Expanding the chiral pool: oxidation of *meta*-bromobenzoic acid by *R. eutrophus* B9 allows access to new reaction manifolds.


Metabolism of *meta*-bromobenzoic acid by the blocked mutant *Ralstonia eutrophus* B9 affords an enantiopure dearomatised halodiene-diol which we demonstrate is a versatile chiron for organic synthesis. The presence of the halogen leads to reactivity that is distinct to that observed for the non-halogenated analogue and also serves as a synthetic handle for further functionalization.

Introduction

The dearomatising dihydroxylation of an aromatic substrate by a microorganism was first reported by Gibson over 40 years ago. It was subsequently recognised that the resultant diene diols were useful starting materials for synthesis owing to their densely-packed, differentiated functionality. Indeed, their synthetic value is enhanced further by the fact that substituted arenes give rise to enantiopure diols in most instances. The production and utilisation of these arene-derived diols has become established methodology and the field has been the subject of several excellent reviews.

Thus far, over 400 arene cis-diols products have been reported. The vast majority of these are produced by organisms expressing tolune dioxygenase (TDO), naphthalene dioxygenase (NDO) and biphenyl dioxygenase (BPDO) enzymes. These metabolise substituted arene substrates in a regio- and stereoselective fashion. A reliable predictive model has been reported for such transformations and the sense of enantiomutation is conserved across organisms and substrates (Scheme 1a, *ortho-meta* oxygenation). In contrast, organisms expressing benzoate dioxygenase (BZDO) enzymes oxidise benzoic acids in a process that exhibits not only different regioselectivity, but also the opposite sense of enantiomutation. For example, *R. eutrophus* B9, *P. putida* U103 and *P. putida* KTSY01 (pSYM01) oxidise benzoic acid to benzoate 1,2-*cis* dihydrodiol 4 (Scheme 1b, *ipso-ortho* oxygenation).

The ability of derivatives of 4 to participate in [4+2] cycloaditions with various dienophiles was also demonstrated. In 2001, Myers *et al.* described multiple approaches for elaborating 4, demonstrating that each position on the ring could be functionalised in a selective fashion through judicious choice of reaction sequence. They also described in detail a large-scale preparation of 4. That the derivatives described therein are of synthetic utility was first shown by the report in 2004 of the synthesis of carbocyclic analogues of topiramate. In this work, Parker *et al.* described a route to analogues of this anticonvulsant agent, requiring between 3 and 4 steps from one of Myers’ chirons; the authors also describe the first use of 4 to access a carbohydrate target, carba-β-1-fructopyranose. Also in 2004, Mihovilovic *et al.* reported intramolecular Diels–Alder reactions of derivatives of 4 bearing tethered dienophiles. In 2005, Myers *et al.* disclosed the total synthesis of natural and unnatural tetracycline antibiotics via a synthetic sequence commencing from a derivative of 4 they had described four years previously. It is noteworthy that whilst the first stereocentre in the target tetracyclines was set in the arene dihydroxylation step, all subsequent stereocentres were installed under substrate control; all stereocchemical information in the final products is thus of ultimate enzymatic origin. In 2010, the Mihovilovic group published a full paper on intramolecular Diels–Alder reactions with 4 and, most recently, the chemistry of 4 has been augmented by our report that simple derivatives of 4 are amenable to complexation to form tricarbonyliron(0)dienyl complexes.

We have been able to demonstrate that such complexation permits the synthesis of otherwise inaccessible moieties. Most notably a diene possessing the *ortho-meta* pattern of oxygenation found in 2 but *antipodal* to 2 may be accessed from 4 by means of such organoniron complexes.

The unique synthetic versatility of 4 has thus been demonstrated. However, we sought to enhance further the utility of the BZDO-mediated benzoate dihydroxylation by use of substituted benzoate substrates. The viability of metabolising substituted benzoates by this approach has been established previously. Studies on *Ralstonia eutrophus* B9† have shown that a variety of mono- and disubstituted benzoates are acceptable substrates. It was noted that turnover...
rates decreased in accordance with the steric demand of a substituent, with the ortho position being least tolerant of substitution (only ortho-fluorobenzoate underwent dioxygenation) and the meta position being the most tolerant. Many other organisms expressing BZDOs have been evaluated for their ability to process substituted benzoates, as have organisms expressing TADO (toluate dioxygenase), TERDOS (terephthalate dioxygenase), IPADO (isophthalate dioxygenase). To our knowledge, however, there is just one example where the use of a halide in the synthesis of an arenedihydrodiol derived from a substituted benzoate: Banwell’s use of a metabolite derived from meta-ethyltoluene in an approach to vinblastine. In this same study, 

\[
\begin{align*}
\text{EII} & \quad \text{P. putida B50XMT} & \quad \text{ES} \quad \text{P. putida B50XMT} \\
\text{Et} & \quad \text{H} & \quad \text{Et} & \quad \text{H} \\
\text{5} & \quad \text{Me} & \quad \text{5} & \quad \text{Me} \\
\end{align*}
\]

Scheme 2 Banwell’s access to aren diol 7 via meta-ethylbenzoic acid.

In the present study, we opted to exploit arenedioils derived from the metabolism of meta-bromobenzoic acid by *R. eutrophus* B9 (Scheme 3). We anticipated a twofold effect due to incorporation of a bromine in the products. Firstly, this halide would modulate the electron density of the diene such that it would exhibit reactivities distinct from those of the parent system 4. Secondly, a bromodiene would be amenable to diverse cross-coupling reactions to permit further functionalization in a manner that would not be possible for unsubstituted diene 4.

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\begin{align*}
\text{Br} & \quad \text{COOH} & \quad \text{R. eutrophus B9} & \quad \text{BZDO} \\
\text{8} & \quad \text{Br} & \quad \text{COOH} & \quad \text{9} & \quad \text{Br} & \quad \text{COOH} & \quad \text{10} \\
\end{align*}
\]

Scheme 3 Arenediol metabolites of meta-bromobenzoic acid.

As is the case for any meta-substituted benzoate, metabolism of 8 may give rise to two regioisomeric diols, 9 and 10, reflecting the possibility of the substrate being accommodated in two possible orientations in the BZDO active site. In the specific case of substrate 8, literature precedent was ambiguous. In their original report on *R. eutrophus* B9, Reiner and Hegeman describe the isolation of both 3- and 5-substituted arenedioils from the metabolism of meta-substituted benzoates, but product ratios were not quantified. Subsequently, Knackmuss and Reineke quantified product formation and found that 5-substituted diols analogous to 10 were formed more rapidly than the corresponding 3-substituted regioisomers analogous to 9. In both of the above studies, however, only meta-chloro- and meta-methyl benzoate were employed as substrates, not metabromobenzoate 8. Reineke and co-workers’ subsequent study has been the only one thus far to specifically address the metabolism of meta-bromobenzoate 8 by *R. eutrophus* B9. Whilst the production of both 9 and 10 is described, the product ratio was not determined. In this same study, it is stated that when using meta-methylbenzoate, the 5-methyl analogue of 10 is “accessible only with difficulty”, whereas the 3-methyl analogue of 9 is “isolable in good yield”; such statements seem to contradict the earlier study. Thus, it was unclear at the outset what the regiochemical outcome of metabolism of 8 by *R. eutrophus* B9 would be.

**Results and Discussion**

We undertook the biooxidation of 8 in accordance with the procedure of Myers et al., but on a 15 L scale. *R. eutrophus* B9 cells were induced with a small quantity of benzoate before addition of sodium meta-bromobenzoate solution portionwise over 48 h; disodium succinate solution was added as sole carbon source. The fermentation broth was then centrifuged to remove cellular material and the supernatant was concentrated under reduced pressure. The concentrate was acidified to pH 3.0 and extracted numerous times with ethyl acetate. Organic washings were dried, then concentrated under reduced pressure to give a crude mixture of 8, 9, and 10. NMR analysis indicated that unreacted 8 was the major constituent of the crude mixture. *meta*-Bromobenzoate 8 had been introduced to the fermentation vessel at a rate comparable to that used previously in the non-halogenated case, but *R. eutrophus* B9 metabolised the brominated substrate much more slowly, as expected, leading to accumulation of unreacted 8. Formation of 3-bromo product 9 was found to have predominated (>10:1) over the 5-bromo isomer 10.

Purification of this crude material was effected by repeated trituration with dichloromethane; this served to remove both the starting material 8 and also the traces of 5-bromo product 10. After 7 triturations, NMR analysis showed the residual quantity of 8 to be negligible. By this means, pure 3-bromo product 9 was obtained in a yield of 65 mg per litre of fermentation broth, approximately two orders of magnitude lower than those reported for the unsubstituted benzoate.

With access to 9 secured we sought to verify the absolute stereochemistry through X-ray analysis of a crystalline derivative. The configuration of 4 was originally determined by Widdowson et al. through formation of the corresponding para-bromobenzoylmethyl ester and subsequent X-ray analysis; the same approach was adopted to determine the configuration of 7. However, as 9 already incorporates a heavy atom, we opted instead to target a crystalline cycloaduct. Esterification of 9 by means of (trimethylsilyl) diazomethane to give 11 was followed by diol protection as acetone 12. Upon treatment with 4-phenyl-1,2,4-triazoline-3,5-dione, 12 underwent [4+2] cycloaddition to afford crystalline adduct 13 (Scheme 4).

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\begin{align*}
\text{Br} & \quad \text{COOH} & \quad \text{TMS-CH}_{2} & \quad \text{MeOH} / \text{Benzene} : 1:1 \\
\text{9} & \quad \text{Br} & \quad \text{OH} & \quad \text{11} & \quad \text{Br} & \quad \text{COOH} \\
\end{align*}
\]

Scheme 4 Formation of crystalline derivative.
Single crystals of 13 suitable for X-ray structure determination were obtained from diffusion of petroleum ether into a solution of 13 in ethyl acetate. The structure of 13 is depicted in Figure 1 and confirms the absolute stereochemistry as (1S,25). Thus, the sense of enantiomeric in the formation of 9 is the same as in the formation of 4 and 7, as expected.

![Fig. 1 Solid state structure of 13. Ellipsoids are represented at 50% probability. H atoms are shown as spheres of arbitrary radius.]

That the sole cycloadduct formed (13) is that in which the dienophile approaches anti to the acetonide is in keeping with Widdowson’s precedent.7 Aside from that report, all literature examples of heterodienophile cycloadditions with arene diol derived acetonides involve substrates with the ortho-meta pattern of oxygenation (c.f. 2). Here also, every report describes dienophile addition anti to the acetonide.23b,c,24 In such substrates the original arene substituent is attached to the diene. In contrast, in 12 (which has the ipso-ortho pattern of oxygenation, c.f. 4), the original arene substituent is attached to an sp3 centre and will not be coplanar with the diene. If the substituent is of sufficient steric bulk, approach of the dienophile to both diene faces may be hindered. Exclusive formation of 13 in this instance is indicative of the steric bulk of the acetonide being the controlling factor in determining regioselectivity of cycloaddition, not steric bulk of the ester.

The above considerations are also relevant in the context of Diels–Alder dimerisation. Arene diol derived acetonides with the ortho-meta pattern of oxygenation (c.f. 2) are known to undergo spontaneous dimerization in many instances; this has been reported with halodienes, e.g. R = Br23c,24a-c Cl23d,c and also with many other substituents on the diene (R = CF323b,24a-d, R = C2H523b,24a,d, R = CN24d, R = COOME24d, R = COOEt23w, R = COOCH2CH223w, R = SiMe2H24b,h, R = SiMe2C2H524b,h and R = Me244). In each instance, the dimerisation is reportedly totally selective for formation of the adduct which is anti both with respect to the diene and also the dienophile. (The only reported exception to this trend is dimerisation of the acetonide of the parent unsubstituted diene 2, R = H25 – a minor cycloadduct deriving from syn diene addition and anti dienophile addition has also been reported22,25). The behaviour of the bromodiene acetonide 12 reported here is in marked contrast to the above cases, in that we have observed no dimerisation of 12 upon prolonged storage at room temperature or below. Dimerisation of the non-brominanalogue of 12 has also not been reported. We ascribe this inertness to dimerisation to the fact that such acetonides with the ipso-ortho pattern of oxygenation (c.f. 4) present sterically demanding substituents on both sides of the diene, hence retarding the formation of all possible isomeric dimers.

We next examined the susceptibility of the bromodiene to oxidative elaboration. Neither 11 nor 12 gave tractable products upon attempted epoxidation with mCPBA, in contrast to the corresponding non-halogenated dienes.8 However, osmium tetroxide-mediated dihydroxylation of 12 proceeded smoothly to afford diol 14 as the sole regio- and diastereoisomer (Scheme 5). Such selectivity is also in contrast to the non-halogenated series, wherein the corresponding diene 15 undergoes dihydroxylation to afford a 5:1 mixture of regioisomers favouring the other olefin.8 The installation of the free diol functionality in 14 on the β-face was confirmed by NOESY NMR experiments.7 Reductive elaboration of bromodiene 12 also proceeded in a markedly different fashion to the non-halogenated analogue 15. Whereas LiAlH4-mediated reduction of the ester in 15 to give 16 is reportedly7,10,12 high-yielding (Scheme 6a), the corresponding reduction of bromodiene 12 affords the primary alcohol 17 in only 11% yield. We ascribe this low yield partly to the concomitant formation of appreciable amounts of debrominated product 16, which we have isolated (Scheme 6b).

![Scheme 5 Dihydroxylation on the β-face. Selected NOESY correlations in 14 shown with double-headed arrows.]

The complexation of arene diols of type 2 with a tricarboxyliron fragment has been extensively studied.27 We have reported previously13 on complexation of 18 (the methyl ester of 4) to afford 19 as the sole product, with iron complexed to the α-face (Scheme 7a). A later attempt14 to reverse the facial selectivity in this complexation by use of an acetonide 15 to block the α-face did indeed result in complexation of iron to the diene β-face, but the isolated product 20 was that in which acetonide migration had occurred (Scheme 7b). We were therefore keen to determine the outcome of complexation of bromodiene acetonide 12 as an iron carbonyl in order to shed light on the mechanism of formation of 20. In the event, treatment of 12 with nonacarbonyldiiron in THF afforded isomeric complexes 21 and 22, in which 21 was the major isomer (Scheme 7c). The
structure of 21 was determined unambiguously by X-ray crystallography (Figure 2). (The minor isomer 22, which was appreciably less stable than 21, could not be crystallised successfully; its structure was assigned by 2D NMR experiments and by comparison with 21.) In this instance, the sole products are those in which acetonide migration has not occurred. This may be explained by the fact that in 12, the carbon to which an acetonide oxygen would be bonded upon rearrangement already bears the bromine substituent. We had previously concluded that the formation of 20 arose via “clockwise” migration of the acetonide, rather than “anticlockwise” migration of the ester. The absence of any such rearrangement in the case of bromodiene 12 is in keeping with this conclusion. The presence of the bromine in 12 also retards the rate of complexation with respect to unsubstituted analogue 15 – identical reaction conditions result in consumption of all of 15, but recovery of 60% unreacted 12 (Scheme 7).

The above transformations of bromodiene 12 represent cases in which the presence of the halogen modulates the reactivity of the system with respect to the unsubstituted diene case. However, we wished to exploit further the value inherent in the bromine substituent by using transformations that would not be possible for the unsubstituted case. A dienyl bromide is suggestive of applications in cross-coupling chemistry and in the more common ortho,meta arene dihydrodiol series, both 2 (R = Br)\(^{24a,28}\) and 2 (R = I)\(^{28a,b,n,29}\) have indeed been exploited in this context. To date, such cross-couplings have not been reported for derivatives of 4.

We first examined a Suzuki-Miyaura coupling\(^{30}\) of protected bromodiene 12 with para-tolylboronic acid. Union of these fragments under palladium catalysis was indeed achieved, although it was found that the reaction conditions also affected ester hydrolysis. The free acid 23, recovered from the aqueous phase, was re-subjected to esterification with (trimethylsilyl)diazomethane to afford the originally targeted methyl ester 24, albeit in moderate yield (Scheme 8). A much more straightforward cross-coupling was the Sonogashira\(^{31}\) reaction of 12 with (triisopropylsilyl)acetylene, which afforded the dienyne 25 in near-quantitative yield (Scheme 8).

Dienyne 25 is a useful intermediate for further functionalization. Desilylation with TBAF affords terminal acetylene 26, which is amenable to the Huisgen\(^{32}\) copper-catalysed azide-alkyne cycloaddition protocol. We have demonstrated this through the reaction of 26 with benzyl azide to afford triazole 27 (Scheme 9).

**Conclusions**

We have demonstrated the versatility of a halobenzoate-derived ipso,ortho-oxygenated arene dihydrodiol in various synthetic contexts and shown that the presence of the halogen fundamentally alters the course of the reaction in many instances. The formation of cross-coupling products 23-27 is especially significant, as these structures are arene dihydrodiol derivatives that would not be accessible by direct metabolism of the corresponding arene precursors. Thus, for
example, accessing 23 by biotransformation of biaryl carboxylic acid 28 would not be expected to succeed as the steric bulk of the tolyl substituent would likely preclude docking of 28 in the R. eutrophus B9 BZDO active site. Compound 23 is nevertheless accessible, by means of the indirect route described in this work (Scheme 10).

Scheme 10

Current efforts in our laboratory are focused on optimising the production process for 9 and incorporating this versatile chiron in the synthesis of more complex targets, including appropriate natural products. The results of these endeavours will be reported subsequently.

Experimental

General

Reactions which required the use of anhydrous, inert atmosphere techniques were carried out under an atmosphere of nitrogen. Nonacarbonyldiiron was dispersed in a glovebox. Solvents were dried and degassed by passing through anhydrous alumina columns using an Innovative Technology Inc. PS-400-7 solvent purification system. Petrol refers to petroleum ether, bp 40-60 °C. TLCs were performed using aluminium-backed plates precoated with Alugram 8 SIL G/UV and visualized by UV light (254 nm) and/or KMnO4 followed by gentle warming. Flash column chromatography was carried out using Davissil LC 60Å silica gel (35-70 micron) purchased from Fisher Scientific. IR spectra were recorded on Perkin-Elmer 1600 FT IR spectrometer with absorbances quoted as ν in cm⁻¹. NMR spectra were run in CDC13 (unless otherwise specified) on Bruker Avance 250, 300, 400 or 500 MHz instruments at 298 K. Mass spectra were recorded with a micrOTOF electrospray time-of-flight (ESI-TOF) mass spectrometer (Bruker Daltonik).

Biotransformation of meta-bromobenzoic acid

This was performed in accordance with a literature procedure, substituting meta-bromobenzoate for benzoate in the biotransformation step. A sterile pipette tip was streaked across the surface of a frozen glycerol stock solution of R. eutrophus B9 cells to produce small shards (approx. 10 mg). The frozen shards were added to a sterile 250 mL Erlenmeyer flask containing 100 mL of Hutner’s mineral base medium (1.5 M) supplemented with 50 mL of a 1.0 M solution of benzoic acid (50 mL of a 1.0 M solution) added to initiate BZDO expression. After 3 h, sodium meta-bromobenzoate (50 mL of a 1.0 M solution) and sodium succinate (25 mL of a 1.5 M solution) were added. Over the next 48 h, sodium meta-bromobenzoate solution (1.0 M) and sodium succinate solution (1.5 M) were added portionwise. In total 60.3 g (270 mmol) sodium meta-bromobenzoate and 48.6 g (300 mmol) sodium succinate were introduced. The culture was incubated for a further 72 h, then the fermentation broth was centrifuged at 6000 rpm for 25 min and the supernatant liquid decanted. The concentrated supernatant was then carefully acidified to pH 3.0 with concentrated hydrochloric acid. The acidified solution was then extracted with ethyl acetate (20 x 1 L). After each two extractions, the aqueous phase was re-acidified to pH 3.0. Each organic extract was dried over MgSO4 and filtered.

Combined fractions were concentrated under reduced pressure to give 51.4 g of crude material, of which unreacted meta-bromobenzoic acid was the major constituent. This material was repeatedly trituated with copious quantities of dichloromethane, resulting in dissolution of most of the material. (Toluene was also determined to be a suitable tritutant.) After 7 such triturations and drying under vacuum, the residual solid material was shown by NMR to be pure (15,6S)-5-bromo-1,6-dihydroxycyclohexa-2,4-diencarboxylic acid 9, a brown powder; 979 mg (4.17 mmol), 1.5%, 65 mg dm⁻³, [α]D²⁵ -0.16° (c 0.1, CHCl₃); δBr (300 MHz, CDCl₃) 6.30 (1H, dd, J = 6.0, 1.0 Hz, CBr–CH), 5.89 (1H, dd, J = 9.5, 6.0 Hz, CBr=CH–CH), 5.75 (1H, d, J = 9.5 Hz, CBr=CH=CH), 4.69 (1H, d, J = 1.0 Hz, HO–CH); δC (75 MHz, CDCl₃) 177.3 (C=O), 130.6 (CBr), 128.5 (CBr=CH), 127.0 (CBr=CH), 126.7 (CBr), 77.6 (C–COOH), 74.6 (HO–CH); vmax (film) 3220, 1698, 1417, 1358, 1229, 1091, 1028, 992, 671 cm⁻¹; HRMS (ESI–) m/z calculated for (C₁₇H₁₅BrO₂–C₂H₂O–H)⁺ 170.9446, 172.9425; found 170.9446, 172.9422.

(15,6S)-Methyl 5-bromo-1,6-dihydroxycyclohexa-2,4-diencarboxylate (11)

To a stirred solution of 9 (61 mg, 0.260 mmol, 1 equiv) in MeOH / benzene (1:1, 32 mL) at room temperature was added dropwise (trimethylsilyl)diazomethane (1.5 mL, 2.0 M in hexanes) until the yellow colour persisted and effervescence ceased. The solution was stirred for 2 h then concentrated under reduced pressure to give crude (15,6S)-methyl 5-bromo-1,6-dihydroxycyclohexa-2,4-diene carboxylate 11 (61 mg, 94%) as a brown oil, sufficiently pure to be used without further purification: Rf 0.73 (50% EtOAc–petrol); [α]D²⁵ -40° (c 0.175, CH₂Cl₂); δBr (250 MHz) 6.38 (1H, ddd, J = 6.0, 2.5, 0.5 Hz, CBr=CH), 5.99 (1H, dd, J = 9.5, 6.0 Hz CBr=CH=CH), 5.76 (1H, d, J = 9.5 Hz, CBr=CH=CH), 4.79 (1H, br s, C(OH)H); δC (75 MHz, CDCl₃) 175.5 (C=O), 130.3 (CBr), 127.9, 127.3, 126.8, 78.0 (C–COOMe), 74.6 (C(OH)H), 53.9 (O–CH₃); vmax (film) 3451, 2953, 1734, 1587, 1417, 1359, 1229, 1091, 1028, 992, 671 cm⁻¹; HRMS (ESI–) 254.1082 calculated for (C₁₆H₁₀BrO₂CH₂O–H)⁺, 255.0585 found.

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(3aS,7aS)-Methyl 7-bromo-2,2-dimethyl-3a,7a-dihydrobenzo[d][1,3]dioxole-3-carboxylate (12)

To diol 11 (84 mg, 0.337 mmol, 1 equiv) and para-toluenesulfonic acid (2.0 mg, 0.01 mmol, 0.03 equiv) in acetone (30 mL) was added 2,2-dimethoxypropane (600 μL, 4.88 mmol, 14.5 equiv). The reaction mixture was stirred at room temperature for 48 h, transferred to a separating funnel, washed with saturated NaCl(aq) then extracted with EtOAc. The organic phase was dried over MgSO₄ and filtered. The filtrate was concentrated under reduced pressure to give [(3aS,7aS)-methyl 7-bromo-2,2-dimethyl-3a,7a-dihydrobenzo[d][1,3]dioxole-3-carboxylate (12) (68 mg, 70%) as a brown oil, sufficiently pure to be used without further purification: Rf 0.82 (25% EtOAc–petrol); [α]D²⁵ -192° (c 0.65, CH₂Cl₂); δH (250 MHz) 6.45 (1H, d, J = 6.0 Hz, CBr=CH), 5.90 (1H, d, J = 9.0 Hz, CBr=CH=CH(CH₃)), 5.08 (1H, s, CH(OH)), 3.81 (3H, s, OCH₃), 1.47 (3H, s, CH₃), 1.43 (3H, s, CH₃); δC (75 MHz) 171.3 (C=O), 125.9 (CBr), 124.2, 124.1, 123.4, 81.5 (O–C–O), 78.5 (CH=O), 53.2 (–OCH₃), 26.9 (–CH₃), 25.5 (–CH₃); vmax (film) 2971, 1775, 1706, 1602, 1358, 1240, 1211, 1159, 1099, 957, 758, 723, 687 cm⁻¹; HRMS (ESI+) m/z calcd for (C₁₇H₁₄BrO₆Na)+, 310.985, 312.987; found 310.985, 312.987.

(3aS,4R,5R,7aS)-Methyl 7-bromo-4,5-dihydroxy-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxole-3-carboxylate (14)

To a solution of 11 (14.0 mg, 0.048 mmol, 1 equiv) and NMO (6.6 mg, 0.048 mmol, 1 equiv) in aceton/H₂O 4:1 (3 mL) was added OsO₄ (10 μL, 2.5 wt% in rBuOH, 2 mol%). The reaction mixture was stirred at room temperature for 24 h, then diluted with Na₂S₂O₄(aq) and extracted with EtOAc. The organic extracts were dried over MgSO₄ and filtered. The filtrate was concentrated under reduced pressure and purified by column chromatography (40% EtOAc–petrol) to give (3aS,4R,5R,7aS)-methyl 7-bromo-4,5-dihydroxy-2,2-dimethyl-3a,4,5,7a-tetrahydrobenzo[d][1,3]dioxole-3-carboxylate (14) (11 mg, 80%) as a colourless oil: Rf 0.32 (40% EtOAc–petrol); [α]D²⁵ -5.5° (c 0.55, CH₂Cl₂); δH (500 MHz) 6.23 (1H, d, J = 2.5 Hz, CBr=CH), 5.08 (1H, d, J = 0.5 Hz, CBr=CH-O), 4.42 (1H, br s, CH(OH)COOCH₃), 4.32 (1H, br s, CH(OH)COOCH₃), 3.87 (3H, s, −OCH₃), 2.80 (1H, br s, −OH); 7.5 mg, 14.5% yield) as a white crystalline solid: m.p. 172-174 °C (EtOAc–Petrol); Rf 0.54 (50% EtOAc–petrol); [α]D²⁵ -11.5° (c 0.78, CH₂Cl₂); δH (500 MHz) 7.44-7.37 (5H, m, Ar-H), 6.62 (1H, dt, J = 10.0, 0.5 Hz, CBr=CH=CH), 6.37 (1H, dd, J = 10.0, 7.0 Hz, CBr=CH=CH), 5.39 (1H, dd, J = 7.0, 1.5 Hz, N–CH–C–C–O–O–), 5.33 (1H, d, J = 1.5 Hz, CBr=CH-O), 3.93 (3H, s, −OCH₃), 1.42 (3H, s, CH₃), 1.36 (3H, s, CH₃); δC (500 MHz) 169.7 (O–C=O), 154.3 (N–C=O), 154.0 (N–C=O), 135.4, 130.7, 129.2, 128.8, 127.6, 125.8, 114.3 (–O–C–O–), 84.2, 82.9, 67.7, 53.9, 53.8, 26.2, 26.1; vmax (film) 2993, 2786, 1721, 1559, 1501, 1457, 1400, 1260, 1214, 1149, 1088, 974, 880, 754 cm⁻¹; HRMS (ESI+) m/z calcd for (C₁₇H₁₂BrN₃O₆+H)⁺, 464.0457, 466.0437; found 464.0449, 466.0429.
(4S)-Tricarbonyl(η₄-(3aS,7aS)-methyl 7-bromo-2,2-dimethyl-3a,7a-dihydrobenzod[d][1,3]dioxole-3a-carboxylate)-iron(0) (21) and (4R)-Tricarbonyl(η₄-(3aS,7aS)-methyl 7-bromo-2,2-dimethyl-3a,7a-dihydrobenzod[d][1,3]dioxole-3a-carboxylate)-iron(0) (22).

To a flask containing 12 (185 mg, 0.59 mmol, 1 equiv) in a glovebox was added nonacarbonyliron (440 mg, 1.21 mmol, 2 equiv). THF (40 mL) was added and the reaction mixture was stirred at room temperature for 7 d. The reaction mixture was then concentrated under reduced pressure (Care! Toxic pentacarbonyliron distilled at this point) and purified by column chromatography (10% EtOAc–petrol) to give (4S)-tricarbonyl(η₄-(3aS,7aS)-methyl 7-bromo-2,2-dimethyl-3a,7a-dihydrobenzod[d][1,3]dioxole-3a-carboxylate)-iron(0) (21) as fine brown needles (43 mg, 17%) and (4R)-tricarbonyl(η₄-(3aS,7aS)-methyl 7-bromo-2,2-dimethyl-3a,7a-dihydrobenzod[d][1,3]dioxole-3a-carboxylate)-iron(0) (22) as a brown oil (36 mg, 14%). Unreacted 12 (100 mg, 60%) was also isolated.

Complex 21: m.p. 95–97 °C (EtOAc–petrol); Rf 0.32 (10% EtOAc–petrol); [α]₀²⁵ -10° (c 0.2, CH₂Cl₂); δ η (300 MHz) 5.95 (1H, dt, J = 4.5, 1.5 Hz, CBr=CH), 5.47 (1H, dd, J = 6.5, 4.5 Hz, CBr=CH-H$_{2}$), 5.36 (1H, d, J = 1.5 Hz, CH-0), 3.88 (3H, s, O-CH$_{3}$), 3.06 (1H, dd, J = 6.5, 1.0 Hz, CBr=CH=CH=H), 1.45 (3H, s, C-CH$_{3}$), 1.23 (3H, s, C-CH$_{3}$); δ$_{C}$ (75 MHz) 171.3 (-COOme), 117.8 (-O-C-0), 89.9 (CBr=CH), 87.0 (-COOme), 86.7 (CH-0), 83.6 (CBr=CH=CH=), 70.2 (CBr), 54.0 (CBr=CH=CH=CH=), 53.3 (O, CH$_{3}$), 28.1 (C-CH$_{3}$), 27.3 (C-CH$_{3}$); ν$_{max}$ (film) 2981, 2068, 2003, 1730, 1437, 1375, 1261, 1214, 1162, 1062, 978, 865, 752, 687, 636 cm$^{-1}$; HRMS (ESI+) m/z calcd for (C$_{21}$H$_{18}$BrFeO$_{2}$Na)$_{+}$, 450.9909, 452.9071; found 450.9106, 452.9164.

Complex 22: Rf 0.26 (10% EtOAc–petrol); [α]₀²⁵ -90° (c 0.6, CH₂Cl₂); η (500 MHz) 5.76 (1H, d, J = 3.5 Hz, CBr=CH), 5.15 (1H, dd, J = 6.0, 4.5 Hz, CBr=CH-H$_{2}$), 4.60 (1H, s, CH-O), 3.75 (3H, s, O-CH$_{3}$), 2.97 (1H, d, J = 6.5 Hz, CBr=CH=CH=H), 1.71 (3H, s, C-CH$_{3}$), 1.20 (3H, s, C-CH$_{3}$); δ$_{C}$ (75 MHz) 207.1 (Fe C=O), 173.3 (-COOme), 109.7 (-O-C-0), 89.3 (CBr=CH), 85.4 (-COOme), 84.8 (-CH-O), 80.5 (CBr=CH=CH=), 73.6 (CBr), 61.2 (CBr=CH=CH=CH=), 53.1 (O-CH$_{3}$), 25.0 (C-CH$_{3}$), 24.2 (C-CH$_{3}$); ν$_{max}$ (film) 2980, 2061, 1995, 1729, 1460, 1380, 1252, 1207, 1168, 1070, 1030 cm$^{-1}$; HRMS (ESI+) m/z calcd for (C$_{21}$H$_{13}$BrFeO$_{2}$Na)$_{+}$, 450.9909, 452.9071; found 450.9091, 452.9073.

(3aS,7aR’)-Methyl 2,2-dimethyl-7-(triisopropylsilyl)ethynyl)-3a,7a-dihydrobenzod[d][1,3]dioxole-3a-carboxylate 25

To a solution of bromodiene 12 (81 mg, 0.28 mmol, 1 equiv), tetrais(triphenylphosphine)palладium (16 mg, 0.014 mmol, 5 mol %), copper(I) iodide (3.7 mg, 0.0196 mmol, 7 mol %) dissolved in THF (20 mL), was added by syringe n-butyamine (110 μL, 1.12 mmol, 4 equiv) and (triisopropyl)acetelylene (100 μL, 0.45 mmol, 1.6 equiv). The reaction mixture was stirred at room temperature for 24 h, then diluted with EtOAc and washed with NH$_{4}$Cl$_{aq}$. The organic phase was dried over MgSO$_{4}$ and filtered. The filtrate was concentrated under reduced pressure and purified by column chromatography (10% EtOAc–petrol) to give (3aS,7aR’)-methyl 2,2-dimethyl-7-(triisopropylsilyl)ethynyl)-3a,7a-dihydrobenzod[d][1,3]dioxole-3a-carboxylate (25) (108 mg, 98%) as a yellow oil: Rf 0.45 (10% EtOAc–petrol); [α]₀²⁵ -176° (c 0.89, CH$_{2}$Cl$_{2}$); δ η (250 MHz) 6.37 (1H, d, J = 6.0 Hz, SiC=C=C=CH=H), 6.12 (1H, dd, J = 9.5, 6.0 Hz, SiC=C=C=CH=CH=H), 5.85 (1H, dd, J = 9.5, 0.5 Hz, SiC=C=C=CH=CH=H), 4.91 (1H, d, J = 0.5 Hz, CH-O), 3.78 (3H, s, O-CH$_{3}$), 1.45 (3H, s, C-CH$_{3}$), 1.39 (3H, s, C-CH$_{3}$), 1.08 (21H, br s, Si-CH and Si-CH=CH=H); δ$_{C}$ (75 MHz) 171.7 (-C=O), 129.0 (alkene C), 125.0 (alkene C), 124.4 (alkene C), 120.7 (alkene C), 108.2 (-O-C-0), 105.5 (alkyne C), 96.7 (alkyne C), 80.0 (-COOme), 75.6 (-CH-O), -53.0 (O-CH$_{3}$), 26.9 (-CH$_{3}$), 25.6 (-CH$_{3}$), 18.6 (-SiC=CH$_{3}$), 11.3 (-Si-C=CH$_{3}$); ν$_{max}$ (film) 2943, 2865, 2158, 2032, 1741, 1462, 1381, 1243, 1039, 883, 677 cm$^{-1}$; HRMS (ESI+) m/z calcd for (C$_{21}$H$_{18}$O$_{2}$Si$_{2}$Na)$_{+}$, 413.2124; found 413.2127.

(3aS,7aR’)-Methyl 7-ethyl-2,2-dimethyl-3a,7a-dihydrobenzod[d][1,3]dioxole-3a-carboxylate 26
To a stirred solution of terminal alkyne 26 (13.0 mg, 0.05 mmol, 1 equiv) in EtOH/H₂O (5:1) (25 mL) were added benzyl azide (7.9 mg, 0.06 mmol, 1.2 equiv), CuSO₄ (1.1 mg, 1 mol %) and ascorbic acid (5.9 mg, 10 mol%). The solution was stirred at room temperature for 48 h, then diluted with NaCl(aq) and extracted with EtOAc. The organic layer was dried over MgSO₄ and filtered. The filtrate was concentrated under reduced pressure and purified by column chromatography (10% EtOAc–petrol) to give 26 (7.8 mg, 66%) and (3aS,7aR)-methyl 7-((1-benzyl-1H-1,2,3-triazol-4-yl)-2,2-dimethyl-3a,7a-dihydrobenzo[d][1,3]dioxole-3-carboxylate (27) (6.3 mg, 34%) as a pale brown oil: Rf 0.48 (50% EtOAc–petrol); [α]₂₅D -40° (c 0.18, CH₂Cl₂); δH (300 MHz) 7.62 (1H, s, Het-Ar-H), 7.37-7.29 (5H, m, Ph-H) 6.94 (1H, d, J = 6.0 Hz, Het-Ar-C=CH₂), 6.28 (1H, dd, J = 9.0, 6.0 Hz, Het-Ar-C=CH₂), 5.92 (1H, d, J = 9.0 Hz, Het-Ar-C=CH₂), 5.61 (1H, d, J = 15.0 Hz, Ph-CH₂), 5.49 (1H, d, J = 15.0 Hz, Ph-CH₂) 5.27 (1H, s, CH-O-), 3.78 (3H, s, O-CH₃), 1.48 (3H, s, C-CH₃), 1.34 (3H, s, C-CH₃); δC (75 MHz) 172.0 (C=O), 134.8, 129.3, 128.8, 128.1, 127.8, 126.1, 124.9, 124.5, 121.0 (3° Het-Ar), 119.7 (Het-Ar=C=CH₂), 108.7 (-O-C=O), 80.6 (C-OMe), 74.2 (CH₂-C=O), 54.3 (Ph-CH₃), 53.2 (O-CH₃), 27.1 (C-CH₃), 25.8 (C-CH₃) νmax (film) 2995, 2917, 1857, 1739, 1496, 1457, 1258, 1066, 887, 799, 727 cm⁻¹; HRMS (ESI+) m/z calcd for (C₁₅H₁₄O₄Na)⁺ 390.1429; found 390.1440.

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Notes and references

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5. Previously known as Alcaligenes eutrophus B9.
6. Electrostatic Supplementary Information (ESI) available: H and 13C NMR spectra for all novel compounds. 2D NMR spectra for selected compounds. Crystallographic data for 13 and 21. CCDC 808270 and 808269. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/b000000x.
Dimerisation of derivatives of 2 (R = H) incorporating more sterically demanding acetal has also been studied; the anti,anti adduct was again the sole dimer formed. See ref. 22; also see (a) R. Gillard and D. J. Burnell, *J. Chem. Soc., Chem. Commun.*, 1989, 1439; (b) R. J. Gillard and D. J. Burnell, *Can. J. Chem.*, 1992, 70, 296.


