ARTICLE

OBC
www.rsc.org/obc

Enantiospecific synthesis of the phospholipase A_2 inhibitors (−)-cinatrin C_1 and (+)-cinatrin C_3

Anthony N. Cuzzupe, Romina Di Florio, Jonathan M. White and Mark A. Rizzacasa *
School of Chemistry, The University of Melbourne, Victoria, 3010, Australia.
E-mail: masr@unimelb.edu.au

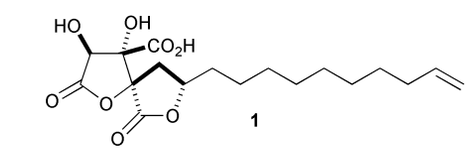
Received 16th July 2003, Accepted 1st September 2003
First published as an Advance Article on the web 22nd September 2003

The enantiospecific synthesis of (−)-cinatrin C_1 (**3**) and (+)-cinatrin C_3 (**5**) from the D-arabinose derivative **9** is described. The stereochemistry at C2 was introduced *via* a chelation-controlled addition of a carbanion to α-hydroxy ketone **8**. The best selectivity was achieved by use of the Grignard reagent derived from trimethylsilylacetylene. Transformation of the terminal alkyne into methyl ester **17** followed by acetal hydrolysis and selective lactol oxidation gave cinatrin C_1 dimethyl ester (**7**). Base hydrolysis and acid induced relactonization then gave a 1 : 1 mixture of cinatrins C_1 (**3**) and C_3 (**5**).

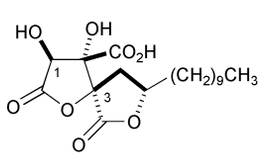
Introduction

Endogenous eicosanoids such as the prostanoids (prostaglandins and thromboxanes) and leukotrienes, are powerful mediators in all types of inflammation.[1] The main source of eicosanoids is arachidonic acid, a 20 carbon unsaturated fatty acid. The bulk of arachidonate in mammalian cells is found esterified in the fatty acyl chains of glycerophospholipids, almost exclusively in the 2-position.[2] The rate limiting step in the biosynthesis of eicosanoids is catalysed by the lipolytic enzyme, phospholipase A_2 (PLA_2) which specifically hydrolyses the 2-acyl position of a glycerophospholipid.[1] PLA_2 also mediates the formation of lyso-PAF, a precursor to platelet activating factor (PAF). PAF is released from many inflammatory cells when activated and is responsible for producing most phenomena of inflammation.[3]

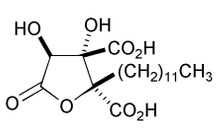
In a screening program for microbial products which possess pharmacological activity, Itazaki and co-workers isolated a family of PLA_2 inhibitors, cinatrins A (**1**), B (**2**), C_1 (**3**), C_2 (**4**) and C_3 (**5**), from the fermentation broth of the microorganism *Circinotrichum falcatisporum* RF-641.[4] Since inhibition of the enzymatic activity of PLA_2 could be therapeutically beneficial, the cinatrins show potential for use as anti-inflammatory agents.



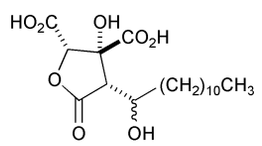
1



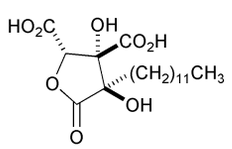
2



3



4



5

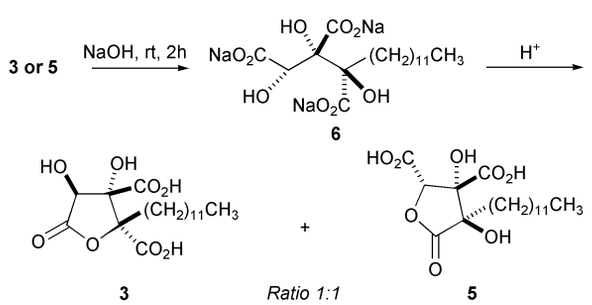
The structures of all members of the cinatrin family were determined by elemental analysis, SI-mass spectrometry, various NMR techniques and analysis of the respective methyl esters, which also served to confirm the number of carboxylic acid groups present.[4] The relative configuration of cinatrin C_3 (**5**) was determined from its single crystal X-ray structure while the absolute stereochemistry was obtained by CD spectroscopy.

In 1997, Evans and co-workers reported the first total synthesis of (−)-cinatrin C_1 (**3**) and (+)-cinatrin C_3 (**5**) using a tartrate aldol methodology.[5] This work revealed the structures of the cinatrins to be enantiomeric to those originally reported.[4] The novel structure and biological activity of this group of natural products makes them interesting targets, and we have embarked on a synthetic program towards these compounds. In 2002 we reported the first enantiospecific synthesis of (−)-cinatrin B (**2**) from D-arabinose.[6] We now report the enantiospecific synthesis of (−)-cinatrin C_1 (**3**) and (+)-cinatrin C_3 (**5**) from a common intermediate used in the (−)-cinatrin B (**2**) synthesis.[6]

Results and discussion

Retrosynthetic analysis

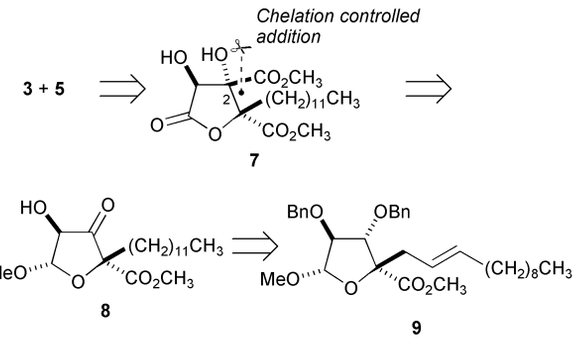
Structural studies on cinatrins C_1 (**3**) and C_3 (**5**) revealed that the two natural products could be obtained in a 1 : 1 ratio by acidification of the seco acid salt **6** produced by hydrolysis of either **3** or **5** with aqueous sodium hydroxide (Scheme 1).[4]



Scheme 1 Conversion of **3** or **5** into a 1 : 1 mixture of **3** and **5**.[4]

In light of the above result, it was envisaged that synthesis of the methyl ester of either natural product might provide access to both **3** and **5**. Therefore, it was proposed that cinatrins C_1 (**3**) and C_3 (**5**) could be obtained by hydrolysis and acidification of the cinatrin C_1 dimethyl ester (**7**)[4] (Scheme 2). A chelation controlled[7] carbanion addition to α-hydroxy ketone **8** in preference to the ester functionality should provide a method for the

DOI: 10.1039/b308028e



Scheme 2 Retrosynthetic analysis of cinatrins C_1 (**3**) and C_3 (**5**).

introduction of C2† acid functionality. An analagous addition reaction was utilized successfully in the synthesis of cinatrin B (**2**)[6] where the free hydroxyl group delivered an appropriate nucleophile to the ketone from the β-face. The α-hydroxy ketone **8** could be synthesised from ester **9** which in turn is obtained from D-arabinose.[6]

Enantiospecific synthesis of cinatrins C_1 (3) and C_3 (5)

The route towards cinatrins C_1 (**3**) and C_3 (**5**) began with the known methyl ester **9**, available in eight steps from the chiral pool starting material D-arabinose (Scheme 3).[6] The key step in the synthesis of **9** involved an Ireland–Claisen rearrangement of chiral ester **10** in the presence of a β-leaving group, by treatment with $TMSCl/NEt_3$ and LDA at −100 °C.[8,9] Warming to room temperature followed by base hydrolysis and esterification afforded esters **9** and **11** in excellent yield and in 73 : 27 ratio. The stereochemistry of the major ester **9** was assigned on the basis of an NOE observed between the allylic protons and H2 in the NOESY spectrum of **9** (Scheme 3).[6]

Debenzylation and saturation of the double bond in **9** by exposure to 60 psi H_2 gas and $Pd(OH)_2$ gave diol **12** as a crystalline solid (Scheme 4). Initial work carried out in the enantiomeric series derived from L-arabinose provided *ent*-**12**, the enantiomer of diol **12** (Fig. 1) using an identical sequence. An X-ray structure of *ent*-**12** was obtained which confirmed the stereochemistry of diol **12** and therefore **9** to be as depicted in Scheme 3.

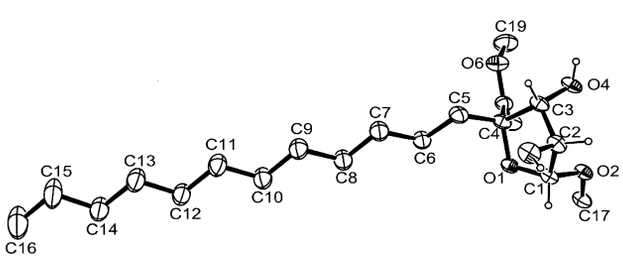
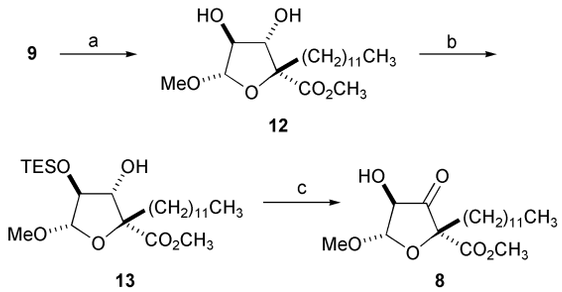


Fig. 1 X-Ray structure of *ent*-**12**. Some protons are omitted for clarity.

† Cinatrin numbering is used throughout.

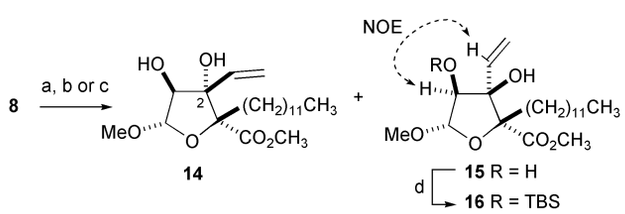


Scheme 4 *Reagents and conditions*: (a) 60 psi H_2, $Pd(OH)_2$/C, MeOH, 88%; (b) TESCl, imidazole, DMF, 84%; (c) (i) Dess–Martin periodinane, CH_2Cl_2, pyridine; (ii) 48% HF/H_2O/MeCN, rt.

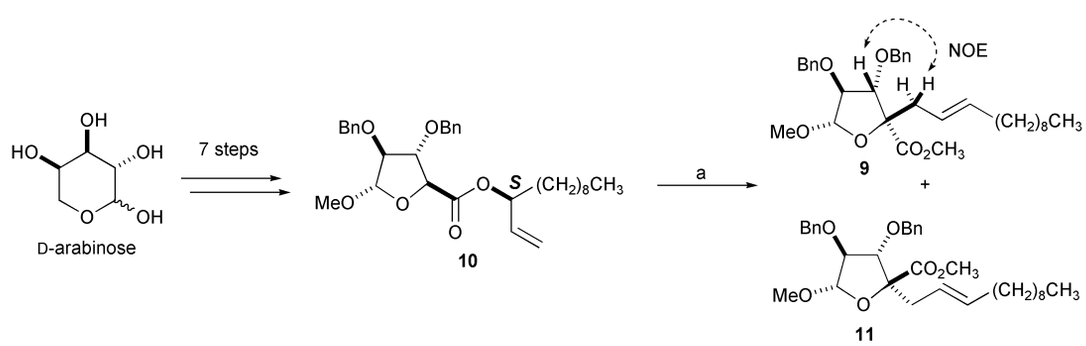
Protection of the less hindered alcohol in **12** by treatment with TESCl and imidazole provided monoether **13**. Oxidation with Dess–Martin periodinane[10] buffered with pyridine followed by HF induced desilylation gave labile α-hydroxy ketone **8**, which was utilized immediately in the addition reaction.

With the α-hydroxy ketone **8** in hand, attention then turned to introduction of the C2 acid functionality. A number of reagents were trialed for the chelation-controlled addition[11–13] to **8** as outlined in Scheme 5. Treatment of **8** with vinylmagnesium bromide in THF at −78 °C gave a separable mixture of C2 epimeric alkenes **14** and **15** in good overall yield. These were obtained in a 1.6:1 ratio favouring **14**, which was found to possess correct configuration at the newly formed stereocentre (see Scheme). On the other hand, treatment of **8** with lithium trimethylsilylacetylide followed by desilylation and partial reduction of the resultant alkyne with hydrogen gas in the presence of Lindlar catalyst gave **14** with a higher ds but in lower yield. Treatment of **8** with the Grignard reagent derived from TMS acetylene in THF at −78–0 °C followed by desilylation and partial reduction gave **14** in highest ds and excellent overall yield. The stereoselectivity of this reaction was sensitive to the rate of addition of a solution of the anion to ketone **8** and how long the reaction was maintained at low temperature.

Protection of the less hindered alcohol by treatment of minor epimer **15** with TBSOTf and 2,6-lutidine provided monoether **16** (Scheme 5). Observation of an NOE between the vinylic



Scheme 5 *Reagents and conditions*: (a) $H_2C{=}CHMgBr$, THF, −78 °C, 59% overall from **13**, *Ratio 1.6 : 1*, **14** : **15**; (b) (i) $TMSC{\equiv}CLi$, THF, −78 °C; (ii) TBAF, THF, rt; (iii) H_2, Lindlar, MeOH, 27% overall from **13**, *Ratio 3 : 1*, **14** : **15**; (c) (i) $TMSC{\equiv}CMgBr$, THF, −78–0 °C; (ii) TBAF, THF, rt; (iii) H_2, Lindlar, MeOH, 57% overall from **13**, *Ratio 5 : 1*, **14** : **15**; (d) TBSOTf, 2,6-lutidine, 0 °C, 79%.



Scheme 3 *Reagents and conditions*: (a) LDA, $TMSCl/NEt_3$, HMPA/THF, −100 °C–rt then aqueous NaOH, CH_2N_2, 97%, *Ratio 73 : 27*, **9** : **11**.[6]

Table 1 Comparison of ^{1}H NMR data δ_H (400 MHz, CD_3OD) for natural[5] and synthetic cinatrin C_1 (**3**)

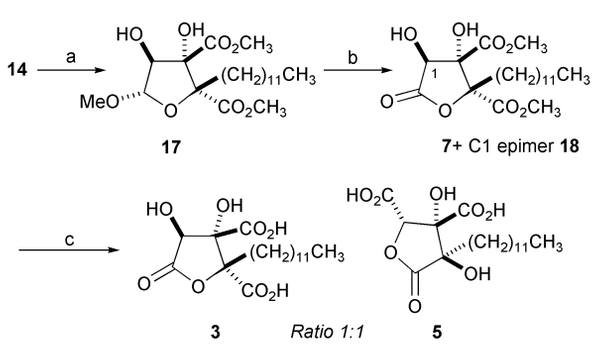
Position	Natural (m, *J*/Hz)	Synthetic (m, *J*/Hz)
C_1H	4.69 (s)	4.73 (s)
C_4H	2.14 (app. t, 8.4)	2.16 (dd, 13.6, 4.4)
C_4H	1.70 (m)	1.68 (dd, 13.6, 4.8)
C_5H	1.50 (m)	1.50 (m)
C_5H	1.39 (m)	1.37 (m)
$(CH_2)_9CH_3$	1.28 (m)	1.28 (m)
CH_3	0.89 (app. t, 6.9)	0.89 (app. t, 6.8)

Table 2 Comparison of ^{1}H NMR data δ_H (400 MHz, CD_3OD) for natural[5] and synthetic cinatrin C_3 (**5**)

Position	Natural (m, *J*/Hz)	Synthetic (m, *J*/Hz)
C_1H	5.40 (s)	5.40 (s)
C_4H_2	1.83 (app. t, 8.4)	1.83 (app. t, 8.4)
C_5H_2	1.55–1.41 (m)	1.56–1.42 (m)
$(CH_2)_9CH_3$	1.28 (m)	1.28 (m)
CH_3	0.89 (app. t, 6.9)	0.89 (app. t, 6.8)

proton and H1 confirmed that ether **16** derived from **15** possesses incorrect configuration at C2. This in turn confirmed major epimer **14** had the desired configuration.

The final steps of the synthesis are outlined in Scheme 6. Cleavage of the terminal alkene in **14** by ozonolysis followed by oxidation with sodium chlorite and methylation yielded the methyl ester **17**. One-step oxidation[14] to form the lactone failed, so a two step sequence was employed. Acid hydrolysis of the methyl ketal followed by selective lactol oxidation[15] gave (−)-cinatrin C_1 dimethyl ester (**7**). Some of the C1 epimer **18** was also isolated. The data obtained for synthetic **7** compared well with that reported for the dimethyl ester derived from natural cinatrin C_1 (**3**).[4] Base hydrolysis of **7** followed by relactonization and purification by reverse-phase HPLC afforded (−)-cinatrin C_1 (**3**) and (+)-cinatrin C_3 (**5**) in a 1 : 1 ratio. The data obtained for the synthetic compounds compared well with that for the natural products[4,5] (see Tables 1, 2 and 3). In addition, reverse phase HPLC retention times of the synthetic samples of **3** and **5** compared well with those of the natural materials.



Scheme 6 *Reagents and conditions*: (a) (i) O_3, Me_2S, CH_2Cl_2/MeOH, −78 °C; (ii) $NaClO_2$/NaH_2PO_4·H_2O; (iii) excess CH_2N_2, 33% overall; (b) 80% aq. AcOH/10% aq. HCl, 100 °C, 3.5 h; (ii) I_2/$CaCO_3$, MeOH/H_20, 70 °C, 16 h, 41% **7**; 10% **18** for 2 steps; (c) 2 M aq. NaOH/THF, 40 °C, 22 h then 2 M aq. HCl, 68%.

In conclusion, we have completed the total synthesis of (−)-cinatrin C_1 (**3**) and (+)-cinatrin C_3 (**5**) from the common precursor, cinatrin C_1 dimethyl ester (**7**). This was synthesised from a common intermediate also utilised for the synthesis of (−)-cinatrin B (**2**).[6] A key step was the chelation-controlled addition to an α-hydroxy ketone in the presence of an ester to introduce the C2 acid functionality.

Table 3 Comparison of ^{13}C NMR data δ_C (100 MHz, d_6-DMSO) for natural[4] and synthetic cinatrins C_1 (**3**) and C_3 (**5**)

	Cinatrin C_1 (**3**)		Cinatrin C_3 (**5**)	
Position	Natural	Synthetic	Natural	Synthetic
1	73.1	73.0	79.4	79.5
1-*C*O	173.6	173.2	167.5	167.5
2	84.0	83.9	81.3	81.4
2-*C*O	170.4	170.0	170.4	170.5
3	86.5	86.4	78.7	78.8
3-*C*O	170.6	170.2	174.6	174.6
4	30.8	30.8	30.5	30.6
5	23.5	23.5	21.0	21.1
6	28.7	28.7	29.6	29.7
7	28.8	28.9	28.9	29.0
8	29.2	29.2	29.0	29.0
9	29.0	29.0	29.0	29.0
10	29.0	29.0	29.0	29.0
11	29.0	29.0	29.0	29.1
12	28.7	28.7	28.7	28.7
13	31.3	31.3	31.2	31.3
14	22.1	22.1	22.0	22.1
15	13.9	13.9	13.9	14.0

Experimental

General

Optical rotations were recorded in a 10 cm microcell. High resolution mass spectra (HRMS) electrospray ionisation (ESI) were run at Monash University, Clayton, Victoria. Proton nuclear magnetic resonance (^{1}H NMR, 300 and 400 MHz) and proton decoupled carbon nuclear magnetic resonance spectra (^{13}C NMR, 75.5 and 100 MHz) were recorded for deuteriochloroform solutions with residual chloroform as internal standard unless otherwise stated. Microanalyses were carried out by the Campbell Microanalytical Laboratory at the University of Otago, Dunedin, New Zealand. Analytical thin layer chromatography (TLC) was conducted on aluminium backed 2 mm thick silica gel GF_{254}. Compounds were visualised with solutions of 20% w/w phosphomolybdic acid in ethanol, 20% w/w potassium permanganate in water or under UV (365 nm). Anhydrous THF and diethyl ether were distilled from sodium benzophenone ketyl and sodium metal under a nitrogen atmosphere. Petrol refers to the fraction boiling at 40–60 °C. All other commercial reagents were used as received. All air and moisture sensitive reactions were performed in glassware that was either flame dried under an atmosphere of dry argon or oven dried at 150 °C.

Synthesis

Methyl methyl-4-*C*-dodecanyl-β-L-xylofuranosiduronate (12). To a solution of the dibenzyl ether **9**[6] (463 mg, 0.859 mmol) in MeOH (14 mL) was added $Pd(OH)_2$ on C, 20 wt% Pd (dry basis) (158 mg, 0.297 mmol) and the resulting suspension was stirred vigorously under a H_2 atmosphere at 60 psi for 22 h. The suspension was then filtered through Celite and the eluent was concentrated under reduced pressure. The residue was purified by flash chromatography with 40% EtOAc/petrol as eluent to give the diol **12** (302 mg, 97%) as a crystalline solid: mp 60–61 °C; $[\alpha]_D^{24}$ +75.5 (*c* 0.41, $CHCl_3$) (Found: C, 63.11; H, 9.86%; $C_{19}H_{36}O_6$ requires C, 63.30; H, 10.07%); ν_{max} (Nujol) 3451, 2915, 1721, 1466, 1219, 1096, 1052, 967, 722 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 0.88 (t, *J* = 6.3 Hz, 3H), 1.24 (m, 20H), 1.59 (br s, 1H), 1.97 (m, 2H), 3.35 (d, *J* = 11.1 Hz, 1H), 3.51 (s, 3H), 3.78 (s, 3H), 3.92 (d, *J* = 10.0 Hz, 1H), 4.19 (br s, 1H), 4.96 (s, 1H); δ_C (75.5 MHz, $CDCl_3$) 14.0, 22.6, 24.0, 29.2, 29.4, 29.46, 29.53, 29.6, 31.8, 37.3, 52.0, 55.3, 80.1, 81.8, 91.7, 109.3, 173.1.

Methyl methyl-2-*O*-(triethylsilyl)-4-*C*-dodecanyl-β-L-xylofuranosiduronate (13). To a solution of the diol **12** (406 mg, 1.13 mmol) and imidazole (152.8 mg, 2.24 mmol) in DMF

(11 mL) at −40 °C under argon was added chlorotriethylsilane (207 μL, 1.23 mmol) and the solution was stirred for 1 h. Water and Et_2O were added and the aqueous phase was extracted with Et_2O. The combined extracts were washed with water, brine and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was purified by flash chromatography with 5–10% EtOAc/petrol as eluent to give the TES ether **13** (451 mg, 84%) as a colourless oil: $[\alpha]_D^{19}$ +44.2 (*c* 1.04, $CHCl_3$); ν_{max} (thin film) 3510, 2927, 2856, 1758, 1464, 1115 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 0.62 (q, *J* = 7.8 Hz, 6H), 0.86 (t, *J* = 6.6 Hz, 3H), 0.95 (t, *J* = 7.8 Hz, 9H), 1.06–1.34 (m, 19H), 1.42–1.56 (m, 1H), 1.95 (m, 2H), 3.22 (d, *J* = 11.1 Hz, 1H), 3.49 (s, 3H), 3.75 (s, 3H), 3.80 (d, *J* = 11.1 Hz, 1H), 4.07 (d, *J* = 1.2 Hz, 1H), 4.86 (s, 1H); δ_C (75.5 MHz, $CDCl_3$) 4.6, 6.6, 14.1, 22.7, 24.1, 29.3, 29.5, 29.6, 29.7, 31.9, 37.1, 52.0, 55.2, 80.5, 82.2, 93.2, 110.0, 172.7; HRMS (ESI) *m*/*z*: Calc. for $C_{25}H_{50}NaO_6Si$ $[M + Na]^+$ 497.3274, found 497.3274.

Methyl methyl-4-*C*-dodecanyl-D-erythro-3-pentulofuranosiduronate (8). To a solution of the alcohol **13** (333 mg, 0.701 mmol) in CH_2Cl_2 (8.6 mL) at 0 °C under argon was added pyridine (103 μL, 3.5 mmol) followed by Dess–Martin reagent (0.59 g, 1.4 mmol). The mixture was allowed to warm to rt and stirred for 1 h. Et_2O (30 mL), saturated aqueous $NaHCO_3$ (30 mL) and 1.5 M aqueous $Na_2S_2O_3$ (30 mL) were added and the mixture was stirred until two clear layers formed. The aqueous phase was extracted with Et_2O and the combined extracts were washed with water, saturated aqueous $CuSO_4$, water, brine and dried ($MgSO_4$). The solvent was removed under reduced pressure to give the ketone as a yellow oil (340 mg) which was treated with a solution (7 mL) containing MeCN (19 mL), water (2 mL) and 48% aqueous HF (1 mL) and the mixture was stirred vigorously at rt for 1 h. Et_2O was added and the reaction was quenched with saturated aqueous $NaHCO_3$. The aqueous phase was extracted with Et_2O and the combined extracts were washed with water, brine and dried ($MgSO_4$). The solvent was removed under reduced pressure to give the α-hydroxy ketone **8** as a yellow oil (265 mg) which was utilized immediately in the next step.

Methyl methyl-3-*C*-vinyl-4-*C*-dodecanyl-β-L-xylofuranosiduronate (14) and methyl methyl-3-*C*-vinyl-4-*C*-dodecanyl-β-L-ribofuranosiduronate (15). *Method A.* To a solution of the crude ketone **8** (708 mg) in THF (11.1 mL) at −78 °C under argon was added vinylmagnesiumbromide (1.0 M solution in THF, 13.9 mL, 13.9 mmol). The mixture was stirred at −78 °C for 1 h after which time more vinylmagnesiumbromide was added (1.0 M solution in THF, 5.9 mL, 5.9 mmol). Stirring was continued for 1 h before the mixture was allowed to warm to 0 °C and quenched with saturated aqueous NH_4Cl. The aqueous phase was extracted with Et_2O and the combined extracts were washed with water, brine and dried ($MgSO_4$). The solvent was removed under reduced pressure to give a mixture of alkenes **14** and **15** in a 1.6 : 1 ratio which were separated by flash chromatography. Elution with 20% EtOAc/petrol gave the major C2 epimer **14** as a pale yellow oil (290 mg, 39% from **13**): $[\alpha]_D^{17}$ +42.8 (*c* 1.16, $CHCl_3$); ν_{max} (thin film) 3474, 2926, 2855, 1755, 1738, 1445, 1105, 1037 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 0.86 (t, *J* = 6.6 Hz, 3H), 0.94–1.11 (m, 1H), 1.23 (m, 18H), 1.44–1.60 (m, 1H), 1.79–1.91 (m, 2H), 2.47 (d, *J* = 4.2 Hz, 1H), 3.55 (s, 3H), 3.72 (s, 3H), 3.93 (d, *J* = 3.9 Hz, 1H), 4.17 (s, 1H), 5.00 (s, 1H), 5.41 (dd, *J* = 11.0, 1.8 Hz, 1H), 5.61 (dd, *J* = 17.1, 1.8 Hz, 1H), 6.13 (dd, *J* = 17.1, 11.1 Hz, 1H); δ_C (75.5 MHz, $CDCl_3$) 14.1, 22.7, 24.4, 29.3, 29.4, 29.5, 29.62, 29.64, 29.8, 31.9, 36.5, 51.9, 55.4, 82.4, 82.7, 95.6, 108.4, 118.2, 131.9, 172.1; HRMS (ESI) *m*/*z*: Calc. for $C_{21}H_{38}NaO_6$ $[M + Na]^+$ 409.2566, found 409.2565.

Further elution afforded the minor C2 epimer **15** as a pale yellow oil (140 mg, 19% from **13**): $[\alpha]_D^{19}$ +31.5 (*c* 1.03, $CHCl_3$); ν_{max} (thin film) 3468, 2925, 2855, 1737, 1467, 1109 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 0.86 (t, *J* = 6.6 Hz, 3H), 0.96–1.13 (m, 1H), 1.23 (m, 18H), 1.38–1.55 (m, 1H), 1.86 (m, 2H), 3.52 (s, 3H), 3.68 (s, 3H), 3.99 (d, *J* = 2.7 Hz, 1H), 4.96 (d, *J* = 3.0 Hz, 1H), 5.18 (d, *J* = 10.8 Hz, 1H), 5.43 (d, *J* = 17.1 Hz, 1H), 5.95 (dd, *J* = 17.1, 10.8 Hz, 1H); δ_C (75.5 MHz, $CDCl_3$) 14.1, 22.6, 23.8, 29.3, 29.4, 29.5, 29.6, 29.64, 29.9, 31.9, 33.4, 52.0, 56.3, 79.6, 81.1, 91.9, 108.2, 114.9, 137.0, 172.6; HRMS (ESI) *m*/*z*: Calc. for $C_{21}H_{38}NaO_6$ $[M + Na]^+$ 409.2566, found 409.2573.

Method B. To a solution of trimethylsilylacetylene (73 μL, 0.525 mmol) in dry THF (1.1 mL) at −78 °C under argon was added *n*-BuLi in hexanes (197 μL, 0.473 mmol, 2.4 M) dropwise. The solution was stirred at −78 °C for 30 min and a solution of the crude ketone **8** (40 mg) in dry THF (0.5 mL) was added *via* cannula. The reaction was then stirred for a further 15 min and quenched with saturated aqueous NH_4Cl. The aqueous phase was extracted with Et_2O and the combined extracts were washed with water, brine and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was purified by flash chromatography with 10–20% EtOAc/petrol as eluent to give an inseparable 3 : 1 mixture of TMS acetylenes as a yellow oil (17.6 mg, 37% from **13**). To a solution of the mixture of TMS acetylenes (17.6 mg, 0.039 mmol) in THF (1 mL) at rt was added TBAF·$3H_2O$ (17.8 mg, 0.056 mmol) and the mixture was stirred for 1 h. Water was added and the aqueous phase was extracted with Et_2O and the combined extracts were washed with water, brine and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was dissolved in MeOH (1 mL). Lindlar catalyst (5 mg, 2.38 μmol) was added and the suspension was stirred under a H_2 atmosphere for 1.5 h. The suspension was filtered through Celite and the eluent was concentrated under reduced pressure to give a mixture of alkenes **14** and **15** which were separated by flash chromatography. Elution with 20% EtOAc/petrol gave **14** (8.3 mg, 21% from **13**) and **15** (2.5 mg, 6% from **13**) as pale yellow oils.

Method C. To a solution of ethylmagnesiumbromide (1.0 M in THF, 4.2 mL, 4.2 mmol) in THF (0.68 mL) at 0 °C under argon was added TMS acetylene (0.6 mL, 4.25 mmol) and the mixture was stirred for 30 min before being allowed to warm to 30 °C and stirred for a further 10 min. The mixture was then cooled to −78 °C and a solution of the crude ketone **8** (265 mg) in THF (1.36 mL) was added dropwise by cannula and the mixture was stirred for 3 h. The mixture was allowed to warm to 0 °C and stirring was continued for a further 10 min then quenched with saturated aqueous NH_4Cl. The aqueous phase was extracted with Et_2O and the combined extracts were washed with water, brine and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was dissolved in THF (13 mL). TBAF·$3H_2O$ (320 mg, 1.01 mmol) was added and the mixture was stirred at rt for 1 h. Water was added and the aqueous phase was extracted with Et_2O and the combined extracts were washed with water, brine and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was purified by flash chromatography with 30% EtOAc/petrol as eluent to give an 83 : 17 inseparable mixture of alkynes as a yellow oil (179 mg, 66% from **13**). To a solution of the alkynes (179 mg, 0.466 mmol) in MeOH (8.4 mL) was added Lindlar catalyst (33.6 mg, 0.016 mmol) and the suspension was stirred under a H_2 atmosphere for 1 h. The suspension was filtered through Celite and the eluent was concentrated under reduced pressure to give a mixture of alkenes **14** and **15** which were separated by flash chromatography. Elution with 20% EtOAc/petrol gave **14** (128 mg, 47% from **13**) and **15** (28 mg, 10% from **13**) as pale yellow oils.

Methyl methyl-2-*O*-(*tert*-butyldimethylsilyl)-3-*C*-vinyl-4-*C*-dodecanyl-β-L-ribofuranosiduronate (16). To a solution of the alcohol **15** (30 mg, 0.084 mmol) and 2,6-lutidine (58.7 μL, 0.504 mmol) in CH_2Cl_2 (1.09 mL) at 0 °C under argon was added

tert-butyldimethylsilyl trifluoromethanesulfonate (57.9 μL, 0.252 mmol) and the solution was stirred for 40 min. Saturated aqueous $NaHCO_3$ was added and the aqueous phase was extracted with Et_2O. The combined extracts were washed with water, saturated aqueous $CuSO_4$, water, brine and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was purified by flash chromatography with 10% EtOAc/petrol as eluent to give the TBS ether **16** as a colourless oil (33 mg, 79%): $[\alpha]_D^{22}$ +12.1 (*c* 1.12, $CHCl_3$); ν_{max} (thin film) 3524, 2919, 2851, 1747, 1460, 1251, 1127, 1061, 1023 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 0.07 (s, 3H), 0.10 (s, 3H), 0.87 (t, *J* = 6.6 Hz, 3H), 0.89 (s, 9H), 1.23 (m, 20H), 1.85 (m, 2H), 3.14 (s, 1H), 3.50 (s, 3H), 3.69 (s, 3H), 3.94 (d, *J* = 3.0 Hz, 1H), 4.83 (d, *J* = 3.0 Hz, 1H), 5.16 (dd, *J* = 10.8, 1.8 Hz, 1H), 5.47 (dd, *J* = 17.1, 1.8 Hz, 1H), 5.89 (dd, *J* = 17.1, 10.8 Hz, 1H); δ_C (75.5 MHz, $CDCl_3$) −5.0, −4.7, 14.1, 18.0, 22.7, 23.7, 25.6, 29.3, 29.4, 29.5, 29.6, 29.7, 30.0, 31.9, 33.0, 51.9, 56.4, 80.9, 81.3, 91.8, 108.8, 115.2, 137.0, 172.6; HRMS (ESI) *m/z*: Calc. for $C_{27}H_{52}O_6SiNa$ $[M + Na]^+$ 523.3431, found 523.3428.

Methyl methyl-3-*C*-carboxymethyl-4-*C*-dodecanyl-β-L-xylo-furanosiduronate (17). Ozone gas was bubbled through a solution of the alkene **14** (152 mg, 0.39 mmol) in CH_2Cl_2 (8.82 mL) and MeOH (306 μL) at −78 °C until a pale blue colour persisted. Me_2S (145 μL, 1.95 mmol) was added and the solution was allowed to warm to rt and stirring was continued for a further 20 min. Water and Et_2O were added and the aqueous phase was extracted with Et_2O. The combined extracts were washed with water, brine and dried ($MgSO_4$) and the solvent was removed under reduced pressure. The resulting crude aldehyde (178 mg) was dissolved in *tert*-butanol (2.59 mL) and treated with 2-methyl-2-butene (414 μL). A solution of $NaH_2PO_4 \cdot H_2O$ (108.8 mg, 0.79 mmol) and $NaClO_2$ (142.6 mg, 1.59 mmol) in water (0.91 mL) was then added and the mixture was stirred at rt for 16 h. Water and EtOAc were added and the aqueous phase was extracted with EtOAc and the combined extracts were washed with water, brine and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was treated with an excess of CH_2N_2. Purification by flash chromatography with 20–30% EtOAc/petrol as eluent gave the dimethyl ester **17** as a yellow oil (54.3 mg, 33%): $[\alpha]_D^{19}$ +26.9 (*c* 1.09, $CHCl_3$); ν_{max} (thin film) 3464, 2927, 2855, 1743, 1440, 1238, 1126, 1020 cm^{-1}; δ_H (300 MHz, $CDCl_3$) 0.87 (t, *J* = 6.6 Hz, 3H), 0.96–1.12 (m, 1H), 1.23 (m, 18H), 1.48–1.62 (m, 1H), 1.70 (m, 1H), 1.82 (m, 1H), 3.57 (s, 3H), 3.79 (s, 3H), 3.91 (s, 3H), 4.27 (s, 1H), 4.29 (br s, 1H), 4.50 (br s, 1H), 5.07 (s, 1H); δ_C (75.5 MHz, $CDCl_3$) 14.1, 22.7, 24.4, 29.3, 29.4, 29.5, 29.56, 29.61, 29.7, 31.9, 35.8, 52.4, 53.3, 55.9, 81.1, 82.8, 93.8, 108.6, 170.9, 171.2; HRMS (ESI) *m/z*: Calc. for $C_{21}H_{38}NaO_8$ $[M + Na]^+$ 441.2464, found 441.2467.

Cinatrin C_1 dimethyl ester (7). A solution of the methyl ketal **17** (106 mg, 0.253 mmol) in 80% aqueous acetic acid (12.2 mL) and 10% aqueous HCl (428 μL) was heated at 100 °C for 3.5 h then cooled to rt. Water was added and the aqueous phase was extracted with Et_2O and the combined extracts were washed with water, saturated aqueous $NaHCO_3$, brine and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was dissolved in MeOH (1.58 mL) and water (159 μL). $CaCO_3$ (190 mg, 1.90 mmol) and I_2 (394 mg, 1.52 mmol) were then added and the resulting brown suspension was stirred at 70 °C for 16 h then cooled to rt. Water was added and the aqueous phase was extracted with Et_2O. The combined extracts were washed with 1.5 M aqueous $Na_2S_2O_3$, brine and dried ($MgSO_4$). The solvent was removed under reduced pressure and the residue was purified by flash chromatography with 30–40% EtOAc/petrol as eluent to give the C2 epimer of cinatrin C_1 dimethyl ester (**18**) as a colourless oil (10.1 mg, 10%): $[\alpha]_D^{20}$ +55.4 (*c* 0.43, $CHCl_3$); ν_{max} (thin film) 3473, 2957, 2926, 2855, 1799, 1749, 1440, 1275 cm^{-1}; δ_H (400 MHz, d_6-DMSO) 0.84 (t, *J* = 6.0 Hz, 3H), 1.23 (m, 20H), 1.40 (m, 1H), 1.69 (m, 1H), 3.65 (s, 3H), 3.72 (s, 3H), 5.50 (s, 1H), 6.40 (s, 1H), 6.72 (s, 1H); δ_C (75.5 MHz, $CDCl_3$) 14.1, 21.6, 22.7, 29.33, 29.35, 29.5, 29.58, 29.62, 29.63, 29.7, 31.9. 32.0, 52.9, 54.2, 78.4, 80.9, 166.4, 170.8, 173.7; HRMS (ESI) *m/z*: Calc. for $C_{20}H_{34}NaO_8$ $[M + Na]^+$ 425.2151, found 425.2146.

Further elution gave cinatrin C_1 dimethyl ester (**7**) as a colourless oil (42.2 mg, 41%): $[\alpha]_D^{20}$ −5.9 (*c* 0.31, $CHCl_3$); ν_{max} (thin film) 3466, 2926, 2855, 1801, 1749, 1439, 1261, 1122 cm^{-1}; δ_H (400 MHz, d_6-DMSO) 0.84 (t, *J* = 6.6 Hz, 3H), 1.22 (m, 20H), 1.42 (m, 1H), 2.05 (m, 1H), 3.69 (s, 3H), 3.71 (s, 3H), 4.50 (d, *J* = 6.8 Hz, 1H), 6.59 (d, *J* = 6.8 Hz, 1H), 7.07 (s, 1H); δ_C (75.5 MHz, d_6-DMSO) 13.9, 22.0, 23.4, 28.5, 28.6, 28.7, 28.9, 30.7, 31.2, 52.3, 52.8, 73.0, 84.4, 86.8, 168.8, 169.0, 172.6; HRMS (ESI) *m/z*: Calc. for $C_{20}H_{34}NaO_8$ $[M + Na]^+$ 425.2151, found 425.2148.

Cinatrin C_1 (3) and C_3 (5). A solution of the dimethyl ester (**7**) (15.5 mg, 0.038 mmol) in THF (0.5 mL) and 2 M aqueous NaOH (0.5 mL, 1.0 mmol) was stirred at 40 °C for 22 h. The solution was acidified with 2 M aqueous HCl (0.7 mL, 1.4 mmol) and stirring was continued for a further 1 h. The solution was allowed to cool to rt and stirring was continued for a further 3.5 h. Water was added and the mixture was extracted with EtOAc. The combined extracts were washed with brine and dried (Na_2SO_4). The solvent was removed under reduced pressure to give a mixture of cinatrin C_1 (**3**) and cinatrin C_3 (**5**) in a 1 : 1 ratio by integration of the ^1H NMR spectrum. Purification by preparative reverse phase HPLC (C-18 5 μm, 250 × 10 mm, 0.1% TFA–80% MeCN/H_2O as eluent, flow rate: 2 mL min^{-1}; synthetic **3**, t_R 7.33 min; natural **3**, t_R 7.37 min; synthetic **5**, t_R 8.06 min; natural **5**, t_R 8.12 min) gave cinatrin C_1 (**3**) as a white powder (5.1 mg, 36%): $[\alpha]_D^{26}$ −1.6 (*c* 0.11, MeOH); lit.,[4] $[\alpha]_D^{24}$ −11.2 (*c* 0.31, MeOH); See Tables 1 and 3 for NMR data; HRMS (ESI) *m/z*: Calc. for $C_{18}H_{29}O_8$ $[M - H]^-$ 373.1862, found 373.1863. Further elution gave cinatrin C_3 (**5**) as a white powder (4.5 mg, 32%): $[\alpha]_D^{27}$ +59.7 (*c* 0.26, MeOH); lit.,[5] $[\alpha]_D^{23}$ +73.2 (*c* 0.17, MeOH); See Tables 2 and 3 for NMR data; HRMS (ESI) *m/z*: Calc. for $C_{18}H_{29}O_8$ $[M - H]^-$ 373.1862, found 373.1864.

Crystallography

Data for *ent*-**12** was collected on an Enraf-Nonius CAD-4 diffractometer using Ni-filtered Cu-Kα radiation (λ = 1.5418 Å. The data were corrected for Lorentz and polarization effects[16] for absorption (ABSORB)[17] and extinction.[18] The structure was solved by direct methods (SHELXS-86)[19] and refined on F^2 (SHELXL-97).[18] *ent*-**12** Exists in the solid state with two independent molecules in the asymmetric unit. All non-hydrogens were refined anisotropically, while the hydrogen atoms were constrained in idealised positions. Thermal ellipsoids plots were generated using the program ORTEP-3.[20] All programs were implemented within the suite WINGX.[20]

Crystal data for *ent*-12

$C_{19}H_{36}O_6$, *M* = 360.48, *T* = 298 (1) K, λ = 1.5418 Å, triclinic, space group *P*1, *a* = 5.7655(3), *b* = 9.7432(4), *c* = 19.338(1) Å, α = 83.041(5), β = 85.348(6), γ = 85.954(4)°, *V* = 1072.72(9) $Å^3$, *Z* = 2, D_x = 1.116 Mg m^{-3}, μ(Cu-Kα) = 0.663 mm^{-1}, *F*(000) = 396, crystal size 0.60 × 0.15 × 0.08 mm, max. min. transmission 0.96 and 0.86 respectively, 4048 reflections measured, 4048 independent reflections and the final $wR(F^2)$ was 0.1033 and final *R* was 0.0398 for 2196 unique data with $[I > 2\sigma(I)]$.

CCDC reference number 214283.

See http://www.rsc.org/suppdata/ob/b3/b308028e/ for crystallographic data in CIF or other electronic format.

Acknowledgements

We thank Dr Toshiyuki Kamigauchi (Shinogi Research Laboratories, Osaka, Japan) for authentic samples of cinatrin C_1 (**3**) and cinatrin C_3 (**5**) as well as copies of the NMR spectra of cinatrin C_1 dimethyl ester (**7**). This work was financially supported by the Australian Research Council Large Grants Scheme.

References

1 H. P. Rang and M. M. Dale, *Pharmacology*, Churchill Livingstone, Edinburgh, 1991.
2 R. F. Irvine, *Biochem. J.*, 1982, **204**, 3.
3 P. Vadas and W. Pruzanski, *Lab. Invest.*, 1986, **55**, 391.
4 H. Itazaki, K. Nagashima, Y. Kawamura, K. Matsumoto, H. Nakai and Y. Terui, *J. Antibiot.*, 1992, **45**, 38.
5 D. A. Evans, B. W. Trotter and J. C. Barrow, *Tetrahedron*, 1997, **53**, 8779.
6 A. N. Cuzzupe, R. Di Florio and M. A. Rizzacasa, *J. Org. Chem.*, 2002, **67**, 4392.
7 A. Mengel and O. Reiser, *Chem. Rev.*, 1999, **99**, 1191.
8 R. Di Florio and M. A. Rizzacasa, *J. Org. Chem.*, 1998, **63**, 8595.
9 R. K. Mann, J. G. Parsons and M. A. Rizzacasa, *J. Chem. Soc., Perkin Trans. 1*, 1998, 1283.
10 D. B. Dess and J. C. Martin, *J. Am. Chem. Soc.*, 1991, **113**, 7277.
11 D. A. Evans, S. W. Kaldor, T. K. Jones, J. Clardy and T. Stout, *J. Am. Chem. Soc.*, 1990, **112**, 7001.
12 W. C. Still and J. H. McDonald, III, *Tetrahedron Lett.*, 1980, **21**, 1031.
13 W. C. Still and J. A. Schneider, *Tetrahedron Lett.*, 1980, **21**, 1035.
14 P. A. Grieco, T. Oguri and Y. Yokoyama, *Tetrahedron Lett.*, 1978, **20**, 419.
15 E. J. Corey, M. Kang, M. C. Desai, A. K. Ghosh and I. N. Houpis, *J. Am. Chem. Soc.*, 1988, **110**, 649.
16 R. W. Gable, B. F. Hoskins, A. Linden, I. A. S. McDonald and R. J. Steen, PROCESS_DATA. A Program for the Processing of CAD-4 Diffractometer Data, The University of Melbourne, Australia, 1993.
17 A. L. Spek, PLATON for windows. A Program for Absorption Corrections, University of Utrecht, The Netherlands, 1999.
18 G. M. Sheldrick, SHELXL-97; A Program for the Refinement of Crystal Structures, University of Göttingen, Germany, 1997.
19 G. M. Sheldrick, SHELXS-86; Crystallographic Computing 3, University of Göttingen, Germany, 1986.
20 L. J. Farrugia, *J. Appl. Crystallogr.*, 1999, **32**, 837.