Synthetic application of biotransformations: absolute stereochemistry and Diels–Alder reactions of the (1*S*,2*R*)-1,2-dihydroxycyclohexa-3,5-diene-1-carboxylic acid from *Pseudomonas putida*

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1,*cis*-2-Dihydroxycyclohexa-3,5-diene-1-carboxylic acid, **2**, produced by *Pseudomonas putide*, U103, was shown *via* X-ray analysis of its *p*-bromobenzoylmethyl ester to have (1*S* 2*R*) absolute stereochemistry. The stereo- and regio-selectivity of a series of cycloadditions of the methyl ester **3** (R = Me) the methyl ester acetonide **5** and the hydroxymethylacetonide **6** (R = H) with singlet oxygen, 4-phenyl-4,5-dihydro-3*H*-1,2,4-triazole-3,5-dione, *N*-phenylmaleimide and nitrosobenzene have been established. The stereochemistry of the oxygen adduct **7** of **5** was unambiguously assigned by X-ray analysis of **7**. The acetonides **5** and **6** (R = H) were attacked by the dienophiles *anti* to the acetonide group. The diol ester **3** underwent attack on the face *syn* to the diol groups. The regiochemistry of the addition of nitrosobenzene was predominantly with the *N*–phenyl group distal to the ester function in **3** and **5**, but proximal to the hydroxymethyl group in **6** (R = H).

Microbial degradation of benzene and benzene rings functionalised by lipophilic groups[1,2] is commonly initiated by a 2,3-*cis*-dihydroxylation of the ring to generate diols **1** (Scheme 1).[3–8] This is the first, extensively studied step of the degradative pathway which provides pyruvic acid and acetaldehyde for incorporation into metabolic processes.[3–7,9]

Mutant strains of *P. putida* which are blocked in the subsequent dehydrogenase (rearomatisation) step, accumulate diols **1**[3–8] and have proved to be a rich source of *cis*-cyclohexa-3,5-diene-1,2-diols of high ee[10–12] for asymmetric synthesis.[13–29] 4-Substituted benzoic acids have been shown to be converted by other mutants of *P. putida* (strains JT 101 and JT810) into *cis*-2,3-diols which are antipodal to **1** with respect to the R group/4-substituent.[30,31]

In contrast the 1,2-dihydroxylation of benzoic acid (Scheme 2), first recorded by Hegeman for a mutant strain of *Alcaligenes eutrophus*[32] but subsequently reported in the U103 strain of *P. putida* by Ribbons,[33] is a much less described process. At the outset of this work, although a number of substrates had been used,[34] the products **2** were of unknown absolute stereochemistry[35] and were unexploited in synthesis. We therefore sought to characterise fully the parent diol **2** (R_n = H) and explore its reactivity as a basis for synthetic application. We report here the results of this investigation.

Results and discussions

The fermentation conditions for the large scale production (0.1 kg) of the diol **2** had been established by Taylor[34] and are given in the Experimental section. The diol was secreted into the reaction medium and no cell disruption was necessary. The cell mass was, therefore, removed by centrifugation and the aqueous supernatant was carefully evaporated under reduced pressure, with a bath temperature of ≤40 °C, to 2–5% of the initial volume. Optimal extraction efficiency of the product was achieved by saturation of the concentrate with sodium sulfate, acidification to pH 4 and a gentle stirring of the medium with ethyl acetate. The ethyl acetate extract yielded the diol as a pale yellow solid, [α] −106, which was stable at −20 °C for up to a year without significant rearomatisation or dimerisation.[13]

For the determination of the absolute stereochemistry of **2**, the acid was converted into the *p*-bromobenzoylmethyl ester **3** (R = $CH_2COC_6H_4Br$) under standard conditions and subjected to analysis by X-ray diffraction. The structure of **3** is shown in Figs. 1 and 2 and establishes the stereochemistry as (1*S* 2*R*).

To facilitate the study of the reactivity of the diol **2**, a stable derivative was required. On the basis of the reports on the 2,3-diol series,[36] the diprotected acetonide methyl ester **5** was chosen. The protecting groups could be introduced in either order *via* **3** (R = Me or **4**), but esterification–ketalisation proved to be the optimal sequence with an overall yield of 87% (Scheme 3).

Additionally, reduction of the ester **5** with lithium aluminium hydride to produce the alcohol **6** (R = H) (84%) and the protection of this as the *tert*-butyldiphenylsilyl ether **6** (R = $SiBu^tPh_2$) (53%), extended the range of dienes available for exploitation.

The Diels–Alder chemistry of the diene moiety was studied as a means to amplify the chirality of the bioproduct. The cycloadditions of the 2,3-diols **1** in the neutral series of bioproducts have been extensively studied[9,11,14,15,36–40] and the general sense of the stereoselectivities was established. Thus, the free diols **1** tend to undergo hydroxyl-directed cycloaddition to the face *syn* to the diol but the acetonides of **1** are attacked on the open face, *anti* to the acetonide group.

In the benzoate series **2**, the situation is less clear as both faces of the diene possess functionality capable of influencing the approach of a dienophile by steric or electrostatic effects. The stereo- and regio-chemical outcomes of the Diels–Alder reactions were, therefore, not reliably predictable.

In the event, reaction of **5** with photogenerated singlet

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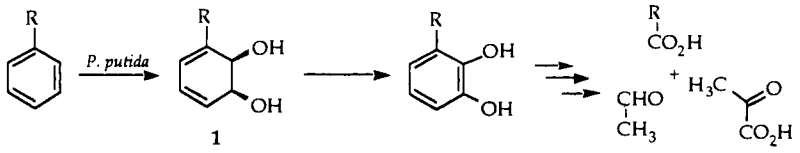
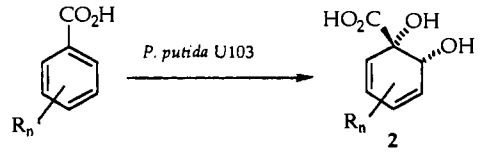


R = H, F, Cl, Br, I, Me, Et, alkyl, vinyl, aryl, CF_3

Scheme 1



Scheme 2

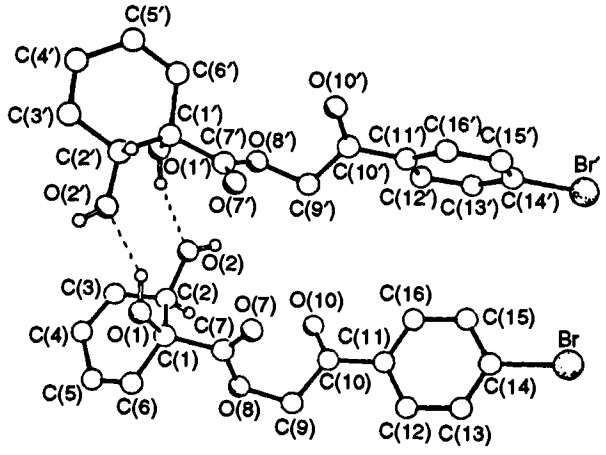


Fig. 1 The hydrogen bonded pair of crystallographically independent molecules of **3** (R = $CH_2COC_6H_4Br$). H-bond parameters are: O(1)···O(2') 2.72 Å, H(1)···O(2') 1.90 Å, O–H···O angle 149°; O(1')···O(2) 2.78 Å, H(1')···O(2) 1.91 Å, O–H···O angle 161°.

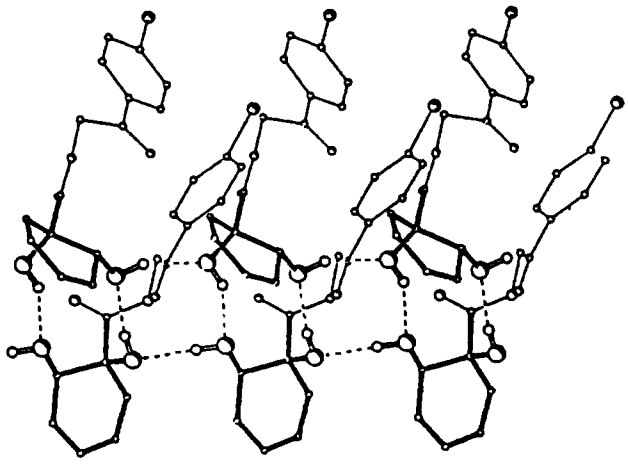
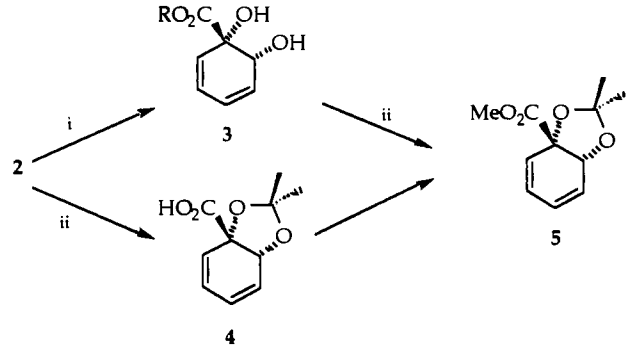
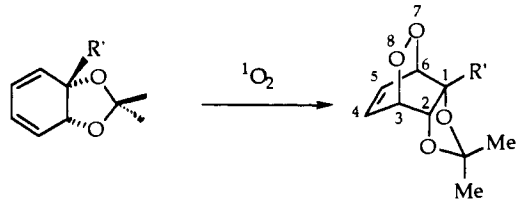


Fig. 2 A hydrogen bonded chain of linked pairs of molecules of **3** (R = $CH_2COC_6H_4Br$) that extend in the *a* direction in the crystal. H-bond parameters are O···O 2.81, 2.77 Å; H···O 1.91, 1.92 Å; O–H···O angles 174, 157°.



Scheme 3 *Reagents:* i, CH_2N_2; ii, $Me_2C(OMe)_2$, Me_2CO, toluene-*p*-sulfonic acid room temp.



5, R' = CO_2Me
6, R' = CH_2OR

7, R' = CO_2Me
8, R' = CH_2OR

Scheme 4

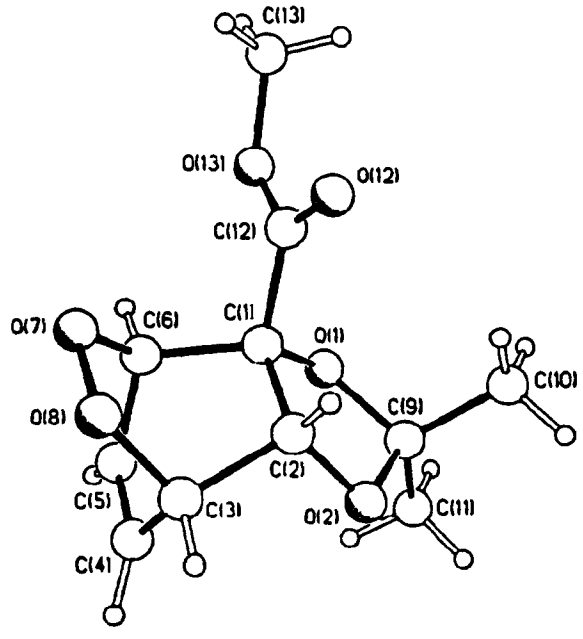
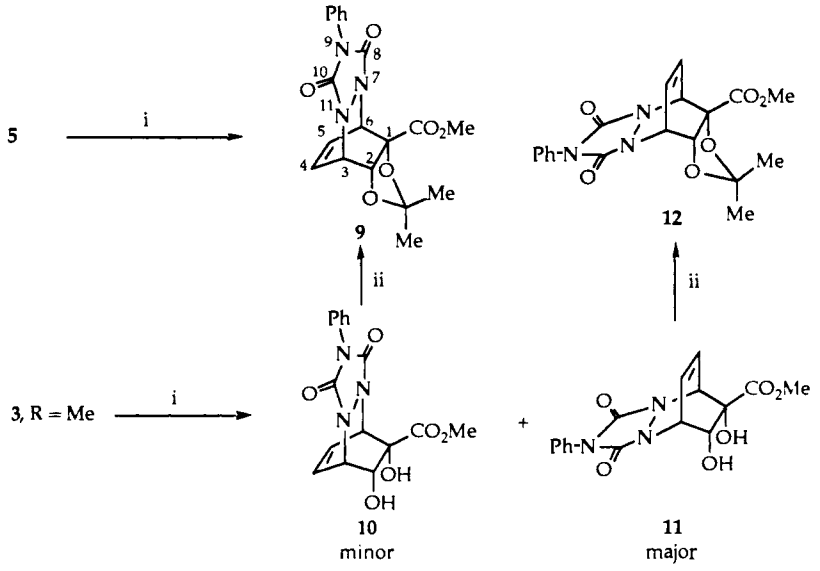


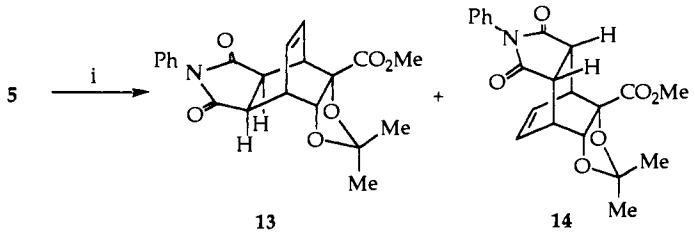
Fig. 3

oxygen gave a single Diels–Alder adduct **7** (Scheme 4), the stereochemistry of which was not immediately apparent from the spectral data. The crystalline compound was therefore subjected to X-ray diffraction analysis, the result of which is given in Fig. 3.

Similarly, the hydroxymethyl and the siloxymethyl analogues **6** (R = H and $SiPh_2Bu^t$) each gave a single adduct **8** (R = H or $SiPh_2Bu^t$), respectively. With the X-ray structure of **7** established, it was possible to interpret the NMR data with a high degree of certainty. Thus, the alkene protons of **7** and **8**



Scheme 5 *Reagents:* i, 4-phenyl-4,5-dihydro-3*H*-1,2,4-triazole-3,5-dione; ii, $Me_2C(OMe)_2$, toluene-*p*-sulfonic acid



Scheme 6 *Reagent:* i, *N*-phenylmaleimide

appear close to δ_H 6.60 when the 4,5-etheno bridge ‡ is *syn* to the isopropylidene group. As will be seen below, when the 4,5-etheno bridge is *syn* to the methoxycarbonyl or hydroxymethyl groups, the alkene protons are differentiated by 0.1–0.5 ppm with the difference always being greater than that of the isomeric *syn* isopropylideneethene unit. The products **8**, which have undifferentiated 4- and 5-H protons by ^1H NMR can therefore be assigned the (3*S*,6*R*) stereochemistry as shown. Adverse lone pair-lone pair interactions between the acetonide oxygen atoms and the incoming heteroene (oxygen or nitrogen) is presumably the principal determinant of the facial selectivity and surprisingly, the stereospecificity was not altered by the presence of the very bulky TBDPS ether group in **6** (R = $SiPh_2Bu^t$).

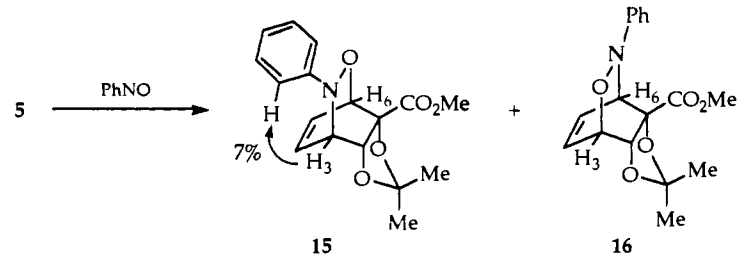
Similarly, reaction of the ester **5** with the 4-phenyl-4,5-dihydro-3*H*-1,2,4-triazole-3,5-dione gave a single adduct **9**‡ (Scheme 5) with the same facial selectivity, *i.e.* with the dienophile adding *syn* to the methoxycarbonyl group. In contrast, the free diol **3** (R = Me) gave an inseparable mixture of the adducts **10** and **11** in a 1:6 ratio (by NMR analysis). Chromatographic separation was achieved by conversion of the adducts into their isopropylidene derivatives [$Me_2C(OMe)_2$–TsOH]. The minor isomer (12%) proved to be the *syn* adduct isolated before. The major isomer (58%) was the *anti* adduct **12**, derived from **11**. This must arise, as in the neutral diol series,[11] by hydrogen bonding of the hydroxy groups with the incoming dienophile.

‡ In order to allow ready comparison and avoid confusion, the numbering system used for the cycloadducts is derived from the parent diol numbering as shown in **7** and **9** and not the IUPAC system.

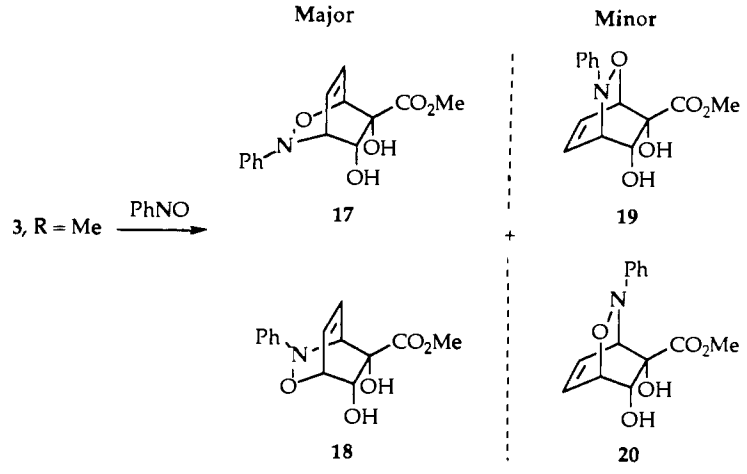
Reaction of the diene **5** with *N*-phenylmaleimide was much more sluggish. After 4 d at room temperature a 20:1 mixture of two *endo*-adducts **13** and **14** (Scheme 6) was produced. The structure of the major adduct **13** was determined by an NOE experiment (see Experimental section) which demonstrated the proximity of 7-H/11-H to the isopropylidene methyl groups. The formulation of the minor adduct as in **14** then follows from the NMR spectrum. The alkene protons 4-H and 5-H are undifferentiated, implying their *syn* relationship to the isopropylidenedioxy group, and the 7-H (δ_H 2.98), 11-H (δ_H 3.10) protons are well differentiated, consistent with their *syn* relationship with the 1-methoxycarbonyl group rather than with the symmetrical 4,5 etheno bridge.

The cycloaddition of nitrosobenzene[39,41] was also studied as a route to functionally differentiated substitution on the cyclohexane ring. Reaction of **5** with nitrosobenzene in toluene at room temperature gave two adducts **15** and **16** in a 10:1 ratio after 4–6 h (Scheme 7). The structure of the adducts was assigned as shown from a NOE experiment and considering the δ_H values of the bridgehead protons 3-H and 6-H. Therefore, on the basis of the functional array around the bridgeheads C-3 and C-6, the 3-H/6-H pair with closer δ_H values can be assigned to **15** and the more separated 3-H/6-H pair to **16**. The NOE correlation of 3-H and the phenyl ring protons confirms the proximity of the PhN group to the 3-H bridgehead.

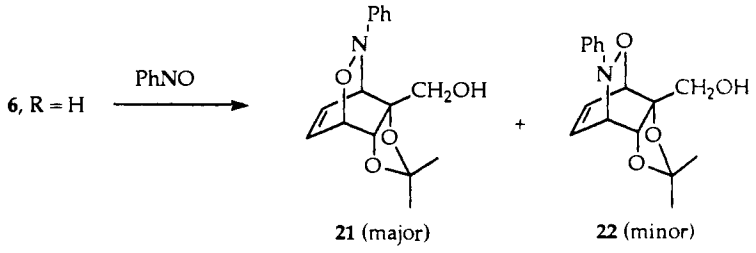
In contrast, the free diol ester **3** (R = Me) gave a more complex mixture of all four possible adducts (Scheme 8). These were readily separated by chromatography into a 6:1 ratio of two pairs of adducts. Again NMR analysis (see Experimental section) allowed the identity of the adducts to be established. The less polar major pair (ratio 3:1) proved to be the products, **17** and **18**, from attack *syn* to the diol functionality with the



Scheme 7



Scheme 8



Scheme 9

major isomer **17** the result of the attachment of oxygen to C-6. The more polar, minor pair, were the alternate adducts **19** and **20** from attack *anti* to the diol.

The major products are presumably the result of hydrogen bonding between the diol and the incoming dienophile.[11] As with the *anti* adducts **15** and **16**, there is a preference for the PhN group to be remote from the quaternary C-1 centre even when the bulky methoxycarbonyl group is *anti* to the attacking dienophile.

Finally, the reduced diene **6** (R = H) was treated with nitrosobenzene as before to produce two chromatographically separable regioisomers, in a 4.7 : 1 ratio, resulting from a facially specific attack on the diene (Scheme 9).

For compounds **21** and **22**, the NMR spectral characteristics used previously to distinguish regioisomers were unresolved. The identity of the minor isomer as **22** was therefore established by reduction of the major product **15** of Scheme 7 with lithium aluminium hydride to produce material identical with **22**.

The facial selectivity was therefore as for the ester **5**, but the regioselectivity was reversed as a result of the relative effects of the ester and hydroxymethyl functions on the orbital coefficients of the diene HOMO.[17]

We have thus developed a route to a range of stereodefined polyfunctionalised cyclohexenes which provides the opportunity to apply these biogenerated synthons to the synthesis of enantiopure targets. This will be exemplified in the next paper in this series.

Experimental

^{1}H and ^{13}C NMR were recorded on JEOL GSX 270, Bruker WM-250 or Bruker AM-500 NMR spectrometers using $CDCl_3$ as solvent unless otherwise stated. ^{1}H NMR spectra were referenced using residual protic solvent, $CHCl_3$ (δ_H 7.26, s) and ^{13}C NMR spectra using $CDCl_3$ (δ_C 77.0, t) with broad band decoupling. All $J_{n,m}$ values are given in Hz. IR spectra were recorded on a Perkin-Elmer 1710 Infrared Fourier Transform spectrometer as Nujol mulls or thin film as indicated. UV spectra were recorded on a Perkin-Elmer Lambda 2 UV–VIS spectrometer and the values for the extinction coefficient, ε are given in $dm^3\ mol^{-1}\ cm^{-1}$. Optical rotations were measured using a Perkin-Elmer 141 Polarimeter and $[\alpha]_D$ values are given in 10^{-1} deg $cm^2\ g^{-1}$. Melting points were determined using a Reichert hot-stage microscope and are uncorrected. Mass spectra were recorded under EI conditions, unless otherwise stated, using VG-7070E or VG AUTOSPEC-Q instruments in

the Imperial College Chemistry Department, or VG 12–253 and VG ZAB-E instruments at the SERC Mass Spectrometry Service in Swansea. Microanalyses were performed in the Imperial College Chemistry Department Microanalysis Laboratory.

Diethyl ether (referred to as ether) and tetrahydrofuran. (THF) were distilled from sodium-benzophenone ketyl. Light petroleum refers to the fraction with bp 40–60 °C which was distilled prior to use. Other solvents and reagents were purified by standard procedures.

Analytical thin layer chromatography was performed using pre-coated glass-backed plates (Merck Kieselgel $60F_{254}$) and visualised by UV light (254 nm), iodine or acidic ammonium molybdate(VI) as appropriate. Flash chromatography was performed on Sorbsil C60 silica under pressure.

(1*S*,2*R*)-1,2-Dihydroxycyclohexa-3,5-dienecarboxylic acid 2 (R_n = H)

Inoculum. Nutrient broth (50 cm^3) was inoculated with *Pseudomonas putida* U103 and incubated with shaking at 30 °C overnight. This culture was then divided and used to inoculate two flasks of nutrient broth (200 cm^3 each) which were also incubated at 30 °C overnight. The inoculum was introduced into the fermenter using a sterile 1 dm^3 aspirator.

Batch feed fermentation. A 14 dm^3 New Brunswick Microferm (Model MF-114) fermenter system was used controlling the temperature, agitation rate, pH and dissolved oxygen (airflow). A mineral salts mixture [11.4 dm^3: containing 10 g dm^{-3} glucose; 0.25 g dm^{-3} $MgSO_4 \cdot 7H_2O$; 3 g dm^{-3} $(KH_2PO_4$; 1 g dm^{-3} $(NH_4)_2SO_4$; 1 g dm^{-3} polypropyleneglycol antifoam] was sterilised at 121 °C for 25 min before a trace element solution (114 cm^3: containing 4.38 g dm^{-3} $CaCl_2 \cdot 2H_2O$; 8 g dm^{-3} $FeSO_4 \cdot 7H_2O$; 0.2 g dm^{-3} $ZnSO_4 \cdot 5H_2O$; 0.4 g dm^{-3} $CuSO_4 \cdot 5H_2O$; 0.4 g dm^{-3} $MnSO_4$ hydrate; 0.04 g dm^{-3} $CoCl_2 \cdot 6H_2O$; 0.004 g dm^{-3} H_3BO_4 100 g dm^{-3} citric acid) was added. After cooling to 30 °C, the pH was adjusted to 7.00 ± 0.1 using ammonia solution (10 mol dm^{-3}). The pH was controlled automatically throughout the fermentation by addition of ammonia (10 mol dm^{-3}) or phosphoric acid (4 mol dm^{-3}) solutions. The oxygen probe was calibrated at 30 °C with 1 dm^3 min^{-1} N_2 as 0% and 1 dm^3 min^{-1} O_2 as 100% at an agitation speed of 1000 rpm.

After inoculation with the *P. putida* U103, the culture was grown overnight at 30 °C, pH 7, with the dissolved oxygen concentration automatically controlled at 40% by changing the speed of agitation (the sterile air was introduced at a rate of 1 dm^3 min^{-1}). With a glucose feed of 13 mmol h^{-1}, sodium benzoate salt (5 mmol) was added to induce the oxygenase and once biotransformation had been initiated, a feed of sodium benzoate was initiated at 5 mmol h^{-1}. The feed was terminated when benzoate began to accumulate in the medium.

The fermenter culture was centrifuged and the supernatant concentrated under reduced pressure to between 1/20th and 1/50th of its original volume at ≤40 °C before being acidified with dilute hydrochloric acid to pH 4.0. The concentrated broth was then repeatedly stirred with ethyl acetate portions (150 cm^3) for 15–20 min until UV analysis of the broth indicated complete extraction of the diol. The pH of the broth was monitored and adjusted when necessary and sodium sulfate was added to aid extraction of the diol. The combined ethyl acetate extracts were dried over $MgSO_4$ and the solvent was removed from the mixture under reduced pressure to give a yellow solid. Much of the yellow colouration was removed by washing the solid with dichloromethane (2 × 25 cm^3) to leave (1*S*,2*R*)-1,2-dihydroxycyclohexa-3,5-dienecarboxylic acid **2** (R_n = H) as a pale beige solid in a yield of 2–3 g dm^{-3} of the original fermentation broth; $[\alpha]_D$ −106 (*c* 0.5 in EtOH) [lit.,[32] (Na^+ salt) −123.8 (*c* 1.78 in H_2O)]; λ_{max}(EtOH) 261.0 nm (ε 3400) [lit.,[32] λ_{max}(H_2O, pH 7.0) 262 nm (ε 3350) (Na^+ salt)]; ν_{max}(Nujol)/cm^{-1} 3271, 1706 and 1640; δ_H(270 MHz, $CDCl_3$ + [$^2H_6$] − DMSO) 5.4 (1 H, br m, 3-H) 5.2 (1 H, d m, 6 H) 5.2 (2 H, br d, 4-H, 5-H) 4.1 (1 H, br s, 2 H).

***p*-Bromobenzoylmethyl (1*S*,2*R*)-1,2-dihydroxycyclohexa-3,5-dienecarboxylate 3 (R = $CH_2COC_6H_4Br$)**

A solution of redistilled triethylamine (0.3 cm^3, 2.15 mmol) in dry acetone (4 cm^3) was neutralised by portionwise addition of compound **2** (R_n = H). After this a solution of p-bromobenzoylmethyl bromide (701.6 mg, 1.2 equiv.) in acetone (5 cm^3) was added to it and the mixture kept at room temperature for 3 h during which time triethylammonium bromide was precipitated. The solution was diluted with water (10 cm^3), stirred and the resultant precipitate filtered off. The crude solid (609.5 mg, 1.73 mmol, 68%) was recrystallised from ethanol–water to give colourless needles of the title compound **3** (R = CH_2CO-C_6H_4Br), mp 100–102 °C; δ_H(270 MHz) 7.79 (2 H, d, *J* 8.5, Ar, 2′,6′-H), 7.67 (2 H, d, *J* 8.5, Ar, 3′,5′-H), 6.23 (1 H, m, diene), 6.01 (2 H, m, diene), 5.87 (1 H, m, diene), 5.57 (1 H, d, *J* 16.6, OC*H*HCO), 5.46 (1 H, d, *J* 16.6, OCH*H*CO) and 5.06 (1 H, br d, *J* 7.3, 2-H).

X-Ray crystallographic analysis of 3 (R = $CH_2COC_6H_4Br$)

Single crystals suitable for X-ray analysis were grown from ethanol–water.

Crystal data. $C_{15}H_{13}BrO_5$, *M* = 353.2, orthorhombic, *a* = 5.467(2), *b* = 18.505(4), *c* = 28.973(6) Å, *V* = 2931(2) $Å^3$, space group $P2_12_12_1$, *Z* = 8 (two crystallographically independent molecules), D_c = 1.60 g cm^{-3}, μ(Cu-Kα) = 40.1 cm^{-1}, *F*(000) = 1424. Data were measured on a Nicolet R3m/E diffractometer (2θ < 116°) with Cu-Kα radiation (graphite monochromator) using ω-scans. 2354 Independent reflections were measured and of these, 1960 had $|F_o| > 4\sigma(|F_o|)$ and were considered to be observed. The data were corrected for Lorentz and polarisation factors and for absorption (face indexed numerical, for a crystal of dimensions 0.04 × 0.09 × 0.50 mm, minimum and maximum transmission factors 0.576 and 0.862, respectively). The structure was solved by direct methods and the non-hydrogen atoms were refined anisotropically. The positions of the hydrogen atoms were determined from a Δ*F* map. The positions of the C–H atoms were idealised, (C–H = 0.96 Å), assigned isotropic thermal parameters, *U*(H) = $1.2U_{eq}$(C), and allowed to ride on their parent carbon atoms. The hydroxy hydrogen atoms were refined isotropically subject to an O–H distance constraint (0.90 Å). Refinement was by full-matrix least squares to give *R* = 0.034, R_ω = 0.035 ($\omega^{-1} = \sigma^2(F) + 0.0005F^2$). The absolute configuration was determined by refinement of a free variable η which multiplies all *f*‴. This parameter refined to a value of 1.17(6) providing a definitive assignment of the absolute stereochemistry. The maximum residual electron density in the final Δ*F* map was 0.25 e $Å^{-3}$. Computations were carried out on a 486 PC using the SHELXTL-PC program system.[42] Atomic coordinates, bond lengths, angles and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre.

Methyl (1*S*,2*R*)-1,2-dihydroxycyclohexa-3,5-dienecarboxylate 3 (R = Me)

A solution of Diazald® (11 g, 1 equiv. assuming 1 g generates ≈3 mmol CH_2N_2) in ethanol (50 cm^3) was stirred in a conical flask fitted with side-arm, rubber septum and magnetic stirrer bar. Nitrogen was bubbled through the solution and into a reaction flask containing a solution of compound **2** (R_n = H) (5 g, 33 mmol) in dichloromethane–ethanol (10:1). Small amounts of sodium hydroxide (5 mol dm^{-3}) were syringed into the conical flask and the diazomethane produced was swept into the reaction flask. Sodium hydroxide was added dropwise

to the reaction mixture until the yellow colouration of the Diazald had disappeared. The nitrogen sweep was continued until the reaction flask also lost its yellow colouration after which the solvents were removed from the mixture under reduced pressure to give the methyl ester **3** (R = Me) as a pale yellow solid (5.3 g, 95%), mp 50–52 °C [lit.,[43] 51.5–52.5 °C from light petroleum] $[\alpha]_D^{20} = -75 \pm 2$ (*c* 0.5 in EtOH); λ_{max}(EtOH) 261.0 nm (ε 2770) [lit.,[43] (MeOH) 260.5 nm (ε 3100)]; ν_{max}(film)/cm^{-1} 3446, 3048, 2956, 1733, 1644 and 1579; δ_H(270 MHz) 6.15 (1 H, dd, $J_{5,6}$ 9.5, $J_{5,4}$ 5.0, 5-H), 5.96 (1 H, dddd, $J_{4,3}$ 9.8, $J_{4,5}$ 5.0, $J_{4,6}$ 1.0, $J_{4,2}$ 0.5, 4-H), 5.82 (1 H, ddd, $J_{3,4}$ 9.8, $J_{3,2} \approx 1.2$, 3-H), 5.76 (1 H, dd, $J_{6,5}$ 9.5, $J_{6,4}$ 1.0, 6-H), 4.85 (1 H, br d, $J_{2,OH} \approx 9$, $J_{2,3} \approx 1.2$, $J_{2,4}$ 0.5, 2-H), 3.88 (3 H, s, OMe), 3.58 (1 H, s, 3°-OH) and 2.67 (1 H, d, $J_{HO,2} \approx 9$, 2°-OH); δ_C(68 MHz) 175.6, 132.0, 126.9, 124.8, 122.8, 74.0, 71.0 and 53.7; *m*/*z* 170 [M$^+$], 152 [M$^+$ – H_2O], 120 [152 – CH_3OH], 111 [M$^+$ – CO_2CH_3], 110 [M$^+$ – HCO_2CH_3], 93 [111 – CO_2Me], 82 [110 – HCO_2Me] and 65 [93 – H_2O] (Found: C, 56.6; H, 5.9. $C_8H_{10}O_4$ requires C, 56.47; H, 5.92%).

(1*S*,2*R*)-1,2-Isopropylidenedioxycyclohexa-3,5-dienecarboxylic acid 4

A solution of compound **2** (R_n = H) (1.058 g, 6.96 mmol) in 2,2-dimethoxypropane (5 cm^3) and acetone (1 cm^3) was stirred with a catalytic amount of toluene-*p*-sulfonic acid (4 mg) for 8 h after which the solvents were removed under reduced pressure and the crude residue was dissolved in the minimum quantity of water. Addition of ethanol to the aqueous solution precipitated the sodium salt which was recrystallised from aqueous ethanol before being converted into the free acid with dilute hydrochloric acid. The mixture was extracted with ethyl acetate, and the extract dried ($MgSO_4$) and evaporated under reduced pressure to produce the *title compound* **4**, as a white solid (1.091 g, 80%); ν_{max}(Nujol)/cm^{-1} 3200 and 1738; δ_H(270 MHz) 6.14 (2 H, m, diene), 6.02 (1 H, m, diene) 5.81 (1 H, m, diene), 4.95 (1 H, dd, $J_{2,4}$ 0.5, $J_{2,3}$ 4.2, 2-H), 1.48 (3 H, s, CH_3) and 1.42 (3 H, s, CH_3).

Methyl (1*S*,2*R*)-1,2-isopropylidenedioxycyclohexa-3,5-diene-carboxylate 4 (R = Me)

A solution of compound **3** (R = Me) (5 g, 29 mmol) in 2,2-dimethoxypropane (20 cm^3) was stirred with a catalytic amount of toluene-*p*-sulfonic acid (4 mg) for 4 h after which TLC analysis indicated complete conversion. Sodium acetate (10 mg) was added to the solution to reduce its acidity after which it was filtered and evaporated under reduced pressure to give a crude yellow–orange oil. This was purified by column chromatography (eluent: 30% ether in light petroleum) to give the *title compound* **5** as a colourless liquid (5.7 g, 92%); ν_{max}(film)/cm^{-1} 2991 and 1737; δ_H(270 MHz) 6.08 (1 H, m, diene), 6.00 (1 H, m, diene), 5.79 (2 H, m, 4-H, 5-H), 4.94 (1 H, d, $J_{2,3}$ 4, 2-H), 3.77 (3 H, s, CO_2Me), 1.41 (3 H, s, CH_3) and 1.39 (3 H, s, CH_3); δ_C(125.8 MHz) 172.2, 124.7, 124.5, 124.0, 106.8, 79.4, 72.7, 52.9, 26.9 and 25.1; *m*/*z* 210.23 [M$^+$], 195 [M – Me], 152 [195 – CH_2=C=O], 151 [M – CO_2Me], 150 [151 – H], 135 [150 – Me], 93 [135 – CH_2=C=O] and 65 [93 – H_2O] (Found: C, 62.6; H, 6.8. $C_{11}H_{14}O_4$ requires C, 62.85; H, 6.71%).

(1*R*,2*R*)-1-Hydroxymethyl-1,2-isopropylidenedioxycyclohexa-3,5-diene 6 (R = H)

To a solution of lithium aluminium hydride (40 mg, 1 equiv.) in dry ether (2 cm^3) under nitrogen, was added a solution of compound **5** (2.10 g, 1 mmol) in ether (2 cm^3), dropwise over 5 min. After 20 min, TLC analysis indicated that the reaction was complete. Ethyl acetate was added to react with any excess of $LiAlH_4$ and after a further 10 min, dilute hydrochloric acid was added dropwise until a precipitate formed. The solution was filtered through Celite and the filter cake further washed with ethyl acetate. The combined organic filtrates were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was purified by column chromatography (30% ether–light petroleum) to give the *title compound* **6** (R = H) as a crystalline solid (1.53 g, 84%), mp 41–44 °C; ν_{max}(Nujol)/cm^{-1} 3392, 1643 and 1594; δ_H(270 MHz) 6.09–5.99 (3 H, m, diene protons) 5.69 (1 H, dd, $J_{6,5}$ 9.5, $J_{6,4}$ 0.5, 6-H), 4.49 (1 H, d, $J_{2,3}$ 4.2, 2-H), 3.59, 3.36 (2 H, ABq, J_{AB} 11.7 -CH_AH_BOH), 1.45 (3 H, s, CH_3) and 1.37 (3 H, s, CH_3); δ_C(126 MHz) 128.93 (diene), 125.20 (diene), 124.42 (diene), 123.49 (diene), 106.39 (O*C*Me_2O-), 80.51 (C-1), 70.93 (C-2), 64.22 (CH_2OH), 27.08 (CH_3) and 26.67 (CH_3); *m*/*z* 182 [M$^+$] 181 [M – H], 167 [M – Me], 151 [181 – CH_2O], 124 [167 – CH_2=C=O], 107 [124 – OH], 95 [124 – CO], 93 [151 – Me_2CO] and 65 [93 – H_2O] (Found: C, 65.8; H, 7.9. $C_{10}H_{14}O_3$ requires C, 65.92; H, 7.74%).

(1*R*,2*R*)-1-*tert*-Butyldiphenylsiloxymethyl-1,2-isopropylidene-dioxycyclohexa-3,5-diene 6 (R = $SiPh_2Bu^t$)

tert-Butylchlorodiphenylsilane (186.7 mg, 1.1 equiv.) was added to a stirred solution of compound **6** (R = H) (112.5 mg, 0.62 mmol) and imidazole (95.3 mg, 2.3 equiv.) in DMF (5 cm^3). After 48 h, TLC analysis indicated complete conversion and the solution was poured into water and extracted repeatedly with dichloromethane. The combined organic extracts were washed with saturated aqueous sodium hydrogen carbonate, dried ($MgSO_4$) and evaporated under reduced pressure. Purification of the residue by column chromatography (5% ether–light petroleum) afforded the *title compound* **6** (R = $SiPh_2Bu^t$) as a clear oil (138 mg, 53%); δ_H(270 MHz) 7.67 (4 H, m, Ph_2Si), 7.40 (6 H, m, Ph_2Si), 6.02 (3 H, m, alkene), 5.68 (1 H, br d, *J* 9.8, 6-H), 4.62 (1 H, d, *J* 4.2, 2-H), 3.67 (1 H, d, *J* 10.5, OC*H*H), 3.52 (1 H, d, *J* 10.5, OCH*H*), 1.44 (3 H, s, CH_3), 1.37 (3 H, s, CH_3) and 1.05 (9 H, s, $(H_3C)_3CSi$); δ_C(126 MHz) 135.7 (Ph), 135.6 (Ph) 133.3 (Ph) 133.2 (Ph) 129.69 (Ph) 129.66 (Ph), 127.6 (alkene), 125.2 (alkene) 124.7 (alkene), 122.9 (alkene), 106.1 (O*C*Me_2O), 80.2 (C-1) 71.9 (C-2), 67.1 (OCH_2), 30.9 (1 C, Me_3*C*Si), 27.2 (CH_3), 26.8 (3 C, Me_3CSi) and 26.7 (CH_3); *m*/*z* 363 [M – Bu^t], 362 [M – Me_2CO], 305 [363 – Me_2CO; 362 – Bu^t], 275 [305 – H_2C=O], 199 [Ph_2Si=OH], 151 [363 – Ph_2SiOCH_2], 93 [151 – Me_2CO] and 65 [93 – CO].

Methyl (1*R*,2*R*,3*S*,6*R*)-1,2-isopropylidenedioxy-7,8-dioxa-bicyclo[2.2.2]oct-4-enecarboxylate 7

A solution of compound **5** (4.21 g, 20 mmol) and tetraphenylporphyrin (3 mg) in tetrachloromethane (100 cm^3) was irradiated externally with a 500 W tungsten light bulb while oxygen was bubbled through the solution. After 4–6 h, the solvents were removed under reduced pressure and the crude crystalline product was purified by column chromatography (eluent: 38% EtOAc–light petroleum) to afford the *title compound* **7** as pale yellow crystals (3.98 g, 82%), mp 66–68 °C; ν_{max}(Nujol)/cm^{-1} 1748; δ_H(270 MHz) 6.60 (1 H, d, *J* 3, 4/5-H), 6.60 (1 H, d, *J* 4, 5/4-H), 5.18 (1 H, d, $J_{2,3}$ 4.9, 2-H), 5.12 (1 H, m, 6-H), 4.98 (1 H, m, 3-H), 3.88 (3 H, s, OCH_3), 1.33 (3 H, s, CH_3) and 1.31 (3 H, s, CH_3); δ_C(125.8 MHz) 171.0, 131.1, 129.9, 112.3, 80.6, 73.5, 73.2, 72.1, 53.2, 26.2 and 26.1; *m*/*z* 242 [M$^+$], 227 [M – Me], 183 [M – H_3C–C=O], 158 and 143 (Found: C, 54.4; H, 5.7. $C_{11}H_{14}O_6$ requires C, 54.54; H, 5.83%).

X-Ray crystallographic analysis of 7

Single crystals suitable for X-ray analysis were grown from dichloromethane–light petroleum.

Crystal data. $C_{11}H_{14}O_6$, *M* = 242.2, monoclinic, *a* = 7.855(3), *b* = 8.568(4), *c* = 8.853(4) Å, β = 93.06(3) °, *V* = 594.9(3) Å^3, space group $P2_1$, *Z* = 2, D_c = 1.35 g cm^{-3}, μ(Mo-Kα) = 1.1 cm^{-1}, *F*(000) = 256. Data were measured on a Siemens P4/PC diffractometer ($2\theta < 55$ °) with Mo-Kα radiation (graphite monochromator) using ω-scans. 1539

Reflections were measured, 1450 were independent (R_{int} = 0.017) and 1234 had $|F_o| > 4\sigma(|F_o|)$ and were considered to be observed. The data were corrected for Lorentz and polarisation factors but not for absorption. The structure was solved by direct methods and the non-hydrogen atoms were refined anisotropically. The positions of all the hydrogen atoms were determined from a ΔF map. These positions were idealised, (C–H = 0.96 Å) and the atoms assigned isotropic thermal parameters, $U(H) = 1.2U_{eq}(C)$, and allowed to ride on their parent carbon atoms. Refinement was by full-matrix least squares to give $R = 0.039$, $R_\omega = 0.048$ ($\omega^{-1} = \sigma^2(F) + 0.0007F^2$). The absolute configuration was assigned by internal reference to a known centre. The maximum residual electron density in the final ΔF map was 0.21 e Å^{-3}. Computations were carried out on a 486 PC using the SHELXTL-PC program system.[42] Atomic coordinates, bond lengths, angles and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre.

(1*R*,2*R*,3*S*,6*R*)-1-Hydroxymethyl-1,2-isopropylidenedioxy-7,8-dioxabicyclo[2.2.2]oct-4-ene 8 (R = H)

A solution of compound **6** (R = H) (75.1 mg, 0.414 mmol) and tetraphenylporphyrin (1.5 mg, 2.4 × 10^{-3} mmol) in carbon tetrachloride (12 cm^3) was placed in a small chromatography column fitted with a sintered frit. Oxygen was bubbled up through the frit whilst the solution was externally irradiated with a 500 W tungsten light bulb. After 12 h (complete reaction by TLC analysis) the solution was treated with activated charcoal, filtered through Celite and the solvent removed under reduced pressure. Purification of the residue by column chromatography (eluent: 30% ether–light petroleum) afforded the *title compound* **8** (R = H) (60 mg, 68%); ν_{max}(Nujol)/cm^{-1} 3375; δ_H(270 MHz) 6.67 (1 H, ddd, $J_{5,4}$ 8.1, $J_{5,6}$ 6.2, $J_{5,3}$ 1.5, 5-H), 6.55 (1 H, ddd, $J_{4,5}$ 8.0, $J_{4,3}$ 6.1, $J_{4,6}$ 1.7, 4-H), 4.96 (1 H, dt(ddd), $J_{6,5}$ 6.4, $J_{6,4}$ 1.7, $J_{6,2}$ 1.7, 6-H), 4.87 (1 H, ddd, $J_{3,4}$ 6.1, $J_{3,2}$ 4.6, $J_{3,5}$ 1.5, 3-H), 4.21 (1 H, d, $J_{2,3}$ 4.9, 2-H), 4.01 (1 H, d, *J* 11.7, C*H*HOH), 3.89 (1 H, d, *J* 11.7, CH*H*OH), 1.41 (3 H, s, CH_3) and 1.316 (3 H, s, CH_3).

(1*R*,2*R*,3*S*,6*R*)-1-*tert*-Butyldiphenylsiloxymethyl-1,2-isopropylidenedioxy-7,8-dioxabicyclo[2.2.2]oct-4-ene 8 (R = $SiPh_2Bu^t$)

A solution of compound **6** (R = $SiPh_2Bu^t$) (100 mg, 0.24 mmol) and tetraphenylporphyrin (1.5 mg, 2.4 × 10^{-3} mmol) in carbon tetrachloride (12 cm^3) was placed in a small chromatography column fitted with a sintered frit. Oxygen was bubbled up through the frit whilst the solution was externally irradiated with a 500 W tungsten light bulb. The reaction was followed by TLC analysis and was complete after 12 h. The solution was treated with activated charcoal, filtered through Celite and the solvent removed under reduced pressure. Purification of the oil by column chromatography (eluent: 10% ether–light petroleum) produced the *title compound* **8** (R = $SiPh_2Bu^t$) as an oil (90 mg, 83%); δ_H(270 MHz) 7.80–7.73 (4 H, m, $SiPh_2$ *meta*), 7.49–7.40 (6 H, m, $SiPh_2$ *para*/*ortho*), 6.74 (1 H, ddd, $J_{5,4}$ 8.3, $J_{5,6}$ 6.1, $J_{5,3}$ 1.5, 5-H), 6.56 (1 H, ddd, $J_{4,5}$ 8.3, $J_{4,3}$ 4.6, $J_{4,6}$ 1.5, 4-H), 5.22 (1 H, dd, $J_{6,5}$ 6.1, $J_{6,4}$ 1.5, 6-H), 4.85 (1 H, ddd, $J_{3,2}$ 5.9, $J_{3,4}$ 4.6, $J_{3,5}$ 1.5, 3-H), 4.07 (1 H, d, $J_{2,3}$ 5.9, 2-H), 4.07 and 4.93 (1 H, d, *J* 11.0, C*H*HOSi), 4.93 (1 H, d, *J* 11.0, CH*H*OSi), 1.34 (3 H, s, CH_3), 1.25 (3 H, s, CH_3) and 1.10 (9 H, s, Bu^{t}Si).

Methyl (1*S*,2*R*,3*S*,6*R*)-1,2-isopropylidenedioxy-8,10-dioxo-9-phenyl-7,9,11-triazatricyclo[5.2.2.0^{7,11}]undec-4-ene-1-carboxylate 9

A solution of 4-phenyl-4,5-dihydro-3*H*-1,2,4-triazole-3,5-dione (284.4 mg, 1 equiv.) in acetone (2 cm^3) was added dropwise with stirring to a solution of compound **5** (341.4 mg, 1.62 mmol) in acetone (2 cm^3), until the red colour of the triazoledione just persisted. The mixture was stirred for a further 20 min after which the solvent was removed under reduced pressure and the crude crystalline product purified by column chromatography (eluent: 25% EtOAc–light petroleum) to afford white crystals of the *title compound* **9** (486.3 mg, 78%); mp 132–133 °C; $[\alpha]_D^{20}$ +25.7 (*c* 1.0 in EtOH); ν_{max}(Nujol)/cm^{-1} 1739 and 1713; δ_H(270 MHz) 7.45–7.36 (5 H, m, N-Ph), 6.48 (2 H, 2 × dd, $J_{4,3}$ 4.4, $J_{5,6}$ 4.4, $J_{4,6}$ 3.2, $J_{5,3}$ 3.2, 4-H and 5-H), 5.36 (1 H, dd, $J_{6,5}$ 4.4, $J_{6,4}$ 3.4, 6-H), 5.31 (1 H, d, $J_{2,3}$ 4.2, 2-H), 5.23 (1 H, ddd, $J_{3,4}$ 4.4, $J_{3,2}$ 4.2, $J_{3,5}$ 3.2, 3-H), 3.92 (3 H, s, CO_2Me), 1.37 (3 H, s, Me) and 1.32 (3 H, s, Me); δ_C(68 MHz) 170.6, 155.6, 155.4, 131.2, 129.7, 129.2, 128.6, 128.2, 125.6, 114.0, 83.4, 75.9, 54.6, 53.7, 52.8, 26.1 and 26.0; *m*/*z* 385 [M$^+$], 371 [M – Me], 327 [M – CO_2Me], 298, 296 [327 – OMe], 268 [296 – CO], 240 [268 – O], 227 [240 – CH], 119, 80 and 65 (Found: C, 59.4; H, 5.25; N, 10.8. $C_{19}H_{19}N_3O_6$ requires C, 59.22; H, 4.97; N, 10.90%).

Methyl (1*S*,2*R*,3*R*,6*S*)-1,2-isopropylidenedioxy-8,10-dioxo-9-phenyl-7,9,11-triazatricyclo[5.2.2.0^{7,11}]undec-4-ene-1-carboxylate 12

A solution of 4-phenyl-4,5-dihydro-3*H*-1,2,4-triazole-3,5-dione (331.7 mg, 1 equiv.) in acetone (2 cm^3) was added, dropwise with stirring, to a solution of compound **3** (R = Me) (322.2 mg, 1.894 mmol) in acetone (3 cm^3) until the red colour of the triazoledione just persisted. The mixture was stirred for a further 20 min after which the solvent was removed under reduced pressure and the crude crystalline product purified by column chromatography (eluent: 40% EtOAc–light petroleum) to afford a mixture of two inseparable adducts **10** and **11**.

The mixture was stirred in dimethoxypropane (5 cm^3) in the presence of a catalytic amount of toluene-*p*-sulfonic acid, to produce the isopropylidene derivatives which were separable by column chromatography (eluent: 25% EtOAc–light petroleum). The less polar, minor product (85.1 mg, 12%) proved to be identical with the adduct **9** produced above. The more polar, major product proved to be the isomeric *title compound* **12** (425.6 mg, 58%), mp 60–63 °C; δ_H(270 MHz) 7.55 (2 H, d, *J* 7.8, *N*-Ph *ortho*), 7.45 (2 H, t, *J* 7.8, *N*-Ph *meta*), 7.34 (1 H, t, *J* 7.8, *N*-Ph *para*), 6.61 (1 H, ddd, $J_{4,5}$ 8.0, $J_{4,3}$ 6.0, $J_{4,6}$ 1.5, 4 H), 6.51 (1 H, ddd, $J_{4,5}$ 8.1, $J_{5,6}$ 5.9, $J_{5,3}$ 1.5, 5 H), 5.31 (1 H, ddd, $J_{6,5}$ 5.9, $J_{6,4}$ 1.4, $J_{6,2}$ 0.5, 6 H), 5.19 (1 H, ddd, $J_{3,4}$ 6.0, $J_{3,2}$ 3.5 $J_{3,5}$ 1.5, 3 H), 4.89 (1 H, dd, $J_{2,3}$ 3.5, $J_{2,6}$ 0.6, 2 H), 3.83 (3 H, s, OCH_3), 1.54 (3 H, s, CH_3) and 1.42 (3 H, s, CH_3).

Methyl (1*S*,2*R*,3*S*,6*R*,7*S*,11*R*)-1,2-isopropylidenedioxy-8,10-dioxo-9-phenyl-9-azatricyclo[5.2.2.0^{7,11}]undec-4-ene-1-carboxylate 13 and methyl (1*S*,2*R*,3*R*,6*S*,7*R*,11*S*)-1,2-isopropylidenedioxy-8,10-dioxo-9-phenyl-9-azatricyclo-[5.2.2.0^{7,11}]undec-4-ene-1-carboxylate 14

A solution of *N*-phenylmaleimide (200 mg, 1.15 mmol) in acetone (1.5 cm^3) was added to a solution of compound **5** (243.0 mg, 1.16 mmol) in acetone (1.5 cm^3) and the resulting solution stirred at room temperature for 4 days before removal of the solvent under reduced pressure the crude oil was separated and purified by column chromatography (20% EtOAc–light petroleum). The first product eluted was unchanged starting material (23 mg, 8%).

The second was the major, syn-*adduct* **13** (281 mg, 63%); mp 138–140 °C; ν_{max}(Nujol)/cm^{-1} 1739 and 1713; δ_H(500.1 MHz) 7.44 (2 H, m, *N*-Ph *meta*), 7.37 (1 H, m, *N*-Ph *para*), 7.17 (2 H, m, *N*-Ph *ortho*), 6.30 (1 H, ddd, $J_{4,5}$ 8.1, $J_{4,3}$ 6.5, $J_{4,6}$ 1.0, 4-H), 6.15 (1 H, ddd, $J_{5,4}$ 8.1, $J_{5,6}$ 6.3, $J_{5,3}$ 1.0, 5-H), 4.80 (1 H, d, $J_{2,3}$ 4.2, 2-H), 3.80 (3 H, s, CO_2CH_3), 3.75 (1 H, ddd, $J_{6,5}$ 6.3, $J_{6,7}$ 2.8, $J_{6,4}$ 0.9, 6-H), 3.65 (1 H, dddd, $J_{3,4}$ 6.5, $J_{3,2}$ 4.2, $J_{3,11}$ 3.0, $J_{3,5}$ 1.0, 3-H), 3.57 (1 H, dd, $J_{11,7}$ 8.3, $J_{11,3}$ 3.0, 11-H), 3.54 (1 H, dd, $J_{7,11}$ 8.3, $J_{7,6}$ 2.8, 7-H), 1.55 (3 H, s, α-CH_3) and 1.37 (3 H, s, β-CH_3); NOE: α-Me of isopropylidene/H7 + H11, 2%; *m*/*z* 383

[M^+], 368 [M − Me], 325 [368 − H_3C–C=O], 294 [325 − OMe], 280, 265 [294 − CO], 237, 209, 173, 158, 143, 119 and 91 (Found: C, 65.6; H, 5.5; N, 3.8. $C_{21}H_{21}NO_6$ requires C, 65.79; H, 5.52; N, 3.65%).

Finally, the third component eluted was the minor, *anti-adduct* **14** (54 mg, 12%); mp 84–86 °C; ν_{max}(Nujol)/cm^{-1} 1742 and 1714; δ_H(270 MHz) 7.47–7.34 (3 H, m, N-Ph *meta/para*), 7.15 (2 H, m, *N*-Ph *ortho*), 6.22 (2 H, m, 4-H, 5-H), 4.83 (1 H, d, $J_{2,3}$ 3.2, 2-H), 3.87 (3 H, s, CO_2CH_3), 3.62 (2 H, m, 3-H and 6-H), 3.10 (1 H, dd, $J_{11,7}$ 8.2, $J_{11,3}$ 2.7, 11-H), 2.98 (1 H, dd, $J_{7,11}$ 8.2, $J_{7,6}$ 3.2, 7-H), 1.36 (3 H, s, CH_3) and 1.23 (3 H, s, CH_3) (Found: C, 66.0; H, 5.5; N, 3.4. $C_{21}H_{21}NO_6$ requires C, 65.79; H, 5.52; N, 3.65%).

Methyl (1*R*,2*R*,3*S*,6*R*)-1,2-isopropylidenedioxy-8-phenyl-7-oxa-8-azabicyclo[2.2.2]oct-4-ene-1-carboxylate 15 and methyl (1*S*,2*R*,3*S*,6*R*)-1,2-isopropylidenedioxy-8-phenyl-8-oxa-7-azabicyclo[2.2.2]oct-4-ene-1-carboxylate 16

Nitrosobenzene (257.6 mg, 1 equiv.) was added to a solution of compound on **5** (505.7 mg, 2.41 mmol) in toluene (10 cm^3) and the resulting blue solution was stirred until the solution had lost its colour and TLC analysis showed complete consumption of the diene. The solvent was removed from the mixture under reduced pressure and the resulting crude viscous oil was purified by column chromatography (20% ether–light petroleum). The less polar minor *adduct* **16** was isolated as a colourless gum (52.7 mg, 7%); ν_{max}(Nujol)/cm^{-1} 1740; δ_H(270 MHz) 7.26–7.18 (3 H, m, *N*-Ph *meta/para*), 6.98–6.93 (2 H, m, *N*-Ph *ortho*), 6.53 (1 H, ddd, $J_{4,5}$ 8.2, $J_{4,3}$ 6.8, $J_{4,6}$ 1.5, 4-H), 6.11 (1 H, ddd, $J_{5,4}$ 8.2, $J_{5,6}$ 6.1, $J_{5,3}$ 1.5, 5-H), 5.28 (1 H, d, $J_{2,3}$ 4.6, 2-H), 4.99 (1 H, dd, $J_{6,5}$ 6.1, $J_{6,4}$ 1.5, 6-H), 4.97 (1 H, ddd, $J_{3,4}$ 6.8, $J_{3,2}$ 4.6, $J_{3,5}$ 1.5, 3-H), 3.93 (3 H, s, CO_2CH_3) and 1.34 [6 H, s, $C(CH_3)_2$].

The more polar major *adduct* **15** was isolated as a colourless gum (520.9 mg, 68%); ν_{max}(Nujol)/cm^{-1} 1738; δ_H(250 MHz) 7.24–7.18 (3 H, m, *N*-Ph *meta/para*), 7.01–6.93 (2 H, m, *N*-Ph *ortho*), 6.97 (1 H, ddd, $J_{5,4}$ 8.1, $J_{5,6}$ 6.2, $J_{5,3}$ 1.8, (5-H), 6.51 (1 H, dddd, $J_{4,5}$ 8.1, $J_{4,3}$ 5.9, $J_{4,6}$ 1.4, $J_{4,2}$ 1.2, 4-H), 5.36 (1 H, dd, $J_{2,3}$ 4.3, $J_{2,4}$ 1.2, 2-H), 5.14 (1 H, dd, $J_{6,5}$ 6.2, $J_{6,4}$ 1.4, 6-H, 4.79 (1 H, ddd, $J_{3,4}$ 5.9, $J_{3,2}$ 4.3, $J_{3,5}$1.8, 3-H), 3.90 (3 H, s, CO_2CH_3) and 1.34 (6 H, s, $C(CH_3)_2$]; δ_C(125.8 MHz) 171.1, 150. 6, 129.3, 128.6, 128.4, 122.8, 117.7, 112.6, 82.7, 75.8, 71.4, 60.3, 53.1, 26.3 and 26.2; *m/z* 317 [M^+], 302 [M − CH_3], 259 [302 − H_3C–C=O] and 201 [259 − CO_2Me] (Found: C, 64.1; H, 6.1; N, 4.15. $C_{17}H_{19}NO_5$ requires C, 64.34; H, 6.03; N, 4.41%).

Methyl (1*R*,2*R*,3*R*,6*S*)-1,2-dihydroxy-8-phenyl-7-oxa-8-azabicyclo[2.2.2]oct-4-ene-1-carboxylate 17, methyl (1*S*,2*R*,3*R*,6*S*)-1,2-dihydroxy-8-phenyl-8-oxa-7-azabicyclo-[2.2.2]oct-4-enecarboxylate 18, methyl (1*R*,2*R*,3*S*,6*R*)-1,2-dihydroxy-8-phenyl-7-oxa-8-azabicyclo[2.2.2]oct-4-ene-1-carboxylate 19 and methyl (1*S*,2*R*,3*S*,6*R*)-1,2-dihydroxy-8-phenyl-8-oxa-7-azabicyclo[2.2.2]oct-4-ene-1-carboxylate 20

Nitrosobenzene (95.2 mg, 1 equiv.) was added to a solution of compound **3** (R = Me) (151.3 mg, 0.720 mmol) in toluene (3 cm^3) and the mixture stirred for 6 h. It was then evaporated under reduced pressure to give a yellow oil containing all four possible isomers (by NMR analysis). Partial separation by column chromatography (eluent: 10% EtOAc–dichloromethane) produced two pairs of inseparable adducts as gums. The major portion of the product consisted of the less polar, *syn-adducts* **17** and **18** (128 mg, 51%, ratio 3:1 respectively); for **17**:δ_H(270 MHz) 7.25 (2 H, t, *J* 8.3, *N*-Ph *meta*), 7.06 (3 H, d, *J* 8.3, *N*-Ph *para*, *ortho*), 6.59 (1 H, ddd, $J_{5,4}$ 8.1, $J_{5,6}$ 5.9, $J_{5,3}$ 1.7, 5-H), 6.11 (1 H, ddd, $J_{4,5}$ 8.3, $J_{4,3}$ 6.1, $J_{4,6}$ 1.4, 4-H), 4.78 (1 H, br d, $J_{6,5}$ 5.9, 6-H), 4.56 (1 H, ddd, $J_{3,4}$ 6.1, $J_{3,2}$ 3.4, $J_{3,5}$ 1.7, 3-H), 4.25 (1 H, d, $J_{2,3}$ 3.4, 2-H) and 3.75 (3 H, s, OCH_3); for **18**:δ_H(270 MHz) 6.57 (1 H, m, 5-H), 6.03, (1 H, m, 4-H), 4.73 (1 H, br d, $J_{6,5}$ 6.4, 6-H) 4.64 (1 H, dd, $J_{3,4}$ 6.1, $J_{3,2}$ 2.1, 3-H) and 3.81 (3 H, s, OCH_3). The aromatic protons and 2-H signals of this minor component are hidden under the analogous signals for **17**.

The more polar pair of products were the minor, *anti-adducts* **19** and **20** (21 mg, 9%, ratio 3:1 respectively); for **19**:δ_H(270 MHz) 7.22 (2 H, t, *J* 8.3, *N*-Ph *meta*), 7.04 (1 H, t, *J* 8.3, m, *N*-Ph *para*), 6.96 (2 H, t, *J* 8.3, *N*-Ph *ortho*), 6.59 (1 H, ddd, $J_{5,3}$ 1.7, $J_{5,6}$ 6.2, $J_{5,4}$ 8.2, 5-H), 6.16 (1 H, br m, $J_{4,6} \approx 1$, $J_{4,3}$ 6.8, 4-H), 4.91 (1 H, d, $J_{2,3}$ 4.1, 2-H), 4.91 (1 H, dd, $J_{6,5}$ 6.4, $J_{6,4}$ 1.2, 6-H) 4.63 (1 H, ddd, $J_{3,4}$ 6.4, $J_{3,2}$ 4.5, $J_{3,5}$ 1.9, 3-H) and 3.90 (3 H, s, $-OCH_3$); for **20**:δ_H(270 MHz) 5.01 (1 H, d, $J_{2,3}$ 4.2, 2-H), 4.79 (1 H, ddd, $J_{3,4}$ 6.5, $J_{3,2}$ 4.5, $J_{3,5}$ 2.0, 3-H), 4.85 (1 H, dd, $J_{6,5}$ 6.1, 6-H) and 3.82 (3 H, s, OCH_3). The aromatic proton signals of 4-H and 5-H of this minor component are hidden under the analogous signals for **19**.

(1*R*,2*R*,3*S*,6*R*)-1-Hydroxymethyl-1,2-isopropylidenedioxy-8-phenyl-8-oxa-7-azabicyclo[2.2.2]oct-4-ene 21 and (1*R*,2*R*,3*S*,6*R*)-1-hydroxymethyl-1,2-isopropylidenedioxy-8-phenyl-7-oxa-8-azabicyclo[2.2.2]oct-4-ene 22

Nitrosobenzene (25.1 mg, 1 equiv.) was added to a solution of compound **6** (R = H) (42.1 mg, 0.234 mmol) in toluene (10 cm^3) and the resulting blue solution was stirred until the solution became colourless and TLC analysis showed complete consumption of the diene. The solvents were removed from the mixture under reduced pressure to give a crude viscous oil, column chromatography (30% ether–light petroleum) of which gave the less polar major *adduct* **21** (44.3 mg, 66%); δ_H(270 MHz) 7.27–7.19 (2 H, m, *N*-Ph *meta*), 7.01–6.93 (3 H, m, *N*-Ph *para/ortho*), 6.48 (1 H, ddd, $J_{4,5}$ 5.9, $J_{4,3}$ 5.9, $J_{4,6}$ 1.8, 4-H), 6.15 (1 H, ddd, $J_{5,4}$ 5.9, $J_{5,6}$ 5.7, $J_{5,3}$ 1.5, 5-H), 4.92 (1 H, ddd, $J_{3,4}$ 5.9, $J_{3,2}$ 4.6, $J_{3,5}$ 1.5, 3-H), 4.84 (1 H, dd, $J_{6,5}$ 5.7, $J_{6,4}$ 1.8, 6-H), 4.40 (1 H, d, $J_{2,3}$ 4.6, 2-H), 4.17 (1 H, d, *J* 11.5, C*H*HOH), 4.02 (1 H, d, *J* 11.5, CH*H*OH) 1.42 (3 H, s, β-CH_3) and 1.32 (3 H, s, α-CH_3); NOE: CH_2OH/2 H 11%; CH_2OH/6 H, 4%; CH_2OH/β-CH_3, 2%; 5-H/4-H, 5%; 5-H/6-H, 5%; 5-H/α-CH_3, < 1%; 4-H/5-H, 6%; 4-H/3-H, 5%; 4-H/α-CH_3, < 1%.

Further elution gave the minor *adduct* **22** (9.1 mg, 14%); δ_H(270 MHz) 7.24–7.20 (2 H, m, *N*-Ph *meta*), 7.04–6.95 (3 H, m, N-Ph *para/ortho*), 6.59 (1 H, ddd, $J_{5,4}$ 8.1, $J_{5,6}$ 6.0, $J_{5,3}$ 1.7, 5-H), 6.09 (1 H, ddd, $J_{4,5}$ 8.1, $J_{4,3}$ 5.6 $J_{4,6}$ 1.4, 4-H), 4.91 (1 H, dd, $J_{6,5}$ 6.0, $J_{6,4}$ 1.4, 6-H), 4.66 (1 H, ddd, $J_{3,4}$ 5.6, $J_{3,2}$ 4.6, $J_{3,5}$ 1.7, 3-H), 4.42 (1 H, d, $J_{2,3}$ 4.6, 2-H), 4.09 (1 H, d, *J* 11.5, C*H*HOH), 3.93 (H, d, *J* 11.5, CH*H*OH), 1.43 (3 H, s, CH_3) and 1.33 (3 H, s, CH_3).

Lithium aluminium hydride reduction of the major nitroso adduct 15

The nitrosyl adduct **15** (48.2 mg, 0.152 mmol) in ether (2 cm^3) was added, dropwise over 5 min, to a solution of lithium aluminium hydride (40 mg, excess) in dry ether (2 cm^3) under nitrogen. After 20 min, TLC analysis indicated complete reaction and ethyl acetate was added to the mixture to react with any excess of $LiAlH_4$. After a further 10 min, standard aqueous work-up gave a crude product which was purified by column chromatography (30% EtOAc–light petroleum) to reveal a product spectroscopically identical with the minor adduct **22** from reaction between the compound **6** (R = H) and nitrosobenzene.

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