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Target Directed Enediyne Prodrugs: Cytotoxic Estrogen Conjugates

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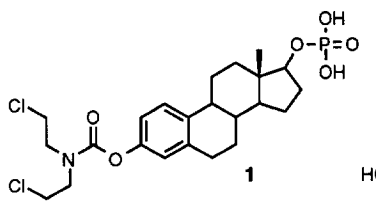
Abstract: Enediyne estrogen conjugates have been prepared from readily available precursors. Enediyne **12** causes significant DNA strand scission at 10^{-3}M, and has demonstrated cytotoxicity against the ER rich MCF-7 human breast cancer cell line. Copyright © 1996 Elsevier Science Ltd

The utilization of hormones as delivery vehicles for cytotoxic agents has long been established.[1] Recent examples, targeted at the estrogen receptor (ER) include estramustine phosphate (NSC-89197) **1**,[2] nitrosourea **2**,[3] and the hexestrol derived agent **3**.[4] The agents were designed to encourage selective accumulation of the drug in estrogen responsive tumors by facilitating transport into these cells, presumably via the ER transporter. In the case of **1** and **2**, once inside the cell, release of the cytotoxic moieties is achieved by hydrolysis of the ester and carbamate groups respectively. Ideally, transport to form a nuclear ER:agent complex would allow release of the cytotoxin to its presumed target, DNA, where alkylation may then inhibit cell proliferation.



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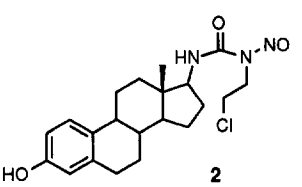

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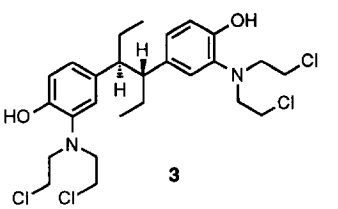

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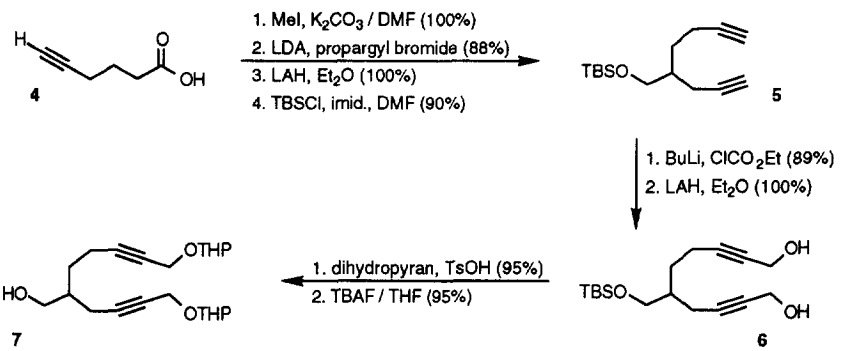
A number of tumors have been shown to possess high concentrations of ER including breast cancer, prostatic carcinoma, melanoma, ovarian adenocarcinoma, colon adenocarcinoma, hypernephroma and endometrial carcinoma.[1] This data makes the area of ER targeted cytotoxins an attractive prospect for drug design, and has been the central feature of a number of investigations.[1]

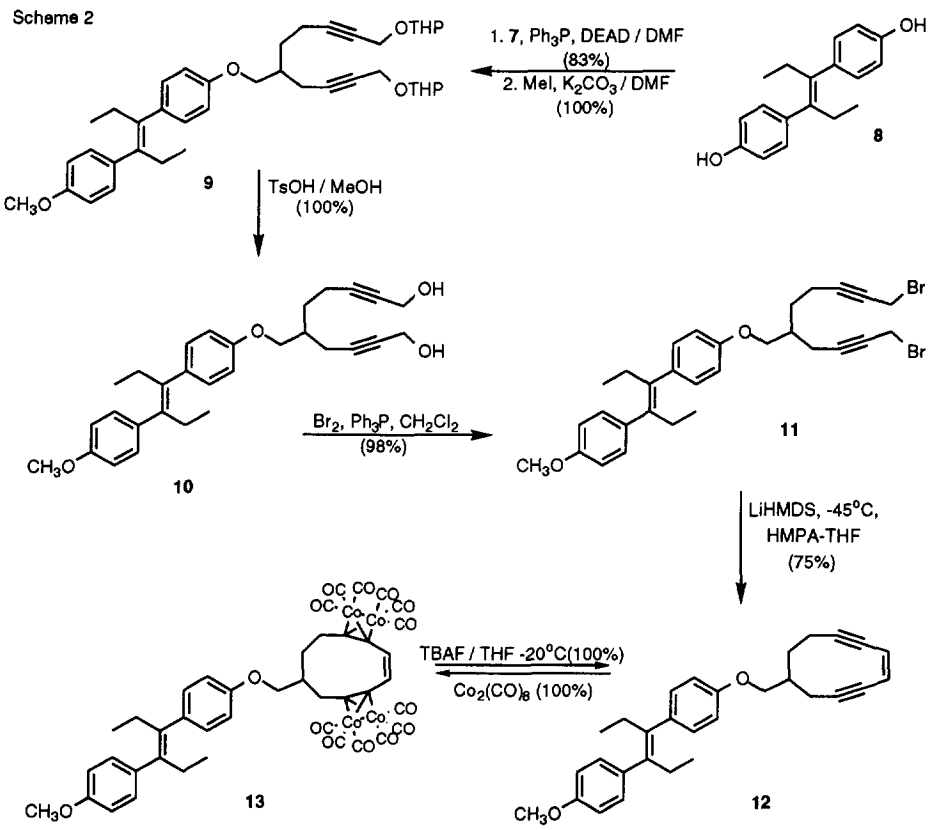
We wished to explore the potential of coupling a bioactive cyclic enediyne[5] function to a delivery vehicle capable of accumulating this cytotoxic group in ER rich cell lines, and envisioned phenolic estrogen agonists to be the most versatile family to test this hypothesis.[1] We recently developed a new synthetic route to linear[6] and cyclic enediynes in this laboratory,[7,8] and wished to extend its versatility by coupling cyclic enediyne *precursors* to the desired phenol. Ultimately, it was decided to employ Mitsonobu coupling of the desired phenol with alcohol **7**, which was prepared in turn from commercially available hexynoic acid **4** (Scheme 1). Esterification followed by propargylation, ester reduction and protection gave bis alkyne **5** in good yield. Chain extension and reduction then gave diol **6**, which was protected, then the silyloxy alcohol was selectively deprotected to give **7** in high overall yield from **4**.

Scheme 1



The Mitsonobu coupling of alcohol **7** with a representative phenolic estrogen was then investigated. We initially wished to demonstrate coupling using the powerful ER agonist diethylstilbestrol (DES) **8**,[1] which proceeded in good yield, and was followed by phenol protection to give **9** (Scheme 2).

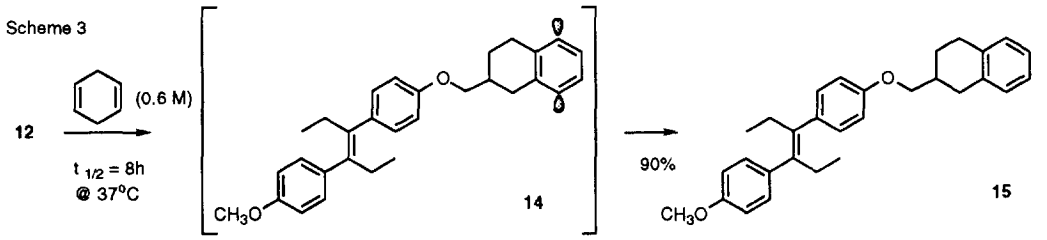
Scheme 2



Mild deprotection of the masked propargyl alcohols yielded **10**, and was followed by bis-bromination, best achieved using triphenylphosphonium bromide (98%) to give **11**. With quantities of the requisite propargylic bromide in hand, we were able to evaluate our intramolecular cyclization-elimination route to enediynes.[7,8] Accordingly, under traditional conditions, smooth conversion to **12** was observed after only 2h at -45°C; for practical purposes the enediyne was immediately protected as the thermally stable bis cobalt carbonyl complex **13**.

Regeneration of **12** from **13** was achieved quantitatively using TBAF in THF (Scheme 2), allowing the chemistry of enediyne-estrogen conjugate **12** to be studied at will. Cyclization of **12** was conducted under controlled conditions (sealed tube, D_6 DMSO, 37°C) which, in the presence of 1,4 cyclohexadiene (20 equiv.) gave conjugate adduct **15** (Scheme 3) in high yield, presumably via the intermediacy of diyl **14**.

Scheme 3



The antiproliferative effect of **12** versus the ER rich MCF-7 human breast cancer cell line was then determined using a standard thymidine uptake assay, with a 24 hr incubation time. The IC_{50} values obtained for enediyne conjugate **12** when compared to its parent enediyne core (cyclodec-3-ene-1,5-diyne)[8] clearly shows that the delivery vehicle contributes substantially to the cytotoxicity of the enediyne moiety (Table 1).[9] Also of note is the relative activity of **12** compared to the established antitumor agent mitomycin C under the assay conditions employed.[9]

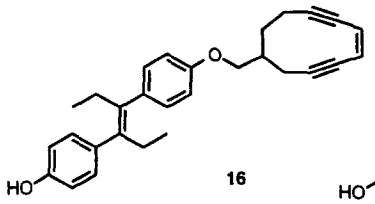
Table 1. Growth Inhibition Assay with MCF-7 breast cancer cells[9,*]

Compound	IC_{50}
12	2.2×10^{-5}
cyclodec-3-ene-1,5-diyne	1.5×10^{-3}
mitomycin C	1.1×10^{-5}

* Cells split, grown to 50% confluence, then treated with candidate compounds (in triplicate, with concentrations 10^{-3} through 10^{-11} M) and ^3H thymidine. Cell growth determined by the relative rates of thymidine incorporation into DNA.

The origin of the cytotoxicity of agent **12** was investigated further. Significant strand scission was only observed when **12** was incubated with ΦX174 RfI DNA at concentrations as high as 10^{-3}M, and this was confined to single stranded cutting events.[10] Though it is tempting to speculate that the target of diyl **14** is indeed DNA, it is also possible that other interactions of the diyl radical with the transcriptional machinery, including the ER hormone binding domain (region E) is occurring, and has become the focus of ongoing research.[11] To assist in these investigations, it becomes desirable to assemble chemical libraries of related enediyne-estrogen conjugates.[12] Thus, using appropriate substrates, and the procedures outlined in Scheme 2, analogs **16-18** were

recently prepared. Full details of the biological studies of these agents and their metabolites will be reported in due course.



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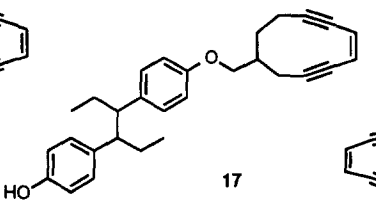

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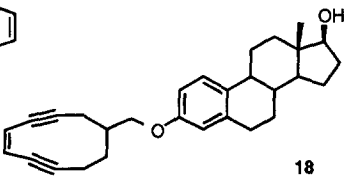

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9. Cells were maintained in phenol red-free medium containing charcoal stripped serum to mitigate any endogenous estrogen effects. Full details of bioassay protocols for this and other related enediynes will be published elsewhere. For details of the thymidine assay see: McConnuaghie, A. W. ; Jenkins, T. C. ; *J. Med. Chem.* **1995**, *38*, 3488.
10. All incubations were performed with ΦX174 Type I DNA (New England Biolabs, 50 μM in base pairs) at pH 8.5 in tris-HCl buffer. Electrophoresis was conducted on a 1% agarose gel and stained with ethidium bromide. Conversion to type II form was evident at agent (**12**) concentration of 50 μM, and pronounced at 500 μM, with traces of type III form evident.
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12. For a recent example of a potentially useful enediyne-steroid hybrid see: Wang, J. ; DeClercq, P. J. ; *Angew. Chem. Intl. Ed.* **1995**, *34*, 1749.

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