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Total Synthesis of the Potent Anticancer *Aglaia* Metabolites (−)-Silvestrol and (−)-Episilvestrol and the Active Analogue (−)-4′-Desmethoxyepisilvestrol

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Abstract: Total synthesis of the anticancer 1,4-dioxane containing natural products silvestrol (**1**) and episilvestrol (**2**) is described by an approach based on the proposed biosynthesis of these novel compounds. The key steps included an oxidative rearrangement of the protected D-glucose derivative **11** to afford the 1,4-dioxane **12**, which could be elaborated to the coupling partner **5** and a photochemical [3 + 2]-cycloadditon between the 3-hydroxyflavone **27** and methyl cinnamate followed by base-induced α-ketol rearrangement and reduction to give the cyclopentabenzofuran core **33**. The core (−)-**6** and 1,4-dioxane fragment **5** were united by a highly stereoselective Mitsunobu coupling with the modified azodicarboxylate DMEAD to afford the axial coupled product **36**. Deprotection then gave episilvestrol (**2**). Silvestrol (**1**) was synthesized by a coupling between core (−)-**6** and the dioxane **44** followed by deprotection. Compound **1** was also synthesized from episilvestrol (**2**) by a Mitsunobu inversion. In addition, the analogue 4′-desmethoxyepisilvestrol (**46**) was synthesized via the same route. It was found that **46** and episilvestrol **2** displayed an unexpected concentration-dependent chemical shift variation for the nonexchangeable dioxane protons. Synthetic compounds **1**, **2**, **38**, **46**, and **54** were tested against cancer cells lines, and it was found that the stereochemistry of the core was critical for activity. Synthetic analogue 4′-desmethoxyepisilvestrol (**46**) was also active against lung and colon cancer cell lines.

Introduction

Aglaia is a genus of the family Meliaceae, which comprises a large group of mostly woody plants found in Malaysia, Indonesia, and parts of the Western Pacific region. Extracts of these plants have been used for the treatment of fever, inflammation, and abdominal tumors and as bactericides and insecticides.[1] The crude extract of the shrub *Aglaia leptantha* Miq. (Meliaceae) was shown to possess potent cytotoxic activity, which was eventually attributed to two new molecules **1** and **2** (Figure 1).[2] Compounds **1** and **2** are diastereoisomers that are epimeric at 5‴ and contain a common cyclopenta[*b*]benzofuran with five contiguous stereogenic centers as well as a novel 1,4-dioxanyloxy or *pseudosugar* substituent.[3] A number of cyclopenta[*b*]benzofuran natural products[4] have been found in several *Aglaia* species, with some examples being aglafoline (methyl rocaglate) (**3**)[5−7] and rocaglamide (**4**).[8] Two metabolites isolated from the dried fruits and twigs of *Aglaia foveolata* (initially incorrectly identified as *Aglaia silvestris*) by Kinghorn and co-workers[9,10] were found to be identical to **1** and **2** and were named silvestrol and episilvestrol, respectively. The structure of silvestrol (**1**) was determined by NMR spectroscopy and X-ray analysis of the 5‴, 6‴-bis-*p*-bromobenzoate derivative of silvestrol, which served to confirm the relative and absolute configuration of this compound. Several total syntheses of rocaglamide[11−13] and methyl rocaglate/aglafolin[12,13] and a number of approaches to the rocaglates[14] have been reported to date, while two independent syntheses of silvestrol (**1**) were communicated in 2007.[15−17]

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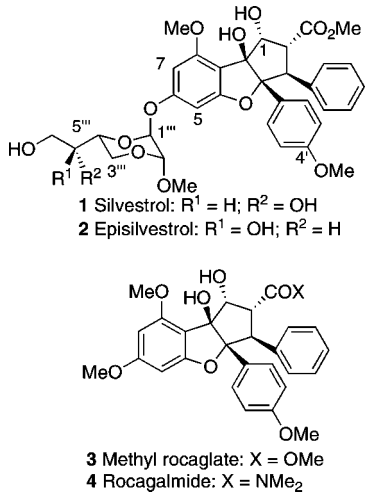
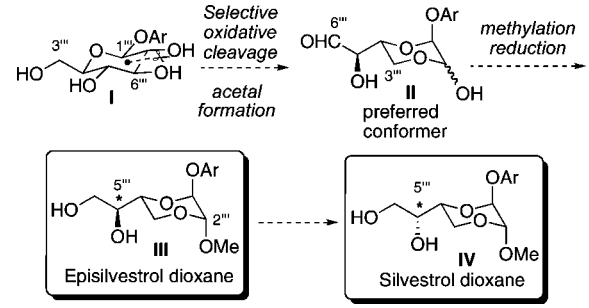


Figure 1. Structures of silvestrol (**1**) and episilvestrol (**2**).

Silvestrol (**1**) displays potent cytoxicity comparable to that for paclitaxel and camptothecin against several human cancer cells lines including lung (Lu1,[9] $ED_{50} = 1.2$ nM; A549,[2] $LC_{50} = 15$ nM), prostate (LNCaP,[9] $ED_{50} = 1.5$ nM; PC3,[2] $LC_{50} = 12$ nM), breast (MCF-7,[9] $ED_{50} = 1.2$ nM) and leukemia (K562,[2] $GI_{50} = 12$ nM). Episilvestrol (**2**) shows similar activity as that for silvestrol (**1**) against some cell lines (K562,[2] $GI_{50} = 15$ nM) but has been reported as ~3 times less active than **1** in other assays (Lu1,[9] $ED_{50} = 3.8$ nM). This demonstrates that the 5‴ stereochemistry does not have a substantial effect on the activity of these compounds. Silvestrol **1** also inhibits protein biosynthesis with $IC_{50} \sim 30$ nM for THP-1 cells. More importantly, compound **1** shows potent in vivo activity against tumor models in mice. Administration of silvestrol (**1**) into athymic mice implanted with PC3 cells (human prostate cancer) by intraperitoneal injection of 3 mg/kg three times a week for 29 days resulted in a reduction of the mean tumor weight by ~60% while body weight remained unaffected.[2] Similarly, in an independent study, doses of up to 5 mg/kg silvestrol caused up to 63% inhibition in the growth of KB cells (human nasopharynx cancer) implanted in mice and over 82% inhibition of LNCaP cells (human prostate cancer).[9] Silvestrol was also active against the iv P388 murine leukemia model. Administration of a maximum tolerated dose of 2.5 mg/kg by intraperitoneal injection daily for 5 days resulted in a maximum increase in lifespan reflected by the mean survival time of treated (T) versus control (C) groups corresponding to a T/C of 150%.[9]

Scheme 1. Possible Biosynthetic Origin of the 1,4-Dioxanyloxy Fragments of **1** and **2**



It is interesting to note that the activities of **1** and **2** do not appear to be shared by related cyclopenta[*b*]benzofuran-type natural products that lack the unusual 1,4-dioxylanoxy substituent.[2,18] Mode of action studies have shown that silvestrol (**1**) arrests human prostate cancer (LNCaP) cells at the G2/M transition and this effect was independent of P53 activity.[19] In addition, compound **1** induces apoptosis in LNCaP cells through the mitochondrial/apoptosome pathway which appears to involve caspases 2, 9, and 10 but not caspases 3 and 7.[18]

Clearly, these in vivo studies demonstrate that silvestrol (**1**) displays a biological profile which certainly warrants further investigation for its potential as a chemotherapeutic agent. However, the paucity of these compounds from natural sources [yield of **1** was 0.01% (w/w) from dried fruits or 0.008% (w/w) from dried twigs of *A. folveolata*] led us to investigate a total synthesis of these important targets. In this paper we present the full details of our total synthesis of **1** and **2**, including an improved route to that initially reported,[16] as well as synthesis of the novel potent analogue 4′-desmethoxyepisilvestrol.

Retrosynthetic Analysis. At the outset, we elected to adopt a synthetic approach inspired by the possible biogenesis of these compounds. It was hoped that a short, so-called biomimetic route to **1** and **2** could be developed, which would be amenable to cost efficient scale-up. With this in mind, some time ago we suggested a biosynthetic origin for the novel 1,4-dioxane of episilvestrol (**2**) that is summarized in Scheme 1.[3] The sequence begins with β-D-glycopyranoside **I**, which undergoes selective oxidative cleavage of the C2‴−C6‴ bond (episilvestrol numbering) and concomitant acetal formation to give the lactols **II**. Subsequent stereoselective methylation and reduction then yields the episilvestrol dioxane **III**. Inversion at the C5‴ stereocenter would give silvestrol dioxane **IV**; however, this route could also begin with a β-D-galactopyranoside analogue of **I**, which would provide 1,4-dioxane **IV** directly. Inversion of this affords the episilvestrol configured dioxane **III**.

A biosynthetic rationale for the origin of the cyclopentabenzofuran core of **1** and **2** is based on that suggested by Proksch and co-workers[4,20] as shown in Scheme 2. This hypothesis

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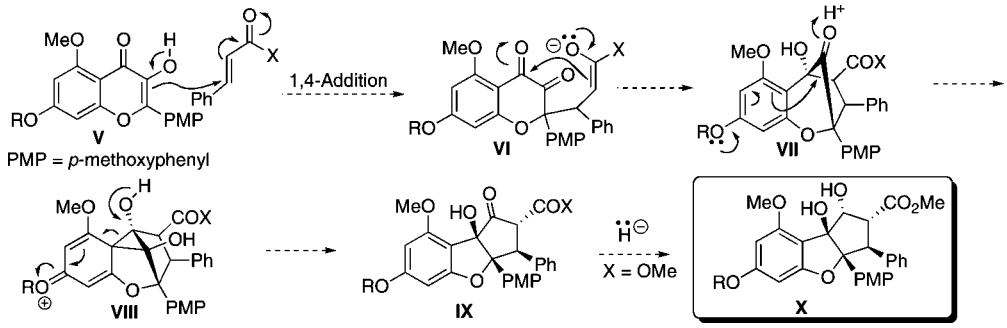
Scheme 2. Proposed Biosynthetic Origin of the Cyclopenta[*b*]benzofuran Core of **1** and **2**



begins with a Michael-type conjugate addition of the 3-hydroxyflavone **V** into a cinnamate electrophile to give the enolate **VI**. An intramolecular aldol between the enolate **VI** and the C4 carbonyl group forms a cyclopentane ring and gives the aglain precursor **VII**. Reduction of the ketone in this intermediate would provide an aglain-type natural product. In addition, **VIII** serves as a precursor to the rocaglate-type natural products (formally an α-ketol-type rearrangement), which could initially involve an electrophilic ipso substitution to give intermediate cyclopropane **VIII**, which is transformed into the α-hydroxyketone **IX**. Compound **IX** is a *β*-keto ester and this serves as the thermodynamic sink in the sequence.[21] Subsequent antispecific reduction would then afford the cyclopenta[*b*]benzofuran **X**.

The above proposals are supported by the occurrence of the ubiquitous flavinone populnin[22] in many natural sources (Figure 2). Populnin is the 7-O-glycoside of kaempferol and is a viable biosynthetic precursor to episilvestrol (**2**) via the pathways proposed above. While adopting populnin as a starting material was an intriguing prospect, problems with cost, availability, and protecting group issues would render this substrate unviable as an episilvestrol (**2**) precursor, so we elected to pursue a convergent route as shown in Scheme 3.

It was envisaged that episilvestrol could be formed from a coupling between 1,4-dioxane lactols **5** and the cyclopenta[*b*]benzofuran core phenol **6** via a Mitsunobu reaction.[23] A Mitsunobu glycosylation approach was applied by Roush and Lin[24,25] for the stereoselective synthesis of *O*-aryl *β*-glycosides, and this could be easily adapted to the present case for forming the 1,4-dioxylanoxy *pseudoglycoside*. It was hoped that the stereoselective formation of the required C1‴ axial isomer (or α-anomer in this case) might arise by a coupling between **6** and α,*β*-lactol mixture **5** via an oxonium ion intermediate by an S_N1-type mechanism rather than a direct S_N2 displacement.[25] This coupling approach was attractive in that it does not require the synthesis of an activated dioxylanoxy donor and would permit the recovery of unreacted lactol. Dioxane lactols **5** could be produced from commercially available D-glucose derivative α-D-glucopyranosyl bromide **7** by following a route based on the biosynthesis proposed above. In turn, the cyclopentabenzofuran core **6** could then be synthesized from commercially available 4′, 5,7-trihydroxyflavanone or (±)-naringenin (**8**)

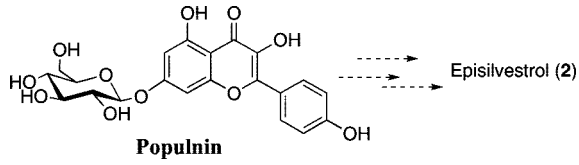
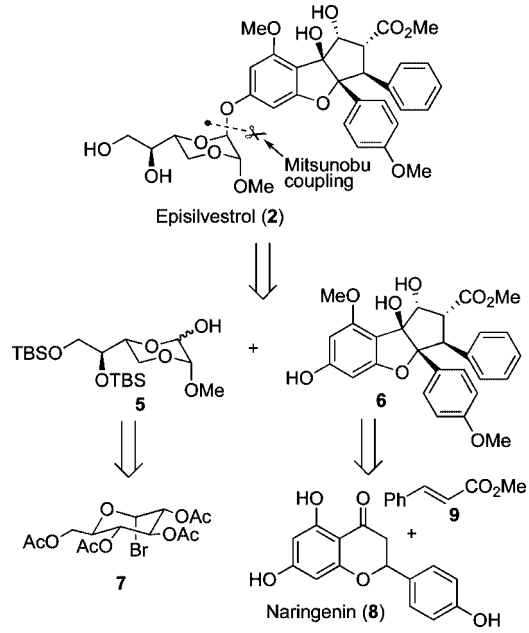


Figure 2. Populnin as a biosynthetic precursor of episilvestrol (**2**).

Scheme 3. Retrosynthetic Analysis of Episilvestrol (**2**)



[~$10 Australian dollars (AUD)/g] and methyl cinnamate (**9**) via a sequence similar to that described in Scheme 2.

Results and Discussion

Synthesis of 1,4-Dioxylanoxy Fragment 5. The route to the 1,4-dioxylanoxy fragment begins with Koenigs−Knorr glycosylation[26] of glycosyl bromide **7** with *p*-methoxybenzyl alcohol (Scheme 4). The resultant glycoside was subjected to methanolysis and the crude pentol was converted into the O4−6 benzylidene acetal **10**[27] in good overall yield for the three steps. Selective cleavage of the O6−C acetal bond was achieved with BH_3·THF in the presence of $Cu(OTf)_2$.[28] With the O-1,4 protected glucopyranoside **11** in hand, we subjected this to $NaIO_4$,[29] which cleanly provided the 1,4-dioxane aldehyde **12**

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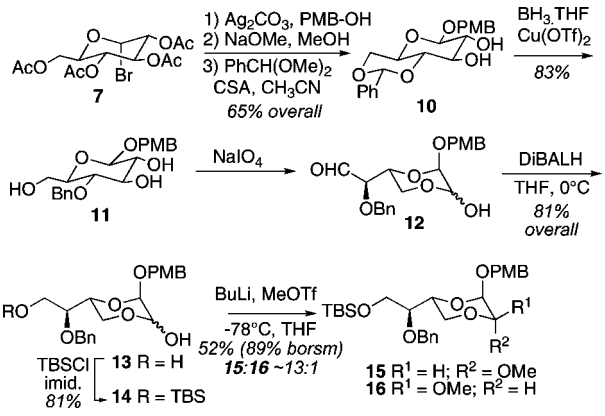
Scheme 4. Synthesis of 1,4-Dioxane **15**



as a ∼3:1 mixture of anomers in quantitative yield as a result of concomitant acetal formation involving the C6 primary alcohol. Reduction of the aldehyde **12** was achieved with diisobutylaluminum hydride (DiBALH) to afford the alcohol **13**, which was selectively protected to give the *tert*-butyldimethylsilyl (TBS) ether **14** as a ∼1:1 mixture of lactols. After some experimentation, we found that methylation of the lactol **14** was best achieved via the lithium alkoxide formed by treatment with BuLi or lithium hexamethyldisilazide (LiHMDS) followed by MeOTf[30] as the methylating agent. This gave good selectivity for the desired axial acetal **15** over the equatorial isomer **16**. The use of Na as a counterion or MeI as methylating agent gave inferior selectivity.

The Mitsunobu coupling was then investigated using a model core phenol as shown in Scheme 5. Oxidative removal of the *p*-methoxybenzyl (PMB) group in **15** was plagued by competitive debenzylation affording the lactols **17** in low yield. We therefore elected to remove the benzyl group at this stage and replace this with a TBS group. This would then only necessitate one deprotection step at the end of the synthesis. Hydrogenolysis of **15** selectively removed the benzyl ether to give alcohol **18**, which upon reprotection gave the bis-TBS ether **19**. PMB group removal now proceeded in acceptable yield to afford the lactol **5**. The coupling between the lactol **17** and model core 3-methoxyphenol in the presence of diisopropyl azodicarboxylate (DIAD) and PPh_3 provided only minuscule amounts of the desired product. We eventually found that the coupling progressed in the presence of powdered 4 Å molecular sieves to give axial and equatorial coupled products **20** and **21** in 71% yield and a ratio of 2.6:1. The coupling between 3-methoxyphenol and lactol **5** also gives a similar ratio (2:1) of axial to equatorial products **22** and **23** but in a lower combined yield of 54%.

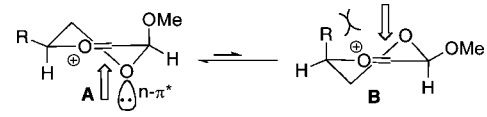
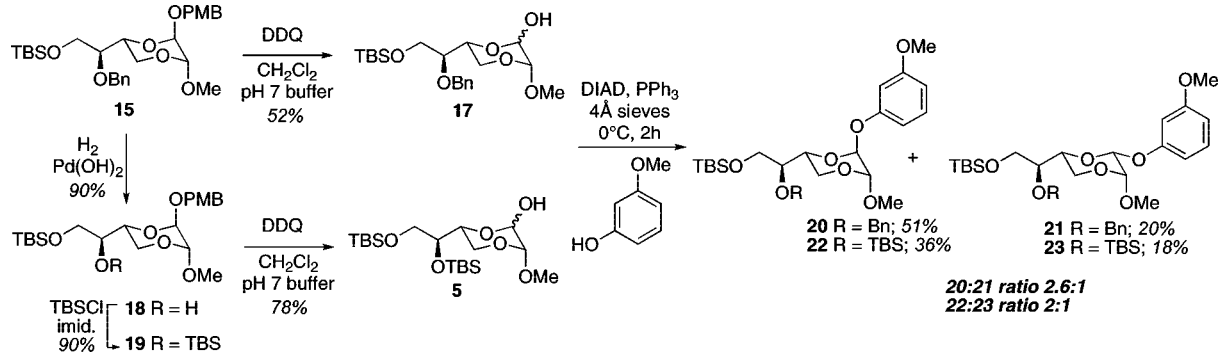


Figure 3. Rationale for Mitsunobu coupling selectivity.

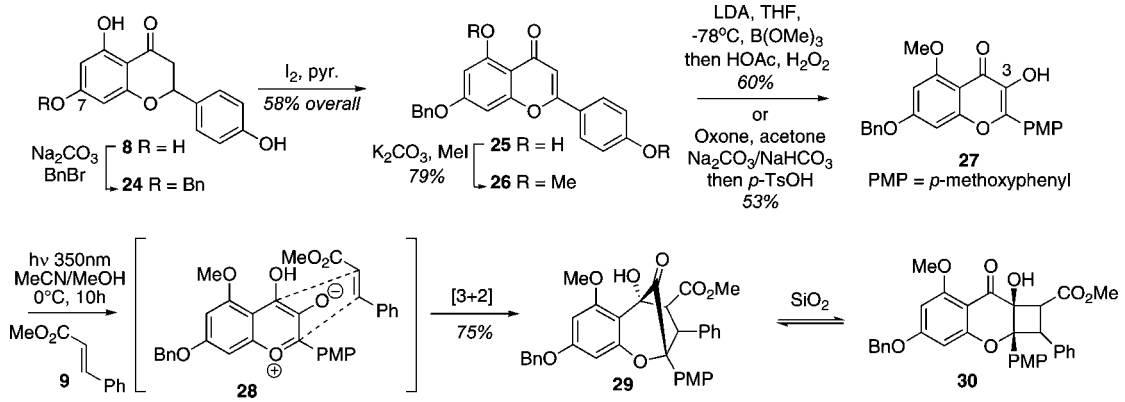
The stereochemical outcome of these Mitsunobu couplings does not appear to correlate with the original stereochemistry of the lactols observed for the synthesis of *O*-aryl glucosides.[24,25] For example, compound **17** was a 1.4:1 mixture of axial and equatorial (α and β) hemiacetals, respectively (^{1}H NMR in $CDCl_3$). If the coupling is proceeding via an S_N2-type mechanism, one would expect the ratio of the axial α-anomer **20** to the equatorial β-anomer **21** to be in *favor of isomer* **21**, which is not the case. A similar result is seen for the coupling of **5** where the lactol ratio was 1.13:1 (ax:eq) by NMR. While the lactol mixture could vary under the reaction conditions, which therefore does not discount an S_N2 mechanism in the Mitsunobu reaction, the above results do lend support to an alternative S_N1-type mechanism. First, the extra oxygen atom in the 1,4-dioxane could stabilize an oxocarbenium ion by n_O-π_{C-O}* homoconjugation.[31] Second, the 1,2-trans selectivity could result from axial attack of the nucleophile on the oxocarbenium ion in the preferred half-chair conformation **A** rather than **B** due to the interaction with the pseudoaxial R substituent (Figure 3). This selectivity is opposite to that observed for simple 3-alkoxy-substituted six-membered oxocarbenium ions in which a cis preference is observed (via conformer **B** with no axial R group).[32]

Synthesis of Cyclopentabenzofuran Fragment 6. With the 1,4-dioxane fragment in hand as well as a viable coupling method, we next investigated the synthesis of the cyclopentabenzofuran core (Scheme 6). This began with selective benzylation of naringenin (**8**) on the more acidic C7 phenol to afford benzyl ether **24**. Iodination followed by base-induced elimination gave the flavone **25** in a reasonable yield for the two steps. Methylation of the remaining phenols afforded ether **26**. Oxidation of **26** to the 3-hydroxyflavone **27** proved to be challenging. The first method involved deprotonation of **26** with lithium diisopropylamide (LDA) at the C3 position, followed by quenching with trimethylborate.[33] Oxidation and hydrolysis of the intermediate boronate provided **27**, which could be isolated by crystallization from the crude product in methanol. An alternative preferred method was the oxidation[34] of flavone **26** with dimethyldioxirane (DMDO) generated in situ from oxone and acetone,[35] followed by acid-induced rearrangement that gave the hydroxyflavone **27** in comparable yield. We found this method to be superior to oxidation with prepared DMDO in acetone followed by rearrangement.[34] The sequence from

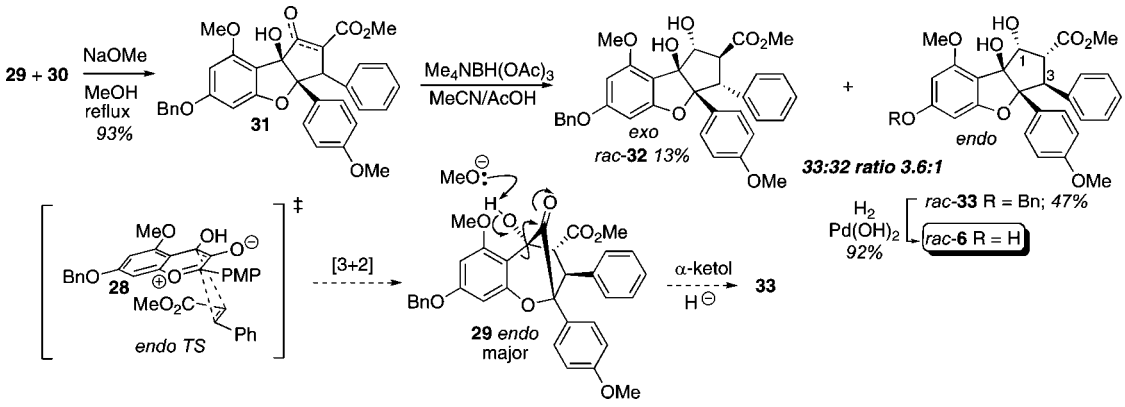
Scheme 5. Model Mitsunobu Couplings of **17** and **20**



Scheme 6. Synthesis of Cyclopentabenzofuran Core Precursor **29**



Scheme 7. Synthesis of Cyclopenta[*b*]benzofuran (*rac*-**6**)



naringenin (**8**) to hydroxyflavone **27** was easily conducted on a large scale, and each intermediate could be purified by recrystallization.

Numerous attempts to induce the conjugate addition/cyclization with hydroxyflavone **27** and methyl cinnamate (**9**) as proposed in Scheme 2 failed to produce any aglain-type intermediate under a myriad of conditions. We therefore adopted the elegant photochemical [3 + 2]-cycloaddition approach for the synthesis of rocaglate-type natural products as pioneered by Porco and co-workers[12,13] (Scheme 6). This involved irradiation of **27** with a 450-W medium-pressure mercury lamp to induce an excited-state intramolecular proton transfer[36] to form the intermediate oxidopyrillium dipole **28**, which undergoes a facile [3 + 2]-cycloaddition with methyl cinnamate[12] to provide the desired product **29** as a mixture of exo and endo adducts. This may indeed be the biosynthetic pathway from a flavone to the aglains rather than the conjugate addition/cyclization cascade outlined in Scheme 2; that is, the aglains may form by a sunlight-induced [3 + 2]-cycloaddition between a 3-hydroxyflavone and a cinnamate dipolarophile. Purification of the crude product from the photochemical reaction on silica gel gave an inseparable mixture of the endo/exo adducts of the desired bicycle **29** along with stereoisomers of cyclobutane **30**.

We initially reasoned that cyclobutane **30** was formed by a photoinduced [2 + 2]-cycloaddition between hydroxyflavone **27** and methyl cinnamate. A photochemical [2 + 2]-cycloaddition reaction between a flavone and cinnamate has also been proposed as a possible biogenetic route to the aglains and rocaglates;[37] however, this product apparently did not form in the initial photochemical reaction, as it was not detected in the ^{1}H NMR spectrum of the crude photochemical reaction mixture. Therefore, it seems that cyclobutane **30** was produced by α-ketol rearrangement upon purification on silica gel.[12,13,17] Although the adduct **29** and cyclobutane isomer **30** had different R_f values on thin-layer chromatography (TLC), they were not separable since repeated attempts at chromatographic purification yielded mixtures.

The interconversion of **29** and **30** was of no consequence since subjection of the mixture to base-mediated α-ketol rearrangement[38] provided the β-keto esters[21] as a mixture of keto−enol tautomers **31** (Scheme 7). Immediate anti-selective reduction[39] gave the cyclopentabenzofurans *rac*-**33** and *rac*-**32** in 60% combined yield (42% over three steps from ∼0.6 g of hydroxyflavone **27**) and a ratio of 3.6:1, respectively, favoring

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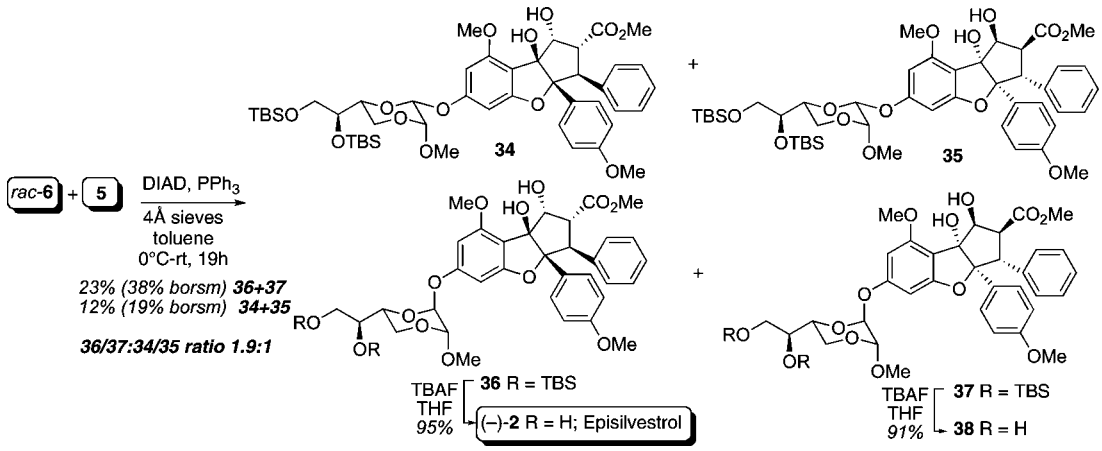
(39) Evans, D. A.; Chapman, K. T.; Carreira, E. M. *J. Am. Chem. Soc.* **1988**, *110*, 3560–3578.

Scheme 8. Synthesis of (−)-Episilvestrol (**2**)

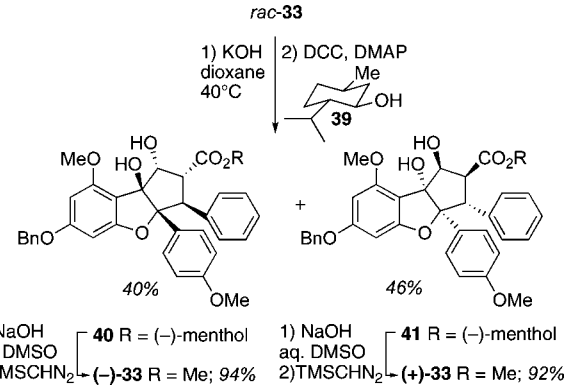


the required product after separation by flash chromatography. On a smaller scale (<100 mg), a **33**:**32** ratio of 4.6:1 was obtained due to more effective temperature control in the cycloaddition reaction. The stereochemistry for the major endo product *rac*-**33** was assigned on the basis of ^{1}H NMR coupling constants for H1−H3, which indicated a 1,2-cis-2,3-trans orientation for these protons.[16] On the other hand, the ^{1}H NMR spectrum of compound *rac*-**32** displayed couplings indicative of a 1,2-trans-2,3-trans orientation as shown. Thus, the original [3 + 2]-cycloaddition reaction favors an endo-type transition state as shown in Scheme 7, resulting in *endo*-**29** as the major adduct.[12] Subsequent base-induced α-ketol rearrangement on **29** proceeds as shown, and anti-selective reduction of the resultant *β*-keto ester affords cyclopentabenzofuran **33** as the major product. Hydrogenolysis of *rac*-**33** then provided the core phenol *rac*-**6** in excellent yield.

Coupling and Total Synthesis of (−)-Episilvestrol (2) and (−)-Silvestrol (1). With the racemic cyclopentabenzofuran *rac*-**6** and optically pure dioxane **5** (lactol mixture) in hand, we then tested the Mitsunobu coupling reaction (Scheme 8). Treatment of a mixture of *rac*-**6** and **5** with DIAD and PPh_3 in the presence of 4 Å molecular sieves afforded the equatorial (*β*-anomers) and axial (α-anomers) coupled products **36/37** and **34/35** in a 1.9:1 ratio respectively in disappointing overall yield (35%, 57% based on recovered starting material). This reaction was sluggish and had to be warmed to room temperature to proceed at a reasonable rate. The axial and equatorial isomers could be separated by flash chromatography but were still, of course, mixtures of two diastereoisomers as a result of utilizing racemic **6**. Each of these mixtures was then separated by preparative HPLC to provide pure equatorial *β*-isomers **34** and **35** as well as the axial α-isomers **36** and **37**. The axial isomers displayed singlets ($J_{eq,eq} \sim 0$ Hz) for H1‴ and H2‴ in their respective ^{1}H NMR spectra, and in the equatorial isomers, the same protons resonated as doublets ($J_{ax,eq} = 1.5$ Hz).

Each of the axial isomers was then differentiated by conversion of one to episilvestrol **2**. Treatment of the faster-eluting isomer, namely, **36**, with tetrabutylammonium fluoride (TBAF) induced efficient deprotection to afford synthetic episilvestrol (**2**), which has spectroscopic and chiroptical data ($[\alpha]_D$ −91.3° (*c* 0.06, $CHCl_3$)) comparable to the natural material[9] ($[\alpha]_D$ −94.5° (*c* 0.43, $CHCl_3$)). On the other hand, deprotection of isomer **37** gave the diastereosomer of episilvestrol **38**, which was epimeric at all stereocenters of the cyclopentabenzofuran. Compound **38** had a different specific rotation ($[\alpha]_D$ −66.3° (*c* 0.205, $CHCl_3$)), and the spectra (especially the ^{13}C NMR spectrum) were slightly different to that for episilvestrol (**2**).

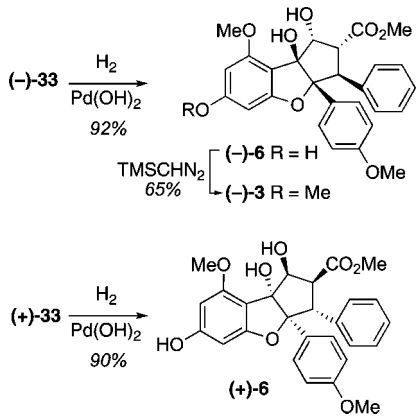
Scheme 9. Resolution of *rac*-**33**



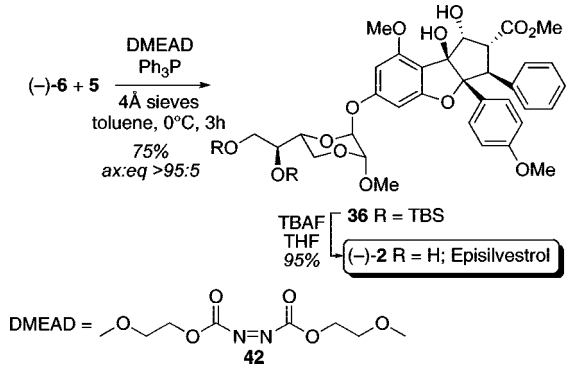
Although total synthesis of episilvestrol (**2**) was achieved and enough material had been isolated to fully characterize, the final steps of the route were far from efficient. The first problem that needed to be addressed was the production of optically pure cyclopentabenzofuran core **6** in order to circumvent the tedious HPLC separation. In addition, the yield and selectivity of the coupling reaction had to be improved. We first investigated an asymmetric version of the photoinduced [3 + 2]-cycloaddition[13] reaction with a number of chiral cinnamates including amides and esters such as the menthol ester. Rather surprisingly, all these proved fruitless with the [3 + 2]-cycloaddition failing to proceed. Eventually, we opted for a simple and efficient resolution of the racemic cyclopentabenzofuran *rac*-**33** (Scheme 9). Hydrolysis of the methyl ester *rac*-**33** followed by esterification with (−)-menthol (**39**) produced menthol esters **40** and **41**, which were easily separated by conventional flash chromatography. The menthol esters proved resistant to methanolysis, so the slower-eluting pure diastereoisomeric menthol ester **40** was hydrolyzed to the acid with powdered KOH in wet dimethyl sulfoxide (DMSO), and subsequent methylation then afforded (−)-**33** in optically pure form. The absolute configuration of (−)-**33** was determined by its conversion into the natural product (−)-methyl rocaglate (**3**).

Hydrogenolysis of the benzyl ether (−)-**33** gave phenol (−)-**6** in excellent yield (Scheme 10). Methylation of (−)-**6** then afforded synthetic methyl rocaglate (−)-**3**, the spectroscopic data of which was identical to the natural product.[6,7] In addition, the sign and magnitude of the specific rotation of the synthetic

Scheme 10. Synthesis of Methyl Rocaglate (−)-**3**



Scheme 11. Improved Mitsunobu Coupling with DMEAD

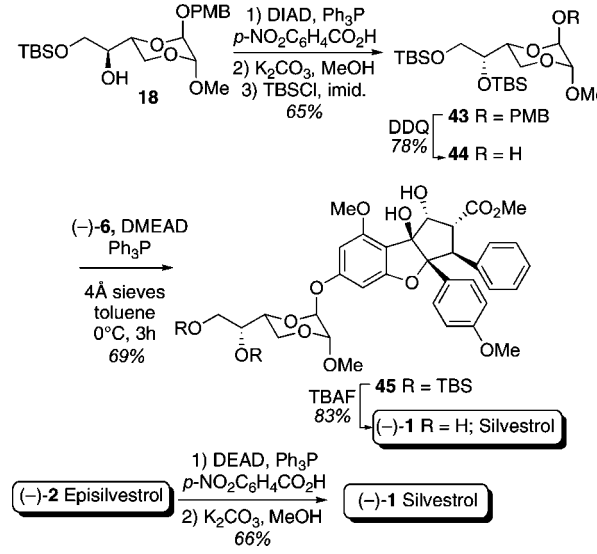


material ([α]$_D$ −47.0° (*c* 0.165, $CHCl_3$)) matched that of natural **3**[7] ([α]$_D$ −48.0° (*c* 0.69, $CHCl_3$)), allowing assignment of the absolute configuration of (−)-**6** and its precursors. The faster-eluting menthol ester diastereoisomer **41** was also treated in a similar manner to that described for **40** to afford an enantiomerically pure sample of (+)-**6.** With an efficient route to optically pure cyclopentabenzofuran (−)-**6** in hand, we next examined the Mitsunobu coupling.

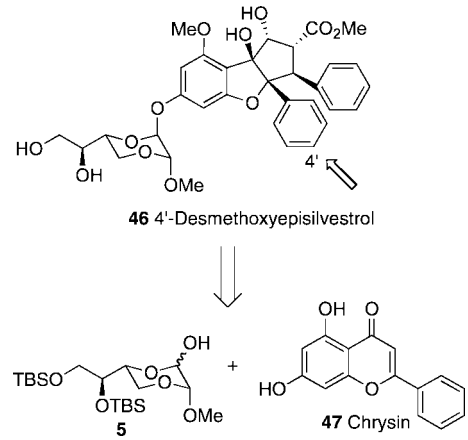
Our first foray into optimizing the coupling proved successful. When the azodicarboxylate was changed from DIAD to the more reactive diethyl azodicarboxylate (DEAD), a marked increase in reaction rate and stereoselectivity was observed (>95:5 ax:eq) and the yield was improved. Unfortunately, the coupled product **36** could not be separated from the reduced diethyl hydrazinedicarboxylate byproduct. To resolve this, the alternative crystalline Mitsunobu coupling reagent DMEAD (di-2-methoxyethyl azodicarboxylate) (**42**)[40] was utilized, which affords a water-soluble hydrazinedicarboxylate byproduct. Coupling of (−)-**6** and an excess of **5** (2.5 equiv) with DMEAD (**42**) afforded pure **36** as the only isomer in 75% yield along with unreacted **5** after aqueous workup and flash chromatography (Scheme 11). Thus, the reactivity of DEAD and DMEAD are comparable in this case, with the coupling complete after 3 h at 0 °C. At room temperature, the reaction was faster but the axial/equatorial selectivity was reduced, giving compounds **36** and **34** in a 3:1 ratio. It appears that the steric bulk of the cyclopentabenzofuran core and lower temperature accounts for the improvement in diastereoselectivity for this critical reaction compared to the examples with DIAD and between the real and model cores (see Schemes 5 and 8). Deprotection of **36** again provided episilvestrol (**2**).

(40) Sugimura, T.; Hagiya, Z. *Chem. Lett.* **2007**, *36*, 566–567.

Scheme 12. Total Synthesis of Silvestrol (**1**)



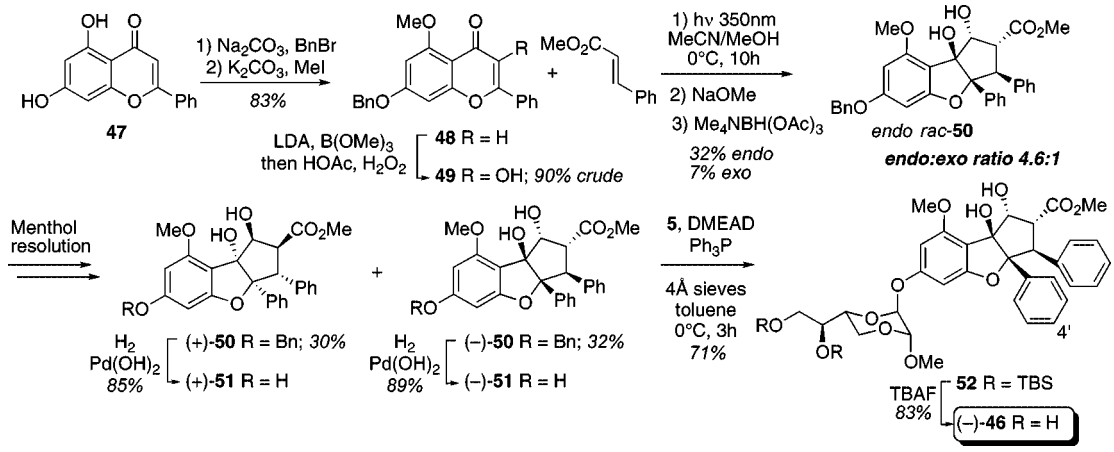
Scheme 13. Retrosynthesis of 4′-Desmethoxyepisilvestrol (**46**)



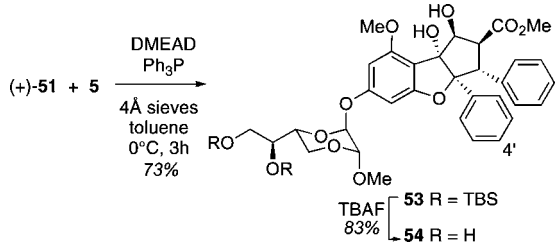
For the total synthesis of silvestrol (**1**), a Mitsunobu coupling between silvestrol dioxane **44**, the C5‴ epimer of **5**, and (−)-**6** was conducted as shown in Scheme 12. The coupling partner **44** was synthesized from dioxane **18** (see Scheme 5). Mitsunobu inversion of **18** and protection of the resultant alcohol gave silyl ether **43**. Oxidative removal of the PMB ether then provided the silvestrol dioxane fragment **44**. Mitsunobu coupling between (−)-**6** and an excess of **44** with DMEAD gave the adduct **45** as the only isomer in 69% yield, which upon deprotection provided (−)-silvestrol (**1**). Data for the synthetic material ([α]$_D$ −159° (*c* 0.12, MeOH)) again compared well to that for the natural product[9] ([α]$_D$ −137° (*c* 0.2, MeOH)). We also synthesized compound **1** by a selective double Mitsunobu inversion conducted on synthetic episilvestrol (**2**), which resulted in inversion of the C5‴ stereocenter as the C1 secondary alcohol was too hindered to react (Scheme 12). Methanolysis then provided silvestrol (**1**).

Synthesis of 4′-Desmethoxyepisilvestrol (46). The improved route to episilvestrol (**2**) and silvestrol (**1**) led us to investigate a synthesis of the analogue 4′-desmethoxyepisilvestrol (**46**) devoid of the C4′ methoxy group (Scheme 13). The starting material for this would be chrysin (**47**), which is much less expensive (~$3 AUD/g) than naringenin (**8**) and requires one less step to afford the cyclopentabenzofuran core. It was envisaged that the subtle change in 4′-desmethoxyepisilvestrol

Scheme 14. Synthesis of (−)-4′-Desmethoxyepisilvestrol (**46**)



Scheme 15. Synthesis of 4′-Desmethoxyepisilvestrol Isomer **54**



(**46**) relative to the natural product **2** would not greatly affect the biological activity but would allow for a large-scale total synthesis of an active analogue that would be difficult to obtain from the natural product.

The route to the cyclopentabenzofuran core of 4′-desmethoxyepisilvestrol is outlined in Scheme 14 and begins with the selective benzylation and subsequent methylation of chrysin **47** to give the flavone **48**. The oxidation protocol with oxone and acetone that was successful previously (see Scheme 6) failed in the case of flavone **48**, probably due to the decreased electron density of the alkene in **48** compared to **26**. However, the alternative procedure[33] involving deprotonation, borate quench, and oxidative workup gave the hydroxyflavone in good crude yield. Purification of this compound on silica gel resulted in significant loss of material (23% yield). Photochemical [3 + 2]-cycloaddition[12,17] followed by α-ketol rearrangement and reduction[39] gave the endo adduct *rac*-**50** as the major product along with exo product in a 4.6:1 ratio favoring the desired product. Resolution via the menthol esters in a manner similar to that described before gave optically pure (+)-**50** and the correct core enantiomer (−)-**50**. Debenzylation of each enantiomer gave phenols (+)-**51** and (−)-**51**, and DMEAD-mediated Mitsunobu coupling of the levorotatory isomer with **5** afforded adduct **52** as the only isomer detected. TBAF-mediated desilylation then gave 4′-desmethoxyepisilvestrol **46**, the NMR spectrum of which was similar to that for episilvestrol (**2**). We also synthesized the diastereoisomer **54** by coupling of (+)-**51** and **5** to afford **53** followed by deprotection (Scheme 15).

On several occasions, we noticed some differences in the ^{1}H NMR spectra of **46** when run at different concentrations in $CDCl_3$ (i.e., more concentrated for ^{13}C NMR spectra: **46** decomposed considerably when left as a concentrated solution in $CDCl_3$ for a few hours at room temperature). More specifically, the shifts of signals for the protons on the 1,4-dioxane fragment varied considerably, so we investigated this phenomenon further and found that the chemical shifts of H1‴ and H2‴ (easiest to observe) changed in a similar fashion according to concentration (Figure 4). This was also observed in the ^{1}H NMR spectra for varying concentrations of solutions of synthetic episilvestrol (**2**) in $CDCl_3$, albeit not as large as that for **46**, and we had noticed some slight differences in the dioxane chemical shifts for natural **2**.

Chemical shift differences for the 1,4-dioxane protons were also observed in the ^{1}H NMR spectra for natural silvestrol (**1**) before and after chromatography on silica gel with EtOAc as solvent (H1‴ br s, 5.27 ppm before, 5.31 ppm after); however, no explanation for this change was suggested.[17] In our case, these shift changes are clearly an interesting example of concentration-dependent chemical shift variation of *nonexchangable protons*.[41] In the case of **46** and **2**, the plots in Figure 4 show a *upfield* change in the chemical shift of both H1‴ and H2‴ as well as other 1,4-dioxane signals upon increasing concentration. This is most prominent for 4′-desmethoxyepisilvestrol **46** (H1‴, $\Delta\delta$ 0.2 ppm, Δc 6.1 × 10^{-2} M; H2‴, $\Delta\delta$ 0.09 ppm, Δc 6.1 × 10^{-2} M). In addition, the same protons in silvestrol (**1**) show chemical shift concentration dependence of the dioxane protons comparable to episilvestrol (**2**). Clearly, compounds **46** and episilvestrol (**2**) and silvestrol (**1**) have some intermolecular association, which probably involves hydrogen bonding in the highly oxygenated 1,4-dioxylanoxy fragment that affects the conformation of this moiety, resulting in the chemical shift concentration dependence trend observed. In addition, the presence of multiple aromatic rings in a sterically congested environment might affect the chemical shift of the dioxane protons to a larger extent with only slight conformational variations. These observations reiterate the need in some cases for solute concentrations to be reported for NMR spectra.[41]

Anticancer Assays. Compounds **1**, **2**, and **46** and the corresponding diastereoisomers **38** and **54** were then tested for their anticancer activity in a A549 lung cancer proliferation assay (Figure 5). Both synthetic silvestrol (**1**) and episilvestrol (**2**) were potent inhibitors with similar IC_{50} values. Gratifyingly, 4′-desmethoxyepisilvestrol (**46**) was also active with an IC_{50} value around 4 times that for **2**. The episilvestrol diastereoisomer **38**, however, was considerably less active, while the desmethoxy isomer **54** was essentially inactive. A similar activity profile was seen for episilvestrol **2** and isomer **38** in a preliminary assay[16] against epidermal growth factor- (EGF-) treated colon

(41) Mitra, A.; Seaton, P. J.; Assarpour, R. A.; Williamson, T. *Tetrahedron* **1998**, *54*, 15489–15498.

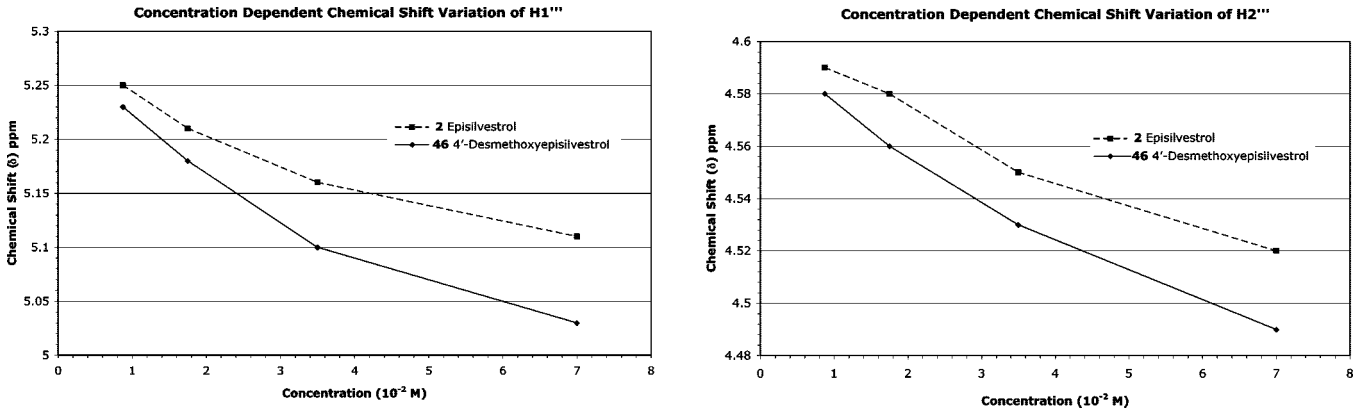


Figure 4. Concentration variance of ^{1}H NMR chemical shifts for H1‴ and H2‴ in episilvestrol (**2**) and 4′-desmethoxyepisilvestrol (**46**).

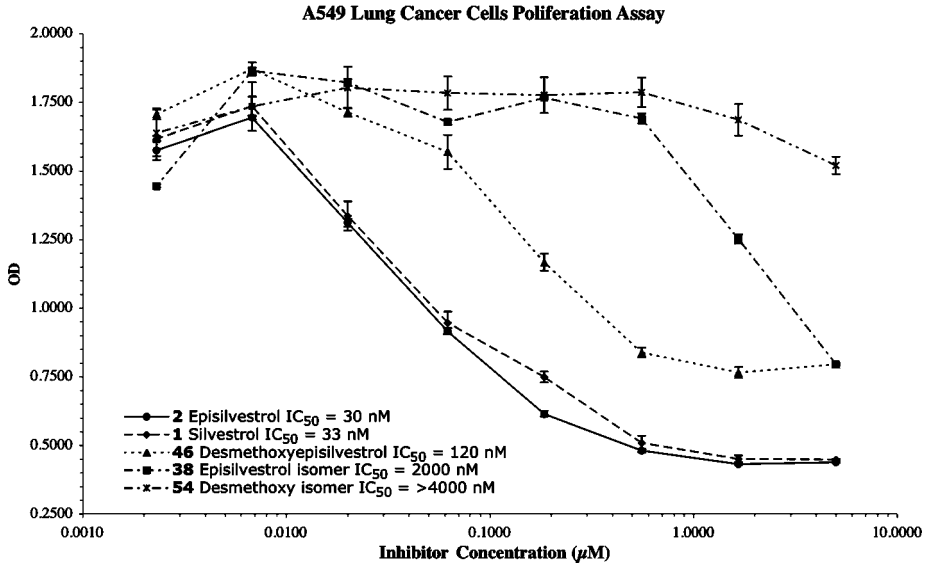


Figure 5. A549 lung cancer proliferation assays.

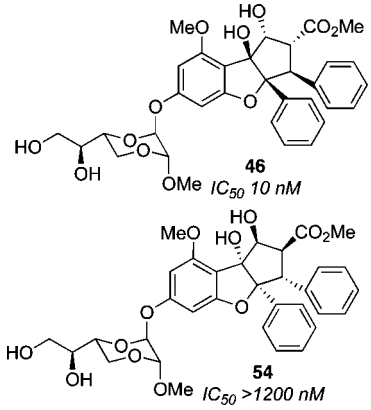


Figure 6. LIM1215 colon cancer proliferation assay results.

cancer cells (LIM 1215) (**2**, IC_{50} = 2 nM; **38**, IC_{50} = 56 nM), and this was also observed for desmethoxyepisilvestrol **46** and the isomer **54** to an even greater extent as shown in Figure 6. These biological results clearly indicate which isomers have the correct stereochemistry and show the importance of the natural cyclopentabenzofuran core stereochemistry for activity. In addition, the dioxane stereochemistry is important, as the H1‴ equatorial (β-anomer) analogue of silvestrol (**1**) also shows a lower activity.[17]

Conclusion

In summary, we have developed a short synthesis of the potent anticancer natural products silvestrol (**1**) and episilvestrol (**2**) based on their proposed biogenesis. Highlights of the approach include the oxidative rearrangement of a D-glucose derivative to afford the 1,4-dioxane, which could be elaborated into the coupling partner **5**, and the adaptation of a photochemical [3 + 2]-cycloaddition followed by α-ketol rearrangement, reduction, and resolution to provide the cyclopentabenzofuran fragment (−)-**6**. The modified Mitsunobu coupling between dioxane **5** and core (−)-**6** mediated by DMEAD afforded only the desired axial isomer **36**, which upon deprotection gave episilvestrol (**2**), and a similar route was then utilized to synthesize silvestrol (**1**) and the potent analogue 4′-desmethoxyepisilvestrol (**46**). It is envisaged that the synthesis of **46** described will provide enough material for further in vivo biological evaluation of this novel analogue.

Acknowledgment. We are indebted to Dr. Murray Tait (formally of Cerylid Biosciences) and Professor A. Douglas Kinghorn (Ohio State University) for authentic samples of silvestrol and episilvestrol. We also thank Mr. Kazutake Hagiya (Toyo Kasei Kogyo Co., Ltd, Japan) for bringing DMEAD to

our attention and providing a generous sample and Professor Tony Burgess and Dr Francesca Walker (Ludwig Cancer Institute, Melbourne) for conducting the preliminary colon cancer assays. This research was funded by the Australian Research Council-Discovery Grants program (DP0770408), Cerylid Biosciences, and CSIRO MHT.

Supporting Information Available: Experimental procedures, characterization data, and copies of NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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