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3-HYDROXYMUSCARINES FROM L-RHAMNOSE

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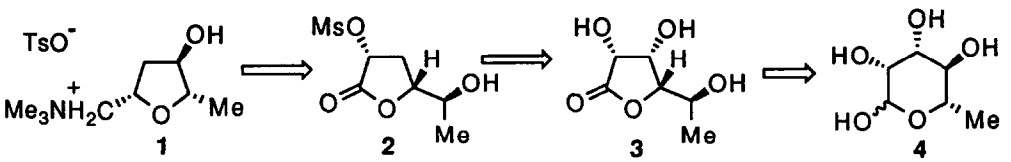
Abstract: No protection is necessary for the synthesis of the muscarine analogue 3R-3-hydroxymuscarine from L-rhamnose; a sole silyl ether protecting group is required for the synthesis of 3S-3-hydroxymuscarine. Efficient construction of the tetrahydrofuran ring can be achieved either by ring contraction of the α-triflate of δ-rhamnonolactone, or by ring opening and closing of the α-triflate of γ-rhamnonolactone.

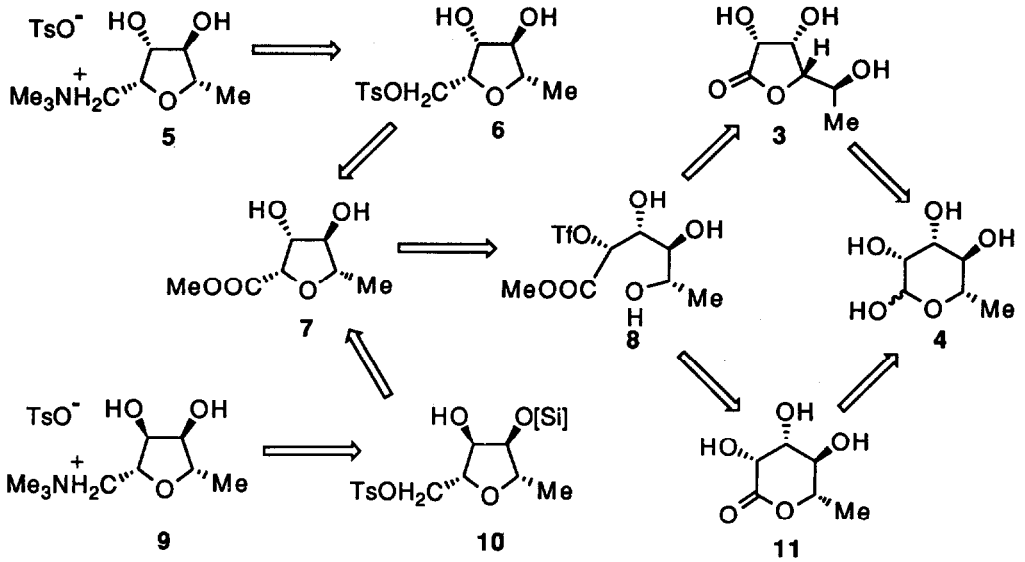
There remains much interest in the synthesis of muscarine (1) and of muscarine analogues in the hope that such agents may provide clues to the nature of selective interactions with individual muscarinic receptors and thus to chemotherapeutically useful compounds as specific muscarinic agonists or antagonists.[1] The selective degeneration of cholinergic neurons that project from the basal forebrain to the cerebral cortex and the hippocampus forms the basis of the cholinergic hypothesis of Alzheimer's disease;[2] substantial investigations have been reported on the synthesis of cholinomimetics[3] as potential drugs for the treatment of Alzheimer's disease.[4] The synthesis of muscarine and of many of its analogues have been reviewed;[5] however, no optically active analogues of muscarine with substituents in the methylene group of the muscarine ring have been described. Such materials may be of interest for studying their interactions with muscarinic receptor subtypes. This paper describes the synthesis of the epimeric 3-hydroxymuscarines (5) and (9) from L-rhamnose (4); part of this work has appeared in a preliminary communication.[6]



Scheme 1.

L-Rhamnose (4) [Scheme 1] is an excellent starting material for the synthesis of muscarine itself, since it has the correct functionality and absolute and relative stereochemistry at C-6, C-5, C-4 and C-2 if the tetrahydrofuran ring is constructed by an approach in which the oxygen at C-5 of the sugar induces nucleophilic displacement of a leaving group at C-2.[7] Save for the need to remove the hydroxyl group at C-3, the

stereochemistry and functionality at all the carbons of rhamnose is suitable for the transformation to muscarine.[8] Thus, oxidation of rhamnose gave the γ-lactone (3) which allowed selective mesylation of the C-2 OH alcohol functionality and removal of the C-3 hydroxyl group to give (2); reduction of (2) gave an open chain dihydroxymesylate which closed with inversion of configuration at C-2 to give a tetrahydrofuran suitable for subsequent elaboration to muscarine.

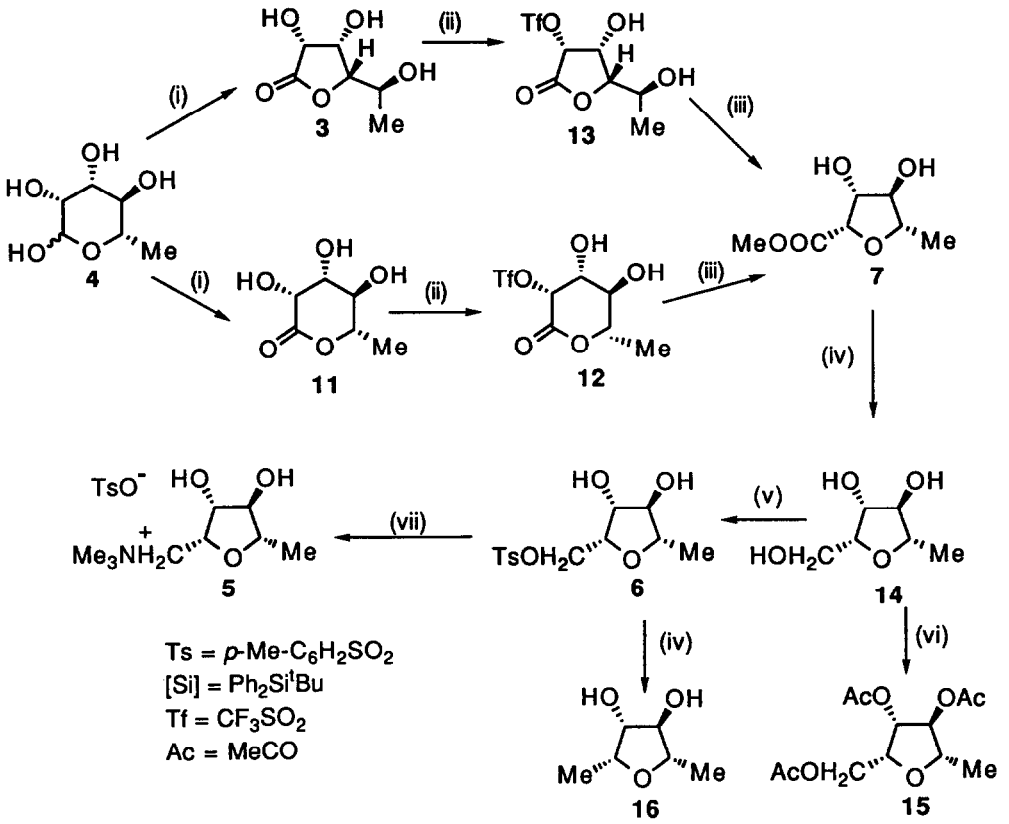


Scheme 2.

If the oxygen at C-3 of rhamnose is not removed, the same basic synthetic strategy [Scheme 2] allows easy access to both 3-hydroxymuscarines (5) and (9) *via* the tetrahydrofuran methyl ester (7); the key intermediate (7) is derived from ring closure of the open chain hydroxytriflate (8) generated from either the γ- (3) or δ- (11) lactones. Reduction of (7) allows the synthesis of the tosylate (6) which with trimethylamine gives 3R-3-hydroxymuscarine in a sequence which does not require the use of any protecting group. Alternatively, the C-4 hydroxyl group in (7) may readily be protected to allow inversion of the C-3 hydroxyl group and elaboration to (10); this sequence, with only a silyl ether protection of the C-4 hydroxyl group, allows the synthesis of 3-S-3-hydroxymuscarine (9).

For the synthesis of the tetrahydrofuran ester (7) [Scheme 3], oxidation of rhamnose (4) with bromine buffered with barium carbonate gave a mixture of the γ- (3) and δ- (11) lactones[9] in 75% yield. The δ-lactone (11) was the major product (about 65% as judged by the integration of the methyl peaks in the 1H NMR) in the mixture formed under kinetic conditions and could be isolated in about 35% overall yield by a single crystallisation from acetone. The remaining mixture could be quantitatively converted into the thermodynamically more stable γ-lactone (3) by treatment with aqueous trifluoroacetic acid. In general, α-hydroxy groups in lactones are more nucleophilic than any other secondary alcohol functions. Thus esterification of (3) with triflic anhydride in pyridine:tetrahydrofuran gave the stable 2-O-triflate (13) in 85% yield. Treatment of methanolic solution of (13) with pyridine gave, after three days at room temperature, the required tetrahydrofuran (7) in 79% yield [67% yield from (3)]. Alternatively, treatment of the δ-lactone (11) with triflic anhydride gave predominantly the α-

triflate (12); unlike the γ-lactone isomer, (12) was unstable and was worked up in the presence of methanol to give (7) in 57% overall yield. This base-induced ring contraction of the δ-lactone triflate (12) is an example of a general procedure for the synthesis of highly functionalised tetrahydrofurans.[10,11]



Scheme 3. (i) Br_2, $BaCO_3$, H_2O, see text (ii) $(CF_3SO_2)_2O$, pyridine, THF (iii) MeOH, pyridine (iv) $LiAlH_4$, THF (v) *p*-Me-C_6H_4-SO_2Cl, DMAP, pyridine (vi) Ac_2O, DMAP, pyridine (vii) Me_3N, MeOH

The conversion of the δ-lactone triflate (12) to (7) is much faster than that of the γ-lactone isomer (13), presumably because the ring opening of the lactones to the open chain hydroxytriflate (8) is more rapid for the six-ring lactone. Because of the instability of the δ-ester (12), larger scale preparations of (7) are more conveniently carried out via the γ-lactone route which gives (7) in an overall yield of 50% from L-rhamnose. The stereochemistry of the carboxylate in (7) was firmly established by X-ray crystallography [Figure] which showed that the tetrahydrofuran ring had been formed with inversion of configuration at C-2.

Reduction of the methyl ester (7) with lithium aluminum hydride in tetrahydrofuran afforded the glucitol (14) [77% yield] which was very hygroscopic and difficult to purify completely; (14) was fully characterised as the triacetate (15), formed by reaction of (14) with acetic anhydride in pyridine in the presence of 4-(N,N-dimethylamino)pyridine (DMAP). Treatment of the triol (14) with tosyl chloride in pyridine with DMAP resulted in selective esterification of the primary hydroxyl function to give the tosylate (6) in 38% yield. It was just

possible that reduction of the ester (7) to the triol (14) had been accompanied by a change in configuration at the ester-bearing carbon; however, reduction of the tosylate (6) with lithium aluminum hydride in tetrahydrofuran afforded (16) which has six different signals for carbon atoms in the ^{13}C NMR. This demonstrates that epimerisation at C-2 did not occur during the reduction of the ester as the alternative product would have possessed a two-fold axis of symmetry. Reaction of the tosylate (6) with trimethylamine in methanol gave 3-R-3-hydroxymuscarine in 82% yield [23% yield from (7)].

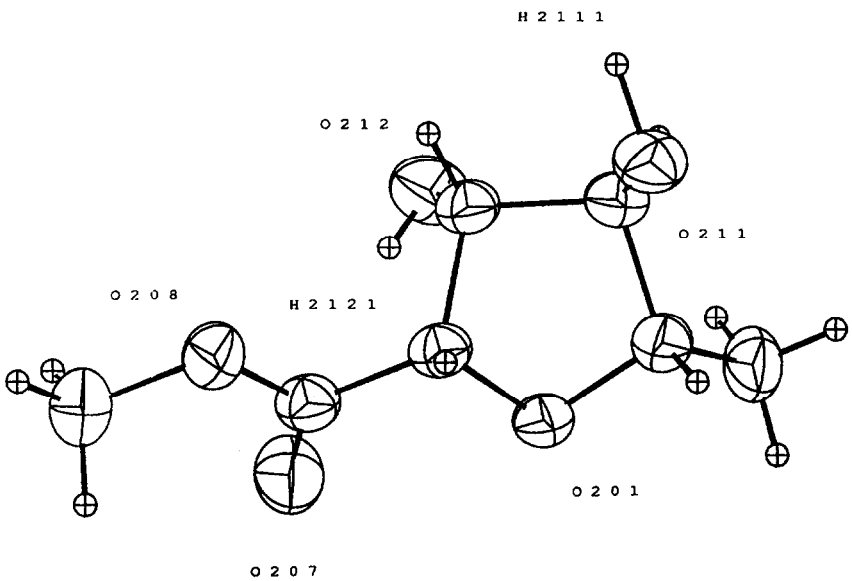
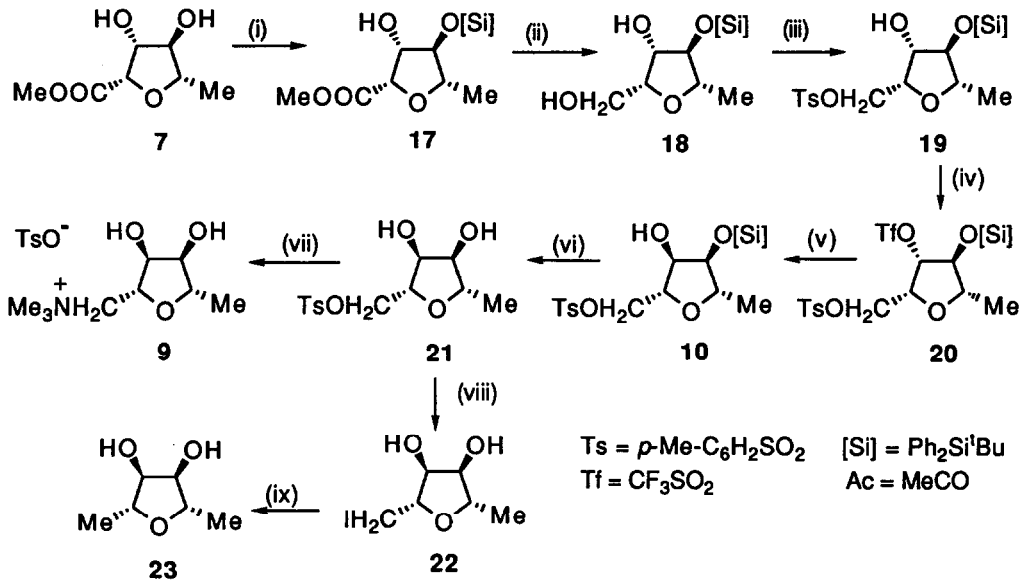


Figure: X-Ray Crystal Structure of Methyl 2,5-Anhydro-6-deoxy-L-gluconate

For the synthesis of 3S-3-hydroxymuscarine (9) [Scheme 4], it is necessary to protect the C-4 hydroxyl group in (7). Reaction of (7) with *tert*-butyldimethylsilyl chloride gave a mixture of silyl ethers; however, treatment of (7) with the highly bulky *tert*-butyldiphenylsilyl chloride in dimethylformamide in the presence of imidazole resulted in the selective formation of (17) in 60% yield. Reduction of the ester (17) with lithium borohydride in tetrahydrofuran gave the diol (18) [76% yield] which underwent selective esterification of the primary hydroxyl group by tosyl chloride in pyridine to afford (19) [85% yield]. In order to invert the configuration of at C-3, the remaining free hydroxyl group in (19) was converted to the corresponding triflate (20) [67% yield]. Treatment of (20) with sodium trifluoroacetate in dimethylformamide resulted in displacement

of the secondary triflate function while leaving the primary tosylate unaffected to give, after work-up in methanol, the inverted alcohol (10) [72% yield]. The silyl protecting group in (10) was removed by the action of tetra-*n*-butylammonium fluoride in tetrahydrofuran to form the tosylate (21) in 78% yield. The overall stereochemistry in (21) was firmly established by reaction of (21) with sodium iodide in butanone to give the iodide (22) [90% yield] which on hydrogenation gave the optically inactive (23) [85% yield]. Additionally, (23) only had three signals in the ^{13}C NMR, indicating the compound has a plane of symmetry; these results clearly demonstrate that the configuration at C-2 has remained the same during these interconversions, but that the stereochemistry of the hydroxyl group at C-3 has been inverted. Reaction of the tosylate (21) with trimethylamine in methanol gave the target 3S-3-hydroxymuscarine (9) in 82% yield [12% from (7)]



Scheme 4. (i) *tert*$BuPh_2SiCl$, imidazole, DMF (ii) $LiBH_4$, THF (iii) *p*-Me-C_6H_4-SO_2Cl, DMAP, pyridine (iv) $(CF_3SO_2)_2O$, pyridine, THF (v) CF_3COONa, DMF; then MeOH, AcOH (vi) Bu_4NF, THF (vii) Me_3N, MeOH (viii) NaI, MeCOEt (ix) H_2, 10% Pd/C, NaOAcMeOH

In summary, this paper further demonstrates the value of L-rhamnose in the synthesis of muscarine and its analogues; 3R-3-hydroxymuscarine (5) has been prepared in a sequence in which no protection of any of the oxygen functions is necessary. The tetrahydrofuran (7) is a highly divergent intermediate for the synthesis of muscarine analogues with functional groups at C-3 of muscarine and this is illustrated in the synthesis of the epimeric 3S-3-hydroxymuscarine (9) and clearly can be used for the synthesis of muscarine derivatives with other functional groups in the ring methylene position. The biological properties of the hydroxymuscarines and of other analogues will be reported elsewhere.

Acknowledgements: This work has been supported by a SERC graduate award (S. J. M.).

Experimental

Melting points were recorded on a Kofler hot block. Proton nuclear magnetic resonance (δ_H) spectra were recorded on Varian Gemini 200 (at 200 MHz) or Bruker AM 500 (500 MHz) spectrometers. Carbon nuclear magnetic resonance (δ_C) spectra were recorded on a Varian Gemini 200 (50 MHz) or a Bruker AM 500 (125 MHz) spectrometer. Multiplicities were assigned using DEPT sequence. Spectra run in D_2O were referenced to dioxan (δ 67.3) or methanol (δ_C 49.7) as internal standards. All chemical shifts are quoted on the δ-scale. Infrared spectra were recorded on a Perkin-Elmer 150 Fourier Transform spectrophotometer. Mass spectra were recorded on VG Micromass 30F, ZAB 1F, Masslab 20-250 or Trio-1 GCMS (DB-5 column) spectrometers using desorption chemical ionisation (NH_3 DCI), electron impact (EI), chemical ionisation (NH_3 CI) and fast atom bombardment (FAB) techniques, as stated. Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a path length of 1 dm. Concentrations were given in g/100 ml. Hydrogenations were run under an atmosphere of hydrogen gas maintained by inflated balloon. Microanalyses were performed by the microanalysis service of the Dyson Perrins laboratory. Thin layer chromatography (t.l.c.) was carried out on aluminium sheets coated with $60F_{254}$ silica. Plates were developed using 0.2% w/v cerium (IV) sulphate and 5% ammonium molybdate in 2M sulphuric acid. Flash chromatography was carried out using Sorbsil C60 40/60 silica. Solvents and commercially available reagents were dried and purified before use according to standard procedures; methanol was distilled from magnesium methoxide, pyridine was distilled from calcium hydride and stored over potassium hydroxide and tetrahydrofuran was distilled from a purple solution of sodium benzophenone ketyl immediately before use. *p*-Toluenesulphonyl chloride and imidazole were recrystallised from hexane and ethanol respectively. Hexane was distilled at 68°C before use to remove involatile fractions. L-Rhamnose (4) was obtained from Sigma Chemical Company.

L-Rhamnono-1,5-lactone (11) and L-Rhamnono-1,4-lactone (3). Barium carbonate (81.40 g, 275.0 mmol, 1.5 equiv) was added to a solution of L-rhamnose monohydrate (4) (50.0 g, 275.0 mmol) in distilled water (350 ml). Bromine (15.4 ml, 302.5 mmol, 1.1 equiv) was added (3 x 5 ml) at twenty minute intervals to the stirred solution at 0°C. The reaction mixture was stirred at this temperature for 1 h and then for 6 h at room temperature. T.l.c. (methanol : ethyl acetate 1: 9) indicated that the starting material (R_f 0.1) had been replaced by two products (R_f 0.35) and (R_f 0.4). Excess barium carbonate was filtered off through Celite and the residual solid washed with distilled water (2 x 50 ml). Compressed air was passed through the filtered solution until all the colour had disappeared. The solvent was removed to give a white solid which was extracted with boiling acetone (3 x 1 l). Solvent removal afforded a mixture of title lactones (11) and (3) (33.10 g, 75% yield) in approximately 2:1 ratio as estimated by ^1H NMR integration of the methyl protons in the two lactones. A portion of the mixture was recrystallised from acetone to give L-rhamnono-1,5-lactone (11) (13.0 g). m.p. indeterminate due to phase change; $[\alpha]_D^{20}$ -104.9 (*c*, 3.0 in H_2O) {lit[9] $[\alpha]_D^{24}$ -98 (*c*, 3.0 in H_2O)}; υ_{max} (KBr): 1727 (s, C=O) cm^{-1}; δ_H (200MHz; D_2O): 1.23 (3H, d, *J* 6.5, H-6), 3.44 (1H, dd, *J* 1.5, 9, H-4), 3.95 (1H, dd, *J* 1.5, 4, H-3), 4.13 (1H, m, *J* 6.5, 9, H-5), 4.55 (1H, d, *J* 4, H-2); δ_C (50.3MHz; D_2O): 18.6 (q, C-6), 68.8, 75.1, 76.3, 77.0 (4xd, C-2, C-3, C-4, C-5), 176.1 (s, C-1); m/z (CI NH_3): 180 ($M+NH_4^+$, 100), 163 ($M+H^+$, 5%).

Aqueous trifluoroacetic acid (100 ml, 1:1 w/v) was added to the residual mixture of lactones (20 g), the reaction mixture was warmed until all the solid dissolved and then stirred at room temperature for 4 h at which point t.l.c. (methanol : ethyl acetate 1:9) indicated that conversion to the 1,4-lactone (3) (R_f 0.35) was complete. The solvent was removed to give L-rhamnono-1,4-lactone (3) (quantitative yield) which could be used in subsequent

experiments without further purification, m.p. indeterminate due to phase change. $[\alpha]_D^{20}$ -39.4 (*c*, 2.0 in water) {lit.[9] m.p. 148-150°C, $[\alpha]_D^{24}$ -40 (*c*, 2.0 in water)}. υ_{max} (KBr): 3300 (br, OH), 1776 (C=O) cm^{-1}; δ_H (200MHz; D_2O): 1.14 (3H, d, *J* 6, H-6), 3.74-3.97 (1H, m, H-5), 4.05 (1H, dd, *J* 3, 8, H-4), 4.41 (1H, dd, *J* 3, 4.5, H-3), 4.52 (1H, d, *J* 4.5, H-2); δ_C (D_2O; 50.3MHz): 19.5 (q, C-1), 64.5, 70.1, 71.6 (3xd C-2, C-3, C-5), 83.8 (d, C-4), 179.3 (s, C-1); m/z (CI NH_3): 180 ($M+NH_4^+$, 36), 163 ($M+H^+$, 100%).

2-O-Trifluoromethanesulphonyl-L-rhamnono-1,4-lactone (13). Trifluoromethanesulphonic anhydride (5.23 ml, 28.5 mmol, 1.1 equiv) was added dropwise to a stirred solution of L-rhamnono-1,4-lactone (3) (4.20 g, 25.9 mmol) at -20°C in pyridine : tetrahydrofuran (1:3) (90 ml). After two hours at -20°C, further trifluoromethanesulphonic anhydride (1.3 ml, 7.8 mmol, 0.3 equiv) was added at -20°C and then stirred at 0°C for a further hour, at which point t.l.c. indicated nearly complete conversion to product (R_f 0.8). The reaction mixture was partitioned between ethyl acetate (100 ml) and aqueous hydrochloric acid (2N, 100 ml); the aqueous phase was extracted with ethyl acetate (2 x 100 ml) and the combined organic extracts were washed with brine (50 ml) and dried (magnesium sulphate). The solvent was removed *in vacuo* to give a brown amorphous solid which was purified by flash chromatography (ethyl acetate : petrol 2:3) to give *2-O-trifluoromethanesulphonyl-L-rhamnono-1,4-lactone (13)* (6.45 g, 21.9 mmol, 85% yield), a white crystalline solid, m.p. 128-130°C; $[\alpha]_D^{20}$ -7.3 (*c*, 1 in CH_3CN); (Found: C, 28.63; H, 3.03. $C_7H_9SO_7F_3$ requires C, 28.58; H, 3.08%); υ_{max} (KBr): 1801 (s, C=O) cm^{-1}; δ_H (200MHz; CD_3CN): 1.27 (3H, d, *J* 6, H-6), 3.97-4.09 (1H, m, *J* 6, 8, H-5), 4.15 (1H, dd, *J* 2.5, 8, H-3), 4.76 (1H, dd, *J* 2.5, 4.5, H-3), 5.67 (1H, d, *J* 4.5, H-2); δ_C (50.3MHz; CD_3CN): 19.9 (q, C-6), 64.5, 69.3 (2xd, C-3, C-5), 82.0, 83.6 (2xd, C-2, C-4), 169.7 (s, C-1); m/z (CI NH_3): 312 ($M+NH_4^+$, 100) 194 (8%).

Methyl 2,5-Anhydro-6-deoxy-L-gluconate (7). (i) From 2-O-trifluoromethanesulphonyl-L-rhamnono-1,4-lactone (13). Pyridine (10.6 ml, 132.0 mmol, 6 equiv) was added to a solution of 2-*O*-trifluoromethanesulphonyl-L-rhamnono-1,4-lactone (13) (6.45 g, 22.0 mmol) in methanol (120 ml). The solution was allowed to stand at room temperature for three days until t.l.c. (ethyl acetate) indicated the disappearance of starting material (R_f 0.6) and the appearance of the major product (7) (R_f 0.3) and a trace of a minor product (R_f 0.35) . The solvent was removed *in vacuo*, the residue pre-absorbed onto silica and purified by flash chromatography (ethyl acetate : petrol 1:1, then 3:1) to give *methyl 2,5-anhydro-6-deoxy-L-gluconate (7)* (2.84 g, 79% yield), a white crystalline compound, m.p. 83-84°C; $[\alpha]_D^{20}$ -12.4 (*c*, 1.0 in CH_3CN); (Found: C, 47.99; H, 7.00. $C_7H_{12}O_5$ requires C, 47.73; H, 6.87%); υ_{max} (KBr): 3420 (OH), 1746 (C=O) cm^{-1}; δ_H (500MHz; CD_3OD): 1.36 (3H, d, *J* 6.5, H-6), 3.74 (3H, s, CO_2Me), 3.75-3.77 (1H, m, H-4), 3.83 (1H, dq, *J* 4.2, 6.5, H-5), 4.23 (1H, dd, *J* 2.7, 5.1, H-3), 4.56 (1H, d, *J* 5.1, H-2); δ_C (50.3MHz; CD_3CN): 18.2 (q, C-6), 51.2 (q, CO_2Me), 78.9, 80.3, 81.3, 82.1 (d, C-2, C-3, C-4, C-5), 170.4 (s, C-1); m/z (DCI NH_3): 194 ($M+NH_4^+$, 93), 177 ($M+H^+$, 100%).

(ii) From L-rhamnono-1,5-lactone (11). Trifluoromethanesulphonic anhydride (8.1 ml, 47.5 mmol, 1.1 equiv) was added to a stirred solution of L-rhamnono-1,5-lactone (11) (7.00 g, 43.2 mmol) in tetrahydrofuran : pyridine (2:1, 60 ml) at -30°C . The reaction was stirred as the temperature was allowed to rise to -5°C. After 0.3 h, t.l.c. (ethyl acetate) showed a reduction in starting material (R_f 0.15) and the appearance of the triflate (12) (R_f 0.6). The solution was cooled to -30°C and further trifluoromethanesulphonic anhydride (1.0 ml, 5.6 mmol, 0.13 equiv) was added. The reaction mixture was allowed to warm to -5°C and stirred for 0.5 h when t.l.c. indicated that all starting material had disappeared. The solution was cooled to -30°C and methanol (100 ml) was added.

After 2 h at room temperature, t.l.c. (ethyl acetate) indicated the absence of the triflate (12) and formation major product (7) (R_f 0.3). The solvent was removed *in vacuo* to give a thick brown gum which was flushed through a silica plug (ethyl acetate); the solvent was removed and the residue was purified by flash chromatography (ethyl acetate : petrol 3:1) to give *methyl 2,5-anhydro-6-deoxy-L-gluconate* (7) (4.35 g, 24.7 mmol, 57% yield), identical to material produced by method (i) above.

2,5-Anhydro-6-deoxy-L-glucitol (14). Methyl 2,5-anhydro-6-deoxy-L-gluconate (7) (0.900 g, 5.49 mmol) was added portionwise to a stirred suspension of lithium aluminium hydride (0.620 g, 16.5 mmol, 3.2 equiv) in tetrahydrofuran at 0°C. After effervescence had ceased, the reaction was warmed to room temperature and t.l.c. (methanol : ethyl acetate 1:9) indicated starting material disappearance (R_f 0.5) and the formation of (14) (R_f 0.3). The reaction was quenched by the careful dropwise addition of methanol until gas evolution ceased. The solution pH was then adjusted to 2 with aqueous hydrochloric acid (2N) and the solvent removed *in vacuo*. The residue was redissolved in methanol and flushed through a Celite plug. Solvent evaporation gave a yellow oil which was purified by flash chromatography [ethyl acetate, then methanol : ethyl acetate (1:19)] to give *2,5-anhydro-6-deoxy-L-glucitol (14)* (0.578 g, 3.91 mmol, 77% yield) as an impure yellow gum, υ_{max} (thin film): 3369 (s, OH) cm^{-1}; δ_H (500MHz; CD_3OD): 1.31 (3H, d, *J* 6.2, H-6), 3.67 (1H, dd, *J* 2.6, 4.7), 3.69-3.73 (2H, m), 3.79 (1H, dd, *J* 4.6, 11.7), 3.95 (1H, dt, *J* 4.6, 6.3), 4.05 (1H, dd, *J* 2.6, 4.7); δ_C (50.3MHz; CD_3CN): 19.0 (q, C-6), 61.6 (t, C-1), 74.9, 79.7, 82.6x2 (4xd, C-2, C-3, C-4, C-5); m/z (CI NH_3): 166 ($M+NH_4^+$, 100), 149 ($M+H^+$, 12%). Since it was not possible to purify (14), it was characterised as the triacetate (15).

1,3,4-O-Acetyl-2,5-anhydro-6-deoxy-L-glucitol (15). Acetic anhydride (1 ml, 10.1 mmol, 10 equiv) and a catalytic amount of N,N-dimethylaminopyridine was added to 2,5-anhydro-6-deoxy-L-glucitol (14) (0.15 g, 1.0 mmol) in pyridine (5 ml) at 0°C. The reaction mixture was stirred for 16 h at room temperature when t.l.c. (ethyl acetate) showed two spots at (R_f 0.6) and the triacetate (15) (R_f 0.65). More acetic anhydride (0.5 ml, 5.0 mmol, 5 equiv) was then added at room temperature. After stirring for a further 6 h, the reaction was quenched by the addition of methanol (3 ml). The solvent was removed *in vacuo* and the residue partitioned between ethyl acetate (80 ml) and aqueous hydrochloric acid (2N, 20 ml). The organic phase was washed with brine (10 ml) and dried (magnesium sulphate). Evaporation of the solvent produced a yellow oil which was purified by flash chromatography (ethyl acetate : petrol, 3:7) to yield *1,3,4-O-acetyl-2,5-anhydro-6-deoxy-L-glucitol (15),* oil, (0.105g, 0.38 mmol, 38% yield), $[\alpha]_D^{20}$ -15.7 (*c*, 1.2 in $CHCl_3$); (Found: C, 52.59; H, 6.73. $C_{12}H_{18}O_7$ requires C, 52.55; H, 6.61%); υ_{max} (thin film): 1746 (C=O) cm^{-1}; δ_H (500MHz; $CDCl_3$): 1.40 (3H, d, *J* 6.4, H-6), 2.08, 2.09, 2.10 (3 x 3H, s, 3 x $C\underline{H}_3CO$), 3.93 (1H, dq, *J* 4.1, 6.4, H-5), 4.15 (1H, dd, *J* 6.6, 11.1, H-1), 4.24-4.28 (1H, m, H-2), 4.30 (1H, dd, *J* 4.5, 11.1, H-1'), 4.79 (1H, dd, *J* 1.7, 4.1), 5.30 (1H, dd, *J* 1.7, 4.1); δ_C (50.3MHz; $CDCl_3$): 18.7 (q, C-6), 20.5, 20.6 (q, 3x$CO\underline{C}H_3$), 62.1 (t, C-1), 77.2, 77.4 (2 x d, C-2, C-5), 80.0, 82.3 (2 x d, C-3, C-4), 169.9, 170.1, 170.9 (3 x s, 3 x $CH_3\underline{C}O$); m/z (CI NH_3): 292 ($M+NH_4^+$, 38), 275 ($M+H^+$, 100%).

2,5-Anhydro-1-O -p-toluenesulphonyl-6-deoxy-L-glucitol (6). *p*-Toluenesulphonyl chloride (0.722 g, 3.79 mmol, 0.8 equiv) in tetrahydrofuran (2 ml) was added to 2,5-anhydro-6-deoxy-L-glucitol (14) (0.694 g, 4.75 mmol) in the presence of a catalytic amount of N,N-dimethylaminopyridine in pyridine (4 ml) at 0°C. The reaction was stirred for 2 h at 0°C and then at room temperature for 3 h. T.l.c. (ethyl acetate) indicated

disappearance of starting material (R_f 0.25) and the formation of product (6) (R_f 0.6). The reaction was quenched by the addition of methanol (2 ml). The solvent was removed *in vacuo* and the brown gum azeotroped with toluene (2 x 3 ml). The resulting residue was purified by flash chromatography (ethyl acetate : petrol, 1:1 then 3:1) to give *2,5-anhydro-1-O -p-toluenesulphonyl-6-deoxy-L-glucitol (6)*, a white solid (0.535 g, 1.77 mmol, 38% yield), m.p. 132-133°C; $[\alpha]_D^{20}$ +4.0 (*c*, 0.9 in CH_3CN); (Found: C, 51.50; H, 6.07. $C_{13}H_{18}O_6S$ requires C, 51.64; H, 6.00%); υ_{max} (KBr): 3426 (OH) cm^{-1}; δ_H (500MHz; CD_3OD): 1.23 (3H, d, *J* 6.4, H-6), 2.45 (3H, s, ArMe), 3.62 (1H, dd, *J* 2.7, 4.6, H-4), 3.68 (1H, qd, *J* 4.6, 6.4, H-5), 3.99 (1H, dd, *J* 2.8, 4.2, H-3), 4.05 (1H, d, *J* 2.6, H-1), 4.07 (1H, s, H-1'), 4.27 (1H, m, H-3), 7.44 (2H, d, *J* 8.4, ArH), 7.80 (2H, d, *J* 8.4, ArH); δ_C (50.3MHz; CD_3CN): 18.3 (q, C-6), 20.5 (q, ArMe), 70.1 (t, C-1), 77.6, 78.0, 80.7, 83.1 (4xd, C-2, C-3, C-4, C-5), 128.1 (d, ArCH), 130.2 (d, ArCH), 132.9 (s, ArC), 145.7 (s, ArC); m/z (CI NH_3): 320 ($M+NH_4^+$, 100), 303 ($M+H^+$, 6%).

3R-3-Hydroxymuscarine [(2S,3R,4R,5S)-3,4-Dihydroxy-tetrahydro-N,N,N,5-tetramethyl-2-furanmethanimium tosylate] (5). 2,5-Anhydro-1-*O*-*p*-toluenesulphonyl-6-deoxy-L-glucitol (6) (0.240 g, 0.80 mmol) was dissolved in trimethylamine : methanol (15 ml, 3:1). The solution was heated under pressure in a Fisher Porter bottle to 70°C for 16 h after which time t.l.c. (ethyl acetate) showed the formation of UV active baseline products and no starting material (R_f 0.6). The solvent was removed *in vacuo* and the oily residue partitioned between ethyl acetate (20 ml) and distilled water (40 ml). The organic layer was removed and the aqueous phase extracted again with ethyl acetate (20 ml). The aqueous layer was evaporated to give *3R-3-hydroxymuscarine (5)* a colourless oil (0.235 g, 0.65 mmol, 82% yield). $[\alpha]_D^{20}$ -0.1 (*c*, 1.8 in MeOH); (Found: C, 53.19; H, 7.45; N, 4.18. $C_{16}H_{27}NO_6S$ requires C, 53.17; H, 7.53; N, 3.88%); υ_{max} (thin film): 3370 (OH) cm^{-1}; δ_H (500MHz; D_2O): 1.22 (3H, d, *J* 6.4, H6), 2.26 (3H, s, ArMe), 3.08 (9H, s, NMe), 3.39 (1H, dd, *J* 9.1, 14.3, H-1), 3.59 (1H, dd, *J* 1.9, 14.3, H-1'), 3.69 (1H, dd, *J* 1.9, 4.4, H-4), 3.72 (1H, qd, *J* 6.4, 4.4, H-5), 4.06 (1H, dd, *J* 1.9, 4.1, H-3), 4.28 (1H, m, H-2), 7.23 (2H, d, *J* 8.3, ArH), 7.55 (2H, d, *J* 8.3, ArH); δ_C (50.3MHz; D_2O): 19.0 (q, C-6), 21.2 (q, ArMe), 54.9 (q, NMe), 67.2 (t, C-1), 75.0, 79.6, 82.5, 83.0 (4xd, C-2, C-3, C-4, C-5), 126.2 (d, ArCH), 130.2 (d, ArCH), 140.6 (s, ArC), 143.1 (s, ArC); m/z (DCI NH_3): 190 (M^+, 100), 58 (22%).

2,5-Anhydro-1,6-dideoxy-L-glucitol (16). Lithium aluminium hydride (0.050 g, 1.33 mmol, 4 equiv) was added to a stirred solution of 2,5-anhydro-1-*O*-*p*-toluenesulphonyl-6-deoxy-L-glucitol (6) (0.100 g, 0.31 mmol) in tetrahydrofuran (3 ml) at 0°C. After 24 h, t.l.c. (ethyl acetate) indicated the disappearance of starting material (R_f 0.4) and the formation of a product (16) (R_f 0.2). The reaction was quenched by the addition of methanol (1 ml). The solution was filtered through a Celite plug and the filtrate preabsorbed onto silica and purified by flash chromatography (ethyl acetate : petrol 3:1, then neat ethyl acetate) to *2,5-anhydro-1,6-dideoxy-L-glucitol (16)* (0.040 g) in approximately quantitative yield, oil, $[\alpha]_D^{20}$ -28.8 (*c*, 0.9 in $CHCl_3$); (Found: C, 54.54; H, 8.93. $C_6H_{12}O_3$ requires C, 54.53; H, 9.15%); υ_{max} (thin film): 3401(OH) cm^{-1}; δ_H (200MHz; $CDCl_3$): 1.26 (3H, d, *J* 6.5, CH_3), 1.34 (3H, d, *J* 6, CH_3), 3.65-3.76 (2H, m), 3.91 (1H, dd, *J* 1.5, 4), 4.01-4.13 (1H, m); δ_C (50.3MHz; $CDCl_3$): 13.9 (q), 19.3 (q), 76.5 (d), 80.4 (d), 80.5 (d), 84.5 (d); m/z (CI NH_3): 150 ($M+NH_4^+$, 100), 133 ($M+H^+$, 20).

Methyl 2,5-Anhydro-6-deoxy-4-O-tert-butyldiphenylsilyl-L-gluconate (17). *tert*-Butyldiphenylsilyl chloride (45.8 mmol, 12 ml, 1.5 equiv) was added dropwise to a stirred solution of methyl 2,5-anhydro-6-deoxy-L-gluconate (7) (30.5 mmol, 5.00 g), N,N-dimethylaminopyridine (0.1 g) and imidazole (76.2 mmol, 5.24 g, 2.5 equiv) in N,N-dimethylformamide (40 ml). The reaction was stored at -10°C for 16 h after which time t.l.c. (ethyl acetate : petrol 1:1) indicated disappearance of starting material (R_f 0.05) and the formation of a product (R_f 0.6). Methanol (5 ml) was added to quench the reaction. The solvent was evaporated, and the residue partitioned between ethyl acetate (100 ml) and aqueous hydrochloric acid (2N, 75 ml). The aqueous phase was extracted once more with ethyl acetate (100 ml). The combined organic layers were washed with brine (30 ml) and dried (magnesium sulphate). Evaporation of solvent gave a brown, oily residue which was purified by flash chromatography (ethyl acetate : petrol 1:9, then 1:3) to give *methyl 2,5-anhydro-6-deoxy-4-O-tert-butyldiphenylsilyl-L-gluconate (17),* (7.52 g, 18.1 mmol, 60% yield) a white crystalline solid, m.p. 105-106°C; $[\alpha]_D^{20}$ -38.7 (*c*, 1 in $CHCl_3$); (Found: C, 66.39; H 7.16. $C_{23}H_{30}O_5Si$ requires C 66.64, H 7.29%); υ_{max} (KBr): 1750 (C=O) cm^{-1}; δ_H (500MHz; $CDCl_3$): D_2O exchange 1.08 (3H, d, *J* 6.6, H-6), 1.09 (9H, s, CMe_3), 3.79 (3H, s, CO_2Me), 3.89 (1H, dd, *J* 1.5, 2.9, H-4), 3.96 (1H, qd, *J* 2.9, 6.6, H-5), 4.28 (1H, dd, *J* 1.5, 3.9, H-3), 4.66 (1H, d, *J* 3.9, H-2), 7.38-7.48 (6H, m, ArH), 7.63-7.67 (4H, m, ArH); δ_C (50.3MHz; $CDCl_3$): 18.6 (q, C-6), 19.0 (s, $\underline{C}Me_3$), 26.8 (q, $C\underline{Me}_3$), 52.3 (q, CO_2Me), 79.8, 80.5, 80.6, 83.6 (4xd, C-2, C-3, C-4, C-5), 128.1, 130.3, 136.0 (4xd, ArCH), 133.3 (s, ArC), 170.3 (s, C-1); m/z (CI NH_3): 432 ($M+NH_4^+$, 45), 337 (100%).

2,5-Anhydro-4-O-tert-butyldiphenylsilyl-6-deoxy-L-glucitol (18). Lithium borohydride (40.0 mmol, 20 ml of 2N solution in tetrahydrofuran, 2 equiv) was added to a solution of methyl 2,5-anhydro-6-deoxy-4-O-*tert*-butyldiphenylsilyl-L-gluconate (17) (8.0 g, 19.3 mmol) in tetrahydrofuran (50 ml) at 0°C. Vigorous effervescence and clouding of the solution occurred as the reaction temperature was allowed to rise to room temperature. After effervescence had ceased, a t.l.c. (ethyl acetate : petrol 1:1) showed loss of starting material (R_f 0.65) and formation of (18) (R_f 0.25). The reaction was quenched by the addition of solid ammonium chloride (6 g) and the careful dropwise addition of methanol until no more gas was evolved. The reaction mixture was diluted with ethyl acetate (250 ml) and aqueous hydrochloric acid (2N, 100 ml) added slowly. The phases were shaken together and left to stand until gas evolution ceased. The aqueous phase was washed with ethyl acetate (150 ml). The combined organic phases were washed with brine (50 ml) and dried (magnesium sulphate). Solvent removal left an oily residue which was purified by flash chromatography (ethyl acetate : hexane 1:4, then 3:7) to give *2,5-anhydro-4-O-tert-butyldiphenylsilyl-6-deoxy-L-glucitol (18)* (5.89 g, 15.3 mmol, 76% yield) as a white solid, m.p. 75-76°C; $[\alpha]_D^{20}$ -17.0 (*c*, 1 in $CHCl_3$); (Found: C, 68.52; H, 8.06. $C_{22}H_{30}O_4Si$ requires C, 68.36; H, 7.82%); δ_H (500MHz; $CDCl_3$): D_2O exchanged 1.05 (3H, d, *J* 6.5, H-6), 1.09 (9H, s, CMe_3), 3.81 (1H, dd, *J* 2.1, 3.6), 3.89-3.92 (2H, m), 3.97 (1H, dd, *J* 3.9, 12.3, H-1), 4.08-4.10 (1H, m), 4.18 (1H, dd, *J* 2.1, 4.3), 7.39-7.48 (6H, m, ArH), 7.64-7.69 (4H, m, ArH); δ_C (50.3MHz; $CDCl_3$): 18.7 (q, C-6), 19.0 (s, $\underline{C}Me_3$), 26.8 (q, $C\underline{Me}_3$) 61.5 (t, C-1), 79.3, 80.7, 82.0, 85.0 (4xd, C-2, C-3, C-4, C-5), 128.0 (d, ArCH), 130.2 (d, ArCH), 133.7 (s, ArC), 136.0 (d, ArCH); m/z (CI NH_3): 404 ($M+NH_4^+$, 53), 387 ($M+H^+$, 2), 235 (100%).

2,5-Anhydro-4-tert-butyldiphenylsilyl-6-deoxy-1-O-p-toluenesulphonyl-L-glucitol (19). *p*-Toluenesulphonyl chloride (6.7 mmol, 1.27 g, 1.5 equiv) was added to a solution of 2,5-anhydro-4-O-*tert*-butyldiphenylsilyl-6-

deoxy-L-glucitol (18) (4.5 mmol, 1.72 g), pyridine (13.4 mmol, 1.1 ml, 3 equiv) and N,N-dimethylaminopyridine (0.1 g) in dichloromethane (5 ml) at 0°C. The solution was stirred at room temperature for 60 h at which point a white precipitate had developed and t.l.c. (ethyl acetate : hexane 1:1) showed some starting material (R_f 0.2) but mostly a product (R_f 0.55). The reaction was quenched by the addition of methanol (5 ml). The solvent was removed *in vacuo* and the residue redissolved between ether (100 ml) and water (50 ml). The aqueous phase pH was adjusted to 2 with aqueous hydrochloric acid (2N) and washed with ether (100 ml). The combined organic layers were washed with brine (30 ml) and dried (magnesium sulphate). Solvent removal gave a brown, oily residue which was purified by flash chromatography (ethyl acetate : petrol 1:9, then 1:3) to give *2,5-anhydro-4-O-tert-butyldiphenylsilyl-6-deoxy-1-O-p-toluenesulphonyl-L-glucitol (19)* (2.03 g, 3.8 mmol, 85% yield) as a white crystalline solid, m.p. 111-112°C; $[\alpha]_D^{20}$ -12.8 (*c*, 1 in $CHCl_3$); (Found: C, 64.45; H, 6.86. $C_{29}H_{36}O_6SSi$ requires C, 64.41; H 6.71%); δ_H (500MHz; $CDCl_3$): 0.97 (3H, d, *J* 6.5, H-6), 1.08 (9H, s, CMe_3), 1.41 (1H, d, *J* 5.1, OH), 2.44 (3H, s, ArMe), 3.77 (1H, dd, *J* 1.7, 3.4, H-4), 3.84 (1H, dq, *J* 3.4, 6.5, H-5), 4.05-4.09 (2H, m) 4.20 (1H, dt, *J* 3.9, 6.0), 4.27 (1H, dd, *J* 6.1, 10.1), 7.33 (2H, d, *J* 8.1, ArH), 7.38-7.49 (6H, m, ArH), 7.62-7.67 (4H, m, ArH), 7.77-7.80 (2H, m, ArH); δ_C (50.3MHz; $CDCl_3$): 18.6 (q, C-6), 18.9 (s, $\underline{C}Me_3$), 21.6 (q, ArMe), 26.8 (q, $C\underline{Me}_3$), 67.9 (t, C-1), 78.0, 78.5, 82.6, 84.8 (4xd, C-2, C-3, C-4, C-5), 128.1, 130.1, 130.3 (3xd, ArCH), 132.7, 133.4, 133.5 (3xs, ArC), 136.0 (d, ArCH), 145.3 (s, ArC); m/z (CI NH_3): 558 ($M+NH_4^+$, 8), 386 (23), 95 (100%).

2,5-Anhydro-4-O-tert-butyldiphenylsilyl-6-deoxy-1-O-p-toluenesulphonyl-3-O-trifluoro methanesulphonyl-L-glucitol (20). Trifluoromethanesulphonic anhydride (2.16 mmol, 0.37 ml, 1.3 equiv) was added to a stirred solution of 2,5-anhydro-4-O-*tert*-butyldiphenylsilyl-6-deoxy-1-O-*p*-toluenesulphonyl-L-glucitol (19) (1.66 mmol, 0.90 g) and pyridine (5.00 mmol, 0.4 ml, 3 equiv) at -5°C. After 1 h of stirring at room temperature, t.l.c. [(ethyl acetate : petrol, 3:7] showed starting material (R_f 0.2) and the formation of (20) (R_f 0.45). Pyridine (1.33 mmol, 0.1 ml, 0.8 equiv) and further trifluoromethanesulphonic anhydride (0.73 mmol, 0.12 ml, 0.4 equiv) were added at 0°C. The solution was stirred for a further 1.5 h at room temperature after which t.l.c. indicated almost complete conversion to product. The reaction mixture was partitioned between ether (70 ml) and aqueous hydrochloric acid (2N, 20 ml). The organic layer was washed with brine (10 ml) and dried (magnesium sulphate). The solvent was removed to give a brown oily residue which was purified by flash chromatography (ethyl acetate : petrol, 1:9, then 1:4) to give *2,5-anhydro-4-O-tert-butyldiphenylsilyl-6-deoxy-1-O-p-toluenesulphonyl-3-O-trifluoromethanesulphonyl-L-glucitol (20)* (0.743 g, 1.0 mmol, 67% yield) as an oil. $[\alpha]_D^{20}$ -21.1 (*c*, 1.1 in $CHCl_3$); (Found: C, 53.59; H, 4.99. $C_{30}H_{35}O_8SiSF_3$ requires C, 53.56; H; 5.24%); δ_H (200MHz; $CDCl_3$): 0.73 (3H, d, *J* 6.5, H-6), 1.08 (9H, s, CMe_3), 3.89 (1H, qd, *J* 1.5, 6.5, H-5), 4.08 (1H, bs, H-4), 4.16 (1H, dd, *J* 6.5, 10.5, H-1), 4.24 (1H, dd, *J* 6, 10.5, H-1), 4.51 (1H, dt, *J* 2.5, 6.5, H-2), 5.18 (1H, d, *J* 2.5, H-3), 7.36-7.57 (8H, m, ArH), 7.60-7.64 (4H, m, ArH), 7.82-7.86 (2H, m, ArH); δ_C (50.3MHz; $CDCl_3$): 18.1 (q, C-6), 18.9 (s, $\underline{C}Me_3$), 21.6 (q, ArMe), 26.7 (q, $C\underline{Me}_3$), 65.9 (t, C-1), 76.9, 81.7, 83.9, 90.7 (4xd, C-2, C-3, C-4, C-5), 128.2 (d, ArCH), 130.2, 130.6, (2xd, ArCH), 131.8, 132.5, 132.7 (3xs, ArC), 136.0 (d, ArCH), 145.6 (s, ArC); m/z (CI NH_3): 690 ($M+NH_4^+$, 9), 95 (100%).

2,5-Anhydro-4-O-tert-butyldiphenylsilyl-6-deoxy-1-O-p-toluenesulphonyl-L-allitol (10). Sodium trifluoroacetate (5.29 mmol, 0.72 g, 9.5 equiv) was added to a stirred solution of 2,5-anhydro-4-O-*tert*-butyldiphenylsilyl-6-deoxy-4-*O*-*p*-toluenesulphonyl-3-*O*-trifluoromethane sulphonyl-L-glucitol (20) (0.55 mmol, 0.370 g) in N,N-

dimethylformamide (3 ml) at room temperature. After 16 h t.l.c. (ethyl acetate : petrol 3:7) showed a spot that co-ran with starting material the triflate (R_f 0.45) but which did not discolour on heating without staining, and another (10) (R_f 0.35). The reaction was quenched by the addition of methanol (3 ml) and acetic acid (1ml) and the solution left to stand at room temperature for 3 h at which point t.l.c. showed only one compound (R_f 0.35) was present. The solvents were removed and the residue was partitioned between ethyl acetate (70 ml) and water (20 ml). The organic layer was washed with brine (10 ml) and dried (magnesium sulphate). Traces of acetic acid were removed *in vacuo* prior to purification by flash chromatography (ethyl acetate : petrol 1:9, then 1:3) to give *2,5-anhydro-4-O-tert-butyldiphenylsilyl-6-deoxy-1-O-p-toluenesulphonyl-L-allitol (10)* (0.215 g, 0.40 mmol, 72% yield) as a white crystalline solid. m.p. 81-83°C; $[\alpha]_D^{20}$ +12.6 (*c*, 1.1 in $CHCl_3$); υ_{max} 3468 (OH) cm^{-1}; δ_H (500MHz; $CDCl_3$): 0.69 (3H, d, *J* 6.0, H-6), 1.11 (9H, s, CMe_3), 2.43 (3H, s, ArMe), 3.79-3.81 (3H, m), 3.93-3.94 (1H, m), 4.05 (1H, dd, *J* 4.6, 10.7, H-1), 4.17 (1H, dd, *J* 3.0, 10.7, H-1), 7.30 (2H, d, *J* 8.2, ArH), 7.39-7.50 (6H, m, ArH), 7.63-7.66 (4H, m, ArH), 7.73-7.75 (2H, m, ArH); δ_C (50.3MHz; $CDCl_3$): 18.0 (q, C-6), 19.1 (s, $\underline{C}Me_3$), 21.5 (q, ArMe), 26.9 (q, $C\underline{Me}_3$), 69.7 (t, C-1), 71.7, 77.6, 79.7, 80.8 (4xd, C-2, C-3, C-4, C-5), 128.2, 128.3 (2xd, ArCH), 130.0, 130.5 (2xd, ArCH), 132.4, 133.0 (2xs, ArC), 135.9, 136.0 (2xd, ArCH), 145.1 (s, ArC); m/z (CI NH_3): 558 ($M+NH_4^+$, 38), 480 (100%).

2,5-Anhydro-6-deoxy-1-O-p-toluenesulphonyl-L-allitol (21). Tetra-*n*-butyl ammonium fluoride (0.47 mmol, 0.5 ml of 1N solution in tetrahydrofuran, 1.5 equiv) was added to 2,5-anhydro-4-*O-tert*-butyldiphenylsilyl-6-deoxy-1-*O-p*-toluenesulphonyl-L-allitol (10) (0.31 mmol, 0.170 g) in tetrahydrofuran (1 ml). After 5 h of stirring at room temperature t.l.c. (ethyl acetate : petrol 3:7) indicated disappearance of starting material (R_f 0.35) and formation of a product (21)(R_f 0.05) and a non-staining material (R_f 0.9). The reaction was quenched by the addition of methanol (1 ml) and acetic acid (0.1 ml) then partitioned between ethyl acetate (80 ml) and water (20 ml). The organic phase was washed with brine (10 ml), dried (magnesium sulphate) and the solvent removed to give a yellow oil which was purified by flash chromatography (ethyl acetate : petrol 1:1, then 3:1) to give *2,5-anhydro-6-deoxy-1-O-p-toluenesulphonyl-L-allitol (21)* (0.070g, 0.23 mmol, 78% yield), a clear oil. $[\alpha]_D^{20}$ +17.2 (*c*, 1 in $CHCl_3$); (Found: C, 51.67 H, 6.38. $C_{13}H_{18}O_6S$ requires C, 51.64 H, 6.38%); υ_{max} 3401 (b, OH) cm^{-1}; δ_H (200MHz; CD_3CN): 1.22 (3H, d, *J* 6.5, H-6), 2.45 (3H, s, ArMe), 3.52 (1H, t, *J* 5.5), 3.64-3.77 (1H, m), 3.77-3.85 (2H, m), 4.02 (1H, dd, *J* 4.5, 10.5, H-1), 4.09 (1H, dd, *J* 3, 10.5, H-1'), 7.45 (2H, d, *J* 8, ArH), 7.80 (2H, m, ArH); δ_C (50.3MHz; $CDCl_3$): 18.0 (q, C-6), 20.6 (q, ArMe), 70.6 (t, C-1), 71.1, 75.8, 79.3, 80.9 (4xd, C-2, C-3, C-4, C-5), 128.0, 130.2 (2xd, ArCH), 132.8 (s, ArC), 145.8 (s, ArC); m/z (CI NH_3): 320 ($M+NH_4^+$, 100), 303 ($M+H^+$, 6%).

3S-3-Hydroxymuscarine [(2S,3S,4R,5S)-3,4-Dihydroxy-tetrahydro-N,N,N,5-tetramethyl-2-furanmethaniminium tosylate] (9). A solution of 2,5-anhydro-6-deoxy-1-*O-p*-toluenesulphonyl-L-allitol (21) (0.20 mmol, 0.061 g) in methanol : trimethylamine (2:1, 15 ml) in a Fisher Porter bottle was heated to 75°C for 6 h whn t.l.c. (ether) most of starting material (R_f 0.2) had been replaced by a baseline product. The solvent was removed *in vacuo*, the residue co-evaporated with methanol (2 x 5 ml) and then dissolved in water (20 ml), and the resulting solution extracted with ethyl acetate (2 x 20 ml). The aqueous phase was freeze dried to yield *3S-3-hydroxymuscarine (9)* (0.060 g, 0.17 mmol, 82% yield) as a white crystalline solid, m.p. 101-102°C; $[\alpha]_D^{20}$ +25.3 (*c*, 0.45 MeOH); (Found: C, 52.99; H, 7.57; N, 4.05. C16H27O6NS requires C, 53.17; H, 7.57; N, 3.88%); υ_{max} (KBr): 3391 (OH) cm^{-1}; δ_H (500MHz; D_2O): 1.19 (3H, d, *J* 6.5, H-6), 2.32 (3H, s, ArMe), 3.12

(9H, s, NMe), 3.46 (1H, dd, *J* 9.5, 14.0, H-1), 3.57 (1H, dd, *J* 1.6, 14.0, H-1), 3.77 (1H, dd, *J* 3.9, 5.6, H-4), 3.89 (1H, dd, *J* 5.6, 7.2, H-3), 3.97 (1H, dq, 3.9, 6.5, H-5), 4.13 (1H, t, *J* 8, H-2), 7.28-7.31 (2H, m, ArH), 7.60-7.63 (2H, m, ArH); δ_C (50.3MHz; D_2O): 19.4 (q, C-6), 21.2 (q, ArMe), 54.8 (q, NMe), 70.0, 73.9, 75.3, 76.7, 82.4 (4xd, C-2, C-3, C-4, C-5), 126.4, 130.6 (2xd, ArCH), 140.7, 143.6 (2xs, ArC); m/z (FAB$^+$): 190 (M^+, 100%).

2,5-Anhydro-1,6-dideoxy-1-iodo-L-allitol (22). Sodium iodide (0.360 g, 2.40 mmol, 5 equiv) was added to a solution of 2,5 anhydro-6-deoxy-1-*O*-*p*-toluenesulphonyl-L-allitol (21) (0.145 g, 0.48 mmol) in butanone (5 ml). The resultant solution was heated to reflux for 6 h. The solvent was removed *in vacuo* and the residue partitioned between water (20 ml) and ethyl acetate (50 ml). The aqueous layer was washed with ethyl acetate (2x50 ml). The combined organic layers were washed with brine (15 ml) and dried (magnesium sulphate). Solvent evaporation gave a yellow oil which was purified by flash chromatography (ethyl acetate : petrol 1:1) to give *2,5-anhydro-1,6-dideoxy-1-iodo-L-allitol (22)* (0.110 g, 0.43 mmol, 90% yield) as a white solid, m.p. 26-28°C; υ_{max} 3392 (OH) cm^{-1}; $[\alpha]_D^{20}$ -4.1 (*c*, 1 $CHCl_3$); (Found: C, 28.08; H, 4.25. $C_6H_{11}O_3I$ requires C, 27.93; H, 4.30%); δ_H (500MHz; $CDCl_3$): 1.35 (3H, d, *J* 6.3, H-6), 2.42 (1H, bs, OH), 2.51 (1H, bs, OH), 3.35 (2H, d, *J* 5.2, H-1), 3.75 (1H, q, *J* 5.1, H-2), 3.78 (1H, bt, *J* 6.1), 3.91 (1H, q, *J* 6.3, H-5), 3.95 (1H, bt, *J* 5.5); δ_C (50.3MHz; $CDCl_3$): 7.7 (t, C-1), 18.7 (q, C-6), 75.2, 76.3, 79.7, 82.4 (4xd, C-2, C-3, C-4, C-5); m/z (CI NH_3): 276 ($M+NH_4^+$, 100%), 113 (100%).

2,5-Anhydro-1,6-dideoxy-L-allitol (23). Sodium acetate (0.065 g, 0.78 mmol, 4 equiv) was added to 10% palladium on carbon (0.01 g) suspended in a solution of 2,5 anhydro-1,6-dideoxy-1-iodo-L-allitol (22) (0.068 g, 0.27 mmol) in methanol (2 ml). The reaction was placed under an atmosphere of hydrogen gas and stirred at room temperature. A t.l.c. (ethyl acetate) was run after 16 h which showed only a non-UV active product at (R_f 0.3). The hydrogen was removed, magnesium sulphate (0.3 g) added and the mixture filtered through a magnesium sulphate plug. Solvent removal and flash chromatography [(ethyl acetate : hexane 1:1) then 3:1] gave *2,5-anhydro-1,6-dideoxy-L-allitol (23)* (0.030 g, 0.23 mmol, 85% yield) as a clear oil. $[\alpha]_D^{20}$ 0.0 (*c*, 1 in $CHCl_3$); (Found: C, 54.45; H, 9.36. $C_6H_{12}O_3$ requires C, 54.53; H, 9.15%); υ_{max} (thin film): 3392 (OH) cm^{-1}; δ_H (200MHz; $CDCl_3$): 1.33 (6H, d, *J* 6, H-1, H-6), 3.69-3.81 (4H, m, H-2, H-3, H-4, H-5); δ_C (50.3MHz; $CDCl_3$): 18.9 (q, C-1, C-6), 76.2, 79.6 (2xd, C-2, C-3, C-4, C-5); m/z (CI NH_3): 150 ($M+NH_4^+$, 100), 97 (32%).

X-Ray Crystal Structure Analysis.

The structure of methyl 2,5-anhydro-6-deoxy-L-gluconate (7) (crystallised from ethyl acetate/hexane) was established by single crystal X-ray analysis. Cell dimensions and intensity data were measured with an Enraf-Nonius CAD4-F diffractometer up to $\theta = 75°$ (Cu-Kα radiation). The data were corrected for absorption, Lorentz and polarisation effects. All calculations were carried out on a Microvax 3800 computer using SHELXS-86[12] for direct methods and CRYSTALS[13] for all other calculations. Atomic scattering factors were taken from International Tables.[14] Atomic coordinates for this compound have been deposited at the Cambridge Crystallographic Data Centre.[15] The coordinates of all non-hydrogen atoms were given by SHELXS-86. The hydrogen atoms were placed geometrically except for the hydroxyl hydrogens which were found by Fourier difference maps. The structure was refined by full-matrix least-squares with isotropic temperature factors for the

hydrogen atoms and anisotropic temperature factors for all other atoms using data with merged Friedel pairs. Corrections for secondary extinction were applied,[16] and the model refined almost to convergence. The data were refined using Chebychev weighting schemes[17] to give a final value of R = 3.21 %. The resulting structure contained two identical molecules in the asymmetric unit, hence the atomic position data given below includes twice as many atoms as the formula unit.

Crystal Data for : methyl 2,5-anhydro-6-deoxy-L-gluconate

Molecular formula $C_7H_{12}O_5$ Formula weight 176.17

Crystal data:

Crystal system Primitive Monoclinic

a/Å 5.891(2) α/° 90

b/Å 16.530(3) β/° 97.69(2)

c/Å 8.920(1) γ/° 90

space group P1 2_1 1 D_C/g cm^{-3} 1.36

linear absorption coeff. /cm^{-1} 9.58

Crystal size /mm 0.20 x 0.50 x 0.60

Data collection:

X-radiation λ = 1.5418 Å Cu-Kα

θ min., max. /° 0, 75

ω–2θ scan parameters: A, B (°) (A + B tanθ) A = 1.0 B = 0.35

Horizontal aperture parameters: A, B (mm) (A + B tanθ) A =3.5 B = 0

Scan speed/° min^{-1} 1.7 (min.) to 6.7 (max.)

Total data 1747

Observed data 1598 for [I > nσ(I)] where n = 3

Absorption correction: min 1.42 , max 1.16 Merging R 2.90 %

Programs: Solved by SHELXS-86, refined by CRYSTALS

Weighting Scheme type Chebychev 3 coefficients 12.07 1.22 9.99

Extinction parameter 68.27

Maximum residual electron density/ eÅ^{-3} 0.71

Final R 3.21 % R_w 4.04 %

Fractional atomic coordinates and equivalent isotropic temperature factors U(equ) with standard deviations in parentheses for methyl 2,5-anhydro-6-deoxy-L-gluconate (7)

Atom	x/a	y/b	z/c	U(equ)
O(101)	0.4392(2)	0.8630(2)	0.1850(2)	0.0423
C(102)	0.5796(3)	0.9348(3)	0.1801(3)	0.0431
C(103)	0.8178(3)	0.9109(3)	0.2576(3)	0.0435
C(104)	0.7797(3)	0.8303(3)	0.3328(2)	0.0382
C(105)	0.5890(3)	0.7961(3)	0.2190(2)	0.0377
C(106)	0.4618(4)	0.7260(3)	0.2762(2)	0.0427
O(107)	0.2639(3)	0.7242(3)	0.2899(3)	0.0675
O(108)	0.6069(3)	0.6658(2)	0.3091(2)	0.0539
C(109)	0.5204(6)	0.5921(3)	0.3650(4)	0.0668
C(110)	0.4641(5)	1.0022(3)	0.2546(4)	0.0617

O(111)	0.9590(3)	0.9005(3)	0.1414(2)	0.0556
O(112)	0.6892(3)	0.8407(3)	0.4716(2)	0.0483
O(201)	0.9912(3)	0.2877(2)	0.2158(2)	0.0432
C(202)	0.8472(3)	0.2185(3)	0.2359(2)	0.0411
C(203)	0.6171(3)	0.2533(3)	0.2718(2)	0.0406
C(204)	0.6564(3)	0.3448(3)	0.2761(2)	0.0392
C(205)	0.8392(3)	0.3529(3)	0.1713(2)	0.0370
C(206)	0.9666(3)	0.4317(3)	0.1850(2)	0.0413
O(207)	1.1569(3)	0.4420(2)	0.2503(2)	0.0581
O(208)	0.8359(3)	0.4899(2)	0.1156(2)	0.0476
C(209)	0.9423(5)	0.5686(3)	0.1068(4)	0.0626
C(210)	0.9754(4)	0.1650(3)	0.3565(3)	0.0522
O(211)	0.4438(3)	0.2314(2)	0.1532(2)	0.0497
O(212)	0.7528(3)	0.3724(3)	0.4224(2)	0.0544

Final anisotropic temperature factors with standard deviations in parentheses for methyl 2,5-anhydro-6-deoxy-L-gluconate (7)

Atom	U(11)	U(22)	U(33)	U(23)	U(13)	U(12)
O(101)	0.0322(7)	0.0407(8)	0.0579(8)	-0.0013(6)	-0.0066(5)	0.0010(6)
C(102)	0.035(1)	0.043(1)	0.052(1)	0.0043(9)	0.0019(8)	-0.0019(8)
C(103)	0.0304(9)	0.055(1)	0.049(1)	0.0042(9)	0.0026(7)	-0.0037(8)
C(104)	0.0284(9)	0.051(1)	0.0381(9)	0.0024(8)	0.0033(6)	0.0013(7)
C(105)	0.0377(9)	0.039(1)	0.0372(9)	-0.0029(8)	0.0044(7)	0.0052(8)
C(106)	0.041(1)	0.040(1)	0.050(1)	-0.0082(8)	0.0065(8)	0.0006(8)
O(107)	0.043(1)	0.059(1)	0.138(2)	0.005(1)	0.028(1)	-0.0003(8)
O(108)	0.0489(9)	0.0423(9)	0.078(1)	0.0082(8)	0.0104(8)	0.0035(7)
C(109)	0.073(2)	0.045(2)	0.098(2)	0.012(1)	0.018(2)	-0.004(1)
C(110)	0.052(1)	0.044(1)	0.108(2)	-0.016(1)	0.006(1)	0.002(1)
O(111)	0.0365(8)	0.089(1)	0.066(1)	0.022(1)	0.0179(7)	0.0026(8)
O(112)	0.0431(8)	0.075(1)	0.0357(7)	-0.0045(7)	0.0043(5)	-0.0091(7)
O(201)	0.0315(7)	0.0428(8)	0.0683(9)	0.0091(7)	0.0151(6)	0.0060(6)
C(202)	0.035(1)	0.038(1)	0.052(1)	-0.0030(9)	0.0071(8)	-0.0007(8)
C(203)	0.0326(9)	0.046(1)	0.047(1)	0.0007(8)	0.0091(8)	-0.0004(8)
C(204)	0.0310(9)	0.044(1)	0.046(1)	-0.0015(8)	0.0080(7)	0.0030(7)
C(205)	0.0321(9)	0.043(1)	0.0381(9)	0.0039(8)	0.0068(7)	0.0055(8)
C(206)	0.0346(9)	0.047(1)	0.044(1)	0.0031(8)	0.0059(7)	0.0038(8)
O(207)	0.0397(8)	0.054(1)	0.092(1)	0.0051(9)	-0.0073(8)	-0.0031(7)
O(208)	0.0451(8)	0.0413(8)	0.0591(9)	0.0049(7)	0.0071(6)	0.0048(6)
C(209)	0.069(2)	0.042(1)	0.089(2)	0.006(1)	0.017(1)	0.001(1)
C(210)	0.046(1)	0.038(1)	0.083(2)	0.012(1)	0.001(1)	0.0017(9)
O(211)	0.0331(7)	0.059(1)	0.0653(9)	-0.0101(8)	0.0052(6)	-0.0064(6)
O(212)	0.059(1)	0.069(1)	0.0462(8)	-0.0146(8)	0.0149(7)	-0.0151(8)

Bond lengths (in Å) for the non-hydrogen atoms, with standard deviations in parentheses, for methyl 2,5-anhydro-6-deoxy-L-gluconate (7)

O(101)- C(102)	1.450(3)	O(101)- C(105)	1.423(2)
C(102)- C(103)	1.531(3)	C(102)- C(110)	1.506(4)
C(103)- C(104)	1.523(3)	C(103)- O(111)	1.423(3)
C(104)- C(105)	1.519(3)	C(104)- O(112)	1.423(2)
C(105)- C(106)	1.505(3)	C(106)- O(107)	1.189(3)
C(106)- O(108)	1.319(3)	O(108)- C(109)	1.436(3)
O(201)- C(202)	1.449(3)	O(201)- C(205)	1.424(2)
C(202)- C(203)	1.545(3)	C(202)- C(210)	1.515(3)
C(203)- C(204)	1.529(3)	C(203)- O(211)	1.415(3)
C(204)- C(205)	1.524(3)	C(204)- O(212)	1.427(3)
C(205)- C(206)	1.500(3)	C(206)- O(207)	1.204(3)
C(206)- O(208)	1.332(3)	O(208)- C(209)	1.451(3)

Bond angles (in degrees) for the non-hydrogen atoms with standard deviations in parentheses for methyl 2,5-anhydro-6-deoxy-L-gluconate (7)

C(105)- O(101)- C(102)	107.6(1)	C(103)- C(102)- O(101)	105.5(2)
C(110)- C(102)- O(101)	107.4(2)	C(110)- C(102)- C(103)	115.6(2)
C(104)- C(103)- C(102)	104.1(2)	O(111)- C(103)- C(102)	107.0(2)
O(111)- C(103)- C(104)	110.4(2)	C(105)- C(104)- C(103)	99.8(2)
O(112)- C(104)- C(103)	111.8(2)	O(112)- C(104)- C(105)	107.3(1)
C(104)- C(105)- O(101)	103.6(2)	C(106)- C(105)- O(101)	110.4(2)
C(106)- C(105)- C(104)	114.7(2)	O(107)- C(106)- C(105)	126.4(2)
O(108)- C(106)- C(105)	108.6(2)	O(108)- C(106)- O(107)	125.0(2)
C(109)- O(108)- C(106)	117.9(2)	C(205)- O(201)- C(202)	106.0(1)
C(203)- C(202)- O(201)	106.1(2)	C(210)- C(202)- O(201)	107.7(2)
C(210)- C(202)- C(203)	116.0(2)	C(204)- C(203)- C(202)	103.8(2)
O(211)- C(203)- C(202)	108.4(2)	O(211)- C(203)- C(204)	111.3(2)
C(205)- C(204)- C(203)	100.9(2)	O(212)- C(204)- C(203)	112.4(2)
O(212)- C(204)- C(205)	107.8(2)	C(204)- C(205)- O(201)	103.4(2)
C(206)- C(205)- O(201)	110.2(1)	C(206)- C(205)- C(204)	114.5(2)
O(207)- C(206)- C(205)	125.9(2)	O(208)- C(206)- C(205)	109.7(2)
O(208)- C(206)- O(207)	124.5(2)	C(209)- O(208)- C(206)	116.4(2)

References

1. Bravo, P.; Resnati, G.; Angeli, P.; Frigerio, M.; Viani, F.; Arnone, A.; Marucci, G.; Cantalamessa, F. *J. Med. Chem.*, 1992, **35**, 3102; Kaiser, C.; Spagnuolo, C. J.; Adams, T. C.; Audia, V. H.; Dupont, A. C.; Hatoum, H.; Lowe, V. C.; Prosser, J. C.; Sturm, B. L.; Naronha-Blob, L. *J. Med. Chem.*, 1992, **35**, 4415.
2. Bartus, R. T.; Dean, R. L.; Beer, B.; Lippa, A. S. *Science*, 1982, **217**, 408.
3. Kozikowski, A. P.; Fauq, A. H.; Miller, J. H.; McKinney, M. *Biol. Med. Chem. Lett.*, 1992, **2**, 797; Sugimoto, H.; Tsuchiya, Y.; Sugumi, H.; Higurashi, K.; Karibe, N.; Iimura, Y.; Sasaki, A.; Araki, S.; Yamanishi, Y.; Yamatsu, K. *J. Med. Chem.*, 1992, **35**, 4542.
4. Jaen, J. C.; Moos, W. H.; Johnson, G. *Biol. Med. Chem. Lett.*, 1992, **2**, 777; Sahpiro, G.; Floersheim, P.; Amstutz, A.; Boddeke, H.; Bolliger, G.; Cottens, S.; Enz, A.; Gmelin, G.; Gull, P.; Supavilai, P. *Biol. Med. Chem. Lett.*, 1992, **2**, 815; Fisher, A.; Gurwitz, D.; Barak, D.; Haring, R.; Karton, I.; Brandeis, R.; Pittel, Z.; Marciano, D.; Meshulam, H.; Vogel, Z.; Heldman, E. *Biol. Med. Chem. Lett.*, 1992, **2**, 839.
5. Wang, P.-C.;Joullie, M. M. *The Alkaloids (Ed. A. Brossi)*, 1984, **23**, 327; Antkowiak, R.; Antkowiak, W. Z. *The Alkaloids (Ed. A. Brossi)*, 1990, **40**, 190; Lewis, J. R. *Nat. Prod. Rep.* 1992, **9**, 81.
6. Mantell, S. J.; Ford, P. S.; Watkin, D. J.; Fleet, G. W. J.; Brown, D.*Tetrahedron Lett.*, 1992, **33**, 4503.
7. Mantell, S. J.; Fleet, G. W. J.; Brown, D. *J. Chem. Soc., Chem. Commun.*, **1991**, 1563.
8. Mantell, S. J.; Fleet, G. W. J.; Brown, D. *J. Chem. Soc., Perkin Trans. 1*, **1992**, 3023
9. Jackson, E.;Hudson,C. J. *J. Am. Chem. Soc.*, 1930, **52**, 1271.
10. Choi, S. S.; Myerscough, P. M.; Fairbanks, A. J.; Skead, B. M.; Bichard, C. J. F.;Mantell, S. J.; Fleet, G. W. J.; Saunders, J.; D. Brown, D. *J. Chem. Soc., Chem. Commun.*, **1992**, 1605.
11. Myerscough, P. M.; Fairbanks, A. J.; Jones, A. H.; Choi, S.-S.; Fleet, G. W. J.; Al-Daher, S. S.; Cenci di Bello, I.; Winchester, B. *Tetrahedron*, 1992, **48**, 10177.
12. Sheldrick, G. M. *Crystallographic Computing 3*, ed. M. Sheldrick, G. M.; Kruger, C.; Goddard, R. Oxford University Press, Oxford, **1985**.
13. Watkin, D. J.; Carruthers, J. R.; Betteridge, P. W.; *CRYSTALS User Guide*, Chemical Crystallography Laboratory, University of Oxford, **1985**.
14. *International Tables for X-Ray Crystallography*, Vol. IV, Kynoch Press, Birmingham, **1974**.
15. The atomic coordinates are available on request from the Cambridge Crystallographic Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW. Any request should be accompanied by the full literature citation for this paper.
16. Larson, A. C. *Crystallographic Computing Techniques*, ed. Ahmed, F. R., Munksgaard, Copenhagen, **1976**.
17. Prince, E. *Mathematical Techniques in Crystallography and Material Sciences*, Springer-Verlag Inc., New York, **1982**.