JOC Article

Synthesis of 17-α-Substituted Estradiol-Pyridin-2-yl Hydrazine Conjugates as Effective Ligands for Labeling with Alberto's Complex *fac*-$[Re(OH_2)_3(CO)_3]^+$ in Water

Jeffrey B. Arterburn,*,† Cesear Corona,† Kalla Venkateswara Rao,† Kathryn E. Carlson,‡ and John A. Katzenellenbogen‡

Department of Chemistry and Biochemistry MSC 3C, New Mexico State University, P.O. Box 30001, Las Cruces, New Mexico 88003, and Department of Chemistry, University of Illinois, 600 South Mathews Avenue, Urbana, Illinois 61801

jarterbu@nmsu.edu

Received June 6, 2003

The development of ^{99m}Tc-estradiol radiopharmaceuticals would be advantageous for the detection of estrogen receptor-positive breast tumors. Estradiol derivatives conjugated to organometallic tricarbonyl-Tc(I) and related Re(I) complexes are capable of achieving high receptor binding affinity, but effective methods for synthesizing radiolabeled complexes in water are not available. Our interest in the synthesis of 2-hydrazinopyridines as ligands for Tc and Re led us to investigate Pd-catalyzed amination reactions of halo-pyridine substrates with di-*tert*-butyl hydrazodiformate. Both 2- and 4-substituted halo-pyridine substrates undergo C−N coupling with di-*tert*-butyl hydrazodiformate to produce Boc-protected pyridine hydrazine derivatives. Only highly electrophilic 3-pyridine halides were converted to the hydrazine. The Boc-protected 5-bromopyridin-2-yl hydrazine substrate **3** was prepared by regioselective substitution at the 2-position of 2,5-dibromopyridine. This bifunctional chelate was attached to ethynyl or vinyl groups at the 17α position of estradiol, using Sonogashira and Suzuki/Miyaura coupling reactions to synthesize **1** and **2** in high yields, respectively. Deprotection of **1** under acidic conditions provided the hydrazine hydrochloride salt **25**. The 17α-estradiol-tricarbonylrhenium(I) complex **4** was synthesized by labeling **25** with *fac*-$[Re(OH_2)_3(CO)_3]^+$ in aqueous ethanol. This complex exhibited excellent stability and high receptor binding affinity for the estrogen receptor, and it is a promising model for evaluation of the analogous Tc-99m complexes as diagnostic imaging agents for breast tumors.

Introduction

The kinetic and thermodynamic stability of certain classes of organometallic complexes in aqueous, aerobic environments contrasts with common assumptions that relegate them to remain in the glovebox. These are fascinating molecules with unique structural, electronic, and chemical properties. Proponents of bioorganometallic chemistry have realized this potential and pursued the development of novel labeling agents, biochemical probes, and new drugs.[1−15] Technetium-99m is a radionuclide of choice for diagnostic imaging due to its favorable nuclear decay properties, a 6-h half-life, low cost, and availability throughout the world. The discovery of kinetically stable, substitution inert d^6-technetium(I) complexes first demonstrated the opportunities for organometallic complexes in nuclear medicine.[16,17] Current interest has focused on developing a new generation of highly selective, target-specific radiopharmaceuticals consisting of a metal complex conjugated to an organic molecule that is a ligand with high binding affinity for a receptor.[18−25] The syn-

* Address correspondence to this author. Phone: (505) 646-2738. Fax: (505) 646-2649.
† New Mexico State University.
‡ University of Illinois.

(1) Jaouen, G.; Vessieres, A.; Butler, I. S. *Acc. Chem. Res.* **1993**, *26*, 361−369.
(2) Salmain, M.; Gunn, M.; Gorfi, A.; Top, S.; Jaouen, G. *Bioconj. Chem.* **1993**, *4*, 425−433.
(3) Severin, K.; Bergs, R.; Beck, W. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 1635−1654.
(4) Fish, R. H. *Coord. Chem. Rev.* **1999**, *186*, 569−584.
(5) Grotjahn, D. B. *Coord. Chem. Rev.* **1999**, *192*, 1125−1141.
(6) Jaouen, G.; Top, S.; Vessieres, A.; Alberto, R. *J. Organomet. Chem.* **2000**, *600*, 23−36.
(7) Albrecht, M.; Rodriguez, G.; Schoenmaker, J.; vanKoten, G. *Org. Lett.* **2000**, *2*, 3461−3464.
(8) Salmain, M.; Licandro, E.; Baldoli, C.; Maiorana, S.; Tran-Huy, H.; Jaouen, G. *J. Organomet. Chem.* **2001**, *617*, 376−382.
(9) Lo, H. C.; Leiva, C.; Buriez, O.; Kerr, J. B.; Olmstead, M. M.; Fish, R. H. *Inorg. Chem.* **2001**, *40*, 6705−6716.
(10) Metzler-Nolte, N. *Angew. Chem., Int. Ed.* **2001**, *40*, 1040.
(11) Jackson, A.; Davis, J.; Pither, R. J.; Rodger, A.; Hannon, M. J. *Inorg. Chem.* **2001**, *40*, 3964−3973.
(12) van Staveren, D. R.; Metzler-Nolte, N. *Chem. Commun.* **2002**, 1406−1407.
(13) Rodriguez, G.; Albrecht, M.; Schoenmaker, J.; Ford, A.; Lutz, M.; Spek, A. L.; van Koten, G. *J. Am. Chem. Soc.* **2002**, *124*, 5127−5138.
(14) Herebian, D.; Sheldrick, W. S. *J. Chem. Soc., Dalton Trans.* **2002**, 966−974.
(15) Cadierno, V.; Conejero, S.; Gamasa, M. P.; Gimeno, J.; Rodriguez, M. A. *Organometallics* **2002**, *21*, 203−209.
(16) Abrams, M. J.; Davison, A.; Jones, A. G.; Costello, C. E.; Pang, H. *Inorg. Chem.* **1983**, *22*, 2798−2800.
(17) Jones, A. G.; Abrams, M. J.; Davison, A.; Brodack, J. W.; Toothaker, A. K.; Adelstein, S. J.; Kassis, A. I. *Int. J. Nucl. Med. Biol.* **1984**, *11*, 225−234.

10.1021/jo034780g CCC: $25.00 © 2003 American Chemical Society
Published on Web 08/14/2003

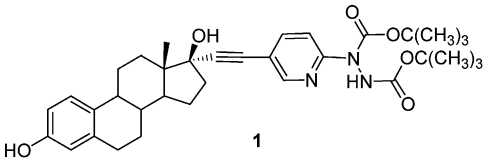
thesis of rhenium analogues is important as stable isotope models for the chemically similar technetium complexes, and as Re-186,188 radiopharmaceuticals for therapeutic applications.[26−28] In this approach the organic targeting groups are connected to a bifunctional chelate that enables coordination of the transition metal. The characteristics of an ideal bifunctional chelate for labeled targeted radiopharmaceuticals include exceptionally strong ligating ability, small size, and a nonpolar backbone. The receptor ligand−chelate conjugate should undergo labeling in water and produce neutral complexes in high yield and radiochemical purity.

The importance of determining the estrogen receptor (ER) content in breast tumors as a prerequisite for effective treatment of this devastating disease and the advantages associated with early detection have stimulated great interest in ^{99m}Tc-estradiol radiopharmaceuticals.[19,29−32] Estradiol conjugates with pendant organometallic cyclopentadienyl tricarbonyl complexes are capable of binding to ER with high affinity; however, no satisfactory methods are available for the synthesis of these radiopharmaceuticals directly in water.[31−41] Estradiol derivatives possessing thioether ligands also form tricarbonyl-technetium(I) and -rhenium(I) complexes; however, the compounds investigated to date exhibit relatively low binding affinity to ER.[11,37,42−44]

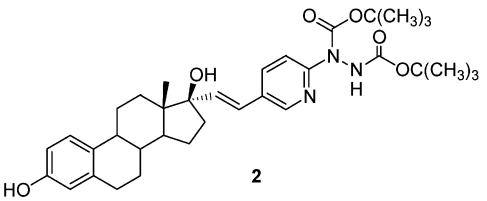
The remarkable synthesis of Alberto's *fac*-[^{99m}Tc(OH$_2$)$_3$(CO)$_3$]$^+$ complex directly from [^{99m}TcO$_4$]$^-$ in water under mild conditions has made this an important precursor for radiopharmaceutical labeling with a variety of chelators.[45−51] A highly stable complex with 2-hydrazinopyridine formed rapidly and quantitatively under dilute (μM) concentrations, which indicates the potential of this ligand class for producing radiopharmaceuticals with high specific activity.[47] Hydrazine ligands are also known to form stable complexes with Tc/Re in higher oxidation states.[52−58] Hydrazinonicotinamide derivatives have become important bifunctional ligands that can be conjugated to substrates possessing an available amino group.[59−74]

(18) Schwochau, K. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2258−2267.
(19) Hom, R. K.; Katzenellenbogen, J. A. *Nucl. Med. Biol.* **1997**, *24*, 485−498.
(20) Johannsen, B.; Spies, H. *Transition Met. Chem.* **1997**, *22*, 318−320.
(21) Jurisson, S. S.; Lydon, J. D. *Chem. Rev.* **1999**, *99*, 2205−2218.
(22) Liu, S.; Edwards, D. S. *Chem. Rev.* **1999**, *99*, 2235−2268.
(23) Dilworth, J. R.; Parrott, S. J. *Chem. Soc. Rev.* **1998**, *27*, 43−55.
(24) Bandoli, G.; Dolmella, A.; Porchia, M.; Refosco, F.; Tisato, F. *Coord. Chem. Rev.* **2001**, *214*, 43−90.
(25) Schibli, R.; Schubiger, P. A. *Eur. J. Nucl. Med. Mol. Imaging* **2002**, *29*, 1529−1542.
(26) Mirzadeh, S.; Knapp, F. F. J. *J. Radioanal. Nucl. Chem.* **1996**, *203*, 471−488.
(27) Srivastava, S. C. In *Current Directions in Radiopharmaceutical Research and Development*; Mather, S. J., Ed.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1996; p 63−79.
(28) Schubiger, P. A.; Alberto, R.; Smith, A. *Bioconj. Chem.* **1996**, *7*, 165−179.
(29) Wust, F.; Spies, H.; Johannsen, B. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2729−2734.
(30) Sugano, Y.; Katzenellenbogen, J. A. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 361−366.
(31) Skaddan, M. B.; Katzenellenbogen, J. A. *Bioconj. Chem.* **1999**, *10*, 119−129.
(32) Mull, E. S.; Sattigeri, V. J.; Rodriguez, A. L.; Katzenellenbogen, J. A. *Bioorg. Med. Chem. Lett.* **2002**, *10*, 1381−1398.
(33) Wenzel, M.; Klinge, C. *J. Labeled Compd. Radiopharm.* **1994**, *34*, 981−987.
(34) Top, S.; El Hafa, H.; Vessieres, A.; Quivy, J.; Vaissermann, J.; Hughes, D. W.; McGlinchey, M. J.; Mornon, J. P.; Thoreau, E.; Jaouen, G. *J. Am. Chem. Soc.* **1995**, *117*, 8372−8380.
(35) Minutolo, F.; Katzenellenbogen, J. A. *J. Am. Chem. Soc.* **1998**, *120*, 13264−13265.
(36) Spradau, T. W.; Katzenellenbogen, J. A. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 3235−3240.
(37) Skaddan, M. B.; Wust, F. R.; Katzenellenbogen, J. A. *J. Org. Chem.* **1999**, *64*, 8108−8121.
(38) Skaddan, M. B.; Wust, F.; Jonson, S.; Syhre, R.; Welch, M. J.; Spies, H.; Katzenellenbogen, J. A. *Nucl. Med. Biol.* **2000**, *27*, 269−278.
(39) Osella, D.; Cavigiolio, G.; Vincenti, M.; Vessieres, A.; Laios, I.; Leclercq, G.; Napolitano, E.; Fiaschi, R.; Jaouen, G. *J. Organomet. Chem.* **2000**, *596*, 242−247.
(40) Le Bideau, F.; Salmain, M.; Top, S.; Jaouen, G. *Chem. Eur. J.* **2001**, *7*, 2289−2294.
(41) Acylcyclopentadienyl Tc/Re tricarbonyl complexes have recently been synthesized in H_2O. Wald, J.; Alberto, R.; Ortner, K.; Candreia, L. *Angew. Chem., Int. Ed.* **2001**, *40*, 3062−3066.
(42) Reisgys, M.; Wust, F.; Alberto, R.; Schibli, R.; Schubiger, P. A.; Pietzsch, H. J.; Spies, H.; Johannsen, B. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2243−2246.
(43) Wust, F.; Spies, H.; Johannsen, B. *Tetrahedron Lett.* **1997**, *38*, 2931−2932.
(44) Wust, F.; Carlson, K. E.; Katzenellenbogen, J. A.; Spies, H.; Johannsen, B. *Steroids* **1998**, *63*, 665−671.
(45) Alberto, R.; Schibli, R.; Egli, A.; Schubiger, A. P.; Abram, U.; Kaden, T. A. *J. Am. Chem. Soc.* **1998**, *120*, 7987−7988.
(46) Alberto, R.; Schibli, R.; Waibel, R.; Abram, U.; Scubiger, A. P. *Coord. Chem. Rev.* **1999**, *192*, 901−919.
(47) Alberto, R.; Schibli, R.; Schubiger, A. P.; Abram, U.; Pietzsch, H.-J.; Johannsen, B. *J. Am. Chem. Soc.* **1999**, *121*, 6076−6077.
(48) Schibli, R.; Bella, R. L.; Alberto, R.; Garcia-Garayoa, E.; Ortner, K.; Abram, U.; Schubiger, P. A. *Bioconj. Chem.* **2000**, *11*, 345−351.
(49) Garcia, R.; Paulo, A.; Domingos, A.; Santos, I.; Ortner, K.; Alberto, R. *J. Am. Chem. Soc.* **2000**, *122*, 11240−11241.
(50) Pietzsch, H.-J.; Gupta, A.; Reisgys, M.; Drews, A.; Seifert, S.; Syhre, R.; Spies, H.; Alberto, R.; Abram, U.; Schubiger, P. A.; Johannsen, B. *Bioconj. Chem.* **2000**, *11*, 414−424.
(51) Correia, J. D. G.; Domingos, A.; Santos, I.; Alberto, R.; Ortner, K. *Inorg. Chem.* **2001**, *40*, 5147−5151.
(52) Hirsch-Kuchma, M.; Nicholson, T.; Davison, A.; Davis, W. M.; Jones, A. G. *Inorg. Chem.* **1997**, *36*, 3237−3241.
(53) Liu, S.; Edwards, D. S.; Harris, A. R.; Singh, P. R. *Appl. Radiat. Isot.* **1997**, *48*, 1103−1111.
(54) Rose, D. J.; Maresca, K. P.; Nicholson, T.; Davison, A.; Jones, A. G.; Babich, J.; Fischman, A.; Graham, W.; DeBord, J. R. D.; Zubieta, J. *Inorg. Chem.* **1998**, *37*, 2701−2716.
(55) daCosta, M. T. A. R. S.; Dilworth, J. R.; Duarte, M. T.; daSilva, J. J. R. F.; Galvao, A. M.; Pombeiro, A. J. L. *J. Chem. Soc., Dalton Trans.* **1998**, 2405−2410.
(56) Perez-Lourido, P.; Romero, J.; Garcia-Vazquez, J. A.; Sousa, A.; Maresca, K. P.; Zubieta, J. *Inorg. Chem.* **1999**, *38*, 1511−1519.
(57) Albertin, G.; Antoniutti, S.; Bacchi, A.; Ballico, G. B. B., E.; Pelizzi, G.; Ranieri, M.; Ugo, P. *Inorg. Chem.* **2000**, *39*, 3265−3279.
(58) Mevellec, F.; Lepareur, N.; Roucoux, A.; Noiret, N.; Patin, H.; Bandoli, G.; Porchia, M.; Tisato. F *Inorg. Chem.* **2002**, *41*, 1591−1597.
(59) Abrams, M. J.; Juweid, M.; TenKate, C. I.; Shwartz, D. A.; Hauser, M. M.; Gaul, E. F.; Fucello, A. J.; Rubin, R. H.; Strauss, H. W.; Fischman, A. J. *J. Nucl. Med.* **1990**, *31*, 2022−2028.
(60) Schwartz, D. A.; Abrams, M. J.; Hauser, M. *Bioconj. Chem.* **1991**, *2*, 333−336.
(61) Larson, S. K.; Solomon, H. F.; Caldwell, G.; Abrams, M. J. *Bioconj. Chem.* **1995**, *6*, 635-.
(62) Edwards, D. S.; Liu, S. *Transition Met. Chem.* **1997**, *22*, 425−426.
(63) Edwards, D. S.; Liu, S.; Harris, A. R.; Poirier, M. J.; Ewels, B. A. *Bioconj. Chem.* **1999**, *10*, 803−807.
(64) Decristoforo, C.; Mather, S. J. *J. Nucl. Biol. Med.* **1999**, *26*, 389−396.
(65) Rennen, H. J. J. M.; Boerman, O. C.; Koenders, E. B.; Oyen, W. J. G.; Corstens, F. H. M. *Nucl. Med. Biol.* **2000**, *27*, 599−604.
(66) Liu, S.; Edwards, D. S.; Harris, A. R. *Bioconj. Chem.* **1998**, *9*, 583−595.
(67) Ono, M.; Arano, Y.; Uehara, T.; Fujioka, Y.; Ogawa, K.; Namba, S.; Mukai, T.; Nakayama, M.; Saji, H. *Bioconj. Chem.* **1999**, *10*, 386−394.
(68) Decristoforo, C.; Mather, S. J. *Bioconj. Chem.* **1999**, *10*, 431−438.
(69) Edwards, D. S.; Liu, S.; Ziegler, M. C.; Harris, A. R.; Crocker, A. C.; Heminway, S. J.; Barrett, J. A.; Bridger, G. J.; Abrams, M. J.; Higgins, J. D. *Bioconj. Chem.* **1999**, *10*, 884−891.

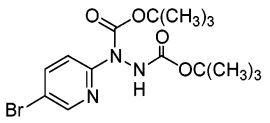
Our interest has focused on the synthesis of pyridin-2-yl hydrazine derivatives of estradiol **1** and **2** as potential ligands for labeling with Alberto's complex.[75] We report here the synthesis of **1** and **2** via palladium-catalyzed C−C coupling of *N*,*N*′-di-*tert*-butyloxycarbonyl-(5-bromopyridin-2-yl)-hydrazine **3**. The tricarbonylrhenium(I) complex **4** was synthesized in high yield from $[Re(OH_2)_3(CO)_3]^+$ in water, and its binding affinity for the estrogen receptor was determined. The chemical similarity of Re to Tc suggests complex **4** should be an effective model for Tc-99m-containing imaging agents for breast cancer.



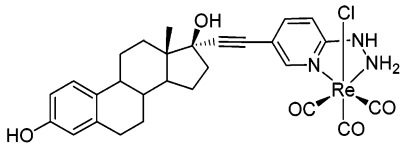
1



2



3

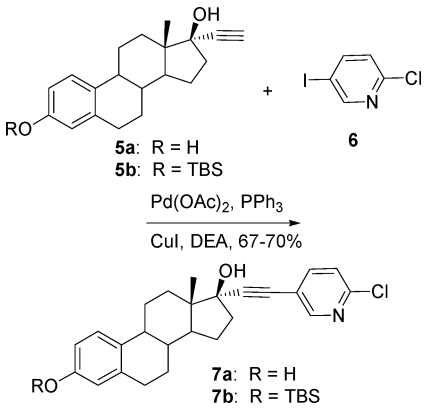


4

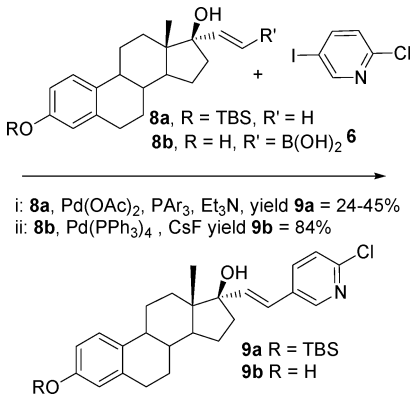
Results and Discussion

The 17α-position of estradiol was chosen for conjugation to the pyridin-2-yl hydrazine group, based on previous examples that demonstrated large groups could be introduced without compromising binding affinity.[11,34,40,44,76−78] We designed our synthetic approach to the protected pyridin-2-yl hydrazine derivatives **1** and **2** with the expectation of using palladium-catalyzed cross-coupling reactions of halogenated pyridines with alkyne and alkene derivatives, respectively. The selective coupling of 17α-ethynylestradiol **5** with 2-chloro-5-iodopyridine **6** to produce **7** was accomplished with use of standard Sonogashira conditions as shown in Scheme 1.[79−81] The mild conditions and functional group tolerance of this reaction resulted in high product yields with use of either the unprotected substrate **5a** or the *O*-*tert*-butyldimethylsilyl (TBS) derivative **5b**.

SCHEME 1



SCHEME 2



The alkene derivative **8a** was synthesized in 85% yield by addition of vinylmagnesium chloride in the presence of cerium(III) chloride to TBS-protected estrone.[82] The standard Heck reaction conditions used to couple **8a** with 2-chloro-5-iodo pyridine **6** gave the desired product **9a** in 24% yield, as shown in Scheme 2. Replacing PPh_3 with the bulky phosphine ligand (*o*-tolyl)$_3$P resulted in a modest improvement in the yield of **9a** to 45%. These relatively low yields with the Heck reaction prompted us to investigate the Suzuki/Miyaura coupling procedure.[83,84] The vinyl boronic acid derivative **8b** was prepared according to the literature procedure from ethynylestradiol and catechol borane, followed by hydrolysis.[85] The Suzuki coupling of **8b** with 2-chloro-5-iodo pyridine **6** was

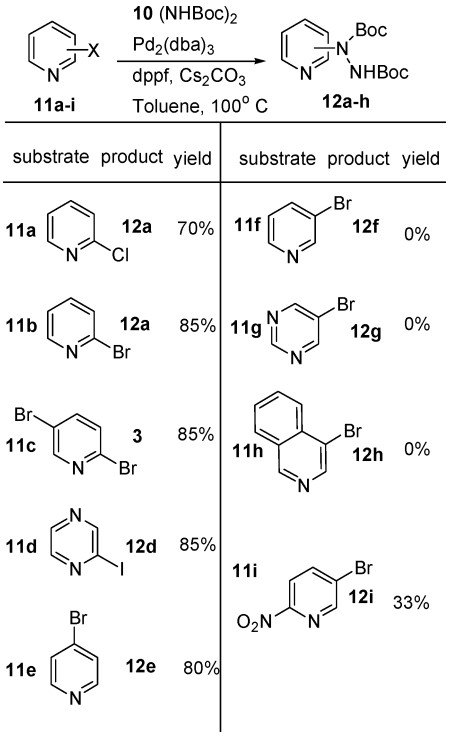
(70) Babich, J. W.; Coco, W. G.; Barrow, S.; Fischman, A. J.; Femia, F. J.; Zubieta, J. *Inorg. Chim. Acta* **2000**, *309*, 123−136.
(71) Liu, S.; Edwards, D. S.; Ziegler, M. C.; Harris, A. R.; Hemingway, S. J.; Barrett, J. A. *Bioconj. Chem.* **2001**, *12*, 624−629.
(72) Rennen, H. J. J. M.; van Eerd, J. E.; Oyen, W. J. G.; Corstens, F. H. M.; Edwards, D. S.; Boerman, O. C. *Bioconj. Chem.* **2002**, *13*, 370−377.
(73) Liu, S.; Harris, A. R.; Williams, N. E.; Edwards, D. S. *Bioconj. Chem.* **2002**, *13*, 881−886.
(74) Graham, K. A. N.; Wang, Q.; Eisenhut, M.; Haberkorn, U.; Mier, W. *Tetrahedron Lett.* **2002**, *43*, 5021−5024.
(75) Arterburn, J. B.; Rao, K. V.; Perry, M. C. *Angew. Chem., Int. Ed.* **2000**, *39*, 771−772.
(76) Amouri, H. I.; Vessieres, A.; Vichard, D.; Top, S.; Gruselle, M.; Jaouen, G. *J. Med. Chem.* **1992**, *35*, 3130−3135.
(77) Foy, N.; Stephan, E.; Jaouen, G. *Tetrahedron Lett.* **2000**, *41*, 8089−8092.
(78) Lee, C. Y.; Hanson, R. N. *Tetrahedron* **2000**, *56*, 1623−1629.
(79) Sonogashira, K.; Tohda, Y.; Hagihara, N. *Tetrahedron Lett.* **1975**, 4467−4470.
(80) Grosshenny, V.; Romero, F. M.; Ziessel, R. *J. Org. Chem.* **1997**, *62*, 1491−1500.
(81) Arterburn, J. B.; Rao, K. V.; Perry, M. C. *Tetrahedron Lett.* **2000**, *41*, 839−842.
(82) Imamoto, T.; Takiyama, N.; Nakamura, K.; Hatajima, T.; Kamiya, Y. *J. Am. Chem. Soc.* **1989**, *111*, 4392−4398.
(83) Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457−2483.
(84) Kotha, S.; Lahiri, K.; Kashinath, D. *Tetrahedron* **2002**, *58*, 9633−9695.

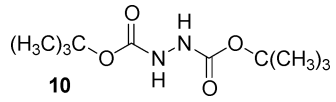
accomplished with $Pd(PPh_3)_4$/CsF.[86] The reaction progressed slowly at 70 °C and produced **9b** in 65% yield. Heating the reaction to 90 °C for 6 h improved the yield of **9b** to 84%.

Hydrazine Synthesis. Various attempts at direct nucleophilic substitution of the chloride in derivatives **7** and **9** with hydrazine were unsuccessful, and under forcing conditions reduction of the alkene/alkyne groups was observed.[87] These results were consistent with our expectations for low S_NAr reactivity in the absence of additional electron-withdrawing substituents on pyridine. Our efforts were then directed toward the application of catalytic methods for C−N bond formation with pyridin-2-yl halide substrates. Recent developments in methods for the catalytic amination of aryl halides have greatly facilitated arylamine synthesis,[88−93] and there has been interest in extending these methods for the synthesis of hydrazines.[75,81,94−101] Halogenated pyridines were originally found to be unsuitable substrates for Pd-catalyzed amination with use of first generation catalysts. The amination of pyridyl substrates with aryl- and alkylamines was accomplished with use of chelating ligands or sterically hindered monodentate biphenylphosphine ligands.[92,102−105] We recently reported that Hartwig's conditions for catalytic amination using $Pd_2(dba)_3$, 1,1′-bis(diphenylphosph1ino)ferrocene (dppf), and Cs_2CO_3 were suitable for the amination of 2-halo-pyridines with di-*tert*-butyl hydrazodiformate **10**.[106] To further define the scope of reactivity of halo-pyridine substrates in the Pd-catalyzed amination with **10**, we conducted a series of experiments using compounds **11a**−**i** (Table 1).

The amination of 2-chloropyridine **11a** and 2-bromopyridine **11b** with **10** produced the hydrazine **12a** in 70% and 85% yields, respectively. The consistently higher yields with bromide **11b** reflect the general reactivity trend for Pd-catalyzed amination of aryl-bromides > aryl-chlorides. 2,5-Dibromopyridine **11c** was selectively aminated at the 2-position to give **3**. This demonstrates the greater reactivity of the bromide ortho to the nitrogen atom. The reaction of 2-chloro-5-iodopyridine **6** under these conditions resulted in a complex mixture that was not characterized further. Iodopyrazine **11d** underwent catalytic amination to produce the hydrazine product **12d** in excellent yield. Similarly, 4-bromopyridine **11e** was converted to hydrazine **12e** in high yield. In contrast, the attempted amination of 3-bromopyridine **11f** with **10** was unsuccessful under these conditions. Related substrates possessing a similar meta-substituted halide, such as 5-bromopyrimidine **11g** and 4-bromoisoquinoline **11h**, also failed to produce the corresponding hydrazine products. The electron-deficient substrate 2-nitro-5-bromopyridine **11i** underwent C−N coupling to form the hydrazine product **12i** in low yield. Bromobenzene **13**, which typically functions as an effective arylating agent for Pd-catalyzed aminations with alkyl- and arylamines, did not react with **10** under these conditions. The electron-deficient aryl substrate 4-nitrobromobenzene **14** was converted to the hydrazine product **15** in 63% yield.

TABLE 1. Coupling of Pyridine Halides with DTBHz

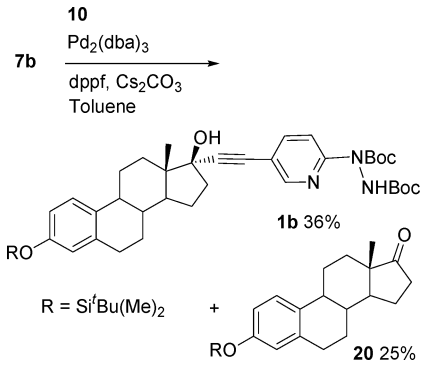




These results demonstrate the regioselectivity of the Pd/dppf-catalyzed amination reaction of **10** for 2- or 4-halo-pyridine substrates. Positions 2 and 4 are also the most reactive in nucleophilic substitution reactions due to stabilization of the intermediate addition products by localization of electron density on nitrogen. The failure

(85) Nakatsuka, I.; Ferreira, N. L.; Eckelman, W. C.; Francis, B. E.; Rzeszotarski, W. J.; Gibson, R. E.; Jagoda, E. M.; Reba, R. C. *J. Med. Chem.* **1984**, *27*, 1287−1291.
(86) Collet, S.; Danion-Bougot, R.; Danion, D. *Synth. Commun.* **2001**, *31*, 249−255.
(87) Corey, E. J.; Mock, W. L.; Pasto, D. J. *Tetrahedron Lett.* **1961**, 347−352.
(88) Hartwig, J. F. *Angew. Chem., Int. Ed.* **1998**, *37*, 2046−2067.
(89) Hartwig, J. F. *Acc. Chem. Res.* **1998**, *31*, 852−860.
(90) Wolfe, J. P.; Wagaw, S.; Marcoux, J. F.; Buchwald, S. L. *Acc. Chem. Res.* **1998**, *31*, 805−818.
(91) Frost, C. G.; Mendonca, P. *J. Chem. Soc., Perkin Trans. 1* **1998**, 2615−2623.
(92) Yang, B. H.; Buchwald, S. L. *J. Organomet. Chem.* **1999**, *576*, 125−146.
(93) Prim, D.; Campagne, J. M.; Joseph, D.; Andrioletti, B. *Tetrahedron* **2002**, *58*, 2041−2075.
(94) Hartwig, J. F. *Angew. Chem., Int. Ed.* **1998**, *37*, 2090−2093.
(95) Wagaw, S.; Yang, B. H.; Buchwald, S. L. *J. Am. Chem. Soc.* **1998**, *120*, 6621−6622.
(96) Wang, Z.; Skerlj, R. T.; Bridger, G. J. *Tetrahedron Lett.* **1999**, *40*, 3543−3546.
(97) Wagaw, S.; Yang, B. H.; Buchwald, S. L. *J. Am. Chem. Soc.* **1999**, *121*, 10251−10263.
(98) Stieber, F.; Grether, U.; Waldmann, H. *Angew. Chem., Int. Ed.* **1999**, *38*, 1073−1077.
(99) Wolfe, J. P.; Tomori, H.; Sadighi, J. P.; Yin, J.; Buchwald, S. L. *J. Org. Chem.* **2000**, *65*, 1158−1174.
(100) Song, J. J.; Yee, N. K. *Org. Lett.* **2000**, *2*, 519−521.
(101) Song, J. J.; Yee, N. K. *Tetrahedron Lett.* **2001**, *42*, 2937−2940.
(102) Wagaw, S.; Buchwald, S. L. *J. Org. Chem.* **1996**, *61*, 7240−7241.
(103) Wagaw, S.; Rennels, R. A.; Buchwald, S. L. *J. Am. Chem. Soc.* **1997**, *119*, 8451−8458.
(104) Cheng, J.; Trudell, M. L. *Org. Lett.* **2001**, *3*, 1371−1374.
(105) Wolfe, J. P.; Ahman, J.; Sadighi, J. P.; Singer, R. A.; Buchwald, S. L. *Tetrahedron Lett.* **1997**, *38*, 6367−6370.
(106) Arterburn, J. B.; Rao, K. V.; Ramdas, R.; Dible, B. R. *Org. Lett.* **2001**, *3*, 1351−1354.

SCHEME 3

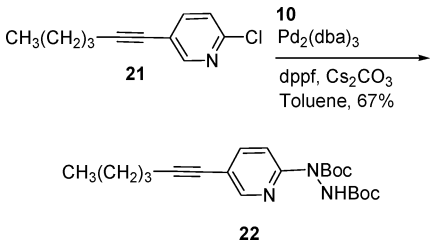


of 3-bromopyridine substrates under the catalytic reaction conditions was not anticipated, since these substrates have previously been used in Pd-catalyzed C−N coupling reactions with nucleophilic amines and hydrazones.[90] Numerous investigations have identified that electron-withdrawing groups on aryl substrates increased the rates of reductive elimination to form C−N, C−S, and C−O bonds. These electronic effects are also evident in the amination with weak *N*-nucleophiles. Sulfoximine and amides react efficiently with electrophilic aryl bromides and poorly with electron-rich aryl halides.[107,108] This emphasizes the importance of matching weak *N*-nucleophiles with electrophilic substrates in Pd-catalyzed C−N coupling reactions.

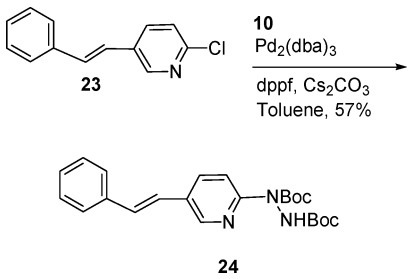
The results from Table 1 can be rationalized by considering resonance effects in the putative pyridine-Pd intermediates. The intermediates resulting from oxidative addition of 2- and 4-pyridine halides are capable of localizing electron density on nitrogen. These resonance effects enhance the electrophilicity of the heterocycle, and increase the reactivity for C−N bond formation with the weak nucleophile **10**. Similar stabilizing resonance effects are not possible for substitution at the 3-position. The more electrophilic 2-nitro-5-bromopyridine **11i** functions in the C−N coupling due to stabilization of the developing charge by the nitro group. The interplay of similar resonance effects is also consistent with our observations that 4-nitrobromobenzene **14** was coupled with **10** under these conditions, whereas no reaction occurred with bromobenzene.

Synthesis of 17α-(pyridin-2-yl)-hydrazine Estradiol Derivatives 1 and 2. The catalytic amination of the TBS-protected estradiol derivative **7b** with **10** under these conditions produced hydrazine **1b** in a moderate 36% yield, as shown in Scheme 3. Estrone **20** was also isolated from the reaction product mixture in 25% yield, and similar amounts of starting **7b** were recovered. Control reactions established that estrone was produced by a competing fragmentation of the tertiary propargyl alcohol under these mildly basic, anhydrous conditions. This fragmentation is analogous to reported cleavage reactions of propargylic alcohols under basic conditions.[109] The unprotected derivative **7a** possesses an acidic phenol group and precipitated as the potassium salt under these

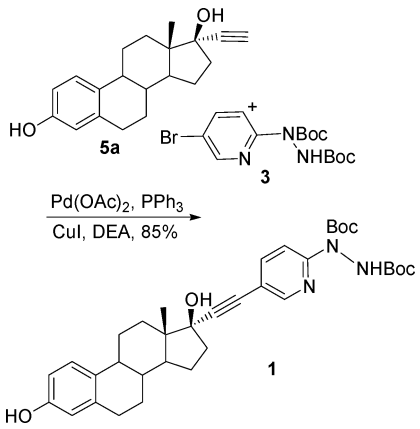
SCHEME 4



SCHEME 5



SCHEME 6



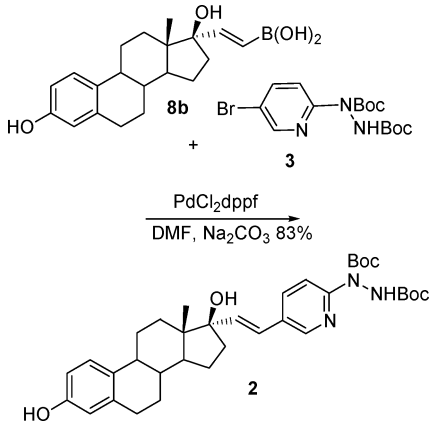
reaction conditions. The reaction of the simple model 2-chloropyridine substrate **21** under the standard conditions produced the corresponding hydrazine product **22** in an improved 67% yield, as shown in Scheme 4. These results indicate the incompatibility of tertiary propargylic alcohol substrates under the C−N coupling conditions.

No reaction of alkene derivative **9b** with **10** was observed under these C−N coupling conditions. The model substrate **23**, which lacks a tertiary allylic alcohol group, produced hydrazine **24** in 57% yield, as shown in Scheme 5. These results demonstrate the incompatibility of the C−N coupling reaction of **10** with allylic alcohol **9b.** We then investigated the direct coupling of a protected pyridine hydrazine group as an alternative approach for the synthesis of **1** and **2**. The protected hydrazine derivative **3** was coupled with 17α-ethynylestradiol **5a** with use of standard Sonogashira conditions to afford the estradiol pyridine hydrazine derivative **1** in 85% yield, as shown in Scheme 6.

The attempted Heck reaction of the vinyl estradiol derivative **8a** with the protected pyridine hydrazine derivative **3** was unsuccessful, and some decomposition of **3** was observed. Employing the Suzuki conditions for coupling the estradiol vinyl boronic acid **8b** with **3** with use of $Pd(PPh_3)_4$/CsF gave a 40% yield of the desired

(107) Bolm, C.; Hildebrand, J. P. *J. Org. Chem.* **2000**, *65*, 169−175.
(108) Yin, J.; Buchwald, S. L. *Org. Lett.* **2000**, *2*, 1101−1104.
(109) (a) Boyal, D.; Lopez, F.; Sasaki, H.; Frantz, D.; Carreira, E. M. *Org. Lett.* **2000**, *2*, 4233−4236. (b) Crisp, G. T.; Jiang, Y. L. *Synth. Commun.* **1998**, *28*, 2571−2576.

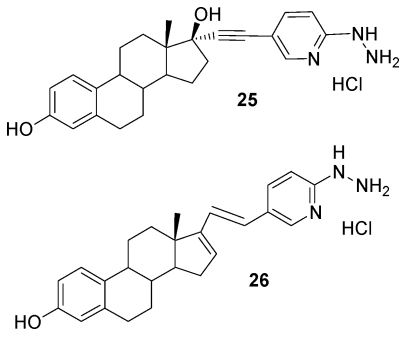
SCHEME 7



product **2**. Under these conditions, protodeboronation of the boronic acid occurred as a side reaction to give 17α-vinylestradiol in 34% yield. The yield of **2** was improved significantly to 83% with use of $PdCl_2$(dppf) in DMF with aqueous Na_2CO_3, as shown in Scheme 7. A second portion of catalyst was added after 4 h to complete the conversion.

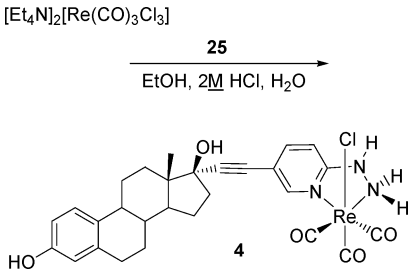
A variety of hydrolysis conditions were investigated for the removal of the Boc-protecting groups from **1** and **2**. The hydrolysis of **1** was incomplete with use of 10% trifluoroacetic acid in 2-propanol at 60 °C. A complicated mixture was obtained from **1** with use of 20% trifluoroacetic acid in CH_2Cl_2 at ambient temperature. The deprotection of **1** was accomplished by using 20% concentrated HCl in ethanol (v/v) at ambient temperature for 6 h to produce the hydrochloride salt **25** in 95% yield. The salt **25** was stored at 4 °C and found to be stable as a solid without observable decomposition after two months. Solutions of **25** in DMSO exhibited decomposition with extended storage.

The HCl/EtOH hydrolysis conditions were then used with the allylic alcohol substrate **2**, but the deprotection was incomplete and the product mixture contained significant amounts of the dehydration product **26**. The



C16−H of the conjugated diene in **26** appeared as a characteristic vinyl signal at δ 5.94 in the ^{1}H NMR, and the other vinyl hydrogens were observed at δ 6.64 and 7.10 with J = 8.0 Hz. Complete deprotection of **2** was accomplished with use of 25% concentrated HCl in ethyl acetate (v/v) at ambient temperature for 1.5 h, but produced the conjugated diene **26** in 97% yield instead of the desired allylic alcohol. Further attempts to deprotect **2** without elimination of the sensitive allylic alcohol under milder hydrolysis conditions were unsuccessful.

SCHEME 8



The elimination reaction producing **26** removes the 17β-hydroxyl group, which is crucial for ER binding; therefore, this compound was not investigated further. Hydrogenation of the alkene in **2** would be expected to facilitate the deprotection by preventing the formation of conjugated diene. This derivative would also be of interest for further evaluation.

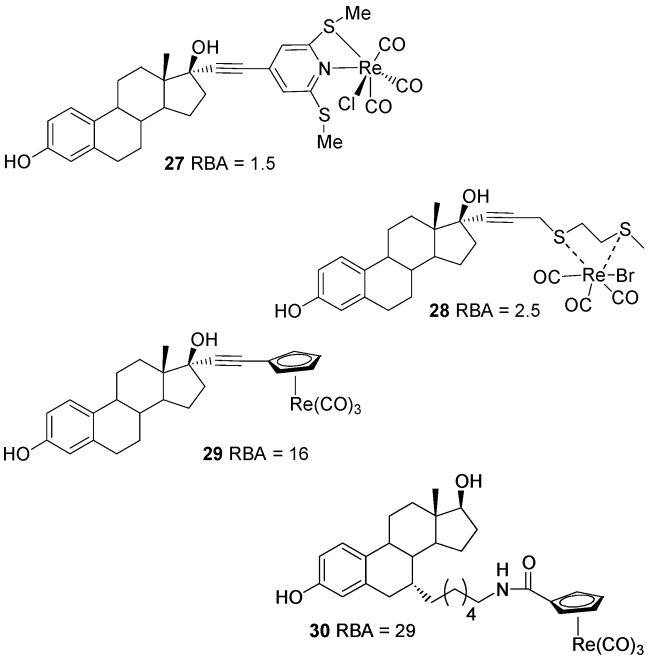
Synthesis of Tricarbonyl-Re(I) Complex 4. The tricarbonylrhenium(I) complex $[Et_4N]_2[ReCl_3(CO)_3]$was prepared from $[Bu_4N][ReO_4]$ as reported in the literature.[110] This complex undergoes rapid hydrolysis of the chloride ligands in water, and is an effective precursor for *fac*-$[Re(OH_2)_3(CO)_3]^+$ in situ. The resulting complex was mixed with the estradiol-hydrazine hydrochloride **25** in ethanol, acidified with 2 M HCl (aq), and stirred at ambient temperature for 30 h, as shown in Scheme 8. The product tricarbonylrhenium(I) complex **4** was isolated as a light pink solid in 92% yield after precipitation from H_2O. The complex was purified by chromatography on silica gel eluted with 5% CH_3OH/CH_2Cl_2 and obtained in 71% yield.

The structure of complex **4** is supported by spectral and elemental analysis data. The complex exhibits UV/vis absorption maxima at 273 and 330 nm. The ^{13}C NMR spectra exhibited three distinct resonances for the carbonyl peaks at δ 198.8, 198.0, and 193.5. The FT-IR absorbances at 2029, 1919, and 1898 cm^{-1} also confirm the presence of carbonyl groups. The ^{13}C NMR signals of the *o*-pyridine positions were shifted significantly downfield upon formation of the complex, such that C-1 shifted by 4.8 ppm, and C-5 by 4.0 ppm, relative to **25**. The *p*-pyridine position C3 shifted downfield by 1.8 ppm. These results are consistent with coordination of the pyridine nitrogen to the electrophilic tricarbonylrhenium(I) complex. The ^{1}H NMR revealed sharp signals for the phenol and 3° alcohol at δ 9.29 and 4.55, respectively, and three distinct hydrazine protons in acetone-d_6 that exchanged with D_2O. The internal hydrazine N−H appeared at δ 7.88, while the two terminal hydrazine protons appeared at δ 7.98 and 6.58 as doublets with a geminal coupling constant J = 8.8 Hz. This assignment was verified by decoupling experiments in which irradiation of the signal at δ 7.98 collapsed the peak at δ 6.58 to a singlet. These data demonstrate that the diastereotopic terminal hydrazine hydrogen atoms do not interconvert on the NMR time scale. This observation is consistent with expectations for a kinetically stable octahedral d^6 Re(I) configuration. The coordination environment of the metal complex is chiral and complex **4**

(110) Alberto, R.; Schibli, R.; Egli, A.; Schubiger, P. A.; Herrmann, W. A.; Artus, G.; Abram, U.; Kaden, T. A. *J. Organomet. Chem.* **1995**, *492*, 217−224.

exists as two diastereomers. The distance of the chiral steroid appendage from the stereogenic complex minimizes the energy difference between the diastereomers and leads to a single set of pyridine ^{1}H and ^{13}C NMR signals. Attempts to grow single crystals suitable for X-ray analysis have not been successful. The proposed structure with the pyridine-N and terminal hydrazine-N oriented trans to the carbonyls is analogous to the recently reported X-ray structure of a similar picolylamine complex [$ReBr(CO)_3$(2-pyridine-CH_2NH_2)].[111] Complex **4** was stable for weeks without observable decomposition as a solid and in solutions of acetone or DMSO exposed to air and moisture.

Estrogen Receptor Binding Affinity of 4. The estrogen binding affinity of complex **4** was measured at 0 °C by a competitive radiometric binding assay, using either cytosol estrogen receptor preparation from lamb uterus or purified human estrogen receptors (ERα or ERβ), and [^{3}H]estradiol as tracer.[112] Affinities are expressed as relative binding affinity (RBA) values, where estradiol has a value of 100. Complex **4** exhibited a high binding affinity for ERα (RBA = 38), though its affinity for ERβ and lamb uterine ER was somewhat lower (RBA's of 5.0 and 5.9, respectively). This affinity compares favorably with other reported estradiol-derived tricarbonyl-Re(I) complexes, measured in uterine cytosol or whole cell extract preparations of ER. The sterically demanding 17α-ethynylpyridine mercaptan complex **27**



exhibited a low RBA = 1.5.[11] The dithioether tricarbonylrhenium(I) complex **28** also showed low affinity for the estrogen receptor with RBA = 2.5.[44] The observed RBA for **4** is also higher than the cyclopentadienyl-$Re(CO)_3$ complexes substituted in the 17α- and 7α-positions of estradiol **29** and **30** that have binding affinities of 16 and 29, respectively.[34,37]

Conclusions

We have described the synthesis of pyridine hydrazine derivatives using Pd-dppf catalyzed C−N coupling of di-*tert*-butyl hydrazodiformate. The amination reaction was successful for 2- and 4-halo-pyridine substrates, but 3-halo-pyridines required an additional electron-withdrawing nitro group for activation. 2-Chloropyridine-estradiol conjugates were synthesized with use of Sonogashira and Suzuki/Miyaura procedures, but the propargylic and allylic alcohol groups were not compatible with the alkaline Pd-catalyzed C−N coupling conditions. The syntheses of novel estradiol pyridine hydrazine derivatives **1** and **2** were accomplished by using **3** as a protected bifunctional chelate in catalytic C−C coupling procedures. The Re(I)-tricarbonyl complex **4** was synthesized directly in water from the hydrazine hydrochloride salt, and exhibited a very high RBA for ER. These results highlight the potential of this structural class of compounds for labeling with Tc-99m to provide new imaging agents for ER+ breast cancers.

Experimental Section

17α-[6-(*N*,*N*′-Bis-*tert*-butyloxycarbonyl-hydrazinopyridin-3-ylethynyl)]-estra-1,3,5(10)-triene-3,17β-diol (1). A solution of $Pd(OAc)_2$ (12 mg, 0.05 mmol) and PPh_3 (27 mg, 0.1 mmol) in diethylamine (5 mL) under an argon atmosphere was stirred for 10 min. CuI (19 mg, 0.1 mmol) and **3** (234 mg, 1 mmol) were added. After the mixture was stirred for 5 min, 17α-ethynylestradiol **5a** (296 mg, 1 mmol) was added and the solution was stirred for 4 h at 55 °C. The volatiles were removed in vacuo, and the residue was chromatographed (5% CH_3OH/CH_2Cl_2) to yield **1** (452 mg, 75%) as a yellow solid. Mp 172−173 °C. IR (KBr) 3419, 2932, 1730, 1290, 1154 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz,) δ 8.45 (s, 1H), 7.71 (s, 2H), 7.15 (d, J = 8 Hz, 1H), 7.07 (s, 1H,), 6.62 (d, J = 8 Hz, 1H), 6.56 (s, 1H), 5.10 (s, 1H), 2.82 (s, 2H), 2.50−1.60 (m, 14H), 1.52 (s, 9H), 1.45 (s, 9H), 0.93 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 154.9, 153.9, 152.9, 152.4, 150.1, 140.2, 137.8, 131.8, 126.3, 117.9, 116.9, 115.3, 112.9, 96.2, 83.2, 82.1, 81.6, 80.2, 49.9, 47.7, 43.6, 39.5, 39.0, 33.1, 29.6, 28.1, 28.0, 27.2, 26.4, 22.9, 12.9. Anal. Calcd for $C_{35}H_{45}N_3O_6$·0.5H_2O: C, 68.60; H, 7.57; N, 6.86. Found: C, 68.48; H, 7.30; N, 6.57.

17α-[6-(*N*,*N*′-Bis-*tert*-butyloxycarbonyl-hydrazinopyridin-3-yl-(*E*)-ethenyl)]-estra-1,3,5(10)-triene-3,17β-diol (2). A Shlenck tube was charged with **5a** (300 mg, 0.88 mmol) and **3** (407.6 mg, 1.05 mmol). $PdCl_2$(dppf)·CH_2Cl_2 (28.7 mg, 0.035 mmol) was added in a glovebox. The tube was purged with argon and the contents were dissolved in DMF (4.4 mL). To the stirred solution was added degassed 2 M Na_2CO_3(aq) (2.2. mL) and the mixture was heated to 80 °C with stirring. After 4 h $PdCl_2$(dppf)·CH_2Cl_2 (28.7 mg, 0.035 mmol) in DMF (0.75 mL) was added to the reaction mixture, which was then stirred with heating for 20 h. Volatiles were removed in vacuo. The residue was dissolved in EtOAc, filtered through Celite, washed with water, and dried over Na_2SO_4. Removal of solvent yielded a brown solid that was purified by flash chromatography (5% MeOH/CH_2Cl_2) to yield **2** (440 mg, 83%) as a white solid. Mp 189−190 °C. IR (KBr) 3385, 2931, 1728, 1480, 1154 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.38 (s, 1H), 7.76 (d, J = 8 Hz, 1H), 7.65 (s, 1H), 7.13 (d, 8 Hz, 1H), 7.00 (s, 1H), 6.68−6.45 (m, 4H), 4.70 (s, 1H), 2.82 (m, 2H), 2.40−1.60 (m, 14H), 1.52 (s, 9H), 1.46 (s, 9H), 0.99 (s, 3H). ^{13}C NMR

(111) Banerjee, S. R.; Levadala, M. K.; Lazarova, N.; Wei, L.; Valliant, J. F.; Stephenson, K. A.; Babich, J. W.; Maresca, K. P.; Zubieta, J. *Inorg. Chem.* **2002**, *41*, 6417−6425.

(112) (a) Katzenellenbogen, J. A.; Johnson, H. J. J.; Myers, H. N. *Biochemistry* **1973**, *12*, 4085−4092. (b) Carlson, K. E.; Choi, I.; Gee, A.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. *Biochemistry* **1997**, *36*, 14897−14905. (c) Stauffer, S. R.; Coletta, C. J.; Tedesco, R.; Sun, J.; Katzenellenbogen, B. S.; Katzenellenbogen, J. A. *J. Med. Chem.* **2000**, *43*, 4934−4947.

($CDCl_3$, 100 MHz) δ 155.0, 154.3, 153.2, 152.4, 145.9, 137.8, 137.1, 134.7, 131.7, 130.4, 128.1, 126.2, 123.1, 118.6, 115.4, 112.9, 84.2, 82.8, 81.4, 49.6, 47.6, 43.6, 39.6, 37.1, 32.6, 31.4, 29.6, 28.1, 27.4, 26.3, 23.4, 14.2. Anal. Calcd for $C_{35}H_{47}N_3O_6 \cdot 0.5H_2O$: C, 68.38; H, 7.87; N, 6.83. Found: C, 67.99; H, 7.99; N, 6.57.

***N,N′*-Bis-*tert*-butyloxycarbonyl-(5-bromo-pyridin-2-yl)-hydrazine (3).** A Schlenk tube was charged with $Pd_2(dba)_3$ (37 mg, 0.04 mmol), dppf (67 mg, 0.12 mmol), Cs_2CO_3 (352 mg, 1 mmol), and **10** (186 mg, 0.8 mmol) in a drybox. The tube was purged with argon, then toluene (2 mL) and 2,5-dibromopyridine (388.2 mg, 1 mmol) were added. The reaction mixture was heated at 100 °C with stirring until **10** had been consumed, as judged by TLC. The reaction mixture was cooled, diluted with CH_2Cl_2, washed with water, concentrated under vacuum, and chromatographed (10% EtOAc/Hex) to yield **3** (792 mg,85%) as a white solid. Mp 75−77 °C. IR (KBr) 3212, 1730, 1582, 1462, 1156, 838 cm^{-1}. ^1H NMR ($CDCl_3$, 400 MHz) δ 8.43 (d, J = 2.2 Hz, 1H), 7.78 (dd, J = 8 Hz, 2.2 Hz, 1H), 7.75 (d, J = 8 Hz, 1H), 7.32 (s, 1H), 1.52 (s, 9H), 1.47 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz) 154.8, 152.9, 152.2, 148.5, 139.80, 119.5, 116.1, 82.9, 81.6, 28.1. FAB MS (M + 1) calcd for $C_{15}H_{22}BrN_3O_4$ 388.0871, found 388.0853.

Tricarbonyl-chloro-[(6-(hydrazino-pyridin-3-ylethynyl)-estra-1,3,5(10)-triene-3,17β-diol-17α-yl)]-rhenium(I) (4). $[ReCl_3(CO)_3][NEt_4]_2$ (230 mg, 0.36 mmol) was stirred in 4 mL of H_2O for 1 h. To the solution was added **25** (116 mg, 0.24 mmol) in absolute ethanol (8 mL) as a clear yellow solution and 2 M HCl(aq) (4 mL), then the mixture was allowed to stir for 30 h. The product was precipitated by the addition of 90 mL of cold H_2O and was collected by vacuum filtration. The resulting product was purified by flash chromatography (10% MeOH/CH_2Cl_2) to yield (130 mg, 75%) **4** as a pink solid. IR (KBr) 3254, 2029, 1919, 1898, 1625, 1503 cm^{-1}. ^1H NMR (acetone-d_6, 400 MHz) δ 9.32 (s, 1H), 8.34 (d, J = 2 Hz, 1H), 7.98 (d, J = 8.8 Hz, 1H), 7.91 (s, 1H), 7.73 (dd, J = 8.8, 2.0 Hz, 1H), 7.10 (d, J = 8.4 Hz, 1H), 7.02 (d, J = 8.8 Hz, 1H), 6.64 (d, J = 8.8 Hz, 1H), 6.58 (dd, J = 8.4, 2.4 Hz, 1H), 6.52 (d, J = 2.4 Hz, 1H), 4.59 (s, 1H), 3.00 (s, 1H), 2.80−2.74 (m, 2H), 2.55−2.20 (m, 2H), 2.05−1.80 (m, 3H), 1.79 (m, 4H), 1.40 (m, 4H), 0.93 (s, 3H). ^{13}C NMR (acetone-d_6, 100 MHz) δ 198.78, 197.99, 193.51, 160.16, 156.30, 152.69, 143.00, 138.83, 132.41, 127.45, 116.42, 114.08, 112.78, 109.10, 97.44, 81.36, 80.22, 51.19, 48.89, 45.04, 41.09, 40.41, 34.54, 28.59, 27.83, 24.04, 13.82. Anal. Calcd for $C_{28}H_{29}ClN_3O_5Re$: C, 47.42; H, 4.12; N, 5.92; Cl, 5.00. Found: C, 47.42; H, 4.39; N, 6.03; Cl, 5.04.

Estrogen Receptor Binding. Binding affinities of each compound for ERα and ERβ, and uterine cytosol were determined in a radiometric competitive binding assay, using [^{3}H]-estradiol as tracer and charcoal dextran to adsorb free tracer (uterine cytosol ER)[112a] or hydroxylapatite to adsorb ligand−receptor complex (human ERα and ERβ).[112b,112c] Receptor preparation was either lamb uterine cytosol, which consists of nearly exclusively ERα,[113] or human ERα and ERβ, expressed in baculovirus and purified (PanVera, Madison, WI). Values given represent the mean of 2−3 repeat determinations which have a coefficient of variation of 0.3.

Acknowledgment. The authors thank the US-AMRC Breast Cancer Research Program DAMD17-97-1-7120 (to J.B.A.) and National Institutes of Health (GM 08136 to J.B.A. and CA25563 to J.A.K.) for support of this work. C.C. was supported by NIH/RISE (GM61222).

Supporting Information Available: General experimental methods, experimental methods for compounds **7a**,**b**, **9b**, **12a**−**i**, **15**, **16**, and **21**−**26** as well as ^1H NMR and ^{13}C NMR spectra of compounds **1**−**4**, **7a**,**b**, **9b**, **12a**−**i**, **15**, **16**, and **21**−**26**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO034780G

(113) Pettersson, K.; Gustafsson, J. A. *Annu. Rev. Physiol.* **2001**, *63*, 165−192.