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Unique spirocyclopiperazinium salt III: Further investigation of monospirocyclopiperazinium (MSPZ) salts as potential analgesics

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Abstract—Two novel classes of monospirocyclopiperazinium salts were designed, synthesized, and evaluated for their in vivo analgesic activities. Some interesting structure–activity relationships are revealed: (1) Spirocyclopiperazinium moiety is favorable to improve the analgesic activity; (2) The size and conformation of spirocyclopiperazinium moiety significantly affects the analgesic activity; (3) Phenylethyl group of **3d** is a crucial pharmacophore. Among the compounds synthesized, **3d** exhibited the most potent activity with low toxicity. Further antinociceptive mechanism studies of **3d** showed that these compounds will be a new kind of analgesics.

The discovery of compounds that can be used to treat both acute and chronic pain without the side effect of drug dependency would be an important advance in pain management. Since the epibatidine was reported to possess strong analgesic properties as nicotinic acetylcholine receptor (nAChR) agonist,[1] the studies on the nAChR ligands have drawn great attention.[2] Naturally, N^1,N^1-dimethyl-N^4-phenylpiperazinium iodide (DMPP, Fig. 1),[3] a well-known unique nAChR agonist, attracted the interest of medicinal chemists. Numerous DMPP analogues with substituted phenyl and heteroaromatic groups have been synthesized and subjected to physiological studies.[4]

Our group has engaged in the study on the synthesis and biological activity of quaternary ammonium salts for many years.[5] Recently, we have reported a novel class of monospirocyclopiperazinium salts (MSPZ, Fig. 1)[6] and dispirocyclopiperazinium salts (DSPZ, Fig. 1)[7] with potent analgesic activities. These results have received much attention due to their peculiar structure related to the DMPP.[8] Furthermore, we also found that dipiperazinium salts (**1**, **97-9-G4**, Fig. 1) showed excellent analgesic activity.[9]

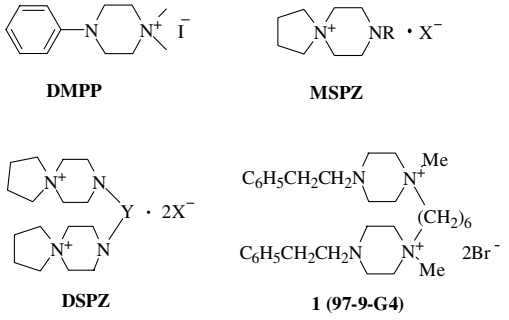

Figure 1. Structures of DMPP, MSPZ, DSPZ, and 97-9-G4.

On the bases of our research results and Gualtieri's report of 3-NO_2 and 4-MeO substituted aryl DMPP derivatives with excellent affinity for the nicotinic receptor,[4a] we designed and synthesized two series of novel monospirocyclopiperazinium salts (**2** and **3**, Fig. 2) to

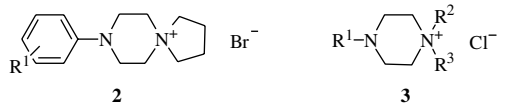

Figure 2. General structures of compounds **2** and **3**.

Keywords: Monospirocyclopiperazinium salts; Analgesic; Structure–activity relationship; Synthesis.
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get additional SAR information and improve the pharmacological properties. All newly synthesized compounds were evaluated for their in vivo analgesic activities and some of them exhibited potent analgesic activities. Here we report the design, synthesis and analgesic activities of the new spirocyclopiperazinium derivatives.

The synthesis of compounds **2a**–**d** is outlined in Scheme 1. Reaction of piperazine with different substituted phenyl iodides[10] in the presence of CuI and K_3PO_4 gave the key intermediate 1-phenyl-piperazines **4** at room temperature. The intermediates **4a**–**d** were treated with 1,4-dibromobutane in refluxing ethanol using $NaHCO_3$ as acid-adsorbent affording the corresponding monospirocyclopiperazinium salts **2a**–**d**. The DMPP derivatives **2e** and **2f** were prepared from the quaternarization of **5** with methyl bromide and methyl iodine, respectively. The synthesis of intermediate **5** is similar to compound **4**.

The synthesis of compounds **3a**–**l** is illustrated in Scheme 2. Diethanolamine was N-alkylated with various halides R^1X **6**, and then chlorinated with $SOCl_2$ in chloroform to give the key intermediate **8**. Reaction of **8** with different secondary amines afforded the desired products **3a**–**l**.

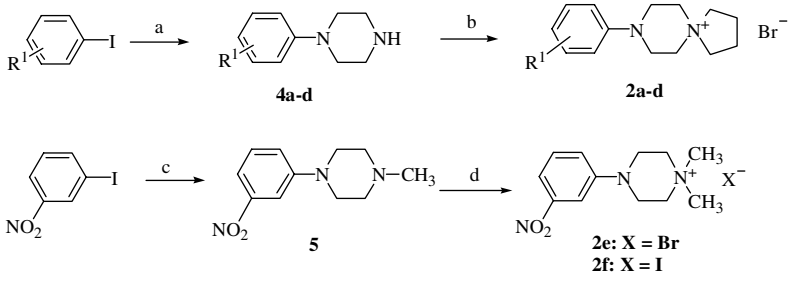
All the target compounds were purified by recrystallization and characterized using NMR and elemental analysis.[11] Their analgesic activities were assessed by acetic acid writhing test,[5c] and the results are summarized in Tables 1 and 2.

The data in Table 1 indicate that the most potent compound is **2c** (3-NO_2, 61% analgesic activity at the dose of 31 μmol/kg) among compounds **2a**–**f**. However, the DMPP derivative **2f**, which was reported to have high affinity for the nicotinic receptor,[4a] did not show any in vivo analgesic activity. Considering the difference of anion between **2c** and **2f**, we also synthesized bromide salt **2e** to compare with **2c** and **2f**. Though **2e** exhibits better analgesic activity than **2f**, it is still weaker than **2c**. Therefore, it was suggested that spirocyclopiperazinium structure might be favorable to improve the analgesic activity.

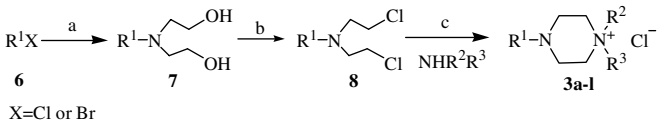
It was also found from the Table 1 that the property and position of the substituents on the benzene ring have significant influence on the analgesic activities. For example, compound **2b** (4-MeO) showed the most potent analgesic activity among the compounds **2a** (H), **2b** (4-MeO), and **2d** (4-NO_2); compound **2c** with electron-withdrawing group (3-NO_2, 61%) exhibited more potent analgesic activity than compound **2d** (16%).

Considering the phenylethyl group as significant pharmacophore in **97-9-G4**, compounds **3a**–**e** in Table 2 were designed and synthesized to explore the effect of cyclic size and steric effect of quaternary ammonium salt moieties. Compound **3d** (65%) showed excellent biological activity, **3a** with five-member ring (−36%), **3b** with six-member ring (5%), **3c** with seven-member ring (4%) and acyclic compound **3e** (18%) did not show measurable analgesic activity. This result demonstrates that the appropriate conformation of the compound was critical for the interaction between ligand and receptor.

With the biological result of **3d** in hand, we synthesized compounds **3f**–**l** by replacing the phenylethyl of **3d** with various substituted groups R^1 and maintaining the spirocyclopiperazinium moiety of **3d** to get more potential compounds. For the substitution on the phenyl ring, both electron-withdrawing group (**3g**, NO_2–, −24%) and electron-donating group (**3h**, MeO–, −47%) were definitely detrimental to the analgesic activity. Comparing the compound **3i** (21%) and **3j** (−19%) with **3d**, it is clear that the distance of two methylene units between phenyl and N-atom is suitable for the analgesic activity. By introducing an allyl group according to previous work,[6] compound **31** exhibited potent activity. However, other compounds almost completely lost the activity. These results indicated that phenylethyl group of **3d** was a crucial pharmacophore for the analgesic activity.

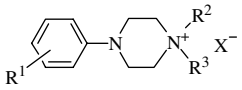


Scheme 1. Synthesis of compounds **2a**–**f**. Reagents and conditions: (a) piperazine, CuI, K_3PO_4, *i*-PrOH, rt; (b) $Br(CH_2)_4Br$, $NaHCO_3$, ethanol, reflux; (c) methyl piperazine, CuI, K_3PO_4, *i*-PrOH, rt; (d) CH_3X, acetone, rt.



Scheme 2. Synthesis of compounds **3a**–**l**. Reagents and conditions: (a) bis(hydroxyethyl)amine, K_2CO_3, ethanol, reflux; (b) $SOCl_2$, $CHCl_3$; (c)$NaHCO_3$, ethanol, reflux.

Table 1. The analgesic activities of compounds **2a–f**



Compound	R^1	R^2, R^3	X	Analgesic activities[a,b] (%)
2a	H		Br	20*
2b	4-MeO–		Br	56**
2c	3-NO_2–		Br	61**
2d	4-NO_2–		Br	16*
2e	3-NO_2–	Me, Me	Br	22*
2f	3-NO_2–	Me, Me	I	−16*

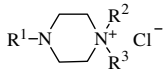
[a] Drugs were dissolved in isotonic saline solution (NaCl 0.9%) immediately before use. Drug concentrations were prepared in such a way that the necessary dose could be administered in a volume of 10 ml/kg. Acetic acid writhing test was used on mice (eight per each group), and drugs were administered at the dose of 31 μmol/kg intraperitoneally (ip).

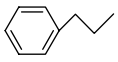
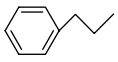
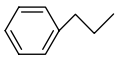
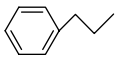
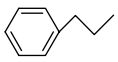
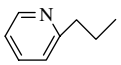
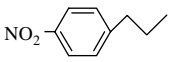
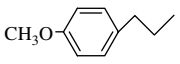
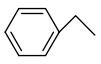
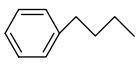
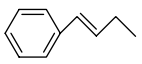
[b] % Inhibition = 100 − (A/B × 100), where *A* = incidence of writhing in the treated group and *B* = incidence of writhing in the control group, occurring from the 5th to 10th min after administration of the noxious agents.

* $P > 0.05$.

** $P < 0.01$.

Table 2. The analgesic activities of compounds **3a–l**



Compound	R^1	R^2, R^3	Analgesic activity[a,b] (%)
3a			−36*
3b			5*
3c			4*
3d			65**
3e		Me, Me	18*
3f			−2*
3g			−24*
3h			−47*
3i			21*
3j			−19*
3k			−43*
3l			49**

[a,b] See footnotes in Table 1.

* $P > 0.05$.

** $P < 0.01$.

On the bases of above results, we have chosen the most potential compound **3d** (65% inhibition in acetic acid writhing test) to further investigate its pharmacological efficacy. The results show that the compound **3d** produced antinociception in chemical and thermal models of nociception in mice without significant side effects (LD_{50} = 1.55 mmol/kg, ip), and the antinociceptive effect was achieved by activating peripheral neuronal nicotinic acetylcholine and muscarinic acetylcholine receptors, but the effect did not relate to opioid receptors or α-adrenoreceptors.[12] Meanwhile, we also completed the binding test of compound **3d** with nAChR(α4β2). It was found that the IC_{50} value of compound **3d** (>10 μM) was far higher than that of epibatidine (0.00106 μM), which suggested that the antinociceptive effect of **3d** might not closely relate with nAChR(α4β2). There results coincided with our recent results.[12] Further pharmacological study is in progress.

In summary, we have designed and synthesized two series of novel monospirocyclopiperazinium salts and evaluated their in vivo analgesic activity. Some compounds showed good analgesic activities. Especially, the compound **3d** exhibited not only good activity but also low toxicity. Meanwhile, some important structure–activity relationships were revealed. These results will provide insight into new kind of analgesics.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.09.026.

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11. Compound **2a**: white powder, mp: 208–209 °C. ^{1}H NMR (D_2O, 300 MHz): 7.25 (dd, J = 7.2 Hz, 8.7 Hz, 2H, ArH), 7.00 (d, J = 8.4 Hz, 1H, ArH), 6.92 (t, J = 7.5 Hz, 2H, ArH), 3.46–3.54 (m, 8H, N^+–CH_2), 3.39 (t, J = 4.5 Hz, 4H, N–CH_2), 2.01 (br s, 4H, N^+–C–CH_2–CH_2–C–N^+). Anal. Calcd for $C_{14}H_{21}BrN_2 \cdot 0.3H_2O$: C, 55.56; H, 7.19; N, 9.26. Found: C, 55.55; H, 6.91; N, 9.26. Compound **2b**: orange slice, mp: 201–203 °C. ^{1}H NMR (D_2O, 300 MHz): 6.93–6.99 (m, 2H, ArH), 6.84–6.88 (m, 2H, ArH), 3.65 (s, 3H, OCH_3), 3.44–3.52 (m, 8H, N^+–CH_2), 3.28 (br s, 4H, N–CH_2), 2.07 (br s, 4H, N^+–C–CH_2–CH_2–C–N^+). Anal. Calcd for $C_{15}H_{23}BrN_2O$: C, 55.05; H, 7.08; N, 8.56. Found: C, 55.00; H, 7.07; N, 8.55. Compound **2c**: yellow powder, mp: 246–249 °C. ^{1}H NMR (D_2O, 300 MHz): 7.72 (s, 1H, ArH), 7.65 (d, J = 8.1 Hz, 1H, ArH),7.36 (t, J = 8.1 Hz, 1H, ArH), 7.28 (d, J = 9.0 Hz, 1H, ArH), 3.50–3.54 (m, 12H, N^+–CH_2, N–CH_2), 2.08 (br s, 4H, N^+–C–CH_2–CH_2–C–N^+). Anal. Calcd for $C_{14}H_{20}Cl_2N_3 \cdot 0.2H_2O$: C, 48.62; H, 5.95; N, 12.15. Found: C, 48.42; H, 5.74; N, 11.99. Compound **2d**: white powder, mp: 278–280 °C. ^{1}H NMR (D_2O, 300 MHz): 8.00 (d, J = 9.6 Hz, 2H, ArH), 6.90 (d, 2H, J = 9.6 Hz, ArH),3.67 (br s, 4H, Ph–N–CH_2), 3.48–3.57 (m, 8H, N^+–CH_2), 2.11 (br s, 4H, N^+–C–CH_2–CH_2–C–N^+). Anal. Calcd for $C_{14}H_{20}BrN_3O_2 \cdot 0.1H_2O$: C, 48.88; H, 5.92; N, 12.21. Found: C, 48.73; H, 6.28; N, 11.94. Compound **2e**: white solid, mp: 234–237 °C. ^{1}H NMR (D_2O, 300 MHz): 7.74 (s, 1H, ArH), 7.68–7.70 (d, J = 7.2 Hz, 1H, ArH), 7.38 (t, J = 8.4 Hz, 1H, ArH), 7.30 (t, J = 8.4 Hz, 1H, ArH), 3.52–3.53 (m, 8H, N^+–CH_2–CH_2–N), 3.11 (s, 6H, CH_3). Anal. Calcd for $C_{12}H_{18}BrN_3O_2 \cdot 0.2H_2O$: C, 45.07; H, 5.80; N, 13.14. Found: C, 44.98; H, 5.95; N,13.10. Compound **2f**: yellow solid, mp: 210–212 °C. ^{1}H NMR (D_2O, 300 MHz): 7.70 (t, J = 2.1 Hz, 1H, ArH), 7.64 (dq, J = 8.1 Hz, 0.9 Hz, 1H, ArH), 7.36 (t, J = 8.4 Hz, 1H, ArH), 7.28 (dq, 1H, J = 8.7 Hz, 0.9 Hz, ArH), 3.52 (s, 8H, N^+–CH_2–CH_2–N), 3.13 (s, 6H, CH_3). Anal. Calcd for $C_{12}H_{18}IN_3O_2$: C, 39.68; H, 5.00; N, 11.57. Found: C, 39.46; H, 5.21; N, 11.42. Compound **3a**: white powder, mp: 220 °C (dec). ^{1}H NMR (D_2O, 300 MHz): 7.11–7.25 (m, 5H, ArH), 3.30–3.51 (m, 8H, N^+–CH_2), 2.77 (m, 4H, N–CH_2), 2.58–2.73 (m, 4H, Ph–CH_2–CH_2), 2.03–2.05 (d, 4H, C–CH_2–CH_2–C). Compound **3b**: white slice, mp: 221–225 °C. ^{1}H NMR (D_2O, 300 MHz): 7.10–7.23 (m, 5H, ArH), 3.27 (t, J = 6.0 Hz, 8H, N^+–CH_2), 2.63–2.66 (m, 4H, N–CH_2), 2.74 (s, 4H, Ph–CH_2–CH_2), 1.67 (t, J = 5.7 Hz, 4H, N^+–C–CH_2–C), 1.49 (t, J = 6.0 Hz, 2H, N^+–C_2–CH_2–C_2–N^+). Anal. Calcd for $C_{17}H_{27}ClN_2 \cdot H_2O$: C, 65.26; H, 9.34; N, 8.95. Found: C, 65.60; H, 9.28; N, 8.70. Compound **3c**: white powder, mp: 220 °C (dec). ^{1}H NMR (D_2O, 300 MHz): 7.10–7.24 (m, 5H, ArH), 3.31 (t, J = 4.5 Hz, 8H, N^+–CH_2), 2.76 (br s, 4H, N–CH_2), 2.64–2.67 (m, 4H, Ph–CH_2–CH_2), 1.73 (br s, 4H, N^+–C–CH_2–C), 1.53 (br s, 4H, N^+–C–C–CH_2). Anal. Calcd for $C_{18}H_{29}ClN_2 \cdot 0.5H_2O$: C, 68.01; H, 9.51; N, 8.81. Found: C, 67.97; H, 9.70; N, 8.73. Compound **3d**: white powder, mp: 220 °C (dec). ^{1}H NMR (D_2O, 300 MHz): 7.12–7.26 (m, 5H, ArH), 4.05–4.14 (m, 2H, O–CH), 3.30–3.64 (m, 8H, N^+–CH_2), 2.79–2.95 (m, 4H, N–CH_2), 2.61–2.74 (m, 4H, Ph–CH_2–CH_2), 1.05 (d, J = 11.7 Hz, 6H, CH_3). Anal. Calcd for $C_{18}H_{29}ClN_2O \cdot 1.1H_2O$: C, 62.72; H, 9.12; N, 8.13. Found: C, 62.50; H, 9.12; N, 8.05. Compound **3e**: white powder, mp: 230 °C (dec). ^{1}H NMR (D_2O, 300 MHz): 7.12–7.26 (m, 5H, ArH), 3.30 (t, J = 8.1 Hz, 4H, N^+–CH_2), 3.04 (s, 6H, CH_3), 2.79 (br s, 4H, N–CH_2), 2.62–2.69 (m, 4H, Ph–CH_2–CH_2). Compound **3f**: pink powder, mp: 247 °C. ^{1}H NMR (D_2O, 300 MHz): 8.26 (d, J = 4.8 Hz, 1H, ArH), 7.62 (t, J = 7.8 Hz, 1H, ArH), 7.20 (d, J = 4.8 Hz, 1H, ArH), 7.13 (t, J = 6.6 Hz, 1H, ArH), 4.05–4.11 (m, 2H, O–CH), 3.49–3.62 (m, 4H, N^+–CH_2), 3.34 (t, J = 5.1 Hz, 2H, Ar–CH_2), 2.71–2.93 (m, 10H, N^+–CH_2, N–CH_2), 1.06 (d, J = 6.0 Hz, 6H, CH_3). Anal. Calcd for $C_{17}H_{28}ClN_3O$: C, 62.66; H, 8.66; N, 12.89. Found: C, 62.38; H, 8.63; N, 12.68. Compound **3g**: buff solid, mp: 288–294 °C. ^{1}H NMR (D_2O, 300 MHz): 8.01 (d, J = 8.4 Hz, 2H, ArH), 7.30 (d, 2H, J = 8.4 Hz, ArH), 4.05–4.11 (m, 2H, O–CH), 3.59–3.62 (m, 4H, N^+–CH_2), 3.35 (t, J = 4.8 Hz, 2H, Ar–CH_2), 2.64–2.94 (m, 10H, N^+–CH_2, N–CH_2), 1.07 (d, J = 6.3 Hz, 6H, CH_3). Anal. Calcd for $C_{18}H_{28}ClN_3O_3$: C, 58.45; H, 7.63; N, 11.36. Found: C, 58.75; H, 7.67; N, 11.31. Compound **3h**: yellow powder, mp: 265–269 °C. ^{1}H NMR (D_2O, 300 MHz): 7.06 (d, J = 8.4 Hz, 2H, ArH), 6.78 (d, 2H, J = 8.4 Hz, ArH), 4.04–4.10 (m, 2H, O–CH), 3.64 (s, 3H, OCH_3), 3.57–3.62 (m, 4H, N^+–CH_2), 3.33 (t, J = 4.8 Hz, 2H, Ar–CH_2), 2.64–2.94 (m, 10H, N^+–CH_2, N–CH_2), 1.06 (d, J = 6.3 Hz, 6H, CH_3). Anal. Calcd for $C_{19}H_{31}ClN_2O_2 \cdot 0.5H_2O$: C, 62.71; H, 8.86; N, 7.70.

Found: C, 62.68; H, 8.98; N, 7.38. Compound **3i**: white powder, mp: 280 °C (dec). ^{1}H NMR (D_2O, 300 MHz): 7.18–7.29 (m, 5H, ArH), 4.02–4.06 (m, 2H, O–*CH*), 3.53–3.63 (m, 6H, N^+–*CH*$_2$), 3.31 (t, *J* = 5.1 Hz, 2H, Ar–*CH*$_2$), 2.86–2.90 (m, 2H, N^+–*CH*$_2$), 2.71–2.76 (m, 4H, N–*CH*$_2$), 1.04 (d, *J* = 6.0 Hz, 6H, *CH*$_3$). Anal. Calcd for $C_{17}H_{27}ClN_2O$: C, 65.68; H, 8.75; N, 9.01. Found: C, 65.49; H, 8.47; N, 8.95. Compound **3j**: white powder, mp: 220 °C (dec). ^{1}H NMR (D_2O, 300 MHz): 7.09–7.24 (m, 5H, ArH), 4.04–4.10 (m, 2H, O–*CH*–CH_3), 3.56–3.64 (m, 4H, N^+–*CH*$_2$), 3.33 (t, *J* = 4.8 Hz, 2H, Ph–*CH*$_2$), 2.84 (t, *J* = 12.0 Hz, 2H, Ph–C–C–*CH*$_2$), 2.71 (br s, 4H, N–*CH*$_2$), 2.84 (t, *J* = 7.5 Hz, 2H, N–*CH*$_2$), 2.37 (t, *J* = 7.5 Hz, 2H, N–*CH*$_2$), 1.64 (p, *J* = 7.8 Hz, 2H, Ph–C–*CH*$_2$), 1.05 (d, *J* = 11.7 Hz, 6H, *CH*$_3$). Anal. Calcd for $C_{19}H_{31}ClN_2O$: C, 67.33; H, 9.22; N, 8.27. Found: C, 67.29; H, 9.12; N, 8.11. Compound **3k**: white powder, mp: 266–268 °C. ^{1}H NMR (D_2O, 300 MHz): 7.15–7.35 (m, 5H, ArH), 6.51 (d, *J* = 15.9 Hz, 1H, Ph–*CH*═), 6.06–6.16 (m, 1H, Ph–C═*CH*), 4.03–4.08 (m, 2H, O–*CH*) 2.77–3.58 (m, 14H, N^+–*CH*$_2$, N–*CH*$_2$), 1.06 (d, *J* = 6.0 Hz, 6H, *CH*$_3$). Anal. Calcd for $C_{19}H_{29}ClN_2O \cdot 0.4H_2O$: C, 66.32; H, 8.73; N, 8.14. Found: C, 66.57; H, 8.90; N, 7.70. Compound **3l**: white powder, mp: 236 °C (dec). ^{1}H NMR (D_2O, 300 MHz): 5.60–5.72 (m, 1H, ═C*H*—), 5.12–5.18 (m, 2H, ═*CH*$_2$), 4.05–4.10 (m, 2H, O–*CH*–CH_3), 3.58–3.64 (m, 4H, N^+–*CH*$_2$), 3.34 (t, 2H, ═C—*CH*$_2$), 2.74–3.01 (m, 8H, N–*CH*$_2$, N^+–*CH*$_2$), 1.08 (d, *J* = 12.0 Hz, 6H, *CH*$_3$). Anal. Calcd for $C_{13}H_{25}ClN_2O$: C, 59.87; H, 9.66; N, 10.74. Found: C, 59.62; H, 9.61; N, 10.60.

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