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Synthesis of novel estrone analogs by incorporation of thiophenols via conjugate addition to an enone side chain

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ABSTRACT

Functionalized estrogen analogs have received interest due to their unique and differing biological activity compared to their parent compounds. The synthesis of a new class of 3-methoxyestrone analogs functionalized at the C17 position possessing both alkyl and aryl substituted α,β-unsaturated ketones is described, along with their thiophenol conjugate addition products.

1. Introduction

Estrogens (estrone (**1**) (Fig. 1), estradiol, and estriol) are a set of common steroids found in both women and men that play important roles in various physiological processes [1]. Known more for their hormonal activities and contribution to the progression of estrogen-dependent breast cancer [2], estrogens and particularly their analogs have gained an increased interest due to their ability to affect other biological processes without producing the negative side effects associated with estrogen treatment.

The fact that minor modifications of the estrane structure can result in extensive changes in biological activity has led to the development of various estrane derivatives. These can be classified into two different categories. The first consists of modification of the steroid ring system itself, either through substitution of an estrane core carbon atom with a heteroatom [3], or modification of the ring systems through expansion, contraction, or additional cyclic features [4]. The second category involves addition of one or more functional groups to the estrane core structure. The most common of which tends to be the latter due to the extensive synthetic work that is necessary for the incorporation of heteroatoms into the steroid structure.

A number of research groups have been able to expand upon the known biological activity of estrogen compounds by combining them with important structural features from other natural products, creating new hybrid molecules for testing [5]. Additional estrogen hybrids or conjugates have also been synthesized to allow for receptor specific targeted delivery of a known drug candidate in an effort to increase its efficacy and minimize side effects [6]. While incorporating important chemical features of estrogens and various natural products can be effective, this is not always necessary to see evidence of modified activity.

The estradiol metabolite 2-methoxyestradiol is an example of one of these types of compounds (Fig.1) [7]. It has been reported to exhibit anticancer capabilities via microtubule disruption, anti-angiogenesis activity, and upregulation of apoptotic pathways while no longer exhibiting estrogenic characteristics [8]. However, as a result of 2-methoxyestradiol's (**2**) low bioavailability, research has continued into the synthesis of analogs concentrating on modifications at C2, C3, and C17 of the estrogen skeleton in an effort to optimize their activities (Fig.1) [9].

Even simple functionalization at the C2 position of estradiol with an adamantyl moiety provides a substrate that exhibits estrogen receptor-independent neuroprotection and vasoactive effects [10].

While modification of the A-ring of estrone (**1**) has received substantial attention, it is not the only site shown to produce interesting biological activity. Incorporation of various appendages at C17 of ring D has produced analogs that induce apoptosis in prostate cancer cell lines [11], while another is capable of simultaneous induction of autophagy and apoptosis in breast cancer cells [12] and others have shown modest cytotoxicity in human breast, lung and epidermoid carcinoma cell lines [13].

In an attempt to investigate further the biological activity associated with the C17 functionalized estrone (**1**) structure and its

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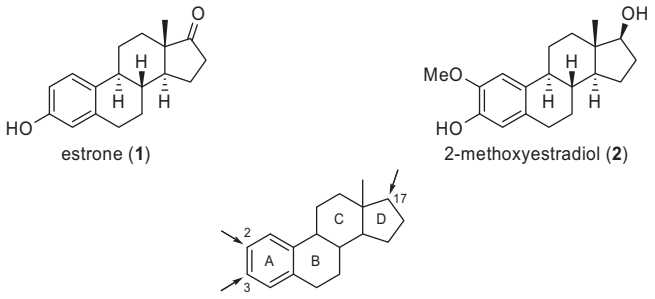


Fig. 1. Estrone (**1**), 2-methoxyestradiol (**2**), significant sites for activity.

side chain, a new set of estrogen-derived targets were identified for synthesis (Scheme 1). A key intermediate in the progression of this understanding is enone **4**. The utility of the enone functionality in organic synthesis is widely demonstrated by its use in conjugate addition [14], cycloaddition [15], organometallic [16] and many other types of synthetic reactions. The usefulness of the enone here is in the formation of β-substituted aromatic thioethers via a thiol addition to alkyl and phenyl β-substituted enones[17].

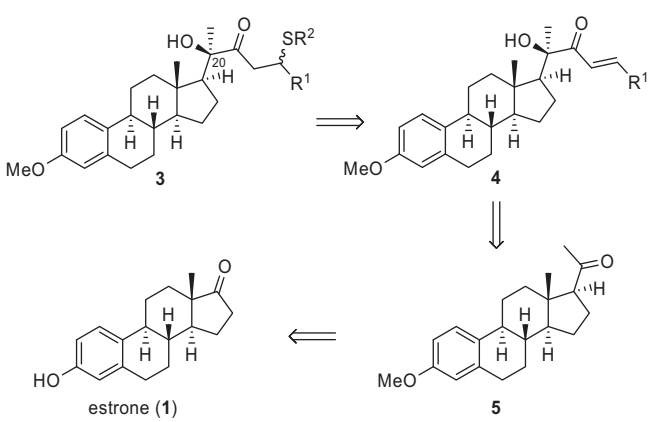
2. Experimental

2.1. General

^{1}H and ^{13}C NMR spectra were acquired on a Bruker AVANCE-400 MHz NMR spectrometer, in $CDCl_3$ using TMS (δ = 0 ppm) as the internal standard for ^{1}H NMR and $CDCl_3$ (δ = 77.16 ppm) for ^{13}C NMR, with the reporting of coupling constants in Hz and the signal multiplicities are reported as singlet (s), doublet (d), triplet (t), quartet (q), doublet of doublets (dd), doublet of triplets (dt), multiplet (m), or broad (br). HRMS data was obtained using EI ionization on a ThermoFinnigan MAT 95 XL mass spectrometer. X-ray crystal structure of **8** was obtained on a Bruker APEXII diffractometer. TLC analysis was performed using pre-coated silica gel PE sheets. Products were purified via column chromatography using silica gel 40–63um (230–400 mesh), prep-plates, and reverse phase HPLC (Alltech preparative column, Econosil C18 10u, length 250 mm, ID 22 mm). All reagents and solvents were obtained from commercial suppliers and used as received. All chemical reactions requiring anhydrous conditions were performed with oven-dried glassware under an atmosphere of nitrogen.

2.2. Alkene *6*

To a stirred solution of ethyltriphenylphosphonium bromide (26.1 g, 70.2 mmol) in THF (140 mL) was added potassium *tert*-butoxide in 2 portions (7.36 g, 65.5 mmol) at room temperature for 1 h. To this solution was added estrone 3-methyl ether (6.66 g, 23.4 mmol) in a mixture of THF:DMSO (1:1, 200 mL) and then allowed to stir at 70 °C for 5 h. After cooling to room temperature, saturated NH_4Cl (150 mL) was added to quench the reaction mixture and the aqueous layer was extracted with Et_2O (3 × 200 mL) and the combined organic layers were washed with saturated NaCl (1x75 mL), dried (Na_2SO_4), and concentrated *in vacuo*. The crude material was purified by silica gel column chromatography with hexanes/EtOAc (gradient; 95:5–9:1–4:1) to yield alkene **6** (6.1 g, 88%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (d, J = 8.6 Hz, 1H), 6.70 (dd, J = 8.7, 2.8 Hz, 1H), 6.62 (d, J = 2.7 Hz, 1H), 5.15 (qt, J = 7.2, 2.0 Hz, 1H), 3.77 (s, 3H), 2.96–2.78 (m, 2H), 2.47–2.17 (m, 5H), 1.97–1.88 (m, 1H), 1.79–1.67 (m, 2H), 1.69 (dt, J = 7.2, 2.0 Hz, 3H), 1.61–1.23 (m, 5H), 0.91 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.5, 150.4, 138.1, 133.0, 126.4, 113.9, 113.5, 111.6, 55.3, 55.3, 44.7, 43.9, 38.5, 37.4, 31.6, 30.0, 27.7, 27.1, 24.3, 17.1, 13.3.



Scheme 1. Retrosynthetic analysis of estrone analogs.

2.3. Alcohol *7*

A solution of 9-BBN (0.5 M in THF, 52 mL, 26 mmol) was added to Wittig product **6** (2.0 g, 6.7 mmol) at room temperature. The solution was stirred until the solid starting material was dissolved, then allowed to stir at 60 °C for 18 h. After cooling to 0 °C, 50 mL of 10% NaOH and 90 mL of 30% H_2O_2 were slowly added and stirred for 1 h at 0 °C, followed by 1 h at room temperature. The aqueous layer was extracted with ethyl acetate (3 × 100 mL) and the combined organic layers were washed with saturated sodium thiosulfate (1 × 75 mL), dried (Na_2SO_4), and concentrated *in vacuo*. The crude material was purified by silica gel column chromatography with hexanes/EtOAc (4:1) to yield alcohol **7** (2.0 g, 94%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (d, J = 8.7 Hz, 1H), 6.70 (dd, J = 8.5, 2.7 Hz, 1H), 6.62 (d, J = 2.6 Hz, 1H), 3.77–3.68 (m, 1H), 3.76 (s, 3H), 2.94–2.77 (m, 2H), 2.32–2.12 (m, 3H), 1.83–1.68 (m, 1H), 1.68–1.18 (m, 8H), 1.25 (d, 3H), 0.69 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.5, 138.1, 132.9, 126.3, 113.8, 111.5, 70.4, 58.7, 55.4, 55.3, 43.8, 42.1, 39.0, 38.5, 30.0, 27.8, 26.5, 26.0, 23.9, 23.6, 12.7.

2.4. Ketone *5*

To a stirred solution of alcohol **7** (2.0 g, 6.36 mmol) in CH_2Cl_2 (30 mL) was added 4 Å powdered molecular sieves (2.5 g), NaOAc (2.5 g, 30.5 mmol), and PCC (2.7 g, 12.7 mmol). The reaction mixture was stirred for 2 h and then Et_2O was added and the mixture was filtered over a pad of silica gel, washing with Et_2O. The filtrate was concentrated *in vacuo* to give the crude material that was purified by silica gel column chromatography with hexanes/EtOAc (4:1) to yield ketone **5** (1.9 g, 95%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (d, J = 8.6 Hz, 1H), 6.71 (dd, J = 8.6, 2.8 Hz, 1H), 6.63 (d, J = 2.7 Hz, 1H), 3.77 (s, 3H), 2.95–2.78 (m, 2H), 2.61 (dd, J = 9.2, 9.2 Hz, 1H), 2.34 (dddd, J = 12.9, 3.6, 3.6, 3.6 Hz, 1H), 2.29–2.19 (m, 2H), 2.19–2.12 (m, 1H), 2.15 (s, 3H), 1.94–1.85 (m, 1H), 1.84–1.24 (m, 8H), 0.65 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 209.6, 157.6, 138,0, 132.5, 126.3, 113.9, 111.6, 63.9, 55.8, 55.3, 44.5, 43.8, 39.1, 38.8, 31.6, 29.9, 27.8, 26.8, 24.2, 23.0, 13.5.

2.5. OTMS cyanohydrin *8*

To a stirred solution of ketone **5** (1.8 g, 5.76 mmol) in CH_2Cl_2 (12 mL) was added ZnI_2 (0.054 g, 0.17 mmol) and TMSCN (1.0 mL, 7.4 mmol). The reaction mixture was stirred for 3 h. Most of the solvent was evaporated and the remaining slurry was taken up in a water/EtOAc mixture (1:2, 90 mL) and the aqueous layer was subsequently extracted with EtOAc (2 × 50 mL), dried with Na_2-

SO_4, and concentrated *in vacuo* to give the crude product. This material was purified by silica gel column chromatography with hexanes/EtOAc (9:1) to yield OTMS cyanohydrin **8** (1.75 g, 82%) as a white solid. X-ray quality crystals were prepared by recrystallization from hexanes. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 (d, J = 8.6 Hz, 1H), 6.68 (dd, J = 8.6, 2.7 Hz, 1H), 6.61 (d, J = 6.7 Hz, 1H), 3.75 (s, 3H), 2.94–2.76 (m, 2H), 2.32–2.09 (m, 3H), 1.92–1.14 (m, 11H), 1.60 (s, 3H), 0.97 (s, 3H), 0.27 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.5, 137.9, 132.7, 126.3, 122.1, 113.8, 111.5, 72.4, 60.5, 55.2, 54.9, 43.8, 43.8, 40.0, 38.2, 30.8, 29.8, 27.7, 26.5, 24.9, 23.9, 12.8, 1.5, 1.5, 1.5; HRMS calcd for $C_{25}H_{37}NO_2$-Si 4111.2594, found 411.2596.

2.6. Hydroxy ketone **9**

To a stirred solution of OTMS cyanohydrin **8** (1.7 g, 4.13 mmol) in Et_2O (10 mL) was added MeLi (1.6 M in Et_2O, 8.0 mL, 12.4 mmol) drop-wise at 0 °C. The reaction mixture was stirred for 2 h at 0 °C before quenching the reaction mixture with glacial acetic acid (1.6 mL), followed by stirring for 30 min at 0 °C. The acetic acid was neutralized with saturated $NaHCO_3$, the aqueous layer was extracted with CH_2Cl_2 (3 × 50 mL), dried with Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography with hexanes/EtOAc (9:1) to yield α-hydroxy ketone **9** (1.4 g, 82%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.16 (d, J = 8.6 Hz, 1H), 6.70 (dd, J = 8.5, 2.6 Hz, 1H), 6.62 (d, J = 2.6 Hz, 1H), 3.97 (s, 1H), 3.76 (s, 3H), 2.93–2.75 (m, 2H), 2.36–2.11 (m, 3H), 2.22 (s, 3H), 1.94–1.15 (m, 11H), 1.46 (s, 3H), 0.92 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.8, 157.5, 138.0, 132.6, 126.2, 113.8, 111.5, 80.1, 55.7, 55.2, 55.1, 44.2, 43.8, 40.7, 38.1, 29.9, 27.7, 26.7, 24.6, 23.7, 23.3, 22.1, 13.5; HRMS calcd for $C_{23}H_{32}O_3$ 356.2351, found 356.2348.

2.7. Alkyl enone **10**

To a stirred solution of α-hydroxy ketone **9** (0.50 g, 1.36 mmol) in a 1:1 mixture of THF:water (10 mL) was added $LiOH{\cdot}H_2O$ (0.50 g, 7.0 mmol) and pivaldehyde (0.30 mL, 2.73 mmol). The reaction mixture was stirred at reflux for 6 h. The reaction mixture was cooled to room temperature and the organic solvents were removed. The remaining slurry was taken up in a water/CH_2Cl_2 mixture (1:1, 80 mL) and the aqueous layer was subsequently extracted with CH_2Cl_2 (2 × 40 mL), dried with Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography with hexanes/EtOAc (9:1) to yield alkyl enone **10** (0.35 g, 59%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (d, J = 8.2 Hz, 1H), 7.14 (d, J = 15.5 Hz, 1H), 6.71 (dd, J = 8.5, 2.6 Hz), 6.63 (d, J = 2.5 Hz, 1H), 4.18 (s, 1H), 3.78 (s, 3H), 2.96–2.76 (m, 2H), 2.40–2.16 (m, 3H), 1.92–1.09 (m, 11H), 1.48, (s, 3H), 1.12 (s, 9H), 0.92 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.5, 160.9, 157.6, 138.1, 132.9, 126.4, 117.2, 113.9, 111.6, 79.1, 55.8, 55.3, 55.1, 44.4, 44.0, 40.8, 38.3, 34.3, 30.0, 28.8, 28.8, 28.7, 27.8, 26.8, 24.4, 23.8, 22.1, 13.7; HRMS calcd for $C_{28}H_{40}O_3H$ 424.2977, found 424.2972.

2.8. Alkyl thioether **11**

To a stirred solution of 4-methylthiophenol (0.116 g, 0.94 mmol) in petroleum ether (4.0 mL) was added Et_3N (0.127 mL, 0.94 mmol). This reaction mixture was then added to a solution of alkyl enone **10** (0.20 g, 0.468 mmol) dissolved in petroleum ether (4 mL), followed by stirring for 24 h. The solvent was removed and the remaining slurry was taken up in a water/CH_2Cl_2 mixture (1:1, 50 mL) and the aqueous layer was subsequently extracted with CH_2Cl_2 (2 × 25 mL), dried with Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by silica gel column chromatography with hexanes/EtOAc (9:1) to yield two diastereomers, with alkyl thioether **11a** (100 mg, 39%) eluting faster than alkyl thioether **11b** (110 mg, 43%) and both recovered as a white solid. **alkyl thioether11a** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.35 (d, J = 8.1 Hz, 2H), 7.20 (d, J = 8.6 Hz, 1H), 7.06 (d, J = 8.0 Hz, 2H), 6.71 (dd, J = 8.5, 2.6 Hz, 1H), 6.62 (d, J = 2.5 Hz, 1H), 3.92 (s, 1H), 3.77 (s, 3H), 3.60 (dd, J = 9.9, 2.8 Hz, 1H), 3.02 (dd, J = 18.0, 10.0 Hz, 1H), 2.94–2.77 (m, 2H), 2.69 (dd, J = 17.9, 2.8 Hz, 1H), 2.38–2.14 (m, 3H), 1.91–0.99 (m, 11H), 1.42 (s, 3H), 1.01 (s, 9H), 0.92 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 212.0, 157.6, 138.0, 136.6, 134.0, 132.8, 131.4, 131.4, 129.7, 129.7, 126.4, 113.9, 111.6, 80.4, 56.3, 55.7, 55.3, 55.3, 44.4, 43.9, 40.7, 38.8, 38.2, 36.2, 29.9, 27.8, 27.8, 27.8, 27.7, 26.7, 24.6, 23.8, 22.3, 21.2, 13.7; HRMS calcd for $C_{35}H_{48}O_3S$ 548.3324, found 548.3318; **alkyl thioether 11b** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, J = 8.1 Hz, 2H), 7.19 (d, J = 8.6 Hz, 1H), 7.08 (d, J = 8.3 Hz, 2H), 6.70 (dd, J = 8.6, 2.6 Hz, 1H), 6.62 (d, J = 2.6 Hz, 1H), 3.82 (s, 1H), 3.77 (s, 3H), 3.62 (dd, J = 8.0, 4.1 Hz, 1H), 2.89–2.77 (m, 3H), 2.38–2.14 (m, 3H), 2.31 (s, 3H), 1.91–0.99 (m, 11H), 1.48 (s, 3H), 1.00 (s, 9H), 0.90 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 212.3, 157.5, 138.1, 136.7, 133.8, 132.8, 131,5, 131.5, 129.8, 129.8, 126.3, 113.9, 111.6, 80.5, 55.6, 55.4, 55.4, 55.3, 44.4, 43.9, 40.7, 38.9, 38.2, 36.2, 30.0, 27.8, 27.8, 27.8, 27.7, 26.8, 24.7, 23.7, 21.7, 21.2, 13.6; HRMS calcd for $C_{35}H_{48}O_3S$ 548.3324, found 548.3307.

2.9. Aryl enone **12**

To a stirred solution of diisopropylamine (0.57 mL, 3.7 mmol) in THF (11.6 mL) at −78 °C was added *n*-BuLi (2.5 M in hexanes, 1.52 mL, 3.7 mmol) drop-wise and stirred for 1 h at −78 °C. A solution of α-hydroxy ketone **9** (0.40 g, 1.09 mmol) in THF (2.18 mL) was then added to the reaction and stirred for an additional 1 h at −78 °C. A solution of *p*-methoxybenzaldehyde (0.29 g, 2.18 mmol) in THF (14.5 mL) was then added at −78 °C and the solution was allowed to slowly warm to room temperature and stirred for 20 h. The reaction was quenched by the addition of saturated NH_4Cl (20 mL), followed by extraction of the aqueous layer with EtOAc (3 × 50 mL), dried with Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by silica gel prep-plate chromatography, developing 6× with hexanes/EtOAc (95:5) to yield aryl enone **12** (0.30 g, 57%) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 15.5 Hz, 1H), 7.57 (d, J = 8.6 Hz, 2H), 7.20 (d, J = 8.6 Hz, 1H), 6.92 (d, J = 8.7 Hz, 2H), 6.92 (d, J = 15.4 Hz, 1H), 6.71 (dd, J = 11.1 Hz, 1H), 6.62 (d, J = 2.6 Hz, 1H), 4.27 (s, 1H), 3.84 (s, 3H), 3.77 (s, 3H), 2.95–2.75 (m, 2H), 2.41–2.12 (m, 3H), 1.97–1.16 (m, 11H), 1.55 (s, 3H), 0.96 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 201.9, 162.1, 157.5, 145.6, 138.0, 132.8, 130.6, 130.6, 127.1, 126.3, 116.0, 114.5, 114.5, 113.8, 111.5, 79.1, 55.8, 55.5, 55.3, 55.3, 44.3, 43.9, 40.8, 38.2, 29.9, 27.7, 26.7, 24.5, 23.7, 22.1, 13.7; HRMS calcd for $C_{31}H_{38}O_4$ 474.2770, found 474.2763.

2.10. Aryl thioether **13**

To a stirred solution of 4-methylthiophenol (0.104 g, 0.84 mmol) in petroleum ether (2.0 mL) was added Et_3N (0.116 mL, 0.84 mmol). This reaction mixture was then added to a solution of aryl enone **12** (0.20 g, 0.42 mmol) dissolved in a 1:1 solution of THF:petroleum ether (2 mL), followed by stirring for 24 h. The solvent was removed and the remaining slurry was taken up in a water/CH_2Cl_2 mixture (1:1, 50 mL) and the aqueous layer was subsequently extracted with CH_2Cl_2 (2 × 25 mL), dried with Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by reverse phase HPLC using MeOH:H_2O to yield two diastereomers, with aryl thioether **13a** (45 mg, 36%) eluting faster than aryl thioether **13b** (56 mg, 44%) and both recovered as a white solid. **aryl thioether13a** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24–7.17 (m,

5H), 7.05 (d, *J* = 8.0 Hz, 2H), 6.80 (d, *J* = 8.7 Hz, 2H), 6.71 (dd, *J* = 8.5, 2.6 Hz, 1H), 6.62 (d, *J* = 2.7 Hz, 1H), 4.69 (dd, *J* = 8.4, 5.8 Hz, 1H), 3.77 (s, 3H), 3.77 (s, 3H), 3.72 (s, 1H), 3.18 (dd, *J* = 17.6 8.7 Hz, 1H), 3.04 (dd, *J* = 17.8, 5.9 Hz, 1H), 2.92–2.76 (m, 2H), 2.33–2.12 (m, 3H), 2.30 (s, 3H), 1.85–1.76 (m, 1H), 1.60–0.77 (m, 9H), 1.43 (s, 3H), 0.84 (s, 3H), 0.47–0.34 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.1, 159.0, 157.6, 138.1, 137.8, 133.0, 133.0, 133.0, 132.8, 130.8, 129.8, 129.8, 129.0, 129.0, 126.4, 113.9, 113.9, 113.9, 111.6, 80.3, 55.7, 55.3, 55.3, 55.2, 48.0, 44.4, 43.9, 42.8, 40.7, 38.2, 30.0, 27.8, 26.7, 24.1, 23.8, 22.1, 21.3 13.6; HRMS calcd for [$C_{38}H_{46}O_4S$, $-H_2O$, $-HSC_6H_4CH_3$] 456.2664 (**13a**), found 456.2658; HRMS calcd for $C_{38}H_{46}O_4S$ 598.3117 (mixture of **13a** and **13b**),found 598.3099;**aryl thioether13b** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.24–7.15 (m, 5H), 7.06 (d, *J* = 7.9 Hz, 2H), 6.78 (d, *J* = 8.6 Hz, 2H), 6.71 (dd, *J* = 8.6, 2.9 Hz, 1H), 6.63 (d, *J* = 2.5 Hz, 1H), 4.68 (dd, *J* = 9.4, 5.0 Hz, 1H), 3.78 (s, 3H), 3.77 (s, 3H), 3.73 (s, 1H), 3.21 (dd, *J* = 17.7, 5.3 Hz, 1H), 2.93–2.77 (m, 2H), 2.36–2.13 (m, 3H), 2.30 (s, 3H), 1.90–1.81 (m, 1H), 1.74–1.10 (m, 9H), 1.16 (s, 3H), 0.91–0.82 (m, 1H), 0.86 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 211.1, 159.0, 157.6, 138.1, 137.8, 133.0, 133.0, 133.0, 132.8, 130.8, 129.8, 129.8, 129.0, 129.0 126.4, 113.9, 113.9, 113.9, 111.6, 80.3, 55.7, 55.3, 55.3, 55.2, 48.0, 44.4, 43.9, 42.8, 40.7, 38.2, 30.0, 27.8, 26.7, 24.1, 23.8, 22.1, 21.3, 13.6; HRMS calcd for [$C_{38}H_{46}O_4S$, $-H_2O$, $-HSC_6H_4CH_3$] 456.2664 (**13b**), found 456.2664; HRMS calcd for $C_{38}H_{46}O_4S$ 598.3117 (mixture of **13a** and **13b**),found 598.3099.

3. Results and discussion

The final step in the strategy for the synthesis of the estrone analogs was designed to be a conjugate addition of an aryl thiol to the α,β-unsaturated ketone of compound **4** (Scheme 1). The enone of **4** can be envisioned forming through an aldol coupling reaction between the desired ketone and various aldehydes. The required ketone for use in the aldol reaction can be formed from either methyl Grignard or methyl lithium addition to a nitrile, with the nitrile resulting from the OTMS cyanohydrin formation from ketone **5**. Intermediate **5** can be synthesized in 4-steps from estrone (**1**) through the use of modified literature procedures.

The synthesis of ketone **9** began with protection of the C3 hydroxyl of commercially available estrone (**1**) as a methyl ether [18], followed by a Wittig reaction with the ketone on ring D (Scheme 2) [19]. This reaction provided the required olefin for installation of the C17 side chain and C20 oxygenation. Hydroboration of alkene **6** with 9-borabicyclo[3.3.1]nonane (9-BBN) proceeded selectively upon heating to 60 °C in THF, opposite of the angular methyl to give alcohol **7**. This was followed by an oxidation with PCC to form ketone **5** in 92% yield [20]. Treatment of ketone **5** with catalytic zinc iodide and TMSCN resulted in the formation of desired nitrile **8** in 90% yield, as a single diastereomer [21]. Although this stereocenter has previously been made via other nucleophilic processes [22], this is the first known report being formed via an OTMS cyanohydrin formation. The configuration of the new stereocenter at C20 was shown to be (*R*) through X-ray crystallography (Fig. 2) [23].

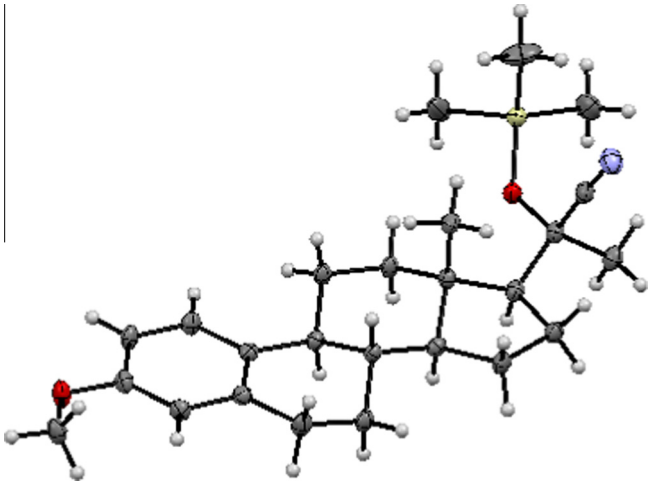
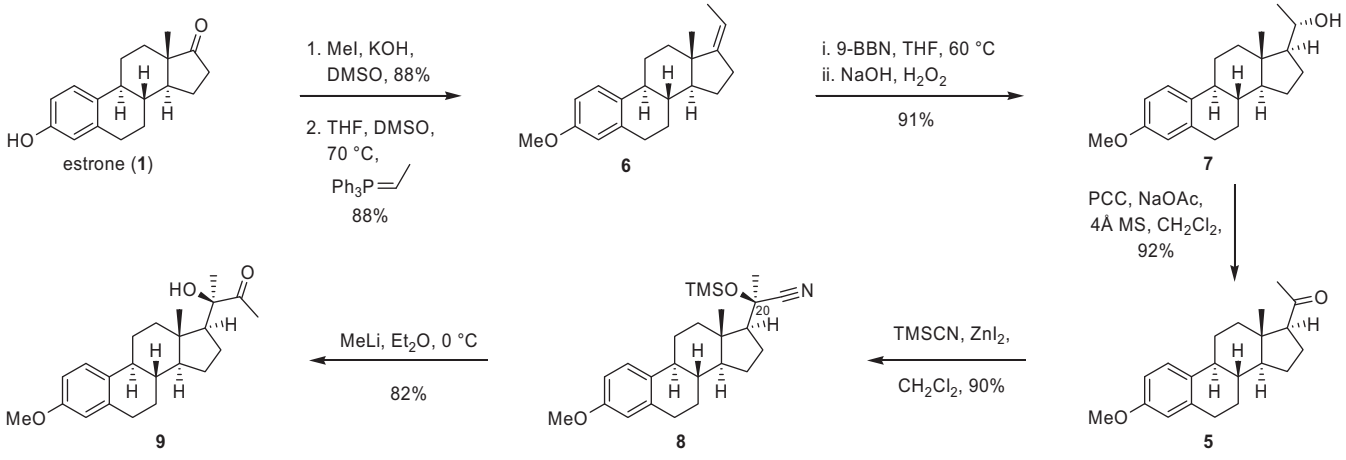


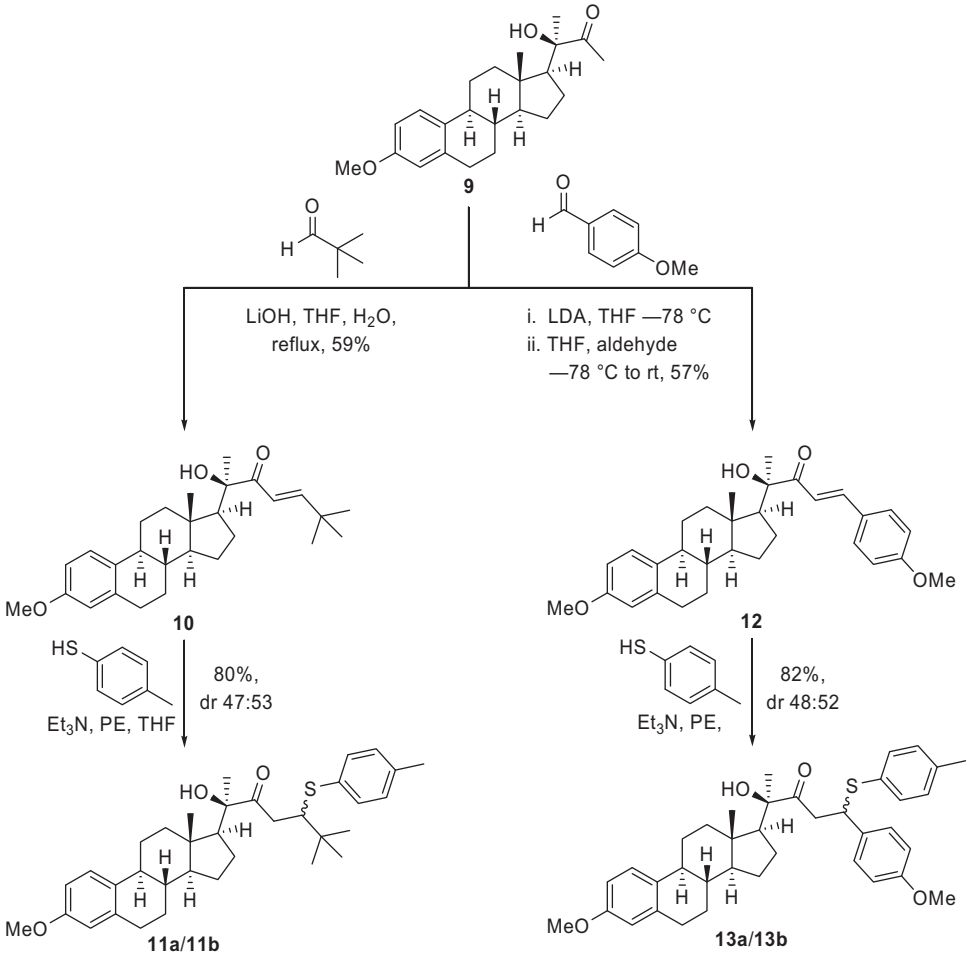
Fig. 2. X-ray crystal structure of **8**.

Initial trials to extend the estrone side chain by one carbon commenced by treatment of hindered nitrile **8** with methylmagnesium bromide with heating to 60 °C [24], failing to provide any of the desired ketone **9**. Changing reagents to the more nucleophilic methyl lithium reagent allowed for the addition to nitrile **8**, providing the α-hydroxyl ketone **9** after an acetic acid work-up (Scheme 2) [25].

This set the stage for diverging the syntheses of the estrone analogs by reacting ketone **9** with either an alkyl or aromatic aldehyde through an aldol reaction, with *in situ* elimination to provide the desired enone. Optimized reaction conditions in the aldol reaction of ketone **9** and pivaldehyde required the use of lithium hydroxide as a base in a refluxing mixture of THF:H_2O to produce enone **10** in 59% yield (Scheme 3) [26]. Formation of the lithium enolate derived from ketone **9** and treatment with pivaldehyde only resulted in



Scheme 2. Synthesis of hydroxy ketone **9**.



Scheme 3. Analog synthesis via an aldol reaction and 1,4-conjugate addition of 4-methylthiophenol.

recovery of unreacted starting material. With material in hand, the sulfa-Michael addition of 4-methylthiophenol to the acyclic α,β-unsaturated ketone of **10** was attempted using triethylamine as the base and a solvent mixture of THF and petroleum ether [17]. This resulted in an easily separable mixture of thioether diastereomers **11** in an almost 1:1 diastereomeric ratio and good yield.

Returning to ketone **9** and *p*-anisaldehyde, enolate formation using lithium diisopropylamine for the aldol reaction and *in situ* elimination was determined to be optimal here compared to the lithium hydroxide procedure previously described (Scheme 3) [26]. These reaction conditions resulted in a greater percent conversion and a more respectable yield. The α,β-unsaturated ketone **12** was subjected to a similar thiol addition procedure as before, with modification of the solvent system. Again, an almost 1:1 diastereomeric mixture of thioether products **13** were obtained like before, this time requiring separation by reverse-phase HPLC [17]. Although the sulfa-Michael addition of 4-methylthiophenol to either enone proved to be non-selective, once the stereochemistry needed for activity is identified, this problem has the potential to be resolved in the future by applying known ligands for asymmetric conjugate addition of thiols [27].

In conclusion, novel estrone analogs featuring either alkyl or aromatic substituted enones were synthesized and reacted with 4-methylthiophenol to form β-substituted thioethers. The biological activity of these new estrone derived compounds are currently being investigated and will be reported in due course, along with additional analogs, taking advantage of the synthesized enone functionality and the previously described reaction sequence.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.steroids.2013.07.005.

References

[1] (a) Gruber CJ, Tschugguel W, Schneeberger C, Huber JC. Mechanisms of disease: production and actions of estrogens. N Engl J Med 2002;346:340–52;
(b) Nilsson S, Mäkelä S, Treuter E, Tujague M, Thomsen J, Andersson G, et al. Mechanisms of estrogen action. Physiol Rev 2001;81:1535–65.
[2] (a) Jordan VC, Brodie AMH. Development and evolution of therapies targeted to the estrogen receptor for the treatment and prevention of breast cancer. Steroids 2007;72:7–25;
(b) Yager JD, Davidson NE. Mechanisms of disease: estrogen carcinogenesis in breast cancer. N Engl J Med 2006;354:270–82;
(c) Kuhl H. Pharmacology of estrogens and progestogens: influence of different routes of administration. Climacteric 2005;8:3–63;
(d) Turgeon JL, McDonnell DP, Martin KA, Wise PM. Hormone therapy:

physiological complexity belies therapeutic simplicity. Science 2004;304:1269–73.
[3] (a) Ibrahim-Ouali M, Rocheblave L. Recent advances in azasteroids chemistry. Steroids 2008;73:375–407;
(b) Ibrahim-Ouali M. Recent advances in oxasteroids. Steroids 2007;72:475–508;
(c) Ibrahim-Ouali M, Santelli M. Recent advances in thiasteroids chemistry. Steroids 2006;71:1025–44;
(d) Ibrahim-Ouali M. First total synthesis of 11-tellura steroids. Tettrahedron Lett 2010;51:3610–2.
[4] (a) Panda S, Panda G. A new example of a steroid–amino acid hybrid: construction of constrained nine membered D-ring steroids. Org Biomol Chem 2007;5:360–6;
(b) Kürti L, Czakó B, Corey EJ. A short, scalable synthesis of the carbocyclic core of the anti-angiogenic cortistatins from (+)-estrone by B-ring expansion. Org Lett 2008;10:5247–50;
(c) Baranovsky AV, Bolibrukh DA, Bull JR, Lyakhov AS, Khripach VA. Synthesis and molecular structure of 14,15-pyrrolidino-and 14,16-ethano derivatives of estrone. Steroids 2008;73:585–93;
(d) Frank E, Körtvélyesi T, Czugler M, Mucsi Z, Keglevich G. New steroid-fused *P*-heterocycles: Part I. Synthesis and conformational study of dioxaphosphorino[16,17-*d*]estrone derivatives. Steroids 2007;72:437–45;
(e) Singh V, Lahiri S, Kane VV, Stey T, Stalke D. Efficient stereoselective synthesis of novel steroid-polyquinane hybrids. Org Lett 2003;5:2199–202;
(f) Zhang Z, Baubet V, Ventocilla C, Xiang C, Dahmane N, Winkler JD. Stereoselective synthesis of F-ring saturated estrone-derived inhibitors of hedgehog signaling based on cyclopamine. Org Lett 2011;13:4786–9.
[5] (a) Saxena HO, Faridi U, Kumar JK, Lugman S, Darokar MP, Shanker K, et al. Synthesis of chalcone derivatives on steroidal framework and their anticancer activities. Steroids 2007;72:892–900;
(b) Parihar S, Gupta A, Chaturvedi AK, Agarwal J, Luqman S, Changkija B, et al. Gallic acid based steroidal phenstatin analogues for selective targeting of breast cancer cells through inhibiting tubulin polymerization. Steroids 2012;77:878–86;
(c) Acebedo SL, Alonso F, Ramírez JA, Galagovsky LR. Synthesis of aromatic stigmastanes: application to the synthesis of aromatic analogs of brassinosteroids. Tetrahedron 2012;68:3685–91;
(d) Matsuya Y, Masuda S, Ohsawa N, Adam S, Tschamber T, Eustache J, et al. Synthesis and antitumor activity of the estrane analogue of OSW-1. Eur J Org Chem 2005:803–8;
(e) Tietze LF, Scheneider G, Wölfling J, Fecher A, Nöbel T, Petersen S, et al. A novel approach in drug discovery: synthesis of estrone-talaromycin natural product hybrids. Chem Eur J 2000;6:3755–60;
(f) Zhao L, Jin C, Mao Z, Gopinathan MB, Rehder K, Brinton RD. Design, synthesis, and estrogenic activity of a novel estrogen receptor modulator–a hybrid structure of 17β-estradiol and vitamin E in hippocampal neurons. J Med Chem 2007;50:4471–81.
[6] (a) Saha P, Fortin S, Leblanc V, Parent S, Asselin E, Bérubé G. Design, synthesis, cytocidal activity and estrogen receptor α affinity of doxorubicin conjugates at 16α-position of estrogen for site-specific treatment of estrogen receptor positive breast cancer. Steroids 2012;77:1113–22;
(b) Oaksmith JM, Ganem B. Synthesis of a COMC-estradiol conjugate for targeted, tissue-selective cancer chemotherapy. Tetrahedron Lett 2009;50:3497–8;
(c) Jones GB, Wright JM, Hynd G, Wyatt JK, Yancisin M, Brown MA. Protein-degrading enediynes: library screening of Bergman cycloaromatization products. Org Lett 2000;2:1863–6.
[7] Kraychy S, Gallagher TF. 2-Methoxyestrone, a metabolite of estradiol-17β in the human. J Am Chem Soc 1957;79:754.
[8] (a) Mueck AO, Seeger H. 2-Methoxyestradiol–biology and mechanism of action. Steroids 2010;75:625–31;
(b) Prakasham AP, Shanker K, Negi AS. A simple and convenient synthesis of 2-methoxyestradiol from estrone. Steroids 2012;77:467–70;
Kirches E, Warich-Kirches M. 2-methoxyestradiol as a potential cytostatic drug in gliomas. Anticancer Agents Med Chem 2009;9:55–65;
Hou Y, Meyers CY, Akomeah M. A short, economical synthesis of 2-methoxyestradiol, and anticancer agent in clinical. J Org Chem 2009;74:6362–4.
[9] (a) Cushman M, He HM, Katzenellenbogen JA, Varma RK, Hamel E, Lin CM, et al. Synthesis of analogs of 2-methoxyestradiol with enhanced inhibitory effects on tubulin polymerization and cancer cell growth. J Med Chem 1997;40:2323–34;
(b) Panchapakesan G, Dhayalan V, Moorthy ND, Saranya N, Mohanakrishnan AK. Synthesis of 2-substituted 17β-hydroxy/17-methylene estratrienes and their *in vitro* cytotoxicity in human cancer cell cultures. Steroids 2011;76:1491–504;
(c) Shah JH, Agoston GE, Suwandi L, Hunsucker K, Pribluda V, Zhan XH, et al. Synthesis of 2- and 17-substituted estrone analogs and their antiproliferative structure–activity relationships compared to 2-methoxyestradiol. Bioorg Med Chem 2009;17:7344;
(d) Stander A, Joubert F, Joubert A. Docking, synthesis, and *in vitro* evaluation of antimitotic estrone analogs. Chem Biol Drug Des 2011;77:173–81;
(e) Jourdan F, Leese MP, Dohle W, Ferrandis E, Newman SP, Chander S, et al. Structure-activity relationships of C-17-substituted estratriene-3-O-sulfamates as anticancer agents. J Med Chem 2011;54:4863–79;
(f) Leese MP, Jourdan FL, Gaukroger K, Mahon MF, Newman SP, Foster PA, et al. Structure-activity relationships of C-17 cyano-substituted estratrienes as anticancer agents. J Med Chem 2008;51:1295–308;
(g) Jourdan F, Leese MP, Dohle W, Hamel E, Ferrandis E, Newman SP, et al. Synthesis, antitubulin, and antiproliferative SAR of analogues of 2-methoxyestradiol-3,17-O,O-bis-sulfamate. J Med Chem 2010;53:2942–51;
(h) LaVallee TM, Burke PA, Swartz GM, Hamel E, Agoston GE, Shah J, et al. Significant antitumor activity in vivo following treatment with the microtubule agent ENMD-1198. Mol Cancer Ther 2008;7:1472–82;
(i) Maltais R, Ayan D, Poirier D. Crucial role of 3-bromoethyl in removing the estrogenic activity of 17β- HSD1 inhibitor 16β-(m-carbamoylbenzyl)estradiol. Med Chem Lett 2011;2:678–81.
[10] (a) Simpkins JW, Yang S, Liu R, Perez E, Cai ZY, Covery DF, et al. Estrogen-like compounds for ischemic neuroprotection. Stroke 2004;35:2648–51;
(b) Liu R, Yang S, Perez E, Yi KD, Wu SS, Eberest K, et al. Neuroprotective effects of a novel non–receptor-binding estrogen analogue. Stroke 2002;33:2485–91.
[11] Mobley JA, L'Esperance JO, Wu M, Friel CJ, Hanson RH, Ho S. The novel estrogen 17α-20Z-21-[(4-amino)phenyl]-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol induces apoptosis in prostate cancer cell lines at nanomolar concentrations *in vitro*. Mol Cancer Ther 2004;3:587–96.
[12] Sinha S, Roy S, Reddy BS, Pal K, Sudhakar G, Iyer S, et al. A lipid-modified estrogen derivative that treats breast cancer independent of estrogen receptor expression through simultaneous induction of autophagy and apoptosis. Mol Cancer Res 2011;9:364–74.
[13] Bunyathaworn P, Boonananwong S, Kongkathip B, Kongkathip N. Further study on synthesis and evaluation of 3,16,20-polyoxygenated steroids of marine origin and their analogs as potent cytotoxic agents. Steroids 2010;75:432–44.
[14] (a) Alexakis A, Bäckvall JE, Krause N, Pàmies O, Diéguez M. Enantioselective copper-catalyzed conjugate addition and allylic substitution reactions. Chem Rev 2008;108:2796–823;
(b) Zhong C, Wang Y, Hung AW, Schreiber SL, Young DW. Diastereoselective control of intramolecular aza-Michael reaction using achiral catalysts. Org Lett 2011;13:5556–9;
(c) Brown MK, Corey EJ. Catalytic enantioselective formation of chiral-bridged dienes which are themselves ligands for enantioselective catalysis. Org Lett 2010;12:172–5;
(d) Mei K, Jin M, Zhang S, Li P, Liu W, Chen X, et al. Simple cyclohexanediamine-derived primary amine thiourea catalyzed highly enantioselective conjugate addition of nitroalkanes to enones. Org Lett 2009;11:2864–7.
[15] (a) Crimmins MT. Synthetic applications of intramolecular enone-olefin photocycloadditions. Chem Rev 1988;88:1453–73;
(b) Chen Y, Zhong C, Petersen JL, Akhmedov NG, Shi X. One-pot asymmetric synthesis of substituted piperidines by exocyclic chirality induction. Org Lett 2009;11:2333–6;
(c) Huang YR, Katzenellenbogen JA. Regioselective synthesis of 1,3,5-triaryl-4-alkylpyrazoles: novel ligands for the estrogen receptor. Org Lett 2000;2:2833–6;
(d) Hawkins JM, Nambu M, Loren S. Asymmetric Lewis acid-catalyzed Diels-Alder reactions of α, β-unsaturated ketones and α, β-unsaturated acid chlorides. Org Lett 2003;5:4293–5.
[16] (a) Alfonsi M, Arcadi A, Aschi M, Bianchi G, Marinelli F. Gold-catalyzed reactions of 2-alkynyl-phenylamines with α, β-enones. J Org Chem 2005;70:2265–73;
(b) Sato K, Omote M, Ando A, Kumadaki I. Rhodium-catalyzed novel trifluoromethylation at the α-position of α, β-unsaturated ketones. Org Lett 2004;6:4359–61;
(c) Zhao W, Zhang J. Rhodium-catalyzed tandem heterocyclization and carbonylative [(3+2)+1] Cyclization of diyne-enones. Org Lett 2011;13:688–91;
(d) Oshita M, Yamashita K, Tobisu M, Chatani N. Catalytic [4+1] cycloaddition of α, β-unsaturated carbonyl compounds with isocyanides. J Am Chem Soc 2005;127:761–6.
[17] (a) Cherkauskas JP, Cohen T. Carbonyl-protected beta-lithio aldehydes and ketones via reductive lithiation. A general preparative method for remarkably versatile homoenolate equivalents. J Org Chem 1992;57:6–8;
(b) Nishide K, Ohsugi S, Miyamoto T, Kumar K, Node M. Development of odourless thiols and sulfides and their applications to organic synthesis. Monatsh Chem 2004;135:189–200;
(c) Kumar A, Tripathi VD, Kumar P, Gupta LP, Akanksha, Trivedi R. Design and synthesis of 1,3-biarylsulfanyl derivative as new anti-breast cancer agents. Bioorg Med Chem 2011;19:5409–19;
(d) Khatik GL, Kumar R, Chakraborti AK. Catalyst-free conjugated addition of thiols to α, β-unsaturated carbonyl compounds in water. Org Lett 2006;8:2433–6.
[18] Montenegro HE, Ramírez-López P, De La Torre MC, Asenjo M, Sierra MA. Two versatile and parallel approaches to highly symmetrical open and closed natural product-based structures. Chem Eur J 2010;16:3798–814.
[19] (a) Kim HK, Blye RP, Rao PN, Cessac JW, Acosta CK, Simmons AM. U.S. Patent 6, 900, 193 B1, 2005.;
(b) Labrie F, Singh S, Gauthier S, Frechette Y, Chenard S, Breton R. W.O. Patent 2005/066194 A1, 2005.;
(c) Houston TA, Tanaka Y, Koreeda M. Stereoselective construction of 22-oxygenated steroid side chains by dimethylaluminum chloride-mediated ene reactions of aldehydes. J Org Chem 1993;58:4287–92.
[20] Corey EJ, Suggs W. Pyridinium chlorochromate. An efficient reagent for oxidation of primary and secondary alcohols to carbonyl compounds. Tetrahedron Lett 1975;16:2647–50.

[21] Evans DA, Truesdale LK, Carrol GL. Cyanosilylation of aldehydes and ketones. A convenient route to cyanohydrin derivatives. J Chem Soc Chem Commun 1973:55–6.

[22] (a) Mauvais A, Hetru C, Roussel JP, Luu B. Inhibition of ecdysone biosynthesis: preparation of acetylenic intermediates. Tetrahedron 1993;49:8597–604;
(b) Sondheimer F, Danieli N, Mazur Y. Synthesis and reactions of 3β-acetoxy-20-ethynyl-5-pregnen-20-ol. J Org Chem 1959;24:1278–80;
(c) Litvinovskaya RP, Drach SV, Khripach VA. Synthesis and transformations of 2β,3β-diacetoxy-20-(4,5-dihydroisoxazol-5-yl)-6-oxo steroids.Russ. J Org Chem 2004;40:1450–5;
(d) Hazra BG, Basu S, Bahule BB, Pore VS, Nyas BN, Ramraj VM. Stereoselective synthesis of a new hexanor(C23–C28)castasterone-20,22-ethyl diether from 16-dehydropregnenolone acetate and its plant growth promoting activity. Tetrahedron 1997;53:4909–20.

[23] CCDC 908720 (8) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via. Available from: www.ccdc.cam.ac.uk/data_request/cif.

[24] Claudel E, Arbez-Gindre C, Berl V, Lepoittevin J. An efficient hemisynthesis of 20- and 21-[^{13}C]-labeled cortexolone: a model for the study of skin sensitization to corticosteroids. Synthesis 2009:3391–8.

[25] Bañuelos P, García JM, Gómez-Bengoa E, Odriozola JM, Oiarbide M, Palomo C, et al. (1R)-(+)-camphor and acetone derived α′-hydroxy enones in asymmetric Diels-Alder reaction: catalytic activation by Lewis and Brønsted acids, substrate scope, applications in syntheses, and mechanistic studies. J Org Chem 2010;75:1458–73.

[26] Palomo C, Oiarbide M, Gómez-Bengoa E, Mielgo A, González-Rego MC, García JM, et al. Asymmetric propionate aldol reactions of a chiral lithium enolate accessible from direct enolization with n-butyllithium. ARKIVOC 2005;vi:377–92.

[27] (a) Bonollo S, Lanari D, Pizzo F, Vaccaro L. Sc(III)-catalyzed enantioselective addition of thiols to α, β-unsaturated ketones in neutral water. Org Lett 2011;13:2150–2;
(b) Rana NK, Selvakumar S, Singh VK. Highly enantioselective organocatalytic sulfa-Michael addition to α, β-unsaturated ketones. J Org Chem 2010;75:2089–91;
(c) Ricci P, Carlone A, Bartoli G, Bosco M, Sambri L, Melchiorre P. Organocatalytic asymmetric sulfa-Michael addition to α, β-unsaturated ketones. Adv Synth Catal 2008;350:49–53;
(d) Tian X, Cassani C, Liu Y, Moran A, Urakawa A, Galzerano P, et al. Diastereodivergent asymmetric sulfa-Michael additions of α-branched enones using a single chiral organic catalyst. J Am Chem Soc 2011;133:17934–41.