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Improved synthesis of unique estradiol-linked platinum(II) complexes showing potent cytocidal activity and affinity for the estrogen receptor alpha and beta

*Caroline Descôteaux, Valérie Leblanc, Geoffroy Bélanger, Sophie Parent, Éric Asselin, Gervais Bérubé**

Groupe de Recherche en Biopathologies Cellulaires et Moléculaires, Département de Chimie-Biologie, Université du Québec à Trois-Rivières, C.P. 500, Trois-Rivières, Québec, G9A 5H7 Canada

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ABSTRACT

We have recently reported the synthesis of a platinum(II) complex, made of estradiol, the female sex hormone, and a cisplatin analog, an anticancer drug, linked together by an eleven carbon atoms chain. The novel estradiol-Pt(II) hybrid molecule was synthesized in nine chemical steps with 10% overall yield. This new compound has been tested *in vitro* on estrogen-dependent (MCF-7) and -independent (MDA-MD-231) (ER^+ and ER^-) cell lines. Interestingly, the biological activity was quite significant, more potent than that of cisplatin, the compound currently used in chemotherapy. The estrogen receptor binding affinity (ERBA) of this compound was very similar to that of 17β-estradiol (E_2) on both estrogen receptors (ERs), α and β. In order to further study this type of molecule, we have decided to synthesize several analogs with the same estrogenic scaffold but with various chain lengths separating the estradiol from the toxic part of the molecule. This was planned in order to study the effect of the length of the linking chain on the biological activity of the hybrids. Four E_2-Pt(II) hybrid molecules having 6–14 carbon atoms linking chain have been synthesized using a new synthetic methodology. They are synthesized in only eight chemical steps with 21% overall yield. The 17β-estradiol-linked platinum(II) complexes have been tested for their receptor binding affinity as well as for their cytocidal activity on several breast cancer cell lines. The synthesis and biological results are reported herein.

1. Introduction

Cisplatin (*cis*-diamminedichloroplatinum(II)) (**1**) is a small anticancer agent useful in the treatment of several type of cancers, including lung, head and neck, ovarian, bladder and testicular tumors [1]. Cisplatin (**1**) binds to the DNA of fast growing cells, such as cancerous cells, and stops cell proliferation leading to apoptosis [2,3]. This compound is very active but presents no specificity. Thus, when administered, it spreads throughout the whole body and affects healthy organs, causing numerous side effects such as nausea and vomiting, kidney toxicity, blood test anomalies, anaemia and increased risk of infection [4].

Bearing in mind the severe side effects following chemotherapy with platinum-based drugs and other chemotherapeutic agents, the search for site specific treatment is still, to this day, underway. The specific delivery of anticancer drugs to the sites needing treatment would, not

* *Corresponding author.*
E-mail address: Gervais.Berube@uqtr.ca (G. Bérubé).
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only improve efficacy, but also minimize toxic side effects. The strategy of drug targeting and delivery could be applied to tumours that exhibit biochemical differences (biomarkers) from normal tissues [5].

Most of breast cancer diseases are classified as hormone-dependent (60–70%) due to an overexpression of estrogen receptors (ERs) at mammalian cells level [6]. The number of estrogen receptor (ER) in hormone-dependent (ER^+) breast cancer cells is in the range of 10 000–30 000 (20 000 on average) [7]. The ERα is well characterized and is present in mammalian gland, as well as in ovarian and in uterine tissues [8]. The ERα is present at different levels of expression in a variety of normal and cancer tissues. For examples, ER^+ positive breast cancer is an example of a high expression level tissue while the expression level in normal mammary gland is relatively low [9]. Considering those facts, the ERα has already been used as a biological target in cancer therapies [10]. Thus, using estradiol (E_2) (**2**) as a carrier molecule, an anticancer drug could, theoretically, be directed accurately to hormone-dependent breast, uterus and ovarian cancer cells.

Several estrogenic compounds have already been used to link a platinum(II)-based toxic moiety at different position of the steroid nucleus [10]. Only a few of the molecules reported in the review paper are described herein in order to exemplify the general concept of estrogen-linked platinum(II) strategy for the treatment of hormone-dependent cancers. For example, more than a decade ago, compounds (**3**, **4**) have been synthesized using the alcohol function (either at position 3 or 17) as an anchor point for adding a cytotoxic moiety [11,12]. Relative binding affinities (RBA) for those complexes were relatively low, with RBA values of 0.53 for compound **3** and of 6.0 (compound **4**). Taking into account that the hydroxyl functions were playing an important role in receptor recognition, another compound (**5**) was then synthesized in order to maintain these functions free to bind to the estrogen receptor [13]. In this case, the cisplatin component was added to the 17α position. Unfortunately, the RBA values were less than 1%, yet again probably due to steric hindrance at the 17β-hydroxy function. Cisplatin dimers (**6**) have also been designed in order to target estrogen receptor-positive cells [14]. Hence, several estrogen-tethered orally active platinum(IV) complexes were designed to release the toxic moiety by intracellular esterases. The reducing environment of the cell converts the platinum(IV) to platinum(II), in this case cisplatin itself. The design rationale was inspired by the observation that ER^+ cells exposed to the hormone are sensitized to cisplatin (**1**). This type of molecule (dimer **6**) seems to show substantial activity *in vitro*. These molecules are a few examples of estrogen-linked platinum(II) complexes, additional examples are provided in the review manuscript [10].

As one can noticed, an important requirement for high affinity for the ER is to maintain the integrity of the hydroxyl functions on the estrogenic nucleus. In fact, experiments have revealed that the replacement of the hydroxyl groups at positions 3 and 17β of the steroid backbone is generally detrimental to the receptor interaction as they play a crucial role in the receptor recognition. Thus, it is important to reduce steric hindrance at both the 3- and 17-hydroxyl binding pocket of the receptor. Furthermore, it was reported that a rigid spacer kept the platinum core well away from the two hydroxyl groups responsible for binding to the receptors [5]. An estradiol ligand modified in the 16 position of the steroid backbone, the 17β-estradiol-platinum(II) complexe (**7**, $n = 9$, Fig. 1), was described in an early report [15].

The distance separating the natural ligand from the cytotoxic moiety seems to play an important role in the biological activity. It was observed that, on several non-steroidal estrogen-Pt(II) complexes, the length of the side chain bearing the cytotoxic Pt(II) portion should be 11 or 12 carbon atoms long for optimal biological activity [16]. The 17β-estradiol-platinum(II) (**7**, $n = 9$) reported earlier has an 11 carbon atoms chain at position 16β of the steroid nucleus [15].

The new 17β-estradiol-platinum(II) derivative (**7**, $n = 9$) showed high affinity for the estrogen receptor α even better than 17β-estradiol itself [15]. Hybrid **7** ($n = 9$) showed an IC_{50} of 0.35 nM compared to 4.79 nM for the natural ligand **2**. This strong affinity confirms that the modified structure could theoretically target the ERα-expressing cancer cells in an *in vivo* experiment and, consequently, carry the cytotoxic moiety at the sites needing treatment. Also, the *in vitro* biological activity studies revealed a very active compound. The new estradiol-platinum(II) complex is 32 times more active than cisplatin against MCF-7 ER-dependent cells (**1**, $IC_{50} = 16.1\ \mu M$; **7c**, $IC_{50} = 0.5\ \mu M$) and 26 times more active with MDA-MB-231 ER-independent cells (**1**, $IC_{50} = 12.8\ \mu M$; **7c**, $IC_{50} = 0.5\ \mu M$) [15]. These results were obtained using the Sulforhodamine B colorimetric assay.

Taking into consideration the structure of the 17β-estradiol-platinum(II) complex (**7c**), its great cytotoxic activity and its potential for the selective treatment of ER^+ cancers, this hybrid molecule was synthesised with a shorter and more efficient methodology, resulting in an increase overall yield. Three other 17β-estradiol-platinum(II) complexes bearing 6, 8 and 14 carbon atoms in the alkyl chain (compounds **7a**, **7b** and **7d**) were also synthesized in order to study the influence of the length of the alkyl chain upon the biological activity.

This manuscript gives the detailed description of the synthesis of four 17β-estradiol-platinum(II) complexes, bearing an hexyl, octyl, undecyl and tetradecyl tether chain. The cytocidal activity on four neoplastic human breast cancer cell lines, MCF-7 (ER^+), MDA-MB-231, MDA-MB-468 and MDA-MB-436 (ER^-) is reported herein. The estrogen receptor binding affinities (ERBA) (ERα and ERβ) are also reported for three of the four hybrids.

2. Experimental

2.1. *Chemistry*

All reactions were performed with ACS Fisher solvents. In some cases, solvent, as well as starting materials and reactants, were first purified and dried by standard means [17]. Anhydrous reactions required an inert atmosphere of dry nitrogen. Estrone was purchased from Steraloids Inc., Wilton, NH, USA and the *n*-alkyl chains were purchased from Sigma–Aldrich Canada Ltd., Oakville, Ontario, Canada. All reactions were monitored by UV fluorescence or staining with iodine on Sigma T 6145 commercial TLC plates (polyester silica gel 60 Å, 0.25 mm). Purification were done using flash column

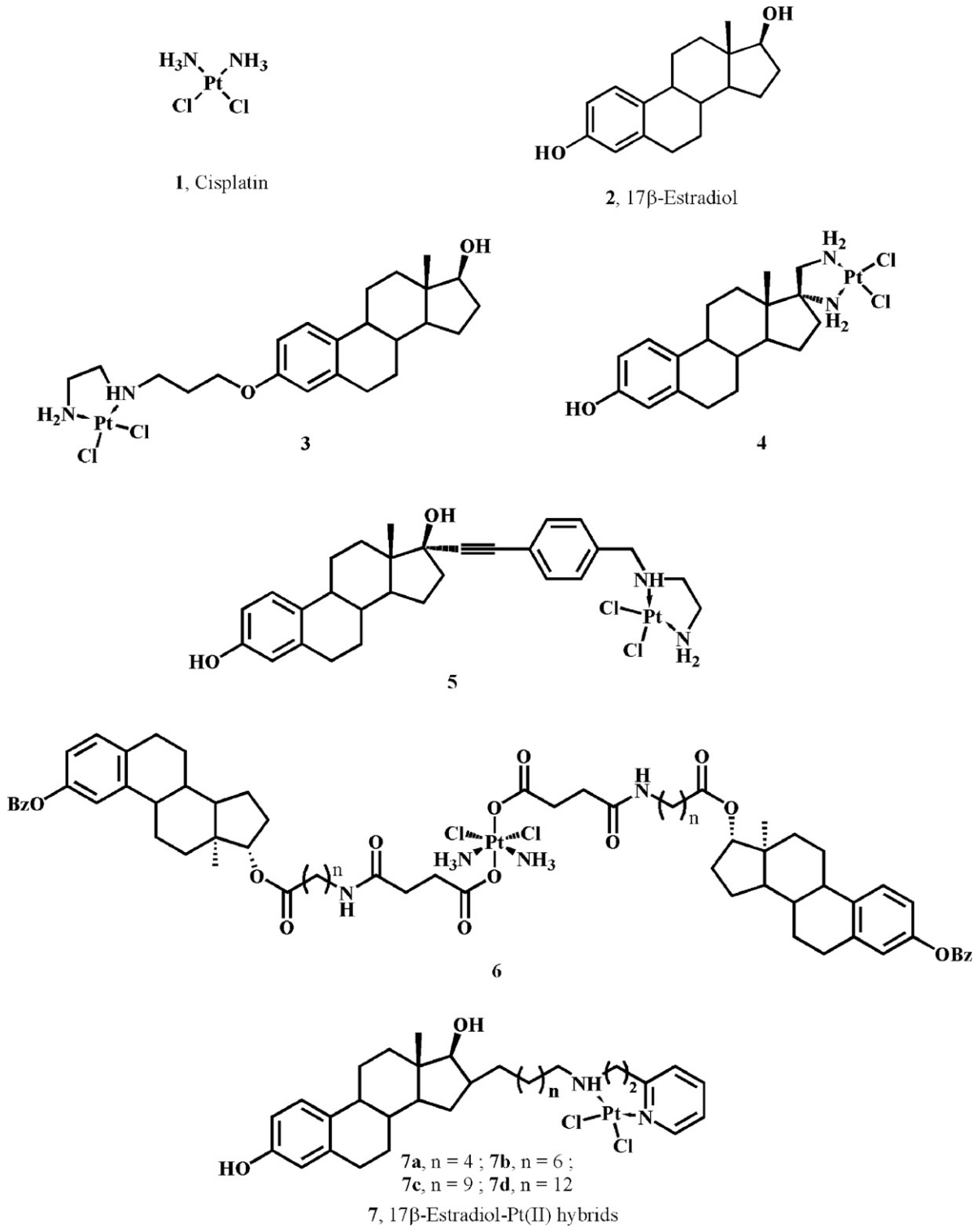


Fig. 1 – Structure of cisplatin (1), 17β-estradiol (2) and estrogen-linked platinum(II) compounds (3–7).

chromatography according to the method of Still et al. [18] on Silicycle UltraPure Flash Silica Gel, 40–63 μm mesh. Hexanes and acetone were distilled before their use as chromatography eluant.

The infrared spectra were taken on a Nicolet Impact 420 FT-IR. Sodium chloride or potassium bromide pellets were used for analysis. Mass spectral assays were obtained using a VG Micromass 7070 HS instrument using ionization energy of 70 eV (University of Sherbrooke). Chemical ionization (NH_3) was required for a few compounds.

Nuclear magnetic resonance (NMR) spectra were recorded on a Varian 200 MHz NMR apparatus. Samples were dissolved in deuterochloroform ($CDCl_3$) or in deuteroacetone (acetone-d_6) for data acquisition using tetramethylsilane as internal

standard (TMS, δ 0.0 ppm for ^{1}H NMR and ^{13}C NMR). Carbon identification was sometimes clarified by using DEPT (Distortionless Enhancement by Polarization Transfert) technique. Chemical shifts (δ) are expressed in parts per million (ppm), the coupling constants (J) are expressed in hertz (Hz). Multiplicities are described by the following abbreviations: s for singlet, d for doublet, dd for doublet of doublets, t for triplet, q for quartet, m for multiplet, #m for several multiplets and br s for broad singlet.

2.1.1. General procedure for the preparation of the THP chains ***10a,b*** *and* ***11a,b***

To a solution of an appropriate *n*-alkyl chain (**8a**, **8b**, **9a** or **9b**) (6.66 mmol) in dichloromethane, 3,4-dihydro-2*H*-pyran (2.81 mL, 30.8 mmol) and pyridinium *p*-toluenesulfonate (100 mg, 0.40 mmol), were added. The resulting mixture was stirred at room temperature (21 °C) for 18 h. Afterwards, solution was neutralised with a small amount of solid sodium bicarbonate. Magnesium sulfate was added for drying and the mixture was vigorously stirred. The mixture was filtered on a silica gel (3 cm) and celite (1 cm) pad using hexanes as the eluant. The resulting filtrate was evaporated to an oil. The THP-alkyl chains (**10a**, **10b**, **11a** or **11b**) were obtained with 100% yield.

*2.1.1.1. Spectral data for 1-tetrahydropyrannyloxy-6-chlorohexane (**10a**).* IR (NaCl, ν_{max}, cm^{-1}): 1137 and 1020 (C–O–C). ^{1}H NMR (Acetone-d_6, δ ppm): 4.54 (1H, t, J = 2.3 Hz, OC**H**O), 3.78–3.27 (4H, 4m, 2× C**H**$_2$O), 3.58 (2H, t, J = 3.3 Hz, C**H**$_2$Cl), 1.78 (2H, m, C**H**$_2$CH$_2$Cl). ^{13}C NMR (Acetone-d_6, δ ppm): 98.8 (O**C**HO), 67.4 (**C**H$_2$O, THP), 61.9 (**C**H$_2$O, chain), 45.5 (**C**H$_2$Cl). MS (m/e), $C_{11}H_{21}ClO_2$: 219 (M−H)$^+$, 119 (M$^+$−$C_5H_9O_2$). Exact mass: calculated for $C_{11}H_{21}ClO_2 - H$ = 219.1152; found = 219.1148.

*2.1.1.2. Spectral data for 1-tetrahydropyrannyloxy-8-chlorooctane (**10b**).* IR (NaCl, ν_{max}, cm^{-1}): 1143 and 1025 (C–O–C). ^{1}H NMR ($CDCl_3$, δ ppm): 4.46 (1H, t, J = 3.5 Hz, OC**H**O), 3.79–3.21 (4H, 4m, 2× C**H**$_2$O), 3.41 (2H, t, J = 6.6 Hz, C**H**$_2$Cl). ^{13}C NMR ($CDCl_3$, δ ppm): 98.9 (O**C**HO), 67.6 (**C**H$_2$O, THP), 62.3 (**C**H$_2$O, chain), 45.1 (**C**H$_2$Cl). MS (m/e), $C_{13}H_{25}ClO_2$: 247 (M−H)$^+$, 147 (M$^+$−$C_5H_9O_2$). Exact mass: calculated for $C_{13}H_{25}ClO_2 - H$ = 247.1465; found = 247.1470.

*2.1.1.3. Spectral data for 1-tetrahydropyrannyloxy-11-bromoundecane (**11a**).* IR (NaCl, ν_{max}, cm^{-1}): 1143 and 1040 (C–O–C). ^{1}H NMR ($CDCl_3$, δ ppm): 4.52 (1H, t, J = 3.3 Hz, OC**H**O), 3.81–3.26 (4H, 4m, 2× C**H**$_2$O), 3.34 (2H, t, J = 6.8 Hz, C**H**$_2$Br). ^{13}C NMR ($CDCl_3$, δ ppm): 99.0 (O**C**HO), 67.8 (**C**H$_2$O, THP), 62.4 (**C**H$_2$O, chain), 35.7 (**C**H$_2$Br). MS (m/e), $C_{16}H_{31}BrO_2$: 335 (M+H)$^+$, 333 (M−H)$^+$, 233 (M$^+$−$C_5H_9O_2$). Exact mass: calculated for $C_{16}H_{31}BrO_2 - H$ = 333.1429; found = 333.1432.

*2.1.1.4. Spectral data for 1-tetrahydropyrannyloxy-14-bromotetradecane (**11b**).* IR (NaCl, ν_{max}, cm^{-1}): 1148 and 1040 (C–O–C). ^{1}H NMR ($CDCl_3$, δ ppm): 4.53 (1H, t, J = 3.3 Hz, OC**H**O), 3.82–3.27 (4H, 4m, 2× C**H**$_2$O), 3.35 (2H, t, J = 6.8 Hz, C**H**$_2$Br). ^{13}C NMR ($CDCl_3$, δ ppm): 99.0 (O**C**HO), 67.8 (**C**H$_2$O, THP), 62.4 (**C**H$_2$O, chain), 34.1 (**C**H$_2$Br). MS (m/e), $C_{19}H_{37}BrO_2$: 377 (M+H)$^+$, 375 (M−H)$^+$, 305 (M$^+$−C_4H_8O) and 303 (M$^+$−C_4H_9O), 275 (M$^+$−$C_5H_9O_2$). Exact mass: calculated for $C_{19}H_{37}BrO_2 - H$ = 375.1898; found = 375.1901.

2.1.1.5. General procedure for the preparation of the iodide THP chains ***12a–d***. Anhydrous acetone (70 mL) and sodium iodide (6.41 g, 42.8 mmol) were added to the protected alkyl chain (**10** or **11**). The resulting mixture was stirred at reflux, under nitrogen atmosphere, sheltered from daylight, for 3 days. Acetone was then evaporated. The mixture was diluted with diethyl ether (30 mL) and washed first with a 5% sodium thiosulfate solution (30 mL) and after with water (4 × 75 mL). The organic phase was dried with magnesium sulfate, filtered and evaporated to yield the desired compound with 98% yield.

*2.1.1.6. Spectral data for 1-tetrahydropyrannyloxy-6-iodohexane (**12a**).* IR (NaCl, ν_{max}, cm^{-1}): 1132 and 1035 (C–O–C). ^{1}H NMR (Acetone-d_6, δ ppm): 4.56 (1H, t, J = 2.7 Hz, OC**H**O), 3.85–3.35 (4H, #m, 2× C**H**$_2$O), 3.29 (2H, t, J = 6.8 Hz, C**H**$_2$I). ^{13}C NMR (Acetone-d_6, δ ppm): 98.4 (O**C**HO), 67.0 (**C**H$_2$O, THP), 61.5 (**C**H$_2$O, chain), 7.3 (**C**H$_2$I). MS (m/e), $C_{11}H_{21}IO_2$: 311 (M−H)$^+$, 211 (M$^+$−$C_5H_9O_2$). Exact mass: calculated for $C_{11}H_{21}IO_2 - H$ = 311.0508; found = 311.0510.

*2.1.1.7. Spectral data for 1-tetrahydropyrannyloxy-8-iodooctane (**12b**).* IR (NaCl, ν_{max}, cm^{-1}): 1143 and 1025 (C–O–C). ^{1}H NMR ($CDCl_3$, δ ppm): 4.46 (1H, t apparent, J = 3.9 Hz, OC**H**O), 3.76–3.21 (4H, 4m, 2× C**H**$_2$O), 3.08 (2H, t, J = 7.0 Hz, C**H**$_2$I). ^{13}C NMR ($CDCl_3$, δ ppm): 98.9 (O**C**HO), 67.6 (**C**H$_2$O, THP), 62.4 (**C**H$_2$O, chain), 7.3 (**C**H$_2$I). MS (m/e), $C_{13}H_{25}IO_2$: 339 (M−H)$^+$, 239 (M$^+$−$C_5H_9O_2$). Exact mass: calculated for $C_{13}H_{25}IO_2 - H$ = 339.0821; found = 339.0816.

*2.1.1.8. Spectral data for 1-tetrahydropyrannyloxy-11-iodoundecane (**12c**).* IR (NaCl, ν_{max}, cm^{-1}): 1127 and 1040 (C–O–C). ^{1}H NMR ($CDCl_3$, δ ppm): 4.47 (1H, t, J = 3.3 Hz, OC**H**O), 3.75–3.20 (4H, 4m, 2× C**H**$_2$O), 3.07 (2H, t, J = 7.0 Hz, C**H**$_2$I). ^{13}C NMR ($CDCl_3$, δ ppm): 98.8 (O**C**HO), 67.7 (**C**H$_2$O, THP), 62.3 (**C**H$_2$O, chain), 7.3 (**C**H$_2$I). MS (m/e), $C_{16}H_{31}IO_2$: 382 (M$^+$), 381 (M−H)$^+$, 183 (M$^+$−$C_{12}H_{23}O_2$). Exact mass: calculated for $C_{16}H_{31}IO_2 - H$ = 381.1290; found = 381.1296.

*2.1.1.9. Spectral data for 1-tetrahydropyrannyloxy-14-iodotetradecane (**12d**).* IR (NaCl, ν_{max}, cm^{-1}): 1137 and 1030 (C–O–C). ^{1}H NMR ($CDCl_3$, δ ppm): 4.52 (1H, t, J = 3.5 Hz, OC**H**O), 3.69–3.29 (4H, 4m, 2× C**H**$_2$O), 3.12 (2H, t, J = 7.0 Hz, C**H**$_2$I). ^{13}C NMR ($CDCl_3$, δ ppm): 98.9 (O**C**HO), 67.8 (**C**H$_2$O, THP), 62.4 (**C**H$_2$O, chain), 7.4 (**C**H$_2$I). MS (m/e), $C_{19}H_{37}IO_2$: 424 (M$^+$), 423 (M−H)$^+$, 296 (M−HI)$^+$. Exact mass: calculated for $C_{19}H_{37}IO_2 - H$ = 423.1760; found = 423.1757.

2.1.2. Synthesis of 17β-estradiol-platinum(II) complexes ***7a–d***

*2.1.2.1. Synthesis of 3-tetrahydropyrannyloxy-1,3,5(10)-estratrien-17-one (**14**).* To a solution of estrone (**13**) (10.12 g, 37.43 mmol) in dichloromethane, pyridinium *p*-toluenesulfonate (1.0 g, 4.0 mmol) and 3,4-dihydro-2*H*-pyran (8.54 mL, 93.6 mmol), were added. The resulting mixture was stirred at room temperature (21 °C) for 20 h. Afterwards, solution was neutralised with a small amount of solid

sodium bicarbonate. Magnesium sulfate was added for drying and the mixture was stirred vigorously. The mixture was filtered on a silica gel (3 cm) and celite (1 cm) pad using hexanes as the eluant. The resulting filtrate was evaporated and set to vacuum for an hour to give a beige solid, 100% yield.

*2.1.2.2. Spectral data for 3-tetrahydropyrannyloxy-1,3,5(10)-estratrien-17-one (**14**).* IR (NaCl, ν_{max}, cm^{-1}): 1744 (C=O), 1613 (C=C), 1247 and 1043 (C–O). ^1H NMR ($CDCl_3$, δ ppm): 7.19 (1H, d, J = 8.6 Hz, 1-C**H**), 6.85 (1H, dd, J = 2.3 Hz and J = 8.6 Hz, 2-C**H**), 6.81 (1H, d, J = 2.7 Hz, 4-C**H**), 5.39 (1H, t, J = 2.9 Hz, OC**H**O), 3.98–3.55 (2H, 2m, C**H**$_2$O), 2.90 (2H, m, 6-C**H**$_2$), 2.57–1.37 (19H, #m, 3× C**H** and 8× C**H**$_2$), 0.90 (3H, s, 18-C**H**$_3$). ^{13}C NMR ($CDCl_3$, δ ppm): 220.9 (17-C), 155.3 (3-C), 137.8 (5-C), 133.2 (10-C), 126.4 (1-C), 116.8 (4-C), 114.4 (2-C), 96.6 (O**C**HO), 62.2 (**C**H$_2$O), 50.7, 48.2, 44.3, 38.6, 36.1, 31.8, 30.7, 29.8, 26.8, 26.1, 25.5, 21.8, 19.0, 14.1 (C-18). MS (m/e), $C_{23}H_{30}O_3$: 354 (M^+), 271 ($M^+-C_5H_7O$). Exact mass: calculated for $C_{23}H_{30}O_3$ = 354.2195; found = 354.2200.

*2.1.2.3. Synthesis of 3-tetrahydropyrannyloxy-16α,β-(methoxycarbonyl)-1,3,5(10)-estratrien-17-one (**15**).* In a double necked flask, tetrahydrofuran (30 mL) was added to potassium hydride (30% in oil, 15.06 g, 112.7 mmol) under nitrogen conditions. The solution was stirred at room temperature. Dimethylcarbonate (7.91 mL, 93.9 mmol) was then added to the potassium hydride suspension. Separately, protected estrone (**14**) (13.3 g, 37.6 mmol) was dissolved in tetrahydrofuran (70 mL) under nitrogen atmosphere. The estrone solution was then added to reaction mixture and the overall was heated to reflux for 2.5 h. Afterwards, the mixture was cooled down. Tert-butanol (10 mL), methyl alcohol (20 mL) and water were added and the resulting solution was stirred for 15 min between each adding. The mixture was then diluted with ethyl acetate (80 mL) and the organic phase was washed with saturated NH_4Cl solution (2× 90 mL) as well as with water (4× 90 mL). The organic phase was dried with anhydrous magnesium sulfate, filtered and evaporated to give the crude product. The residue was first triturated with hexanes and purified by flash chromatography with a mixture of hexanes and acetone (92:8) to give the activated estrone in 90% yield.

*2.1.2.4. Spectral data for 3-tetrahydropyrannyloxy-16α,β-(methoxycarbonyl)-1,3,5(10)-estratrien-17-one (**15**).* IR (NaCl, ν_{max}, cm^{-1}): 1763 (C=O, ester), 1740 (C=O, ketone), 1605 (C=C), 1232 (C–O), 1155 and 1035 (C–O–C). ^1H NMR ($CDCl_3$, δ ppm): 7.18 (1H, d, J = 8.6 Hz, 1-C**H**), 6.85 (1H, dd, J = 2.7 Hz and J = 8.6 Hz, 2-C**H**), 6.81 (1H, d, J = 2.3 Hz, 4-C**H**), 5.39 (1H, t, J = 2.9 Hz, OC**H**O), 3.96–3.54 (2H, 2m, C**H**$_2$O), 3.76 (3H, s, OC**H**$_3$), 3.20 (1H, t, J = 9.2 Hz, 16-C**H**), 2.88 (2H, m, 6-C**H**$_2$), 2.42–1.39 (17H, #m, 3× C**H** and 7× C**H**$_2$), 0.98 and 0.95 (3H, 2s, 18-C**H**, 16α,β (2.5: 1)). ^{13}C NMR ($CDCl_3$, δ ppm): 212.4 (17-C), 170.1 (**C**OOCH$_3$), 155.3 (3-C), 137.8 (5-C), 132.9 (10-C), 126.5 (1-C), 116.7 (4-C), 114.3 (2-C), 96.5 (O**C**HO), 62.2 (**C**H$_2$O), 54.3, 52.8 (O**C**H$_3$), 49.2, 48.1, 44.3, 38.1, 32.2, 30.6, 29.8, 26.6, 25.5, 19.0, 14.6 (18-C). MS (m/e), $C_{23}H_{32}O_5$: 413 $(M+H)^+$, 381 $(M-CH_3O)$, 328 $(M-C_5H_9O)$. Exact mass: calculated for $C_{25}H_{32}O_5 + H$ = 413.2328; found = 413.2319.

*2.1.2.5. Synthesis of 3-THP-16β-(methoxycarbonyl)-16α-(THP-alkylchain)-estrone (**16a–d**).* Activated estrone (**15**) (0.500 g, 1.22 mmol), dichloromethane (8 mL), triethylbenzylammonium chloride (100 mg), 1-tetrahydropyrannyloxy-*n*-iodohexane (4.89 mmol) and a 10% (w/v) NaOH solution (5 mL) were put together in a flask. The mixture was heated to reflux and strongly stirred for 24 h. The resulting solution was diluted with diethyl ether (60 mL). The organic phase was washed with saturated NH_4Cl solution (2× 70 mL) as well as with water (4× 70 mL). The organic phase was dried with anhydrous magnesium sulfate, filtered and evaporated. Flash chromatography with a mixture of hexanes and acetone (92:8) gave yellow oil with 92% optimized yield.

*2.1.2.6. Spectral data for 3-tetrahydropyrannyloxy-16β-(methoxycarbonyl)-16α-(6′-tetrahydropyrannyloxyhexyl)-1,3,5(10)-estratrien-17-one (**16a**).* IR (NaCl, ν_{max}, cm^{-1}): 1757 (C=O, ester), 1731 (C=O, ketone), 1609 and 1573 (C=C), 1050 (C–O). ^1H NMR (Acetone-d_6, δ ppm): 7.16 (1H, d, J = 8.6 Hz, 1-C**H**), 6.80 (1H, d, J = 8.6 Hz, 2-C**H**), 6.75 (1H, m, 4-C**H**), 5.37 (1H, m, OC**H**O, steroid), 4.55 (1H, m, OC**H**O, chain), 3.84–3.27 (6H, 6m, 3× C**H**$_2$O), 3.68 (3H, s, OC**H**$_3$), 2.82 (2H, m, 6-C**H**$_2$), 2.38–1.12 (33H, #m, 3× C**H**, 15× C**H**$_2$), 0.98 and 0.93 (3H, 2s, 18-C**H**$_3$, 16α,β (12: 1)). ^{13}C NMR (Acetone-d_6, δ ppm): 213.0 (17-C), 171.7 (**C**OOCH$_3$), 155.4 (3-C), 137.6 (5-C), 133.0 (10-C), 126.3 (1-C), 116.8 (4-C), 114.2 (2-C), 98.4 (O**C**HO, chain), 96.2 (O**C**HO, steroid), 67.1 (**C**H$_2$O, THP, side chain), 61.6 (**C**H$_2$O, hexyl chain), 61.5 (**C**H$_2$O, THP, steroid), 60.2, 52.0 (O**C**H$_3$), 49.4, 46.1, 44.2, 38.1, 35.3, 32.4, 30.88, 30.73, 30.56, 29.83, 26.6, 26.2, 25.9, 25.8, 25.4 19.6, 15.6, 13.8 (18-C). MS (m/e), $C_{36}H_{52}O_7$: 596 (M^+), 529 ($M^+-C_4H_3O$), 513 ($M^+-C_5H_7O$). Exact mass: calculated for $C_{36}H_{52}O_7$ = 596.3713; found = 596.3704.

*2.1.2.7. Spectral data for 3-tetrahydropyrannyloxy-16β-(methoxycarbonyl)-16α-(8′-tetrahydropyrannyloxyoctyl)-1,3,5(10)-estratrien-17-one (**16b**).* IR (NaCl, ν_{max}, cm^{-1}): 1762 (C=O, ester), 1726 (C=O, ketone), 1614 and 1573 (C=C), 1250 and 1040 (C–O). ^1H NMR ($CDCl_3$, δ ppm): 7.18 (1H, d, J = 8.6 Hz, 1-C**H**), 6.85 (1H, dd, J = 2.3 Hz and J = 8.6 Hz, 2-C**H**), 6.81 (1H, d, J = 2.3 Hz, 4-C**H**), 5.39 (1H, t, J = 3.1 Hz, OC**H**O, steroid), 4.57 (1H, t, J = 3.1 Hz, OC**H**O, chain), 3.92–3.35 (6H, 6m, 3× C**H**$_2$O), 3.72 (3H, s, OC**H**$_3$), 2.89 (2H, m, 6-C**H**$_2$), 2.38–1.13 (37H, #m, 3× C**H**, 17× C**H**$_2$), 0.92 and 0.88 (3H, 2s, 18-C**H**$_3$, 16α,β (7: 1)). ^{13}C NMR ($CDCl_3$, δ ppm): 214.3 (17-C), 172.1 (**C**OOCH$_3$), 155.3 (3-C), 137.8 (5-C), 133.0 (10-C), 126.4 (1-C), 116.8 (4-C), 114.3 (2-C), 99.1 (O**C**HO, chain), 96.5 (O**C**HO, steroid), 67.9 (**C**H$_2$O, THP, side chain), 62.5 (**C**H$_2$O, octyl chain), 62.2 (**C**H$_2$O, THP, steroid), 60.4, 52.8 (O**C**H$_3$), 49.7, 46.2, 44.3, 38.1, 35.8, 32.3, 30.99, 30.72, 30.62, 29.93, 29.77, 29.61, 29.51, 26.8, 26.4, 25.9, 25.7, 25.6, 25.5, 19.6, 18.9, 15.5, 14.3 (18-C). MS (m/e), $C_{38}H_{56}O_7$: 624 (M^+), 594 (M^+-CH_2O), 542 ($M^+-C_5H_6O$). Exact mass: calculated for $C_{38}H_{56}O_7$ = 624.4026; found = 624.4014.

*2.1.2.8. Spectral data for 3-tetrahydropyrannyloxy-16β-(methoxycarbonyl)-16α-(11′-tetrahydropyrannyloxyundecyl)-1,3,5(10)-estratrien-17-one (**16c**).* IR (NaCl, ν_{max}, cm^{-1}): 1759 (C=O, ester), 1721 (C=O, ketone), 1617 (C=C), 1231 (C–O). ^1H NMR ($CDCl_3$, δ ppm): 7.16 (1H, d, J = 8.6 Hz, 1-C**H**), 6.84 (1H, dd, J = 2.3 Hz and J = 10.9 Hz, 2-C**H**), 6.79 (1H, s, 4-C**H**), 5.37 (1H, t, J = 3.1 Hz, OC**H**O, steroid), 4.56 (1H, t, J = 3.1 Hz, OC**H**O,

chain), 3.90–3.34 (6H, 6m, 3× C**H**$_2$O) 3.71 (3H, s, OC**H**$_3$), 2.87 (2H, m, 6-C**H**$_2$), 2.37–1.25 (43H, #m, 3× C**H**, 20× C**H**$_2$), 0.90 and 0.87 (3H, 2s, 18-C**H**$_3$, 16α,β (3.5: 1)). ^{13}C NMR ($CDCl_3$, δ ppm): 214.3 (17-C), 172.1 (**C**OOCH$_3$), 155.3 (3-C), 137.8 (5-C), 133.0 (10-C), 126.4 (1-C), 116.8 (4-C), 114.3 (2-C), 99.0 (O**C**HO, chain), 96.5 (O**C**HO, steroid), 67.9 (**C**H$_2$O, THP, side chain), 62.5 (**C**H$_2$O, undecyl chain), 62.1 (**C**H$_2$O, THP, steroid), 60.4, 52.8 (O**C**H$_3$), 49.7, 46.2, 44.3, 38.1, 35.8, 32.3, 31.8, 31.0, 30.72, 30.62, 29.97(2× C), 29.75 (3× C), 29.57, 26.75, 26.46, 25.91, 25.72, 25.65, 25.49, 22.9, 19.9, 19.0, 14.34 (18-C). MS (m/e), $C_{41}H_{62}O_7$: 666 (M^+), 635 (M^+-CH_3O), 582 ($M^+-C_5H_8O$), 498 ($M^+-2C_5H_8O$). Exact mass: calculated for $C_{41}H_{62}O_7$ = 666.4496; found = 666.4488.

*2.1.2.9. Spectral data for 3-tetrahydropyrannyloxy-16β-(methoxycarbonyl)-16α-(14′-tetrahydropyrannyloxytetradecyl)-1,3,5(10)-estratrien-17-one (**16d**)*. IR (NaCl, ν_{max}, cm^{-1}): 1757 (C=O, ester), 1726 (C=O, ketone), 1619 (C=C), 1224 and 1030 (C–O). ^{1}H NMR ($CDCl_3$, δ ppm): 7.18 (1H, d, *J* = 8.6 Hz, 1-C**H**), 6.84 (1H, dd, *J* = 2.7 Hz and *J* = 8.6 Hz, 2-C**H**), 6.81 (1H, s, 4-C**H**), 5.39 (1H, t, *J* = 3.1 Hz, OC**H**O, steroid), 4.57 (1H, t, *J* = 3.1 Hz, OC**H**O, chain), 3.91–3.31 (6H, 6m, 3× C**H**$_2$O), 3.72 (3H, s, OC**H**$_3$), 2.88 (2H, m, 6-C**H**$_2$), 2.38–1.15 (49H, #m, 3× C**H**, 23× C**H**$_2$), 0.91 and 0.88 (3H, 2s, 18-C**H**$_3$, 16α,β (4: 1)). ^{13}C NMR ($CDCl_3$, δ ppm): 214.7 (17-C), 172.2 (**C**OOCH$_3$), 155.3 (3-C), 137.8 (5-C), 133.0 (10-C), 126.4 (1-C), 116.7 (4-C), 114.3 (2-C), 99.1 (O**C**HO, chain), 96.5 (O**C**HO, steroid), 67.9 (**C**H$_2$O, THP, side chain), 62.6 (**C**H$_2$O, tetradecyl chain), 62.2 (**C**H$_2$O, THP, steroid), 60.4, 52.8 (O**C**H$_3$), 49.7, 46.2, 44.3, 38.1, 35.8, 34.9, 32.3, 31.8, 31.0, 30.6, 29.98, 29.84, 29.73, 29.61, 26.8, 26.5, 25.92, 25.73, 25.67, 25.49, 22.9, 19.9, 19.0, 14.35, 14.28 (18-C). MS (m/e), $C_{44}H_{68}O_7$: 726 $(MNH_4)^+$, 624 $(M-C_5H_8O)^+$. Exact mass: calculated for $C_{44}H_{68}O_7 + NH_4$ = 726.5308; found = 726.5326.

*2.1.2.10. General procedure for the synthesis of 3-hydroxy-16α,β-(hydroxyalkyl)-1,3,5(10)-estrone (**17a–d**)*. Alkylated estrone **16a–d** (2.62 mmol), lithium chloride (2.43 g, 57.6 mmol) and water (1.04 mL, 57.6 mmol) were made soluble in DMF (15 mL). The solution was stirred to reflux for 20 h. Afterwards, ethyl acetate was added (90 mL) and the organic phase was washed with 10% (v/v) HCl solution (2× 70 mL) as well as with water (4× 70 mL). The organic phase was then dried with anhydrous magnesium sulfate, filtered and evaporated. Purification was done by flash chromatography with a mixture of hexanes and acetone (70:30). We obtained a decarboalkoxylated and bis-deprotected compound with 71% optimized yield.

*2.1.2.11. Spectral data for compound **17a***. IR (NaCl, ν_{max}, cm^{-1}): 3200–3600 (O–H), 1711 (C=O), 1624 and 1578 (C=C), 1255 and 1076 (C–O). ^{1}H NMR (Acetone-d_6, δ ppm): 7.10 (1H, d, *J* = 8.2 Hz, 1-C**H**), 6.60 (1H, dd, *J* = 2.7 Hz and *J* = 8.2 Hz, 2-C**H**), 6.55 (1H, s, 4-C**H**), 3.52 (2H, t, *J* = 5.9 Hz, C**H**$_2$OH 16β), 2.83 (2H, m, 6-C**H**$_2$), 2.26–1.32 (24H, #m, 4× C**H**, 9× C**H**$_2$, 2× O**H**), 0.94 and 0.85 (3H, 2s, 18-C**H**$_3$, 16α,β (1: 1.9)). ^{13}C NMR (Acetone-d_6, δ ppm): 221.0 (17-C), 155.4 (3-C), 137.7 (5-C), 131.0 (10-C), 126.4 (1-C), 115.3 (4-C), 113.0 (2-C), 61.8 (**C**H$_2$OH), 49.18, 49.01, 48.25, 44.37, 38.69, 38.33, 33.07, 32.46, 32.28, 29.5, 28.6, 28.2, 26.9, 26.04, 25.92, 13.7 (18-**C**H$_3$). MS (m/e), $C_{24}H_{34}O_3$: 370 (M^+), 353 (M^+-OH), 270 (M^+-H_2O and C_6H_{10}). Exact mass: calculated for $C_{24}H_{34}O_3$ = 370.2508; found = 370.2512.

*2.1.2.12. Spectral data for compound **17b***. IR (NaCl, ν_{max}, cm^{-1}): 3200–3600 (O–H), 1711 (C=O), 1629 and 1583 (C=C), 1255 and 1071 (C–O). ^{1}H NMR (Acetone-d_6, δ ppm): 7.10 (1H, d, *J* = 8.2 Hz, 1-C**H**), 6.60 (1H, dd, *J* = 2.7 Hz and *J* = 8.2 Hz, 2-C**H**), 6.55 (1H, s, 4-C**H**), 3.51 (2H, t, *J* = 5.9 Hz, C**H**$_2$OH, 16β), 2.82 (2H, m, 6-C**H**$_2$), 2.40-1.33 (28H, #m, 4× C**H**, 11× C**H**$_2$, 2× O**H**), 0.94 and 0.85 (3H, 2s, 18-C**H**$_3$, 16α,β (1: 4)). ^{13}C NMR (Acetone-d_6, δ ppm): 220.9 (17-C), 155.4 (3-C), 137.7 (5-C), 131.0 (10-C), 126.4 (1-C), 115.3 (4-C), 113.0 (2-C), 61.8 (**C**H$_2$OH), 49.17, 49.01, 48.24, 44.38, 38.33, 33.13, 32.49, 32.28, 29.5, 26.9, 26.0, 13.7 (18-C). MS (m/e), $C_{26}H_{38}O_3$: 398 (M^+), 383 (M^+-CH_3), 270 (M^+-H_2O and C_8H_{14}). Exact mass: calculated for $C_{26}H_{38}O_3$ = 398.2821; found = 398.2824.

*2.1.2.13. Spectral data for compound **17c***. IR (NaCl, ν_{max}, cm^{-1}): 3200–3600 (O–H), 1732 (C=O), 1670 (C=C), 1255 and 1093 (C–O). ^{1}H NMR (Acetone-d_6, δ ppm): 7.10 (1H, d, *J* = 8.2 Hz, 1-C**H**), 6.60 (1H, dd, *J* = 2.7 Hz and *J* = 8.2 Hz, 2-C**H**), 6.55 (1H, s, 4-C**H**), 3.51 (2H, t, *J* = 5.9 Hz, C**H**$_2$OH, 16β), 2.82 (2H, m, 6-C**H**$_2$), 2.40–1.33 (28H, #m, 4× C**H**, 11× C**H**$_2$, 2× O**H**), 0.94 and 0.85 (3H, 2s, 18-C**H**$_3$, 16α,β (1: 4)). ^{13}C NMR (Acetone-d_6, δ ppm): 220.9 (17-C), 155.4 (3-C), 137.7 (5-C), 131.0 (10-C), 126.4 (1-C), 115.3 (4-C), 113.0 (2-C), 61.8 (**C**H$_2$OH), 49.17, 49.01, 48.24, 44.38, 38.33, 33.13, 32.49, 32.28, 29.5, 26.9, 26.0, 13.7 (18-C). MS (m/e), $C_{29}H_{44}O_3$: 440 (M^+), 422 (M^+-H_2O), 270 (M^+-H_2O and $C_{11}H_{20}$). Exact mass: calculated for $C_{29}H_{44}O_3$ = 440.3290; found = 440.3297.

*2.1.2.14. Spectral data for compound **17d***. IR (NaCl, ν_{max}, cm^{-1}): 3200–3600 (O–H), 1737 (C=O), 1511 (C=C), 1214 (C–O). ^{1}H NMR (Acetone-d_6, δ ppm): 7.12 (1H, d, *J* = 8.6 Hz, 1-C**H**), 6.60 (1H, dd, *J* = 2.7 Hz and *J* = 8.6 Hz, 2-C**H**), 6.56 (1H, s, 4-C**H**), 3.51 (2H, t, *J* = 5.9 Hz, C**H**$_2$OH, 16β), 2.81 (2H, m, 6-C**H**$_2$), 2.38–1.13 (40H, #m, 4× C**H**, 17× C**H**$_2$, 2× O**H**), 0.95 and 0.86 (3H, 2s, 18-C**H**$_3$, 16α,β (1: 1.2)). ^{13}C NMR (Acetone-d_6, δ ppm): 220.4 (17-C), 155.4 (3-C), 137.8 (5-C), 131.3 (10-C), 126.4 (1-C), 115.3 (4-C), 112.9 (2-C), 62.0 (**C**H$_2$OH), 49.20, 49.01, 48.24, 44.38, 38.33, 33.13, 33.00, 32.49, 32.28, 31.45, 31.14, 29.5, 26.9, 26.0, 13.7 (18-C). MS (m/e), $C_{32}H_{50}O_3$: 482 (M^+), 464 (M^+-H_2O) and 270 (M^+-H_2O and $C_{14}H_{26}$). Exact mass: calculated for $C_{32}H_{50}O_3$ = 482.3760; found = 482.3755.

*2.1.2.15. General procedure for the synthesis of 16α,β-(hydroxyalkyl)-17β-estradiol (**18a–d**)*. Compound **17a–d** (1.3 mmol) was dissolved in diethyl ether (10 mL), dichloromethane (3 mL) and anhydrous THF (5 mL) under nitrogen atmosphere. The resulting solution was cooling down with an ice and water bath, afterwards lithium borohydride (0.17 g, 7.9 mmol) was added. The mixture was kept at 0 °C for 3 h and then, at room temperature for 3 days. When reaction completed, sodium sulfate decahydrate (0.5 g) was added. Work-up was done by diluting with diethyl ether (40 mL) and washing the organic phase with saturated NH_4Cl solution (2× 20 mL) and with water (4× 50 mL). The organic phase was dried with anhydrous magnesium sulfate, filtered and evaporated. A white solid was obtained quantitatively and was used without further purification at the next step.

2.1.2.16. Spectral data for compound ***18a***. IR (NaCl, ν_{max}, cm^{-1}): 3200–3600 (O–H), 1621 (C=C), 1255 and 1072 (C–O). ^1H NMR (Acetone-d_6, δ ppm): 7.90 (1H, s, 3-O**H**), 7.09 (1H, d, J = 8.2 Hz, 1-C**H**), 6.60 (1H, dd, J = 2.7 Hz and J = 8.2 Hz, 2-C**H**), 6.53 (1H, s, 4-C**H**), 3.62 (1H, m, 17-O**H**), 3.52 (2H, t, J = 6.1 Hz, C**H**$_2$OH), 3.40 (1H, m, 17-C**H**), 2.86 (1H, s, CH$_2$O**H**), 2.75 (2H, m, 6-C**H**$_2$), 2.30–1.29 (22H, #m, 4× C**H**, 9× C**H**$_2$), 0.81 and 0.78 (3H, 2s, 18-C**H**$_3$, 16α,β (1: 2)). ^{13}C NMR (Acetone-d_6, δ ppm): 155.3 (3-C), 137.8 (5-C), 131.5 (10-C), 126.4 (1-C), 115.3 (4-C), 112.9 (2-C), 81.7 (17-C), 61.9 (**C**H$_2$OH), 49.0, 44.34, 44.30, 40.7, 38.9, 38.1, 33.2, 32.7, 32.0, 30.2, 29.7, 29.0, 27.7, 26.6, 26.1, 12.5 (18-C). MS (m/e), $C_{24}H_{36}O_3$: 372 (M^+), 354 (M^+-H_2O), 285 ($M^+-C_5H_{11}O$). Exact mass: calculated for $C_{24}H_{36}O_3$ = 372.2664; found = 372.2662.

2.1.2.17. Spectral data for compound ***18b***. IR (NaCl, ν_{max}, cm^{-1}): 3200–3600 (O–H), 1619 and 1506 (C=C), 1245 and 1066 (C–O). ^1H NMR (Acetone-d_6, δ ppm): 7.95 (1H, s, 3-O**H**), 7.09 (1H, d, J = 8.6 Hz, 1-C**H**), 6.56 (1H, dd, J = 2.7 Hz and J = 8.6 Hz, 2-C**H**), 6.53 (1H, s, 4-C**H**), 3.68-3.47 (2H, 2m, 17-O**H** and 17-C**H**), 3.52 (2H, t, J = 5.5 Hz, C**H**$_2$OH), 2.96 (1H, s, CH$_2$O**H**), 2.75 (2H, m, 6-C**H**$_2$), 2.23–1.07 (26H, #m, 4× C**H**, 11× C**H**$_2$), 0.81 and 0.78 (3H, 2s, 18-C**H**$_3$, 16α,β (1: 2.7)). ^{13}C NMR (Acetone-d_6, δ ppm): 155.3 (3-C), 137.8 (5-C), 131.5 (10-C), 126.4 (1-C), 115.3 (4-C), 112.9 (2-C), 81.7 (17-C), 61.9 (**C**H$_2$OH), 48.9, 44.34, 44.30, 40.7, 38.9, 33.1, 32.75, 32.09, 30.4, 30.12, 30.07, 29.90, 29.85, 29.74, 29.69, 29.03, 26.07, 12.5 (18-C). MS (m/e), $C_{26}H_{40}O_3$: 400 (M^+), 382 (M^+-H_2O), 354 ($M^+-C_2H_6O$). Exact mass: calculated for $C_{26}H_{40}O_3$ = 400.2977; found = 400.2964.

2.1.2.18. Spectral data for compound ***18c***. IR (NaCl, ν_{max}, cm^{-1}): 3200–3600 (O–H), 1600 and 1506 (C=C), 1245 and 1066 (C–O). ^1H NMR ($CDCl_3$, δ ppm): 7.27 (1H, s, 3-O**H**), 7.14 (1H, d, J = 7.8 Hz, 1-C**H**), 6.61 (1H, dd, J = 2.7 Hz and J = 8.6 Hz, 2-C**H**), 6.56 (1H, s, 4-C**H**), 3.85–3.45 (1H, m, 17-C**H**), 3.64 (2H, m, C**H**$_2$OH), 2.80 (2H, m, 6-C**H**$_2$), 2.70–0.88 (34H, #m, 4× C**H**, 14× C**H**$_2$, 2× O**H**), 0.79 and 0.76 (3H, 2s, 18-C**H**$_3$, 16α,β (1: 1.2)). ^{13}C NMR ($CDCl_3$, δ ppm): 153.6 (3-C), 138.5 (5-C), 132.9 (10-C), 126.7 (1-C), 115.5 (4-C), 112.9 (2-C), 82.8 (17-C), 63.4 (**C**H$_2$OH), 44.35, 44.20, 40.2, 38.6, 33.0, 32.6, 30.54, 30.12, 29.90, 29.85, 29.74, 29.63, 28.9, 25.9, 12.6 (18-C). MS (m/e), $C_{29}H_{46}O_3$: 442 (M^+), 424 (M^+-H_2O). Exact mass: calculated for $C_{29}H_{46}O_3$ = 442.3447; found = 442.3449.

2.1.2.19. Spectral data for compound ***18d***. IR (NaCl, ν_{max}, cm^{-1}): 3650–3100 (O–H), 1609 (C=C), 1230 and 1061 (C–O). ^1H NMR (Acetone-d_6, δ ppm): 7.94 (1H, s, 3-O**H**), 7.09 (1H, d, J = 8.2 Hz, 1-C**H**), 6.58 (1H, dd, J = 2.7 Hz and J = 8.6 Hz, 2-C**H**), 6.53 (1H, s, 4-C**H**), 3.69–3.42 (2H, 2m, 17-O**H** and 17-C**H**), 3.52 (2H, m, C**H**$_2$OH), 2.94 (1H, s, CH$_2$O**H**), 2.76 (2H, m, 6-C**H**$_2$), 2.23–1.03 (40H, #m, 4× C**H**, 18× C**H**$_2$), 0.81 and 0.79 (3H, 2s, 18-C**H**$_3$, 16α,β (1: 4.5)). ^{13}C NMR (Acetone-d_6, δ ppm): 155.2 (3-C), 137.8 (5-C), 131.5 (10-C), 126.4 (1-C), 115.3 (4-C), 112.9 (2-C), 81.7 (17-C), 61.9 (**C**H$_2$OH), 49.0, 44.34, 44.31, 40.7, 38.9, 38.1, 33.1, 32.8, 32.1, 30.15, 29.86, 29.79, 29.66, 29.45, 29.03, 27.7, 26.6, 26.1, 25.5, 12.5 (18-C). MS (m/e), $C_{32}H_{52}O_3$: 484 (M^+), 466 (M^+-H_2O) and 397 ($M^+-C_5H_{11}O$). Exact mass: calculated for $C_{32}H_{52}O_3$ = 484.3916; found = 484.3922.

*2.1.2.20. General procedure for the synthesis of 16α,β-(bromoalkyl)-17β-estradiol (****19a–d****)*. Compound **18a–d** (1.4 mmol) was dissolved in dichloromethane (15 mL). After complete dissolution, carbon tetrabromide (1.81 g, 5.44 mmol) and triphenylphosphine (1.46 g, 5.44 mmol) were added. The mixture was stirred at room temperature (21 °C), under nitrogen atmosphere, for 5 h. The organic phase was then extracted with diethyl ether (50 mL) and washed with a saturated NH_4Cl solution (2× 60 mL) as well as with water (2× 60 mL). The ethereal phase was dried, filtered and evaporated. Flash chromatography with a mixture of hexanes and acetone (80:20) gave the desired product in 62% optimised yield.

2.1.2.21. Spectral data for compound ***19a***. IR (NaCl, ν_{max}, cm^{-1}): 3650–3150 (O–H), 1624 and 1501 (C=C), 1230 and 1071 (C–O). ^1H NMR (Acetone-d_6, δ ppm): 7.90 (1H, s, 3-O**H**), 7.09 (1H, d, J = 8.6 Hz, 1-C**H**), 6.58 (1H, dd, J = 2.3 Hz and J = 8.2 Hz, 2-C**H**), 6.53 (1H, s, 4-C**H**), 3.74 (1H, m, 17-C**H**), 3.49 (2H, t, J = 6.6 Hz, C**H**$_2$Br), 2.90 (1H, s, 17-O**H**), 2.76 (2H, m, 6-C**H**$_2$), 2.25–1.03 (22H, #m, 4× C**H**, 9× C**H**$_2$), 0.81 and 0.78 (3H, 2s, 18-C**H**$_3$, 16α,β (1: 23)). ^{13}C NMR (Acetone-d_6, δ ppm): 155.3 (3-C), 137.8 (5-C), 131.5 (10-C), 126.4 (1-C), 115.3 (4-C), 112.9 (2-C), 81.7 (17-C, 16β), 49.0, 44.33, 44.31, 40.7, 38.9, 38.1, 34.2, 33.0, 32.7, 32.0, 29.73, 29.45, 29.06, 28.26, 27.73, 26.6, 12.5 (18-C). MS (m/e), $C_{24}H_{35}BrO_2$: 434 (M^+). Exact mass: calculated for $C_{24}H_{35}BrO_2$ = 434.1820; found = 434.1815.

2.1.2.22. Spectral data for compound ***19b***. IR (NaCl, ν_{max}, cm^{-1}): 3650–3150 (O–H), 1614 and 1501 (C=C), 1255 and 1066 (C–O). ^1H NMR (Acetone-d_6, δ ppm): 7.94 (1H, s, 3-O**H**), 7.09 (1H, d, J = 8.6 Hz, 1-C**H**), 6.58 (1H, dd, J = 2.7 Hz and J = 8.2 Hz, 2-C**H**), 6.53 (1H, s, 4-C**H**), 3.69 (1H, m, 17-C**H**), 3.49 (2H, t, J = 6.6 Hz, C**H**$_2$Br), 2.88 (1H, s, 17-O**H**), 2.75 (2H, m, 6-C**H**$_2$), 2.23–1.02 (26H, #m, 4× C**H**, 11× C**H**$_2$), 0.81 and 0.78 (3H, 2s, 18-C**H**$_3$, 16α,β (1: 16)). ^{13}C NMR (Acetone-d_6, δ ppm): 155.3 (3-C), 137.8 (5-C), 131.5 (10-C), 126.4 (1-C), 115.3 (4-C), 112.9 (2-C), 81.7 (17-C, 16β), 48.9, 44.34, 44.30, 40.7, 38.9, 38.1, 34.2, 33.0, 32.7, 32.1, 29.82, 29.72, 29.43, 28.94, 28.16, 27.72, 26.6, 12.5 (18-C). MS (m/e), $C_{26}H_{39}BrO_2$: 462 (M^+). Exact mass: calculated for $C_{26}H_{39}BrO_2$ = 462.2133; found = 462.2129.

2.1.2.23. Spectral data for compound ***19c***. IR (NaCl, ν_{max}, cm^{-1}): 3650–3150 (O–H), 1614 and 1516 (C=C), 1255 and 1071 (C–O). ^1H NMR ($CDCl_3$, δ ppm): 7.26 (1H, s, 3-O**H**), 7.15 (1H, d, J = 8.2 Hz, 1-C**H**), 6.62 (1H, dd, J = 2.7 Hz and J = 8.2 Hz, 2-C**H**), 6.56 (1H, s, 4-C**H**), 3.74 (1H, d, J = 9.8 Hz, 17-C**H**), 3.41 (2H, t, J = 7.0 Hz, C**H**$_2$Br), 2.80 (2H, m, 6-C**H**$_2$), 2.60–1.02 (33H, #m, 4× C**H**, 14× C**H**$_2$, 1× O**H**), 0.80 and 0.76 (3H, 2s, 18-C**H**$_3$, 16α,β (1: 3.5)). ^{13}C NMR ($CDCl_3$, δ ppm): 153.6 (3-C), 138.5 (5-C), 133.0 (10-C), 126.7 (1-C), 115.5 (4-C), 112.9 (2-C), 82.8 (17-C, 16β), 48.8, 44.35, 44.21, 40.2, 38.6, 37.9, 34.3 (2× C), 33.1 (2× C), 32.6, 31.7, 30.1, 28.99 (2× C), 28.87, 28.41 (2× C), 27.63, 27.42, 26.52, 12.6 (18-C). MS (m/e), $C_{29}H_{45}BrO_2$: 504 (M^+). Exact mass: calculated for $C_{29}H_{45}BrO_2$ = 504.2603; found = 504.2592.

2.1.2.24. Spectral data for compound ***19d***. IR (NaCl, ν_{max}, cm^{-1}): 3650–3150 (O–H), 1619 and 1506 (C=C), 1260 and 1081 (C–O). ^1H NMR (Acetone-d_6, δ ppm): 7.92 (1H, s, 3-O**H**), 7.09 (1H, d, J = 8.5 Hz, 1-C**H**), 6.58 (1H, dd, J = 2.7 Hz and J = 8.2 Hz, 2-C**H**), 6.53 (1H, s, 4-C**H**), 3.71 (1H, m, 17-C**H**), 3.48 (2H, t, J = 6.6 Hz, C**H**$_2$Br), 2.98, (1H, s, 17-O**H**), 2.76 (2H, m, 6-C**H**$_2$), 2.23–1.03 (38H, #m, 4× C**H**, 17× C**H**$_2$), 0.81 and 0.79 (3H, 2s, 18-C**H**$_3$, 16α,β (1: 11.5)). ^{13}C NMR (Acetone-d_6, δ ppm): 155.3 (3-C), 137.7 (5-C),

131.5 (10-C), 126.4 (1-C), 115.3 (4-C), 112.9 (2-C), 81.7 (17-C, 16β), 49.0, 44.35, 44.31, 40.7, 38.9, 38.1, 34.2, 33.0, 32.8, 32.1, 30.19, 29.91, 29.83, 29.77, 29.70, 29.07, 28.2, 27.8, 26.6, 12.5 (18-C). MS (m/e), $C_{32}H_{51}BrO_2$: 546 (M^+). Exact mass: calculated for $C_{32}H_{51}BrO_2$ = 546.3072; found = 546.3079.

*2.1.2.25. General procedure for the synthesis of 16α,β-[n′-(2″pyridylethylamino)alkyl]-1,3,5(10)-estratrien-3,17β-diol compound (**20a–d**).* The bromide **19a–d** (0.46 mmol) was dissolved in anhydrous methanol (7 mL). 2-(2′-Aminoethyl)pyridine (0.56 mL, 4.6 mmol) was added to the bromide solution. The mixture was stirred and heated to reflux under nitrogen atmosphere for 4 days. The solvent was then evaporated and the residue was diluted in diethyl ether (30 mL). The organic phase was washed with water (6× 50 mL). Resulting aqueous layers were washed again with diethyl ether. Organic phases were combined, dried, filtered and evaporated to a yellow viscous oil. The crude amino intermediates were obtained quantitatively with excellent purity (>95%). No further purification was needed.

*2.1.2.26. Spectral data for compound **20a**.* IR (NaCl, ν_{max}, cm^{-1}): 3550–3050 (O–H and N–H), 1598 (C=C), 1255 and 1086 (C–O). ^{1}H NMR (Acetone-d_6, δ ppm): 8.48 (1H, dm, *J* = 5.1 Hz, *a*′-C**H**), 8.02 (1H, s, 3-O**H**), 7.66 (1H, td, *J* = 1.6 Hz and *J* = 7.5 Hz, *c*′-C**H**), 7.25 (1H, dd, *J* = 1.2 Hz and *J* = 7.8 Hz, *d*′-C**H**), 7.16 (1H, td, *J* = 1.2 Hz and *J* = 5.1 Hz, *b*′-C**H**), 7.08 (1H, d, *J* = 8.6 Hz, 1-C**H**), 6.58 (1H, dd, *J* = 2.3 Hz and *J* = 8.2 Hz, 2-C**H**), 6.52 (1H, d, *J* = 2.3 Hz, 4-C**H**), 3.72 (1H, d, *J* = 9.8 Hz, 17-C**H**, 16β), 3.26 (1H, d, *J* = 4.3 Hz, 17-C**H**, 16α), 2.94 (4H, m, NHC$\mathbf{H}_2$C$\mathbf{H}_2$pyridyl), 2.75 (2H, m, 6-C$\mathbf{H}_2$), 2.60 (2H, t, *J* = 6.6 Hz, $(CH_2)_5$C$\mathbf{H}_2$NH), 2.24–1.90 (3H, m, 16-C**H**, 17-O**H**, N**H**), 1.46–1.06 (21H, m, 3× C**H**, 9× C$\mathbf{H}_2$), 0.78 (3H, s, 18-C$\mathbf{H}_3$, 16β). ^{13}C NMR (Acetone-d_6, δ ppm): 161.0 (pyridyl-C), 155.4 (3-C), 149.3 (*a*′-C), 137.7 (5-C), 136.4 (*c*′-C), 131.4 (10-C), 126.4 (1-C), 123.3 (*d*′-C), 121.3 (*b*′-C), 115.4 (4-C), 113.0 (2-C), 81.6 (17-C, 16β), 49.8, 49.6, 49.0, 44.35, 44.30, 40.7, 38.9, 38.3, 38.1, 32.8, 32.1, 30.14, 30.08, 29.84, 29.75, 29.45, 27.51, 26.6, 12.5 (18-C). MS (m/e), $C_{31}H_{44}N_2O_2$: 476 (M^+), 398 ($M^+ - C_5H_4N$), 384 ($M^+ - C_6H_5N$). Exact mass: calculated for $C_{31}H_{44}N_2O_2$ = 476.3403; found = 476.3408.

*2.1.2.27. Spectral data for compound **20b**.* IR (NaCl, ν_{max}, cm^{-1}): 3550–3050 (O–H and N–H), 1609 (C=C), 1219 and 1061 (C–O). ^{1}H NMR (Acetone-d_6, δ ppm): 8.48 (1H, dm, *J* = 4.7 Hz, *a*′-C**H**), 7.65 (1H, tt, *J* = 2.0 Hz and *J* = 7.4 Hz, *c*′-C**H**), 7.25 (1H, dd, *J* = 1.2 Hz and *J* = 7.8 Hz, *d*′-C**H**), 7.16 (1H, dd, *J* = 1.2 Hz and *J* = 4.7 Hz, *b*′-C**H**), 7.09 (1H, d, *J* = 8.6 Hz, 1-C**H**), 6.58 (1H, dd, *J* = 2.7 Hz and *J* = 8.6 Hz, 2-C**H**), 6.52 (1H, d, *J* = 2.3 Hz, 4-C**H**), 3.72 (1H, d, *J* = 10.0 Hz, 17-C**H**, 16β), 3.26 (1H, d, *J* = 4.3 Hz, 17-C**H**, 16α), 3.60–2.81 (4H, m, NHC$\mathbf{H}_2$C$\mathbf{H}_2$pyridyl), 2.75 (2H, m, 6-C$\mathbf{H}_2$), 2.59 (2H, t, *J* = 6.6 Hz, $(CH_2)_7$C$\mathbf{H}_2$NH), 2.36–1.87 (3H, m, 16-C**H**, 17-O**H**, N**H**), 1.46–1.02 (25H, m, 3× C**H**, 11× C$\mathbf{H}_2$), 0.81 and 0.78 (3H, 2s, 18-C$\mathbf{H}_3$, 16α,β (1:15)). ^{13}C NMR (Acetone-d_6, δ ppm): 161.0 (pyridyl-C), 155.4 (3-C), 149.3 (*a*′-C), 137.7 (5-C), 136.4 (*c*′-C), 131.4 (10-C), 126.4 (1-C), 123.3 (*d*′-C), 121.3 (*b*′-C), 115.4 (4-C), 113.0 (2-C), 81.6 (17-C, 16β), 49.8, 49.6, 49.0, 44.35, 44.30, 40.7, 38.9, 38.3, 38.1, 32.8, 32.1, 30.14, 30.08, 29.84, 29.75, 29.45, 27.51, 26.6, 12.5 (18-C). MS (m/e), $C_{33}H_{48}N_2O_2$: 504 (M^+). Exact mass: calculated for $C_{33}H_{48}N_2O_2$ = 504.3716; found = 504.3726.

*2.1.2.28. Spectral data for compound **20c**.* The spectral data for this particular compound were reported elsewhere and are in agreement with the literature [15]. The proportion of the 16α,β epimers for derivative **20c** is 1:4.

*2.1.2.29. Spectral data for compound **20d**.* IR (NaCl, ν_{max}, cm^{-1}): 3550–3050 (O–H and N–H), 1598 (C=C), 1224 (C–O). ^{1}H NMR (Acetone-d_6, δ ppm): 8.48 (1H, dd, *J* = 2.0 Hz and *J* = 4.7 Hz, *a*′-C**H**), 7.65 (1H, tt, *J* = 2.0 Hz and *J* = 7.4 Hz, *c*′-C**H**), 7.25 (1H, dd, *J* = 1.2 Hz and *J* = 7.8 Hz, *d*′-C**H**), 7.16 (1H, tt, *J* = 1.6 Hz and *J* = 4.7 Hz, *b*′-C**H**), 7.09 (1H, d, *J* = 8.2 Hz, 1-C**H**), 6.96 (1H, s, 3-O**H**), 6.58 (1H, dd, *J* = 2.7 Hz and *J* = 8.2 Hz, 2-C**H**), 6.52 (1H, d, *J* = 2.7 Hz, 4-C**H**), 3.72 (1H, d, *J* = 9.4 Hz, 17-C**H**, 16β), 3.61–2.82 (6H, #m, NHC$\mathbf{H}_2$C$\mathbf{H}_2$pyridyl, 16-C**H**, 17-C**H** 16α), 2.76 (2H, m, 6-C$\mathbf{H}_2$), 2.59 (2H, t, *J* = 6.6 Hz, $(CH_2)_{13}$C$\mathbf{H}_2$NH), 2.24–1.06 (39H, #m, 3× C**H**, 17× C$\mathbf{H}_2$, 17-O**H**, N**H**), 0.81 and 0.79 (3H, 2s, 18-C$\mathbf{H}_3$, 16α,β (1:9)). ^{13}C NMR (Acetone-d_6, δ ppm): 161.1 (pyridyl-C), 155.3 (1-C), 149.3 (*a*′-C), 137.7 (5-C), 136.3 (*c*′-C), 131.4 (10-C), 126.4 (1-C), 123.3 (*d*′-C), 121.3 (*b*′-C), 115.3 (4-C), 112.9 (2-C), 81.5 (17-C, 16β), 65.5, 51.2, 49.7, 49.6, 49.0, 44.35, 44.28, 40.7, 39.6, 38.9, 38.4, 38.1, 34.3, 32.7, 32.1, 30.18, 30.06, 29.72, 29.44, 29.06, 28.98, 27.74, 27.44, 26.6, 15.0, 12.5 (18-C). MS (m/e), $C_{39}H_{60}N_2O_2$: 588 (M^+). Exact mass: calculated for $C_{39}H_{60}N_2O_2$ = 588.4655; found = 588.4658.

*2.1.2.30. General procedure for the synthesis of 16α,β-[n′-(2″pyridylethylamino)alkyl]-1,3,5(10)-estratien-3,17β-diol dichloroplatinate(II) (**7a–d**).* To a solution of 2-pyridylethylamino-17β-estradiol **20a–d** (0.37 mmol) in DMF (3 mL) at room temperature (21 °C), was added potassium tetrachloroplatinate(II) (0.16 g, 0.39 mmol) dissolved in mixture of DMF/H_2O (3.0 mL:1.5 mL). The starting pH value was 9. After 5 days stirring in darkness, pH reached 5. Then, 5 drops of DMSO were added to the resulting mixture to destroy the excess of K_2PtCl_4 and the solution was stirred for 3 h more. Next, a saturated KCl solution (4 mL) was added followed by solid KCl (1 g). The mixture was strongly stirred overnight in order to pulverize the lumps of precipitated Pt(II) complex. The solid suspension was then filtered, washed with water and dried in a desiccator for 1 day. The residue was adsorbed on silica gel and purified by flash chromatography with hexanes and acetone (1:1) as the eluant to give 17β-estradiol-platinum(II) **7** (**a–d**) as yellow crystals with 58% optimized yield.

*2.1.2.31. Spectral data for compound **7a**.* IR (NaCl, ν_{max}, cm^{-1}): 3600–3100 (O–H and N–H), 1614 (C=C), 1240 and 1076 (C–O). ^{1}H NMR (Acetone-d_6, δ ppm): 9.15 (1H, dd, *J* = 1.2 Hz and *J* = 6.3 Hz, *a*′-C**H**), 8.04 (1H, td, *J* = 1.6 Hz and *J* = 7.8 Hz, *c*′-C**H**), 7.90 (1H, s, 3-O**H**), 7.54 (1H, d, *J* = 7.8 Hz, *d*′-C**H**), 7.43 (1H, td, *J* = 1.6 Hz and *J* = 7.4 Hz, *b*′-C**H**), 7.08 (1H, d, *J* = 8.6 Hz, 1-C**H**), 6.58 (1H, dd, *J* = 2.7 Hz and *J* = 8.2 Hz, 2-C**H**), 6.53 (1H, d, *J* = 2.7 Hz, 4-C**H**), 6.05 (1H, br s, N**H**), 3.78–3.68 (1H, m, 17-C**H**, 16β), 3.50–2.81 (7H, #m, C$\mathbf{H}_2$NHC$\mathbf{H}_2$C$\mathbf{H}_2$ pyridyl, and 17-C**H**, 16α), 2.78–2.74 (2H, m, 6-C$\mathbf{H}_2$), 2.40–0.84 (23H, #m, 4× C**H**, 9× C$\mathbf{H}_2$ and 17-O**H**), 0.80 and 0.77 (3H, 2s, 18-C$\mathbf{H}_3$, 16α,β (1: 22)). ^{13}C NMR (Acetone-d_6, δ ppm): 160.0 (pyridyl-C), 155.2 (*a*′-C), 153.7 (3-C), 139.4 (*c*′-C), 137.8 (5-C), 131.5 (10-C), 127.8 (1-C), 126.4 (*d*′-C), 124.9 (*b*′-C), 123.9, 115.3 (4-C), 112.9 (2-C), 81.7 (17-C, 16β), 56.6, 49.0, 45.9, 44.33, 44.30, 40.6, 39.9, 38.9, 38.1, 32.7, 31.9, 29.7, 28.7, 27.93, 27.75, 26.68, 26.61, 12.5 (18-C). MS (m/e), $C_{31}H_{44}Cl_2N_2O_5Pt$: 742.2 $(M+H)^+$. Exact mass: calculated

for $C_{31}H_{44}Cl_2N_2O_5Pt$ = 742.25003 $(M+H)^+$; found = 742.24692 and calculated for $C_{31}H_{44}Cl_2N_2O_5Pt$ = 764.23198 $(M+Na)^+$; found = 764.23100.

2.1.2.32. *Spectral data for compound* **7b**. IR (NaCl, ν_{max}, cm^{-1}): 3600–3100 (O–H and N–H), 1624 (C=C), 1250 and 1076 (C–O). ^1H NMR (Acetone-d_6, δ ppm): 9.15 (1H, dd, J = 1.2 Hz and J = 5.9 Hz, a'-C**H**), 8.02 (1H, td, J = 1.6 Hz and J = 7.8 Hz, c'-C**H**), 7.90 (1H, br s, 3-O**H**), 7.53 (1H, d, J = 7.8 Hz, d'-C**H**), 7.43 (1H, td, J = 1.6 Hz and J = 7.4 Hz, b'-C**H**), 7.08 (1H, d, J = 8.6 Hz, 1-C**H**), 6.58 (1H, dd, J = 2.7 Hz and J = 8.2 Hz, 2-C**H**), 6.53 (1H, d, J = 2.7 Hz, 4-C**H**), 6.11–6.06 (1H, br s, N**H**), 3.83–3.70 (1H, m, 17-C**H**, 16β), 3.45–2.81 (7H, #m, $CH_2NHCH_2CH_2$ pyridyl and 17-C**H**, 16α), 2.75 (2H, m, 6-CH_2), 2.40–0.97 (27H, #m, 4× C**H**, 11× CH_2 and 17-O**H**), 0.80 and 0.77 (3H, 2s, 18-CH_3, 16α,β (1: 14)). ^{13}C NMR (Acetone-d_6, δ ppm): 160.0 (pyridyl-C), 155.2 (a'-C), 153.7 (3-C), 139.5 (c'-C), 137.8 (5-C), 131.5 (10-C), 126.4 (1-C), 124.9 (d'-C), 123.9 (b'-C), 115.3 (4-C), 112.9 (2-C), 81.7 (17-C, 16β), 56.6, 54.4, 48.9, 45.9, 44.3, 39.9, 38.9, 38.1, 32.8, 32.1, 29.90, 29.76, 29.51, 29.37, 29.00, 27.97, 27.77, 26.71, 26.63, 12.6 (18-C). MS (m/e), $C_{33}H_{48}Cl_2N_2O_5Pt$: 770.3 $(M+H)^+$. Exact mass: calculated for $C_{33}H_{48}Cl_2N_2O_5Pt$ = 770.28133 $(M+H)^+$; found = 770.28107 and calculated for $C_{33}H_{48}Cl_2N_2O_5Pt$ = 792.26328 $(M+Na)^+$; found = 792.26258.

2.1.2.33. *Spectral data for compound* **7c**. The spectral data for this particular compound were reported elsewhere and are in agreement with the literature [15]. The proportion of the 16α,β epimers for derivative **7c** is 1:9. MS (m/e), $C_{36}H_{54}Cl_2N_2O_5Pt$: 812.3 $(M+H)^+$. Exact mass: calculated for $C_{36}H_{54}Cl_2N_2O_5Pt$ = 812.32828 $(M+H)^+$; found = 812.33215 and calculated for $C_{36}H_{54}Cl_2N_2O_5Pt$ = 834.31023 $(M+Na)^+$; found = 834.30901.

2.1.2.34. *Spectral data for compound* **7d**. IR (NaCl, ν_{max}, cm^{-1}): 3600–3100 (O–H and N–H), 1600 (C=C), 1212 and 1076 (C–O). ^1H NMR (Acetone-d_6, δ ppm): 9.14 (1H, dd, J = 0.8 Hz and J = 5.9 Hz, a'-C**H**), 8.03 (1H, td, J = 1.6 Hz and J = 7.8 Hz, c'-C**H**), 7.91 (1H, s, 3-O**H**), 7.54 (1H, d, J = 7.8 Hz, d'-C**H**), 7.42 (1H, td, J = 1.6 Hz and J = 5.9 Hz, b'-C**H**), 7.09 (1H, d, J = 8.6 Hz, 1-C**H**), 6.59 (1H, dd, J = 2.7 Hz and J = 8.2 Hz, 2-C**H**), 6.54 (1H, d, J = 2.7 Hz, 4-C**H**), 6.04 (1H, br s, N**H**), 3.84–2.80 (8H, #m, $CH_2NHCH_2CH_2$pyridyl and 17-C**H**, 16α and 16β), 2.76 (2H, m, 6-CH_2), 2.31–1.08 (39H, #m, 4× C**H**, 17× CH_2 and 17-O**H**), 0.80 and 0.77 (3H, 2s, 18-CH_3, 16α,β (1: 15)). ^{13}C NMR (Acetone-d_6, δ ppm): 159.9 (pyridyl-C), 155.3 (a'-C), 153.7 (3-C), 139.4 (c'-C), 137.8 (5-C), 131.5 (10-C), 126.4 (1-C), 124.9 (d'-C), 123.8 (b'-C), 115.3 (4-C), 112.9 (2-C), 81.7 (17-C, 16β), 56.6, 49.0, 45.9, 44.34, 44.30, 40.7, 39.8, 38.9, 38.1, 32.8, 32.1, 30.1, 29.85, 29.76 (4× C), 29.34, 29.02, 27.96, 27.75, 26.67, 26.62, 12.5 (18-C). MS (m/e), $C_{39}H_{60}Cl_2N_2O_5Pt$: 854.4 $(M+H)^+$. Exact mass: calculated for $C_{39}H_{60}Cl_2N_2O_5Pt$ = 854.37524 $(M+H)^+$; found = 854.37162 and calculated for $C_{39}H_{60}Cl_2N_2O_5Pt$ = 876.35718 $(M+Na)^+$; found = 876.35655.

2.2. *Biology*

2.2.1. *In vitro cytotoxic activity*

The cytotoxicity of the 17β-estradiol-platinum(II) complexes (**7a–d**) was evaluated on MCF-7 (ER^+), MDA-MD-231 (ER^-), MDA-MB-468 (ER^-) and MDA-MB-436 (ER^-) breast cancer cell lines. MTT (3-(4,5-dimethylthiazol-2-yl)-phenyl-tetrazolium bromide) assay, a standard colorimetric test, was used for measuring cellular proliferation [19]. Tumor cell lines were added into 96-well tissue culture plates in culture medium and incubated at 37 °C in a 5% CO_2 atmosphere. Dilution were done using cremophore:ethanol (1:1) solution. Cells were incubated with or without drugs for 24 h, 48 h and 72 h. Culture plates were processed using MTT for 3.5 h afterwards SDS solubilisation solution (HCl 0.010 M, sodium dodecyl solution 10%) was added. The absorbance was read using a scanning multiwell spectrophotometer (FLUOStar OPTIMA) at 565 nm. All measurements were carried in triplicates. The results were compared with those of a control reference plate fixed on the treatment day, and the growth inhibition percentage was calculated for each drug contact period.

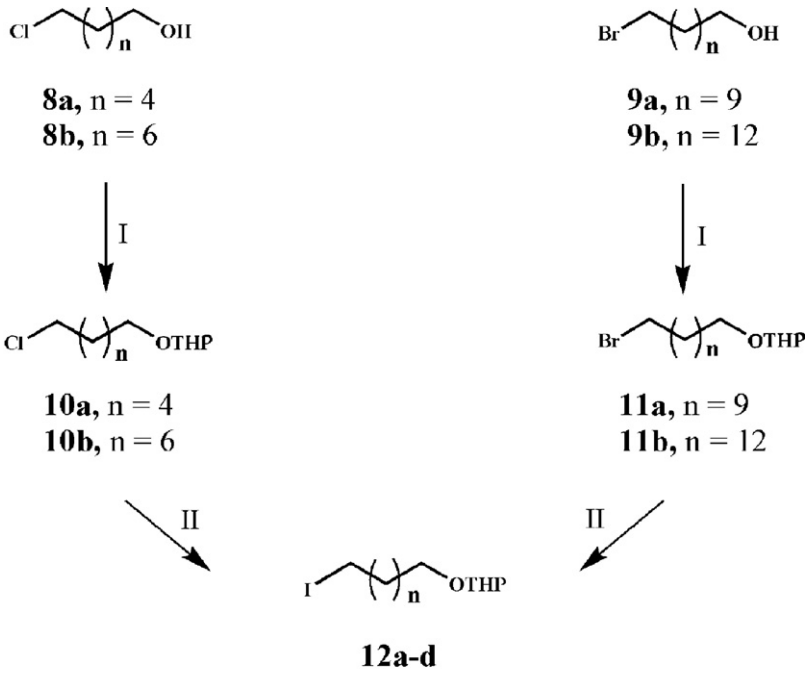
2.2.2. *Estrogen receptor binding affinity*

The estrogen receptor alpha and beta (ERα and ERβ) affinity assay was performed using recombinant hERα and hERβ (Calbiochem/EMD BioSciences, Darmstadt, Germany) and the HitHunter™ Enzyme Fragment Complementary (EFC) Estrogen Receptor Assay kit (Discoverex Corporation, Fremont, CA) according to manufacturer's protocol [20]. HitHunter™ EFC technology is based on a genetically engineered β-galactosidase enzyme that consists of two fragments termed Enzyme Acceptor (EA) and Enzyme Donor (ED). Briefly, different concentration of estrogen analogs (**7a–c**) were added to wells containing ES (Estrogen Steroid) Receptor + ED in a 96-well black plate. Incubation provided competition for the estrogen receptor binding against labelled Enzyme Donor–Estrogen Steroid hormone conjugate (ED–ES conjugate), a small peptide fragment of β-galactosidase (β-gal). Then, EA, an inactive β-gal protein fragment, and a fluorescent substrate were added to each well. Unbound ED–ES bind to EA to form an active enzyme, which subsequently hydrolyse the fluorescent substrate for EFC detection by a microplate reader (FLUOStar OPTIMA). The excitation wave is 530 nm and luminosity is detected at 620 nm. The amount of free ED conjugate in the assay is proportional to the concentration of estrogen analogs bound to the estrogen receptor [20]. A standard curve of 17β-estradiol was run in parallel. All assays were done in triplicates.

3. Results and discussion

3.1. *Synthesis of 17β-estradiol-platinum(II) complexes (7a–d)*

As shown in Scheme 1, the ω-chloro-alcohols (**8a**,**b**) and the ω-bromo-alcohols (**9a**,**b**) were first transformed into the corresponded THP protected derivatives **10** and **11** (**a–b**) under standard reaction conditions. These derivatives were converted to their respective iodo analogs **12a–d**. Thus, the alkyl chains were treated with 3,4-dihydro-2*H*-pyran and pyridinium *p*-toluenesulfonate in dichloromethane. After an appropriate work-up, the crude THP-products were treated with sodium iodide in acetone at reflux for 3 days to give the

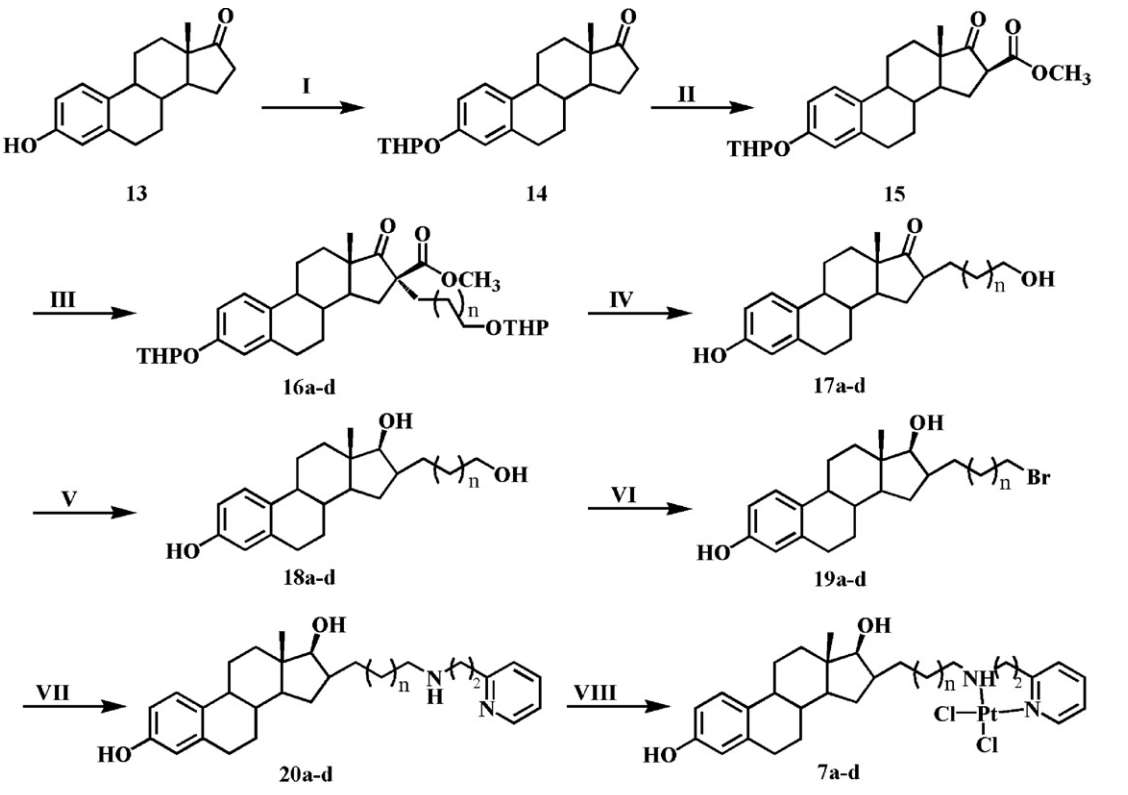


Scheme 1 – Synthesis of protected iodo alkyl side chains.

desired protected ω-iodo-THP chains **12a–d** with an average overall yield of 98%.

As shown in Scheme 2, four 17β-estradiol-platinum(II) complexes (**7a–d**) bearing a tether chain of different length were obtained using a straightforward reaction sequence. Initially, estrone (**13**) was protected as a tetrahydropyrannyl (THP) ether. Thus, estrone was treated with 3,4-dihydro-2*H*-pyran and pyridinium *p*-toluenesulfonate in dichloromethane. The yield of the protection reaction is 100%. The THP is the right choice of protecting group as it was also used for the protection of the ω-iodo-THP chains. It was rationalized that, at a later step, it will be possible to deprotect them both at the same time. Derivative **14** was further transformed into the β-ketoester **15** upon treatment with dimethylcarbonate in the presence of KH in dry tetrahydrofuran. Compound **15** was obtained with 90% yield. The pure activated estrone was then alkylated using phase transfer catalysis (PTC) methodology [21]. Accordingly, derivative **15** was treated with protected iodo-chain (**12a–d**) and triethylbenzylammonium chloride in dichloromethane in the presence of a 10% aqueous sodium hydroxide solution, to give the desired product **16a–d** in 92% optimised yield. The next chemical step led to the concurrent transformations of three functional groups on the molecule. Hence, decarboalkoxylation and two THP deprotections were achieved upon treatment with lithium chloride in DMF and water at reflux for 20 h. We were aware that the cleavage of the tetrahydropyrannyl ether under these reaction conditions is a known process [22]. The diol derivatives **17a–d** were obtained with an overall yield of 71%. The pure compounds were then reduced in the presence of lithium borohydride in dichloromethane and tetrahydrofuran to provide quantitatively the appropriate triols **18a–d** as a single. It is worth mentioning that the hydride reduction of either pure 16α-substituted or 16β-substituted estrone derivatives gave stereospecifically the corresponding 17β-estradiol derivatives, without any trace of the formation of the 17α-estradiol derivatives [23]. This is also what was observed in our experiments as only the 17β-estradiol derivatives (**18a–d**) were obtained. The intermediates were subjected to selective bromation of the primary alcohol. Compounds **18a–d** were treated with carbon tetrabromide and triphenylphosphine in dichloromethane to give the hydroxy-bromides **19a–d** with 62% optimised yield. S_N2 nucleophilic substitution of the primary bromide upon treatment with an excess 2-aminoethylpyridine in methanol gave the desired amino-pyridine products **20a–d** in 97% yield. The amino-pyridines were then treated with potassium tetrachloroplatinate in a mixture of DMF and water to give the corresponding 17β-estradiol-linked Pt(II) complexes **7a–d** with 58%. The estradiol-platinum(II) complexes were obtained in eight chemical steps with an optimized overall yield of 21%. This new synthetic methodology represents a great improvement of the initial synthesis of **7c** done in nine chemical steps and 11% overall yield. All the new compounds were fully characterized by their respective IR, ^{1}H NMR, ^{13}C NMR and mass spectra.

The final products and the intermediates are a mixture of only two stereoisomers: 16α and 16β. However, the final E_2-Pt(II) hybrid molecules were greatly enrich by flash chromatography to the major 16β stereoisomer, the main orientation of the tether chain. It was established that the proportion



I. DHP, PPTs, CH_2Cl_2, 21°C, 20h.
II. Dimethylcarbonate, KH, THF anhydrous, N_2, 2.5h.
III. Iodo alkyl chain, triethylbenzylammonium chloride, CH_2Cl_2, NaOH 10% aq., reflux, darkness, 24h.
IV. LiCl, DMF, H_2O, reflux, 20h.
V. $LiBH_4$, Et_2O, CH_2Cl_2, THF anhydrous , 0°C, 3h, 21°C, 3 days, N_2.
VI. CBr_4, PPh_3, CH_2Cl_2, N_2, 21°C, 5h.
VII. Pyridine-2-$CH_2CH_2NH_2$, MeOH anhydrous, reflux N_2, 21°C, 4 days.
VIII. K_2PtCl_4, DMF:H_2O (4:1), 21°C, darkness, 5 days.

Scheme 2 – Synthesis of estradiol-platinum(II) complexes 7a–d.

of the 16α,β-side chain varied from 1:9 to 1:22. This is a level of purity adequate to perform the biological assays and to get meaningful results on these experimental anticancer agents. It is possible to easily establish the 16-C side chain orientation (α or β) using the 17-C and 18-C chemical shifts reported in the literature [23]. Substituted 17β-estradiol at the 16α position gives a 17-C and 18-C signals at δ 87.11 ppm and 12.20 ppm, respectively, whereas those same carbons appear at δ 81.91 ppm and 12.93 ppm when it is 16β substituted. Moreover, in ^{1}H NMR the 18-CH_3 singlet appear at δ 0.80 ppm for the 16α isomer and δ 0.77 ppm for the 16β isomer. It was then possible to establish the ratio of the two stereoisomers with two of the most distinctive NMR features: 16α, ^{13}C NMR for 17-C; δ 87.11 ppm, ^{1}H NMR for 18-CH_3; δ 0.80 ppm and 16β, ^{13}C NMR for 17-C; δ 81.91 ppm, ^{1}H NMR for 18-CH_3; δ 0.77 ppm.

According to the spectral data, it was establish that the alkylation step (**15→16**) was done with the side chain added mainly to the less hindered α face of the molecule (16α,β; 4:1–12:1). This was shown by the presence of two singlets for the 18-CH_3 at δ 0.92 ppm and 0.88 ppm. The subsequent decarboalkoxylation reaction gave mainly the 16-β stereoisomers as the final product since the protonation of the enolate intermediate occurs at the α side of the steroid. For all molecules, the stereochemistry of the 16-α or 16-β side chain orientation was confirmed by comparison with ^{13}C NMR spectral data of known 16-α and 16-β substituted 17β-estradiol derivatives [23].

3.2. *In vitro cytotoxic activity*

The cytotoxicity of the complexes was evaluated on several tumor cell lines using the MTT colorimetric assay [19]. The cytotoxicity of the compounds was tested along with cisplatin as the control on both estrogen-receptor positive (ER^+, MCF-7) and estrogen-receptor negative (ER^-, MDA-MB-231, MDA-MB-468 and MDA-MB-436) human mammary carcinomas [24].

As shown by the MTT assays, the 17β-estradiol-platinum(II) hybrid molecules **7a–d** were more cytotoxic than cisplatin (4–9 times), on MCF-7 and MDA-MB-231 breast cancer cell lines (Table 1). However, for the MDA-MB-468 and MDA-MB-436 breast cancer cell lines the hybrid molecules **7a–d** are mostly less cytotoxic than cisplatin itself. At best, hybrid **7c**

Table 1 – Inhibitory concentrations of cisplatin and the hybrids (7a–d) on both ER$^+$ and ER$^-$ breast cancer cell lines

Compounds	MCF-7 (ER$^+$) IC_{50} (μM)[a]	MDA-MB-231 (ER$^-$) IC_{50} (μM)	MDA-MB-468 (ER$^-$) IC_{50} (μM)	MDA-MB-436 (ER$^-$) IC_{50} (μM)	Chain length ($n+2$)
Cisplatin (**1**)	18.97 ± 0.43	17.33 ± 2.28	0.99 ± 0.06	3.28 ± 0.38	–
7a	4.22 ± 0.18	3.59 ± 0.17	1.81 ± 0.05	3.66 ± 0.18	6
7b	2.18 ± 0.11	2.16 ± 0.16	1.48 ± 0.08	1.85 ± 0.16	8
7c	2.29 ± 0.13	2.06 ± 0.06	1.08 ± 0.01	1.68 ± 0.03	11
7d	3.81 ± 0.05	1.88 ± 0.04	1.02 ± 0.06	2.21 ± 0.13	14

[a] Inhibitory concentration (IC_{50}, μM) as obtained by the MTT assay. Experiments were performed in duplicates and the results represent the mean ± S.E.M. of three independent experiments. The cells were incubated for a period of 72 h.

is about twice as cytotoxic as cisplatin on the MDA-MB-436 cells. Generally, the hybrid **7c** presents a useful cytotoxic activity throughout the different cancer cells tested with potential for *in vivo* evaluation. This is in agreement with our previous results [15]. Overall, the addition of a toxic moiety at the 16β position of the steroid nucleus does not interfere with the cytocidal activity of the molecule.

This study was undertaken to verify the influence of the length of the alkyl chain on the biological activity. For these particular E_2-Pt(II) hybrid molecules, the length of the alkyl chain does not influence very much the activity. However, we can observed that the Pt(II) complexes bearing the shortness chain **7a** is the least effective towards ER$^+$ as well as ER$^-$ breast cancer cell lines. This observation can be rationalized by the hypothesis that a short linkage can cause additional steric hindrance around the 17-hydroxyl group of the steroid, which is necessary for binding to the estrogen receptor [6]. Unfortunately, the *in vitro* assays show no clear specific action on estrogen-dependent cells as compared to estrogen-independent cells. Interestingly, the *in vivo* studies show that this class of E_2-Pt(II) hybrid molecules are specific towards homone-dependent breast and ovarian cancers. All the *in vivo* results will be soon reported in a subsequent paper.

3.3. *Estrogen receptor binding affinity*

The estrogen receptor alpha and beta (ERα and ERβ) affinity assay was performed using the HitHunter™ EFC Estrogen Fluorescence assay kit (Discoverx, Fremont, CA) according to manufacturer's instructions [20]. The results are presented in Fig. 2. The binding affinities were measured for only for hybrids **7a**, **7b** and **7c**. As expected, the estrogen receptor binding studies showed strong affinities for 17β-estradiol with EC_{50} of 0.40 nM and 0.93 nM, respectively, for the ERα and the ERβ. Interestingly, those three E_2-Pt(II) hybrids demonstrated similar binding affinity for the ERα and ERβ, close to that of 17β-estradiol. They have EC_{50} varying from 1.52 nM to 2.75 nM for ERα and from 1.49 nM to 1.83 nM for ERβ. Hence, those results demonstrate that the length of the tether chain does not interfere that much with the estrogen receptor binding affinity. It is noteworthy that cisplatin has no affinity for the ER [15]. Consequently, this type of E_2-Pt(II) hybrids have the potential to target the ER in an *in vivo* model and show selective anticancer activity as well as reduce systemic toxicities. The strong cytocidal activity of E_2-Pt(II) hybrids **7a**, **7b** and **7c** may be, at least partly, attributed to their interactions with the ERα or ERβ.

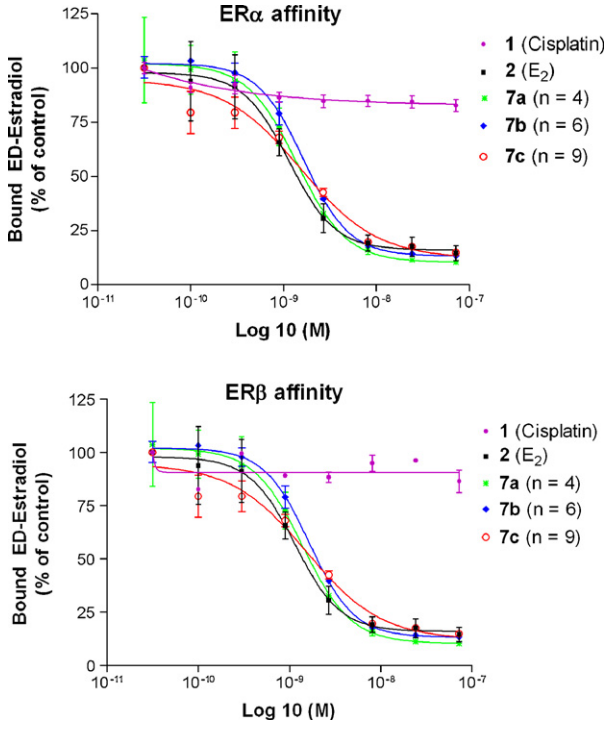


Fig. 2 – Estrogen receptor binding affinity (ERα and ERβ). ED-estradiol: enzyme donor-estradiol; E_2: 17β-estradiol. Estradiol and 17β-estradiol-platinum(II) complexes (7a–7c) present similar affinity for ERs. Cisplatin presents no affinity for the ERs.

4. Conclusion

In summary, this manuscript presents a new and improved methodology for the synthesis of E_2-Pt(II) hybrid molecules. The novel compounds are made at position 16 of the steroid nucleus. There are 6, 8, 11 or 14 carbon atoms separating the cytotoxic Pt(II) moiety from the steroid nucleus. The E_2-Pt(II) hybrids were made from estrone in only 8 chemical steps with an overall yield of 21%. This represents a huge improvement from an earlier synthesis of hybrid **7c** which was made in 9 steps with only 10% overall yield. The THP protective group at the beginning of the synthesis proved to be a judicious choice as it allowed in a single synthetic step three chemical transformations (**16a–d** to **17a–d**); decarboalkoxylation and bis-deprotection. The previous synthesis used a benzyl protective group which required an additional synthetic step [15].

Moreover, the new synthetic methodology allowed the enrichment of the final hybrids to the 16β stereoisomer. Nevertheless, work is in progress in our laboratory to isolate and test the pure 16α and 16β isomers for derivative **7c**, the best E_2-Pt(II) hybrid. This work will be published in a complementary study.

As anticipated, all hybrids tested, **7a**, **7b** and **7c**, presented high affinity for the ERα, basically the same as 17β-estradiol. Interestingly, those three compounds also showed great affinity for the ERβ. *In vitro* cytotoxicity assay revealed that the E_2-Pt(II) hybrids showed potent cytocidal activity on ER^+ (MCF-7) and ER^- (MDA-MB-231) breast cancer cell lines. They are from four to nine times more cytotoxic than cisplatin itself. The length of the alkyl chain separating the estradiol from the cytotoxic part does not seem to play a crucial role for *in vitro* biological activity. Based on the cytotoxic assay as well as on the binding affinity assay, we can anticipate that the hybrids bearing an octyl and an undecyl tether chain, compounds **7b** and **7c**, are the best candidates to be further studied *in vivo* for site specific treatment of hormone-dependent breast cancers. Further investigation will be necessary to assess the full biological potential of these new E_2-Pt(II) hybrid molecules. Several *in vivo* assays have been carried out with hybrid **7c** on nude mouse xenografted model. Hybrid **7c** show strong and selective anticancer activity on both, hormone-dependent breast and ovarian cancers and the results will be reported elsewhere.

Acknowledgments

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