Stereocontrolled Synthesis of (2*R*,3*S*)-2-Methylisocitrate, a Central Intermediate in the Methylcitrate Cycle

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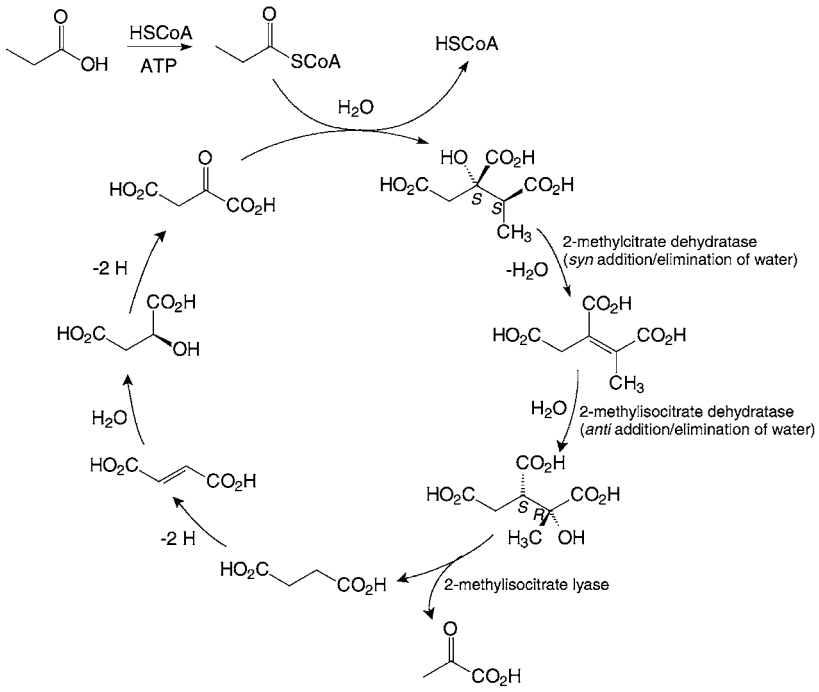
Dedicated to *Duilio Arigoni* on the occasion of his 75th birthday

2-Methylisocitrate (= 3-hydroxybutane-1,2,3-tricarboxylic acid) is an intermediate in the oxidation of propanoate to pyruvate (=2-oxopropanoate) *via* the methylcitrate cycle in both bacteria and fungi (*Scheme 1*). Stereocontrolled syntheses of (2*R*,3*S*)- and (2*S*,3*R*)-2-methylisocitrate (98% e.e.) were achieved starting from (*R*)- and (*S*)-lactic acid (=(2*R*)- and (2*S*)-2-hydroxypropanoic acid), respectively. The dispiroketal (6*S*,7*S*,15*R*)-15-methyl-1,8,13,16-tetraoxadispiro[5.0.5.4]hexadecan-14-one (**2a**) derived from (*R*)-lactic acid was deprotonated with lithium diisopropylamide to give a carbanion that was condensed with diethyl fumarate (*Scheme 3*). The configuration of the adduct diethyl (2*S*)-2-[(6*S*,7*S*,14*R*)-14-methyl-15-oxo-1,8,13,16-tetraoxadispiro[5.0.5.4]hexadec-14-yl]butanedioate (**3a**) was assigned by consideration of possible transition states for the fumarate condensation (*cf. Scheme 2*), and this was confirmed by a crystal-structure analysis. The adduct was subjected to acid hydrolysis to afford the lactone **4a** of (2*R*,3*S*)-2-methylisocitrate and hence (2*R*,3*S*)-2-methylisocitrate. Similarly, (*S*)-lactic acid led to (2*S*,3*R*)-2-methylisocitrate. Comparison of 2-methylisocitrate produced enzymatically with the synthetic enantiomers established that the biologically active isomer is (2*R*,3*S*)-2-methylisocitrate.

1. Introduction. – 2-Methylisocitrate (= 3-hydroxybutane-1,2,3-tricarboxylate) is a key intermediate in the methylcitrate cycle of both bacteria [1–4] and fungi [4–6]. In this cycle (*Scheme 1*), propanoate is oxidised to pyruvate (=2-oxopropanoate) at the expense of oxaloacetate (=oxobutanedioate), which is reduced to succinate (=butanedioate). The oxaloacetate is regenerated by oxidation of succinate, mediated by enzymes of the *Krebs* cycle. The reactions of the methylcitrate cycle are closely related to those of the glyoxylate cycle, in which acetate is oxidised to the α-oxo acid glyoxylate (=oxoacetate). In the methylcitrate cycle, propanoyl-CoA condenses with oxaloacetate to yield (2*S*,3*S*)-2-methylcitrate (=(2*S*,3*S*)-2-hydroxybutane-1,2,3-tricarboxylate) [7], which is probably dehydrated to (*Z*)-2-methylaconitate (=(2*Z*)-but-2-ene-1,2,3-tricarboxylate) in a *syn* manner catalysed by methylcitrate dehydratase (PrpD) [3][4]. Pig-heart aconitase catalyses the hydration of the double bond of (*Z*)-2-methylaconitate with the opposite regioselectivity to that of methylcitrate dehydratase [3][8]. The addition of H and OH in an *anti* manner would yield either (2*R*,3*S*)- or (2*S*,3*R*)-2-methylisocitrate. By optical-rotation and circular-dichroism studies, the enantiomer formed by aconitase has been tentatively assigned as (2*R*,3*S*)-2-methyl-

Scheme 1. *The Methylcitrate Cycle*. The configuration of 2-methylisocitrate was determined as (2*R*,3*S*) in the present paper.



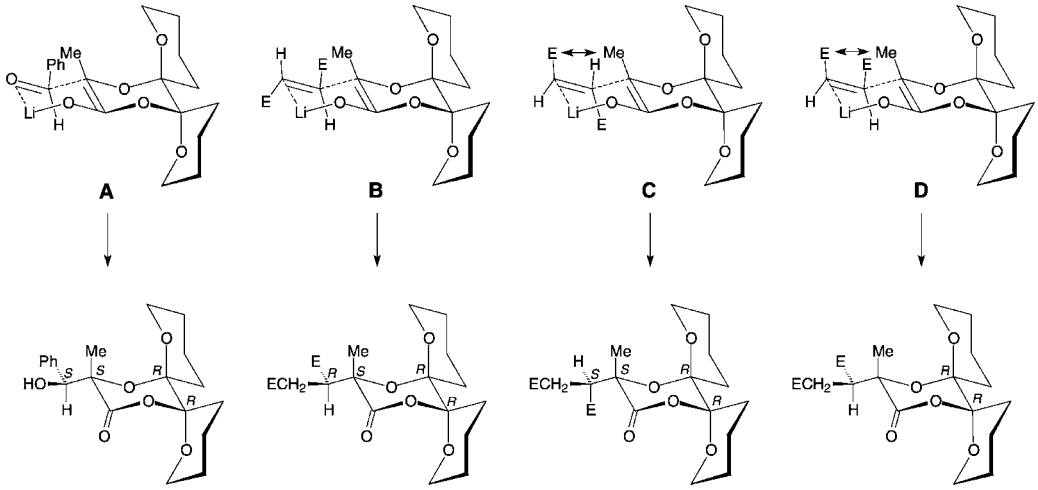
isocitrate (=(2*S*,3*R*)-3-hydroxybutane-1,2,3-tricarboxylate) [9]. Recently, it was shown that the 2-methylisocitrate lyases from *Escherichia coli* and *Aspergillus nidulans* accept only one of the *threo*-diastereoisomer pair (2*R*,3*S*)- and (2*S*,3*R*)-2-methylisocitrate as substrate, with the *erythro*-diastereoisomer pair (2*R*,3*R*)- and (2*S*,3*S*)-2-methylisocitrate being enzymatically inactive. Amino acid sequence comparisons have revealed that 2-methylisocitrate lyase is homologous with isocitrate lyase, which catalyses the cleavage of (2*R*,3*S*)-isocitrate [10][11] to glyoxylate and succinate. It has been proposed, therefore, that 2-methylisocitrate lyase stereospecifically mediates the cleavage of (2*R*,3*S*)-2-methylisocitrate to pyruvate and succinate. A definite answer to this stereochemical problem requires, however, an independent synthesis of biologically active 'monochiral' (one-handed; enantiomerically pure) [12] 2-methylisocitrate.

A number of synthetic methods have been developed for 2-methylisocitrate, which lead either to a mixture of all possible isomers [13] or to distinct diastereoisomer pairs ((2*R*,3*R*)/(2*S*,3*S*) or (2*R*,3*S*)/(2*S*,3*R*)) [14]. Although these methods provided a substrate that was useful for assaying 2-methylisocitrate lyase [4], the configurationally nonhomogeneous nature of the materials limited their usefulness. Furthermore, none of the procedures can be adapted for generating single enantiomers of 2-methylisocitrate. 'Monochiral' 2-methyliso-citrate has been synthesised in small amounts enzymatically [4][15], but this did not permit the unambiguous assignment of

configuration. In this paper, we describe highly diastereoselective routes to (2*R*,3*S*)- and (2*S*,3*R*)-2-methylisocitrate from (*R*)- and (*S*)-lactic acid (=(2*R*)- and (2*S*)-2-hydroxypropanoic acid), respectively. Comparison of 2-methylisocitrate produced enzymatically [4][15] with these synthetic enantiomers established that the biologically active isomer is (2*R*,3*S*)-2-methylisocitrate.

2. Results and Discussion. – *Ley* and co-workers devised a method for coupling one enantiomer of lactic acid at the C(α) atom with an electrophile, which in the case of an aldehyde gave rise to a product that contains two adjacent chiral centres in a configurationally defined relationship. In this method, the enolate derived by deprotonation of a dispiro ketal of lactic acid (*e.g.*, **2b** from (*S*)-lactic acid) was condensed with an electrophile (*e.g.*, an aldehyde) to give, after acidic deprotection, a chiral product of high enantiomer purity (see **A** in *Scheme 2*) [16]. The lactate Me group occupied a pseudo-equatorial position and dictated the dispiro ketal configurations as (6*R*,7*R*) from (*S*)-lactic acid and (6*S*,7*S*) from (*R*)-lactic acid. Upon enolate formation, the dispiro ketal configurations were preserved, and this determined the mode of attack by the electrophile. We found that *Ley*'s dispiro-ketal-protected lactates [16] can be used to enable efficient syntheses of 'monochiral' 2-methylisocitrates.

Scheme 2. *Proposed transition states for the addition of prochiral electrophiles* (E = CO_2Et) *to the dispiro ketal lithium enolate.* **A**: Addition of benzaldehyde to the least sterically hindered orientation with the Ph group adopting a pseudo-equatorial position (adapted from *Ley* and co-workers [16]). **B**: Addition of diethyl fumarate with both ester groups adopting pseudo-equatorial positions in the transition state. **C**: Addition of diethyl fumarate with both ester groups occupying pseudo-axial positions in the transition state resulting in a 1,3-diaxial interaction. **D**: One of the two possible modes of addition of diethyl maleate.

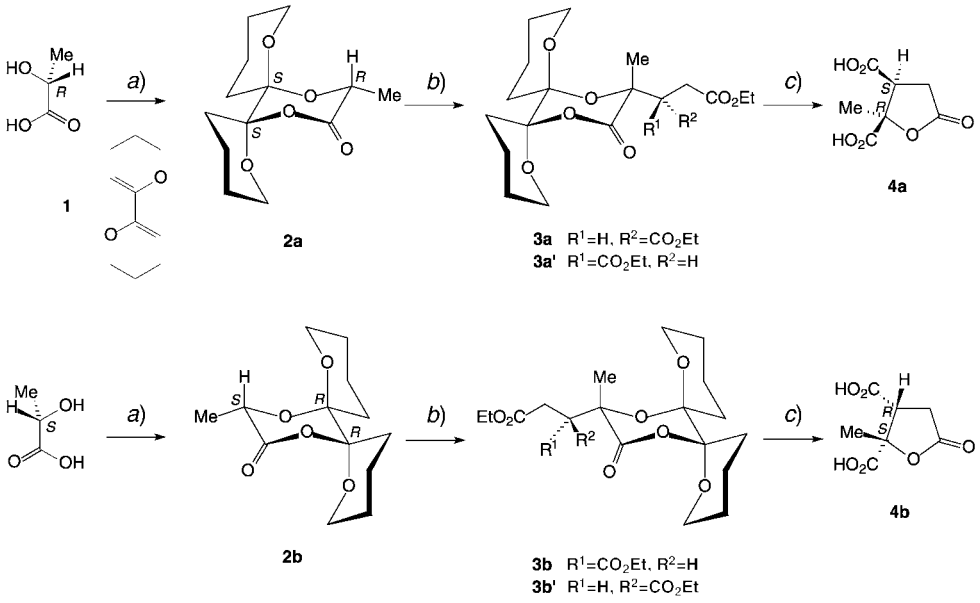


We envisaged that coupling the enolate of the dispiro ketal **2a** from (*R*)-lactic acid and **1** with a suitable succinate (= butanedioate) or fumarate (=(2*E*)-but-2-enedioate derivative would lead, with control of configuration at both newly formed stereogenic centres, to a precursor **3a** or **3a′** of 2-methylisocitrate. In practice, we obtained only precursor **3a** of (2*R*,3*S*)-2-methylisocitrate starting from (*R*)-lactic acid, and solely

precursor **3b** of (2*S*,3*R*)-2-methylisocitrate from (*S*)-lactic acid (*Scheme 3*). Thus, when the enolate of the spiro ketal **2b** from (*S*)-lactic acid was reacted with diethyl fumarate (or *rac*-diethyl bromosuccinate; see below), a single adduct **3b** was obtained, which, after deprotection by treatment with hydrochloric acid, afforded lactone **4b**. The configuration of **4b** was assigned as either (2*R*,3*S*) or (2*S*,3*R*) by comparison of its ^{1}H- and ^{13}C-NMR spectra with data for the authentic diastereoisomer pairs of 2-methylisocitrates [4][14]. This conclusion was supported by reversed-phase-HPLC analysis, which showed that lactone **4b** eluted with the same retention time as the (2*R*,3*S*)/(2*S*,3*R*) diastereoisomer pair (data not shown). The lactone **4b** was also analysed by chiral HPLC, with a system known to separate the four stereoisomers of 2-methylisocitrate [4]. A single peak (*Fig. 1,c*) was observed that corresponded to the earlier-eluting peak of the synthetic (2*R*,3*S*)/(2*S*,3*R*)-diastereoisomer pair (*cf. Fig. 1,b*), but did not match either peak of the synthetic (2*R*,3*R*)/(2*S*,3*S*) pair (*cf. Fig. 1,a*).

Condensation of the dispiro ketal **2a** derived from (*R*)-lactic acid with diethyl fumarate (or *rac*-diethyl bromosuccinate; see below) gave a single adduct **3a** that by acid-catalysed deprotection gave lactone **4a**, analysis of which by chiral HPLC showed a single peak corresponding to the later-eluting peak of the (2*R*,3*S*)/(2*S*,3*R*) pair (*Fig. 1,d*). These results are consistent with those reported for reactions of the dispiro ketal enolate with aldehydes, whereby predominantly one diastereoisomeric product arises [16][17]. For these types of reaction, *Ley* and co-workers proposed a 6-membered, chair-like transition state, in which the Li^{+} ion coordinated to both the carbonyl and enolate O-atom. The orientation of the aldehyde with respect to *Re* or *Si*-

Scheme 3. *Synthesis of the Lactones* **4a** *and* **4b** *of (2*R,3S*)- and (2*S,3R*)-2-Methylisocitrate, Respectively*



a) 1M HCl, Et_2O, r.t., 25 h (*cf.* [15]). *b*) LDA, *rac*-diethyl bromobutanedioate, THF, −78°, 1 h; 76%. *c*) 6M HCl, reflux 3 h, r.t., 12 h, 91%

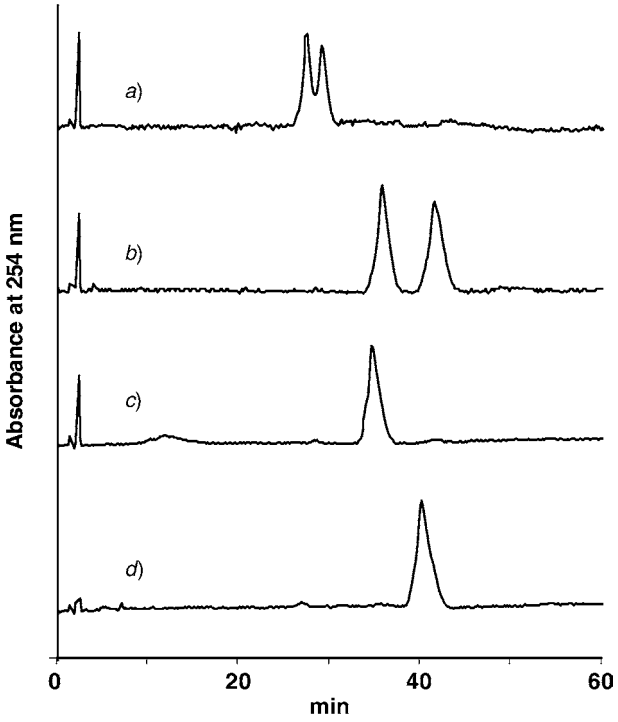


Fig. 1. *Elution profiles of 2-methylisocitrate samples:* a) *(2*R,*3*R*)/(2*S,*3*S*)*, b) *(2*R,*3*S*)/(2*S,*3*R*)*, c) *(2*S,*3*R*), and* d) *(2*R,*3*S*) from a* Chirex-D-*penicillamine column*. The sharp, early eluting peaks are injection artefacts stemming from acid-induced leaching of Cu^{2+} ions from the column.

addition to the enolate is dictated by the steric influence of the group attached to the aldehyde, with this group adopting a pseudo-equatorial orientation in the transition state. This model explains why steric control operates at both stereogenic centres. Indeed, of the four possible stereoisomers that could arise from the addition of the dispiro ketal **2b** enolate to benzaldehyde, only the (*S*,*S*)-enantiomer was observed. For the addition of diethyl fumarate to a dispiro ketal enolate, an analogous chair-like 6-membered-ring transition state can be formulated. Assuming that the C_{2h}-symmetric fumarate attacks the enolate from the less-hindered face, thus avoiding the 1,3-diaxial interaction with the C−O of the pyran ring, then there are two possible transition states, as shown by **B** and **C** in *Scheme 2*. In **B**, the fumarate approaches the enolate in an orientation that results in both ester groups adopting pseudo-equatorial positions as the chair-like transition state develops. Models indicate very little steric crowding of the ester groups in this transition state. By contrast, when the fumarate approaches the enolate in the alternative orientation **C**, both ester groups adopt pseudo-axial positions in the transition state, resulting in an unfavourable 1,3-diaxial interaction between the distal fumarate ester group and the Me group of the dispiro ketal. Such an interaction would raise the energy of this transition state **C** relative to the alternative form **B** (*cf. Scheme 2*). The transition state **B** would lead to either (2*R*,3*S*)- or (2*S*,3*R*)-2-

methylisocitrate depending on the chirality of the precursor lactate, whereas the alternative **C** would yield either the (2*R*,3*R*)- or (2*S*,3*S*)-enantiomer. This analysis leads to the conclusion that the dispiro ketal **2a** of (*R*)-lactic acid gave the lactone **4a** of (2*R*,3*S*)-2-methylisocitrate, whereas the dispiro ketal **2b** of (*S*)-lactic acid gave the lactone **4b** of (2*S*,3*R*)-2-methylisocitrate. Gratifyingly, these conclusions were confirmed by crystal structure analyses of dispiro ketals **3a** and **3b**.

In the absence of significant anomalous scattering effects (no elements heavier than O), the crystallographic data do not permit the determination of the absolute configuration, but the relative configuration at all the chiral centres was established and found to be the same for **3a** and **3b**, confirming that they are enantiomers. Indeed, all the crystallographic results for both compounds are essentially identical, the two compounds crystallising with opposite chirality in the same space group with the same cell parameters. However, as the configuration of the lactate defines the dispiro ketal configurations (*e.g.*, as in **2a**) and these are not affected in the condensation with diethyl fumarate, the configurations of the newly formed stereogenic centres in **3a** are defined by reference to those at dispiroketal centres. The molecular structure of **3a**, with the absolute configuration derived from that of the starting material, is shown in *Fig. 2*. All conformational parameters are as expected. It was also confirmed by X-ray analysis that the relative configuration of the starting material was retained in the conversion of dispiro ketal **3** into lactone **4** (results not shown).

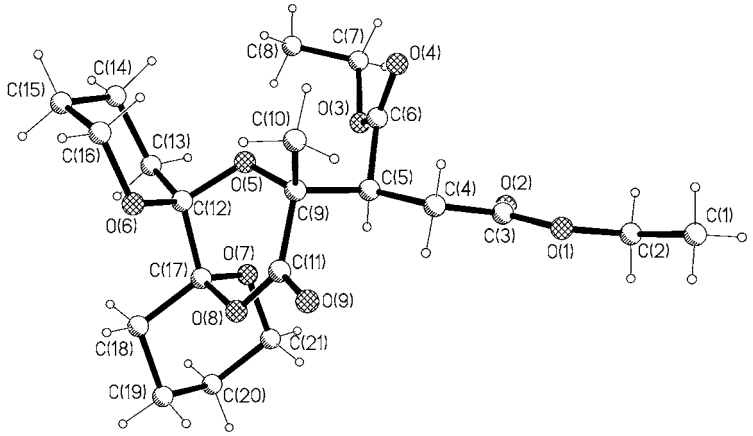


Fig. 2. *Molecular structure of* **3a** *determined by X-ray crystallography.* Arbitrary numbering.

When diethyl fumarate was substituted by diethyl maleate (=diethyl (2*Z*)-but-2-enedioate), no product was observed from the reaction with the lithium enolate of the dispiro ketal **2b**. According to the model proposed above, both possible diethyl maleate orientations with a chair transition state would result in one of the ester groups adopting a pseudo-axial orientation (see, *e.g.*, **D** in *Scheme 2*), thus raising the transition-state energy.

An alternative approach to a 'monochiral' 2-methylisocitrate was attempted through reaction of diethyl bromosuccinate with the lithium enolate of dispiro ketal **2b**. If this reaction proceeded through inversion at the chiral centre of the bromosuccinate,

condensing (for example) diethyl (*R*)-bromosuccinate with dispiro ketal **2b** from (*S*)-lactic acid would lead to the lactone of (2*S*,3*S*)-2-methylisocitrate. However, regardless of which diethyl bromosuccinate was employed ((*R*), (*S*), or *rac*), lactone **4b** of (2*S*,3*R*)-2-methylisocitrate was the exclusive product in every case. These results suggested that, under the basic conditions of the reaction, diethyl fumarate was being generated *in situ* by dehydrobromination of the diethyl bromosuccinate. TLC and ^{1}H-NMR Analysis of the crude reaction mixture showed that diethyl fumarate was formed during the reaction (data not shown). However, the isolated yield (76%) of the dispiro ketal **3b** was significantly higher than that obtained from the direct addition of fumarate (44%). Possibly, *in situ* formation of diethyl fumarate limits the extent of its polymerisation during the reaction.

Hydrolysis of the lactone **4a** as described for the diastereomeric lactones ((2*R*,3*S*)/(2*S*,3*R*) and (2*R*,3*R*)/(2*S*,3*S*)) [4] gave (2*R*,3*S*)-2-methylisocitrate. This isomer, but not (2*S*,3*R*)-2-methylisocitrate derived from hydrolysis of **4b**, was a substrate for both 2-methylisocitrate lyase and 2-methylisocitrate dehydratase. By enzymatic determination with 2-methylisocitrate lyase and lactate dehydrogenase, the synthesised (2*S*,3*R*)-2-methylisocitrate was found to contain 1.0% of (2*R*,3*S*)-enantiomer (*i.e.*, 98% e.e.).

3. Conclusions. – We have shown that lactone **4a** of (2*R*,3*S*)-2-methylisocitrate can be obtained with very high diastereoselectivity by addition of diethyl fumarate to the enolate of dispiro ketal **2a** of (*R*)-lactic acid. Similarly, its enantiomeric lactone **4b** of (2*S*,3*R*)-2-methylcitrate can be derived from (*S*)-lactic acid. This selectivity can be explained by a six-membered, chair-like transition state. The failure of diethyl maleate to react in an analogous way precludes the possible extension of the method to the synthesis of the lactones of either (2*R*,3*R*)- or (2*S*,3*S*)-2-methylisocitrate. The synthetic (2*R*,3*S*)-2-methylisocitrate obtained by hydrolysis of lactone **4a** was a substrate for 2-methylisocitrate lyase and was chromatographically identical to 2-methylisocitrate enzymatically produced from succinate and pyruvate *via* the methylcitrate cycle [4]. Hence, it is (2*R*,3*S*)-2-methylisocitrate that participates in the methylcitrate cycle, which can now be completed with respect to stereochemistry as shown in *Scheme 1*.

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Experimental Part

1. *General*. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl (= diphenylmethanone radical ion(1 −)). TLC: aluminium sheets pre-coated with silica gel *60* F_{254} (0.2 mm). Column chromatography (CC): silica gel *60* ('flash'). Reversed-phase and chiral HPLC: as described in [4]. M.p.: *Gallenkamp* apparatus; uncorrected. Optical rotations: *Perkin-Elmer 241* polarimeter. ^{1}H- and ^{13}C-NMR Spectra: chemical shifts δ in ppm with residual protons of the deuterated solvents as internal standard, coupling constants J in Hz.

2. *Enzyme Assays and Enantiomer Purity*. The enzyme assays were performed spectrophotometrically at 340 nm ($\varepsilon = 6.3$ mM^{-1} cm^{-1}) with 2-methylisocitrate lyase and lactate dehydrogenase as described [4]. To determine the amount of (2*R*,3*S*)-2-methylisocitrate in the synthesised sample of the (2*S*,3*R*)-enantiomer, the lactone was hydrolysed with NaOH and neutralised. The cuvette ($d = 1$ cm) contained, in a total volume of 1.0 ml, 50 mM potassium phosphate pH 7.0, 2 mM $MgCl_2$, 2 mM dithiothreitol, 0.2 mM NADH, 1.5 U of lactate dehydrogenase, and 1.0 mM of the sample. The measured $\Delta E_{340} = 0.06$ indicated that the (2*S*,3*R*)-2-methylisocitrate sample contained 1.0% of the (2*R*,3*S*)-enantiomer. Unfortunately this method does not allow

the determination of the enantiomer purity of the synthesised (2*R*,3*S*)-2-methylisocitrate with the same precision. In a kinetic assay under saturating substrate conditions with 1 mM (2*R*,3*S*)-2-methylisocitrate, the specific activity of 2-methylisocitrate lyase was 28 U/mg, 32% higher than that determined with the equal mixture of (2*R*,3*S*)- and (2*S*,3*R*)-2-methylisocitrate [4].

3. *Diethyl Bromosuccinates* (= *Diethyl Bromobutanedioates*). A soln. of *rac*-bromosuccinic acid (2.0 g, 10.2 mmol) in 3% (*v*/*v*) HBr in EtOH was stirred at r.t. overnight. The solvent and HBr were removed, and the residual oil was taken up in Et_2O (50 ml). The Et_2O soln. was washed with 5% $NaHCO_3$ soln. (2 × 25 ml), dried ($MgSO_4$), and evaporated to give *rac*-diethyl bromosuccinate (2.1 g, 81%) as a pale yellow oil that was used directly for condensations.

(*R*)- and (*S*)-Bromosuccinic acid were prepared as described [18] and were converted to (*R*)- and (*S*)-diethyl bromosuccinate, respectively, as described above for *rac*-bromosuccinic acid.

4. *Diethyl (2*S*)-2-[(6*S*,7*S*,14*R*)- and (2*R*)-2-(6*R*,7*R*,14*S*,14-Methyl-15-oxo-1,8,13,16-tetraoxadispiro[5.0.5.4]hexadec-14-yl]butanedioate* (**3a** and **3b**, resp.). To $^{i}Pr_2NH$ (0.4 g, 0.6 ml, 4.0 mmol) in THF (10 ml) at −78°, 2.2M BuLi in THF (1.8 ml, 4.5 mmol) was added dropwise, and the soln. was allowed to warm to r.t. over 20 min. The resulting mixture was cooled to −78° and the (6*S*,7*S*,15*R*)-15-methyl-1,8,13,16-tetraoxadispiro[5.0.5.4]hexadecan-14-one (**2a**; from (*R*)-lactic acid) or (6*R*,7*R*,15*S*)-15-methyl-1,8,13,16-tetraoxadispiro[5.0.5.4]hexadecan-14-one (**2b**; from (*S*)-lactic acid) (1.0 g, 3.9 mmol) in THF (10 ml) at −78° was added *via* cannula. The mixture was stirred for 1 h at −78°. Diethyl bromosuccinate (0.75 ml, 1.0 g, 4.0 mmol) or diethyl fumarate (0.65 ml, 0.68 g, 4.0 mmol) was added, and the soln. was stirred for 1 h at −78°. The mixture was quenched by the addition of H_2O (10 ml). The resulting mixture was extracted with Et_2O (3 × 25 ml), the combined org. layer dried ($MgSO_4$) and evaporated, and the crude yellow oil purified by medium-pressure CC (silica gel, petroleum ether/Et_2O 3:2): **3a** or **3b** (1.5 g, 88%) as a colourless oil that was crystallised from petroleum ether (40–60°): colourless crystals (1.3 g, 76%). ^{1}H-NMR ($CDCl_3$, 300 MHz): 4.11 (*q*, *J* = 7.2, 4 H); 4.00–3.65 (*m*, 4 H); 3.43 (*t*, *J* = 7.6, 1 H); 2.80 (*d*, *J* = 7.6, 2 H); 2.04–1.50 (*m*, incl. *s* at 1.63 (3 H), 15 H); 1.25 (*t*, *J* = 7.1, 3 H); 1.23 (*t*, *J* = 7.2, 3 H). ^{13}C-NMR ($CDCl_3$ 300 MHz): 172.2; 171.4; 170.6; 104.5; 96.3; 76.3; 62.7; 62.3; 61.01; 60.94; 49.6; 32.5; 29.4; 29.0; 25.2; 24.6; 18.5; 17.8; 14.49; 14.48. EI-MS: 228, 199, 168 (100, 154, 111, 98.

Data of **3a**: M.p. 74–76°. $[\alpha]_D^{25} = +77.4$ (*c* = 1.0, $CHCl_3$). Anal. calc. for $C_{21}H_{32}O_9$: C 58.87, H 7.53; found: C 59.00, H 7.78.

Data of **3b**: M.p. 74–76°. $[\alpha]_D^{25} = -77.8$ (*c* = 1.0, $CHCl_3$). Anal. calc. for $C_{21}H_{32}O_9$: C 58.87, H 7.53; found: C 58.79, H 7.80.

5. *Lactones of (2*R*,3*S*)- and (2*S*,3*R*)-2-Methylisocitrate (=2*R*,3*S*)- and (2*S*,3*R*)-Tetrahydro-2-methyl-5-oxofuran-2,3-dicarboxylate;* **4a** and **4b**, resp.). A soln. of **3a** or **3b** (100 mg, 0.25 mmol) in 6M HCl (20 ml) was refluxed for 3 h, cooled, and allowed to stand at r.t. overnight. The H_2O and HCl were evaporated. The residual oil was taken up in H_2O and evaporated 3 times to remove traces of HCl. The obtained yellow oil was crystallised from acetone/benzene: **4a** or **4b** (41 mg, 91%). Colourless crystals. ^{1}H-NMR ((D_6) acetone, 300 MHz): 3.63 (*dd*, ^3J = 10.6, 9.7, 1 H); 3.06 (*dd*, ^2J = 17.6, ^3J = 10.6, 1 H); 2.82 (*dd*, ^2J = 17.6, ^3J = 9.7, 1 H); 1.79 (*s*, 3 H). ^{13}C-NMR ((D_6) acetone, 300 MHz): 174.9; 171.6; 171.3; 85.4; 51.1; 32.8. MS-EI: 189 ($[M+1]^+$), 143, 115, 98, 55, 43 (100).

Data of **4a**: M.p. 142–144°. $[\alpha]_D^{25} = -25.0$ (*c* = 1.0, acetone).

Data of **4b**: M.p. 140–142°. $[\alpha]_D^{25} = +25.3$ (*c* = 1.0, acetone). Anal. calc. for $C_7H_8O_6$: C 44.69, H 4.29; found: C 45.09, H 4.68.

6. *X-Ray Crystallography.* Crystals of **3a** and **3b** were examined on a *Bruker SMART-CCD* diffractometer at 150 K, with Mo-*Kα* radiation (*λ* 0.71073 Å). Results for **3a** are presented here. Crystal data: $C_{21}H_{32}O_9$, *M* 428.5, monoclinic, space group *C*2, *a* = 25.7317(14), *b* = 8.3941(4), *c* = 10.3023(5) Å, *β* = 93.712(2)°, *V* = 2220.57(19) Å^3, *Z* = 4; *R* = 0.037 for refinement of 275 parameters from 2810 unique data. In the absence of significant anomalous scattering effects, *Friedel* pairs were merged. The crystallographic results for **3b** are essentially identical within experimental error, since the absolute configuration is undetermined[1]).

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[1]) CCDC-220257 and -220256 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/conts/retrieving.html (or from the *Cambridge Crystallographic Data Centre*, 12 Union Road, Cambridge CB2 1EZ; fax +44 1223 336033; e-mail deposit@ccdc.cam.ac.uk).

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