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The Chemistry Of Pseudomonic Acid Part 131. Modifications At C-12 To C-14

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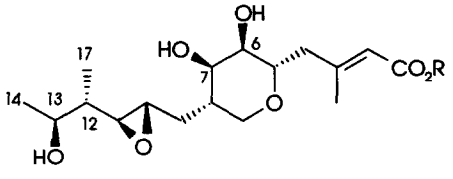
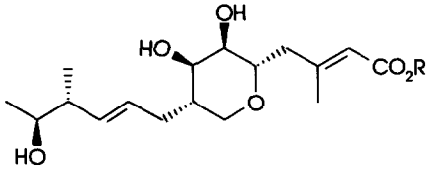
Abstract: The novel antibiotic, pseudomonic acid, binds tightly to its target enzyme, isoleucyl t-RNA synthetase. The C12 to C14 region of the molecule is thought to bind to the isoleucine binding site of the enzyme. Semisynthetic analogues in which functionality present in this region have been systematically modified are reported here: all the derivatives prepared showed weak enzyme binding.

Pseudomonic acid A (**1**) is a novel antibiotic isolated from *Pseudomonas fluorescens* NCIB 10586[2], the mechanism of action of which is competitive inhibition of isoleucine t-RNA synthetase[3]. It has been postulated that the C-12 to C-14 part of the molecule occupies the site on the enzyme which normally binds isoleucine[3], and since this enzyme is known to accept as substrates a variety of aminoacids other than isoleucine[4], it is of interest to see how modifications made in this region affect biological activity. This paper describes such modifications.



(**1**) R = $(CH_2)_8CO_2H$

(**2**) R = Et

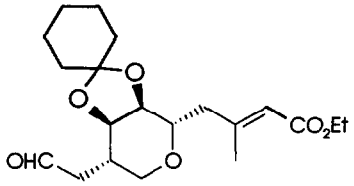


(**3**) R = $(CH_2)_8CO_2H$

(**4**) R = Et

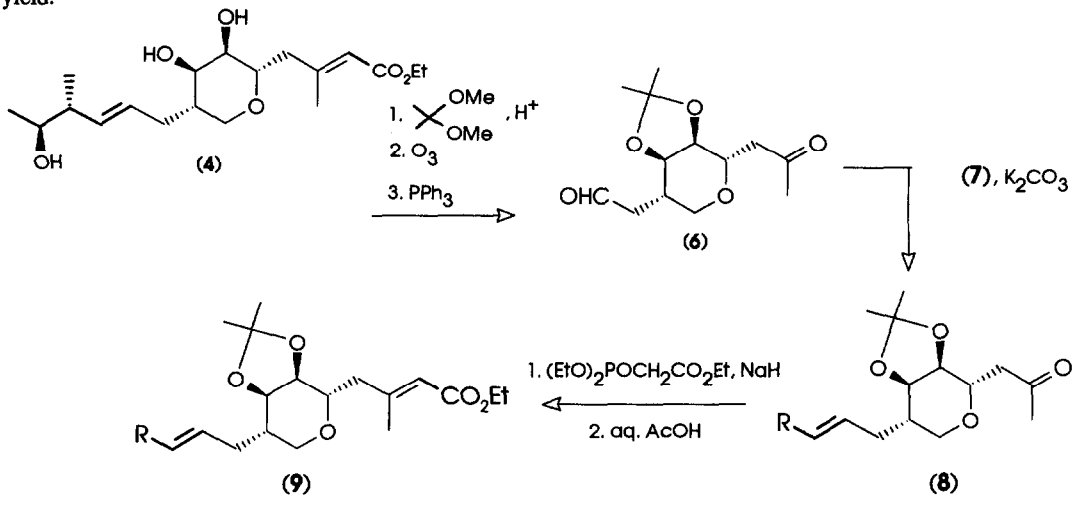
It has previously been shown that ethyl monate C (**4**) prepared from naturally occurring pseudomonic acid C (**3**) has antibacterial activity of the same order as (**1**) and is significantly more chemically stable[5], and so direct analogues of ethyl monate C were chosen as the primary targets of this study. We have previously reported modification of the 13-hydroxyl groups of compounds (**1**) and (**4**)[6]; their inversion and removal, and conversion into the corresponding (*R*) and (*S*) chlorides and amines. However all these derivatives showed poor antibacterial activity suggesting that the 13-hydroxyl is necessary for enzyme binding. We have therefore targeted derivatives containing such a group.

Kozikowski *et al.* have reported the total synthesis of racemic ethyl monate C *via* racemic aldehyde (**5**), attaching the C-11 to C-14 fragment by means of a Wittig reaction[7]. Subsequently Fleet *et al.* have reported a preparation of chiral (**5**) starting from D-arabinose[8]. We have therefore investigated the retrosynthesis of intermediates related to aldehyde (**5**), from semi-synthetic ethyl monate C and subsequent olefinations leading to the desired C-12 to C-14 modifications in which the 13-(*S*) alcohol is retained.



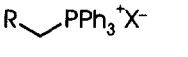
(**5**)

A practical route to (**4**) was however required, as the only previously reported method for deoxygenation of the epoxide in this series used prolonged heating with potassium selenocyanate and gave only a variable yield of the olefin[6]. We have now shown that (**4**) may be prepared from the corresponding epoxide (**2**), ethyl monate A, in good yield using trifluoroacetyl iodide in acetonitrile[9], with temporary protection of the three hydroxyl groups as their trichloroacetate esters. Ester (**2**) can be prepared from the readily available (**1**) in good yield.[10]



(**7**) - (**9**):

	R	X⁻
a.	Ph	Cl⁻
b.	2-py	Cl⁻



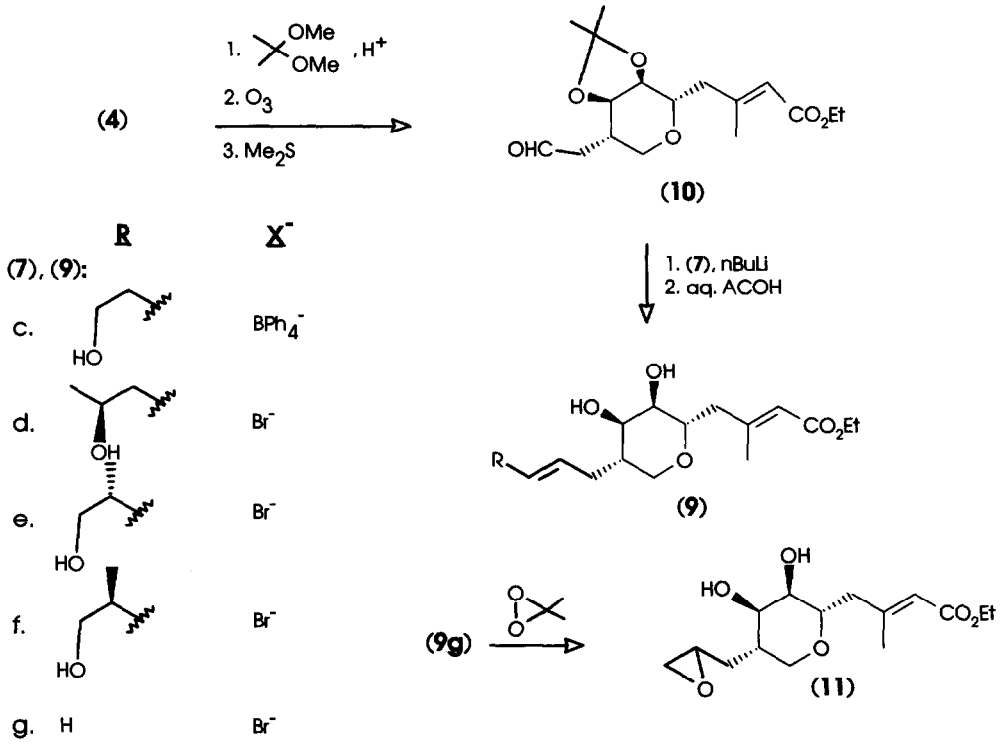
(**7**)

Scheme 1

Our first approach to the desired derivatives consisted of protection of the 1,2-diol in (**4**) and subsequent ozonolysis to give the novel intermediate, ketoaldehyde (**6**). This intermediate could be used to prepare, for example, the aromatic derivatives (**9a**) and (**9b**). Thus, selective reaction of the aldehyde group with semi-stabilised ylids derived from (**7a**) and (**7b**) gave olefins (**8a**) and (**8b**) as isomer mixtures, from which the predominant *E*-isomers could be obtained pure. Subsequent reactions of the C-3 ketones with the anion derived

from triethyl phosphonoacetate gave the desired αβ-unsaturated esters (**9a**) and (**9b**). As expected these were predominantly as the desired *E*-isomers[10], from which the unwanted *Z*-isomer could be removed chromatographically after acetonide deprotection. Similar products containing an appropriately sited phenolic hydroxyl which would mimic the 13-alcohol could not however be obtained in this way.

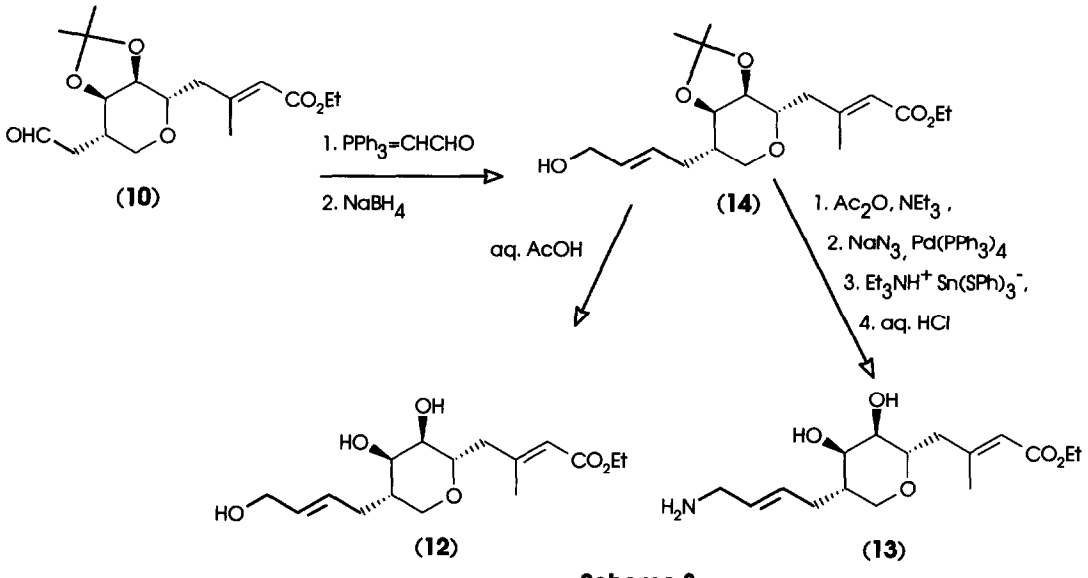
The preparation of hydroxyalkyl derivatives more closely related to (**4**) but lacking one or both of the C-14 and C-17 methyl groups could not be achieved by this method, as it required the use of unstabilised ylids which would not be expected to react selectively with the aldehyde of (**6**). The differential reactivity of the two double bonds in (**4**) was therefore used to provide, by carefully controlled ozonolysis, aldehyde (**10**) in acceptable yield.



Scheme 2

Reaction of (**10**) with the Wittig reagents derived from (**7c**),[12] (**7d**),[13] and (**7e**) and subsequent deprotection gave the bisnormethyl derivative (**9c**) and the two normethyl derivatives (**9d**) and (**9e**) respectively. As valine is known to bind to isoleucyl tRNA synthetase,[4] (**9e**) was of particular interest since this derivative corresponds to the change from an isoleucine equivalent to a valine equivalent. In the same way the ylid from (**7f**) gave the unnatural diastereoisomer (**9f**): this is the first example of a pseudomonic acid derivative with inverted stereochemistry at position-12 i.e. *R* not *S*. Although a predominance of *E*-isomer was expected from these reactions[14], in all four cases approximately equal amounts of *E*- and *Z*-isomers were obtained. Except for (**9f**) these isomers were inseparable. Attempts to improve the stereoselectivity by using the Julia reaction were unsuccessful.

Finally we prepared the truncated derivative (**9g**) in which the C-12 to C-14 side-chain has been completely removed; (**9g**) was prepared by reaction of (**10**) with methylene triphenylphosphorane. To check that the C-10,11 epoxide was not necessary for activity in this group of compounds, (**9g**) was epoxidised using dimethyldioxirane prepared *in situ* from Oxone® and acetone[15] to give (**11**) as a 1:1 mixture of epoxide diastereomers.



Scheme 3

We then prepared compounds in which the C-12 to C-14 side-chain was shortened, derivatives (**12**) and (**13**). Attempts to introduce a hydroxyl group at C-12 using a direct Wittig reaction on (**10**) were unsuccessful, but such a hydroxyl could be introduced using formylmethylenetriphenylphosphorane followed by reduction. This had the added advantage of producing only the *E* double bond isomer (**14**). The C-12 alcohol of (**14**) was converted into the corresponding amine (**13**) *via* the azide which was introduced using an allyl palladium intermediate to minimise allylic transposition.[16] Of a number of reducing agents tried for the reduction of the azide to the amine, only triethylammoniun *tris*(phenylthio)stannate (-1)[17] was found to be succesful, albeit in low yield, and this led to a low overall yield for the conversion of only 9%.

The antibacterial activity of each of the compounds described above was compared with that of (**4**) and in every case was much poorer. Compared to a Minimum Inhibitory Concentration (MIC) value of 1 μg/ml for (4) against *Staphylococcus Aureus* Oxford, all the above derivatives showed MIC values of greater than or equal to 64 μg/ml.Binding of selected derivatives to the isoleucyl t-RNA synthetase enzyme from *S. aureus* Oxford was measured as the concentration of compound required to reduce the catalytic activity of an enzyme preparation by half (I_{50}). As for the antibacterial activity, in each case the binding was found to be similarly weaker than the binding of (**4**). This was surprising in view of the known lack of aminoacid substrate specificity of this enzyme,[4] and may indicate that the binding requirements for the inhibitor are stricter than those for the aminoacid substrate.

Experimental

^{1}H n.m.r. data were recorded at 250MHz on a Bruker AC-250F spectrometer, infrared data on a Perkin-Elmer PE 983 machine, ultraviolet data on a Beckman DU 68, and mass spectra on a VG-ZAB spectrometer. Coupling constants *J* are given in Hertz. The silicagel used for both thin layer and column chromatography was Merck type 60. Tetrahydrofuran was distilled before use from sodium-benzophenone. Solutions were dried with anhydrous sodium sulfate. The atom numbering system used throughout in the spectral analysis of semisynthetic derivatives is that of pseudomonic acid (**1**).

Ethyl 4-{(2S,3R,4R,5S)-3,4-dihydroxy-5-[(4R,5S)-5-hydroxy-4-methylhex-2(E)-enyl]tetrahydropyran-2-yl}-3-methylbut-2(E)-enoate (**4**) - Trichloroacetyl chloride (3.3cm^3, 30mmol) was added dropwise to a solution containing the corresponding epoxide (**2**) (2.8g, 7.5mmol) in a mixture of dichloromethane (50cm^3) and pyridine (2.6cm^3, 32.5mmol) at 20°C. After 1h at 20°C the solution was washed consecutively with 1M aqueous sodium hydrogen carbonate (50cm^3), 0.5M aqueous citric acid (2x50cm^3), and brine (50cm^3), dried, and evaporated to dryness to give the crude tris(trichloroacetate).

Trifluoroacetic anhydride (1.0cm^3, 7mmol) was added to a mixture of sodium iodide (3.5g, 25mmol) and acetonitrile (20cm^3) at 20°C. After 5min at 20°C the above tris(trichloroacetate) in acetonitrile (10cm^3) was added and the solution stirred at 20°C for 18h. Diethyl ether (100cm^3) was added and the mixture then washed with 1M aqueous sodium hydrogen sulphite (2x20cm^3), 1M aqueous sodium hydrogen carbonate (2x20cm^3), and brine (20cm^3), then dried and evaporated to dryness to give the crude protected olefin.

This crude material was dissolved in ethanol (50cm^3) and potassium carbonate (7g, 50mmol) was added. After stirring for 2h at 20°C, ethyl acetate (200cm^3) was added and the mixture washed with water (100cm^3) then brine (50cm^3), dried, and evaporated to dryness. Chromatography on silica gel eluting with ethyl acetate-hexane (1:1) gave the title olefin (**4**) (2.0g, 75%), m.p.96-97°C (from diethyl ether) (lit.[5], 96.5-97°C), identical in all ways to that prepared from pseudomonic acid C.

(3aS,4S,7S,7aR)-2,2-Dimethyl-7-(2-oxoethyl)-4-(2-oxopropyl)-3a,6,7,7a-tetrahydro-4H-1,3-dioxolo[4,5-c]pyran (**6**) - A mixture of olefin (**4**) (1.5g, 4.2mmol), 2,2-dimethoxypropane (20cm^3), tetrahydrofuran (20cm^3) and *p*-toluenesulphonic acid (0.1g) was stirred for 1h at 20°C. Ethyl acetate (100cm^3) was then added and the solution washed with 1M aqueous sodium hydrogen carbonate (100cm^3), dried, and evaporated to dryness to give the crude 6,7-acetonide (1.7g, quant.).

The above acetonide (1.7g, 4.2mmol) in methanol (40cm^3) and dichloromethane (40cm^3) was ozonized at -70°C until a permanent blue colour was obtained then purged with oxygen and treated with triphenylphosphine (2.2g, 8mmol). The mixture was warmed to 20°C and evaporated to dryness. Chromatography twice on silica gel, first with methanol-dichloromethane (1:9) then with ethyl acetate-hexane (1:1) gave ketoaldehyde (**6**) (0.84g, 78%) as a colourless oil; υ_{max} (neat)/cm^{-1} 3400 (OH) and 1720 (C=O); δ_H ($CDCl_3$) 1.36 and 1.53 (6H, 2s, CMe_2), 2.20 (3H, s, COMe), 2.5-2.85 (5H, m, 7-H, 2 x CH_2CO), 3.59 (1H, d, *J* 12, 6-H), 3.7-3.9 (3H, m, 4-H, 6-H and 7a-H), 4.05 (1H, bs, 3a-H), and 9.81 (1H, s, CHO); *m/z* (EI) 241 (M^+-Me, 2%) and 43 (100).

Ethyl 4-[(2S,3R,4R,5S)-3,4-dihydroxy-5-(3-phenylprop-2(E)-enyl)tetrahydropyran-2-yl]-3-methylbut-2(E)-enoate (**9a**) - A mixture of ketoaldehyde (**6**) (0.18g, 0.7mmol), benzyltriphenylphosphonium chloride (0.31g, 0.8mmol), potassium carbonate (0.28g, 2.0mmol), and N,N-dimethylformamide ($3cm^3$) was stirred at 20°C for 3h and then 3M aqueous ammonium chloride ($10cm^3$) and ethyl acetate ($10cm^3$) were added. The organic layer was dried and evaporated to dryness. Chromatography on silica gel eluting with diethyl ether-hexane (2:1) gave the *E* keto olefin (30mg, 13%), which was dissolved in tetrahydrofuran ($1cm^3$) and added to a solution prepared from triethyl phosphono-acetate (0.22g, 1mmol) and sodium hydride (80% in mineral oil; 0.03g, 1mmol) in tetrahydrofuran ($2cm^3$). After 18h at 20°C, 3M aqueous ammonium chloride ($20cm^3$) was added and the mixture extracted with ethyl acetate ($2x20cm^3$), and the combined extracts were dried and evaporated to dryness. Chromatography on silica gel eluting with diethyl ether-hexane (1:1) gave a mixture of *E* and *Z* αβ-unsaturated esters as a colourless oil (25mg, 74%). This mixture was dissolved in acetic acid ($1.6cm^3$) - water ($0.4cm^3$). After 16h at 20°C, evaporation to dryness and chromatography on silica gel eluting with ethyl acetate-hexane (1:1) gave *E,E*-diene (**9a**) as a colourless oil (8mg, 31%); υ_{max} (neat)/cm^{-1} 1710 and 1690 (C=O) and 1640 (C=C); λ_{max} (EtOH)/nm 246; δ_H ($CDCl_3$) 1.28 (3H, t, *J* 7, ester-Me), 1.95 (1H, m, 8-H), 2.1-2.5 (8H, m, 4-H, 9-H_2, 15-H_3, 2 x OH), 2.66 (1H, dd, *J* 2 and 15, 4-H), 3.50 (1H, bd, *J* 9, 6-H), 3.59 (1H, d, *J* 12, 16-H), 3.71 (1H, dt, *J* 3 and 9, 5-H), 3.85 (1H, dd, *J* 3 and 12, 16-H), 3.96 (1H, t, *J* 3, 7-H), 4.13 (2H, q, *J* 7, ester-CH_2), 5.80 (1H, s, 2-H), 6.17 (1H, dt, *J* 16 and 7, 10-H), 6.44 (1H, d, *J* 16, 11-H), and 7.2-7.4 (5H, m, aryl); *m/z* (EI) 360 (M^+, 8%), 233 (61), and 117 (100) (Found: M^+ 360.1950. $C_{21}H_{28}O_5$ requires M, 360.1937).

Ethyl 4-[(2S,3R,4R,5S)-3,4-dihydroxy-5-(3-2-pyridylprop-2(E)-enyl)tetrahydropyran-2-yl]-3-methylbut-2(E)-enoate (**9b**) - Prepared in the same fashion as (**9a**) from ketoaldehyde (**6**) (0.13g, 0.5mmol) and 2-pyridylmethyltriphenylphosphonium chloride.[11] Final purification was by silica gel chromatography eluting with methanol-dichloromethane (1:9) to give *E, E* diene (**9b**) as a colourless oil (15mg overall, 8%); υ_{max} (neat)/cm^{-1} 1710 (C=O) and 1650 (C=C); δ_H ($CDCl_3$) 1.27 (3H, t, *J* 7, ester-Me), 2.00 (1H, bs, 8-H), 2.22 (3H, s, 15-H_3), 2.29 (1H, dd, *J* 9 and 13, 4-H), 2.40 (2H, d, *J* 7, 9-H_2), 2.66 (1H, d, *J* 14, 4-H), 3.47 (1H, dd, *J* 3 and 9, 6-H), 3.58 (1H, d, *J* 12, 16-H), 3.73 (1H, dt, *J* 3 and 9, 5-H), 3.88 (1H, dd *J* 3 and 12, 16-H), 3.98 (1H, t, *J* 3, 7-H), 4.14 (2H, q, *J* 7, ester-CH_2), 5.79 (1H, s, 2-H), 6.50 (1H, d, *J* 16, 11-H), 6.66 (1H, dt, *J* 15 and 7, 10-H), 7.13 (1H, dd, *J* 5 and 7, 5 pyridyl), 7.21 (1H, d, *J* 8, 3-pyridyl), 7.65 (1H, dt, *J* 2 and 8, 4-pyridyl), and 8.52 (1H, d, *J* 6, 6-pyridyl); *m/z* (EI) 361 (M^+, 10%) and 234 (100) (Found: M^+ 361.1893. $C_{20}H_{27}NO_5$ requires M, 361.1889).

General method for the reaction of aldehyde (**10**) *with* β-*oxidoylids* - n-Butyllithium (1.6M in hexane; $1.25cm^3$, 2.0mmol) was added to a suspension of the appropriate β-hydroxyalkyl phosphonium salt (1.0mmol) in dry tetrahydrofuran ($5cm^3$) under argon at -30°C. After about 30min at -30°C, when a clear red solution had been obtained, a solution of aldehyde (**10**) (0.16g, 0.5mmol) in dry tetrahydrofuran ($1cm^3$) was added dropwise. After 1h at -30°C and 2h at 0°C, 3M aqueous ammonium chloride ($20cm^3$) was added. The mixture was extracted with ethyl acetate ($2x20cm^3$), and the combined extracts were dried and evaporated to dryness. The residue was purified by chromatography on silica gel, eluting with ethyl acetate-hexane (1:1 or 1:2) to give an oil, which was dissolved in a mixture of water ($0.4cm^3$) and acetic acid ($1.6cm^3$). After 18h at 20°C the

solution was evaporated to dryness. Chromatography on silica gel, eluting with methanol-dichloromethane (1:9 or 1:19) gave olefins (**9c-f**).

Ethyl 4-[(2S,3R,4R,5S)-3,4-dihydroxy-5-(5-hydroxypent-2-enyl)tetrahydropyran-2-yl]-3-methylbut-2(E)-enoate (**9c**) - Prepared from 3-hydroxypropyltriphenylphosphonium tetraphenylborate (**7c**)[12], giving 60mg (37%) of a colourless oil as a 2:1 *E*:*Z* mixture of isomers; υ_{max} (neat)/cm^{-1} 3400 (OH), 1695 and 1710 (C=O), and 1645 (C=C); λ_{max} (EtOH)/nm 220; δ_H ($CDCl_3$) 1.26 (3H, t, *J* 7, ester-Me), 1.83 (1H, bs, 8-H), 2.2-2.4 (8H, m, 4-H, 9-H_2, 12-H_2, and 15-H_3), 2.63 (1H, d, *J* 15, 4-H); 3.5-3.8 (6H, m, 5-H, 6H, 13-H_2, and 16-H_2), 3.90 (1H, bs, 7-H), 4.15 (2H, q, *J* 7, ester-CH_2), 5.45-5.55 (2H, m, 10-H and 11-H), and 5.78 (1H, s, 2-H); *m/z* (EI) 329 (MH^+, 1%) and 95 (100) (Found: MH^+ 329.1962. $C_{17}H_{29}O_6$ requires 329.1964). The isomer ratio was determined as 2:1 by h.p.l.c., and the major isomer was shown to be *E* by irradiation of the protected intermediate at 2.3 δ, which collapsed the major part of H-10 to a doublet *J* 15.

Ethyl 4-[(2S,3R,4R,5S)-3,4-dihydroxy-5-(5(S)-hydroxyhex-2-enyl)tetrahydropyran-2-yl]-3-methylbut-2(E)-enoate (**9d**) - Prepared from (*S*)-3-hydroxybutyltriphenylphosphonium bromide (**7d**)[13] giving 60mg (36%) of a colourless oil as a 2:1*Z*:*E* mixture, assigned by comparison with the other compounds in this series; υ_{max} (neat)/cm^{-1} 3400 (OH), 1705 and 1695 (C=O), and 1645 (C=C); λ_{max} (EtOH)/nm 220; δ_H ($CDCl_3$) 1.18 and 1.22 (3H, 2d, *J* 6, 14-H_3), 1.27 (3H, t, *J* 7, ester-Me), 1.84 (1H, bs, 8-H), 2.1-2.4 (8H, m, 4-H, 9-H_2), 12-H_2, and 15-H_3), 2.61 (1H, d, *J* 13, 4-H), 3.4-3.9 (6H, m, 5-H, 6-H, 7-H, 13-H, and 16-H_2), 4.15 (2H, q, *J* 7, ester-CH_2), 5.45-5.55 (2H, m, 10-H and 11-H), and 5.75 (1H, s, 2-H); *m/z* (EI) 343 (MH^+, 1%) and 111 (100) (Found: MH^+ 343.2125. $C_{18}H_{31}O_6$ requires 343.2121).

Ethyl 4-[(2S,3R,4R,5S)-3,4-dihydroxy-5-(5-hydroxy-4(R)-methylpent-2-enyl)tetrahydropyran-2-yl]-3-methylbut-2(E)-enoate (**9e**) - Prepared from (*S*)-3-hydroxy-2-methylpropyltriphenylphosphonium bromide (**7e**), giving 60mg (36%) of a colourless oil as a 2:1 *E*:*Z* mixture of isomers; υ_{max} (neat)/cm^{-1} 3400 (OH), 1705 and 1695 (C=O), and 1645 (C=C); λ_{max} (EtOH)/nm 221; δ_H ($CDCl_3$) 0.92 and 0.99 (3H, 2d, *J* 7, 17-H_3), 1.27 (3H, t, *J* 7, ester-Me), 1.63 (1H, bs, 8-H), 2.1-2.4 (7H, m, 4-H, 9-H_2, 12-H and 15-H_3), 2.62 (1H, bd, *J* 14, 4-H), 3.3-3.9 (7H, m, 5-H, 6-H, 17-H, 13-H_2, and 16-H_2), 4.15 (2H, q, *J* 7, ester-CH_2), 5.2-5.6 (2H, m, 10-H and 11-H), and 5.77 (1H, s, 2-H); *m/z* (EI) 343 (MH^+, 9%) and 111 (100) (Found: MH^+ 343.2106. $C_{18}H_{31}O_6$ requires 343.2121). Irradiation at δ2.4 simplified H-10 to a major doublet *J* 15 and a minor doublet *J* 11.

Ethyl 4-[(2S,3R,4R,5S)-3,4-dihydroxy-5-(5-hydroxy-4(S)-methylpent-2-enyl)tetrahydropyran-2-yl]-3-methylbut-2(E)-enoate (**9f**) - Prepared from (*R*)-3-hydroxy-2-methylpropyltriphenylphosphonium chloride (**7f**) giving the *E*-isomer (20mg, 12%) and *Z*-isomer (32mg, 19%) as colourless oils. *E*-isomer: υ_{max} (neat)/cm^{-1} 3400 (OH), 1705 and 1690 (C=O), and 1640 (C=C); λ_{max} (EtOH)/nm 220; δ_H ($CDCl_3$) 0.96 (3H, d, *J* 7, 17-H_3), 1.27 (3H, t, *J* 7, ester-Me), 1.62 (1H, bs, 8-H), 2.1-2.4 (7H, m, 4-H, 9-H_2, 12-H and 15-H_3), 2.6-2.7 (3H, m, 4-H and OH_2), 3.3-3.9 (7H, m, 5H, 6-H, 7-H_2, and 16H_2), 4.14 (2H, q, *J* 7, ester-CH_2), 5.32 (1H, dd, J 15 and 8, 11-H), 5.47 (1H, dt, *J* 15 and 8, 11-H), and 5.77 (1H, s, 2-H); *m/z* (EI) 343 (MH^+, 46%) and 83 (100). *Z*-isomer; υ_{max} (neat)/cm^{-1} 3400 (OH), 1710 and 1695 (C=O), and 1645 (C=C); λ_{max} (EtOH)/nm 220; δ_H ($CDCl_3$) 0.93 (3H, d, J 7, 17-H_3), 1.27 (3H, t, *J* 7, ester-Me), 1.65 (1H, bs, 8-H), 2.1-2.4 (7H, m, 4-H, 9-H_2,

12-H and 15-H_3), 2.61 (1H, d, *J* 14, 4-H), 3.35 (1H, ddd, J 5, 8, and 10, 13-H), 3.5 (2H, m, 6-H, and 13-H), 3.60 (1H, dd, J 2 and 12, 16-H), 3.72 (1H, dt, *J* 3 and 9, 5-H), 3.77 (1H, dd, J 3 and 12, 16-H), 3.91 (1H, bs, 7-H), 4.14 (2H, q, *J* 7, ester-CH_2), 5.28 (1H, dd, *J* 10 and 11, 11-H), 5.44 (1H, dt, *J* 8 and 11, 10-H), and 5.75 (1H, s, 2-H); *m/z* (NH_3 CI) 343 (MH^+).

Ethyl 4-[(2S,3R,4R,5S)-3,4-dihydroxy-5-(prop-2-enyl)tetra-hydropyran-2-yl]-3-methylbut-2(E)-enoate (**9g**) - *n*-Butyllithium (1.6M in hexane; 0.25ml, 0.40mmol) was added to a suspension of methyltriphenylphosphonium bromide (0.14g, 0.40mmol) in tetrahydrofuran (2cm^3) at 0°C. After 1h at 0°C, a solution of aldehyde (**10**) (70mg, 0.21mmol) in more tetrahydrofuran (1cm^3) was added. After 10min at 0°C 3M aqueous ammonium chloride (20cm^3) was added, and the mixture extracted with ethyl acetate (2x20cm^3). The combined extracts were dried and evaporated to dryness. Chromatography on silica gel eluting with hexane-diethyl ether (1:1) gave the protected olefin.

This was dissolved in acetic acid (2cm^3) and water (0.5cm^3). After 40h at 20°C evaporation and chromatography on silica gel, eluting with methanol-dichloromethane (1:9) gave (**9g**) as a colourless oil (18mg, 41%); υ_{max} (neat)/cm^{-1} 3500 (OH), 1710 and 1695 (C=O), and 1640 (C=C); λ_{max} (EtOH)/nm 220; δ_H ($CDCl_3$) 1.29 (3H, t, *J* 7, ester-Me), 1.88 (1H, bs, 8-H), 2.15-2.35 (6H, m, 4-H, 9-H_2, and 15-H_3), 2.65 (1H, d, *J* 14, 4-H), 3.47 (1H, dd, *J* 3 and 9, 6-H), 3.53 (1H, d, *J* 12, 16-H), 3.72 (1H, dt, *J* 3 and 9, 5-H), 3.81 (1H, dd, *J* 3 and 12. 16-H), 3.93 (1H, t, *J* 3, 7-H), 4.14 (2H, q, *J* 7, ester-CH_2), 5.0-5.1 (2H, m, 11-H_2), 5.7-5.9 (2H, m, 2-H and 10-H); *m/z* (EI) 285 (MH^+, 12%) and 239 (82) (Found MH^+ 285.1707. $C_{15}H_{25}O_5$ requires 285.1702).

Ethyl 4-[(3aS,4S,7S,7aR)-2,2-dimethyl-7-(2-oxoethyl)-3a,6,7,7a-tetrahydro-4H-1,3-dioxolo[4,5-c]pyran-4-yl]-3-methylbut-2(E)-enoate (**10**) - Olefin (**4**) (1.5g, 4.2mmol) was protected as its acetonide as in the preparation of ketoaldehyde (**6**). This was dissolved in dichloromethane (30cm^3)/ethanol (20cm^3) and ozonized at -70°C examining the reaction mixture periodically by t.l.c. until all the starting material had just disappeared; in this way over-oxidation to the keto-aldehyde was minimized. Dimethyl sulphide (0.6cm^3, 8.4mmol) was added and the solution then stirred for 1h at 20°C and evaporated to dryness. Chromatography on silica gel eluting with ethyl acetate-hexane (2:1) gave the aldehyde (**10**) (1.1g, 79%); υ_{max} (neat)/cm^{-1} 1720 (C=O) and 1650 (C=C); λ_{max} (EtOH)/nm; δ_H ($CDCl_3$) 1.27 (3H, t, *J* 7, ester-Me), 1.35 and 1.50 (6H, 2s, CMe_2), 2.15-2.25 (4H, m, =C-CH_2 and =C-CH_3), 2.50 (1H, d, J 13, =C-CH_2), 2.58 (1H, dd, *J* 5 and 17, CH_2-C=O), 2.6-2.7 (1H, m, 7-H), 2.79 (1H, dd, *J* 7 and 17, CH_2-C=O), 3.46 (1H, dt, *J* 3 and 9, 4-H), 3.59 (1H, d, *J* 12, 6-H), 3.69 (1H, dd, *J* 5 and 9, 3a-H), 3.75 (1H, dd, *J* 3 and 12, 6-H), 4.06 (1H, bs, 7a-H), 4.16 (2H, q, *J* 7, ester-CH_2), 5.72 (1H, s, =CH), and 9.81 (1H, s, CHO); *m/z* (EI) 327 (MH^+, 1%) and 199 (63).

Ethyl 4-[(2S,3R,4R,5S)-3,4-dihydroxy-5-oxiranylmethyltetra-hydropyran-2-yl]-3-methylbut-2(E)-enoate (**11**) - Oxone® (90mg, 0.3mmol) was added to a mixture of water (1.0cm^3), acetone (0.7cm^3), and sodium hydrogen carbonate (45mg, 0.6mmol). After 15min at 20°C, olefin (**9g**) (10mg, 0.035mmol) in more acetone (0.3cm^3) was added, and after a further 20min ethyl acetate (10cm^3)was added and the solution washed with 1M aqueous sodium hydrogen sulphite (10cm^3), dried, and evaporated to dryness. Chromatography on silica gel, eluting with ethyl acetate-hexane (2:1) gave (**11**) (6mg, 57%); as a 1:1 diastereoisomeric mixture; δ_H ($CDCl_3$) 1.30 (3H, t, *J* 7, ester-Me), 1.7-2.1 (3H, m, 8-H and 9-H_2), 2.21 (3H, s, 15-H_3), 2.2 (1H, m, 4-H), 2.4-2.7 (2H, m, 4-H and 10-H), 2.8-2.9 and 3.0-3.1 (2H, 2m, 11-H_2), 3.45-4.05 (5H, m, 5-H, 6-H, 7-H, and 16-

H_2), 4.14 (2H, q, *J* 7, ester-CH_2), and 5.75 (1H, s, 2-H); *m/z* (EI) 300 (M^+, 1%) and 111 (100%) (Found: M^+ 300.1573. $C_{15}H_{24}O_6$ requires 300.1572).

Ethyl 4-[(2S,3R,4R,5S)-3,4-dihydroxy-5-(4-hydroxybut-2(E)-enyl)tetrahydropyran-2-yl]-3-methylbut-2(E)-enoate (**12**) - A solution containing aldehyde (**10**) (0.39g, 1.2mmol), formylmethylene-triphenylphosphorane (0.36g, 1.2mmol) and dichloromethane (5cm^3) was stood at 20°C for 3d. The product was isolated by chromatography on silica, eluting with hexane-ethyl acetate (1:1) to give crude allylic aldehyde which was immediately dissolved in methanol (5cm^3) and sodium borohydride (20mg, 0.5mmol) added. After 30min at 20°C, acetic acid (0.5cm^3) was added and after a further 30min at 20°C the solution was evaporated to dryness. Chromatography on silica gel eluting with ethyl acetate-hexane (2:1) gave allylic alcohol (**14**) which was treated with acetic acid (3cm^3) and water (1cm^3) for 18h at 20°C then evaporated to dryness. Chromatography on silica gel eluting with dichloromethane-methanol (9:1) gave (**12**) as a colourless oil (57mg, 15%); υ_{max} (neat)/cm^{-1} 3400 (OH), 1705 and 1690 (C=O), and 1645 (C=C); λ_{max} (EtOH)/nm 220; δ_H ($CDCl_3$) 1.28 (3H, t, *J* 7, ester-Me), 1.87 (1H, bs, 8-H), 2.1-2.4 (6H, m, 4-H, 9-H_2, and 15-H_3), 2.65 (1H, d, *J* 15, 4-H), 3.44 (1H, d, *J* 9, 6-H), 3.52 (1H, d, *J* 13, 16-H), 3.72 (1H, dt, *J* 3 and 9, 5-H), 3.83 (1H, dd, *J* 3 and 12, 16-H), 3.90 (1H, t, *J* 3, 17-H), 4.05-4.2 (4H, m, ester-CH_2 and 12-H_2), 5.71 (2H, bs, 10-H and 11-H), and 5.79 (1H, s, 2-H); *m/z* (EI) 315 (MH^+, 1%) and 81 (100) (Found MH^+ 315.1796. $C_{16}H_{27}O_6$ requires 315.1808). The assignment of stereochemistry at C10-C11 was based on a 15Hz coupling constant between 10-H and 11-H in the aldehyde intermediate described herein.

Ethyl 4-[(2S,3R,4R,5S)-5-(4-aminobut-2(E)-enyl)-3,4-dihydroxytetrahydropyran-2-yl]-3-methylbut-2(E)-enoate (**13**) - A solution of alcohol (**14**) (60mg, 0.17mmol), prepared as in the previous procedure, in dichloromethane (2cm^3), was treated with triethylamine (0.14cm^3, 1.0mmol) acetic anhydride (0.10cm^3, 1.0mmol) and 4-dimethylaminopyridine (10mg). After 1h at 20°C, more dichloromethane (20cm^3) was added and the solution washed with 0.5M aqueous citric acid (20cm^3), 1M aqueous sodium hydrogen carbonate (20cm^3), and brine(20cm^3), dried, and evaporated to dryness giving the acetate as an oil.

This was dissolved in tetrahydrofuran (3cm^3) and water (1cm^3), and tetrakis(triphenylphosphine)-palladium (12mg, 0.01mmol) and sodium azide (13mg, 0.20mmol) were added. After 18h at 20°C, water (20cm^3) and diethyl ether (20cm^3) were added, then the organic layer was separated, dried, and evaporated to dryness. Purification by chromatography on silica gel eluting with diethyl ether-hexane (1:1) gave the azide as a colourless oil.

This was dissolved in acetonitrile (1.5cm^3) and triethylammonium *tris*(phenylthio)stannate (1-)[17] (0.1M in acetonitrile; 1.5cm^3, 0.15mmol) was added. After 30min at 20°C the solution was evaporated to dryness. The residue was dissolved in ethanol (10cm^3) and potassium carbonate (50mg) was added. After stirring at 20°C for 20min the mixture was filtered and the filtrate evaporated to dryness. Chromatography on silica gel eluting with methanol-dichloromethane (1:9) gave the amine acetonide which was dissolved in aqueous hydrochloric acid (0.5M, 2cm^3). After 30min at 20°C evaporation to dryness gave amine (**13**) as the hydrochloride (5mg, 9%); δ_H (CD_3OD) 1.27 (3H, t, *J* 7, ester-Me), 1.83 (1H, bs, 8-H), 2.15-2.4 (6H, m, 4-H, 9-H_2 and 15-H_3), 2.67 (1H, d, *J* 14, 4-H), 3.45-3.85 (7H, m, 5-H, 6-H, 7-H, 12-H_2, and 16-H_2), 4.11 (2H, q, *J* 7, ester-CH_2), 5.61 (1H, dt, J 15 and 7, 10-H), 5.73 (1H, s, 2-H), and 5.89m (1h, dt, *J* 15 and 7, 11-H); *m/z* (glycerol flow FAB) 314 (MH^+).

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