TIN MEDIATED SYNTHESIS OF *lyso*-PHOSPHOLIPIDS

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SUPPLEMENTARY DATA

Table of content:

EXPERIMENTAL

HPLC analyses were performed on a apparate fitted with a diol 5 µm column, length/internal diameter 125/4 and an evaporative light scattering detector . The operating conditions of the light scattering were 60 °C and 3.5 bar with a column temperature was 55 °C. A binary solvent system of hexane/isopropanol/acetic acid/triethylamine (84.4/14/1.5/0.08, v/v/v/v) (solvent A) and isopropanol/water/acetic acid/triethylamine (84.4/14/1.5/0.08, v/v/v/v) (solvent B) was used in a gradient mode starting from 60% A, 40% B ramping to 100% B in 10 minutes. 20 µl samples of a methanolic solution 1 mg/ml were injected into the column at a mobile phase flow rate of 1.5 ml/min. Rt: DPPC 1.3 min; 1-acyl-2-*lyso*PA 4.6 min; 2-acyl-1-*lyso*PC 4.9 min; 1-acyl-2-*lyso*PC 5,1; GPC 12; GPA 9,8. ^{1}H NMR, ^{13}C NMR were recorded in $CDCl_3$ or CD_3OD on a 250 or 400 MHz apparate with TMS as an internal std. ^{1}H NMR, ^{13}C NMR spectra were identical with the one reported in the literature [9e, 20]

1-O-hexadecanoyl-*sn*-glycero-3-phosphocholine (1-O-Palm-PC)

a) in methanol with 2 eq of acylating agent

To a stirred solution of GPC (10g, 0.039 mol) in methanol (200 ml), di-butyl tin oxide (10.65 g, 0.043 mol) was added at room temperature. The mixture was stirred under reflux until the reaction became clear (1 h). The resulting solution was cooled to 0° C in an ice bath and TEA (10.83 ml, 0.078 mol) and PalmCl (21.45g, 0.078 mol) were added dropwise to the solution.

After addition was complete the reaction was stirred at room temperature for a period of 15 min. A sample of the reaction mixture was analysed (HPLC) and shown to contain 1-palmitoyl-*sn-2-lyso*-phosphatidylcholine (66%) and GPC (34%) as the only phospholipids.

b) in 2-propanol with the intermediate preformed in methanol with 1.2 eq of acylating agent

To a stirred solution of GPC (10g, 39 mmol) in methanol (200 ml), di-butyl tin oxide (10.65 g, 43 mmol) was added at room temperature. The mixture was stirred under reflux until the reaction became clear (1 h). The solvent was removed under vacuum and the residue suspended in 2-PrOH (200 ml). The solvent was again removed in vacuum and the residue resuspended in 2-PrOH (400 ml). The mixture was then treated at rt with TEA (6.5 ml, 47 mmol, 1.2 eq) and palmitoyl chloride (12.8 g, 47 mmol, 1.2 eq.). After the addition was complete the reaction was stirred at room temperature for a period of 15 min. A sample of the reaction mixture was analysed (HPLC) and shown to contain 1-palmitoyl-*sn-2-lyso*-phosphatidylcholine (97%) and GPC (3%) as the only phospholipids.

c) in 2-propanol with 1.2 eq of acylating agent

GPC (10 g, 39 mmol) and DBTO (10.65 g, 43 mmol) were suspended in 400 ml of 2-propanol and refluxed for 1h. The mixture was cooled at 25°C and treated with TEA (6.5 ml, 47 mmol) and palmitoyl chloride (12.8 g, 47 mmol). The formation of the lysoPC and the disappearance of GPC was followed by HPLC. After 15 min at rt, the conversion was 97%.

The solution was treated with 400 ml of water and extracted with heptane (400 ml). The acqueous/alcoholic solution was extracted again with heptane (3 x 250 ml) dried and evaporated. The residue was dissolved and precipitated in acetone to give 15 g of a white powder (80%). A sample of the reaction mixture was analysed (HPLC) and shown to contain 1-palmitoyl-*sn-2-lyso*-phosphatidylcholine (97%) and GPC (3%) as the only phospholipids.

d) in 2-propanol with 1.2 eq of acylating agent and a catalytic amount of tin species (DBTO,5 mol %)

A suspension of GPC 10 g (0.039 mol) and dibutyltin oxide (0.5 g, 2 mmol) in 2-propanol (400 ml) was heated under reflux for 1h. The mixture was cooled at 25°C and treated with TEA (6.5 ml, 47 mmol) and palmitoyl chloride (12.8 g, 47 mmol). After addition was complete the reaction was

stirred at room temperature for a period of 15 min. A sample of the reaction mixture was analysed (HPLC) and shown to contain 1-palmitoyl-*sn-2-lyso*-phosphatidylcholine and GPC in a ratio 88 : 12.

e) in 2-propanol with 1.2 eq of acylating agent and a catalytic amount of tin species (DMTDMeO), 5 mol %)

A suspension of GPC 10 g (0.039 mol) and dibutyl-tin dimethoxide (2.8 g, 0.008 mol) in 2-propanol (400 ml) was heated under reflux for 1h. The mixture was cooled at 25°C and treated with TEA (6.5 ml, 47 mmol) and palmitoyl chloride (12.8 g, 47 mmol). After addition was complete the reaction was stirred at room temperature for a period of 15 min. A sample of the reaction mixture was analysed (HPLC) and shown to contain 1-palmitoyl-lyso-phosphatidylcholine and GPC in a ratio 89 : 11. Analytical samples were obtained by column chromatography.

^{1}HNMR (400 MHz, CD_3OD) 0.90 (t, J = 6.7 Hz, 3H), 1.20 (m, 24H), 1.62 (m, 2H), 2.35 (t, J =7.4 Hz, 2H), 3.23 (s, 9H), 3.65 (m, 2H), 3.89 (m, 2H), 3.97 (m, 1H), 4.11 (dd, J = 11.5 and 6 Hz 1H), 4.18 (dd, J = 11.5 and 4.5 Hz, 1H) 4.30(m, 2H).

^{13}CNMR (100 MHz CD_3OD 14.4, 23.6, 26.0, 30.2, 30.3, 30.5, 30.6, 32.9, 34.9, 54.9 (t, J= 3.8 Hz), 60.4 (d, J = 5.2 Hz) 66.3, 67.6 (dt, J = 7.0 and 3.5 Hz), 67.9 (d, J= 5.6 Hz), 70.0 (d, J = 7.2 Hz) , 175.2.

1-O-octadecanoyl-*sn*-glycero-3-phosphocholine

A suspension of GPC 1.85 g (7.2 mmol) and dibutyltin oxide (1.90 g, 7.6 mmol) in 2-propanol (30 ml) was heated under reflux for 1h. The resulting suspension was then cooled to 25° C in an ice bath and TEA (1.2 ml, 7.9 mmol) and stearoylchloride (2.3 g 7.9 mmol) were added. The reaction was stirred for 35 min at room temperature. A sample of the reaction mixture was analysed (HPLC) and shown to contain 1-stearoyl-LPC and GPC in a ratio 95 : 5. Isolation of the product was effected as previously described for the palmitoyl analogue. ^{1}HNMR (400 MHz, CD_3OD) 0.90

(t, J = 6.7 Hz, 3H), 1.20-1.40 (m, 28H), 1.62 (m, 2H), 2.35 (t, J =7.4 Hz, 2H), 3.23 (s, 9H), 3.65 (m, 2H), 3.89 (m, 2H), 3.97 (m, 1H), 4.11 (dd, J = 11.5 and 6 Hz 1H), 4.18 (dd, J = 11.5 and 4.5 Hz, 1H), 4.30(m, 2H).

^{13}CNMR (62.9 MHz, CD_3OD) 14.7, 23.7, 26.0, 30.2, 30.5, 30.6, 30.8, 33.1, 34.9, 54.7 (t, J= 3.5 Hz), 60.5 (d, J = 3.8 Hz), 66.2, 67.4 (dt, J = 7.0 and 3.5 Hz), 67.9 (d, J= 6.0 Hz), 69.8 (d, J = 8.0 Hz) , 175.3.

1-O-oleoyl-*sn*-glycero-3-phosphocholine

A suspension of GPC 1.85 g (7.2 mmol) and DBTO (1.90 g, 7.6 mmol) in 2-propanol (30 ml) was heated under reflux for 6h. The resulting suspension was then cooled to 25° C in an ice bath and TEA (1.2 ml, 7.9 mmol) and oleoyl chloride (2.1 g, 7.9 mmol) were added. The reaction was stirred for 35 min at room temperature. A sample of the reaction mixture was analysed (HPLC) and shown to contain 1-oleoyl-LPC and GPC in a ratio 95 : 5. Isolation of the product was effected as previously described for the palmitoyl analogue.

^{1}HNMR (400 MHz, $CDCl_3$) 0.90 (t, J = 6.7 Hz, 3H), 1.20- 1.40 (m, 24H), 1.58 (m, 2H), 2.01 (m, 4H), 2.31 (t, J =7.4 Hz, 2H), 3.36 (s, 9H), 3.82 (m, 2H), 3.87 (m, H), 3.96 (m, 2H), 4.09 (m, 2H), 4.37(m, 2H), 5.35 (m, 2H).

^{13}CNMR (62.9 MHz, $CDCl_3$) 14.1, 22.7, 25.0, 27.2, 29.2, 29.3, 29.5, 29.8, 31.9, 34.1, 54.3, 59.4, 65.1, 66.1, 67.2, 68.6, 129.6, 130.0, 173.9.

1-O-dodecanoyl-*sn*-glycero-3-phosphocholine

A suspension of GPC (1.85 g, 7.2 mmol) and dibutyltin oxide (1.90 g, 7.6 mmol) in 2-propanol (30 ml) was heated under reflux for 2h. The resulting suspension was then cooled to 25° C in an ice bath and TEA (1.2 ml, 7.9 mmol) and lauroyl chloride (1.6 g 7.9 mmol) were added. The reaction was stirred for 35 min at room temperature. A sample of the reaction mixture was analysed (HPLC) and shown to contain 1-lauroyl-LPC and GPC in a ratio 95 : 5. Isolation of the product was

effected as previously described for the palmitoyl analogue.

^{1}HNMR (400 MHz, CD_3OD) 0.90 (t, J = 6.7 Hz, 3H), 1.20 (m, 16 H), 1.62 (m, 2H), 2.35 (t, J =7.4 Hz, 2H), 3.23 (s, 9H), 3.65 (m, 2H), 3.89 (m, 2H), 3.97 (m, 1H), 4.11 (dd, J = 11.5 and 6 Hz 1H), 4.18 (dd, J = 11.5 and 4.5 Hz, 1H), 4.30 (m, 2H).

^{13}CNMR (62.9 MHz CD_3OD) 14.5, 23.8, 25.9, 30.2, 30.5, 30.6, 30.7, 33.0, 34.8, 54.6 (t, J= 3.5 Hz), 60.4 (d, J = 5.0 Hz), 66.2, 67.3 (dt, J = 7.0 and 3.5 Hz), 67.8 (d, J= 5.6 Hz), 69.7 (d, J = 5.6 Hz) , 175.2.

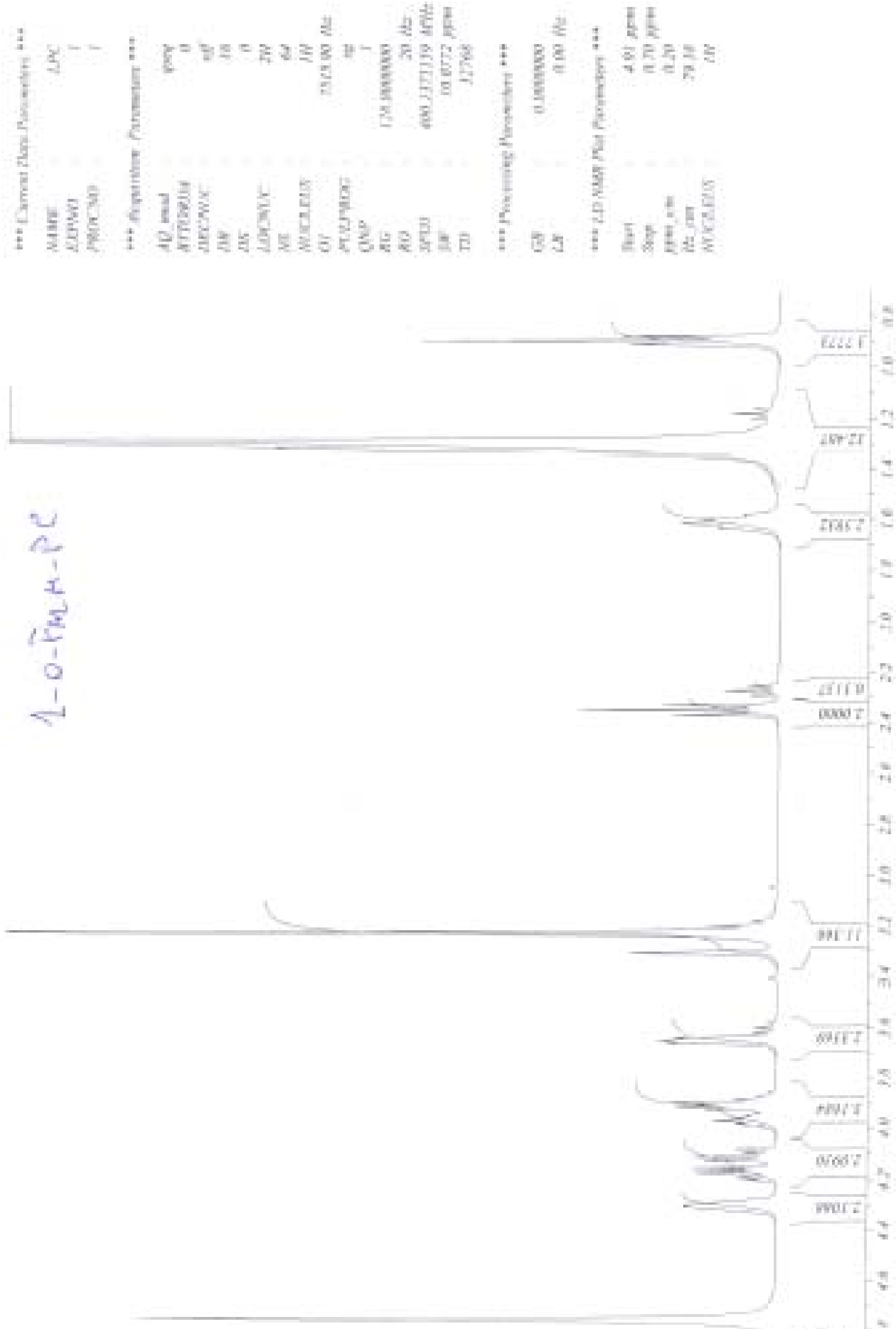
1-O-hexadecanoyl-*sn*-glycero-3-phosphoric acid DMAP salt

10 g of glycerol-3-phosphate disodium salt was dissolved in 20 ml of H_2O and passed through a column of sulphonic resin DOWEX 50X8 in the acidic form. The solution was treated with DMAP to pH =6 and the solution lyophilised giving glycerol-3-phosphate DMAP mono salt.

GPA DMAP mono salt (1 g, 5.4 mmol) and DBTO (1.35 g, 3.4 mmol) in 2-propanol (50 ml) was heated under reflux for 2h. The resulting suspension was then cooled to 25° C in an ice bath and TEA (1,3 ml, 8.1 mmol) and palmitoyl chloride (2.2 g, 8.1 mmol) were added dropwise. The reaction was stirred for 15 min at room temperature. A sample of the reaction mixture was analysed (HPLC) and shown to contain 1-palmitoyl-LPA and GPA in a ratio 91 : 9. An analytical sample was obtained by column chromatography.

^{1}HNMR (400 MHz, CD_3OD) 0.90 (t, J = 6.7 Hz, 3H), 1.20-1.40 (m, 24H), 1.62 (m, 2H), 2.34 (t, J =7.4 Hz, 2H), 3.26 (s, 6H), 3.91 (m, 2H), 3.97 (m, 1H), 4.11 (dd, J = 11.5 and 6 Hz, 1H), 4.18 (dd, J = 11.5 and 4.5 Hz, 1H), 7.00 (m, 2H), 8.13 (m, 2H).

^{13}CNMR (100 MHz CD_3OD)14.8, 23.6, 25.9, 30.2, 30.30, 30.5, 30.6, 32.9, 34.9, 40.3, 47.8, 66.4, 67.4, 70.1, (dt, J = 7.0 and 3.5 Hz), 108.3 , 140.3, 158.5, 175.2.



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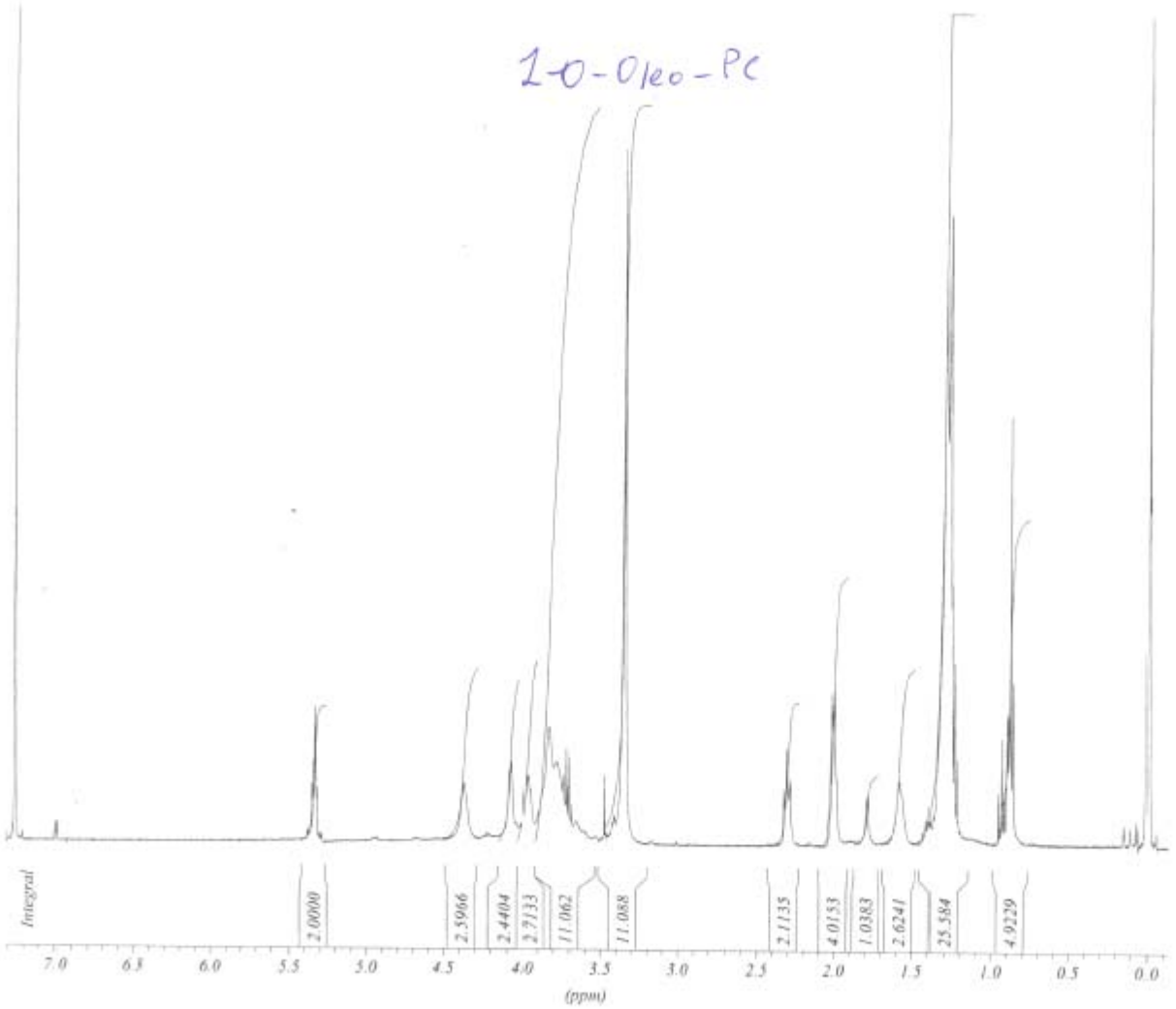
(ppm)



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*** Processing Parameters ***

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*** 1D NMR Plot Parameters ***

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*** Processing Parameters ***

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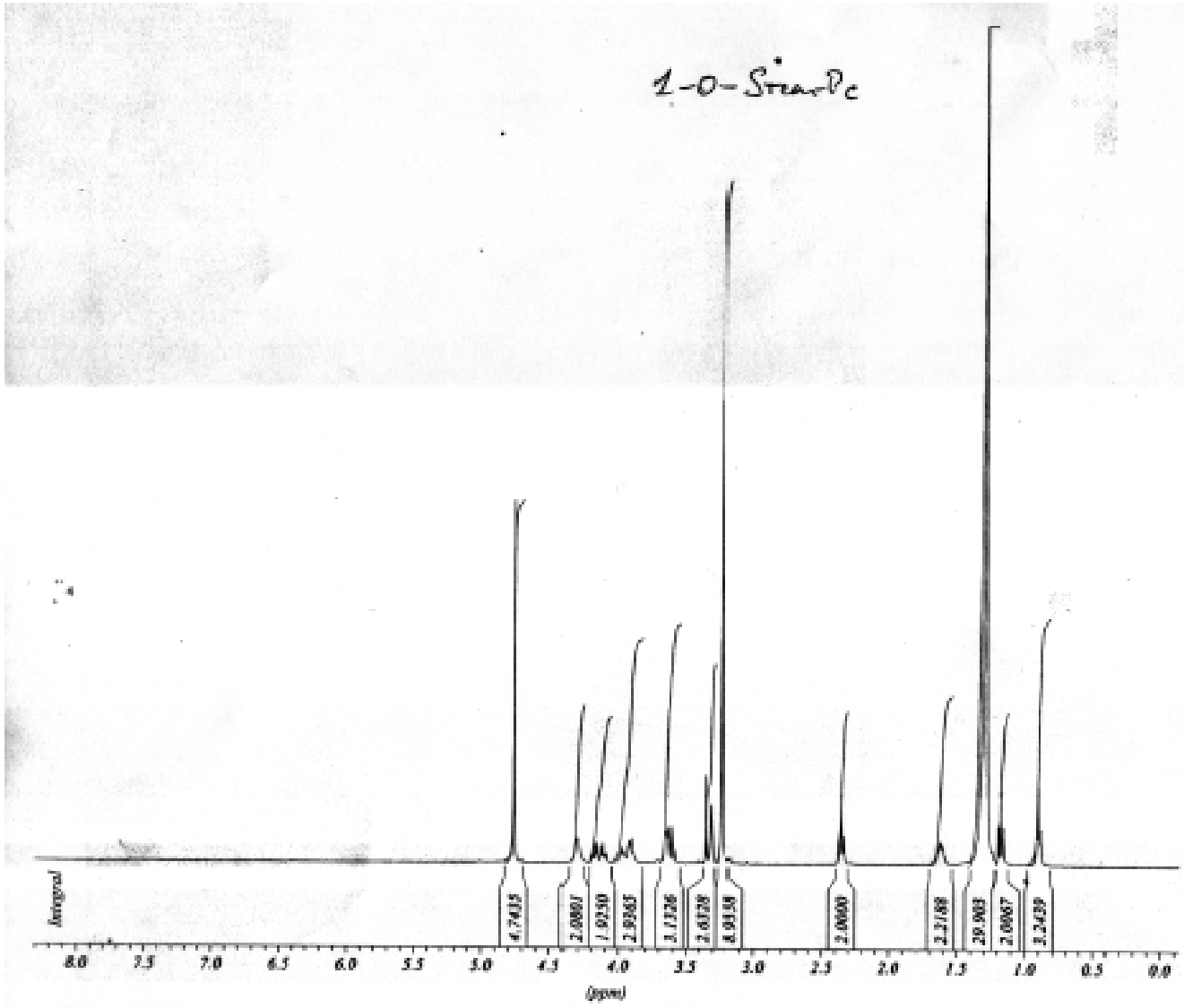

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NUCLEUS	:	1H
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PULPROG	:	zg
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RO	:	20 Hz
SFO1	:	400.1375159 MHz
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TD	:	32768
TE	:	300.0 K

*** Processing Parameters ***

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*** 1D NMR Plot Parameters ***

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*** Processing Parameters ***

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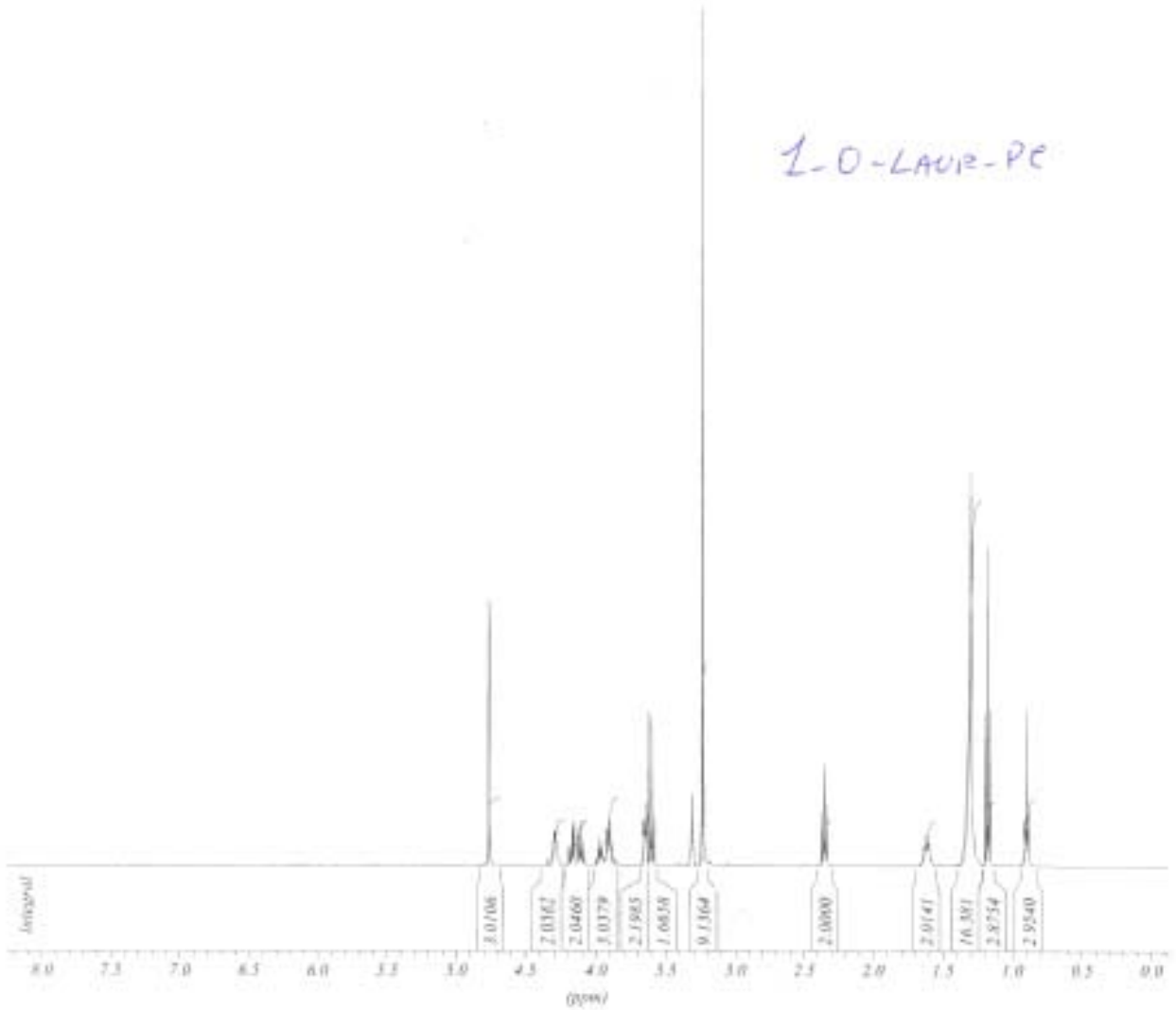

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*** Current Data Parameters ***

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PROCNO	1

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RO	20 Hz
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SW	220.8236 ppm
TD	16384
TE	303 K

*** Processing Parameters ***

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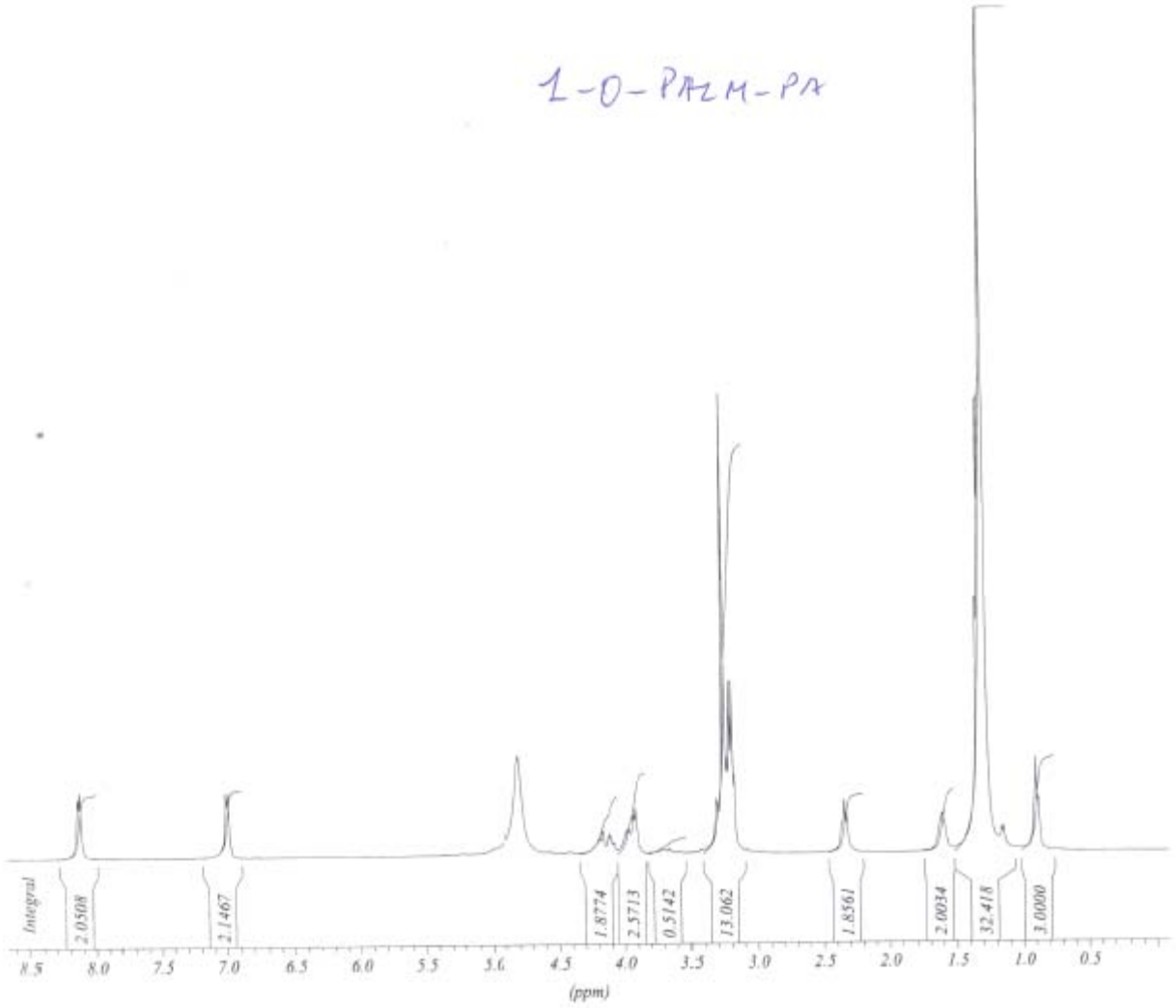

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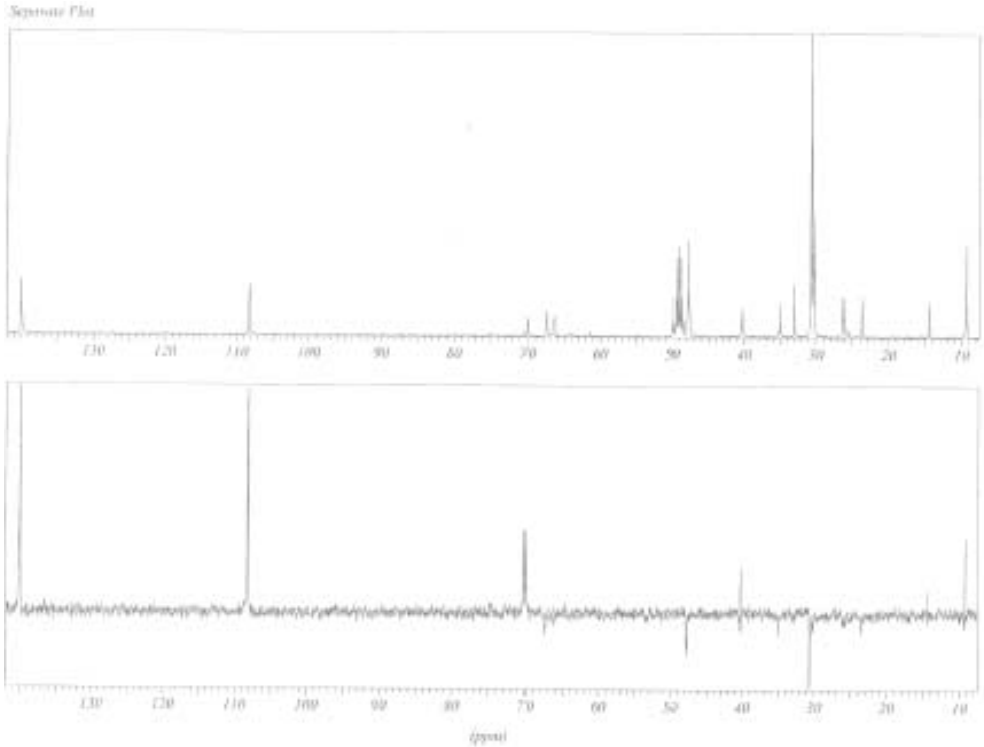

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1-O-PMB, Pd

^{13}C DEPT

Display Report

Analysis Info

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Sample Name	se_oleyl-lpc	Method	Copy of _awolf2.MS	Instrument	esquire3000plus
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Acquisition Parameter

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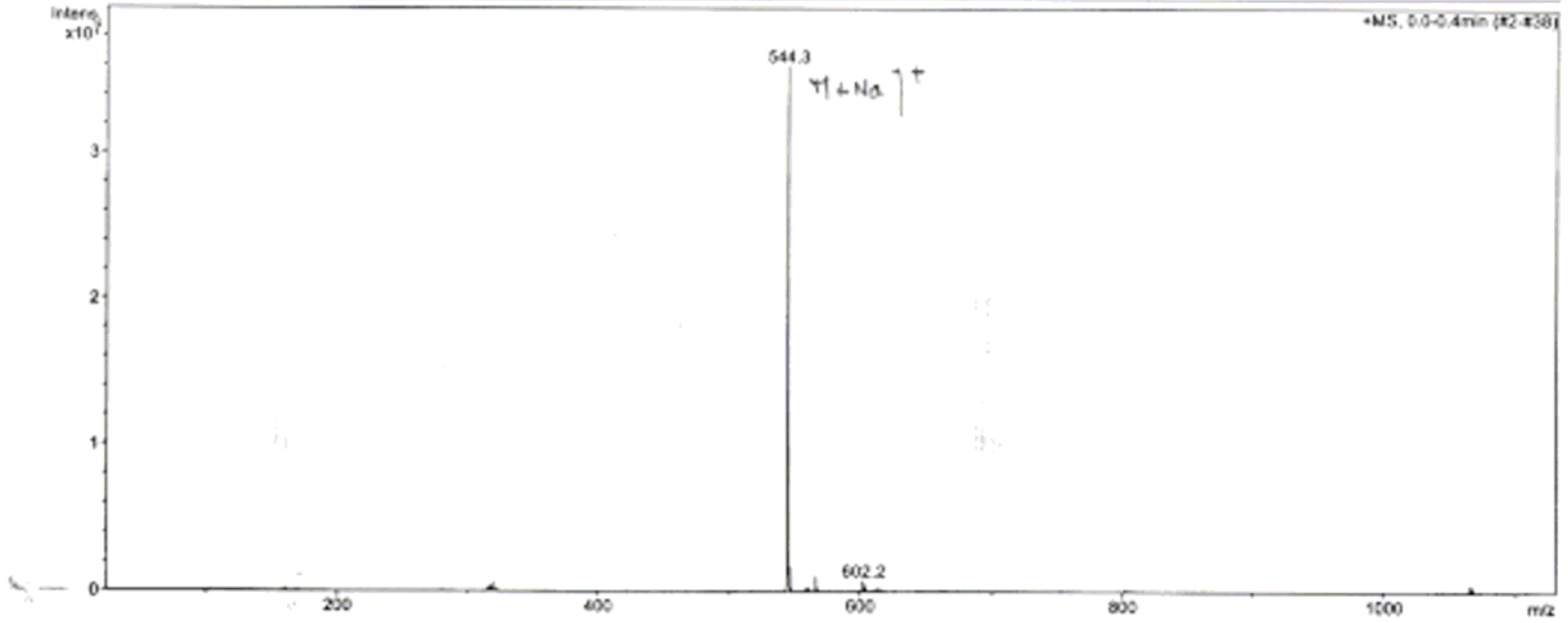

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Analysis Info

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Sample Name	se_laurlpc_	Method	Copy of _awalt2.MS	Instrument	esquire3000plus
Comment	se_laurlpc_ 1 mL--> 10 microL/1 mL MeOH+HCOOH 0.1% Richiedente D'arrigo				

Acquisition Parameter

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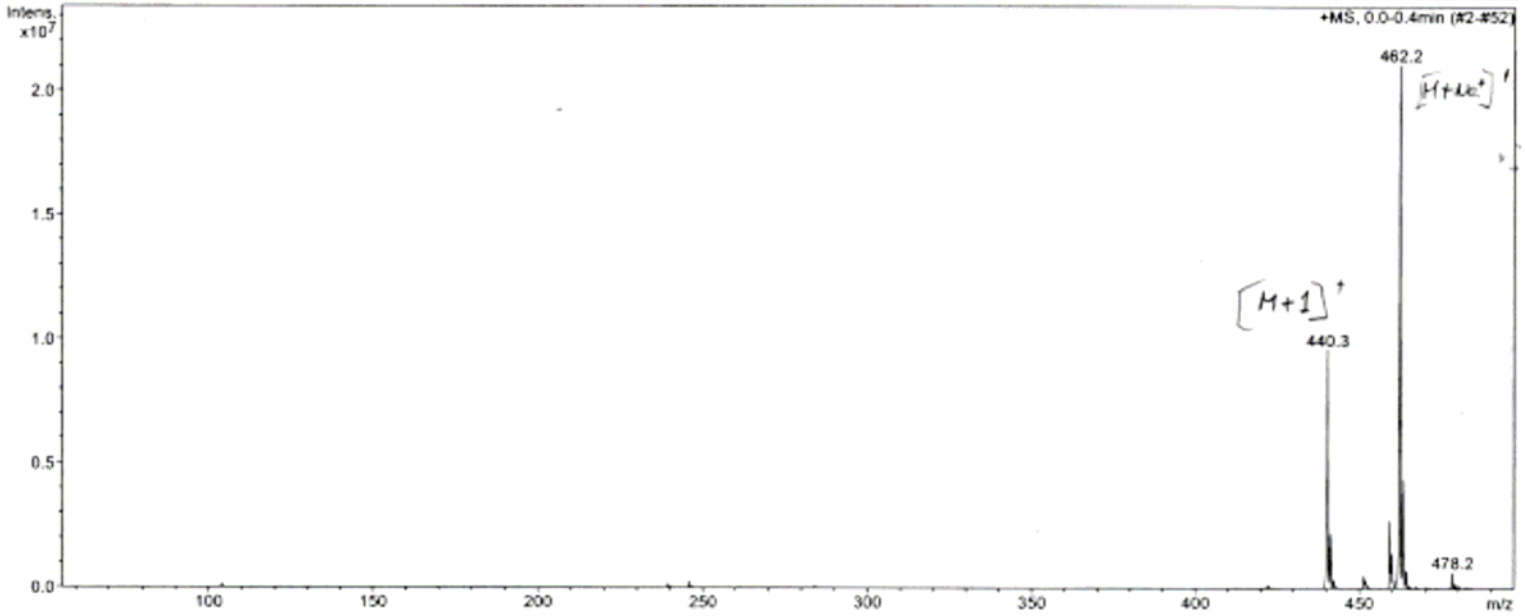

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Acquisition Parameter

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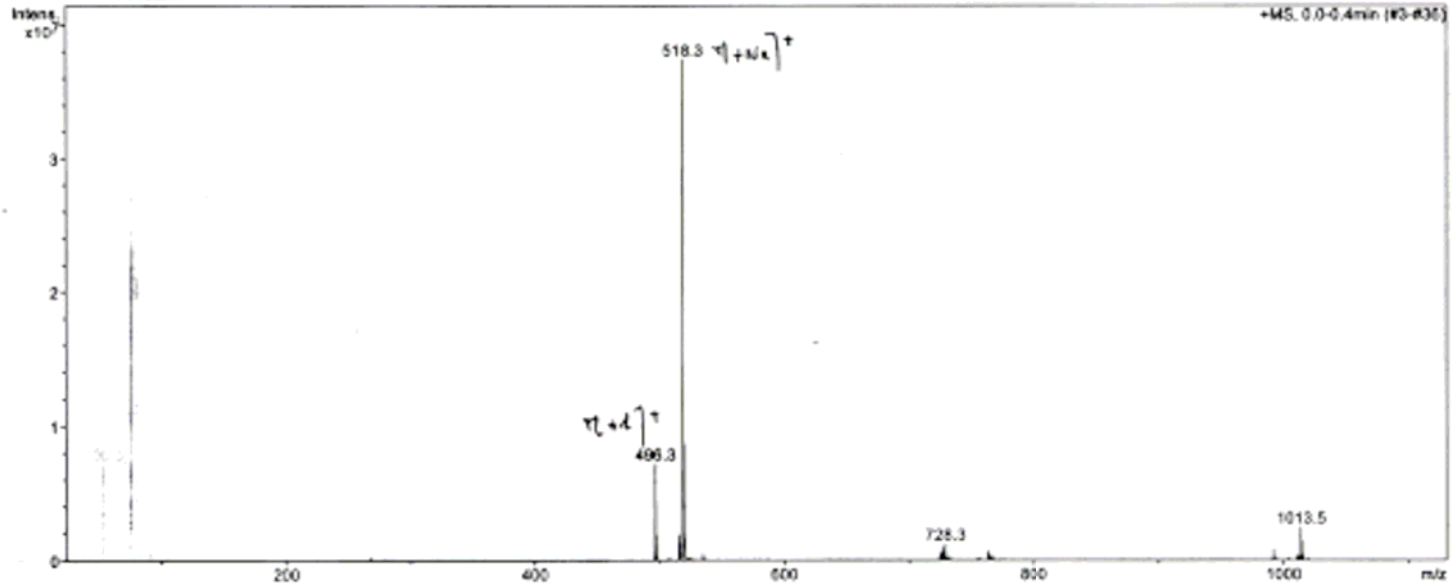

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Acquisition Parameter

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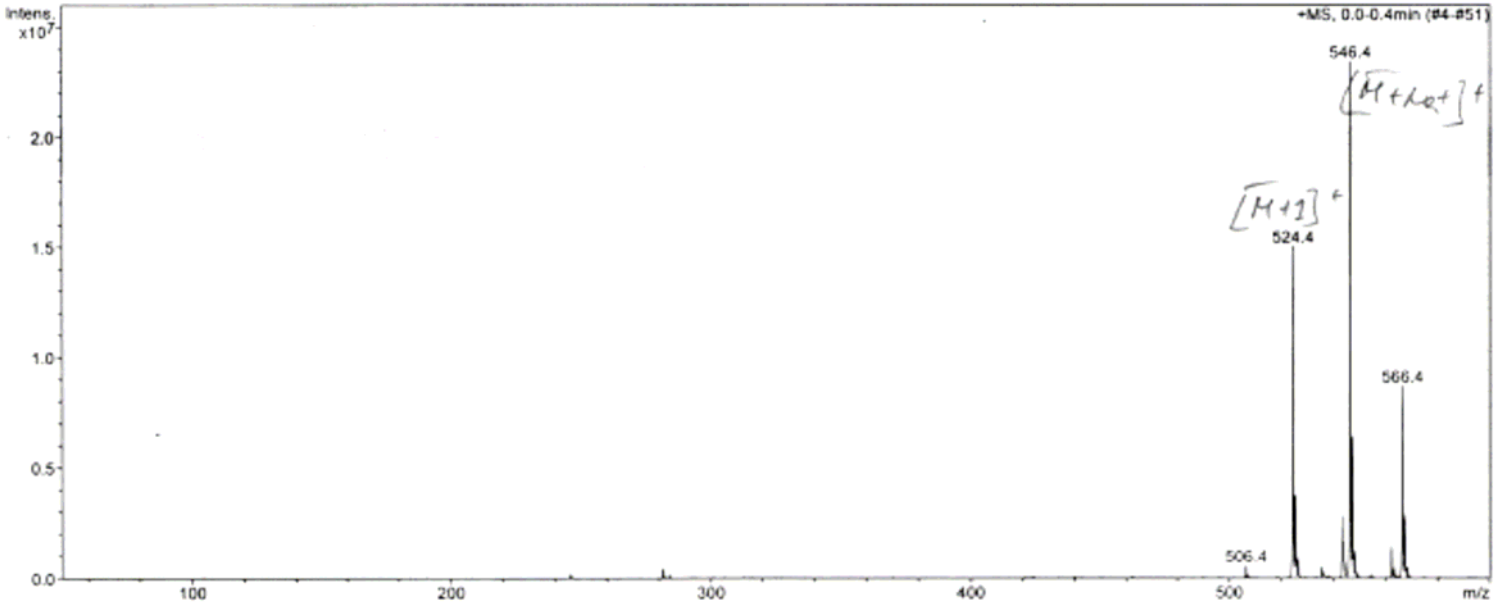

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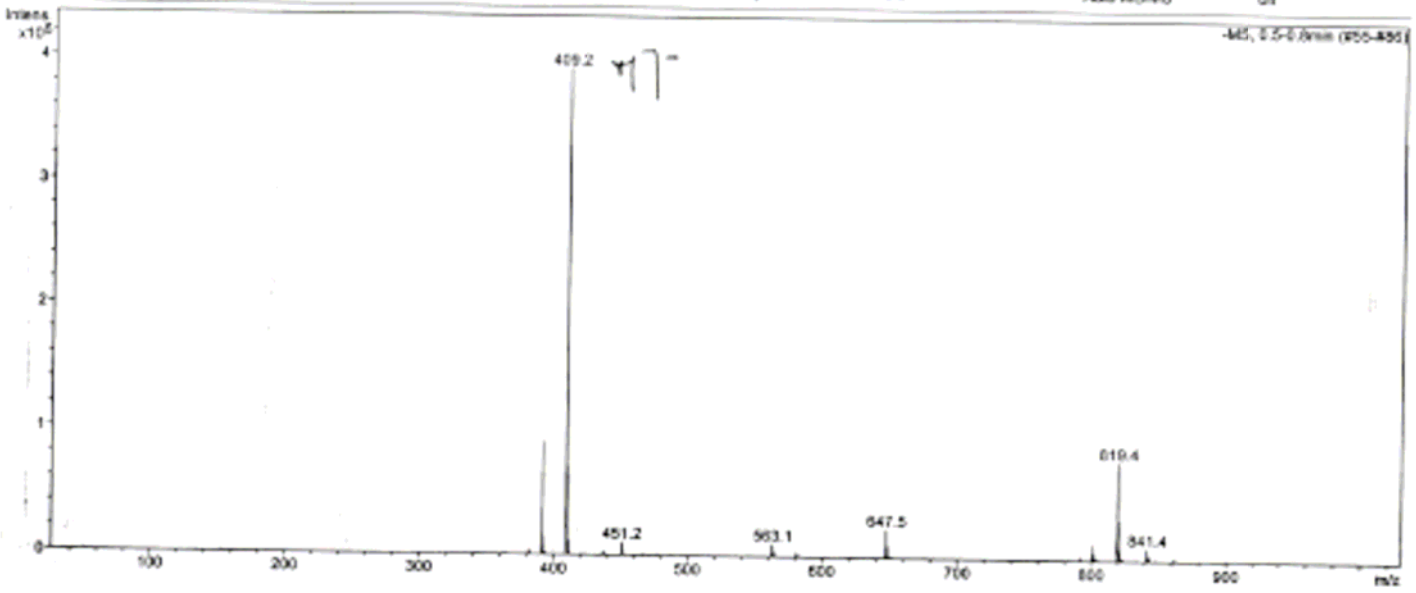
Analysis Info

Analysis Name	se_palmipa_.d	Acquisition Date	05/29/06 14:12:05	Operator	Walter Panzeri
Sample Name	se_palmipa	Method	Copy of _small2.MS	Instrument	esquire3000plus
Comment	se_palmipa 1 mL--> 10 microL/1 mL MeOH + HCOOH 0.1% Richiedente D'amigo				

Acquisition Parameter

Ion Source Type	ESI	Mass Range Mode	Std/Normal	Ion Polarity	Positive	Alternating Ion Polarity	off
Scan Begin	50 m/z	Scan End	700 m/z	Averages	5 Spectra	Accumulation Time	109 µs
Capillary Exit	122.7 Volt	Skim 1	40.0 Volt	Trap Drive	42.0	Auto MS/MS	off



HPLC trace example

```
Sample Name      : cat155
Acq. Operator    : ezio
Acq. Method      : C:\HPCHEM\1\METHODS\FOSFOLIP.M
Last changed     : 11/02/03 14.29.01  by ezio
                   (modified after loading)
Analysis Method  : C:\HPCHEM\1\METHODS\FOSFOLIP.M
Last changed     : 22/12/05 11.08.25  by paola
                   (modified after loading)
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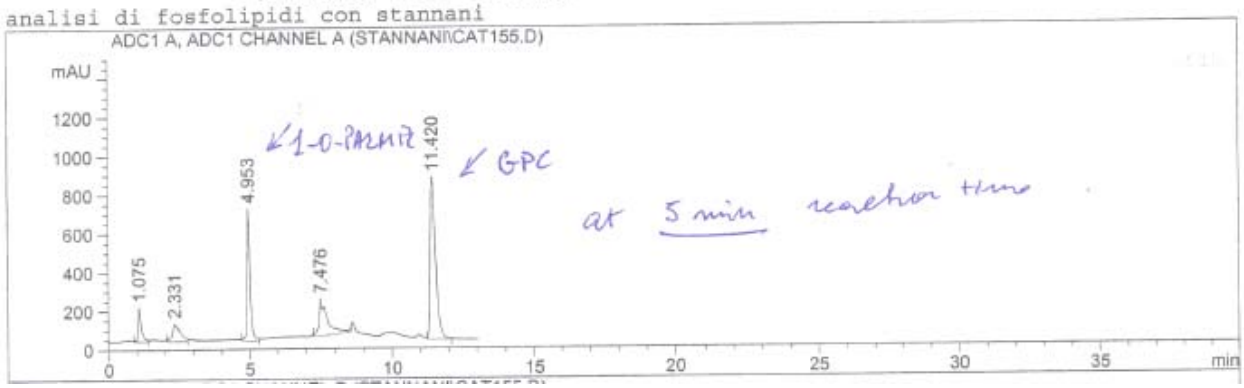
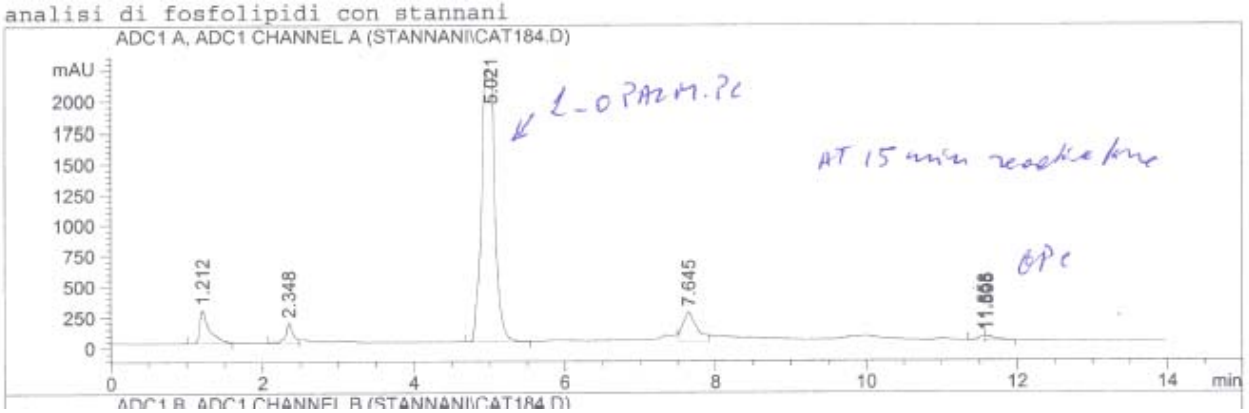
analisi di fosfolipidi con stannani



analisi di fosfolipidi con stannani



analisi di fosfolipidi con stannani