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Efficient synthesis of the ketone body ester (R)-3-hydroxybutyryl-(R)-3-hydroxybutyrate and its (S,S) enantiomer

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ABSTRACT

The ketone body ester (R)-3-hydroxybutyryl-(R)-3-hydroxybutyrate and its (S,S) enantiomer were prepared in a short, operationally simple synthetic sequence from racemic β-butyrolactone. Enantioselective hydrolysis of β-butyrolactone with immobilized *Candida antarctica* lipase-B (CAL-B) results in (R)-β-butyrolactone and (S)-β-hydroxybutyric acid, which are easily converted to (R) or (S)-ethyl-3-hydroxybutyrate and reduced to (R) or (S)-1,3 butanediol. Either enantiomer of ethyl-3-hydroxybutyrate and 1,3 butanediol are then coupled, again using CAL-B, to produce the ketone body ester product. This is an efficient, scalable, atom-economic, chromatography-free, and low cost synthetic method to produce the ketone body esters.

1. Introduction

The ketone bodies (R)-β-hydroxybutyrate (βHB) and acetoacetate (AcAc) are produced from fatty acids in the liver under glucose deprivation conditions, such as fasting or adherence to a high fat/low carbohydrate "ketogenic" diet (Fig. 1). The brain relies on carbohydrate as a fuel source, so in its absence, ketone body production serves as an alternative, fat-derived fuel source for the brain that is converted to acetyl CoA and enter the citric acid cycle [1].

There is evidence that the ketogenic state is therapeutically useful to treat a variety of neurological diseases. The ketogenic diet is an effective and widely used epilepsy treatment [2]. Ketosis is being explored for a variety of other neurological conditions as well. The brain of patients with Alzheimer's disease shows impaired glucose uptake and utilization, which correlates with disease progression [3,4]. However, ketone body uptake and utilization is unaffected, making it reasonable to suspect that ketosis is useful to treat Alzheimer's disease [5]. Similarly, ketone bodies have been shown to be protective of neurons in Parkinson's disease [6,7]. Clinical trials of the ketogenic diet are ongoing for these and other medical conditions, including as an adjuvant to chemotherapy for cancer treatment [8].

Despite the utility and promise of the ketogenic diet, adherence to the diet is difficult. It is uncomfortable, has a social cost, and side effects. Ketone body esters (KE) are an alternative approach to induce ketosis. Ketone body esters are taken as nutritional supplements—they release ketone bodies directly after simple metabolic reactions, and avoid the salt/acid load if βHB or AcAc were ingested directly [9]. The most well-known and effective ketone body ester, (R)-3-hydroxybutyryl-(R)-3-hydroxybutyrate, was recently shown to safely induce serum ketone body concentrations similar to prolonged fasting or the ketogenic diet when taken orally by humans on a normal diet [10,11]. It is proposed that this KE is converted to two equivalents of βHB by hydrolysis of the ester followed by liver oxidation of the resulting (R)-1,3 butanediol, a known metabolic precursor of βHB (Fig. 2) [12].

Ketosis via ingestion of KEs produces a unique metabolic state in which glucose and ketone bodies, normally mutually exclusive, exist simultaneously in the bloodstream. This unusual metabolic state was shown to enhance human athletic performance. Indeed, it was found that ingestion of this KE improved performance in a cycling time-trial test compared to subjects who consumed the equivalent number of calories as carbohydrate [11]. This and similar ketone body esters also have significant promise to replace the ketogenic diet for treating the medical conditions that respond to ketosis.

The synthetic scheme by which this KE was originally made is a one-step, *Candida antarctica* lipase-B (CAL-B) mediated transesterification of (R)-ethyl-3-hydroxybutyrate with (R)-1,3 butanediol [13]. Our interest in ketone body esters led us to seek an efficient and scalable synthesis of this KE which begins with inexpensive, achiral starting materials. Considering that one dose of this KE in humans may be 20–30 g of material, cost becomes an issue for longitudinal studies of its effects on ketosis-treatable diseases, athletic performance, and the emerging signaling properties of βHB [14]. Synthesis of the (S,S) enantiomer is also of interest due to its less-well understood metabolism. It is plausible that it is also converted to the natural ketone bodies AcAc and (R)-βHB with different kinetics and may well be a useful tool to achieve ketosis.

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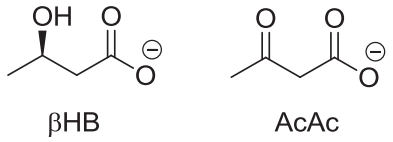


Fig. 1. Chemical structures of the ketone bodies β-hydroxybutyrate and acetoacetate.

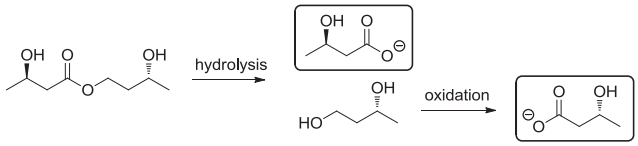


Fig. 2. Proposed metabolism of the KE (R)-3-hydroxybutyryl-(R)-3-hydroxybutyrate to two molar equivalents of βHB by ester hydrolysis and oxidation of 1,3 butanediol.

2. Results and discussion

Our synthetic approach towards (R)-3-hydroxybutyryl-(R)-3-hydroxybutyrate and its (S,S) enantiomer is based on having both halves of the ester and both stereocenters originate from racemic β-butyrolactone. It was previously reported that immobilized *Candida antarctica* lipase B (CAL-B) can enantioselectively trans-esterify racemic β-butyrolactone with methanol to give (S)-methyl-3-hydroxybutyrate and (R)-β-butyrolactone with quantitative conversion and > 99% ee [15]. We found that altering this protocol for enzymatic hydrolysis of racemic β-butyrolactone with immobilized CAL-B and 0.6 M equivalents of water in MTBE yielded (R)-β-butyrolactone and (S)-3-hydroxybutyric acid with identical enantioselectivity as the previously reported methanolysis reaction (Fig. 3).

Monitoring the reaction via chiral GC–MS showed that (S)-butyrolactone was converted to (S)-3-hydroxybutyric acid in < 8 h with considerably slower hydrolysis of the (R) enantiomer (Fig. 4).

Washing the reaction mixture with saturated aqueous sodium bicarbonate easily removed (S)-3-hydroxybutyric acid and allowed recovery of pure (R)-β-butyrolactone in high yield and > 99% ee. (S)-3-Hydroxybutyric acid can be recovered by acidifying the aqueous bicarbonate solution to pH 2 followed by continuous liquid-liquid extraction with diethyl ether. We found that both the MTBE solvent and CAL-B enzyme can be recycled.

2.1. Synthesis of (R)-3-hydroxybutyryl-(R)-3-hydroxybutyrate

With pure (R)-β-butyrolactone in hand, we found that it underwent smooth reduction with sodium borohydride in ethanol, unusual reactivity presumably due to ring strain. The resulting (R)-1,3 butanediol was purified by extraction into 2-propanol from water containing 25% K_2HPO_4 [16]. (R)-β-Butyrolactone was easily converted to (R)-ethyl 3-hydroxybutyrate by treatment with catalytic sulfuric acid in ethanol. The synthesis was completed using the previously reported transesterification of (R)-ethyl 3-hydroxybutyrate with (R)-1,3 butanediol catalyzed by CAL-B under reduced pressure and solvent-free conditions to yield the ketone body ester product (R,R)-3-hydroxybutyryl-3-hydroxybutyrate (Scheme 1) [13].

2.2. Attempted stereochemical inversion

We attempted to convert (S)-3-hydroxy butyric acid into (R)-ethyl-3-hydroxy butyrate, the starting material to generate the more valuable (R,R) ketone body ester. This route involves esterification, mesylation of the hydroxyl group followed by S_n2 inversion by cesium acetate (Scheme 2) [17]. In our hands, this route resulted in substantial amounts of the acrylate elimination product, requiring chromatography to recover the (R) ester. As such, these steps did not conform to our goal of accessing the ketone body ester product in an operationally simple, scalable manner.

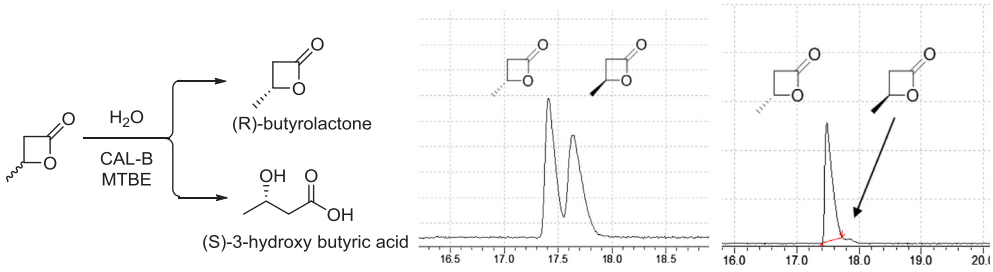


Fig. 3. Enantioselective hydrolysis of racemic β-butyrolactone by CAL-B. Chiral GC–MS chromatogram of the reaction prior to CAL-B addition (left) and after overnight incubation (right).

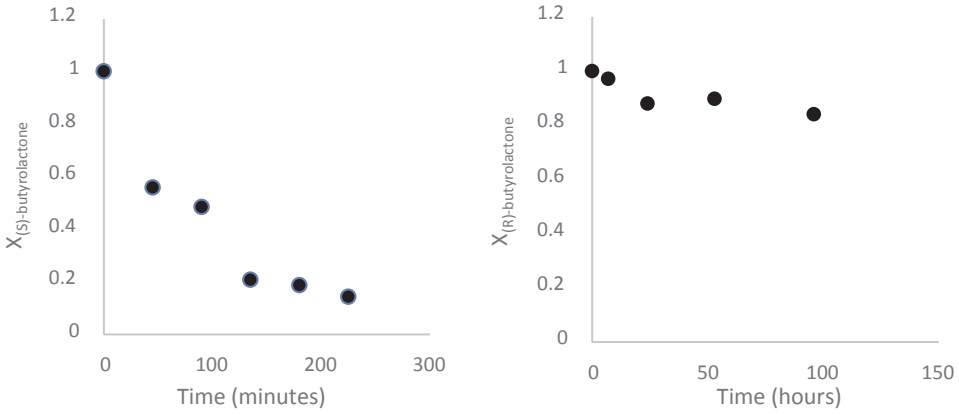
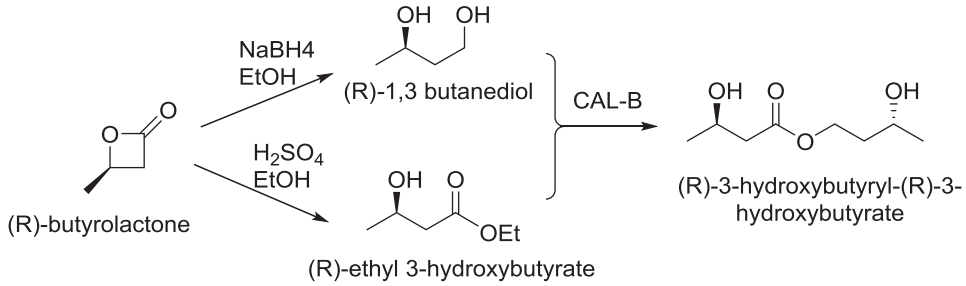
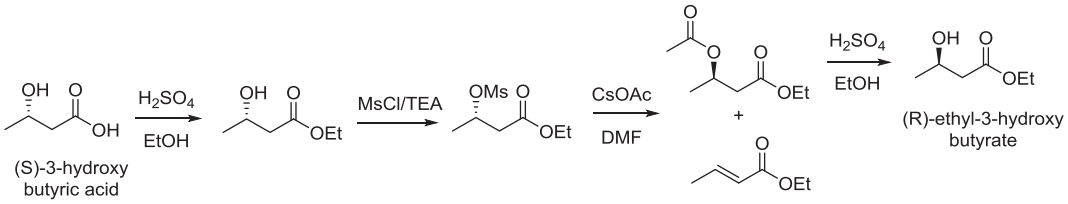


Fig. 4. Kinetics of CAL-B hydrolysis of (S) and (R) β-butyrolactone.



Scheme 1. Synthesis of (R)-3-hydroxybutyryl-(R)-3-hydroxybutyrate from (R)-β-butyrolactone.



Scheme 2. Attempted route to convert (S)-3-hydroxy butyric acid into (R)-ethyl-3-hydroxy butyrate.

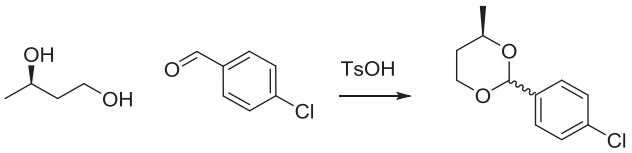
2.3. Synthesis of (S)-3-hydroxybutyryl-(S)-3-hydroxybutyrate

(S)-3-Hydroxybutyrate from the original chiral resolution can be converted to (S)-3-hydroxybutyryl-(S)-3-hydroxybutyrate using similar chemistry. First, esterification with ethanol gives (S)-ethyl-3-hydroxybutyrate which allows subsequent reduction to (S)-1,3 butanediol with lithium borohydride [18]. The resulting (S)-1,3 butanediol can be coupled to (S)-ethyl-3-hydroxybutyrate with CAL-B under reduced pressure and solvent-free conditions to give (S)-3-hydroxybutyryl-(S)-3-hydroxybutyrate (Scheme 3).

2.4. Confirmation of stereochemistry

The stereocenters of the ketone body esters all derive from the stereoselectivity of CAL-B hydrolysis of racemic β-butyrolactone. In order to confirm the stereochemistry of hydrolysis by CAL-B, we generated the acetal of presumed (R)-1,3 butanediol with 4-chlorobenzaldehyde to generate solid, crystalline material to determine the absolute stereochemistry by X-ray crystallography. The formation of the acetal creates a second stereogenic carbon, but we hoped that the equilibrium conditions of acetal formation would produce the *cis* diastereomer allowing both substituents to be equatorial (Scheme 4).

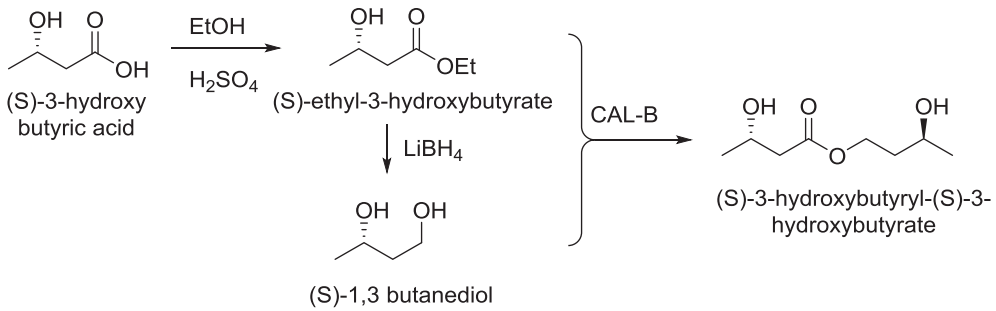
Recrystallization of the acetal from hexane generated needle-like crystals that were subjected to X-ray crystallography. Indeed, the configuration of the stereogenic carbon from 1,3 butanediol was (R), giving us confidence that CAL-B hydrolysis of racemic β-butyrolactone is selective for the (S) enantiomer (Fig. 5).



Scheme 4. Acetal formation of (R) 1,3 butanediol with 4-chloro benzaldehyde.

3. Conclusion

We developed a low cost, efficient method to produce the ketone body ester (R)-3-hydroxybutyryl-(R)-3-hydroxybutyrate and its (S,S) enantiomer from racemic β-butyrolactone. The enzyme CAL-B catalyzes enantioselective hydrolysis of racemic β-butyrolactone, and the resulting (R)-β-butyrolactone and (S)-3-hydroxybutyric acid can each be easily converted to (R) or (S) ethyl-3-hydroxybutyrate and reduced to (R) or (S) 1,3 butanediol. Both enantiomers of ethyl-3-hydroxybutyrate and 1,3 butanediol can be joined to form the two enantiomeric KE products again using immobilized CAL-B. Surprisingly, while CAL-B has a pronounced enantioselectivity for hydrolysis of (S) versus (R) β-butyrolactone, it is effective in catalyzing transesterification of either enantiomer of ethyl-3-hydroxybutyrate and 1,3 butanediol to make the (R,R) or (S,S) final ketone body esters. En route, we developed efficient methods to prepare the useful chiral synthons (R)-β-butyrolactone, (R) or (S) 1,3 butanediol and (R) or (S)-ethyl-3-hydroxybutyrate. This synthetic method is scalable, involves no column chromatography, uses mild reaction conditions, and is atom-economic. This method should help provide cost-effective access to large amounts of this important ketone body ester for research into its effects on medical conditions that



Scheme 3. Synthesis of (S,S)-3-hydroxybutyryl-3-hydroxybutyrate from (S)-3-hydroxybutyric acid.

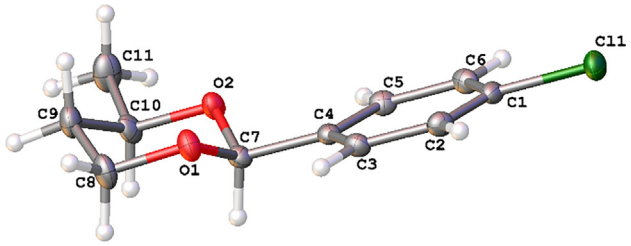


Fig. 5. X-ray crystal structure of the acetal of (R) 1,3 butanediol and 4-chlorobenzaldehyde.

are treatable by nutritional ketosis as well as the effects of ketosis on human athletic performance.

4. Materials and methods

Candida antarctica Lipase B immobilized on Immobead 150 was purchased from Sigma. Racemic β-butyrolactone was purchased from TCI. Sodium borohydride was purchased from EMD-Millipore. Lithium borohydride was purchased from Fluka. MTBE was purchased from VWR. All reagents and solvents were used without further purification. Chiral GC–MS was carried out on a Shimadzu GC-2010/QP2010S with an Agilent J&W HP-CHIRAL-10B column. NMR spectra were recorded on a 400 MHz JEOL spectrometer. X-ray crystallography data was recorded on a Bruker single crystal X-ray diffractometer. Accurate mass measurement/HRMS was carried out by the mass spectrometry facility of the University of Iowa.

4.1. (R)-β-Butyrolactone and (S)-β-hydroxybutyrate

Racemic β-butryrolactone (50 g, 0.58 mol) was dissolved in MTBE (3 L) in a 5 L round-bottomed flask charged with a stir bar. Water (6.3 g, 0.35 mol) was added, followed by immobilized CAL-B (3.5 g, 7000 U). The reaction mixture was capped and stirred at room temperature. Aliquots (150 μL) were removed at indicated times and added to 1.0 mL of methanol for chiral GC–MS analysis. After complete conversion of (S)-β-butyrolactone, the beads were filtered, washed with MTBE, and the solvent volume reduced to 1 L by rotary evaporation. The reaction mixture was extracted with 400 mL of saturated sodium bicarbonate. The organic phase was dried over anhydrous sodium sulfate which was then filtered and washed with MTBE. The solvent was removed by rotary evaporation to yield (R)-β-butyrolactone as a clear oil (23 g, 92%). Both the CAL-B and MTBE from this step can be recovered and reused.

^{1}H NMR: (400 MHz, $CDCl_3$): δ = 4.64–4.72 (m, 1H), 3.55 (ddd, J = 16.0, 6.0, 1.4 Hz, 2H) 3.05 (ddd, J = 16.5, 4.2, 1.4 Hz, 2H) 1H), 1.56 dd J = 6.1 Hz, 1.8 Hz, 3H).
^{13}C NMR: (100 MHz, $CDCl_3$): δ = 168.2, 68.0, 44.4, 20.8 Hz.

The aqueous phase was carefully acidified to pH 2 with conc. HCl and subjected to continuous liquid-liquid extraction with 500 mL diethyl ether for 18 h. The ether layer was dried with magnesium sulfate, filtered, and the solvent removed by rotary evaporation to yield (S)-3-hydroxybutyric acid as a clear oil (19 g, 63%).

^{1}H NMR: (400 MHz, $CDCl_3$): δ = 4.19–4.27 (m, 1H), 2.44–2.58 (m, 2H), 1.25 (d, J = 6.4 Hz, 3H)
^{13}C NMR: (100 MHz, $CDCl_3$): δ = 177.69, 64.39, 42.57, 22.46

4.2. (R)-Ethyl-3-hydroxybutyrate

(R)-β-Butyrolactone (20 g, 230 mmol) was dissolved in 500 mL ethanol with 1.0 mL H_2SO_4 and stirred at room temperature. Aliquots (100 μL) were added to 1.0 mL of methanol and analyzed by GC–MS for conversion of the lactone. Upon completion (> 48 h), a small amount of solid sodium bicarbonate was added and the solvent removed by rotary evaporation. The residue was taken up in dichloromethane and washed with water. The water layer was extracted twice with ethyl acetate. The pooled organic layers were dried with magnesium sulfate, which was then filtered and the solvent removed by rotary evaporation to yield (R)-ethyl-3-hydroxybutyrate as a clear oil (20.2 g, 66%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.1–4.2 (m, 3H), 2.35–2.48 (m, 2H), 1.20 (d, J = 6.4 Hz, 3H), 1.25 (t, J = 6.9 Hz, 3H)
^{13}C NMR: (100 MHz, $CDCl_3$): δ = 173.04, 64.30, 60.84, 42.89, 22.48, 14.24.

4.3. (R)-1,3 butanediol

(R)-β-Butryrolactone (18.1 g, 210 mmol) was dissolved in 100% ethanol (100 mL) in an Erlenmeyer flask and cooled in an ice bath. Sodium borohydride (17 g, 450 mmol) was added in small portions over 30 min with stirring and allowed to warm to room temperature overnight. The reaction was quenched by dropwise addition of 10% HCl until effervescence ceased and the solvent removed by rotary evaporation. The residue was taken up in 200 mL 25% aqueous K_2HPO_4 and extracted twice with 250 mL isopropanol. The isopropanol layers were combined and solvent was removed by rotary evaporation to yield a cloudy oil. The material was suspended in dichloromethane, dried over anhydrous sodium sulfate, filtered, and the solvent removed by rotary evaporation to yield (R)-1,3 butanediol as a clear oil. (15.2 g, 79%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.02–4.12 (m, 1H), 3.84–3.92 (m, 1H), 3.75–3.83 (m, 1H), 1.64–1.74 (m, 2H), 1.20–1.25 (d, J = 6.4 Hz, 3H).
^{13}C NMR: (100 MHz, $CDCl_3$): δ = 68.30, 61.59, 39.96, 23.81.

4.4. (S)-Ethyl-β-hydroxybutyrate

(S)-3-Hydroxybutyric acid (9.6 g, 92 mmol) was dissolved in 100 mL ethanol with 10 drops of sulfuric acid and stirred in an Erlenmeyer flask. Aliquots (100 μL) were added to 1.0 mL of methanol and analyzed by GC–MS for conversion to product. Upon completion, a small amount of solid sodium bicarbonate was added and the solvent removed by rotary evaporation. The residue was taken up in dichloromethane and washed with water. The water was extracted once with additional dichloromethane and the pooled organic layers dried over magnesium sulfate, filtered, and the solvent removed by rotary evaporation to yield (S)-ethyl-3-hydroxybutyrate as a clear oil (8.9 g, 73%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.1–4.2 (m, 3H), 2.35–2.48 (m, 2H), 1.20 (d, J = 6.4 Hz, 3H), 1.25 (t, J = 6.9 Hz, 3H)
^{13}C NMR: (100 MHz, $CDCl_3$): δ = 173.04, 64.30, 60.84, 42.89, 22.48, 14.24.

4.5. (S)-1,3 butanediol

(S)-Ethyl-β-hydroxybutyrate (2.0 g, 15 mmol) was dissolved in diethyl ether (80 mL) and methanol (1 mL) in a covered Erlenmeyer flask and placed in an ice bath. Lithium borohydride (0.66 g, 30 mmol) was added, allowed to warm to room temperature and stirred overnight. The reaction was quenched by addition of a small amount of water ice followed by dropwise addition of 10% HCl until effervescence ceased. Aqueous 25% K_2HPO_4 (50 mL) was added and extracted twice with 50 mL isopropanol. The isopropanol layers were combined and solvent was removed by rotary evaporation to yield a cloudy oil. The material was suspended in dichloromethane, dried over anhydrous sodium sulfate, filtered, and the solvent removed by rotary evaporation to yield (S)-1,3 butanediol as a clear oil. (1.0 g, 74%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.02–4.12 (m, 1H), 3.84–3.92 (m, 1H), 3.75–3.83 (m, 1H), 1.64–1.74 (m, 2H), 1.20–1.25 (d, J = 6.4 Hz, 3H).
^{13}C NMR: (100 MHz, $CDCl_3$): δ = 68.30, 61.59, 39.96, 23.81.

4.6. (R,R)-3-Hydroxybutyryl-3-hydroxybutyrate/(S,S)-3-hydroxybutyryl-3-hydroxybutyrate

(R)-Ethyl 3-hydroxybutyrate (2.1 g, 16 mmol) and (R)-1,3 butanediol (1.0 g, 11 mmol) were combined and incubated with CAL-B (0.2 g, 400 U) at 80 torr without solvent in a rotary evaporator. The reaction was monitored by withdrawing 5 µL portions of the reaction mixture, which were dissolved in 1.0 mL methanol for analysis by GC–MS. Upon consumption of the diol, the reaction mixture was taken up in dichloromethane, the beads were filtered and washed, and the solvent removed by rotary evaporation. Excess (R)-ethyl 3-hydroxybutyrate was removed by heating to 60 deg C under reduced pressure (1 torr). The residue was suspended in ethyl acetate, treated with activated carbon and filtered to yield (R)-3-hydroxybutyryl-(R)-3-hydroxybutyrate as a clear oil (1.2 g, 62%).

^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.30–4.39 (m, 1H), 4.13–4.21 (m, 2H), 3.84–3.93 (m, 1H), 2.78 (br s, 2H), 2.36–2.50 (m, 2H), 1.66–1.84 (m, 2H), 1.21 (d, J = 6.4 Hz, 6H)
^{13}C NMR: (100 MHz, $CDCl_3$): δ = 173.13, 65.12, 64.33, 62.19, 43.11, 37.80, 23.69, 22.61

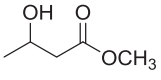
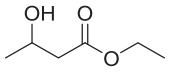
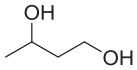
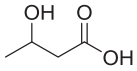
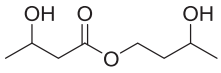
HRMS: calc for $C_8H_{16}O_4Na$ 199.0941; found 199.0947

4.7. (R)-1,3 Butanediol-4-chlorobenzaldehyde acetal

(R)-1,3 Butanediol (1.0 g, 11 mmol) and 4-chlorobenzaldehyde (0.31 g, 2.2 mmol) were mixed with p-toluene sulfonic acid (38 mg, 0.22 mmol) and heated to reflux in 15 mL benzene for 18 h using a Dean-Stark apparatus. After cooling, ethyl acetate (30 mL) was added and the mixture washed with 5 portions of 20 mL water. The organic layer was dried with magnesium sulfate, filtered and the solvent removed by rotary evaporation to yield the acetal as a white solid (1.6 g, 68%). The material was dissolved in a minimal volume of hexanes at room temperature and cooled to −20 deg C until crystals formed. The crystal data have been deposited in the Cambridge Crystallographic Data Centre, deposition number CCDC 1843902.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.43 (d, J = 8.2 Hz, 2H), 7.32 (d, J = 8.2 Hz, 2H), 5.47 (s, 1H), 4.23 (dd, J = 5.0 Hz, 11.4 Hz, 1H), 3.90–3.96 (m, 2H), 1.73–1.84 (dq, J = 5.0, 12.2 Hz, 1H), 1.50–1.55 (m, 1H), 1.30 (d, J = 6.4 Hz, 3H)

GC method and retention times:
Hold at 40 degrees for 3 min
Ramp to 60 degrees at 1 deg/min
Ramp to 190 degrees at 18 degrees/min
Hold at 190 degrees for 5 min

Retention time (min)	Compound
17.4	
17.7	
24.1	
26.1	
27.0	
29.1	
31.3	

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.bioorg.2018.07.010.

References

[1] G.F. Cahill, Starvation in man, New Engl. J. Med. 282 (1970) 668–675.
[2] M. Balietti, T. Gasoli, G. DiStefano, B. Giorgetti, G. Aicardi, P. Pattovetti, Ketogenic diets: an historical antiepileptic therapy with promising potentialities for the aging brain, Ageing Res. Rev. 9 (2010) 273–279.
[3] L. Mosconi, R. Mistur, R. Switalski, W.H. Tsui, L. Glodzik, Y. Li, E. Pirraglia, S. De Santi, B. Reisberg, T. Wisniewski, et al., FDGPET changes in brain glucose metabolism from normal to pathologically verified Alzheimer's disease, Eur. J. Nucl. Med. Mol. Imaging 36 (2009) 811–822.
[4] I.A. Simpson, K.R. Chundu, T. Davies-Hill, W.G. Honer, P. Davies, Decreased concentrations of GLUT 1 and GLUT 3 in brains of patients with Alzheimer's disease, Ann. Neurol. 35 (1994) 546–551.
[5] Y. Liu, F. Liu, C.A. Castellano, S. Nugent, S. Tremblay, M. Fortier, N. Pacquet, C. Bocti, M. Lepage, E. Turcotte, T. Fulop, S.C. Cunnane, In contrast to lower glucose uptake, brain ketone uptake is unchanged in mild Alzheimer's disease. A dual tracer study comparing 18FDG and 11C-acetoacetate, J. Nutr. Health Aging. 17 (2013) 810–811.
[6] K. Tieu, C. Perier, C. Casperson, P. Teismann, D.C. Wu, S.D. Yan, A. Naini, M. Vila, Ramasamy VR Jackson-Lewis, et al., D-beta-hydroxybutyrate rescues mitochondrial respiration and mitigates features of Parkinson's disease, J. Clin. Invest. 112 (2003) 892–901.
[7] Y. Kashiwaya, T. Takeshima, N. Mori, K. Nakashima, K. Clarke, and Veech RL. D-beta-hydroxybutyrate protects neurons of Alzheimer's and Parkinson's disease, Proc. Natl. Acad. Sci. USA 97 (2000) 5440–5444.
[8] N. Erickson, A. Boscheri, B. Linke, J. Huebner, Systematic review: isocaloric ketogenic dietary regimes for cancer patients, Med. Oncol. 34 (5) (2017) 72, https://doi.org/10.1007/s12032-017-0930-5 Epub 2017 Mar 28.
[9] S.A. Hashim, T.B. VanItallie, Ketone body therapy: from the ketogenic diet to the oral administration of ketone ester, J. Lipid Res. 55 (9) (2014) 1818–1826.
[10] K. Clarke, K. Tchabanenko, R. Pawlosky, E. Carter, M. Todd King, K. Musa-Veloso, M. Ho, A. Roberts, J. Robertson, T.B. Vanitallie, R.L. Veech, Kinetics, Safety and tolerability of (R)-3-hydroxybutyl (R)-3-hydroxybutyrate in healthy adult subjects, Regul. Toxicol. Pharmacol. 63 (3) (2012) 401–408.
[11] P.J. Cox, T. Kirk, T. Ashmore, K. Willerton, R. Evans, A. Smith, A.J. Murray, B. Stubbs, J. West, S.W. McLure, M.T. King, M.S. Dodd, C. Holloway, S. Neubauer, S. Drawer, R.L. Veech, J.L. Griffin, K. Clarke, Nutritional ketosis alters fuel preference and thereby endurance performance in athletes, Cell Metab. 24 (2) (2016) 256–268.
[12] R.B. Tobin, L.H. Garthoff, M.A. Mehlman, R.L. Veech, Metabolite levels, redox states, and gluconeogenic enzyme activities in livers of rats fed diets containing 1,3-butanediol, J. Environ. Pathol. Toxicol. 2 (1978) 389–398.
[13] K. Clarke, R.L. Veech, Preparation of (3R)-hydroxybutyl (3R)-hydroxybutyrate as a drug and food additive, PCT Int. Appl. 2010021766 (Feb 2010) 25.
[14] J.C. Newman, E. Verdin, Ketone bodies as signaling metabolites, Trends Endocrinol. Metab. 25 (1) (2014) 42–52.
[15] Y.K. Huang, S.W. Tsai, Kinetic and thermodynamic analysis of Candida Antarctica lipase B-catalyzed alcoholytic resolution of (R, S) β-butyrolactone in organic solvents, Appl. Microbiol. Biotechnol. 98 (2014) 621–628.
[16] L.H. Sun, B. Jiang, Z.L. Xiu, Aqueous two-phase extraction of 2,3-butanediol from fermentation broths by isopropanol/ammonium sulfate system, Biotechnol. Lett. 31 (3) (2009) 371–376, https://doi.org/10.1007/s10529-008-9874-3.
[17] M.C. Turcu, E. Kiljunenb, L.T. Kanervaa, Transformation of racemic ethyl 3-hydroxybutanoate into the (R)-enantiomer exploiting lipase catalysis and inversion of configuration, Tetrahedron Asymmetry 18 (2007) 1682–1687.
[18] K. Soai, A. Ookawa, Mixed solvents containing methanol as useful reaction media for unique chemoselective reductions within lithium borohydride, J. Org. Chem. 51 (21) (1986) 4000–4005.