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A Short, Economical Synthesis of 2-Methoxyestradiol, an Anticancer Agent in Clinical Trials†

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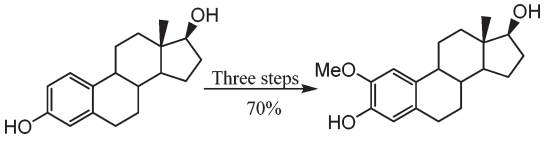

2-Methoxyestradiol, a natural metabolite of estradiol and potential therapeutic agent for many types of cancers, has been synthesized successfully in three steps, starting from estradiol and cumyl methyl peroxide.

Estradiol (**1**), the major female hormone in humans, is primarily oxidized into 2-hydroxyestradiol by P450 enzymes in the liver and converted into 2-methoxyestradiol (**2**) through the action of catechol-*O*-methyl transferase.[1] Recently, it was reported that **2** binds to the colchicine binding site, inhibiting the polymerization of tubulin and, thereby, cell division.[2] Thus, **2** inhibits the growth of new blood vessels, i.e., it is antiangiogenic, leading to the possibility that **2** may be useful in treating cancers by inhibiting the formation of new blood vessels around cancer tissues, thereby cutting off the nutrition supply to the cancer cells. This strategy has proven practical by the FDA's approval of the first antiangiogenic drug for the treatment of advanced-stage colorectal cancer with Avastin (bevacizumab), introduced by Genentech.[3] Currently, **2** has been granted an "orphan drug" status by the FDA, and is in clinical trials for many types of cancers, including breast and prostate cancers, by Entremed, Inc., in association with leading medical institutions in the United States under the trade name Panzem.[4] Unlike many other chemotherapeutic agents for cancer, **2** has shown few side effects in numerous studies, which is a remarkable advantage for a potential chemotherapeutic agent.[5]

The commercial and research interest in **2** is obvious from the many reports on its synthesis, mostly starting from **1**. The shortest reported synthesis requires only two steps: halogenation of C-2, followed by a catalyzed substitution of the halide with methoxide.[6] However, the first step suffers poor selectivity between C-2 and C-4, and the complete separation of 2-halo- and 4-haloestradiols is very difficult to achieve. A much more selective method involves the use of organoiridium complex to direct the methoxylation process; thus, **2** was obtained from **1** in three steps with a good yield, although a stoichiometric amount of iridium complex is required.[7] More recently, oxidation of 2-substituted estradiol with peroxide has proven successful in the preparation of **2** with high selectivity; however, five or more steps were needed to convert **1** into **2**.[8] We wish to report an improved synthesis of **2** from **1** by directly introducing a methoxy group onto C-2, using similar MOM-directed C-2 lithiation chemistry followed by oxidation with cumyl methyl peroxide.[9]

Although it had been known that alkyl peroxides could alkoxylate carbanions,[10] this methodology has not been widely used; a search on SciFinder Scholar showed only a handful of references for this type of reaction.[11] Interestingly, Kochi *et al.*[10c] carried out a detailed mechanistic study more than three decades ago and concluded that, in the case of sterically encumbered dialkyl peroxides, these reactions went through electron-transfer mechanisms. The limited usage of this methodology might therefore be attributed to the explosiveness of these alkyl peroxides, particularly the low molecular weight analogues such as dimethyl peroxide.[10a] It has been reported that cumyl methyl peroxide

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(**4**)[9] was easily made by methylating commercially available cumyl hydroperoxide (**3**) under basic condition (Scheme 1) and purified via chromatography and rotary evaporation without decomposition. Therefore, we postulate that **4** could be used as a safe, convenient methoxylating reagent for carbanions due to the difference in bulkiness of the two alkyl groups on the peroxide functionality, *i.e.*, methyl *vs.* cumyl.

To test our hypothesis, we chose to synthesize **2** via MOM-protected estradiol.[8d,8h] First, **1** was treated with a solution of MOMCl and DIPEA in THF to produce the doubly MOM-protected estradiol, **5**. When treated with *t*-BuLi in THF at −40 °C, **5** was lithiated on C-2 exclusively, directed by the MOM protecting group attached on the phenolic oxygen. This anion was subsequently methoxylated on C-2 by treatment with cumyl methyl peroxide. Hydrolysis of the two MOM protecting groups on **6** with 6 N HCl in THF provided compound **2** in high yield. The overall yield of the three steps was 70%. Other reported methods using C-2 lithiated MOM-protected estradiol to synthesize **2**[8c,8d,8h] did not introduce the methoxy group directly onto the lithiated C-2, but went through a combination of formylation/Bayer−Villiger oxidation/methylation or boration/oxidation/methylation (Scheme 2). Clearly, our method is a shorter and more efficient method than the reported methods.

In conclusion, taking advantage of the reaction between a carbanion and cumyl methyl peroxide, we have designed and executed a three-step synthesis of 2-methoxyestradiol (**2**, Panzem) in an overall yield of 70%, a key step being the direct methoxylation of C-2 lithiated **5**.

Experimental Section

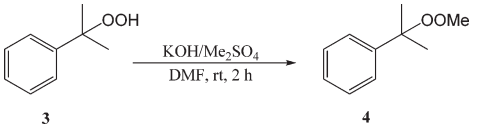
Preparation of Cumyl Methyl Peroxide (4). To a colorless solution of cumyl hydroperoxide (20.0 g, 80% aq solution, 105 mmol) in DMF (50 mL) was added freshly pulverized KOH (7.40 g, 112 mmol), which immediately turned the solution into a slightly purple suspension. To this suspension was added dimethyl sulfate (15.0 mL, 158 mmol). The mixture faded to colorless, producing an exothermic reaction. The mixture was stirred for 2 h, water was added to quench the reaction, and this mixture was extracted with hexanes, washed with water, and dried over $MgSO_4$. After the solvent was removed in vacuo, the residual oil was chromatographed (silica gel, hexanes/ethyl acetate 50:1) to provide a colorless oil (12.4 g; 71.0%). ^{1}H NMR δ 1.59 (s, 6 H), 3.78 (s, 3 H), 7.25 (m, 1 H), 7.35 (m, 2 H), 7.47 (m, 2 H), agreeing with literature data.[9] (**Caution**: Cumyl methyl peroxide might be shock sensitive. Careful handling is recommended, although no problem was encountered in this lab.)

Preparation of 3,17β-*O*-Bis(Methoxymethyl)estradiol (5).[8d,8h] A mixture of estradiol (4.0 g, 14.7 mmol), methoxymethyl chloride (5.3 mL, 69 mmol), and diisopropylethylamine (13.0 mL) in dry THF (30 mL) was refluxed overnight under argon before being cooled to rt. Water was added to destroy excess methoxymethyl chloride. Product was extracted with ether, washed with 10% AcOH solution, water, and aq $NaHCO_3$, and dried over $MgSO_4$. Evaporation of solvent in vacuo left a yellow viscous oil (5.4 g, 102%). ^{1}H NMR showed that it was essentially pure product. The product was further purified through column chromatography to provide a pale-yellow oil (5.1 g; 96%). ^{1}H NMR δ 0.81 (s, 3 H), 1.14−1.76 (m, 8 H), 1.87 (m, 1 H), 1.96−2.32 (m, 4 H), 2.82−2.88 (m, 2 H), 3.38 (s, 3 H), 3.47 (s, 3 H), 3.62 (dd, 1 H, J = 8.1, 8.7 Hz), 4.66 (ABq, 2 H, J = 6.6 Hz), 5.15 (s, 2 H), 6.77 (d, 1 H, J = 2.7 Hz), 6.83 (dd, 1 H, J = 2.7, 8.4 Hz), 7.20 (d, 1 H, J = 8.7 Hz), agreeing with the literature data.[8h]

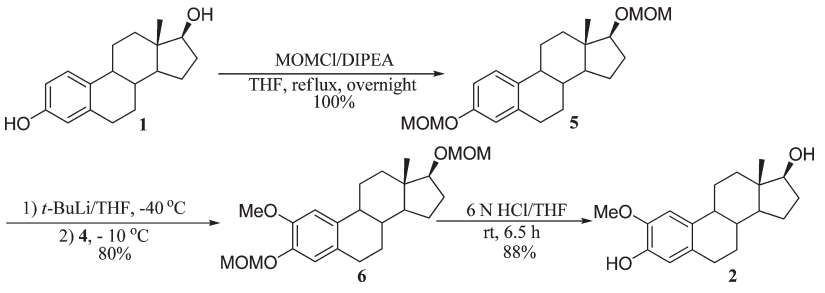
Preparation of 2-Methoxy-3,17β-*O*-bis(methoxymethyl)estradiol (6). Compound **5** (0.727 g, 2.00 mmol) was dissolved in dry THF (2 mL). The flask was sealed with a rubber septum, flushed with argon, and cooled in a −40 °C liquid N_2−acetone bath. The solution was stirred for 5 min before *t*-BuLi (1.5 M, 3 mL, 4.5 mmol) was injected dropwise, which rapidly turned the mixture yellow. The mixture was stirred for 30 min, during which time the temperature rose to −10 °C. Cumyl methyl peroxide (0.552 g, 3.32 mmol) was injected slowly, which turned the mixture back to colorless. The reaction was quenched with water and the product was extracted with ether and dried over $MgSO_4$. Removal of solvent on rotavap provided a pale yellow oil, which was subjected to high vacuum at 100 °C to remove cumyl alcohol and residual cumyl methyl peroxide to afford a pale yellow thick oil (0.744 g). Chromatography (silica gel, hexanes/ether 10:1 to 4:1) provided **6** as a thick oil (0.444 g—80% based on consumed **5**) and **5** (0.216 g). Crystallization of **6** from methanol provided colorless crystals (0.302 g, 54.6%). Mp 73−75 °C (lit.[8c] mp 68−70 °C). ^{1}H NMR δ 0.82 (s, 3 H), 1.14−1.76 (m, 8 H), 1.87 (m, 1 H), 1.96−2.32 (m, 4 H), 2.76−2.82 (m, 2 H), 3.38 (s, 3 H), 3.51 (s, 3 H), 3.62 (dd, 1 H, J = 8.1, 8.7 Hz), 3.86 (s, 3 H), 4.66 (ABq, 2 H, J = 6.6 Hz), 5.20 (s, 2 H), 6.84 (s, 1 H), 6.87 (s, 1 H).

Preparation of 2-Methoxyestradiol (2). Compound **6** (0.241 g, 0.61 mmol) in a mixture of THF (6 mL), water (1.5 mL), and 12 N HCl (1.5 mL) was stirred at rt under argon for 6.5 h. The product was extracted with ether, dried over $MgSO_4$, and evaporated to dryness to provide a white solid (0.162 g, 88%). Recrystallization from acetone/hexanes provided colorless crystals (0.143 g, 77.7%). Mp 185−187 °C. Next 0.06 g of the crystals was further recrystallized from ethyl acetate to provided 0.031 g of colorless crystals. Mp 187−188 °C (lit.[8g] mp 186−187 °C). ^{1}H NMR δ 0.79 (s, 3 H), 1.14−1.56 (m, 8 H), 1.70 (m, 1 H),

SCHEME 1. Preparation of Cumyl Methyl Peroxide



SCHEME 2. Preparation of 2-Methoxyestradiol (2)



1.86 (m, 1 H), 1.96 (m, 1 H), 2.08−2.32 (m, 3 H), 2.74−2.82 (m, 2 H), 3.74 (dd, 1 H, J = 8.1, 8.7 Hz), 3.86 (s, 3 H), 6.65 (s, 1 H), 6.80 (s, 1 H). ^{13}C NMR δ 11.3, 23.3, 26.9, 27.5, 29.2, 30.9, 37.0, 39.0, 43.5, 44.5, 50.3, 56.3, 82.2, 108.3, 114.8, 129.8, 132.0, 143.7, 144.8, agreeing with literature data.[8g]

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Supporting Information Available: General experimental conditions, ^{1}H NMR spectra of compounds **4**, **5**, **6**, and **2**, and ^{13}C NMR spectrum of **2**. This material is available free of charge via the Internet at http://pubs.acs.org.