Supporting Information

Roles of the Synergistic Reductive *O*-Methyltransferase GilM and of *O*-Methyltransferase GilMT in the Gilvocarcin Biosynthetic Pathway

*Nidhi Tibrewal,[1] Theresa E. Downey,[1] Steven G. Van Lanen,[1] Ehesan Ul Sharif,[2] George A. O'Doherty[2] and Jürgen Rohr[1]**

[1]Department of Pharmaceutical Sciences, College of Pharmacy, University of Kentucky, 789 South Limestone Street, Lexington, KY 40536-0596, USA. [2]Department of Chemistry and Chemical Biology, Northeastern University, 360 Huntington Ave., Boston, MA 02115, USA.

jrohr2@email.uky.edu

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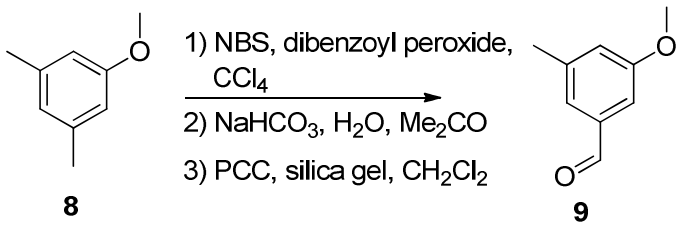
Section A: General Information

General Remarks: ^{1}H and ^{13}C spectra were recorded using Agilent instruments (^{1}H frequencies 300, 400 and 500 MHz, corresponding ^{13}C frequencies are 75, 100, 125 MHz, respectively). Chemical shifts are quoted in parts per million (ppm) relative to TMS. *J* values are recorded in Hz. A photodiode array detector (Waters 2996) along with a Micromass ZQ 2000 mass spectrometer (Waters Corporation) equipped with an electrospray ionization (ESI) probe was used to detect the molecular ions and analyze the compounds. 60-200 mesh silica gel was used for flash column chromatography. Thin layer chromatography was carried out using aluminum backed plates coated with silica gel. The plates were visualized under UV light at 254 NM and /or vanillin stain. R_f values were obtained by elution in the stated solvent ratios (v/v). All small-scale dry reactions were carried out under Nitrogen using standard syringe-septum technique.

Section B: Synthesis of 1

3-Methoxy-5-methylbenzaldehyde (9)[1]: This was prepared in 3 steps.

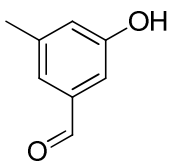


To a magnetically stirred solution of the dimethyl anisole (500 mg, 3.68 mmol) in CCl_4 (40 mL) was added NBS (622 mg, 3.5 mmol) and benzoyl peroxide (10 mg). After refluxing for 1h, the reaction mixture was filtered and the filtrate was successively washed with aqueous HCl (3 N), saturated aqueous $NaHCO_3$, H_2O and brine. The organic layer was dried over Na_2SO_4 and the solvent was evaporated to give a residue that was purified on a silica gel column (30% CH_2Cl_2–hexane, R_f = 0.56) to furnish the bromide (70%).[1]

To a stirred solution of the above bromide (2.15 g, 9.95 mmol) in acetone (60 mL) and H_2O (100 mL) was added $NaHCO_3$ (1.05 g, 12.5 mmol). The reaction mixture was refluxed for 4h then cooled to r.t. and extracted with EtOAc (3×50 mL). The combined organic layer was washed with brine and dried (Na_2SO_4). Evaporation of the solvent and purification of the residue on a silica gel column (20% EtOAc–hexane, R_f = 0.2) furnished the alcohol (86%).[1]

To a stirred suspension of pyridinium chlorochromate (2.0 g, 14.4 mmol) and silica gel (2.0 g) in anhydrous CH_2Cl_2 (10 mL) was added a solution of the above alcohol (1.3 g, 9.6 mmol) in CH_2Cl_2 (5 mL). After stirring the reaction mixture for 1h at r.t., it was filtered through a small silica gel column. The column was eluted with excess CH_2Cl_2 and the solvent was evaporated under reduced pressure to furnish the benzaldehyde[1] as oil (quant.) (40% CH_2Cl_2–hexane, R_f = 0.45). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.90 (s, 1H), 7.25 (bs, 1H), 7.18 (bs, 1H), 6.97 (bs, 1H), 3.82 (s, 3H), 2.38 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 192.3, 160.1, 140.4, 137.7, 124.4, 122.2, 109.5, 55.6, 21.5.

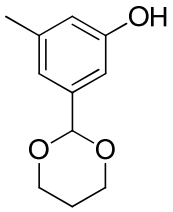
3-Hydroxy-5-methylbenzaldehyde (10)[2]



1M BBr_3 in CH_2Cl_2 (6.6 mL, 6.6 mmol) was added dropwise to a stirred solution of **9** (500 mg, 3.3 mmol) in anhydrous CH_2Cl_2 (10 mL) under nitrogen at r.t. After stirring for 2h, 3 mL of each HCl and AcOH were added and reaction mixture was refluxed for 10h. The reaction was cooled, diluted with H_2O and extracted with CH_2Cl_2. The combined organic layer was dried over Na_2SO_4 and the solvent was evaporated to give a residue that was purified on silica gel (20% EtOAc-hexane, R_f = 0.25) to give deprotected derivative as yellowish solid in 60% yield. ^{1}H NMR (CD_3OD, 300 MHz) δ 9.76 (s, 1H), 7.09 (bs, 1H), 7.04 (bs, 1H), 6.87 (bs, 1H), 2.27 (s, 3H); ^{13}C NMR (CD_3OD, 75 MHz) δ 194.2, 158.8, 141.4, 138.9, 123.5, 113.1, 54.7, 21.3.

2-(3-Methoxymethoxy-5-methylphenyl)-1,3-dioxane (11)[3]**:** This protected benzaldehyde was prepared in 2 steps.

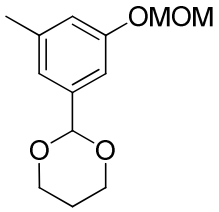
(a) 2-(3-Hydroxy-5-methylphenyl)-1,3-dioxane[3]



A mixture of **10** (200 mg, 1.5 mmol), 1,3-propanediol (0.16 mL, 2.2 mmol) and $TsOH.H_2O$ (6 mg, 0.03 mmol) in toluene was refluxed with the azeotropic removal of H_2O. After starting material was consumed completely, the reaction mixture was cooled and the solvent was removed under pressure to give a residue. The crude residue was purified on silica (EtOAc-hexane, R_f = 0.32) to give the protected aldehyde as a white solid in 75% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.84 (bs, 1H), 6.70 (bs, 1H), 6.49 (bs, 1H), 6.45 (bs, 1H), 5.39 (s, 1H), 4.27–4.21 (m, 2H), 3.99–3.92 (m, 2H), 2.22 (s, 3H), 2.20–2.14 (m,

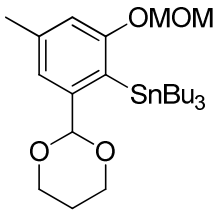
1H), 1.44–1.38 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 155.7, 139.6, 139.4, 118.7, 116.8, 110.4, 101.7, 67.4, 25.8, 21.5; HRMS (+EI): Calc'd for $C_{11}H_{14}O_3$: 194.0943, observed: 194.0944.

(b) 2-(3-Methoxymethoxy-5-methylphenyl)-1,3-dioxane (11)[3]



To a stirred solution of the above phenyl acetal (165 mg, 0.85 mmol) and diisopropylethylamine (0.23 mL, 1.35 mmol) in anhydrous CH_2Cl_2 was dropwise added chloromethyl methyl ether (0.14 mL, 1.69 mmol) and the mixture was heated at 40 °C. After 24h, reaction mixture was washed with H_2O and brine. The combined organic layer was dried over Na_2SO_4 and the solvent was removed under vacuum to give MOM ether as colorless oil in 95% yield. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.97 (bs, 1H), 6.82 (bs, 1H), 5.42 (s, 1H), 5.15 (s, 2H), 4.26–4.20 (m, 2H), 3.98–3.89 (m, 2H), 3.44 (s, 3H), 2.32 (s, 3H), 2.28–2.12 (m, 1H), 1.42–1.36 (m, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 157.0, 139.8, 139.3, 120.1, 117.2, 110.8, 101.4, 94.2, 67.3, 55.9, 25.8, 21.5; HRMS (+EI): Calc'd for $C_{13}H_{18}O_4$: 238.1205, observed: 238.1204.

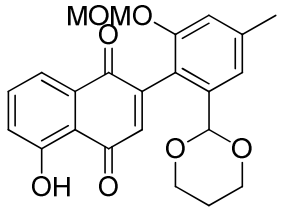
(2-(1,3-dioxan-2-yl)-6-(methoxymethoxy)-4-methylphenyl)tributylstannane (12)[4]



The above MOM ether (300 mg, 1.26 mmol) was co-evaporated twice with anhydrous toluene and was dissolved in freshly dried hexane (10 mL). At 0 °C, *n*-BuLi (0.6 mL, 2.5M in hexane, 1.5 mmol) was added and the mixture was stirred for 30 min. A white precipitate indicates the formation of lithiated species. After 30 min, *n*-Bu_3SnCl (0.5 mL, 1.89 mmol) was added and the mixture was further stirred for 30 min. It was then diluted with hexane-Et_2O (1:1, v/v), followed by addition of saturated aqueous $NaHCO_3$. The mixture was stirred at 0 °C for 30 min. After separation, the aqueous was extracted twice with hexane-Et_2O (1:1, v/v). The combined organic layer was washed with brine and dried over Na_2SO_4.

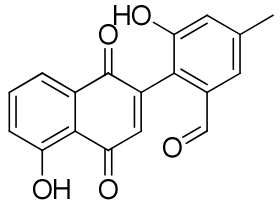
The solvent was removed under vacuum and the residue was subjected to purification on silica to give the required stannane (3% EtOAc-hexane, $R_f = 0.5$) as colorless oil (88%). ^{1}H NMR ($CDCl_3$, 300 MHz) 7.24 (bs, 1H), 6.90 (bs, 1H), 5.38 (s, 1H), 5.11 (s, 2H), 4.26-4.21 (bs, 2H), 3.99 (bs, 2H), 3.45 (s, 3H), 2.34 (s, 3H), 2.30-2.12 (m, 1H), 1.56-0.87 (m, 28H); δ ^{13}C NMR ($CDCl_3$, 75 MHz) δ 161.9, 146.6, 140.3, 125.8, 120.6, 113.7, 102.5, 94.5, 67.4, 56.0, 29.4, 27.7, 26.0, 21.8, 14.0, 12.4; HRMS (ESI): Calc'd for $[C_{25}H_{44}O_4Sn+H]^+$: 529.2339, observed: 529.2342.

2-(2-(1,3-dioxan-2-yl)-6-(methoxymethoxy)-4-methylphenyl)-5-hydroxy-1,4-napthaquinone (14) [4]



To a solution of 2-Bromo-8-hydroxy-1,4-naphthaquinone **13** (96 mg, 0.38 mmol), stannane **12** (200 mg, 0.38 mmol), and CuI (7.6 mg, 0.04 mmol) in THF (5 mL) was added a solution of $Pd_2(dba)_3$•$CHCl_3$ (9.8 mg, 0.009 mmol) and PPh_3 (10.5 mg, 0.04 mmol) in THF (1 mL). The mixture was heated at 75 °C for 12h. The mixture was cooled to 0 °C and diluted with EtOAc (25 mL). Saturated aqueous $NaHCO_3$ (25 mL) was added and the mixture was stirred for 30 min. The layers were separated and the aqueous layer was extracted twice with EtOAc (50 mL). The combined organic layer was washed with brine and dried over Na_2SO_4. The solvent was removed under vacuum and the resulting residue was subjected to flash silica gel column chromatography to provide the coupled product (35% EtOAc-hexane, R_f = 0.6) as orange solid (75%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.33 (s, 1H), 7.80 (dd, J = 8.5, 7.5 Hz, 1H), 7.61 (dd, J = 7.5, 1 Hz, 1H), 7.39 (bs, 1H), 7.36 (dd, J = 8.5, 1 Hz, 1H), 7.18 (bs, 1H), 6.99 (s, 1H), 2.43 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 190, 5, 184.5, 162.5, 156.1, 147.5, 142.1, 138.7, 137.7, 137.3, 133.5, 126.4, 124.8, 124.7, 123.3, 119.2, 116.4, 21.1.

3-hydroxy-2-(5-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-5-methylbenzaldehyde (15)[4]



To **14** (60 mg, 0.14 mmol) in a 250 mL flask at r.t. was added a solution of concentrated HCl (2 mL) in 10 mL CH_3CN. The mixture was stirred for 4 min and then was quenched with $NaHCO_3$ (60 mL) followed by addition of EtOAc (50 mL). After separation of the layers, the aqueous layer was extracted with EtOAc twice. The combined organic layer was washed with brine, dried over Na_2SO_4 and the solvent was removed under vacuum. The resulting residue was subjected to purification by flash silica gel column chromatography to provide the desired product (35% EtOAc-hexane, R_f = 0.25) as orange solid (74%). ^{1}H NMR (acetone-d_6, 500 MHz) δ 12.06 (bs, 1H), 9.90 (s, 1H), 7.80 (dd, J = 8.5, 7.5 Hz, 1H), 7.61 (dd, J = 7.5, 1 Hz, 1H), 7.39 (bs, 1H), 7.36 (dd, J = 8.5, 1 Hz, 1H), 7.18 (bs, 1H), 6.99 (s, 1H), 2.43 (s, 3H); ^{13}C NMR (acetone-d_6, 100 MHz) δ 193.0, 190.5, 184.5, 162.5, 156.1, 147.5, 142.1, 138.7, 137.7, 137.3, 133.5, 126.4, 124.8, 124.7, 123.3, 119.2, 116.4, 21.1; HRMS (+EI): Calc'd for $C_{18}H_{12}O_5$: 308.0685, observed: 308.0686.

Section C: Expression and Purification of Enzymes

*gil*M, *gil*MT and *gil*R genes were expressed using pET28a-expression constructs in *E. coli* BL21 (DE3) with *N*-terminal polyhistidine tag. A single colony was transferred to 10 mL LB supplemented with 50 µg/mL kanamycin and grown at 37 °C and 250 rpm for 5h. Subsequently, 500 mL LB supplemented with 50 µg/mL kanamycin was inoculated with 5 mL of the culture and was grown at 37 °C until OD600 reached to 0.5. Gene expression was induced with Isopropyl-β-D-1-thiogalactopyranoside (IPTG, 0.2 mM final concentration) and the culture was allowed to grow at 18 °C for 16h. The cell pellets were collected by centrifugation (4000 × g, 15 min) and were washed twice with 20 mL of lysis buffer (50 mM KH_2PO_4, 300 mM KCl, 10 mM imidazole, and pH 8.0). The cells were lysed using a French Press and the crude soluble enzyme fractions were collected through centrifugation (16000 × g, 1h). The crude enzymes were loaded onto Talon metal affinity resin (BD Biosciences) column and were washed thrice with lysis buffer. The enzymes were then eluted with elution buffer (50 mM KH_2PO4, 300 mM KCl, 250 mM imidazole, pH 8.0). The purified proteins were concentrated using an Amicon Ultra centrifugal filter (Millipore Corp.) and stored as 25% glycerol stocks at -20 °C. Concentrations of proteins were determined by the Bradford method using a calibration curve of the known concentrations of BSA. The concentrations for GilM, GilMT and GilR were found to be 6.7, 12.5 and 2.4 mg mL^{-1} respectively.

Section D: Cofactor Analysis of GilM

GilM catalyzes reductive methylation without any external cofactor. However, the Blast analysis showed a very vague similarity to thiopurine-S-methyltransferases. To analyze any bound cofactor, the enzyme was boiled for 5 minutes and centrifuged (12000 ×g, 5 min). The supernatant was then subjected to LC-MS analysis. A linear gradient of acetonitrile and 0.1% formic acid-water (solvent A = 0.1% formic acid-H_2O; solvent B = acetonitrile; 0-15 min 25% B to 100% B; 16-24 min 100% B; 25-26 min 100% to 25% B; 27-29 min 25% B) with flow rate of 0.5 mL/min was used to separate the compounds in a Waters Symmetry C_{18} (4.6 × 250 mm, 5µm) column. The supernatant showed UV-absorption at 260, typical of adenosine spectrum. To further verify presence of loosely bound S-adenosylmethionine, standard solution of S-adenosylmethionine was prepared in 50 mM phosphate buffer and was used in parallel for comparison. GilM was found to be co-purifying with S-adenosylmethionine.

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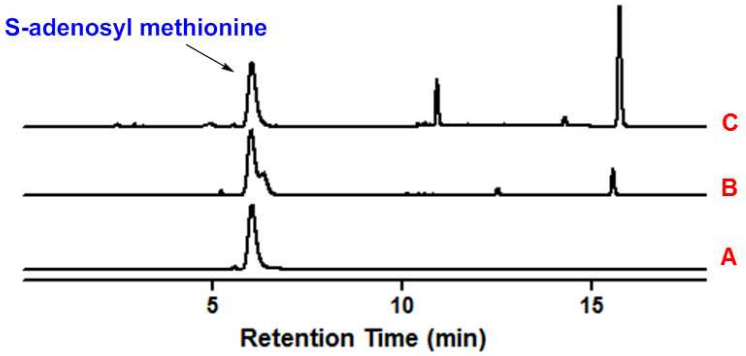
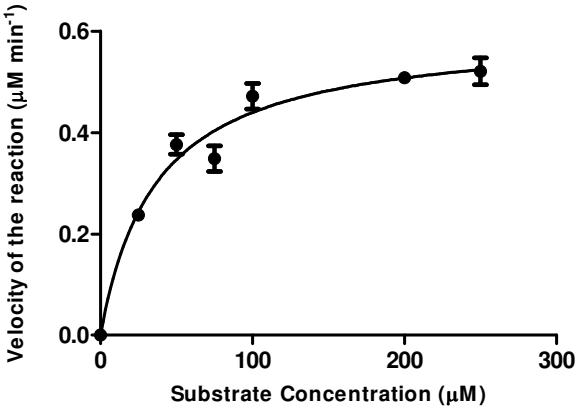


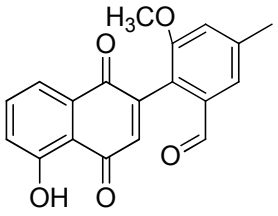
Figure 1. HPLC traces of the released cofactor: (A) standard S-adenosylmethionine; (B) Cofactor released from GilM; (C) S-adenosylmethionine boiled for 5 min.

Section E: Kinetic Profile

GilM

A typical reaction mixture (50 µM) composed of substrate (**5**), 50 mM phosphate buffer, 20 µM enzyme (final concentration) was incubated at 25 °C. After 5 min reaction was extracted twice with EtOAc (300 µl) and combined organic layers were dried under vacuum. The residue was then dissolved in 50 µl acetonitrile and 20 µl was injected onto HPLC following the protocol described in Section D. Amount of product formed was estimated by plotting the peak area in the standard calibration curve. The data resulting from incubating 7 different substrate concentrations with enzyme were fit to the Michael-Menten equation with nonlinear regression. k_{cat} and K_M values were calculated using GraphPad Prism 5.0. The analysis was done in triplicate and the average was taken.





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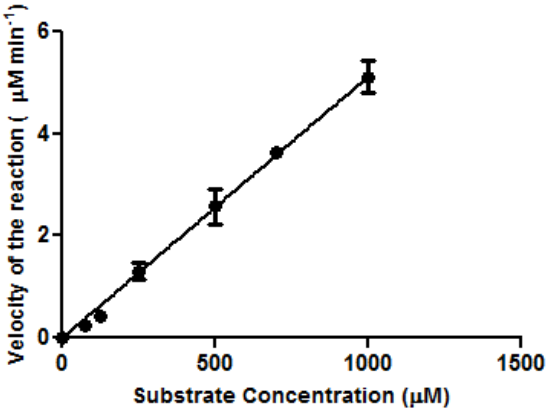
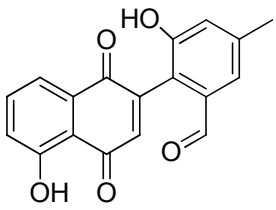
GilMT

A typical reaction mixture (100 μM) composed of substrate (**15**), 50 mM phosphate buffer, S-adenosylmethionine (excess), 13.7 μM GilMT (final concentration) was incubated at 25 °C for 5–15 min to optimize the analysis condition. Kinetic profile for GilMT could not be generated due to solubility issues. The substrate concentration above 1 mM resulted in substrate precipitation while less enzyme concentration failed to produce substantial amount of product to be monitored by HPLC.





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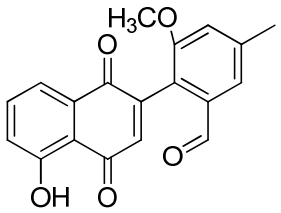
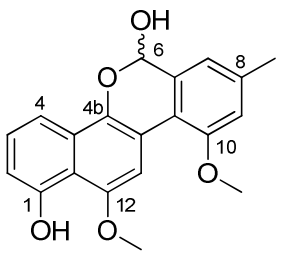
Section F: In vitro enzymatic reactions

Enzymatic synthesis of 2-(5-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-3-methoxy-5-methylbenzaldehyde (5)



An assay mixture (5 mL) composed of phosphate buffer (pH 6.5, 50 mM), substrate **15** (100 μM), S-adenosylmethionine (excess), GilMT (10 μM) was incubated at 30 °C for 12h. The reaction was extracted with EtOAc (2 × 10 mL). The organic solvent was dried at low pressure and the crude product was dissolved in CH_3CN. The product showed UV-absorbance at 420 and was purified through HPLC using the conditions as described in Section D. The reaction produced the desired methylated product in 40% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 12.03 (s, 1H), 9.85 (s, 1H), 7.64-7.60 (m, 2H), 7.33 (bs, 1H), 7.28 (dd, J = 7.5, 2 Hz, 1H), 7.05 (bs, 1H), 6.82 (s, 1H), 3.77 (s, 3H), 2.48 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 191.7, 190.2, 183.3, 161.5, 157.3, 147.5, 141.6, 137.1, 136.4, 135.8, 132.7, 125.7, 124.2, 119.8, 117.7, 115.6, 56.3, 21.8; HRMS (+EI): Calc'd for $C_{19}H_{14}O_5$: 322.0841, observed: 322.0850.

Enzymatic synthesis of defuco-pregilvocarcin M (6)

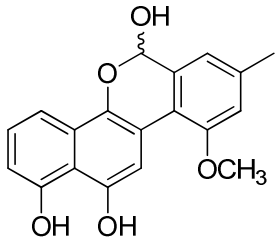


An assay mixture (1 mL) composed of phosphate buffer (pH 6.5, 50 mM), substrate **5** (100 μM), S-adenosylmethionine (excess), GilM (8 μM) was incubated at 30 °C for 4h. The reaction was extracted with EtOAc (2 × 2 mL). The organic solvent was dried at low pressure and the crude product was dissolved in CH_3CN. The cyclized product showed a sharp absorbance at 375 nm and was purified through HPLC (conditions as described in Section D). The reaction produced the desired product in 60% yield. HRMS (+EI): Calc'd for $C_{20}H_{18}O_5$: 338.1154, observed: 338.1160.

Table 1. ^{1}H and HSQC data for defuco-pregilvocarcinM (**6**) in $CDCl_3$ (500 MHz, relative to internal TMS, J in Hz).

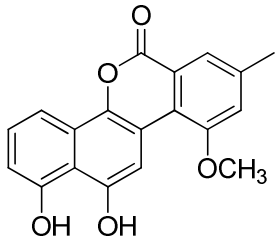
Position	**^{1}H NMR**	**Multiplicity (Hz)**	**HSQC**
1-OH	9.43	s	
2	6.88	d (1)	113.6
3	7.38	dd (8.5, 7.5)	125.4
4	7.83	dd (8.5, 1)	
6	6.40	d (6.5)	93.1
6-OH	3.02	d (6.5)	
7	6.89	d (2)	119.4
8-CH_3	2.41	s	21.8
9	6.86	bs	111.2
10-OCH_3	3.97	s	56.0
11	8.04	s	103.6
12-OCH_3	4.08	s	56.2

Enzymatic synthesis of demethyl-defuco-pregilvocarcin M (16)



An assay mixture (50 μl) composed of phosphate buffer (pH 6.5, 50 mM), substrate **5** (0.8 μM), GilM (0.04 μM) was incubated at 30 °C for 5 min. The reaction was extracted with EtOAc (2 × 2 mL). The organic solvent was dried at low pressure and the crude product was dissolved in CH_3CN. The solution was injected onto HPLC (conditions described in Section D). The reaction produced the desired intermediate in 10 % yield. HRMS (ESI): Calc'd for $[C_{19}H_{16}O_5\text{-}H]^-$ 323.0919, observed: 323.0919.

Enzymatic synthesis of demethyl-defuco-gilvocarcin M (17)[3]

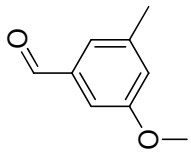


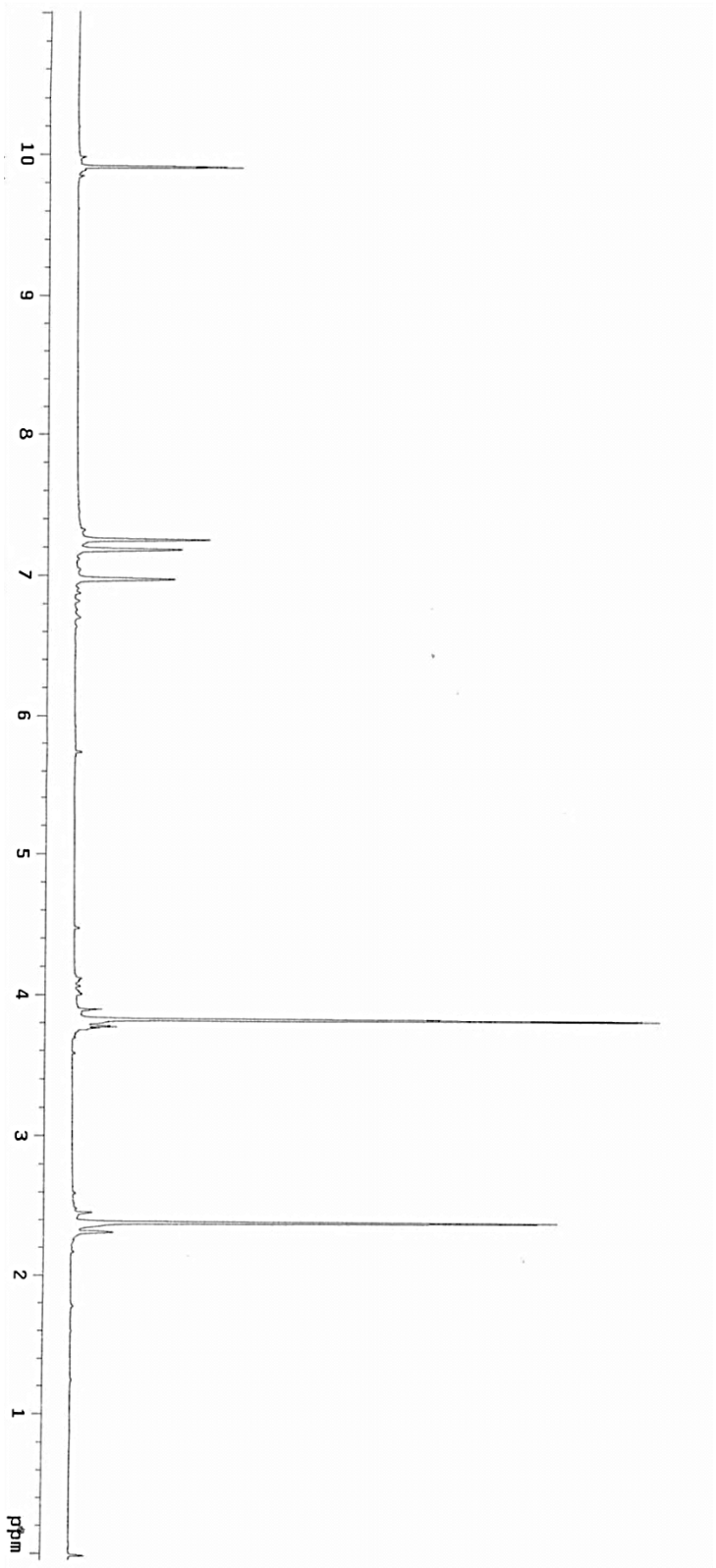
An assay mixture (100 μl) composed of phosphate buffer (pH 6.5, 50 mM), substrate **5** (2 μM), GilM (0.1 μM) and GilR (0.2 μM) was incubated at 30 °C for 1h. The reaction was extracted with EtOAc (2 × 2 mL). The organic solvent was dried at low pressure and the crude product was dissolved in CH_3CN. The solution was injected onto HPLC (conditions described in Section D). The reaction produced the desired product in 31 % yield. HRMS (+EI): Calc'd for $C_{19}H_{14}O_5$ 322.0841, observed: 322.0846.

Section G: Spectral Data

3-Methoxy-5-methylbenzaldehyde (9)

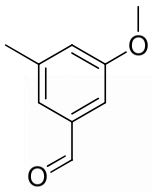
^{1}H NMR ($CDCl_3$, 300 MHz)

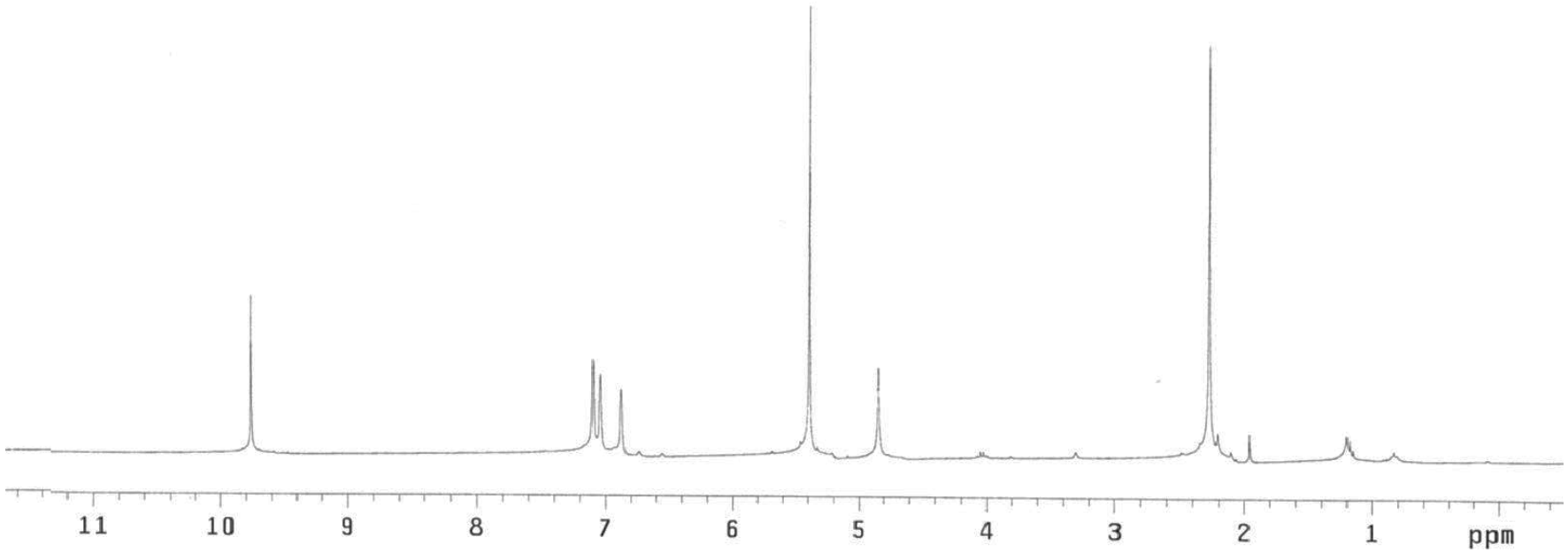




3-Methoxy-5-methylbenzaldehyde (9)

^{13}C NMR ($CDCl_3$, 75 MHz)



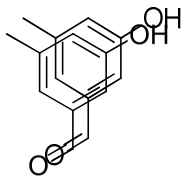

11
10
9
8
7
6
5
4
3
2
1
ppm

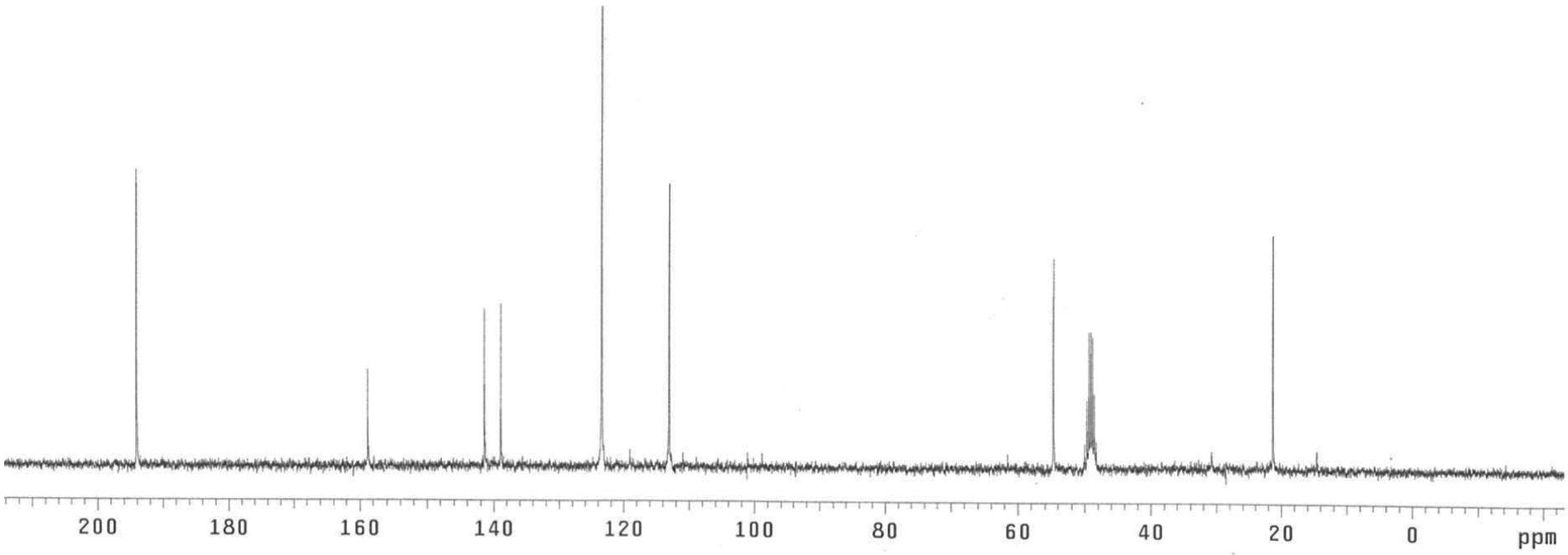
3-Hydroxy-5-methylbenzaldehyde (10)

3-Hydroxy-5-methylbenzaldehyde (10)

^{13}C NMR (CD_3OD, 75 MHz)

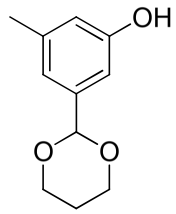
^{1}H NMR (CD_3OD, 300 MHz)

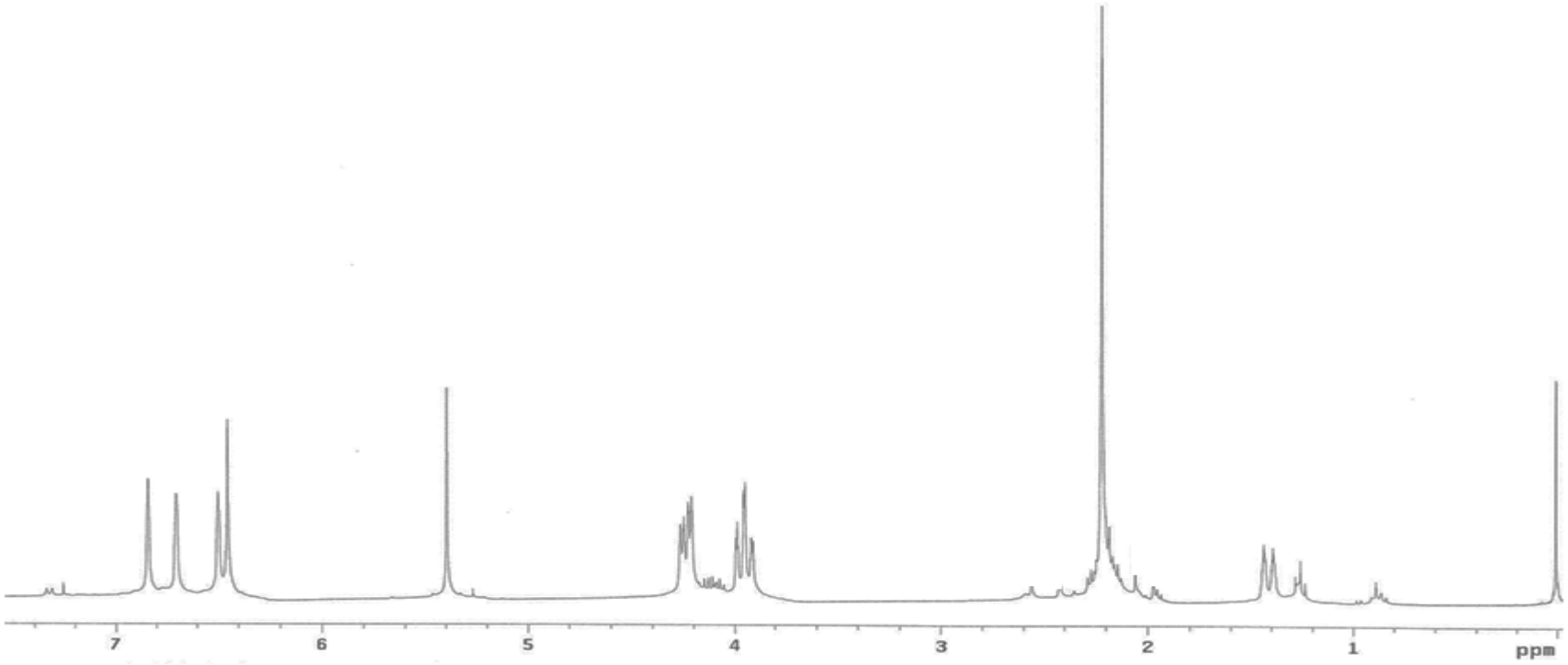




2-(3-Hydroxy-5-methylphenyl)-1,3-dioxane

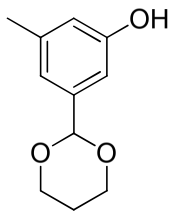
^{1}H NMR ($CDCl_3$, 300 MHz)

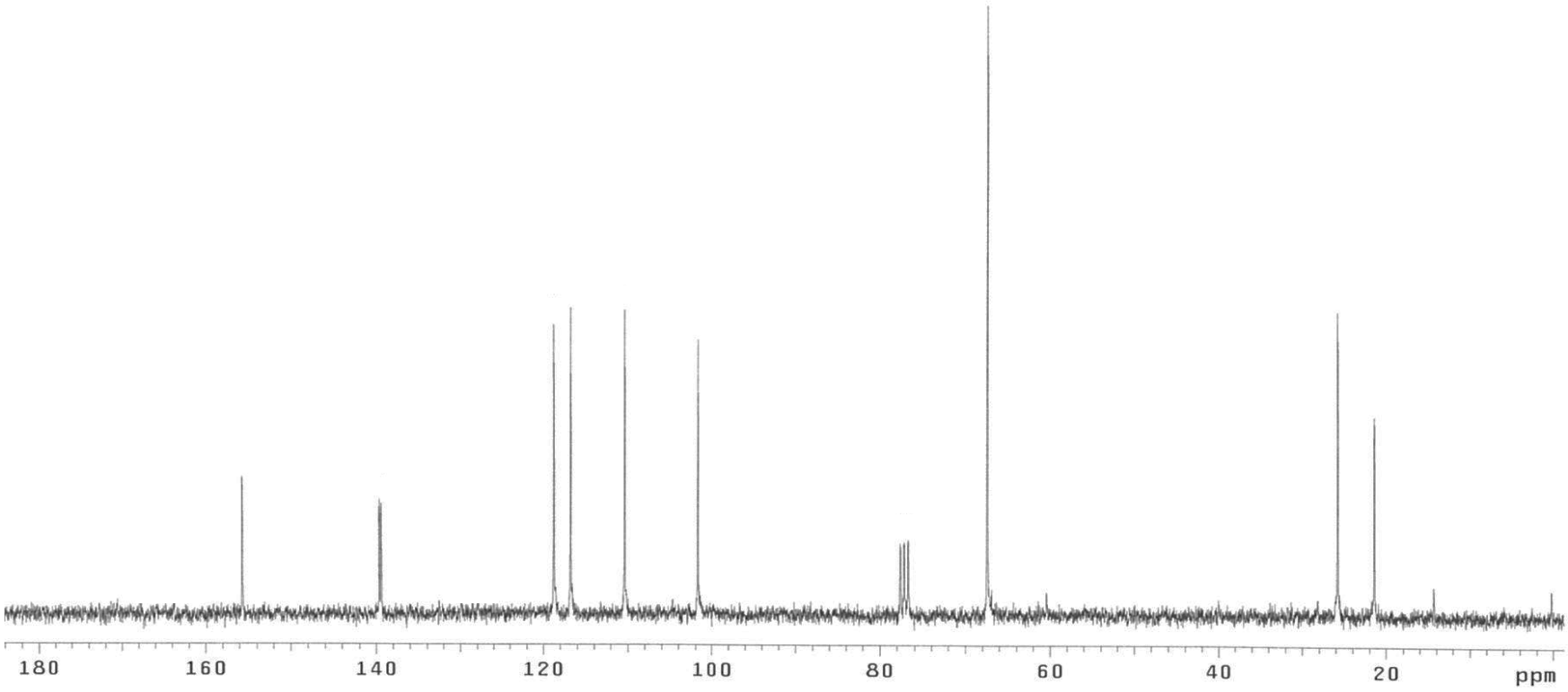




2-(3-Hydroxy-5-methylphenyl)-1,3-dioxane

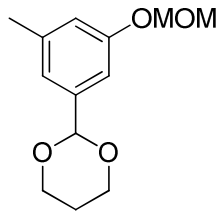
^{13}C NMR ($CDCl_3$, 75 MHz)

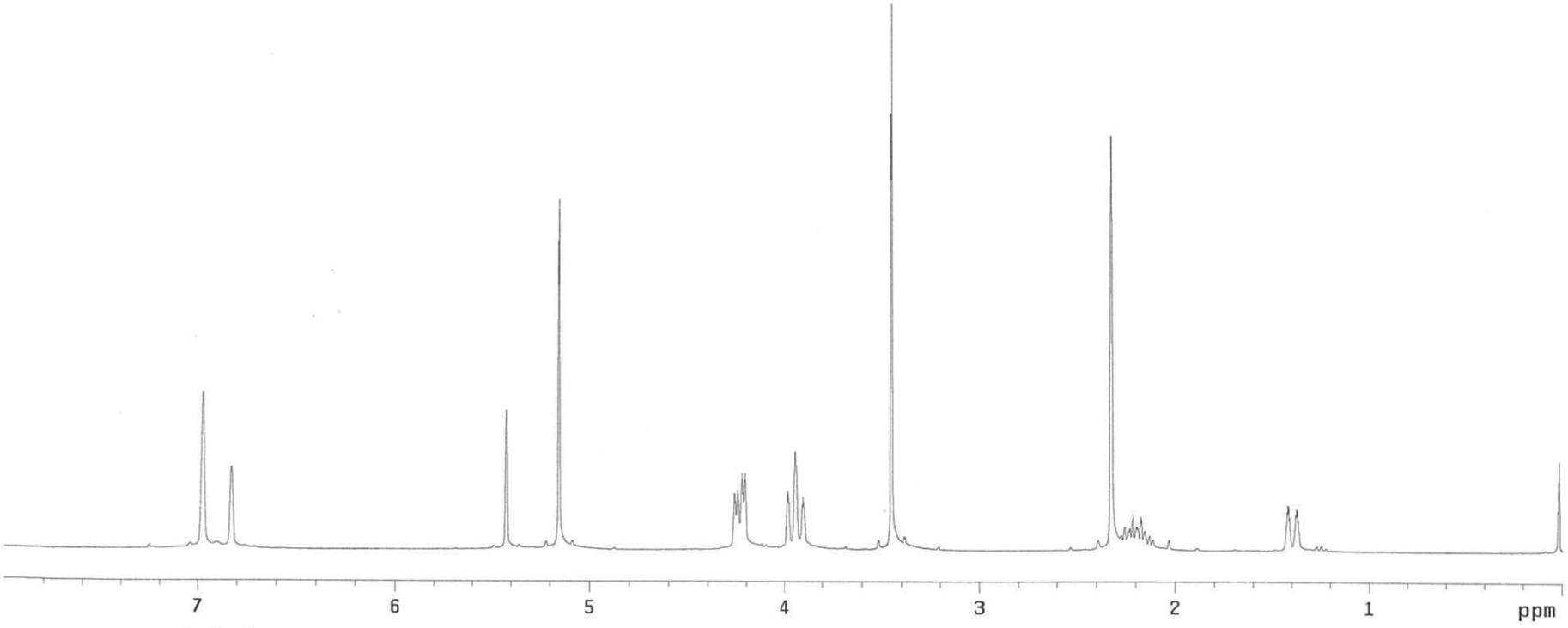




2-(3-Methoxymethoxy-5-methylphenyl)-1,3-dioxane (11)

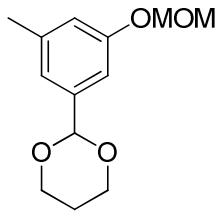
^{1}H NMR ($CDCl_3$, 300 MHz)

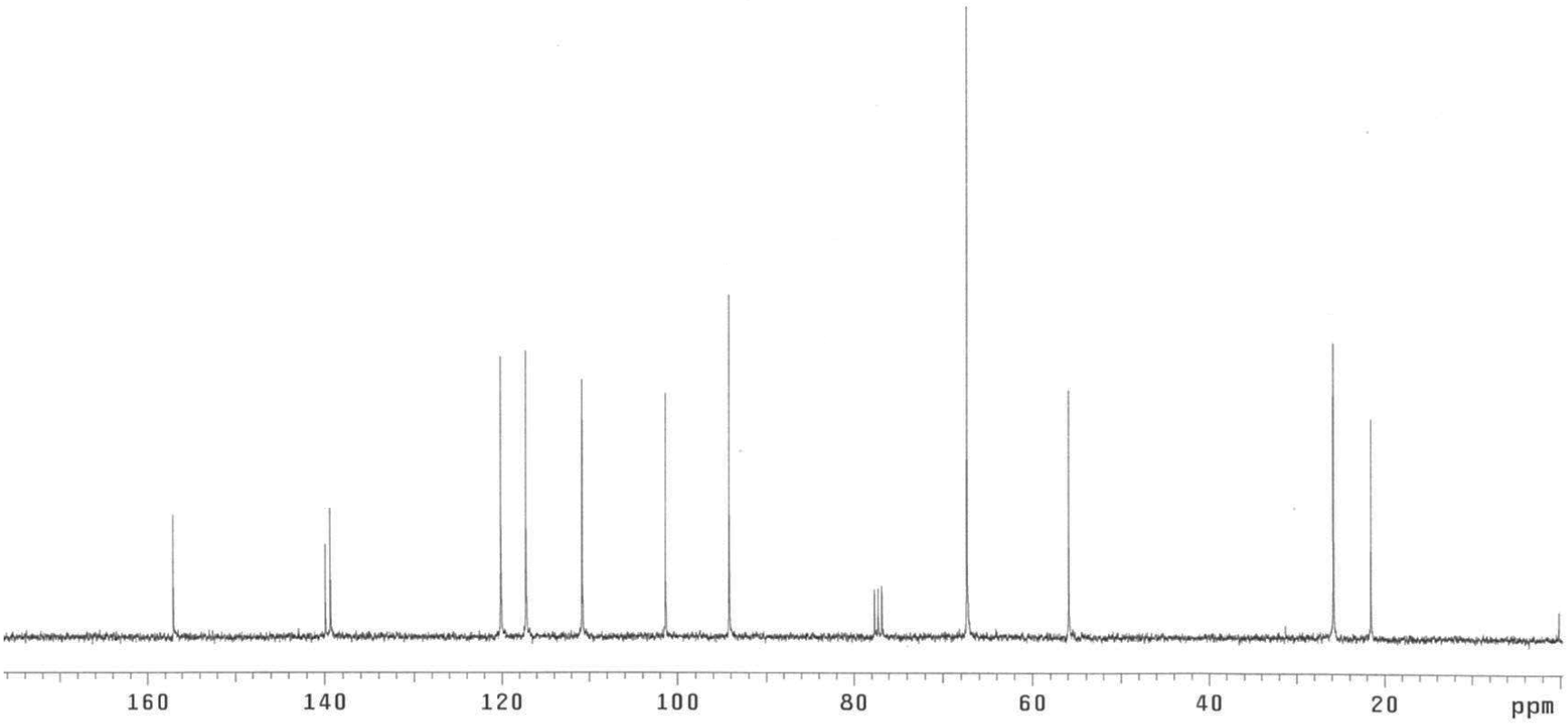




2-(3-Methoxymethoxy-5-methylphenyl)-1,3-dioxane (11)

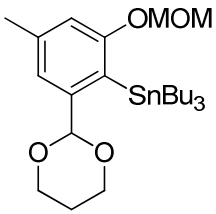
^{13}C NMR ($CDCl_3$, 75 MHz)

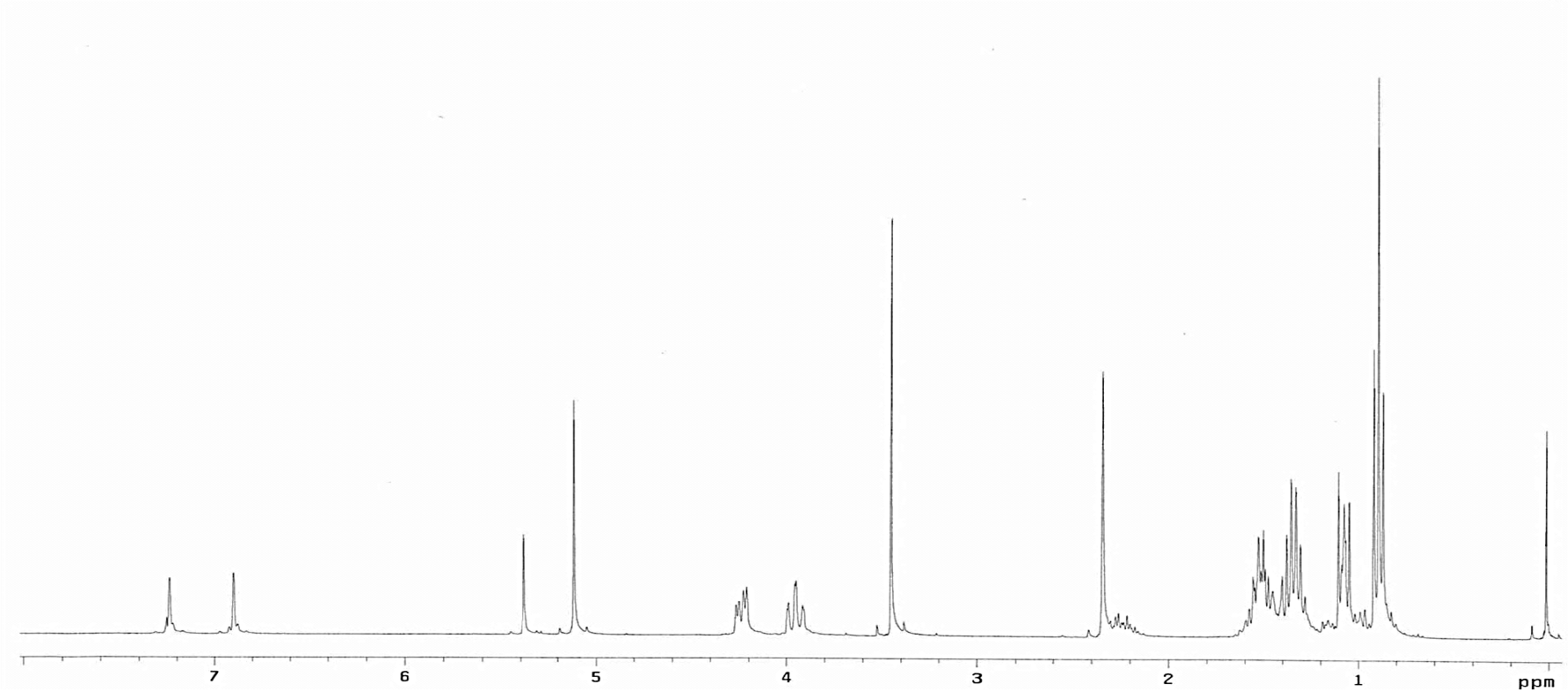




(2-(1,3-dioxan-2-yl)-6-(methoxymethoxy)-4-methylphenyl)tributylstannane (12)

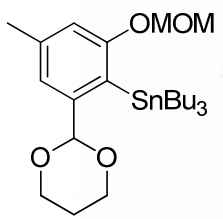
^{1}H NMR ($CDCl_3$, 300 MHz)





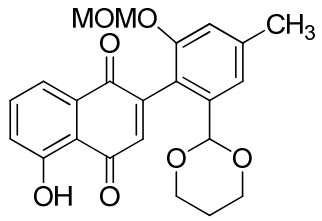
(2-(1,3-dioxan-2-yl)-6-(methoxymethoxy)-4-methylphenyl)tributylstannane (12)

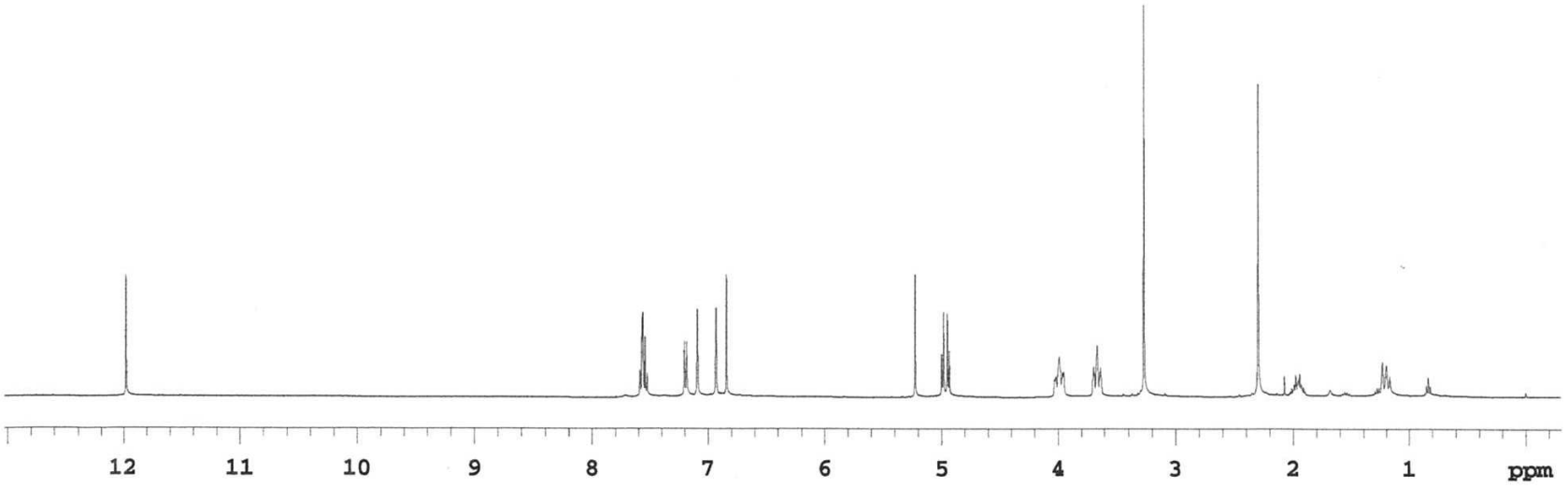
^{13}C NMR ($CDCl_3$, 75 MHz)



2-(2-(1,3-dioxan-2-yl)-6-(methoxymethoxy)-4-methylphenyl)-5-hydroxy-1,4-napthaquinone (14)

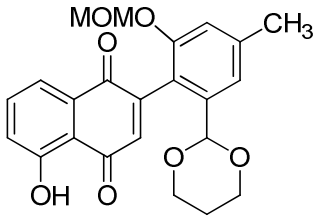
^{1}H NMR ($CDCl_3$, 400 MHz)

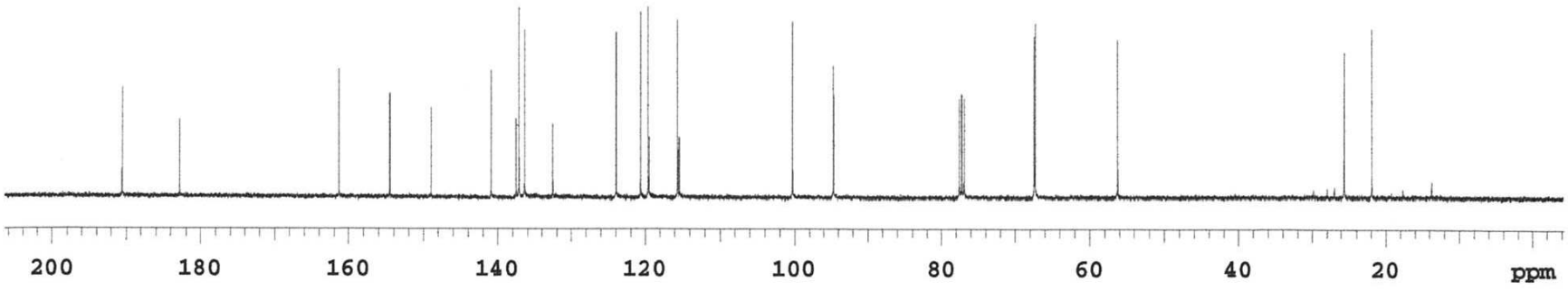




2-(2-(1,3-dioxan-2-yl)-6-(methoxymethoxy)-4-methylphenyl)-5-hydroxy-1,4-napthaquinone (14)

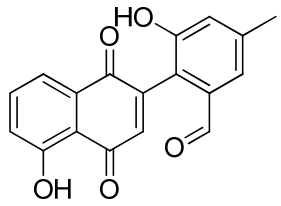
^{13}C NMR ($CDCl_3$, 100 MHz)

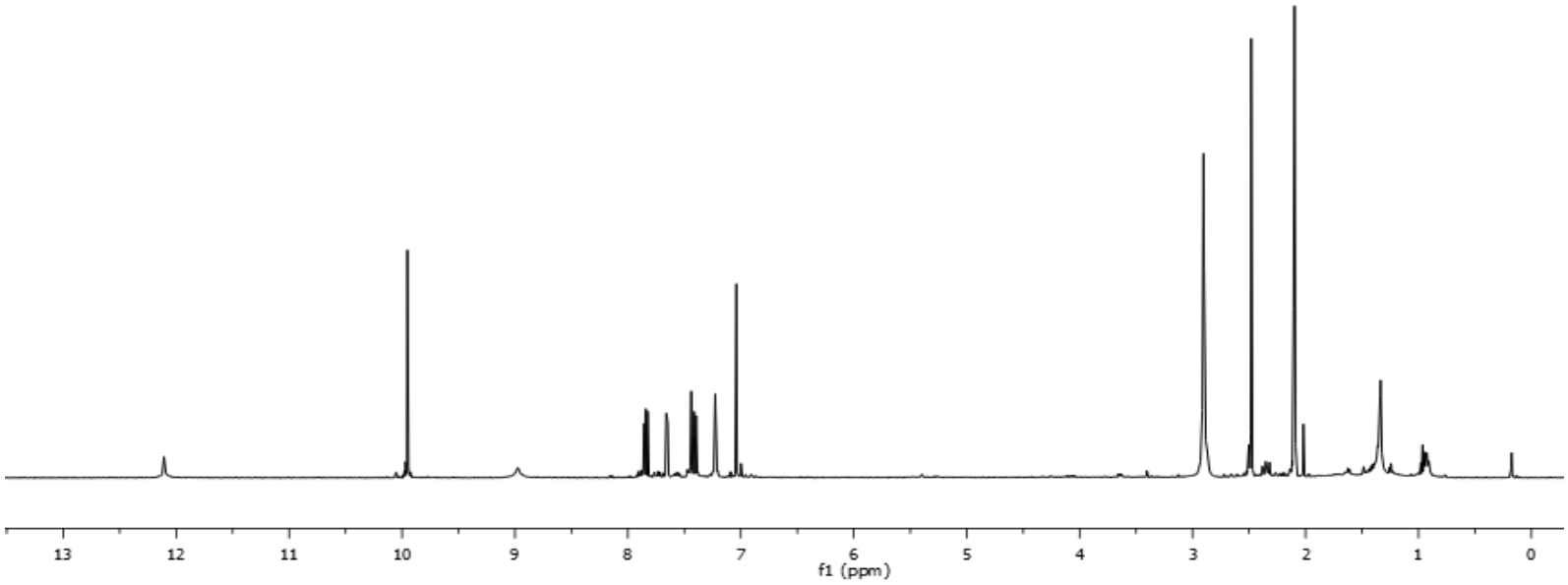




3-Hydroxy-2-(5-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-5-methylbenzaldehyde (15)

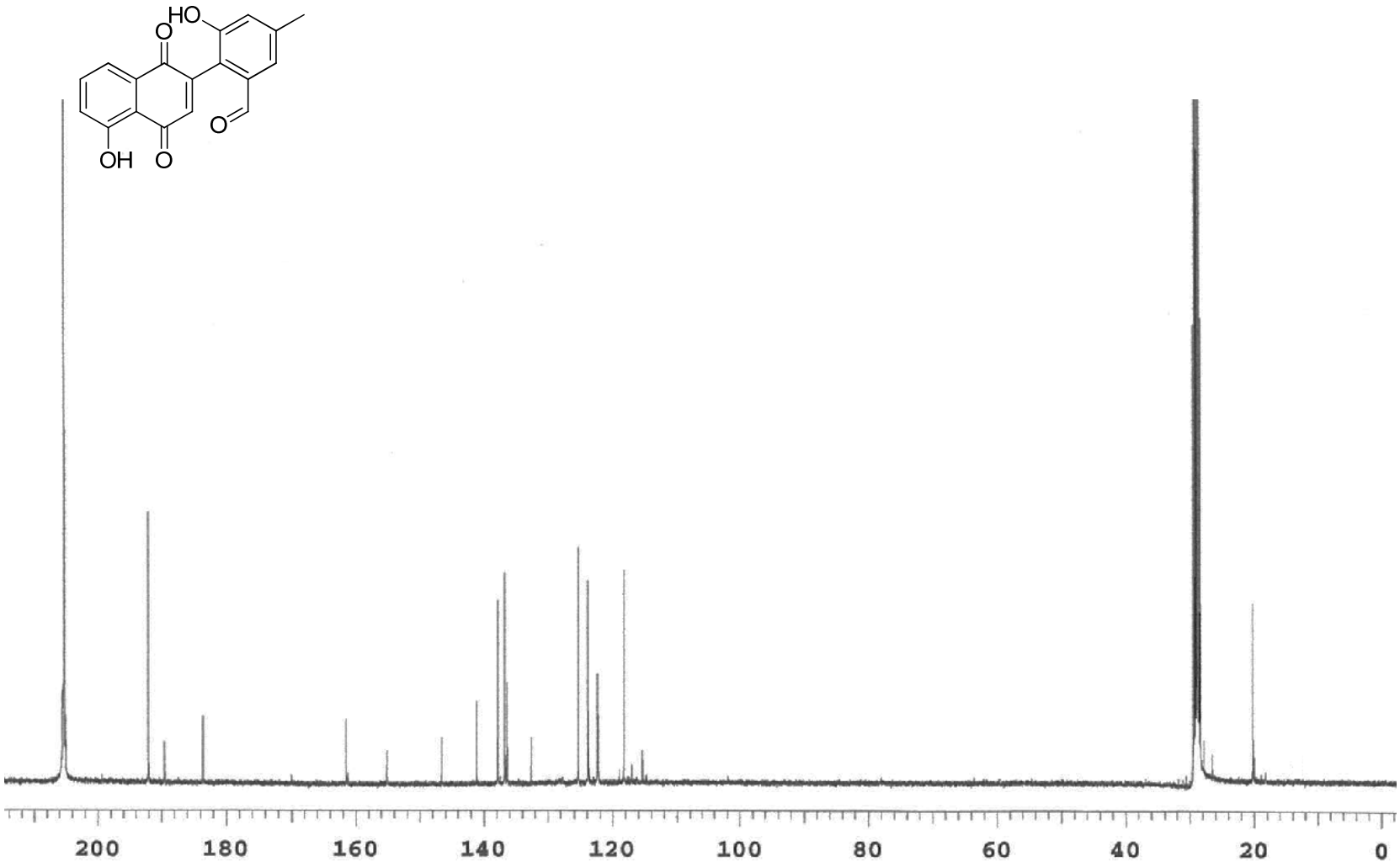
^{1}H NMR (acetone-d_6, 500 MHz)





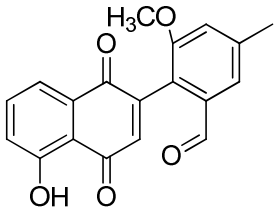
3-Hydroxy-2-(5-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-5-methylbenzaldehyde (15)

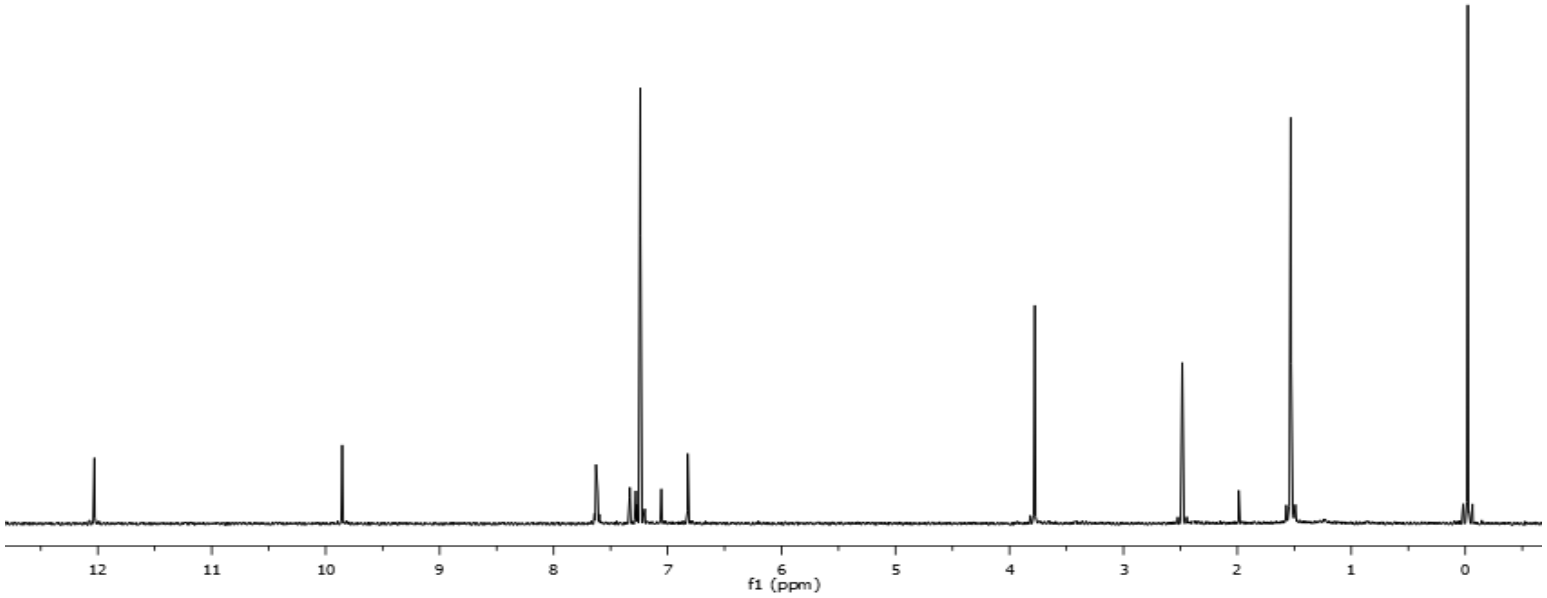
^{13}C NMR (acetone-d_6, 100 MHz)



2-(5-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-3-methoxy-5-methylbenzaldehyde (5)

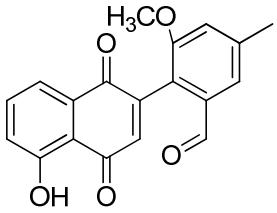
^{1}H NMR ($CDCl_3$, 400 MHz)





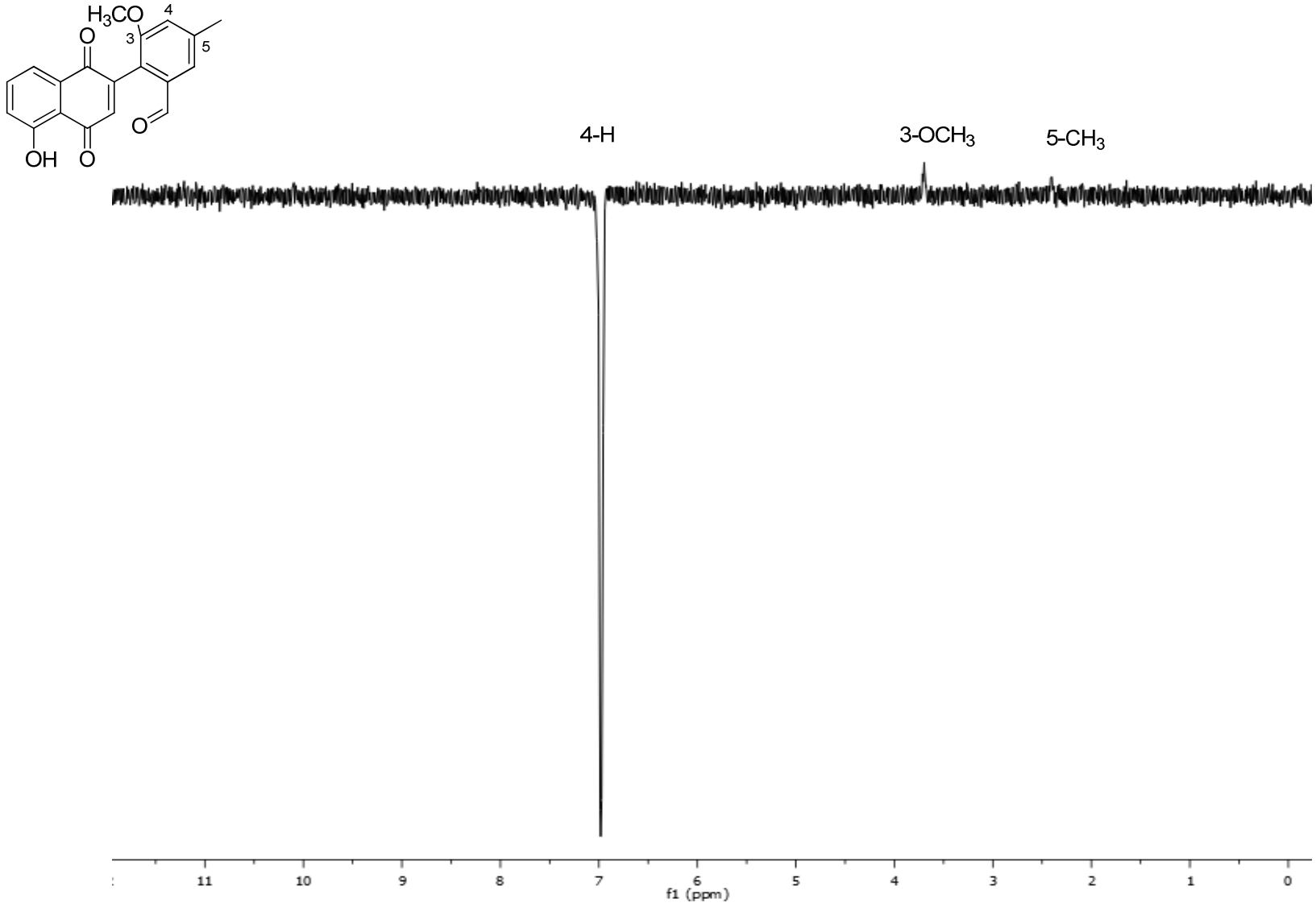
2-(5-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-3-methoxy-5-methylbenzaldehyde (5)

^{13}C NMR ($CDCl_3$, 100 MHz)



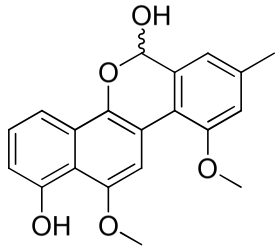
2-(5-hydroxy-1,4-dioxo-1,4-dihydronaphthalen-2-yl)-3-methoxy-5-methylbenzaldehyde (5)

NOE ($CDCl_3$, 400 MHz)



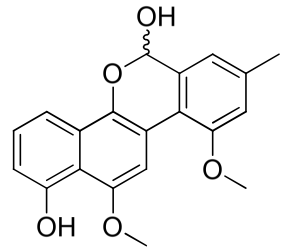
Defuco-pregilvocarcin M (6)

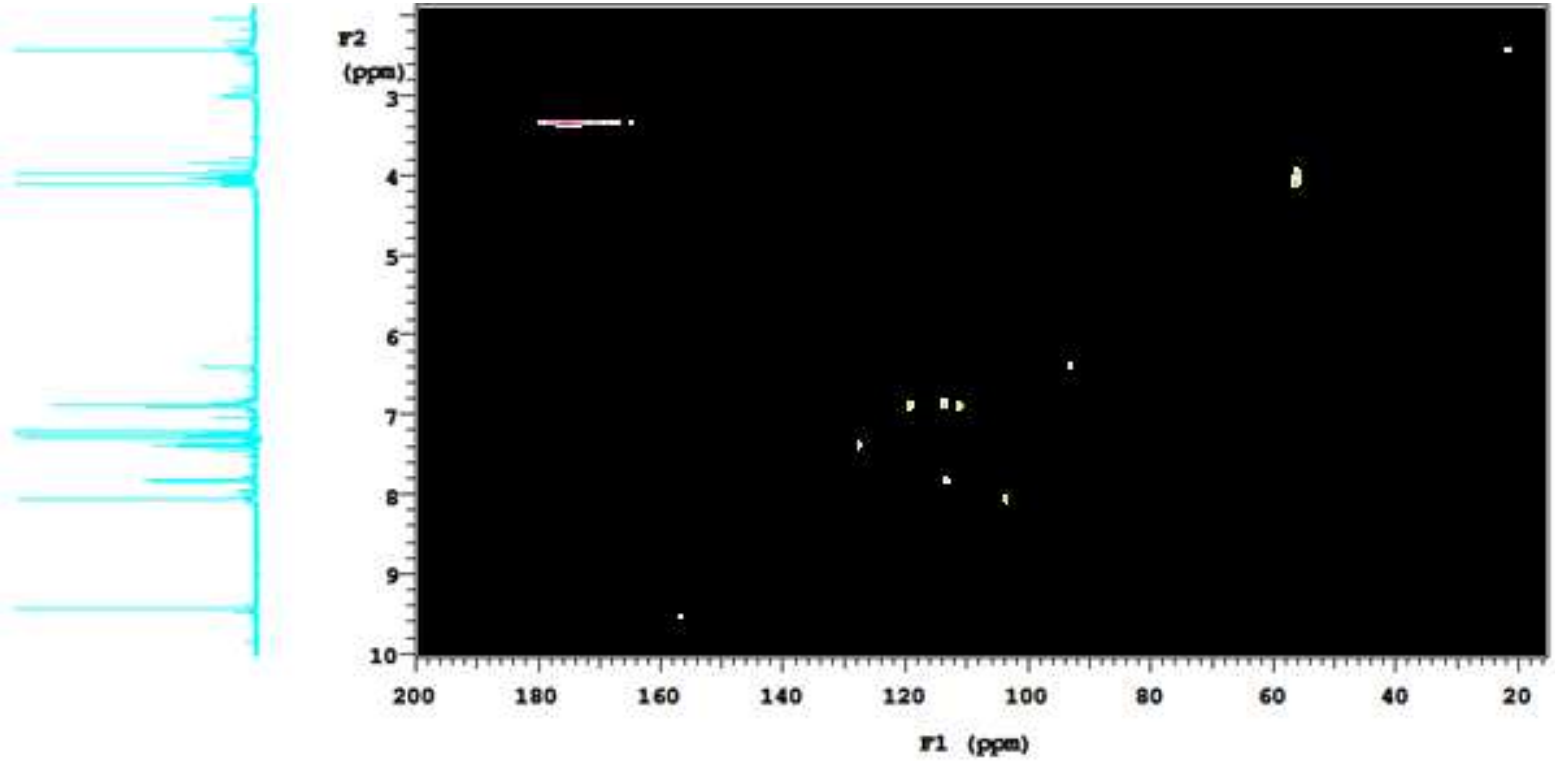
^{1}H NMR ($CDCl_3$, 500 MHz)



Defuco-pregilvocarcin M (6)

gHSQC ($CDCl_3$, 500 MHz)

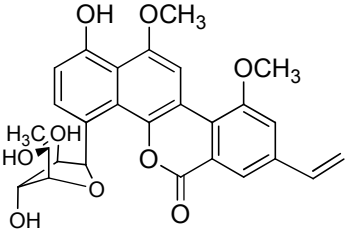
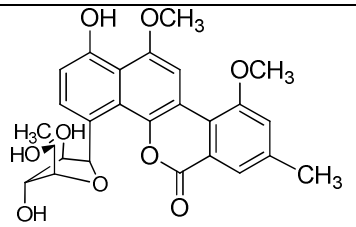
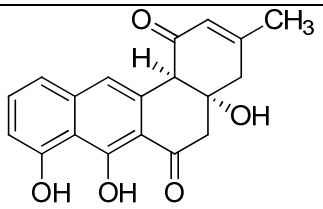
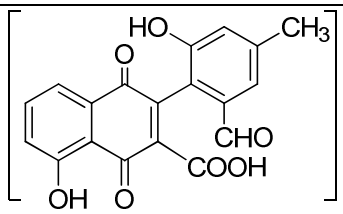
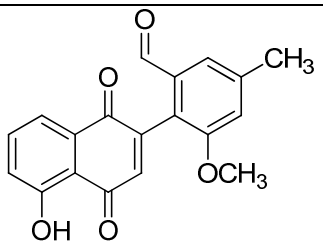


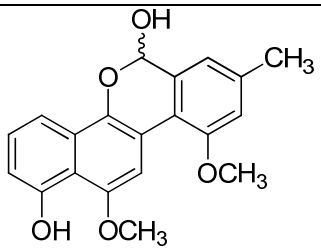
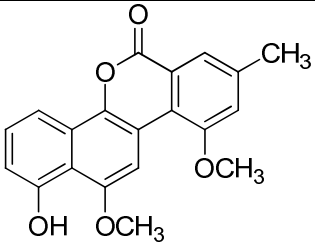
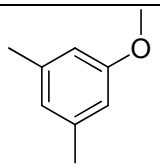
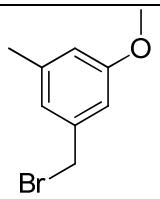
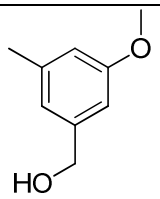
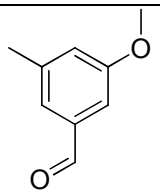
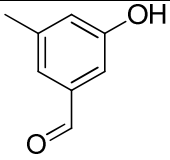


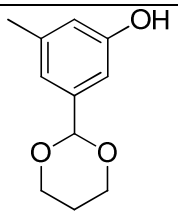
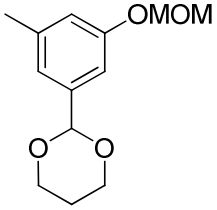
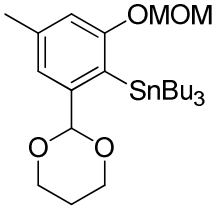
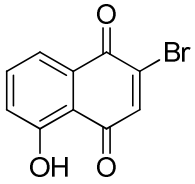
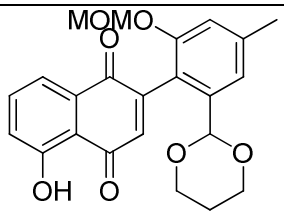
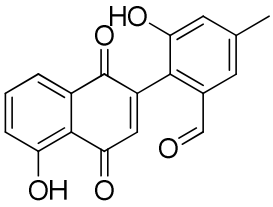
Section H: References

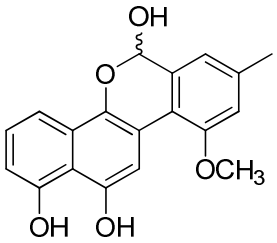
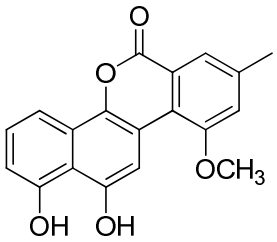
1) Srikrishna, A.; Ravikumar, P. C. *Synthesis-Stuttgart* **2007**, 65.
2) Brown, P. M.; Thomson, R. H. *J. Chem. Soc., Perkin Trans. 1*, **1976**, 997.
3) de Frutos, Ó.; Atienza, C.; Echavarren, A. M. *Eur. J. Org. Chem.* **2001**, 163–171.
4) Shan, M.; Sharif, E. U.; O'Doherty, G. A. *Angew. Chem. Int. Ed.* **2010**, *49*, 9492.

Section I: Compound Characterization

Compound	Structure	^{1}H NMR	^{13}C NMR	HRMS/ Elemental Analysis	Previously Characterized	
Gilvocarcin V (1)		-	-	-	Yes	Hosoya, T.; Takashiro, E.; Matsumoto, T.; Suzuki, K. *J. Am. Chem. Soc.* **1994**, *116*, 1004.
Gilvocarcin M (2)		-	-	-	Yes	Hosoya, T.; Takashiro, E.; Matsumoto, T.; Suzuki, K. *J. Am. Chem. Soc.* **1994**, *116*, 1004.
Prejadomycin (3)		-	-	-	Yes	Rix, U.; Wang, C. C.; Chen, Y. H.; Lipata, F. M.; Rix, L. L. R.; Greenwell, L. M.; Vining, L. C.; Yang, K. Q.; Rohr, J. *Chembiochem* **2005**, *6*, 838.
4		-	-	-	Proposed Intermediate	
5		✓	✓	✓	No	

6		✓	HSQC	✓	No	
Defuco-gilvocarcin M (7)		-	-	-	Yes	Takemura, I.; Imura, K.; Matsumoto, T.; Suzuki, K. *Org. Lett.* **2004**, *6*, 2503.
8		-	-	-	Purchased from Fisher Scientific.	
		-	-	-	Yes	Srikrishna, A.; Ravikumar, P. C. *Synthesis-Stuttgart* **2007**, 65.
		-	-	-	Yes	Srikrishna, A.; Ravikumar, P. C. *Synthesis-Stuttgart* **2007**, 65.
9		✓	✓	-	Yes	Srikrishna, A.; Ravikumar, P. C. *Synthesis-Stuttgart* **2007**, 65.
10		✓	✓	-	Yes	Brown, P. M.; Thomson, R. H. *J. Chem. Soc. Perkin Trans. 1*, **1976**, 997.

		✓	✓	✓	Yes	de Frutos, Ó.; Atienza, C.; Echavarren, A. M. *Eur. J. Org. Chem.* **2001**, 163.
11		✓	✓	✓	Yes	de Frutos, Ó.; Atienza, C.; Echavarren, A. M. *Eur. J. Org. Chem.* **2001**, 163.
12		✓	✓	✓	Yes	Shan, M.; Sharif, E. U.; O'Doherty, G. A. *Angew. Chem. Int. Ed.* **2010**, *49*, 9492.
Bromojuglone (13)		-	-	-	Yes	Kitani, Y.; Morita, A.; Kumamoto, T.; Ishikawa, T. *Helv. Chim. Acta* **2002**, *33*, 1186.
14		✓	✓	-	Yes	Shan, M.; Sharif, E. U.; O'Doherty, G. A. *Angew. Chem. Int. Ed.* **2010**, *49*, 9492.
15		✓	✓	✓	Yes	Shan, M.; Sharif, E. U.; O'Doherty, G. A. *Angew. Chem. Int. Ed.* **2010**, *49*, 9492.

16		-	-	✓	No	-
17		-	-	✓	Yes	de Frutos, Ó.; Atienza, C.; Echavarren, A. M. *Eur. J. Org. Chem.* **2001**, 163.