

# Synthesis and characterization of novel amphiphilic *N*-benzyl 1,4-dihydropyridine derivatives – evaluation of lipid monolayer and self-assembling properties

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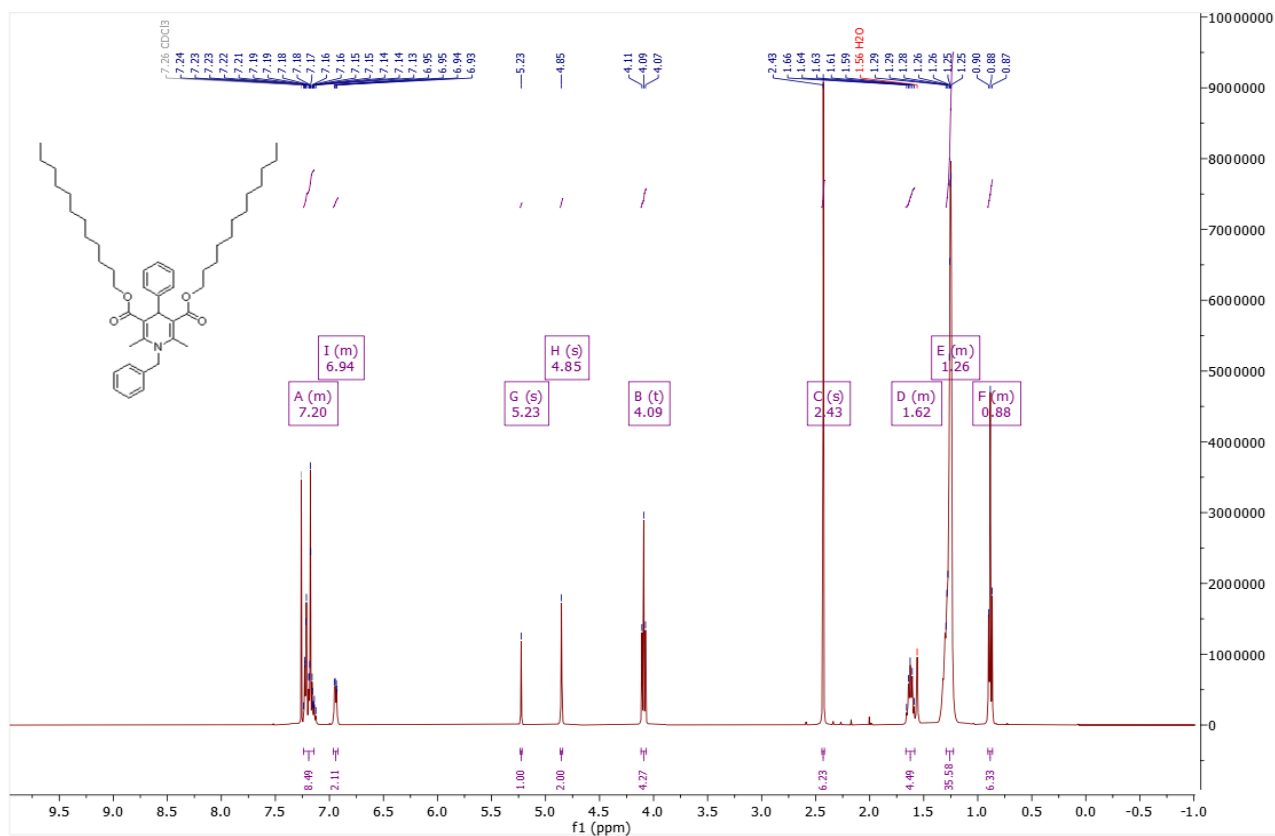
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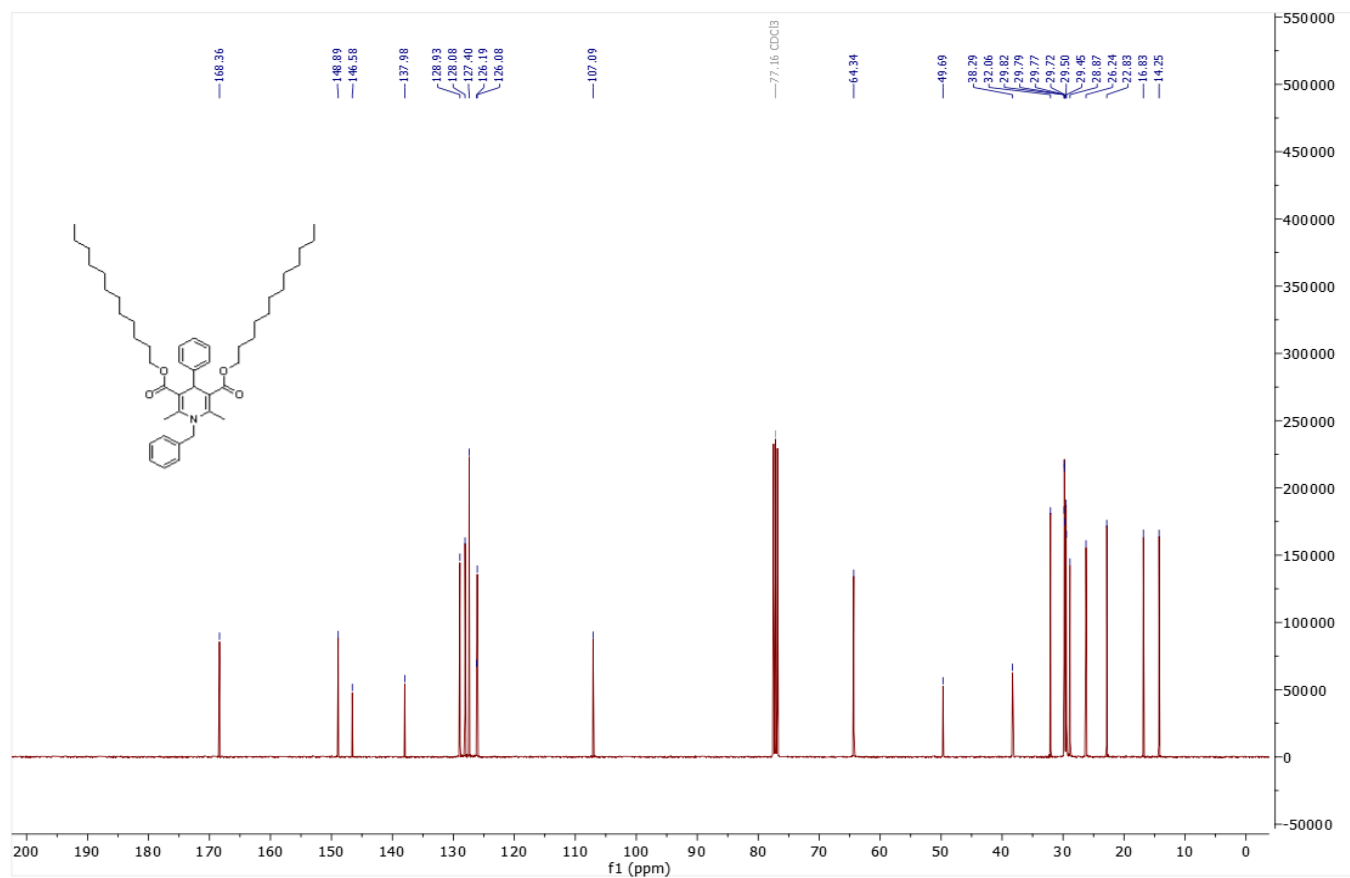
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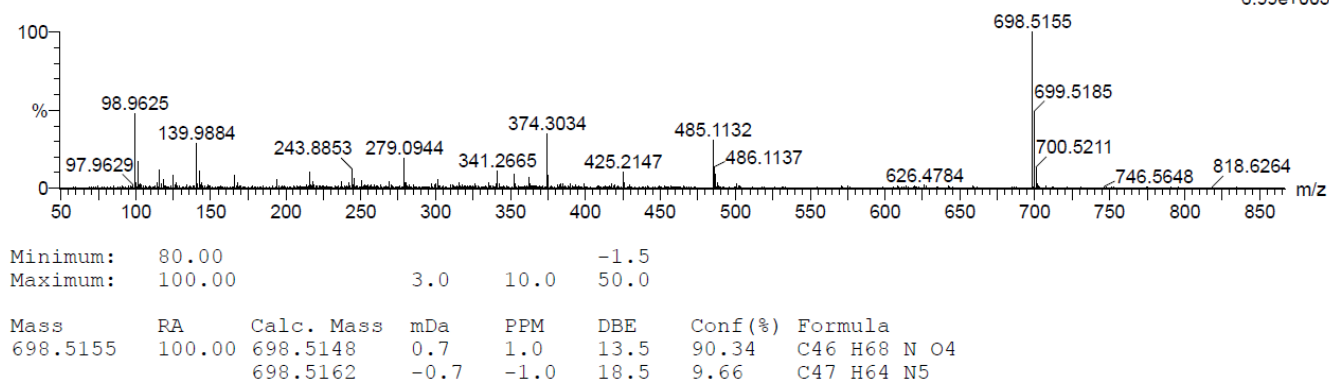
**Figure S1.**  $^1\text{H}$  NMR spectrum of didodecyl 1-benzyl-2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-dicarboxylate (2).



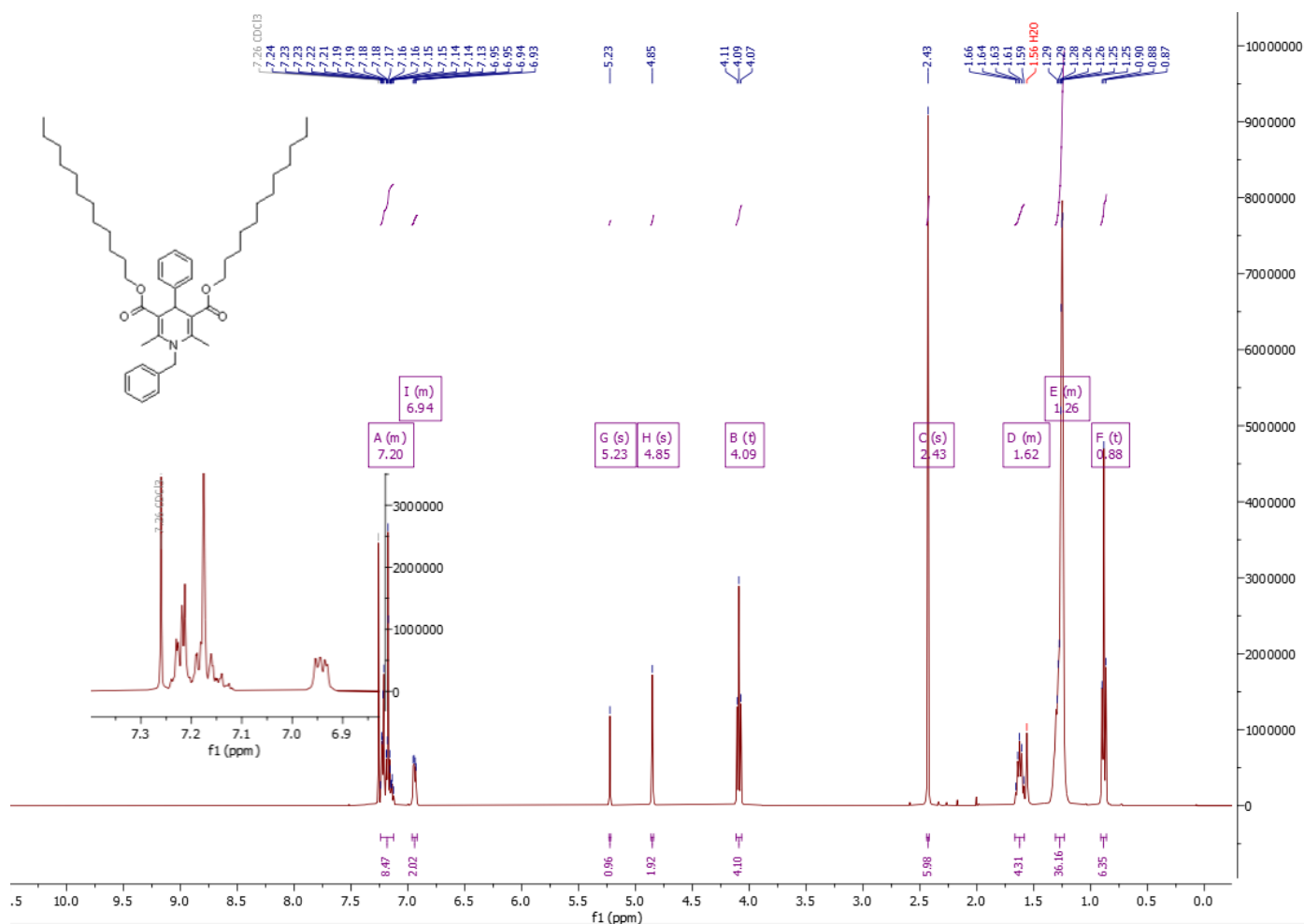
**Figure S2.**  $^{13}\text{C}$  NMR spectrum of didodecyl 1-benzyl-2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-dicarboxylate (2)

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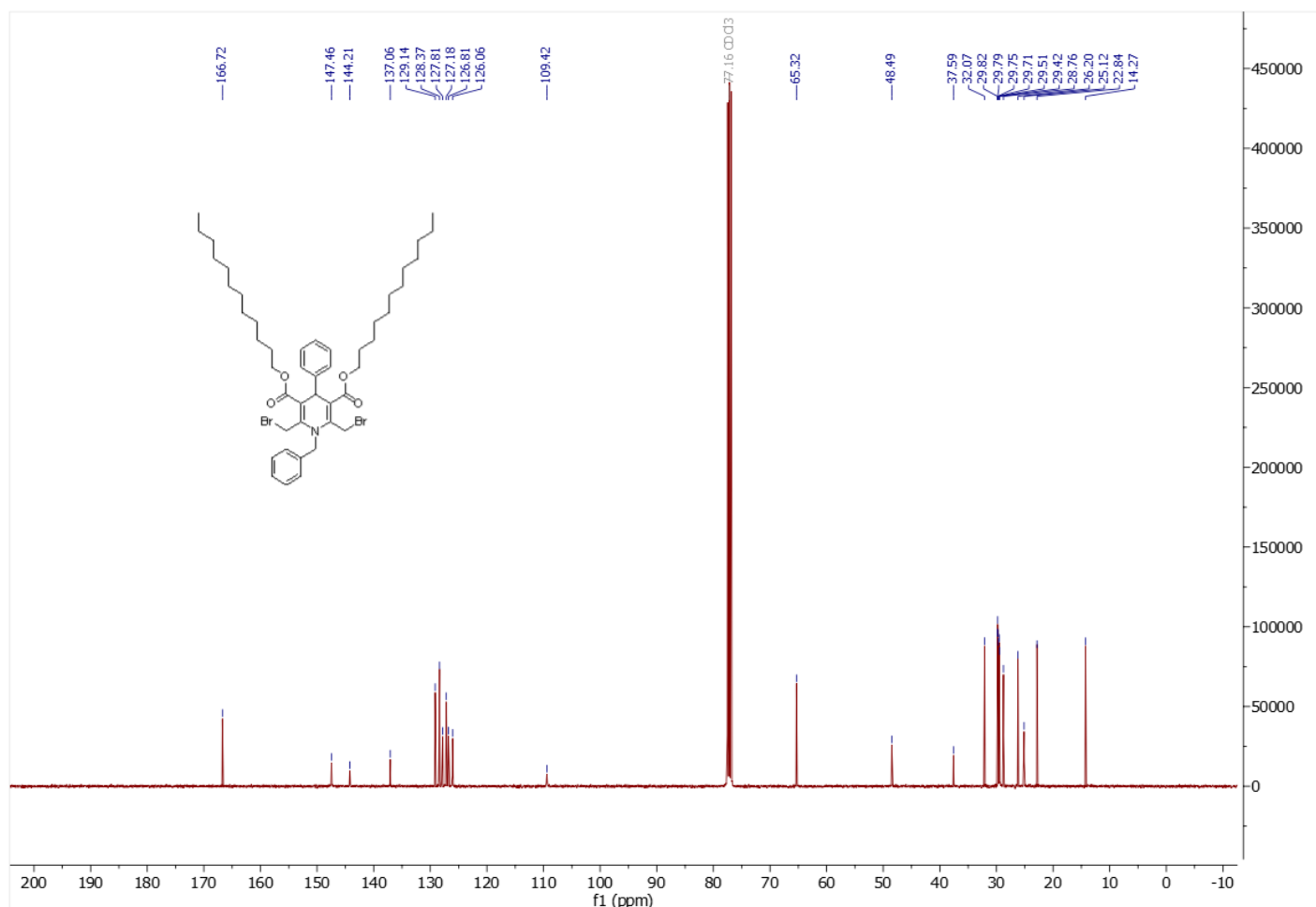
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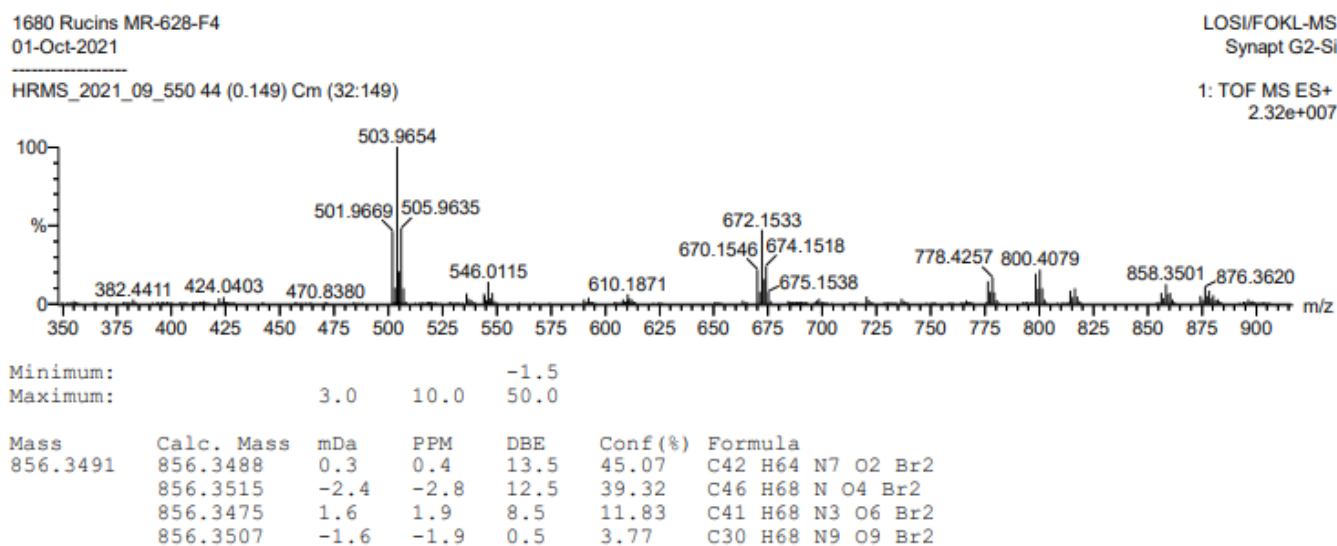
**Figure S3.** HRMS data of 1 didodecyl 1-benzyl-2,6-dimethyl-4-phenyl-1,4-dihydropyridine-3,5-dicarboxylate (**2**)



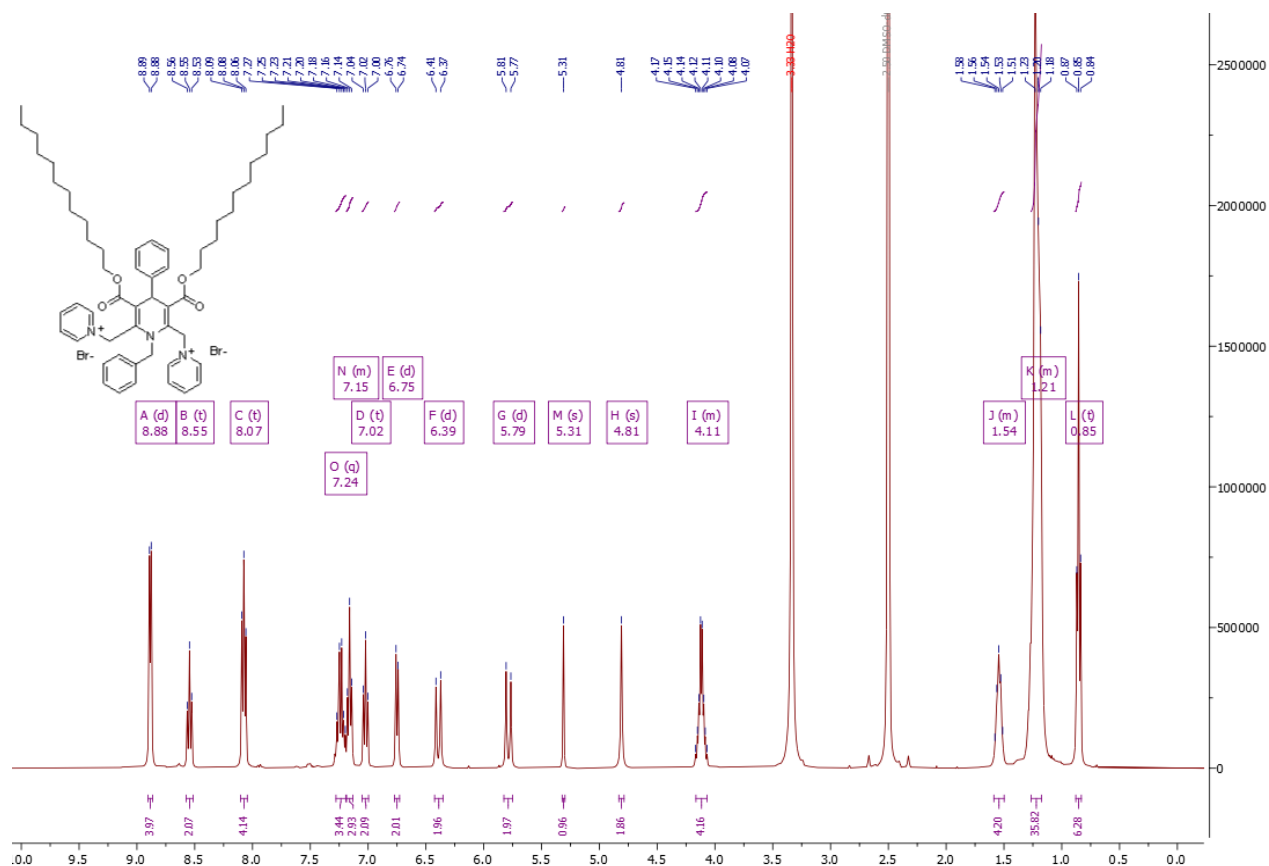
**Figure S4.**  $^1\text{H}$  NMR spectrum of didodecyl 1-benzyl-2,6-bis(bromomethyl)-4-phenyl-1,4-dihydropyridine-3,5-dicarboxylate (**3**)



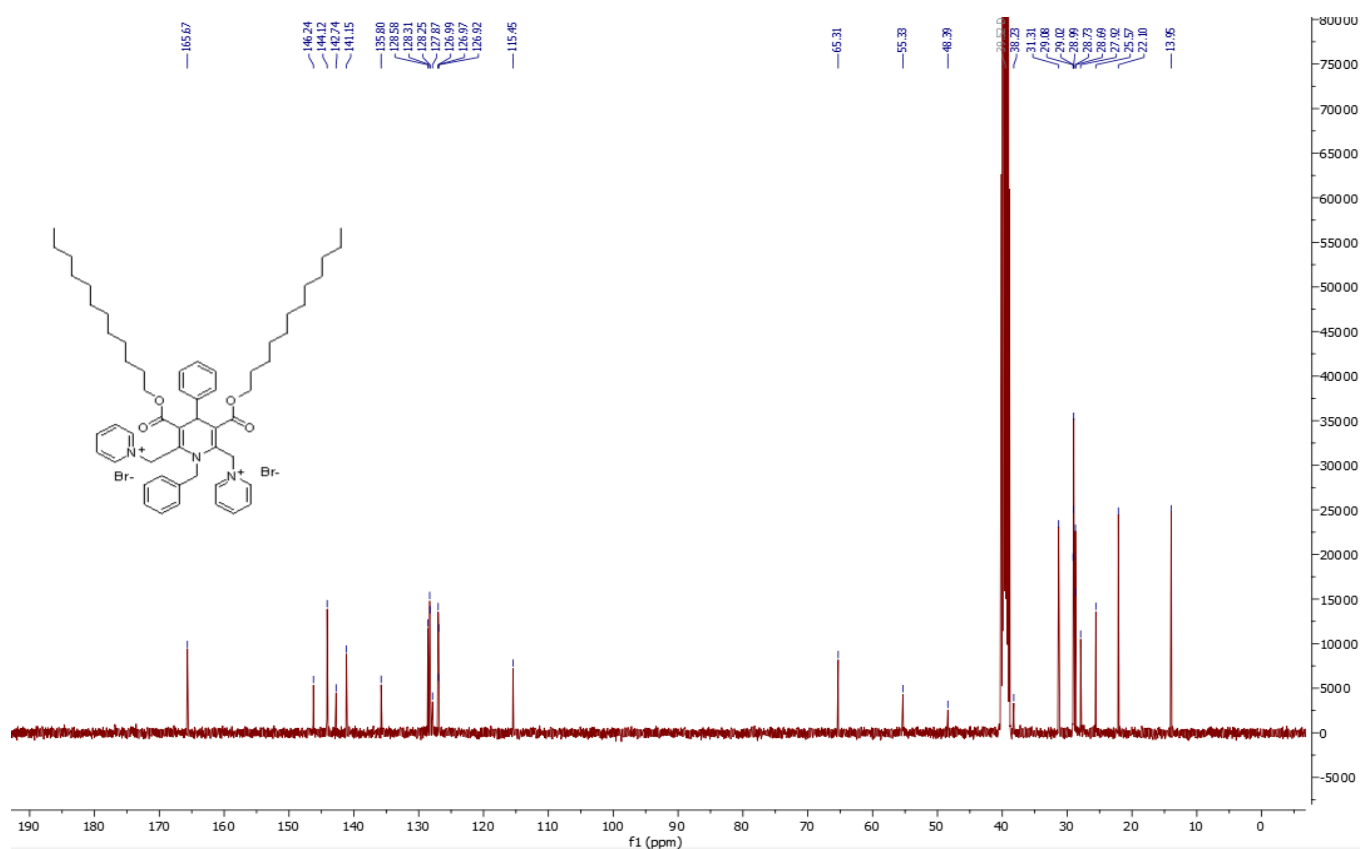
**Figure S5.**  $^{13}\text{C}$  NMR spectrum of didodecyl 1-benzyl-2,6-bis(bromomethyl)-4-phenyl-1,4-dihydropyridine-3,5-dicarboxylate (**3**)



**Figure S6.** HRMS data of didodecyl 1-benzyl-2,6-bis(bromomethyl)-4-phenyl-1,4-dihydropyridine-3,5-dicarboxylate (**3**)



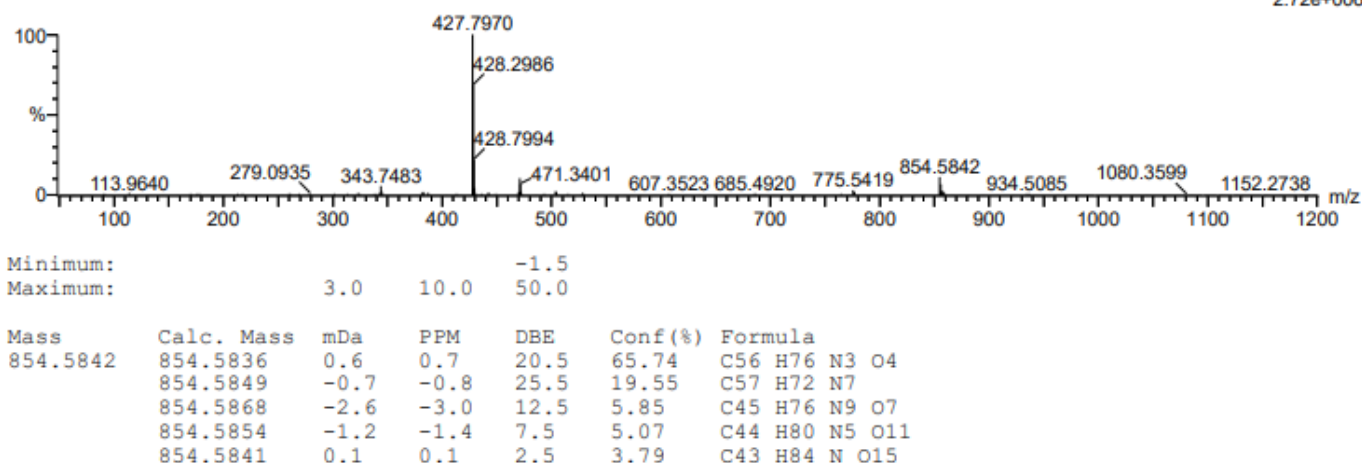
**Figure S7.** <sup>1</sup>H NMR spectrum of 1,1'-((1-benzyl-3,5-bis((dodecyloxy)carbonyl)-4-phenyl-1,4-dihydropyridine-2,6-diyl)bis(methylene))bis(pyridin-1-ium) dibromide (**5a**)



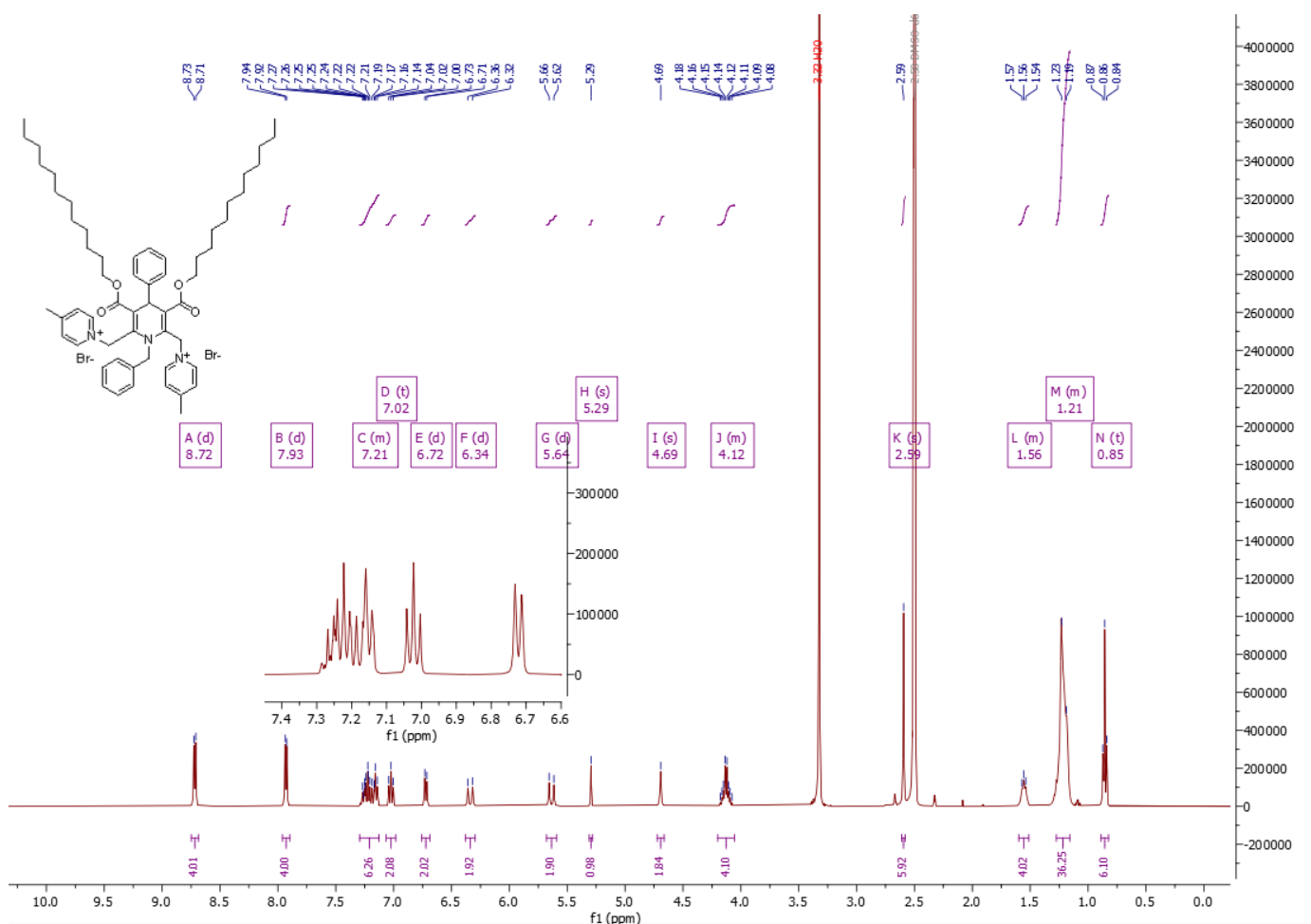
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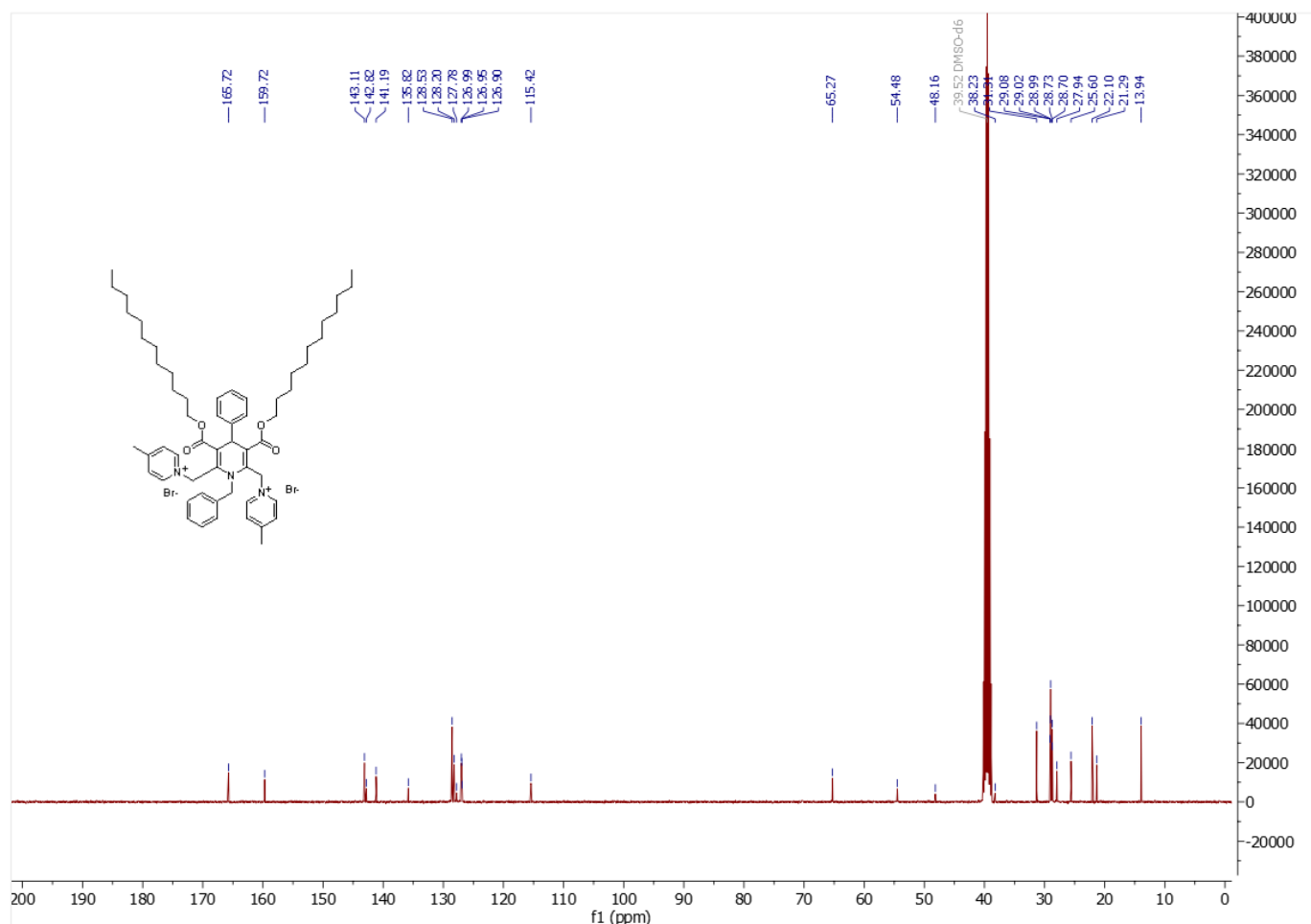
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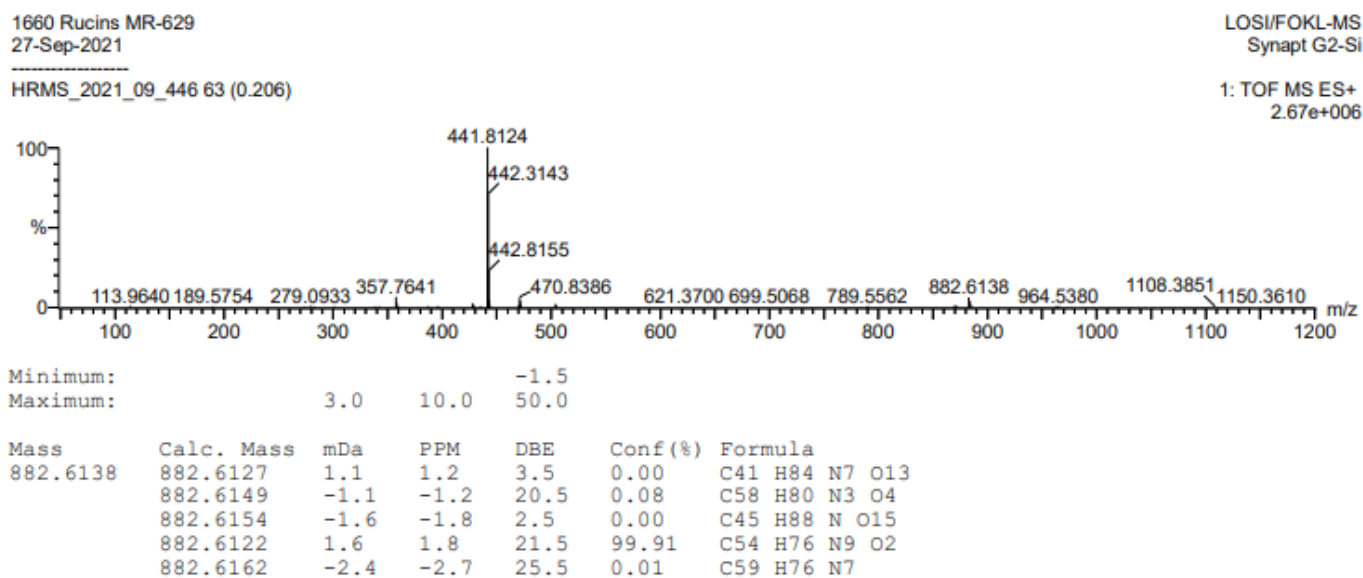
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**Figure S10.**  $^1\text{H}$  NMR spectrum of 1,1'-((1-benzyl-3,5-bis((dodecyloxy)carbonyl)-4-phenyl-1,4-dihydropyridine-2,6-diyl)bis(methylene))bis(pyridin-1-ium) dibromide (**5b**)



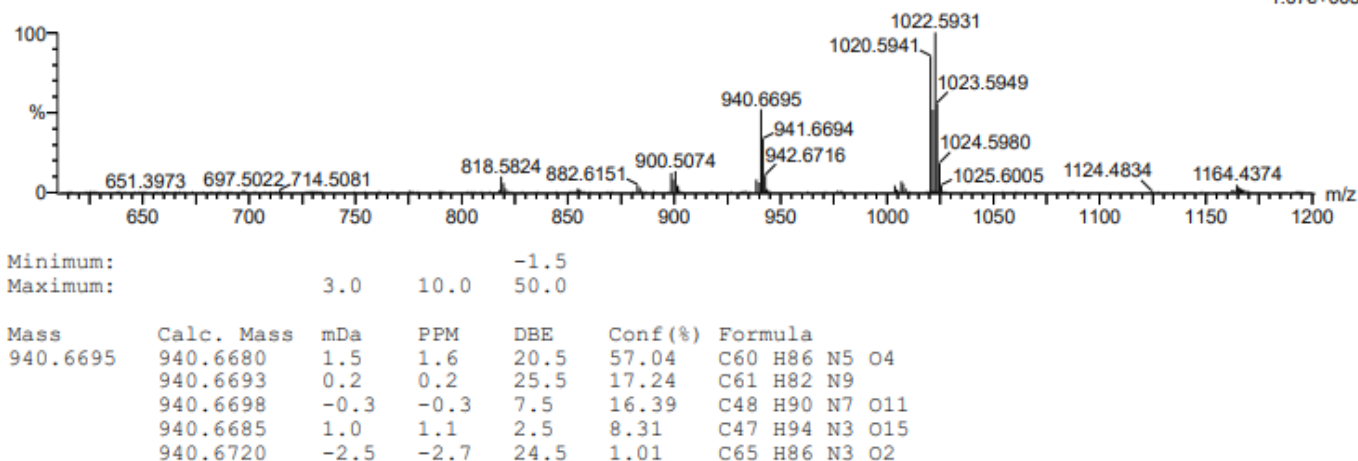
**Figure S11.**  $^{13}\text{C}$  NMR spectrum of 1,1'-((1-benzyl-3,5-bis((dodecyloxy)carbonyl)-4-phenyl-1,4-dihydropyridine-2,6-diyl)bis(methylene))bis(pyridin-1-ium) dibromide (**5b**)



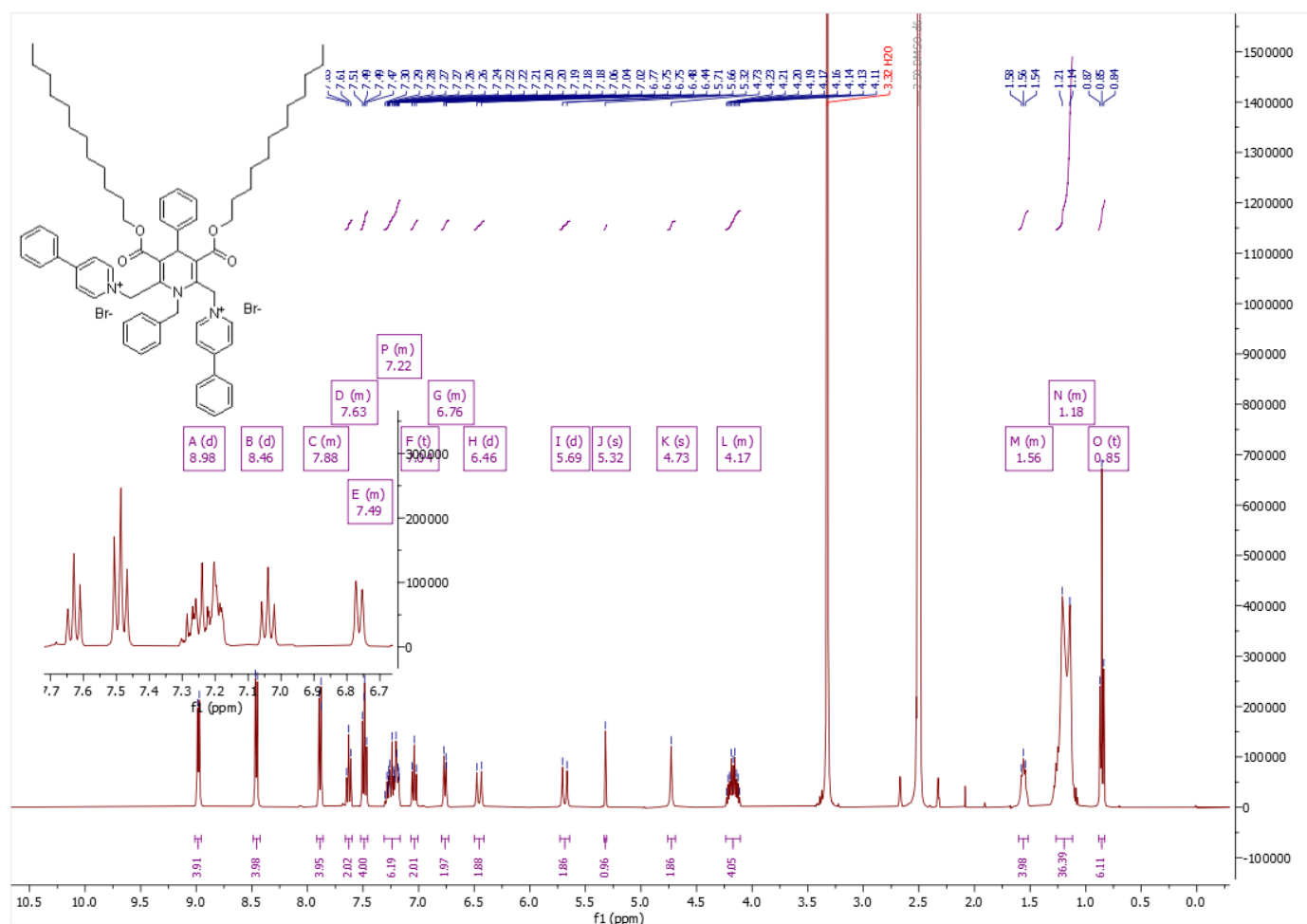
**Figure S12.** HRMS data of 1,1'-((1-benzyl-3,5-bis((dodecyloxy)carbonyl)-4-phenyl-1,4-dihydropyridine-2,6-diyl)bis(methylene))bis(pyridin-1-ium) dibromide (**5b**)



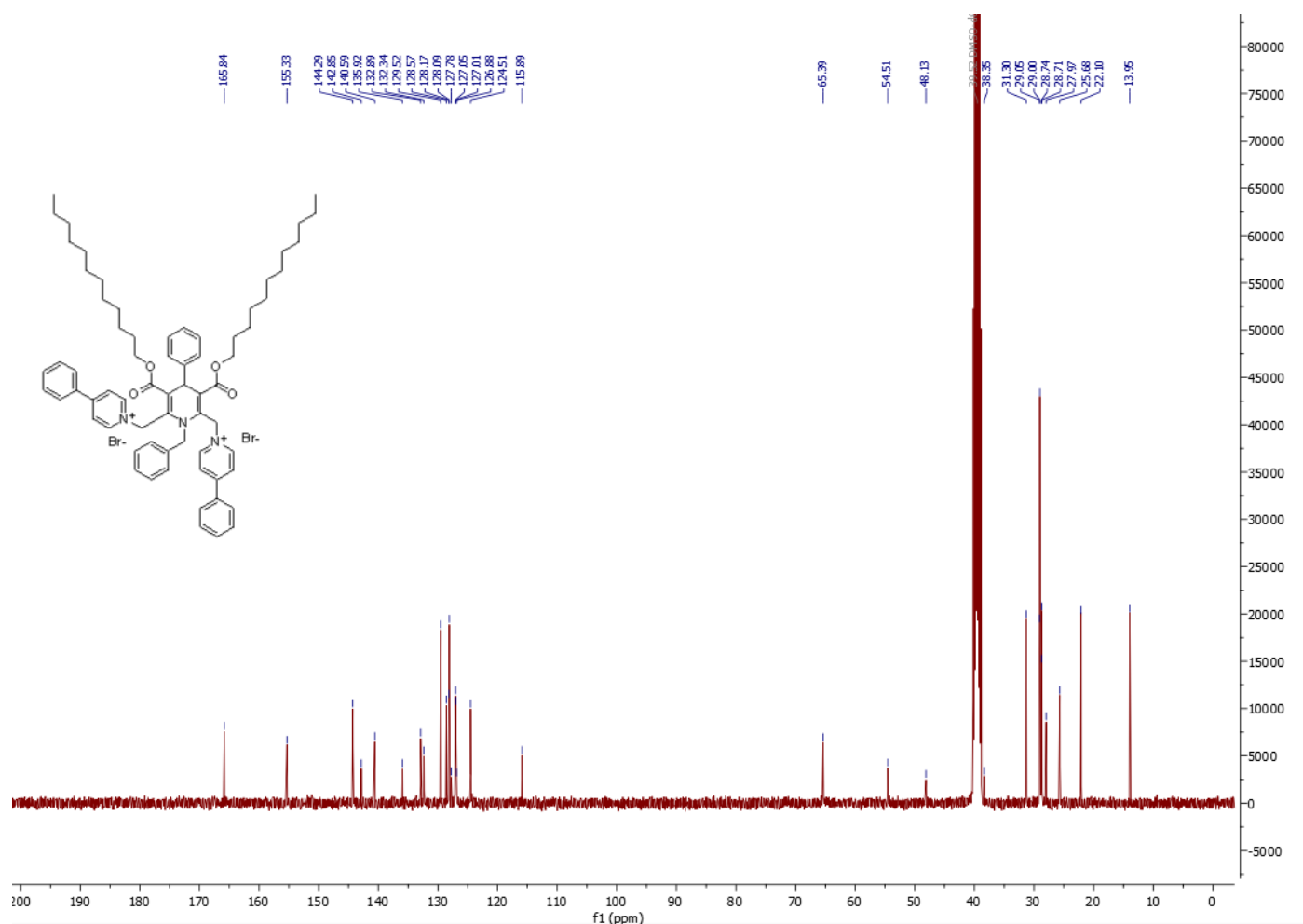




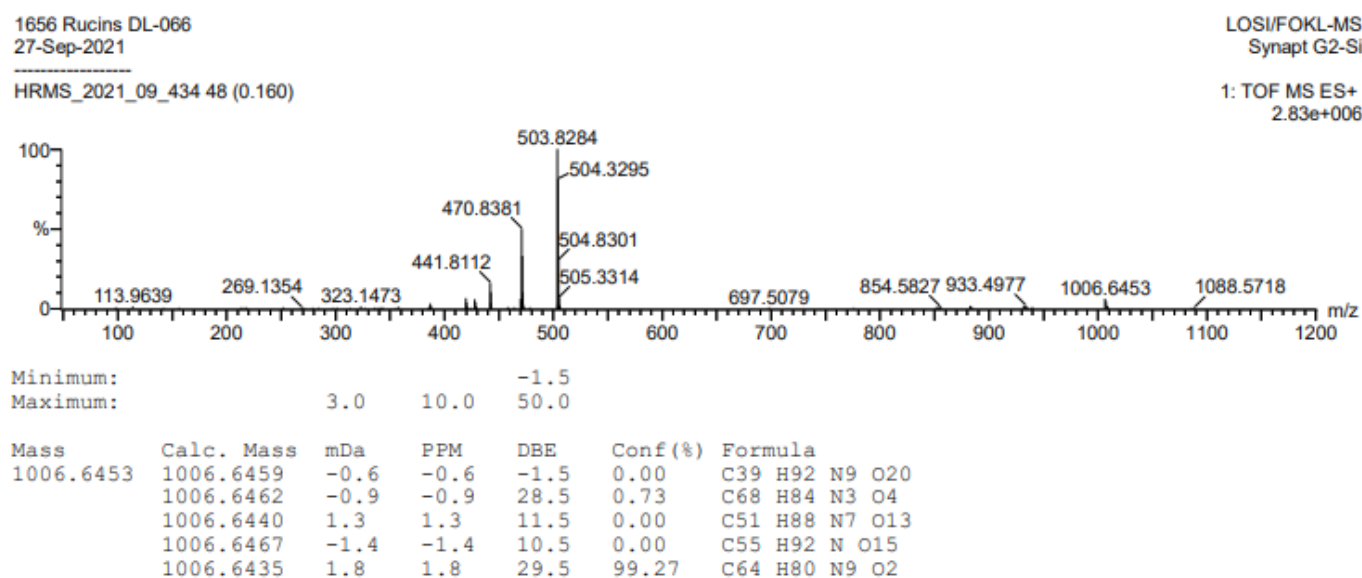
**Figure S15.** HRMS data of 1,1'-((1-benzyl-3,5-bis((dodecyloxy)carbonyl)-4-phenyl-1,4-dihydropyridine-2,6-diyl)bis(methylene))bis(4-(dimethylamino)pyridin-1-ium) (**5c**)



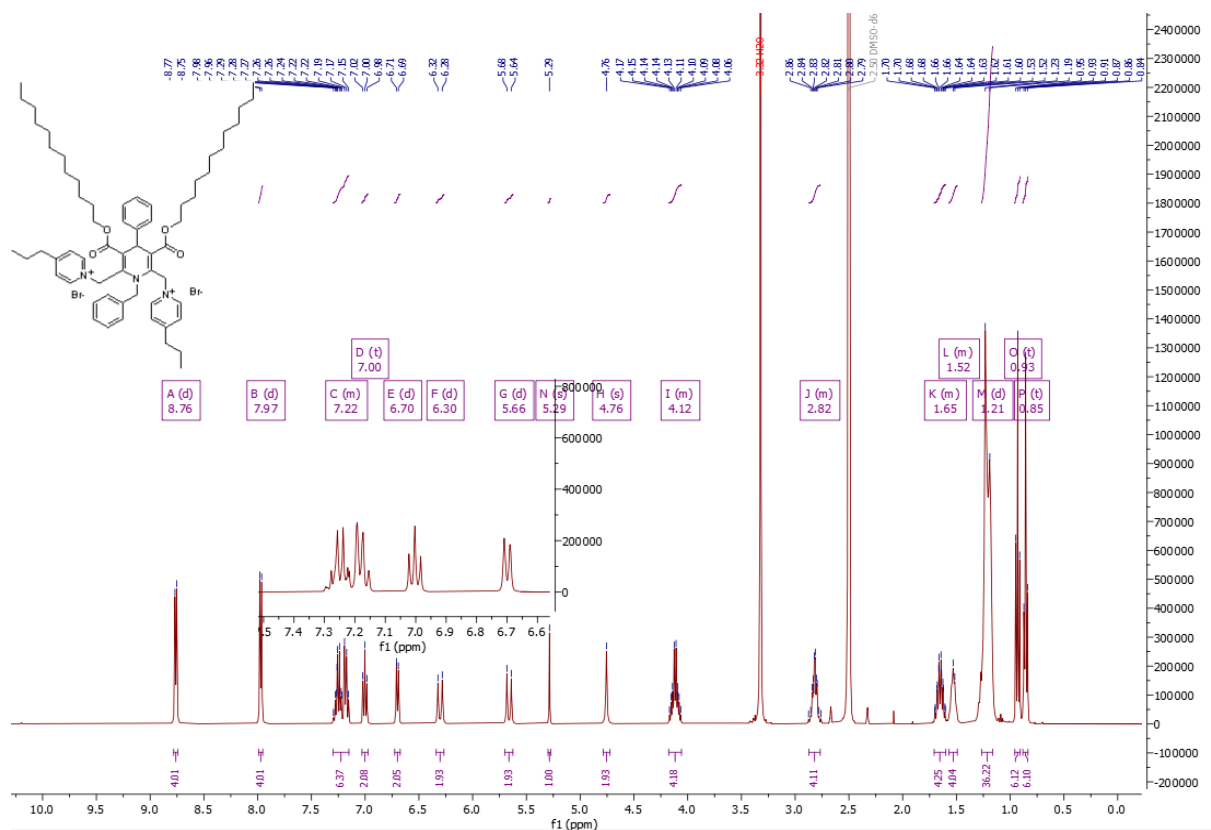
**Figure S16.**  $^1\text{H}$  NMR spectrum of 1,1'-((1-benzyl-3,5-bis((dodecyloxy)carbonyl)-4-phenyl-1,4-dihydropyridine-2,6-diyl)bis(methylene))bis(4-phenylpyridin-1-ium) (**5d**)



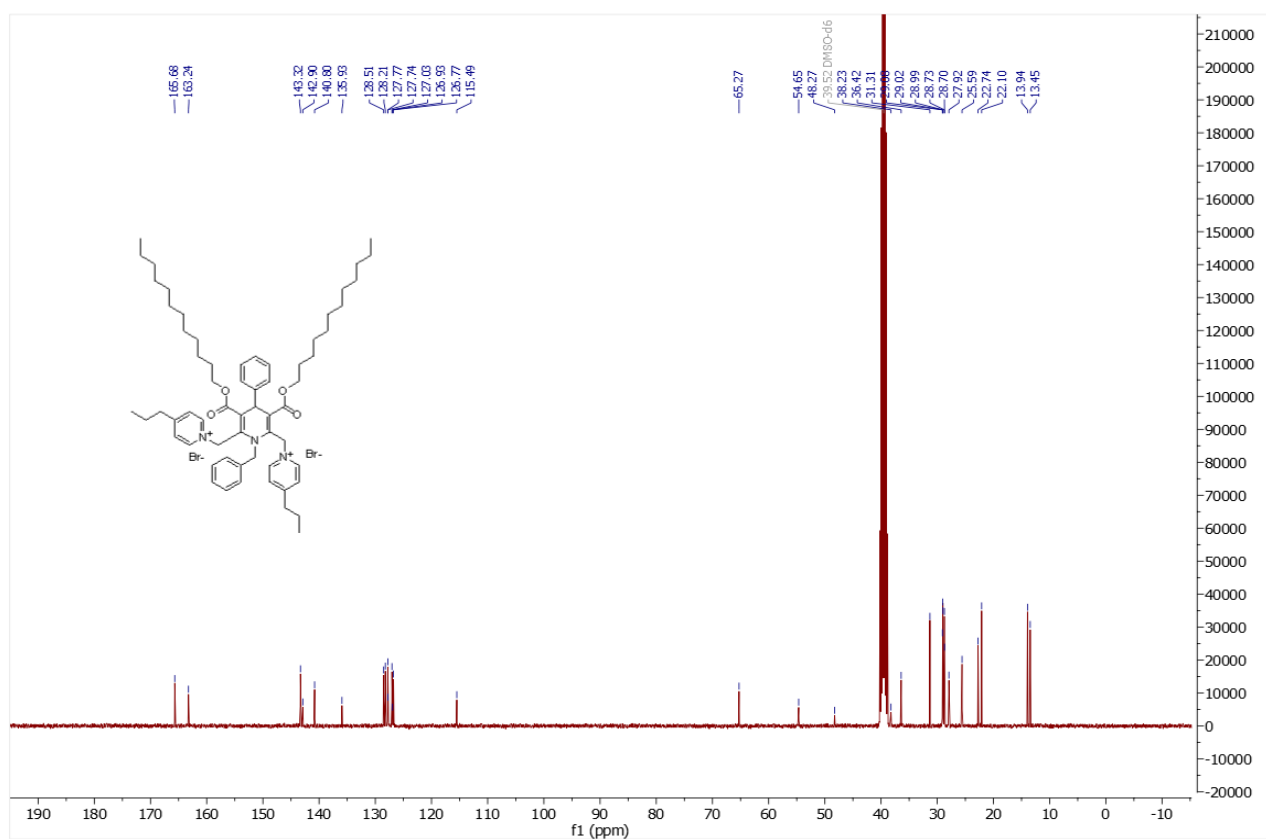
**Figure S17.**  $^{13}\text{C}$  NMR spectrum of 1,1'-((1-benzyl-3,5-bis((dodecyloxy)carbonyl)-4-phenyl-1,4-dihydropyridine-2,6-diyl)bis(methylene))bis(4-phenylpyridin-1-ium) (**5d**)



**Figure S18.** HRMS data of 1,1'-((1-benzyl-3,5-bis((dodecyloxy)carbonyl)-4-phenyl-1,4-dihydropyridine-2,6-diyl)bis(methylene))bis(4-phenylpyridin-1-ium) (**5d**)

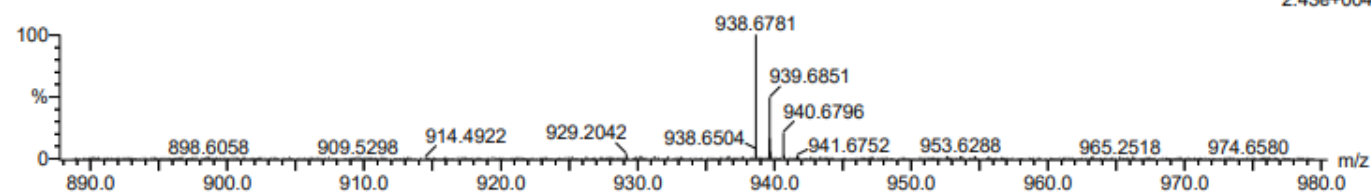


**Figure S19.** <sup>1</sup>H NMR spectrum of 1,1'-((1-benzyl-3,5-bis((dodecyloxy)carbonyl)-4-phenyl-1,4-dihydropyridine-2,6-diyl)bis(methylene))bis(4-propylpyridin-1-ium) (**5e**)



**Figure S20.** <sup>13</sup>C NMR spectrum of 1,1'-((1-benzyl-3,5-bis((dodecyloxy)carbonyl)-4-phenyl-1,4-dihydropyridine-2,6-diyl)bis(methylene))bis(4-propylpyridin-1-ium) (**5e**)

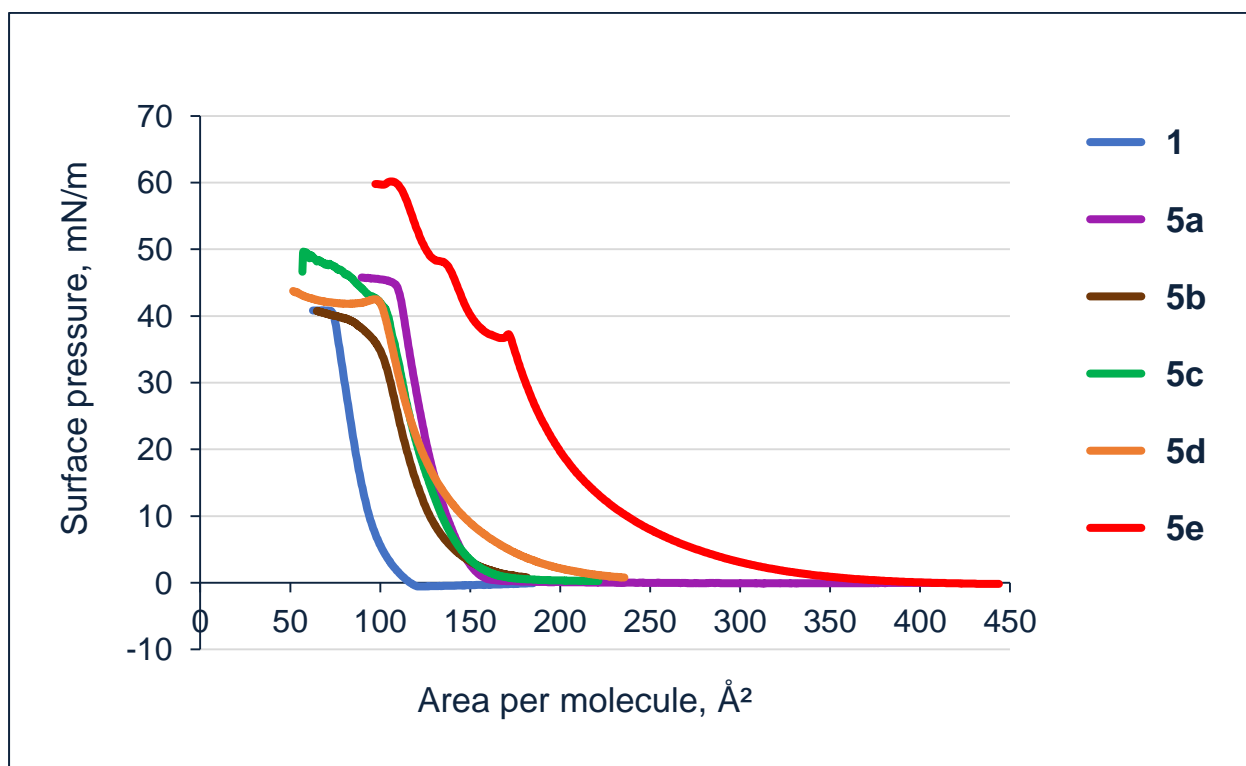
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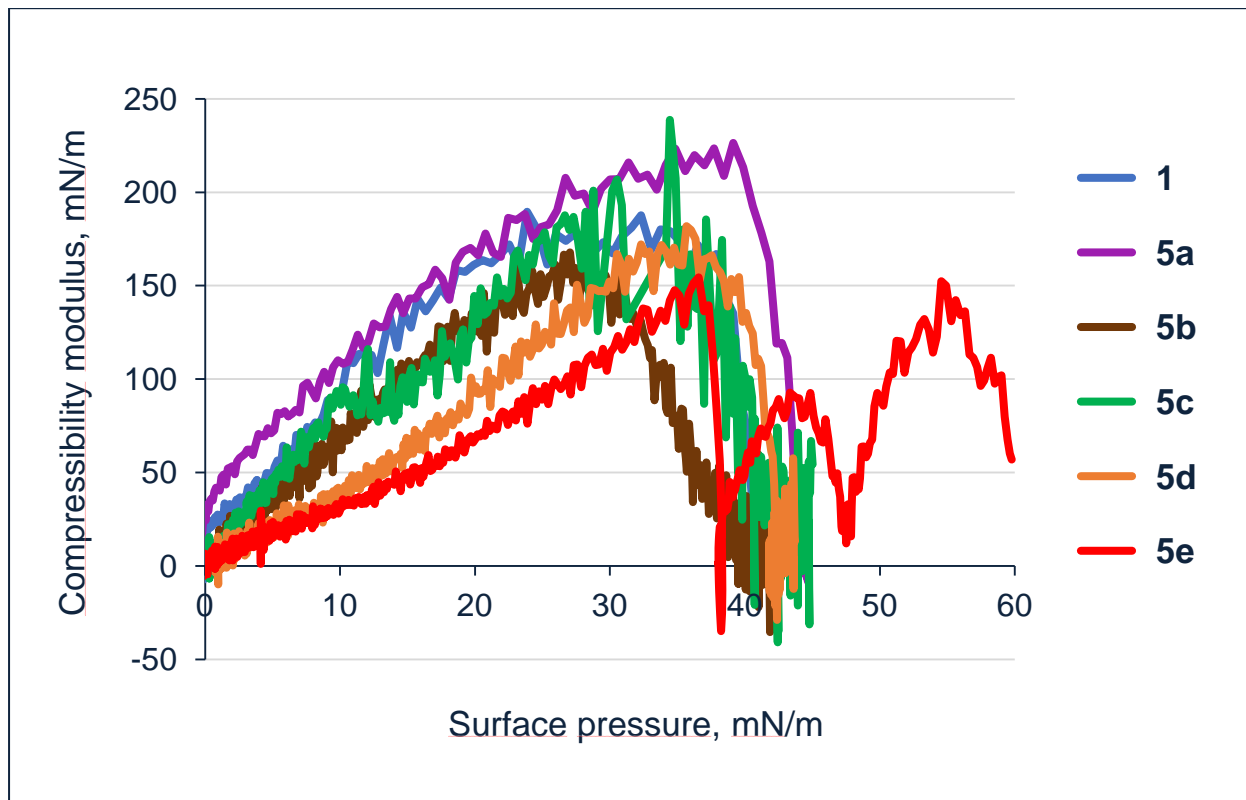
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Maximum: 100.00

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		938.6788	-0.7	-0.7	25.5	488.4	1.803	16.48	C63 H84 N7
		938.6748	3.3	3.5	21.5	487.1	0.496	60.92	C58 H84 N9 O2

**Figure S21.** HRMS data of 1,1'-((1-benzyl-3,5-bis((dodecyloxy)carbonyl)-4-phenyl-1,4-dihydropyridine-2,6-diyl)bis(methylene))bis(4-propylpyridin-1-ium) (**5e**)



**Figure S22.** Surface pressure – mean molecular area isotherms of tested 1,4-DHP amphiphiles **1** and **5** at  $25\pm 1^\circ\text{C}$ .



**Figure S23.** Compressibility modulus-surface pressure dependences obtained for the 1,4-DHP amphiphiles **1** and **5** monolayers.

**Table S1.** Values of Z-average ( $Z_{av}D_H$ ) diameter and polydispersity index (PDI) of nanoparticles formed by 1,4-DHP amphiphiles **1** and **5** at the 0.1mM concentration obtained by DLS measurements for freshly prepared samples and after 1 month storage. The PDI value describes polydispersity of the sample; the  $Z_{av}$  diameter represents the average hydrodynamic diameter of all nanoparticles in the sample.

Sample concentration – 0.1 mM					
Entry	Comp.	Fresh samples		After 1 month storage	
		$Z_{av} D_H$ , nm	PDI	$Z_{av} D_H$ , nm	PDI
1	<b>1</b>	395 ± 28	0.342 ± 0.047	346 ± 3	0.305 ± 0.042
2	<b>5a</b>	781 ± 73	0.116 ± 0.112	873 ± 114	0.391 ± 0.027
3	<b>5b</b>	529 ± 258	0.521 ± 0.284	430 ± 44	0.443 ± 0.043
4	<b>5c</b>	715 ± 34	0.414 ± 0.087	458 ± 16	0.445 ± 0.02
5	<b>5d</b>	2572 ± 153	0.680 ± 0.328	1195 ± 148	0.731 ± 0.081
6	<b>5e</b>	462 ± 174	0.526 ± 0.251	1003 ± 566	0.782 ± 0.163

**Table S2.** Values of Z-average ( $Z_{av}D_H$ ) diameter and polydispersity index (PDI) of lipoplexes formed by 1,4-DHP amphiphiles **1** and **5** and mRNA at different N/P charge ratios obtained by DLS measurements for freshly prepared samples. The PDI value describes polydispersity of the sample; the  $Z_{av}D_H$  diameter represents the average hydrodynamic diameter of all nanoparticles in the sample.

Lipoplex size (fresh samples) at different N/P charge ratios							
Entry	Comp.	N/P = 1		N/P = 2		N/P = 5	
		$Z_{av} D_H$ , nm	PDI	$Z_{av} D_H$ , nm	PDI	$Z_{av} D_H$ , nm	PDI
1	<b>1</b>	806 ± 60	0.246 ± 0.122	582 ± 90	0.127 ± 0.085	298 ± 49	0.266 ± 0.031
2	<b>5a</b>	1112 ± 169	0.48 ± 0.087	1325 ± 336	0.492 ± 0.083	143 ± 7	0.295 ± 0.071
3	<b>5b</b>	809 ± 88	0.349 ± 0.098	386 ± 9	0.42 ± 0.503	148 ± 7	0.179 ± 0.045
4	<b>5c</b>	1039 ± 215	0.357 ± 0.196	986 ± 75	0.514 ± 0.444	139 ± 2	0.215 ± 0.039
5	<b>5d</b>	2959 ± 2010	1 ± 0	1540 ± 198	0.373 ± 0.199	1551 ± 237	0.318 ± 0.233
6	<b>5e</b>	1535 ± 125	1 ± 0	1228 ± 266	0.439 ± 0.283	1208 ± 419	0.653 ± 0.305