Design, Synthesis and *In Vitro* Evaluation of 4-Androstene-3,17-dione/Adenosine Hybrid Compounds as Bisubstrate Inhibitors of Type 3 17β-Hydroxysteroid Dehydrogenase

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Abstract: Steroidogenic enzyme type 3 17β-hydroxysteroid dehydrogenase (17β-HSD) is an important therapeutic target for androgen-sensitive diseases. This enzyme selectively reduces the C17 ketone of 4-androstene-3,17-dione (Δ^4-dione), thus producing testosterone (T) using NADPH as cofactor. Our group previously synthesized hybrid (estradiol/adenosine) inhibitors that successfully inhibit the biosynthesis of the potent estrogen estradiol by type 1 17β-HSD. To similarly lower the level of the potent androgen testosterone, inhibitors of type 3 17β-HSD were designed and synthesized applying the same hybrid (substrate/cofactor) strategy. Two chemical approaches were developed to join the three components of the bisubstrate inhibitor (the substrate Δ^4-dione, an alkyl spacer and the cofactor moiety adenosine). An alkylation in the α position of steroidal 17-ketone or a cross-metathesis was used as a key step to efficiently join the substrate and the alkyl spacer, whereas an esterification was employed to link the spacer to adenosine. An enzymatic assay in homogenated HEK-293 cells overexpressing type 3 17β-HSD revealed that the best inhibitors of that series are those bearing an alkyl side-chain spacer of 11 or 12 methylenes: inhibition of 69 and 78% at 1 μM were respectively observed. As expected, these bisubstrate inhibitors were less potent in intact cells than in homogenated cells. However, both enzymatic assays revealed that the strategy of substrate/cofactor dual inhibitors seems to work for type 3 17β-HSD, although the inhibitors designed have not been optimized yet.

Key Words: Inhibitor, enzyme, 17β-hydroxysteroid dehydrogenase, type 3 17β-HSD, steroid, hormone, androgen, cross-metathesis.

INTRODUCTION

Estrogens and androgens, female and male sex steroids, are essential to the reproductive process. They are also involved in general metabolic activities in a variety of peripheral tissues. However, despite their positive effect in both reproductive and non-reproductive organs, these hormones are involved in the development of estrogen- and androgen-sensitive diseases such as breast and prostate cancers [1,2]. The last step in the biosynthesis of potent estrogens and androgens is catalyzed by 17β-hydroxysteroid dehydrogenases (17β-HSDs) [3-8]. More precisely, these enzymes modulate the biological potency of estrogens and androgens by interconversion between 17-ketosteroids and 17β-hydroxysteroids. Until now, twelve different isoforms have been identified [3]. Although these enzymes catalyze both oxidative and reductive reactions in homogenated cells, it was observed that, in intact cells, they drive the reaction unidirectionally [9]. Human types 1, 3, 5, 7 and 12 are reductive enzymes, whereas types 2, 4, 8, 10 and 11 are oxidative ones. Types 6 and 9 have not yet been cloned from man [6]. Furthermore, 17β-HSDs use the nicotinamide-adenine dinucleotide, reduced [NAD(P)H] or not [$NAD(P)^+$], as cofactor. Interestingly, the isozymes that catalyze the reductive reaction prefer a phosphorylated cofactor (NADPH), whereas the oxidative enzymes prefer NAD^+ [7]. In addition, 17β-HSD activity is not only present in classical steroidogenic tissues such as ovaries, placenta and testes, but it is also found in a large series of peripheral tissues such as liver, adipose tissue, skin, breast and cancer cells [7]. Considering the key role of steroidogenic enzymes of the 17β-HSD family, several research groups, including our laboratory, are very interested in developing inhibitors of 17β-HSDs [10-12].

In our project to develop potent inhibitors of steroidogenic enzymes, we were interested in developing a new class of inhibitors of type 3 17β-HSD. This enzyme, also called androgenic 17β-HSD or testicular 17β-HSD, is a protein of 310 amino acids with a molecular mass of 34 513 [7,8,13]. Out of the 16 tissues tested by Northern blot analysis, the type 3 mRNA species was detected only in the testes [14]. It is noteworthy that mutations in the HSD17B3 gene, and consequently a deficiency of the enzyme, cause an autosomal recessive form of male pseudohermaphroditism [15]. Type 3 17β-HSD is a microsomal enzyme using NADPH as cofactor. It stereoselectively reduces the C17 ketone of 4-androstene-3,17-dione (Δ^4-dione) into testosterone (T), a potent androgenic compound, which is further converted to dihydrotestosterone (DHT), the most potent androgen, by 5α-reductase (Fig. (**1**)) [11,16-18]. The third type of human 17β-HSD thus plays a crucial role in the biosynthesis of the most potent androgens (T and DHT). The development of

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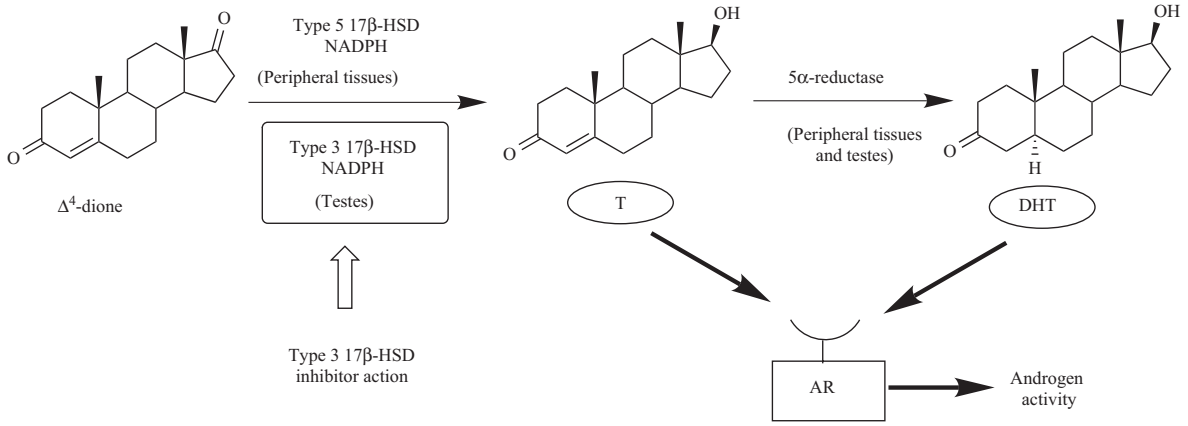


Fig. (1). Role of type 3 17β-HSD in the transformation of 4-androstene-3,17-dione (Δ^4-dione, inactive androgen) into active androgens testosterone (T) and dihydrotestosterone (DHT).

selective inhibitors against it constitutes a new therapeutic approach for the treatment of androgen-sensitive diseases [19,20]. These include, but are not limited to, benign prostatic hyperplasia [21], prostate cancer [22,23], acne [24,25], hirsutism [26], and male-pattern baldness [27,28]. Also, because type 3 17β-HSD is found almost exclusively in the testes, this enzyme could be an interesting target for blocking spermatogenesis and an inhibitor could be used as an anti-fertility agent for males [29]. Pittaway, in 1983, was the first to report a study on the inhibition of type 3 17β-HSD [30]. Until now, other compounds reported as inhibitors have consisted in steroidal pyrazoles and isoxazoles [31], phytoestrogens [32,33] and androsterone derivatives [34-39]. Now, we wish to report a new class of type 3 17β-HSD inhibitors: the Δ^4-dione/adenosine hybrid compounds **1**-**7** (Fig. (**2**)). These bisubstrate inhibitors were designed to interact with both the substrate (Δ^4-dione) and the cofactor (NADPH) binding sites of the enzymes.

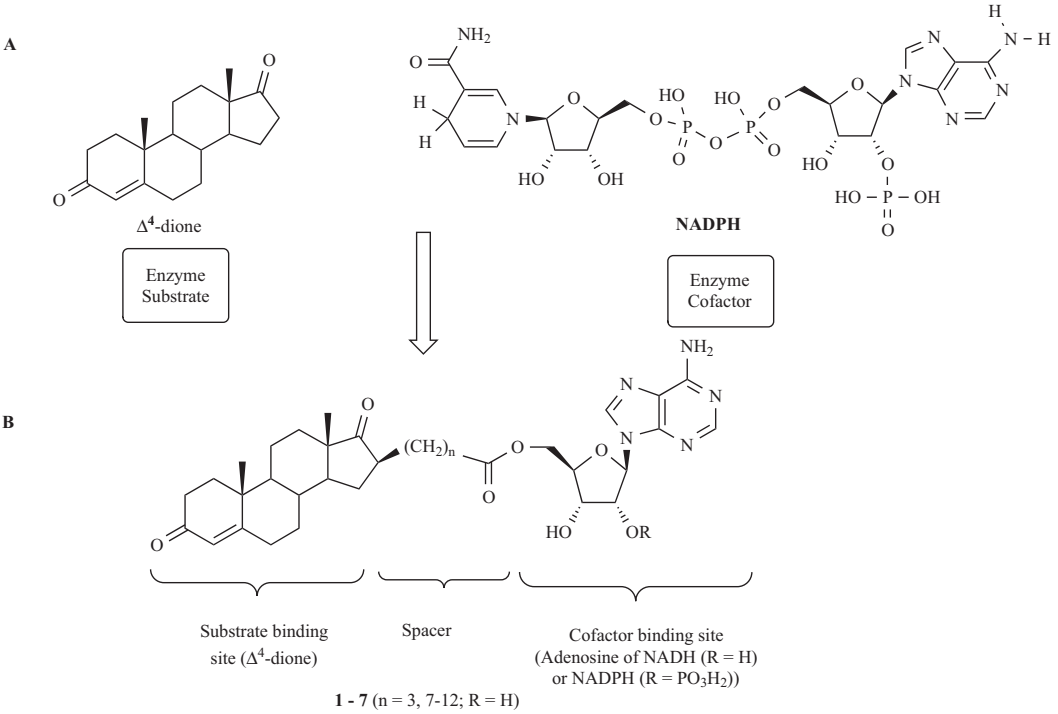


Fig. (2). *A:* Representation of the natural substrate Δ^4-dione and the cofactor NADPH during the enzymatic process catalyzed by type 3 17β-HSD. *B:* Proposed bisubstrate inhibitors of type 3 17β-HSD.

Inhibitors of the bisubstrate type have already been described for several enzymes such as adenylosuccinate synthetase [40], farnesyltransferase [41,42], glycosyltransferases [43], catechol-*O*-methyltransferase [44], serotonin N-acetyltransferase [45], protein kinases [46,47] and estrogen sulfotransferase [48]. Bisubstrate inhibitors were also developed by our research group for type 1 17β-HSD [49, 50]. For this steroidogenic enzyme, estradiol (E_2) and adenosine were chosen to interact with their respective binding site and the moieties were linked by an alkyl side-chain spacer. After optimization of the orientation and length of the spacer, EM-1745 (**8**) was found to be a potent *in vitro* inhibitor of type 1 17β-HSD with an IC_{50} of 52 nM and a K_i of 3 nM (Fig. (**3**)). Furthermore, the compound was crystallized inside the enzyme and X-ray analysis confirmed that it interacts with both binding sites [49]. Because type 3 17β-HSD belongs to the same family as type 1, the short chain dehydrogenase/reductase (SDR) family [51], we hypothesized that the same hybrid (substrate/cofactor) strategy could be applied to the development of potent type 3 inhibitors. We thus designed a series of Δ^4-dione/adenosine hybrid compounds (Fig. (**2**)). As for EM-1745, the two moieties (Δ^4-dione and adenosine) of the compound are linked by an alkyl side-chain spacer, the length of which yet remains to be optimized. The main structural difference between bisubstrate inhibitors of type 3 and type 1 17β-HSD is the nature of the substrate part: Δ^4-dione was chosen for compounds **1**-**7** instead of E_2 because type 3 is an androgenic enzyme. In both cases, cofactor NADH was chosen for the adenosine moiety instead of NADPH in order to simplify the chemical synthesis. We report herein the two chemical approaches developed for preparing these new bisubstrate inhibitors of type 3 17β-HSD. The *in vitro* biological evaluation of these compounds will also be described and discussed.

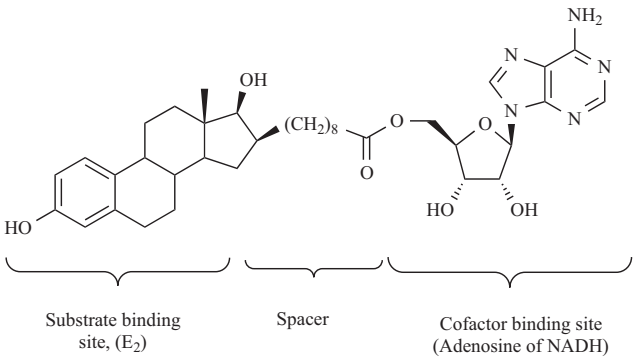
RESULTS AND DISCUSSION

Chemical Synthesis

Bisubstrate inhibitors of type 3 17β-HSD (compounds **1**-**7**) are composed of three moieties: the substrate (Δ^4-dione as the steroid nucleus), the spacer (an alkyl side-chain) and the cofactor (NADH as the adenosine moiety). Because the alkyl side-chain spacer of the most potent hybrid inhibitor of type 1 17β-HSD (EM-1745, **8**) is composed of 8 methylenes, we assumed that the best spacer for the type 3 inhibitor should have about the same length. Thus, we decided to synthesize bisubstrate inhibitors with an alkyl side-chain spacer of 7 to 9 methylenes (compounds **2**-**4**). A compound with a spacer of only 3 methylenes (compound **1**) was also prepared for comparison purposes.

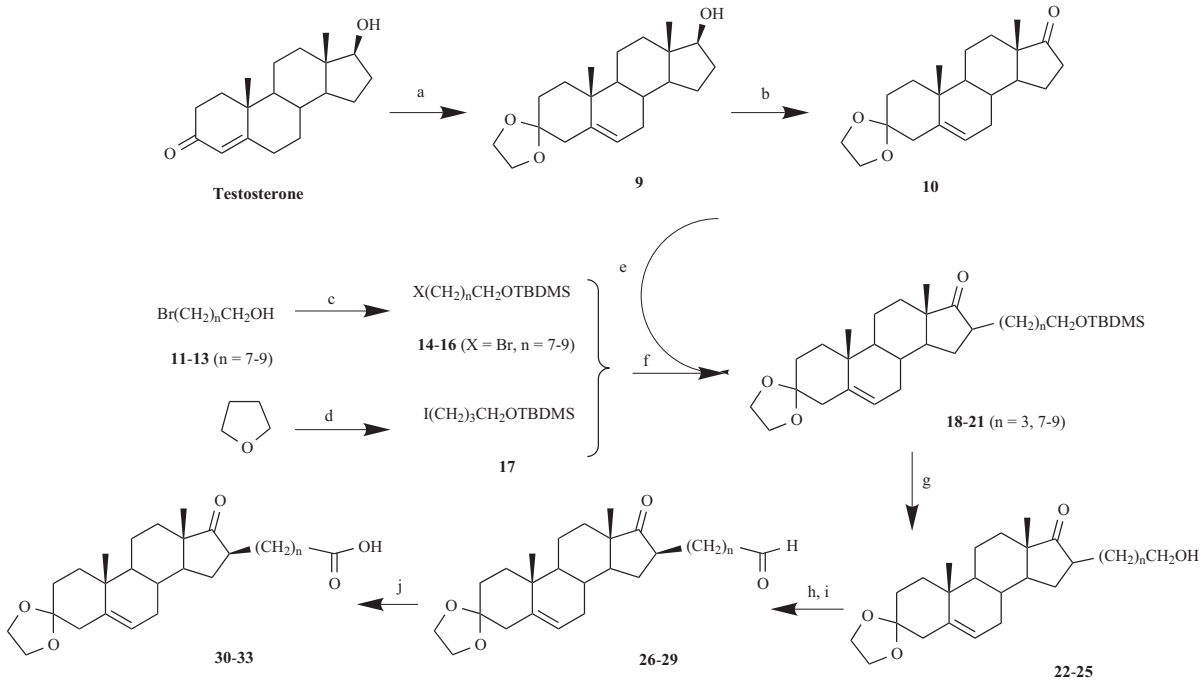
Compounds **1**-**4** were synthesized through an approach similar to that previously used to prepare type 1 17β-HSD hybrid inhibitors [50,52]. First, carboxylic acids **30**-**33** bearing an alkyl side-chain spacer of 3, 7, 8 and 9 methylenes were synthesized as shown in Scheme (**1**). Starting from commercially available testosterone, the C3 ketone was protected as a ketal using ethylene glycol, *p*-TSA and benzene. Under these conditions, the Δ^4-olefin is isomerised to a Δ^5-olefin affording compound **9** in 89% yield. The 17β-hydroxy group of **9** was next subjected to oxidation under TPAP conditions giving **10** in an excellent 86% yield. The next step, an alkylation in the α position of steroidal 17-ketone could only have been achieved with an activated electrophile if using LDA as base [53]; this methodology is thus not appropriate for introducing long alkyl side chains needed for the synthesis of **2**-**4**. A three-step strategy (activation, alkylation in the α position of 17-ketone and decarboalkoxylation) for introducing long alkyl side-chains was also known [54,55]. However, a direct alkylation in the α position of the 17-ketone steroid of **10**, using the strong base LiHMDS, was preferred for generating **18**-**21** because fewer steps were necessary. A TBDMS protective group was chosen instead of the THP used in the synthesis of type 1 17β-HSD hybrid inhibitors, because the ketal group of the steroid nucleus could not resist under the acid conditions used to deprotect the side-chain alcohol.

Bromoalcohols **11**-**13** were thus protected as TBDMS derivatives **14**-**16** in good 65-83% yields using classical conditions. Protected 4-iodobutanol (**17**) was alternatively obtained from THF by a treatment with TBDMSCl and NaI under reflux (88% yield) [56]. Alkylation in the α position of the 17-ketone of steroid **10** was done using LiHMDS as base



EM-1745 (**8**)

Fig. (3). Hybrid inhibitor EM-1745: a potent inhibitor of type 1 17β-HSD.



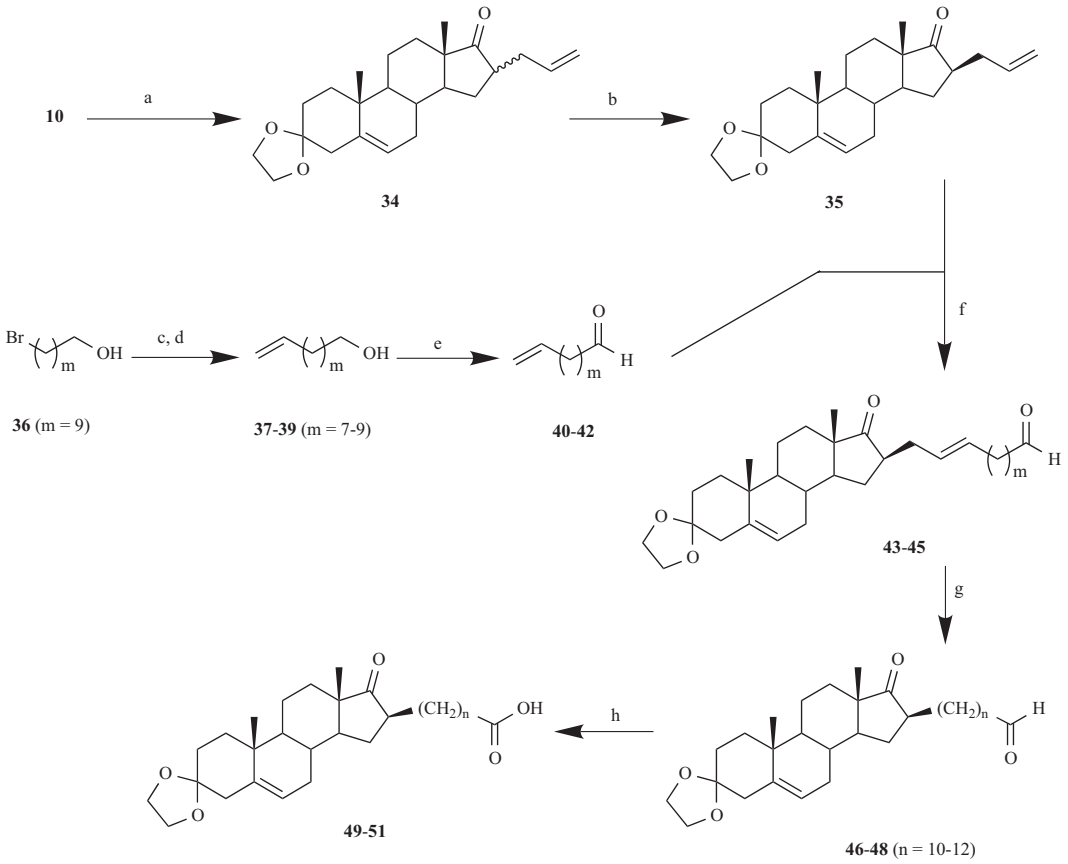
Scheme (1). Synthesis of carboxylic acids **30**-**33**. Reagents, conditions and yields: (a) $(CH_2OH)_2$, *p*-TSA, benzene, Dean-Stark, 24 h (89%); (b) TPAP, NMO, molecular sieves, DCM, rt, 1 h (86%); (c) TBDMSCl, imidazole, DMF, 0°C to rt, 1 h (65-83%); (d) TBDMSCl, NaI, THF, reflux, 16 h (88%); (e) LiHMDS, THF, –78°C to rt, 30 min; (f) THF, -78°C to reflux, 16 h (21-36%); (g) TBAF, THF, rt, 3 h (54-74%); (h) *i.* LDA, THF, -78°C to 0°C, 1 h. *ii*. MeOH, -78°C, 1 h; (i) TPAP, NMO, molecular sieves, DCM, rt, 2 h (52-80%, for two steps); (j) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH, THF, rt, 15 min (97-100%).

and protected halogenoalcohols **14**-**17** as electrophiles. A mixture of 16β/16α-epimers **18**-**21** in a 62-77/38-23 ratio was obtained in low 21-36% yields. A dialkylated steroid and **10** were also part of the reaction mixture. The optimum quantity of base was determined to be 1.8 equivalent. Indeed, using more LiHMDS resulted in the dialkylated product being the major compound, and less resulted in starting steroid **10** being the major product.

After deprotection of **18**-**21** with TBAF to gain free alcohols **22**-**25**, an asymmetric protonation at C16 was done to exclusively afford the 16β epimer. It is well known that the enolate (generated from LDA) will attack on the electrophile proton of MeOH by the less hindered α face of the steroid [53,57]. In order to only obtain the 16β-epimer, the addition of MeOH on the enolate needs to be performed slowly at –78°C. From the 16β-epimer of alcohols **22**-**25**, carboxylic acids **30**-**33** could have been generated in one step using Jones' reagent. However, the ketal group would not resist under the acid conditions. A two-step approach was then considered. Alcohols **22**-**25** were first oxidized with TPAP to gain aldehydes **26**-**29**, which were further oxidized to carboxylic acids **30**-**33** using mild conditions ($NaClO_2$, NaH_2PO_4, 2-methyl-2-butene and *t*-BuOH at room temperature).

As will be described below, the first biological result revealed that other bisubstrate inhibitors with a longer alkyl side-chain (10 to 12 methylenes) needed to be synthesized. However, because, in the approach illustrated in Scheme **1**, low alkylation yields (21-36%) result in a low 7% overall yield of carboxylic acid derivatives **30**-**33** (7 steps from testosterone), a new and more convergent approach was developed. This strategy had, in part, previously been used to synthesize simplified hybrid inhibitors of type 1 17β-HSD [58]. Briefly, alkylation in the α position of steroidal 17-ketone with LDA and allyl bromide, an activated electrophile, and a cross-metathesis with an olefin aldehyde were used as key steps to generate carboxylic acid derivatives **49**-**51** in better overall yields and fewer steps for the introduction of the spacer diversity.

As described in Scheme (**2**), starting from **10**, an alkylation in the α position of steroidal 17-ketone was done using LDA as base and allyl bromide as the activated electrophile to obtain a mixture of 16α/16β-allyl (88/12) protected Δ^5-dione **34** in an excellent 90% yield. The 16β-epimer **35** was easily obtained from the mixture using the epimerisation procedure described above. At that point, each spacer could be joined to the steroid by coupling the appropriate olefin aldehyde using Grubbs cross-metathesis [59]. These olefinic aldehydes needed to be previously synthesized from alcohols **37**-**39** since they are not commercially available. To afford **39** (m = 9) from **36**, a Br-I exchange was first done using Finkelstein conditions, followed by addition of vinyl cuprate. Olefin aldehydes **40**-**42** were prepared by TPAP oxidation of the corresponding alcohols **37**-**39**. A cross-metathesis of allyl steroid **35** with olefins **40**, **41** or **42** and 2nd generation Grubbs catalyst

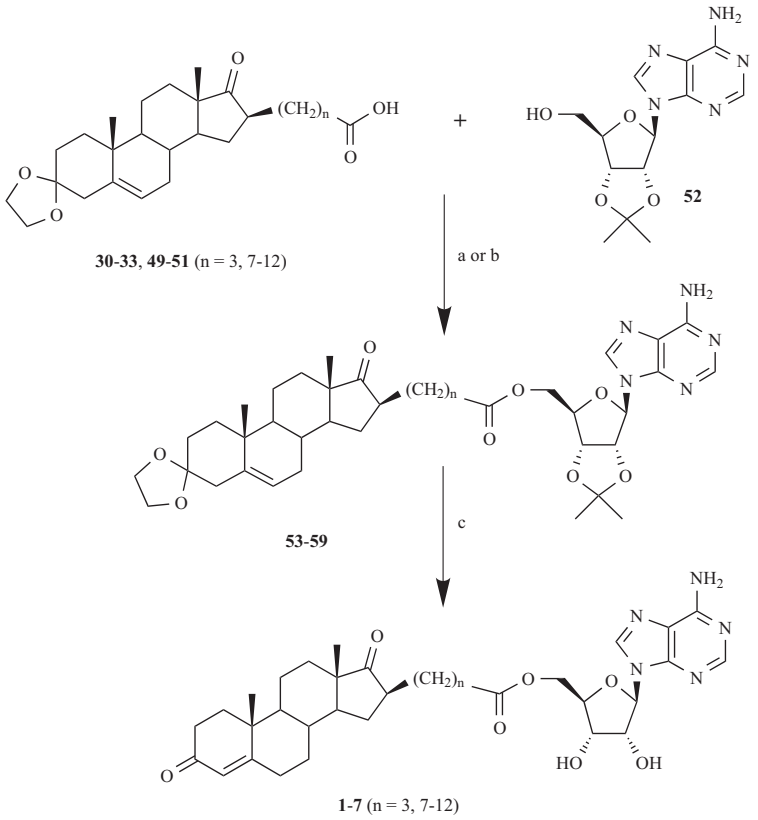


Scheme (2). Synthesis of carboxylic acids **49-51**. Reagents, conditions and yields: (a) *i*. LDA, THF, -78°C to 0°C, 1 h. *ii*. allyl bromide, -78°C to rt, 16 h (90%); (b) *i*. LDA, THF, -78°C to 0°C, 1 h. *ii*. MeOH, -78°C, 1 h (82%); (c) NaI, acetone, reflux, 16 h; (d) vinylMgBr, CuI, HMPA, $P(OEt)_3$, THF, -40°C, 2 h (60% for two steps); (e) TPAP, NMO, molecular sieves, DCM, rt, 1 h (65-93%); (f) 2nd generation Grubbs catalyst, DCM, reflux, 16 h (38-53%); (g) H_2, 10% Pd/C, EtOAc, rt, 16 h (96-99%); (h) $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *t*-BuOH, THF, rt, 15 min (74-99%).

afforded aldehydes **43-45** in 38-53% yields. Formation of a side product resulting from the cross-metathesis of two molecules of steroid **35** was also observed. It is noteworthy that aldehydes are unstable and are rapidly oxidized into carboxylic acid derivatives. Furthermore, since 2nd generation Grubbs catalyst is not compatible with carboxylic acids, resulting in lower cross-metathesis yield, freshly prepared olefin aldehydes are necessary. Selective reduction of the secondary alkene on the side chain upon the tertiary 5,6-olefin (from steroid backbone) was achieved by hydrogen action catalyzed by 10% Pd/C in EtOAc to gain **46-48**. Finally, mild oxidation of aldehydes **46-48** afforded carboxylic acid derivatives **49-51** in excellent yield. Using this synthetic strategy, all carboxylic acid derivatives were obtained in a better 22% overall yield and in only three steps for the introduction of the spacer diversity instead of the 10% overall yield and five steps for the first approach.

As described in Scheme (**3**), carboxylic acid derivatives **30-33**, and **49-51** obtained from both approaches were next linked to the adenosine moiety **52**. Instead of adding the anionic form of isopropylidene adenosine on acid chloride as we did for preparing EM-1745 [50,52], an esterification procedure (EDCI, DMAP or PyBOP, HOBt, DIPEA) was preferred to gain protected bisubstrate compounds **53-59**. After removing ketal and isopropylidene protective groups using acidic conditions (TFA/H_2O, 9/1), Δ^4-dione/adenosine hybrids **1-7** were obtained in good yield.

In addition, in order to confirm that our hybrid inhibitors interact with both the substrate and the cofactor binding sites, we synthesized two compounds that interact only with one binding site (Scheme (**4**)). Alkylation in the α position of 17-ketone of steroid **10** using LiHMDS as base and 1-bromononane as electrophile led to **60** in a low 17% non-optimized yield after isolation of the 16β-epimer from the mixture by flash chromatography. Compound **61** was next obtained under acid treatment (*p*-TSA, MeOH, DCM). This compound is structurally composed of the substrate (Δ^4-dione) and of an alkyl spacer and should thus only interact with the substrate-binding site. The second compound **62**, synthesized as previously reported [50], is composed of the adenosine moiety of the cofactor NADH and of an alkyl spacer, and should interact only with the cofactor-binding



Scheme (3). Synthesis of Δ^4-dione/adenosine hybrids **1**-**7**. Reagents, conditions and yields: (a) EDCI, DMAP, DMF, rt, 16 h (44-69% for n = 3, 7-9); (b) HOBt, PyBOP, DIPEA, DMF, rt, 16 h (45-52% for n = 10-12); (c) TFA/H_2O (9/1), THF, rt, 30 min (52-85%).

site. Compounds **1**-**7**, **61** and **62** were properly purified for enzymatic assay and fully characterized by IR, NMR and HRMS analysis to validate their structure.

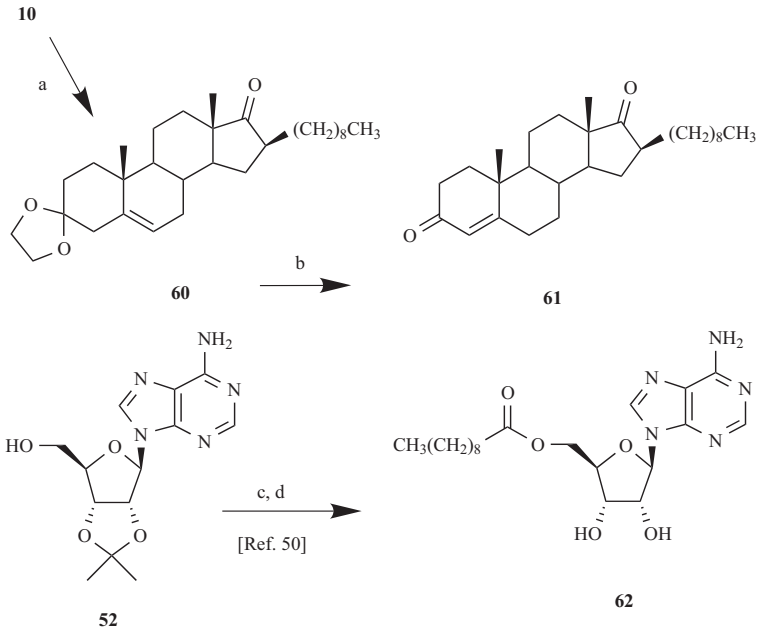
Inhibition of Type 3 17β-HSD

The structure-activity relationship (SAR) study was performed using a homogenate of human embryonic kidney (HEK)-293 cells, which were chosen because they do not display intrinsic 17β-HSD activities. For the enzymatic assay, the cells were transfected with cDNA encoding type 3 17β-HSD [9]. The ability of the synthesized compounds to inhibit type 3 17β-HSD activity was tested on the transformation of [^{14}C]-labeled Δ^4-dione (0.05 μM) into [^{14}C]-labeled T. The reaction was carried out at 37°C and controlled pH (pH = 7.4) to mimic physiological conditions. Because, in homogenated cells, type 3 17β-HSD is a reversible (reductive and oxidative) enzyme, the cofactor NADPH (500 μM) was used in excess to induce the reductive activity of the enzyme.

The first assay showed that compounds **1**-**4** (n = 3, 7-9) were not as good as expected, with percentages of inhibition varying from 4 to 34% at a concentration of 1 μM (Fig. (**4**)). However, these first biological results suggested that the inhibitory capacity of these bisubstrate inhibitors increases with spacer length. In fact, with an alkyl side-chain spacer of three methylenes, at 1 μM compound **1** inhibited only 4% of type 3 17β-HSD activity, while compounds **2**-**4**, with an alkyl side-chain spacer of 7, 8 and 9 methylenes, respectively inhibited 21, 24 and 34% of the enzyme activity at 1 μM.

With these results in hand, we decided to synthesize additional bisubstrate inhibitors with a longer alkyl side-chain spacer. Compounds **5**-**7** were thus prepared with spacers of 10, 11 and 12 methylenes, respectively, using the new and improved synthetic approach described above. Bisubstrate inhibitors **5**-**7** were also tested to determine their inhibitory potential and the results are presented in Fig. (**4**). This enzymatic assay confirmed that better inhibition is obtained with a longer alkyl side chain. Compounds **6** and **7** with spacers of 11 and 12 methylenes are the best bisubstrate inhibitors of type 3 17β-HSD with percentages of inhibition of 69 and 78% at 1 μM, and 44 and 46% at 0.1 μM, respectively. Compound **5** (n = 10) also showed an interesting percentage of inhibition at 1 μM (73%). However, no inhibition effect was observed at 0.1 μM. Given the flexibility of the alkyl chain (11 or 12 methylenes), we do not think that better inhibitors could have been obtained with a longer spacer. In fact, inhibition reaches a plateau for compounds **6** and **7** at both 1 and 0.1 μM.

In order to confirm that the bisubstrate inhibitor strategy applies to type 3 17β-HSD (the Δ^4-dione moiety interacts



Scheme (4). Synthesis of monosubstrate compounds **61** and **62**. (a) *i.* LiHMDS, THF, –78°C to rt, 30 min. *ii*.1-bromononane, -78°C to reflux, 16 h (17%); (b) *p*-TSA, MeOH, DCM, rt, 1 h (77%); (c) NaH, nonanoyl chloride, THF, -20°C, 2 h (78%); (d) HCl*g* in DCM, rt, 100 min (28%).

with the substrate-binding site, while the adenosine moiety interacts with the cofactor-binding site), two compounds (**61** and **62**) bearing only one of the components and an alkyl side-chain spacer of 9 carbons were synthesized. The enzymatic assay, presented in Fig. (**4**), revealed that the presence of two components (Δ^4-dione and adenosine) is an important requirement for good enzyme inhibition. In fact, 16β-nonyl-Δ^4-dione (**61**) and unlabeled Δ^4-dione used as inhibitor gave lower inhibition (60 and 68% at 1 μM and 21 and 25% at 0.1 μM) than the best bisubstrate inhibitors **6**-**7**. With 5-nonanoyl-*O*-adenosine (**62**) which should interact only with the cofactor-binding site, much lower inhibition was obtained with 18 and 17% at 1 and 0.1 μM respectively.

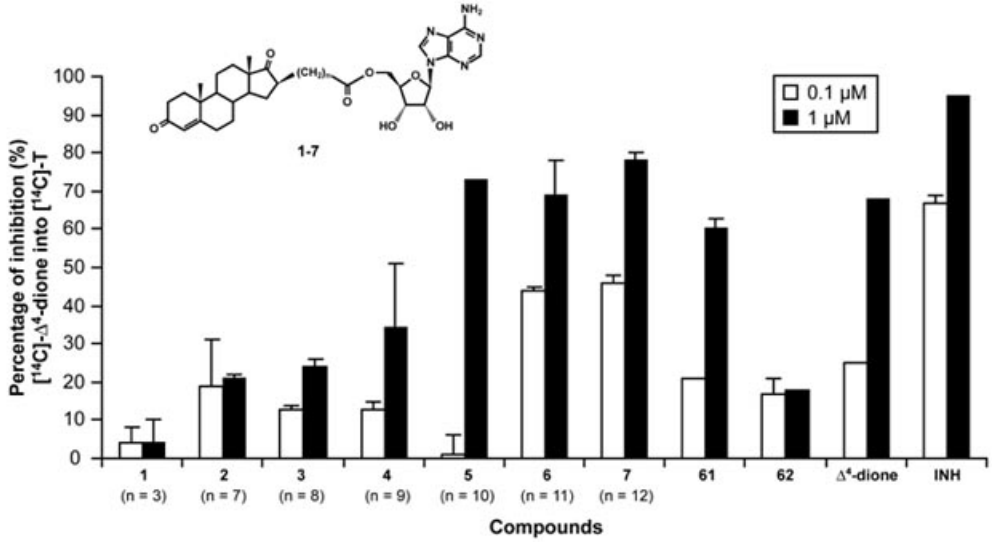


Fig. (4). Inhibition of the transformation of [^{14}C]-Δ^4-dione into [^{14}C]-testosterone by compounds **1**-**7**, **61**, **62**, unlabeled Δ^4-dione and a known inhibitor (INH) [39] at two concentrations of 0.1 and 1 μM in homogenated HEK-293 cells overexpressing type 3 17β-HSD. See Experimental Section for more details. INH = 3β-[*N*-(1-adamantylmethyl-*N*-butyl)amino-methyl]-3α-hydroxy-5α-androstan-17-one, compound **20** in reference [39].

In order to evaluate the compounds in conditions closer to physiological ones, compounds **1**-**7**, **61** and **62** were also tested on intact HEK-293 cells transfected with a vector encoding for type 3 17β-HSD [9,36]. The inhibitors were tested at two concentrations (1 and 10 μM) for screening purposes and the results are presented in Fig. (**5**). At 10 μM, compounds **3**-**7** bearing an alkyl side-chain spacer of 8 to 12 methylenes strongly inhibit type 3 17β-HSD in intact cells with a percentage of inhibition of 85 to 97%. The best bisubstrate inhibitors in intact cells are **5** and **6** (10 and 11 methylenes respectively). Furthermore, at this concentration lower inhibitions were obtained with monosubstrate inhibitors **61** and **62,** unlabeled Δ^4-dione, and compound **1** (the alkyl side-chain of which is too short) with a percentage of inhibition of 51, 0, 75 and 11% at 10 μM, respectively. All results obtained with intact cells agreed well with the SAR study performed in homogenated cells. However, at the lower 1 μM concentration, almost no inhibition of type 3 17β-HSD is observed. This could be explained by a difficult access to cells of this type of compounds in low concentration. On the other hand, when the best bisubstrate inhibitors **6** and **7** are compared with one of the best inhibitors of type 3 17β-HSD (INH) [39], a better inhibition is also obtained in homogenated cells than in intact ones at 1 μM.

If we compare the results obtained above with **6** and **7** for type 3 17β-HSD and those obtained previously with EM-1745 (**8**) for type 1 17β-HSD [49,50], less potent bisubstrate inhibitors are obtained for type 3. Furthermore, the optimal type 1 17β-HSD inhibition was obtained with the E_2/adenosine hybrid **8** in which the steroid and adenosine were linked by a side chain of 8 methylenes, whereas in type 3 17β-HSD the chain separating Δ^4-dione and adenosine required at least 3-4 more methylenes for significant inhibition (see compounds **6** and **7**). Since both enzymes belong to the same SDR family, it is unlikely that the distances between E_2 and adenosine in type 1 and Δ^4-dione and adenosine in type 3 are enough different to explain these two results [60]. One hypothesis to explain such a difference in optimal side-chain length is that the steroid (Δ^4-dione) moiety of inhibitors **6** and **7** do not bind the type 3 17β-HSD as expected for the natural substrate (Δ^4-dione). Indeed, it is known that the C18-steroid E_2 and the C19-steroid Δ^4-dione (or its reduced form testosterone) display different binding modes in the type 1 17β-HSD binding pocket [61,62]. The PDB model 1QYX [63] shows that Δ^4-dione is positioned exactly in the reverse orientation when compared to E_2 (i.e. C3 of Δ^4-dione approximately on C17 of E_2). The distance from the C17-O of E_2 to the C2'-phosphate of adenosine is 11.03 Å for type 1 (1FDT) [60], although the same distance for Δ^4-dione extends to 19.94 Å for type 3 (1QYX) [63]. Thus, if the same difference in orientation between estrogens and androgens applies for the type 3 17β-HSD as well, this might explain the need for a longer chain between the steroid and adenosine moieties of hybrid inhibitors. In that case, it will be interesting to investigate bisubstrate hybrids for type 3 17-HSD where adenosine is linked to the A ring instead of the D ring of Δ^4-dione. It is also possible that the steroid (Δ^4-dione) moiety of inhibitors **6** and **7** does not bind the type 3 17β-HSD exactly as the natural substrate (Δ^4-dione) does or exactly in a fully reversed orientation (as observed for type 1 17β-HSD). In this alternative, the side-chain should be necessary longer to allow both Δ^4-dione and adenosine moiety to interact efficiently with both the substrate and cofactor binding sites. Crystallizing inhibitor **6** or **7** with type 3 17β-HSD would be the best way to check these hypotheses; unfortunately, the crystallization of a membrane enzyme like type 3 17β-HSD remains a great challenge.

When we compare hybrid inhibitors of type 1 and type 3 17β-HSDs, we have to be careful because different cofactors were used in the enzymatic assays. NADH was used as

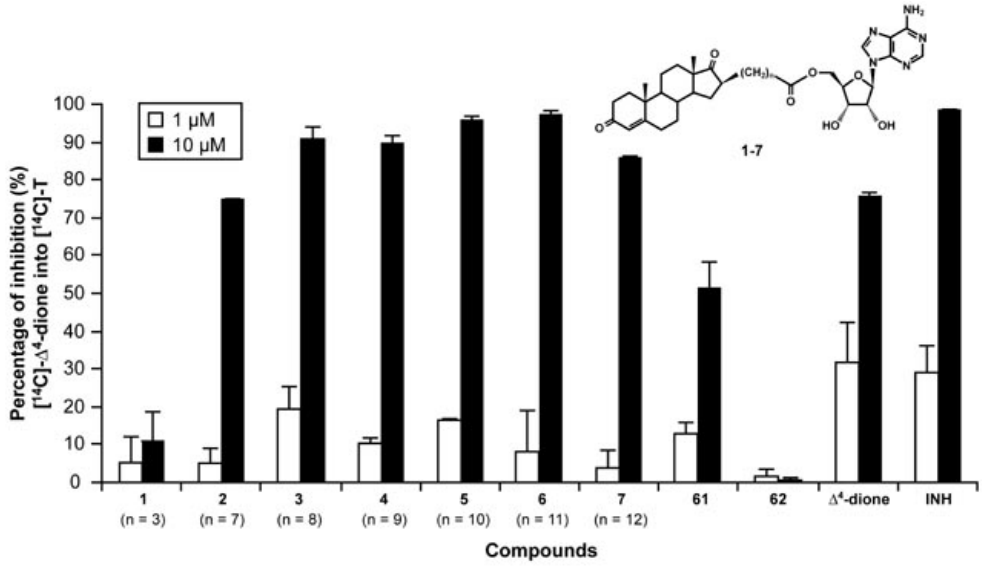


Fig. (5). Inhibition of the transformation of [^{14}C]-Δ^4-dione into [^{14}C]-testosterone by compounds **1**-**7**, **61**, **62**, unlabeled Δ^4-dione and a known inhibitor (INH) [39] at two concentrations of 1 and 10 μM in intact HEK-293 cells overexpressing type 3 17β-HSD. See Experimental Section for more details. INH = 3β-[*N*-(1-adamantylmethyl-*N*-butyl)amino-methyl]-3α-hydroxy-5α-androstan-17-one, compound **20** in reference [39].

cofactor for type 1 17β-HSD whereas NADPH was used for type 3 17β-HSD. Furthermore, it is known that both enzymes prefer NADPH as cofactor [7]. The structural difference between these two entities is the presence of a phosphate group on C2' of adenosine for NADPH. Thus, in the design of bisubstrate inhibitors for both enzymes, the adenosine moiety from the cofactor NADH was used to simplify the synthesis. In the type 1 17β-HSD enzymatic assay, the bisubstrate inhibitor **8** was in competition with the cofactor NADH, but this was not the case in the type 3 17β-HSD enzymatic assay. The adenosine moiety of **6** and **7**, which is structurally equivalent to the adenosine part of NADH, is in competition with NADPH that shows a much higher affinity for the cofactor-binding site of the enzyme than NADH. To prove our hypothesis, we compared the effect of NADH and NADPH on the ability of **8** to inhibit the transformation of [^{14}C]-labeled estrone into [^{14}C]-labeled E_2 in homogenated HEK-293 cells overexpressing type 1 17β-HSD activity. We found that **8** strongly inhibits type 1 17β-HSD when the cofactor NADH is used (91% at 1 μM), but its inhibitory potency dropped when NADPH was used as cofactor with only 19% at 1 μM (data not shown).

We tried to use NADH in the type 3 17β-HSD enzymatic assay but, as expected, we lost all enzyme activity. It appears that the preference of this enzyme for NADPH come from the amino acid arginine 80 found in the cofactor-binding site [64]. Mutation of this amino acid inactivates this enzyme. It was found that the arginine 80 residue forms part of a hydrophobic pocket for the purine ring of the adenosine while its guanidinium moiety interacts with the 2'-phosphate to both stabilize cofactor binding and neutralize its intrinsic negative charge through two hydrogen bonds. To overcome this problem, new bisubstrate inhibitors of type 3 17β-HSD should be designed. These inhibitors should be composed of three components: Δ^4-dione to interact with the substrate-binding site, a 2'-phosphate adenosine moiety (providing the NADPH structure) to interact with the cofactor-binding site and an alkyl side chain spacer of appropriate length (see Fig. (**2B**): R = PO_3H_2).

CONCLUSION

Seven bisubstrate inhibitors of type 3 17β-HSD (compounds **1**-**7**) were synthesized using two chemical synthetic approaches. Because direct alkylation in the α position of steroidal 17-ketone with unactivated electrophiles results in low yield, a new and improved strategy was developed using Grubbs cross-metathesis as a key step to introduce the alkyl side-chain spacer. Using this second strategy, the key intermediate carboxylic acids **49**-**51** were prepared in a better 22% overall yield and in three steps to introduce the spacer diversity compared to 10% overall yield and five steps for the synthesis of carboxylic acids **30**-**33**. An enzymatic assay in homogenated HEK-293 cells overexpressing type 3 17β-HSD revealed that the best bisubstrate inhibitors of type 3 17β-HSD are compounds **6** and **7** with an alkyl side-chain spacer of 11 or 12 methylenes. As expected, these hybrid inhibitors were less potent in intact cells than in homogenated cells. Although the inhibitor design was not optimized, both series of results suggest that the strategy of substrate/cofactor dual inhibitors works for type 3 17β-HSD, but this conclusion remains to be confirmed. Furthermore, Δ^4-dione/adenosine hybrids inhibit weakly the type 3 17β-HSD when compared to the estradiol/adenosine hybrids, which inhibit the type 1 17β-HSD much more efficiently. Maybe adding a phosphate group on the C2' of adenosine moiety of the bisubstrate inhibitor **6** or **7** would increase the inhibition of type 3 17β-HSD. This way, the adenosine-phosphate moiety of the inhibitor will be more adapted to competition with the NADPH cofactor needed for the enzymatic reaction. The synthesis of this second generation of bisubstrate inhibitors of type 3 17β-HSD is under progress and synthesis and biological evaluation will be reported elsewhere shortly.

EXPERIMENTAL

General Methods

Reagents were obtained from Sigma-Aldrich Canada Co. (Oakville, ON, Canada) except for benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) which was purchased from NovaBiochem (EMD Biosciences Inc, La Jolla, CA, USA), tricyclohexylphosphine [1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene] [benzylidine] ruthenium(IV) dichloride from Strem Chemicals (Newburyport, MA, USA) and testosterone from Steraloids (Newport, RI, USA). Usual solvents were obtained from Fisher Scientific (Montreal, Qc, Canada) and VWR (Ville Mont-Royal, Qc, Canada) and were used as received. Anhydrous solvents were purchased from Aldrich and VWR in SureSeal bottles, which were conserved under positive argon pressure. Tetrahydrofuran (THF) was distilled from sodium/benzophenone under argon. All anhydrous reactions were performed under positive argon pressure in oven-dried glassware. Thin-layer chromatography (TLC) was performed on 0.25-mm silica gel 60 F_{254} plates from Whatman (distributed by Fisher Scientific) and compounds were visualized by exposure to UV light (254 nM) and/or with a solution of ammonium molybdate/sulphuric acid/water (with heating). Flash chromatography was performed on Silicycle 60 (Québec, Qc, Canada) 230-400 mesh silica gel. Infrared (IR) spectra were obtained neat or from a thin film of the solubilized compound on NaCl pellets (usually in CH_2Cl_2). They were recorded on a Perkin-Elmer series 1600 FT-IR spectrometer (Norwalk, CT, USA); only significant bands are reported (in cm^{-1}). ^{1}H- and ^{13}C-NMR spectra were recorded with a Bruker AC/F 300 spectrometer (Billerica, MA, USA) at 300 and 75 MHz, respectively, and a Bruker AVANCE 400 spectrometer at 400 (^{1}H) and 100 (^{13}C) MHz. The chemical shifts (δ) are expressed in ppm and referenced to chloroform (7.26 and 77.0 ppm), acetone (2.07 and 206.0 ppm), or dimethylsulfoxide (2.51 and 39.5 ppm) for ^{1}H and ^{13}C respectively. Assignment of NMR signals was done using 1D- and 2D-NMR experiments (COSY, HSQC, HMBC, APT) and made easier using literature data [65,66]. Low-resolution mass spectra (LRMS) were recorded with an LCQ Finnigan apparatus (San Jose, CA, USA) equipped with an atmospheric pressure chemical ionisation (APCI) source on positive or negative mode. High-resolution mass spectra (HRMS) were provided by the Regional Laboratory for Instrumental Analysis (Université de Montréal, Montréal, Canada).

Synthesis of 3,3-(ethylenedioxy)-androst-5-en-17β-ol (9) [67]

Ethylene glycol (116 mL, 2.08 mol) and *p*-TSA (131 mg, 0.693 mmol) were added to a solution of testosterone (20 g, 69.3 mmol) in benzene (1000 mL) in a flask equipped with a Dean-Stark trap. The mixture was refluxed for 24 h until no more water distilled from the reaction. Half of the benzene was evaporated before a saturated solution of $NaHCO_3$ was added to quench the reaction. The product was extracted with EtOAc, washed with brine, dried over $MgSO_4$ and evaporated to dryness. Purification by flash chromatography (hexanes/acetone, 85:15) afforded **9** (20.5 g, 61.7 mmol, 89%) as a white solid. IR (film) 3408 (OH); ^{1}H-NMR (400 MHz, $CDCl_3$) 0.76 (s, 18-CH_3), 0.95 to 2.15 (m, 18H, CH and CH_2 of steroid skeleton), 1.04 (s, 19-CH_3), 2.57 (m, 4-CH), 3.65 (t, J = 8.5 Hz, 17α-CH), 3.95 (m, CH_2 of ketal), 5.35 (m, 6-CH); ^{13}C-NMR (75 MHz, $CDCl_3$) 11.0, 18.9, 20.6, 23.4, 30.4, 31.0, 31.3, 31.9, 36.3, 36.5, 36.7, 41.8, 42.7, 49.8, 51.3, 64.2, 64.4, 81.8, 109.4, 121.9, 140.2; LRMS calculated for $C_{21}H_{33}O_3$ $[M+H]^+$ 333.2, found 333.1 m/z.

Synthesis of 3,3-(ethylenedioxy)-androst-5-en-17-one (10) [67]

Protected testosterone **9** (15 g, 45.1 mmol) was dissolved in dry DCM (90 mL) under argon atmosphere at room temperature. 4-Methylmorpholine *N*-oxide (NMO) (7.9 g, 67.5 mmol) and molecular sieves (22 g) were added and the reaction was stirred for 15 min. Then, tetrapropylammonium perruthenate (TPAP) (540 mg, 2.2 mmol) was added and the mixture was stirred for 1 h. DCM was removed under reduced pressure and the crude compound was filtered through a pad of silica gel using DCM as eluent to afford **10** (12.8 g, 38.7 mmol, 86%) as a white solid. IR (film) 1738 (C=O, ketone); ^{1}H-NMR (400 MHz, $CDCl_3$) 0.87 (s, 18-CH_3), 1.03 (s, 19-CH_3), 1.05 to 2.15 (m, 17H, CH and CH_2 of steroid skeleton), 2.44 (dd, J_1 = 19.2 Hz, J_2 = 8.5 Hz, 16-CH), 2.57 (m, 4-CH), 3.93 (m, CH_2 of ketal), 5.36 (m, 6-CH); ^{13}C-NMR (75 MHz, $CDCl_3$) 13.5, 18.8, 20.2, 21.8, 30.5, 30.9, 31.3, 31.4, 35.7, 36.2, 36.6, 41.7, 47.5, 49.7, 51.6, 64.2, 64.4, 109.2, 121.3, 140.3, 221.1; LRMS calculated for $C_{21}H_{31}O_3$ $[M+H]^+$ 331.2, found 331.2 m/z.

Synthesis of Key Intermediate Carboxylic Acids 30 - 33 (First Strategy)

General Procedure for Protection of Alcohol as Silyl Ether

To a solution of alcohols **11-13** (5.05-10.00 g, 21.2-44.8 mmol) in dry DMF (300-600 mL) under argon atmosphere at 0°C was added *tert*-butyldimethylsilyl chloride (TBDMSCl) (6.42-13.51 g, 42.6-89.6 mmol) and imidazole (7.20-15.24 g, 106-224 mmol). The reaction mixture was stirred 1 h at room temperature and then poured into cold water. After extraction with EtOAc, the organic phase was washed with brine, dried over $MgSO_4$ and evaporated to dryness. Purification of the crude compound by flash chromatography (hexanes/EtOAc, 99:1 to 97:3) gained protected alcohols **14-16** (6.13-12.54 g, 17.4-37.1 mmol, 65-83%).

8-Bromo-1-tert-butyldimethylsilyloxyoctane (14)

Colourless oil (65%); IR (neat) No OH band, 1255 (CH_2Br), 1100 (Si-O-C); ^{1}H-NMR (400 MHz, $CDCl_3$) 0.05 (s, $Si(CH_3)_2$), 0.89 (s, $SiC(CH_3)_3$), 1.31 (m, 3 x CH_2 of chain), 1.42 (m, C$\underline{H}_2$CH$_2$CH$_2$Br), 1.50 (m, C$\underline{H}_2$CH$_2$OTBDMS), 1.85 (m, C$\underline{H}_2$CH$_2$Br), 3.40 (t, J = 6.9 Hz, CH$_2$Br), 3.59 (t, J = 6.6 Hz, C$\underline{H}_2$OTBDMS); ^{13}C-NMR (75 MHz, $CDCl_3$) -5.3 (2x), 18.3, 25.7, 25.9 (3x), 28.1, 28.7, 29.2, 32.8 (2x), 33.9, 63.2.

9-Bromo-1-tert-butyldimethylsilyloxynonane (15)

Colourless oil (83%); IR (neat) No OH band, 1255 (CH_2Br), 1100 (Si-O-C); ^{1}H-NMR (300 MHz, $CDCl_3$) 0.03 (s, $Si(CH_3)_2$), 0.88 (s, $SiC(CH_3)_3$), 1.29 (m, 4 x CH_2 of chain), 1.40 (m, C$\underline{H}_2$CH$_2$CH$_2$Br), 1.49 (m, C$\underline{H}_2$CH$_2$OTBDMS), 1.84 (m, C$\underline{H}_2$CH$_2$Br), 3.38 (t, J = 6.7 Hz, CH$_2$Br), 3.58 (t, J = 6.6 Hz, C$\underline{H}_2$OTBDMS); ^{13}C-NMR (75 MHz, $CDCl_3$) -5.3 (2x), 18.3, 25.7, 25.9 (3x), 28.1, 28.7, 29.3, 29.4, 32.8 (2x), 33.9, 63.2.

10-Bromo-1-tert-butyldimethylsilyloxydecane (16)

Colourless oil (82%); IR (neat) No OH band, 1256 (CH_2Br), 1099 (Si-O-C); ^{1}H-NMR (400 MHz, $CDCl_3$) 0.04 (s, $Si(CH_3)_2$), 0.89 (s, $SiC(CH_3)_3$), 1.28 (m, 5 x CH_2 of chain), 1.41 (m, C$\underline{H}_2$CH$_2$CH$_2$Br), 1.50 (m, C$\underline{H}_2$CH$_2$OTBDMS), 1.84 (m, C$\underline{H}_2$CH$_2$Br), 3.39 (t, J = 6.9 Hz, CH$_2$Br), 3.59 (t, J = 6.6 Hz, C$\underline{H}_2$OTBDMS); ^{13}C-NMR (75 MHz, $CDCl_3$) -5.3 (2x), 18.3, 25.7, 25.9 (3x), 28.1, 28.7, 29.2 (2x), 32.8 (3x), 33.9, 63.2.

Synthesis of 4-bromo-1-tert-butyldimethylsilyloxybutane (17)

To a suspension of NaI (13 g, 82 mmol) in anhydrous THF (50 mL) under argon was added a solution of TBDMSCl (4.1 g, 27 mmol) in dry THF (10 mL). The mixture was stirred at reflux for 16 h and purified by flash chromatography (diethyl ether) to afford protected alcohol **17** (7.6 g, 24 mmol, 88%) as light-sensitive colourless oil. The NMR data agreed with those reported in the literature [56].

General Procedure for Introducing the Alkyl Spacer (Synthesis of 18-21)

Steroid **10** (1.00-2.00 g, 3.03-6.00 mmol) was dissolved in dry THF (75-125 mL) under argon atmosphere and the solution was cooled at –78°C. A solution of lithium bis(trimethylsilyl)amide (LiHMDS) (1.0 M in THF) (5.4-10.9 mL, 5.4-10.9 mmol) was added and the reaction mixture was stirred 30 min at room temperature. The mixture was then cooled at –78°C followed by the addition dropwise of the appropriate protected bromoalcohols **14-17** (3.18-6.12 g, 9.1-18.1 mmol) previously filtered through a pad of aluminium oxide 60 (70-230 Mesh, EMD Chemicals, Gibbstown, NJ, USA) using diethyl ether as solvent. The solution was refluxed and stirred overnight. Then, a saturated aqueous solution of NH_4Cl was added and the mixture was extracted with EtOAc, washed with brine and dried over $MgSO_4$. Solvent was evaporated and the crude product was purified by flash chromatography (hexanes/EtOAc, 98:2) to gain a mixture of 16α- and 16β-alkylated Δ^4-dione derivatives **18-21** (0.45-1.28 g, 0.78-2.18 mmol, 21-36%) in variable proportions. It is noteworthy that dialkylated compounds and starting materials **10** were eliminated during the chromatography process.

4-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'α/β]-1-(tert-butyldimethylsilyloxy)-butane (18)

Colourless viscous oil (0.66 g, 21%); IR (film) 1736 (C=O, ketone); ^{1}H-NMR (400 MHz, $CDCl_3$) 0.04 (s, $Si(CH_3)_2$), 0.84 and 0.92 (2s, 18'-CH_3, 16'β:16'α / 62:38), 0.89 (s, $SiC(CH_3)_3$), 1.05 (s, 19'-CH_3), 1.10 to 2.20 (m, 23H, CH and CH_2 of steroid skeleton and alkyl chain), 2.58 (m, 4'-CH), 3.60 (t, J = 6.4 Hz, C<u>H</u>$_2$OTBDMS), 3.95 (m, CH_2 of ketal), 5.39 (m, 6'-CH); LRMS calculated for $C_{31}H_{53}O_4Si$ $[M+H]^+$ 517.4, found 517.0 m/z.

8-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'α/β]-1-(tert-butyldimethylsilyloxy)-octane (19)

Colourless viscous oil (1.11 g, 32%); IR (film) 1736 (C=O, ketone); ^{1}H-NMR (400 MHz, $CDCl_3$) 0.05 (s, $Si(CH_3)_2$), 0.84 and 0.92 (2s, 18'-CH_3, 16'β:16'α / 66:34), 0.89 (s, $SiC(CH_3)_3$), 1.05 (s, 19'-CH_3), 1.10 to 2.20 (m, 31H, CH and CH_2 of steroid skeleton and alkyl chain), 2.58 (m, 4'-CH), 3.59 (t, J = 6.6 Hz, C<u>H</u>$_2$OTBDMS), 3.95 (m, CH_2 of ketal), 5.39 (m, 6'-CH); LRMS calculated for $C_{35}H_{61}O_4Si$ $[M+H]^+$ 573.4, found 573.3 m/z.

9-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'α/β]-1-(tert-butyldimethylsilyloxy)-nonane (20)

Colourless viscous oil (1.28 g, 36%); IR (film) 1726 (C=O, ketone); ^{1}H-NMR (400 MHz, $CDCl_3$) 0.05 (s, $Si(CH_3)_2$), 0.84 and 0.92 (2s, 18'-CH_3, 16'β:16'α / 73:27), 0.89 (s, $SiC(CH_3)_3$), 1.05 (s, 19'-CH_3), 1.10 to 2.20 (m, 33H, CH and CH_2 of steroid skeleton and alkyl chain), 2.59 (m, 4'-CH), 3.59 (t, J = 6.6 Hz, C<u>H</u>$_2$OTBDMS), 3.95 (m, CH_2 of ketal), 5.39 (m, 6'-CH); LRMS calculated for $C_{36}H_{63}O_4Si$ $[M+H]^+$ 587.4, found 587.4 m/z.

10-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'α/β]-1-(tert-butyldimethylsilyloxy)-decane (21)

Colourless viscous oil (579 mg, 33%); IR (film) 1735 (C=O, ketone); ^{1}H-NMR (400 MHz, $CDCl_3$) 0.05 (s, $Si(CH_3)_2$), 0.84 and 0.92 (2s, 18'-CH_3, 16'β:16'α / 77:23), 0.89 (s, $SiC(CH_3)_3$), 1.05 (s, 19'-CH_3), 1.10 to 2.20 (m, 35H, CH and CH_2 of steroid skeleton and alkyl chain), 2.59 (m, 4'-CH), 3.59 (t, J = 6.6 Hz, C<u>H</u>$_2$OTBDMS), 3.95 (m, CH_2 of ketal), 5.39 (m, 6'-CH); LRMS calculated for $C_{37}H_{65}O_4Si$ $[M+H]^+$ 601.5, found 601.4 m/z.

General Procedure for Hydrolysis of Silyl Ether (Synthesis of 22-25)

A solution of steroids **18**-**21** (0.53-1.11 g, 0.93-1.93 mmol) in dry THF (20-40 mL) under argon atmosphere was treated with TBAF (1M in THF) (1.39-2.91 mL, 1.39-2.91 mmol) and stirred 1-2 h at room temperature. The reaction was quenched by addition of water and the mixture was extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$ and evaporated to dryness. The crude material was purified by flash chromatography (hexanes/EtOAc, 7:3 to 5:5) to yield alcohols **22**-**25** (296-635 mg, 0.65-1.38 mmol, 57-74%).

4-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'α/β-yl]-butanol (22)

White solid (352 mg, 74%); IR (film) 3427 (OH), 1733 (C=O, ketone); ^{1}H-NMR (400 MHz, $CDCl_3$) 0.84 and 0.93 (2s, 18'-CH_3, 16'β:16'α / 70:30), 1.05 (s, 19'-CH_3), 1.10 to 2.20 (m, 23H, CH and CH_2 of steroid skeleton and alkyl chain), 2.59 (m, 4'-CH), 3.66 (t, J = 6.5 Hz, C<u>H</u>$_2$OH), 3.95 (m, CH_2 of ketal), 5.39 (m, 6'-CH); LRMS calculated for $C_{25}H_{39}O_4$ $[M+H]^+$ 403.3, found 403.2 m/z.

8-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'α/β-yl]-octanol (23)

White solid (635 mg, 72%); IR (film) 3398 (OH), 1726 (C=O, ketone); ^{1}H-NMR (400 MHz, $CDCl_3$) 0.84 and 0.92 (2s, 18'-CH_3, 16'β:16'α / 70:30), 1.05 (s, 19'-CH_3), 1.10 to 2.20 (m, 31H, CH and CH_2 of steroid skeleton and alkyl chain), 2.59 (m, 4'-CH), 3.64 (t, J = 6.6 Hz, C<u>H</u>$_2$OH), 3.95 (m, CH_2 of ketal), 5.39 (m, 6'-CH); LRMS calculated for $C_{29}H_{47}O_4$ $[M+H]^+$ 459.3, found 459.3 m/z.

9-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'α/β-yl]-nonanol (24)

White solid (477 mg, 57%); IR (film) 3470 (OH), 1726 (C=O, ketone); ^{1}H-NMR (400 MHz, $CDCl_3$) 0.84 and 0.92 (2s, 18'-CH_3, 16'β:16'α / 70:30), 1.05 (s, 19'-CH_3), 1.10 to 2.20 (m, 33H, CH and CH_2 of steroid skeleton and alkyl chain), 2.59 (m, 4'-CH), 3.64 (t, J = 6.6 Hz, C<u>H</u>$_2$OH), 3.95 (m, CH_2 of ketal), 5.39 (m, 6'-CH); LRMS calculated for $C_{30}H_{49}O_4$ $[M+H]^+$ 473.4, found 473.3 m/z.

10-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'α/β-yl]-decanol (25)

White solid (296 mg, 70%); IR (film) 3425 (OH), 1719 (C=O, ketone); ^{1}H-NMR (400 MHz, $CDCl_3$) 0.83 and 0.91 (2s, 18'-CH_3, 16'β:16'α / 82:18), 1.04 (s, 19'-CH_3), 1.10 to 2.20 (m, 35H, CH and CH_2 of steroid skeleton and alkyl chain), 2.59 (m, 4'-CH), 3.62 (t, J = 6.6 Hz, C<u>H</u>$_2$OH), 3.93 (m, CH_2 of ketal), 5.38 (m, 6'-CH); LRMS calculated for $C_{31}H_{51}O_4$ $[M+H]^+$ 487.4, found 487.3 m/z.

General Procedure for Synthesis of Aldehydes 26-29

Epimerisation of the C16 Configuration. A solution of diisopropylamine (381-650 μL, 2.72-4.64 mmol) in dry THF (5-10 mL) was stirred under argon at 0°C, and *n*-BuLi (2.5 M in hexanes) (1.1-1.8 mL, 2.72-4.64 mmol) was added dropwise. After 30 minutes, the solution was cooled at –78°C and steroids **22**-**25** (250-438 mg, 0.546-0.927 mmol), dissolved in dry THF (5-10 mL), were added dropwise to the LDA solution. The mixture was stirred for 1 h at 0°C before cooling at –78°C. Then, MeOH (155-160 μL, 3.82-6.49 mmol) was added very slowly to the solution, which was stirred at –78°C for 1 h. After addition of water, the mixture was extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$ and evaporated to dryness. ^{1}H-NMR analysis of the crude product showed only one peak for 18'-CH_3 at 0.84 ppm (alkyl chain in C16'β) and disappearance of the peak at 0.92 ppm (alkyl chain in C16'α). *Oxidation*. Molecular sieves (222-436 mg) and 4-methylmorpholine N-oxide (NMO) (78-153 mg, 0.66-1.30 mmol) were added to a solution of crude alcohols (210-400 mg, 0.44-0.87 mmol) in dry DCM (5-10 mL) under argon atmosphere at room temperature. The reaction mixture was stirred for 15 minutes. Then, tetrapropylammonium perruthenate (TPAP) (8-15 mg, 0.022-0.044 mmol) was added and the mixture was stirred for 1 h. DCM was removed under reduced pressure and the crude product were purified

by flash chromatography (hexanes/EtOAc, 6:4) to gain aldehydes **26-29** (160-231 mg, 0.35-0.51 mmol, 52-80%).

4-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'β-yl]-butanal (26)

White solid (180 mg, 58%, two steps); IR (film) 2708 (C-H, aldehyde), 1719 (C=O, ketone and aldehyde); ^{1}H-NMR (400 MHz, $CDCl_3$) δ = 0.84 (s, 18'-CH_3), 1.05 (s, 19'-CH_3), 1.10 to 2.20 (m, 21H, CH and CH_2 of steroid skeleton and alkyl chain), 2.47 (m, C$\underline{H}_2$CHO), 2.58 (m, 4'-CH), 3.95 (m, CH_2 of ketal), 5.38 (m, 6'-CH), 9.78 (t, *J* = 1.5 Hz, 1H, CHO).

8-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'β-yl]-octanal (27)

White solid (231 mg, 58%, two steps); IR (film) 2726 (C-H, aldehyde), 1726 (C=O, ketone and aldehyde); ^{1}H-NMR (400 MHz, $CDCl_3$) 0.84 (s, 18'-CH_3), 1.05 (s, 19'-CH_3), 1.10 to 2.20 (m, 29H, CH and CH_2 of steroid skeleton and alkyl chain), 2.42 (dt, J_1 = 7.3 Hz, J_2 = 1.8 Hz, C$\underline{H}_2$CHO), 2.58 (m, 4'-CH), 3.95 (m, CH_2 of ketal), 5.39 (m, 6'-CH), 9.76 (t, *J* = 1.8 Hz, CHO).

9-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'β-yl]-nonanal (28)

White solid (160 mg, 80%, two steps); IR (film) 2726 (C-H, aldehyde), 1726 (C=O, ketone and aldehyde); ^{1}H-NMR (400 MHz, $CDCl_3$) 0.84 (s, 18'-CH_3), 1.05 (s, 19'-CH_3), 1.10 to 2.20 (m, 31H, CH and CH_2 of steroid skeleton and alkyl chain), 2.42 (dt, J_1 = 7.3 Hz, J_2 = 1.8 Hz, C$\underline{H}_2$CHO), 2.58 (m, 4'-CH), 3.95 (m, CH_2 of ketal), 5.39 (m, 6'-CH), 9.76 (t, *J* = 1.8 Hz, CHO).

10-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'β-yl]-decanal (29)

White solid (130 mg, 52%, two steps); IR (film) 2725 (C-H, aldehyde), 1725 (C=O, ketone and aldehyde); ^{1}H-NMR (400 MHz, acetone-d_6) 0.84 (s, 18'-CH_3), 1.08 (s, 19'-CH_3), 1.10 to 2.20 (m, 33H, CH and CH_2 of steroid skeleton and alkyl chain), 2.44 (t, *J* = 7.3 Hz, C$\underline{H}_2$CHO), 2.49 (m, 4'-CH), 3.89 (m, CH_2 of ketal), 5.31 (m, 6'-CH), 9.74 (s, CHO).

General Procedure for Oxidation of Aldehydes into Carboxylic Acids 30-33

Aldehydes **26-29** (130-209 mg, 0.285-0.458 mmol) were dissolved in a minimum of THF (~1 mL) followed by addition of *t*-BuOH (8-13 mL) and 2-methyl-2-butene (3-5 mL) in a proportion of 8: 3. An oxidative solution freshly prepared by dissolving $NaClO_2$ (300-500 mg) and NaH_2PO_4 (300-500 mg) in H_2O (3-5 mL) was added and the reaction mixture was allowed to stir for 10 to 20 minutes. The reaction was quenched by addition of water and the extraction was performed with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$ and evaporated to dryness under reduced pressure to afford carboxylic acids **30-33** (135-212 mg, 0.285-0.449 mmol, 97-100%) in good purities without purification.

4-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'β-yl]-butanoic acid (30)

White solid (166 mg, 98%); IR (film) 3500-2600 (OH, carboxylic acid), 1732 (C=O, ketone), 1710 (C=O, carboxylic acid); ^{1}H-NMR (400 MHz, $CDCl_3$) 0.84 (s, 18'-CH_3), 1.05 (s, 19'-CH_3), 1.10 to 2.20 (m, 21H, CH and CH_2 of steroid skeleton and alkyl chain), 2.38 (m, C$\underline{H}_2$COOH), 2.58 (m, 4'-CH), 3.95 (m, CH_2 of ketal), 5.39 (m, 6'-CH); ^{13}C-NMR (75 MHz, $CDCl_3$) 13.7, 18.9, 20.2, 23.2, 28.6, 30.8, 31.0, 31.6, 31.7, 32.6, 33.9, 36.2, 36.8, 41.8, 47.9, 49.3, 49.9, 50.2, 64.2, 64.4, 109.3, 121.4, 140.4, 177.5, 221.6; LRMS calculated for $C_{25}H_{37}O_5$ $[M+H]^+$ 417.3, found 417.1 m/z.

8-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'β-yl]-octanoic acid (31)

White solid (212 mg, 98%); IR (film) 3600-2500 (OH, carboxylic acid), 1732 (C=O, ketone), 1712 (C=O, carboxylic acid); ^{1}H-NMR (400 MHz, $CDCl_3$) 0.84 (s, 18'-CH_3), 1.05 (s, 19'-CH_3), 1.10 to 2.20 (m, 29H, CH and CH_2 of steroid skeleton and alkyl chain), 2.35 (t, *J* = 7.5 Hz, C$\underline{H}_2$COOH), 2.59 (m, 4'-CH), 3.95 (m, CH_2 of ketal), 5.39 (m, 6'-CH); ^{13}C-NMR (75 MHz, $CDCl_3$) 13.7, 18.9, 20.2, 24.6, 28.0, 28.9, 29.0 (2x), 29.2, 30.8, 30.9, 31.1, 31.7, 32.4, 33.7, 36.2, 36.8, 41.8, 47.9, 49.3, 49.9, 50.2, 64.2, 64.4, 109.3, 121.5, 140.4, 178.2, 223.1; LRMS calculated for $C_{29}H_{45}O_5$ $[M+H]^+$ 473.3, found 473.3 m/z.

9-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'β-yl]-nonanoic acid (32)

White solid (155 mg, 97%); IR (film) 3600-2500 (OH, carboxylic acid), 1726 (C=O, ketone), 1712 (C=O, carboxylic acid); ^{1}H-NMR (400 MHz, $CDCl_3$) 0.84 (s, 18'-CH_3), 1.05 (s, 19'-CH_3), 1.10 to 2.20 (m, 31H, CH and CH_2 of steroid skeleton and alkyl chain), 2.35 (t, *J* = 7.5 Hz, C$\underline{H}_2$COOH), 2.58 (m, 4'-CH), 3.95 (m, CH_2 of ketal), 5.39 (m, 6'-CH); ^{13}C-NMR (75 MHz, $CDCl_3$) 13.7, 18.9, 20.2, 24.6, 28.1, 28.9, 29.0, 29.1, 29.2, 29.3, 30.8, 31.0, 31.1, 31.8, 32.5, 33.7, 36.2, 36.8, 41.8, 47.9, 49.3, 49.9, 50.2, 64.2, 64.4, 109.3, 121.5, 140.4, 178.2, 223.1; LRMS calculated for $C_{30}H_{47}O_5$ $[M+H]^+$ 487.3, found 487.3 m/z.

10-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'β-yl]-decanoic acid (33)

White solid (135 mg, 100%); IR (film) 3600-2400 (OH, carboxylic acid), 1726 (C=O, ketone), 1704 (C=O, carboxylic acid); ^{1}H-NMR (400 MHz, $CDCl_3$) 0.84 (s, 18'-CH_3), 1.05 (s, 19'-CH_3), 1.10 to 2.20 (m, 33H, CH and CH_2 of steroid skeleton and alkyl chain), 2.35 (t, *J* = 7.5 Hz, C$\underline{H}_2$COOH), 2.59 (m, 4'-CH), 3.95 (m, CH_2 of ketal), 5.39 (m, 6'-CH); ^{13}C-NMR (75 MHz, $CDCl_3$) 13.7, 18.9, 20.2, 24.7, 28.0, 28.7, 28.9, 29.0, 29.1, 29.3 (2x), 30.8, 30.9, 31.1, 31.8, 32.4, 33.8, 36.2, 36.8, 41.8, 47.9, 49.3, 49.9, 50.2, 64.2, 64.4, 109.3, 121.5, 140.4, 178.3, 223.2; LRMS calculated for $C_{31}H_{49}O_5$ $[M+H]^+$ 501.4, found 501.3 m/z.

Synthesis of Key Intermediate Carboxylic Acids 49-51 (Second Strategy)

Synthesis of 16-allyl-3,3-(ethylenedioxy)-androst-5-en-17-one (34)

To a solution of diisopropylamine (1.3 mL, 9.5 mmol) in dry THF (100 mL) under argon at 0°C was added dropwise *n*-BuLi (1.4 M in hexanes) (6.8 mL, 9.5 mmol). After 30 minutes, the LDA solution was cooled at –78°C and a solution of **10** (2.84 g, 8.59 mmol) in dry THF (25 mL) was

added. The mixture was stirred 1 h at 0°C before another cooling at –78°C. Then, allyl bromide (2.2 mL, 25 mmol) was slowly added to the reaction mixture, which was allowed to stir from –78°C to room temperature for 16 h. The reaction was quenched by addition of water and the crude product was extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$ and evaporated to dryness. Purification by flash chromatography (hexanes/EtOAc, 85:15) afforded **34** (2.87 g, 7.8 mmol, 90%) as a colourless viscous oil. IR (film) 1737 (C=O, ketone); ^{1}H-NMR (300 MHz, acetone-d_6) 0.82 and 0.94 (2s, 18-CH_3, 16β:16α / 12:88), 1.07 (s, 19-CH_3), 1.10 to 2.60 (m, 20H, CH and CH_2 of steroid skeleton and alkyl chain), 3.87 (m, CH_2 of ketal), 5.03 (m, CH=C$\underline{H}_2$), 5.28 (m, 6-CH), 5.79 (m, C$\underline{H}$=CH_2); ^{13}C-NMR (75 MHz, acetone-d_6) 14.5, 19.2, 20.9, 27.4, 29.0, 31.2, 31.8, 32.5, 35.7, 36.9, 37.4, 42.3, 44.4, 48.7, 50.0, 51.0, 64.6, 64.7, 109.7, 116.5, 121.7, 137.7, 141.2, 220.1.

C16 Epimerisation of 34 to Obtain 16β-allyl-3,3-(ethylenedioxy)-androst-5-en-17-one (35)

A solution of diisopropylamine (3.2 mL, 23 mmol) in dry THF (100 mL) was stirred under argon at 0°C, and *n*-BuLi (1.4 M in hexanes) (16 mL, 23 mmol) was added dropwise. After 30 minutes, the solution was cooled at –78°C and steroid **34** (2.8 g, 7.6 mmol), dissolved in dry THF (20 mL), was added dropwise to the LDA solution. The mixture was stirred for 1 h at 0°C before cooling at –78°C. Then, MeOH (3 mL) was added very slowly to the solution, which was stirred at –78°C for 1 h. After addition of water, the crude product was extracted with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$ and evaporated under reduced pressure. The crude residue was purified by flash chromatography (hexanes/EtOAc, 85: 15) to gain **35** (2.3 g, 6.2 mmol, 82%) as a white solid. IR (film) 1737 (C=O, ketone); ^{1}H-NMR (300 MHz, acetone-d_6) 0.82 (s, 18-CH_3), 1.06 (s, 19-CH_3), 1.10 to 2.60 (m, 20H, CH and CH_2 of steroid skeleton and alkyl chain), 3.88 (m, CH_2 of ketal), 5.05 (m, CH=C$\underline{H}_2$), 5.29 (m, 6-CH), 5.82 (m, C$\underline{H}$=CH_2); ^{13}C-NMR (75 MHz, acetone-d_6) 14.0, 19.3, 21.0, 29.0, 29.1, 31.6, 31.86, 31.91, 32.8, 36.8, 36.9, 42.4, 48.3, 49.3, 50.9, 51.2, 64.7 (2x), 109.7, 116.3, 121.8, 137.5, 141.3, 220.4. LRMS calculated for $C_{24}H_{35}O_3$ $[M+H]^+$ 371.3, found 371.3 m/z.

Synthesis of 11-dodecen-1-ol (39)

Halogen Exchange Br-I. To a solution of 10-bromo-1-decanol (**36**) (12 g, 50.6 mmol) in acetone (100 mL) was added NaI (30 g, 202 mmol). The solution was heated to reflux and stirred 16 h. After addition of water, the product was extracted with EtOAc and the organic phase was washed with a saturated solution of $Na_2S_2O_3$, dried over $MgSO_4$ and evaporated to dryness. 10-iodo-1-decanol (14.3 g, 50.6 mmol, 99%) was used for the next step without further purification. *Vinylation*. CuI (9.65 g, 50.7 mmol) was suspended in anhydrous THF (100 mL) under argon. The mixture was cooled down to –40°C and vinylmagnesium bromide (1 M in THF) (250 mL, 250 mmol) was added. The reaction was stirred for 15 min at –40°C. Then, HMPA (17.6 mL, 100 mmol) and triethyl phosphite (17.4 mL, 100 mmol) were added and the mixture was stirred 5 minutes at –40°C. 10-iodo-1-decanol (14.3 g, 50.6 mmol) was then added and the reaction mixture was stirred 1 h at –40°C and 2 h at room temperature. The reaction was quenched by addition of a saturated solution of NH_4Cl. The crude product was extracted with EtOAc, washed with brine, dried over $MgSO_4$ and evaporated under reduced pressure. Purification by flash chromatography (hexanes/EtOAc, 85:15) yielded **39** (5.5 g, 30.0 mmol, 60%) as a colourless oil. IR (film) 3334 (OH); ^{1}H-NMR (300 MHz, $CDCl_3$) 1.23 (m, 7 x CH_2), 1.50 (m, C$\underline{H}_2$CH$_2$OH), 1.99 (m, C$\underline{H}_2$CH=CH_2), 2.57 (s, OH), 3.55 (t, J = 6.7 Hz, C$\underline{H}_2$OH), 4.91 (m, CH=C$\underline{H}_2$), 5.75 (m, C$\underline{H}$=CH_2); ^{13}C-NMR (75 MHz, $CDCl_3$) 25.7, 28.9, 29.1, 29.4 (2x), 29.5, 29.6, 32.7, 33.8, 62.7, 114.0, 139.1.

General Procedure for Synthesizing Aldehydes 40-42

4-Methylmorpholine-*N*-oxide (0.43-1.23 g, 3.7-10.5 mmol) and molecular sieves (0.7-2.0 g) were added to a solution of alcohols **37**-**39** (0.45-1.10 g, 2.44-7.04 mmol) in dry DCM (5-15 mL) under argon at room temperature. The mixture was stirred for 15 min. Then, tetrapropylammonium perruthenate (TPAP) (43-123 mg, 0.12-0.35 mmol) was added. After the reaction mixture was stirred for 1 h, the solvent was removed under reduced pressure. Crude products were purified by flash chromatography (hexanes/EtOAc, 9:1) to yield aldehydes **40**-**42** (289-986 mg, 1.58-6.52 mmol, 65-93%).

9-decenal (40)

Colourless oil (986 mg, 93%); IR (film) 3077 (C-H, alkene), 2717 (C-H, aldehyde), 1728 (C=O, aldehyde), 1641 (C=C, alkene); ^{1}H-NMR (300 MHz, $CDCl_3$) 1.29 (m, 4 x CH_2), 1.60 (m, C$\underline{H}_2$CH$_2$CHO), 2.02 (m, C$\underline{H}_2$CH=CH_2), 2.40 (dt, J_1 = 7.4 Hz, J_2 = 1.8 Hz, C$\underline{H}_2$CHO), 4.94 (m, CH=C$\underline{H}_2$), 5.78 (m, C$\underline{H}$=CH_2), 9.74 (t, J = 2.0 Hz, CHO); ^{13}C-NMR (75 MHz, $CDCl_3$) 22.0, 28.77, 28.83, 29.0, 29.1, 33.7, 43.8, 114.2, 139.0, 202.9.

10-undecenal (41)

Colourless oil (708 mg, 80%); IR (film) 3077 (C-H, alkene), 2715 (C-H, aldehyde), 1728 (C=O, aldehyde), 1641 (C=C, alkene); ^{1}H-NMR (300 MHz, $CDCl_3$) 1.28 (m, 5 x CH_2), 1.61 (m, C$\underline{H}_2$CH$_2$CHO), 2.02 (m, C$\underline{H}_2$CH=CH_2), 2.40 (dt, J_1 = 7.3 Hz, J_2 = 1.5 Hz, C$\underline{H}_2$CHO), 4.95 (m, CH=C$\underline{H}_2$), 5.79 (m, C$\underline{H}$=CH_2), 9.74 (t, J = 1.9 Hz, CHO); ^{13}C-NMR (75 MHz, $CDCl_3$) 22.0, 28.8, 29.0, 29.1, 29.3 (2x), 33.7, 43.9, 114.1, 139.1, 202.9.

11-dodecenal (42)

Colourless oil (289 mg, 65%); IR (film) 3076 (C-H, alkene), 2715 (C-H, aldehyde), 1728 (C=O, aldehyde), 1640 (C=C, alkene); ^{1}H-NMR (300 MHz, $CDCl_3$) 1.27 (m, 6 x CH_2), 1.62 (m, C$\underline{H}_2$CH$_2$CHO), 2.03 (m, C$\underline{H}_2$CH=CH_2), 2.41 (dt, J_1 = 7.3 Hz, J_2 = 1.8 Hz, C$\underline{H}_2$CHO), 4.96 (m, CH=C$\underline{H}_2$), 5.80 (m, C$\underline{H}$=CH_2), 9.75 (s, CHO); ^{13}C-NMR (75 MHz, $CDCl_3$) 22.0, 28.9 (2x), 29.1 (2x), 29.3 (2x), 33.7, 43.9, 114.1, 139.2, 203.0.

General Procedure for Grubbs' Cross-metathesis (Synthesis of 43-45)

A mixture of **35** (250-700 mg, 0.67-1.89 mmol), freshly prepared aldehydes **40**-**42** (244-885 mg, 1.33-5.73 mmol) and tricyclohexylphosphine [1,3-bis(2,4,6-trimethyl-phenyl)-

4,5-dihydroimidazol-2-ylidene] [benzylidine] ruthenium(IV) dichloride (Grubbs's catalyst) (57-160 mg, 0.067-0.189 mmol) in dry DCM (15-30 mL) was refluxed for 16 h under argon atmosphere. The crude mixture was preadsorbed on silica gel and a flash chromatography was performed with hexanes/EtOAc, 9:1 to 7:3 to afford the desired steroids **43-45** (183-360 mg, 0.349-0.725 mmol, 38-53%).

11-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'β-yl]-9-undecenal (43)

Colourless viscous oil (360 mg, 38%); IR (film) 2713 (C-H, aldehyde), 1732 (C=O, ketone); ^{1}H-NMR (300 MHz, acetone-d_6) 0.81 (s, 3H, 18'-CH_3), 1.05 to 2.15 (m, 30H, CH and CH_2 of steroid skeleton and alkyl chain), 1.06 (s, 3H, 19'-CH_3), 2.45 (m, 4'-CH, 11-CH, C$\underline{H}_2$CHO), 3.87 (m, CH_2 of ketal), 5.30 (m, 6'-CH), 5.44 (m, CH=CH), 9.72 (s_{app}, CHO); ^{13}C-NMR (75 MHz, acetone-d_6) 13.9, 19.2, 21.0, 22.7, 28.8, 29.1, 29.3 to 30.4 (3C under solvent peaks), 30.6, 31.6, 31.8, 32.7, 33.1, 35.3, 36.9, 37.5, 42.4, 44.3, 48.3, 49.9, 50.9, 51.1, 64.7 (2x), 109.7, 121.8, 128.6, 132.8, 141.3, 202.8, 220.7.

12-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'β-yl]-10-dodecenal (44)

Colourless viscous oil (367 mg, 53%); IR (film) 2714 (C-H, aldehyde), 1732 (C=O, ketone); ^{1}H-NMR (300 MHz, acetone-d_6) 0.81 (s, 18'-CH_3), 1.05 to 2.15 (m, 32H, CH and CH_2 of steroid skeleton and alkyl chain), 1.07 (s, 19'-CH_3), 2.45 (m, 4'-CH, 12-CH, C$\underline{H}_2$CHO), 3.87 (m, CH_2 of ketal), 5.29 (m, 6'-CH), 5.45 (m, CH=CH), 9.72 (s_{app}, CHO); ^{13}C-NMR (75 MHz, acetone-d_6) 13.9, 19.3, 21.0, 22.7, 28.8, 29.1, 29.4 to 30.4 (4C under solvent peaks), 30.6, 31.7, 31.9, 32.8, 33.2, 35.4, 36.9, 37.5, 42.4, 44.3, 48.3, 49.9, 50.9, 51.2, 64.7 (2x), 109.7, 121.8, 128.6, 132.9, 141.3, 202.8, 220.8.

13-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'β-yl]-11-tridecenal (45)

Colourless viscous oil (183 mg, 52%); IR (film) 2715 (C-H, aldehyde), 1732 (C=O, ketone); ^{1}H-NMR (300 MHz, acetone-d_6) 0.82 (s, 18'-CH_3), 1.05 to 2.15 (m, 34H, CH and CH_2 of steroid skeleton and alkyl chain), 1.07 (s, 19'-CH_3), 2.45 (m, 4'-CH, 13-CH, C$\underline{H}_2$CHO), 3.88 (m, CH_2 of ketal), 5.29 (m, 6'-CH), 5.45 (m, CH=CH), 9.72 (t, J = 1.6 Hz, CHO); ^{13}C-NMR (75 MHz, acetone-d_6) 13.9, 19.3, 21.1, 22.8, 28.9, 29.0, 29.1, 29.4 to 30.4 (4C under solvent peaks), 30.7, 31.7, 31.9, 32.8, 33.2, 35.4, 36.9, 37.5, 42.4, 44.3, 48.3, 49.9, 50.9, 51.2, 64.7 (2x), 109.7, 121.8, 128.6, 132.9, 141.3, 202.8, 220.7.

General Procedure for the Synthesis of 46-48

A suspension of **43-45** (187-347 mg, 0.356-0.679 mmol) and 10% Pd/C (20-35 mg) in EtOAc (7-15 mL) was stirred under hydrogen atmosphere at room temperature. After 16 h, the resulting suspension was filtered through celite, washed with EtOAc and evaporated to dryness to afford **46-48** (179-346 mg, 0.340-0.675 mmol, 96-99%) in good purities without purification.

11-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'β-yl]-undecanal (46)

Colourless viscous oil (280 mg, 99%); ^{1}H-NMR (300 MHz, $CDCl_3$) 0.82 (s, 18'-CH_3), 1.00 to 2.15 (m, 35H, CH and CH_2 of steroid skeleton and alkyl chain), 1.03 (s, 19'-CH_3), 2.41 (m, C$\underline{H}_2$CHO), 2.58 (m, 4'-CH) 3.94 (m, CH_2 of ketal), 5.37 (m, 6'-CH), 9.74 (s_{app},, CHO).

12-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'β-yl]-dodecanal (47)

Colourless viscous oil (346 mg, 99%); ^{1}H-NMR (300 MHz, acetone-d_6) 0.83 (s, 18'-CH_3), 1.05 to 2.20 (m, 37H, CH and CH_2 of steroid skeleton and alkyl chain), 1.07 (s, 19'-CH_3), 2.43 (dt, J_1 = 7.3 Hz, J_2 = 1.3 Hz, C$\underline{H}_2$CHO), 2.49 (m, 4'-CH), 3.87 (m, CH_2 of ketal), 5.29 (m, 6'-CH), 9.72 (s_{app},, CHO).

13-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'β-yl]-tridecanal (48)

Colourless viscous oil (179 mg, 96%); ^{1}H-NMR (300 MHz, acetone-d_6) 0.83 (s, 18'-CH_3), 1.05 to 2.20 (m, 39H, CH and CH_2 of steroid skeleton and alkyl chain), 1.07 (s, 19'-CH_3), 2.43 (t, J = 7.3 Hz, C$\underline{H}_2$CHO), 2.49 (m, 4'-CH), 3.87 (m, CH_2 of ketal), 5.29 (m, 6'-CH), 9.72 (s_{app},, CHO).

Oxidation of Aldehydes to Carboxylic Acids 49-51

Oxidation of aldehydes **46-48** to carboxylic acids **49-51** was achieved following the same procedure as described above for the synthesis of carboxylic acids **30-33**.

11-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'β-yl]-undecanoic acid (49)

Colourless solid (312 mg, 99%); IR (film) 3600-2400 (OH, carboxylic acid), 1726 (C=O, ketone), 1710 (C=O, carboxylic acid); ^{1}H-NMR (300 MHz, $CDCl_3$) 0.82 (s, 18'-CH_3), 1.04 (s, 19'-CH_3), 1.05 to 2.20 (m, 35H, CH and CH_2 of steroid skeleton and alkyl chain), 2.32 (t, J = 7.5 Hz, C$\underline{H}_2$COOH), 2.58 (m, 4'-CH), 3.93 (m, CH_2 of ketal), 5.38 (m, 6'-CH); ^{13}C-NMR (75 MHz, $CDCl_3$) 13.6, 18.9, 20.2, 24.7, 28.1, 28.9, 29.0, 29.1, 29.4 (2x), 30.8, 30.9, 31.0 (2x), 31.1, 31.7, 32.5, 33.9, 36.2, 36.8, 41.8, 47.9, 49.3, 49.9, 50.2, 64.2, 64.4, 109.3, 121.5, 140.3, 178.3, 223.2; LRMS calculated for $C_{32}H_{51}O_5$ $[M+H]^+$ 515.4, found 515.3 m/z.

12-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'β-yl]-dodecanoic acid (50)

White solid (251 mg, 74%); IR (film) 3600-2400 (OH, carboxylic acid), 1726 (C=O, ketone), 1710 (C=O, carboxylic acid); ^{1}H-NMR (300 MHz, $CDCl_3$) 0.83 (s, 18'-CH_3), 1.04 (s, 19'-CH_3), 1.05 to 2.20 (m, 37H, CH and CH_2 of steroid skeleton and alkyl chain), 2.32 (t, J = 7.4 Hz, C$\underline{H}_2$COOH), 2.59 (m, 4'-CH), 3.95 (m, CH_2 of ketal), 5.37 (m, 6'-CH); ^{13}C-NMR (75 MHz, $CDCl_3$) 13.6, 18.9, 20.2, 24.7, 28.1, 28.9, 29.0, 29.2, 29.4 (2x), 29.5 (3x), 30.8, 30.9, 31.1, 31.8, 32.5, 33.9, 36.2, 36.8, 41.8, 47.9, 49.3, 49.9, 50.2, 64.2, 64.4, 109.3, 121.5, 140.3, 179.0, 223.2; LRMS calculated for $C_{33}H_{53}O_5$ $[M+H]^+$ 529.4, found 529.3 m/z.

13-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'β -yl]-tridecanoic acid (51)

White solid (177 mg, 99%); IR (film) 3600-2400 (OH, carboxylic acid), 1726 (C=O, ketone), 1711 (C=O, carboxylic acid); ^{1}H-NMR (400 MHz, $CDCl_3$) 0.84 (s, 18'-CH_3), 1.05 (s, 19'-CH_3), 1.05 to 2.20 (m, 39H, CH and CH_2 of steroid

skeleton and alkyl chain), 2.35 (t, J = 7.5 Hz, CH_2COOH), 2,58 (m, 4'-CH), 3.95 (m, CH_2 of ketal), 5.39 (m, 6'-CH); ^{13}C-NMR (75 MHz, $CDCl_3$) 13.7, 18.9, 20.2, 24.7, 28.1, 28.9, 29.0, 29.2 (2x), 29.5 (4x), 29.7, 30.8, 31.0, 31.1, 31.8, 32.5, 33.8, 36.2, 36.8, 41.8, 47.9, 49.3, 49.9, 50.2, 64.2, 64.4, 109.3, 121.5, 140.4, 178.3, 223.2; LRMS calculated for $C_{34}H_{55}O_5$ $[M+H]^+$ 543.4, found 543.3 m/z.

General Procedure for Synthesizing Compounds 53-39

Method A

A mixture of carboxylic acids **30**-**33** (100-150 mg, 0.212-0.308 mmol), DMAP (38-56 mg, 0.317-0.462 mmol) and 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (61-89 mg, 0.317-0.462 mmol) in dry DMF (2 mL) under argon atmosphere was stirred for 30 minutes at room temperature. Then, a solution of 2',3'-isopropylidene adenosine (**52**) (130-189 mg, 0.423-0.617 mmol) in dry DMF (1 mL) was added and stirred for 24 h at room temperature. The reaction was quenched by addition of water and extraction was performed with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated to dryness. The crude product was purified by flash chromatography (DCM/MeOH, 98:2) to afford esters **53**-**56** (71-120 mg, 0.093-0.154 mmol, 44-69%).

Method B

To a solution of carboxylic acids **49**-**51** (137-248 mg, 0.259-0.482 mmol) in dry DMF (3-5 mL) under argon atmosphere at room temperature was added benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP) (202-376 mg, 0.388-0.723 mmol), 1-hydroxy-benzotriazole (HOBt) (52-98 mg, 0.388-0.723 mmol) and diisopropylethylamine (135-250 µL, 0.78-1.45 mmol). The reaction mixture was stirred for 5 minutes. Then, a solution of 2',3'-isopropylidene adenosine (159-296 mg, 0.518-0.964 mmol) in dry DMF (0.5-1.0 mL) was added and stirred for 16 h at room temperature. Water was added to quench the reaction and the extraction was performed with EtOAc. The organic phase was washed with brine, dried over $MgSO_4$ and evaporated under reduced pressure. Purification of the crude residue by flash chromatography (hexanes/EtOAc, 5:5 followed by DCM/MeOH 98:2 to 96:4) gained esters **57**-**59** (108-173 mg, 0.132-0.215 mmol, 45-52%).

5'-O-{4-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'β-yl]-butanoyl}2',3'-O-isopropylidene-adenosine (53)

White foam (119 mg, 55%); IR (film) 3332 and 3174 (NH_2), 1732 (C=O, ester and ketone), 1644 (C=N); ^{1}H-NMR (400 MHz, $CDCl_3$) 0.82 (s, 18'-CH_3), 1.05 (s, 19'-CH_3), 1.05 to 2.20 (m, 21H, CH and CH_2 of steroid skeleton and alkyl chain), 1.41 and 1.63 (2s, 2 x CH_3 of isopropylidene), 2.21 (m, CH_2COO), 2.56 (m, 4'-CH), 3.95 (m, CH_2 of ketal), 4.29 (m, 5'-CH_2 of ribose), 4.52 (m, 4'-CH of ribose), 5.00 (dd, J_1 = 6.3 Hz, J_2 = 3.3 Hz, 3'-CH of ribose), 5.37 (m, 2'-CH of ribose and 6'-CH), 6.16 (d, J = 2.1 Hz, 1'-CH of ribose), 8.09 and 8.37 (2s, 2 x CH of adenine); ^{13}C-NMR (100 MHz, $CDCl_3$) 13.7, 18.9, 20.2, 23.3, 25.3, 27.1, 28.8, 30.8, 30.97, 31.05, 31.75, 31.78, 33.6, 36.2, 36.8, 41.8, 48.0, 48.9, 49.9, 50.2, 63.8, 64.3, 64.4, 81.5, 84.5, 85.1, 91.4, 109.3, 114.8, 120.0, 121.4, 139.6, 140.4, 148.8, 153.8, 155.5, 172.7, 223.0; LRMS calculated for $C_{38}H_{52}N_5O_8$ $[M+H]^+$ 706.4, found 706.3 m/z.

5'-O-{8-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'β-yl]-octanoyl}2',3'-O-isopropylidene-adenosine (54)

White foam (71 mg, 44%); IR (film) 3332 and 3166 (NH_2), 1732 (C=O, ester and ketone), 1645 (C=N); ^{1}H-NMR (300 MHz, $CDCl_3$) 0.79 (s, 18'-CH_3), 1.00 to 2.20 (m, 31H, CH and CH_2 of steroid skeleton and alkyl chain), 1.01 (s, 19'-CH_3), 1.36 and 1.58 (2s, 2 x CH_3 of isopropylidene), 2.54 (m, 4'-CH), 3.91 (m, CH_2 of ketal), 4.16 to 4.35 (m, 5'-CH_2 of ribose), 4.44 (m, 4'-CH of ribose), 5.01 (dd, J_1 = 6.2 Hz, J_2 = 3.3 Hz, 3'-CH of ribose), 5.34 (m, 6'-CH), 5.44 (dd, J_1 = 6.1 Hz, J_2 = 1.8 Hz, 2'-CH of ribose), 6.08 (d, J = 1.8 Hz, 1'-CH of ribose), 6.17 (s, NH_2), 7.88 and 8.31 (2s, 2 x CH of adenine); ^{13}C-NMR (75 MHz, $CDCl_3$) 13.5, 18.7, 20.1, 24.6, 25.2, 27.0, 27.9, 28.8, 28.9 (2x), 29.1, 30.7, 30.8, 30.9, 31.6, 32.3, 33.7, 36.1, 36.6, 41.6, 47.8, 49.1, 49.8, 50.0, 63.7, 64.1, 64.3, 81.5, 84.1, 84.8, 90.9, 109.2, 114.4, 120.1, 121.4, 139.5, 140.2, 149.1, 152.8, 155.4, 173.1, 222.9; LRMS calculated for $C_{42}H_{60}N_5O_8$ $[M+H]^+$ 762.4, found 762.3 m/z.

5'-O-{9-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'β-yl]-nonanoyl}2',3'-O-isopropylidene-adenosine (55)

White foam (120 mg, 50%); IR (film) 3340 and 3173 (NH_2), 1732 (C=O, ester and ketone), 1644 (C=N); ^{1}H-NMR (400 MHz, $CDCl_3$) 0.84 (s, 18'-CH_3), 1.05 (s, 19'-CH_3), 1.05 to 2.25 (m, 33H, CH and CH_2 of steroid skeleton and alkyl chain), 1.41 and 1.64 (2s, 2 x CH_3 of isopropylidene), 2.58 (m, 4'-CH), 3.95 (m, CH_2 of ketal), 4.22 to 4.38 (m, 5'-CH_2 of ribose), 4.54 (m, 4'-CH of ribose), 4.97 (dd, J_1 = 6.2 Hz, J_2 = 3.2 Hz, 3'-CH of ribose), 5.35 (m, 2'-CH of ribose and 6'-CH), 6.14 (d, J = 2.2 Hz, 1'-CH of ribose), 8.04 and 8.36 (2s, 2 x CH of adenine); ^{13}C-NMR (75 MHz, acetone-d_6) 13.9, 19.1, 20.9, 25.4, 27.4, 28.6, 29.0 to 30.5 (6C under solvent peaks) 31.5, 31.8 (2x), 32.6, 33.0, 34.2, 36.8, 37.4, 42.3, 48.3, 49.6, 50.8, 51.1, 64.4, 64.6 (2x), 82.6, 84.8, 85.4, 91.0, 109.6, 114.5, 120.0, 121.7, 140.7, 141.2, 150.9, 153.6, 157.1, 173.2, 221.5; LRMS calculated for $C_{43}H_{62}N_5O_8$ $[M+H]^+$ 776.4, found 776.4 m/z.

5'-O-{10-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'β-yl]-decanoyl}2',3'-O-isopropylidene-adenosine (56)

White foam (111 mg, 69%); IR (film) 3332 and 3167 (NH_2), 1732 (C=O, ester and ketone), 1645 (C=N); ^{1}H-NMR (400 MHz, $CDCl_3$) 0.84 (s, 18'-CH_3), 1.05 (s, 19'-CH_3), 1.10 to 2.25 (m, 35H, CH and CH_2 of steroid skeleton and alkyl chain), 1.41 and 1.63 (2s, 2 x CH_3 of isopropylidene), 2.59 (m, 4'-CH), 3.95 (m, CH_2 of ketal), 4.22 to 4.37 (m, 5'-CH_2 of ribose), 4.50 (m, 4'-CH of ribose), 4.97 (dd, J_1 = 6.2 Hz, J_2 = 3.3 Hz, 3'-CH of ribose), 5.39 (m, 2'-CH of ribose and 6'-CH), 6.13 (d, J = 2.1 Hz, 1'-CH of ribose), 8.00 and 8.36 (2s, 2 x CH of adenine); ^{13}C-NMR (75 MHz, $CDCl_3$) 13.7, 18.9, 20.2, 24.7, 25.3, 27.1, 28.1, 28.9, 29.0, 29.1, 29.3 (3x), 30.8, 31.0, 31.1, 31.8, 32.5, 33.9, 36.2, 36.8, 41.8, 47.9, 49.3, 49.9, 50.2, 63.8, 64.2, 64.4, 81.6, 84.3, 85.0, 91.2, 109.3, 114.7, 120.2, 121.5, 140.2, 140.4, 149.0, 151.2, 154.5, 173.2, 223.1; LRMS calculated for $C_{44}H_{64}N_5O_8$ $[M+H]^+$ 790.5, found 790.4 m/z.

5'-O-{11-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'β-yl]-undecanoyl}2',3'-O-isopropylidene-adenosine (57)

White foam (173 mg, 45%); IR (film) 3326 and 3172 (NH_2), 1733 (C=O, ester and ketone), 1645 (C=N); ^{1}H-NMR (300 MHz, $CDCl_3$) 0.81 (s, 18'-CH_3), 1.00 to 2.22 (m, 37H, CH and CH_2 of steroid skeleton and alkyl chain), 1.02 (s, 19'-CH_3), 1.38 and 1.60 (2s, 2 x CH_3 of isopropylidene), 2.56 (m, 4'-CH), 3.93 (m, CH_2 of ketal), 4.18 to 4.37 (m, 5'-CH_2 of ribose), 4.46 (m, 4'-CH of ribose), 5.01 (dd, J_1 = 6.2 Hz, J_2 = 3.3 Hz, 3'-CH of ribose), 5.37 (m, 6'-CH), 5.43 (dd, J_1 = 6.1 Hz, J_2 = 1.5 Hz, 2'-CH of ribose), 6.09 (d, J = 1.8 Hz, 1'-CH of ribose), 6.27 (s, NH_2), 7.91 and 8.32 (2s, 2 x CH of adenine); ^{13}C-NMR (75 MHz, $CDCl_3$) 13.6, 18.9, 20.2, 24.7, 25.3, 27.1, 28.1, 28.9, 29.0, 29.2, 29.37 (2x), 29.42 (2x), 30.8, 30.9, 31.0, 31.7, 32.5, 33.9, 36.2, 36.7, 41.8, 47.9, 49.3, 49.9, 50.2, 63.8, 64.2, 64.4, 81.6, 84.3, 85.0, 91.1, 109.3, 114.6, 120.1, 121.5, 139.8, 140.3, 149.1, 152.3, 155.2, 173.2, 223.0; LRMS calculated for $C_{45}H_{66}$ N_5O_8 $[M+H]^+$ 804.5, found 804.8 m/z.

5'-O-{12-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'β-yl]-dodecanoyl}2',3'-O-isopropylidene-adenosine (58)

White foam (108 mg, 51%); IR (film) 3332 and 3185 (NH_2), 1734 (C=O, ester and ketone), 1653 (C=N); ^{1}H-NMR (400 MHz, acetone-d_6) 0.83 (s, 18'-CH_3), 1.08 (s, 19'-CH_3), 1.15 to 2.15 (m, 37H, CH and CH_2 of steroid skeleton and alkyl chain), 1.38 and 1.58 (2s, 2 x CH_3 of isopropylidene), 2.25 (t, J = 2.6 Hz, CH_2COO), 2.48 (m, 4'-CH), 3.89 (m, CH_2 of ketal), 4.21 to 4.35 (m, 5'-CH_2 of ribose), 4.43 (m, 4'-CH of ribose), 5.16 (dd, J_1 = 6.2 Hz, J_2 = 3.2 Hz, 3'-CH of ribose), 5.30 (m, 6'-CH), 5.57 (m, 2'-CH of ribose), 6.23 (d, J = 1.9 Hz, 1'-CH of ribose), 6.69 (s, NH_2), 8.20 and 8.23 (2s, 2 x CH of adenine); ^{13}C-NMR (75 MHz, acetone-d_6) 13.9, 19.1, 20.8, 25.4, 26.7, 26.8, 27.3, 28.6, 28.9 to 30.4 (7C under solvent peaks) 31.4, 31.7 (2x), 32.6, 33.0, 34.1, 36.7, 37.3, 42.2, 48.2, 49.6, 50.8, 51.0, 63.1, 64.3, 64.5, 82.5, 84.7, 85.3, 90.9, 109.5, 114.4, 119.9, 121.7, 140.7, 141.1, 150.9, 153.6, 157.0, 173.2, 221.4; LRMS calculated for $C_{46}H_{68}N_5O_8$ $[M+H]^+$ 818.5, found 818.3 m/z.

5'-O-{13-[3',3'-(ethylenedioxy)-androst-5'-en-17'-oxo-16'β-yl]-tridecanoyl}2',3'-O-isopropylidene-adenosine (59)

White foam (148 mg, 52%); IR (film) 3325 and 3176 (NH_2), 1734 (C=O, ester and ketone), 1653 (C=N); ^{1}H-NMR (300 MHz, acetone-d_6) 0.82 (s, 18'-CH_3), 1.07 (s, 19'-CH_3), 1.15 to 2.20 (m, 39H, CH and CH_2 of steroid skeleton and alkyl chain), 1.37 and 1.57 (2s, 2 x CH_3 of isopropylidene), 2.25 (m, CH_2COO), 2.50 (m, 4'-CH), 3.88 (m, CH_2 of ketal), 4.18 to 4.34 (m, 5'-CH_2 of ribose), 4.42 (m, 4'-CH of ribose), 5.15 (dd, J_1 = 6.3 Hz, J_2 = 3.3 Hz, 3'-CH of ribose), 5.29 (m, 6'-CH), 5.57 (m, 2'-CH of ribose), 6.22 (d, J = 2.1 Hz, 1'-CH of ribose), 6.65 (s, NH_2), 8.18 and 8.21 (2s, 2 x CH of adenine); ^{13}C-NMR (75 MHz, acetone-d_6) 13.8, 19.1, 20.8, 25.3, 26.6, 26.7, 27.2, 28.6, 28.9 to 30.4 (8C under solvent peaks) 31.4, 31.7 (2x), 32.6, 32.9, 34.1, 36.7, 37.3, 42.2, 48.2, 49.5, 50.7, 51.0, 63.0, 64.3, 64.5, 82.5, 84.7, 85.3, 90.9, 109.5, 114.4, 119.9, 121.6, 140.6, 141.1, 150.9, 153.5, 157.0, 173.2, 221.4; LRMS calculated for $C_{47}H_{70}N_5O_8$ $[M+H]^+$ 832.5, found 832.4 m/z.

General Procedure for the Final Deprotection (Synthesis of 1-7)

Esters **53-59** (68–110 mg, 0.089-0.144 mmol) in dry THF (1 mL) were treated with a TFA/H_2O (9/1, v/v) solution (4.5-7.3 mL). The reaction mixture was stirred for 30 minutes and then quenched by addition of a saturated aqueous $NaHCO_3$ solution. The crude product was extracted with EtOAc and the organic phase was washed with brine, dried over $MgSO_4$ and evaporated under reduce pressure. The crude residue was purified by flash chromatography (DCM/MeOH, 95:5) to afford bisubstrate compounds **1**-**7** (42-83 mg, 0.058-0.123 mmol, 52-85%).

5'-O-{4-[androst-4'-en-3',17'-dioxo-16'β-yl]-butanoyl}adenosine (1)

White solid (44 mg, 52%); IR (film) 3331 and 3178 (OH and NH_2), 1731 (C=O, ester and ketone), 1652 (C=N and C=O conjugated ketone); ^{1}H-NMR (400 MHz, DMSO-d_6) 0.74 (s, 18'-CH_3), 0.80 to 2.50 (m, 22H, CH and CH_2 of steroid skeleton and alkyl chain), 1.17 (s, 19'-CH_3), 2.32 (m, CH_2COO), 4.08 (m, 4'-CH of ribose), 4.19 to 4.36 (m, 3'-CH and 5'-CH_2 of ribose), 4.63 (m, 2'-CH of ribose), 5.38 and 5.59 (2m, 2 x OH), 5.66 (s, 4'-CH), 5.91 (d, J = 4.8 Hz, 1'-CH of ribose), 7.35 (s, NH_2), 8.15 and 8.32 (2s, 2 x CH of adenine); ^{13}C-NMR (75 MHz, DMSO-d_6) 13.5, 16.9, 19.8, 22.9, 28.0, 30.5, 31.0, 31.3, 31.8, 33.2, 33.6, 34.0, 35.1, 38.3, 47.3, 48.1, 48.3, 53.3, 63.8, 70.2, 73.0, 81.4, 87.7, 119.1, 123.3, 139.6, 149.3, 152.7, 156.0, 170.8, 172.6, 198.0, 221.1; HRMS calculated for $C_{33}H_{44}N_5O_7$ $[M+H]^+$ 622.32353, found 622.32361 m/z.

5'-O-{8-[androst-4'-en-3',17'-dioxo-16'β-yl]-octanoyl}adenosine (2)

White solid (83 mg, 85%); IR (film) 3330 and 3188 (OH and NH_2), 1732 (C=O, ester and ketone), 1645 (C=N and C=O conjugated ketone); ^{1}H-NMR (400 MHz, DMSO-d_6) 0.78 (s, 18'-CH_3), 0.85 to 2.50 (m, 30H, CH and CH_2 of steroid skeleton and alkyl chain), 1.16 (s, 19'-CH_3), 2.28 (t, J = 6.6 Hz, CH_2COO), 4.07 (m, 4'-CH of ribose), 4.19 to 4.32 (m, 3'-CH and 5'-CH_2 of ribose), 4.66 (m, 2'-CH of ribose), 5.38 (d, J = 5.5 Hz, OH), 5.59 (d, J = 5.8 Hz, OH), 5.66 (s, 4'-CH), 5.90 (d, J = 4.9 Hz, 1'-CH of ribose), 7.33 (s, NH_2), 8.14 and 8.31 (2s, 2 x CH of adenine); ^{13}C-NMR (75 MHz, DMSO-d_6) 13.6, 16.9, 19.8, 24.4, 27.3, 28.2, 28.3, 28.5, 28.7, 30.6, 31.3, 31.7, 31.8, 33.3, 33.6, 34.0, 35.1, 38.3, 47.3, 48.4 (2x), 53.3, 63.7, 70.2, 72.9, 81.4, 87.7, 119.1, 123.3, 139.7, 149.3, 152.6, 156.0, 170.7, 172.8, 198.0, 221.4; HRMS calculated for $C_{37}H_{52}N_5O_7$ $[M+H]^+$ 678.38613, found 678.38596 m/z.

5'-O-{9-[androst-4'-en-3',17'-dioxo-16'β-yl]-nonanoyl} adenosine (3)

White solid (69 mg, 80%); IR (film) 3332 and 3190 (OH and NH_2), 1733 (C=O, ester and ketone), 1652 (C=N and C=O conjugated ketone); ^{1}H-NMR (400 MHz, DMSO-d_6) 0.78 (s, 18'-CH_3), 0.90 to 2.50 (m, 32H, CH and CH_2 of steroid skeleton and alkyl chain), 1.17 (s, 19'-CH_3), 2.28 (t, J = 7.3 Hz, CH_2COO), 4.07 (m, 4'-CH of ribose), 4.15 to 4.37 (m, 3'-CH and 5'-CH_2 of ribose), 4.66 (m, 2'-CH of ribose), 5.37 and 5.58 (2m, 2 x OH), 5.66 (s, 4'-CH), 5.90 (d, J = 4.8

Hz, 1'-CH of ribose), 7.35 (s, NH_2), 8.15 and 8.31 (2s, 2 x CH of adenine); ^{13}C-NMR (75 MHz, DMSO-d_6) 13.5, 16.8, 19.8, 24.3, 27.3, 28.1, 28.3 (2x), 28.7 (2x), 30.5, 31.2, 31.6, 31.8, 33.2, 33.6, 33.9, 35.0, 38.2, 47.2, 48.3 (2x), 53.2, 63.6, 70.2, 72.8, 81.4, 87.7, 119.1, 123.2, 139.7, 149.2, 152.4, 155.9, 170.6, 172.7, 198.0, 221.3; HRMS calculated for $C_{38}H_{54}N_5O_7$ $[M+H]^+$ 692.40170, found 692.40219 m/z.

5'-O-{10-[androst-4'-en-3',17'-dioxo-16'β-yl]-decanoyl} adenosine (4)

White solid (44 mg, 73%); IR (film) 3332 and 3178 (OH and NH_2), 1725 (C=O, ester and ketone), 1649 (C=N and C=O conjugated ketone); ^{1}H-NMR (400 MHz, DMSO-d_6) 0.78 (s, 18'-CH_3), 0.90 to 2.50 (m, 34H, CH and CH_2 of steroid skeleton and alkyl chain), 1.17 (s, 19'-CH_3), 2.28 (t, J = 7.6 Hz, CH_2COO), 4.07 (m, 4'-CH of ribose), 4.16 to 4.35 (m, 3'-CH and 5'-CH_2 of ribose), 4.66 (t, J = 4.8 Hz, 2'-CH of ribose), 5.38 (d, J = 5.5 Hz, OH), 5.58 (d, J = 5.7 Hz, OH), 5.66 (s, 4'-CH), 5.90 (d, J = 4.8 Hz, 1'-CH of ribose), 7.32 (s, NH_2), 8.14 and 8.31 (2s, 2 x CH of adenine); ^{13}C-NMR (75 MHz, DMSO-d_6) 13.6, 16.9, 19.8, 24.4, 27.4, 28.2, 28.4, 28.6, 28.8 (3x), 30.6, 31.3, 31.7, 31.8, 33.3, 33.6, 34.0, 35.1, 38.3, 47.3, 48.4 (2x), 53.3, 63.7, 70.2, 72.8, 81.4, 87.7, 119.1, 123.3, 139.7, 149.3, 152.6, 156.0, 170.7, 172.8, 198.0, 221.4; HRMS calculated for $C_{39}H_{56}N_5O_7$ $[M+H]^+$ 706.41743, found 706.41721 m/z.

5'-O-{11-[androst-4'-en-3',17'-dioxo-16'β-yl]-undecanoyl} adenosine (5)

White solid (42 mg, 54%); IR (film) 3334 and 3198 (OH and NH_2), 1732 (C=O, ester and ketone), 1647 (C=N and C=O conjugated ketone); ^{1}H-NMR (300 MHz, DMSO-d_6) 0.78 (s, 18'-CH_3), 0.90 to 2.50 (m, 36H, CH and CH_2 of steroid skeleton and alkyl chain), 1.17 (s, 19'-CH_3), 2.28 (t, J = 7.3 Hz, CH_2COO), 4.08 (m, 4'-CH of ribose), 4.16 to 4.35 (m, 3'-CH and 5'-CH_2 of ribose), 4.66 (m, 2'-CH of ribose), 5.38 (d, J = 5.3 Hz, OH), 5.59 (d, J = 5.8 Hz, OH), 5.66 (s, 4'-CH), 5.90 (d, J = 4.9 Hz, 1'-CH of ribose), 7.33 (s, NH_2), 8.14 and 8.31 (2s, 2 x CH of adenine); ^{13}C-NMR (75 MHz, DMSO-d_6) 13.6, 16.9, 19.8, 24.4, 27.4, 28.2, 28.4, 28.7, 28.9 (4x), 30.6, 31.3, 31.7, 31.8, 33.3, 33.6, 34.0, 35.1, 38.3, 47.3, 48.4 (2x), 53.3, 63.7, 70.3, 72.9, 81.4, 87.8, 119.2, 123.3, 139.7, 149.3, 152.6, 156.1, 170.7, 172.8, 198.1, 221.4; HRMS calculated for $C_{40}H_{58}$ N_5O_7 $[M+H]^+$ 720.43308, found 720.43295 m/z.

5'-O-{12-[androst-4'-en-3',17'-dioxo-16'β-yl]-dodecanoyl} adenosine (6)

White solid (50 mg, 61%); IR (film) 3332 and 3188 (OH and NH_2), 1732 (C=O, ester and ketone), 1651 (C=N and C=O conjugated ketone); ^{1}H-NMR (400 MHz, DMSO-d_6) 0.78 (s, 18'-CH_3), 0.90 to 2.50 (m, 38H, CH and CH_2 of steroid skeleton and alkyl chain), 1.16 (s, 19'-CH_3), 2.28 (t, J = 7.8 Hz, CH_2COO), 4.07 (m, 4'-CH of ribose), 4.16 to 4.34 (m, 3'-CH and 5'-CH_2 of ribose), 4.65 (m, 2'-CH of ribose), 5.39 (d, J = 5.5 Hz, OH), 5.59 (d, J = 5.8 Hz, OH), 5.65 (s, 4'-CH), 5.90 (d, J = 4.9 Hz, 1'-CH of ribose), 7.33 (s, NH_2), 8.14 and 8.31 (2s, 2 x CH of adenine); ^{13}C-NMR (75 MHz, DMSO-d_6) 13.5, 16.8, 19.8, 24.3, 27.4, 28.2, 28.4, 28.6, 28.8 (3x), 28.9 (2x), 30.6, 31.3, 31.6, 31.8, 33.3, 33.6, 33.9, 35.0, 38.2, 47.3, 48.4 (2x), 53.3, 63.7, 70.2, 72.8, 81.4, 87.7, 119.1, 123.2, 139.6, 149.3, 152.6, 156.1, 170.7, 172.7, 198.1, 221.3; HRMS calculated for $C_{41}H_{60}$ N_5O_7 $[M+H]^+$ 734.44870, found 734.44818 m/z.

5'-O-{13-[androst-4'-en-3',17'-dioxo-16'β-yl]-tridecanoyl} adenosine (7)

White solid (48 mg, 53%); IR (film) 3334 and 3218 (OH and NH_2), 1733 (C=O, ester and ketone), 1657 (C=N and C=O conjugated ketone); ^{1}H-NMR (400 MHz, DMSO-d_6) 0.78 (s, 18'-CH_3), 0.90 to 2.50 (m, 40H, CH and CH_2 of steroid skeleton and alkyl chain), 1.16 (s, 19'-CH_3), 2.28 (t, J = 7.5 Hz, CH_2COO), 4.07 (m, 4'-CH of ribose), 4.16 to 4.35 (m, 3'-CH and 5'-CH_2 of ribose), 4.65 (m, 2'-CH of ribose), 5.37 (d, J = 5.5 Hz, OH), 5.58 (d, J = 5.7 Hz, OH), 5.65 (s, 4'-CH), 5.90 (d, J = 4.8 Hz, 1'-CH of ribose), 7.31 (s, NH_2), 8.14 and 8.30 (2s, 2 x CH of adenine); ^{13}C-NMR (75 MHz, DMSO-d_6) 13.6, 16.9, 19.8, 24.4, 27.4, 28.2, 28.4, 28.7, 28.9 (3x), 29.0 (3x), 30.6, 31.3, 31.7, 31.8, 33.3, 33.6, 34.0, 35.1, 38.3, 47.3, 48.4 (2x), 53.3, 63.7, 70.3, 72.9, 81.4, 87.7, 119.1, 123.3, 139.7, 149.3, 152.6, 156.1, 170.7, 172.8, 198.0, 221.3; HRMS calculated for $C_{42}H_{62}$ N_5O_7 $[M+H]^+$ 748.46436, found 748.46473 m/z.

Synthesis of 16β-nonyl-3,3-(ethylenedioxy)-androst-5-en-17-one (60)

Steroid **10** (1.00 g, 3.03 mmol) was dissolved in dry THF (60 mL) under argon atmosphere and the solution was cooled at –78°C. A solution of lithium bis(trimethylsilyl) amide (LiHMDS) (1.0 M in THF) (5.4 mL, 5.4 mmol) was added and the reaction mixture was stirred 30 min at room temperature. The mixture was then cooled at –78°C followed by the addition dropwise of 1-bromononane (1.8 mL, 9.08 mmol). The solution was heated to reflux and stirred overnight. Then, a saturated aqueous solution of NH_4Cl was added and the mixture was extracted with EtOAc, washed with brine and dried over $MgSO_4$. The solvent was evaporated and the crude product was purified by flash chromatography (hexanes/EtOAc, 98:2) to yield **60** (230 mg, 0.503 mmol, 17%) as a colourless viscous oil. IR (film) 1726 (C=O, ketone); ^{1}H-NMR (400 MHz, $CDCl_3$) 0.84 (s, 18-CH_3), 0.88 (t, J = 6.8 Hz, CH_3 of alkyl chain), 1.05 (s, 19-CH_3), 1.05 to 2.20 (m, 33H, CH and CH_2 of steroid skeleton and alkyl chain), 2.57 (m, 4-CH), 3.96 (m, CH_2 of ketal), 5.39 (m, 6-CH); ^{13}C-NMR (100 MHz, $CDCl_3$) 13.7, 14.1, 18.9, 20.3, 22.7, 28.1, 28.9, 29.3, 29.47, 29.50, 29.6, 30.8, 31.0, 31.1, 31.8, 31.9, 32.5, 36.2, 36.8, 41.8, 47.9, 49.4, 50.0, 50.2, 64.2, 64.4, 109.3, 121.5, 140.4, 223.0; LRMS calculated for $C_{30}H_{49}O_3$ $[M+H]^+$ 457.4, found 457.2 m/z.

Synthesis of 16β-nonyl-androst-4-en-3,17-dione (61)

To steroid **60** (85 mg, 0.186 mmol) dissolved in DCM (0.5 mL) were added MeOH (3.5 mL) and *p*-TSA (106 mg, 0.558 mmol). After 1 h at room temperature, the reaction was quenched by addition of a saturated $NaHCO_3$ aqueous solution. The crude product was extracted with DCM, washed with brine, dried over $MgSO_4$ and evaporated to dryness. Purification by flash chromatography (hexanes/EtOAc, 85:15) afforded **61** (60 mg, 0.145 mmol, 77%) as a colourless viscous oil. IR (film) 1735 (C=O, ketone), 1676 (C=O, conjugated ketone); ^{1}H-NMR (400 MHz, $CDCl_3$) 0.87 (s, 18-CH_3), 0.88 (t, J = 6.7 Hz, CH_3 of alkyl chain), 0.95 to

2.50 (m, 34H, CH and CH_2 of steroid skeleton and alkyl chain), 1.21 (s, 19-CH_3), 5.76 (s, 4-CH); ^{13}C-NMR (75 MHz, $CDCl_3$) 13.8, 14.1, 17.4, 20.2, 22.7, 28.1, 28.7, 29.3, 29.5 (2x), 29.6, 30.9, 31.6, 31.9, 32.5, 32.6, 33.9, 34.8, 35.7, 38.7, 47.9, 49.29, 49.34, 53.9, 124.1, 170.4, 199.4, 222.4; HRMS calculated for $C_{28}H_{45}O_2$ $[M+Na]^+$ 435.32335, found 435. 32296 m/z.

Enzymatic Assay

Inhibition of Type 3 17β-HSD in Homogenated Cells

This enzymatic assay was performed as described previously [9,35]. Briefly, an expression vector encoding for type 3 17β-HSD was transfected into human embryonic kidney (HEK)-293 cells using the Exgen 500 procedure (Fermentas, Burlington, ON, Canada). Heat shock was performed in 50 mM sodium phosphate buffer (pH 7.4), containing 20% glycerol and 1 mM EDTA, to obtain cellular fragmentation (-80°C to 37°C, three times, 5 minutes). The crude enzymes were used without further purification. 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-^{14}C]-4-androstene-3,17-dione in ethanol (53.6 mCi / mmol, Perkin Elmer Life Sciences Inc., Boston, MA, USA), 10 µL of indicated inhibitor dissolved in ethanol and 20 µL of diluted enzymatic source in phosphate buffer. Each inhibitor was assessed in duplicate. Afterward, radiolabelled steroids were extracted twice from the reaction mixture with 1 mL of diethyl ether. The organic phases were pooled and evaporated to dryness with nitrogen. Residues were dissolved in 50 µL of DCM, applied on silica gel 60 F_{254} thin-layer chromatography plates (EMD Chemicals Inc., Gibbstown, NJ, USA) and eluted with a mixture of toluene/acetone (4:1). Substrate ([^{14}C]-Δ^4-dione) and metabolite ([^{14}C]-T) were identified by comparison with reference steroids and quantified using the Storm 860 system (Molecular Dynamics, Sunnyvale, CA, USA). The percentage of transformation of [^{14}C]-Δ^4-dione into [^{14}C]-T was calculated as follows: % transformation = 100 x ([^{14}C]-T / ([^{14}C]-T + [^{14}C]-Δ^4-dione)), and subsequently, % inhibition = 100 x ((% trans-formation without inhibitor - % transformation with inhibitor) / % transformation without inhibitor).

Inhibition of Type 3 17β-HSD in Intact Cells

HEK-293 cells were plated at 200 000 cells/well in a 12-well Falcon flask at 37°C under 95% air 5% CO_2 humidified atmosphere in minimum essential medium (MEM) containing non-essential amino acids (0.1 mM), glutamine (2 mM), sodium pyruvate (1 mM), 10% foetal bovine serum, penicillin (100 IU/mL) and streptomycin (100 µg/mL). The expression vector encoding for type 3 17β-HSD [9] was transfected using the Exgen 500 procedure (Fermentas, Burlington, ON, Canada) with 2 µg of recombinant plasmid per well. For the inhibitory activity assay, 50 nM of [4-^{14}C]-4-androstene-3,17-dione in ethanol (53.6 mCi / mmol, Perkin Elmer Life Sciences Inc., Boston, MA, USA), and an ethanol solution of inhibitor (0.5% v/v) at a concentration of 1 or 10 µM were added to freshly changed culture medium and incubated for 16 h. Each inhibitor was assessed in duplicate. After incubation, culture medium was removed and radiolabelled steroids were extracted and treated as described above for the enzymatic assay in homogenated cells. Percentage of inhibition was also calculated as described above.

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