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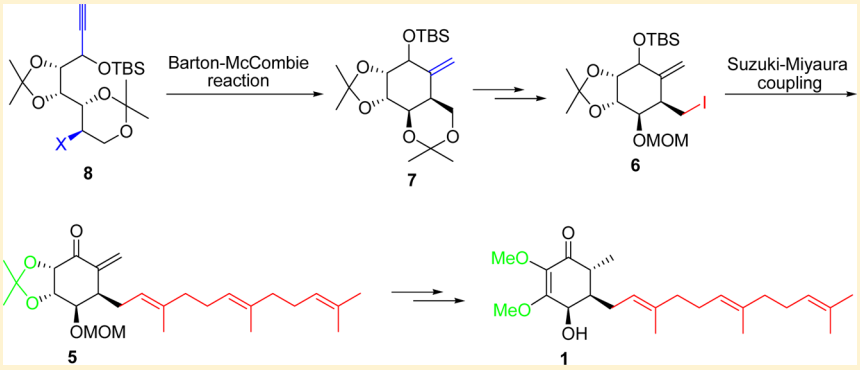
Synthesis of (+)-Antroquinonol: An Antihyperglycemic Agent

Rohidas S. Sulake,[†,§] Hsiao-Han Lin,[†] Chia-Yu Hsu,[‡,§] Ching-Feng Weng,*[,‡] and Chinpiao Chen*[,†]

[†]Department of Chemistry, National Dong Hwa University, Hualien 97401, Taiwan

[‡]Institute of Biotechnology, National Dong Hwa University, Hualien 97401, Taiwan

Supporting Information



ABSTRACT: The total synthesis of antroquinonol has been accomplished through Suzuki−Miyaura cross-coupling and Barton−McCombie reaction, and the α,β-unsaturation was achieved through selenylation and oxidation protocols. *In vitro* and *in vivo* studies on the glucose-lowering properties of antroquinonol indicate that it is a potential antidiabetic agent.

INTRODUCTION

Antrodia camphorata (*A. camphorata*) has been utilized as a folk medicine for defense against assorted health-related problems. Antroquinonol (**1**), antroquinonol B (**2**), and C (**3**), as well as many other bioactive components (anticin, antrodioxolone, antrocamphin, zhankuic acid), were isolated[1] from such expensive and rarely found medicinal fungal species (Figure 1). Antroquinonols are tetrahydro ubiquinone (**4**) derivatives and are considered responsible for the biological properties of *A. camphorata*. Ubiquinone, also known as coenzyme Q, is a critical component of electron transport pathways and has been investigated extensively because it is present in almost every cell and plays an essential role in the human body. Different types of ubiquinones[2] are distinguished according to the number of isoprenoid units occurring in different homologous forms in nature, and numerous synthetic methods have been reported for ubiquinone and its analogues.[3]

Antroquinonol has received considerable attention because of its high *in vitro* and *in vivo* activity against numerous cancer cells. It possesses the cytotoxic activities of several cancer cell lines, MCF-7, MDA-MB-231, Hep3B, HepG2, DU145, and LNCaP, with the IC_{50} (half-maximal inhibitory concentration) ranging from 0.13 to 6.09 μM.[1] Antroquinonol also significantly suppressed the levels of TNF-α and IL-1β by 75% and 78%, respectively, in RAW 264.7 cells.[4] Moreover, antroquinonol acts as a potent inhibitor of HBsAg and HBeAg[5] synthesis, and it inhibits the proliferation of nonsmall cell lung cancer by the alteration of proteins and miRNA expression profiles in PI3K/mTOR.[6] Antroquinonol (Hocena) capsule was recognized as a breakthrough anticancer drug and has been proven to be a potential anticancer agent under FDA Phase II review.

In addition to possessing potential anticancer properties, *A. camphorata* is used as a traditional medicine for treating diabetes mellitus; however, the effects remain unclear. Because antroquinonol is isolated from such fungal species, it may possess glycemic control properties in *A. camphorata*; hence, it could be a potential applicant for new medication of antidiabetes. Although antroquinonol is commonly used as an antitumor agent, no studies have reported on its impending use in the controlling of human diabetes. Because the natural abundance of antroquinonol is low, the total synthesis of (+)-antroquinonol is particularly critical for preparing sufficient quantities for biological evaluation. Amid these efforts, the total synthesis of (±)-antroquinonol D was achieved through the Michael addition of cyclohexadienone, followed by a diastereomeric reduction of cyclohexenone and the synthesis of the sesquiterpene side chain.[7a] Another approach to the asymmetric total synthesis of (+)-antroquinonol and (+)-antroquinonol D was achieved through iridium-catalyzed olefin isomerization, Claisen rearrangement reaction, lactonization, and Grubbs olefin metathesis.[7b,c] In this study, we report the efficient total synthesis and glucose-lowering properties and reducing of obesity of antroquinonol.

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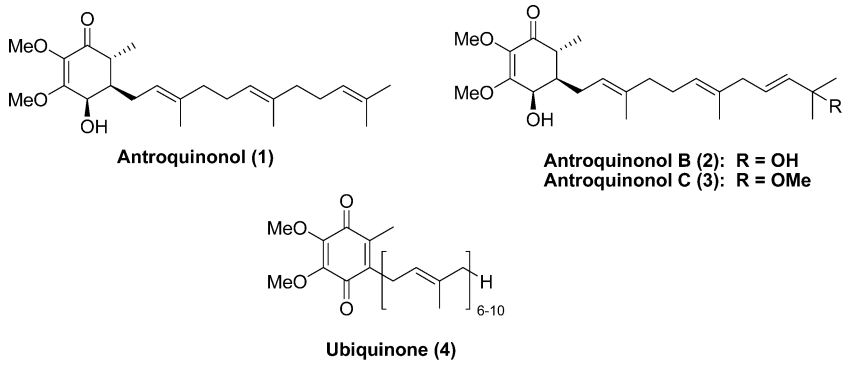
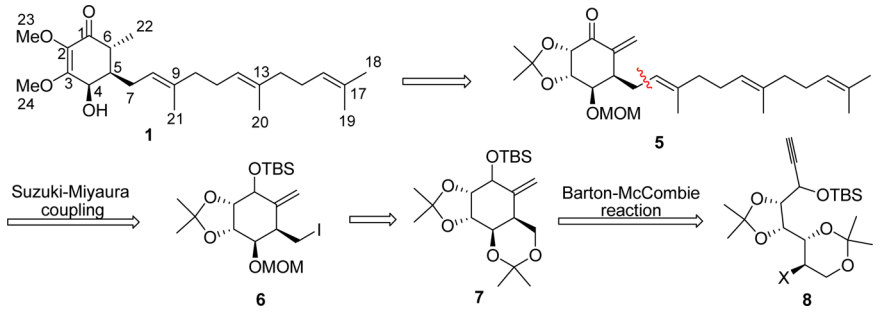


Figure 1. Naturally occurring quinolic derivatives and ubiquinone.

Scheme 1. Retrosynthetic Analysis of Antroquinonol 1



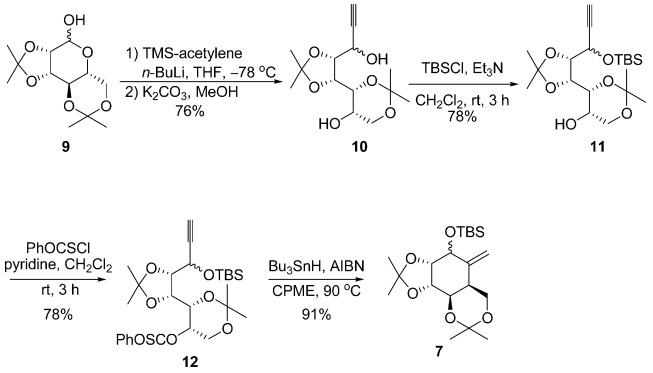
RESULT AND DISCUSSION

Retrosynthetic Analysis of Antroquinonol 1. Retrosynthetically, the enone system of (+)-antroquinonol (**1**) can be achieved through the selenylation and oxidation protocols of the corresponding ketone[7b] available from precursor **5** (Scheme 1). A sesquiterpene side chain of **5** could, in turn, be synthesized using Suzuki–Miyaura cross-coupling between the corresponding vinyl iodide and boronate available from iodo **6**. The *syn* configuration between C4 and C5 could be generated through the 6-exo-digonal radical cyclization of a cyclic radical generated from **8** by the Barton–McCombie deoxygenation.[8]

Total Synthesis of Antroquinonol 1. Antroquinonol synthesis was commenced by adding lithium trimethylsilyl acetylide to lactol **9** (which can be synthesized in a single step by using D-mannose)[9] and slightly modifying the process developed by López.[10] We observed that K_2CO_3/MeOH treatment is essential for the complete dissociation of the trimethylsilyl group, and that the reaction afforded an inseparable mixture (2:1) of the epimeric isomers of diol **10** (Scheme 2).

A steric bulk presented by the acetonide moiety enabled the mono protection of **10** at C1 hydroxy with TBSCl in CH_2Cl_2 in the presence of triethylamine to afford TBS ether **11** in 78% yield. Subsequently, the other hydroxyl group was transformed to thionocarbonate **12** with excess phenyl chlorothionoformate and pyridine in DCM. Radical ring closure of the cyclic radical generating an alkene or alkyne is known to afford predominantly *cis* fused systems[11] and is applied in the synthesis of carbasugars.[10] Accordingly, when acetylenethionocarbonate **12** was treated with Bu_3SnH and AIBN as a radical initiator in cyclopentyl methyl ether,[8] deoxygenative radical cyclization produced *exo*-methylene cyclohexane **7** in 91% yield with excellent diastereoselectivity (*cis*:*trans*, 14:1). Stereoselective hydrogenation of *exo*-methylene at this stage was hampered due to the different orientation of cyclopentane, cyclohexane rings, and the bulky OTBS group.

Scheme 2. Synthesis of *exo*-Methylene Cyclohexene (7)



After successfully generating *syn* geometry between C4 and C5, attachment of the sesquiterpene side chain was the next target. For this purpose, chemoselective deprotection of the six-membered isopropylidene group of **7** was achieved using pyridinium *p*-toluenesulfonate (PPTS) in MeOH to afford diol **13** (81% yield). The primary hydroxyl group was transformed to iodo **6** through selective tosylation, followed by iodination with NaI in acetone. Subsequently, the secondary hydroxyl group was protected using MOM ether to provide an iodo precursor (**6**) in 76% yield over three steps. To accomplish the crucial coupling of **6** and polyenic vinyl iodide **14**,[12] we decided to use the Marshall protocol[13] for a Suzuki–Miyaura cross-coupling,[14] which provided the desired coupling product **15** in 90% yield (Scheme 3).

Chemoselective and stereoselective conjugate reduction of α,β-unsaturated carbonyl compounds is well-established.[15] Consequently, the deprotection of TBS ether with TBAF in

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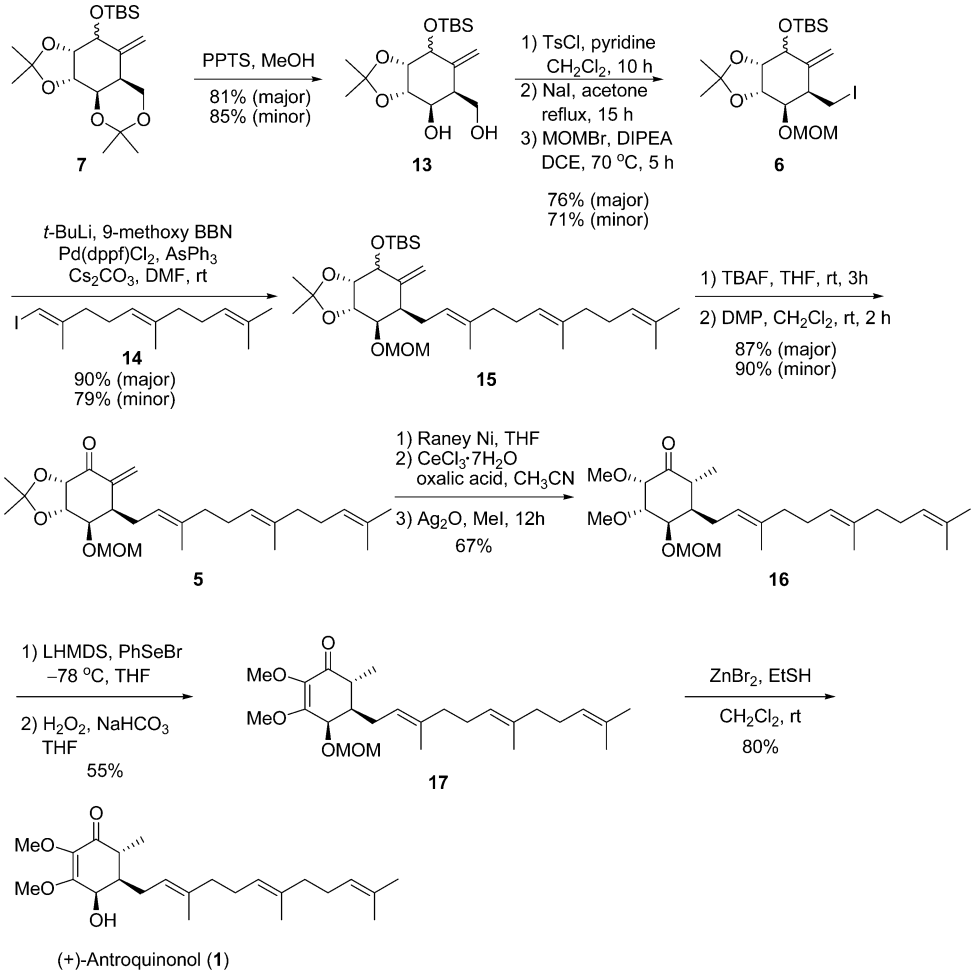
Scheme 3. Total Synthesis of Antroquinonol



THF and the oxidation of the resultant alcohol with Dess–Martin periodinane provided the requisite enone **5** in 80% yield. Most of the metal hydrides described[16] for the α,β-conjugate reduction of enone predominantly yielded 1,2-reduction with or without conjugate reduction. Chemoselective reduction of the conjugated double bond of **5** was achieved using Raney nickel[17] to give a mixture (4:1) of diastereomers at C6. Deprotection of the cyclic acetonide was performed using $CeCl_3$ and oxalic acid in acetonitrile[18] to afford the diol intermediate, which was later dimethylated with a Purdie reagent (Ag_2O, MeI) to provide a keto compound[7b] **16** in a 67% yield over three steps. We postulate that keto–enol tautomerization during reduction of the α,β-conjugate double bond preferably gives a *trans* orientation of methyl and sesquiterpene side chain. Thus, the development of an α,β-unsaturated ketone can proceed. Regioselective phenylselenation of **16** with LHMDS in THF at −78 °C afforded α-phenylselenyl ketone at methoxy carbon. This was then followed by oxidative elimination with 30% H_2O_2 to give α,β-unsaturated ketone **17**. The final stage of synthesizing antroquinonol required the deprotection of MOM ether. The deprotection of the MOM group of **17** was efficiently achieved using dry $ZnBr_2$[19] and ethanethiol in CH_2Cl_2 to afford (+)-antroquinonol in favorable yield.

The spectroscopic data of the synthesized (+)-antroquinonol were consistent with those described for the natural product.[1] Additionally, the optical rotation of the synthetic material is in favorable agreement with the authentic material.[7b,20] Synthetic (+)-antroquinonol exerts dual efficacy for insulin resistance by eliciting the activities of AMPK and anti-DPP IV activities.[7c]

Antroquinonol Promoted the Glucose Uptake. The overall lowering of glucose is pivotal in the treatment of diabetes, with proven beneficial effects on micro- and macrovascular outcomes. Furthermore, evidence indicates that "glucose variability" is a high risk factor for cardiovascular complications in diabetes. The therapeutic challenge entails the need for intensive glycemic control and the maintenance of glycaemia within a strict normal narrow range. Recently, extensive glycemic control is applied to reduce the exacerbation of insulin resistance and type 2 diabetes mellitus (T2DM) in long-term mega trials are demonstrated.[21] Therefore, the development of new agents for glycemic control is gaining considerable attention.

The uptake of glucose arises from the enployment of GLUT4 translocation into the plasma membrane, where these GLUT4 transporters expedite the glucose uptake. The ability of glucose uptake is determined by cellular levels that control the amount of GLUT4 glucose transporters present in the plasma membrane. First, we determined the efficiency of antroquinonol in cellular glucose uptake by measuring the glucose uptake through a standard procedure (Supporting Information); Figure 2 provides the results of the glucose uptake assay for insulin (Ins) and antroquinonol (Ant). The results indicate that antroquinonol has a beneficial effect on GLUT4 translocation,

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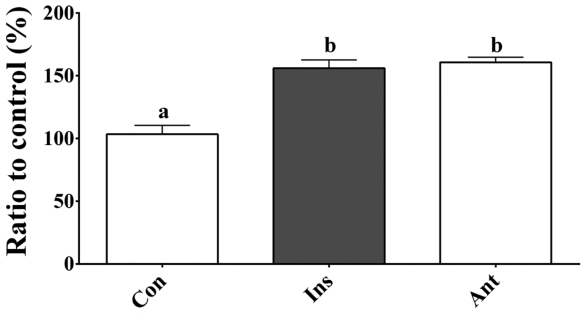

Figure 2. Antroquinonol promoted the glucose uptake. Differentiated L6 cell after treatment of 1 μM insulin and 10 nM antroquinonol (Ant) for 30 min (mean ± SEM, n = 5 in each group). A different letter represents the significant difference (p < 0.05) among various treatments.

and its effects on glucose uptake are similar to those on insulin (10 nM of antroquinonol was equivalent to 1 μM insulin).

Hypoglycemic Efficacy of Antroquinonol and Metformin on OGTT Glucose Levels in C57BL/6 Mice. To test the glycemic control effect of antroquinonol on mice with hyperglycemia, the mice were induced by an insulin receptor antagonist; S961 was employed to induce mice for mimicking hyperglycemia. The mice were subjected to fasting for 12 h before the test and were oral gavage given D-glucose (2 g/kg Bwt, p.o.). At approximately 0, 30, 60, 90, and 120 min, blood was sampled using venipuncture from the tail vein for determining blood glucose, which was immediately measured using the glucose oxidase method with a glucose analyzer (Accu-Chek, Roche). To investigate the entire change of blood glucose, the area under the curve (AUC) was calculated and is shown as a bar chart.

Figure 3 shows the oral glucose tolerance test (OGTT) and the AUC (ratio to the control) of antroquinonol (Ant) and metformin (Met) (administrated as a clinical drug for mediating glucose uptake) determined through the oral glucose tolerance test (OGTT), wherein the blood glucose change was indicated by the AUC (area under curve) of mice treated with S961 (an antagonist for insulin receptor, used to mimic DM) at 40 nmol/kg Bwt (Body weight), followed by metformin (Met) at 100 mg/kg Bwt as a positive control and antroquinonol (Ant) at 50 mg/kg Bwt. The results showed that antroquinonol has a significant hypoglycemic efficacy similar to that of metformin under the condition of insulin resistance. Antroquinonol showed satisfactory efficacy in lowering blood glucose (Figures 2 and 3), and only a half dose of routine was required for glycemic control.

CONCLUSION

In summary, the total synthesis of (+)-antroquinonol has been accomplished from a readily available mannose diacetonide in 16 steps and in 5.9% overall yield. Key features of the synthesis include (i) *cis* geometry (C_4-OH, C_5−C_7) was achieved through the 6-*exo*-*dig* cyclization of a cyclic radical generated by Barton−McCombie deoxygenation and (ii) Suzuki−Miyaura coupling for the synthesis of the sesquiterpene side chain. This strategy allows for the preparation of sufficient quantities of natural as well as its analogues for detailed biological studies. The results of *in vitro* and *in vivo* studies revealed that antroquinonol is a potential candidate for glycemic control through the enhancement of glucose transporter 4 translocation, thereby improving glucose uptake. Therefore, antroquinonol can be developed as a drug for treating diabetes mellitus, particularly T2DM.

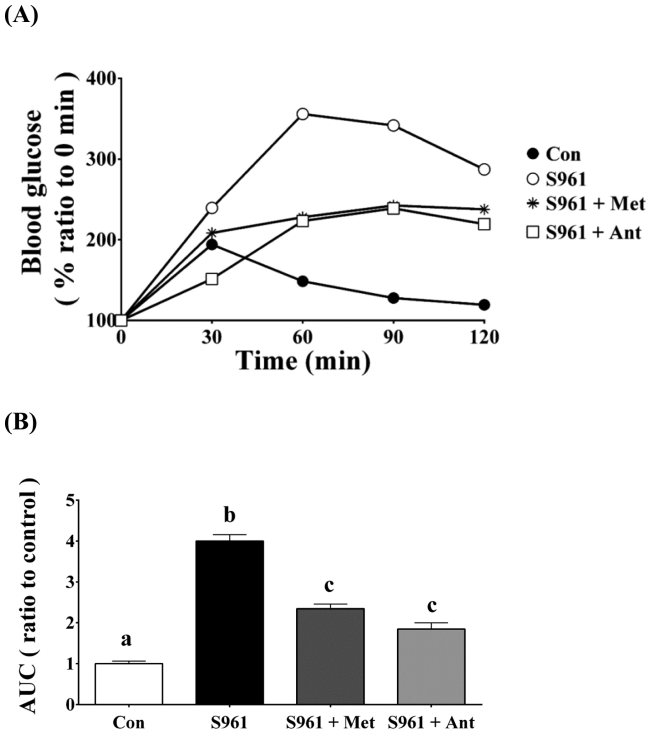

Figure 3. Hypoglycemic efficacy of antroquinonol and Metformin on OGTT glucose levels in C57BL/6 mice in (A) oral glucose tolerance test (OGTT) and (B) AUC (area under curve) mice after administration of S961 (insulin receptor antagonist to mimic DM), followed by antroquinonol (Ant), Metformin (Met), and then glucose in each group. Data are expressed as means with standard errors of mean (mean ± SEM; n = 5 in each group). A different letter represents the significant difference (p < 0.05) among various treatments.

EXPERIMENTAL SECTION

General Methods. Unless specified otherwise, all starting materials and reagents were obtained from commercial suppliers and used without further purification. All reactions were conducted in oven-dried glassware, in an argon atmosphere with anhydrous solvents, which were dried and distilled before use according to standard procedures. Solvents used for isolating products and chromatography were glass distilled. Reactions were monitored by performing thin-layer chromatography with precoated silica gel 60 glass plates and an F254 indicator. Visualization was accomplished using UV light (254 nm) combined with iodine, potassium permanganate, or anisaldehyde staining solutions. The products were purified using neutral column chromatography on 230−400 mesh silica gels. Yields refer to a chromatographically and spectrographically pure material, unless otherwise noted. ^{1}H NMR and ^{13}C NMR spectra were obtained using 400 and 100.6 MHz NMR spectrometers, respectively. Chemical shifts (δ) are reported in parts per million (ppm) relative to $CDCl_3$ (7.26 and 77.00 ppm); coupling constants are reported in hertz (Hz); and multiplicities are indicated as br = broad, s = singlet, d = doublet, dd = doublet of doublet, t = triplet, and m = multiplet. Infrared spectra were recorded using an FT/IR spectrometer. High-resolution mass spectra (HRMS-EI) were determined using a mass spectrometer. HRMS spectra were recorded with a TOF detector. Optical rotations were measured using a polarimeter at the indicated temperature with a sodium lamp (D line, 589 nm). The insulin receptor antagonist S961 was a generous gift from Dr. Lauge Schäffer (Novo-Nordisk, Denmark). Male C57BL/6 mice were purchased from the National

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Laboratory Animal Center (Taipei, Taiwan) and kept under controlled environmental conditions at 22 ± 2 °C and humidity (50 ± 10%). The photocycle of 12 h light (0600 am to 1800 pm) and dark was maintained throughout the study. Animal experiments were approved by the Animal Ethics Committee of National Dong Hwa University, and the animals were used according to the "Guide for the Care and Use of Laboratory Animals" of National Dong Hwa University.

(4*R*,5*S*)-4-((4*R*,5*R*)-5-(1-Hydroxyprop-2-ynyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyl-1,3-dioxan-5-ol (10). *n*-BuLi (131.43 mmol, 65.71 mL of 2.0 M solution in cyclohexane) was added to the solution of trimethylsilylacetylene (19.70 mL, 138.36 mmol) in dry THF (150 mL) at −78 °C and stirred for 5 min. A solution of lactol **9** (9.00 g, 34.59 mmol) in dry THF (30 mL) was added dropwise, and the reaction mixture was stirred for 1 h at −78 °C and then allowed to reach room temperature; after that, stirring was continued for 12 h. The reaction mixture was then concentrated, and the residue obtained was dissolved in MeOH (50 mL). K_2CO_3 (4.70 g, 34.60 mmol) was added, and the mixture was stirred for 1 h at rt. Methanol was removed and the residue obtained was diluted with Et_2O (200 mL), and then it was washed with water. The organic layer was separated, dried, and concentrated, and the residue obtained was purified by flash chromatography (hexane/EtOAc, 4:1) to give the diol **10** as an inseparable mixture of diastereomers (7.50 g, 26.13 mmol): yield 76%; R_f (hexane/EtOAc, 3:2) 0.3; IR (film) 3306, 2118, 1634, 1372, 1222 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.61 (m, 1H), 4.55 (m, 1H), 4.44 (m. 1H), 4.21 (m, 1H), 4.04 (m, 1H), 3.80 (m, 2H), 3.67 (m, 1H), 3.56 (m, 1H), 2.52 (m, 1H), 1.95 (bs, 1H), 1.43 (m, 6H), 1.30 (m, 6H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 109.7, 109.0, 99.1, 98.5, 82.6, 81.0, 79.9, 78.4, 75.0, 74.3, 74.1, 73.7, 72.1, 71.5, 64.5, 64.3, 62.3, 62.1, 61.0, 60.8, 60.4, 28.3, 28.2, 26.19, 26.18, 25.7, 25.4, 20.8, 19.0, 13.9; HRMS-EI (m/z) calcd for $C_{14}H_{23}O_6$ $[M + H]^+$ 287.1495, found 287.1494.

(4*R*,5*S*)-4-((4*S*,5*S*)-5-(1-(*tert*-Butyldimethylsilyloxy)prop-2-ynyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyl-1,3-dioxan-5-ol 11 (Major Isomer). TBSCl (2.10 g, 13.97 mmol) was added to a solution of diol **10** (4.00 g, 13.97 mmol) and Et_3N (2.11 g, 20.92 mmol) in CH_2Cl_2 (50 mL), and the mixture was stirred for 3 h at rt. The reaction mixture was washed with water (2 × 50 mL) and brine and dried over $MgSO_4$. CH_2Cl_2 was removed, and the residue obtained was purified by flash column chromatography (hexane/EtOAc, 4:1) to give the mono OTBS compound **11** (4.46 g, 11.14 mmol): yield 78%; R_f (hexane/EtOAc, 4:1) 0.4; IR (film) 3415, 2930, 2122, 1475, 1265, 1095, 1035 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 4.71 (dd, J = 9.2, 1.9 Hz, 1H), 4.25 (dd, J = 9.2, 6.7 Hz, 1H), 3.86 (m, 3H), 3.59 (m, 1H), 2.66 (bs, 1H), 2.57 (d, J = 1.9 Hz, 1H), 1.52 (s, 3H), 1.46, (s, 3H), 1.38 (s, 6H), 0.88 (s, 9H), 0.22 (s, 3H), 0.19 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 110.0, 98.4, 83.5, 79.3, 75.0, 74.6, 72.4, 64.7, 62.5, 61.9, 28.5, 26.3, 26.1, 26.0, 25.6, 19.6, 18.0, −3.3, −4.4; HRMS-EI (m/z) calcd for $C_{20}H_{36}O_6Si$ $[M]^+$ 400.2281, found 400.2286.

***O*-(4*R*,5*S*)-4-((4*S*,5*S*)-5-(1-(*tert*-Butyldimethylsilyloxy)prop-2-ynyl)-2,2-dimethyl-1,3-dioxolan-4-yl)-2,2-dimethyl-1,3-dioxan-5-yl *O*-Phenyl Carbonothioate 12.** To a solution of alcohol **11** (5.00 g, 12.50 mmol) in CH_2Cl_2 (50 mL) were added pyridine (2.01 mL, 25.03 mmol) and phenyl chlorothionoformate (5.18 mL, 37.52 mmol). The mixture was stirred at room temperature for 3 h. The solution was washed with brine and dried over anhydrous $MgSO_4$. After concentration, the residue was purified by flash chromatography on silica gel (hexane/EtOAc, 4/1) to give thionocarbonate **12** (5.23 g, 9.35 mmol): yield 78%; major isomer: R_f (hexane/EtOAc, 19:1) 0.5; IR (film) 2930, 2119, 1781, 1591, 1490, 1248 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.41 (m, 2H), 7.29 (m, 1H), 7.09 (m, 2H), 5.51 (m, 1H), 4.75 (m, 1H), 4.29 (m, 3H), 4.19 (m, 1H), 3.93 (m, 1H), 2.61 (m, 1H), 1.55 (s, 3H), 1.52 (s, 3H), 1.42 (m, 6H), 0.92 (m, 9H), 0.25 (m, 3H), 0.23 (m, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 194.4, 153.5, 129.5, 126.6, 121.9, 110.4, 99.9, 83.3, 79.2, 77.2, 75.7, 75.2, 68.6, 62.0, 61.5, 52.8, 29.7, 27.8, 26.2, 25.7, 22.1, 18.1, −3.1, −4.2; HRMS-EI (m/z) calcd for $C_{27}H_{40}NaO_7SSi$ $[M + Na]^+$ 559.2162, found 559.2160.

***tert*-Butyldimethyl(((3a*S*,5a*S*,9a*R*,9b*S*)-2,2,8,8-tetramethyl-5-methylenehexahydro-3a*H*-[1,3]dioxolo[40,50:5,6]benzo[1,2-*d*][1,3]dioxin-4-yl)oxy)silane 7.** To a degassed solution of the thiocarbonate **12** (5.00 g, 9.33 mmol) in CPME (250 mL) at 90 °C under an argon atmosphere was added a solution of tri-*n*-butyltin hydride (3.76 mL, 13.99 mmol) and AIBN (153 mg, 0.93 mmol) in CPME (15 mL) over 3 h. After addition was finished, stirring was continued for 6 h, and then the reaction mixture was concentrated under reduced pressure. The resultant residue obtained was purified by flash chromatography (hexane/EtOAc, 19:1) to get separable *cis*-fused diastereomers **7**; major (2.25 g, 5.86 mmol): yield 63%, minor diastereomer (1.00 g, 2.60 mmol): yield 28%; Major. R_f (hexane/EtOAc, 15:1) 0.7; $[\alpha]_D^{25}$ +160.0° (c 1.3, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.46 (s, 1H), 5.10 (s, 1H), 5.05 (m, 1H), 4.41 (dd, J = 7.7, 2.8 Hz, 1H), 4.26 (dd, J = 7.4, 2.3 Hz, 1H), 4.13 (dd, J = 11.6, 3.6 Hz, 1H), 4.08 (t, J = 3.0 Hz, 1H), 3.9 (dd, J = 11.7, 2.3 Hz, 1H), 2.51 (m, 1H), 1.43 (s, 3H), 1.39 (s, 3H), 1.33 (s, 3H), 1.32 (s, 3H), 0.95 (s, 9H), 0.16 (s, 3H), 0.13 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 145.0, 110.7, 109.1, 98.6, 76.9, 75.8, 70.6, 67.7, 65.9, 35.0, 29.2, 26.2, 26.1, 24.0, 19.0, 18.7, −4.5, −5.2; HRMS-EI (m/z) calcd for $C_{20}H_{36}O_5Si$ $[M]^+$ 384.2332, found 384.2338.

Minor. R_f (hexane/EtOAc, 15:1) 0.8; $[\alpha]_D^{25}$ +68.0° (c 1.05, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 5.46 (m, 1H), 5.33 (m, 1H), 4.49 (m, 1H), 4.12 (m, 2H), 4.02 (m, 2H), 3.89 (dd, J = 7.48, 5.45 Hz, 1H), 2.18 (m, 1H), 1.51 (s, 3H), 1.46 (s, 3H), 1.36 (s, 3H), 1.33 (s, 3H), 0.94 (s, 9H), 0.12 (s, 3H), 0.05 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 143.1, 110.8, 108.0, 99.0, 82.3, 77.4, 76.0, 69.3, 60.8, 36.4, 29.5, 28.4, 26.2, 25.9, 18.7, 18.3, −4.5, −4.8; HRMS-EI (m/z) calcd for $C_{20}H_{36}O_5Si$ $[M]^+$ 384.2332, found 384.2330.

(3a*S*,4*R*,5*S*,7a*S*)-7-(*tert*-Butyldimethylsilyloxy)-5-(hydroxymethyl)-2,2-dimethyl-6-methylene-hexahydrobenzo[*d*][1,3]-dioxol-4-ol 13. A solution of diacetonide **7** (2.25 g, 5.86 mmol) in MeOH (20 mL) was treated with PPTS (219 mg, 0.48 mmol), and the solution was stirred for 48 h. The mixture was concentrated, diluted with EtOAc (30 mL), washed with a saturated aqueous solution of sodium bicarbonate and then with brine. EtOAc was removed, and the residue obtained was purified by flash chromatography (hexane/EtOAc, 3:2) to yield diol **13** (1.63 g, 4.72 mmol): yield 81%; Major. R_f (hexane/EtOAc, 3:2) 0.35; $[\alpha]_D^{25}$ +68.3° (c 1.1, $CHCl_3$); IR (film) 3400, 2929, 1733, 1599, 1383 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.55 (s, 1H), 5.19 (s, 1H), 4.89 (m, 1H), 4.40 (dd, J = 7.4, 2.5 Hz, 1H), 4.28 (dd, J = 7.1, 3.1 Hz, 1H), 4.21 (m, 1H), 4.15 (m, 1H), 3.95 (m, 1H), 3.47 (m, 1H), 2.82 (m, 1H), 2.13 (m, 1H), 1.60 (bs, 2H), 1.43 (s, 3H), 1.33 (s, 3H), 0.94 (s, 9H), 0.15 (s, 3H), 0.12 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 143.5, 111.4, 109.3, 76.6, 72.3, 70.4, 65.0, 40.8, 26.6, 26.0, 24.2, 18.5, −4.6, −4.9. HRMS-EI (m/z) calcd for $C_{17}H_{33}O_5Si$ $[M + H]^+$ 345.2097, found 345.2103.

Minor. R_f (hexane/EtOAc, 3:2) 0.35; $[\alpha]_D^{25}$ +50.0° (c 1.2, $CHCl_3$); IR (film) 3410, 2929, 2860, 1623, 1586, 1248 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.24 (s, 1H), 5.13 (s, 1H), 4.47 (d, J = 6.6 Hz, 1H), 4.23 (dd, J = 6.6, 3.3 Hz, 1H), 4.15 (m, 2H), 4.12 (d, J = 3.4 Hz, 1H), 3.94 (dd, J = 11.0, 9.1 Hz, 1H), 3.77 (dd, J = 11.0, 5.5 Hz, 1H), 2.84, (m, 1H), 2.70 (bs, 1H), 1.36 (s, 3H), 1.31 (s, 3H), 0.88 (s, 9H), 0.13 (s, 3H), 0.08 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 142.4, 116.7, 108.8, 78.5, 76.4, 75.2, 68.2, 63.8, 41.8, 26.7, 25.6, 24.6, 18.0, −4.8, −5.0; HRMS-EI (m/z) calcd for $C_{17}H_{33}O_5Si$ $[M + H]^+$ 345.2097, found 345.2103.

***tert*-Butyl((3a*S*,6*R*,7*R*,7a*S*)-6-(iodomethyl)-7-(methoxymethoxy)-2,2-dimethyl-5-methylene-hexahydrobenzo[*d*][1,3]-dioxol-4-yloxy)dimethylsilane 6.** To a solution of diol **13** (1.50 g, 4.35 mmol) and pyridine (0.71 mL, 8.70 mmol) in CH_2Cl_2 (20 mL) at 0 °C was added TsCl (0.91 g, 4.79 mmol). The reaction mixture was then stirred at rt for 10 h. It was washed with 1 M HCl and then with a saturated aqueous solution of sodium bicarbonate. CH_2Cl_2 was removed, and the residue obtained was dissolved in acetone (20 mL). NaI (1.17 g, 7.80 mmol) was added, and the solution was refluxed for 15 h. Acetone was removed and diluted with ether, washed with water, dried over $MgSO_4$, and concentrated to a give residue, which was used as it is for further steps.

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The crude residue was dissolved in EDC (20 mL), and diisopropylethylamine (1.53 mL, 8.80 mmol) and bromomethyl methyl ether (0.83 g, 6.60 mmol) were added under an argon atmosphere at 0 °C. After stirring at 70 °C for 5 h, this solution was separated with dichloromethane and 0.5 M aqueous hydrochloric acid solution. The organic layer was washed with brine and dried over $MgSO_4$. The solvent was evaporated, and the resulting residue was purified by flash column chromatography (hexane/EtOAc, 9:1) to obtain iodo compound **6** (1.65 g, 3.31 mmol): yield 76%; Major. R_f (hexane/EtOAc, 9:1) 0.80; $[\alpha]_D^{25}$ +65.6° (*c* 1.4, $CHCl_3$); IR (film) 2904, 1755, 1658, 1446, 1259, 787 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.46 (s, 1H), 5.11 (s, 1H), 4.80 (m, 3H), 4.52 (dd, *J* = 7.7, 3.5 Hz, 1H), 4.38 (dd, *J* = 7.3, 3.2 Hz, 1H), 4.02 (t, *J* = 3.4 Hz, 1H), 3.59 (dd, *J* = 9.7, 5.4 Hz, 1H), 3.51 (t, *J* = 10.2 Hz, 1H), 3.37 (s, 3H), 3.05 (m, 1H), 1.40 (s, 3H), 1.31 (s, 3H), 0.92 (s, 9H), 0.12 (s, 3H), 0.09 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 143.8, 112.7, 109.5, 98.4, 77.1, 75.5, 74.9, 69.8, 55.7, 42.2, 32.9, 26.3, 24.2, 18.6, 7.0, −4.7, −5.1; HRMS-EI (*m*/*z*) calcd for $C_{19}H_{36}IO_5Si$ $[M + H]^+$ 499.1377, found 499.1373.

Minor. R_f (hexane/EtOAc, 9:1) 0.80; $[\alpha]_D^{25}$ +61.7° (*c* 1.1, $CHCl_3$); IR (film) 2928, 1654, 1576, 1450, 1170, 1079 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.34 (s, 1H), 4.88 (s, 1H), 4.73 (m, 2H), 4.25 (m, 2H), 4.10 (d, *J* = 6.0 Hz, 1H), 3.95 (t, *J* = 6.0 Hz, 1H), 3.47 (m, 2H), 3.44 (s, 3H), 2.71 (m, 1H), 1.52 (s, 3H), 1.38 (s, 3H), 0.93 (s, 9H), 0.13 (s, 3H), 0.07 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 144.7, 110.4, 109.1, 97.3, 82.7, 76.8, 75.8, 75.7, 56.0, 45.1, 28.4, 26.4, 25.9, 18.2, 4.1, −4.6, −4.8; HRMS-EI (*m*/*z*) calcd for $C_{19}H_{36}IO_5Si$ $[M + H]^+$ 499.1377, found 499.1373.

(1*E*,5*E*)-1-Iodo-2,6,10-trimethylundeca-1,5,9-triene 14. To a solution of zirconocene dichloride (6.63 g, 22.69 mmol) in CH_2Cl_2 (20 mL) at rt under an argon atmosphere was added dropwise a solution of trimethylaluminum in heptane (2 M in heptane, 12.48 mL, 24.96 mmol). After 15 min, the solution was cooled to 0 °C, and a solution of (*E*)-6,10-dimethylundeca-5,9-dien-1-yne (2.00 g, 11.34 mmol) dissolved in CH_2Cl_2 (10 mL) was added to the above solution. The reaction mixture was stirred at 0 °C for 6 h and then cooled to −30 °C. Iodine (1.80 g, 14.18 mmol) in THF (15 mL) was added. The resulting brown slurry was raised to 0 °C and poured slowly with stirring into an ice cold saturated aqueous $NaHCO_3$. The aqueous layer was extracted with ether (3 × 100 mL). The combined organic layer was washed with saturated aqueous $NaHCO_3$ and dried over $MgSO_4$. Concentration, followed by flash chromatography on silica gel with 2:1 hexane/ether as eluent, provided the desired product **14** as a colorless oil (2.89 g, 9.08 mmol): yield 80%; R_f (hexane/EtOAc, 9:1) 0.7; ^1H NMR (400 MHz, $CDCl_3$) δ 5.87 (d, *J* = 0.7 Hz, 1H), 5.09 (t, *J* = 5.8 Hz, 2H), 2.22 (m, 2H), 1.97−2.13 (m, 6H), 1.84 (s, 3H), 1.69 (s, 3H), 1.60 (s, 3H), 1.59 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 147.9, 136.1, 131.4, 124.2, 123.0, 74.7, 39.7, 39.5, 26.7, 26.3, 25.7, 24.0, 17.7, 16.0.

***tert*-Butyl((3a*S*,6*R*,7*R*,7a*S*)-7-(methoxymethoxy)-2,2-dimethyl-5-methylene-6-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienyl)-hexahydrobenzo[*d*][1,3]dioxol-4-yloxy)dimethylsilane 15.** To a stirring solution of alkyl iodide **6** (0.60 g, 1.20 mmol) in Et_2O (10 mL) was added 9-MeO-9-BBN (4.81 mL, 1.0 M solution in hexane), and the mixture was cooled to −78 °C. To this solution was rapidly added *tert*-butyllithium (2.48 mL, 1.7 M solution in pentane). The mixture was stirred for 5 min; then, THF (5.0 mL) was added and the reaction mixture was warmed to 25 °C for 1 h. In a separate flask, to the solution of vinyl iodide **14** (0.38 g, 1.20 mmol) in DMF (5 mL), $Pd(dppf)Cl_2$ (44 mg, 0.6 mmol), $AsPh_3$ (55 mg, 0.18 mmol), $CsCO_3$ (1.56 g, 4.81 mmol) and water (0.52 mL, 28.8 mmol) were added. The ethereal mixture of the alkylboronate was cannulated into the DMF solution and stirred overnight. The reaction mixture was then diluted with water and extracted with Et_2O. The organic extracts were washed with brine and worked up as usual, and the residue was purified by column chromatography on silica gel (hexane/EtOAc, 19:1) to give coupled product **15** (1.01 g, 1.80 mmol): yield 90%; Major. R_f (hexane/EtOAc, 15:1) 0.60; $[\alpha]_D^{25}$ +25.8° (*c* 1.2, $CHCl_3$); IR (film) 2929, 1645, 1464, 1090, 1035 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.35 (s, 1H), 5.10 (m, 4H), 4.68 (m, 2H), 4.62 (d, *J* = 2.8 Hz, 1H), 4.37 (m, 2H), 3.71 (m, 1H), 3.37 (s, 3H), 2.72 (m, 1H), 2.32 (m, 1H), 2.19 (m, 1H), 1.95−2.05 (m, 8H), 1.68 (s, 3H), 1.60 (s, 6H), 1.57 (s, 3H), 1.46 (s, 3H), 1.33 (s, 3H), 0.94 (s, 9H), 0.14 (s, 3H), 0.11 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 146.0, 136.4, 135.1, 131.3, 124.4, 124.1, 122.3, 110.9, 109.4, 97.2, 81.3, 77.6, 77.5, 76.1, 69.8, 55.6, 41.5, 39.8, 39.7, 29.7, 27.6, 27.0, 26.7, 26.65, 26.0, 25.7, 24.9, 18.6, 17.7, 16.3, 16.0, −4.6, −5.0; HRMS-EI (*m*/*z*) calcd for $C_{33}H_{58}O_5Si$ $[M]^+$ 562.4054, found 562.4056.

Minor. R_f (hexane/EtOAc, 15:1) 0.30; $[\alpha]_D^{25}$ +25.1° (*c* 0.7, $CHCl_3$); IR (film) 2930, 1640, 1610, 1584, 1471, 1154, 837 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.32 (s, 1H), 5.18 (m, 1H), 5.10 (m, 2H), 4.89 (s, 1H), 4.69 (d, *J* = 6.7 Hz, 1H), 4.61 (d, *J* = 6.7 Hz, 1H), 4.25 (dd, *J* = 5.3, 2.8 Hz, 1H), 4.10 (dt, *J* = 6.9, 1.9 Hz, 1H), 3.99 (t, *J* = 2.6 Hz, 1H), 3.94 (dd, *J* = 6.5, 5.4 Hz, 1H), 3.36 (s, 3H), 2.39 (m, 2H), 2.28 (m, 1H), 1.97−2.09 (m, 8H), 1.68 (s, 3H), 1.64 (s, 3H), 1.60 (s, 6H), 1.53 (s, 3H), 1.38 (s, 3H), 0.93 (s, 9H), 0.13 (s, 3H), 0.06 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 146.3, 136.4, 135.1, 131.2, 124.4, 124.1, 122.4, 112.2 108.9, 97.2, 83.2, 76.9, 76.4, 76.38, 55.8, 41.5, 39.8, 39.7, 28.5, 26.7, 26.6, 26.5, 25.9, 25.8, 25.7, 18.3, 17.7, 16.3, 16.0, −4.5, −4.8; HRMS-EI (*m*/*z*) calcd for $C_{33}H_{58}O_5Si$ $[M]^+$ 562.4054, found 562.4057.

(3a*S*,6*R*,7*R*,7a*S*)-7-(Methoxymethoxy)-2,2-dimethyl-5-methylene-6-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienyl)-tetrahydrobenzo[*d*][1,3]dioxol-4(3a*H*)-one 5. A solution of **15** (0.60 g, 1.07 mmol) in THF (10 mL) was treated with TBAF (2.13 mL, 1 M in THF, 2.14 mmol) at rt. The reaction mixture was stirred for 3 h at rt, and then THF was removed and the residue obtained was diluted with ether (20 mL), washed successively with water and brine, dried over anhydrous $MgSO_4$, filtered, and concentrated to afford the crude product, which was subjected to oxidation.

To a solution of the above crude alcohol in CH_2Cl_2 (10 mL) was added Dess−Martin periodinane (0.67 g, 1.60 mmol), and stirring continued at rt for 2 h. The reaction mixture was then diluted with CH_2Cl_2, quenched with a saturated aqueous solution of sodium bicarbonate, and washed with brine. The usual workup and flash column chromatography (hexane/EtOAc, 9:1) afforded keto compound **5** (0.41 g, 0.92 mmol, over two steps): yield 87%; R_f (hexane/EtOAc, 4:1) 0.40; $[\alpha]_D^{25}$ −4.8° (*c* 1.0, $CHCl_3$); IR (film) 2928, 1736, 1455, 1375, 1259, 1095, 919 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 6.24 (s, 1H), 5.45 (s, 1H), 5.16 (m, 1H), 5.09 (m, 2H), 4.69 (d, *J* = 6.7 Hz, 1H), 4.67 (dd, *J* = 7.7, 4.2 Hz, 1H), 4.63 (d, *J* = 6.7 Hz, 1H), 4.46 (d, *J* = 7.7 Hz, 1H), 3.86 (dd, *J* = 3.7, 2.0 Hz, 1H), 3.34 (s, 3H), 2.99 (m, 1H), 2.35 (m, 2H), 1.97−2.08 (m, 8H), 1.68 (s, 3H), 1.61 (s, 3H), 1.60 (s, 6H), 1.49 (s, 3H), 1.38 (s, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 195.4, 143.6, 137.7, 135.2, 131.3, 124.3, 123.9, 123.6, 121.4, 111.0, 97.0, 77.0, 76.7, 76.3, 55.8, 40.0, 39.7, 29.7, 27.1, 26.7, 26.5, 25.7, 17.7, 16.3, 16.0; HRMS-EI (*m*/*z*) calcd for $C_{27}H_{42}O_5$ $[M]^+$ 446.3032, found 446.3038.

(2*S*,3*S*,4*R*,5*R*,6*R*)-2,3-Dimethoxy-4-(methoxymethoxy)-6-methyl-5-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienyl)cyclohexanone 16. Under vigorous stirring, 0.5 mL of a 50% suspension of Raney Ni in water was added to a cold (0 °C) solution of **5** (0.30 g, 0.67 mmol) in THF (10 mL). The mixture was stirred for 30 min at 0 °C. Ether (20 mL) was added, and the resultant mixture was washed with brine, dried over $MgSO_4$, and concentrated to give a product which was used as it is for further steps.

To a stirred solution of the above crude product and $CeCl_3{\cdot}7H_2O$ (0.75 g, 2.02 mmol) in acetonitrile (5 mL) was added oxalic acid (4.0 mg, 0.05 mmol) at ambient temperature. Stirring was continued for 3 h at rt, and then the mixture was cooled to 0 °C. Solid sodium bicarbonate was added to neutralize the pH of the reaction mixture, which was then concentrated in vacuo. The residue was treated with EtOAc (10 mL) and filtered through Celite. The filtrate was then concentrated to get a diol compound.

The diol compound was dissolved in iodomethane (5 mL). Ag_2O (0.47 g, 2.02 mmol) was added, and the mixture was stirred for 12 h at reflux. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and filtered through Celite. The filtrate was then concentrated, and the resulting product was subjected to flash chromatography (hexane/EtOAc, 4:1) to afford keto compound **16** (196 mg, 0.45 mmol): yield

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67%; R_f (hexane/EtOAc, 7:3) 0.50; $[\alpha]_D^{25}$ −67.7° (*c* 1.3, $CHCl_3$); IR (film) 2927, 1726, 1655, 1457, 1119, 1031, 919 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.09 (m, 3H), 4.74 (d, *J* = 6.8 Hz, 1H), 4.69 (d, *J* = 6.8 Hz, 1H), 4.25 (d, *J* = 2.8 Hz, 1H), 4.10 (t, *J* = 3.7 Hz, 1H), 3.87 (t, *J* = 3.3 Hz, 1H), 3.48 (s, 3H), 3.44 (s, 3H), 3.41 (s, 3H), 2.46 (m, 1H), 2.19 (m, 2H), 1.96−2.09 (m, 8 H), 1.91 (m, 1H), 1.36 (s, 3H), 1.33 (s, 3H), 1.32 (s, 6H), 1.07 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 207.7, 136.8, 135.2, 131.3, 124.3, 124.0, 122.1, 98.3, 84.0, 83.0, 76.3, 59.0, 58.5, 56.1, 44.5, 44.0, 39.8, 39.7, 27.3, 26.7, 26.6, 25.7, 17.7, 16.2, 16.0, 11.1; HRMS-EI (*m*/*z*) calcd for $C_{26}H_{44}O_5$ $[M]^+$ 436.3189, found 436.3181.

(4*R*,5*R*,6*R*)-2,3-Dimethoxy-4-(methoxymethoxy)-6-methyl-5-((2*E*,6*E*)-3,7,11-trimethyldodeca-2,6,10-trienyl)cyclohex-2-enone 17. To a cold (−78 °C) solution of ketone **16** (0.19 g, 0.43 mmol) in THF (5 mL) was added dropwise LHMDS (1 M solution in THF, 0.43 mL). After 20 min of stirring at this temperature, a solution of phenylselenenyl bromide (0.10 g, 0.43 mmol) in THF (1 mL) was added. The mixture was stirred for an additional 40 min at −78 °C and then quenched with a saturated ammonium chloride solution. The aqueous layer was extracted with Et_2O. The combined organic phases were washed with brine and dried over $MgSO_4$, and the solvents were evaporated. The crude product was used as it is for the next step.

The above crude product was dissolved in THF (10 mL). This was treated with $NaHCO_3$ (3 mL) and slow addition of 1 mL of hydrogen peroxide (30 wt % solution in water). The reaction mixture was allowed to warm and then stirred for 2 h at rt. The reaction was quenched with a $Na_2S_2O_3$ solution, and the aqueous layer was extracted with Et_2O. The combined organic phases were washed with brine and dried over $MgSO_4$, and the solvents were evaporated. Purification by flash chromatography on silica gel (hexane/EtOAc, 19:1) afforded unsaturated ketone **17** (0.11 g, 0.25 mmol): yield 55%; R_f (hexane/EtOAc, 4:1) 0.60; $[\alpha]_D^{25}$ +108.3° (*c* 1.2, $CHCl_3$); IR (film) 2929, 1671, 1629, 1458, 1250, 1027, 922 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.16 (m, 1H), 5.10 (m, 2H), 4.76 (d, *J* = 6.6 Hz, 1H), 4.66 (d, *J* = 6.6 Hz, 1H), 4.11 (d, *J* = 3.0 Hz, 1H), 4.07 (s, 3H), 3.65 (s, 3H), 3.35 (s, 3H), 2.55 (m, 1H), 2.18−2.22 (m, 2H), 1.97−2.10 (m, 8H), 1.75 (m, 1H), 1.61 (s, 3H), 1.59 (s, 6H), 1.18 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 197.7, 161.5, 137.4, 136.0, 135.2, 131.3, 124.3, 124.0, 121.4, 96.5, 74.2, 60.7, 59.9, 55.9, 44.9, 41.2, 39.8, 39.7, 27.0, 26.7, 26.5, 25.7, 17.7, 16.2, 16.0, 13.1; HRMS-EI (*m*/*z*) calcd for $C_{26}H_{42}O_5$ $[M]^+$ 434.3032, found 434.3034.

Antroquinonol (1). To a stirred solution of MOM ether **17** (0.10 g, 0.23 mmol) in dry CH_2Cl_2 (5 mL) were added dry $ZnBr_2$ (57 mg, 0.25 mmol) and EtSH (36 μL, 0.50 mmol). After stirring for 1 h at room temperature, the resulting mixture was diluted with CH_2Cl_2 (10 mL). Sat. $NaHCO_3$ (5 mL) was added slowly at 0 °C, and the mixture was filtered through Celite. The aqueous layer was separated and further extracted with CH_2Cl_2 (10 mL). The combined organic layer was washed with brine (3 mL), dried over $MgSO_4$, and concentrated under reduced pressure. The crude product obtained was purified by flash column chromatography on silica gel (hexane/EtOAc, 4/1) to afford antroquinonol **1** (72 mg, 0.18 mmol): yield 80%; R_f (hexane/EtOAc, 7:3) 0.45; $[\alpha]_D^{25}$ +44.6° (*c* 1.2, $CHCl_3$); IR (film) 3435, 2969, 1662, 1621, 1451, 1358, 1240, 1016, 944 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 5.17 (m, 1H), 5.10 (m, 2H), 4.35 (d, *J* = 3.3 Hz, 1H), 4.06 (s, 3H), 3.67 (s, 3H), 2.52 (m, 1H), 2.24 (t, *J* = 7.6 Hz, 2H), 1.95−2.11 (m, 9H), 1.75 (m, 1H), 1.67 (s, 3H), 1.66 (s, 3H), 1.60 (s, 6H), 1.17 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 197.1, 160.4, 138.1, 136.0, 135.4, 131.3, 124.3, 123.9, 121.0, 68.0, 60.6, 59.2, 43.4, 40.3, 39.8, 39.7, 27.0, 26.8, 26.5, 25.7, 17.7, 16.1, 16.0, 12.3; HRMS-EI (*m*/*z*) calcd for $C_{24}H_{38}O_4$ $[M]^+$ 390.2770, found 390.2776.

Glucose Uptake Assay. The glucose uptake assay was performed as explained in a previous study, with a minor modification and generous assistance of Professor Hitoshi Ashida (Kobe University, Kobe, Japan). Insulin was administered as a clinical drug to control the glucose uptake. The differentiated L6 myotubes were seeded in a 96-well microplate and treated with 100 μL/well of α-MEM with 0.25% BSA adding insulin and antroquinonol for 30 min. After treatment, the cells were washed twice with KRH. Next, the L6 myotubes were incubated with a KRH buffer containing 1 mM 2-deoxyglucose (2DG, Sigma-Aldrich, St. Louis, MO USA) and 60 μL of 0.1% BSA in 5% CO_2 at 37 °C for 20 min. After incubation, the cells were washed twice with the KRH buffer again, and 50 μL of 0.1 N NaOH was then added. The microplate was dried at 85 °C for 90 min. Then, the reactants were neutralized by adding 50 μL of 0.1 N HCl, and 50 μL of 50 mM triethanolamine hydrochloride (TEA) buffer (200 mM KCl, 200 mM TEA pH 8.1) was then added. The uptake of 2-deoxy-D-glucose (2DG) into the cells was measured using the enzymatic fluorescence assay. The fluorescence assay buffer was composed of 50 mM TEA buffer, 0.1% BSA, 2.5 mM β-NADP (Wako Pure Chemical, Osaka, Japan), 0.05 units of diaphorase (Wako), and 150 units of *L. Mesenteroides* G6PDH (Sigma), and 0.5 mM resazurin sodium salt (Sigma). Next, 10 μL of a 2DG sample with 100 μL of the fluorescence assay buffer was reacted at 37 °C for 30 min. At the end of incubation, fluorescence was measured at 570 nm emission with an 540 nm excitation using a spectrophotometer (EnSpire 2300 Multilabel Reader, PerkinElmer, Waltham, MA, USA).

Oral Glucose Tolerance Test (OGTT). All protocols of animal experiments were according to the "Guide for the Care and Use of Laboratory Animals" approved by the National Dong Hwa University Animal Ethics Committee. Eight week old male C57 BL/6 mice were purchased from National Laboratory Animal Center (Taipei, Taiwan) and kept at 22 ± 2 °C and humidity (50 ± 10%). The 12 h light (0600 am to 1800 pm) and 12 h dark cycle was maintained throughout the entire study. Mice had free access to food and water and were maintained on a standard laboratory diet (carbohydrates; 60%, proteins; 28%, lipids; 12%, vitamins; 3%). Mice were employed to this test after fasting for 12 h. Mice were induced with insulin receptor antagonist-S961 (40 nmol/kg Bwt, i.p.) to mimic hyperglycemia at 30 min prior to oral glucose tolerance test (OGTT). Next, mice were given antroquinonol (25 mg/kg Bwt) or Metformin (100 mg/kg Bwt) 15 min prior to oral gavaged (p.o.) with D-glucose (2 g/kg Bwt). At approximately 0, 30, 60, 90, and 120 min, blood was sampled by venipuncture from the tail vein for determining blood glucose. Blood glucose was immediately determined by the glucose oxidase method using a glucose analyzer (Accu-Chek, Roche). To investigate the whole blood glucose change, the area under the curve (AUC) was calculated and shown as a bar chart. Data were expressed as means ± SEM. Statistical comparisons of the data were determined by one-way analysis of variance (ANOVA). This means within each column followed by the different letters are significantly different at $p < 0.05$ with the Tukey's test.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR spectra of all new compounds. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.5b00345.

■ AUTHOR INFORMATION

Corresponding Authors

*Phone: +886-3-863-3597. Fax: +886-3-863-0475. E-mail: chinpiao@mail.ndhu.edu.tw (C.C.).

*Phone: +886-3-863-3637. Fax: +886-3-863-3630. E-mail: cfweng@mail.ndhu.edu.tw (C.-F.W.).

Author Contributions

§The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. R.S.S. and C.-Y.H. contributed equally.

Notes

The authors declare no competing financial interest.

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