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Total Synthesis of 2‴,5‴-Diepisilvestrol and Its C1‴ Epimer: Key Structure Activity Relationships at C1‴ and C2‴

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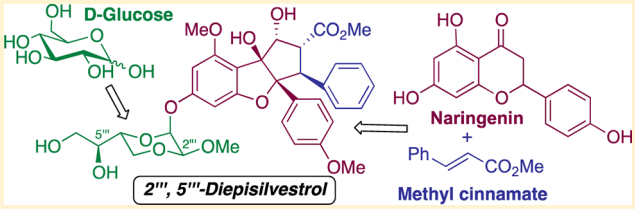
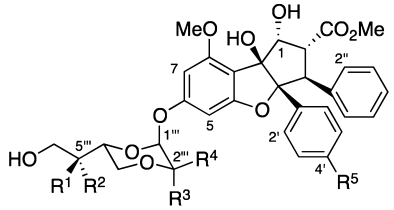
Supporting Information

ABSTRACT: The first total synthesis of the low-abundance natural product 2‴,5‴-diepisilvestrol (**4**) is described. The key step involved a Mitsunobu coupling between cyclopenta[*b*]-benzofuran phenol **7** and dioxane lactol **6**. Deprotection then gave a 1:2.6 ratio of natural product 2‴,5‴-diepisilvestrol (**4**) and its C1 epimer 1‴,2‴,5‴-triepisilvestrol (**15**) in 50% overall yield. An in vitro protein translation inhibition assay showed that 2‴,5‴-diepisilvestrol (**4**) was considerably less active than episilvestrol (**2**), while the unnatural isomer 1‴,2‴,5‴-triepisilvestrol (**15**) was essentially inactive, showing that the configuration at C1‴ and C2‴ has a large effect on the biological activity.



Aglaia is a large genus of the family Meliaceae that consists of over 100 species of mostly woody trees and shrubs found throughout the rainforests of Indomalaysia. Assay-guided extraction of *Aglaia leptantha* and *Aglaia foveolata* afforded two active metabolites, named silvestrol (**1**) and 5‴-episilvestrol (**2**), in low yield (0.02% w/w).[1,2] Compounds **1** and **2** contain a common cyclopenta[*b*]benzofuran core,[3] as well as a novel, unprecedented 1,4-dioxanyloxy *pseudosugar* substituent.[4] Both silvestrol (**1**) and episilvestrol (**2**) showed comparable potent cytotoxic activity against several human tumor cell lines including lung (ED_{50} value of 1.2 nM) and breast cancer (ED_{50} value of 1.5 nM).[2] Recently, silvestrol (**1**) has shown a remarkable potent and selective activity against B-cells from patients with chronic lymphocytic leukemia (LC_{50} value of 6.9 nM) as well as in vivo activity against acute lymphoblastic leukemia in a mouse xenograft model (dose 1.5 mg/kg).[5]



1 $R^1 = R^4 = H$; $R^2 = OH$; $R^3 = R^5 = OMe$: **Silvestrol**
2 $R^1 = OH$; $R^2 = R^4 = H$; $R^3 = R^5 = OMe$: **5‴-Episilvestrol**
3 $R^1 = R^3 = H$; $R^2 = OH$; $R^4 = R^5 = OMe$: **2‴-Episilvestrol**
4 $R^1 = OH$; $R^2 = R^3 = H$; $R^4 = R^5 = OMe$: **2‴, 5‴-Diepisilvestrol**
5 $R^1 = OH$; $R^2 = R^4 = R^5 = H$; $R^3 = OMe$: **4'-Desmethoxyepisilvestrol**

Further phytochemical investigation of *A. foveolata* resulted in the isolation of the minor isomers 2‴-episilvestrol (**3**) and 2‴,5‴-diepisilvestrol (**4**) in very low yield (0.8 mg of **3** and 0.9 mg of **4** from 40 to 45 kg of stem bark, ~2×10^{-6} %).[6] Compound **4** was tested against only one cell line (HT-29 colon cancer) and showed lower activity (ED_{50} 1.07 μM) than episilvestrol (**2**), which had an ED_{50} of 0.001 μM, suggesting that the configuration at C2‴ has a strong influence on the biological activity.[6]

The mechanism of action for the cytoxicity of this family of compounds is not entirely clear. A dramatic reduction in the antiapoptotic protein Mcl-1 in the presence of **1** is often reported,[5,7] and Pelletier and co-workers have demonstrated that this is due to direct inhibition of translation initiation by depletion of the helicase eIF4A from the eIF4F complex, as opposed to indirectly via the mTOR and ER stress pathways.[7,8]

The total synthesis of silvestrol (**1**)[9−11] and episilvestrol (**2**)[10,11] has been reported as well as the analogue 4′-desmethoxyepisilvestrol (desmethstrol) (**5**).[11] Analogue **5** is also a potent cytotoxic compound [A549 lung cancer (IC_{50} 130 nM) and colon cancer (IC_{50} 10 nM)].[11] Herein, we report the total synthesis of the minor constituent 2‴,5‴-diepisilvestrol (**4**) and its unnatural C1‴ epimer as well their activities in a protein translation inhibition assay. Our synthetic approach to **4** is summarized in Scheme 1. We envisaged that the C2‴ isomer **4** could be constructed in a convergent manner as utilized by us for the total synthesis of silvestrol (**1**) and

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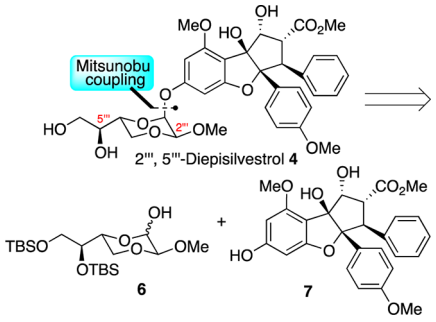
Scheme 1. Retrosynthetic Analysis of 4

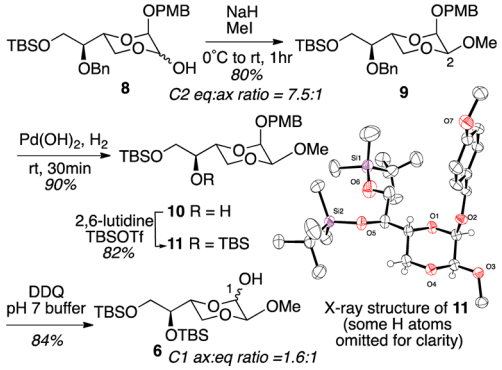


episilvestrol (**2**).[10,11] Thus, a modified Mitsunobu coupling between the 1,4-dioxane lactol **6** and known phenol (−)-**7**[11] followed by a final deprotection would then afford 2‴,5‴-diepisilvestrol (**4**).

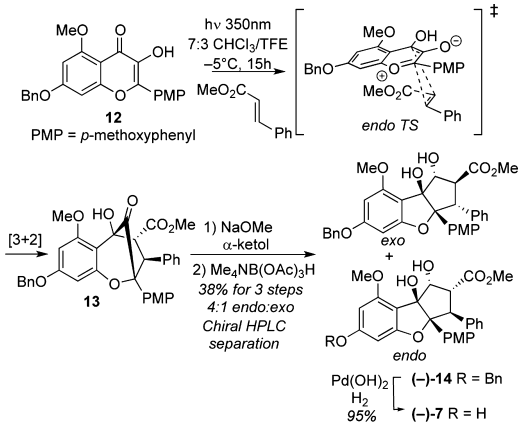
RESULTS AND DISCUSSION

Synthesis of the requisite 1,4-dioxane lactol **6** is shown in Scheme 3. Lactol **8** is available in eight steps from β-D-glucose

Scheme 2. Synthesis of Lactol 6



Scheme 3. Synthesis of Phenol 7

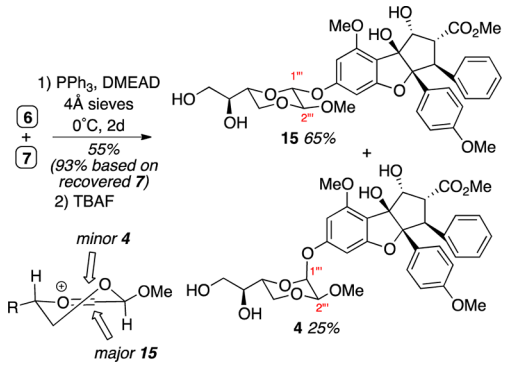


pentaacetate.[11] Methylation of **8** using NaH as base and MeI gave good selectivity (7.5:1) for the desired C2 (equatorial) acetal **9**. Previously, when a lithium base was used with MeOTf as the electrophile, the selectivity was reversed and the major isomer formed was the C2 (axial) epimer of **9**.[11] Removal of the benzyl ether under carefully controlled conditions afforded the alcohol **10**, which was silylated to provide the crystalline bis-TBS ether **11**. A single-crystal X-ray structure[12] analysis of this compound then confirmed the absolute stereochemistry. Removal of the PMB group then gave the lactol **6** fragment as a 1.6:1 mixture of anomers favoring the axial isomer.

The known phenol **7**[11] was synthesized utilizing the [3+2]-photocycloaddition/α-ketol approach pioneered by Porco for the production of methylrocaglate and related compounds.[13,14] In this case, an O6 benzyl protecting group was utilized as reported,[11] but the approach has been modified by using an alternative solvent mixture and separation method (Scheme 3). Thus, the naringenin derivative **12**[11] was irradiated with a 7:3 mixture of $CHCl_3$ and trifluoroethanol (TFE) as solvent[14] to afford an oxidopyrilium species, which underwent a [3+2]-cycloaddition[13] with methyl cinnamate to give aglaian **13**. Base-induced α-ketol[13] rearrangement and selective *anti*-reduction[15] gave the *endo*-adduct (±)-**14** as well as the corresponding *exo*-product[11] in a 4:1 ratio, respectively. This was a slightly better yield and higher *endo*-selectivity (38% over three steps on a 500 mg scale) than we had reported using a MeCN/MeOH solvent mixture.[11] We had previously resolved (±)-**14** via the menthol ester derivative, but this process was not high yielding (26% average overall yield of (−)-**14**).[11] Porco has also reported that the [3+2]-photocycloaddition can be conducted in an enantioselective manner using an equimolar amount of a tetraaryl-1,3-dioxolane-4,5-dimethanol derivative as a chiral Brønsted acid source.[6b] However, we elected to separate the enantiomers by chiral HPLC (45% average yield of (−)-**14**) as an operationally simple and more economic alternative. Debenzylation as described previously then gave the phenol (−)-**7** in high yield.[11]

The final stages of the synthesis are shown in Scheme 4. Mitsunobu coupling between lactol **6** and phenol **7** using di-2-

Scheme 4. Total Synthesis of 2‴,5‴-Diepisilvestrol (4)



methoxyethyl azodicarboxylate (DMEAD)[16] and PPh_3 in the presence of 4 Å molecular sieves[11] afforded the coupling products in 55% yield as a ~2.5:1 mixture (as determined by ^1H NMR spectroscopy), which could not be separated. Deprotection of the mixture with TBAF gave 1‴,2‴,5‴-triepisilvestrol (**15**) and 2‴,5‴-diepisilvestrol (**4**) as the minor product, which was separated by HPLC in a combined yield of 50% for the two steps. Synthetic 2‴,5‴-diepisilvestrol (**4**) was identical to the natural product in all respects (see Table 1) {$[\alpha]^{25}_D$ −62.3 (*c* 0.25, MeOH); lit.[6] $[\alpha]^{20}_D$ −53.0 (*c* 0.05, MeOH)}, thus confirming the originally assigned structure.[6]

The selectivity in the Mitsunobu coupling was lower than that observed when the C2‴ OMe group is axial.[11] This suggested an S_N1-type mechanism rather than an S_N2 inversion process. In the present case, if the reaction proceeds by an S_N1

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Table 1. ^{1}H NMR (600 MHz, $CDCl_3$) and ^{13}C NMR Data (150 MHz, $CDCl_3$) for Synthetic and Natural[6] 2‴,5‴-Diepisilvestrol (4) Run at 8.6 mM.[17]

	^{1}H NMR data: δ, mult. (J in Hz)		^{13}C NMR data: δ	
position	synthetic	natural[6]	synthetic	natural[6]
1	5.04 dd (6.8, 1.5)	5.04 d (7.2)	79.8	79.7
2	3.89 dd (14.0, 6.8)	3.90 dd (14.4, 6.6)	50.5	50.2
3	4.27 d (14.2)	4.28 d (14.4)	55.27	55.0[a]
3a			102.3	101.9
4a			160.5	160.6[a]
5	6.48 d (1.8)	6.48 d (1.8)	93.1	92.9
6			160.4	160.0[a]
7	6.40 d (1.8)	6.40 d (1.8)	95.5	95.4
8			157.2	157.1
8a			109.5	109.6
8b			93.6	93.4
1′			126.3	126.6[a]
2′, 6′	7.09 d (9.0)	7.09 d (9.0)	129.1	129.0
3′, 5′	6.68 d (9.0)	6.69 d (9.0)	113.0	112.8
4′			159.0	158.8
1″			136.9	136.7
2″, 6″	6.85 m	6.85 m	127.94	127.8
3″, 5″	7.05 m	7.05 m	127.92	127.8[a]
4″	7.06 m	7.06 m	126.8	126.6
1‴	5.34 brs	5.36 brs	92.9	92.7
2‴	4.62 d (1.5)	4.63 d (1.2)	99.3	98.9
3‴α	4.24 dd (12.0, 2.6)	4.24 dd (11.4, 1.8)	67.4	67.2
3‴β	3.83 dd (10.9, 11.9)	3.82 t (11.4)		
4‴	4.04 ddd	4.05 ddd	67.5	67.4
5‴	3.59 dt (10.8, 4.8)	3.58 dd (10.8, 6.0)	71.3	71.2
6‴	3.66 − 3.69 m	3.59−3.61 m	62.8	62.7
	3.73 dt (11.0, 4.0)	3.74 brd (10.8)		
2-CO_2CH_3			170.9	170.6
2-CO_2CH_3	3.65 s	3.65 s	52.2	52.1
8-OCH_3	3.86 s	3.87 s	56.1	56.0
4′-OCH_3	3.71 s	3.72 s	55.28	55.1
2‴-OCH_3	3.62 s	3.63 s	57.4	57.3

[a]Chemical shifts were assigned based on 2D NMR analysis and were not observed in the reported ^{13}C NMR spectrum of 4.[6]

pathway, the low selectivity could be attributed to the small stereofacial difference in the nucleophilic attack of the intermediate oxonium ion (see Scheme 4). Alternatively, an S_N2 mechanism would also give a preference for the C1‴ equatorial isomer **15** via the major axial lactol anomer of **7**; however, the lactol ratio (1.6:1) is not reflected in the selectivity of the Mitsunobu coupling (1:2.6).[18]

Both compounds **4** and **15** along with synthetic episilvestrol (**2**)[11] were tested for inhibition of protein synthesis. In vitro assays were performed essentially as previously described[19] in rabbit reticulocyte lysate. As shown in Figure 1, episilvestrol (**2**) was a potent inhibitor of translation, while 2‴,5‴-diepisilvestrol (**4**) was considerably less active and compound **15** was essentially inactive. These results demonstrate that for potent activity the diaxial orientation of the C1‴ and C2‴ groups on the dioxane ring is required. This supports the initial observation that 2‴,5‴-diepisilvestrol (**4**) is several orders of magnitude less active than episilvestrol (**2**) in a tumor cell line assay.[6] Additionally, the C1‴ epimer of silvestrol (**1**) is less active in a translation assay.[9]

In conclusion, we have achieved the first total synthesis of the low-abundant natural product 2‴,5‴-diepisilvestrol (**4**) using an approach based on its proposed biogenesis. The biological activity of this family of compounds appears to be extremely sensitive to stereochemistry at the C1‴ and C2‴stereocenters but not the C5‴ position of the 1,4-dioxane fragment. These results will allow for more focused SAR studies in the future.

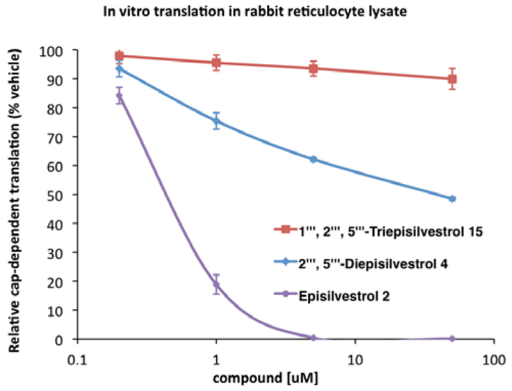

Figure 1. Translation assay results for compounds **2**, **4**, and **15**.

EXPERIMENTAL SECTION

General Experimental Procedures. ^{1}H NMR (400, 500, 600 MHz) and ^{13}C NMR (100, 125, and 150 MHz) spectra were obtained in deuterochloroform with residual chloroform as internal standard unless otherwise noted. Chemical shifts (δ) are followed by multiplicity, coupling constant(s) (J, Hz), integration, and assignments where possible. Optical rotations were recorded for 1 mL solutions, and units are deg·cm^2 g^{-1}. Flash chromatography was carried out on silica gel 60. Analytical thin-layer chromatography was conducted on aluminum-backed 2 mm thick silica gel 60 GF_{254}, and chromatograms were visualized with 20% w/w phosphomolybdic acid in ethanol. High-resolution mass spectra (HRMS) were obtained by ionizing samples via electron spray ionization (ESI). Anhydrous THF and CH_2Cl_2 were used from a solvent cartridge system. Dry methanol was distilled from magnesium methoxide. All other solvents were purified by standard methods. Petrol used refers to petroleum ether in the 40−60 °C boiling range. All other commercially available reagents were used as received. The standard workup refers to extraction with a particular solvent (3×), washing with water and brine, drying with $MgSO_4$, and concentration under reduced pressure.

Methyl Ketal 9. A solution of the lactol **8**[11] (187.5 mg, 0.372 mmol) in DMF (3 mL) was added dropwise to a suspension of NaH (87.0 mg, 3.625 mmol) in DMF (1 mL) at 0 °C. The reaction was allowed to stir at 0 °C for 10 min, then at rt for 1 h. Diethyl ether and saturated aqueous $NaHCO_3$ were added, and the organic layer was subjected to the standard workup. Purification via flash chromatography using a gradient elution of 10% to 30% EtOAc/petrol to give the C2 axial isomer[11] (20.5 mg, 9%) followed by methyl ketal **9** (153.4 mg, 80%) as a colorless oil: $[\alpha]_D^{24}$ −31.0 (*c* 0.95, CH_2Cl_2), lit.[11] $[\alpha]_D^{20}$ −28.3 (*c* 2.11, CH_2Cl_2); ^{1}H NMR (500 MHz) δ 7.36−7.28 (m, 7H), 6.86 (d, J = 8.7 Hz, 2H), 4.72 (ABq, J = 11.9 Hz, 2H), 4.60 (ABq, J = 4.0 Hz, 2H), 4.58 (d, J = 8.5 Hz, 1H), 4.39 (d, J = 1.7 Hz, 1H), 4.18−4.10 (m, 2H), 3.79 (s, 3H), 3.78 (dd, J = 10.9, 4.1 Hz, 1 H), 3.67 (dd, J = 11.3, 5.6 Hz, 1H) 3.66 (t, J = 11.0 Hz, 1H), 3.50 (s 3H), 3.46−3.43 (m 1H), 0.91 (s, 9H), 0.070 (s, 3H), 0.066 (s, 3H); ^{13}C NMR (125 MHz) δ 159.3, 138.5, 129.8, 129.4, 128.4, 127.8, 127.7, 113. 8, 99.3, 93.3, 93.2, 79.5, 72.9, 68.7, 67.0, 66.3, 62.8, 56.80, 56.79, 55.2, 26.0, 18.3, −5.34, −5.36.

Alcohol 10. $Pd(OH)_2$ (20%) on C (22.4 mg, 0.032 mmol) was added to a solution of methyl ketal **9** (75.6 mg, 0.146 mmol) in distilled EtOH (3 mL), and the resulting mixture was stirred under a hydrogen atmosphere for 30 min. The catalyst was removed by filtration through Celite, and the crude product obtained after evaporation of the filtrate was purified by flash chromatography with 20% EtOAc/petrol as eluent to give the alcohol **10** (56.2 mg, 90%) as

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a colorless oil: $[\alpha]_D^{24}$ −41.6 (*c* 0.30, CH_2Cl_2); IR ν_{max} (film) 3480, 2929, 2857, 1614, 1515, 1463, 1361, 1302, 1250, 1217, 1172, 1120, 1102, 1062, 985, 898, 837, 779, 679 cm^{-1}; ^1H NMR (500 MHz) δ 7.30 (d, *J* = 8.7 Hz, 2H), 6.86 (d, *J* = 8.7 Hz, 2H), 4.70 (d, *J* = 12.1 Hz, 1H), 4.60 (d, *J* = 1.7 Hz, 1H), 4.57 (d, *J* = 12.1 Hz, 1H), 4.41 (d, *J* = 1.8 Hz, 1H), 4.17 (dd, *J* = 11.9, 2.9 Hz, 1H), 3.99 (ddd, *J* = 10.2, 7.4, 2.9 Hz, 1H), 3.80 (s, 3H), 3.70 (d, *J* = 1.7 Hz, 1H), 3,69 (ABq, *J* = 10.2 Hz, 1H), 3.62 (dd, *J* = 10.0, 5.3 Hz, 1H), 3.57−3.53 (m, 1H), 3.51 (s, 3H), 0.90 (s, 9H), 0.092 (s, 3H), 0.089 (s, 3H); ^{13}C NMR (125 MHz) δ 159.4, 129.9, 129.4, 113.8, 99.3, 93.4, 71.4, 68.9, 67.3, 66.6, 63.4, 56.9, 55.3, 26.0, 18.4, −5.23, −5.29; HRESIMS *m/z* 451.2107 (calcd for $C_{21}H_{36}NaO_7Si$ $[M + Na]^+$, 451.2123).

Bis-TBS Ether 11. 2,6-Lutidine (195 μL, 1.674 mmol) and TBSOTf (255 μL, 1.110 mmol) were added to a solution of the alcohol **10** (118.7 mg, 0.277 mmol) in CH_2Cl_2 (4 mL) at 0 °C. The reaction was then stirred at rt for 2.5 h; then saturated $NaHCO_3$ and ether were added. The organic layer was subjected to the standard workup. Purification via flash chromatography followed using a gradient elution of 10% to 15% EtOAc/petrol to give bis-TBS ether **11** (123.7 mg, 82%) as a white, crystalline solid: mp 64.5−64.9 °C; $[\alpha]_D^{26}$ −30.7 (*c* 0.75, CH_2Cl_2); IR ν_{max} (film) 2955, 2929, 2858, 1614, 1515, 1464, 1361, 1303, 1251, 1217, 1108, 1064, 1005, 940, 901, 833, 778, 680 cm^{-1}; ^1H NMR (500 MHz) δ 7.31 (d, *J* = 8.6 Hz, 2H), 6.86 (d, *J* = 8.6 Hz, 2H), 4.71 (d, *J* = 12.0 Hz, 1H), 4.57 (d, *J* = 12.0 Hz, 1H), 4.57 (d, *J* = 1.3 Hz, 1H), 4.37 (d, *J* = 1.7 Hz, 1H), 4.11−4.08 (m, 1H), 4.05 (dd, *J* = 11.7, 2.9 Hz, 1H), 3.79 (s, 3H), 3.72 (dd, *J* = 11.5, 10.5 Hz, 1H), 3.66 (q, *J* = 5.1 Hz, 1H), 3.60−3.52 (m, 2H), 3.50 (s, 3H), 0.90 (s, 9H), 0.88 (s, 0.9H), 0.08 (s, 6H), 0.060 (s, 3H), 0.058 (s, 3H); ^{13}C NMR (125 MHz) δ 159.3, 129.9, 129.5, 113.8, 99.4, 93.2, 73.7, 68.5, 67.0, 66.7, 64.9, 56.9, 55.3, 26.1, 25.9, 18.4, 18.2, −4.3, −4.7, −5.29, −5.31; HRESIMS *m/z* 565.2980 (calcd for $C_{27}H_{50}NaO_7Si_2$ $[M + Na]^+$ 565.2987).

Lactol 6. Buffer (pH 7, 0.5 mL) and DDQ (42.0 mg, 0.185 mmol) were added to a solution of the bis-TBS ether **11** (70.0 mg, 0.129 mmol) in CH_2Cl_2 (4 mL), at 0 °C. The reaction mixture was stirred at rt for 17 h, then filtered through Celite, and the filtrate was concentrated. Purification by flash chromatography with 15% EtOAc/petrol as eluent gave the mixture of 1.6:1 C1 ax:eq lactols **6** (46.0 mg, 84%) as a colorless oil: IR ν_{max} (film) 3435, 2954, 2929, 2886, 2857, 1472, 1463, 1389, 1361, 1252, 1213, 1147, 1092, 1004, 987, 955, 829, 813, 774 cm^{-1}; ^1H NMR (500 MHz) δ 4.89 (dd, *J* = 7.0, 1.6 Hz, 1H, minor), 4.59 (t, *J* = 4.9 Hz, 1H, major), 4.40 (d, *J* = 1.7 Hz, 1H, minor), 4.10 (d, *J* = 5.2 Hz, 1H, major), 4.08−4.05 (m, 1H, minor), 4.00 (dd, *J* = 11.7, 2.9 Hz, 1H, major and minor), 3.87 (q, *J* = 11.1, 5.2 Hz, 1H, major), 3.79−3.71 (m, 2H, major and minor), 3.66−3.54 (m, 3H, major and minor), 3.522 (s, 3H, major), 3.521 (s, 3H, minor), 3.25 (d,, *J* = 4.7 Hz, 1H, major), 3.21 (d, *J* = 7.0 Hz, 1H, minor); ^{13}C NMR (125 MHz) δ 159.3, 129.9, 129.5, 113.8, 99.4, 93.2, 73.7, 68.5, 67.0, 66.7, 64.9, 56.9, 55.3, 26.1, 25.9, 18.4, 18.2, −4.30, −4.7, −5.3, −5.3; HRESIMS *m/z* 445.2412 (calcd for $C_{19}H_{42}NaO_6Si_2$ $[M + Na]^+$ 445.2412).

***endo*-Cyclopentabenzofuran (−)-14.** To a solution of **12**[11] (539.0 mg, 1.33 mmol) in freshly distilled $CHCl_3$ (25.6 mL) and trifluoroethanol (11.0 mL) was added methyl *trans*-cinnamate (3.062 g, 18.880 mmol). After degassing for 5 min, the mixture was irradiated (450 W Hanovia UV lamp, Pyrex filter) at −5 °C under a nitrogen atmosphere for 15 h. The solvent was removed in vacuo, and the residue was purified by flash chromatography with 20% EtOAc/petrol followed by 50% EtOAc/petrol as eluent to yield the desired cycloadduct **13** (709.1 mg, 94%) as a bright orange foam. To a solution of **13** (709.1 mg, 1.252 mmol) in MeOH (34 mL) was added a NaOMe solution (0.5 M, 7.1 mL, 3.550 mmol). The reaction was heated to reflux for 40 min and was quenched with saturated NH_4Cl. The standard workup with EtOAc provided the α-ketoesters as a mixture of keto−enol tautomers (661.9 mg, 93%) as a brown, glassy oil. A solution of tetramethylammonium triacetoxyborohydride (1.559 g, 5.926 mmol) and acetic acid (550 μL, 9.608 mmol) in acetonitrile (24.0 mL) was stirred under argon for 5 min. A solution of keto−enol tautomers (522.9 mg, 0.923 mmol) in acetonitrile (16.0 mL) was cannulated, and the mixture was stirred at rt for 19 h. The reaction was quenched with saturated NH_4Cl and 0.5 M (+)-sodium tartrate. The standard workup with CH_2Cl_2 and purification by flash chromatography with 40% EtOAc/petrol as eluent gave the racemic *endo* isomer **14** (232.1 mg, 44%, 38% over three steps) as a colorless oil. Racemic **14** was then subjected to chiral HPLC separation (5 μm Phenomenex Lux Cellulose-1 semipreparative column: 10 × 250 mm, 70% MeCN/H_2O eluent, flow rate: 2.00 mL/min) to provide compound (−)-**14** (104.4 mg, 45%) (t_R = 18.03 min) as a colorless oil: $[\alpha]_D^{24}$ −51.2 (*c* 0.48, CH_2Cl_2), lit.[11] $[\alpha]_D^{23}$ −51.5 (*c* 0.86, CH_2Cl_2); ^1H NMR (500 MHz) δ 7.47−7.35 (m, 5H), 7.11 (d, *J* = 8.9 Hz, 2H), 7.08−7.04 (m, 3H), 6.89−6.87 (m, 2H), 6.67 (d, *J* = 8.9 Hz, 2H), 6.36 (d, *J* = 1.9 Hz, 1H), 6.22 (d, *J* = 1.9 Hz, 1H), 5.09 (d, *J* = 2.0 Hz, 1H), 5.03 (dd, *J* = 6.7, 1.2 Hz, 1H), 4.32 (d, *J* = 14.1 Hz, 1H), 3.91 (dd, *J* = 14.1, 6.2 Hz, 1H), 3.86 (s, 3H), 3.70 (s, 3H), 3.66 (brs, 1H), 3.65 (s, 3H), 1.86 (s, 1H), 1.58 (brs, 1H). Further elution provided the other isomer, (+)-**14** (105.0 mg, 45%) (t_R = 19.45 min), as a colorless oil: $[\alpha]_D^{24}$ +55.9 (*c* 0.95, CH_2Cl_2), lit.[11] $[\alpha]_D^{23}$ +48.2 (*c* 0.21, CH_2Cl_2).

Phenol (−)-7. Following the reported procedure,[11] 20% $Pd(OH)_2$ on C (31.8 mg, 0.0453 mmol) was added to a solution of the benzyl ether (−)-**14** (63.2 mg, 0.111 mmol) in ethanol (5.0 mL), and the mixture was stirred vigorously under a hydrogen atmosphere for 3 h and then filtered through Celite. After removal of solvent, the crude product was purified by flash chromatography with 60% EtOAc/petrol as eluent to give the phenol (−)-7 (50.7 mg, 95%) as a white solid: $[\alpha]_D^{25}$ −27.2 (*c* 0.70, CH_2Cl_2), lit.[11] $[\alpha]_D^{23}$ −27.0 (*c* 0.71, CH_2Cl_2); ^1H NMR (500 MHz, d_6-acetone) δ 8.70 (s, 1H), 7.12 (d, *J* = 8.9 Hz, 2H), 7.05−6.99 (m, 3H), 6.91 (d, *J* = 7.4 Hz, 2H), 6.63 (d, *J* = 9.0 Hz, 2H), 6.17 (d, *J* = 1.8 Hz, 1H), 6.12 (d, *J* = 1.7 Hz, 1H), 4.93 (dd, *J* = 6.5, 2.7 Hz, 1H), 4.28 (d, *J* = 14.1 Hz, 1H), 4.17 (dd, *J* = 2.8, 0.8 Hz, 1H), 3.97 (s, 1H), 3.94 (ddd, *J* = 14.0, 6.5, 0.7 Hz, 1H), 3.83 (s, 3H), 3.66 (s, 3H), 3.56 (s, 3H).

2‴,5‴-Diepisilvestrol (4) and 1‴,2‴,5‴-Triepisilvestrol (15). PPh_3 (82.3 mg, 0.314 mmol) and powdered 4 Å molecular sieves (~350 mg) were added to a stirred solution of the lactols **6** (75.8 mg, 0.179 mmol) and phenol (−)-7 (33.4 mg, 0.070 mmol) in toluene (1.5 mL). After 20 min, DMEAD[16] (79.8 mg, 0.341 mmol) was then added at 0 °C, and the suspension was stirred at 0 °C for 2 d. The reaction mixture was filtered through Celite, and the cake washed with toluene. The filtrate was washed with water to remove the hydrazine byproduct, and the organic layer was dried and concentrated. The crude residue was subjected to column chromatography, and gradient elution with 20% to 50% EtOAc/petrol provided the bis-TBS coupled products as an inseparable mixture of axial and equatorial isomers (33.9 mg, 0.038 mmol). The mixture of bis-TBS isomers (31.0 mg, 0.035 mmol) was dissolved in THF (4.0 mL) and cooled to 0 °C. TBAF in 1.5 mL of THF (37.5 mg, 0.119 mmol) was added dropwise, and the mixture was allowed to stir at rt for 1 h before the solvent was removed in vacuo. The crude product was subjected to flash chromatography with 100% EtOAc on a plug of silica to give a 2.4:1 mixture of isomers **15** and **4**, which was purified using HPLC (5 μm Phenomenex Lux Cellulose-1 semipreparative column: 10 × 250 mm, 70% MeCN/H_2O eluent, flow rate: 2.00 mL/min) to provide 2‴,5‴-diepisilvestrol (**4**) (5.8 mg, 25%) (t_R = 9.33 min) as a white solid: $[\alpha]_D^{25}$ −62.3 (*c* 0.25, MeOH), lit.[6] $[\alpha]_D^{23}$ −53.0 (*c* 0.05, MeOH); IR ν_{max} (film) 3424, 3325, 2937, 2845, 2254, 1746, 1611, 1515, 1497, 1452, 1339, 1251, 1217, 1168, 1144, 1120, 1042, 978, 912, 825, 730, 700, 664 cm^{-1}; ^1H NMR (600 MHz, 8.6 mM in $CDCl_3$) δ 7.09 (d, *J* = 9.0 Hz, 2H), 7.06 (m, 1H), 7.05 (m, 2H), 6.85 (m, 2H), 6.68 (d, *J* = 9.0 Hz, 2H), 6.48 (d, *J* = 1.8 Hz, 1H), 6.40 (d, *J* = 1.8 Hz, 1H), 5.34 (brs, 1H), 5.04 (dd, *J* = 6.8, 1.5 Hz, 1H), 4.62 (d, *J* = 1.5 Hz, 1H), 4.27 (d, *J* = 14.2 Hz, 1H), 4.24 (dd, *J* = 12.0, 2.6 Hz, 1H), 4.04 (ddd, *J* = 10.6, 7.0, 2.7 Hz, 1H), 3.89 (dd, *J* = 14.0, 6.8 Hz, 1H), 3.86 (s, 3H), 3.83 (dd, *J* = 11.9, 10.9 Hz, 1H), 3.77 (brs, 1H), 3.73 (dt, *J* = 11.0, 4.0, 1H), 3.71 (s, 3H), 3.69−3.66 (m, 1H), 3.65 (s, 3H), 3.62 (s, 3H), 3.59 (dt, *J* = 10.8, 4.8 Hz, 1H), 2.41 (d, *J* = 5.8 Hz, 1H), 2.02 (t, *J* = 4.8 Hz, 1H), 1.88 (s, 1H); ^{13}C NMR (150 MHz) δ 170.9, 160.5, 160.4, 159.0, 157.2, 136.9, 129.1, 127.94, 127.92, 126.8, 126.3, 113.0, 109.5, 102.0, 99.0, 95.5, 93.6, 93.1, 92.9, 79.8, 71.3, 67.5, 67.4, 62.8, 57.4, 56.1, 55.28, 55.27, 52.2, 50.5; HRESIMS *m/z* 677.2164 (calcd for $C_{34}H_{38}NaO_{13}$ $[M + Na]^+$ 677.2205).

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Further elution provided 1‴,2‴,5‴-triepisilvestrol (**15**) (15.0 mg, 65%) (t_R = 10.38 min) as a white solid: $[\alpha]_D^{24}$ +14.3 (*c* 0.42, MeOH); IR ν_{max} (film) 3338, 2953, 2916, 2869, 2841, 1742, 1603, 1514, 1498, 1453, 1435, 1378, 1344, 1299, 1250, 1214, 1170, 1153, 1117, 1017, 950, 899, 827, 771, 732, 700 cm^{-1}; ^1H NMR (500 MHz, 16.7 mM in $CDCl_3$) δ 7.09 (d, *J* = 9.0 Hz, 2H), 7.05 (m, 3H), 6.84 (m, 2H), 6.67 (d, *J* = 9.0 Hz, 2H), 6.38 (d, *J* = 1.9 Hz, 1H), 6.21 (d, *J* = 1.9 Hz, 1H), 5.06 (d, *J* = 3.4, 1H), 5.01 (dd, *J* = 6.7, 1.9 Hz, 1H), 4.48 (d, *J* = 3.4 Hz, 1H), 4.27 (d, *J* = 14.2 Hz, 1H), 4.24 (dd, *J* = 12.0, 2.6 Hz, 1H), 4.09 (dd, *J* = 11.9, 3.3 Hz, 1H), 3.93−3.88 (m, 1H), 3.88 (ddd, *J* = 14.2, 6.7, 0.7 Hz, 1H), 3.86 (s, 3H), 3.77 (dd, *J* = 11.9, 5.9 Hz, 1H), 3.70−3.67 (m, 5H), 3.64 (s, 3H), 3.60 (dt, *J* = 11.4, 3.4 Hz, 1H), 3.53 (s, 3H), 3.50 (dd, *J* = 11.5, 3.7 Hz, 1H), 2.62 (d, *J* = 5.7 Hz, 1H), 2.25 (s, 1H), 2.05 (t, *J* = 4.8 Hz, 1H); ^{13}C NMR (125 MHz) δ 170.7, 160.8, 160.7, 158.9, 157.3, 136.9, 129.1, 128.0, 127.9, 126.8, 126.4, 112.9, 109.8, 102.0, 98.3, 95.5, 94.1, 93.7, 92.5, 79.7, 77.36, 72.6, 70.7, 63.5, 56.07, 56.05, 55.3, 55.1, 52.2, 50.5; HRESIMS *m/z* 677.2164 (calcd for $C_{34}H_{38}NaO_{13}$ $[M + Na]^+$ 677.2205).

In Vitro Translation Inhibition Assays. Assays were performed essentially as previously described.[19] Capped FF/HCV/Ren mRNA was transcribed using Sp6 RNA polymerase (Promega) from pSP/$(CAG)_{33}$/FF/HCV/Ren.pA_{51} linearized with *Bam*HI. Reactions were performed in rabbit reticulocyte lysate following the manufacturer's instructions (Promega) with a final concentration of 135 mM KCl and programmed with 8 μg/mL mRNA. Luciferase activity was detected using the Dual Glo Luciferase Assay system (Promega).

X-ray Crystallography. Intensity data for compound **11** were collected on an Oxford Diffraction SuperNova CCD diffractometer using Cu Kα radiation; the temperature during data collection was maintained at 130.0(1) using an Oxford Cryostream cooling device. The structure was solved by direct methods and difference Fourier synthesis.[20] A thermal ellipsoid plot was generated using the program ORTEP-3[21] integrated within the WINGX[22] suite of programs. The absolute configuration of **11** is set by the configuration of the starting material D-glucose and is supported by the Flack parameter, which was 0.02(3).

Crystal data for compound ***11****:* $C_{27}H_{50}O_7Si_2$, *M* = 542.85, *T* = 130.0 K, λ = 1.54180, orthorhombic, space group $P2_12_12_1$, *a* = 8.2450(2), *b* = 10.6777(3), *c* = 36.136(1) Å. *V* = 3181.4(2) $Å^3$, *Z* = 4, D_c = 1.133 Mg m^{-3}, μ(Cu Kα) = 1.324 mm^{-1}, *F*(000) = 1184, crystal size 0.38 × 0.25 × 0.04 mm^3, 10 320 reflections measured, 5849 independent reflections [*R*(int) = 0.0248], final *R* = 0.0436 [$I > 2\sigma(I)$] and $wR(F^2)$ = 0.1208 (all data).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

HPLC traces and copies of the NMR spectra of all new compounds as well as the CIF for the X-ray structure of compound **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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