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# Synthesis of novel dimeric cationic lipids based on an aromatic backbone between the hydrocarbon chains and headgroup

Bishwajit Paul,[a] Avinash Bajaj,[a] S. S. Indi[b] and Santanu Bhattacharya[a,*]

[a]*Department of Organic Chemistry, Indian Institutue of Science, Bangalore 560 012, India*
[b]*Department of Microbiology and Cell Biology, Indian Institutue of Science, Bangalore 560 012, India*

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**Abstract**—Seven dimeric cationic lipids possessing an aromatic anchor between the hydrocarbon chains and cationic headgroup have been synthesized. The spacers in these lipids vary in length, hydrophobicity and flexibility. The synthesis, membrane-forming properties and complexation with plasmid DNA (lipoplex formation) are briefly described.


The use of genes as therapeutic materials holds much promise for practical clinical applications. Indeed, many clinical trials in this field are currently in progress.[1] Towards this end, engineered viruses have been shown to be highly efficient vectors,[2] but a number of severe setbacks have recently imposed serious limits on their medical use.[3] This has intensified the search for synthetic vectors such as cationic lipids[4] which do not elicit immunogenic reactions when administered.

Cationic lipid suspensions readily form complexes with negatively charged DNA (the gene) under ambient conditions. The molecular structure of cationic lipids is an important parameter that controls their DNA

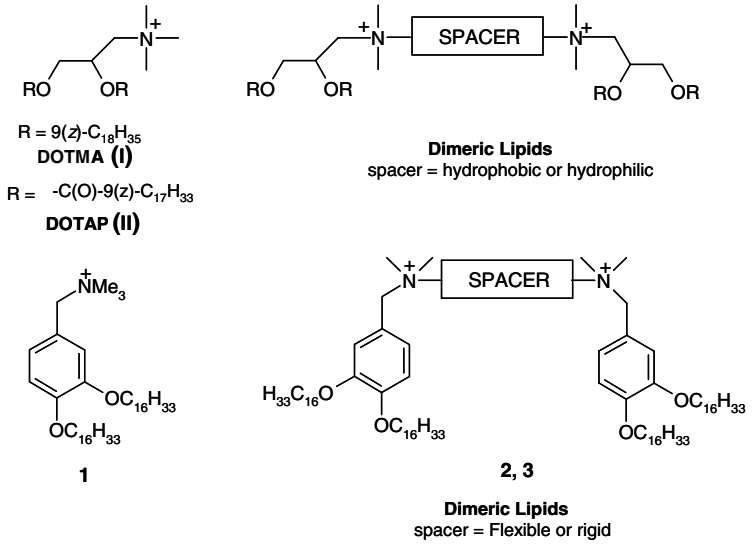


**Figure 1.** Schematic of the Gemini lipids based on glycerol or aromatic backbone. Transfection active lipids, for example, DOTMA (I) and DOTAP (II) and monomeric counterpart of the aromatic gemini are also shown.

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* Corresponding author. Tel.: +91 80 22932664; e-mail: sb@orgchem.iisc.ernet.in

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complexation and gene transfection activity. For instance, the functional group that links the polar head group and the hydrocarbon chains of such lipid molecules plays a crucial role in their utilization in gene transfer events. Thus, *N*-[1-(2,3-dioleyloxy)-*N*,*N*,*N*-trimethylammonium chloride (DOTMA) (**I**), which contains an ether linkage between the head group and the long alkyl chains shows greater in vivo transfection efficiency than the corresponding lipid possessing an ester *N*-[1-(2,3-dioleyl)-*N*,*N*,*N*-trimethylammonium chloride (DOTAP) (**II**),[5] (Fig. 1).

Recently, another class of lipids (in the dimeric form) known as dimeric lipids, have also received attention due to their better gene transfection abilities compared to their monomeric counterparts.[6] Clearly the design and syntheses of new lipid systems with alternative structural types are crucial for the development of potent synthetic vectors for such applications.

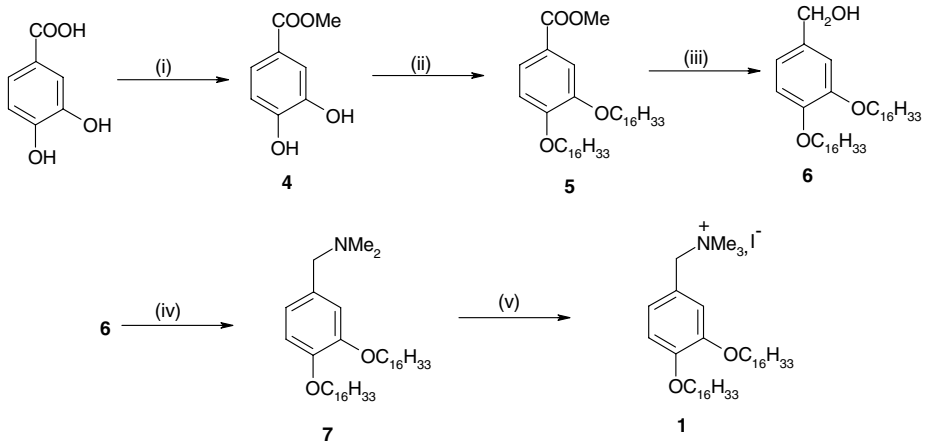
In search of new targets for lipid design, we envisioned developing dimeric lipids based on aromatic backbones.[7] We thought that it would be of interest to design and synthesize lipids based on such structural anchors and investigate their membrane-forming properties. In particular, the presence of $sp^2$ hybridized planar aromatic hydrocarbon rings at the hydrocarbon chain-polar headgroup linkage region pose interesting situations pertaining to their inter-lipidic interactions in membranes as opposed to those based on pseudoglyceryl backbones, which associate with each other and the interfacial water via hydrogen bonding and dipolar interactions.

Lipids based on aromatic backbones are rare in eukaryotic organisms, although their presence has been demonstrated in marine sponges.[8] Among aromatic ring compounds, those considered as lipids are, however, formed in some plants and contain a catechol, resorcinol or hydroquinone nucleus alkylated with a long hydrocarbon chain. Such resorcinolic lipid molecules have a dual, aromatic and acyclic character, demonstrated by the presence of the aromatic ring and straight hydrocarbon chain of a length depending on the source of such lipids.[8]

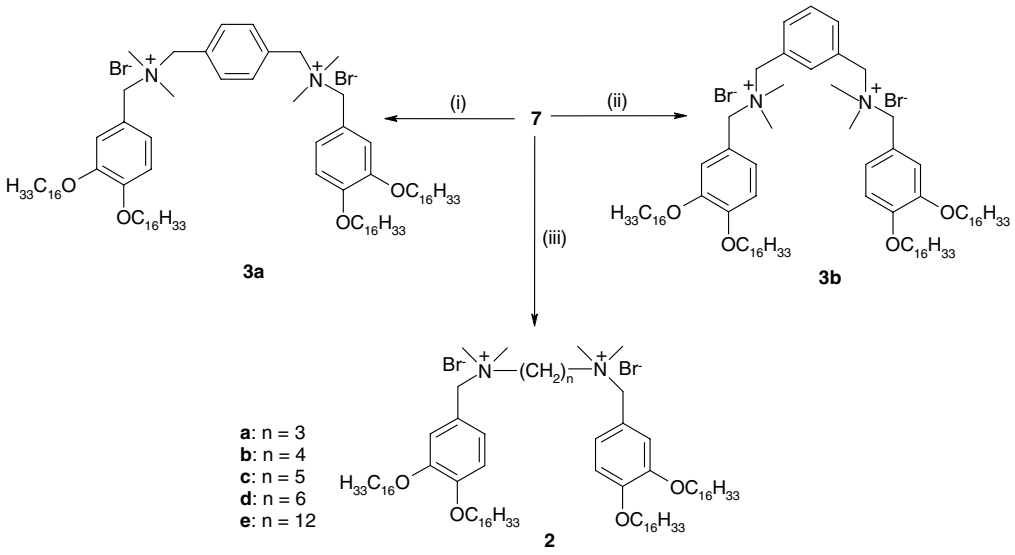
For the last few years, we have been examining the role of various molecular level modifications on the membrane forming properties of different lipids.[9,10] Most of these lipid designs included molecules based on natural glycerol based architectures. Herein, we describe a convenient synthesis of the above type of cationic lipids **1**–**3** (Fig. 1) and briefly report their membrane forming and DNA binding properties.

The synthesis of the key precursor molecule **7** for all the lipids is outlined in Scheme 1. Firstly the $-CO_2H$ moiety of 3,4-dihydroxybenzoic acid was protected via esterification in the presence of concd. HCl and dry MeOH under reflux conditions to afford the corresponding ester **4** in 90% yield. The hydroxyl groups of ester **4** were then subjected to alkylation with *n*-hexadecyl bromide by reflux in acetone in the presence of $K_2CO_3$ to furnish the dialkylated product **5** in 77% yield. The product **5** was then reduced with $LiAlH_4$ in dry THF, under reflux over a period of 12 h, to afford **6** in 97% isolated yield. Next, the conversion of **6** to the corresponding benzylic bromide was attempted. However, under Appel conditions ($CBr_4/PPh_3$ in $CH_2Cl_2$), although TLC indicated satisfactory conversion, during the isolation, the product decomposed. The conversion of the alcohol to the corresponding bromide by $PBr_3$ in $CH_2Cl_2$, appeared to be complete as judged by TLC. However, again it was not possible to isolate the corresponding bromide presumably because of its high instability. Therefore upon confirming the formation of the bromide after reaction with $PBr_3$ in $CH_2Cl_2$ for 24 h by TLC, a methanolic solution of dimethylamine was introduced directly into the reaction mixture and the mixture was stirred at room temperature for 12 h. After work-up, compound **7** was obtained in 86% yield.

The lipid **1** was prepared upon refluxing the tertiary amine **7** with an excess of MeI in EtOH in quantitative




**Scheme 1.** Reagents, conditions and yields: (i) HCl, MeOH, 80 °C, 12 h, 90%; (ii) *n*-$C_{16}H_{33}Br$, $K_2CO_3$, dry acetone, 65 °C, 84 h, 77%; (iii) $LiAlH_4$, dry THF, 70 °C, 12 h, 97%; (iv) (a) $PBr_3$, $CH_2Cl_2$, rt, 24 h; (b) $Me_2NH$ (excess), MeOH, rt, 12 h (overall yield = 86% for steps (a) and (b)) and (v) MeI, EtOH, 80 °C, 24 h, screw-top pressure tube, quantitative yield.




**Scheme 2.** Reagents, conditions and yields (i) α,α–dibromo-*p*-xylene, MeOH–EtOAc (2:1), 80 °C, screw-top pressure tube, 24 h, 60%; (ii) α,α-dibromo-*m*-xylene, MeOH–EtOAc (2:1), 80 °C, screw-top pressure tube, 24 h, 60% and (iii) $Br(CH_2)_nBr$, MeOH–EtOAc (2:1), 80 °C, screw-top pressure tube, 2–4 days, 30–40%.

yield (Scheme 1). The dimeric cationic lipids with polymethylene spacers, **2a–e** were synthesized by heating **7** with the appropriate α,ω-dibromo alkanes to 80 °C in a mixture of MeOH–EtOAc for 2–4 days in a screw-top pressure tube (Scheme 2). After repeated crystallizations from a mixture of MeOH–EtOAc, the isolated yields of the dimeric lipids ranged from 30% to 40%. Compound **7** on reaction with α,α–dibromo-*p*-xylene in a mixture of MeOH–EtOAc afforded the dimeric lipid **3a** with a hydrophobic rigid spacer (Scheme 2). Similarly, the reaction of **7** with α,α-dibromo-*m*-xylene afforded the dimeric lipid **3b** in 60% yield (Scheme 2). Lipids **3a** and **3b** are of interest as cyclophane macrocycles built with *para*- and *meta*-xylenediamine.[11]

All the new lipids were purified by repeated crystallizations from MeOH–EtOAc and isolated as white solids. All the numbered intermediates and the final products were characterized from their IR, $^1$H NMR, $^{13}$C NMR and mass spectra.[12]

Sonication (15 min) of each of the newly synthesized lipids above 70–80 °C with an aqueous buffer afforded stable translucent suspensions. The existence of vesicle-like organizations in these suspensions was evident from transmission electron microscopy (Fig. 2). Gel retardation studies with the plasmid EGFP-c3 at different N/P ratios, showed the retardation of the DNA-plasmid at an N/P ratio of 1.0–2.0 for all the lipids (Fig. 3).

In summary, we have synthesized a series of novel cationic dimeric lipids, which will help in understanding the effect of modulation of different spacers between the headgroups in membranes. The method developed is simple and efficient for the synthesis of a series of cationic dimeric lipids based on an aromatic backbone. The process is scalable from the gram to kilogram scale. These cationic dimeric lipids will be useful for the preparation of a range of therapeutic formulations, including gene transfection agents as well as for antisense DNA technology. The application of these cationic liposomes to deliver genetic materials into targeted cells in vitro

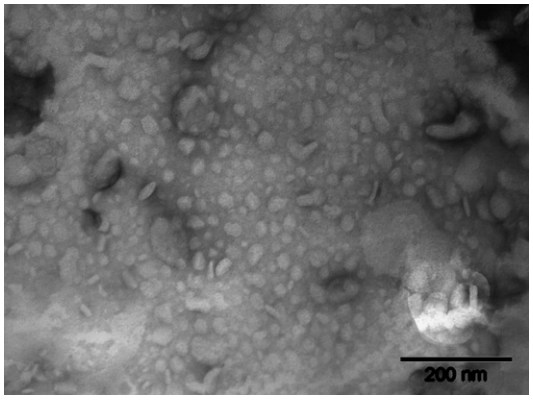



**Figure 2.** Transmission electron micrograph of an aqueous suspension of the dimeric lipid **2d** showing the vesicular nature of the dimeric lipid.

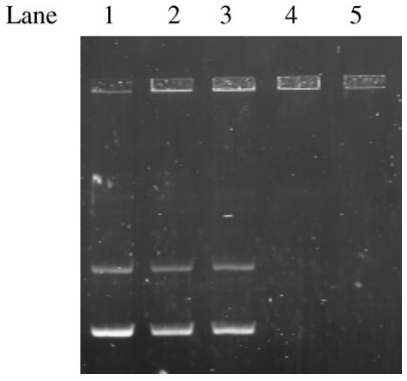



**Figure 3.** Gel retardation assay of dimeric lipid **2c** at different N/P ratios. (Lane 1: Plasmid alone, Lane 2: N/P = 0.5, Lane 3: N/P = 1.0, Lane 4: N/P = 2.0, Lane 5: N/P = 4.0.)

and in vivo is under investigation and will be reported in due course.

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12. All new compounds exhibited spectral characteristics consistent with their given structures. Selected spectral data for the final compounds are as follows: Lipid **1**. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 0.85 (t, 6H, $J = 7.0$ Hz, 2 × –$CH_3$), 1.25–1.46 (m, 52H, 26 × –$CH_2$), 1.78–1.80 (m, 4H, 2 × –O–$CH_2$–$CH_2$–), 3.35 (s, 9H, 3 × –$N^+$–$CH_3$), 3.97–4.05 (m, 4H, –O–$CH_2$–$CH_2$–), 4.91 (s, 2H, –$N^+$–$CH_2$–Ar), 6.87 (d, 1H, $J = 8.1$ Hz, ArH), 7.11 (d, 1H, $J = 8.1$ Hz, ArH), 7.21 (s, 1H, ArH). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 14.10, 22.67, 26.02, 26.11, 29.15, 29.35, 29.43, 29.51, 29.71, 31.91, 52.77, 69.11, 69.86, 113.08, 117.93, 119.01, 126.10, 149.27, 151.27. ESIMS: 631.8 ($M^+$). Lipid **2a**. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 0.87 (t, 12H, $J = 7.0$ Hz, 4 × –$CH_3$), 1.25–1.46 (m, 104H, 52 × –$CH_2$), 1.82–1.86 (m, 8H, 4 × –O–$CH_2$–$CH_2$–), 3.01–3.10 (bm, 2H, –$N^+$–$CH_2$–$CH_2$–), 3.21 (s, 12H, 4 × –$N^+$–$CH_3$), 3.96–4.01 (m, 12H, –O–$CH_2$–$CH_2$– and –$N^+$–$CH_2$–$CH_2$), 4.72 (s, 4H, 2 × –$N^+$–$CH_2$–Ar), 6.85–6.88 (d, 2H, $J = 8.1$ Hz, ArH), 7.07–7.10 (m, 4H, ArH). $^{13}$C NMR (75 MHz, $CDCl_3$ + $CD_3OD$) $\delta$: 13.87, 22.49, 25.91, 28.97, 29.16, 29.26, 29.35, 29.53, 31.73, 49.43, 61.80, 68.43, 68.96, 69.65, 113.04, 118.06, 118.62, 126.20, 149.05, 151.40. ESIMS: 701.7 ($M^+ - CH_2Ar(OC_{16}H_{33})$), 571.7. Lipid **2b**. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 0.87 (t, 12H, $J = 7.0$ Hz, 4 × –$CH_3$), 1.25–1.46 (m, 104H, 52 × –$CH_2$), 1.76–1.86 (m, 8H, 4 × –O–$CH_2$–$CH_2$–), 2.29–3.10 (m, 4H, 2 × –$N^+$–$CH_2$–$CH_2$–), 3.12 (s, 12H, 4 × –$N^+$–$CH_3$), 3.99 (t, 8H, $J = 6.0$ Hz, 4 × –O–$CH_2$–$CH_2$–), 4.02–4.11 (m, 4H, –$N^+$–$CH_2$–$CH_2$), 4.62 (s, 4H, 2 × –$N^+$–$CH_2$–Ar), 6.88 (d, 2H, $J = 8.1$ Hz, ArH), 7.05–7.12 (m, 4H, ArH). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 14.06, 26.06, 26.12, 29.20, 29.33, 29.48, 29.56, 29.71, 31.89, 49.33, 64.12, 68.05, 69.04, 69.81, 113.09, 118.12, 118.88, 126.37, 149.17, 151.16. ESIMS: 644.2 ($M^{+2}$/2), 715.8. Lipid **2c**. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 0.88 (t, 12H, $J = 7.0$ Hz, 4 × –$CH_3$), 1.25–1.47 (m, 106H, 52 × –$CH_2$ and –$N^+$–$CH_2$–$CH_2$–$CH2$–), 1.79–1.85 (m, 8H, 4 × –O–$CH_2$–$CH_2$–), 2.10–2.24 (bm, 4H, 2 × –$N^+$–$CH_2$–$CH_2$–), 3.18 (s, 12H, 4 × –$N^+$–$CH_3$), 3.99–4.01 (m, 12H, 4 × –O–$CH_2$–$CH_2$– and 2 × –$N^+$–$CH_2$–$CH_2$), 4.75 (s, 4H, 2 × –$N^+$–$CH_2$–Ar), 6.87 (d, 2H, $J = 8.1$ Hz, ArH), 7.13 (m, 4H, ArH). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 14.03, 22.60, 26.07, 29.30, 29.43, 29.66, 31.85, 49.20, 64.25, 67.51, 68.97, 69.73, 112.98, 118.17, 119.18, 126.35, 149.05, 150.99. ESIMS: 731.2, 650.2 ($M^{+2}$/2), 571.7, Lipid **2d**. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 0.88 (t, 12H, $J = 7.0$ Hz, 4 × –$CH_3$), 1.25–1.46 (m, 108H, 52 × –$CH_2$ and 2 × –$N^+$–$CH_2$–$CH_2$–$CH_2$–), 1.78–1.82 (m, 8H, 4 × –O–$CH_2$–$CH_2$–), 2.24–2.40 (m, 4H, 2 × –$N^+$–$CH_2$–$CH_2$–), 3.21 (s, 12H, 4 × –$N^+$–$CH_3$), 3.84–3.95 (m, 4H, –$N^+$–$CH_2$–$CH_2$), 3.97–4.03 (m, 8H, 4 × –O–$CH_2$–$CH_2$–), 4.62 (s, 4H, –$N^+$

–$CH_2$–Ar), 6.87 (d, 2H, $J = 8.1$ Hz, ArH), 7.13–7.19 (m, 4H, ArH). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 14.06, 21.86, 22.63, 24.67, 26.02, 26.09, 29.16, 29.31, 29.43, 29.51, 29.62, 31.88, 49.51, 64.56, 67.62, 69.04, 69.76, 113.07, 118.22, 119.24, 126.33, 149.12, 151.06. ESIMS: 657.2 ($M^{+2}$/2), 616.5, 571.3. Lipid **2e**. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 0.86 (t, 12H, $J = 7.0$ Hz, 4 × –$CH_3$), 1.16–1.40 (m, 124H, 52 × –$CH_2$ and 2 × –$N^+$–$CH_2$–$CH_2$–$CH_2$–$CH_2$–$CH_2$–$CH_2$–), 1.76–1.78 (m, 8H, 4 × –O–$CH_2$–$CH_2$–), 3.17 (s, 12H, 4 × –$N^+$–$CH_3$), 3.62–3.70 (m, 4H, 2 × –$N^+$–$CH_2$–$CH_2$), 3.94–3.99 (m, 8H, 4 × –O–$CH_2$–$CH_2$–), 4.79 (s, 4H, –$N^+$–$CH_2$–Ar), 6.82 (d, 2H, $J = 8.1$ Hz, ArH), 7.09 (d, 2H, $J = 8.1$ Hz, ArH), 7.15–7.21 (m, 2H, ArH). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 14.05, 22.62, 26.04, 26.07, 28.65, 29.15, 29.31, 29.41, 29.48, 29.67, 31.86, 49.36, 64.04, 67.62, 69.01, 69.70, 112.98, 118.14, 119.14, 126.27, 149.10, 151.04. ESIMS: 700.3 ($M^{+2}$/2). Lipid **3a**. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 0.88 (t, 12H, $J = 7.0$ Hz, 4 × –$CH_3$), 1.25–1.46 (m, 104H, 52 × –$CH_2$), 1.78–1.90 (m, 8H, 4 × –O–$CH_2$–$CH_2$–), 3.13 (s, 12H, 4 × –$N^+$–$CH_3$), 3.99–4.13 (m, 8H, 4 × –O–$CH_2$–$CH_2$–), 4.87 (s, 4H, –$N^+$–$CH_2$–Ar), 5.33 (s, 4H, –$N^+$–$CH_2$–Ar), 6.87 (d, 2H, $J = 8.1$, ArH), 7.21–7.25 (m, 4H, ArH), 7.81 (s, 4H, –$CH_2$–$C_6H_6$–$CH_2$–). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 14.20, 22.88, 26.29, 26.34, 29.38, 29.56, 29.66, 29.72, 29.87, 29.92, 32.13, 48–49 (mixed with $CD_3OD$), 66.67, 68.23, 69.42, 70.13 113.52, 118.57, 119.05, 126.73, 130.18, 134.36, 149.54, 151.63. ESIMS: 763.5, 668.5 ($M^{+2}$/2), 616.7, 571.3. Lipid **3b**. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 0.88 (t, 12H, $J = 7.0$ Hz, 4 × –$CH_3$), 1.25–1.46 (m, 104H, 52 × –$CH_2$), 1.78–1.85 (m, 8H, 4 × –O–$CH_2$–$CH_2$–), 3.11 (s, 12H, 4 × –$N^+$–$CH_3$), 3.98–4.00 (m, 8H, 4 × –O–$CH_2$–$CH_2$–), 4.84 (s, 4H, –$N^+$–$CH_2$–Ar), 5.14 (s, 4H, –$N^+$–$CH_2$–Ar), 6.85 (d, 2H, $J = 8.1$ Hz, ArH), 7.13–7.24 (m, 4H, ArH), 7.27 (m, 1H, ArH), 7.40–7.48 (m, 1H, ArH), 7.79–7.84 (m, 2H, ArH). $^{13}$C NMR (75 MHz, $CDCl_3$) $\delta$: 14.05, 22.65, 26.04, 26.10, 29.18, 29.31, 29.46, 29.53, 29.69, 31.86, 48.48, 66.67, 68.23, 68.99, 69.73, 112.98, 118.14, 118.90, 126.51, 126.80, 129.95, 135.25, 138.42, 149.15, 151.11. ESIMS: 763.3, 668.5 ($M^{+2}$/2), 571.7.