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Synthesis and Estrogen Receptor Affinity of a 4-Hydroxytamoxifen-Labeled Ligand for Diagnostic Imaging

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Abstract—A 10-step synthesis of a novel 4-hydroxytamoxifen-DTPA ligand (HOTam-DTPA) is reported. Tamoxifen and its primary metabolite 4-hydroxytamoxifen are common estrogen receptor ligands. Consequently, tamoxifen has found utility as the targeting component of various diagnostic agents for selective imaging of estrogen receptor-rich tissue, specifically breast cancer. An L-aspartic acid-derived DTPA analogue was attached to the ethyl side chain of 4-hydroxy-tamoxifen using *N*,*N*′-dimethylethylenediamine as a hydrophilic linker. A competitve estrogen receptor binding assay using [^{3}H]-17β-estradiol was performed to determine the effect of the ethyl side chain modification on estrogen receptor affinity. The results show that while the relative affinity of HOTam-DTPA for the estrogen receptor is ~10-fold lower than that of tamoxifen, it still remains a potent ligand at relatively low concentrations.

Introduction

Breast cancer is the leading form of cancer in women today, affecting as many as one in eight women.[1] Depending on the type of breast cancer, one therapy is to administer an anti-estrogen to compete with estradiol (Fig. 1) for binding the estrogen receptor (ER).[2] The ER is a 66 kDa protein member of the nuclear hormone receptor family of transcription factors[3] that exists in two forms, ERα and ERβ.[4] The ER is over-expressed in cancerous cells relative to healthy tissues and high ER concentrations are associated with decreased overall breast cancer survival.[5,6] In addition, changes in the relative levels of expression of each form of ER are reported to occur in cancer cells, with decreases in ERβ expression common in mammary and other tumors.[7] The abundance of ER in forms of breast cancer suggests that this protein is a candidate for preferential targeting of cancer cells, an important consideration for any form of chemotherapy. Indeed, anti-estrogen therapy affects principally tissues rich in ER.[2] Tamoxifen (Fig. 1), the prototypical anti-estrogen, has been employed over the course of three decades for the treatment of ER-positive tumors.[8] Since FDA approval for tamoxifen in 1978, efforts to elucidate the mechanism of action,[9] the associated metabolic pathways,[10] and structure–activity relationships[11] have led to an understanding of ER-ligand interactions. Tamoxifen is reported to bind the ER ligand binding site with a relative affinity of between

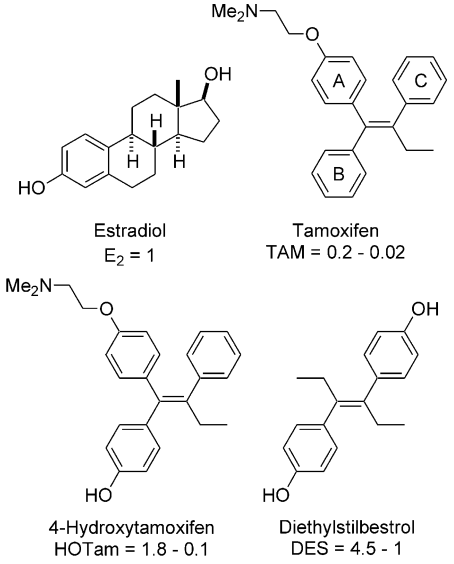

Figure 1. Structures and approximate relative binding values of common estrogen receptor ligands.[4,12,13]

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20–200-fold lower than that of 17β-estradiol, while 4-hydroxytamoxifen, the primary metabolite of tamoxifen, is significantly more potent, with reported affinities ranging between 1.8-fold more potent to 10-fold less potent than 17β-estradiol.[4,12,13] Although the two ER forms appear to differ somewhat in their tissue distribution and in their specificity and relative affinity for ligands, tamoxifen binds both subtypes with similar affinity.[4]

An emerging area of research that seeks to exploit the tamoxifen affinity for the ER is the use of tamoxifen as a targeting element of diagnostic and imaging agents.[14] Since the initial observation by Bloomer et al. that an ^{125}I-labeled tamoxifen analogue was cytotoxic to various cells,[15] several isotopically enriched, halogenated analogues of tamoxifen have been synthesized and tested for their ability to localize in ER-rich tissue (e.g., **1**–**3**, Fig. 2).[16,17] For a similar purpose, tamoxifen also has been fitted with moieties for chelation of radionuclides and magnetic resonance imaging (MRI) elements (e.g., **4**–**6**).[18–20] Positron emission tomography (PET) and single photon emission computed tomography (SPECT) studies in animals using analogues **2** and **3** confirmed the central hypothesis underlying the design of tamoxifen-based imaging agents, namely that uptake of the agents occurred via an ER-mediated process.[17] The possibility

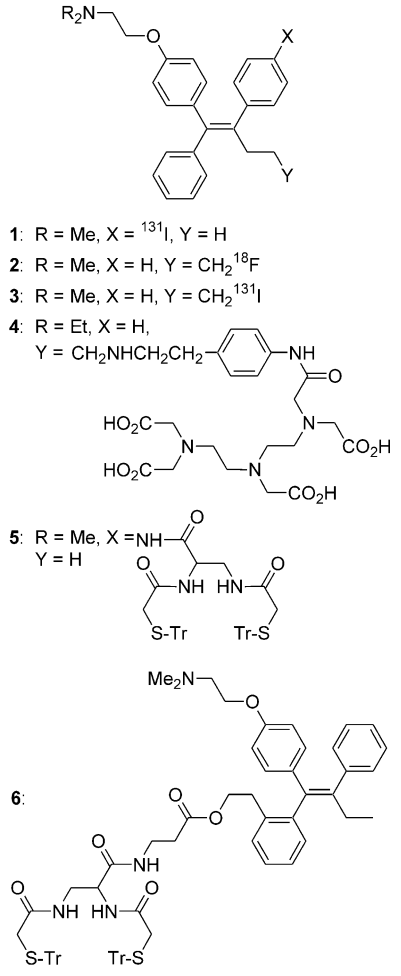


Figure 2. Tamoxifen radionuclides and chelates for imaging. Tr = CPh_3.

that sex hormone-binding globulin (SHBG), a serum glycoprotein that specifically binds testosterone and 17β-estradiol,[21] may have contributed to the uptake of these tamoxifen-based agents exists since SHBG also binds membranes of estrogen-dependent tissues such as breast cancer.[22] However, tamoxifen-based agents generally have failed to provide sufficient contrast between tissues rich in ER and surrounding tissues to warrant their diagnostic use in humans. To underscore this problem, Podoloff et al. suggested that clearance of the ^{111}In-chelate of **4** from tissues surrounding ER-rich tumors might be improved if the hydrophilicity of the agent were increased.[18]

With these considerations in mind, we set out to design and synthesize the first 4-hydroxytamoxifen (HOTam) analogue containing a hydrophilic link to diethylenetriaminepentaacetic acid (DTPA), a common[23] ligand for chelation of radionuclides and MRI-elements. The synthesis and ER-binding measurements of HOTam-DTPA are described here. Our rationale for using 4-hydroxytamoxifen as the targeting element is two-fold: (i) the greater ER-binding affinity of 4-hydroxytamoxifen relative to tamoxifen is expected to improve uptake and retention of HOTam-derived agents by ER-rich tissue, while (ii) the 4-hydroxyl group, combined with a hydrophilic ethylenediamine linker,[24] is expected to improve clearance from tissue that expresses lower levels of ER. Thus, the increased hydrophilicity and ER-binding affinity of this new anti-estrogen-labeled DTPA are designed to improve retention of the agent in ER-rich tissue.

Of greatest concern was whether an appended DTPA would significantly affect interaction of the HOTam-DTPA conjugate with the ER. It is reasonable to expect that factors such as linker composition and length, and site of attachment could alter the ER-binding affinity of the conjugate. The knowledge that the A-ring of tamoxifen cannot be modified, including the A-ring β-(dimethylamino)ethoxy substituent,[25] without altering the pharmacological activity helped guide our selection for a site of 'chelate arm' attachment onto HOTam.[26] Furthermore, the recent crystallographic findings of Brzozowski et al.[27] and Shiau et al.[28] indicate that tamoxifen and HOTam bind the ER in a manner that places the B- and C-rings firmly within a binding pocket. In agreement with this model for ER-binding, in vitro and in vivo studies of **5**, a substituted C-ring analogue, indicated very limited ER-binding.[20] In contrast to the binding requirements for the aryl rings, the ethyl side chain of tamoxifen protrudes out of the ER binding pocket and thus appears to be a suitable position for functionalization. This assumption is supported by the results of an [^{3}H]-17β-estradiol competition binding assay that indicated the ethyl side chain derivative **4** bound ER.[18] Although no other ER-binding data for ethyl side chain derivatives is available, we felt that functionalization of hydroxytamoxifen at this site is the most attractive option for attachment of DTPA.

A final consideration in our design of HOTam-DTPA involves the mode of DTPA attachment. Recent studies

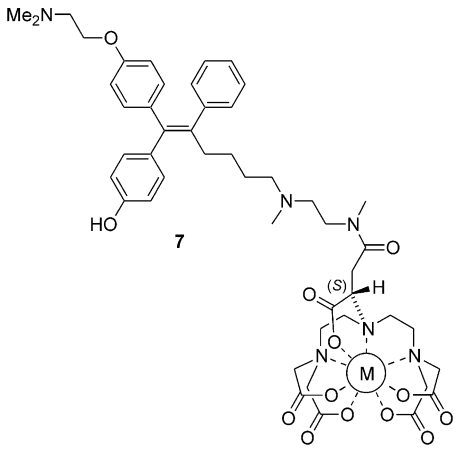


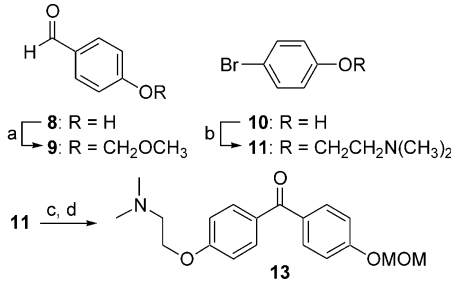
Figure 3. Hydroxytamoxifen-linked chelate.

point toward maintaining the full complement of DTPA-carboxylates for maximum stability of the metal-ligand complex.[29] Several investigators have synthesized functionalized DTPA derivatives so that covalent attachment of the chelate need not occur through one of the five DTPA carboxylic acid groups.[30] With this in mind, we selected a hydrophilic L-aspartic acid-derived DTPA which can be conjugated without consuming any of the DTPA-carboxylates.[31] This feature and the aforementioned considerations led to the design of HOTam-DTPA, the target chelate represented by structure **7** (Fig. 3).

Results and Discussion

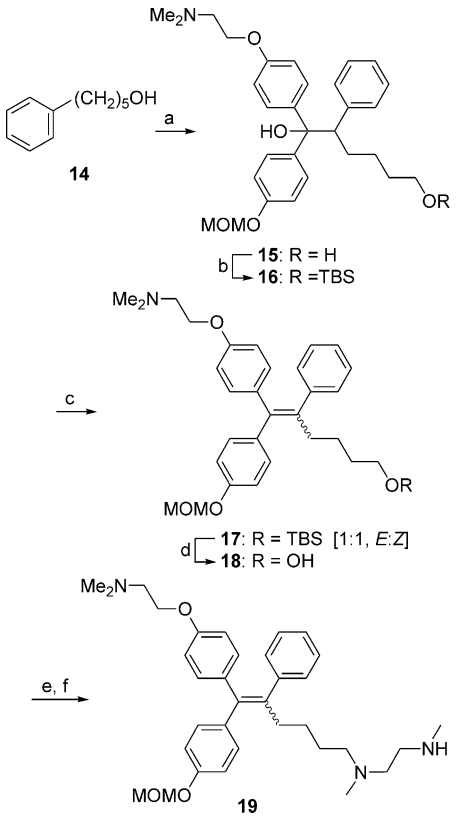
Synthesis

We envisioned a convergent synthesis of **7** wherein the alkene would result from dehydration of a benzylic alcohol. Fragment coupling to form the benzylic alcohol suggested the synthesis of an A,B-ring benzophenone. Thus, we prepared benzophenone derivative **13** (Scheme 1) from methoxymethyl protected *p*-anisaldehyde (**9**)[32] and aryl bromide **11**.[33] Treatment of **9** with the Grignard reagent derived from **11** gave the corresponding alcohol, **12**, and subsequent MnO_2 oxidation provided benzophenone **13** in near quantitative yield.

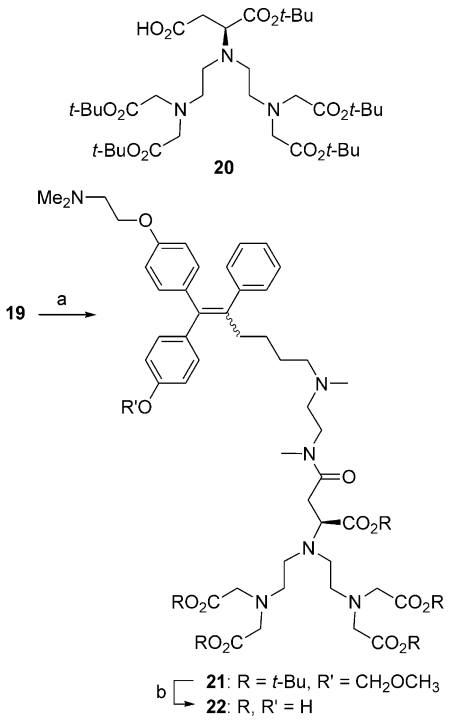


Scheme 1. (a) NaH (1.1 equiv), MOMCl (1 equiv), DMF, 0 °C–rt; 4.5 h, 74%; (b) K_2CO_3 (4 equiv), $Me_2NCH_2CH_2Cl{\cdot}HCl$ (2 equiv), DMF, rt, 24 h, 80%; (c) (i) Mg, THF, reflux 2 h, (ii) **9**, THF, 0 °C–rt, 3.5 h, 97%; (d) MnO_2 (20 equiv), pentane, rt, 18 h, 98%.

We modified the procedure for assembly of the tamoxifen core first described by Olier-Reuchat et al.[34] by reacting benzophenone **13** with the 1,6-dianion generated from 5-phenyl-1-pentanol (**14**) (Scheme 2). This unique example of 1,2-addition by a 1,6-dianion gave adduct **15** as a mixture of diastereomers in good yield. However, all attempts to selectively dehydrate the tertiary benzylic alcohol of **15** resulted in the formation of an undesired cyclized product.[35] We circumvented this problem by silylating the primary alcohol and then effecting dehydration. Treatment of silyl ether **16** with thionyl chloride in pyridine gave alkene **17** as a separable 1:1 mixture of *E* and *Z* isomers in quantitative yield. The two isomers are differentiated by the ^1H NMR chemical shifts of the respective methylene groups adjacent to the A-ring side chain oxygen.[36] Although the isomeric forms of tamoxifen and its metabolites have different therapeutic effects, both isomers bind the ER, which, for imaging and diagnostic purposes, obviates the need for isomer separation.[12a] Furthermore, the observation that the *E* and *Z* isomers of hydroxytamoxifen readily isomerize in vivo also suggests that **17** may be elaborated as a mixture of isomers.[37] We therefore used **17** as obtained and pursued conversion of its silyl ether into a suitable leaving group for nucleophilic attachment of the ethylenediamine linker.



Scheme 2. (a) (i) *t*-BuOK (1 equiv), pentane, rt, 5 min, (ii) *s*-BuLi (2 equiv), TMEDA (2 equiv), 0 °C–rt, 1 h, (iii) **13** (0.17 equiv), THF, 0 °C–rt, 4 h, 80%; (b) TBS-Cl, imidazole, DMAP (cat.), CH_2Cl_2, rt, 12 h, 79%; (c) $SOCl_2$, pyridine, −10 °C, 4.5 h, 99%; (d) TBAF, THF, rt, 6 h, 98%; (e) CBr_4 (1.2 equiv), PPh_3 (1.2 equiv), CH_2Cl_2, 0 °C–rt, 1 h; (f) $MeNHCH_2CH_2NHMe$ (10 equiv), CH_3CN, rt, 12 h, 73% two steps.



Scheme 3. (a) DCC, HOBT, **20**, $CHCl_3$, 64%; (b) TFA, Anisole (30 equiv), CH_2Cl_2, −10 °C – rt, 12 h.

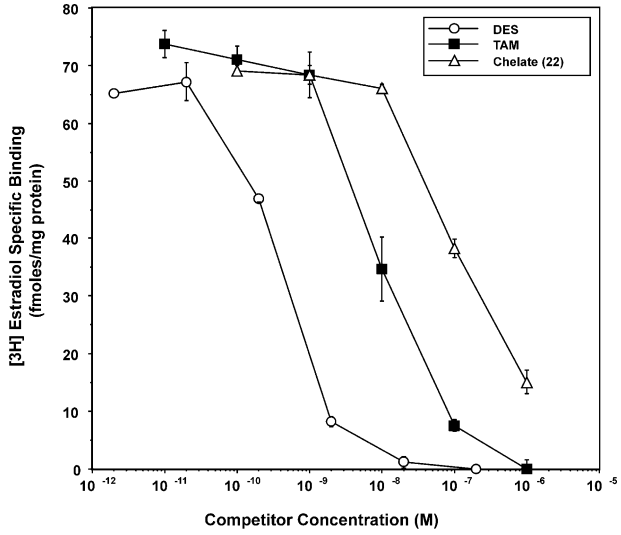


Figure 4. Competition by diethylstilbestrol, tamoxifen and chelate **22** for [^{3}H]-17β-estradiol binding to the ER. Calf uterine cytosol was incubated with 3 nM [^{3}H]-17β-estradiol in the absence or presence of increasing concentrations of diethylstilbestrol (DES), tamoxifen (TAM) or HOTam–DTPA (**22**) for 2 h at 4 °C. Specific binding and competitive displacement of [^{3}H]-17β-estradiol was determined using the dextran-coated charcoal binding assay as described in the material and methods. Values are expressed as the mean ± SD of incubations performed in triplicate.

Silyl ether deprotection using *n*-Bu_4NF gave alcohol **18** in near quantitative yield. The alcohol was converted to its tosylate derivative using standard conditions, but this tosylate was prone to decomposition. Attempts to directly convert silyl ether **17** to the corresponding bromide (PPh_3, Br_2) also failed.[38] We were gratified, however, when application of a literature method[39] for alcohol to bromide conversion transformed **18** to the corresponding bromide. Not surprisingly, the bromide was also unstable and reacted within hours at room temperature. Thus, immediately after its formation, the crude bromide was passed through a short SiO_2 column and then immediately treated with an acetonitrile solution of *N*,*N*′-dimethylethylenediamine. In this way, we obtained amine **19** from alcohol **18** in a two-step 73% yield.

At this stage in the synthesis, we opted to attach the DTPA-chelate to the ethylenediamine moiety of **19**. To reduce DTPA chelation of undesired metals during preparation and handling, we attached the *t*-butyl-protected DTPA analogue **20** (Scheme 3).[31] HOBT-mediated DCC coupling of **19** and **20** gave hydroxytamoxifen analogue **21**. Purification of **21** was achieved using normal silica gel chromatography. The MOM group and all *t*-butyl esters were cleaved by treatment of **21** with TFA and anisole[40] to liberate HOTam-DTPA chelate **22**. The average concentration of free chelating groups in a given solution of **22**, determined using the ^{57}Co-method developed by Meares et al.[41] indicated that 73% of the theoretical titer of **22** was available for chelation.

With HOTam-DTPA fully assembled, we next evaluated the effect of the ethyl side chain modifications on the ability of **22** to bind to the ER by performing a competitive ligand binding analysis. Equilibrium binding of [^{3}H]-17β-estradiol to the ER in the presence of increasing concentrations of a competitor provides a means of estimating the relative binding affinity of the competitor to the ER. Comparison of the resulting competitive binding curves (Fig. 4) revealed that the relative binding affinity of HOTam-DPTA (**22**) was ~10-fold lower than that of tamoxifen and ~200 fold lower than that of the most potent ER ligand diethylstilbestrol. While these data suggest that ligand **22** binds ER with greater affinity than other tamoxifen ligands for which ER-binding data is available, the in vivo sufficiency of its metal chelates in diagnostic studies needs to be determined experimentally. Considering that the ER content in estrogen-responsive cells is roughly 100,000 ERs per cell,[42] it is unlikely that the corresponding gadolinium chelate of **22** would afford the detection sensitivity needed to image ER positive tumors using MRI methods. However, since signal detection in scintigraphy is approximately 2–3 orders of magnitude more sensitive than in MRI, the corresponding indium and technetium chelates of **22** may be used for this purpose. Work is in progress to evaluate the in vivo diagnostic capability of chelate **7** (M = ^{111}In).

Conclusion

To our knowledge, no reported imaging agents have been prepared using 4-hydroxy-tamoxifen as the targeting element. The investigations reported here describe a method for attaching DTPA to the ethyl side chain of HOTam and the influence of this structural modification on ER-binding. Although modification of the ethyl side chain with a hydrophilic EDA–DTPA substituent does

decrease its affinity for the ER, the ability of the conjugate to effectively compete with [^{3}H]-17β-estradiol for binding to the ER at relatively low concentrations demonstrates that it still remains a relatively potent ligand. In fact, the relative binding affinity of HOTam–DTPA is still greater than that of numerous ER ligands that have been shown to produce effects in cells and animals in vivo.[4,12b]

Experimental procedures

All reactions were carried out under an atmosphere of nitrogen. CH_2Cl_2 was distilled from calcium hydride immediately prior to use. THF and Et_2O were distilled from sodium benzophenone ketyl immediately prior to use. Column chromatography was carried out using 230–400 mesh silica gel, slurry packed in glass columns, eluting with the solvents indicated. Yields were calculated for material judged to be homogeneous by TLC and NMR. TLC was performed on kieselgel 60 F_{254} plates. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) were recorded in $CDCl_3$. High resolution mass spectrometry was performed by Mass Spectrometry Service Lab, UC Riverside and combustion analyses were performed by Midwest Microlab, Indianapolis, IN. Infrared (IR) data were obtained on neat samples.

[4-(2-Dimethylamino-ethoxy)-phenyl]-(4-methoxymethoxy-phenyl)-methanol (12). A three necked flask fitted with a reflux condenser and a pressure equalizing addition funnel was flame dried and charged with magnesium turnings (1.34 g, 55.2 mmol) and THF (80.0 mL). The suspension was heated to 60 °C and a mixture of **11** (11.2 g, 46.0 mmol) and 1,2-dibromoethane (0.60 mL, 7.00 mmol) in dry THF (10 mL) was added dropwise via the addition funnel. The resulting suspension was heated at the reflux temperature until the metal was consumed and then cooled to room temperature. The reaction solution was transferred via cannula into an empty, flame-dried flask charged with argon and then cooled to 0 °C. To the cooled solution was added a chilled solution of **9** (5.35 g, 32.2 mmol) in THF (15 mL) via cannula over 90 min. After addition, the mixture was stirred for 1 h at 0 °C, and then warmed to room temperature and stirred for 1 h. The reaction was quenched by slowly pouring into saturated aqueous $NaHCO_3$ (100 mL). The aqueous layer was extracted with diethyl ether (3×50 mL), the combined organic fraction was washed with water (100 mL), brine (100 mL) and dried (Na_2SO_4). The solvents were removed in vacuo to give **12** (10.4 g, 97%) as a light yellow oil; R_f = 0.15 (CH_2Cl_2/MeOH, 9:1); IR 3360, 2780, 1609, 1508 cm^{-1}; ^{1}H NMR δ 7.29–7.33 (m, 4H), 7.02 (d, J = 6.4 Hz, 2H), 6.90 (d, J = 8.8 Hz, 2H), 5.73 (s, 1H), 5.18 (s, 2H) 4.03 (t, J = 5.9 Hz, 2H), 3.89 (br. s, 1H), 3.50 (s, 3H), 2.73 (t, J = 5.9 Hz, 2H), 2.31 (s, 6H); ^{13}C NMR δ 157.6, 156.1, 138.0, 136.8, 127.5, 127.4, 94.1, 74.7, 65.4, 57.7, 55.7, 45.4; MS (EI) m/z (rel intens) 332 (MH$^+$, 26), 58 (100), 72 (10); HRMS (EI) calcd for $C_{19}H_{25}NO_4$ 331.1764, found 332.1850 (MH$^+$).

[4-(2-Dimethylamino-ethoxy)-phenyl]-(4-methoxymethoxy-phenyl)-methanone (13). To a solution of carbinol **12** (10.63 g, 32.07 mmol) in pentane (640 mL) at room temperature was added MnO_2 (55.77 g, 642 mmol). The suspension was stirred at room temperature for 18 h and then filtered by passing through a bed of Celite. The retentate was washed with pentane (200 mL) and then diethyl ether (200 mL). The combined solvents were removed in vacuo to give **13** (10.35 g, 98%) as a reddish oil that was used without further purification; R_f = 0.26 (CH_2Cl_2/MeOH, 9:1); IR 2944, 1648, 1603, 1507 cm^{-1}; ^{1}H NMR δ 7.69–7.73 (m, 4H), 7.03 (d, J = 8.8 Hz, 2H), 6.91 (d, J = 8.2 Hz, 2H), 5.18 (s, 2H), 4.08 (t, J = 5.9 Hz, 2H), 3.43 (s, 3H), 2.71 (t, J = 5.9 Hz, 2H), 2.29 (s, 6H); ^{13}C NMR δ 194.5, 162.3, 160.5, 132.4, 132.2, 131.8, 130.7, 115.6, 114.2, 94.2, 66.3, 58.3, 56.4, 46.0; MS (EI) m/z (rel intens) 330 (MH$^+$ 100), 259 (50); HRMS (EI) calcd for $C_{19}H_{23}NO_4$ 329.1627, found 330.1692 (MH$^+$).

[4-(2-Dimethylamino-ethoxy)-phenyl]-(4-methoxymethoxy-phenyl)-2-phenyl-hexane-1,6-diol (15). To a stirred suspension of *t*-BuOK (4.03 g, 36.0 mmol) in pentane (36 mL) at room temperature was added 5-phenyl-1-pentanol (**14**) (6.06 mL, 36.0 mmol). The mixture was cooled to 0 °C and *s*-BuLi (55.3 mL of a 1.3 M solution, 71.9 mmol) was added dropwise. To the resultant red suspension was added TMEDA (10.9 mL, 72.0 mmol) followed by warming to room temperature. After stirring 1 h, the dark red solution was recooled to 0 °C and an ice cold solution of the ketone **13** (1.98 g, 6.00 mmol) in dry THF (20 mL) was added dropwise via cannula. After the addition was complete, the mixture was stirred for 2 h at room temperature and then quenched by pouring over saturated aqueous $NaHCO_3$ (50 mL). The aqueous layer was extracted three times with diethylether (25 mL). The combined organics were washed successively with water and brine, then dried (Na_2SO_4). The solvents were evaporated and the residue was purified by flash chromotography, eluting with a gradient (CH_2Cl_2 to CH_2Cl_2/MeOH, 19:1), to obtain **15** (2.55 g, 80%) as a 1:1 mixture of diastereomers; R_f = 0.35 (CH_2Cl_2/MeOH, 4:1); IR 3400, 2942, 1607, 1508, 1235 cm^{-1}; ^{1}H NMR δ 7.33–7.37 (m, 4H), 6.92–7.05 (m, 16H), 6.80 (d, J = 6.8 Hz, 2H), 6.66 (d, J = 6.8 Hz, 2H), 6.55 (d, J = 9.0 Hz, 2H), 5.09 (s, 2H), 4.97 (s, 2H), 3.99 (t, J = 5.9 Hz, 2H), 3.87 (t, J = 5.9 Hz, 2H), 3.55 (s, 1H), 3.52 (s, 1H), 3.40 (s, 3H), 3.38 (m, 4H), 3.31 (s, 3H), 2.66 (t, J = 5.9 Hz, 2H), 2.57 (t, J = 5.9 Hz, 2H), 2.40 (bs, 2H), 2.26 (s, 6H), 2.23 (bs, 2H), 2.21 (s, 6H), 1.6–1.8 (m, 4H), 1.3–1.5 (m, 4H), 1.0–1.2 (m, 4H); ^{13}C NMR δ 157.3, 156.9, 155.8, 155.3, 140.1, 139.9, 139.4, 138.8, 138.1, 130.05, 130.03, 127.7, 127.4, 126.9, 126.8, 126.3, 115.6, 115.1, 113.9, 113.4, 94.39, 94.30, 80.3, 65.7, 65.5, 62.6, 58.2, 58.1, 56.0, 55.8, 54.4, 45.8, 45.7, 32.6, 30.08, 30.00, 24.1; MS (EI) m/z (rel intens) 494 (MH$^+$, 100), 332 (45), 58, (40); HRMS (EI) calcd for $C_{30}H_{39}NO_5$ 493.2828, found 494.2895 (MH$^+$).

6-(*tert*-Butyl-dimethyl-silanyloxy)-1-[4-(2-dimethylamino-ethoxy)-phenyl]-(4-methoxymethoxy-phenyl)-2-phenyl-hexan-1-ol (16). To a stirred solution of diol **15** (4.01 g, 8.13 mmol) in CH_2Cl_2 (30 mL) at room temperature was added imidazole (1.13 g, 16.7 mmol) and DMAP (50.0 mg, 0.40 mmol). After 5 min TBSCl (1.29 g, 8.54 mmol) was added and the reaction mixture was stirred overnight at room temperature. The reaction

mixture was poured over saturated aqueous $NaHCO_3$ (30 mL), the organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated in vacuo. The crude oil was purified by flash chromotography, eluting with a gradient (CH_2Cl_2–CH_2Cl_2/MeOH, 9:1, v/v), to give **16** (3.89 g, 79%) as a pale yellow oil; R_f = 0.33 (CH_2Cl_2/MeOH, 9:1); IR 2950, 1607, 1507, 1257 cm^{-1}; ^1H NMR δ 7.42 (m, 4H), 6.99–7.16 (m 16H), 6.88 (d, J = 5.9 Hz, 2H), 6.75 (d, J = 5.9 Hz, 2H), 6.63 (d, J = 5.9 Hz, 2H) 5.17 (s, 2H), 5.05 (s, 2H), 4.07 (t, J = 5.9 Hz, 2H), 3.93 (t, J = 5.9 Hz, 2H), 3.62 (s, 1H), 3.59 (s, 1H), 3.48 (s, 3H), 3.40–3.47 (m, 4H), 3.39 (s, 3H), 2.74 (t, J = 5.9 Hz, 2H), 2.65 (t, J = 5.9 Hz, 2H), 2.34 (s, 6H), 2.32 (bs, 2H), 2.29 (s, 6H), 1.69–1.83 (m, 4H), 1.33–1.49 (m, 4H), 1.1–1.15 (m, 4H); 0.88 (s, 18H), 0.00 (s, 12H); ^{13}C NMR δ 158.6, 157.3, 156.8, 155.7, 155.2, 140.2, 140.0, 139.5, 138.9, 138.2, 130.0, 129.9, 127.5, 127.3, 126.9, 126.8, 126.1, 120.6, 115.5, 115.0, 114.4, 113.8, 113.4, 94.3, 94.2, 80.2, 65.7, 65.69, 65.60, 62.8, 58.19, 58.13, 55.8, 55.7, 54.3, 53.3, 45.8, 45.7, 32.6, 30.0, 29.9, 25.8, 23.9, 18.1, −5.4; MS (EI) m/z (rel intens) 608 (MH^+ 100), 330 (65), 259 (40); HRMS (EI) calcd for $C_{36}H_{53}NO_5Si$ 607.3693, found 608.3788 (MH^+).

(2-{4-[6-(*tert*-Butyl-dimethyl-silanyloxy)-1-[4-(2-dimethylamino-ethoxy)-phenyl]-(4-methoxymethoxy-phenyl)-2-phenyl-hex-1-enyl]-phenoxy}-ethyl)-dimethyl-amine (17). To a solution of carbinol **16** (108 mg, 0.18 mmol) in dry pyridine (0.4 mL) at −10 °C was added $SOCl_2$ (0.023 mL, 0.32 mmol) via syringe, and the reaction was stirred at this temperature 4.5 h. The reaction mixture was then poured into water and extracted with diethyl ether. The organic layer was dried (Na_2SO_4) and then concentrated *in* vacuo to give the crude product. The crude, orange oil was purified by column chromotography, eluting with a gradient (CH_2Cl_2 to CH_2Cl_2/MeOH, 9:1), to afford **17** (104 mg, 99%) as a 1:1 mixture of diastereomers; R_f = 0.27 (CH_2Cl_2/MeOH, 9:1); IR 2951, 1609, 1507, 1240 cm^{-1}; ^1H NMR δ 7.07–7.14 (m, 14H), 6.97 (d, J = 11.7 Hz, 2H), 6.86 (d, J = 11.1 Hz, 2H), 6.74–6.77 (m, 4H), 6.63 (d, J = 11.7 Hz, 2H), 6.52 (d, J = 11.7 Hz, 2H), 5.18 (s, 2H), 5.04 (s, 2H), 4.13 (t, J = 5.9 Hz, 2H), 3.97 (t, J = 5.9 Hz, 2H), 3.51 (s, 3H), 3.47 (m, 4H), 3.40 (s, 3H), 2.84 (t, J = 5.9 Hz, 2H), 2.75 (t, J = 5.9 Hz, 2H), 2.42 (s, 6H), 2.38 (m, 4H), 2.36 (s, 6H), 1.28–1.40 (m, 8H), 0.84 (s, 18H), −0.02 (s, 12H); ^{13}C NMR δ 157.4, 156.6, 155.8, 255.0, 142.6, 139.8, 139.7, 138.0, 137.4, 136.8, 136.1, 135.6, 131.7, 130.5, 129.5, 127.7, 125.8, 115.6, 114.9, 113.9, 113.2, 94.4, 94.3, 65.7, 65.5, 62.9, 58.3, 58.2, 56.0, 55.9, 45.88, 45.84, 35.6, 32.9, 25.9, 25.1, 18.2, −5.3; MS (EI) m/z (rel intens) 590 (MH^+ 100); HRMS (EI) calcd for $C_{36}H_{51}NO_4Si$ 589.3587, found 590.3652 (MH^+).

6-[4-(2-Dimethylamino-ethoxy)-phenyl]-6-(4-methoxy-methoxy-phenyl)-5-phenyl-hex-5en-1-ol (18). To a solution of silyl ether **17** (1.43 g, 2.4 mmol) in anhydrous THF (10 mL) at 0 °C was added TBAF (4.8 mL of a 1.0 M solution in THF, 4.8 mmol). The reaction was allowed to room temperature and stirred for 6 h. The reaction mixture was poured over water (10 mL) and extracted several times with diethyl ether. The combined organic layer was washed with water (30 mL), brine (30 mL), and then dried (Na_2SO_4). The solvents were concentrated in vacuo, and the residue was purified by column chromotography, eluting with a gradient (CH_2Cl_2–CH_2Cl_2/MeOH, 19:1), to afford **18** (1.12 g, 98%) as a pale yellow oil; IR 3393, 2932, 1601, 1498, 1236 cm^{-1}; ^1H NMR δ 7.08–7.15 (m, 14H), 7.01 (d, J = 0.03 Hz, 2H), 6.89 (d, J = 0.03 Hz, 2H), 6.75–6.87 (m, 4H), 6.65 (d, J = 0.03 Hz, 2H), 6.52 (d, J = 0.03 Hz, 2H), 5.18 (s, 2H), 5.03 (s, 2H), 4.07 (t, J = 5.9 Hz, 2H), 3.91 (t, J = 5.9 Hz, 2H), 3.50 (s, 3H), 3.46–3.48 (m, 4H), 3.39 (s, 3H), 2.74 (t, J = 5.9 Hz, 2H), 2.63 (t, J = 5.9 Hz, 2H), 2.43–2.47 (m, 4H), 2.33 (s, 6H), 2.27 (s, 6H), 1.65 (bs, 2H), 1.44–1.49 (m, 4H), 1.33–139 (m, 4H); ^{13}C NMR δ 157.4, 156.6, 155.8, 155.0, 142.5, 139.5, 139.4, 138.2, 137.2, 136.7, 136.0, 135.5, 131.74, 131.71, 130.47, 130.44, 129.4, 127.8, 125.9, 125.8, 115.7, 114.9, 114.0, 113.2, 94.4, 94.2, 65.6, 65.4, 62.3, 58.1, 58.0, 55.9, 55.8, 45.7, 45.6, 35.5, 35.4, 32.6; MS (EI) m/z (rel intens) 476 (MH^+, 100), 215 (10); HRMS (EI) calcd for $C_{30}H_{37}NO_4$ 475.2723, found (MH^+) 476.2786.

***N*-[6-[4-(2-Dimethylamino-ethoxy)-phenyl]-6-(4-methoxy-methoxy-phenyl)-5-phenyl-hex-5enyl]-*N*,*N*′-dimethyl-ethane-1,2-diamine (19).** To a solution of **18** (1.01 g, 2.13 mmol) and CBr_4 (0.85 g, 2.55 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added dropwise a solution of PPh_3 (0.67 g, 2.55 mmol) in CH_2Cl_2 (5 mL) over 30 min. The reaction mixture was warmed to room temperature and stirred 30 min whereupon the solvent was removed by rotary evaporation. The residue was dissolved in a minimal quantity of ethyl acetate and passed through a short column of silica gel, eluting with CH_2Cl_2/MeOH (9:1, v/v). The fractions were concentrated to afford the corresponding bromide which was immediately dissolved in acetonitrile (10 mL) and treated at room temperature with *N*,*N*′-dimethylethylenediamine (2.3 mL, 21 mmol). After stirring 12 h, the reaction mixture was poured into water (50 mL) and the resultant mixture was extracted using CH_2Cl_2 (3×50 mL). The combined organic layer was washed with water, brine, and then dried (Na_2SO_4). The solvent was removed and the residue purified by column chromotography, eluting with a gradient (CH_2Cl_2/MeOH/TEA, 19/1/0.1 to 4/1/0.1) to give **19** (0.85 g, 73%) as a viscous yellow oil; R_f = 0.12 (CH_2Cl_2/MeOH, 4:1); IR 2941, 1604, 1501, 1236 cm^{-1}; ^1H NMR δ 7.16–7.07 (m, 14H), 6.9 (d, J = 6.6 Hz, 2H), 6.68 (d, J = 8.4 Hz, 2H), 6.76–6.73 (m, 4H), 6.66 (d, J = 6.6, 2H), 6.54 (d, J = 8.6, 2H), 5.18 (s, 2H), 5.03 (s, 2H), 4.07 (t, J = 5.9 Hz, 2H), 3.90 (t, J = 5.9 Hz, 2H), 3.50 (s, 3H), 3.39 (s, 3H), 2.73 (t, J = 5.9 Hz, 2H), 2.62 (t, J = 5.9 Hz, 2H), 2.61–2.56 (m, 2H), 2.45–2.41 (m, 10H), 2.38 (s, 6H), 2.34 (s, 6H), 2.27 (s, 6H), 2.20–2.16 (m, 6H), 2.11 (s, 6H), 1.34–1.28 (m, 8H); ^{13}C NMR δ 157.4, 156.6, 155.8, 155.0, 142.6, 139.7, 139.6, 138.1, 137.3, 136.8, 136.1, 135.6, 131.8, 131.7, 130.5, 130.4, 129.5, 127.7, 125.8 (2), 115.7, 114.9, 114.0, 113.6, 94.4, 94.3, 65.8, 65.5, 58.3, 58.2, 57.8, 56.7, 56.0, 55.9, 49.2, 45.8 (2), 42.1, 36.3, 35.8, 35.7, 27.3, 26.7; MS (EI) m/z (rel intens) 546 (MH^+, 100), 501 (55), 147 (45); HRMS (EI) calcd for $C_{34}H_{47}N_3O_3$ 545.3617, found (MH^+) 546.3714.

***tert*-Butyl(2*S*)-3-(*N*-{2-[((5*Z*)-6-{4-[2-(dimethylamino)-ethoxy]phenyl}-5,6-diphenylhex-5-enyl)methylaino]ethyl}-*N*-methylcarbamoyl)-2-{bis[2-(bis{[(*tert*-butyl)oxycarbonyl]methyl}amino)ethyl]amino}propanoate (21).** To a solution

of **20** (0.877 g, 1.20 mmol) and amine **19** (0.658 g, 1.20 mmol) in $CHCl_3$ (8.0 mL) at 0 °C were added DCC (0.272 g, 1.318 mmol) and HOBT (0.238 g, 1.56 mmol). The reaction was warmed to room temperature and stirred overnight. The reaction mixture was poured into water (20 mL) and extracted several times with $CHCl_3$. The combined extracts were dried (Na_2SO_4), and the solvent was removed in vacuo. The crude yellowish oil was purified using column chromotography using a gradient elution (CH_2Cl_2/MeOH/TEA (19/1/0.1, v/v to CH_2Cl_2/MeOH:TEA, 4/1/0.1) to give **21** (0.953 g, 64%) as a viscous yellow oil; R_f = 0.05 (CH_2Cl_2/MeOH, 4:1), IR 2974, 1724, 1642, 1153 cm^{-1}; ^1H NMR δ 7.08–7.14 (m, 8H), 6.99 (d, J = 8.7 Hz, 2H), 6.74 (d, J = 8.1 Hz, 2H), 6.54 (8.7 Hz, 2H), 5.18 (s, 2H), 3.94 (t, J = 5.4 Hz, 2H) 3.80 (m, 1H), 3.50 (s, 3H), 3.422 (s, 8H) 2.98 (s, 2H), 2.82–2.85 (m, 2H), 2.69–2.72 (m, 10H), 2.37–2.41 (m, 5H), 2.32 (s, 8H), 2.17–2.23 (m, 4H), 1.80 (m, 4H) 1.44 (s, 45H); ^{13}C NMR δ 171.3,171.2, 170.6, 156.1, 155.9, 143.6, 142.3, 139.1, 137.0, 135.8, 131.8, 130.4, 129.4, 127.9, 127.5, 126.0, 124.5, 123.5, 118.7, 115.8, 114.9, 113.2. 110.6, 94.4, 64.6, 61.2, 57.5, 56.8, 56.1, 56.0, 53.7, 51.2, 45.0, 40.8, 35.8, 35.4, 33.9, 29.6, 28.19, 28.14, 28.0; MS (EI) m/z (rel intens) 1282 (MNa^+, 100), 560 (25), 825 (15); HRMS (EI) calcd for $C_{70}H_{110}N_6O_{14}$ 1258.8080, found (MNa^+) 1281.7943. Anal. calcd for $C_{70}H_{110}N_6O_{14}$: C, 66.74; H, 8.80; N, 6.67. Found C, 66.27; H, 8.61; N, 6.66.

(2*S*)-3-(*N*-{2-[((5*Z*)-6-{4-[2-(dimethylamino)ethoxy]phenyl}-6-(4-hydroxyphenyl)-5-phenylhex-5-enyl)methylamino]-ethyl}-*N*-methylcarbamoyl)-2-(bis{2-[bis(carboxymethyl) amino]ethyl}amino)propanoic acid (22). Prior to removal of the MOM and *tert*-butyl protecting groups, all glassware was soaked in a 6M HCl bath overnight. The glassware was then rinsed with nano pure water and dried. To a solution of **21** (0.060 g, 0.047 mmol) in CH_2Cl_2 (0.5 mL) at −10 °C was added anisole (0.155 mL, 1.43 mmol). After 5 min, TFA (0.11 mL, 1.43 mmol) was added dropwise. The reaction was stirred for 2 h at −10 °C and then was warmed to room temperature. The anisole and the TFA were removed by reduced pressure distillation. The residue was dissolved in CH_2Cl_2 (0.5 mL) and again treated with TFA (0.11 mL, 1.43 mmol) followed by stirring at room temperature overnight. The solvent and excess TFA were removed by bulb-to-bulb distillation, and the residue was dissolved in a 19:1 mixture of CH_2Cl_2/MeOH (5.5 mL) to obtain a stock solution of **22**. The titer of **22** in the stock solution was established using the method of analysis developed by Meares.[41] Measurement of bound (**7**, M = ^{57}Co) versus unbound (**22**) material was performed using Selecto Scientific Silica Gel 60, F-254 TLC plates eluting with normal saline. The results of the ^{57}Co assay counted using a LKB Wallac 1282 universal gamma counter determined the effective concentration of **22** equaled 6.25 mM (73%).

ER ligand binding analysis. [^3H]Estradiol binding was determined using dextran-coated charcoal (DCC). Calf uterine cytosol was prepared as previously described[43] and diluted to 3 mg/mL in HEDGM buffer (25 mM Hepes, pH 7.4, 1 mM EDTA, 1 mM dithiothreitol, 10% (v/v) glycerol and 20 mM sodium molybdate). Diluted cytosol (0.5 mL) was incubated with 3 nM [^3H] 17β-estradiol (33.5 Ci/mmol, NEN Life Sciences, Boston, MA) in the absence or presence of the indicated concentration of each competitor, for 2 h at 4 °C. Diethylstilbestrol and tamoxifen were obtained from Sigma Chemical Company (St. Louis). Incubations were then mixed with a dextran-coated charcoal pellet that had been prepared by centrifuging 0.5 mL of a DCC solution (0.5 mg charcoal (Norit A, Fisher Scientific)/ 0.05 mg dextran (average MW 127,000)/mL water) at 3500 rpm for 15 min and removing the supernatant. The sample/DCC mixture was incubated for 15 min at 4 °C, followed by centrifugation at 3500 rpm for 15 min and the supernatant collected. Radioactivity in an aliquot of the supernatant (300 μL) was then determined by liquid scintillation counting. Specific binding of [^3H] estradiol to the ER was computed by subtracting the amount of [^3H] estradiol bound in the presence of competitor (diethylstilbestrol, tamoxifen or **22**) from the amount of total [^3H] estradiol bound in the absence of excess competitor. Specific binding values were expressed as fmoles [^3H] estradiol specific binding/mg protein.

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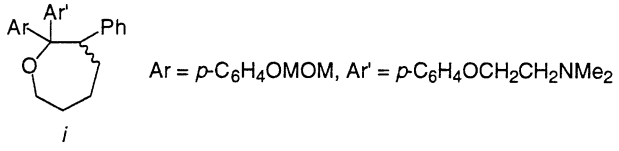

i

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