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Synthesis of the Reported Pyranonaphthoquinone Structure of the Indoleamine-2,3-dioxygenase Inhibitor Annulin B by Regioselective Diels–Alder Reaction

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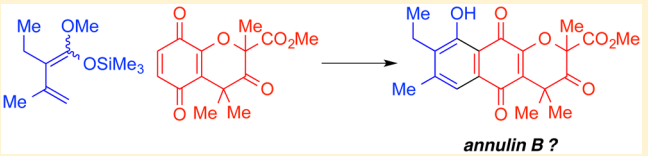
Supporting Information

ABSTRACT: Annulin B, isolated from the marine hydroid isolated from *Garveia annulata*, is a potent inhibitor of the tryptophan catabolizing enzyme indoleamine-2,3-dioxygenase (IDO). A synthesis of the reported pyranonaphthoquinone structure is described, in which the key step is a regioselective Diels–Alder reaction between a pyranobenzoquinone dienophile and a silyl ketene acetal diene.



INTRODUCTION

The metabolism of the essential amino acid tryptophan plays an important role in the regulation of the immune system. One of the key catabolic routes, the kynurenine pathway,[1] begins with the oxidative cleavage of the indole C2–C3 double bond, catalyzed by the heme-containing enzyme indoleamine 2,3-dioxygenase (IDO). The products of this pathway have been shown to exhibit powerful immunosuppressive effects, while depletion of tryptophan inhibits the proliferation of T-cells required for an effective immune response.[2−6] Evidence thus supports the possibility that inhibition of the kynurenine pathway, and of IDO in particular, may be a viable mode of action for cancer chemotherapy.[7,8] As the structure of IDO is known,[9] and its activity has been confirmed in many human tumor types,[10] it represents an attractive target for small molecule drug development.[11−15] Indeed the first compounds in this class are now entering the clinic.[16,17]

Several natural and synthetic IDO inhibitors have been reported (Figure 1), largely divisible into two categories: first, indole derivatives, such as the widely studied but poorly active 1-methyltryptophan **1** (K_i = 62 μM),[18] and brassinin **2** (K_i = 98 μM);[19] second, quinones, such as the annulins **3**–**5** (A, K_i = 0.21 μM; B, K_i = 0.12 μM; C, K_i = 0.14 μM),[20,21] and the heterocyclic quinones **6** (IC_{50} = 71 nM)[22] and **7** (IC_{50} = 0.18 μM),[23] recently reported from our laboratory. Some IDO inhibitors, such as exiguamine A **8** (K_i = 0.21 μM), fit into both categories.[24]

As one of the most active naturally occurring IDO inhibitors reported, annulin B **4**, isolated from *Garveia annulata*,[20] attracted our attention, and we now report a concise and flexible route to the pyranonaphthoquinone structure reported for the natural product based on a quinone Diels–Alder reaction.

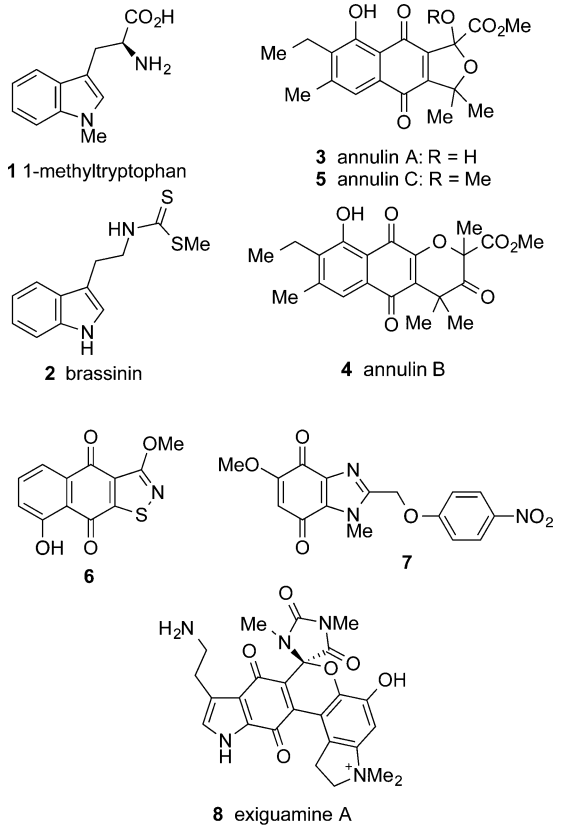


Figure 1. Selected inhibitors of indoleamine-2,3-dioxygenase (IDO).

RESULTS AND DISCUSSION

The Diels–Alder reaction of quinones has a distinguished history in total synthesis,[25] and its regiochemistry is known to be determined by the localization of charge in both the quinone and the diene, or strictly the orbital coefficients in the $LUMO_{quinone}$ and $HOMO_{diene}$. Thus, the atom bearing the greatest partial positive charge of the quinone forms a bond with the end of the

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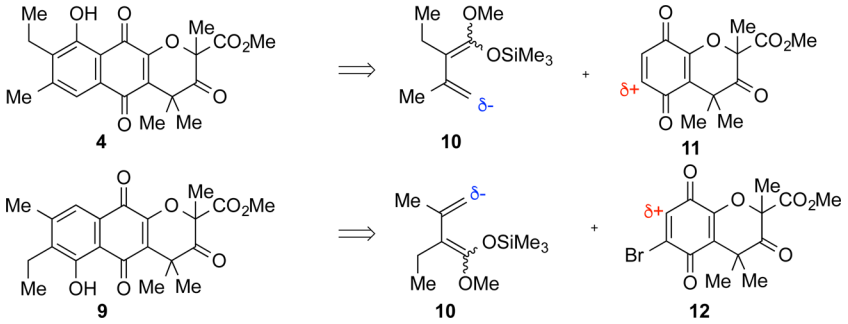
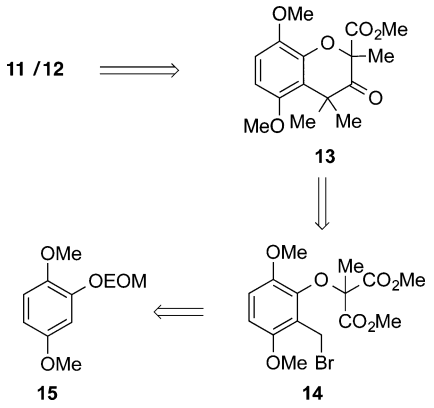
Scheme 1. Retrosynthesis of Annulin B 4 and Its Regioisomer 9



diene bearing the greatest negative charge. Therefore, we envisaged that both annulin B and its regioisomer **9** could be synthesized via Diels–Alder reactions of diene **10** with the quinones **11** and **12**, respectively (Scheme 1). The electron-donating properties of the oxygen atom in the pyran **11** result in the greatest positive charge being located at C-6, and hence Diels–Alder reaction should give annulin B **4** selectively. According to the rules described by Brassard et al.[26] the presence of a halogen at C-6 in the quinone **12** would localize the positive charge onto C-5 and thus result in the formation of the isomeric compound **9**.

The synthesis of quinones **11** and **12** ought to proceed along a single route, with a late-stage bromination providing divergent syntheses of both compounds; we hypothesized that an intramolecular Grignard reaction of benzylic bromide **14** would provide rapid access to these quinones (Scheme 2). Retrosynthesis of the diene **10** led via silylation and alkylation to methyl senecioate **16** as a convenient starting material.

Scheme 2. Retrosynthesis of Quinone Dienophiles 11 and 12



In order to access quinones **11** and **12**, a single synthetic route was developed starting from the commercially available 2,5-dimethoxyphenol **17**, which was EOM-protected in quantitative yield (Scheme 3). Directed lithiation and methylation, followed by deprotection and alkylation of the liberated phenol with methyl 2-bromopropionate, gave the intermediate **18** smoothly and on a ca. 5 g scale. The ester **18** was then converted into the diester **19** by reaction with LDA and methyl chloroformate. Bromination under radical conditions afforded the desired benzylic bromide **14** in good yield.

Various conditions were employed in an attempt to convert the bromide to either the corresponding Grignard, organozinc or organolithium reagent, and thus to effect ring closure, but these did not meet with any success. To circumvent this problem, the bromide was subjected to substitution with sodium benzenesulfinate to give the sulfone **21**, which underwent cyclization to the ketoester **22** in excellent yield upon deprotonation with LDA. Subsequent desulfonylation with zinc powder proceeded well, giving the desired ketoester **20** in 76% over three steps. Dimethylation of the resulting ketoester proved troublesome, with decarboxylation occurring when even relatively non-nucleophilic bases, such as sodium hydride or potassium *tert*-butoxide, were used. Eventually it was found that the use of excess iodomethane and DBU in DMF gave an excellent yield of the quinone precursor **13** (Scheme 4). Bromination of this compound proceeded as expected on the 6-position, as evinced by NOE experiments. The regiochemistry was confirmed by X-ray crystallography of the bromoquinone **12**, derived by oxidation of bromoarene **23** with cerium(IV) ammonium nitrate (Figure 2). Meanwhile, oxidation of **13** with silver(II) oxide gave the quinone **11**.

With both quinones in hand, our attention turned to the diene **10** (Scheme 5). Alkylation of methyl senecioate **16** with iodoethane gave the known β,γ-unsaturated ester **24**.[27] As direct silylation of this compound proved problematic, it was instead treated with potassium *tert*-butoxide to move the double bond back into conjugation with the ester. The resulting ester **25** proved more amenable to silylation, giving the diene **10** in good yield as a mixture of *E* and *Z* isomers. Notably, all attempts to synthesize the unsaturated ester **25** directly by Horner–Wadsworth–Emmons, Wittig, or aldol condensation chemistry were unsuccessful.

The diene **10** was used immediately after preparation, as it proved unstable to prolonged storage. Diels–Alder reactions were attempted under various conditions with both quinones. Gratifyingly, reaction of quinone **11** with diene **10** in toluene, followed by an oxidative workup with iron(III) chloride, resulted in the isolation of a single pyranonaphthoquinone **4**, after aromatization of the initial Diels–Alder adduct, albeit in modest yield (Scheme 6). Likewise, reaction of quinone **12** with diene **10** in the presence of triethylamine gave only one product, although again in moderate yield. In both cases, no trace of the alternative product could be detected by thin layer chromatography or NMR, with the balance of the material consisting of degradation products.

The spectroscopic data for compound **4** were entirely consistent with the structure; furthermore, X-ray crystallography (Figure 3) confirmed that the synthetic material **4** matched the proposed structure for annulin B, although the natural product was reported as an oil.[20] However, there were some discrepancies with the spectroscopic data reported for the natural compound, particularly in the ^{13}C NMR spectra. Thus, the peaks for carbons 1, 4, and 4′ were found at 152.6, 126.9, and 183.1, respectively,

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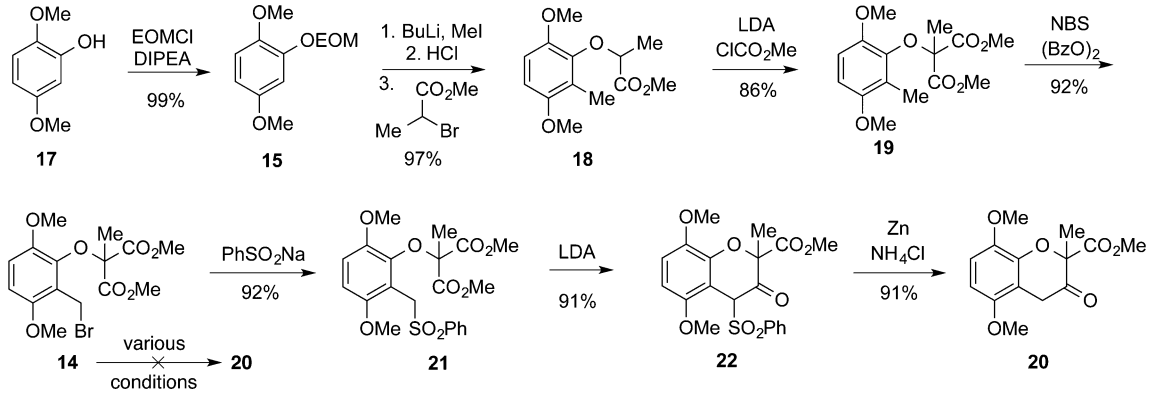
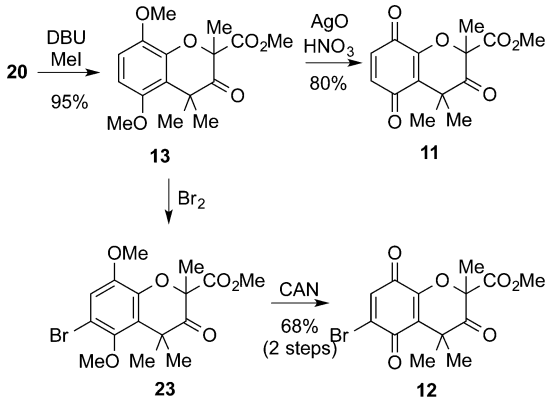
Scheme 3. Synthesis of Intermediate Chromanone 20



Scheme 4. Synthesis of Quinone Dienophiles 11 and 12



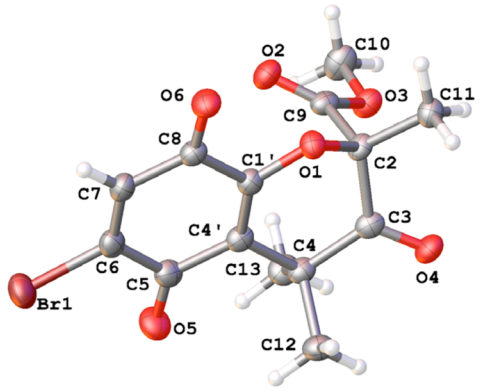
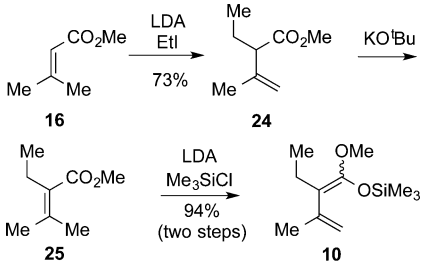


Figure 2. X-ray crystal structure of methyl 6-bromo-2,4,4-trimethyl-3,5,8-trioxo-3,4,5,8-tetrahydro-2*H*-chromene-2-carboxylate **12**. Ellipsoid contour percent probability level is 50%.

Scheme 5. Synthesis of Diene 10



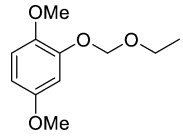
compared to 163.8, 119.1, and 178.6 in the natural material (see Supporting Information for a full comparison). Variation of the concentration and pH of the NMR sample did not result in any significant change in the NMR spectra. These discrepancies throw into doubt the structure of annulin B; the regioisomer **9** also does not match the reported data, excluding one obvious possible alternative structure.

In summary, a short and convergent synthetic route to the proposed structure of annulin B **4** is reported, in 19% yield over 12 steps. The regioselectivity of the key Diels–Alder reaction can be completely controlled, allowing for a flexible synthesis of analogues.

■ EXPERIMENTAL SECTION

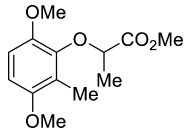
For general details of experimental procedures, see Supporting Information.

2-(Ethoxymethoxy)-1,4-dimethoxybenzene 15. Ethyl chloromethyl ether (8.35 mL, 90 mmol) was added to a solution of 2,5-



dimethoxyphenol (9.42 g, 60 mmol) and *N*,*N*-di*iso*propylethylamine (15.24 mL, 90 mmol) in chloroform (250 mL) at room temperature. The resulting solution was stirred at 60 °C for 20 h, cooled to room temperature and concentrated. Column chromatography eluting with ethyl acetate and light petroleum (1:19) gave the title compound as a colorless oil (12.64 g, 99%); (Found: M+Na$^+$, 235.0941. $C_{11}H_{16}O_4Na^+$ requires: 235.0941); ν_{max} ($CHCl_3$)/cm^{-1} 3008, 2980, 1598, 1511, 1393; δ_H (400 MHz; $CDCl_3$) 6.86 (1H, d, *J* 3.0), 6.84 (1H, d, *J* 8.8), 6.51 (1H, dd, *J* 8.8, 3.0), 5.29 (2H, s), 3.86 (3H, s), 3.80 (2H, q, *J* 7.1), 3.79 (3H, s), 1.25 (3H, t, *J* 7.1); δ_C (75 MHz; $CDCl_3$) 154.1, 147.5, 144.1, 112.5 (CH), 105.5 (CH), 104.5 (CH), 94.2 (CH_2), 64.3 (CH_2), 56.6 (Me), 55.7 (Me), 15.1 (Me); *m*/*z* (ESI) 235 (M+Na$^+$, 100%).

Methyl 2-(3,6-Dimethoxy-2-methylphenoxy)propanoate 18. *n*-Butyllithium (2.25 M in hexanes; 10.67 mL, 24 mmol) was added



dropwise to a solution of compound **15** (4.24 g, 20 mmol) and *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (5.99 mL, 40 mmol) in THF (100 mL) at −15 °C. The solution was stirred at −15 °C for 1.5 h, then iodomethane (2.74 mL, 44 mmol) was added dropwise. The resulting white suspension was stirred at room temperature for 2 h, diluted with water (100 mL), and extracted with ether (3 × 60 mL). The combined

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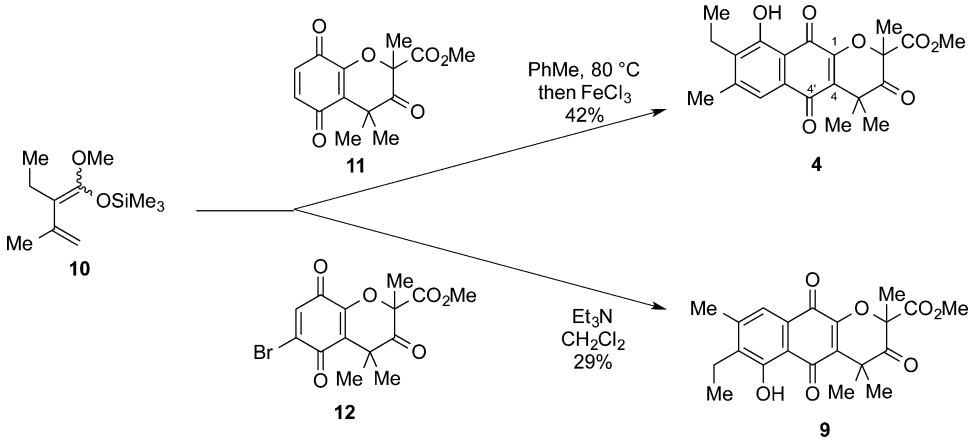
Scheme 6. Diels-Alder Reaction To Give Pyranonaphthoquinones 4 and 9



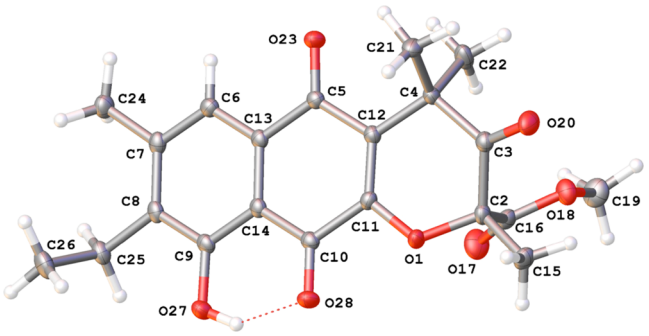


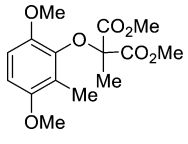
Figure 3. X-ray crystal structure of methyl 8-ethyl-9-hydroxy-2,4,4,7-tetramethyl-3,5,10-trioxo-3,4,5,10-tetrahydro-2*H*-benzo[*g*]chromene-2-carboxylate **4**, the reported structure of annulin B. Ellipsoid contour percent probability level is 50%.

organic phases were washed with brine (60 mL), dried ($MgSO_4$), filtered, and concentrated to give the methylated intermediate as a colorless oil, used without further purification.

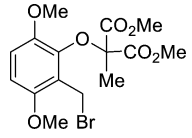
The residue was dissolved in THF (40 mL) and methanol (40 mL), and hydrochloric acid (1 M; 40 mL, 40 mmol) was added. The resulting mixture was stirred at room temperature for 17 h, diluted with water (300 mL), and extracted with ether (3 × 100 mL). The combined organic phases were washed with brine (100 mL), dried ($MgSO_4$), filtered, and concentrated to give the phenol as a colorless oily solid, used directly in the next step.

The residue was dissolved in acetonitrile (150 mL); potassium carbonate (5.52 g, 40 mmol) and methyl 2-bromopropionate (4.46 mL, 40 mmol) were added, and the resulting mixture was stirred at reflux for 18 h, cooled to room temperature, diluted with dichloromethane (150 mL), filtered, and concentrated. Column chromatography eluting with ether and light petroleum (1:7) gave the title compound as a colorless oil (4.921 g, 97%); (Found: M+Na^+, 277.1059. $C_{13}H_{18}O_5Na^+$ requires: 277.1046); ν_{max} ($CHCl_3$)/cm^{-1} 3007, 2955, 1751, 1600, 1488, 1257; δ_H (400 MHz; $CDCl_3$) 6.70 (1H, d, *J* 8.9), 6.55 (1H, d, *J* 8.9), 4.78 (1H, q, *J* 6.8), 3.80 (6H, s), 3.78 (3H, s), 2.21 (3H, s), 1.56 (3H, d, *J* 6.8); δ_C (75 MHz; $CDCl_3$) 173.0, 152.5, 146.3, 145.8, 121.2, 109.4 (CH), 105.1 (CH), 76.9 (CH), 56.3 (Me), 55.9 (Me), 52.0 (Me), 18.8 (Me), 9.6 (Me); *m*/*z* (ESI) 277 (M+Na^+, 100%).

Dimethyl 2-(3,6-Dimethoxy-2-methylphenoxy)-2-methylmalonate 19. *n*-Butyllithium (2.25 M in hexanes; 10.16 mL, 22.8 mmol) was added dropwise to a solution of di*iso*propylamine (4.00 mL, 28.6 mmol) in THF (55 mL) at −78 °C. The resulting mixture was stirred at 0 °C for 20 min then cooled to −78 °C; a solution of the ester **18** (4.84 g, 19.1 mmol) in THF (55 mL) was added over 10 min, and the resulting mixture was stirred at −78 °C for 45 min. Methyl chloroformate (2.95 mL, 38.1 mmol) was added, and the mixture was stirred at −78 °C for a further 2h, then quenched with saturated aqueous ammonium chloride (50 mL), followed by water (200 mL). The mixture was extracted with ether (3 × 80 mL) and the combined organic phases were washed with brine (80 mL), dried ($MgSO_4$), filtered, and concentrated. Column chromatography eluting with ether and light petroleum (1:2) gave the title compound as a colorless solid (5.12 g, 86%); mp 62−64 °C; (Found: M+imidazolium$^+$, 381.1664. $C_{18}H_{25}N_2O_7^+$ requires: 381.1656); ν_{max} ($CHCl_3$)/cm^{-1} 3007, 2955, 1746, 1490, 1259; δ_H (400 MHz; $CDCl_3$) 6.63 (1H, d, *J* 8.9), 6.58 (1H, d, *J* 8.9), 3.88 (6H, s), 3.80 (3H, s), 3.69 (3H, s), 2.28 (3H, s), 1.45 (3H, s); δ_C (75 MHz; $CDCl_3$) 170.1, 152.5, 147.2, 141.7, 123.2, 108.1 (CH), 105.9 (CH), 82.9, 55.9 (Me), 55.7 (Me), 52.9 (Me), 18.7 (Me), 9.6 (Me); *m*/*z* (ESI) 381 (M+imidazolium$^+$, 100%).

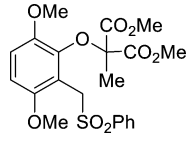


Dimethyl 2-(2-(Bromomethyl)-3,6-dimethoxyphenoxy)-2-methylmalonate 14. *N*-Bromosuccinimide (3.055 g, 17.16 mmol)



was added to a solution of diester **19** (5.100 g, 16.35 mmol) and benzoyl peroxide (0.198 g, 0.82 mmol) in carbon tetrachloride (80 mL) at room temperature. The resulting mixture was stirred at reflux for 4 h, cooled to room temperature, and concentrated. Column chromatography eluting with ether and light petroleum (1:2) gave the title compound as a colorless solid (5.871 g, 92%); mp 117−119 °C; (Found: M+Na^+, 413.0219. $C_{15}H_{19}{}^{79}BrO_7Na^+$ requires: 413.0206); ν_{max} ($CHCl_3$)/cm^{-1} 3011, 2956, 1747, 1601, 1493, 1266; δ_H (400 MHz; $CDCl_3$) 6.89 (1H, d, *J* 8.9), 6.63 (1H, d, *J* 8.9), 4.93 (2H, s), 3.91 (6H, s), 3.89 (3H, s), 3.71 (3H, s), 1.62 (3H, s); δ_C (75 MHz; $CDCl_3$) 169.8, 152.5, 146.7, 141.8, 122.4, 111.8 (CH), 106.6 (CH), 83.4, 56.1 (Me), 55.7 (Me), 53.1 (CH_2), 23.6 (Me), 18.7 (Me); *m*/*z* (ESI) 413/415 (M+Na^+, 100/98%).

Dimethyl 2-(3,6-Dimethoxy-2-((phenylsulfonyl)methyl)-phenoxy)-2-methylmalonate 21. Sodium benzenesulfinate (2.165



g, 13.2 mmol) was added to a solution of bromide **14** (4.301 g, 11.0 mmol) in DMF (55 mL), and the resulting mixture was stirred at room

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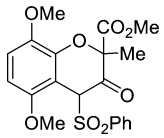
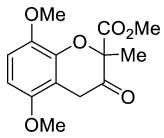
temperature for 22 h. Water (300 mL) was added, and the mixture was extracted with ethyl acetate (4 × 100 mL). The combined organic phases were washed with water (100 mL), dried ($MgSO_4$), filtered, and concentrated. Flash column chromatography eluting with ether gave the title compound as a colorless solid (4.562 g, 92%); mp 128−130 °C; (Found: M+Na^+, 475.1060. $C_{21}H_{24}SO_9Na^+$ requires: 475.1033); ν_{max} ($CHCl_3$)/cm^{-1} 3008, 2955, 1747, 1602, 1493, 1318, 1270, 1141; δ_H (400 MHz; $CDCl_3$) 7.82 (2H, d, *J* 7.9), 7.61 (1H, t, *J* 7.5), 7.50 (2H, dd, *J* 7.9, 7.5), 6.79 (1H, d, *J* 8.9), 6.46 (1H, d, *J* 8.9), 4.91 (2H, s), 3.89 (6H, s), 3.69 (3H, s), 3.36 (3H, s), 1.70 (3H, s); δ_C (75 MHz; $CDCl_3$) 169.8, 152.6, 146.3, 143.3, 140.8, 132.9 (CH), 128.6 (CH), 128.4 (CH), 113.2, 112.4 (CH), 105.5 (CH), 83.9, 55.7 (Me), 55.3 (Me), 53.1 (CH_2), 52.3 (Me), 18.8 (Me); *m*/*z* (ESI) 475 (M+Na^+, 100%).

Methyl 5,8-Dimethoxy-2-methyl-3-oxo-4-(phenylsulfonyl)-chromane-2-carboxylate 22. *n*-Butyllithium (2.25 M in hexanes;



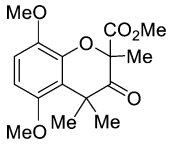
8.89 mL, 20.0 mmol) was added to a solution of *N*,*N*-di*iso*propylamine (3.08 mL, 22.0 mmol) in THF (50 mL) at −78 °C. The resulting mixture was stirred at 0 °C for 20 min, then cooled to −78 °C. A solution of sulfone **21** (4.52 g, 10.0 mmol) in THF (50 mL) was added over 10 min, and the mixture was stirred at −78 °C for a further 2 h, quenched with saturated aqueous ammonium chloride (75 mL), diluted with water (75 mL), and extracted with ethyl acetate (3 × 50 mL). The combined organic phases were dried ($MgSO_4$), filtered, and concentrated. Column chromatography eluting with ether and light petroleum (2:1) gave the title compound as a colorless solid (3.821 g, 91%); mp 141−143 °C; (Found: M+Na^+, 443.0796. $C_{20}H_{20}SO_8Na^+$ requires: 443.0771); δ_H (400 MHz; $CDCl_3$) 7.74 (2H, d, *J* 8.0), 7.64 (1H, t, *J* 7.5), 7.49 (2H, dd, *J* 8.0, 7.5), 6.93 (1H, d, *J* 8.9), 6.37 (1H, d, *J* 8.9), 5.51 (1H, s), 3.91 (3H, s), 3.61 (3H, s), 3.50 (3H, s), 1.97 (3H, s); δ_C (75 MHz; $CDCl_3$) 195.5, 167.7, 151.2, 144.9, 144.2, 138.2, 134.1 (CH), 129.3 (CH), 128.6 (CH), 114.9 (CH), 105.7, 104.3 (CH), 85.8, 69.3 (CH), 57.2 (Me), 55.5 (Me), 53.3 (Me), 21.6 (Me); ν_{max} ($CHCl_3$)/cm^{-1} 3008, 2957, 1737, 1597, 1500, 1328, 1272, 1151, 1101; *m*/*z* (ESI) 443 (M+Na^+, 100%).

Methyl 5,8-Dimethoxy-2-methyl-3-oxochromane-2-carboxylate 20. Zinc powder (1.76 g, 27.0 mmol) was added to a solution



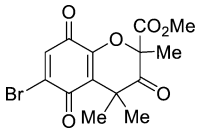
of sulfone **22** (3.78 g, 9.0 mmol) in THF (100 mL), water (100 mL), and saturated aqueous ammonium chloride (100 mL). The resulting mixture was stirred at room temperature for 20 h, allowed to stand for 10 min, decanted from the excess zinc, and extracted with ethyl acetate (3 × 60 mL). The combined organic phases were dried ($MgSO_4$), filtered, and concentrated. Flash column chromatography eluting with ether and light petroleum (1:1) gave the title compound as a colorless solid (2.297 g, 91%); mp 113−115 °C; (Found: M+Na^+, 303.0848. $C_{14}H_{16}O_6Na^+$ requires: 303.0839); ν_{max} ($CHCl_3$)/cm^{-1} 3011, 2942, 1756, 1730, 1490, 1263, 1137; δ_H (400 MHz; $CDCl_3$) 6.82 (1H, d, *J* 8.9), 6.50 (1H, d, *J* 8.9), 3.91 (3H, s), 3.80 (3H, s), 3.76 (1H, d, *J* 21.5), 3.71 (3H, s), 3.43 (1H, d, *J* 21.5), 1.79 (3H, s); δ_C (75 MHz; $CDCl_3$) 201.3, 168.9, 150.8, 143.7, 143.0, 111.4 (CH), 110.7, 103.5 (CH), 84.3, 57.1 (Me), 55.6 (Me), 53.1 (Me), 33.7 (CH_2), 19.9 (Me); *m*/*z* (ESI) 303 (M+Na^+, 33%); 583 (2M+Na^+, 100).

Methyl 5,8-Dimethoxy-2,4,4-trimethyl-3-oxochromane-2-carboxylate 13. DBU (3.88 mL, 26.0 mmol) was added dropwise to a stirred solution of ketone **20** (0.729 g, 2.60 mmol) and iodomethane (1.62 mL, 26.0 mmol) in DMF (15 mL) at 0 °C. The resulting mixture was stirred at 40 °C for 16 h, cooled to room temperature, diluted with hydrochloric acid (1M; 100 mL), and extracted with ether (4 × 50 mL). The combined organic phases were washed with brine (50 mL), dried



($MgSO_4$), filtered, and concentrated. Flash column chromatography eluting with ether and light petroleum (1:3) gave the title compound as a colorless solid (0.762 g, 95%); mp 90−92 °C; (Found: M+H^+, 309.1336. $C_{16}H_{21}O_6^+$ requires: 309.1333); ν_{max} ($CHCl_3$)/cm^{-1} 3043, 2957, 1757, 1737, 1680, 1590, 1260, 1175; δ_H (400 MHz; $CDCl_3$) 6.82 (1H, d, *J* 8.9), 6.53 (1H, d, *J* 8.9), 3.91 (3H, s), 3.80 (3H, s), 3.70 (3H, s), 1.80 (3H, s), 1.60 (3H, s), 1.53 (3H, s); δ_C (75 MHz; $CDCl_3$) 206.4, 169.1, 152.3, 143.8, 142.6, 119.6, 111.3 (CH), 104.8 (CH), 82.8, 57.1 (Me), 55.4 (Me), 52.8 (Me), 44.9, 26.9 (Me), 24.4 (Me), 20.7 (Me); *m*/*z* (ESI) 309 (M+H^+, 40%), 639 (2M+Na^+, 100).

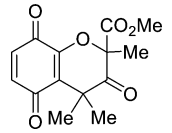
Methyl 6-Bromo-2,4,4-trimethyl-3,5,8-trioxo-3,4,5,8-tetrahydro-2H-chromene-2-carboxylate 12. Bromine (0.139 mL, 2.72



mmol) in dichloromethane (2.5 mL) was added to a stirred solution of arene **13** (0.762 g, 2.47 mmol) in dichloromethane (37.5 mL). The resulting mixture was stirred at room temperature for 3 h, washed with saturated aqueous sodium thiosulfate (40 mL), dried ($MgSO_4$), filtered, and concentrated to give the bromoarene **23** as a colorless solid (quant), used without purification.

Cerium(IV) ammonium nitrate (4.067 g, 7.42 mmol) in water (20 mL) was added to a stirred solution of the crude bromoarene **23** in acetonitrile (20 mL). The resulting mixture was stirred at room temperature for 2 h, diluted with water (50 mL), and extracted with ethyl acetate (3 × 50 mL). The combined organic phases were dried ($MgSO_4$), filtered, and concentrated. Flash column chromatography eluting with ether and light petroleum (1:5) gave the title compound as a yellow solid (0.600 g, 68%); mp 88−90 °C; (Found: M+Na^+, 378.9780. $C_{14}H_{13}{}^{79}BrO_6Na^+$ requires: 378.9793); ν_{max} ($CHCl_3$)/cm^{-1} 3011, 2958, 1738, 1601, 1497, 1264, 1108; δ_H (400 MHz; $CDCl_3$) 7.31 (1H, s), 3.79 (3H, s), 1.84 (3H, s), 1.53 (3H, s), 1.51 (3H, s); δ_C (75 MHz; $CDCl_3$) 201.9, 178.1, 177.9, 167.0, 151.1, 139.6, 135.2 (CH), 123.9, 84.8, 53.6 (Me), 44.0, 26.1 (Me), 24.1 (Me), 20.1 (Me); *m*/*z* (ESI) 379/381 (100/98%, M+Na^+).

Methyl 2,4,4-Trimethyl-3,5,8-trioxo-3,4,5,8-tetrahydro-2H-chromene-2-carboxylate 11. Silver(II) oxide (1.329 g, 10.71



mmol) and nitric acid (6 M, 2.14 mL, 12.85 mmol) were added sequentially to a stirred solution of arene **13** (0.660 g, 2.14 mmol) in 1,4-dioxane (30 mL). The resulting mixture was stirred at room temperature for 3.5 h, diluted with water (150 mL), and extracted with dichloromethane (3 × 50 mL). The combined organic phases were dried ($MgSO_4$), filtered, and concentrated; column chromatography eluting with ether and light petroleum (1:4) gave the title compound as a yellow solid (0.477 g, 80%); mp 91−93 °C; (Found: M+Na^+, 301.0672. $C_{14}H_{14}O_6$+Na^+ requires 301.0683); ν_{max} ($CHCl_3$)/cm^{-1} 3037, 3029, 2957, 1757, 1736, 1682, 1650, 1599, 1383, 1333; δ_H (400 MHz; $CDCl_3$) 6.77 (1H, d, *J* 10.1), 6.71 (1H, d, *J* 10.1), 3.78 (3H, s), 1.84 (3H, s), 1.53 (3H, s), 1.50 (3H, s); δ_C (75 MHz; $CDCl_3$) 202.3, 186.3, 180.4, 167.2, 150.8, 138.3 (CH), 133.5 (CH), 124.1, 84.6, 53.5 (Me), 43.3, 26.1 (Me), 24.2 (Me), 20.2 (Me); *m*/*z* (ESI) 301 (M+Na^+, 49%); 205 (100).

Methyl 2-Ethyl-3-methylbut-3-enoate 24.[27] A mixture of methyl senecioate (1.28 mL, 10.5 mmol) in THF (4.4 mL) was added to a stirring solution of LDA (12.0 mmol), prepared from *n*-butyllithium

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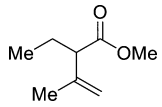
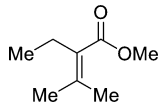
J. Org. Chem. 2016, 81, 7924−7930



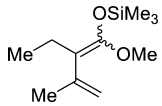
and di*iso*propylamine in THF (13 mL) at −78 °C, and the stirring was continued for 1 h. The solution was allowed to warm to 0 °C over 1 h, and iodoethane (1.56 mL, 19.4 mmol) was added dropwise. The mixture was warmed to room temperature, over 1 h, and stirred for 2 h, before being poured into water (10 mL) and extracted with dichloromethane (3 × 10 mL). The combined organic extracts were then washed with water (20 mL), dried ($MgSO_4$), and concentrated *in vacuo* without heating. The residue was subjected to flash chromatography on silica gel, using light petroleum/ethyl acetate (25:1) to give the title compound as a colorless oil (1.09 g, 73%); (Found: M+Na^+, 165.0884. $C_8H_{14}O_2$+Na^+ requires 165.0891); ν_{max} ($CHCl_3$)/cm^{-1} 3081, 2954, 1731, 1646, 1601, 1457, 1376, 1249, 1170, 902; δ_H (300 MHz; $CDCl_3$) 4.89−4.86 (2H, m), 3.67 (3H, s), 2.95−2.90 (1H, m), 1.73 (3H, s); 1.60−1.87 (2H, m); 0.87 (3H, t, *J* 7.5); δ_C (100 MHz; $CDCl_3$) 174.2 (C), 142.4 (C), 113.7 (CH_2), 54.8 (CH), 51.7 (Me), 23.2 (CH_2), 20.0 (Me), 11.9 (Me); *m*/*z* (ESI) 165 (M+Na^+, 21%). Data in agreement with literature values.[27]

Methyl 2-Ethyl-3-methylbut-2-enoate 25.[27] To a solution of ester **24** (5.00 g, 35.2 mmol) in THF (40 mL) at −78 °C was added



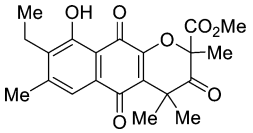
potassium *tert*-butoxide (4.34 g, 38.6 mmol) and the reaction mixture was allowed to reach 0 °C over the course of 2 h. The mixture was washed with water (30 mL) and extracted with dichloromethane (30 mL). The organic phase was dried ($MgSO_4$) and evaporated *in vacuo* without heating. Evaporation of the solvent gave the title compound as a yellow oil (4.92 g) contaminated with *t*-BuOH. The material was used in the next step without further purification; ν_{max} ($CHCl_3$)/cm^{-1} 3010, 1707, 1435, 876; δ_H (300 MHz; $CDCl_3$) 3.72 (3H, s), 2.29 (2H, q, *J* 7.5), 1.94 (3H, s), 1.79 (3H, s), 0.97 (3H, t, *J* 7.5); δ_C (75 MHz; $CDCl_3$) 170.2 (C), 141.6 (C), 129.1 (C), 51.1 (Me), 23.1 (CH_2) 22.9 (Me), 21.4 (Me), 13.4 (Me); *m*/*z* (EI) 142 (M^+). Data in agreement with literature values.[27]

((2-Ethyl-1-methoxy-3-methylbuta-1,3-dien-1-yl)oxy)-trimethylsilane 10. *n*-Butyllithium (2.25 M in hexanes; 0.978 mL, 2.20



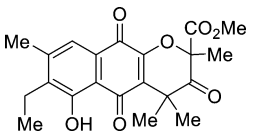
mmol) was added dropwise to a stirring solution of di*iso*propylamine (0.336 mL, 2.40 mmol) in THF (2 mL) at −78 °C. The resulting solution was stirred at 0 °C for 30 min and then cooled to −78 °C. A solution of ester **25** (0.284 g, 2.0 mmol) in THF (3 mL) was added dropwise and the mixture was stirred at −78 °C for 1.5 h. Freshly distilled chlorotrimethylsilane (0.406 mL, 3.2 mmol) was added and the reaction mixture was stirred for another 1 h and then warmed to room temperature over 1.5 h. The solvent was removed under reduced pressure and the resulting slurry was suspended in pentane (10 mL), filtered, and the filtrate was concentrated *in vacuo* to give the desired compound as a colorless oil (1:1 *E*:*Z*; 0.404 g, 94%) used without purification; ν_{max} ($CHCl_3$)/cm^{-1} 3081, 3011, 1726, 1661, 1636, 1438, 1253, 1175, 1115, 1060, 860, 850; δ_H (400 MHz; $CDCl_3$) (*Z*)-isomer: 4.94−4.92 (1H, m), 4.83−4.82 (1H, m), 3.55 (3H, s), 2.13 (2H, q, *J* 7.6), 1.89 (3H, s), 0.94 (3H, t, *J* 7.6), 0.23 (9 H); (*E*)-isomer: 4.94−4.92 (1H, m), 4.81−4.80 (1H, m), 3.57 (3H, s), 2.17 (2H, q, *J* 7.4), 1.91 (3H, s), 0.96 (3H, t, *J* 7.5), 0.27 (9H, s); *m*/*z* (FT-MS) 214 (M^+, 22%). The carbonyl peak in the IR spectrum is likely due to some hydrolysis occurring.

Methyl 8-Ethyl-9-hydroxy-2,4,4,7-tetramethyl-3,5,10-trioxo-3,4,5,10-tetrahydro-2H-benzo[*g*]chromene-2-carboxylate (Annulin B) 4. A solution of diene **10** (0.042 g, 0.2 mmol) in toluene (0.5 mL) was added to the quinone **11** (0.028 g, 0.1 mmol), and the resulting



mixture was stirred at 80 °C for 18 h, then cooled to room temperature. Aqueous iron(III) chloride solution (0.5 M; 1.0 mL, 0.5 mmol) was added, and the resulting mixture was stirred at room temperature for 2 h, diluted with water (5 mL), and extracted with dichloromethane (3 × 5 mL). The combined organic phases were dried ($MgSO_4$), filtered, and concentrated. Column chromatography eluting with ether and light petroleum (1:7) gave the title compound as an orange solid (0.016 g, 42%); mp 144−146 °C (lit.,[20] oil); (Found: M+Na^+, 409.1254. $C_{21}H_{22}O_7Na^+$ requires: 409.1258); ν_{max} ($CHCl_3$)/cm^{-1} 3036, 3015, 2959, 1735, 1644, 1610, 1458, 1303, 1218; δ_H (400 MHz; $CDCl_3$) 12.13 (1H, s), 7.45 (1H, s), 3.78 (3H, s), 2.79 (2H, q, *J* 7.0), 2.45 (3H, s), 1.88 (3H, s), 1.59 (6H, s), 1.18 (3H, t, *J* 7.0); δ_C (75 MHz; $CDCl_3$) 202.9, 183.2, 183.1, 167.4, 160.1, 152.6, 146.2, 138.1, 129.6, 126.9, 121.6 (CH), 111.6, 84.4, 53.5 (Me), 44.0, 26.3 (Me), 24.2 (Me), 20.3 (Me), 20.2 (Me), 19.4 (CH_2), 12.8 (Me); *m*/*z* (ESI) 409 (100%, M+Na^+).

Methyl 7-Ethyl-6-hydroxy-2,4,4,8-tetramethyl-3,5,10-trioxo-3,4,5,10-tetrahydro-2H-benzo[g]chromene-2-carboxylate 9. A



solution of the diene **10** (0.075 g, 0.30 mmol) in dichloromethane (1 mL) was added to a solution of the quinone **12** (0.054 g, 0.15 mmol) and triethylamine (0.022 mL, 0.16 mmol) in dichloromethane (1 mL). The resulting mixture was stirred at room temperature for 19 h, diluted with hydrochloric acid (1 M; 5 mL), and extracted with dichloromethane (3 × 5 mL). The combined organic phases were concentrated to give a mixture of the desired product and its trimethylsilyl ether. This mixture was dissolved in dichloromethane (2 mL) and methanol (2 mL). Hydrochloric acid (1 M; 1 mL) was added, and the mixture was stirred at room temperature for 30 min, diluted with water (10 mL), and extracted with dichloromethane (3 × 5 mL). The combined organic phases were dried ($MgSO_4$), filtered, and concentrated. Column chromatography eluting with ether and light petroleum (1:7) gave the title compound as an orange solid (0.017 g, 29%); mp 136−138 °C; (Found: M+Na^+, 409.1262. $C_{21}H_{22}O_7Na^+$ requires: 409.1258); ν_{max} ($CHCl_3$)/cm^{-1} 3029, 2960, 1734, 1678, 1627, 1605, 1295; δ_H (400 MHz; $CDCl_3$) 12.74 (1H, s), 7.53 (1H, s), 3.77 (3H, s), 2.79 (2H, q, *J* 7.0), 2.44 (3H, s), 1.87 (3H, s), 1.62 (6H, s), 1.19 (3H, t, *J* 7.0); δ_C (75 MHz; $CDCl_3$) 202.8, 189.9, 178.2, 167.3, 159.9, 153.1, 144.4, 140.0, 127.5, 125.7, 121.4 (CH), 112.5, 84.4, 53.5 (Me), 43.8, 26.5 (Me), 24.2 (Me), 22.2 (Me), 19.9 (Me), 19.6 (CH_2), 12.7 (Me); *m*/*z* (ESI) 409 (100%, M+Na^+).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.6b01622.

General experimental details, comparison of NMR data for natural and synthetic material, copies of ^1H and ^{13}C NMR spectra, and X-ray crystallographic data (PDF)
X-ray crystallographic data for compound **4** (CIF)
X-ray crystallographic data for compound **12** (CIF)

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Notes

The authors declare no competing financial interest.

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