Communication

Synthesis and Evaluation of Self-Assembling Properties of 3-(3,5-Difluoro-3,5-bis((alkoxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methylpyridin-1-ium Iodides

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Abstract: A synthesis of 3-(3,5-difluoro-3,5-bis((alkoxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methylpyridin-1-ium iodides with ethyl or nonyl ester groups at positions 3 and 5 was performed. Treatment of the corresponding 2',6'-dimethyl-1',4'-dihydro-[3,4'-bipyridine]-3',5'-dicarboxylates with Selectfluor® followed by quaternization of pyridine moiety in the obtained dialkyl 2,4-diacetyl-2,4-difluoro-3-(pyridin-3-yl)pentanedioates with methyl iodide gave the desired 3-(3,5-difluoro-3,5-bis((alkoxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methylpyridin-1-ium iodides. This type of compound would be useful as synthetic lipids for further development of the delivery systems. The obtained target compounds were fully characterized by ^{1}H NMR, ^{19}F NMR, ^{13}C NMR, HRMS, IR and UV data. The estimation of self-assembling properties and characterization of the nanoparticles obtained by ethanol solution injection in an aqueous media were performed by dynamic light scattering (DLS) measurements. DLS measurement data showed that 3-(3,5-difluoro-3,5-bis((nonyloxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methylpyridin-1-ium iodide created liposomes with the average diameter of 300–400 nm and polydispersity index (PDI) value around 0.30–0.40, while 3-(3,5-difluoro-3,5-bis((ethyloxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methylpyridin-1-ium iodide formed a heterogeneous sample with PDI value 1, which was not prospective for delivery system development.

Keywords: 2,4-diacetyl-2,4-difluoro-3-pyridinylpentanedioates; Selectfluor®; pyridinium; DLS; nanoparticles; self-assembling properties; synthetic lipids

1. Introduction

N-Heterocyclic compounds represent a large class of organic molecules, where many representatives possess various biological activities and are widely applied in medicine. Pyridine and structurally related molecules—dihydropyridine and pyridinium derivatives—are suggested as prevalent structural units for pharmaceutical targets [1]. According to the US Food and Drug Administration (FDA) database, a pyridine and dihydropyridine system containing drugs reaches almost 18% of the *N*-heterocyclic drugs approved by the agency in the major therapeutic areas—infectious diseases, inflammation, the nervous system and oncology [2]. Additionally, structurally diverse pyridinium salts are quite common structures in various pharmaceuticals and many natural compounds. Pyridinium salts are usable in a wide range of research topics. Pyridinium ionic liquids and pyridinium ylides are used in synthetic chemistry and in material science and biological issues related to gene delivery, antimicrobial, anticancer and antimalarial activities [3]. Different SAINT pyridinium salts with alkyl chain variations at the quaternized pyridine *N*-atom or as substituents at the pyridinium cycle were proposed as active gene delivery agents by several

research groups [4–6]. Additionally, gemini dioleylbispyridinium-based amphiphiles were elaborated for nucleic acid transfection [7].

Pyridinium salts based on 1,4-dihydropyridine (1,4-DHP) core possessed self-assembling properties, formed liposomes, and some of them were found to be active in DNA delivery [8–10]. These liposomes filled with magnetic iron oxide nanoparticles formed magnetoliposomes [11]. Magnetic nanoparticles functionalized by pyridinium moieties containing 1,4-DHP demonstrated bactericidal and immunomodulatory properties [12]. It was also found that amphiphilic 1,4-DHP, depending on the structure, possessed selective cytotoxicity—significant cytotoxicity toward cancer cell lines HT-1080 and MH-22A with still very low cytotoxicity in noncancerous NIH3T3 cells [13]. It was recently demonstrated that 4-(*N*-alkylpyridinium)-1,4-DHP showed toxicity in Gram-positive and Gram-negative bacteria species and eukaryotic microorganisms [14], and they also demonstrated calcium channel blocking and antioxidant activities [15]. It was shown that 4-(*N*-dodecylpyridinium)-1,4-dihydropyridine crossed the blood–brain barrier and blocked neuronal and vascular calcium channels [16].

The interest in fluorinated surfactants has increased due to their chemical and biological inertness and their hydrophobic and, at the same time, lipophobic character. Fluorous-containing amphiphiles are important for the formation of uniform nanoparticles, avoiding protein denaturation, efficient endocytosis and maintaining low cytotoxicity [17,18]. Several new fluorinated surfactants on the base of pyridinium salts were recently synthesized and studied as drug carriers and gene delivery systems with very promising results [19–22].

Herein, we report the synthesis and full characterization of new original synthetic lipid-like compounds: 3-(3,5-difluoro-3,5-bis((ethyloxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methylpyridin-1-ium iodide (**4a**) and 3-(3,5-difluoro-3,5-bis((nonyloxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methylpyridin-1-ium iodide (**4b**). The evaluation of the self-assembling properties of target iodides **4** and characterization of formed nanoparticles were performed by DLS measurements in an aqueous solution.

2. Results and Discussion

Taking into account the fact that the introduction of fluorine atoms in the synthetic cationic lipid structure may lead to the formation of the original delivery systems with more pronounced properties, the synthesis of 3-(3,5-difluoro-3,5-bis((alkoxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methylpyridin-1-ium iodides **4** was performed.

The desired target products—3-(3,5-difluoro-3,5-bis((alkoxy)carbonyl)-2,6-dioxo heptan-4-yl)-1-methylpyridin-1-ium iodides **4**—due to cationic moiety and lipophilic substituents would be useful as synthetic lipids for further development of delivery systems.

3-(3,5-Difluoro-3,5-bis((ethoxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methylpyridin-1-ium iodide (**4a**) and 3-(3,5-difluoro-3,5-bis((nonyloxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methylpyridin-1-ium iodide (**4b**) were synthesized via a three-step procedure. The synthetic procedure and the structures of all compounds were depicted in Scheme 1.
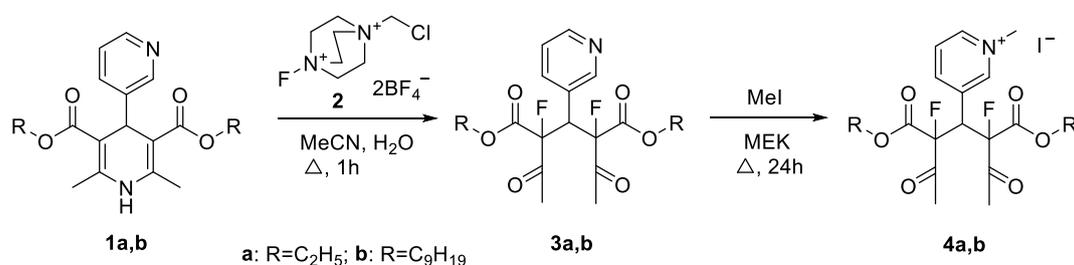
Scheme 1. Synthesis of 3-(3,5-difluoro-3,5-bis((alkoxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methylpyridin-1-ium iodides **4a,b**.

Firstly, the parent 2′,6′-dimethyl-1′,4′-dihydro-[3,4′-bipyridine]-3′,5′-dicarboxylates **1a,b** were obtained in good yields by three-component Hantzsch-type one-pot cyclocondensation reaction of the corresponding acetoacetate, 3-pyridinecarbaldehyde and ammonia

solution in ethanol under reflux for 8 h according to the previously described procedures [23,24]. The next step—electrophilic fluorination of 1,4-DHPs **1** with Selectfluor® (**2**)—was performed following a procedure elaborated by our research group [25,26] and resulted in the formation of fluorine-containing dialkyl 3-(pyridin-3-yl)pentanedioates **3a** and **3b** with yields of 98 and 94%, respectively. The electrophilic fluorination of aromatic heterocyclic systems has been less studied than the fluorination of arenes. However, a number of heterocycles, including 1,2-dihydropyridines [27], quinolines [28] and indoles [29,30] were modified either by direct fluorination or by fluorodecarboxylation using mainly Selectfluor® or *N*-fluorobenzenesulfonimide. The last step—treatment of dialkyl 2,4-diacetyl-2,4-difluoro-3-(pyridin-3-yl)pentanedioates **3** with an excess of methyl iodide—led to quaternization of the pyridine moiety and formation of target compounds— 3-(3,5-difluoro-3,5-bis((alkoxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methylpyridin-1-ium iodides **4** with 56 and 43% yields, respectively. Quaternization reactions of 4-pyridyl-1,4-dihydropyridines were usually carried out in acetone or in an acetone–chloroform mixture if the starting substance was insoluble in acetone [23] or in methyl ethyl ketone (MEK), which decreased the reaction time and increased the yield of product [24]. In this work, quaternization reaction was performed in MEK, and the average yields of the product can be explained by steric hindrance.

The structures of compounds **3a,b** and **4a,b** were established and confirmed on the basis of one-dimensional ^{1}H, ^{19}F, ^{13}C NMR spectral data (Supplementary Materials).

In the ^{19}F NMR spectra of compounds **3a,b** and **4a,b**, a signal of the fluorine atom appeared as a doublet in the range of -166.1 ppm to -166.5 ppm with the constants around $^3J_{F-H} = 29$ Hz for compounds **3a,b** and in the range of -163.2 ppm to -163.3 ppm with the constants around $^3J_{F-H} = 25$ Hz for compounds **4a,b**. The corresponding constants were also observed in the ^{1}H NMR spectra for the proton attached to the carbon between the two CF groups, which appeared as a triplet in the range of $4.92-4.93$ ppm for compounds **3a,b** and at $5.10-5.11$ ppm for **4a,b**.

CF carbon atoms appeared characteristically as doublet multiplets in the ^{13}C NMR spectra at $99.2-101.4$ ppm for compounds **3a,b** and at $97.7-99.9$ ppm for **4a,b** with the constants around $^1J_{C-F} = 213$ Hz and $^1J_{C-F} = 210$ Hz, respectively.

The self-assembling properties of synthetic lipid-like compounds, including cationic moieties containing 1,4-DHPs, are their characteristic feature. The hydrodynamic average diameters (Z_{av}) and polydispersity index (PDI) and stability of nanoparticles formed by 3-(3,5-difluoro-3,5-bis((alkoxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methylpyridin-1-ium iodides **4a,b** in aqueous medium were determined by the DLS method. The results are summarized in Table 1. The DLS measurements were performed for a freshly prepared sample and after storage for 1 and 5 days at room temperature.

Table 1. Values of polydispersity index (PDI) and *Z*-average (Z_{av}) diameter of nanoparticles formed by 3-(3,5-difluoro-3,5-bis((alkoxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methylpyridin-1-ium iodides **4a,b** obtained by DLS measurements. The PDI value describes polydispersity of the sample; the Z_{av} diameter represents the average hydrodynamic diameter of all nanoparticles in the sample.

Comp.	PDI			Z_{av} D_H, nm		
	Fresh *	1 Day **	5 Days ***	Fresh *	1 Day **	5 Days ***
4a	1.00 ± 0.01	-	-	736 ± 84	-	-
4b	0.31 ± 0.05	0.42 ± 0.04	0.40 ± 0.06	371 ± 49	335 ± 66	427 ± 53

* Freshly prepared sample; ** After storage for 1 day at r. t.; *** After storage for 5 days at r. t.

It was demonstrated that 3-(3,5-difluoro-3,5-bis((nonyloxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methylpyridin-1-ium iodide (**4b**) formed nanoparticles with the average diameter around 370 nm for a freshly prepared sample; after 5 days of storage, the average diameter of the nanoparticles increased to about 430 nm. The values of PDI were 0.30 for a freshly prepared sample and around 0.40 for the samples after the storage. These values confirmed

that the sample can be considered as homogenous. It is suggested that for lipid-based formulations in drug delivery applications, PDI values of 0.3 and below are considered as acceptable and indicate homogenous populations of particles [31,32]. A sample of 3-(3,5-difluoro-3,5-bis((ethyloxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methylpyridin-1-ium iodide (**4a**) formed particles with a PDI value of 1, which demonstrated the heterogeneity of the sample and confirmed that the compound was not prospective for delivery system development. The evaluation of the sample formed by compound **4a** will not be tested and discussed further. Previously, we obtained similar data for nanoparticles formed by 4-(*N*-alkylpyridinium)-1,4-DHP derivatives; compounds containing short alkyl chains at quaternized pyridinium moiety and/or 3,5-ester moieties of the 1,4-DHP cycle were not prospective as delivery systems [14]. Additionally, the results regarding membranotropic effects of 4-(*N*-alkylpyridinium)-1,4-DHP derivatives demonstrated the influence of the length of alkyl chain at quaternized pyridinium moiety upon the incorporation of 4-(*N*-alkylpyridinium)-l,4-DHP derivatives in the liposomal membranes and the influence on bilayer fluidity [33]. We confirmed the conclusion that variation in the *N*-alkylpyridinium chain length also impacted the self-assembling properties of the molecules for 3-(3,5-difluoro-3,5-bis((alkoxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methylpyridin-1-ium iodides having ethyl (**4a**) or nonyl (**4b**) ester groups.

3. Materials and Methods

All reagents were purchased from Acros Organics (Geel, Belgium), Sigma-Aldrich/Merck KGaA (Darmstadt, Germany) or Alfa Aesar (Lancashire, UK) and used without further purification. TLC was performed on silica gel 60 F_{254} aluminum sheets 20 cm $\times$ 20 cm (Merck KGaA, Darmstadt, Germany). The melting points were recorded on an OptiMelt digital melting point apparatus (Stanford Research Systems, Sunnyvale, CA, USA) and were uncorrected. ^{1}H, ^{19}F and ^{13}C NMR spectra were recorded on a Bruker Avance Neo 400 MHz (Bruker Biospin Gmbh, Rheinstetten, Germany). Chemical shifts of the hydrogen, carbon and fluorine atoms are presented in parts per million (ppm) and referred to the residual signals of the undeuterated $CDCl_3$ (δ: 7.26) solvent for the ^{1}H NMR spectra and $CDCl_3$ (δ: 77.16) solvent for the ^{13}C NMR, respectively. For the ^{19}F-NMR experiments, indirect referencing (Bruker standard referencing) was used. The coupling constants J were reported in hertz (Hz). High-resolution mass spectra (HRMS) were determined on an Acquity UPLC H-Class system (Waters, Milford, MA, USA) connected to a Waters Synapt GII Q-ToF operating in the ESI positive or negative ion mode on a Waters Acquity UPLC® BEH C18 column (1.7 µm, 2.1 mm $\times$ 50 mm, using gradient elution with acetonitrile (0.1% formic acid) in water (0.1% formic acid)). Infrared spectra were recorded with a Prestige-21 FTIR spectrometer (Shimadzu, Kyoto, Japan). UV spectra were recorded on a UV-Vis Spectrophotometer (501 UV-Vis CamSpec Spectrophotometer; Spectronic CamSpec Ltd., Leeds, UK). The DLS measurements of the nanoparticles in aqueous solution were carried out on a Zetasizer Nano ZSP (Malvern Panalytical Ltd., Malvern, UK) instrument with Malvern Instruments Ltd. Software 8.01.4906.

3.1. General Procedure for the Synthesis of Dialkyl 2,4-Diacetyl-2,4-difluoro-3-(pyridin-3-yl)pentanedioates **3a,b**

To a stirred solution of 4-pyridyl-1,4-dihydropyridines **1a,b** [23,24] (1.5 mmol) in acetonitrile (15 mL), water (2 mL) was added, after which Selectfluor® (**2**, 1.34 g, 3.8 mmol) was added in portions at room temperature. After being stirred at reflux for 1 h, the solvents were evaporated in vacuo. Then, the remaining residue was diluted with dry diethyl ether (30 mL), and the precipitate was filtered off. The filtrate was concentrated in vacuo. Compound **3a** was obtained as solid; compound **3b** was obtained as oil. Compound **3a** was recrystallized from EtOH. Compounds **3a,b** were obtained in 94–98% yields.

3.2. Diethyl 2,4-Diacetyl-2,4-difluoro-3-(pyridin-3-yl)pentanedioate (3a)

Yield: 98%. Pale yellow solid; m.p. 84–85 °C (EtOH). ^{1}H NMR (400 MHz, CDCl$_3$) δ: 8.60 (s, 1H, Py), 8.51 (dd, $^3J_{H-H}$ = 4.8 Hz, $^4J_{H-H}$ = 1.5 Hz, 1H, Py), 7.71 (d, $^3J_{H-H}$ = 7.6 Hz, 1H, Py), 7.22 (dd, $^3J_{H-H}$ = 8.0 Hz, $^3J_{H-H}$ = 4.8 Hz, 1H, Py), 4.92 (t, $^3J_{H-F}$ = 29.3 Hz, 1H, CH), 4.33−4.19 (m, 4H, 2 × OCH_2CH$_3$), 2.02 (m, 6H, 2× COCH_3), 1.31 (t, $^3J_{H-H}$ = 7.2 Hz, 6H, 2 × OCH$_2$CH_3) ppm. ^{19}F NMR (376 MHz, CDCl$_3$) δ: −166.5 (d, $^3J_{F-H}$ = 29.3 Hz, 2F) ppm. ^{13}C NMR (101 MHz, CDCl$_3$) δ: 200.5 (dd, $^2J_{C-F}$ = 28.7 Hz, $^4J_{C-F}$ = 12.8 Hz, 2 × C(O)CH$_3$), 164.6 (dd, $^2J_{C-F}$ = 25.5 Hz, $^4J_{C-F}$ = 15.6 Hz, 2 × C(O)OC$_2$H$_5$), 152.2 (s, Py), 149.8 (s, Py), 139.2 (s, Py), 128.3 (s, Py), 123.3 (s, Py), 100.3 (dd, $^1J_{C-F}$ = 213.0 Hz, $^3J_{C-F}$ = 3.4 Hz, 2 × CF), 63.6 (s, 2 × OCH$_2$CH$_3$), 49.9 (t, $^2J_{C-F}$ = 18.3 Hz, CH), 26.4 (s, 2 × CH$_3$), 13.9 (s, 2 × OCH$_2$CH$_3$) ppm. IR ν$_{max}$ (film) 2987 (w), 2941 (m), 1757 (s), 1737 (s), 1570 (s) cm^{-1}. UV-Vis λ$_{max}$ (EtOH): 210 (log ε 4.70), 260 (3.53) nm. HRMS TOF ESI$^+$ of [C$_{18}$H$_{21}$F$_2$NO$_6$ + H]$^+$ (*m/z*) 386.1420; calcd: 386.1415.

3.3. Dinonyl 2,4-Diacetyl-2,4-difluoro-3-(pyridin-3-yl)pentanedioate (3b)

Yield: 94%. Pale brownish oil. ^{1}H NMR (400 MHz, CDCl$_3$) δ: 8.60 (s, 1H, Py), 8.51 (dd, $^3J_{H-H}$ = 4.8 Hz, $^4J_{H-H}$ = 1.7 Hz, 1H, Py), 7.70 (d, $^3J_{H-H}$ = 8.0 Hz, 1H, Py), 7.21 (dd, $^3J_{H-H}$ = 8.0 Hz, $^3J_{H-H}$ = 4.8 Hz, 1H, Py), 4.93 (t, $^3J_{H-F}$ = 29.1 Hz, 1H, CH), 4.23−4.11 (m, 4H, 2 × CH_2CH$_2$(CH$_2$)$_6$CH$_3$), 2.03 (m, 6H, 2 × COCH_3), 1.70−1.63 (m, 4H, 2 × OCH$_2$CH_2(CH$_2$)$_6$CH$_3$), 1.30−1.26 (m, 24H, 2 × OCH$_2$CH$_2$(CH_2)$_6$CH$_3$), 0.87 (m, 6H, 2 × OCH$_2$CH$_2$(CH$_2$)$_6$CH_3) ppm. ^{19}F NMR (376 MHz, CDCl$_3$) δ: −166.3 (d, $^3J_{F-H}$ = 29.1 Hz, 2F) ppm. ^{13}C NMR (101 MHz, CDCl$_3$) δ: 200.5 (dd, $^2J_{C-F}$ = 28.7 Hz, $^4J_{C-F}$ = 12.2 Hz, 2 × C(O)CH$_3$), 164.7 (dd, $^2J_{C-F}$ = 25.0 Hz, $^4J_{C-F}$ = 15.6 Hz, 2 × C(O)OC$_9$H$_{19}$), 152.2 (s, Py), 149.8 (s, Py), 139.2 (s, Py), 128.4 (s, Py), 123.2 (s, Py), 100.3 (dd, $^1J_{C-F}$ = 212.9 Hz, $^3J_{C-F}$ = 3.5 Hz, 2 × CF), 67.6 (s, 2 × OCH$_2$CH$_2$(CH$_2$)$_6$CH$_3$), 49.9 (t, $^2J_{C-F}$ = 18.2 Hz, CH), 32.0 (s, 2 × CH$_2$), 29.6 (s, 2 × CH$_2$), 29.3 (s, 2 × CH$_2$), 29.2 (s, 2 × CH$_2$), 28.3 (s, 2 × CH$_2$), 26.4 (s, 2 × CH$_3$), 25.8 (s, 2 × CH$_2$), 22.8 (s, 2 × CH$_2$), 14.3 (s, 2 × CH$_3$) ppm. IR ν$_{max}$ (film) 3181 (w), 2955 (m), 2928 (s), 2856 (m), 1761 (s), 1730 (s), 1576 (s) cm^{-1}. UV-Vis λ$_{max}$ (EtOH): 210 (log ε 4.94), 260 (4.48) nm. HRMS TOF ESI$^+$ of [C$_{32}$H$_{49}$F$_2$NO$_6$ + H]$^+$ (*m/z*) 582.3621; calcd: 582.3606.

3.4. General Procedure for the Synthesis of 3-(3,5-Difluoro-3,5-bis((alkoxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methylpyridin-1-ium Iodide 4a,b

To a stirred solution of 2,4-diacetyl-2,4-difluoro-3-(pyridin-3-yl) pentanedioates **3a,b** (1.0 mmol) in methyl ethyl ketone (MEK), methyl iodide (5.0 mmol) was added in portions over 3 h, after which the reaction mixture was refluxed for 24 h. After overnight cooling of the reaction mixture to + 4 °C, the formed precipitates were filtered off (for compound **4a**). In the case of product **4b**, the solvent was evaporated, after which the residue was treated with diethyl ether, and the formed precipitates were filtered off. The obtained products were recrystallized from acetone.

3.5. 3-(3,5-Difluoro-3,5-bis((ethoxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methylpyridin-1-ium Iodide (4a)

Yield: 56%. Yellow solid; m.p. 153–154 °C (acetone). ^{1}H NMR (400 MHz, CDCl$_3$) δ: 9.65 (d, $^3J_{H-H}$ = 6.0 Hz, 1H, Py), 8.65 (m, 1H, Py), 8.38 (d, $^3J_{H-H}$ = 8.1 Hz, 1H, Py), 8.15 (dd, $^3J_{H-H}$ = 8.1 Hz, $^3J_{H-H}$ = 6.0 Hz, 1H, Py), 5.11 (t, $^3J_{H-F}$ = 24.7 Hz, 1H, CH), 4.70 (s, 3H, CH$_3$), 4.34−4.20 (m, 4H, 2 × OCH_2CH$_3$), 2.27 (m, 6H, 2 × C(O)CH_3), 1.30 (m, 6H, 2 × OCH$_2$CH_3) ppm. ^{19}F NMR (376 MHz, CDCl$_3$) δ: −163.2 (d, $^3J_{F-H}$ = 24.7 Hz, 2F) ppm. ^{13}C NMR (101 MHz, CDCl$_3$) δ: 200.7−200.2 (m, 2 × C(O)CH$_3$), 163.9−163.5 (m, 2 × C(O)OCH$_2$CH$_3$), 146.3 (m, Py), 133.9 (s, Py), 127.9 (s, Py), 98.8 (dd, $^1J_{C-F}$ = 209.6 Hz, $^3J_{C-F}$ = 1.6 Hz, 2 × CF), 64.2 (s, 2 × OCH$_2$CH$_3$), 50.3 (s, CH$_3$), 49.2 (t, $^2J_{C-F}$ = 19.2 Hz, CH), 27.0 (s, 2 × C(O)CH$_3$), 13.9 (s, 2 × OCH$_2$CH$_3$) ppm. IR ν$_{max}$ (film) 3452 (b, w), 2995 (b, w), 2939 (b, w), 1756 (s), 1730 (m), 1636 (w), 1511 (w) cm^{-1}. UV-Vis λ$_{max}$ (EtOH): 214 (log ε 5.40), 265 (4.85) nm. HRMS TOF ESI$^+$ of [C$_{19}$H$_{24}$F$_2$NO$_6$]$^+$ (*m/z*) 400.1579; calcd: 400.1572.

*3.6. 3-(3,5-Difluoro-3,5-bis((nonyloxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methylpyridin-1-ium Iodide (**4b**)*

Yield: 43%. Pale yellow solid; m.p. 129–130 °C (acetone). ^{1}H NMR (400 MHz, CDCl$_3$) δ: 9.66 (d, $^3J_{\text{H-H}}$ = 6.1 Hz, 1H, Py), 8.53 (m, 1H, Py), 8.37 (d, $^3J_{\text{H-H}}$ = 8.4 Hz, 1H, Py), 8.12 (dd, $^3J_{\text{H-H}}$ = 8.4 Hz, $^3J_{\text{H-H}}$ = 6.1 Hz, 1H, Py), 5.10 (t, $^3J_{\text{H-F}}$ = 25.0 Hz, 1H, CH), 4.68 (s, 3H, CH$_3$), 4.26−4.13 (m, 4H, 2 × OC*H*$_2$CH$_2$(CH$_2$)$_6$CH$_3$), 2.27 (m, 6H, 2 × COC*H*$_3$), 1.66 (m, 4H, 2 × OCH$_2$C*H*$_2$(CH$_2$)$_6$CH$_3$), 1.29−1.25 (m, 24H, 2 × OCH$_2$CH$_2$(C*H*$_2$)$_6$CH$_3$), 0.87 (m, 6H, 2 × OCH$_2$CH$_2$(CH$_2$)$_6$C*H*$_3$) ppm. ^{19}F NMR (376 MHz, CDCl$_3$) δ: −163.3 (d, $^3J_{\text{F-H}}$ = 25.0 Hz, 2F) ppm. ^{13}C NMR (101 MHz, CDCl$_3$) δ: 200.8−200.4 (m, 2 × *C*(O)CH$_3$), 164.1−163.2 (m, 2 × *C*(O)OCH$_2$CH$_2$(CH$_2$)$_6$CH$_3$), 146.4 (m, Py), 134.1 (s, Py), 128.0 (s, Py), 98.9 (dm, $^1J_{\text{C-F}}$ = 210.1 Hz, 2 × CF), 68.3 (s, 2 × OCH$_2$CH$_2$(CH$_2$)$_6$CH$_3$), 50.2 (s, CH$_3$), 49.3 (t, $^2J_{\text{C-F}}$ = 19.2 Hz, CH), 31.9 (s, 2 × CH$_2$), 29.5 (s, 2 × CH$_2$), 29.3 (s, 2 × CH$_2$), 29.1 (s, 2 × CH$_2$), 28.2 (s, 2 × CH$_2$), 26.8 (s, 2 × C(O)*C*H$_3$), 22.7 (s, 2 × CH$_2$), 14.2 (s, 2 × OCH$_2$CH$_2$(CH$_2$)$_6$*C*H$_3$) ppm. IR ν$_{\text{max}}$ (film) 3470 (b, w), 3033 (w), 2955 (m), 2924 (m), 2855 (m), 1756 (s), 1728 (m), 1510 (w) cm^{-1}. UV-Vis λ$_{\text{max}}$ (EtOH): 214 (log ε 5.43), 265 (4.94) nm. HRMS TOF ESI$^+$ of [C$_{33}$H$_{52}$F$_2$NO$_6$]$^+$ (*m/z*) 596.3773; calcd: 596.3763.

3.7. Self-Assembling Properties by Dynamic Light Scattering Measurements

Samples of 3-(3,5-difluoro-3,5-bis((alkoxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methylpyridin-1-ium iodides **4a,b** for dynamic light scattering (DLS) studies were prepared utilizing the ethanol injection technique. A solution of compound **4a** or compound **4b** (300 μL, 1 mM in EtOH 96%) was injected into 2.70 mL deionized water with maximum stirring (IKA Vortex 2 (IKA, Staufen, Germany)) to give a final concentration of the compound—100 μM. The sample was sonicated for 60 min at 50 °C using a bath-type sonicator (Cole Parmer Ultrasonic Cleaner 8891CPX (Vernon Hills, IL, USA)).

The DLS measurements of the nanoparticles in aqueous solution were carried out on a Zetasizer Nano ZSP (Malvern Panalytical Ltd., Malvern, UK) instrument with Malvern Instruments Ltd. Software 8.01.4906, using the following specifications: medium—water; refractive index—1.33; viscosity—0.8872 cP; temperature—25 °C; dielectric constant—78.5; nanoparticles—liposomes; refractive index of materials—1.60; detection angle—173°; wavelength—633 nm. Data were analyzed using the multimodal number distribution software that was included with the instrument. The measurements were performed in triplicate in order to check their reproducibility. The measurements were performed in triplicate in order to check their reproducibility. Example of DLS data for freshly prepared nanoparticles of 4b see in the Supplementary Materials.

3.8. Statistical Analysis

Results are expressed as mean standard deviation (SD). All experiments were performed in triplicate.

4. Conclusions

Synthesis of the ethyl or nonyl ester containing 3-(3,5-difluoro-3,5-bis((alkoxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methylpyridin-1-ium iodides **4a** and **4b** was performed after treatment of the corresponding 2′,6′-dimethyl-1′,4′-dihydro-[3,4′-bipyridine]-3′,5′-dicarboxylates with Selectfluor®, which gave dialkyl 2,4-diacetyl-2,4-difluoro-3-(pyridin-3-yl) pentanedioates. Quaternization of pyridine moiety of dialkyl 2,4-diacetyl-2,4-difluoro-3-(pyridin-3-yl) pentanedioates with methyl iodide resulted in target compounds. The compounds were characterized by fully characterized by ^{1}H NMR, ^{19}F NMR, ^{13}C NMR, HRMS, IR and UV data. DLS measurements demonstrated that 3-(3,5-difluoro-3,5-bis((nonyloxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methylpyridin-1-ium iodide (**4b**) formed nanoparticles with the average diameter around 370/430 nm and PDI values 0.3/0.4 for a freshly prepared sample and sample after 5 days of storage, respectively. Meanwhile, a sample of 3-(3,5-difluoro-3,5-bis((ethyloxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methyl-pyridin-1-ium iodide (**4a**) formed particles with a PDI value of 1, which indicated the heterogeneity of the

sample. It was demonstrated that variation in the *N*-alkylpyridinium chain length also impacted the self-assembling properties of the molecules in the case of 3-(3,5-difluoro-3,5-bis((alkoxy)carbonyl)-2,6-dioxoheptan-4-yl)-1-methylpyridin-1-ium iodides.

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