Organometallic Routes to Novel Steroids Containing Heterocyclic C-17 Side-Chains

|  |  |
| --- | --- |
| Lucia Vitellozzi aGraeme D. McAllister aThorsten GenskibRichard J. K. Taylor\*aa Department of Chemistry, University of York, Heslington, York, YO10 5DD, UKb AnalytiCon Discovery GmbH, Hermannswerder Haus 17, 14473 Potsdam, Germany\* indicates the main/corresponding author.richard.taylor@york.ac.uk |  |
|  |

Received:
Accepted:
Published online:
DOI:

Abstract A range of novel steroid analogues bearing a C-17 side-chain containing a 20(*R*)-hydroxyl group and a variety of heterocyclic substituents have been prepared by organometallic additions to 3-methoxy-pregnenolone. X-ray crystallography has been used to establish that the organometallic additions proceed with excellent Felkin-Anh control. This methodology has been extended, by use of the Achmatowicz rearrangement and ring-closing metathesis approaches, to prepare pyran-dione and δ-lactone steroidal analogues reminiscent of the withanolide natural products.

**Key words** Withanolides, steroid analogues, heterocycles, organometallic, metathesis, Achmatowicz

The *Solanaceae*, or nightshades, are a widespread family of flowering plants which include important agricultural crops, spices and components of traditional medicinal remedies. The psychotropic alkaloids and poisons found in mandrake, datura, deadly nightshade etc. are well-known, but there is increasing interest in the steroidal withanolides isolated from this family (Figure 1). The first withanolide structure to be elucidated was withaferin A **1**,extracted from winter cherry (*Withania somnifera*, indian ginseng, also known as *Ashwagandha* in ayurvedic medicine) in 1965 by Lavie *et al*.1 Since then 3-400 withanolides have been isolated from various nightshades, all based on polyoxygenated steroidal frameworks with most containing a C-17 side chain linked to a -lactone or lactol as in withaferin A. A small sub-set of withanolides possess a C-17 side chain bearing a -lactone or lactol, *e.g.* ixocarpalactone A **2** isolated from the Mexican tomatillo (*Physalis philadelphica*).3 The withanolides have attracted significant attention because of their diverse biological activities and consequent potential as biological probes and drug leads/candidates.2 For example, withaferin A **1** acts as an anti-angiogenic by inhibiting transcription factors Sp1 and NF-κB,4 ixocarpalactone A **2** possesses potent antiproliferative and apoptotic activity in colon cancer cells,3 and other withanolides exhibit anti-stress, anti-inflammatory, immunosuppressive, anti-microbial, anti-cancer, leishmanocidal/trypanocidal, phytotoxic and anti-feedant activities.2 In recent years, interest in the withanolides has accelerated with the discovery that withanolide A **3**, also isolated from *Withania somnifera*,5 has shown dramatic neurological effects in mice (neurite outgrowth, memory enhancement etc.)6,7 offering promise in Alzheimer's research8,9 and elsewhere.10



**Figure 1** Representative Withanolide Natural Products

We initiated a research programme to prepare a wide range of novel steroid analogues inspired by the withanolides in which readily-available steroid starting materials **4** were modified to generate withanolide-like products **5** and **6** (Scheme 1).



**Scheme 1**

This approach to the preparation of a library of novel withanolide analogues is illustrated herein using the readily-available11 3-methoxy-pregnenolone **7** as starting material for organometallic additions.12-14 It should be noted that the 20*R-*configuration (as shown) is believed to be optimal for bioactivity15 - and the Felkin-Anh model indicates that these should be the major products from organometallic additions to methyl ketones of this type (and this is well-precedented12-14).

The addition of simple lithiated heterocycles to the C-20 ketone of steroid **7** were studied first (Table 1).

**Table 1** Organometallic Additions to Ketone **7**

|  |
| --- |
|  |
|  | **Het-Li** | **Conditions** | **Product (Yield)** |
| i |  | i) thiazole (2.5 eq), *n-*BuLi (2.5 eq), -30 °C, 30 minii) **7**, -20 °C to rt, 2 h | **8** (84%), (*R:S*, 9:1) |
| ii |  | i) thiophene (1.1 eq), *n-*BuLi (1.1 eq), TMEDA (1.1 eq), 0 °C to rt, 1 hii) **7**, rt, 3 h | **9** (51%), (*R:S*, >98:2) a,b |
| iii |  | i) furan (2 eq), *n-*BuLi (2.1 eq), TMEDA (2.5 eq), -50 °C, 40 minii) **7**, -50 to -30 °C, 30 min | **10** (84%), (*R:S*, >98:2)a |
| iv |  | i) furan-2-CH2OTBS (2 eq), *n-*BuLi (2.1 eq), TMEDA (2 eq), 0 °C, 40 minii) **7**, 0 °C to rt, 18 h | **11** (75%), (*R:S*, >98:2) |
| v |  | i) 2-Br-pyridine (2.2 eq), *n-*BuLi (2.5 eq), -78 °C, 10 minii) **7**, 0 °C to rt, 3.5 h | **12** (68%), (*R:S*, 9:1) |
| vi |  | i) benzothiophene (2.5 eq), *n-*BuLi (2.5 eq), -30 °C to rt, 30 minii) **7**, rt, 3 h | **13** (47%), (*R:S*, >98:2) |
| vii |  | i) benzofuran (2.5 eq), *n-*BuLi (2.5 eq), -30 to -10 °C, 30 minii) **7**, -10 °C to rt, 4 h | **14** (56%), (*R:S*, >98:2) |
| viii |  | i) *N*-Me-indole (1.1 eq), *n-*BuLi (1.1 eq), reflux, 4 hii) **7**, rt, 18 h | **15** (12%)(*R:S*, >98:2) |

a In the absence of TMEDA, a mixture of products was formed

b Readily undergoes dehydration under acidic conditions

Given promising precedent in the literature,13 2-lithiothiazole was studied first (entry i). Thus, 3β-methoxy-pregnenolone **7** was added to a cooled solution of2-lithiothiazole, prepared *in situ*, and a chromatographically inseparable mixture of adducts **8*R*** and **8*S*** (84%, 9:1) was obtained; however, recrystallization of the mixture from methanol gave the required *R*-diastereomeric adduct **8*R*** in 48% isolated yield.

We next looked at the addition of other lithiated 5-membered heterocycles on to ketone **7** (entries ii-iv). Using thiophene, furan and TBS-protected furfurol, lithiation was aided by the addition of TMEDA, although the isolated yields of adducts **9**-**11** were depressed by the ease with which the products, particularly **9**, underwent dehydration. Nevertheless, the reactions proceeded with complete diastereoselectivity giving **9*R***-**11*R*** with no sign of the corresponding *S*-isomers by NMR spectroscopy. The diastereoselective addition of 2-lithio-pyridine (prepared from 2-bromopyridine) is well-precedented13,14 and the use of 3β-methoxy-pregnenolone **7** gave a 9:1 mixture of adducts **12*R*:12*S*** (entry v); recrystallisation from methanol/dichloromethane gave the pure *R*-diastereomer **12*R*** in 53% yield.

We next examined the addition of lithiated benzothiophene, benzofuran and *N*-methyl-indole (entries vi-viii); to the best of our knowledge organometallic addition of such reagents to 20-keto steroids have not previously been reported. All additions proceeded diastereoselectively, although the yields of the adducts were modest (**13*R***,47% **14*R***, 56%) or low (**15*R***, 12%). In each of these examples, *n*-butyllithiumwas used for the metallation reactions; the use of stronger bases would almost certainly improve these yields, particularly for *N*-methyl-indole where *t*-butyllithium is often employed.

The configuration of the newly formed stereocentre (C-20) was assigned based upon the earlier mentioned Felkin-Anh model, ample literature precedent12-14 and comparability of NMR data across all of the adducts (see experimental section).  These stereochemical assignments were confirmed by X-ray crystallography in the cases of the thiazole (**8*R***) and pyridine adducts (**12*R***) as shown in Figure 2.

**Figure 2** X-ray structures of (a) thiazolyl-steroid **8*R*** (CCDC 1019833) and (b) pyridyl-steroid **12*R*** (CCDC 1019835).

|  |  |
| --- | --- |
| (a)**8*R*** |  |
| (b)**12*R*** |  |

We next proceeded to explore routes to systems **6** containinga pyran-dione or lactone ring in the side-chain to mimic the withanolide (and bufadienolide) natural products (Scheme 2). The pyran-dione analogue **17** was readily obtained from furan **10*R*** using the Achmatowicz rearrangement17 in the key step (Scheme 2). This sequence, originally developed by Kametani *et al.* on a closely related system,18 proceeded efficiently using *N*-bromosuccinimide for the furan ring elaboration,19 and tetrapropylammonium perruthenate / *N*-methylmorpholine *N*-oxide20 for the oxidation of the lactol **16** to lactone **17**. The structure and stereochemistry of compound **17** was again confirmed by X-ray crystallography (Scheme 2 and Figure 3).



**Scheme 2**

Having successfully prepared the pyran-dione analogue **17**, we next targeted the lactones **20** and **23** as interesting withanolide mimics. Inspired by recent research on the use of ring-closing metathesis for -lactone formation,21 3-methoxy-pregnenolone **7** was first treated with allylmagnesium bromide to generate exclusively the 20*S*-alcohol **18** in 72% yield (Scheme 3). Subsequent esterification of the sterically hindered tertiary alcohol in **18** with acryloyl chloride proved difficult but ester **19** was isolated in 33% yield, albeit along with recovered starting material **18**. The use of second-generation Hoveyda-Grubbs catalystthenled to the formation of the desired
α,β-unsaturated lactone **20** in near-quantitative yield. X-ray crystallography (Figure 3) confirmed the structure of lactone **20** and therefore established, once again, that the organometallic addition to generate alcohol **18** had occurred with complete Felkin-Anh control.



**Scheme 3**

Finally, we wanted to investigate whether similar reaction conditions could be applied to the synthesis of the α,β-dimethyl-α,β-unsaturated C-20-δ-lactone **23**, i.e.possessing a decorated lactone moiety typical of withanolide A. Thus (Scheme 3), addition of 2-methylallylmagnesium chloride to ketone **7** at -78 °C gave gave β-alcohol **24** in 75% yield (a mixture of products was obtained at 0 °C). Esterification using methacryloyl chlorideagain proved very slow but diene **25** was isolatedin 12% unoptimised yield along with recovered starting material. The enhanced steric hindrance (compared to **19**) affected the efficiency of the intramolecular ring-closure, but we were delighted to find that treatment with the second-generation Hoveyda-Grubbs catalyst, gave dimethyl-α,β-unsaturated lactone **23** in 24% yield.



**Figure 3** X-ray structures of dione **17** (CCDC 1019836) and lactone-steroid **20** (CCDC 1019837).

In summary, we have established that organometallic additions to 3-methoxy-pregnenolone **7** proceed with excellent Felkin-Anh control and that this procedure can be employed to obtain a range of novel steroid analogues bearing a C-17 side-chain containing a 20*R*-hydroxyl group and a variety of heterocyclic substituents. This methodology has been extended, by use of the Achmatowicz rearrangement and ring-closing metathesis approaches, to prepare pyran-dione and δ-lactone analogues reminiscent of the withanolides. With the basic protocols established, preparative procedures well described and stereoselectivity confirmed by several X-ray studies, this methodology is now ripe for application to other steroidal ketone precursors in order to generate lead compounds of therapeutic interest.

The experimental section has no title; please leave this line here.

Reactions were monitored by Thin Layer Chromatography (TLC) and/or LC-MS (Liquid Chromatography-Mass Spectrometry). TLC was performed using pre-coated aluminium foil TLC-sheets Xtra SIL G/UV254 (MACHERAY-NAGEL), layer 0.20 mm, silica gel 60 with fluorescent indicator UV254, and on Merck silica gel 60F254. Visualisation was carried out using UV light at 254 nm and basic aqueous potassium permanganate or ethanolic *p*-anisaldehyde as stains. Chemical reactions were carried out with magnetic stirring. All air and moisture sensitive reactions were performed in flame-dried glassware and under a nitrogen or argon atmosphere. Water refers to distilled water. Reagents were purchased from commercial sources and used without any further purification. Tetrahydrofuran was distilled from sodium-benzophenone ketyl immediately before use, and dichloromethane was dried with an Innovative Technology Inc. PureSolv® solvent purification system. Flash column chromatography was performed on a Biotage Isolera Four with a UV-VIS detector, using Fluka silica gel 60 (SiO2) or using slurry packed Fluka silica gel, 35-70 µm, 60 Å, with the specific eluent. Petroleum ether (PE) is the fraction with the boiling point range 40-60 °C. Nuclear Magnetic Resonance (NMR) spectra were recorded on a Bruker AVANCE spectrometer or on a Jeol ECX400 spectrometer operating at 400 MHz for 1H and at 100 MHz for 13C or at 500 MHz for 1H and 125 MHz for 13C. Chemical shifts (δ) are quoted in parts per million (ppm) from tetramethylsilane calibrated to the residual nondeuterated solvent peak as internal standard. Coupling constants (*J*) are quoted in Hertz. Structural assignment was verified by two dimensional NMR (HSQC, HMBC, COSY) and nOe where necessary. High resolution mass spectra were obtained by the University of York Mass Spectrometry Service using electrospray ionisation (ESI) on a Bruker Daltonics, Micro-Tof spectrometer. LC-MS spectra were recorded at AnalytiCon Discovery GmbH using API165, API150, API365, AB Sciex (UV, ELSD and DAD detectors), gradient A: 5mM ammonium formate + 0.1% formic acid, B: methanol/acetonitrile = 1/1 + ammonium monohydrogen carbonate. CHN elemental analyses were obtained by the University of York CHN Service using an Exeter Analytical CE440 Elemental Analyser. Optical Rotations were measured on a JASCO DIP-370 polarimeter using a sodium lamp and a 2 mL cell with 1 dm path length, or a 1 mL cell with 10 mm path length. Data are reported as follows: [α]DT (c in g/100 mL, solvent). Infrared spectra were recorded on a ThermoNicolet IR-100 spectrometer with NaCl plates as a thin film dispersed from a CH2Cl2 solution, or a PerkinElmer UATR spectrometer. (3β)-3-methoxypregn-5-en-20-one **7** was prepared following a literature procedure.11

**General procedure for the addition of lithiated heterocycles**

The relevant heterocycle (and where necessary, TMEDA) was dissolved in THF and cooled to the temperature given in Table 1, and *n*-BuLi (2.5 M or 1.6 M solution in hexane) added dropwise. Stirring was continued for the times given in Table 1, before a solution of 3β-methoxy-pregnenolone **7** (1 eq) in THF was added. After stirring for the time given, the reaction was quenched with H2O (20 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (20 mL), dried over MgSO4, filtered and evaporated under reduced pressure. Purification by recrystallization or column chromatography gave the desired products.

 **3-Methoxy-20*R*-(1,3-thiazol-2-yl)-pregnen-20-ol (8*R*)**

Following the general procedure, thiazole (54.0 µL, 0.76 mmol) in THF (500 µL), cooled to -30 °C, was treated with *n*-BuLi (2.5 M solution in hexane, 303 µL, 0.76 mmol) and ketone **7** (100 mg, 0.32 mmol) in THF (1 mL). The crude product was purified by flash column chromatography (SiO2, PE/AcOEt = 4/1) to afford the diasteromeric mixture of thiazolyl-steroids **8*R*/8*S*** (9/1, 105 mg, 84%) as white solids. Recrystallisation from methanol gave thiazolyl steroid **8*R*** (64.0 mg, 48%) as white needles. Evaporation of the filtrate under reduced pressure gave a mixture of **8*R*/8*S*** (77/23, 35.0 mg) as a white solid.

**8*R***:Mp 190-193 °C (MeOH); [α]D24 = -42 (*c* 0.40, CHCl3); *R*f: 0.14 (PE/AcOEt = 9/1).

IR (neat): 3500, 2927, 1441, 1097 cm-1.

1H-NMR (500 MHz, CDCl3) δ: 7.65 (1H, d, *J* = 3.2 Hz, H-2b), 7.22 (1H, d, *J* = 3.2 Hz, H-3b), 5.35-5.31 (1H, m, H-6), 3.34 (3H, s, 3H-1a), 3.12 (1H, brs, OH), 3.09-3.01 (1H, m, H-3), 2.41-2.34 (1H, m, H-4), 2.19-2.07 (2H, m, H-4, H-12), 2.04 (1H, “t”, *J* = 9.8 Hz, H-17), 1.99-1.88 (2H, m, H-4, H-7), 1.85 (1H, “dt”, *J* = 13.3, 3.1 Hz, H-1), 1.82-1.73 (1H, m, H-16), 1.70 (3H, s, 3H-21), 1.60-1.22 (8H, m, H-2, H-7, H-8, 2H-11, H-12, H-15, H-16), 1.18-0.97 (3H, m, H-1, H-14, H-15,), 0.99 (3H, s, 3H-19), 0.97-0.89 (1H, m, H-9), 0.86 (3H, s, 3H-18).

13C-NMR (100 MHz, CDCl3) δ: 180.6 (C, C-1b), 141.8 (CH, C-2b), 141.0 (C, C-5), 121.5 (CH, C-6), 118.9 (CH, C-3b), 80.4 (CH, C-3), 77.8 (C, C-20), 60.4 (CH, C-17), 56.8 (CH, C-14), 55.7 (CH3, C-1a), 50.2 (CH, C-9), 43.3 (C, C-13), 40.0 (CH2, C-12), 38.8 (CH2, C-4), 37.3 (CH2, C-1), 37.0 (C, C-10), 31.9 (CH2, C-7), 31.4 (CH, C-8), 30.0 (CH3, C-21), 28.1 (CH2, C-2), 23.8 (CH2, C-15), 22.9 (CH2, C-16), 21.0 (CH2, C-11), 19.5 (CH3, C-19), 13.4 (CH3, C-18).

HRMS (ESI): *m/z* [M+Na]+ calcd for C25H37NNaO2S: 438.2437, found 438.2403 (8.0 ppm error); *m/z* [M+H]+ calcd for C25H38NO2S: 416.2618, found 416.2586 (7.3 ppm error).

**3-Methoxy-20*R*-(thiophen-2-yl)-pregnen-20-ol 9*R***

Following the general procedure, thiophene (19.0 µL, 0.24 mmol) and TMEDA (36.0 µL, 0.24 mmol) in THF (0.5 mL) cooled to 0 °C was treated with *n*-BuLi (1.6 M solution in hexane, 150 µL, 0.24 mmol) and ketone **7** (72 mg, 0.22 mmol) in THF (1 mL). The crude product was purified by flash column chromatography (SiO2, PE/AcOEt = 9/1) followed by recrystallisation in methanol to afford diastereomerically pure thiophenyl-steroid **9*R*** (46.0 mg, 51%) as a white solid.

Mp 190-192 °C (MeOH); [α]D24: -67 (*c* 0.40, CH2Cl2); *R*f: 0.40 (PE/AcOEt = 9/1).

IR (neat): 3454, 2932, 2902, 2861, 1096 cm-1.

1H-NMR (400 MHz, CD2Cl2) δ: 7.13 (1H, dd, *J* = 5.0, 1.1 Hz, H-4b), 6.92 (1H,dd, *J* = 5.0, 3.6 Hz, H-3b), 6.84 (1H, dd, *J* = 3.6, 1.1 Hz, H-2b), 5.37-5.30 (1H, m, H-6), 3.30 (3H, s, 3H-1a), 3.06-2.96 (1H, m, H-3), 2.39-2.31 (1H, m, H-4), 2.16-2.06 (1H, m, H-4), 2.05-1.81 (5H, m, H-1, H-2, H-7, H-12, H-17), 1.80-1.69 (1H, m, H-16), 1.68 (3H, s, H-21), 1.62-1.22 (8H, m, H-2, H-7, H-8, 2H-11, H-12, H-15, H-16), 1.19-0.89 (4H, m, H-1, H-9, H-14, H-15), 1.00 (3H, s, 3H-19), 0.86 (3H, s, 3H-18).

13C-NMR (100 MHz, CD2Cl2) δ: 156.6 (C, C-1b), 141.5 (C, C-5), 126. 9 (CH, C-3b), 123.2 (CH, C-4b), 121.8 (CH, C-2b), 121.6 (CH, C-6), 80.7 (CH, C-3), 76.6 (C, C-20), 61.9 (CH, C-17), 57.3 (CH, C-14), 55.7 (CH3, C-1a), 50.5 (CH, C-9), 43.3 (C, C-13), 40.1 (CH2, C-12), 39.1 (CH2, C-4), 37.6 (CH2, C-1), 37.2 (C, C-10), 32.2 (CH2, C-7), 31.7 (CH, C-8), 31.4 (CH3, C-21), 28.4 (CH2, C-2), 23.9 (CH2, C-15), 23.6 (CH2, C-16), 21.3 (CH2, C-11), 19.5 (CH3, C-19), 13.4 (CH3, C-18).

HRMS (ESI): *m/z* [M+Na]+ calcd for C26H38NaO2S: 437.2485, found 437.2470 (2.6 ppm error).

 **3-Methoxy-20*R*-(furan-2-yl)-pregnen-20-ol 10*R***

Following the general procedure, furan (162 µL, 2.24 mmol) and TMEDA (401 µL, 2.73 mmol) in THF (0.2 mL) cooled to -50 °C, was treated with *n*-BuLi (1.6 M solution in THF, 1.47 mL, 2.35 mmol) and ketone **7** (368 mg, 1.12 mmol) in THF (2 mL). The crude product was purified by flash column chromatography (SiO2, PE/AcOEt = 9/1, 1% Et3N) to afford the title compound **10*R*** (364 mg, 84%) as a white solid.

Mp 152 °C (MeOH); [α]D24: -57 (*c* 0.82, CH2Cl2); *R*f: 0.30 (PE/AcOEt = 9/1, 1% Et3N).

IR (thin film): 3378, 2889, 2856, 2806, 1347, 1138, 1081, 995 cm-1.

1H-NMR (400 MHz, C6D6) δ: 7.08 (1H, brs, H-4b), 6.11 (1H, dd, *J* = 3.0, 1.6 Hz, H-3b), 6.06 (1H, d, *J* = 3.0 Hz, H-2b), 5.39-5.34 (1H, m, H-6), 3.22 (3H, s, 3H-1a), 3.11-2.99 (1H, m, H-3), 2.57-2.49 (1H, m, H-4), 2.41-2.30 (1H, m, H-4), 1.98-1.77 (5H, m, H-2, H-7, H-12, H-16, H-17), 1.71 (1H, “dt”, *J* = 13.2, 3.3 Hz, H-1), 1.60-1.28 (7H, m, H-2, H-7, H-8, 2H-11, H-15, H-16), 1.54 (3H, s, 3H-21), 1.18-0.82 (5H, m, H-1, H-9, H-12, H-14, H-15), 0.93 (3H, s, 3H-19), 0.78 (3H, s, 3H-18).

13C-NMR (100 MHz, C6D6) δ: 162.1 (C, C-1b), 141.2 (C, C-5), 140.7 (CH, C-4b), 121.7 (CH, C-6), 110.4 (CH, C-3b), 104.0 (CH, C-2b), 80.6 (CH, C-3), 73.9 (C, C-20), 59.5 (CH, C-17), 57.0 (CH, C-14), 55.4 (CH3, C-1a), 50.6 (CH, C-9), 42.8 (C, C-13), 39.7 (CH2, C-12), 39.4 (CH2, C-4), 37.6 (CH2, C-1), 37.2 (C, C-10), 32.2 (CH2, C-7), 31.7 (CH, C-8), 28.6 (CH2, C-2), 27.6 (CH3, C-21), 24.0 (CH2, C-15), 23.4 (CH2, C-16), 21.3 (CH2, C-11), 19.5 (CH3, C-19), 13.2 (CH3, C-18).

HRMS (ESI): *m/z* [M+Na]+ calcd for C26H38NaO3: 421.2713, found 421.2699 (3.3 ppm error).

**3-Methoxy-20*R*-[5-(*t*-butyldimethylsilyloxy)methylfuran-2-yl]pregnen-20-ol 11*R***

Following the general procedure, the furfurol TBS ether22 (720 mg, 3.39 mmol) and TMEDA (420 µL, 3.39 mmol) in THF (3 mL) cooled to 0 °C was treated with *n*-BuLi (1.6 M in hexane, 2.23 mL, 3.56 mmol and ketone **7** (560 mg, 1.70 mmol) in THF (2 mL). The crude product was purified by flash column chromatography (SiO2, PE/AcOEt = 9/1) to afford furfuryl-steroid **11*R*** (688 mg, 75%) as a yellow gum.

[α]D24: -32 (*c* 0.98, CHCl3); *R*f: 0.11 (PE/AcOEt = 9/1).

IR (neat): 3453, 2932, 1074, 835, 777, 497 cm-1.

1H-NMR (400 MHz, C6D6) δ: 6.04 (2H, brs, H-2b, H-3b), 5.39-5.34 (1H, m, H-6), 4.51 (2H, s, 2H-5b), 3.20 (3H, s, 3H-1a), 3.10-3.00 (1H, m, H-3), 2.57-2.48 (1H, m, H-4), 2.40-2.30 (1H, m, H-4), 2.00-1.79 (5H, m, H-2, H-7, H-12, H-16, H-17), 1.77-1.68 (1H, m, H-1), 1.62-1.34 (7H, m, H-2, H-7, H-8, 2H-11, H-15, H-16), 1.58 (3H, s, 3H-21), 1.24-0.75 (5H, m, H-1, H-9, H-12, H-14, H-15), 0.97 (9H, s, 3H-9b, 3H-10b, 3H-11b), 0.93 (3H, s, 3H-19), 0.81 (3H, s, 3H-18), 0.09 (6H, s, 3H-6b, 3H-7b).

13C-NMR (100 MHz, C6D6) δ: 161.7 (C, C-1b), 153.0 (C, C-4b), 141.2 (C, C-5), 121.7 (CH, C-6), 108.2 (CH, C-3b), 104.6 (CH, C-2b), 80.6 (CH, C-3), 73.8 (C, C-20), 59.5 (CH, C-17), 58.4 (CH2, C-5b), 57.1 (CH, C-14), 55.4 (CH3, C-1a), 50.6 (CH, C-9), 42.8 (C, C-13), 39.7 (CH2, C-12), 39.4 (CH2, C-4), 37.6 (CH2, C-1), 37.2 (C, C-10), 32.2 (CH2, C-7), 31.7 (CH, C-8), 28.6 (CH2, C-2), 27.6 (CH3, C-21), 26.1 (3CH3, C-9b, C-10b, C-11b), 24.0 (CH2, C-15), 23.4 (CH2, C-16), 21.3 (CH2, C-11), 19.5 (CH3, C-19), 18.5 (C, C-8b), 13.4 (CH3, C-18), -5.0 (2CH3, C-6b, C-7b).

HRMS (ESI): *m/z* [M+Na]+ calcd for C33H54NaO4Si: 565.3684, found 565.3674 (1.8 ppm error).

**3-Methoxy-20*R*-(pyridin-2-yl)-pregnen-20-ol 12*R***

Following the general procedure, 2-bromopyridine (64.0 µL, 0.67 mmol) in THF (1 mL) cooled to -78 °C, was treated with *n*-BuLi (2.5 M solution in hexane, 303 µL, 0.76 mmol) and ketone **7** (100 mg, 0.30 mmol) in THF (1 mL). The crude product was purified by flash column chromatography (SiO2, hexane/AcOEt = 9/1) to afford a mixture of **12*R*** and its minor diastereomer **12*S*** (9/1, 80.0 mg, 68%) as a white solid. Recrystallisation from MeOH and CH2Cl2 gave compound **12*R*** (65.0 mg, 53%) as white needles.

Mp 223-225 °C (MeOH/CH2Cl2); [α]D24: -90 (*c* 0.49, CHCl3); *R*f: 0.24 (PE/AcOEt = 9/1).

IR (liquid film): 3329, 2891, 2324, 1366, 1341, 1082 cm-1.

1H-NMR (400 MHz, CDCl3), δ: 8.46 (1H, d, *J* = 4.4 Hz, H-5b), 7.68 (1H, “td”, *J* = 7.7, 1.5 Hz, H-3b), 7.33 (1H, d, *J* = 8.1 Hz, H-2b), 7.16 (1H, dd, *J* = 6.6, 5.1 Hz, H-4b), 5.53 (1H, s, OH), 5.37-5.31 (1H, m, H-6), 3.35 (3H, s, 3H-1a), 3.13-3.00 (1H, m, H-3), 2.43-2.34 (1H, m, H-4), 2.23 (1H, “dt”, *J* = 12.1, 3.3 Hz, H-12), 2.29-2.10 (1H, m, H-4), 2.19-1.84 (3H, m, H-1, H-2, H-7), 1.82-1.75 (1H, “t”, *J* = 9.8 Hz, H-17), 1.67-1.27 (8H, m, H-2, H-7, H-8, 2H-11, H-12, H-15, H-16), 1.59 (3H, s, 3H-21), 1.17-0.88 (4H, m, H-1, H-9, H-14, H-15), 1.01 (3H, s, 3H-19), 0.94 (3H, s, 3H-18), 0.88-0.76 (1H, m, H-16).

13C-NMR (100 MHz, CDCl3), δ: 166.2 (C, C-1b), 146.7 (CH, C-5b), 141.1 (C, C-5), 137.1 (CH, C-3b), 121.6 (2CH, C-6, C-4b), 119.5 (CH, C-2b), 80.5 (CH, C-3), 75.1 (C, C-20), 60.5 (CH, C-17), 57.2 (CH, C-14), 55.7 (CH3, C-1a), 50.4 (CH, C-9), 43.5 (C, C-13), 40.5 (CH2, C-12), 38.8 (CH2, C-4), 37.4 (CH2, C-1), 37.1 (C, C-10), 32.0 (CH2, C-7), 31.5 (CH, C-8), 28.9 (CH3, C-21), 28.2 (CH2, C-2), 23.9 (CH2, C-15), 22.6 (CH2, C-16), 21.2 (CH2, C-11), 19.5 (CH3, C-19), 13.4 (CH3, C-18).

HRMS (ESI): *m/z* [M+H]+ calcd for C27H40NO2: 410.3054, found 410.3032 (4.6 ppm error).

Anal. Calcd for C27H39NO2: C, 79.17; H, 9.60; N, 3.42. Found: C, 78.82; H, 9.54; N, 3.42.

 **3-Methoxy-20*R*-(1-benzothiophen-2-yl)-pregnen-20-ol 13*R***

Following the general procedure, benzothiophene (87.0 µL, 0.74 mmol) in THF (2 mL) cooled to -30 °C was treated with *n*-BuLi (1.6 M in hexane, 463 µL, 0.74 mmol) and ketone **7** (98 mg, 0.30 mmol) in THF (1 mL). The crude product was purified by flash column chromatography (SiO2, PE/AcOEt = 95/5 to 4/1) to afford benzothiophenyl steroid **13*R*** (65 mg, 47%) as a white solid.

Mp 194-196 °C (MeOH); [α]D24: -70 (*c* 0.61, CHCl3); *R*f: 0.30 (PE/AcOEt = 4/1).

IR (thin film): 3361, 2887, 1411, 1343, 1074 cm-1.

1H-NMR (400 MHz, CDCl3), δ: 7.78 (1H, d, *J* = 7.9 Hz, H-7b), 7.68 (1H, d, *J* = 7.7 Hz, H-4b), 7.35-7.23 (2H, m, H-5b, H-6b) 7.10 (1H, s, H-2b), 5.37-5.32 (1H, m, H-6), 3.36 (3H, s, 3H-1a), 3.12-3.02 (1H, m, H-3), 2.44-2.36 (1H, m, H-4), 2.20-2.06 (2H, m, H-4, H-12), 2.02-1.77 (5H, m, H-1, H-2, H-7, H-16, H-17), 1.76 (3H, s, 3H-21), 1.63-1.29 (8H, m, H-2, H-7, H-8, 2H-11, H-12, H-15, H-16), 1.21-0.89 (4H, m, H-1, H-9, H-14, H-15), 1.01 (3H, s, 3H-19), 0.92 (3H, s, 3H-18).

13C-NMR (100 MHz, CDCl3), δ: 156.7 (C, C-1b), 141.0 (C, C-3b), 140.1 (C, C-5), 139.1 (C, C-8b), 124.2 (CH, C-5b), 123.7 (CH, C-6b), 123.3 (CH, C-4b), 122.3 (CH, C-7b), 121.6 (CH, C-6), 117.9 (CH, C-2b), 80.4 (CH, C-3), 76.6 (C, C-20), 61.1 (CH, C-17), 57.0 (CH, C-14), 55.8 (CH3, C-1a), 50.2 (CH, C-9), 43.2 (C, C-13), 40.0 (CH2, C-12), 38.8 (CH2, C-4), 37.3 (CH2, C-1), 37.0 (C, C-10), 31.9 (CH2, C-7), 31.4 (CH, C-8), 31.2 (CH3, C-21), 28.1 (CH2, C-2), 23.7 (CH2, C-15), 23.3 (CH2, C-16), 21.0 (CH2, C-11), 19.5 (CH3, C-19), 13.5 (CH3, C-18).

HRMS (ESI): *m/z* [M+Na]+ calcd for C30H40NaO2S: 487.2641, found 487.2657 (-3.2 ppm error).

**3-Methoxy-20*R*-(1-benzofuran-2-yl)-pregnen-20-ol 14*R***

Following the general procedure, benzofuran (92.0 µL, 0.83 mmol) in THF (1 mL) cooled to -30 °C was treated with *n*-BuLi (1.6 M in hexane, 520 µL, 0.83 mmol) and ketone **7** (100 mg, 0.33 mmol) in THF (1.5 mL). The crude product was purified by flash column chromatography (SiO2, PE/AcOEt = 4/1) to afford benzofuranyl steroid **14*R*** (83 mg, 56%) as a white solid.

Mp 187-189 °C (MeOH/CH2Cl2); [α]D24: -37 (*c* 1, CHCl3); *R*f: 0.30 (PE/AcOEt = 4/1).

IR (thin film): 3371, 2873, 1431, 1078 cm-1.

1H-NMR (400 MHz, C6D6), δ: 7.44 (1H, d, *J* = 7.1 Hz, H-4b), 7.40 (1H, d, *J* = 8.1 Hz, H-7b), 7.14-7.09 (2H, m, H-5b, H-6b), 6.41 (1H, s, H-2b), 5.39-5.31 (1H, m, H-6), 3.22 (3H, s, 3H-1a), 3.12-2.99 (1H, m, H-3), 2.58-2.48 (1H, m, H-4), 2.40-2.30 (1H, m, H-4), 2.04-1.79 (5H, m, H-2, H-7, H-12, H-16, H-17), 1.69 (1H, “dt”, *J* = 13.2, 3.3 Hz, H-1), 1.62-1.27 (7H, m, H-2, H-7, H-8, 2H-11, H-15, H-16), 1.60 (3H, s, 3H-21), 1.23-1.12 (1H, m, H-12), 1.09-0.80 (4H, m, H-1, H-9, H-14, H-15), 0.92 (3H, s, 3H-9), 0.82 (3H, s, 3H-18).

13C-NMR (100 MHz, C6D6), δ: 165.1 (C, C-1b), 155.2 (C, C-8b), 141.2 (C, C-5), 129.1 (C, C-3b), 124.0 (C, C-6b), 123.1 (CH, C-5b), 121.6 (CH, C-6), 121.2 (CH, C4b), 111.4 (CH, C-7b), 100.9 (CH, C-2b), 80.6 (CH, C-3), 74.1 (C, C-20), 58.5 (CH, C-17), 57.0 (CH, C-14), 55.4 (CH3, C-1a), 50.6 (CH, C-9), 42.9 (C, C-13), 39.9, (CH2, C-12), 39.4 (CH2, C-4), 37.6 (CH2, C-1), 37.2 (C, C-10), 32.2 (CH2, C-7), 31.7 (CH, C-8), 28.6 (CH2, C-2), 27.8 (CH3, C-21), 24.0 (CH2, C-15), 23.3 (CH2, C-16), 21.2 (CH2, C-11), 19.5 (CH3, C-19), 13.4 (CH3, C-18).

HRMS (ESI): *m/z* [M+Na]+ calcd for C30H40NaO3: 471.2870, found 471.2892 (-4.8 ppm error).

**3-Methoxy-20*R*-(1-methyl-1*H*-indol-2-yl)-pregnen-20-ol 15*R***

Following the general procedure, *N*-methyl-indole (37.0 µL, 0.30 mmol) in THF (1 mL) was treated with *n*-BuLi (1.6 M in hexane, 0.27 mL, 0.30 mmol) and ketone **7** (90 mg, 0.27 mmol) in THF (1 mL). The crude product was purified by flash column chromatography (SiO2, PE/AcOEt = 9/1 to 4/1) to afford indolyl steroid **15*R*** (83 mg, 56%) as a white solid.

Mp 182-184 °C (MeOH); [α]D24: -50 (*c* 0.21, CHCl3); *R*f: 0.20 (PE/AcOEt = 4/1).

IR (thin film): 3376, 2889, 1411, 1354, 1080, 742 cm-1.

1H-NMR (400 MHz, C6D6), δ: 7.74 (1H, d, *J* = 7.3 Hz, H-4b), 7.33-7.22 (2H, m, H-5b, H-6b), 7.19 (1H, d, *J* = 8.1 Hz, H-7b), 6.33 (1H, s, H-2b), 5.39-5.32 (1H, m, H-6), 3.68 (3H, s, 3H-9b), 3.19 (3H, s, 3H-1a), 3.08-2.95 (1H, m, H-3), 2.55-2.46 (1H, m, H-4), 2.38-2.27 (1H, m, H-4), 2.18-2.10 (1H, m, H-17), 1.96-1.73 (4H, m, H-2, H-7, 2H-16), 1.62-1.27 (5H, m, H-1, H-2, H-7, H-8, H-15), 1.56 (3H, s, 3H-21), 1.10-0.61 (8H, m, H-1, H-9, 2H-11, 2H-12, H-14, H-15), 0.83 (3H, s, 3H-19), 0.71 (3H, s, 3H-18).

13C-NMR (100 MHz, C6D6), δ: 145.4 (C, C-1b), 141.4 (C, C-5), 139.0 (C, C-8b), 127.6 (C, C-3b), 121.9 (CH, C-6b), 121.5 (CH, C-6), 120.9 (CH, C-4b), 120.1 (CH, C-5b), 109.5 (C, C-7b), 100.2 (C, C-2b), 80.6 (CH, C-3), 74.1 (C, C-20), 58.6 (CH, C-17), 57.3 (CH, C-14), 55.4 (CH3, C-1a), 50.7 (CH, C-9), 42.5 (C, C-13), 39.4 (CH2, C-4), 37.7 (CH2, C-12), 37.5 (CH2, C-1), 37.1 (C, C-10), 32.3 (CH3, C-9b), 32.2 (CH2, C-7), 31.8 (CH, C-8), 28.5 (CH2, C-2), 27.0 (CH3, C-21), 23.9 (CH2, C-15), 23.4 (CH2, C-16), 20.9 (CH2, C-11), 19.4 (CH3, C-19), 13.3 (CH3, C-18).

HRMS (ESI): *m/z* [M+Na]+ calcd for C31H43NNaO2: 484.3186, found 484.3182 (0.6 ppm error).

**2*H*-Pyran-2,5(6*H*)-dione analogue 17**

(a) A solution of furyl steroid **10*R*** (630 mg, 1.58 mmol) in a mixture of THF/H2O (4/1 ratio, 12.5 mL) was cooled to 0 °C. NBS (281 mg, 1.58 mmol) was added portionwise (the yellow colour of the reaction mixture faded before addition of a second portion of NBS), while the temperature was carefully maintained at 0 °C. After the addition of the last portion of NBSthe reaction was stirred for a further 10 min at 0 °C and diluted with AcOEt (20 mL). The organic phase was washed successively with aq 10% KI (20 mL), sat aq Na2S2O4 (20 mL) and H2O (20 mL), dried over MgSO4, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO2, PE/AcOEt = 4/1) to afford the intermediate lactol **16** as a mixture of diastereomers (3/2, 510 mg, 78%) as a white solid which was fully characterised by NMR and HRMS.

(b) The above lactol **16** (451 mg, 1.09 mmol) was dissolved in CH2Cl2 (10 mL), NMO (446 mg, 3.82 mmol) was added and the mixture was stirred at rt for 10 min. TPAP (38.0 mg, 0.11 mmol) was added and the reaction mixture was stirred at rt for 2 h, before being diluted with CH2Cl2 (20 mL) and filtered through a pad of Celite®. The filtrate was evaporated under reduced pressure and the residue was purified by flash column chromatography (SiO2, PE/AcOEt = 9/1) to afford lactone **17** (240 mg, 53%) as a white solid.

Mp 240-250 °C (decomposition); [α]D24: -43 (*c* 0.46, CHCl3); *R*f: 0.28 (hexane/AcOEt = 4/1).

IR (neat): 2971, 2948, 1721, 1683, 1301, 1101, 867 cm-1.

1H-NMR (400 MHz, CDCl3), δ = 6.87 (1H, d, *J* = 10.1 Hz, H-2b), 6.67 (1H, d, *J* = 10.1 Hz, H-3b), 5.36-5.31 (1H, m, H-6), 3.35 (3H, s, 3H-1a), 3.09-3.01 (1H, m, H-3), 2.42-2.34 (1H, m, H-4), 2.20-2.09 (1H, m, H-4), 2.07-1.80 (5H, m, H-1, H-2, H-7, H-12, H-17), 1.70-1.29 (9H, m, H-2, H-7, H-8, 2H-11, H-12, H-15, 2H-16), 1.64 (3H, s, 3H-21), 1.25-1.12 (1H, m, H-15), 1.09-0.96 (2H, m, H-1, H-14), 0.99 (3H, s, 3H-19), 0.96-0.89 (1H, m, H-9), 0.87 (3H, s, 3H-18).

13C-NMR (100 MHz, CDCl3), δ: 196.3 (C, C-4b), 160.8 (C, C-1b), 141.1 (C, C-5), 137.7 (CH, C-3b), 135.3 (CH, C-2b), 121.3 (CH, C-6), 92.4 (C, C-20), 80.4 (CH, C-3), 58.4 (CH, C-17), 56.3 (CH, C-14), 55.8 (CH3, C-1a), 50.1 (CH, C-9), 43.3 (C, C-13), 39.8 (CH2, C-12), 38.8 (CH2, C-4), 37.3 (CH2, C-1), 37.0 (C, C-10), 31.8 (CH2, C-7), 31.4 (CH, C-8), 28.1 (CH2, C-2), 25.6 (CH3, C-21), 23.8 (CH2, C-15), 23.0 (CH2, C-16), 21.0 (CH2, C-11), 19.5 (CH3, C-19), 13.8 (CH3, C-18).

HRMS (ESI): *m/z* [M+Na]+ calcd for C26H36NaO4: 435.2506, found 435.2511 (-0.8 ppm error).

**3-Methoxy-20*R*-(1-allyl)-pregnen-20-ol** **18**

Ketone **7** (83.0 mg, 0.25 mmol) was dissolved in THF (1 mL), cooled to 0 °C and allylmagnesium bromide (1 M solution in Et2O, 503 µL, 0.50 mmol) added. The reaction mixture was stirred at 0 °C for 5 min, then at rt for 4 h. The reaction was quenched with H2O (10 mL) and the resulting mixture was diluted with AcOEt (20 mL) and filtered through a pad of Celite®. The organic layer was isolated and the aqueous phase was extracted with AcOEt (3 x 5 mL). The combined organic layers were dried over MgSO4, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO2, PE/AcOEt = 9/1) to afford the title compound **21** (67.0 mg, 72%) as a white solid.

Mp 103-105 °C (MeOH); [α]D24: -62 (*c* 0.27, CHCl3); *R*f: 0.46 (hexane/AcOEt = 4/1).

IR (neat): 3486, 2973, 2931, 2901, 2871, 2841, 1458, 1434, 1374, 1102, 909 cm-1.

1H-NMR (400 MHz, CDCl3), δ: 5.89-5.73 (1H, m, H-2b), 5.35-5.31 (1H, m, H-6), 5.14-5.02 (2H, m, 2H-3b), 3.33 (3H, s, 3H-1a), 3.11-2.99 (1H, m, H-3), 2.42-2.33 (1H, m, H-4), 2.23-2.04 (4H, m, H-4, H-12, 2H-1b), 2.02-1.34 (12H, m, H-1, 2H-2, 2H-7, H-8, 2H-11, H-15, 2H-16, H-17), 1.32-1.09 (2H, m, H-12, H-15), 1.27 (3H, s, H-21), 1.08-0.82 (3H, m, H-1, H-9, H-14), 0.98 (3H, s, H-19), 0.85 (3H, s, H-18).

13C-NMR (100 MHz, CDCl3), δ: 141.0 (C, C-5), 134.5 (CH, C-2b), 121.6 (CH, C-6), 118.3 (CH2, C-3b), 80.4 (CH,C-3), 74.7 (C, C-20), 58.0 (CH, C-17), 56.9 (CH, C-14), 55.7 (CH3, C-1a), 50.2 (CH, C-9), 48.3 (CH2, C-1b), 42.8 (C, C-13), 40.2 (CH2, C-12), 38.8 (CH2, C-4), 37.3 (CH2, C-1), 37.0 (C, C-10), 31.9 (CH2, C-7), 31.4 (CH, C-8), 28.1 (CH2, C-2), 26.7 (CH3, C-21), 23.9 (CH2, C-15), 22.4 (CH2, C-16), 21.0 (CH2, C-11), 19.5 (CH3, C-19), 13.7 (CH3, C-18).

HRMS (ESI): *m/z* [M+Na]+ calcd for C25H40NaO2: 395.2921, found 395.2930 (-2.5 ppm error).

**3-Methoxy-20*R*-(1-allyl)-pregnen-20-yl** **prop-2-enoate 19**

Alcohol **18** (474 mg, 1.27 mmol) was dissolved in THF (6 mL) and Et3N (442 µL, 3.18 mmol), acryloyl chloride (205 µL, 2.55 mmol) and DMAP (1.55 mg, 0.013 mmol) were added. The resulting suspension was stirred at 40 °C for 48 h, cooled to rt and quenched with H2O (30 mL). The organic compounds were extracted with AcOEt
(3 x 40 mL). The combined organic layers were dried over MgSO4, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO2, PE/AcOEt = 95/5 to 9/1) to afford ester **19** (176 mg, 33%) as a colourless gum.

[α]24D: -61 (*c* 0.26, CHCl3); *R*f: 0.63 (hexane/AcOEt = 4/1).

IR (neat): 2937, 2901, 2853, 1720, 1398, 1205, 1102, 806 cm-1.

1H-NMR (400 MHz, CDCl3), δ: 6.28 (1H, dd, *J* = 17.3, 1.4 Hz, H-6bb), 6.02 (1H, dd, *J* = 17.3, 10.4 Hz, H-5b), 5.80-5.67 (1H, m, H-2b), 5.72 (1H, dd, *J* = 10.4, 1.4 Hz, H-6ba), 5.35 (1H, m, H-6), 5.14-5.00 (2H, m, 2H-3b), 3.34 (3H, s, 3H-1a), 3.14 (1H, dd, *J* = 13.5, 6.4 Hz, H-1b), 3.10-3.00 (1H, m, H-3), 2.42-2.28 (2H, m, H-4, H-1b), 2.20-2.09 (1H, m, H-4), 2.07-1.80 (5H, m, H-1, H-2, H-7, H-12, H-16), 1.79-1.66 (1H, m, H-17), 1.64 (3H, s, H-21), 1.57-1.09 (9H, m, H-2, H-7, H-8, 2H-11, H-12, 2H-15, H-16), 1.09-0.80 (3H, m, H-1, H-9, H-14), 0.99 (3H, s, 3H-19), 0.84 (3H, s, 3H-18).

13C-NMR (100 MHz, CDCl3), δ: 165.5 (C, C-4b), 141.0 (C, C-5), 134.0 (CH, C-2b), 130.7 (CH, C-5b), 129.4 (CH2, C-6b), 121.6 (CH, C-6), 118.0 (CH2, C-3b), 87.2 (C, C-20), 80.4 (CH, C-3), 57.0 (CH, C-17), 56.7 (CH, C-14), 55.7 (CH3, C-1a), 50.1 (CH, C-9), 43.1 (CH2, C-1b), 42.7 (C, C-13), 40.0 (CH2 C-12), 38.8 (CH2, C-4), 37.3 (CH2, C-1), 37.0 (C, C-10), 31.9 (CH2, C-7), 31.5 (CH, C-8), 28.1 (CH2, C-2), 23.9 (CH2, C-15), 23.7 (CH3, C-21), 22.5 (CH2, C-16), 21.0 (CH2, C-11), 19.5 (CH3, C-19), 14.1 (CH3, C-18).

HRMS (ESI): *m/z* [M+Na]+ calcd for C28H42NaO3: 449.3026, found 449.3036 (-2.1 ppm error).

**5,6-Dihydro-2*H*-pyran-2-one analogue 20**

Diene **19** (10.0 mg, 0.02 mmol) was dissolved in CH2Cl2 (2 mL) and the 2nd generation Hoveyda-Grubbs catalyst (1.50 mg, 0.002 mmol) was added. The reaction mixture was stirred at rt overnight, diluted with CH2Cl2 (20 mL) and filtered through a pad of Celite®. The filtrate was evaporated under reduced pressure, and the residue was purified by flash column chromatography (SiO2, PE/AcOEt = 4/1) to afford **20** (9.00 mg, 98%) as a white solid.

Mp 177-179 °C; [α]D24: -54 (*c* 0.44, CHCl3); *R*f: 0.16 (hexane/AcOEt = 8/2).

IR (neat): 2974, 2929, 2828, 1721, 1381, 1136, 1102 cm-1.

1H-NMR (400 MHz, CDCl3), δ: 6.71 (1H, ddd, *J* = 9.7, 5.9, 2.4 Hz, H-2b), 6.00 (1H, dd, *J* = 9.7, 1.8 Hz, H-3b), 5.38-5.31 (1H, m, H-6), 3.35 (3H, s, 3H-1a), 3.10-2.99 (1H, m, H-3), 2.75-2.65 (1H, m, H-1b), 2.43-2.33 (1H, m, H-4), 2.22-2.06 (3H, m, H-4, H-12, H-1b), 2.05-1.72 (5H, m, H-1, H-2, H-7, 2H-16), 1.71-1.33 (7H, m, H-2, H-7, H-8, 2H-11, H-15, H-17), 1.51 (3H, s, 3H-21), 1.30-1.11 (2H, m, H-12, H-15), 1.10-0.82 (3H, m, H-1, H-9, H-14), 1.00 (3H, s, 3H-19), 0.91 (3H, s, 3H-18).

13C-NMR (100 MHz, CDCl3), δ: 163.9 (C, C-4b), 143.7 (CH, C-2b), 141.1 (C, C-5), 121.5 (CH, C-6), 120.9 (CH, C-3b), 84.9 (C, C-20), 80.4 (CH, C-3), 59.1 (CH, C-17), 57.1 (CH, C-14), 55.8 (CH3, C-1a), 50.2 (CH, C-9), 43.0 (C, C-13), 40.2 (CH2, C-12), 38.8 (CH2, C-4), 37.3 (CH2, C-1), 37.0 (C, C-10), 34.5 (CH2, C-1b), 31.9 (CH2, C-7), 31.4 (CH, C-8), 28.1 (CH2, C-2), 24.8 (CH3, C-21), 23.8 (CH2, C-15), 23.5 (CH2, C-16), 21.0 (CH2, C-11), 19.5 (CH3, C-19), 13.7 (CH3, C-18).

HRMS (ESI): *m/z* [M+Na]+ calcd for C26H38NaO3: 421.2713, found 421.2709 (1.8 ppm error).

**3-Methoxy-20*R*-(methallyl)-pregnen-20-ol** **21**

Ketone **7** (700 mg, 2.12 mmol) was dissolved in THF (20 mL) and cooled to -78 °C. 2-Methylallylmagnesium chloride (0.5 M solution in THF, 6.36 mL, 3.18 mmol) was added and the reaction mixture was stirred for 2 h during which time the temperature reached -30 °C. Due to the presence of residual starting material, the reaction was re-cooled to -78 °C and a further portion of 2-methylallylmagnesium bromide (0.5 M solution in THF, 2.12 mL, 1.06 mmol) was added. The reaction mixture was stirred for a further 2 h during which time the temperature reached -30 °C, quenched with H2O (20 mL) and extracted with AcOEt (3 x 20 mL). The combined organic layers were dried over MgSO4, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO2, PE/AcOEt = 95/5) to afford **21** (610 mg, 75%) as a white solid.

Mp 124 °C (MeOH); [α]D24: -50 (*c* 0.1, CHCl3); *R*f: 0.38 (hexane/AcOEt = 4/1).

IR (neat): 3492, 2931, 2895, 2865, 1368, 1090, 902, 885, 734 cm-1.

1H-NMR (400 MHz, CDCl3), δ: 5.33 (1H, m, H-6), 4.88 (1H, brs, H-3b), 4.69 (1H, brs, H-3b), 3.33 (3H, s, H-1a), 3.08-2.98 (1H, m, H-3), 2.40-2.32 (1H, m, H-4), 2.22 (1H, d, *J* = 13.5 Hz, H-1b), 2.18-2.06 (2H, m, H-4, H-12), 2.05 (1H, d, *J* = 13.5 Hz, H-1b), 2.00-1.33 (12H, m, H-1, 2H-2, 2H-7, H-8, 2H-11, H-15, 2H-16, H-17), 1.80 (3H, s, H-4b), 1.31-1.09 (2H, m, H-12, H-15), 1.26 (3H, s, H-21), 1.05-0.78 (3H, m, H-1, H-9, H-14), 0.98 (3H, s, H-19), 0.84 (3H, s, H-18).

13C-NMR (100 MHz, CDCl3), δ: 143.0 (C, C-2b), 140.9 (C, C-5), 121.6 (CH, C-6), 114.9 (CH2, C-3b), 80.4 (CH, C-3), 74.9 (C, C-20), 59.4 (CH, C-17), 57.0 (CH, C-14), 55.7 (CH3, C-1a), 50.9 (CH2, C-1b), 50.2 (CH, C-9), 42.9 (C, C-13), 40.3 (CH2, C-12), 38.7 (CH2, C-4), 37.2 (CH2, C-1), 36.9 (C, C-10), 31.9 (CH2, C-7), 31.4 (CH, C-8), 28.1 (CH2, C-2), 26.8 (CH3, C-21), 25.3 (CH3, C-4b), 23.9 (CH2, C-15), 22.7 (CH2, C-16), 21.0 (CH2, C-11), 19.4 (CH3, C-19), 13.5 (CH3, C-18).

HRMS (ESI): *m/z* [M+Na]+ calcd for C26H42NaO2: 409.3077, found 409.3081 (-0.9 ppm error).

Anal. Calcd for C26H42O2: C, 80.77; H, 10.95. Found: C, 80.86; H 11.05.

**3-Methoxy-20*R*-(methallyl)-pregnen-20-ol** **2-methylprop-2-enoate 22**

Alcohol **21** (90.0 mg, 0.23 mmol) was dissolved in CH2Cl2 (2 mL) and Et3N (130 µL, 0.93 mmol), 2-methylacryloyl chloride (68.0 µL, 0.70 mmol) and DMAP (0.28 mg, 0.002 mmol) were added. The reaction mixture was stirred at 40 °C for 18 h, cooled to rt and quenched with H2O (10 mL). The organic layer was isolated, diluted with CH2Cl2 (5 mL) and then washed with sat aq NaHCO3 (3 x 5 mL), dried over MgSO4, filtered and evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO2, PE/AcOEt = 95/5) to afford the title compound **22** (13.0 mg, 12%) as a colourless gum.

[α]D24: -54 (*c* 0.13, CHCl3); *R*f: 0.65 (hexane/AcOEt = 4/1).

IR (neat): 2933, 2829, 1710, 1171, 1144, 1101 cm-1.

1H-NMR (400 MHz, CDCl3), δ: 6.00 (1H, brs, H-7b), 5.48 (1H, brs, H-7b), 5.38-5.32 (1H, m, H-6), 4.85 (1H, brs, H-3b), 4.78 (1H, brs, H-3b), 3.35 (3H, s, 3H-1a), 3.11-3.00 (2H, m, H-3, H-1b), 2.42-2.33 (2H, m, H-4, H-1b), 2.20-2.10 (1H, m, H-4), 2.09-1.58 (8H, m, H-1, H-2, H-7, H-12, H-15, 2H-16, H-17), 1.90 (3H, s, H-8b), 1.79 (3H, s, H-4b), 1.67 (3H, s, H-21), 1.57-1.38 (5H, m, H-2, H-7, H-8, 2H-11), 1.28-1.11 (2H, m, H-12, H-15), 1.08-0.96 (2H, m, H-1, H-14), 0.99 (3H, s, 3H-19), 0.96-0.81 (1H, m, H-9), 0.85 (3H, s, 3H-18).

13C-NMR (100 MHz, CDCl3), δ: 166.8 (C, C-5b), 142.2 (C, C-2b), 141.0 (C, C-5), 138.4 (C, C-6b), 124.6 (CH2, C-7b), 121.6 (CH, C-6), 115.5 (CH2, C-3b), 88.0 (C, C-20), 80.4 (CH, C-3), 56.9 (CH, C-17), 56.7 (CH, C-14), 55.8 (CH3, C-1a), 50.1 (CH, C-9), 46.0 (CH2, C-1b), 42.8 (C, C-13), 39.8 (CH2, C-12), 38.8 (CH2, C-4), 37.3 (CH2, C-1), 37.0 (C, C-10), 31.9 (CH2, C-7), 31.5 (CH, C-8), 28.1 (CH2, C-2), 24.7 (CH3, C-21 or C-4b), 24.5 (CH3, C-21 or C-4b), 23.9 (CH2, C-15), 23.1 (CH2, C-16), 21.0 (CH2, C-11), 19.5 (CH3, C-19), 18.8 (CH3, C-8b), 14.2 (CH3, C-18).

HRMS (ESI): *m/z* [M+Na]+ calcd for C30H46NaO3: 477.3339, found 477.3332 (1.3 ppm error).

**3,4-Dimethyl-5,6-dihydro-2*H*-pyran-2-one analogue 23**

Diene **22** (58.0 mg, 0.13 mmol) was dissolved in toluene (10 mL) and 2nd generation Hoveyda-Grubbs catalyst (8.00 mg, 0.01 mmol) was added. The reaction mixture was stirred at 120 °C for 24 h. At that time TLC still showed the presence of unreacted starting material, so another portion of catalyst (40 mg, 0.06 mmol) was added and the mixture was stirred at 120 °C for further 18 h. After cooling to rt, the mixture was diluted with CH2Cl2 (10 mL) and filtered through a pad of Celite®. The filtrate was evaporated under reduced pressure and the residue was purified by flash column chromatography (SiO2, PE/AcOEt = 9/1 to 4/1) and then recrystallisation from methanol to afford lactone **23** (13.0 mg, 24%) as a colourless solid.

Mp 168-170 °C (MeOH) [α]D24: -65 (*c* 0.15, CHCl3); *R*f: 0.18 (hexane/AcOEt = 4/1).

IR (neat): 2925, 2850, 1704, 1463, 1381, 1102 cm-1.

1H-NMR (400 MHz, CDCl3), δ: 5.38-5.32 (1H, m, H-6), 3.35 (3H, s, 3H-1a), 3.11-2.99 (1H, m, H-3), 2.71 (1H, d, *J* = 17.8 Hz, H-1b), 2.43-2.33 (1H, m, H-4), 2.22-2.05 (2H, m, H-4, H-12), 2.05-1.69 (6H, m, H-1, H-2, H-7, 2H-16, H-1b), 1.88 (6H, s, 3H-5b, 3H-6b), 1.69-1.36 (7H, m, H-2, H-7, H-8, 2H-11, H-15, H-17), 1.44 (3H, s, 3H-21), 1.31-1.09 (2H, m, H-12, H-15), 1.08-0.96 (2H, m, H-1, H-14), 1.00 (3H, s, H-19), 0.95-0.80 (1H, m, H-9), 0.89 (3H, s, H-18).

13C-NMR (100 MHz, CDCl3), δ: 165.9 (C, C-4b), 146.5 (C, C-2b), 141.1 (C, C-5), 121.5 (CH, C-6), 121.4 (C, C-3b), 82.6 (C, C-20), 80.4 (CH, C-3), 59.0 (CH, C-17), 57.1 (CH, C-14), 55.8 (CH3, C-1a), 50.2 (CH, C-9), 42.9 (C, C-13), 41.1 (CH2, C-1b), 40.2 (CH2, C-12), 38.8 (CH2, C-4), 37.3 (CH2, C-1), 37.0 (C, C-10), 31.9 (CH2, C-7), 31.4 (CH, C-8), 28.1 (CH2, C-2), 24.6 (CH3, C-21), 23.8 (CH2, C-15), 23.4 (CH2, C-16), 21.0 (CH2, C-11), 20.8 (CH3, C-6b), 19.5 (CH3, C-19), 13.7 (CH3, C-18), 12.5 (CH3, C-5b).

HRMS (ESI): *m/z* [M+Na]+ calcd for C28H42NaO3: 449.3026, found 449.3034 (-1.3 ppm error).

Acknowledgment

We thank EU-FP7-Healing-Project (Marie Currie ITN) (LV) and the University of York (GDM) for funding and Dr. A. C. Whitwood (University of York) for X-ray crystallography.

Supporting Information

YES (this text will be updated with links prior to publication)

Primary Data

NO (this text will be deleted prior to publication)

References

1. D. Lavie, E. Glotter and Y. Shvo, *J. Org. Chem.* **1965**, *30*, 1774 and *J. Chem. Soc.* **1965**, 7517.
2. For a review see L-X. Chen, H. He and F. Qiu, *Nat. Prod. Rep.* **2011**, *28*, 705 and references therein.
3. J. K. Choi, G. Murillo, B.-N. Su, J. M. Pezzuto, A. D. Kinghorn and

R. G. Mehta, *FEBS J.* **2006**, *273*, 5714.

1. R. Mohan, H. J. Hammers, P. Bargagna-Mohan, X. H. Zhan, C. J. Herbstritt, A. Ruiz, L. Zhang, A. D. Hanson, B. P. Connor, J. Rougas and V. S. Priluba, *Angiogenesis* **2004**, *7,* 115.
2. S. S. Subramanian, P. D. Sethi, E. Glotter, I. Kirson and D. Lavie, *Phytochemistry* **1971**, *10*, 685.
3. For a review on the cognitive enhancement properties of *ashwagandha*: S. Jain, S. D. Shukla, K. Sharma and M. Bathnagar, *Phytother. Res.* **2001**, *15*, 544.
4. (a) T. Kuboyama, C. Tohda, J. Zhao, N. Nakamura, M. Hattori

and K. Komatsu, *NeuroReport* **2002**, *13*, 1715. (b) J. Zhao, N. Nakamura, M. Hattori, T. Kuboyama, C. Tohda and K. Komatsu, *Chem. Pharm. Bull.* **2002**, *50*, 760; T. Kuboyama, C. Tohda and K. Komatsu, *Br. J. Pharmacol.* **2005**, *144*, 961.

1. C. K. Jana, J. Hoecker, T. M. Woods, H. J. Jessen, M. Neuburger

and K. Gademann, *Angew. Chem., Int. Ed.* **2011**, *50*, 8407.

1. R. Liffert, J. Hoecker, C. K. Jana, T. M. Woods, P. Burch, H. J. Jessen, M. Neuburger and K. Gademann, *Chem. Sci.* **2013**, *4*, 2851.
2. J. Svenda, M. Sheremet, L. Kremer, L. Maier, J. O. Bauer, C. Strohmann, S. Ziegler, K. Kumar and H. Waldmann, *Angew. Chem., Int. Ed.* **2015**, *54*, 5596.
3. Y. Hirayama, K. Okuzumi, H. Masubuti, H. Uekusa, J.-P. Girault and Y. Fujimoto, *J. Org. Chem.* **2014**, *79*, 5471 and references therein.
4. D. M. Piatak and J. Wicha, *Chem. Rev.*, **1978**, *78*, 199.
5. B. B. Shingate, B. G. Hazra, D. B. Salunke, V. S. Pore, F. Shirazi and M. V. Deshpande, *Eur. J. Med. Chem.* **2011**, *46,* 3681.
6. J. Heer and K. Hoffmann, *Helv. Chim. Acta*, 1956, **39**, 1814-1820.
7. (a) S. Nachtergaele, L. Mydock, K. Krishnan, J. Rammohan, P. H. Schlesinger, D. F. Covey and R. Rohatgi, *Nat. Chem. Biol.*, **2012**, *8,* 211. (b) D. Nedelcu, J. Liu, Y. Xu, C. Jao and A. Salic, *Nat. Chem. Biol.* **2013**, *9,* 557.
8. M. Ishikura and M. Terashima, *J. Chem. Soc., Chem. Commun.*, **1991**, 1219.
9. O. Achmatowicz, P. Bukowski, B. Szechner, Z. Zwierzchowska and A. Zamojski, *Tetrahedron* **1971**, *27*, 1973.
10. T. Kametani, M. Tsubuki, H. Furuyama and T. Honda *J. Chem. Soc., Perkin Trans. 1* **1985**, 557.
11. M. P. Georgiadis and E. A. Couladouros, *J. Org. Chem.*, **1986**, *51*, 2725.
12. J. Robertson, C. North, J. E. R. Sadig, *Tetrahedron*, **2011**, *67*, 5011.
13. Y. Matsuya, Y.-i. Yamakawa, C. Tohda, K. Teshigawara, M.Yamada and H. Nemoto, *Org. Lett.* **2009**, *11*, 3970; see also reference 10.
14. E. J. Corey and M. C. Noe, *J. Am. Chem. Soc.* **1996**, *118,* 319.

**Checklist (have these on hand for manuscript submission in ScholarOne):**

* cover letter, including a statement of the work’s significance
* full mailing address, telephone and fax numbers, and e-mail address of the corresponding author
* email address for each author
* original Word file
* original graphics files zipped into one zip file
* eye-catching graphical abstract as an individual file
* 5–8 key words
* separate Supporting Information file
* separate zipped Primary Data files including cover sheet (optional)

**Useful links:**

* [SYNTHESIS homepage](http://www.thieme.de/de/synthesis/journal-information-55920.htm)
* [SYNTHESIS information and tools for authors](http://www.thieme.de/de/synthesis/authors-55962.htm)
* [Graphical abstract samples](https://www.thieme.de/statics/dokumente/thieme/final/de/dokumente/zw_synthesis/CFZ-Sample-Graphical-Abstracts.pdf) (PDF file download)
* [What is “Primary Data”](http://www.thieme.de/de/synthesis/author-guidelines-58874.htm)?
* [ScholarOne](https://mc.manuscriptcentral.com/synthesis) (manuscript submission)