Estrogen-Derived Steroidal Metal Complexes: Agents for Cellular Delivery of Metal Centers to Estrogen Receptor-Positive Cells

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Targeted cellular delivery of drugs to specific tissues is an important goal in biomedical chemistry. Achieving this requires harnessing and applying molecular-level recognition events prevalent in (or specific to) the desired tissue type. Tissues rich in estrogen receptors (ERs), which include many types of breast cancer, accumulate molecules that have high binding affinities for these receptors. Therefore, molecules that (i) bind to the ER, (ii) have favorable cellular transport properties, and (iii) contain a second functionality (such as a center that may be used for diagnostic imaging or medical therapy) are exciting synthetic targets in the field of drug delivery. To this end, we have prepared a range of metallo-estrogens based on 17α-ethynylestradiol and examined their binding to the ER both as isolated receptor and in whole cell assays (ER positive MCF-7 cells). Estrogens functionalized with metal binding units are prepared by palladium-catalyzed cross-coupling reactions and a wide range of metal centers introduced readily. All the compounds prepared and tested exhibit effective binding to the estrogen receptor and are delivered across the cell membrane into MCF-7 cells. In the whole cell assays, despite their monocationic nature, the palladium and platinum complexes prepared exhibit similar (and even enhanced) receptor binding affinities compared to their corresponding neutral free ligands. It is unprecedented for a higher ER binding affinity to be observed for a cationic complex than for its metal-free ligand.

Introduction

Transition-metal centers are widely being utilized as agents for diagnostic imaging[1] and medical therapeutic[2−6] (including anti-cancer and anti-viral) treatment[7] both in the clinic and in research programs. Modes of therapeutic action include binding to the bases of DNA to interfere with replication (as seen in the action of *cis*-platin and other potential anti-cancer platinum therapeutics)[8−20] and the use of radioisotopes for site-specific radiation damage.[21] Such strategies require intracellular location of the metal complex. The ability to deliver metal centers across the cellular membrane and into specific cells is consequently an important goal. Since the cell membrane provides a barrier to nonspecific transport of the free hydrophilic metal ions, achieving this goal in a systematic, predictable, and reliable fashion requires delivery vectors that facilitate the controlled transport of metal ions. At the simplest level, such a vector might be a lipophilic ligand that encapsulates the metal ion within a lipophilic shell. However, it is difficult to systematically design and control tissue or cell specificity using such a simple approach.

A more sophisticated approach is to use a remote delivery vector that harnesses an existing cellular transport pathway through which the metal can be delivered into a cell. An advantage of this approach is that a variety of different metal centers might be delivered into a cell using the same delivery vector. The approach therefore has potential as a generic strategy through which different radio-imaging and luminescent-imaging

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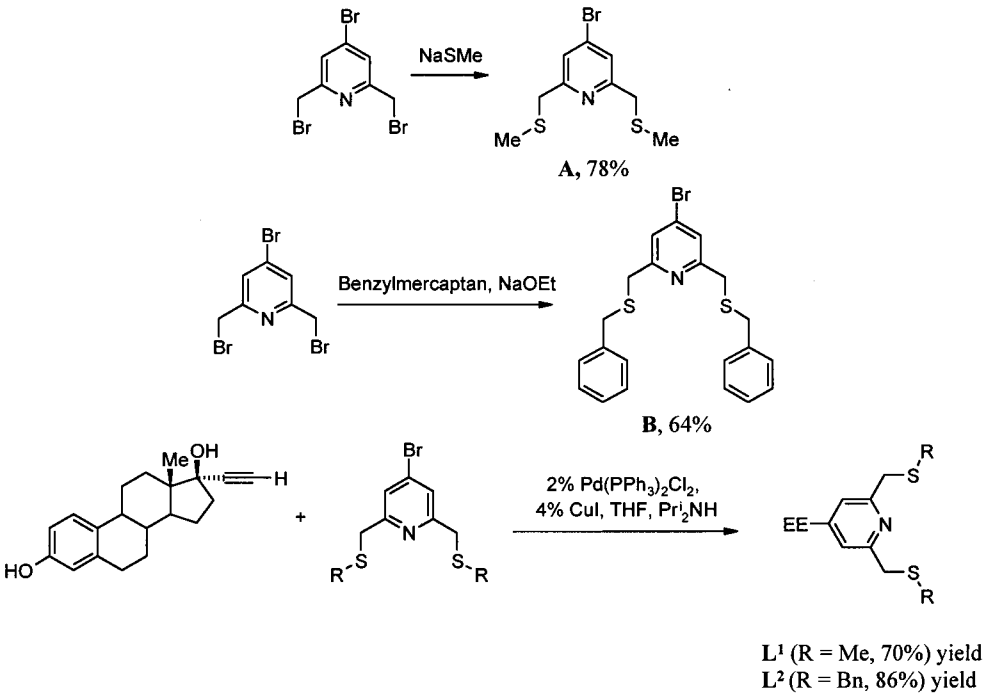
Scheme 1. Synthesis of $\mathbf{L^1}$ and $\mathbf{L^2}$



metal complexes or radio-therapeutic nuclei of different energies might be systematically delivered into a cell. Estrogenic steroid conjugates containing metal chelates represent an attractive delivery vector for such a strategy. It is known that estrogenic steroids are transported through the blood (bound to steroid transport proteins) and across the cellular membrane[22] and that by binding to the estrogen receptor (ER) in the cytoplasm, they are finally transported into the cell nucleus. Moreover, they localize in specific tissues (e.g., breast), which affords a level of targeting.[23] We now wish to report the preparation of a variety of estrogen based steroidal metal complexes based on 17α-functionalized estrogens and relative binding affinities of the compounds with isolated ERs and with receptors in ER-positive MCF-7 viable cells.

Molecular Design. Our design strategy to achieve this goal was stimulated by the results of Jaouen et al., who have prepared estrogenic steroids labeled with quite bulky organometallic species mainly at the 17α-position.[24,25] Substituting bulky groups onto a steroid generally reduces its affinity for its receptor; however, Jaouen et al. showed that it is possible to append a range of moieties, organic and organometallic, at the 17α-position of estradiol while maintaining affinity for the ER.[24,25] In the case of organometallic-substituted estrogens, it was shown that a short linker group between the estrogen 17α-position and the organometallic moiety was required to maintain an effective ER binding affinity.[24] One of the more successful linkers used was an ethynyl group, because it gives an effective separation of the steroid and organometallo substituent without introducing excessive conformational flexibility.[25] Jaouen et al. concluded that directing the substituent away from the β-face (the side of the 17β-hydroxy group) of the estrogen reduced steric interference with the 17β-hydroxyl binding pocket of the receptor. They found that molecules with charged and/or large substituents exhibited reduced binding affinities. Katzenellenbogen et al. have separately demonstrated that receptor binding can also be retained if the 7α position is used to attach bulky substituents.[26] However, substitution is more readily effected at the 17α position and so we selected this as the site with which to initiate our studies.

Two synthetic routes to substituted 17α-ethynyl estradiols have previously been described. One involves enantioselective attack of a nucleophile at the 17-position carbonyl of an estrone molecule to give the 17α-estradiol derivative.[24,25] This method allows the use of organic and inorganic nucleophiles and is generally high yielding. The other method involves a palladium-catalyzed cross-coupling reaction between 17α-ethynylestradiol and aryl bromo, iodo, or triflato groups and is also usually high yielding.[27−29] The latter synthetic strategy is more flexible if the potential to incorporate a radioactive metal ion into the compound as a final step is required and consequently was the one we chose to focus on.

Results and Discussion: Chemistry

Compounds $\mathbf{L^1}$ and $\mathbf{L^2}$ were selected as the initial synthetic targets for estrogenic metal binding agents because their chelating groups have a compact shape and show a wide variety of coordination chemistry.[30−33] Many of the metal complexes formed by these ligands are cationic, which is usually detri-

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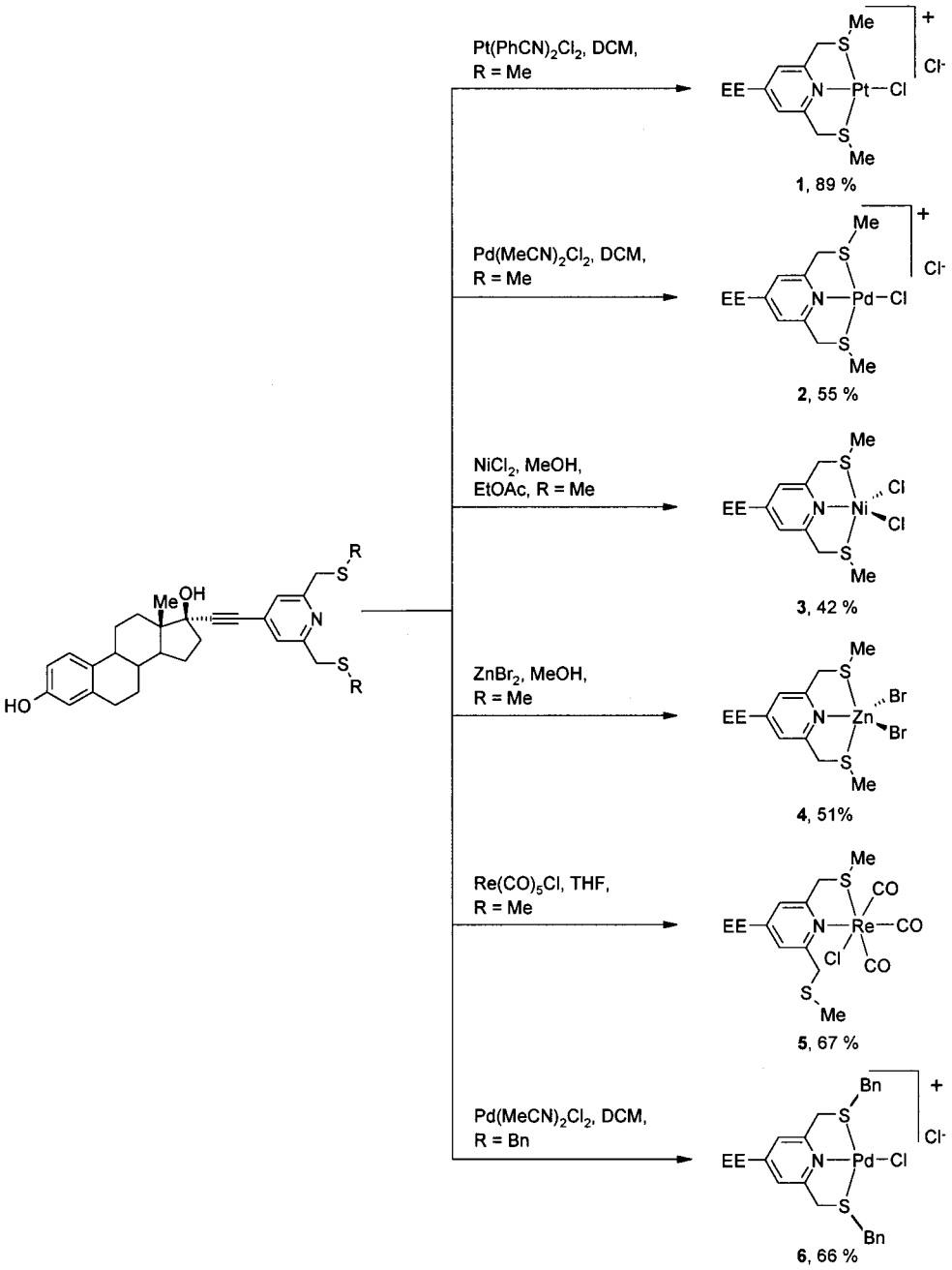
Scheme 2. Synthesis of the Complexes **1**−**6**



mental to ER binding[24,25] (as noted above). However, we considered that measuring the ER relative binding affinities (RBA) for a range of metallo-estrogens comprising a common ligand system with different metal centers would be a useful first step in our investigations. This would allow us to test our basic design premise (that estrogenic systems could be used for generic delivery of a range of metal centers into cells) and, we anticipated, would naturally lead to ligand systems designed to give neutral complexes with higher ER binding affinities.

Synthesis and Characterization. Two 4-bromo-2,6-bis-((alkylthio)methyl)pyridines (**A**, alkylthio group = SMe and **B**, alkylthio group = SCH_2Ph) were synthesized through the reactions of the appropriate thiolate with 4-bromo-2,6-bis-(bromomethyl)pyridine[34] in methanol or ethanol (Scheme 1). The 4-bromo substituent on the pyridyl ring is stable to a wide range of chemical conditions, making this a versatile molecular core from which a large set of chelating ligands can be synthesized.

Palladium-catalyzed cross-coupling of **A** with 17α-ethynylestradiol proceeds to completion at room temperature in less than 24 h to give $\mathbf{L^1}$ contaminated only with catalyst residues and trace starting materials (Scheme 1). Purification by column chromatography afforded $\mathbf{L^1}$ as a cream solid in 70% yield. The ^1H and ^{13}C NMR spectra of $\mathbf{L^1}$ were assigned using a combination of one and two-dimensional techniques and confirmed the expected structure of the compound. A variety of different

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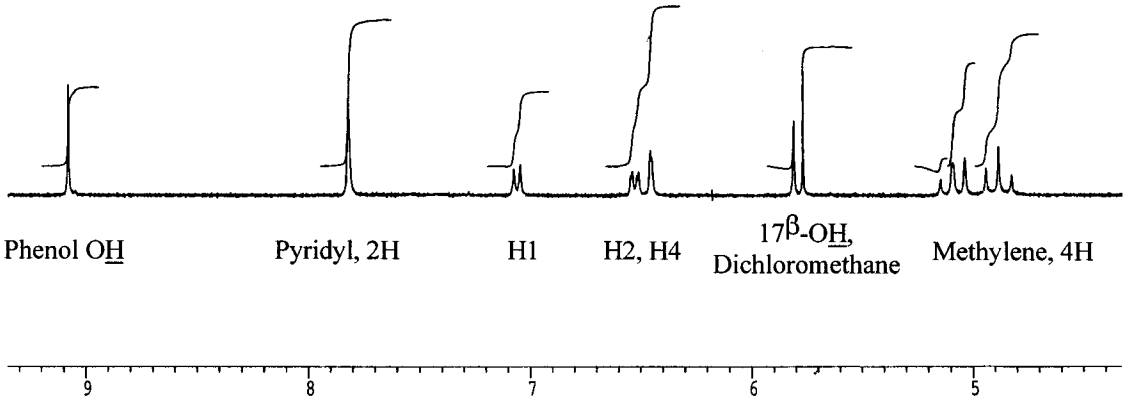


Figure 1. Expansion of the aromatic−methylene region in the ^{1}H NMR spectrum of **1** in DMSO-d_6.

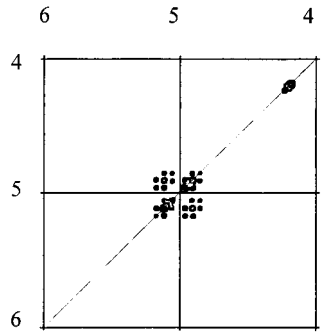


Figure 2. Expansion of methylene region in the COSY spectrum of **1** in DMSO-d_6.

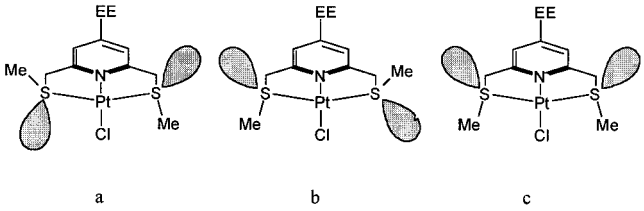


Figure 3. Possible orientations of the Me groups in **1**. Isomers a and b are formally rendered diastereoisomers by the chiral estrogen attached at the back of the pyridine.

metal complexes may readily be prepared by mixing the ligand with solutions of the metal salts (Scheme 2).

Mixing dichloromethane solutions of $\mathbf{L^1}$ and $[Pt(PhCN)_2Cl_2]$ and heating under reflux for 3 h afforded $[Pt(\mathbf{L^1})Cl]Cl$ (**1**) in 89% yield. Similarly, mixing a dichloromethane solution of $\mathbf{L^1}$ and $[Pd(MeCN)_2Cl_2]$ affords the analogous palladium complex **2** in 55% yield. Compounds **1** and **2** are very soluble in DMSO, soluble in methanol, and sparingly soluble in water. The ^{1}H NMR spectrum of **1** in DMSO-d_6 is complicated by the appearance of a multiplet assignable to the methylene protons on the chelating moiety (Figures 1 and 2). This led us to conclude that diastereoisomers resulting from different orientations of the ligand thiomethyl groups (Figure 3) were present. Two of the possible isomers (a and b) are enantiomeric at the metal complex, and it is only the presence of the chiral steroid that renders them formally diastereoisomeric. The steroid chirality is remote from the metal center and thiomethyl groups, and a and b appear as a single set of resonances in the ^{1}H NMR spectrum (it is highly unlikely that the distant chiral steroid induces formation of only one enantiocenter (a or b) at the metal). The isomers are present in an approximate 1:1 ratio of a,b/c. The resulting inequivalence of the methylene protons gave

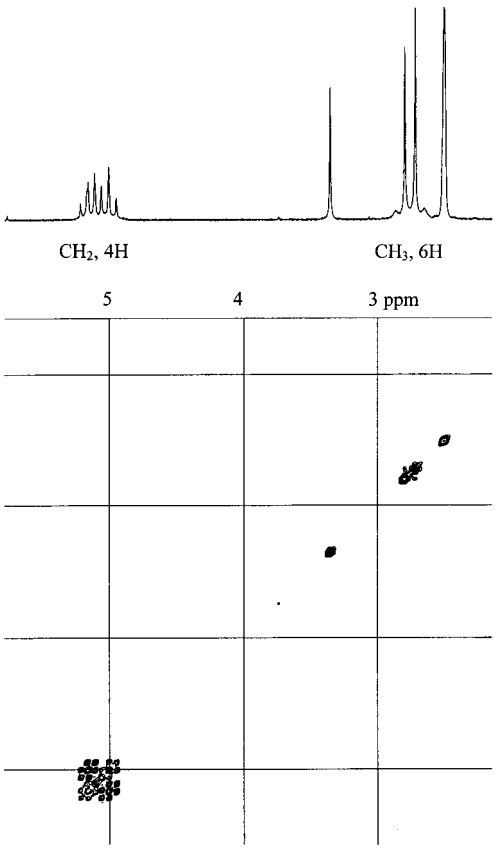
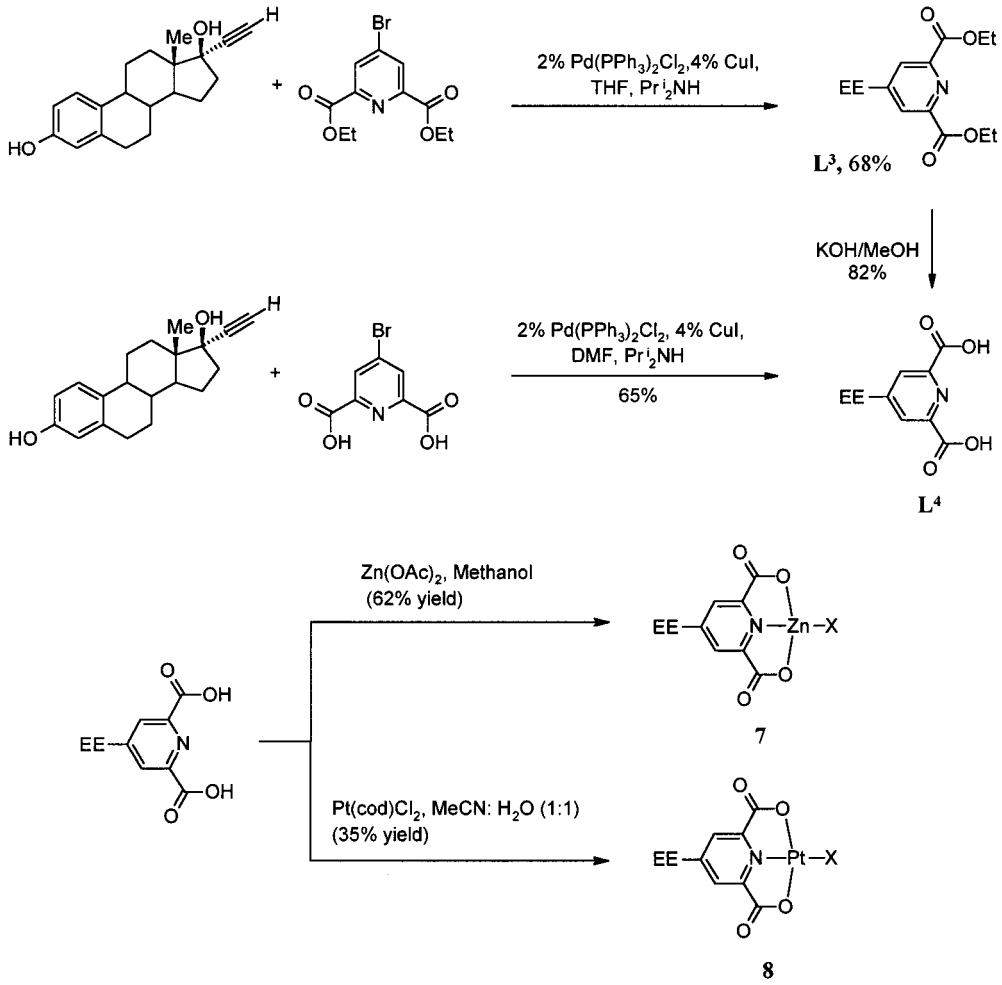


Figure 4. Expansion of the methyl−methylene region in the ^{1}H NMR and COSY spectra of (**10**) [PtLCl] (L = 2,6-bis(thiomethyl)pyridine in DMSO-d_6).

rise to a series of overlapping doublets (^2J = 17 Hz) consistent with a geminal coupling. To establish more firmly that this effect is not one induced by the presence of the chiral steroid, the analogous platinum complex (**10**) without the steroid [PtLCl]-Cl (L = 2,6-bis((methylthio)methyl)pyridine, which does not bear the ethynyl estradiol substituent) was also prepared. The methylene protons of this complex showed a similar splitting pattern (Figure 4). The ligand chelate methyl protons of complexes **1** and [PtLCl]Cl are also inequivalent with their ^{1}H NMR shifts differing by 0.08 ppm. Variable-temperature ^{1}H NMR studies revealed that the methylene signals from the two diastereoisomers remain resolved up to 363 K. The methylene proton NMR signals from the chelating groups of **2** are broad but not resolved into the series of doublets observed at room

Scheme 3. Synthesis of $\mathbf{L^3}$ and $\mathbf{L^4}$



temperature for the platinum complex **1**. This indicates the existence of similar diastereoisomers but with a rapid interconversion process between the isomers.

Slow evaporation of a methanol/ethyl acetate solution of nickel(II) chloride and $\mathbf{L^1}$ afforded the complex [Ni($\mathbf{L^1}$)Cl_2] (**3**) as a pale green solid in 42% yield. Compound **3** is highly soluble in methanol and ethanol. Although the complex has a formal neutral charge, it is likely that one or two of the coordinated chlorides exchange with solvent in solution to give a cationic solution species. Similarly slow evaporation of solvent from a methanol solution of zinc(II) bromide and $\mathbf{L^1}$ afforded [Zn($\mathbf{L^1}$)Br_2] (**4**) as a white solid in 51% yield. This complex also has good solubility in methanol/ethanol.

The reaction of ligand $\mathbf{L^1}$ with [$Re(CO)_5Cl$] to give **5** was followed by monitoring the carbonyl IR signal in a tetrahydrofuran (THF) solution. The starting material [$Re(CO)_5Cl$] exhibits two bands in the carbonyl region at 2040 (broad) and 1980 cm^{-1}. After heating under reflux for 1 h, the bands due to [$Re(CO)_5Cl$] almost disappear and three new bands of equal intensity at 2027, 1928, and 1901 cm^{-1} may be observed. After 3 h, the bands due to the starting material completely disappear. The three carbonyl stretching bands of the product suggest that a *fac* configuration is adopted.[33] This is anticipated from the chemistry of the unfunctionalized ligand 2,6-bis((methylthio)methyl)-pyridine. Upon reaction with [$Re(CO)_5Cl$] in THF, a complex is formed where the pyridyl, one of the methylthio groups, three carbonyls, and a chloride ion coordinate to the rhenium(I) center.[33] The high degree of complexity of the signals in the ^1H NMR of **5** (CD_2Cl_2) is consistent with the presence of two exchanging invertomer species (with the thiomethyl groups coordinated cis and trans to the chloride ligand) and an asymmetric arrangement around the rhenium(I) atom. In DMSO-d_6 the ^1H NMR is simplified, revealing a simple symmetrical system, presumably as a result of increased exchange between the two invertomers.

B was coupled to 17α-ethynyl estradiol under the same conditions as for the preparation of $\mathbf{L^1}$. Purification by column chromatography afforded $\mathbf{L^2}$ as an orange wax in 86% yield. Again, the ^1H NMR and ^{13}C NMR were assigned using a combination of one- and two-dimensional techniques and confirmed the expected structure of the compound.

The palladium complex of $\mathbf{L^2}$, [Pd($\mathbf{L^2}$)Cl]Cl (**6**), was prepared in a manner analogous to that of **2**. At room temperature in the ^1H NMR (DMSO-d_6) spectrum, the methylene protons adjacent to the pyridine ring are not resolved into doublets but are again broad. The analogous platinum complex, [Pt($\mathbf{L^2}$)Cl]Cl, could not be prepared by the same method. Treatment of $\mathbf{L^2}$ with [$Pt(PhCN)_2Cl_2$] in dichloromethane yielded a mixture of products that would only fully dissolve in DMSO. ^1H NMR (DMSO-d_6) showed that at least two compounds were present. Varying the reaction time gave little change in the composition of the product, and no further attempts were made to isolate the components of the mixture. However, the desired product could be obtained by heating $\mathbf{L^2}$ with [$Pt(PhCN)_2Cl_2$] or [Pt(cod)Cl_2] (cod = 1,5-cyclooctadiene) in methanol. The ^1H NMR spectrum (DMSO-d_6) showed the characteristic series of overlapping

Table 1. ER Binding Data for Compounds **L[1]**, **L[2]**, **L[3]**, **L[4]**, **1**, **2**, and **5**−**8**a

compd	**L[1]**	**L[2]**	**1**	**2**	**5**	**6**	**L[3]**	**L[4]**	**7**	**8**
isolated receptor (IC_{50}/nM)	−	−	39¥	39¥	83¥	11¥	144‡	875‡	5600‡	137‡
RBA (%)	−	−	3.3	3.3	1.5	11.7	1	0.17	0.027	1.08
viable cell (IC_{50}/nM)	15.5‡	1.5†	11.1‡	6.3‡	32†	11†	18‡	2000‡	13000‡	140‡

[a] The relative binding affinities were measured over a total of three experiments. In each case the control compound diethylstilbestrol was shown to have the following relative affinities: †DES IC_{50} = 0.2, ‡DES IC_{50} = 0.22 nM, ¥DES = 0.19 nM. A dash indicates where the value has not been measured.

doublets arising from cis/trans isomerism as observed for **1** (see above).

The pyridine-2,6-dicarboxylic acid group has both great potential breadth of coordination chemistry and a similar compact shape to the chelating groups of **L[1]** and **L[2]**. It commonly coordinates in a tridentate chelating mode with deprotonated carboxylic acid groups, giving complexes of neutral charge. Anticipating that complexes with neutral charge might exhibit enhanced receptor binding, **L[4]** (Scheme 3) was our next target ligand.

Palladium-catalyzed cross-coupling of 4-bromo-2,6-pyridinedicarboxylate diethyl ester with 17α-ethynylestradiol proceeds to completion at room temperature in less than 24 h to give **L[3]** (Scheme 3). Purification by column chromatography afforded **L[3]** as a white glassy solid in 68% yield. The ^{1}H and ^{13}C NMR spectra of **L[3]** were assigned using a combination of one and two-dimensional techniques and confirmed the expected structure of the compound. Base hydrolysis of the diester **L[3]** afforded the diacid **L[4]** in 55% yield. **L[4]** could also be prepared directly from 4-bromo-pyridine-2,6-dicarboxylic acid and 17α-ethynylestradiol in 65% yield under similar reaction conditions using DMF as solvent instead of THF. Purification of **L[4]** from the reaction mixture was difficult and therefore it proved preferable to proceed via the diester **L[3]** since its purification is straightforward and overall yields are similar.

A zinc complex of **L[4]** was prepared by adding a methanolic solution of zinc(II) acetate to a methanol solution of the ligand. A white solid **7** formed which proved to be soluble only in DMSO and was therefore difficult to characterize. Attempts to prepare more soluble complexes by introducing a range of additional monodentate ligands into the reaction mixture were unsuccessful and the same complex resulted on each occasion. The platinum chemistry proved to be more tractable, and a platinum complex of **L[4]** was readily prepared by heating a solution of [Pt(cod)Cl_2] (cod = 1,5-cyclooctadiene) and the potassium salt of **L[4]** in an acetonitrile/water mixture at 60 °C for 3 h to yield a cream solid **8** of 1:1 stoichiometry as evidenced by electrospray ionization mass spectrometry. Although again soluble in DMSO, the compound exhibited poor solubility in most other organic solvents and in water.

Literature reports on the complexation of pyridine dicarboxylic acids to zinc are scarce,[35−37] and the low solubility leads us to suspect that an extended array is formed in the solid state. This could happen either through hydrogen bonding between the carboxylate and steroid hydroxyl groups or by the formation of a coordination polymer. The poor solubility of the metal complexes **7** and **8** can also be attributed to the fact that upon complexation to dicationic metals pyridine-2,6-dicarboxylate often doubly deprotonates, resulting in neutral complexes. As noted, complexes **3** and **4** also have neutral charge but show good solubility in methanol and ethanol. We attribute this to their ability to undergo a ligand replacement with the solvent giving a soluble species. Since in the ligand **L[4]** the anionic ligand donors are integral to the chelating group, they cannot easily be displaced, and therefore, it is unlikely that charged, soluble species can easily be formed.

Results and Discussion: Biological Assays

Having established that we could prepare vectors into which a range of metal centers could readily be incorporated, we investigated whether these steroidal compounds would retain binding affinity for the estrogen receptor and whether they would be transported across cell membranes effectively.

Isolated ER and Viable Cell ER Binding. The relative binding affinities of the steroidal ligands and metal complexes for isolated ERs (obtained from human breast cancer MCF-7 cells) and for ERs in viable MCF-7 cells were determined by competitive radiometric binding assays. The assays involve competition of the steroidal conjugates with 16α-[^{125}I]estradiol. The procedures involve incubation of isolated receptors or viable cells in a medium containing a fixed concentration of 16α-[^{125}I]-estradiol and various concentrations of the competing steroidal ligand or metal complex. For the whole cell assays, the bound 16α-[^{125}I]estradiol is then extracted from the cells and measured using a γ counter. For the free receptor assays, the receptors are isolated by filtration and the bound 16α-[^{125}I]estradiol again measured using a γ counter. From these data, the concentration of competing chelate or metal complex required to displace half of the 16α-[^{125}I]estradiol that would be bound to the ER (isolated or in a cell) in the absence of the test compound is calculated. This quantity is presented as an IC_{50} displacement measurement for the compound. Low IC_{50} values correlate to high binding affinities. In each experiment, the compound diethylstilbestrol is used as a control. Since the percentage binding affinity of diethylstilbestrol, relative to 17β-estradiol, for isolated ER is known, the test compound's IC_{50} displacement values can be converted into percentage binding affinities compared to 17β-estradiol (100%) (Table 1).

The two assays (free receptor and whole cell) measure different properties. While the free estrogen receptor assay assesses only the receptor binding, the whole cell assay will also reflect (i) the ability of the steroidal conjugate to be transported into the cell, (ii) the ability of the steroidal conjugate to be transported into the nucleus (possibly prebound into the receptor) since many of the ERs are located in the cell nucleus,[22] and (iii) any binding to other biomolecules in the cell which interferes with or prevents its binding to the ER (e.g., random metalation of protein amino acid residues).

The binding results are presented in Table 1. The whole-cell assay results for the metal-free conjugates **L[1]**, **L[2]**, **L[3]**, and **L[4]** can be interpreted in terms of the lipophilicity of the chelating

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group with $\mathbf{L^1}$ and $\mathbf{L^2}$ exhibiting high binding affinities comparable to that of 17β-estradiol. Steric effects appear to play a less important role: $\mathbf{L^2}$ has a higher affinity than compound $\mathbf{L^1}$ despite the greater size of benzyl groups compared with methyl groups. The benzyl group is more hydrophobic and appears to be better tolerated in the ER ligand binding domain.[38] As would be expected, in the viable cell assay where membrane transport is required, the ester compound $\mathbf{L^3}$ competes much more effectively with 16α-[^{125}I]estradiol than does the corresponding hydrophilic acid $\mathbf{L^4}$. The free receptor assays for these two compounds indicate that neither binds strongly to the receptor. We speculate that the low IC_{50} value for $\mathbf{L^3}$ in the viable cell assay results from transport of the lipophilic ester across the cell membrane into the cell where it hydrolyses to the acid compound $\mathbf{L^4}$ which is not transported back across the membrane effectively, leading to a high concentration of steroidal conjugate inside the cell.

The binding results for the metal complexes revealed an unexpected (and pleasant) surprise. In the whole cell assays, despite their monocationic nature, the palladium and platinum complexes **1** and **2** exhibit similar (and even enhanced) receptor binding affinities compared to their corresponding neutral free ligand $\mathbf{L^1}$, and **6** also exhibits a similar receptor binding affinity compared to its neutral free ligand $\mathbf{L^2}$. We are not aware of any precedent for a higher ER binding affinity of a cationic complex when compared to its neutral metal-free ligand. Indeed, it is usual to observe a considerable drop in ER relative binding affinity (RBA) in cationic compounds over structurally similar neutral ones.[24] The platinum and palladium complexes of **1** and **2** bind to isolated ERs with very similar affinities. This is to be expected since they have the same charge and are very similar in size and bulk. In the whole cell assay, the palladium complex **2** shows approximately double the affinity of the platinum complex **1** and this may reflect competitive random platination of other biomolecules within the cell. Platination of sulfur residues on proteins, for example, is believed to be a major cause of the unpleasant side-effects associated with the anti-cancer drug cisplatin.[9] Palladation of sulfur residues is also possible but the bonds formed are likely to be reversible allowing ultimate delivery to the steroid binding site of the receptor.

Just as in the metal-free conjugates, the increased lipophilicity afforded by the benzyl groups on the metal chelating unit is beneficial for receptor binding: the thiobenzyl palladium complex **6** binds three times as strongly as the corresponding thiomethyl palladium complex **2** in the free receptor binding assay. To confirm that the binding to the receptor is indeed a feature of the steroidal unit of the complex, the analogous palladium (**9**) and platinum (**10**) complexes of 2,6-bis((methylthio)methyl)pyridine, which do not bear an appended steroid, were prepared and tested in the free receptor competition assay. In neither case were we able to detect any evidence of competition with 16α-[^{125}I]estradiol, indicating that, as anticipated, the metal complexes themselves show no inherent receptor binding ability.

The rhenium complex (**5**) of ligand $\mathbf{L^1}$ is neutral but possesses more steric bulk than either $\mathbf{L^1}$ or the corresponding palladium and platinum complexes. This complex binds less effectively than these related species, implying again that charge is not a major determining factor in the binding of these complexes to the ER; steric factors appear more important.

The implication that charge is not a serious barrier to either transport or receptor binding negates our original view that effective binding would require neutral species. The neutral zinc and platinum compounds **7** and **8** achieved only poor binding, which we attribute to the more hydrophilic nature of the metallo-unit introduced by the dicarboxylate binding site. Both of these compounds are poor ER binders; however, compound **8** does show an order of magnitude higher ER RBA than its parent ligand $\mathbf{L^4}$ in both assays.

Thus, the ligands $\mathbf{L^1}$ and $\mathbf{L^2}$ and their complexes **1**, **2**, **5**, and **6** show strong binding to the ER in both isolated ER and for ER in viable MCF-7 cells, while much lower binding affinities are observed for $\mathbf{L^3}$ and $\mathbf{L^4}$ and the complexes **7** and **8**. The comparison of the results from the free ER assays and those from the whole-cell assays confirm that all the compounds prepared exhibit effective binding to the estrogen receptor and are delivered across the cell membrane and into MCF-7 cells. The compounds based on $\mathbf{L^1}$ and $\mathbf{L^2}$ are effective delivery vehicles for different metal ions and mediate cellular transport while retaining strong ER binding.

Conclusion

We have shown that estrogenic steroids are effective delivery vectors that can be used to mediate the transport of a variety of different metal ions into cells. By linking metal-binding domains to estrogen through the 17α-position, strong affinity for the estrogen receptor is retained. All the compounds prepared and tested have both exhibited effective binding to the estrogen receptor and, more importantly, been delivered across the cell membrane and into MCF-7 cells. The palladium(II) metalloestrogen **6** shows transport and subsequent binding affinity that is of the same order as estradiol itself. In the whole cell assays, despite their monocationic nature, the palladium(II) and platinum(II) complexes prepared have exhibited similar (and even enhanced) receptor binding affinities compared to their corresponding neutral free ligands; in these systems, it appears that attention to hydrophobicity and size of the appended metal complex is more important than charge. This is a surprising result, as to the best of our knowledge it is unprecedented for a higher ER binding affinity to be observed for a cationic complex than for its metal-free ligand.

A key feature of the approach developed and the compounds described in this paper are that a single estrogen conjugate may be used to bind a range of metals and, correspondingly, that a series of ligands may be used to bind a given metal. In this way, we can systematically tune the molecular properties of conjugate complexes through which we may introduce desired properties including identity of the metal, charge, size, hydrophobicity, etc. and have those species delivered into cells to achieve a desired biological effect.

Experimental Section

General Methods. ^{1}H NMR and ^{13}C NMR spectra were recorded on Brüker ACF250, DPX300, or AMX400 spectrometers operated in Fourier transform mode. Chemical shifts were referenced to tetramethylsilane as an internal standard or to residual solvent peaks (CH^*Cl_3 at 7.25 ppm in $CDCl_3$ and C^*DCl_3 at 77.0 ppm, $CHDCl_2$ at 5.31 in DCM, and $C^*D_2Cl_2$ at 53.73, $CD_3S(O)CHD_2$ at 2.52 ppm in DMSO-d_6). Electron impact (EI) mass spectra and fast atom bombardment (FAB) spectra (using *m*-nitrobenzyl alcohol (NOBA) or glycerol as matrix) were recorded on a Micromass Autospec spectrometer either at the University of Warwick or by the EPSRC National Mass Spectrometry Service Centre, Swansea. Infrared spectra were measured as solid samples on a Brüker Vector 22 spectrometer. Solution-phase IR spectra were measured using a CaF_2 cell in THF at approximately 2 mM concentration on a JASCO J-620 spectrometer. Microanalyses were performed by Warwick Analytical Service on a Leeman Labs Inc. CE440 elemental analyzer. Analytical thin-layer chromatography

(TLC) was performed on aluminum plates precoated with Merck silica gel 60 (F_{254}). Flash chromatography was carried out using Merck 9385 Kieselgel 60 silica (0.040−0.063 mm, 230−400 mesh). Tetrahydrofuran, dimethylformamide, and diisopropylamine used in reactions were the best available commercial anhydrous grades. Other solvents and reagents were standard grades available from commercial suppliers and were used without any further purification. The compounds 4-bromo-2,6-bis(bromomethyl)pyridine, 4-bromo-2,6-pyridinedicarboxylate diethyl ester,[39] and $[Pt(cod)Cl_2]$[40] were prepared according to literature procedures. The compounds **9** and **10**, [MLCl]Cl (M = Pd, Pt; L = 2,6-bis((methylthio)methyl)pyridine), were prepared from dichloromethane solutions of $[Pd(MeCN)_2Cl_2]$ or $[Pt(MeCN)_2Cl_2]$ and 2,6-bis((methylthio)methyl)pyridine (methods described previously).[41]

4-Bromo-2,6-bis((methylthio)methyl)pyridine (A). 4-Bromo-2,6-bis(bromomethyl)pyridine (2 g, 5.36 mmol) and sodium methanethiolate (0.784 g, 10.68 mmol) were dissolved in ethanol (60 mL) and heated to reflux for 2 h. The yellow solution was reduced to dryness in vacuo, and the organic residues were dissolved in chloroform (50 mL), washed with water (3 × 30 mL), and dried over anhydrous sodium sulfate. The chloroform was removed in vacuo to yield an oil that was purified by flash column chromatography (silica gel 60/ dichloromethane). The product was eluted as a narrow band (R_f = 0.35). The colorless oil crystallized upon standing overnight (1.17 g, 78%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.44 (s, 2H, 3-pyridyl), 3.74 (s, 4H, methylene), 2.06 (s, 6H, methyl). ^{13}C NMR: 160.0 (2-pyridyl), 134.3 (4-pyridyl), 125.7 (3-pyridyl), 40.0 (methylene), 15.7 (methyl). EI MS m/z = 278 and 280 $[M]^+$, 231 and 233 $[M - SMe]^+$, 184 and 186 $[M - 2(SMe)]^+$. Anal. Calcd for $C_9H_{12}BrNS_2$: C, 38.99; H, 4.37; N, 5.06. Found: C, 38.75; H, 4.27; N, 4.73. IR: 3056, 2977, 2918, 2356, 2337, 1556, 1415, 1393, 1277, 1259, 1212, 1157, 1141, 1100, 974, 962, 872, 852, 810, 765, 649, 589 cm^{-1}.

4-Bromo-2,6-bis((benzylthio)methyl)pyridine (B). A solution of benzyl mercaptan (5.35 mmol, 0.63 mL) and sodium methoxide (5.35 mmol, 0.289 g) in dry methanol (10 mL) was stirred for 10 min and then added to a solution of 4-bromo-2,6-bis(bromomethyl)pyridine (1 g, 2.67 mmol) in dry methanol (20 mL). The mixture was heated at reflux for 3 h and then reduced to dryness in vacuo to give a yellow oil. The oil was dissolved in diethyl ether, washed with water (3 × 30 mL), and dried over sodium sulfate. Evaporation in vacuo yielded the product, which was purified by flash column chromatography (silica gel 60/ gradient elution: hexane/dichloromethane 3:2−2:2) (R_f = 0.25) to give as a pale yellow oil (0.74 g, 64%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.2 (m, 12H, pyridyl and phenyl), 3.65 (s, 4H, ethylene), 3.61 (s, 4H, ethylene). ^{13}C NMR: δ 160.07 (2-pyridyl), 138.22, 134.09 (4-pyridyl and 1-phenyl), 129.50, 128.93 (2,3-phenyl), 127.54, 124.82 (3-pyridyl and 4-phenyl), 37.30 (2-pyridyl methylene), 36.42 (benzyl methylene). EI MS m/z = 430 and 432 $[M]^+$, 308 and 310 $[M - SCH_2Ph]^+$. Anal. Calcd for $C_{21}H_{20}BrNS_2 \cdot 0.5CH_3OH$: C, 57.84; H, 4.97; N, 3.14. Found: C, 57.51; H, 4.57; N, 3.24.

17α-[(2,6-Bis((methylthio)methyl)pyridyl)-4-ethynyl]estra-1,3,5-(10)-triene-3,17β-diol (L^1). Compound **A** (0.25 g, 0.947 mmol), 17α-ethynylestradiol (0.281 g, 0.947 mmol), $Pd(PPh_3)_2Cl_2$ (0.0133 g, 0.0189 mmol), and CuI (0.0072 g, 0.0397 mmol) were placed under a nitrogen atmosphere, and dry degassed THF (5 mL) was added followed by Pr^i_2NH (5 mL). The mixture was stirred under a nitrogen atmosphere in the dark at room temperature for 24 h. The brown mixture was filtered and reduced to dryness in vacuo to give the crude product. The glassy compound was purified by flash column chromatography (silica gel 60, gradient elution: neat dichloromethane to dichloromethane/methanol 30:1) (R_f = 0.24) to give $\mathbf{L^1}$ as a yellow solid (0.33 g, 70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28 (s, 2H, pyridyl), 7.15 (d, 1H, J = 8.5 Hz, H1), 6.62 (dd, 1H, J = 8.5 Hz, 2.7 Hz, H2), 6.56 (1H, d, J = 2.7 Hz, H4), 5.25 (s, 1H, hydroxyl), 3.78 (s, 4H, methylene), 2.82 (2H, H6), 2.35 and 2.05 (m, 4H, H15/16), 2.35 (m, 1H, H9), 1.84 and 1.44 (m, 2H, H11), 1.85 and 1.32 (m, 2H, H7), 1.83 (m, 2H, H12), 2.07 (s, 6H, methyl), 1.82 (m, 1H, H8), 1.65 (m, 1H, H14), 0.93 (s, 3H, H18).^{13}C NMR: δ 158.3 (4-pyridyl, C21), 153.8 (C3), 138.0 (C5), 132.7 (C10), 131.8 (C23), 126.4 (C1), 123.2 (3-pyridyl, C22), 115.4 (C4), 112.9 (C2),97.8 (C17), 83.5 and 80.3 (alkyne C19/20), 49.9 (C14), 47.7 (C13), 43.5 (C9), 39.4 (ligand methylene, C24), 39.3 (C8), 39.0 (C16), 33.0 (C12), 29.5 (C6), 27.1 (C7), 25.5 (C11), 20.9 (C15), 15.2 (ligand methyl, C25), 12.8 (methyl, C18). Low-resolution CI MS m/z = 494 $[M]^+$, 447 $[M - SMe]^+$, 401 $[M - 2(SMe)]^+$. High-resolution CI MS calcd for $[M^+]$ ($C_{29}H_{35}NO_2S_2$) 494.2187, found 494.2203. Anal. Calcd for $C_{29}H_{35}NO_2S_2 \cdot 0.45CH_2Cl_2$: C, 66.50; H, 6.80; N, 2.63. Found: C, 66.48; H, 6.77; N, 2.42. IR: 2970, 2915, 2867, 1597, 1540, 1498, 1434, 1354, 1286, 1234, 1062, 1048, 998, 874, 817 cm^{-1}.

17α-[(2,6-Bis((benzylthio)methyl)pyridyl)-4-ethynyl)]estra-1,3,5-(10)-triene-3,17β-diol (L^2). Compound **B** (0.588 g, 1.37 mmol), 17α-ethynylestradiol (0.405 g, 1.37 mmol), $Pd(PPh_3)_2Cl_2$ (0.0192 g, 0.0273 mmol), and CuI (0.0104 g, 0.0546 mmol) were placed under a nitrogen atmosphere, and dry degassed THF (10 mL) was added followed by Pr^i_2NH (5 mL). The mixture was stirred under a nitrogen atmosphere in the dark at room temperature for 24 h. The orange mixture was filtered and reduced to dryness in vacuo to give the crude product. The glassy compound was purified by flash column chromatography (silica gel 60, gradient elution, neat dichloromethane to dichloromethane/methanol 40:1) (R_f = 0.27) to give $\mathbf{L^2}$ as a yellow wax (0.76 g. 86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31 (m, 13H, H1, pyridyl H22, phenyl and H1), 6.62 (dd, 1H, J = 8, 2 Hz, H2), 6.55 (d, 1H, J = 2 Hz, H4), 3.71 (s, 4H, ethylene), 3.69 (s, 4H, ethylene), 2.46 and 2.15 (m, 2H, H16), 2.42 and 1.95 (m, 2H, H6), 2.29 (m, 1H, H9), 1.95 and 1.82 (m, 2H, H12), 1.9 and 1.55 (m, 2H, H11), 1.9 and 1.5 (m, 2H, H15), 1.9 and 1.42 (m, 2H, H7), 1.77 (m, 1H, H14), 1.65 and 1.35 (m, 1H, H8), 0.98 (s, 3H, H18). ^{13}C NMR: δ 158.11 (4-pyridyl, C21), 153.97 (C3), 137.7 and 137.6 (C5 and phenyl C26), 132.39 (C10), 131.35 (2-pyridyl, C23), 128.85 and 128.3 (phenyl C27/28), 126.9 (3-pyridyl, C22), 126.2 (C1), 123.1 (phenyl C29), 115.4 (C4), 112.9 (C2), 97.8 (C17), 50.3 (C14), 44.0 (C9), 39.9 (C8), 39.5 (C16), 37.5 and 36.5 (C24/25), 33.6 (C12), 27.7 (C7), 26.4 (C6), 26.0 (C11), 23.4 (C15), 13.3 (C18). Low-resolution CI MS m/z = 646 $[MH]^+$, 524 $[M - SCH_2Ph]^+$, 402 $[M - 2(SCH_2Ph)]^+$. High-resolution CI MS calcd for $[M^+]$ ($C_{41}H_{43}NO_2S_2$) 646.2813, found 646.2800. Anal. Calcd for $C_{41}H_{35}NO_2S_2$ $Pr^i_2NH \cdot 2H_2O$: C, 72.83; H, 7.02. Found: C, 73.10; H, 7.00. IR: 2924, 2867, 1597, 1540, 1495, 1453, 1286, 1235, 1141, 1065, 1019, 1001, 913, 871, 697.

[Pt(L^1)Cl]Cl (1). A solution of $[Pt(PhCN)_2Cl_2]$ (0.0191 g, 0.0405 mmol) in dichloromethane (5 mL) was added dropwise with stirring to a solution of $\mathbf{L^1}$ (0.02 g, 0.0405 mmol) in dichloromethane (10 mL). The solution was heated to reflux for 2 h with light exclusion during which time a yellow precipitate formed. The precipitate was collected by filtration, washed with dichloromethane (20 mL), and dried in vacuo (0.0274 g, 89%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.06 (s, 1H, phenol), 7.80 (s, 2H, pyridyl), 7.06 (d, 1H, J = 8.5 Hz, H1), 6.51 (dd, 1H, J = 8.5, 2 Hz, H3), 6.44 (d, 1H, H4, J = 2 Hz), 5.80 (s, 1H, 17β-hydroxyl), 5.14, 5.09, 5.03, 4.92, 4.87, 4.81 (4 × d, 4H, ligand methylene), 2.78, 2.70 (2 × s, 6H, ligand methyl), 3−1.0 (m, 15H, steroid), 0.83 (s, 3H, H18). +FAB MS (NBA matrix) m/z = 724 $[M - Cl]^+$. Anal. Calcd for $C_{29}H_{35}Cl_2NO_2S_2Pt \cdot 0.5CH_2Cl_2$: C, 44.17; H, 4.52; N, 1.75. Found: C, 44.13; H, 4.52; N, 1.38. IR: 2916, 2862, 2217, 1614, 1497, 1443, 1427, 1418, 1286, 1142, 1066, 1049, 1020, 1005, 984, 866, 823 cm^{-1}.

[Pd(L^1)Cl]Cl (2). A solution of $[Pd(MeCN)_2Cl_2]$ (0.0155 g, 0.0405 mmol) in dichloromethane (5 mL) was added dropwise with stirring to a solution of $\mathbf{L^1}$ (0.02 g, 0.0405 mmol) in dichloromethane (10 mL). A cream precipitate formed immediately and was collected by filtration, washed with dichloromethane (10 mL), and dried in vacuo (0.015 g, 55%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.04 (s, 1H, phenol), 7.74 (s, 2H, pyridyl), 7.06 (d, 1H, J = 9 Hz, H1), 6.46 (d, 1H, J = 9 Hz, H2), 6.44 (s, 1H, H4), 5.78 (s, 1H, 17β-hydroxyl), 4.86 (s, 4H, ligand methylene), 2.73 (s, 6H, ligand methyl), 2.5−1.0 (m, 15H, steroid), 0.83 (s, 3H, H18). +FAB MS m/z = 636 $[M - Cl]^+$. Anal. Calcd for $C_{29}H_{35}Cl_2NO_2S_2Pd \cdot CH_2Cl_2$: C, 47.66; H, 4.93; N, 1.85. Found: C, 47.96; H, 4.99; N, 1.59. IR: 2921, 2864, 1609, 1498, 1444, 1418, 1346, 1286, 1248, 1227, 1142, 1068, 1046, 969 cm^{-1}.

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Ni(L^1)Cl$_2$ (3). A solution of **L^1** (0.06 g, 0.122 mmol) in ethyl acetate/methanol (1:1, 3 mL) was added with stirring to a solution of nickel(II)chloride hexahydrate (0.029 g, 0.122 mmol) in methanol (2 mL). Slow evaporation of the bright green solution gave a green precipitate (0.012 g, 42%). ^{1}H NMR (400 MHz, MeOH-d_4): δ 133.48 (broad s, 4H, ligand methylene), 53.28 (s, 2H, pyridyl), 7.10 (m, 1H, H1), 6.52 (m, 2H, H2 and 4), 4−0.8 (m, 15H, steroid), 3.34 (s, 6H, ligand methyl). +FAB MS m/z = 586 [M − Cl]$^+$, 551 [M − 2Cl]$^+$. Anal. Calcd for $C_{29}H_{35}NO_2S_2Cl_2Ni\cdot 2H_2O$: C, 52.83; H, 5.96; N, 2.12. Found: C, 53.27; H, 5.64; N, 1.97. IR: 2923, 2866, 1607, 1542, 1498, 1447, 1416, 1385, 1286, 1231, 1048 cm^{-1}.

Zn(L^1)Br$_2$ (4). A solution of **L^1** (0.03 g, 0.061 mmol) in methanol (2 mL) was added with stirring to a solution of zinc(II) bromide (0.0.137 g, 0.061 mmol) in methanol (1 mL). Slow evaporation yielded a white precipitate that was collected by filtration (0.025 g, 51%). ^{1}H NMR (MeOH-d_4): δ 7.34 (s, 2H, pyridyl), 7.12 (d, 1H, J = 8 Hz, H1) 6.56 (d, 1H, J = 8 Hz, H2), 6.50 (s, 1H, H4), 3.77 (s, 4H, ligand methylene), 3−1 (15H, steroid), 2.04 (s, 6H, ligand methyl), 0.96 (s, 3H, H18). +FAB MS: m/z = 638 [M − Br]$^+$, 494 Anal. Calcd for $C_{29}H_{35}NO_2S_2Br_2Zn$: C, 47.27; H, 5.06; N, 1.90. Found: C, 47.45; H, 4.83; N, 1.70. IR: 2928, 2868, 2360, 2230, 1612, 1496, 1421, 1284, 1247, 1141, 1065, 1018, 819 cm^{-1}.

[Re(L^1)(CO)$_3$Cl] (5). **L^1** (0.02 g, 0.0405 mmol) and [Re(CO)$_5$Cl] (0.0146 g, 0.0405 mmol) were dissolved in tetrahydrofuran (15 mL) and heated to reflux for 4 h. The solution was reduced to dryness in vacuo, and the cream residue recrystallized from dichloromethane/toluene (0.022 g, 67%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.02 (s, 1H, phenol hydroxyl), 7.20 (s, 2H, pyridyl), 7.07 (dd, 1H, J = 8 Hz, 3 Hz, H1), 6.54 (dd, 1H, J = 8 Hz, 3 Hz, H2), 6.43 (d, 1H, J = 3 Hz, H4), 5.63 (s, 1H, 17β-hydroxyl), 3.74 (s, 4H, ligand methylene), 3−1 (m, 15H, steroid), 2.04 (s, 6H, ligand methyl), 0.83 (s, 3H, H18). ^{1}H NMR (300 MHz, CD_2Cl_2): δ 7.81 (m, 1H, pyridyl), 7.47 (m, 1H, pyridyl), 7.16 (m, 1H, H1), 6.62 (dd, 1H, H2, J = 3, 8 Hz), 5.31, 5.26, 4.64, 4.59, 4.55, 4.50, 4.42, 4.40, 4.37, 4.36, 4.35, 4.25, 4.20 (m, 4H, ligand methylene), 2.83 (m, 2H, H6), 2.6−1.2 (m, 13H, steroid), 2.56, 2.30, 2.20, 2.18 (m, 6H, ligand methylene), 0.97 (s, 3H, H18). ^{13}C NMR (400 MHz, CD_2Cl_2): 163.5, 162.7, 160.0, 159.3, 153.8, 138.5, 134.9, 132.5, 129.2, 127.0, 126.6, 126.3, 125.5, 125.0, 115.3, 112.8, 103.1, 82.4, 80.7, 50.4, 48.2, 45.8, 45.7, 44.4, 43.8, 39.7, 39.3, 33.5, 29.8, 27.8, 26.7, 23.3, 23.1, 16.0, 15.8, 15.4, 12.9. Low-resolution +FAB MS m/z = 764 [M − Cl]$^+$. High-resolution +FAB MS calcd for [(M − Cl)$^+$] ($C_{32}H_{36}NO_5Re$) 762.1486, found 762.1463. Anal. Calcd for ($C_{32}H_{35}NS_2O_5ClRe$) $C_7H_8\cdot 1.75(CH_2Cl_2)$: C, 47.05; H, 4.51; N, 1.35. Found: C, 47.13; H, 4.24; N, 1.38. Solution IR: 2219 (w), 2027 (s), 1929 (s), 1901 (s), 1779 (m), 1727 (m), 1612 (m), 1460 cm^{-1} (m).

[Pd(L^2)Cl]Cl (6). A solution of [Pd(MeCN)$_2$Cl$_2$] (0.0201 g, 0.0744 mmol) in dichloromethane (5 mL) was added dropwise with stirring to a solution of **L^2** (0.05 g, 0.0744 mmol) in dichloromethane (10 mL). A cream precipitate formed immediately and was collected by filtration, washed with dichloromethane (10 mL), and dried in vacuo (0.04 g, 66%). ^{1}H NMR (300 MHz, DMSO-d_6): δ 9.06 (s, 1H, phenol), 7.58 (d, 4H, J = 6.6 Hz, phenyl H27), 7.49 (s, 2H, 3-pyridyl (H22)), 7.28 (m, 6H, H28/29), 5.91 (s, 1H, 17β-OH), 4.71 (broad s, 4H, ligand methylene), 4.43 (s, 4H, benzyl-CH$_2$), 3−1 (m, 15H, steroid), 0.833 (s, 3H, H18). +FAB MS m/z = 786 [M − Cl]$^+$. Anal. Calcd for $C_{41}H_{43}NO_2S_2Cl_2Pd\cdot CH_2Cl_2$: C, 57.7; H, 5,14; N, 1.62. Found: C, 57.80; H, 5.12; N, 1.53. IR: 2924, 2865, 2362, 2342, 2185, 1608, 1496, 1454, 1286, 1246, 1069, 912 cm^{-1}.

17α-[Diethyl(pyridine-2,6-bis-carboxylate)-4-ethynyl]estra-1,3,5(10)-triene-3,17β-diol (L^3). 4-Bromo-2,6-pyridinedicarboxylate diethyl ester (0.3 g, 0.993 mmol), 17α-ethynylestradiol (0.295 g, 0.993 mmol), bis-triphenylphosphine palladium(II)chloride (0.014 g, 0.02 mmol), and copper(I) iodide (0.008 g, 0.041 mmol) were placed under an atmosphere of nitrogen. Tetrahyrofuran (4 mL) was added followed by diisopropylamine (4 mL). The solution was stirred under nitrogen for 24 h at room temperature with light exclusion. Diisopropylamine hydrobromide was removed by filtration and the filtrate reduced in vacuo. The solid was dissolved in chloroform (15 mL), and the solution washed with water (3 × 5 mL) and dried over anhydrous MgSO$_4$. The chloroform was removed in vacuo to give a cream solid that was purified by flash column chromatography (silica gel 60; chloroform/methanol, 30:1). The product (R_f = 0.34) was eluted after an initial orange band to give a white solid (0.35 g, 68%, mp 130−132 °C). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.24 (2H, s, py), 7.14 (1H, d, J = 8 Hz, H1), 6.62 (1H, dd, J = 3, 8 Hz, H2), 6.56 (1H, d, J = 3 Hz, H4), 4.48 (4H, q, J = 7 Hz, H25), 2.85 (2H, H6), 2.45 and 2.15 (2H, H10), 2.25 (1H, H9), 1.90 (2H, H7), 1.9 and 1.8 (2H, H15), 1.8 (2H, H12), 1.75 (1H, H14), 1.54 and 2.42 (2H, H11), 1.48 (1H, H8), 1.44 (6H, t, J = 7 Hz, H26), 0.94 (3H, s, H18). ^{13}C NMR: δ 164.7 (acyl-C, C24), 153.9 (C3), 149.2 (2-pyridyl, C23), 138.5 (C5), 132.59 (C10), 134.36 (4-pyridyl, C21), 130.1 (3-pyridyl, C22), 126.9 (C1), 115.8 (C4), 113.2 (C2), 100.98 (C17), 82.9/81.0 (alkyne C19/20), 63.0 (ethoxy methylene, C25), 50.5 (C14), 48.3 (C13), 43.9 (C9), 39.85 (C8), 39.5 (C16), 33.6 (C12), 30.0 (C6), 27.6 (C7), 26.8 (C11), 23.4 (C15), 13.25 (ethoxy methyl, C26). +FAB MS (glycerol/methanol) m/z = 518 [M]$^+$. Anal. Calcd for $C_{31}H_{35}NO_6\cdot 0.55CH_2Cl_2$: C, 67.15; H, 6.45; N, 2.48. Found: C, 67.02; H, 6.32; N, 2.29. IR: 2978, 2930, 2869, 1723, 1595, 1375, 1344, 1287, 1235, 1218, 1177, 1155, 1123, 1082, 1063, 1049, 1019 cm^{-1}.

17α-[(Pyridine-2,6-bis-carboxylic acid)-4-ethynyl]estra-1,3,5(10)-triene-3,17β-diol (L^4). **L^3** (0.7 g, 1.35 mmol) was warmed in a solution of potassium hydroxide (20 mL, 0.5 M in ethanol) for 2 h. Water (30 mL) was added, and the solution was acidified to pH 2 using dilute aqueous hydrochloric acid. The cream precipitate was collected by filtration, washed with a little cold water, recrystallized from methanol/water, and dried in vacuo over phosphorus pentaoxide (0.34 g, 55%). ^{1}H NMR (250 MHz, DMSO-d_6): δ 13.60 (s, 2H, acid), 9.00 (s, 1H, phenol), 8.00 (s, 2H, pyridyl), 7.06 (d, 1H, J = 8 Hz, H1), 6.50 (d, 1H, J = 8 Hz, H2), 6.44 (s, 1H, H4), 5.72 (s, 1H, hydoxyl), 3.00−1 (15H, m, steroid), 0.83 (s, 3H, H18). IR: 1734 cm^{-1}, (C═O). Low-resolution +FAB MS (glycerol/methanol): m/z = 462 [MH$^+$]. High-resolution +FAB MS calcd for MH$^+$ ($C_{27}H_{28}NO_6$) 462.1916, found 462.1915. IR: 2929, 2914, 2865, 1574, 1454, 1430, 1415, 1387, 1350, 1286, 1246, 1216, 1173, 1143, 1119, 1107, 1071, 1052, 1021 cm^{-1}.

[Zn(L^4)X] (7). Zinc acetate (0.05 g, 0.25 mmol) was dissolved in a hot methanolic solution (30 mL) of **L^4** (0.115 g, 0.25 mmol). The solution was heated at 50 °C for 1 h and cooled and the white precipitate collected by filtration. The cream solid was washed with a little cold methanol and dried in vacuo (0.086 g, 62%). ^{1}H NMR (250 MHz, DMSO-d_6): δ 9.01 (1H, s, phenol), 8.02 (2H, s, pyridyl), 7.07 (1H, d, J = 9 Hz, H1), 6.50 (1H, dd, J = 9, 2 Hz, H2), 6.44 (1H, d, J = 2 Hz, H4), 5.75 (1H, s, hydroxyl), 3.00−1 (m, 15H, steroid), 0.84 (3H, s, H18). IR: 2928, 2866, 2630, 1638, 1556, 1423, 1393, 1335, 1286, 1248, 1133, 1065, 1042, 1015 cm^{-1}.

[Pt(L^4)X] (8). **L^4** (0.03 g, 0.065 mmol) and potassium hydroxide (7.28 mg, 0.13 mmol) were dissolved in methanol (10 mL), and the solution was reduced to dryness in vacuo. The white solid was heated with platinum(cod)Cl$_2$ (0.024 g, 0.065 mmol) in acetonitrile/water (1:1) (50 mL) with stirring in the dark at 60 °C for 3 h. The cream solid that precipitated from the cooled solution was collected by filtration and dried in vacuo over phosphorus pentaoxide (0.031 g, 35%). ^{1}H NMR (DMSO-d_6): δ 9.01 (1H, s, phenol), 7.87 (H, s, hydroxyl), 7.06 (1H, d, J = 9 Hz, H1), 6.50 (1H, d, J = 9 Hz, H2), 6.43 (1H, s, H4), 5.59 (2H, s, pyridyl), 3.0−1.0 (m, 15H, steroid), 0.83 (3H, s, H18). IR: 2928, 2866, 1575, 1504, 1434, 1407, 1344, 1286, 1064, 1054, 1011 cm^{-1}.

Estrogen Binding Radioligand Binding Assay. Materials and Methods. Compound Preparation. Compounds were dissolved to give a stock solution that was subsequently further diluted with assay buffer to give the desired range of concentrations used for determination of binding affinity. Compounds **L^1**, **L^2**, **1**, **2**, **5**−**8**, **L^3**, **L^4**, **9**, and **10** were used for determination of binding affinity. Preparations of 10 mM stock solutions were as follows. **L^1** (1.9 mg) was dissolved in ethanol (385 μL), **L^2** (3.5 mg) was dissolved in ethanol (548 μL), **5** (3.6 mg) was dissolved in methanol (450 μL), **6** (2.4 mg) was dissolved in methanol (305 μL), **7** (1 mg) was dissolved methanol (215 μL), **8** (3 mg) was dissolved in methanol (797.8 μL), **9** (2.4 mg) was dissolved in DMSO (442 μL), and **10** (2.1 mg) was dissolved in DMSO (318.8 μL). Preparations of 5 mM stock solutions were as follows. **1** (1.2 mg) was dissolved in ethanol (284 μL), and **2** (2.7 mg) was dissolved in methanol (714 μL). Solutions were sonicated to ensure thorough mixing. A 2 mM stock solution of diethystilbestrol (DES) was prepared in ethanol.

Cell Line Growth Conditions. The estrogen receptor positive MCF-7 human breast cancer cell line was obtained from the American Type Culture Collection. Cells were routinely grown at 37 °C in 5% carbon dioxide−95% air and maintained by weekly passage in minimum essential medium (MEM) (Gibco) supplemented with 2 mM L-glutamine, 50 IU/50 μg/mL penicillin/streptomycin, 1% nonessential amino acids, and 10% fetal bovine serum. For radioligand binding assays, MCF-7 cells (breast adenocarcinoma) were seeded in growth media at a concentration of 1×10^4 cells per well (1 mL) into 24-well culture plates. After a period of 24 h, medium was removed from the wells and the cell monolayer was washed with PBS to remove residual medium and serum. Estrogen-free medium (1 mL), which consisted of MEM without phenol red (Gibco) supplemented with 2 mM L-glutamine, 50 IU/50 μg/mL penicillin/streptomycin, 1% nonessential amino acids, and controlled process serum replacement-1 (CPSR-1, Sigma), was added to each well. Incubation was continued for a further 3 days, after which time cells were used in radioligand binding studies.

Estrogen Receptor Preparation. Media was removed from MCF-7 cells routinely growing in 162 cm^2 flasks and replaced with estrogen-free media following washing of the monolayer with PBS. Incubation was continued for a further 3 days prior to harvesting. To harvest, cells were scraped into ice-cold PBS and recovered cells centrifuged at 1000 rpm for 5 min. The supernatant was decanted, and the pellets were resuspended in lysis buffer (10 mM HEPES, 1.5 mM $CaCl_2$, 2 mM dithiothreitol, pH 7.4). Resuspended pellets were passed through a 21 G needle three times to ensure disruption of the cells. Disrupted cells were centrifuged at 3000 rpm for 10 min at 2 °C. Supernatant was collected for use in radioligand binding assays.

Radioligand Binding Assays. Binding affinities for each compound were determined from a minimum of two assays.

Viable Cell Assay. Medium was removed from 24 well plate and monolayer washed with phosphate-buffered saline (PBS) (1 mL). Medium was replaced with 200 μL of assay buffer (MEM without phenol red containing 0.1% BSA) containing 0.1 nM 16α[^{125}I]-iodo-3,17β-estradiol NEN (NEX-144) plus or minus competing compound at desired concentration. The cells were incubated at 37 °C for 1 h, after which time assay reagents were removed and replaced with 500 μL of equilibration buffer (5 mM sodium phosphate, 0.25 M sucrose, 0.5% BSA, 10% glycerol, pH 7.5). Cells were further incubated at 37 °C for 30 min. Buffer was removed, and cells were washed rapidly with ice-cold equilibration buffer. Ethanol (1 mL) was added to each well to extract the radioactivity. After 30 min at room temperature, ethanol was transferred to counting vials and radioactivity counted by a Wallac Wizard γ counter.

Isolated Receptor Assay. Isolated receptor was incubated in assay buffer containing 0.1 nM 16α[^{125}I]-iodo-3,17β-estradiol, plus or minus competing compound at desired concentration. Assay reagents were incubated at room temperature for 3 h, after which time receptor bound radioactivity was separated from free by filtration methods. Filters were transferred to counting vials and radioactivity counted by a Wallac Wizard γ counter.

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