Absolute Configuration of Tautomycin, a Protein Phosphatase Inhibitor from a Streptomycete

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The absolute configurations of all the chiral centres of tautomycin, a new protein phosphatase inhibitor from *Streptomyces spiroverticillatus*, have been determined. Because of its non-crystallinity and flexibility, the absolute configurations of tautomycin, which possesses 13 chiral centres were determined on the basis of chemical degradation and spectroscopic evidence, with aid from conformational calculations.

An antifungal antibiotic, tautomycin, was isolated in our laboratory from a culture of *Streptomyces spiroverticillatus*.[1] Besides its antifungal activity, tautomycin induced a morphological change (bleb formation) in human leukaemia cells K562, which was shown to be correlated with the inhibition of protein phosphatase.[2] Afterwards, it was found that tautomycin is a specific inhibitor of protein phosphatase 1 and 2A obtained from various sources.[3,4] Although we have reported the structure of tautomycin,[5,6] its stereochemistry remains unknown. The hygroscopic amorphous nature and the flexibility of the molecule have been major obstacles to the stereochemical assignment. However, because of the increasing importance of this compound as a biological probe, it is an urgent requirement to establish its stereochemistry. We now report the absolute configuration of tautomycin **1** determined through chemical transformation, spectroscopic analyses, and conformational calculations.

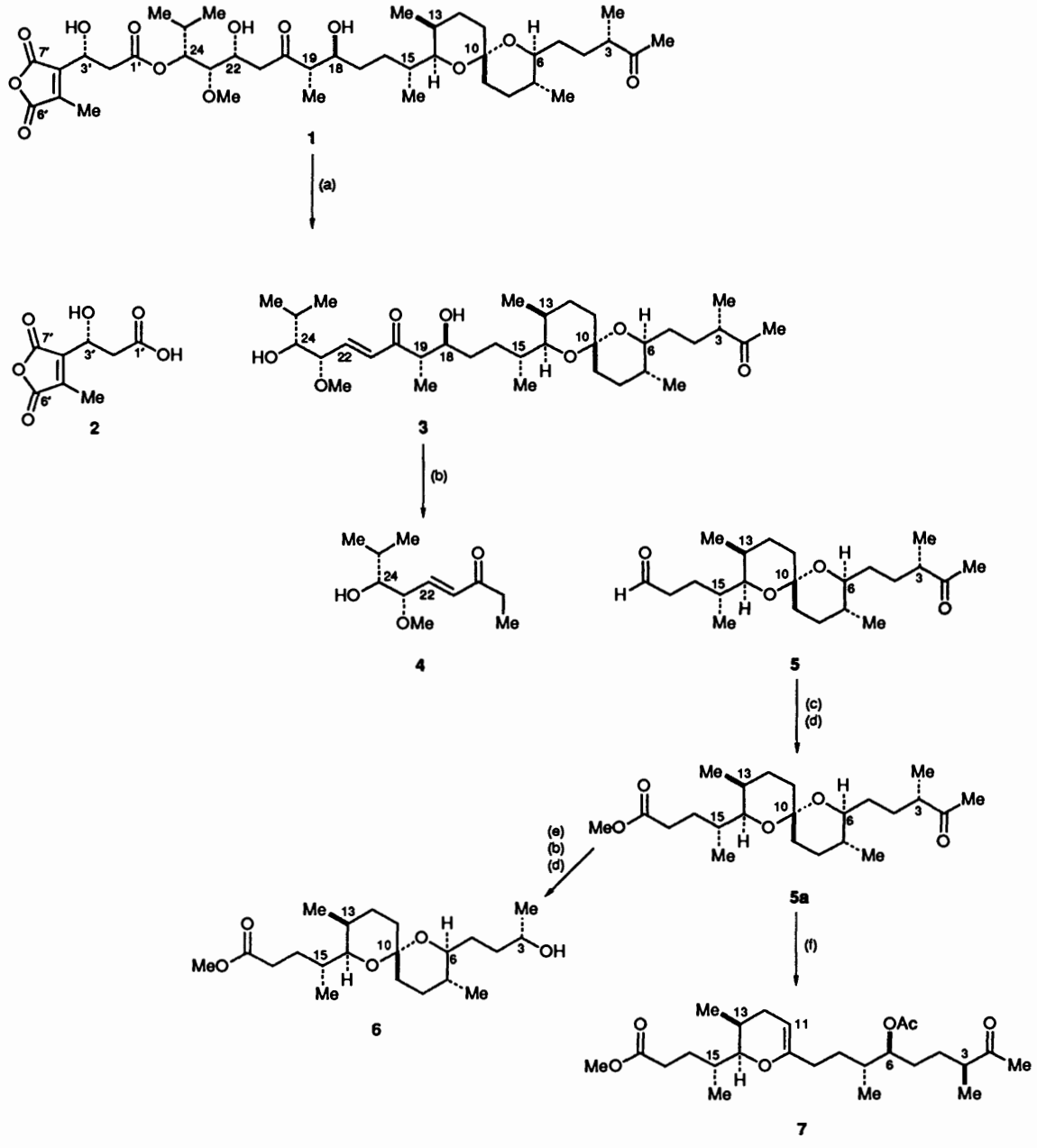
Results and Discussion

The absolute configuration of tautomycin **1** was fully assigned on the basis of an analysis of the degradation products **2–9** obtained as outlined in Schemes 1–4.[7] Key reactions involve alkaline hydrolysis, retro-aldol reaction, ozonolysis, reductive ester cleavage, Baeyer–Villiger oxidation and mixed anhydride ketal cleavage. With respect to the tautomycin numbering system, which is used throughout the Discussion for clarity, the 13 stereogenic centres are C-3′ in structure **2b**, C-24 and -18 in **3**, C-19 in **8**, C-22 and -23 in **9**, C-3 in **6**, C-6 in **7**, and C-7, -10, -13, -14 and -15 in **5**.

Absolute Configuration at C-3′ in Compound **2b**.—The trimethyl ester **2b** and the corresponding anhydride **2a** were obtained as major products under the reaction conditions shown in Scheme 2. The absolute configuration at C-3′ was established by a variation of Trost's method[8] which is comparable to an advanced Mosher method.[9] There have previously been few reported applications of Trost's method.[10] In the present study, high-field FT NMR application of Trost's method using computer-aided conformational analyses[7] was performed. Alcohol **2b** was converted into its (*S*)-*O*-methylmandelate **2d** and (*R*)-*O*-methylmandelate **2e** as shown in Scheme 2. The chemical-shift differences ($\Delta\delta = \Delta\delta_{2d} - \delta_{2e}$) of protons of diastereoisomers **2d** and **2e** and the absolute configuration *R* at C-3′, which were determined by a modified Trost method, are shown in Fig. 1. To clarify the reason for the unusual positive $\Delta\delta$-value of the methyl proton, sequential COSMIC (COmputation and Structure Manipulation In Chemistry)[11] force field calculations on structure **2d** were performed on the Nemesis program. One of the most stable conformations of **2d** was found by torsion-driving around each bond of structure **2d** and energy-minimization of the conformer in the COSMIC force field was carried out. The conformational search suggested that the C-1′ ester methyl moiety is folded below the double bond as shown in Fig. 2. The conformation obtained in this way was consistent with ^{1}H NMR data. In comparison with the C-1′ ester methyl (δ 3.72) of the corresponding *p*-bromobenzoate **2c**, the methyl protons of compounds **2d** and **2e** are shielded by the double bond and appear at high field (δ 3.41 for **2d** and δ 3.39 for **2e**) in the ^{1}H NMR spectrum. Since the C-1′ ester methyl exists on the same side of the *O*-methylmandelate plane as the other ester methyls and the vinyl methyl, the positive $\Delta\delta$-value of the methyl protons is reasonably explained.

Absolute Configurations at C-18 and C-24 in Compound **3**.—The absolute configurations at C-18 and -24 in compound **3** were clarified by the advanced Mosher method. The hydrolysis product **3** was converted into the corresponding (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic acid [(*R*)-(+)-MTPA] ester **3a** and (*S*)-(−)-MTPA ester **3b**. The absolute configurations *S*/*R* at C-18 and -24 in compound **3** were determined from the chemical-shift differences ($\Delta\delta = \delta_{3b} - \delta_{3a}$) by the advanced Mosher method as shown in Fig. 3. In order to rationalize the positive $\Delta\delta$-values of the C-1-to-C-3 portion, conformational analyses of the corresponding bis-[(*S*)-α-methoxy-α-(methyl)phenylacetate] [(*S*)-MTPA model] and bis-[(*R*)-α-methoxy-α-(methyl)phenylacetate] [(*R*)-MTPA model] of compound **3** were performed after the absolute configurations of all the chiral centres of compound **3** had been assigned (*vide infra*). A stable conformation of the corresponding bis-[(*S*)-α-methoxy-α-(methyl)phenylacetate] [(*S*)-MTPA model] calculated by the Nemesis program suggested that the C-1-to-C-3 portion is deshielded by the phenyl ring of the C-18 ester. Thus, the positive $\Delta\delta$-values of this part of the molecule were reasonably explained.

Absolute Configuration at C-19 in Compound **8**.—Diastereoisomeric acetonides **8** were prepared from compound **3** in four steps as shown in Scheme 3. A pair of large coupling constants ($J_{18,19}$ 10.3 Hz, $J_{19,20ax}$ 12.5 Hz) for the *trans*-diaxial protons indicated that the 1,3-dioxane ring adopts a chair conformation

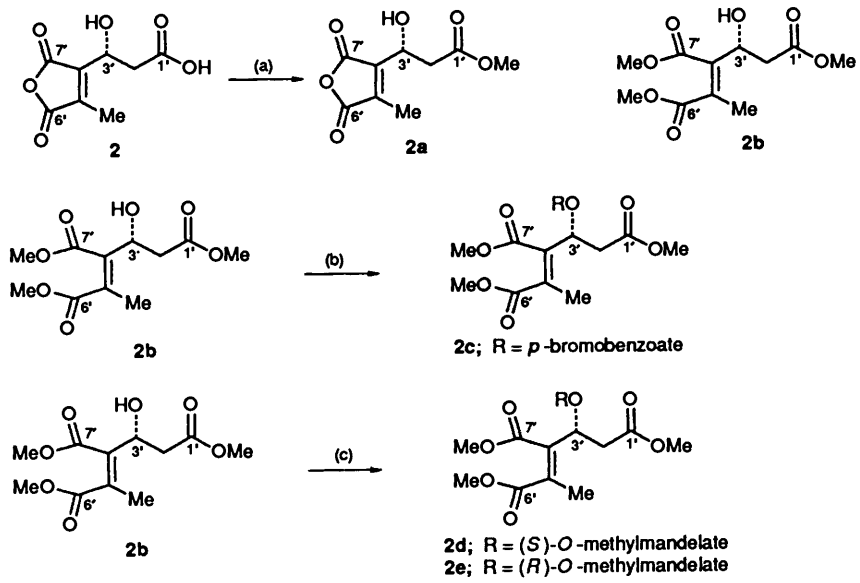


Scheme 1 Degradation of tautomycin **1** and structures of products **2–7**. *Reagents and conditions:* (a) 20% aq. Cs_2CO_3, MeOH, pH 9; (b) 20% aq. Cs_2CO_3, MeOH, pH 10; (c) Jones oxidation; (d) CH_2N_2; (e) MCPBA; (f) *p*-TsOH, Ac_2O, AcOH.

and that the relative configuration at C-18 and -19 in compound **8** is *S*/*R* (*R*/*S*) as shown in Fig. 4. Since the absolute configuration at C-18 had already been determined as being *S*, the absolute configuration at C-19 in compound **8** should be *R*.

Absolute Configurations at C-22 and C-23 in Compound **9**.—To determine the absolute configurations at C-22 and C-23, isopropylidene derivatives **9** were prepared by stereoselective reduction of the C-20 carbonyl in tautomycin **1**, followed by formation of the isopropylidene ketals (Scheme 4). Although reduction at the C-2 carbonyl was not stereoselective, the diastereoisomeric mixture at C-2 did not prevent assignment of the acetonide parts by NMR spectroscopy. The ^{13}C NMR acetonide resonances (ketal carbon: δ_C 100.2, 100.4; methyl carbons: δ_C 25.3, 25.3, 23.7, 24.0) showed that both 1,3-dioxane rings exist as *anti*-1,3-diol acetonides.[12,13] The vicinal coupling constants between protons 22/23/24 indicated that this 1,3-dioxane ring exists in twist-boat conformation. Since the absolute configuration at C-24 is *R*, the absolute configuration at C-22 is *R* as shown in Fig. 5. To confirm the relative configuration at C-23, a conformational search (200 structures searched) of the model acetonides (C-16–C-26) with C-23 *R* and C-23 *S* configurations was performed on the Biograf program using the Monte Carlo method. After energy minimization of each of the 200 conformers in the Dreiding force field,[14] each conformer that possessed relatively low potential energy was selected carefully and the co-ordinates of the selected conformers were transferred to MacroModel. The coupling constants ($J_{22,23}$ and $J_{23,24}$) of each conformer were calculated in MacroModel. In the case of the acetonide with C-23 *R*, a suitable conformer having coupling constants comparable with the observed *J* values could not be found. However, a stable conformer [PE value: 76.37 kcal/cm^{-3},* dihedral angle 24-H/

* 1 cal = 4.184 J.



Scheme 2 Preparation of the *p*-bromobenzoate **2c** and the *O*-methylmandelates **2d** and **2e**. *Reagents and conditions:* (a) MeOH, H_2SO_4, 2 h for **2a**, 48 h for **2b**; (b) *p*-bromobenzoyl chloride, DMAP; (c) (*S*)-*O*-methylmandelic acid or (*R*)-*O*-methylmandelic acid, DCC, DMAP.

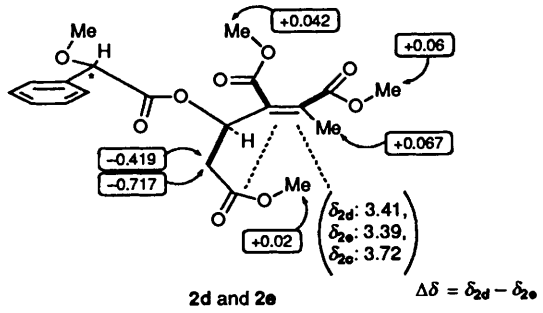


Fig. 1 A stable conformation of the (*S*)-*O*-methylmandelate derivative **2d** and the (*R*)-*O*-methylmandelate derivative **2e** and Δδ-values obtained from diastereoisomers **2d** and **2e**. The symbols δ_{2d}, δ_{2e} and δ_{2c} represent chemical shifts of C-1′ ester methyl protons in species **2d**, **2e** and **2c**, respectively.

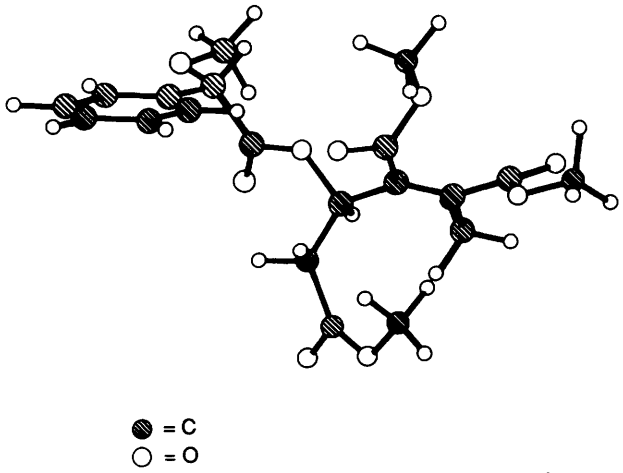


Fig. 2 A stable conformation of compound **2d** obtained from the COSMIC force field calculations

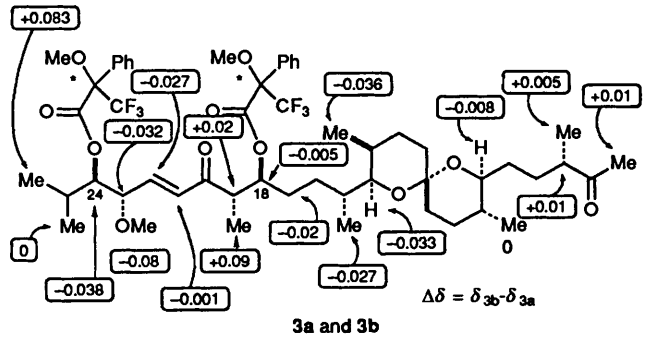
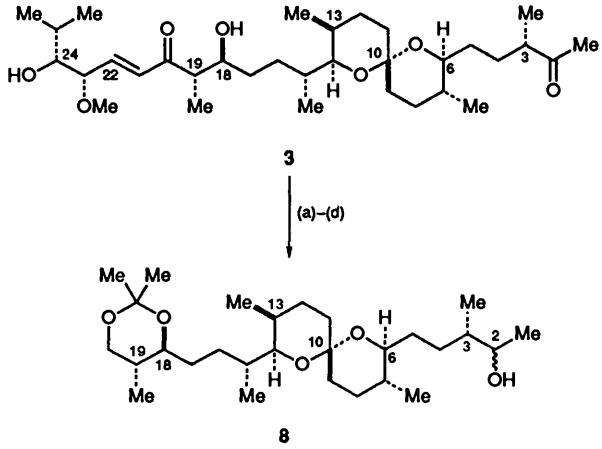


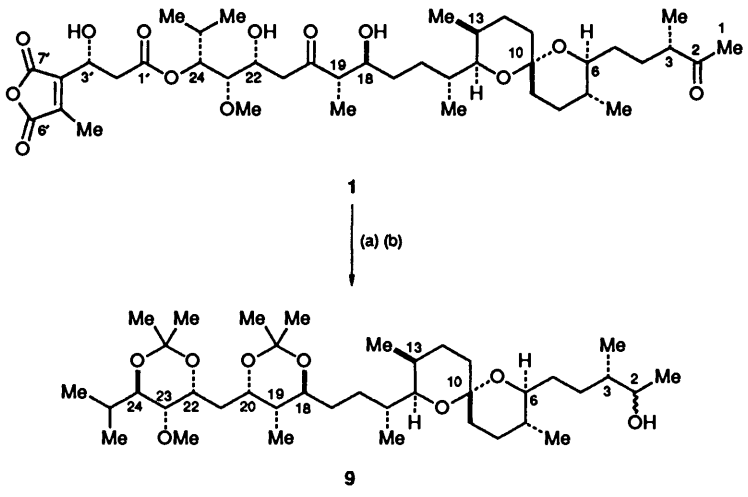
Fig. 3 Determination of absolute configurations at C-18 and C-24 in compound **3**: Δδ-values obtained from (*R*)-MTPA diester **3a** and (*S*)-MTPA diester **3b**.



Scheme 3 Preparation of the acetonide **8**. *Reagents:* (a) O_3, 30% H_2O_2; (b) CH_2N_2; (c) LAH; (d) 2,2-dimethoxypropane, *p*-TsOH.

23-H (−140°), 23-H/22-H (36°)] of the C-23 *S* acetonide showed comparable coupling constants ($J_{22,23}$ 3.4 Hz, $J_{23,24}$ 6.1 Hz) with the observed *J*-values ($J_{22,23}$ 3.3 Hz, $J_{23,24}$ 6.1 Hz) (Fig. 5 and Fig. 6). Thus the absolute configuration *S* at C-23 was confidently assigned.

Absolute Configuration at C-3 in Compound **6**.—The absolute configuration at C-3 in compound **6** was deduced from an analysis using Mosher's method. Aldehyde **5** obtained by retro-aldol reaction from compound **3** was converted into compound **6** in five steps as shown in Scheme 1. Alcohol **6** was esterified with *R*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride *R*-(+)-MTPACl] and *S*-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride [*S*-(−)-MTPACl] in pyridine in the presence of 4-dimethy(amino)pyridine (DMAP) to give the



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Scheme 4 Preparation of the diacetonide **9**. *Reagents:* (a) $LiBH_4$; (b) 2,2-dimethoxypropane, *p*-TsOH.

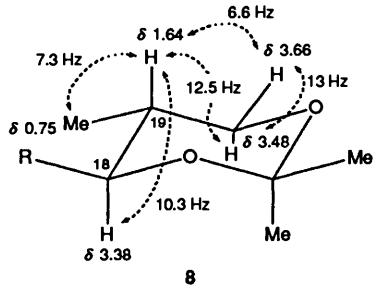

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Fig. 4 Assignments of relative and absolute configurations at C-18/C-19 in compound **8**

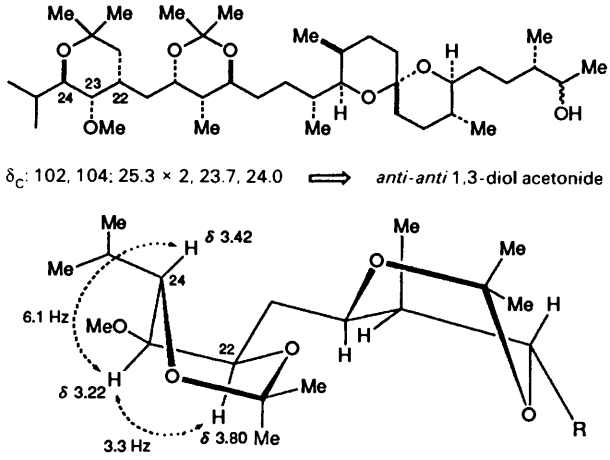

Fig. 5 Assignments of relative and absolute configurations at C-22/C-23/C-24 in compound **9**

R-(+)-MTPA ester **6a** and the *S*-(−)-MTPA ester **6b**, respectively (Fig. 7). Baeyer–Villiger oxidation of compound **5a** with *m*-chloroperbenzoic acid (MCPBA) under alkaline conditions gave an inseparable diastereoisomeric mixture of acetates at C-3 (major : minor 61 : 39). Since racemization at C-3 in compound **5** was observed under alkaline conditions (20% Cs_2CO_3, pH 10–11, 7 h) and also at C-3 in compound **5a** under the spiroketal ring-cleavage condition (*vide infra*), it was considered that the major diastereoisomeric product from the Baeyer–Villiger oxidation retains the original configuration at C-3 whereas the minor diastereoisomer is produced by epimerization at C-3. The chemical shifts of the major peaks of the C-3 methyl protons of diesters **6a** and **6b** were unambiguously determined by spin-decoupling experiments, with irradiation at the frequency of the C-3 protons of compounds **6a** and **6b** in the ^1H NMR spectrum. The chemical-shift differences of the major peaks ($\Delta\delta = \delta_{6b} - \delta_{6a}$) between compounds **6b** and **6a** are shown in Fig. 7. The absolute configuration *S* for C-3 was thus established by Mosher's method.

Absolute Configuration at C-6 in Compound **7**.—The absolute configuration at C-6 in compound **7** was established by the advanced Trost method. Spiroketal ring cleavage of compound **5a** with mixed sulfonic–acetic anhydride[15,16] afforded the C-6 acetate **7** (Scheme 1). The ^1H NMR resonance at C-1 was observed at high field (δ_H 1.63) due to the shielding effect of the acetyl carbonyl, which was supported by COSMIC force field calculations and the standard chemical shift (δ_H 2.1) of the corresponding alcohol **7a** obtained by methanolysis (Fig. 8). The C-6 alcohol **7a** was converted into the (*S*)-*O*-methylmandelate derivative **7b** and (*R*)-*O*-methylmandelate derivative **7c**. The chemical-shift differences ($\Delta\delta = \delta_{7b} - \delta_{7c}$) are shown in Fig. 8 and the absolute configuration *S* for C-6 was determined by the advanced Trost method.

Absolute Configurations at C-7, C-10, C-13, C-14 and C-15 in Compound **5**.—Retro-aldol reaction of compound **3** gave compounds **4** and **5** as shown in Scheme 1.[5,6] By analysis of the ^{13}C NMR resonances (7-Me: δ_C 17.9; 13-Me; δ_C 10.9) of compound **5**, the orientations of the 7-Me and 13-Me groups were revealed to be equatorial and axial, respectively. The relative configuration at C-6, -7, -10, -13 and -14 was established by the assignment of dihedral angles which in turn were determined from coupling-constant data and nuclear Overhauser effects (NOEs) obtained from the NOESY spectrum and difference NOE specta of **5** as shown in Fig. 9. The absolute configuration *S* for C-6 leads to *R*/*R*/*S*/*S* for C-7, -10, -13 and -14, respectively. To determine the absolute configuration at C-15, sequential Dreiding force field calculations on both the 15*R* and 15*S* epimers were performed on the Biograf program, and calculations of the coupling constants were accomplished on the MacroModel program. The dihedral angles O–C(14)/C(15)–C(16) for each epimer were varied clockwise every 4° from 0° to 360° and the minimum energy of each of the 90 rotamers was calculated. Fig. 10 shows the relationships between dihedral angles in C-15*R* and C-15*S* aldehydes and the corresponding potential energies. In the case of the C-15*R* aldehyde, the *anti*-type **I** [dihedral angle O–C(14)/C(15)–C(16): 180°; 55.8 kcal mol^{-1}] is more stable than the other two, *gauche*-type **II** (268°; 58.0 kcal mol^{-1}) and **III** (52°; 58.5 kcal mol^{-1}). The distance (3.15 Å) between 15-Me and 6-H in **I** is short enough to be detected by a difference-NOE

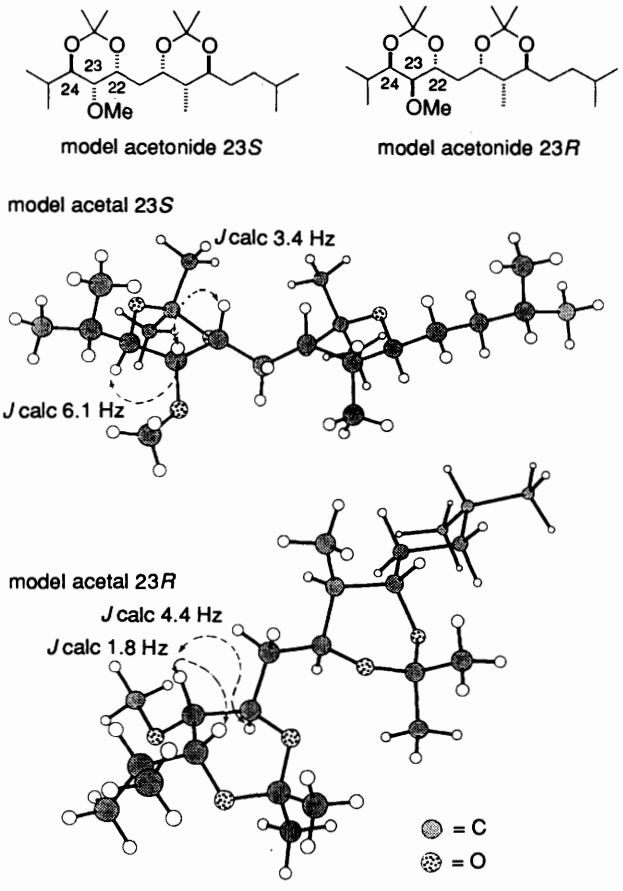

Fig. 6 Stable conformations of 23-*R* and 23-*S* models obtained by the Monte Carlo method and Dreiding force field calculations, and the coupling constants $J_{22,23}$ and $J_{23,24}$ calculated by the MacroModel program.

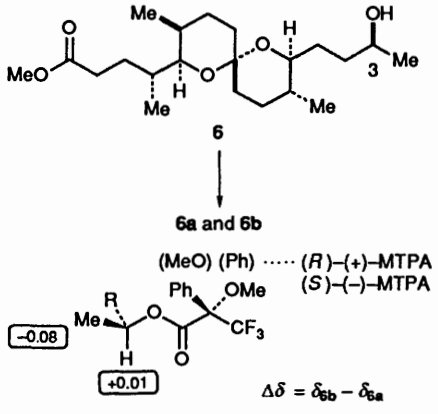

Fig. 7 Determination of absolute configuration at C-3 in compound **6**: Δδ-values obtained from (*R*)-MTPA ester **6a** and (*S*)-MTPA ester **6b**. *Reagents:* (*R*)-(+)-MTPACl, pyridine, DMAP and (*S*)-(−)-MTPACl, pyridine, DMAP.

experiment between these protons. Although the most stable rotamer of the C-15 *S* epimer is also *anti*-type **I'**, 15-Me exists near to 13-H rather than to 6-H (Fig. 9 and Fig. 11). On the basis of the calculated coupling constant ($J_{14,15}$ 9.11 Hz) of conformer **I** coinciding with the observed value ($J_{14,15}$ 9.76 Hz), and the distinct NOE enhancement between 15-Me and 6-H but not between 15-Me and 13-H being observed (Fig. 9 and Fig. 11), the absolute configuration *R* for C-15 was assigned.

Conclusions.—From the data described above, the stereostructure of tautomycin was determined as being that shown

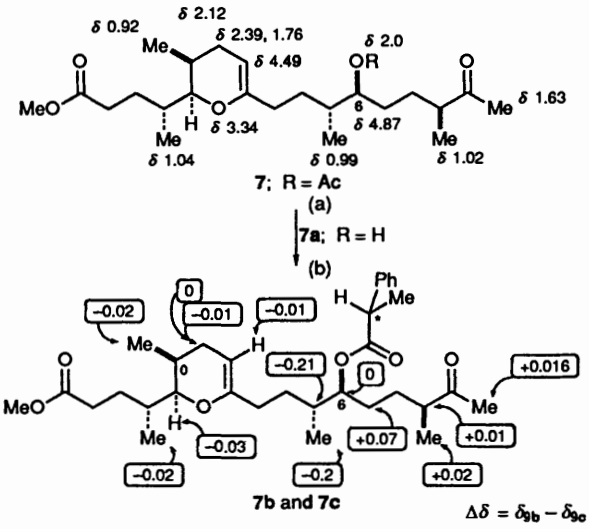

Fig. 8 Determination of absolute configuration at C-6 in compound **7**: Δδ-values obtained from (*S*)-*O*-methylmandelate derivative **7b** and (*R*)-*O*-methylmandelate derivative **7c**. *Reagents:* (a) NaOMe, MeOH; (b) (*S*)- or (*R*)-*O*-methylmandelic acid, DMAP, DCC.

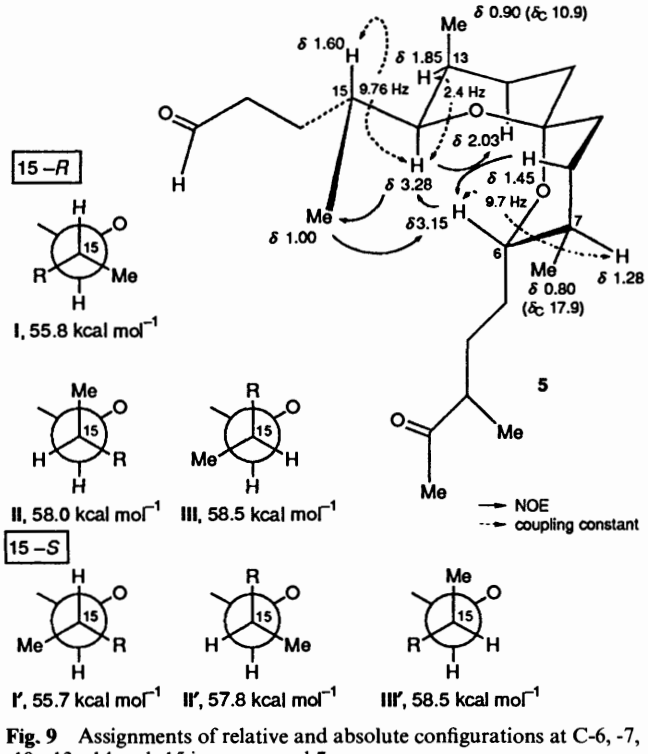

Fig. 9 Assignments of relative and absolute configurations at C-6, -7, -10, -13, -14 and -15 in compound **5**

in structure **1**. Since tautomycin **1** is a specific inhibitor of protein phosphatase 1 and 2A[3,4] and also competes with [^{3}H]okadaic acid in the binding assay to protein phosphatases,[2] the binding sites of each inhibitor may be identical.

Approximately 60% of the tautomycin molecule may exist as a dicarboxylic acid under aqueous conditions[5,6] and the carboxylic acid moieties may mimic the phosphate moiety on serine or threonine of phospho-proteins. The major difference between tautomycin **1** and okadaic acid is that okadaic acid is a strong inhibitor of protein phosphatase 2A and a weak inhibitor of protein phosphatase 1, whereas tautomycin **1** inhibits strongly both protein phosphatases 1 and 2A.

Conformational flexibility of tautomycin **1** as compared with that of the more rigid okadaic acid may account for its different specificity to protein phosphatases. The establishment of the absolute configuration of tautomycin **1** presented in this report

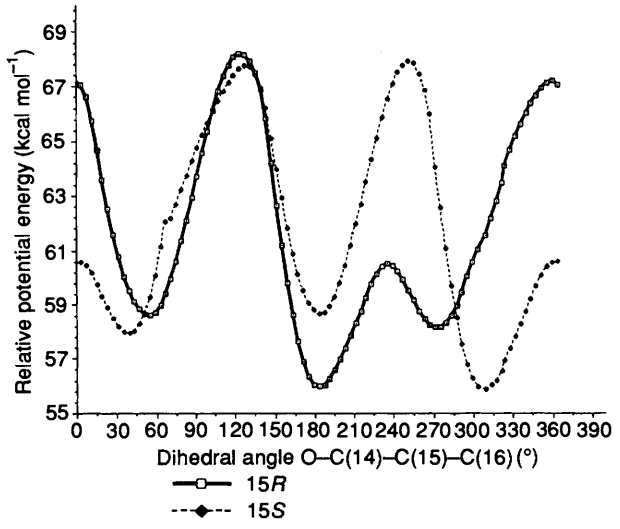

Fig. 10 Plots indicating the relationships between the dihedral angles O–C(14)/C(15)–C(16) of the 15*R* and 15*S* aldehydes **5** and their corresponding energies

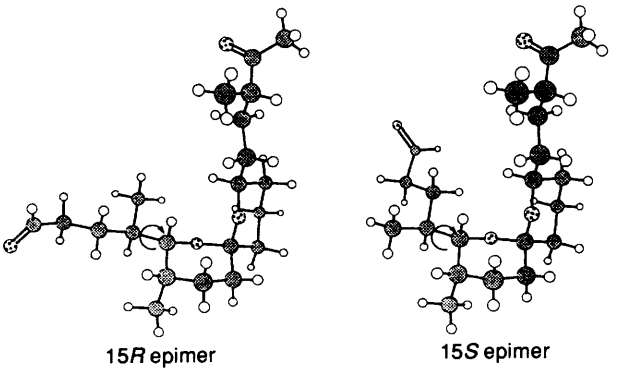

Fig. 11 Stable conformations of 15*R* and 15*S* aldehydes **5** obtained from Dreiding force field calculations

may promote a greater understanding of the biological mode of action of tautomycin **1** and increase its utility as a biological probe.[2-4]

Experimental

General Procedure.—All moisture-sensitive reactions were carried out under argon. Optical rotations were measured on a Perkin-Elmer 141 polarimeter and $[\alpha]_D$-values are given in 10^{-1} deg cm^2 g^{-1}. CD spectra were recorded on a JASCO J-20 Automatic Recording Spectropolarimeter. EI-MS and SI-MS were recorded on a Hitachi M-80 spectrometer. FAB-MS were recorded on a JMS-HX110 spectrometer. ^{1}H NMR and 2D-NMR spectra were measured on JMN GX-400 FT NMR and JMN GSX-500 FT NMR instruments. *J* Values are given in Hz. The steric conformations were built up by three-dimensional graphics on a Macintosh II$_{fx}$ computer. COSMIC force field energy calculations were performed by the Nemesis program V 1.1 (Oxford Molecular Limited). Delicate conformational analyses of structures **7** and **5** were calculated by the Monte Carlo method and the Dreiding force field method by using the Biograf program, and the calculation of *J* values by Macro-Model were performed on a Power IRIS GTX200BII (Silicongraphics Limited).

p-*Bromobenzoylation of Compound* **2b**.—To a stirred solution of tautomycin **1** (259 mg, 0.33 mmol) in methanol (5 cm^3) was added dropwise aq. 20% caesium carbonate until the mixture solution reached pH 9. The reaction mixture was stirred at room temperature for 3 h. The solution was then adjusted to pH 4 with 0.1 mol dm^{-3} hydrochloric acid, and the methanol was evaporated off. Water was added to the resulting mixture. After extraction with ethyl acetate, the aqueous layer was lyophilized. The dry powder was suspended in methanol (5 cm^3). To the stirred suspension at 0 °C was added dropwise conc. sulfuric acid (0.5 cm^3) and, after 48 h at room temperature, the methanol was removed by evaporation. The aqueous solution was extracted with ethyl acetate. The organic solution was washed with water and dried over anhydrous sodium sulfate. After filtration, the filtrate was concentrated. The residue was subjected to preparative TLC (PLC) [silica gel; MeOH–$CHCl_3$ (1:25)] to give compound **2b** (48.5 mg, 56%); $[\alpha]_D^{25}$ − 16.3 (*c* 0.93, $CHCl_3$); EI-MS *m/z* 261 (M + H)$^+$; δ_H(400 MHz; $CDCl_3$) 2.00 (3 H, s, 5'-Me), 2.58 (1 H, dd, *J* 4.0 and 17, 2'-H), 2.95 (1 H, dd, *J* 10 and 17, 2'- H), 3.72 (6 H, s, OMe × 2), 3.80 (3 H, s, OMe) and 5.10 (1 H, dd, *J* 4.0 and 10.0, 3'-H).

To a solution of compound **2b** (5 mg, 0.02 mmol) in dichloromethane (1 cm^3) at 0 °C were added *p*-bromobenzoyl chloride (4.4 mg, 0.024 mmol) and DMAP (3.6 mg, 0.03 mmol). The reaction mixture was stirred for 15 min. After removal of solvent under reduced pressure, ethyl acetate was added to the residue. The organic solution was washed successively with 0.1 mol dm^{-3} hydrochloric acid, aq. sodium hydrogen carbonate, and water, dried over Na_2SO_4, filtered, and concentrated. Purification *via* PLC [silica gel; benzene–EtOAc (4:1)] provided compound **2c** (4.4 mg, (50%); CD λ_{ext} nm ($\Delta\varepsilon$) (EtOH) 244 (−2.4); δ_H(400 MHz, $CDCl_3$) 2.18 (3 H, s, 5'-Me), 2.90 (1 H, dd, *J* 5.6 and 17, 2'-H), 3.22 (1 H, dd, *J* 9.7 and 17, 2'-H), 3.685 (3 H, s, OMe), 3.69 (3 H, s, OMe), 3.72 (3 H, s, OMe), 6.32 (1 H, dd, *J* 5.6 and 9.7, 3'-H), 7.58 (2 H, d, *J* 9.1, ArH) and 7.83 (2 H, d, *J* 9.1, ArH).

(S)-O-*Methylmandelate* **2d**.—To a stirred solution of compound **2b** (12.4 mg, 0.048 mmol) prepared as previously described,[6] in CH_2Cl_2 (2 cm^3) were added (*S*)-*O*-methylmandelic acid (15.83 mg, 0.095 mmol), 1,3-dicyclohexylcarbodiimide (DCC) (19.67 mg, 0.095 mmol) and DMAP (11.65 mg, 95.4 μmol). After 19 h, the reaction mixture was filtered. The filtrate was subjected to PLC [silica gel; $CHCl_3$–MeOH (25:1)] to give compound **2d** (11.2 mg, 58%); SI-MS *m/z* 409 (M + H)$^+$; δ_H(500 MHz; $CDCl_3$) 2.09 (3 H, s, 5'-Me), 2.67 (1 H, dd, *J* 4.6 and 16.5, 2'-H), 2.97 (1 H, dd, *J* 9.8 and 16.5, 2'-H), 3.41 (3 H, s, OMe), 3.42 (3 H, s, OMe), 3.71 (3 H, s, OMe), 3.74 (3 H, s, OMe), 4.69 (1 H, s, CH), 6.05 (1 H, dd, *J* 4.6 and 9.8, 3'-H) and 7.29–7.4 (5 H, m, Ph).

(R)-O-*Methylmandelate* **2e**.—This compound was obtained in 53% yield from compound **2b** with (*R*)-*O*-methylmandelic acid by essentially the same procedure as for the preparation of diastereoisomer **2d**; SI-MS *m/z* 409 (M + H)$^+$; δ_H(500 MHz; $CDCl_3$) 2.03 (3 H, s, 5'-Me), 2.78 (1 H, dd, *J* 4.6 and 16.8, 2'-H), 3.11 (1 H, dd, *J* 10 and 16.8, 2'-H), 3.39 (6 H, s, OMe), 3.65 (3 H, s, OMe), 3.70 (3 H, s, OMe), 4.71 (1 H, s, CH), 6.12 (1 H, dd, *J* 4.6 and 10, 3'-H) and 7.3–7.4 (5 H, m, Ph).

(R)-(+)-*MTPA Diester* **3a**.—To a stirred solution of compound **3** (40 mg, 0.07 mmol), prepared as previously described,[6] in tetrachloromethane (1 cm^3) were added DMAP (18 mg, 0.15 mmol), pyridine (0.5 cm^3) and (*R*)-(+)-MTPACl (60 mg, 0.26 mmol). After 3 days, the reaction mixture was concentrated under reduced pressure. The residue was dissolved in ethyl acetate and the organic solution was washed successively with water, aq. copper(II) sulfate, and water, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was subjected to PLC [silica gel; $CHCl_3$–MeOH (50:1)] to give compound **3a** (40 mg, 57%); FAB-MS

m/z 999 $(M + H)^+$; δ_H(400 MHz; $CDCl_3$) 0.8 (3 H, d, *J* 7.3, 7-Me), 0.806 (3 H, d, *J* 7.3, 13-Me), 0.863 (3 H, d, *J* 7.3, 26-H_3), 0.93 (3 H, d, *J* 7.3, 25-Me), 0.96 (3 H, d, *J* 7.3, 15-Me), 0.96 (3 H, d, *J* 7.3, 19-Me), 1.087 (3 H, *J* 7.3, 3-Me), 1.686 (1 H, m, 13-H), 1.90 (1 H, m, 25-H), 2.125 (3 H, s, 1-H_3), 2.54 (1 H, m, 3-H), 3.13 (1 H, dt, *J* 2.6 and 9, 6-H), 3.2005 (1 H, dd, *J* 2.8 and 9.7, 14-H), 3.3 (3 H, s, 23-OMe), 3.47 (3 H, s, OMe), 3.54 (3 H, s, OMe), 3.926 (1 H, dd, *J* 5.9 and 7.8, 23-H), 5.18 (1 H, dd, *J* 5.6 and 8.3, 24-H) 5.37 (1 H, dt, *J* 4.7 and 7.7, 18-H), 6.24 (1 H, d, *J* 16.2, 21-H) and 6.689 (1 H, dd, *J* 7.8 and 16.2, 22-H).

(S)-(−)-*MTPA Ester* **3b**.—This compound was obtained in 41% yield from compound **3** with (*S*)-(−)-MTPACl by essentially the same procedure as for the preparation of compound **3a**; FAB-MS *m/z* 999 $(M + H)^+$; δ_H(400 MHz; $CDCl_3$) 0.77 (3 H, d, *J* 7.3, 13-Me), 0.8 (3 H, d, *J* 7.3, 7-Me), 0.946 (3 H, d, *J* 7.3, 26-H_3), 0.93 (3 H, d, *J* 7.3, 25-Me), 0.93 (3 H, d, *J* 7.3 15-Me), 1.05 (3 H, d, *J* 7.3, 19-Me), 1.092 (3 H, d, *J* 7.3, 3-Me), 1.625 (1 H, m, 13-H), 1.96 (1 H, m, 25-H), 2.135 (3 H, s, 1-H_3), 2.55 (1 H, m, 3-H), 3.125 (1 H, dt, *J* 2.6 and 9, 6-H), 3.1675 (1 H, dd, *J* 2.8 and 9.7, 14-H), 3.22 (3 H, s, 23-OMe), 3.47 (3 H, s, OMe), 3.49 (3 H, s, OMe), 3.894 (1 H, dd, *J* 5.4 and 7.8, 23-H), 5.142 (1 H, dd, *J* 5.4 and 8.4, 24-H), 5.375 (1 H, dt, *J* 4.2 and 8.3, 18-H), 6.239 (1 H, d, *J* 16, 21-H) and 6.662 (1 H, dd, *J* 7.8 and 16, 22-H).

Acetonide **8**.—Ozone was passed through a solution of compound **3** (250 mg, 0.44 mmol) in CH_2Cl_2 (10 cm^3) at −78 °C for 1 h. Excess of ozone was removed by concentration under reduced pressure, and the residue was dissolved in a mixture of CH_2Cl_2 (5 cm^3), methanol (2.5 cm^3) and water (2.5 cm^3). To the above solution was added 30% hydrogen peroxide (300 cm^3) at −10 °C. The reaction mixture was adjusted to pH 4 with 0.1 mol dm^{-3} hydrochloric acid and kept at 10 °C for 2 h. The mixture was then concentrated under reduced pressure. The aqueous solution was extracted with ethyl acetate. The organic solution was concentrated under reduced pressure. The residue was subjected to PLC [silica gel; $CHCl_3$–MeOH (25:1)] to give an acid (**3-1**) (120 mg) (R_f 0.2).

To a stirred solution of the intermediate **3-1** (120 mg) in diethyl ether (5 cm^3) at 0 °C was added an excess of diazomethane (CH_2N_2) in diethyl ether solution. The reaction mixture was stirred at 0 °C for 3 h. After concentration to dryness under reduced pressure the residue was subjected to PLC [silica gel; benzene–EtOAc (4:1)] to give a methyl ester **3-2** (35 mg) (R_f 0.3).

To a stirred solution of the methyl ester **3-2** (26 mg, 0.057 mmol) in tetrahydrofuran (THF) (5 cm^3) was added lithium aluminium hydride (LAH) (10 mg, 0.26 mmol). The reaction mixture was stirred at room temperature for 1 h. After dilution with ethyl acetate, the resulting organic solution was washed successively with 1 mol dm^{-3} hydrochloric acid and water, dried over Na_2SO_4, and concentrated under reduced pressure. The residue was subjected to PLC [silica gel; $CHCl_3$–MeOH (10:1)] to give a diastereoisomeric mixture of a triol (12 mg) **3-3** (R_f 0.3).

To a solution of the intermediate **3-3** (12 mg) in 2,2-dimethoxypropane (1 cm^3) was added a catalytic amount of toluene-*p*-sulfonic acid (*p*-TsOH). The reaction mixture was stirred overnight at room temperature and then diluted with ethyl acetate. The organic solution was washed successively with 1 mol dm^{-3} hydrochloric acid and water, and then dried over Na_2SO_4. After filtration, the solvent was evaporated off under reduced pressure. The residue was subjected to PLC [silica gel; benzene–EtOAc (4:1)] to give a diastereoisomeric mixture of acetonides **8** (7.4 mg); SI-MS *m/z* 469 $(M + H)^+$; δ_H(400 MHz; $CDCl_3$) 0.75 (3 H, d, *J* 7.3, 19-Me), 0.82 (3 H, *J* 7.3, 7-Me), 0.9 (6 H, d, *J* 7.3, 3- and 13-Me), 1.00 (3 H, *J* 7.3, 15-Me), 1.14 and 1.18 [3 H (1:1), d × 2, 1-H_3], 1.36 and 1.41 (each 3 H, each s, acetonide Me_2), 1.64 (1 H, m, 19-H), 3.20 (1 H, dt, *J* 3.3 and 10.4, 6-H), 3.30 (1 H, dd, *J* 2.5 and 10.8, 14-H), 3.38 (1 H, dt, *J* 3.2 and 10.3, 18-H), 3.48 (1 H, dd, *J* 12.5 and 14, 20-H_{ax}), 3.66 (1 H, dd, *J* 6.6 and 13, 20-H_{eq}) and 3.68 and 3.71 [1 H (1:1), m, 2-H].

Aldehyde **5**.—Aldehyde **5** was prepared by the method previously described.[6] 2D-NOESY and differential NOE spectra were run with a JMN GX-400 FT NMR spectrometer. For aldehyde **5**: $[\alpha]_D^{25}$ −45.8 (*c* 1.35 in $CHCl_3$); FAB-MS *m/z* 367 $(M + H)^+$; δ_H(400 MHz; $CDCl_3$) 0.8 (3 H, d, *J* 7.6, 7-Me), 0.9 (3 H, d, *J* 7.6, 13-Me), 1.0 (3 H, d, *J* 7.6, 15-Me), 1.1 (3 H, d, *J* 7.6, 3-Me), 1.25 and 1.60 (each 1 H, m, 5-H_2), 1.25 and 1.78 (each 1 H, m, 16-H_2), 1.28 (1 H, m, 7-H), 1.40 and 1.60 (each 1 H, m, 11-H_{ax} and 11-H_{eq}), 1.40 (1 H, m, 12-H_{eq}), 2.03 (1 H, dddd, *J* 4.3, 4.6, 13.9 and 14, 12-H_{ax}), 1.45 (1 H, m, 8-H_{ax}), 1.60 (each 1 H, m, 8-H_{eq}), 1.45 and 1.63 (each 1 H, m, 9-H_{ax} and 9-H_{eq}), 1.60 (1 H, m, 15-H), 1.60 and 1.72 (each 1 H, m, 4-H_{ax} and 4-H_{eq}), 1.85 (1 H, m, 13-H), 2.15 (3 H, s, 1-H_3), 2.40 and 2.55 (each 1 H, m, 17-H_2), 2.55 (1 H, m, 3-H), 3.15 (1 H, dt, *J* 3 and 9.7, 6-H), 3.28 (1 H, dd, *J* 2.4 and 9.76, 14-H) and 9.8 (1 H, t, *J* 2.5, 18-H).

Diacetonide **9**.—To a solution of crude tautomycin **1** (200 mg, ~50% purity) in THF (5 cm^3) was added lithium borohydride ($LiBH_4$) (20 mg). The reaction mixture was stirred for 1 h. After the usual work-up, the residue was subjected to silica gel column chromatography [$CHCl_3$–MeOH (10:1)] to give crude pentanols (34 mg). The crude pentanols were treated with pyridine (0.5 cm^3) and acetic anhydride (0.5 cm^3). After the usual work-up, the residue was subjected to silica gel column chromatography [$CHCl_3$–MeOH (50:1)] to give a penta-acetate mixture (26 mg). To the above mixture in methanol (0.5 cm^3) was added dropwise 20% aq. Cs_2CO_3 until the solution reached pH 10. The reaction mixture was stirred for 12 h at room temperature. The solution was then adjusted to pH 4 with 0.1 mol dm^{-3} hydrochloric acid, the methanol was evaporated off, and the residue was extracted with ethyl acetate. The organic solution was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was subjected to silica gel column chromatography [$CHCl_3$–MeOH (10:1)] to give a pure pentanol (10 mg). The pentanol was treated with 2,2-dimethoxypropane (1 cm^3) and a catalytic amount of *p*-TsOH. The reaction mixture was stirred for 1 h and then diluted with ethyl acetate. The organic solution was washed successively with 1 mol dm^{-3} hydrochloric acid and water, and was then dried over Na_2SO_4. After filtration, the filtrate was concentrated under reduced pressure. The residue was subjected to PLC [silica gel; benzene–EtOAc, (4:1)] to give a diastereoisomeric mixture of diacetonides **9** (6.4 mg); EI-MS, *m/z* 668 M^+; δ_H(400 MHz; $CDCl_3$) 0.85 (3 H, d, *J* 7.3, 19-Me), 0.9 (3 H, d, *J* 7.3, 7-Me), 0.87 and 1.01 (3 H, 1:1, each d, *J* 7.3, 3-Me), 0.9 (3 H, d, *J* 7.3, 13-Me), 0.94 and 0.98 (each 3 H, each d, *J* 7.3, 25-Me_2), 1.14 and 1.16 (3 H, 1:1, each d, *J* 7.3, 1-H_3), 1.27, 1.33, and 1.35 × 2 (each 3 H, each s, acetonide Me_4), 1.6 (2 H, m, 15- and 3-H), 1.8 (2 H, m, 13- and 25-H), 3.18 and 3.2 (2 H, overlapping, 6- and 18-H), 3.22 (1 H, dd, *J* 3.3 and 6.1, 23-H), 3.32 (1 H, dd, *J* 1.9 and 10, 14-H), 3.39 (3 H, s, 23-OMe), 3.40 (1 H, d or t, *J* 6.1, 24-H), 3.68 and 3.71 (1 H, 1:1, each m, 2-H), 3.79 (1 H, dt, *J* 3.3 and 7.6, 22-H) and 4.0 (1 H, dt, *J* 6 and 7.3, 20-H).

(R)-(+)-*MTPA Ester* **6a**.—To a stirred solution of compound **5a** (20 mg, 0.05 mmol), prepared as previously described,[6] in CH_2Cl_2 (10 cm^3) was added MCPBA (10 mg, 0.06 mmol) and $NaHCO_3$ (30 mg) and the mixture was stirred for 48 h. After the usual work-up, the crude material was subjected to PLC [silica gel; benzene–EtOAc (8:1)] to give inseparable diastereoisomeric acetates **5a-1** (14 mg, 68%) (major : minor 61 : 39).

To a stirred solution of acetates **5a-1** (14 mg, 0.04 mmol) in methanol (5 cm^3) was added 20% aq. Cs_2CO_3 until the solution reached pH 10. After 48 h the methanol was removed under reduced pressure. The aqueous solution was extracted with ethyl acetate. The organic layer was dried over Na_2CO_3. After filtration, the solution was concentrated and the residue was subjected to PLC [silica gel; $CHCl_3$–MeOH (10:1)] to give the acids **5a-2** (10 mg, 70%) (major:minor 61:39).

To a stirred solution of acids **5a-2** (10 mg, 0.028 mmol) in diethyl ether (2 cm^3) at 0 °C was added an excess of ethereal diazomethane. The solution was stirred at room temperature for 3 h. After concentration to dryness under reduced pressure the residue was subjected to PLC [silica gel; $CHCl_3$–$MeOH_2$ (10:1)] to give the methyl esters **6** (7 mg, 68%) (major:minor 61:39).

To a stirred solution of compounds **6** (3.5 mg, 0.01 mmol) in CCl_4 (0.5 cm^3) were added pyridine (0.5 cm^3), DMAP (3 mg, 0.02 mmol) and (*R*)-(+)-MTPACl (10 mg, 0.044 mmol) and the mixture was stirred for 36 h. After the usual work-up, the crude material was subjected to PLC [silica gel; benzene–EtOAc (4:1)] to give (*R*)-(+)-MTPA esters **6a** (3.0 mg, 50%) (major:minor 61:39); δ_H(400 MHz; $CDCl_3$; major) 0.68 (3 H, d, *J* 7), 0.89 (3 H, d, *J* 7), 0.96 (3 H, d, *J* 7), 1.37 (3 H, d, *J* 7, 3-Me), 2.29 (1 H, m), 2.38 (1 H, m), 3.06 (1 H, dt, *J* 3.3 and 10, 6-H), 3.17 (1 H, dd, *J* 3.4 and 10, 14-H), 3.57 (3 H, br s, OMe), 3.66 (3 H, s, CO_2Me), 5.18 (1 H, m, 3-H) and 7.39 and 7.53 (5 H, overlapping, Ph).

(S)-(−)-*MTPA Ester* **6b**.—To a stirred solution of compound **6** (3.5 mg, 0.01 mmol) in CCl_4 (0.5 cm^3) were added pyridine (0.5 cm^3), DMAP (3 mg, 0.02 mmol) and (*S*)-(−)-MTPACl (10 mg, 0.044 mmol) and the mixture was stirred for 36 h. After the usual work-up, the crude material was subjected to PLC [silica gel; benzene–EtOAc (4:1)] to give (*S*)-(−)-MTPA ester **6b** (3.8 mg, 63%) (major:minor 61:39); δ_H(400 MHz; $CDCl_3$; major) (0.78 (3 H, d, *J* 7), 0.9 (3 H, d, *J* 7), 0.98 (3 H, d, *J* 7), 1.29 (3 H, d, *J* 7, 3-Me), 2.29 (1 H, m), 2.36 (1 H, m), 3.13 (1 H, overlapping, 6-H), 3.2 (1 H, dd, *J* 3.2 and 10, 14-H), 3.55 (3 H, br s, OMe), 3.67 (3 H, s, CO_2Me), 5.19 (1 H, m, 3-H) and 7.39 and 7.54 (5-H, overlapping, Ph).

Spiro-ketal Cleavage of Compound **5a**.—To a stirred solution of spiro-ketal **5a** (37 mg, 0.093 mmol) in Ac_2O (1.85 cm^3) were added acetic acid (0.185 cm^3) and *p*-TsOH (35 mg). The reaction mixture was heated at 70 °C for 30 min. After cooling to room temperature, the resulting solution was poured onto ice and extracted with ethyl acetate. The organic solution was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was subjected to PLC [silica gel; toluene–EtOAc (9:1)] to give the acetate **7** (6.7 mg, 16.4%); δ_H(500 MHz; $CDCl_3$) 0.92 (3 H, d, *J* 7, 13-Me), 0.99 (3 H, d, *J* 7, 7-Me), 1.02 (3 H, d, *J* 7, 3-Me), 1.04 (3 H, d, *J* 7, 15-Me), 1.29 and 1.66 (each 1 H, m, 4-H_2), 1.63 (3 H, s, 1-H_3), 1.71 (1 H, m, 15-H), 1.76 and 2.39 (each 1 H, 12-H_2), 2.0 (3 H, s, Ac), 2.12 (1 H, m, 13-H), 2.13 (1 H, m, 7-H), 3.34 (1 H, br d, *J* 10.4, 14-H), 3.68 (3 H, s, CO_2Me), 4.49 (1 H, dd, *J* 2.6 and 5, 11-H) and 4.87 (1 H, m, 6-H).

(S)-O-*Methylmandelate* **7b**.—To a stirred solution of compound **7** (6.5 mg, 0.015 mmol) in methanol (2 cm^3) was added sodium methoxide (70 mg). After being stirred for 12 h the reaction mixture was quenched with ammonium chloride, and water, and extracted with ethyl acetate. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure to give crude alcohol **7a** (5.8 mg). The unstable product **7a** was subjected to the next reaction without purification.

To a stirred solution of crude alcohol **7a** (2.9 mg, 0.0073 mmol) in CH_2Cl_2 (0.6 cm^3) were added (*S*)-*O*-methylmandelic acid (2.5 mg, 0.015 mmol), DCC (3.5 mg, 0.017 mmol) and DMAP (0.2 mg, 0.0016 mmol). The reaction mixture was stirred at room temperature for 8 h before being subjected to PLC [silica gel; toluene–EtOAc (9:1)] to give the (*S*)-*O*-methylmandelate derivative **7b** (800 μg, 20% from **7**); FAB-MS: *m/z* 545 $(M + H)^+$; δ_H(500 MHz; $CDCl_3$) 0.75 (3 H, d, *J* 7, 7-Me), 0.89 (3 H, d, *J* 7, 13-Me), 1.0 (3 H, d, *J* 7, 3-Me), 1.01 (3 H, d, *J* 7, 15-Me), 1.61 (3 H, s, 1-H_3), 1.68 (1 H, m, 15-H), 1.76 and 2.37 (each 1-H, overlapping, 12-H_a and -H_b), 1.84 (2 H, m, 5-H_2), 1.95 (1 H, m, 7-H), 2.1 (1 H, m, 13-H), 2.73 (1 H, m, 3-H), 3.29 (1 H, br d, *J* 10.2, 14-H), 3.4 (3 H, s, OMe), 3.68 (3 H, s, CO_2Me), 4.45 (1 H, dd, *J* 2.9 and 6.12, 11-H), 4.7 [1 H, s, COC*H*(OMe)Ph], 4.91 (1 H, br dd, *J* 4.93 and 8.84, 6-H) and 7.25–7.43 (5 H, Ph).

(R)-O-*Methylmandelate* **7c**.—To a stirred solution of *compound* **7a** (2.9 mg, 0.0073 mmol) in CH_2Cl_2 (0.6 cm^3) were added (*R*)-*O*-methylmandelic acid (2.7 mg, 0.016 mmol), DCC (4 mg, 0.019 mmol) and DMAP (0.23 mg, 0.0019 mmol). The resulting solution was subjected to PLC [silica gel; toluene–EtOAc (9:1)] to give (*R*)-*O*-methylmandelate derivative **7c** (500 μg, 12% from **7**): δ_H(500 MHz; $CDCl_3$) 0.91 (3 H, d, *J* 7, 13-Me), 0.95 (3 H, d, *J* 7, 7-Me), 0.98 (3 H, d, *J* 7, 3-Me), 1.03 (3 H, *J* 7, 15-Me), 1.597 (3 H, s, 1-Me), 1.69 (1 H, m, 15-H), 1.77 (2 H, m, 5-H_2), 1.77 and 2.37 (each 1 H, overlapping, 12-H_a and -H_b), 2.1 (1 H, m, 13-H), 2.16 (1 H, m, 7-H), 3.32 (1 H, br d, *J* 10.5, 14-H), 3.41 (3 H, s, OMe), 3.68 (3 H, s, 18-OMe), 4.46 (1 H, dd, *J* 3 and 6.12, 11-H), 4.7 [1 H, s, COC*H*(OMe)Ph], 4.91 (1 H, br dd, *J* 4.8 and 8.1, 6-H) and 7.25–7.43 (5 H, Ph).

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