Supporting Information for

Identification of (8*S*,9*S*,10*S*)-8,10-dimethyl-1-octalin, a key intermediate in the biosynthesis of geosmin in bacteria

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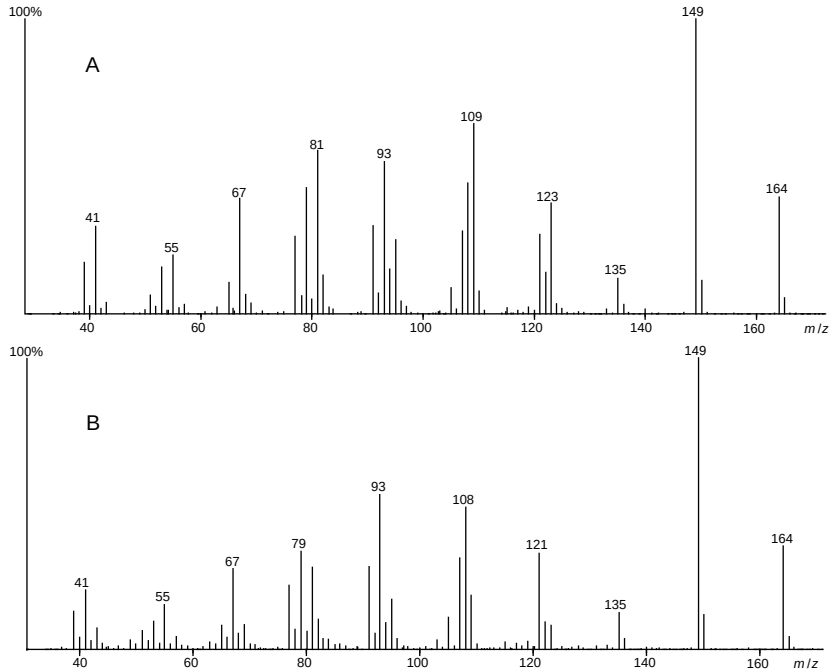

Figure S1. Mass spectra of the natural octalins **A** (8,10-dimethyl-1-octalin, **3**), and **B** (8,10-dimethyl-1(9)-octalin, **4**)

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Materials and methods.

The cultivation of the strains *Stigmatella aurantiaca* DW4/3-1 and *Myxococcus xanthus* DK1622 have been described previously.[S1a,S1b] Volatile organic compounds emitted by agar plate cultures were collected using the CLSA technique as described previously.[S1a] GC-MS analyses were carried out on a HP 6890 Series GC System connected to a HP 5973 Mass Selective Detector (Hewlett-Packard, Wilmington, USA) fitted with a BPX5 fused-silica capillary column (25 m x 0.22 mm i. d., 0.25 μm film, SGE, Melbourne, Australia). Conditions were as follows: inlet pressure: 77.1 kPa, He 23.3 mL min^{-1}; injection volume: 1 μL; transfer line: 300°C; electron energy: 70 eV. The GC was programmed as follows: 5 min at 50°C increasing at 5°C min^{-1} to 320°C, and operated in splitless mode (60 s valve time). The carrier gas was He at 1 mL min^{-1}. Retention indices *I* were determined from a homologous series of *n*-alkanes ($C_8 - C_{38}$). Identification of compounds was performed by comparison of mass spectra and retention indices as well as coinjection experiments. Chiral GC was performed with a Hydrodex-6-TBDMS-phase (35 m x 0.25 mm i. d., 0.25 μm phase thickness, Macherey-Nagel, Düren, Germany) programmed as follows: 70°C, 5 min, then 0.1°C/min to 100°C. Recombinant germacradienol/geosmin synthase, encoded by the *sco6073* gene of *S. coelicolor* A3(2) was expressed from *E. coli* BL21(DE3)pLysS/pRW31, resolubilized from inclusion bodies, purified to homogeneity, and assayed as previously described.[S2,S3] Incubation of germacradienol/geosmin synthase with farnesyl diphosphate and isolation of the pentane–methylene chloride-soluble products has been previously described.[S2,S3]

Synthesis (general methods). Chemicals: Chemicals were purchased from Fluka Chemie (Buchs, Switzerland) or Sigma-Aldrich Chemie (Steinheim, Germany); ^{1}H NMR and ^{13}C NMR: Spectra were obtained using a Bruker AMX400 (400 MHz/100MHz) or a Bruker AV II-600 (600 MHz/150MHz) spectrometer with TMS as an internal standard; Column chromatography: Column chromatography was carried out using Merck Kieselgel 60; Thin layer chromatography: TLC was carried out using 0.2 mm pre-coated plastic sheets Polygram Sil G/UV_{254} (Marcherey-Nagel, Düren, Germany); Solvents: Solvents were purified by distillation and dried according to standard methods.

Synthesis of (8*S*,10*S*)-8,10-dimethyl-1-octalin *(cis*- and *trans*-3)

*(8*S*,10*S*)-8,10-dimethyl-1(9)-octalin-2-tosylhydrazone* (**11**). Three drops of boron trifluoride diethyl etherate are added to a suspension of toluene-4-sulfonohydrazide (313 mg,

1.63 mmol) and octalone **10** (300 mg, 1.63 mmol) in toluene (13.5 ml) according to the general procedure by Liu et al.[S4] The reaction mixture was stirred for 15 h at room temperature. Then diethyl ether (30 ml) was added and the mixture was washed twice with water and brine. The organic layer was separated, dried with $MgSO_4$, and the solvent was evaporated under vacuum. Column chromatography on silica gel using diethyl ether as solvent gave pure **12** (155 mg, 0.45 mmol, 27%). m. p. 122°C (decomp.)

^{1}H-NMR ($CDCl_3$): δ = 1.06 (s, 3H, CH_3), 1.37 (d, 3H, $^3J_{H,H}$ = 5.5 Hz, CH_3), 1.51-1.66 (m, 8H, 4xCH_2), 2.05-2.23 (m, 2H, CH_2), 2.42 (s, 3H, CH_3), 2.42-2.49 (m, 1H, CH), 5.86 (m, 1H, CH), 7.30-7.33 (m, 2H, 2xCH), 7.85-7.89 (m, 2H, 2xCH).

^{13}C-NMR ($CDCl_3$): δ = 18.4 (CH_3), 20.5 (CH_2), 21.6 (CH_3), 21.8 (CH_2), 23.1 (CH_3), 33.7 (CH), 35.4 (C), 36.3 (CH_2), 37.1 (CH_2), 41.6 (CH_2), 118.4 (CH), 128.0 (2xCH), 129.6 (2xCH), 135.6 (C), 137.0 (C), 143.9 (C), 187.2 (C).

EI-MS (*m*/*z*): = 162 (32), 147 (28), 133 (7), 119 (23), 106 (33), 105 (62), 92 (26), 91 (100), 77 (16), 65 (9), 51 (5), 41 (12), 39 (10).

*(8*S*,10*S*)-8,10-dimethyl-1-octalin* (**3**). The tosylhydrazone **12, (**274 mg, 0.79 mmol) was dissolved in 5.5 ml conc. acetic acid and $NaBH_4$ (828 mg, 21.8 mmol) was added portionwise over a period of 25 minutes, so that gas formation was not too strong.[4] The mixture was then stirred for 30 min at room temperature and subsequently for 2 h under reflux conditions. The reaction was quenched by pouring the mixture onto crushed ice followed by addition of 1 M NaOH until the solution was basic. The aqueous layer was extracted three times with diethyl ether. The combined organic layers were dried with $MgSO_4$ and concentrated to give an inseparable mixture of *cis*- and *trans*-**3** (67.5 mg, 0.41 mmol, 52%). d. r. = 80: 20, The e. r. was 78:22 for both diastereomers.

^{1}H-NMR ($CDCl_3$): δ = 0.81 (s, 3H, CH_3, *trans*-**3**), 0.90 (d, 3H, $^3J_{H,H}$ = 6.4 Hz, CH_3, *trans*-**3**), 0.93 (d, 3H, $^3J_{H,H}$ = 7.0 Hz, CH_3, *cis*-**3**), 0.98 (s, 3H, CH_3, *cis*-**3**), 1.30-1.53 (m, 16H, 8xCH_2), 1.82-1.84 (m, 2H, 2xCH), 1.87-1.94 (m, 2H, 2xCH), 1.94-2.13 (m, 4H, 2xCH_2), 5.58-5.69 (m, 2H, 2xCH), 5.67-5.71 (m, 2H, 2xCH) (*cis*-**3** and *trans*-**3**).

^{13}C-NMR ($CDCl_3$): δ = 15.7 (CH_3, *trans*-**3**), 19.3 (CH_3), 19.5 (CH_3, *trans*-**3**), 21.4 (CH_2, *trans*-**3**), 22.0 (CH_2), 22.3 (CH_2), 23.1 (CH_2, *trans*-**3**), 26.7 (CH_3), 29.0 (CH_2), 29.6 (CH_2), 29.7 (CH, *trans*-**3**), 30.3 (CH), 31.9 (C), 32.4 (C, *trans*-**3**), 36.6 (CH_2, *trans*-**3**), 36.9 (CH_2), 37.9 (CH_2, *trans*-**3**), 40.1 (CH_2, *trans*-**3**), 46.1 (CH), 50.6 (CH, *trans*-**3**), 126.0 (CH), 126.3 (CH, *trans*-**3**), 127.1 (CH), 127.5 (CH, *trans*-**3**).

EI-MS (*m*/*z*) = 164 (38) $[M]^+$, 149 (100), 135 (14), 123 (45), 109 (77), 108 (46), 93 (61), 81 (62), 79 (64), 67 (43), 55 (29), 41 (48), 39 (30).

Synthesis of (8*S*,10*S*)-8,10-dimethyl-1-octalin *(trans*-3)

*Diethyl-(8*S*,10*S*)-8,10-dimethyl-1(9)-octalin-2-yl phosphate* (**11**). Compound **11** was prepared using a method described by Grieco et al.[S5]. Lithium metal (134 mg, 19.1 mmol) was added to distilled ammonia (27 ml) at -78 °C. A solution of octalone **10** (670 mg, 3.76 mmol) and *tert*-butyl alcohol (217 µl, 3.76 mmol) in THF (14 mL) was added. After 1 h, the reaction was quenched by the addition of 2 ml freshly distilled isoprene. The reaction mixture was warmed to 0 °C and parts of the ammonia were evaporated under vacuum conditions (~800 mbar). A 1:1 mixture of THF and *N*,*N*,*N'*,*N'*-tetramethylethylenediamine (10 mL) was then added. This was followed by the addition of diethyl chloro phosphate (2.12 g, 12.3 mmol). The reaction mixture was stirred for 30 min at 0 °C and then for 3 h at room temperature, and finally poured into cold sat. $NaHCO_3$ solution (80 ml). The layers were separated and the aqueous layer was extracted three times with diethyl ether. The combined extracts were washed with brine, dried with anhydrous magnesium sulfate, and concentrated in vacuo. Chromatography on silica gel using diethyl ether as solvent afforded 1.001 g (3.17 mmol; 84%) of the enol phosphate **11**.[17]

^{1}H-NMR ($CDCl_3$): δ = 0.84 (s, 3H, CH_3), 0.88 (d, 3H, $^3J_{H,H}$ = 6.3 Hz, CH_3), 1.32-1.38 (m, 6H, 2xCH_3), 1.39-1.75 (m, 9H, 1xCH, 4xCH_2), 2.14-2.37 (m, 3H, 1xCH, 1xCH_2), 4.02-4.21 (m, 4H, 2xCH_2), 5.49 (d, 1H, $^3J_{H,H}$ = 0.81 Hz, CH).

^{13}C-NMR ($CDCl_3$): δ = 15.8 (CH_3), 16.0 (CH_3), 16.1 (CH_3), 21.3 (CH_3), 25.5 (CH_2), 30.0 (CH), 31.1 (CH_3), 32.7 (C), 36.2 (CH_2), 37.9 (CH_2), 39.3 (CH_2), 49.6 (CH), 63.9 (CH_2), 64.0 (CH_2), 111.4/111.5 (CH), 147.2/147.3 (C).

EI-MS (*m*/*z*): = 316 (49) $[M]^+$, 301 (6), 287 (13), 179 (31), 162 (45), 155 (82), 147 (100), 133 (24), 127 (44), 119 (24), 105 (34), 99 (42), 91 (46), 81 (33), 55 (15).

*(8*S*,10*S*)-8,10-dimethyl-1-octalin* (**3**). Lithium (11 mg, 1.57 mmol) was added to anhydrous ethylamine (1.5 mL) at 0 °C. After stirring for 45 min, a solution of **11 (**50 mg, 0.16 mmol) and *tert*-butyl alcohol (26 µl, 0.25 mmol) in THF (1.5 mL) was added dropwise. Stirring was continued for 2 h at 0 °C, and the reaction mixture was then quenched with isoprene followed by slow addition of 0.5 ml methanol. The ethylamine was allowed to evaporate and the residue was taken up in 10 ml of water. The layers were separated and the aqueous layer was extracted three times with diethyl ether. The combined organic layers were washed with brine,

dried over magnesium sulfate, and concentrated in vacuo.[S5] Column chromatography on silica gel using pentane as solvent gave 3 mg (0.02 mmol; 10%) of *trans*-**3**.

^{1}H-NMR ($CDCl_3$): δ = 0.81 (s, 3H, CH_3), 0.90 (d, 3H, $^3J_{H,H}$ = 6.2 Hz, CH_3), 0.91-1.01 (m, 1H, CH_2), 1.08-1.14 (m, 1H, CH_2), 1.28-1.39 (m, 4H, 1xCH, 2x CH_2), 1,46-1.52 (m, 1H, CH), 1.55-1.56 (m, 1H, CH_2), 1.68-1.75 (m, 2H, 2xCH_2), 1.98-2.18 (m, 2H, CH_2), 5.55-5.64 (m, 1H, CH), 5.67-5.69 (m, 1H, CH).

^{13}C-NMR ($CDCl_3$): δ = 15.9 (CH_3), 19.8 (CH_3), 21.6 (CH_2), 23.3 (CH_2), 29.9 (CH), 32.6 (C), 36.8 (CH_2), 38.1 (CH_2), 40.3 (CH_2), 50.8 (CH), 126.5 (CH), 127.7 (CH).

EI-MS (*m*/*z*) = 164 (38) $[M]^+$, 149 (100), 135 (15), 123 (35), 109 (65), 93 (48), 81 (49), 67 (36), 55 (21), 41 (28).

Synthesis of (8*S*,10*S*)-8,10-dimethyl-1(9)-octalin (4)

*(8*S*,10*S*)-8,10-dimethyl-1(9)-octalin-2-one dithioketal* (**13**). A mixture of 100 mg (0.56 mmol) **10**, 324 mg (3 mmol) propane-1,3-dithiol, and 0.2 ml of boron trifluoride diethyl etherate in 1.5 ml methanol was stirred at room temperature. After 4 h the reaction mixture was poured onto 25 ml of ice-cooled aq. NaOH (10% w/w). The aqueous layer was extracted three times with a 1:1 mixture of pentane/diethyl ether. The combined organic layer was dried over $MgSO_4$, and all solvents were evaporated under vacuum to yield 145.8 mg (0.54 mmol, 97%) of **13**. The product was sufficiently pure for the next step.

^{1}H-NMR ($CDCl_3$): δ = 1.01 (d, 3H, $^3J_{H,H}$ = 6.5 Hz, CH_3), 1.09 (s, 3H, CH_3), 1.26-1.32 (m, 2H, CH_2), 1.50-2.42 (m, 9H, 4xCH_2, CH), 2.66-3.06 (m, 6H, 3xCH_2), 5.34 (m, 1H, CH).

^{13}C-NMR ($CDCl_3$): δ = 18.6 (CH_3), 22.0 (CH_2), 24.8 (CH_3), 25.1 (CH_2), 26.7 (CH_2), 27.1 (CH_2), 32.2 (CH_2), 33.2 (CH), 35.8 (C), 36.9 (CH_2), 37.1 (CH_2), 41.9 (CH_2), 49.6 (C), 119.2 (CH), 151.9 (C).

EI-MS (*m*/*z*): 268 (81) $[M]^+$, 253 (3), 235 (16), 194 (100), 161 (19), 137 (22), 125 (54), 112 (91), 105 (44), 91 (42), 41 (25).

*(8*S*,10*S*)-8,10-dimethyl-1(9)-octalin* (**4**). As described by Gutierrez et al.[S6] 330 mg (1.23 mmol) dithioketal **13** were dissolved in benzene (5 ml) and heated to 80 °C. To this solution Bu_3SnH (717 mg, 2.46 mmol) and then AIBN (30 mg, 0.18mmol) dissolved in benzene (1.5 ml) were added dropwise under stirring. The progress of the reaction was observed by TLC. After 3 h the reaction was complete and the solvent was evaporated. The crude product was purified by column chromatography on silica gel using hexane as solvent to give pure **4 (**102 mg, 0.62 mmol, 50%) as a colorless liquid.

^{1}H-NMR ($CDCl_3$): δ= 0.96 (d, 3H, $^{3}J_{H,H}$ = 6.4 Hz, CH_3), 1.07 (s, 3H, CH_3), 1.28-1.59 (m, 10H, 5xCH_2), 1.66 (tt, 1H, $^{3}J_{H,H}$ = 3.7 Hz), 1.72-1.78 (m, 1H), 1.97-2.02 (m, 1H, CH), 5.34 (dt, 1H, $^{3}J_{H,H}$ = 3.9 Hz, CH).

^{13}C-NMR ($CDCl_3$): δ = 18.8 (CH_3), 18.9 (CH_2), 22.3 (CH_2), 25.2 (CH_3), 26.4 (CH_2), 32.9 (CH), 35.1 (C), 37.5 (CH_2), 40.7 (CH_2), 42.4 (CH_2), 116.4 (CH), 147.5 (C).

EI-MS (*m*/*z*): 164 (40) $[M]^+$, 149 (100), 135 (13), 121 (34), 108 (48), 93 (56), 79 (36), 67 (28), 55 (14), 41 (23).

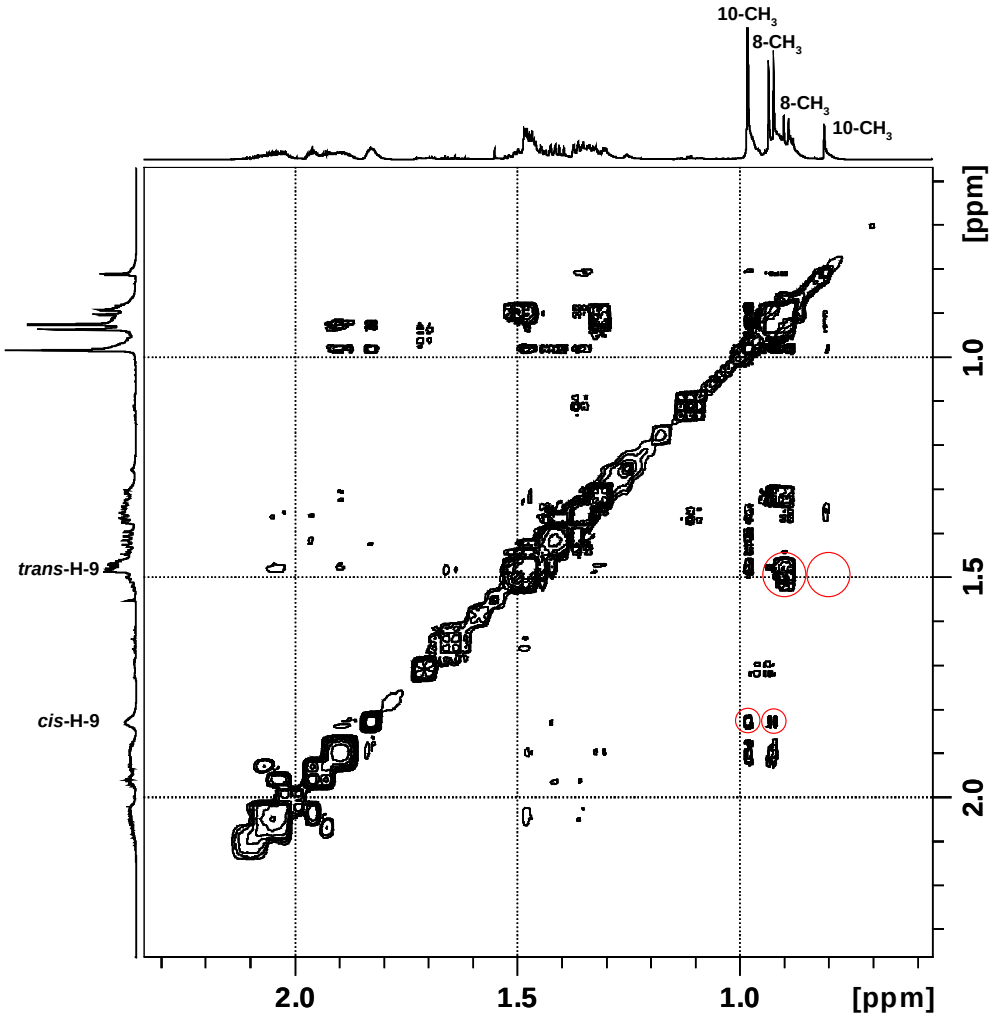

Figure S2. NOESY-spectrum of the diastereomeric mixture of *cis*- and *trans*-**3**. The interactions of the bridgehead hydrogen atom (H-9) with the methyl groups at C-8 and C-10 are annotatated.

Gas chromtatographic separation of octalins 3 on a chiral cyclodextrin phase

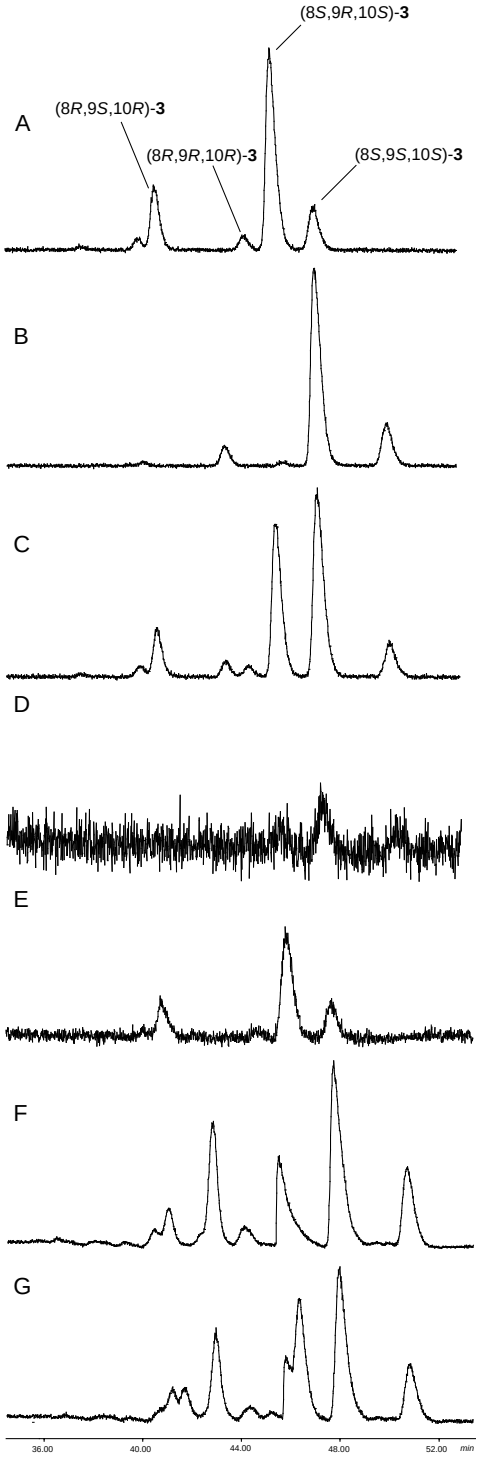

Figure S3. Chiral GC-experiments. A) Synthetic sample of **3**, B) enzyme extract including **3**, and C) coninjection synthetic sample/enzyme extract, D) headspace extract of *M. xanthus*, E) coinjection synthetic sample/headspace extract of *M. xanthus*, F) headspace of extract *Sorangium cellulosum*, G) coinjection synthetic sample/headspace extract *S. cellulosum*. Phase: 35 m Hydrodex-6-TBDMS.

References

(S1) a) Pollack, F. C.; Berger, R. G. *Appl. Env. Microbiol.* **1996**, *62*, 1295-1299. b) Dickschat, J. S.; Wenzel, S. C.; Bode, H. B.; Müller, R.; Schulz, S. *ChemBioChem* **2004**, *5*, 778-787.

(S2) Cane, D. E.; Watt, R. M. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 1547-1551.

(S3) Jiang, J.; He, X.; Cane, D. E. *J. Am. Chem. Soc.* **2006**, *128*, 8128-8129.

(S4) Liu, L.; Xiong, Z.; Nan, F.; Li, T.; Li, Y *Synth. Commun.* **1995**, *25*, 1971-1975.

(S5) Grieco, P. A.; Nargund, R. P.; Parker, D. T. *J. Am. Chem. Soc.* **1989**, *111*, 6287-6294

(S6) Gutierrez, C. G.; Stringham, R. A.; Nitasaka, T.; Glasscock, K. G. *J. Org. Chem.* **1980**, *45*, 3393-3395.