the propargylic alcohol exposed and then converted to the corresponding allene,[9] and finally the phenol unmasked to give **7** in moderate yield. As with **5**, this ligand had appreciable affinity for hERα, (Table 1) but also proved stable even after prolonged incubation at physiological temperature and even in refluxing THF. Suspecting that the steroid core may inhibit the (reversible) cycloaromatization process, we opted to install a spacing linker between the two entities and prepared **10**. Coupling of the pre-assembled alkynyl enediyne to **3** proved impossible, warranting synthesis via intermediate iodoalkyne **8**, which was then coupled with enediyne **9**[8] then the allene moiety elaborated. As was the case with **7**, cycloaromatization of **10** could not be induced even under forcing conditions. Intriguingly, prompted by earlier investigations, photochemical cycloaromatization of **10** was attempted, resulting in trace (>10%) conversion to the expected arene product. Encouraged by this finding, we sought to couple a more efficient photoactivated group to the steroid, and based on prior findings in the design of photoproteases, selected a carbocyclic enediyne.[10] Specifically, enediyne **11** which is available via an efficient carbenoid coupling reaction,[10] was coupled to **3** via its organocerium derivative, then unmasked to give **12** (Scheme 3). Unfortunately this compound, which had an RBA of >100 nM, proved stable at physiological temperature, and did not undergo the expected photo-Bergman cycloaromatization even on prolonged irradiation.[11] Following the strategy adopted previously, we elected to insert a spacer unit in the system, and pursued *m*-disubstituted ligand **15**. Coupling of **11** with iodoalkyne **13** followed by 1,2 addition and deprotection gave **15** in good yield. To our surprise, the affinity of **15** was greatly superior to **12**, supporting the notion that the LBD of hERα has considerable tolerance for lipophilic functionality at the

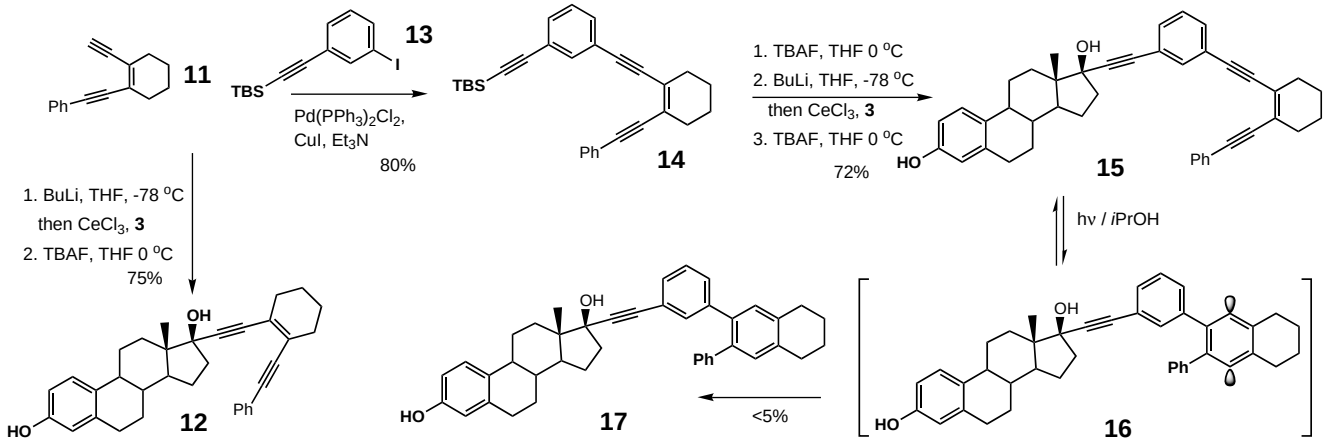
Table 1. Relative binding affinity of steroid–enediynes for hER[a]

Entry	Substrate	RBA (nM)
1	**2**	510
2	**5**	8
3	**7**	45
4	**10**	75
5	**12**	103
6	**15**	14
7	**21**	12

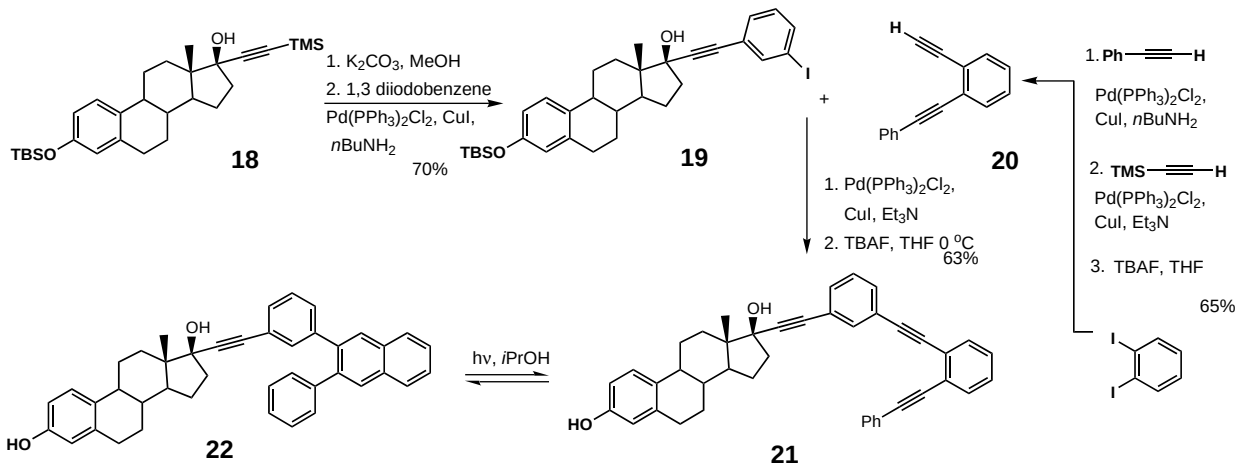
[a] Candidate+^{3}H β-estradiol incubated with cytosol at 4°C. Unbound agents removed (DCC), and bound ^{3}H β-estradiol measured by scintillation counter. RBA corresponds to concentration required to reduce hER bound ^{3}H β-estradiol by 50%.[12,15]



Scheme 2. Alkynyl linked estrogen-enediynes and estrogen–eneyne–allenes.



Scheme 3. High affinity carbocyclic enediyne-estrogens.



Scheme 4. High affinity photoactivated enediyne-estrogens.

steroidal 17α position.[12] Though the binding affinity of **15** was spectacular, to our disappointment its photo-Bergman cycloaromatization profile was poor, giving only trace amounts of the expected product **17** even after prolonged irradiation. We elected to improve the efficiency of the critical process by extending the conjugation of the ligand, and pursued **21** as a refined target. Though this required complete re-synthesis, an efficient process was developed via pre-assembly of enediyne template **20**, in turn available by sequential coupling to 1,2-diiodobenzene (Scheme 4). This was coupled in turn to iodoarene **19**, produced by arylation of the unmasked alkyne derived from **18**, which could be produced either from **3**, or the commercially available 17α-ethynyl estradiol. Though the binding affinity of **21** did not differ markedly from **15**, this ligand underwent smooth photocycloaromatization to give complete conversion to product **22** within 3 h.[13] We have thus succeeded in designing an enediyne-estrogen with nM affinity, and which can be activated on irradiation.[14] Given the promising data obtained with the μM binder **2**, numerous applications of this molecular probe can be envisioned, and we are actively pursuing experiments designed to maximize its potential.

In summary, the convergent synthesis of a photoactivated enediyne-estrogen conjugate has been accomplished. The ligand binds to the ligand binding domain of the human estrogen receptor with low nM affinity, and more importantly, undergoes efficient photo-Bergman cycloaromatization. We anticipate this ligand will serve as a useful probe for the receptor, and may permit site selective modification via diyl radical atom transfer chemistry.[16]

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