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Total Synthesis of Gymnoconjugatins A and B

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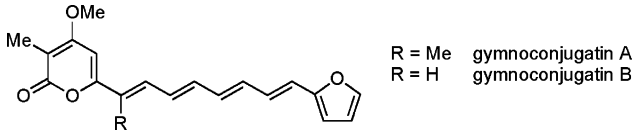


Convergent and efficient syntheses of the microbial natural products gymnoconjugatin A and B are reported and were based on a linchpin coupling strategy using a boron/tin hetero-bis-metallated butadiene system.

In early 2006, a report from Capon and co-workers[1] detailed the isolation and structure determination of two polyenylfurans, named gymnoconjugatins A and B, from the soil microbe *Gymnoascus reessii* (Figure 1). These new compounds were isolated along with several known polyenyl-pyrroles, including rumbrin[2] and auxarconjugatin A.[3] (This same species produces the structurally unrelated prenylated diketopiperazine roquefortine E[4] and the butenolides gymnoascolides A−C.)[5] Structure determination and bioassays were hampered by the small quantities of compounds that were isolated, and gymnoconjugatin B was not fully characterized. There has been no published synthetic work on any of these natural products to date. We now report the stereocontrolled total synthesis of gymnoconjugatins A (**1**) and B (**2**).

Our synthetic approach to the gymnoconjugatins (Scheme 1) relied on disconnection of the central tetraene of **1**/**2** at the C7/C8 and C11/C12 sp^2-sp^2 single bonds to give the vinyl halide fragments **3** and **5** and the central butadiene connector **4**. The hypothesis that hetero-bis-metallated reagent **4**[6] could be incorporated within a polyene chain via *sequential* Stille and Suzuki−Miyaura cross-coupling reactions was previously demonstrated in the total synthesis of the antitumor agent lucilactaene.[7] A related pentadiene system was used in a total synthesis of the antifungal agent strobilurin B, where Suzuki−Miyaura

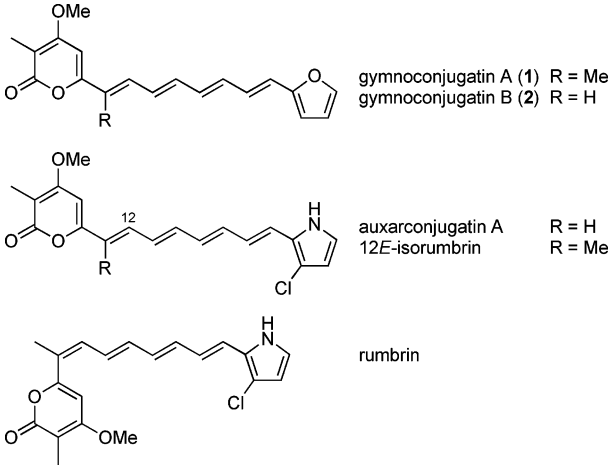
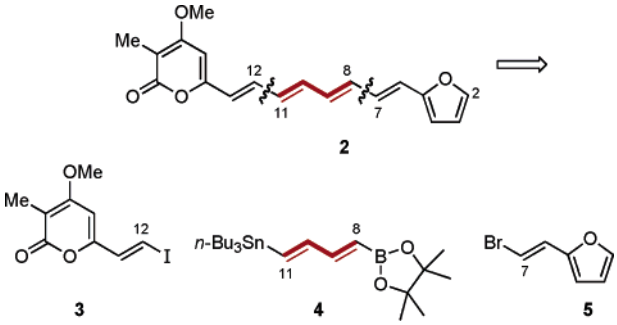
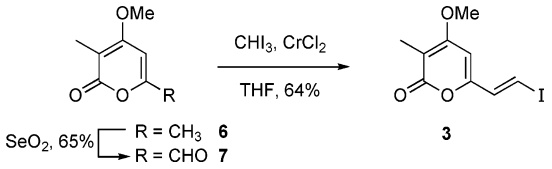


FIGURE 1. Structures of *Gymnoascus reessii* natural products.

SCHEME 1



SCHEME 2



cross-coupling was chemoselective in the presence of the vinyl stannane.[8] We now report the implementation of this synthetic strategy with boron/tin diene **4** in a brief total synthesis of gymnoconjugatins A and B. In addition, we provide preliminary biological evaluation of these two natural products.

Vinyl iodide **3** was synthesized in two steps from pyrone **6** by oxidation with selenium dioxide[9] (190 °C, 3 h) followed by Takai olefination[10] (Scheme 2). Attempts to use IBX[11] rather than SeO_2 for oxidation of the methyl group of **6** were less successful, and although aldehyde **7** was present as the major product of this reaction, it was accompanied by the over- and underreduction products (e.g., the corresponding acid and

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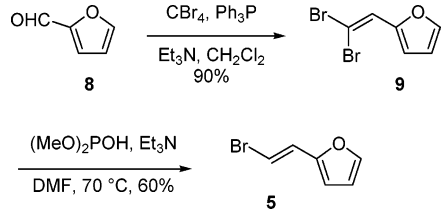
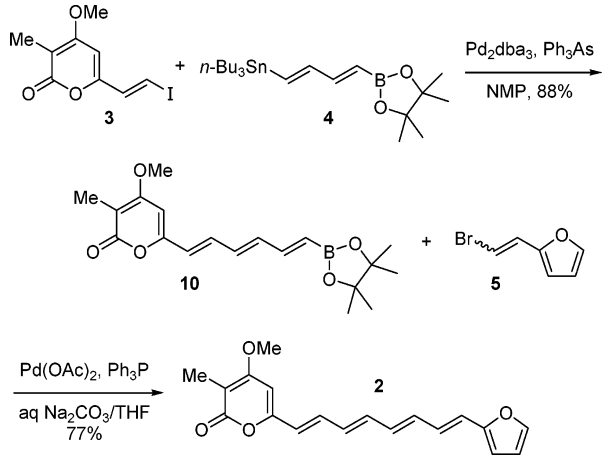
SCHEME 3



SCHEME 4



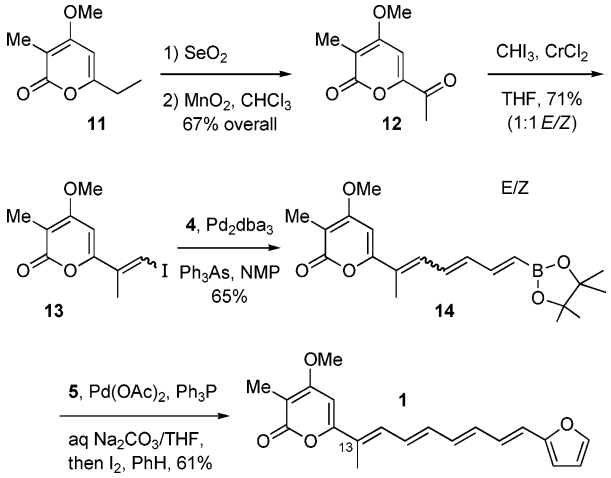
alcohol). Pyrone **6** is synthesized from dehydroacetic acid by known protocols.[12]

The second coupling partner, 2-(2-bromovinyl)furan **5**, was prepared in two steps from furfural (**8**) by Corey−Fuchs olefination to afford **9**[13] followed by selective reduction of the *cis*-bromide to afford **5** (Scheme 3).[14] Vinyl bromide **5** was produced as a 70:30 mixture of *E*- and *Z*-stereoisomers, which proved inconsequential due to subsequent isomerization (Scheme 4).

Stille coupling[15] of vinyl iodide **3** with the vinyl stannane of hetero-bis-metallated diene **4** afforded the triene **10** (Scheme 4). Subsequent Suzuki−Miyaura coupling[16] of the vinyl boronate of **10** with the bromovinyl furan **5** afforded gymnoconjugatin B (**2**). Isomerization of stereoisomers about the furan vinyl group occurred during Suzuki−Miyaura cross-coupling, and **2** was produced as a single stereoisomer. Spectroscopic data of synthetic **2**, including the UV spectrum, were identical with those published for natural **2**[1] and provided confirmation of the structure of gymnoconjugatin B. The synthesis of **2** was accomplished from pyrone **6**, furan **5**, and hetero-bis-metallated diene **4**, in four linear steps without the use of protecting groups.

The synthesis of gymnoconjugatin A (**1**) presented an additional challenge in the form of the C13-methyl group (Scheme 5). Methyl ketone **12** was synthesized from pyrone **11**[17] by two-step oxidation with SeO_2 and MnO_2, in good overall

SCHEME 5



yield. In this system, Takai olefination of **12** produced an inseparable mixture of vinyl iodide stereoisomers **13**. This stereoisomeric mixture was propagated through the subsequent Stille coupling of **13** with **4** to afford vinyl boronate **14**. Final Suzuki−Miyaura coupling between **14** and **5**, with subsequent iodine-promoted isomerization,[18] afforded gymnoconjugatin A (**1**), which provided spectral data identical with natural **1**.

In vitro cytotoxicity assays of gymnoconjugatin A (**1**) and B (**2**) were performed against two breast cancer cell lines: hormone-dependent MCF-7 cells and hormone-independent MDA-MB-231 cells. Disappointingly, preliminary cell antiproliferation assays[19,20] showed no significant activity for **1** after exposure of cells to agent for 48 h at 25 μM.[21] Although gymnoconjugatin B (**2**) was never isolated in sufficient quantities to permit full characterization, in the present cytotoxicity assay this compound was also without significant activity under the same conditions. These results point to the important role that the 3-chloropyrrole of auxarconjugatin and 12*E*-isorumbrin must play in effecting cytotoxic activity, as these compounds were reported to be significantly more active than **1** against murine myeloma NS-1 cells,[1] despite sharing such close structural homology.

Experimental Section

4-Methoxy-5-methyl-6-oxo-6*H*-pyran-2-carbaldehyde (7). Selenium dioxide (1.03 g, 9.34 mmol) was added in a single portion to a solution of pyrone **6** (240 mg, 1.56 mmol) in dioxane (8 mL) in a sealed tube. The reaction mixture was warmed at 180 °C and was stirred rapidly at this temperature for 3 h. The reaction mixture was cooled to room temperature and filtered, and the filtrate was concentrated. The residue was purified by flash chromatography (silica, 5% acetone/chloroform) to afford aldehyde **7** (171 mg, 65%) as a light yellow solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.56 (s, 1H), 7.00 (s, 1H), 3.97 (s, 3H), 2.03 (s, 3H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 183.4, 163.3, 162.4, 152.3, 111.2, 101.6, 56.8, 9.6; IR (KBr) ν_{max} 3080, 2957, 1682, 1636, 1553, 1451, 1340, 1256, 1132, 1017, 857, 747 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_8H_8O_4Na$ 191.0320, found 191.0319.

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6-[(*E*)-2-Iodovinyl]-4-methoxy-3-methyl-2*H*-pyran-2-one (3). A solution of aldehyde 7 (25 mg, 0.15 mmol) and CHI_3 (117 mg, 0.30 mmol) in dioxane (1 mL) were added by syringe to a solution of anhydrous $CrCl_2$ (110 mg, 0.89 mmol) in tetrahydrofuran (THF) (1 mL) at 0 °C under argon. The reaction mixture was stirred at 0 °C for 3 h, poured onto a large excess of water, and extracted with Et_2O. The combined organic extracts were washed with brine, dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography (2% acetone/chloroform) to afford vinyl iodide **3** (28 mg, 64%) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 (d, J = 14.8 Hz, 1H), 7.83 (d, J = 14.8 Hz, 1H), 4.90 (s, 1H), 3.88 (s, 3H), 1.91 (s, 3H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 164.9, 164.1, 155.9, 135.8, 104.2, 95.7, 86.6, 56.3, 8.9; IR (KBr) ν_{max} 3463, 3089, 2919, 1684, 1552, 1465, 1374, 1348, 1289, 1254 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_9H_9IO_3Na$ 314.9494, found 314.9496.

4-Methoxy-3-methyl-6-[(1*E*,3*E*,5*E*)-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)hexa-1,3,5-trienyl)-2*H*-pyran-2-one (10). In a dry box, Pd_2dba_3 (1.2 mg, 1.3 μmol) and Ph_3As (0.8 mg, 2.6 μmol) were added to a Schlenk flask equipped with a stir bar, which was capped with a septum and removed from the dry box. A solution of diene **4** (44 mg, 94 μmol) and vinyl iodide **3** (25 mg, 86 μmol) in *N*-methylpyrrolidone (NMP) (0.9 mL) was added by syringe at 23 °C. The flask was wrapped with foil and stirred at 23 °C for 24 h. The reaction mixture was diluted with Et_2O and poured onto saturated aqueous NH_4Cl. The aqueous layer was extracted with Et_2O, and the combined organic extracts were dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography (2% acetone/chloroform) to afford **10** (26 mg, 88%) as an orange-red solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (dd, J = 15.2, 10.8 Hz, 1H), 7.05 (dd, J = 17.2, 10.4 Hz, 1H), 6.53 (dd, J = 14.8, 10.4 Hz, 1H), 6.42 (dd, J = 14.8, 10.2 Hz, 1H), 6.13 (d, J = 15.2, 1H), 6.07 (s, 1H), 5.71 (d, J = 17.6 Hz, 1H), 3.88 (s, 3H), 1.95 (s, 3H), 1.29 (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.2, 164.7, 157.0, 148.5, 139.3, 135.9, 134.1, 123.9, 117.0, 96.2, 83.4, 56.2, 24.8; IR (KBr) ν_{max} 3425, 3143, 2990, 2896, 2249, 1813, 1790, 1649, 1561, 1472, 1378, 1096, 914, 750 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{19}H_{25}BO_5Na$ 367.1693, found 367.1692.

Gymnoconjugatin B (2). A reaction vessel was charged with $Pd(OAc)_2$ (1.7 mg, 7.6 μmol) and Ph_3P (4.0 mg, 15 μmol) and was flushed with argon. A solution of boronate **10** (26 mg, 70 μmol) and vinyl bromide **5** (14 mg, 83 μmol) in THF (1 mL) was added by syringe, followed by aqueous Na_2CO_3 (1 M, 0.15 mL, 0.15 mmol) in one portion. The flask was wrapped with foil and the reaction mixture was stirred at 23 °C until thin-layer chromatography (TLC) indicated the starting halide was consumed. The reaction mixture was concentrated and the residue was purified by preparative TLC (7% acetone/chloroform) to afford gymnoconjugatin B (18 mg, 77%) as a red solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.68 (br s, 1H), 7.06 (dd, J = 15.2, 11.5 Hz, 1H), 6.76 (dd, J = 15.6, 10.0 Hz, 1H), 6.74 (dd, J = 15.1, 9.6 Hz, 1H), 6.67 (s, 1H), 6.59 (d, J = 15.2 Hz, 1H), 6.59 (dd, J = 14.6, 10.0 Hz, 1H), 6.55 (dd, J = 14.6, 10.0 Hz, 1H), 6.54 (br s, 1H), 6.53 (br s, 1H), 6.51 (dd, J = 14.6, 11.5 Hz, 1H), 6.36 (d, J = 15.2 Hz, 1H), 3.89 (s, 3H), 1.81 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.9, 163.4, 156.9, 152.7, 143.5, 138.3, 135.6, 133.2, 131.6, 128.7, 127.2, 122.6, 121.3, 112.4, 110.1, 100.7, 96.8, 56.8, 8.9; UV (MeOH) λ_{max} 442.5, 423.5, 330.5, 319.5, 264.0, 224.0 nm; IR (KBr) ν_{max} 3154, 2990, 2896, 2249, 1813, 1790, 1649, 1561, 1467, 1384, 1167, 1091, 908, 744 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{19}H_{18}O_4Na$ 333.1103, found 333.1109.

6-Acetyl-4-methoxy-3-methyl-2*H*-pyran-2-one (12). Selenium dioxide (219 mg, 1.99 mmol) was added in a single portion to a solution of pyrone **11** (51 mg, 0.33 mmol) in dioxane (1.3 mL) in a sealed tube. The reaction mixture was warmed at 190 °C and was stirred rapidly at this temperature for 3 h. The reaction mixture was cooled to room temperature and filtered, and the filtrate was concentrated. The crude product was treated with MnO_2 (720 mg, 8.3 mmol) in $CHCl_3$ (2 mL), and the reaction mixture was stirred for 24 h. The reaction mixture was filtered and concentrated, and the residue was purified by flash chromatography (3% acetone/chloroform) to afford ketone **12** (40 mg, 67%) as an off-yellow solid: ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.05 (s, 1H), 3.95 (s, 3H), 2.54 (s, 3H), 2.01 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 191.7, 164.1, 163.3, 153.0, 109.7, 98.1, 56.7, 25.9, 9.4,; IR (KBr) ν_{max} 3460, 2955, 2849, 1684, 1619, 1396, 1343, 1261, 1161 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_9H_{10}O_4Na$ 205.0477, found 205.0477.

6-(1-Iodoprop-1-en-2-yl)-4-methoxy-3-methyl-2*H*-pyran-2-one (13). A solution of ketone **12** (40 mg, 0.22 mmol) and CHI_3 (173 mg, 0.44 mmol) in dioxane (1 mL) were added by syringe to a solution of anhydrous $CrCl_2$ (162 mg, 1.32 mmol) in THF (1 mL) at 0 °C under argon. The reaction mixture was stirred at 0 °C for 3 h, poured onto a large excess of water, and extracted with Et_2O. The combined organic extracts were washed with brine, dried ($MgSO_4$), and concentrated. The residue was purified by flash chromatography (2% acetone/chloroform) to afford a 50:50 *E*/*Z* mixture of vinyl iodide **13** (48 mg, 71%) as an off-white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.57 (s, 1H), 6.68 (s, 1H), 6.60 (dd, J = 4.8, 2.4 Hz, 1H) 6.25 (s, 1H), 3.93 (s, 3H), 3.91 (s, 3H), 2.18 (d, J = 2.4 Hz, 3H), 2.13 (d, J = 1.6 Hz, 3H), 1.95 (s, 3H), 1.92 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.2, 164.0, 139.4, 136.9, 98.0, 97.5, 93.4, 89.9, 78.8, 56.4, 56.2, 23.2, 20.6, 8.8, 8.8; IR (KBr) ν_{max} 3472, 2943, 2907, 2849, 1713, 1666, 1631, 1543, 1455, 1384, 1349, 1243, 1149 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_9H_9IO_3Na$ 328.9651, found 328.9652.

4-Methoxy-3-methyl-6-[(6*E*)-7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)hepta-2,4,6-trien-2-yl]-2*H*-pyran-2-one (14). In a dry box, Pd_2dba_3 (1.6 mg, 2.0 μmol) and Ph_3As (1.3 mg, 4.0 μmol) were added to a Schlenk flask equipped with a stir bar, which was capped with a septum and removed from the dry box. A solution of diene **4** (61 mg, 0.13 mmol) and vinyl iodide **13** (32 mg, 0.11 mmol) in NMP (1.5 mL) was added by syringe at 23 °C. The flask was wrapped with foil and stirred at 23 °C for 24 h. The reaction mixture was diluted with Et_2O and poured onto saturated aqueous NH_4Cl. The aqueous layer was extracted with Et_2O, and the combined organic extracts were dried ($MgSO_4$) and concentrated. The residue was purified by flash chromatography (2% acetone/chloroform) to afford a 50:50 *E*/*Z* mixture of triene **14** (24 mg, 65%) as an orange-red solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.20 (d, J = 2.4 Hz, 1H), 7.16 (s, 1H), 7.12−7.15 (m, 1H), 7.08−7.10 (m, 1H), 6.69 (dd, J = 14.8, 11.6 Hz, 1H), 6.58 (dd, J = 14.4, 10.4 Hz, 1H), 6.39 (dd, J = 14.8, 10.8 Hz, 1H), 6.31 (d, J = 12 Hz, 1H), 6.20 (s, 1H), 6.15 (s, 1H), 5.71 (d, J = 17.6 Hz, 1H), 5.64 (d, J = 17.6 Hz, 1H), 3.91 (s, 3H), 3.89 (s, 3H), 2.07 (s, 3H), 2.00 (s, 3H), 1.28 (s, 12H), 1.27 (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 165.6, 164.7, 159.3, 159.3, 149.4, 149.0, 139.4, 138.6, 134.2, 132.6, 131.3, 131.1, 128.4, 127.5, 103.1, 96.1, 93.1, 83.4, 83.3, 56.2, 56.1, 29.7, 27.8, 24.9, 24.8, 21.8, 17.5, 13.6, 12.6, 8.8, 8.7; IR (KBr) ν_{max} 3425, 3143, 2990, 2896, 2249, 1631, 1531, 1384, 995 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{20}H_{27}BO_5Na$ 381.1853, found 381.1849.

Gymnoconjugatin A (1). A reaction vessel was charged with $Pd(OAc)_2$ (1.0 mg, 4.6 μmol) and Ph_3P (2.4 mg, 9.2 μmol) and was flushed with argon. A solution of boronate **14** (17 mg, 46 μmol) and vinyl bromide **5** (8.8 mg, 51 μmol) in THF (1 mL) was added by syringe, followed by aqueous Na_2CO_3 (1 M, 0.1 mL, 0.09 mmol) in one portion. The flask was wrapped with foil and stirred at 23 °C until TLC indicated the starting halide was consumed. The reaction mixture was concentrated and the residue was purified by preparative TLC (7% acetone/chloroform) to afford a 50:50 *E*/*Z* mixture of gymnoconjugatin A. This mixture was treated with a catalytic amount of I_2 in benzene (1 mL) at 25 °C. After 24 h, the mixture was concentrated and purified by preparative TLC (7% acetone/chloroform) to afford stereoisomerically pure gymnoconjugatin A (10 mg, 61%) as a red solid: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.67 (br s, 1H), 7.05 (d, J = 10.0 Hz, 1H), 6.72−

6.78 (m, 3H), 6.59 (d, $J = 11.2$ Hz, 1H), 6.56−6.59 (m, 2H), 6.50−6.53 (m, 1H), 6.54 (br s, 1H), 6.53 (br s, 1H), 3.95 (s, 3H), 2.05 (s, 3H), 1.81 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 166.0, 163.3, 158.7, 152.7, 143.4, 138.3, 135.2, 133.6, 130.9, 128.8, 126.5, 121.1, 112.3, 100.5, 94.0, 56.8, 12.4, 8.7; IR (KBr) ν_{max} 3154, 2990, 2896, 2364, 1813, 1772, 1653, 1560, 1465, 1374, 1142, 744 cm^{-1}; HRMS (ESI) *m*/*z* calcd for $C_{19}H_{18}O_4Na$ 333.1103, found 333.1109.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of intermediates and products. This material is available free of charge via the Internet at http://pubs.acs.org.

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