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Synthesis of a COMC–estradiol conjugate for targeted, tissue-selective cancer chemotherapy

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ABSTRACT

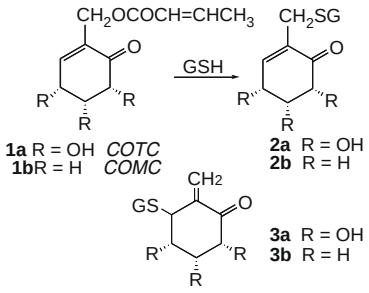
Guided by a revised mechanism of action for the antitumor agent 2-crotonyloxymethyl-(4*R*,5*R*,6*R*)-4,5,6-trihydroxy-2-cyclohexenone (COTC) recently advanced by our laboratory, an estradiol conjugate of the bioactive COTC analog, COMC, has been synthesized.

In 1975, Umezawa et al. described a substance extracted from *Streptomyces griseosporeus* that exhibited cancerostatic activity with low toxicity.[1] Its structure was determined to be 2-crotonyloxymethyl-(4*R*,5*R*,6*R*)-4,5,6-trihydroxycyclohex-2-enone **1a** (COTC, Scheme 1). The antitumor activity of COTC was originally thought to arise from competitive inhibition of the enzyme glyoxalase I by the glutathione (GSH) adduct **2a**. Recently, using the simpler and equally bioactive synthetic analog 2-crotonyloxymethyl-2-cyclohexenone (COMC) **1b**, we showed that neither **1b** nor **2b** inhibited glyoxalase I, and that GSH adduct **2b** was devoid of biological activity.[2]

Since then, our laboratory has advanced an alternative hypothesis for antitumor activity, suggesting that COMC, and by extension COTC, are prodrugs that are activated by an initial human glutathione-*S*-transferase (hGSTP1-1)-dependent Michael addition of GSH to afford the exocyclic enone **3b**.[3,4] Enone **3b** is a potent electrophile and has been shown to react with GSH (to form **2b**), as well as with other nucleophilic groups in proteins and/or in nucleic acids to form adducts structurally analogous to **2**.[5] A related family of GSH-sensitive daunomycin-releasing prodrugs has been developed to overcome drug resistance mechanisms.[6]

This alternative hypothesis postulates that the crotonate ester group functions mainly as a leaving group in COTC and COMC. Consequently, replacement of the crotonate ester with other carboxylate esters might be used to attach a ligand (e.g., a hormone) that could deliver the prodrug to a particular tissue (e.g., via the hormone receptor) prior to activation of the prodrug.

Here we describe a short, stereoselective synthesis of a COMC–estradiol conjugate **4** (Fig. 1) that was designed for selectivity toward tissues expressing the estrogen receptor (ER). Estradiol displays potent affinity for the ER, which is overexpressed in breast, ovary, and gonad tissues.



Scheme 1. Reactivity of COTC and COMC.

The design of conjugate **4** was based on extensive crystallographic analysis of the ER,[7] together with structure–activity studies on estradiol analogs indicating that all four rings, as well as the C-3 and C-17 hydroxyl groups, were important for binding.

Earlier work on estradiol–drug conjugates suggested several possible loci for a linker to the hormone. Linking to C-16, Kuduk et al. synthesized estradiol conjugates **5** and **6** (Fig. 2) of geldanamycin,[8] an ansamycin antibiotic that binds to the Hsp90 molecular chaperone and induces degradation of Hsp90 substrates. Both **5** and **6** were selective in degrading proteins expressing ER (ER and HER2). Conjugates with saturated linkers were inactive.

Alternatively, linking a porphyrin to C-11 of estradiol selectively localized the conjugate in ER-positive breast cancer cell lines for

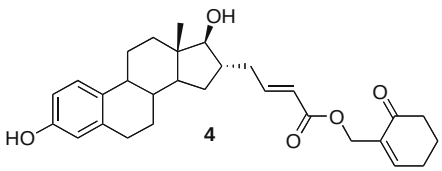


Figure 1. Proposed COMC–estradiol conjugate.

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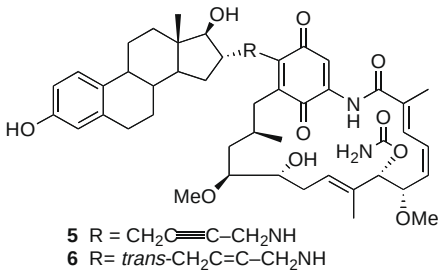
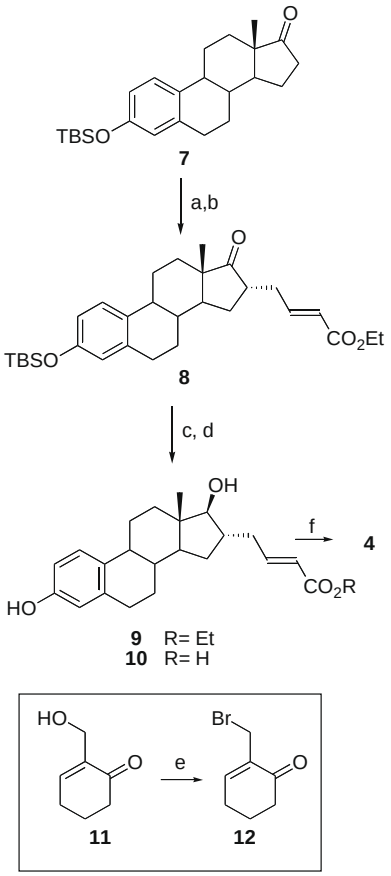


Figure 2. Related estradiol conjugates of geldanamycin.

photodynamic therapy.[9] In the case of paclitaxel, conjugates of estradiol at either C-11 or C-16 displayed weaker anticancer activity than the drug itself.[10]

We concluded that linking COMC to the C-16α position of the hormone was synthetically practical, based on numerous reports of stereoselective alkylations of estrone ethers.[11–13] Moreover, the experience with geldanamycin conjugates **5** and **6** suggested that **4** should exhibit significant ER binding. Scheme 2 depicts the synthetic route to **4**.

The synthesis began with the known[12] estrone *tert*-butyldimethyl silyl ether **7**. Following Katzenellenbogen's procedure, **7** was deprotonated (0.95 equiv of LDA, ⩽−20 °C) and was then alkylated with ethyl 4-bromocrotonate. The desired product **8** was obtained in 70% yield and no C-16β isomer could be detected by ^{1}H NMR. Interestingly, alkylation using 2 equiv of LDA afforded a 5:1 mixture of C-16α and C-16β epimers, with the β-isomer displaying, as expected,[13,14] the more shielded C-18 methyl resonance.



Scheme 2. Synthesis of COMC–estradiol conjugate **4**. Reagents and conditions: (a) LDA (0.95 equiv), THF, 0 °C; (b) $BrCH_2CH{=}CHCO_2Et$, −45 to −20 °C, 12 h, 70%; (c) LAH, −78 °C, 30 min, 37%; (d) 2 M KOH, MeOH, THF, 99%; (e) PBr_3, THF, 0 °C, 1 min, 48%; (f) **12**, CsF-Celite, CH_3CN, acetone, reflux, 2 d, 48%.

After experimenting with several different metal hydrides, chemo- and stereoselective reduction of the ketone function in **8** to the 17β-hydroxyester **9** were best achieved using $LiAlH_4$,[12] which afforded a 9:1 mixture of **9** and the corresponding 17α alcohol in a combined yield of 70%. Pure **9** could be obtained by chromatography and crystallization in 37% yield. When exposed to aqueous KOH in a mixture of methanol and THF, both hydrolysis of the ester group and deprotection of the TBS ether in **9** occurred, quantitatively affording hydroxy acid **10**.

The final stage of the synthesis required esterification of **10** with the known[3] 2-hydroxymethyl-2-cyclohexenone **11**. However, standard methods of direct esterification (carbodiimide; acylimidazole coupling) failed using **11** as the nucleophile. Lee and Choi have reported the preparation of esters from acids and alkyl halides using cesium fluoride on Celite.[15] To implement that approach, alcohol **11** was transformed using PBr_3 into the corresponding bromide **12**. Bromide **12** was a highly reactive electrophile that decomposed with a half-life of ca. 1 h at rt. Despite its instability, reaction of freshly prepared **12** (4 equiv) with **10** in the presence of CsF on Celite (3 equiv) using acetone/acetonitrile (1:8) at reflux furnished the target ester **4** in 48% yield.

The antitumor activity of COMC–estradiol conjugate **4**, as well as toxicological and pharmacological evaluation of its properties in the presence of hGSTP1-1 is currently being investigated. At the clinical level, differential expression of hGSTP1-1 activity in normal cells and tumor cells will likely have a major influence on the differential release of the active agent **3b**, and thus affect the potency and safety profiles that **4** and its congeners may demonstrate in the treatment of tumors.

Acknowledgments

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Supplementary data

Experimental procedures for the synthesis of **4** as well as supporting spectroscopic data. Supplementary data associated with this Letter can be found, in the online version, at doi:10.1016/j.tetlet.2009.03.083.

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