

## Supporting materials

### **Cationic lipophosphoramidates with two different lipid chains: synthesis and evaluation as gene carriers.**

Stéphanie S. Le Corre, Mathieu Berchel, Nawal Belmadi, Caroline Denis, Jean-Pierre Haelters, Tony Le Gall, Pierre Lehn, Tristan Montier, Paul-Alain Jaffrès\*

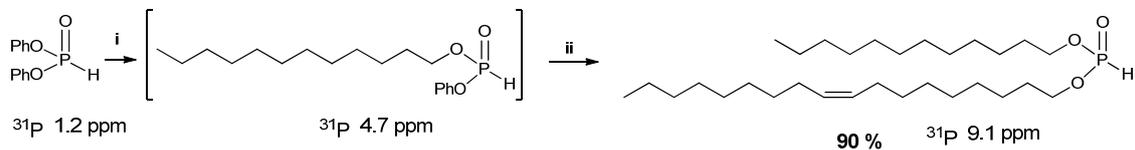
#### **Summary**

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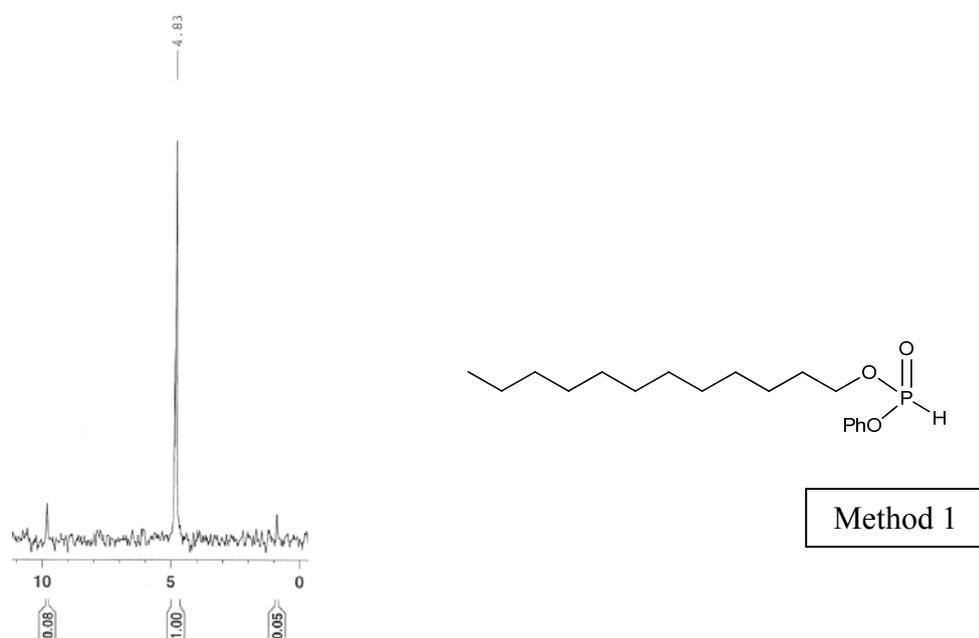
## Supporting materials

### S1 NMR spectra (non-symmetric phosphite – method 1 and 2)

#### Method 1



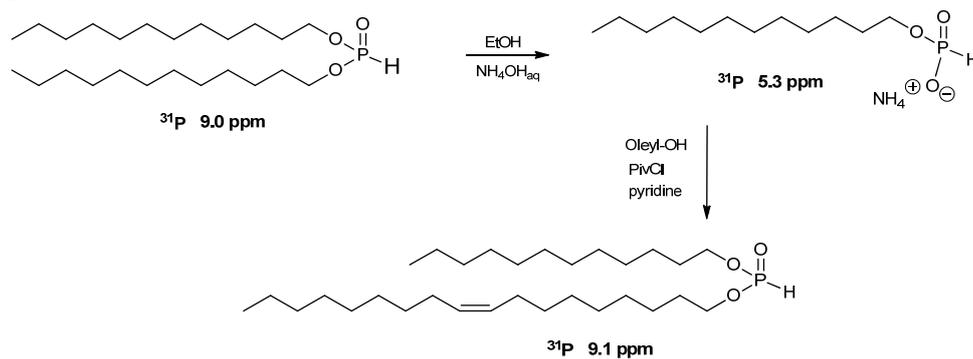
**Figure S1-1:** Attempts to produce non-symmetric dialkylphosphite from diphenylphosphite. i) pyridine (0.9 eq) ;  $\text{C}_{12}\text{H}_{25}\text{OH}$  (0.9 eq), 20°C, 2h ; ii) pyridine (1 eq) ; Oleyl-OH (1 eq) 3h, 20°C.



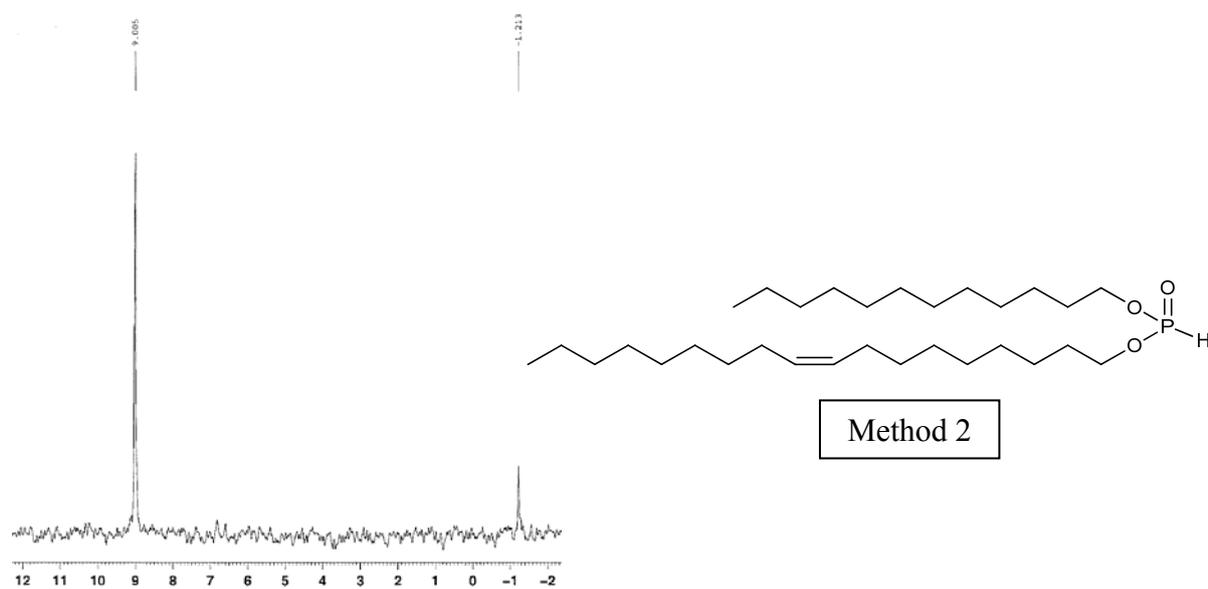
**Figure S1-2 :**  $^{31}\text{P}$  NMR of alkylarylphosphite synthesised by method 1.

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### Method 2



**Figure S1-3** : Synthesis of non-symmetric dialkylphosphite from symmetric dialkylphosphite.



**Figure S1-4** :  $^{31}\text{P}$  NMR of non-symmetric dialkylphosphite synthesised by method 2.

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## S2 NMR spectra (POCl<sub>3</sub> method)

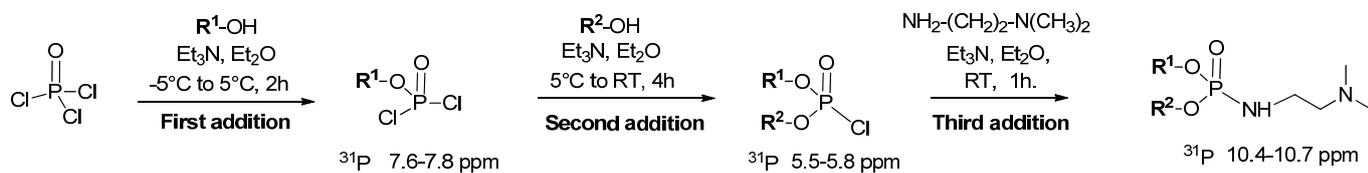


Figure S2-1 : One-pot procedure for the synthesis of non-symmetric lipophosphoramidate (R<sup>1</sup> ≠ R<sup>2</sup>).

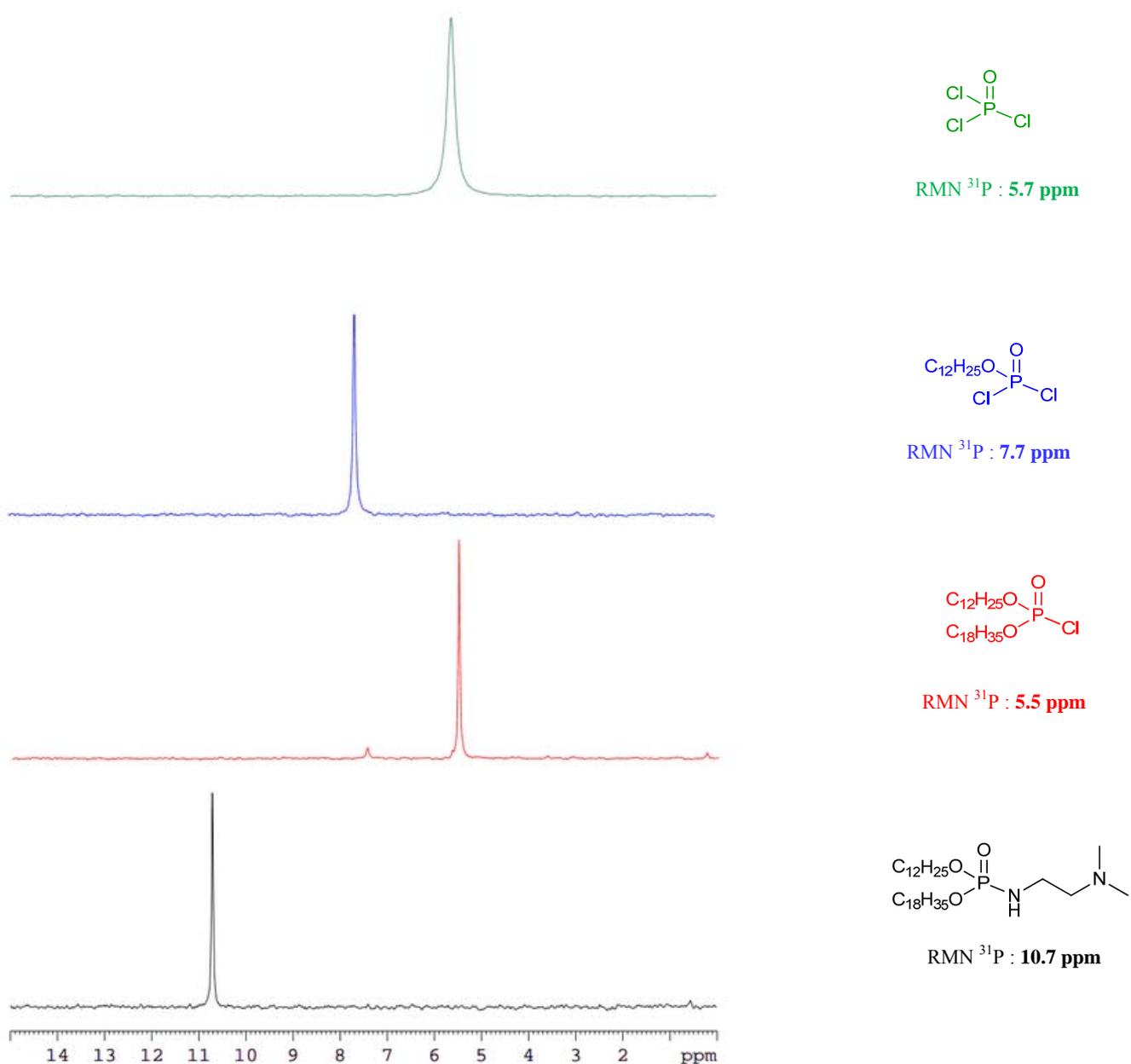


Figure S2-2 : <sup>31</sup>P NMR (CDCl<sub>3</sub>) spectrum of POCl<sub>3</sub> method

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### S3 NMR spectra

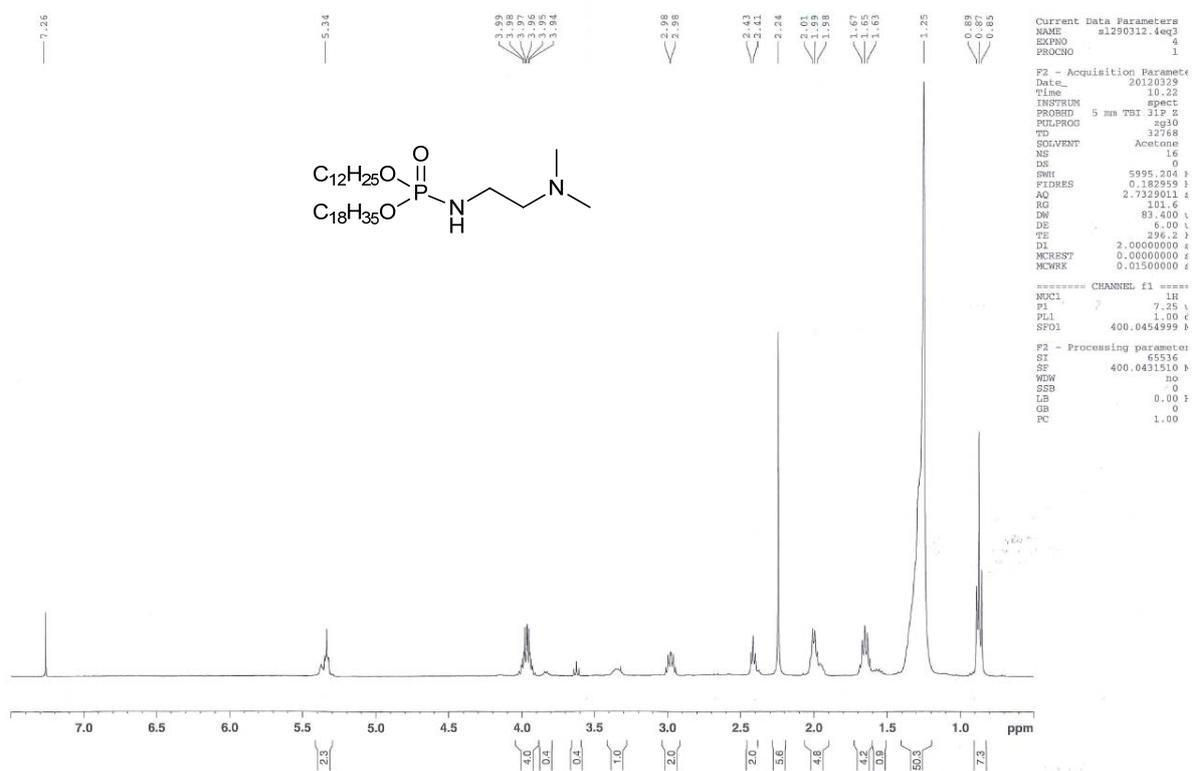


Figure S3-1: <sup>1</sup>H NMR (CDCl<sub>3</sub>) spectrum of compound 1.

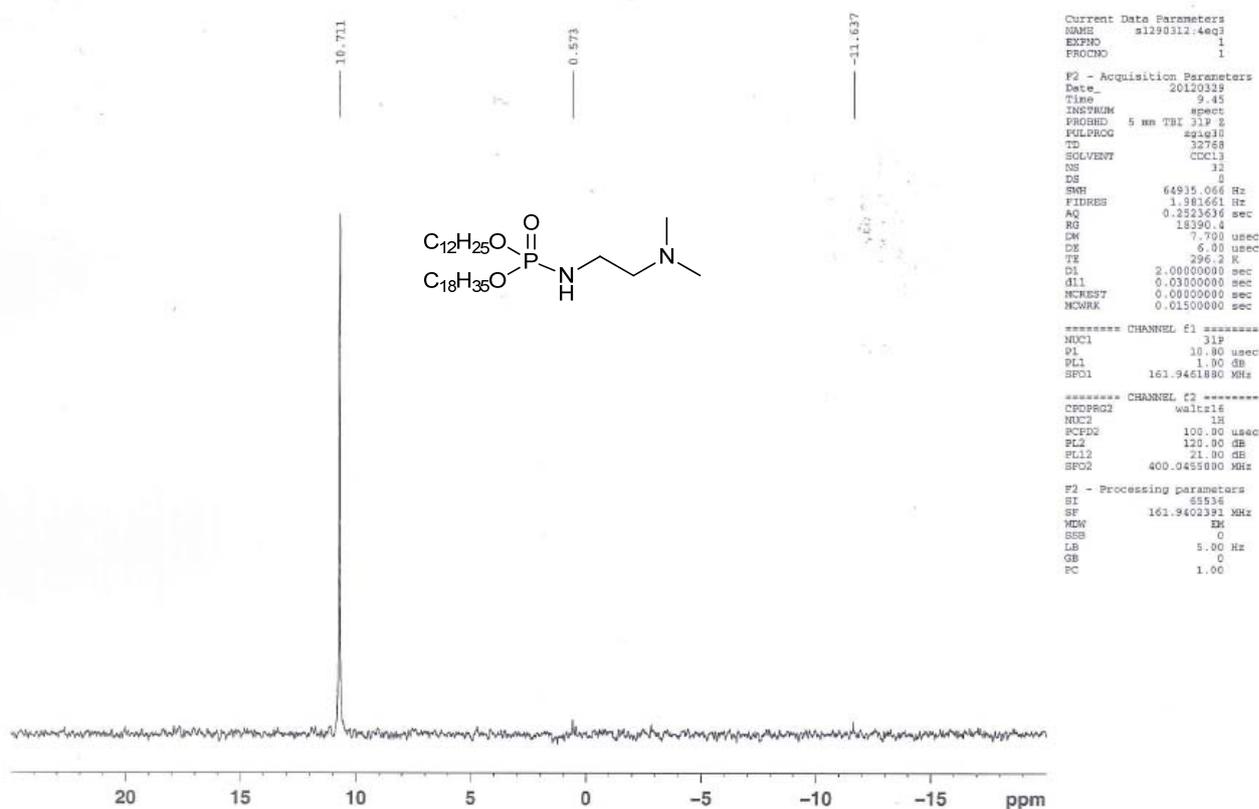


Figure S3-2: <sup>31</sup>P NMR (CDCl<sub>3</sub>) spectrum of compound 1.

Supporting materials

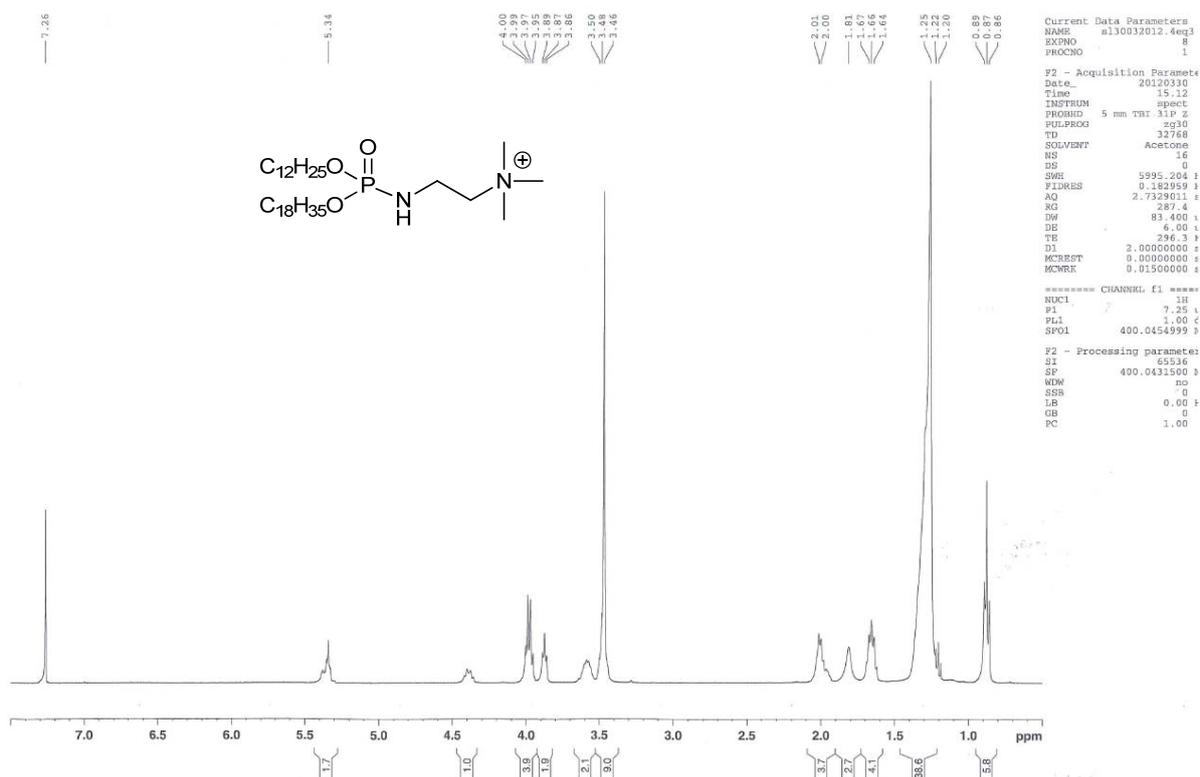


Figure S3-3: <sup>1</sup>H NMR (CDCl<sub>3</sub>) spectrum of compound 2.

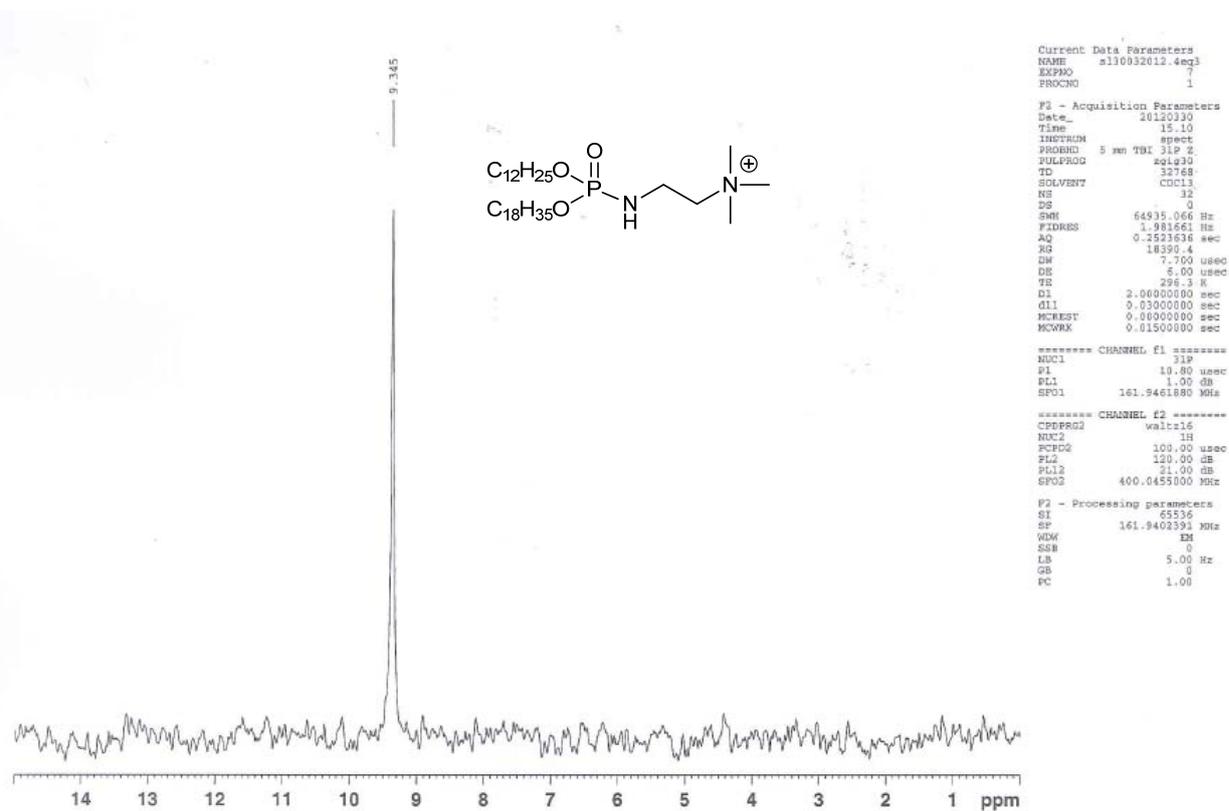


Figure S3-4: <sup>31</sup>P NMR (CDCl<sub>3</sub>) spectrum of compound 2.

Supporting materials

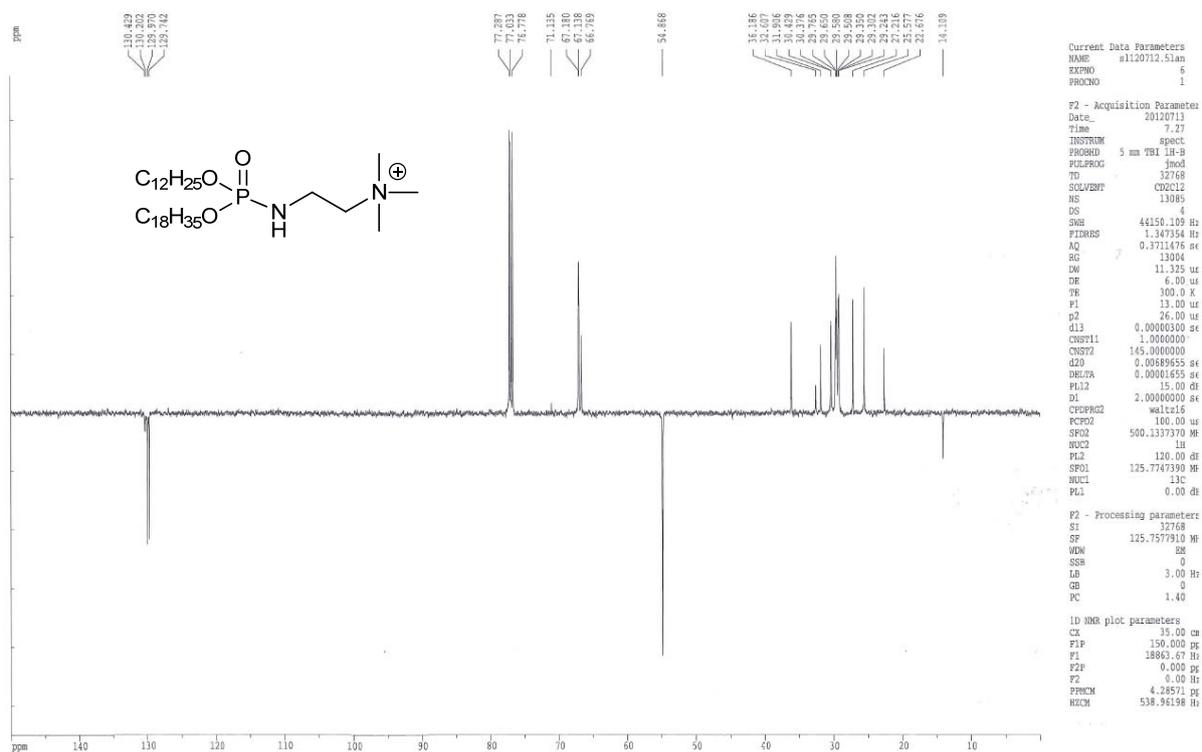


Figure S3-5: <sup>13</sup>C jmod (CDCl<sub>3</sub>) spectrum of compound 2.

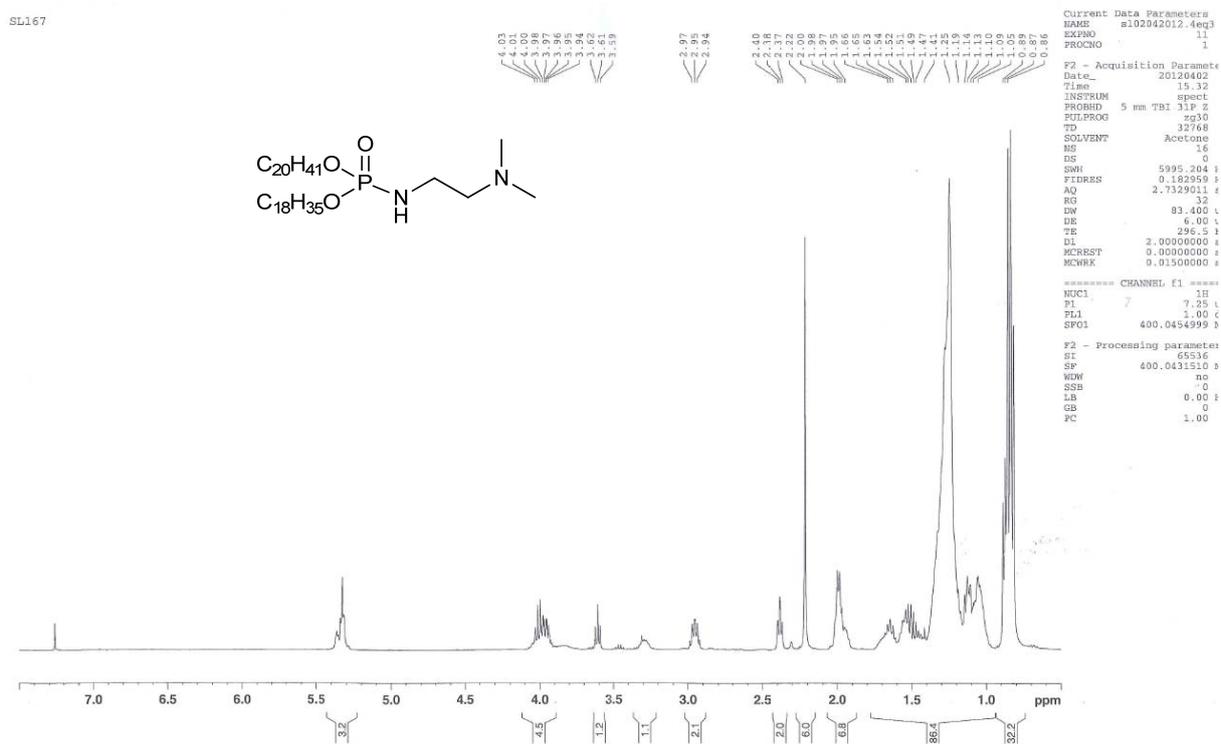


Figure S3-6: <sup>1</sup>H NMR (CDCl<sub>3</sub>) spectrum of compound 3.

Supporting materials

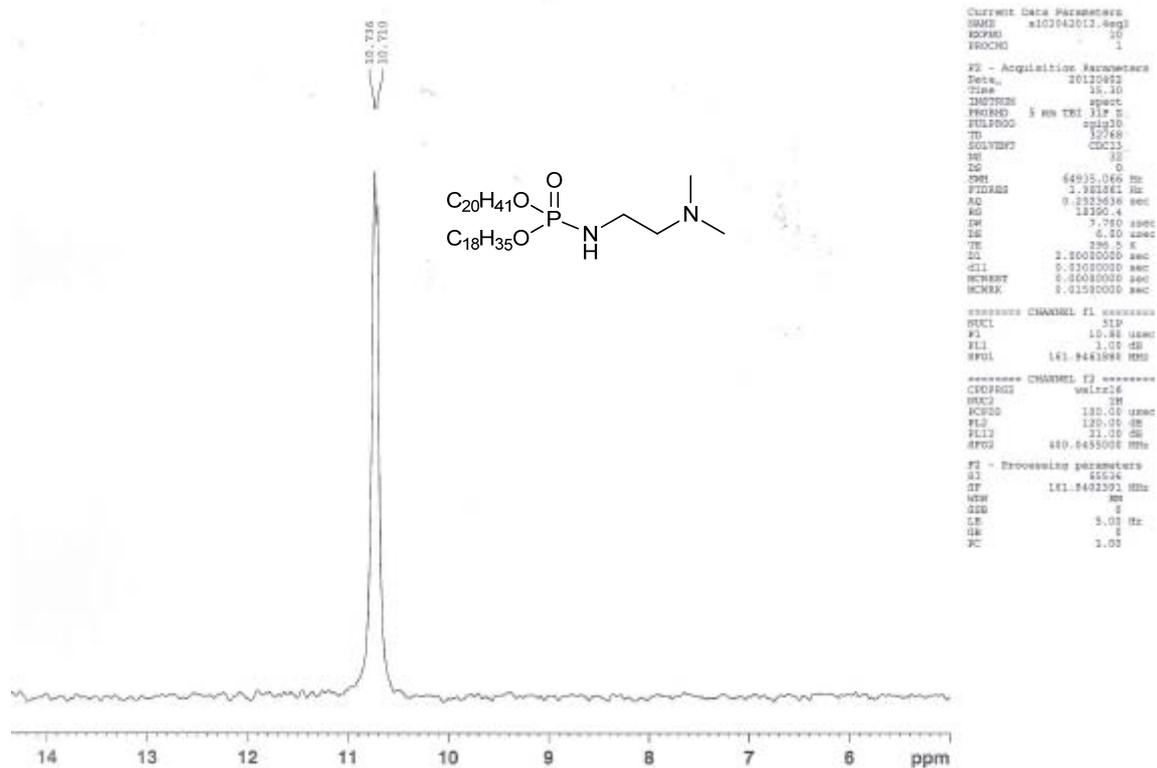


Figure S3-7: <sup>31</sup>P NMR (CDCl<sub>3</sub>) spectrum of compound 3.

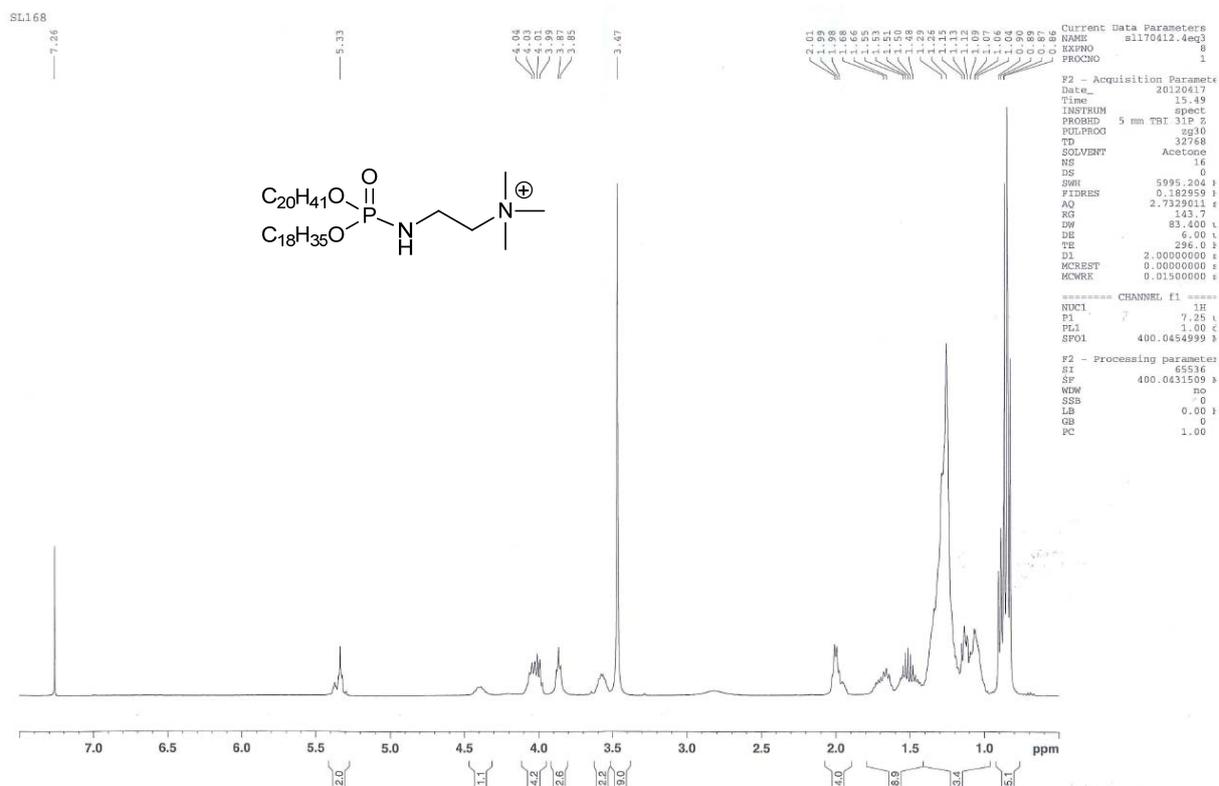


Figure S3-8: <sup>1</sup>H NMR (CDCl<sub>3</sub>) spectrum of compound 4.



Supporting materials

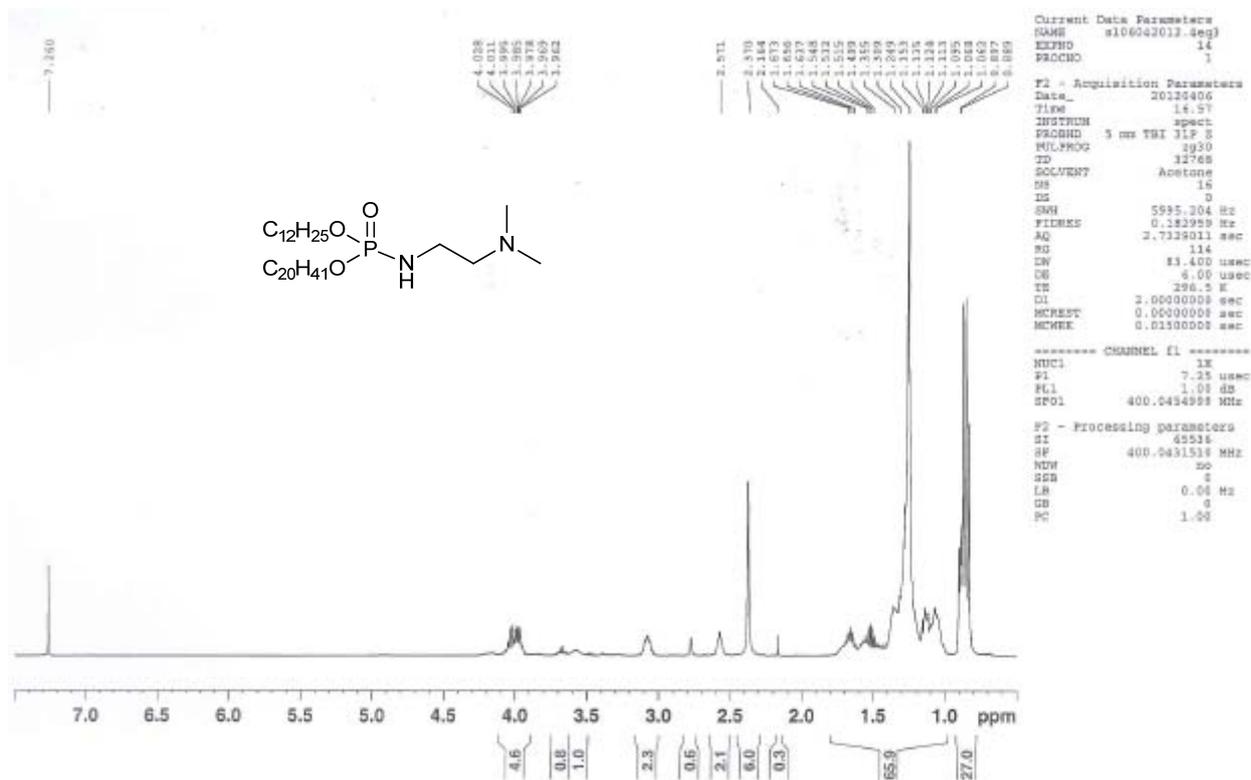


Figure S3-11: <sup>1</sup>H NMR (CDCl<sub>3</sub>) spectrum of compound 5.

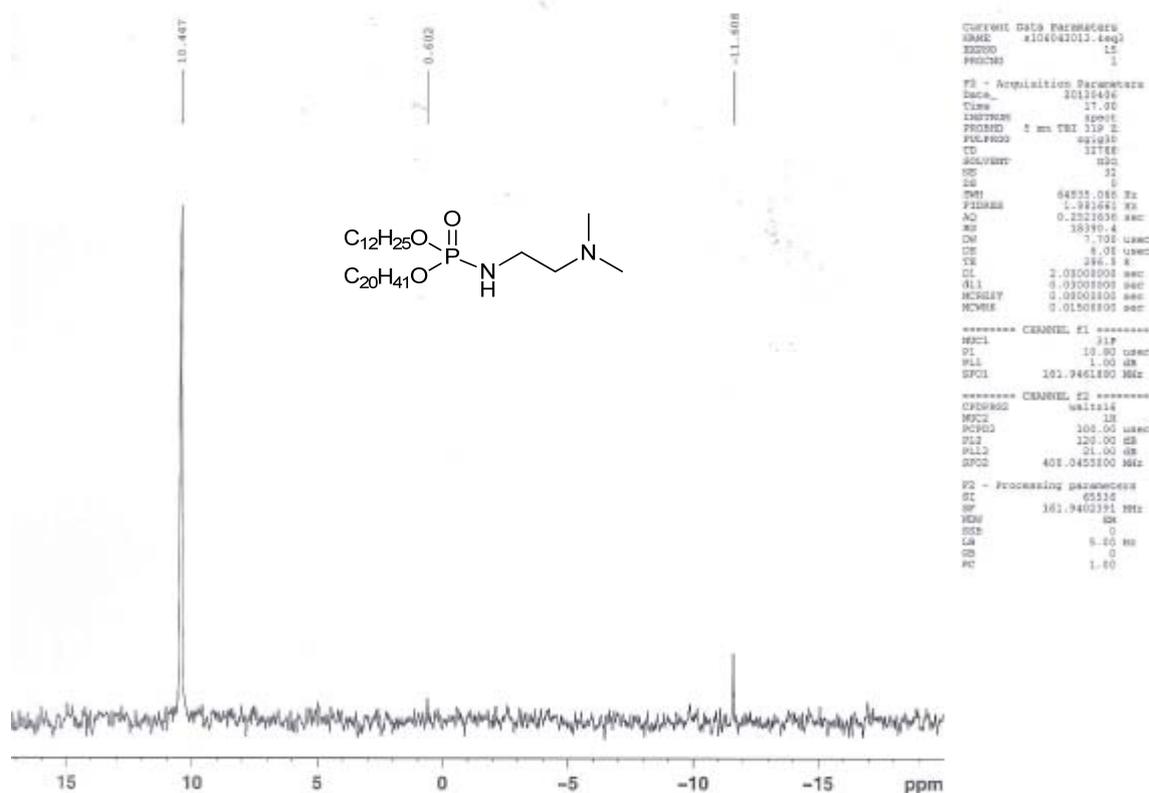


Figure S3-12: <sup>31</sup>P NMR (CDCl<sub>3</sub>) spectrum of compound 5.

Supporting materials

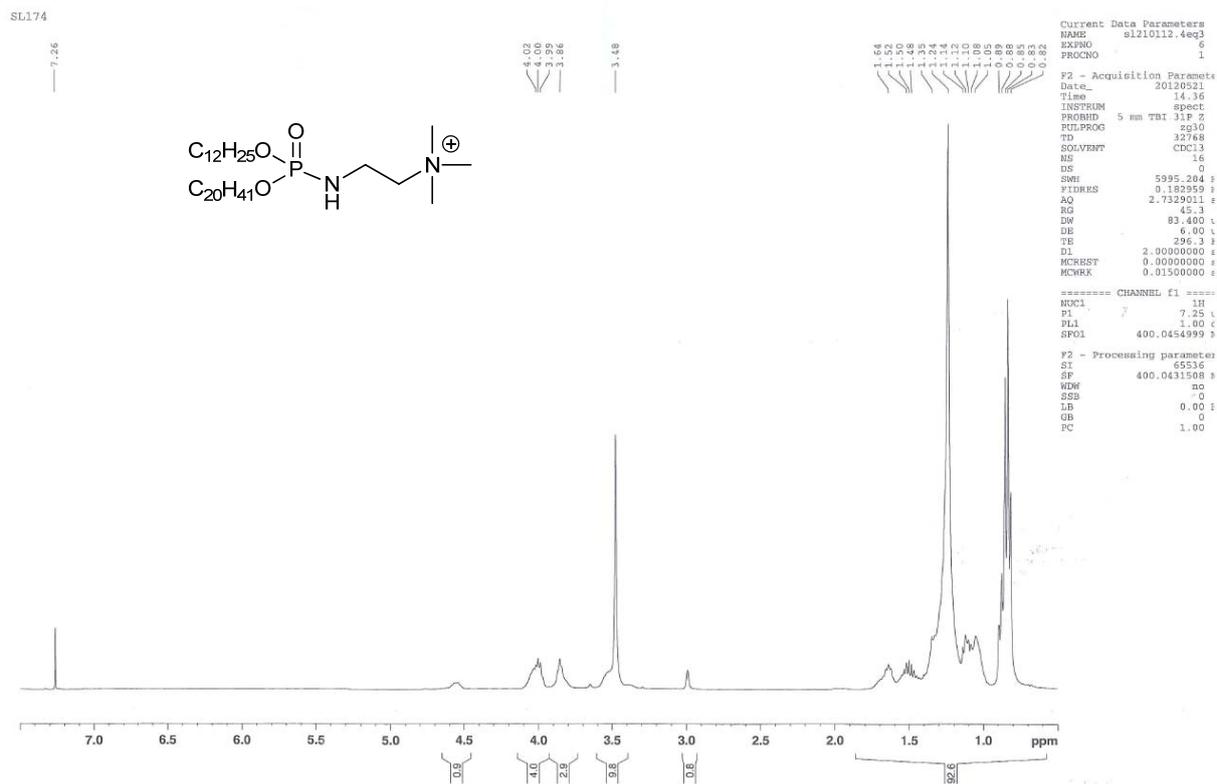


Figure S3-13: <sup>1</sup>H NMR (CDCl<sub>3</sub>) spectrum of compound 6.

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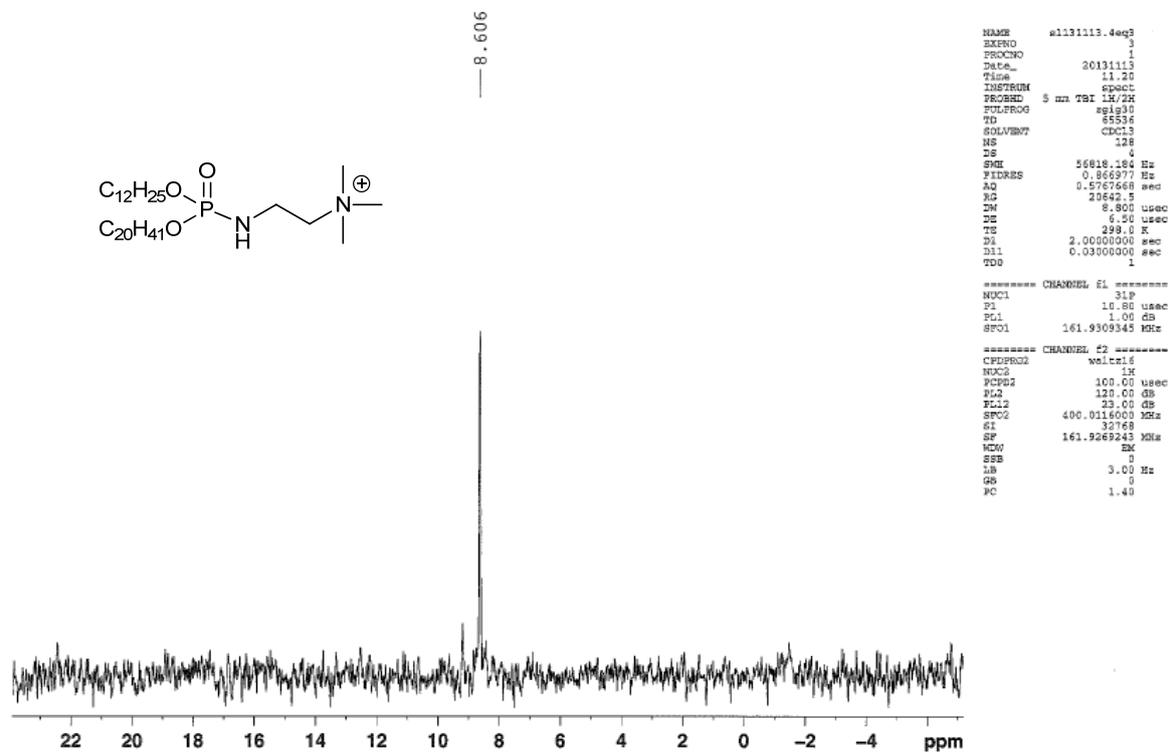


Figure S3-14: <sup>31</sup>P NMR (CDCl<sub>3</sub>) spectrum of compound 6.

Supporting materials

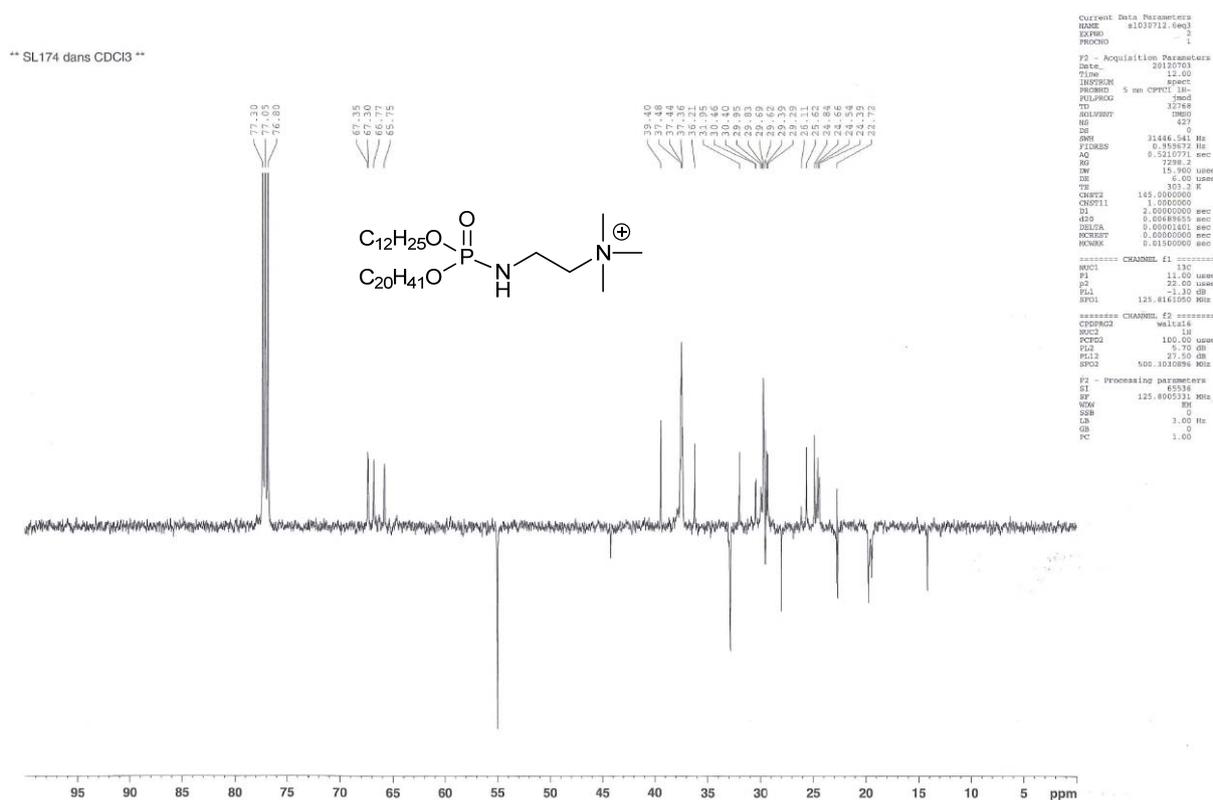


Figure S3-15: <sup>13</sup>C jmod (CDCl<sub>3</sub>) spectrum of compound 6.

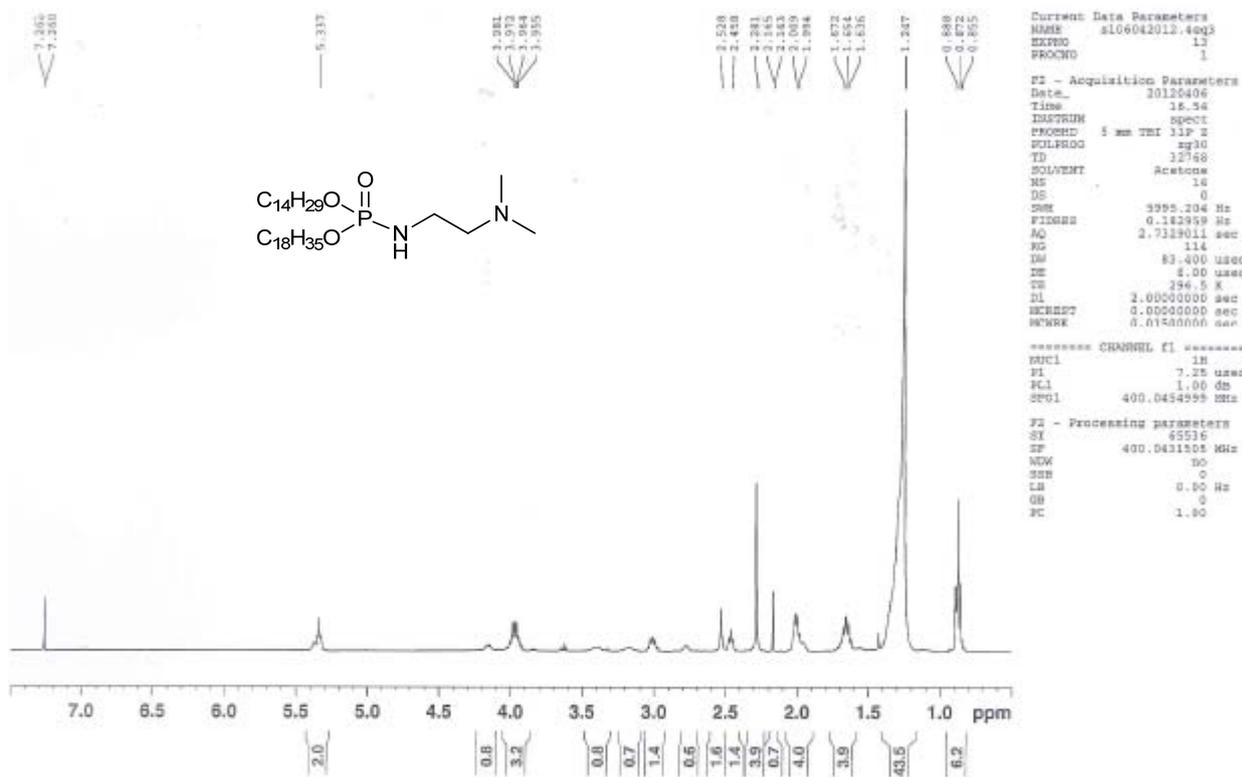


Figure S3-16: <sup>1</sup>H NMR (CDCl<sub>3</sub>) spectrum of compound 7.

Supporting materials

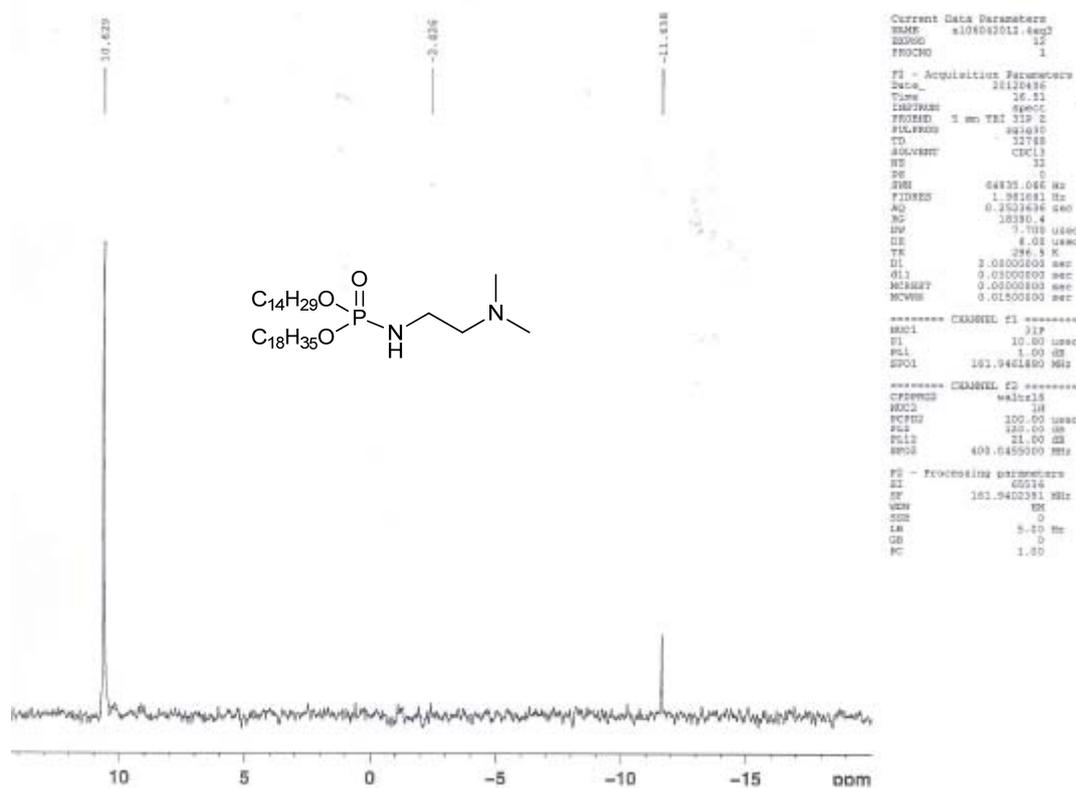


Figure S3-17: <sup>31</sup>P NMR (CDCl<sub>3</sub>) spectrum of compound 7.

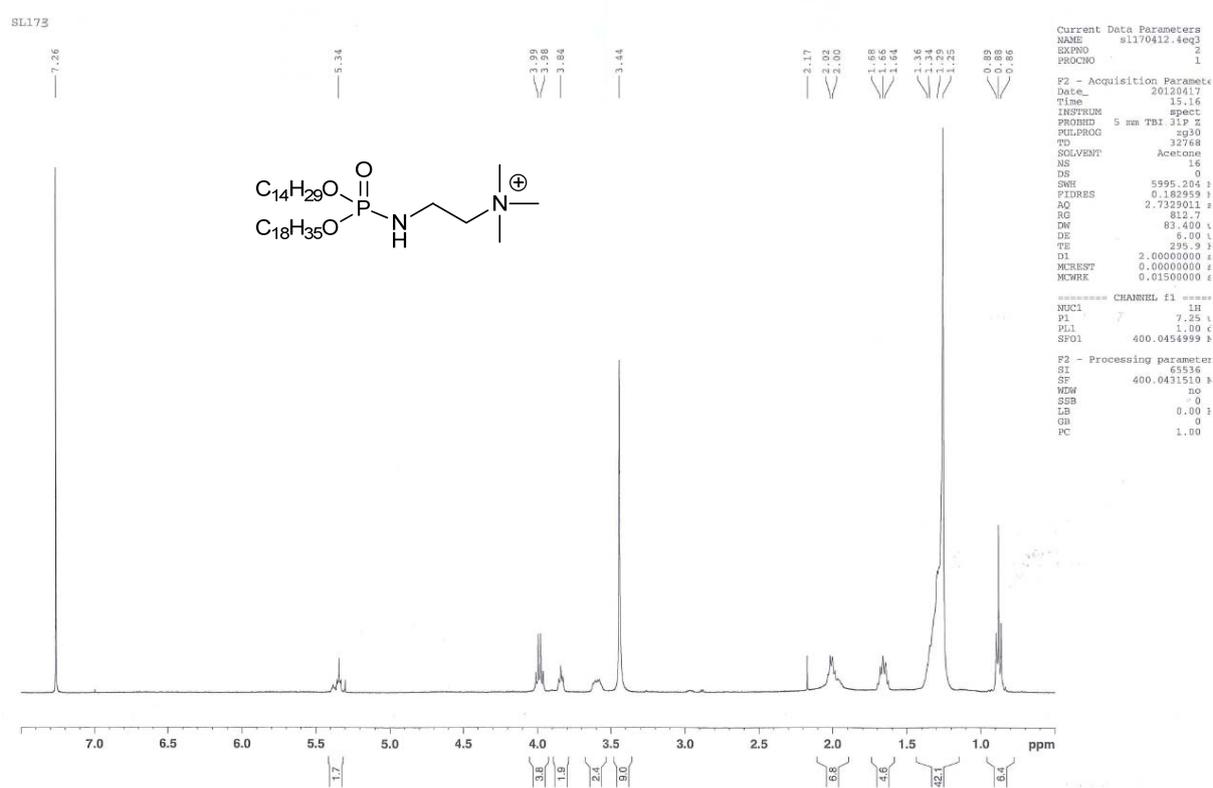


Figure S3-18: <sup>1</sup>H NMR (CDCl<sub>3</sub>) spectrum of compound 8.

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SL 173

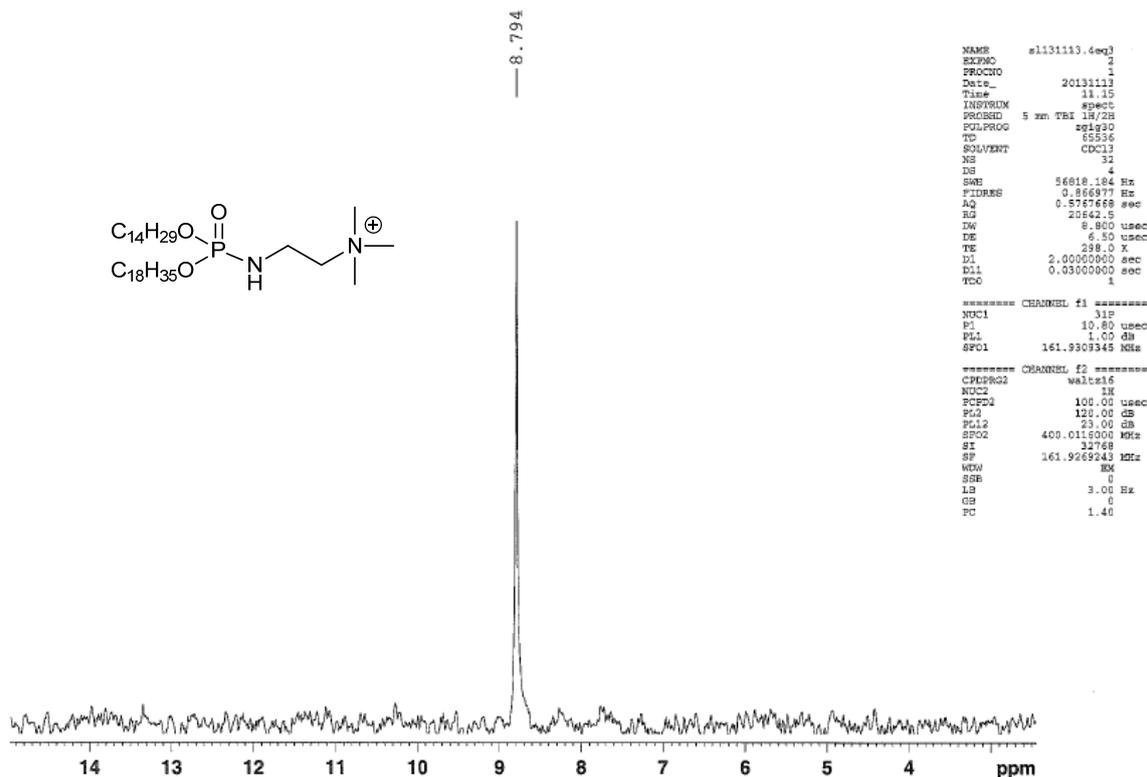


Figure S3-19 : <sup>31</sup>P NMR (CDCl<sub>3</sub>) spectrum of compound 8.

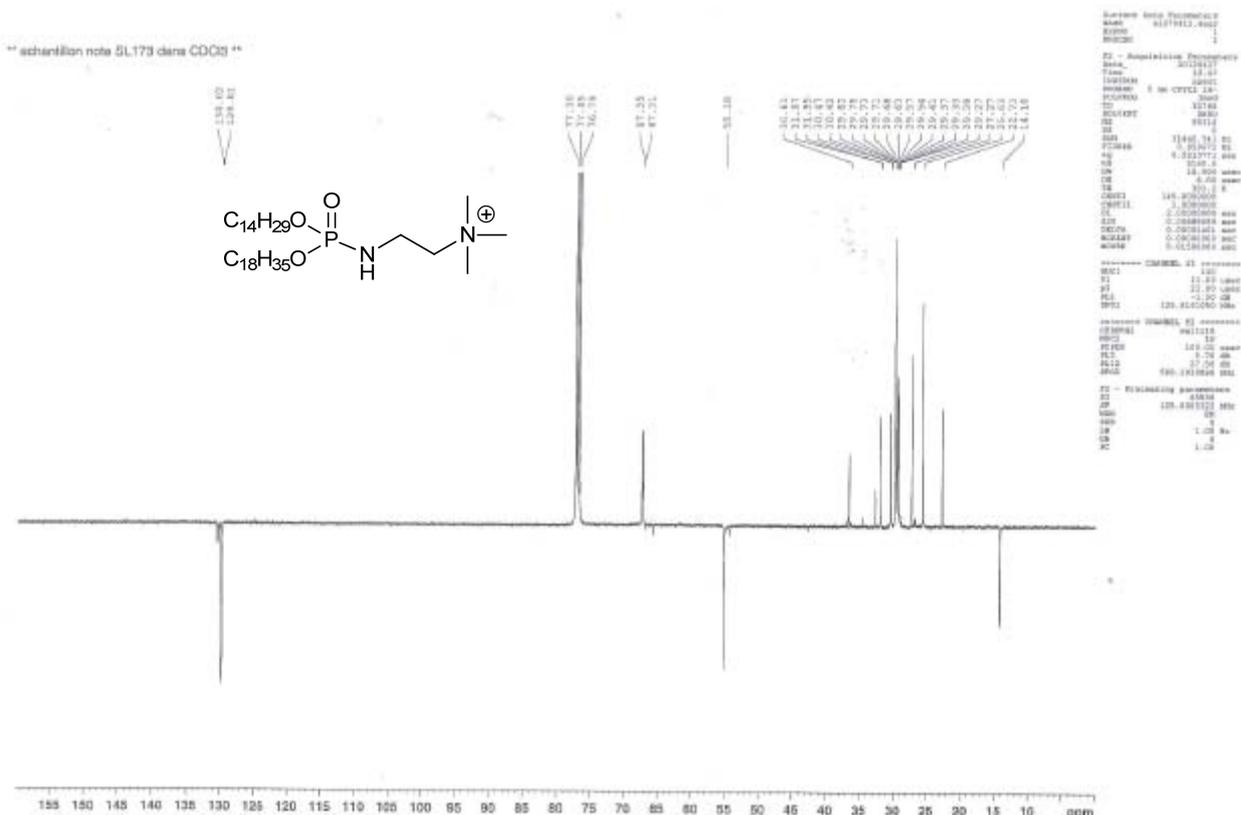


Figure S3-20: <sup>13</sup>C jmod (CDCl<sub>3</sub>) spectrum of compound 8.



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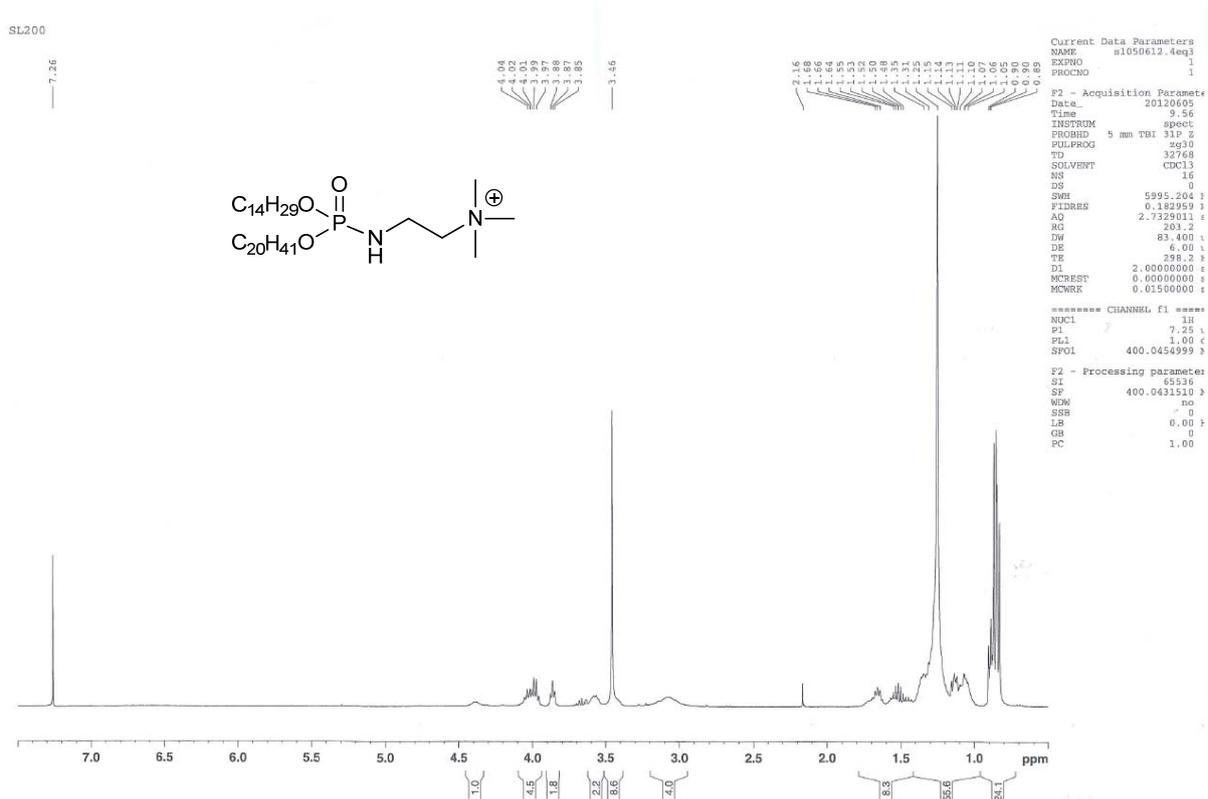


Figure S3-23:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) spectrum of compound 10.

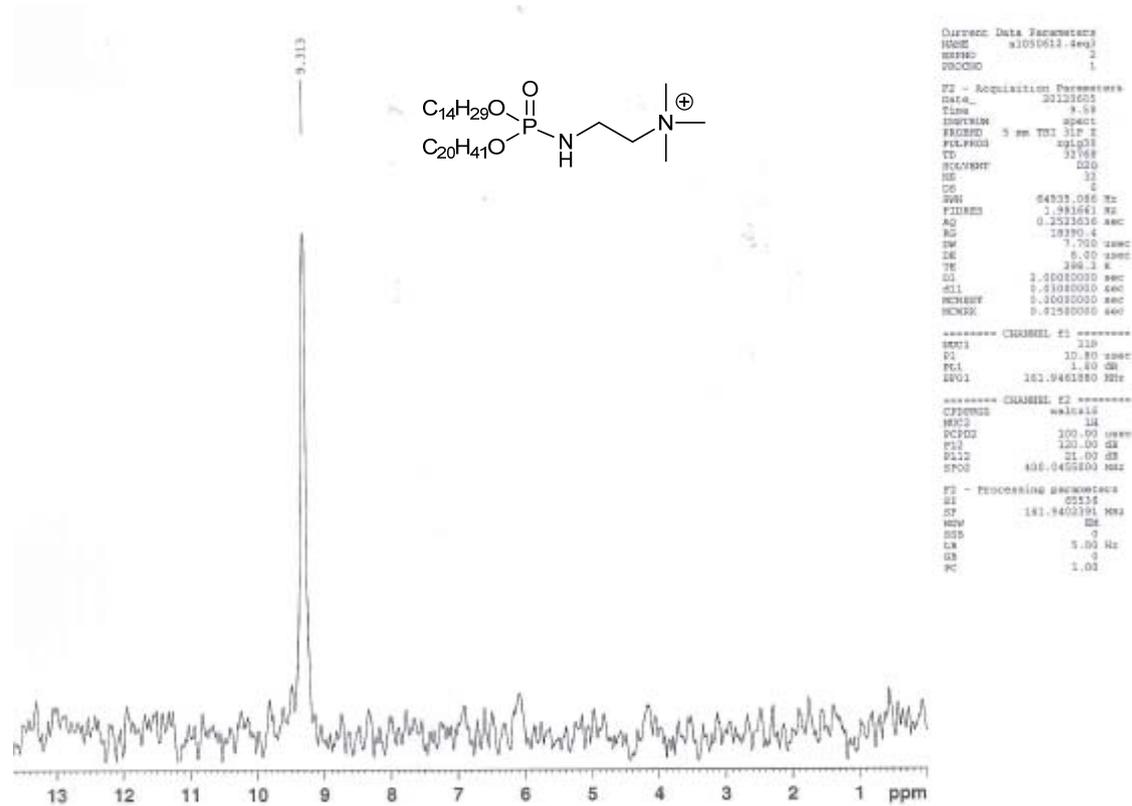


Figure S3-24:  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ ) spectrum of compound 10.



Supporting materials

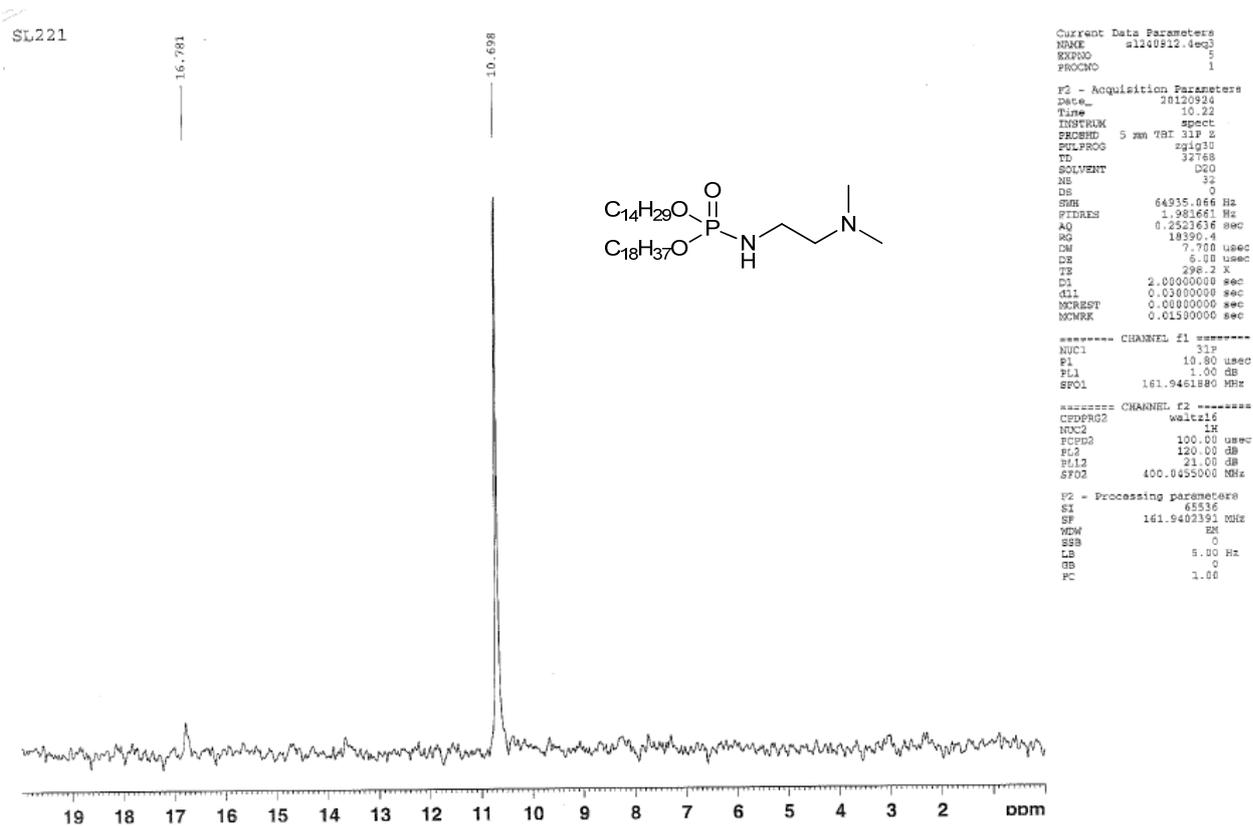


Figure S3-27:  $^{31}\text{P}$  NMR ( $\text{CDCl}_3$ ) spectrum of compound 11.

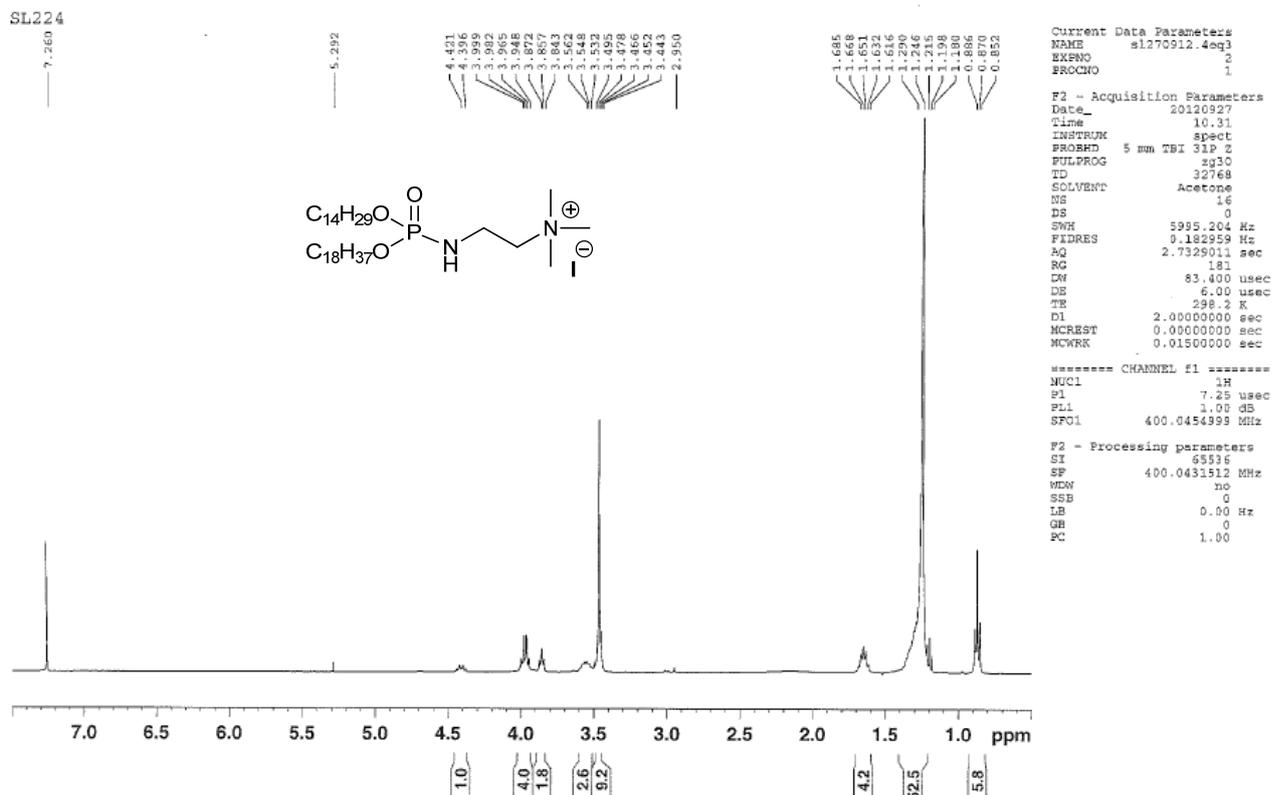


Figure S3-28:  $^1\text{H}$  NMR ( $\text{CDCl}_3$ ) spectrum of compound 12.

Supporting materials

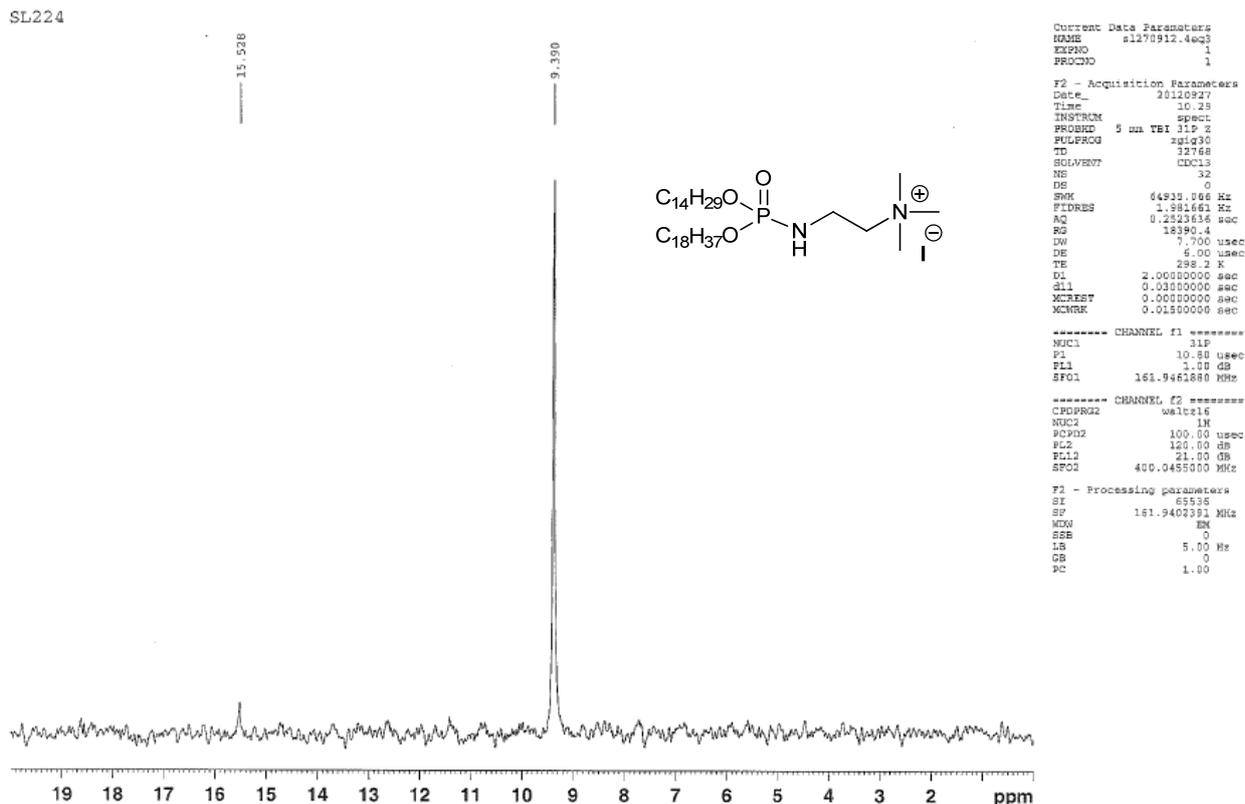


Figure S3-29: <sup>31</sup>P NMR (CDCl<sub>3</sub>) spectrum of compound 12.

\*\* echantillon note SL224 dans CDCl3 \*\*

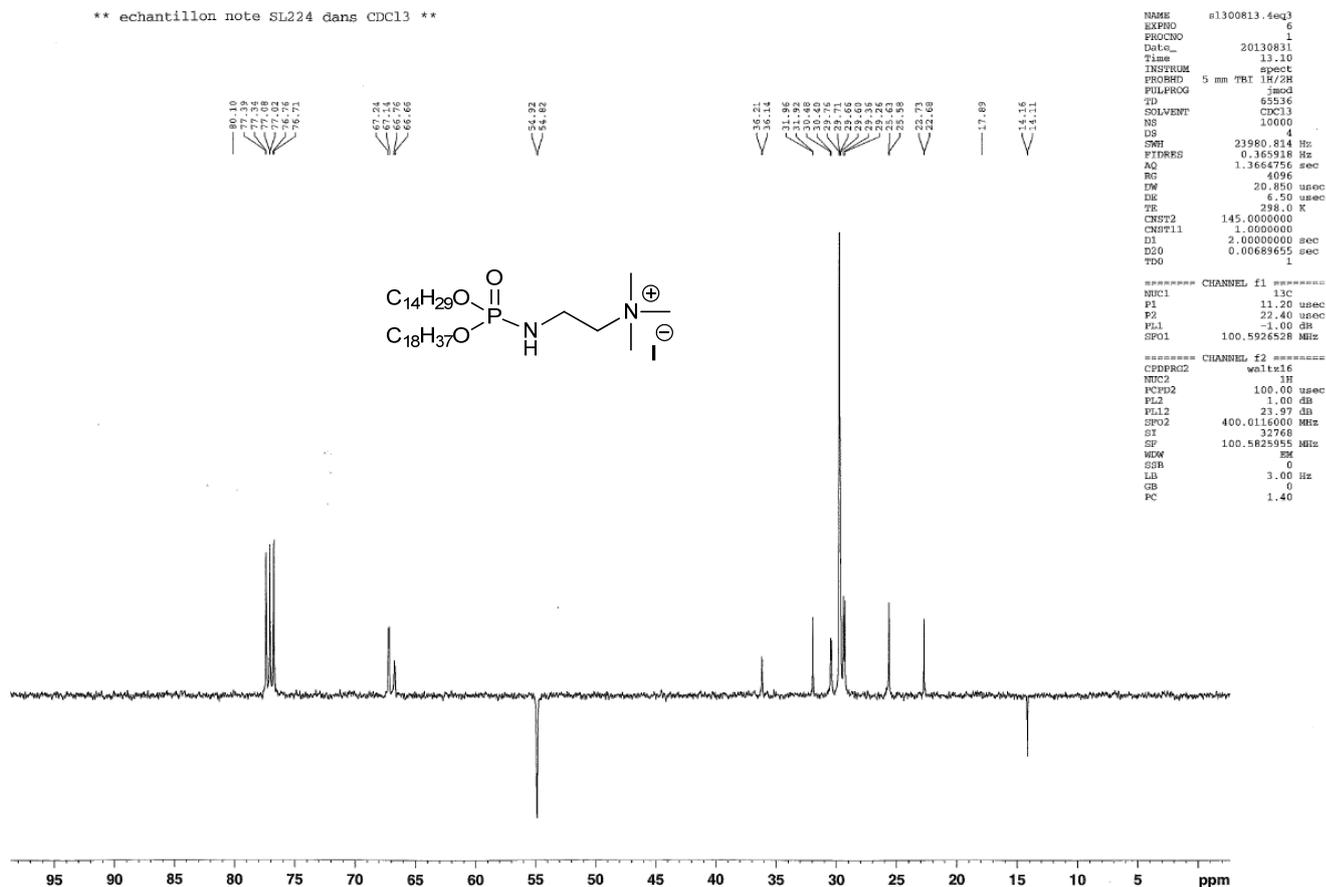


Figure S3-30: <sup>13</sup>C jmod (CDCl<sub>3</sub>) spectrum of compound 12.



Supporting materials

\*\* echantillon note SL205 dans CDCl3 \*\*

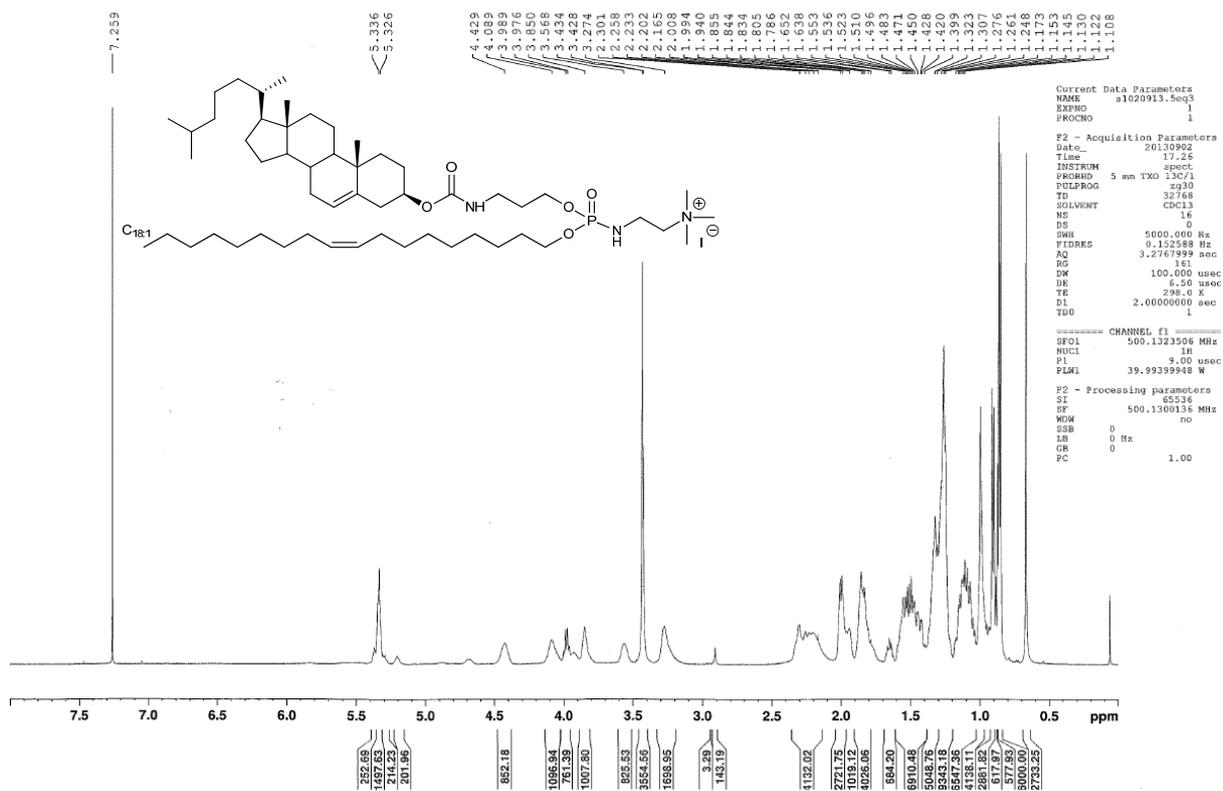


Figure S3-33: <sup>1</sup>H NMR (CDCl<sub>3</sub>) spectrum of compound 14.

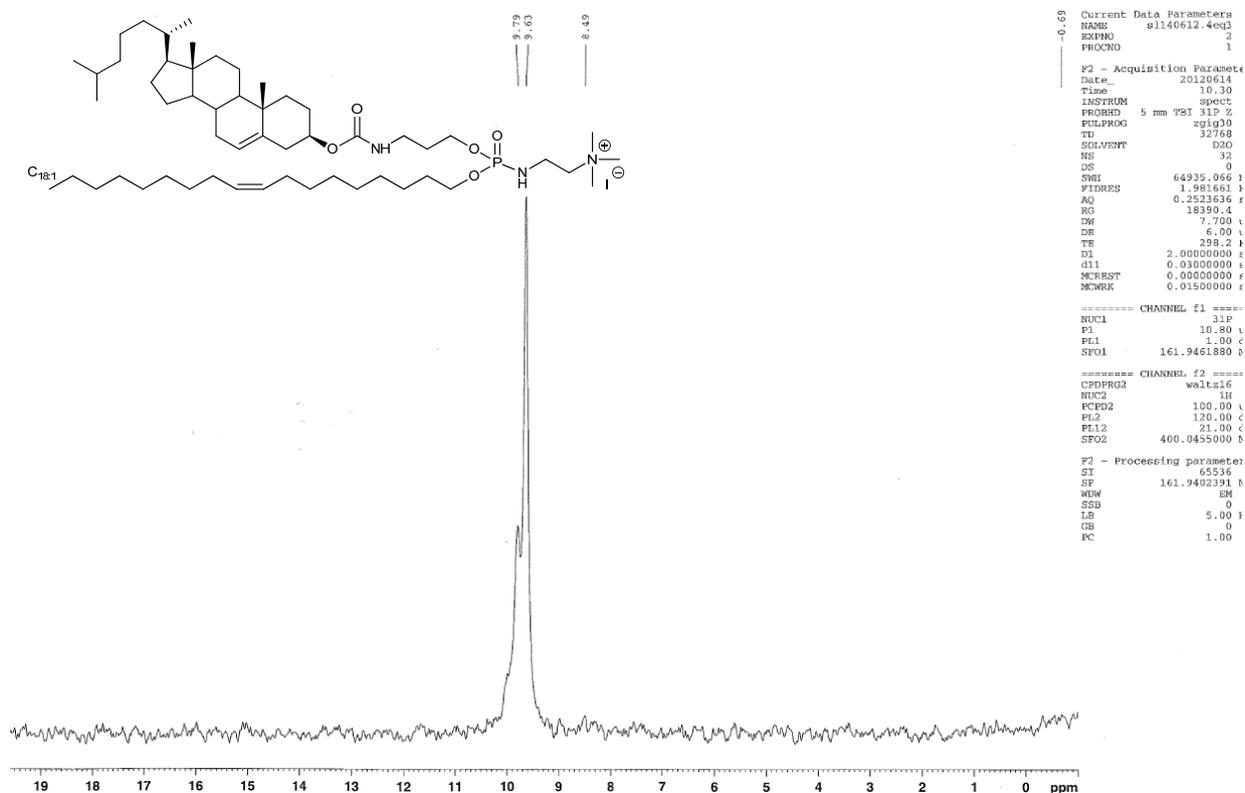


Figure S3-34: <sup>31</sup>P NMR (CDCl<sub>3</sub>) spectrum of compound 14.

Supporting materials

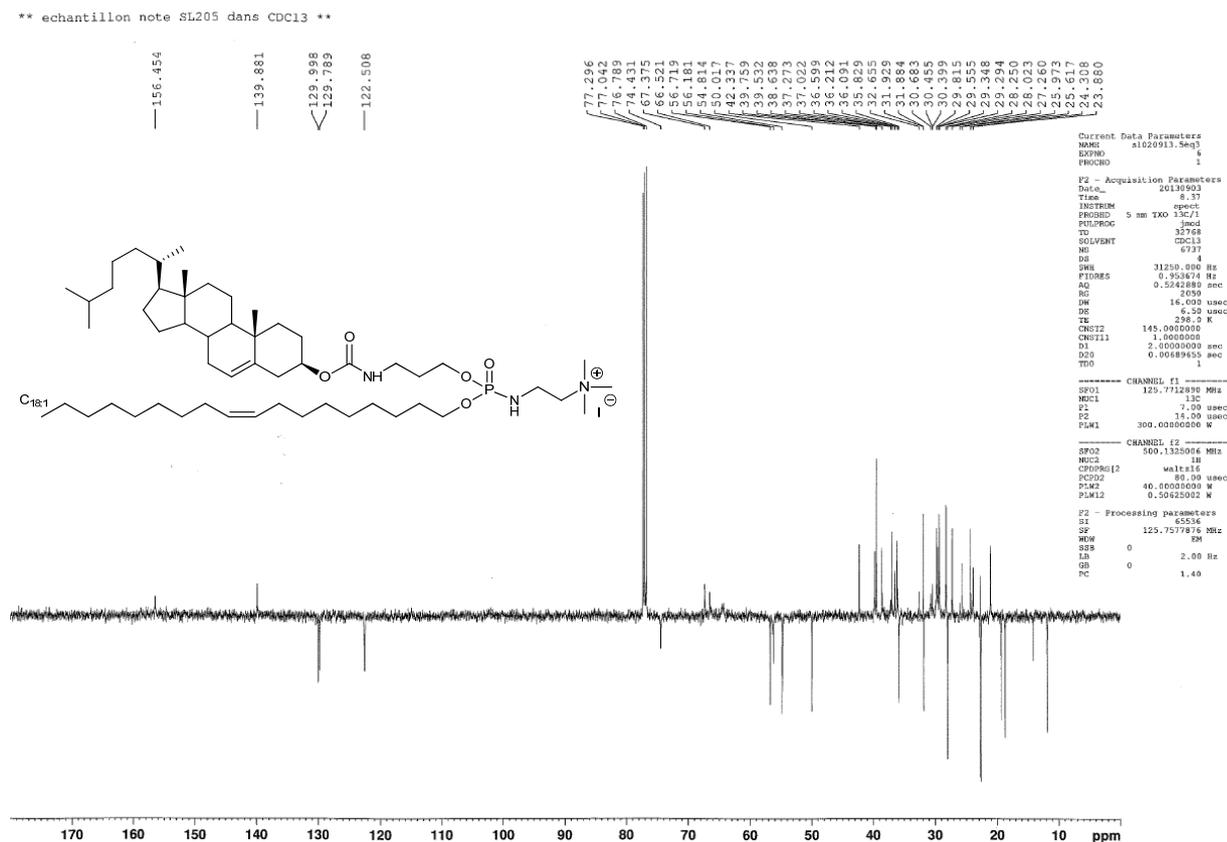


Figure S3-35: <sup>13</sup>C jmod (CDCl<sub>3</sub>) spectrum of compound 14.

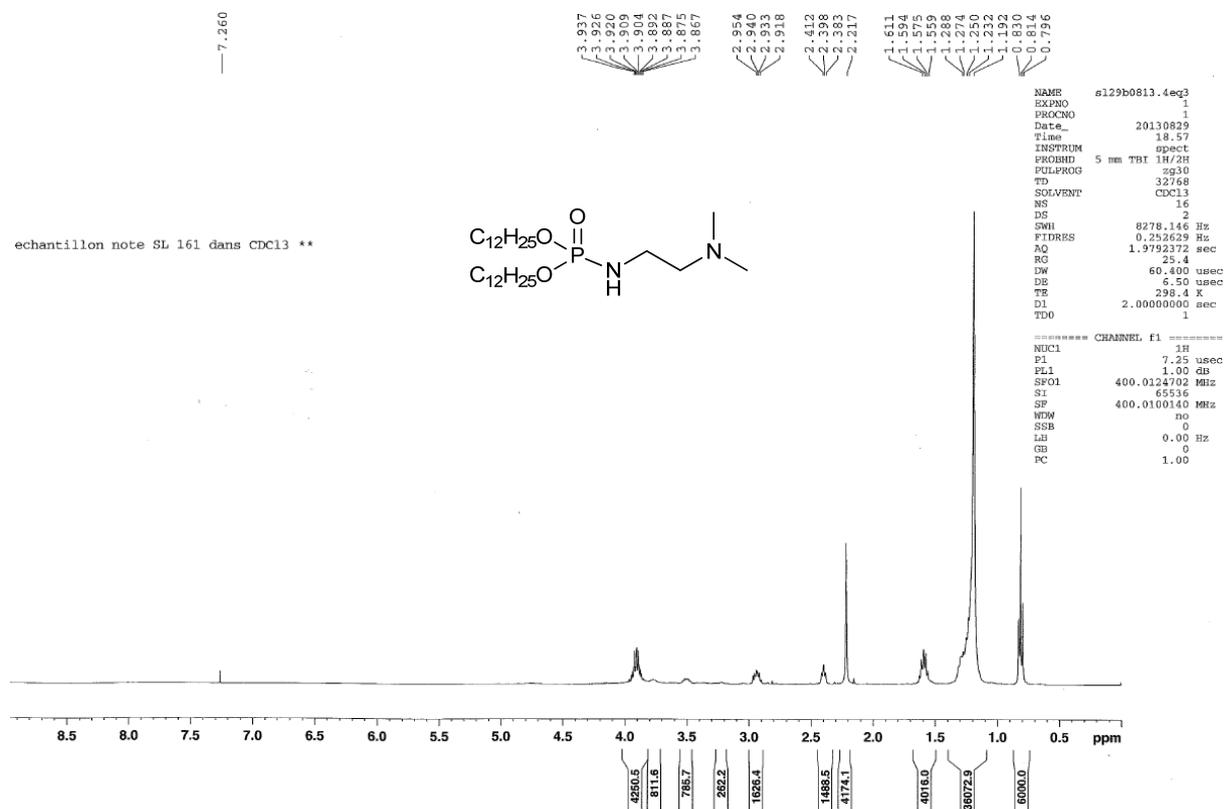


Figure S3-36: <sup>1</sup>H NMR (CDCl<sub>3</sub>) spectrum of compound 15.

### Supporting materials

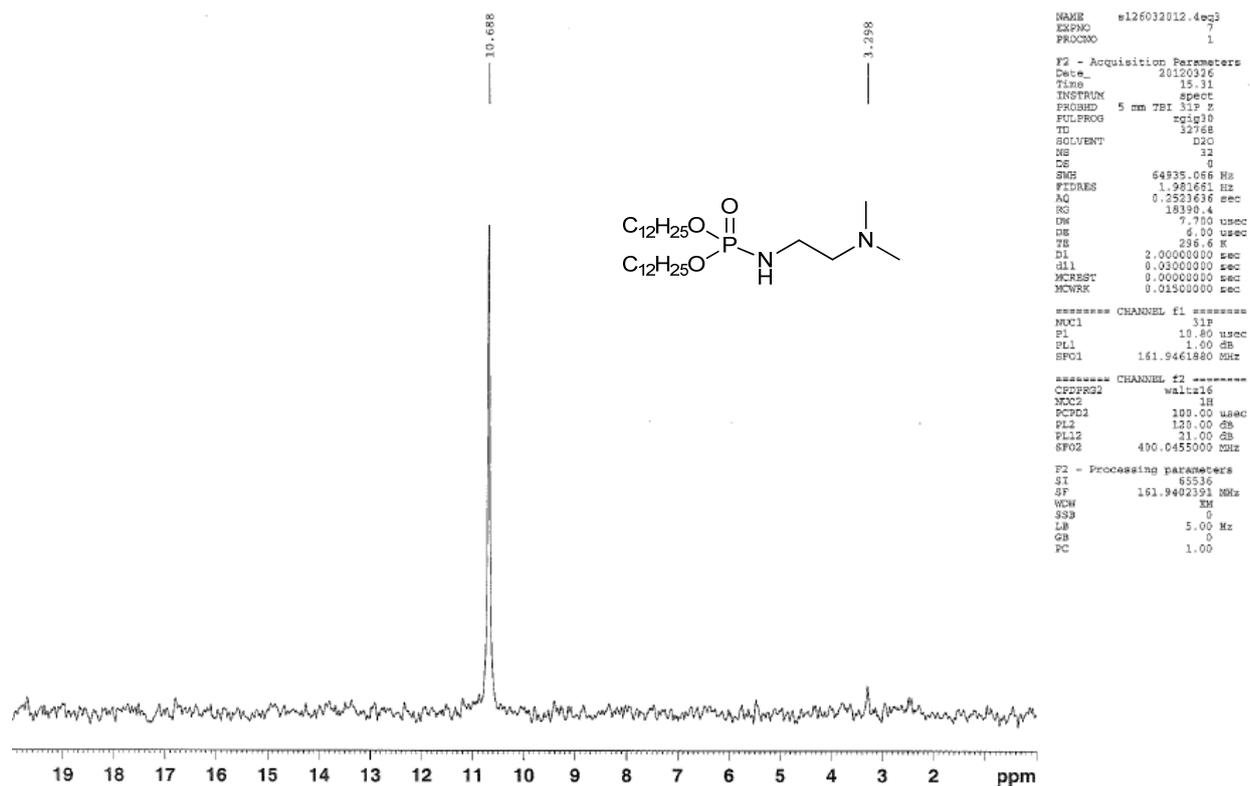


Figure S3-37: <sup>31</sup>P NMR (CDCl<sub>3</sub>) spectrum of compound 15.

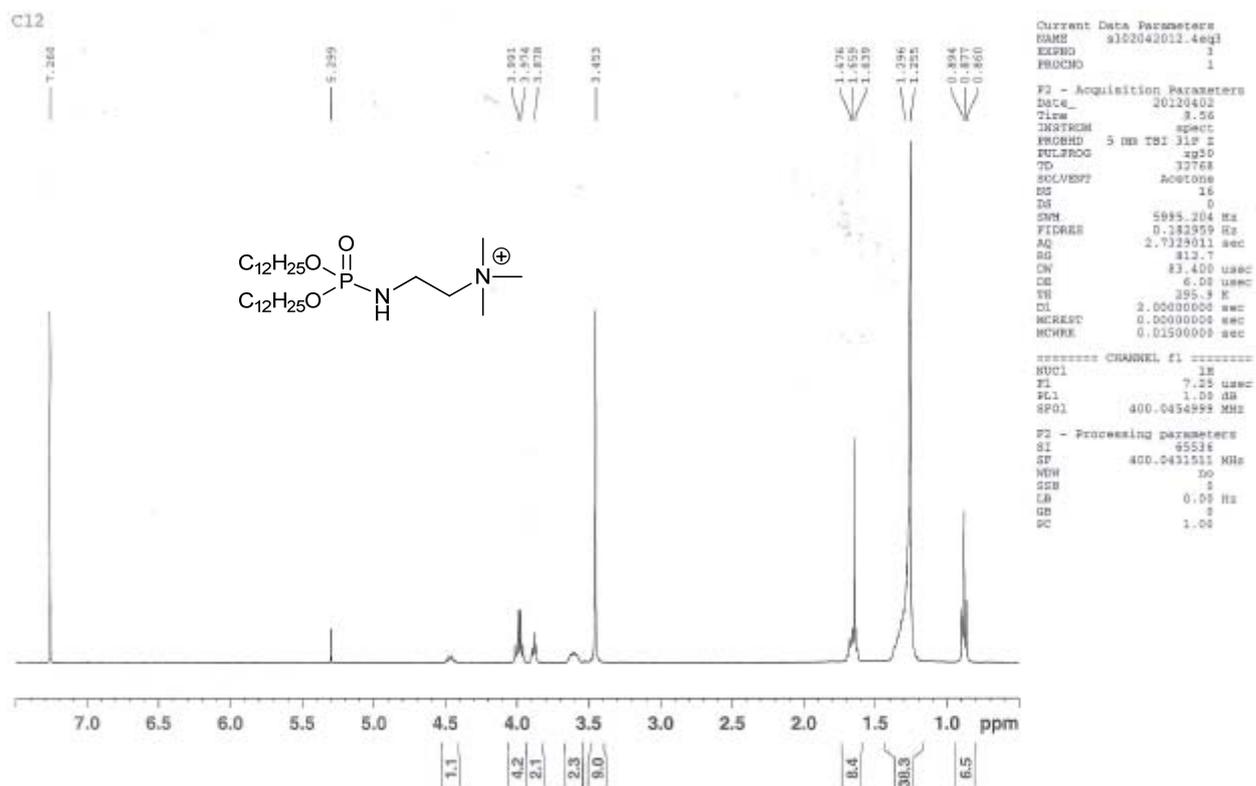


Figure S3-38: <sup>1</sup>H NMR (CDCl<sub>3</sub>) spectrum of compound 16.

Supporting materials

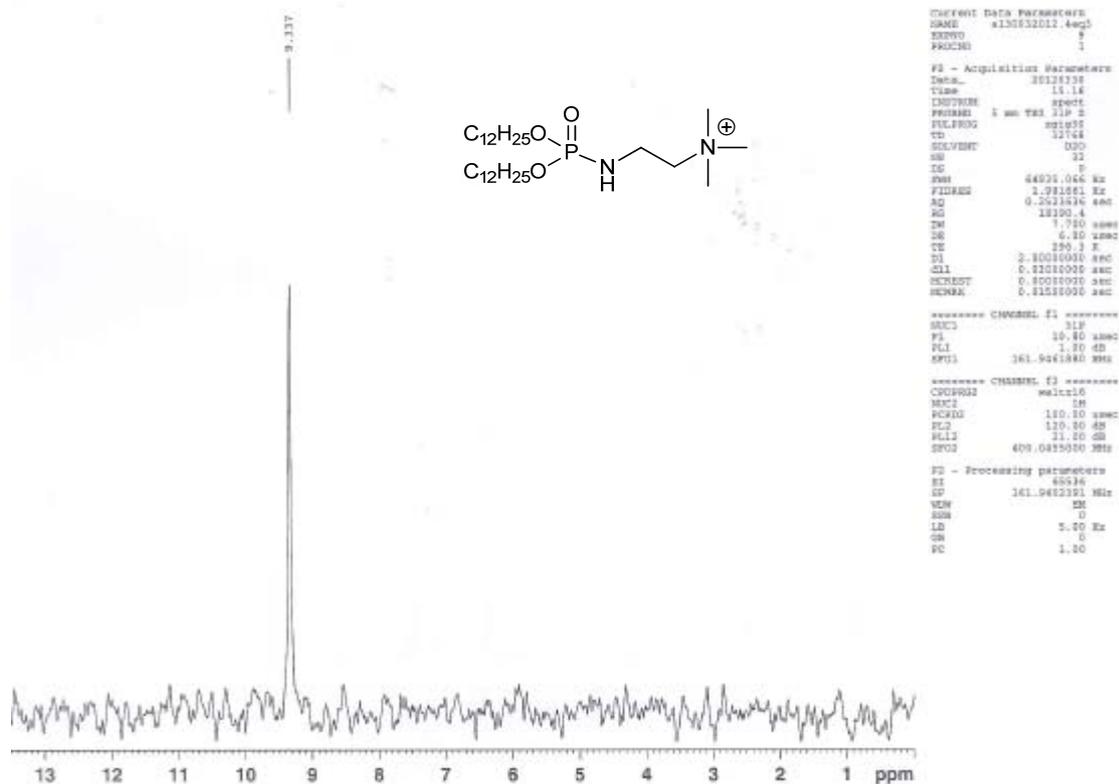


Figure S3-39: <sup>31</sup>P NMR (CDCl<sub>3</sub>) spectrum of compound 16.

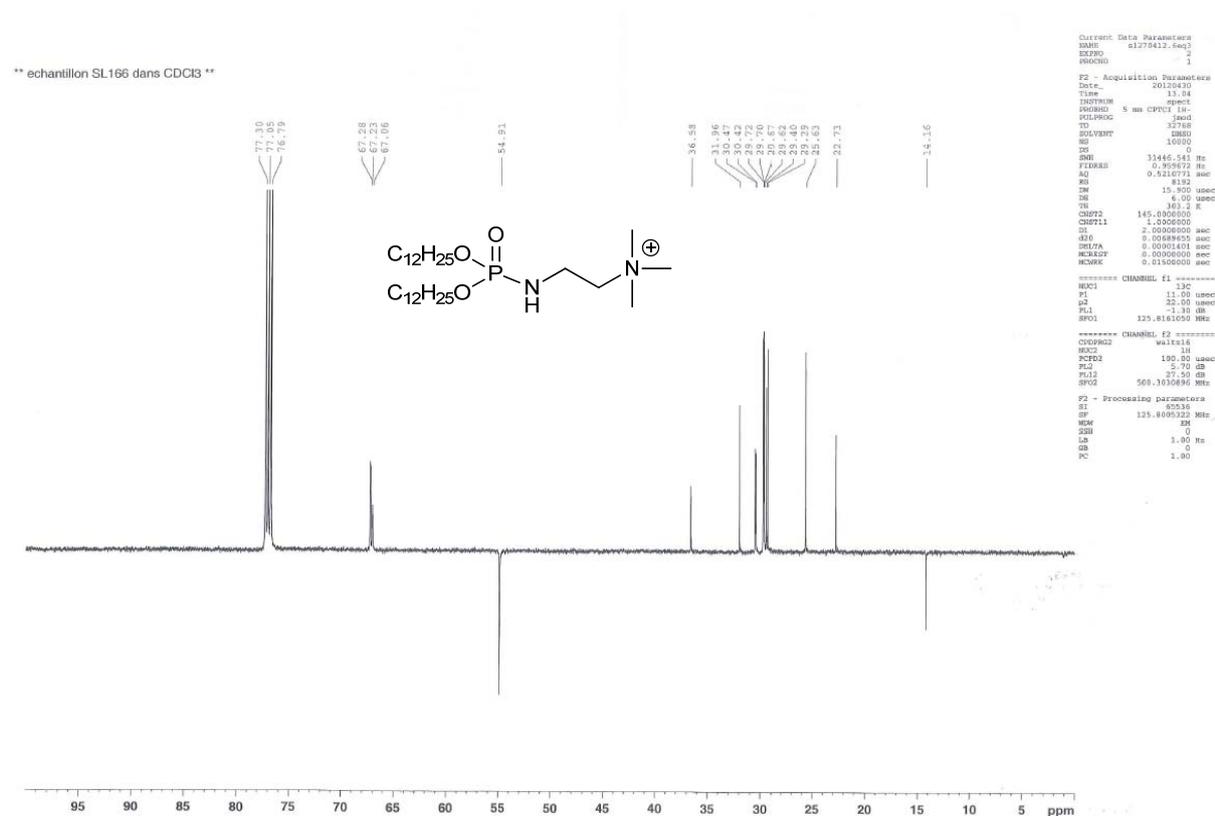


Figure S3-40: <sup>13</sup>C jmod (CDCl<sub>3</sub>) spectrum of compound 16.

Supporting materials

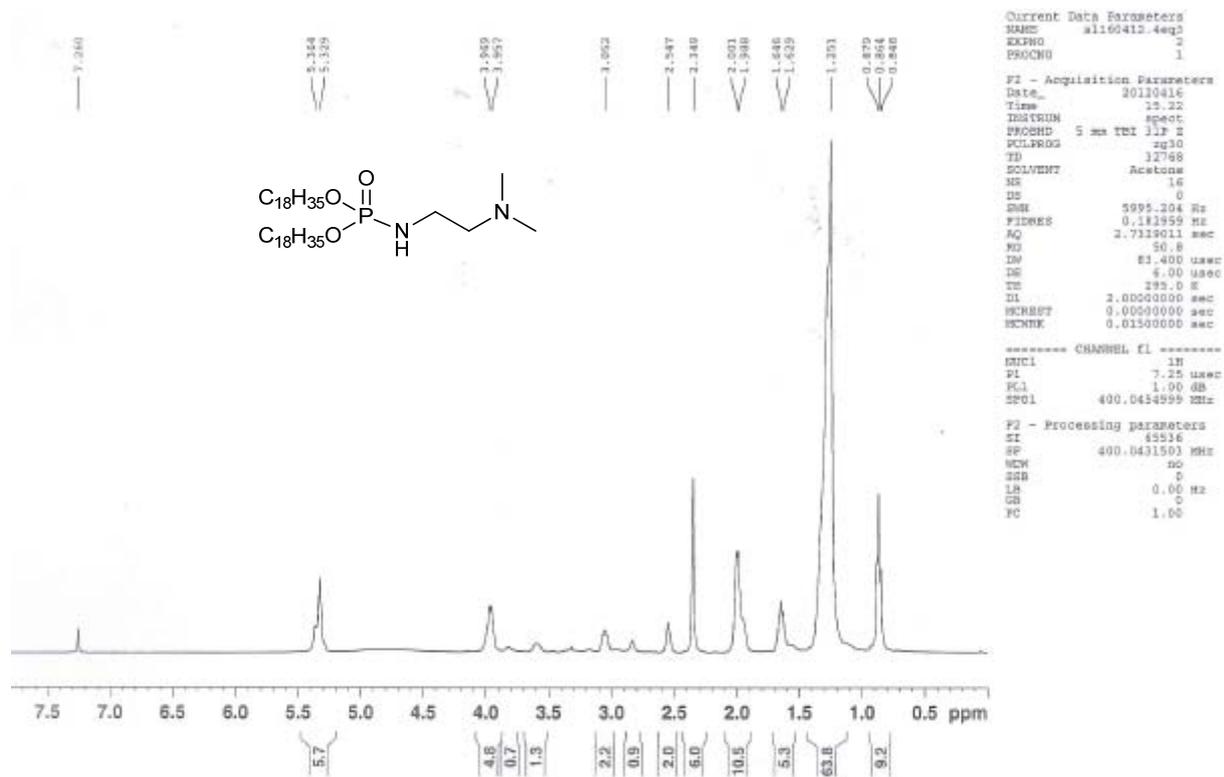


Figure S3-41: <sup>1</sup>H NMR (CDCl<sub>3</sub>) spectrum of compound 17.

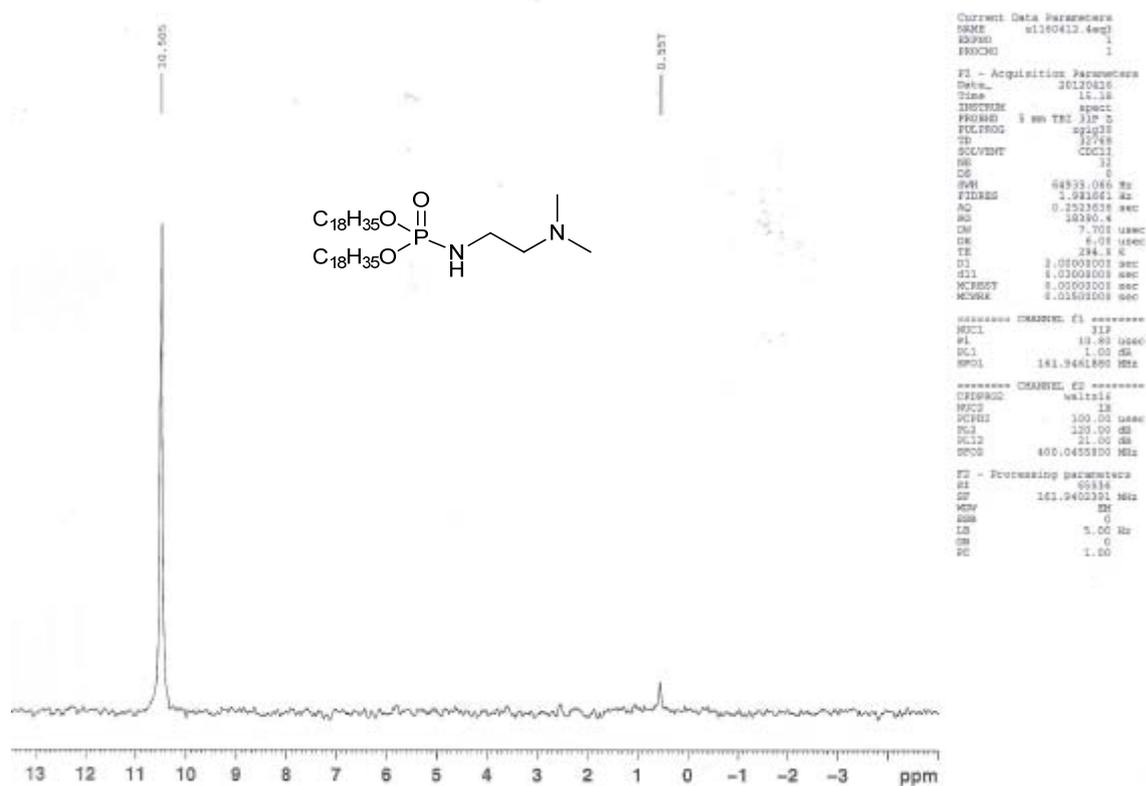


Figure S3-42: <sup>31</sup>P NMR (CDCl<sub>3</sub>) spectrum of compound 17.

Supporting materials

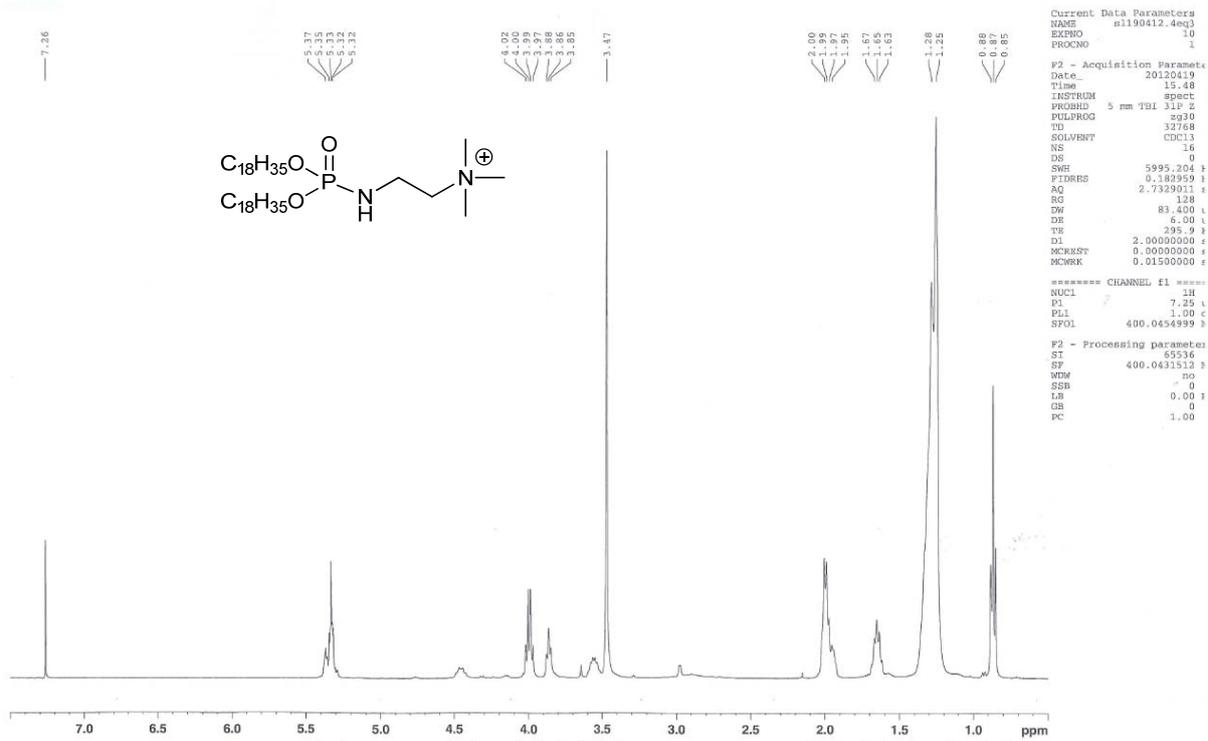


Figure S3-43: <sup>1</sup>H NMR (CDCl<sub>3</sub>) spectrum of compound 18.

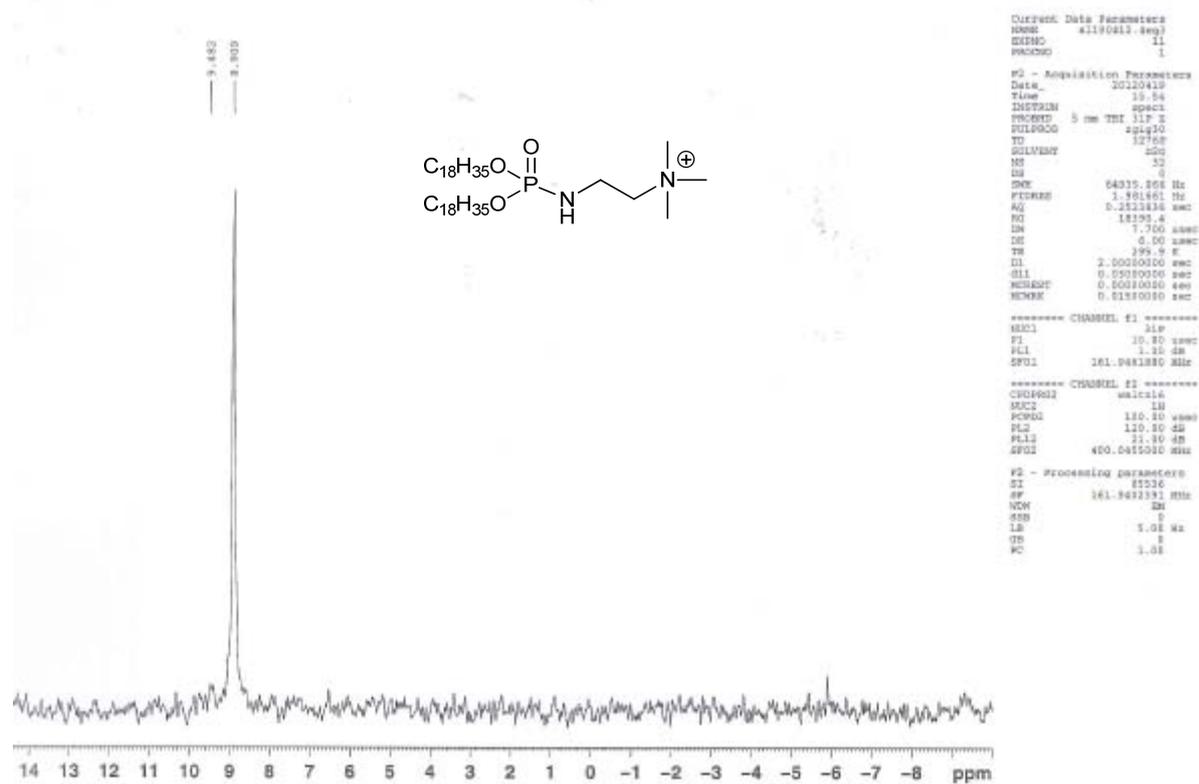


Figure S3-44: <sup>31</sup>P NMR (CDCl<sub>3</sub>) spectrum of compound 18.

Supporting materials

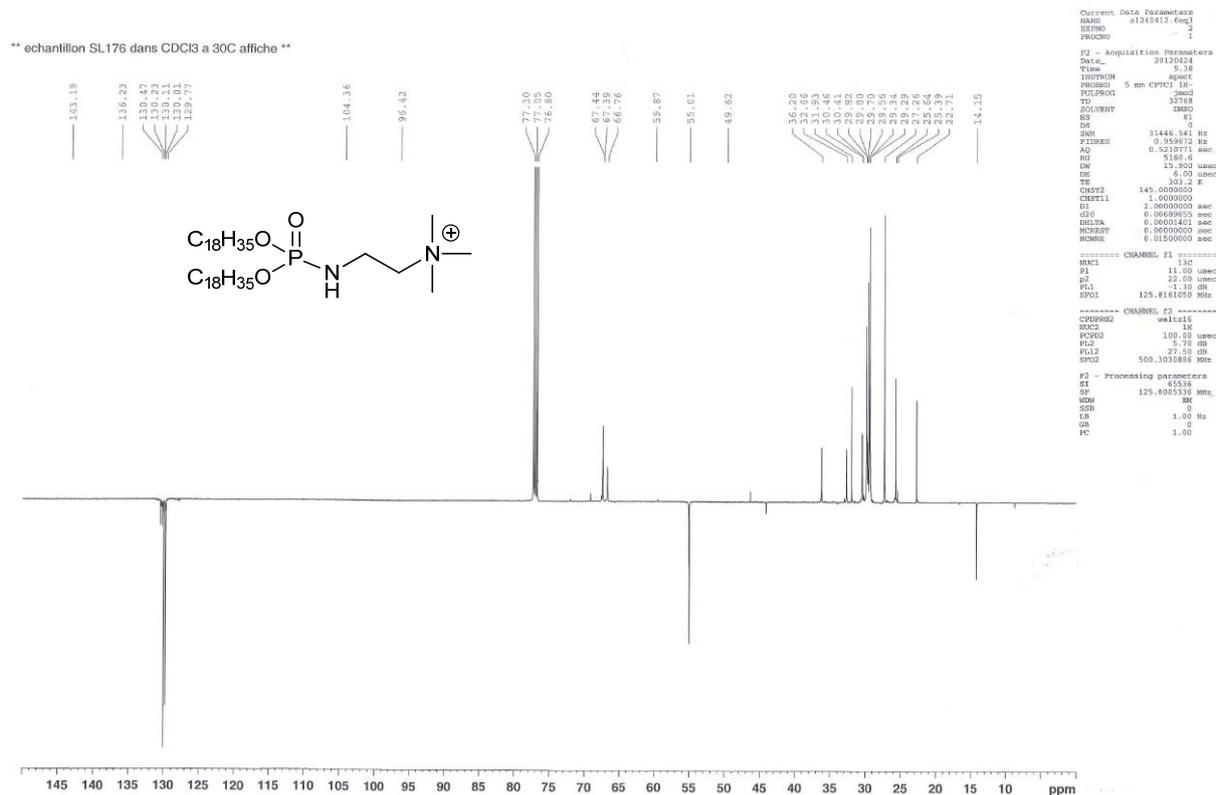


Figure S3-45: <sup>13</sup>C jmod (CDCl<sub>3</sub>) spectrum of compound 18.

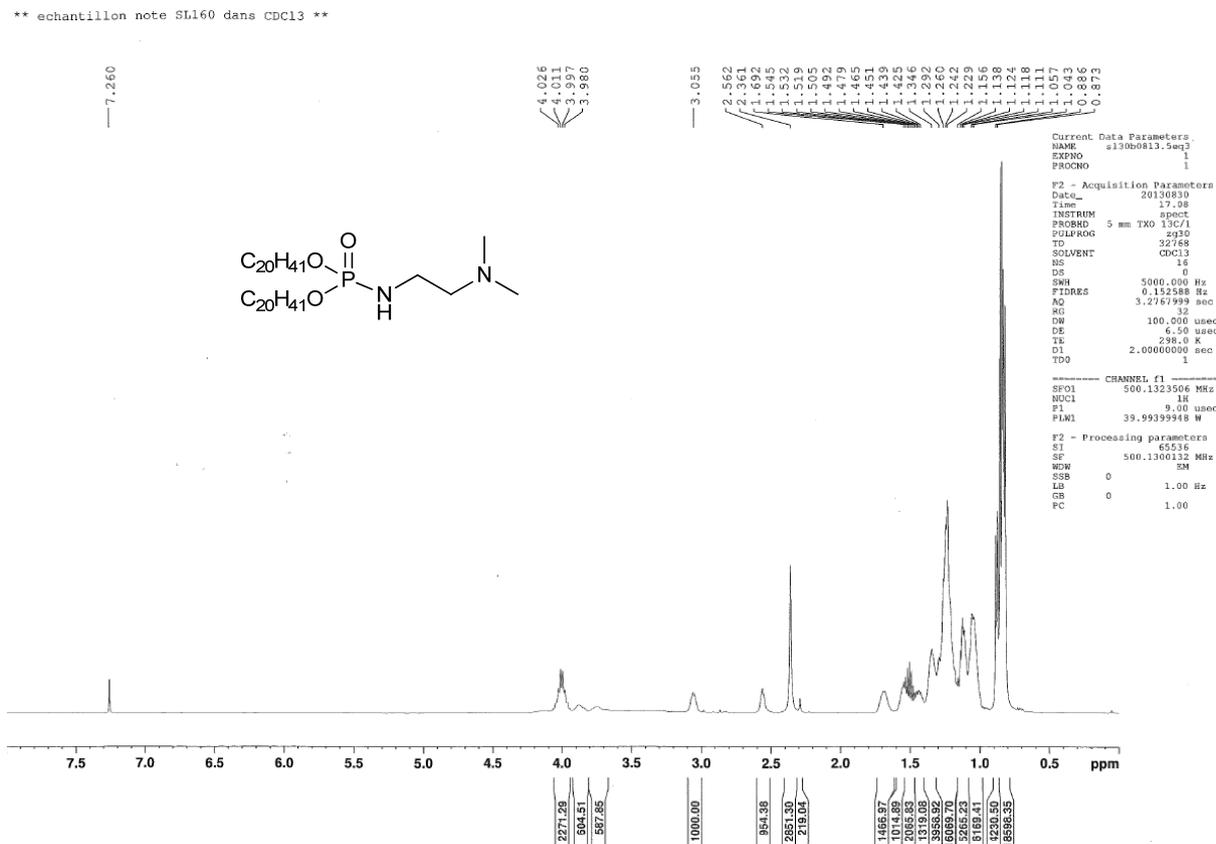


Figure S3-46: <sup>1</sup>H NMR (CDCl<sub>3</sub>) spectrum of compound 19.

Supporting materials

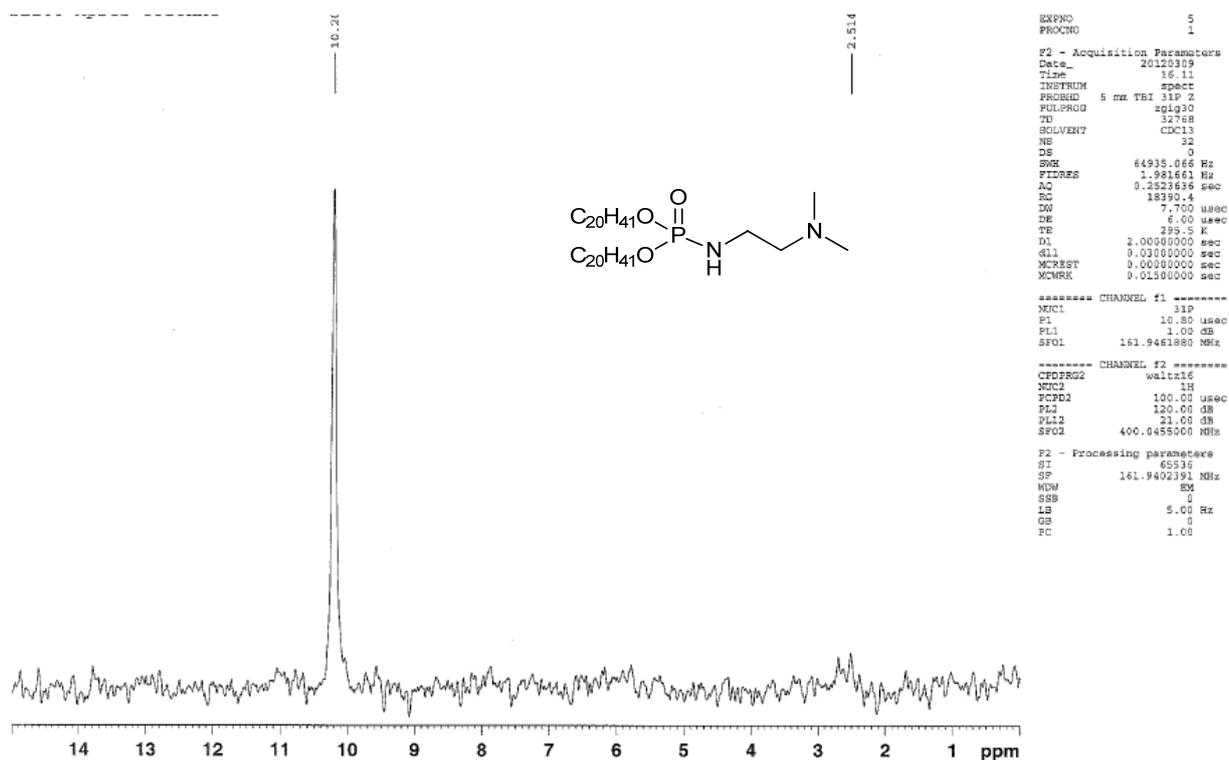


Figure S3-47: <sup>31</sup>P NMR (CDCl<sub>3</sub>) spectrum of compound 19.

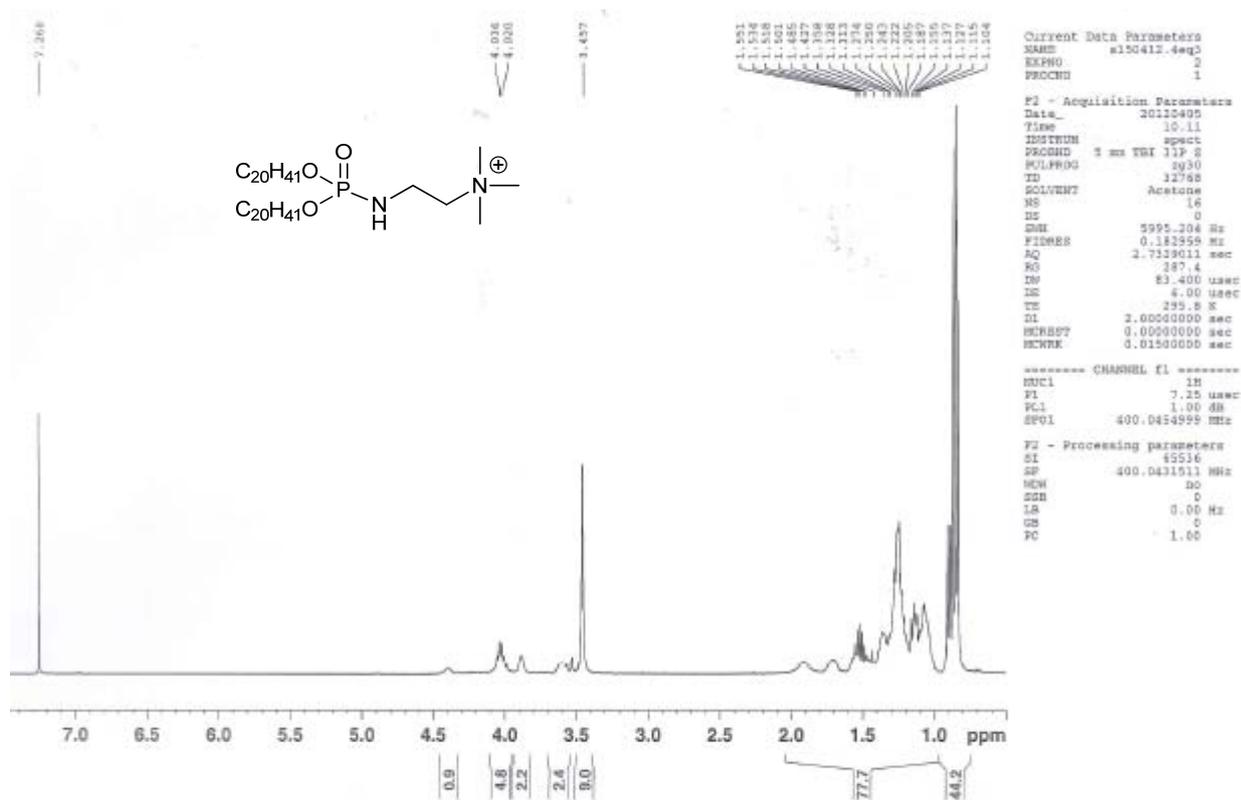


Figure S3-48: <sup>1</sup>H NMR (CDCl<sub>3</sub>) spectrum of compound 20.

Supporting materials

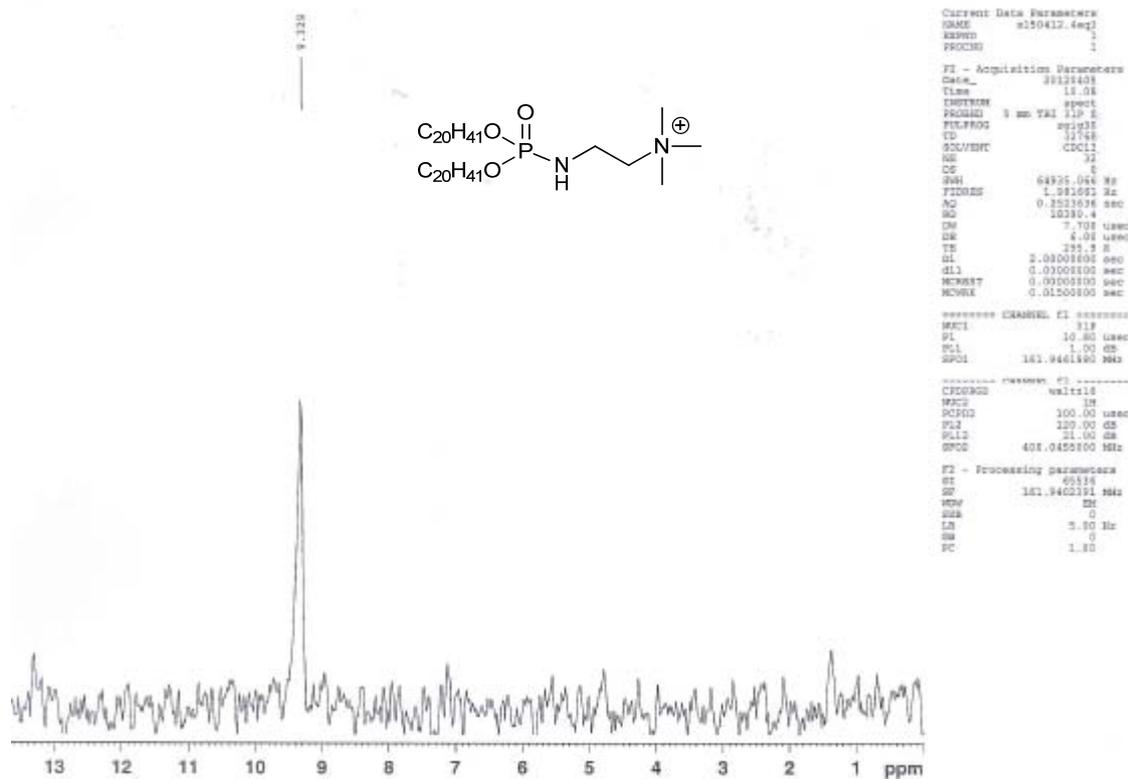


Figure S3-49: <sup>31</sup>P NMR (CDCl<sub>3</sub>) spectrum of compound 20.

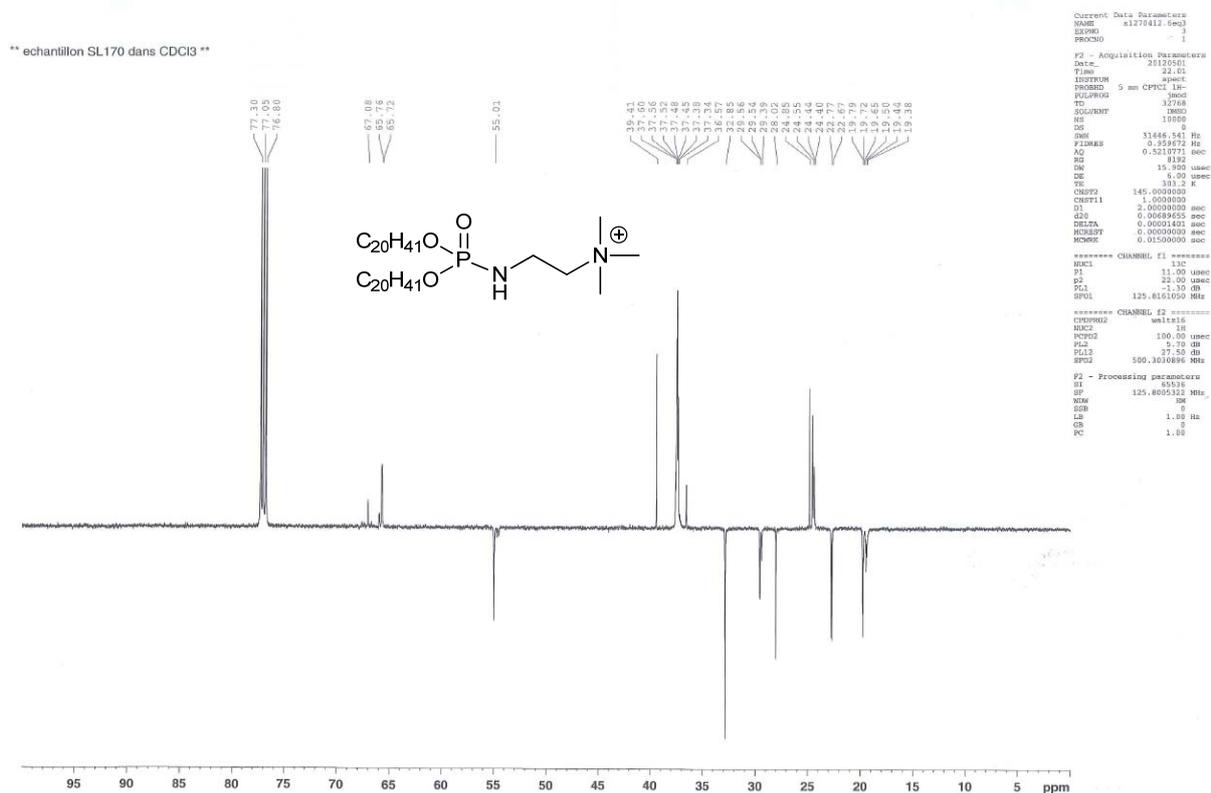


Figure S3-50: <sup>13</sup>C jmod (CDCl<sub>3</sub>) spectrum of compound 20.

Supporting materials

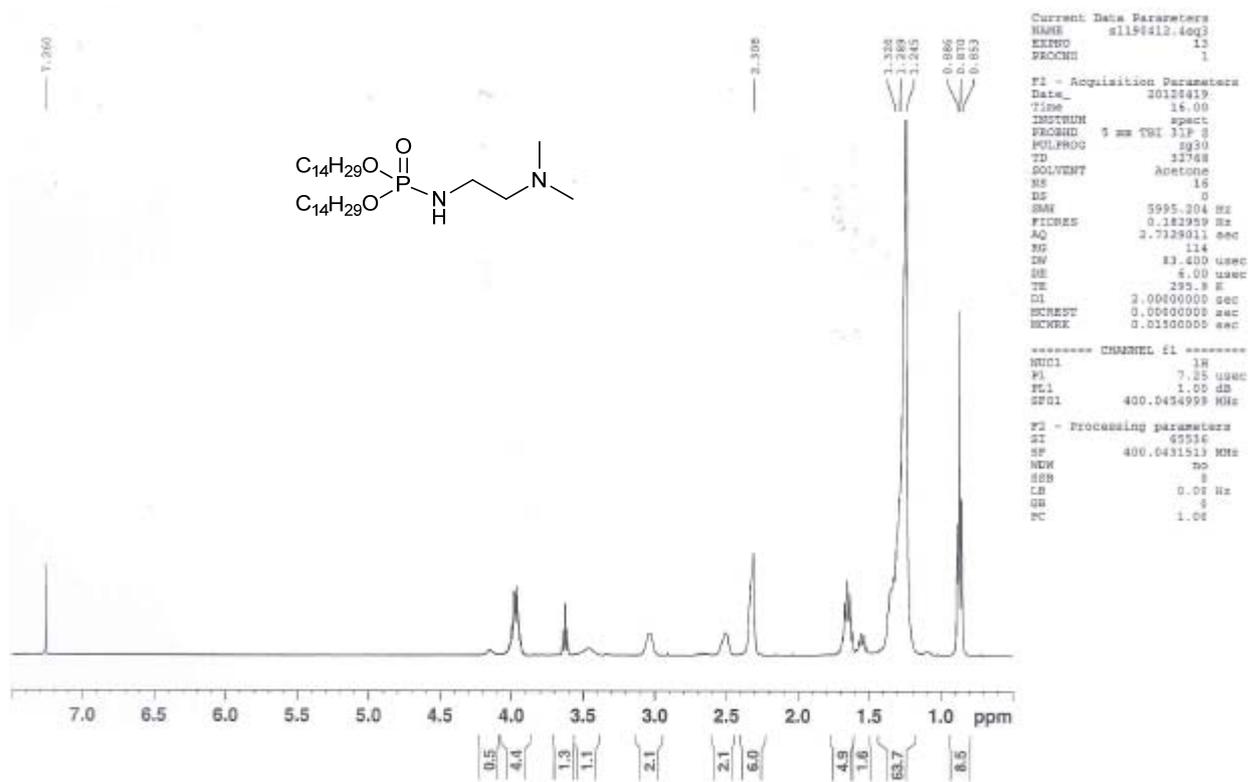


Figure S3-51: <sup>1</sup>H NMR (CDCl<sub>3</sub>) spectrum of compound 21.

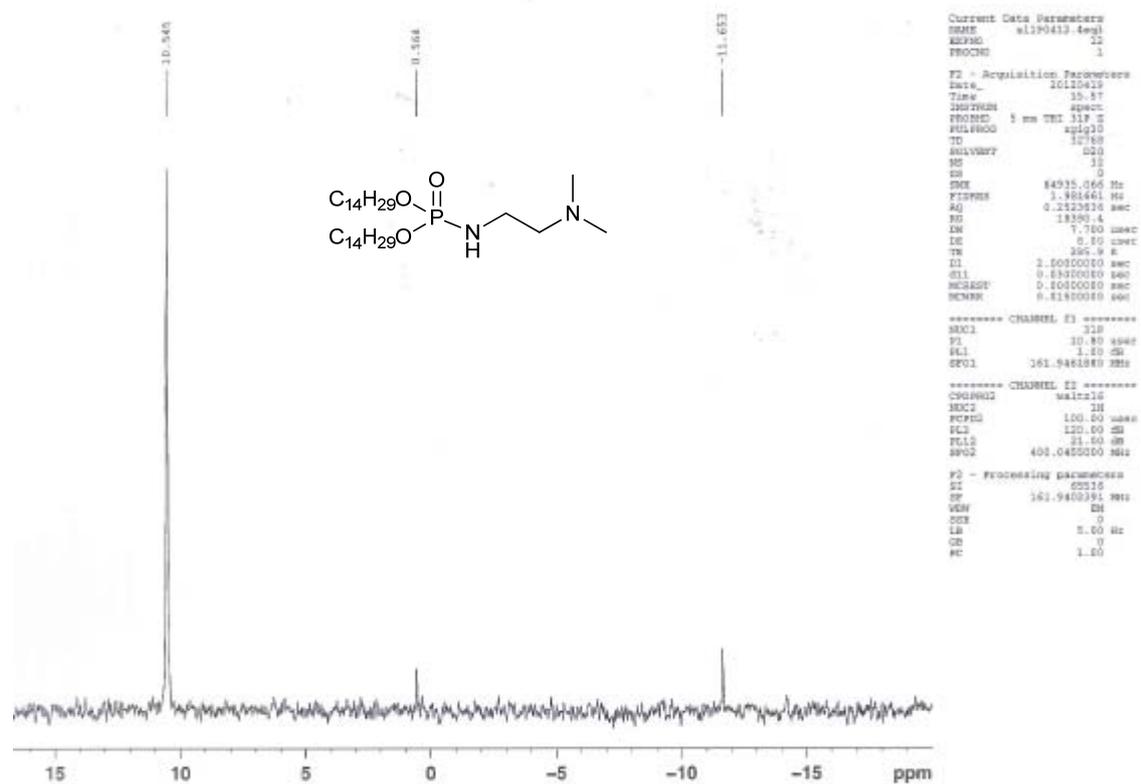


Figure S3-52: <sup>31</sup>P NMR (CDCl<sub>3</sub>) spectrum of compound 21.

Supporting materials

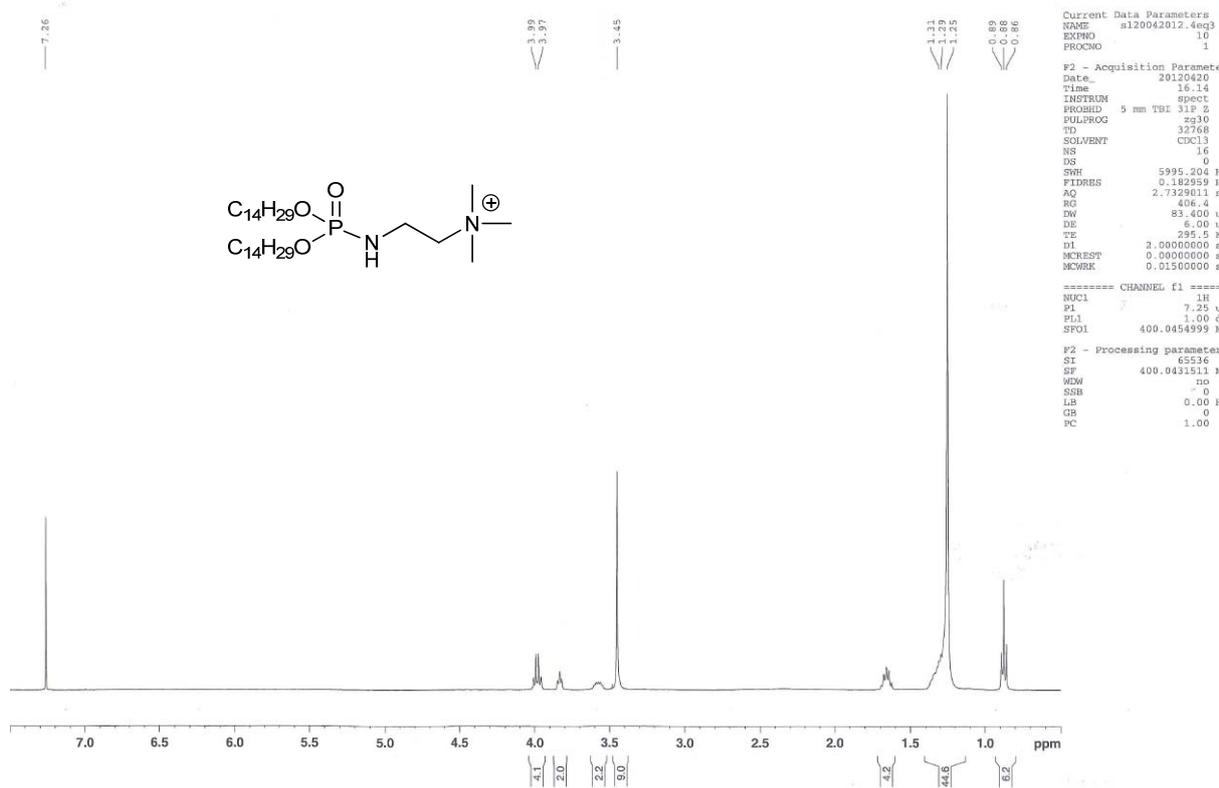


Figure S3-53: <sup>1</sup>H NMR (CDCl<sub>3</sub>) spectrum of compound 22.

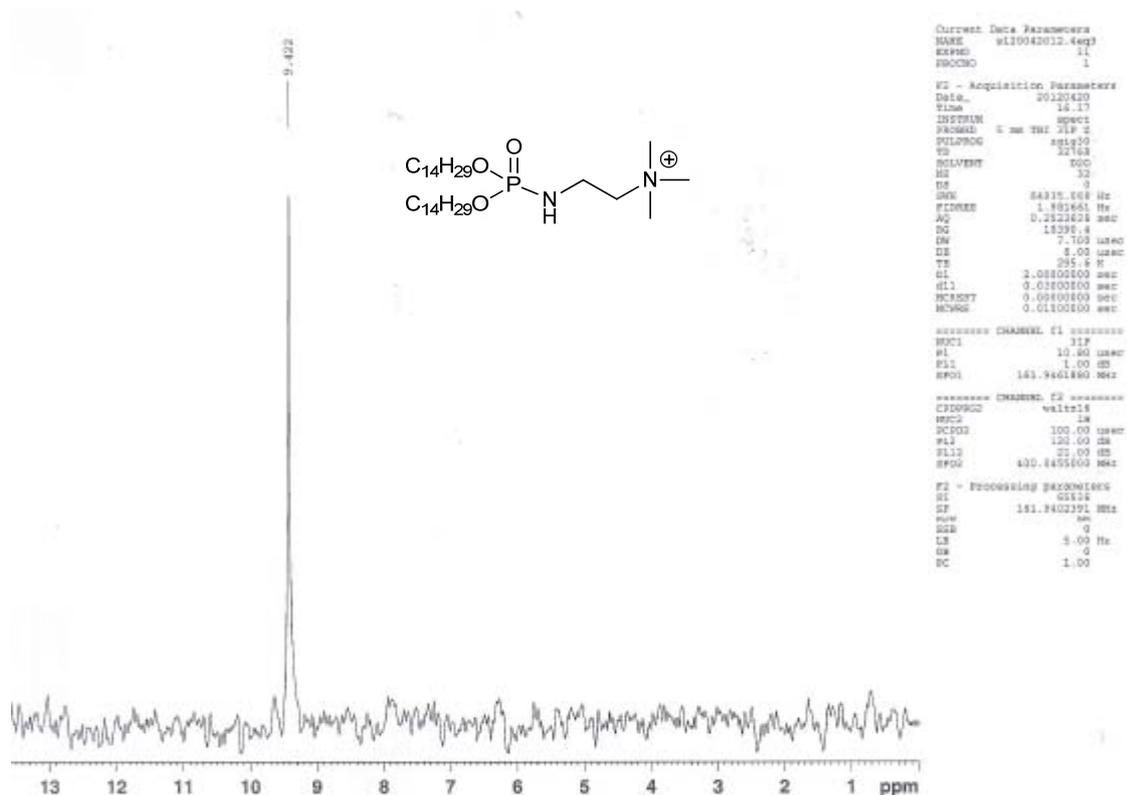


Figure S3-54: <sup>31</sup>P NMR (CDCl<sub>3</sub>) spectrum of compound 22.

Supporting materials

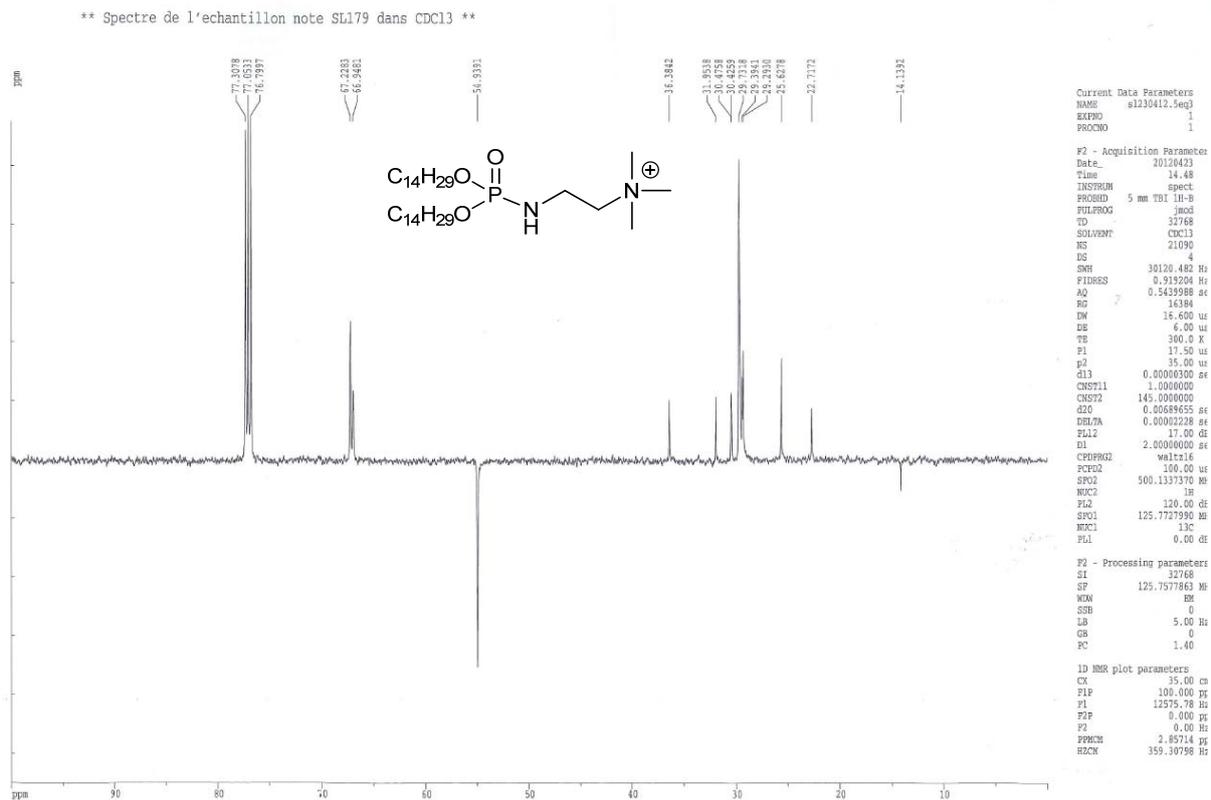


Figure S3-55: <sup>13</sup>C jmod (CDCl<sub>3</sub>) spectrum of compound 22.

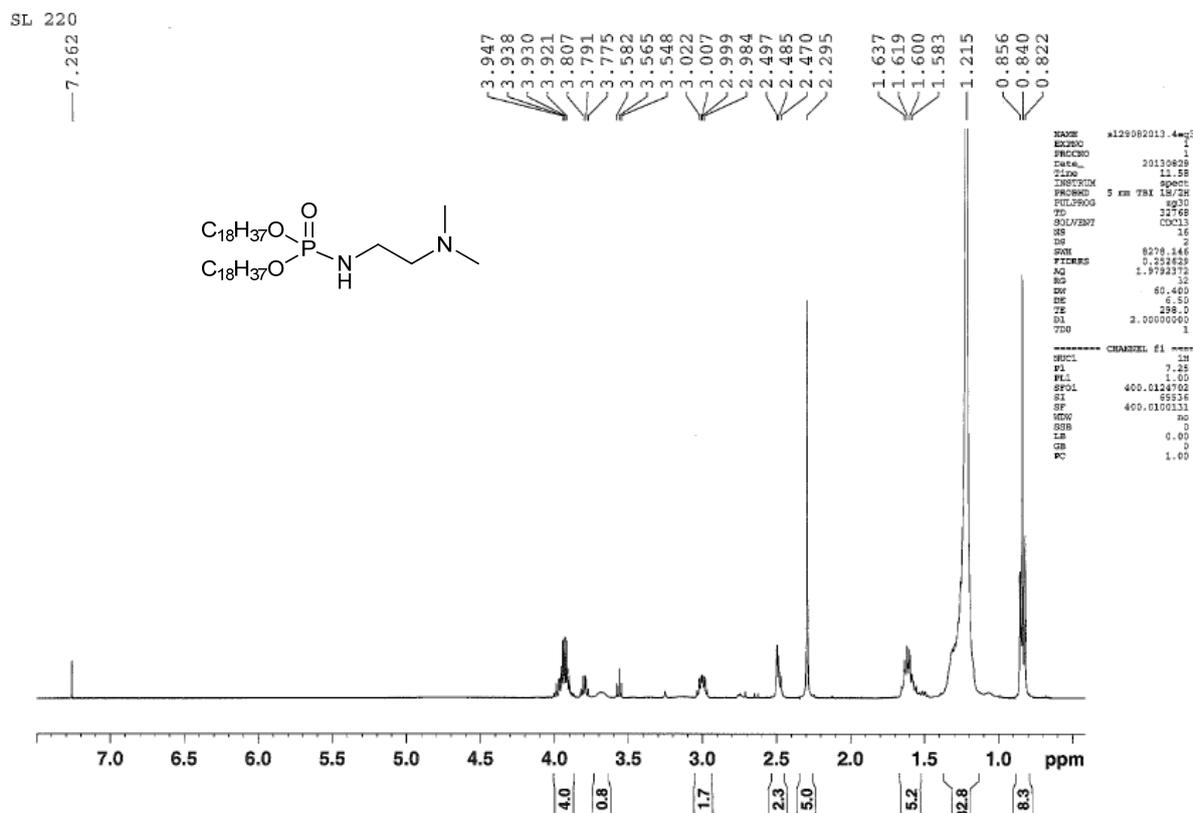


Figure S3-56: <sup>1</sup>H NMR (CDCl<sub>3</sub>) spectrum of compound 23.

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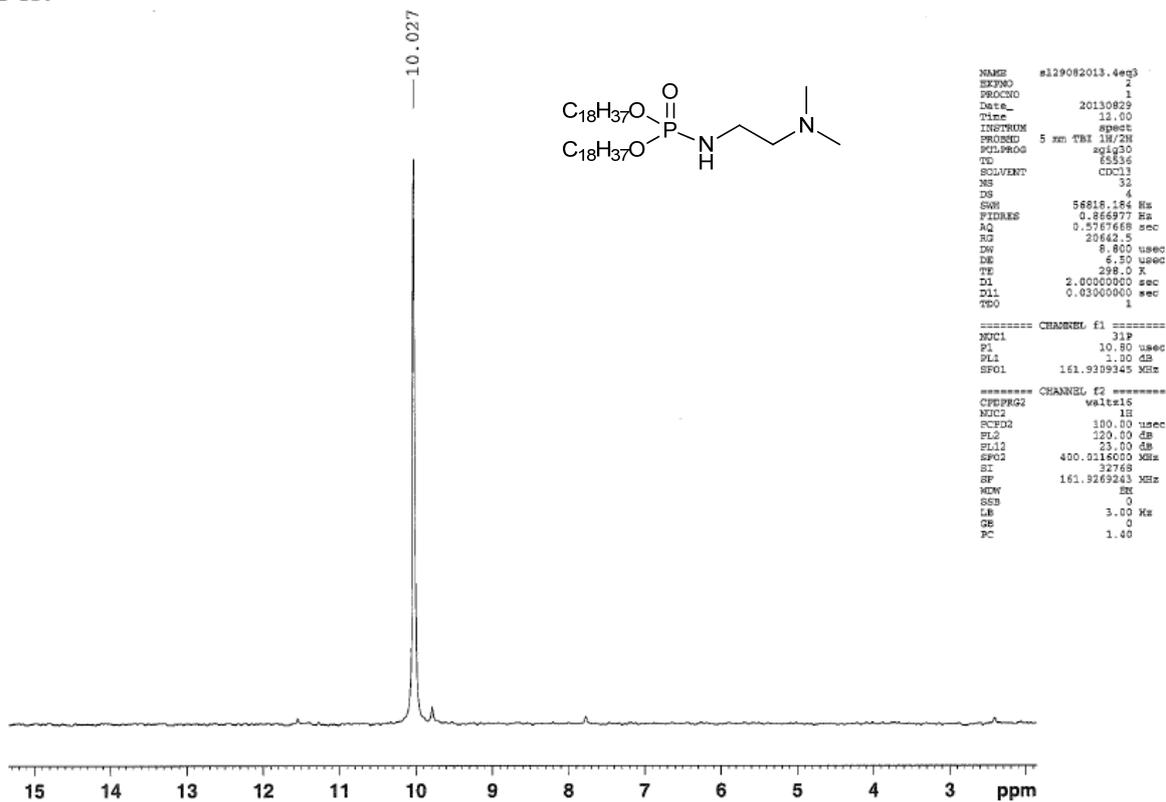


Figure S3-57: <sup>31</sup>P NMR (CDCl<sub>3</sub>) spectrum of compound 23.

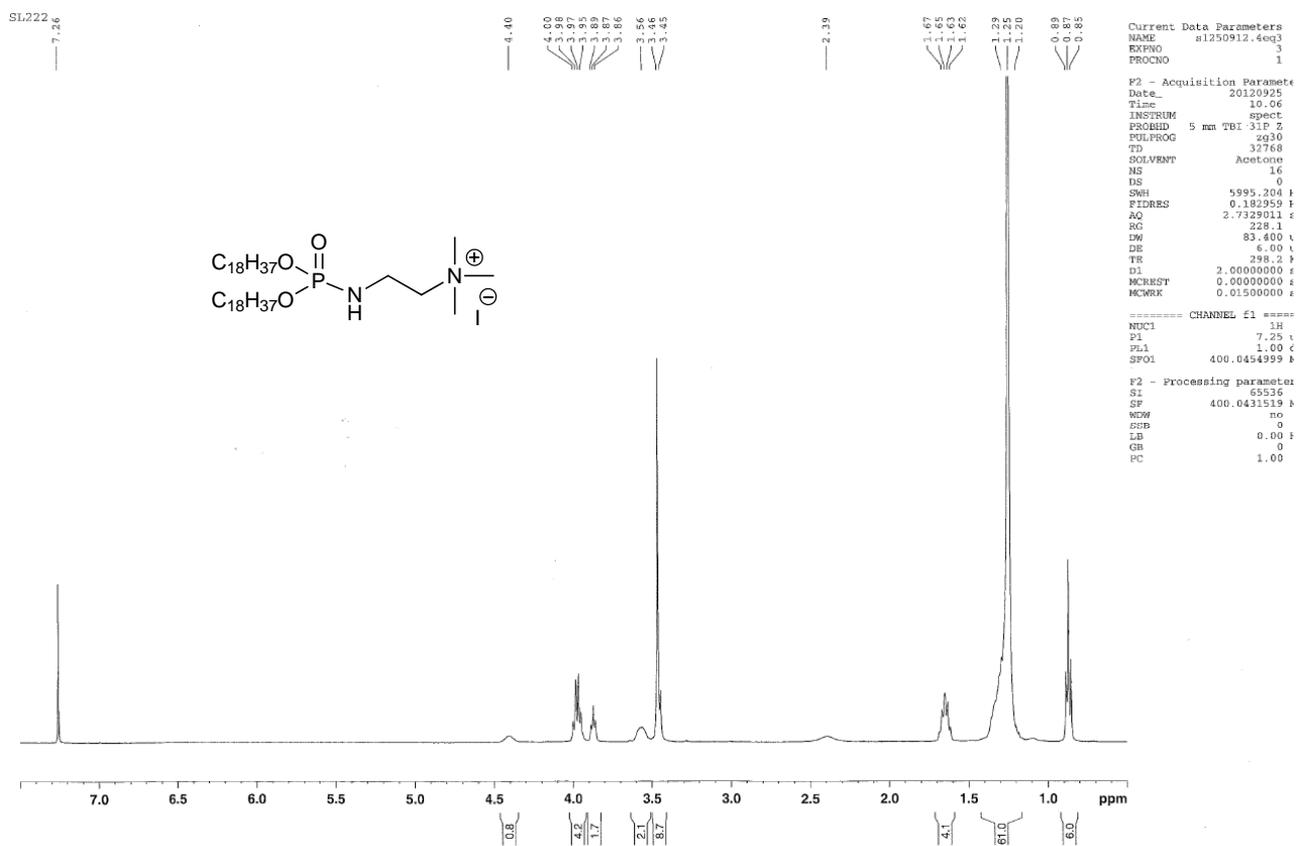
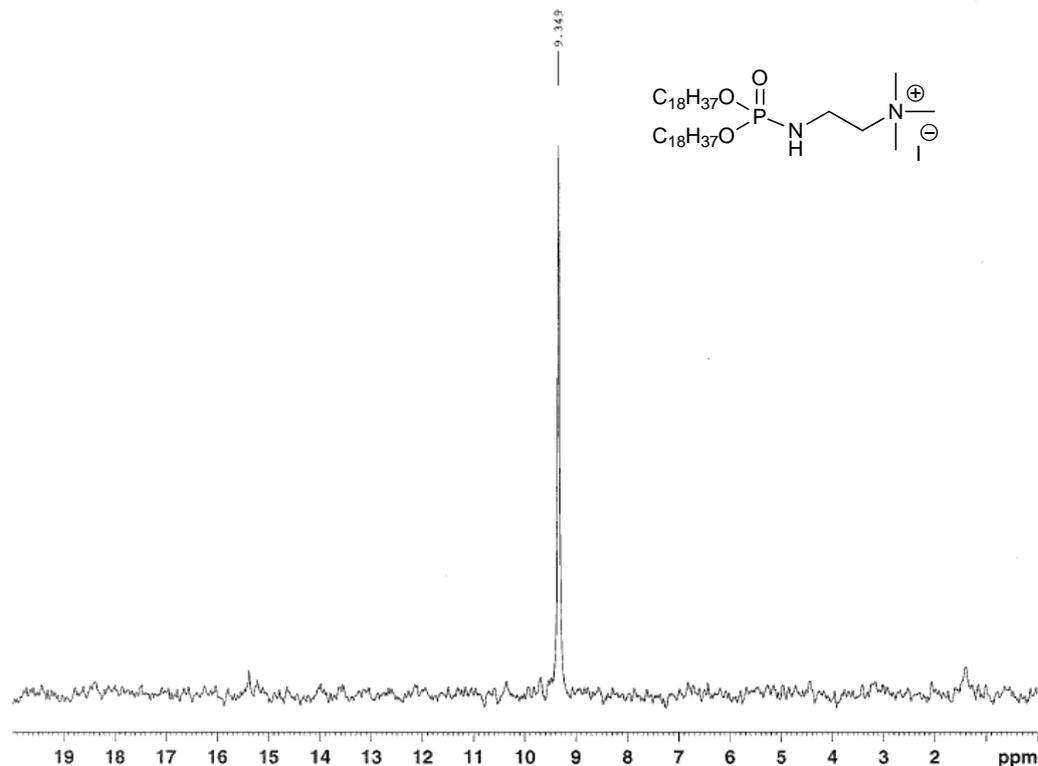


Figure S3-58: <sup>1</sup>H NMR (CDCl<sub>3</sub>) spectrum of compound 24.

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SL222



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PROCNO   1

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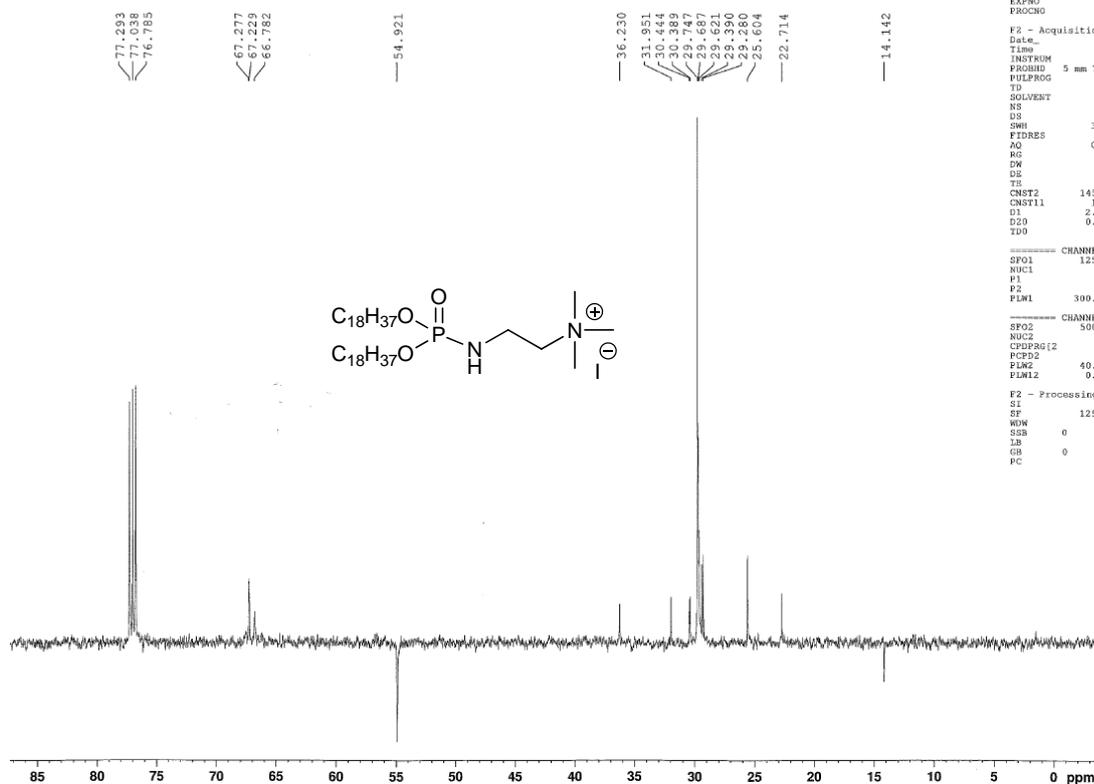
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Figure S3-59: <sup>31</sup>P NMR (CDCl<sub>3</sub>) spectrum of compound 24.

\*\* echantillon note SL222 dans CDCl3 \*\*



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D11       2.00000000 sec
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P2        14.00 usec
PLM1     300.00000000 W

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PCPD2    80.00 usec
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PLM12    0.50825002 W

F2 - Processing parameters
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Figure S3-60: <sup>13</sup>C jmod (CDCl<sub>3</sub>) spectrum of compound 24.

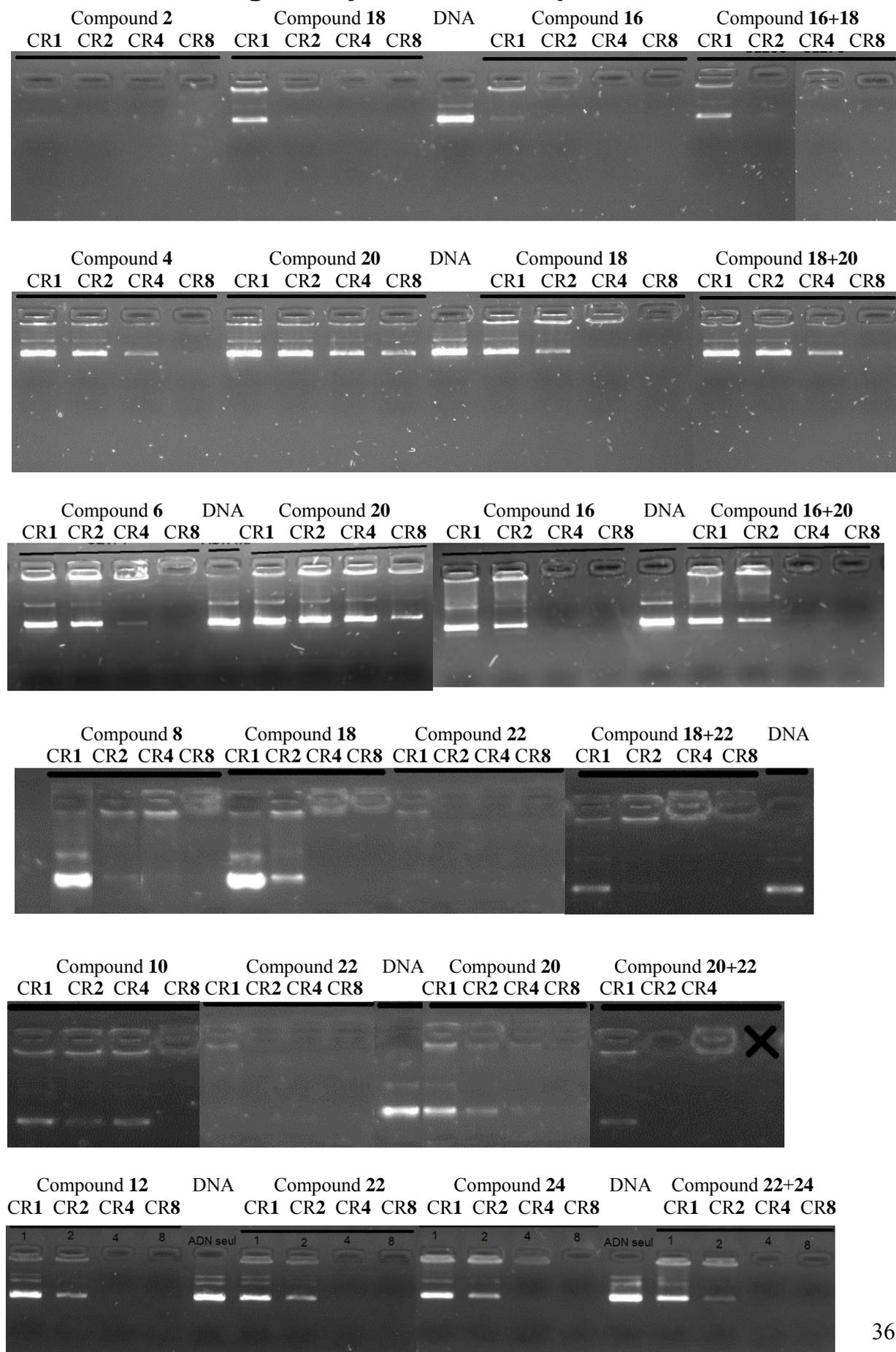
Supporting materials

**S4 Size, Zeta**

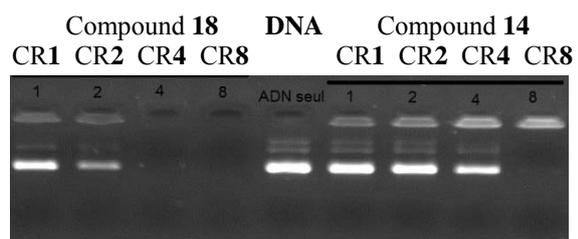
Series	Compound <sup>a</sup>	Size (nm)	Index Poly	Zeta (mV)
<b>1</b>	Non-symmetric C <sub>12:0</sub> /C <sub>18:1</sub> ( <b>2</b> )	112.8	0.563	49.3
	Symmetric C <sub>12:0</sub> ( <b>16</b> )	162.8	0.742	63.5
	Symmetric C <sub>18:1</sub> ( <b>18</b> )	66.7	0.352	37.3
	Mixture of <b>16</b> and <b>18</b> (1/1 ratio)	183.7	0.504	50.8
<b>2</b>	Non-symmetric C <sub>20:0</sub> /C <sub>18:1</sub> ( <b>4</b> )	131.5	0.311	9.1
	Symmetric C <sub>18:1</sub> ( <b>18</b> )	63.9	0.393	70.2
	Symmetric C <sub>20:0</sub> ( <b>20</b> )	194.7	0.304	45.1
	Mixture of <b>18</b> and <b>20</b> (1/1 ratio)	204.0	0.269	53.7
<b>3</b>	Non-symmetric C <sub>12:0</sub> /C <sub>20:0</sub> ( <b>6</b> )	170.0	0.242	39.1
	Symmetric C <sub>12:0</sub> ( <b>16</b> )	120.8	0.362	51.7
	Symmetric C <sub>20:0</sub> ( <b>20</b> )	178.4	0.315	31.2
	Mixture of <b>16</b> and <b>20</b> (1/1 ratio)	266.7	0.488	46.0
<b>4</b>	Non-symmetric C <sub>14:0</sub> /C <sub>18:1</sub> ( <b>8</b> )	154.8	0.204	52.3
	Symmetric C <sub>18:1</sub> ( <b>18</b> )	125.0	0.273	50.1
	Symmetric C <sub>14:0</sub> ( <b>22</b> )	242.3	0.376	66.9
	Mixture of <b>18</b> and <b>22</b> (1/1 ratio)	107.3	0.390	49.1
<b>5</b>	Non-symmetric C <sub>14:0</sub> /C <sub>20:0</sub> ( <b>10</b> )	262.1	0.387	46.7
	Symmetric C <sub>20:0</sub> ( <b>20</b> )	156.4	0.193	33.5
	Symmetric C <sub>14:0</sub> ( <b>22</b> )	242.3	0.376	66.9
	Mixture of <b>20</b> and <b>22</b> (1/1 ratio)	173.6	0.255	49.1
<b>6</b>	Non-symmetric C <sub>14:0</sub> /C <sub>18:0</sub> ( <b>12</b> )	169.9	0.277	44.4
	Symmetric C <sub>18:0</sub> ( <b>24</b> )	172.7	0.349	44.3
	Symmetric C <sub>14:0</sub> ( <b>22</b> )	213.4	0.302	40.6
	Mixture of <b>24</b> and <b>22</b> (1/1 ratio)	179.9	0.409	53.1
<b>7</b>	Non-symmetric C <sub>cholest</sub> /C <sub>18:1</sub> ( <b>14</b> )	210.3	0.406	38.3
	Symmetric C <sub>18:1</sub> ( <b>18</b> )	<b>208.7</b>	<b>0.372</b>	<b>40.5</b>

Supporting materials

## S5 DNA binding ability of cationic lipids



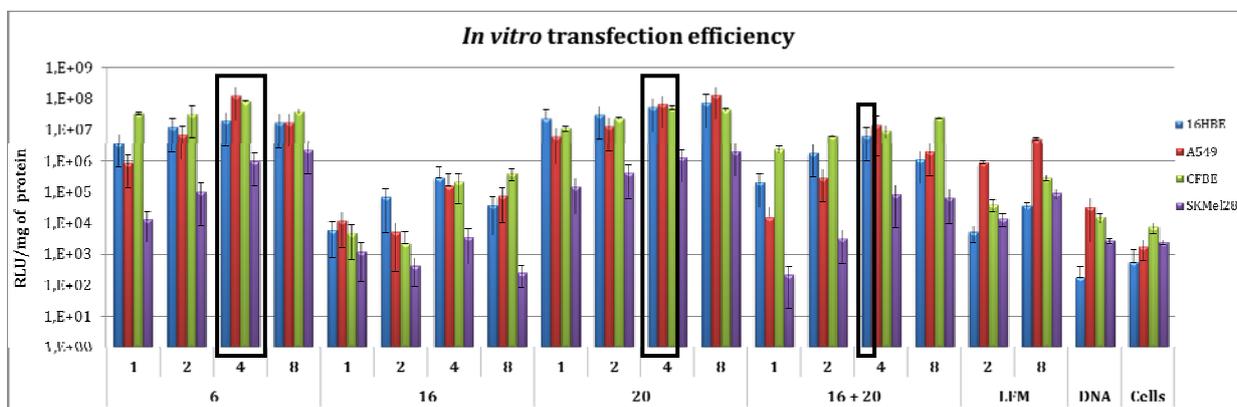
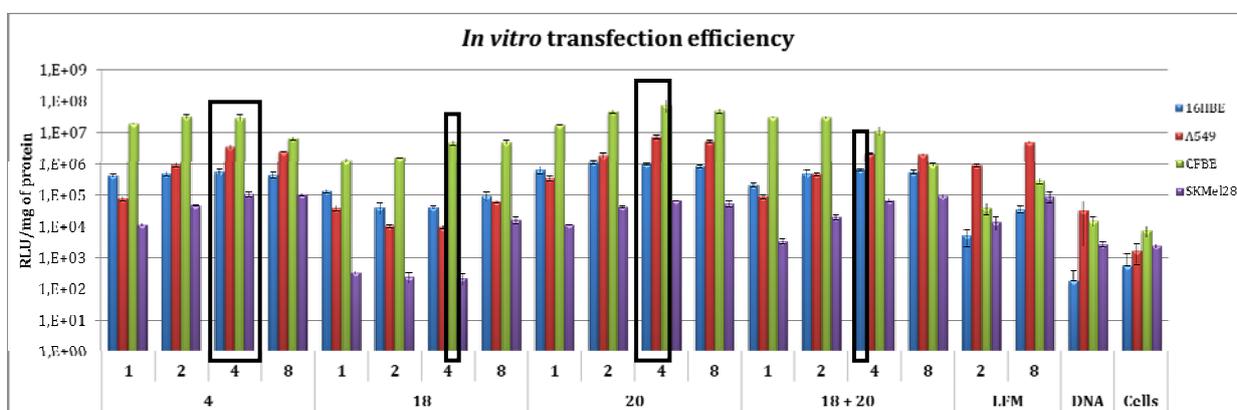
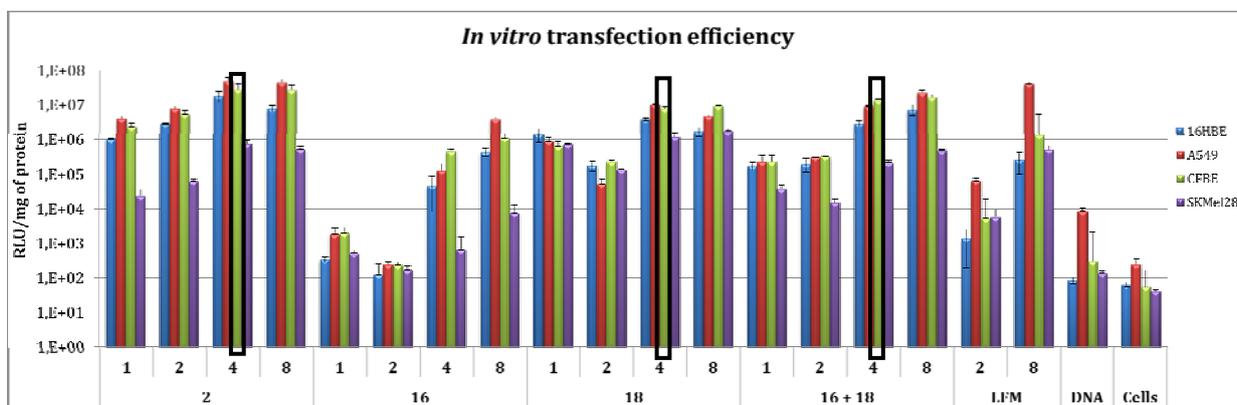
## Supporting materials



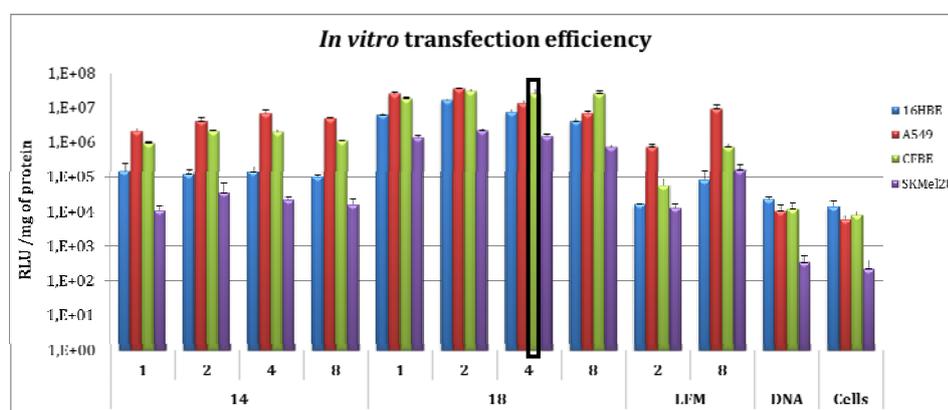
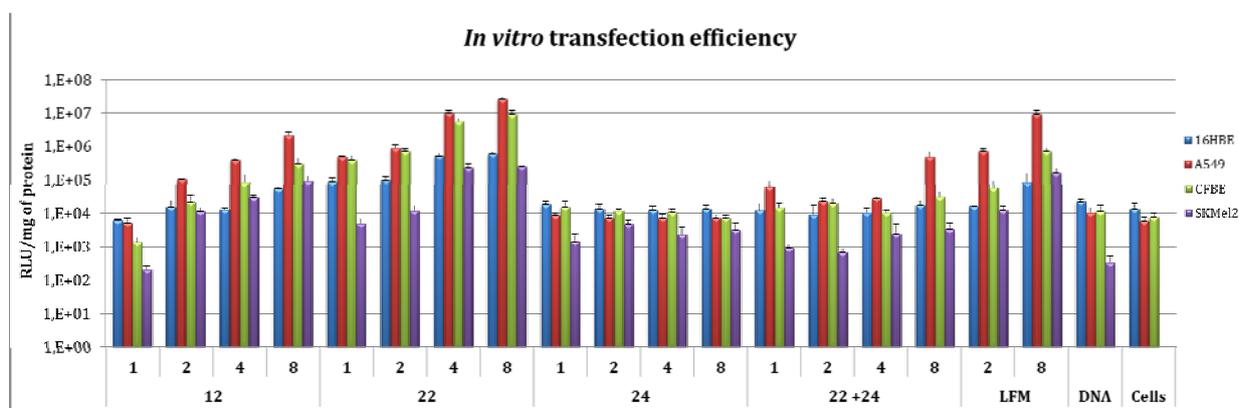
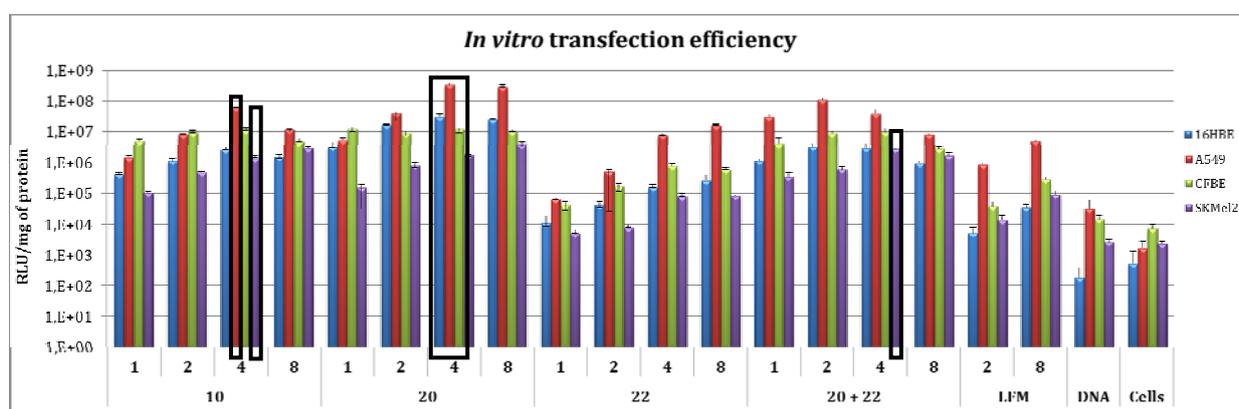
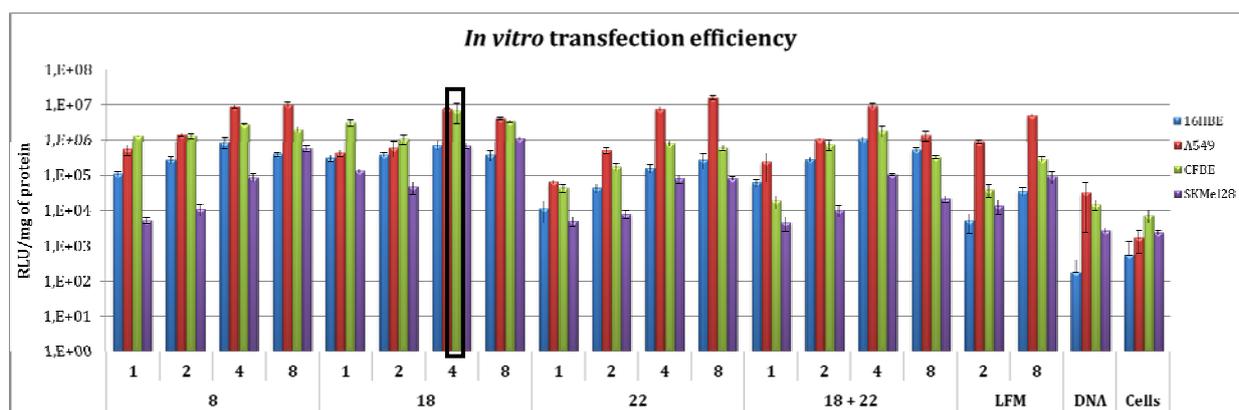
Supporting materials

## S6 *In vitro* transfection assays

For details see the experimental procedure.



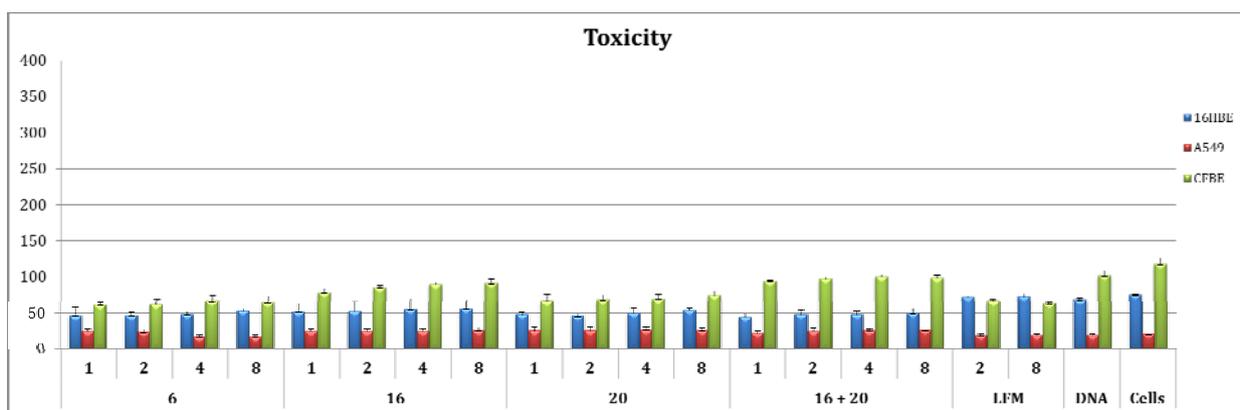
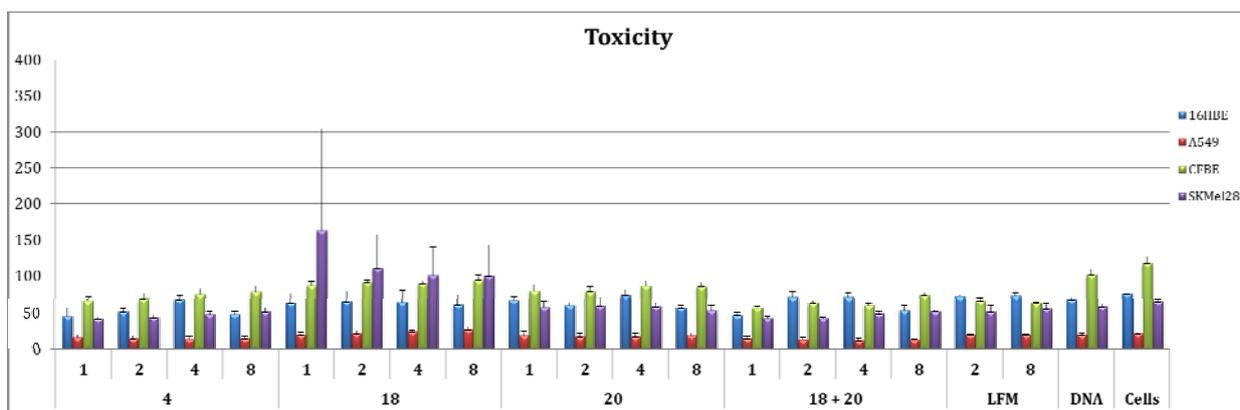
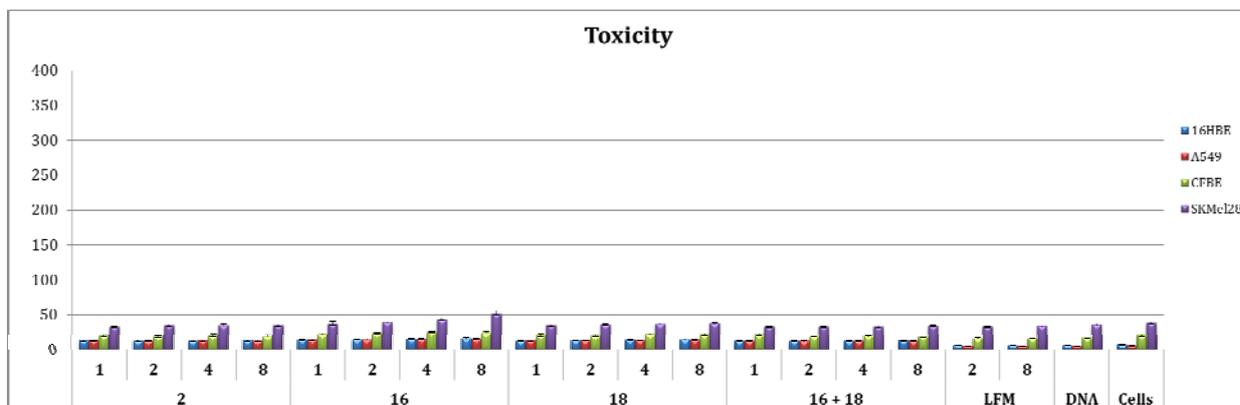
## Supporting materials



## Supporting materials

### S7 Evaluation of toxicity

The toxicity of the different lipid/DNA complexes was determined by using a chemiluminescent assay (Toxilight - Cambrex, Liège, Belgium). For details see experimental procedure.



## Supporting materials

