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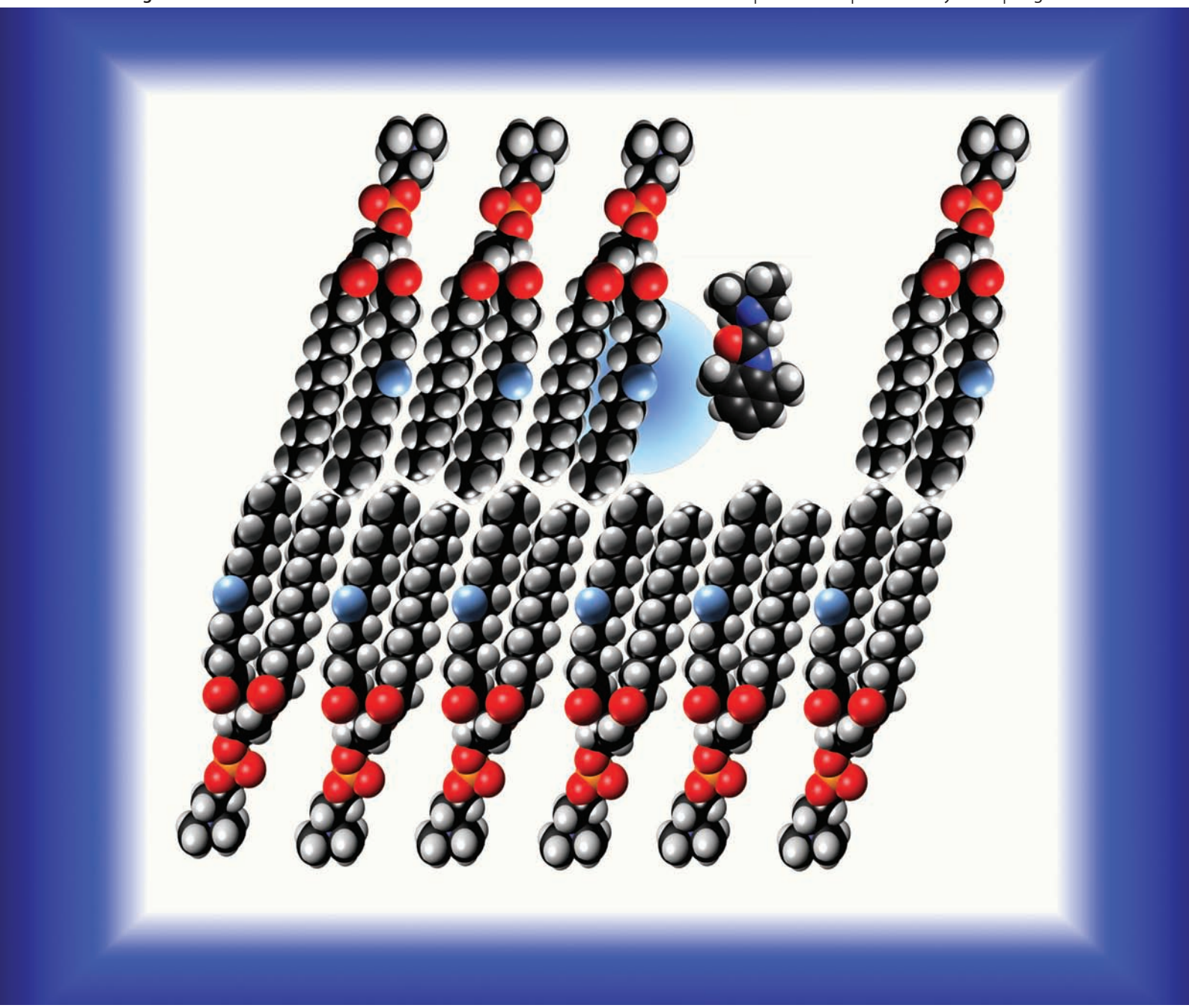

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COMMUNICATION

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COMMUNICATION

Synthesis and properties of monofluorinated dimyristoylphosphatidylcholine derivatives: Potential fluorinated probes for the study of membrane topology†

Jonathan Guimond-Tremblay,[a,b] Marie-Claude Gagnon,[a,b] Jozy-Ann Pineault-Maltais,[a,b] Vanessa Turcotte,[a,b] Michèle Auger*[a] and Jean-François Paquin*[b]

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The synthesis of three monofluorinated dimyristoylphosphatidylcholine derivatives (F-DMPC), with the fluorine atom located on the acyl chain in position 2 of the glycerol (*sn*-2) is described. The synthetic strategy relies on the coupling of 1-myristoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (lyso-PC) and three different fluorinated fatty acids. The latter were obtained from two different and complementary synthetic routes. Preliminary FTIR studies suggest that the presence of the fluorine atom does not significantly perturb the lipid conformational order and phase transition temperature and that these monofluorinated PC derivatives could be used as probes for the study of membrane topology, *i.e.* the location of drugs, peptides or proteins in membranes.

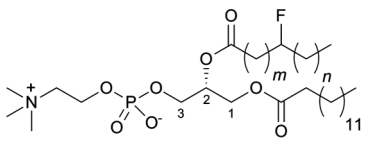
Introduction

Understanding the interactions between lipid membranes and drugs, peptides or proteins is of primary importance to determine their mechanism of action.[1] For example, several drugs with diverse structures and applications (anticancer, local anaesthetic, non-steroidal anti-inflammatory, antibiotic, *etc.*) can interact with cell membranes and modulate their physical properties. The membranes can also affect the pharmacological properties of the drugs being administered.

Biological membranes are made of a variety of lipids differing in terms of their headgroup, chain length and degree of saturation. For example, eukaryotic membranes are composed mainly of zwitterionic (neutral) lipids such as phosphatidylcholine (PC) and phosphatidylethanolamine (PE) while prokaryotic cell membranes also contain negatively charged lipids such as cardiolipin (CL) and phosphatidylglycerol (PG). A key factor in determining drug and peptide selectivity is the composition of the membranes. For examples, many antimicrobial peptides are cationic and bind to the surface of negatively charged membranes through electrostatic interactions, thus conferring selectivity towards bacterial membranes.[2]

Several techniques have been developed to investigate the interactions between drugs or peptides and lipid membranes.[1a] Among these, solid-state nuclear magnetic resonance (NMR) is a method of choice to study the effects of drugs and peptides on model membranes.[3] In addition, the measurement of internuclear distances between specifically labelled nuclei such as ^{13}C and ^{15}N can help to probe local structure at the molecular level.[4] However, these nuclei suffer from their low natural abundance and sensitivity. The use of ^{19}F offers several advantages for NMR studies, including its high sensitivity and 100% natural abundance.[3c] In addition, the absence of native ^{19}F in membrane components allows the selective detection of ^{19}F labelled sites.[3c] It is therefore of great interest to develop efficient methods for the synthesis of fluorinated lipids labelled both on the lipid acyl chains and the polar head groups.

We report herein the synthesis of three monofluorinated dimyristoylphosphatidylcholine derivatives (F-DMPC) with the fluorine atom located on one of the two acyl chains (Fig. 1). The synthetic strategy relies on the coupling of 1-myristoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (lyso-PC) and three different fluorinated fatty acids. The latter were obtained from two different and complementary synthetic routes. Characterization of the three monofluorinated DMPC derivatives by FTIR is also reported.



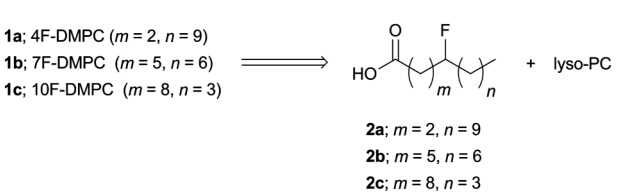


Fig. 1 Targeted monofluorinated 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (F-DMPC) and their retrosynthetic analysis.

[a]*Department of Chemistry, PROTEO, CERMA, 1045 avenue de la Médecine, Université Laval, Québec, Canada. E-mail: michele.auger@chm.ulaval.ca; Fax: +1-418-656-7916; Tel: +1-418-656-3393*
[b]*Canada Research Chair in Organic and Medicinal Chemistry, Department of Chemistry, PROTEO, 1045 avenue de la Médecine, Université Laval, Québec, Canada. E-mail: jean-francois.paquin@chm.ulaval.ca; Fax: +1-418-656-7916; Tel: +1-418-656-2131 ext. 11430*

† Electronic supplementary information (ESI) available: Experimental procedures, isolation and spectroscopic information for the new compounds prepared. See DOI: 10.1039/c2ob06570c

Results and discussion

We initially decided to focus on fluorinated derivatives of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) as this lipid is commonly used as model membrane for eukaryotic cells.[5] We focused on the introduction of only one fluorine atom at different specific locations on the acyl chain located in position 2 of the glycerol (*sn*-2) as it has been shown that having two fluorinated acyl chains led to very different physical properties of non-fluorinated lipids preventing their use as model membranes.[6] Also, the side chain will bear only one fluorine atom because it has been shown that two fluorine atoms (*vs* CH_2) led to an underestimation of the order parameters,[7] again limiting their use in model membranes. Finally, two fluorinated derivatives of 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) has been described previously in the literature[8] but to the best of our knowledge, monofluorinated derivatives of DMPC have not yet been reported.

The synthesis of 4F-DMPC (**1a**) starts with commercially available or readily prepared 4-benzyloxy-1-butanol (**3**)[9] which is first oxidized to the aldehyde **4**[10] under Albright–Onodera conditions using Taber's modification (Scheme 1).[11] Addition of a freshly prepared solution of decanemagnesium bromide gave the secondary alcohol in good yield. Deoxofluorination using diethylaminosulfur trifluoride (DAST)[12] furnished compound **6** in 80% isolated yield. The latter is however contaminated by *ca.* 10% of an unexpected isomer, 4-benzyloxy-1-fluorotetradecane (**9**) and a possible mechanism for its formation is shown in Fig. 2. Upon activation of the alcohol (**5**) with DAST, two pathways would be possible. First, as expected for a deoxofluorination reaction, fluoride could displace the activated alcohol on **7** through a S_N2 reaction leading to compound **6** (pathway not shown). In addition, the oxygen of the protected alcohol could displace the leaving group *via* an intramolecular S_N2 reaction, thus leading to the intermediate **8**. Fluoride ion could then attack either carbon (C1 or C4) where attack at C4 would lead to the expected product (**6** *via* path b) and attack at C1 would give the isomeric compound (**9** *via* path a). Nevertheless, the impure fluoro compound **6** is carried on by removing the benzyl protecting group. The primary alcohol is then directly oxidized to the carboxylic acid using TEMPO[13] to give compound **2a** in 86% yield. Finally, the fluorinated fatty acid **2a** is coupled with 1-myristoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (lyso-PC) to give pure 4F-DMPC (**1a**) in 72% yield after purification by flash chromatography on silica gel using a 1 : 1 $MeOH/CH_2Cl_2$ mixture as the eluent.[14] An overall yield of 33% was obtained for the 6 synthetic steps.

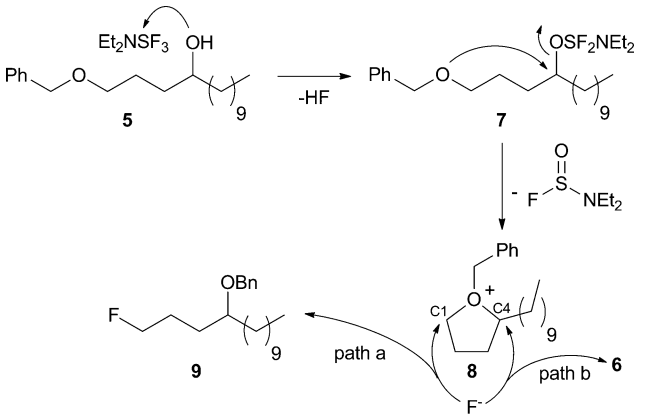
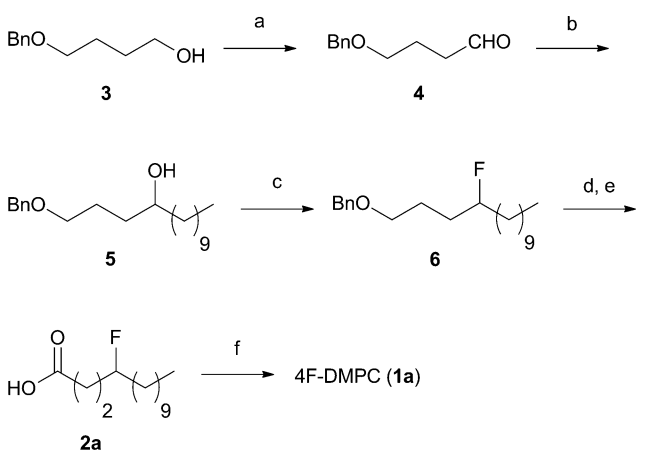
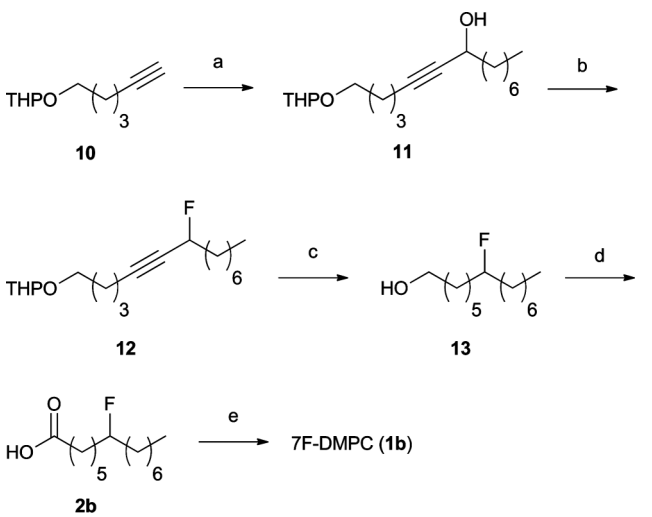


Fig. 2 Possible mechanism for the formation of **9**.

As shown in Scheme 2, the synthesis of 7F-DMPC (**1b**) starts with readily available 5-hexynyl tetrahydro-2*H*-pyran-2-yl ether (**10**).[15] Deprotonation of the alkyne and addition of the resulting alkynyl anion onto octanal produced alcohol **11**[16] in 70% yield. Deoxyfluorination of the alcohol with DAST[12] gave the fluoro compound **12** in good yield. Hydrogenation of the alkyne under palladium catalysis and simultaneous deprotection of the THP ether[17] gave the fluorinated alcohol **13** in 91% yield. Oxidation of the primary alcohol to the carboxylic acid was achieved using TEMPO[13] and gave the desired acid in 80% yield. Finally, the latter



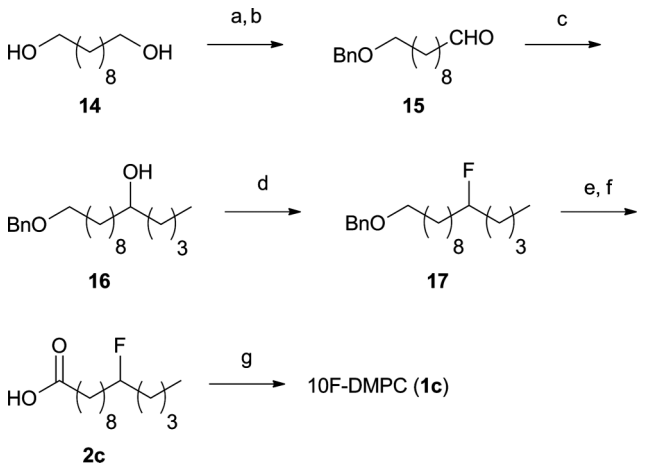
Scheme 1 Synthesis of 4F-DMPC: *Reagents and conditions*: a) DMSO, P_2O_5, Et_3N, CH_2Cl_2, 0 °C to rt, 98%; b) 1-bromodecane, Mg, THF, 0 °C to rt, 83%; c) DAST, CH_2Cl_2, −78 °C to rt, 80% (contaminated with *ca.* 10% of **9**); d) H_2 (2 atm), 10% Pd/C, AcOH/THF (1/1), 82%; e) TEMPO (7 mol%), $NaClO_2$, NaOCl (2 mol%), phosphate buffer (0.67 M, pH = 6.7), CH_3CN, 86%; f) Lyso-PC, 2,6-dichlorobenzoyl chloride, 1-methylimidazole, $CHCl_3$, 72%.



Scheme 2 Synthesis of 7F-DMPC: *Reagents and conditions*: a) i) *n*-BuLi, THF, −78 °C, ii) Octanal, THF, 0 °C to rt, 70%; b) DAST, CH_2Cl_2, −78 °C to rt, 64%; c) H_2 (1 atm), 10% Pd/C, $PdCl_2$ (5 mol%), MeOH/hexane (1/1), 91%; d) TEMPO (7 mol%), $NaClO_2$, NaOCl (2 mol%), phosphate buffer (0.67 M, pH = 6.7), CH_3CN, 80%; e) Lyso-PC, 2,6-dichlorobenzoyl chloride, 1-methylimidazole, $CHCl_3$, 85%.

was coupled with lyso-PC to give 7F-DMPC (**1b**) after purification by flash chromatography on silica gel using a 1 : 1 $MeOH/CH_2Cl_2$ mixture as the eluent. An overall yield of 28% was obtained for the 5 synthetic steps.

Finally, the synthesis of 10F-DMPC, conducted similarly to 4F-DMPC, starts with 1,10-decanediol (**14**) which is first monobenzylated followed by oxidation of the remaining alcohol to the aldehyde **15** (Scheme 3).[18] Addition of butylmagnesium bromide gave the secondary alcohol.[19] Deoxofluorination using DAST furnished compound **17** in good yield. Removal of the benzyl group[20] followed by oxidation of the newly revealed alcohol using TEMPO gave compound **2c** in 77% yield.[20] Finally, the fluorinated fatty acid **2c** is coupled with 1-myristoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (lyso-PC) to give pure 10F-DMPC (**1c**) after purification by flash chromatography on silica gel using a 1 : 1 $MeOH/CH_2Cl_2$ mixture as the eluent. An overall yield of 19% was obtained for the 7 synthetic steps.



Scheme 3 Synthesis of 10F-DMPC: *Reagents and conditions*: a) BnBr, Ag_2O, $CH_2Cl_2/CHCl_3$ (1/1), 64%; b) DMSO, P_2O_5, Et_3N, CH_2Cl_2, 0 °C to rt, 64%; c) 1-bromobutane, Mg, THF, 0 °C to rt, 94%; d) DAST, CH_2Cl_2, –78 °C to rt, 81%; e) H_2 (2 atm), 10% Pd/C, AcOH/THF (1/1), 98%; f) TEMPO (7 mol%), $NaClO_2$, NaOCl (2 mol%), phosphate buffer (0.67 M, pH = 6.7), CH_3CN, 77%; g) Lyso-PC, 2,6-dichlorobenzoyl chloride, 1-methylimidazole, $CHCl_3$, 80%.

We have used FTIR spectroscopy to determine the effect of the introduction of a fluorine atom on the acyl chain conformational order. More specifically, the study of the CH_2 symmetric (2850 cm^{-1}) stretching vibration, which is sensitive to *trans/gauche* isomerization in the lipid acyl chains, can provide valuable information about the order of the acyl chains and the lipid gel-to-fluid phase transition temperature.[21]

Fig. 3 shows the wavenumbers of the CH_2 stretching vibration as a function of temperature for pure commercial DMPC and pure 4F-DMPC, 7F-DMPC and 10F-DMPC. These results indicate that the presence of a fluorine atom in the three derivatives does not significantly perturb the properties of the lipid bilayer. However, it induces a small decrease in the lipid phase transition temperature (by about 1–2 °C), a small broadening (by about 1–2 °C) of the phase transition and a small increase in the CH_2 stretching vibration wavenumber (~0.5–1 cm^{-1}) associated with a small increase in the number of *gauche* conformers. This effect is more important for the 7F-DMPC derivative then for the 10F-DMPC derivative and the 4F-derivative. However, a single phase transition temperature is observed for all three derivatives, indicating that the lipids form a homogeneous mixture.

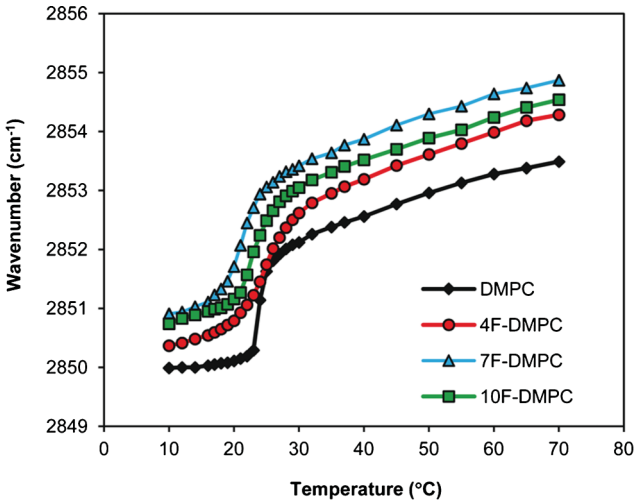


Fig. 3 Temperature dependence of the wavenumber of the CH_2 symmetric stretching vibration for DMPC, 4F-DMPC, 7F-DMPC and 10F-DMPC. The error is estimated to be ±0.1 cm^{-1}.

Our results are in agreement with previous studies on lipids monofluorinated on both acyl chains which have shown that the introduction of the fluorine atom does not significantly perturb the order of the lipid bilayer.[8a] However, the introduction of more than one fluorine atom on one or two acyl chains has been shown to induce a significant decrease of the lipid gel-to-fluid phase transition temperature.[7b,22] More specifically, the phase transition temperature is decreased by about 5–11 °C with the introduction of two fluorine atoms on the *sn*-2 acyl chains[7b] and by ~21 °C with the introduction of six fluorine atoms on both acyl chains.[22] In addition, the introduction of six fluorine atoms on both acyl chains results in an increase of the CH_2 stretching vibration wavenumber of ~2.5 cm^{-1} in the gel phase and a significant broadening (by about 12 °C) of the phase transition.[22] It is also interesting to note that the introduction of two fluorine atoms at position 4 of the *sn*-2 acyl chain has less effect than the introduction of two fluorine atoms at positions 8 and 12, which is in agreement with the results obtained in the present study with monofluorinated lipids.[7b]

The wavenumbers of the CH_2 stretching vibration as a function of temperature obtained for pure commercial DMPC and for 4F-DMPC incorporated in DMPC at different percentages ranging from 2.5 to 100% are shown in Fig. 4. These results indicate that the presence of the fluorine atom does not significantly perturb the lipid phase transition temperature and conformational order at percentages between 2.5 and 25%. A small decrease of the lipid phase transition temperature and lipid order is observed at higher ratios of fluorinated lipids (percentages of 50 and 100%). Similar results have also been obtained as a function of the fluorinated lipid molar ratio with the 7F-DMPC and 10F-DMPC derivatives (results not shown).

Conclusions

We have described a practical synthesis of three monofluorinated dimyristoylphosphatidylcholine derivatives in 5–7 synthetic steps

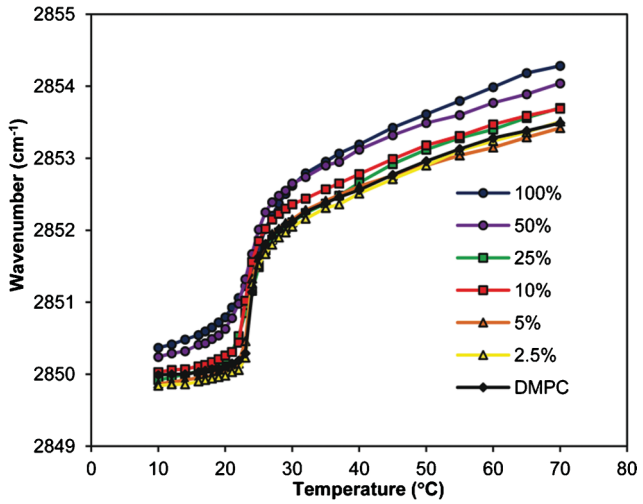


Fig. 4 Temperature dependence of the wavenumber of the CH_2 symmetric stretching vibration for various mixtures of 4F-DMPC and DMPC. The error is estimated to be ±0.1 cm^{-1}.

and 19–33% overall yields from commercially or readily available starting materials. Preliminary data obtained using FTIR indicate that the presence of the fluorine atom does not significantly perturb the lipid conformational order and phase transition temperature. This suggests that these novel monofluorinated lipids could potentially be used as NMR probes for the study of membrane topology and for the investigation at the molecular level of the interaction between drugs or peptides and lipid membranes.

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