

Article

Synthesis of 5 α -Androstane-17-spiro- δ -lactones with a 3-Keto, 3-Hydroxy, 3-Spirocarbamate or 3-Spiromorpholinone as Inhibitors of 17 β -Hydroxysteroid Dehydrogenases

Guy Bertrand Djigoué [†], Béatrice Tchédam Ngatcha [†], Jenny Roy and Donald Poirier ^{*}

Laboratory of Medicinal Chemistry, CHU de Québec (CHUL)—Research Center and Faculty of Medicine, Laval University, 2705 Laurier Boulevard, Québec (Québec), G1V 4G2, Canada

[†] These authors contributed equally to this work.

^{*} Author to whom correspondence should be addressed; E-Mail: donald.poirier@crchul.ulaval.ca; Tel.: +1-418-654-2296; Fax: +1-418-654-2761.

Received: 3 December 2012; in revised form: 24 December 2012 / Accepted: 5 January 2013 /

Published: 11 January 2013

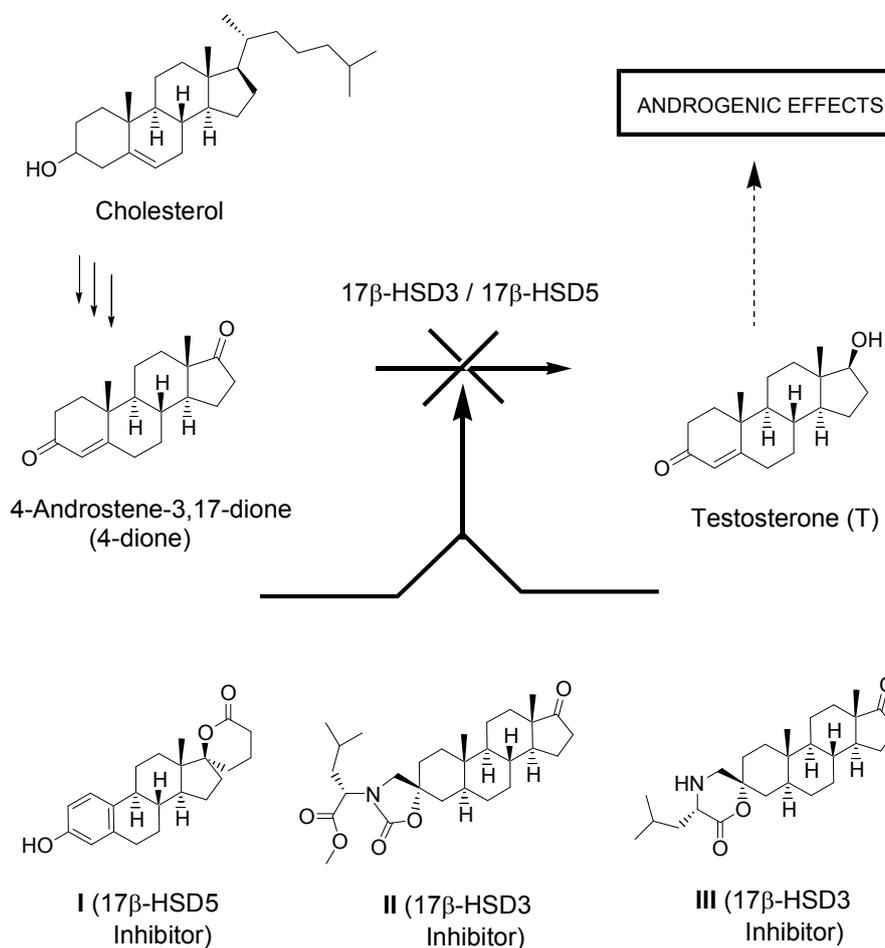
Abstract: We synthesized two series of androstane derivatives as inhibitors of type 3 and type 5 17 β -hydroxysteroid dehydrogenases (17 β -HSDs). In the first series, four monospiro derivatives at position C17 were prepared from androsterone (ADT) or *epi*-ADT. After the protection of the alcohol at C3, the C17-ketone was alkylated with the lithium acetylide of tetrahydro-2-(but-3-ynyl)-2-H-pyran, the triple bond was hydrogenated, the protecting groups hydrolysed and the alcohols oxidized to give the corresponding 3-keto-17-spiro-lactone derivative. The other three compounds were generated from this keto-lactone by reducing the ketone at C3, or by introducing one or two methyl groups. In the second series, two dispiro derivatives at C3 and C17 were prepared from *epi*-ADT. After introducing a spiro- δ -lactone at C17 and an oxirane at C3, an aminolysis of the oxirane with L-isoleucine methyl ester provided an amino alcohol, which was treated with triphosgene or sodium methylate to afford a carbamate- or a morpholinone-androstane derivative, respectively. These steroid derivatives inhibited 17 β -HSD3 (14–88% at 1 μ M; 46–94% at 10 μ M) and 17 β -HSD5 (54–73% at 0.3 μ M; 91–92% at 3 μ M). They did not produce any androgenic activity and did not bind steroid (androgen, estrogen, glucocorticoid and progestin) receptors, suggesting a good profile for prostate cancer therapy.

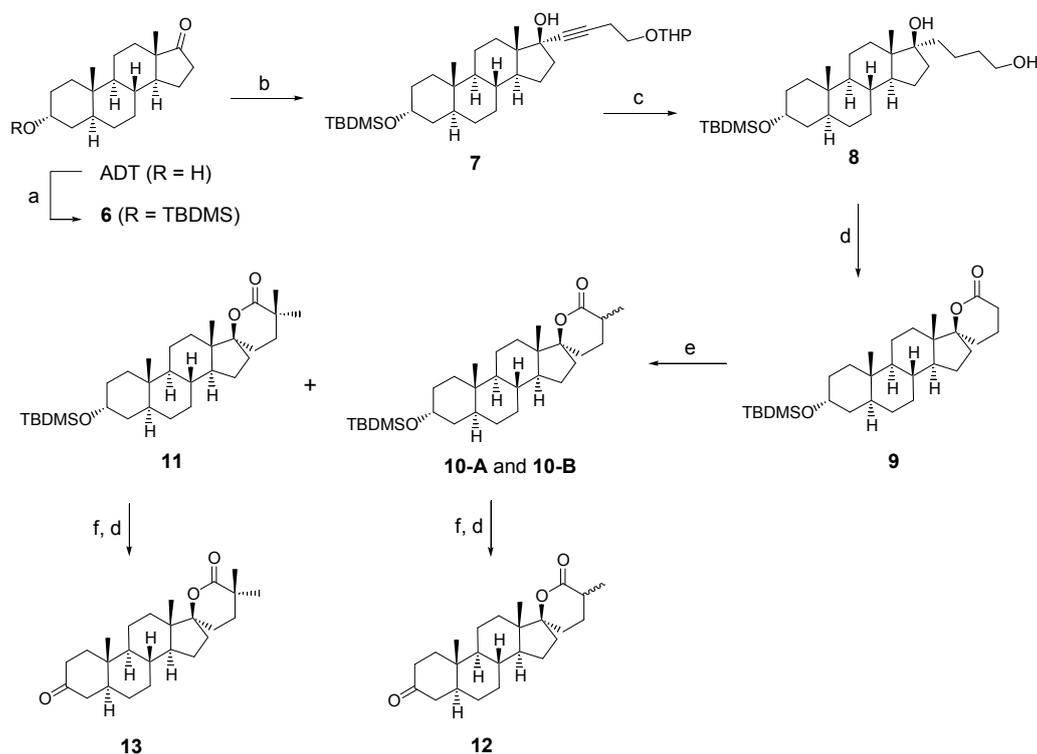
Keywords: synthesis; steroids; spiro lactone; enzyme inhibitor; 17β -hydroxysteroid dehydrogenase

1. Introduction

Prostate cancer is an androgen-dependent disease that is well known for its high sensitivity to androgen deprivation. In fact, for over 50 years, the exclusive treatment of advanced metastatic prostate cancer was androgen deprivation achieved through castration, as it was believed that 95% of androgens were of testicular origin [1,2]. However, it is now well known that peripheral tissues represent another important source of androgens [3,4]. In fact, the prostatic tissues efficiently convert the hormone precursor dehydroepiandrosterone (DHEA) into the active androgens testosterone (T) and dihydrotestosterone (DHT) [5–10]. Both type 3 and type 5 17β -hydroxysteroid dehydrogenases (17β -HSD3 and 17β -HSD5, respectively) catalyse the reduction of 4-androstene-3,17-dione (4-dione) to testosterone (T) (Figure 1). However, whereas type 3 is located mainly in the testis, type 5 is expressed in the peripheral tissues [10]. In order to control the peripheral formation of active androgens, which could enhance the efficacy of endocrine therapy (such as the use of a pure antiandrogen with an LHRH agonist), we focused on the development of inhibitors of 17β -HSD3 and 17β -HSD5.

Figure 1. Role of 17β -HSD3 and 17β -HSD5 in the synthesis of the androgenic hormone testosterone.

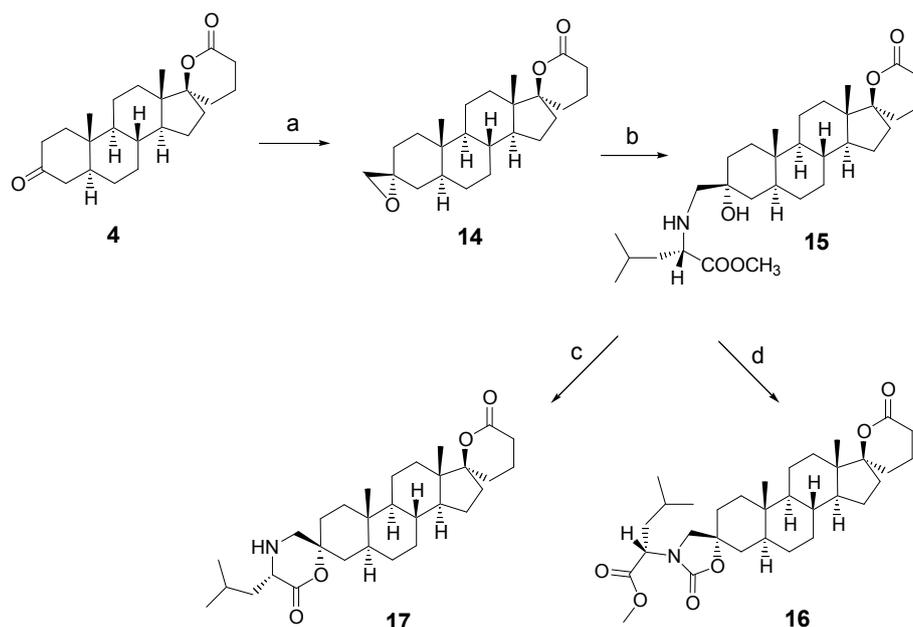


Scheme 2. Synthesis of methylated spiro- δ -lactones **12** and **13**.

Reagents and conditions: (a) TBDMS-Cl, imidazole, DMF, rt; (b) $\text{HC}\equiv\text{C}(\text{CH}_2)_2\text{OTHP}$, *n*-BuLi, THF -78°C ; (c) *i.* H_2 , 10% Pd/C, 5% Pd/CaCO₃, EtOAc, rt, *ii.* *p*-TSA, MeOH, rt; (d) Jones' reagent (2.7 M), acetone, 0°C ; (e) LDA, CH₃I, THF, 0°C ; (f) TBAF, THF, reflux.

2.3. Synthesis of Spiro-Carbamate **16** and Spiro-Morpholinone **17** (Scheme 3)

Following a slightly modified method reported previously in our laboratory [21], the 3-oxo-spiro- δ -lactone **4** was reacted with trimethylsulfoxonium iodide (four equivalents rather than two) to yield oxirane **14**. The 3β -CH₂ orientation of **14** was confirmed by a correlation with methyl 19 in NOESY spectrum. Compound **14** was then submitted to an aminolysis with L-leucine methyl ester to generate the amino alcohol **15**. The amino group of L-leucine methyl ester was previously generated from the commercially available chlorhydrate [21]. The spiro-carbamate **16** was obtained from the reaction of **15** with triphosgene. Because the reaction was very slow with only 0.5 equivalent of triphosgene, the quantity reported to produce a similar carbamate [14], we used one equivalent to complete the reaction generating **16**. The formation of a carbamate group was confirmed by a characteristic signal at 157.64 ppm in ¹³C-NMR. The spiro-morpholinone **17** was obtained from the lactonization of the amino alcohol **15**. During this step, unknown products are formed; this explains the poor yield of this reaction. This is probably due to the polymerization of the starting amino alcohol or the partial aminolysis of the spiro- δ -lactone. HPLC chromatogram showed four resolved peaks, integrating for 39% (expected compound **17**), 18% (the starting amino alcohol **15**) and two other peaks representing unknown products. The formation of the spiro-morpholinone moiety was confirmed by a characteristic signal at 171.99 ppm in ¹³C-NMR.

Scheme 3. Synthesis of dispirolactone **16** (carbamate) and spiromorpholinone **17**.

Reagents and conditions: (a) $(\text{CH}_3)_3\text{SOI}$, NaH, DMSO/THF, rt; (b) L-Leucine, MeOH, 90 °C; (c) CH_3ONa , THF, rt; (d) $(\text{Cl}_3\text{CO})_2\text{CO}$, DIPEA, DCM, 0 °C to rt.

2.4. Biological Evaluation of Monospiro Derivatives **4**, **5**, **12** and **13**

Compounds **4**, **5**, **12** and **13** were evaluated for their ability to inhibit the 17 β -HSD5 activity found in transfected HEK-293 cells by measuring the amount of labelled testosterone (T) formed from labelled natural substrate 4-dione (Table 1). All compounds inhibited the 17 β -HSD5 (91–92% at 3 μM), but the C19-steroid (androstane) backbone seem to be less efficient than the C18-steroid (estrane) backbone. In fact, the androstane spiro- δ -lactone **4** is a less potent inhibitor than the corresponding estrane compound **I** (64 and 92% of inhibition at 0.3 μM , respectively). The monomethylation of lactone **4** (compound **12**) brought a slight increase in the inhibitory activity (73% at 0.3 μM), whereas the dimethylation (compound **13**) brought a small decrease of inhibition (54% at 0.3 μM). The spiro- δ -lactone bearing a hydroxyl at position 3, compound **5**, gave a 56% inhibition of 17 β -HSD5 at 0.3 μM , a value lower than that of the corresponding keto compound **4** (64% of inhibition). Compound **4**, **5**, **12** and **13** were also tested as inhibitor of 17 β -HSD3 by measuring the transformation of labelled 4-dione to labelled T by a microsomal preparation of rat testis. No significant inhibition was observed at concentrations of 0.1 and 1 μM for all compounds, and only small inhibitory activities (46–58%) were obtained at the higher concentration of 10 μM . This is fully in accord with our first structure-activity relationship (SAR) results that identified the importance of a hydrophobic group at position 3 of ADT, instead of at position 16, to inhibit 17 β -HSD3 [13,16].

After we determined the inhibitory potency of a spiro- δ -lactone at position 17 of an androstane backbone on 17 β -HSD5 and 17 β -HSD3, we evaluated their proliferative activity on Shionogi androgen-sensitive cell lines. In fact, for a potential use in prostate cancer, an enzyme inhibitor should be devoid of proliferative androgenic activity. The behaviour of compounds **4**, **5**, **12** and **13** on an androgen-sensitive Shionogi cell line was then evaluated and compared to that of hydroxyflutamide, a well known antiandrogen (Table 2) [22,23].

Table 1. Inhibition of 17 β -HSD5 and 17 β -HSD3 by compounds **4**, **5**, **12**, **13**, **16** and **17**.

Compounds (characteristics) ^a	Inhibition of 17 β -HSD5 at 0.3 μ M (%) ^b	Inhibition of 17 β -HSD5 at 3 μ M (%) ^b	Inhibition of 17 β -HSD3 at 0.1 μ M (%) ^c	Inhibition of 17 β -HSD3 at 1 μ M (%) ^c	Inhibition of 17 β -HSD3 at 10 μ M (%) ^c
I (C18/17-lactone/3-OH)	92	95	--	--	--
II (C19/17-oxo/3-carbamate)	--	--	66.0 \pm 1.7	88.3 \pm 1.1	93.7 \pm 0.8
III (C19/17-oxo/3-morpholinone)	--	--	63.2 \pm 2.6	81.0 \pm 1.6	88.7 \pm 4.0
4 (C19/17-lactone/3-oxo)	64	92	4.9 \pm 4.8	15.0 \pm 0.9	56.7 \pm 2.2
5 (C19/17-lactone/3-OH)	56	91	0.6 \pm 5.3	22.4 \pm 2.8	45.7 \pm 1.5
12 (C19/17-lactone; mono-CH ₃ /3-oxo)	73	91	1.0 \pm 3.3	22.2 \pm 4.9	53.4 \pm 5.0
13 (C19/17-lactone; bis-CH ₃ /3-oxo)	54	91	1.0 \pm 7.2	14.4 \pm 3.3	58.0 \pm 3
16 (C19/17-lactone/3-carbamate)	--	--	32.0 \pm 3.3	60.4 \pm 4.4	60.9 \pm 0.7
17 (C19/17-lactone/3-morpholinone)	--	--	11.2 \pm 2.6	51.0 \pm 1.5	87.2 \pm 0.5

^a C18: estrane nucleus (18 carbons) and C19: androstane nucleus (19 carbones); ^b For the transformation of [¹⁴C]-4-dione to [¹⁴C]-T by HEK-293 cells overexpressing human 17 β -HSD5 (transfected cells in culture); ^c For the transformation of [¹⁴C]-4-dione to [¹⁴C]-T by rat testicular 17 β -HSD3 (microsomal fraction).

Table 2. Proliferative and antiproliferative activities of monospiro-compounds **4**, **5**, **12** and **13** on Shionogi (AR⁺) cells.

Compounds	Proliferative activity at 0.1 μ M (%) ^a	Proliferative activity at 1 μ M (%) ^a	Antiproliferative activity at 0.1 μ M (%) ^b	Antiproliferative activity at 1 μ M (%) ^b
4	12 \pm 2	0 \pm 5	23 \pm 1	100 \pm 1
5	19 \pm 4	0 \pm 5	10 \pm 1	61 \pm 3
12	9 \pm 5	0 \pm 3	0 \pm 5	58 \pm 2
13	0 \pm 9	0 \pm 5	1 \pm 2	49 \pm 7
OH-Flu ^c	0 \pm 6	0 \pm 2	69 \pm 3	100 \pm 3

^a The proliferative activity expressed in percentage was calculated in comparison to the stimulation (100%) induced by 0.3 nM of potent androgen dihydrotestosterone (DHT); ^b The antiproliferative activity expressed in percentage is the ability of a compound to inhibit the 0.3 nM DHT-induced proliferation of cells; ^c The antiandrogen hydroxyflutamide was used as a reference compound [22,23].

Except for the α -dimethylated lactone **13** which did not show any proliferative (androgenic) activity, the C19-steroid spiro- δ -lactones **4**, **5** and **12** exhibited a slight proliferative activity at 0.1 μ M on Shionogi cells (12, 19 and 9%, respectively). However, at the higher concentration of 1 μ M, no proliferative activity was observed for spiro- δ -lactones **4**, **5**, **12** and **13** suggesting that proliferative

effects observed at 0.1 μM were not significant. The antiproliferative (antiandrogenic) activity was measured by the inhibition of DHT (0.3 nM)-induced proliferation on Shionogi cells. All the target compounds **4**, **5**, **12** and **13** showed an antiproliferative activity at 1 μM , the most important effect being observed with the spiro- δ -lactone **4** (100% antiproliferative activity). Its activity dropped down at lower concentration: 23% at 0.1 μM , compared to 69% for hydroxyflutamide at the same concentration.

To discriminate between two possible antiproliferative effects: an antiandrogenic activity mediated by the androgen receptor (AR) and a cytotoxic activity not mediated by AR, we measured the binding affinity on AR for each compound (Table 3). In fact, the C19-steroids **4**, **5**, **12** and **13** were expected to show some affinities with androgen receptor, as their chemical structures are similar to that of the natural substrate T and DHT. Compounds **4**, **5** and **12** showed a weak binding on AR suggesting an antiandrogenic effect instead of a cytotoxic effect. It is however possible that the antiproliferative effect we have observed on Shionogi cells was a mixture of both antiandrogenic and cytotoxic activities. Interestingly, the spiro-lactones **4**, **5**, **12** and **13** did not show affinities with other steroid (estrogen, glucocorticoid and progestin) receptors.

Table 3. Binding affinity (%) of compounds **4**, **5**, **12** and **13** on steroid receptors.

Cpds	Androgen receptor (%) ^a		Estrogen receptor- α (%) ^a		Glucocorticoid receptor (%) ^a		Progestin receptor (%) ^a	
	10 nM	1 μM	10 nM	1 μM	10 nM	1 μM	10 nM	1 μM
4	4 \pm 1	9 \pm 2	0 \pm 1	1 \pm 1	0 \pm 3	0 \pm 1	4 \pm 2	0 \pm 2
5	7 \pm 1	5 \pm 1	0 \pm 1	0 \pm 1	0 \pm 1	1 \pm 2	4 \pm 1	1 \pm 2
12	1 \pm 2	4 \pm 2	0 \pm 2	1 \pm 3	0 \pm 3	0 \pm 1	0 \pm 2	0 \pm 1
13	0 \pm 2	0 \pm 2	0 \pm 2	0 \pm 3	0 \pm 2	1 \pm 3	0 \pm 3	1 \pm 3
DHT ^b	70 \pm 1	100 \pm 1	2 \pm 2	4 \pm 1	2 \pm 2	6 \pm 2	3 \pm 2	40 \pm 2
E2 ^b	0 \pm 2	34 \pm 1	75 \pm 1	100 \pm 1	5 \pm 2	12 \pm 2	6 \pm 3	25 \pm 2
DEX ^b	0 \pm 1	2 \pm 1	0 \pm 3	0 \pm 1	66 \pm 2	99 \pm 1	0 \pm 3	1 \pm 2
R5050 ^b	1 \pm 4	28 \pm 2	5 \pm 2	4 \pm 1	9 \pm 2	85 \pm 2	65 \pm 2	99 \pm 2

^a Data are expressed in percentage (%) of the binding affinity obtained with a natural or synthetic ligand tested at a concentration of 1 μM (100% of binding); ^b Natural and synthetic ligands used for each receptor: DHT, dihydrotestosterone; E2, estradiol; DEX, dexamethasone; R5050, synthetic progestin.

2.5. Biological Evaluation of Dispiro Derivatives **16** and **17**

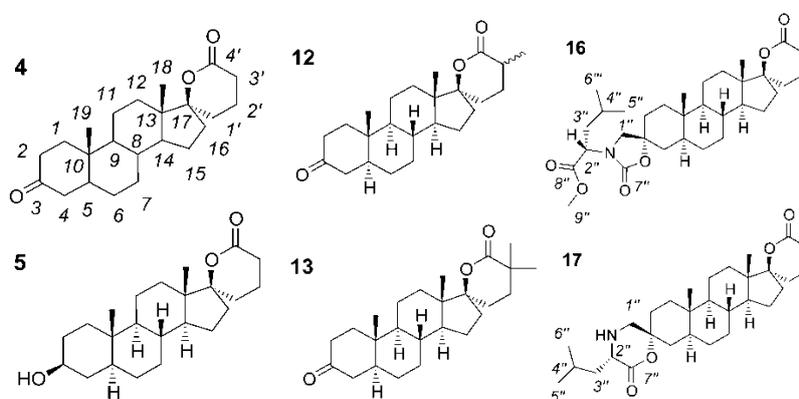
Compounds **16** and **17** were synthesized to determine the impact of two spiro-functionalities on the inhibition of 17 β -HSD3 (Table 1). As mentioned above, a microsomal preparation of rat testis was used as source of enzyme activity transforming 4-dione to T. In the first series of monospiro derivatives (compounds **4**, **5**, **12** and **13**), the presence of a spiro- δ -lactone at position C-17 resulted in a very weak inhibition (1–5% at 0.1 μM). At the opposite, the presence of a carbamate or a morpholinone moiety at position C-3 generated a very good inhibitory activity (66 and 63% at 0.1 μM) for monospiro derivatives II and III, respectively. When we introduced a carbamate or a morpholinone at position C-3 of compound **4**, both dispiro derivatives **16** and **17** produced a moderate inhibitions of 17 β -HSD3 (32 and 11% at 0.1 μM ; 60 and 51% at 1 μM), which are less important than those of known inhibitors II and III (only C-3 derivatives) and more important than those of **4** and **5** (only C-17 derivatives).

3. Experimental

3.1. General

Chemical reagents as well as DMF and CH_2Cl_2 , 99.8% anhydrous grade, were purchased from Aldrich Chemical Company (Milwaukee, WI, USA). Androsterone and *epi*-androsterone were obtained from Steraloids (Wilton, NH, USA). THF, used in anhydrous conditions, was distilled from sodium benzophenone ketyl. Solvents for chromatographies were purchased from BDH Chemicals (Montréal, QC, Canada) or Fisher Chemicals (Montréal, QC, Canada). Thin-layer chromatography (TLC) was performed on 0.20 mm silica gel 60 F₂₅₄ plates (E. Merck, Darmstadt, Germany) and 230–400 mesh ASTM silica gel 60 (E. Merck) was used for flash chromatography. Infrared spectra (IR) are reported in cm^{-1} and obtained on a Perkin-Elmer 1600 (FT-IR series) spectrophotometer. Nuclear magnetic resonance spectra (NMR) were recorded with a Bruker AC/F 300 spectrometer (Billerica, MA, USA) at 300 (^1H) and 75 (^{13}C) MHz or a Bruker AVANCE 400 spectrometer at 400 (^1H) and 100 (^{13}C) MHz. The chemical shifts (δ) are expressed in ppm and referenced to chloroform (7.26 ppm for ^1H and 77.00 ppm for ^{13}C). All ^{13}C -NMR signals of final compounds **4**, **5**, **12**, **13**, **16** and **17** (Table 4) were fully assigned using a series of NMR experiments (APT, HSQC, HMBC, COSY and NOESY) and data reported in literature [20,21,24–27]. High-resolution mass spectra (HRMS) were provided by Pierre Audet at the Laval University Chemistry Department (Québec, QC, Canada). The names of steroid derivatives were generated using ACD/Labs (Chemist' version) software (Toronto, ON, Canada).

Table 4. ^{13}C -NMR data of final compounds **4**, **5**, **12**, **13**, **16** and **17** dissolved in CDCl_3 .



Cpds	4	5	12	13	16	17
C1	38.55	36.98	38.60	38.58	33.86	32.98
C2	38.07	31.38	38.12	38.11	32.75	31.48
C3	211.67	71.07	211.79	211.84	79.74	82.37
C4	44.59	38.03	44.63	44.62	39.48	38.11
C5	46.71	44.84	46.75	46.74	40.86	39.29
C6	28.72	28.47	28.77	28.75	28.01	28.00
C7	31.40	31.72	31.44	31.41	31.47	31.38
C8	35.75	35.82	35.81	35.81	35.84	35.85
C9	53.61	54.12	53.67	53.63	53.61	53.50
C10	35.75	35.49	35.81	35.81	35.31	36.02
C11	20.90	20.65	20.91 (20.98)	20.97	20.43	20.46

Table 4. Cont.

Cpds	4	5	12	13	16	17
C12	31.86	31.92	31.93	31.92	31.93	31.96
C13	46.99	46.95	46.96	47.01	46.99	46.99
C14	49.64	49.77	49.45 (49.61)	49.49	49.77	49.83
C15	23.77	23.73	23.94 (23.72)	23.61	23.76	23.78
C16	33.93	33.90	33.99 (33.50)	34.77	33.95	33.92
C17	93.21	93.34	92.78 (93.65)	93.70	93.40	93.42
C18	14.40	14.37	14.52	14.56	14.43	14.45
C19	11.43	12.24	11.47	11.45	11.40	11.36
C1'	27.86	27.82	27.21 (28.58)	25.51	27.91	27.89
C2'	15.83	15.78	24.41 (24.21)	31.55	15.84	15.84
C3'	29.40	29.37	34.64 (36.16)	37.76	29.47	29.47
C4'	171.98	172.13	174.90 (175.89)	177.90	172.22	172.25
C5'	---	---	17.35 (17.26)	27.65	---	---
C6'	---	---	---	27.75	---	---
C1''	---	---	---	---	52.89	52.41
C2''	---	---	---	---	53.61	55.58
C3''	---	---	---	---	37.69	41.41
C4''	---	---	---	---	24.93	24.46
C5''/C6''	---	---	---	---	21.06/23.14	20.97/23.40
C7''	---	---	---	---	157.99	171.99
C8''	---	---	---	---	171.99	---
C9''	---	---	---	---	52.22	---

3.2. Synthesis of δ -Lactones 4 and 5 (Scheme 1)

(3 β ,5 α)-3-(Tetrahydro-2H-pyran-2-yloxy)androstan-17-one (**1**). To a solution of *epi*-androsterone (2.0 g, 6.89 mmol) in dry CH₂Cl₂ (150 mL) was added 3,4-dihydropyran (1.9 mL, 3 eq) and *p*-toluene-sulfonic acid (0.130 g, 0.1 eq). The mixture was stirred for 2 h at 0 °C under an atmosphere of argon. The reaction was stopped by adding a saturated NaHCO₃ solution. Extraction was done with EtOAc, the organic layer was washed with water, then dried over MgSO₄ and evaporated to dryness under reduced pressure. The yellow oil obtained was purified by flash chromatography, using a mixture of hexanes and EtOAc (8/2) as eluent, to give the compound **1** as a white amorphous solid in 82% yield. IR (film) ν 1740 (C=O, ketone); ¹H-NMR (CDCl₃) δ 0.83 and 0.85 (2s, CH₃-18 and CH₃-19), 0.60–2.10 (unresolved CH and CH₂), 2.43 (dd, $J_1 = 19.0$ Hz and $J_2 = 8.8$ Hz, CH-16 β), 3.48 and 3.92 (2m, CH₂O of THP), 3.58 (m, CH-3 α), 4.71 (t, $J = 3.7$ Hz, CH of THP); ¹³C-NMR (CDCl₃) δ 12.28, 13.81, 20.04, 20.49, 21.78, 25.52, 27.79, 28.42, (28.56), 29.41, (29.70), 30.94, 31.30, 31.59, 34.29, 35.09, 35.85, (36.15), 36.96, (37.17), 44.80, (45.14), 47.80, 51.46, 54.52, 62.81, 75.09, (75.45), 96.60, (96.96), 221.32.

17 β -Hydroxy-3 β -(tetrahydro-2H-pyran-2-yloxy)-17 α -[4'-(tetrahydro-2H-pyran-2-yloxy)butynyl]-5 α -androsterane (**2**). To a solution of 2-(3-butynyloxy)-tetrahydro-2H-pyran (3.1 mL, 19.86 mmol) in dry THF (100 mL) under an argon atmosphere was added a 1.6 M solution of *n*-BuLi in hexanes (12.4 mL, 19.65 mmol) and the reaction mixture was stirred at –78 °C for 20 min. A solution of ketone **1** (1.85 g,

4.95 mmol) in dry THF (50 mL) was then added dropwise over a period of 15 min and the mixture was allowed to react overnight, the temperature going from $-78\text{ }^{\circ}\text{C}$ to room temperature. A saturated NaHCO_3 solution was added and the extraction was done with EtOAc. The organic layer was washed with brine, dried over MgSO_4 and evaporated to dryness under reduced pressure. The crude product was purified by column chromatography using a mixture of hexanes and EtOAc (8/2) as eluent to give the alkylated compound **2** as an amorphous white solid in 68% yield. IR (film) ν 3429 (OH, alcohol); $^1\text{H-NMR}$ (CDCl_3) δ 0.76 and 0.77 (2s, CH_3 -18 and CH_3 -19), 0.55–2.20 (unresolved CH and CH_2), 2.49 (t, $J = 6.9\text{ Hz}$, $\text{C}\equiv\text{CCH}_2$), 3.50 and 3.84 (2m, $2 \times \text{CH}_2\text{O}$ of THPs), 3.75 (m, CH-3 α), 4.63 and 4.68 (2t, $J = 3.1\text{ Hz}$, $2 \times \text{CH}$ of THPs); $^{13}\text{C-NMR}$ (CDCl_3) δ 12.18, 12.79, 19.13, 19.79, 19.85, 20.25, 20.79, 23.04, 25.36, 25.41, 27.70, 28.50, (28.65), 29.31, 30.44, 31.16, 31.50, 32.72, 34.22, 35.65, (36.06), 36.85, (37.07), 38.94, 44.66, (45.01), 46.78, 50.24, 53.90, 61.79, 62.49, (62.59), 65.71, 75.08, (75.41), 79.75, 82.76, 84.73, 96.37, (96.64), 98.43.

(5*S*,8*R*,9*S*,10*S*,13*S*,14*S*,17*S*)-10,13-Dimethylhexadecahydrospiro[cyclopenta[*a*]phenanthrene-17,2'-pyran]-3,6'-(2*H*,3'*H*)-dione (**4**). To a solution of alkyne **2** (1.5 g, 2.94 mmol) in EtOAc (100 mL) was added a 1:1 mixture of palladium on charcoal (10%) and palladium on calcium carbonate (5%) (150 mg). The reaction mixture was stirred overnight at room temperature under an atmosphere of hydrogen. The mixture was filtered through a pad of celite and the solvent evaporated to dryness under reduced pressure. Without purification, the white solid corresponding to hydrogenated compound **3** was directly used for the next step. To a stirred solution of **3** (1.4 g) in acetone (100 mL) stirred at $0\text{ }^{\circ}\text{C}$ was added dropwise a 2.7 M solution of Jones' reagent (3.5 mL). The reaction was monitored by TLC and was completed after 30 min. Isopropyl alcohol was then added until a persistent green colour remained. Organic solvents were removed under reduced pressure and the resulting green concentrate dissolved in water. Extraction was done with EtOAc and the combined organic layers were washed with brine, dried over MgSO_4 and evaporated to dryness. Purification by column chromatography using a mixture of hexanes and EtOAc (9/1) gave the spiro- δ -lactone **4** as white solid in 98% yield (for the two steps). IR (film) ν 1714 ($\text{C}=\text{O}$, ketone and lactone); $^1\text{H-NMR}$ (CDCl_3) δ 0.98 (s, CH_3 -19), 1.02 (s, CH_3 -18), 0.60–2.58 (unresolved CH and CH_2); $^{13}\text{C-NMR}$ (CDCl_3) δ 11.43, 14.40, 15.83, 20.90, 23.77, 27.86, 28.72, 29.40, 31.40, 31.86, 33.93, 35.75 ($2\times$), 38.07, 38.55, 44.59, 46.71, 46.99, 49.64, 53.61, 93.21, 171.98, 211.67; HRMS for $\text{C}_{23}\text{H}_{38}\text{NO}_3$ [$\text{M}+\text{NH}_4$] $^+$: calculated 376.2846, found 376.2850.

(3*S*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*,17*S*)-3-Hydroxy-10,13-dimethyloctadecahydrospiro[cyclopenta[*a*]phenanthrene-17,2'-pyran]-6'(3'*H*)-one (**5**). Ketone **4** (200 mg, 0.549 mmol) was dissolved in MeOH (30 mL) and NaBH_4 (23 mg, 1.1 eq) was added. The solution was stirred at room temperature for 3 h. Water was then added, MeOH was evaporated under reduced pressure and the product was extracted with EtOAc. The organic layer was dried over MgSO_4 , the solvent evaporated under reduced pressure and the crude product was purified by column chromatography using a mixture of hexanes and EtOAc (8/2) to give the alcohol **5** as an epimeric mixture at position 3 (3 β -OH/3 α -OH: 85/15, evaluated by $^1\text{H-NMR}$). White solid (85% yield); IR (film) ν 3418 (OH, alcohol), 1716 ($\text{C}=\text{O}$, lactone); $^1\text{H-NMR}$ (CDCl_3) δ 0.77 and 0.79 (2s, CH_3 -19 of both epimers), 0.93 (s, CH_3 -18), 0.50–2.00 (unresolved CH and CH_2), 2.43 (m, CH_2COO), 3.55 and 4.01 (2m, CH-3 β and CH-3 α in proportions 85/15); $^{13}\text{C-NMR}$ (CDCl_3) *minor compound signals indicated between* [] δ [11.10], 12.24, 14.37, 15.78, 20.65, 23.73, 27.82,

28.47, 29.37, [29.61], 31.38, 31.72, 31.92, [32.15], 33.90, 35.49, 35.82, [36.08], 36.98, 38.03, [39.05], 44.84, 46.95, 49.77, 54.12, [66.31], 71.07, 93.34, 172.13; HRMS for $C_{23}H_{40}NO_3$ $[M+NH_4]^+$: calculated 378.3003, found 378.3008.

3.3. Synthesis of α -Methylated δ -lactones **12** and **13** (Scheme 2)

(3 α ,5 α)-3- $\{[tert$ -Butyl(dimethyl)silyl]oxy $\}$ androstan-17-one (**6**). The hydroxy group of androsterone (1.5 g, 5.17 mmol) was protected as a silylated ether in a mixture of dry DMF (100 mL), imidazole (1.76 g, 5 eq) and TBDMS-Cl (2.34 g, 3 eq). TBDMS-ADT (**6**) was thus obtained as a white solid in 94% yield and the IR, NMR and MS data are in accord with those reported in literature [28].

3 α -(*tert*-Butyldimethylsilyloxy)-17 β -hydroxy-17 α -[4'-(*tetrahydro-2H*-pyran-2-yloxy)butynyl]-5 α -androstane (**7**). The carbonyl group of TBDMS-ADT (**6**) was alkylated with 2-(3-butynyloxy)tetrahydro-2*H*-pyran as described above for the synthesis of compound **2**. Compound **7** was obtained as colourless oil in 70% yield. IR (film) ν 3444 (OH, alcohol); 1H -NMR ($CDCl_3$) δ 0.02 (s, Si(CH₃)₂), 0.76 (s, CH₃-19), 0.81 (s, CH₃-18), 0.89 (s, SiC(CH₃)₃), 0.70–2.25 (unresolved CH and CH₂), 2.55 (t, J = 6.8 Hz, C \equiv CCH₂), 3.55 and 3.84 (2m, 2 \times CH₂O of THP and side chain), 3.95 (t, J = 2.1 Hz, CH-3 β), 4.68 (t, J = 3.0 Hz, CH of THP); ^{13}C -NMR ($CDCl_3$) δ -4.85 (2 \times), 11.40, 12.90, 18.12, 19.26, 20.44, 23.15, 25.49, 25.63, 25.89 (3 \times), 28.59, 29.72, 30.57, 31.62, 32.35, 32.83, 36.09, 36.22, 36.78, 39.06, 46.24, 46.93, 50.37, 53.87, 61.92, 65.81, 66.86, 80.11, 83.16, 84.69, 98.62.

3 α -(*tert*-Butyldimethylsilyloxy)-17 β -hydroxy-17 α -(4'-hydroxybutyl)-5 α -androstane (**8**). Compound **7** was submitted to hydrogenation conditions as described in the first part of the synthesis of **4**. The crude product was then used without purification for the next step, the hydrolysis of the THP group. The crude hydrogenated product (3.6 g, 6.406 mmol) was dissolved in MeOH and *p*-TSA (122 mg, 0.1 eq) was added. The reaction mixture was stirred at room temperature for 1 h. Water was added, the MeOH evaporated under reduced pressure and the mixture extracted with EtOAc. The organic layer was washed with brine, dried over MgSO₄ and evaporated to dryness. The crude product was purified by column chromatography using a mixture of hexanes and EtOAc (5/5) as eluent to give the diol **8** as a white solid in 80% (for the two steps). IR (film) ν 3351 (OH, alcohol); 1H -NMR ($CDCl_3$) δ 0.01 (s, Si(CH₃)₂), 0.77 (s, CH₃-19), 0.85 (s, CH₃-18), 0.89 (s, SiC(CH₃)₃), 0.62–2.05 (unresolved CH and CH₂), 3.69 (m, CH₂OH), 3.95 (t, J = 2.2 Hz, CH-3 β); ^{13}C -NMR ($CDCl_3$) δ -4.86 (2 \times), 11.41, 14.41, 18.10, 19.80, 20.45, 23.65, 25.86 (3 \times), 28.56, 29.72, 31.61, 31.90, 32.43, 33.41, 34.51, 36.04, 36.16, 36.41, 36.74, 39.08, 46.45, 50.53, 54.36, 62.93, 66.83, 83.59.

(3*S*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*,17*S*)-3- $\{[tert$ -Butyl(dimethyl)silyl]oxy $\}$ -10,13-dimethyloctadecahydrospiro [cyclopenta[*a*]phenanthrene-17,2'-pyran]-6'(3'*H*)-one (**9**). The diol **8** was oxidized with Jones' reagent as described in the second part of the synthesis of **4**. After purification by column chromatography using a mixture of hexanes and EtOAc (8/2) as eluent, lactone **9** was obtained in 95% yield as a white solid. IR (film) ν 1734 (C=O, lactone); 1H -NMR ($CDCl_3$) δ 0.01 (s, Si(CH₃)₂), 0.77 (s, CH₃-19), 0.89 (s, SiC(CH₃)₃), 0.97 (s, CH₃-18), 0.62–2.00 (unresolved CH and CH₂), 2.45 (m, CH₂CO), 3.96 (t, J = 2.2 Hz, CH-3 β); ^{13}C -NMR ($CDCl_3$) δ -4.85 (2 \times), 11.40, 14.45, 15.91, 18.12, 20.26, 23.78, 25.86

(3×), 27.91, 28.47, 29.50, 29.69, 31.87, 31.99, 32.44, 34.02, 35.92, 36.03, 36.69, 39.08, 47.04, 49.90, 54.22, 66.80, 93.55, 172.25.

Methylation of Lactone **9**

A solution of diisopropylamine (0.45 mL, 3.5 eq) in dry THF (2 mL) was stirred at 0 °C under an argon atmosphere and a 1.6 M solution of *n*-BuLi in hexanes (2.36 mL, 4.02 eq) was added dropwise. After 30 min, the resulting LDA solution was cooled at −78 °C and lactone **9** (0.445 g, 0.938 mmol) in dry THF (50 mL) was added. The mixture was allowed to stir 1 h at 0 °C and then cooled again at −78 °C before the addition of methyl iodide (4.02 mL, 6 eq) dropwise. The reaction mixture was stirred overnight from −78 °C to room temperature. Water was added to quench the reaction and the crude product was extracted with EtOAc. The organic phase was washed with a saturated NaCl solution, dried over MgSO₄ and evaporated under reduced pressure. A column chromatography using a mixture of hexanes and EtOAc (9/1) as eluent allowed us to separate the three reaction products: the monomethylated lactone **10A**, the monomethylated lactone **10B** and the dimethylated lactone **11**, in proportions 2:2:1, respectively, in 70% yield.

(3*S*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*,17*R*)-3-*{tert-Butyl(dimethyl)silyl}oxy*}-5',10,13-trimethyloctadecahydrospiro [cyclopenta[*a*]phenanthrene-17,2'-pyran]-6'(3'*H*)-one (**10**). **10A**: White solid; IR (film) ν 1729 (C=O, lactone); ¹H-NMR (CDCl₃) δ 0.01 (s, Si(CH₃)₂), 0.76 (s, CH₃-19), 0.88 (s, SiC(CH₃)₃), 0.96 (s, CH₃-18), 1.26 (d, *J* = 7.1 Hz, CH₃-CH), 0.62–2.00 (unresolved CH and CH₂), 2.38 (m, CHCO), 3.95 (t, *J* = 2.0 Hz, CH-3 β); ¹³C-NMR (CDCl₃) δ −4.86 (2×), 11.38, 14.52, 17.35, 18.08, 20.31, 23.65, 25.23, 25.85 (3×), 28.46, 28.56, 29.68, 31.85, 31.97, 32.43, 34.65, 35.93, 36.02, 36.12, 36.68, 39.07, 46.94, 49.80, 54.21, 66.78, 93.93, 175.06. **10B**: White solid; IR (film) ν 1729 (C=O, lactone); ¹H-NMR (CDCl₃) δ 0.004 (s, Si(CH₃)₂), 0.76 (s, CH₃-19), 0.88 (s, SiC(CH₃)₃), 0.95 (s, CH₃-18), 1.22 (d, *J* = 6.8 Hz, CH₃-CH), 0.60–2.20 (unresolved CH and CH₂), 2.52 (m, CHCO), 3.95 (t, *J* = 2.0 Hz, CH-3 β); ¹³C-NMR (CDCl₃) δ −4.88 (2×), 11.37, 14.49, 17.25, 18.06, 20.21, 23.87, 24.43, 25.84 (3×), 27.23, 28.45, 29.66, 31.84, 31.99, 32.33, 33.50, 34.02, 35.88, 35.99, 36.66, 39.02, 46.99, 49.63, 54.18, 66.77, 93.02, 176.01.

(3*S*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*,17*R*)-3-*{tert-Butyl(dimethyl)silyl}oxy*}-5',5',10,13-tetramethyloctadecahydrospiro [cyclopenta[*a*]phenanthrene-17,2'-pyran]-6'(3'*H*)-one (**11**). White solid; IR (film) ν 1718 (C=O, lactone); ¹H-NMR (CDCl₃) δ 0.002 (s, Si(CH₃)₂), 0.76 (s, CH₃-19), 0.87 (s, SiC(CH₃)₃), 0.95 (s, CH₃-18), 1.23 and 1.25 (2s, 2 × CH₃), 0.62–2.15 (unresolved CH and CH₂), 3.94 (s, CH-3 β); ¹³C-NMR (CDCl₃) δ −4.88 (2×), 11.37, 14.54, 18.05, 20.29, 23.53, 25.52, 25.83 (3×), 27.61, 27.73, 28.44, 29.66, 31.58, 31.80, 31.99, 32.40, 34.82, 35.91, 36.00, 36.66, 37.71, 39.04, 47.01, 49.69, 54.17, 66.76, 93.91, 177.96.

Hydrolysis of the Silylated Ethers of **10A**, **10B** and **11**

Lactones **10A**, **10B** and **11** were respectively dissolved in dry THF. A 1 M solution of TBAF in THF (2 eq) was added and the resulting mixture was stirred overnight at refluxing temperature under an argon atmosphere. Water was then added and extraction was done with EtOAc. The organic phase was washed with a saturated NaCl solution and dried over MgSO₄. The crude products were respectively

submitted to Jones' reagent as described above for the synthesis of **4**. A column chromatography using a mixture of hexanes and EtOAc (9/1) afforded the same mixture of monomethylated lactones **12**, in the case of **10A** and **10B**, and the dimethylated lactone **13** in the case of **11**.

(5*S*,8*R*,9*S*,10*S*,13*S*,14*S*,17*R*)-5',10,13-Trimethylhexadecahydrospiro[cyclopenta[*a*]phenanthrene-17,2'-pyran]-3,6'(2*H*,3'*H*)-dione (**12**). White solid; 90% yield; IR (film) ν 1714 (C=O, ketone and lactone); ¹H-NMR (CDCl₃) δ 0.99 (s, CH₃-19), 1.03 (s, CH₃-18), 1.23 and 1.27 (2d, *J* = 7.0 Hz and *J* = 7.2 Hz, CH₃-CH in proportions 1/1), 0.62–2.60 (unresolved CH and CH₂); ¹³C-NMR (CDCl₃) δ 11.47, 14.52, (17.26), 17.35, 20.91, (20.98), (23.72), 23.94, (24.21), 24.41, 27.21, (28.58), 28.77, 31.44, 31.93, (33.50), 33.99, 34.64, 35.81 (2 ×), (36.15), 38.12, 38.60, 44.63, 46.75, 46.96, 49.45, (49.61), 53.67, 92.78, (93.65), 174.90, (175.89), 211.79; HRMS for C₂₄H₃₇O₃ [M+H]⁺: calculated 373.2737, found 373.2739.

(5*S*,8*R*,9*S*,10*S*,13*S*,14*S*,17*R*)-5',5',10,13-Tetramethylhexadecahydrospiro[cyclopenta[*a*]-phenanthrene-17,2'-pyran]-3,6'(2*H*,3'*H*)-dione (**13**). White solid; 92% yield; IR (film) ν 1716 (C=O, ketone and lactone); ¹H-NMR (CDCl₃) δ 0.99 (s, CH₃-19), 1.03 (s, CH₃-18), 1.25 and 1.26 (2s, 2 × CH₃), 0.63–2.50 (unresolved CH and CH₂); ¹³C-NMR (CDCl₃) δ 11.45, 14.56, 20.97, 23.61, 25.51, 27.65, 27.75, 28.75, 31.41, 31.55, 31.92, 34.77, 35.81, 37.76, 38.11, 38.58, 44.62, 46.74, 47.01, 49.49, 53.63, 93.70, 177.90, 211.84; HRMS for C₂₅H₃₉O₃ [M+H]⁺: calculated 387.2894, 387.2900.

3.4. Synthesis of the Dispiro Compounds **16** and **17** (Scheme 3)

(2*R*,5'*S*,8'*R*,9'*S*,10'*S*,13'*S*,14'*S*,17'*S*)-10',13'-Dimethylhexadecahydro-2'*H*-dispiro[oxirane-2,3'-cyclopenta[*a*]phenanthrene-17,2''-pyran]-6''(3''*H*)-one (**14**). Trimethyl sulfoxonium iodide (1.3 g, 5.9 mmol) and sodium hydride 60% in mineral oil (236 mg, 5.9 mmol) was dissolved in DMSO (15 mL) and the mixture was stirred for 1 h at room temperature under an argon atmosphere. Compound **4** (0.5 g, 1.4 mmol) dissolved in THF (10 mL) was then added and the mixture stirred for 3 h. The reaction was quenched with a saturated solution of NH₄Cl (35 mL) and the crude product was extracted with EtOAc and evaporated under reduced pressure. A column chromatography using a mixture of hexanes and EtOAc (8/2) afforded compound **14** (450 mg, 1.2 mmol) as a white solid in 72% yield. IR (film) ν 1720 (C=O, ketone and lactone); ¹H-NMR (CDCl₃) δ 0.86 (s, CH₃-19), 0.98 (s, CH₃-18), 0.70–2.15 (unresolved CH and CH₂), 2.45 (m, CH₂CO), 2.62 (s, CH₂O); ¹³C-NMR (CDCl₃) δ 11.26, 14.43, 15.84, 20.48, 23.78, 27.89, 28.38, 29.12, 29.45, 31.53, 31.96, 33.95, 35.51, 35.82, 35.85, 35.90, 43.72, 46.98, 49.79, 53.54, 53.84, 58.49, 93.42, 172.19.

Methyl-*N*-{[(13*S*)-3-hydroxy-10,13-dimethyl-6'-oxoicosahydrospiro[cyclopenta[*a*]phenanthrene-17,2'-pyran]-3-yl]methyl}leucinate (**15**). To a solution of the oxirane **14** (200 mg, 0.54 mmol) dissolved in MeOH (8 mL) was added L-leucine methyl ester (782 mg, 5.4 mmol) and the mixture was stirred in a Schlenck tube. After 22 h at 90 °C the mixture was dissolved in CH₂Cl₂ and concentrated under reduced pressure. The crude product was purified by a column chromatography using a mixture of hexanes and EtOAc (8/2) to give the amino alcohol **15** (276 mg) in 98% yield. IR (film) ν 3464 and 3333 (OH and NH); 1736 (C=O, ketone and lactone); ¹H-NMR (CDCl₃) δ 0.75 (s, CH₃-19), 0.90 and 0.92 (2d, *J* = 6.6 Hz, 2 × CH₃ from *i*-Pr), 0.96 (s, CH₃-18), 0.70–1.98 (unresolved CH and CH₂), 2.17

and 2.61 (2d of AB system, $J = 11.9$ Hz, CH_2N), 2.43 (m, CH_2CO), 3.22 (t, $J = 7.3$ Hz, $\text{CHC}=\text{O}$), 3.72 (s, CH_3O); ^{13}C -NMR (CDCl_3) δ 11.23, 14.44, 15.86, 20.42, 21.94, 22.87, 23.79, 24.83, 27.90, 28.46, 29.47, 31.67, 31.71, 32.01, 33.80, 33.96, 35.91, 35.99, 38.52, 40.64, 42.77, 44.10, 47.01, 49.88, 51.71, 53.99, 59.25, 60.87, 69.88, 93.48, 172.24, 176.33.

Methyl-(2S)-2-[(5R,5'S,8'R,9'S,10'S,13'S,14'S,17'S)-10',13'-dimethyl-2,6''-dioxooctadecahydro-2'H,3H-dispiro[1,3-oxazolidine-5,3'-cyclopenta[a]phenanthrene-17',2''-pyran]-3-yl]-4-methylpentanoate (16). The amino alcohol **15** (69 mg, 0.13 mmol) was dissolved in CH_2Cl_2 (4 mL) and DIPEA (47.5 μL) was added. The solution was stirred for 10 min at 0 °C and triphosgene (20 mg, 0.07 mmol) was added in two portions to the mixture, which was stirred for 5 min at 0 °C and for 2.5 h at room temperature. Another portion of triphosgene (20 mg, 0.07 mmol) was then added and the mixture was stirred for 2 h at room temperature. A saturated solution of NaHCO_3 was used to quench the reaction and the crude product was extracted with CH_2Cl_2 . The organic phase was evaporated under reduced pressure and the crude product purified by column chromatography with hexanes and EtOAc (90/10 and 85/15) as eluent to give **16** (40 mg, 0.07 mmol) in 55% yield as a white solid. IR (film) ν 1740 ($\text{C}=\text{O}$, ketone, lactone and carbamate); ^1H -NMR (CDCl_3) δ 0.81 (s, CH_3 -19), 0.96 (s, CH_3 -18 and $2 \times \text{CH}_3$ from *i*-Pr), 0.70–2.00 (unresolved CH and CH_2), 2.44 (m, CH_2CO), 3.13 and 3.42 (2d of AB system, $J = 8.1$ Hz, CH_2N), 3.72 (s, CH_3O), 4.59 (m, $\text{CHC}=\text{O}$); ^{13}C -NMR (CDCl_3) δ 11.40, 14.43, 15.84, 20.43, 21.06, 23.14, 23.76, 24.93, 27.91, 28.01, 29.47, 31.47, 31.93, 32.75, 33.87, 33.95, 35.31, 35.84, 37.69, 39.48, 40.86, 46.99, 49.77, 52.22, 52.89, 53.6 (2 \times), 79.74, 93.40, 157.64, 171.99, 172.22; HRMS for $\text{C}_{32}\text{H}_{50}\text{NO}_6$ $[\text{M}+\text{H}]^+$: calculated 544.3633, found 544.3643.

(2R,5S,5'S,8'R,9'S,10'S,13'S,14'S,17'S)-10',13'-Dimethyl-5-(2-methylpropyl)hexadecahydro-2'H,6H-dispiro[1,4-oxazinane-2,3'-cyclopenta[a]phenanthrene-17',2''-pyran]-6,6''(3''H)-dione (17). To a solution of sodium methoxide (33 mg, 0.61 mmol) in dry THF (17.5 mL) was added the amino alcohol **15** (100 mg, 0.19 mmol) and the reaction mixture was stirred for 2 h at room temperature. The reaction was stopped by adding a saturated solution of NH_4Cl and the crude product was extracted with EtOAc and purified by HPLC to generate the starting amino alcohol **15** (18%) and compound **17** (24 mg, 39%) as a white solid. IR (film) ν 1724 ($\text{C}=\text{O}$, lactone); ^1H -NMR (CDCl_3) δ 0.79 (s, CH_3 -19), 0.92 and 0.95 (2d, $J = 6.2$ Hz, $2 \times \text{CH}_3$ from *i*-Pr), 0.96 (s, CH_3 -18), 0.75–2.02 (unresolved CH and CH_2), 2.43 (m, CH_2CO), 2.80 and 2.88 (2d of AB system, $J = 13.5$ Hz, CH_2N), 3.50 (m, $\text{CHC}=\text{O}$); ^{13}C -NMR (CDCl_3) δ 11.36, 14.45, 15.84, 20.46, 23.40, 23.78, 24.46, 27.89, 28.00, 29.47, 31.38, 31.48, 31.96, 32.98, 33.92, 35.85, 36.02, 38.11, 39.29, 41.41, 46.99, 49.83, 52.41, 53.50, 55.58, 82.37, 93.42, 171.99, 172.25; HRMS $\text{C}_{30}\text{H}_{48}\text{NO}_4$ $[\text{M}+\text{H}]^+$: calculated 486.3578, found 486.3590.

3.5. Inhibition of 17 β -HSD5

The enzymatic assay was performed using transfected (17 β -HSD5) human embryonal kidney (HEK)-293 cells provided by Dr. Van Luu-The (CHUQ (CHUL)—Research Center) [29]. Briefly, 0.1 μM of the natural substrate [^{14}C]-4-androstene-3,17-dione (Dupont Inc., Mississauga, ON, Canada) and 10 μL of an ethanolic solution of inhibitor were added to freshly changed culture medium in a 6-well culture plate containing HEK-293 cells overexpressing human 17 β -HSD5. After incubation for 18 h, the reaction was stopped by adding a solution of unlabelled 4-androstene-3,17-dione (4-dione) and

testosterone (T) before extracting twice with 2 mL of diethyl ether. The organic phase was pooled and evaporated to dryness. The metabolites were solubilised in dichloromethane, applied to silica gel 60 thin layer chromatography (TLC) plate (Merck, Darmstadt, GE), and then separated by migration in the toluene/acetone (4/1) solvent system. Substrates and metabolites were identified by comparing them to reference steroids, revealed by autoradiography, and quantified using the Phosphoimager system (Molecular Dynamics, Sunnyvale, CA, USA). The percentage of transformation (% *Transf*) and then the percentage of inhibition (% *Inh*) were calculated using the following equations: % *Transf* = $100 \times [^{14}\text{C}]\text{-T (cpm)} / ([^{14}\text{C}]\text{-T (cpm)} + [^{14}\text{C}]\text{-4-dione (cpm)})$ and % *Inh* = $100 \times [\% \text{Transf (without inhibitor)} - \% \text{Transf (with inhibitor)}] / \% \text{Transf (without inhibitor)}$. To avoid the enzyme inhibition by the resulting product of reaction (T), the quantity of enzyme (intact cells) and the incubation time were both selected to give a percentage of transformation below 30%, which is in a linear range.

3.6. Inhibition of 17 β -HSD3 (Microsomal Fraction of Rat Testes)

A microsomal preparation of rat testes was obtained using slightly modified previously described procedures [30–32]. In brief, rat testes were homogenized on ice with a Polytron in cold phosphate buffer (20 mM KH₂PO₄, 0.25 M sucrose, 1 mM EDTA, pH 7.5) containing protease inhibitors mini-complete (Roche Diagnostics, Laval, QC, Canada) and centrifugated at 12,500g for 15 min to remove the mitochondria, plasma membranes, and cell fragments. The supernatant was further centrifugated at 100,000g for 45 min using an ultracentrifuge equipped with a 70.1 Ti rotor. The microsomal pellet was washed three times with phosphate buffer and centrifugated at 100,000g for 15 min. All these operations were conducted at 4 °C. The protein concentration of the supernatant was determined by the Bradford method using bovine serum albumin as standard [33]. The enzymatic assay was performed at 37 °C for 2 h in 1 mL of a solution containing 860 μL of 50 mM sodium phosphate buffer (pH 7.4, 20% glycerol and 1 mM EDTA), 100 μL of 5 mM NADPH in phosphate buffer, 10 μL of 5 μM [4-¹⁴C]-4-androstene-3,17-dione in ethanol (53.6 mCi/mmol, Perkin Elmer Life Sciences Inc., Boston, MA, USA), 10 μL of inhibitor dissolved in ethanol and 20 μL of diluted enzymatic source in phosphate buffer. Each inhibitor was assessed in triplicate. Afterwards, radiolabelled steroids were extracted from the reaction mixture with diethyl ether. The organic phases evaporated to dryness with nitrogen stream. Residue was dissolved in 50 μL of dichloromethane and dropped on silica gel 60 F₂₅₄ thin layer chromatography plates (EMD Chemicals Inc., Gibbstown, NJ, USA) and eluted with a mixture of toluene/acetone (4:1) solvent system. Substrate ([¹⁴C]-4-dione) and metabolite ([¹⁴C]-T) were identified by comparison with reference steroids and quantified using the Storm 860 System (Molecular Dynamics, Sunnyvale, CA, USA). The percentage of transformation and then the percentage of inhibition were calculated as reported above (Section 3.5).

3.7. Proliferative and Antiproliferative Shionogi (AR⁺) Cell Assay

Assay for the proliferation of androgen-sensitive Shionogi mammary carcinoma cells as well as the inhibition of 0.3 nM DHT-induced proliferation was carried out according to the procedure described by Bydal and co-workers [11]. Calculations were performed according to the following equations and expressed as percentages: (a) Proliferative or androgenic activity = $[(B - A) / (C - A)] \times 100$ and

(b) Antiproliferative or antiandrogenic activity = $[(C - D)/(C - A)] \times 100$, where A is the DNA content on cells incubated with control medium (μg), B is the DNA content of cells treated with the tested compound (μg), C is the DNA content of DHT-stimulated cells (μg) and D is the DNA content of DHT-stimulated cells treated with the tested compound (μg).

3.8. Steroids Receptor Binding Assays

The binding affinity assays on estrogen and progesterin receptors from rat uterine tissue were carried out under the standard procedure established in our laboratory [34]. Assay for androgen receptor from rat ventral prostate was performed according to the procedure described by Luo and co-workers [35]. In the case of glucocorticoid receptors from rat liver tissue, the affinity binding assay was done using a slightly modified procedure described by Asselin and co-workers [36]. A dextran-coated charcoal adsorption, instead of a protamine sulfate precipitation, was used to achieve the separation of bound and free steroids.

4. Conclusions

Monospiro and dispiro steroid derivatives were efficiently synthesized from ADT or *epi*-ADT and characterized by IR, $^1\text{H-NMR}$, $^{13}\text{C-NMR}$ and MS spectroscopies. Careful analysis of NMR data, especially $^{13}\text{C-NMR}$ spectra, allowed the full assignment of all carbons for the series of monospiro and dispiro steroid derivatives. When tested as inhibitors of $17\beta\text{-HSD5}$ and $17\beta\text{-HSD3}$, the monospiro derivatives inhibited the enzyme according to the positioning and in accord with previously reported SAR studies. Thus, $17\beta\text{-HSD5}$ was inhibited by the monospiro derivative at position C-17 whereas $17\beta\text{-HSD3}$ was inhibited by a monospiro derivative at position C-3. For the first time, the presence of two spiro-functionalities was investigated as inhibitors of $17\beta\text{-HSD3}$, but this strategy resulted in a lower inhibitory potency. Additional SAR results were generated for inhibiting $17\beta\text{-HSD3}$ and $17\beta\text{-HSD5}$, two key steroidogenic enzymes involved in biosynthesis of testosterone and in prostate cancer.

Acknowledgments

We thank the Canadian Institutes of Health Research (CIHR) for an operating grant and le Fonds de la recherche en santé du Québec (FRSQ) for a fellowship (DP). We also thank Van Luu-The, Guy Reimnitz and Mei Wang for the enzymatic assay with $17\beta\text{-HSD5}$ as well as Fernand Labrie, Diane Michaud and Gilles Leblanc for the proliferative/antiproliferative assay on Shionogi cells and for receptor binding affinity assays. Careful reading of this manuscript by Micheline Harvey is also greatly appreciated.

References

1. Huggins, C.; Hodges, C.V. Studies of prostate cancer. I Effect of castration, estrogen and androgen injections on serum phosphatases in metastatic carcinoma of the prostate. *Cancer Res.* **1941**, *1*, 293–307.
2. Gittes, R.F. Carcinoma of the prostate. *N. Engl. J. Med.* **1991**, *24*, 236–245.
3. Labrie, F. Intracrinology. *Mol. Cell. Endocrinol.* **1991**, *78*, C113–C118.

4. Labrie, F.; Bélanger, A.; Simard, J.; Luu-The, V.; Labrie, C. DHEA and peripheral androgen and estrogen formation: Intracrinology. *NY Acad. Sci.* **1995**, *774*, 16–28.
5. Poirier, D. 17 β -Hydroxysteroid dehydrogenase inhibitors: A patent review. *Exp. Opin. Ther. Patents* **2010**, *20*, 1123–1145.
6. Mohler, M.L.; Narayanan, R.; He, Y.; Miller, D.D.; Dalton, J.T. Hydroxysteroid dehydrogenase (17 β -HSD3, 17 β -HSD5, and 3 α -HSD3) inhibitors: Extragonadal regulation of intracellular sex steroid hormone levels. *Recent Pat. Endocr. Metab. Immune Drug Discov.* **2007**, *1*, 103–118.
7. Day, J.M.; Tutill, H.J.; Purohit, A.; Reed, M.J. Design and validation of specific inhibitors of 17 β -hydroxysteroid dehydrogenases for therapeutic application in breast and prostate cancer, and in endometriosis. *Endocr. Relat. Cancer* **2008**, *15*, 665–692.
8. Penning, T.M. Human hydroxysteroid dehydrogenases and pre-receptor regulation: Insights into inhibitor design and evaluation. *J. Steroid Biochem. Mol. Biol.* **2011**, *125*, 46–56.
9. Marchais-Oberwinkler, S.; Henn, C.; Moller, G.; Klein, T.; Negri, M.; Oster, A.; Spadaro, A.; Werth, R.; Wetzel, M.; Xu, K.; *et al.* 17 β -Hydroxysteroid dehydrogenases (17 β -HSDs) as therapeutic targets: Protein structures, functions, and recent progress in inhibitor development. *J. Steroid Biochem. Mol. Biol.* **2011**, *125*, 66–82.
10. Labrie, F.; Luu-The, V.; Lin, S.-X.; Labrie, C.; Simard, J.; Breton, R.; Bélanger, A. The key role of 17 β -hydroxysteroid dehydrogenase in sex steroid biology. *Steroids* **1997**, *62*, 148–158.
11. Bydal, P.; Luu-The, V.; Labrie, F.; Poirier, D. Steroidal lactones as inhibitors of 17 β -hydroxysteroid dehydrogenase type 5: Chemical synthesis, enzyme inhibitory activity, and assessment of estrogenic and androgenic activities. *Eur. J. Med. Chem.* **2009**, *44*, 632–644.
12. Maltais, R.; Fournier, M.A.; Poirier, D. Development of 3-substituted-androsterone derivatives as potent inhibitors of 17 β -hydroxysteroid dehydrogenase type 3. *Bioorg. Med. Chem.* **2011**, *19*, 4652–4668.
13. Tchédam Ngatcha, B.; Luu-The, V.; Labrie, F.; Poirier, D. Androsterone 3 α -ether-3 β -substituted and androsterone 3 β -substituted derivatives as inhibitors of type 3 17 β -hydroxysteroid dehydrogenase: Chemical synthesis and structure-activity relationship. *J. Med. Chem.* **2005**, *48*, 5257–5268.
14. Maltais, R.; Luu-The, V.; Poirier, D. Synthesis and optimization of a new family of type 3 17 β -hydroxysteroid dehydrogenase inhibitors by parallel liquid-phase chemistry. *J. Med. Chem.* **2002**, *45*, 640–653.
15. Maltais, R.; Luu-The, V.; Poirier, D. Parallel solid-phase synthesis of 3 β -peptido-3 α -hydroxy-5 α -androstan-17-one derivatives for inhibition of type 3 17 β -hydroxysteroid dehydrogenase. *Bioorg. Med. Chem.* **2001**, *9*, 3101–3111.
16. Tchédam Ngatcha, B.; Luu-The, V.; Poirier, D. Androsterone derivatives substituted at position 16: Chemical synthesis, inhibition of type 3 17 β -hydroxysteroid dehydrogenase, binding affinity for steroid receptors and proliferative/antiproliferative activity on Shionogi (AR⁺) cells. *J. Enzyme Inhib. Med. Chem.* **2002**, *17*, 155–165.
17. Djigoué, G.B.; Simard, M.; Kenmogne, L.C.; Poirier, D. Two androsterone derivatives as inhibitors of androgen biosynthesis. *Acta Cryst.* **2012**, *c68*, o231–o234.
18. Salman, M.; Stotter, P.L.; Chamness, G.C. 125I-ligand for progesterone receptor: 17 Alpha-(6'-iodohex-1'-ynyl)-19-nortestosterone. *J. Steroid Biochem.* **1989**, *33*, 25–31.

19. Sam, K.-M.; Labrie, F.; Poirier, D. *N*-Butyl-*N*-methyl-11-(3'-hydroxy-21',17'-carb lactone-19'-nor-17' α -pregna-1',3',5'(10')-trien-7' α -yl)-undecanamide: An inhibitor of type 2 17 β -hydroxysteroid dehydrogenase that does not have estrogenic or androgenic activity. *Eur. J. Med. Chem.* **2000**, *35*, 217–225.
20. Bydal, P.; Auger, S.; Poirier, D. Inhibition of type 2 17 β -hydroxysteroid dehydrogenase by estradiol derivatives bearing a lactone on the D-ring: Structure-activity relationships. *Steroids* **2004**, *69*, 325–342.
21. Rouillard, F.; Roy, J.; Poirier, D. Chemical synthesis of (*S*)-spiro(estradiol-17,2'-[1,4]oxazinan)-6'-one derivatives bearing two levels of molecular diversity. *Eur. J. Org. Chem.* **2008**, *2008*, 2446–2453.
22. Neri, R.; Florance, K.; Koziol, P.; van Cleave, S. A biological profile of a nonsteroidal antiandrogen, SCH1352 (4'-nitro-3'-trifluoromethyl-*iso*-butyranilide). *Endocrinology* **1972**, *91*, 427–437.
23. Poyet, P.; Labrie, F. Comparison of the antiandrogenic/androgenic activities of flutamide, cyproterone acetate and megestrol acetate. *Mol. Cell. Endocrinol.* **1985**, *42*, 283–288.
24. Claridge, T.D.W. *High-Resolution NMR Techniques in Organic Chemistry*; Elsevier Science LTD.: Oxford, UK, 1999.
25. Tchédam Ngatcha, B.; Trottier, M.C.; Poirier, D. ¹³C Nuclear magnetic resonance spectroscopy data of a variety of androsterone and epi-androsterone derivatives substituted at position 3 β or/and 3 α . *Curr. Top. Steroids Res.* **2011**, *8*, 35–45.
26. Blunt, J.W.; Stothers, S.B. ¹³C-NMR spectra of steroids—a survey and commentary. *Org. Magn. Reson.* **1977**, *9*, 439–464.
27. Dionne, P.; Poirier, D. ¹³C nuclear magnetic resonance study of 17 α -substituted estradiols. *Steroids* **1995**, *60*, 830–836.
28. Maltais, R.; Mercier, C.; Labrie, F.; Poirier, D. Solid-phase synthesis of model libraries of 3 α ,17 β -dihydroxy-16 α -(aminoethyl-*N*-substituted)-5 α -androstanes for the development of steroidal therapeutic agents. *Mol. Divers.* **2005**, *9*, 67–79.
29. Dufort, I.; Rheault, P.; Huand, X.F.; Soucy, P.; Luu-The, V. Characteristics of a highly labile human type 5 17 β -hydroxysteroid dehydrogenase. *Endocrinology* **1999**, *140*, 568–574.
30. Hu, G.; Zhou, H.Y.; Li, X.W.; Chen, B.B.; Xiao, Y.C.; Lian, Q.Q.; Kim, H.H.; Zheng, Z.Q.; Hardy, D.O.; Ge, R.S. The (+)- and (–)-gossypols potently inhibit both 3 β -hydroxysteroid dehydrogenase and 17 β -hydroxysteroid dehydrogenase 3 in human and rat testes. *J. Steroid Biochem. Mol. Biol.* **2009**, *115*, 14–19.
31. Blomquist, C.H.; Bonenfant, M.; McGinley, D.M.; Posalaky, Z.; Lakatua, D.J.; Tuli-Puri, S.; Bealka, D.G.; Tremblay, Y. Androgenic and estrogenic 17 β -hydroxysteroid dehydrogenase/17-ketosteroid reductase in human ovarian epithelial tumors: Evidence for the type 1, 2 and 5 isoforms. *J. Steroid Biochem. Mol. Biol.* **2002**, *81*, 343–351.
32. Moutaouakkil, M.; Prost, O.; Dahan, N.; Adessi, G.L. Estrone and dehydroepiandrosterone sulfatase activities in guinea-pig uterus and liver: Estrogenic effect of estrone sulphate. *J. Steroid Biochem.* **1984**, *21*, 321–328.
33. Bradford, M.M. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.

34. Luo, S.; Martel, C.; Sourla, A.; Gauthier, S.; Merand, Y.; Bélanger, A.; Labrie, C. Comparative potencies effects of 28-day treatment with the new anti-estrogen EM-800 and tamoxifen on estrogen sensitive parameters in intact mice. *Int. J. Cancer* **1997**, *73*, 381–391.
35. Luo, S.; Martel, C.; Leblanc, G.; Candas, B.; Singh, S.M.; Labrie, C.; Simard, J.; Bélanger, A.; Labrie, F. Relative potencies of flutamide and casodex: Preclinical studies. *Endocr. Relat. Cancer* **1996**, *3*, 229–241.
36. Asselin, J.; Melançon, R.; Moachon, G.; Bélanger, A. Characteristics of binding to estrogen, androgen, progestin, and glucocorticoid receptors in 7,12-dimethylbenz(*a*)anthracene-induced mammary tumors and their hormonal control. *Cancer Res.* **1980**, *40*, 1612–1622.

Sample Availability: Samples of the final compounds **4**, **5**, **12**, **13**, **16** and **17** are available from the authors.

© 2013 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/3.0/>).