An Alkylbenzoquinone Involved in Development of Cellular Slime Molds

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Supporting Information

Contents

Generals

UV and IR spectra were recorded on JASCO Ubest V-560 (cell length 10 mm) and FT-IR-410 spectrophotometers, respectively. ^{1}H and ^{13}C NMR spectra were recorded on JEOL ECA-500 (^{1}H:500 MHz and ^{13}C: 125 MHz), JEOL ECA-600 (^{1}H:600 MHz and ^{13}C: 150 MHz), and Bruker Avance III HD600 (^{1}H:600 MHz and ^{13}C: 150 MHz) spectrometers. Chemical shifts for ^{1}H and ^{13}C NMR are given in parts per million (δ) relative to TMS (δ_H 0.00) or solvent signals (methanol-d_4: δ_H 3.30, δ_C 49.0, and chloroform-*d*: δ_H 7.26, δ_C 77.0) as internal standards, respectively. Positive and negative LC-ESI-MS was obtained with JEOL JMS-T100LP equipped with Agilent-1100 HPLC system, which was performed with the following conditions: 30% methanol–H_2O for 3 min followed by linear gradient upto 70% methanol–H_2O for 5 min at a flow rate of 0.2 mL/min by using CAPCELL PAK UG120 C18 3μm (ϕ2.0 × 50 mm, Shiseido, Tokyo) column. LR- and HR-EIMS was obtained on JMS MS-700 (JEOL). GC-MS analysis was carried out on GCMS-GP2010 system equipped with GC-2010 GC system. Analytical TLC was performed on silica gel 60 F254 (Merck). Column chromatography was carried out on silica gel BW-820MH (Fuji Silysia Chemicals, Co. Ltd, Seto, Japan). ODS (Develosil ODS UG-5, ϕ20 × 250 mm), C-8 (Develosil C8-5, ϕ20 × 250 mm), and C-30 (Develosil C8-5, ϕ20 × 250 mm) columns were used for preparative HPLC, and C-8 (Develosil C8-5, ϕ4.6 × 250 mm) column were used for analytical HPLC. All columns for HPLC are products of Nomura Chemical, Seto, Japan.

Assay for prespore cell-inducing activity

Bioassays were carried out basically as described previously.[18] Amoebae of *D. discoideum* strain V12M2 were used as tester cells. After washing in Bonner's standard salt solution,[19] vegetative amoebae were plated in 3.5 cm Nunc culture dishes containing 1 mL of buffered salt solution (10 mM KCl, 10 mM NaCl, 3 mM $CaCl_2$, 1 mM $MgCl_2$ 15 μg/mL tetracycline, 5 mM KH_2PO_4/K_2HPO_4 [pH 6.3]) in the presence of 2.5 mM Na-cAMP and prespore cell inducing factor (PsiA)[18] at a density of 5 x $10^2/cm^2$. As PsiA, we used 20 μL of HL5 medium conditioned by growth of a *psiA*^{oe} transformant. The transformant releases PsiA into HL5 medium during growth.[17] Before use, conditioned HL5 was dialyzed with

Microcon (10 kDa cutoff, Millipore) and was treated with Amberlite XAD-2 resin to eliminate low molecular-weight factors involved in cell differentiation such as DIF-1,[21] MPBD[9] and dictyoquinone.[18] Various concentrations of compounds **A** or **B** diluted with 20 mM Tris-HCl buffer (pH 9.3) were added at the beginning of incubation.

After incubation at 24 °C for 22 h, cells[18] were fixed *in situ* with methanol and stained with FITC-conjugated anti-*D. mucoroides* spore serum. Cells that contained FITC-stained granules (PSVs) in their cytoplasm were considered to be prespore cells.

For bioassays, synthetic **A** and **B** was dissolved in dry dimethyl sulfoxide (DMSO) at 50 μg/mL and stored at −20 °C under argon until use.

Assay for D-factor activity

The strain A586 (*tsg-119 cyc-1 aggA586*) is a non-aggregating *aggA* mutant of *Polysphondylium violaceum*.[22] A586 cells grown for 2 days on *K. aerogenes* were washed free of bacteria in 20 mM phosphate buffer (pH 6.0) and spotted on phosphate buffered agar plate. Stock solutions of compounds **A** and **B**, and DMSO were diluted as indicated in the phosphate buffer (pH 6.0) just before use. One hour after the start of starvation, small pieces of filter paper (2.5 mm × 2.5 mm) soaked with 2 μL each of the test solutions were placed around the A586 cells, and the development was monitored at intervals for over 2 days.

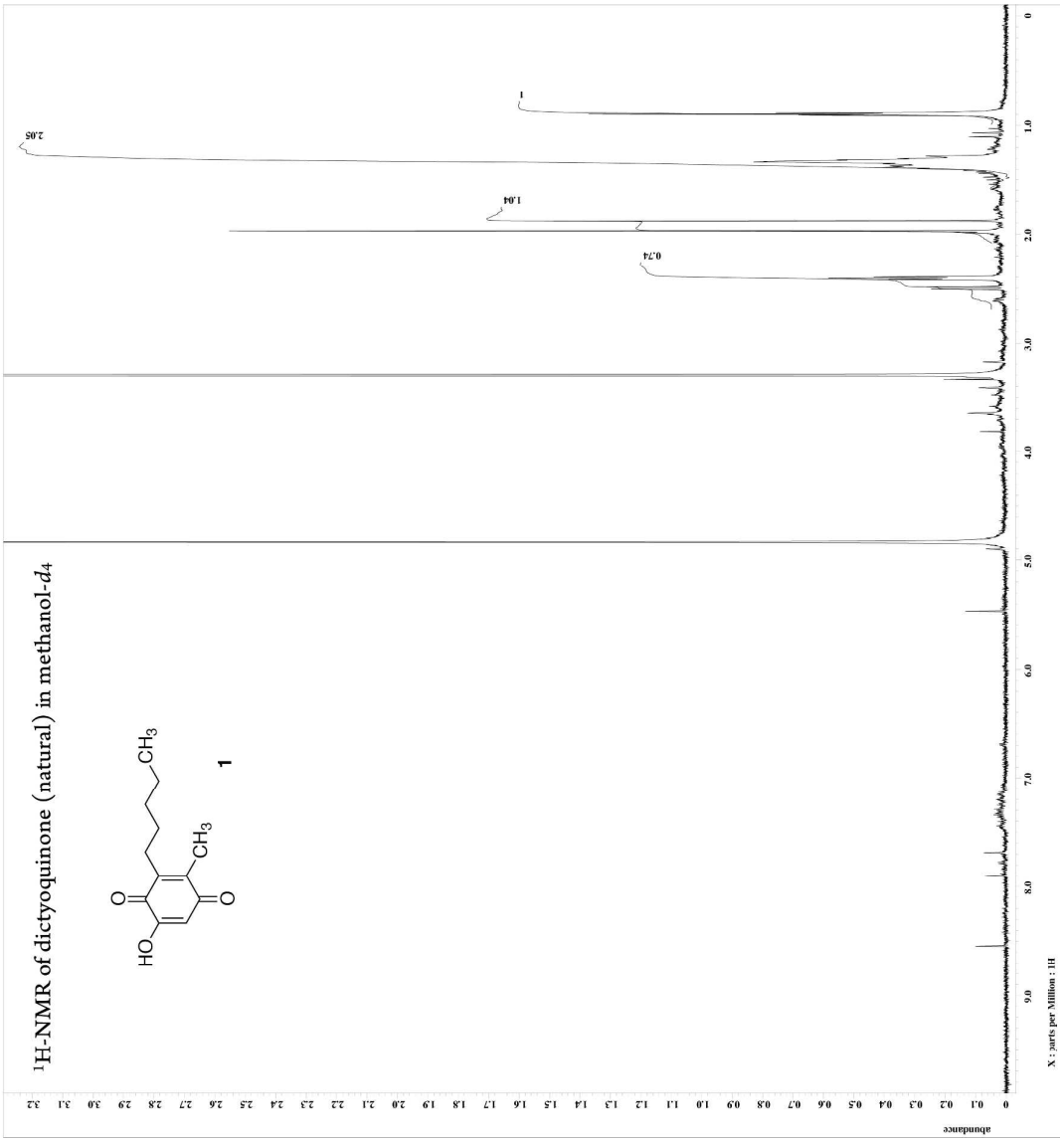
*Preparation of conditioned medium (CM) and purification of the dialyzable prespore-cell inducing factor, dictyoquinone (**1**)*

The preparation of CM and isolation procedure of dictyoquinone (**1**) were carried out as described in our previous paper.[14] Less than 400 μg (estimated from absorbance at 491 nm) of dictyoquinone (**1**) was obtained from 12 L of CM.

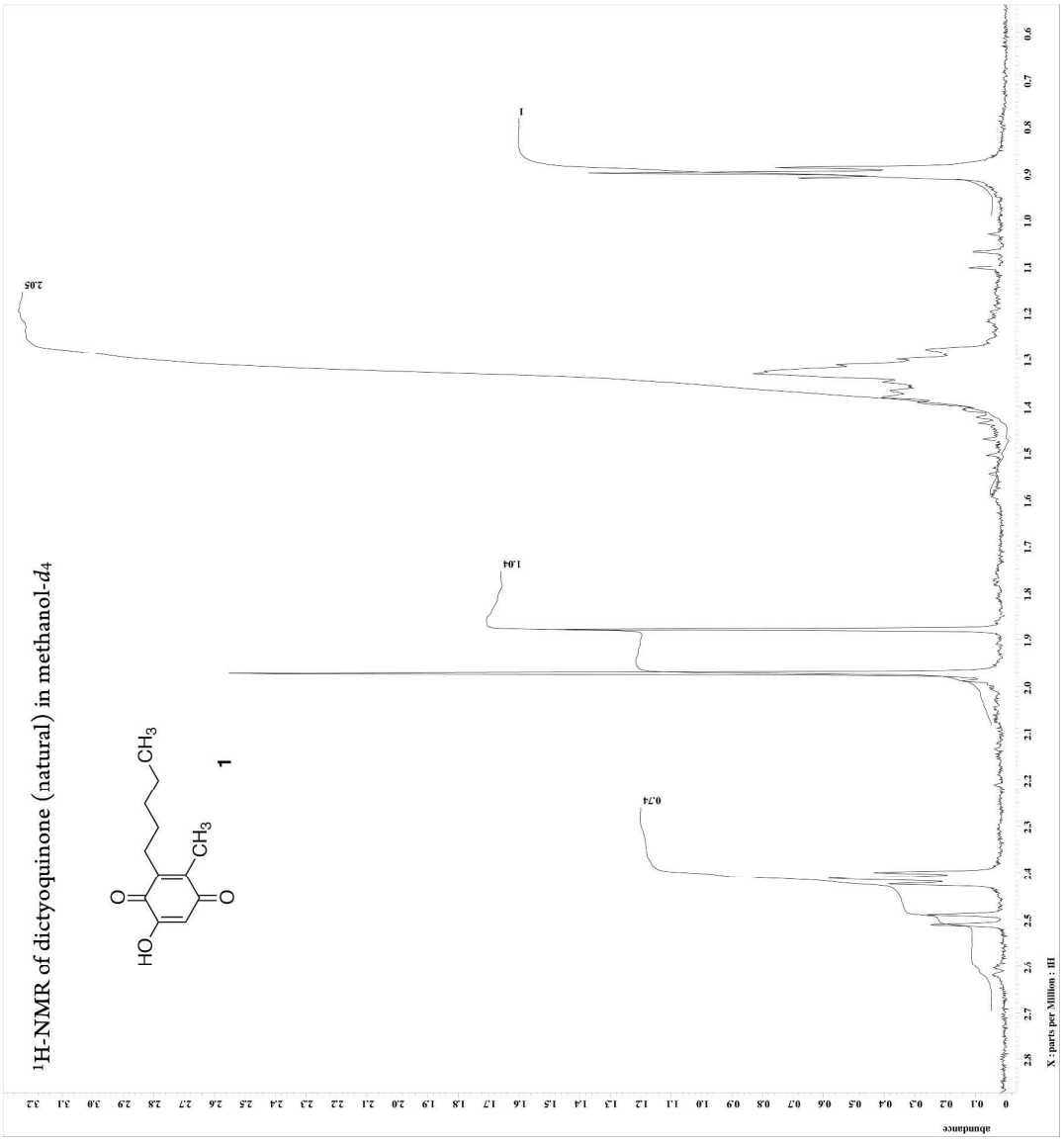
There are signals at δ_H 2.50 and δ_H 1.88. The ratio of the integration value of each signal is different comparing to other signals. Accordingly, we judged that those are signals of different impurities. Further purification of the compound was given up since we observed lability of the active principle in the process of purification as well as in the assay conditions as described in our previous report.[14]

Dictyoquinone (**1**): ^{1}H-NMR (600 MHz, methanol-d_4, δ) 2.413 (2H, t, J = 7.6 Hz), 1.972 (3H, s), 1.41–1.27 (6H, m), 0.899 (3H, t, J = 6.5 Hz), HR-ESI-MS m/z 208.1084 $[M]^+$ (calcd. 208.1099 for $H_{12}H_{16}O_3$), UV (MeOH) λ_{max} nm 283, 351, 491.

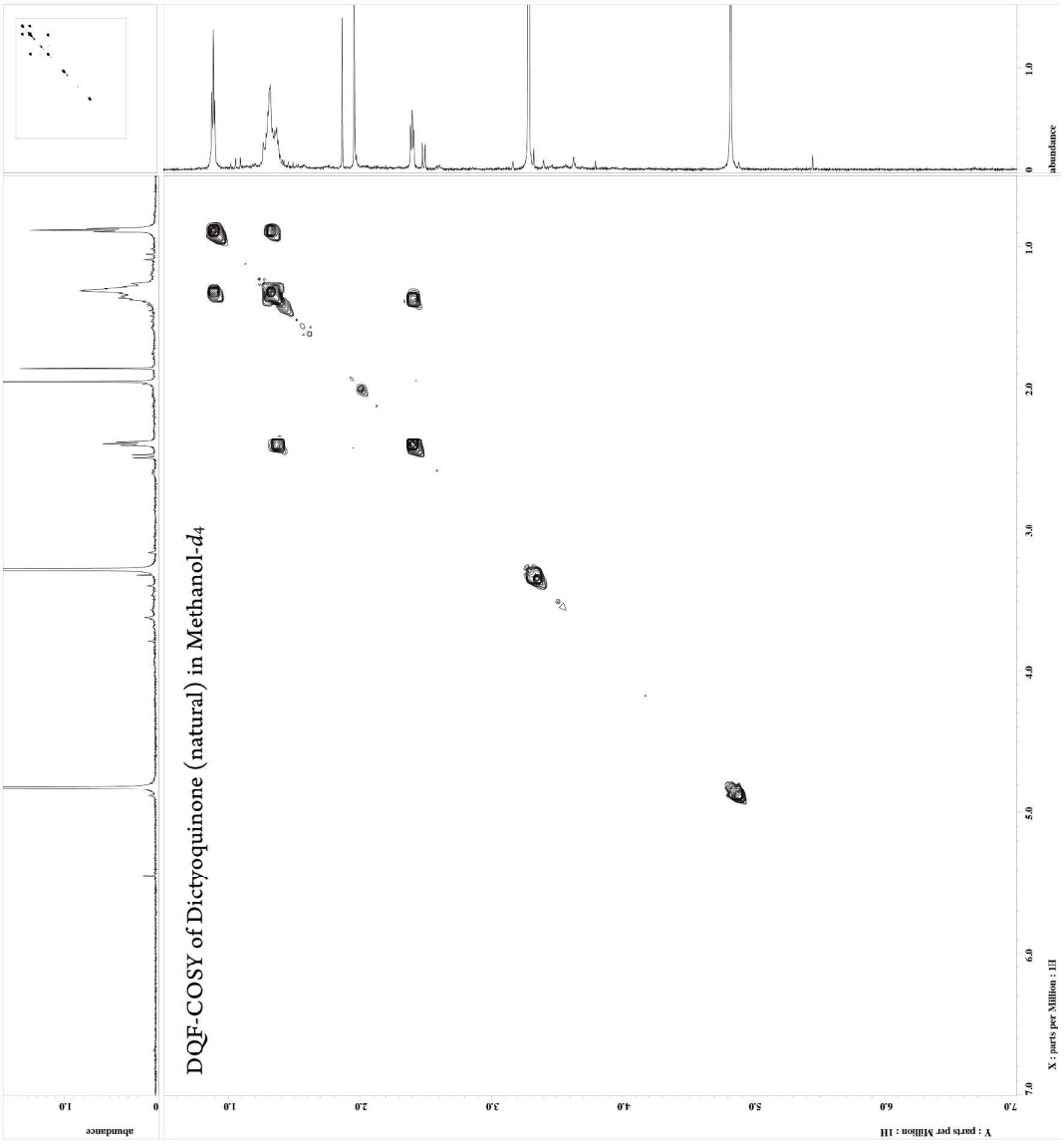
^{1}H-NMR spectrum of dictyoquinone (natural) (600 MHz, methanol-d_4)



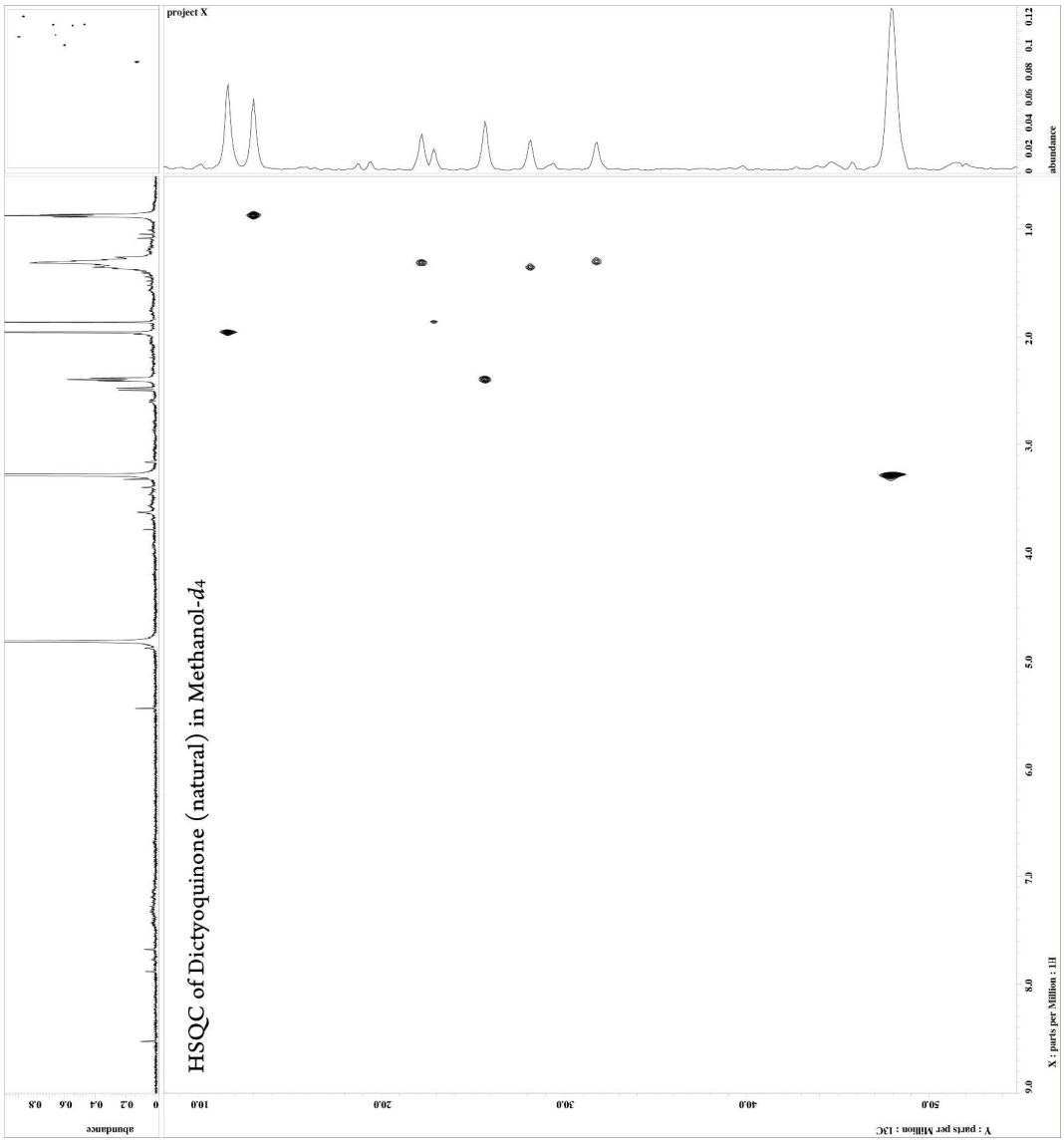
^{1}H-NMR spectrum of dictyoquinone (natural) (600 MHz, methanol-d_4) (expanded)



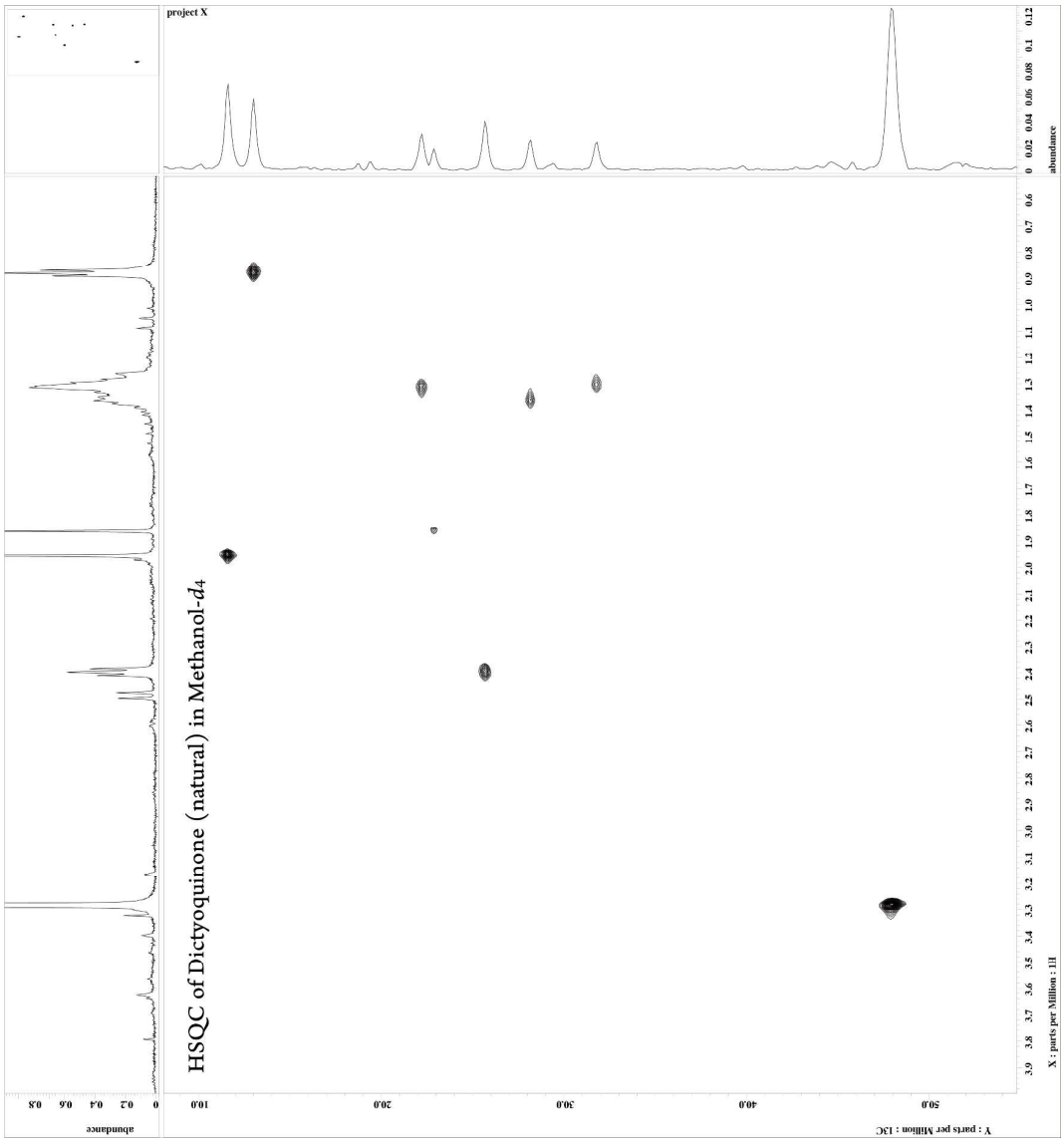
DQF-COSY spectrum of dictyoquinone (natural) in methanol-d_4



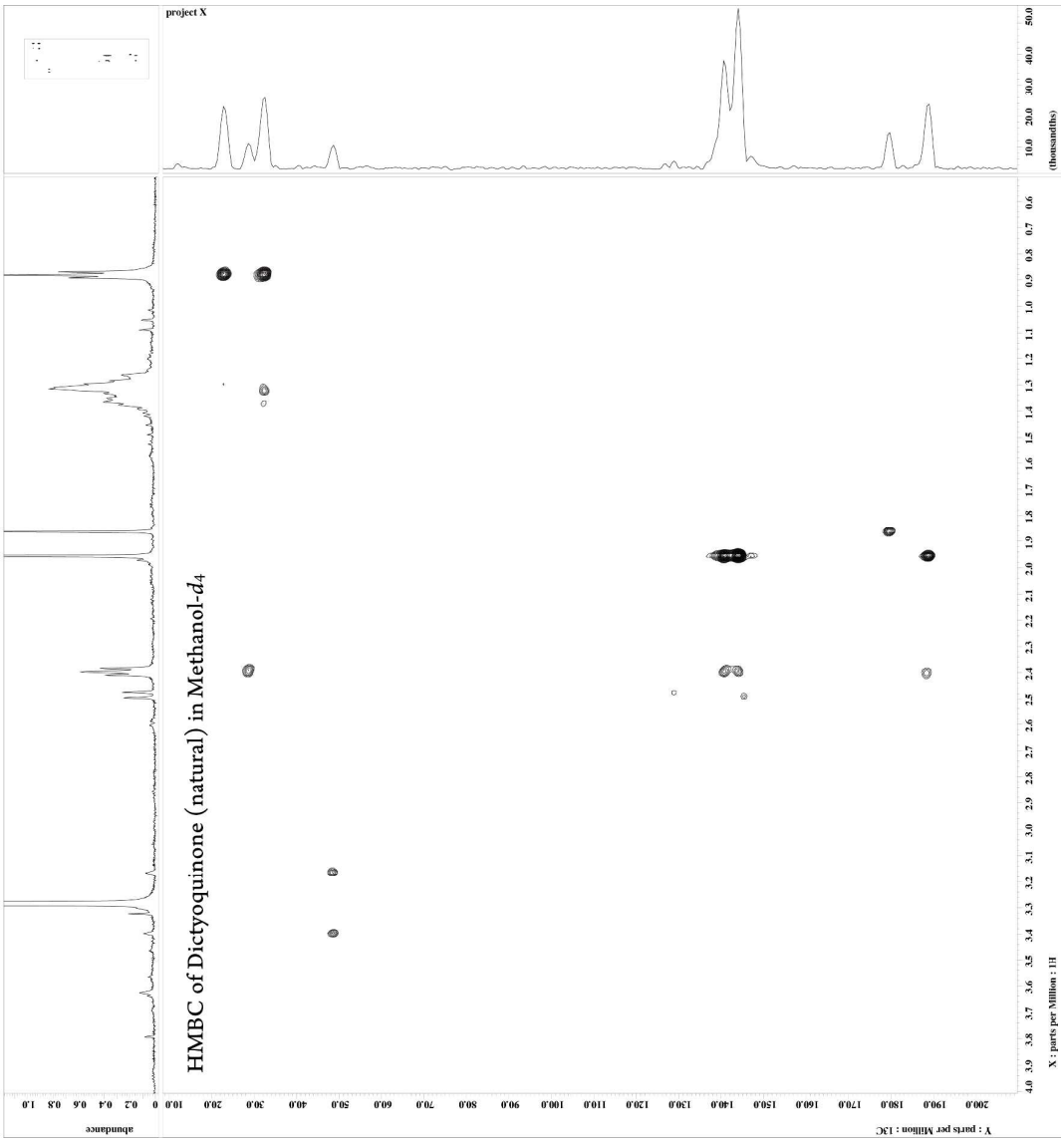
HSQC spectrum of dictyoquinone (natural) in methanol-d_4



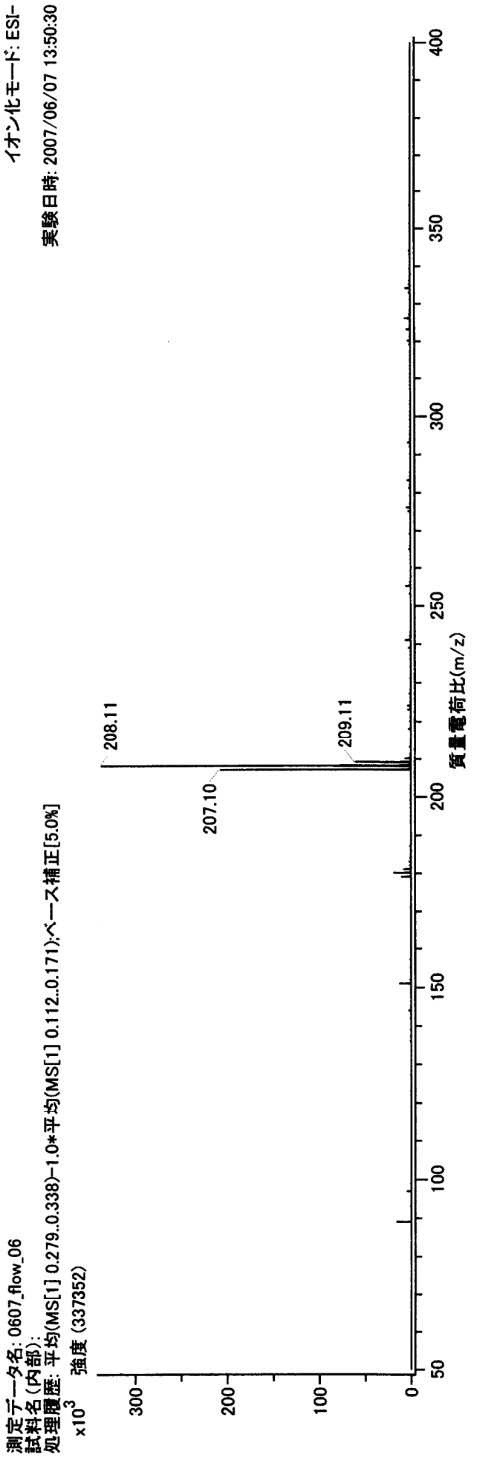
HSQC spectrum of dictyoquinone (natural) in methanol-d_4 (expanded)



HMBC spectrum of dictyoquinone (natural) in methanol-d_4

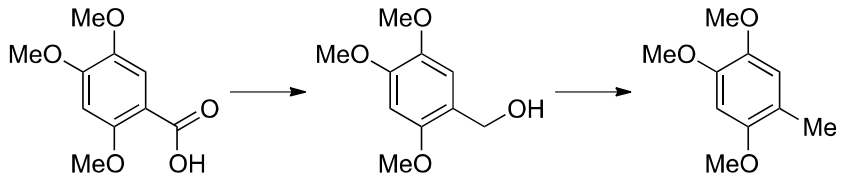


ESI-MS of dictyoquinone (natural)



Synthesis of Compound A

*Preparation of 2,4,5-trimethoxytoluene (**5**)*

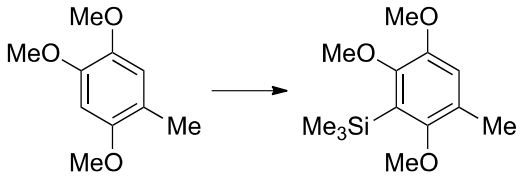


4 **5**

To a solution of 2,4,5-trimethoxybenzoic acid (**4**) (2.0 g, 9.43 mmol) in dry THF (10 mL), 1.0 M BH_3-THF complex (20 mL) was added over 30 min at 0 °C under Ar atmosphere. Then the solution was stirred at room temperature for 30 min. Methanol was added to the solution to quench the excess BH_3 till the generation of gas was no longer observed. The reaction mixture was then evaporated after another 20 mL of methanol was added to the solution. The residue was dissolved to methanol (10 mL), and evaporated into dryness. This procedure was repeated three times. The resulted residue was purified by a chromatography with SiO_2 using a mixture of hexane–EtOAc (9:1) to afford 2,4,5-trimethoxybenzyl alcohol. The alcohol (451 mg, 2.28 mmol) in 3 mL of CH_2Cl_2 was treated with triethylsilane (2.2 mL, 13.8 mmol) in the presence of TMSOTf (0.42 mL, 2.32 mmol) at 0 °C under Ar atmosphere. The reaction mixture was washed with satd. $NaHCO_3$, and extracted with chloroform. The organic layer was washed with water and brine, and evaporated. The residue was chromatographed with SiO_2 to afford **5**. Yield: 88.4% from **4**.

2,4,5-Trimethoxybenzyltoluene (**5**)(CAS 14894-74-7): ^{1}H-NMR ($CDCl_3$, δ) 6.63 (1H, s), 6.44 (1H, s), 3.80 (3H, s), 3.76(3H, s), 3.73 (3H, s), 2.10 (3H, s), IR (KBr) ν_{max} 2955, 2833 cm^{-1}, GC-MS *m*/*z* 182 $[M]^+$, UV (MeOH) λ_{max} nm (log ε) 208 (sh, 3.74), 227 (sh, 3.15), 292 (2.68).

*Preparation of 2,4,5-trimethoxy-3-trimethylsilyltoluene (**6**)*



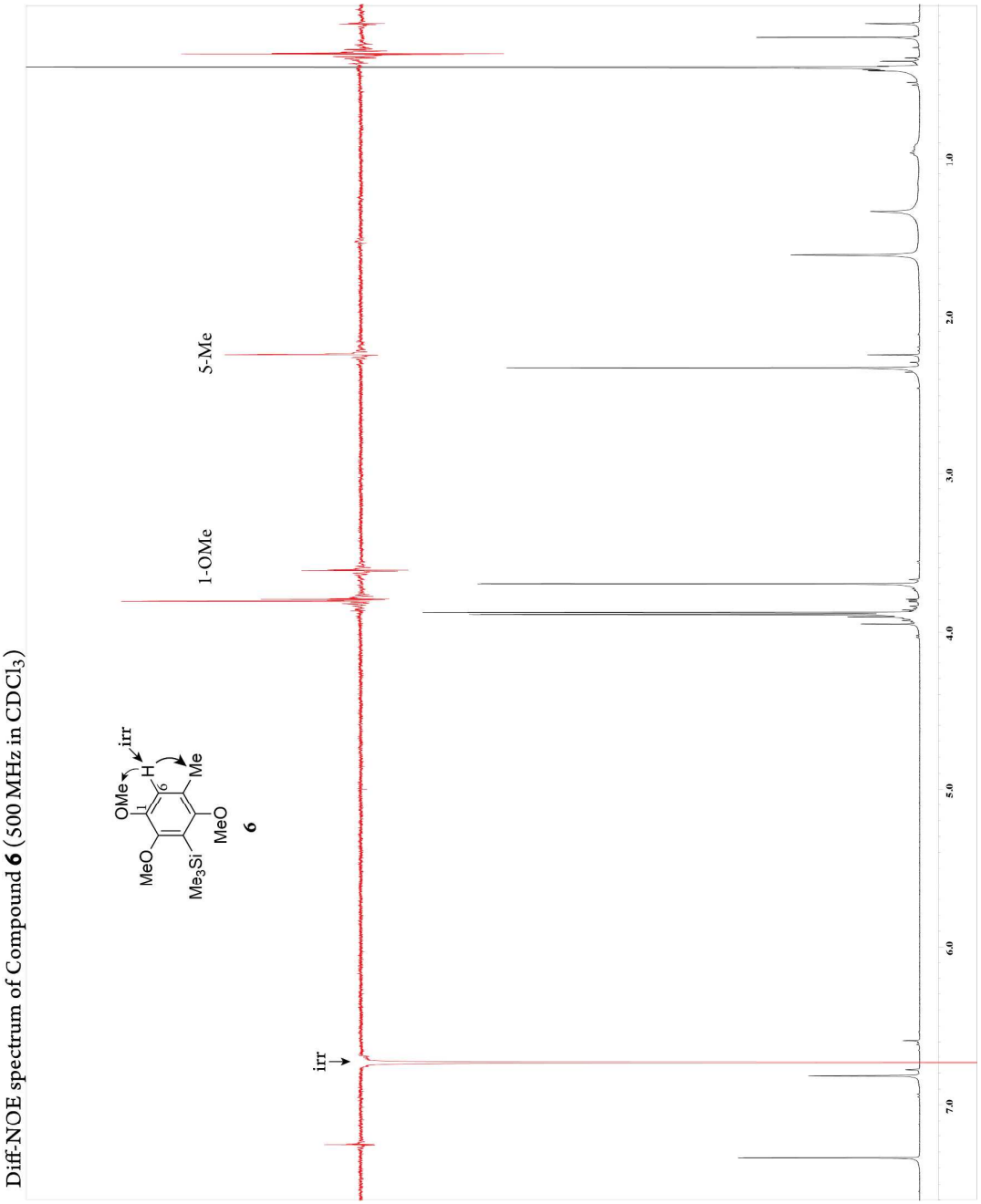
5 **6**

To a solution of **5** (440 mg, 2.42 mmol) in dry THF (24 mL), 1.1 mL of *N,N,N′,N′*-tetramethylethylenediamine (TMEDA) (7.35 mmol) was added at room temperature, and *sec*-BuLi (0.1 M in cyclohexane) (7.3 ml) was added at –78 °C. After the reaction mixture was kept at the temperature for 30 min, TMSCl (1.2 ml, 9.5 mmol) was added. The temperature was raised up to room temperature over 90 min. The reaction was quenched by adding water and the reaction solution was extracted with chloroform. The organic layer was washed with water and evaporated. The residue was purified by SiO_2 chromatography to give **6** (550 mg, yield 89.5%).

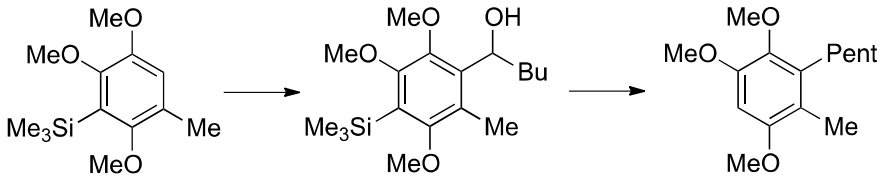
The position of silylation was determined on the basis of NOE experiments.

2,4,5-Trimethoxy-3-trimethylsilyltoluene (**6**)(CAS 138865-90-4): ^{1}H-NMR ($CDCl_3$, δ) 6.74 (1H, s), 3.81 (3H, s), 3.80 (3H, s), 3.62 (3H, s), 2.25 (3H, s), 0.34 (9H, s), ^{13}C-NMR ($CDCl_3$, δ) IR (KBr) ν_{max} 2940, 2900, 2836, 1590 cm^{-1}, GC-MS *m*/*z* 254 $[M]^+$, UV (MeOH) λ_{max} nm (log ε) 204 (3.74), 229 (3.38), 293 (sh, 3.06), 290 (3.09).

*Difference NOE spectrum of **6** (500 MHz, in $CDCl_3$)*



*Preparation of 1,2,4-trimethoxy-5-methyl-6-pentylbenzene (**7**)*

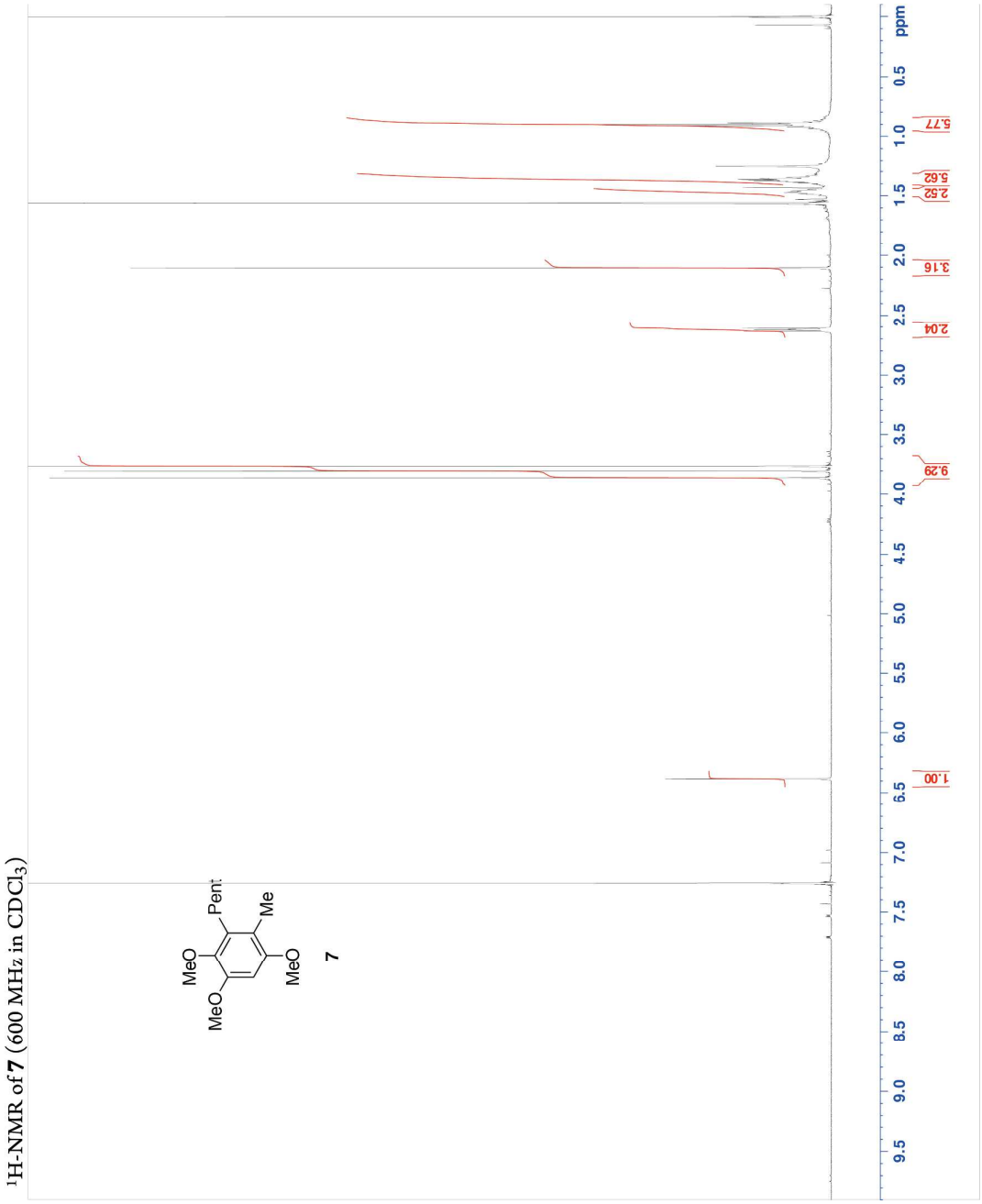


6 **7**

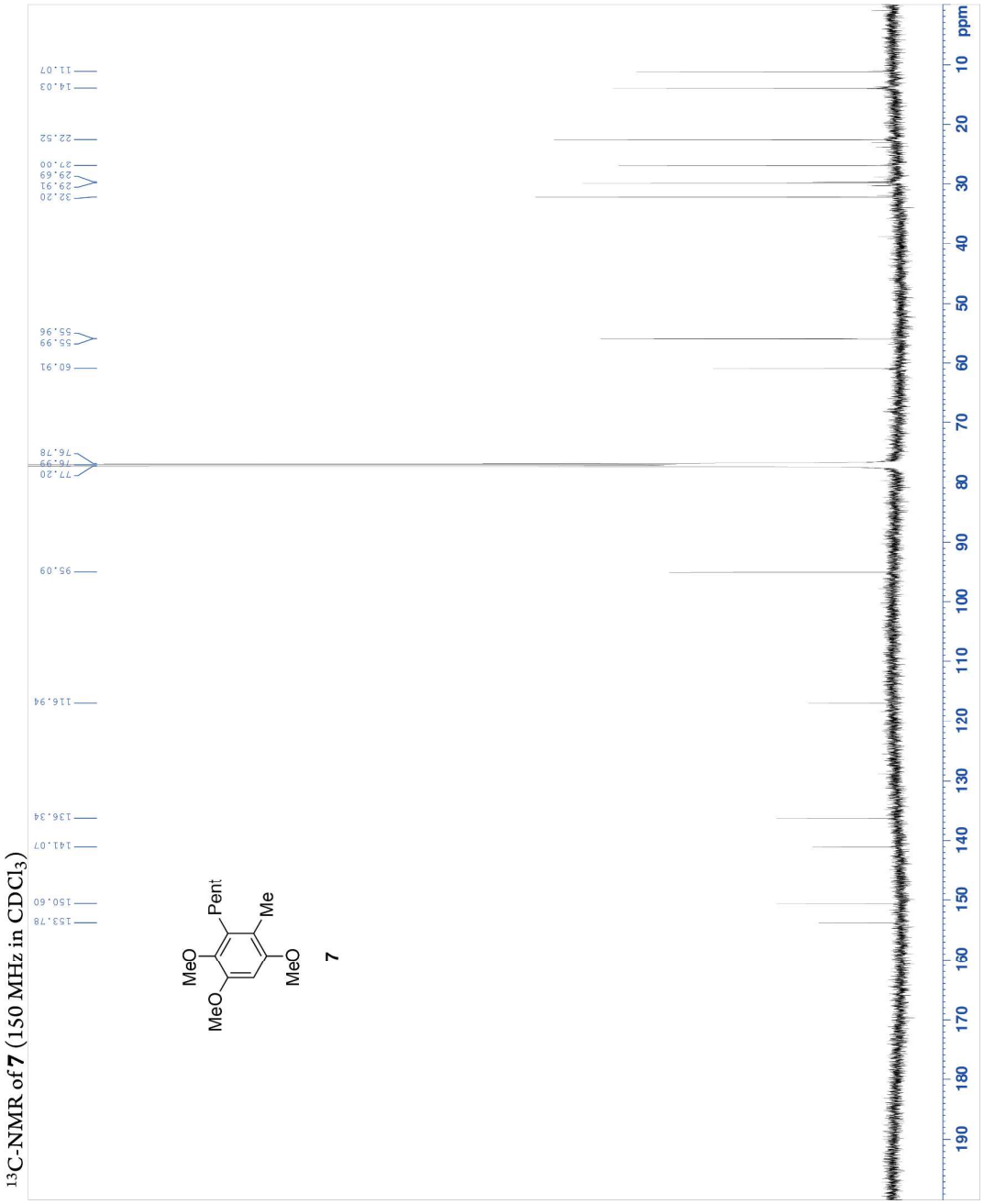
To a solution of **6** (550 mg, 2.16 mmol) in THF, 1 ml of TMEDA (6.7 mmol) was added at room temperature followed by addition of 6.2 ml of *sec*-BuLi (0.1 M in cyclohexane) at –78 °C. After 30 min, 1.7 ml of valeraldehyde (16.0 mmol) was added, and the reaction temperature was raised up to room temperature for 90 min. The reaction was quenched by adding water, and the products were extracted with chloroform, washed with water and brine, and evaporated. The product was purified by SiO_2 chromatography to give 1-(2,3,5-trimethoxy-6-methyl-4-(trimethylsilyl)phenyl)pentan-1-ol (600 mg, yield 81.5%) as a colorless oil. To a solution of the previous product (90 mg, 0.26 mmol) in CH_2Cl_2, 0.3 ml of triethylsilane (1.9 mmol) was added at 0 °C followed by addition of TMSOTf (59 µl). After 5 min, the reaction was quenched with satd. $NaHCO_3$. The product was extracted by chloroform, and purified by SiO_2 chromatography to give **7** (23 mg, yield 35.1%) as a colorless oil.

1,2,4-Trimethoxy-5-methyl-6-pentylbenzene (**7**): ^{1}H-NMR (600 MHz, $CDCl_3$, δ) 6.382 (1H, s), 3.856 (3H, s), 3.800 (3H, s), 3.760 (3H, s), 2.623 (2H, t, *J* = 8.0 Hz), 2.104 (3H, s), 2.07 (2H, m), 1.32 (2H, m), 0.90 (3H, t, *J* = 7.4 Hz), ^{13}C-NMR (150 MHz, $CDCl_3$, δ) 153.8, 150.6, 141.1, 136.3, 116.9, 95.1, 60.9, 55.99 55.96, 32.2, 29.9, 29.7 27.0, 22.5, 14.0, 11.1 IR (KBr) ν_{max} 2956, 2834, 1606, 1523 cm^{-1}, EI-MS *m*/*z* 252 $[M]^+$(base), HR-EIMS *m*/*z* 252.1714 (calcd. 252.1725 for $C_{15}H_{24}O_3$), UV (MeOH) λ_{max} nm (log ε) 206 (4.63), 229 (4.07), 290nm (3.79).

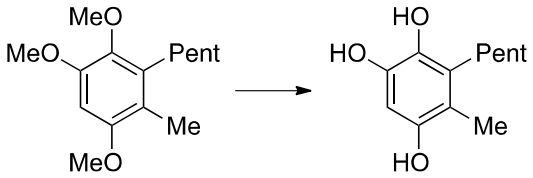
*^{1}H-NMR spectrum of **7** (600 MHz, in $CDCl_3$)*



*^{13}C-NMR spectrum of **7** (150 MHz, in $CDCl_3$)*



*Preparation of 5-methyl-6-pentylbenzene-1,2,4-triol (**8**)*

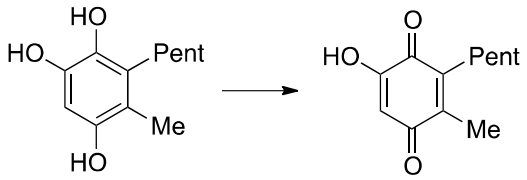


7 **8**

To a solution of **7** (35 mg, 0.14 mmol) in CH_2Cl_2 (5 ml), 1.5 ml of 1 M BBr_3 in CH_2Cl_2 was added, and the reaction solution was stirred at the temperature for 1 h. The temperature of the reaction was raised up to 0 °C for 1 h. Another 1.5 ml of 1 M BBr_3 in CH_2Cl_2 was added. After 30 min, the reaction was quenched by adding methanol. The product was purified by SiO2 column chromatography to give **8** (6.6 mg, yield 22.6%).

5-Methyl-6-pentylbenzene-1,2,4-triol (**8**): ^{1}H-NMR ($CDCl_3$, δ) 6.27 (1H, s), 2.61 (2H, t, *J* = 7.5 Hz), 2.09 (3H, s), 1.50–1.25 (6H, m), 0.89 (3H, t, *J* = 7.2 Hz), ^{13}C-NMR ($CDCl_3$, δ) 143.9, 141.2, 107.7, 32.1, 28.3, 26.3, 22.6, 14.1, 12.7 IR (KBr) ν_{max} 3383, 2929 cm^{-1}, GC-MS *m*/*z* 210 $[M]^+$, UV (MeOH) λ_{max} nm (log ε) 209 (3.85), 223 (sh,3.65), 280 (3.62), 436 (2.98).

*Preparation of compound **A***

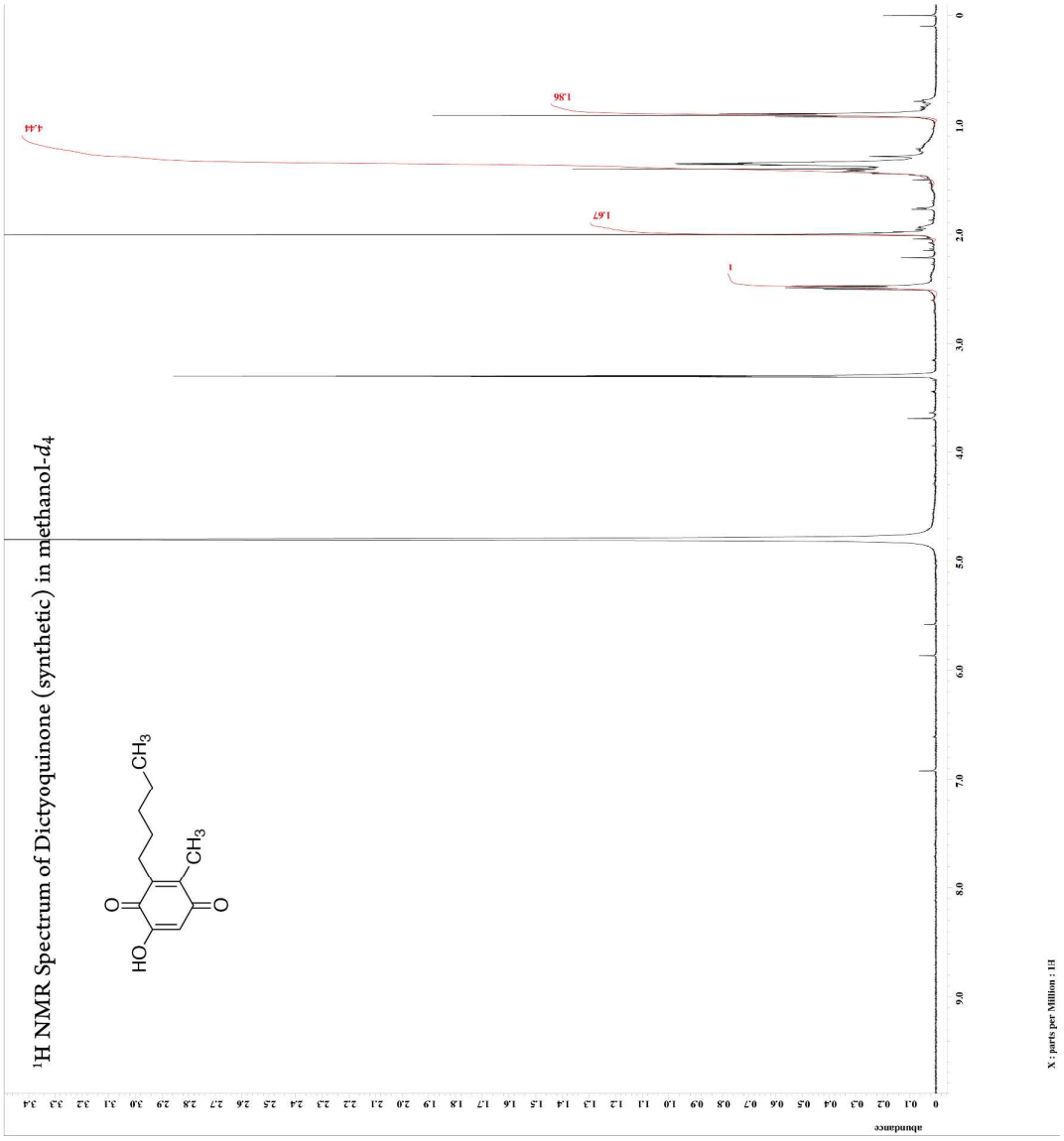


8 **compound A**

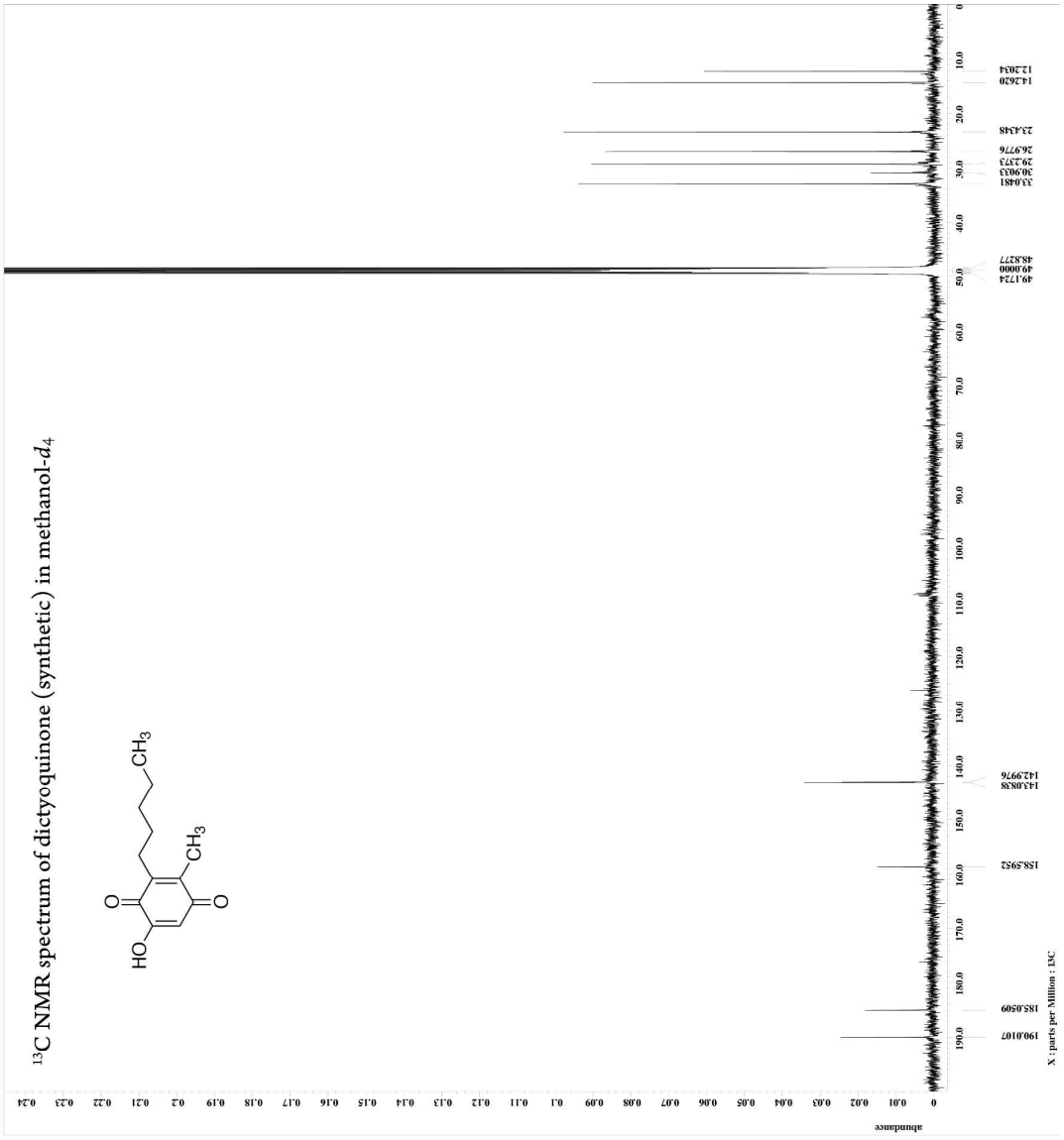
Under Ar atmosphere, compound **8** (1.5 mg, 7.14 μmol) was dissolved to 3 ml of THF, and the solution was stirred at room temperature for 30 min after addition of a little portion of celite. Ag_2CO_3 (55 mg, 0.20 mmol) was added to the reaction mixture and it was stirred for 1.5 h. The reaction mixture was filtered and the filtrate was purified by SiO_2 chromatography to give compound **A** (1.4 mg, yield 94.2%).

Compound **A**: ^{1}H-NMR (500 MHz, methanol-d_4, δ) 2.50 (2H, t, J = 7.6 Hz), 2.01 (3H, s), 1.27–1.47 (6H, m), 0.93 (3H, t, J = 7.6 Hz), ^{13}C-NMR (125 MHz, methanol-d_4, δ) 190.0, 185.1, 158.6, 143.1, 143.0, 33.0, 29.2, 27.0, 23.4, 14.3, 12.2 ^{1}H-NMR ($CDCl_3$, δ) 6.03 (1H, s), 2.48 (2H, t, J = 7.9 Hz), 2.04 (3H, s), 1.25–1.43 (6H, m), 0.88 (3H, t, J = 7.9 Hz), ^{13}C-NMR ($CDCl_3$, δ) 187.7, 183.8, 154.0, 143.7, 140.9, 107.5, 31.8, 28.1, 26.1, 22.3, 13.8, 12.4 IR (KBr) ν_{max} 2925, 1644 cm^{-1}, EIMS m/z 208 $[M]^+$, HR-EIMS m/z 208.1087 (calcd. 208.1099 for $C_{12}H_{16}O_3$), UV (MeOH) λ_{max} nm (log ε) 203 (3.94), 274 (3.56), 492 (2.36).

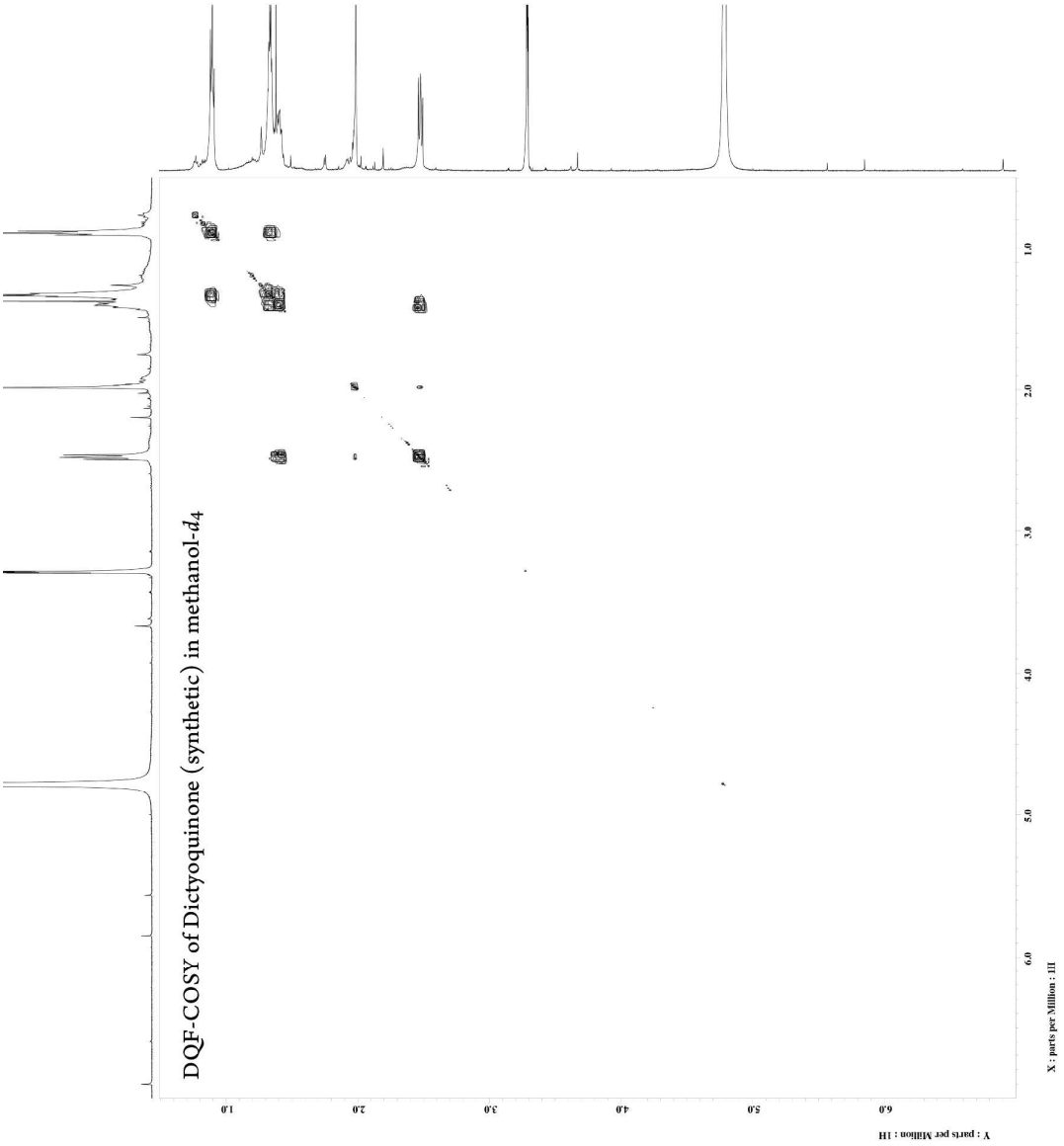
^{1}H-NMR spectrum of dictyoquinone (synthetic) (500 MHz in methanol-d_4)



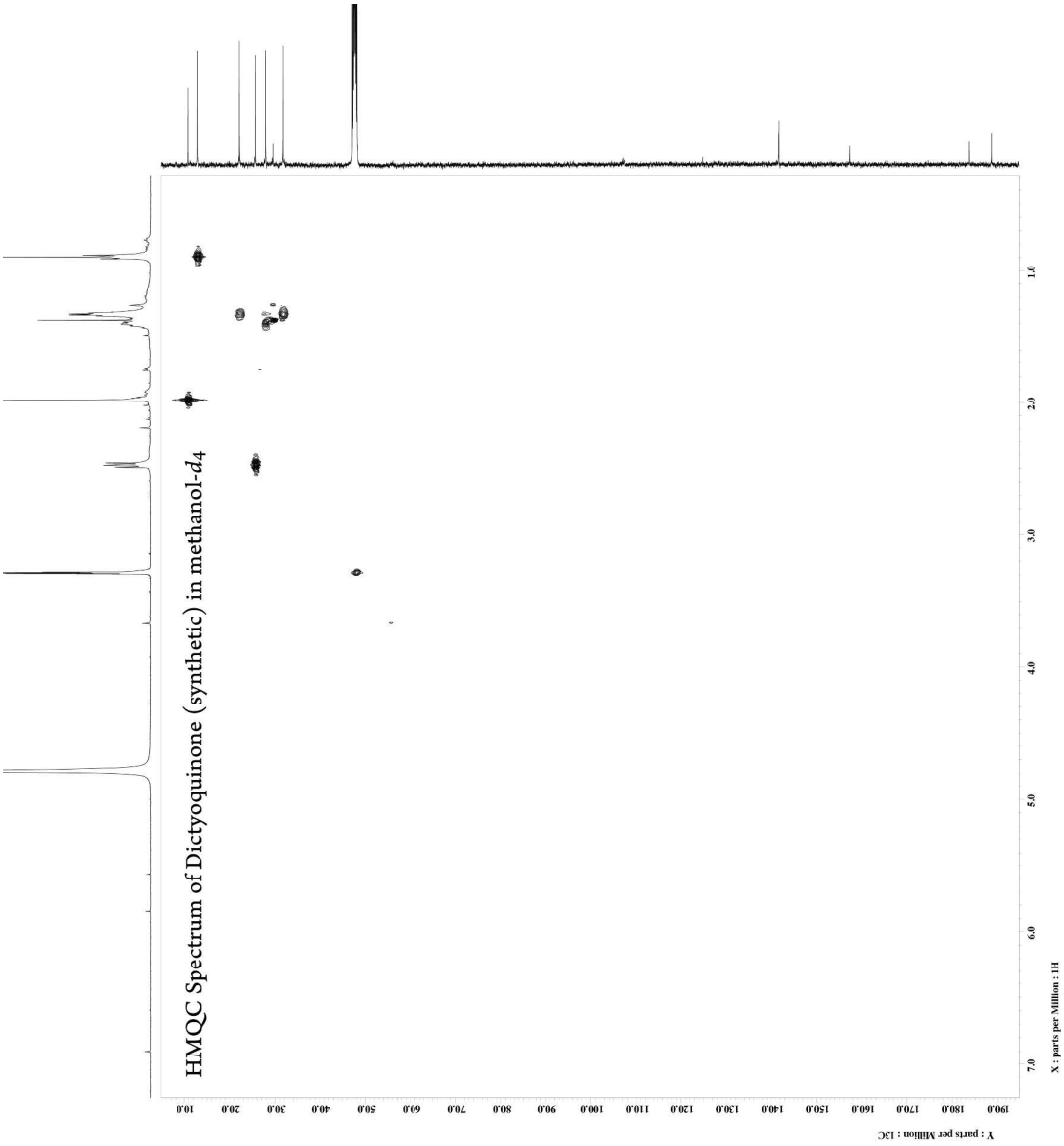
^{13}C-NMR spectrum of dictyoquinone (synthetic) (125 MHz in methanol-d_4)



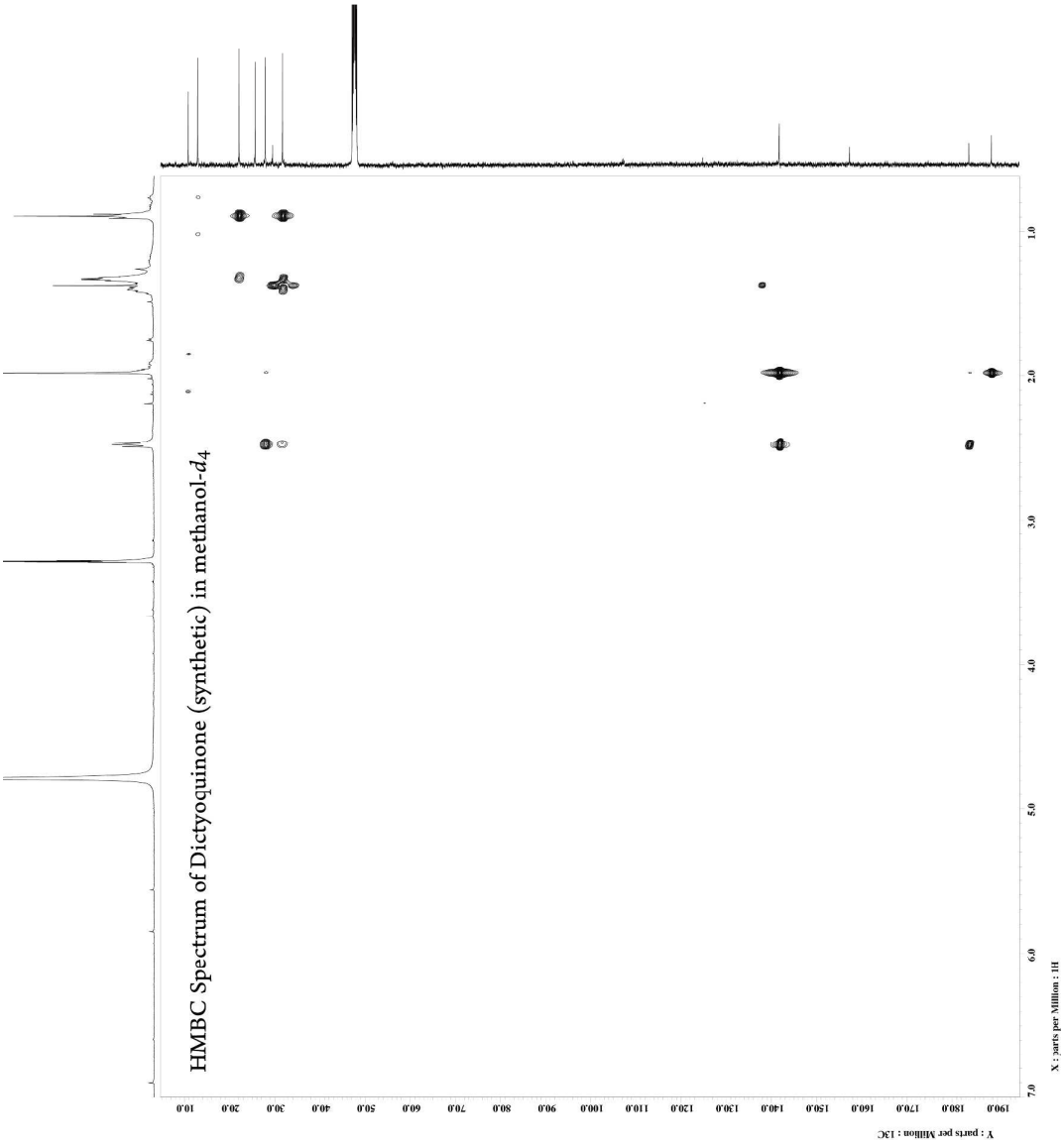
DQF-COSY spectrum of dictyoquinone (synthetic) in methanol-d_4



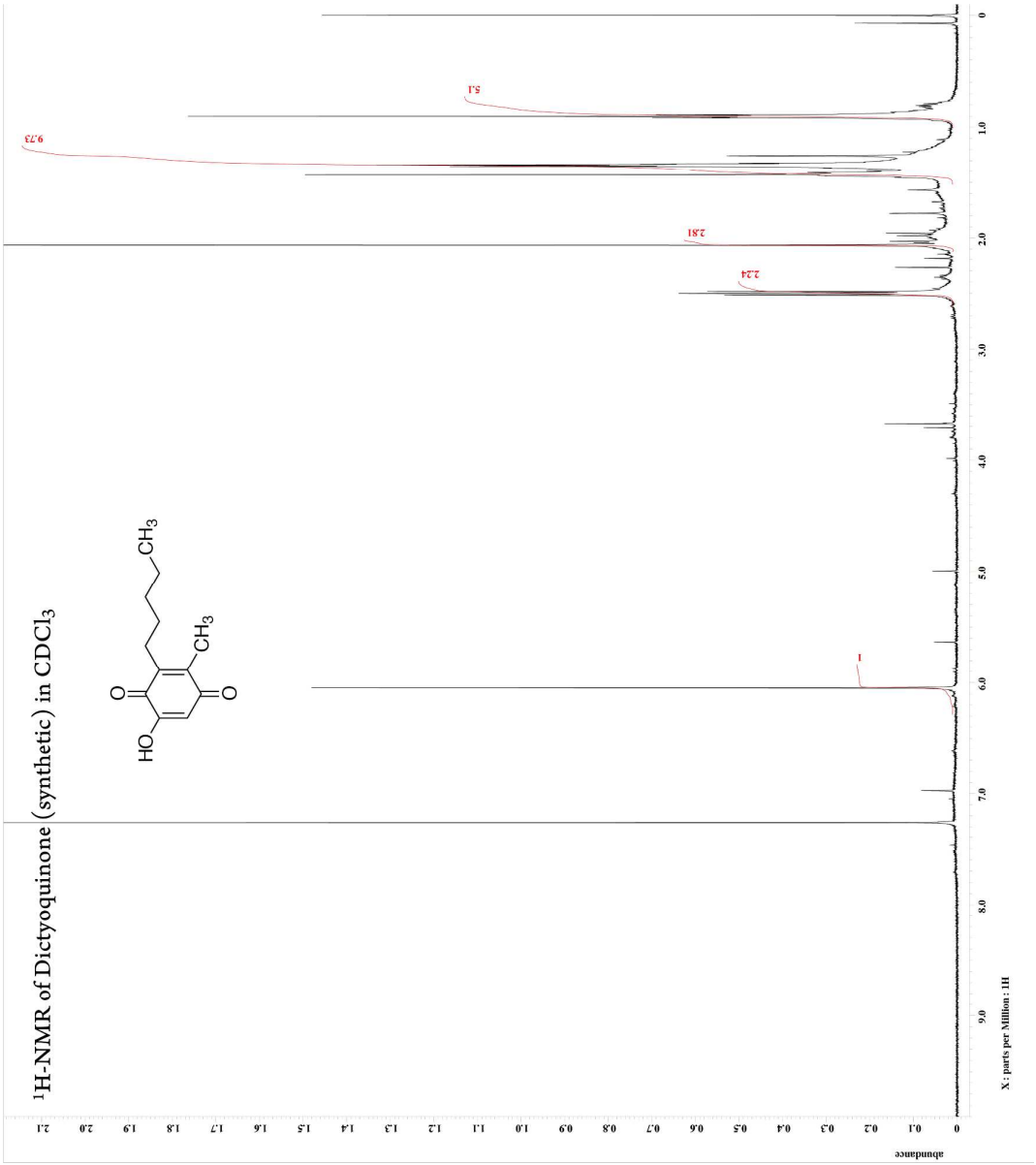
HMQC spectrum of dictyoquinone (synthetic) in methanol-d_4



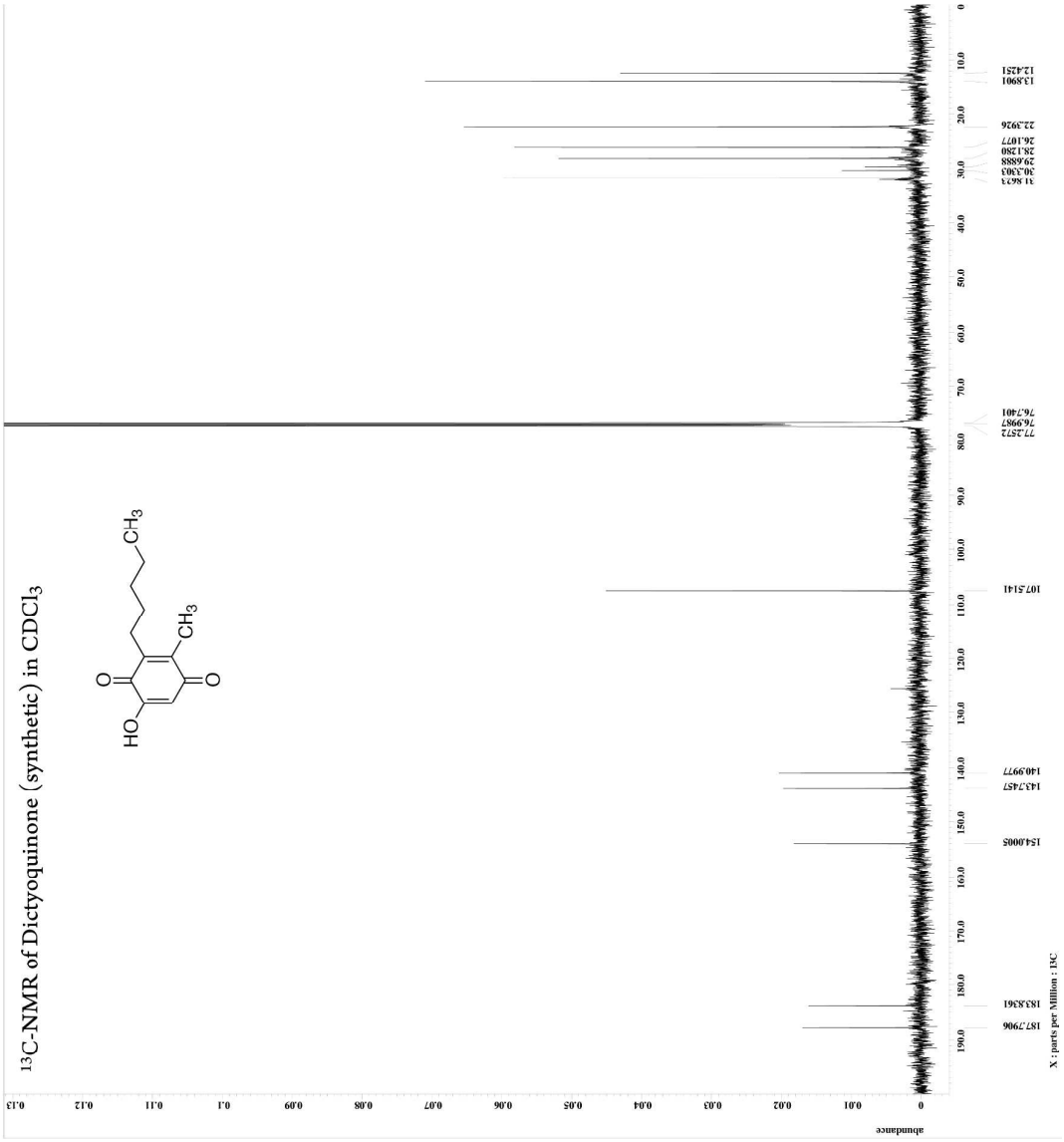
HMBC spectrum of dictyoquinone (synthetic) in methanol-d_4



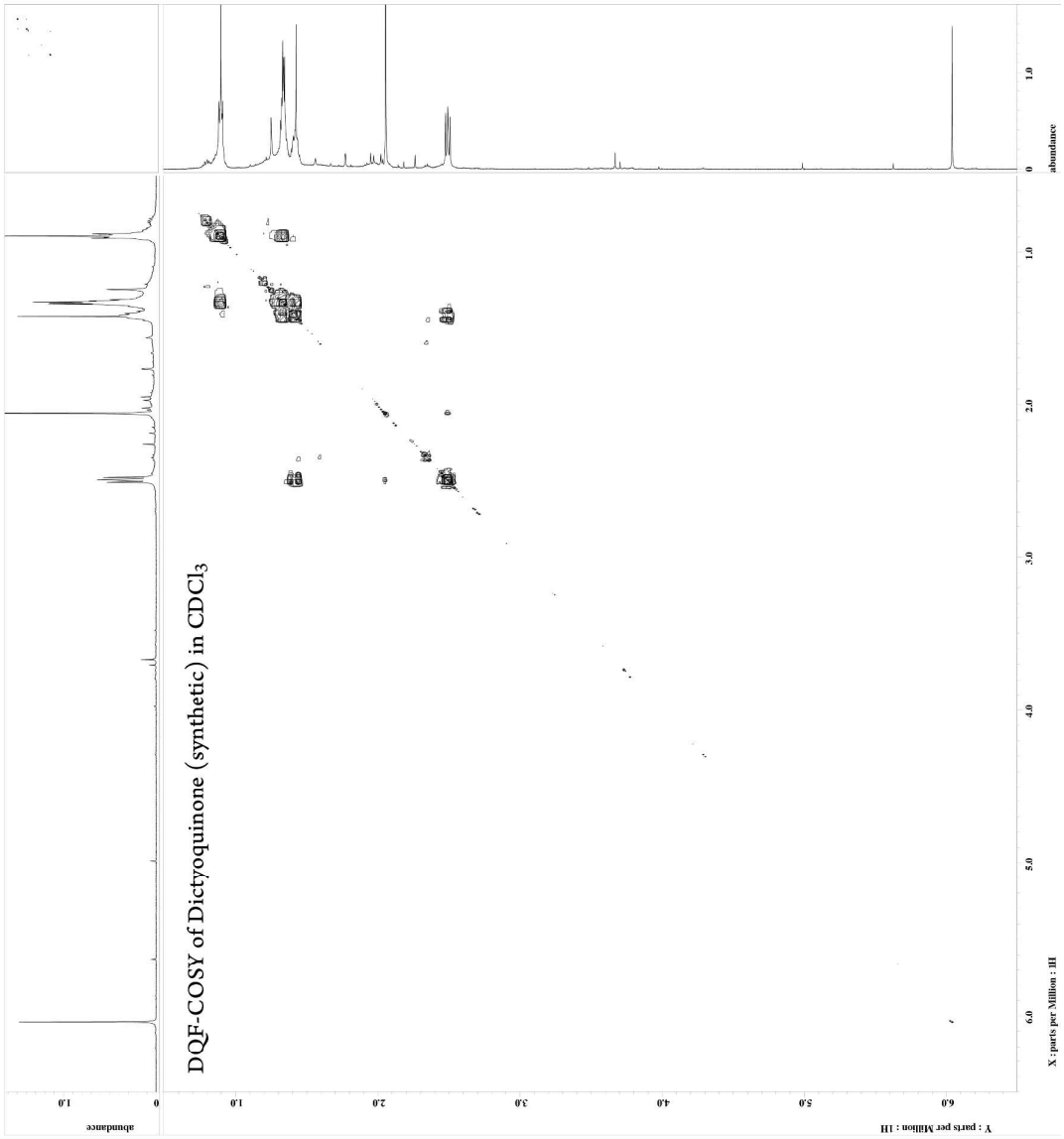
^{1}H-NMR spectrum of dictyoquinone (synthetic) in $CDCl_3$



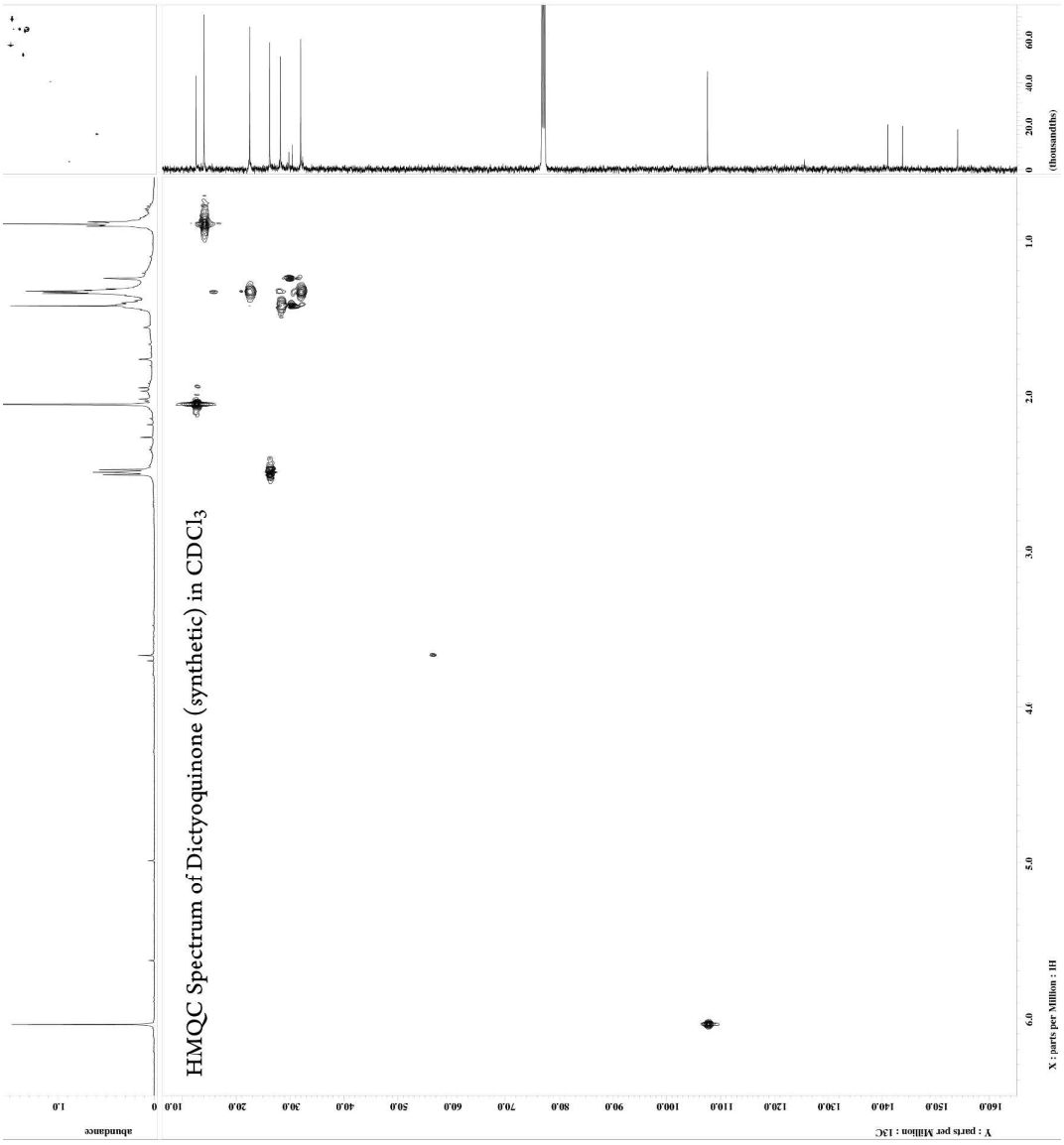
^{13}C-NMR spectrum of dictyoquinone (synthetic) in $CDCl_3$



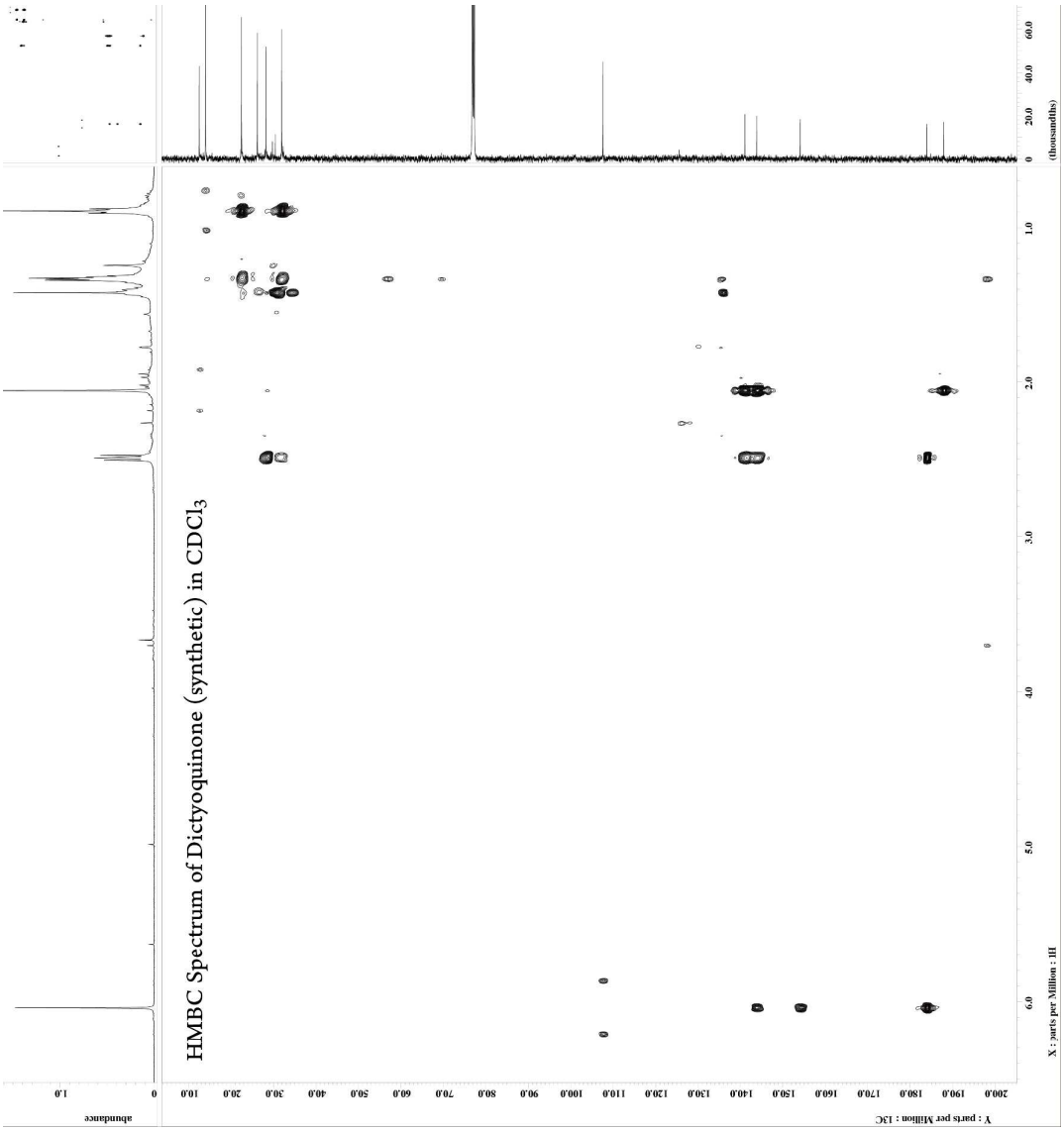
DQF-COSY spectrum of dictyoquinone (synthetic) in $CDCl_3$



HMQC spectrum of dictyoquinone (synthetic) in $CDCl_3$

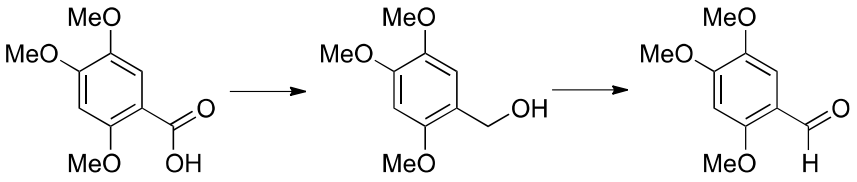


HMBC spectrum of dictyoquinone (synthetic) in $CDCl_3$



Synthesis of Compound B

*Preparation of 2,4,5-trimethoxybenzaldehyde (**9**)*

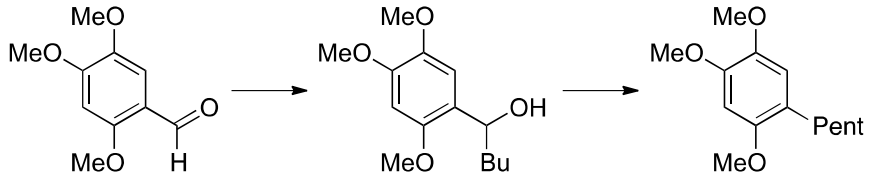


4 **9**

To a solution of 2,4,5-trimethoxybenzoic acid (**4**) (1.06 g, 5.02 mmol) in dry THF (5 mL), 0.93 M BH_3-THF complex (10.8 mL) was added slowly at 0 °C under Ar atmosphere. After 30 min, the ice bath was removed and the solution was stirred at room temperature for 60 min. Methanol was added to the solution to quench the excess BH_3 till the generation of gas was not observed. The reaction mixture was then evaporated after another 20 mL of methanol was added to the solution. The residue was dissolved to methanol (20 mL), and evaporated to dryness. This procedure was repeated three times. The resulted residue was purified by a chromatography with SiO_2 using a mixture of hexane–EtOAc (1:1) to afford 2,4,5-trimethoxybenzyl alcohol (707 mg, yield 71%). 2,4,5-Trimethoxybenzyl alcohol (219 mg, 1.11mmol), 4-methylmorpholine *N*-oxide (205 mg, 1.75 mmol) and molecular sieves 4A (0.5 g) was mixed in 2 ml of CH_2Cl_2 at 0 °C under Ar atmosphere. To this mixture, 19 mg (0.15 mmol) of tetrapropylammonium perruthenate (TPAP) was added. After 1.5 h, the reaction mixture was applied on SiO_2 column to afford 2,4,5-trimethoxybenzaldehyde (**9**) (209 mg, yield 96%).

2,4,5-Trimethoxybenzaldehyde (**9**)(CAS: 4460-86-0): ^{1}H-NMR ($CDCl_3$, δ) 10.29 (1H, s), 7.30 (1H, s), 6.48 (1H, s), 3.95 (3H, s), 3.90 (3H, s), 3.85 (3H, s), ^{13}C-NMR ($CDCl_3$, δ) 187.9 158.6, 155.7 143.5, 117.3, 109.0, 95.9, 56.2, 56.2, 56.1, GC-MS *m*/*z* 196 $[M]^+$.

*Preparation of 1,2,4-trimethoxy-5-pentylbenzene (**10**)*



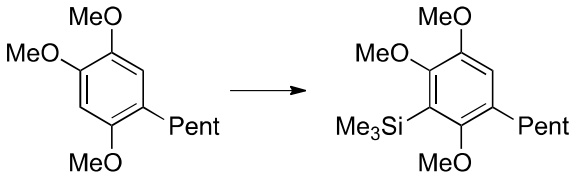
9 **10**

To a solution of **9** (440 mg, 2.24 mmol) in THF (13 ml), 1M BuMgCl in THF (2.86 mL) was added at 0 °C. After 15 min, the product was extacted with chloroform, and was purified by SiO_2 column chromatography to give 1-(2,4,5-trimethoxyphenyl)pentan-1-ol (501 mg, yield 87.9%). To a solution of the alcohol (501 mg, 1.97 mmol) in CH_2Cl_2 (2 ml), 2 ml of triethylsilane (12.5 mmol) was added at 0 °C followed by addition of TMSOTf (0.45 ml). After 5 min, the reaction was quenched with satd. $NaHCO_3$. The product was extracted by chloroform, and purified by SiO_2 chromatography to give 1,2,4-trimethoxy-5-pentylbenzene (**10**) (454 mg, yield 96.6%) as a colorless oil.

1-(2,4,5-Trimethoxyphenyl)pentan-1-ol: ^{1}H-NMR ($CDCl_3$, δ) 6.84 (1H, s), 6.36 (1H, s), 4.70 (1H, t, *J* = 6.4 Hz), 3.82, 3.64, 3.63 (each 3H, s), 1.69 (2H, m), 1.25-1.38 (4H, m), 0.88 (3H, t, *J* = 7.2 Hz), ^{13}C-NMR ($CDCl_3$, δ) 150.4, 147.9, 143.0, 124.2, 111.2, 97.2, 73.8, 56.3, 56.2, 56.1, 36.4, 27.7, 22.7, 14.1, GC-MS *m*/*z* 254 $[M]^+$, 197(base).

1,2,4-Trimethoxy-5-pentylbenzene (**10**)(CAS: 392286-94-1): ^{1}H-NMR ($CDCl_3$, δ) 6.69, 6.52 (each 1H, s), 3.87, 3.83, 3.79 (each 3H, s), 2.53 (2H, t, *J* = 7.6 Hz), 1.55 (2H, quint, *J* = 7.5 Hz), 1.31–1.34 (4H, m), 0.90 (3H, t, *J* = 6.8 Hz), ^{13}C-NMR ($CDCl_3$, δ) 151.5, 147.5, 142.8, 123.1, 114.2, 98.1, 56.7, 56.5, 56.2, 31.7, 30.0, 29.6, 22.5, 14.0, GC-MS *m*/*z* 238 $[M]^+$, 181(base).

*Preparation of 1,2,4-trimethoxy-3-trimethylsilyl-5-pentylbenzene (**11**)*



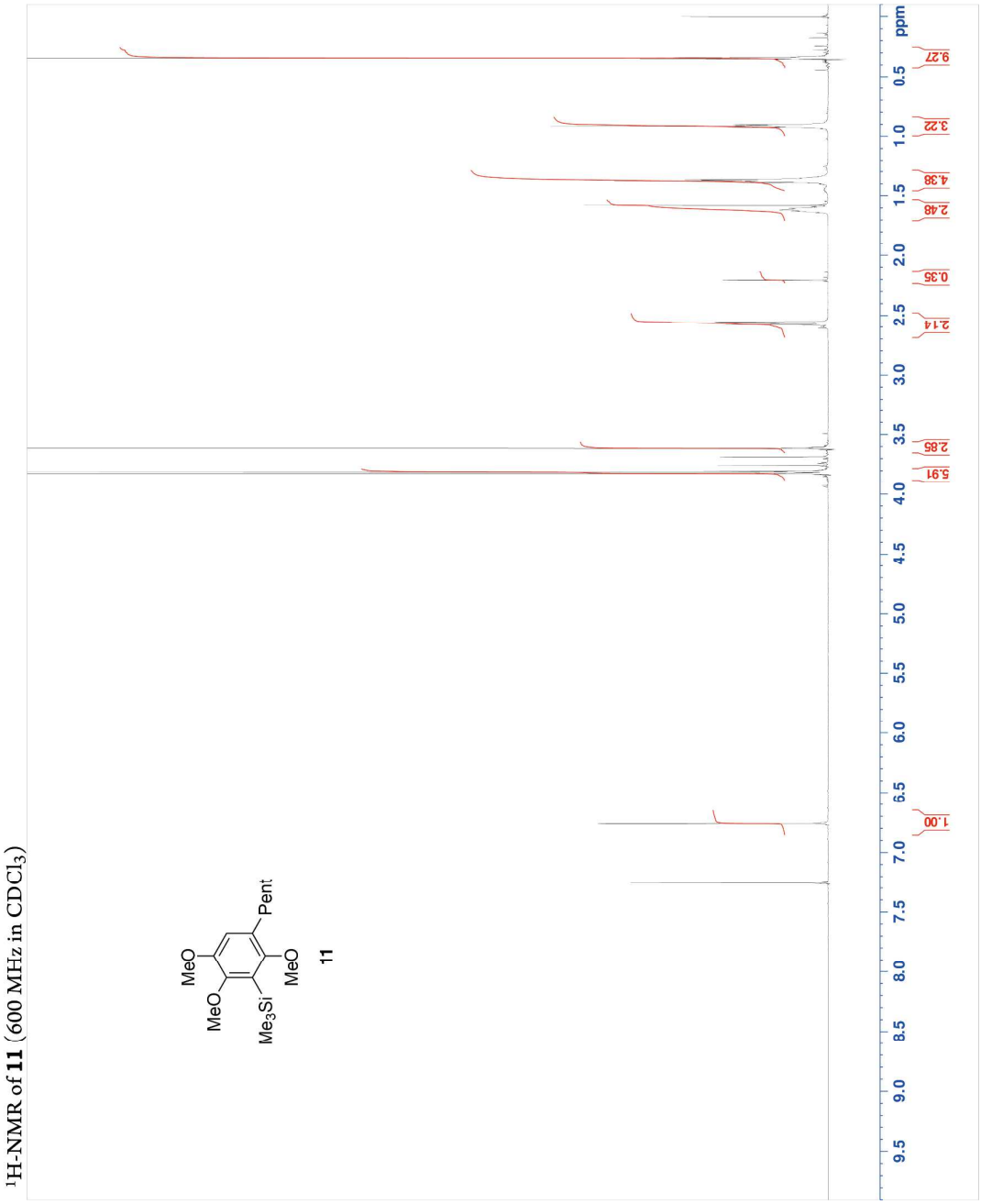
10 **11**

To a solution of **10** (157 mg, 0.66 mmol) in dry THF (4 mL), 0.4 mL of *N,N,N′,N′*-tetramethylethylenediamine (TMEDA) (2.7 mmol) was added at room temperature, and *sec*-BuLi (0.1 M in cyclohexane) (2.5 ml) was added at –78 °C. After the reaction mixture was kept at the temperature for 30 min, TMSCl (0.7 ml, 5.5 mmol) was added. The temperature was raised up to room temperature for 90 min. The reaction was quenched by adding water and the reaction solution was extracted with chloroform. The organic layer was washed with water and evaporated. The residue was purified by SiO_2 chromatography to give 1,2,4-trimethoxy-3-trimethylsilyl-5-pentylbenzene (**11**)(168 mg, yield 82.5%).

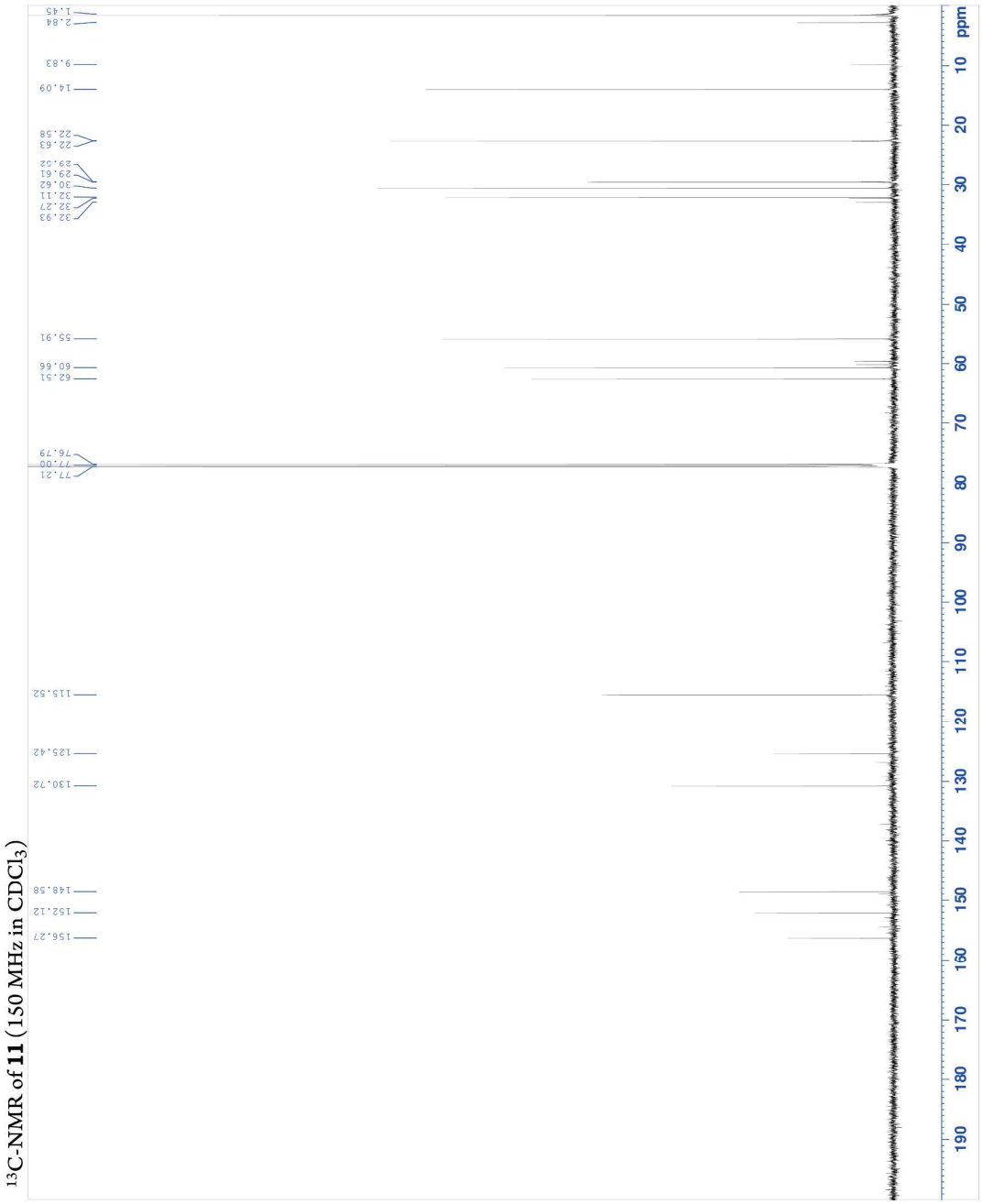
The position of silylation was determined on the basis of NOE experiments.

1,2,4-Trimethoxy-3-trimethylsilyl-5-pentylbenzene (**11**): ^{1}H-NMR (600 MHz, $CDCl_3$, δ) 6.760 (1H, s), 3.822, 3.809, 3.613 (each 3H, s), 2.566 (2H, t, J = 8.1 Hz), 1.615 (2H, m), 1.39–1.36 (4H, m), 0.914 (3H, t, J = 7.1 Hz), 0.344 (9H, s), ^{13}C-NMR (150MHz, $CDCl_3$, δ) 156.3, 152.1, 148.6, 130.7, 125.4, 115.5, 62.5, 60.7 55.9, 32.1, 30.6, 29.6, 22.6, 14.1, 1.5, IR (KBr) ν_{max} 2934, 2859 cm^{-1}, EIMS m/z 310 $[M]^+$ (base), HR-EIMS m/z 310.1937 (calcd. 310.1964 for $C_{17}H_{30}O_3Si$), UV (MeOH) λ_{max} nm (log ε) 206 (4.62), 226 (sh, 3.99), 291(3.53).

*^{1}H-NMR spectrum of compound **11** (600 MHz in $CDCl_3$)*

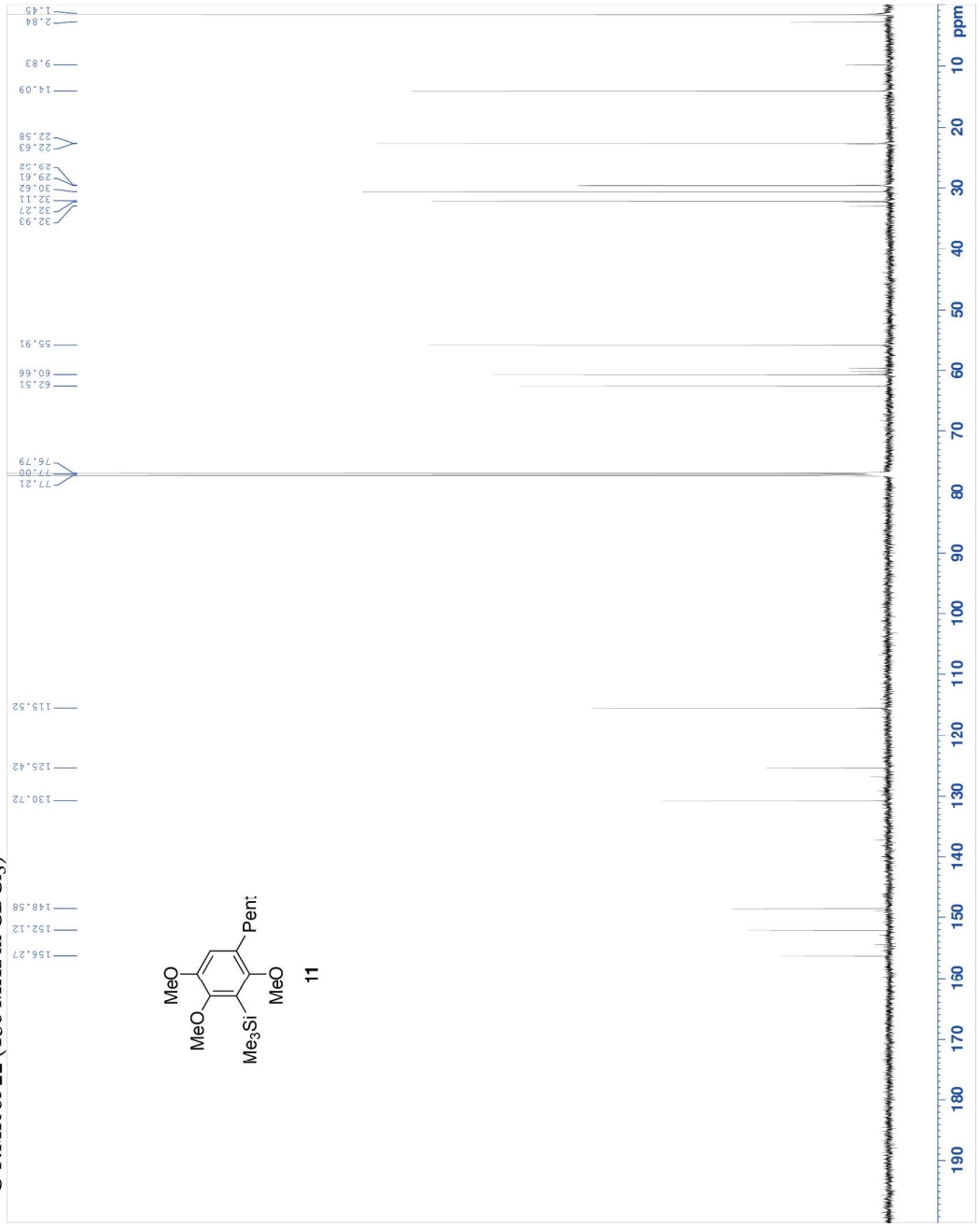


*^{13}C-NMR spectrum of compound **11** (150 MHz in $CDCl_3$)*

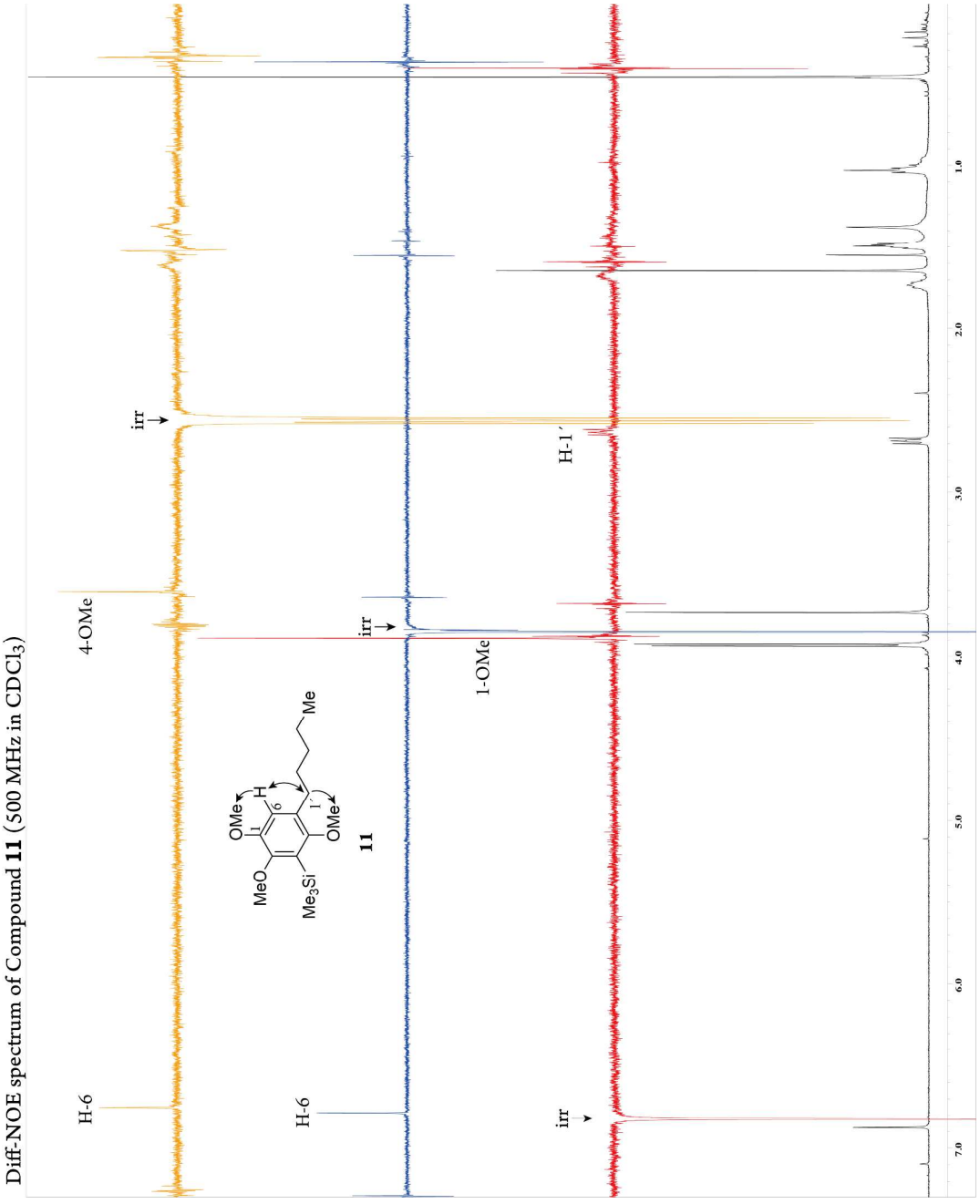


^{13}C-NMR of **11** (150 MHz in $CDCl_3$)

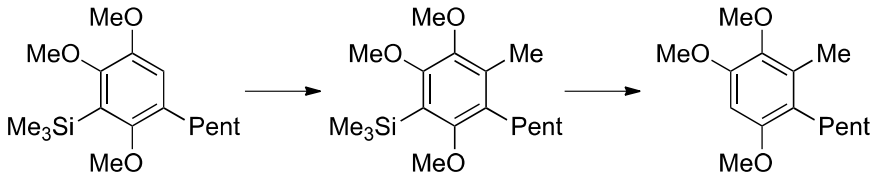
^{13}C-NMR of **11** (150 MHz in $CDCl_3$)



*7.Difference NOE spectrum of **11** (500 MHz, in $CDCl_3$)*



*Preparation of 1,2,5-trimethoxy-3-methyl-4-pentylbenzene (**12**)*

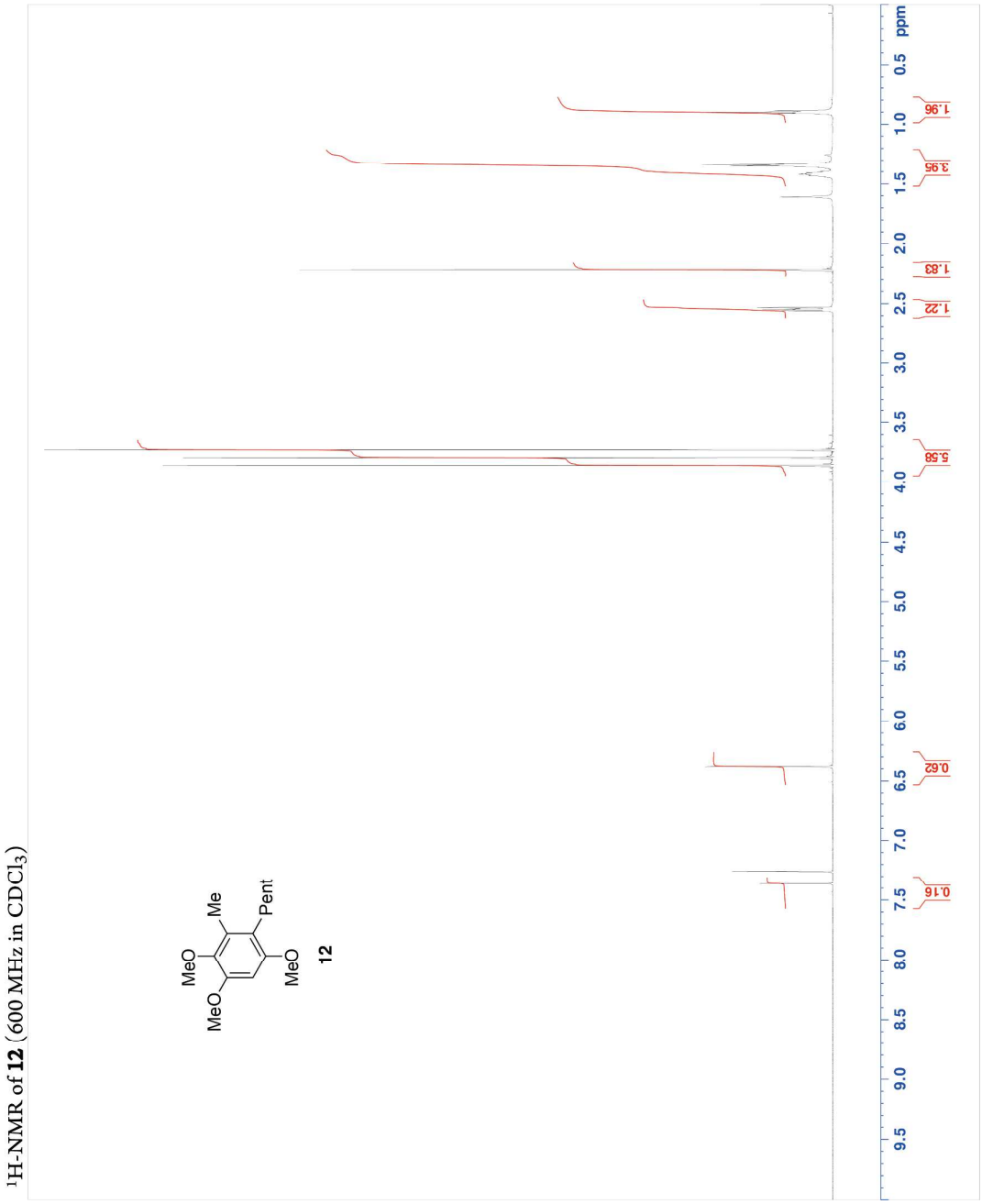


11 **12**

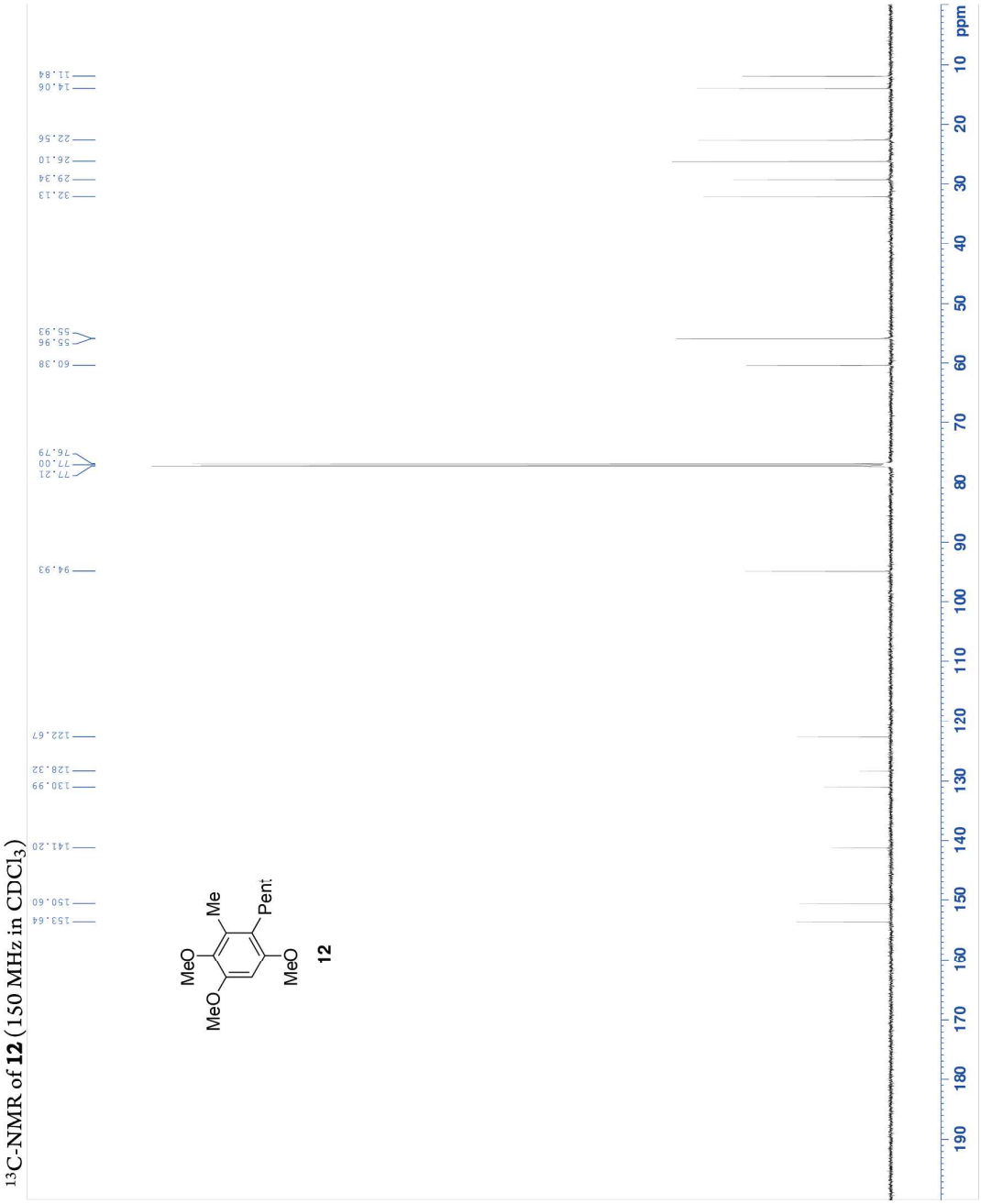
To a solution of **11** (168 mg, 0.54 mmol) in 2 ml of THF, 0.7 ml of TMEDA (4.7 mmol) was added and the reaction solution wad cooled to –78 °C. 1M *sec*-BuLi in cyclohexane (5.0 ml) was added to the solution. After 40 min, MeI (0.3 ml, 4.8 mmol) was added, and the temperature of the reaction solution was raised up to room temperature over 1.5 h. The reaction was quenched by methanol, and the product was extracted with chloroform, and purified by preparative TLC using a mixture of hexane–EtOAc as a mobile phase to afford 1,2,4-trimethoxy-3-trimethylsilyl-6-methyl-5-pentylbenzene (15 mg, yield 25.6 %). The product (115 mg, 0.35 mmol) and KI (330 mg, 1.99 mmol) was mixed in 7 ml of acetonitrile, and water (1.5 ml) was added to this mixture so as to dissolve KI. TMSCl (1.0 ml, 7.88 mmol) was added to the reaction solution at room temperature, after 1 h, another 1 ml of TMSCl was added. After 30 min, the product was extracted with chloroform and purified by SiO_2 column chromatography to give 1,2,5-trimethoxy-3-methyl-4-pentylbenzene (**12**) (63 mg, yield 71.3%).

1,2,5-Trimethoxy-3-methyl-4-pentylbenzene (**12**): ^{1}H-NMR (600MHz, $CDCl_3$, δ) 6.378 (1H, s), 3.855, 3.790, 3.726 (each 3H, s), 2.544 (2H, t, J = 7.9 Hz), 2.220 (3H, s), 1.421 (2H, m), 1.37–1.32 (4H, m), 0.910 (3H, t, J = 7.0 Hz), ^{13}C-NMR (150MHz, $CDCl_3$, δ) 153.6, 150.6, 141.2, 131.0, 128.3, 122.7, 94.9, 60.4, 56.0, 55.9, 32.1, 29.3, 26.1, 22.6, 14.1, 11.8, IR (KBr) ν_{max} 2930, 2858 cm^{-1}, EIMS m/z 252 $[M]^+$, 195 $[M\text{-}Bu]^+$ (base), HR-EIMS m/z 252.1750 (calcd. 252.1725 for $C_{15}H_{24}O_3$), UV (MeOH) λ_{max} nm (log ε) 204 (4.73), 225 (sh, 4.01), 285 (3.60).

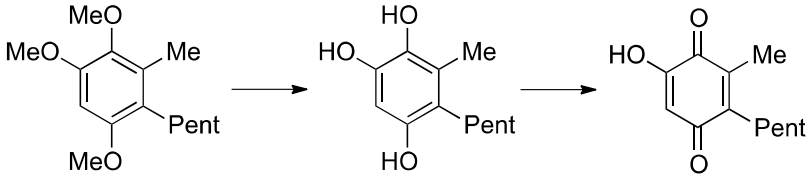
*^{1}H-NMR spectrum of compound **12** (600 MHz in $CDCl_3$)*



*^{13}C-NMR spectrum of compound **12** (150 MHz in $CDCl_3$)*



*Preparation of compound **B***



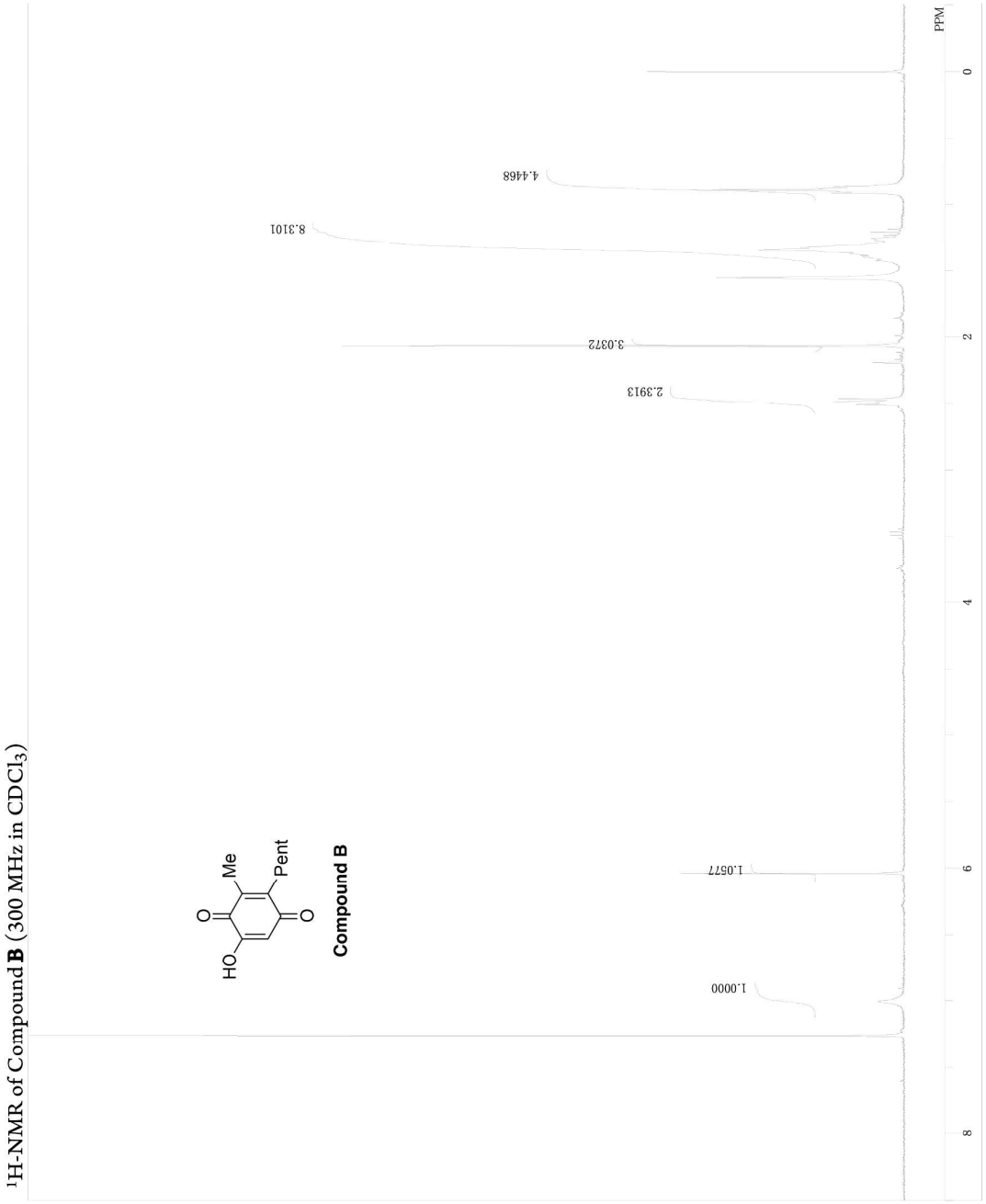
12 **compound B**

To a solution of **12** (34.6 mg, 0.14 mmol) in CH_2Cl_2 (4 ml), 0.65 ml of 1 M BBr_3 in CH_2Cl_2 was added at –78 °C. After 1 h the reaction mixture was stirred at 0 °C for 1h. 1 M BBr_3 (1 ml) was added again at –78 °C, and the solution was kept at the temperature for 30 min, and then was stirred at 0 °C for 30min. The reaction was quenched by adding methanol. The product was purified by SiO_2 column chromatography to give 6-methyl-5-pentylbenzene-1,2,4-triol (10.3 mg, yield 35.1%). Under Ar atmosphere, the triol (10.8 mg, 51 μmol) was dissolved to 2 ml of THF, and the solution was stirred at room temperature for 30 min after addition of a little portion of celite. Ag_2CO_3 (128 mg, 0.46 mmol) was added to the reaction mixture and it was stirred for 1 h. The reaction mixture was filtered and the filtrate was purified by SiO_2 chromatography to give compound **B** (8.8 mg, yield 82.3%).

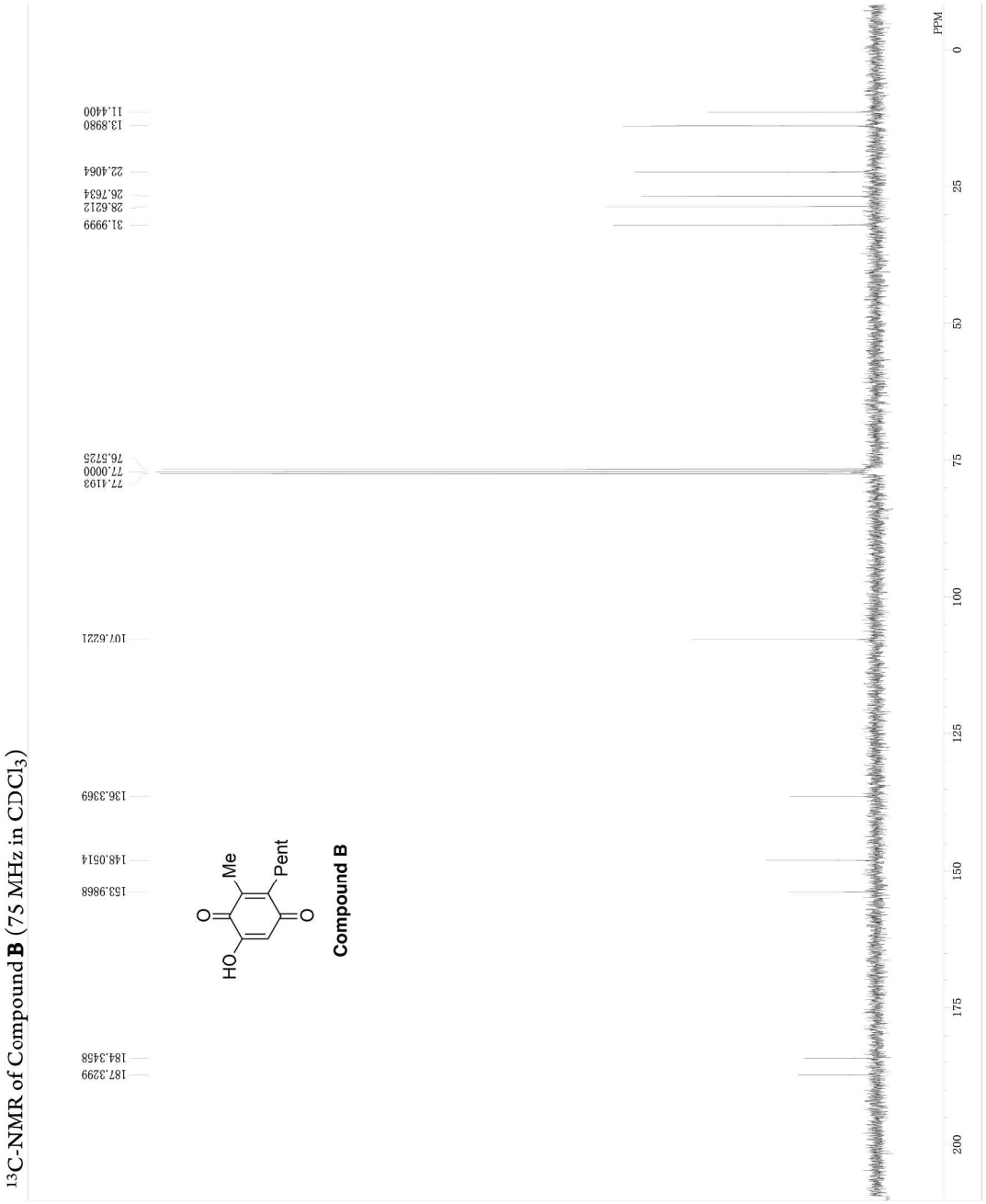
6-Methyl-5-pentylbenzene-1,2,4-triol: ^{1}H-NMR ($CDCl_3$, δ) 6.27 (1H, s), 2.53 (2H, t, *J* = 7.6 Hz), 2.18 (3H, s), 1.26–1.45 (6H, m), 0.89 (3H, t, *J* = 5.7 Hz), ^{13}C-NMR ($CDCl_3$, δ) 147.0 141.7, 135.7, 124.2, 120.0, 100.8, 32.0, 29.4, 26.2, 22.6, 14.0, 11.8, IR (KBr) ν_{max} cm^{-1}, GC-MS *m*/*z* 210 $[M]^+$.

Compound **B**: ^{1}H-NMR (300MHz, $CDCl_3$, δ) 6.04 (1H, s), 2.49 (2H, t, *J* = 7.6 Hz), 2.07 (3H, s), 1.32–1.40 (6H, m), 0.89 (3H, t, *J* = 7.2 Hz), ^{13}C-NMR (75 MHz, $CDCl_3$, δ) 187.3 184.3, 154.0, 148.1, 136.3, 107.6, 32.0, 28.6, 26.8, 22.4, 13.9, 11.4, IR (KBr) ν_{max} 3348, 2926, 2852, 1726, 1657 cm^{-1}, GC-MS *m*/*z* 208 $[M]^+$, UV (MeOH) λ_{max} nm (log ε) 210 (sh, 4.22), 278 (4.23), 494 (3.02).

*^{1}H-NMR spectrum of compound **B** (300 MHz in $CDCl_3$)*



*^{13}C-NMR spectrum of compound **B** (75 MHz in $CDCl_3$)*



*Transformation of MPBD (**3**) into dictyoquinone (**1**)*

3 **1**

MPBD (**3**, 19 mg) was dissolved to 10 mL of acetone. To this solution, 5 mL of 0.2 mM KH_2PO_4 aqueous solution, Frémy salt (55 mg) were added, and the reaction mixture was stirred at room temperature for 20 min. Another 12 mg of Frémy salt was added to the solution and after 20 min, the reaction mixture was extracted with 50 mL of ethyl acetate twice. The organic layer was washed with water and brine, and dried over anhydrous $MgSO_4$. After evaporation, the product was obtained by SiO_2 column chromatography using a mixed solvent of chloroform–MeOH (9:1) as a solvent (**1:** 6 mg, yield 32.9%).

Stalk cell induction in vitro

Amoebae of V12M2 were incubated as described for the prespore-cell induction assay except that 1 mM Na-cAMP was used. One nM DIF-1 and/or 1 nM compound A/B were added at the start of incubation. Stalk cells were scored microscopically at intervals.

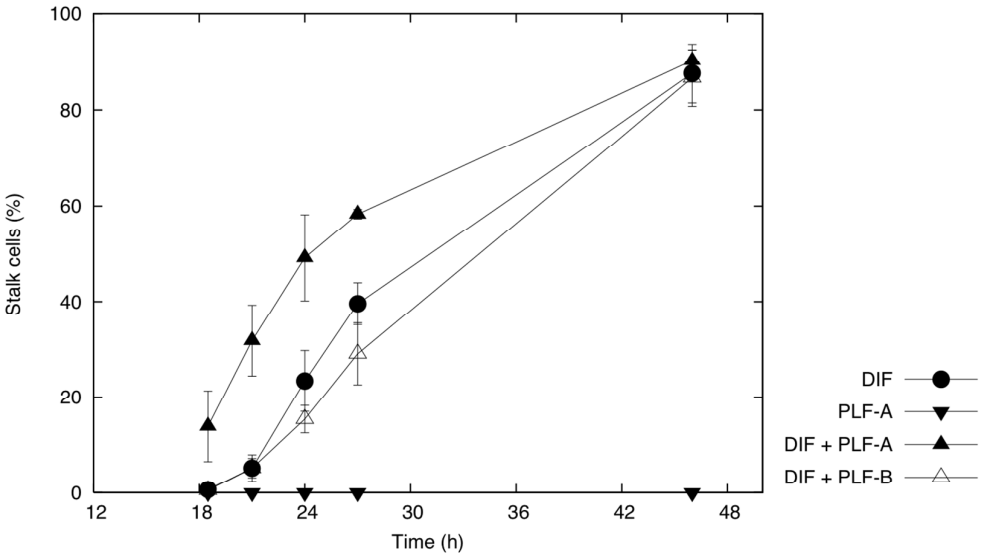


Figure SI-1. Effects of compounds A and B on the time-course of stalk cell differentiation in vitro. Stalk cell differentiation can be induced in vitro by DIF-1 in the presence of cAMP. Compound A but not compound B accelerated this process by ca. 4.5 h. The results shown are the means and SDs of three independent experiments.

Table SI-1 ^{1}H NMR data of natural and synthetic compounds

		^{1}H NMR in methanol-d_4		^{1}H NMR in $CDCl_3$	
Positions	Natural	Compound **A**	Compound **B**	Compound **A**	Compound **B**
3	ND	ND	ND	6.03 (1H, s)	6.04 (1H, s)
1′	2.41 (2H, t, *J*= 7.6)	2.50 (2H, t, *J*= 7.6)	2.46 (2H, t, *J*= 7.4)	2.48 (2H, t, *J*= 7.9)	2.49 (2H, t, *J*= 7.6)
2′–4′	1.41–1.27 (6H, m)	1.47–1.27 (6H, m)	1.46–1.17 (6H, m)	1.43–1.25 (6H, m)	1.40–1.32 (6H, m)
5′	0.90 (3H, t, *J*= 6.5)	0.93 (3H, t, *J*= 7.6)	0.90 (3H, t, J= 7.6)	0.88 (3H, t, *J*= 7.9)	0.89 (3H, t, *J*= 7.2)
1′′	1.97 (3H, s)	2.01 (3H, s)	2.01 (3H, s)	2.04 (3H, s)	2.07 (3H, s)

ND denotes "not detected"

Table SI-2 ^{13}C NMR data of natural and synthetic compounds

		^{13}C NMR in methanol-d_4		^{13}C NMR in $CDCl_3$	
Positions	Natural (HMBC)	Compound **A**	Compound **B**	Compound **A**	Compound **B**
1	189.2	185.1	185.4	183.8	184.3
4		190.0	189.6	187.7	187.3
2	ND	158.6	158.3	154.0	154.0
3	ND	ND	ND	107.5	107.6
5	143.9	143.1	147.0	143.7	148.1
6	140.6	143.0	138.7	140.9	136.3
1′	26.7	27.0	27.3	26.1	26.8
2′	29.3	29.2	29.6	28.1	28.6
3′	32.9	33.0	33.1	31.8	32.0
4′	23.3	23.4	23.5	22.3	22.4
5′	14.1	14.3	14.3	13.8	13.9
1′′	12.7	12.2	11.6	12.4	11.4