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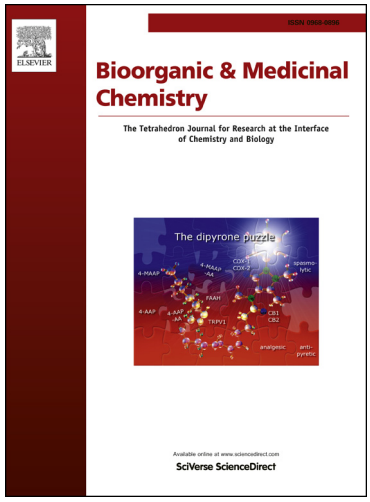

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Design, chemical synthesis and biological evaluation of 3-spiromorpholinone/3-spirocarbamate androsterone derivatives as inhibitors of 17β-hydroxysteroid dehydrogenase type 3

G. B. Djigoué; L. C. Kenmogne; J. Roy; R. Maltais; D. Poirier*

Laboratory of Medicinal Chemistry, CHU de Québec – Research Center (CHUL, T4)
and Laval UNiversity (Faculty of Medicine), Québec, Qc, Canada

* Address correspondence to this author to:

Laboratory of Medicinal Chemistry
CHU de Québec-Research Center (CHUL, T4-42)
2705 Laurier Boulevard, Québec, QC, G1V 4G2, Canada
Phone: (418) 654-2296
Fax: (418) 654-2298
E-mail: donald.poirier@crchul.ulaval.ca

Abstract

17β-Hydroxysteroid dehydrogenase type 3 (17β-HSD3) is a key enzyme involved in the biosynthesis of testosterone and dihydrotestosterone. These hormones are known to stimulate androgen-dependent prostate cancer. In order to generate effective inhibitors of androgen biosynthesis without androgenic effect, we synthesized a new family of 3-spiromorpholinone and 3-spirocarbamate androsterone derivatives bearing diversified hydrophobic groups. We also tested their inhibitory activity in a microsomal fraction of 17β-HSD3-containing rat testes, and their androgenic effect on androgen-sensitive LAPC-4 cells. From our first structure-activity relationship (SAR) study, we noted that compound **7e** inhibited 17β-HSD3 (77 % at 0.1 µM) compared to our reference compound RM-532-105 (76 % at 0.1 µM), but exhibited a residual androgenic effect. A library of **7e** analogues was next synthesized in order to generate compounds with reduced androgenic activity. In this new SAR study, the sulfonamide compound **7e21** and the carboxamide compound **7e22** inhibited 17β-HSD3 (IC_{50} = 28 and 88 nM, respectively). These two compounds were not androgenic and not cytotoxic even at the highest concentration tested, but their inhibitory activity decreased in intact LNCaP cells overexpressing 17β-HSD3 (LNCaP[17β-HSD3]). Structural modifications of these two lead compounds could however be tested to produce a second generation of 17β-HSD3 inhibitors.

Key words: Enzyme inhibitor, Androgen, Spiromorpholinone, Chemical synthesis, Prostate cancer, 17β-Hydroxysteroid dehydrogenase

Abbreviations: ADT: androsterone; AR: androgen receptor; Ar: aryl group; DBD: DNA binding domain; DCM: dichloromethane; DHT: dihydrotestosterone; DIPEA: N,N-diisopropylethylamine; DMF : dimethylformamide; DMSO: dimethylsulfoxide; Δ^4-dione: 4-androstene-3,17-dione; EtOAc: ethyl acetate; FBS: fetal bovine serum; 17β-HSD: 17β-hydroxysteroid dehydrogenase; IR: infrared spectroscopy; KO*t*-Bu: potassium *tert*-butylate; LDA: lithium diisopropylamide; LR-MS: low-resolution mass spectrometry; NADPH: nicotinamide adenosine dinucleotide phosphate reduced form; NaHMDS: sodium bis(trimethylsilyl)amide; NaOMe: sodium methoxide; NMO: *N*-methylmorpholine N-oxide; NMR: nuclear magnetic resonance; Pd(dppf)Cl_2: 1,1'-bis(diphenylphosphino)ferrocene-palladium(II)dichloride; Ph: phenyl group; R_f: Retardation or retention factor; RT: room temperature; SAR: structure-activity relationship; TLC: thin-layer chromatography; *p*-TSA: para-toluene sulfonic acid; T: testosterone; TEA: triethylamine; THF: tetrahydrofurane; TPAP: tetrapropylamonium perruthenate.

1. Introduction

Prostate cancer is the second leading cause of death in American men,[1] and androgens are well known to play an important role in the development, growth and progression of prostate cancer.[2-4] Among the enzymes involved in the biosynthesis of androgens in testes,[5-8] 17β-hydroxysteroid dehydrogenase type 3 (17β-HSD3) converts 4-androstene-3,17-dione (Δ^4-dione) into the potent and major circulating androgen testosterone (T), in the presence of cofactor NADPH.[9] Both T and its metabolite dihydrotestosterone (DHT) bind the androgen receptor (AR), which dimerizes, translocalizes in the nucleus and activates cell proliferation (Fig. 1). Androgen deprivation has been recognized to be one of the most efficient treatments for advanced stage prostate cancer.[10] In fact, up to 80 % of patients with metastatic prostate cancer respond to androgen deprivation treatments.[11] Although many endocrine therapies are now available to block either the formation of testicular T and the action of T and DHT on AR,[12-16] there are still some unmet medical needs for treatments using this kind of approach or for new ones. In fact, several side effects such as hot flushes, erectile dysfunction, decreased muscle mass, hypertension and hypokalemia are usually observed.[17,18] As an example, a recently approved cytochrome P450 17A1 (CYP17) inhibitor used for patients with metastatic castration-resistant prostate cancer, abiraterone acetate, affects the formation of both androgens and glucocorticoids, and replacement therapy is necessary when using this CYP17 inhibitor.[19] According to these observations, and given that 17β-HSD3 acts in the last step of the biosynthesis of T, we hope that the development of a potent inhibitor of 17β-HSD3 without an androgenic effect is a good therapeutic option for prostate cancer and other androgen-dependent diseases.

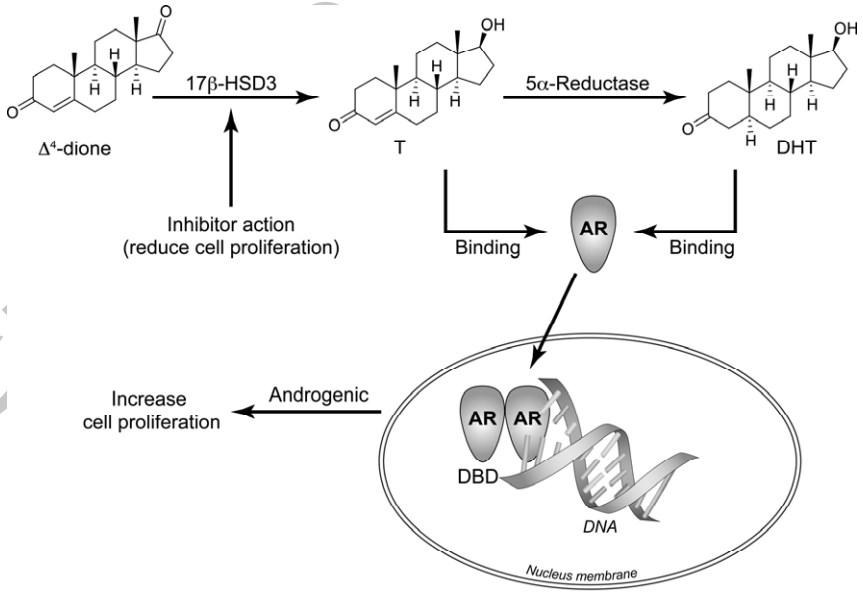


Figure 1: Biosynthesis of testosterone (T) and dihydrotestosterone (DHT) from 4-androstene-3,17-dione (Δ^4-dione) and their androgenic action. T and DHT bind to the androgen receptor (AR) that dimerizes and translocalizes in the cell nucleus, it then binds to the DNA binding domain (DBD) to co-activate cell proliferation.

From previous studies in our laboratory, androsterone (ADT) derivatives substituted at position C-3 were identified as new inhibitors of 17β-HSD3, and many important criteria for the inhibition of 17β-HSD3 were established.[20-27] Although these inhibitors showed strong blockage of the enzymatic activity, many of them produced an undesirable androgenic effect on Shionogi (AR^+) cells. In order to develop novel inhibitors of 17β-HSD3, we decided to build a new ring system (cycle E) at position 3 of the ADT nucleus. In fact, introducing a 3-spiroheterocyclic moiety is our strategy for adding rigidity and introducing diversified hydrophobic groups with different orientations in space. This will enable the exploration of the hydrophobic pocket identified from our studies as important for 17β-HSD3 inhibition. Preliminary reports have shown the impact of the stereochemistry on the carbon adjacent to the carbonyl of morpholinone.[28,29] We herein present full details of the chemical synthesis and characterization of new ADT derivatives substituted at position C-3 by a spiromorpholinone or a spirocarbamate; the assessment of their ability to block the transformation of Δ^4-dione to T by 17β-HSD3, and the evaluation of their androgenic effect on androgen-sensitive cells.

2. Material and methods

2.1. Chemical synthesis

Androsterone was purchased from Steraloids (Wilton, NH, USA). Benzyl bromide, benzyl bromide derivatives, 4-(trifluoromethyl)benzenesulfonyl chloride, allyl bromide and propargyl bromide were obtained from Sigma–Aldrich Canada Ltd. (Oakville, ON, Canada). Solvents were obtained from Fisher Scientific (Montréal, QC, Canada). 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 using 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 DCM and deposited upon a NaCl pellet. They were recorded with a Horizon MB 3000 ABB FTIR spectrometer (ABB, Canada) and only characteristic bands are reported. Nuclear magnetic resonance (NMR) spectra were recorded with a Bruker Avance 400 digital spectrometer (Billerica, MA, USA) and reported in ppm. The residual ^{1}H and ^{13}C NMR signals of chloroform (7.26 and 77.00 ppm respectively) and acetone (2.05 and 28.9 ppm, respectively) were used as internal references. Low-resolution mass spectra (LR-MS) were recorded on a Shimadzu prominence apparatus (Kyoto, Japan) equipped with a Shimadzu LCMS-2020. The name of compounds was generated using ACD/Labs (chemist version) software (Toronto, ON, Canada).

2.1.1. General procedure for the synthesis of 3a-3d

In a Schlenk tube, the oxirane **2**[26] (1.7 mmol) was dissolved in dry MeOH (15 mL) and the freed amino acid methyl ester[30] (17.2 mmol) was added. The Schlenk tube was screwed down hermetically, stirred and heated at 90 °C for 21 h. The MeOH was then evaporated, the crude reaction mixture dissolved in DCM and pre-adsorbed on silica gel, and then purified by flash column chromatography (hexanes/EtOAc/TEA, 89:10:1).

2.1.1.1. Methyl *N*-{[(3*R*,5*S*,8*R*,9*S*,10*S*,13*S*,14*S*)-3-hydroxy-10,13-dimethylhexadecahydrospiro [cyclopenta[*a*]phenanthrene-17,2'-[1,3]dioxolan]-3-yl]methyl}-L-leucinate (3a)

Amorphous solid (99 % yield). R_f = 0.6 (hexanes/EtOAc, 1:1). IR (film) ν 3479 and 3333 (OH and NH), 1736 (C=O) cm^{-1}. ^{1}H NMR (acetone-d_6) δ 0.74 (m, 1H), 0.78 (s, CH_3-19), 0.82

(s, 3H, CH_3-18), 0.91 and 0.92 (2d, J = 6.9 Hz, $(CH_3)_2$- from *i*Pr), 1.10-1.95 (unassigned CH and CH_2), 2.21 and 2.57 (2d of AB system, J = 11.6 Hz, CH_2N), 3.23 (dd, J_1 = 6.4 Hz, J_2 = 8.2 Hz, CHC=O), 3.67 (s, OCH_3), 3.83 (m, OCH_2CH_2O) ppm. ^{13}C NMR ($CDCl_3$) δ 11.2, 14.4, 20.3, 21.9, 22.6, 22.9, 24.8, 28.5, 30.7, 31.2, 31.7, 33.8, 34.2, 35.8, 36.0, 38.6, 40.6, 42.8, 46.0, 50.3, 51.7, 53.9, 59.3, 60.9, 64.5, 65.1, 69.9, 119.5, 176.3 ppm. LR-MS: calcd. for $C_{29}H_{50}NO_5$ $[M+H]^+$ 492.36, found 492.35.

2.1.1.2. Methyl *N*-{[(3R,5S,8R,9S,10S,13S,14S)-3-hydroxy-10,13-dimethylhexadecahydrospiro [cyclopenta[a]phenanthrene-17,2'-[1,3]dioxolan]-3-yl]methyl}-D-leucinate (3b)

Amorphous solid (99 % yield). R_f = 0.3 (hexanes/EtOAc, 1:1). IR (film) ν 3479 and 3333 (OH and NH), 1736 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.73 (s, CH_3-19), 0.82 (m, 1H), 0.83 (s, CH_3-18), 0.90 and 0.93 (2d, J = 6.6 Hz, $(CH_3)_2$- from *i*Pr), 0.96-2.00 (unassigned CH and CH_2), 2.17 and 2.62 (2d of AB system, J = 11.9 Hz, CH_2N), 3.23 ($t_{app.}$, J = 6.7 Hz, CHC=O), 3.72 (s, OCH_3), 3.87 (m, OCH_2CH_2O) ppm. ^{13}C NMR ($CDCl_3$) δ 11.2, 14.4, 20.3, 21.9, 22.6, 22.8, 24.8, 28.4, 30.7, 31.2, 31.5, 33.6, 34.1, 35.8, 35.9, 38.6, 40.6, 42.7, 45.9, 50.3, 51.6, 53.8, 59.3, 60.8, 64.5, 65.1, 69.9, 119.4, 176.3 ppm. LR-MS: calcd. for $C_{29}H_{50}NO_5$ $[M+H]^+$ 492.36, found 492.45.

2.1.1.3. Methyl *N*-{[(3R,5S,8R,9S,10S,13S,14S)-3-hydroxy-10,13-dimethylhexadecahydrospiro [cyclopenta[a]phenanthrene-17,2'-[1,3]dioxolan]-3-yl]methyl}-L-phenylalaninate (3c)

Amorphous solid (77 % yield). R_f = 0.5 (hexanes/EtOAc, 1:1). IR (film) ν 3472 and 3340 (OH and NH), 1736 (C=O) cm^{-1}. ^{1}H NMR (acetone-d_6) δ 0.72 (m, 1H), 0.75 (s, CH_3-19), 0.82 (s, CH_3-18), 0.95 (m, 1H), 1.12-1.97 (unassigned CH and CH_2), 2.24 and 2.56 (2d of AB system, J = 11.6 Hz, CH_2N), 2.92 (m, $\underline{CH_2}$-Ph), 3.45 (t, J = 6.6 Hz, CHC=O), 3.62 (s, OCH_3), 3.84 (m, OCH_2CH_2O), 7.25 (m, Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.2, 14.4, 20.3, 22.7, 28.5, 30.7, 31.2, 31.5, 33.7, 34.2, 35.8, 35.9, 38.5, 39.8, 40.5, 46.0, 50.3, 51.7, 53.8, 59.3, 63.9, 64.5, 65.1, 70.0, 119.5, 126.7, 128.4 (2C), 129.1 (2C), 137.3, 175.0 ppm. LR-MS: calcd. for $C_{32}H_{48}NO_5$ $[M+H]^+$ 526.35, found 526.50.

2.1.1.4. Methyl *N*-{[(3R,5S,8R,9S,10S,13S,14S)-3-hydroxy-10,13-dimethylhexadecahydrospiro [cyclopenta[a]phenanthrene-17,2'-[1,3]dioxolan]-3-yl]methyl}-D-phenylalaninate (3d)

Amorphous solid (87 % yield). R_f = 0.43 (hexanes/EtOAc, 1:1). IR (film) ν 3472 and 3340 (OH and NH), 1736 (C=O) cm^{-1}. ^{1}H NMR (acetone-d_6) δ 0.72 (m, 1H), 0.75 (s, CH_3-19), 0.82 (s, CH_3-18), 0.95 (m, 1H), 1.15-1.97 (unassigned CH and CH_2), 2.24 and 2.56 (2d of AB system, J = 11.6 Hz, CH_2N), 2.92 (m, $\underline{CH_2}$-Ph), 3.43 (t, J = 6.6 Hz, CHC=O), 3.62 (s, OCH_3), 3.84 (m, OCH_2CH_2O), 7.25 (m, Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.2, 14.4, 20.4, 22.7, 28.4, 30.7, 31.2, 31.5, 33.6, 34.2, 35.8, 35.9, 38.4, 39.8, 40.5, 45.9, 50.3, 51.7, 53.8, 59.3, 64.0, 64.5, 65.1, 70.0, 119.4, 126.7, 128.4 (2C), 129.1 (2C), 137.3, 175.0 ppm. LR-MS: calcd. for $C_{32}H_{48}NO_5$ $[M+H]^+$ 526.35, found 526.50.

2.1.2. Synthesis of methyl *N*-{[(3R,5S,8R,9S,10S,13S,14S)-3-hydroxy-10,13-dimethylhexadecahydrospiro[cyclopenta[a]phenanthrene-17,2'-[1,3]dioxolan]-3-yl]methyl}glycinate (3e)

To a mixture of glycine methyl ester hydrochloride (1.1 g, 8.8 mmol) and DIPEA (2.2 g, 17.5 mmol) was added anhydrous MeOH (20 mL) and the solution was stirred for 30 min in a Schlenk tube at room temperature. Oxirane **2**[26] (0.3 g, 0.9 mmol) was then added and the solution heated to 95 °C for 22 h. The solution was cooled to room temperature, filtered on a Büchner and the filtrate was evaporated. The crude mixture (1.8 g) was dissolved in DCM, adsorbed on silica gel (7 g) (dry-pack) and then eluted with a mixture of hexanes/EtOAc/TEA

(70:30:1) to give **3e**. Amorphous solid (53 % yield). R_f = 0.17 (hexanes/EtOAc, 3:7); IR (film) ν 3464 and 3348 (OH and NH), 1744 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.74 (s, CH_3-19), 0.83 (s, CH_3-18), 0.85-1.97 (unassigned CH and CH_2), 2.50 (s, CH_2N), 3.45 (s, $NCH_2C{=}O$), 3.73 (s, OCH_3), 3.83-3.95 (m, OCH_2CH_2O) ppm. ^{13}C NMR (acetone-d_6) δ 10.8, 13.9, 20.3, 22.4, 30.7, 31.4, 31.5, 33.3, 33.7, 33.9, 35.8, 35.9, 38.5, 40.5, 45.8, 50.3, 50.7, 51.2, 54.4, 61.1, 64.2, 64.8, 70.0, 118.9, 172.7 ppm. LR-MS: calcd. for $C_{25}H_{41}NO_5$ $[M+H]^+$ 436.30, found 436.35.

2.1.3. Synthesis of the spiromorpholinones 4a-4e

To a solution of MeONa (0.37 mmol) in anhydrous THF (24 mL) at 0 °C was added a solution of the amino alcohol (**3a-3e)** (0.61 mmol) in dry THF (28 mL) and under an argon atmosphere. The solution was stirred for 2 h at room temperature and the reaction mixture was quenched with a saturated ammonium chloride aqueous solution. The crude product was extracted four times with EtOAc and the organic layer was dried with anhydrous $MgSO_4$, filtered over vacuum and dried under reduced pressure. The residue was adsorbed on silica gel and purified by flash column chromatography with a mixture of hexanes/EtOAc/TEA (80:20:1).

2.1.3.1. (3'R,5'S,5''S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-5''-(2-methylpropyl) tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (4a)

Amorphous solid (89 % yield). R_f = 0.21 (hexanes/EtOAc, 8:2). IR (film) ν 3340 (NH) and 1720 (C=O) cm^{-1}. ^1H NMR (acetone-d_6) δ 0.79 (m, 1H), 0.83 (s, CH_3-19 and CH_3-18), 0.89 and 0.92 (2d, J = 6.6 Hz, $(CH_3)_2$- from *i*Pr), 0.95-1.98 (unassigned CH and CH_2), 2.83 and 2.93 (2d of AB system, J = 13.5 Hz, CH_2N), 3.43 (dd, J_1 = 4.0 Hz, J_2 = 9.6 Hz, NCHC=O), 3.85 (m, OCH_2CH_2O) ppm. ^{13}C NMR ($CDCl_3$) δ 11.3, 14.4, 20.3, 21.0, 22.6, 23.4, 24.4, 28.1, 30.6, 31.5, 32.9, 34.1, 35.7, 36.0, 38.2, 39.2, 39.6, 41.4, 45.9, 50.1, 52.5, 53.3, 55.5, 64.5, 65.1, 82.3, 119.4, 171.9 ppm. LR-MS: calcd. for $C_{28}H_{46}NO_4$ $[M+H]^+$ 460.33, found 460.45.

2.1.3.2. (3'R,5'S,5''R,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-5''-(2-methylpropyl) tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (4b)

Amorphous solid (84 % yield). R_f = 0.23 (hexanes/EtOAc, 8:2). IR (film) ν 3340 (NH) and 1720 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.76 (s, CH_3-19), 0.83 (s, CH_3-18), 0.92 and 0.96 (2d, J = 6.3 Hz, $(CH_3)_2$- from *i*Pr), 0.80-1.99 (unassigned CH and CH_2), 2.80 and 2.88 (2d of AB system, J = 13.5 Hz, CH_2N), 3.49 (dd, J_1 = 3.5 Hz, J_2 = 10.0 Hz, NCHC=O), 3.89 (m, OCH_2CH_2O) ppm. ^{13}C NMR ($CDCl_3$) δ 11.3, 14.4, 20.3, 20.9, 22.7, 23.4, 24.5, 28.2, 30.6, 31.5, 32.6, 34.2, 35.7, 36.0, 38.3, 39.3, 39.7, 41.5, 45.9, 50.1, 52.6, 53.4, 55.5, 64.5, 65.1, 82.3, 119.4, 171.9 ppm. LR-MS: calcd. for $C_{28}H_{46}NO_4$ $[M+H]^+$ 460.33, found 460.45.

2.1.3.3. (3'R,5'S,5''S,8'R,9'S,10'S,13'S,14'S)-5''-benzyl-10',13'-dimethyltetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (4c)

Amorphous solid (62 % yield). R_f = 0.15 (hexanes/EtOAc, 8:2). IR (film) ν 3340 (NH) and 1720 (C=O) cm^{-1}. ^1H NMR (acetone-d_6) δ 0.75 (m, 1H), 0.78 (s, CH_3-19), 0.82 (s, CH_3-18), 0.90-1.99 (unassigned CH and CH_2), 2.93 (m, CH_2N), 3.05 and 3.17 (2m, 2H, $\underline{CH_2}Ph$), 3.72 (dd, J_1 = 3.9 Hz, J_2 = 8.1 Hz, NCHC=O), 3.84 (m, OCH_2CH_2O), 7.22 (m, 1H of Ph), 7.29 (m, 4H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.3, 14.4, 20.3, 22.6, 28.0, 30.6, 30.9, 31.1, 32.9, 34.2,

35.7, 35.9, 38.0 (2C), 39.2, 45.9, 50.1, 52.7, 53.3, 58.7, 64.5, 65.1, 82.8, 119.4, 127.0, 128.7 (2C), 129.5 (2C), 137.2, 170.8 ppm. LR-MS: calcd. for $C_{31}H_{44}NO_4$ $[M+H]^+$ 494.32, found 494.45.

2.1.3.4. (3'R,5'S,5''R,8'R,9'S,10'S,13'S,14'S)-5''-benzyl-10',13'-dimethyltetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (4d)

Amorphous solid (65 % yield). R_f = 0.09 (hexanes/EtOAc 8:2). IR (film) ν 3340 (NH) and 1720 (C=O) cm^{-1}. ^{1}H NMR (acetone-d_6) δ 0.77 (s, CH_3-19), 0.82 (s, CH_3-18), 0.85 (m, 1H), 0.88-1.98 (unassigned CH and CH_2), 2.85 (m, CH_2N), 3.05 and 3.15 (2m, $\underline{CH_2}Ph$), 3.74 (dd, J_1= 4.1 Hz, J_2 = 7.6 Hz, NCHC=O), 3.84 (m, OCH_2CH_2O), 7.24 (m, 1H of Ph), 7.27 (m, 4H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.3, 14.4, 20.3, 22.7, 28.0, 30.6, 31.0, 31.3, 32.5, 34.2, 35.7, 35.9, 37.7, 37.9, 39.6, 45.9, 50.1, 52.7, 53.3, 58.7, 64.5, 65.1, 82.7, 119.4, 127.1, 128.8 (2C), 1.6 (2C), 137.2, 170.8 ppm. LR-MS: calcd. for $C_{31}H_{44}NO_4$ $[M+H]^+$ 494.32, found 494.50.

2.1.3.5. (3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyltetradecahydro-2'H,6''H-dispiro [1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (4e)

Amorphous solid (52 % yield). R_f = 0.16 (hexanes/EtOAc, 3:7). IR (film) ν 3340 (NH) and 1728 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.78 (s, CH_3-19), 0.83 (s, CH_3-18), 0.84-1.99 (unassigned CH and CH_2), 2.82 (s, CH_2N), 3.63 (s, NCH_2C=O), 3.90 (m, OCH_2-CH_2O) ppm. ^{13}C NMR ($CDCl_3$) δ 11.3, 14.4, 20.4, 22.7, 28.1, 30.6, 31.0, 31.3, 32.7, 34.2, 35.7, 36.0, 38.0, 39.4, 45.9, 47.6, 50.1, 53.1, 53.4, 64.6, 65.2, 82.5, 119.4, 168.8 ppm. LR-MS: calcd. for $C_{24}H_{38}NO_4$ $[M+H]^+$ 404.27, found 404.35.

2.1.4. Synthesis of *N*-substituted spiromorpholinones 6a-6e and 6e1-6e22

To a solution of **4a-4e** (0.1 mmol) in dry DCM (5 mL) was added drop-wise DIPEA (0.17 mmol) in a Schlenk tube. The tube was screwed down, stirred and heated at 75 °C for 10 min, and the reaction mixture was cooled to room temperature and benzyl bromide (1.7 mmol) was added to the solution. The reaction mixture was stirred and heated at 75 °C for 48 h. After cooling the mixture, silica gel was added to the crude mixture, the solvent was evaporated under reduced pressure and the residue was purified by flash chromatography with a mixture of hexanes/EtOAc/TEA (95:5:1).

2.1.4.1. (3'R,5'S,5''S,8'R,9'S,10'S,13'S,14'S)-4''-benzyl-10',13'-dimethyl-5''-(2-methyl propyl)tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6a)

Amorphous solid (63 % yield). R_f = 0.83 (hexanes/EtOAc, 1:1). ^{1}H NMR (acetone-d_6) δ 0.74 (s, CH_3-19), 0.81 (s, CH_3-18), 0.93 and 0.98 (2d, J = 6.7 Hz, $(CH_3)_2$- from *i*Pr), 1.00-2.00 (unassigned CH and CH_2), 2.27 and 2.74 (2d of AB system, J = 12.5 Hz, CH_2N), 3.17 (dd, J_1 = 2.7 Hz, J_2 = 7.0 Hz, NCHC=O), 3.23 and 4.07 (2d of AB system, J = 13.7 Hz, $N\underline{CH_2}$-Ph), 3.83 (m, OCH_2CH_2O), 7.28 (m, 1H of Ph), 7.37 (m, 4H of Ph) ppm.

2.1.4.2. (3'R,5'S,5''R,8'R,9'S,10'S,13'S,14'S)-4''-benzyl-10',13'-dimethyl-5''-(2-methyl propyl)tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6b)

Amorphous solid (49 % yield). R_f = 0.83 (hexanes/EtOAc, 1:1). ^{1}H NMR ($CDCl_3$) δ 0.68 (s, CH_3-19), 0.82 (s, CH_3-18), 0.95 and 0.99 (2d, J = 6.7 Hz, $(CH_3)_2$- from *i*Pr), 0.80-2.11 (unassigned CH and CH_2), 2.16 and 2.64 (2d of AB system, J = 12.4 Hz, CH_2N), 3.12 and 4.02 (2d of AB system, J = 13.5 Hz, N$\underline{CH_2}$-Ph), 3.18 (dd, J_1 = 2.8 Hz, J_2 = 7.0 Hz, NCHC=O), 3.89 (m, OCH_2CH_2O), 7.33 (m, Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.1, 22.7, 23.9, 25.0, 28.3, 30.7, 31.1, 32.0, 32.7, 34.2, 35.7, 36.0, 38.3, 38.6, 39.8, 45.9, 50.1, 53.4, 57.8, 58.2, 63.0, 64.6, 65.1, 81.3, 119.4, 127.4, 128.5 (4C), 137.8, 171.4 ppm.

2.1.4.3. (3'R,5'S,5''S,8'R,9'S,10'S,13'S,14'S)-4'',5''-dibenzyl-10',13'-dimethyltetra deca hydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6c)

Amorphous solid (70 % yield). R_f = 0.75 (hexanes/EtOAc, 1:1). ^{1}H NMR (acetone-d_6) δ 0.62 (s, CH_3-19), 0.68 (m, 1H), 0.79 (s, CH_3-18), 0.88-1.95 (unassigned CH and CH_2), 2.21 and 2.58 (2d of AB system, J = 12.5 Hz, CH_2N), 3.28 and 4.39 (2d of AB system, J = 13.8 Hz, N$\underline{CH_2}$-Ph), 3.36 (m, $\underline{CH_2}$Ph), 3.51 (dd, J_1 = 3.0 Hz, J_2 = 5.1 Hz, NCHC=O), 3.85 (m, OCH_2CH_2O), 7.33 (m, Ph) ppm. ^{13}C NMR (acetone-d_6) δ 10.7, 13.9, 20.2, 22.4, 27.9, 29.5, 30.6, 31.2, 33.1, 33.9, 35.2, 35.7, 37.9, 39.5, 45.7, 50.2, 54.0, 57.5, 57.7, 61.0, 64.2, 64.8, 65.9, 80.5, 118.8, 126.4, 127.1, 127.8 (2C), 128.3 (2C), 128.5 (2C), 130.4 (2C), 138.0, 138.1, 169.5 ppm.

2.1.4.4. (3'R,5'S,5''R,8'R,9'S,10'S,13'S,14'S)-4'',5''-dibenzyl-10',13'-dimethyltetra deca hydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6d)

Amorphous solid (61 % yield). R_f = 0.75 (hexanes/EtOAc, 1:1). ^{1}H NMR (acetone-d_6) δ 0.62 (s, CH_3-19), 0.68 (m, 1H), 0.78 (s, CH_3-18), 0.80-1.97 (unassigned CH and CH_2), 2.21 and 2.59 (2d of AB system, J = 12.4 Hz, CH_2N), 3.28 and 4.41 (2d of AB system, J = 13.8 Hz, N$\underline{CH_2}$-Ph), 3.38 (m, $\underline{CH_2}$Ph), 3.50 (dd, J_1 = 3.0 Hz, J_2 = 5.1 Hz, NCHC=O), 3.83 (m, OCH_2CH_2O), 7.30 (m, Ph) ppm. ^{13}CNMR (acetone-d_6) δ 10.8, 13.9, 20.2, 22.4, 27.6, 29.5, 30.6, 31.1, 31.2, 32.7, 33.9, 35.0, 35.7, 37.3, 39.9, 45.7, 50.2, 54.1, 57.5, 57.8, 64.2, 64.8, 65.8, 80.5, 118.8, 126.5, 127.1, 127.8 (2C), 128.4 (2C), 128.6 (2C), 130.5 (2C), 137.9, 138.1, 169.4 ppm.

2.1.4.5. (3'R,5'S,8'R,9'S,10'S,13'S,14'S)-4''-benzyl-10',13'-dimethyltetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6e)

Amorphous solid (70 % yield). R_f = 0.83 (hexanes/EtOAc, 1:1). ^{1}H NMR ($CDCl_3$) δ 0.72 (s, CH_3-19), 0.82 (s, CH_3-18), 0.80-1.99 (unassigned CH and CH_2), 2.41 (s-broad, CH_2N), 3.26 (s, NCH_2C=O), 3.50 and 3.54 (2d of AB system, J = 13.3 Hz, N$\underline{CH_2}$Ph), 3.89 (m, OCH_2CH_2O), 7.31 (m, Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.7, 28.1, 30.7, 31.0, 31.8, 32.9, 34.2, 35.7, 36.0, 38.4, 39.6, 45.9, 50.1, 53.4, 55.4, 59.8, 61.4, 64.6, 65.1, 82.5, 119.4, 127.6, 128.5 (2C), 128.7 (2C), 136.7, 168.3 ppm.

2.1.4.6. (3'R,5'S,8'R,9'S,10'S,13'S,14'S)-4''-(4-bromobenzyl)-10',13'-dimethyltetradeca hydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6e1)

Amorphous solid (87 % yield). R_f = 0.7 (hexanes/EtOAc, 1:1). IR (film) ν 1724 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.73 (s, CH_3-19), 0.83 (s, CH_3-18), 0.80-2.00 (unassigned CH and CH_2),

2.39 (s, CH_2N), 3.26 (s, $NCH_2C{=}O$), 3.46 (s, $\underline{CH_2}$-Ph), 3.89 (m, OCH_2CH_2O), 7.19 (d, J = 8.3 Hz, 2H of Ph), 7.47 (d, J = 8.3 Hz, 2H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.7, 28.1, 30.6, 31.0, 31.8, 32.9, 34.2, 35.7, 36.0, 38.4, 39.6, 45.9, 50.1, 53.4, 55.4, 59.7, 60.6, 64.5, 65.1, 82.4, 119.4, 121.5, 130.3 (2C), 131.7 (2C), 135.8, 167.9 ppm. LR-MS: calcd. for $C_{31}H_{43}{}^{79}BrNO_4$ $[M+H]^+$ 572.23, found 572.35.

2.1.4.7. (3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-[4-(trifluoromethoxy) benzyl]tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6e2)

Amorphous solid (95 % yield). R_f = 0.6 (hexanes/EtOAc, 1:1). IR (film) ν 1732 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.74 (s, CH_3-19), 0.83 (s, CH_3-18), 0.80-1.99 (unassigned CH and CH_2), 2.43 (s, CH_2N), 3.25 (s, $NCH_2C{=}O$), 3.51 (d, J = 2.4 Hz, $\underline{CH_2}$-Ph), 3.89 (m, OCH_2CH_2O), 7.19 (d, J = 8.0 Hz, 2H of Ph), 7.34 (d, J = 8.6 Hz, 2H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.6, 28.1, 30.6, 31.0, 31.8, 32.9, 34.2, 35.7, 36.0, 38.4, 39.6, 45.9, 50.1, 53.4, 55.3, 59.8, 60.5, 64.5, 65.1, 82.4, 119.4, 120.4 (d, $J_{C\text{-}F}$ = 257.1 Hz), 121.0 (2C), 129.9 (2C), 135.5, 148.6, 167.9 ppm. LR-MS: calcd. for $C_{32}H_{43}F_3NO_5$ $[M+H]^+$ 578.30, found 578.40.

2.1.4.8. (3'R,5'S,8'R,9'S,10'S,13'S,14'S)-4''-(biphenyl-4-ylmethyl)-10',13'-dimethyltetra decahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6e3)

Amorphous solid (91 % yield). R_f = 0.6 (hexanes/EtOAc, 1:1). IR (film) ν 1728 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.73 (s, CH_3-19), 0.83 (s, CH_3-18), 0.82-1.99 (unassigned CH and CH_2), 2.46 (s, CH_2N), 3.30 (s, $NCH_2C{=}O$), 3.56 (d, J = 2.2 Hz, $\underline{CH_2}$-Ph), 3.89 (m, OCH_2CH_2O), 7.36 (m, 3H of Ar), 7.45 (m, 2H of Ar), 7.59 (m, 4H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.4, 22.7, 28.1, 30.7, 31.0, 31.9, 32.9, 34.2, 35.8, 36.1, 38.5, 39.6, 46.0, 50.2, 53.4, 55.5, 59.8, 61.0, 64.6, 65.1, 82.5, 119.4, 127.0 (2C), 127.3 (2C), 127.4, 128.8 (2C), 129.1 (2C), 135.7, 140.5, 140.7, 168.2 ppm. LR-MS: calcd. for $C_{37}H_{48}NO_4$ $[M+H]^+$ 570.35, found 570.45.

2.1.4.9. (3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-(2-phenylethyl)tetradeca hydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6e4)

Amorphous solid (41 % yield). R_f = 0.7 (hexanes/EtOAc, 1:1). IR (film) ν 1732 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.77 (s, CH_3-19), 0.83 (s, CH_3-18), 0.75-2.05 (unassigned CH and CH_2), 2.50 (s, CH_2N), 2.61 ($t_{app.}$, J = 7.5 Hz, $\underline{CH_2}$-Ph), 2.78 ($t_{app.}$, J = 7.6 Hz, $N\underline{CH_2}CH_2Ph$), 3.29 (s, $NCH_2C{=}O$), 3.89 (m, OCH_2CH_2O), 7.20 (m, 3H of Ph), 7.28 (m, 2H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.4, 22.7, 28.1, 30.6, 31.0, 31.8, 32.9, 33.2, 34.2, 35.8, 36.0, 38.5, 39.6, 45.9, 50.2, 53.4, 55.3, 58.6, 60.4, 64.6, 65.1, 82.3, 119.4, 126.3, 128.4 (2C), 128.6 (2C), 139.5, 168.3 ppm. LR-MS: calcd. for $C_{32}H_{46}NO_4$ $[M+H]^+$ 508.33, found 508.45.

2.1.4.10. (3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-(3-phenylpropyl)tetradeca hydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6e5)

Amorphous solid (73 % yield). R_f = 0.9 (hexanes/EtOAc, 1:1). IR (film) ν 1732 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.78 (s, CH_3-19), 0.83 (s, CH_3-18), 0.80-2.00 (unassigned CH and CH_2), 2.34 (t, J = 7.0 Hz, $N\underline{CH_2}CH_2CH_2Ph$), 2.43 (s, CH_2N), 2.66 (t, J = 7.5 Hz, $NCH_2CH_2\underline{CH_2}Ph$), 3.21 (s, $NCH_2C{=}O$), 3.89 (m, OCH_2CH_2O), 7.18 (m, 3H of Ph), 7.28 (m, 2H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.4, 22.7, 28.1, 28.2, 30.7, 31.0, 31.9, 32.9, 33.0, 34.2, 35.8,

36.1, 38.6, 39.6, 46.0, 50.2, 53.4, 55.5, 56.0, 60.3, 64.6, 65.1, 82.3, 119.4, 125.9, 128.4 (4C), 141.7, 168.4 ppm. LR-MS: calcd. for $C_{33}H_{48}NO_4$ $[M+H]^+$ 522.35, found 522.45.

2.1.4.11. (3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-{2-[4-(trifluoromethyl) phenyl]ethyl}tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a] phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6e6)

Amorphous solid (53 % yield). R_f = 0.7 (hexanes/EtOAc, 1:1). IR (film) ν 1732 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.79 (s, CH_3-19), 0.83 (s, CH_3-18), 0.80-2.00 (unassigned CH and CH_2), 2.49 (s, CH_2N), 2.63 (t, J = 7.3 Hz, $\underline{CH_2}$-Ph), 2.83 (t, J = 7.3 Hz, $N\underline{CH_2}CH_2Ph$), 3.28 (d, J = 1.5 Hz, $NCH_2C{=}O$), 3.89 (m, OCH_2CH_2O), 7.30 (d, J = 8.0 Hz, 2H of Ph), 7.54 (d, J = 8.1 Hz, 2H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.6, 28.1, 30.6, 30.6, 31.0, 31.8, 32.9, 33.0, 34.2, 35.7, 36.0, 38.5, 39.6, 45.9, 50.1, 53.4, 55.2, 60.4, 64.5, 65.1, 82.3, 119.4, 124.2 (q, $J_{C\text{-}F}$ = 272.0 Hz), 125.3 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 3.6 Hz) (2C), 128.7 ($J_{C\text{-}C\text{-}F}$ = 32.5 Hz), 129.0 (2C), 143.6, 168.0 ppm. LR-MS: calcd. for $C_{33}H_{45}F_3NO_4$ $[M+H]^+$ 576.32, found 576.40.

2.1.4.12. (3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-[4-(trifluoromethyl)benzyl] tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6e7)

Amorphous solid (85 % yield). R_f = 0.7 (hexanes/EtOAc, 1:1). IR (film) ν 1732 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.73 (s, CH_3-19), 0.83 (s, CH_3-18), 0.82-2.00 (unassigned CH and CH_2), 2.42 (s, CH_2N), 3.28 (s, $NCH_2C{=}O$), 3.57 (d, J = 2.7 Hz, $\underline{CH_2}$-Ph), 3.89 (m, OCH_2CH_2O), 7.44 (d, J = 8.0 Hz, 2H of Ph), 7.61 (d, J = 8.1 Hz, 2H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.6, 28.1, 30.6, 31.0, 31.9, 32.9, 34.2, 35.7, 36.0, 38.4, 39.6, 45.9, 50.1, 53.4, 55.4, 59.8, 60.8, 64.5, 65.1, 82.4, 119.4, 124.1 (q, $J_{C\text{-}F}$ = 272.0 Hz), 125.5 ($J_{C\text{-}C\text{-}C\text{-}F}$ = 3.8 Hz) (2C), 128.8 (2C), 130.0 ($J_{C\text{-}C\text{-}F}$ = 32.4 Hz), 140.9, 167.8 ppm. LR-MS: calcd. for $C_{32}H_{43}F_3NO_4$ $[M+H]^+$ 562.31, found 562.40.

2.1.4.13. (3'R,5'S,8'R,9'S,10'S,13'S,14'S)-4''-[4-(benzyloxy)benzyl]-10',13'-dimethyltetra decahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6e8)

Amorphous solid (70 % yield). R_f = 0.6 (hexanes/EtOAc, 1:1). IR (film) ν 1732 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.73 (s, CH_3-19), 0.83 (s, CH_3-18), 0.82-2.00 (unassigned CH and CH_2), 2.40 (s, CH_2N), 3.24 (s, $NCH_2C{=}O$), 3.45 (d, J = 2.6 Hz, $\underline{CH_2}$-Ph), 3.89 (m, OCH_2CH_2O), 5.07 (s, CH_2O), 6.95 (d, J = 8.6 Hz, 2H of Ph), 7.21 (d, J = 8.6 Hz, 2H of Ph), 7.39 (m, 5H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.7, 28.1, 30.7, 31.0, 31.9, 32.9, 34.2, 35.8, 36.0, 38.4, 39.6, 46.0, 50.1, 53.4, 55.4, 59.7, 60.7, 64.6, 65.1, 70.0, 82.5, 114.8 (2C), 119.4, 127.5 (2C), 128.0, 128.6 (2C), 128.9, 129.9 (2C), 136.9, 158.3, 168.3 ppm. LR-MS: calcd. for $C_{38}H_{50}NO_5$ $[M+H]^+$ 600.36, found 600.45.

2.1.4.14. (3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-(4-phenylbutyl)tetradeca hydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6e9)

Amorphous solid (69 % yield). R_f = 0.8 (hexanes/EtOAc, 1:1). IR (film) ν 1732 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.77 (s, CH_3-19), 0.83 (s, CH_3-18), 0.82-2.00 (unassigned CH and CH_2), 2.34 (t, J = 7.0 Hz, $N\underline{CH_2}CH_2$), 2.38 (s, CH_2N), 2.62 (t, J = 7.6 Hz, $CH_2\underline{CH_2}Ph$), 3.18 (s, $NCH_2C{=}O$), 3.89 (m, OCH_2CH_2O), 7.18 (m, 3H of Ph), 7.28 (m, 2H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.4, 22.7, 25.9, 28.1, 28.8, 30.7, 31.0, 31.9, 32.9, 34.2, 35.6, 35.8, 36.0, 38.5, 39.6, 46.0, 50.2, 53.4, 55.5, 56.7, 60.4, 64.6, 65.1, 82.3, 119.4, 125.8, 128.3 (2C), 128.4 (2C), 142.2, 168.5 ppm. LR-MS: calcd. for $C_{34}H_{50}NO_4$ $[M+H]^+$ 536.37, found 536.45.

2.1.4.15. (3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-[2-(trifluoromethyl)benzyl] tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6e10)

Amorphous solid (57 % yield). R_f = 0.8 (hexanes/EtOAc, 1:1). IR (film) ν 1732 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.73 (s, CH_3-19), 0.82 (s, CH_3-18), 0.80-1.98 (unassigned CH and CH_2), 2.48 (s, CH_2N), 3.28 (d, J = 2.5 Hz, NCH_2C=O), 3.67 (s, $\underline{CH_2}$-Ph), 3.88 (m, OCH_2CH_2O), 7.39 (t_{app}, J = 7.6 Hz, 1H of Ar), 7.55 (t, J = 7.5 Hz, 1H of Ar), 7.68 (m, 2H of Ar) ppm. ^{13}CNMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.7, 28.1, 30.6, 31.0, 31.8, 32.9, 34.2, 35.7, 36.0, 38.4, 39.5, 45.9, 50.1, 53.4, 55.3, 57.1, 60.1, 64.6, 65.1, 82.4, 119.4, 124.3 (q, J_{C-F} = 273.9 Hz), 126.1 (q, $J_{C-C-C-F}$ = 5.8 Hz), 127.6, 128.9 (q, J_{C-C-F} = 32.8 Hz), 130.4, 132.0, 135.7, 167.9 ppm. LR-MS: calcd. for $C_{32}H_{43}F_3NO_4$ $[M+H]^+$ 562.31, found 562.40.

2.1.4.16. (3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-(naphthalen-2-ylmethyl) tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4] oxazinan]-6''-one (6e11)

Amorphous solid (74 % yield). R_f = 0.7 (hexanes/EtOAc, 1:1). IR (film) ν 1732 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.69 (s, CH_3-19), 0.82 (s, CH_3-18), 0.80-1.99 (unassigned CH and CH_2), 2.44 (s, CH_2N), 3.33 (s, NCH_2C=O), 3.67 (d, J = 2.8 Hz, $\underline{CH_2}$-Ph), 3.89 (m, OCH_2CH_2O), 7.48 (m, 3H), 7.72 (s, 1H of Ar), 7.83 (m, 3H of Ar) ppm. LR-MS: calcd. for $C_{35}H_{46}NO_4$ $[M+H]^+$ 544.33, found 544.45.

2.1.4.17. (3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-[3-(trifluoromethyl)benzyl] tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6e12)

Amorphous solid (92 % yield). R_f = 0.7 (hexanes/EtOAc, 1:1). IR (film) ν 1732 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.73 (s, CH_3-19), 0.83 (s, CH_3-18), 0.85-2.00 (unassigned CH and CH_2), 2.43 (s, CH_2N), 3.29 (s, NCH_2C=O), 3.57 (s, $\underline{CH_2}$-Ph), 3.89 (m, OCH_2CH_2O), 7.52 (m, 4H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.6, 28.1, 30.6, 31.0, 31.8, 32.9, 34.2, 35.7, 36.0, 38.4, 39.6, 45.9, 50.1, 53.4, 55.4, 59.8, 60.7, 64.5, 65.1, 82.4, 119.4, 124.0 (q, J_{C-F} = 272.3 Hz), 124.5 (q, $J_{C-C-C-F}$ = 3.6 Hz), 125.3 (q, $J_{C-C-C-F}$ = 3.7 Hz), 129.0, 131.0 (q, J_{C-C-F} = 32.3 Hz), 131.9, 137.9, 167.8 ppm. LR-MS: calcd. for $C_{32}H_{43}F_3NO_4$ $[M+H]^+$ 562.31, found 562.35.

2.1.4.18. (3'R,5'S,8'R,9'S,10'S,13'S,14'S)-4''-[3,5-bis(trifluoromethyl)benzyl]-10',13'-dimethyltetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenan threne-3',2''-[1,4]oxazinan]-6''-one (6e13)

Amorphous solid (88 % yield). R_f = 0.8 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.73 (s, CH_3-19), 0.83 (s, CH_3-18), 0.85-2.00 (unassigned CH and CH_2), 2.43 (s, CH_2N), 3.33 (s, NCH_2C=O), 3.65 (s, $\underline{CH_2}$-Ph), 3.89 (m, OCH_2CH_2O), 7.82 (s, 3H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.3, 14.4, 20.3, 22.6, 28.0, 30.6, 31.0, 31.7, 32.8, 34.2, 35.7, 36.0, 38.5, 39.5, 45.9, 50.1, 53.4, 55.3, 59.7, 60.0, 64.5, 65.1, 82.4, 119.4, 121.7 (q, $J_{C-C-C-F}$ = 3.7 Hz), 123.2 (q, J_{C-F} = 272.3 Hz) (2C), 128.5 (2C), 132.0 (q, J_{C-C-F} = 33.6 Hz) (2C), 139.8, 167.3 ppm. LR-MS: calcd. for $C_{33}H_{42}F_6NO_4$ $[M+H]^+$ 630.29, found 630.30.

2.1.4.19. (3'R,5'S,8'R,9'S,10'S,13'S,14'S)-4''-[2,4-bis(trifluoromethyl)benzyl]-10',13'-dimethyltetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenan threne-3',2''-[1,4]oxazinan]-6''-one (6e14)

Amorphous solid (71 % yield). R_f = 0.8 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.74 (s, CH_3-19), 0.83 (s, CH_3-18), 0.82-2.02 (unassigned CH and CH_2), 2.49 (s, CH_2N), 3.32 (d, J = 2.0 Hz, NCH_2C=O), 3.73 (s, $\underline{CH_2}$-Ph), 3.89 (m, OCH_2CH_2O), 7.83 (d, J = 8.2 Hz, 1H of Ar), 7.92 (d, J = 7.5 Hz, 2H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.6, 28.1, 30.6, 31.0, 31.9, 32.8, 34.2, 35.7, 36.0, 38.4, 39.5, 45.9, 50.1, 53.4, 55.3, 56.6, 60.1, 64.5, 65.1, 82.3, 119.4, 121.9, 123.3 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = ~3 Hz), 123.4 (q, $J_{C\text{-}F}$ = 274.1 Hz) (2C), 128.8 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 3.2 Hz), 129.5 (q, $J_{C\text{-}C\text{-}F}$ = 31.1 Hz), 132.2 (q, $J_{C\text{-}C\text{-}F}$ = ~32.8 Hz), 140.2, 167.4 ppm. LR-MS: calcd. for $C_{33}H_{42}F_6NO_4$ $[M+H]^+$ 630.29, found 630.30.

2.1.4.20. (3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-(naphthalen-1-ylmethyl) tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6e15)

Amorphous solid (89 % yield). R_f = 0.7 (hexanes/EtOAc, 1:1). IR (film) ν 1732 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.68 (s, CH_3-19), 0.81 (s, CH_3-18), 0.80-2.05 (unassigned CH and CH_2), 2.49 and 2.54 (2d of AB system, J = 12.1 Hz, CH_2N), 3.25 and 3.32 (2d of AB system, J = 17.4 Hz, NCH_2C=O), 3.89 (m, OCH_2CH_2O), 3.93 (d, J = 3.5 Hz, $\underline{CH_2}$-Ph), 7.40 (m, 2H of Ar), 7.51 (m, 2H of Ar), 7.84 (m, 2H of Ar), 8.23 (m, 2H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.7, 28.1, 30.6, 31.0, 31.8, 32.9, 34.2, 35.7, 36.0, 38.4, 39.5, 45.9, 50.1, 53.4, 55.4, 60.1, 60.1, 64.5, 65.1, 82.5, 119.4, 124.6, 125.1, 125.9 (2C), 127.6, 128.5, 128.7, 132.1, 132.2, 133.9, 168.2 ppm. LR-MS: calcd. for $C_{35}H_{46}NO_4$ $[M+H]^+$ 544.33, found 544.40.

2.1.4.21. (3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-(prop-2-en-1-yl)tetradeca hydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6e16)

Amorphous solid (86 % yield). R_f = 0.8 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.77 (s, CH_3-19), 0.83 (s, CH_3-18), 0.75-2.00 (unassigned CH and CH_2), 2.44 (s, CH_2N), 2.99 (d, J = 6.2 Hz, $N\underline{CH_2}CH{=}CH_2$), 3.22 (d, J = 2.6 Hz, NCH_2C=O), 3.89 (m, OCH_2CH_2O), 5.22 (m, $CH{=}\underline{CH_2}$), 5.78 (m, $\underline{CH}{=}CH_2$) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.7, 28.1, 30.6, 31.0, 31.9, 32.9, 34.2, 35.7, 36.0, 38.5, 39.6, 45.9, 50.1, 53.4, 55.2, 59.9, 59.9, 64.5, 65.1, 82.4, 118.8, 119.4, 133.6, 168.3 ppm. LR-MS: calcd. for $C_{27}H_{42}NO_4$ $[M+H]^+$ 444.30, found 444.35.

2.1.4.22. (3'R,5'S,8'R,9'S,10'S,13'S,14'S)-4''-(4-iodobenzyl)-10',13'-dimethyltetradeca hydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6e17)

Amorphous solid (93 % yield). R_f = 0.7 (hexanes/EtOAc, 1:1). IR (film) ν 1732 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.73 (s, CH_3-19), 0.82 (s, CH_3-18), 0.80-2.00 (unassigned CH and CH_2), 2.39 (s, CH_2N), 3.26 (s, NCH_2C=O), 3.45 (s, $\underline{CH_2}$-Ph), 3.89 (m, OCH_2CH_2O), 7.06 (d, J = 8.2 Hz, 2H of Ar), 7.67 (d, J = 8.3 Hz, 2H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.7, 28.1, 30.6, 31.0, 31.8, 32.9, 34.2, 35.7, 36.0, 38.4, 39.6, 45.9, 50.1, 53.4, 55.4, 59.7, 60.7, 64.5, 65.1, 82.4, 93.0, 119.4, 130.6 (2C), 136.5, 137.7 (2C), 167.9 ppm. LR-MS: calcd. for $C_{31}H_{43}INO_4$ $[M+H]^+$ 620.22, found 620.25.

2.1.4.23. (3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-(prop-2-yn-1-yl)tetradeca hydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6e18)

To a solution of amine **4e** (0.15 mmol) in DMF (4 mL) was added K_2CO_3 (0.22 mmol) and a solution of propargyl bromide (0.45 mmol) in toluene (53 μL). The mixture was refluxed for 17 h and filtered. The DMF was then co-evaporated several times with EtOAc,

and the oily crude product was purified by flash chromatography with a mixture of hexanes/EtOAc/TEA (93:6:1) to give **6e18**. Amorphous solid (65 % yield). R_f = 0.4 (hexanes/EtOAc, 7:3). IR (film) ν 1732 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.77 (s, CH_3-19), 0.82 (s, CH_3-18), 0.80-2.04 (unassigned CH and CH_2), 2.31 (t, J = 2.2 Hz, CH of alkyne), 2.55 (s, CH_2N), 3.35 (s, NCH_2C=O), 3.37 (d, J = 2.3 Hz, N$\underline{CH_2}$CCH), 3.88 (m, OCH_2CH_2O) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.7, 28.1, 30.6, 31.0, 31.8, 32.9, 34.2, 35.7, 36.0, 38.5, 39.6, 45.2, 45.9, 50.1, 53.0, 53.4, 58.8, 64.5, 65.1, 74.7, 76.5, 82.0, 119.4, 168.1 ppm. LR-MS: calcd. for $C_{27}H_{40}NO_4$ $[M+H]^+$ 442.29, found 442.35.

2.1.4.24. (3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-{[3'-(trifluoromethyl)biphenyl-4-yl]methyl}tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6e19)

The tertiary amine **6e17** (0.07 mmol), 3-fluoromethylphenyl boronic acid (0.11 mmol), [1,1'-bis(diphenylphosphino)ferrocene]-dichloro palladium (II) (8 mol %) and Na_2CO_3 (0.11 mmol) were mixed in a 3:1 dioxane/water solution (4 mL) and the mixture refluxed under argon atmosphere for 5h. The reaction mixture was then washed with water and extracted with EtOAc. The organic layer was dried with anhydrous Na_2SO_4, filter and the crude mixture was purified by flash chromatography to give **6e19**. Amorphous solid (23 % yield). R_f = 0.2 (hexanes/EtOAc, 7:3). IR (film) ν 1732 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.73 (s, CH_3-19), 0.83 (s, CH_3-18), 0.80-2.05 (unassigned CH and CH_2), 2.46 (s, CH_2N), 3.30 (s, NCH_2C=O), 3.57 (d, J = 2.0 Hz, $\underline{CH_2}$-Ph), 3.89 (m, OCH_2CH_2O), 7.42 (d, J = 8.1 Hz, 2H of Ar), 7.58 (m, 4H of Ar), 7.78 (d, J = 7.5 Hz, 1H of Ar), 7.84 (s, 1H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.7, 28.1, 30.6, 31.0, 31.9, 32.9, 34.2, 35.7, 36.0, 38.5, 39.6, 45.9, 50.1, 53.4, 55.5, 59.8, 60.9, 64.6, 65.1, 82.5, 119.4, 123.8 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 3.7 Hz), 124.0 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 3.6 Hz), 127.2, 127.3 (2C), 129.3 (2C), 130.3, 131.2 (q, $J_{C\text{-}C\text{-}F}$ = 32.1 Hz), 136.7, 139.1, 141.5, 149.7, 168.1 ppm. LR-MS: calcd. for $C_{38}H_{47}F_3NO_3$ $[M+H]^+$ 638.34, found 638.40.

2.1.4.25. (3'R,5'S,8'R,9'S,10'S,13'S,14'S)-4''-[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]-10',13'-dimethyltetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6e20)

To a solution of amine **6e18** (0.06 mmol) in a 1:1 mixture of *n*-butanol/water (3 mL) was added (azidomethyl)benzene (0.11 mmol), a molar solution of cupper (II) sulfate pentahydrate (63 µL) and a molar solution of sodium ascorbate (125 µL). The reaction mixture was stirred at room temperature for 3 h, washed with water and extracted with EtOAc. The organic layer was dried with anhydrous Na_2SO_4 and filtered. The crude product was purified by flash chromatography to give **6e20**. Amorphous solid (82 % yield). R_f = 0.1 (hexanes/EtOAc, 1:1). IR (film) ν 1728 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.71 (s, CH_3-19), 0.82 (s, CH_3-18), 0.70-2.04 (unassigned CH and CH_2), 2.47 (s, CH_2N); 3.26 (s, NCH_2C=O), 3.67 (s, N$\underline{CH_2}$C=C-N), 3.88 (m, OCH_2CH_2O), 5.53 (s, N$\underline{CH_2}$Ph), 7.27 (s, 1H of triazole), 7.37 (m, 5H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.3, 22.6, 28.1, 30.6, 31.0, 31.8, 32.8, 34.2, 35.7, 36.0, 38.4, 39.5, 45.9, 50.1, 51.6, 53.4, 54.2, 54.9, 59.6, 64.5, 65.1, 82.3, 119.4, 122.5, 128.0 (2C), 128.9, 129.2 (2C), 134.5, 167.9 ppm. LR-MS: calcd. for $C_{34}H_{47}N_4O_4$ $[M+H]^+$ 575.35, found 575.40.

2.1.4.26. (3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-{[4-(trifluoromethyl)phenyl]sulfonyl}tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenanthrene-3',2''-[1,4]oxazinan]-6''-one (6e21)

To a solution of the amine **4e** (0.14 mmol) in DCM was added TEA (0.41 mmol and the mixture was stirred 5 min under an argon atmosphere. 4-(Trifluoromethyl) benzenesulfonyl chloride (0.22 mmol) was then added and the solution stirred at room temperature. After 3 h, DCM is partially evaporated and silica gel added to make a dry-pack with the crude mixture. The residue was purified by flash chromatography with a mixture of hexanes/EtOAc/TEA (90:10:1) to give **6e21**. Amorphous solid (78% yield). R_f = 0.8 (hexanes/EtOAc, 1:1). IR (film) ν 1728 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.79 (s, CH_3-19), 0.83 (s, CH_3-18), 0.80-2.00 (unassigned CH and CH_2), 3.12 (d, J = 2.6 Hz, CH_2N), 3.89 (m, OCH_2CH_2O and NCH_2C=O), 7.85 (d, J = 8.4 Hz, 2H of Ar), 7.91 (d, J = 8.4 Hz, 2H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.4, 22.6, 27.9, 30.6, 30.9, 30.9, 32.6, 34.1, 35.7, 35.8, 37.4, 39.6, 45.9, 46.1, 50.1, 52.1, 53.4, 64.5, 65.2, 82.4, 119.3, 123.0 (q, J_{C-F} = 273 Hz), 126.8 (q, $J_{C-C-C-F}$ = 3.4 Hz) (2C), 128.1 (2C), 135.4 (q, J_{C-C-F} = 33.3 Hz), 138.9, 163.9 ppm. LR-MS: calcd. for $C_{31}H_{41}F_3NO_6S$ $[M+H]^+$ 612.25, found 612.35.

2.1.4.27. (3'R,5'S,8'R,9'S,10'S,13'S,14'S)-10',13'-dimethyl-4''-{[4-(trifluoromethyl) phenyl]}tetradecahydro-2'H,6''H-dispiro[1,3-dioxolane-2,17'-cyclopenta[a]phenan threne-3',2''-[1,4]oxazinan]-6''-one (6e22)

This compound was synthesized as reported for **6e21** except that 4-trifluoromethyl)benzensulfonylchloride was used to give **6e22**. Amorphous solid (86 % yield). R_f = 0.5 (hexanes/EtOAc, 1:1). IR (film) ν 1740 (C=O, morpholinone), 1647 (C=O, amide) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.82 (s, CH_3-19 and CH_3-18), 0.75-2.04 (unassigned CH and CH_2), 3.42 and 3.85 (2m, CH_2N), 3.89 (m, OCH_2CH_2O), 4.19 and 4.53 (2m, NCH_2C=O), 7.54 (d, J = 8.0 Hz, 2H of Ar), 7.74 (d, J = 8.0 Hz, 2H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 14.4, 20.4, 22.6, 28.0, 30.5, 30.9, 32.6, 34.1, 35.7, 35.8, 37.1, 39.5, 45.9, 50.1, 53.4, 64.5, 65.1, 82.2, 119.3, 123.5 (q, J_{C-F} = 272.6 Hz), 126.0 (q, $J_{C-C-C-F}$ = 3.5 Hz) (2C), 127.6 (2C), 132.6 (q, J_{C-C-F} = 35.9 Hz), 137.7, 168.9 (2C) ppm. LR-MS: calcd. for $C_{32}H_{43}F_3NO_4$ $[M+H]^+$ 576.29, found 576.35.

2.1.5. General procedure to generate the spiromorpholinones 5a-5e and the *N*-benzylated spiromorpholinones 7a-7e and 7e1-7e22

To a solution of compounds **4a**-**4e**, **6a**-**6e and 6e1**-**6e22** (30 - 60 mg) in dioxane (2 mL) was added an aqueous solution of sulfuric acid (5%) (2 mL) and the mixture was stirred at room temperature (2 h – 5 h). A saturated aqueous solution of Na_2CO_3 (12 mL) was added and the reaction mixture extracted with EtOAc. The organic phase was collected, dried with anhydrous $MgSO_4$ and evaporated under reduced pressure. The purification was performed by flash column chromatography.

2.1.5.1. (3R,5S,5'S,8R,9S,10S,13S,14S)-10,13-dimethyl-5'-(2-methylpropyl)tetradeca hydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (5a)

Amorphous solid (71 % yield). R_f = 0.5 (hexanes/EtOAc, 1:1). IR (film) ν 3448 and 3333 (NH) and 1736 (C=O) cm^{-1}. ^1H NMR (acetone-d_6) δ 0.84 and 0.85 (2s, CH_3-18 and CH_3-19), 0.90 and 0.93 (2d, J = 6.7 Hz, $(CH_3)_2$- from *i*Pr), 1.05 (m, 1H), 1.10-2.02 (unassigned CH and CH_2), 2.38 (m, CH-16β), 2.82 and 2.95 (2d of AB system, J = 13.4 Hz, CH_2N), 3.44 (dd, J_1 = 4.1 Hz, J_2 = 9.6 Hz, NCHC=O) ppm. ^{13}C NMR (acetone-d_6) δ 10.7, 13.2, 20.1, 20.6, 21.4, 22.9, 24.2, 27.9, 30.7, 31.2, 31.7, 33.2, 35.0, 35.1, 36.1, 37.9, 39.8, 41.3, 47.3, 51.3, 51.9, 54.3, 55.5, 81.8, 170.8, 219.0 ppm. LR-MS: calcd. for $C_{26}H_{42}NO_3$ $[M+H]^+$ 416.31, found 416.35.

2.1.5.2. (3R,5S,5'R,8R,9S,10S,13S,14S)-10,13-dimethyl-5'-(2-methylpropyl)tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (5b)

Amorphous solid (92 % yield). R_f = 0.44 (hexanes/EtOAc, 1:1). IR (film) ν 3448 and 3340 (NH) and 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.80 (s, CH_3-19), 0.86 (s, CH_3-18), 0.93 and 0.97 (2d, J = 6.4 Hz, $(CH_3)_2$- from *i*Pr), 0.99-1.97 (unassigned CH and CH_2), 2.08 (m, CH-16α), 2.42 (m, CH-16β), 2.82 and 2.89 (2d of AB system, J = 13.5 Hz, CH_2N), 3.50 (dd, J_1 = 3.6 Hz and J_2 = 9.8 Hz, NCHC=O) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.0, 21.8, 23.4, 24.5, 28.0, 30.5, 31.4, 31.5, 32.6, 35.0, 35.8, 36.1, 38.2, 39.7, 41.4, 47.8, 51.4, 52.5, 53.8, 55.6, 82.2, 171.8, 221.2 ppm. LR-MS: calcd. for $C_{26}H_{42}NO_3$ $[M+H]^+$ 416.31, found 416.35.

2.1.5.3. (3R,5S,5'S,8R,9S,10S,13S,14S)-5'-benzyl-10,13-dimethyltetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (5c)

Amorphous solid (97 % yield). R_f = 0.7 (hexanes/EtOAc, 3:7). IR (film) ν 3333 (NH) and 1736 (C=O) cm^{-1}. ^1H NMR (acetone-d_6) δ 0.81 (s, CH_3-19), 0.84 (s, CH_3-18), 0.80-1.98 (unassigned CH and CH_2), 2.38 (m, CH-16β), 2.79 and 2.90 (2d of AB system, J = 13.3 Hz, CH_2N), 3.02 and 3.16 (2m, $\underline{CH_2}Ph$), 3.73 (dd, J_1 = 4.0 Hz and J_2 = 8.1 Hz, NCHC=O), 7.24 (m, 1H of Ph), 7.29 (m, 4H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.3, 13.8, 20.2, 21.7, 27.8, 30.5, 31.0, 31.5, 32.8, 35.0, 35.8, 36.0, 38.0, 39.2, 47.8, 51.4, 52.6, 53.7, 53.8, 58.6, 58.7, 82.7, 127.1, 128.8 (2C), 129.5 (2C), 137.2, 170.8, 221.3 ppm. LR-MS: calcd. for $C_{29}H_{40}NO_3$ $[M+H]^+$ 450.29, found 450.35.

2.1.5.4. (3R,5S,5'R,8R,9S,10S,13S,14S)-5'-benzyl-10,13-dimethyltetradecahydro-6'H-spiro [cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (5d)

Amorphous solid (56 % yield). R_f = 0.6 (hexanes/EtOAc, 3:7). IR (film) ν 3448 and 3325 (NH), and 1728 (C=O) cm^{-1}. ^1H NMR (acetone-d_6) δ 0.80 (s, CH_3-19), 0.83 (s, CH_3-18), 0.80-2.00 (unassigned CH and CH_2), 2.37 (m, CH-16β), 2.79 and 2.88 (2d of AB system, J = 13.3 Hz, CH_2N), 3.10 (m, $\underline{CH_2}Ph$), 3.75 (dd, J_1 = 4.1 Hz and J_2 = 7.5 Hz, NCHC=O), 7.27 (m, Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.3, 13.8, 20.2, 21.8, 27.8, 30.5, 31.3, 31.5, 32.5, 35.0, 35.8, 36.0, 37.7, 38.0, 39.6, 47.8, 51.4, 52.7, 53.8, 58.8, 82.7, 127.1, 128.8 (2C), 129.6 (2C), 137.2, 170.7, 221.2 ppm. LR-MS: calcd. for $C_{29}H_{40}NO_3$ $[M+H]^+$ 450.29, found 450.35.

2.1.5.5. (3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyltetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (5e)

Amorphous solid (54 % yield). R_f = 0.29 (hexanes/EtOAc, 1:1). IR (film) ν 3448 and 3333 (NH) and 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.80 (s, CH_3-19), 0.86 (s, CH_3-18), 0.80-1.99 (unassigned CH and CH_2), 2.07 (m, CH-16α), 2.43 (dd, J_1 = 8.4 Hz, J_2 = 19.2 Hz, CH-16β), 2.83 (s, CH_2N), 3.63 (s, NCH_2C=O) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 27.9, 30.5, 31.2, 31.5, 32.7, 35.0, 35.8, 36.1, 37.9, 39.4, 47.6, 47.8, 51.4, 52.9, 53.8, 82.5, 168.7, 221.3 ppm. LR-MS: calcd. for $C_{22}H_{34}NO_3$ $[M+H]^+$ 360.25, found 360.25.

2.1.5.6. (3R,5S,5'S,8R,9S,10S,13S,14S)-4'-benzyl-10,13-dimethyl-5'-(2-methylpropyl) tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7a)

Amorphous solid (81 % yield). R_f = 0.77 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR (acetone-d_6) δ 0.77 (s, CH_3-19), 0.83 (s, CH_3-18), 0.93 and 0.98 (2d, J = 6.4 Hz, $(CH_3)_2$- from *i*Pr), 0.80-1.99 (unassigned CH and CH_2), 2.27 and 2.75 (2d of AB system, J = 12.5 Hz, CH_2N), 2.37 (dd, J_1 = 8.7 Hz, J_2 = 18.3 Hz, CH-16β), 3.17 (dd, J_1 = 2.7 and J_2 = 6.9 Hz, 1H, NCHC=O), 3.21 and 4.07 (2d of AB system, J = 13.6 Hz, $\underline{CH_2}$-Ph), 7.34 (m, 1H of

Ph), 7.37 (m, 4H of Ph) ppm. ^{13}C NMR (acetone-d_6) δ 10.8, 13.2, 20.1, 21.4, 21.6, 23.4, 24.9, 27.8, 30.6, 31.5, 31.7, 33.3, 34.9, 35.1, 36.0, 38.2, 38.3, 39.7, 47.3, 51.3, 54.3, 57.6, 57.7, 63.1, 80.5, 127.2, 128.4 (2C), 128.6 (2C), 138.2, 169.8, 218.7 ppm. LR-MS: calcd. for $C_{33}H_{48}NO_3$ $[M+H]^+$ 506.36, found 506.40.

2.1.5.7. (3R,5S,5'R,8R,9S,10S,13S,14S)-4'-benzyl-10,13-dimethyl-5'-(2-methylpropyl) tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7b)

Amorphous solid (92 % yield). R_f = 0.85 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.71 (s, CH_3-19), 0.84 (s, CH_3-18), 0.95 and 0.99 (2d, J = 6.6 Hz, $(CH_3)_2$-from *i*Pr), 0.80-2.15 (unassigned CH and CH_2), 2.18 and 2.65 (2d of AB system, J = 12.4 Hz, CH_2N), 2.43 (dd, J_1 = 8.7 Hz, J_2 = 19.2 Hz, CH-16β), 3.12 and 4.03 (2d of AB system, J = 13.5 Hz, $\underline{CH_2}$-Ph), 3.18 (dd, J_1 = 2.9 Hz and J_2 = 6.8 Hz, NCHC=O), 7.33 (m, Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 22.1, 24.0, 25.0, 28.1, 30.6, 31.5, 31.9, 32.7, 35.0, 35.8, 36.1, 38.2, 38.5, 39.8, 47.8, 51.4, 53.8, 57.7, 58.1, 63.1, 80.2, 127.4 , 128.5 (2C), 128.6 (2C), 137.7, 171.3, 221.3 ppm. LR-MS: calcd. for $C_{33}H_{48}NO_3$ $[M+H]^+$ 506.36, found 506.40.

2.1.5.8. (3R,5S,5'S,8R,9S,10S,13S,14S)-4',5'-dibenzyl-10,13-dimethyltetradecahydro-6'H-spiro [cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7c)

Amorphous solid (70 % yield). R_f = 0.61 (hexanes/EtOAc, 8:2). IR (film) ν 1728 (C=O) cm^{-1}. ^{1}H NMR (acetone-d_6) δ 0.65 (s, CH_3-19), 0.80 (s, CH_3-18), 0.75-1.99 (unassigned CH and CH_2), 2.22 and 2.59 (2d of AB system, J = 12.5 Hz, CH_2N), 2.36 (dd, J_1 = 8.7 Hz, J_2 = 18.5 Hz, CH-16β), 3.27 and 4.40 (2d of AB system, J = 13.9 Hz, $N\underline{CH_2}$-Ph), 3.37 (m, $\underline{CH_2}$Ph); 3.51 (dd, J_1 = 2.9 Hz and J_2 = 5.1 Hz, NCHC=O), 7.31 (m, 2xPh) ppm. ^{13}C NMR (acetone-d_6) δ 10.7, 13.1, 20.0, 21.4, 27.7, 30.6, 30.6, 31.6, 33.0, 34.9, 35.1, 35.2, 35.8, 37.9, 39.6, 47.3, 51.2, 54.1, 57.4, 57.7, 65.9, 80.5, 126.5, 127.1, 127.8 (2C), 128.4 (2C), 128.5 (2C), 130.4 (2C), 138.0, 138.1, 169.5, 218.7 ppm. LR-MS: calcd. for $C_{36}H_{46}NO_3$ $[M+H]^+$ 540.34, found 540.40.

2.1.5.9. (3R,5S,5'R,8R,9S,10S,13S,14S)-4',5'-dibenzyl-10,13-dimethyltetradecahydro-6'H-spiro [cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7d)

Amorphous solid (81 % yield). R_f = 0.59 (hexanes/EtOAc, 8:2). IR (film) ν 1728 (C=O) cm^{-1}. ^{1}H NMR (acetone-d_6) δ 0.65 (s, CH_3-19), 0.80 (s, CH_3-18), 0.75-1.99 (unassigned CH and CH_2), 2.22 and 2.60 (2d of AB system, J = 12.4 Hz, CH_2N), 2.37 (dd, J_1 = 8.6 Hz, J_2 = 18.4 Hz, CH-16β), 3.29 and 4.42 (2d of AB system, J =13.8 Hz, $N\underline{CH_2}$-Ph), 3.41 (m, $\underline{CH_2}$Ph); 3.51 (dd, J_1 = 3 Hz and J_2 = 5.0 Hz, NCHC=O), 7.30 (m, 10H, 2xPh) ppm. ^{13}C NMR (acetone-d_6) δ 10.7, 13.2, 20.1, 21.4, 27.4, 30.6, 31.2, 31.7, 32.6, 34.9, 35.0, 35.1, 35.8, 37.2, 40.0, 47.3, 51.2, 54.2, 57.4, 57.8, 65.8, 80.5, 126.6, 127.1, 127.8 (2C), 128.4 (2C), 128.6 (2C), 130.5 (2C), 137.9, 138.1, 169.4, 218.7 ppm. LR-MS: calcd. for $C_{36}H_{46}NO_3$ $[M+H]^+$ 540.34, found 540.40.

2.1.5.10. (3R,5S,8R,9S,10S,13S,14S)-4'-benzyl-10,13-dimethyltetradecahydro-6'H-spiro [cyclopenta [a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7e)

Amorphous solid (71 % yield). R_f = 0.7 (DCM/MeOH, 39:1). IR (film) ν 1725 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.75 (s, CH_3-19), 0.85 (s, CH_3-18), 0.85-1.99 (unassigned CH and CH_2), 2.11 (m, CH-16α), 2.43 (m, CH_2N and CH-16β), 3.27 (s, NCH_2C=O), 3.50 and 3.54 (2d of AB system, J = 4.4 Hz, $\underline{CH_2}$-Ph), 7.33 (m, Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 27.9, 30.6, 31.5, 31.8, 32.9, 35.0, 35.8, 36.1, 38.3, 39.6, 47.8, 51.4, 53.8, 55.4, 59.7,

61.4, 82.4, 127.6, 128.5 (2C), 128.7 (2C), 136.7, 168.2, 221.3 ppm. LR-MS: calcd. for $C_{29}H_{40}NO_3$ $[M+H]^+$ 450.29, found 450.35.

2.1.5.11. (3R,5S,8R,9S,10S,13S,14S)-4'-(4-bromobenzyl)-10,13-dimethyltetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7e1)

Amorphous solid (78 % yield). R_f = 0.6 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.75 (s, CH_3-19), 0.85 (s, CH_3-18), 0.84-1.99 (unassigned CH and CH_2), 2.07 (m, CH-16α), 2.40 (s, CH_2N), 2.42 (dd, J_1 = 8.6 Hz, J_2 = 19.5 Hz, CH-16β), 3.26 (s, NCH_2C=O), 3.46 (d, J = 2.5 Hz, $\underline{CH_2}$-Ph), 7.19 (d, J = 8.3 Hz, 2H of Ar), 7.47 (d, J = 8.3 Hz, 2H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.9, 30.6, 31.5, 31.8, 32.8, 35.0, 35.8, 36.1, 38.3, 39.6, 47.8, 51.4, 53.8, 55.4, 59.5, 60.6, 82.3, 121.5, 130.3 (2C), 131.7 (2C), 135.8, 167.9, 221.3 ppm. LR-MS: calcd. for $C_{29}H_{39}{}^{81}BrNO_3$ $[M+H]^+$ 530.20, found 530.15.

2.1.5.12. (3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-[4-(trifluoromethoxy)benzyl] tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7e2)

Amorphous solid (70 % yield). R_f = 0.6 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.76 (s, CH_3-19), 0.85 (s, CH_3-18), 0.85-1.97 (unassigned CH and CH_2), 2.05 (m, CH-16α), 2.42 (dd, J_1 = 8.6 Hz, J_2 = 19.2 Hz, CH-16β); 2.44 (s, CH_2N), 3.25 (s, NCH_2C=O), 3.51 (d, J = 3.8 Hz, $\underline{CH_2}$-Ph), 7.19 (d, J = 8.1 Hz, 2H of Ar), 7.33 (d, J = 8.5 Hz, 2H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.9, 30.6, 31.5, 31.8, 32.8, 35.0, 35.8, 36.1, 38.3, 39.6, 47.8, 51.4, 53.8, 55.3, 59.7, 60.5, 82.3, 120.4 (d, J_{C-F} = 257 Hz) 121.0 (2C), 129.9 (2C), 130.3, 135.4, 148.6, 167.9, 221.3 ppm. LR-MS: calcd. for $C_{30}H_{39}F_3NO_4$ $[M+H]^+$ 534.28, found 534.15.

2.1.5.13. (3R,5S,8R,9S,10S,13S,14S)-4'-(biphenyl-4-ylmethyl)-10,13-dimethyltetradeca hydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7e3)

Amorphous solid (94 % yield). R_f = 0.7 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.76 (s, CH_3-19), 0.85 (s, CH_3-18), 0.80-1.98 (unassigned CH and CH_2), 2.05 (m, CH-16α), 2.43 (dd, J_1 = 8.6 Hz, J_2 = 19.2 Hz, CH-16β), 2.47 (s, CH_2N), 3.30 (s, NCH_2C=O), 3.56 (d, J = 3.6 Hz, $\underline{CH_2}$-Ph), 7.36 (m, 3H of Ar), 7.45 (m, 2H of Ar), 7.60 (m, 4H of Ar) ppm. ^{13}CNMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 27.9, 30.6, 31.5, 31.8, 32.9, 35.0, 35.8, 36.1, 38.4, 39.6, 47.8, 51.4, 53.8, 55.5, 59.7, 61.0, 82.4, 127.0 (2C), 127.3 (2C), 127.4, 128.8 (2C), 129.1 (2C), 135.7, 140.5, 140.6, 168.2, 221.3 ppm. LR-MS: calcd. for $C_{35}H_{44}NO_3$ $[M+H]^+$ 526.32, found 526.30.

2.1.5.14. (3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-(2-phenylethyl)tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7e4)

Amorphous solid (60 % yield). R_f = 0.7 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.79 (s, CH_3-19), 0.85 (s, CH_3-18), 0.75-2.00 (unassigned CH and CH_2), 2.08 (m, CH-16α), 2.43 (dd, J_1 = 8.6 Hz, J_2 = 19.3 Hz, CH-16β), 2.50 (s, CH_2N), 2.62 ($t_{app.}$, J = 7.5 Hz, $\underline{CH_2}$-Ph), 2.78 ($t_{app.}$, J = 7.6 Hz, $N\underline{CH_2}CH_2Ph$), 3.28 (d, J = 1.8 Hz, NCH_2C=O), 7.20 (m, 3H of Ph), 7.28 (m, 2H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 27.9, 30.6, 31.5, 31.7, 32.9, 33.2, 35.0, 35.8, 36.1, 38.4, 39.6, 47.8, 51.4, 53.8, 55.3, 58.6, 60.3, 82.2, 126.3, 128.4 (2C), 128.6 (2C), 139.5, 168.3, 221.3 ppm. LR-MS: calcd. for $C_{30}H_{42}NO_3$ $[M+H]^+$ 464.31, found 464.25.

2.1.5.15. (3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-(3-phenylpropyl)tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7e5)

Amorphous solid (51 % yield). R_f = 0.6 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.80 (s, CH_3-19), 0.86 (s, CH_3-18), 0.80-1.96 (unassigned CH and CH_2), 2.08 (m, CH-16α), 2.34 (t, J = 7.0 Hz, N$\underline{CH_2}CH_2CH_2Ph$), 2.43 (m, CH-16β), 2.44 (s, CH_2N), 2.66 (t_{app}, J = 7.6 Hz, $NCH_2CH_2\underline{CH_2}Ph$), 3.21 (s, $NCH_2C{=}O$), 7.18 (m, 3H of Ph), 7.29 (m, 2H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 27.9, 28.2, 30.6, 31.5, 31.9, 32.9, 33.0, 35.0, 35.8, 36.1, 38.5, 39.6, 47.8, 51.4, 53.8, 55.4, 56.0, 60.1, 82.2, 126.0, 128.4 (4C), 141.6, 168.4, 221.3 ppm. LR-MS: calcd. for $C_{31}H_{44}NO_3$ $[M+H]^+$ 478.32, found 478.25.

2.1.5.16. (3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-{2-[4-(trifluoromethyl)phenyl] ethyl}tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7e6)

Amorphous solid (36 % yield). R_f = 0.5 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.79 (s, CH_3-19), 0.86 (s, CH_3-18), 0.80-2.02 (unassigned CH and CH_2), 2.08 (m, CH-16α), 2.43 (dd, J_1 = 8.5 Hz, J_2 = 19.2 Hz, CH-16β), 2.50 (s, CH_2N), 2.64 (t_{app}., J = 7.3 Hz, $\underline{CH_2}$-Ph), 2.84 (t_{app}., J = 7.4 Hz, N$\underline{CH_2}CH_2Ph$), 3.29 (d, J = 4.1 Hz, $NCH_2C{=}O$), 7.31 (d, J = 8.0 Hz, 2H of Ar), 7.55 (d, J = 8.1 Hz, 2H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.8, 30.5, 31.5, 31.7, 32.8, 33.0, 35.0, 35.8, 36.1, 38.1, 39.6, 44.6, 47.8, 51.4, 53.8, 55.2, 57.9, 60.3, 82.2, 125.3 ($J_{c\text{-}c\text{-}c}$ = 3.6 Hz) (2C), 129.0 (2C), 133.1, 143.6, 167.9, 221.3 ppm. LR-MS: calcd. for $C_{31}H_{41}F_3NO_3$ $[M+H]^+$ 532.30, found 532.20.

2.1.5.17. (3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-[4-(trifluoromethyl)benzyl]tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7e7)

Amorphous solid (78 % yield). R_f = 0.6 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.76 (s, CH_3-19), 0.85 (s, CH_3-18), 0.80-2.01 (unassigned CH and CH_2), 2.08 (m, CH-16α), 2.42 (m, CH-16β), 2.43 (s, CH_2N), 3.28 (s, $NCH_2C{=}O$), 3.57 (d, J = 2.7 Hz, $\underline{CH_2}$-Ph), 7.44 (d, J = 8.0 Hz, 2H of Ar), 7.61 (d, J = 8.1 Hz, 2H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.9, 30.6, 31.5, 31.8, 32.8, 35.0, 35.8, 36.1, 38.3, 39.6, 47.8, 51.4, 53.8, 55.4, 59.7, 60.8, 82.3, 124.0 (q, J = 270.3 Hz), 125.5 ($J_{C\text{-}C\text{-}C\text{-}F}$ = 3.7 Hz) (2C), 128.8 (2C), 130.0 ($J_{C\text{-}C\text{-}F}$ = 32.3 Hz), 140.9, 167.8, 221.3 ppm. LR-MS: calcd. for $C_{30}H_{39}F_3NO_3$ $[M+H]^+$ 518.28, found 518.25.

2.1.5.18. (3R,5S,8R,9S,10S,13S,14S)-4'-[4-(benzyloxy)benzyl]-10,13-dimethyltetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7e8)

Amorphous solid (88 % yield). R_f = 0.7 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.75 (s, CH_3-19), 0.85 (s, CH_3-18), 0.85-1.97 (unassigned CH and CH_2), 2.08 (m, CH-16α), 2.41 (s, CH_2N), 2.43 (dd, J_1 = 8.5 Hz, J_2 = 19.2 Hz, CH-16β), 3.24 (s, $NCH_2C{=}O$), 3.46 (d, J = 3.8 Hz, $\underline{CH_2}$-Ph), 5.07 (s, CH_2O), 6.95 (d, J = 8.6 Hz, 2H of Ph), 7.21 (d, J = 8.6 Hz, 2H of Ph), 7.39 (m, 5H, Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 27.9, 30.6, 31.5, 31.8, 32.9, 35.0, 35.8, 36.1, 38.4, 39.6, 47.8, 51.4, 53.8, 55.4, 59.6, 60.7, 70.1, 82.4, 114.8 (2C), 127.5 (2C), 128.0, 128.6 (2C), 128.9, 129.9 (2C), 136.9, 158.3, 168.3, 221.3 ppm. LR-MS: calcd. for $C_{36}H_{46}NO_4$ $[M+H]^+$ 556.33, found 556.25.

2.1.5.19. (3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-(4-phenylbutyl)tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7e9)

Amorphous solid (77 % yield). R_f = 0.7 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.79 (s, CH_3-19), 0.86 (s, CH_3-18), 0.80-1.97 (unassigned CH and CH_2), 2.08 (m, CH-16α), 2.34 (t, J = 7.0 Hz, N$\underline{CH_2}CH_2CH_2CH_2Ph$), 2.39 (s, CH_2N), 2.43 (dd, J_1 = 8.7 Hz, J_2 = 19.4 Hz, CH-16β), 2.63 (t, J = 7.5 Hz, $NCH_2CH_2\underline{CH_2}Ph$), 3.18 (d, J = 1.9 Hz, NCH_2C=O), 7.17 (m, 3H of Ph), 7.28 (m, 2H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 25.9, 27.9, 28.8, 30.6, 31.5, 31.8, 32.9, 35.0, 35.6, 35.8, 36.1, 38.5, 39.6, 47.8, 51.4, 53.8, 55.5, 56.7, 60.3, 82.2, 125.8, 128.3 (2C), 128.4 (2C), 142.2, 168.4, 221.3 ppm. LR-MS: calcd. for $C_{32}H_{46}NO_3$ $[M+H]^+$ 492.34, found 492.25.

2.1.5.20. (3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-[2-(trifluoromethyl)benzyl]tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7e10)

Amorphous solid (67 % yield). R_f = 0.6 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.76 (s, CH_3-19), 0.85 (s, CH_3-18), 0.86-2.03 (unassigned CH and CH_2), 2.06 (m, CH-16α), 2.43 (dd, J_1 = 8.6 Hz, J_2 = 19.2 Hz, CH-16β), 2.49 (s, CH_2N), 3.29 (d, J = 3.9 Hz, NCH_2C=O), 3.68 (s, $\underline{CH_2}$-Ph), 7.40 (t, J = 7.6 Hz, 1H of Ar), 7.55 (t, J = 7.5 Hz, 1H of Ar), 7.67 (m, 2H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 27.9, 30.6, 31.5, 31.8, 32.8, 35.0, 35.8, 36.1, 38.3, 39.6, 47.8, 51.4, 53.8, 55.3, 57.1, 60.0, 82.3, 124.3 (q, $J_{C\text{-}F}$ = 273.8 Hz), 126.1 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 5.7 Hz), 127.6, 128.9 (q, $J_{C\text{-}C\text{-}F}$ = 30.3 Hz), 130.5, 132.0, 135.7, 167.8, 221.2 ppm. LR-MS: calcd. for $C_{30}H_{39}F_3NO_3$ $[M+H]^+$ 518.28, found 518.50.

2.1.5.21. (3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-(naphthalen-2-ylmethyl)tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7e11)

Amorphous solid (86 % yield). R_f = 0.6 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.71 (s, CH_3-19), 0.84 (s, CH_3-18), 0.80-2.00 (unassigned CH and CH_2), 2.08 (m, CH-16α), 2.44 (m, CH-16β), 2.45 (s, CH_2N), 3.34 (s, NCH_2C=O), 3.67 (d, J = 4.3 Hz, $\underline{CH_2}$-Ph), 7.50 (m, 3H of Ar), 7.71 (s, 1H of Ar), 7.83 (m, 3H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 27.9, 30.6, 31.5, 31.8, 32.9, 34.9, 35.8, 36.1, 38.4, 39.6, 47.8, 51.4, 53.8, 55.6, 59.6, 61.5, 82.4, 126.0, 126.3, 126.7, 127.6, 127.7 (2C), 128.4, 133.0, 133.2, 134.2, 168.2, 221.3 ppm. LR-MS: calcd. for $C_{33}H_{42}NO_3$ $[M+H]^+$ 500.31, found 500.20.

2.1.5.22. (3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-[3-(trifluoromethyl)benzyl]tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7e12)

Amorphous solid (79 % yield). R_f = 0.6 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.75 (s, CH_3-19), 0.84 (s, CH_3-18), 0.82-2.00 (unassigned CH and CH_2), 2.07 (m, CH-16α), 2.42 (m, CH-16β), 2.43 (s, CH_2N), 3.28 (s, NCH_2C=O), 3.57 (s, $\underline{CH_2}$-Ph), 7.52 (m, 4H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.8, 30.5, 31.5, 31.7, 32.8, 35.0, 35.8, 36.1, 38.4, 39.6, 47.8, 51.4, 53.8, 55.4, 59.7, 60.7, 82.3, 124.0 (q, $J_{C\text{-}F}$ = 272.3 Hz), 124.5 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 3.6 Hz), 125.3 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 3.7 Hz), 129.1, 131.0 (q, $J_{C\text{-}C\text{-}F}$ = 32.4 Hz), 131.9, 137.9, 167.8, 221.3 ppm. LR-MS: calcd. for $C_{30}H_{39}F_3NO_3$ $[M+H]^+$ 518.28, found 518.50.

2.1.5.23. (3R,5S,8R,9S,10S,13S,14S)-4'-[3,5-bis(trifluoromethyl)benzyl]-10,13-dimethyltetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7e13)

Amorphous solid (85 % yield). R_f = 0.7 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.75 (s, CH_3-19), 0.84 (s, CH_3-18), 0.87-1.99 (unassigned CH and CH_2),

2.07 (m, CH-16α), 2.42 (m, CH-16β), 2.44 (s, CH_2N), 3.33 (d, J = 1.7 Hz, NCH_2C=O), 3.65 (s, $\underline{CH_2}$-Ph), 7.81 (s, 3H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.3, 13.8, 20.2, 21.7, 27.8, 30.5, 31.5, 31.6, 32.8, 35.0, 35.8, 36.1, 38.4, 39.6, 47.8, 51.3, 53.8, 55.3, 59.6, 60.0, 82.3, 121.7 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 3.7 Hz), 123.2 (q, $J_{C\text{-}F}$ = 272.7 Hz) (2C), 128.5 (2C), 132.0 (q, $J_{C\text{-}C\text{-}F}$ = 33.4 Hz) (2C), 139.8, 167.3, 221.2 ppm. LR-MS: calcd. for $C_{31}H_{38}F_6NO_3$ $[M+H]^+$ 586.27, found 586.15.

2.1.5.24. (3R,5S,8R,9S,10S,13S,14S)-4'-[2,4-bis(trifluoromethyl)benzyl]-10,13-dimethyl tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7e14)

Amorphous solid (28 % yield). R_f = 0.8 (hexanes/EtOAc, 1:1). IR (film) ν 1740 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.77 (s, CH_3-19), 0.85 (s, CH_3-18), 0.80-1.97 (unassigned CH and CH_2), 2.07 (m, CH-16α), 2.43 (dd, J_1 = 8.6 Hz, J_2 = 19.3 Hz, CH-16β), 2.49 (s, CH_2N), 3.32 (d, J = 3.1 Hz, NCH_2C=O), 3.74 (s, $\underline{CH_2}$-Ph), 7.84 (d, J = 8.1 Hz, 1H of Ar), 7.91 (d, J = 8.7 Hz, 1H of Ar), 7.93 (s, 1H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.9, 30.5, 31.5, 31.8, 32.8, 35.0, 35.8, 36.1, 38.3, 39.6, 47.8, 51.4, 53.8, 55.3, 56.6, 60.0, 82.2, 123.3 (q, $J_{c\text{-}F}$ = 270.5 Hz), 123.5 (q, $J_{c\text{-}c\text{-}c\text{-}F}$ = 3.7 Hz), 124.4 (q, $J_{C\text{-}F}$ = 276.2 Hz), 124.8, 128.8, 129.6 (q, $J_{C\text{-}C\text{-}F}$ = 31.3 Hz), 130.2 ($J_{c\text{-}c\text{-}F}$ = 33.1 Hz), 131.0, 140.1, 167.3, 221.2 ppm. LR-MS: calcd. for $C_{31}H_{38}F_6NO_3$ $[M+H]^+$ 586.27, found 586.25.

2.1.5.25. (3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-(naphthalen-1-ylmethyl)tetradeca hydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7e15)

Amorphous solid (86 % yield). R_f = 0.6 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.69 (s, CH_3-19), 0.83 (s, CH_3-18), 0.80-1.93 (unassigned CH and CH_2), 2.06 (m, CH-16α), 2.41 (dd, J_1 = 8.6 Hz, J_2 = 19.2 Hz, CH-16β), 2.52 (d, J = 5.5 Hz, CH_2N), 3.29 (d, J = 7.5 Hz, NCH_2C=O), 3.92 (d, J = 4.8 Hz, $\underline{CH_2}$-Ph), 7.40 (m, 2H of Ar), 7.51 (m, 2H of Ar), 7.85 (m, 2H of Ar), 8.22 (m, 1H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.8, 30.5, 31.5, 31.7, 32.8, 35.0, 35.8, 36.1, 38.3, 39.6, 47.8, 51.4, 53.8, 55.4, 59.9, 60.1, 82.4, 124.6, 125.1, 125.9 (2C), 127.6, 128.5, 128.8, 132.1, 132.2, 133.9, 168.2, 221.3 ppm. LR-MS: calcd. for $C_{33}H_{42}NO_3$ $[M+H]^+$ 500.31, found 500.20.

2.1.5.26. (3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-(prop-2-en-1-yl)tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7e16)

Amorphous solid (55 % yield). R_f = 0.6 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.79 (s, CH_3-19), 0.85 (s, CH_3-18), 0.80-2.00 (unassigned CH and CH_2), 2.08 (m, CH-16α), 2.43 (m, CH-16β), 2.44 (s, CH_2N), 2.99 (d, J = 3.8 Hz, $N\underline{CH_2}CH{=}CH_2$), 3.21 (d, J = 6.3 Hz, NCH_2C=O), 5.21 (m, $CH{=}\underline{CH_2}$), 5.76 (m, $\underline{CH}{=}CH_2$) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 27.9, 30.6, 31.5, 31.8, 32.9, 35.0, 35.8, 36.1, 38.5, 39.6, 47.8, 51.4, 53.8, 55.2, 59.8, 59.9, 82.3, 118.9, 133.6, 168.3, 221.3 ppm. LR-MS: calcd. for $C_{25}H_{38}NO_3$ $[M+H]^+$ 400.28, found 400.15.

2.1.5.27. (3R,5S,8R,9S,10S,13S,14S)-4'-(4-iodobenzyl)-10,13-dimethyltetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7e17)

Amorphous solid (59 % yield). R_f = 0.6 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^{1}H NMR ($CDCl_3$) δ 0.75 (s, CH_3-19), 0.84 (s, CH_3-18), 0.84-1.96 (unassigned CH and CH_2), 2.07 (m, CH-16α), 2.39 (s, CH_2N), 2.42 (dd, J_1 = 8.6 Hz, J_2 = 19.4 Hz, CH-16β), 3.26 (s, NCH_2C=O), 3.45 (d, J = 2.5 Hz, $\underline{CH_2}$-Ph), 7.06 (d, J = 8.2 Hz, Ph), 7.67 (d, J = 8.2 Hz, Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.9, 30.6, 31.5, 31.8, 32.8, 35.0, 35.8, 36.1,

38.3, 39.6, 47.8, 51.4, 53.8, 55.4, 59.5, 60.7, 82.3, 93.1, 130.6 (2C), 136.4, 137.7 (2C), 167.9, 221.2 ppm. LR-MS: calcd. for $C_{29}H_{39}INO_3$ $[M+H]^+$ 576.19, found 576.10.

2.1.5.28. (3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-(prop-2-yn-1-yl)tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7e18)

Amorphous solid (67 % yield). R_f = 0.3 (hexanes/EtOAc, 7:3). IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.80 (s, CH_3-19), 0.86 (s, CH_3-18), 0.75-2.04 (unassigned CH and CH_2), 2.09 (m, CH-16α), 2.31 (d, J = 2.3 Hz, CH of alkyne), 2.43 (dd, J_1 = 8.6 Hz, J_2 = 19.2 Hz, CH-16β), 2.57 (s, CH_2N), 3.36 (d, J = 1.0 Hz, $NCH_2C{=}O$), 3.38 (d, J = 2.4 Hz, $N\underline{CH_2}CCH$) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 27.9, 29.7, 30.6, 31.5, 32.8, 35.0, 35.8, 36.1, 38.4, 39.6, 45.2, 47.8, 51.4, 53.1, 53.8, 58.7, 74.7, 76.5, 82.0, 168.1, 221.3 ppm. LR-MS: calcd. for $C_{25}H_{36}NO_3$ $[M+H]^+$ 398.26, found 398.15.

2.1.5.29. (3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-{[3'-(trifluoromethyl)biphenyl-4-yl]methyl}tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7e19)

Amorphous solid (59 % yield). R_f = 0.7 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.76 (s, CH_3-19), 0.85 (s, CH_3-18), 0.85-2.04 (unassigned CH and CH_2), 2.09 (m, CH-16α), 2.43 (dd, J_1 = 8.6 Hz, J_2 = 19.2 Hz, CH-16β), 2.47 (s-broad, CH_2N), 3.31 (s, $NCH_2C{=}O$), 3.58 (d, J = 3.4 Hz, $\underline{CH_2}$-Ph), 7.42 (d, J = 8.1 Hz, 2H of Ar), 7.59 (m, 4H of Ar), 7.78 (d, J = 7.5 Hz, 1H of Ar), 7.84 (s, 1H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.8, 27.9, 30.6, 31.5, 31.8, 32.9, 35.0, 35.8, 36.1, 38.4, 39.6, 47.8, 51.4, 53.8, 55.5, 59.7, 60.9, 82.4, 123.0 (q, $J_{c\text{-}F}$ = 270 Hz), 123.8 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 3.9 Hz), 124.0 (q, $J_{c\text{-}c\text{-}c\text{-}F}$ = 3.9 Hz), 127.3 (2C), 129.3 (2C), 130.3, 131.2 (q, $J_{c\text{-}c\text{-}F}$ = 34.0 Hz), 136.7, 139.1, 141.5, 149.7, 168.1, 221.3 ppm. LR-MS: calcd. for $C_{36}H_{43}F_3NO_3$ $[M+H]^+$ 594.31, found 594.20.

2.1.5.30. (3R,5S,8R,9S,10S,13S,14S)-4'-[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]-10,13-dimethyltetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7e20)

Amorphous solid (90 % yield). R_f = 0.6 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O), 1454 (C-O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.75 (s, CH_3-19), 0.86 (s, CH_3-18), 0.80-1.97 (unassigned CH and CH_2), 2.06 (m, CH-16α), 2.43 (dd, J_1 = 8.6 Hz, J_2 = 19.2 Hz, CH-16β), 2.50 (s, CH_2N), 3.27 (s, $NCH_2C{=}O$), 3.69 (s, $N\underline{CH_2}C{=}C\text{-}N$), 5.54 (s, $N\underline{CH_2}Ph$), 7.27 (m, 2H of Ph), 7.34 (m, 4H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.8, 30.5, 31.5, 31.7, 32.8, 35.0, 35.8, 36.1, 38.3, 39.6, 47.8, 51.4, 51.6, 53.8, 54.2, 54.9, 59.5, 82.2, 122.5, 128.0 (2C), 128.9, 129.2 (2C), 134.6, 143.6, 167.8, 221.2 ppm. LR-MS: calcd. for $C_{32}H_{43}N_4O_3$ $[M+H]^+$ 531.33, found 531.25.

2.1.5.31. (3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-{[4-(trifluoromethyl)phenyl] sulfonyl}tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7e21)

Amorphous solid (42 % yield). R_f = 0.7 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.82 (s, CH_3-19), 0.86 (s, CH_3-18), 0.80-2.03 (unassigned CH and CH_2), 2.08 (m, CH-16α), 2.44 (dd, J_1 = 8.6 Hz, J_2 = 19.3 Hz, CH-16β), 3.10 and 3.18 (2d of AB system, J = 12.5 Hz, CH_2N), 3.81 and 3.90 (2d of AB system, J = 17.5 Hz, $NCH_2C{=}O$), 7.87 and 7.91 (2d of AB system, J = 8.2 Hz, 4H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.7, 30.5, 30.8, 31.5, 32.6, 35.0, 35.8, 36.0, 37.4, 39.6, 46.1, 47.7, 51.3, 52.0, 53.7, 82.3, 124.3 (q, $J_{C\text{-}F}$ = 273 Hz), 126.8 (q, $J_{C\text{-}C\text{-}C\text{-}F}$ = 3.4 Hz) (2C), 128.1 (2C), 135.4 (q, $J_{C\text{-}C\text{-}F}$ =

33.4 Hz), 138.9, 163.8, 221.0 ppm. LR-MS: calcd. for $C_{29}H_{37}F_3NO_5S$ $[M+H]^+$ 568.23, found 568.10.

2.1.5.32. (3R,5S,8R,9S,10S,13S,14S)-10,13-dimethyl-4'-{[4-(trifluoromethyl)phenyl] carbonyl}tetradecahydro-6'H-spiro[cyclopenta[a]phenanthrene-3,2'-[1,4]oxazinane]-6',17(2H)-dione (7e22)

Amorphous solid (83 % yield). R_f = 0.4 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O), 1647 (C=O, amide) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.85 (s-broad, CH_3-19 and CH_3-18), 0.80-1.96 (unassigned CH and CH_2), 2.07 (m, CH-16α), 2.43 (dd, J_1 = 8.7 Hz, J_2 = 19.3 Hz, CH-16β), 3.44 and 3.84 (2m, CH_2N), 4.20 and 4.53 (2m, NCH_2C=O), 7.54 (d, J = 8.0 Hz, 2H of Ar), 7.74 (d, J = 8.1 Hz, 2H of Ar) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.8, 30.5, 31.4, 32.5, 34.9, 35.8, 35.9, 36.9, 39.5, 47.7, 51.3, 53.7, 57.8, 64.5, 66.1, 123.5 (q, J_{C-F} = 273 Hz), 126.0 (q, $J_{C-C-C-F}$ = 3.5 Hz) (2C), 127.6 (2C), 132.8 (q, J_{C-C-F} = 35.9 Hz), 137.7, 168.9, 221.2 ppm. LR-MS: calcd. for $C_{30}H_{37}F_3NO_4$ $[M+H]^+$ 532.26, found 532.20.

2.1.6. Synthesis of carbamates 9a-9e

To a solution of the amino alcohol **3a**, **3b**, **3c**, **3d** or **3e** (0.12 mmol) in DCM (3 mL) was added DIPEA (0.24 mmol) at 0 °C, and the solution was stirred under an argon atmosphere for 10 min. Triphosgene (0.06 mmol) was added and the reaction mixture stirred for 5 h at room temperature. An acidic solution (2 mL) of HCl/MeOH (10:90) was then added to the reaction mixture which was stirred overnight at room temperature. The reaction was stopped by adding a saturated $NaHCO_3$ aqueous solution (10 mL) and the product extracted with DCM. The organic layer was dried using anhydrous Na_2SO_4 and evaporated under reduced pressure. The purification was done by flash chromatography, using hexanes/EtOAc/TEA (95:5:1) for the elution.

2.1.6.1. Methyl (2S)-2-[(3R,8R,9S,10S,13S,14S)-10,13-dimethyl-2',17-dioxohexadeca hydro-3'H-spiro[cyclopenta[a]phenanthrene-3,5'-[1,3]oxazolidin]-3'-yl]-4-methyl pentanoate (9a)

Amorphous solid (73 % yield). R_f = 0.71 (hexanes/EtOAc, 1:1). IR (film) ν 1744 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.82 (s, CH_3-19), 0.85 (s, CH_3-18), 0.96 and 0.97 (2d, J = 1.9 Hz, $(CH_3)_2$- from *i*Pr), 0.99-1.97 (unassigned CH and CH_2), 2.11 (m, CH-16α), 2.43 (dd, J_1 = 8.4 Hz, J_2 = 19.3 Hz, CH-16β), 3.14 and 3.42 (2d of AB system, J = 8.1 Hz, CH_2N), 3.72 (s, OCH_3), 4.58 (dd, J_1 = 4.8 Hz and J_2 = 11.1 Hz, NCHCO) ppm. ^{13}CNMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.1, 21.8, 23.2, 24.9, 27.9, 30.6, 31.5, 32.7, 33.8, 35.0, 35.4, 35.8, 37.7, 39.5, 40.8, 47.7, 51.3, 52.2, 52.9, 53.6, 53.9, 79.6, 157.6, 172.0, 221.2 ppm. LR-MS: calcd. for $C_{28}H_{44}NO_5$ $[M+H]^+$ 474.31, found 474.35.

2.1.6.2. Methyl (2R)-2-[(3R,8R,9S,10S,13S,14S)-10,13-dimethyl-2',17-dioxohexadeca hydro-3'H-spiro[cyclopenta[a]phenanthrene-3,5'-[1,3]oxazolidin]-3'-yl]-4-methyl pentanoate (9b)[28]

Amorphous solid (67 % yield). R_f = 0.57 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.83 (s, CH_3-19), 0.86 (s, CH_3-18), 0.97 (d, J = 6.5 Hz, $(CH_3)_2$- from *i*Pr), 0.80-1.97 (unassigned CH and CH_2), 2.05 (m, CH-16α), 2.44 (dd, J_1 = 8.4 Hz, J_2 = 19.3 Hz, CH-16β), 3.14 and 3.44 (2d of AB system, J = 8.1 Hz, CH_2N), 3.72 (s, OCH_3), 4.60 (dd, J_1 = 4.8 Hz and J_2 = 11.1 Hz, NCHCO) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.1, 21.7, 23.1, 24.9, 27.8, 30.6, 31.5, 32.9, 33.9, 35.0, 35.4, 35.8, 37.7, 39.3, 40.8, 47.7, 51.3, 52.2, 52.8, 53.6, 53.9, 79.6, 157.5, 172.0, 221.1 ppm. LR-MS: calcd. for $C_{28}H_{44}NO_5$ $[M+H]^+$ 474.31, found 474.35.

2.1.6.3. Methyl (2S)-2-[(3R,8R,9S,10S,13S,14S)-10,13-dimethyl-2',17-dioxohexadecahydro-3'H-spiro[cyclopenta[a]phenanthrene-3,5'-[1,3]oxazolidin]-3'-yl]-3-phenyl propanoate (9c)

Amorphous solid (61 % yield). R_f = 0.40 (hexanes/EtOAc, 1:1). IR (film) ν 1744 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.76 (s, CH_3-19), 0.84 (s, CH_3-18), 0.75-1.95 (unassigned CH and CH_2), 2.06 (m, CH-16α), 2.42 (dd, J_1 = 8.5 Hz, J_2 = 19.3 Hz, CH-16β), 2.95 and 3.35 (2m, $\underline{CH_2}Ph$), 3.09 and 3.33 (2d of AB system, J = 8.0 Hz, CH_2N), 3.76 (s, OCH_3), 4.88 (dd, J_1 = 5.5 Hz and J_2 = 11.3 Hz, NCHCO), 7.22 (m, 3H of Ph), 7.30 (m, 2H of Ph) ppm. ^{13}C NMR ($CDCl_3$) δ 11.3, 13.8, 20.2, 21.7, 27.7, 30.5, 31.5, 32.4, 33.8, 35.0, 35.1, 35.3, 35.8, 38.8, 40.6, 47.7, 51.3, 52.4, 53.2, 53.8, 55.8, 79.7, 127.1, 128.6 (2C), 128.7 (2C), 136.0, 157.3, 171.0, 221.2 ppm. LR-MS: calcd. for $C_{31}H_{42}NO_5$ $[M+H]^+$ 508.30, found 508.35.

2.1.6.4. Methyl (2R)-2-[(3R,8R,9S,10S,13S,14S)-10,13-dimethyl-2',17-dioxohexadecahydro-3'H-spiro[cyclopenta[a]phenanthrene-3,5'-[1,3]oxazolidin]-3'-yl]-3-phenyl propanoate (9d)

Amorphous solid (75 % yield). R_f = 0.50 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.77 (s, CH_3-19), 0.84 (s, CH_3-18), 0.75-1.96 (unassigned CH and CH_2), 2.07 (m, CH-16α), 2.42 (dd, J_1 = 8.5 Hz, J_2 = 19.3 Hz, CH-16β), 2.96 and 3.35 (2m, $\underline{CH_2}Ph$), 3.10 and 3.33 (2d of AB system, J = 8.0 Hz, CH_2N), 3.76 (s, OCH_3), 4.87 (dd, J_1 = 5.4 Hz and J_2 = 11.2 Hz, 1H, NCHCO), 7.22 (m, 3H of Ph), 7.29 (m, 2H of Ph), ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.7, 30.5, 31.5, 32.4, 33.7, 35.0, 35.1, 35.3, 35.8, 39.1, 40.8, 47.7, 51.3, 52.4, 53.3, 53.8, 55.9, 79.7, 127.1, 128.5 (2C), 128.7 (2C), 136.0, 157.3, 171.0, 221.2 ppm. LR-MS: calcd. for $C_{31}H_{42}NO_5$ $[M+H]^+$ 508.30, found 508.40.

2.1.6.5. Methyl [(3R,8R,9S,10S,13S,14S)-10,13-dimethyl-2',17-dioxohexadecahydro-3'H-spiro[cyclopenta[a]phenanthrene-3,5'-[1,3]oxazolidin]-3'-yl]acetate (9e)

Amorphous solid (32 % yield). R_f = 0.48 (hexanes/EtOAc, 1:1). IR (film) ν 1736 (C=O) cm^{-1}. ^1H NMR ($CDCl_3$) δ 0.82 (s, CH_3-19), 0.85 (s, CH_3-18), 0.80-1.97 (unassigned CH and CH_2), 2.10 (m, CH-16α), 2.43 (dd, J_1 = 8.4 Hz, J_2 = 19.3 Hz, CH-16β), 3.33 (s, CH_2N), 3.75 (s, OCH_3), 3.99 and 4.04 (2d, J = 18.0 Hz, NCHCO) ppm. ^{13}C NMR ($CDCl_3$) δ 11.4, 13.8, 20.2, 21.7, 27.8, 30.6, 31.5, 32.7, 33.8, 35.0, 35.4, 35.8, 39.3, 40.8, 45.0, 47.7, 51.3, 52.3, 53.9, 56.5, 79.6, 157.6, 169.0, 221.2 ppm. LR-MS: calcd. for $C_{24}H_{36}NO_5$ $[M+H]^+$ 418.25, found 418.30.

2.2. Biological evaluation

2.2.1. Inhibition of 17β-HSD3 (microsomal fraction of rat testes)

A microsomal preparation of rat testes was obtained using a previously described, but slightly modified procedure.[31] In brief, rat testes were homogenized on ice with a Polytron in cold phosphate buffer (20 mM KH_2PO_4, 0.25 M sucrose, 1 mM EDTA, pH 7.5) containing protease inhibitors mini-complete (Roche Diagnostics, Laval, QC, Canada) and the mixture was centrifuged at 12,500g for 15 min to remove the mitochondria, plasma membranes, and cell fragments. The supernatant was further centrifuged at 100,000g for 45 min using an ultracentrifuge equipped with a 70.1 Ti rotor. The microsomal pellet was washed three times with phosphate buffer and centrifuged at 100,000g for 15 min. All these operations were conducted at 4 ℃. The protein concentration of the supernatant was determined by the Bradford method using bovine serum albumin as standard. The enzymatic assay was

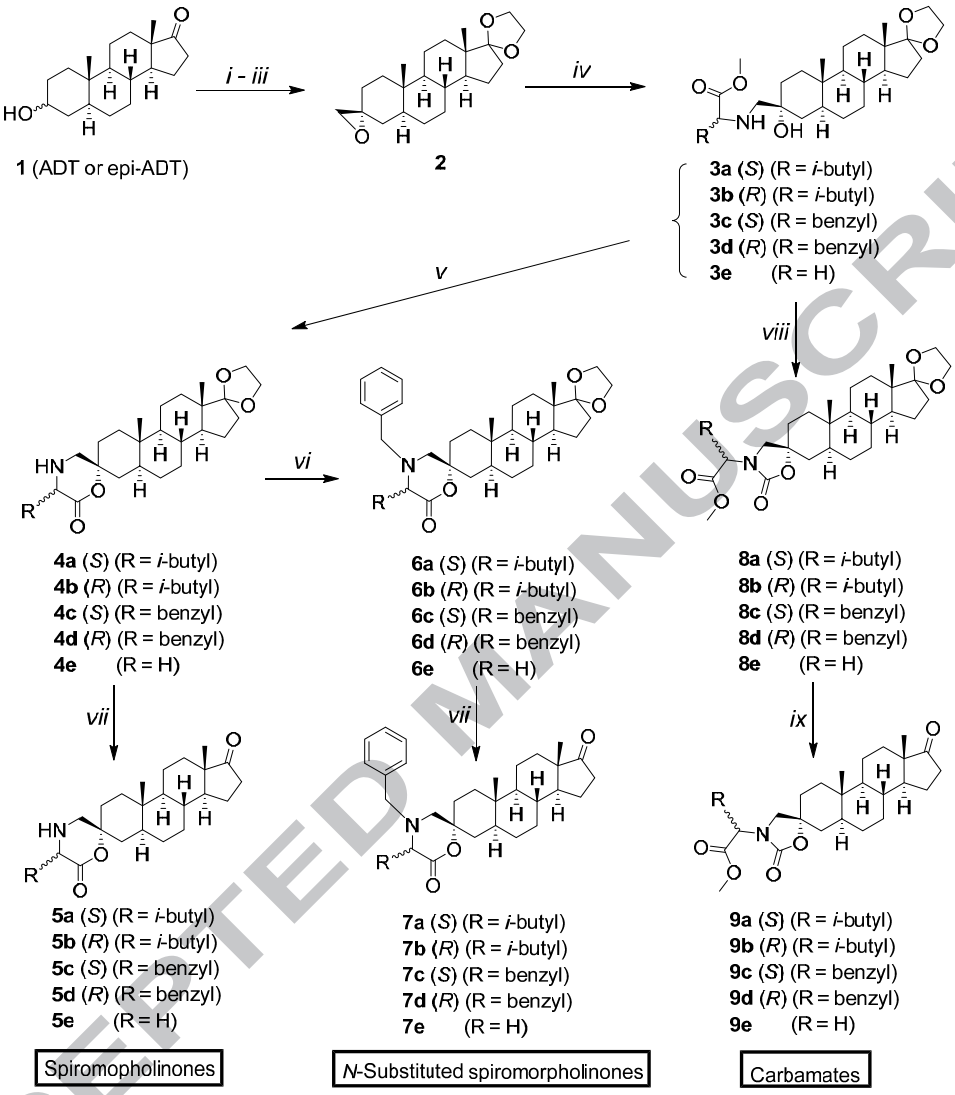
performed at 37 °C for 2 h in 1 mL of a solution containing 860 µL of 50 mM sodium phosphate buffer (pH 7.4, 20 % glycerol and 1 mM EDTA), 100 µL of 5 mM NADPH in phosphate buffer, 10 µL of 5 µM [^{14}C]-4-androstene-3,17-dione in ethanol (53.6 mCi/mmol, Perkin Elmer Life Sciences Inc., Boston, MA, USA), 10 µL of inhibitor dissolved in DMSO and 20 µL of diluted enzymatic source in phosphate buffer. Each inhibitor was assessed in triplicate. Afterwards, radiolabelled steroids were extracted from the reaction mixture with diethyl ether. The organic phase was evaporated to dryness with nitrogen stream. Residue was dissolved in 50 µL of DCM and dropped 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) as solvent system. Substrate ([^{14}C]-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 and then the percentage of inhibition were calculated.

2.2.2. Inhibition of 17β-HSD3 (intact LNCaP cells overexpressing 17β-HSD3)

LNCaP transfected cells (LNCaP[17β-HSD3]) kindly provided by IPSEN INNOVATION (France) were maintained at 37 °C under 5 % CO_2 humidified atmosphere. Cells were grown in RPMI-1640 medium supplemented (v/v) with 10% fetal bovine serum (FBS), 1 % penicillin/streptomycin, 2 mM L-glutamine, 4.5 g/L D-glucose, 10 mM Hépès, 1 mM sodium pyruvate and 250 µg/mL hygromycin. For enzymatic assays, the protocol medium had the same composition, but hygromycin, used to maintain the clone selection, was not included. LNCaP[17β-HSD3] cells were plated in 24-well culture at 15,000 cells per well, in protocol medium. After incubation for 3 days, 15 nM of [^{14}C]-4-androstene-3,17-dione and 10 µL of inhibitor dissolved in DMSO were added. The final DMSO concentration in each well was adjusted to 0.05 %. After 3 h, the culture medium was removed from wells. Steroids were extracted, separated by TLC, quantified, and percentage of inhibition calculated as described earlier for enzymatic assay with microsomal fraction of rat testes.

2.2.3. Proliferative activities on LAPC-4 (AR^+) cells

Androgen-sensitive human prostate cancer LAPC-4 cells were kindly provided by Robert E. Reiter from University of California (Los Angeles, CA, USA) and maintained at 37 °C under 5 % CO_2 humidified atmosphere. Cells were grown in RPMI-1640 medium supplemented (v/v) with 10 % fetal bovine serum (FBS), 1 % L-glutamine, 1 % insulin and 1 % penicillin/streptomycin. To determine the effect of novel compounds on cell proliferation, LAPC-4 cells were suspended with the medium supplemented with 5 % dextran-coated charcoal treated FBS rather than 10 % FBS to remove the remaining hormones. Triplicate cultures of 10,000 cells in a total of 100 µL medium in 96-well microtiter plates (Becton–Dickinson Company, Lincoln Park, NJ, USA) were pre incubated for 24 h at 37 °C under 5 % CO_2 humidified atmosphere. Tested compounds were dissolved in DMSO to prepare the stock solution of 10^{-2} M, the compounds were then diluted at several concentrations with culture medium, added to corresponding wells and incubated for 3 days. The final DMSO concentration in each well was adjusted to 0.05 %. Control wells were treated with vehicle DMSO. Quantification of cell growth was determined by MTS method, using CellTitter 96® AQueous Solution Cell Proliferation Assay (Promega, Nepean, ON, Canada) and following the manufacturer's instructions. The proliferative (androgenic) activity was expressed as difference between the cell proliferation (in %) caused by a given compound and the basal cell proliferation fixed at 100 %.



Scheme 1: Partial numbering for compound **1** and synthesis of target compounds **5a-5e**, **7a-7e** and **9a-9e**. Reagents and conditions: *i.* $HOCH_2CH_2OH$, *p*-TSA, toluene, reflux, overnight; *ii.* NMO, molecular sieves, TPAP, DCM, rt, 3 h; *iii.* $(CH_3)_3SOI$, NaH, DMSO/THF, rt; *iv.* Methyl ester of L- or D-Leucine, L- or D-phenylalanine or glycine methyl esters, MeOH, 90 °C, overnight; *v.* NaOMe, THF, rt, 2 h; *vi.* DIPEA, $C_6H_5CH_2Br$, DCM, 75 °C, 22 h; *vii.* H_2O:H_2SO_4, 1,4-dioxane, rt, 2 h to 5 h; *viii.* $(Cl_3CO)_2CO$; DCM; 0 °C to rt, 5 h; *ix.* HCl:MeOH (1:9), overnight.

3. Results and discussion

3.1. Chemical synthesis

3.1.1. Chemical synthesis of steroidal compounds (1st Series, Schemes 1 and 2)

The oxirane **2** (3α-O) was obtained from a well-known procedure already described in previous work (Scheme 1).[26] Briefly, the C-17 ketone of ADT or epi-ADT (**1**) was protected as a dioxolane group, the C-3 alcohol was oxidized and the corresponding ketone transformed to oxirane **2**. The inverse stereoisomer (3β-O) was the minor product isolated from the major product (stereoisomer 3α-O) after recrystallization in acetone. Aminolysis of oxirane **2** in methanol yielded the amino alcohols **3a**-**3e** with good to excellent yields (53-99 %). A similar aminolysis was previously done with other amino acids at position C-17 of an estrane nucleus by Rouillard et al.,[30] but in this work, since glycine methyl ester has high polarity, the authors failed to isolate the free amino acid and to use it for the aminolysis of the C-17-oxirane. To overcome this problem, we generated glycine methyl ester *in situ* from its hydrochloride salt, and the epoxide ring was opened successfully to form the glycine derivative **3e** in 53 % yield.

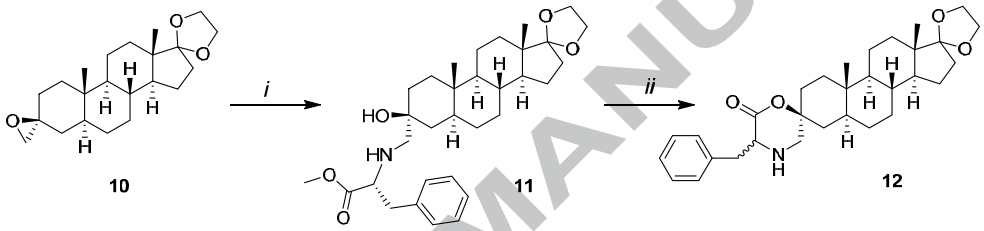
Table 1: Optimization of the lactonization process

Entry	Substrate[a]	Base	Base Equiv.	THF (mL)	T (°C)	Crude mass isolated (mg)	Total mass of pure *R/S*-isomers (mg)	Yield of *R/S*-isomers (%)	*R/S*-ratio[b]
1	**3d**	NaH	0.9	1.5	22	129	17	17	[53:4
2	**11**	NaH	1.1	5	22	50	17	24	[54:4
3	**11**	*t*-BuOK	1.1	5	22	66	12	17	[49:5
4	**11**	NaHMD	1.1	5	22	55	25	36	[66:3
5	**11**	LDA[c]	1.1	5	22	73	50	71	[52:4
6	**11**	LDA[c]	2.2	5	22	----[e]	----[e]	----[e]	----[e]
7	**11**	LDA[c]	3.3	5	22	----[e]	----[e]	----[e]	----[e]
8	**11**	LDA[d]	<1.1	5	22	70	23	33	[86:1
9	**11**	NaOMe	1.1	5	22	83	63	90	[73:2
10	**11**	NaOMe	0.5	5	0	82	69	98	[77:2
11	**11**	NaOMe	0.6	12	22	85	69	98	[80:2
12	**11**	NaOMe	0.5	12	-10	84[f]	42	60	[76:2

(a) We used 114 mg of starting 3α-OH-amino alcohol **3d** (Scheme 1) for entry 1 and 75 mg of 3β-OH-amino alcohol **11** (Scheme 2) for entries 2-12. (b) Two spots with variable importance on TLC plates. The ratio was calculated from ^{1}H NMR considering the integration of C-18 singlet. (c) LDA was generated separately in a large scale. (d) LDA was generated *in situ* in a small scale (345 µL total volume of solution). (e) Products were formed but degraded readily; TLC showed total degradation after 4 h of reaction time and no further work-up was done. (f) 25 % of starting amino alcohol was observed.

The spiromorpholinones **4a**-**4e** were prepared by the intramolecular transesterification of the amino alcohols **3a**-**3e**, respectively. In our first assay with 0.9 equivalent of sodium

hydride (Table 1, entry 1), we observed a poor overall reaction yield (17 %), probably due to undesired intermolecular side reactions. TLC analysis showed two spots for two major products, while ^{1}H NMR characterization showed fingerprints for both isolated products, with a very slight difference on the chemical shift of HC-α, CH_2N, $\underline{CH_2}Ph$ and 18-CH_3, suggesting that a racemization occurred. In fact, since the HC-α of the lactone ring is slightly acid, especially when the geminal group is a benzyl, racemization can occur in the presence of NaH used as a base. In order to optimize this crucial step and to reduce racemization, several cyclisation conditions were performed (Table 1, entries 2-12) using the amino alcohol **11**, obtained from the minor oxirane **10** (isomer 3β-O) (Scheme 2). The mixture of the two major products formed were isolated, purified by chromatography to give the yield, and the *R/S*-ratio of both isomers was measured from the integration of the ^{1}H NMR spectra, which showed two distinctive singlets for 18-CH_3 (Fig. 2A). Yield and *R/S*-ratio were not significantly improved using the same base (NaH) in a more diluted solution (entry 2). In order to investigate the effect of a more hindered base on the yield and *R/S*-ratio, we used potassium *tert*-butoxide (KO*t*-Bu), sodium bis(trimethylsilyl)amide (NaHMDS) and lithium diisopropylamide (LDA).



Scheme 2: Chemical synthesis of **11** and **12** from oxirane **10**. Reagents and conditions: *i.* D-Phenylalanine, MeOH, 90 °C, overnight; *ii.* NaH, KO*t*-Bu, NaHMDS, LDA or NaOMe, THF, -10 °C, 0 °C or 22 °C, 2 h (see Table 1).

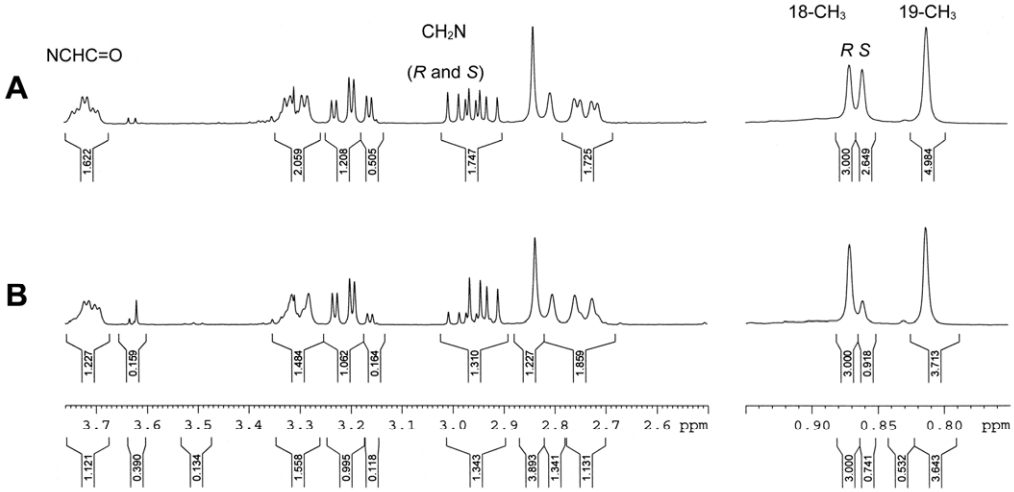


Figure 2: Fragments of ^{1}H NMR spectra showing that racemization occurs during the lactonization of **11** to **12**. Distinctive 18-CH_3 peaks of the two isomers (*R* and *S*) were used to calculate the *R/S*-ratio of the isomeric mixture; (A) [52:48] and (B) [77:23] (entries 5 and 10, respectively, Table 1).

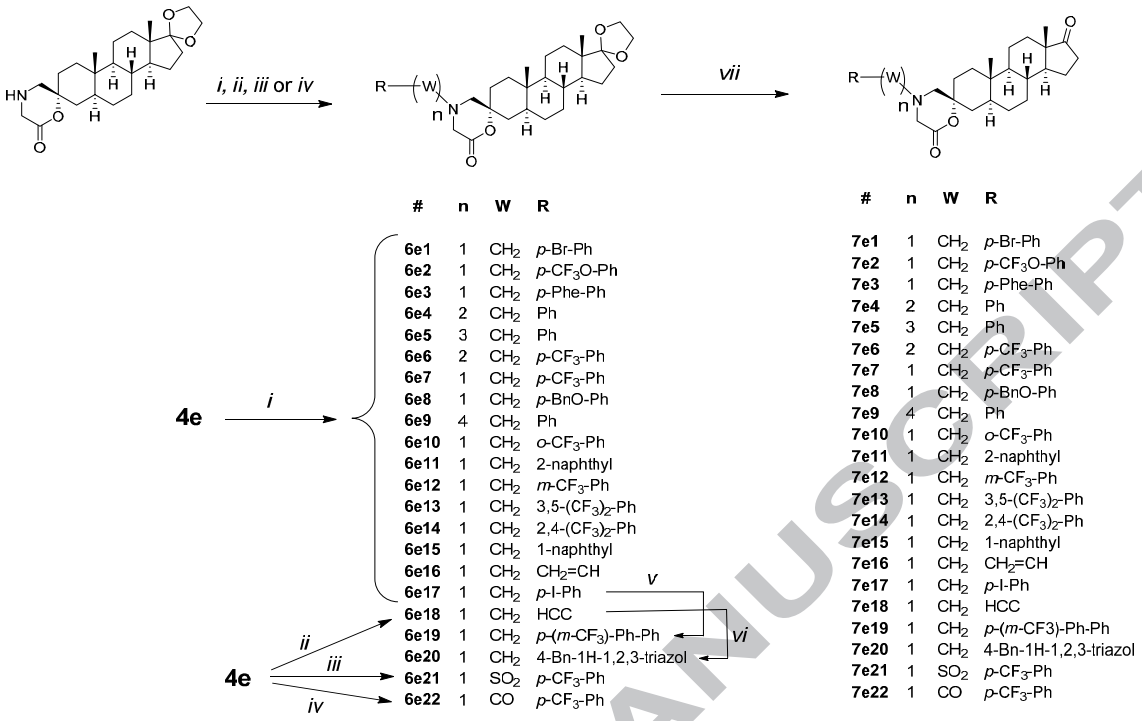
KO*t*-Bu caused a total racemization with only 17 % yield of both isomers (entry 3). Although the global yield was improved with NaHMDS ([66:34] *R/S*-ratio) and LDA ([52:48] *R/S*-ratio) when using these bases in the same conditions (36 % and 71 %, respectively)

(entries 4 and 5), the *R/S*-ratio did not increase. In fact a drastic loss of global yield was observed with 2.2 and 3.3 equivalents of LDA (entries 6 and 7), whereas a moderate yield of 33 % and a very good *R/S*-ratio were obtained with less than 1 equivalent of LDA (entry 8). Sodium methoxide (NaOMe) gave the best yield and least racemization (entries 9-12, Fig. 2B). Among all conditions tested, NaOMe (0.6 equivalent) used at 22 °C in 12 mL of THF (entry 11) was selected for the lactonization of **3a**-**3e** providing **4a**-**4e**. The high volume of THF and low quantity of base favor the intramolecular transesterification and avoid undesired intermolecular side reactions, thus providing the best yields of spiromorpholinones.

The *N*-benzylated spiromorpholinones **6a**-**6e** were obtained by a nucleophilic substitution of the secondary amines **4a**-**4e**. Finally, the C-17-dioxolane group of secondary amines **4a**-**4e** and tertiary amines **6a**-**6e** were hydrolyzed to provide the final compounds **5a**-**5e** and **7a**-**7e**. The spirocarbamates **8a**-**8e** were synthesized from the amino alcohols **3a**-**3e**, respectively, following a treatment with triphosgene.[26] Intermediates **8a**-**8e** were not isolated from the reaction mixture and their C-17-dioxolane group was directly hydrolyzed to obtain **9a**-**9e** in 32 to 73 % yields.

3.1.2. Chemical synthesis of steroidal compounds (2nd Series, Scheme 3)

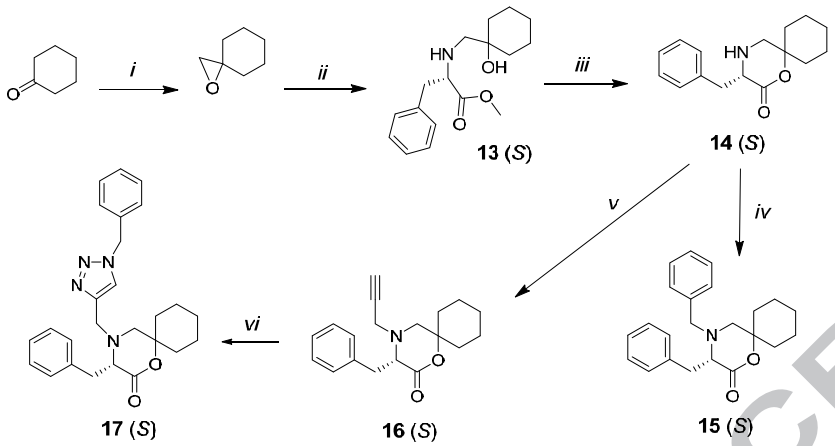
For our SAR study, different benzyl derivatives were also used to introduce molecular diversity on the spiromorpholinone ring. Starting from compound **4e**, a series of tertiary amines **6e1**-**6e18** were obtained by nucleophilic substitution (Scheme 3). We also investigated the Suzuki-Miyaura coupling[32] as an additional strategy to add diversity. In fact, when compound **6e17** was treated with 3-(trifluoromethyl)benzeneboronic acid, no reaction was observed whith $Pd(OAc)_2$ as catalyst, but using 1,1'-bis(diphenylphosphino)ferrocene-palladium(II)dichloride ($Pd(dppf)Cl_2$) yielded the biaryl compound **6e19** in 23 % yield. This poor yield was probably due to the opening of the spirocycle E in aqueous Na_2CO_3 solution, but this reaction could be optimized. Starting from **6e18** we also experienced the "click chemistry" as a mean to diversify the *N*-substituent. Thus, the steroidal triazolic compound **6e20** was obtained by 1,3-dipolar cycloaddition of the terminal alkyne **6e18** with benzyl azide.[33] It is well known that the 1,4-triazole regioisomer is exclusively obtained from catalysis with copper (I).[34-36] The sulfonamide **6e21** and the carboxamide **6e22** were also synthesized by nucleophilic substitution under conditions depicted in Scheme 3 and using benzyl sulfonyl chloride and benzoyl chloride, respectively. The C-17-dioxolane group of the tertiary amines **6e1**-**6e22** was finally hydrolyzed to generate the corresponding 17-keto products **7e1**-**7e22**.



Scheme 3: Reagents and conditions. *i.* DIPEA, X-Ph-CH_2Br, DCM, 65-75 °C, 22h; *ii.* K_2CO_3, propargyl bromide, DMF, reflux, overnight; *iii.* TEA, 4-(trifluoromethyl)benzenesulfonylchloride, DCM, rt, 3 h; *iv*. TEA, 4-(trifluoromethyl)benzoylchloride, DCM, rt, 3 h; *v.* 3-(Trifluoromethyl)phenylboronic acid, Pd(dppf)Cl_2, Na_2CO_3, H_2O:1,4-dioxane (3:1), 110 °C, 5 h; *vi.* Benzyl azide, aqueous $CuSO_4.5H_2O$, sodium ascorbate, *n*-BuOH:H_2O (1:1), rt, 3 h; *vii.* H_2O:H_2SO_4 (95:5), 1,4-dioxane, rt, 2 to 5 h.

3.1.3. Chemical synthesis of non-steroidal compounds (Scheme 4)

In order to verify the importance of the steroid scaffold for the inhibition of 17β-HSD3, non-steroidal spiromorpholinones **14**, **15** and **17** were synthesized starting from cyclohexanone and following the sequence of reactions depicted in Scheme 4. Compound **14** was also used to prepare its propargylic derivative **16**, which was submitted to a 1,3-dipolar cycloaddition, under the same conditions used for the preparation of compound **6e20**, to generate compound **17**. The preparation of **17** using "click chemistry" represents an interesting additional strategy to the nucleophilic substitution for introducing molecular diversity on the spiromorpholinone ring.



Scheme 4: Reagents and conditions. *i.* $(CH_3)_3SOI$, NaH, DMSO/THF, rt, 24 h; *ii.* L-Phenylalanine methyl ester, MeOH, 90 °C, overnight; *iii.* MeONa, THF, rt, 2 h; *iv.* DIPEA, $C_6H_5CH_2Br$, DCM, 75 °C, 22 h; *v.* K_2CO_3, propargyl bromide, DMF, reflux, overnight; *vi.* Benzyl azide, aqueous $CuSO_4.5H_2O$, sodium ascorbate, *n*-BuOH:H_2O (1:1), rt, 3 h.

3.2. Biological activities

3.2.1. Inhibitory activity on 17β-HSD3 (screening study)

With the knowledge that the presence of a hydrophobic group on the C-3 position of ADT enhances the inhibition of 17β-HSD3, our choice of a rigid spirocycle at this position was our strategy to control the orientations of hydrophobic substituents. The amino acid used in the elaboration of the spirocycle at C-3 contains two diversifying factors, the first being the amino acid residue and the second being its stereochemistry. Finally, the presence of a free amino group in the spiromorpholinone moiety is a third point of diversification. Thus, the new synthesized compounds can be grouped according to whether the stereochemistry on the C-α of the starting amino acid methyl ester was (*S*) or (*R*), whether they contain a secondary amino group on the spiromorpholinone (compounds **5a**-**5e**), whether the amino group is substituted (compounds **7a**-**7e**, **7e1**-**7e22**) and finally whether it is a non-steroidal nucleus compound **14-17**. For screening purposes, the ability of all these compounds to inhibit the 17β-HSD3 contained in a microsomal fraction of homogenated rat testes was assessed by measuring the amount of [^{14}C]-T formed from natural substrate [^{14}C]-Δ^4–dione in the presence of NADPH as cofactor, and the result expressed as percent of inhibitory activity for each given compound (Tables 2 and 3).

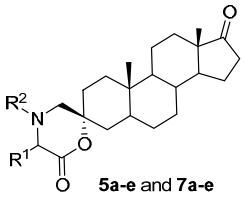
In the series **5a**-**5d** (secondary amines), the glycine derivative **5e** is clearly the less potent inhibitor of 17β-HSD3 and compounds with the (*S*) stereochemistry on the spiromorpholinone are better inhibitors than their corresponding (*R*) analogs. In fact compound **5a** (39.4 % inhibition at 0.1 µM) with (*S*) stereochemistry is better than the analog **5b** (17.9 % inhibition at 0.1 µM) with (*R*) stereochemistry. Similarly, compound **5c** (48.8 % inhibition at 0.1 µM) is better than **5d** (18.5 % inhibition at 0.1 µM). This tendency is maintained in the *N*-benzylated spiromorpholinone series **7a**-**7d** (tertiary amines). In fact, compounds **7a** and **7c** (62.9 % and 58.2 % inhibition at 0.1 µM) with (*S*) stereochemistry are better than the analogs **7b** and **7d** (45.9 % and 25.6 % inhibition at 0.1 µM) with the (*R*) stereochemistry. Interestingly, the mono-benzyl glycine derivative **7e** is as potent an inhibitor

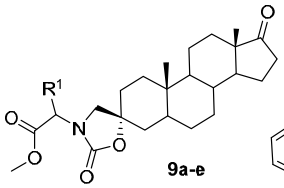
(56.0% inhibition at 0.1 μM) as the two-benzyl derivative **7c** (58.2% inhibition at 0.1 μM). As we mentioned in our previous report supported by the crystalline structure of **9b**,[28] the stereochemistry on the C-α of the amino acid methyl ester moiety does not significantly affect the inhibitory potency of spirocarbamates **9a-9d**. This is probably due to the high degree of liberty of the methyl 4-methylpentanoate (**9a** and **9b**) and methyl 3-phenylpropanoate (**9c** and **9d**) that allows the free rotation around the exocyclic bond of the *N* atom. Thus, compounds **9a** and **9c** (57.6 % and 48.3 % inhibition at 0.1 μM) with the (*S*) stereochemistry are not significantly different from their analogues **9b** and **9d** (55.4 % and 56.1 % inhibition at 0.1 μM) with the (*R*) stereochemistry. As observed above with the spiromorpholinone **5e** (secondary amine), glycine derivative **9e** is the less potent inhibitor in the series of spirocarbamates.

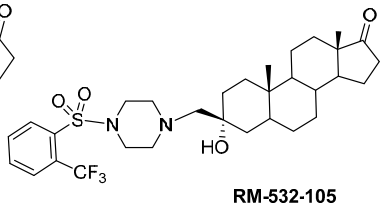
The *N*-benzylated compounds **7a**-**7e** showed better 17β-HSD3 inhibition than their respective NH-analogs **5a**-**5e**, suggesting the importance of a substituent at that position (tertiary amines instead of secondary amines). Thus, the secondary amines **5a** and **5b** (39.4 % and 17.9 % inhibition at 0.1 μM) are weaker inhibitors than the tertiary analogs **7a** and **7b** (62.9 % and 45.9 % inhibition at 0.1 μM). In our screening assay, many of the new compounds have an inhibitory potency that is comparable to the reference inhibitors RM-532-105 and D-5-2 (47.3 % and 51.5 % inhibition at 0.1 μM). As examples, the spiromorpholinones **7a, 7c** and **7e** are even better inhibitors (62.9 %, 58.2 % and 56.0 % inhibition at 0.1 μM, respectively) than the two reference compounds.

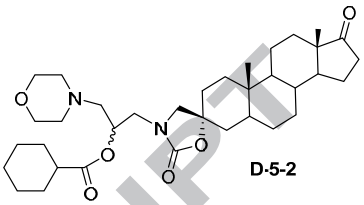
The clogP value seems to correlate with the inhibitory potential in some cases. For example, glycine-derived spiromorpholinone **5e** (clogP = 3.29; 44.7 % inhibition at 1 μM) and spirocarbamate **9e** (clogP = 3.72; 54.6 % inhibition at 1 μM) that have the lowest clogP values are the less potent inhibitors in these series. In fact, compound **5e** (clogP = 3.29; 0 % inhibition at 0.1 μM) is a very poor inhibitor when compared to its *N*-benzylated analog **7e** (clogP = 5.60; 56.0 % inhibition at 0.1 μM). In other cases, however, the clogP values did not correlate with the inhibitory activity. For example, the mono-benzyl and di-benzyl derivatives **7e** and **7c** produced the same inhibitory activity (56.0% and 58.2% at 0.1 μM) although different clogP values (5.60 and 7.77, respectively). Thus, the hydrophobicity of the R^1 and R^2 groups represented by clogP is not the only parameter that correlates with the inhibitor potency, and other parameters, such as the orientation of the substituents (R^1 and R^2) and steric hindrance, should also be taken in consideration.

Table 2: Biological results for the first library of compounds (spiromorpholinones **5a-e**, **7a-e** and spirocarbamates **9a-e)**









					Assay 1		Assay 2			
#	***R/S*[a]**	**R^1**	**R^2**	**clogP[b]**	**17β-HSD3 inhibition (%)[c, d]**		**17β-HSD3 inhibition (%)[c, e]**		**Androgenicity (%)[f]**	
					0.1 µM	**1 µM**	**1 µM**	**10 µM**	**0.1 µM**	**1 µM**
5a	*S*	*i*Bu	H	5.21	39.4 ± 2.4	84.9 ± 1.1	69.3 ± 3.4	83.8 ± 2.6	14.5 ± 6.3	11.1 ± 10
5b	*R*	*i*Bu	H	5.21	17.9 ± 6.8	---	50.2 ± 2.4	77.7 ± 3.5	7.9 ± 2.2	23.6 ± 3.1
5c	*S*	Bn	H	5.66	48.8 ± 2.0	92.0 ± 1.6	75.0 ± 3.3	88.2 ± 1.6	3.9 ± 1.7	36.5 ± 1.6
5d	*R*	Bn	H	5.66	18.5 ± 26.9	---	63.2 ± 5.8	70.6 ± 3.1	0	15.3 ± 1.3
5e	-	H	H	3.49	0	---	44.7 ± 2.8	82.0 ± 3.5	9.7 ± 0.2	32 ± 10.5
7a	*S*	*i*Bu	Bn	7.32	62.9 ± 5.2	93.1 ± 0.6	77.3 ± 5.4	87.1 ± 3.1	8.4 ± 2.4	44.2 ± 8.4
7b	*R*	*i*Bu	Bn	7.32	45.9 ± 5.3	---	56.5 ± 1.3	82.3 ± 3.1	6.2 ± 7.3	47.7 ± 11.1
7c	*S*	Bn	Bn	7.77	58.2 ± 1.7	90.4 ± 0.7	81.0 ± 1.4	83.6 ± 3.1	6.1 ± 2.1	53.7 ± 4
7d	*R*	Bn	Bn	7.77	25.6 ± 5.7	87.3 ± 2.8	61.7 ± 4.4	84.7 ± 3.4	9.9 ± 5	50.1 ± 9
7e	-	H	Bn	5.60	56.0 ± 6.8	89.2 ± 1.8	90.9 ± 1.3	95.2 ± 2.2	8.1 ± 2.1	30.5 ± 11.1
9a	*S*	*i*Bu	---	5.45	57.6 ± 3.8	93.6 ± 1.2	82.3 ± 1.0	88.4 ± 3.5	0	8.4 ± 7.8
9b	*R*	*i*Bu	---	5.45	55.4 ± 3.2	73.6 ± 1.0	76.9 ± 1.8	67.8 ± 4.8	1.5 ± 1.4	21.9 ± 2.1
9c	*S*	Bn	---	5.89	48.3 ± 1.9	---	72.1 ±5.8	87.7 ± 2.0	0	0
9d	*R*	Bn	---	5.89	56.1 ± 2.6	---	74.3 ± 7.3	80.8 ± 3.5	0	29.2 ± 4.2
9e	-	H	---	3.72	9.0 ± 6.4	---	54.6 ± 1.4	80.8 ± 5.6	11 ± 3.5	33.8 ± 1
RM-532-105[g]	-	---	---	6.45	47.3 ± 12.4	92.1 ± 0.4	96.4 ± 0.6	97.1 ± 0.2	0	0
D-5-2[g]	-	---	---	5.92	51.5 ± 2.2	93.1 ± 1.1	93.9 ± 0.9	97.2 ± 0.4	4.8 ± 1.3	9.4 ± 15.5

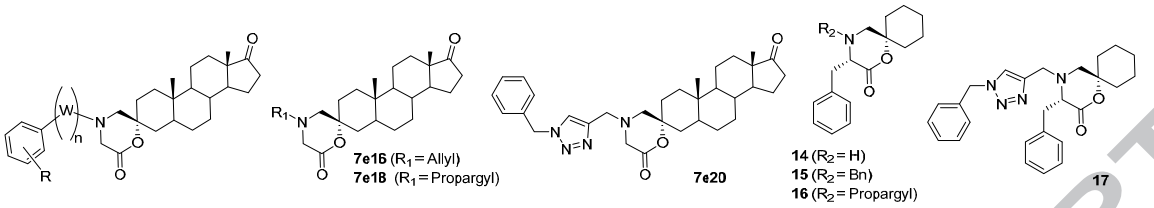
(a) (*S*) or (*R*) Stereochemistry on the asymmetric carbone in the spiroheterocycle. (b) clogP calculated with ChemDraw Ultra 9.0. (c) Transformation of [^{14}C]-Δ^4-dione (50 nM) to [^{14}C]-T by 17β-HSD3 of homogenated rat testes. (d) 18 % of initial enzyme transformation. (e) 4 % of initial enzyme transformation. (f) The androgenicity is the difference between the LAPC-4 cell proliferation (in %) caused by a given compound and the basal proliferation fixed at 100 %. (g) Known inhibitors of 17β-HSD3: D-5-2 (see reference 26) and RM-532-105 (see compound **15b** in reference 21).

Because of the important increase of 17β-HSD3 inhibition due to the *N*-benzylation of compounds **5e** to **7e** (from 0 % to 56.0 % inhibition at 0.1 µM), we decided to diversify the spiromorpholinone **5e** with various alkyl and aryl groups (compounds **7e1**-**7e20** in Table 3). The compounds in this second library were studied regarding the nature of the substituent (R), its position (*ortho*, *meta* or *para*) on the phenyl group and the length of the methylenic chain (n). In order to obtain a less flexible spacer between the *N*-atom and the aryl group, the methylene group was replaced by a sulfonyl (SO_2) in compound **7e21** and a carbonyl (C=O) in compound **7e22**. The results with compounds **7e1-7e9** showed that they have comparable 17β-HSD3 inhibitory potency to our reference inhibitor RM-532-105. Although the 0.1 µM concentration tested did not allow a good comparison of compounds **7e1-7e9**, as their inhibition potential was quite similar, the nature of the substituent (R) on the phenyl group and the length of the methylenic chain (n) slightly affect the inhibition potency (Table 3). Furthermore, the clogP did not correlate with enzyme inhibition. In fact, compound **7e** (R = H, n = 1) with 80.8 % inhibition at 0.1 µM inhibited 17β-HSD3 better than **7e4** (R = H, n = 2) with 78.5 % and **7e5** (R = H, n = 3) with 74.9 % inhibition. The same tendency was observed with compounds **7e7** (R = *p*-CF_3, n = 1) with 83.4 % inhibition at 0.1 µM, compared to **7e6** (R = *p*-CF_3, n = 2) with 70.7 % inhibition at 0.1 µM. Following these observations, we decided to further investigate the inhibitory activity optimization with a single methylene (n = 1) spacer.

Compounds **7e10-7e20** include *N*-benzyl derivatives with different substituents (R) and orientations (*ortho*, *meta* or *para*) on the phenyl ring. In order to refine our lead compound selection, the inhibitory potency was compared at a lower concentration (0.01 µM). At that specific concentration, the *ortho*-substituted compound **7e10** (24.4 % inhibition) is better than it *meta* analog **7e12** (17.7 % inhibition). A disubstituted (CF_3) phenyl ring (**7e13** and **7e14**) and a fused naphthyl ring (**7e11** and **7e15**) did not improve the inhibitory potency at 0.01 µM. Adding a *para*-iodide on the benzyl group (compound **7e17**) reduced its inhibitory potency, but adding a *para* CF_3-phenyl on the benzyl (compound **7e19**) provided an inhibition similar to that of the lead compound **7e** (26.1 % and 27.2 % respectively). In this series of *N*-benzylated derivatives, the clogP values did not correlate with the enzyme inhibition. For example, the mono and di-CF_3-benzyl compounds **7e12**, **7e13** and **7e14** (clogP = 6.52, 7.44 and 7.44) are less potent (17.7, 15.1 and 18.3 % inhibition at 0.01 µM) than unsubstituted benzyl compound **7e** (clogP = 5.60 and 27.2 % inhibition at 0.01 µM). The less hydrophobic unbenzylated compounds **7e16** and **7e18** (clogP = 4.56 and 4.58) are however clearly less potent inhibitors (0.1 and 5.1 % at 0.01 µM). Two compounds (**7e21** and **7e22**) in which the CH_2 (W) group is respectively replaced by a sulfonyl group or a carbonyl group were also tested. Interestingly, at a concentration of 0.01 µM, the sulfonamide compound **7e21** (32.9 % inhibition) and carboxamide compound **7e22** (24.3 % inhibition) inhibited 17β-HSD3 compared to the reference compound RM-532-105 (32.4 % inhibition).

In order to verify the role of the ADT scaffold, we also assessed the inhibitory potency of non-steroidal spiromorpholinones **14**-**17**. These compounds did not inhibit the conversion of Δ^4-dione into T by the 17β-HSD3 (6.2, 24.2, 0 and 9.4 % inhibition at 0.1 µM), proving the usefulness of the androgen core. They are also compounds having the lower clogP values (2.75, 4.86, 3.34 and 4.95, respectively). This result suggests that the steroidal inhibitor competes better with the natural steroid substrate (Δ^4-dione) than the non-steroidal derivative, which probably produces fewer interactions within the enzyme catalytic site. In fact, the non-steroidal compounds did not possess a carbonyl group mimicking the C-17 ketone of the natural substrate, and this carbonyl is necessary for good inhibition of 17β-HSD3.[26]

Table 3: Biological results for the second library of compounds (*N*-derivatives **7e1-7e22** and **14-17**)



R	#	n	w	clogP[a]	17β-HSD3 inhibition (%)[b]				Androgenicity[c]	
					0.01 µM	0.1 µM	1 µM	10 µM	0.1 µM	1 µM
Assay 3[d]										
H	**7e**	**1**	CH_2	5.60	----	80.8 ± 0.5	91.6 ± 1.3	94.3 ± 1.1	----	----
p-Br	**7e1**	**1**	CH_2	6.43	----	79.1 ± 2.7	92.9 ± 1.6	91.6 ± 1.6	21.8 ± 4.5	45.7 ± 4.5
p-OCF_3	**7e2**	**1**	CH_2	7.13	----	79.0 ± 2.4	93.8 ± 0.9	95.7 ± 2.2	45.9 ± 4.0	74.9 ± 5.2
p-Ph	**7e3**	**1**	CH_2	7.27	----	81.9 ± 3.6	93.1 ± 0.2	95.2 ± 0.7	47.7 ± 4.7	55.1 ± 5.7
H	**7e4**	**2**	CH_2	5.88	----	78.5 ± 1.5	92.2 ± 1.3	95.2 ± 1.1	48.9 ± 2.0	79.7 ± 2.2
H	**7e5**	**3**	CH_2	6.30	----	74.9 ± 2.3	90.5 ± 0.9	92.3 ± 0.6	64.9 ± 5.3	55.5 ± 5.2
p-CF_3	**7e6**	**2**	CH_2	6.80	----	70.7 ± 1.1	92.1 ± 0.8	93.3 ± 1.3	40.2 ± 1.4	76.1 ± 4.9
p-CF_3	**7e7**	**1**	CH_2	6.52	----	83.4 ± 2.2	94.0 ± 0.2	91.2 ± 2.1	12.3 ± 3.9	31.7 ± 5.2
p-OBn	**7e8**	**1**	CH_2	7.20	----	80.4 ± 1.3	90.8 ± 2.0	93.4 ± 0.7	0 ± 6.1	16.7 ± 6.0
H	**7e9**	**4**	CH_2	6.71	----	79.9 ± 2.4	91.1 ± 0.1	91.3 ± 1.4	5.3 ± 5.4	19.2 ± 5.2
----	**14**	**-**	**----**	2.75	----	6.2 ± 6.3	19.5 ± 3.8	20.6 ± 1.3	----	----
----	**15**	**-**	**----**	4.86	----	24.2 ± 3.2	24.3 ± 4.2	29.2 ± 2.7	----	----
----	**DHT**	**-**	**----**	3.85	----	----	----	----	28.8 ± 4.7	51.5 ± 8.0
RM-532-105[e]		**-**	**----**	6.45	----	89.4 ± 2.3	94.4 ± 1.6	94.0 ± 0.9	11.1 ± 4.0	0.4 ± 4.9
Assay 4[f]										
H	**7e**	**1**	CH_2	5.60	27.2 ± 14.3	77.5 ± 2.2	95.0 ± 1.3	----	----	----
o-CF_3	**7e10**	**1**	CH_2	6.52	24.4 ± 8.3	77.9 ± 3.5	83.0 ± 0.6	----	0.5 ± 8.9	36.9 ± 2.3
2-naphthyl	**7e11**	**1**	CH_2	6.60	0	88.3 ± 7.1	93.2 ± 2.3	----	22.3 ± 5.8	16.5 ± 2.2
m-CF_3	**7e12**	**1**	CH_2	6.52	17.7 ± 6.8	54.1 ± 7.2	94.2 ± 1.1	----	0 ± 8.2	27.0 ± 9.7
3,5-bis(CF_3)	**7e13**	**1**	CH_2	7.44	15.1 ± 3.0	61.6 ± 13.5	94.4 ± 1.2	----	12.0 ± 4.6	5.9 ± 19.7
2,4-bis(CF_3)	**7e14**	**1**	CH_2	7.44	18.3 ± 41.9	64.1 ± 4.5	89.6 ± 3.5	----	0 ± 2.6	27.0 ± 3.5
1-naphthyl	**7e15**	**1**	CH_2	6.60	12.8 ± 2.8	79.1 ± 5.2	94.5 ± 0.8	----	3.8 ± 1.2	9.3 ± 0.3
----	**7e16**	**-**	**----**	4.56	8.4 ± 12	48.6 ± 7.2	89.0 ± 0.5	----	31.0 ± 4.5	11.3 ± 4.8
p-I	**7e17**	**1**	CH_2	6.96	12.2 ± 3.8	79.0 ± 2.6	95.3 ± 0.6	----	0.9 ± 3.7	15.9 ± 2.4
----	**7e18**	**1**	**----**	4.08	0.1 ± 4.1	34.1 ± 2.6	80.7 ± 1.4	----	20.1 ± 3.5	25.5 ± 7.8
p-(*m*-CF_3-phenyl)	**7e19**	**1**	CH_2	8.19	26.1 ± 11.6	84.8 ± 3.5	84.9 ± 2.2	----	13.5 ± 4.2	14.3 ± 4.2
----	**7e20**	**-**	**----**	5.70	5.1 ± 3.1	42.2 ± 7.9	85.3 ± 3.0	----	10.0 ± 3.3	17.7 ± 6.6
p-CF_3	**7e21**	**1**	SO_2	5.86	32.9 ± 7.6	48.6 ± 10.9	85.1 ± 1.8	----	0 ± 3.1	0 ± 2.6
p-CF_3	**7e22**	**1**	CO	5.96	24.3 ± 15.8	45.4 ± 9.7	87.1 ± 2.9	----	0 ± 3.8	0 ± 3.3
----	**16**	**-**	**----**	3.34	0	0	0	----	11.1 ± 7.4	0 ± 3.5
----	**17**	**-**	----	4.95	0	9.4 ± 1.6	13.3 ± 31.4	----	2.5 ± 3.5	12.4 ± 4.0
----	**DHT**	**-**	**----**	3.85	----	----	----	----	28.8 ± 4.7	51.5 ± 8.0
RM-532-105[e]		**-**	**----**	6.45	32.4 ± 3.2	75.7 ± 6.3	95.2 ± 0.2	----	11.1 ± 4.0	0.4 ± 4.9

(a) clogP calculated with ChemDraw Ultra 9.0. (b) Transformation of [^{14}C]-Δ^4-dione (50 nM) to [^{14}C]-T by 17β-HSD3 of homogenated rat testes. (c) The androgenicity is the difference between the LAPC-4 cell proliferation (in %) caused by a given compound and the basal proliferation fixed at 100 %. (d) 33 % of initial enzyme transformation. (e) Known inhibitor of 17β-HSD3 (see compound **15b** in reference 21). (f) 18 % of initial enzyme transformation.

3.2.2. Proliferative (androgenic) activity on LAPC-4 cells (screening study)

An inhibitor of androgen biosynthesis for prostate cancer therapy should be devoid of any proliferative (androgenic) activity. Since most of our compounds similarly inhibited 17β-HSD3, we added another criterion, the androgenicity, to discriminate the new synthesized molecules. To achieve this, a prostate cancer cell line (LAPC-4 cells) expressing a non-mutated AR was chosen and all compounds were screened for their ability to stimulate cell proliferation or not (Tables 2 and 3). The 17β-HSD3 inhibitor RM-532-105 and the natural androgen DHT were added as reference compounds and tested at two concentrations (0.1 and 1 μM). The androgenicity (in %) is the difference between the cell proliferation caused by a given inhibitor and the basal cell proliferation fixed at 100 %. Interestingly, only the sulfonamide derivative **7e21** and the carboxamide derivative **7e22**, which are the most rigid compounds in the *N*-substituted series, did not exhibit any androgenic (proliferative) effect at the two concentrations tested. These compounds were selected for further *in vitro* studies.

3.2.3. IC_{50} values (homogenated rat testes) for non-androgenic compounds 7e21 and 7e22

The IC_{50} values of **7e21** and **7e22** have been determined for the inhibition of 17β-HSD3 and compared to the reference compound RM-532-105, Δ^4-dione and ADT (Table 4, see also Fig. S1-S3 in Supplementary data). Although, both compounds are less potent than RM-532-105 (IC_{50} = 13 nM), **7e21** (IC_{50} = 28 nM) is 6-fold better than the unlabeled natural substrate Δ^4-dione (IC_{50} = 169 nM) and **7e22** (IC_{50} = 88 nM) is almost 2-fold better than Δ^4-dione.

Table 4: Inhibition of 17β-HSD3 by compounds **7e21**, **7e22**, RM-532-105, ADT and Δ^4-dione

#	IC_{50} (nM)[a]			
	Assay 1	**Assay 2**	**Assay 3**	**Average ± SEM**
7e21[b]	19	36	-	28 ± 9
7e22	69	106	-	88 ± 19
RM -532-105	16	~10	14	13 ± 2
ADT	69	111	-	90 ± 21
Δ^4-dione	122	216	-	169 ± 47

(a) Transformation of [^{14}C]-Δ^4-dione to [^{14}C]-T by rat testicular 17β-HSD3 (microsomal fraction) (see Fig. S1-S3, Sections 5-7 in Supplementary data). (b) Contamined with 30 % of compound **19** (see Section 3 in Supplementary data).

3.2.4. Inhibitory activity on intact LNCaP[17β-HSD3] cells

Lead compounds **7e21** and **7e22** were evaluated for their ability to inhibit 17β-HSD3 activity found in transfected LNCaP cells overexpressing 17β-HSD3, by measuring the amount of labeled T formed from labeled natural substrate Δ^4-dione. As previously reported,[37-39] wild type LNCaP cells only very slightly transform Δ^4-dione into T because the former steroid is rapidly reduced by 5α-reductase into 5α-androstane-3,17-dione. For this reason, LNCaP cells transfected with the *c*-DNA vector for 17β-HSD3 (LNCaP[17β-HSD3]) are a better cell model to test 17β-HSD3 inhibitors than wild type LNCaP cells. Contrary to a microsomal fraction of homogenated rat testes used, where the enzyme is readily available to the inhibitor, the intact cell model has the advantage of being closer to what actually happens *in vivo*, where the inhibitors must cross the cell barrier. In this intact cell model, the inhibitory activity of all compounds decreased (Fig. 3 and Supplementary data) when compared to the earlier reported activity in homogenated rat testes. Weak inhibitory activities (10 and 40 %) were obtained at 1 μM for **7e21** and **7e22**, respectively. The carboxamide derivative is however a better inhibitor than the sulfonamide derivative, but it is less potent than RM-532-105 (100 % inhibition at 0.5 μM). Thus, the estimated IC_{50} values for these two inhibitors increased from a nanomolar to a micromolar level. This might be due in part to the inhibitor's difficulty to cross the LNCaP[17β-HSD3] cell membrane. Optimization of the balance between hydrophobicity (for 17β-HSD3 inhibition) and hydrophilicity (for cell penetration) could lead us to another generation of molecules that are able to maintain their inhibitory activity in intact cells.

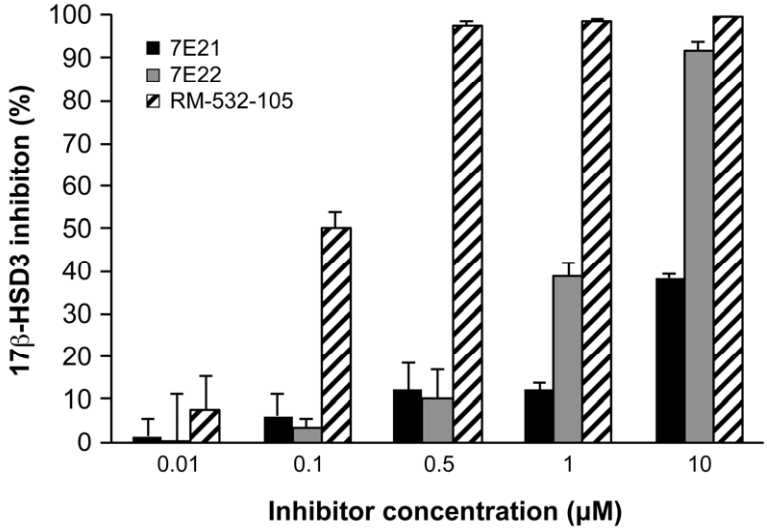


Figure 3: Inhibitory activity of lead compounds **7e21** and **7e22** in intact LNCaP[17β-HSD3] cells.

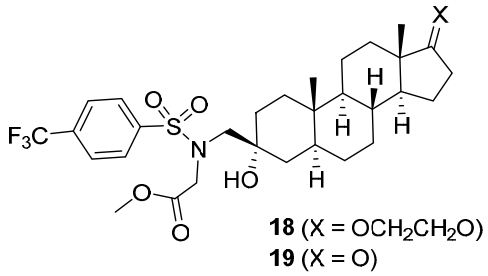


Figure 4: Chemical structure of the residual contaminant **19** and its intermediate **18** (also see Scheme S2 in Supplementary data for the synthesis of **18** and **19**).

4. Conclusions

Thirty-two 3-spiromorpholinone ADT derivatives, five 3-spirocarbamate ADT derivatives and four non-steroidal spiromorpholinones were synthesized. The chemical structure of all the final products and intermediates were determined from ^{1}H NMR, ^{13}C NMR, IR and LR-MS. All target compounds were tested for their ability to inhibit the transformation of [^{14}C]-Δ^4-dione (50 nM) to [^{14}C]-T by 17β-HSD3 and their androgenic effect was assessed on androgen-sensitive LAPC-4 cells. Structure-activity relationship analysis showed the necessity of the steroidal (ADT) backbone in addition to a hydrophobic moiety for 17β-HSD3 inhibition. Stereochemistry, clogP and steric hindrance of the E-ring substituent all affect the inhibition in a complex way. Although we did not succeed in generating an inhibitor that was more potent than RM-532-105, different synthetic strategies were developed to introduce new elements of molecular diversity added on a steroid backbone as a rigid spiromorpholinone E-ring, thus providing some interesting optimization alternatives. Our study did however enable us to identify two compounds (the sulfonamide **7e21** and the amide **7e22**) with inhibitory potency on rat homogenated testes that is comparable to the reference compound RM-532-105 (IC_{50} = 28, 88 and 13 nM, respectively) and does not show any proliferative (androgenic) activity. However, the inhibitory activity of all compounds decreased in intact cell models (LNCaP[17β-HSD3]). Structural modifications are thus necessary to improve the 17β-HSD3 inhibitory activity of spiromorpholinone ADT derivatives **7e21** and **7e22** in intact cells and to identify an analogue compound that could be a good candidate for further *in vitro* and *in vivo* studies.

Supplementary data

Experimental details for the chemical synthesis and characterization of compound **11**, non-steroidal compounds **13-17**, and the residual contaminant (compound **19**). ^{1}H and ^{13}C NMR spectra of compounds **5a-5e**, **7a-7e**, **7e1-7e22**, **9a-9e** and **14-17** (tested as 17β-HSD3 inhibitors). 17β-HSD3 inhibitory curves used for determination of IC_{50} values of compounds **7e21**, **7e22**, RM-532-105, ADT and Δ^4-dione tested in homogenated rat testes and reported in Table 4. 17β-HSD3 inhibitory data for compounds tested at 1 and 5 µM in intact LNCaP[17β-HSD3] cells.

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References

1. Siegel, R.; Ma, J.; Zou, Z.; Jemal, A. Cancer statistics, 2014. CA: a cancer journal for Clinicians 2014, 64, 9-29.
2. Labrie, F.; Bélanger, A.; Cusan, L.; Simard, J.; Luu-The, V.; Diamond, P.; Gomez, J. L.; Candas, B. History of LHRH agonist and combination therapy in prostate cancer. Endocr. Rel. Cancer 1996, 3, 243-278.
3. Brooke, C. N.; Bevan, C. L. The role of androgen receptor mutations in prostate cancer progression. Curr. Genomics 2009, 9, 18-25.
4. Mohler, M. L.; Narayanan, R.; He, Y.; Miller, D. D.; Dalton, J. T. Hydroxysteroid dehydrogenase (17β-HSD3, 17β-HSD5, and 3α-HSD3) inhibitors: Extragonadal regulation of intracellular sex steroid hormone levels. Recent Pat. Endocr. Metab. Immune Drug Discov. 2007, 1, 103-118.
5. Moeller, G.; Adamski, J. Integrated view on 17beta-hydroxysteroid dehydrogenase. Mol. Cell. Endocrinol. 2009, 301, 7-19.
6. Poirier, D. 17β-Hydroxysteroid dehydrogenase inhibitors: a patent review. Expert. Opin. Ther. Patents 2010, *20*, 1123-1145.
7. Day, J. M.; Tutill, H. J.; Purohit, A. 17β-Hydroxysteroid dehydrogenase inhibitors. Ninerva Endocrinol. 2010, 35, 87-108.
8. Marchais-Oberwinkler, S.; Henn, C.; Moller, G.; Klein, T.; Negri, M.; Oster, A.; Spadaro, A.; Werth, R.; Wetzel, M.; Xu, K.; Frotscher, M.; Hartmann, R. W.; Adamski, J. 17β-Hydroxysteroid dehydrogenases (17β-HSDs) as therapeutic target: Protein, structures, functions, and recent progress in inhibitor development. J. Steroid Biochem. Mol. Biol. 2011, 125, 66-82.
9. Luu-The, V.; Zang, Y.; Poirier, D.; Labrie, F. Characteristics of human types 1, 2 and 3 17β-hydroxysteroid dehydrogenase activities: oxidation/reduction and inhibition. J. Steroid Biochem. Mol. Biol. 1995, 55, 581-587.
10. Shellhammer, P. F.; Sharifi, R.; Block, N. L.; Soloway, M. S.; Venner, P. M.; Patterson, A. L.; Sarosdy, M. F.; Vogelzang, N. J.; Chen, Y.; Kolvenbag, G. J. A controlled trial of bicalutamide versus flutamide, each in combination with luteinizing hormone-releasing hormone analogue therapy, in patients with advanced prostate carcinoma. Analysis of time to progression. CASODEX Combination Study Group. Cancer 1996, 78, 2164-2169.
11. Crawford, E. D.; Eisenberger, M. A.; McLeod, D. G.; Spaulding; J. T.; Benson, R.; Dorr, F. A.; Blumenstein, B. A.; Davis, M. A.; Goodman, P. J. A controlled trial of leuprolide with and without flutamide in prostatic carcinoma. N. Engl. J. Med. 1989, 321, 419-424.

12. Salvador, J. A. R.; Pinto, R. M. A.; Silvestre, S. M. Steroidal 5α-reductase and 17α-hydroxylase/17,20-lyase (CYP17) inhibitors useful in the treatment of prostatic diseases. J. Steroid Biochem. Mol. Biol. 2013, 137, 199-222.

13. Adeniji, A. O.; Chen, M.; Penning, T. M. AKR1C3 as a target in castrate resistant prostate cancer. J. Steroid Biochem. Mol. Biol. 2013, 137, 136-149.

14. Seidenfeld, J.; Samson, D. J.; Hasselblad, V.; Aronson, N.; Albertsen, P. C.; Bennett, C. L.; Wilt, T. J. Single-therapy androgen suppression in men with advanced prostate cancer: a systematic review and meta-analysis. Ann. Intern. Med. 2000, 132, 566-577.

15. Singh, S. M.; Gauthier, S.; Labrie, F. Androgen, receptor antagonists (antiandrogens): Structure-activity relationships. Curr Med. Chem 2000, 7, 211-247.

16. Santen, R. J. Clinical review 37: Endocrine treatment of prostate cancer. J. Clin. Endo. Metab. 1992, 75, 685-689.

17. Smith, M. R.; Saad, F.; Egerdie, B.; Sieber, P. R.; Tammela, T. L.; Ke, C. Leder, Z. B.; Sarcopenia during androgen-deprivation therapy for prostate cancer. J. Clin. Oncol. 2012, 30, 3271-3276.

18. Gillessen, S.; Templeton, A.; Marra, G.; Kuo, Y. F.; Valtorta, E.; Shahinian, V. B. Risk of colorectal cancer in men on long-term androgen deprivation therapy for prostate cancer. J. Natl. Cancer Inst. 2010,102, 1760-1770.

19. Poirier, D. Recent patents on new steroid agents targeting the steroidogenesis for endocrine cancer treatments. Recent Patents Endocr. Metab. Immune Drug Discov. 2015, 9, 15-23.

20. Djigoué, G. B.; Ngatcha, B. T.; Roy, J.; Poirier, D. Synthesis of 5α-androstane-17-spiro-δ-lactones with a 3-keto, 3-hydroxy, 3-spirocarbamate or 3-spiromorpholinone as inhibitors of 17β-hydroxysteroid dehydrogenases. Molecules 2013, 18, 914-933.

21. Maltais, R.; Fournier, M-A.; Poirier, D. Development of 3-substituted-androsterone derivatives as potent inhibitors of 17β-hydroxysteroid dehydrogenase type 3. Bioorg. Med. Chem. 2011, 19, 4652-4668.

22. Berube, M.; Poirier, D. Chemical synthesis and in vitro biological evaluation of a phosphorylated bisubstrate inhibitor of type 3 17β-hydroxysteroid dehydrogenase. J. Enzyme Inhib. Med. Chem. 2007, 22, 201-211.

23. Berube, M.; Laplante, Y.; Poirier, D. Design, synthesis and in vitro evaluation of 4-androstene-3,17-dione/adenosine hybrid compounds as bisubstrate inhibitors of type 3 17beta-hydroxysteroid dehydrogenase. Med. Chem. 2006, 2, 329-347.

24. Tchedam-Ngatcha, B.; Laplante, Y.; Labrie, F.; Luu-The, V.; Poirier, D. 3beta-alkyl-androsterones as inhibitors of type 3 17β-hydroxysteroid dehydrogenase: inhibitory potency in intact cells, selectivity towards isoforms 1, 2, 5 and 7, binding affinity for steroid receptors, and proliferative/antiproliferative activities on AR^+ and ER^+ cell lines. Mol. Cell. Endocrinol. 2006, 248, 225-232.

25. Tchedam-Ngatcha, B.; Luu-The, V.; Labrie, F.; Poirier, D. Androsterone 3α-ether-3β-substituted and androsterone 3β-substituted derivatives as inhibitors of type 3 17β-hydroxysteroid dehydrogenase: chemical synthesis and structure-activity relationship. J. Med. Chem. 2005, 48, 5257-5268.

26. Maltais, R.; Luu-The, V.; Poirier, D. Synthesis and optimization of a new family of type 3 17 beta-hydroxysteroid dehydrogenase inhibitors by parallel liquid-phase chemistry. J. Med. Chem. 2002, 45, 640-653.

27. Maltais, R.; Luu-The, V.; Poirier, D. Parallel solid-phase synthesis of 3*β*-peptido-3α-hydroxy-5α-androstane-17-one derivatives for inhibition of type 3 17*β*-hydroxysteroid dehydrogenase. Bioorg. Med. Chem. 2001, 9, 3101-3111.

28. Djigoué, G. B.; Simard, M.; Kenmogne, L. C.; Poirier, D. Two androsterone derivatives as inhibitors of androgen biosynthesis. Acta Cryst. 2012, c68, o231-o234.

29. Djigoué, G. B.; Kenmogne, L. C.; Roy, J.; Poirier, D. Synthesis of 3-spiromorpholinone androsterone derivatives as inhibitors of 17β-hydroxysteroid dehydrogenase type 3. Bioorg. Med. Chem. Lett. 2013, 23, 6360-6362.

30. Rouillard, F.; Roy, J.; Poirier, D. Chemical synthesis of (*S*)-spiro(estradiol-17,2'-[1,4]oxazinan)-6'-one derivatives bearing two levels of molecular diversity Eur. J. Org. Chem. 2008, 14, 2446-2453.

31. Blomquist, C. H.; Bonenfant, M.; McGinley, D. M.; Posalaky, Z.; Lakatua, D. J.; Tuli-Puri, S.; Bealka, D. G.; Tremblay, Y. Androgenic and estrogenic 17β-hydroxysteroid dehydrogenase/17-ketosteroid reductase in human ovarian epithelial tumors: Evidence for the type 1, 2 and 5 isoforms. J. Steroid Biochem. Mol. Biol. 2002, 81, 343–351.

32. Ohmura, T.; Kijima, A. and Suginome, M. synthesis of 1-borylisoindoles via palladium-catalyzed dehydrogenation/C-H borylation of isoindolines. J. Am. Chem. Soc. 2009, 131, 6970-6971.

33. Acquaah-Harrison, G.; Zhou, S.; Hines, J. V.; Bergmeier, S. C. Library of 1,2,3-triazole analogs of oxazolidinone RNA-binding agents. J. Comb. Chem. 2010, 12, 491-496.

34. Rostovtsev, V. V.; Green, L. C.; Fokin, V. V.; Sharpless, K. B. A stepwise Huisgen cycloaddition process: Copper(I)-catalyzed regioselective ligation of azides and terminal alkynes. Angew. Chem., Int. Ed. Engl. 2002, *41,* 2596–2599.

35. Jiang,Y.; Kuang, C.; Yang, Q. The synthesis of 1-monosubstituted aryl 1,2,3-triazoles was achieved in good yields using calcium carbide as a source of acetylene. The copper-catalyzed 1,3-dipolar cycloaddition reactions were carried out without nitrogen protection and in a MeCN-H_2O mixture. Synlett 2009, 3163-3166.

36. Yamada, Y. M. A.; Sarkar, S. M.; Uozumi, Y. Amphiphilic self-assembled polymeric copper catalyst to parts per million levels: click chemistry. J. Am. Chem. Soc. 2012, 134, 9285-9286.

37. Laplante, Y.; Poirier, D. Proliferative effect of androst-4-ene-3,17-dione and its metabolites in the androgen-sensitive LNCaP cell line. Steroids 2008, 73, 266-271.

38. Day, J.M.; Tutill, H.J.; Purohit, A.; Reed, M.J. Design and validation of specific inhibitors of 17β-hydroxysteroid dehydrogenases for therapeutic application in breast and prostate cancer, and in endometriosis. Endocr.-Related Cancer 2008, 15, 665-692.

39. Luu-The, V. Assessment of steroidogenesis and steroidogenic enzyme functions. J. Steroid Biochem. Mol. Biol. 2013, 137, 176-182.

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