Lipase-Catalyzed Enantiomer Separation of 3-Hydroxy-4-(tosyloxy)butanenitrile: Synthesis of (*R*)-GABOB (=(3*R*)-4-Amino-3-hydroxybutanoic Acid) and (*R*)-Carnitine Hydrochloride (=(2*R*)-3-Carboxy-2-hydroxy-*N*,*N*,*N*-trimethylpropan-1-aminium Chloride)

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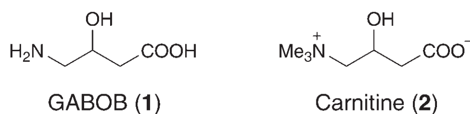
Enzymatic resolution of racemic 3-hydroxy-4-(tosyloxy)butanenitrile ((±)-**5**) by using various lipases in different solvents were studied. The obtained optically pure (3*R*)-3-(acetyloxy)-4-(tosyloxy)-butanenitrile ((*R*)-**6**), upon treatment with aqueous ammonia followed by conc. HCl solution, provided (*R*)-GABOB (=(3*R*)-4-amino-3-hydroxybutanoic acid; (*R*)-**1**). Similarly, reaction of (*R*)-**6** with aqueous trimethylamine solution followed by conc. HCl solution provided (*R*)-carnitine hydrochloride (=(2*R*)-3-carboxy-2-hydroxy-*N*,*N*,*N*-trimethylpropan-1-aminium chloride; (*R*)-**2**·HCl) in an expeditious manner.

Introduction. – γ-Amino-β-hydroxybutanoic acid (GABOB; **1**) is a compound of great pharmacological importance because of its biological function as a neuromodulator in the mammalian central nervous system [1–6]. The (*R*)-form of GABOB is shown to have greater biological activity than its (*S*)-enantiomer [1b][7]. Moreover, the (*R*)-isomer of GABOB serves as a precursor for (*R*)-carnitine ((*R*)-**2**), a compound having a high level of medical significance. Carnitine is a vitamin-like substance and plays an important role in converting stored body fat into energy. Its primary physiological function is to transport long-chain fatty acids through the mitochondrial membrane [5a] [8] into the cellular compartments for oxidation where these fats can be converted into energy. Moreover, there are several medical indications for which carnitine has been prescribed [9][10]. These include its usefulness as a pharmaceutical for hemodialysis [11] (hypolipidemic agent), heart diseases [9], and myopathic deficiencies [5a] [8e,f], *etc.*



The discovery of the adverse effects of (*S*)-carnitine [12] ((*S*)-**2**) and the questionable effectiveness of the racemic mixture in treating angina pectoris [13], coupled with the recent growing interest in therapeutic application of carnitine, has prompted its use in only the (*R*)-form. The high biological importance associated with (*R*)-GABOB ((*R*)-**1**) and (*R*)-carnitine ((*R*)-**2**) continue to stimulate the interest of

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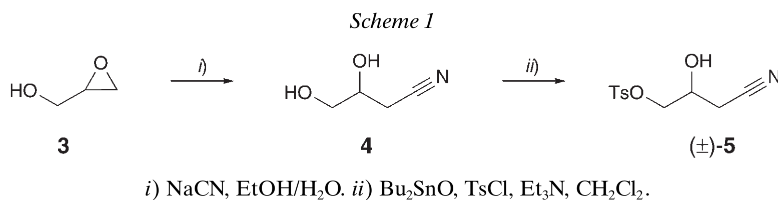
many research groups [14a] for the development of a facile, efficient, and economic approach to their preparation.

Results and Discussion. – On the basis of a *retro*-synthetic pathway, 3-hydroxy-4-(tosyloxy)butanenitrile (**5**), a *β*-hydroxy nitrile, was conceived as an interesting precursor for the synthesis of GABOB and carnitine. History reveals that *β*-hydroxynitriles are important both as technical products and as reagents in organic chemistry [15]. They have been extensively investigated and employed in the preparation of various intermediates for the naturally occurring bioactive compounds [16]. In continuation of our earlier efforts towards the preparation of biologically important compounds or their intermediates by employing biocatalysts [17–20], the present investigation describes an efficient chemoenzymatic preparation of optically pure 3-hydroxy-4-(tosyloxy)butanenitrile and its application towards the synthesis of optically pure (*R*)-GABOB and (*R*)-carnitine. (*R*)-GABOB has been prepared previously from 3-hydroxy-4-(tosyloxy)butanenitrile obtained from D-glucitol [21a] and also by opening of the epoxide ring of glycidyl tosylate by Et_2AlCN [21b]. These reported methods are tedious, employ expensive reagents, and offer low yields. Therefore, the newly developed method is not only versatile but provides higher yields with excellent enantioselectivity.

The preparation of racemic 3-hydroxy-4-(tosyloxy)butanenitrile ((±)-**5**) required for its enzymatic resolution has not been that trivial. Neither the nucleophilic ring opening of glycidyl tosylate with NaCN nor the nucleophilic displacement of the Cl-atom in 1-chloro-3-(tosyloxy)propan-2-ol with NaCN under mild or drastic conditions afforded the desired product. Instead, an indirect approach was adopted to obtain the required *β*-hydroxynitrile. Racemic glycidol (**3**) was subjected to ring opening by NaCN in aqueous alcohol to afford 3,4-dihydroxybutanenitrile (**4**). Compound **4**, without further purification, was selectively monoprotected at the primary OH group by *p*-toluenesulfonyl chloride (TsCl) to give the desired 3-hydroxy-4-(tosyloxy)butanenitrile ((±)-**5**) (*Scheme 1*). However, the reaction of 3,4-dihydroxynitrile with TsCl in the presence of Et_3N and other bases in different solvents afforded only low yields of the desired product. In an interesting development, the yield of the desired monoprotected (±)-**5** was increased to 65% by employing dibutyltin oxide [22] in catalytic amounts. The use of dibutyltin oxide not only improved the rate of tosylation but was also instrumental in selective monoprotection of the primary alcohol (*via* a cyclic intermediate).

The resolution of racemic 3-hydroxy-4-(tosyloxy)butanenitrile ((±)-**5**) was carried out by employing lipases. The preparation of optically pure *β*-hydroxynitriles has generally been carried out by enzymatic approaches like the reduction of the carbonyl

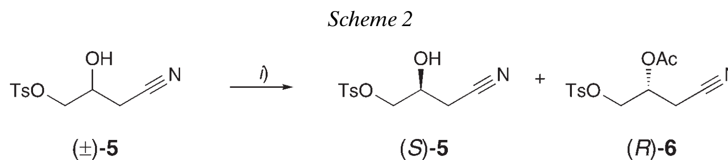
Scheme 1



i) NaCN, $EtOH/H_2O$. *ii*) Bu_2SnO, TsCl, Et_3N, CH_2Cl_2.

group of a ketonitrile by microbes [23] or the lipase-mediated enantioselective hydrolysis of their corresponding acetates [24]. Lipase-mediated resolution of α-hydroxy tosylates [25] has been reported, while this is the first report for the enzymatic resolution of 3-hydroxy-4-(tosyloxy)butanenitrile ((±)-**5**) employing lipases. Therefore, in the present investigation, the lipase-mediated esterification process was applied as well as examined in detail to obtain the best results (*Scheme 2*).

Scheme 2



i) Lipase, vinyl acetate (6 equiv.), diisopropyl ether, r.t.

In the first series of experiments, the efficiency of different commercially available lipases to catalyze the esterification of 3-hydroxy-4-(tosyloxy)butanenitrile ((±)-**5**) was investigated. A variety of lipases including the immobilized ones were screened for the esterification in the presence of vinyl acetate in diisopropyl ether. Among the lipases studied, the immobilized lipase on diatomite from *Pseudomonas cepacia* (PS-D) afforded good yields of both (*S*)-alcohol (*S*)-**5** and (*R*)-acetate (*R*)-**6** in high enantiomer excess (*Table 1*).

Table 1. *Transesterification of (±)-3-Hydroxy-4-(tosyloxy)butanenitrile* ((±)-**5**) *with Various Lipases in Diisopropyl Ether*

Lipase	Time [h]	Alcohol (*S*)-**5**		Acetate (*R*)-**6**		Conversion *C* [%]	Enantiomer ratio *E*
		yield[a]) [%]	ee[b]) [%]	yield[a]) [%]	ee[b]) [%]		
PS-D	13	44	> 99	46	> 99	0.5	1046
PS	24	55	66	37	97	0.4	130
PS-C	7	52	50	40	93	0.35	58.4
AK	25	44	90	47	90	0.5	106
P	67	80	15	12	98	0.13	102
Lipozyme	67	88	06	08	89	0.06	18.28
AYS	67	69	13	23	54	0.2	3.78
CRL	67	68	14	25	51	0.22	4
CAL-B	67	90	03	02	23	0.12	1.4

[a]) Isolated yield. [b]) Determined by chiral HPLC (chiral column *ADH*; *Daicel*) employing hexane/^{i}PrOH 93 : 07 as a mobile phase at 0.7 ml/min and monitored by UV (235 nm).

The stereochemical preference of PS-D during kinetic resolution was in line with *Kazlauskas*' rule (see *Figure*) and further, this can also be predicted based on the existing 3D models of the lipase from *Pseudomonas cepacia* reported by *Theil* and co-workers [26]. According to this model, the substrate binding and orientation is due to two hydrophobic pockets, which are varying by shape – one with a very limited diameter and the other with a spherical shape near the active serine.

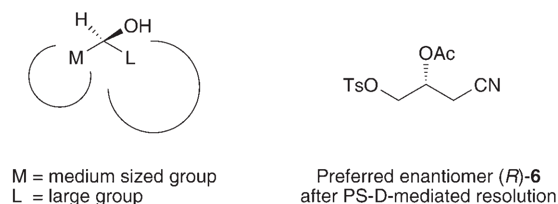


Figure. Kazlauskas' *rule for kinetic resolution*

The enantiomer excess was calculated from the enantiomer ratios obtained by employing a *Chiralpak-ADH* column. The absolute configuration of the alcohol and the acetate was determined by comparison of their chiroptical and chromatographic properties with those of the corresponding known compounds. In this process, comparison of the specific rotation of the unreacted alcohol (*S*)-**5** with that of the reported [16] hydroxy nitrile (*S*)-**5** established the (*S*)-configuration of the alcohol and (*R*)-configuration of the acetate.

As the solvent variation in many cases of lipase-catalyzed kinetic resolution influences the enantiomer or enantiotopic-face selectivity as well as the reaction rates, the effect of the solvents on the substrate (±)-**5**, was also studied (*Table 2*). The solvent effect can be rationalized by the polarity of the solvent. Among the solvents tested for the esterification of (±)-**5**, the hydrophobic solvents gave better results compared to the hydrophilic solvents in terms of enantioselectivity and conversion rates. As the polarity of the solvent increases, the catalytic activity of enzyme diminishes. Hydrophilic solvents may denature enzymes by penetrating into the hydrophobic core of proteins. The use of diisopropyl ether as the solvent provided the best results (*Table 2*). Empirical rules have been developed for the optimization of biocatalytic activity in different organic solvents. The quantitative measurement of solvent can be determined by the parameter *P*, the partition coefficient of the solvent between octanol and H_2O, and the log *P* values for the solvents examined are also given in *Table 2*. The catalytic activity is low in polar solvents having a $\log P < 2$, moderate in solvents having a log *P* between 2 and 4, and high in apolar solvents having a $\log P > 4$.

Optically pure (3*R*)-3-(acetyloxy)-4-(tosyloxy)butanenitrile ((*R*)-**6**) is a highly functionalized chiral compound. It was utilized in the present investigation for the preparation of (*R*)-GABOB ((*R*)-**1**) and (*R*)-carnitine hydrochloride ((*R*)-**2** · HCl). In this process, (*R*)-**1** and (*R*)-**2** were prepared in a single pot starting from optically pure (*R*)-**6** by treating with aqueous ammonia in refluxing EtOH and then treating with conc. HCl solution at 80° to afford (*R*)-**1**, which upon methylation gave (*R*)-**2**. In a similar manner, the treatment of (*R*)-**6** with aqueous trimethylamine and then with conc. HCl solution at 80° yielded optically pure (*R*)-carnitine hydrochloride ((*R*)-**2** · HCl) (*Scheme 3*).

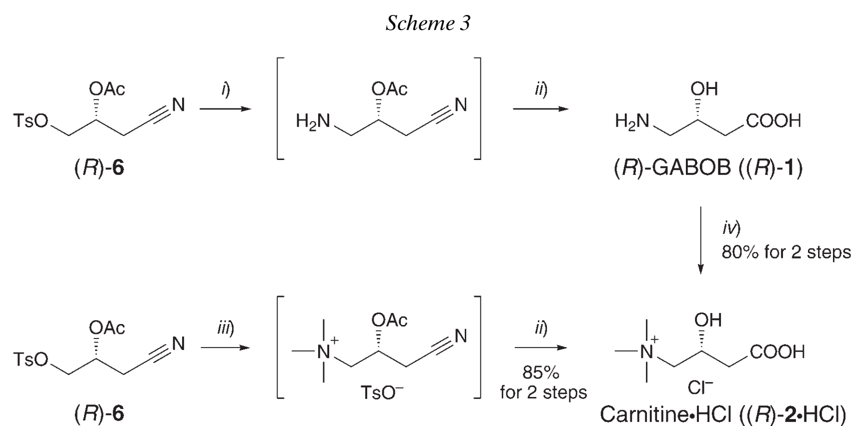
Conclusion. – In summary, an efficient method for the preparation of racemic 3-hydroxy-4-(tosyloxy)butanenitrile ((±)-**5**) and its successful enzymatic resolution employing lipase-catalyzed esterification was reported. This esterification process employing lipase PS-D in diisopropyl ether provided a good conversion with high

Table 2. *Effect of Solvents on the Transesterification of (±)-3-Hydroxy-4-(tosyloxy)butanenitrile* ((±)-**5**) *by Lipase PS-D*

Solvent	Log *P*	Time [h]	Alcohol (*S*)-**5**		Acetate (*R*)-**6**		Conversion *C* [%]	Enantiomeric ratio *E*
			yield[a]) [%]	ee[b]) [%]	yield[a]) [%]	ee[b]) [%]		
$^{i}Pr_2O$	1.9	13	44	> 99	46	> 99	0.5	1046
Hexane	3.5	15	44	86	46	86	0.5	37
Et_2O	0.85	19	48	83	44	86	0.49	1.5
Toluene	2.5	19	40	99	53	84	0.54	59
$CHCl_3$	2.0	72	54	69	38	95	0.42	86
Acetone	– 0.23	76	86	11	07	> 99	0.1	223
Tetrahydrofuran	0.49	69	90	04	03	> 99	0.04	397
Dioxane	– 1.1	78	91	04	03	> 99	0.04	397

[a]) Isolated yield. [b]) Determined by chiral HPLC (chiral column *ADH*; *Daicel*) employing hexane/^{i}PrOH 93 : 07 as a mobile phase at 0.7 ml/min and monitored by UV (235 nm).

Scheme 3



i) Aq. NH_3 soln., EtOH, reflux. *ii*) Conc. HCl soln. *iii*) Aq. Me_3N soln., EtOH, reflux. *iv*) MeI, KOH, $MeOH/H_2O$.

enantioselectivities. Moreover, this investigation also established that immobilized lipases are better than nonimmobilized lipases for this process. Further, optically pure (3*R*)-3-(acetyloxy)-4-(tosyloxy)butanenitrile ((*R*)-**6**) was employed in a short and efficient synthesis of the biologically important (*R*)-GABOB ((*R*)-**1**) and (*R*)-carnitine hydrochloride ((*R*)-**2** · HCl). This chemoenzymatic protocol involves very few steps compared to earlier methods, hence it is efficient and practical.

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Experimental Part

1. *General.* Unless specified, all solvents and reagents were reagent grade and used without purification. Reactions involving moisture-sensitive reagents were performed under N_2 in glassware that

had been oven-dried. M.p.: electrothermal melting-point apparatus; uncorrected. Anal. TLC: *Merck* prepared plates (silica gel *60 F-254* on glass). Column chromatography (CC): *Acme* silica gel (100–200 mesh). Chiral HPLC: *Shimadzu LC-10AT* system controller, *SPD-10A* fixed-wavelength UV monitor as detector; chiral column *ADH* (*Daicel*), hexane/^{i}PrOH 93:07 as mobile phase, flow 0.7 ml/min, UV monitoring at 235 nm. Optical rotations: *SEPA-300* (*Horiba*) digital polarimeter. IR Spectra: *Perkin-Elmer 683* or *1310* spectrometer; cm^{-1}. ^{1}H-NMR Spectra: *Gemini 200* (200 MHz) or *AV 300* (300 MHz) instrument; $CDCl_3$ or (D_6)DMSO solns.; chemical shifts δ in ppm with Me_4Si as an internal standard, coupling constants J in Hz. Low-resolution MS: *CEC-21-100B Finnigan Mat-1210* or *VG-7070 H* micromass mass spectrometer; in m/z.

Lipases. Pseudomonas cepacia lipase immobilized on diatomite (PS-D), *Pseudomonas cepacia* (PS), *Pseudomonas cepacia* lipase immobilized on modified ceramic particles (PS-C), and *Pseudomonas fluorescens* lipase (AK) were obtained from *Amano Pharmaceutical Company*, Japan, *Pseudomonas fluorescens* lipase immobilized in Sol-Gel-AK on sintered glass (P), lipase immobilized from *Mucor meihei* (MML), and *Candida antartica* lipase immobilized in Sol-Gel-AK on sintered glass (CAL B) were from *Fluka*, and *Candida rugosa* lipase (CRL) was from *Sigma*.

2. *3-Hydroxy-4-{[(4-methylphenyl)sulfonyl]oxy}butanenitrile* ((±)-**5**). To a stirred soln. of glycidol (=oxirane-2-methanol; **3**; 14.80 g, 200.00 mmol) in EtOH (120 ml) were added H_2O (240 ml) and NaCN (9.80 g, 200.00 mmol), and stirring was continued overnight. After completion of the reaction, the solvent was evaporated and the residue filtered through a silica gel pad. Evaporation of the solvent of the filtrate afforded 3,4-dihydroxybutanenitrile (**4**). To **4** (10.10 g, 100.00 mmol) dispersed in CH_2Cl_2 (250 ml) was added dibutyltin oxide (4.98 g, 20.00 mmol), Et_3N (25.25 g, 250.00 mmol), and *p*-toluenesulfonyl chloride (=4-methylbenzenesulfonyl chloride; 20.95 g, 110.00 mmol) under N_2. The resultant mixture was stirred at r.t. After completion of the reaction (2 h; TLC monitoring), H_2O (150 ml) was added, and the org. layer was separated. The aq. layer was extracted with CH_2Cl_2 (3 × 150 ml), the combined org. layer dried (Na_2SO_4) and concentrated, and the residue purified by CC (AcOEt/hexane 35:65): pure **5** (16.5 g, 65%). IR (neat): 3474, 3059, 2933, 2902, 2220, 1584, 1349, 1169, 1098, 996. ^{1}H-NMR (300 MHz, $CDCl_3$): 2.48 (*s*, 3 H); 2.52–2.67 (*m*, 2 H); 4.06 (*d*, J=5.4, 2 H); 4.15–4.22 (*m*, 1 H); 7.38 (*d*, J=8.3, 2 H); 7.80 (*d*, J=8.3, 2 H). EI-MS: 255 (M^+), 173, 155, 139, 122, 91.

3. *3-(Acetyloxy)-4-{[(4-methylphenyl)sulfonyl]oxy}butanenitrile* ((±)-**6**). To (±)-**5** (1.27 g, 5.00 mmol) under N_2 were added Ac_2O (2.04 g, 20.00 mmol) and pyridine (0.43 g, 5.50 mmol), and the resultant mixture was stirred at r.t. overnight. After completion of the reaction (TLC), the mixture was diluted with AcOEt (25 ml) and treated with 1N HCl (20 ml). The org. layer was washed with brine and dried (Na_2SO_4), the solvent evaporated, and the residue purified by CC (AcOEt/hexane 25:75): **6** (90%, 1.33 g). ^{1}H-NMR (200 MHz, $CDCl_3$): 2.09 (*s*, 3 H); 2.49 (*s*, 3 H); 2.75 (*d*, J=5.9, 2 H); 3.67–4.18 (*m*, 2 H); 5.09–5.15 (*m*, 1 H); 7.38 (*d*, J=8.0, 2 H); 7.79 (*d*, J=8.0, 2 H). EI-MS: 297 (M^+), 255, 185, 172, 155, 139, 91.

4. *Kinetic Resolution of* (±)-**5**. To a soln. of (±)-**5** (1.5 g, 5.88 mmol) in $^{i}Pr_2O$ (15 ml) were successively added lipase PS-D (1.20 g) and vinyl acetate (6 equiv.) and shaken at r.t. in an orbital shaker. After *ca.* 50% completion of the reaction as indicated by HPLC analysis, the mixture was filtered and the residue washed thrice with $^{i}Pr_2O$. The combined org. layer was concentrated and the residue purified by CC (AcOEt/hexane 30:70): (*R*)-**6** followed by (*S*)-**5**.

*Data of (3*S*)-3-Hydroxy-4-{[(4-methylphenyl)sulfonyl]oxy}butanenitrile* ((*S*)-**5**): $[\alpha]_D^{26} = -14.5$ (c = 1.45, EtOH) ([14b]: $[\alpha]_D^{25} = -14.2$ (c = 1.72, EtOH)). IR, NMR, and MS: identical to those of (±)-**5**.

*Data of (3*R*)-3-(Acetyloxy)-4-{[(4-methylphenyl)sulfonyl]oxy}butanenitrile* ((*R*)-**6**): $[\alpha]_D^{28} = +22.6$ (c = 1.0, $CHCl_3$). NMR and MS: identical to those of (±)-**6**.

5. *(3*R*)-4-Amino-3-hydroxybutanoic Acid* ((=*R*)-GABOB; (*R*)-**1**). To a soln. of (*R*)-**6** (4.07 g, 13.70 mmol) in EtOH (40 ml) was added excess aq. NH_3 soln., the mixture refluxed overnight, and then the solvent evaporated. To the resulting residue, conc. HCl soln. was added, and the mixture was heated to 80° for 6 h. After evaporation, the residue containing crude (*R*)-**1** was purified by an ion-exchange chromatography (*Amberlite IR-120 H*$^+$, H_2O until neutral, then 10% NH_4OH soln.). Concentration of the basic fractions gave a thick oil, which was dissolved in a minimum amount of H_2O, and abs. EtOH was added to provide (*R*)-**1** as a white solid (84%). Recrystallization from H_2O/EtOH provided pure (*R*)-**1** (73%). White crystals. M.p. 211–213° ([14b]: 206–208°). $[\alpha]_D^{28} = -20.7$ (c = 1.0, H_2O) ([14b]: $[\alpha]_D^{25}$ =

-18.6 ($c = 0.5$, H_2O)). ^{1}H-NMR (200 MHz, D_2O): 2.43 (*d*, $J = 5.9$, 2 H); 2.95 (*dd*, $J = 13.4, 9.7$, 1 H); 3.18 (*dd*, $J = 13.4, 3.7$, 1 H); 4.10 – 4.30 (*m*, 1 H). ^{13}C-NMR (50 MHz, D_2O): 42.3; 44.0; 65.5; 178.5. EI-MS: 118 ($[M-H]^+$), 74, 60, 43.

6. *(*R*)-Carnitine Hydrochloride* (= *(2*R*)-3-Carboxy-2-hydroxy*-N,N,N-*trimethylpropan-2-aminium Chloride* (*R*)-**2** · HCl). To a soln. of (*R*)-**6** (4.07 g, 13.70 mmol) in EtOH (40 ml) was added Me_3N (25 – 27 wt.-% in H_2O; 26 ml, 109.6 mmol). The mixture was refluxed overnight and then concentrated. To the resulting residue, conc. HCl soln. was added, and the mixture heated at 80° for 6 h. After evaporation, the residue containing crude (*R*)-**2** was purified by ion-exchange chromatography (*Amberlite IR-120 H*$^+$, H_2O until neutral, then 10% NH_4OH soln.). Concentration of the basic fractions gave a thick oil, which was again acidified with conc. HCl soln. Evaporation of the solvent yielded carnitine hydrochloride. Trituration with ^{i}PrOH afforded (*R*)-**2** · HCl (85%). Colorless solid.

In another approach, MeI (6.82 g, 48.00 mmol) in MeOH (60 ml) was added to a soln. of (*R*)-**1** (0.95 g, 7.98 mmol) and KOH (2.69 g, 48.00 mmol) in H_2O (25 ml). After stirring overnight at r.t., the mixture was concentrated, the residue dissolved in H_2O (60 ml), and the soln. extracted with $CHCl_3$ (3×60 ml). The combined org. phase was again extracted with H_2O (2×30 ml), and the combined aq. phase containing crude carnitine was evaporated to *ca.* 20% of the total volume and then treated with 6N HCl (20 ml) for 2 h. Then the mixture was concentrated and the residue purified by ion-exchange chromatography (*Amberlite IR-120 H*$^+$, H_2O until neutral, then 10% NH_4OH soln.). Concentration of the basic fractions gave a thick oil, which was again acidified with conc. HCl soln. Evaporation of the solvent afforded carnitine hydrochloride in 80% yield. Trituration with ^{i}PrOH afforded (*R*)-**2** · HCl. Colorless solid. M.p. 144 – 146° ([14b]: 144 – 146°). $[\alpha]_D^{30} = -22.4$ ($c = 1.5$, H_2O) ([14b]: $[\alpha]_D^{20} = -20.6$ ($c = 2.1$, H_2O)). ^{1}H-NMR (200 MHz, D_2O): 2.60 – 2.70 (*m*, 2 H); 3.24 (*s*, 9 H); 3.48 – 3.55 (*m*, 2 H); 4.60 – 4.75 (*m*, 1 H). ^{13}C-NMR (50 MHz, D_2O): 45.8; 56.9; 66.9; 73.0; 180.9.

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