DOI: 10.1002/ejoc.200701077

Chemical Synthesis of (*S*)-Spiro(estradiol-17,2′-[1,4]oxazinan)-6′-one Derivatives Bearing Two Levels of Molecular Diversity

François Rouillard,[a] Jenny Roy,[a] and Donald Poirier*[a]

Keywords: Steroids / Lactones / Amino acids / Estradiol derivatives / Enzyme inhibitors

The present study shows the development of a strategy that can afford different estradiol derivatives bearing a 17-spiro-δ-lactone moiety with a nitrogen atom inserted in the lactone ring. Such [1,4]oxazinan-6′-one derivatives contain two levels of molecular diversity introduced to modulate biological activity. The strategy employed to prepare these compounds includes the stereoselective formation of a 17β-oxirane from the carbonyl group of estrone, the regioselective opening of the resulting oxirane with various hydrophobic amino acids (giving the first level of diversity), the spirolactonization, the alkylation of nitrogen by different functional groups (producing the second level of diversity), and final deprotection, when necessary. A series of (*S*)-spiro-(estradiol-17,2′-[1,4]oxazinan)-6′-one derivatives was generated to illustrate the usefulness of this diversification strategy, which could easily be extended to other steroidal and nonsteroidal ketone scaffolds for interacting with additional biological targets.

Introduction

17β-hydroxysteroid dehydrogenases (17β-HSDs) play an important role in the regulation of steroid hormones, such as estrogens and androgens, by catalyzing the reduction of 17-ketosteroids or the oxidation of 17β-hydroxysteroids by using NAD(P)H or $NAD(P)^+$ as cofactor, respectively.[1] Because it is involved in the last step of sex steroid hormone biosynthesis from cholesterol, the 17β-HSD enzyme family is an interesting target for controlling the concentration of estrogens and androgens. The 17β-HSDs activities are widespread in human tissues, not only in classic steroidogenic tissues, such as testis, ovary, and placenta, but also in a large series of peripheral intracrine tissues.[2] Since the 1990s, new types of 17β-HSDs were reported,[3] indicating that a fine regulation is carried out. In a therapeutic perspective, 17β-HSD inhibitors are useful tools in the treatment of estrogen-sensitive pathologies (breast, ovarian, and endometrium cancers) and androgen-sensitive pathologies (prostate cancer, benign prostatic hyperplasia, hirsutism, etc.).[4] Synthetic inhibitors can also help to clarify their role when not fully understood.

In a previous study,[5] a spiro-γ-lactone derivative of C18-steroid estradiol (E2) was reported to inhibit type 2 17β-HSD (Figure 1A). It was later demonstrated that this lactone was the most potent inhibitor among a series of spiro-γ-lactones having a C19-steroid scaffold.[6] From these results, it was established that a spiro-γ-lactone is an important pharmacophore for the inhibition of type 2 17β-HSD. The same group later reported that the spiro-δ-lactone of E2 (Figure 1B) demonstrated the best inhibition over a series of differently substituted lactones.[7] It has also been shown that spiro-δ-lactones inhibited type 5 17β-HSD.[8] More interestingly, introduction of a chemical group in the 4′- and 5′-positions of the lactone ring can modulate biological activity.[9] In light of these results, our group has developed a strategy that can afford different compounds bearing a δ-lactone moiety with a nitrogen atom inserted in the spirolactone ring. Such [1,4]oxazinan-6′-one derivatives (Figure 2) contain two levels of chemical diversity represented by the R^1 and R^2 groups. The strategy employed

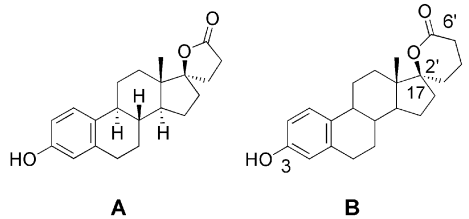


Figure 1. Chemical structures of two type-2 17β-HSD inhibitors with a C18-steroid (estradiol) nucleus; one contains a spiro-γ-lactone (**A**) and the other one contains a spiro-δ-lactone (**B**) as pharmacophore. The stereogenic centers are illustrated only for steroid **A**, but they are the same for all the other steroid derivatives reported in this paper. Partial numbering of carbon atoms is represented on steroid **B**.

[a] Medicinal Chemistry Division, Oncology and Molecular Endocrinology Laboratory, CHUL Research Center and University Laval,
CHUQ – Pavillon CHUL, Québec, Québec G1V 4G2, Canada
Fax: +1-418-654-2761
E-mail: donald.poirier@crchul.ulaval.ca
Supporting information for this article is available on the WWW under http://www.eurjoc.org or from the author.

to prepare these compounds included the formation of a steroidal C17-oxirane, the opening of the resulting oxirane with various hydrophobic amino acid derivatives to give the first level (R^1) of diversity, the spirolactonization, the alkylation of nitrogen by different groups to produce the second level (R^2) of diversity, and the final deprotection. We thus report the development of a versatile route that allows the chemical synthesis of different (*S*)-spiro(estradiol-17,2′-[1,4]oxazinan)-6′-one derivatives with two sites of diversification suitable for modulating biological activities.

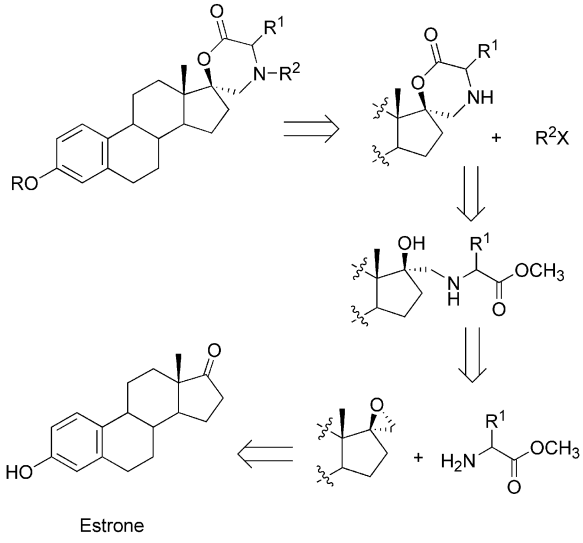
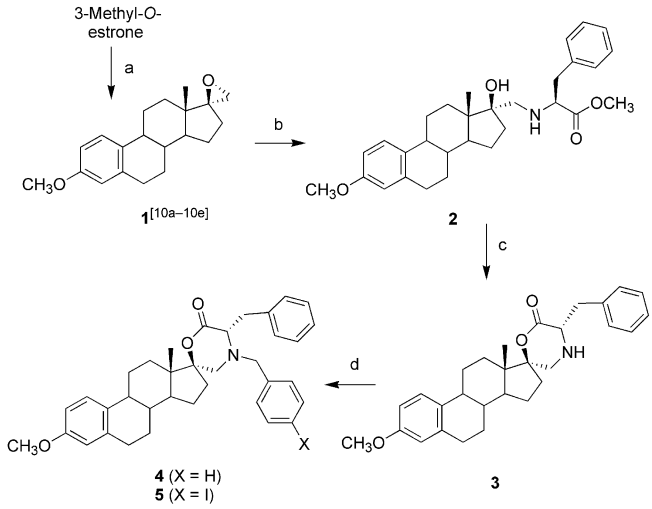


Figure 2. The retrosynthesis of (*S*)-spiro(estradiol-17,2′-[1,4]oxazinan)-6′-one derivatives containing two levels of molecular diversity; one (R^1) coming from the amino acid used for the oxirane opening and the second (R^2) coming from N-alkylation upon the secondary amine.

Results and Discussion

The synthesis of [1,4]oxazinan-6′-one derivatives targeted in our study started from ketosteroid estrone (E1). For chemical strategy development purposes, the phenol of E1 was protected as a methyl ether, which is very stable under various reaction conditions and therefore well indicated for the first part of our work (Scheme 1). The 17-carbonyl of 3-methyl-*O*-E1 was thus treated with dimethylsulfonium methylide to generate the corresponding oxirane **1** with the appropriate (17*S*)-stereochemistry according to a known methodology.[10] A regioselective aminolysis of this oxirane was next performed to introduce the first level of molecular diversity by using an amino acid methyl ester as a versatile building block.



Scheme 1. Synthesis of compounds **4** and **5**. Reagents and conditions: (a) $(CH_3)_3SI$, DMSO, NaH; (b) L-phenylalanine methyl ester, MeOH, Schlenk tube, 90–100 °C; (c) NaH, THF; (d) $(iPr)_2EtN$, benzyl bromide or 4-iodobenzylbromide, CH_2Cl_2, Schlenk tube, 70 °C.

Preparation of Free Amino Acids (Neutralization of the Corresponding Salt)

The trickiest step to achieve in this methodology development was to find a method that would be efficient to obtain the free amino acid methyl ester from its hydrochloride salt (Table 1). We first tried organic bases in organic solvents. We thought that the free amino acid methyl ester would become soluble in the organic solvent and after a simple washing step of the organic layer with water to remove the new salt formed, we would retrieve the desired free amino acid methyl ester by simple evaporation. This strategy proved unsuccessful (Table 1, Entries 1 and 2) regardless of the kind of amino acid methyl ester hydrochloride salt, type of solvent, and strength of base used (Et_3N or DIPEA in EtOAc, CH_2Cl_2, or acetone). We achieved better results by dissolving the amino acid methyl ester hydrochloride salt in water with the use of a weak inorganic

Table 1. Preparation of free amino acid methyl ester from corresponding HCl salt.[a]

Entry	Experimental conditions[b]	Gly	Ala	Val	Leu	Met	Phe
1	Et_3N/EtOAc or CH_2Cl_2	–	N/C[c]	–	–	–	N/C[c]
2	DIPEA/EtOAc/CH_2Cl_2/acetone	–	N/C[c]	–	–	–	N/C[c]
3	Cs_2CO_3 (1.5 equiv.)/H_2O/EtOAc/extraction	0	0	34	44	71	75
4	PMP resin (4 equiv.)/THF/12 h/strong agitation	4	6	45	49	–	–
5	PMP resin (4 equiv.)/THF/48 h/strong agitation	6	37	57	63	–	–
6	EPMP resin (4 equiv.)/THF/48 h/strong agitation	–	–	61	68	75	79
7	EPMP resin (8 equiv.)/THF/48 h/strong agitation	–	–	–	67	–	76

[a] Data expressed as % of free L-amino acid methyl ester recovered after several extractions (Entry 3) or filtrations (Entries 4–7). [b] PMP resin: piperidinomethyl polystyrene resin; EPMP resin: ethylpiperazinomethyl polystyrene resin. [c] N/C: Nonconclusive assay.

base such as Cs_2CO_3 (Table 1, Entry 3). Thus, the free amino acid methyl ester was extracted by using CH_2Cl_2 or EtOAc as solvent. EtOAc was the solvent that offered the best yields. Unfortunately, the yield of extraction dropped in a linearly dependent manner as the hydrophobic character of the amino acid methyl ester used decreased with no possibility at all of extracting the glycine methyl ester or the alanine methyl ester. The third strategy used was the best of all three and involved the use of scavenger base resins and THF as aprotic solvent (Table 1, Entries 4–7). The two scavengers used were piperidinomethyl polystyrene and ethylpiperazinomethyl polystyrene resins. Different quantities of resin were used with different reaction times. The best combination of all the parameters tested required the use of 4 equiv. of ethylpiperazinomethyl polystyrene resin in THF and its reaction with the amino acid methyl ester hydrochloride salt over the course of 48 h with strong agitation (Table 1, Entry 6). We also increased the amount of ethylpiperazinomethyl polystyrene up to 8 equiv. but without any improvement in the yields. The workup of the reaction only required filtration of the resin followed by evaporation of THF. However, the concentrated free amino acid methyl ester starts to polymerize after 24 h, with complete polymerization after 72 h. Therefore, it is important to use the free amino acid methyl ester as soon as obtained to perform the next reaction step (oxirane opening) in order to avoid this polymerization issue.

Oxirane Opening

Two procedures were first attempted to perform the opening of the (17*S*)-oxirane moiety of **1** with simple primary amine to verify how the opening of an oxirane at this position behaves. Indeed, as reported for the low reactivity of steroidal 17-ketones,[11] it is known that the hindered steroidal (17*S*)-oxirane is much-less reactive to aminolysis than other oxiranes.[12] The first method tried was a regioselective opening of the oxirane with heptylamine and lithium perchlorate for 4 d heated at reflux in acetonitrile.[13] Contrary to the opening of an oxirane at the steroidal 3-position,[14] we were not able to achieve the reaction unless we used more then 20 equiv. of amine. We also tried the oxirane opening using the amine in anhydrous EtOH at reflux for 4 d but, unfortunately, with the same result. This last procedure was next tested with an amino acid methyl ester. Explicitly, the procedure tested was to dissolve the oxirane starting material in EtOH and use a large excess of the L-phenylalanine methyl ester (up to 30 equiv., in our case, to have full completion of the reaction) and to heat the reaction mixture at reflux (60 °C) for at least 72 h. We could retrieve the desired compound from this procedure, but the excess amount of amino acid methyl ester used was a serious drawback for further purification of the mixture and also for the waste of precious amino acid methyl ester starting material, particularly because this starting material cannot be recovered owing to the polymerization problem. Conditions using a Lewis acid (boron trifluoride diethyl ether) in an aprotic solvent (toluene)[15] were also tried, but without success. In fact, the yields were substantially lower when the Lewis acid was used than the previous procedures without catalysis. The possibility that the use of a Lewis acid promotes the polymerization of the free amino acid methyl ester is a credible hypothesis to explain the very low yields of oxirane opening. At this point, finding conditions that can lower the amount of amino acid methyl ester for the opening of oxirane becomes an important issue.

The opening of oxirane **1** (Scheme 1) was next performed in a Schlenk tube so that we could substantially raise the temperature without losing the solvent by evaporation and increasing the pressure in the flask. With this procedure, we were able to lower the amount of amino acid methyl ester needed from 30 to 10 equiv. by heating the Schlenk tube containing the reactants in EtOH at 90–100 °C for a period of 2–4 d. One problem encountered was that some transesterification of the methyl ester occurred in EtOH giving a mixture of two compounds; one having a methyl ester and the other one having an ethyl ester. This problem was easily resolved by performing the reaction in MeOH instead of EtOH with an acceptable yield of 73% for **2**. According to another study,[16] it was possible to lower the amount of amine needed for the opening of a hindered epoxide at the 2,3-position of a steroid skeleton to a quantity as low as 3 equiv. by using gadolinium triflate [$Gd(OTf)_3$] as a catalyst. We tried this procedure, but it did not give us the expected results. Contrary to the amines used in this study, the amino acid methyl ester contains oxygen atoms that can chelate the catalyst. Consequently, this procedure probably increases the polymerization problem. In summary, the best oxirane opening conditions were 10 equiv. of freshly generated amino acid methyl ester dissolved in MeOH and heated (90–100 °C) in a Schlenk tube for 2–4 d.

Lactonization

Different lactonization conditions were tested with hydroxy ester **2** as a model compound. Two acidic conditions using *p*-toluenesulfonic acid in toluene or 5–10% aqueous HCl in THF at room temperature to 65 °C were first tried, but they both gave an unwanted reaction: a suprafacial 1,2-migration of the 18-CH_3 with dehydration of the alcohol at the 17-position of the steroid skeleton, which is a well-known side reaction in steroid chemistry.[17] The basic conditions using sodium methylate/MeOH or sodium ethanolate/EtOH in benzene at 65 °C gave also inconclusive results. Finally, the only conditions that gave desired compound **3** in one step was the use of NaH in THF at room temperature. In this case, the alcoholate generated from tertiary alcohol **2** attacks the methyl ester to generate the spiro-[1,4]oxazinan-6′-one derivative **3** in 55% yield.

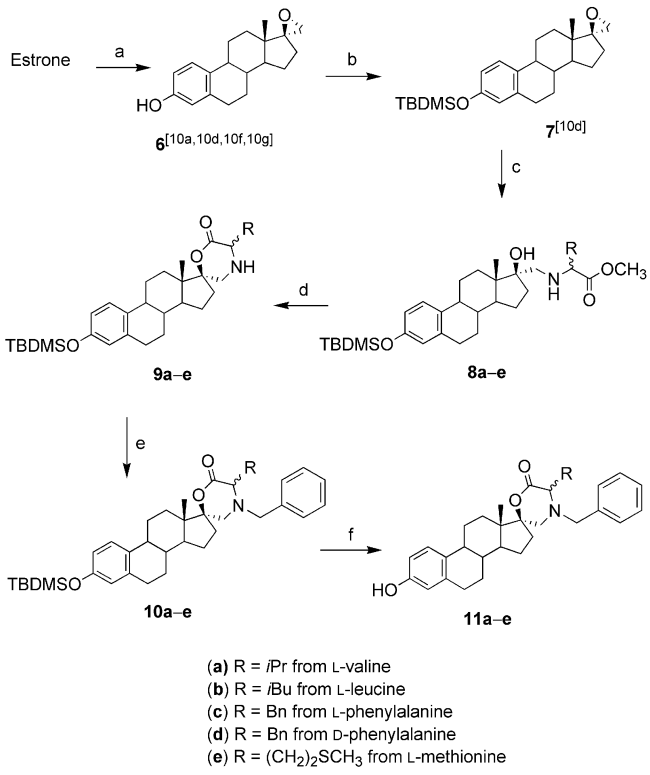
N-Diversification of [1,4]Oxazinan-6′-one Derivative 3

The presence of a secondary amine inserted into the spiro-δ-lactone ring is an interesting way to introduce dif-

ferent types of functional groups. As examples, we now report the alkylation of the NH of **3** with benzyl bromide or 4-iodobenzyl bromide to generate tertiary amines **4** or **5**, but diversity can be extended to other halogenoalkyls, acyl chlorides, activated carboxylic acids, sulfonyl chlorides, cyanates, and thiocyanates in order to obtain tertiary amines, amides, sulfonamides, ureas, and thioureas, respectively. Only one condition was tried to introduce the benzyl group, because the yields were good enough (76–91%) when using benzyl bromide and diisopropylethylamine as base. The introduction of a second level of diversity upon the secondary amine was easily performed and it led us to believe that it would be feasible to link other kinds of groups upon this amine to obtain other kinds of functionality, as mentioned above.

Synthesis of a Series of (*S*)-Spiro(estradiol-17,2′-[1,4]oxazinan)-6′-ones

Phenolic steroids generally have better affinity on steroidogenic receptors and enzymes than their corresponding methyl ether derivatives. Consequently, the same strategy reported above for the synthesis of 3-methyl-*O*-E2 derivatives was applied to synthesize E2 derivatives with a 3-OH group and an [1,4]oxazinan-6′-one moiety at the 17-position (Scheme 2). Estrone was first treated with dimethylsulfonium methylide to afford oxirane **6**, which was next protected as TBDMS derivative **7**. The synthesis of the oxirane group has to be performed before protection of the phenolic group of E1 otherwise the TBDMS group is removed while doing the oxirane synthesis.[10d] The opening of oxirane **7** was easily achieved to give alcohols **8a**–**e** in good yields ranging from 69 to 80%. For the lactonization of **8a**–**e** with NaH in THF, no more than 1 equiv. of NaH should be used because the TBDMS is removed and this protecting group is absolutely necessary during the next step, which is the diversification of the secondary amine of **9a**–**e** into **10a**–**e**. As a final step, the removal of the TBDMS protecting group of **10a**–**e** was easily achieved by a treatment with 2% HCl in methanol. The overall unoptimized yields of **11a**–**e** for the sequence of reactions (six steps) ranged from 18 to 20%. These yields could, however, be improved by optimizing the lactonization, which is the limiting step with yields of 52–55%.



Scheme 2. Synthesis of compounds **11a**–**11e**. Reagents and conditions: (a) $(CH_3)_3SI$, DMSO, NaH; (b) TBDMSCl, imidazole; (c) L- or D-amino acid methyl ester, MeOH, Schlenk tube, 90–100 °C; (d) NaH, THF; (e) $(iPr)_2EtN$, benzyl bromide, CH_2Cl_2, Schlenk tube, 70 °C; (f) 2% HCl in MeOH.

NMR Spectroscopic Analysis of a Representative Final Compound

The compound **4** was analyzed by NMR spectroscopy to confirm its expected structure. The assignment of all carbon signals and assignment of key proton signals are reported in Figure 3. In the ^{13}C NMR spectrum, the signals of the E2 nucleus (C-1 to C-18) were easily identified on the basis of literature data[18] and confirmed by a series of 2D NMR spectroscopic experiments (COSY, TOCSY, APT, HSQC, HMBC, and NOESY).[19] The C-17 (or C-2′) signal at δ = 91.9 ppm and C-6′ signal at δ = 170.7 ppm are very characteristic of the expected spiro[1,4]oxazinan-6′-one ring, and these two values are closely related to reported data of the spiro-δ-lactone shown in Figure 1B.[7] After we identified the aromatic signals of the two benzyl groups, the only remaining CH signal at δ = 64.7 ppm was necessarily CH-5′. From this signal and by using 2D NMR spectroscopic experiments, it was then possible to identify the CH_2-1′′ (δ = 35.0 ppm), a value that corresponds very well to a benzylic methylene linked to a carbon atom. The two other CH_2 signals remaining to be assigned were located at δ = 53.2 and 58.5 ppm; such chemical shifts are characteristic to an NCH_2 group. In the ^1H NMR spectra, the two protons of each NCH_2 are nonequivalent and their chemical shifts are very different. To discriminate which signals are from CH_2-3′ and which signals are from CH_2-1′′′, the HMBC experiment was useful. In fact, the two proton signals associated with the carbon signal at δ = 53.2 ppm (from HSQC experiment) showed no long-range ($^2J_{C,H}$ and $^3J_{C,H}$) correlations with aromatic carbon signals in HMBC experiment then identifying the CH_2-3′. On the other hand, the two proton signals associated with the carbon signal at δ = 58.5 ppm (from HSQC experiment) clearly showed long-range ($^2J_{C,H}$ and $^3J_{C,H}$) correlations with aromatic carbon signals in the HMBC experiment then confirming the CH_2-1′′′.

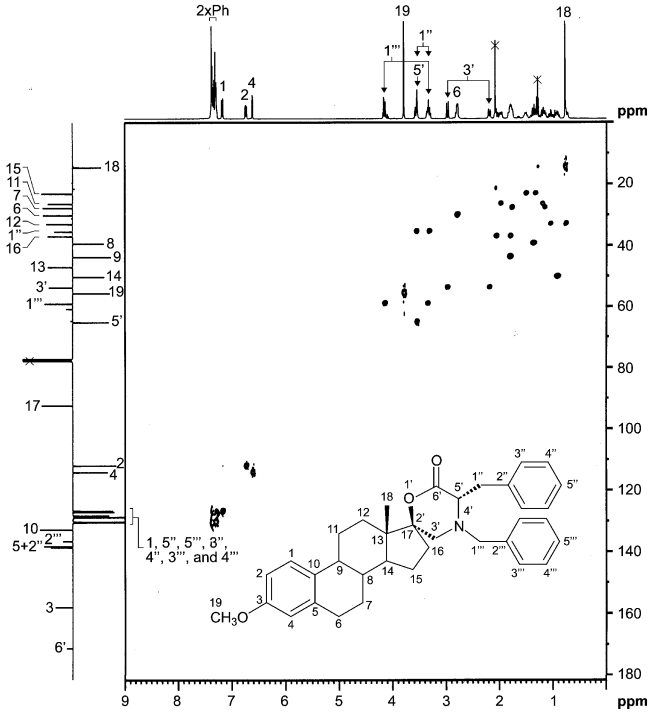


Figure 3. NMR (HSQC) spectra of the representative final compound **4** showing $^1J_{C,H}$ correlations between the protons and carbon atoms. The assignment of all carbon atoms and key protons was indicated on the spectra. The experiment was done with $CDCl_3$ as solvent by using a 400 MHz NMR apparatus.

X-ray Analysis of a Representative Final Compound

Compound **11d** was crystallized and its 3D structure determined by X-ray analysis.[20] As expected, X-ray analysis showed the typical shape of an estra-1,3,5(10)-triene steroid core (C-1 to C-18) and an [1,4]oxazinan-6′-one moiety (O-1′ to C-6′) linked at spirocarbon 17 (Figure 4a). The (17*S*) stereochemistry is in accord with the sequence of reactions starting from (17*S*)-oxirane **7** and needed for the synthesis of **11d**. The (5′*R*) stereochemistry observed corresponds with the D-phenylalanine methyl ester used as a building block. The configuration of the [1,4]oxazinan-6′-one moiety and the orientation of the two benzyl groups at N-4′ and C-5′ are better viewed with the 3D-structure representation reported in Figure 4b. The spiro[1,4]oxazinan-6′-one ring adopts a pseudochair configuration with the two benzyl groups in *trans*-4′,5′-diequatorial position. Being rigid, this pseudochair conformer is a good scaffold that can direct the two elements of molecular diversity (herein represented by the benzyl groups) in two different positions according to the selected amino acid (D or L). As can be seen in Figure 4c, the benzyl groups of **11d** are both positioned in the same right side of the molecule but are perpendicular to each other. However, in the case of **11c**, which is obtained with L-Phe, the two benzyl groups would be in opposite directions: one on the left side and the other on the right side. The positioning of the two elements of molecular diversity differently and in proximity to the steroid D-ring, is an interesting characteristic, because there are several reports in the literature showing that an interacting group added in proximity to the D-ring of E2 can modulate the biological activity of 17β-HSDs inhibitors, steroid sulfatase inhibitors, estrogen receptor antagonists, and estrogen receptor antagonists.[4–7,21]

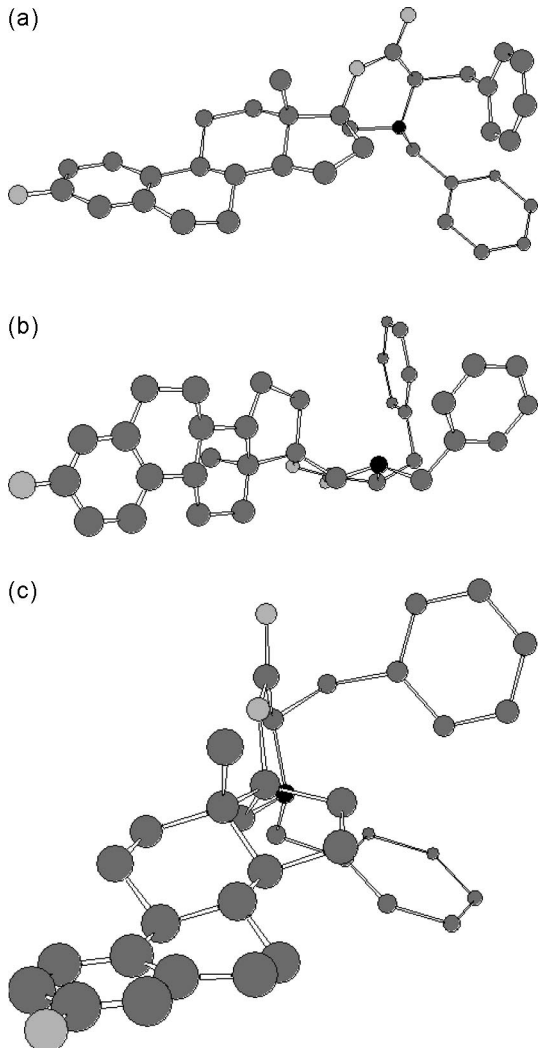


Figure 4. X-ray 3D-structure analysis of the representative final compound **11d** crystallized in EtOH.[20] Three different pictures (**a**–**c**) were represented by using the CS Chem3D Std software (Cambridge Soft Corporation) and the pdb data file obtained from the X-ray data.

Conclusions

A strategy was reported for the synthesis of (*S*)-spiro-(estradiol-17,2′-[1,4]oxazinan)-6′-one derivatives having two levels of molecular diversity. The first level of diversity was introduced by an amino acid for opening an oxirane easily obtained from a steroidal ketone. The second level of diversity was generated by an alkylation of the NH functionality inserted in the spiro-δ-lactone ring. Although the aminolysis step generating the first level of diversity is restricted to L- and D-amino acids that can be extracted in organic solvent after the neutralization of their HCl salt,

the reactivity of the secondary amine offers great possibility of diversification (tertiary amines, amides, sulfonamides, and ureas) for the second level of diversity. The spiro[1,4]-oxazinan-6′-one ring also offers a rigidity that directs the elements of diversity introduced by the two levels of diversity. The strategy was exemplified with a 17-ketosteroid (estrone) as starting material, but the strategy could be advantageously used at other steroidal positions or with other steroid nuclei. Furthermore, all types of ketones could be used, thus constituting a third level of diversity. This additional level of diversity greatly extends the usefulness of our strategy of diversification, not only for developing inhibitors of steroidogenic enzymes but also for interacting with a large series of biological targets.

Experimental Section

General Remarks: Estrone and 3-methyl-*O*-estrone were purchased from Steraloids Inc. (Newport, RI, USA). Chemical reagents of highest purity and anhydrous solvents were obtained from Sigma–Aldrich Canada Ltd. (Oakville, ON, Canada) and Fisher Scientific (Montréal, QC, Canada). The two resins piperidinomethyl polystyrene (3.5 mmol g^{-1}, 100–200 mesh) and ethylpiperazinomethyl polystyrene (3.7 mmol g^{-1}, 100–200 mesh) were obtained from Matrix Innovation (Montréal, QC, Canada). Tetrahydrofuran used in anhydrous reactions was distilled from benzophenone ketyl. Reactions were run under an inert (argon) atmosphere in oven-dried glassware. Analytical thin-layer chromatography (TLC) was performed on 0.20-mm silica gel 60 F254 plates (Fisher Scientific), and compounds were visualized by using UV light or ammonium molybdate/sulfuric acid/water (with heating). Flash column chromatography was performed with Silicycle R10030B 230–400 mesh silica gel (Québec, QC, Canada). Infrared spectra (IR) were obtained from a thin film of compound usually solubilized in $CDCl_3$ and deposited upon a NaCl pellet. They were recorded with a Perkin–Elmer 1600 FTIR spectrometer (Norwalk, CT, USA) and only characteristic bands are reported. Nuclear magnetic resonance (NMR) spectra were recorded at 300 MHz (^{1}H) and 75.5 MHz (^{13}C) with a Bruker AC/F300 spectrometer (Billerica, MA, USA) or at 400 MHz (^{1}H) and 100.6 MHz (^{13}C) with a Bruker Avance 400 digital spectrometer. The chemical shifts are expressed in ppm and are referenced to residual chloroform (δ = 7.26 ppm for ^{1}H NMR and 77.0 ppm for ^{13}C NMR). Low-resolution mass spectra (LRMS) were recorded with a PE Sciex API-150 ex apparatus (Foster City, CA, USA). High-resolution mass spectra (HRMS) were provided by The Mass Spectrometry Unit (McGill University, Montréal, QC, Canada). The X-ray analysis was performed at the Laboratoire de diffraction des rayons-X de l'Université de Montréal (Montréal, QC, Canada). The names of new compounds were obtained by using the ACD/I-Lab Web service (ACD/IUPAC Name 8.05).

(8*R*,9*S*,13*S*,14*S*,17*S*)-3-Methoxy-13-methyl-6,7,8,9,11,12,13,14,15,16-decahydrospiro(cyclopenta[*a*]phenanthrene-17,2′-oxirane) (1):[10a–10e] In a 2-liter flask under an argon atmosphere was added DMSO (175 mL) followed by NaH (60% in oil, 4.94 g, 123.60 mmol). The mixture was heated at 75 °C for 1 h and then cooled to room temperature and anhydrous THF (175 mL) was then added. The mixture was cooled to 0 °C and trimethylsulfonium iodine (25.22 g, 123.60 mmol) dissolved in DMSO (175 mL) was added, followed by 3-methyl-*O*-estrone (5.85 g, 20.60 mmol) dissolved in anhydrous THF (175 mL). The mixture was kept at 0 °C for 0.5 h and then warmed to room temperature and left to react for another 0.5 h before completion. The reaction was quenched at 0 °C with a saturated NH_4Cl aqueous solution and extracted with EtOAc. The organic layers were combined and washed several times with water before drying over Na_2SO_4, filtered and evaporated under reduced pressure. The crude material was then purified by flash column chromatography (hexanes/EtOAc, 9:1). White solid (4.85 g, 79%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.92 (s, 3 H, 18-CH_3), 0.90–2.35 (residual CH and CH_2), 2.65 and 2.97 (2 d, J = 5.0 Hz, 2 H, 17α-CH_2), 2.88 (m, 2 H, 6-CH_2), 3.78 (s, 3 H, CH_3O), 6.64 (d, J = 2.6 Hz, 1 H, 4-CH), 6.72 (dd, J_1 = 8.6 Hz, J_2 = 2.7 Hz, 1 H, 2-CH), 7.20 (d, J = 8.6 Hz, 1 H, 1-CH) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.3, 23.3, 26.0, 27.2, 29.1, 29.7, 33.9, 38.9, 40.4, 43.9, 51.8, 53.7, 55.2, 70.6, 111.5, 113.8, 126.3, 132.4, 137.9, 157.4 ppm. LRMS: calcd. for $C_{20}H_{27}O_2$ $[M + H]^+$ 299.2; found 298.7.

Opening of Oxirane 1. Synthesis of Methyl *N*-{[(17β)-17-Hydroxy-3-methoxyestra-1,3,5(10)trien-17-yl]methyl}-L-phenylalaninate (2)

Step 1, Method A: L-Phenylalanine methyl ester·HCl was freed from the salt by dissolving the salt in H_2O. Cs_2CO_3 (1.5 equiv.) was then added and the mixtures was stirred for 0.5 h before several extractions with EtOAc. After evaporation of the solvent, the free L-phenylalanine methyl ester was recovered (see Table 1, Entry 3).

Step 1, Method B: L-Phenylalanine methyl ester·HCl was freed from the salt by dissolving the salt in THF. The THF solution was then transferred to a Schlenk tube containing ethylpiperazinomethyl polystyrene scavenger resin (4 equiv.). The mixture was strongly agitated for 48 h. Then, the mixture was filtered, and the filtrate was evaporated under reduced pressure to obtain the free L-phenylalanine methyl ester (see Table 1, Entry 6).

Step 2: Oxirane **1** (304 mg, 0.76 mmol) was dissolved in dry MeOH in a Schlenk tube. After bubbling argon to saturation, L-phenylalanine methyl ester (1.11 g, 7.6 mmol) was added, and the solution was heated at 90–100 °C for 48 h. After completion of the reaction, the mixture was left to cool to room temperature before silica gel was added and the solvent evaporated under reduced pressure. The crude material was purified by flash column chromatography (hexanes/EtOAc, 9:1). Viscous oil (265 mg, 73%). IR (film): $\tilde{\nu}$ = 3447 (OH), 3327 (NH, weak), 1737 (C=O, ester) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.90 (s, 3 H, 18-CH_3), 1.20–2.30 (residual CH and CH_2), 2.53 and 2.75 (2 d, J = 11.4 Hz, 2 H, 17α-CH_2), 2.84 (m, 2 H, 6-CH_2), 2.95 and 3.05 (2 m, 2 H, CH_2Ph), 3.53 (t, J = 3.8 Hz, NCHCOO), 3.72 (s, 3 H, CH_3O from ester), 3.78 (s, 3 H, 3-CH_3O), 6.63 (d, J = 2.6 Hz, 1 H, 4-CH), 6.71 (dd, J_1 = 8.6 Hz, J_2 = 2.4 Hz, 2-CH), 7.18 (d, J = 8.5 Hz, 1 H, 1-CH), 7.24 (m, 5 H, CH_2*Ph*) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.0, 23.3, 26.0, 27.3, 29.6, 31.9, 34.8, 39.0, 39.6, 43.5, 45.2, 49.6, 51.7, 54.0, 55.0, 63.4, 81.3, 111.2, 113.5, 126.1, 126.7, 128.3 (2×), 129.0 (2×), 132.4, 137.0, 137.7, 157.2, 174.7 ppm. LRMS: calcd. for $C_{30}H_{40}NO_4$ $[M + H]^+$ 478.3; found 478.2.

Lactonization of 2. Synthesis of (5′*S*,8*R*,9*S*,13*S*,14*S*,17*S*)-5′-Benzyl-3-methoxy-13-methyl-6,7,8,9,11,12,13,14,15,16-decahydro-6′*H*-spiro(cyclopenta[*a*]phenanthrene-17,2′-[1,4]oxazinan)-6′-one (3): To compound **2** (454 mg, 0.835 mmol) dissolved in dry THF (33 mL) was added NaH (60% in oil, 33 mg, 0.835 mmol). The reaction was kept at room temperature and completed after 1 h. The reaction was then quenched at 0 °C with a saturated NH_4Cl aqueous solution and extracted with EtOAc. The organic layers were combined and washed with water, dried with Na_2SO_4, filtered, and evaporated under reduced pressure. The crude material was purified by flash column chromatography (hexanes/EtOAc, 8:2). White solid (55%). IR (film): $\tilde{\nu}$ = 3350 (NH, weak), 1727 (C=O, lactone) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.89 (s, 3 H, 18-CH_3), 1.10–2.20

(residual CH and CH_2), 2.83 and 3.24 (2 m, 4 H, CH_2Ph and 17α-CH_2), 2.85 (m, 2 H, 6-CH_2), 3.78 (s, 3 H, CH_3O), 3.80 (m, 1 H, NCHCOO), 6.62 (d, J = 2.4 Hz, 1 H, 4-CH), 6.71 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1 H, 2-CH), 7.19 (d, J = 8.4 Hz, 1 H, 1-CH), 7.30 (m, 5 H, CH_2*Ph*) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.2, 22.9, 25.9, 27.3, 29.6, 32.4, 35.6, 37.5, 39.0, 43.4, 46.8, 47.9, 49.6, 55.1, 57.7, 93.4, 111.5, 113.7, 126.2, 127.3, 128.8 (2×), 129.6 (2×), 131.9, 137.0, 137.7, 157.4, 172.0 ppm. LRMS: calcd. for $C_{29}H_{36}NO_3$ [M + H]$^+$ 446.3; found 446.1.

(5′*S*,8*R*,9*S*,13*S*,14*S*,17*S*)-4′,5′-Dibenzyl-3-methoxy-13-methyl-6,7,8,9,11,12,13,14,15,16-decahydro-6′*H*-spiro(cyclopenta[*a*]phenanthrene-17,2′-[1,4]oxazinan)-6′-one (4): To compound **3** (90 mg, 0.201 mmol) dissolved in dry CH_2Cl_2 (5 mL) was added DIPEA (60 μL, 0.342 mmol) followed by benzyl bromide (41 μL, 0.342 mmol). The resulting mixture was heated in a Schlenk tube at 75 °C for 12 h. The reaction quenched with a saturated NH_4Cl aqueous solution, extracted with CH_2Cl_2, and washed with water. The organic layers were combined, dried with Na_2SO_4, filtered, and evaporated under reduced pressure. The resulting crude material was purified by flash column chromatography (hexanes/EtOAc, 9:1). White solid (82 mg, 76%). IR (film): $\tilde{\nu}$ = 1724 (C=O, lactone) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.76 (s, 3 H, 18-CH_3), 0.70–2.10 (residual CH and CH_2), 2.16 and 2.95 (2 d, J = 13.1 Hz, 2 H, 17α-CH_2), 2.77 (m, 2 H, 6-CH_2), 3.31 (m, 2 H, 1 H of CH_2Ph and 1 H of NCH_2Ph), 3.53 (m, 2 H, NCHCOO and 1 H of CH_2Ph), 3.78 (s, 3 H, CH_3O), 4.13 (m, 1 H of NCH_2Ph), 6.60 (d, J = 2.4 Hz, 1 H, 4-CH), 6.72 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1 H, 2-CH), 7.15 (d, J = 8.4 Hz, 1 H, 1-CH), 7.30 (m, 10 H, 2×CH_2*Ph*) ppm. ^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 14.1, 22.6, 25.9, 27.2, 29.6, 32.5, 35.0, 36.5, 38.9, 43.3, 46.5, 49.7, 53.2, 55.1, 58.5, 64.7, 91.9, 111.4, 113.6, 126.2, 126.6, 127.7, 128.2 (2×), 128.3 (2×), 129.7 (2×), 129.9 (2×), 132.3, 136.1, 137.7, 138.1, 157.4, 170.7 ppm. LRMS: calcd. for $C_{36}H_{42}NO_3$ [M + H]$^+$ 536.3; found 536.3. HRMS: calcd. for $C_{36}H_{42}NO_3$ [M + H]$^+$ 536.31592; found 536.31552.

(5′*S*,8*R*,9*S*,13*S*,14*S*,17*S*)-5′-Benzyl-3-methoxy-4′-(4-iodobenzyl)-13-methyl-6,7,8,9,11,12,13,14,15,16-decahydro-6′*H*-spiro(cyclopenta[*a*]phenanthrene-17,2′-[1,4]oxazinan)-6′-one (5): To compound **3** (79 mg, 0.177 mmol) dissolved in dry CH_2Cl_2 (5 mL) was added DIPEA (52 μL, 0.300 mmol) followed by 4-iodobenzylbromide (89 mg, 0.300 mmol). The resulting mixture was heated in a Schlenk tube at 75 °C for 12 h. The reaction was quenched with a saturated NH_4Cl aqueous solution, extracted with CH_2Cl_2, and washed with water. The organic layers were combined, dried with Na_2SO_4, filtered, and evaporated under reduced pressure. The resulting crude material was purified by flash column chromatography (hexanes/EtOAc, 9:1). White solid (69 mg, 63% yield). IR (film): $\tilde{\nu}$ = 1721 (C=O, lactone) cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.79 (s, 3 H, 18-CH_3), 0.85–2.10 (residual CH and CH_2), 2.20 and 2.95 (2 d, J = 12.9 Hz, 2 H, 17α-CH_2), 2.79 (m, 2 H, 6-CH_2), 3.25 and 3.50 (2 dd, J_1 = 14.1 Hz, J_2 = 5.3 Hz, 2 H, CHCH_2Ph), 3.40 and 3.97 (2 d, J = 13.2 Hz, 2 H, NCH_2PhI), 3.56 (t, J = 5.1 Hz, 1 H, NCHCH$_2$Ph), 3.77 (s, 3 H, CH_3O), 6.60 (d, J = 2.4 Hz, 1 H, 4-CH), 6.72 (dd, J_1 = 8.4 Hz, J_2 = 2.4 Hz, 1 H, 2-CH), 7.01 (d, J = 8 Hz, 2 H, 2×CH from CH_2PhI), 7.16 (d, J = 8.4 Hz, 1 H, 1-CH), 7.29 (m, 5 H, CH_2*Ph*), 7.65 (d, J = 8.3 Hz, 2 H, 2×CH from CH_2PhI) ppm. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 14.2, 22.7, 25.9, 27.2, 29.6, 32.4, 34.8, 36.4, 38.9, 43.3, 46.6, 49.7, 52.7, 55.1, 58.0, 64.2, 92.0, 93.2, 111.4, 113.6, 126.2, 126.7, 128.4 (2×), 129.9 (2×), 131.3 (2×), 132.2, 135.8, 137.4 (2×), 137.7, 138.0, 157.4, 172.6 ppm. LRMS: calcd. for $C_{36}H_{41}INO_3$ [M + H]$^+$ 662.2; found 662.7. HRMS: calcd. for $C_{36}H_{41}INO_3$ [M + H]$^+$ 622.21256; found 622.21238.

Supporting Information (see also the footnote on the first page of this article): Experimental procedures and spectroscopic data for compounds **6**, **7**, **8a**–**8e**, **9a**–**9e**, **10a**–**10e** and **11a**–**11e**, ^{13}C NMR spectra for final compounds **4**, **5**, **11a**–**11e**.

Acknowledgments

We would like to thank the Canadian Institutes of Health Research (CIHR) for their financial support and Le Fonds de la Recherche en Santé du Québec (FRSQ) for a Senior Scholarship (D. P.). We are grateful to Orval Mamer and Alain Lesimple from The Mass Spectrometry Unit (McGill University) for HRMS analyses. Careful reading of the manuscript by Micheline Harvey is also greatly appreciated.

[1] a) V. Luu-The, *J. Steroid Biochem. Mol. Biol.* **2001**, *76*, 143–151; b) F. Labrie, V. Luu-The, S. X. Lin, J. Simard, M. El-Alfy, G. Pelletier, *J. Mol. Endocr.* **2000**, *25*, 1–16; c) F. Labrie, V. Luu-The, S. X. Lin, J. Simard, C. Labrie, *TEM* **2000**, *11*, 421–427; d) N. Moghrabi, S. Andersson, *TEM* **1998**, *9*, 265–270; e) F. Labrie, V. Luu-The, S. X. Lin, J. Simard, C. Labrie, R. Breton, *Steroids* **1997**, *62*, 148–158.

[2] C. Martel, E. Rheaume, M. Takahashi, C. Trudel, J. Couet, V. Luu-The, J. Simard, F. Labrie, *J. Steroid Biochem. Mol. Biol.* **1992**, *41*, 597–603.

[3] a) H. Peltoketo, V. Isomaa, O. Maentausta, R. Vihko, *J. Mol. Endocr.* **1999**, *23*, 1–11; b) V. Luu-The, Y. Zhang, D. Poirier, F. Labrie, *J. Steroid Biochem. Mol. Biol.* **1995**, *55*, 581–587.

[4] a) D. Poirier, *Curr. Med. Chem.* **2003**, *10*, 453–477; b) D. Poirier, R. Maltais, *Mini-Rev. Med. Chem.* **2006**, *6*, 37–52; c) D. Poirier, P. Bydal, M. R. Tremblay, K. M. Sam, V. Luu-The, *Mol. Cell Endocrinol.* **2001**, *171*, 119–128; d) M. R. Tremblay, D. Poirier, *J. Steroid Biochem. Mol. Biol.* **1998**, *66*, 179–191.

[5] a) S. Auger, V. Luu-The, K. M. Sam, D. Poirier, *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2045–2048; b) K. M. Sam, F. Labrie, D. Poirier, *Eur. J. Med. Chem.* **2000**, *35*, 217–225.

[6] a) K. M. Sam, S. Auger, V. Luu-The, D. Poirier, *J. Med. Chem.* **1995**, *38*, 4518–4528; b) M. R. Tremblay, V. Luu-The, G. Leblanc, P. Noel, E. Breton, F. Labrie, D. Poirier, *Bioorg. Med. Chem.* **1999**, *7*, 1013–1023.

[7] P. Bydal, S. Auger, D. Poirier, *Steroids* **2004**, *69*, 325–342.

[8] W. Qiu, M. Zhou, M. Mazumdar, A. Azzi, D. Ghanmi, V. Luu-The, F. Labrie, S. X. Lin, *J. Biol. Chem.* **2007**, *282*, 8368–8379.

[9] a) F. Labrie, A. Belanger, S. Gauthier, V. Luu-The, Y. Merand, D. Poirier, L. Provencher, S. M. Singh (Endorecherche Inc., Canada), US 6541463, **2003**; b) P. Bydal, M.Sc. Thesis, University Laval, Québec (Québec), Canada, **1997**, ch. 3 and 4, pp. 98–153.

[10] a) C. E. Cook, R. C. Corley, M. E. Wall, *J. Org. Chem.* **1968**, *33*, 2789–2793; b) K. Ponsold, M. Hubner, H. Kasch, I. Noack, *Z. Chem.* **1971**, *11*, 106–107; c) M. Hubner, I. Noack, *J. Prakt. Chem.* **1972**, *314*, 667–668; d) T. Arunachalam, E. Caspi, *J. Org. Chem.* **1981**, *46*, 3415–3420; e) J. L. Zhang, H. B. Zhou, J. S. Huang, C. M. Che, *Chem. Eur. J.* **2002**, *8*, 1554–1562; f) M. Salman, B. R. Reddy, P. Delgado, P. L. Stotter, L. C. Fulcher, G. C. Chamness, *Steroids* **1991**, *56*, 375–387; g) S. Top, H. El Hafa, A. Vessieres, J. Quivy, J. Vaissermann, D. W. Hughes, M. J. McGlinchey, J. P. Mornon, E. Thoreau, G. Jaouen, *J. Am. Chem. Soc.* **1995**, *117*, 8372–8380.

[11] a) T. L. Feviq, J. A. Katzenellenbogen, *J. Org. Chem.* **1987**, *52*, 247–251; b) M. R. Tremblay, S. Auger, D. Poirier, *Synth. Commun.* **1995**, *25*, 2483–2495; c) D. Poirier, R. P. Boivin, M. R. Tremblay, M. Berube, W. Qiu, S. X. Lin, *J. Med. Chem.* **2005**, *48*, 8134–8147.

[12] R. Maltais, M. R. Tremblay, D. Poirier, *J. Comb. Chem.* **2000**, *2*, 604–614.

[13] M. Chini, P. Crotti, F. J. Macchia, *J. Org. Chem.* **1991**, *56*, 5939–5942.

[14] a) R. Maltais, V. Luu-The, D. Poirier, *Bioorg. Med. Chem.* **2001**, *9*, 3001–3011; b) R. Maltais, V. Luu-The, D. Poirier, *J. Med. Chem.* **2002**, *45*, 640–653.
[15] a) J. Iqbal, A. Pandey, *Tetrahedron Lett.* **1990**, *31*, 575–576; b) G. Sekar, V. K. Singh, *J. Org. Chem.* **1999**, *64*, 287–289; c) L. R. Reddy, M. A. Reddy, N. Bhanumathi, K. R. Rao, *Synlett* **2000**, *3*, 339–340.
[16] D. Thibeault, D. Poirier, *Synlett* **2003**, *8*, 1192–1194.
[17] P. Bydal, K. M. Sam, D. Poirier, *Steroids* **1996**, *61*, 349–353.
[18] a) J. D. Pelletier, F. Labrie, D. Poirier, *Steroids* **1994**, *59*, 536–547; b) P. Dionne, D. Poirier, *Steroids* **1995**, *60*, 830–836.
[19] T. D. W. Claridge, *Tetrahedron Organic Chemistry Series Vol. 19: High-Resolution NMR Techniques in Organic Chemistry* (Eds.: J. E. Baldwin, R. M. Williams), Pergamon, New York, **1999**, pp. 382.
[20] CCDC-676120 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
[21] a) G. M. Allan, H. R. Lawrence, J. Cornet, C. Bubert, D. D. Fischer, N. Vicker, A. Smith, H. J. Tutill, A. Purohit, J. M. Day, M. F. Mahon, M. J. Reed, B. V. L. Potter, *J. Med. Chem.* **2006**, *49*, 1325–1345; b) N. Vicker, H. R. Lawrence, G. M. Allan, C. Bubert, A. Smith, H. J. Tutill, A. Purohit, J. M. Day, M. F. Mahon, M. J. Reed, B. V. L. Potter, *ChemMedChem* **2006**, *1*, 464–481; c) J. Messinger, H. H. Thole, B. Husen, B. J. Van Steen, G. Schneider, J. B. E. Hulshof, P. Koskimies, N. Johansson (Solvay pharmaceuticals GMBH), WO 2005/047303, **2005**; d) D. Poirier, R. Maltais, *Mini-Rev. Med. Chem.* **2006**, *6*, 37–52; e) R. Maltais, M. R. Tremblay, L. C. Ciobanu, D. Poirier, *J. Comb. Chem.* **2004**, *6*, 443–456.

Received: November 13, 2007
Published Online: March 25, 2008

