Synthesis of carnitine benzyl esters as prodrugs

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The introduction of C-2 acyl groups and a benzyl ester onto L-carnitine generated a series of carnitine benzyl esters as prodrug with improved liposolubility, stability and bioavailability.

Keywords: carnitine benzyl esters, prodrug, synthesis, neuroprotective

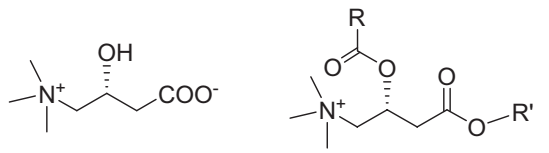
Carnitine (4-*N*-trimethylammonium-3-hydroxybutyric acid), an essential amino acid-like inner salt, is widespread in the tissues of animals, plants and microorganisms.[1] Of its two optically active enantiomers (D and L), only L-carnitine (LC, Fig. 1), a natural occurring betaine with vitamin properties, has attracted considerable attention in recent years. It is non-toxic, non-mutagenic, and has almost no skin irritation properties.[2] It also plays important role in improving myocardial function, pregnancy rate and spermatogenesis in patients affected by male infertility.[3]

Another interesting property of L-carnitine is its bioavailability based on its transport across membranes *via* some carnitine transporters, *e.g*. OCTN1, OCTN2, OCTN3, CT2, *etc*. and the amino acid transporter $ATB^{0,+}$, which can recognise neutral as well as cationic amino acids based on the quaternary amine of carnitine.[4] These transporters, mostly found in peripheral tissues, are responsible for the high-affinity uptake of carnitine across the plasma membrane, and also transport carnitine in other specialised tissues such as sperm.[5] This characteristic makes carnitine particularly useful for the transport of drugs directly to the site where they can exert their pharmacological activity, preventing the drug being metabolised by the liver.[6]

Because of the importance of L-carnitine in pharmaceutical sciences, a number of synthetic derivatives of L-carnitine have been developed with improved therapeutically function or effect over carnitine itself. They are mainly hydroxyl- and/or carboxyl-based derivatives (Fig. 1).[7-9] Nevertheless, these water-soluble quaternary ammonium salts are extremely hydrophilic. The hydroxyl-based carnitine esters do not have a significant improvement in liposolubility,[10] which is a disadvantage for pharmaceutical preparations. Only capsules, tablets and freeze-dried powder injections, have appeared on the market as drugs at present.[11] This increases the potential for designing a wide variety of carboxyl-based carnitine prodrugs that can utilise $ATB^{0,+}$ as drug delivery system.

To obtain improved oral bioavailability *via* introduction of hydrophobic esters, the benzyl ester was selected owing to its solution-stable and biolabile properties.[12,13] Thus, our efforts in developing L-carnitine derivatives as appropriate pharmaceuticals led us to synthesise a series of novel benzyl carnitine esters (**4a**–**4j**), serving as potential prodrug of carnitine.

The synthetic route is summarised in Scheme 1. Briefly, the acyl chorides **1** of different carboxylic acids, were reacted with the commercial available L-carnitine in the presence of trifluoroacetic acid (TFA) at 40–50°C to generate acyl carnitine derivatives **2**. The carboxyl then was converted to the acid chloride with oxalyl chloride and and coupled with benzyl alcohol to give target compounds **4a**–**j**. The ^{1}H NMR, IR and high resolution mass spectra data for the final products are in good agreement with the title compound and exhibit no discernible impurities.



(*L*)-carnitine **1** hydroxyl- and carboxyl-based carnitine derivatives

Fig. 1 Chemical structures of L-carnitine **1** and its derivatives.

Results and discussion

The synthetic methods for preparing carnitine derivatives include ion exchange resin,[14] 1-iodooctadecane/DMF-dioxane,[15] condensation reaction by using dicyclohexy-carbodiimmide (DCC) and 4-(dimethylammino)pyridine (DMAP),[8] and 1-bromo-1-chloroethene method.[16] However, these methods almost involve tedious work-up process for the final products, which is unsatisfactory for commercial production. Our improved work-up is relatively simple by using appropriate recrystallisation solvents with good yields.

Studies have shown over several decades that L-carnitine and its derivatives may play a role in neuroprotection and neurotransmission.[17] Hence, compounds **4a**–**j** were evaluated for their neuroprotective effect *in vitro* by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) colourimetric assay on $CoCl_2$-induced apoptosis in PC12 cell line (pheochromocytoma), and the results were summarised in Table 1. Parallel experiments for the corresponding debenzylated compounds **2a**–**j** were also conducted.

The OD_{630} values were calculated as the ratio of the IC_{50} in the presence or absence of carnitine derivatives (10 mg/l) before addition of $CoCl_2$ (300 μM), using a reference wavelength of 630 nm and a test wavelength of 570 nm. All the data are mean ± SD of three independent experiments.

From Table 1, we can see that almost all the tested carnitine benzyl esters (**4a**–**j**) could block $CoCl_2$-induced apoptosis in PC12 cells.[18] The results indicated, that the newly synthesised carnitine derivatives have a neuroprotective activity. More importantly, with the alteration of their physicochemical properties, they may prolong their effective time and will be devoid of side effects, through increasing the liposolubility, stability and bioavailability. They may be used as potential prodrugs.

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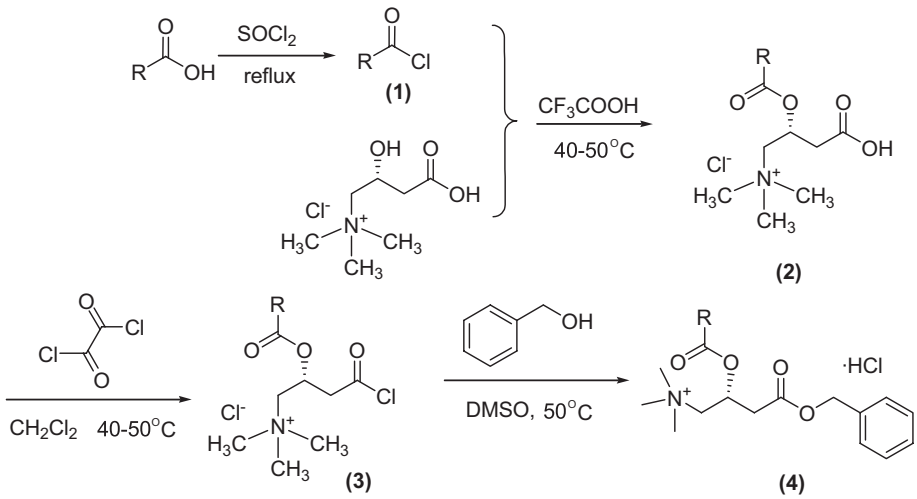
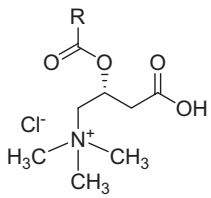
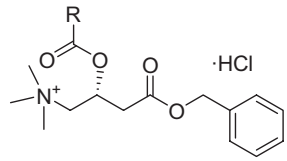


Scheme 1

Table 1 Effect of derivatives of **2a–j** and **4a–j** on the PC12 cells viability induced by $CoCl_2$



Acyl carnitine **2**



Acyl carnitine benzyl ester **4**

Entry	R	M.p./°C	Yield/%	OD_{630} (acyl carnitine **2**)	OD_{630} (carnitine benzoate ester **4**)
Control				1	1
$CoCl_2$ (alone)				0.4589	0.4589
4a		180.5~183.0	58	0.4696	0.4630
4b	CH_3	146.0~148.0	68	0.5574	0.5602
4c	CH_2CH_3	108.0~110.5	71	0.6039	0.6054
4d	$CH_2CH_2CH_3$	112.0~114.0	66	0.7143	0.7042
4e	$CH_2CH(CH_3)_2$	134.0~136.0	69	0.7026	0.7027
4f	$CH_2CH_2CH_2CH_3$	136.0~138.0	70	0.7186	0.7185
4g	$CH_2CH_2CH(CH_3)_2$	117.5~120.0	74	0.7339	0.7355
4h	$CH_2CH_2CH_2CH_2CH_3$	129.5~131.0	73	0.5030	0.4959
4i	$CH_2(CH_2)_5CH_3$	115.5~118.0	62	0.5342	0.5467
4j	$(CH_2)_7CH{=}CH(CH_2)_7CH_3$	65.0~67.0	52	0.5226	0.5153

*$P<0.05$; **$P<0.01$ *vs* negative control (300 μM $CoCl_2$ alone).

It is likely that the esters will be converted to biologically active carboxylic acid. Hence their the metabolic stability and effective blood drug levels might be improved. The corresponding experimental evaluation is underway, and the results will be reported in the near future.

Conclusion

In conclusion, novel carnitine benzyl esters were prepared by acylation and esterification using L-carnitine and different substituted carboxylic acids as starting materials. The neuroprotective effect of final compounds (**4a–j**) and their corresponding debenzylated compounds (**2a–j**) *in vitro* showed that they may be potential prodrugs with improved neuroprotective effect.

Experimental

The course of the reaction and products were routinely monitored by thin-layer chromatography (TLC) on silica gel (precoated GF_{254} plates) and visualised with iodine. All commercially available compounds were directly used without further purification. Yields refer to isolated products after purification. Melting points were determined with capillary apparatus and uncorrected. IR spectra were recorded in the range of 4000–600 cm^{-1} using a Nicolet Nexus 470FT-Spectrometer with KBr disks. ^1H NMR spectra was determined in D_2O on a Bruker 400 MHz spectrometer with TMS as an internal standard. Electrospray ionisation mass spectrometry (ESI–MS) was performed on an API 4000 spectrometer. Elemental analyses were carried out on an Elementar Elvario III C, H, and N elemental analyser.

General procedure for the preparation of compounds **4a–j**

A mixture of the carboxylic acid (1 mol) and thionyl chloride (4.2 mol) was stirred under reflux until the disappearance of the starting materials as evidenced by TLC (about 4 h). After reaction, the excess thionyl chloride was removed in *vacuum,* and the yellow oily product **1** was directly used in the following reaction without any purification.

L-Carnitine hydrochloride (30.06 g, 0.125 mol) was dissolved in anhydrous trifluoroacetic acid 100 ml, followed by the dissolution of

the above compound **1** in an ice bath. The resulting mixture was stirred under 45–50°C until the starting material disappeared as shown by TLC (about 20 h). After the reaction was completed, the solvent was removed *in vacuo* and acetone 400 ml was added. After stirring for another 2 h, the white precipitate was removed, and then diethyl ether 800 ml was added to the filtrate. The resultant mixture was cooled to room temperature and filtered to give the white precipitate which was then collected and recrystallised from ethanol–diethyl ether (V : V = 1 : 4) to give compounds **2a**–**j** as colourless needles.

Compound **2** (0.1 mol) was dissolved in of dichloromethane 100 ml, below 0°C and freshly distilled oxalyl chloride (15 ml, 0.12 mol) was added dropwise. The resulting mixture was reacted at 45–50°C until the reaction was complete. The solvent was removed *in vacuo* and the white residue was dissolved in of anhydrous dimethyl sulfoxide (DMSO) 100 ml followed by the addition of benzyl alcohol (0.18 mol) at room temperature. The resulting mixture reacted at 50°C until the disappearance of starting materials as evidenced by TLC. After adding diethyl ether(80 ml) and stirring for 2 h, the white precipitate was collected and dried *in vacuo* below 40°C. The crude product was recrystallised by the mixture of ethyl acetate/diethyl ether (*V*: *V* = 1 : 2, 75 ml) to give the target compounds **4a**–**j**.

(E)-4-(benzyloxy)-2-(cinnamoyloxy)-N,N,N-trimethyl-4-oxobutan-1-ammonium chloride **(4a):** M.p. 180.5–183.0°C; ^{1}H NMR δ 2.71–2.76 (2H, m, –$\underline{CH_2}$COO–), 2.98 (9H, s, ($\underline{CH_3}$)$_3$N–), 3.58 (2H, d, *J* = 6.9 Hz, –N$\underline{CH_2}$–), 4.79 (2H, s, –$\underline{CH_2}C_6H_5$), 5.60 (1H, m, –N$\underline{CH_2}$CH–), 6.05 (1H, d, *J* = 15.9 Hz, –$\underline{CH}$=CHCOO–), 6.05 (1H, d, *J* = 15.9 Hz, –CH=$\underline{CH}$COO–), 6.84–7.00 (5H, m, –$CH_2\underline{C_6H_5}$), 7.10–7.4 (5H, m, –CH=CH$\underline{C_6H_5}$); IR cm^{-1} (KBr plate): 3067, 3027 (γ_{CH}), 1729, 1695 ($\gamma_{C=O}$), 1669, 1628 ($\gamma_{C=C}$), 1504, 1588 (γ_{Ar-C}), 1486, 1431 ($\delta_{CH2,CH3}$), 1208 (ν_{C-N}), 681, 695, 723, 758, 917 (aromatic ring); ESI–MS (*m/z*): 436.2 [M + H]$^+$. Anal. Calcd. for $C_{23}H_{28}ClNO_4$: C, 66.1; H, 6.8; N, 3.4. Found: C, 65.9; H, 6.9; N, 3.4%.

(R)-2-acetoxy-4-(benzyloxy)-N,N,N-trimethyl-4-oxobutan-1-ammonium chloride **(4b):** M.p. 146.0–148.0°C; ^{1}H NMR δ 2.01 (3H, s, $\underline{CH_3}$COO–), 2.62–2.65 (2H, m, –$\underline{CH_2}$COO–), 3.12 (9H, s, ($\underline{CH_3}$)$_3$N–), 3.58 (2H, m, –N$\underline{CH_2}$–), 4.56 (2H, s, –$\underline{CH_2}C_6H_5$), 5.53 (1H, m, –N$\underline{CH_2}$CH–), 7.35–7.39 (5H, m, –$CH_2\underline{C_6H_5}$); IR cm^{-1} (KBr plate): 3013 (γ_{CH}), 1733 ($\gamma_{C=O}$) 1478, 1411 ($\delta_{CH2,CH3}$), 1215 (ν_{C-N}), 709, 726, 768, 934 (aromatic ring); ESI–MS (*m/z*): 294.4 [M + H]$^+$. Anal. Calcd. for $C_{16}H_{24}ClNO_4$: C, 58.3; H, 7.3; N, 4.3. Found: C, 58.3; H, 7.3; N, 4.3%.

(R)-4-(benzyloxy)-N,N,N-trimethyl-4-oxo-2-(propionyloxy)butan-1-ammonium chloride **(4c):** M.p. 108.0–110.5°C; ^{1}H NMR δ 1.00–1.05 (3H, t, $\underline{CH_3}CH_2COO$–), 2.51–2.58 (2H, m, –$CH_3\underline{CH_2}$COO–), 3.03–3.08 (2H, m, –$\underline{CH_2}$COO–), 3.19 (9H, s, ($\underline{CH_3}$)$_3$N–), 3.58 (2H, dd, *J* = 8.4, 12.8 Hz, –N$\underline{CH_2}$–), 4.49 (2H, s, –$\underline{CH_2}C_6H_5$), 5.01 (1H, m, –N$\underline{CH_2}$CH–), 7.25–7.30 (5H, d, *J* = 2.8 Hz, –$CH_2\underline{C_6H_5}$); IR cm^{-1} (KBr plate): 3061, 2926 (γ_{CH}), 1725 ($\gamma_{C=O}$), 1479, 1403 ($\delta_{CH2,CH3}$), 1177 (ν_{C-N}), 727, 768, 876, 933 (aromatic ring), ESI–MS (*m/z*): 308.7 [M + H]$^+$. Anal. Calcd. for $C_{17}H_{26}ClNO_4$: C, 59.4; H, 7.6; N, 4.1. Found: C, 59.5; H, 7.7; N, 4.0%.

(R)-4-(benzyloxy)-2-(butyryloxy)-N,N,N-trimethyl-4-oxobutan-1-ammonium chloride **(4d):** M.p. 112.0–114.0°C; ^{1}H NMR δ 0.75–0.81 (3H, t, $\underline{CH_3}CH_2$–), 1.40–1.47 (2H, m, –$CH_3\underline{CH_2}$–), 2.18–2.22 (2H, m, –$CH_2\underline{CH_2}$COO–), 3.14–3.27 (2H, m, –$CH\underline{CH_2}$COO–), 3.05 (9H, s, ($\underline{CH_3}$)$_3$N–), 3.58 (2H, dd, *J* = 8.4, 12.8 Hz, –N$\underline{CH_2}$–), 5.10 (2H, s, –$\underline{CH_2}C_6H_5$), 5.57 (1H, m, –NCH$_2\underline{CH}$–), 7.31–7.37 (5H, d, –$CH_2\underline{C_6H_5}$); IR cm^{-1} (KBr plate): 2966(γ_{CH}), 1739 ($\gamma_{C=O}$), 1690 ($\gamma_{C=C}$), 1504, 1588 (γ_{Ar-C}), 1483, 1456 ($\delta_{CH2,CH3}$), 1173 (ν_{C-N}), 681, 699, 752, 968 (aromatic ring); ESI–MS (*m/z*): 322.4 [M + H]$^+$. Calcd. for $C_{18}H_{28}ClNO_4$: C, 60.4; H, 7.9; N, 3.9. Found: C, 60.5; H, 7.8; N, 3.9%.

(R)-4-(benzyloxy)-2-(isobutyryloxy)-N,N,N-trimethyl-4-oxobutan-1-ammonium chloride **(4e):** M.p. 134.0–136.0°C; ^{1}H NMR δ 0.97–1.09 (6H, m, ($\underline{CH_3}$)$_2$CH–), 2.46–2.51 (1H, m, (CH_3)$_2\underline{CH}$–), 2.75–2.78 (2H, m, –$\underline{CH_2}$COO–), 2.83 (9H, s, ($\underline{CH_3}$)$_3$N–), 3.56 (2H, dd, *J* = 3.6, 14.4 Hz, –N$\underline{CH_2}$–), 5.12 (2H, s, –$\underline{CH_2}C_6H_5$), 5.59 (1H, m, –NCH$_2\underline{CH}$–), 7.32–7.38 (5H, m, –$CH_2\underline{C_6H_5}$); IR cm^{-1} (KBr plate): 3229, 2971 (γ_{CH}), 1732 ($\gamma_{C=O}$), 1480, 1391 ($\delta_{CH2,CH3}$), 1179 (ν_{C-N}), 700, 718, 755, 799, 935 (aromatic ring); ESI–MS (*m/z*): 422.6 [M + H]$^+$. Calcd. for $C_{18}H_{28}ClNO_4$: C, 60.4; H, 7.9; N, 3.9. Found: C, 60.3; H, 7.9; N, 3.9%.

(R)-4-(benzyloxy)-N,N,N-trimethyl-4-oxo-2-(pentanoyloxy)butan-1-ammonium chloride **(4f):** M.p. 136.0–138.0°C; ^{1}H NMR δ 0.77–0.80 (3H, t, $\underline{CH_3}CH_2$–), 0.88–0.93 (2H, m, –$CH_3\underline{CH_2}$–), 1.45–1.47 (2H, m, –$CH_2\underline{CH_2}CH_2$–), 2.54–2.62 (2H, m, –$CH_2\underline{CH_2}$COO–), 3.04–3.07 (2H, m, –$CH\underline{CH_2}$COO–), 3.13 (9H, s, ($\underline{CH_3}$)$_3$N–), 3.58 (2H, dd, *J* = 2.8, 10.2 Hz, –N$\underline{CH_2}$–), 5.08 (2H, s, –$\underline{CH_2}C_6H_5$), 5.55 (1H, m, –NCH$_2\underline{CH}$–), 7.34 (5H, d, *J* = 8.4 Hz, –$CH_2\underline{C_6H_5}$); IR cm^{-1} (KBr plate): 3020, 2959 (γ_{CH}), 1732 ($\gamma_{C=O}$), 1480, 1455 ($\delta_{CH2,CH3}$), 1173 (ν_{C-N}), 616, 700, 753, 936 (aromatic ring); ESI–MS (*m/z*): 337.4 [M + H]$^+$. Calcd. for $C_{19}H_{30}ClNO_4$: C, 61.4; H, 8.1; N, 3.8. Found: C, 61.3; H, 8.3; N, 3.7%.

(R)-4-(benzyloxy)-N,N,N-trimethyl-2-(3-methylbutanoyloxy)-4-oxobutan-1-ammonium chloride **(4g):** M.p. 117.5–120.0°C; ^{1}H NMR δ 0.78–0.81(3H, d, *J* = 6.6 Hz, ($\underline{CH_3}$)$_2$CH–), 0.85–0.87 (3H, d, *J* = 6.6 Hz, ($\underline{CH_3}$)$_2$CH–), 2.15–2.19(1H, m, (CH_3)$_2\underline{CH}$–), 2.77–2.84 (2H, m, –$\underline{CH_2}$COOCH–), 3.10–3.15 (2H, m, –$\underline{CH_2}$COOCH$_2$–), 3.07 (9H, s, ($\underline{CH_3}$)$_3$N–), 3.56–3.76 (2H, dd, *J* = 10.2, 14.4 Hz, –N$\underline{CH_2}$–), 5.11 (2H, s, –$\underline{CH_2}C_6H_5$), 5.59 (1H, m, –NCH$_2\underline{CH}$–), 7.34–7.38 (5H, m, –$CH_2\underline{C_6H_5}$). IR cm^{-1} (KBr plate): 3020, 2957 (γ_{CH}), 1736, 1704 ($\gamma_{C=O}$), 1488, 1412 ($\delta_{CH2,CH3}$), 1181 (ν_{C-N}), 665, 699, 752, 880, 938 (aromatic ring); ESI–MS (*m/z*): 336.5 [M + H]$^+$. Calcd. for $C_{19}H_{30}ClNO_4$: C, 61.4; H, 8.1; N, 3.8. Found: C, 61.3; H, 8.2; N, 3.6%.

(R)-4-(benzyloxy)-2-(hexanoyloxy)-N,N,N-trimethyl-4-oxobutan-1-ammonium chloride **(4h):** M.p. 129.5–131.0°C; ^{1}H NMR δ 0.62–0.64 (3H, m, $\underline{CH_3}CH_2$–), 0.96–1.02 (4H, m, –$CH_3\underline{CH_2}CH_2$–), 1.98–2.07 (2H, m, –$\underline{CH_2}CH_2$COO–), 2.53–2.62 (2H, m, –$CH_2\underline{CH_2}$COO–), 3.07–3.12 (2H, m, –$CH\underline{CH_2}$COO–), 3.04 (9H, s, ($\underline{CH_3}$)$_3$N–), 3.56 (2H, m, –N$\underline{CH_2}$–), 4.90 (2H, s, –$\underline{CH_2}C_6H_5$), 5.50–5.57 (1H, m, –NCH$_2\underline{CH}$–), 7.29–7.31 (5H, m, –$CH_2\underline{C_6H_5}$); IR cm^{-1} (KBr plate): 3014, 2957 (γ_{CH}), 1734 ($\gamma_{C=O}$), 1480, 1413 ($\delta_{CH2,CH3}$), 1174 (ν_{C-N}), 700, 727, 768, 935 (aromatic ring); ESI–MS (*m/z*): 350.4 [M + H]$^+$. Anal. Calcd. for $C_{20}H_{32}ClNO_4$: C, 62.3; H, 8.4; N, 3.6. Found: C, 62.3; H, 8.4; N, 3.6%.

(R)-4-(benzyloxy)-N,N,N-trimethyl-2-(octanoyloxy)-4-oxobutan-1-ammonium chloride **(4i):** M.p. 115.5–118.0°C; ^{1}H NMR δ 0.76–0.78 (3H, t, $\underline{CH_3}CH_2$–), 1.08–1.24 (8H, m, –$CH_3(\underline{CH_2})_2$–), 2.35–2.40 (2H, m, –$\underline{CH_2}CH_2$COO–), 2.75–2.78 (2H, m, –$CH_2\underline{CH_2}$COO–), 3.10–3.16 (2H, m, –$CH\underline{CH_2}$COO–), 3.13 (9H, s, ($\underline{CH_3}$)$_3$N–), 3.60 (2H, dd, *J* = 8.7, 14.4 Hz, –N$\underline{CH_2}$–), 5.08 (2H, s, –$\underline{CH_2}C_6H_5$), 5.57–5.63 (1H, m, –NCH$_2\underline{CH}$–), 7.33–7.39 (5H, m, –$CH_2\underline{C_6H_5}$); IR cm^{-1} (KBr plate): 3017, 2927 (γ_{CH}), 1735, 1698 ($\gamma_{C=O}$), 1646 ($\gamma_{C=C}$), 1482, 1416 ($\delta_{CH2,CH3}$), 1174 (ν_{C-N}), 666, 726, 880, 938 (aromatic ring); ESI–MS (*m/z*): 378.6 [M + H]$^+$. Anal. Calcd. for $C_{22}H_{36}ClNO_4$: C, 63.8; H, 8.8; N, 3.4. Found: C, 63.8; H, 6.8; N, 3.3%.

(R,E)-4-(benzyloxy)-N,N,N-trimethyl-2-(octadec-9-enoyloxy)-4-oxobutan-1-ammonium chloride **(4j):** M.p. 65.0–67.0°C; ^{1}H NMR δ 0.86–0.90 (3H, t, $\underline{CH_3}CH_2$–), 1.18–1.42 (20H, m, –($\underline{CH_2}$)$_6$CH$_2$CHCHCH$_2$($\underline{CH_2}$)$_4$), 1.68 (4H, m, –$\underline{CH_2}$CHCH$\underline{CH_2}$–), 2.22–2.29 (2H, m, –$\underline{CH_2}CH_2$COO–), 2.75–2.80 (2H, m, –$CH_2\underline{CH_2}$COO–), 3.03–3.10 (2H, m, –$CH\underline{CH_2}$COO–), 3.24 (9H, s, ($\underline{CH_3}$)$_3$N–), 3.87 (2H, m, –N$\underline{CH_2}$–), 5.10 (2H, s, –$\underline{CH_2}C_6H_5$), 5.51–5.62 (1H, m, –NCH$_2\underline{CH}$–), 6.43 (1H, d, *J* = 14.7 Hz, –$\underline{CH}$=CH–), 6.87 (1H, d, *J* = 12.5 Hz, –CH=$\underline{CH}$–), 7.27–7.36 (5H, m, –$CH_2\underline{C_6H_5}$); IR cm^{-1} (KBr plate): 3034, 3028 (γ_{CH}), 1735, 1688 ($\gamma_{C=O}$), 1479, 1468 ($\delta_{CH2,CH3}$), 1184 (ν_{C-N}), 705, 712, 753, 799, 914 (aromatic ring); ESI–MS (*m/z*): 552.7 [M + H]$^+$. Anal. Calcd. for $C_{32}H_{54}ClNO_4$: C, 69.6; H, 9.9; N, 2.5. Found: C, 69.6; H, 9.9; N, 2.6%.

Data for **2a–j**

(R,E)-3-carboxy-2-(cinnamoyloxy)-N,N,N-trimethylpropan-1-ammonium chloride **(2a):** M.p. 206.0–208.0; ^{1}H NMR δ 2.70–2.72(2H, m, –$\underline{CH_2}$COO–), 3.03 (9H, s, ($\underline{CH_3}$)$_3$N–), 3.54 (2H, m, –N$\underline{CH_2}$–), 5.57 (1H, m, –NCH$_2\underline{CH}$–), 6.30 (1H, d, *J* = 15.9 Hz, –$\underline{CH}$=CHCOO–), 7.24–7.42 (5H, m, –$CH_2\underline{C_6H_5}$), 7.53 (1H, d, *J* = 15.9 Hz, –CH=$\underline{CH}$COO–). IR cm^{-1} (KBr plate): 2500–3000 (γ_{OH}), 1729, 1701 ($\gamma_{C=O}$), 1637, 1577 ($\gamma_{C=C}$), 1486, 1450 ($\delta_{CH2,CH3}$), 1196 (ν_{C-N}), 712, 768 (aromatic ring). ESI–MS (*m/z*): 292.4 [M + H]$^+$. Anal. Calcd. for $C_{16}H_{22}ClNO_4$: C, 58.6; H, 6.8; N, 4.3 Found: C, 58.7; H, 6.8; N, 4.2%. These analytical data were agreement with the literature.[19]

(R)-2-acetoxy-3-carboxy-N,N,N-trimethylpropan-1-ammonium chloride **(2b):** M.p. 187.0–189.0. The analytical data were shown in literature.[20]

(R)-3-carboxy-N,N,N-trimethyl-2-(propionyloxy)propan-1-ammonium chloride **(2c):** M.p. 161.0–163.0. The analytical data were agreement with literature.[21]

(R)-2-(butyryloxy)-3-carboxy-N,N,N-trimethylpropan-1-ammonium chloride **(2d):** M.p. 159.0–162.0; ^{1}H NMR δ 0.81 (3H, d, *J* = 14.7 Hz, $\underline{CH_3}CH_2$–), 1.48–1.60 (2H, m, –$CH_3\underline{CH_2}$–), 2.32–2.37 (2H, m, –$CH_2\underline{CH_2}$COO–), 2.75 (2H, m, –$CH\underline{CH_2}$COO–), 3.12 (9H, s, ($\underline{CH_3}$)$_3$N–), 3.58 (2H, m, –N$\underline{CH_2}$–), 5.57 (1H, m, –NCH$_2\underline{CH}$–). IR cm^{-1} (KBr plate): 2500–3000 (γ_{OH}), 1735 ($\gamma_{C=O}$), 1479, 1413 ($\delta_{CH2,CH3}$), 1175 (ν_{C-N}). ESI–MS (*m/z*): 232.4 [M + H]$^+$. Anal. Calcd. for $C_{11}H_{22}ClNO_4$: C, 49.4; H, 8.3; N, 5.2. Found: C, 49.5; H, 8.3, N, 5.1%.

(R)-3-carboxy-N,N,N-trimethyl-2-(3-methylbutanoyloxy)propan-1-ammonium chloride **(2e):** M.p. 160.0–162.5; ^{1}H NMR δ 1.06 (6H, dd, *J* = 2.4, 7.2 Hz, ($\underline{CH_3}$)$_2$CH–), 2.55–2.65 (1H, m, (CH_3)$_2\underline{CH}$–), 2.74 (2H, d, *J* = 6.3 Hz, –$\underline{CH_2}$COO–), 3.12 (9H, s, ($\underline{CH_3}$)$_3$N–), 3.59–3.90 (2H, m, –N$\underline{CH_2}$–), 5.57–5.64 (1H, m, –NCH$_2\underline{CH}$–).

IR cm^{-1} (KBr plate): 2500–3000 (γ_{OH}), 1734, 1701 ($\gamma_{C=O}$), 1479, 1443 ($\delta_{CH2,CH3}$), 1186 (ν_{C-N}). ESI–MS (*m/z*): 232.4 $[M + H]^+$. Anal. Calcd. for $C_{11}H_{22}ClNO_4$: C, 49.4; H, 8.3; N, 5.2. Found: C, 49.3; H, 8.4; N, 5.2%.

(R)-3-carboxy-N,N,N-trimethyl-2-(pentanoyloxy)propan-1-ammonium chloride **(2f):** M.p. 146.0–148.0; ^1H NMR δ 0.78 (3H, t, J = 14.7 Hz, $\underline{CH_3}CH_2$–), 1.18–1.30 (2H, m, –$CH_3\underline{CH_2}$–), 1.46–1.56 (2H, m, –$CH_2\underline{CH_2}CH_2$–), 2.35 (2H, t, J = 15.3 Hz, –$CH_2\underline{CH_2}COO$–), 2.75 (2H, d, J = 6.3 Hz, –$CH\underline{CH_2}COO$–), 3.12 (9H, s, $(\underline{CH_3})_3N$–), 3.59–3.88 (2H, m, –$N\underline{CH_2}$–), 5.57–5.64 (1H, m, –$NCH_2\underline{CH}$–). IR cm^{-1} (KBr plate): 2500–3000 (γ_{OH}), 1736 ($\gamma_{C=O}$), 1480, 1412 ($\delta_{CH2,CH3}$), 1170 (ν_{C-N}). ESI–MS (*m/z*): 246.5 $[M + H]^+$. Anal. Calcd. for $C_{12}H_{24}ClNO_4$: C, 51.2; H, 8.6; N, 5.0. Found: C, 51.1; H, 8.6; N, 4.9%.

(R)-3-carboxy-N,N,N-trimethyl-2-(4-methylpentanoyloxy)propan-1-ammonium chloride **(2g):** M.p. 166.0–168.0; ^1H NMR δ 0.85 (6H, d, J = 6.6 Hz, $(\underline{CH_3})_2CH$–), 2.91–2.04 (1H, m, $(CH_3)_2\underline{CH}$–), 2.24 (2H, t, J = 12.9 Hz, –$\underline{CH_2}COOCH$–), 2.77 (2H, d, J = 6.0 Hz, –$\underline{CH_2}COOCH_2$–), 3.13 (9H, s, $(\underline{CH_3})_3N$–), 3.60–3.89 (2H, m, –$N\underline{CH_2}$–), 5.57–5.64 (1H, m, –$NCH_2\underline{CH}$–). IR cm^{-1} (KBr plate): 2500–3000 (γ_{OH}), 1736, 1715 ($\gamma_{C=O}$), 1484, 1412 ($\delta_{CH2,CH3}$), 1182 (ν_{C-N}). ESI–MS (*m/z*): 246.5 $[M + H]^+$. Anal. Calcd. for $C_{12}H_{24}ClNO_4$: C, 51.2; H, 8.6; N, 5.0. Found: C, 51.5; H, 8.6; N, 4.9%.

(R)-3-carboxy-2-(hexanoyloxy)-N,N,N-trimethylpropan-1-ammonium chloride **(2h):** M.p. 170.0–172.0; ^1H NMR δ 0.76 (3H, t, J = 13.8 Hz, $\underline{CH_3}CH_2$–), 1.18–1.23 (4H, m, –$CH_3\underline{CH_2}\underline{CH_2}$–), 1.48–1.58 (2H, m, –$\underline{CH_2}CH_2COO$–), 2.35 (2H, t, J = 15.3 Hz, –$CH_2\underline{CH_2}COO$–), 2.75 (2H, d, J = 6.0 Hz, –$CH\underline{CH_2}COO$–), 3.12 (9H, s, $(\underline{CH_3})_3N$–), 3.59–3.89 (2H, m, –$N\underline{CH_2}$–), 5.57–5.63 (1H, m, –$NCH_2\underline{CH}$–). IR cm^{-1} (KBr plate): 2500–3000 (γ_{OH}), 1736 ($\gamma_{C=O}$), 1480, 1415 ($\delta_{CH2,CH3}$), 1170 (ν_{C-N}). ESI–MS (*m/z*): 260.5 $[M + H]^+$. Anal. Calcd. for $C_{13}H_{26}ClNO_4$: C, 52.8; H, 8.9; N, 4.7. Found: C, 52.8; H, 8.9, N, 4.7%.

(R)-3-carboxy-N,N,N-trimethyl-2-(octanoyloxy)propan-1-ammonium chloride **(2i):** M.p. 172.0–174.0; ^1H NMR δ 0.76 (3H, t, J = 13.5 Hz, $\underline{CH_3}CH_2$–), 1.19–1.21 (8H, m, –$CH_3(\underline{CH_2})_2$–), 1.51–1.56 (2H, m, –$\underline{CH_2}CH_2COO$–), 2.35–2.40 (2H, m, –$CH_2\underline{CH_2}COO$–), 2.75 (2H, d, J = 6.0 Hz, –$CH\underline{CH_2}COO$–), 3.12 (9H, s, $(\underline{CH_3})_3N$–), 3.60–3.89 (2H, m, –$N\underline{CH_2}$–), 5.57–5.63 (1H, m, –$NCH_2\underline{CH}$–). IR cm^{-1} (KBr plate): 2500–3000 (γ_{OH}), 1735, 1716 ($\gamma_{C=O}$), 1484, 1416 ($\delta_{CH2,CH3}$), 1174 (ν_{C-N}). ESI–MS (*m/z*): 288.4 $[M + H]^+$. Anal. Calcd. for $C_{15}H_{30}ClNO_4$: C, 55.6; H, 9.3; N, 4.3. Found: C, 55.7; H, 9.4, N, 4.3%.

(R,E)-3-carboxy-N,N,N-trimethyl-2-(octadec-9-enoyloxy)propan-1-ammonium chloride **(2j):** M.p. 121.0–124.0; ^1H NMR δ 0.81 (3H, t, J = 6.9 Hz, $\underline{CH_3}CH_2$–), 1.12–1.18 (20H, m, –$(\underline{CH_2})_6CH_2CHCHCH_2(\underline{CH_2})_4$), 1.50–1.52 (4H, m, –$\underline{CH_2}CHCH\underline{CH_2}$–), 2.10–2.14 (2H, m, –$\underline{CH_2}CH_2COO$–), 2.56 (2H, t, J = 12.6 Hz, –$CH_2\underline{CH_2}COO$–), 3.08–3.13 (2H, m, –$CH\underline{CH_2}COO$–), 3.10 (9H, s, $(\underline{CH_3})_3N$–), 3.60 (2H, m, –$N\underline{CH_2}$–), 5.51–5.53 (2H, m, –$NCH_2\underline{CH}$–), 5.53–5.63 (1H, m, –$NCH_2\underline{CH}$–). IR cm^{-1} (KBr plate): 2500–3000 (γ_{CH}), 1716 ($\gamma_{C=O}$), 1482, 1396 ($\delta_{CH2,CH3}$), 1183 (ν_{C-N}). ESI–MS (*m/z*): 426.6 $[M + H]^+$. Anal. Calcd. for $C_{25}H_{48}ClNO_4$: C, 65.0; H, 10.5; N, 3.0. Found: C, 65.0; H, 10.5; N, 3.0%.

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References

1 I.B. Fritz, *Current Concepts in Carnitine Research*, ed. A.L. Carter CRC Press, Boca Raton, FL, 1992, pp. 107.
2 B. Kamm, M. Kamm, A. Kiener and H.P. Meyer, *Appl. Microbiol. Biotechnol.,* 2005, **67**, 1.
3 X. Zhou, F. Liu and S. Zhai, *Asia Pac. J. Clin. Nutr*., 2007, **16** Suppl, 383.
4 K.A. NaBcz, D. Miecz, V. Berezowski and R. Cecchelli, *Mol. Aspects Med*., 2004, **25**, 551.
5 W. Xuan, A.M. Lamhonwah, C. Librach, K. Jarvi, and I. Tein, *Biochem. Biophys. Res. Commun*., 2003, **306**, 121.
6 A.P. Aprilia, M.O.T. Roma, M.S. Nettuno, L.C. Pomezia and G.S. Rome, 2004, US 6797281B1.
7 S.R. Srinivas, P.D. Prasad, N.S. Umapathy, V. Ganapathy and P.S. Shekhawat, *Am. J. Physiol. Gastrointest. Liver Physiol*., 2007, **293**, G1046.
8 O. Piermatti, F. Fringuelli, L. Pochini, C. Indiveri and C.A. Palmerini, *Bioorg. Med. Chem*., 2008, **16**, 1444.
9 E.J. Tadeusiak, *Bioorg. Chem.,* 2004, **32**, 473.
10 A. Inano, Y. Sai, H. Nikaido, N. Hasimoto, M. Asano, A. Tsuji and I. Tamai, *Biopharm. Drug Dispos*., 2003, **24**, 357.
11 Index by database: MICROMEDEX(R) Healthcare Series Vol. 121. (or Thomson micromedex Vol.121)
12 T. Kawaguchi, T. Hasegawa, T. Seki, K. Juni, Y. Morimoto, A. Miyakawa and M. Saneyoshi, (DDI): *Chem. Pharm. Bull. (Tokyo)*., 1992, **40**, 1338.
13 H. Bundgaard, E. Jensen and E. Falch, *Pharm. Res.,* 1991, **8**, 1087.
14 C. Cavazza and M.O. Tinti, Esters of alkoxy–acyl derivatives of carnitine and pharmaceutical compositions containing same. 1985, US 4,551,477.
15 M. Calvani, L. Critelli, G. Gallo, F. Giorgi, G. Gramiccioli, M. Santaniello, N. Scafetta, M.O. Tinti and F. De Angelis, *J. Med. Chem.,* 1998, **41**, 2227.
16 C. Pisano, M.O. Tinti, M. Santaniello, L. Critelli and G. Salvatori, Esters of L–carnitine or alkanoyl L–carnitine. 2004, US 6,797,281 B1.
17 A. Virmani, F. Gaetani and Z. Binienda, *Ann. NY Acad. Sci*., 2005, **1053**, 183.
18 J.Y. Jung, H.C. Mo, K.H. Yang, Y.J. Jeong, H.G. Yoo, N.K. Choi, W.M. Oh, H.K. Oh, S.H. Kim, J.H. Lee, H.J. Kim and W.J. Kim, *Life Sci*., 2007, **80**, 1355.
19 de W. Paolo, Novel acyl–derivatives of cartine and process for their preparation. 1980, UK GB 2,028,826 A.
20 H.P. Weber, R.C. Anderson, M. Shapiro, E.B. Villhauer and T. Gullion, *Acta. Cryst.,* 1995, **C51**, 2570.
21 C. Cavazza, Amides of acyl–carnitines, process for preparing same and pharmaceutical compositions containing such amides. 1980, UK GB 2,048,268 A.