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A Synthetic Precursor of Maleylacetic Acid

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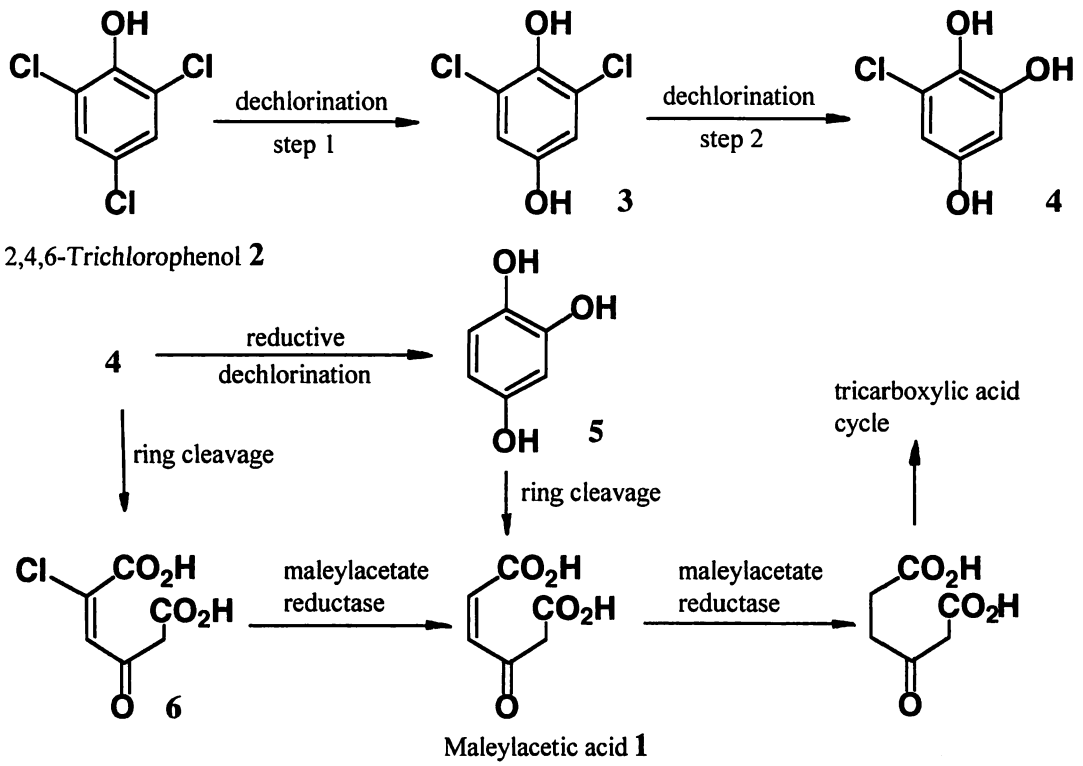
Abstract: **They say that maleylacetic acid (1) is one of the key metabolites when bacteria metabolize polychlorinated phenols. We succeeded to synthesize maleylacetic acid dimethyl ester (7), which is a synthetic precursor of 1, from *cis*-2-butene-1,4-diol (8) as the starting material. Hydrolysis of the ester 7 failed to afford maleylacetic acid 1 under usual basic and phosphate buffer conditions.**

Polychlorinated phenols are troubled as one of environmental pollutions because a large amount of them is used as an antiseptic for woods in the world.[1] Bacteria which metabolize polychlorinated phenols were isolated, and it is very interesting to analyze the bacteria's genes. Maleylacetic acid (**1**) is one of the metabolites by the bacteria. For example, pathway for degradation of 2,4,6-trichlorophenol (**2**) in *Ralstonia picketti* DTP0602 is proposed as shown in Scheme 1.[2] Dechlorination twice of **2** gives 6-chloro-1,2,4-benzenetriol (**4**).[3] Metabolism of **4** is indicated as two routes of reductive dechlorination and ring cleavage to give 1,2,4-benzenetriol (**5**) and chloromaleylacetic acid (**6**), respectively. Then, ring cleavage of **5** or reductive dechlorination of **6** affords maleylacetic acid **1**. Synthesis of maleylacetic acid **1**[4] leads to identify the genes quickly. In this paper, we describe synthesis of maleylacetic acid dimethyl ester (**7**), which is a synthetic precursor of **1**, and results of hydrolysis of the ester **7** under usual basic and phosphate buffer conditions.

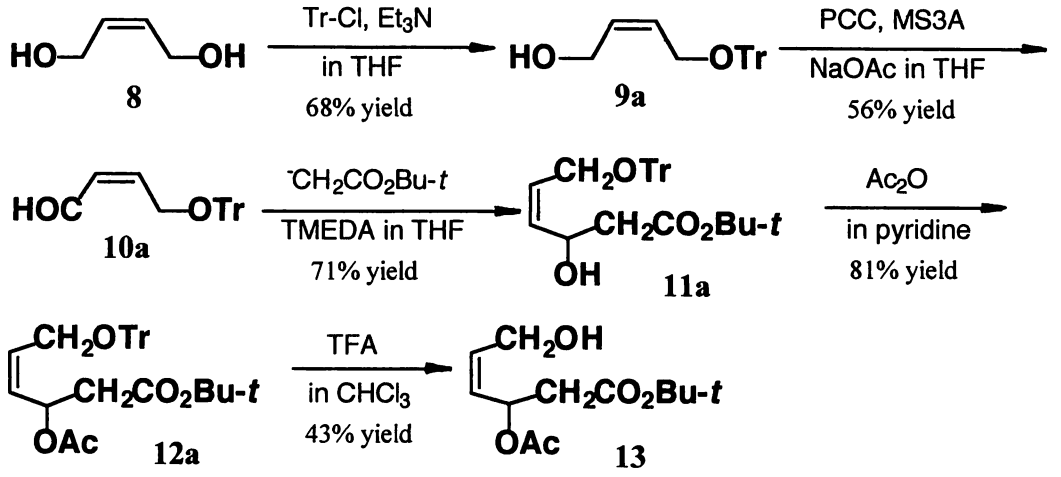


Scheme 1. Proposed Pathway for Degradation of 2,4,6-Trichlorophenol **2**

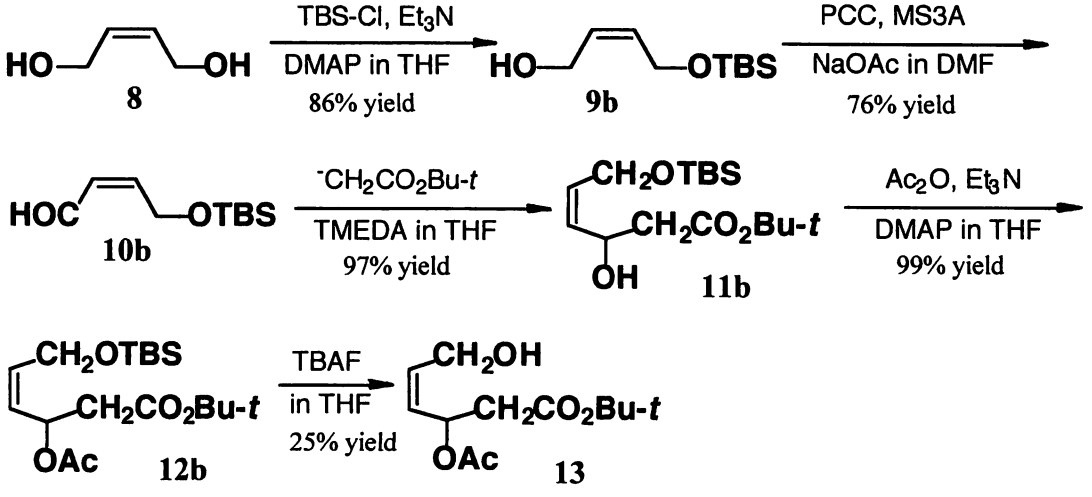
cis-2-Butene-1,4-diol (**8**) was chosed as the starting material for synthesis of maleylacetic acid **1**. Initially, triphenylmethyl (trityl, Tr) group was tried to use as a protecting group. The diol **8** was treated with trityl chloride (Tr-Cl) and triethylamine in tetrahydrofuran (THF) to afford the corresponding alcohol (**9a**) in 68% yield. The alcohol **9a** was oxidized with pyridium chlorochromate (PCC), molecular sieves 3A (MA3A), and sodium acetate in THF to give the aldehyde (**10a**) in 56% yield. Then, the aldehyde **10a** was reacted with the anion which was prepared from *tert*-butyl acetate and litium diisopropylamide (LDA) in the presence of *N,N,N',N'*-tetramethylethylenediamine (TMEDA) in THF to afford the secondary alcohol (**11a**) in 71% yield. The alcohol **11a** was converted to **12a** in 81% yield by acetic anhydride in pyridine. Deprotection of **12a** with trifluoroacetic acid (TFA) in chloroform afforded the alcohol (**13**) in 43% yield. These reactions were summarized in Scheme 2.

Next, in the case of using *tert*-butyldimethylsilyl (TBS) group for protection, the aldehyde (**10b**) was obtained in 65% overall yield in two steps. Namely, **8** was protected by TBS group using chloro-*tert*-butyldimethylsilane (TBS-Cl) in the presence of triethylamine and *N,N*-dimethylaminopyridine (DMAP) in THF (86% yield), followed by oxidation with PCC, MA3A, and sodium acetate in *N,N*-dimethylformamide (DMF) (76% yield). Then, the aldehyde **10b** was reacted with the anion which was prepared from *tert*-butyl acetate and LDA in THF to afford the secondary alcohol (**11b**) in 97% yield. The alcohol **11b** was converted to **13** by protection of **11b** with acetic anhydride in the

presence of triethylamine in THF (99% yield) and by deprotection of the TBS group on **12b** with tetrabutylammonium fluoride (TBAF) in THF (25% yield). These reactions were summarized in Scheme 3.



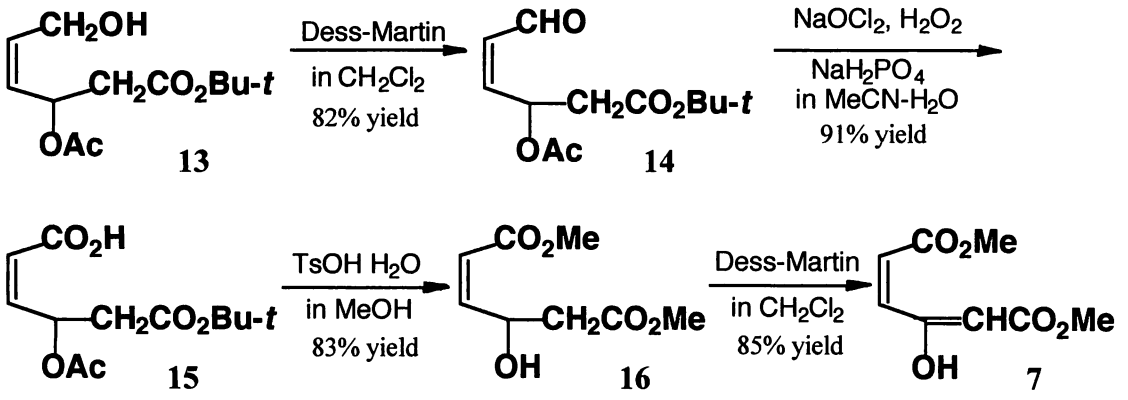
Scheme 2. Synthetic Route of Maleylacetic Acid **1** *via* Tr-Protected Intermediates



Scheme 3. Synthetic Route of Maleylacetic Acid **1** *via* TBS-Protected Intermediates

The primary alcohol **13** was converted to the aldehyde (**14**) in 82% yield by Dess-Martin oxidation.[5, 6] The aldehyde **14**[7] was then oxydized with $NaOCl_2$, H_2O_2, NaH_2PO_4 in MeCN-H_2O[8] to the carboxylic acid (**15**) in 91% yield. Treatment of **15** with *p*-toluenesulfonic acid (TsOH) in MeOH afforded the secondary alcohol (**16**) in 83% yield. Finally, Dess-Martin oxidation[6] of **16** in CH_2Cl_2 gave maleylacetic acid dimethyl ester **7** in 85% yield as the enol-form. Hydrolysis of maleylacetic acid dimethyl ester **7** failed to afford maleylacetic acid **1** under basic (2*N aq.*

NaOH in THF-MeOH) and neutral (porcine pancreas lipase (PPL) in 1/10 phosphate buffer-THF) conditions. It is guessed that decomposition of **1** proceeded under these conditions. These reactions were summarized in Scheme 4.



Scheme 4. Synthesic of Maleylacetic Acid Dimethyl Ester **7** from the Alcohol **13**

It is noted that maleylacetic acid dimethyl ester **7** was synthesized in nine steps from *cis*-2-butene-1,4-diol **8** and that hydrolysis of maleylacetic acid dimethyl ester **7** failed to obtain maleylacetic acid **1** under usual basic and neutral conditions. Maleylacetic acid **1** would be very unstable at the room temparature.

Experimental Section

^{1}H NMR and ^{13}C NMR spectra were measured with a JEOL JNM-GSX400 (400MHz and 100MHz, respectively). The chemical shifts are expressed in ppm downfield from tetramethylsilane, using tetramethylsilane (δ = 0) and/or residual chloroform (δ = 7.25) as an internal standard. Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad signal. For thin layer chromatographic (TLC) analyses, Merck precoated TLC aluminum plates (silica gel 60 F254, Art 5554) were used.

4-(Triphenyl)methoxy-*cis*-2-butenol (9a):

To a solution of 2.79 g (10 mmol, 1 eqiv.) of Tr-Cl in 5 mL of dried THF were added 0.88 mL (10 mmol, 1 equiv.) of *cis*-2-butene-1,4-diol **8** and added dropwise at 0 °C 2.09 mL (15 mmol, 1.5 equiv.) of triethylamine. After stirring for 17 h at rt, the reaction mixture was diluted with 50 mL of EtOAc, washed with 10 mL of brine, and dried over $MgSO_4$. The crude product was chromatographed on silica gel with a 1 : 3 mixture of EtOAc and hexane to afford 2.25 g (68% yield) of **9a**. **9a**: ^{1}H NMR ($CDCl_3$) δ 1.59 (1H, brs, OH), 3.70, 4.02 (2H, 2H, d, d, J = 5.6, 6.0 Hz, CH_2 x2), 5.67-5.83 (2H, m, CH=CH), 7.13-7.48 (15H, m, C_6H_5 x3).

4-(Triphenyl)methoxy-*cis*-2-butenal (10a):

To a solution of 159 mg (1.95 mmol, 0.75 equiv.) of sodium acetate, 860 mg of MS3A, and 842 mg (3.91 mmol, 1.5 equiv.) of PCC in 15 mL of dried THF was added a solution of 860 mg (2.61 mmol, 1 equiv.) of **9a** in 10 mL of dried THF. After stirring for 7 h at rt, the reaction suspension was filtered through a Celite pad, diluted with 30 mL of EtOAc, washed with 30 mL of half brine and 10 mL of brine, and dried over $MgSO_4$. The crude product was chromatographed on silica gel with a 1 : 9 mixture of EtOAc and hexane to afford 481 mg (56% yield) of **10a**. **10a**: ^{1}H NMR ($CDCl_3$) δ 3.94 (2H, d, J = 3.2 Hz, CH_2), 6.64 (1H, dd, J = 8.0, 16.0 Hz, CH=C*H*CHO), 6.79 (1H, dt, J = 3.2, 16.0 Hz, C*H*=CHCHO), 7.17-7.53 (15H, m, C_6H_5 x3), 9.56 (1H, d, J = 8.0 Hz, CHO).

***tert*-Butyl 3-Hydroxy-6-(triphenyl)methoxy-*cis*-4-hexenate (11a):**

To a solution of a freshly prepared LDA from 276 μL (2.00 mmol, 1.3 equiv.) of diisopropylamine and 1.14 mL (1.80 mmol, 1.2 equiv.) of a 1.59M solution of butyl lithium in hexane in the presence of 343 μL (2.30 mmol, 1.5 equiv.) of TMEDA in 10 mL of dried THF at 0 °C for 30 min was added dropwise 245 μL (1.80 mmol, 1.2 equiv.) of *tert*-butyl acetate. After stirring for 1 h at -78 °C, a solution of 500 mg (1.50 mmol, 1 equiv.) of **10a** in 2 mL of dried THF was added dropwise at -78 °C to the solution of the freshly prepared anion. The reaction mixure was stirred for 6 h at -78 °C, quenched at -78 °C with 30 mL of 2*N. aq.* NH_4Cl, extracted with 40 mL of EtOAc, washed with 15 mL of brine, and dried over $MgSO_4$. The crude product was chromatographed on silica gel with a 1 : 7 mixture of EtOAc and hexane to afford 479 mg (71% yield) of **11a**. **11a**: ^{1}H NMR ($CDCl_3$) δ 1.46 (9H, s, $OC(CH_3)_3$), 2.43-2.56 (2H, m, CH_2O), 3.14 (1H, brs, OH), 3.61 (2H, d, J = 3.2 Hz, C*H*$_2$CH), 4.48-4.60 (1H, m, C*H*OH), 5.80-5.92 (2H, m, CH=CH), 7.19-7.52 (15H, m, C_6H_5 x3).

***tert*-Butyl 3-Acetoxy-6-(triphenyl)methoxy-*cis*-4-hexenate (12a):**

To a solution of 205 mg (0.460 mmol, 1 equiv.) of **11a** in 4 mL of dried pyridine was added at 0 °C 87 μL (0.923 mmol, 2 equiv.) of acetic anhydride. After stirring for 16 h at rt, the reaction mixture was quenched with 5 mL of water, extracted with 40 mL of EtOAc, washed with 20 mL of brine, and dried over $MgSO_4$. The crude product was chromatographed on silica gel with a 1 : 10 mixture of EtOAc and hexane to afford 182 mg (81% yield) of **12a**. **12a**: ^{1}H NMR ($CDCl_3$) δ 1.44 (9H, s, $OC(CH_3)_3$), 2.05 (3H, s, CH_3C=O), 2.54 (1H, dd, J = 5.6, 16.6 Hz, C*H*$_A$CH), 2.64 (1H, dd, J = 8.0, 16.6 Hz, C*H*$_B$CH), 3.61 (2H, s, CH_2O), 5.61-5.71 (1H, m, C*H*OAc), 5.78-5.91 (2H, m, CH=CH), 7.17-7.50 (15H, m, C_6H_5 x3).

***tert*-Butyl 3-Acetoxy-6-hydroxy-*cis*-4-hexenate (13):**

Prepared from 12a: A colorless clear solution of 34 mg (0.07 mmol, 1 equiv.) of **12a** and 6.5 μL (0.08 mmol, 1.2 equiv.) of TFA in 2 mL of chloroform was stirred at rt for 2 h and evaporated. The crude residue was chromatographed on silica gel with a 1 : 5 mixture of EtOAc and hexane to afford 7 mg (43% yield) of **13**. **13**: ^{1}H NMR ($CDCl_3$) δ 1.44 (9H, s, $OC(CH_3)_3$), 1.64 (1H, brs, OH), 2.05 (3H, s, CH_3C=O), 2.52 (1H, d, J = 5.8, 15.2 Hz, C*H*$_A$CH), 2.62 (1H, d, J = 8.0, 15.2 Hz, C*H*$_B$CH), 4.17 (2H, d, J = 4.0 Hz, C*H*$_2$OH), 5.60-5.77 (3H, m, C*H*OAc, C*H*=CHCH$_2$OH), 5.94 (1H, dt, J = 5.8, 15.3 Hz, CH=C*H*CH$_2$OH).

Prepared from 12b: A yellow clear solution of 1.68 g (4.69 mmol, 1 equiv.) of **12b** and 9.39 mL (9.39 mmol, 2 equiv.) of a 1M solution of TBAF in THF was stirred at 0 °C for 4.5 h and evaporated. The residual brown oil was dissolved in 70 mL of EtOAc, washed with 20 mL of water and 10 mL of brine, and dried over $MgSO_4$. The crude product was chromatographed on silica gel with a 1 : 1 mixture of EtOAc and hexane to afford 293 mg (25% yield) of **13**.

4-(*tert*-Butyldimethyl)siloxy-*cis*-2-butenol (9b):

To a solution of 1.65 mL (20 mmol, 2 equiv.) of **8** in 10 mL of dried THF were added a solution of 1.50 g (10 mmol, 1 eqiv.) of TBS-Cl in 3 mL of dried THF and added dropwise at 0 °C a solution of 244 mg (2 mmol, 0.2 equiv.) of DMAP in 2 mL of dried THF, then 1.40 mL (10 mmol, 1 equiv.) of triethylamine. After stirring for 22 h at rt, the reaction mixture was diluted with 60 mL of EtOAc, washed with 10 mL of brine, and dried over $MgSO_4$. The crude product was chromatographed on silica gel with a 1 : 9 mixture of EtOAc and hexane to afford 1.73 g (86% yield) of **9b**. **9b**: ^{1}H NMR ($CDCl_3$) δ 0.09 (6H, s, $Si(CH_3)_2$), 0.91 (9H, s, $SiC(CH_3)_3$), 4.20, 4.26 (2H, 2H, d, d, J = 5.2, 5.2 Hz, CH_2 x2), 5.66-5.74 (2H, m, CH=CH).

4-(*tert*-Butyldimethyl)siloxy-*cis*-2-butenal (10b):

To a solution of 2.20 g (10.9 mmol, 1 equiv.) of **9b** in 15 mL of dried DMF were added 1.07 g (13.1 mmol, 1.2 equiv.) of sodium acetate, 2.2 g of MS3A, and 2.83 g (13.1 mmol, 1.2 equiv.) of PCC. After stirring for 3.5 h at rt, the reaction suspension was filtered through a Celite pad, diluted with 40 mL of EtOAc, washed with 40 mL of half brine and 10 mL of brine, and dried over $MgSO_4$. The crude product was chromatographed on silica gel with a 1 : 5 mixture of EtOAc and hexane to afford 1.67 g (76% yield) of **10b**. **10b**: ^{1}H NMR ($CDCl_3$) δ 0.09 (6H, s, $Si(CH_3)_2$), 0.93 (9H, s, $SiC(CH_3)_3$), 4.46 (2H, d, J = 7.2 Hz, CH_2), 6.42 (1H, dd, J = 10.8, 20.5 Hz, CH=C*H*CHO), 6.90 (1H, dt, J = 4.4, 20.5 Hz, C*H*=CHCHO), 9.61 (1H, d, J = 10.8 Hz, CHO).

***tert*-Butyl 6-(*tert*-Butyldimethyl)siloxy-3-hydroxy-*cis*-4-hexenate (11b):**

To a solution of a freshly prepared LDA from 1.22 mL (8.71 mmol, 1.3 equiv.) of diisopropylamine and 5.06 mL (8.04 mmol, 1.2 equiv.) of a 1.59M solution of butyl lithium in hexane in the presence of 1.52 mL (10.1 mmol, 1.5 equiv.) of TMEDA in 15 mL of dried THF at 0 °C for 1 h was added dropwise 1.08 mL (8.04 mmol, 1.2 equiv.) of *tert*-butyl acetate. After stirring for 1 h at -78 °C, a solution of 1.34 g (6.70 mmol, 1 equiv.) of **10b** in 7 mL of dried THF was added dropwise at -78 °C to the solution of the freshly prepared anion. The reaction mixure was stirred for 2 h at -78 °C, the reaction mixture was quenched at -78 °C with 20 mL of 2*N*. *aq*. NH_4Cl, extracted with 50 mL of EtOAc, washed with 20 mL of brine, and dried over $MgSO_4$. The crude product was chromatographed on silica gel with a 1 : 4 mixture of EtOAc and hexane to afford 2.06 g (97% yield) of **11b**. **11b**: ^{1}H NMR ($CDCl_3$) δ 0.06 (6H, s, $Si(CH_3)_2$), 0.90 (9H, s, $SiC(CH_3)_3$), 1.46 (9H, s, $OC(CH_3)_3$), 2.38-2.53 (2H, m, C*H*$_2$CH), 3.10 (1H, brs, OH), 4.17 (2H, d, J = 3.6 Hz, CH_2O), 4.47-4.56 (1H, m, C*H*OH), 5.72 (1H, dd, J = 8.0, 16.0 Hz, C*H*=CHCH$_2$O), 5.83 (1H, dt, J = 3.6, 16.0 Hz,

CH=C*H*CH$_2$O); ^{13}H NMR ($CDCl_3$) δ -4.6 (Si(CH_3)$_2$), 19.1 (Si*C*Me$_3$), 26.6, 28.8 (C(*C*H$_3$)$_3$ x2), 43.0 (*C*H$_2$CO$_2$), 63.7 (CH$_2$O), 69.1 (*C*HOH), 82.0 (O*C*Me$_3$), 131.0, 131.2 (*C*H=*C*HCH$_2$OTBS), 172.5 (C=O).

***tert*-Butyl 3-Acetoxy-6-(*tert*-butyldimethyl)siloxy-*cis*-4-hexenate (12b):**

To a pale yellow clear solution of 1.66 g (5.25 mmol, 1 equiv.) of **11b**, 2.19 mL (15.8 mmol, 3 equiv.) of triethylamine, and 128 mg (1.05 mmol, 0.2 equiv.) of DMAP in 20 mL of dried THF was added dropwise at rt 0.99 mL (10.5 mmol, 2 equiv.) of acetic anhydride. After stirring for 20 h at rt, the yellow clear solution was diluted with 100 mL of EtOAc, washed with 20 mL of brine, and dried over $MgSO_4$. The crude product was chromatographed on silica gel with a 1 : 4 mixture of EtOAc and hexane to afford 1.87 g (99% yield) of **12b**. **12b**: ^{1}H NMR ($CDCl_3$) δ 0.05 (6H, s, Si(CH_3)$_2$), 0.90 (9H, s, SiC(CH_3)$_3$), 1.43 (9H, s, OC(CH_3)$_3$), 2.03 (3H, s, CH_3C=O), 2.50 (1H, dd, *J* = 5.6, 13.8 Hz, C*H*$_A$CH), 2.60 (1H, dd, *J* = 8.4, 13.8 Hz, C*H*$_B$CH), 4.16 (2H, d, *J* = 3.6 Hz, CH$_2$O), 5.60-5.73 (1H, m, C*H*=CHCH$_2$O), 5.84 (1H, dt, *J* = 3.6, 16.0 Hz, CH=C*H*CH$_2$O); ^{13}H NMR ($CDCl_3$) δ -4.6 (Si(CH_3)$_2$), 19.1 (Si*C*Me$_3$), 21.8 (*C*H$_3$C=O), 26.6, 28.7 (C(*C*H$_3$)$_3$ x2), 41.7 (*C*H$_2$CO$_2$), 63.4 (CH$_2$O), 71.3 (*C*HOAc), 81.7 (O*C*Me$_3$), 127.0 (*C*H=CHCH$_2$O), 133.8 (CH=*C*HCH$_2$O), 169.7, 170.5 (C=O x2).

***tert*-Butyl 3-Acetoxy-5-hormyl-*cis*-4-pentenate (14):**

To a colorless clear solution of 293 mg (1.20 mmol, 1 equiv.) of **13** in 10 mL of dried CH_2Cl_2 was added 610 mg (1.44 mmol, 1.2 equiv.) of 1,1,1-tris(acetyloxy)-1,1-dihydro-1,2-benziodoxol-3-(1*H*)-one (Dess-Martin reagent).[6] After stirring for 3 h at rt, the colorless suspension was filtered through a Celite pad. The filtrate was cencentrated and the residue was dissolved in 50 mL of EtOAc, washed with 10 mL of 2*N aq.* $NaHCO_3$ and 5 mL of brine, and dried over $MgSO_4$. The crude product was chromatographed on silica gel with a 1 : 4 mixture of EtOAc and hexane to afford 238 mg (82% yield) of **14**. **14**: ^{1}H NMR ($CDCl_3$) δ 1.43 (9H, s, C(CH_3)$_3$), 2.11 (3H, s, CH_3C=O), 2.63 (1H, dd, *J* = 6.4, 16.0 Hz, C*H*$_A$CH), 2.70 (1H, dd, *J* = 7.4, 16.0 Hz, C*H*$_B$CH), 5.82-5.87 (1H, m, C*H*OAc), 6.23 (1H, dd, *J* = 8.0, 16.0 Hz, CH=C*H*CHO), 6.81 (1H, dd, *J* = 4.8, 16.0 Hz, C*H*=CHCHO), 9.57 (1H, d, *J* = 8.0 Hz, CHO); ^{13}H NMR ($CDCl_3$) δ 21.4 (*C*H$_3$C=O), 28.7 (C(*C*H$_3$)$_3$), 40.5 (CH$_2$), 69.5 (*C*HOAc), 82.5 (*C*Me$_3$), 132.6 (CH=*C*HCHO), 152.8 (*C*H=CHCHO), 168.8, 170.2 (C=O x2), 193.5 (CHO).

***tert*-Butyl Hydrogen 3-Acetoxy-*cis*-4-hexene-1,6-dicarboxylate (15):**

To a colorless clear solution of 237 mg (0.979 mmol, 1 equiv.) of **14** in 2 mL of MeCN were added 32 mg (0.260 mmol, 0.27 equiv.) of NaH_2PO_4, 115 μL (1.02 mmol, 1.04 equiv.) of 30% *aq.* H_2O_2, and 0.5 mL of water and added dropwise at 0 °C a solution of 155 mg (1.37 mmol, 1.4 equiv.) of $NaClO_2$ in 2 mL of water.[8] After stirring for 3.5 h at rt, the colorless clear solution was quenched at 0 °C with 2 mL of *sat. aq.* Na_2SO_3, acidified at 0 °C with 2 mL of 2*N aq.* HCl, extracted with 30 mL of EtOAc, washed with 5 mL of brine, and dried over $MgSO_4$. After removal of the solvent, 229 mg (91% yield) of a crude **15** was obtained. The crude **15** was used for the next step without purification. **15**: ^{1}H NMR ($CDCl_3$) δ 1.45 (9H, s, C(CH_3)$_3$), 2.11 (3H, s, CH_3), 2.60 (1H, dd, *J* = 6.4, 16.0 Hz, C*H*$_A$CH), 2.66 (1H, dd, *J* = 7.6, 16.0 Hz, C*H*$_B$CH), 5.74-5.85 (1H, m, C*H*OAc), 5.99 (1H, d, *J* = 16.0 Hz, CH=C*H*CO$_2$H), 6.99 (1H,

dd, J = 5.0, 16.0 Hz, C*H*=CHCO$_2$H); ^{13}H NMR ($CDCl_3$) δ 21.4 (*C*H$_3$C=O), 28.6 (C(*C*H$_3$)$_3$), 40.5 (CH_2), 69.5 (*C*HOAc), 82.3 (*C*Me$_3$), 122.1 (CH=*C*HCO$_2$H), 147.1 (*C*H=CHCO$_2$H), 168.9, 170.2, 171.4 (C=O x3).

Dimethyl 4-Hydroxy-*cis*-2-hexene-1,6-dicarboxylate (16):

A colorless clear solution of 228 mg (0.884 mmol, 1 equiv.) of **15** and 31 mg (0.177 mmol, 0.2 equiv.) of TsOH in 5 mL of MeOH was refluxed for 28 h and evaporated. The residue was dissolved in 30 mL of EtOAc, washed with 10 mL of 2*N aq.* $NaHCO_3$ and 5 mL of brine, and dried over $MgSO_4$. The crude product was chromatographed on silica gel with a 1 : 1 mixture of EtOAc and hexane to afford 138 mg (83% yield) of **16**. **16**: ^{1}H NMR ($CDCl_3$) δ 2.55 (1H, dd, J = 8.4, 16.8 Hz, C*H*$_A$CH), 2.67 (1H, dd, J = 4.0, 16.8 Hz, C*H*$_B$CH), 3.73 (3H, s, CH_3), 3.75 (3H, s, CH_3), 4.71-4.76 (1H, m, C*H*OH), 6.15 (1H, d, J = 15.6 Hz, CH=C*H*CO$_2$Me), 6.92 (1H, dd, J = 4.4, 15.6 Hz, C*H*=CHCO$_2$Me); ^{13}H NMR ($CDCl_3$) δ 40.8 (CH_2), 52.3, 52.7 (CH_3 x2), 67.7 (CHOH), 121.5 (CH=*C*HCO$_2$Me), 148.3 (*C*H=CHCO$_2$Me), 167.4, 172.8 (C=O x 2).

Dimethyl 4-Hydroxy-*cis*,*cis*-2,4-hexadiene-1,6-dicarboxylate (Maleylacetic Acid Dimethyl Ester, 7):

The procedure described for preparation of **14** was repeated with 137 mg (0.729 mmol, 1 equiv.) of **16** and 371 mg (0.874 mmol, 1.2 equiv.) of Dess-Martin reagent in 6 mL of dried CH_2Cl_2. The filtrate was cencentrated and the residue was dissolved in 30 mL of EtOAc, washed with 5 mL of 2*N aq.* $NaHCO_3$ and 5 mL of brine, and dried over $MgSO_4$. The crude product was chromatographed on silica gel with a 1 : 2 mixture of EtOAc and hexane to afford 116 mg (85% yield) of **7**. **7**: ^{1}H NMR ($CDCl_3$) δ 3.79 (3H, s, CH_3), 3.80 (3H, s, CH_3), 5.31 (1H, s, CH=C<), 6.66, 6.94 (1H, 1H, d, d, J = 15.4 Hz, CH=CH); ^{13}H NMR ($CDCl_3$) δ 52.3, 52.7 (CH_3 x2), 96.9 (*C*H=C<), 126.1 (CH=*C*HCO$_2$Me), 138.1 (*C*H=CHCO$_2$Me), 166.6 (CH=*C*<), 166.9, 173.0 (C=O x 2).

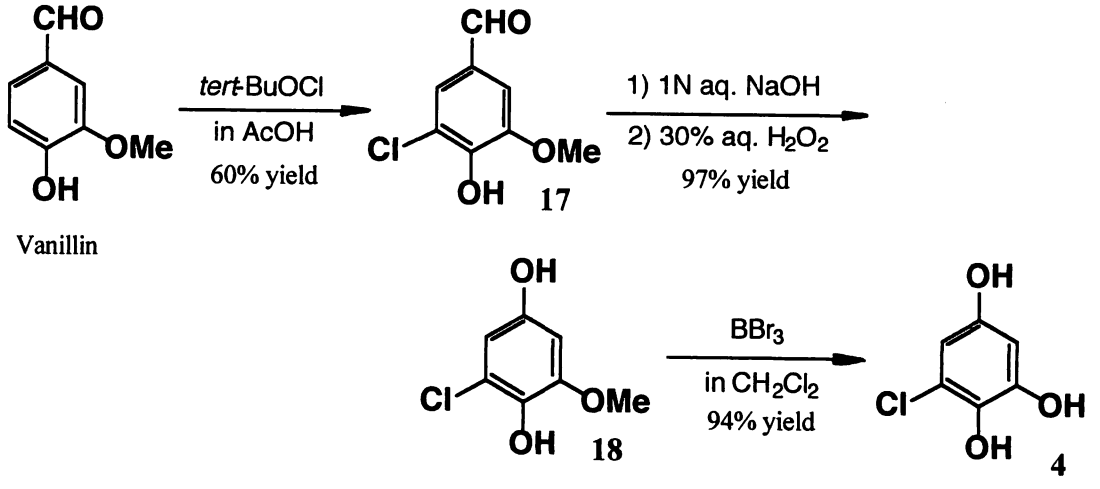
References and Notes

1) Apajalhti, J. H. A.; Karpanoja, P.; Salkinoja-Salonen, M. S. *Int. J. Syst. Bacteriol.*, **1986**, *36*, 246.

2) a) Hatta, T.; Nakano, O.; Imai, N.; Takizawa, N.; Kiyohara , H. *J. Biosci. Bioeng.*, **1999**, *87*, 267; b) Hatta, T.; Miyabe, K.; Tsukiashi, Y; Imai, N.; Takizawa, N.; Kiyohara, H. *Bull. Res. Inst. of Tech., Okayama Univ. of Sci.*, **1998**, *16*, 16; c) Hatta, T.; Nakano, O.; Imai, N.; Takizawa, N.; Kiyohara, H. *Bull. Res. Inst. of Tech., Okayama Univ. of Sci.*, **1997**, *15*, 13; d) Kiyohara, H.; Hatta, T.; Ogawa, Y.; Kakuda, T.; Yokoyama, H.; Takizawa, N. *Appl. Environ. Microbiol.*, **1992**, *58*, 1276; e) Schmidt, E.; Knackmuss, H. -J. *Biochem. J.*, **1980**, *192*, 339.

3) We failed to synthesize **4** by Lutus' procedure[3a] in our hands. Therefore, we improved the procedure for preparation of **4** as follows. Vanilin was chlorinated by *tert*-BuOCl in AcOH to give **17** in 60% yield.[3b] The chlorinated vanillin **17** was converted to **18** in 97% yield by treatment with 1*N aq.* NaOH and 30% *aq.* H_2O_2.[3a, 3c] Finally, deprotection of the methoxy group on **18** was proceeded with BBr_3 in CH_2Cl_2 to afford **4** in 94% yield.[3d] It is found that 6-chloro-1,2,4-benzenetriol **4** is unstable under light or basic conditions.

a) Lutus, M; Seitz, H. -J.; Eberspacher, J.; Lingens, F. *Appl. Environ. Microbiol.*, **1995**, *61*, 2453; b) Giusburg, D. *J. Am. Chem. Soc.*, **1951**, 73, 702; c) Dakin, H. D. *Org. Syn.* I, 149; d) McOmie, J. F. W.; Watts, M. L.; West, D. E. *Tetrahedron*, **1968**, *24*, 2289.



4) We failed to synthesize **1** in our hands by the procedures described by Lutus[4a] and Kaschabek.[4b]
a) Lutus, M; Seitz, H. -J.; Eberspacher, J.; Lingens, F. *Appl. Environ. Microbiol.*, **1995**, *61*, 2453; b) Kaschabek, S.R.; Reineke, W. *J. Bacteriol.*, **1995**, *177*, 320.

5) Oxidation of the primary alcohol **13** with PCC, MS3A, sodium acetate in DMF afforded **14** in 72% yield.

6) Dess, D. B.; Martin, J. C. *J. Org. Chem.*, **1983**, *48*, 4155.

7) The aldehyde **14** was also converted to the nitrile **19** in 13% yield with $HONH_3Cl$, MS4A in triethylamine-benzene, followed by treatment of *p*-toluenesulfonyl chloride.
a) Saednya, A. *Synthesis*, **1982**, 190; b) Dauzonne, D.; Demerseman, P.; Royer, R. *Synthesis*, **1981**, 739.

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