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Title:
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Date:
2024

Citation:
Rossouw, N. P. & Rizzacasa, M. A. (2024). The cyclobutene diester approach to alkyl citrate natural products. AUSTRALIAN JOURNAL OF CHEMISTRY, 77 (9), https://doi.org/10.1071/CH24088.

Persistent Link:
https://hdl.handle.net/11343/350380

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CORNFORTH REVIEW
https://doi.org/10.1071/CH24088

AUSTRALIAN JOURNAL OF CHEMISTRY

The cyclobutene diester approach to alkyl citrate natural products

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Handling Editor:
Curt Wentrup

Received: 3 July 2024
Accepted: 24 July 2024
Published: 22 August 2024

Cite this: Rossouw NP and Rizzacasa MA (2024) The cyclobutene diester approach to alkyl citrate natural products. *Australian Journal of Chemistry* **77**, CH24088. doi:10.1071/CH24088

ABSTRACT

This review outlines the synthesis of alkyl citrate natural products using cyclobutene diester precursors. The approach is efficient and stereoselective and provides the correct oxidation state of the citrate core of these compounds. The synthesis of a number of alkyl citrates along with some higher oxidised members of this family is detailed.

Keywords: alkyl citrates, cyclobutenes, natural products, oxidation, rearrangement, total synthesis.

The alkyl citrate family of natural products

The alkyl citrate family of natural products are a group of secondary fungal metabolites that share a common citric acid core (Fig. 1). Alkyl citrate natural products contain two contiguous asymmetric centres at the C3 and C4 positions and an alkyl chain, ranging from simple aliphatic chains as found in (−)-CJ-13,982[1] and L-731,120[2] to more highly oxidised compounds such as viridiofungin A[3] and trachyspic acid.[4] Additionally, the C4 carboxylic acid can be incorporated into a lactone or amide as found in citrafungin A[5] and viridiofungin A. Further oxidation of the citrate core at positions C2 and C4 gives compounds such as cinatrins C_1 and C_3[6,7] as well as the zaragozic acid family.[8–12] Alkyl citrates exhibit a broad range of biological properties. This, coupled with their complex and densely functionalised structures has resulted in a number total syntheses of a range of alkyl citrates.[13–15] The most well known are the zaragozic acids, which are potent squalene synthase (SSase) inhibitors. SSase is a key cholesterol biosynthesis enzyme and a target of interest for the treatment of cardiovascular disease resulting from hypercholesteremia. The key challenge in the synthesis of this class of natural product is the highly oxidised triacid moiety in a step and redox economical manner.[16] This review details our efforts towards the synthesis of alkyl citrates using cyclobutene diesters as key intermediates.

Challenges in alkyl citrate natural product total synthesis

The main challenge encumbering the total syntheses of these natural products is the generation of the highly oxidised triacid core with appropriate protecting group strategy. Additionally, the stereoselective installation of the two contiguous asymmetric centres is difficult. A number of strategies have been employed to overcome this, but the oxidative manipulations required increase the number of steps resulting in low quantities of synthetic material for biological testing. An analysis of our earlier synthesis of cinatrins C_1 and C_3 exemplifies some of these issues (Scheme 1).[17] Most steps in the partial sequence shown are protecting group manipulations, non-strategic oxidative or reductive manipulations and few skeletal or strategic bond disconnections. Obviously, it is not possible to do away with non-strategic steps entirely, but we sought a more direct approach to accessing the highly oxidised triacid citrate core using a common approach to access all members of this class of interesting natural products.

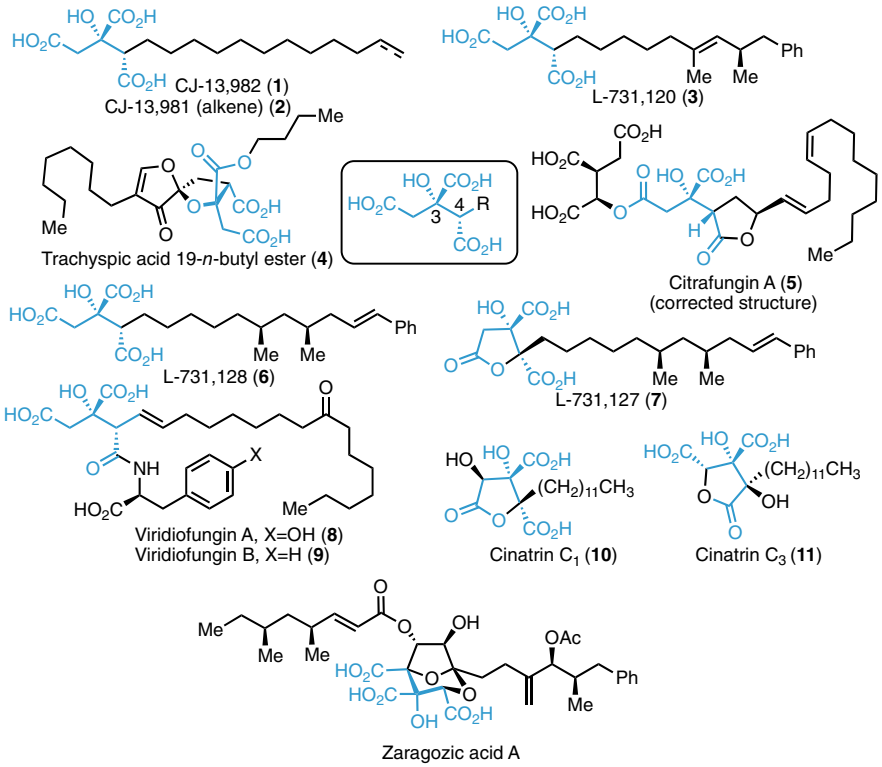
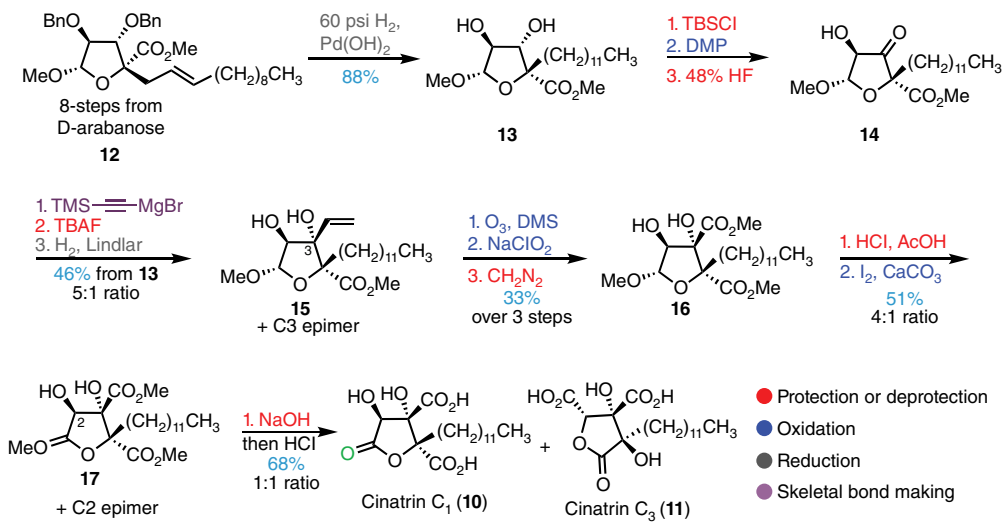
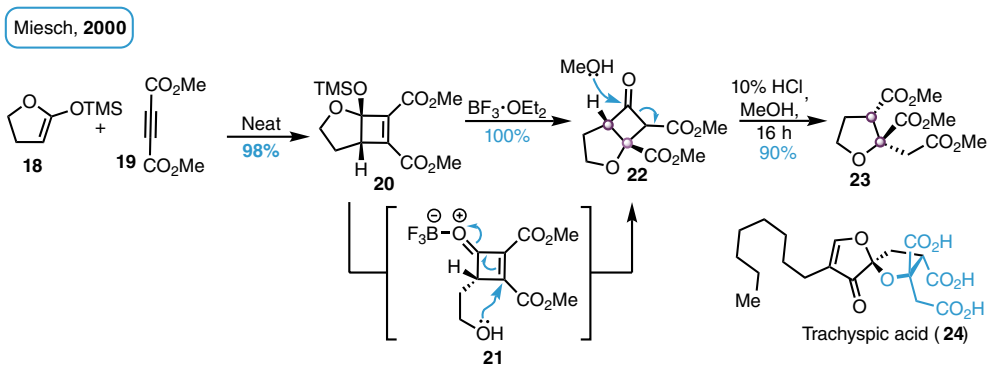


Fig. 1. Alkyl citrate natural products.

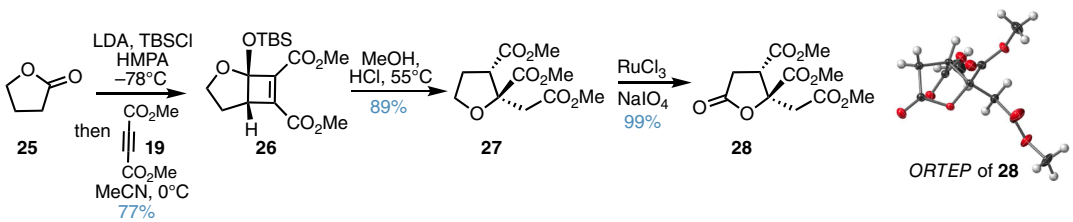


Scheme 1. Previous total synthesis of cinatrins C_1 and C_3 (TBSCl, *tert*-butyldimethylsilyl chloride; DMP, Dess–Martin periodinane; TBAF, tetrabutylammonium fluoride; TMS, trimethylsilane; DMS, dimethylsulfide).

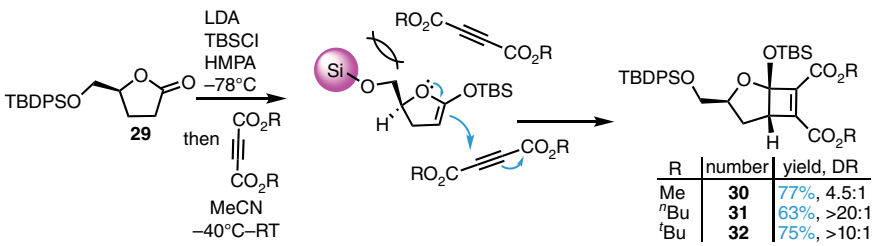
Miesch *et al.*[18] and Miesch and Wendling[19] reported that the cyclic ketene silyl acetal **18** undergoes an uncatalysed formal [2 + 2]-cycloaddition with dimethylacetylene dicarboxylate **19** (DMAD) to furnish the cyclobutene diester **20** in high yield. The resultant adduct then underwent a Lewis-acid-mediated rearrangement to the corresponding cyclobutanone **22** by a mechanism involving an intramolecular oxa–Michael reaction. Subsequent methanolysis of the fused cyclobutanone then afforded the trimethyl ester tetrahydrofuran **23** (Scheme 2), which maps onto the core of trachyspic acid. This sequence would be particularly valuable in providing direct access to the triacid core of the alkyl citrates such as trachyspic acid (**24**) from simple precursors and we wondered whether this cascade could deliver triester **23** directly from the corresponding cyclobutene diester **20**.



Scheme 2. Formal [2 + 2]-cycloaddition and rearrangement of cyclobutene diester **20**.



Scheme 3. Model studies (LDA, lithium diisopropylamide; HMPA, hexamethylphosphoramide).



Scheme 4. Diastereoselective formal [2 + 2] (TBDPS, *tert*-butyldiphenylsilyl; DR, diastereomeric ratio; RT, room temperature).

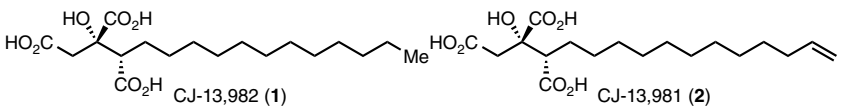


Fig. 2. CJ-13,982 (**1**) and CJ-13,981 (**2**).

A model study[20] found that the more stable *tert*-butyldimethylsilyl (TBS) ketene silyl acetal was superior and this underwent the formal [2 + 2]-cycloaddition in acetonitrile solvent to deliver the diester **26** in good yield. An acid-mediated acetal cleavage, oxa–Michael addition and methanolysis cascade by simple refluxing a solution of **26** in methanolic HCl delivered the triester **27** in one pot. THF oxidation then gave crystalline lactone **28** and X-ray analysis confirmed the relative configuration of the triester core (Scheme 3).

Several formal [2 + 2] cycloadditions were then performed with the chiral ketene silyl acetal derived from optically pure lactone **29** and a range of acetylene diesters (Scheme 4). This gave the adducts **30–32** in good yields with stereoselectivities ranging from 4.5:1 to 20:1. The diastereoselectivity is proposed to arise from top face approach of the Michael acceptor involving the higher energy transition state, thus the addition occurs from the bottom face, which rapidly undergoes ring closure from the zwitterionic intermediate. Cyclobutene diesters **30–32** were then utilised in the syntheses of all the alkyl citrates shown above (Fig. 1).

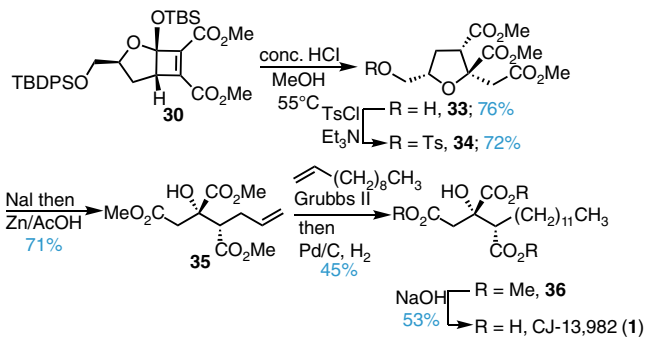
Total synthesis of CJ-13,982 and CJ-13,981

In 2001 researchers at Pfizer Japan isolated CJ-13,982 (**1**) and CJ-13,981(**2**) from an unidentified fungus (CL15036) (Fig. 2).[1]

They are the least complex examples of alkyl citrates, with a simple C2 alkyl chain and the citrate triacid moiety and are fairly potent inhibitors of squalene synthase (SQS).

To date, Calo *et al.*[21] and our group[20,22] are the only ones to have synthesised these compounds. Barrett reported the synthesis of the enantiomer of natural CJ-13,982 and CJ-13,981, which confirmed the absolute configuration of this compound. Both total syntheses required over 20 steps to reach the desired natural products and thus an improved route was investigated by a cyclobutene diester intermediate.

The synthesis began with exposure of cyclobutene diester **30** to methanolic HCl, which induced a silyl acetal cleavage, oxa–Michael, methanolysis cascade to furnish trimethyl ester **33** in excellent yield as a single stereoisomer (Scheme 5).



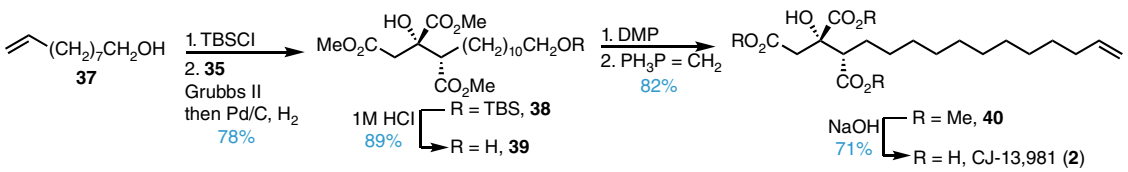
Scheme 5. Total synthesis of CJ-13,982 (**1**) (TsCl, toluenesulfonyl chloride).

Trimethyl ester **33** was converted into the corresponding iodide in good yield over two steps and treatment with zinc powder in acetic acid induced a Boord olefination and gave the citrate triester core **35** in excellent yield over a total of seven steps with no oxidative adjustments required. We have also established a flow chemistry protocol for the synthesis of this key intermediate.[23] Grubbs II catalysed cross metathesis with 1-undecene followed by hydrogenation gave the trimethyl ester **36** and base-promoted hydrolysis furnished the natural product CJ-13,982 (**1**).

The synthesis of CJ-13,981 (**2**) commenced from primary alcohol **37**, which was protected as the TBS silyl ether, and underwent Grubbs II catalysed cross metathesis with triester **35** followed by hydrogenation (Scheme 6). After deprotection, Dess–Martin periodinane (DMP) oxidation and Wittig olefination installed the terminal alkene in good yield. Base-promoted ester hydrolysis gave CJ-13,981 (**2**) in good yield following reverse-phase–high-performance liquid chromatography (RP-HPLC).

Total synthesis of citrafungin A

In 2004 a group at Merck (Rahway) isolated citrafungins A (**5**) and B (**41**) from cow dung-derived sterile mycelia MF6339 (Fig. 3).[5] CJ-15,183 (**42**)[24] is a proposed isoester of citrafungin B; however, the published spectroscopic data are identical, suggesting they are the same compound. Citrafungin A is an inhibitor of the membrane promotor



Scheme 6. Total synthesis of CJ-13,981 (**2**).

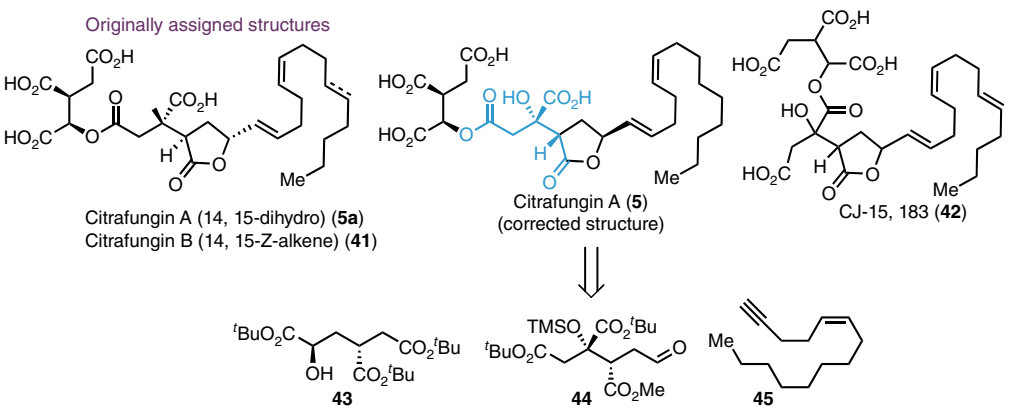
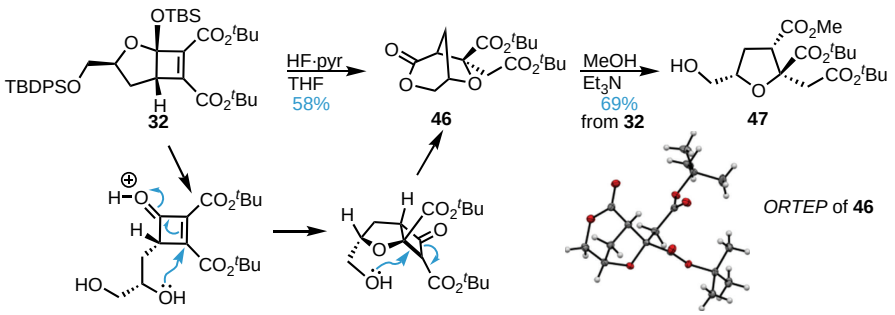
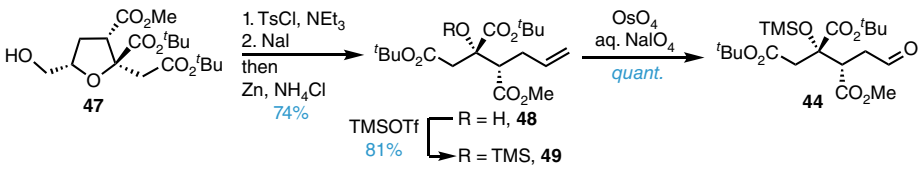


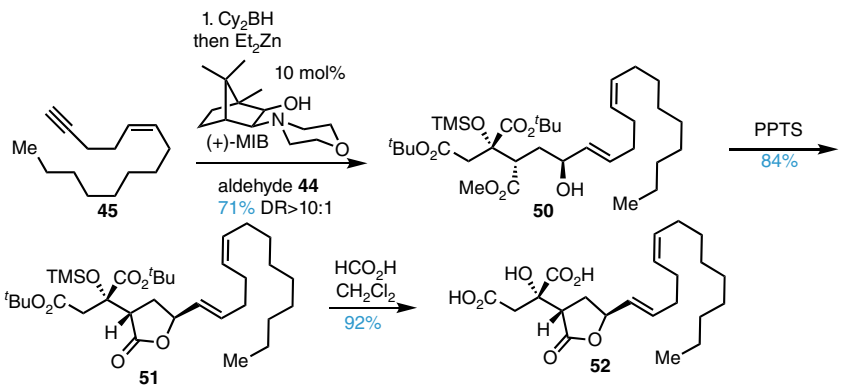
Fig. 3. Structures of the citrafungins and retrosynthesis.



Scheme 7. Acid-mediated rearrangement.



Scheme 8. Synthesis of aldehyde **44**.



Scheme 9. Diastereoselective vinyl zinc addition (PPTS, pyridinium *p*-toluenesulfonate; DR, diastereomeric ratio).

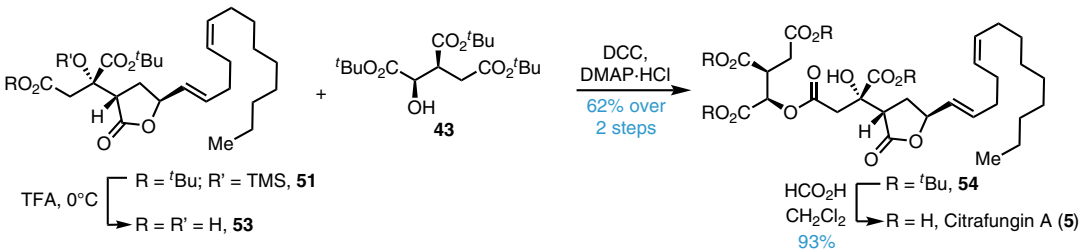
GGTase I and thus exhibits antifungal properties. In the Merck group's chemical degradation studies, a Mosher ester analysis led to an incorrect assignment of the natural absolute configuration. There are two total syntheses by Amer *et al.*[25] and Calo *et al.*[26] and a formal total synthesis[27] of the original incorrect stereoisomer was reported by our group. We then corrected the absolute stereochemistry by the total synthesis of the correct isomer some 15 years later.[28]

Our synthesis began with cyclobutene di-*t*-butyl ester **32**, which, upon treatment with a HF·pyridine complex, furnished bicyclic lactone **46** as a crystalline solid that allowed for X-ray analysis (Scheme 7). The mechanism of this reaction involved desilylation, intramolecular oxa–Michael addition and concomitant cyclobutanone ring opening by the pendant primary alcohol. The intermediate bicyclic lactone **46** proved pivotal for the synthesis of a number of alkyl citrates by allowing for orthogonal protection of the C4 carboxylic acid. Methanolysis under basic conditions afforded THF intermediate **47** in high yield.

Functional group manipulation to the corresponding iodide, followed by Boord olefin synthesis furnished alkene **48** in high yield over three steps (Scheme 8). Protection of the C3 tertiary alcohol as the TMS silyl ether and oxidative cleavage of the terminal alkene yielded aldehyde **44** in good yield.

A (+)-MIB (2-methylisborneol)[29]-mediated diastereoselective vinylzinc (formed from alkyne **45**) addition reaction with aldehyde **44** afforded allylic alcohol **50** in good yield and high diastereoselectivity that was converted into the corresponding lactone **51** in high yield (Scheme 9). Deprotection of **51** with formic acid gave citrafungin A diacid degradation product **52** that had identical spectral and chiroptical properties to the naturally derived material, and this confirmed the absolute configuration of the lactone fragment was enantiomeric to that originally proposed.

Selective C1 *tert*-butyl ester deprotection of **51** was mediated by brief exposure to trifluoroacetic acid (TFA), and the crude mono acid was coupled with the tri-*tert*-butyl isocitrate fragment **43** in good yield (Scheme 10). Exposure to formic acid cleanly afforded citrafungin A in high yield with identical spectroscopic properties and optical rotation to the reported material, confirming the stereochemical reassignment.



Scheme 10. Completion of the Rizzacasa group synthesis of citrafungin A (DCC, *N,N'*-dicyclohexylcarbodiimide; DMAP, 4-dimethylaminopyridine).

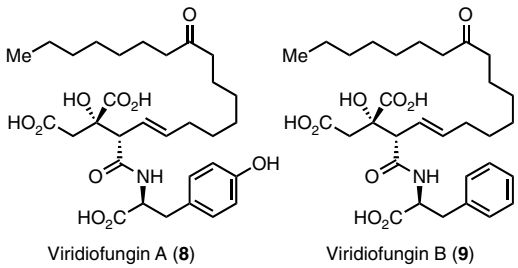


Fig. 4. Viridiofungins A (**8**) and B (**9**).

Total synthesis of viridiofungins A and B

The viridiofungins are a large group of alkyl citrate-type natural products. Viridiofungins A (**8**) and B (**9**) were isolated from *Trichoderma viride* in 1993 by a group at Merck (Rahway) while screening for novel SQS inhibitors (Fig. 4).[3]

They were found to be broad spectrum anti-fungal compounds as well as inhibitors of SQS and serine palmitoyl transferase. Their structures were determined by 1- and 2-D ^{1}H- and ^{13}C-NMR spectroscopy and mass spectrometry fragmentation, which located the amino acid residue on C4. Several syntheses of the viridiofungins and their derivatives have been reported.[30–38]

Our synthesis[39] began with bicyclic lactone **46** (Scheme 11). Ring-opening with allyl alcohol in the presence of Et_3N followed by tosylation gave tosylate **55**. Iodide displacement followed by zinc-mediated reductive elimination gave the corresponding alkene and after allyl ester removal using Wilkinson's catalyst the C4-carboxylic acid **56** was obtained. Intramolecular Steglich reaction gave β-lactone **57** in high yield and the terminal double bond was then isomerised with Grubbs II in refluxing methanol to give the disubstituted alkene **58**, the structure of which was confirmed by X-ray crystallography.

Cross metathesis of lactone **58** with alkene **59** using Grubbs–Hoveyda II in the presence of 2,6-dichloro-1,4-benzoquinone (DCBQ) (to retard undesired alkene isomerisation)[40] proceeded in good yield and stereoselectivity (Scheme 12). Immediate treatment of the intermediate alkene with either the *tert*-butyl ester of tyrosine hydrochloride (**61**) or **62** in the presence of base **60** effected ring opening of the lactone to form the amides **63** and **64** high yield. Formic-acid-mediated global deprotection gave either viridiofungin A (**8**) or B (**9**) in high yield.

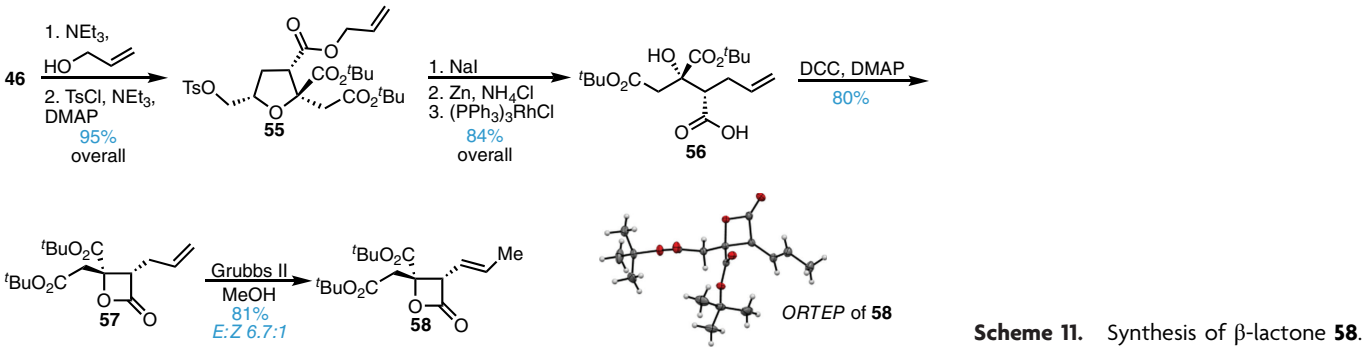
Total synthesis of trachyspic acid 19-*n*-butyl ester

Trachyspic acid (**24**) was isolated in 1995 from the fermentation broth of *Talaromyces treachyspermus* during a search for new heparinase inhibitors (Fig. 5).[4] Later, in 2017 a group at RIKEN in Japan re-isolated trachyspic acid as well as trachyspic acid 19-butyl ester from the uncharacterised fungus RKGS-F2684 while screening for polo-like kinase 1 inhibitors (Plk1).[41]

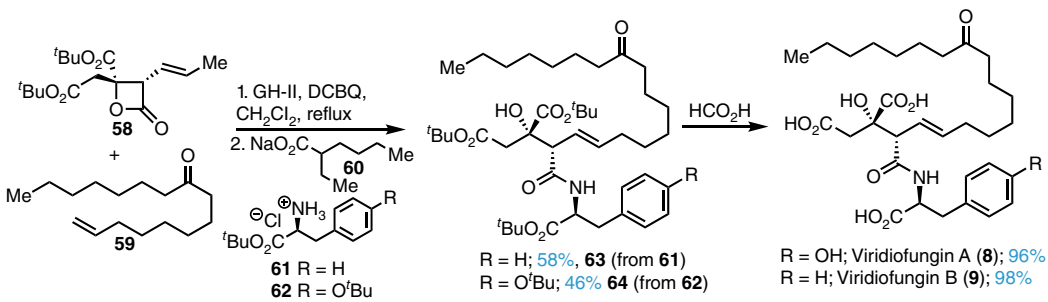
Both trachyspic acid (**24**) and trachyspic acid 19-*n*-butyl ester (**4**) are weak inhibitors of Plk1, whereas trachyspic acid is also active against heparanase, thus both molecules show potential as anti-cancer compounds. The total synthesis of trachyspic acid has been completed by Zammit *et al.*,[42,43] Hirai *et al.*[44] and Morokuma *et al.*,[45] whereas a formal synthesis was completed by Calo *et al.*[46] and the Johnson group has reported a synthesis of trachyspic acid dimethyl ester.[47]

Our total synthesis[48] of trachyspic acid 19-*n*-butyl ester began with di-*n*-butyl cyclobutene diester **31**, which underwent the HF·pyridine-mediated rearrangement to give the corresponding bicyclic lactone (Scheme 13). Lactone ring-opening with allyl alcohol and tosylation gave intermediate **65**. Functional group manipulation to the iodide, followed by zinc-mediated reductive elimination gave alkene **66** in high yield. Base-promoted cleavage of the C1 *n*-butyl ester, palladium-mediated allyl ester cleavage and reprotection as the di-*tert*-butyl ester gave the orthogonally protected di-*tert*-butyl-mono-*n*-butyl ester **67**. Trimethylsilane (TMS) protection of the C3 hydroxy group and oxidative cleavage of the terminal double bond gave aldehyde **69** in high yield.

Synthesis of the sidechain began with Markovnikov iodoboration alkyne **70**, and protodeborylation gave vinyl iodide **71** (Scheme 14). Fluoride-mediated desilylation, DMP oxidation and acetalisation with ethylene glycol then gave protected aldehyde **72**. Kishi–Nozaki coupling with aldehyde **69** and Swern oxidation afforded enone **73** in good yield as a mixture of diastereoisomers. Acidic cleavage of the ketal and TMS ether caused spiroacetal formation and following acetate formation, ozonolysis and base-promoted



Scheme 11. Synthesis of β-lactone **58**.



Scheme 12. Total synthesis of viridiofungins A (**8**) and B (**9**) (GH-II, Grubbs–-Hoveyda II catalyst).

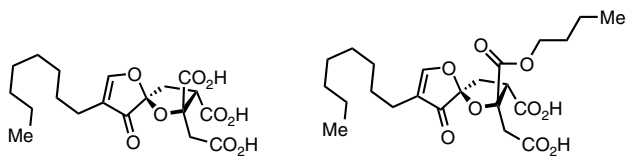


Fig. 5. Trachyspic acid (**24**) and trachyspic acid 19-butyl ester (**4**).

elimination, **74** was obtained in high yield and in good diastereoselectivity for the desired spiroisomer. Exposure to TFA cleaved the *tert*-butyl esters to give the trachyspic acid 19-*n*-butyl ester in good yield.

Total synthesis of L-731,120, L-731,128 and L-731,127

In 1995, Harris *et al*. at Merck research laboratories reported three new alkyl citrates, L-731,120 (**3**), L-731,128 (**6**) and L-731,127 (**7**) (Fig. 6).[2]

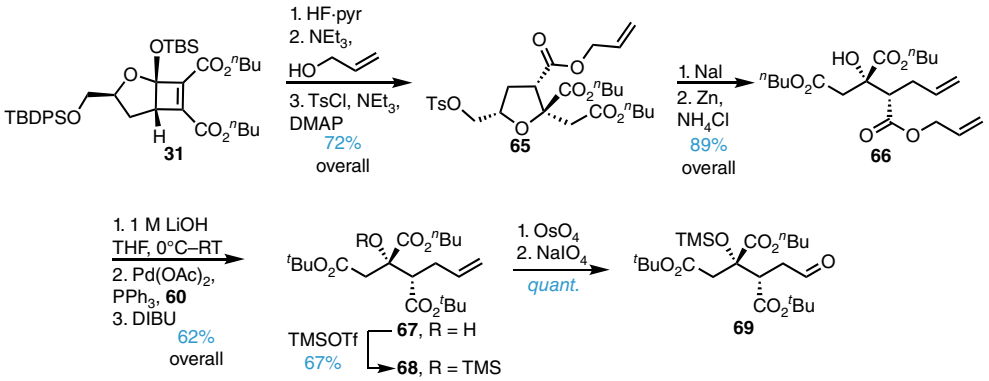
Both **6** and **7** are from the fermentation broth of *Sporormiella intermedia* and **3** was isolated from the broth of *Phoma* sp. MF5453. L-731,120 and L-731,128 are also inhibitors of SQS but no half maximal inhibitory concentration (IC_{50}) value was quoted for L-731,127. To date, the total synthesis of these compounds has only been reported by our group.[20,49]

The synthesis of the alkene sidechain for L-731,120 began with known alcohol **75**,[50] which was converted into the corresponding aldehyde by DMP oxidation. Grignard addition of isopropenylmagnesium bromide gave secondary alcohol **76** as a mixture of diastereomers and subsequent Johnson–Claisen rearrangement gave the methyl ester **77**. Ester reduction, oxidation and Wittig homologation then gave alkene **78** in good yield over three steps (Scheme 15).

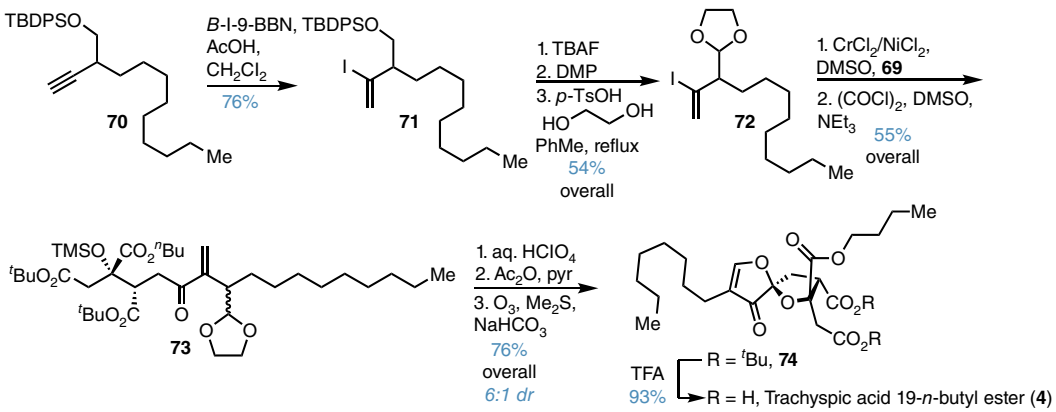
Cross metathesis between the trimethyl citrate **35** and alkene **78** gave a mixture of alkene isomers and regioselective alkene reduction using a modified Myers diimide reduction[51] gave the trisubstituted alkene (Scheme 16). Methyl ester deprotection then afforded the desired natural product L-731,120 (**3**) and this confirmed the absolute configuration.

Since the stereochemistry of the sidechain for L-731,128 was unknown, we synthesised both *syn*- and *anti*- diastereomers. This began with a Myers diastereoselective alkylation[52] of propionamide **80** with iodide **81**, which afforded the syn-alkylated product **82** in excellent yield and diastereomeric ratio (DR) (Scheme 17). Reductive removal of the chiral auxiliary and conversion into the tosylate followed by cuprate displacement with allyl magnesium bromide gave alkene **83** in high yield over three steps. The *anti*-stereodiad **84** was also synthesised in good yield from *ent*-**80** utilising a similar sequence.

Cross metathesis of tri-*tert*-butyl ester **85** and alkene **83** followed by alkene reduction and benzyl ester hydrogenolysis gave the diol **86** (Scheme 18). Installation of the styrene moiety was achieved by primary alcohol oxidation followed by Horner–Wadsworth–Emmons (HWE) reaction using the anion derived from phosphonate **87**. Formic-



Scheme 13. Synthesis of aldehyde **69** (DIBU, diisopropyl-*O*-*tert*-butyisourea; RT, room temperature).



Scheme 14. Total synthesis of trachyspic acid 19-*n*-butyl ester (*B*-I-9-BBN, 9-iodo-9-borabicyclo[3.3.1] nonane; DMSO, dimethyl sulfoxide).

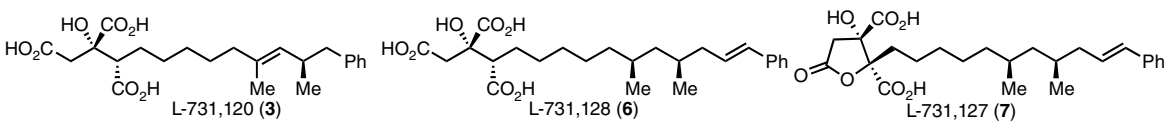
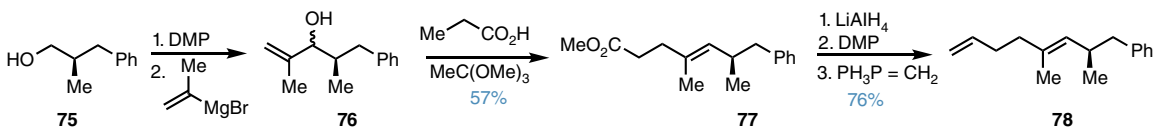
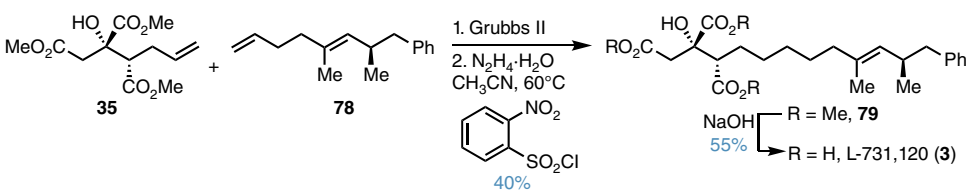


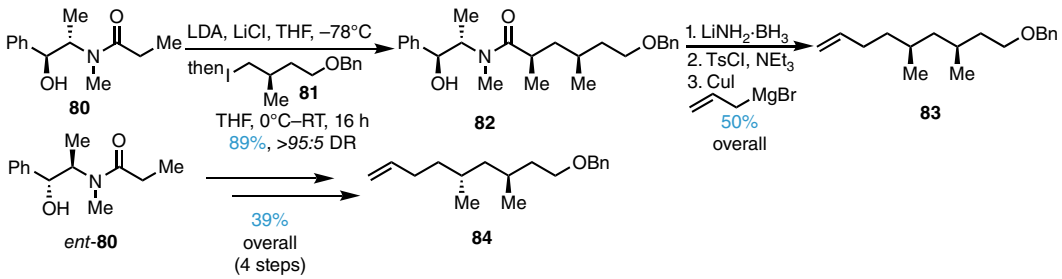
Fig. 6. L-731,120 (**3**), L-731,128 (**6**) and L-731,127 (**7**).



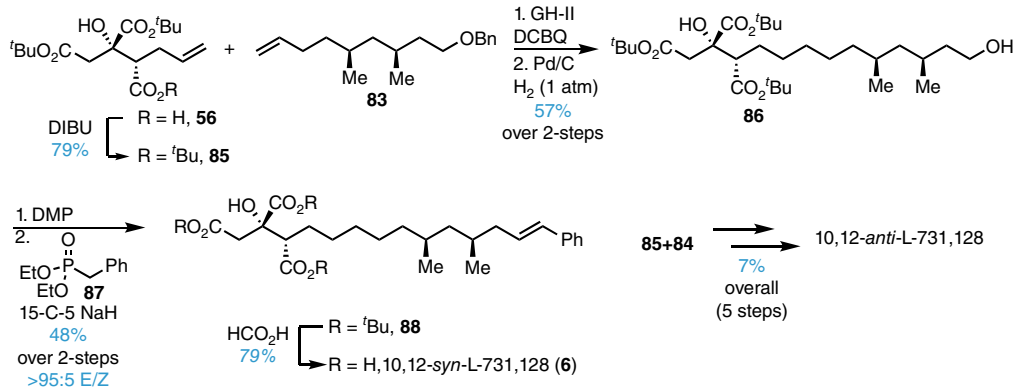
Scheme 15. Synthesis of alkene **78**.



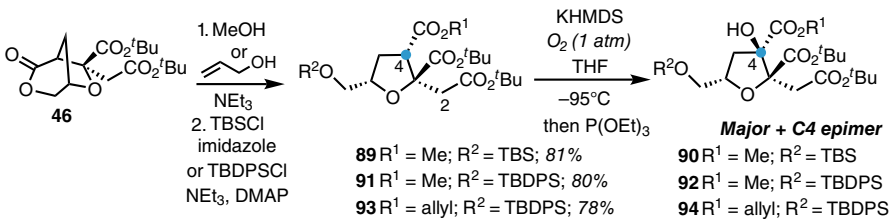
Scheme 16. Total synthesis of L-731,120.



Scheme 17. Synthesis of alkenes **83** and **84** (DR, diastereomeric ratio; RT, room temperature).



Scheme 18. Total synthesis of L-731,128.



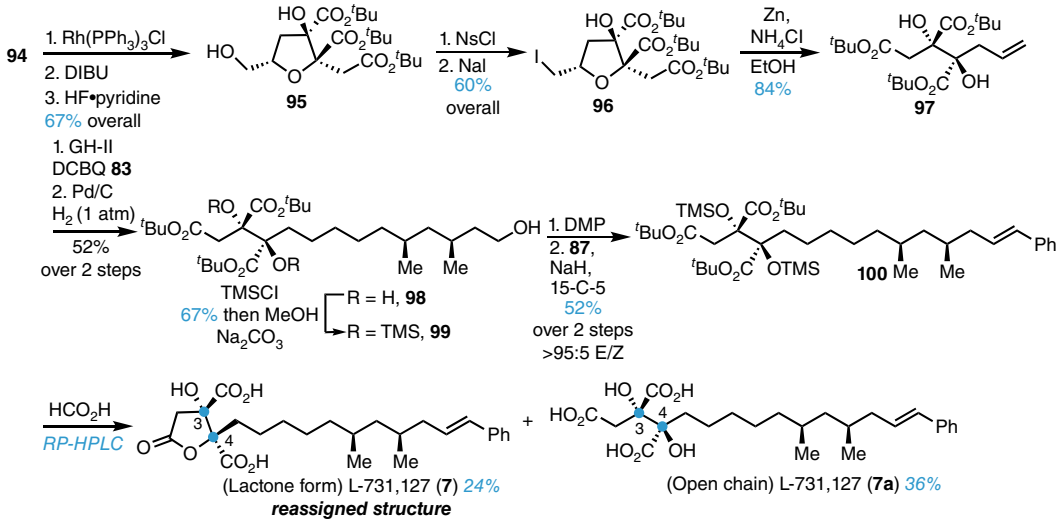
Entry	Substrate	Additive	Yield	DR
1	**89**	-	60%	1.2: 1
2	**91**	-	56%	2.3:1
3	**91**	18-crown-6	82%	2.3:1
4	**93**	-	72%	3:1

Scheme 19. Regioselective direct oxidation of THF intermediates. DR, diastereomeric ratio.

acid-mediated deprotection gave 10,12-*syn*-L-731,128 (**6**) that possessed identical spectroscopic data to that reported for the natural compound. The synthesis was repeated with *anti*-alkene **84** and the final 10,12-*anti*-L-731,128 showed significant deviations in the spectroscopic data, thus confirming the stereochemistry as 10,12-*syn*.

With the stereochemistry of L-731,128 determined, the synthesis of the C4 oxidised congener L-731,127 commenced with bicyclic lactone **46** (Scheme 19). Conversion into the corresponding methyl or allyl ester and protection with either a TBS or TBDPS silyl ether gave substrates **89**, **90** and **91**. Treatment of the silyl ethers with potassium hexamethyldisilazide (KHMDS) at −95°C under an oxygen atmosphere resulted in formation of the C4 tertiary alcohol in good yields with some diastereoselectivity. Improvements in yield were observed with the addition 18-crown-6 (Scheme 19). Remarkably, this oxidation was completely regioselective, with no C2 oxidation observed. This reaction enabled access to higher oxidised members of the alkyl citrates, such as L-731,127.

Conversion of **94** into the corresponding tri-*tert*-butyl ester and silyl deprotection gave diol **95** in good yield (Scheme 20). Functional group manipulation to the iodide **96** and reductive elimination gave tri-*tert*-butyl ester **97** in good yield over three steps. Cross metathesis of triester **95** and alkene **83** followed by concomitant alkene reduction and benzyl ether hydrogenolysis gave triol **98**. Exhaustive TMS silylation and primary deprotection gave alcohol **99**,



Scheme 20. Total synthesis of L-731,127.

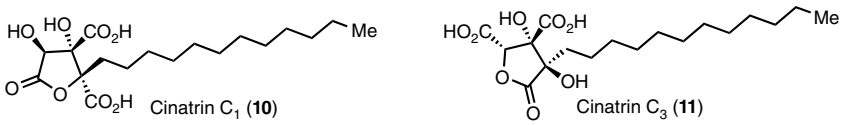
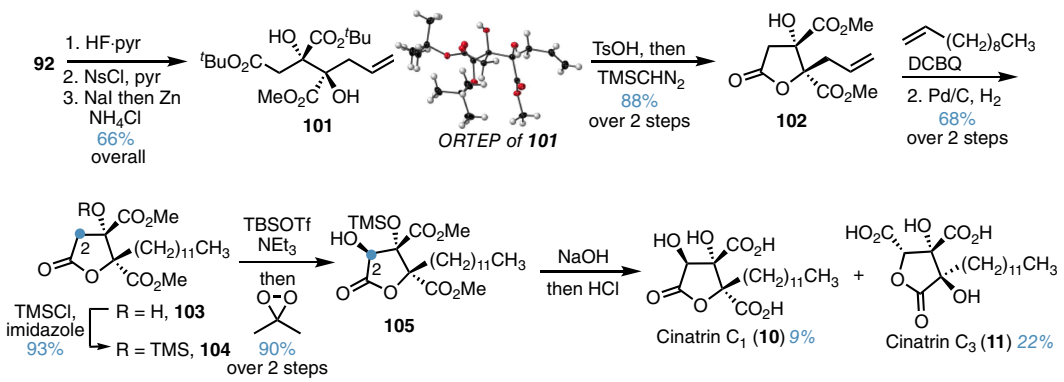


Fig. 7. Cinatrins C_1 (**10**) and C_3 (**11**).



Scheme 21. Total synthesis of cinatrins C_1 and C_3 (NsCl, nitrobenzenesulfonyl chloride).

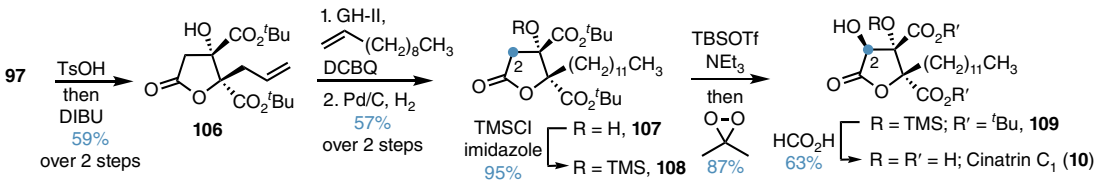
which was homologated to the corresponding styrene in a manner similar to that described for L-731-128 above. Formic-acid-mediated deprotection and purification by RP-HPLC gave both the open chain triacid **7a**, which was the assigned structure for L-731,127, as well lactone **7**. The data for lactone **7** matched more closely to the partial data reported L-731,127 and thus the structure was reassigned as **7**.

Total synthesis of cinatrins C_1 and C_3

The cinatrin family of alkyl citrates were first isolated in 1992 during screening for phospholipase A2 (PLA_2) inhibitors (Fig. 7).[6,7]

The cinatrins were isolated from the fermentation broth of *Circinotrichum falcatisporum* and were found to be weak inhibitors of PLA_2. The first total synthesis of cinatrins C_1 and C_3 was reported by the Evans group[53] and this confirmed their structures as enantiomeric to those originally proposed. The Rizzacasa group also completed the total synthesis of cinatrins C_1, C_3[17,49] and B,[54] whereas the Hatakeyama group completed the synthesis of C_1[55] and the Yukura group completed the synthesis of C3-*epi*-dimethyl cinatrin C_1.[56]

Our synthesis proceeded with deprotection of alcohol **92**, conversion into the iodide and reductive elimination gave crystalline alkene **101**, which confirmed the stereochemical outcome of the oxidation (Scheme 21). Acid catalysed



Scheme 22. Selective synthesis of cinatrin C_1.

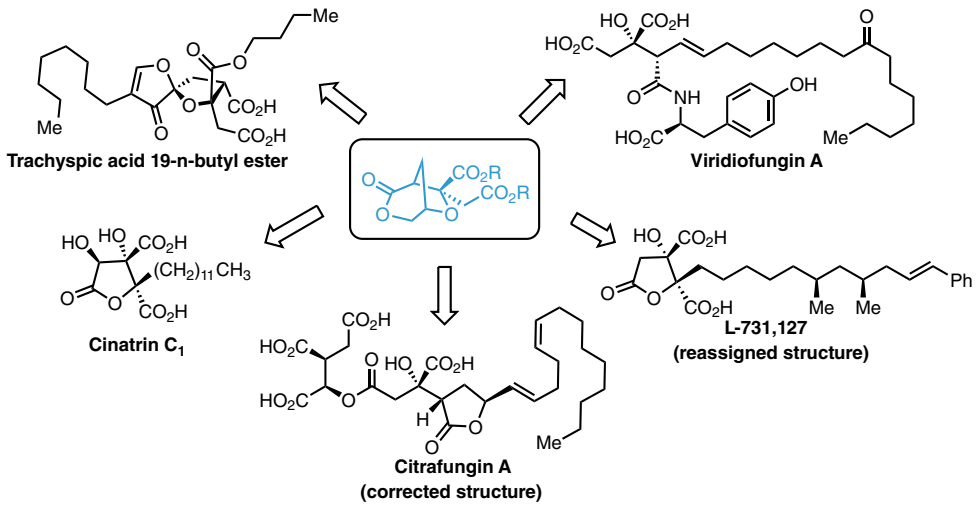


Fig. 8. Synthesis of alkyl citrates from a bicyclic lactone.

lactonisation of alkene **101** and reprotection afforded lactone **102**, which underwent cross metathesis with 1-undecene and reduction installed the chain to give lactone **103**. Protection of the C3 tertiary alcohol and formation of the corresponding silyl ketene acetal under soft enolisation conditions enabled efficient and diastereoselective Rubottom type oxidation at the C2 position giving **105** in high yield. Subsequent base-mediated ester hydrolysis and acidification gave cinatrins C_1 and C_3 in moderate yield after RP-HPLC.

A more direct route to cinatrin C_1 was undertaken, and this began with tri-*tert*-butyl ester **97** (Scheme 22). Lactone formation followed by cross metathesis and reduction using a similar sequence to that described above gave di-*tert*-butyl-lactone **106** in good yield. C2 oxidation proceeded in high yield and DR to give **109**, and subsequent formic-acid-mediated deprotection gave cinatrin C_1 in high yield.

Conclusion

Cyclobutene diesters serve as useful precursors for the synthesis of the core of the alkyl citrate natural products. These are easily accessed from a simple silyl ketene acetal and various acetylene diesters by formal [2 + 2]-cycloadditions. More specifically, the derived bicyclic lactones are key intermediates for the synthesis of a large number of these natural products including the higher oxidised metabolites (Fig. 8). This robust methodology can provide the citrate core with no further oxidative manipulations required and, in essence, access to the entire family of these interesting natural products.

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Data availability. Data sharing is not applicable as no new data were generated or analysed during this study.

Conflicts of interest. The authors declare that they have no conflicts of interest.

Declaration of funding. The authors thank the Australian Research Council Discovery Grants Scheme for funding (DP DP200100722 to M. A. Rizzacasa).

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