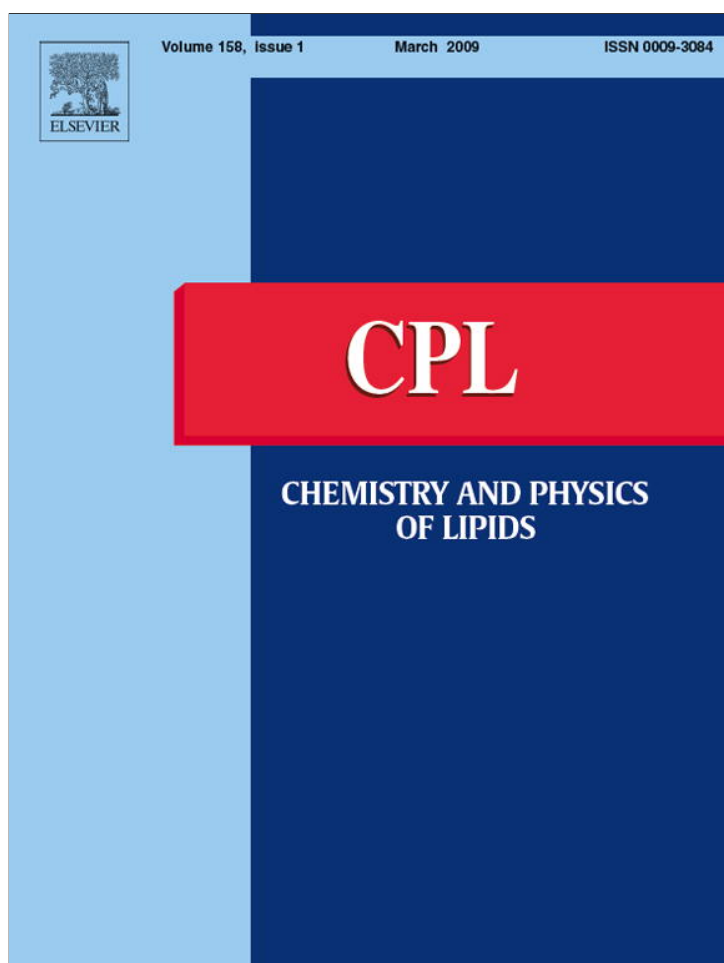


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## Polycations. 18. The synthesis of polycationic lipid materials based on the diamine 1,4-diazabicyclo[2.2.2]octane

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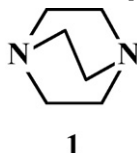
## ABSTRACT

Herein is reported the preparation of several series of polyammonium salts that serve as cationic lipids or precursors thereof, and are structurally based on the parent diamine 1,4-diazabicyclo[2.2.2]octane (dabco). Through selective alkylation of dabco a variety of di- and tetracationic lipid species and precursors thereof have been prepared. The resultant materials are of significant interest for a variety of purposes, including serving as antimicrobial agents and antihydrophobic species, the details of which are provided in separate reports.

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### 1. Introduction

The di-tertiary amine 1,4-diazabicyclo[2.2.2]octane (dabco) constitutes a most useful species for the construction of intriguing series of cationic lipids. The two tertiary amine sites, initially identical and highly reactive as nucleophiles, are readily differentiated in their reactivities upon alkylation of one through judicious choice of solvent and electrophilic substrate so as to allow selective alkylation at each of the two sites. Judicious choice of solvent and electrophilic reagents used provides a wide range of cationic lipids and other useful materials that can be prepared from dabco.



One particularly intriguing aspect of cationic lipids prepared using dabco is that a polar head group can be generated in which two positive charges are present, as well as the possibility of prepar-

ing cationic lipids with variable positive charge depending on the pH of the medium. Further, gemini lipids may be constructed readily using dabco bearing these characteristics at each end of the structure.

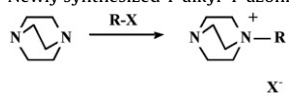
Prior efforts of these laboratories have been reported in which a variety of polycationic salts have been constructed from dabco and used for the preparation of room temperature ionic liquids (Lall et al., 2000, 2002a,b; Engel et al., 2002; Cohen et al., 2002; Wishart et al., 2005), as well as for their activity as specific ion binders (Cohen et al., 2000), antimicrobial agents (Abel et al., 2002, 2004; Cohen et al., 2004; Fabian et al., 1997), modifiers for chromatographic applications (Behaj et al., 2002), modifiers of DNA interactions (Strekas et al., 1999), antihydrophobic agents for modification of solubility (Cohen et al., 1998) and ion channel regulators (Gordon et al., 2006). Additional summaries of these efforts have also been published (Cohen and Engel, 1998, 2002).

The antimicrobial (antibacterial and antifungal) activity of the cationic lipids and related cationic large molecular species has been investigated and is understood in some instances (bacteria and biofilms) as involving invasion and distortion of the cell wall leading to lysis of the bacterial cell (Russell and Chopra, 1996; Cabral, 1992; Kugler et al., 2005; Endo et al., 1987; Fidal et al., 1997; Friedrich et al., 2000; Isquith et al., 1972; Tapias et al., 1994; Sicchierolli et al., 1995; Campanhã et al., 2001; Lincopan et al., 2003; Lincopan et al., 2005;

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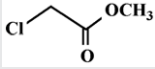
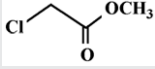
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**Table 1**  
Newly synthesized 1-alkyl-1-azonia-4-azabicyclo[2.2.2]octane salts.



Compound number	R-X	Solvent	Yield	<sup>1</sup> H NMR (solvent) (δ)	<sup>13</sup> C NMR (δ)	Analysis
1	<i>n</i> -C <sub>3</sub> H <sub>7</sub> Br	EtOAc	76%	(D <sub>2</sub> O) 0.83 (3H) <i>t</i> , 1.71 (2H) <i>br</i> , 3.10 (6H) <i>br</i> , 3.15 (2H) <i>br</i> , 3.29 (6H), <i>br</i>	13.2, 21.7, 44.2, 52.1, 64.7	Calcd: C <sub>9</sub> H <sub>19</sub> N <sub>2</sub> Br(H <sub>2</sub> O) C: 42.70%; H: 8.36% Found: C: 42.57%; H: 8.50%
2	<i>i</i> -C <sub>3</sub> H <sub>7</sub> Br	EtOAc	54%	(D <sub>2</sub> O) 0.94 (6H) <i>d</i> , 3.11 (6H) <i>br</i> , 3.28 (6H) <i>br</i> , 3.45 (1H) <i>m</i>	14.4, 45.1, 53.0, 66.3	Calcd: C <sub>9</sub> H <sub>19</sub> N <sub>2</sub> Br(H <sub>2</sub> O) C: 42.70%; H: 8.36% Found: C: 42.63%; H: 8.42%
3	<i>i</i> -C <sub>4</sub> H <sub>9</sub> Br	EtOAc	61%	(D <sub>2</sub> O) 0.77 (6H) <i>d</i> , 1.65 (1H) <i>br</i> , 3.11, (6H) <i>br</i> , 3.45 (1H) <i>m</i>	13.1, 22.0, 44.6, 52.4, 64.4	Calcd: C <sub>10</sub> H <sub>21</sub> N <sub>2</sub> Br(H <sub>2</sub> O) C: 44.95%; H: 8.68% Found: C: 44.88%; H: 8.83%
4	<i>n</i> -C <sub>5</sub> H <sub>11</sub> Br	EtOAc	53%	(D <sub>2</sub> O) 0.79 (3H) <i>t</i> , 1.24 (4H) <i>br</i> , 1.66 (2H) <i>br</i> , 3.11 (6H) <i>br</i> , 3.17 (2H), <i>br</i> , 3.23 (6H) <i>br</i>	13.0, 20.8, 21.5, 22.2, 42.0, 44.2, 64.6	Calcd: C <sub>11</sub> H <sub>23</sub> N <sub>2</sub> Br (2H <sub>2</sub> O) C: 44.15%; H: 9.09% Found: C: 44.22%; H: 9.00%
5		EtOAc	41%	(D <sub>2</sub> O) 0.68 (3H) <i>t</i> , 0.91 (3H) <i>d</i> , 1.22 (2H) <i>br</i> , 1.63 (1H) <i>br</i> , 3.12 (6H), <i>br</i> , 3.18 (2H) <i>br</i> , 3.33, (6H) <i>br</i>	12.7, 13.6, 22.7, 28.1, 44.5, 51.8, 64.4	Calcd: C <sub>18</sub> H <sub>30</sub> N <sub>2</sub> O <sub>3</sub> S C: 60.98%; H: 8.53% Found: C: 60.79%; 8.67%
6	<i>n</i> -C <sub>6</sub> H <sub>13</sub> Br	EtOAc	64%	(D <sub>2</sub> O) 0.78 (3H) <i>t</i> , 1.28 (6H) <i>br</i> , 1.67 (2H) <i>br</i> , 3.10 (6H) <i>br</i> , 3.17 (2H), <i>br</i> , 3.31 (6H) <i>br</i> , 1.63 (1H) <i>br</i> , 3.12 (6H), <i>br</i> , 3.18 (2H) <i>br</i> , 3.33, (6H) <i>br</i>	13.2, 21.0, 21.7, 25.2, 30.4, 44.2, 52.0, 64.7, 64.4	Calcd: C <sub>12</sub> H <sub>25</sub> N <sub>2</sub> Br(H <sub>2</sub> O) C: 48.81%; H: 9.22% Found: C: 48.71%; H: 9.23% Found: C: 60.79%; 8.67%
7	<i>n</i> -C <sub>7</sub> H <sub>15</sub> I	EtOAc	49%	(D <sub>2</sub> O) 0.79 (3H) <i>t</i> , 1.28 (8H) <i>br</i> , 1.69 (2H) <i>br</i> , 3.13 (6H) <i>br</i> , 3.18 (2H), <i>br</i> , 3.33 (6H) <i>br</i>	13.4, 21.2, 21.9, 25.6, 27.8, 30.8, 44.3, 52.1, 64.8	Calcd: C <sub>13</sub> H <sub>27</sub> N <sub>2</sub> I C: 46.02%; H: 8.04% Found: C: 46.02%; H: 8.15%
8	<i>n</i> -C <sub>10</sub> H <sub>21</sub> Br	EtOAc	96%	(D <sub>2</sub> O) 0.80 (3H) <i>t</i> , 1.13–1.32 (14H) <i>br</i> , 1.72 (2H) <i>br</i> , 3.14 (6H) <i>m</i> , 3.25, (2H) <i>m</i> , 3.43 (6H) <i>m</i>	13.9, 21.7, 22.7, 26.3, 29.1, 29.4, 29.51, 29.55, 31.9, 44.2, 52.1, 64.4	Calcd: C <sub>16</sub> H <sub>33</sub> N <sub>2</sub> Br C: 57.65%; H: 9.98% Found: C: 57.60%; H: 10.02%
9	<i>n</i> -C <sub>18</sub> H <sub>37</sub> Br	EtOAc	53%	(CDCl <sub>3</sub> ) 0.81 (3H) <i>t</i> , 1.19–1.27 (30H) <i>br</i> , 1.76 (2H) <i>br</i> , 3.21 (6H) <i>br</i> , 3.44 (2H) <i>br</i> , 3.59 (6H), <i>br</i>	14.1, 22.2, 22.7, 26.4, 28.9, 29.36, 29.41, 29.43, 29.47, 29.51, 29.59, 29.63, 29.68, 29.72, 29.77, 29.85, 29.94, 31.93, 42.2, 45.4	Calcd: C <sub>24</sub> H <sub>49</sub> N <sub>2</sub> Br C: 64.69%; H: 11.08% Found: C: 64.73%; H: 11.00%
10	<i>n</i> -C <sub>22</sub> H <sub>45</sub> Br	EtOAc	58%	(CDCl <sub>3</sub> ) 0.80 (3H) <i>t</i> , 1.17–1.30 (38H) <i>br</i> , 1.75 (2H) <i>br</i> , 3.20 (6H) <i>br</i> , 3.42 (2H) <i>br</i> , 3.60 (6H), <i>br</i>	13.0, 22.3, 22.8, 26.3, 28.66, 28.93, 29.34, 29.40, 29.44, 29.47, 29.50, 29.52, 29.58, 29.61, 29.67, 29.70, 29.73, 29.75, 29.84, 29.90, 29.95, 32.00, 45.3, 52.4	Calcd: C <sub>28</sub> H <sub>57</sub> N <sub>2</sub> Br C: 67.04%; H: 11.48% Found: C: 66.95%; H: 11.48%
11		EtOAc	73%	(D <sub>2</sub> O) 3.05 (6H) <i>br</i> , 3.27 (6H) <i>br</i> , 3.75 (2H) <i>br</i> , 5.57 (2H) <i>m</i> , 5.83 (1H), <i>m</i>	44.1, 51.9, 66.5, 123.6, 129.0	Calcd: C <sub>9</sub> H <sub>17</sub> N <sub>2</sub> Cl(2H <sub>2</sub> O) C: 48.10%; H: 9.42% Found: C: 46.17%; H: 9.38%
12		EtOAc	44%	(D <sub>2</sub> O) 0.91 (3H) <i>d</i> , 1.27 (2H) <i>br</i> , 1.63 (2H) <i>br</i> , 3.25 (6H) <i>br</i> , 3.71 (9H), <i>br</i>	13.7, 21.6, 22.3, 44.7, 52.1, 63.3, 68.2	Calcd: C <sub>11</sub> H <sub>23</sub> N <sub>2</sub> OCl(H <sub>2</sub> O) C: 52.27%; H: 9.99% Found: C: 51.98%; H: 10.10%
13		EtOAc	61%	(D <sub>2</sub> O) 1.85 (2H) <i>m</i> , 2.01 (3H) <i>s</i> , 2.55 (2H) <i>br</i> , 3.10 (8H) <i>br</i> , 3.34 (6H) <i>br</i>	17.3, 31.1, 46.0, 53.9, 64.4, 65.1, 165.0	Calcd: C <sub>11</sub> H <sub>21</sub> N <sub>2</sub> OCl C: 56.76%; H: 9.09% Found: C: 56.61%; H: 9.21%
14		EtOAc	73%	(D <sub>2</sub> O) 3.09 (6H) <i>t</i> , 3.28 (2H) <i>m</i> , 3.40–3.52 (8H) <i>m</i> , 4.23–4.29 (1H) <i>m</i>	44.1, 53.2, 63.6, 65.2, 66.6	Calcd: C <sub>9</sub> H <sub>19</sub> N <sub>2</sub> O <sub>2</sub> Cl C: 48.54%; H: 8.60% Found: C: 48.14%; H: 8.36%

Table 1 (Continued)

Compound number	R-X	Solvent	Yield	<sup>1</sup> H NMR (solvent) (δ)	<sup>13</sup> C NMR (δ)	Analysis
15		EtOAc	65%	(D <sub>2</sub> O) 1.99 (2H) br, 2.35	16.8, 30.0, 44.1,	Calcd: C <sub>11</sub> H <sub>21</sub> N <sub>2</sub> O <sub>2</sub> Cl(H <sub>2</sub> O)
				(2H) br, 3.10 (6H) br, 3.19 (2H) br, 3.35 (6H), br, 3.71 (3H) s	52.0, 52.3, 63.2, 175.9	C: 49.53%; H: 8.69% Found: C: 49.71%; H: 8.72%
16		EtOAc	86%	(D <sub>2</sub> O) 3.13 (3H) s, 3.59	47.8, 51.6, 52.1,	Calcd: C <sub>9</sub> H <sub>17</sub> N <sub>2</sub> O <sub>2</sub> Cl
				(12H) m, 3.73 (2H) s	60.2, 164.0	C: 45.91%; H: 10.70% Found: C: 46.02%; H: 10.85%

Thome et al., 2003; Kanazawa et al., 1993; Popa et al., 2003), and is at least one instance with fungi (*C. albicans*) has been shown to be the result of changing the charge nature of the cell wall without lysis, but leading to cell death (Vieira and Carmona-Ribeiro, 2006). Such investigations have been performed using cationic ionene polymeric species bearing monocationic regions or simple monocationic lipid species. The incorporation of higher charge densities within the polar portions of the cationic lipids would be anticipated to provide corresponding effects at lower concentrations owing to increased electrostatic disruption of the normal cell wall structure. Indeed, when incorporated into surfaces, such polycationic species exhibit very efficient cell lysis as determined with the Gram positive bacterium *S. aureus* (Abel et al., 2002, 2004; Cohen et al., 2004; Melkonian, 2008). Specifically, *S. aureus* cells stained with Gram stain when placed on such treated surfaces immediately release the stain to the surroundings, the change in color and dispersal of dye indicating immediate cell lysis.

Numerous additional cationic lipids and related salts based on the dabco molecule have more recently been synthesized. These have found use in a variety of biological systems for specific biological regulatory purposes. Among these purposes are their uses as antiviral agents (Melkonian, 2008), antiparasitic agents (Yarlett, 2008), cardiac ion channel regulators (Abbott, 2008), and neuronal ion channel regulators (Brumberg, 2008). The particular biological characteristics of these polycationic lipids in these systems will be discussed in reports concerning the specific biological phenomena. The current report is concerned with the syntheses of new polycationic lipids based on dabco and related salts that are used therein. Continuing efforts will be concerned with the use of such materials for generating artificial membranes with characteristics quite different from those commonly found with biological lipids.

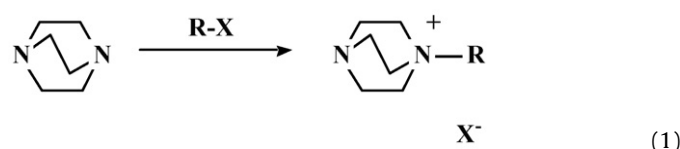
## 2. Results and discussion

Syntheses and descriptions of quaternary ammonium polycationic lipids and their precursors, as well as derived species, are organized herein according to structural types. The fundamental structural building blocks are presented first, with further constructed materials following according to detailed structural type.

### 2.1. 1-Alkyl-1-azonia-4-azabicyclo[2.2.2]octane salts

These materials bearing a single quaternary ammonium site within the dabco framework, along with a free tertiary amine site on the same dabco unit, are produced by simple nucleophilic substitution reaction involving one of the two reactive dabco tertiary amine sites on an alkyl unit bearing a reasonable leaving group, as

is shown in Eq. (1).



The materials prepared in this manner can serve as simple cationic lipids (with a wide range in chain lengths and attendant functionality) and as precursors to structurally more complex species. The full range of compounds (1–16) thus synthesized is shown in Table 1 with data concerning the reaction yield, reaction solvent, analytical data, and NMR (<sup>1</sup>H and <sup>13</sup>C) spectra. Along with other monoalkylated dabco salts previously reported (Cohen et al., 2000), species 7–10 are of particular interest for direct binding to pre-existing surfaces to render them antimicrobial, and are of interest in establishing specific structural relationships with bacteria and fungi studied. These materials by themselves are not observed to have antiviral effects, although other types of dabco derivatives (*vide infra*) have exhibited such effects (Melkonian, 2008). Other species are of particular use for further structural elaboration.

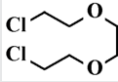
The principal difficulty in accomplishing these syntheses in good yield is related to the proper choice of solvent that allows reaction to be limited to monoalkylation with minimal formation of the dialkylated product. As anticipated many of these species are quite hydrophilic and become hydrated upon standing for even brief times open to the surrounding atmosphere. Anhydrous materials can be obtained by drying under high vacuum with immediate use, although this is generally not necessary for continuing syntheses involving them as discussed here.

While ethyl acetate is the most common solvent used for efficient monoalkylation of dabco, other solvent systems can be used, including methylene chloride and simple alcohols. The use of ethyl acetate facilitates the precipitation of the monoalkylated product and thus prevents extensive dialkylation. With methylene chloride, while there is some solubility of the monoalkylated material, the reactivity difference between dabco and the monoalkylated species is such that selectivity can be attained simply by limiting the amount of alkylating reagent used. Acetonitrile, on the other hand, should be avoided for the solvent to allow maximization of monoalkylation; the monoalkylated salts exhibit significant solubility and reactivity in acetonitrile.

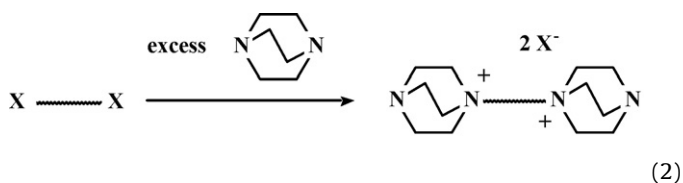
### 2.2. 4-(ω'-{1''-Azonia-4''-azabicyclo[2.2.2]octyl}-α'-alkyl)azonia-1-azabicyclo[2.2.2]octane salts

These materials constitute a building block both for more complex salts derived from dabco, as well as gemini lipids via simple

**Table 2**  
Newly synthesized 4-( $\omega'$ -{1''-azonia-4''-azabicyclo[2.2.2]octyl}- $\alpha'$ -alkyl)azonia-1-azabicyclo[2.2.2]octane salts.

Compound number	X–X	Solvent	Yield	<sup>1</sup> H NMR (solvent) ( $\delta$ )	<sup>13</sup> C NMR ( $\delta$ )	Analyses
17	Br(CH <sub>2</sub> ) <sub>3</sub> Br	CH <sub>3</sub> CN	74%	(D <sub>2</sub> O) 2.32 (2H) <i>m</i> , 3.19 (12H) <i>br</i> , 3.35 (4H) <i>br</i> , 3.45 (12H), <i>br</i>	14.0, 42.8, 51.1, 59.2	Calcd: C <sub>15</sub> H <sub>30</sub> N <sub>4</sub> Br <sub>2</sub> C: 42.27%; H: 7.09% Found: C: 42.51%; H: 7.23% <b>17</b>
18	Cl(CH <sub>2</sub> ) <sub>4</sub> Cl	CH <sub>3</sub> CN	71%	(D <sub>2</sub> O) 1.34 (4H) <i>br</i> , 3.18 (16H) <i>br</i> , 3.38 (12H) <i>br</i>	17.9, 45.5, 53.2, 65.7	Calcd: C <sub>16</sub> H <sub>32</sub> N <sub>4</sub> Cl <sub>2</sub> C: 54.69%; H: 9.18% Found: C: 54.50%; H: 9.01%
19	Cl(CH <sub>2</sub> ) <sub>6</sub> Cl	CH <sub>3</sub> CN	82%	(D <sub>2</sub> O) 1.29 (4H) <i>br</i> , 1.66 (4H) <i>br</i> , 3.00–3.16 (16H) <i>br</i> , 3.26 (12H), <i>br</i>	27.8, 31.9, 50.8, 58.7, 71.0	Calcd: C <sub>18</sub> H <sub>36</sub> N <sub>4</sub> Cl <sub>2</sub> (H <sub>2</sub> O) C: 54.40%; H: 9.64% Found: C: 54.31%; H: 9.72%
20	Cl(CH <sub>2</sub> ) <sub>8</sub> Cl	CH <sub>3</sub> CN	77%	(D <sub>2</sub> O) 1.31 (8H) <i>br</i> , 1.71 (4H) <i>br</i> , 3.14 (16H) <i>br</i> , 3.35 (12H), <i>br</i>	21.4, 25.7, 28.1, 31.2, 44.4, 64.7	Calcd: C <sub>20</sub> H <sub>40</sub> N <sub>4</sub> Cl <sub>2</sub> C: 58.95%; H: 9.90% Found: C: 58.79%; H: 10.03%
21	Br(CH <sub>2</sub> ) <sub>9</sub> Br	CH <sub>3</sub> CN	92%	(D <sub>2</sub> O) 1.25 (10H) <i>br</i> , 1.63–1.65 (4H) <i>br</i> , 3.12–3.17 (16H) <i>br</i> , 3.27–3.31 (12H) <i>br</i>	19.3, 23.6, 26.2, 26.3, 42.4, 50.2, 62.8	Calcd: C <sub>21</sub> H <sub>42</sub> N <sub>4</sub> Br <sub>2</sub> C: 49.42%; H: 8.29% Found: C: 49.21%; H: 8.45%
22	Cl(CH <sub>2</sub> ) <sub>10</sub> Cl	CH <sub>3</sub> CN	92%	(D <sub>2</sub> O) 1.25 (12H) <i>br</i> , 1.65 (4H) <i>br</i> , 3.05–3.10 (16H) <i>br</i> , 3.25–3.31, (12H) <i>br</i>	22.7, 27.1, 29.7, 29.9, 45.7, 53.6, 66.2	Calcd: C <sub>22</sub> H <sub>44</sub> N <sub>4</sub> Cl <sub>2</sub> (1.5H <sub>2</sub> O) C: 57.13%; H: 10.24% Found: C: 57.22%; H: 10.51%
23		CH <sub>3</sub> CN	82%	(D <sub>2</sub> O) 3.14 (12H) <i>br</i> , 3.44 (16H) <i>m</i> , 3.66 (4H) <i>s</i> , 3.93 (4H) <i>br</i>	46.2, 55.1, 65.3, 65.4, 71.6	Calcd: C <sub>18</sub> H <sub>36</sub> N <sub>4</sub> O <sub>2</sub> Cl <sub>2</sub> (H <sub>2</sub> O) C: 50.34%; H: 8.92% Found: C: 50.08%; H: 9.04%

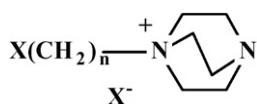
alkylation of the remaining tertiary amine sites. Each of these salts bears two positively charged sites (quaternary ammonium) as well as two tertiary amine sites at opposite ends of the molecule. Preparations are generally performed as shown in Eq. (2), with acetonitrile as the solvent, which allows the initially formed intermediate mono-salt to remain substantially in solution while the second alkylation occurs.



Dicationic species of this type generally are insoluble in ethyl acetate, but with slight solubility in acetonitrile. This character allows the isolation of the materials in good yield, and the slight solubility in acetonitrile, while not preventing their isolation, also allows further reactions to be performed upon them. An excess of dabco is used in these syntheses, the substrate bearing two displaceable units being added dropwise to facilitate optimal formation of the desired dicationic salt.

The new compounds of this type presently synthesized for use in further preparation of more complex species, including gemini lipids, are listed in Table 2 with yields, <sup>1</sup>H and <sup>13</sup>C NMR data, and elemental analyses.

Numerous attempts have been made to perform *monoalkylation* reactions on substrates of the  $\alpha$ ,  $\omega$ -dihaloalkane type used herein for attachment of dabco units at each end of the chain. These were universally unsuccessful for the efficient preparation of species of the type shown below.

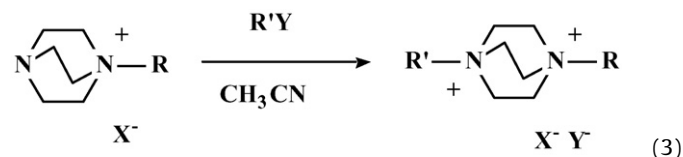


Preparation of this category of species is best accomplished through monoalkylation of dabco using an  $\omega$ -halo-1-alkanol followed by conversion of the remaining residual primary alcohol site to a halide by standard alcohol-to-halide conversion processes (*vide infra*). This halide site then can be utilized in reaction with another mono-alkylated dabco species to generate unsymmetrical di-dabco species.

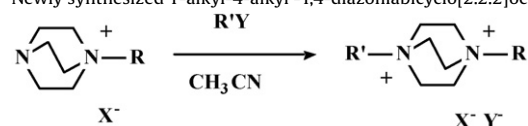
### 2.3. 1-Alkyl-4-alkyl'-1,4-diazoniabicyclo[2.2.2]octane salts

These unsymmetrical dicationic derivatives of dabco are an intriguing set of species that can serve a in a variety of roles depending on the specific natures of the attached alkyl groups. With one attached alkyl group relatively small, they can represent a relatively simple group of ordinary cationic lipids with a highly charged unit as the polar head group. With two large attached groups which have the capability of folding toward each other they can serve as a unique type of cationic lipid, again with a highly charged polar head group.

They are readily constructed by the alkylation of the previously noted 1-alkyl-1-azonia-4-azabicyclo[2.2.2]octane salts with an additional electrophilic reagent (providing alkyl'). As the precursor mono-quaternary salts exhibit significant solubility in acetonitrile, while the dicationic target species exhibits only much more limited solubility in that solvent, isolation of the pure target species is relatively simple. Following the occurrence of the alkylation, the product precipitates from the acetonitrile solution. In some instances other solvent systems were used that allow control of the two separate alkylation procedures. The fundamental reaction system is shown in Eq. (3).



**Table 3**  
Newly synthesized 1-alkyl-4-alkyl'-1,4-diazoniabicyclo[2.2.2]octane salts.



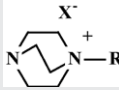
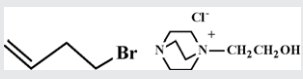
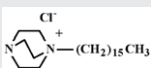
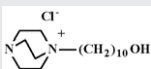
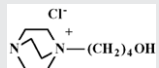
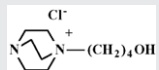
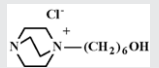
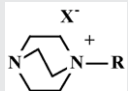
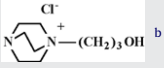
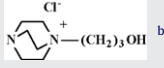
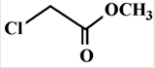
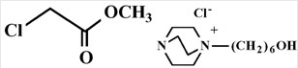
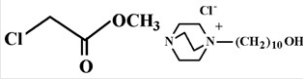
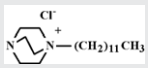
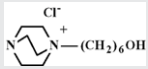
Compound number	R'Y 	Yield	<sup>1</sup> H NMR (solvent) (δ)	<sup>13</sup> C NMR (δ)	Analyses	
24		72 %	(D <sub>2</sub> O) 1.25 (2H) <i>m</i> , 2.71 (2H) <i>br</i> , 2.88 (2H) <i>br</i> , 3.52–4.11 (14H) <i>br</i> , 5.24, (2H) <i>m</i> , 5.76 (1H) <i>br</i>	(D <sub>2</sub> O) 1.25 (2H) <i>m</i> , 2.71 (2H) <i>br</i> , 2.88 (2H) <i>br</i> , 3.52–4.11 (14H) <i>br</i> , 5.24, (2H) <i>m</i> , 5.76 (1H) <i>br</i>	26.2, 51.3, 52.0, 55.0, 65.4, 66.4, 119.5, 131.4	Calcd: C <sub>12</sub> H <sub>24</sub> N <sub>2</sub> OClBr Found: C: 44.11%; H: 7.53%
25	C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> Cl <b>11</b>	63 %	(D <sub>2</sub> O) 2.77 (2H) <i>br</i> , 3.79–3.95 (12H) <i>br</i> , 4.77 (2H) <i>s</i> , 5.68 (2H) <i>m</i> , 5.91 (1H) <i>m</i> , 7.51, (5H) <i>br</i>	(D <sub>2</sub> O) 2.77 (2H) <i>br</i> , 3.79–3.95 (12H) <i>br</i> , 4.77 (2H) <i>s</i> , 5.68 (2H) <i>m</i> , 5.91 (1H) <i>m</i> , 7.51, (5H) <i>br</i>	44.0, 50.8, 67.1, 68.9, 122.3, 122.5, 129.7, 131.1, 131.4, 131.1, 131.4	Calcd: C <sub>16</sub> H <sub>24</sub> N <sub>2</sub> Cl <sub>2</sub> Found: C: 60.78%; H: 7.91%
26	C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> Cl 	79 %	(D <sub>2</sub> O) 0.81 (3H) <i>t</i> , 1.15–1.33 (16H) <i>br</i> , 1.75 (2H) <i>br</i> , 3.05–3.51 (6H) <i>m</i>	(D <sub>2</sub> O) 0.81 (3H) <i>t</i> , 1.15–1.33 (16H) <i>br</i> , 1.75 (2H) <i>br</i> , 3.05–3.51 (6H) <i>m</i>	13.2, 21.2, 21.9, 27.9, 28.2, 28.3, 28.4, 28.5, 28.6, 29.0, 29.7, 31.0, 44.0, 50.6, 51.0, 65.1, 124.7, 129.5, 131.5, 132.7	Calcd: C <sub>24</sub> H <sub>44</sub> N <sub>2</sub> BrCl Found: C: 60.56%; H: 9.32%
27	C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> Cl 	81 %	(D <sub>2</sub> O) 1.13–1.36 (16H) <i>br</i> , 1.77 (2H) <i>br</i> , 3.06–3.49 (4H) <i>br</i> , 3.88–4.00 (12H) <i>m</i> , 7.46–7.88 (5H) <i>m</i>	(D <sub>2</sub> O) 1.13–1.36 (16H) <i>br</i> , 1.77 (2H) <i>br</i> , 3.06–3.49 (4H) <i>br</i> , 3.88–4.00 (12H) <i>m</i> , 7.46–7.88 (5H) <i>m</i>	21.3, 21.8, 25.1, 28.0, 28.3, 28.4, 28.7, 31.1, 34.6, 44.1, 50.7, 50.9, 65.2, 124.9, 129.4, 131.6, 132.9	Calcd: C <sub>23</sub> H <sub>40</sub> N <sub>2</sub> OCl <sub>2</sub> Found: C: 60.28%; H: 8.03%
28	ClC <sub>12</sub> H <sub>25</sub> 	81 %	(D <sub>2</sub> O) 0.84 (3H) <i>t</i> , 1.14–1.38 (20H) <i>m</i> , 1.77–2.08 (4H) <i>m</i> , 3.72 (6H) <i>m</i> , 4.03 (12H) <i>m</i>	(D <sub>2</sub> O) 0.84 (3H) <i>t</i> , 1.14–1.38 (20H) <i>m</i> , 1.77–2.08 (4H) <i>m</i> , 3.72 (6H) <i>m</i> , 4.03 (12H) <i>m</i>	13.9, 21.9, 22.6, 24.6, 25.8, 28.8, 29.3, 29.4, 29.6, 29.7, 31.9, 34.2, 41.9, 51.2, 57.7, 58.4, 62.9, 65.1	Calcd: C <sub>22</sub> H <sub>46</sub> N <sub>2</sub> OCl <sub>2</sub> Found: C: 61.96%; H: 11.01%
29	ClC <sub>16</sub> H <sub>33</sub> 	72 %	(D <sub>2</sub> O) 0.85 (3H) <i>t</i> , 1.28–1.41 (28H) <i>br</i> , 1.85–2.26 (4H) <i>br</i> , 3.65 (6H) <i>m</i> , 4.12, (12H) <i>m</i>	(D <sub>2</sub> O) 0.85 (3H) <i>t</i> , 1.28–1.41 (28H) <i>br</i> , 1.85–2.26 (4H) <i>br</i> , 3.65 (6H) <i>m</i> , 4.12, (12H) <i>m</i>	13.9, 22.0, 22.7, 24.6, 26.0, 28.9, 29.0, 29.1, 29.3, 29.6, 29.7, 29.9, 30.0, 30.2, 32.0, 34.1, 41.8, 51.2, 57.7, 58.4, 62.9, 65.1	Calcd: C <sub>26</sub> H <sub>54</sub> N <sub>2</sub> OCl <sub>2</sub> Found: C: 64.58%; H: 11.42%
30	ClC <sub>16</sub> H <sub>33</sub> 	63 %	(D <sub>2</sub> O) 0.79 (3H) <i>t</i> , 1.14–1.63 (30H) <i>br</i> , 1.70–1.85 (6H) <i>m</i> , 3.51 (6H) <i>m</i> , 5.00 (12H) <i>m</i>	(D <sub>2</sub> O) 0.79 (3H) <i>t</i> , 1.14–1.63 (30H) <i>br</i> , 1.70–1.85 (6H) <i>m</i> , 3.51 (6H) <i>m</i> , 5.00 (12H) <i>m</i>	13.8, 21.5, 22.0, 22.6, 24.5, 24.7, 25.0, 26.1, 26.2, 29.1, 29.6, 29.8, 29.9, 30.1, 32.2, 45.4, 51.1, 61.4, 61.6, 65.1, 65.2	Calcd: C <sub>28</sub> H <sub>58</sub> N <sub>2</sub> OCl <sub>2</sub> Found: C: 65.83%; H: 11.52%

Table 3 (Continued)

Compound number	R'Y 	Yield	<sup>1</sup> H NMR (solvent) (δ)	<sup>13</sup> C NMR (δ)	Analyses	
31	 <sup>b</sup>	ClC <sub>12</sub> H <sub>25</sub>	77 %	(D <sub>2</sub> O) 0.81 (3H) <i>t</i> , 1.12–1.33 (20H) <i>br</i> , 1.50–1.92 (2H) <i>m</i> , 3.34–3.82 (18H) <i>br</i>	13.6, 16.9, 18.4, 18.9, 27.2, 27.4, 29.2, 32.4, 33.0, 33.2, 33.4, 35.1, 44.0, 51.1, 58.0, 61.3, 61.4	Calcd: C <sub>21</sub> H <sub>44</sub> N <sub>2</sub> OCl <sub>2</sub> C: 61.30%; H: 10.78% Found: C: 61.25%; H: 10.84%
32	 <sup>b</sup>	C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> Cl	83 %	(D <sub>2</sub> O) 1.38 (2H) <i>m</i> , 1.90 (2H) <i>br</i> , 3.02–3.51 (4H) <i>br</i> , 3.91–4.07 (12H) <i>br</i> , 7.44–7.81 (5H) <i>m</i>	26.2, 34.2, 44.9, 50.8, 51.2, 65.3, 125.4, 132.7, 131.3, 132.7	Calcd: C <sub>16</sub> H <sub>26</sub> N <sub>2</sub> OCl <sub>2</sub> C: 57.66%; H: 7.86% Found: C: 57.50%; H: 7.99%
33	 <sup>c</sup>		80 %	(D <sub>2</sub> O) 3.78 (6H) <i>s</i> , 4.30 (12H) <i>br</i> , 4.63 (4H) <i>s</i>	51.3, 52.5, 60.3, 163.2	Calcd: C <sub>12</sub> H <sub>22</sub> N <sub>2</sub> O <sub>4</sub> Cl <sub>2</sub> C: 45.98%; H: 10.77% Found: C: 46.11%; H: 10.91%
34	 <sup>a,c</sup>		76 %	(D <sub>2</sub> O) 1.23–1.45 (8H) <i>br</i> , 3.31 (4H) <i>br</i> , 3.71 (3H) <i>s</i> , 4.11 (6H) <i>br</i> , 4.20 (6H) <i>br</i> , 4.49 (2H) <i>br</i>	21.5, 25.6, 28.7, 28.8, 28.9, 32.2, 52.4, 54.0, 62.2, 65.8, 164.0	Calcd: C <sub>15</sub> H <sub>30</sub> N <sub>2</sub> O <sub>3</sub> Cl <sub>2</sub> C: 45.70%; H: 10.87% Found: C: 45.81%; H: 10.99%
35	 <sup>c,d</sup>		68 %	(D <sub>2</sub> O) 1.20–1.41 (16H) <i>br</i> , 3.29 (4H) <i>br</i> , 3.76 (3H) <i>s</i> , 4.07 (6H) <i>br</i> , 4.16 (6H) <i>br</i> , 4.53 (2H) <i>br</i>	21.7, 25.3, 25.5, 28.4, 28.6, 28.7, 28.8, 31.6, 44.3, 51.7, 52.4, 54.0, 62.2, 65.8, 165.0	Calcd: C <sub>19</sub> H <sub>38</sub> N <sub>2</sub> O <sub>3</sub> Cl <sub>2</sub> C: 55.20%; H: 9.26% Found: C: 55.08%; H: 9.37%
36	 <sup>a</sup>	C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> Cl	82 %	(D <sub>2</sub> O) 0.78 (3H) <i>t</i> , 1.08–1.32 (20H) <i>br</i> , 1.70 (2H) <i>m</i> , 3.41 (2H) <i>br</i> , 3.82–3.97 (12H) <i>br</i> , 7.59 (5H) <i>m</i>	13.6, 21.5, 22.2, 25.4, 26.2, 28.3, 28.6, 28.7, 28.8, 29.0, 31.4, 50.7, 51.1, 65.2, 68.8, 124.8, 129.6, 131.6, 132.9	Calcd: C <sub>25</sub> H <sub>44</sub> N <sub>2</sub> BrCl C: 61.53%; H: 9.09% Found: C: 61.44%; H: 9.25%
37	 <sup>c,d</sup>	C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub> Cl	71 %	(D <sub>2</sub> O) 1.29 (4H) <i>br</i> , 1.38 (2H) <i>br</i> , 1.73 (2H) <i>br</i> , 3.31–3.57 (6H) <i>br</i> , 3.78–3.90 (12H) <i>br</i> , 7.44 (5H) <i>m</i>	24.5, 24.9, 25.0, 30.8, 50.7, 51.0, 61.6, 65.2, 68.9, 124.8, 129.6, 131.6, 132.9	Calcd: C <sub>19</sub> H <sub>32</sub> N <sub>2</sub> OCl <sub>2</sub> C: 60.79%; H: 8.59% Found: C: 60.70%; H: 8.64%

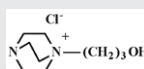
<sup>a</sup> Cohen et al. (2000).<sup>b</sup> Fabian et al. (1997).<sup>c</sup> Methylene chloride was used as the solvent in this reaction.<sup>d</sup> Cohen and Engel (2002).

The characteristics and reaction data for these species, both for structures within which R=R' and R ≠ R', are shown in Table 3. References for reagent species previously reported are indicated as well in Table 3.

Further derivatization of the products of these reactions has been performed to provide multifunctional species that have particular capabilities for specific binding. Of particular note is the conversion of a hydroxyl group to a halide, allowing continued alkylation and extension of the polycationic "string" species. An

example of this extension process is shown with the species listed in Table 4. This specific derivatization involved the conversion of **32** to the chloride **36** by reaction under commonly used conditions (Fabian et al., 1997) with thionyl chloride. The resultant **36** is then extended (unsymmetrically) by reaction with 1-(3'-hydroxypropyl)-1-azonia-4-azabicyclo[2.2.2]octane chloride to generate **37**. This two-step general approach can provide access to the class of polycationic "string" species that are unsymmetrical (have unlike termini).

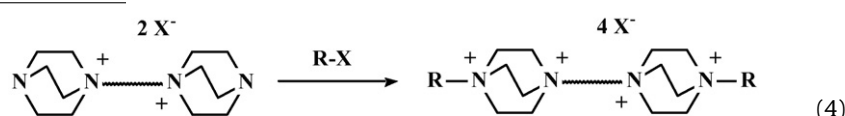
**Table 4**  
Newly synthesized derivatives of fundamental dabco salts.

Compound number	Precursor	Reactant	Yield	<sup>1</sup> H NMR (solvent) (δ)	<sup>13</sup> C NMR (δ)	Analysis
<b>38</b>	<b>32</b>	SOCl <sub>2</sub>	87%	(D <sub>2</sub> O) 1.43 (2H) <i>m</i> , 1.88 (2H) <i>br</i> , 3.45–4.07 (16H) <i>br</i> , 7.43–7.82 (5H) <i>m</i>	23.5, 41.1, 63.4, 69.4, 51.2, 51.9, 125.5, 128.9, 131.1, 132.7	Calcd: C <sub>16</sub> H <sub>26</sub> N <sub>2</sub> OCl <sub>2</sub> C: 54.63%; H: 7.16% Found: C: 54.59%; H: 7.33%
<b>39</b>	<b>38</b>		72%	(D <sub>2</sub> O) 1.32 (2H) <i>m</i> , 1.45 (2H) <i>m</i> , 1.91 (2H) <i>br</i> , 3.22–3.41 (8H) <i>br</i> , 3.51–4.11 (24H) <i>br</i> , 7.40–7.79 (5H) <i>m</i>	23.5, 23.6, 49.8, 62.1, 68.1, 123.9, 128.8, 130.8, 132.0	Calcd: C <sub>25</sub> H <sub>44</sub> N <sub>4</sub> OCl <sub>4</sub> C: 53.77%; H: 7.94% Found: C: 53.82%; H: 7.83%

<sup>a</sup> Cohen et al. (2000).

Symmetrical extension of termini for derivatives of 4-(ω'-{1''-azonia-4''-azabicyclo[2,2,2]octyl}-α'-alkyl)azonia-1-azabicyclo[2.2.2]octane salts. These symmetrical derivatives of the

precursors is illustrated in Eq. (4), and new compounds of this type presently synthesized are illustrated in Table 5 with their particular preparation, NMR, and analytical data.



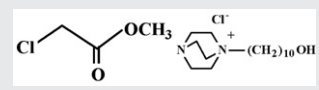
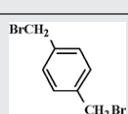
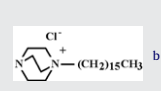
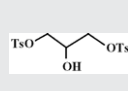
4-(ω'-{1''-azonia-4''-azabicyclo[2,2,2]octyl}-α'-alkyl)azonia-1-azabicyclo[2.2.2]octane salts have been prepared to generate cationic lipid species and their precursors that incorporate not only additional cationic sites within a defined structure, but at the same time provide a variety of terminal functionality. This process for construction of tetracationic lipids and their pre-

### 3. Experimental

#### 3.1. General

All chemicals and solvents used in these syntheses and purifications were of commercial reagent quality and used without

**Table 5**  
Newly synthesized symmetrical tetracationic derivatives of dabco.

Compound number	Precursor	Reactant	Yield	<sup>1</sup> H NMR (solvent) (δ)	<sup>13</sup> C NMR (δ)	Analysis
<b>40</b>			51%	(D <sub>2</sub> O) 1.15–1.31 (28H) <i>br</i> , 1.35 (4H) <i>m</i> , 1.67 (4H) <i>br</i> , 3.36 (8H) <i>br</i> , 3.82 (12H) <i>br</i> , 3.94, (12H) <i>br</i> , 7.68 (4H) <i>s</i>	21.3, 24.9, 25.1, 28.0, 28.2, 28.3, 28.4, 31.2, 44.1, 50.9, 51.1, 61.8, 65.4, 128.2, 134.1	Calcd: C <sub>40</sub> H <sub>74</sub> N <sub>4</sub> O <sub>2</sub> Br <sub>2</sub> Cl <sub>2</sub> C: 54.98%; H: 8.54% Found: C: 54.87%; H: 8.59%
<b>41</b>			81%	(D <sub>2</sub> O) 0.91 (6H) <i>t</i> , 0.95–1.38 (56H) <i>br</i> , 2.11 (6H) <i>s</i> , 3.38–3.71 (33H) <i>br</i> , 7.30 (8H) AA'BB'	14.0, 20.7, 21.0, 22.8, 26.5, 27.5, 28.2, 29.1, 29.6, 29.8, 29.9, 30.1, 30.2, 30.4, 31.2, 32.2, 43.4, 43.8, 50.6, 62.4, 72.0, 125.6, 129.0, 140.1, 141.3	Calcd: C <sub>61</sub> H <sub>110</sub> N <sub>4</sub> O <sub>7</sub> S <sub>2</sub> Cl <sub>2</sub> C: 63.90%; H: 9.67% Found: C: 63.75%; H: 9.91%
<b>42</b>	HO(CH <sub>2</sub> ) <sub>2</sub> Cl	<b>19</b>	68%	(D <sub>2</sub> O) 1.38 (4H) <i>br</i> , 1.82 (4H) <i>br</i> , 3.31–3.79 (12H) <i>br</i> , 3.85–4.15, (24H) <i>br</i>	21.4, 24.7, 51.3, 52.0, 54.9, 64.9, 66.3	Calcd: C <sub>22</sub> H <sub>46</sub> N <sub>4</sub> O <sub>2</sub> Cl <sub>4</sub> C: 48.89%; H: 8.58% Found: C: 48.60%; H: 8.77%
<b>43</b>	HO(CH <sub>2</sub> ) <sub>3</sub> Cl	<b>19</b>	64%	(D <sub>2</sub> O) 1.34 (4H) <i>br</i> , 1.77 (4H) <i>br</i> , 1.98 (4H) <i>br</i> , 3.41–3.69 (12H) <i>br</i> , 3.98 (24H) <i>br</i>	21.3, 24.4, 24.7, 51.1, 51.2, 57.7, 62.8, 64.8	Calcd: C <sub>24</sub> H <sub>50</sub> N <sub>4</sub> O <sub>2</sub> Cl <sub>4</sub> C: 50.71%; H: 6.67% Found: C: 50.66%; H: 8.92%
<b>44</b>	TsO(CH <sub>2</sub> ) <sub>13</sub> CH <sub>3</sub>	<b>19</b>	63%	(D <sub>2</sub> O) 0.81 (6H) <i>t</i> , 0.91–1.36 (56H) <i>br</i> , 2.14 (6H) <i>s</i> , 3.02 (4H) <i>br</i> , 3.37–3.76 (28H) <i>br</i> , 7.40 (8H), AA'BB'	13.8, 20.8, 21.6, 22.7, 26.2, 28.8, 29.0, 29.5, 29.6, 29.7, 29.8, 29.9, 30.0, 32.0, 32.2, 43.9, 50.8, 63.1, 69.1, 70.7, 125.7, 129.0, 140.1, 141.2	Calcd: C <sub>60</sub> H <sub>108</sub> N <sub>4</sub> O <sub>6</sub> S <sub>2</sub> Cl <sub>2</sub> C: 64.54%; H: 9.75% Found: C: 64.38%; H: 9.81%
<b>45</b>	HO(CH <sub>2</sub> ) <sub>10</sub> Cl	<b>21</b>	83%	(D <sub>2</sub> O) 1.15–1.30 (36H) <i>br</i> , 1.39 (4H) <i>br</i> , 1.71, (8H) <i>br</i> , 3.38 (12H) <i>br</i> , 3.85 (24H) <i>br</i>	21.3, 21.4, 24.9, 25.1, 25.2, 28.0, 28.1, 28.2, 28.3, 28.4, 28.5, 31.2, 43.9, 50.8, 51.0	Calcd: C <sub>42</sub> H <sub>86</sub> N <sub>4</sub> O <sub>2</sub> Cl <sub>4</sub> C: 61.44%; H: 10.56% Found: C: 61.22%; H: 10.73%

<sup>a</sup> Cohen et al. (2000).

<sup>b</sup> Fabian et al. (1997).

further purification. All  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were measured with samples in commercial deuterated solvents using a Bruker 400 MHz DPX400 instrument. Elemental analyses were performed by Schwarzkopf Microanalytical Services of Woodside, NY.

### 3.2. Preparation of 1-alkyl-1-azonia-4-azabicyclo[2.2.2]octane salts—general procedure (1–16)

The diamine dabco (1 equivalent amount) and the appropriate haloalkane (0.5 equivalent amount) were dissolved in ethyl acetate (4 × the combined volumes of the reagents) in a round-bottomed flask and stirred at ambient temperature with a magnetic stirrer for 24 h. After this time the white precipitate was collected by suction filtration through sintered glass, washed with ethyl acetate (3 × 35 mL) and anhydrous ether (3 × 30 mL), and dried under high vacuum. Excess dabco and any unreacted haloalkane were washed away in the purification process. Typical yields of products and analytical data are given in Table 1. In the instance of compound 5, rather than the haloalkane the alcohol, 2-methyl-1-butanol was converted to its tosylate ester by reaction with *p*-toluenesulfonyl chloride in pyridine using a standard procedure (Cohen et al., 2000), and the resultant tosylate ester used as the electrophile.

### 3.3. Preparation of 4-( $\omega'$ -{1''-azonia-4''-azabicyclo[2.2.2]octyl}- $\alpha'$ -alkyl)azonia-1-azabicyclo[2.2.2]octane salts (17–23)—general procedure

The diamine dabco (1 equivalent amount) was dissolved in acetonitrile (4 × the combined volumes of the reagents) in a round-bottomed flask and the appropriate  $\alpha, \omega$ -dihaloalkane (0.25 equivalent amount) dissolved in acetonitrile (2 × the combined volumes of the reagents) was added dropwise with stirring. The reaction was stirred at ambient temperature for 24 h after which time the resultant white precipitate was collected by suction filtration through sintered glass and was washed with ethyl acetate (3 × 30 mL) and anhydrous ether (3 × 30 mL), and dried under high vacuum. Typical yields of products and analytical data are shown in Table 2.

### 3.4. Preparation of 1-alkyl-4-alkyl-1,4-diazoniabicyclo[2.2.2]octane salts (24–36)—general procedure

The appropriate 1-alkyl-1-azonia-4-azabicyclo[2.2.2]octane salt (1 equivalent amount) was dissolved in acetonitrile (4 × the combined volume of the reagents) and to it was added the appropriate haloalkane (1 equivalent amount). The reaction mixture was stirred for 3 days at ambient temperature after which time the white precipitate was collected by suction filtration through sintered glass, washed with ethyl acetate (3 × 30 mL) and anhydrous ether (3 × 30 mL), and dried under high vacuum. Typical yields of products and analytical data are shown in Table 3.

### 3.5. Preparation of 1-benzyl-4-(3'-chloropropyl)-1,4-diazoniabicyclo[2.2.2]octane dichloride (38)

The dicationic salt 1-benzyl-4-(3'-hydroxypropyl)-1,4-diazoniabicyclo[2.2.2]octane dichloride (32) (3.0 g; 9.0 mmol) was added to  $\text{CHCl}_3$  (25 mL) in a round bottomed flask (50 mL) fitted with magnetic stirrer, oil bath and reflux condenser. To it was added an excess of thionyl chloride (6.6 g; 56 mmol) and the reaction mixture was stirred with heating at reflux for 16 h. After this time ethanol (8 mL) was added to destroy the excess thionyl chloride. After 2 h stirring the solvent was evaporated under reduced pressure and the solid residue was washed with

ethyl acetate (3 × 50 mL) and the solid material was dried under high vacuum. Yield and analytical data are shown in Table 4.

### 3.6. Preparation of 1-benzyl-4-(3'-{4''-[3'''-hydroxypropyl]-1'',4''-diazoniabicyclo[2.2.2]octane}-1,4-diazoniabicyclo[2.2.2]octane tetrachloride (39)

The dicationic salt 1-benzyl-4-(3'-chloropropyl)-1,4-diazoniabicyclo[2.2.2]octane dichloride (38) (2.0 g; 5.6 mmol) along with 4-(3'-hydroxypropyl)-1-aza-4-azoniabicyclo[2.2.2]octane chloride (1.1 g; 5.6 mmol) were mixed in acetonitrile (50 mL) and heated at reflux with stirring for 3 days. After cooling, the solvent was evaporated and the solid residue was washed with ethyl acetate (3 × 30 mL) and anhydrous ether (3 × 30 mL) and dried under high vacuum. Yield and analytical data are shown in Table 4.

### 3.7. Preparation of 1-alkyl-4-( $\omega'$ -{4''-alkyl-1'',4''-diazoniabicyclo[2.2.2]octane}alkyl)-1,4-diazoniabicyclo[2.2.2]octane tetraanion salts—general procedure by reaction of a 1-alkyl-1-azonia-4-azabicyclo[2.2.2]octane salt with an $\alpha, \omega$ -disubstituted alkylidene (40–41)

The  $\alpha, \omega$ -ditosylalkane (or corresponding dihalo xylylidene) (1 equivalent amount) was dissolved in acetonitrile (4 × the volume of the combined reagents) and to it was added the appropriate 1-alkyl-1-azonia-4-azabicyclo[2.2.2]octane salt (2 equivalent amounts) and the mixture was stirred with heating at reflux for 3 days. After this time the reaction mixture was cooled, the solvent evaporated under reduced pressure, and the residual solid was washed with ethyl acetate (3 × 30 mL) and anhydrous ether (3 × 30 mL) and dried under high vacuum. Yields and analytical data are shown in Table 5.

### 3.8. Preparation of 1-alkyl-4-( $\omega'$ -{4''-alkyl-1'',4''-diazoniabicyclo[2.2.2]octane}alkyl)-1,4-diazoniabicyclo[2.2.2]octane tetraanion salts - general procedure by reaction of a 4-( $\omega'$ -{1''-azonia-4''-azabicyclo[2.2.2]octyl}- $\alpha'$ -alkyl)azonia-1-azabicyclo[2.2.2]octane salt with a 1-haloalkane or 1-alkane tosylate (42–45)

The 1-alkyl-4-( $\omega'$ -{4''-alkyl-1'',4''-diazoniabicyclo[2.2.2]octane}alkyl) salt (1 equivalent amount) was dissolved in acetonitrile (4 × the combined volume of reagents) and to it was added the 1-haloalkane (or 1-alkane tosylate) (2 equivalent amounts) and the reaction mixture stirred with heating at reflux for 3 days. After this time the reaction mixture was cooled, the solvent evaporated under reduced pressure, and the residual solid washed with ethyl acetate (3 × 30 mL) and anhydrous ether (3 × 30 mL) and dried under high vacuum. Yields and analytical data are shown in Table 5.

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## References

- Abbott, 2008. Personal communication.
- Abel, T., Cohen, J.I., Engel, R., Filshinskaya, M., Melkonian, A., Melkonian, K., 2002. Carbohydr. Res. 337, 2496.

- Abel, T., Cohen, J.I., Escalera, J., Engel, R., Filshtinskaya, M., Fincher, R., Melkonian, A., Melkonian, K., 2004. *J. Text. Apparel Technol. Manage.* 3, 1.
- Behaj, V., Cohen, J.I., Engel, R., 2002. *Anal. Lett.* 35, 1715.
- Brumberg, J., 2008. Personal communication.
- Cabral, J.P.S., 1992. *Can. J. Microbiol.* 38, 115.
- Campanhã, M.T.N., Mamizuka, E.M., Carmona-Ribeiro, A.M., 2001. *Phys. Chem. B* 105, 8230.
- Cohen, J.I., Engel, R., 1998. *Trends Org. Chem.* 7, 151.
- Cohen, J.I., Engel, R., 2002. *Curr. Org. Chem.* 6, p453.
- Cohen, J.I., Traficante, L., Schwartz, P.W., Engel, R., 1998. *Tetrahedron Lett.* 39, 8617.
- Cohen, J.I., Castro, S., Han, J.-A., Shteto, V., Engel, R., 2000. *Heteroatom. Chem.* 11, 546.
- Cohen, J.I., Thomas, M., Rikin, A., Castro, S., Behaj, V., Massone, C., Gaillard, J., Lall, S., Engel, R., 2002. *Phosphorus Sulfur* 177, 2265.
- Cohen, J.I., Abel, T., Burkett, D., Engel, R., Escalera, J., Filshtinskaya, M., Hatchett, R., Leto, M., Melgar, Y., Melkonian, K., 2004. *Lett. Drug Des. Discov.* 1, 1.
- Endo, Y., Tani, T., Kodama, M., 1987. *Appl. Environ. Microbiol.* 53, 2050.
- Engel, R., Cohen, J.I., Lall, S., 2002. *Phosphorus Sulfur* 177, 1441.
- Fabian, J., October, T., Cherestes, A., Engel, R., 1997. *Synlett*, 1007.
- Fidal, S., Farer, S.W., Hancock, R.E.W., 1997. *Methods Mol. Biol.* 78, 187.
- Friedrich, G.L., Moyles, D., Beverige, T.J., Hancock, R.E.W., 2000. *Antimicrob. Agents Chemother.* 44, 2086.
- Gordon, E., Cohen, J.I., Engel, R., Abbott, G.W., 2006. *Mol. Pharmacol.* 69, 718.
- Isquith, A.J., Abbott, E.A., Walters, P.A., 1972. *Appl. Microbiol.* 24, 859.
- Kanazawa, A., Ikeda, T., Endo, T., 1993. *J. Polym. Sci. Part A: Polym. Chem.* 31, 3003.
- Kugler, R., Bouloussa, O., Rondelez, F., 2005. *Microbiology* 151, 1341.
- Lall, S.I., Mancheno, D., Castro, S., Behaj, V., Cohen, J.I., Engel, R., 2000. *J. Chem. Soc., Chem. Commun.*, 2413.
- Lall, S.I., Behaj, V., Mancheno, D., Castano, R., Thomas, M., Rikin, A., Gaillard, J., Raju, R., Scumpia, A., Castro, S., Engel, R., Cohen, J.I., 2002a. *Synthesis*, 1430.
- Lall, S., Mancheno, D., Casiano, R., Castro, S., Rikin, A., Cohen, J.I., Engel, R., 2002b. *Phosphorus and Sulfur* 177, 2267.
- Lincopan, N., Mamizuka, E.M., Carmona-Ribeiro, A.M., 2003. *J. Antimicrob. Chemother.* 52, 412.
- Lincopan, N., mamizuka, E.M., Carmona-Ribeiro, A.M., 2005. *J. Antimicrob. Chemother.* 55, 727.
- Melkonian, K., 2008. Personal communication.
- Popa, A., Davidescu, C.M., Trif, R., Ilia, G., Iliescu, S., Dehelean, G., 2003. *React. Funct. Polym.* 55, 151.
- Russell, A.D., Chopra, I., 1996. *Understanding Antibacterial Action and Resistance*. Ellis Harwood, Chichester.
- Sicchierolli, S.M., Mamizuka, E.M., Carmona-Ribeiro, A.M., 1995. *Langmuir* 11, 2991.
- Strekas, T., Engel, R., Locknauth, K., Cohen, J., Fabian, J., 1999. *Arch. Biochem. Biophys.* 364, 129.
- Tapias, G.N., Sicchierolli, E.M., Mamizuka, A.M., Carmona-Ribeiro, 1994. *Langmuir* 10, 3461.
- Thome, J., Höllander, A., Jaeger, W., Trick, I., Oehr, C., 2003. *Surf. Coatings Technol.* 174/175, 584.
- Vieira, D.B., Carmona-Ribeiro, A.M., 2006. *J. Antimicrob. Chemother.* 58, 760.
- Wishart, J., Lall-Ramnarine, S., Raju, R., Scumpia, A., Bellvue, A., Ragbir, R., Engel, R., 2005. *Radiat. Phys. Chem.* 72, 99.
- Yarlett, N., 2008. Personal communication.