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Stereoselective Routes to Chiral 2-Hydroxy-4-oxo Acids and Substituted 2-Hydroxybutyrolactones using Lactate Dehydrogenases

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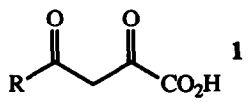
Key Words: Lactate dehydrogenase; 2,4-Dioxo acids; 2-Hydroxy-4-oxo acids; 2-Hydroxybutyrolactones

Abstract: The enantioselective reduction 2,4-dioxo acids catalysed by lactate dehydrogenases provides access to 2-hydroxy-4-oxo acids of both *S* and *R* configuration. Subsequent diastereoselective chemical reduction affords 4-substituted 2-hydroxybutyrolactones.

The convergent synthesis of complex biologically important compounds in enantiomerically pure form frequently relies on the availability of polyfunctional chiral building blocks. Efficient routes to such precursors can often be accomplished by the use of enzymes, which enable selective transformations to be effected under mild conditions and without resort to circuitous protection and deprotection of individual functional groups. For example, the use of lactate dehydrogenases in conjunction with reagents for cofactor recycling provides a practical method for the enantioselective synthesis of both *S*- and *R*-2-hydroxy acids.[1]



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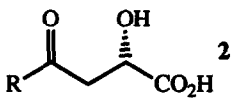

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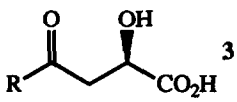

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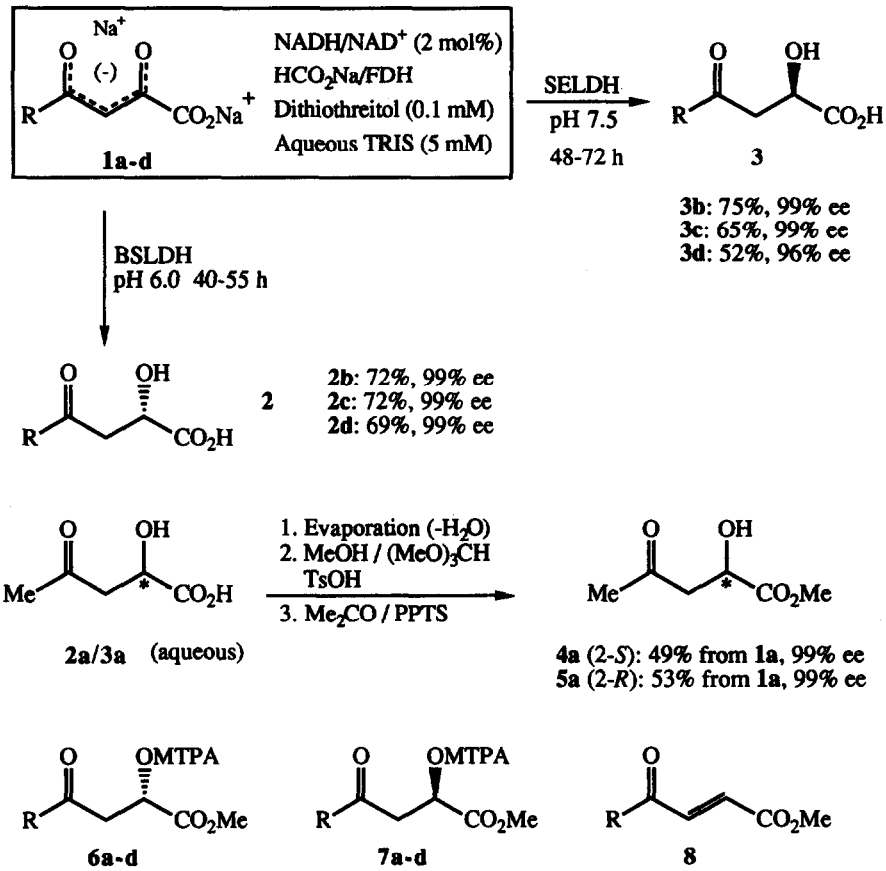
This letter discloses a novel application of this methodology, the impetus for which was a 1950 kinetic study by Meister,[2] concerned with the reduction of 2,4-dioxoalkanoic acids **1** catalysed by the *S*-LDH isolated from beef heart. The major finding of this work was that the deleterious effect of increased chain length on catalytic rate was significantly less pronounced with these compounds than that observed in the corresponding series of compounds lacking the C-4 carbonyl group. To illustrate the synthetic potential of this phenomenom, it can now be reported that a structurally diverse range of 2,4-dioxo acids can be reduced on a preparative scale using *S*-LDH from *Bacillus stearothermophilus* (BSLDH) to provide (*S*)-2-hydroxy-4-oxo acids **2** in high optical purity. Furthermore, for all the substrates investigated, complementary routes to the opposite enantiomers **3** can be effected using *R*-LDH from *Staphylococcus epidermidis* (SELDH). Although alternative enzyme-based routes to both cyclic[3] and acyclic[4] 2-hydroxy-4-oxo acid derivatives have been reported, these do not usually provide access to both enantiomers of a target molecule and the stereocontrol is variable. Despite containing a versatile array of functionality, 2-hydroxy-4-oxo acids have received only limited attention as synthetic intermediates.[5] As an initial foray into this area, a preliminary study of chemical reduction of enzyme-derived products is also described.

The dioxo acids employed as enzyme substrates were readily prepared as the disodium salts **1a-d** by a two-step literature procedure[6] commencing from diethyl oxalate and the appropriate methyl ketone. Reductions of substrates **1a-d** catalysed by BSLDH were carried out on a 2-15 mmol scale at pH 6.0, using the formate

dehydrogenase/formate method[7] for continuous cofactor regeneration *in situ* (scheme 1). A similar protocol was employed for reductions using SELDH at pH 7.5. Once complete reduction was observed, the 2-hydroxy-4-oxo acids **2b-d** and **3b-d** were isolated by acidification to pH 2 followed by extractive work-up with ethyl acetate.[8] In contrast, attempts to isolate (*S*)- and (*R*)-2-hydroxy-4-oxopentanoic acid (**2a** and **3a**) by this method failed. However, the corresponding methyl esters **4a** and **5a** could be obtained by removal of water *in vacuo* from the acidified reaction mixture prior to acid-catalysed methanolysis.[9]

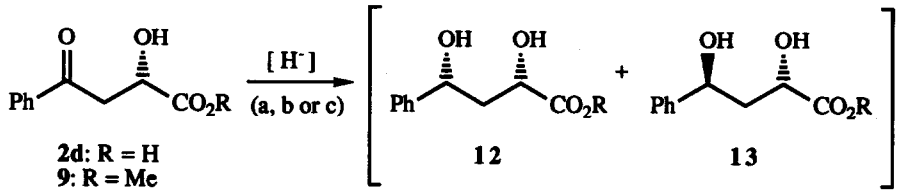


Scheme 1 **a**: R = Me; **b**: R = *i*-Pr; **c**: R = *n*-C_6H_{13}; **d**: R = Ph

^{1}H NMR analysis of the (*R*)-MTPA (Mosher[10]) derivatives **6a-d** and **7a-d** was used to measure the enantiomeric purity of each product, which was uniformly high.[11] Despite the reported propensity of these derivatives to undergo facile β-elimination,[4] contamination of compounds **6** and **7** with the alkenic by-products **8** was limited to 1-5% (w/w) and did not interfere with NMR measurements. In each case, chemical shift differences between diastereoisomers[12] were entirely consistent with the expected absolute configuration at C-2 (as represented in scheme 1), according to the correlation models of Mosher[10] and Yamaguchi.[13] However, it remains a key objective of future work to provide unambiguous evidence for configurational assignments, using either chemical or crystallographic methods.

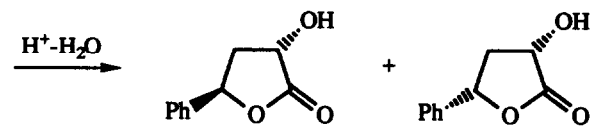
Preliminary investigation of the synthetic utility of these products has focused on hydride reductions of compound **2d** and the corresponding methyl ester **9** (scheme 2). Simple chemoselective reduction of the C-4 carbonyl group was effected using sodium borohydride in aqueous alkali (reagents a). Acidification to pH 2

followed by extraction afforded a diastereoisomeric mixture of the 2-hydroxy lactones **10** and **11**,[14] arising from cyclisation of the intermediate *syn*- and *anti*-diol derivatives **12** and **13** respectively. Compounds **10** and **11** were were isolated in a combined yield of 79% and were separated by column chromatography. The assignment of relative stereochemistry was made on the basis of ^{1}H NMR measurements, including an N.O.E. enhancement of 4% between signals for the C-2 methine hydrogen and aromatic protons of compound **10**, which was absent from the spectrum of compound **11**.[15] In an evaluation of established methods[16] for diastereoselective reduction of β-hydroxy ketone derivatives, excellent *syn*-selectivity (reagents b) was observed in the reaction of compound **2d**. A reversal in this selectivity, albeit to a modest level, was achieved by reacting compound **9** under Evans' conditions (reagents c),[16b] prior to sequential treatment with aqueous alkali and dilute acid to effect ester hydrolysis and lactonisation.



2d: R = H
9: R = Me

12 **13**



10 **11**

(a) **2d**; $NaBH_4$ / NaOH / H_2O	2	:	3	79%
(b) **2d**; 3 eq. DIBAL-H / THF -78°C	50	:	1	65%
(c) **9**; 1. Me_4N^+ $(AcO)_3BH^-$ AcOH / MeCN -20°C 2. NaOH / H_2O	1	:	6	90%

Scheme 2

In summary, enzymatic reduction of 2,4-dioxo acids provides the basis for versatile stereoselective routes to 2-hydroxy-4-oxo acids and substituted 2-hydroxybutyrolactones. These results suggest that a broad range of dioxo acid salts, exemplified by compounds **1a-d**, could be readily converted to any one of four diastereoisomeric lactones, depending on the choice of conditions employed for enzymatic (*S*- or *R*-LDH) and chemical (*syn*- or *anti*-selective) reduction. Studies are currently underway to investigate alternative chirality-transfer reactions of 2-hydroxy-4-oxo acids.

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8. $[\alpha]_D^{24}$ values (*c*, Me_2CO): **2b**, -14.8° (2.24); **3b**, 14.2° (2.08); **2c**, -9.3° (2.39); **3c**, 9.4° (2.05); **2d**, -1.6° (2.08); **3d**, 1.5° (2.58); **4a**, -11.6° (3.35); **5a**, 11.3° (3.22). Melting points: **2b**, 67-68°C; **2c**, 69-70°C; **2d**, 132-134°C. Spectroscopic data for **4/5a** and the methyl esters of **2/3b,d** have been reported previously.[17] Data for **2/3c**: υ_{max} (nujol) 3508 and 1704 cm^{-1}; δ [270 MHz, $(CD_3)_2CO$] 4.55 (1H, dd, J 4.6 and 6.9 Hz, C-2-H), 2.96-2.79 (2H, m, C-3-H_2), 2.53-2.48 (2H, m, C-5-H_2), 1.57-1.49 (2H, m, C-6-H_2), 1.34-1.27 (6H, m, C-7,8,9-H_2) and 0.88 (3H, t, J 6.6 Hz, C-10-H_3); δ_C (67.9 MHz) 207.55 (C-3), 174.80 (C-1), 66.79 (C-2), 46.32 (C-3), 42.95 (C-5), 31.70, 28.79, 23.39, 22.49 and 13.65 (remainder); m/z 202 (1%; M^+), 157 (3%) and 114 (100%).
9. This also resulted in acetalisation of the C-4 carbonyl group. Treatment with acetone in a separate operation was required to effect a reversal of this process.
10. J. A. Dale and H. S. Mosher, *J. Am. Chem. Soc.* **1973**, *95*, 512.
11. Minimum % ee figures are quoted, based on an NMR detection limit of ca. 0.5% for the minor diastereoisomer present in a mixture.
12. In the spectra of each pair of diastereoisomers, the two methoxy signals for compound **6** appear upfield relative to those for compound **7**. Diagnostic ^1H NMR (270 MHz; $CDCl_3$) chemical shifts for CO_2Me protons: 3.75 (**6a-c**), 3.78 (**6d**), 3.80 (**7a**), 3.79 (**7b,c**) and 3.83 (**7d**); $MeOCCF_3$ protons: 3.54 (**6a**), 3.52 (**6b**), 3.53 (**6c,d**) and 3.64 (**7a-d**).
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14. Spectroscopic data: **10**: υ_{max} (film) 3422 br and 1776 cm^{-1}; δ [$(CD_3)_2CO$] 7.42-7.34 (5H, m, Ph), 5.72-5.68 (1H, m, C-4-H), 5.25 (1H, d, J 4.8 Hz, OH), 4.57-4.50 (1H, m, C-2-H), 2.67-2.46 (2H, m, C-3-H_2); δ_C 176.3 (C-1), 140.2, 128.9, 128.4, 125.7 (Ph), 78.9, 67.4 (C-2 and C-4) and 39.2 (C-3); m/z 178 (M^+; 6%), 134 (89%), 105 (39%) and 92 (100%). **11**: υ_{max} (nujol) 3369 br and 1762 cm^{-1}; δ [$(CD_3)_2CO$] 7.45-7.33 (5H, m, Ph), 5.43 (1H, dd, J 5.4 and 10.9 Hz, C-4-H), 5.12 (1H, d, J 5.5 Hz, OH), 4.78 (1H, ddd, J 5.5, 8.1 and 11.2 Hz, C-2-H), 3.01 (1H, ddd, J 5.3, 8.1 and 12.3 Hz, C-3-H_β) and 2.18-2.05 (1H, m, C-3-H_α); δ_C 176.45 (C-1), 139.5, 128.8, 128.7, 126.1 (Ph), 76.8, 68.7 (C-2 and C-4) and 40.15 (C-3); m/z 178 (12%; M^+),134 (68%) and 92 (100%).
15. Irradiation of C-4-H in compound **10** gave rise to an unexpected, albeit small (1%), enhancement of C-2-H. For compound **11** a larger enhancement (7%) was observed.
16. (a) S. Kiyooka, H. Kuroda and Y. Shimasaki, *Tetrahedron Lett.* **1986**, *26*, 3009; (b) D. A. Evans, K. T. Chapman and E. M. Carriera, *J. Am. Chem. Soc.* **1988**, *110*, 3560.
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