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Synthesis of Chimeric 7α-Substituted Estradiol Derivatives Linked to Cholesterol and Cholesterylamine

Stephen L. Hussey, Enfei He, and Blake R. Peterson*

Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802

brpeters@chem.psu.edu

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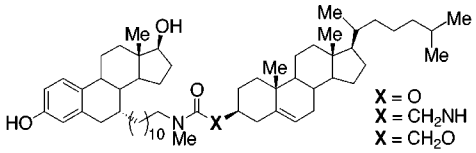
ABSTRACT



We report the synthesis of 7α-substituted *β*-estradiol derivatives bearing side chains terminated with cholesterol and 3*β*-cholesterylamine. These chimeric compounds were designed to exhibit high affinity for estrogen receptors (ERs) and cellular plasma membranes to potentially enable regulated uptake of ERs by mammalian cells. Evaluation with recombinant yeast reporting compound-mediated ER dimerization revealed potencies similar to the antiestrogen ICI 182780. Compounds that efficiently deliver dominant negative ERs into cells may provide novel therapeutics against breast cancers.

Compounds that enable the regulated delivery of small molecules, proteins, and DNA to mammalian cells are critical to the effectiveness of therapeutics and molecular probes.[1] Since macromolecules do not efficiently penetrate cell membranes, the delivery of these compounds to cells is typically mediated by lipids[2−5] or cationic polymers[6−9] that modulate the chemical properties of their cargo prior to addition of complexes or conjugates to cells. Alternatively, cellular membranes can be chemically altered to facilitate macromolecular uptake.[10−12] As an example of this latter approach, we recently reported[13] the synthesis of the fluorescent "memtigen" (membrane-anchored antigen) **1** (Figure 1), which strongly associates with cellular plasma membranes. When added to mammalian cells, **1** efficiently promotes uptake of macromolecular antifluorescein antibodies and associated protein complexes in nearly 100% of viable cells.

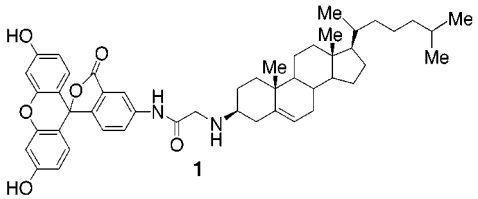


Figure 1. Structure of a previously reported fluorescein−cholesteramine chimera (**1**) that enables uptake of antifluorescein antibodies and associated protein complexes by mammalian cells.

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We report here the synthesis of structurally related 7α-substituted derivatives of β-estradiol (**2**) linked to cholesterol and cholesterylamine. The antiestrogens ICI 182780 (**3**)[14] and ICI 164384 (**4**)[15] (Figure 2) provided models for the

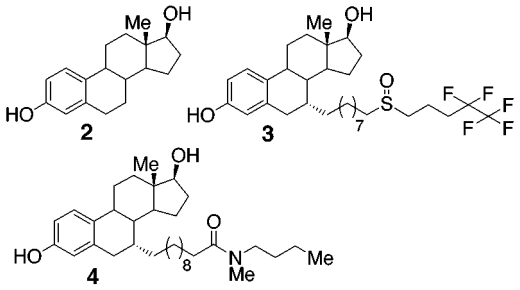


Figure 2. Structures of the estrogenic steroid hormone β-estradiol (**2**), the antiestrogen ICI 182780 (**3**), and the antiestrogen ICI 164384 (**4**).

design of compounds with high affinity for estrogen receptor (ER) proteins. Antiestrogens such as tamoxifen, raloxifene, **3**, and **4**, are employed clinically to treat hormonally responsive breast cancers dependent on estrogens such as **2** to proliferate.[16] The proliferation of these cancers is controlled by ERs α and β, which are transcription factor proteins that regulate gene expression in the cell nucleus.[16−18]

Although antiestrogens **3** and **4** comprise potent competitive antagonists of estrogen receptors,[14,15] most breast cancers eventually become resistant to these types of antihormone therapeutics.[19] As an alternative therapeutic approach, the delivery of transcriptionally altered ERs, termed dominant negative mutants, to breast cancer cells holds promise as a strategy to treat breast cancer.[20] However, current methods of delivery of estrogen receptors are either inefficient or require the use of a recombinant virus, which limits the therapeutic potential of this approach.[20,21]

As a preliminary step directed at investigating whether small molecules might be employed to deliver ERs into mammalian cells, we report here the synthesis of compounds **5**−**8** (Figure 3). Recent X-ray crystal structures of ERβ-bound **4** revealed that whereas the steroid is buried in the protein interior, the amide nitrogen atom of the side chain is solvent-exposed,[22] suggesting that this would be an excellent point of attachment for cholesterol derivatives. Cholesterol (**9**) derivatives were chosen for investigation because cholesterol is an abundant plasma-membrane-associated steroid that controls membrane fluidity[23] and is covalently linked to proteins involved in cellular signaling[24] and synthetic derivatives can enable protein uptake by mammalian cells.[13]

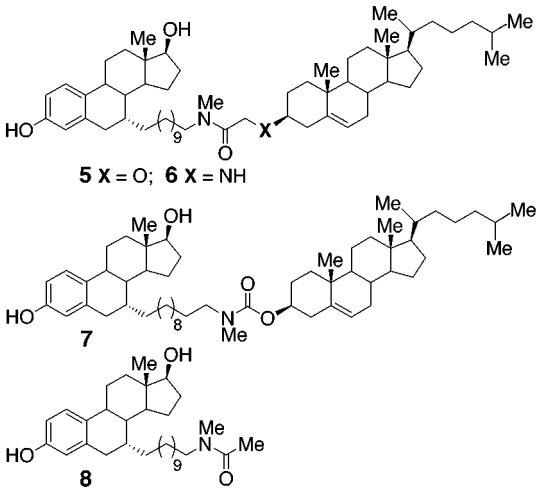
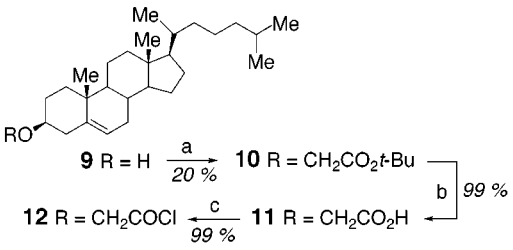


Figure 3. Structures of synthetic targets.

The synthesis of compounds **5**−**7** required cholesterol-derived electrophiles **12**−**14**. The acid chloride **12** was prepared from cholesterol (**9**) in three steps as shown in Scheme 1. As shown in Figure 4, cholesteryl chloroformate (**13**) was commercially available, and the protected cholesterylamine (**14**) was prepared as previously described.[13]

Scheme 1[a]



[a] Reagents and conditions: (a) NaH, *tert*-butylbromoacetate, THF, reflux. (b) HCO_2H, Et_2O, 65 °C. (c) $SOCl_2$, CH_2Cl_2, reflux.

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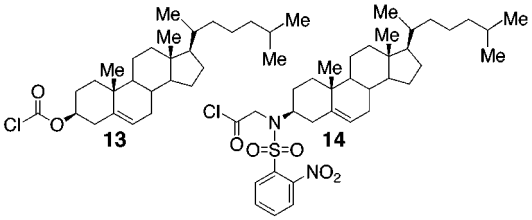
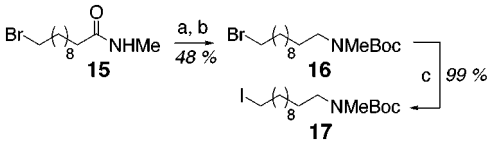


Figure 4. Structures of commercially available cholesteryl chloroformate (**13**) and the previously described acid chloride (**14**).

Steroid side chain precursors were prepared from the known[25] *N*-methylamide **15** as shown in Scheme 2. Reduc-

Scheme 2[a]

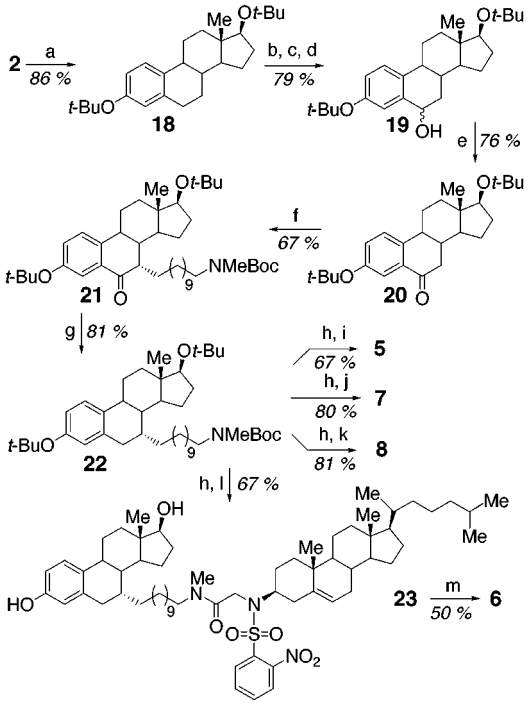


[a] Reagents and conditions: (a) DIBAL, CH_2Cl_2, 25 °C. (b) $(Boc)_2O$, DIEA, CH_2Cl_2, 25 °C. (c) NaI, acetone, reflux.

tion of the amide, protection of the resulting amine, and conversion to the iodide under Finkelstein conditions afforded the *tert*-butylcarbamate-protected *N*-methylamine **17** (Scheme 2).

In contrast to other syntheses of 7α-substituted estradiol derivatives,[26,27] which have primarily employed tetrahydropyranyl (THP) ethers for alcohol protection,[28−34] we installed *tert*-butyl ether protecting groups for the preparation of this class of compounds. This approach avoided generation of mixtures of diastereomers that limit facile assignment of side chain stereochemistry by NMR. Protection of **2** as the di-*tert*-butyl ether[35] was accomplished in good yield using a heterogeneous mixture of isobutylene and acidic Amberlyst resin in a sealed flask (Scheme 3).

Scheme 3[a]



[a] Reagents and conditions: (a) Isobutylene, acidic Amberlyst-15, CH_2Cl_2, 25 °C. (b) LDA, *t*-BuOK, THF, −78 °C. (c) $B(OMe)_3$, 0 °C. (d) H_2O_2, H_2O, 25 °C. (e) NaOCl, TEMPO, KBr, H_2O, CH_2Cl_2, 25 °C. (f) *t*-BuOK, **17**, THF, 0 °C. (g) 10% Pd (C), H_2, ethanol, 65 °C. (h) Trichloroacetic acid, CH_2Cl_2, 25 °C. (i) **12**, DIEA, THF, 25 °C. (j) **13**, DIEA, THF, 25 °C. (k) Ac_2O, DIEA, THF, 25 °C. (l) **14**, DIEA, THF, 25 °C. (m) PhSH, K_2CO_3, CH_3CN, 25 °C.

Compound **18** was deprotonated under well precedented[28,36,37] "superbase" conditions employing a 1:1 ratio of potassium *tert*-butoxide and lithium diisopropylamide. This anion was trapped with trimethyl borate to yield an intermediate borate ester that was oxidized with hydrogen peroxide to epimeric alcohols **19**. These epimers were further oxidized to ketone **20** with aqueous sodium hypochlorite, including TEMPO free radical and KBr as catalysts.[38]

Deprotonation of **20** with potassium *tert*-butoxide generated the corresponding enolate, which was alkylated with iodoalkane **17** to furnish ketone **21** as a single epimer in good yield. A minor O-alkylation product could also be isolated. Two-dimensional NMR experiments unambiguously confirmed the configuration of the 7α-side chain of **21** by detection of a nuclear Overhauser effect (NOE) between protons H_7 and H_8 and the absence of a NOE between H_7 and H_9 (Figure 5).

Hydrogenolysis of **21** over palladium on carbon afforded **22** in high yield. Complete removal of the acid-labile

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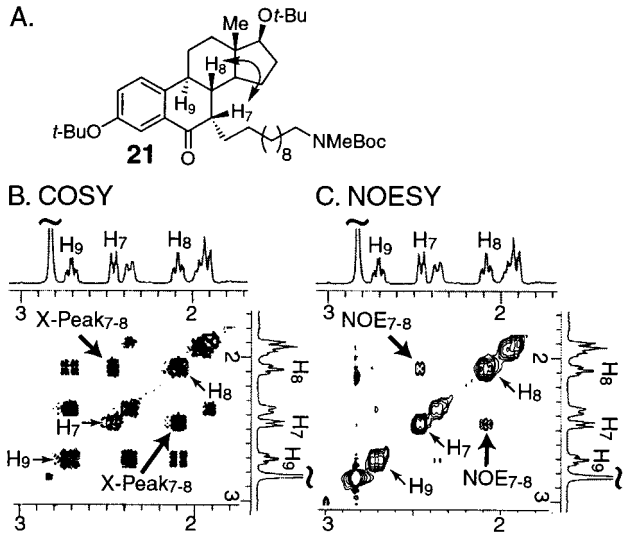

Figure 5. Assignment of 7α-stereochemistry to compound **21** by 2-D NMR. (A) Structure of **21** depicting the diagnostic NOE between H_7 and H_8. (B) COSY spectrum of B-ring proton resonances. (C) NOESY spectrum of this region.

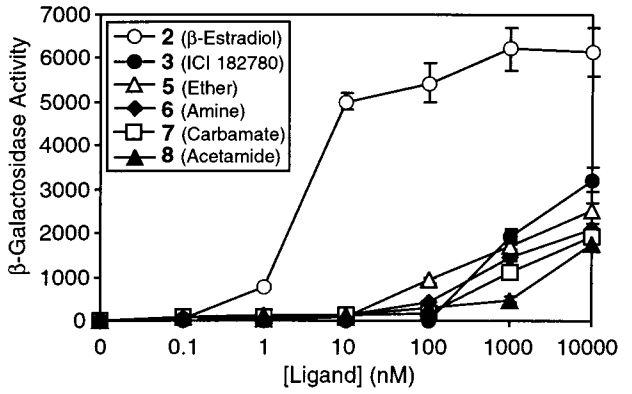

Figure 6. Dose-dependent activation of gene expression in whole yeast cells engineered to report compound-induced dimerization of ERα.

protecting groups with 10% trichloroacetic acid was followed by acylation of the side chain amine with **12**, **13**, acetic anhydride, and **14** to yield **5**, **7**, **8**, and **23** as shown in Scheme 3. The 2-nitrobenzene sulfonamide protecting group on **23** was removed with deprotonated thiophenol to provide **6**.

The ability of these 7α-substituted *β*-estradiol derivatives to interact with ERα was assessed by comparison with *β*-estradiol (**2**) and the antiestrogen ICI 182780 (**3**) in a yeast whole cell assay[39,40] that reports both estrogen- and antiestrogen-induced dimerization of ERα by activating expression of the enzyme *β*-galactosidase. Although competitive binding assays with purified ERα and radiolabeled or fluorescent estrogen probes might have provided a more quantitative comparison, recombinant yeast provide well-precedented assays[39,40] for analysis of compound-mediated ER dimerization. This yeast-based assay provided a simple, rapid, inexpensive, and nonradioactive method for generation of initial biological activity data.

As shown in Figure 6, these experiments revealed that compounds **5**−**8** exhibit activities nearly identical to that of ICI 182780 (**3**). Remarkably, the cholesterol derivatives **5**−**7** did not differ from the acetylated control compound **8** in ability to induce ERα dimerization, indicating that these compounds exhibit substantial affinity for estrogen receptors. Future studies will investigate the ability of lipidic estrogens to promote uptake of estrogen receptors by mammalian cells.

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Supporting Information Available: Experimental procedures and characterization data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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