CHEMISTRY
A EUROPEAN JOURNAL

DOI: 10.1002/chem.200902794

N,*N*′-Methylenediimidazolium Salts: From Self-Assembly to an Efficient DNAse Protection System

Christophe Pardin,[a] Loïc Leclercq,[b] and Andreea R. Schmitzer*[a]

Abstract: We have developed *N*,*N*′-dialkylmethylenediimidazolium salts ($[C_nC_nDIM][X]_2$) that self-organize into multilayered cationic vesicles and can interact with DNA. These preorganized systems form complexes with linear DNA and protect it from DNase I cleavage.

Keywords: diimidazolium salts · DNA interactions · enzymes · self-assembly · vesicles

Introduction

The imidazole group, which is an effective structural unit found at the active site of various proteins and nucleic acids, plays a key role in the biological function of these macromolecules when in its nonprotonated or protonated form.[1] The imidazolium cation has similar properties to the pyridinium cation in that it retains a positive charge and its salts possess ionic liquid properties.[2] Moreover, it has been demonstrated that the presence of imidazolium groups in a lipid can be beneficial for DNA complexation and many polyimines or polyamines have shown efficacy in nonviral gene therapy.[3] In analogy with the imidazolium cation, *N*,*N*′-disubstituted methylene diimidazolium salts seem to be an interesting recognition motif for the assembly of novel architectures with tunable physicochemical properties. For a better understanding of diimidazolium self-organization and to develop new applications, we have synthesized and studied *N*,*N*′-disubstituted diimidazolium derivatives and investigated their interaction with DNA as potential gene therapy systems.

Results and Discussion

The choice of surfactant needed to obtain a well-defined, self-organized system remains an important question because it can be difficult to predict. In general, the type of aggregate is dependent on the effective van der Waals envelope around the wide polar head group and the narrow apolar tail(s). For example, a conical-shaped envelope gives a micelle, whereas a cylindrical one gives a planar bilayer. The most common types of aggregates are 1) micelles, 2) cylindrical micelles, 3) vesicles, 4) bilayers, and 5) inverted micelles (Table 1). The packing parameter (*PP*) concept was developed over thirty years ago to predict the aggregate structure (Table 1). This parameter is defined as the ratio of the surfactant tail volume, v_0, to the surface area of the head group, a, times the chain length, l_0, of the tail of the surfactant [Eq. (1)].[4]

Table 1. Relationship between packing parameter (*PP*) and the aggregate structure.

PP	Aggregate structure
$<1/3$	micelle
1/3–1/2	cylindrical micelle
1/2–1	vesicle
1	bilayer
>1	inverted micelle

$$PP = \frac{v_0}{a l_0} \quad (1)$$

In general, double-tailed surfactants (for example, di-*n*-dodecyl-dimethylammonium salts) are known to form vesi-

[a] Dr. C. Pardin, Prof. Dr. A. R. Schmitzer
Department of Chemistry, Université de Montréal
C.P. 6128 Succursale Centre-ville, Montréal
Québec, H3C 3J7 (Canada)
Fax: (+1) 514-343-7586

[b] Dr. L. Leclercq
Present address: Université Lille1, UFR Chimie
LCOM, UMR CNRS 8009, Bât. C6
59655 Villeneuve d'Ascq Cedex (France)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/chem.200902794.

cles readily.[5] On the other hand, among reported applications of imidazolium salts,[6] we demonstrated previously that *N*-alkyl-*N*-methylimidazolium can be very useful in micellar catalysis.[7] The methylene diimidazolium cation is useful in orienting the lateral alkyl chains. In analogy with the conventional double-tailed surfactants, *N*,*N*′-dialkylmethylenediimidazolium dication salts ($[C_nC_nDIM][X]_2$) can be versatile building blocks to obtain stable vesicles. To guide us in the selection of promising surfactant molecules, we calculated the *PP* for several diimidazolium salts using MOPAC2009.[8] To gain better insight into their geometrical parameters in aqueous solution, we performed a computational study using the PM6/SCF-MO method, at the restricted Hartree–Fock level, with COSMO (conductor-like screening model) parameters, since the COSMO method is useful for determining the stability of various species in an aqueous environment.[9]

As shown in Figure 1, the effective van der Waals envelope of the diimidazolium cation (without anions) gives a cylinder and a planar bilayer is likely to be obtained in the aggregate. However, the proximity of the counterion is an important parameter. Addition of the anion results in the formation of a truncated cone that may allow the formation of bilayer vesicles. In addition to forming electrostatic interactions with the anions, the imidazolium ring can also be involved in hydrogen bonds. By molecular modeling, we observed that the two triflate anions (four hydrogen bonds, three of which are very strong, Table 2) are in closer proximity to the dication than the two bromide ions. By increasing the length of the alkyl chains, more hydrophobic contacts were obtained (see Figure 1). Moreover, as seen in Figure 1c, for the more hydrophobic CF_3 group of the triflate anion, more van der Waals interactions occur with the alkyl chain compared with the case in which bromide is the counterion.

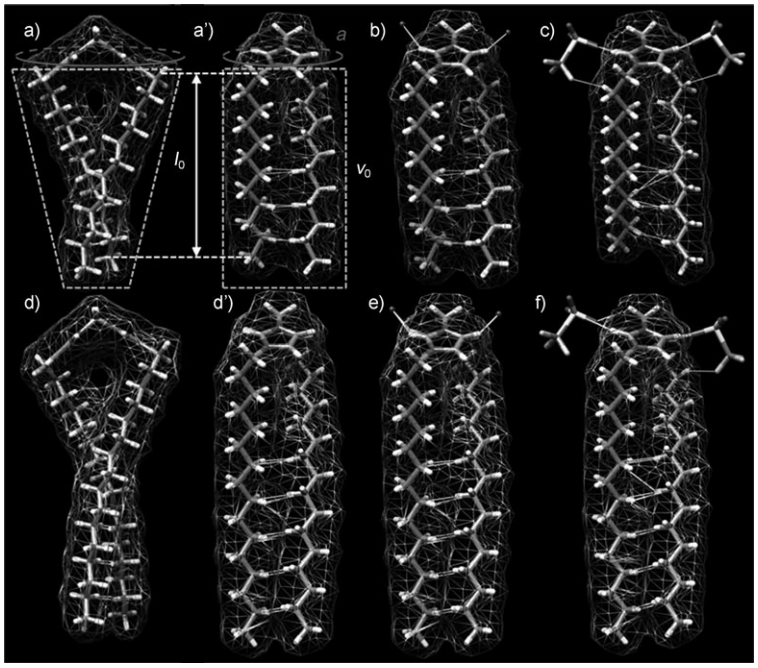


Figure 1. Minimized structures (PM6/COSMO, MOPAC2009TM) of *N*,*N*′-dialkylmethylenediimidazolium salts: a) $[C_{12}C_{12}DIM]$, a′) $[C_{12}C_{12}DIM]$ side view, b) **2**, c) **4**, d) $[C_{16}C_{16}DIM]$, d′) $[C_{16}C_{16}DIM]$ side view, e) **3**, f) **5** (the dashed lines represent the van der Waals interactions (overlap ≥ -0.4 Å)).

Table 2. Hydrogen-bonding analysis after energy minimization for various *N*,*N*′-dialkylmethylenediimidazolium salts.[a]

Compound	$H_2\cdots X$ [Å]	$H_4\cdots X$ [Å]	$C_2{-}H_2\cdots X$ [°]	$C_4{-}H_4\cdots X$ [°]
2	2.47	2.79	142.8	141.3
	2.41	2.83	146.3	141.2
4	1.76	1.79	156.9	156.5
	1.75	2.68	149.5	130.3
3	2.51	2.58	140.5	143.0
	2.51	2.57	140.1	143.2
5	1.76	1.79	157.8	155.7
	1.76	2.69	150.1	129.2

[a] PM6/COSMO, MOPAC2009.

To gain better insight into the plausible aggregates for each optimized structure, the *PP* value was calculated by taking into consideration the hydrophobic volume, the chain length, and the head group surfaces obtained after energy minimization (Table 3).[10]

Knowing the PP and the geometrical parameters can also be useful for predicting certain parameters for a spherical bilayer vesicle [Eqs. (2) and (3) and Table 3].[11]

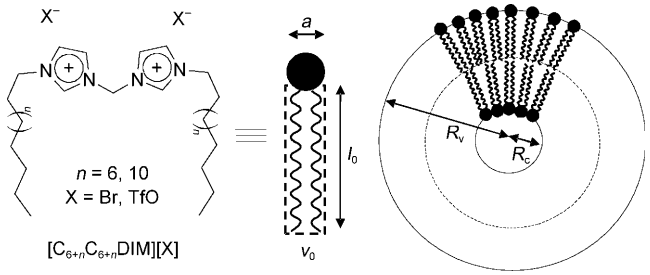
$$R_v = \frac{l_0(3+\sqrt{3(4PP-1)})}{6(1-PP)} \quad (2)$$

$$R_c = \sqrt[3]{\frac{3a(R_v-2l_0)}{12\pi}} \quad (3)$$

The results presented in Table 3 show that in the absence of the anions, the *PP* is very close to one (i.e., planar bilayers are predicted). However, the presence of anions close to the dication sufficiently enhances the surface area of the head group to allow vesicle formation. The predicted size of the bilayer vesicles decreases as the size of the anions increases.

The last parameter that can be estimated from molecular modeling is the solubility of diimidazolium salts in aqueous solution. This method compares the calculated hydration enthalpies of salts [Eq. (4) and Table 4].

Table 3. *PP* values of various *N,N*-dialkylmethylenediimidazolium salts.



	Single molecule parameter				Bilayer vesicle parameter	
	a [Å^2]	l_0 [Å]	v_0 [Å^3]	PP[a]	R_v[b] [Å]	R_c[c] [Å]
[$C_{12}C_{12}$DIM]	40	13.8	556	0.98	–	–
2	50	13.8	556	0.81	66	5
3	50	18.8	730	0.78	77	5
4	60	13.8	556	0.67	37	4
[$C_{16}C_{16}$DIM]	40	18.8	730	1.01	–	–
5	60	18.8	730	0.65	46	3

[a] See Equation (1). [b] See Equation (2). [c] See Equation (3).

Table 4. Calculated hydration enthalpies, entropies, and free energies ($\Delta H_{hydration}$, $\Delta S_{hydration}$, $\Delta G_{hydration}$) of various *N,N'*-dialkylmethylenediimidazolium salts. (The estimated error limits by MOPAC are 3 cal mol^{-1} for ΔS and 8 kcal mol^{-1} for ΔH and ΔG.)

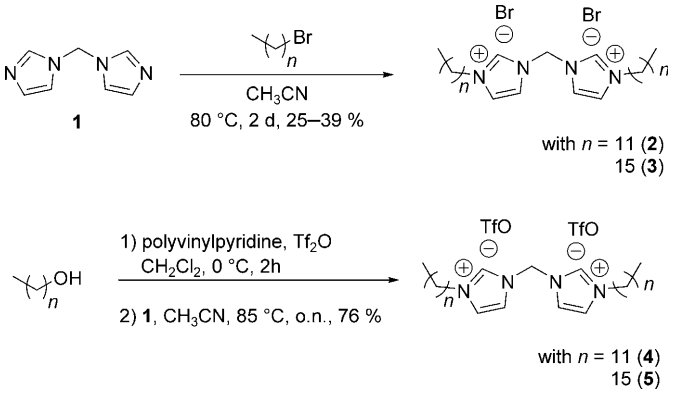
Compound	$\Delta H_{hydration}$ [kcal mol^{-1}]	$\Delta S_{hydration}$ [cal mol^{-1}]	$\Delta G_{hydration}$ [kcal mol^{-1}]
2	63.7	−15.7	68.4
4	45.7	−27.7	53.9
3	56.7	−31.1	66.0
5	47.9	−8.1	50.3

$$\Delta G_{hydration} = (\Delta H_{f(gas)} - \Delta H_{f(water)}) - T(S_{(gas)} - S_{(water)}) \quad (4)$$

The value of $\Delta G_{hydration}$ decreases following the order [$C_{12}C_{12}$DIM][Br]$_2$ (**2**) > [$C_{16}C_{16}$DIM][Br]$_2$ (**3**) > [$C_{12}C_{12}$DIM][TfO]$_2$ (**4**) > [$C_{16}C_{16}$DIM][TfO]$_2$ (**5**). Thus, compound **2** possesses the best affinity for water. All of the salts presented above seem to have the ability to form vesicles. However, a possible limitation to this is their water solubility, especially for the salts with triflate as the counterion.

The *N,N'*-dialkylmethylenediimidazolium bromide salts **2** and **3** were synthesized from methylene diimidazole (**1**)[12] by using 1-bromododecane or 1-bromohexadecane in CH_3CN at 80 °C over 2 days (Scheme 1). Compounds **4** and **5** were synthesized by using methylene diimidazole (**1**) and dodecyl- or hexadecyltrifluoromethanesulfonate in CH_3CN at 85 °C, overnight. The triflate derivatives were obtained from dodecan-1-ol or hexadecane-1-ol by using triflate anhydride and polyvinylpyridine in CH_2Cl_2 for 2 h at 0 °C.[13]

The in silico predictions were confirmed by surface-tension measurements (critical vesicle concentration CVC), zeta potential (ζ), and electron microscopy studies. As predicted by molecular modeling, compound **5** was completely water insoluble. The surface tension curves of **2**, **3**, and **4** are presented in the Supporting Information. The CVC of **4** (0.9 μM) decreases due to the increased hydrophobicity of



Scheme 1. Synthesis of [C_nC_nDIM][Br]$_2$ and [C_nC_nDIM][TfO]$_2$.

the triflate counterion compared with **2** (2 μM). It should be noted that the surface tension values and CVC are not influenced by the alkyl chain length, since the same CVC values were obtained for **2** and **3**.

The morphology of the organized aggregates formed by **2**, **3**, and **4** above the CVC were investigated by dynamic light scattering (DLS) at 633 nm and scanning electron microscopy (SEM). The same size distributions of the aggregates were obtained at different concentrations for all the diimidazolium salts. The hydrodynamic diameter of the aggregates increased with the alkyl chain length (C_{12} versus C_{16}, Table 5) and with the size of the anion (Br versus TfO). The

Table 5. Hydrodynamic diameter and ζ potential of **2**, **3**, and **4** without (−) and with (+) linear DNA.[a]

Compound	DNA (20 ng)	Hydrodynamic diameter [nm]	Polydispersity index (PI)	ζ potential [mV]
2	–	179 ± 19	0.42 ± 0.06	+49 ± 6
	+	234 ± 4	0.41 ± 0.06	+23 ± 4
3	–	262 ± 7	0.45 ± 0.02	+72 ± 3
	+	768 ± 13	0.54 ± 0.05	+32 ± 3
4	–	515 ± 16	0.38 ± 0.01	+11 ± 1
	+	693 ± 32	0.59 ± 0.05	−9 ± 1

[a] Compounds **2**, **3**, and **4** were used in concentrations of 57 μM.

three derivatives exhibit positive ζ potentials (Table 5), since the electric charge distribution on the surface of the vesicles is positive. The vesicles formed by **4** had a lower ζ potential than **2** and **3**. This is probably due to a stronger interaction between the triflate anion and the imidazolium cation.

The distributions of the hydrodynamic diameters, centered at about 180 nm for **2**, 260 nm for **3**,and 515 nm for **4**, correspond to large aggregates, which were proven to be vesicles by SEM. SEM images revealed that spherical vesicles were indeed formed in aqueous solution at concentrations higher than their CVC for all three salts (Figure 2). The diameters of these vesicles were between 2.1 (Figure 2 A) and 3.2 μm (Figure 2 B and C), in accordance with the observation of the large aggregates in the DLS plot. It can be suggested that these vesicles possess a multilayered

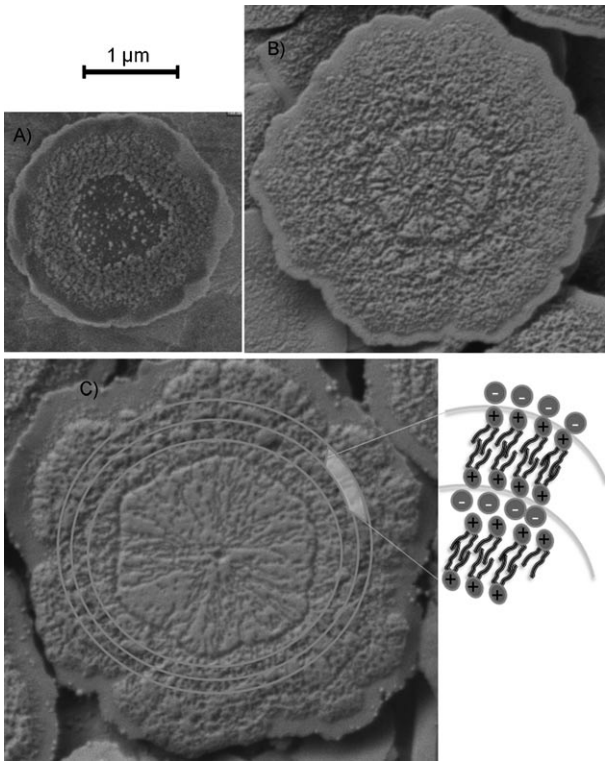


Figure 2. Electron microscopy images of **2** (A), **3** (B), and **4** (C) at a concentration of 10 μM. The inset shows a schematic representation of the multilayered structure of the vesicles.

structure, in which the alkyl tails are slightly tilted and intercalate with each other to form a bilayer cationic structure, alternating with an anionic layer, as shown in the inset of Figure 2. With a longer spacer or a different anion, similar results were obtained for compounds **3** and **4**.

When these vesicles are formed, the positively charged diimidazolium groups are located at the surface of the bilayer, as indicated by the positive ζ potentials of the aggregates. As a result, ionic interaction with a negatively charged molecule (e.g., DNA) should be more difficult for the triflate derivative **4** than for the bromide derivatives **2** and **3**. To test this hypothesis, complexation assays with linear DNA were performed.[14]

DNA complexation was undertaken with a linear DNA fragment obtained by digestion of the circular pQE32-gTG plasmid with the restriction enzyme Xho1. The linearized pQE32-gTG DNA was incubated for 30 min at 37 °C with 50 μM of **2** or **3** or with 100 μM of **4**. The formation of complexes between the linear DNA and the cationic [C_nC_nDIM][X] vesicles can be observed in Figure 3. A commonly used method to characterize cationic vesicle/DNA complexes is to measure their migration in agarose gel electrophoresis. The DNA band completely disappeared in the presence of **2** and **3** indicating the formation of a strong complex (Figure 3). Compound **4** shows a less intense DNA band than the reference DNA lane, indicating a partial complexation. The formation of the DNA complexes with **2**, **3**, and **4**

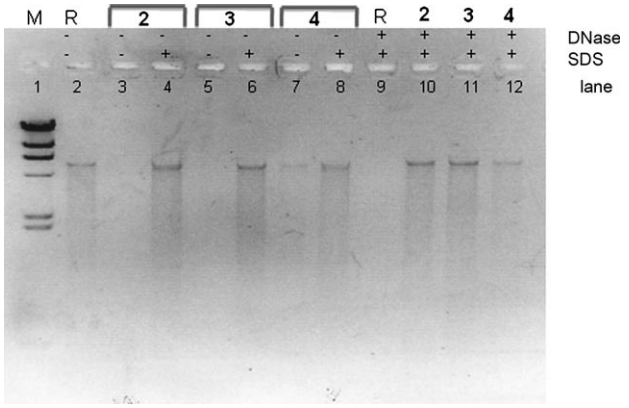


Figure 3. Gel electrophoresis for DNA complexation, protection, and release. Lane 1: marker [M]; lane 2: pQE32-gTG [R]; lanes 3 and 4: complex pQE32-gTG·**2** before and after SDS treatment; lanes 5 and 6: complex pQE32-gTG·**3** before and after SDS; lanes 7 and 8: complex pQE32-gTG·**4** before and after SDS; lane 9: pQE32-gTG + DNase I; lanes 10–12: complexes pQE32-gTG·**2**–**4** incubated with DNase I then released with SDS.

were also studied by DLS. Of note in Table 5 is the increased vesicle size in the presence of DNA and the changes in surface charge; all of the complexes possess inferior ζ potentials than those in the absence of DNA. The hydrodynamic diameter for the three DNA/*N,N′*-dialkylmethylenediimidazolium complexes are higher than the diameter of the vesicles obtained in the absence of DNA (Table 5). The DNA complexes formed with **2** and **3** have a decreased positive charge on the surface. As the final charge is still positive, we assume that at this stoichiometry the entire DNA is complexed by the diimidazolium cationic vesicles. In the case of the complex of **4** with DNA, a negative surface charge is observed, indicating that the DNA is not totally bound. This weaker interaction between the triflate derivative and DNA was also observed by electrophoresis.

The morphological changes in these complexes compared with the free vesicles were investigated by SEM (Figure 4). A more compact aggregate was observed in the presence of DNA. We propose that DNA is predominantly entrapped

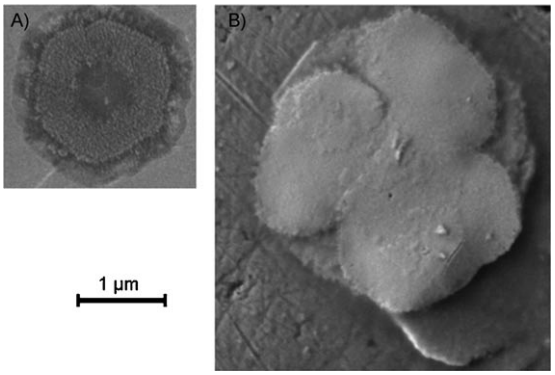


Figure 4. Electron microscopy images of **2** (A) and **3** (B) with linear DNA.

within the bilayers of the vesicles, presumably bound to the inner cationic charges with only a minor portion of DNA interacting with surface cationic charges of the vesicles, given the observed changes in surface ζ potential (Table 5).

A prerequisite for use as a DNA vector is that liposomes or other DNA carriers should not only have the ability to bind DNA, but also to release DNA from the complex. It was previously shown that sodium dodecylsulfate (SDS) is able to electrostatically compete with DNA bound to cationic surface charges and release it into the medium.[3] From Figure 3 it appears that incubation of the complexes with SDS readily released the DNA from the vesicles.

Since the DNA binding ability of these diimidazolium salts has been demonstrated, the capacity of the diimidazolium salts to protect bound DNA from enzymatic cleavage was studied. The DNA/diimidazolium complexes were treated with DNase I and then released from the vesicles with SDS. As seen in Figure 3 (lanes 9–12), we observed the complete disappearance of the free DNA band in the absence of any diimidazolium salt, due to digestion with the DNase I. In contrast, DNA entrapped within diimidazolium salt vesicles was protected from nuclease attack in biological media. The intact DNA band was released in the presence of SDS, with the same intensity for **2** and **3**, and a weaker intensity for **4**. These results show that DNA complexation was probably not complete in the case of **4**, whereas cationic vesicles formed by the diimidazolium bromide salts offer an efficient DNA protection system. This result also confirmed that DNA was entrapped within the bilayer and it was not complexed exclusively on the vesicle surface.

The ease with which the physicochemical and biological properties of these imidazolium salts can be modified encourages us to study their potential for gene transfer in vitro. Each new system has advantages and disadvantages and the selection of a suitable vector depends on the context of a given therapeutic strategy. Bearing in mind the limitations of current cationic systems, it is important to gain a deeper understanding of the complex correlation of physicochemistry and biology to be able to rationally design vectors that overcome systemic, cellular, and molecular barriers to genetic therapy.

Conclusion

N,*N*′-Dialkylmethylenediimidazolium salts were synthesized and showed self-organization into multilayer vesicles that exhibited hydrodynamic diameters varying according to alkyl chain length or the nature of the anion. These vesicles possess positive charges on their surface, allowing them to interact with DNA. Linear DNA was complexed by these self-organized systems to give stable complexes that protected the DNA from enzymatic cleavage. Complete release of the bound DNA was obtained by dissociating the complex with SDS. These diimidazolium dibromide salts are the first step towards the development of nonviral cationic systems containing multiple imidazolium groups.

Experimental Section

General: Imidazole, 1-bromododecane, 1-bromohexadecane, triflate anhydride, polyvinylpyrine, $MgSO_4$, and $NaHCO_3$ were obtained from Aldrich. Acetonitrile (CH_3CN), dichloromethane and diethyl ether were purchased from EMD. SDS was obtained from Bio-rad and Agar from Bioshop. MilliQ water was used for the electronic microscopy assays and the DNA studies. DNase I was obtained from Invitrogen and plasmid pQE32-gTG[14] from Joelle N. Pelletier (Université de Montréal). ^{1}H and ^{13}C NMR spectra were recorded on a Bruker spectrometer at 400 and 300, or100 and 75 MHz, respectively, in the indicated solvent. Chemical shifts are reported in ppm with internal reference to TMS. High-resolution mass spectra were recorded on a LC-MSD-TOF instrument from Agilent technologies in positive electrospray mode in general. Either protonated molecular ions $[M]^{2+}$ or sodium adducts $[M+Na]^+$ were used for empirical formula confirmation. The microscopic images were taken by a JSM-7600F scanning electron microscope with a field-emission gun (JEOL). Each wet sample was deposited on the polished surface of a brass stub and dried under vacuum prior to the SEM observations. The hydrodynamic diameters, the polydipersity indices and the ζ potentials were measured by dynamic light scattering at 633 nm on a Zetamaster S dynamic light scattering instrument (Malvern Instruments).

Molecular modeling: All calculations were performed on a Windows Vista platform. To assess the energy content for various molecules designed, semiempirical quantum calculations were undertaken by using the PM6 method in gas phase or in aqueous solution (MOPAC2009; Stewart Computational Chemistry). For the solution, an implicit model was created, namely, the COSMO model. The COSMO method (conductor-like screening model) is useful for determining the stability of various species in a solvent environment, particularly in water. Theoretical calculations were carried out at the restricted Hartree–Fock level (RHF) using the PM6 semi-empirical SCF-MO methods. For water simulation, a relative permissivity of 78.4 was employed with up to 92 surface segments per atom for the COSMO model being used to construct a solvent accessible surface area based on van der Waals radii. All structures were optimized to a gradient inferior to 0.1 using the eigenvector following method. The enthalpies and entropies were calculated from a thermodynamic calculation after geometric minimization (recycling of the final geometry with the THERMO keyword). The free hydration energy is defined as the difference between the gas and solution phases. For the calculation of the *PP* values, after the geometric optimization, molecular volumes were calculated as COSMO volumes, and tail lengths were measured directly from the optimized geometry as the length from the N atom to the terminal atom of the tail. Head group areas (with anions) were calculated by assuming a spherical head geometry. The MOPAC Cartesian coordinates were generated with OpenBabel 2.2.0 graphical interface (Chris Morley) from the geometries obtained with ArgusLab UFF. After MOPAC geometry optimization, the output files were opened with Molda 6.5 and final pictures were obtained with UCSF Chimera (Regents of the University of California).

***N*,*N*′-Didodecylmethylenediimidazolium dibromide (2)**: 1-Bromododecane (13.4 mmol) was added to a solution of methylene diimidazole **1** (6.7 mmol) in CH_3CN (15 mL) and the mixture was stirred for 48 h at 80 °C. The mixture was filtered and the white solid was washed with Et_2O to give the pure product as a white solid in an isolated yield of 25 %. M.p. > 200 °C; ^{1}H NMR (300 MHz, [D$_6$]DMSO): $\delta = 9.74$ (s, 2H), 8.17 (d, 2H, $J = 1.5$ Hz), 7.94 (d, 2H, $J = 1.2$ Hz), 6.79 (s, 2H), 4.23 (t, 4H, $J = 7.2$ Hz), 1.74–1.85 (m, 4H), 1.21–1.29 (m, 36H), 0.84 ppm (t, 6H, $J = 6.6$ Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): $\delta = 137.5$, 123.1, 122.1, 57.9, 49.3, 31.3, 29.2, 29.1, 29.0, 28.9, 28.8, 28.7, 28.4, 25.5, 22.1, 13.9; HRMS (ESI): *m*/*z* calcd for $C_{31}H_{58}N_4^{2+}$ $[M]^{2+}$: 243.2325; found: 243.2329.

***N*,*N*′-Dihexadecylmethylenediimidazolium dibromide (3)**: 1-Bromohexadecane (13.4 mmol) was added to a solution of methylene diimidazole (6.7 mmol) in CH_3CN (15 mL) and the mixture was stirred for 48 h at 80 °C. The mixture was filtered and the white solid was washed with Et_2O to give the pure product as a white solid in an isolated yield of 39 %. M.p. 198–200 °C (dec.); ^{1}H NMR (300 MHz, CD_3OD): $\delta = 8.04$ (d, 2H, $J = 2.1$ Hz), 7.81 (d, 2H, $J = 2.1$ Hz), 6.81 (s, 2H), 4.30 (t, 4H, $J = 7.6$ Hz),

1.81–1.99 (m, 4H), 1.25–1.39 (m, 52H), 0.90 ppm (t, 6H, J= 6.6 Hz);^{13}C NMR (75 MHz, CD_3OD): δ=139.2, 124.8, 123.5, 59.9, 51.6, 33.1, 30.9, 30.8, 30.7, 30.6, 30.5, 30.2, 30.1, 27.3, 27.2, 23.7, 14.5 ppm; HRMS (ESI): m/z calcd for $C_{39}H_{74}N_4^{2+}$ $[M]^{2+}$: 299.2951; found: 299.2949.

***N,N′*-Didodecylmethylenediimidazolium ditriflate (4)**: Dodecan-1-ol (5.4 mmol) and polyvinylpryridine (6.21 mmol) were added to CH_2Cl_2 (50 mL). The mixture was cooled to 0°C and sulfonic trifluoromethane anhydride (1.36 mL, 1.5 equiv) was added dropwise. The mixture was stirred for 2 h at 0°C and filtered. The solid was washed with CH_2Cl_2 (5 mL)and the filtrate was washed with a saturated aqueous solution of $NaHCO_3$, dried with $MgSO_4$, and concentrated under reduced pressure to give a yellow oil. CH_3CN (25 mL) then methylene bisimidazole (2.7 mmol) were added to the oil. The mixture was stirred at 85°C overnight. Then the mixture was filtered and the white solid was washed with Et_2O to give the pure product as a white solid in an isolated yield of 76%. M.p. 161–163°C (dec.); ^{1}H NMR (400 MHz, CD_3OD): δ=7.82 (s, 2H), 7.90 (s, 2H), 6.66 (s, 2H), 4.27 (s, 4H), 1.92–1.95 (m, 4H), 1.27 (s, 36H), 0.88 ppm (t, 6H, J=6.7 Hz); ^{13}C NMR (100 MHz, CD_3OD): δ= 138.7, 124.5, 124.4, 124.0, 123.4, 123.1, 119.8, 58.4, 50.6, 41.5, 41.3, 41.2, 41.0, 39.8, 32.6, 30.2, 30.1, 30.0, 29.9, 29.7, 29.6, 26.7, 23.4, 15.2 ppm; HRMS (ESI): m/z calcd for $C_{31}H_{58}N_4^{2+}$ $[M]^{2+}$: 243.2325; found: 243.2318.

***N,N′*-Dihexadecylmethylenediimidazolium ditriflate (5)**: Hexadecan-1-ol (4 mmol) and polyvinylpryridine (4.6 mmol) were added to CH_2Cl_2 (50 mL). The mixture was cooled to 0°C and sulfonic trifluoromethane anhydride (1.00 mL, 1.5 equiv) was added dropwise. The mixture was stirred 3 h to 0°C at RT and filtered. The solid was washed with CH_2Cl_2 (5 mL) and the filtrate was washed with a saturated aqueous solution of $NaHCO_3$, dried with $MgSO_4$, and concentrated under reduced pressure to give a yellow oil. CH_3CN (25 mL) then methylene bisimidazole (2 mmol) were added to the oil. The mixture was stirred at 85°C overnight. Then the mixture was filtered and the white solid was washed with Et_2O to give the pure product as a white solid in an isolated yield of 23%. M.p. 143–145°C (dec.); ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ=9.40 (s, 2H), 7.97 (t, 2H. J=1.7 Hz), 7.88 (t, 2H, J=1.7 Hz), 6.60 (s, 2H), 4.20 (t, 4H, J=7.2 Hz), 1.76–1.81 (m, 4H), 1.23 (s, 54H), 0.84 ppm (t, 6H, J= 6.4 Hz); ^{13}C NMR (75 MHz, [D$_6$]DMSO): δ=136.9, 122.7, 121.7, 57.9, 48.8, 30.8, 28.6, 28.4, 28.3, 28.0, 25,0, 21.6, 13.4 ppm; HRMS (ESI): m/z calcd for $C_{39}H_{74}N_4^{2+}$ $[M]^{2+}$: 299.2951; found: 299.2963.

Electron microscopy: Diimidazolium salts were dissolved in water to give a 10 μM solution. The sample was obtained by evaporation of a drop of the solution under reduced pressure. The observations were done with a JSM-7600F scanning electron microscope at 2 kV and the pictures were taken with a conventional secondary electron detector (SEI) with a topography contrast.

Surface tension measurements: The processor tensiometer DCAT 11 (Dataphysics) and the Wilhelmy plate method were used for the surface tension measurements. A concentrated solution was installed in a syringe and the addition of small volumes to ultrapure water (double-distilled water, σ=72.0 mN m^{-1} at 25°C) was used to increase the solution concentration. After each addition, the solution was gently stirred for 30 s. Equilibrium surface tension was measured at each concentration. All surface tension values were mean quantities of at least three measurements. The standard deviation of the mean never deviated more than ±1.5%. The precision of the force transducer of the surface tension apparatus was 0.1 mN m^{-1} and before each experiment, the platinum plate was cleaned in a red/orange-colored flame.

Particle size and ζ-potential measurements: The hydrodynamic diameters of the vesicles formed from the derivatives as well as that of the corresponding complexes were measured by dynamic light scattering at 633 nm on a Zetamaster S dynamic light scattering instrument (Malvern Instruments) at 25°C and at a detection angle of 173°. All of the measurements were performed in triplicate. The ζ-potential measurements were also performed using the same instrument at a detection angle of 173° in triplicate.

Preparation of complexes from diimidazolium salts and DNA: The DNA plasmid pQE32-gTG, used for the preparation of the DNA complexes is a plasmid-encoding guinea pig transglutaminase. The circular plasmid (15 μL at 50 ng μL^{-1}) was digested with XhoI restriction enzyme (12.5 μL) in lyse buffer (50 μL total volume) furnished by the supplier. Following incubation for 1 h at 37°C, the linear plasmid was obtained and this solution (10 μL) was incubated with diimidazolium derivative (10 μL) for 30 min at 37°C to form the complex. Finally, an aliquot (8 μL) of each sample was subjected to agarose gel electrophoresis (1% agarose) in Tris-acetate-EDTA (TAE; EDTA=ethylenediaminetetraacetic acid) buffer.

DNase protection assays: pQE32-gTG (10 μL at 2 ng μL^{-1}) was mixed with an equal volume of stock solution of diimidazolium derivative and incubated for 30 min at 37°C. A solution of DNase I (1 μL, 0.02 U μL^{-1}) was added to the mixture and incubated for 1 h at 37°C. After digestion, a 10% SDS solution (2 μL) was added and the samples were incubated for 15 min at 65°C. Finally, an aliquot (8 μL) of each sample was subjected to agarose gel electrophoresis (1% agarose) in TAE buffer.

Acknowledgements

This work was supported by the Natural Sciences and Engineering Research Council of Canada, the Fonds Québécois de la Recherche sur la Nature et les Technologies, the Canada Foundation for Innovation and the Université de Montréal. We thank Professor J. Pelletier for the pQE32-gTG plasmid and Professor K. C. Waldron for careful reading and discussion of this manuscript.

[1] a) *Comprehensive Supramolecular Chemistry, Vol. 11* (Eds.: J. L. Atwood, J. E. D. Davies, D. D. MacNicol, F. Vögtle), Pergamon, Oxford, **1996**; b) H.-J. Schneider, A. Yatsimirsky in *Principles and Methods in Supramolecular Chemistry*, Wiley, New York, **2000**.

[2] a) M. R. Sambrook, P. D. Beer, J. A. Wisner, R. L. Paul, A. R. Cowley, F. Szemes, M. G. B. Drew, *J. Am. Chem. Soc.* **2005**, *127*, 2292–2302; b) B. Tomapatanaget, T. Tuntulani, J. A. Wisner, P. D. Beer, *Tetrahedron Lett.* **2004**, *45*, 663–666; c) J. A. Wisner, P. D. Beer, N. G. Berry, B. Tomapatanaget, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 4983–4986.

[3] a) M. Mével, G. Breuzard, J.-J. Yaouanc, J. C. Clément, P. Lehn, C. Pichon, P.-A. Jaffrès, P. Midoux, *ChemBioChem* **2008**, *9*, 1462–1471; b) P. Midoux, C. Pichon, J.-J. Yaouanc, P.-A. Jaffrès, *Br. J. Pharmacol.* **2009**, *157*, 166–178.

[4] a) J. N. Israelachvili, D. J. Mitchell, B. W. Ninham, *J. Chem. Soc. Faraday Trans.* **1976**, *72*, 1525–1568; b) J. N. Israelachvili in *Intermolecular and Surface Forces*, 2nd ed., Academic Press, London, **1991**, p. 371.

[5] a) H. Kunieda, K. Shinoda, *J. Phys. Chem.* **1978**, *82*, 1710–1714; b) Y. Talmon, D. Evans, B. W. Ninham, *Science* **1983**, *221*, 1047–1049; c) T. Kunitake, Y. Okahata, *J. Am. Chem. Soc.* **1977**, *99*, 3860–3861; d) D. D. Miller, D. F. Evans, *J. Phys. Chem.* **1989**, *93*, 323–333; e) D. D. Miller, L. J. Magid, D. F. Evans, *J. Phys. Chem.* **1990**, *94*, 5921–5930; f) T. F. Svitova, Y. P. Smirnova, S. A. Pisarev, N. A. Berezina, *Colloids Surf. A* **1995**, *98*, 107–115; g) L. Leclercq, V. Rataj-Nardello, M. Turmine, N. Azaroual, J.-M. Aubry, *Langmuir* **2010**, 1716–1723.

[6] a) H. Zhao, S. V. Malhorta, *Aldrichim. Acta* **2002**, *35*, 75–83; b) Y. Chauvin, H. Olivier-Bourbigou, *CHEMTECH* **1995**, *25*, 26–30; c) K. R. Seddon, *J. Chem. Technol. Biotechnol.* **1997**, *68*, 351–356; d) K. R. Seddon, *Chem. Commun.* **2001**, 2399–2407; e) C. Baudequin, J. Baudoux, J. Levillain, D. Cahard, A. C. Gaumont, J. C. Plaquevent, *Tetrahedron: Asymmetry* **2003**, *14*, 3081–3093; f) L. Leclercq, I. Suisse, F. Agbossou-Niedercorn, *Chem. Commun.* **2008**, 311–313; g) H.-U. Blaser, M. Studer, *Green Chem.* **2003**, *5*, 112–117; h) C. E. Song, *Chem. Commun.* **2004**, 1033–1043; i) S. Park, R. J. Kazlauskas, *Curr. Opin. Biotechnol.* **2003**, *14*, 432–437; j) U. Kragl, M. Eckstein, N. Kaftzik, *Curr. Opin. Biotechnol.* **2002**, *13*, 565–571; k) S. K. Poole, S. Patel, K. Dehring, H. Workman, C. F. Poole, *J. Chromatogr. A* **2004**, *1037*, 445–454; l) A. M. Stalcup, B. Cabovska, *J. Liq. Chromatogr.* **2004**, *27*, 1443–1459; m) M. Antonietti, D. B. Kuang, B. Smarsly, Z. Yong, *Angew. Chem.* **2004**, *116*,

5096–5100; *Angew. Chem. Int. Ed.* **2004**, *43*, 4988–4992; n) S. Geetha, D. C. Trivedi, *Bull. Electrochem.* **2003**, *19*, 37–48.
[7] L. Leclercq, M. Lacour, S. H. Sanon, A. R. Schmitzer, *Chem. Eur. J.* **2009**, *15*, 6327–6331.
[8] J. J. P. Stewart, Stewart Computational Chemistry, Version 7.213W, http://openmopac.net/.
[9] A. Klamt, G. Schüümann, *J. Chem. Soc. Perkin Trans. 2* **1993**, 799–805.
[10] For an example of calculated *PP* values using MOPAC, see: X. Li, J. Turanek, P. Knotigova, H. Kudlackova, J. Masek, D. B. Pennington, S. E. Rankin, B. L. Knutsonc, H.-J. Lehmler, *New J. Chem.* **2008**, *32*, 2169–2179.
[11] R. Nagarajan, *Langmuir* **2002**, *18*, 31–38.
[12] a) H. Kim, J. Kang, *Tetrahedron Lett.* **2005**, *46*, 5443–5445; b) S. Samsam, L. Leclercq, A. R. Schmitzer, *J. Phys. Chem. B* **2009**, *113*, 9493–9498.
[13] L. Leclercq, I. Suisse, G. Nowogrocki, F. Agbossou-Niedercorn, *Green Chem.* **2007**, *9*, 1097–1103.
[14] J. W. Keillor, R. A. Chica, N. Chabot, V. Vinci, C. Pardin, E. Fortin, S. M. F. G. Gillet, Y. Nakano, M. Kaartinen, J. N. Pelletier, W. D. Lubell, *Can. J. Chem.* **2008**, *86*, 271–276.

Received: October 9, 2009
Published online: March 12, 2010

