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Bioorganic Chemistry 32 (2004) 473–482
BIOORGANIC CHEMISTRY
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Mini-Review

Synthesis of phosphonic analogues of carnitine and γ-amino-β-hydroxybutyric acid

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Received 3 March 2004
Available online 2 July 2004

Abstract

The involvement of carnitine and γ-amino-β-hydroxybutyric acid in the biology of mammalian cells, the physiology of the human body, and some important aspects of medicinal treatment has induced many research groups to develop their pharmacologically potent analogues. Among them are the very important phosphonic analogues: phosphocarnitine and γ-amino-β-hydroxypropylphosphonic acid. This mini-review describes the various methodologies used for the synthesis of these compounds.

Keywords: γ-Amino-β-hydroxypropylphosphonic acid; Carnitine; γ-Amino-β-hydroxybutyric acid; Phosphocarnitine

1. Introduction

(*R*)-Carnitine **1** and (*R*)-γ-amino-β-hydroxybutyric acid (GABOB) **2** have attracted considerable attention in recent years, owing to their interesting biological properties and usefulness as pharmaceuticals. Their (*S*)-enantiomers do not have these properties (see Fig. 1).

GABOB is a colorless crystalline solid with a melting point of 210–212 °C. It has been found to be a remarkable antiepileptic and hypotensive drug [1]. Many research

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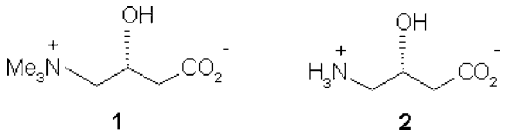
doi:10.1016/j.bioorg.2004.05.009



1 2

Fig. 1. (R)-carnitine **1** and (R)-γ-amino-β-hydroxybutyric acid (GABOB) **2**.

groups described its synthesis using natural products [2], asymmetric methods [3], biological processes [4], and other methodologies [5]. GABOB is a very useful compound because it may be easily converted to carnitine [6].

R(−)-Carnitine **1** [7], a vitamin-like compound (vitamin B_T), plays an important role in the transport of long-chain fatty acids through the membranes of mitochondria [8]. It is also an effective drug to improve myocardial function and to treat carnitine deficiency [9] and myopathic deficiencies [10]. (*S*)-(+)-Carnitine **3** is a competitive inhibitor of the (*R*) isomer [11] (see Fig. 2).

(*R*)-Carnitine, the growth factor vitamin B_T of *Teneribo molitor* [12], was isolated from meat in 1905 [13]. It is a white, hygroscopic powder with a melting point of 198 °C and an optical rotation of −31° (c 10, H_2O). A number of preparations for racemic carnitine and its enantiomers have been described. These methods involve the formation of chiral salts and the separation of the resulting diastereomers [14], biological processes [4,15], asymmetric synthesis from chiral pool materials [2,16], catalytic asymmetric synthesis [17], electrochemical oxidative decarboxylation [5a], and other methods [3a,5b,18]. Some synthesis of carnitine analogues have been described too. Among these are the cyclic analogues [18b], α-, β-, γ-substituted analogues [3a,14b,18a,19], which contain one or two methyl groups in these positions, and analogues with another atom instead of a carbon in the carboxylic group [20] or in the oxygen or hydrogen position in the hydroxyl group [21]. This report collects the synthetic methodologies for phosphonic analogues of carnitine and GABOB.

2. Synthesis of phosphocarnitine

The replacement of the carboxylic acid functional group in biologically important molecules by phosphoric acid continues to attract much interest in bioorganic and medicinal chemistry [22]. Much of the progress in this field has been associated with the phosphorus analogues of amino acids. These compounds have a tetrahedral configuration due to the presence of the phosphorous atom so they serve as stable analogues of the unstable tetrahedral intermediate formed in enzymatic processes. Many of them act as enzyme inhibitors. Others are very interesting because they increase

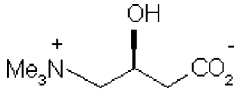


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Fig. 2. (S)-(+)-carnitine **3**.

our knowledge about the function of biologically active compounds in living organisms. One very interesting compound is the phosphonic analogue of carnitine, phosphocarnitine **4**, in which the carboxylate group is replaced with a phosphate group. The ^{31}P nucleus is a very attractive probe for structural studies of phosphoroorganic compounds because of the high sensitivity and 100% natural abundance [23]. New analogues of carnitine may be analyzed by ^{31}P NMR spectroscopy (see Fig. 3).

Phosphocarnitine as potassium salt was first obtained by Tadeusiak [20] in 1999 (Scheme 1).

The starting material was phosphonic ester **7** obtained from epichlorohydrin **5** and dimethyl (trimethylsilyl)phosphite **6** according to Azuhata and Okamoto [24]. Phosphonic ester **7** underwent dealkylation to 3-chloro-2-hydroxypropylphosphonic acids (**8**), which after treatment with a large excess of trimethylamine gave salt **9**. This salt, after treatment with an equimolar amount of potassium carbonate yielded the potassium salt of carnitine **10.** Starting from *R*-(−)-epichlorohydrin $[\alpha]_D^{20}-44.5$ (neat), it was possible to obtain the (*S*)-(−)-potassium salt of **10**.

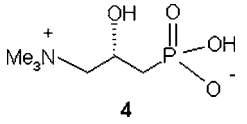

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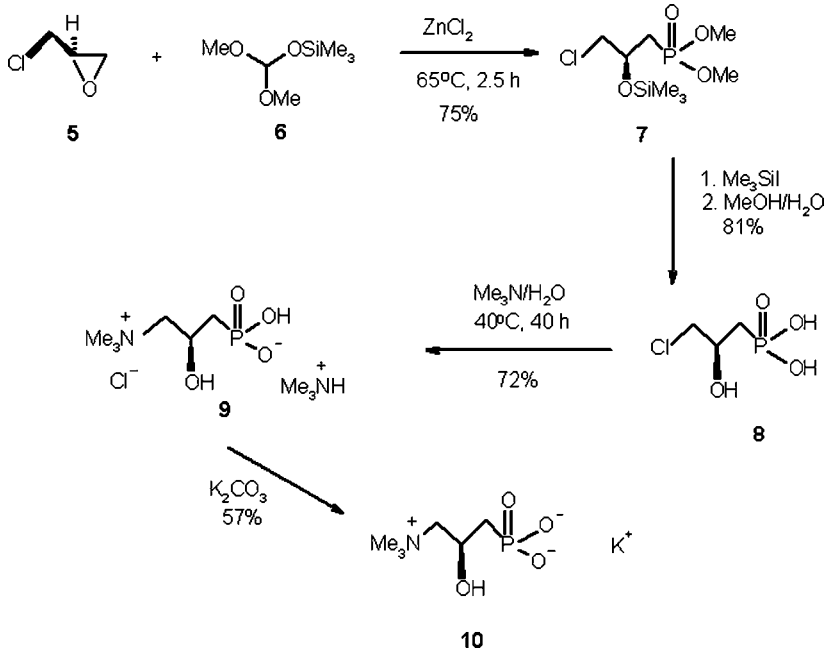
Fig. 3. Phosphocarnitine **4**.



5 **6** **7** **8** **9** **10**

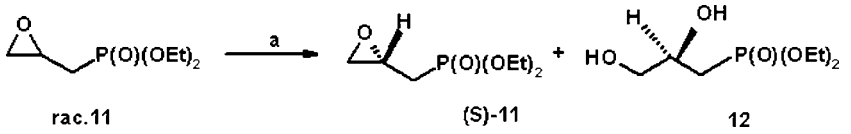
Scheme 1.

^{31}P NMR spectroscopy showed a characteristic signal at δ 16.90 ppm (D_2O). The *S*-isomer had an optical rotation of −11.7° (c 0.75, MeOH/H_2O, 1:1).

Since the discovery of this interesting biologically active phosphonic acid derivative, the interest in practical syntheses of this compound has grown. The next effective synthesis of (*S*)-phosphocarnitine was based on Jacobsen's hydrolytic kinetic resolution of the racemic epoxyphosphonate **11**, as described by Wróblewski and Halajewska-Wosik [25]. The racemic epoxyphosphonate **11** was transformed in the presence of 0.2 mol% of (*R*,*R*)-scalen-Co^{III}-OAc into a mixture (*S*)-**11** and diethyl (*R*)-(−)-2,3-dihydroxypropylphosphonate **12** (Scheme 2). The epoxide (*S*)-**11** was reacted with $MgBr_2$ in diethyl ether to afford the bromohydrin (*R*)-**13** (Scheme 3), which was transformed into the ammonium salt (−)-**14**. Hydrolysis of (*S*)-**14** was accomplished with concentrated HCl under reflux to give (*S*)-**4**, which after crystallization from water/acetone gave a white solid in 78% yield. ^{31}P NMR spectroscopy showed a signal at δ 17.8 ppm (D_2O).

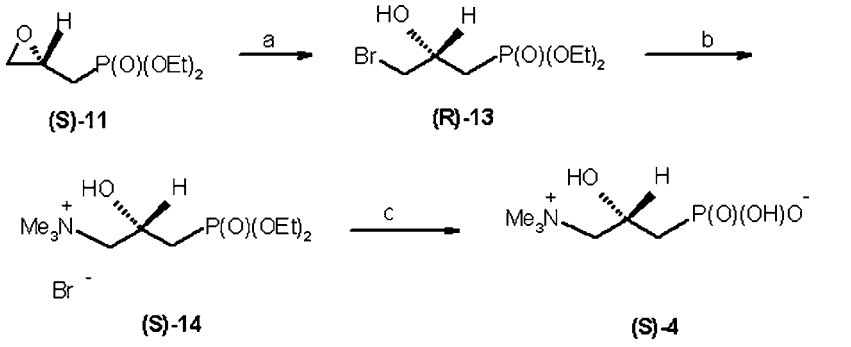
In 2002, Mikolajczyk et al. [26] obtained the *R* and *S* enantiomers of phosphocarnitine in high yields using a chemoenzymatic approach. Carnitine precursors (*R*)-(+)-**15** and (*S*)-(−)-**15** were obtained from racemic **15** used lipase AH-S AMANO (*Burkholderia cepacia*). Next, each one was dealkylated using trimethylsilyl bromide and the resulting phosphonic acid **16** was treated with an aqueous solution of trimethylamine to afford the trimethylammonium salt **17**. This salt was then subjected to column chromatography on silica gel to give (*S*) or (*R*) phosphocarnitine **4**. ^{31}P NMR spectroscopy showed signals at δ 18.84 ppm (D_2O) and δ 18.95 ppm (D_2O), respectively (Scheme 4).

Quite recently, Yuan and co-workers [27] described a very interesting enzymatic synthesis of phosphocarnitine using lipase-mediated kinetic resolution in the key step. Phosphocarnitine was obtained with a total yield of 45% starting from diethyl



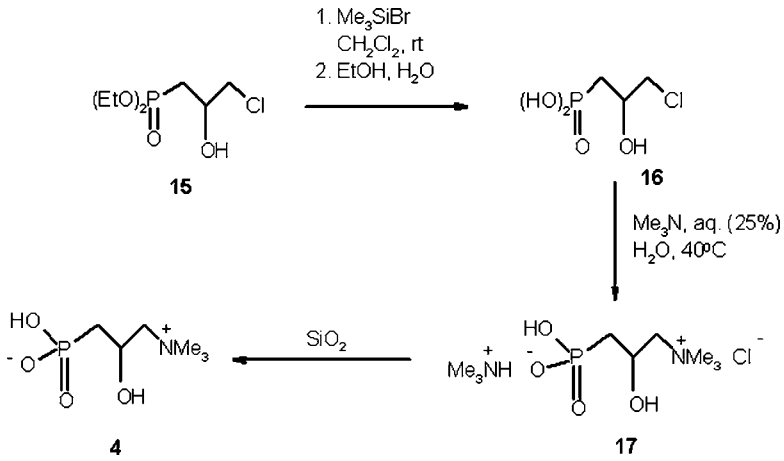
rac.11 (S)-11 12

Scheme 2. Reagents and conditions: (a) (*R*,*R*)-scalen-Co^{III}-OAc (0.2 mol%), H_2O (0.55 equiv.).

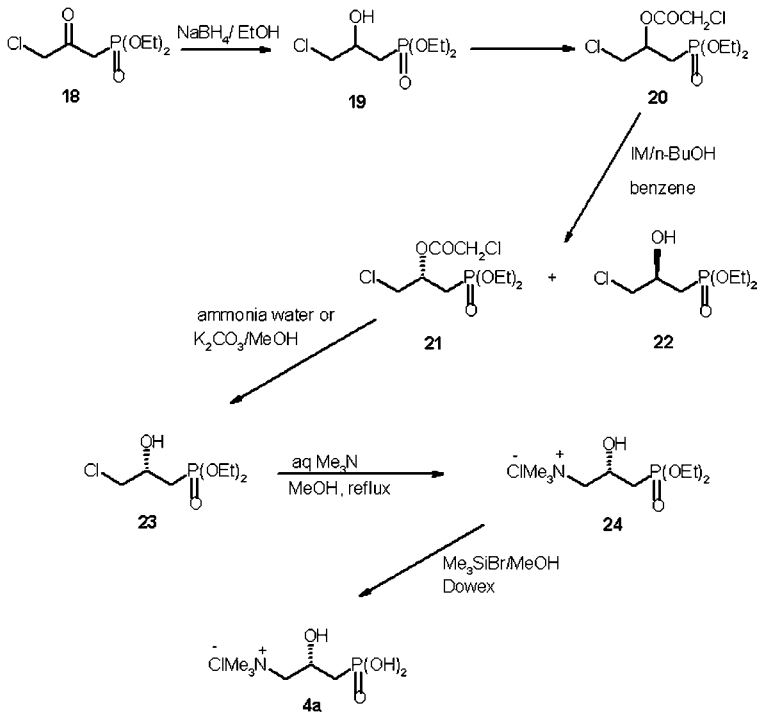


(S)-11 (R)-13 (S)-14 (S)-4

Scheme 3. Reagents and conditions: (a) $MgBr_2$, diethyl ether; (b) 45% Me_3N in ethanol/water; and (c) 12 M HCl, H_2O.

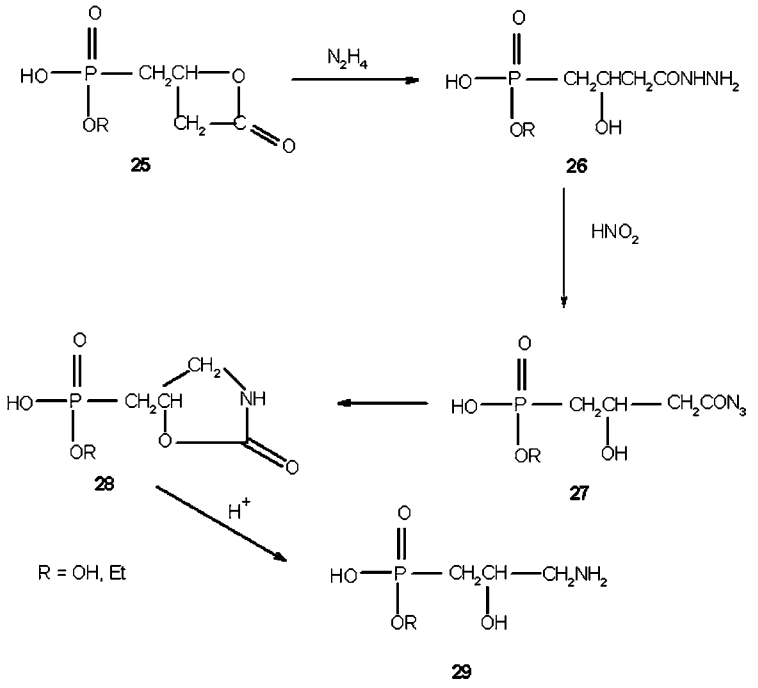


Scheme 4.

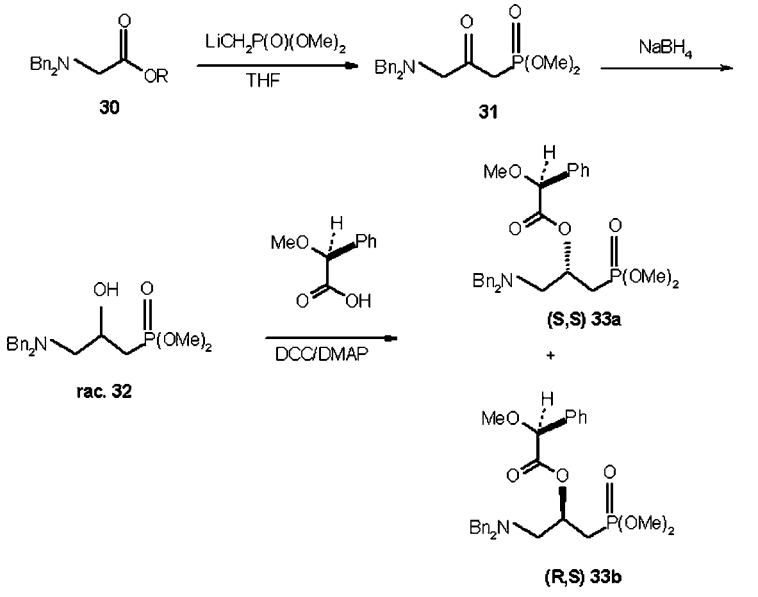


Scheme 5.

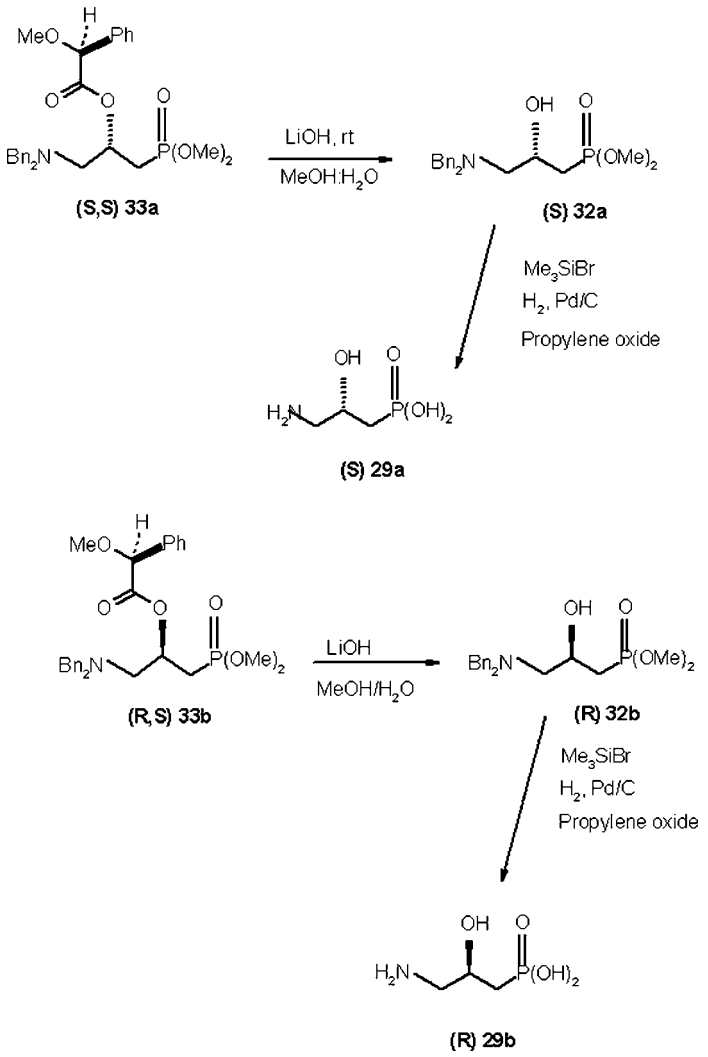
3-chloro-2-oxopropanephosphonate (**18**). Subsequent reduction using sodium borohydride, followed by esterification produced **20**, which was treated with *Mucor miehei* lipase (IM) to achieve a kinetic resolution. Amination of **23** and dealkylation produced **4a** (Scheme 5).



Scheme 6.



Scheme 7.



Scheme 8.

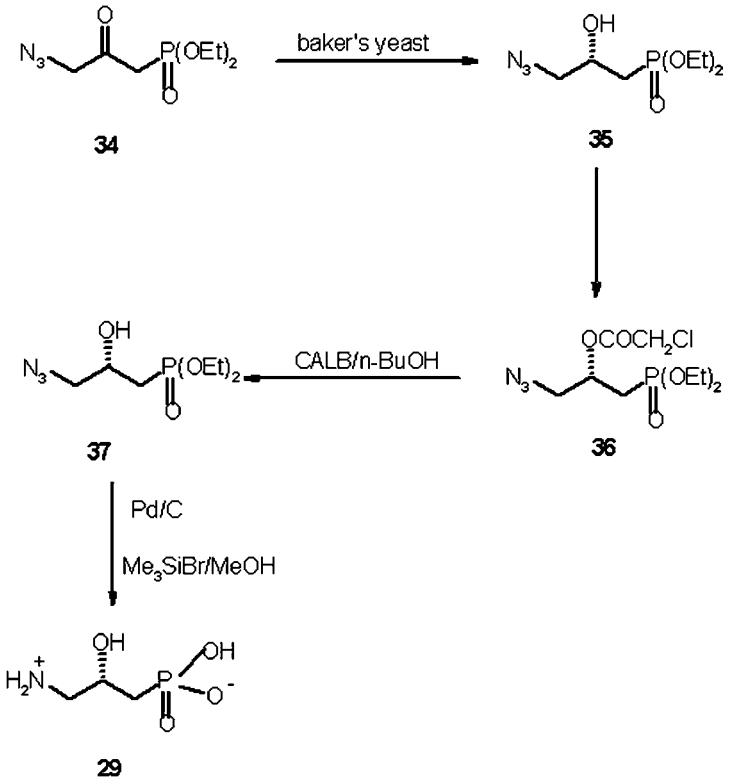
3. Synthesis of γ-amino-β-hydroxypropylphosphonic acid

Many biologically active phosphorus containing amino acids isolated from natural sources are known. Among these is the family of antibiotic γ-amino acids derived from *Streptomyces* species [28]. One very interesting compound is the phosphonic analogue of γ-amino-β-hydroxybutyric acid (GABOB), which was synthesized by Dingwall [29]. The β-lactone **25** was used as an intermediate and was prepared by free radical-catalyzed addition of phosphonite to diketene [30]. Ring opening with hydra-

zine followed by a Curtius reaction led to the oxazolidinone **28**, which upon acid hydrolysis gave the acid **29** in overall yield of about 80% based on the diketene (Scheme 6). The phosphonic analogue of GABOB, γ-amino-β-hydroxypropylphosphonic acid (**29**) is readily transformed into phosphocarnitine. This is similar to the facile conversion of GABOB into carnitine.

The first synthesis of enantiomerically pure compound **29** was presented by Ordóñez et al. [31] in 2003. According to Scheme 7, the main starting material was obtained by the reaction of *N*,*N*-dibenzylglycinate **30** with two equivalents of the lithium salt of dimethyl methylphosphonate to give the dimethyl 3-(*N*,*N*-dibenzylamino)-2-ketopropylphosphonate **31**. Reduction with $NaBH_4$ in methanol at room temperature afford the racemic mixture of the dimethyl 3-(*N*,*N*-dibenzylamino)-2-hydroxypropylphosphonate **32**. This mixture was resolved by (*S*)-*O*-methylmandelic acid in the presence of DCC and DMAP, which afforded the respective mandelates, **33a** and **33b**. The diastereomers were then separated by column chromatography.

Treatment of each with LiOH, followed by hydrolysis with bromotrimethylsilane and reduction gave (*S*)- or (*R*)-γ-amino-β-hydroxypropylphosphonic acid as white solids in 78% (**29a**) and 76% (**29b**) yields, respectively (Scheme 8). The ^{31}P NMR spectroscopy showed signals at δ 19.94 ppm (D_2O) and δ 19.94 ppm (D_2O), respectively.



Scheme 9.

Recently, the enzymatic synthesis of the phosphonic analogue of GABOB was described by Yuan and co-workers [27]. They used baker's yeast [32] and CALB (*Candida antarctica* lipase B). Baker's yeast (*Saccharomyces cerevisiae*) was used for the bioreduction of **34.** CALB shows satisfactory enantioselectivity toward molecules bearing an azido moiety so it was used in the next step for the CALB-mediated kinetic resolution of **36**. The resulting compound, **37**, was converted in betaine (**29**) in high yield (Scheme 9).

4. Conclusion

Because of the importance of (*R*)-carnitine and (*R*)-γ-amino-β-hydroxybutyric acid in pharmaceutical sciences, many synthetic routes have been developed. The phosphonic analogues of these compounds are still under investigation. These biologically active compounds may be useful for increasing our knowledge about the function and effect of carnitine and GABOB. They may lead to a better understanding of 4-aminobutanoic acid derivatives as neuromodulators in the mammalian central nervous system and a better recognition of a variety of clinical conditions including schizophrenia, epilepsy, and other illness that result in severe convulsions. Finally, they may lead to effective drugs to improve myocardial function and myopathic deficiencies.

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