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Synthetic routes to L-carnitine and L-gamma-amino-beta-hydroxybutyric acid from (*S*)-3-hydroxybutyrolactone by functional group priority switching

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Abstract

(*R*)-3-Hydroxy-4-trimethylaminobutyric acid (L-carnitine) and (*R*)-4-amino-3-hydroxybutyric acid (GABOB) are two compounds with a very high level of medical significance. They can be prepared from (*R*)-3-hydroxy-γ-butyrolactone which is not readily available in significant quantities. The corresponding (*S*)-lactone is available in large quantities but attempts at inverting the stereochemistry of the hydroxyl group lead to elimination to give the furanone. Here we describe a straightforward route to these two compounds, starting from (*S*)-3-hydroxy-γ-butyrolactone by adding a highly oxidized carbon at one end whilst removing one carbon from the other, thus switching the functional group priorities. In this method, the lactone is transformed to an (*R*)-4-cyano-3-hydroxybutyric acid ester which is then converted to an acyl hydrazide by treatment with hydrazine. This stable, crystalline hydrazide has not been described before. It is readily converted to (*R*)-4-amino-3-hydroxybutyronitrile, a precursor of L-carnitine and GABOB, by Curtius rearrangement under conditions that do not result in deamination.

1. Introduction

(*R*)-3-Hydroxy-4-trimethylaminobutyric acid (L-carnitine) **1** and (*R*)-4-amino-3-hydroxybutyric acid (GABOB) **2** are two compounds with a very high level of medical significance (Fig. 1). L-Carnitine is a very important intermediate in lipid biosynthesis. It functions as a carrier for transporting fatty acids into mitochondria for oxidation. Since fatty acid oxidation is a critical step by which cells derive energy, carnitine is important for cellular energetics. Deficiencies in the biosynthesis of carnitine lead to severe neurological problems. The two major uses of carnitine are in sports medicine and infant nutrition. There are several medical indications for which carnitine can be prescribed.[1–3] (*R*)-4-Amino-3-hydroxybutyric acid is a well known drug substance that functions as an agonist of gamma-aminobutyric acid (GABA). It

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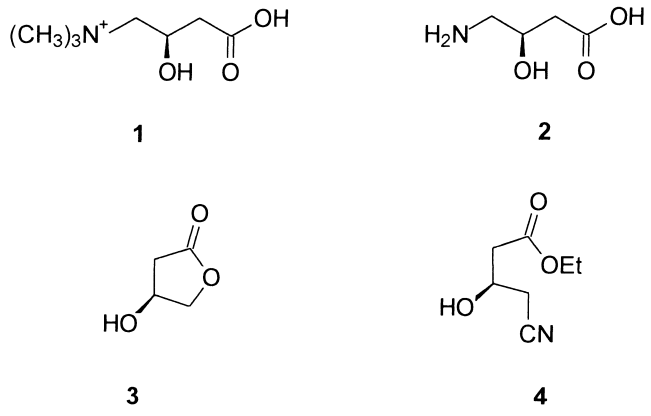


Figure 1.

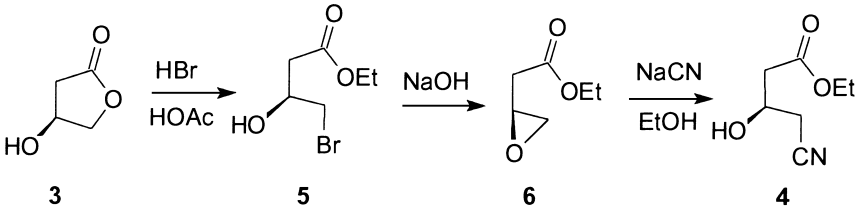
has been demonstrated to be effective in managing a variety of clinical conditions including schizophrenia and other character-based illnesses,[4,5] epilepsy and other illnesses that result in severe convulsions.[6,7] Its use for the correction of some clinical conditions observed in children has also been explored.[8] Because of the importance of L-carnitine and L-GABOB in medical science, many synthetic routes have been developed including optical resolution,[9,10] fermentation,[11] asymmetric synthesis from natural products,[12] and catalytic asymmetric synthesis.[13,14] There is still a need, however, for straightforward syntheses that have significant practical value.

(*S*)-3-Hydroxy-γ-butyrolactone **3** is a four-carbon chiral intermediate that can be obtained in high yield and very high enantiomeric purity from a variety of carbohydrate raw materials including lactose, maltose and maltodextrins.[15–17] The functionalities present in this molecule make it easily amenable to conversion to carnitine and GABOB by placing a trimethylammonium group in the 4-position after ring opening the lactone with hydrogen bromide to form the 4-bromo acid and then displacing the bromo group with trimethylamine. However, the configuration at the 3-position is not the desired one. Synthesizing these molecules with the correct configuration from (*S*)-3-hydroxy-γ-butyrolactone requires inversion of the 3-hydroxyl group or some equivalent transformation. Because of its position relative to the carbonyl group, attempts at inverting the 3-hydroxyl group by activation and displacement readily lead to elimination to yield 2-(5*H*) furanone. The alcohol group could not be modified even under the mildest of basic conditions. It was therefore necessary to explore some other equivalent of an inversion reaction. One possibility is to switch the priorities of the 1- and 4-position in the four-carbon intermediate represented by (*S*)-3-hydroxy-γ-butyrolactone. This would require removal of the 1-carbon and addition of a new high-priority carbon at position 4. This can be obtained either by removing the 1-carbon first then adding one more carbon at position 4, or introducing one more carbon to the 4-position then removing the 1-carbon. The first approach has already been described.[18] Here we describe the second approach.

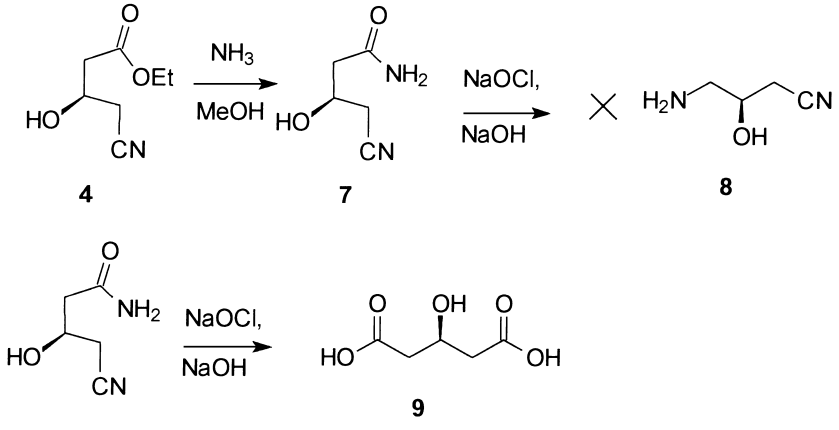
2. Results and discussions

The lactone **3** (Scheme 1) was transformed to (*R*)-4-cyano-3-hydroxybutyric acid ester **4**, a very useful synthetic intermediate which has been used for the synthesis of other natural products such as HMG-coA reductase inhibitors.[19] HMG-coA reductase is the rate limiting enzyme in cholesterol biosynthesis. In the earlier preparation of the nitrile **4**,[19] (*S*)-4-bromo-3-hydroxybutyric acid ethyl ester was an intermediate and was prepared from ascorbic acid by a very circuitous route. Here it was prepared from **3** simply by treatment with HBr in acetic acid followed by deacylation of the acetylated bromohydroxy acid with

acidic ethanol. This also converted the acid to an ethyl ester group. In the first approach (Scheme 2), the ester was transformed to the corresponding amide by treatment with ammonia in methanol solution. Interestingly, attempts using aqueous ammonia gave a complex mixture of products. An attempt to convert the amide group **7** to an amino group by Hoffman rearrangement using hypochlorite failed. 3-Hydroxypentanedioic acid **9** was obtained instead. Protecting the free hydroxyl group in the amide **7** with a variety of functional groups such as methoxymethyl ether and methoxyisopropyl ether also did not result in a successful transformation. In each case the nitrile group was hydrolyzed to a carboxylic acid function. The intermediate amide[20] has been converted to *R*-carnitine by other Hoffman rearrangement reagents such as I,I-bis-trifluoroacetyloxy-iodobenzene.[21,22] The reagents for this transformation are expensive, and this method is therefore not very practical on a commercially relevant scale. A simple process using simple reagents is desirable.

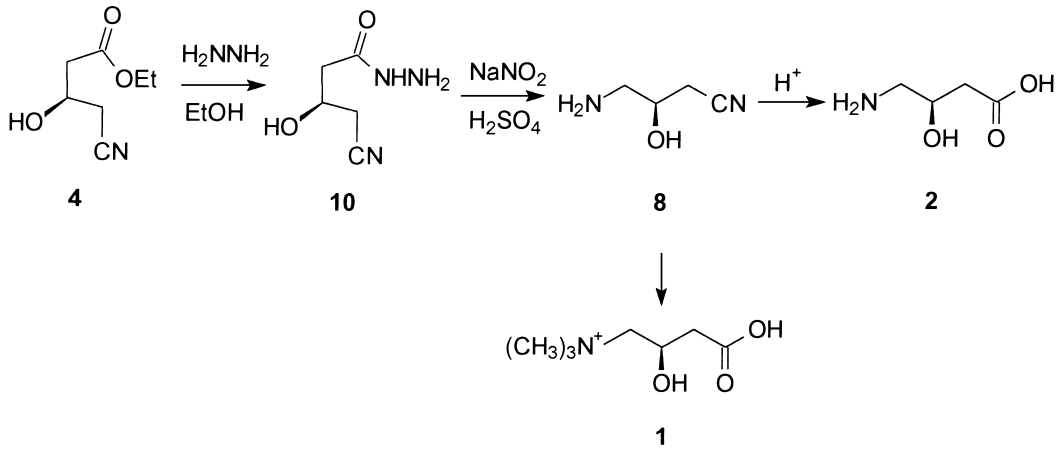


Scheme 1. Synthesis of cyano ester **4** from 3-hydroxy-γ-butyrolactone **3**



Scheme 2. Proposed scheme for Hoffman rearrangement on the amide and protected amide

The Curtius reaction avoids the oxidative alkaline conditions that characterize the Hoffman reaction. There is the possibility, however, that the amino group, once formed can be further deaminated to give a hydroxyl group. In principle, this could be avoided by controlling the stoichiometry of the reagents. It successfully gave the desired product in a short simple sequence (Scheme 3). The conversion was carried out by treating the cyano ester **4** with hydrazine. The resulting acyl hydrazide **10** was then treated with sodium nitrite and sulfuric acid at 60°C for 16–18 h. This reaction was followed by ^{1}H NMR spectroscopy. The acyl hydrazide is described here for the first time. It is a white crystalline material that is quite stable at room temperature for several weeks to a few months if kept away from light. The conversion from this hydrazide to the amine proceeded in excellent conversion (>95%). The resulting cyano amine **8** has been converted to GABOB by refluxing it with an acid and also to carnitine by methylation followed by hydrolysis of the cyano group.[11a,12g] These conversions are straightforward and well documented in the literature.[23,24]



Scheme 3. Synthesis to L-carnitine and *R*-GABOB intermediate by Curtius rearrangement

The process just described provides a general route to L-carnitine or (*R*)-GABOB and indeed other four-carbon chiral compounds such as hydroxy-pyrrolidinones (from the cyclization of GABOB) from a readily available chiral material with the undesirable enantiostructure. The route utilizes an intermediate cyano ester that is already a desired material for use in the synthesis of other drug substances to prepare a new stable intermediate, 4-cyano-3-hydroxybutanoic acid hydrazide. The conversion of intermediate **8** to carnitine and GABOB is simple and straightforward and the starting lactone material is readily available from carbohydrates such as maltose and lactose on a large scale. The route brings about an effective inversion at the stereogenic center by switching the priorities of two groups, thus overcoming the stereochemical bias in the hexose pool and circumventing the difficult elimination problems that attend a direct inversion.

3. Experimental

*3.1. (S)-4-Bromo-3-hydroxybutyric acid ethyl ester **5***

A mixture of 20.4 g (0.2 mol) of lactone **3** was stirred with 60 ml (0.3 mol) of 30% hydrogen bromide in acetic acid at 60°C for 4 h. Ethanol (300 ml) was added to the reaction mixture and it was left stirring at the same temperature for another 4–6 h. The mixture was concentrated to remove the solvent and ethyl acetate formed during the reaction. The residue was taken up in toluene and treated with 10% sodium bicarbonate solution followed with water until the water phase was neutral. The toluene layer was dried with sodium sulfate, and after removal of the solvent, the product ester **5** was obtained as a dark yellow liquid. Yield was 38 g (90%). It can be further purified by Kugelrohr distillation to yield a light yellow oil >95% pure by gas chromatography. $[\alpha]_D^{598}=-14.0$ (*c* 1.1, $CHCl_3$) (lit.[25], $[\alpha]_D^{598}=-11$, *c* 1, ethanol); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm, 4.20 (m, 1H), 4.17 (q, 2H, *J*=7.2 Hz), 3.50 (dd, 1H, *J*=5.1, 10.5 Hz), 3.45 (dd, 1H, *J*=5.7, 10.5 Hz), 2.63 (m, 2H) 1.26 (t, *J*=7.2 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm, 171.7, 67.4, 61.0, 39.3, 37.3, 14.0.

3.2. (R)-4-Cyano-3-hydroxybutyric acid ethyl ester **4**

The bromo ester **5** (42.2 g, 0.2 mol) was dissolved in a vigorously stirred 4:1 ethanol:water mixture (80 ml). The solution was heated to 50°C and 11.8 g (0.24 mol) of NaCN was added. Vigorous stirring was continued at this temperature for 3 h. The reaction mixture was then cooled, solvent was removed by rotatory evaporation and the residue was extracted with ethyl acetate (300 ml). The ethyl acetate layer was filtered through Celite mixed with silica gel, and the solvent was then removed to give the product **4** as a light yellow liquid. Yield was 29.8 g (95%). It could be further purified by distillation (b.p. 108°C, 0.5 mmHg). $[\alpha]_D^{598}$=−31.3 (*c* 1.0, $CHCl_3$) (lit.[19], $[\alpha]_D^{598}$=−33.1, *c* 1.2, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ ppm, 4.32 (m, 1H), 4.18 (q, 2H, *J*=7.2 Hz), 2.70–2.50 (m, 4H), 1.26 (t, *J*=7.2 Hz) (lit.[19]); ^{1}H NMR ($CDCl_3$, 200 MHz) δ 4.36 (m, 1H), 4.19 (q, 2H, *J*=7.1 Hz), 2.64 (m, 4H), 1.29 (t, *J*=7.1 Hz); ^{13}C NMR ($CDCl_3$, 75 MHz) δ ppm, 171.3, 117.2, 63.8, 61.0, 40.1, 40.0, 24.9, 13.9.

3.3. (R)-4-Cyano-3-hydroxybutyramide **7**

The cyano ester **4** (15.7 g, 0.10 mol) was stirred with 30% ammonium hydroxide (21 g, 0.18 mol), and 20 ml methanol for 10 h, after which time the reaction was essentially completed. Salts and other ions were removed by passing the mixture through a mix-bed resin in methanol and water as the eluting solvent. Removal of the solvent gave the amide as a yellow crystalline solid. Yield was 10.6 g (83%); m.p. 124–126°C; $[\alpha]_D^{598}$=−10.6 (*c* 1.0, MeOH); ^{1}H NMR (D_2O, 300 MHz) δ ppm, 4.25 (m, 1H), 2.68 (dd, 1H, *J*=4.8, 17.1 Hz), 2.60 (dd, 1H, *J*=6.6, 17.1 Hz), 2.36 (d, 2H, *J*=6.6 Hz); ^{13}C NMR (CD_3OD, 75 MHz) δ ppm, 176.4, 119.8, 65.1, 42.5, 26.1. IR absorption cm^{-1}, 3387, 3100, 1665, 1410, 1208, 1084.

3.4. (R)-4-Cyano-3-hydroxybutyric acid hydrazide **10**

Cyano ester **4** (15.7 g, 0.10 mol) was dissolved in absolute ethanol (30 ml) and the mixture was added to 4.8 g (0.15 mol) of anhydrous hydrazine in absolute ethanol (10 ml). It was left stirring for 2 h over which time a white solid precipitated. The white solid was filtered by vacuum filtration and washed twice with 5 ml ethanol and dried. Yield was 14 g (98%); m.p. 134–136°C; $[\alpha]_D^{598}$=−13.2 (*c* 1.0, H_2O); ^{1}H NMR (D_2O, 300 MHz) δ ppm, 4.24 (m, 1H), 2.70 (dd, 1H, *J*=4.5, 17.1 Hz), 2.58 (dd, 1H, *J*=6.3, 17.1 Hz), 2.36 (m, 2H); ^{13}C NMR (CD_3OD, 75 MHz) δ ppm, 172.4, 119.8, 65.0, 41.3, 26.1; CHN elemental analysis (Galbraith Laboratories, Knoxville, TN): C, 41.89%; H, 6.34%; N, 29.37% (calcd: C, 41.95%; H, 6.34%; N, 29.35%).

3.5. (R)-4-Amino-3-hydroxybutyronitrile **8**

The hydrazide **10** (1.43 g, 0.01 mol) was dissolved in 10 ml water, and 1.2 g concentrated sulfuric acid diluted in 10 ml water was added to the stirred solution. The mixture was cooled in an ice bath and then 1.36 g (0.02 mol) of $NaNO_2$ was added. It was stirred at 60°C for 14 h, after which time the reaction was essentially completed as determined by ^{1}H NMR spectroscopy (>95% conversion). The reaction mixture was then concentrated to dryness and then taken up in ethanol. It was stirred for 1 h, filtered to remove salts and other insoluble material and the ethanol was removed by rotatory evaporation to give the product as a light yellow liquid. The crude product was treated with an anion exchange resin, chloride form, to convert the amine to its hydrochloride salt. Water was removed and the product was obtained as a light yellow liquid which, upon cooling to room temperature, yielded a light yellow crystalline solid, the hydrochloride salt of compound **8**. Yield was 1.1 g (80%); ^{1}H NMR

(D_2O, 300 MHz) δ ppm, 4.90 (m, 1H), 3.73 (dd, *J*=9.0, 9.9 Hz), 3.30 (dd, 1H, *J*=5.7, 9.9 Hz), 2.94 (dd, 1H, *J*=4.2, 17.4 Hz), 2.84 (dd, 1H, *J*=5.7, 17.4 Hz); ^{13}C NMR (CD_3OD, 75 MHz) δ ppm, 117.3, 73.1, 45.8, 23.8. IR (CaF_2 cell) cm^{-1}, 3306 (broad), 2255, 1491, 1078; $[\alpha]_D^{598}$=+63.2 (*c* 1.0, MeOH) (hydrochloride salt). The enantiomeric excess of the cyano amine was determined by chiral HPLC as the 3,4-dinitrobenzoyl derivative. Conditions for chiral HPLC: Phenomenex (*S*)-ICA+R, 250×4.0 mm, mobile phase (hexane:dichloroethane:ethanol=6:3:1), flow rate 0.8 ml/min. The enantiomeric excess was greater than 99%.

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