The β-Lactone Route to a Totally Stereoselective Synthesis of Carnitine Derivatives

Ida Bernabei, Roberto Castagnani, Francesco De Angelis, Enrico De Fusco, Fabio Giannessi,* Domenico Misiti, Sandra Muck, Nazareno Scafetta and Maria Ornella Tinti

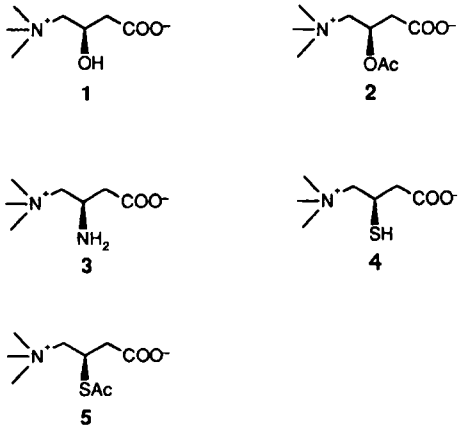
Abstract: The syntheses of the enantiomerically pure, carnitine-related β-lactones **10** and **12** starting from various carnitine precursors of opposite configuration (or carnitine itself) are described. (*R*)-3-Chlorocarnitine (**20**) has also been directly prepared from (*S*)-carnitine (**14**) and has been cyclized to **12** by a second inversion of configuration of the stereogenic centre. By nucleophilic attack at the carbonyl carbon, the β-lactone carnitine derivatives have been converted into esters, amides and guanidino congeners. Following this route, it is possible to obtain the biologically active isomer (*R*)-carnitine (**1**) starting from the otherwise useless industrial by-product (*S*)-carnitine (**14**). Nucleophilic attack by selected ambident nucleophiles at the β-carbon of the same β-lactone derivatives results in a second inversion of configuration of the stereogenic centre. Besides aminocarnitine (**3**), chiral acetylcarnitine (**2**) and acetylthiocarnitine (**5**) have been synthesized in homochiral forms following this latter procedure.

Keywords
asymmetric ring-opening · carnitine · cyclizations · β-lactones · nucleophilic substitutions

Introduction

(*R*)-(−)-Carnitine (**1**)[1] is an essential cofactor for the transport of acyl groups across the mitochondrial membrane and is therefore involved primarily in fatty acid metabolism. (*R*)-Carnitine is ubiquitous in mammalian tissues; it is an endogenous metabolite that is also obtained by dietary uptake and is present in biological materials as free carnitine and as acylcarnitines; the latter are metabolic products of reactions that utilize acylCoA, catalysed by carnitine acyltranferases.[2]

Among carnitine derivatives, (*R*)-(−)-acetylcarnitine (**2**), the major acylcarnitine found in animal cells, has been shown to possess important biological properties: it has been reported to increase both spontaneous and stimulated discharges in rat brainstem neurons and to potentiate cholinergic and serotonergic responses;[3] furthermore, it has been shown to induce a statistically significant improvement of the behaviour profile of attention and of psychomotricity in demented patients.[4]



(*R*)-(−)-Aminocarnitine (**3**, emeriamine) and its *N*-acyl derivatives are inhibitors of fatty acid oxidation as well as hypoglycaemic and antiketogenic compounds.[5, 6] In particular, they inhibit carnitine acetyl transferase (CAT)[6] and carnitine palmitoyl transferase (CPT).[6–8] The other derivatives (*R*)-(−)-thiocarnitine (**4**) and (*R*)-(−)-acetylthiocarnitine (**5**) were also found to be substrates for CAT.[9]

The involvement of carnitine and its derivatives in the biology of mammalian cells, the physiology of the human body and some important aspects of medical treatment has induced many research groups to develop a large number of stereocontrolled efficient syntheses of the natural compound and of its pharmacologically potent analogues (for derivatives **4** and **5** the synthesis of racemic forms is reported).[10] We have also been engaged

[*] Dr. F. Giannessi, Dipl.-Chem. I. Bernabei, Dr. R. Castagnani, Dipl.-Chem. E. De Fusco, Dr. S. Muck, Dipl.-Chem. N. Scafetta, Dr. M. O. Tinti
Dipartimento Ricerca Chimica, Sigma-Tau
Via Pontina Km 30400, 00040 Pomezia (Roma) (Italy)
Fax: Int. code +(6)91-393638
Prof. Dr. F. De Angelis
Dipartimento di Chimica, Ingegneria Chimica e Materiali
Università di L'Aquila (Italy)
Prof. Dr. D. Misiti
Dipartimento di Studi di Chimica e Tecnologia
delle Sostanze Biologicamente Attive
Università "La Sapienza", Roma (Italy)

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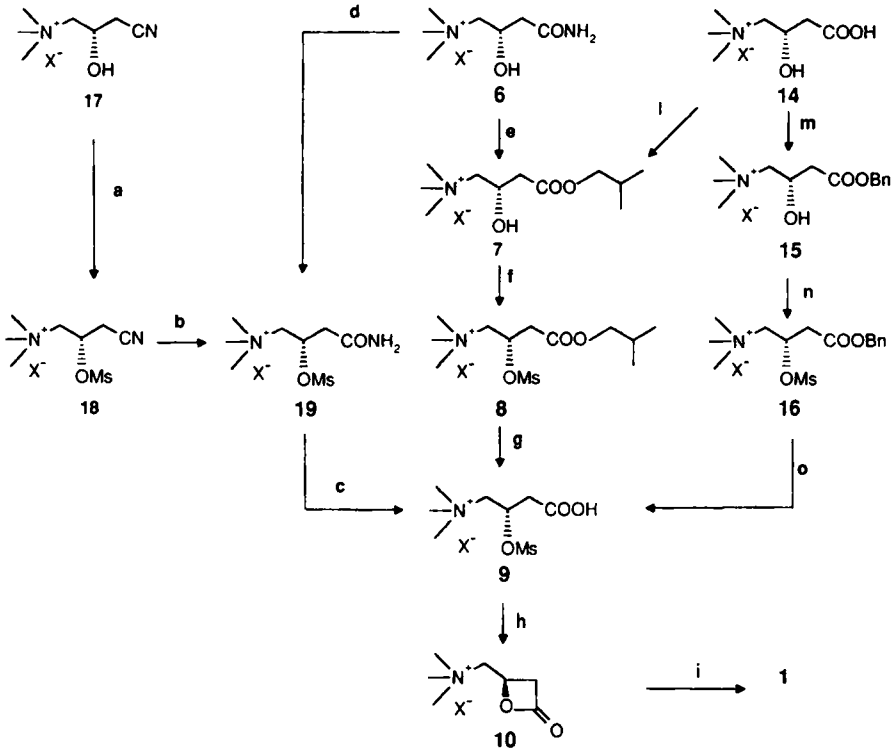
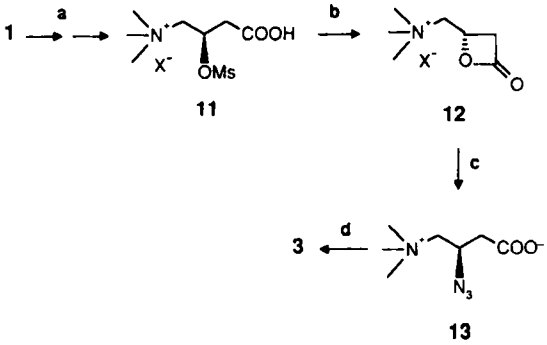
in this effort for many years,[11] and have recently published as a preliminary communication the high-yielding, totally enantioselective synthesis of (*R*)-(−)-carnitine starting from waste (*S*)-(+)-carnitinamide (**6**), obtained as a by-product in the industrial production of **1** (Scheme 1).[12] A method has also been developed for the stereochemically controlled transformation of (*R*)-(−)-carnitine into the pharmacologically active (*R*)-(−)-aminocarnitine **3** (Scheme 2).[13] In both processes, the key step is the stereospecific formation of a β-lactone derivative (**10** or its enantiomer **12**, respectively), obtained from carnitine or its precursors, with total inversion of configuration of the stereogenic centre. In continuation of these studies, we decided to explore systematically the chemistry of the β-lactone, its formation and its reactivity towards nucleophiles, with the aim of using this molecule as a chiral synthon for the synthesis of biologically interesting carnitine derivatives.



Scheme 1. Synthetic routes to (*S*)-carnitine mesylate (**9**) and to the (*R*)-β-lactone **10**. a) MsCl (3 equiv), RT, Py, 1 h (71%); b) 12N HCl, 50°C, 2 h (not isolated); c) 12N HCl, 50°C, 36 h (94%); d) MsCl (1.5 equiv), RT, Py, 75 min (52%); e) *i*BuOH, HCl gas, reflux (90%); f) methanesulfonic anhydride (3 equiv), 80°C, 24 h (99%); g) 2M HCl, 55°C, 20 h (90%); h) $NaHCO_3$ (1 equiv), RT, 6, 20 and 48 h, respectively, in DMSO, H_2O and CH_3CN (respectively 90%, almost quantitative and 80% based on NMR); i) $NaHCO_3$ (1 equiv), RT, H_2O, 24 h (99% from **9**; 99% *ee*); l) *i*BuOH, HCl gas, reflux, 3 h (99%); m) BnOH, HCl; IRA-402, ClO_4^- form (95%); n) MsCl (3 equiv), RT, Py, 105 min (70%); o) Pd/C 10%, H_2, 45 psi, 4 h (99%). The counterion X^- is specified for each structure in the experimental section.



Scheme 2. Synthesis of (*R*)-aminocarnitine **3** from (*R*)-carnitine **1**. a) See Scheme 1, steps from **14** to **9**; b) $NaHCO_3$ (1 equiv), RT, DMSO, 6 h (almost 90% based on NMR); c) NaN_3 (1 equiv), RT, DMSO, 2 h (not isolated); d) Pd/C 10%, H_2, 45 psi, 20 h (global yield 55% from **11**; >99% *ee*). $X^- = MsO^-$.

Results and Discussion

Synthetic routes to the β-lactones 10 and 12 via carnitine derivatives: Carnitine mesylate (**9**) is the preferred intermediate for cyclization to the β-lactone **10**; it is easily prepared and can be obtained in various ways from carnitine itself (**14**) or carnitine analogues (**6**, **17**) (Scheme 1). As already reported,[12] the carnitine isobutyl ester **7** (as chloride salt) was treated with methanesulfonic anhydride by direct fusion of the reagents at 80°C to give the mesylate isobutyl ester **8**, which was in turn hydrolysed to give **9** in 80% overall yield.

In a more traditional way, starting from (*S*)-(+)-carnitine hydrochloride (**14**) itself (the "wrong" isomer) a derivative soluble in organic solvents can be obtained by forming the carnitine benzyl ester perchlorate (**15**). After a routine mesylation reaction with mesyl chloride in pyridine (to give **16**) the benzyl ester moiety was cleaved off by catalytic hydrogenation to give **9** in 70% overall yield.[14] Also, carnitine nitrile (**17**) and carnitine amide (**6**), used as perchlorate salts in order to improve their solubility in organic solvents, were treated with mesyl chloride in pyridine to give the corresponding mesylates **18** and **19**, respectively, which after acidic hydrolysis were converted into carnitine mesylate **9** (actually, **6** afforded a mixture of both mesyl derivatives **18** and **19**).[15]

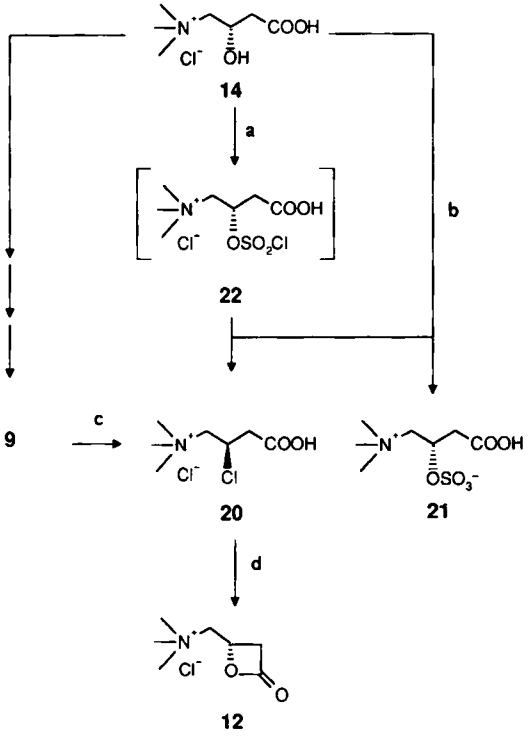
The (*R*)-(−)-β-lactone **10** was finally obtained by simple treatment of the mesylate **9** with one equivalent of $NaHCO_3$ in various solvents (e.g., DMSO, H_2O or CH_3CN for 6, 20 and 48 h, respectively), at room temperature. An analytically pure sample of **10** was obtained by transforming **9** into its inner salt with an ion-exchange resin activated in the basic form. The zwitterionic derivative cyclizes directly with total stereoselectivity, giving rise to the β-lactone **10** as a methanesulfonic salt. As to the ring closure reaction, it might be hypothesized that the involvement of the trimethylammonium moiety could favour the intramolecular approach of the carboxylate function to the β-carbon to form the product.

The NMR pattern of the two C2 hydrogens and the shift of the C3 hydrogen signal are fully consistent with those expected for a β-lactone structure like **10**.[16] So the C2 and the C3 hydrogens present an AA'B pattern, with the signal of the C2 proton *cis* with respect to the proton on C3 at $\delta = 3.94$ ($^3J_{cis} = 6.5$ Hz) overlapping with the signal of the exocyclic methylene, and the signal of the C2 proton *trans* with respect to the proton on C3 at $\delta = 3.55$ presenting a $^2J = 16.8$ Hz and a $^3J_{trans} = 4.8$ Hz. The C3 proton has a shift of $\delta = 5.3$, comparable to that of acetylcarnitine **2** ($\delta = 5.6$).

As to the determination of the absolute configuration of the β-carbon, the sign of the specific rotation of **10** ($[\alpha]_D^{25} = -24.7$ ($c = 1$ in MeOH)) as compared with that of (*R*)-(−)-acetylcarnitine **2** ($[\alpha]_D^{25} = -27.3$ ($c = 1$ in MeOH)), is of some help, being the same for both compounds. Above all, its chemical genesis (**10** must be formed by nucleophilic attack of the carboxylate function from the back side of the carbon atom bearing the mesylate group, with inversion of configuration) and its stereochemical fate (vide infra) suggest the (*R*) absolute configuration.

Any attempt to cyclize carnitine to the β-lactone by carbonyl group activation (for example with benzenesulfonyl chloride/pyridine to form the mixed anhydride)[16, 17] or by hydroxy group activation (Mitsunobu conditions)[16, 18] failed. In the first case, elimination and degradation products predominated, in the latter, probably the steric hindrance and the charge interaction between the reaction centre of the putative intermediate and the trimethylammonium moiety were responsible for the substrate's complete survival.

By using a different kind of approach, it is possible to prepare the enantiomer **12** of the β-lactone **10** starting from (*S*)-(+)-carnitine hydrochloride **14** by double inversion of configuration. The overall strategy is described in Scheme 3. We found that **14**



Scheme 3. Synthetic route to the (*S*)-β-lactone **12** from (*S*)-carnitine by double inversion of configuration. a) SO_2Cl_2 (1 equiv), RT, CH_3CN, 18 h (37%); b) $ClSO_3H$ (1.1 equiv), RT, CH_3CN, 20 h (98%); c) DMSO, 80 °C, 7 d (not isolated); d) $NaHCO_3$ (1 equiv), RT, DMSO, 24 h (65% based on NMR).

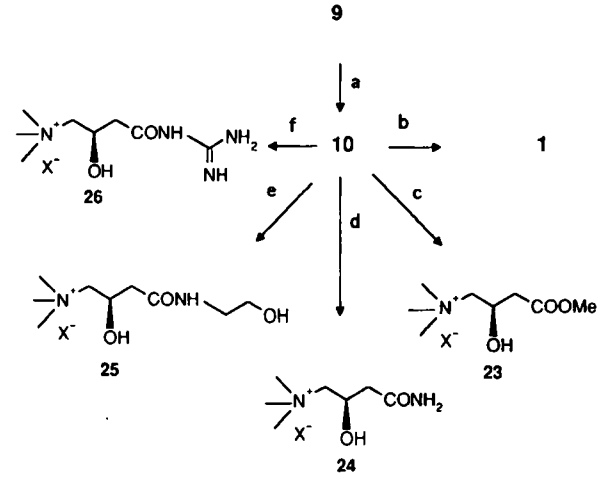
reacts with one mole of SO_2Cl_2 in CH_3CN to give an almost equimolecular amount of the β-chloroderivative **20** and of the carnitine sulfate **21** in 40% and 55% isolated yield, respectively. Sulfate **21** was also obtained in quantitative yield by simply treating **14** with chlorosulfonic acid, thus confirming for **21** the same chirality as **14**. The chloroderivative **20** underwent base-catalysed cyclization to the β-lactone **12** by treatment with one equivalent of $NaHCO_3$ in DMSO. The absolute configuration of the β-carbon in **12** was deduced by observing the optical rotation of this compound, which is opposite in sign to that of the β-lactone **10**; this also agrees with the reasonable hypothesis that, since ring closure in **20** must occur by the internal attack of the carboxylate nucleophile, the β-carbon in the chloroderivative must have absolute configuration opposite to that of the final product and of its carnitine precursor.

These results deserve some comments. From the mechanistic point of view, treatment of **14** with SO_2Cl_2 should initially form the chlorosulfonic intermediate **22**. The chlorosulfonyloxy group then undergoes nucleophilic displacement, with inversion of configuration, by the chloride ion present in solution as a side product of the first reaction step (and also as the counterion of carnitine itself). Chlorosulfonic acid is thus produced, which is then consumed by carnitine still present in the reaction mixture to give the sulfonyl monoester **21** cited above. This mechanistic hypothesis is supported by the observation that carnitine mesylate **9** (when X = Cl) can undergo substitution by the chloride ion.

In conclusion, this last reaction sequence can be used to synthesize the carnitine β-lactone derivative starting from carnitine itself, with overall *retention* of configuration, while the sequence described in Scheme 1 yields the β-lactone with *inversion* of configuration. The easy access to both carnitine enantiomers, together with the reaction strategies described here, opens the way to a versatile synthesis and subsequent exploitation of the chiral β-lactone synthon.

Reactions of 10 and 12 with nucleophiles: We systematically explored the reactivity of the β-lactones **10** and **12** towards different nucleophiles. It was gratifying to observe that these substrates react cleanly and with total regioselectivity at the carbonyl carbon or at C3 to afford useful carnitine derivatives in excellent yields and isomeric purities. Also, since only some nucleophiles appear to be effective in the β-lactone opening, a possible theory to explain this peculiar behaviour has been advanced.

O–Acyl Fission (Scheme 4): As we have already seen (Scheme 1), the (*R*)-β-lactone **10** reacts in the presence of one equivalent of $NaHCO_3$ in water to give (*R*)-carnitine (**1**) in practically quantitative yield.[12] Nucleophilic attack of the hydrox-



Scheme 4. Reactions of the β-lactone **10** with nucleophiles: *O*-acyl fission. a) $NaHCO_3$ (1 equiv), RT, 6 or 20 h, respectively, in DMSO and H_2O (respectively 90% and almost quantitative based on NMR); b) $NaHCO_3$ (1 equiv), RT, H_2O, 24 h (99% from **9**; >99% *ee*); c) MeOH (excess), RT, DMSO, 20 h (80% based on NMR); d) NH_3 gas, RT, DMSO, 20 h (75% based on NMR); e) ethanolamine (1 equiv), RT, DMSO, 20 h (80% based on NMR); f) guanidine (1 equiv), RT, DMSO, 20 h (85% based on NMR). $X^- = MsO^-$.

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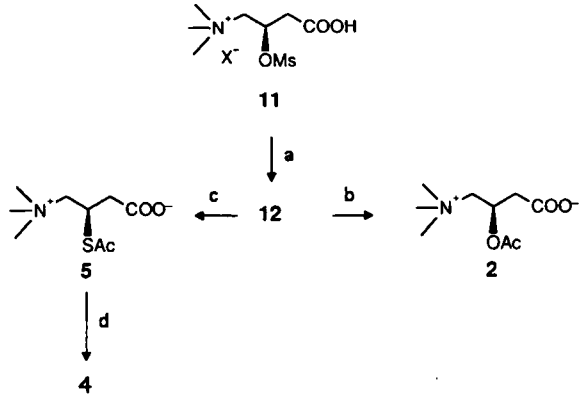
ide ion at the acyl carbon atom results in the totally regioselective opening of the ring, as expected for oxygen–acyl bond fission in a slightly basic medium.[16] The entire reaction sequence from **6** (or alternatively from **14** and **17**) therefore allows the formation of biologically active (*R*)-carnitine starting from carnitine derivatives or carnitine itself of opposite configuration.

It is worth pointing out that all these compounds (**6**, **14** and **17**) are by-products of the industrial processes for the production of (*R*)-carnitine, each of them formed in equimolar amount with respect to the target compound, depending on the stage at which the racemate resolution is performed. In fact, it has to be considered that the routes for industrial production of (*R*)-carnitine start from achiral precursors, and require the separation of diastereoisomeric salts to obtain the enantiomerically pure final product.

The (*R*)-β-lactone **10** reacts also with alcohols and amines to give carnitine esters and amides exclusively in excellent yields. Addition of excess methyl alcohol, for example, to preformed **10** in DMSO afforded (*R*)-carnitine methyl ester **23** after 24 h in 80% yield (NMR; impurities result from elimination and hydrolysis products). As to the reaction of nitrogen nucleophiles with β-lactones, opening of the serine β-lactone with ammonia is reported to proceed towards the formation of amide or amine derivatives (the latter by attack at C3), depending on the polarity of the solvent, less polar solvents allowing the formation of 2,3-diaminopropanoic acid.[19] In our case the same reaction yields only carnitine amide **24**, essentially because of the insolubility of **10** in nonpolar solvents. Additionally, treatment of **10** with ethanolamine or guanidine in DMSO allows the preparation of the corresponding acyl *N*-substituted derivatives **25** and **26**.

It is worth pointing out that the reactions of the carnitine β-lactone with amines and alcohols, far from being just a complicated way to synthesize carnitine amides and esters, make it possible to obtain these compounds in the absolute (*R*) configuration starting from waste (*S*)-carnitine derivatives. Alternatively, starting from the industrial product, (*R*)-carnitine, it is possible to obtain (*S*)-carnitine amides and esters via the (*S*)-β-lactone **12**.

O–Alkyl Fission (Scheme 5): The (*S*)-β-lactone **12** (and its (*R*)-enantiomer **10**) reacts with various nucleophiles at C3 with inversion of configuration, thus, because of the overall double inversion of configuration at the stereogenic carbon, giving products with the same chirality as the starting carnitine used to form the intermediate lactone. In general, it is convenient to carry out the lactone formation and the following reaction with the nucleophile in sequence, without isolation of the cyclic intermediate.



Scheme 5. Reactions of the β-lactone **12** with nucleophiles: *O*-alkyl fission. a) $NaHCO_3$ (1 equiv), RT, DMSO, 6 h (almost 90% based on NMR); b) AcONa (1 equiv), RT, DMSO, 20 h (37% from **11**); c) AcSK (1 equiv), RT, DMSO, 2 h (34% from **11**); d) KOH (excess), 0 °C, 2 min, HCl (ref. [10]). $X^- = MsO^-$.

Thus, treatment of a diluted DMSO solution of the (*S*)-β-lactone **12** (obtained from the (*R*)-mesylcarnitine derivative **11** by reaction with sodium hydrogencarbonate) with sodium acetate gives (*R*)-acetylcarnitine (**2**) in 37% yield. In the same way, potassium thioacetate furnishes (*R*)-acetylthiocarnitine (**5**) in 34% yield; simple hydrolysis of **5** gives (*R*)-thiocarnitine (**4**) in significant yield.[10] It should be noted that the reaction sequence described in Scheme 3 to give **12** via the (*R*)-chloro derivative **20** can also be followed. Accordingly, starting from (*S*)-carnitine, (*R*)-acetylcarnitine of high optical purity (98% *ee*) but in low yields was obtained after a triple inversion of configuration of the chiral centre.

As already described[13] (Scheme 2), reaction of **12** with NaN_3 affords azidocarnitine **13**, which after catalytic reduction gives (*R*)-aminocarnitine **3**. In spite of the considerable pharmacological interest of this compound and the efforts made for its synthesis (see ref. [1] cited in [13]), this reaction sequence is so far the only known convenient route to an enantiomerically pure product starting from chiral carnitine itself.

The (*R*)-β-lactone **10** also reacts with sodium carbonate in DMSO solution giving, as the final products, an almost equimolar mixture of (*R*)- and (*S*)-carnitine (44:56, see Experimental). The formation of the (*S*)-isomer can be explained as the initial attack of the carbonate ion at the C3 atom followed by hydrolysis, while the (*R*)-form should merely result from alkaline hydrolysis of the β-lactone ring.

Despite the results we have obtained, the reactivity of the carnitine β-lactone is not so general as expected on the grounds of β-lactone chemistry.[16, 19, 20] With other nucleophiles, in fact, things are less straightforward. For instance, we were not able to obtain the expected products from reaction with thiourea, sodium hydrosulfide or mercaptoethylamine hydrochloride. In all cases the unreacted lactone was recovered or elimination and/or hydrolysis products were formed. It should also be noted that, on our β-lactone substrate, the reactivity in general and regioselectivity in particular referred to the carboxyl carbon or to the β-carbon can be hardly discussed in terms of the HSBA principle.

In all the cases where a reaction takes place at the β-carbon, charged ambident nucleophiles were used. From a mechanistic point of view, a possible explanation for this particular behaviour is indicated in Figure 1. The trimethylammonium moiety appears a potential candidate to rapidly interact with the charged nucleophile to form the ion couple. In an ambident nucleophile where the pairs of electrons are distributed on three atoms, the other potentially attacking atom, which is not engaged by the ammonium moiety, can attack the β-carbon opposite to the leaving oxygen, forming a six-membered intermediate. This could be the case for the reaction with salts of carboxylic acids as well as with the azide ion. In the case of sodium thioacetate, it is observed that substitution occurs through the softer sulfur atom.

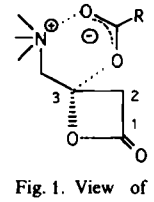


Fig. 1. View of the possible interaction between the β-lactone **12** and an ambident nucleophile leading to substitution at C3.

Conclusions

The ready formation of enantiomerically pure carnitine β-lactones starting from carnitine and carnitine precursors of opposite configuration, or even from carnitine of the same configuration by a different stereochemically controlled route, appears to

be of particular interest for two reasons: i) hydrolysis and solvolysis of the β-lactone moiety opens the way to an overall inversion of configuration of the stereogenic centre, thus permitting complete recovery of the biologically useful from the useless carnitine isomer; ii) nucleophilic attack at the β-carbon of the β-lactone by certain nucleophiles results in a second inversion of configuration of the stereogenic centre for the synthesis of more elaborate and biologically important carnitine derivatives. The electrophilic behaviour of the β-carbon of the β-lactone ring towards only certain nucleophiles with two potentially attacking atoms has been tentatively explained in the light of a possible reaction intermediate involving the charged trimethylammonium moiety.

To our knowledge, the "β-lactone route" to carnitine derivatives described in this paper represents the most efficient and general method of stereocontrolled syntheses of selected carnitine derivatives.

Experimental Procedure

Melting points were determined by the capillary method on an electrothermal apparatus (Buchi 535) and are uncorrected. ^{1}H NMR spectra were recorded on a Varian VXR 300 MHz FT spectrometer; chemical shifts were expressed in δ values downfield from DSS (in D_2O) or TMS; the following abbreviations are used: s, singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet(s); br, broad. Elemental analyses were performed by an EA 1108 elemental analyzer (Carlo Erba) apparatus. IR spectra were recorded on a Nicolet 20SCX FT-IR spectrometer. Rotation was measured on a Perkin–Elmer 241 polarimeter. MS (FAB) spectra were recorded on a VG MASSLAB TRIO-2 apparatus.

Preparation of **3**, together with that of **7** from **14**, is described in ref. [13]. Preparation of **15** from **14** is described in ref. [21]. Analytical data for **2** are consistent with those reported in ref. [22]. Compounds **6** and **17** are intermediates in the industrial production of **14**. The isolation and purification procedures for products **23**, **24**, **25** and **26** (Scheme 4) were not optimized: only NMR and MS-FAB spectra of the crude products are reported.

(*S*)-Carnitine isobutyl ester (7): A stirred suspension of **6** ($X^- = Cl^-$, 10 g, 0.05 mol) in isobutyl alcohol (50 mL) was cooled at 4 °C and saturated with HCl gas. The reaction mixture was refluxed for 1 h and then filtered while still hot to remove NH_4Cl. The filtrate was concentrated under vacuum, and the residue was taken up twice into isobutyl alcohol (2 × 50 mL) and concentrated to dryness. The residue was triturated with acetone to give, after filtration, **7** ($X^- = Cl^-$, 11.6 g, 90%). M.p. 150 °C (decomp.); $[\alpha]_D^{25} = +15.0$ ($c = 1$ in H_2O); ^{1}H NMR (300 MHz, D_2O, 25 °C): $\delta = 4.7$ (m, 1H), 4.0–3.9 (m, 2H), 3.5 (m, 2H), 3.2 (s, 9H), 2.7 (m, 2H), 2.0–1.9 (m, 1H), 0.9 (d, $^3J = 7$ Hz, 6H); IR (KBr): $\tilde{\nu} = 1730$ (C=O) cm^{-1}; MS (FAB): $m/z = 218$ [M^+]; $C_{11}H_{24}ClNO_3$ (253.77): calcd C 52.06, H 9.53, Cl 13.97, N 5.52; found C 51.89, H 9.86, Cl 13.67, N 5.13.

(*S*)-Carnitine mesylate isobutyl ester (8): A mixture of **7** ($X^- = Cl^-$, 2.5 g, 0.01 mol) and methanesulfonic anhydride (5.2 g, 0.03 mol) was heated at 80 °C for 24 h. The mixture was taken up into CH_2Cl_2 (20 mL), and the product was precipitated with Et_2O. This procedure was repeated three times in order to thoroughly eliminate the residual anhydride to give **8** ($X^- = MsO^-$, 3.9 g, 99%). M.p. 137–140 °C (decomp.); $[\alpha]_D^{25} = +24.7$ ($c = 1$ in H_2O); ^{1}H NMR (300 MHz, D_2O, 25 °C): $\delta = 5.5$ (m, 1H), 3.9–3.8 (m, 3H), 3.6 (dd, $^2J = 16$ Hz, $^3J = 1.3$ Hz, 1H), 3.2 (s, 3H), 3.1 (s, 9H), 3.0 (m, 2H), 2.7 (s, 3H), 1.8 (m, 1H), 0.8 (d, $^3J = 7$ Hz, 6H); IR (KBr): $\tilde{\nu} = 1731$ (C=O) cm^{-1}, 1341, 1200 ($CH_3SO_3^-$); MS (FAB): $m/z = 296$ [M^+]; $C_{13}H_{29}NO_8S_2$ (391.50): calcd C 39.88, H 7.47, N 3.58, S 16.38; found C 39.55, H 7.43, N 3.75, S 16.24.

(*S*)-Carnitine mesylate (9): A solution of **8** ($X^- = MsO^-$, 3.9 g, 0.01 mol) in HCl (2N, 65 mL) was heated at 50 °C for 20 h. The solution was then concentrated to dryness under vacuum, and the residue was triturated with acetone to give, after filtration, pure **9** ($X^- = MsO^-$, 3.3 g, 90%). M.p. 148–150 °C (decomp.); $[\alpha]_D^{25} = +22.0$ ($c = 1$ in H_2O); ^{1}H NMR (300 MHz, D_2O, 25 °C): $\delta = 5.5$ (m, 1H), 3.9 (dd, $^2J = 16$ Hz, $^3J = 10.6$ Hz, 1H), 3.6 (dd, $^2J = 16$ Hz, $^3J = 1.3$ Hz, 1H), 3.2 (s, 3H), 3.1 (s, 9H), 2.9 (m, 2H), 2.7 (s, 3H); IR (KBr): $\tilde{\nu} = 1713$ (C=O), 1332, 1200 ($CH_3SO_3^-$) cm^{-1}; MS (FAB): $m/z = 240$ [M^+]; $C_9H_{21}NO_8S_2$ (335.39): calcd C 32.23, H 6.31, N 4.17, S 19.12; found C 31.98, H 6.12, N 4.03, S 18.97.

(*R*)-β-Lactone 10: A solution of **9** ($X^- = MsO^-$, 1.5 g, 4.47 mmol) in H_2O was transferred onto Amberlite IRA-402 (30 g, activated as HCO_3^-) at 5 °C and eluted with cold water (5 °C). The eluate was allowed to stand for 4 h at room temperature and, after water evaporation under vacuum, gave a crude product, which was taken up into CH_3CN and filtered. The filtrate was evaporated to dryness to give **10** ($X^- = MsO^-$, 0.855 g, 80%) as a white solid. M.p. 160 °C (decomp.); $[\alpha]_D^{25} = -24.7$ ($c = 1$ in MeOH); ^{1}H NMR (300 MHz, D_2O, 25 °C): $\delta = 5.35–5.25$ (m, 1H), 3.98–3.89 (m, 3H), 3.54–3.46 (dd, $^2J = 16.8$ Hz, $^3J = 4.8$ Hz, 1H), 3.26 (s, 9H), 2.81 (s, 3H); IR (KBr): $\tilde{\nu} = 1835$ (C=O) cm^{-1}; MS (FAB): $m/z = 144$ [M^+]; $C_8H_{17}NO_3S$ (239.29): calcd C 40.16, H 7.16, N 5.85, S 13.40; found C 39.81, H 7.13, N 5.77, S 13.23.

(*S*)-Carnitine mesylate nitrile (18): *Methanesulfonyl chloride (14.2 g, 123 mmol)* was added in 5 min to (*S*)-carnitine nitrile perchlorate **17** (10 g, 41 mmol) in dry pyridine (200 mL) and the solution was stirred for 1 h and then poured into a flask containing Et_2O (800 mL). The obtained precipitate was crystallized from CH_3CN/*i*PrOH (filtering the insoluble residue in warm CH_3CN) and the crystals were triturated with warm *i*PrOH to give **18** as perchlorate (9.4 g, 71%). M.p. 155 °C; $[\alpha]_D^{20} = +43$ ($c = 1$ in H_2O); ^{1}H NMR (300 MHz, D_2O, 25 °C): $\delta = 5.78–5.70$ (m, 1H), 4.10 (dd, $^2J = 15.4$ Hz, $^3J = 8.4$ Hz, 1H), 3.84 (dd, $^2J = 15.4$ Hz, $^3J = 1.9$ Hz, 1H), 3.42 (s, 3H), 3.41 (dd, $^2J = 18.0$ Hz, $^3J = 5.7$ Hz, 1H), 3.30 (s, 9H), 3.25 (dd, $^2J = 18.0$ Hz, $^3J = 3.8$ Hz, 1H); IR (KBr): $\tilde{\nu} = 2256$ (CN), 1351 and 1175 (CH_3SO_3) cm^{-1}; $C_8H_{17}ClN_2O_5S$ (320.74): calcd C 29.96, H 5.34, N 8.73, Cl 11.05; found C 30.21, H 5.35, N 8.47, Cl 10.97.

Preparation of 9 from 18: A stirred solution of **18** (2 g, 6.23 mmol) in 12N HCl (40 mL) was heated at 50 °C for 36 h (the reaction proceeds through the formation of **19**, as evidenced by HPLC analysis after 2 h) and then evaporated under vacuum to give an oily product. CH_3CN was added, the insoluble material was separated by filtration and the filtrate was poured into a flask containing Et_2O. The precipitate thus obtained was triturated with ether and dried under vacuum to give **9** (2 g) as a raw product, which could be used without further purification in the formation of β-lactone **10**.

(*S*)-Carnitine mesylate amide (19): Methanesulfonyl chloride (9.88 g, 86.31 mmol) was added over 5 min to (*S*)-carnitinamide perchlorate **6** (15 g, 57.54 mmol) in dry pyridine (300 mL); the solution was stirred for 75 min and then poured into a flask containing Et_2O (2.5 L). The precipitate obtained was triturated with warm *i*PrOH to afford **19** as perchlorate (10.2 g, 52%). M.p. 156–158 °C; $[\alpha]_D^{20} = +21.5$ ($c = 1$ in H_2O); ^{1}H NMR (300 MHz, [D$_6$]DMSO, 25 °C): $\delta = 7.4$ (s, 1H), 7.2 (s, 1H), 5.4 (m, 1H), 3.9 (dd, $^2J = 14.0$ Hz, $^3J = 7.6$ Hz, 1H), 3.68 (dd, $^2J = 14.0$ Hz, $^3J = 1.2$ Hz, 1H), 3.35 (s, 3H), 3.15 (s, 9H), 2.8–2.7 (m, 2H); IR (KBr): $\tilde{\nu} = 1696$ (CO), 1333 and 1174 (CH_3SO_3) cm^{-1}; $C_8H_{19}ClN_2O_6S$ (338.75): calcd C 28.36, H 5.65, N 8.31, Cl 10.46, S 9.46; found C 28.74, H 5.60, N 7.89, Cl 10.26, S 9.25.

Preparation of 9 from 19: **9** was obtained from **19** as described starting from **18**.

(*S*)-Carnitine mesylate benzyl ester (16): Methanesulfonyl chloride (25.77 g, 225 mmol) was added over 5 min to (*S*)-carnitine benzyl ester perchlorate **15** (24.4 g, 75 mmol) in dry pyridine (100 mL); the solution was stirred for 105 min and then poured into a flask containing Et_2O (500 mL). The oily precipitate thus obtained was dissolved in CH_2Cl_2 (300 mL), washed with HCl (2N, 4 × 50 mL) and brine (1 × 20 mL) and dried on Na_2SO_4 to give **16** as perchlorate after evaporation under vacuum (22 g, 70%). M.p. 180 °C (decomp); $[\alpha]_D^{25} = +20.0$ ($c = 1$ in MeOH); ^{1}H NMR (300 MHz, $(CD_3)_2CO$, 25 °C): $\delta = 7.45–7.30$ (m, 5H), 5.71–5.62 (m, 1H), 5.20 (m, 2H), 4.20 (dd, $^2J = 15.2$ Hz, $^3J = 7.7$ Hz, 1H), 4.05 (dd, $^2J = 15.2$ Hz, $^3J = 1.7$ Hz, 1H), 3.47 (s, 9H), 3.30 (s, 3H), 3.20 (d, $^3J = 6.0$ Hz, 2H); IR (KBr): $\tilde{\nu} = 1735$ (CO), 1341 and 1174 (CH_3SO_3) cm^{-1}; $C_{13}H_{24}ClNO_9S$ (429.86): calcd C 41.91, H 5.63, N 3.25, Cl 8.25; found C 41.81, H 5.72, N 3.28, Cl 8.10.

Preparation of 9 from 16: Pd/C 10% (300 mg) was added to a solution of **16** ($X^- = ClO_4^-$, 3 g, 7 mmol) in MeOH (50 mL) and the mixture was hydrogenated at 45 psi in a Parr apparatus for 4 h. Subsequent filtration of the catalyst and evaporation of the filtrate under vacuum gave **9** as perchlorate (2.3 g, quantitative yield). M.p. 170 °C (decomp.); $[\alpha]_D^{25} = +19.6$ ($c = 1$ in MeOH); ^{1}H NMR (300 MHz, D_2O, 25 °C): $\delta = 5.68–5.59$ (m, 1H), 4.05–3.75 (m, 2H), 3.33 (s, 3H), 3.27 (s, 9H), 3.15–3.00 (m, 2H); IR (KBr): $\tilde{\nu} = 1731$ (C=O), 1340–1174 (CH_3SO_3) cm^{-1}; $C_8H_{18}ClNO_9S$ (339.75): calcd C 28.28, H 5.34, N 4.12 Cl 10.43, S 9.43; found C 28.48, H 5.34, N 4.15, Cl 10.23, S 9.27.

(*R*)-3-Chlorocarnitine (20): To a suspension of **14** ($X^- = Cl^-$, 10 g, 50.56 mmol) in CH_3CN (100 mL) was added SO_2Cl_2 (6.28 g, 50.56 mmol), and the mixture was stirred for 18 h. The formed precipitate was collected by filtration to give **21** (5 g, 41% yield, see preparation of **21** for analytical data). After precipitation and repeated treatments with Et_2O the filtrate gave a raw product, which was triturated with warm acetone and then washed with *i*PrOH at 35 °C to give **20** (4 g, 37%). M.p. 104–106 °C; $[\alpha]_D^{20} = -41.2$ ($c = 1$ in H_2O); ^{1}H NMR (300 MHz, D_2O, 25 °C): $\delta = 4.85$ (m, 1H), 3.95 (dd, $^2J = 14.9$ Hz, $^3J = 8.1$ Hz, 1H), 3.85 (dd, $^2J = 14.9$ Hz, $^3J = 2.4$ Hz, 1H), 3.3 (s, 9H), 3.14–2.98 (m, 2H); $C_7H_{15}Cl_2NO_2$ (216.1): calcd C 38.90, H 6.99, N 6.39, Cl 32.35; found C 38.63, H 7.02, N 6.44, Cl 32.58.

(*S*)-Carnitine sulfate (21): To a suspension of **14** ($X^- = Cl^-$, 2 g, 10.12 mmol) in CH_3CN (20 mL) chlorosulfonic acid (1.3 g, 11.15 mmol) was added and the mixture was stirred for 20 h. The formed precipitate was collected to give **21** in 98% yield (2.4 g). M.p. 180 °C (decomp.); $[\alpha]_D^{20} = +49.5$ ($c = 1$ in H_2O); ^{1}H NMR (300 MHz,

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D_2O, 25 °C): δ = 5.2 (m, 1H), 3.85 (dd, ^2J = 14.8 Hz, ^3J = 9.5 Hz, 1H), 3.63 (dd, ^2J = 14.8 Hz, ^3J = 1.6 Hz, 1H), 3.26 (s, 9H), 3.09 (dd, ^2J = 17.2 Hz, ^3J = 3.6 Hz, 1H), 2.93 (dd, ^2J = 17.2 Hz, ^3J = 8.3 Hz, 1H); $C_7H_{15}NO_6S$ (241.264): calcd C 34.85, H 6.27, N 5.81, S 13.29; found C 34.67, H 6.14, N 5.66, S 13.03.

(*R*)-Carnitine (1): Sodium hydrogencarbonate (375.5 mg, 4.47 mmol) was added to a solution of **9** (X^- = MsO^-, 1.5 g, 4.47 mmol) in H_2O (25 mL), and the solution was allowed to stand at room temperature for 20 h with stirring. Additional $NaHCO_3$ (375.5 g, 4.47 mmol) was added and the solution was stirred at room temperature for 24 h. The solution was then transferred into Amberlite IRA-402 (20 g, OH form), and eluted with deionized water till pH = 7. The collected eluate was concentrated (10 mL) and transferred onto Amberlite IRC-50 (20 g), and **1** was eluted completely. Evaporation under vacuum gave **1** in practically quantitative yield (720 mg, >99%). The enantiomeric purity (>99% *ee*) was established by treating the final product with (+)-1-(9-fluorenyl)ethylchloroformate [(+)-FLEC] and examining the resulting ester by HPLC [23].

(*R*)-Carnitine methyl ester (23): Sodium hydrogencarbonate (250 mg, 2.98 mmol) was added to **9** (X^- = MsO^-, 1 g, 2.98 mmol) in DMSO (60 mL) and the resulting solution was stirred at room temperature for 6 h. Methyl alcohol (30 mL) was added, the solution was stirred for another 24 h and the excess alcohol was evaporated under vacuum. Following precipitation with Et_2O a raw product was obtained (80% yield based on NMR). ^{1}H NMR (300 MHz, D_2O, 25 °C): δ = 4.65 (m, 1H), 3.75 (s, 3H), 3.45 (m, 2H), 3.2 (s, 9H), 2.65 (m, 2H); MS (FAB): m/z = 176 [M^+].

(*R*)-Carnitinamide (24): Sodium hydrogencarbonate (250 mg, 2.98 mmol) was added to **9** (X^- = MsO^-, 1 g, 2.98 mmol) in DMSO (60 mL) and the solution was stirred at room temperature for 6 h. NH_3 gas was bubbled through for 20 min and the solution was stirred overnight. After precipitation with Et_2O a raw product was obtained (75% yield based on NMR). ^{1}H NMR (300 MHz, D_2O, 25 °C): δ = 4.62 (m, 1H), 3.5 (m, 2H), 3.2 (s, 9H), 2.55 (d, 2H); MS (FAB): m/z = 161 [M^+].

(*R*)-*N*-(2-Hydroxy)ethyl carnitinamide (25): Sodium hydrogencarbonate (250 mg, 2.98 mmol) was added to **9** (X^- = MsO^-, 1 g, 2.98 mmol) in DMSO (60 mL) and the solution was stirred for 6 h. Ethanolamine (182 mg, 2.98 mmol) was added and the solution was stirred for a further 20 h. Following precipitation with Et_2O a raw product was obtained (80% yield based on NMR). ^{1}H NMR (300 MHz, D_2O, 25 °C): δ = 4.68 (m, 1H), 3.67 (t, ^3J = 5.4 Hz, 2H), 3.47 (m, 2H), 3.37 (t, ^3J = 5.4 Hz, 2H), 3.22 (s, 9H), 2.54 (d, ^3J = 6.4 Hz, 2H) (in [D_6]DMSO the amidic NH triplet was detected at δ = 8); MS (FAB): m/z = 205 [M^+].

(*R*)-*N*-(Aminoiminomethyl) carnitinamide (26): Sodium hydrogencarbonate (250 mg, 2.98 mmol) was added to **9** (X^- = MsO^-, 1 g, 2.98 mmol) in DMSO (60 mL) and the solution was stirred at room temperature for 6 h. Guanidine (176 mg, 2.98 mmol) was added and the solution was stirred for an additional 2 h. Following precipitation with Et_2O a raw material (containing 85% of **26** based on NMR) was obtained that gave **26** contaminated by about 8% residual guanidine after reverse-phase C_{18} HPLC with H_2O as eluant. ^{1}H NMR (300 MHz, D_2O, 25 °C): δ = 4.6 (m, 1H), 3.4 (m, 2H), 3.15 (s, 9H), 2.45 (m, 2H); MS (FAB): m/z = 203 [M^+].

(*R*)-Acetylcarnitine (2) from 20: To **20** (5 g, 23.15 mmol) in dry DMSO (1.5 L) was added $NaHCO_3$ (3.88 g, 23.15 mmol) and the solution was stirred for 24 h till complete formation of β-lactone **12** (crude **12** showed positive optical rotation). Sodium acetate (1.9 g, 23.15 mmol) was added, the solution was stirred for 24 h and following precipitation with acetone a raw material was obtained that gave **2** as inner salt (1.28 g, 27.3%) after reverse-phase C_{18} HPLC with H_2O as eluant. ^{1}H NMR (300 MHz, D_2O, 25 °C): δ = 5.6 (m, 1H), 3.85 (dd, ^2J = 13.6 Hz, ^3J = 8.7 Hz, 1H), 3.6 (dd, ^2J = 13.6 Hz, ^3J = 1 Hz, 1H), 3.2 (s, 9H), 2.65 (dd, ^2J = 15.3 Hz, ^3J = 5.7 Hz, 1H), 2.5 (dd, ^2J = 15.3 Hz, ^3J = 7.9 Hz, 1H), 2.12 (s, 3H).

(*R*)-Acetylcarnitine (2) from 11: Sodium hydrogencarbonate (0.5 g, 5.96 mmol) was added to **11** (X^- = MsO^-, 2 g, 5.96 mmol) in DMSO (120 mL) and the resulting solution was stirred at room temperature for 6 h. Sodium acetate was added (489 mg, 5.96 mmol) and the solution was stirred for a further 20 h. Following precipitation with acetone and Et_2O a raw material was obtained that gave **2** as inner salt (450 mg, 37%) after reverse-phase C_{18} HPLC with H_2O as eluant.

(*R*)-Acetylthiocarnitine (5) from 11: Sodium hydrogencarbonate (1.245 g, 14.82 mmol) was added to **11** (X^- = MsO^-, 5 g, 14.82 mmol) in DMSO (300 mL) and the resulting solution was kept under stirring at room temperature for 6 h. Potassium thioacetate was added (1.693 g, 14.82 mmol) and the solution was stirred for another 2 h. Following precipitation with Et_2O and treatment with CH_3CN/Et_2O, a raw material was obtained that gave (*R*)-**5** inner salt (1.1 g, 34%) after reverse-phase C_{18} HPLC with H_2O as eluant. $[\alpha]_D^{20}$ = −28.7 (c = 1 in H_2O); ^{1}H NMR (300 MHz, D_2O, 25 °C): δ = 4.20 (m, 1H), 3.80 (dd, ^2J = 14.2 Hz, ^3J = 6.6 Hz, 1H), 3.65 (dd, ^2J = 14.2 Hz, ^3J = 3.1 Hz, 1H), 3.20 (s, 9H), 2.70 (d, ^3J = 6.6 Hz, 2H), 2.40 (s, 3H); IR (neat): $\tilde{\nu}$ = 1688 (C=OS), 1589 (C=OO); $C_9H_{17}NSO_3$ (219.302) cm^{-1}: calcd C 49.29, H 7.81, N 6.39, S 14.62; found C 49.10, H 7.93, N 6.01, S 14.28.

Reaction of 10 with Na_2CO_3: Sodium hydrogencarbonate (250 mg, 2.98 mmol) was added to **9** (X^- = MsO^-, 1 g, 2.98 mmol) in DMSO (60 mL) and the solution was stirred at room temperature for 6 h. Sodium carbonate (316 mg, 2.98 mmol) was added and the solution was stirred for an additional 20 h. Following precipitation with Et_2O a raw material containing carnitine was obtained (70% yield based on NMR). The enantiomeric ratio (44:56, (*R*):(*S*)) was established by treating the raw product with (+)-1-(9-fluorenyl)ethylchloroformate [(+)-FLEC] and examining the resulting ester by HPLC [23].

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