Synthesis and Radiosynthesis of 17α-[*p*-(Iodophenylethynyl)]estra-3,17β-diols

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Estrones have been subjected to an addition reaction with [(4-ethynylphenyl)azo]pyrrolidine to provide 17 α-{4-[(pyrrolidin-1-yl)-azo]phenylethynyl}estra-3,17 β-diols. A subsequent iodination with iodotrimethylsilane, produced *in situ* from chlorotrimethylsilane and NaI, leads to 17α-(*p*-iodophenylethynyl)estra-3,17 β-diols. This reaction can also be carried out with Na^{125}I to give radiolabelled estra-3,17 β-diols, which are potentially useful radiodiagnostic agents for the detection of estrogen positive breast cancer. The stability of the radiolabelled compounds is exemplified in a stability study of 3-*O*-methyl 17 α-(*p*-[^{125}I]iodophenylethynyl)estra-1,3,5(10),6-tetraene-17 β-ol in acetonitrile.

Key words: Steroids, Radioiodination, Triazenes

Radiolabelled estrogens and antiestrogens with a high selective binding affinity to the estrogen receptor ER α have been shown to have potential as radiodiagnostica for the detection of minimal estrogen positive breast cancer [1]. Although a number of nuclides have been forwarded as radiolabels in this context, such as ^{99m}Tc [2] and ^{18}F [3], ^{123}I [4] and ^{125}I [5] remain attractive because they are medically well-studied radioisotopes for radioimaging. Previously, the authors have investigated the iodovinyl derivatives **1a** and **1b** [6]. While especially the *Z*-iodovinyl compound **1b** showed a promising biodistribution in mice [6a], it was evident that the iodovinyl compounds metabolized too quickly, as a large amount of iodine was found in the thyroid rather quickly. The stability of the corresponding iodoethynyl derivatives **1c** is also low, even *in vitro*.

Additionally, although described for a number of compounds [7] in our hands, the radioiodination of a terminal acetylene did not proceed to satisfaction. Recently, the preparation of 17 α-(*p*-iodophenyl)estra-3,17 β-diols has been reported [8]. In the following, the synthesis of 17α-(*p*-iodophenylethynyl)estra-3,17 β-diols **2** will be described, representative examples of radioiodination will be given and exemplary results of studies on the stability of the radiolabelled compounds will be disclosed.

As ultimately compounds **2** are to carry a radiolabel [^{125}I], the iodination has to be the last step of the synthetic sequence and the iodo function cannot be introduced with the phenylethynyl moiety, for instance by addition of the readily available *p*-iodophenylacetylene to estrones **7**. A critical point in the iodination is the sensitivity of the C-17 hydroxy group, which easily undergoes dehydration. Thus, it was not possible to exchange other groups at the *para* position of the phenyl unit, such as the trimethylsilyl group (*e. g.*, with NCS, NaI, AcOH, or with ICl, CH_2Cl_2 [9] or with benzyltrimethylammonium dichloroiodate [BTMAICl_2], $ZnCl_2$, AcOH) or the tributylstannyl function (I_2, THF) to an iodo functionality.

It is known that a pyrrolidinylazo group as a triazene moiety can be exchanged effectively to an iodo function. Thus, an introduction of the iodo function in 17 α-{4-[(pyrrolidin-1-yl)-azo]phenylethynyl}estra-3, 17 β-diols of type **8** seemed viable. The starting material should be easily accessible via addition of the [(4-ethynylphenyl)azo]pyrrolidine **6** to estrones **7**. [(4-

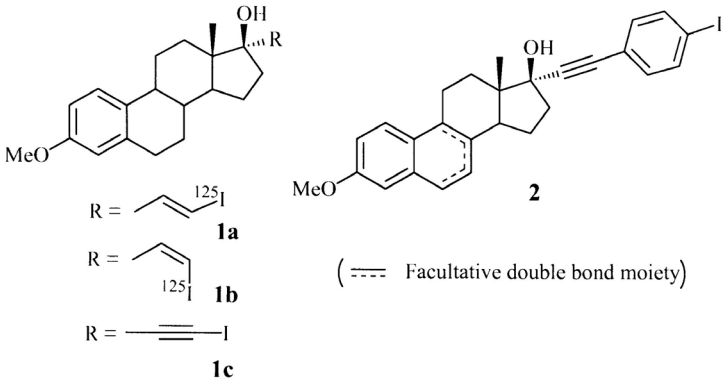
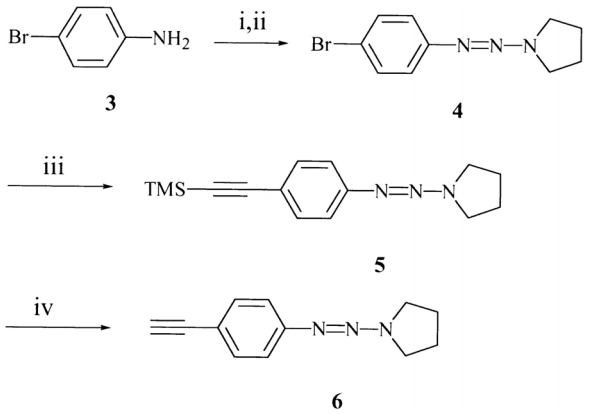


Fig. 1. Iodinated estradiols as potential diagnostic agents for estrogen positive breast cancer.



Scheme 1. Synthesis of **6**, reaction conditions: i. $NaNO_2$, HCl; ii. pyrrolidine, KOH (64%); iii. $(Ph_3P)_2PdCl_2$, Et_3N, 48 h (91%); iv. KOH, MeOH, 5 h (58%).

Ethynylphenyl)azo]pyrrolidine **6** itself can be synthesized from *p*-bromoaniline (**3**) in a number of ways involving the transformation of the aniline function to the triazene, a Sonogashira coupling and a desilylation of the ethynyl terminus. While the order of the Sonogashira coupling and the triazene formation can be changed, *i. e.*, the triazene formation can be performed as the second or even as the final step, the best yield of **6** is achieved by initial reaction of the aniline **3** to the triazene **4**, subsequent ethynylation to **5** and desilylation. The yield of the Sonogashira coupling could be increased from 67% [12] to 91% by changing the published reaction conditions [$(Ph_3P)_2PdCl_2$, CuI, Et_2NH, rt] to $(Ph_3P)_2PdCl_2$, Et_3N, CuI, 70 °C, 2 d (Scheme 1).

The addition of **6** to ketones **7** proceeds in fair yields (Scheme 2). The above mentioned reactions were carried out with methoxyestra-1,3,5(10)-triene **7a**, but also with estranes with further insaturations within the B-ring such as with 3-*O*-methyl-equilinine (**7b**), with 3-*O*-methyl-equiline (**7c**) and with 3-*O*-methyl-estra-1,3,5(10),6-tetraen-17-one (**7d**). Especially derivatives of **7b** and **7c** have been used in combination with other substances in hormone replacement therapy and are known to bind to the estrogen receptor. The iodophenylethynyl derivatives of **7d** are analogs to the iodovinyl compounds **1a** and **1b**, which have been studied by the authors in *in vivo* biodistribution experiments [6a].

Iodination of the triazenes is carried out with *in situ* produced iodotrimethylsilane (Scheme 3). It is not an advantage to use an excess of reagent as then the 17 β-hydroxy group will be silylated. Most likely, the mechanism of the iodination involves an initial attack of the iodotrimethylsilane on the lone electron pair of the pyrrolidine nitrogen followed by a nucleophilic displacement reaction by the iodide (Scheme 4).

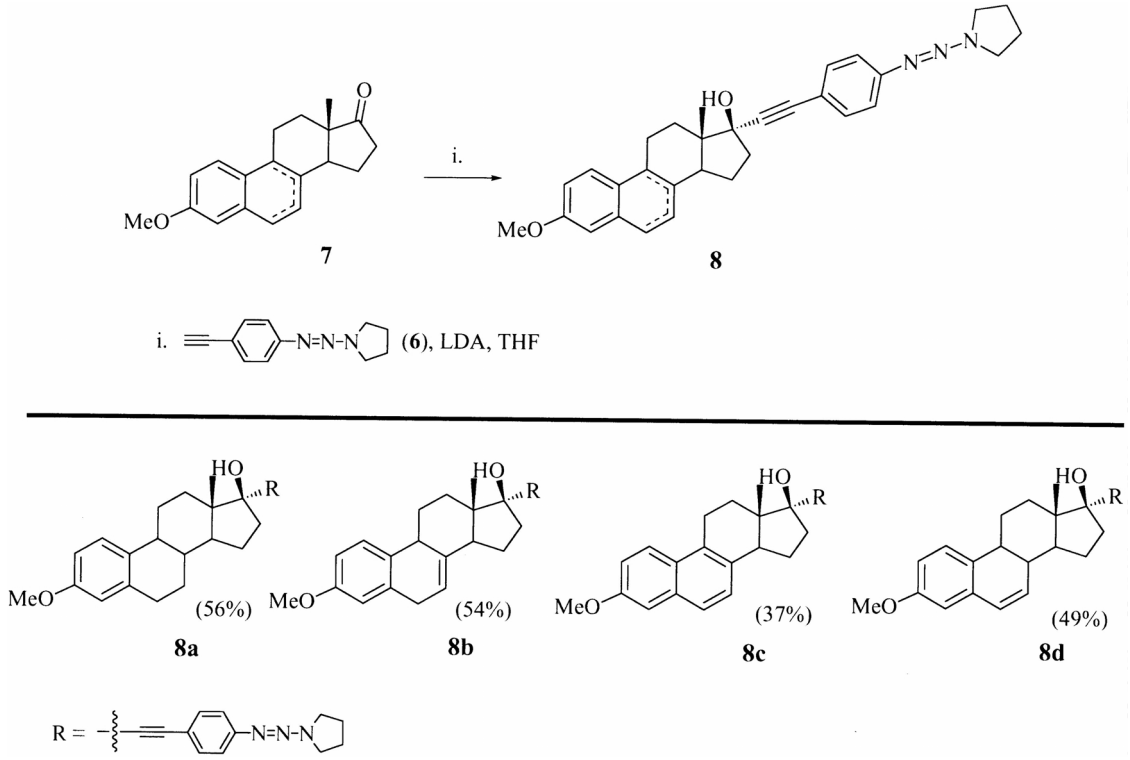
The 3-hydroxy group of equilinine can be protected as a silyloxy group without affecting the addition of **6**. After the addition and subsequent iodination, the silyloxy group is cleaved under fluoride catalysis in the usual way (Scheme 5).

The radioiodination [14] can be carried out in a similar way as the cold iodination (Scheme 6).

The stability of the iodinated compounds in acetonitrile is good. This has also been determined for the radiolabelled compounds by RP-HPLC analysis of **2a** $-^{125}$**I** in acetonitrile 6 days and 21 days after labelling (Fig. 2). Stability tests in PBS, PBS/PEG, *in vitro* binding affinity assays to ER α and *in vivo* biodistribution studies of the compounds are underway.

Experimental Section

General remarks: Synthesis. Melting points were measured on a Yanaco microscopic hotstage and are uncorrected. Infrared spectra were measured with JASCO IR-700 and



Scheme 2. Synthesis of **8**.

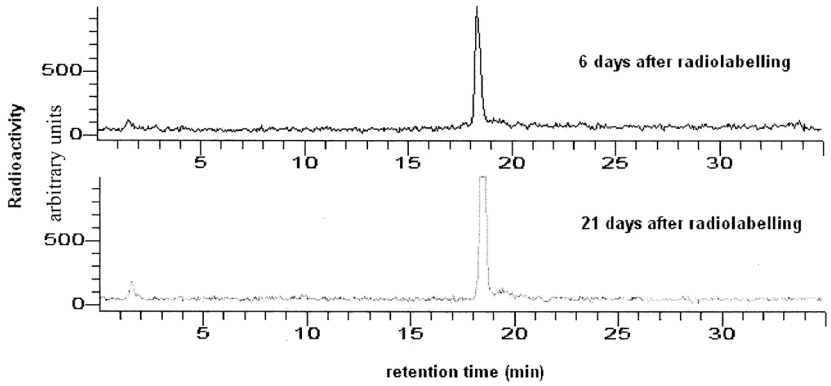
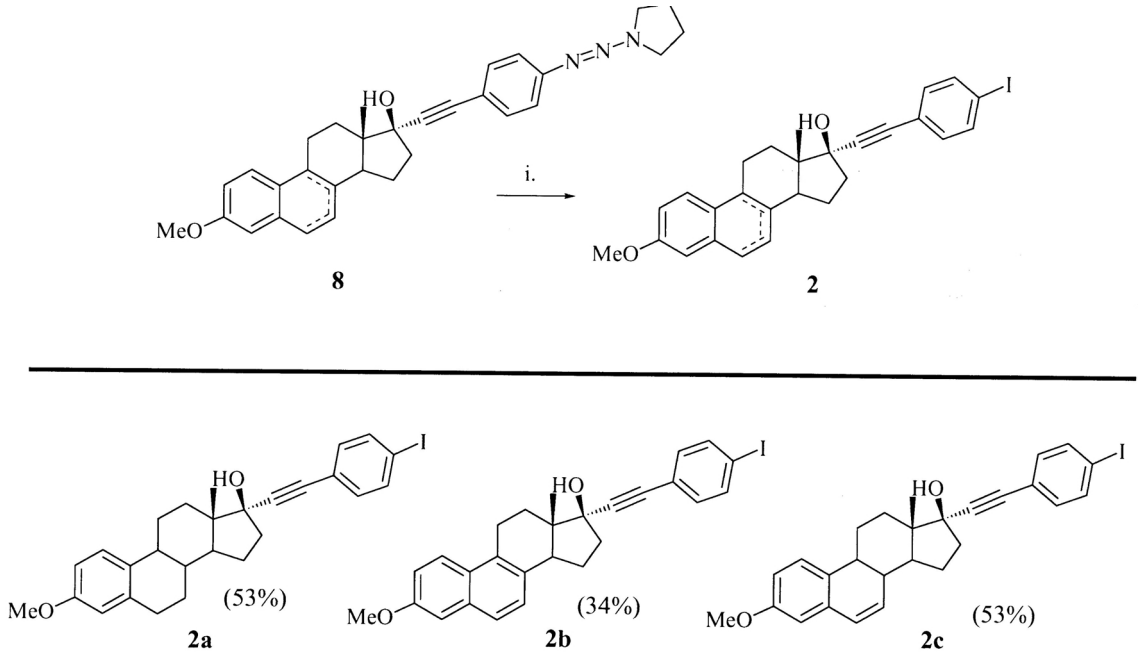


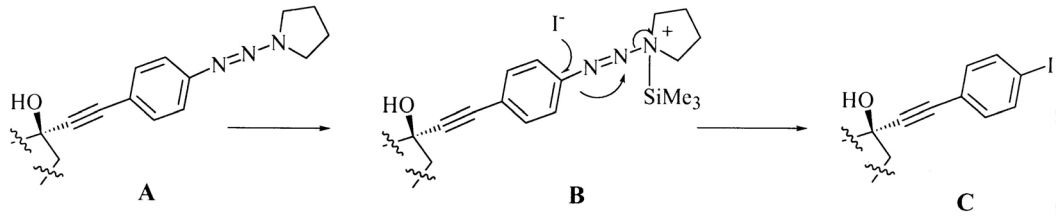
Fig. 2. Stability of **2a**—^{125}**I**, acetonitrile, shown by reversed phase HPLC analysis (see Exp. Section for details).

Nippon Denshi JIR-AQ2OM instruments. ^{1}H and ^{13}C NMR spectra were recorded with a JEOL EX-270 spectrometer. The chemical shifts are relative to TMS (solvent $CDCl_3$, unless otherwise noted). Mass spectra were measured with a JMS-01-SG-2 spectrometer (EI, 70 eV). Column chromatography was carried out on Wakogel 300. All experiments were purged with argon at the start.

All chemical reagents were of reagent grade. THF was dried over sodium ketyl. 3-*O*-Methylestrone (**7a**) and 3-*O*-methylequiline (**7b**) were obtained by methylation (KOH, DMSO, MeI) [14] of commercial material. 3-*O*-Methylequilinine (**7c**) has been obtained from 3-*O*-methylestrone (**7a**) *via* 3-*O*-methyl-9-hydroxy-6-ketoestra-1,3,5(10)-trien-17-one 17,17-(2'-[5',5'-dimethyl-1',3'-dioxane]) and 3-*O*-methyl-6-ketoestra-1,3,5(10),9(11)-tetraen-17-one 17,17-(2'-[5',5'-dimethyl-1',3'-dioxane]) in a four-step procedure (i. $KMnO_4$, Adogen, benzene, reflux; ii. *p*-TsOH, neopentylglycol, benzene, reflux; iii. $NaBH_4$, MeOH; iv. *p*-TsOH, benzene, reflux) [15].



Scheme 3. Synthesis of **2**, reaction conditions: i. TMSCl, NaI, CH_3CN, 1 h.

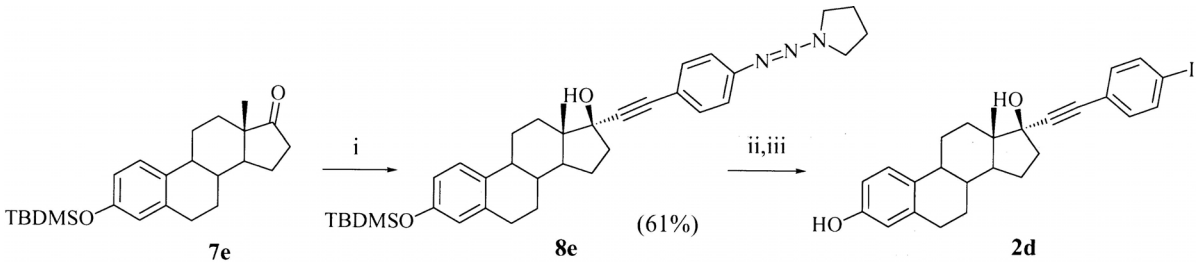


Scheme 4. Mechanism of the iodination.

Radiosynthesis: Acetonitrile was dried over P_2O_5 under N_2. The radioiodinated product was purified by reversed phase HPLC on a C18 Nucleosil column (250/4 10 μm) with a flow of 1 ml/min using a gradient of acetonitrile-water (70 : 30 to 100 : 0). The compounds were detected by their UV absorption at 254 nm with an UV detector SPD-10AV from Shimadzu and by their γ-radiation using a γ-Radioflow detector LB 509 from Berthold. The commercially available [^{125}I] in NaOH solution (pH = 7.0 – 11) was used as purchased from Amersham.

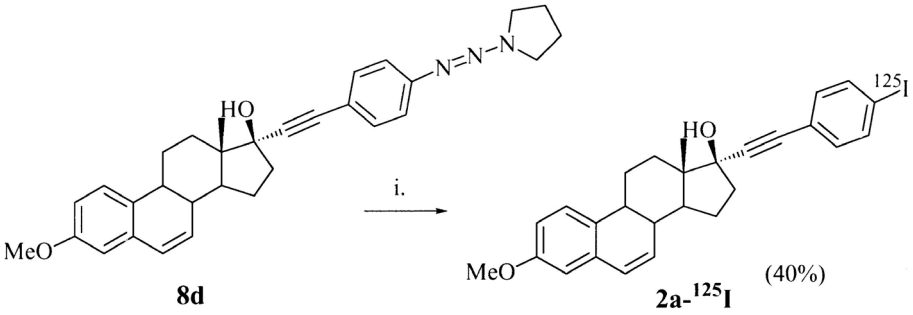
Typical procedures: [(4-Trimethylsilylethynylphenyl)-azo]pyrrolidine (**5**): To a solution of [(4-bromophenyl)-azo]pyrrolidine **4** (1.0 g, 3.94 mmol) in anhydrous Et_3N (10 ml) was added trimethylsilylacetylene (463 mg, 4.73 mmol) and $(PPh_3)_2PdCl_2$ (55 mg, 0.08 mmol) and CuI (15 mg, 0.08 mmol) under an inert atmosphere. The medium was heated at 70 °C for 2 d. Then, the solution was cooled and poured into water (50 ml) and extracted with dichloromethane (3 × 50 ml). The organic layer was washed with water and brine and subsequently dried over anhydrous $MgSO_4$. Concentration of the solution *in vacuo* and column chromatography (hexane/ether 1:1) gave **5** [see also lit. 12] (972 mg, 91%) as colorless crystals; IR (KBr) ν = 2146, 1650, 1596, 904, 759 cm^{-1}. – ^{1}H NMR (67.8 MHz, $CDCl_3$) δ = 0.24 (s, 9H, 3 CH_3), 2.00 (m, 4H), 3.78 (bs, 4H, 2 CH_2N), 7.32 (d, 2H, ^3J = 8.6 Hz), 7.39 (d, 2H, ^3J = 8.6 Hz). – ^{13}C NMR (67.8 MHz, $CDCl_3$, DEPT 90, DEPT 135) δ = 0.07 (3C, $SiMe_3$), 23.72 (2C, CH_2), 49.15 (2C, NCH_2, very broad signal), 93.56 (C≡), 105.68 (C≡), 117.68 (C_{quat}), 120.09 (CH), 132.68 (CH), 151.19 (C_{quat}). – MS (EI, 70 eV): *m/z* (%) = 271 (6), 233 (100), 173 (20), 121 (7), 77(3).

3-O-Methyl-17α-{4-[(pyrrolidin-1-yl)-azo]phenylethynyl}estra-1,3,5(10)-trien-3,17β-diol (**8a**): To a solution of *N*-[*p*-ethynylphenylazo]pyrrolidine (**6**) (88 mg, 0.44 mmol) in dry THF (2 ml) was added LDA (2M LDA in a mixture of heptane / THF / ethylbenzene, 0.24 ml, 0.48 mmol) at −78 °C under an Ar atmosphere. The resulting solution



7e **8e** **2d**

Scheme 5. Synthesis of **2d**, reaction conditions: i. LDA, THF (**6**); ii. TMSCl, NaI, Et_3N, 1 h (32%); iii. *n*-Bu_4NF, THF, 0 °C, 15 min (84%).



8d **2a-^{125}I**

Scheme 6. Radiosynthesis of **2a**–^{125}**I**, reaction conditions: i. TMSCl, $Na^{125}I$, CH_3CN, Δ, 3 h.

was stirred at −78 °C for 1 h. Then, 3-*O*-methylestrone (**7a**) (114 mg, 0.4 mmol) was added to the solution. The reaction mixture was warmed slowly to r.t. and stirring was continued for 24 h. Thereafter, the mixture was poured into water (15 ml) and extracted with ether (3 × 20 ml). The organic phase was dried over anhydrous $MgSO_4$ and concentrated *in vacuo*. The resulting residue was subjected to chromatography on silica gel (hexane/ether 2:1) to give **8a** (102 mg, 53%) as colorless needles, m.p. 237 – 238 °C. – IR (KBr): ν = 3436, 2928, 2866, 1610, 1498, 1423, 1398, 1340, 1315, 843, 754 cm^{-1}. – ^{1}H NMR (270 MHz, $CDCl_3$): δ = 0.93 (s, 3H, CH_3), 1.35 – 2.45 (m, 14H), 2.02 – 2.05 (m, 4H), 2.87 (m, 2H), 3.78 (s, 3H, OCH_3), 3.70 – 3.93 (bs, 4H), 6.63 (d, 1H, ^4J = 2.6 Hz), 6.71 (dd, 1H, ^3J = 8.9 Hz, ^4J = 2.6 Hz), 7.23 (d, 1H, ^3J = 8.9 Hz), 7.35 (d, 2H, ^3J = 8.6 Hz), 7.41 (d, 2H, ^3J= 8.6 Hz). – ^{13}C NMR (67.8 MHz, $CDCl_3$): δ = 12.90, 22.93, 23.77, 26.50, 27.24, 29.87, 30.96, 33.06, 39.06, 39.51, 43.61, 47.60, 49.68, 55.18, 80.39, 86.40, 92.34, 111.46, 113.75, 119.12, 120.21 (2C), 126.39, 132.38 (2C), 132.61, 138.00, 151.17, 157.37. – MS (FAB, 3-nitrobenzyl alcohol): *m/z* (%) = 484 (2.1) [MH^+]. – HRMS (Found) 484.2963. Calcd. for $C_{31}H_{38}O_2N_3$: 484.2964. – $C_{31}H_{37}0_2N_3$ (483.6): calcd. C 76.98; H 7.71; N 8.69; found: C 77.09; H 7.84; N 8.29.

3-O-Methyl 17α-(p-iodophenylethynyl)estra-1,3,5(10),6-tetraene-3,17 β-diol (**2c**): Under an argon atmosphere, NaI (70 mg, 0.47 mmol) was dissolved in dry acetonitrile (0.6 ml). TMSCl (25 mg, 0.23 mmol) was added and the solution was stirred at r.t. for several minutes. A solution of **8d** (50 mg, 0.10 mmol) in acetonitrile (3.3 ml) was added and the mixture was stirred for 1 h. After hydrolysis (sat. $NaHCO_3$, 10 ml) and evaporation of the acetonitrile, the residue was extracted with dichloromethane (2 × 15 ml). The organic phase was washed with brine and dried over anhydrous $MgSO_4$. Concentration of the solution *in vacuo* and column chromatography on silica gel (hexane/ether 1:1) gave **2c** (28 mg, 53%) as a colorless solid; IR (KBr): ν = 3434, 3026, 2356, 1603, 1147, 1037, 1005, 818 cm^{-1}. – ^{1}H NMR (270 MHz, $CDCl_3$): δ = 0.93 (s, 3H, CH_3), 1.32 – 2.45 (m, 12H), 3.80 (s, 3H, OCH_3), 6.00 (dd, 1H, ^3J = 9.6 Hz, ^4J = 1.7 Hz, C6), 6.46 (d, 1H, ^3J = 9.6 Hz, ^3J = 2.6 Hz, C7), 6.65 (d, 1H, ^4J = 2.6 Hz, C4), 6.75 (dd, 1H, ^4J = 2.6 Hz, ^3J = 8.6 Hz), 7.15 (d, 2H, ^3J = 8.6 Hz), 7.18 (d, 1H, ^3J = 8.6 Hz), 7.63 (d, 2H, ^3J = 8.6 Hz). – ^{13}C NMR (67.8 MHz, $CDCl_3$): δ = 12.63, 22.79, 24.24, 32.60, 38.90, 39.32, 41.78, 48.07, 48.16, 55.31, 80.18, 85.22, 94.12, 111.79, 122.32, 124.29, 127.96, 128.28, 131.30, 131.62, 132.70, 133.15 (2C), 135.34, 137.45 (2C), 158.13. – MS (FAB, 3-nitrobenzyl alcohol): *m/z* (%) = 510 (30), 329 (37), 309 (27), 273 (29), 242 (26), 178 (18). – HRMS (FAB) Found: 510.1056. Calcd. for $C_{17}H_{27}IO_2$: 510.1056.

3-O-Methyl 17α-(p-[^{125}I]iodophenylethynyl)estra-1,3,5(10),6-tetraene-17β-ol (**2a**–^{125}**I**): To a solution of **8d** (120 μg) in dry acetonitrile (50 μl) were added 50 μl of a trimethylsilyl chloride solution (5 μl of dry trimethylsilyl chloride in 5 ml of acetonitrile) and 15 μl (≃ 600 μCi) of sodium iodide (^{125}I). The reaction mixture was diluted in 70 μl of acetonitrile and the capped vial was stirred at 80 –

90 °C for 2.5 h in an oil bath. The mixture was purified by reversed phase HPLC and the fractions corresponding to the radioiodinated compound were eluted in a volume of $\sim$ 2 ml. The radiochemical yield was 40%.

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