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7α- and 17α-Substituted Estrogens Containing Tridentate Tricarbonyl Rhenium/Technetium Complexes: Synthesis of Estrogen Receptor Imaging Agents and Evaluation Using MicroPET with Technetium-94m

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Abstract—To develop technetium and rhenium-labeled imaging agents for estrogen receptor (ER) positive breast tumors, we have prepared tridentate metal tricarbonyl chelates substituted at the 7α- and 17α-positions of estradiol. Some of the $Re(CO)_3$ conjugates have high binding for the ER in vitro. The in vivo biodistribution of the highest affinity of these novel metal tricarbonyl conjugates, prepared as the ^{94m}Tc labeled analogue, was evaluated by tissue dissection and microPET imaging. Although target tissue-selective uptake was not apparent, it is notable that microPET imaging identified the stomach as a major site of activity deposition, a site that might have been missed by standard tissue distribution studies.
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Introduction

Selecting the most effective therapy for women with breast cancer requires early detection and accurate staging of the disease. This includes determining the estrogen receptor (ER) status of the tumor, because hormone therapy with antiestrogens is beneficial only in tumors that are ER positive (ER+).[1] Imaging of the ER in vivo using an ER binding radiopharmaceutical has the potential for determining the ER status of all tumor sites simultaneously and non-invasively. Previously, we and others have labeled ER ligands with fluorine-18 and other cyclotron-produced short-lived radionuclides to develop such in vivo imaging agents.[2–14] In particular, we have used 16α-[^{18}F]fluoro-estradiol (FES) to image primary and metastatic breast tumors, and we have shown that these images have value in predicting the clinical effectiveness of hormone therapy with tamoxifen.[15,16]

Short-lived, cyclotron-produced radionuclides, however, are expensive and are not widely available, and this limits the utility of ER imaging agents such as FES for routine clinical studies. By contrast, technetium-99m is the most commonly used radioisotope in nuclear medicine, because of its favorable nuclear emission characteristics and its wide availability at low cost from a convenient generator system. A number of attempts have been made to conjugate technetium (or its non-radioactive congener rhenium) with estradiol, the natural ligand for the ER, to create an agent suitable for imaging ER in vivo that would be more widely available than FES.[17–26] Many of these estradiol complexes showed excellent in vitro binding to the ER; however, none of them gave favorable tissue distribution in vivo.

Recently, organometallic technetium and rhenium complexes in low oxidation states have been described and have been applied in receptor-targeted radiopharmaceutical

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development.[24,25] Advances in the preparation of the technetium-99m tricarbonyl core from generator-produced pertechnetate has provided a method of radiolabelling using a convenient aqueous-based kit.[27] The organometallic precursor formed is *fac*-$[^{99m}Tc(OH_2)_3(CO)_3]^+$, a stable, water-soluble precursor that can be readily coordinated to a variety of ligand systems via substitution of the coordinated water molecules.[28,29] Similarly, the organometallic aqua species *fac*-$[Re(OH_2)_3(CO)_3]^+$ can be readily formed,[30,31] and it may hold promise for application using the therapeutic radionuclide ^{188}Re.[32]

We are interested in exploring the use of *tridentate* chelators, conjugated to estradiol, that would enable us to form Tc(I) or Re(I) tricarbonyl complexes as estradiol–radiometal complexes. When appropriately designed, these estradiol complexes would be stable and neutral, and they would have a substantially different lipophilicity than previously reported estradiol complexes containing the Tc(I) or Re(I) tricarbonyl moiety. In addition, it has been reported in other systems that such *tridentate* complexes have better pharmacokinetic characteristics than *bidentate* complexes; the latter suffer from interaction with cellular nucleophiles through their labile third coordination site.[33] As is typically the case, in these exploratory studies we have used rhenium as a congener for technetium to facilitate the complete characterization and safe handling of the estrogen derivatives, and the measurement of their binding affinity to the ERs.

The generator-produced technetium-99m isotope has nuclear characteristics ($t_{1/21/2}=6$ h, $E\gamma=140.5$ keV, $I\gamma=87.2\%$) that make it an ideal isotope for single photon emission computed tomography (SPECT). Technetium-94m has the same chemistry as ^{99m}Tc, but has nuclear characteristics ($t_{1/2}=52$ min, $E^{+}_{\beta max}=2.44$ MeV, $I\beta^{+}=72\%$) suitable for imaging using positron emission tomography (PET), with which more quantitative measurements of radiopharmaceutical uptake, biodistribution and clearance can be made. Such studies have been performed by imaging small numbers of animals at multiple time points on a microPET scanner (a small animal PET imaging device),[34] rather than by traditional, time-consuming tissue dissection biodistribution studies.[35–41]

There are two components to the investigations presented in this report: The first component involves the development of four estradiol derivatives containing the rhenium tricarbonyl moiety tethered through two novel *tridentate* chelates, one having a typical amide linkage between the biomolecule (estradiol) and the metal complex, and the other being unique in having an all carbon linkage, potentially representing a more stable biomolecule-chelator junction. The second component of this study involves the labeling of the highest affinity estrogen–technetium conjugate with technetium-94m. This has enabled us to perform both in vivo tissue distribution and microPET imaging studies in the rat, thus expediting the evaluation of novel technetium-labeled radiopharmaceuticals.

Results and Discussion

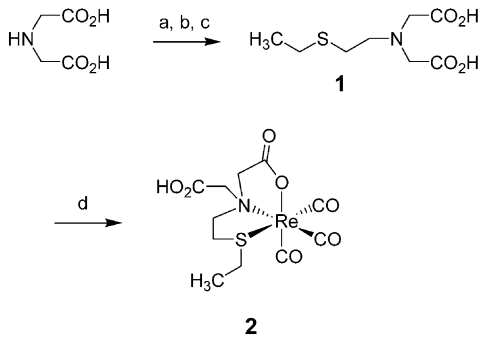
Tridentate chelation units to form rhenium(I) and technetium(I) tricarbonyl complexes

Two novel chelation systems were prepared to investigate estrogens containing rhenium or technetium tricarbonyl with a tridentate chelation core. The first chelate utilizes iminodiacetic acid and a thioether, the synthesis of which is described in Scheme 1. This chelator, prepared via an alternate synthetic route, has been reported in the literature, but it was used to form Tc(V)–oxo complexes, not as a tridentate chelator for M(I) tricarbonyl complexes (M = Tc or Re).[42] Similar chelators utilizing iminodiacetic acid as part of a chelator system were reported by Schibli et al., but they did not include a thioether as part of the chelation unit.[33] Thioether ligands are believed to be strong coordinating ligands for the metal(I) carbonyl center, due to their π-accepting properties.[43–47]

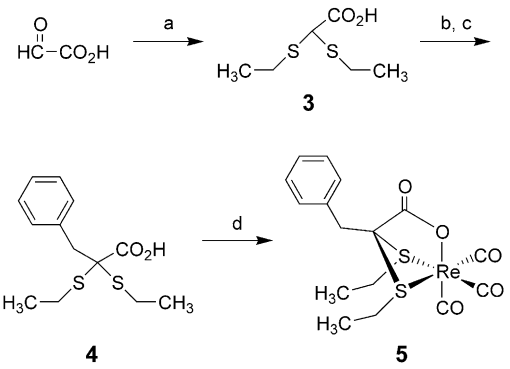
The synthesis starts with the reaction of iminodiacetic acid with ethyl chloromethyl sulfide under basic conditions, giving material, isolated first as a barium salt, and then liberated with sulfuric acid to furnish the aminothioether diacid **1** in 46% yield. This material is ready for conjugation through amide coupling of one of the carboxylic acid groups to an amino group on the biomolecule. To confirm the metal chelating ability of compound **1**, it was complexed with the rhenium tricarbonyl cation under aqueous conditions with one equivalent of base, resulting in the formation of rhenium(I) tricarbonyl complex **2** in 88% yield.

The second chelation system utilizes a carboxylic acid and two thioethers, which upon chelation with rhenium or technetium would give a neutral, and very small metal complex. This system can provide a complex that is achiral, which is a substantial advantage, because it avoids formation of diastereomers when the complex is attached to a chiral biomolecule. Formation of such diastereomers has introduced unnecessary stereochemical complications in metal-labeled receptor-binding radiopharmaceuticals.[48–50]

The dithioether chelator was prepared as outlined in Scheme 2. Glyoxalic acid was reacted with two equivalents of ethanethiol to yield the thioketal acid **3** in 93%



Scheme 1. Reagents and conditions (yield): (a) NaOH, $ClCH_2CH_2SCH_2CH_3$, H_2O, EtOH; (b) $BaCl_2$, H_2O; (c) H_2SO_4, H_2O (46% three steps); (d) $Re(CO)_3^+$, H_2O (88%).



Scheme 2. Reagents and conditions (yield): (a) EtSH, TsOH, benzene (93%); (b) nBuLi, THF; (c) BnBr, THF (68%); (d) $Re(CO)_3^+$, H_2O (79%).

yield. To explore the use of this chelation system for conjugation to biomolecules, a benzyl group was placed adjacent to the carboxylic acid. This was accomplished by forming the anion of **3** followed by addition of benzyl bromide, yielding alkylated product **4** in 68% yield. While a previously described procedure suggests that a mixed cation pair (e.g., sodium–lithium) is required for successful bismetallation of **3**,[51] we found that the use of two equivalents of *n*-butyl lithium gave satisfactory results when benzyl bromide is the alkylation substrate. Chelation with the rhenium tricarbonyl cation under aqueous conditions yielded the complex **5** in 79% yield. This system has the unique ability to provide a rhenium or technetium labeled biomolecule in which the linker is an aliphatic chain, without a more typical amide or ester linkage to the biomolecule. This may prove to be a more stable linkage for in vivo applications.

Design and synthesis of 7α-substituted estradiol derivatives

Previous work from our laboratory and elsewhere has shown that bulky substituents are well tolerated at the 7α-position of estradiol, without a significant decrease in ER binding (Fig. 1).[19,52] In particular, having a hexamethylene spacer between a metal chelator and estradiol results in estrogens having relative binding affinities in vitro that are from 5% to as high as 43% that of estradiol (estradiol = 100%). While the receptor binding assays indicate that these compounds have reasonably high ER binding affinity, the behavior of these bioconjugates in vivo has proved to be disappointing, with little or no ER-specific target tissue uptake being evident. Although the reasons were not fully clear, we ascribed the poor behavior of these compounds in vivo to be the result of their large size and in some cases their very high lipophilicity. *Bidentate* Tc(I)/Re(I) tricarbonyl estradiol systems have also been previously reported,[19,53] but they also proved to be poorly behaved in vivo. In this case, the poor behavior is ascribed to ligand exchange at the open coordination site, as was mentioned earlier. We hoped that by using a *tridentate* tricarbonyl metal complex to label an ER ligand, we might obtain more effective ER imaging agents in vivo, ones that would be labeled through a small, stable, neutral, and moderately lipophilic metal-containing moiety.

Two 7α-substituted estradiol derivatives have been prepared using a common precursor, a protected 7α-(6-hydroxyhexan-1-yl)estradiol **6**, previously described by Skaddan et al., which was prepared from estradiol via a 6-keto-estradiol intermediate.[19,54,55] Conversion of the alcohol **6** to the 6-aminohexyl **7** proceeded following the literature procedure (Scheme 3), with initial Mitsunobu conditions to produce a phthalimide, and then hydrazinolysis to give the amine **7**. Carbodimide coupling to chelator **1** provided conjugate **8** in 62% yield, with concomitant deprotection of the phenolic hydroxy group. Complete deprotection was carried out with HF, providing amine **9** quantitatively . The rhenium(I) tricarbonyl complex **10a** was then prepared by chelation of **9** with aqueous $Re(CO)_3^+$ and one equivalent of base. The neutral rhenium estrogen conjugate was purified by column chromatography, yielding the metal complex **10a** in 48% yield.

The second 7α-substituted estradiol derivative was prepared as outlined in Scheme 4. The alcohol **6** was converted to methanesulfonate **11**, as previously detailed.[19] The anion of ethyl bis(ethylthio)acetate was prepared using potassium *t*-butoxide in a manner similar to that described by Lerner et al.[56] This

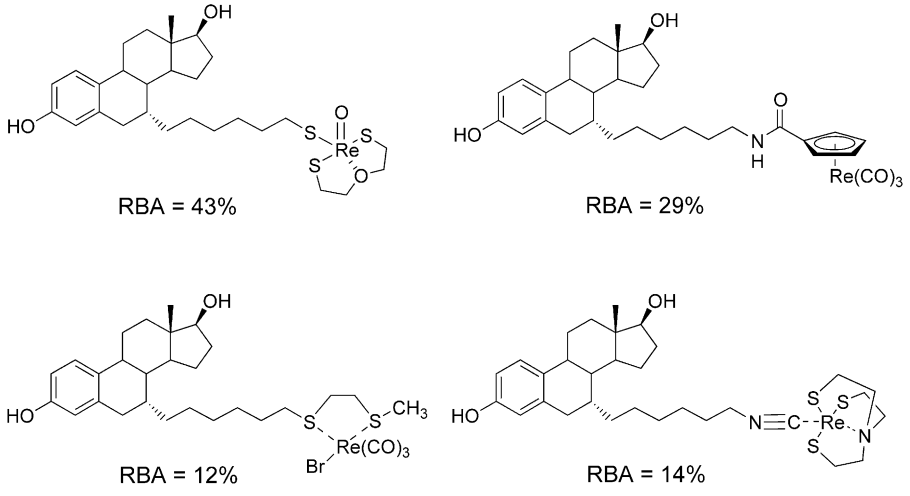
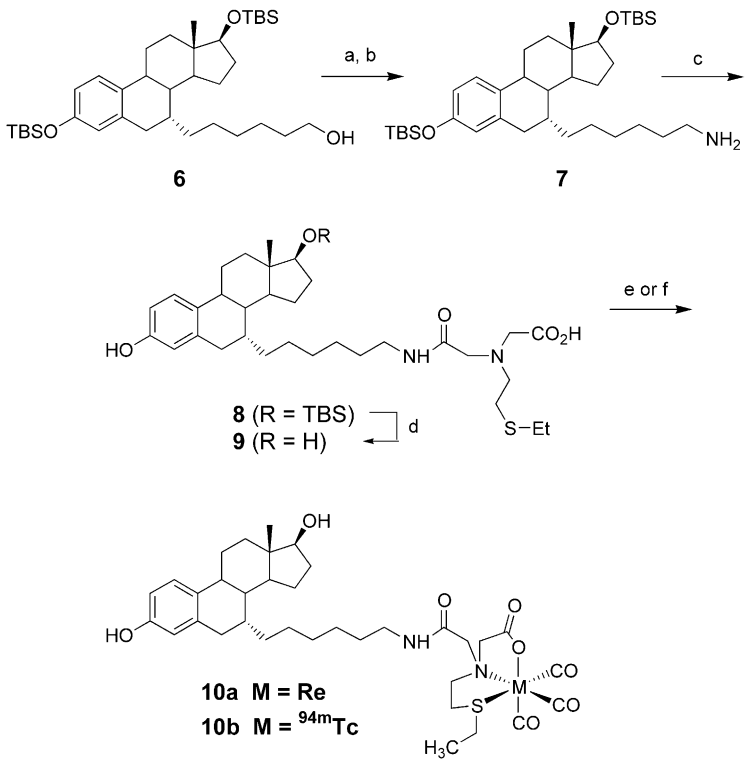
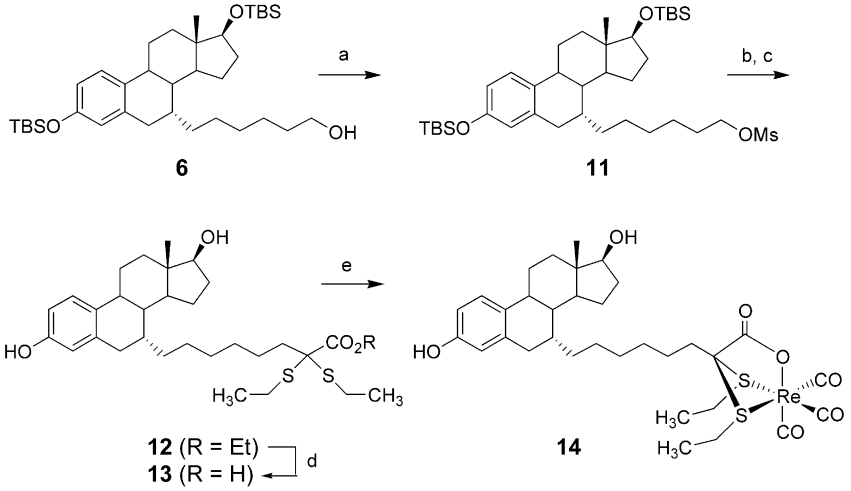


Figure 1. Representative examples of 7α-substituted estradiols with their corresponding relative binding affinity (RBA, estradiol = 100%).



Scheme 3. Reagents and conditions (yield): (a) PPh_3, DEAD, phthalimide, THF;[19] (b) NH_2NH_2, DME, EtOH;[19] (c) **1**, EDC, DMAP, DMF, CH_2Cl_2 (40%); (d) 40% HF, THF, CH_3CN (quant); (e) $Re(CO)_3^+$, H_2O (48%); (f) $^{94m}Tc(CO)_3^+$, H_2O.



Scheme 4. Reagents and conditions (yield): (a) MsCl, NEt_3, THF (quant); (b) $(CH_3CH_2S)_2CHCO_2Et$, KOtBu, THF; (c) 40% HF, THF, CH_3CN (30% two steps); (d) NaOH, H_2O, MeOH (57%); (e) $Re(CO)_3^+$, H_2O (44%).

carbanion then displaced the methanesulfonate, providing the conjugated estradiol as a mixture of di-TBS protected and mono-deprotected material. Attempts to prepare **12** via the alkylation of the anion of 6-keto-estradiol with 8-iodo-2,2-bis(ethanthiol)-octanoic acid ethyl ester, gave a low yield. Complete alcohol deprotection was carried out using HF, providing the estradiol conjugate **12** in moderate yield, followed by base saponification to provide **13**. Rhenium complexation provided the neutral **14**, which was purified by flash chromatography prior to being tested for ER binding affinity.

Design and synthesis of 17α-ethynyl substituted estradiol derivatives

High affinity ligands for the ER can be designed by placing a compact, electron-rich substituent, connected through a rigid linker to estradiol at the 17α-position.[52,57] This concept was extended to Tc/Re estradiol conjugates, examples of which are indicated in Fig. 2.[21,24–26,53]

Attaching the unique bisthioether chelator **3** to estradiol through a rigid alkyne was accomplished by preparing

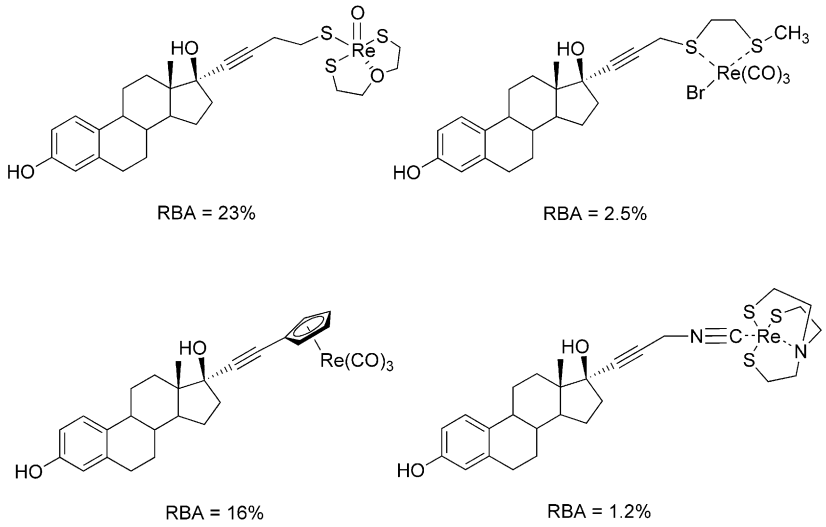
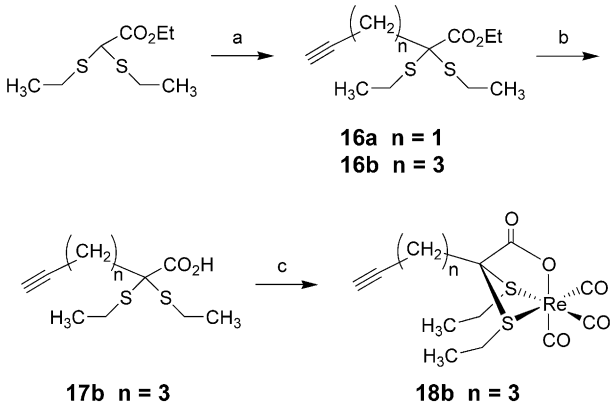


Figure 2. Representative examples of 17α-substituted estradiols with their corresponding relative binding affinity (RBA, estradiol = 100%).



Scheme 5. Reagents and conditions (yield): (a) KOtBu, THF, propargyl bromide or **15** (**16a** 58%, **16b** 57%); (b) NaOH, H_2O, MeOH (90%); (c) $Re(CO)_3^+$, H_2O (49%).

alkynyl–chelator analogues as shown in Scheme 5. We started with commercially available 2,2-bisthioether ethyl acetate and reacted the corresponding anion with propargyl bromide or with 5-iodopentyne to yield the propargylic dithioketal acetate ester **16**. To test the chelation ability of this alkynyl system, the ester **16b** was converted to the acid **17b** in excellent yield. The rhenium(I) tricarbonyl complex **18b** was then prepared by reaction of the chelate system **17b** with triaquatricarbonylrhenium(I) in an aqueous solution.

These two alkynyl compounds provide an excellent means of preparing 17α-substituted estradiol, with a built in rigid linker (Scheme 6). The lithium anions of alkynes **16a** and **16b** were reacted with TBS-protected estrone to provide the conjugated estradiols **19a** and **19b**. Saponification with 3 M NaOH solution gave the carboxylic acid **20a**/**20b** with concomitant deprotection of the phenol. Chelation using triaquatricarbonylrhenium(I) in aqueous medium provided the rhenium(I) complexes **21a** and **21b**.

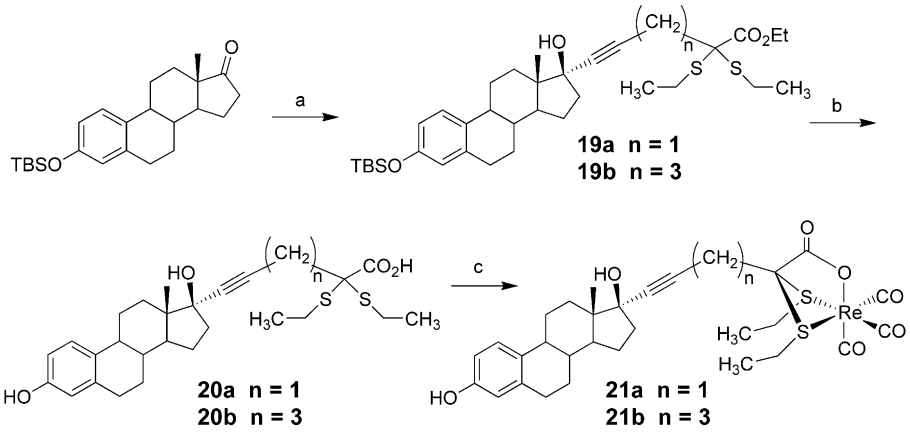
Estrogen receptor binding affinity determination

Table 1 presents the binding affinity of the estrogens developed in this study. Binding affinities were determined by competitive radiometric binding assays with [^{3}H]estradiol, using cytosol preparations of lamb uterus or full-length human ERα and ERβ, as previously described.[58,59] Binding affinities are expressed relative to that of the standard, estradiol, as relative binding affinity (RBA) values, where the affinity of estradiol is 100%.

The binding affinity of the two 7α-substituted estrogen rhenium tricarbonyl tridentate complexes, **10a** and **14**, is very good, being in the nanomolar range. These affinities are very comparable to previously reported 7α-substituted estradiol derivatives, which indicates again the ability of the ER to tolerate a variety of bulky substituents at this position.[19] The binding affinity of these rhenium-labeled estrogens showed no preference for either of the two ER subtypes, ERα or ERβ. The RBA values for the rhenium-chelated 17α-ethynyl substituted estradiol derivatives **21a** and **21b** are much poorer than for the 7α-substituted estradiols, and chain extension from $n = 1$ to $n = 3$ results in a lowering of the RBA. Overall, the 7α-substituted estrogen rhenium conjugates proved to be much better ER ligands than the 17α-substituted ones.

Radiolabelling with technetium-94m, in vivo biodistribution by tissue dissection and microPET imaging in rats

Compound **10a**, which overall had the highest affinity for ER in vitro, was chosen for preliminary in vivo biological assessment. In this manner, we sought to test whether using a neutral, tridentate $M(CO)_3$ chelation unit would result in improved uptake in ER-rich tissues of an animal model. The radiolabelling was carried out with both ^{99m}Tc (data not included) and ^{94m}Tc. Technetium-94m is a positron emitter, which allows for imaging using small-animal PET as well as standard



Scheme 6. Reagents and conditions (yield): (a) **16a** or **16b**, nBuLi, THF (**19a** 53%, **19b** 46%); (b) NaOH, H_2O, MeOH (**20a** 65%, **20b** 78%); (c) $Re(CO)_3^+$, H_2O (**21a** 29%, **21b** 68%).

Table 1. Relative binding affinities (RBA)[a] of rhenium(I) tricarbonyl estrogens

Ligand	RBA (%)		
	Cytosol	ERα	ERβ
Estradiol	100	100	100
10a	18±1	27±3	38±2
14	1.9±0.4	36±9	27±7
21a	0.25±0.04	0.26±0.07	0.14±0.03
21b	0.14±0.01	0.22±0.04	0.072±0.006

[a]Determined by a competitive radiometric binding assay with [^{3}H]estradiol, using cytosol preparations of lamb uterus or full-length human ERα and ERβ; see Experimental for details. Values are reported as the mean±range ($n=2$) or SD ($n>2$). Under these conditions, the K_d for estradiol is 0.2 nM for ERα and 0.5 nM for ERβ.

tissue dissection biodistribution analysis. This radionuclide was produced by proton irradiation (approximately 13 MeV) of an enriched molybdenum target according to the $^{94}Mo(p,n)^{94m}Tc$ nuclear reaction.[60–62] The ^{94m}Tc was separated by sublimation as previously described,[61] and $^{94m}TcO_4^-$ was isolated using a normal phase Sep-Pak® cartridge. We were able to prepare compound **10b**, the technetium-94m-labeled analogue of compound **10a**, by first reducing $^{94m}TcO_4^-$ with sodium borohydride in the presence of CO(g) to give the triaqua cation $^{94m}Tc(CO)_3^+$, and then adding **9** and heating at 80 °C for 20 min. This material (**10b**) was shown to be radiochemically homogeneous and free from chemical impurities after purification by reverse-phase HPLC, and it was used in both tissue distribution and microPET imaging studies of mature female Sprague–Dawley rats.

Table 2 presents the uptake of **10b** in selected tissues of mature (180–200 g) female Sprague–Dawley rats at 1 h post-injection, at 1 h with co-injected estradiol to block the ER, and at 2 h. Uptake in ER-rich, target tissues (uterus and ovaries) was quite low, yet uterus/muscle and ovary/muscle ratios were reasonably high. However, the fact that target tissue uptake and target tissue to muscle ratios did not show a significant reduction when the receptors were blocked by co-injection of a large excess of estradiol indicates that this uptake is not mediated by binding to ER. Of the tissues assayed, highest uptake was found in the liver, though these values are within the ranges reported for estradiol compounds labeled with ^{18}F.[2,4–7]

MicroPET images of **10b** in a mature female Sprague–Dawley rat, at four times after injection, are shown in Figure 3. Although activity was evident in large organs, in these images, we were unable to resolve the smaller organs, including the target tissues (uterus and ovaries). This is due to the long positron range distribution of ^{94m}Tc, which results in a strong contrast reduction of the images. Because ^{94m}Tc has a transaxial resolution of 4.3 mm,[63] it is necessary to perform tissue biodistribution studies in conjunction to the microPET imaging studies. Work is underway on developing reconstruction algorithms that may account for these characteristics and therefore improve image quality of ^{94m}Tc-labeled radiopharmaceuticals.

In the microPET images (Fig. 3), high liver uptake was noted at early times, followed by substantial uptake in the gastrointestinal system. Most striking, however, was the very high stomach activity levels evident in these images. While it is not surprising to find high intestinal activity following injection of lipophilic radiopharmaceuticals that undergo elimination by a hepatobiliary route, the high activity levels in the stomach were unexpected. One should note that this site of activity deposition, clearly apparent on the microPET images, would likely have been missed entirely if the distribution studies had relied solely on tissue dissection experiments, which do not routinely include the stomach as a potential site of uptake. This highlights the utility of using microPET imaging paired with the positron-emitting radionuclide technetium-94m to expedite the evaluation of novel technetium-labeled radiopharmaceuticals.

At this point, we do not know the mechanism by which activity becomes deposited in the stomach after injection of **10b**, nor its chemical form. We believe, however, it is likely that at the site of acidic gastric secretions, the chelate donor heteroatoms become protonated and the complex decomposes, releasing the free $^{94m}Tc(CO)_3$ cation that is then deposited in the surrounding tissue from which it is cleared more slowly than the original

Table 2. Biodistribution of **10b** in female Sprague–Dawley rats[a]

Tissue	1 h	1 h block	2 h
Blood	0.84±0.07	0.56±0.29	0.48±0.02
Lung	1.55±0.15	1.07±0.77	1.38±0.18
Liver	3.55±0.21	2.24±1.54	3.54±0.25
Spleen	1.04±0.04	0.71±0.51	0.97±0.09
Kidney	1.76±0.16	1.08±0.71	1.61±0.26
Heart	0.69±0.05	0.48±0.35	0.67±0.07
Uterus	0.17±0.05	0.15±0.11	0.20±0.05
Ovaries	0.65±0.14	0.58±0.42	0.58±0.08
Muscle	0.06±0.00	0.05±0.03	0.06±0.01
Uterus/blood	0.20±0.06	0.23±0.10	0.41±0.09
Uterus/muscle	2.64±0.86	2.90±0.71	3.10±0.41
Ovaries/blood	0.77±0.13	0.91±0.37	1.19±0.14
Ovaries/muscle	10.02±1.56	11.69±3.23	9.14±0.45

[a]% Injected dose per gram; $n=3$ for each time point; 1 h block indicates co-administration of 15 μg estradiol.

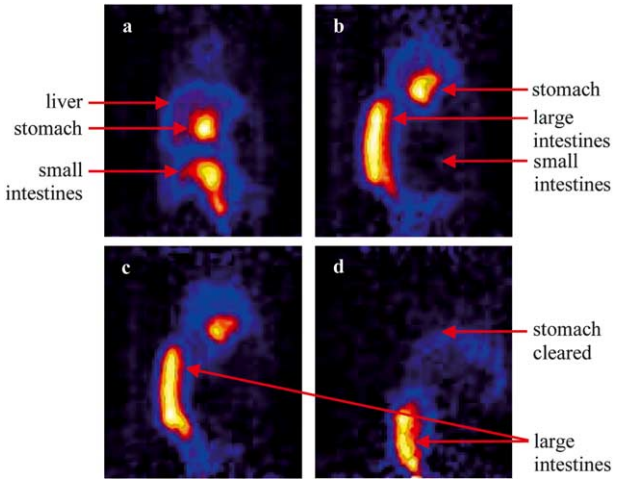


Figure 3. Coronal microPET image slices of a female Sprague–Dawley rat at 5 (a), 30 (b), 60 (c), and 120 (d) min post-injection of compound **10b** (87 μCi). Note: the entire rat was in the imaging plane in panel (a), whereas in panels (b)–(d), the rat was imaged from the neck down.

complex **10b**. If this proves to be the case, then our observation of high stomach activity suggests that this tissue should be routinely examined when the biodistribution of radiolabeled metal chelates is being determined by the commonly used tissue dissection method.

Conclusions

Novel tridentate $Re(CO)_3$ chelates substituted at the 7α- and 17α- positions of estradiol gave estrogen conjugates having good in vitro binding for the ER. Compounds **14**, **21a**, and **22a**, in particular, are unique in their conjugation through an aliphatic chain, with the absence of an amide or ester linkage that might be susceptible to hydrolysis. Despite favorable ER binding of these $Re(CO)_3$ conjugates in vitro, the highest affinity of these novel metal tricarbonyl conjugates, prepared as the ^{94m}Tc labeled analogue, did not show target tissue-directed biodistribution in vivo. An integrated approach to the labeling of estrogens with technetium—in which the metal center is buried more centrally in the core of the ligand structure—may prove to be a more successful approach for developing ER imaging agents.[64] Nevertheless, it is notable that by using microPET to image the biodistribution of the ^{94m}Tc labeled estrogen, we were able to identify a major site of activity deposition, the stomach, that would have been missed by tissue distribution studies as they are routinely performed.

Experimental

Reagents and solvents were purchased from Acros, Aldrich, Fisher, Strem, or TCI. Estrone was purchased from Steraloids (Newport, RI, USA). Methylene chloride and tetrahydrofuran were dried by a Solvent Delivery System (neutral alumina columns) fabricated by J. C. Meyer, based on a design published by Pagborn et al.[65] Methods for the production and the purification of ^{94m}Tc by sublimation have been previously reported.[61] The Re(I) aqua ion, $[Re(H_2O)_3(CO)_3]^+$, was prepared by refluxing $Re(CO)_5Cl$ in water over-night, with the Re concentration being determined by ICP analysis. All air-sensitive reactions were carried out under a nitrogen or argon atmosphere, using flame or oven-dried glassware. Reaction progress was monitored by analytical thin-layer chromatography on silica gel medium. Flash chromatography was performed using Woelm silica gel (0.040–0.063 mm) packing. Hexane for chromatography was distilled prior to use.

^1H and ^{13}C NMR spectra were obtained using a Unity 400, Unity 500, or Unity INOVA 500NB Varian FT-NMR spectrometer. Chemical shifts (δ) are reported in parts per million downfield from internal tetramethylsilane and referenced from solvent resonances. NMR coupling constants are reported as absolute values in Hertz. Low resolution electron impact (EI) mass spectra and high resolution EI mass spectra (HRMS) were obtained on Micromass 70-VSE, or Micromass 70-SE-4F spectrometers. Both low and high resolution fast atom bombardment (FAB) mass spectra were obtained on Micromass ZAB-SE and 70-SE-4F spectrometers, respectively. Melting points were determined on a Thomas Hoover melting point apparatus and are uncorrected.

***N*-(2-Ethanethioethyl)iminodiacetic acid (1)**. Iminodiacetic acid (0.61 g, 4.58 mmol) was dissolved in 10 mL water and 30 mL ethanol, with 4.6 mL of a 1.0 M NaOH solution. To this stirred solution was added an additional 4.6 mL 1.0 M NaOH solution and 540 μL ethyl chloroethyl sulfide (4.6 mmol) portionwise over 10 min. The flask was then heated at 70 °C, with the reagents remaining dissolved. After 12 h, an additional 4.6 mL 1.0 M NaOH solution was added, and heating was continued for 4 h. The reaction was cooled to rt; the solvents were removed by a rotary evaporator, and 20 mL water was added with the slurry being heated to 80 °C. To this was added barium chloride dihydrate (1.12 g, 4.58 mmol) in 3 mL hot water. The mixture was heated at 80 °C for 30 min, and then cooled to 0 °C. The resulting white precipitate was removed by filtration

and dried under vacuum overnight, yielding 0.90 g of the barium salt. A slurry was made with the barium salt in water, to which was added 140 μL concd sulfuric acid, and the mixture was heated at 80 °C for 1 h. The solution was hot filtered, and the product was recovered by evaporation of the filtrate yielding 0.46 g (46%). ^{1}H NMR (D_2O): δ 1.09 (t, 3H, J = 7.3 Hz), 2.49 (q, 2H, J = 7.3 Hz), 2.82 (t, 2H, J = 7.1 Hz), 3.40 (t, 2H, J = 7.1 Hz), 3.89 (s, 4H). ^{13}C NMR (D_2O): δ 13.76, 24.92, 24.92, 54.41, 55.97, 169.45. MS (FAB): m/z 244 (100%, $M^+ + H + Na$), 222 (86%, $M^+ + H$); HRMS calcd $C_8H_{16}NO_4S$, 222.0800, found 222.0799.

***N*-(2-Ethanethioethyl)iminodiacetic acid rhenium(I) tricarbonyl (2)**. The iminodiacetic acid **1** (12 mg, 0.05 mmol) was dissolved in 0.5 mL H_2O and heated to 70 °C. $Re(CO)_3^+$ in an aqueous solution (0.55 mL, 0.098 M) was added, and the reaction was stirred at 70 °C for 30 min with a white ppt forming. The product was filtered, washed with H_2O and dried under vacuum to yield 23 mg (88%). MS (FAB): m/z 492 ($M[^{187}Re]^+ + H$, 100%), 490 ($M[^{185}Re]^+ + H$, 57%); HRMS calcd $C_{11}H_{15}NO_7S^{187}Re$, 492.0127, found 492.0125.

2,2-Bis(ethylthio)acetic acid (3). This compound was prepared as previously described.[51] ^{1}H NMR ($CDCl_3$): δ 1.27 (t, 6H, J = 7.5 Hz), 2.74 (m, 4H,), 4.35 (s, 1H), 11.40 (br s, 1H). ^{13}C NMR ($CDCl_3$): δ 14.01, 25.19, 49.74, 175.87. MS (EI): m/z 180 (M^+, 27%); HRMS calcd $C_6H_{12}O_2S_2$, 180.0279, found 180.0279.

2,2-Bis(ethanethio)-3-phenylpropanoic acid (4). A dry argon filled flask was charged with the thioketal acetic acid **3** (132 mg, 0.74 mmol) and 8 mL THF. The vessel was cooled to −23 °C, and then 0.81 mL of 2.0 M *n*-butyl lithium solution (1.62 mmol) was slowly added, producing a precipitate that soon re-dissolved. The solution was maintained at −23 °C for 1 h, and was then cooled to −78 °C, at which point benzyl bromide (87 μL, 0.74 mmol) was added. The reaction was kept at −78 °C for 50 min, and then warmed to rt and stirred overnight. The reaction was quenched with water, acidified with 1 M HCl, and extracted with ethyl acetate (3×). The combined organics were washed with brine, dried over Na_2SO_4, and evaporated to dryness in vacuo. Purification by flash chromatography (silica, gradient: 0–10% MeOH/CH_2Cl_2) yielded **4** (136 mg, 68%) as a clear colorless oil. ^{1}H NMR ($CDCl_3$): δ 1.25 (t, 6H, J = 7.5 Hz), 2.70 (m, 4H), 3.35 (s, 2H), 7.26–7.33 (5H). ^{13}C NMR ($CDCl_3$): δ 13.34, 24.53, 42.49, 65.17, 127.30, 128.05, 130.59, 135.23, 176.21. MS (EI): m/z 270 (M^+, 8%); HRMS calcd $C_{13}H_{18}O_2S_2$, 270.0748, found 270.0743.

2,2-Bis(ethanethio)-3-phenylpropanoic acid rhenium(I) tricarbonyl (5). The chelator **4** (29 mg, 0.11 mmol) was dissolved in 1 mL water, 0.5 mL MeOH, and 120 μL 1 M NaOH solution. The solution was heated to 60 °C, and then 1.1 mL of a 0.098 M $Re(CO)_3^+$ aqueous solution was added and a ppt formed. After 20 min, the reaction was cooled to rt and the solvents were removed by rotary evaporation. The product was dissolved in $CHCl_3$, filtered, and the filtrate was evaporated to dryness. Purification was carried out by flash chromatography (silica, gradient: 0–2% MeOH/CH_2Cl_2) yielding 46 mg (79%) of **5**. ^{1}H NMR ($CDCl_3$): δ 1.30 (t, 6H, J = 7.5), 2.61 (dq, 2H, J = 7.5, 11.6), 2.79 (dq, 2H, J = 7.5, 11.6), 3.47 (s, 2H), 7.33–7.46 (5H). ^{13}C NMR ($CDCl_3$): δ 12.20, 32.02, 46.54, 80.89, 128.17, 128.79, 131.04, 133.12, 191.53, 194.28, 197.99. MS (FAB, m.b.): m/z 541 ($M[^{187}Re]^+ + H$, 100%), 539 ($M[^{185}Re]^+ + H$, 46%); HRMS calcd $C_{16}H_{18}O_5S_2^{187}Re$, 541.0153, found 541.0153.

7α-(6-(*N*-(acetylimino-*N*′-acetic acid-*N*′-ethylthioethyl)-amino)hexan-1-yl)-estra-1,3,5(10)-triene-3,17β-diol (8). 7α-(6-Aminohexan-1-yl)-3,17α-bis(t-butyldimethylsilanyloxy)-estra-1,3,5(10)-trienediol (**7**, 30.9 mg, 0.052 mmol),[19] **1** (12.5 mg, 0.057 mmol), EDC (10.9 mg, 0.057 mmol), and dimethylaminopyridine (3 mg, 0.02 mmol) were combined in a flask with 4 mL DMF and 2 mL CH_2Cl_2. The mixture was stirred at rt for 2 days, at which time the reaction was deemed complete by tlc (20% MeOH/80% $CHCl_3$). After solvent removal under vacuum, the solids were dissolved in chloroform and separated by flash chromatography (silica, gradient: 10–30% MeOH/$CHCl_3$), yielding 22 mg of crude product (62%). This was partitioned between ethyl acetate and 0.01 M HCl, with two subsequent ethyl acetate extractions. The combined organics were combined, washed with brine, dried with Na_2SO_4, evaporated under vacuum to give a clear colorless glassy solid (14 mg, 40%). ^{1}H NMR ($CDCl_3$): δ 0.02 (s, 3H), 0.03 (s, 3H), 0.74 (s, 3H), 0.89 (s, 9H), 1.16–1.96 (21H), 2.25 (m, 2H), 2.52 (q, 2H, J = 7.6 Hz), 2.62–2.86 (5H), 2.99 (m, 2H), 3.22 (m, 2H), 3.50 (m, 4H), 3.65 (t, 2H, J = 8.1 Hz), 6.58 (m, 1H), 6.63 (m, 1H), 7.11 (d, 1H, J = 8.3 Hz), 7.71 (m, 1H, NH). ^{13}C NMR ($CDCl_3$): δ −4.51, −4.83, 11.37, 14.66, 18.07, 22.75, 25.15, 25.83, 25.97, 26.52, 27.31, 27.65, 28.94, 29.21, 30.90, 33.32, 34.69, 37.34, 38.36, 39.46, 42.04, 43.69, 46.08, 54.16, 55.35, 57.56, 81.83, 113.05, 116.23, 127.03, 131.51, 136.98, 153.87, 164.51, 172.35. MS (FAB): m/z 690 ($M^+ + H$, 78%); HRMS calcd $C_{38}H_{65}N_2O_5SiS$, 689.4383, found, 689.4383.

7α-(6-(*N*-(Acetylimino-*N*′-acetic acid-*N*′-ethylthioethyl)-amino)hexan-1-yl)-estra-1,3,5(10)-triene-3,17β-diol (9). A flask containing **8** (12.4 mg, 0.017 mmol) was charged with 1 mL THF and 0.5 mL acetonitrile. The solution was heated to 50 °C, 0.1 mL 40% HF was added, and the reaction was stirred at 50 °C for 20 min. The reaction was made neutral with satd $NaHCO_3$ and extracted into methylene chloride (3×). Removal of the solvent yielded 11 mg (quant) of **9**. ^{1}H NMR (CD_3OD): δ 0.77 (s, 3H), 1.25 (t, 3H, J = 7.3 Hz), 1.28–1.54 (14H), 1.60 (m, 2H), 1.73 (m, 1H), 1.91 (m, 1H), 2.03 (m, 1H), 2.29 (m, 2H), 2.60 (q, 2H, J = 7.3 Hz), 2.68 (d, 1H, J = 16.6 Hz), 2.81 (dd, 1H, J = 4.9, 16.6 Hz), 2.89 (t, 2H, J = 7.6 Hz), 3.22 (t, 2H, J = 7.1 Hz), 3.52 (t, 2H, J = 7.6 Hz), 3.66 (t, 2H, J = 8.7 Hz), 4.11 (s, 2H), 4.23 (s, 2H), 6.45 (d, 1H, J = 2.4 Hz), 6.54 (dd, 1H, J = 2.4, 8.5 Hz), 7.07 (d, 1H, J = 8.5 Hz). MS (FAB): m/z 575 ($M^+ + H$, 30%); HRMS calcd $C_{32}H_{51}N_2O_5S$, 575.3519, found 575.3517.

7α-(6-(*N*-(Acetylimino-*N*′-acetic acid-*N*′-ethylthioethyl)-amino)hexan-1-yl)-estra-1,3,5(10)-triene-3,17β-diol rhenium(I) tricarbonyl (10a). The estradiol analogue **9** (8.1

mg, 0.014 mmol) was dissolved in 1.5 mL MeOH, 1.5 mL H_2O, and 30 μL of 1.0 M KOH. The solution was heated to 70 °C, and 139 μL of 0.098 M $Re(CO)_3^+$ aqueous cation in solution was added, with a cloudy suspension immediately forming. After 45 min, the mixture was cooled to 0 °C, 2 mL of H_2O was added, the solid was removed by filtration, washed with H_2O, and dried under vacuum. Purification was carried out by flash chromatography (silica, 10% MeOH/90% $CHCl_3$) to give **10a** as a white solid (5.5 mg, 48%). ^{1}H NMR (CD_3OD): δ 0.77 (s, 3H), 1.20–1.53 (18H), 1.60 (m, 2H), 1.74 (m, 1H), 1.91 (m, 1H), 2.03 (m, 1H), 2.29 (m, 2H), 2.66 (m, 2H), 2.78–2.95 (m, 2H), 3.17 (m, 4H), 3.34 (m, 1H), 3.66 (t, 1H, J = 8.5 Hz), 3.80 (m, 1H), 3.96–4.32 (m, 4H), 6.45 (d, 1H, J = 2.4 Hz), 6.53 (dd, 1H, J = 2.6, 8.3 Hz), 7.07 (d, 1H, J = 8.3 Hz). MS (FAB): m/z 845 (M[^{187}Re]$^+$ + H, 3%), 843 (M[^{185}Re]$^+$ + H, 2%); HRMS calcd $C_{35}H_{50}N_2O_8S^{187}Re$, 845.2846, found, 845.2845.

7α-(6-(*N*-(Acetylimino-*N*′-acetic acid-*N*′-ethylthioethyl)-amino)hexan-1-yl)-estra-1,3,5(10)-triene-3,17β-diol technetium-94m(I) tricarbonyl (10b). A pressure vessel was charged with sodium borohydride (5 mg, 0.13 mmol), sodium carbonate (5 mg, 0.05 mmol), and a stir bar. The vessel was flushed with CO (g) for 5 min, and then 14.6 mCi of $^{94m}TcO_4^-$ in 0.4 mL saline was added while under CO flow. The pressure vessel was sealed and heated at 80 °C for 30 min in an aluminum block, behind a safety shield. The vessel was then cooled to 0 °C, opened, and 0.3 mL of a buffer (2 parts 1 M HCl, 1 part 1 M Na_2HPO_4) was added. After brief mixing, the ligand **9** (1 mg, 0.002 mmol), dissolved in 0.1 mL methanol, was added. The pressure vessel was sealed and heated at 80 °C for 20 min (see safety precautions above), followed by cooling to 0 °C. The reaction solution was passed through a syringe filter (0.2 μm) and rinsed with 0.5 mL H_2O. The product was eluted from the filter with 0.5 mL CH_3CN to yield 1.74 mCi (27% decay corrected radiochemical yield) of crude product. Purification was carried out by reversed-phase C_{18} HPLC, using an eluent composition of 65% CH_3CN/35% H_2O. The fractions containing the product (as determined by prior HPLC analysis of **10a**) were combined, extracted with ether (2×), dried over Na_2SO_4, and gently evaporated to dryness yielding 303 μCi of **10b** (8% decay corrected radiochemical yield). The product was dissolved in 20 μL ethanol, and then diluted with 180 μL saline containing 0.1% Tween-80, and the injectable solution was divided into syringes in the required dosage.

3,17β-Bis(*t*-butyldimethylsilanyloxy)-7α-[6-(methanesulfonyloxy)-hexan-1-yl]-estra-1,3,5(10)-triene (11). 3,17β-Bis(*t*-butyldimethylsilanyloxy)-7α-[6-hydroxy-hexan-1-yl]-estra-1,3,5(10)-triene (**6**, 106 mg, 0.18 mmol) was dissolved in 15 mL THF and 49 μL triethylamine (0.35 mmol). The solution was cooled to 0 °C, and then methanesulfonyl chloride (27 μL, 0.35 mmol) was added, and the reaction was stirred overnight with warming to rt. The mixture was partitioned between satd $NaHCO_3$ and $CHCl_3$ (3×), the organics were combined and evaporated in vacuo. Purification by flash chromatography (silica, 10% ethyl acetate/90% hexane) provided **11** (121 mg, 0.18 mmol) in quantitative yield. ^{1}H NMR ($CDCl_3$): δ 0.03 (s, 3H), 0.04 (s, 3H), 0.20 (s, 6H), 0.75 (s, 3H), 0.90 (s, 9H), 0.98 (s, 9H), 1.18–1.75 (18H), 1.84 (m, 1H), 1.94 (m, 1H), 2.27 (m, 2H), 2.68 (d, 1H, J = 16.7 Hz), 2.85 (dd, 1H, J = 4.9, 16.3 Hz), 2.99 (s, 3H), 3.67 (t, 1H, J = 8.4 Hz), 4.20 (t, 2H, J = 6.5 Hz), 6.54 (d, 1H, J = 2.6 Hz), 6.62 (dd, 1H, J = 2.6, 8.6 Hz), 7.12 (d, 1H, J = 8.6 Hz). ^{13}C NMR ($CDCl_3$): δ −4.84, −4.52, −4.43, 11.33, 18.06, 22.75, 25.46, 25.65, 25.82, 27.24, 27.98, 29.08, 29.38, 30.88, 33.24, 34.53, 37.30, 38.22, 41.88, 43.67, 46.04, 70.10, 81.79, 117.18, 120.79, 126.69, 132.51, 136.70, 153.22.

7α-[7-Carboxyethyl-7,7-bis(ethanethio)-heptan-1-yl]-estra-1,3,5(10)-triene-3,17β-diol (12). A flame-dried, nitrogen filled flask was charged with ethyl bis(ethanethio)acetate (41 mg, 0.19 mmol) and 5 mL THF. The solution was cooled to 0 °C, and then 194 μL of 1.0 M potassium *t*-butoxide solution (THF) was added and the reaction was stirred at 0 °C for 30 min. The mesylate **11** (120 mg, 0.18 mmol) was added in 2 mL THF, the mixture was stirred at 0 °C for 2 h, and then at rt overnight. The reaction was quenched with satd NH_4Cl, the organics were extracted into $CHCl_3$ (3×), dried over $MgSO_4$, and evaporated to dryness. Flash chromatography (silica, gradient: 10–20% ethyl acetate/hexanes) provided the desired product as a mixture of mono-protected and di-protected alcohols. Complete deprotection was carried out by dissolving the mixture in 2 mL acetonitrile and 0.2 mL THF, heating to 60 °C, and adding 0.3 mL of 40% HF. After 15 min, the reaction was quenched with sat. $NaHCO_3$, extracted into CH_2Cl_2 (3×), dried over Na_2SO_4, and evaporated in vacuo to give the crude **12**. Purification was carried out by flash chromatography (silica, 30% ethyl acetate/70% hexanes) to give **12** (30 mg, 30% over two steps) as a clear colorless glassy solid. ^{1}H NMR ($CDCl_3$): δ 0.78 (s, 3H), 1.18–1.64 (26H), 1.72 (m, 1H), 1.89 (m, 2H), 2.13 (m, 1H), 2.29 (m, 2H), 2.51 (m, 1H), 2.60 (m, 4H), 2.70 (m, 1H), 2.85 (m, 1H), 3.76 (t, 1H, J = 8.5 Hz), 4.22 (q, 2H, J = 7.1 Hz), 5.05 (br s, 1H), 6.55 (d, 1H, J = 2.4 Hz), 6.63 (dd, 1H, J = 2.7, 8.4 Hz), 7.14 (d, 1H, J = 8.4 Hz). ^{13}C NMR ($CDCl_3$): δ 11.04, 13.58, 14.09, 22.59, 23.64, 25.24, 25.49, 27.19, 28.03, 29.49, 29.54, 29.68, 30.46, 33.10, 34.50, 36.22, 36.80, 37.98, 41.88, 43.33, 46.37, 62.01, 65.44, 82.02, 112.82, 116.13, 127.05, 131.79, 137.07, 153.43, 170.82 . MS (EI) m/z 562 (M^+, 32%); HRMS calcd $C_{32}H_{50}O_4S_2$, 562.3151, found 562.3153.

7α-[7-Carboxy-7,7-bis(ethanethio)-heptan-1-yl]-estra-1,3,5(10)-triene-3,17β-diol (13). The ester **12** (26 mg, 0.046 mmol) was dissolved in 5 mL methanol and 3 mL 3 M NaOH solution. The mixture was heated at reflux for 3 h, cooled to rt, and the methanol was removed by rotary evaporation. The solution was then diluted with water, acidified with 3 M HCl, and then extracted with CH_2Cl_2 (3×). The combined organics were washed with brine, dried over Na_2SO_4, and evaporated to dryness in vacuo. Purification by flash chromatography (silica, ethyl acetate then 20% MeOH/80% $CHCl_3$) yielded the pure **13** (14 mg, 57%). ^{1}H NMR ($CDCl_3$): δ 0.76 (s, 3H), 1.15–1.60 (22H), 1.70–1.82 (m, 4H), 1.94 (m, 1H), 2.03 (m, 1H), 2.27 (m, 2H), 2.57 (m, 4H), 2.67 (m, 1H), 2.80 (m, 1H), 3.66 (t, 1H, J = 8.6 Hz), 6.45 (d, 1H,

$J=2.4$ Hz), 6.53 (dd, 1H, $J=2.5$, 8.6 Hz), 7.06 (d, 1H, $J=8.6$ Hz). ^{13}C NMR ($CDCl_3$): δ 11.72, 14.13, 18.35, 23.66, 24.70, 26.60, 28.62, 29.17, 30.68, 30.75, 31.07, 34.63, 35.75, 38.06, 38.24, 39.61, 43.67, 44.52, 47.78, 58.32, 82.57, 113.95, 116.96, 127.90, 131.81, 137.61, 156.01, 174.00. MS (FAB) m/z 535 ($M^+ + H$, 26%); HRMS calcd $C_{30}H_{46}O_4S_2$, 534.2838, found 534.2840.

7α-[7-carboxy-7,7-bis(ethanethio)-heptan-1-yl]-estra-1,3,5(10)-triene-3,17β-diol rhenium(I) tricarbonyl (14). The estradiol analogue **13** (12 mg, 0.022 mmol) was dissolved in 0.5 mL MeOH, 1 mL H_2O, and 23 μL 1 M NaOH solution. The mixture was heated to 60 °C at which point 202 μL of a 0.122 M $Re(CO)_3^+$ aqueous solution was added, with the solution soon turning turbid. After 10 min tlc (silica, 20% MeOH/80% $CHCl_3$) indicated the complete disappearance of the starting **13** and the formation of a new, less polar product. The solvents were then removed in vacuo, and the product purified by flash chromatography (silica, gradient 5–10% MeOH/$CHCl_3$) to give 8 mg (44%) of **14**. ^{1}H NMR ($CDCl_3$): δ 0.78 (s, 3H), 1.20–1.73 (24H), 1.93 (m, 3H), 2.13 (m, 1H), 2.30 (m, 2H), 2.71 (m, 1H), 2.79 to 3.00 (5H), 3.73 (m, 1H), 6.56 (d, 1H, $J=2.8$ Hz), 6.63 (dd, 1H, $J=2.6$, 8.6 Hz), 7.15 (d, 1H, $J=8.6$ Hz). MS (FAB) m/z 805 ($M[^{187}Re]^+ + H$, 54%), 803 ($M[^{185}Re]^+ + H$, 37%); HRMS calcd $C_{33}H_{46}O_7S_2$, 805.2243, found, 805.2241.

5-Iodopentyne (15). 5-Chloropentyne (1.12 g, 10.9 mmol), sodium iodide (8.2 g, 55 mmol), and acetone were heated at reflux overnight. The mixture was cooled to rt, filtered, and the filtrate was evaporated to dryness. The crude material was partitioned between water and CH_2Cl_2 (3×), dried over Na_2SO_4, and the solvent was removed by rotary evaporation, with final drying under a steady stream of nitrogen to yield 1.9 g (90%) of **15** as a clear and colorless oil. ^{1}H NMR ($CDCl_3$): δ 1.99 (t, 1H, $J=2.7$ Hz), 2.00 (quintet, 2H, $J=6.8$ Hz), 2.34 (dt, 2H, $J=2.7$, 6.8 Hz), 3.31 (t, 2H, $J=6.8$ Hz). ^{13}C NMR ($CDCl_3$): δ 5.06, 19.39, 31.75, 69.45, 82.24.

2,2-Bis-(ethanethio)-pent-4-ynoic acid ethyl ester (16a). A flame-dried flask was charged with 2,2-bis-(ethanethio)acetate (1.00 g, 4.80 mmol) and 6 mL THF, and then cooled to 0 °C. Slowly added 4.8 mL 1.0 M potassium *t*-butoxide solution (in THF) to the flask and warmed the solution to room temperature. After 30 min, propargyl bromide (80% solution, 535 μL, 4.80 mmol) was added, and the mixture was stirred for 5 h. The reaction was quenched with satd NH_4Cl, extracted with diethyl ether (3×), and the combined organics were washed with brine, dried with Na_2SO_4, evaporated and dried under vacuum. The crude material was purified by flash chromatography (silica, 10% ethyl acetate/90% heaxanes) to yield 0.69 g of **16a** as a clear colorless oil (58%). ^{1}H NMR ($CDCl_3$): δ 1.24 (t, 6H, $J=7.5$ Hz), 1.32 (t, 3H, $J=7.2$ Hz), 2.12 (t, 1H, $J=2.6$ Hz), 2.70 (m, 4H), 2.93 (d, 2H, $J=2.6$ Hz), 4.27 (q, 2H, $J=7.2$ Hz). ^{13}C NMR ($CDCl_3$): δ 13.54, 14.01, 24.33, 28.38, 62.41, 62.71, 71.48, 78.85, 169.44 . MS (EI): m/z 246 (M^+, 100%); HRMS calcd $C_{11}H_{18}O_2S_2$, 246.0748, found, 246.0751.

2,2-Bis(ethanethio)-hept-6-ynoic acid ethyl ester (16b). A flame-dried flask was charged with 2,2-bis(ethanethio)-acetate (1.02 g, 4.90 mmol) and 10 mL THF, and then cooled to 0 °C. A potassium *t*-butoxide solution (5.4 mL 1.0 M in THF) was slowly added to the flask and stirred for 30 min at 0 °C. 5-Iodopentyne (601 μL, 4.90 mmol) was then added and the mixture was stirred for 2 h at 0 °C, and then warmed to rt and stirred overnight. The reaction was quenched with satd NH_4Cl, extracted with diethyl ether (3×), the combined organics were washed with brine, dried with Na_2SO_4, evaporated and dried under vacuum. The crude material was purified by flash chromatography (silica, 10% ethyl acetate/90% hexanes) and was then placed under vacuum at 130 °C (0.3 mm Hg) to remove residual 2,2-bisthioetherethyl acetate, yielding 0.76 g of **16b** as a clear colorless oil (57%). ^{1}H NMR ($CDCl_3$): δ 1.22 (t, 6H, $J=7.5$ Hz), 1.30 (t, 3H, $J=7.1$ Hz), 1.70 (m, 2H), 1.96 (t, 1H, $J=2.6$ Hz), 2.05 (m, 2H), 2.23 (dt, 2H, $J=2.6$, 6.9 Hz), 2.63 (dq, 4H, $J=1.5$, 7.5 Hz), 4.23 (q, 2H, $J=7.1$ Hz). ^{13}C NMR ($CDCl_3$): δ 13.52, 14.08, 18.19, 23.62, 24.22, 35.19, 62.08, 64.79, 68.86, 83.65, 170.46. MS (EI): m/z 274 (M^+, 1%), 246 ($M^+ - Et$, 5%); HRMS calcd $C_{13}H_{22}O_2S_2$, 274.1061, found, 274.1059.

2,2-Bis(ethanethio)-hept-6-ynoic acid (17b). The ester **16b** (155 mg, 0.57 mmol) was dissolved in 6 mL MeOH, to which 3 mL 3 M NaOH solution was added. The mixture was heated at 40 °C for 6 h, and then was cooled to rt and diluted with H_2O. Unreacted ester was removed by ether extraction, followed by acidification, and the product was extracted with ether (3×), washed with brine, dried over $MgSO_4$. The solvent was removed by rotary evaporation, and then dried under vacuum to give **17b** as a clear colorless oil (125 mg, 90%). ^{1}H NMR ($CDCl_3$): δ 1.24 (t, 6H, $J=7.5$ Hz), 1.77 (m, 2H), 1.97 (t, 1H, $J=2.7$ Hz), 2.06 (m, 2H), 2.24 (dt, 2H, $J=2.7$, 6.8 Hz), 2.67 (dq, 4H, $J=1.9$, 7.5 Hz). ^{13}C NMR ($CDCl_3$): δ 13.34, 18.17, 23.80, 24.09, 34.81, 64.08, 68.99, 83.51, 176.50 . MS (EI): m/z 247 (M^+, 10%); HRMS calcd $C_{11}H_{18}O_2S_2$, 246.0748, found 246.0747.

2,2-Bis(ethanethio)-hept-6-ynoic acid rhenium(I) tricarbonyl (18b). The acid **17b** (31 mg, 0.13 mmol) was dissolved in 0.5 mL MeOH, 1 mL H_2O, and 126 μL 1.0 M NaOH solution. The mixture was heated to 60 °C, and then 1.24 mL of a 0.122 M $Re(CO)_3^+$ aqueous solution was added. After 15 min tlc (silica, 5% MeOH/95% $CHCl_3$) indicated the absence of s.m., and the reaction was cooled to 0 °C. The beige ppt. was removed by filtration to give 65 mg of crude product. Purification by flash chromatography (silica, gradient: 0–2% MeOH/CH_2Cl_2) yielded 32 mg of **18b** (49%). ^{1}H NMR ($CDCl_3$): δ 1.39 (t, 6H, $J=7.5$ Hz), 1.82 (m, 2H), 2.01 (t, 1H, $J=2.8$ Hz), 2.13 (m, 2H), 2.35 (dt, 2H, $J=2.6$, 6.6 Hz), 2.85 (m, 2H), 3.00 (m, 2H).

17α-(4′,4′-Bis(ethanethio)-4′-ethoxycarbonyl-butyn-1′-yl)-3-*t*butyldimethylsilanyloxy-estra-1,3,5(10)-triene-17β-ol (19a). A flame-dried flask was charged with alkyne **16a** (290 mg, 1.18 mmol) and 20 mL THF, and then was cooled to −78 °C. A 1.2-mL portion of 1.0 M *n*-butyl lithium was slowly added via syringe and the reaction

was stirred for 30 min. A portion of 3-*t*butyldimethylsilanyloxy-estrone (377 mg, 0.98 mmol) dissolved in 5 mL THF was slowly added, and the reaction was maintained at −78 °C for 8 h, and then warmed to room temperature overnight. The reaction was quenched with satd NH_4Cl, the organics were extracted with $CHCl_3$ (3×), the combined organics were washed with brine, dried over Na_2SO_4, evaporated and dried under vacuum. Purification was carried out by flash chromatography (silica, gradient 10% ethyl acetate/90% hexanes to 20% ethyl acetate/80% hexanes) yielding 329 mg (53%) of a clear, pale yellow oil. ^{1}H NMR ($CDCl_3$): δ 0.19 (s, 6H), 0.85 (s, 3H), 0.98 (s, 9H), 1.23 (t, 6H, J = 7.5 Hz), 1.31 (t, 3H, J = 7.3 Hz), 1.32–1.50 (4H), 1.63–2.01 (6H), 2.22–2.33 (3H), 2.68 (m, 4H), 2.79 (m, 2H), 2.96 (d, 2H, J = 3.0 Hz), 4.24 (q, 2H, J = 7.1 Hz), 6.55 (d, 1H, J = 2.6 Hz), 6.61 (dd, 1H, J = 2.6, 8.4 Hz), 7.12 (d, 1H, J = 8.6 Hz). ^{13}C NMR ($CDCl_3$): δ −4.45, 12.65, 13.66, 13.69, 14.08, 18.11, 22.69, 24.20, 24.24, 25.66, 26.38, 27.26, 28.51, 29.60, 32.47, 38.88, 39.35, 43.41, 47.33, 49.00, 62.29, 63.66, 79.95, 80.87, 87.06, 117.09, 119.88, 126.12, 133.17, 137.85, 153.24, 169.59 . MS (EI): *m*/*z* 630 (M^+, 73%); HRMS calcd $C_{35}H_{54}O_4SiS_2$, 630.3233, found, 630.3232.

17α-(6′,6′-Bis(ethanethio)-6′-ethoxycarbonyl-hexyn-1′-yl)-3-*t*butyldimethylsilanyloxy-estra-1,3,5(10)-triene-17β-ol (19b). A flame-dried flask was charged with alkyne **16b** (290 mg, 1.06 mmol) and 10 mL THF, and then was cooled to −78 °C. A 1.1 mL portion of 1.0 M *n*-butyl lithium was slowly added via syringe and the reaction was stirred for 30 min. A portion of 3-*t*butyldimethylsilanyloxy-estrone (333 mg, 0.87 mmol) dissolved in 5 mL THF was slowly added, and the reaction was maintained at −78 °C for 6 h, and then warmed to room temperature overnight. Workup and purification were carried out as described for **19a**, yielding 264 mg (46%) of a clear, colorless oil. ^{1}H NMR ($CDCl_3$): δ 0.18 (s, 6H), 0.87 (s, 3H), 0.97 (s, 9H), 1.20 (t, 6H, J = 7.5 Hz), 1.29 (t, 3H, J = 7.1 Hz), 1.32 to 1.51 (4H), 1.64–1.90 (7H), 2.01 (m, 1H), 2.08 (m, 2H), 2.20–2.36 (5H), 2.63 (m, 4H), 2.80 (m, 2H), 4.22 (q, 2H, J = 7.1 Hz), 6.55 (d, 1H, J = 2.6 Hz), 6.61 (dd, 1H, J = 2.6, 8.4 Hz), 7.12 (d, 1H, J = 8.4 Hz). ^{13}C NMR ($CDCl_3$): δ −4.46, 12.78, 13.49, 14.08, 14.09, 18.11, 18.63, 22.76, 23.63, 23.70, 24.49, 25.65, 26.33, 27.18, 29.62, 32.93, 35.54, 39.05, 39.33, 43.59, 47.14, 49.53, 62.08, 64.63, 80.00, 84.53, 85.33, 117.11, 119.88, 126.14, 133.01, 137.82, 153.22, 170.42 . MS (EI): *m*/*z* (M^+, 1%); HRMS calcd $C_{37}H_{58}O_4SiS_2$, 658.3546, found, 658.3548.

17α-(4′,4′-Bis(ethanethio)-4′-carboxy-butyn-1′-yl)-estra-1,3.5(10)-triene-3, 17β-diol (20a). A flask with attached condenser was charged with **19a** (253 mg, 0.40 mmol), 12 mL MeOH, and 12 mL 3 M NaOH aqueous solution. The mixture was heated at reflux for 5 h, cooled to room temperature, and made slightly acidic with the addition of 1 M HCl. The organics were extracted with $CHCl_3$ (3×), the combined organics were washed with brine, dried over Na_2SO_4, evaporated and dried under vacuum. Purification was carried out by flash chromatography (silica, gradient: 0–30% MeOH/$CHCl_3$) to give a clear, colorless oil (128 mg, 65%). ^{1}H NMR ($CDCl_3$): δ 0.83 (s, 3H), 1.19 (t, 3H, J = 7.5 Hz), 1.20 (t, 3H, J = 7.5 Hz), 1.30–1.40 (4H), 1.65 (m, 1H), 1.72 (m, 1H), 1.85 (m, 1H), 1.90–1.97 (m, 2H), 2.12 (m, 1H), 2.22 (m, 2H), 2.32 (m, 1H), 2.64–2.78 (6H), 2.93/3.01 (AB, 2H, J = 18.7 Hz), 6.45 (d, 1H, J = 2.6 Hz), 6.52 (dd, 1H, J = 2.6, 8.4 Hz), 7.06 (d, 1H, J = 8.4 Hz). ^{13}C NMR ($CDCl_3$): δ 13.41, 14.12, 23.72, 25.29, 25.36, 27.84, 28.67, 30.17, 30.77, 33.88, 39.83, 41.23, 44.88, 48.63, 50.16, 67.62, 80.65, 82.62, 87.61, 113.73, 116.04, 127.26, 132.73, 138.84, 155.87, 176.46. MS (EI): *m*/*z* 488 (M^+, 1%), 487 (M-H, 1%), 470 (M−H_2O, 4%); HRMS calcd $C_{27}H_{35}O_4S_2$, 487.1977, found 487.1975.

17α-(6′,6′-Bis(ethanethio)-6′-carboxy-hexyn-1′-yl)-estra-1,3,5(10)-triene-3,17β-diol (20b). A flask with attached condenser was charged with **19b** (210 mg, 0.32 mmol), 12 mL MeOH, and 12 mL 3 M NaOH aqueous solution. The mixture was heated at reflux overnight, cooled to room temperature, and the methanol was evaporated in vacuo. The aqueous solution was made slightly acidic with the addition of 3 M HCl. The organics were extracted with $CHCl_3$ (4×), the combined organics were washed with brine, dried over Na_2SO_4, evaporated and dried under vacuum. Purification was carried out by flash chromatography (silica, gradient: 0–20% MeOH/$CHCl_3$) to give a clear, colorless oil (129 mg, 78%). ^{1}H NMR (CD_3OD): δ 0.84 (s, 3H), 1.14 (dt, 6H, J = 2.8, 7.5 Hz), 1.36–1.41 (4H), 1.67–1.79 (5H), 1.85 (m, 1H), 1.95 (m, 2H), 2.06 (m, 2H), 2.17–2.26 (2H), 2.30 (t, 2H, J = 6.5), 2.34 (m, 1H), 2.59 (m, 4H), 2.75 (m, 2H), 6.46 (d, 1H, J = 2.6), 6.53 (dd, 1H, J = 2.6, 8.4), 7.07 (d, 1H, J = 8.4). ^{13}C NMR (CD_3OD): δ 13.47, 13.93, 19.32, 23.73, 24.62, 24.70, 25.74, 27.75, 28.56, 30.78, 34.24, 37.17, 39.96, 41.14, 45.05, 48.43, 50.84, 67.97, 80.56, 85.73, 85.86, 113.68, 116.00, 127.31, 132.57, 138.82, 155.85, 175.55.

17α-(4′,4′-Bis(ethanethio)-4′-carboxy-butyn-1′-yl)-estra-1,3,5(10)-triene-3,17β-diol rhenium(I) tricarbonyl (21a). Compound **20a** (27 mg, 0.054 mmol) was dissolved in 1 mL MeOH, 1 mL water, and 100 μL of satd $NaHCO_3$. The solution was heated to 70 °C and 337 μL of a 0.178 M $Re(CO)_3^+$ aqueous solution was added. After 90 min the solution was cooled and evaporated to dryness under vacuum. The crude material was purified by flash chromatography (silica, gradient: 0–20% MeOH/$CHCl_3$) to give 12 mg (29%) of **21a**. ^{1}H NMR (CD_3OD): δ 0.88 (s, 3H), 1.12–1.49 (10H), 1.76–2.07 (6H), 2.16–2.38 (3H), 2.64–2.81 (8H), 6.48 (d, 1H, J = 2.4 Hz), 6.54 (dd, 1H, J = 2.4, 8.1 Hz), 7.09 (d, 1H, J = 8.1 Hz). MS (FAB): *m*/*z* 759 ($M[^{187}Re]^+ + H$, 2.9%), 757 ($M[^{185}Re]^+ + H$, 2.2%), 741 ($M[^{187}Re]^+ - OH$, 2.7%), 739 ($M[^{185}Re]^+ - OH$, 1.7%). HRMS calcd $C_{30}H_{35}O_7S_2Re$, 759.1460, found 759.1457.

17α-(6′,6′-Bis(ethanethio)-6′-carboxy-hexyn-1′-yl)-estra-1,3,5(10)-triene-3,17β-diol rhenium(I) tricarbonyl (21b). Compound **20b** (26 mg, 0.051 mmol) was dissolved in 2 mL methanol, 2 mL water, and 52 μL of 1 M NaOH. The solution was heated to 70 °C and 500 μL of a 0.122 M $Re(CO)_3^+$ aqueous solution was added. After 15 min the solution was cooled and evaporated to dryness under vacuum. The crude material was purified by

flash chromatography (silica, gradient: 5–20% MeOH/$CHCl_3$) to give 27 mg (68%) of **21b** as a as a clear colorless oil. ^1H NMR (CD_3OD): δ 0.85 (s, 3H), 1.10–1.46 (10H), 1.69–2.02 (8H), 2.14–2.46 (7H), 2.56–2.84 (4H), 3.11 (m, 1H), 3.30 (m, 1H), 6.47 (m, 1H), 6.53 (m, 1H), 7.08 (m, 1H). MS (FAB): *m/z* 787 ($M[^{187}Re]^+ + H$, 2%), 769 ($M[^{187}Re]^+ - OH$, 12%), 767 ($M[^{185}Re]^+ - OH$, 6%). HRMS calcd (for $M[^{187}Re]^+ - OH$) $C_{32}H_{38}O_6S_2{}^{187}Re$, 769.1667, found, 769.1671.

Estrogen receptor binding affinity

Relative binding affinities were determined by competitive radiometric binding assays using [^3H]estradiol, following previously published procedures.[58,59] The relative binding affinities (RBA) are expressed as a percentage, with estradiol being set to 100%. The receptor sources were lamb uterine cytosol, purified full-length human ERα, or purified full-length human ERβ (PanVera Inc.). The RBA values are reported as the average of two or more determinations ± the range ($n = 2$) or standard deviation ($n > 2$).

PET imaging

Positron emission tomography imaging was performed on a microPET-R4 system (Concorde Microsystems Inc, Knoxville, TN, USA) which is based on the design of Cherry and colleagues.[34] The microPET-R4 has a field-of-view of 8 cm axially by 11 cm transaxially, and is capable of a spatial resolution of 2.3 mm and an absolute sensitivity of 1020 cps/μCi in the middle of the field-of-view. To obtain the images, the list mode data were sorted into two-dimensional sinograms via the FORE algorithm and subsequently reconstructed by a two-dimensional filtered-back projection. The microPET imaging study was carried out on a mature (200 g) female Sprague–Dawley rat, anesthetized with 1–2% isofluorane/oxygen and placed supine in an immobilizing scanner support. Compound **10b** was administered as a bolus injection via the tail vein. Single position imaging was performed at four timepoints (5, 30, 60 and 120 min post-injection), each consisting of 10-min static data collections.

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