Reduction of 2-Substituted 3-Oxoglutarates Mediated by Baker's Yeast. Variation in Enantioselectivity without Corresponding Variation in Diastereoselectivity

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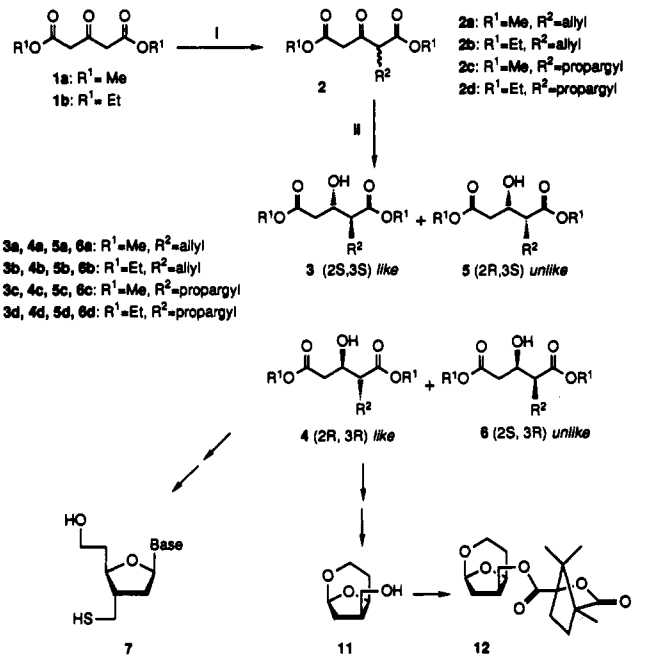
The reduction of 2-substituted 3-oxoglutarates by yeast yields a new class of chiral building blocks, 2-allyl- and 2-propargyl-3-hydroxyglutarates. These are useful as starting points for the synthesis of, inter alia, branched chain analogs of sugars and nucleosides. When allyl is the side chain, the principal product has the absolute configuration (2*S*,3*S*), proven by correlation with a compound whose absolute configuration was established by crystallography. Several features of this yeast-mediated reduction are noteworthy. First, its diastereoselectivity is higher than its enantioselectivity, especially with the propargyl side chain. Further, with all substrates, variation in enantioselectivity is not manifested by a variation in diastereoselectivity. This example therefore serves as a warning for those using yeast-mediated reactions that diastereoselectivity cannot be accepted as a substitute for direct measurements of enantioselectivity, even with analogous substrates and similar reaction conditions. Finally, an unexpected metabolism of impurities in the starting material by the yeast made the overall transformation preparatively useful.

Introduction

Yeast-dependent reactions are widely used to prepare functionalized chiral building blocks for the synthesis of natural and unnatural products.[1] The low expense, environmental friendliness, and ease of use of yeast as a reagent are its principle advantages. The principal disadvantage arises from the fact that yeast contains several (and perhaps many) enzymes that can reduce exogenously added substrates[2] with different stereo- and regiospecificities. This makes it difficult to predict the absolute configurations of products obtained from yeast-mediated reductions in all but the best precedented cases.[3,4] This, in part, explains why many organic chemists are reluctant to try yeast-dependent reactions as part of target-oriented synthetic work.

For example, we have for some time been interested in the synthesis of enantiomerically pure branched-chain nucleosides[5] as building blocks (7) for the synthesis of oligonucleotide analogues bearing dimethylenesulfone groups instead of phosphodiester linkages.[6] A retrosynthetic analysis suggested that these molecules might be obtained via glycosylation of the branched-chain sugar analogs generated from 2-substituted-3-hydroxyglutarates (3–6) obtained via yeast-mediated reduction of the appropriate 2-substituted-3-oxoglutarates (2)[7] (Scheme I). We did not, however, have extensive experience in microbial transformations.

Scheme I[a]



[a] (i) $CH_2{=}CHCH_2Br$, $NaOR_1$, R_1OH, rt; or $HCCHCH_2Br$, $NaOR_1$, R_1OH, rt (28% yield); (ii) nonfermenting baker's yeast, H_2O (see Table II).

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(5) For some recent reports on the synthesis of the branched-chain nucleoside analogues, see: (a) Pudlo, J. S.; Townsend, L. B. *Tetrahedron Lett.* **1990**, *31*, 3101. (b) Haraguchi, K.; Tanaka, H.; Itoh, Y.; Miyasaka, T. *Tetrahedron Lett.* **1991**, *32*, 777. (c) Madsuda, A.; Takenuki K.; Sasaki, T.; Ueda, T. *J. Med. Chem.* **1991**, *34*, 234. (d) Svansson, L.; Kvarnstrom, I.; Classon, B.; Samuelsson, B. *J. Org. Chem.* **1991**, *56*, 2993. (e) Lavallee, J. F.; Just, G. *Tetrahedron Lett.* **1991**, *32*, 3469 and references cited therein.

(6) (a) Huang, Z.; Schneider, K. C.; Benner, S. A. *J. Org. Chem.* **1991**, *56*, 3869. (b) Schneider, K. C.; Benner, S. A. *Tetrahedron Lett.* **1990**, *31*, 335.

(7) (a) To the best of our knowledge, there has not been any report on the microorganism-mediated reduction of 2-substituted-3-oxoglutarates. For the reduction of unsubstituted 3-ketoglutarates, see Ogura, K.; Lihama, T.; Kiuchi, S.; Kajiki, T.; Koshikava, O.; Takahashi, K.; Lida, H. *J. Org. Chem.* **1986**, *51*, 700. (b) For the synthesis of the chiral unsubstituted 3-hydroxyglutaric acid monoesters using hydrolytic enzymes, see Monteiro, J.; Braun, J.; Le Goffic, F. *Synth. Commun.* **1990**, *20*, 315.

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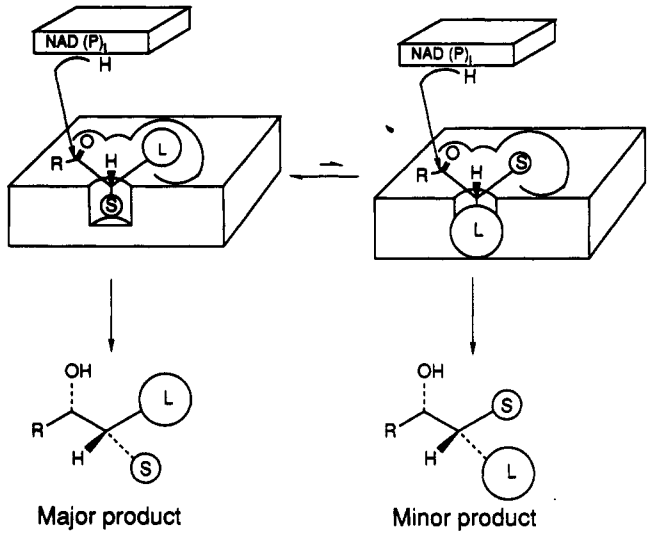


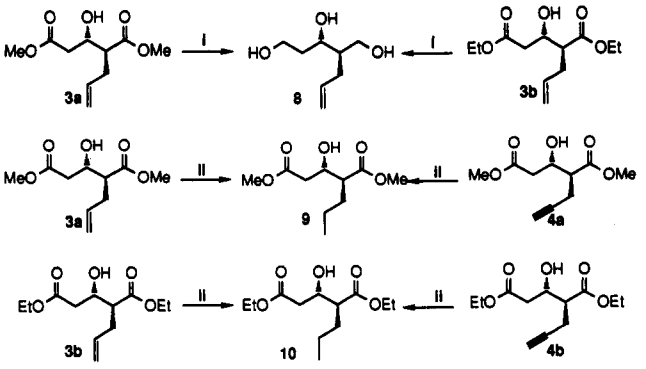
Figure 1. The Vanmiddlesworth–Sih adaptation of the Prelog model for predicting the stereospecificity of yeast-mediated reduction of 2-L = COOMe or COOEt; S = side chain, R = CH_3 (Vanmiddlesworth–Sih), R = COOMe or COOEt (this work).

A model for yeast-mediated reduction of 3-keto esters developed by Vanmiddlesworth and Sih[8] for predicting the absolute configuration of the α-substituted β-hydroxy esters arising from a yeast-catalyzed reduction of the corresponding α-substituted β-keto esters was the most relevant available model for anticipating the stereochemical course of the yeast-catalyzed reduction of such substrates. The parent structure for this system is shown in Figure 1. However, the model is rendered ambiguous by the simple modification of the parent structure to make R = COOMe, as either of the two ester moieties in the oxoglutarate substrate could serve as the "reference" ester in the model. Therefore, we explored the yeast-mediated reduction of 2-allyl-3-oxoglutaric acid and 2-propargyl-3-oxoglutaric acid methyl and ethyl esters. The results expand the predictive power of the Vanmiddlesworth–Sih model. They also provide an unusual example of a yeast-mediated reduction where the *enantioselectivity* but not the *diastereoselectivity* of the reaction is strongly dependent on reaction conditions.

Results and Discussion

Substrates **2a–d** were prepared by reacting the appropriate diester of 3-oxoglutaric acid (**1**) with allyl bromide (for **2a** and **b**) or propargyl bromide (for **2c** and **d**), using the corresponding sodium alkoxide in the appropriate alcoholic solvent (Scheme I).[9] The products of the alkylation reaction were contaminated with substantial amounts of the starting material and dialkylated products; as discussed below, this did not limit the usefulness of the yeast reduction. Crude substrates (ca. 0.27 M) were incubated at 40 °C in four portions in Erlenmeyer flasks (2 L) with baker's yeast (Migros, prepared at the factory at Krauchthalstrasse 2, 3324 Hindelbank, Bern, Switzerland, 500 g) suspended in water (800 mL) with vigorous shaking (200 rpm).

Scheme II[a]



[a] (i) $LiAlH_4$, Et_2O, rt; (ii) H_2, Pd–$BaSO_4$.

The progress of the reaction[10] was followed by GC/MS; diastereoselectivities were determined by capillary gas-liquid chromatography of the crude reaction product. The principal diastereomers of the products of each reaction were obtained pure by flash-chromatography[11,12] and the enantiomeric purities were determined by either ^{19}F-NMR spectroscopy of the Mosher derivative,[13] ^1H-NMR spectroscopy in the presence of Eu(hfc),[14] or capillary GC using a chiral stationary phase.[15]

To assign the absolute configuration of the major diastereomer of the reduction, **3a** was converted to a derivative of (1*S*)-(−)- camphanic acid (Scheme II)[16] and the crystal structure solved by X-ray diffraction.[17] The absolute configuration of the remaining compounds was established by correlation with **3a**, either via reduction ($LiAlH_4$ in ether) to the corresponding triols[18] or via reduction to 2-propyl-3-hydroxglutarates.

The results of yeast-mediated reduction of **2** are collected in Table I. Yeast converted **2a** to **3a** as the primary product (Scheme I). The configuration of the product correlates with that of products obtained by yeast-mediated reduction of ethyl 2-allylacetoacetate, the compound for which the Vanmiddlesworth–Sih model directly applies, where the methyl group of the latter correlates with the COOMe group of the former. The diastereoselectivity (94:6) is, however, higher than with ethyl 2-allylacetoacetate (75:25), indicating a higher preparative value of this reaction

(8) Vanmiddlesworth, F.; Sih, C. J. *Biocatalysis* **1987**, *1*, 117.

(9) A variety of conditions were examined in an effort to obtain monoalkylated products, but a mixture of 2-substituted-3-oxoglutarate, 2,4-dialkylated-3-oxoglutarate, and starting material was invariably obtained. Neither flash chromatography nor distillation proved to be suitable for separation of these on large scales.

(10) During the reduction of diethyl 2-allyl-3-oxoglutarate, GC/MS analysis showed that after 6 h, almost no diethyl 2-allyl-3-oxoglutarate (**2b**) and no diethyl 2,4-diallyl-3-oxoglutarate remained in solution. Reduction product **4b** came back slowly to the solution first after about 27 h.

(11) With the methyl ester, diastereomeric excess depends on the concentration of starting material; the lower is the concentration of starting material, the higher is diastereoselectivity.

(12) The minor diastereomers of the reduction product were not obtained in pure form.

(13) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512.

(14) (a) Fraser, R. R. In *Asymetric Synthesis*; Morrison, J. D. Ed.; Academic Press: New York, **1983**; Vol *1*, p 173. (b) Yamaguchi, S. In *Asymetric Synthesis*; Morrison, J. D., Ed.; Academic Press: New York, **1983**, Vol. *1*, p 125.

(15) Askari, C.; Hener, U.; Schmarr, H. G. Rapp, A.; Mosandl, A. *Fresenius Z. Anal. Chem.* **1991**, *340*, 768.

(16) Via ozonolysis, work up in methanol with Me_2S to yield the internal methoxy acetal, reduction with $LiAlH_4$, protection of the CH_2CH_2OH group as a *tert*-butyldiphenylsilyl ether, benzoylation of the remaining OH group, removal of the TBDPS group with fluoride, formation of the bicyclic acetal under acidic conditions, removal of the benzoyl group under basic conditions, and reaction with camphanic chloride.

(17) Arslan, T.; Schweizer, W. B.; Herradon, B.; Benner, S. A., manuscript in preparation.

(18) Triol **8** from each reaction was contaminated with aluminum salts, which introduced an error into the magnitude, but not the sign, of the specific rotation.

Table I. Products of Yeast-Mediated Reduction of 2-Substituted-3-Oxoglutaric Esters

substrate	diastereomeric ratio[a] (3/5)	enantiomeric excess (%)	yield[b] (%)
2a	94:6	>93[c,d]	44
2b	97:3	>92[e]	50
2c	85:15	>93[f]	35
2d	98:2	54[d,e]	25

[a] Determined by capillary gas-liquid chromatography. [b] Isolated yield of the major diastereomers. [c] Determined with ^{19}F-NMR spectroscopy of the Mosher ester.[13] [d] Determined by ^{1}H-NMR spectroscopy in the presence of Eu(hfc)$_3$.[14] [e] Determined by capillary gas-liquid chromatography using a column coated with perethylated β-cyclodextrin[15] (see Experimental Section). [f] Optical rotations value compared with **3a** (see Scheme II).[18]

Table II. Variation in Enantiomeric Excess

substrate	yeast/ substrate ratio (w/w)	diastereomeric ratio (3/5)	enantiomeric excess (%)	temp (°C)	time (h)
2d	0.006	90:10	33	30	60
2d	0.012	98:2	60	30	18
2d	0.024	99:1	54	30	60
2b	0.008	95:5	58	30	20
2b	0.012	92:8	71	30	20
2b	0.020	94:6	74	30	45
2b	0.020	97:3	92	40	45
2b	0.012	*b*	*b*	30[a]	72

[a] The yeast suspension was shaken at 60 °C for 1 h and cooled 30 °C before adding the substrates. The progress of the reaction was followed with GC/MS. [b] Only diethyl 3-hydroxyglutarate was detected as the sole product in solution.

with oxoglutarates as substrates.[19] The ethyl ester **2b** is reduced with still higher diastereoselectivity.

Repeated runs under various conditions (temperature, ratio of yeast to substrate, shaking rate, and incubation time) showed these diastereoselectivities to be relatively constant (93–97%). Remarkably, however, the enantiomeric excess of the products from **2b** was extremely variable (between 25–95%, results of 30 experiments) under the same set of conditions (Table II). While these findings might be explained in several ways, they are consistent with the presence of several different reductases in yeast with different stereoselectivities and different thermal stabilities or induction properties.[20]

A wide variation in enantioselectivity without a correspondingly large variation in diastereoselectivity in yeast-mediated reductions is not often seen. Indeed, some investigators rely primarily on diastereomeric ratios to characterize the products of yeast reductions, apparently assuming that diastereomeric ratios will always vary more than enantiomeric ratios in compounds with two or more stereogenic centers. These results suggest that assignments of structures to the products of yeast-mediated reactions based on such assumptions and unsupported by direct structure analysis should be viewed with caution.[21]

The propargyl derivatives also yielded compounds with the (2*S*,3*S*) configuration as the major products. However, the enantioselectivity was quite low with the diethyl ester, despite a remarkably high diastereoselectivity. This again shows how minor variations in reaction conditions may produce large changes in enantioselectivity that may not be foreshadowed by a corresponding variation in diastereoselectivity.

Careful attention to experimental conditions made it possible to obtain high and reproducible enantioselectivity at 40 °C with **2b**.[22] An unexpected discovery further enhanced the preparative value of this procedure. Allylation of 1 typically gives **2b** contaminated with diethyl 3-ketoglutarate and diethyl diallyl-3-ketoglutarate. However, essentially no corresponding dialkyl hydroxyglutarate was isolated as a product. Analysis of the products[23] showed the presence of the methyl ester of 3-keto-2-allylhept-6-enoic acid, suggesting that primary fate of dimethyl 2,4-dialkylated glutarates was hydrolysis followed by decarboxylation. Similar products were not observed with diethyl 2,4-dialkylglutarates, suggesting that these were metabolized further by yeast. The adventitious metabolism of undesired impurities in the starting material makes this route especially convenient for the synthesis of the carbon skeleton for building blocks for oligonucleotide analogs incorporating dimethylene sulfides, sulfoxides, and sulfone groups.[7]

Experimental Section

All experiments requiring anhydrous conditions were performed under argon atmosphere. Reactions were done at room temperature rt unless otherwise indicated. All solvents were Fluka p.a and were used without purification unless mentioned otherwise. Diethyl ether was distilled from potassium/benzophenone. For shaking, an Infors HT RS-306 rotary shaking platform was used. The following temperature program was used for all GC/MS measurements; initial temp: 100 °C, rate: 20 °C/min, final temp: 250 °C.

Diethyl 2-Allyl-3-oxoglutarate (2b). NaOEt (72 g, 1 mol) was dissolved in EtOH (450 mL). This solution was added with a dropping funnel to a solution of diethyl 3-oxoglutarate (202 g, 1 mol) and allyl bromide (71.5 g, 50 mL, 590 mmol) at rt over a 2-h period. The solution was stirred overnight. The mixture was cooled to 0 °C. HCl (1 N, 840 mL) was added and the mixture extracted with ether (2 × 2 L). The organic phase was dried over $MgSO_4$. The solvents were evaporated in vacuo and the remaining ethanol removed under high vacuum to give a mixture of starting material, monoallylated product, and diallylated product (220 g) in a ratio of 1:1:1:5: TLC (CH_2Cl_2) R_f = 0.56 for title compound, 0.36 for starting material; GC/MS retention times (5.12 min for title compound, 3.95 min for starting material); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.27 (t, *J* = 7.2 Hz, 6 H), 2.60–2.66 (m, 2 H), 3.60 (dd, *J* = 15.8, 28.3 Hz, 2 H), 3.73 (t, *J* = 7.3 Hz, 1 H), 4.20 (q, *J* = 8.2, 16.3 Hz, 4 H), 5.02–5.16 (m, 2 H), 5.68–5.82 (m, 1 H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 196.9 (s), 169.9 (s), 168.5 (s), 133.9 (d), 117.6 (t), 61.6 (t), 61.4 (t), 58.3 (d), 48.3 (t), 31.9 (t), 14.0 (q); IR ($CHCl_3$) 3120, 2980, 1738, 1718, 1655, 1645, 1370, 1322, 1232, 1185, 1030, 925 cm^{-1}; MS *m/e* 243 (M^+ + 1), 197, 168, 150, 127, 109, 99, 81, 54, 43, 29.

Diethyl 2,4-Diallyl-3-oxoglutarate. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.26 (t, *J* = 7.2 Hz, 6 H), 2.52–2.67 (m, 4 H), 3.75 (dd, *J* = 7.0, 7.7 Hz, 1 H), 3.84 (t, *J* = 7.2 Hz, 1 H), 4.13–4.25 (m, 4 H), 4.99–5.14 (m, 4 H), 5.63–5.79 (m, 2 H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 198.3 (s), 168.3 (s), 134.3 (d), 117.5 (t), 61.7 (t), 58.1 (d), 48.3 (t), 32.0 (t), 14.1 (q). IR ($CHCl_3$) 3020, 2980, 1735, 1725, 1641, 1645, 1440, 1370, 1230, 1180, 1130, 1070, 995, 925 cm^{-1}; MS

(19) Frater, G.; Muller, U.; Gunther, W. *Tetrahedron* **1984**, *40*, 1269.
(20) Ward, P. O.; Young, S. C. *Enzyme Microb. Technol.* **1991**, *12*, 482.
(21) For example, it is reported that although ethyl or methyl esters of 2-allyl-3-oxobutanoic acid are reduced by yeast to mainly (2*R*,3*S*) *unlike* products, if allyl group is replaced with propargyl, yeast reduction give mainly the (2*S*,3*S*) *like* product. Our analysis suggests that all four substrates are transformed by yeast to *like* products. Nakamura, K.; Miyai, T.; Nagar, A.; Oka, S.; Ohno, A. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 1179.
(22) We are indebted to Dr. Andrew Roughton and Dr. Thomas Jenny for their experimental work reproducing these results.
(23) The fate of the glutarates and 2,4-diallylglutarates during the yeast-dependent reduction was followed by GC/MS. Isolable amounts of the methyl esters of 3-hydroxyglutaric acid and the methyl ester of 3-keto-2-allylhept-6-enoic acid were observed, together with trace amounts of the methyl ester of 2-allyl-3-hydroxybutyric acid.

m/e 283 ($M^+ + 1$), 254, 237, 208, 190, 155, 127, 109, 99, 81, 55, 41, 29, TLC (CH_2Cl_2) R_f = 0.76. GC/MS retention time: 6.03 min.

Dimethyl 2-Allyl-3-oxoglutarate (2a). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 2.60–2.66 (m, 2 H), 3.54–3.78 (m, 9 H), 5.04–5.15 (m, 2 H), 5.67–5.81 (m, 1 H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 196.8 (s), 169.1 (s), 167.0 (s), 133.8 (d), 117.8 (t), 58.3 (d), 52.6 (q), 52.4 (q), 48.2 (t), 32.0 (t); IR ($CHCl_3$) 3020, 2960, 1740, 1720, 1660, 1640, 1330, 1170, 995, 925 cm^{-1}; MS *m/e* 215 ($M^+ - 1$), 183, 154, 150, 113, 109, 101, 81, 69, 59, 55, 41, 29; TLC (ether:hexane, 3:2) R_f = 0.5. GC/MS retention time: 4.20 min.

Dimethyl 2,4-Diallyl-3-oxoglutarate (mixture of isomers):* ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 2.54 (t, J = 7.3 Hz, 4 H), 3.65 (s, 6 H), 3.64–3.83 (m, 2 H), 4.94–5.07 (m, 4 H); ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 199 (s), 198.5 (s), 169.1 (s), 169 (s), 134.4 (d), 134.4 (d), 118.1 (t), 117.8 (t), 117.7 (t)*, 115.1 (t)*, 58.2 (d), 58.0 (d)*, 52.6 (q), 52.5 (q)*, 52.1 (q)*, 47.3 (d)*, 33.2 (d)*, 32.5 (t), 32.1 (t), 29.5 (t)*, (*: minor isomer); MS *m/e* 223 ($M^+ - OCH_3$), 190, 162, 141, 135, 109, 61, 59; TLC (ether:hexane, 3:2) R_f = 0.76; GC/MS retention time 5.30 min.

Dimethyl 2-propargyl-3-oxoglutarate (2c): TLC (CH_2Cl_2) R_f = 0.59; GC/MS retention time 4.34 min; MS *m/e* 181 (M – OMe), 152, 139, 121, 84, 69, 59.

Dimethyl 2,4-dipropargyl-3-oxoglutarate: TLC (CH_2Cl_2) R_f = 0.76; GC/MS retention time 5.48 min; MS *m/e* 219 (M^+ – OMe), 179, 150, 139, 131, 111, 79, 59.

Diethyl 2-propargyl-3-oxoglutarate (2d): TLC (CH_2Cl_2) R_f = 0.54; GC/MS retention time 5.12 min; MS *m/e* 197 (M^+ – OEt), 166, 150, 127, 109, 61, 55.

Diethyl 2,4-dipropargyl-3-oxoglutarate: TLC (CH_2Cl_2) R_f = 0.63; GC/MS retention time 6.22 min; MS *m/e* 237 (M^+ – OEt), 206, 190, 155, 127, 109, 61, 55.

Diethyl (2*S*,3*S*)-2-Allyl-3-hydroxyglutarate (3b). Four portions of baker's yeast (500 g, Migros) each were suspended in tap water (800 mL) in Erlenmeyer flasks (2 L). The unseparated products (11 g) of the alkylation reaction was added to each flask and the mixture shaken (200 rpm) at 40 °C for 43 h. The reaction mixtures were separated centrifuged (7000 rpm, 20 min at 10–16 °C) to remove the yeast. The supernatants were extracted in portions with Et_2O (2 × 1.5 L). The Et_2O phases were combined and dried over $MgSO_4$. Solvents were evaporated in vacuo and any traces of volatile material removed under high vacuum. The residue was chromatographed (CH_2Cl_2:CH_3CN 8:2) to give **3b** (6.3 g, 26 mmol, 50%).

To determine enantiomeric ratios, samples of the crude mixture (0.5 mg) were dissolved in ether (1 mL) and injected onto a GC chiral column (perethylated β-cyclodextrin; in OV 1710: 40:60 CD 28; column length = 20.0 m, diameter = 0.27 mm). Under the following conditions (1.2 atm, initial temp 80 °C, rate 0.5 °C/min, final temperature 160 °C), retention times for the enantiomers were 62.33 min for **3b** and 63.13 min for **4b**. Using a slightly different column (in OV 1710 CD 27, length = 17.5 m, diameter = 0.27 mm) under slightly different conditions (0.8 atm initial temp 80 °C, rate 0.5 °C/min, final temperature 160 °C), retention times were 54.65 min for **3b** and 55.28 min for **4b**. Retention times on the second column of **3b** and **5b** corresponded closely to these values (54.72 min and 55.40 min from $NaBH_4$), TLC (CH_2Cl_2:CH_3CN 9:1) R_f = 0.69. GC/MS retention times: 4.23 min for diethyl-3-hydroxyglutarate, 5.38 for diethyl (2*R*,3*S*)-allyl-3-hydroxyglutarate (**5b**), and 5.45 min for diethyl (2*S*,3*S*)-2-allyl-3-hydroxyglutarate. Standards ($NaBH_4$ reduction of **2b**) for GC/MS shows that **5b** emerges after 5.36 min and **3b** after 5.42 min: ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.25 (t, J = 7.4 Hz, 6 H), 2.38–2.63 (m, 5 H), 3.30 (d, J = 5.3 Hz, 1 H), 4.14–4.23 (m, 5 H), 5.03–5.15 (m, 2 H), 5.69–5.83 (m, 1 H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 173.8 (s), 171.9 (s), 134.6 (t), 117.4 (t) 68.5 (d), 60.8 (t), 60.7 (t), 50.1 (d), 39.6 (t), 33.1 (t), 14.3 (q), 14.2 (q); IR ($CHCl_3$) 3680, 3520, 2980, 1725, 1375, 1180, 1025, 925 cm^{-1}; MS *m/e* 245 ($M^+ + 1$), 244 (M^+), 199, 181, 157, 128, 117, 100, 83, 71, 55, 43, 29.

Dimethyl (2*S*,3*S*)-2-Allyl-3-hydroxyglutarate (3a): $[\alpha]_D$ = –11.5 (*c* = 3.3, $CHCl_3$); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 2.31–2.64 (m, 5 H), 3.24 (br, s, OH), 3.70 (s, 3 H), 3.71 (s, 3 H), 4.18–4.24 (m, 1 H), 5.03–5.13 (m, 2 H), 5.67–5.81 (m, 1 H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 174.2 (s), 172.3 (s), 134.5 (d), 117.5 (t), 68.3 (d), 51.6 (q), 51.7 (q), 50.1 (d), 39.3 (t), 33.1 (d); IR ($CHCl_3$) 3690, 3515, 3005, 2950, 1735, 1440, 1200, 1185, 995, 825 cm^{-1}; MS *m/e* 218 (M^+), 217 ($M^+ - 1$), 185, 167, 143, 114, 103, 83, 71, 55, 43, 29, 15; TLC (ether:hexane. 3:2) R_f = 0.35. GC/MS retention times: 4.68 min for dimethyl (2*S*,3*S*)-2-allyl-3-hydroxyglutarate (**3a**), 4.48 min for dimethyl (2*R*,3*S*)-2-allyl-3-hydroxyglutarate (**5a**). To determine enantiomeric ratios, samples (0.5 mg) of the mixture were dissolved in ether (1 mL) and injected onto a chiral GC column (perethylated β-cyclodextrin in OV 1710: 40:60 CD 28; column length = 20.0 m, diameter = 0.27 mm; pressure = 1.2 atm, initial temp 80 °C, rate: 0.5 °C/min, final temperature 160 °C). Retention times were 44.05 min for **3a** and 44.90 min for **5a**.

Dimethyl (2*S*,3*S*)-2-propargyl-3-hydroxyglutarate (3c): ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 2.03 (t, J = 2.6 Hz, 1 H), 2.60–2.79 (m, 5 H), 3.75 (d, J = 9.6 Hz, 6 H), 4.39 (m, 1 H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 172.7 (s), 172.3 (s), 80.7 (d), 70.5 (d), 67.8 (s), 52.1 (q), 51.9 (q), 49.1 (d), 39.2 (t), 18.3 (t); IR ($CHCl_3$) 3680, 3540, 3020, 2950, 2120, 1730, 1440, 1170, 650 cm^{-1}; MS *m/e* 215 ($M^+ + 1$), 185, 167, 143, 112, 103, 81, 71, 59, 53, 43, 29, 15; TLC (ether:hexane, 3:2) R_f = 0.32; GC/MS retention times 4.62 min for dimethyl (2*S*,3*S*)-2-propargyl-3-hydroxyglutarate (**3c**) and 4.70 min for dimethyl (2*R*,3*S*)-2-propargyl-3-hydroxyglutarate (**5c**).

Diethyl (2*S*,3*S*)-2-propargyl-3-hydroxyglutarate (3d): $[\alpha]_D$ = –2.34 (*c* = 3.25, MeOH); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 1.29 (tt, J = 5.3, 7.1 Hz, 6 H), 2.03 (t, J = 2.6 Hz, 1 H), 2.58–2.76 (m, 5 H), 3.30 (d, J = 3.8 Hz, 1 H), 4.20 (qq, J = 7.1, 12.1 Hz, 4 H), 4.37 (m, 1 H); ^{13}C-NMR ($CDCl_3$, 75 MHz) δ 172.4 (s), 171.9 (s), 80.7 (d), 70.4 (s), 67.8 (d), 61.1 (t), 60.9 (t), 49.0 (d), 39.4 (t), 18.3 (t), 14.2 (q), 14.1 (q); IR ($CHCl_3$), 3680, 3540, 3300, 3020, 2980, 2120, 1730, 1375, 1180, 1095, 1020 cm^{-1}; MS *m/e* 243 ($M^+ + 1$), 197, 179, 157, 126, 117, 98, 81, 71, 53, 43, 29. TLC (CH_2Cl_2:CH_3CN, 9:1) R_f = 0.69; GC/MS retention times 5.57 min for diethyl (2*S*,3*S*)-2-propargyl-3-hydroxyglutarate (**3d**) and 5.47 min for diethyl (2*R*,3*S*)-2-propargyl-3-hydroxyglutarate (**5d**). To determine enantiomeric ratios, samples of the crude mixture (0.5 mg) were dissolved in ether (1 mL) and injected onto a chiral GC column (perethylated β-cyclodextrin; in OV 1710 40:60 CD 28; column length = 20.0 m, diameter = 0.27 mm). Under the following conditions (1.2 atm, initial temp 80 °C, rate: 0.5 °C/min, final temperature 160 °C), retention times for enantiomers were 74.29 min for **3d** and 76.48 min for **4d**.

Dimethyl 3-hydroxyglutarate: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.57 (d, J = 6.1 Hz, 4 H), 3.39 (d, 1 H), 3.72 (s, 6 H), 4.43–4.50 (m, 1 H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 172.2 (s), 64.7 (d), 51.9 (q), 40.5 (t); IR ($CHCl_3$) 3550, 3010, 2955, 1735, 1440 cm^{-1}; MS *m/e* 177 ($M^+ + 1$), 145, 127, 116, 103, 84, 71, 61, 43, 29, 18.

2-Allyl-3-oxohept-6-enoic acid methyl ester: ^{1}H-NMR ($CDCl_3$, 400 MHz) δ 2.30–2.36 (m, 2 H), 2.50–2.72 (m, 4 H), 3.56 (t, J = 7.4 Hz, 1 H), 3.72 (s, 3 H), 4.95–5.12 (m, 4 H), 5.68–5.86 (m, 2H); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ 209 (s), 169.6 (s), 136.7 (d), 134.2 (d), 117.6 (t), 115.5 (t), 58.4 (d), 52.3 (q), 41.4 (t), 32.2 (t), 27.4 (t); IR ($CHCl_3$) 3090, 3010, 2952, 1745, 1715, 1645, 1440, 925 cm^{-1}; MS *m/e* 196 (M^+), 164, 155, 141.1, 136, 123, 113, 83, 7, 55, 41, 27, 18.

(2*R*,3*S*)-2-Allyl-1,3,5-trihydroxypentanol (8). As a typical procedure for reduction, diester **3b** (32.1g, 132 mmol) in ether (250 mL) was added dropwise to a suspension of $LiAlH_4$ (10 g, 263 mmol, 2 equiv) in Et_2O (250 ml) at rt under argon and stirred overnight. The solution was cooled to –78 °C, saturated Na_2SO_4 (50 mL) and 1 N HCl (200 mL) were added dropwise giving a white precipitate. The mixture was diluted with THF (800 mL) and filtered through Celite. The organic phase was dried ($MgSO_4$) and the solvent removed by evaporation. The residue was chromatographed (CH_2Cl_2:EtOAc:MeOH 6:3:1), affording triol **8** (18 g, 112 mmol, 85%) as a colorless oil: $[\alpha]_D$ = –5.4 (*c* = 3.5, MeOH) from **3a** and $[\alpha]_D$ = –8.9 (*c* = 3.5, MeOH) from **3b**: ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 1.48–1.74 (m, 3 H), 1.95–2.24 (m, 2 H), 3.52–4.32 (2 m, 7 H), 4.62 (d, J = 4.2 Hz, 1 H), 4.96–5.06 (m, 2 H), 5.63–5.83 (m, 1 H); ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 136.7 (d), 117.1 (t), 74.9 (d), 63.9 (t), 61.6 (t), 44.8 (d), 36.5 (t), 33.1 (t); TLC (CH_2Cl_2–EtOAc–MeOH 6:3:1) R_f = 0.27; GC/MS retention time 4.72 min.

Dimethyl (2*S*,3*S*)-2-Propyl-3-hydroxyglutarate (9). As a typical procedure for hydrogenation, **3a** (100 mg, 0.467 mmol) and Pd–$BaSO_4$ (15 mg, 5%) in MeOH (3 mL) were treated with

hydrogen (1 atm) at rt for 1 h. The solids were removed by filtration and the solvents removed in vacuo. The residue was dried under high vacuum gave **9** (91.8 mg, 0.421 mmol) in 90% yield: $[\alpha]_D$ = −17.4 (c = 2.3, MeOH) from **3a**, and $[\alpha]_D$ = −17.6 (c = 2.4, MeOH) from **3d**; ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.92 (t, J = 7.1 Hz, 3 H), 1.24–1.74 (m, 4 H), 2.42–2.64 (m, 4 H), 3.18 (d, J = 2.6 Hz, 1 H), 3.72 (s, 6 H), 4.14–4.20 (m, 1 H); ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 175.5 (s), 172.8 (s), 69.1 (d), 52.1 (q), 51.9 (q), 50.6 (d), 39.6 (t), 31.2 (t), 20.7 (t), 14.0 (t).

Diethyl (2*S*,3*S*)-2-Propyl-3-hydroxyglutarate (10): $[\alpha]_D$ = −9.0 (c = 2.6, MeOH) from **3d** and $[\alpha]_D$ = −15.5 (c = 15.5, MeOH) from **3b**; ^{1}H-NMR ($CDCl_3$, 200 MHz) δ 0.92 (t, J = 6.7, 3 H), 1.28 (tt, J = 1.7, 7.2 Hz, 6 H), 1.36–1.78 (m, 4 H), 2.41–2.61 (m, 3H), 3.24 (d, J = 6.6 Hz, 1 H), 4.12–4.24 (m, 5 H); ^{13}C-NMR ($CDCl_3$, 50 MHz) δ 175.1 (s), 172.4 (s), 69.2 (d), 61.0 (t), 60.8 (t), 50.6 (d), 39.9 (t), 31.2 (t), 20.7 (t), 14.4 (q), 14.3 (q), 14.0 (q); IR ($CHCl_3$) 3680, 3540, 3020, 2950, 2120, 1730, 1440, 1170, 650; MS *m/e* 215 (M^+ + 1), 185, 167, 143, 112, 103, 81, 71, 59, 53, 43, 29, 15.

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