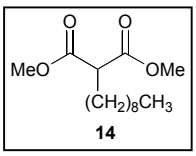
Enantiospecific Synthesis of the Heparanase Inhibitor (+)-Trachyspic Acid and Stereoisomers from a Common Precursor

Steven C. Zammit, Vito Ferro, Edward Hammond and Mark A. Rizzacasa*

Electronic Supplementary Information

Contents

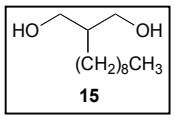
Dimethyl 2-nonylmalonate 14



14

Dimethyl malonate (4.33 cm^3, 37.8 mmol) and nonyl bromide (8.00 cm^3, 41.6 mmol) were added to a solution of sodium methoxide in methanol [sodium metal (0.87 g, 37.8 mmol) in dry MeOH (27 cm^3)]. The reaction mixture was heated under reflux for 16 h and the MeOH was removed under reduced pressure. Water and Et_2O were added and the aqueous phase was acidified with 10% HCl. The aqueous phase was extracted with Et_2O and the combined organic extracts were washed with water, brine, dried and concentrated. Purification by flash chromatography using 2.5-5% EtOAc/petrol as eluent gave **14** (8.44g, 86%) as a colourless oil: ν_{max} 2927, 2856, 1759, 1737, 1436, 1344, 1154 cm^{-1}; δ_H (400 MHz) 0.85 (t, J = 6.8 Hz, 3H), 1.25 (br m, 14H), 1.87 (m, 2H), 3.33 (t, J = 7.2 Hz, 1H), 3.70 (s, 3H), 3.71 (s, 3H); δ_C (100 MHz) 14.0, 22.6, 27.3, 28.8, 29.1, 29.2, 29.2, 29.4, 31.8, 51.6, 52.4, 169.9; HRMS (ESI): Calculated for $C_{14}H_{26}O_4Na$ $[M+Na]^+$ 281.1729, found 281.1725.

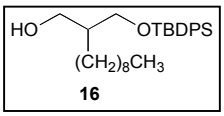
2-Nonylpropane-1,3-diol 15



15

A solution of malonate **14** (6.00 g, 23.2 mmol) in dry Et_2O (30 cm^3) was added to a cooled suspension of $LiAlH_4$ (2.64 g, 69.7. mmol) in dry Et_2O (70 cm^3) at 0°C. The suspension was stirred at 0°C for 2 h, warmed to rt and stirred for another 2 h and 5 M aqueous NaOH was added until the grey coloured suspension turned white. The suspension was filtered and the filtrate concentrated to give the diol **15** (4.18 g, 89%) as a colourless gum pure enough for the next step: ν_{max} 3371, 2928, 2856, 1467, 1029 cm^{-1}; δ_H (400 MHz) 0.89 (t, J = 6.8 Hz, 3H), 1.24 (br s, 16H), 1.72 (sept, J = 3.6 Hz, 1H), 2.27 (br s, 2H), 3.60 (dd, J = 10.4, 7.6 Hz, 2H), 3.76 (dd, J = 10.4, 3.6 Hz, 2H); δ_C (100 MHz) 14.1, 22.6, 27.2, 27.7, 29.3, 29.5, 29.6, 29.9, 31.8, 41.9, 66.2, 66.2; HRMS (ESI): Calculated for $C_{12}H_{26}O_2Na$ $[M+Na]^+$ 225.1830, found 225.1826.

Silyl ether 16

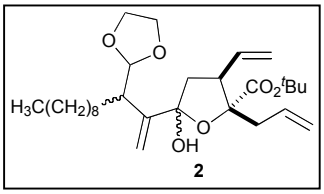


16

A solution of the diol **15** (4.18 g, 20.7 mmol) in dry THF (30 cm^3) was added via cannula to a suspension of NaH (60% dispersion in oil, 0.83 g, 20.7 mmol) in dry THF (45 cm^3). The suspension was cooled to 0°C and TBDPSCl (5.38 cm^3, 20.7 mmol) was added and the mixture was stirred at 0°C for 1 h, then warmed to rt and stirred for another 1 h. Water was added and the aqueous phase was extracted with Et_2O. The combined organic extracts were washed with water, brine, dried and concentrated. The crude product was purified by flash chromatography with 5% EtOAc/petrol as eluent to afford **16** (8.29 g, 91%) as a colourless oil: ν_{max} 3401, 2928, 2856, 1428, 1113 cm^{-1}; δ_H (400 MHz) 0.89 (t, J = 7.2 Hz, 3H), 1.07

(s, 9H), 1.24 (br s, 16H), 1.78 (m, 1H), 2.75 (br s, 1H), 3.62-3.72 (m, 2H), 3.79 (dd, J = 10.4, 4.0 Hz, 2H), 7.43 (m, 4H), 7.70 (m, 4H); δ_C (100 MHz) 14.1, 19.2, 22.7, 26.5, 26.8, 27.1, 27.6, 29.3, 29.5, 29.5, 29.8, 31.9, 40.1, 66.4, 67.5, 127.6, 127.7, 129.5, 129.8, 132.0, 133.0, 135.5, 135.6; HRMS (ESI): Calculated for $C_{28}H_{44}O_2SiNa$ $[M+Na]^+$ 463.3008, found 463.3007.

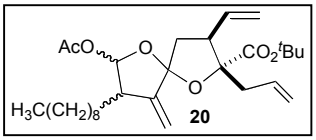
Lactols 2



2

A solution of ^{t}BuLi in hexanes (168 μL, 1.7 M, 0.286 mmol) was added dropwise to a solution of bromoalkene **4** (57 mg, 0.179 mmol) in dry Et_2O (1.0 cm^3) and dry hexane (0.8 cm^3) at −78°C under argon. The resulting solution was stirred at −78°C for 5 min and a solution of lactone **3** (17 mg, 0.0674 mmol) in Et_2O (0.5 cm^3) and hexane (0.4 cm^3) was added dropwise via cannula. The mixture was stirred at −78°C for 1 h, warmed to 0°C and stirred for another 30 min and quenched with water. The aqueous phase was extracted with Et_2O and the combined organic extracts were washed with water, brine, dried and concentrated. Purification of the crude product by flash chromatography with 10% EtOAc/petrol as eluent afforded **2** (18 mg, 54%) as a mixture of anomers: ν_{max} 3505, 2926, 2855, 1719, 1369, 1148 cm^{-1}; δ_H (400 MHz) 0.86 (t, *J* = 7.2 Hz, 3H), 1.21 (br s, 14H), 1.46 (s, 9H), 1.63 (m, 2H), 2.30 (m, 1H), 2.46 (m, 1H), 2.96-3.13 (m, 3H), 3.75-3.91 (m, 4H), 4.79 (d, *J* = 5.0 Hz, 0.5H), 4.82 (d, *J* = 5.0 Hz, 0.5H), 5.07-5.13 (m, 4H), 5.62-5.78 (m, 2H), 5.82 (s, 1H), 6.13 (s, 1H); δ_C (100 MHz) 14.1, 22.7, 27.0, 27.1, 27.9, 28.9, 29.2, 29.3, 29.5, 29.5, 29.5, 29.7, 31.9, 37.9, 38.1, 42.3, 42.9, 47.1, 47.2, 64.7, 64.7, 64.9, 78.5, 78.5, 83.1, 105.9, 106.1, 118.7, 119.0, 119.1, 125.4, 125.5, 132.4, 135.6, 148.0, 148.2, 174.8, 199.4, 199.6; HRMS (ESI): Calculated for $C_{29}H_{48}O_6Na$ $[M+Na]^+$ 515.3349, found 515.3351.

Spiroketals 20

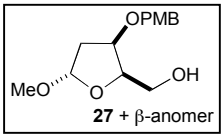


20

A solution of the lactols **2** (32 mg, 0.065 mmol) in THF (1.5 cm^3) was cooled to 0°C and treated with 3 M $HClO_4$ (0.5 cm^3). The solution was stirred at 0°C for 1 h, then quenched with saturated aqueous $NaHCO_3$ and the aqueous phase was extracted with EtOAc. The combined organic extracts were washed with water, brine, dried and concentrated and the residue was dissolved in pyridine (1.25 cm^3) and DMAP (0.80 mg, 6.50 μmol) and acetic anhydride (37 μL, 0.390 mmol) were added. The solution was stirred at rt for 16 h and then diluted with water and the aqueous phase was extracted with EtOAc. The combined organic extracts were washed with saturated aqueous $CuSO_4$, water, brine, dried, and concentrated. The crude product was purified by flash chromatography with 5% EtOAc/petrol as eluent to afford acetate **20** (16.5 mg, 41%) as an inseparable mixture of

diastereoisomers: ν_{max} 2927, 2856, 1750, 1368, 1230 cm^{-1}; δ_H (400 MHz) 0.87 (t, *J* = 7.2 Hz, 3H), 1.25 (br s, 14H), 1.45 (s, 9H), 1.63 (m, 2H), 2.01-2.63 (m, 7H), 2.80-2.89 (m, 0.5H), 3.04-3.11 (m, 0.5H), 3.35 (m, 0.5H), 3.45 (m, 0.5H), 5.01-5.36 (m, 6H), 5.74-5.97 (m, 2H), 5.97 (s, 1H), 6.12 (s, 1H), 6.48 (d, J = 5.2 Hz, 0.5H); δ_C (100 MHz) 14.1, 21.2, 21.5, 21.6, 22.7, 26.3, 26.5, 26.6, 26.9, 27.2, 28.0, 28.0, 29.3, 29.3, 29.3, 29.4, 29.5, 29.5, 29.6, 29.7, 29.7, 31.8, 31.9, 32.3, 32.9, 38.7, 38.9, 39.2, 39.4, 42.2, 42.8, 43.0, 43.7, 45.6, 47.8, 47.9, 48.1, 48.2, 48.6, 48.8, 49.5, 51.2, 80.9, 81.3, 81.4, 86.9, 86.9, 87.5, 87.6, 89.1, 95.7, 100.0, 100.4, 108.1, 110.0, 113.3, 113.7, 113.7, 117.3, 117.5, 117.6, 117.9, 118.4, 118.5, 118.6, 127.7, 132.5, 132.7, 132.8, 132.9, 133.4, 134.8, 134.9, 135.2, 136.0, 148.9, 149.0, 149.4, 150.7, 169.9, 171.1, 171.3, 171.7, 171.8; HRMS (ESI): Calculated for $C_{29}H_{46}O_6Na$ $[M+Na]^+$ 513.3192, found 513.3194.

Alcohol 27



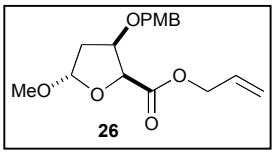
27 + β-anomer

To a solution of **7** (10.0 g, 25.6 mmol) in benzene (225 cm^3) was added *p*-nitrobenzoic acid (8.6 g, 51.2 mmol), triphenylphosphene (16.8 g, 64.0 mmol) and DIAD (12.4 cm^3, 64.0 mmol). The solution was stirred at rt for 16 h and the benzene was removed under reduced pressure and the crude product was purified by flash chromatography with 5% EtOAc/petrol as eluent. The resultant benzoate ester was dissolved in MeOH (500 cm^3) and treated with potassium carbonate (5.3 g, 38.4 mmol) at rt for 16 h and then diluted with water. The aqueous phase was extracted with CH_2Cl_2 and the combined organic extracts were washed with water, brine and dried, and concentrated to give the alcohol (7.2 g, 72%) as a pale yellow oil. A solution of the alcohol (3.2 g, 8.19 mmol) in DMF (45 cm^3) was added via cannula to a suspension of NaH (60% dispersion in oil, 492 mg, 12.3 mmol) in DMF (45 cm^3). PMBCl (1.7 cm^3, 12.3 mmol) was added and the solution was stirred at rt for 4 h and then quenched with water. The aqueous phase was extracted with Et_2O and the combined organic extracts were washed with water, brine, dried and concentrated. The crude product was dissolved in MeOH (500 cm^3) and treated with 10% HCl (0.5 cm^3). The reaction was stirred at rt for 16 h and quenched with saturated aqueous $NaHCO_3$. The MeOH was removed under reduced pressure and Et_2O was added and the organic phase was washed with water, brine, dried and concentrated. The crude product was purified by flash chromatography with 20% EtOAc/petrol as eluent to afford **27** (0.90 g, 41%) as a yellow oil: $[\alpha]_D^{23}$ +25.1 (*c* 1.35, CH_2Cl_2); ν_{max} 3460 2916, 2835, 1612, 1514, 1248, 1037 cm^{-1}; δ_H (500 MHz) 2.16 (m, 2H), 3.33 (s, 3H), 3.80 (s, 3H), 3.82 (m, 2H), 4.11 (dd, *J* = 10.0, 4.0 Hz, 1H), 4.37 (dd, *J* = 11.5, 6.0 Hz, 1H), 4.43 (ABq, *J* = 11.5 Hz, 2H), 5.15 (t, *J* = 3.5 Hz, 1H), 6.87 (d, *J* = 9.0 Hz, 2H), 7.22 (d, *J* = 9.0 Hz, 2H); δ_C (125 MHz) 39.5, 55.0, 55.2, 61.8, 71.4, 78.8, 78.9, 104.0,

113.9, 129.3, 129.4, 159.4; HRMS (ESI): Calculated for $C_{14}H_{20}O_5Na$ $[M+Na]^+$ 291.1208, found 291.1206.

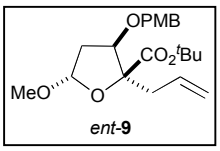
Further elution afforded the β-anomer (0.79 g, 36%) as a yellow oil: $[\alpha]_D^{23}$ –94.8 (*c* 0.34, CH_2Cl_2); ν_{max} 3447 2922, 2851, 1612, 1514, 1248, 1034 cm^{-1}; δ_H (500 MHz) 2.11-2.25 (m, 2H), 3.40 (s, 3H), 3.81 (s, 3H), 3.82 (m, 2H), 4.15 (dd, *J* = 11.0, 5.5 Hz, 1H), 4.22 (m, 1H), 4.48 (ABq, *J* = 11.5 Hz, 2H), 5.02 (dd, *J* = 6.0, 2.0 Hz, 1H), 6.88 (d, *J* = 8.5 Hz, 2H), 7.25 (d, *J* = 8.5 Hz, 2H); δ_C (125 MHz) 38.0, 55.3, 55.5, 62.4, 71.5, 77.8, 80.8, 104.7, 113.9, 129.4, 129.6, 159.4; HRMS (ESI): Calculated for $C_{14}H_{20}O_5Na$ $[M+Na]^+$ 291.1208, found 291.1206.

Allyl ester 26



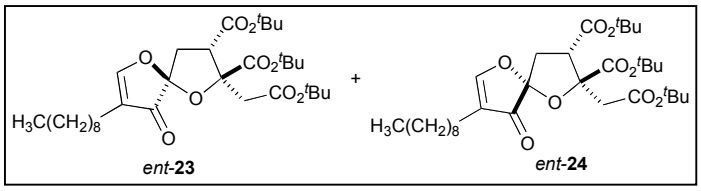
Dess-Martin periodinane (2.23 g, 5.26 mmol) was added to a solution of alcohol **27** (940 mg, 3.50 mmol) in CH_2Cl_2 (40 cm^3) and pyridine (850, 10.5 mmol) and the reaulting solution was stirred at rt for 30 min and quenched with saturated aqueous $NaHCO_3$ and 1.5 M $Na_2S_2O_3$. The biphasic mixture was stirred for 15 min and the aqueous phase was extracted with Et_2O and the combined organic extracts were washed with water, brine, dried, and concentrated. The crude product was dissolved in EtOH (20 cm^3) and a solution of $AgNO_3$ (1.47 g, 8.75 mmol) in water (2.0 cm^3) and KOH (1.32 g, 22.8 mmol) in water (18.0 cm^3) were then added and the dark suspension was stirred at rt for 16 h. The suspension was filtered through celite and the filter cake was washed with 1 M KOH. Et_2O was added and the organic phase was separated and discarded. The aqueous phase was acidified with 10% HCl, extracted with Et_2O and the combined organic extracts were washed with water, brine, dried, and concentrated to give an acid (781 mg, 79%). A solution of the crude acid (781 mg, 2.77 mmol) in dry CH_2Cl_2 (25 cm^3) was cooled to 0°C and DMAP (43 mg, 0.350 mmol), allyl alcohol (262 μL, 3.85 mmol) and DCC (794 mg, 3.85 mmol) were added. The mixture was warmed to rt and stirred for 16 h then filtered and the filtrate was concentrated under reduced pressure. The crude product was purified by flash chromatography with 10% EtOAc/petrol as eluent to afford ester **26** (580 mg, 65%) as a pale yellow oil: $[\alpha]_D^{23}$ +56.2 (*c* 1.03, CH_2Cl_2); ν_{max} 2934, 2835, 1761, 1613, 1514, 1250, 1065 cm^{-1}; δ_H (500 MHz) 2.08 (ddd, *J* = 14.0, 6.5, 2.0 Hz, 1H), 2.26 (m, 1H), 3.36 (s, 3H), 3.79 (s, 3H), 4.44 (ABq, *J* = 11.5 Hz, 2H), 4.48 (m, 1H), 4.62 (dd, *J* = 12.8, 6.0 Hz, 1H), 4.69 (d, *J* = 5.5 Hz, 1H), 4.72 (dd, *J* = 12.8, 6.0 Hz, 1H), 5.21 (m, 1H), 5.29-5.34 (m, 2H), 5.89 (m, 1H), 6.84 (d, *J* = 8.5 Hz, 2H) 7.18 (d, *J* = 8.5 Hz, 2H); δ_C (125 MHz) 38.5, 55.1, 55.4, 65.6, 71.4, 78.3, 79.1, 105.0, 113.6, 118.6, 129.1, 129.5, 131.7, 159.1, 168.6; HRMS (ESI): Calculated for $C_{17}H_{22}O_6Na$ $[M+Na]^+$ 345.1314, found 345.1314.

***tert*-Butyl ester *ent*-9**

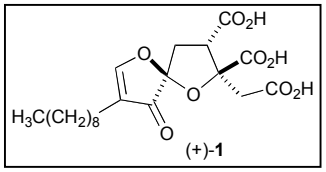


^{n}BuLi in hexanes (3.2 cm^3, 2.1 M, 6.69 mmol) was added dropwise to a cooled solution of $^{i}Pr_2NH$ (0.85 cm^3, 6.08 mmol) in dry THF (15 cm^3) at 0°C under argon. The resultant LDA solution was stirred at 0°C for 5 min, then cooled to –78°C and added dropwise via cannula to a solution of the allyl ester **26** (0.98 g, 3.04 mmol) and the supernatant from a centrifuged mixture of freshly distilled TMSCl (2.2 cm^3, 17.3 mmol) and NEt_3 (2.2 cm^3, 15.8 mmol) in dry THF (22 cm^3) and HMPA (4.0 cm^3) at –100°C. The solution was stirred at –100°C for 10 min and then allowed to warm to rt and stirred for 2 h. The solution was cooled to 0°C and aqueous 1 M NaOH (25 cm^3) was added followed by Et_2O and water. The organic phase was separated and discarded and the aqueous phase was cooled to 0°C, acidified with 10% HCl and extracted with Et_2O. The combined organic extracts were washed with water, brine, dried and concentrated and the crude residue was dissolved in dry CH_2Cl_2 (50 cm^3) and treated with *N,N'*-diisopropyl-*O*-*t*-butylisourea (3.04 g, 15.2 mmol) for 16 h at rt. The white suspension was filtered and the filtrate was concentrated under reduced pressure. The crude product was purified by flash chromatography with 10% EtOAc/petrol as eluent to give *t*-butyl ester *ent*-**9** (0.79 g, 67%) as a colourless oil. The physical data for *ent*-**9** was identical to **9** except for the sign of rotation: $[\alpha]_D^{23}$ +60.1 (*c* 0.98, CH_2Cl_2).

(+)-Trachyspic acid tri-*tert*-butyl ester *ent*-23



Coupling of bromoalkene **4** (100 mg, 0.313 mmol) and triester *ent*-**13** (44 mg, 0.110 mmol) in Et_2O (1.0 cm^3) followed by spirocyclisation, ozonolysis and elimination in a manner similar to that described for **13** gave spiroisomers *ent*-**24** (0.6 mg, 3%) and *ent*-**23** (8.5 mg, 52%). The physical data for *ent*-**24** was identical to **24** except for the sign of rotation: $[\alpha]_D^{24}$ +19.5 (*c* 0.03, CH_2Cl_2); HRMS (ESI): Calculated for $C_{32}H_{52}O_9Na$ $[M+Na]^+$ 603.3509, found 603.3512. The physical data for *ent*-**23** was identical to **23** except for the sign of rotation: $[\alpha]_D^{23}$ +16.4 (*c* 0.40, CH_2Cl_2); HRMS (ESI): Calculated for $C_{32}H_{52}O_9Na$ $[M+Na]^+$ 603.3509, found 603.3509.

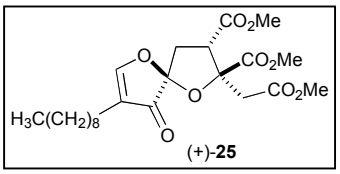


(+)-Trachyspic acid (1)

Deprotection of tri-*t*-butylester *ent*-**23** (8.5 mg, 14.6 μmol) in a manner similar to that described above for **23** afforded (+)-trachyspic acid (**1**)

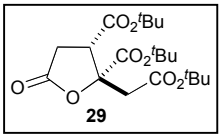
(6.0 mg, 99%) which was identical in all aspects to natural trachyspic acid (**1**): $[\alpha]_D^{22}$ +3.1 (*c* 0.30, MeOH); $[\alpha]_D^{23}$ +7.1 (*c* 0.30, CH_2Cl_2); lit.[3] $[\alpha]_D^{25}$ +3.1 (*c* 1.0, MeOH); HRMS (ESI): Calculated for $C_{20}H_{28}O_9Na$ $[M+Na]^+$ 435.1631, found 435.1638.

(+)-Trachypsic acid trimethyl ester (25)



Methylation of (+)-trachyspic acid (**1**) (6.0 mg, 14.5 μmol) as described above for (–)-*ent*-**1** gave the trimethyl ester **25** (4.0 mg, 61%). The physical data for **25** was identical that for natural **25**: $[\alpha]_D^{23}$ +17.3 (*c* 0.10, CH_2Cl_2); HRMS (ESI): Calculated for $C_{23}H_{34}O_9Na$ $[M+Na]^+$ 477.2101, found 477.2097.

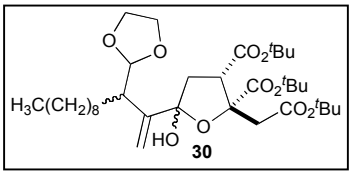
Lactone triester 29



Ozone gas was bubbled through a solution of the alkene **12** (98 mg, 0.388 mmol) in CH_2Cl_2 (9.8 cm^3) and MeOH (600 μL) at –78°C until a pale blue colour persisted. Me_2S (285 μL, 3.88 mmol) was added and the solution was warmed to rt and stirred for 1 h. Water was added and the aqueous phase was extracted with Et_2O and the combined organic extracts were washed with water, brine, dried and concentrated. The crude product was dissolved in a mixture of ^{t}BuOH (9.8 cm^3) and 2-methyl-2-butene (1.6 cm^3) and a solution of $NaH_2PO_4{\cdot}H_2O$ (429 mg, 3.11 mmol) and 80% $NaClO_2$ (702 mg, 6.21 mmol) in water (5.3 cm^3) was then added and the mixture was stirred at rt for 16 h. Water and Et_2O were added and organic layer was separated and discarded. The aqueous phase was acidified with 10% HCl and extracted with Et_2O and the combined organic extracts were washed with water, brine and dried and concentrated. A solution of the crude product in dry CH_2Cl_2 (25 cm^3) was treated with *N,N'*-diisopropyl-*O*-*t*-butylisourea (1.56 g, 7.77 mmol) for 16 h at rt. The suspension was filtered through celite and the filtrate was concentrated and the crude product was purified by flash chromatography with 10% EtOAc/petrol as eluent to give the triester **29** (62 mg, 40%) as a colourless crystalline solid: $[\alpha]_D^{19}$ +3.3 (*c* 0.55, CH_2Cl_2); ν_{max} 2980, 1804, 1736, 1369, 1151 cm^{-1}; δ_H (400 MHz) 1.45 (s, 9H), 1.46 (s, 9H), 1.47 (s, 9H), 2.66 (dd, *J* = 17.6, 9.2 Hz, 1H), 3.07 (ABq, *J* = 16.8 Hz, 2H), 3.12 (dd, *J* = 18.0, 11.6 Hz, 1H), 3.76 (dd, *J* = 11.6, 9.6 Hz, 1); δ_C (100 MHz) 27.8, 27.9, 28.0, 31.0, 40.3, 48.1, 81.9, 82.9, 83.9, 84.3, 167.1, 167.5, 168.0, 174.0; HRMS (ESI): Calculated for $C_{20}H_{32}O_8Na$ $[M+Na]^+$ 423.1995, found 423.1992. Further elution provided the dimethylacetal byproduct (10 mg, 7%) as a yellow oil: $[\alpha]_D^{17}$ +5.8 (*c* 1.05, CH_2Cl_2); ν_{max} 2981, 1798, 1742, 1370, 1260, 1151 cm^{-1}; δ_H (400 MHz) 1.46 (s, 18H), 2.25 (dd, *J* = 14.4, 5.6 Hz, 1H), 2.58 (dd, *J* = 14.4, 5.6 Hz, 1H), 2.61 (dd, *J* = 18.0, 9.6 Hz, 1H), 3.03 (dd, *J* = 17.6, 11.2 Hz, 1H), 3.32 (s, 3H), 3.34 (s, 3H), 3.61 (dd, *J* = 11.6, 9.6 Hz, 1H), 4.64 (t, *J* = 5.6 Hz, 1H); δ_C (100 MHz) 27.8, 27.9, 31.4, 38.0,

49.2, 53.1, 53.9, 82.6, 83.8, 84.7, 101.3, 167.5, 167.7, 174.2; HRMS (ESI): Calculated for $C_{18}H_{30}O_8Na$ $[M+Na]^+$ 397.1838, found 397.1837.

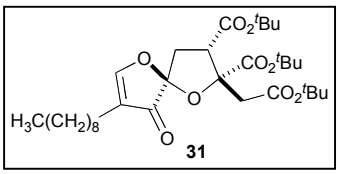
Lactols 30



30

A solution of ^{t}BuLi in hexanes (178 μL, 1.4 M, 0.250 mmol) was added dropwise to a solution of bromoalkene **4** (45 mg, 0.142 mmol) in dry Et_2O (1.0 cm^3) and dry hexane (0.5 cm^3) at −78°C under argon. The anion solution was stirred at −78°C for 5 min and a solution of lactone **29** (20 mg, 49.9 μmol) in Et_2O (0.5 cm^3) and hexane (0.4 cm^3) was added dropwise via cannula. The orange solution was stirred at −78°C for 2 h and quenched with water. The aqueous phase was extracted with Et_2O and the combined organic extracts were washed with water, brine and dried and concentrated. Purification of the crude product by flash chromatography with 10% EtOAc/petrol as eluent gave recovered triester **29** (5 mg). Further elution afforded lactols **30** (10 mg, 31%, 42% based on recovered triester **29**) as a mixture of diastereoisomers: ν_{max} 3485, 2926, 2854, 1732, 1367, 1151 cm^{-1}; δ_H (500 MHz) 0.86 (m, 3H), 1.23 (m, 14H), 1.42 (s, 9H), 1.44 (s, 9H), 1.49 (s, 9H), 1.68 (m, 2H), 2.66-3.25 (m, 4H), 3.72-3.94 (m, 5H), 4.80 (d, J = 4.5 Hz, 0.5H), 4.86 (d, J = 5.0 Hz, 0.5H), 5.83 (d, J = 3.5 Hz, 1H), 6.21 (s, 1H); δ_C (100 MHz) 14.1, 22.7, 27.0, 27.1, 27.8, 27.9, 28.0, 28.0, 28.1, 29.0, 29.2, 29.3, 29.5, 29.5, 29.6, 29.7, 31.9, 31.9, 35.2, 35.3, 42.1, 42.2, 43.0, 43.7, 48.2, 48.3, 49.2, 61.3, 64.7, 64.7, 64.9, 65.0, 74.5, 74.5, 81.3, 81.4, 83.2, 105.8, 106.2, 117.2, 125.1, 125.9, 146.5, 147.2, 147.4, 170.0, 170.2, 172.2, 199.3, 199.5;
HRMS (ESI): Calculated for $C_{35}H_{60}O_{10}Na$ $[M+Na]^+$ 663.4084, found 663.4083.

(–)-3-*epi*-Trachyspic acid tri-*tert*-butyl ester (31)

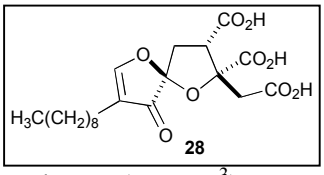


31

A solution of lactols **30** (20 mg, 31.2 μmol) in THF (1.0 cm^3) was cooled to 0°C and treated with 3 M $HClO_4$ (0.5 cm^3). The solution was stirred at 0°C for 1 h, then quenched with saturated aqueous $NaHCO_3$ and the aqueous phase was extracted with Et_2O, washed with water, brine, dried and concentrated. The crude product was dissolved in pyridine (1.0 cm^3) and DMAP (0.76 mg, 6.24 μmol) and acetic anhydride (30 μL, 0.312 mmol) were added. The solution was stirred at rt for 16 h, water was added and the aqueous phase was extracted with Et_2O, washed with water, brine, dried and concentrated. The crude product was dissolved in CH_2Cl_2 (1.0 cm^3) and MeOH (100 μL) and ozone gas was bubbled through the solution at −78°C until a pale blue colour persisted. Me_2S (23 μL, 0.312 mmol) and $NaHCO_3$ (26 mg, 0.312 mmol) were added and the solution was warmed to rt and stirred for 16

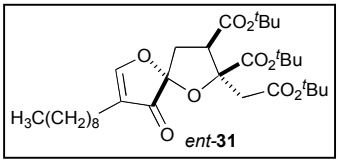
h. Water was added and the aqueous phase was extracted with Et_2O and the combined organic extracts were washed with water, brine, dried and concentrated. The crude product was purified by flash chromatography with 5-10% EtOAc/petrol as eluent to afford **31** (5.5 mg, 31%) as a thin film: $[\alpha]_D^{22}$ –9.7 (*c* 0.18, CH_2Cl_2); ν_{max} 2928, 1732, 1368, 1153 cm^{-1}; δ_H (400 MHz) 0.87 (t, *J* = 7.2 Hz, 3H), 1.25 (br s, 14H) 1.45 (s, 9H), 1.46 (s, 9H), 1.54 (s, 9H), 2.09 (t, *J* = 8.0 Hz, 2H), 2.19 (dd, *J* = 13.2, 7.2 Hz, 1H), 3.03 (ABq, *J* = 15.6 Hz, 2H), 2.88 (t, *J* = 13.2 Hz, 1H), 4.03 (dd, *J* = 12.8, 6.8 Hz, 1H), 7.79 (s, 1H); δ_C (100 MHz) 14.1, 21.2, 22.7, 27.8, 27.9, 28.0, 28.1, 29.3, 29.3, 29.5, 31.8, 37.5, 41.4, 49.2, 81.1, 82.0, 83.3, 88.0, 108.7, 118.3, 168.1, 168.1, 168.9, 171.2, 198.0; HRMS (ESI): Calculated for $C_{32}H_{52}O_9Na$ $[M+Na]^+$ 603.3509, found 603.3505.

(+)-3-*epi*-Trachyspic acid (+)-(28)



A solution of the tri-*tert*-butylester **31** (5.5 mg, 9.5 μmol) in dry CH_2Cl_2 (1.0 cm^3) was cooled to 0°C and treated with TFA (200 μL). The solution was stirred at 0°C for 1 h, then warmed to rt and stirred for 16 h. Toluene (1.0 cm^3) was added and the solvent was removed under reduced pressure to afford 3-*epi*-trachyspic acid (+)-(**28**) (3.7 mg, 94%) as a thin film: $[\alpha]_D^{22}$ +2.9 (*c* 0.10, MeOH); $[\alpha]_D^{23}$ +10.9 (*c* 0.10, CH_2Cl_2); ν_{max} 3425, 2926, 2855, 1720, 1602, 1369, 1150 cm^{-1}; δ_H (500 MHz, d_6-DMSO) 0.84 (t, *J* = 6.5 Hz, 3H), 1.23 (br s, 12H), 1.38 (m, 2H), 2.02 (t, *J* = 7.5 Hz, 2H), 2.21 (dd, *J* = 13.0, 7.0 Hz, 1H), 2.50 (m, 1H), 2.66 (d, *J* = 16.5 Hz, 1H), 3.22 (d, *J* = 16.5 Hz, 1H), 3.51 (dd, *J* = 13.0, 7.0 Hz, 1H), 8.41 (s, 1H); δ_C (100 MHz, d_6-DMSO) 14.0, 20.5, 22.1, 27.5, 28.6, 28.7, 28.9, 31.3, 36.5, 42.9, 50.6, 87.1, 108.6, 117.1, 170.2, 170.3, 170.4, 173.7, 197.3; HRMS (ESI): Calculated for $C_{20}H_{28}O_9H$ $[M-H]^-$ 411.1661, found 411.1650.

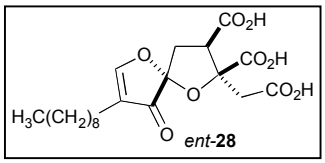
(+)-3-*epi*-Trachyspic acid tri-*tert*-butyl ester (*Ent*-31)



Coupling of bromoalkene **4** (141 mg, 0.441 mmol) and lactone *ent*-**29** (62 mg, 0.155 mmol) followed by spirocyclisation, ozonolysis and elimination in a manner similar to that described for **29** gave spiroketal *ent*-**31** (11 mg, 31%). The physical data for *ent*-**31** was identical to **31** except for the sign of rotation: $[\alpha]_D^{23}$ +6.3 (*c* 0.45, CH_2Cl_2); HRMS (ESI): Calculated for $C_{32}H_{52}O_9Na$ $[M+Na]^+$ 603.3509, found 603.3506.

(–)-3-*epi*-Trachyspic acid (–)-*ent*-(28)

Deprotection of the tri-*tert*-butylester *ent*-**31** (8.0 mg, 13.8 μmol) in a manner similar to that described above for **31** afford (–)-3-*epi*-trachyspic acid (*ent*-**28**) (5.5 mg, 96%). The physical data



ent-**28**

for (–)-3-*epi*-trachyspic acid (*ent*-**28**) was identical to (+)-3-*epi*-trachyspic acid (**28**) except for the sign of rotation: $[\alpha]_D^{24}$ –3.4 (*c* 0.25, MeOH); $[\alpha]_D^{24}$ –20.1 (*c* 0.25, CH_2Cl_2); HRMS (ESI): Calculated for $C_{20}H_{28}O_9H$ $[M-H]^-$ 411.1661, found 411.1645.

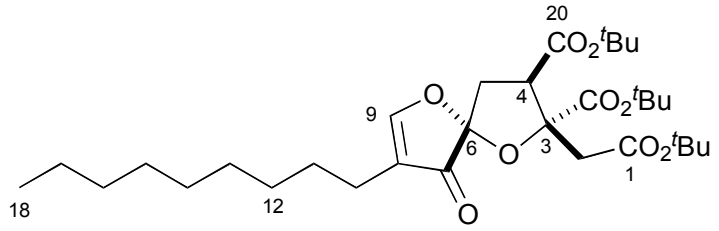
Table 1: ^{1}H (400 MHz, d_6-DMSO) and ^{13}C NMR data (100 MHz, d_6-DMSO) for natural and synthetic trachyspic acid (**1**).

Carbon atom	Natural trachyspic acid[1]			Synthetic trachyspic acid		
	δ_C	δ_H	(mult, *J*)	δ_C	δ_H	(mult, *J*)
1	170.0	-		170.1	-	
2	38.7	2.69 2.87	(1H, d, 16.8) (1H, d, 16.8)	38.7	2.67 2.85	(1H, d, 16.8) (1H, d, 16.8)
3	86.5	-		86.5	-	
4	48.4	3.57	(1H, dd, 7.7, 12.0)	48.4	3.56	(1H, dd, 7.8, 11.8)
5	37.4	2.38	(2H, m)	37.6	2.36	(2H, m)
6	108.0	-		108.1	-	
7	198.1	-		198.2	-	
8	116.7	-		116.7	-	
9	174.3	8.45	(1H, s)	174.5	8.45	(1H, s)
10	20.4	2.03	(2H, t, 7.5)	20.5	2.02	(2H, t, 7.8)
11	27.4	1.40	(2H, m)	27.5	1.39	(2H, m)
12	28.5	1.24	(2H, br s)	28.6	1.23	(2H, br s)
13	28.6	1.24	(2H, br s)	28.7	1.23	(2H, br s)
14	28.6	1.24	(2H, br s)	28.7	1.23	(2H, br s)
15	28.8	1.24	(2H, br s)	28.9	1.23	(2H, br s)
16	31.2	1.24	(2H, br s)	31.3	1.23	(2H, br s)
17	22.0	1.24	(2H, br s)	22.1	1.23	(2H, br s)
18	13.8	0.85	(3H, t, 6.8)	14.0	0.84	(3H, t, 6.6)
19	171.2	-		171.3	-	
20	170.5	-		170.6	-	

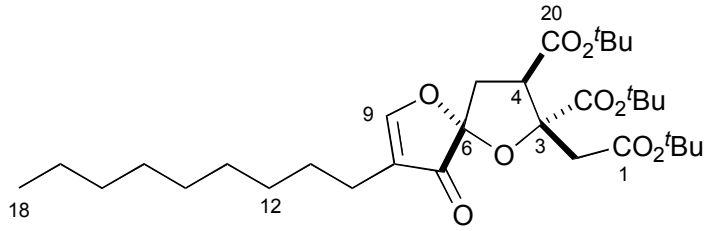
1 H. Shiozawa, M. Takahashi, T. Takatsu, T. Kinishita, K. Tanzawa, T. Hosoya, K. Furuya, S. Takahashi, K. Furihata, and H. Seto, *J. Antibiot.*, 1995, **48**, 357.

Table 2: ^{1}H (400 MHz, d_6-DMSO) and ^{13}C NMR data (100MHz, d_6-DMSO) for synthetic trachyspic acid trimethyl ester (**25**).

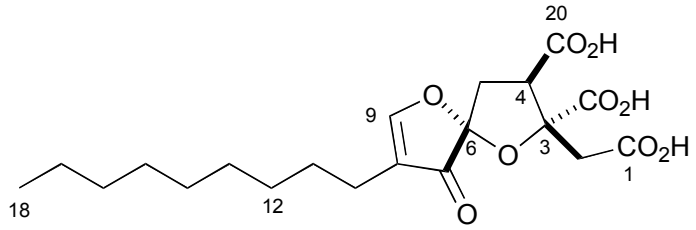
Carbon atom	Naturally derived trachyspic acid trimethyl ester[1]			Synthetic trachyspic acid trimethyl ester		
	δ_C	δ_H	(mult, J)	δ_C	δ_H	(mult, J)
1	169.1	-		169.1	-	
2	38.5	2.87 2.93	(1H, d, 16.5) (1H, d, 16.5)	38.5	2.87 2.93	(1H, d, 16.8) (1H, d, 16.8)
3	86.5	-		86.5	-	
4	47.7	3.81	(1H, dd, 7.5, 12.5)	47.7	3.80	(1H, dd, 7.4, 12.7)
5	37.3	2.40 2.47	(1H, dd, 12.5, 13.5) (1H, dd, 7.5, 13.5)	37.3	2.39 2.47	(1H, dd, 12.7, 13.2) (1H, dd, 7.4, 13.2)
6	107.7	-		107.7	-	
7	197.7	-		197.7	-	
8	117.0	-		117.0	-	
9	174.7	8.45	(1H, s)	174.7	8.47	(1H, s)
10	20.5	2.02	(2H, t, 7.5)	20.5	2.02	(2H, t, 7.6)
11	27.5	1.39	(2H, m)	27.5	1.38	(2H, m)
12	28.7	1.23	(2H, br s)	28.7	1.23	(2H, br s)
13	28.8	1.23	(2H, br s)	28.8	1.23	(2H, br s)
14	28.8	1.23	(2H, br s)	28.8	1.23	(2H, br s)
15	29.0	1.23	(2H, br s)	29.0	1.23	(2H, br s)
16	31.4	1.23	(2H, br s)	31.4	1.23	(2H, br s)
17	22.2	1.23	(2H, br s)	22.2	1.23	(2H, br s)
18	14.1	0.85	(3H, t, 7.0)	14.0	0.84	(3H, t, 6.8)
19	170.0	-		169.8	-	
20	169.5	-		169.5	-	
21	51.9	3.56	(3H, s)	51.9	3.55	(3H, s)
22	53.0	3.71	(3H, s)	53.0	3.70	(3H, s)
23	52.6	3.64	(3H, s)	52.6	3.64	(3H, s)



(–)-Trachyspic acid tri-*t*-butyl ester (**23**)
^{1}H NMR spectrum (400MHz, $CDCl_3$)

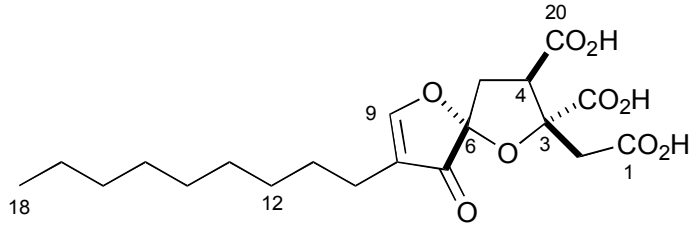


(–)-Trachyspic acid tri-*t*-butyl ester (**23**)
^{13}C NMR spectrum (100MHz, $CDCl_3$)



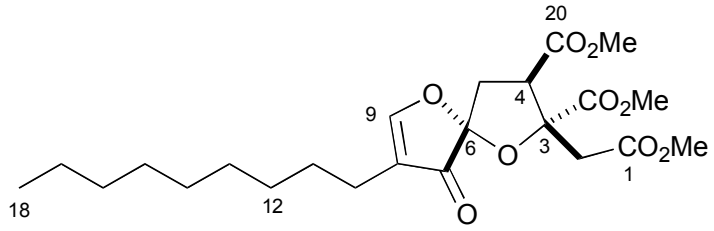
(–)-Trachyspic acid (*ent*-**1**)

^{1}H NMR spectrum (400MHz, d_6-DMSO)

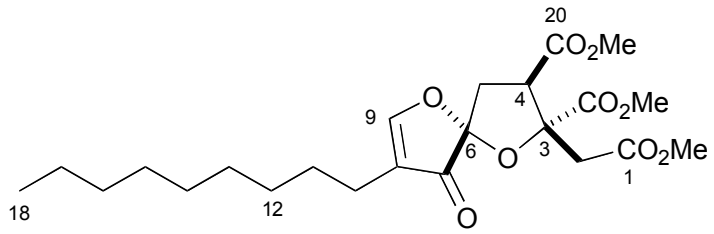


(–)-Trachyspic acid (*ent*-**1**)

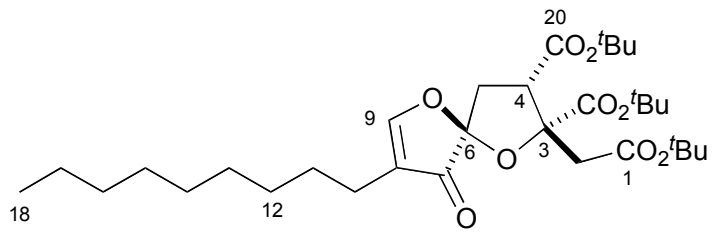
^{13}C NMR spectrum (100MHz, d_6-DMSO)



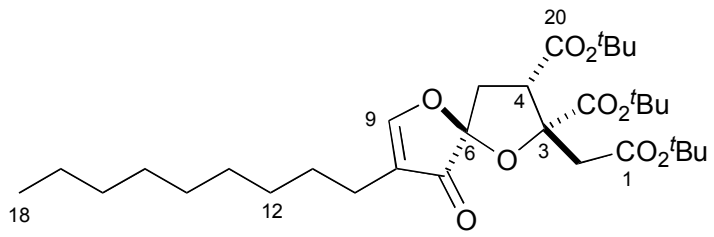
(–)-Trachyspic acid trimethyl ester (*ent*-**25**)
^{1}H NMR spectrum (400MHz, d_6-DMSO)



(–)-Trachyspic acid trimethyl ester (*ent*-**25**)
^{13}C NMR spectrum (100MHz, d_6-DMSO)

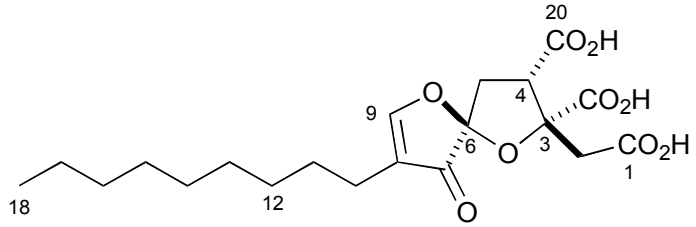


(–)-3-*epi*-Trachyspic acid tri-*t*-butyl ester (**31**)
^{1}H NMR spectrum (400MHz, $CDCl_3$)



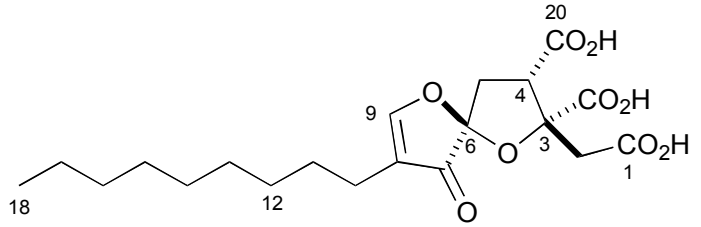
(–)-3-*epi*-Trachyspic acid trimethyl tri-*t*-butyl ester (**31**)

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



(+)-3-*epi*-Trachyspic acid (**28**)

^{1}H NMR spectrum (400MHz, d_6-DMSO)



(+)-3-*epi*-Trachyspic acid (**28**)

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