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 α-glucose derivative 11 to afford the 1,4-dioxane 12, which could be elaborated to the coupling partner 5 and a photochemical [3 + 2]-cycloaddition between the 3-hydroxyflavone 27 and methyl cinnamate followed by base-induced α-ketol rearrangement and reduction to give the cyclopentabenzo furan 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 products4 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-workers9,10 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 rocaglamide11–13 and methyl rocaglate/aglafolin12,13 and a number of approaches to the rocaglates14 have been reported to date, while two independent syntheses of silvestrol (1) were communicated in 2007.15–17

Silvestrol (1) displays potent cytotoxicity comparable to that for paclitaxel and camptothecin against several human cancer cell lines including lung (Lu1, ED50 = 1.2 nM; A549, LC50 = 15 nM), prostate (LNCaP, ED50 = 1.5 nM; PC3, LC50 = 12 nM), breast (MCF-7, ED50 = 1.2 nM) and leukemia (K562, GI50 = 12 nM). Episilvestrol (2) shows similar activity as that for silvestrol (1) against some cell lines (K562, LC50 = 15 nM) but has been reported as ∼3 times less active than 1 in other assays (Lu1, ED50 = 3.8 nM). This demonstrates that the 3′′ stereochemistry does not have a substantial effect on the activity of these compounds. Silvestrol 1 also inhibits protein biosynthesis with IC50 ∼ 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% and this effect was independent of P53 activity.19 In addition, compound 1 induces apoptosis in LNCaP cells through the mitochondria apoptotic pathway which appears to involve caspases 2, 9, and 10 but not caspases 3 and 7.β

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. foliata] led us to investigate a total synthesis of these important targets. In this paper we present the full details of our total synthesis of silvestrol (1) and episilvestrol (2) that is summarized in Scheme 1.β The sequence begins with β-D-galactopyranoside I, which undergoes selective oxidative cleavage of the C2′′′−C6′′′ bond (episilvestrol numbering) and concomitant acetal formation to give the lactols II and III. Subsequent stereoselective methylation and reduction then yields the episilvestrol dioxane IV. Inversion at the C5′′′ stereocenter would give silvestrol 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 cyclopentabenzenofuran core of 1 and 2 is based on that suggested by Proksch and co-workers4,20 as shown in Scheme 2. This hypothesis

![Scheme 1. Possible Biosynthetic Origin of the 1,4-Dioxanyloxy Fragments of 1 and 2](image)

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

Silvestrol (1) was isolated from the Indonesian stem bark of Ficus racemosa L. (Moraceae), which was collected on the island of Bali, Indonesia. The isolation and structure elucidation of silvestrol (1) was carried out by Rizza, et al.21 The molecules were generated from the full details of our total synthesis of silvestrol (1) and episilvestrol (2) that is summarized in Scheme 1.β The sequence begins with β-D-galactopyranoside I, which undergoes selective oxidative cleavage of the C2′′′−C6′′′ bond (episilvestrol numbering) and concomitant acetal formation to give the lactols II and III. Subsequent stereoselective methylation and reduction then yields the episilvestrol dioxane IV. Inversion at the C5′′′ stereocenter would give silvestrol 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 cyclopentabenzenofuran core of 1 and 2 is based on that suggested by Proksch and co-workers4,20 as shown in Scheme 2. This hypothesis

![Scheme 2. Possible Biosynthetic Origin of the 1,4-Dioxanyloxy Fragments of 1 and 2](image)
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 rocallate-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-hydroxy-ketone IX. Compound IX is a β-ketoester and this serves as the thermodynamic sink in the sequence. Subsequent anti-specific reduction would then afford the cyclopenta[b]benzofuran X.

The above proposals are supported by the occurrence of the ubiquitous flavinone populnin22 in many natural sources (Figure 2). Populnin is the 7-O-glycoside of kaempferol and is a viable biosynthetic precursor to episilvestrol (2). While adopting populnin as a starting material would provide an aglain-type natural product. In addition, the cyclopentabenzo-furan core 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-hydroxy-ketone IX. Compound IX is a β-ketoester and this serves as the thermodynamic sink in the sequence. Subsequent anti-specific reduction would then afford the cyclopenta[b]benzofuran X.

It was envisaged that episilvestrol could be formed from a coupling between 1,4-dioxane lactols 5 and the cyclopenta-b[b]benzofuran core phenol 6 via a Mitsunobu reaction. A Mitsunobu glycosylation approach was applied by Roush and Lin24,25 for the stereoselective synthesis of O-ary1 β-glycosides, and this could be easily adapted to the present case for forming the 1,4-dioxlanlyoxy 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 Sn1-type mechanism rather than a direct Sn2 displacement. This coupling approach was attractive in that it does not require the synthesis of an activated dioxylanloxy donor and would permit the recovery of unreacted lactol. Dioxane lactols 5 could be produced from commercially available 4′,5,7-trihydroxyflavanone or (+)-naringenin (8) as a biosynthetic precursor of episilvestrol (2).

Results and Discussion

Synthesis of 1,4-Dioxylanoxy Fragment 5. The route to the 1,4-dioxylanoxy fragment begins with Koenigs–Knorr glycosylation26 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 acetel27 in good overall yield for the three steps. Selective cleavage of the O6–C acetal bond was achieved with BH3·THF in the presence of Cu(OTf)2. With the O-1,4 protected glucopyranoside 11 in hand, we subjected this to NaIO4,29 which cleanly provided the 1,4-dioxane aldehyde 12.


Figure 2. Populnin as a biosynthetic precursor of episilvestrol (2).
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 MeOTf30 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 reprotetion 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 disopropyl azodicarboxylate (DIAD) and PPh3 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%.

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

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 (1H NMR in CDCl3). If the coupling is proceeding via an SN2-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 SN2 mechanism in the Mitsunobu reaction, the above results do lend support to an alternative SN1-type mechanism. First, the extra oxygen atom in the 1,4-dioxane could stabilize an oxocarbenium ion by π-π 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 disopropylamide (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 oxidation34 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

**Figure 3. Rationale for Mitsunobu coupling selectivity.**
rocaglates; however, this product apparently did not form in proposed as a possible biogenetic route to the aglains and to induce an excited-state intramolecular proton transfer to methyl cinnamate. A photochemical \([2+2]\)-cycloaddition with hydroyflavone \([27]\) into the elegant photochemical intermediate under a myriad of conditions. We therefore adopted proposed in Scheme 2 failed to produce any aglain-type cyclization. Large scale, and each intermediate could be purified by recrystalization. Numerous attempts to induce the conjugate addition/cyclization with hydroxyflavone \(27\) and methyl cinnamate \(9\) as \(27\) and \(29\) was produced by \(\alpha\)-ketol rearrangement upon purification on silica gel. \(^{12,13,17}\) Although the adduct \(29\) and cyclobutane isomer \(30\) had different \(R\) 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 \(\alpha\)-ketol rearrangement \(^{38}\) provided the \(\beta\)-ketol esters \(^{21}\) as a mixture of keto–enol tautomers \(31\) (Scheme 7). Immediate anti-selective reduction \(^{33}\) gave the cyclopentabenzofurans \(rac-33\) and \(rac-32\) in 60% combined yield (42% over three steps from \(\sim 0.6\) g of hydroxyflavone \(27\)) and a ratio of 3.6:1, respectively, favoring

Therefore, it seems that cyclobutane \(30\) was produced by \(\alpha\)-ketol rearrangement upon purification on silica gel. \(^{12,13,17}\) Although the adduct \(29\) and cyclobutane isomer \(30\) had different \(R\) values on thin-layer chromatography (TLC), they were not separable since repeated attempts at chromatographic purification yielded mixtures.

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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.
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. 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. Subsequent base-induced $\alpha$-ketol rearrangement on 29 proceeds as shown, and anti-selective reduction of the resultant $\beta$-ketol 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-aminers) and axial (α-aminers) 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 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 ($J_{ax,eq}$ ∼ 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 ([$\alpha$]$_D^{29}$ = -91.3° ($c$ 0.06, CHCl$_3$)) comparable to the natural material ($[\alpha]_D^{29}$ = -94.5° ($c$ 0.43, CHCl$_3$)). On the other hand, deprotection of isomer 37 gave the diastereoisomer of episilvestrol 38, which was epimeric at all stereocenters of the cyclopentabenzofuran. Compound 38 had a different specific rotation ([$\alpha$]$_D^{29}$ = -66.3° ($c$

0.205, CHCl$_3$)), and the spectra (especially the [$^1$C NMR spectrum) were slightly different to that for episilvestrol (2).

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 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 methanalysis, 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. In addition, the sign and magnitude of the specific rotation of the synthetic
model cores (see Schemes 5 and 8). Deprotection of compared to the examples with DIAD and between the real and the improvement in diastereoselectivity for this critical reaction coupling of ( afforded a water-soluble hydrazinedicarboxylate byproduct. Coupling with unreacted 5 (2.5 equiv) with DMEAD gave the adduct 42 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 episolvestrol (2).

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 ([α]D −137° (c 0.2, MeOH)). We also synthesized compound 1 by a selective double Mitsunobu inversion conducted on synthetic episolvestrol (2), which resulted in inversion of the C5′′ stereocenter as the C1 secondary alcohol was too hindered to react (Scheme 12). Methanolation then provided silvestrol (1).

Synthesis of 4′-Desmethoxyepisolvestrol (46). The improved route to episolvestrol (2) and silvestrol (1) led us to investigate a synthesis of the analogue 4′-desmethoxyepisolvestrol (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′-desmethoxyepisolvestrol

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(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 benzylxylation and subsequent methylation of chrys 

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

![Scheme 14](image)

(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 involving deprotonation, borate quench, and oxidative workup gave the hydroxylflavone in good crude yield. Purification of this compound on silica gel resulted in the cor 

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

![Scheme 15](image)

46 enon 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 1H NMR spectra for varying concentrations of solutions of synthetic episilvestrol (2) in CDCl3, 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 1H 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. In our case, these shift changes are clearly an interesting example of concentration-dependent chemical shift variation of nonexchang 

![Image](image)

**Figure 4. Plot of Chemical Shifts (δ ppm) Against Concentration (mM) for (a) 1 and (b) 2.**

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 IC50 values. Gratifyingly, 4'-desmethoxyepisilvestrol (46) was also active with an IC50 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 against epidermal growth factor- (EGF-) treated colon (41) Mitra, A.; Seaton, P. J.; Assarpour, R. A.; Williamson, T. Tetrahedron 1998, 54, 15489–15498.
cancer cells (LIM 1215) (2, IC\textsubscript{50} = 2 nM; 38, IC\textsubscript{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.\textsuperscript{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.

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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.