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First synthesis of separable isomeric testosterone dimers showing differential activities on prostate cancer cells

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ABSTRACT

The synthesis of two separable isomeric testosterone dimers is reported. The dimers are made from testosterone in a 5 step sequence and with 36% overall yield. The key dimerization step was performed using Hoveyda–Grubb's metathesis catalysts on 7α-allyltestosterone with 75% yield. The synthesis led to separable isomeric dimers (*trans* and *cis*, 2:1). X-ray diffraction crystallography, performed on monocrystal of the minor isomer, confirms the *cis* geometry of the double bound between the two testosterone units. MTT assays showed that the *cis* dimer has the highest activity against prostate cancer cell lines. The novel *cis* dimer is more active than the antiandrogen cyproterone acetate indicating the possible therapeutic value of this molecule.

Androgens are important in the development and normal functions of prostate cells. They are implicated in male sexual organ growth and sexual function. Two androgens are known to be active in the cells, testosterone (T) and dihydrotestosterone (DHT). Testosterone is the principal androgen in the blood while DHT is the most potent androgen in the cells.[1] In order to induce their biological effects, androgens have to bind to the androgen receptor (AR): the hormone-receptor complex binds DNA and modulates gene expression.[2] Upon androgen stimulation, the proliferation of prostate cells is increased and a malignant tumor can develop.[2] In addition, the androgen receptor level is higher in prostate cancer cells compared to normal cells.[2] Consequently, androgens are involved not only in prostate tumorigenesis, but also in hormone-dependent cancer progression, supporting the use of androgen deprivation therapy in prostate cancer patients.

Androgens bind the AR by the fixation of two chemical groups to amino acids found on the receptor. The ketone at position 3 of the steroid nucleus can bind to Gln 711 and Arg 752 while the hydroxyl at position 17β binds to Asn 703 and Thr 877. These binding sites are very important as the activation of AR depends on the fixation of androgens on these specific amino acids.[3]

The most interesting position on the testosterone nucleus to perform chemistry is at position 7 (Fig. 1). This is the site of choice as it is located midway between the two functional groups (ketone and hydroxyl) that interact with the AR. These functional groups should remain intact for AR binding. As a result, the steroid-receptor interaction should be significant. However, the major problem for this site (carbon 7) is the absence of a functional group allowing further chemical transformations. Thus, it has to be introduced first before being able to modify this specific site of the steroid.

Our main goal is to synthesize a testosterone dimer that can exhibit antiandrogenic activity. The concept of dimers (or bivalent ligands) as bioactive molecules have attracted considerable attention over the years because of their promising therapeutic value for the treatment of several diseases.[4,5] Indeed, many receptors have to dimerize in order to activate their biological functions. Some studies showed that the increase of selectivity of a bivalent ligand may be due to the presence of two nearby binding sites which can be on different receptors.[4] AR-induced signaling necessitates dimerization of the receptor.[2] The idea of constructing a

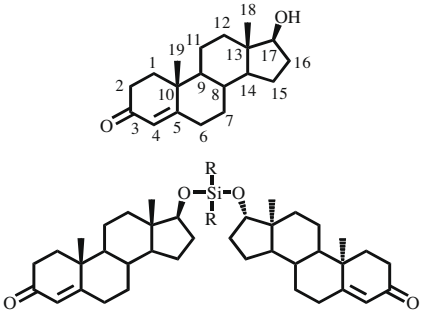

Figure 1. Testosterone structure and known testosterone dimers (R = Me or Ph).

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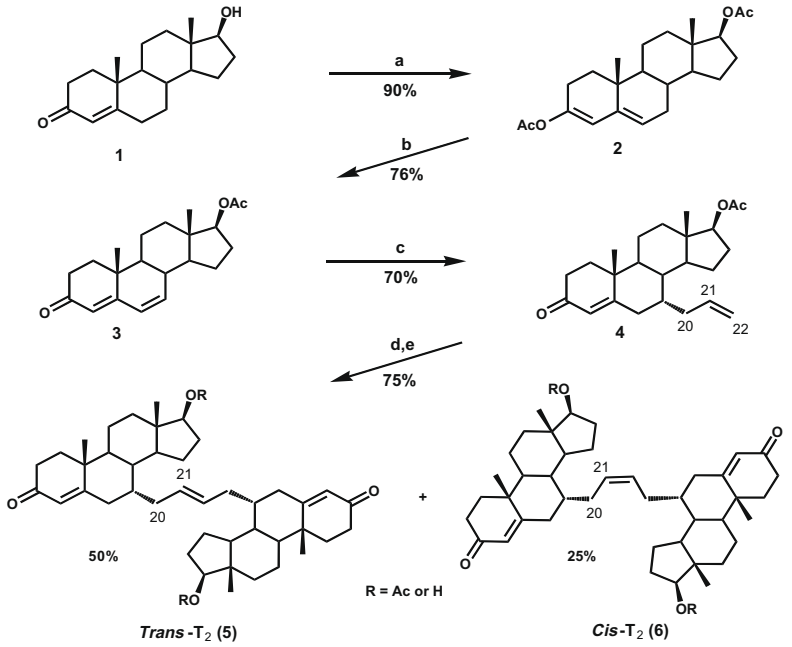

Scheme 1. Reagents and conditions: (a) AcCl, Ac_2O, Pyr, reflux, 4 h; (b) (1) NBS, DMF, 0 °C, 1.5 h, (2) Li_2CO_3, LiBr, DMF, 92°C, 4 h; (c) (1) $TiCl_4$, Pyr, DCM, −78 °C, 5 min, (2) allyltrimethylsilane, −30 °C; 1.5 h; (d) Hoveyda–Grubb's catalyst 2nd generation, CH_2Cl_2 (75%); (e) 10% aqueous HCl, MeOH, (95%).

testosterone dimer that could act as an antiandrogen by simultaneously binding two ARs is quite interesting. Indeed, the size of the spacer between the two moieties of a dimer has a direct impact on its biological activity. In fact, the length, geometry and conformational mobility of the tether chain can influence the orientation of the testosterone heads of the unbound dimer and thus, the affinity for its cognate receptor.[5] It is noteworthy that only a few testosterone dimers were reported in the literature. Some symmetric dimeric silyl ethers of testosterone were designed to act as prodrugs (Fig. 1). Hence the results showed that they were prodrugs of testosterone in an animal model.[6] For obvious reasons, these dimers cannot be used for antiandrogen therapy. The current Letter describes the synthesis of two new separable isomeric testosterone dimers; *trans*-T_2 (**5**) and *cis*-T_2 (**6**) (Scheme 1). The novel molecules are made from testosterone in only 5 chemical steps with an overall yield of 36%.

Testosterone (**1**) was initially functionalized using a known two-step reaction sequence (Scheme 1). For the first reaction, testosterone was treated with acetyl chloride and acetic anhydride in the presence of pyridine.[7] This reaction gave the diacetate **2** with 95% yield. The ^{1}H NMR spectrum showed a new triplet at 5.36 ppm corresponding to the alkene proton on carbon 6. The proton environment next to carbon 17 changed from a hydroxyl group (C–H at 3.62 ppm) to an acetate group (C–H at 4.60 ppm). Compound **2** was further transformed into the dienone acetate **3** upon treatment of NBS, Li_2CO_3 and LiBr at reflux for 2 h in DMF. The double bond migrated back on carbons 4 and 5 and a new double bond was created on the carbon 6 and 7 (6.09 ppm, ^{1}H NMR). Derivative **3** was obtained with 76% yield.[7]

The next step was a Michael addition of an allyl chain on derivative **3** upon treatment with $TiCl_4$ and allyltrimethylsilane in the presence of pyridine. This reaction was stereospecific.[8] The allyl chain was added at position 7α of the steroid nucleus. The 7α-allyltestosterone **4** was obtained with 70% yield. The chain on carbon 7 was identified by ^{1}H NMR showing two distinct signals at 5.00 ppm and at 5.60 ppm corresponding to the three alkene protons of the allyl chain.

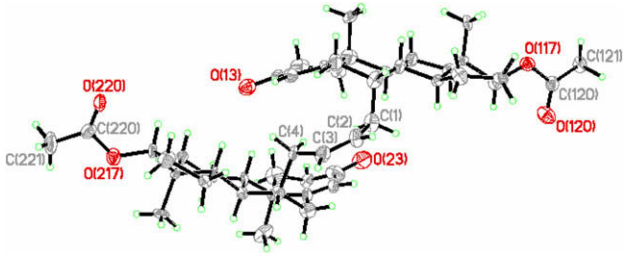

Figure 2. Crystal structure of the diacetate *cis*-T_2 (**6**).

Table 1
Yield obtained with different assays of Grubb's metathesis

Experimental conditions[a]	Yield (%)
2nd generation Grubb's catalyst (0.1 equiv) 9 h reflux	55
2nd generation Grubb's catalyst (0.1 equiv) 15 h reflux	55
2nd generation Grubb's catalyst (0.5 equiv) 9 h reflux	55
2nd generation Hoveyda–Grubb's catalyst (0.1 equiv) 9 h reflux	75

[a] All the reactions were performed in CH_2Cl_2.

Grubb's metathesis[9] was performed with 7α-allyltestosterone **4** to obtain the testosterone dimers *trans*-T_2 (**5**) and *cis*-T_2 (**6**) with 75% yield. The synthesis led to two separable isomeric dimers (*trans*-T_2 (**5**) and *cis*-T_2 (**6**), 2:1). C_2-Symmetry was confirmed by ^{13}C NMR which showed only 23 distinct carbons for both dimers. As anticipated, the minor product obtained is the *cis* isomer as confirmed by X-ray crystallography (Fig. 2).[10] The two testosterone units are linked with an unsaturated four carbon atom chain.

The isomeric dimers were separable by flash column chromatography. This, in itself, constitutes a very interesting result as it

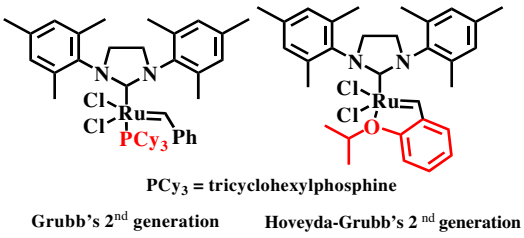

Figure 3. Catalysts used for the Grubb's metathesis.

is quite unusual to separate easily two relatively large olefinic isomers. In fact, the physicochemical properties of large olefinic isomers are so similar that they are normally not separable by standard chromatographic techniques. This dimerization reaction was initially optimized to 75% yield. Table 1 presents some of the results obtained with various experimental conditions. Initially, the Grubb's catalyst 2nd generation was used with various reaction conditions. Regardless of the catalyst's quantity or time of reflux, the total yield was only about 55% (*trans*-T_2 (**5**), 30% and *cis*-T_2 (**6**), 25%). On the other hand, when Hoveyda–Grubb's catalyst 2nd generation was used, the overall yield increased to 75%. The only difference between these two catalysts is the presence of the isopropoxybenzene group (in red) in the Hoveyda–Grubb's catalyst (Fig. 3). As reported in the literature, the higher basicity of this group brings a higher catalytic activity than the other group (PCy_3).[11] A trial reaction was performed with benzene as the solvent, but no metathesis occurred. This could suggest that the choice of the solvent is also important to perform this particular metathesis.

Finally, each of the protected dimers was hydrolyzed with HCl in methanol to give the final derivatives with 95% yield. All new compounds synthesized were characterized by IR, NMR spectroscopy and mass spectrometry.[12]

The second objective of the present study was also to determine the cytotoxic effect of these novel molecules using androgen-dependent (androgen receptor positive; AR^+) and androgen-independent (androgen receptor negative; AR^-) human prostate cancer cells. The biological activity of these compounds was evaluated in vitro using the MTT cell proliferation assay.[13,14] The MTT assay was performed over an incubation period of 72 h.

As shown by the MTT assays (Table 2), the new *cis*-T_2 (**6**) dimer showed higher toxicity towards the two human prostate cancer cell lines used in our study (LNCaP (AR^+) and PC3 (AR^-)) compared to the *trans*-T_2 (**5**) dimer. This supports the idea that the double bond geometry of the dimer influences its biological activity. In fact, for *cis*-T_2 (**6**) we observed IC_{50} values of 30.3 μM and 24.7 μM for, respectively, LNCaP and PC3 while the isomer *trans*-T_2 (**5**) exhibited an IC_{50} of 35.7 μM for PC3 cell line and was completely inactive towards the LNCaP cell line at the maximum dose tested (80 μM, see Table 2). The latter derivative might be useful in the treatment of hormone-independent prostate cancer. Interestingly, the dimer *cis*-T_2 (**6**) is slightly more cytotoxic than cyproterone acetate (CPA) (Fig. 4), a clinically used steroid-based antiandrogen.

Table 2
Inhibitory concentration[a] of cyproterone acetate, *trans*-**5** and *cis*-**6** on both AR^+ and AR^- prostate cancer cell lines

Compounds	LNCap (AR^+) IC_{50}[a] (μM)	PC3 (AR^-) IC_{50}[a] (μM)
Cyproterone acetate	43.0 ± 2.5	32.3 ± 3.7
trans-T_2 (**5**)	NR	35.7 ± 3.7
cis-T_2 (**6**)	30.3 ± 0.7	24.7 ± 1.5

NR: Not reached.
[a] Inhibitory concentration (IC_{50}, μM) as obtained by the MTT assay. Experiments were performed in duplicates and the results represent the mean ± SEM of three independent experiments. The cells were incubated for a period of 72 h.

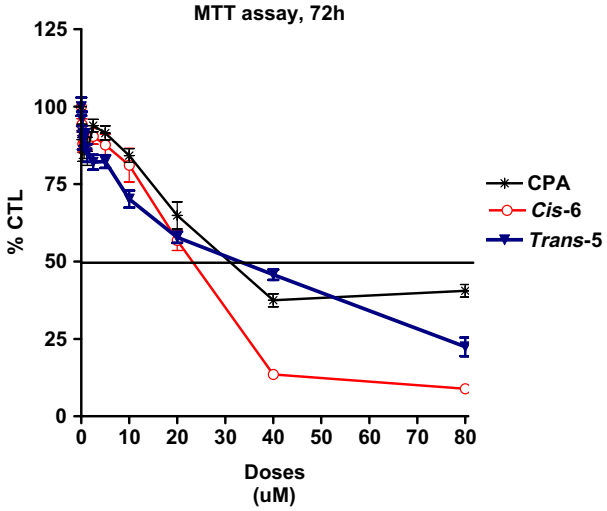

Figure 4. Dose–response curves for cyproterone acetate (CPA), *trans*-T_2 (**5**, R = H) and *cis*-T_2 (**6**, R = H) as obtained by the MTT assay for 72 h treatment on PC3 cell line.

Of note, and contrary to our expectations, both testosterone dimers are more active against the hormone-independent cell line, PC3, than toward hormone-dependent cell line LNCaP. Similarly, cyproterone acetate (CPA) was also more active on the PC3 cells than LNCaP cells (Table 2). This result could be explained by the fact that the androgen receptor of LNCaP cells is mutated in the ligand binding domain.[15] The dimeric molecules could possibly have a lower affinity for the receptor and this could be verified with a receptor affinity assay. However, it should be emphasized that LNCaP and PC3 cells present multiple differences apart from AR status. It is thus possible that LNCaP cells have a higher intrinsic resistance to growth suppression compared to PC3 cells. In vivo biological assays will later allow us to determine the selectivity of these compounds towards hormone-dependent prostate tumors.

In summary, this Letter presents the synthesis of two novel testosterone dimers (*trans*-T_2 (**5**) and *cis*-T_2 (**6**)). They are readily available from testosterone in a 5 steps sequence with overall yields of 36% (24% for *trans*-T_2 (**5**) and 12% for *cis*-T_2 (**6**)). The key dimerization step involved the use of Hoveyda–Grubbs catalyst 2nd generation yielding 75% of a separable mixture of the isomeric dimers. It is noteworthy that such large olefinic isomers can be separated by simple flash chromatography. Also, X-ray diffraction crystallography confirmed the structure of the *cis*-T_2 (**6**) dimer. MTT assays were performed on an androgen-dependent and androgen-independent prostate cancer cell lines, LNCaP and PC3 respectively. The *cis* dimer had higher biological effect than the *trans* dimer. Interestingly, the *trans* dimer is only active on androgen-independent prostate cancer cell (PC3). This demonstrates that the double bond geometry has an important effect on the cytotoxic activity of the two dimers. Furthermore, the *cis* dimers had a potent growth-suppressive effect on androgen-dependent as well as androgen-independent prostate cancer cells in vitro. Further research will be necessary to evaluate the complete biological potential of these two unique testosterone dimers.

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References and notes

1. Jin, Y.; Penning, T. M.. *Best Pract. Res. Clin. Endocrinol. Metab.* **2001**, *15*, 79.
2. Heinlein, C. A.; Chang, C. *Endocr. Rev.* **2004**, *25*, 276.
3. Pereira de Jesus-Tran, K.; Cote, P. L. *Protein Sci.* **2006**, *15*, 987.
4. Portoghese, P. S. *J. Med. Chem.* **1992**, *35*, 1927.
5. Bérubé, G. *Curr. Med. Chem.* **2006**, *13*, 131.
6. Millership, J. S.; Shanks, M. L. *J. Pharm. Sci.* **1988**, *77*, 116.
7. Bucourt, R.; Vignau, M.; Torelli, V.; Richard-Foy, H.; Geynet, C.; Secco-Millet, C.; Redeuilh, G.; Baulieu, E.-E. *J. Biol. Chem.* **1978**, *253*, 8221.
8. Nickisch, K.; Laurent, H. *Tetrahedron Lett.* **1988**, *29*, 1533.
9. Yee, N. K.; Farina, V. *J. Org. Chem.* **2006**, *71*, 7133.
10. Cambridge Crystallographic Data Center (CCDC) deposit #765942. The compound was recrystallized using a mixture of dichloromethane and diethyl ether. The presence of minute amount of chlorine atom in the final resolution of the crystalline structure was observed and is believed to have occurred during the recrystallization process.
11. Trnka, T. M.; Grubbs, R. H. *Acc. Chem. Res.* **2001**, *34*, 18.
12. Anhydrous reactions were performed under an inert atmosphere; the setup was assembled and cooled under nitrogen. Unless otherwise noted, starting material, reactant and solvents were obtained commercially and were used as such or purified and dried by standard means.[16] Organic solutions were dried over magnesium sulfate ($MgSO_4$), filtered and evaporated on a rotary evaporator under reduced pressure. All reactions were monitored by UV fluorescence or staining with iodine. Commercial TLC plates were Sigma T 6145 (polyester silica gel 60 Å, 0,25 mm). Preparative TLC was performed on 1 mm silica gel 60 Å, 20×20 plates (Whatman, 4861 840). Flash column chromatography was performed according to the method of Still et al.[17] on Merck grade 60 Silica Gel, 230–400 mesh. All solvents used in chromatography were distilled.
    The infrared spectra were taken on a Nicolet Impact 420 FT-IR spectrophotometer. Mass spectral assays for derivatives **2**–**4** were obtained using a VG Micromass 7070 HS instrument using an ionization energy of 70 eV (Université de Sherbrooke). Derivatives **5** and **6** (R = H or CO_2CH_3) were analyzed using a MS model 6210, Agilent technology instrument. The high resolution mass spectra (HRMS) were obtained by TOF (time of flight) using ESI (electrospray ionization) using the positive mode (ESI+) (Université du Québec à Montréal). Nuclear magnetic resonance (NMR) spectra were recorded on a Varian 200 MHz NMR apparatus. Samples were dissolved in deuterochloroform ($CDCl_3$), or deuteroacetone (acetone-d_6) for data acquisition using tetramethylsilane or chloroform as internal standard (TMS, δ 0.0 ppm for ^{1}H NMR and $CDCl_3$ δ 77.0 ppm for ^{13}C). 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.
    Synthesis of 3,5-androstadien-3,17β-diol diacetate (**2**): Acetyl chloride (20.9 mL, 281.53 mmol), acetic anhydride (6.24 mL, 77.2 mmol) and pyridine (1.82 mL, 19.3 mmol) were added to testosterone (5.57 g, 19.3 mmol). The solution was stirred 4 h at reflux and then 30 min at room temperature. The solvents were evaporated to dryness under vacuum. The steroid was dissolved in dichloromethane and filtered on silica gel. The solvent were evaporated to obtain 6.46 g of the diacetate **2** (crude yield 90%). No flash chromatography was needed for that step. The crude material showed a single spot on thin layer chromatography and was used as such for the next transformation. **IR** (NaCl, ν_{max}, cm^{-1}): 1736 (C=O), 1666 (C=C), 1248 (C–O); ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) : 5.67 (1H, s, **4-CH**), 5.36 (1H, m, **6-CH**), 4.59 (1H, t, J = 8.2 Hz, **17-CH**), 2.11 (3H, s, **3-OAc**), 2.02 (3H, s, **17-OAc**), 0.99 (3H, s, **19-CH$_3$**), 0.81 (3H, s, **18-CH$_3$**)); ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 171.4 (**17-OAc**), 169.6 (**3-OAc**), 147.3 (**C-3**), 139.7 (**C-5**), 123.7 (**C-6**), 117.1 (**C-4**), 82.9 (**C-17**), 51.4, 48.1, 42.7, 36.9, 35.2, 33.9, 31.8, 31.6, 27.7, 25.0, 23.7, 21.4, 21.3, 20.9, 19.1, 12.3. MS (*m/e*): 372 (M$^+$), 330 (M$^+$–C_2H_2O), exact mass: calcd for $C_{23}H_{32}O_4$: 372.2300; found: 372.2297.
    Synthesis of 4,6-androstadien-17β-ol-3-one acetate (**3**): Under a nitrogen atmosphere, DMF (70 mL) and water (3 mL) were combined with the diacetate **2** (6.46 g, 17.3 mmol) and cooled to 0 °C. NBS was added over a period of 1 h and stirred for an additional 40 min at 0 °C. Li_2CO_3 and LiBr were added to the mixture at room temperature. The mixture was heated for 4 h at 95 °C and then was poured in a water/ice solution containing 150 mL of water and 10 mL of acetic acid. The crude compound **3** was filtered and washed with water and dried. Then, the crude material was purified by flash chromatography with a mixture of hexane/acetone (9:1) to give **3** (4.33 g, 76% yield). IR (NaCl, ν_{max}, cm^{-1}): 1735 (C=O), 1664 (C=O), 1613 (C=C), 1252 (C–O); ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) : 6.09 (2H, s, **6-CH** and **7-CH**), 5.65 (1H, s, **4-CH**), 4.61 (1H, t, J = 7.8 Hz, **17-CH**), 2.03 (3H, s, **17-OAc**) 1.10 (3H, s, **19-CH$_3$**), 0.86 (3H, s, **18-CH$_3$**).; ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 199.3 (**C-3**), 171.3 (**17-OAc**), 163.8 (**C-5**), 140.3 (**C-7**), 128.4 (**C-4**), 124.0 (**C-6**), 82.3 (**C-17**), 50.8, 48.3, 43.6, 37.6, 36.8, 36.7, 36.3, 34.1, 27.7, 23.3, 21.3, 20.4, 16.5, 12.2. MS (*m/e*) 328 (M$^+$), 286 (M$^+$- C_2H_2O), exact mass: calcd for $C_{21}H_{28}O_3$: 328.2038; found: 328.2032.
    Synthesis of 7α-allyl-4-androsten-17β-ol-3-one acetate (**4**): Under an inert atmosphere of nitrogen, the steroid **3** was dissolved in dry dichloromethane and cooled to −78 °C. Then, titanium(IV) chloride (3.58 mL, 32.6 mmol) and pyridine (0.65 mL, 6.39 mmol) were added to the solution. The mixture was stirred for 5 min; allyltrimethylsilane was added, stirred for 1.5 h at −78 °C and 1.5 h at −30 °C. The black mixture was diluted with ether, washed with a 2% HCl solution (2×20 mL) and with water (4×20 mL). The organic phase was dried, filtered and concentrated to a solid. The crude steroid was purified by flash chromatography with hexane/acetone (9:1) as the eluent. The crystalline compound **4** was obtained in good yield (1.67 g, 70%). IR (NaCl, ν_{max}, cm^{-1}): 1736 (C=O) 1678 (C=O), 1616 (C=C), 1243 (C–O); ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) : 5.70 (1H, s, **4-CH**), 5.60 (1H, m, –*CH*=*C*H$_2$), 5.00 (2H, m, –CH=*CH*$_2$), 4.60 (1H, t, J = 8.4 Hz, **17-CH**), 2.03 (3H, s, **17-OAc**), 1.20 (3H, s, **19-CH$_3$**), 0.84 (3H, s, **18-CH$_3$**); ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 199.3 (**C-3**), 171.3 (**17-OAc**), 169.4 (**C-5**), 137.0 (**C-21**), 126.4 (**C-4**), 117.0 (**C-22**), 82.6 (**C-17**), 47.2, 46.2, 42.8, 38.9, 38.5, 36.7, 36.3, 36.2, 36.1, 34.2, 30.4, 27.6, 23.1, 21.4, 20.9, 18.2, 12.1. MS (*m/e*) 370 (M$^+$), 312 (M$^+$–$C_2H_2O_2$), exact mass: calcd for $C_{24}H_{34}O_3$: 370.2508; found: 370.2505.
    Synthesis of dimers of testosterone trans-T$_2$ (**5**) *and cis-T$_2$* (**6**): Under nitrogen, the steroid **4** (0.56 g, 1.51 mmol) was dissolved in dry dichloromethane (8 mL) and Hoveyda–Grubbs 2nd generation (90 mg, 0.15 mmol) was added to that solution. The mixture was stirred overnight at reflux and then 30 min at room temperature. The solvent was evaporated. The product was purified by flash chromatography (hexane/acetone, 95:5). That reaction gave two separable isomeric dimers. The major product (*trans*-T$_2$ (**5**), 0.26 g) was obtained with 50% yield, while the minor product (*cis*-T$_2$ (**6**), 0.13 g) was obtained with 25% yield. Thin layer chromatography using hexane/acetone, 4:1 gave rf: 0.28 for *cis*-T$_2$ (**6**) and rf: 0.24 for *trans*-T$_2$ (**5**). The dimers were hydrolysed, separately, using a 5 N HCl solution in methanol at reflux for 4.5 h. The crude product was washed with a 5% $NaHCO_3$ aqueous solution. The organic phase was washed with water. The solvent was dried, filtered and concentrated to a solid. The dimers were obtained with 95% yield; no purification was needed as the crude material was pure.
    trans-T$_2$ (**5**) (R = $COCH_3$): mp: 123–126 °C; IR (NaCl, ν_{max}, cm^{-1}): 1734 (C=O), 1673 (C=O), 1611 (C=C), 1250 (C–O); ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) : 5.63 (1H, s, **4-CH**), 5.14 (1H, m, **21-CH**), 4.59 (1H, t, J = 8.2 Hz, **17-CH**), 2.03 (3H, s, **17-OAc**), 1.19 (3H, s, **19-CH$_3$**), 0.83 (3H, s, **18-CH$_3$**); ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 199.3 (**C-3**), 171.4 (**17-OAc**), 169.8 (**C-5**), 131.1 (**C-21**), 126.3 (**C-4**), 82.7 (**C-17**), 47.1, 46.2, 42.7, 38.9, 38.3, 36.6, 36.4, 36.1, 34.2, 31.2, 29.3, 27.6, 23.1, 21.4, 20.9, 18.2, 12.1. ESI+ HRMS: (M+H)$^+$ calcd for $C_{46}H_{65}O_6$: 713.4776; found: 713.4773 (M+H)$^+$.
    trans-T$_2$ (**5**) (R = OH): mp: 225–228 °C; IR (NaCl, ν_{max}, cm^{-1}): 3422 (O–H), 1656 (C=O), 1217 (C–O); ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) : 5.65 (1H, s, **4-CH**), 5.17 (1H, m, **21-CH**), 3.65 (1H, t, J = 5.1 Hz, **17-CH**), 1.21 (3H, s, **19-CH$_3$**), 0.80 (3H, s, **18-CH$_3$**); ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 199.3 (**C-3**), 169.9 (**C-5**), 131.1 (**C-21**), 126.3 (**C-4**), 81.9 (**C-17**), 47.3, 46.4, 43.1, 39.0, 38.6, 36.6, 36.4, 36.1, 34.2, 30.6, 29.9, 29.3, 23.0, 21.1, 18.2, 11.1. ESI+HRMS: (M+H)$^+$ calcd for $C_{42}H_{61}O_4$: 629.4564; found: 629.4563 (M+H)$^+$.
    cis-T$_2$ (**6**) (R = $COCH_3$): mp: 241–244 °C; IR (NaCl, ν_{max}, cm^{-1}): 1734 (C=O), 1673 (C=O), 1250 (C–O); ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) : 5.61 (1H, s, **4-CH**), 5.30 (1H, m, **21-CH**), 4.65 (1H, t, J = 8.2 Hz, **17-CH**), 2.03 (3H, s, **17-OAc**), 1.17 (3H, s, **19-CH$_3$**), 0.83 (3H, s, **18-CH$_3$**); ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 198.9 (**C-3**), 171.3 (**17-OAc**), 169.8 (**C-5**), 129.8 (**C-21**), 126.6 (**C-4**), 82.8 (**C-17**), 47.0, 46.1, 42.7, 38.8, 38.5, 37.0, 36.4, 36.7, 36.1, 34.2, 27.7, 24.7, 23.2, 21.4, 21.0, 18.3, 12.1. ESI+HRMS: (M+H)$^+$ calcd for $C_{46}H_{65}O_6$: 713.4776; found: 713.4773 (M+H)$^+$.
    cis-T$_2$ (**6**) (R = OH): mp: 127–130 °C; IR (NaCl, ν_{max}, cm^{-1}): 3424 (O–H), 1658 (C=O), 1217 (C–O); ^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) : 5.64 (1H, s, **4-CH**), 5.29 (1H, m, **21-CH**), 3.78 (1H, t, J = 6.9 Hz, **17-CH**), 1.18 (3H, s, **19-CH$_3$**), 0.79 (3H, s, **18-CH$_3$**); ^{13}C NMR (50 MHz, $CDCl_3$, δ ppm): 199.0 (**C-3**), 170.5 (**C-5**), 129.9 (**C-21**), 126.6 (**C-4**), 81.6 (**C-17**), 47.3, 46.3, 43.1, 38.84, 38.80, 36.9, 36.7, 36.2, 34.2, 30.4, 29.9, 24.9, 23.0, 21.1, 18.3, 11.2. ESI+HRMS: (M+H)$^+$ calcd for $C_{42}H_{61}O_4$: 629.4564; found: 629.4558 (M+H)$^+$.
13. Carmichael, J.; DeGrapp, W. G.; Gazdar, A. F.; Minna, J. D.; Mitchell, J. B. *Cancer Res.* **1987**, *47*, 936.
14. Ford, C. H. J.; Richardson, V. J.; Tsaltas, G. *Cancer Chemother. Pharmacol.* **1989**, *24*, 295.
15. Veldscholte, J.; Berrevoets, C. A. *Biochemistry* **1992**, *31*, 2393.
16. Perrin, D. D.; Armarego, W. L. F. *Purification of Laboratory Chemicals*, 3rd ed.; Pergamon Press: Oxford, 1988.
17. Still, W. C.; Kahn, M. *J. Org. Chem.* **1978**, *43*, 2923.