Journal of Enzyme Inhibition and Medicinal Chemistry, June 2009; 24(3): 832–843

Improved synthesis of EM-1745, preparation of its C17-ketone analogue and comparison of their inhibitory potency on 17β-hydroxysteroid dehydrogenase type 1

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(*Received 2 April 2008; revised 11 July 2008; accepted 20 July 2008*)

Abstract

Endocrine therapies are widely used for the treatment of estrogen-sensitive diseases. 17β-hydroxysteroid dehydrogenase type 1 (17β-HSD1) is involved in the last step of the biosynthesis of potent estrogen estradiol (E_2). This enzyme catalyzes the reduction of the C17-ketosteroid estrone (E_1) into the C17β-hydroxy steroid E_2 using the cofactor NAD(P)H. The X-ray analysis of E_2/adenosine bisubstrate inhibitor EM-1745 proven that this compound interacts with both the substrate- and the cofactor-binding sites. However, E_1 is a better substrate of 17β-HSD1 than E_2. Thus, in order to improve the inhibitory potency of EM-1745, the C17-ketone analogue was prepared. During this work, a new and more efficient method for synthesizing EM-1745 was developed using an esterification and a cross-metathesis as key steps. Contrary to what was expected, the C17-ketone analogue of EM-1745 is a less potent inhibitor ($IC_{50} = 12$ nM) than the C17-alcohol ($IC_{50} = 4$ nM) in homogenated HEK-293 cells overexpressing 17β-HSD1. Our results contribute to the knowledge of an unexpected observation: the C17-ketone steroidal inhibitors of 17β-HSD1 are less potent than their corresponding C17-alcohol derivatives.

Keywords: *17β-Hydroxysteroid dehydrogenase, enzyme, inhibitor, steroid, chemical synthesis, cross-metathesis*

Introduction

Estrogens are well known to be involved in the development of estrogen-sensitive diseases such as breast cancer. In fact, most breast cancers are initially hormone-dependent and estradiol (E_2), the most potent estrogen, plays a crucial role in their development and progression [1,2]. Several endocrine therapies were thus developed since this approach is less toxic than chemotherapy [3]. Consequently, hormonal control should become a predominant choice in the treatment of estrogen-sensitive diseases. Antiestrogens, such as blockers of the estrogen receptor (ER), have been developed and their efficiency has been proved by several clinical trials [4]. Another option in the treatment of ER^+ breast cancer is the use of an inhibitor of a steroidogenic enzyme involved in the biosynthesis of E_2 [5–7]. In fact, three generations of inhibitors of aromatase, an enzyme catalyzing the conversion of androgens to estrogens, have been developed and the third one is now used for treatment of breast carconima [8–10]. Inhibitors of steroid sulfatase, an enzyme which hydrolyses the sulfates from circulating estrogens, androgens and hormone precursors, were also prepared, but are not yet available for clinical use [11–14]. Another group of steroidogenic enzymes, the 17β-hydroxysteroid dehydrogenases (17β-HSDs), catalyze the last step in the biosynthesis of potent estrogens and androgens. However, even if this family of enzymes was discovered more than fifty years ago [15,16], we have yet to obtain potent inhibitors for clinical use.

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ISSN 1475-6366 print/ISSN 1475-6374 online © 2009 Informa UK Ltd.
DOI: 10.1080/14756360802399761

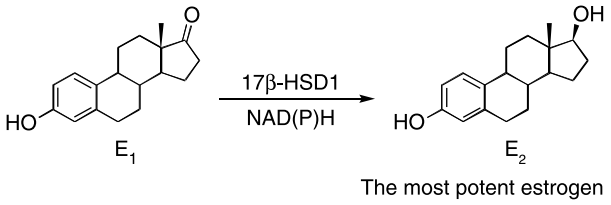


Figure 1. 17β-HSD1 catalyzes the reduction of estrone (E_1) into estradiol (E_2) with the cofactor NADH or NADPH.

Among the 17β-HSDs, we are interested in 17β-HSD1. This reductive enzyme, also called human estradiol dehydrogenase [E.C.1.1.1.62], catalyzes the last step in the biosynthesis of the most potent estrogen, E_2. This enzyme is also a member of the short-chain alcohol dehydrogenase family and exists as a homodimer. It is a protein of 327 amino acids with a subunit mass of 35 kDa [17]. It stereoselectively reduces the C17-ketone of estrone (E_1) preferentially using cofactor NADPH to provide E_2 (Figure 1) [17–20]. 17β-HSD1 also catalyzes, at a lower rate, the transformation of dehydroepiandrosterone (DHEA) into 5-androstene-3β,17β-diol (Δ^5-diol), a weak estrogenic C19 steroid. The reductive activity of 17β-HSDs was found to be present in several steroidogenic and peripheral tissues such as placenta, ovary and breast [21]. Furthermore, a study revealed that the reductive activity (E_1 into E_2) is higher in malignant breast tumours whereas the oxidative pathway (E_2 into E_1) is dominant in normal breast cell [22]. 17β-HSD1 mRNA has been detected in malignant breast tumours by *in situ* hybridization, immunohistochemistry and reverse transcription-PCR [23–25]. It was also demonstrated that 17β-HSD1 plays a role in the local biosynthesis of estrogen in the breast, especially in postmenopausal women, after the ovaries have ceased to produce estrogen [26,27]. Thus, 17β-HSD1 is an interesting target for estrogen-sensitive disease such as breast cancer.

Several inhibitors have been developed for 17β-HSD1 [3,28–37]. Among these inhibitors E_2/adenosine hybrid compounds are bisubstrate inhibitors interacting with both the substrate-binding (E_2) and the cofactor-binding (adenosine) sites and were developed in part based on the three-dimensional structure of the enzyme [38–40]. With an alkyl side chain spacer of 8 methylenes in steroidal position C16β to link the two moieties (E_2 and adenosine), EM-1745 (**1**, Figure 2) is the best of that series of inhibitors with a K_i of 3 nM (E_2 into E_1). In addition, this compound was found to be 13-fold more potent than unlabeled E_1 used as inhibitor in homogenated HEK-293 cells overexpressing 17β-HSD1 [41,42]. Simplified bisubstrate inhibitors were also developed in order to improve the bioavailability of **1** [43]. These are analogues of **1** where the adenosine moiety was replaced by a benzyl group bearing a carboxylic acid function. These inhibitors are, however, less potent than EM-1745.

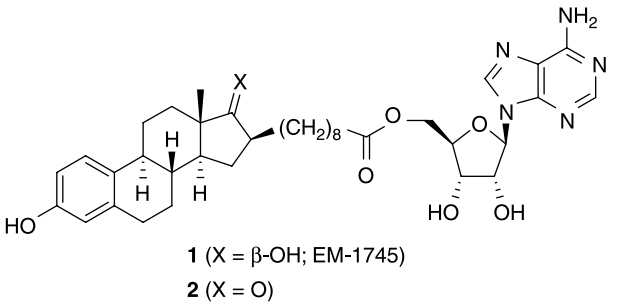


Figure 2. Chemical structure of the bisubstrate inhibitors of 17β-HSD1: EM-1745 (**1**) and its C17-ketone analogue **2**. Illustrated only for **1** and **2**, the stereogenic centers are the same for all other steroid derivatives reported in this paper.

It is known that E_1, a ketosteroid, is a better substrate for the reductive activity of 17β-HSD1 than the corresponding hydroxy-steroid E_2. In fact, the Michaelis constant (K_m) for E_1 is 0.03 and 0.36 μM when using respectively the cofactors NADPH and NADH while the K_m for E_2 is higher with 4.6 and 1.7 μM when using the cofactors $NADP^+$ and NAD^+[44]. In addition, X-ray analysis of the EM-1745/17β-HSD1 complex confirmed that this inhibitor was interacting with both the substrate- and the cofactor-binding sites. It was also shown that the two moieties of EM-1745 interact with most of the amino acids with which E_2 or E_1 interacts in the substrate-binding site and with which adenosine of NAD(P)H interacts in the cofactor-binding site. Furthermore, a Lineweaver-Burk plot demonstrated clearly that EM-1745 is a typical reversible competitive inhibitor [42]. Taken together the results discussed above suggest that replacing the E_2 nucleus by an E_1 moiety in the structure of EM-1745 should give a better enzyme inhibition. Thus, in order to improve the inhibitory effect of EM-1745 (**1**), we prepared the C17-ketone analogue **2** (Figure 2). Herein, we report the chemical synthesis of the C17-ketone analogue of EM-1745 (compound **2**), as well as a more convenient procedure for synthesizing EM-1745 (**1**). Moreover, the inhibitory potency of **2** was evaluated on 17β-HSD1 and compared to that of its corresponding C17-hydroxy analogue (EM-1745, **1**).

Material and methods

General

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) and tricyclohexylphosphine [1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene] [benzylidine] ruthenium(IV) dichloride (2nd generation

Grubbs catalyst) from Strem Chemicals (Newburyport, MA, USA). Usual solvents were obtained from Fisher Scientific (Montréal, 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, from a thin film of the solubilized compound on NaCl pellets (usually in CH_2Cl_2) or from a KBr pellet. 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 methyl sulfoxide (2.51 and 39.5 ppm) for ^{1}H and ^{13}C respectively. Duplication of NMR signals was generally recorded for THP derivatives. In that case, the presence of two stereoisomers increased the complexity of ^{13}C NMR spectra, and additional peaks are written between parentheses. Assignment of NMR signals was made easier using literature data [45–47]. ^{13}C NMR data are reported in Table I. 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.

*Synthesis of 7-octen-1-ol (**4**)*

Halogen exchange (Br-I). To a solution of 6-bromo-1-hexanol (**3**) (2.90 mL, 22.0 mmol) in acetone (50 mL) was added NaI (13.2 g, 88.1 mmol) and the solution was refluxed and stirred for 16 h. After addition of water, the product was extracted with EtOAc and the organic phase was washed with a saturated aqueous solution of $Na_2S_2O_3$, washed with brine, dried over $MgSO_4$, and evaporated to dryness. 6-Iodo-1-hexanol (4.71 g, 94% crude yield) was used for the next step without further purification. *Vinylation.* CuI (3.58 g, 18.8 mmol) was suspended in anhydrous THF (40 mL) under an argon atmosphere. The mixture was cooled to −40°C and vinylmagnesium bromide (1 M in THF) (94.4 mL, 94.4 mmol) was added. The reaction was stirred for 15 min at −40°C. Then, HMPA (6.54 mL, 37.6 mmol) and triethyl phosphite (6.44 mL, 37.6 mmol) were added and the mixture was stirred 5 min at −40°C. 6-Iodo-1-hexanol (4.28 g, 18.8 mmol) was added and the reaction mixture was stirred 1 h at −40°C and 4 h at room temperature. The reaction was quenched by addition of a saturated aqueous solution of NH_4Cl. The crude product was extracted with EtOAc, and the organic phase was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. Purification by flash chromatography (hexanes/EtOAc, 85:15) yielded **4** (1.80 g, 75% yield for two steps) as a colourless oil. IR (neat) 3342 (OH), 3077 (H-C═), 1641 (C═C, alkene); ^{1}H NMR (400 MHz, $CDCl_3$) 1.31 (m, 3 × CH_2), 1.52 (m, CH_2CH_2OH), 2.01 (m, $CH_2CH{=}CH_2$), 2.49 (s_{br}, OH), 3.58 (t, $\mathcal{J} = 6.6$ Hz, CH_2OH), 4.93 (m, $CH{=}CH_2$), 5.77 (m, $CH{=}CH_2$); ^{13}C NMR (75 MHz, $CDCl_3$) 25.5, 28.76, 28.81, 32.5, 33.6, 62.7, 114.1, 138.9; LRMS calculated for $C_8H_{15}O$ $[M\text{-}H]^-$ 127.1, found 127.0 m/z.

*Synthesis of 7-octenal (**5**)*

4-Methylmorpholine-*N*-oxide (NMO) (3.30 g, 28.2 mmol) and molecular sieves (5 g) were added to a solution of alcohol **4** (1.51 g, 11.8 mmol) in dry DCM (100 mL) under an argon atmosphere at room temperature. The mixture was stirred for 15 min and tetrapropylammonium perruthenate (TPAP) (207 mg, 0.59 mmol) was then added. After the reaction mixture was stirred for 90 min, the solvent was removed under reduced pressure. The crude product was purified by flash chromatography (hexanes/EtOAc, 95:5) to afford aldehyde **5** (765 mg, 51% yield) as a colourless oil. IR (neat) 3077 (H-C═), 2718 (C-H, aldehyde), 1727 (C═O, aldehyde), 1641 (C═C, alkene); ^{1}H NMR (400 MHz, $CDCl_3$) 1.37 (m, 2 × CH_2), 1.64 (m, CH_2CH_2CHO), 2.05 (m, $CH_2CH{=}CH_2$), 2.43 (dt, $\mathcal{J}_1 = 7.4$ Hz, $\mathcal{J}_2 = 1.7$ Hz, CH_2CHO), 4.98 (m, $CH{=}CH_2$), 5.79 (m, $CH{=}CH_2$), 9.76 (t, $\mathcal{J} = 1.7$ Hz, CHO); ^{13}C NMR (75 MHz, $CDCl_3$) 21.9, 28.5 (2x), 33.5, 43.8, 114.5, 138.7, 202.8.

Synthesis of 9-[3′-(tert-butyldimethylsilyloxy)-17′β-(tetrahydro-2H-pyran-2-yl-oxy)-estra-1′,3′,5′(10′)-trien-16′β-yl]-7-nonen-1-al (7)

Diprotected 16β-allyl-estradiol **6** was synthesized in five steps from estrone as previously reported [48]. A mixture of **6** (1.00 g, 1.96 mmol), freshly prepared aldehyde **5** (745 mg, 5.90 mmol) and tricyclohexylphosphine [1,3-bis(2,4,6-trimethylphenyl)-4,5-dihydroimidazol-2-ylidene] [benzylidine] ruthenium(IV) dichloride (Grubbs's catalyst 2nd generation) (166 mg, 0.196 mmol) in dry DCM (50 mL) was refluxed for 16 h under an argon atmosphere. The crude mixture was

Table I. ^{13}C NMR data for compounds **2**, **7**-**11**.

C #	2*	7†	8†	9†	10†	11†
C1′	126.0	126.9	126.9	126.9	126.8	126.8
C2′	112.8	117.9	117.8	117.9	113.4	113.4
C3′	155.0	154.0	153.9	153.9	155.7	155.8
C4′	115.0	120.6	120.5	120.5	115.7	115.8
C5′	137.1	138.4	138.4	138.4	138.3	138.2
C6′	29.1	‡	‡	‡	‡	‡
C7′	28.0	28.0	27.9	28.0	28.2	27.32
C8′	c	40.5	40.5	39.2 (40.5)	41.2	38.7
C9′	47.8	44.6 (44.8)	44.2	44.6 (44.76)	44.8	49.4
C10′	130.0	133.9 (134.0)	134.0	133.9 (134.0)	132.0	131.4
C11′	26.3	26.9 (27.0)	26.9 (27.0)	27.1	27.1	26.5
C12′	37.6	38.7 (39.1)	38.7 (39.0)	39.5	38.6	34.1
C13′	48.0	44.6 (44.8)	44.6 (44.7)	44.2 (44.79)	44.7	48.7
C14′	48.5	49.4 (49.6)	49.3 (49.5)	49.4 (49.6)	49.4	49.6
C15′	28.4	32.4 (32.6)	32.8 (32.9)	33.0 (33.1)	33.2	28.5
C16′	43.6	39.2 (39.4)	39.4 (39.5)	39.06 (39.10)	39.3	44.8
C17′	221.7	86.1 (86.4)	86.0 (86.6)	86.1 (86.7)	82.49	221.5
C18′	13.7	13.6	13.4	13.5	12.9	14.2
$(CH_3)_2Si$	–	−4.2	−4.1	−4.4	–	–
$(CH_3)_3C$-Si	–	18.6	18.5	18.5	–	–
$(CH_3)_3C$-Si	–	25.9	25.8	25.9	–	–
CHO of THP	–	98.1 (99.6)	98.0 (99.4)	98.1 (99.4)	–	–
CH_2O of THP	–	62.0 (62.9)	61.9 (62.8)	61.9 (62.8)	–	–
CH_2CHO of THP	–	31.5 (31.6)	31.3 (31.6)	31.4 (31.6)	–	–
CH_2CH_2O of THP	–	26.2 (26.3)	26.2 (26.3)	26.2 (26.3)	–	–
CH_2CH_2CHO of THP	–	20.0 (20.5)	19.9 (20.4)	20.4	–	–
CH=CH	–	130.9 (131.0) 131.3	–	–	–	–
CHO or COO	172.8	202.6	174.4	173.2	173.2	173.2
CH_2CHO or CH_2COO	33.3	44.2	34.0	38.8	34.1	32.9
C1′ of ribose	87.7	–	–	91.0	91.0	90.9
C2′ of ribose	72.9	–	–	84.7	84.7	84.7
C3′ of ribose	70.3	–	–	82.6	82.46	82.5
C4′ of ribose	81.4	–	–	85.4	85.3	85.3
C5′ of ribose	63.7	–	–	64.3	64.3	64.3
$C(CH_3)_2$	–	–	–	25.41 & 27.3	25.4 & 27.3	25.3 & 27.26
$C(CH_3)_2$	–	–	–	114.5	114.4	114.4
C adenine	156.1	–	–	157.1	157.0	157.0
C adenine	152.7	–	–	153.6	153.6	153.6
C adenine	149.3	–	–	150.0	150.0	150.0
C adenine	139.7	–	–	140.5	140.6	140.6
C adenine	119.1	–	–	120.7	120.9	120.6
C alkyl chain	31.6	36.3 (36.5)	33.1	34.2	32.52	32.7
C alkyl chain	28.8	33.0	25.5	30.4	32.48	6C‡
C alkyl chain	28.7	30.5	5C‡	25.37	30.22	–
C alkyl chain	28.6	22.5	–	4C‡	29.1	–
C alkyl chain	27.4	1C‡	–	–	25.3	–
C alkyl chain	25.5	–	–	–	2C‡	–
C alkyl chain	24.4	–	–	–	–	–

* DMSO-d_6 as solvent; † acetone-d_6 as solvent; ‡ under solvent peak.

preadsorbed on silica gel and a flash chromatography was performed with hexanes/EtOAc, 95:5 as eluent to afford the desired steroid **7** (597 mg, 50% yield) as a yellowish viscous oil. IR (film) 2714 (C-H, aldehyde), 1727 (C=O, aldehyde); ^{1}H NMR (400 MHz, acetone-d_6) 0.205 and 0.206 (2s, $Si(CH_3)_2$), 0.83 and 0.87 (2s, 18′-CH_3), 1.00 (s, $SiC(CH_3)_3$), 1.05 to 2.35 (m, 28H, CH and CH_2 of steroid skeleton and alkyl chain), 2.44 (t, $J = 7.3$ Hz, CH_2CHO), 2.81 (m, 6′-CH_2), 3.48 and 3.92 (2m, OCH_2 of THP), 3.76 and 3.81 (2d, $J = 9.8$ Hz, 17′α-CH), 4.66 and 4.72 (2m, CH of THP), 5.44 (m, CH=CH), 6.58 (d, $J = 1.8$ Hz, 4′-CH), 6.64 (d, $J = 8.5$ Hz, 2′-CH), 7.16 (m, 1′-CH), 9.74 (t, $J = 1.5$ Hz, CHO); ^{13}C NMR (75 MHz, acetone-d_6) − 4.2 (2x), 13.6, 18.6, 20.0 (20.5), 22.5, 25.9 (3x), 26.2 (26.3), 26.9 (27.0), 28.0, 29.0 to 30.2 (2C under solvent peaks), 30.5, 31.5 (31.6), 32.4 (32.6), 33.0, 36.3 (36.5), 38.7 (39.1), 39.2 (39.4), 40.5, 44.2, 44.6 (44.8) (2x), 49.4 (49.6), 62.0 (62.9), 86.1 (86.4), 98.1 (99.6), 117.9, 120.6, 126.9, 130.9 (131.0), 131.3, 133.9 (134.0), 138.4, 154.0, 202.6.

*Synthesis of 9-[3′-(tert-butyldimethylsilyloxy)-17′β-(tetrahydro-2H-pyran-2-yl-oxy)-estra-1′,3′,5′(10′)-trien-16′β-yl]-nonanoic acid (**8**)*

Hydrogenation. A suspension of **7** (588 mg, 0.965 mmol) and 10% Pd/C (88 mg) in EtOAc (20 mL) was stirred under a hydrogen atmosphere at room temperature. After 16 h, the resulting suspension was filtered through celite, washed with EtOAc and evaporated to dryness to afford 9-[3′-(*tert*-butyldimethylsilyloxy)-17′β-(tetrahydro-2*H*-pyran-2-yl-oxy)-estra-1′,3′,5′(10′)-trien-16′β-yl]-nonanal (593 mg, quantitative crude yield) in good purity without purification. *Oxidation.* This aldehyde (498 mg, 0.815 mmol) was dissolved in a minimum of THF (~2 mL) followed by addition of *t*-BuOH (29 mL) and 2-methyl-2-butene (11 mL). An oxidative solution freshly prepared by dissolving $NaClO_2$ (860 mg) and NaH_2PO_4 (860 mg) in H_2O (8.6 mL) was added and the reaction mixture was allowed to stir for 30 min. 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. Purification by flash chromatography (hexanes/EtOAc, 8:2) provided carboxylic acid **8** (383 mg, 75% yield for two steps) as a gummy white foam. IR (film) 3600-2300 (OH, carboxylic acid), 1709 (C=O, carboxylic acid); ^{1}H NMR (400 MHz, acetone-d_6) 0.202 and 0.203 (2s, $Si(CH_3)_2$), 0.82 and 0.86 (2s, 18′-CH_3), 1.00 (s, $SiC(CH_3)_3$), 1.00 to 2.35 (m, 32H, CH and CH_2 of steroid skeleton and alkyl chain), 2.30 (t, $\mathcal{J} = 7.4$ Hz, CH_2COOH), 2.81 (m, 6′-CH_2), 3.48 and 3.92 (2m, OCH_2 of THP), 3.74 and 3.79 (2d, $\mathcal{J} = 10.0$ Hz, 17′α-CH), 4.64 and 4.73 (2m, CH of THP), 6.58 (d, $\mathcal{J} = 2.2$ Hz, 4′-CH), 6.64 (d, $\mathcal{J} = 8.4$ Hz, 2′-CH), 7.16 (m, 1′-CH); ^{13}C NMR (75 MHz, acetone-d_6) − 4.1 (2x), 13.4, 18.5, 19.9 (20.4), 25.5, 25.8 (3x), 26.2 (26.3), 26.9 (27.0), 27.9, 28.9 to 30.4 (6C under solvent peaks), 31.3 (31.6), 32.8 (32.9), 33.1, 34.0, 38.7 (39.0), 39.4 (39.5), 40.5, 44.2, 44.6 (44.7), 49.3 (49.5), 61.9 (62.8), 86.0 (86.6), 98.0 (99.4), 117.8, 120.5, 126.9, 134.0, 138.4, 153.9, 174.4; LRMS calculated for $C_{38}H_{61}O_5Si$ $[M-H]^-$ 625.4, found 625.6 m/z.

*Synthesis of 5′-O-{9-[3′-(tert-butyldimethylsilyloxy)-17′β-(tetrahydro-2H-pyran-2-yl-oxy)-estra-1′,3′,5′(10′)-trien-16′β-yl]-nonanoyl}-2′,3′-O-isopropylidene adenosine (**9**)*

To a solution of carboxylic acid **8** (422 mg, 0.673 mmol) in dry DMF (7 mL) under an argon atmosphere at room temperature was added benzotriazole-1-yl-oxy-tris-pyrrolidinophosphonium hexafluorophosphate (PyBOP) (525 mg, 1.01 mmol), hydroxybenzotriazole (HOBt) (136 mg, 1.01 mmol) and diisopropylethylamine (DIPEA) (352 μL, 2.02 mmol). The reaction mixture was stirred for 5 min. Then, 2′,3′-isopropylidene adenosine (414 mg, 1.35 mmol) was added and the mixture was 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 to 4:6) afforded **9** (312 mg, 51% yield) as a white foam. IR (film) 3324 and 3172 (NH_2), 1742 (C=O, ester), 1643 (C=N); ^{1}H NMR (400 MHz, acetone-d_6) 0.20 (s, $Si(CH_3)_2$), 0.81 and 0.85 (2s, 18′-CH_3), 0.99 (s, $SiC(CH_3)_3$), 1.00 to 2.35 (m, 32H, CH and CH_2 of steroid skeleton and alkyl chain), 1.39 and 1.59 (2s, 2 × CH_3 of isopropylidene), 2.26 (dt, $\mathcal{J}_1 = 7.5$ Hz, $\mathcal{J}_2 = 2.7$ Hz, CH_2COO), 2.81 (m, 6′-CH_2), 3.47 and 3.91 (2m, OCH_2 of THP), 3.73 and 3.78 (2d, $\mathcal{J} = 10.0$ Hz, 17′α-CH), 4.24 and 4.31 (2m, 5′-CH_2 of ribose), 4.44 (m, 4′-CH of ribose), 4.63 and 4.72 (2m, CH of THP), 5.17 (m, 3′-CH of ribose), 5.59 (dd, $\mathcal{J}_1 = 6.2$ Hz, $\mathcal{J}_2 = 2.0$ Hz, 2′-CH of ribose), 6.24 (d, $\mathcal{J} = 2.1$ Hz, 1′-CH of ribose), 6.58 (d, $\mathcal{J} = 2.1$ Hz, 4′-CH), 6.63 (d, $\mathcal{J} = 8.4$ Hz, 2′-CH), 6.82 (s_{br}, NH_2), 7.15 (m, 1′-CH), 8.21 and 8.24 (2s, 2 × CH of adenine); ^{13}C NMR (100 MHz, acetone-d_6) − 4.4 (2x), 13.5, 18.5, 20.4, 25.37, 25.41, 25.9 (3x), 26.2 (26.3), 27.1, 27.3, 28.0, 29.1 to 30.2 (5C under solvent peaks), 30.4, 31.4 (31.6), 33.0 (33.1), 34.2, 38.8, 39.06 (39.10), 39.2 (40.5), 39.5, 44.2 (44.79), 44.6 (44.76), 49.4 (49.6), 61.9 (62.8), 64.3, 82.6, 84.7, 85.4, 86.1 (86.7), 91.0, 98.1 (99.4), 114.5, 117.9, 120.5, 120.7, 126.9, 133.9 (134.0), 138.4, 140.5, 150.0, 153.6, 153.9, 157.1, 173.2; LRMS calculated for $C_{51}H_{78}N_5O_8Si$ $[M + H]^+$916.6, found 916.4 m/z.

*Synthesis of 5′-O-{9-[3′,17′β-dihydroxy-estra-1′,3′,5′(10′)-trien-16′β-yl]-nonanoyl} adenosine (**1**)*

Gaseous hydrogen chloride was bubbled for 3 h at room temperature in a solution of **9** (100 mg, 0.109 mmol) in anhydrous DCM (21 mL). The solvent was removed under vacuum and the residue was preadsorbed on silica gel and purified by flash chromatography (DCM/MeOH, 94:6 to 97:3) to provide EM-1745 (**1**) (20 mg, 27% yield) as a white solid. The ^{1}H and ^{13}C NMR data agreed with those reported previously in the literature [41].

*Synthesis of 5′-O-{9-[3′,17′β-dihydroxy-estra-1′,3′,5′(10′)-trien-16′β-yl]-nonanoyl}-2′,3′-O-isopropylidene adenosine (**10**)*

Deprotection of C17′β-OTHP. Compound **9** (132 mg, 0.144 mmol) was dissolved in a minimum of dry DCM (~1 mL) under an argon atmosphere. Dry MeOH (3 mL) and pyridinium *p*-toluenesulfonate (PPTS) (290 mg, 1.15 mmol) were added, and the reaction mixture was refluxed for 5 h. A saturated aqueous solution of $NaHCO_3$ was added to the cooled mixture to quench the reaction and the crude product

was extracted with DCM. The organic phase was washed with an aqueous 1 M $CuSO_4$ solution, then brine, dried over $MgSO_4$, and evaporated to dryness. *Deprotection of C3′-OTBDMS.* To a solution of this crude product (123 mg) in dry THF (3 mL) under an argon atmosphere at 0°C was added TBAF (1 M in THF) (290 μL, 0.290 mmol). The reaction mixture was stirred for 10 min at 0°C and then quenched by addition of a saturated solution of $NaHCO_3$. The crude product was extracted with EtOAc and the organic phase was washed with brine, dried over $MgSO_4$, and evaporated to dryness. Purification by flash chromatography (hexanes/EtOAc, 3:7 to 2:8, then DCM/MeOH, 95:5) afforded **10** (75 mg, 72% yield for two steps) as a light yellow solid. IR (film) 3340 and 3188 (OH and NH_2), 1738 (C=O, ester), 1644 (C=N); ^{1}H NMR (400 MHz, acetone-d_6) 0.80 (s, 18′-CH_3), 0.95 to 2.35 (m, 26H, CH and CH_2 of steroid skeleton and alkyl chain), 1.39 and 1.59 (2s, 2 × CH_3 of isopropylidene), 2.26 (m, CH_2COO), 2.78 (m, 6′-CH_2), 3.72 (d, $\mathcal{J}$ = 9.7 Hz, 17′α-CH), 4.25 and 4.32 (2m, 5′-CH_2 of ribose), 4.44 (m, 4′-CH of ribose), 5.16 (m, 3′-CH of ribose), 5.59 (m, 2′-CH of ribose), 6.24 (d, $\mathcal{J}$ = 1.8 Hz, 1′-CH of ribose), 6.54 (d, $\mathcal{J}$ = 2.0 Hz, 4′-CH), 6.60 (dd, $\mathcal{J}_1$ = 8.4 Hz, $\mathcal{J}_2$ = 2.4 Hz, 2′-CH), 6.69 (s_{br}, NH_2), 7.11 (d, $\mathcal{J}$ = 8.5 Hz, 1′-CH), 7.98 (s, 3′-OH), 8.20 and 8.23 (2s, 2 × CH of adenine); ^{13}C NMR (100 MHz, acetone-d_6) 12.9, 25.3, 25.4, 27.1, 27.3, 28.2, 29.1, 29.3 to 30.2 (3C under solvent peaks), 30.22, 32.48, 32.52, 33.2, 34.1, 38.6, 39.3, 41.2, 44.7, 44.8, 49.4, 64.3, 82.46, 82.49, 84.7, 85.3, 91.0, 113.4, 114.4, 115.7, 120.9, 126.8, 132.0, 138.3, 140.6, 150.0, 153.6, 155.7, 157.0, 173.2; LRMS calculated for $C_{40}H_{56}N_5O_7$ $[M + H]^+$718.5, found 718.9 m/z.

*Synthesis of 5′-O-{9-[3′-hydroxy-17′-oxo-estra-1′,3′,5′(10′)-trien-16′β-yl]-nonanoyl}-2′,3′-O-isopropylidene adenosine (**11**)*

To a solution of **10** (91 mg, 0.127 mmol) in acetone at 0°C was added Jones' reagent (2.7 M CrO_3 in H_2SO_4/H_2O, 2:8) (56 μL, 0.152 mmol). The mixture was stirred for 8 min. Then, the reaction was quenched by addition of isopropanol and a saturated aqueous solution of $NaHCO_3$. The crude product was extracted with EtOAc and the organic phase was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. Purification by flash chromatography (DCM/MeOH, 97:3 to 95:5) provided the starting material **10** (20 mg, 22%) and **11** (47 mg, 52% yield) as a white solid. IR (film) 3343 and 3176 (OH and NH_2), 1732 (C=O, ester and ketone), 1644 (C=N); ^{1}H NMR (400 MHz, acetone-d_6) 0.86 (s, 18′-CH_3), 1.20 to 2.40 (m, 26H, CH and CH_2 of steroid skeleton and alkyl chain), 1.39 and 1.59 (2s, 2 × CH_3 of isopropylidene), 2.26 (m, CH_2COO), 2.82 (m, 6′-CH_2), 4.24 and 4.32 (2m, 5′-CH_2 of ribose), 4.44 (m, 4′-CH of ribose), 5.16 (m, 3′-CH of ribose), 5.59 (m, 2′-CH of ribose), 6.24 (d, $\mathcal{J}$ = 2.0 Hz, 1′-CH of ribose), 6.56 (d, $\mathcal{J}$ = 2.4 Hz, 4′-CH), 6.62 (dd, $\mathcal{J}_1$ = 8.4 Hz, $\mathcal{J}_2$ = 2.6 Hz, 2′-CH), 6.67 (s_{br}, NH_2), 7.12 (d, $\mathcal{J}$ = 8.4 Hz, 1′-CH), 8.02 (s, 3′-OH), 8.19 and 8.23 (2s, 2 × CH of adenine); ^{13}C NMR (75 MHz, acetone-d_6) 14.2, 25.3, 26.5, 27.26, 27.32, 28.5, 28.9 to 30.4 (7C under solvent peaks), 32.7, 32.9, 34.1, 38.7, 44.8, 48.7, 49.4, 49.6, 64.3, 82.5, 84.7, 85.3, 90.9, 113.4, 114.4, 115.8, 120.6, 126.8, 131.4, 138.2, 140.6, 150.0, 153.6, 155.8, 157.0, 173.2, 221.5; LRMS calculated for $C_{40}H_{54}N_5O_7$ $[M + H]^+$716.4, found 716.7 m/z.

*Synthesis of 5′-O-{9-[3′-hydroxy-17′-oxo-estra-1′,3′,5′(10′)-trien-16′β-yl]-nonanoyl} adenosine (**2**)*

Compound **11** (34 mg, 0.048 mmol) in dry THF (500 μL) was treated with a TFA/H_2O (9/1, v/v) solution (2.4 mL). The reaction mixture was stirred for 30 min at room temperature and 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 reduced pressure. The crude residue was purified by flash chromatography (DCM/MeOH, 95:5) to afford **2** (18 mg, 56% yield) as a white solid. IR (KBr) 3338 and 3213 (OH and NH_2), 1737 (C=O, ester), 1725 (C=O, ketone), 1655 (C=N); ^{1}H NMR (400 MHz, DMSO-d_6) 0.77 (s, 18′-CH_3), 1.15 to 2.35 (m, 26H, CH and CH_2 of steroid skeleton and alkyl chain), 2.28 (t, $\mathcal{J}$ = 7.1 Hz, CH_2COO), 2.74 (m, 6′-CH_2), 4.07 (m, 4′-CH of ribose), 4.19 and 4.32 (2m, 5′-CH_2 of ribose), 4.25 (m, 3′-CH of ribose), 4.66 (m, 2′-CH of ribose), 5.39 (d, $\mathcal{J}$ = 5.5 Hz, OH of ribose), 5.60 (d, $\mathcal{J}$ = 4.8 Hz, OH of ribose), 5.90 (d, $\mathcal{J}$ = 4.8 Hz, 1′-CH of ribose), 6.45 (d, $\mathcal{J}$ = 2.0 Hz, 4′-CH), 6.51 (dd, $\mathcal{J}_1$ = 8.3 Hz, $\mathcal{J}_2$ = 2.2 Hz, 2′-CH), 7.05 (d, $\mathcal{J}$ = 8.5 Hz, 1′-CH), 7.33 (s, NH_2), 8.14 and 8.31 (2s, 2 × CH of adenine), 9.04 (s, 3′-OH); ^{13}C NMR (75 MHz, DMSO-d_6) 13.7, 24.4, 25.5, 26.3, 27.4, 28.0, 28.4, 28.6, 28.7, 28.8, 29.1, 31.6, 33.3, 37.6, 38.7 to 40.3 (1C under solvent peaks), 43.6, 47.8, 48.0, 48.5, 63.7, 70.3, 72.9, 81.4, 87.7, 112.8, 115.0, 119.1, 126.0, 130.0, 137.1, 139.7, 149.3, 152.7, 155.0, 156.1, 172.8, 221.7; LRMS calculated for $C_{37}H_{50}N_5O_7$ $[M + H]^+$676.4, found 676.7 m/z.

Enzymatic assay: Inhibition of 17β-HSD1 in homogenated cells

This enzymatic assay on 17β-HSD1 was performed as previously described [49]. Briefly, HEK-293 cells transfected with 17β-HSD1 cDNA fragment were sonicated in 50 mM sodium phosphate buffer (pH 7.4), containing 20% glycerol and 1 mM EDTA to obtain cellular fragmentation. The cytosol fraction

containing the enzyme was isolated as the supernatant after centrifugation (100 000 × g, 5 min, 4°C). The enzymatic reaction was performed at 37°C for 2 h in 1 mL of a solution which included 980 μL of a stock solution containing 50 mM sodium phosphate buffer (pH 7.4, 20% glycerol and 1 mM EDTA), 0.1 mM NADH and 0.1 μM [^{14}C]-estrone (54 mCi/mmol, American Radiolabeled Chemicals Inc., St-Louis, MO, USA), 10 μL of the indicated inhibitor dissolved in ethanol and 10 μL of diluted enzymatic source in phosphate buffer. Each inhibitor was assessed in duplicate. Afterward, radiolabeled steroids were extracted twice from the reaction mixture by 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]-E_1) and metabolite ([^{14}C]-E_2) 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]-E_1 into [^{14}C]-E_2 was calculated as follows: % transformation = 100 × ([^{14}C]-E_2/([^{14}C]-E_2 + [^{14}C]-E_1)), and subsequently, % inhibition = 100 × ((% transformation without inhibitor − % transformation with inhibitor)/% transformation without inhibitor).

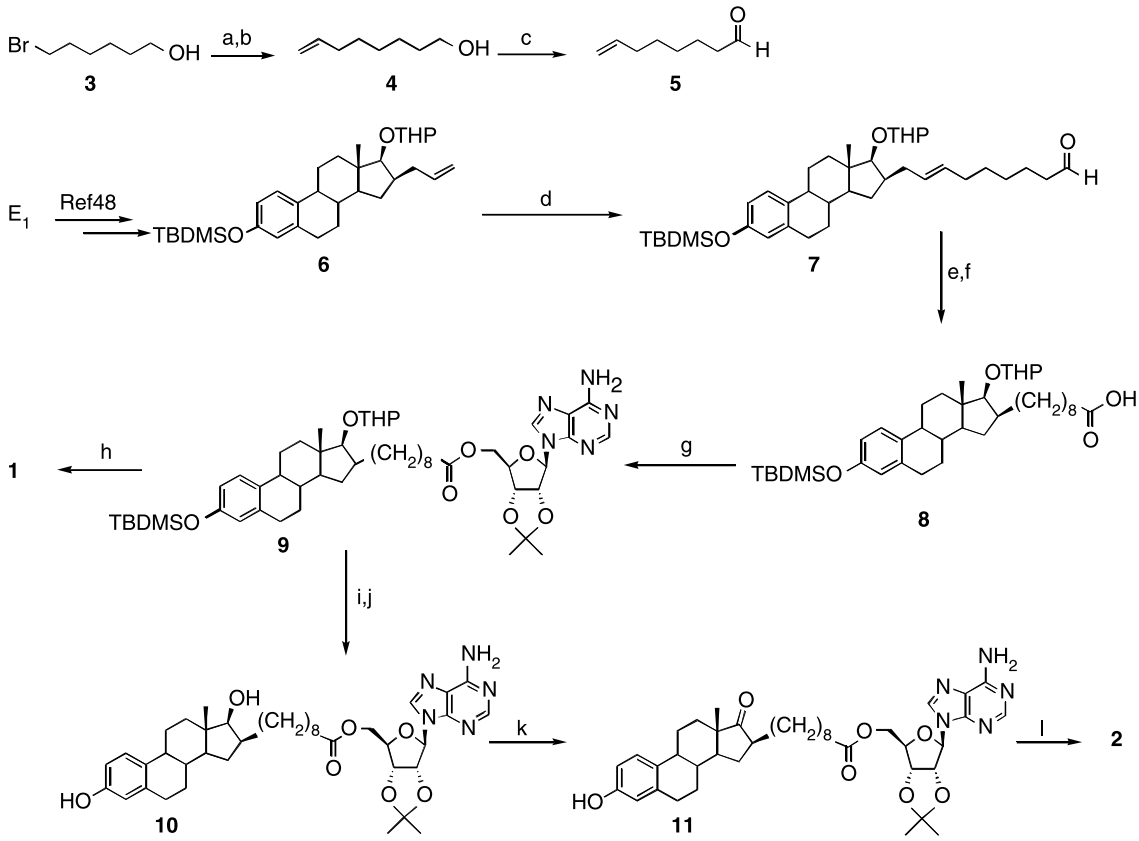
Results

Chemistry

The C17-ketone analogue of EM-1745, compound **2**, was prepared by oxidation of the C17-alcohol of an advanced intermediate in the synthesis of EM-1745. However, the chemical procedure initially developed for the synthesis of **1** was time-consuming and difficult [41]. In fact, the addition of a long alkyl chain in the α position of the C17 ketone gave a mixture of mono (C16α- and C16β-alkylated) and C16-dialkylated E_1 derivatives. Moreover, it was impossible to separate the C16β-alkylated-E_1 derivatives from the mixture using standard purification by flash chromatography. After stereoselective reduction of the C17-ketone, they applied a four-step sequence to isolate the pure β-alkylated-E_2. This sequence was based on selective protection of the C17β-alcohol derivatives with TBDMS. The C17β-alcohol of the 16α-isomer could easily be protected as a TBDMS using classical condition (TBDMSCl, imidazole). However, the C17β-alcohol of the 16β-isomer or the dialkylated derivatives needed more drastic conditions (TBDMSOTf, lutidine). The other weakness of this former procedure was the final deprotection. Even if the three protective groups (two TBDMS and one isopropylidene) can normally be removed in one step under acid conditions, a two-step sequence was necessary to hydrolyse the C17β-OTBDMS. Thus, the protective groups were removed by bubbling hydrogen chloride in dry DCM, except for the C17β-OTBDMS that was taken off by a subsequent treatment with TBAF at 60°C. A new procedure was then developed for preparing EM-1745 (**1**). The strategy that is presented here is roughly the same one developed to prepare the androstenedione/adenosine hybrid inhibitor of 17β-HSD3 [45]. Briefly, an alkyl side chain was added on 16β-allyl-E_2 by a cross-metathesis methodology. Furthermore, a THP ether, more easily removed under acid conditions than a C17-OTBDMS, was chosen as the C17β-protective group.

The chemical synthesis of **1** and **2** is illustrated in Scheme 1. Starting from 6-bromo-1-hexanol (**3**), a Br-I exchange using Finkelstein conditions followed by addition of a vinyl cuprate using HMPA and triethylphosphite as additives afforded vinyl alcohol **4** in 75% yield for two steps. This alcohol was next oxidized into aldehyde **5** with TPAP. The low boiling point of **5** explains the low 51% yield for this oxidation that seemed complete on TLC. Diprotected 16β-allyl-E_2 (**6**) was prepared in five steps from commercially available E_1 as previously reported [48]. A cross-metathesis [50] between steroid **6** and olefin **5** using Grubbs' catalyst afforded **7** in 50% yield. The formation of a side product resulting from the metathesis of two molecules of steroid **6** was also observed. It is noteworthy that these kinds of aldehydes are very unstable and are rapidly oxidized into carboxylic acid. Thus, a freshly prepared olefinic aldehyde **5** is necessary since the Grubbs catalyst is not compatible with carboxylic acids, resulting in a lower cross-metathesis yield. Olefin **7** was next reduced with hydrogen catalyzed by palladium followed by oxidation of the aldehyde under mild conditions ($NaClO_2$, NaH_2PO_4, 2-methyl-2-butene in *tert*-butanol) to provide carboxylic acid **8** in 75% yield.

The adenosine and the steroid moieties were next linked together. Commercially available 2′,3′-isopropylidene-adenosine was used to direct the addition on the C5′-OH of adenosine. A classical esterification procedure (EDCI and DMAP in DMF) resulted in a low 11% coupling yield, and thus, mainly starting materials were recovered after the reaction. However, reagents primarily used for coupling amino acids on solid phase synthesis (PyBOP, HOBt and DIPEA in DMF) gave a better 51% esterification yield and provided the E_2/adenosine hybrid compound **9**. Then, EM-1745 (**1**) was obtained in one deprotection step. Unfortunately, the deprotection conditions developed for the synthesis of the hybrid inhibitors of 17β-HSD3 (TFA/H_2O, 9:1) [45], did not give the expected product. Therefore, the three protective groups were removed by bubbling hydrogen chloride in dry DCM, which afforded EM-1745 (**1**) in a non-optimized 27% yield. Starting from **6**, EM-1745 (**1**)



Scheme 1. A new procedure for preparing EM-1745 (**1**) and synthesizing the C17-ketone analogue of EM-1745 (**2**). Reagents, conditions and yields: (a) NaI, acetone, reflux, 16 h; (b) *i*. CuI, vinylMgBr, THF, −40°C, 15 min; *ii*. HMPA, $P(OEt)_3$, THF, −40°C to rt, 5 h (75% for two steps); (c) TPAP, NMO, molecular sieves, DCM, rt, 90 min (51%); (d) **5**, 2nd generation Grubbs' catalyst, DCM, reflux, 16 h (50%); (e) 10% Pd/C, H_2, EtOAc, rt, 16 h; (f) $NaClO_2$, NaH_2PO_4, THF, 2-methyl-2-butene, *t*-BuOH, rt, 30 min (75% for two steps); (g) 2′,3′-isopropylidene adenosine, PyBOP, HOBt, DIPEA, DMF, rt, 16 h (51%); (h) HCl_g, DCM, rt, 3 h (27%); (i) PPTS, DCM, MeOH, reflux, 5 h; (j) TBAF, THF, 0°C, 10 min (72% for two steps); (k) Jones' reagent, acetone, 0°C, 8 min (52%); (l) TFA/H_2O, 9:1, THF, rt, 30 min (56%).

was obtained in five steps and 5% overall yield (19% overall yield before the final trideprotection).

In order to obtain the C17-ketone analogue **2** of EM-1745, the C17-OTHP protective group needs to be removed from **9** without hydrolysis of the isopropylidene group. In addition, one must take care to avoid hydrolysis of the ester bond. All the following reagents that we tried in various conditions: HCl/MeOH, *p*-TSA/DCM, HCl/dioxane or acid resins DOWEX or Amberlyst, removed both the C17β-OTHP and the C3-OTBDMS protective groups, but unfortunately some hydrolysis of the ester bond was observed. C17-OTHP and C3-OTBDMS were then removed in two steps. First, the THP protective group was removed with pyridinium *p*-toluenesulfonate (PPTS) in refluxed MeOH. With these conditions, part of the C3-OTBDMS was hydrolysed. Then, the remaining C3-OTBDMS group was removed carefully with TBAF in THF at 0°C to afford steroid **10** in 72% yield for two steps.

The last step was to oxidize the C17-alcohol of **10** into the C17-ketone. However, this step proved to be more difficult than expected. Oxidation with TPAP, Dess-Martin or PCC resulted in the decomposition of **10**. Only oxidation with Jones' reagent gave **11** in 52% yield with 22% of the starting material recovered after chromatography. In order to avoid formation of side reactions such as ester hydrolysis and oxidation at C6 steroidal position, the reaction time was limited to 8 minutes. Final deprotection of **11** with TFA/H_2O, 9:1 provided the C17-ketone analogue of EM-1745, compound **2**, in 56% yield. From steroid intermediate **6**, compound **2** was obtained in eight steps (4% overall yield) and was fully characterized by IR, ^{1}H NMR, ^{13}C NMR and LRMS to validate its structure.

Inhibition of 17β-HSD1

The enzymatic assay was performed with homogenated human embryonic kidney (HEK)-293 cells transfected with a vector encoding for 17β-HSD1 as previously described [49]. This test was carried out at 37°C for 2 h using NADH as cofactor to promote the reductive activity of the enzyme. Compounds **1** and **2** were evaluated for their ability to inhibit the transformation of [^{14}C]-E_1 into [^{14}C]-E_2. For this study, the inhibitors were tested in duplicate at four

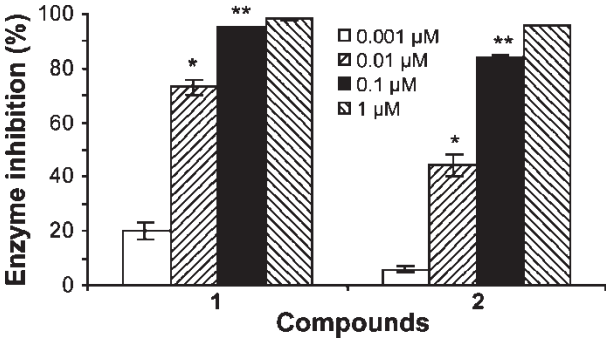


Figure 3. Inhibition of the transformation of [^{14}C]-E_1 into [^{14}C]-E_2 by EM-1745 (**1**) and its C17-ketone analogue **2** in homogenated HEK-293 cells everexpressing 17β-HSD1. See experimental section for more details. Data with the same symbol (⋆ or ⋆⋆) are significantly different ($P < 0.01$).

concentrations: 0.001, 0.01, 0.1 and 1 μM (Figure 3 and Table II). From these data, we estimated the IC_{50} values of 4 and 12 nM for **1** and **2**, respectively.

Discussion

Several families of inhibitors have been developed for 17β-HSD1, such as competitive reversible inhibitors, competitive irreversible inhibitors and bisubstrate inhibitors [28–32]. EM-1745 (**1**), the bisubstrate inhibitor we previously synthesized [41,42], contains an E_2 moiety. The K_m values reported in the literature for E_1 (0.36 μM) and E_2 (1.7 μM) with 17β-HSD1 indicated that E_1 is a much better substrate of 17β-HSD1 [44]. Furthermore, competitive inhibitors are known to interact with the substrate-binding site of the enzyme. We then hypothesized that modifying compound **1** by replacing E_2 with E_1 (a C17-ketone instead of a C17-alcohol) should give a better inhibition of 17β-HSD1. A C17-ketone derivative of EM-1745, compound **2**, was prepared in order to confirm our working hypothesis and we evaluated the ability of **1** and **2** to inhibit the transformation of [^{14}C]-E_1 into [^{14}C]-E_2 on homogenated HEK-293 cells over-expressing 17β-HSD1. However, the C17-ketone (E_1) derivative **2** gave a lower inhibition than the C17-alcohol (E_2) derivative **1**. This result was surprising. In fact, X-ray analysis of the EM-1745/17β-HSD1 complex clearly showed that the substrate moiety of EM-1745 interacts with the same site as E_1 does and kinetic studies demonstrated clearly that it is a reversible competitive inhibitor [42].

After obtaining this unexpected result, we looked the inhibitory potency of some of our 17β-HSD1 steroidal inhibitors available under the C17β-hydroxy and the C17-ketone forms (Figure 4, Table II). For 6β-alkylamide-E_2 and -E_1 derivatives (compounds **12** and **13**) [51], which are analogue to EM-678 inhibitor [52], a better inhibition of 17β-HSD1 was obtained with the C17β-hydroxy analogue, as for the bisubstrate inhibitors **1** and **2**. In fact, the C6β-alkylamide-E_2 (**12**) gave a better inhibition with 46% at 1 μM whereas the C6β-alkylamide-E_1 (**13**) gave almost no inhibition with 23% at 1 μM. The same tendency was also observed for the 16β-*m*-carbamoylbenzyl derivative of E_2, compounds **14** and **15**, recently reported as inhibitors of 17β-HSD1 [53]. At a concentration of 0.1 μM, the C17β-hydroxy compound **14** inhibited 77% the transformation of E_1 into E_2, but only 51% was obtained for the C17-ketone analogue ($IC_{50} = 44$ and 171 nM for **14** and **15**, respectively).

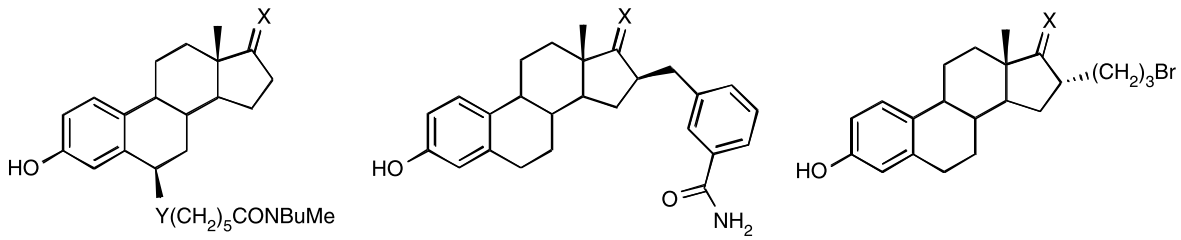
For the competitive irreversible inhibitors **16** and **17** a similar inhibition was reported for the 16α-(bromopropyl)-E_2 (**16**) and the 16α-(bromopropyl)-E_1 (**17**), with a slightly better inhibition (less than 1.5 fold) for the C17-ketone analogue (**17**) [54], whereas unlabeled E_1 gave a better inhibition than unlabeled E_2 with IC_{50} values of 300 nM and >1000 nM, respectively.

In summary, a more efficient procedure to prepare EM-1745 (**1**) was developed. Because the

Table II. Inhibition of 17β-HSD1 by different kinds of inhibitors.

			Inhibition of 17β-HSD1			
Entry	Compounds	C17 (X)	% at 0.01 mM	% at 0.1 mM	% at 1 mM	IC_{50} (nM)
1⋆	**1** (EM-1745)	β-OH	73	95	98	4
	2	O	44	84	96	12
2⋆ [51]	**12**	β-OH	–	15	46	–
	13	O	–	12	23	–
3† [53]	**14**	β-OH	–	77	94	44
	15	O	–	51	88	171
4‡ [54]	**16**	β-OH	–	–	–	460
	17	O	–	–	–	310
5‡ [54]	Unlabeled-E_2	β-OH	–	–	–	>1000
	Unlabeled-E_1	O	–	–	–	300

⋆Inhibition of the transformation of [^{14}C]-E_1 (100 nM) into [^{14}C]-E_2 in homogenated HEK-293 cells everexpressing 17β-HSD1. See experimental section for more details; †Inhibition of the transformation of [^{14}C]-E_1 (60 nM) into [^{14}C]-E_2 by 17β-HSD1 in breast cancer T-47D cells; ‡Inhibition of the transformation of [^{3}H]-E_1 (6 nM) into [^{3}H]-E_2 in partially purified human placenta cytosolic 17β-HSD1.



EM-678 (Y = S; X = β-OH)
12 (Y = CH_2; X = β-OH)
13 (Y = CH_2; X = O)

14 (X = β-OH)
15 (X = O)

16 (X = β-OH)
17 (X = O)

Figure 4. A selection of our previously reported inhibitors of 17β-HSD1.

C17-ketosteroid E_1 is a better substrate for the reductive activity of 17β-HSD1 than the corresponding C17-hydroxy E_2, the C17-ketone analogue of EM-1745 (compound **2**) was also synthesized using this new procedure. Unexpectedly, the C17-ketone derivative **2** gave a lower inhibition of the enzyme than C17-hydroxy derivative **1**. In fact, the presence of a C17-ketone increases the binding affinity of a C18 steroid for 17β-HSD1, but it can also modify the orientation of the 16β-side chain and thus the positioning of the adenosine group interacting with the cofactor binding site of the enzyme. In that case, the potency or number of these key interactions could be reduced with as consequence a diminution of the inhibitory activity of **2**. A lower inhibitory activity of ketone derivatives also holds true for two series of reversible inhibitors (**12, 13** and **14, 15)**, and similar inhibition was obtained for two competitive irreversible inhibitors (**16** and **17)**. Nonetheless, the inhibitory potency of a larger number of inhibitors should be evaluated in a same enzymatic assay in order to confirm this observation (the C17-alcohol analogues are better inhibitors than their corresponding C17-ketone analogues). That knowledge could be applied to the development of new and more potent competitive inhibitors of 17β-HSD1.

Acknowledgements

We thank the Canadian Institutes of Health Research for financial support (DP) and a scholarship (MB). We are also grateful to Dr. Van Luu-The for providing HEK-293 cells everexpressing 17β-HSD1 and Fatima Kamal for chemical synthesis of intermediate compound **6**. Careful reading of the manuscript by Sylvie Méthot is also greatly appreciated.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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