Accepted Manuscript

Relative stereochemical assignment of C-33 and C-35 in the antibiotic gladiolin

Christopher Perry, Jacob R. Sargeant, Lijiang Song, Gregory L. Challis

PII: S0040-4020(18)30775-0

DOI: 10.1016/j.tet.2018.06.060

Reference: TET 29656

To appear in: *Tetrahedron*

Received Date: 7 March 2018

Revised Date: 25 June 2018

Accepted Date: 26 June 2018

Please cite this article as: Perry C, Sargeant JR, Song L, Challis GL, Relative stereochemical assignment of C-33 and C-35 in the antibiotic gladiolin, *Tetrahedron* (2018), doi: 10.1016/j.tet.2018.06.060.

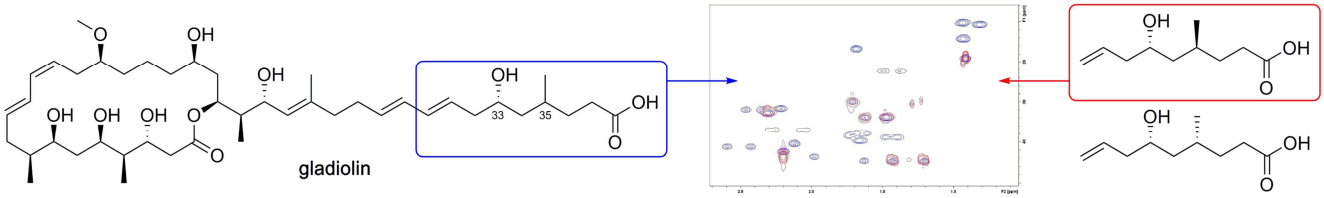
Graphical Abstract

To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

Relative stereochemical assignment of C-33 and C-35 in the antibiotic gladiolin

Leave this area blank for abstract info.

Christopher Perry, Jacob R. Sargeant, Lijiang Song and Gregory L. Challis
Department of Chemistry and Warwick Integrative Synthetic Biology Centre, University of Warwick, UK
Department of Biochemistry and Molecular Biology, Monash University, Australia



Tetrahedron
journal homepage: www.elsevier.com

Relative stereochemical assignment of C-33 and C-35 in the antibiotic gladiolin

Christopher Perry[a], Jacob R. Sargeant[a], Lijiang Song[a], and Gregory L. Challis[a, b, c *]

[a] *Department of Chemistry, University of Warwick, Coventry CV4 7AL, UK*
[b] *Warwick Integrative Synthetic Biology Centre, University of Warwick, Coventry CV4 7AL, UK*
[c] *Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria 3800, Australia*

ARTICLE INFO

Article history:
Received
Received in revised form
Accepted
Available online

Keywords:
Antibiotic
Stereochemistry
Asymmetric allylation
Polyketide
NMR spectroscopy

ABSTRACT

Gladiolin is a macrolide antibiotic isolated from *Burkholderia gladioli* BCC0238 with promising activity against *Mycobacterium tuberculosis*, including several multidrug resistant strains. The configuration of all but one of the stereogenic centers of gladiolin has previously been elucidated using a combination of NOESY NMR experiments and predictive sequence analysis of the polyketide synthase responsible for its assembly. However, it was not possible to assign the configuration of the C-35 methyl group using such methods. Here we report the synthesis of C-33/C-35-*syn* and C-33/C-35-*anti* mimics of the C-30 to C-38 fragment of gladiolin from (*R*) and (*S*)-citronellol, respectively. Comparison of HSQC NMR data for the mimics and the natural product showed that the C-35 methyl is *anti* to the C-33 hydroxyl group, indicating that gladiolin has the 35*S* configuration.

* Corresponding author. Tel.: +44-247-657-4024; fax: +44-247-652-4112; e-mail: g.l.challis@warwick.ac.uk, gregory.challis@monash.edu

1. Introduction

The emergence of wide-spread antimicrobial resistanace in pathogenic microoganisms represents a major global health threat.[1] To combat this, new antimicrobials that overcome resistance to current drugs are urgently needed. Most antimicrobials in clinical use are natural products originating from Gram-positive Actinobacteria and filamentous fungi, or semi-synthetic derivatives.[2] Screening such organisms for antimicrobial activity frequently results in the rediscovery of known compounds. In recent years, Gram-negative bacteria have become increasingly recognised as underexplored producers of antibiotics and other bioactive natural products.[2] We are pursuing a programme of natural product discovery in the *Burkholderia cepacia* complex (BCC),[3] a group of opportunisitic Gram-negative pathogens that freqeuntly infect the lungs of cystic fibrosis patients.

In 2017, we reported the discovery of the novel macrolide antibiotic gladiolin **1** from *Burkholderia gladioli* BCC0238, a clinical isolate from the lung of a boy suffering from cyctic fibrosis (Figure 1).[4] Gladiolin **1** has promising activity against isoniazid and rifampicin resistant clinical isolates of *Mycobacterium tuberculosis*.[4] It inhibits RNA polymerase,[4] the same target as rifampicin, which is widely used for the treatment of tuberculosis. Rifampicin is becoming increasingly ineffective due to resistance-conferring mutations in RNA polymerase. However, the binding sites for rifampicin and gladiolin **1** appear to be distinct, because mutations in RNA polymerase that confer rifampicin resistance do not appear to significantly affect the activity of gladiolin.[4] Moreover, gladiolin **1** shows low activity towards mamalian cell lines and no toxicity was observed in a *Galleria* wax moth model.[4]

The macrolide core of gladiolin **1** is similar to that of etnangien **2** (Figure 1), an antibiotic isolated from *Sorangium cellulosum* that is also active against Mycobacteria.[5] However, the structures of the C-21 side chains of gladiolin **1** and etnangien **2** differ markedly. The C-24 to C-35 portion of the etangien **2** side chain contains a conjugated hexaene that is highly unstable.[5] The corresponding C-24 to C-31 region of the gladiolin **1** side chain does not suffer such stability problems due to its shorter length and higher degree of saturation.[4]

The relative stereochemistry of the C-1 to C-31 portion of gladiolin **1** was initially assigned by NMR spectoscopy using a combination of NOESY and ^{1}H coupling constant data.[4] Sequencing of the *B. gladioli* BCC0238 genome led to identification of the *trans*-acyltransferase modular polyketide synthase (PKS) responsible for gladiolin **1** assembly.[4] Sequence analysis of the ketoreductase (KR) domains in this PKS allowed the absolute configurations of the stereogenic centres in the C-1 to C-33 portion of gladiolin **1** to be predicted.[4] These predictions independently confirmed the NMR-based relative stereochemical assignment of the C-1 to C-31 portion of gladiolin **1**. However, the configuration of the C-35 stereogenic centre in gladiolin **1**, which appears to arise from the action of an enoyl reductase domain in the PKS of undetermined stereospecifity,[4] remains to be elucidated.

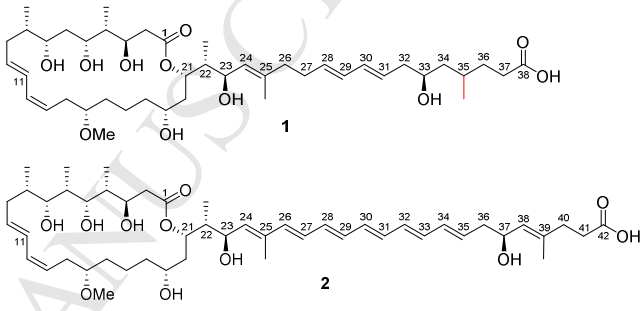
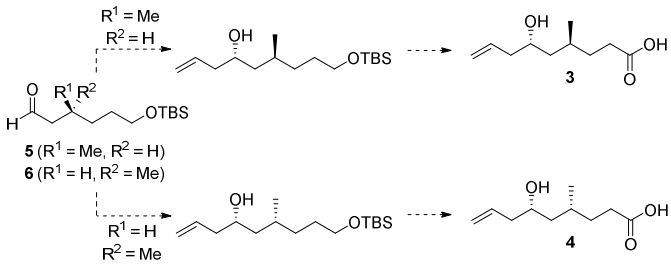


Figure 1: Structures of gladiolin **1** and etnangien **2**, isolated from the Gram-negative bacteria *B. gladioli* and *S. cellulosum*, respectively.

Here we report stereoselective synthesis of *syn*- and *anti*-configured mimics of the C-30 to C-38 portion of the gladiolin C-21 side chain. Comparison of HSQC NMR data for the mimics and the natural product allowed the C-35 stereochemistry of gladiolin **1** to be assigned.

2. Results and discussion

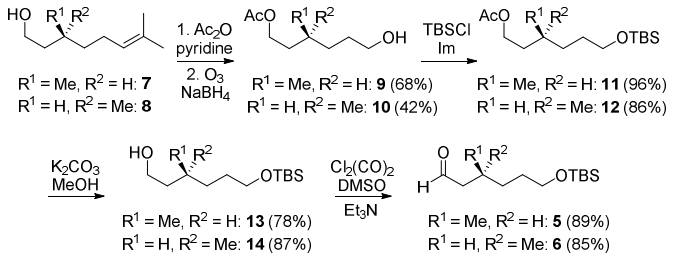
We envisaged synthesising the C-30 to C-38 mimics **3** and **4** of the gladiolin C-21 side chain (with *anti* and *syn* relative configurations) via catalytic asymmetric allylation of the 3*S* and 3*R* isomers of *tert*-butyldimethylsilyl (TBS)-protected 3-methyl-6-hydroxyhexanal, **5** and **6**, respectively (Scheme 1).



Scheme 1: Route envisaged for synthesis of the C-30 to C-38 mimics **3** (*anti*) and **4** (*syn*) of the gladiolin C-21 side chain via asymmetric allylation of **5** / **6**.

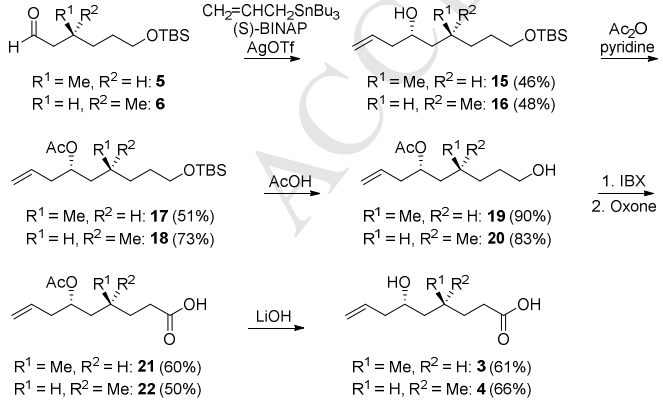
Burke *et al.* have reported a five step synthesis of aldehyde **6** from *(R)*-citronellol **8** and we postulated

that aldehyde **5** could be prepared from *(S)*-citronellol **7** via the same route.[6] Thus, acetylation of *(S)*-citronellol **7**, followed by ozonolytic cleavage employing a reductive work up gave alcohol **9**, which was converted to the corresponding TBS ether **11** (Scheme 2). Based-catalysed transesterification removed the acetyl group from **11** and the resulting alcohol **13** was oxidised to the corresponding aldehyde **5** (Scheme 2). Starting from *(R)*-citronellol **8**, aldehyde **6** was prepared using analogous procedures via intermediates **10**, **12** and **14** (Scheme 2).[6]



Scheme 2: Synthesis of aldehydes **5** and **6** from (*S*)-citronellol **7** and (*R*)-citronellol **8**, respectively, following the route reported by Burke *et al.*[6]

Reaction of aldehyde **5** with allytributyltin in the presence of a catalytic (*S*)-BINAP.Ag(I) complex, under the conditions reported by Yamamoto and coworkers,[7] yielded allylic alcohol **15** as a 9:1 mixture of diasteromers (Scheme 3). To confirm the absolute configuration of the newly created stereogenic centre in the major diastereomer, **15** was separately coupled with *(S)* and *(R)*-α-methoxy-trifluorophenylacetic acid.[8] Analysis of the resulting Mosher's esters using ^{1}H NMR spectroscopy confirmed that this stereogenic centre is *S*-configured (see supplementary material). Acetylation of **15** gave **17**, which was deprotected to yield **19**. Oxidation of this alcohol gave the corresponding carboxylic acid **21**, which was saponified to give **3** (Scheme 3).



Scheme 3: Asymmetric allylation of aldehydes **5** and **6** and converison of the products to C-30 to C-38 mimics **3** and **4** of the gladiolin C-21 side chain.

Reaction of aldehyde **6** with allytributyltin in the presence of a catalytic (*S*)-BINAP.Ag(I) complex gave allylic alcohol **16**, also as a 9:1 mixture of diastereomers (Scheme 3). Mosher's ester analysis confirmed that the the newly created stereogenic centre in the major diastereomer of **16** is also *S*-configured (see supplementary material). Elaboration of **16** to **4**, via intermediates **18**, **20** and **22**, was accomplished using the same sequence of reactions as that employed for the conversion of **15** to **3** (Scheme 3).

The signals due to C-34, C-35, C-36, C-37 and the C-35 methyl group, and their associated hydrogen atoms, in the HSQC NMR spectrum of gladiolin **1** all have essentially identical chemical shifts to the corresponding signals in synthetic C-30 to C-38 mimic **3** (Figure 1). In contrast, the signals in the HSQC spectrum of gladiolin due to C-34, C-35, C-36 and the C-35 methyl group, and their associated hydrogen atoms, are all significantly shifted relative to the *syn*-configured mimic **4** (Figure 1). We thus assign the *anti* relative configuration to the C-33 hydroxyl group and the C-35 methyl group of gladiolin.

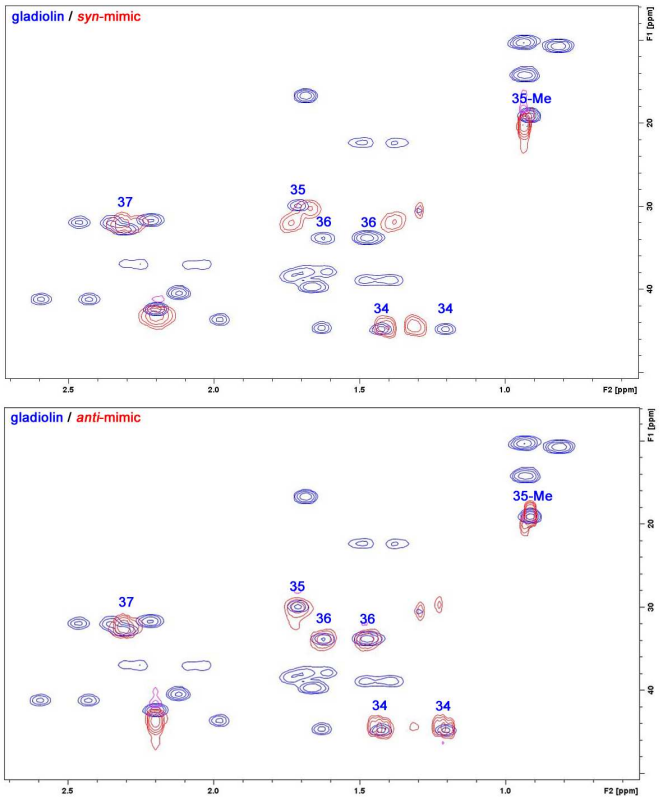


Figure 1: Comparison of HSQC spectra for gladiolin **1** (blue) with *syn*-configured C-30 to C-38 mimic **4** (red, top panel) and *anti*-configured C-30 to C-38 mimic **3** (red, bottom panel). Relevant signals due to carbon atoms of gladiolin and their associated hydrogen atoms are labelled with blue numbers.

3. Conclusion

Stereocontrolled syntheses of *syn* and *anti*-configured mimics of the C-30 to C-38 fragment of gladiolin have been accomplished in 11 steps from (*R*)- and (*S*)-citronellol, respectively. Comparison of NMR spectroscopic data for the synthetic

mimics and gladiolin led us to conclude that the C-33 hydroxyl and C-35 methyl groups have the *anti* relative configuration in the natural product. C-33 of gladiolin has previously been assigned the *R* absolute configuration on the basis of the predicted stereospecificity of the KR domain in module 2 of the PKS responsible for its assembly. We thus propose that C-35 of gladiolin is *S*-configured, implying that the enoyl reductase domain responsible for creation of this stereogenic centre (in module 1 of the PKS) delivers hydride from NAD(P)H to the *re* face of its β-methyl-α, β-unsaturated thioester substrate.

4. Experimental section

*4.1 (S)-6-hydroxy-3-methylhexyl acetate **9***

To (*S*)-citronellol **7** (5.0 g, 32.00 mmol) in dichloromethane (DCM) (30 mL) was added pyridine (3.36 mL, 42.00 mmol) under an argon atmosphere. The reaction mixture was cooled to -10 °C. Acetic anhydride (3.62 mL, 38.00 mmol) was added drop-wise and the solution was left to warm to room temperature over 16 hours. The reaction mixture was diluted with DCM, and washed with $NaHCO_3$ (10 mL), 1 M HCl (10 mL) and brine (10 mL). The organics were dried ($MgSO_4$) and concentrated *in vacuo* and the resulting product was dissolved in DCM (20 mL) then cooled to -78 °C. Ozone was bubbled through the resulting solution until it displayed a blue colour. Compressed air was then bubbled through the solution until the blue colour disappeared. The solution was warmed to 0 °C and diluted methanol (40 mL). Sodium borohydride (4.77 g, 0.126 mol) was added cautiously in small portions and the mixture was left to stir for 90 minutes. DCM and water was then added, the organic layer was separated, then washed with brine (20 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by flash column chromatography (ethyl acetate: diethyl ether, 2:1) to give **9** as a colourless oil (3.80 g, 68%). (*R*)-6-hydroxy-3-methylhexyl acetate **10** (1.40 g, 42%) was synthesized from *(R)*-citronellol **8** using the same procedure.

^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.15-4.05 (m, 2H, C$\underline{H}$CO(CH_3)), 3.63 (t, J = 6.5 Hz, 2H, C$\underline{H}_2$OH), 2.04 (s, 3H, COC$\underline{H}_3$), 1.70-1.36 and 1.25-1.17 (m, 7H, C$\underline{H}_2$C$\underline{H}$(CH_3)C$\underline{H}_2$C$\underline{H}_2$), 0.93 (d, J = 6.5 Hz, 3H, CH_2CH(C$\underline{H}_3$)); HRMS (ESI^+): Found 197.1147 for **9** and 197.1149 for **10**, $C_9H_{18}O_3(Na)^+$ requires 197.1148. These data match those reported previously for (*R*)-6-hydroxy-3-methylhexyl acetate **10**.[6]

*4.2 (S)-6-((tert-butyldimethylsilyl)oxy)-3-methylhexyl acetate **11***

To **9** (3.80 g, 22.00 mmol) in dimethylformamide (20 mL) was added imidazole (2.23 g, 33.00 mmol) and the resulting mixture was stirred for 10 minutes at room temperature. *Tert*-butyldimethylsilyl chloride (3.95 g, 26.00 mmol) was added in one portion and stirring was continued at room temperature overnight. Diethyl ether (20 mL) and water (20 mL) were added. The organics were separated and washed with water (20 mL), brine (20 mL), then dried ($MgSO_4$) and concentrated *in vacuo*. The crude mixture was purified by flash column chromatography (diethyl ether: petroleum ether, 1:9) to give **11** as a colourless oil (6.10 g, 96*%).* (*R*)-6-((tert-butyldimethylsilyl)oxy)-3-methyl-hexyl acetate **12** (1.98 g, 86%) was synthesized from **10** using the same procedure.

^{1}H NMR (500 MHz, $CDCl_3$) δ: 4.13-4.05 (m, 2H, C$\underline{H}_2$COCH_3), 3.59 (t, J = 6.5 Hz, 2H, C$\underline{H}_2$OSi), 2.04 (s, 3H, COC$\underline{H}_3$), 1.69-1.10 (m, 7H, C$\underline{H}_2$C$\underline{H}$(CH_3)C$\underline{H}_2$C$\underline{H}_2$), 0.91 (d, J = 6.5 Hz, 3H, CH(C$\underline{H}_3$), 0.89 (s, 9H, OSi(CH3)$_3$C(C$\underline{H}_3$)$_3$), 0.04 (s, 6H OSi(C$\underline{H}_3$)$_2$C(CH_3)$_3$); HRMS (ESI^+): Found 311.2020 for **11** and 311.2016 for **12**, $C_{15}H_{32}O_3Si(Na)^+$ requires 311.2013. These data match those reported previously for (*R*)-6-((*tert*-butyldimethyl-silyl)oxy)-3-methyl-hexyl acetate **12**.[6]

*4.3 (S)-6-((tert-butyldimethylsilyl)oxy)-3-methylhexan-1-ol **13***

A mixture of **11** (6.10 g, 21 mmol) and potassium carbonate (250 mg) in methanol (50 mL) was stirred at room temperature for 24 hours. The methanol was removed *in vacuo* and the resulting residue was partitioned between ethyl acetate and water. The organics were separated, dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by flash column chromatography (petroleum ether: diethyl ether, 9:1) to give **13** as a colourless oil (4.04 g, 78%). (*R*)-6-((*tert*-butyldimethylsilyl)oxy)-3-methylhexan-1-ol **14** (1.48 g, 87%) was synthesized from **12** using the same procedure.

^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.73-3.64 (m, 2H, C$\underline{H}_2$OH), 3.59 (t, J = 6.5 Hz, 2H, C$\underline{H}_2$OSi), 1.64-1.12 (m, 7H, C$\underline{H}_2$C$\underline{H}$(CH_3)C$\underline{H}_2$C$\underline{H}_2$), 0.91 (d, J = 6.5 Hz, 3H, CH(C$\underline{H}_3$)), 0.89 (s, 9H, OSi(CH_3)$_2$ C(C$\underline{H}_3$)$_3$), 0.05 (s, 6H, OSi(CH_3)$_3$C(C$\underline{H}_3$)$_3$); HRMS (ESI^+): Found 269.1906 for **13** and 269.1907 for **14**, $C_{13}H_{30}O_2Si(Na)^+$ requires 269.1907. These data match those reported previously for (*R*)-6-((*tert*-butyldimethylsilyl)oxy)-3-methylhexan-1-ol **14**.[6]

*4.4 (S)-6-((tert-butyldimethylsilyl)oxy)-3-methylhexanal **5***

To oxalyl chloride (0.697 mL, 8.12 mmol) in DCM (15 mL) at -78 °C was added dimethyl sulphoxide (1.15 mL, 16.0 mmol) drop-wise and the resulting solution was stirred for 20 minutes. Alcohol **13** (1.00 g, 4.06 mmol) in DCM (5 mL) was added and the solution was stirred for 45 minutes. Triethylamine (3.40 mL, 24.0 mmol) was added drop-wise and the reaction mixture was stirred for a further 15 minutes before being allowed to warm to

room temperature. Water (20 mL) was added, and the organic layer was separated and extracted using DCM (2 x 20 mL). The organics were washed with brine (20 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by flash column chromatography (petroleum ether: diethyl ether, 1:1) to give **5** as a colourless oil (885 mg, 89%). (*R*)-6-((*tert*-butyldimethylsilyl)oxy)-3-methylhex-anal **6** (840 mg, 85%) was synthesized from **14** using the same procedure.

^{1}H NMR (500 MHz, $CDCl_3$) δ: 9.76-9.75 (m, 1H, C$\underline{H}$OC$\underline{H}_2$), 3.59 (t, J = 6.5 Hz, 2H, C$\underline{H}_2$OSi), 2.43-2.38 (ddd, J = 1.5, 5.5, 7.0 Hz, 1H, CHOC$\underline{H}_2$), 2.26-2.21 (ddd, J = 2.5, 8.0, 10.0 Hz, 1H, CHOC$\underline{H}_2$), 2.10-2.04 (m, 1H, $CH_2C\underline{H}(CH_3)CH_2$), 1.60-1.46 (m, 2H, $CH_2C\underline{H}_2CH_2OSi$), 1.41-1.34 (m, 1H, C$\underline{H}_2CH_2CH_2OSi$), 1.31-1.23 (m, 1H, C$\underline{H}_2CH_2C\underline{H}_2OSi$), 0.98 (d, J = 6.5 Hz, 3H, C$\underline{H}$(C$\underline{H}_3$)), 0.89 (s, 9H, $OSi(CH_3)_2$ $C(C\underline{H}_3)_3$), 0.04 (s, 6H, $OSi(C\underline{H}_3)_2$ $C(CH_3)_3$); HRMS (ESI$^+$): Found: 267.1751 for **5** and 267.1749 for **6**, $C_{13}H_{28}O_2Si(Na)^+$ requires 267.1751. These data matched thosed reported previously for (*R*)-6-((*tert*-butyldimethylsilyl)oxy)-3-methylhexan-al **6**.[6]

*4.5 (4S, 6S)-9-((tert-butyldimethylsilyl)oxy)-6-methylnon-1-en-4-ol **15***

Silver triflate (32.0 mg, 0.12 mmol) and *(S)*-BINAP (77.0 mg, 0.12 mmol) were added to THF (3 mL) under an argon atmosphere at room temperature. Aldehyde **5** (260 mg, 1.23 mmol) was added and the resulting mixture was stirred for 10 minutes. After cooling to -20 °C, allytributyl stannane (0.76 mL, 2.46 mmol) was added drop-wise and the mixture was stirred at -20 °C for 8 hours. Water was added and the organics were separated, extracted using THF (2x 5 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by flash column chromatography using a potassium carbonate: silica (1:9) stationary phase, eluting with diethyl ether: petroleum ether (1:9), to give **15** as a colorless oil (162 mg, 46%). Mosher's ester analysis showed that this material is an approximately 9:1 mixture of diastereomers and that the newly created stereogenic centre in the major diastereomer is *S*-configured (see supplememtary material).

^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.87-5.79 (m, 1H, H_2C=C$\underline{H}$C$\underline{H}_2$), 5.15 (d, J = 13.0 Hz, 2H, $\underline{H}_2$C=CHC$\underline{H}_2$), 3.78-3.72 (m, 1H, C$\underline{H}$(OH)), 3.59 (t, J = 6.5 Hz, 2H, C$\underline{H}_2$OSi), 2.30-2.25 (m, 1H, H_2C=CHC$\underline{H}$H), 2.17-2.11 (m, 1H, H_2C=CHC$\underline{H}$H), 1.73-1.61 (m, 1H, $CH_2C\underline{H}(CH_3)CH_2$), 1.52-1.46 (m, 2H, $CH(CH_3)CH_2C\underline{H}_2$),1.36-1.28 (m, 2H, CH(OH)C$\underline{H}_2$), 1.22-1.16 (m, 2H, $CH(CH_3)C\underline{H}_2CH_2$), 0.92 (d, J = 6.5 Hz, 3H, CH(C$\underline{H}_3$), 0.89 (s, 9H, $OSi(CH_2)_2C(C\underline{H}_3)_3$, 0.05 (s, 6H, OSi(C$\underline{H}_2$)$(CH_3)$; ^{13}C NMR (125 MHz, $CDCl_3$) δ: 135.0, 118.3, 68.5, 63.7, 44.4, 42.9, 33.9, 30.4, 29.2, 26.1, 19.4, 13.9, -5.1; HRMS (ESI$^+$): Found 309.2221; $C_{16}H_{34}O_2Si(Na)^+$ requires 309.2220.

*4.6 (4S, 6R)-9-((tert-butyldimethylsilyl)oxy)-6-methylnon-1-en-4-ol **16***

The same procedure as that employed for the synthesis of **15** was used starting from aldehyde **6**. This yielded **16** as a colorless oil (170 mg, 48 %). Mosher's ester analysis showed that this material is an approximately 9:1 mixture of diastereomers and that the newly created stereogenic centre in the major diastereomer is *S*-configured (see supplememtary material).

^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.87-5.79 (m, 1H, H_2C=C$\underline{H}$C$\underline{H}_2$), 5.15-5.12 (m, 2H, $\underline{H}_2$C=CHC$\underline{H}_2$), 3.77-3.72 (m, 1H, H_2C=CHC$\underline{H}_2$C$\underline{H}$(OH)), 3.61-3.58 (t, J = 7.0 Hz, C$\underline{H}_2$OSi$(CH_3)_2(CH_3)_3$, 2.32-2.25 (m, 1H, H_2C=CHC$\underline{H}$H), 2.17-2.08 (m, 1H, H_2C=CHCH$\underline{H}$), 1.68-1.60 (m, 1H, CH(OH)$CH_2C\underline{H}(CH_3)$), 1.53-1.45 (m, 2H), $CH_2CH(CH_3)C\underline{H}_2$)), 1.44-1.38 (m, 2H, C$\underline{H}_2CH_2OSi(CH_3)_2(CH_3)_3$), 1.39-1.31 (m, 1H, C$\underline{H}$(OH)C$\underline{H}_2CH(CH_3)$), 1.16-1.09 (m, 1H, CH(OH)C$\underline{H}_2$CH(CH_3), 0.93 (d, J = 6.5 Hz, 3H, $CH_2CH(C\underline{H}_3)CH_2$), 0.89 (s, 9H, $OSi(CH_3)_2(C\underline{H}_3)_3$), 0.05 (s, 6H, $OSi(C\underline{H}_3)_2(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 135.0, 118.4, 68.8, 63.7, 44.5, 42.3, 32.6, 30.2, 29.5, 26.1, 20.5, 18.5, -5.1; HRMS (ESI$^+$): Found 309.2226; $C_{16}H_{34}O_2Si(Na)^+$ requires 309.2220.

*4.6 (4S,6S)-9-((tert-butyldimethylsilyl)oxy)-6-methylnon-1-en-4-yl acetate **17***

To **15** (120 mg, 0.42 mmol) in DCM (3 mL) was added pyridine (44.0 µL, 0.55 mmol). The resulting mixture was stirred at room temperature for 5 minutes, then cooled to -10 °C. Acetic anhydride (48.0 µL, 0.53 mmol) was added and the mixture was stirred at room temperature for 16 hours. DCM (3 mL) was added and the resulting solution was washed with $NaHCO_3$ (5 mL) and brine (5 mL), then dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by flash column chromatography (ethyl acetate: petroleum ether, 1:9) to give **17** as a colourless oil (70 mg, 51%).

^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.78-5.70 (m, 1H, H_2C=C$\underline{H}$C$\underline{H}_2$), 5.08-5.02 (m, 3H, $\underline{H}_2$C=CHC$\underline{H}_2$ and $CH_2C\underline{H}$(O-COC$\underline{H}_3$), 3.58 (t, J = 6.5 Hz, 2H, C$\underline{H}_2$ $OSi(C\underline{H}_2)_2C(CH_3)_3$, 2.29 (t, J = 6.5 Hz, 2H, H_2C=CHC$\underline{H}_2$), 2.02 (s, 3H, CH(O-COC$\underline{H}_3$), 1.62 (ddd, J = 4.0, 5.5, 7.0 Hz, 1H, $CH_2CH(OCOCH_3)C\underline{H}$H), 1.51-1.44 (m, 2H, $CH_2CH(CH_3)CH_2C\underline{H}_2$), 1.37-1.31 (m, 1H, $CH(CH_3)C\underline{H}HCH_2$), 1.30-1.27 (m, 1H, C$\underline{H}(CH_3)C\underline{H}_2CH_2$), 1.26-1.22 (m, 1H, C$\underline{H}_2CH(OCOCH_3)C\underline{H}$H), 1.19-1.13 (m, 1H, $CH(CH_3)C\underline{H}HCH_2$), 0.89 (s, 12H, $CH(C\underline{H}_3)CH_2$ and $OSi(C\underline{H}_3)_2C(C\underline{H}_3)_3$, 0.05 (s, 6H, $OSi(C\underline{H}_3)_2C(CH_3)_3$; ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.9, 133.9, 117.8, 71.4, 63.6, 41.2, 39.7, 33.7, 30.4, 29.2, 27.5, 26.1, 21.4, 19.5, -5.2; HRMS (ESI$^+$): Found 351.2327, $C_{18}H_{36}O_3Si(Na)^+$ requires 351.2326; $[\alpha]_D^{25}$ -0.40° (*c* 0.12, $CHCl_3$).

(4S,6R)-9-((tert-butyldimethylsilyl)oxy)-6-methylnon-1-en-4-yl acetate (14)

The same procedure as that employed for the synthesis of **17** was used starting from compound **14**. This yielded **18** as a colorless oil (100 mg, 73%).

^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.79-5.71 (m, 1H, H_2C=C<u>H</u>CH_2), 5.08-4.98 (m, 3H, <u>H</u>$_2$C=CHC<u>H</u>$_2$ and CH_2C<u>H</u>(CH_3)CH_2), 3.58-3.56 (m, 2H, C<u>H</u>$_2$OSi(CH_3)$_2$(CH_3)$_3$), 2.35-2.29 (m, 1H, H_2C=CHC<u>H</u><u>H</u>), 2.29-2.22 (m, 1H, H_2C=CHCH<u>H</u>), 2.02 (s, 3H, CH_2CH(OCOC<u>H</u>$_3$)CH_2), 1.52-1.40 (m, 4H, C<u>H</u>$_2$C<u>H</u>$_2$C<u>H</u>$_2$OSi(CH_3)$_2$(CH_3)$_3$ and CH_2C<u>H</u>$_2$C<u>H</u>$_2$OSi(CH_3)$_2$(CH_3), 1.40-1.29 (m, 1H, CH(OH)C<u>H</u><u>H</u>), 1.15-1.08 (m, 1H, CH(OH)CH<u>H</u>), 0.91 (d, J = 5.5 Hz, 3H, CH(C<u>H</u>$_3$)CH_2), 0.89 (s, 9H, OSi(CH_3)$_2$(C<u>H</u>$_3$)$_3$), 0.04 (s, 6H, OSi(C<u>H</u>$_3$)$_2$(CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 170.8, 133.9, 117.8, 71.9, 63.7, 41.2, 39.0, 32.8, 30.2, 29.6, 26.1, 21.4, 20.1, 18.5, -5.1; HRMS (ESI^+): Found 351.2334, $C_{18}H_{36}O_3Si(Na)^+$ requires 351.2337; $[\alpha]_D^{25}$ +4.20 (*c* 0.18, $CHCl_3$).

*4.7 (4S,6S)-9-hydroxy-6-methylnon-1-en-4-yl acetate **19***

Compound **17** (70 mg, 0.21 mmol) was stirred at room temperature in a 3:1:1 mixture of acetic acid, THF and H_2O (3 mL) for 16 hours. Saturated $NaHCO_3$ (5 mL) was added and the mixture was extracted using DCM (2 x 3 mL). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo* to yield **19** as a colorless oil (41 mg, 90%).

^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.78-5.70 (m, 1H, H_2C=C<u>H</u>CH_2), 5.08-5.01 (m, 3H, <u>H</u>$_2$C=CHC<u>H</u>$_2$ and CH_2C<u>H</u>($OCOCH_3$)), 3.63 (t, J = 6.60 Hz, 2H, C<u>H</u>$_2$OH), 2.29 (t, J = 6.20 Hz, 2H, H_2C=CHC<u>H</u>$_2$), 2.03 (s, 3H, CH(OCOC<u>H</u>$_3$), 1.66-1.62 (m, 1H, CH(OCOCH$_3$)C<u>H</u><u>H</u>), 1.62-1.60 (m, 2H, CH_2C<u>H</u>$_2$$CH_2$OH), 1.51-1.46 (m, 1H, CH_2C<u>H</u>(CH_3)CH_2), 1.41-1.32 (m, 1H, C<u>H</u><u>H</u>C$H_2$$CH_2$OH), 1.27-1.24 (m, 1H, C<u>H</u>(OCOCH$_3$)CH<u>H</u>), 1.23-1.19 (m, 1H, CH<u>H</u>C$H_2$$CH_2$OH) 0.90 (d, J = 6.60 Hz, 3H, CH_2CH(C<u>H</u>$_3$)); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 171.0, 133.9, 117.9, 71.4, 63.4, 41.1, 39.7, 33.5, 30.2, 29.3, 21.4, 19.5; HRMS (ESI^+): Found 237.1461, $C_{12}H_{22}O_3(Na)^+$ requires 237.1461; $[\alpha]_D^{25}$ -22.7 (*c* 0.06, $CHCl_3$).

*(4S,6R)-9-hydroxy-6-methylnon-1-en-4-yl acetate **20***

The same procedure as that employed for the synthesis of **19** was used starting from compound **18**. This yielded **20** as a colorless oil (54.0 mg, 83%).

^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.79-5.70 (m, 1H, H_2C=C<u>H</u>CH_2), 5.08-5.02 (m, 3H, <u>H</u>$_2$C=CHC<u>H</u>$_2$ and CH_2C<u>H</u>($OCOCH_3$)CH_2), 3.64-3.57 (m, 2H, CH_2C<u>H</u>$_2$OH), 2.34-2.23 (m, 2H, H_2C=CHC<u>H</u>$_2$), 2.03 (s, 3H, CH(OCOC<u>H</u>$_3$), 1.65-1.13 (m, 7H, CH_2C<u>H</u>(CH_3)C<u>H</u>$_2$$CH_2$$CH_2$OH), 0.92 (d, J = 6.0 Hz, 3H, CH_2CH(C<u>H</u>$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 171.1, 133.8, 117.9, 71.7, 63.0, 41.3, 39.2, 32.17, 30.0, 29.2, 21.4, 20.2; HRMS (ESI^+): Found 237.1460, $C_{12}H_{22}O_3(Na)^+$ requires 237.1461; $[\alpha]_D^{25}$ +20.6 (*c* 0.09, $CHCl_3$).

*4.8 (4S,6R)-6-acetoxy-4-methylnon-8-enoic acid **21***

To **19** (41.0 mg, 0.19 mmol) in dimethylsulphoxide (0.50 mL) was added iodoxybenzoic acid (64.0 mg, 0.23 mmol). The resulting mixture was stirred at room temperature for 5 hours, then diluted with ethyl acetate (5 mL) and filtered through celite. The solvent was removed *in vacuo* and the crude product was purified by flash column chromatography (diethyl ether: petroleum ether, 2:1). The resulting aldehyde was dissolved in dimethylformamide (1 mL), Oxone (151 mg, 0.25 mmol) was added and the solution was stirred at room temperature under an argon atmosphere for 2 hours. Water (3 mL) was added and the resulting mixture was extracted with ethyl acetate (2 x 3 mL). The organic phase was dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by flash column chromatography (ethyl acetate: petroleum ether, 1:2) to give **21** as a colorless oil (26.0 mg, 60%).

^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.78-5.70 (m, 1H, H_2C=C<u>H</u>CH_2), 5.09-5.01 (m, 3H, <u>H</u>$_2$C=CHC<u>H</u>$_2$ and CH_2C<u>H</u>($OCOCH_3$)), 2.43-2.33 (m, 2H, C<u>H</u>$_2$COOH), 2.30-2.28 (m, 2H, H_2C=CHC<u>H</u>$_2$), 2.10 (s, 3H, CH(OCOC<u>H</u>$_3$)), 1.68-1.63 (m, 1H, C<u>H</u>HCH_2COOH), 1.63-1.60 (m, 1H, C<u>H</u>(OCOCH$_3$)C<u>H</u><u>H</u>), 1.55-1.52 (m, 1H, CH_2C<u>H</u>(CH_3)), 1.52-1.49 (m, 1H, CH<u>H</u>CH_2COOH), 1.31-1.24 (m, 1H, CH(OCOCH$_3$)CH<u>H</u>), 0.92 (d, J = 6.0 Hz, 3H, CH_2CH(C<u>H</u>$_3$) ; ^{13}C NMR (125 MHz, $CDCl_3$) δ: 177.0, 171.0, 133.7, 118.0, 71.3, 40.8, 39.6, 32.2, 31.2, 29.0, 21.3, 19.0; HRMS (ESI^+): Found 251.1254, $C_{12}H_{20}O_4(Na)^+$ requires 251.1254; $[\alpha]_D^{25}$ -46.4 (*c* 0.06, $CHCl_3$).

*(4S,6R)-6-acetoxy-4-methylnon-8-enoic acid **22***

The same procedure as that employed for the synthesis of **21** was used starting from alcohol **20**. This yielded **22** as a colorless oil (26 mg, 50%).

^{1}H NMR (500 MHz, $CDCl_3$) δ: 5.79-5.70 (m, 1H, H_2C=C<u>H</u>), 5.09-5.00 (m, 3H, <u>H</u>$_2$C=CH and CH_2C<u>H</u>($OCOCH_3$), 2.41-2.23 (m, 4H, H_2C=CHC<u>H</u>$_2$ and C<u>H</u>$_2$COOH), 2.03 (s, 3H, CH(OCOC<u>H</u>$_3$)), 1.79-1.72 (m, 1H, CH_2CH(CH_3)C<u>H</u><u>H</u>), 1.67-1.58 (m, 1H, CH(OCOCH$_3$)C<u>H</u><u>H</u>), 1.58-1.50 (m, 1H, CH_2C<u>H</u>(CH_3), 1.45-1.39 (m, 1H, CH_2CH(CH_3)CH<u>H</u>), 1.33-1.31 (m, 1H, CH(OCOCH$_3$)CH<u>H</u>), 0.94 (d, J = 6.5 Hz, 3H, CH(C<u>H</u>$_3$)CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 177.6, 170.9, 133.7, 118.0, 71.5, 40.8, 39.0, 31.3, 29.8, 29.2, 21.4, 19.7; HRMS (ESI^+): Found

251.1250, $C_{12}H_{20}O_4(Na)^+$ requires 251.1254; $[\alpha]_D^{25}$ +17.3° (*c* 0.06, $CHCl_3$).

*4.10 (4S,6S)-6-hydroxy-4-methylnon-8-enoic acid **3***

Carboxylic acid **21** (20.0 mg, 0.09 mmol) and lithium hydroxide (4.00 mg, 0.18 mmol) were stirred in 2:1 THF/H_2O (1.2 mL) at room temperature for 16 hours. The THF was removed *in vacuo* and 1 M HCl (2 mL) was added to the residue. The resulting mixture was extracted with ethyl acetate (2 x 2 mL), and the organic phase was dried ($MgSO_4$) and concentrated *in vacuo*. The crude product was purified by flash column chromatography (diethyl ether: petroleum ether, 1:2) to give **3** as a thick colorless oil (10.0 mg, 61%).

^{1}H NMR (500 MHz, d_4-MeOH) δ: 5.90-5.82 (m, 1H, H_2C=C<u>H</u>CH_2), 5.08-5.03 (m, 2H, <u>H_2</u>C=CHCH_2), 3.72-2.67 (m, 1H, H_2C=CHCH_2C<u>H</u>(OH)), 2.36-2.25 (m, 2H, C<u>H_2</u>COOH), 2.21-2.19 (t, J = 6.5 Hz, 2H, H_2C=CHC<u>H_2</u>), 1.75-1.68 (m, 1H, CH_2C<u>H</u>(CH_3), 1.66-1.59 (m, 1H, C<u>H</u>HCH_2COOH), 1.51-1.45 (m, 1H, CH<u>H</u>CH_2COOH), 1.44-1.40 (m, 1H, CH_2CH(O<u>H</u>)C<u>H</u>H), 1.23-1.19 (m, 1H, CH_2CH(OH)C<u>H</u><u>H</u>), 0.92 (d, J = 6.5 Hz, 3H, CH_2CH(C<u>H_3</u>)CH_2); ^{13}C NMR (125 MHz, d_4-MeOH) δ: 178.0, 136.5, 117.2, 69.7, 44.9, 43.9, 34.0, 32.8, 30.0, 19.2; HRMS (ESI^+): Found 209.1140, $C_{10}H_{18}O_3(Na)^+$ requires 209.1148; $[\alpha]_D^{25}$ -0.80 (*c* 0.18, $CHCl_3$).

*(4S,6R)-6-hydroxy-4-methylnon-8-enoic acid **4***

The same procedure as that employed for the synthesis of **3** was used starting from compound **22**. This yielded **4** as a thick colorless oil (14.0 mg, 66%).

^{1}H NMR (500 MHz, d_4-MeOH) δ: 5.90-5.83 (m, 1H, H_2C=C<u>H</u>), 5.08-5.03 (m, 2H, <u>H_2</u>C=CH), 3.74-3.69 (m, 1H, CH_2C<u>H</u>(OH)), 2.36-2.25 (m, 2H, C<u>H_2</u>COOH), 2.24-2.14 (m, 2H, H_2C=CHC<u>H_2</u>), 1.77-1.70 (m, 1H, C<u>H</u>HCH_2COOH), 1.69 – 1.64 (m, 1H, CH_2C<u>H</u>(CH_3)), 1.44-1.35 (m, 2H, CH<u>H</u>CH_2COOH and CH(OH)C<u>H</u><u>H</u>) 1.33-1.28 (m, 1H, CH(OH)C<u>H</u><u>H</u>), 0.94 (d, J = 6.5 Hz, 3H, CH_2CH(C<u>H_3</u>)) ; ^{13}C NMR (125 MHz, $CDCl_3$) δ: 177.9, 136.4, 117.3, 69.9, 45.0, 43.3, 32.5, 32.5, 30.3, 20.4; HRMS (ESI^+): Found 209.1143, $C_{10}H_{18}O_3(Na)^+$ requires 209.1148; $[\alpha]_D^{25}$ +3.5° (*c* 0.10, $CHCl_3$).

Acknowledgments

We thank Dr Lona Alkhalaf for critical reading of the manuscript, Dr Douglas Roberts for helpful discussions and Dr Ivan Prokes for assistance with the acquisition of NMR spectroscopic data. This research was supported by the EPSRC through the University of Warwick's doctoral training account.

References and notes

1. Neu, H. C. *Source Sci. New Ser.* **1992**, *257*, 5073, 1064–1073.
2. Masschelein, J.; Jenner, M.; Challis, G. L. *Nat. Prod. Rep.* **2017**, *34*, 7, 712–783.
3. Mahenthiralingam, E.; Song, L.; Sass, A.; White, J.; Wilmot, C.; Marchbank, A.; Boaisha, O.; Paine, J.; Knight, D.; Challis, G. L. *Chem. Biol.* **2011**, *18*, 5, 665–677.
4. Song, L.; Jenner, M.; Masschelein, J.; Jones, C.; Bull, M. J.; Harris, S. R.; Hartkoorn, R. C.; Vocat, A.; Webster, G.; Dunn, M.; Weiser, R.; Paisey, C.; Cole, S. T.; Parkhill, J.; Mahenthiralingam, E.; Challis, G. L. *J. Am. Chem. Soc.* **2017**, *139*, *23*, 7974-7981.
5. Irschik, H.; Schummer, D.; Höfle, G.; Reichenbach, H.; Steinmetz, H.; Jansen, R. *J. Nat. Prod.* **2007**, *70*, 6, 1060–1063.
6. Burke, L. T.; Dixon, D. J.; Ley, S. V; Rodríguez, F. *Org. Biomol. Chem.* **2005**, *3*, 274-280.
7. Yanagisawa, A.; Nakashima, H.; Ishiba, A.; Yamamoto, H. *J. Am. Chem. Soc.* **1996,** *118*, *19*, 4723-4724.
8. Hoye, T. R.; Jeffrey, C. S.; Shao, F. *Nat. Protoc.* **2007**, *2*, 2451-2458.

Supplementary Material

Procedure for the preparation of Mosher's ester derivatives of alcohols **15** and **16**, ^{1}H NMR spectra of the products, and ^{1}H and ^{13}C NMR spectra of all compounds synthesised.