Supporting Information

Complete Stereochemistry of Tetrafibricin

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Synthesis of *syn*- and *anti*-1,5-dihydroxy-3-ketone

(*R*)-4-*O*-Benzyl-hept-1-en-4-ol (**2**)

To a solution of 1.68 g (14.8 mmol) of **1** in 100 ml of DMF at 0 °C was added 1.65 g of 60% NaH. The mixture was stirred at 25 °C for 1 h, cooled to 0 °C, treated with 3.25 g (19 mmol) of BnBr and 20 mg of Bu₄NI, and stirred at 25 °C for 14 h. The reaction was quenched with H₂O, extracted with Et₂O, the organic phase was washed with brine, dried (MgSO₄), and evaporated. Flash column chromatography (pentane/Et₂O 50:1) gave 1.14 g of **2** and mixed fractions which were subjected to chromatography (pentane/Et₂O 80:1) to give another 1.06 g of **2**. 2.2 g (73%) of **2**.

Data of **2**: TLC (pentane/Et₂O 35:1): 0.36. ¹H-NMR (CDCl₃, 300 MHz): 7.39-7.26 (m, 5H), 5.94-5.79 (m, 1H), 5.14-5.03 (m, 2H), 4.58 (d, *J* = 11.4, 1H), 4.49 (d, *J* = 11.4, 1H), 3.50-3.41 (m, 1H), 2.36-2.30 (m, 2H), 1.60-1.09 (m, 4H), 0.90 (t, *J* = 6.9, 3H).

(*R*)-4-*O*-Benzyl-heptane-1,2,4-triols (**2**)

To a solution of 1.46 g (7 mmol) of **2** in 48 ml of THF and 7.5 ml of H₂O were added 1.7 g of NMO, 35 mg of DABCO, and 0.5 ml of 1M OsO₄ in toluene. The reaction mixture was stirred at 25 °C for 3 h, 100 ml of saturated aqueous Na₂S₂O₃ was

added, the mixture stirred at 25 °C for 2 h, then extracted with AcOEt. The organic phase was washed with brine, dried (MgSO₄), and evaporated. Flash column chromatography (hexanes/AcOEt 2:1 to 1:5) gave 460 mg (27%, 2 diastereoisomers ca. 55:45, not optimized) of **3**.

Data of **3**: TLC (hexanes/AcOEt 1:1): 0.22. ¹H-NMR (CDCl₃, 300 MHz): 7.40-7.25 (m, 5H), 4.65 (d, *J* = 11.1, 0.45H), 4.59 (d, *J* = 11.4, 0.55H), 4.50 (d, *J* = 11.7, 0.55H), 4.43 (d, *J* = 11.1, 0.45H), 3.99 (ddd, *J* = 12.6, 6.6, 3.6, 0.55H), 3.89 (ddd, *J* = 12.3, 6.3, 3.0, 0.45H), 3.77-3.67 (m, 1H), 3.61 (dd, *J* = 8.1, 3.6, 0.45H), 3.57 (dd, *J* = 7.8, 3.6, 0.55H), 3.46 (d, *J* = 6.0, 0.55H), 3.42 (d, *J* = 6.9, 0.45H), 2.65-2.20 (br., OH), 1.84-1.46 (m, 4H), 1.44-1.28 (m, 2H), 0.97-0.90 (m, 3H). ¹³C-NMR (CDCl₃, 125 MHz): 138.18, 137.83, 128.57, 128.48, 127.91, 127.90, 127.80, 79.37, 76.62, 71.74, 71.21, 70.59, 69.08, 66.95, 66.80, 36.96, 35.85, 35.60, 35.56, 18.63, 17.87, 14.29, 14.21. (6*R*)-6-

O-Benzyl-non-1-ene-4,6-diols (**4**)

To a solution of 1.13 g (4.75 mmol) of **3** in 60 ml of CH₂Cl₂ were added 5.1 g of K₂CO₃, and, at 0 °C, 3.4 g of Pb(OAc)₄. The reaction was stirred at 0 °C for 15 min, filtered through Celite/MgSO₄, the residue washed with CH₂Cl₂, and the filtrate evaporated at 25 °C. The resulting aldehyde was dissolved in 100 ml of dry THF, cooled to 0 °C, treated with 9.5 ml of 1M AllylMgBr in Et₂O, stirred at 0 °C for 1 h, stirred at 25 °C for 30 min, cooled to 0 °C, quenched with saturated aqueous NH₄Cl, and extracted with Et₂O. The organic phase was washed with brine, dried (MgSO₄), and evaporated. Flash column chromatography (hexanes/AcOEt 6:1) gave 1.04 g (88%, ca. 1:1 mixture of diastereoisomers) of **4**.

Data of **4**: TLC (hexanes/AcOEt 6:1): 0.32. ¹H-NMR (CDCl₃, 300 MHz): 7.40-7.25 (m, 5H), 5.59-5.75 (m, 1H), 5.18-5.05 (m, 2H), 4.64 (d, *J* = 11.1, 0.5H), 4.58 (d, *J* = 11.4, 0.5H), 4.53 (d, *J* = 11.4, 0.5H), 4.43 (d, *J* = 11.1, 0.5H), 4.02-3.90 (m, 0.5H), 3.88-3.78 (m, 0.5H), 3.77-3.64 (m, 1H), 3.57 (d, *J* = 1.2, 0.5H), 2.78 (m, 0.5H), 2.22 (t, *J* = 6.0, 2H), 1.77-1.50 (m, 4H), 1.44-1.28 (m, 2H), 0.94 (t, *J* = 7.5, 1.5H), 0.93 (t, *J* = 7.5, 1.5H). ¹³C-NMR (CDCl₃, 125 MHz): 138.37, 137.98, 134.97 (2x), 128.50, 128.41, 127.87, 127.76, 127.68, 117.49, 117.30, 79.75, 76.86, 71.21, 70.91, 70.53, 67.69, 42.19, 42.13, 40.20, 39.31, 35.70, 35.60, 18.70, 17.90, 14.31, 14.22.

(5*R*)-5-*O*-Benzyl-octane-1,3,5-triols (**5**)

To a solution of 1.0 g (4 mmol) of **4** in 26 ml of THF and 4.1 ml of H₂O were added 0.93 g of NMO, 20 mg of DABCO, and 0.25 ml of 1M OsO₄ in toluene. The reaction was stirred at 25 °C for 2.5 h, treated with 40 ml of saturated aqueous Na₂S₂O₃ for 1 h, extracted with CHCl₃, the organic phase was washed with brine, dried (MgSO₄), and evaporated. The crude triol was dissolved in 60 ml of benzene, treated with 2.3 g of Pb(OAc)₄, stirred at 25 °C for 30 min, treated with 60 ml of saturated aqueous NaHCO₃, and the mixture stirred for 20 min. The reaction was extracted with benzene, the organic phase was washed with brine, dried (MgSO₄), and evaporated. The crude aldehyde was dissolved in 80 ml of MeOH, treated with 400 mg of NaBH₄ at 0 °C, and stirred at 25 °C for 2 h. The solution was diluted with AcOEt, extracted with H₂O and brine, dried (MgSO₄), and evaporated. Flash column chromatography (hexanes/AcOEt 1:2 to AcOEt) gave 870 mg (86%) of **5**.

Data of **5**: TLC (hexanes/AcOEt 2:1): 0.45. ¹H-NMR (CDCl₃, 500 MHz): 7.40-7.20 (m, 5H), 4.66 (d, *J* = 11.5, 0.5H), 4.58 (d, *J* = 11.5, 0.5H), 4.53 (d, *J* = 11.5, 0.5H), 4.43 (d, *J* = 11.0, 0.5H), 4.21-4.15 (m, 0.5H), 4.08-4.03 (m, 0.5H), 3.86-3.76 (m, 2H), 3.75-3.68 (m, 1H), 3.40-2.70 (br., 2OH), 1.88-1.48 (m, 6H), 1.46-1.28 (m, 2H), 0.98-0.90 (m, 3H). ¹³C-NMR (CDCl₃, 125 MHz): 138.11, 137.81, 128.56, 128.50, 128.48, 127.90, 127.87, 127.81, 80.06, 77.00, 72.31, 71.20, 70.52, 69.19, 61.75, 61.40, 40.93, 39.60, 38.72, 38.66, 35.49, 35.45, 18.75, 17.83, 14.30, 14.19.

(5R)- 5-O-Benzyl-1,3-O-(3',4'-dimethoxybenzylidene)-octane-1,3,5-triols (6)

To a solution of 860 mg (3.4 mmol) of **5** in 70 ml of CH₂Cl₂ was added 4Å MS, 2.17 g of 3,4-dimethoxybenzaldehyde, and 11 mg of *p*-TsOH·H₂O. The reaction was stirred at 25 °C for 14 h, solid NaHCO₃ was added, the resulting mixture filtered, and evaporated. Flash column chromatography (hexanes/AcOEt 20:1 to 12:1) gave 300 mg (22%) of pure **6** and 1.7 g of a ca. 1:4 (molar ratio, according to NMR) mixture of the acetals **6** and dimethoxybenzaldehyde which were subjected to another flash column chromatography.

Data of **6**: TLC (hexanes/AcOEt 1:1): 0.73. ¹H-NMR (CDCl₃, 500 MHz): 7.40-7.25 (m, 5H), 7.10-6.98 (m, 2H), 6.90-6.85 (m, 1H), 5.48, 5.41 (2s, 1H), 4.64 (d, *J* = 12.0), 4.57 (d, *J* = 12.0), 4.50 (d, *J* = 11.0), 4.49 (d, *J* = 11.5) 2H, 4.27 (dd, *J* = 11.0, 5.0),

(4*S*,8*R*)- 8-*O*-Benzyl-6-*O*-(3',4'-dimethoxybenzyl)-undec-1-ene-4,6,8-triols (**8a**) and (4*R*,8*R*)- 8-*O*-Benzyl-6-*O*-(3',4'-dimethoxybenzyl)-undec-1-ene-4,6,8-triols (**8b**)

A solution of 190 mg (0.47 mmol) of **7** in 9 ml of CH₂Cl₂ and 6 ml of DMSO was treated with 0.6 ml of *i*-Pr₂NEt and 0.34g SO₃·Pyr, and stirred at 25 °C for 45 min. The reaction was diluted with AcOEt, washed with H₂O and brine, dried (MgSO₄), and evaporated (crude aldehyde).

165 mg (0.52 mmol) of (-)-Ipc₂BOMe was dissolved in 5 ml of Et₂O, cooled to -78 °C, treated with 0.52 ml 1M AllylMgBr (0.52 mmol) in Et₂O, stirred at -78 °C for 15 min, stirred at 25 °C for 1 h, cooled to -78 °C, and treated with a solution of the crude aldehyde in 5 ml of Et₂O dropwise. The reaction was stirred at -78 °C for 1 h 15 min, brought to 25 °C, treated with 0.5 ml 3M NaOH dropwise, treated (slowly!) with 0.2 ml of 30% aqueous H₂O₂, and stirred at 25 °C for 14 h. The reaction was diluted with Et₂O, extracted with H₂O, the organic phase was dried (MgSO₄), and evaporated. Flash column chromatography (hexane/AcOEt 4:1 to 1:1) gave 137 mg (66%) of **8a**.

The diastereoisomer **8b** was obtained analogously using (+)-Ipc₂BOMe (78%).

Data of **8a**: TLC (hexanes/AcOEt 2:1): 0.34, 0.29. ¹H-NMR (CDCl₃, 400 MHz): 7.40-7.25 (m, 5H), 6.92-6.75 (m, 3H), 5.88-5.72 (m, 1H), 5.20-5.00 (m, 2H), 4.60-4.25 (m, 4H), 4.00-3.38 (m, 3H), 3.861, 3.849, 3.846, 3.837 (4s, 6H), 3.20-2.80 (br., OH), 2.30-1.30 (m, 10H), 0.93 (t, *J* = 7.2), 0.92 (t, *J* = 7.2) 3H. ¹³C-NMR (CDCl₃, 100 MHz): 148.91, 148.90, 148.62, 138.75, 138.64, 138.53, 134.82, 130.68, 130.41, 128.32, 128.30, 127.89, 127.81, 127.75, 127.68, 127.59, 127.50, 127.46, 120.62, 120.52, 117.43, 117.19, 111.29, 111.21, 110.89, 110.83, 76.83, 75.66, 75.26, 74.47, 71.45, 70.69, 70.57, 70.55, 70.33, 67.91, 55.86, 55.76, 55.75, 42.17, 41.98, 40.46, 39.43, 39.39, 38.40, 36.19, 36.01, 18.34, 18.10, 14.28, 14.27.

Data of **8b**: TLC (hexanes/AcOEt 2:1): 0.34, 0.29. ¹H-NMR (CDCl₃, 400 MHz): 7.36-7.24 (m, 5H), 6.90-6.78 (m, 3H), 5.86-5.72 (m, 1H), 5.15-5.03 (m, 2H), 4.59-4.25 (m, 4H), 3.96-3.74 (m), 3.60-3.52 (m), 3.46-3.39 (m) 3H, 3.861, 3.849, 3.845, 3.837 (4s, 6H), 3.20-2.95 (br., OH), 2.30-1.30 (m, 10H), 0.93 (t, *J* = 7.6), 0.92 (t, *J* = 6.8) 3H. ¹³C-NMR (CDCl₃, 100 MHz): 148.91, 148.58, 138.62, 134.88, 134.78, 130.69, 130.62, 128.30, 127.88, 127.79, 127.74, 127.66, 127.58, 127.50, 120.53, 120.47, 117.49, 117.41, 111.23, 111.18, 110.88, 110.82, 76.25, 75.87, 75.72, 73.94, 70.86 (2x), 70.61 (2x), 69.78, 67.80, 55.84, 55.73, 55.75, 42.20, 42.07, 41.30, 39.75, 39.34, 38.06, 36.18, 18.40, 18.16, 14.25, 14.20.

(4*S*,8*R*)- 4,8-Di-*O*-benzyl-6-*O*-(3',4'-dimethoxybenzyl)-undec-1-ene-4,6,8-triols (**9a**) and (4*R*,8*R*)- 4,8-Di-*O*-Benzyl-6-*O*-(3',4'-dimethoxybenzyl)-undec-1-ene-4,6,8-triols (**9b**)

A solution of 160 mg (0.36 mmol) of **8b** in 14 ml of DMF was treated with 37 mg of 60% NaH, stirred at 25 °C for 1 h, treated with 74 μ l of BnBr and 2 mg of Bu₄NI, and stirred at 25 °C for 20 h. The reaction was quenched with H₂O, extracted with AcOEt, the organic phase was washed with H₂O and brine, dried (MgSO₄), and evaporated. Flash column chromatography (hexanes/AcOEt 4:1) gave 129 mg (67%) of **9b**.

The diastereoisomer **9a** was obtained analogously.

Data of **9a**: TLC (hexanes/AcOEt 2:1): 0.57. ¹H-NMR (CDCl₃, 400 MHz): 7.40-7.20 (m, 10H), 6.90-6.70 (m, 3H), 5.93-5.75 (m, 1H), 5.16-5.00 (m, 2H), 4.63-4.20 (m, 6H), 3.95-3.43 (m, 9H), 2.43-2.20 (m), 2.05-1.90 (m), 1.80-1.20 (m) 10H, 0.98-0.80 (m, 3H). ¹³C-NMR (CDCl₃, 100 MHz): 148.82, 148.40, 148.62, 138.73, 138.70, 134.53, 134.46, 128.26, 127.72, 127.66, 127.60, 127.55, 127.46, 127.38, 120.55, 120.25, 117.28, 117.24, 111.27, 111.16, 110.82, 110.80, 75.97, 75.77, 75.57, 75.35, 73.22, 72.75, 71.27, 70.77, 70.65, 70.60, 70.45, 70.17, 55.86, 55.83, 55.68, 40.45, 40.44, 38.81, 38.62, 38.32, 38.18, 36.21, 36.17, 18.39, 18.19, 14.31, 14.21.

Data of **9b**: TLC (hexane/AcOEt 2:1): 0.57. ¹H-NMR (CDCl₃, 400 MHz): 7.42-7.20 (m, 10H), 6.93-6.70 (m, 3H), 5.95-5.77 (m, 1H), 5.20-5.00 (m, 2H), 4.63-4.20 (m, 6H), 3.98-3.45 (m, 3H), 3.83 (s, 3H), 3.82 (s, 3H), 2.70-2.25 (m, 2H), 2.10-1.93 (m, 1H), 1.80-1.25 (m, 7H), 1.00-0.85 (m, 3H). ¹³C-NMR (CDCl₃, 100 MHz): 148.79, 148.38, 138.99, 138.83, 138.74, 138.59, 138.46, 134.50, 134.49, 128.32, 128.21, 128.20, 127.70, 127.61, 127.56, 127.51, 127.42, 127.35, 127.25, 120.51, 120.48, 117.30, 117.19, 111.29, 110.76, 76.04, 75.64, 75.53, 72.81, 72.03, 71.05, 70.98, 70.86, 70.73, 70.56, 70.49, 70.14, ca. 55.90 (2x), 40.42, 40.37, 39.31, 39.00, 38.59, 38.46, 36.60, 36.33, 18.40, 18.21, 14.30, 14.25.

(4*S*,8*R*)- 4,8-Di-*O*-benzyl-undec-1-ene-4,6,8-triols (**10a**) and (4*R*,8*R*)- 4,8-Di-*O*-benzyl-undec-1-ene-4,6,8-triols (**10b**)

A solution of 130 mg (0.244 mmol) of **9b** in 25 ml of CH₂Cl₂ and 1.4 ml of H₂O was treated with 85 mg of DDQ, stirred at 25 °C for 30 min, quenched by addition of saturated aqueous NaHCO₃, extracted with CH₂Cl₂, the organic phase was washed with brine, dried (MgSO₄), and evaporated. Flash column chromatography (hexanes/AcOEt 3:1 to 2:1) gave 77 mg (82%) of **10b**.

The diastereoisomer **10a** was obtained analogously.

Data of **10a**: ¹H-NMR (CDCl₃, 400 MHz): 7.40-7.23 (m, 10H), 5.90-5.76 (m, 1H), 5.15-5.01 (m, 2H), 4.72-4.43 (m, 4H), 4.10-4.00 (m), 3.86-3.60 (m) 3H, 2.46-2.33 (m, 2H), 1.80-1.30 (m, 8H), 0.93 (t, *J* = 7.6), 0.92 (t, *J* = 7.2) 3H.

Data of **10b**: ¹H-NMR (CDCl₃, 400 MHz): 7.43-7.20 (m, 10H), 5.92-5.75 (m, 1H), 5.20-5.00 (m, 2H), 4.74-4.40 (m, 4H), 4.23-4.15 (m), 4.11-4.00, 4.00-3.60 (m) 3H, 2.50-2.30 (m, 2H), 1.80-1.25 (m, 8H), 1.02-0.88 (m, 3H).
¹³C-NMR (CDCl₃, 100 MHz): 138.52, 138.40, 138.16, 134.53, 134.14, 128.38, 128.36, 128.30, 127.85, 127.82, 127.72, 127.63, 127.60, 127.57, 117.48, 117.30, 78.05, 77.54, 76.76, 75.95, 71.46, 71.24, 70.63, 70.44, 69.07, 65.27, 41.30, 41.25, 41.19, 40.79, 38.42, 37.89, 35.96, 35.76, 18.62, 18.06, 14.29, 14.23.

(4*S*,8*R*)- 4,8-Di-*O*-benzyloxy-undec-1-en-6-one (**11a**) and (4*R*,8*R*)- 4,8-Di-*O*-benzyloxy-undec-1-en-6-one (**11b**)

A solution of 39 μl of (COCl)₂ in 3 ml of CH₂Cl₂ at -78 °C was treated with 69 μl DMSO, and stirred for 15 min. The mixture was treated with a solution of 77 mg (0.2 mmol) of **10b** in 1.5 ml of CH₂Cl₂ and stirred at -78 °C for 1 h 15 min. 140 μl of NEt₃ was added, and the reaction warmed to 25 °C. The mixture was diluted with CH₂Cl₂,

extracted with brine, dried (MgSO₄), and evaporated. Flash column chromatography (hexanes/AcOEt 8:1 to 4:1) gave 55 mg (72%) of **11b**.

The diastereoisomer **11a** was obtained analogously.

Data of **11a**: ¹H-NMR (CDCl₃, 400 MHz): 7.40-7.20 (m, 10H), 5.85-5.76 (m, 1H), 5.15-5.03 (m, 2H), 4.60-4.42 (m, 4H), 4.10-4.00 (m, 1H), 4.00-3.88 (m, 1H), 2.80-2.70 (m, 2H), 2.60-2.43 (m, 2H), 2.40-2.26 (m, 2H), 1.62-1.20 (m, 4H), 1.00-0.82 (t, *J* = 7.0, 3H). ¹³C-NMR (CDCl₃, 100 MHz): 208.23, 138.51, 138.35, 134.02, 128.30, 127.77, 127.57, 127.50, 117.80, 75.18, 74.65, 71.51, 71.46, 48.73, 48.27, 38.37, 36.63, 18.44, 14.11. ¹³C-NMR (S-DMBA, 100 MHz): 206.48, 74.94, 74.61, 71.14, 71.09, 48.54, 48.05, 38.16, 36.46, 18.23, 13.92, residual peaks under DMBA.

Data of **11b**: ¹H-NMR (CDCl₃, 500 MHz): ¹H-NMR (CDCl₃, 400 MHz): 7.40-7.23 (m, 10H), 5.87-5.77 (m, 1H), 5.14-5.07 (m, 2H), 4.62-4.46 (m, 4H), 4.09-4.02 (m, 1H), 3.98-3.92 (m, 1H), 2.80-2.73 (m, 2H), 2.58-2.50 (m, 2H), 2.40-2.33 (m, 2H), 1.65-1.30 (m, 4H), 0.92 (t, *J* = 7.5, 3H). ¹³C-NMR (CDCl₃, 100 MHz): 208.17, 138.54, 138.36, 133.96, 128.27, 127.73, 127.54, 127.48, 117.80, 75.25, 74.75, 71.60, 71.55, 48.91, 48.41, 38.32, 36.58, 18.41, 14.09. ¹³C-NMR (S-DMBA, 100 MHz): 206.52, 74.92, 74.53, 71.07, 71.03, 48.37, 47.94, 38.20, 36.52, 18.24, 13.92, residual peaks under DMBA.

4,8-Dihydroxy-undecan-6-one (12a) and *(4R,8R)- 4,8-Dihydroxy-undecan-6-one (12b)*

A solution of 27 mg of **11a** in 2 ml of MeOH was treated with Pd-C, the mixture was put on house-vacuum for 3 min, a H₂-balloon was added, and the mixture stirred at 25 °C for 14 h. After exchange of H₂ by N₂, the mixture was filtered over Celite, and evaporated. Flash column chromatography (hexane/AcOEt 3:1) gave 8 mg (56%) of **12a**. The diastereoisomer **12b** was obtained analogously.

Data of **12a**: ¹H-NMR (CDCl₃, 400 MHz): 4.11-4.02 (m, 2H), 3.00-2.80 (br., OH), 2.60-2.54 (m, 4H), 1.60-1.20 (m, 8H), 0.92 (t, *J* = 6.8, 6H). ¹³C-NMR (CDCl₃, 100 MHz): 212.92, 67.43, 50.14, 38.68, 18.61, 13.92. ¹H-NMR (CD₃OD, 400 MHz): 4.08-4.00 (m, 2H), 2.60-2.55 (m, 4H), 1.50-1.35 (m, 8H), 0.93 (t, *J* = 7.2, 6H). ¹³C-NMR (CD₃OD, 100 MHz): 211.54, 68.48, 51.85, 40.56, 19.78, 14.33. ¹³C-NMR (CD₃SOCD₃, 100 MHz): 209.19, 66.23, 51.02, 18.28, 13.98, 1 signal under DMSO.

Data of **12b**: ¹H-NMR (CDCl₃, 400 MHz): 4.12-4.00 (m, 2H), 2.65-2.50 (m, 4H), 1.60-1.20 (m, 8H), 0.92 (t, *J* = 7.0, 6H). ¹³C-NMR (CDCl₃, 100 MHz): 212.78, 67.30, 50.08, 38.63, 18.62, 13.94. ¹H-NMR (CD₃OD, 400 MHz): 4.12-3.98 (m, 2H), 2.80-2.40

(m, 4H), 1.60-1.20 (m, 8H), 0.93 (t, $J = 7.0$, 6H). ^{13}C -NMR (CD_3OD , 100 MHz): 211.48, 68.45, 51.92, 40.56, 19.78, 14.32. ^{13}C -NMR (CD_3SOCD_3 , 100 MHz): 66.24, 51.10, 18.28, 13.97, 1 signal under DMSO, C=O missing.

Comparison of ^{13}C NMR chemical shifts of *syn*- and *anti*-1,5-dihydroxy-3-ketone: Figure shows the difference $\delta\Delta_{(\text{anti-syn})}$ of the ^{13}C -shifts of the carbons C1-C11 of **12a** (*syn*) and **12b** (*anti*) in CDCl_3 and CD_3OD . In both solvents, the shift differences are smaller than 0.2 ppm.

Synthesis of *N*-acetyl-13-dihydrotetrafibricin methyl ester (2) and (3): [Kamiyama, T.; Itezono, Y.; Umino, T.; Satoh, T.; Nakayama, N.; Yokose, K. *J. Antibiot.* **1993**, *46*, 1047-1054.] To a solution of tetrafibricin (**1**) (20 mg) in H₂O (1.5 mL) was added NaBH₄ (10 mg) at 0 °C. After stirring for 1 h at 0 °C, the mixture was neutralized by 0.1N HCl, and directly applied on a C18 reverse phase column (H₂O only to H₂O:acetone = 1:1).

The eluate was concentrated in vacuo to give 13-dihydrotetrafibricin (17 mg).

To a solution of 13-dihydrotetrafibricin in MeOH (10 mL) was added TMSdiazomethane (2.0 mL, 2.0M in hexane) at 0 °C. After stirring for 1 h at 0 °C, the mixture was concentrated in vacuo to give 13-dihydrotetrafibricin methyl ester (20 mg).

To a solution of 13-dihydrotetrafibricin methyl ester in H₂O (0.5 mL) and THF (2.5 mL) was added Ac₂O (100 µL). After stirring for 30 min at room temperature, the mixture was concentrated in vacuo and purified by C18 reverse phase column to give *N*-acetyl-13-dihydrotetrafibricin methyl esters as a mixture of **2** and **3** (12 mg).

Preparation of Perdeuterated DMBA: We have developed practical chromatography-free synthesis of enantiomerically pure perdeuterated DMBA: starting from 100 g of benzene-*d*₆, 25 g of each enantiomer (totally 50 g, >99% atom D by MS analysis) was obtained with 41% of overall yield in 9 steps (The yield was improved from 33% in the original report shown below: F. Song, Y. Kishi, Jan 2002). For the details, see Supporting Information of the following reference: Kobayashi, Y.; Hayashi, N.; Tan, C.-H.; Kishi, Y. *Org. Lett.* **2001**, *3*, 2245-2248.

Structure of monodentate chiral NMR solvent (*R*)-DMBA

NMR experiments in DMBA: A Varian Mercury 400 spectrometer (100 MHz) was used to collect all ¹³C NMR data of *N*-acetyl-13-dihydrotetrafibricin methyl esters (12 mg) in perdeuterated (*R*)-DMBA (400 mg)/DMSO-*d*₆ (40 mg) (10:1 w/w), with acetone-*d*₆ in the co-axial inserts as an external reference (δ 29.800) and a lock-signal, and with readout of NMR spectra being adjusted to 0.001 ppm/point (sw=23980.8, fn=524288). The half-band width was 0.008 ppm. After the chiral solvent was distilled out from the NMR sample, the following experiment was conducted in perdeuterated (*S*)-DMBA by using the recovered *N*-acetyl-13-dihydrotetrafibricin methyl esters (ca.12 mg) under the same condition. After completion of the NMR experiments in both enantiomers of DMBA, *N*-acetyl-13-dihydrotetrafibricin methyl esters were recovered almost quantitatively (ca.12 mg) for the NMR experiments in bidentate chiral solvent. In general, the chiral solvent was readily recovered by distillation (60-65 °C/10 mmHg) after extraction with 1N HCl followed by basification and reextraction of the resulting free amine with AcOEt. Because of the reliability of data (the halfband-width is 0.008 ppm), the Δδ_{R-S} below 0.010 ppm is not included.

Synthesis of compound E and F: The compounds **E** and **F** were prepared by Brown's asymmetric allylation of the corresponding aldehydes: (a) Brown, H. C.; Jadhav, P. K. *J. Am. Chem. Soc.* **1983**, *105*, 2092-2093. (b) Jadhav, P. K.; Bhat, K. S.; Perumal, P. T.; Brown, H. C. *J. Org. Chem.* **1986**, *51*, 432-439.

NMR database E

NMR database F

Preparation of perdeuterated bidentate chiral NMR solvent, BMBA-*p*-Me: Both enantiomers of perdeuterated BMBA-*p*-Me were prepared from perdeuterated DMBA's and perdeuterated 1,3-dibromo-2-methylpropane by following the procedure of synthesis of the corresponding protio-compounds reported from our laboratory: Kobayashi, Y.; Hayashi, N.; Kishi, Y. *Org. Lett.* **2002**, *4*, 411-414.

NMR experiments in BMBA-*p*-Me: A Varian Mercury 400 spectrometer (100 MHz) was used to collect all ¹³C NMR data of *N*-acetyl-13-dihydrotetrafratricin methyl esters (6 mg) in perdeuterated (*R,R*)-BMBA-*p*-Me (350 mg) and CDCl₃ (140 mg)/DMSO-*d*₆ (70 mg) (5:2:1 w/w), (CDCl₃ as an internal reference (δ 77.000) and a lock-signal), and with readout of NMR spectra being adjusted to 0.001 ppm/point (sw=23980.8, fn=524288). Because of the reliability of data (the halfband-width is 0.008 ppm), the Δδ_{R-S} below 0.010 ppm is not included. The NMR experiment in (*S,S*)-BMBA-*p*-Me was also conducted under the same conditions. After completion of the NMR experiment, the

bidentate chiral solvent was removed by SiO₂ column chromatography to recover *N*-acetyl-13-dihydrotetrafibricin methyl esters (9.0 mg). In general, the perdeuterated bidentate chiral NMR solvents were also recovered by distillation (230-240 °C/1 mmHg) after extraction with 1N HCl followed by basification and reextraction of the resulting free amine with AcOEt.

Degradation of *N*-acetyl-13-dihydrotetrafibricin methyl ester: The solution of *N*-acetyl-13-dihydrotetrafibricin methyl esters (9 mg) in MeOH (2 mL) was bubbled at -78 °C with O₃ for 5 mins. After N₂ bubbling for 3 mins, to the reaction mixture was added NaBH₄ (8.0 mg) at -78°C, and it then warmed to room temperature. The mixture was evaporated in vacuo to give the crude mixture, which was acetylated under a standard condition (Ac₂O, Py, DMAP). After evaporation in vacuo, the resulting crude was purified by SiO₂ column chromatography (AcOEt only) to give ca. 8 mg of a mixture containing the target acetates.

To a solution of the acetates in MeOH (1 mL) was added NaOMe (2 mg), and the resulting mixture was stirred at room temperature for 1 h. The mixture was neutralized with Dowex-50w and filtered to remove the acidic resin and concentrated in vacuo. The resulting crude mixture was treated with (*S*)-MTPACl, NEt₃, DMAP in CH₂Cl₂ to give (*R*)-MTPA esters. After evaporation, the crude was purified by preparative TLC (AcOEt only) to furnish both the desired (*R*)-MTPA esters (<0.5 mg) with inseparable impurities.

Each (*R*)-MTPA ester containing C33 and C37 was purified again by preparative TLC (AcOEt only) to give the desired (*R*)-MTPA esters with ca. 50% purity. The amount of (*R*)-MTPA esters thus isolated was estimated to be much smaller than 0.1 mg each. Therefore, these materials were used for ¹H NMR comparison without further purification.

Comparison of ¹H NMR spectra of MTPA esters between the degradation product and the synthetic compound 6-9: On comparison of ¹H NMR spectra, it is found that the (*R*)-MTPA ester derived from the C31-C34 degradation product is identical to the corresponding synthetic (*R*)-MTPA ester **6**, but not (*S*)-MTPA ester **8**. The area around 3.3-3.6 ppm (the protons on the methoxy group in MTPA) and 4.0-4.6 ppm (C.31 and C34) are diagnostic to conclude that as shown below.

On the other hand, it is found that the (*R*)-MTPA ester derived from the C35-C40 degradation product is identical to the corresponding synthetic (*R*)-MTPA ester **7**, but not (*S*)-MTPA ester **9**. The area around 3.0-3.3 ppm (C40) and 4.0-4.4 ppm (C35) is diagnostic to conclude that as shown below.

^1H NMR spectra of Mosher esters derived from the C31-C34 degradation product of tetrafricin (**1**) and authentic compounds (500MHz, CDCl_3)

^1H NMR spectra of Mosher esters derived from the C35-C40 degradation product of tetrafibricin (**1**) and authentic compounds (500MHz, CDCl_3)

Assignment of Linearmycin A (10) by NMR Database Approach: The relative configuration of linearmycin A (**10**) can be addressed on the basis of the reported ^{13}C NMR chemical shifts in the references [(a) Sakuda, S.; Guce-Bigol, U.; Itoh, M.; Nishimura, T.; Yamada, Y. *Tetrahedron Lett.* **1995**, 36, 2777-2780. (b) Sakuda, S.; Guce-Bigol, U.; Itoh, M.; Nishimura, T.; Yamada, Y. *J. Chem. Soc., Perkin Trans. 1* **1996**, 2315-2319.] as follows: (C15,C16,C17) = (*syn/syn* [C16Me, δ 8.8 ppm in CD_3OD]), (C33,C35,C37) = (*anti/syn* or *syn/syn* [C35, δ 69.2]), (C41,C43,C45) = (*antianti* [C43, δ 66.2]), (C43,C45,C47) = (*antianti* [C45, δ 66.3]), (C55,C57) = (*syn* [C57, δ 70.7]), by employing 1,3,5-triol NMR database **A** in CD_3OD [*syn/syn* (70.4 ppm), *syn/anti*- and *anti/syn* (68.3), and *anti/anti* (66.3) (see below)], 2-methyl-1,3-diol NMR database **C** in CD_3OD [*syn/syn* (7.1 ppm), *syn/anti*- and *anti/syn* (10.7), and *antianti* (11.6) (see below)], and saturated 1,3-diol NMR database in CD_3OD [C5 of NMR database **D** (see below), *syn* (71.5 ppm) and *anti* (69.2) (see below)].

Moreover, 1,3-diol NMR database **B** in CD_3OD (see below) is found to be around 71.7 and 69.6 ppm for *syn* and *anti*, respectively. Therefore, C35/C37 and C47/C49 is assigned as *syn* (C37, 72.0) and *anti* (C47, 70.3), respectively. For linearmycin A (**10**), only remaining experiments could be ^{13}C NMR experiment of the 31-dihydroderivative of **10** in chiral solvents, DMBA and BMBA-*p*-Me.

Reported ^{13}C NMR Chemical Shifts (δ in ppm) in CD_3OD of Linearmycin (**10**) and the Predicted Stereochemistry

The predicted relative configuration is indicated below.
The absolute configuration of each stereocluster is chosen arbitrarily.

NMR database A in CD_3OD

NMR database C in CD_3OD

NMR database B in CD_3OD

NMR database D in CD_3OD