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Improved asymmetric syntheses of (*R*)-(−)-homocitrate and (2*R*,3*S*)-(−)-homoisocitrate, intermediates in the α-aminoadipate pathway of fungi

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Abstract

Improved asymmetric syntheses of the title compounds are reported. Both products are produced through diastereoselective alkylation of malic acid; (*R*)-homocitrate synthesis uses the self-regeneration of stereocenters approach. Both procedures represent an improvement in yield over existing methods without loss of stereoselectivity.

(*R*)-(−)-Homocitrate and (2*R*,3*S*)-homoisocitrate are natural products known to be intermediates in the α-aminoadipate pathway of lysine biosynthesis.[1] This pathway is found primarily in fungi, although it has recently been shown to be present in some archaea. Because this pathway is absent from plants and animals, it is considered a promising target for anti-fungal therapy.[2] Fungal infections are a severe medical problem, especially among immunocompromised patients such as AIDS sufferers, organ transplant recipients, and patients undergoing chemotherapy. The need for novel anti-fungal therapies has been expounded upon in recent reviews.[3] (*R*)-Homocitrate is also a compound of interest because it is a required component of the active site of the iron-molybdenum protein of nitrogenase.[4]

One aspect of the α-aminoadipate pathway is poorly understood.[1,5] Homocitrate is converted to homoisocitrate in a series of reactions apparently *in simile* with the conversion of citrate to isocitrate by the enzyme aconitase (Fig. 1). While the intermediates homocitrate, homoaconitate and homoisocitrate have been isolated from *Saccharomyces cerevisiae* mutants, which have been impaired in their ability to produce lysine, the process by which homocitrate is converted to homoisocitrate has been characterized so poorly that it is not clear whether one enzyme or two are involved. Our laboratory is currently investigating this portion of the pathway to resolve this issue. One of the factors which has confounded the study of this process is the lack of

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availability of the compounds shown in Fig. 1: only *racemic* homocitric lactone is available commercially (at >US$1000/g). Nitrogenase research has been similarly affected. While models of the FeMo protein active site continue to be investigated,[6] (*R*)-homocitrate, a required component of the enzyme's reaction center, has not been incorporated into any such models.

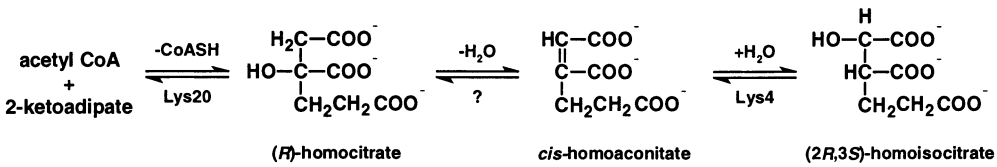
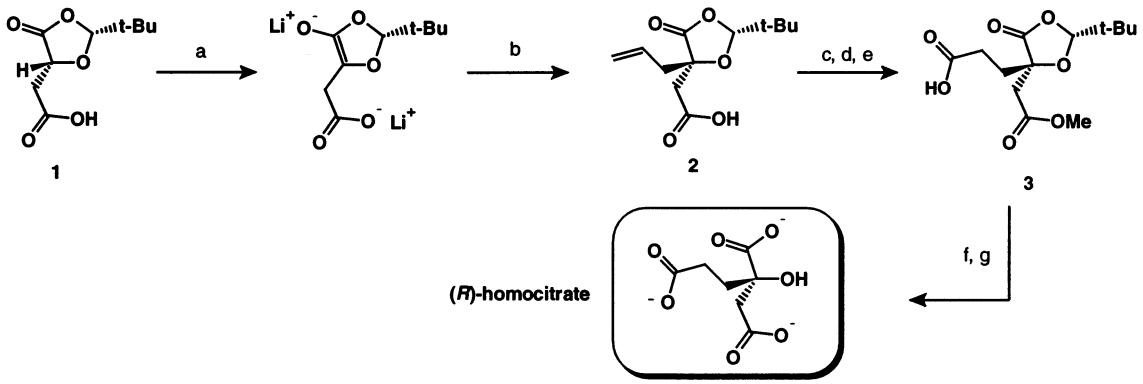


Figure 1. Initial steps of the α-aminoadipate pathway. Lys20 = homocitrate synthase, Lys4 = 'homoaconitate hydratase'. The enzyme catalysing the second step, *perhaps* Lys4, has not been identified experimentally.

Only one asymmetric synthesis of homocitric lactone has been published.[7,8] The initial steps of this synthesis were based on the work of Seebach,[9] using trimethylacetaldehyde as a protecting group to convert L-lactic acid to an asymmetrically hindered chiral alkene. This compound was then converted to (*R*)-(−)-homocitric lactone in four steps with a reported yield of 7.5% (3% from L-lactic acid). The key step in this scheme is a Diels–Alder addition which results in 92% ee.

We felt that the Seebach strategy might be more effective using a diastereoselective alkylation rather than cycloaddition. To this end, we pursued the procedure shown in Scheme 1. Trimethylacetal-protected D-malic acid (**1**) was synthesized and alkylated with allyl bromide.[9] The planar α-carbon of the dilithium enolate can be alkylated stereofacially (>95% diastereoselectivity) by virtue of the bulky protecting group. This is referred to as the self-regeneration of stereocenters (SRS) approach.[10] Esterification of the carboxylic acid, followed by hydroboration of the alkene with oxidative workup gave **3** in 25% yield from **2**. Deprotection gave the trisodium salt of (*R*)-homocitrate.[11] The overall yield from D-malic acid was 12%.



Scheme 1. (a) 2 equiv. of LiHMDS/THF, −78°C. (b) Allyl bromide. (c) AcCl, reflux 1 h; MeOH, reflux 4 h. (d) $BH_3{\cdot}SMe_2/CH_2Cl_2$, 25°C, 4 h. (e) Pyridinium dichromate/DMF, 25°C, 16 h. (f) 50% trifluoroacetic acid, 95°C, 2 h. (g) 3% NaOH, 1 equiv., 25°C, 2 h.

This method, using the SRS approach, represents a substantial improvement over the literature procedure. Considering the potential of SRS it is surprising how rarely this method has been reported by researchers other than Seebach himself.[12] Our results reinforce how practical the SRS approach can be for the synthesis of carboxyl-containing compounds. If low

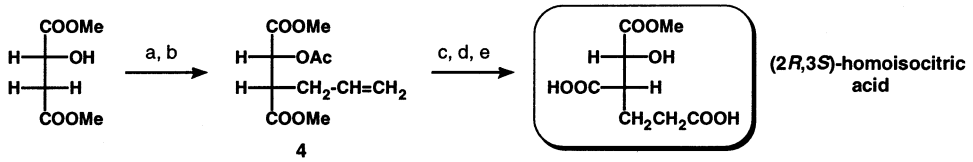
cost is a dominant criterion to enantiomeric purity, the cost of starting material can be defrayed by preparing D-malic acid from D-aspartic acid (about US$1.50/g).[13] Reaction of D-aspartic acid with HNO_2 proceeds with retention of configuration, an ee of >95%, and is purified from the side product 2-chlorosuccinic acid by separation of the dimethyl ester. We can isolate D-malic acid in 80% yield by this method.

It should be noted that the alkylation procedure is only suitable for active electrophiles. Seebach's procedure reports alkylations with methyl iodide, allyl bromide and benzyl bromide,[9] and these reactions have been successfully repeated in our laboratory. A more direct route to (*R*)-homocitrate was sought using less activated electrophiles 3-bromopropionitrile and 2-(2-bromoethyl)-1,3-dioxolane, but in our hands these electrophiles did not react with **1**.

(2*R*,3*S*)-Homoisocitrate is no longer commercially available.[14] The previously reported asymmetric synthesis of this compound[15] used a diastereoselective alkylation of dimethyl D-malate using trimethylsilylpropargyl bromide, resulting in a 51% yield of a 9:1 mixture of diastereomers, which were separated after acetylation by column chromatography. The alkyne was then treated with benzenesulfenyl chloride to give a vinylic addition product from which chlorotrimethylsilane was eliminated using fluoride ion. The resulting alkynyl thioether was cleaved in the presence of Hg^{2+} to give the carboxylic acid. Deprotection gave the desired product in an overall yield from dimethyl D-malate of 21%. In our hands, however, hydrolysis of the alkynyl thioether resulted in a complex mixture.

We have adapted this procedure by substituting allyl bromide for trimethylsilylpropargyl bromide in the alkylation step, as shown in Scheme 2. Note that allyl bromide is less than 1/50 the cost of the trimethylsilylpropargyl bromide, and reacts with the dianion of dimethyl malate with improved diastereoselectivity (19:1) and comparable yield (80%). This 3-allylmalic dimethyl ester was then acetylated in 91% yield to give **4**. Hydroboration of the alkene **4**, followed by deprotection gave the desired compound in an overall yield of 30% from dimethyl D-malate.[16]

In conclusion, we have synthesized two important natural products with high stereoselectivity and improved yields over literature methods. We have purposefully sought a unified approach to minimize the method development time for laboratories wishing to repeat both these preparations. We hope that improved syntheses of these compounds can have an immediate impact on research into the α-aminoadipate pathway of fungi and on the investigation of nitrogenase and nitrogenase models.



Scheme 2. (a) LDA/THF, −78°C; allyl bromide. (b) Ac_2O, Et_3N, DMAP. (c) $BH_3{\cdot}SMe_2/CH_2Cl_2$, 25°C, 2 h. (d) Pyridinium dichromate/DMF, 25°C, 16 h. (e) 2.5% KOH, 1 h; Dowex 50X8-100(H^+).

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References

1. Zabriskie, T. M.; Jackson, M. D. *Nat. Prod. Rep.* **2000**, 85–97.
2. Suvarna, K.; Seah, L.; Bhattacharjee, V.; Bhattacharjee, J. K. *Curr. Genet.* **1998**, *33*, 268–275.
3. See, for example: Alexander, B. D.; Perfect, J. R. *Drugs* **1997**, *54*, 657–678.
4. Hoover, T. R.; Imperial, J.; Ludden, P. W.; Shah, V. K. *Biochemistry* **1989**, *28*, 2768–2771.
5. Gamonet, F.; Lauquin, G. J.-M. *Eur. J. Biochem.* **1998**, *251*, 716–723.
6. Couvouvanis, D.; Konstantinos, D. D.; Chang, G. K.; Dunham, R. W.; Kampf, J. W. *J. Am. Chem. Soc.* **1993**, *115*, 2244–3345.
7. Rodriguez, G. H. R.; Biellmann, J.-F. *J. Org. Chem.* **1996**, *61*, 1822–1824.
8. Preparation of (*R*)-homocitrate by resolution of diastereomeric salts has also been reported, Ancliff, R. A.; Russell, A. T.; Sanderson, A. J. *Tetrahedron: Asymmetry* **1997**, *8*, 3379–3382.
9. Seebach, D.; Naef, R.; Calderari, G. *Tetrahedron* **1984**, *40*, 1313–1324.
10. Seebach, D.; Sting, A. R.; Hoffmann, M. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2708–2748.
11. Note that **1** and **2** are novel compounds only in the sense that they are enantiomers of compounds prepared in Ref. 9. Physical properties matched those reported previously, except for the direction of optical rotation. Compound **3** (oil): ^{1}H NMR ($CDCl_3$) $\delta = 10.91$, 5.05, 3.61, 2.78, 2.42, 2.20, 2.02, 0.87. ^{13}C NMR $\delta = 178.2$, 173.6, 168.7, 108.2, 79.4, 52.3, 39.1, 34.3, 28.3, 27.8, 23.7. $[\alpha]^{25}$ ($CHCl_3$, $c = 2.4$) $= -11.20°$. High resolution mass [M+1] calcd (found) for $C_{13}H_{20}O_7$: 289.1287 (289.1295). Physical properties of trisodium (*R*)-homocitrate and its corresponding lactone matched those previously reported.
12. Other applications reported: Juaristi, E.; Lopez-Ruiz, H.; Madrigal, D.; Ramirez-Quiros, Y.; Escalante, J. *J. Org. Chem.* **1998**, *63*, 4706–4710. Einhorn, J.; Einhorn, C.; Ratajczak, F.; Gautier-Luneau, I.; Pierree, J.-L. *J. Org. Chem.* **1997**, *62*, 9385–9386. O'Donnell, M. J.; Fang, Z.; Ma, X.; Huffman, J. C. *Heterocycles* **1997**, *46*, 617–630.
13. Winitz, M.; Bloch-Frankenthal, L.; Izumiya, N.; Birnbaum, S. M.; Baker, C. G.; Greenstein, J. P. *J. Am. Chem. Soc.* **1956**, *78*, 2423–2430.
14. Homoisocitrate was until recently available from Research Plus, Inc.
15. Schmitz, C.; Rouanet-Dreyfuss, A.-C.; Tueni, M.; Biellmann, J.-F. *J. Org. Chem.* **1996**, *61*, 1817–1821.
16. Compound **4** (oil): ^{1}H NMR ($CDCl_3$) $\delta = 5.65$ (m 1H), 5.15 (d, $J = 4.8$, 1H), 4.94–5.00 (m, 2H), 3.65 (s, 3H), 3.59 (s, 3H), 3.02 (m, 1H), 2.44 (m, 1H) 2.18 (m, 1H), 2.03 (s, 3H). ^{13}C NMR $\delta = 171.2$, 170.0, 169.2, 134.2, 118.3, 71.4, 52.6, 52.2, 46.3, 31.9, 20.5. $[\alpha]^{25}$ ($CHCl_3$, $c = 1.77$) $= +0.71°$. High resolution mass [M+1] calcd (found) for $C_{11}H_{16}O_6$: 245.1025 (245.1020). Physical properties of (2*R*,3*S*)-homoisocitric acid matched those previously reported.