Supporting Information for:

Giant Vesicles from rehydrated crude mixtures containing unexpected mixtures of amphiphiles formed under plausible prebiotic conditions

Michele Fiore,[a,]*Warren Madanamoothoo,[a] Alexandra Berlioz-Barbier,[a] Ofelia Maniti,[a] Agnès Girard-Egrot,[a] René Buchet, [a] and Peter Strazewski [a,]*

Materials and methods

Reagents, solvents and gases: All reagents, purchased from Sigma Aldrich, TCI-Europe or Acros-Organics, including dry solvents, were used without further purification. HPLC solvents were purchased from Thermo-Fischer Scientific (mass spectrometry grade). Thin-layer chromatography (TLC) was carried out on aluminium sheets coated with silica gel 60 F_{254} (Merck). TLC plates were inspected by UV light (λ = 254 nm) and developed by treatment with a mixture of 10% H_2SO_4 in EtOH/H_2O (1 : 1 *v/v*), $KMnO_4$ 10% solution or the *Pancaldi* reagent ($(NH_4)_6MoO_4$, $Ce(SO_4)_2$, H_2SO_4, H_2O), followed by heating. All phosphorylation reactions and control experiments were carried out in unsealed Eppendorf or 2 mL unsealed glass tubes.

Spectroscopy and microscopy: IR data were acquired with a Thermo Scientific Nicolet iS10 spectrometer equipped with a DTGS detector. The IR spectra were recorded with 64 interferograms at 4 cm^{-1} resolution each and then Fourier transformed. NMR spectra were recorded at 293 K using a 300 MHz spectrometer (Bruker) with field strengths for ^1H nuclei 300 MHz, ^{13}C nuclei 70 MHz and ^{31}P nuclei 121.5 MHz. Reference samples for ^{13}C NMR analysis were prepared as 1 mM solutions and dissolved in 660 µL H_2O/D_2O (9:1 *v/v*) or DMSO-d_6. ^1H NMR chemical shifts were referenced to the residual undeuterated solvent signals of $CHCl_3$ (7.26 ppm) and DMSO 2.50 ppm). Signal shape and multiplicities are abbreviated as br (broad), *s* (singlet), *d* (doublet), *t* (triplet), *q* (quartet) and *m* (multiplet). Where possible, a scalar coupling constant *J* is given in Hertz (Hz). ^{31}P NMR chemical shifts were referenced to $NH_4H_2PO_4$ (0.00 ppm). ^{13}C NMR chemical shifts were referenced to DMSO (39.51 ppm) or, in the case of spectra taken in H_2O/D_2O, the signal of cyanamide (**2a**) at 118.10 ppm (considered as internal reference). Electrospray-ionization mass spectra (ESI-MS) were recorded using a Q-Tof Micromass spectrometer. HPLC/ESI-HRMS analyses were performed on a Bruker Impact II equipped with a hybrid mass spectrometer quadrupole type. The columns used were C8 and C18 Agilent 2.7 µm (100 x 2.1 mm). Mobile phases were A: 10mM milli-Q water; B: acetonitrile/isopropanol 9:1 (*v/v*). Micrographs were recorded with a Carl-Zeiss inverted microscope LSM 800 equipped with a 50x oil immersion objective and AxioCam recording. Micrographs were used without any graphical treatment and the image size was adjusted respecting the x/y pixel proportions.

Amphiphile solutions: TLC eluent system A ($CHCl_3$/MeOH/H_2O, 65:45:4 *v/v/v*) was prepared by mixing first MeOH and H_2O and then adding under mechanical agitation $CHCl_3$ to avoid the separation of phases. Eluent System B was pure $CHCl_3$ (10 mL for TLC size 5x4 cm). Buffers were prepared by diluting the desired amount of powdered compound in the necessary amount of water. The pH was adjusted with freshly prepared 1M HCl and 10M NaOH solutions. For samples hydrated at pH 2, we followed the indication reported in reference 28. Standard solutions (10 mM in $CHCl_3$ of pure commercial/synthetic compounds: **1**, **5s**, **7s and 10%$_{mol}$ 5s in 7s**) were prepared by dissolving the corresponding pure dried compound in $CHCl_3$. For IR analysis, amphiphile samples (around 50 mM) in 100 mM Tris-HCl pH 7.82 were deposited between two BaF_2 windows of a demountable thermostated cell (model Harrick) separated with a 12 µm Teflon spacer (approximate sample volume 10 µL).

Undecan-1-ol **(1)**, commercial from Sigma Aldrich. ^1H NMR (DMSO-d_6): δ_H = 0.85 (*t*, *J* = 6.0, 3H), 1.24 (br*s*, 18 H) 1.40 (*t*, , *J* = 6.0,2H), 3.37 (*t*, *J* = 6.0, 3H) 4.38 (*brs*, 1H, OH); ^{13}C NMR (DMSO-d_6): δ_C = 13.7, 22.8, 25.6, 28.9, 29.1, 29.2, 29.3, 29.4, 31.5, 32.6, 60.3.

2-Aminoethyl phosphate **(3b)** ^1H NMR (D_2O): δ_H = 3.17 (*t*, *J* = 3.0 Hz, 3H), 4.0 (*q*, *J* = 6.0 Hz, 2H) ; ^{13}C NMR (D_2O): δ_C = 40.0, 61.4; ^{31}P NMR (D_2O): δ_P = 0.05 ppm.

Undecanol-2-oxo-1,3,2-dioxaphospholane (**6**) To a solution of **1** (0.250 g, 1.45 mmol) in 6 mL of dry THF was added dry triethylamine (0.235 ml, 1.67 mmol) and the mixture was cooled to 0 °C. To this was added 2-chloro-2-oxo-1,3,2-dioxaphospholane (153 µL, 1.67 mmol) in 3 mL of dry toluene in one portion. The mixture was stirred at room temperature for 16h. The heterogeneous mixture was then filtered off through a pad of celite and washed 3 times with 1:1 solution of Et_2O-hexanes. The solvent was evaporated to give **12** as a single phosphate-positive product in the form of a colorless oil. This compound was used as soon as possible for the next reaction without further treatment (0.380 g, 95 %): TLC ($CHCl_3$/MeOH 98:2): R_f = 0.23; ^1H NMR ($CDCl_3$): δ_H = 0.88 (*t*, *J* = 9.0, 3H, -*CH*$_3$), 1.26 (*s*, 16 H, -*CH*$_2$-) 1.67-1.72 (*m*, 2H, β-*CH*$_2$-), 4.16 (*q*, *J* = 9.0, 2H, α-*CH*$_2$-) 4.38 (*m*, 4H, -O-*CH*$_2$-*CH*$_2$-O-); ^{13}C NMR ($CDCl_3$): δ_C = 14.1, 22.7, 25.3, 29.1, 29.3, 29.5, 29.6, 31.9,

65.9, 69.2, 69.2; 69.3; ^{31}P NMR ($CDCl_3$): δ_P = 17.6 ppm. HRMS (ESI): $[M+Na]^+$ calcd. for $C_{13}H_{27}NaO_4P$ *m/z* 301.1545; found 301.1539.

2-Ammonioethyl undecyl phosphate (**5s**) A solution of **6** (380 mg, 1.42 mmol) in 2.5 mL of anhydrous acetonitrile was placed in a pressure bottle, cooled in an ice bath, and to this was bubbled ammonia gas until a white suspension appeared. The pressure bottle was sealed and then heated in an oil bath at 85 °C for 4 h. The reaction mixture was cooled down. Flash chromatography of the crude mixture on dried silica gel using the eluent system A gave 0.160 g of pure **5s** (0.54 mmol, 38 %) as a pale yellow wax. TLC (eluent system A): R_f = 0.38 ^{1}H NMR ($CDCl_3$): δ_H = 0.87 (br *t*, 3H, -*CH*$_3$), 1.25 (br *s*, 16 H, -*CH*$_2$-,), 1.57 (br *s*, 2H, α-*CH*$_2$-); 3.87 (br *t*, 4H, -O-*CH*$_2$-*CH*$_2$-NH-); ^{13}C NMR ($CDCl_3$): δ_C = 14.1, 22.7, 29.4, 29.7, 29.8, 31.9, 45.7; ^{31}P NMR ($CDCl_3$): δ_P = –0.5 ppm; HRMS (ESI): $[M+Na]^+$ calcd. for $C_{13}H_{30}NNaO_4P$ *m/z* 318.1810; found 318.1805.

2-Hydroxyethyl undecyl phosphate (**7s**) A solution of **6** (170 mg, 0.92 mmol) in 1.0 mL of anhydrous acetonitrile was placed in a pressure bottle, cooled in an ice bath, and to this was added an excess of ultrapure water (1 mL). The pressure bottle was sealed and then heated in an oil bath at 65 °C for 16 h. The reaction mixture was then cooled down to ambient T. Flash chromatography of the crude mixture on silica gel eluted with chloroform/methanol/water (65:25:4 *v/v/v*) gave 0.265 g of pure **7** (0.90 mmol, 90 %) as a white wax. TLC (eluent system A): R_f = 0.58. ^{1}H NMR ($CDCl_3$): δ_H = 0.85 (br *t*, 3H, -*CH*$_3$), 1.20 (br *s*, 16 H, -*CH*$_2$-) 3.05 (br *s*, 2H, -*CH*$_2$-*O*); 3.96 (br *s*, 2H, , O-P-O-*CH*$_2$-); ^{13}C NMR): δ_C = 14.1, 22.6, 25.8, 29.3, 29.4, 29.5, 29.6, 29.7, 30.7, 31.9, 45.7; ^{31}P NMR ($CDCl_3$): δ_P = –0.31 ppm; HRMS (ESI): M^+ calcd for $C_{13}H_{28}O_5P$ *m/z* 295.1674; found $C_{13}H_{28}O_5P$ 295.1676.

Preaparation of 13 and 14 from **7s**. In a 2 ml Eppendorf tube 29.5 mg (0.1 mmol) of **7s** were mixed with 60 mg of **2b** (10 mmol). The mixture was suspended in water (1mL) and the opalescent suspension was vortexed (1 minute), sonicated (5 minutes) and was left heated without any cap until dryness of the water/ethanol mixture in a thermo-shaker apparatus set up at 80°C between 24. The yellowish paste was dissolved in pure chloroform (3x2 mL) and each time the obtained susoension was centrifuged. The organic layers were collected, dried and then analysed by NMR and UPLC-HRMS analysis. **14** and **15** (selected signals) ^{1}H NMR ($CDCl_3$), mixtures of tow aliphatic compounds: δ_H = 0.88 (br*t, 4x* -*CH*$_3$), 1.22-1.35 (br*s, 30x* -*CH*$_2$-) 1.58 (*t*, 3x β-*CH*$_2$-), 3.63 (br*t*, *2x* α-*CH*$_2$-); 3.64-3.80 (2xm, 12 H, -*O*-*CH*$_2$-*CH*$_2$-*O*-) 4.05 (br*t*, -*CH*$_2$-); ^{13}C NMR (DMSO-*d*$_6$): δ_C = 14.10, 14.12, 22.69, 22.71, 22.74, 29.34, 29.44, 29.62, 29.71,31.86, 32.81, 63.09; ^{31}P NMR ($CDCl_3$): δ_P = 1.08, 0.43 and –0.02 ppm. HRMS (ESI): $[M–H]^–$ calcd. for $C_{26}H_{55}O_9P_2$ 573.3336; found 573.3326.

Supporting Information Table S1. *Cyanamide and urea under simulated prebiotic conditions*†

Entry	Added compounds	Aspect after 24/48h	Temp/time	pH	Products
1	**2a** + **3a**	**dry**	65°C/24h	**4.5***	**2b**
2	**2a** + **3a**	dry	80°C/48h	7.0	**2c**+**2d**
3	**2a** + **3a**	dry	100°C/48h	7.0	**2a**+**2c**
4	**2a** + **3a**	**wet**	80°C/24h	**4.5***	**2b**
5	**2a** + **3a**	wet	25°C/48h	7.0	n.c.
6	**2a** + **3a**	wet	40°C/48h	4.0#	n.c.
7	**2b** + **3a**	dry	80°C/48h	4.5	**2b** / n.r.

*1mM solution of $NH_4H_2PO_4$ (**3a**) has pH 4.5; #pH 4.0 was obtained by adjusting 10mL of unbuffered Milli-Q water with 1M HCl. Melamine (**2d**) was observed only by ESI-MS analysis and when reactions were carried out at 100°C. In red are the optimized conditions used in the subsequent experiments; n.r. : no reaction.

Supporting Information Table S2. ESI-MS and UPLC-HRMS analysis of crude mixtures A and B

Entry	Conditions	Temp/time	Products	*m/z* in positive ion mode	*m/z* in negative ion mode
1	**1 + 2a + 3a**	65°C/ 24h[a]	**4**		251 / $[M-H]^-$
			2c	85 /$[M+H]^+$	
			2d	127 / $[M+H]^+$	
2	**1 + 2a + 3a**	65°C/ 24h[b]	**10**		213 / $[M-H]^-$
3	**1 + 2a + 3a**	100°C/ 24h[a]	**4**		251 / $[M-H]^-$
			9	317 / $[M+Na]^+$	293 / $[M-H]^-$
4	**1 + 2b + 3a**	100°C/ 24h[a]	**4**		251 / $[M-H]^-$
			8	255 / $[M+Na+H]^{2+}$	
			9	317 / $[M+Na]^+$	293 / $[M-H]^-$
5	**1 + 2b + 3a**	80°C/ 24h[a]	**4**	253 / $[M+H]^+$	251 / $[M-H]^-$
			4	275 / $[M+Na]^+$	
			8	244 / $[M+2H]^{2+}$	
			9	294 / $[M+H]^+$	
6	**1 + 2a + 3b**	100°C/ 24h[a]	**11** or **12**	337 / $[M+H]^+$	
7	**1 + 2a + 3b**	100°C/ 24h[b]	**9**	317 / $[M+Na]^+$	
			10	215 / $[M+H]^+$	
8	**1 + 2a + 3b**	100°C/ 24h[a]	**11** or **12**	337 / $[M+H]^+$	
			13 or **14**	288 / $[M+2H]^{2+}$	
9	**1 + 2a + 3b**	100°C/ 24h[b]	**15**		348 / $[M+Na-H]^-$
10	**1 + 2a + 3b**	80°C/ 48h[b]	**11** or **12**	337 / $[M+H]^+$	
11	**1 + 2a + 3b**	80°C/ 48h[b]	**11** or **12**	337 / $[M+H]^+$	
			15		348 / $[M+Na-H]^-$
12	**1 + 2a + 3b**	80°C/ 24h[b]	**10**	215 / $[M+H]^+$	
13	**1 + 2a + 3b**	80°C/ 24h[a]	**9**	317 / $[M+Na]^+$	293 / $[M-H]^-$
			13 or **14**	597 / $[M+H]^+$	
14	**1 + 2a**	80°C/ 24h[a]	**10**	237 / $[M+Na]^+$	
			2d	127 / $[M+H]^+$	
15*	**1 + 2b + 3b**	80°C/ 48h[a]	**5**		294 / $[M-H]^-$
			7		295 / $[M-H]^-$
			12		337 / $[M-H]^-$
			13 and **14**		573 / $[M-H]^-$
16*	**7s + 3a**	80°C/ 48h[a]	**13** and **14**		573 / $[M-H]^-$

a) dry conditions; b) neat conditions; *analysed by UPLC-HRMS

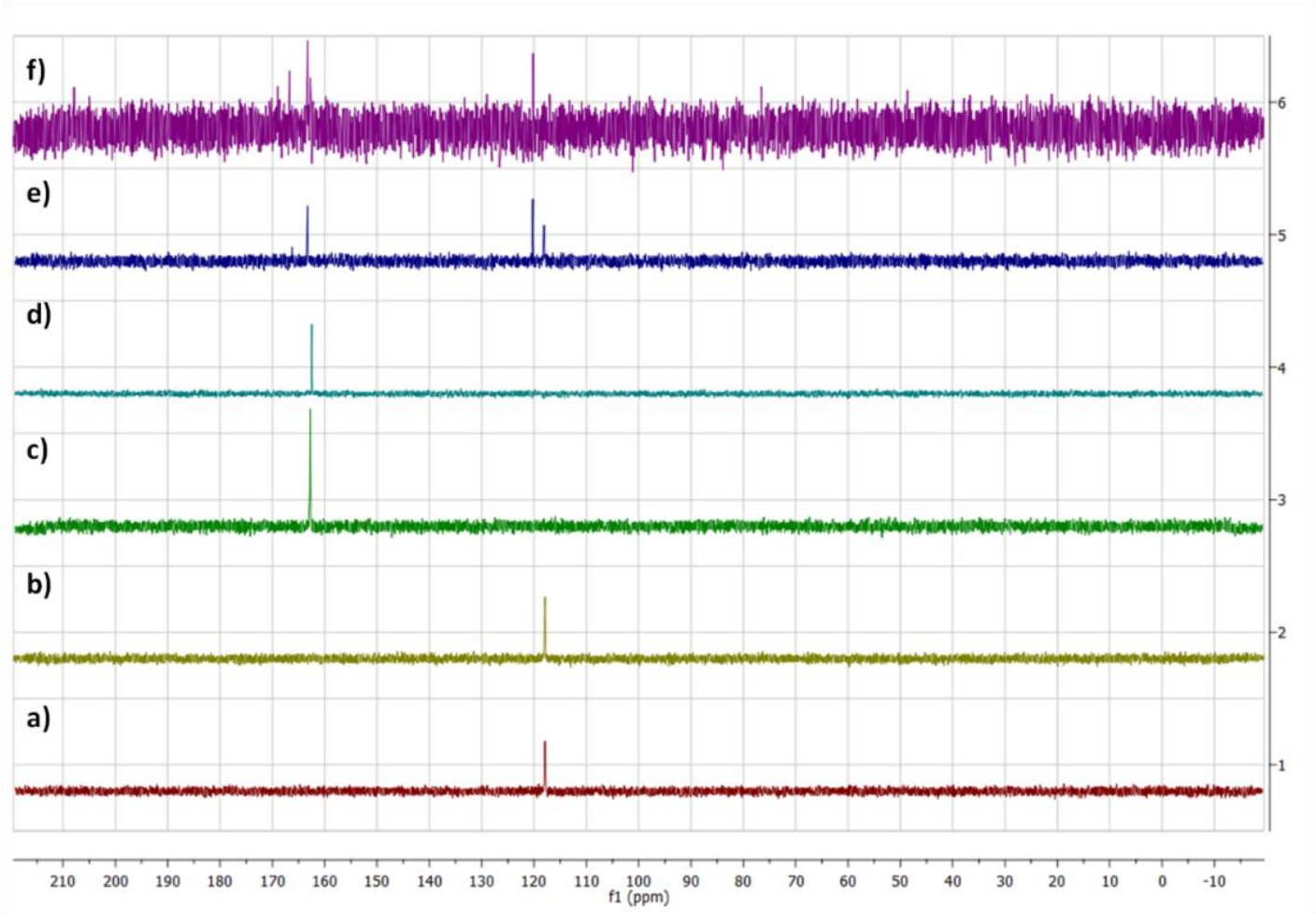


Figure S1. ^{13}C NMR (H_2O D_2O 90:10 *v/v*, 75 MHz) of the crude mixture obtained in control experiments carried out to monitor cyanamide (**2a**) transformation under prebiotic conditions. Values were expressed in ppm. Reference peak: cyanamide at 118.1. Pure compounds were dissolved as purchased. **a**) **2a** + **3a** ($NH_4H_2PO_4$) molar ratio 1:1 (1.0 mM) kept at 25°C for 24h: no conversion; **b**) **2a** + **3a** molar ratio 1:1 (1.0 mM) kept at 40°C for 24h: no conversion; **c**) urea (**2b**): 163.2 ppm, commercial from Sigma Aldrich; **d**) **2a** + **3a** molar ratio 1:1 (1.0 mM) kept at 80°C for 24h, total conversion of **2a** into **2b**; **e**) **2a**, 1.0 mM kept at 80°C for 24h, conversion into cyanoguanidine (**2c)** (120.0 and 163.3 ppm) and melamine (**2d**) (166.0 ppm). f) mix of commercial samples of **2a**, **2b**, **2c** and **2d** with molar ratio 1:1:1:1, signal of urea **2b** at 163.2 ppm close to that of cyanoguanidine **2c** at 163.4 ppm.

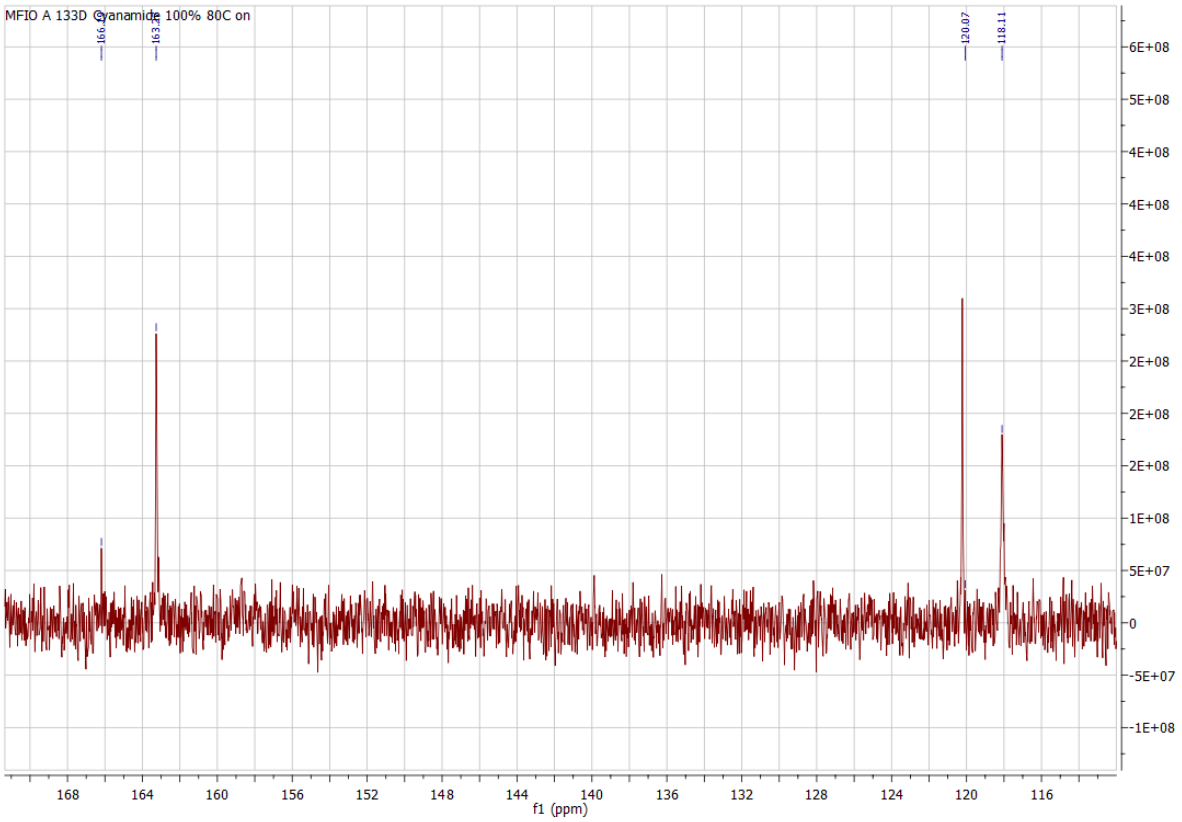


Figure S2. ^{13}C NMR (H_2O D_2O 90:10 *v/v*, 75 MHz) of the crude mixture obtained in control experiments carried out to monitor cyanamide (**2a**) transformation under prebiotic conditions. Values were expressed in ppm. Reference peak: cyanamide at 118.1. Zoom of Fig. S1e) **2a**, 1.0 mM kept at 80°C for 24h, conversion into cyanoguanidine (**2c**) (120. and 163.3 ppm) and melamine (**2d**) (166 ppm). No hydrolysis of cyanamide in urea occurred.

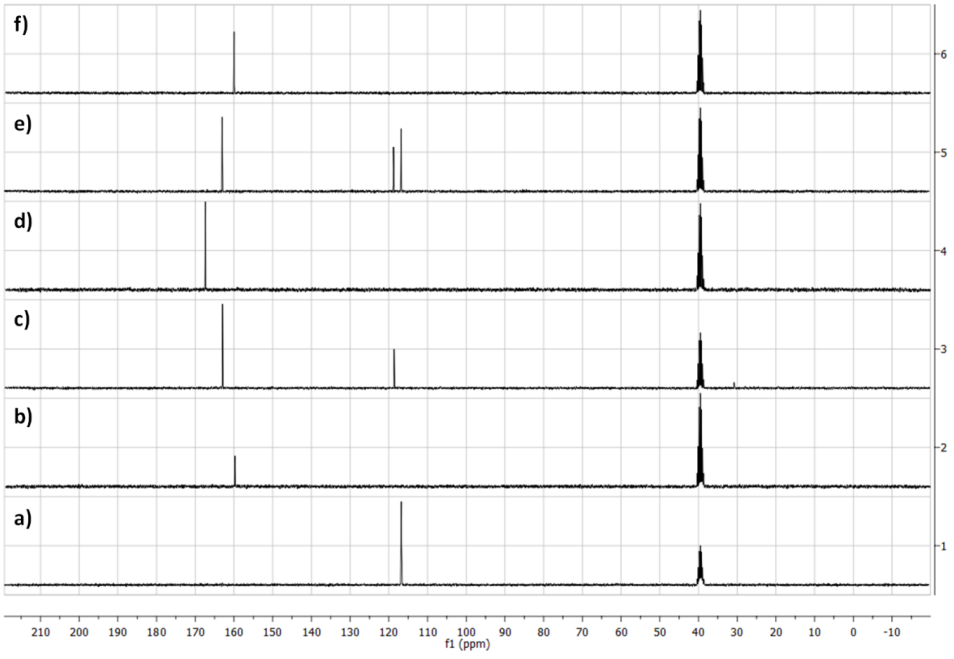


Figure S3. ^{13}C NMR (DMSO-d_6, 75 MHz) of the crude mixture obtained in control experiments carried out to monitor cyanamide (**2a**) transformation under prebiotic conditions. Values were expressed in ppm. Reference peak DMSO-d_6 : 39.51 ppm. Pure compounds were dissolved as purchased. (**a**) cyanamide: 117.2 ; (**b**) urea: 159.3; (**c**) cyanoguanidine 119.1 and 163.4; (**d**) melamine: 167.4; (**e**) reaction mixture containing only **2c** together with **2a**, obtained from heating **2a** at pH 7 to 80°C for 24hrs. (**f**) Total conversion of **2a** into **2b** at pH 4.5 was observed when **2a** was heated at 80°C for 24h.

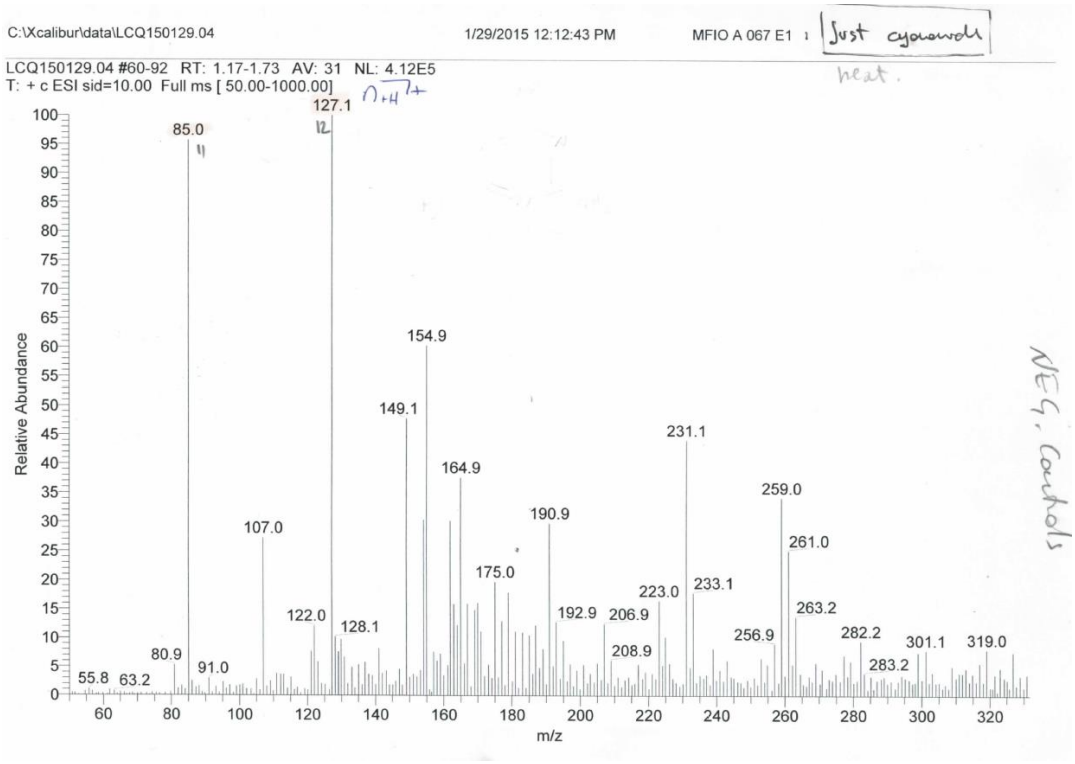


Figure S4: ESI-MS in positive ion mode of the sample presented in Figure S3. Cyanamide (**2a**, as 1.0 mM water solution) was kept at 80°C for 24h. Conversion into cyanoguanidine (**2c**, *m/z* 85.0) and melamine (**2d**, *m/z* 127.1) were recorded as $[M+H]^+$. No highter molecular mass cyanamide products were recorded.

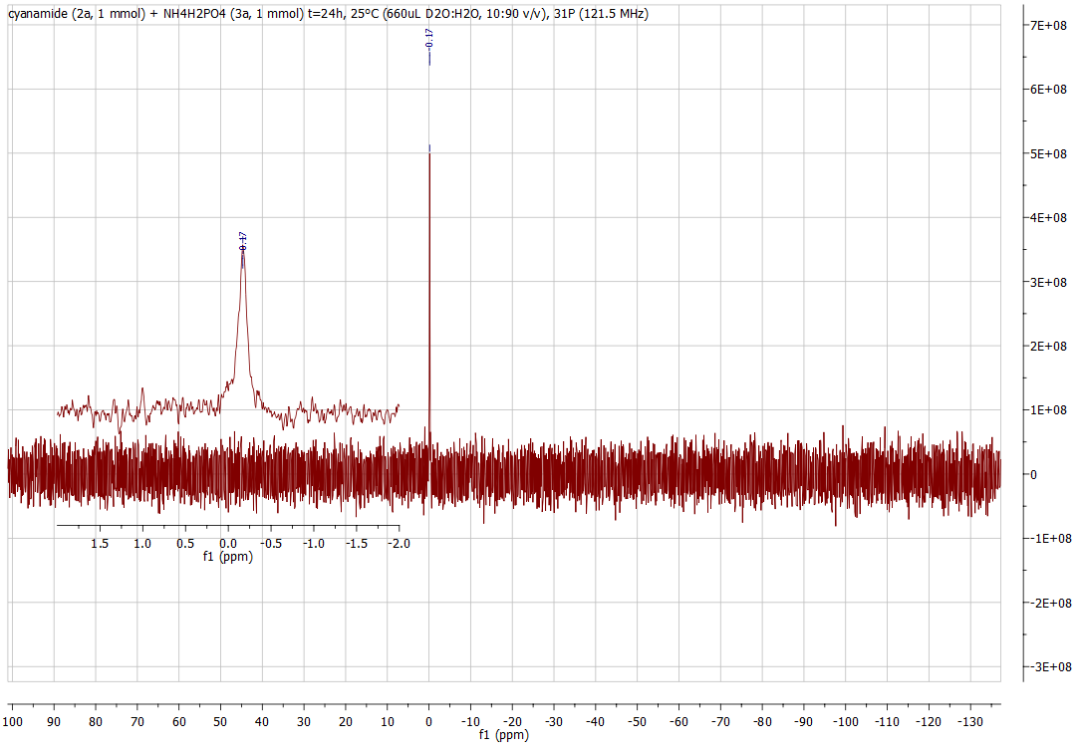


Figure S5: ^{31}P NMR (H_2O:D_2O 9:1 *v/v*, 121.5 MHz) of cyanamide (**2a**, 1 mmol, 42 mg) together with ammonium dihydrogen phosphate (**3a**, 1 mmol, 115 mg) kept at 80°C for 24 hrs at pH 4.5. No other phosphorous species than $H_2PO_4^-$ (–0.17 ppm) were present in the sample.

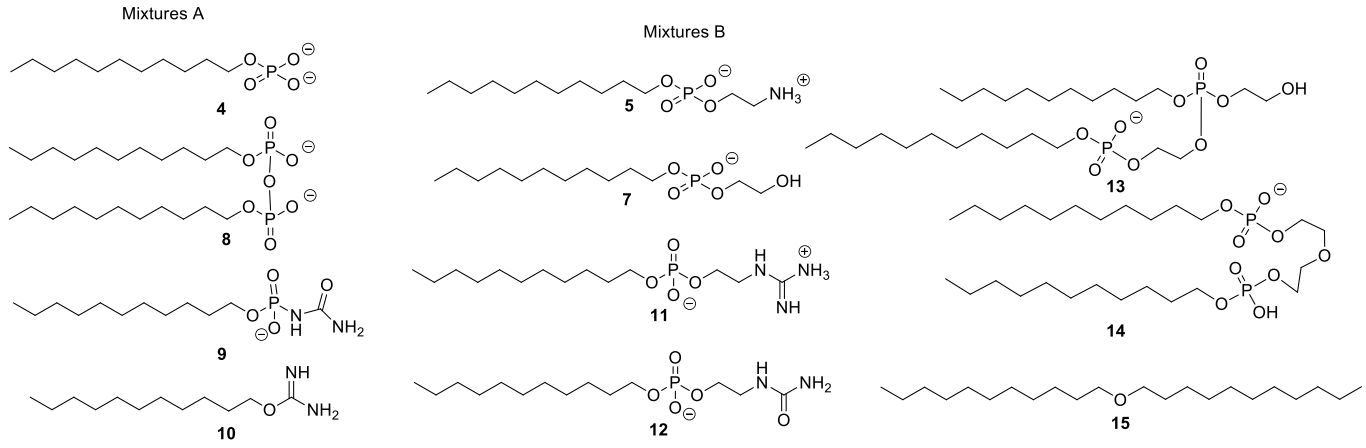


Figure S6. Assumed structures of the products of Mixture A (**4–10**) and Mixture B (**5**, **7** and **10-15**) obtained using cyanamide (**2a**) or urea (**2b**) as condensing agents. Phosphorylation occurred in the presence of added ammonium dihydrogen phosphate (**3a**) or 2-aminoethyl phosphate (**3b**). The structures **4–15** are consistent with the ESI-MS and/or UPLC-HRMS analyses of the crude mixtures.

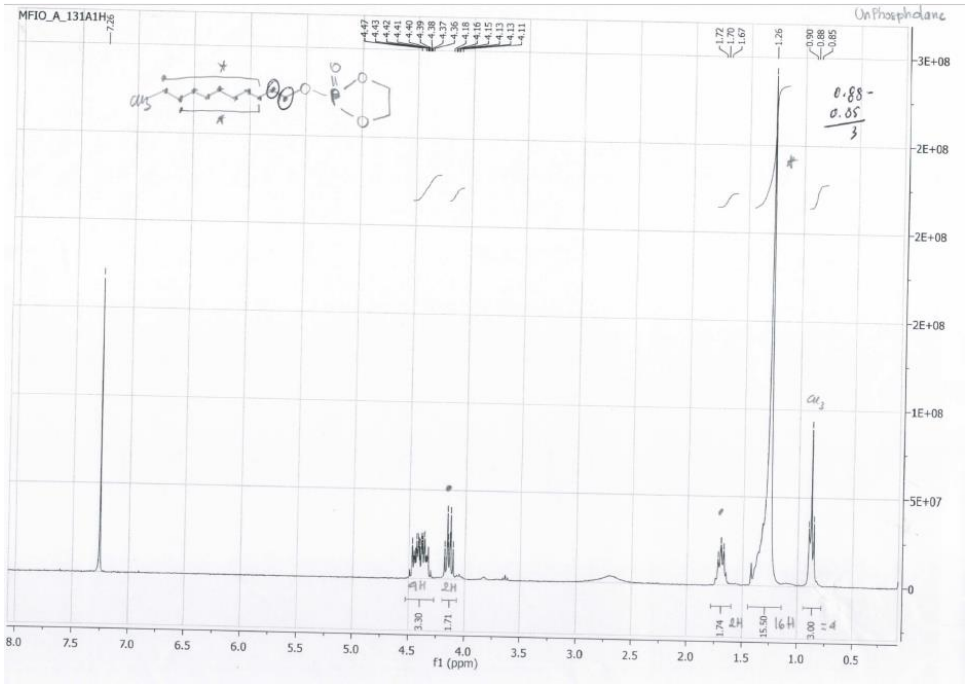


Figure S7: ^{1}H NMR ($CDCl_3$, 300MHz) of product **6**.

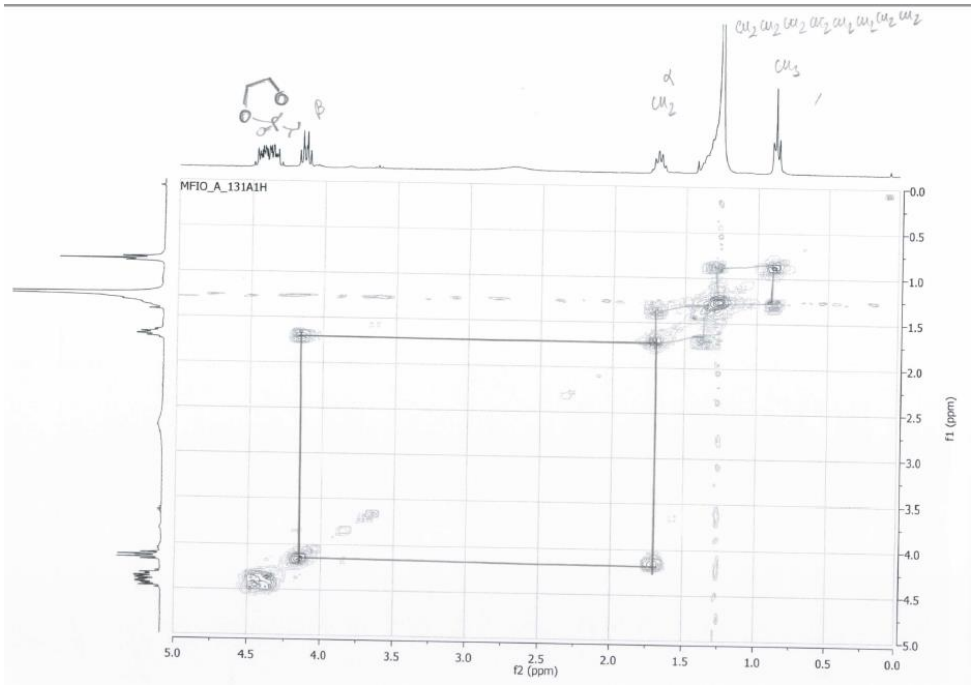


Figure S8: COSY ($CDCl_3$, 300MHz) of product **6**.

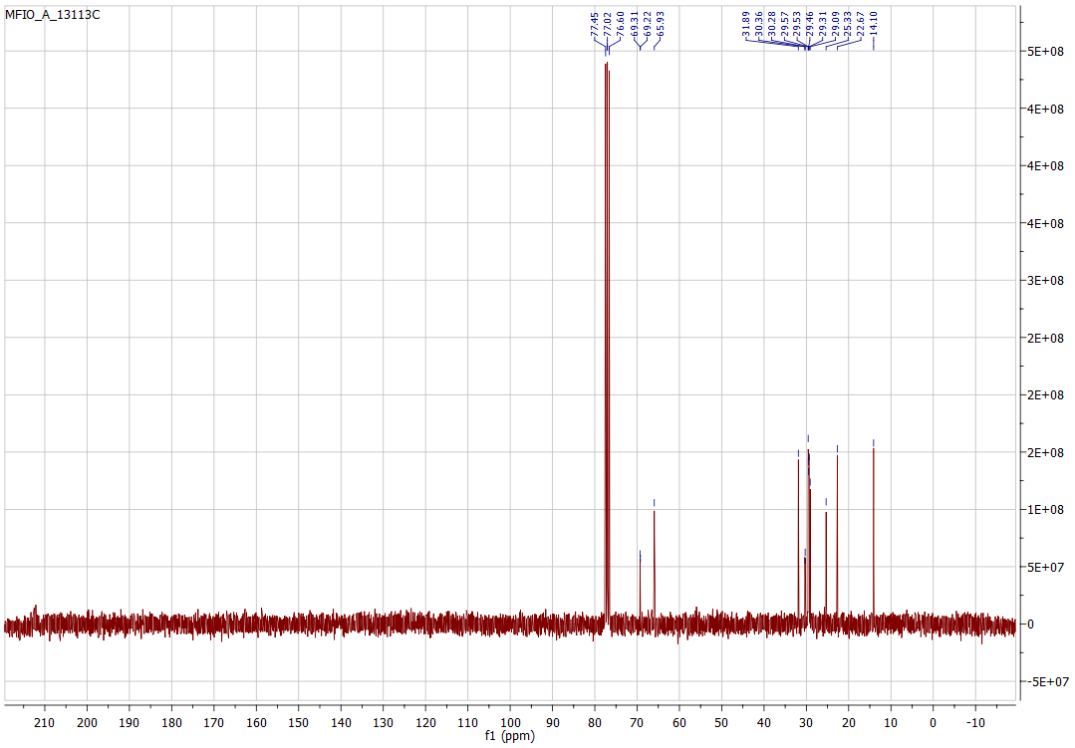


Figure S9: ^{13}C NMR ($CDCl_3$, 75MHz) of product **6**.

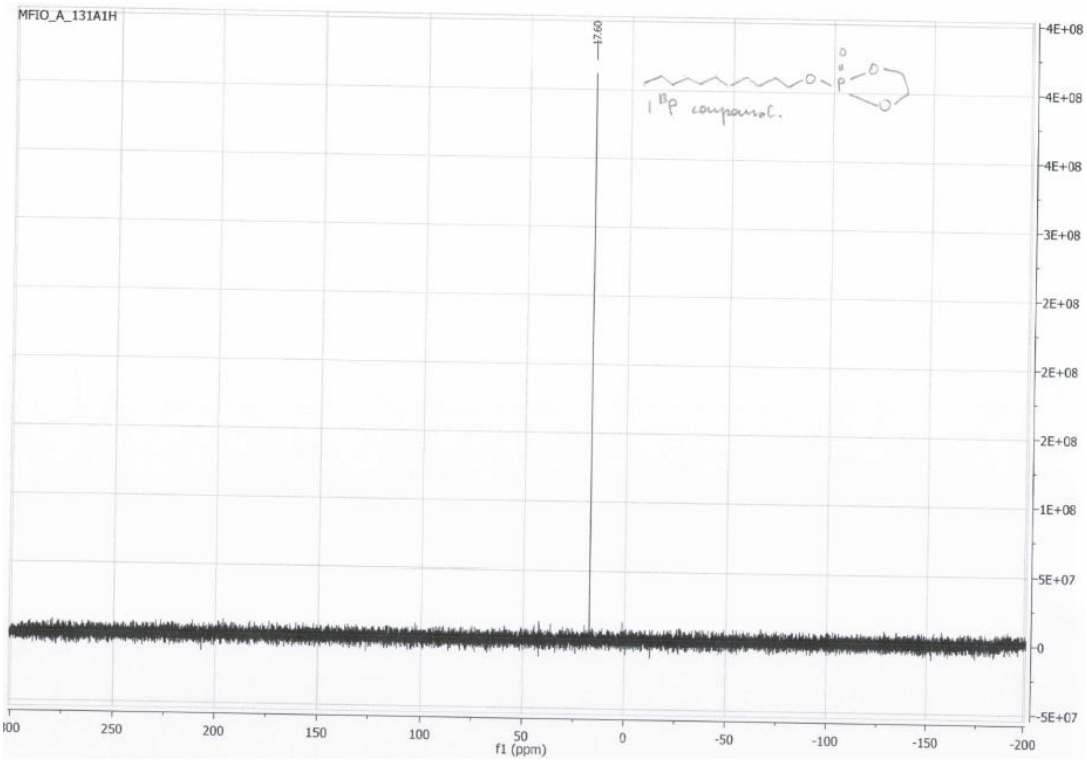


Figure S10: ^{31}P NMR ($CDCl_3$, 121.5 MHz) of product **6**

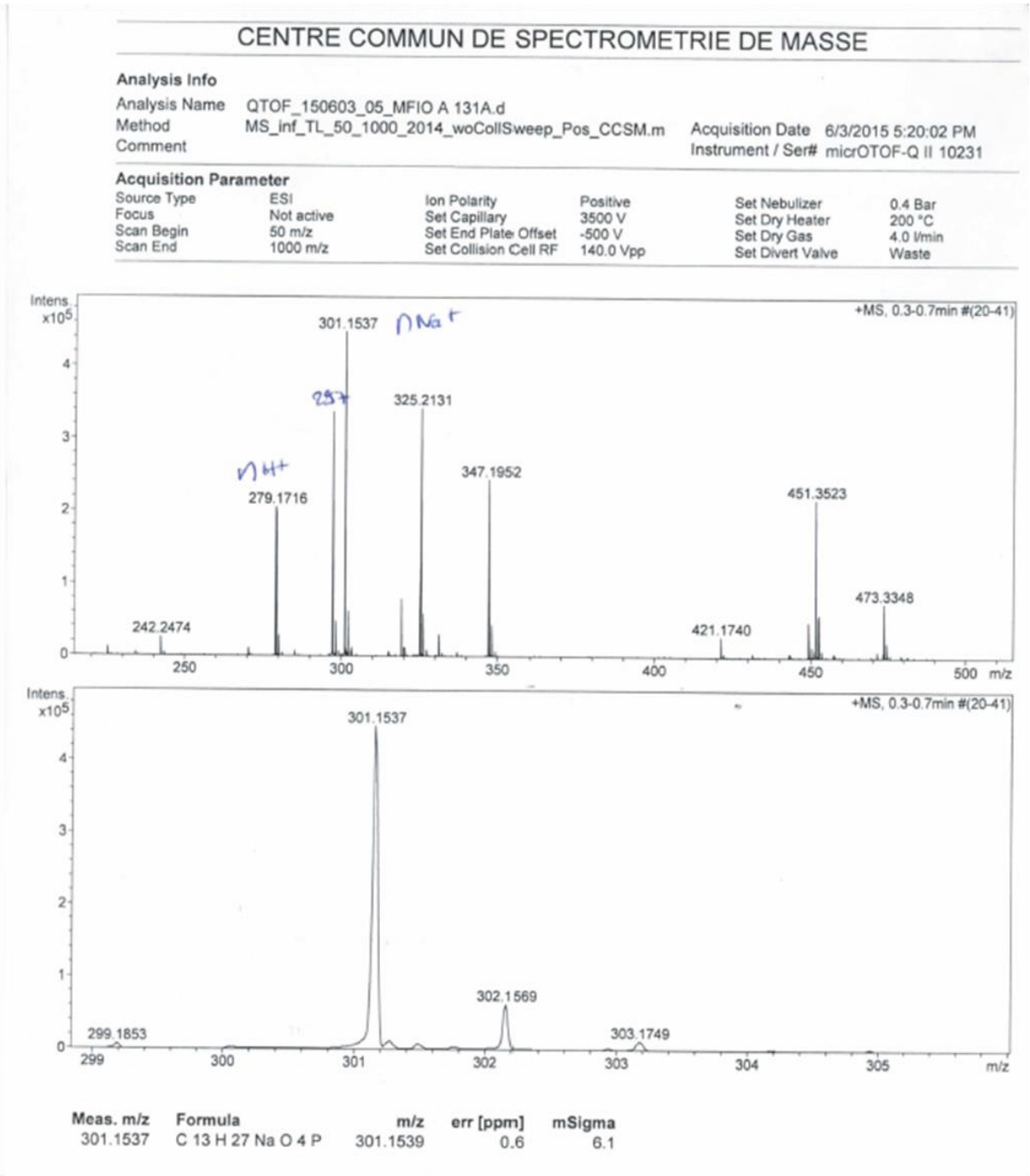


Figure S11: HR-MS of product **6**. Upper lane: full spectrum; lower lane: zoom into $[M+Na]^+$ adduct.

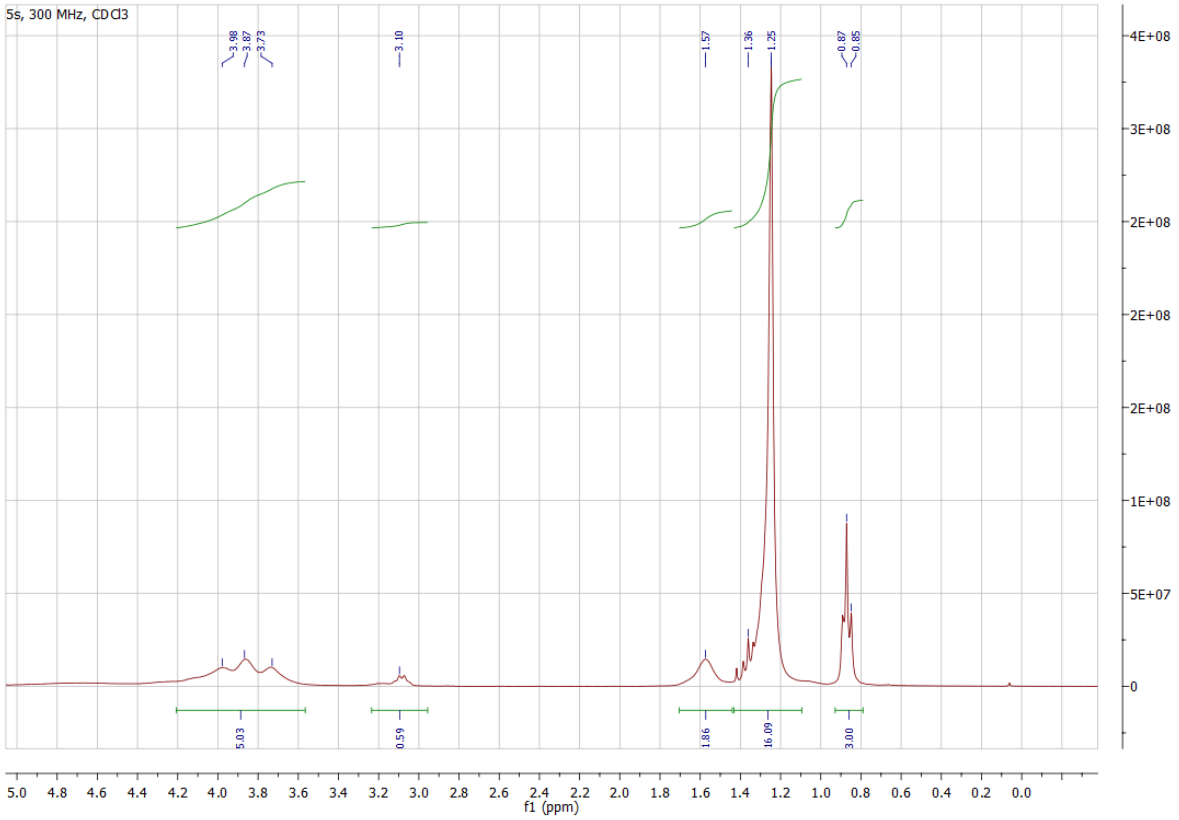


Figure S12 - ^{1}H NMR ($CDCl_3$, 300MHz) of product **5s**

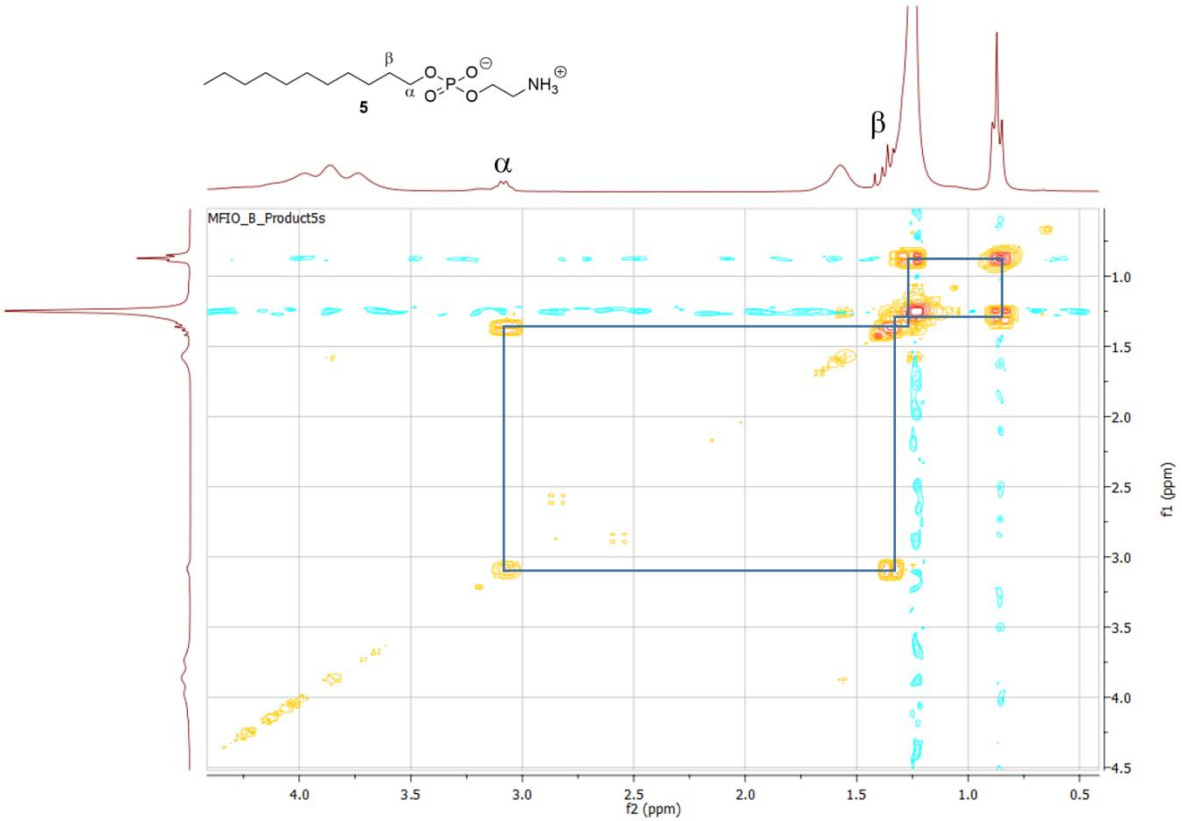


Figure S13: COSY ($CDCl_3$, 300MHz) of product **5s**.

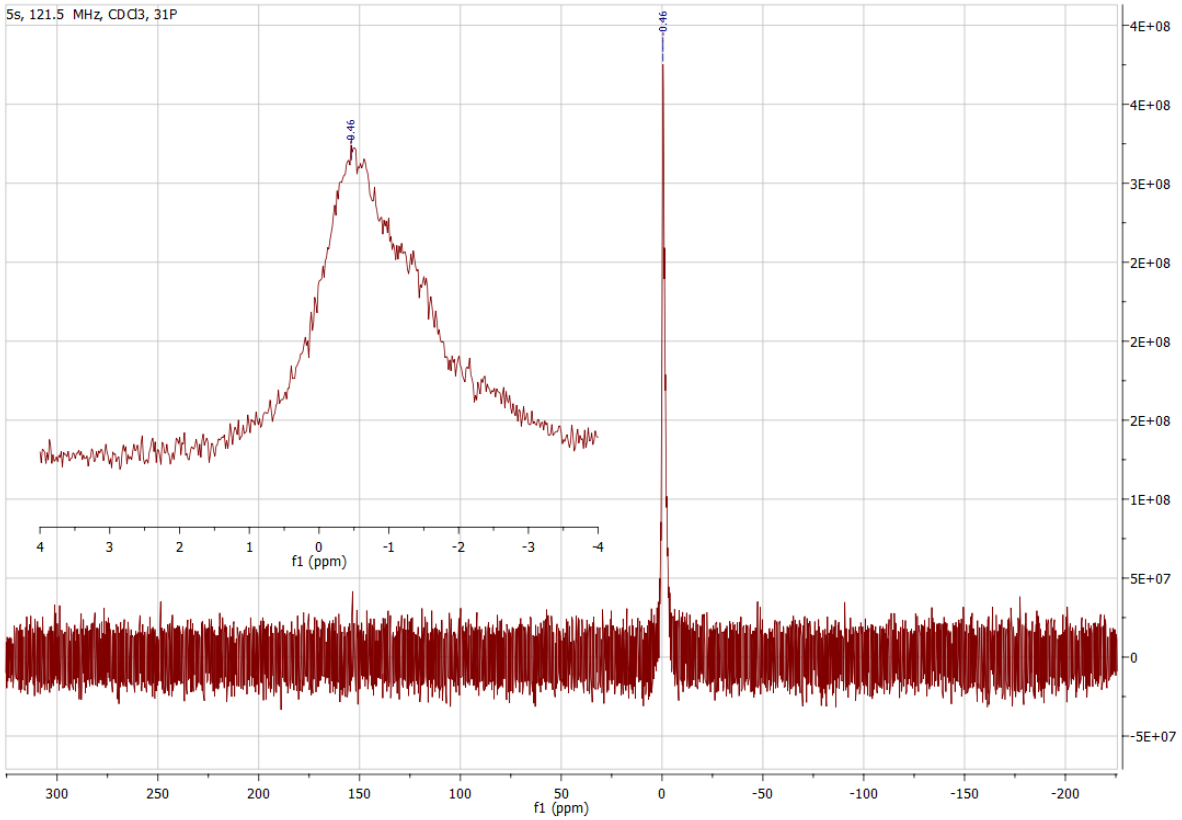


Figure S14: ^{31}P NMR ($CDCl_3$, 121.5 MHz) of product **5s**

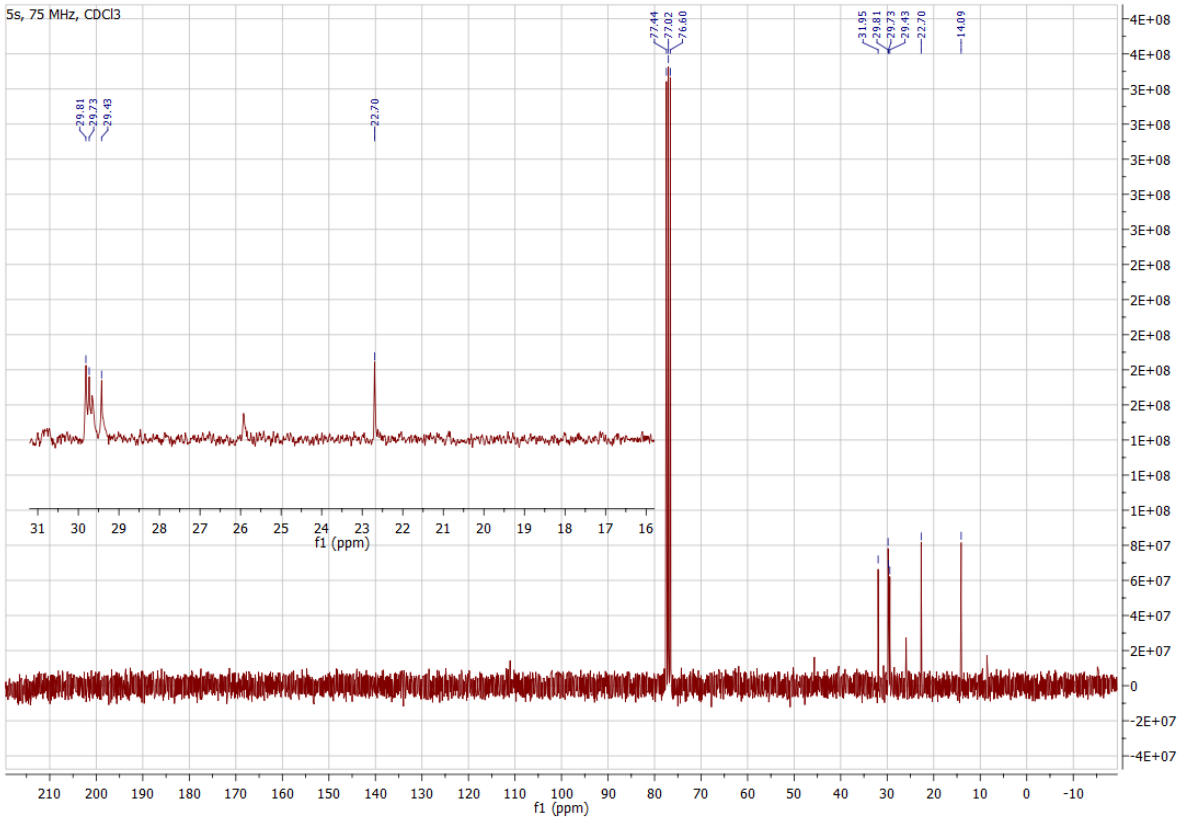


Figure S15 : ^{13}C NMR ($CDCl_3$, 75MHz) of product **5s** (small amounts of **7s** may be present in the sample).

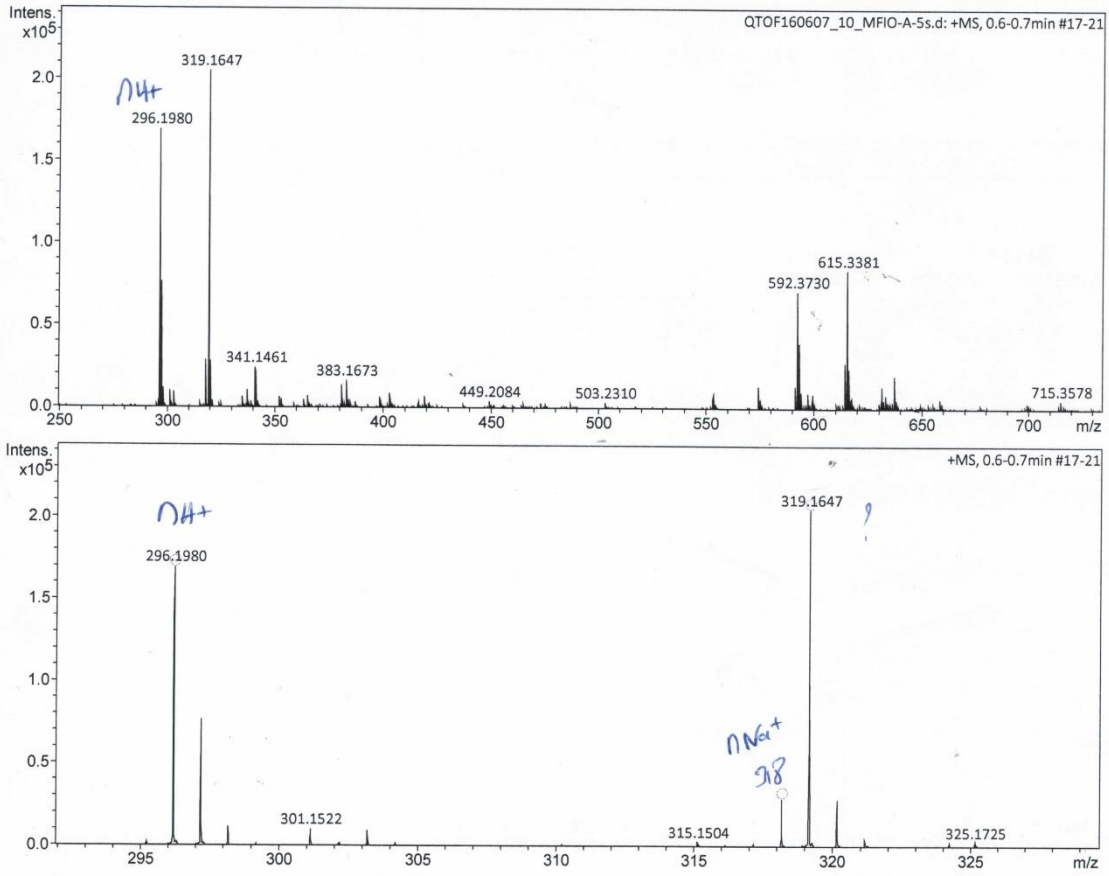
CENTRE COMMUN DE SPECTROMETRIE DE MASSE

Analysis Info

Analysis Name	QTOF160607_10_MFIO-A-5s.d		
Method	2016_03_17_Infusion_50-1000_pos.m	Acquisition Date	6/7/2016 3:47:22 PM
Comment		Instrument / Ser#	micrOTOF-Q 228888.10 231

Acquisition Parameter

Source Type	ESI	Ion Polarity	Positive	Set Nebulizer	0.4 Bar
Focus	Active	Set Capillary	3500 V	Set Dry Heater	200 °C
Scan Begin	50 m/z	Set End Plate Offset	-500 V	Set Dry Gas	4.0 l/min
Scan End	1000 m/z	Set Collision Cell RF	400.0 Vpp	Set Divert Valve	Waste



Meas. m/z	Ion Formula	m/z	err [ppm]	mSigma
296.1980	C13H31NO4P	296.1985	1.7	n.a.
318.1800	C13H30NNaO4P	318.1805	1.5	n.a.
319.1647	C13H29NaO5P	319.1645	-0.6	5.0

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Figure S16: HR-MS of product **5s** (traces of **7s** as $[M+Na]^+$ are present in the sample).

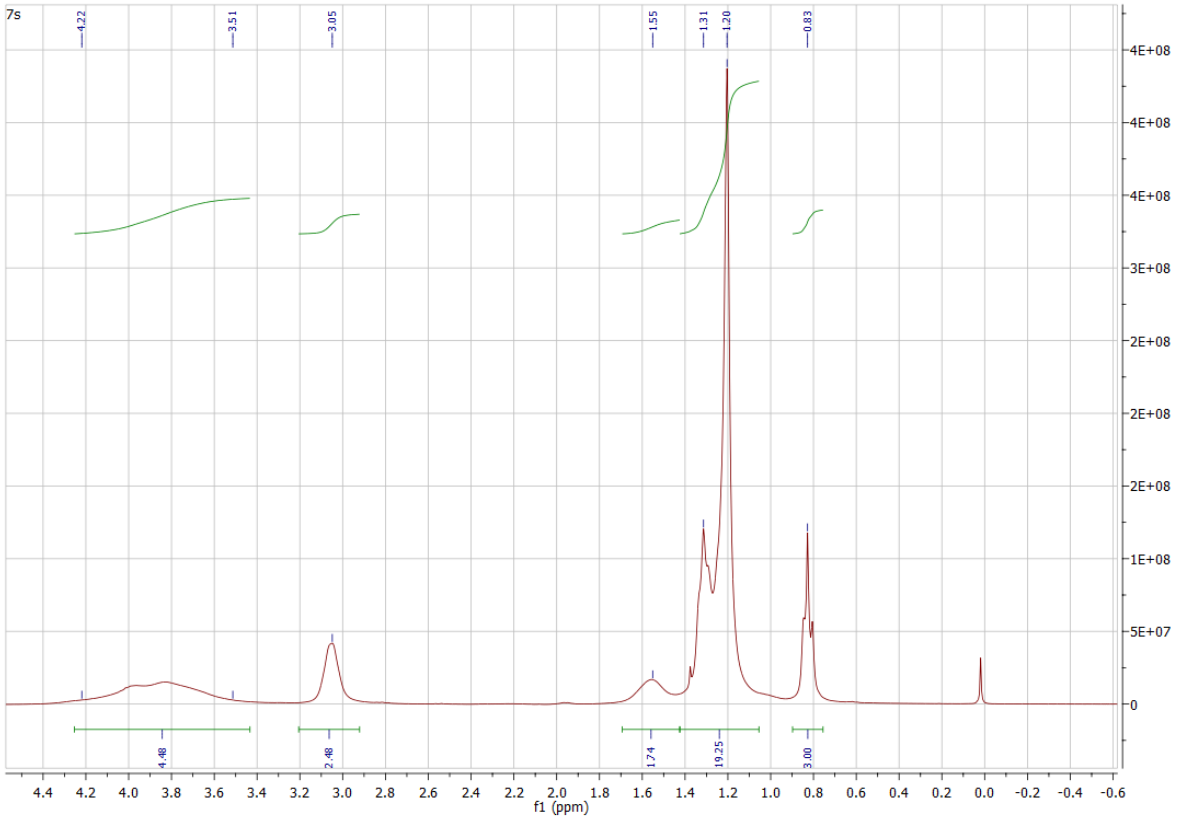


Figure S17: ^{1}H NMR ($CDCl_3$, 300MHz) of product **7s**.

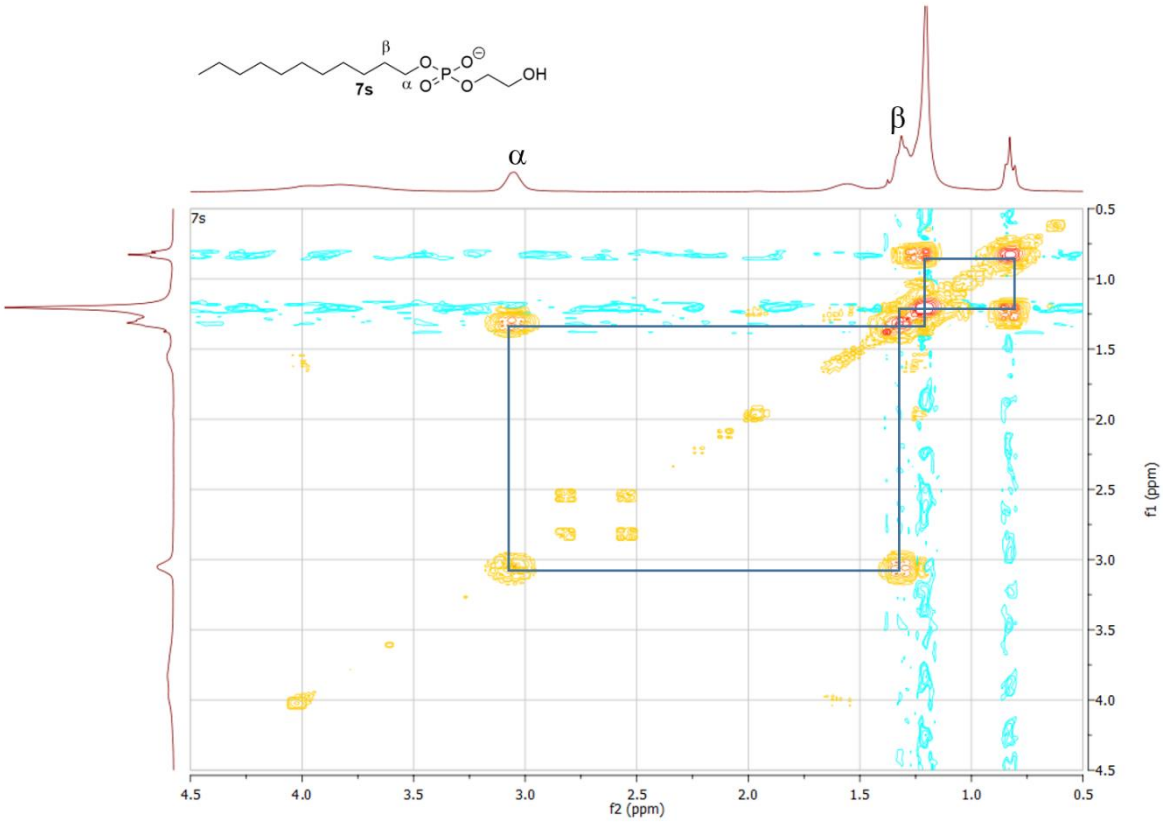


Figure S18: COSY ($CDCl_3$, 300MHz) of product **7s**.

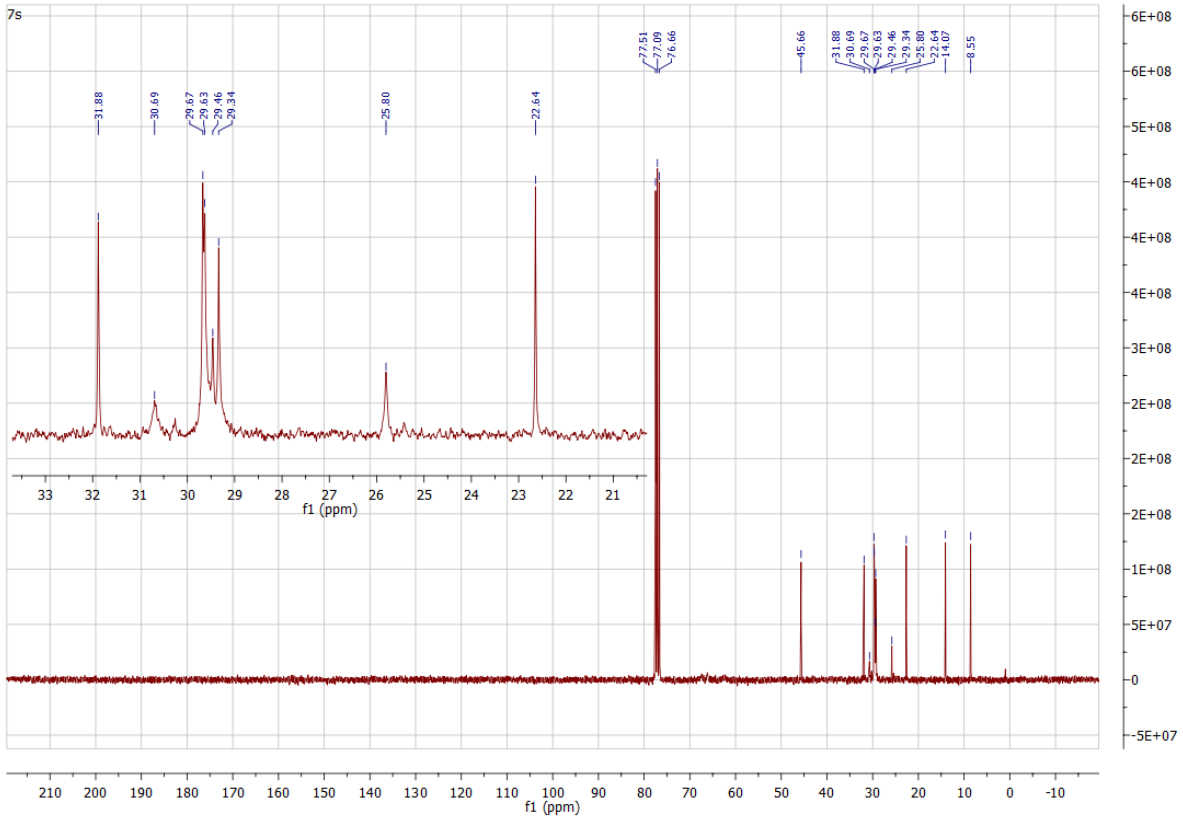


Figure S19: ^{13}C NMR ($CDCl_3$, 75MHz) of product **7s**.

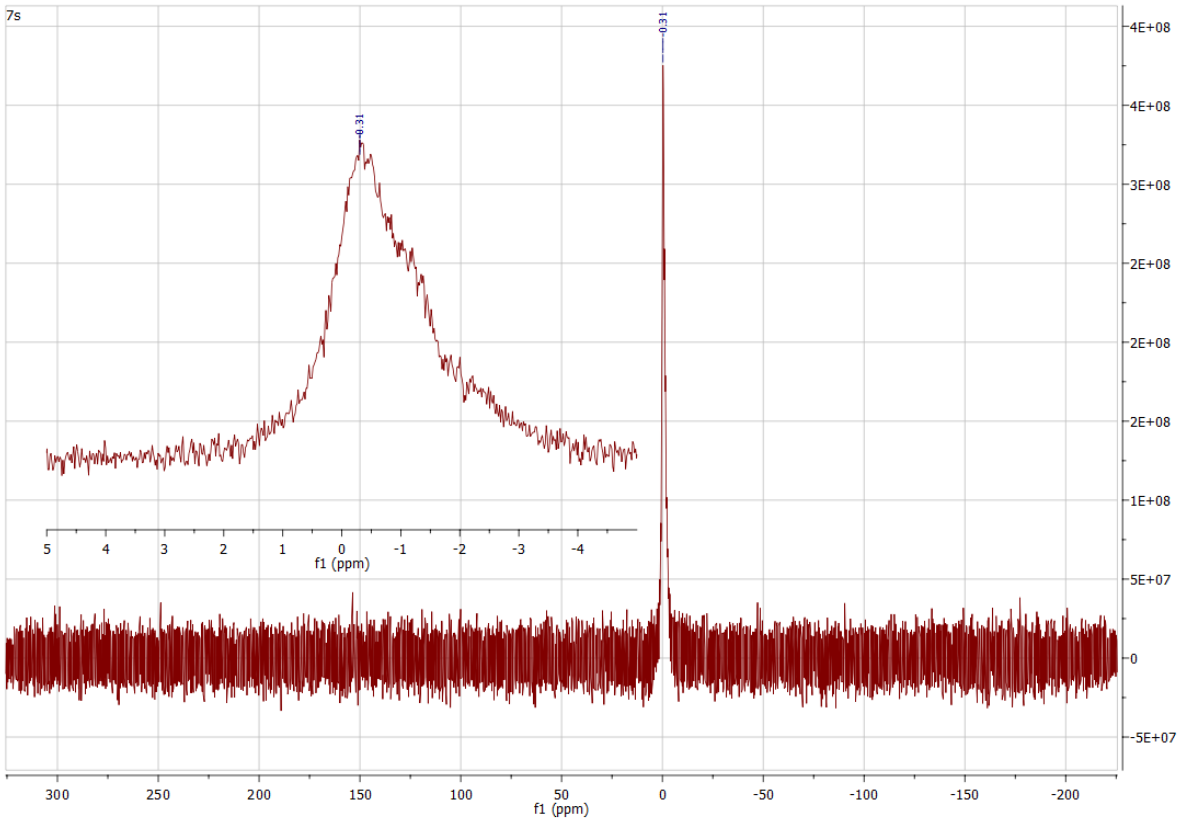


Figure S20: ^{31}P NMR ($CDCl_3$, 121.5 MHz) of product **7s**.

I

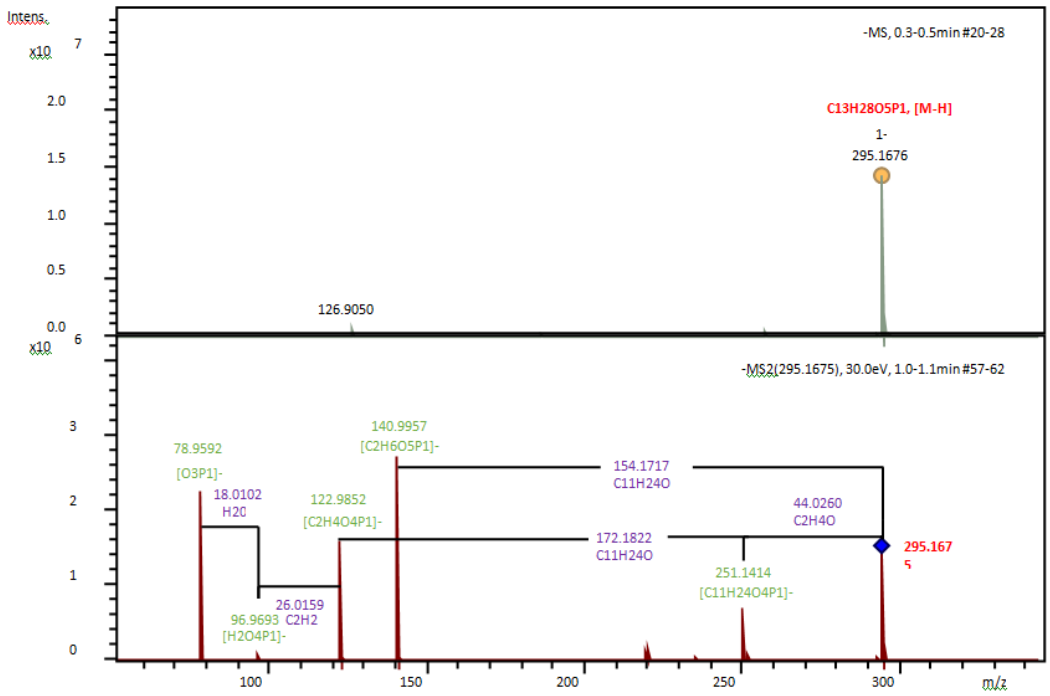


Figure S21: HR-MS of product **7s**.

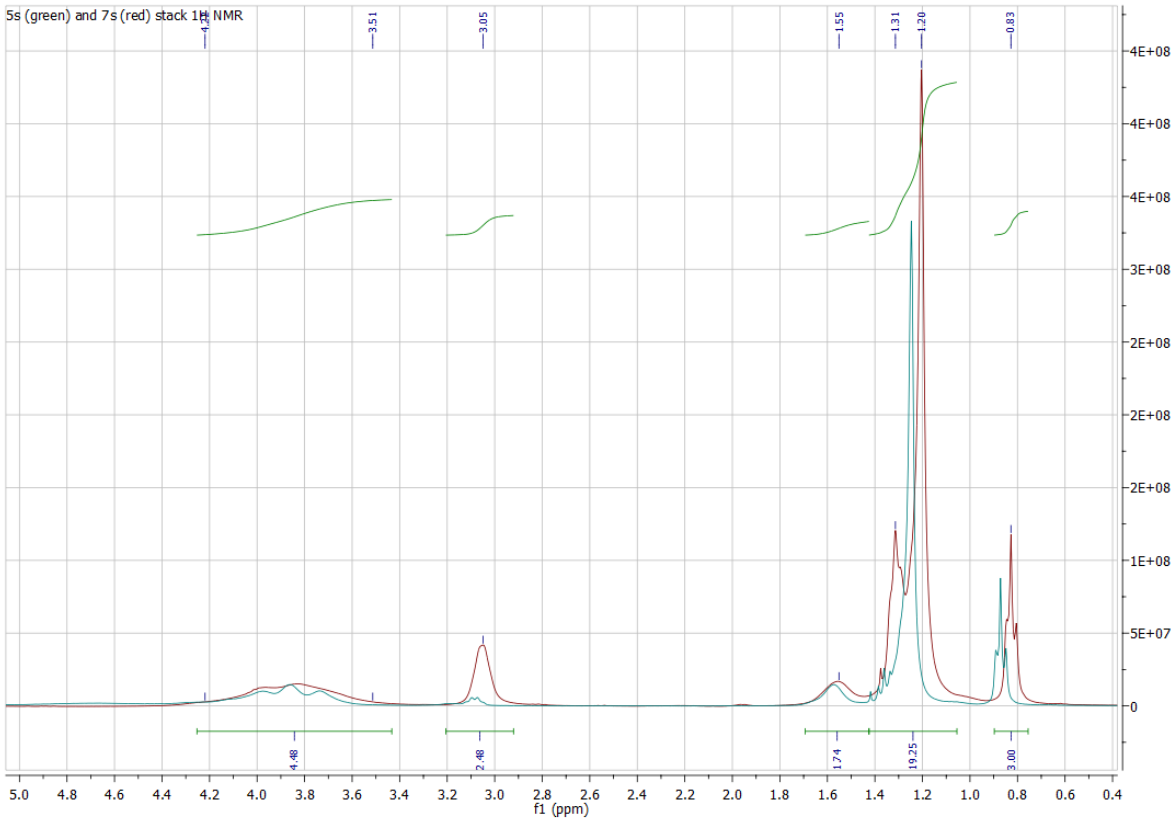


Figure S22: Superposition of ^{1}H NMR spectra of **5s** (green) and **7s** (red) in $CDCl_3$.

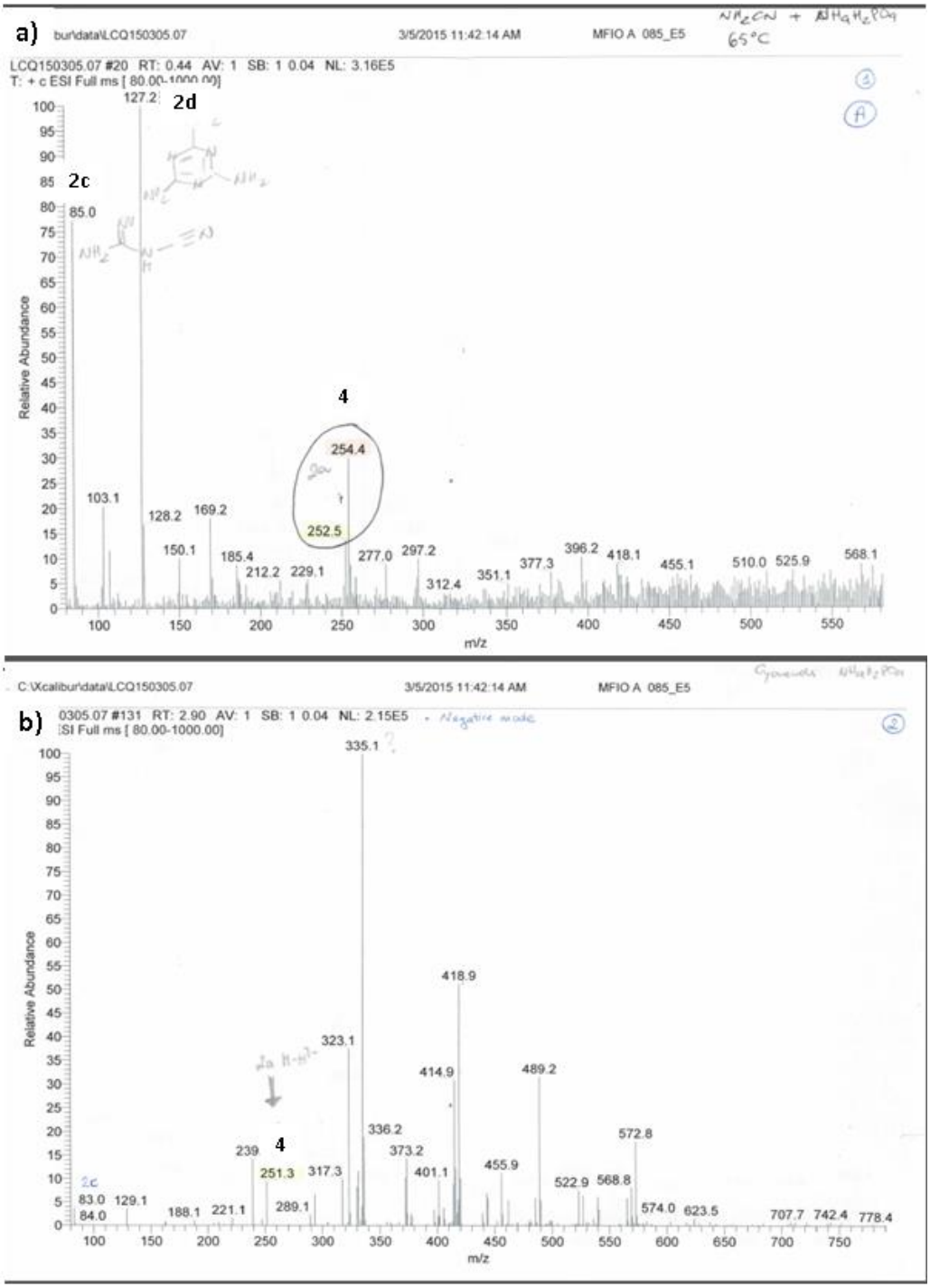


Figure S23: ESI-MS of crude reaction mixture obtained by heating, from wet to dryness, of **1 + 2a + 3a** at 65°C for 24 hr (Table S2, entry 1). Top image: Positive ion mode. Bottom image: Negative ion mode.

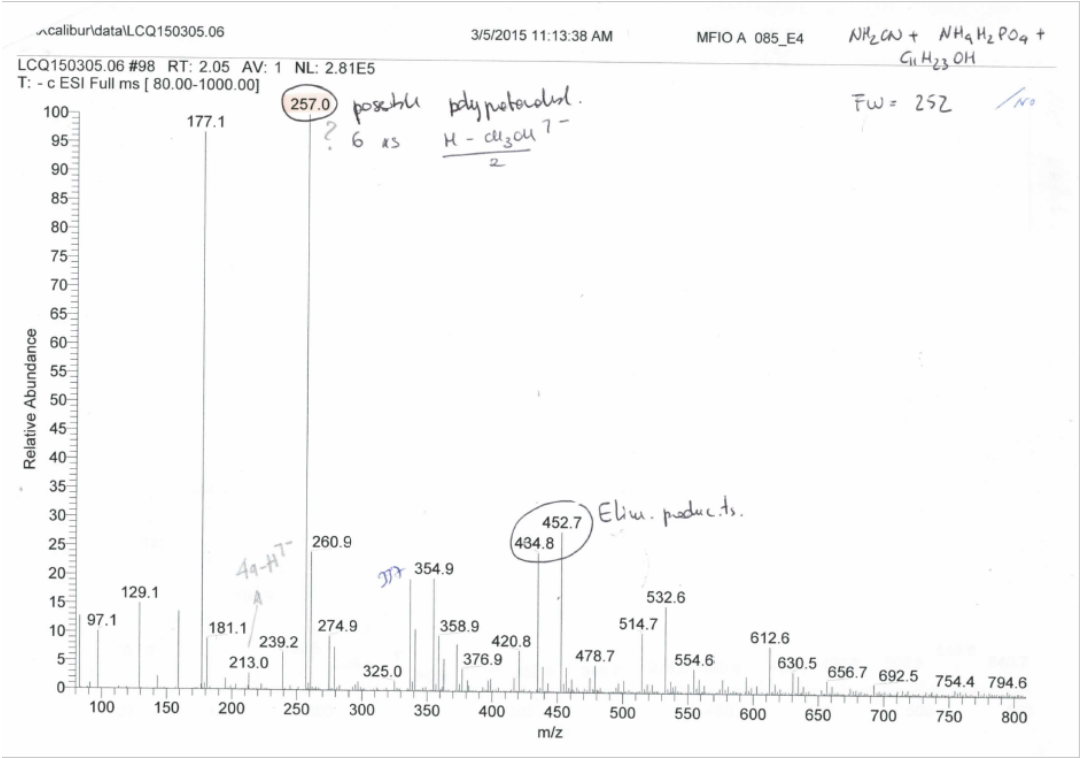


Figure S24: ESI-MS (negative ion mode) of crude reaction mixture obtained by heating **1 + 2a + 3a** at 65°C for 24h under neat conditions (Table S2, entry 2).

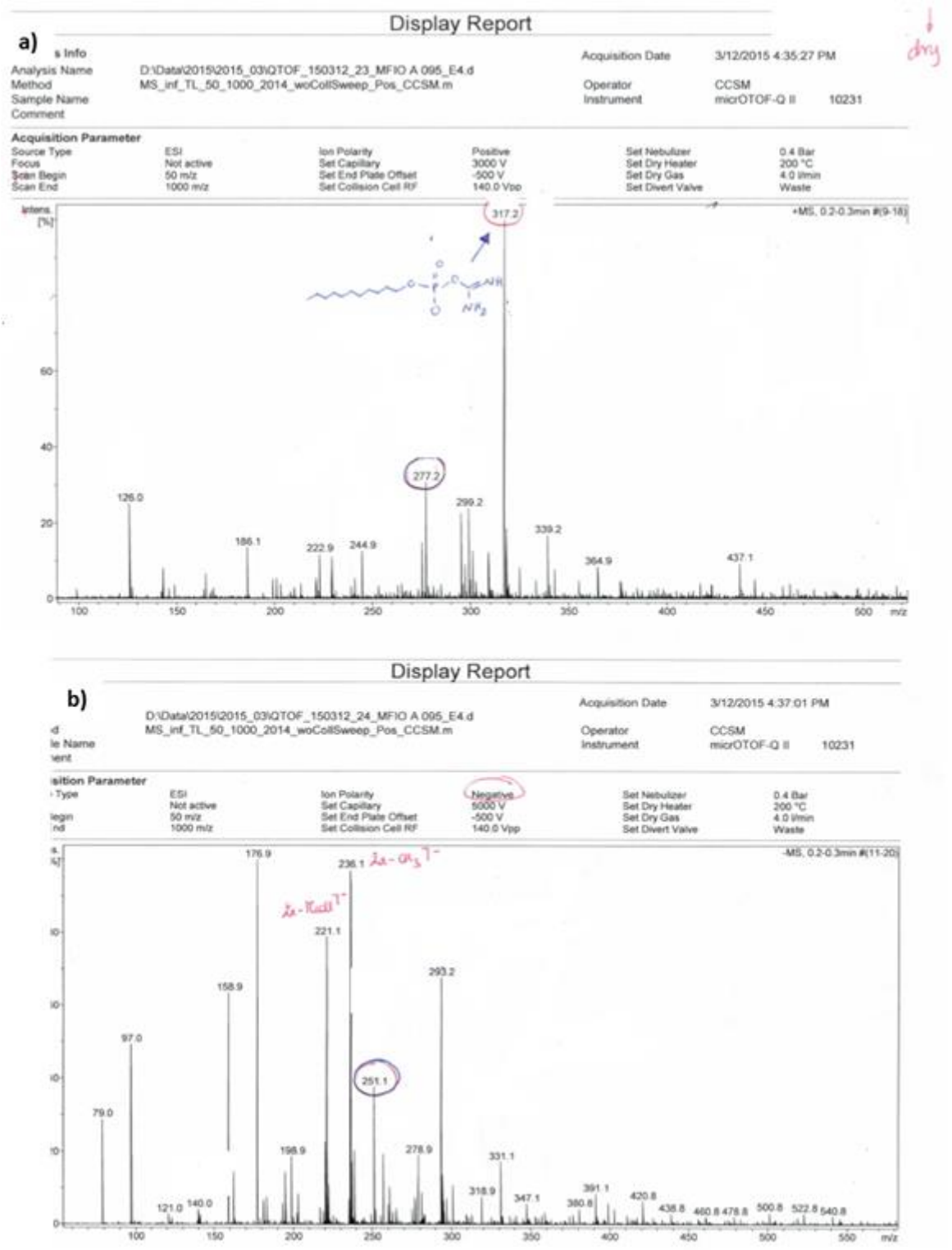


Figure S25: ESI-MS of crude reaction mixture obtained by heating **1 + 2a + 3a** at 100°C for 24h, from wet to dryness (Table S2, entry 3). Top image: Positive ion mode. Bottom image: Negative ion mode.

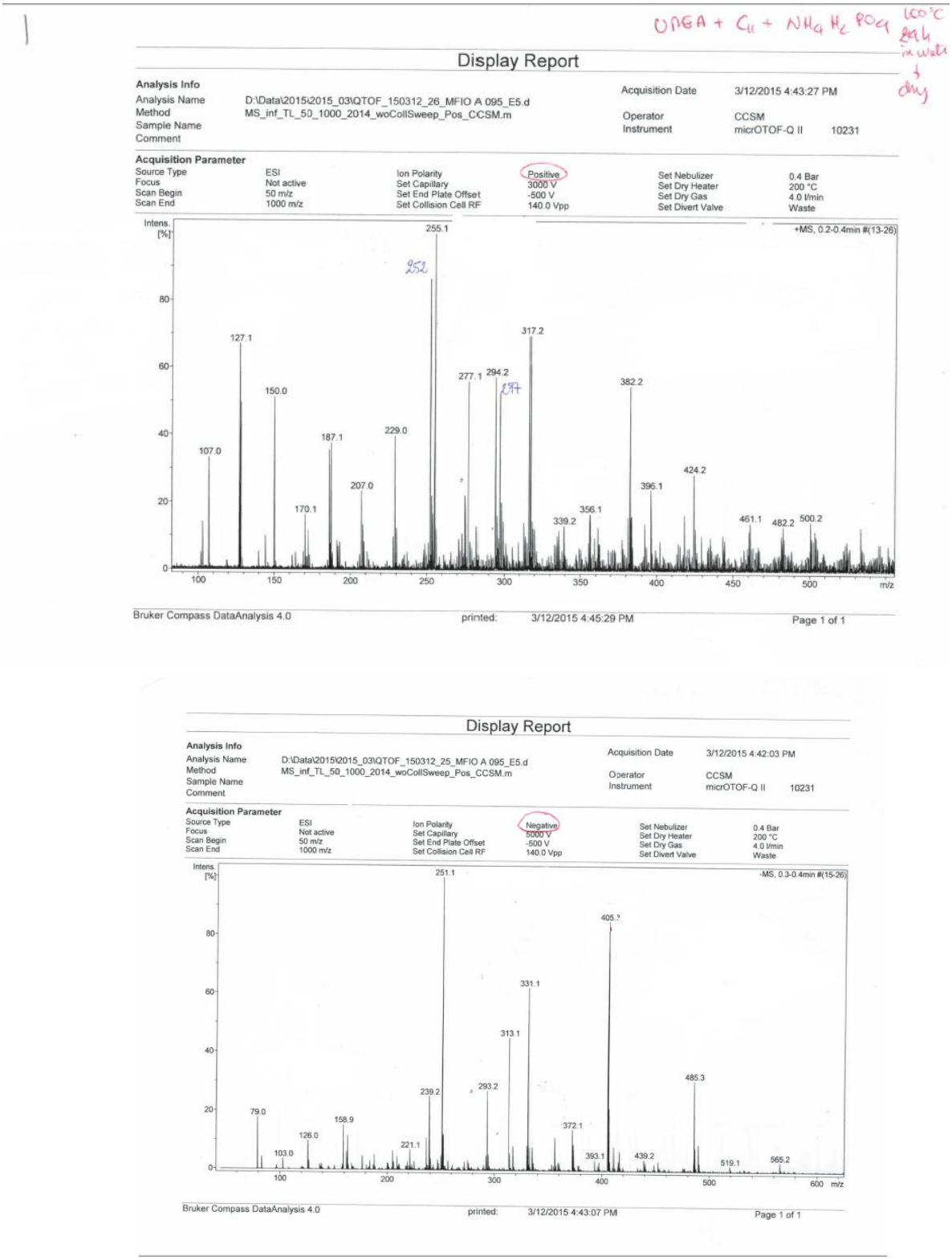


Figure S26: ESI-MS of crude reaction mixture obtained by heating **1 + 2b + 3a** at 100°c for 24h form wet to dryness (Table S2, entry 4). Top image: Positive ion mode. Bottom image: Negative ion mode.

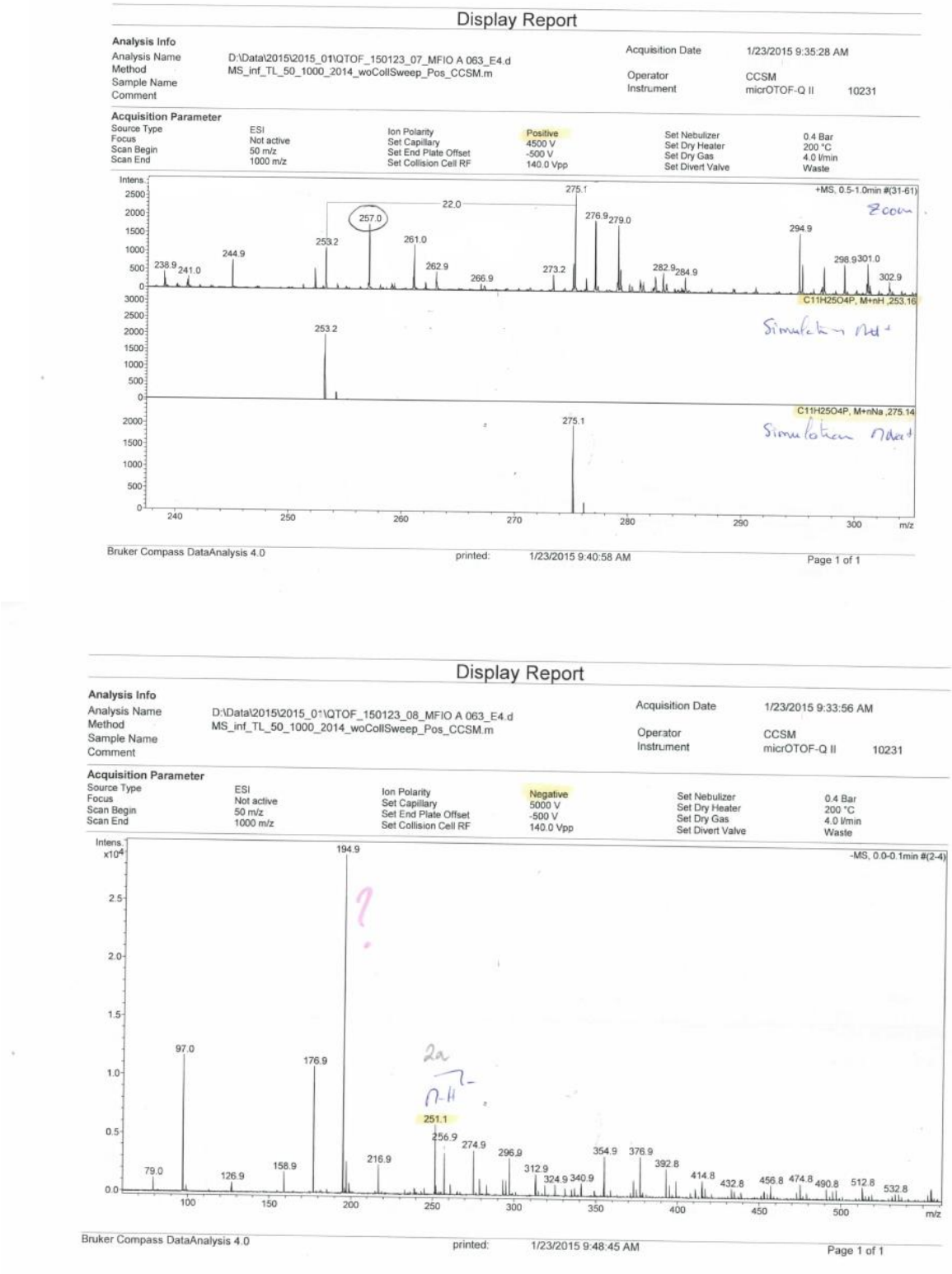


Figure S27: ESI-MS of crude reaction mixture obtained by heating **1 + 2b + 3a** at 80°C for 24h in neat (Table S2, entry 5). Top image: Positive ion mode. Bottom image: Negative ion mode.

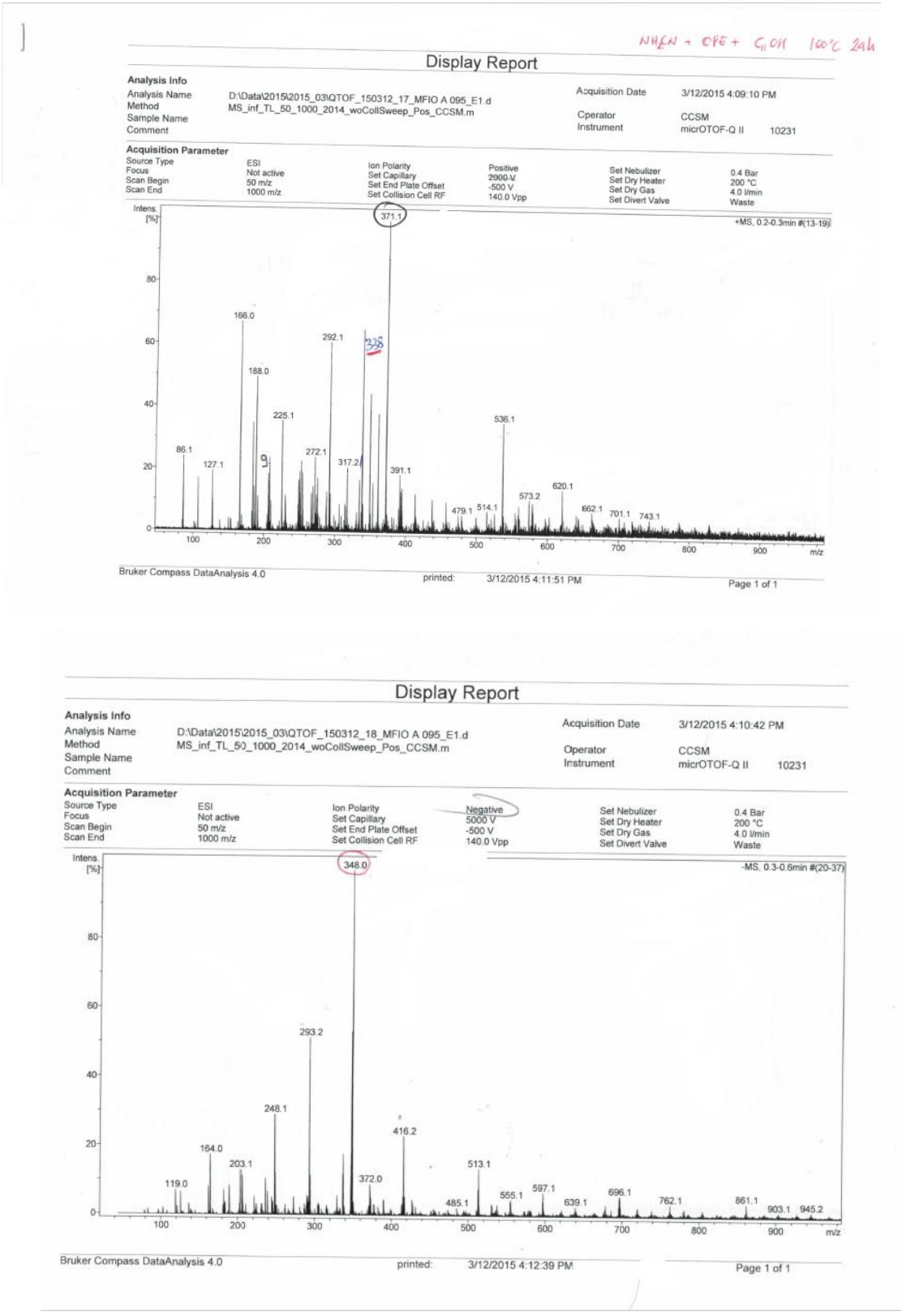
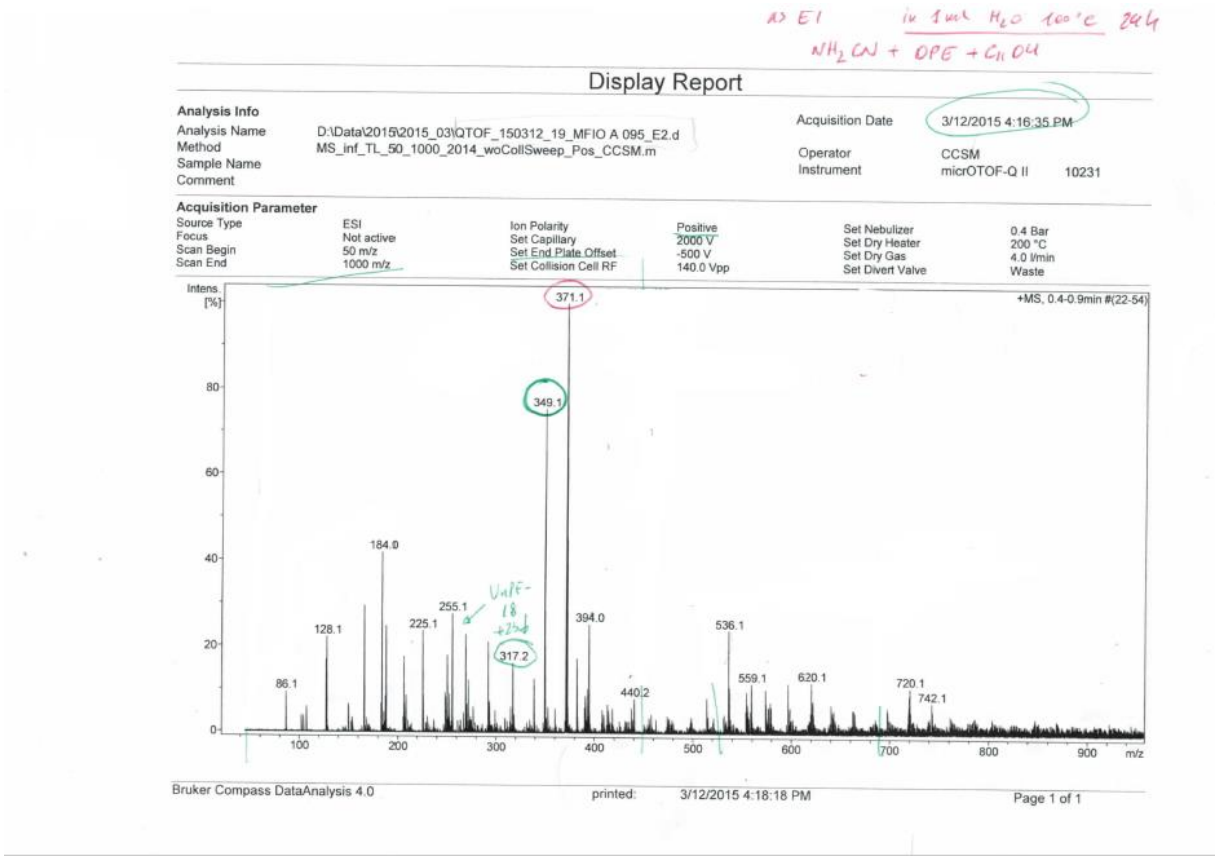


Figure S28: ESI-MS of crude reaction mixture obtained by heating **1 + 2b + 3a** at 100°C for 24h form wet to dryness (Table S2, entry 6). Top image: Positive ion mode. Bottom image: Negative ion mode.



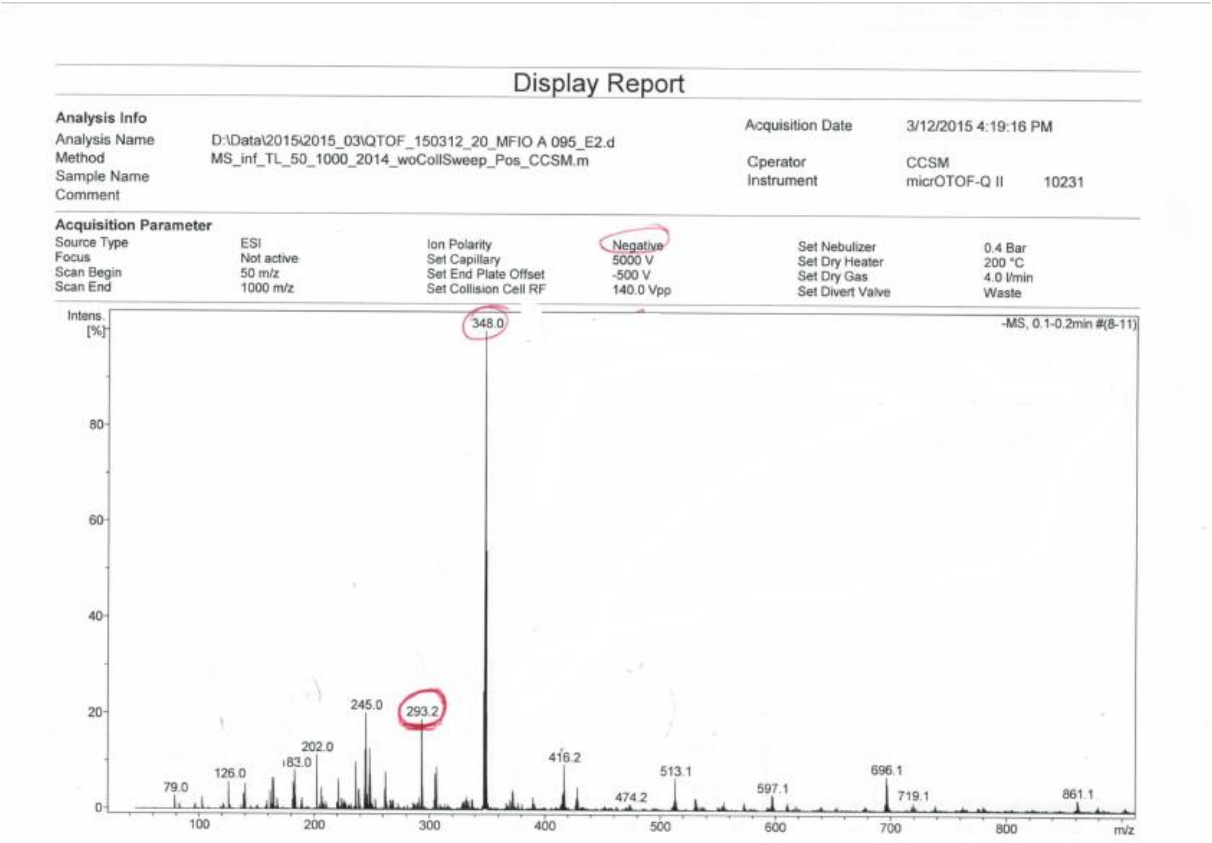


Figure S29. ESI-MS of crude reaction mixture obtained by heating **1 + 2b + 3a** at 100°C for 24h in neat (Table S2, entry 7). Top image: Positive ion mode. Bottom image: Negative ion mode.

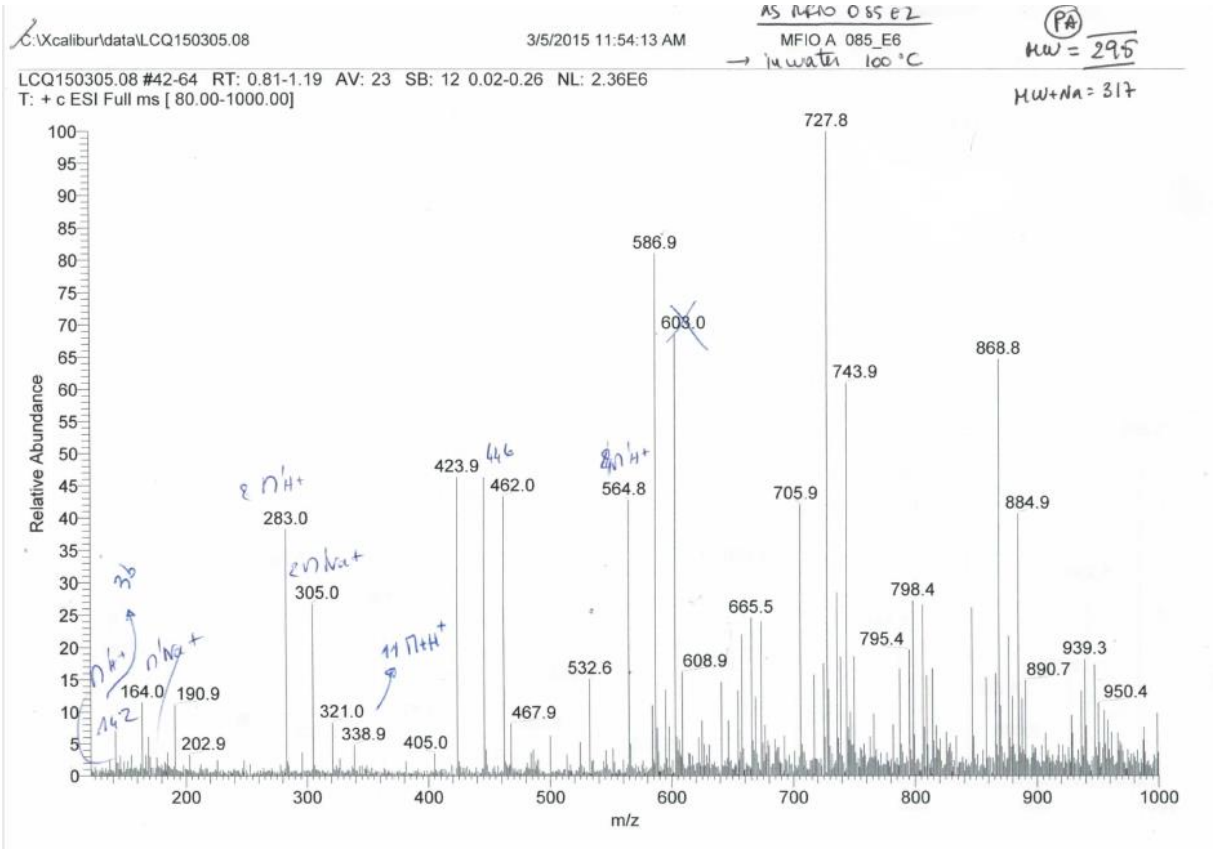


Figure S30: ESI-MS (positive ion mode) of crude reaction mixture obtained by heating **1 + 2a + 3b** at 100°C for 48h, under neat conditions (Table S2 entry 8)

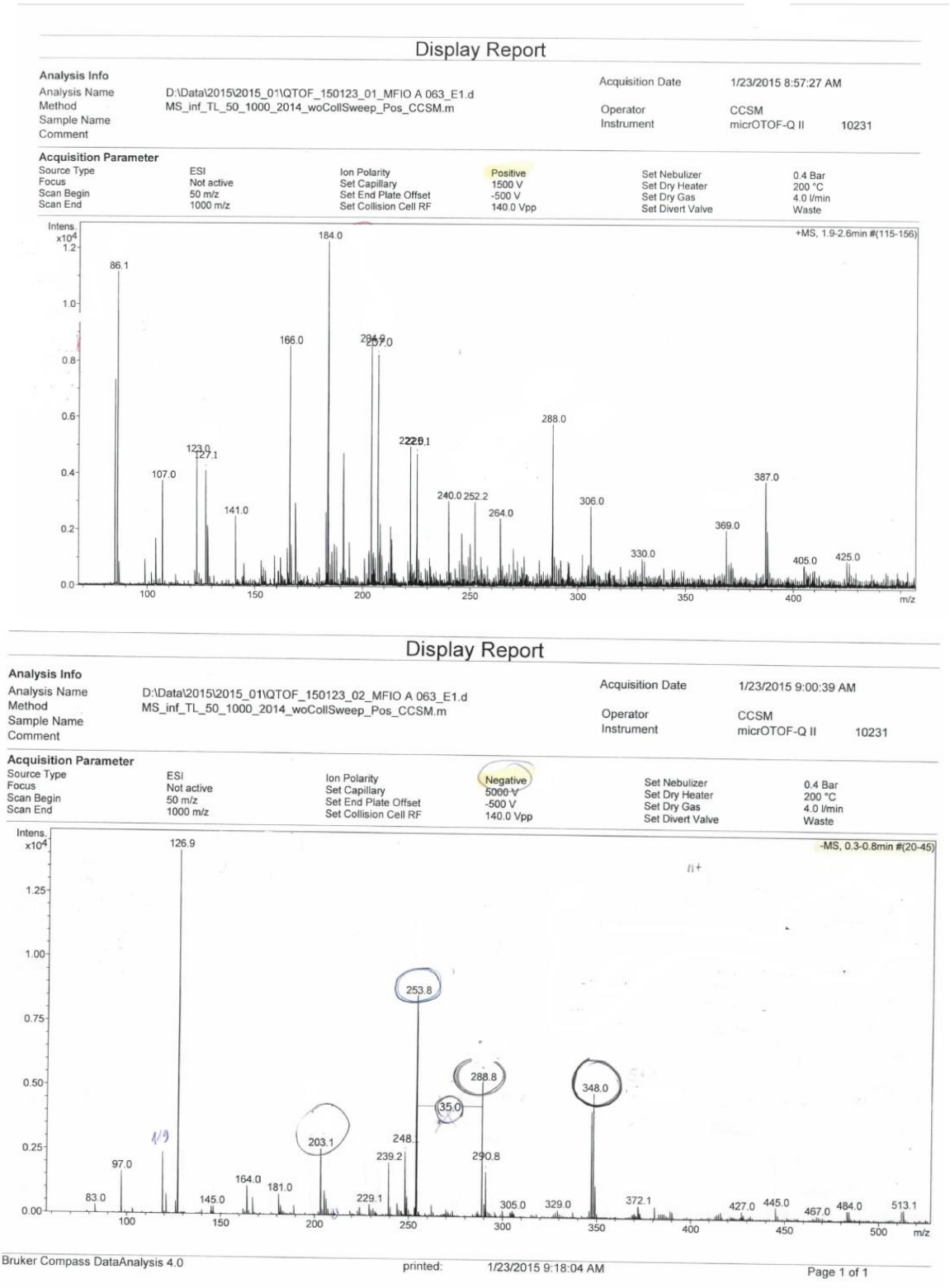


Figure S31: ESI-MS of crude reaction mixture obtained by heating **1 + 2a + 3b** at 80°C for 48h form wet to dryness (Table S2, entry 9). Top image: Positive ion mode. Bottom image: Negative ion mode.

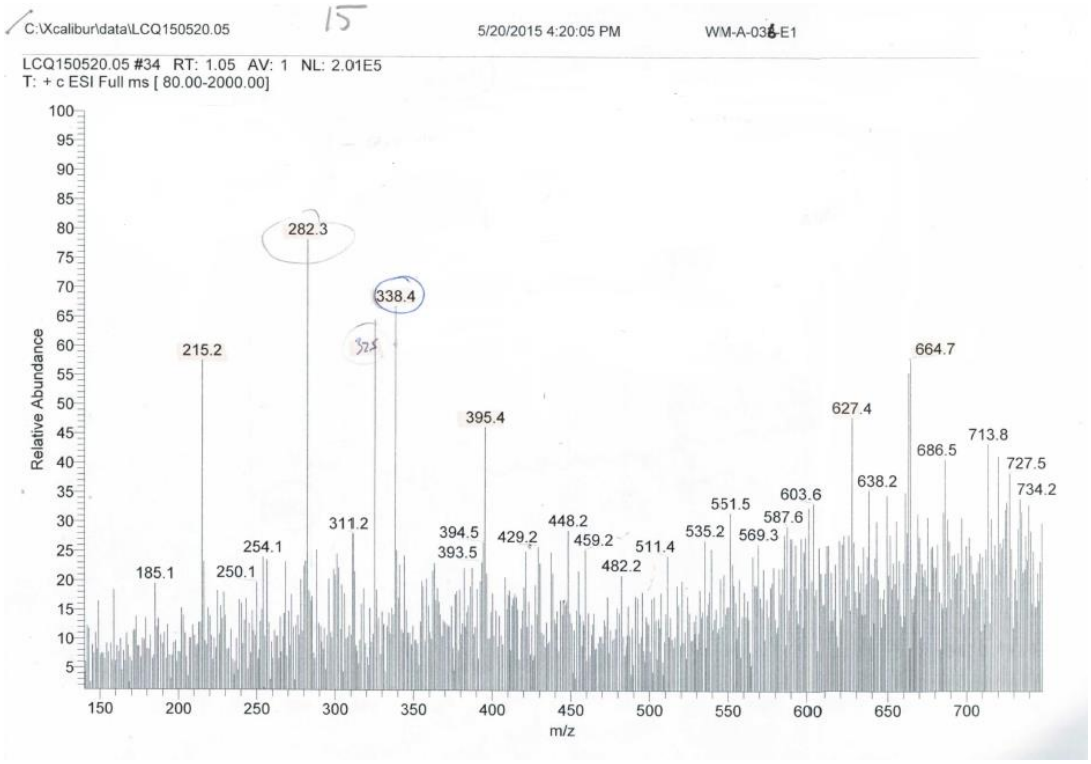


Figure S32. ESI-MS (positive ion mode) of crude reaction mixture obtained by heating **1 + 2a + 3b** at 80°C for 48h under neat conditions (Table S2, Entry 10).

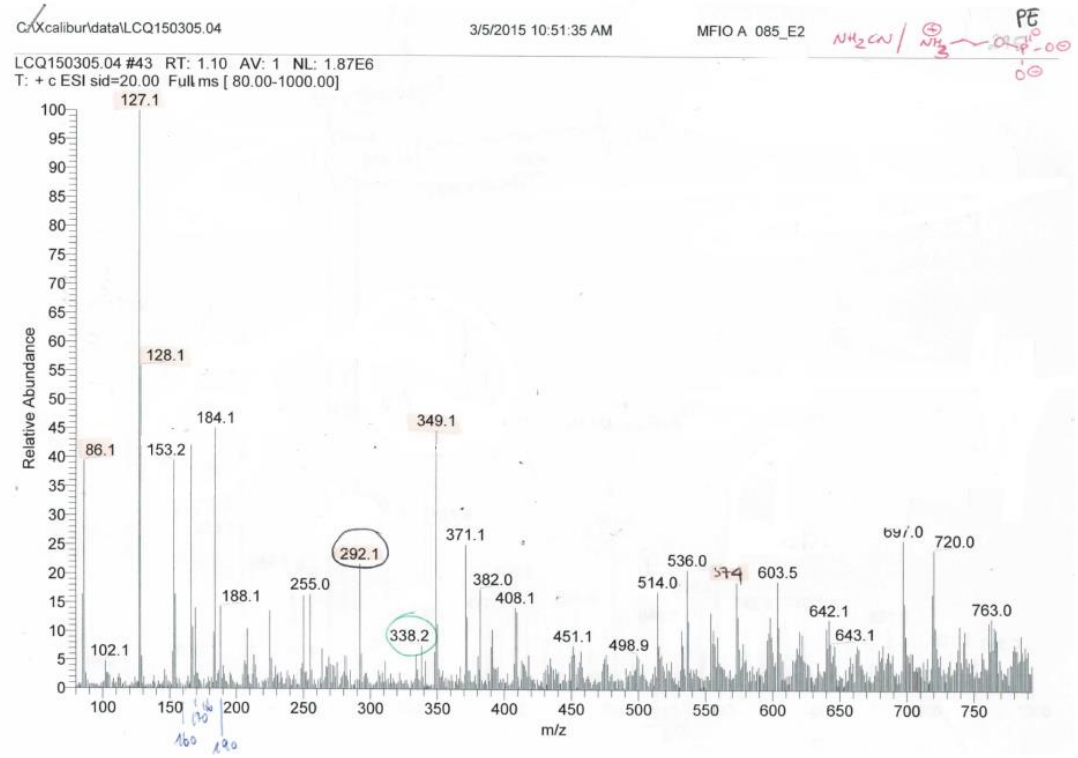


Figure S33. ESI-MS (positive ion mode) of crude reaction mixture obtained by heating **1 + 2a + 3b** at 100°C for 24h under neat conditions (Table S2, entry 11).

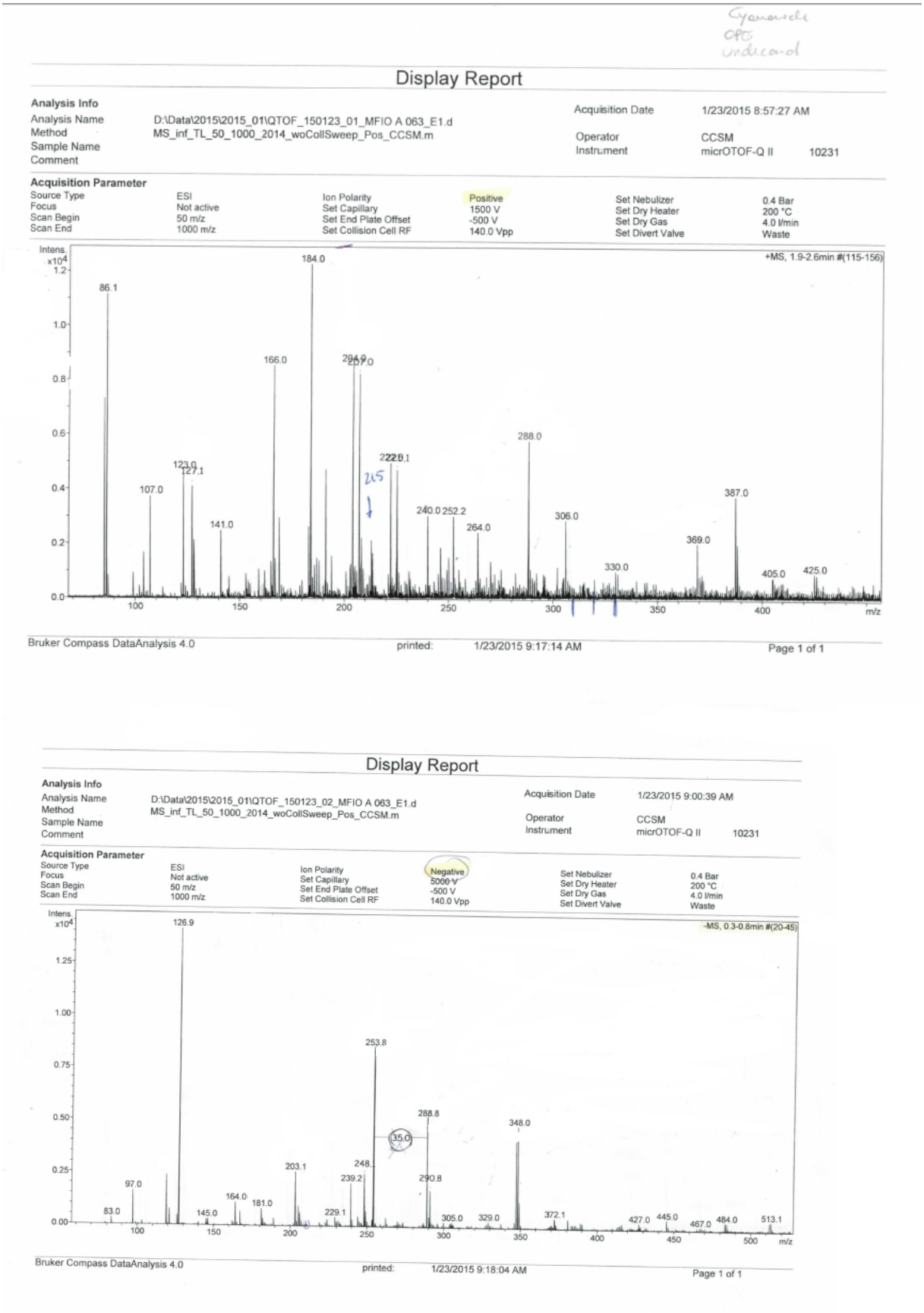
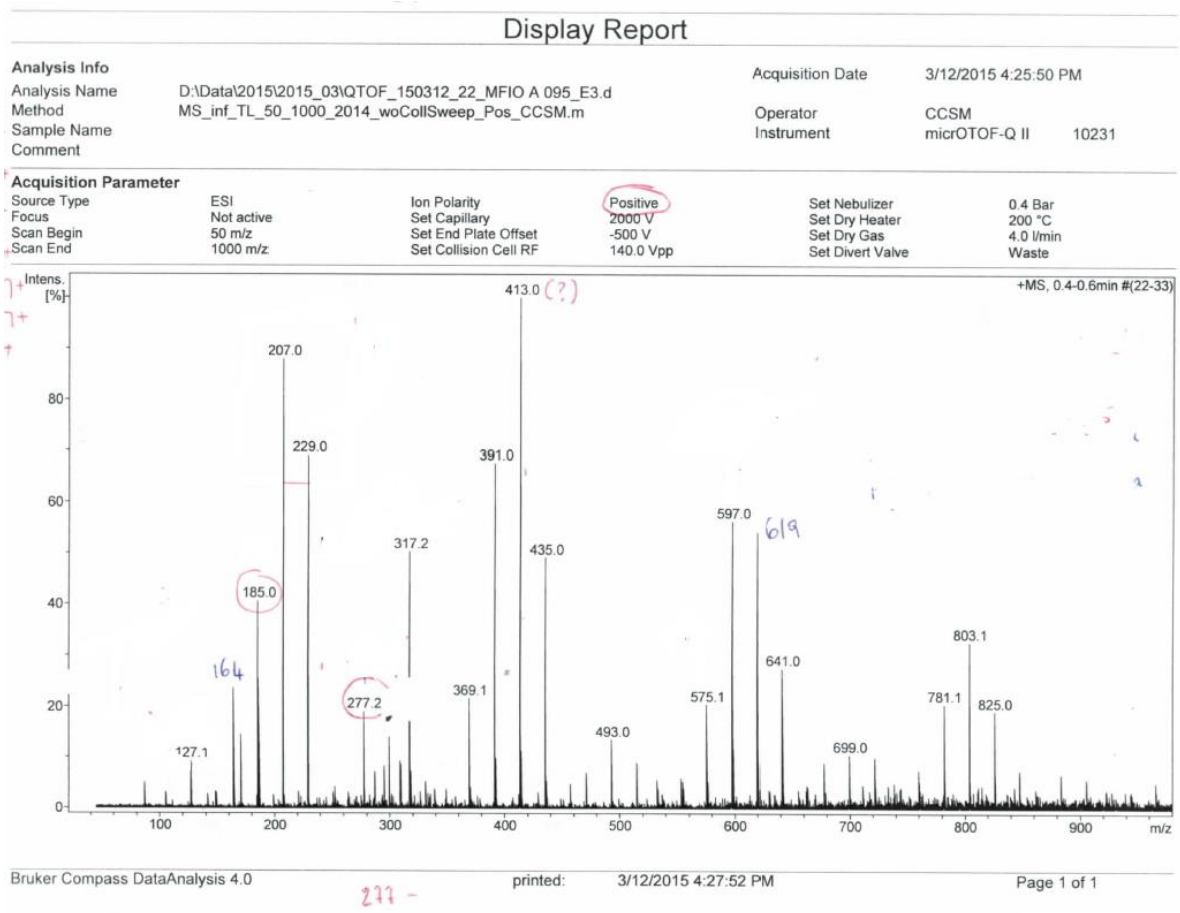


Figure S34. ESI-MS of crude reaction mixture obtained by heating **1 + 2a + 3b** at 80°C for 48h under neat conditions; (Table S2 entry 12). Top image: positive ion mode. Bottom image: Negative ion mode.



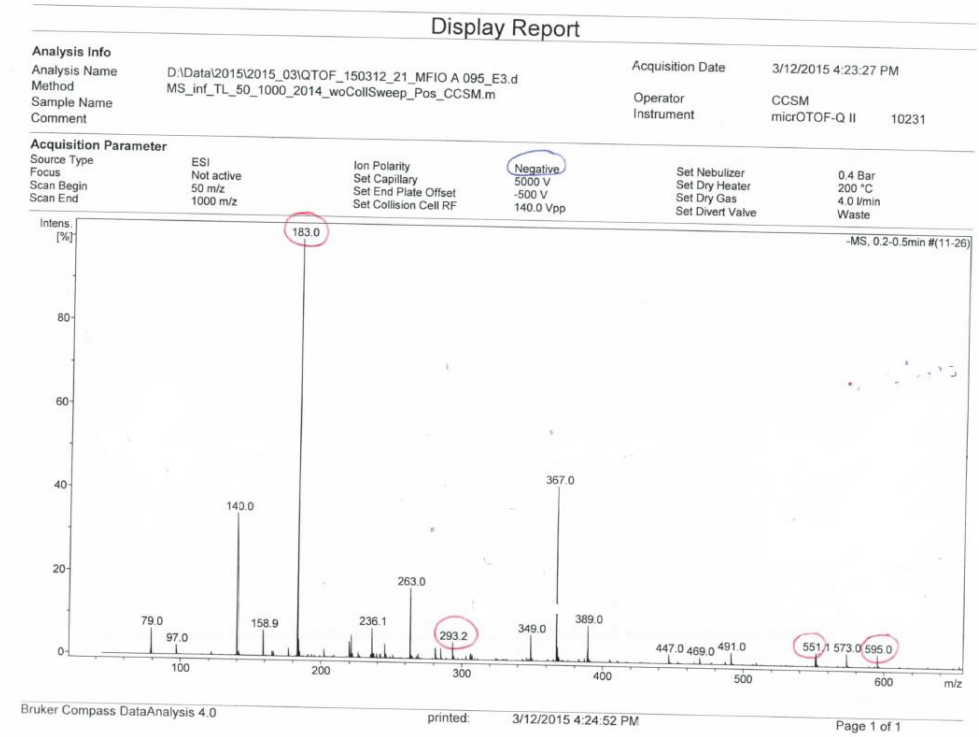


Figure S35. ESI-MS of crude reaction mixture obtained by heating **1 + 2b + 3b** at 80°C for 72h, under neat conditions (Table S2 entry 13). Top image: positive ion mode. Bottom image: negative ion mode.

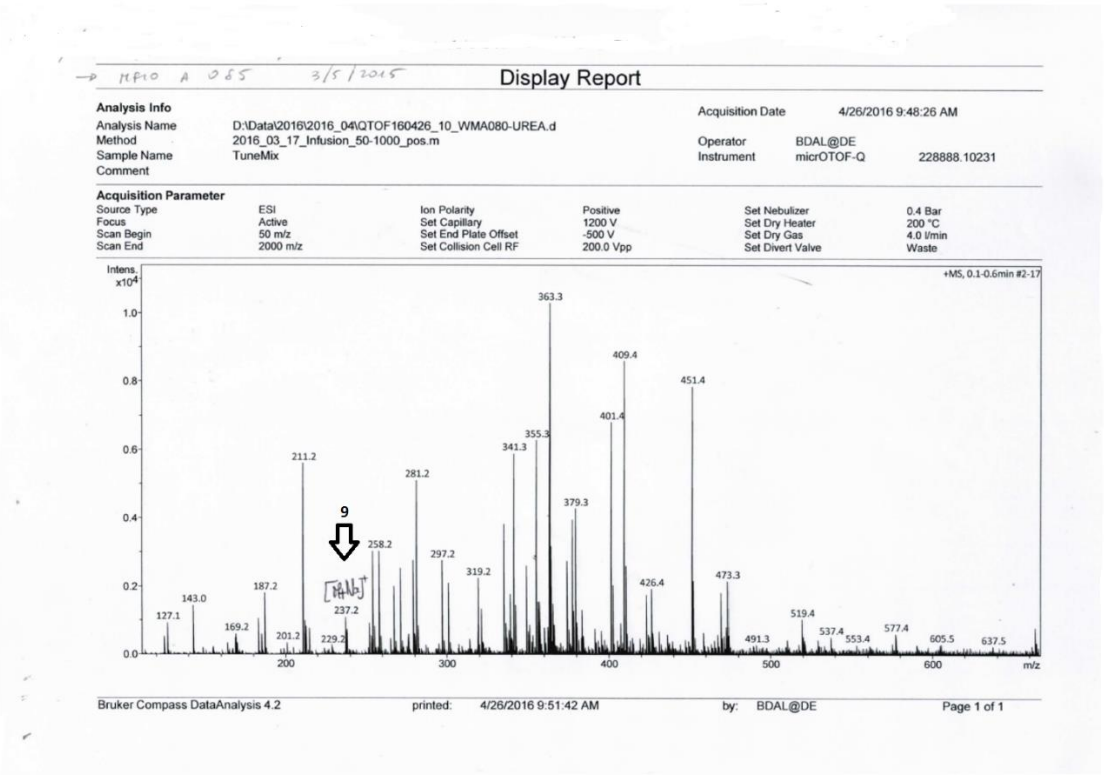


Figure S36. ESI-MS (positive ion mode) of crude reaction mixture obtained by heating **1 + 2a** at 80°C for 48h from wet to dryness (Table S2 entry 14).

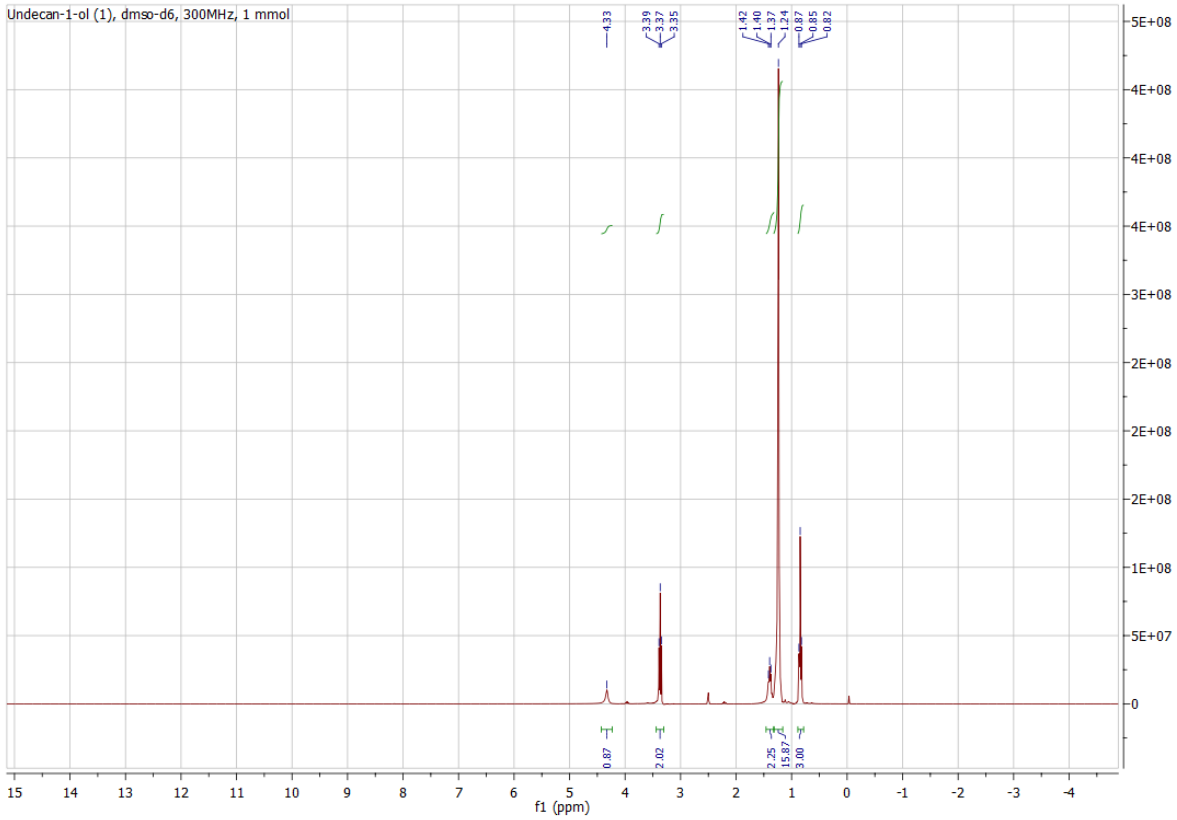


Figure S37. ^{1}H NMR (DMSO-d_6, 300MHz) of undecan-1-ol (**1**).

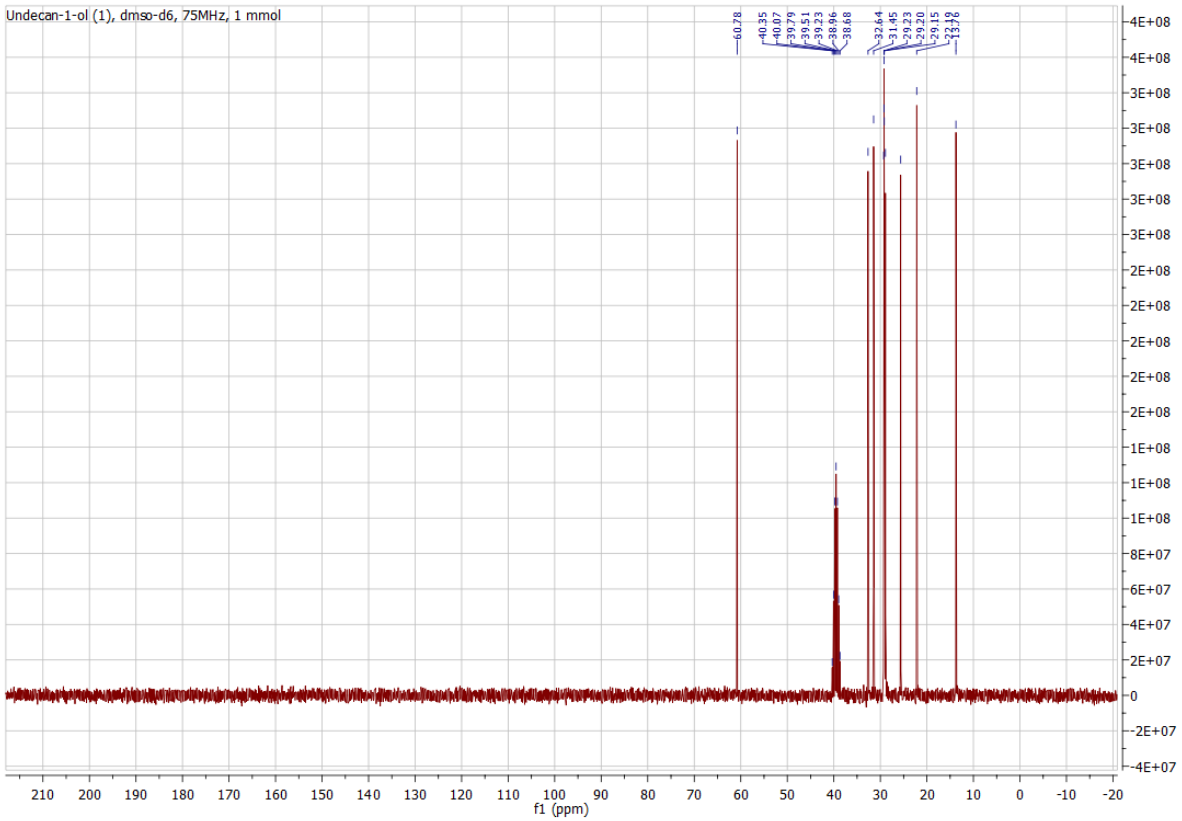


Figure S38. ^{13}C NMR (DMSO-d_6, 75MHz) of undecan-1-ol (**1**).

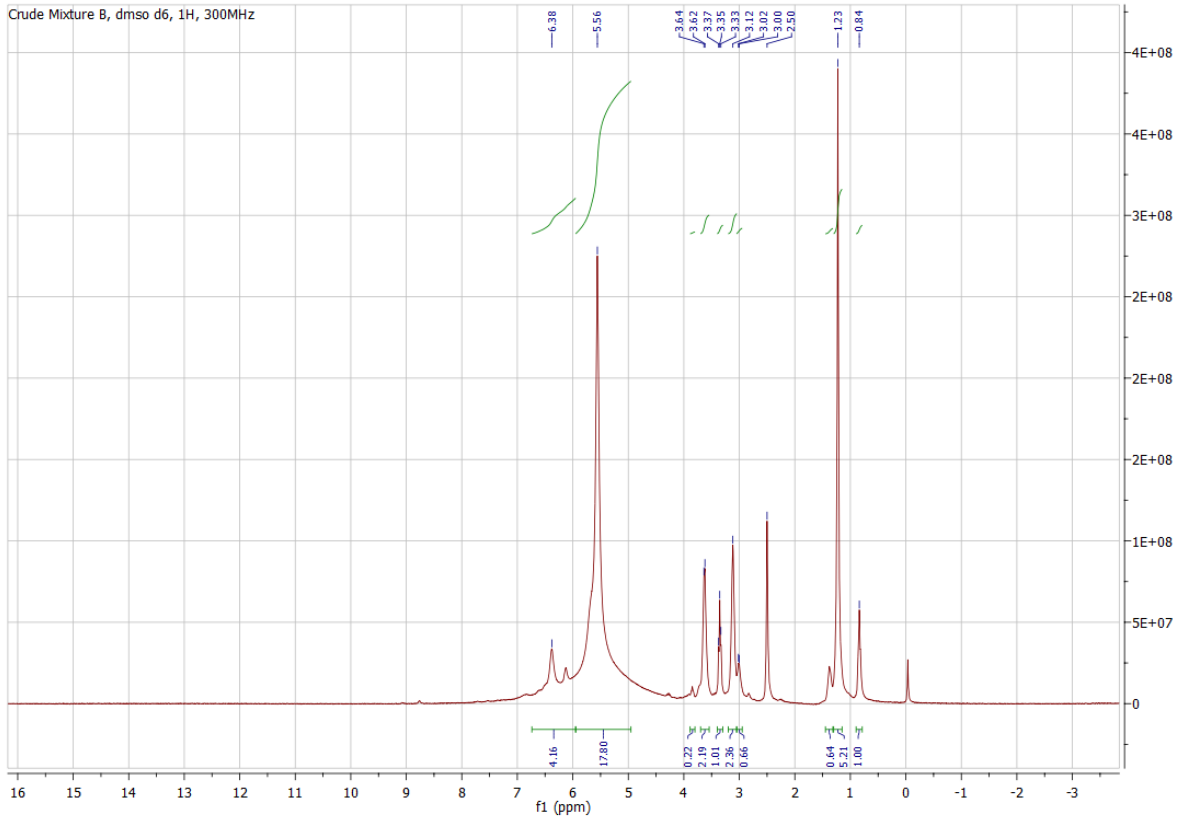


Figure S39. ^{1}H NMR (DMSO-d_6, 300MHz) of crude Mixture B.

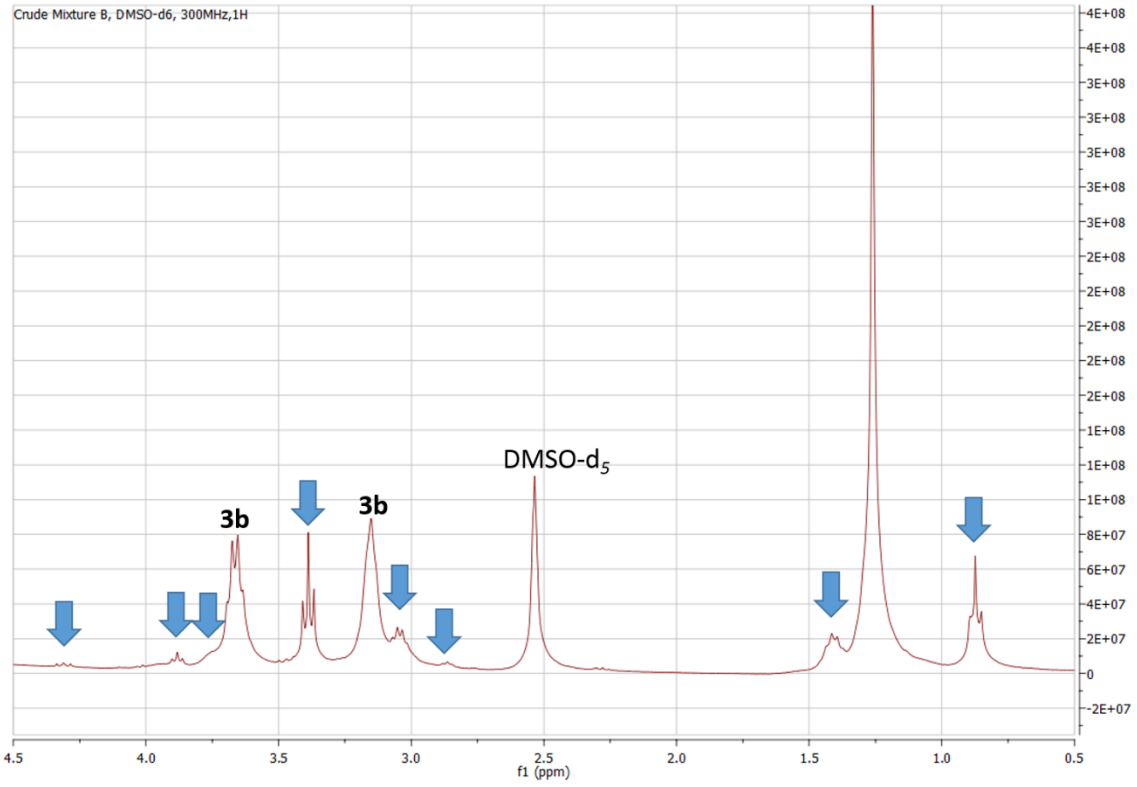


Figure S40. ^{1}H NMR (DMSO-d_6, 300MHz) of crude Mixture B showing the region (4.5 – 0.5 ppm) of the aliphatic chain. Blue arrows indicate signals of different products recognizable in the mixture. Some signals seem to overlap with those of undecan-1-ol (**1**) which is not present in the mixture (not found in by TLC, direct ESI-MS, HR-MS or UPLC-HRMS analysis). A large excess of **3b** (3.6 and 3.3 ppm) is recognizable in the mixture.

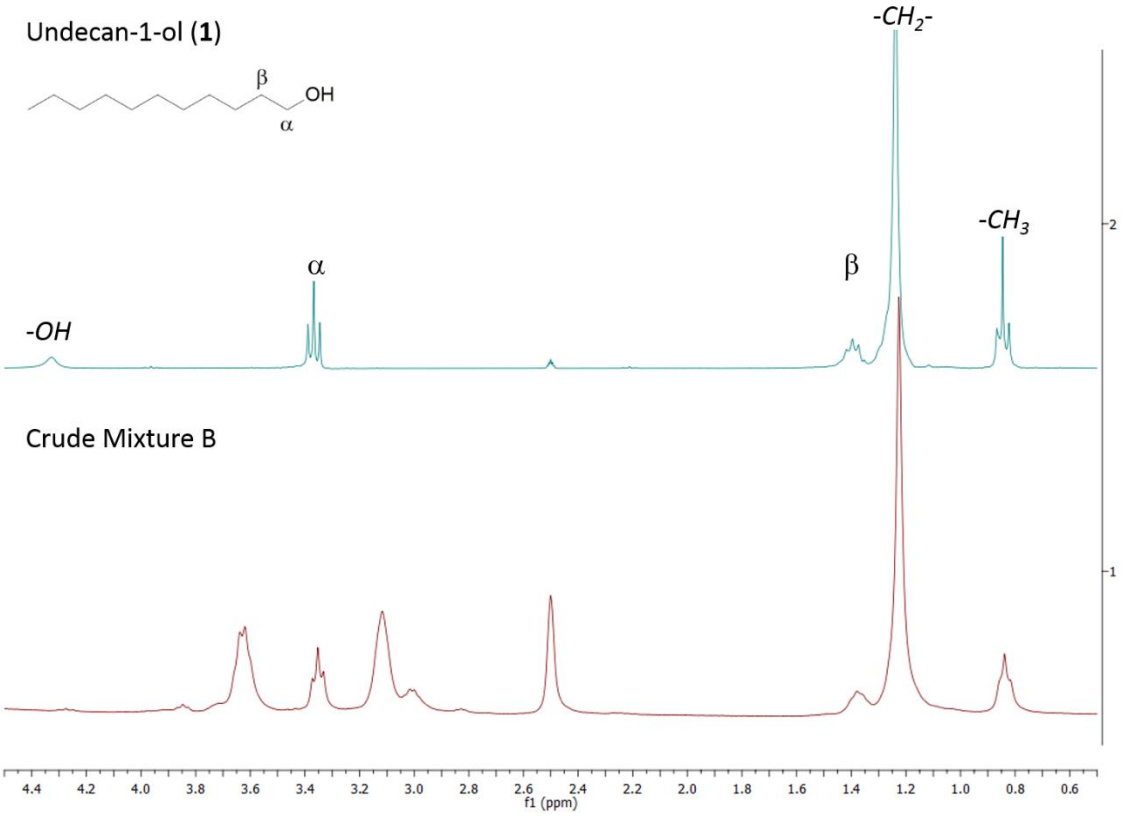


Figure S41. ^{1}H NMR spectra (DMSO-d_6, 300MHz) of crude Mixture B (red, bottom) and undecan-1-ol (green, top).

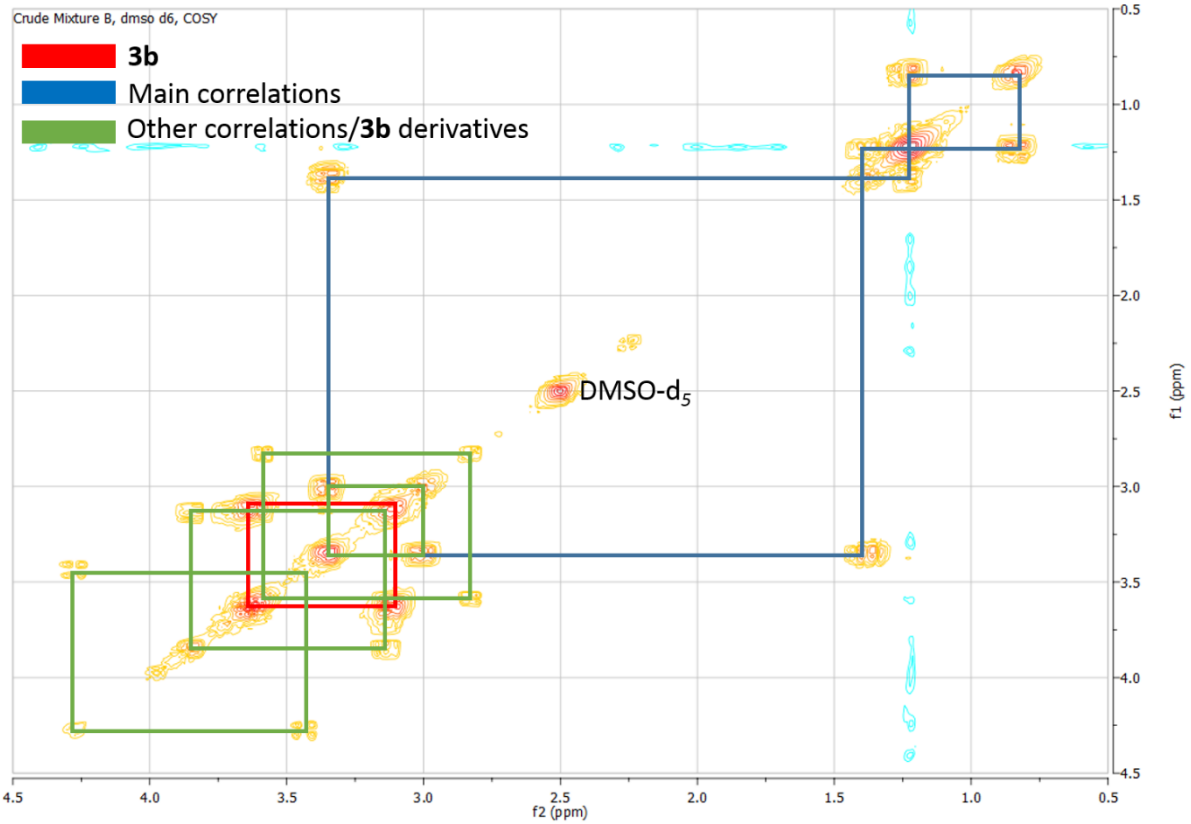


Figure S42. COSY (DMSO-d_6, 300MHz) of crude Mixture B.

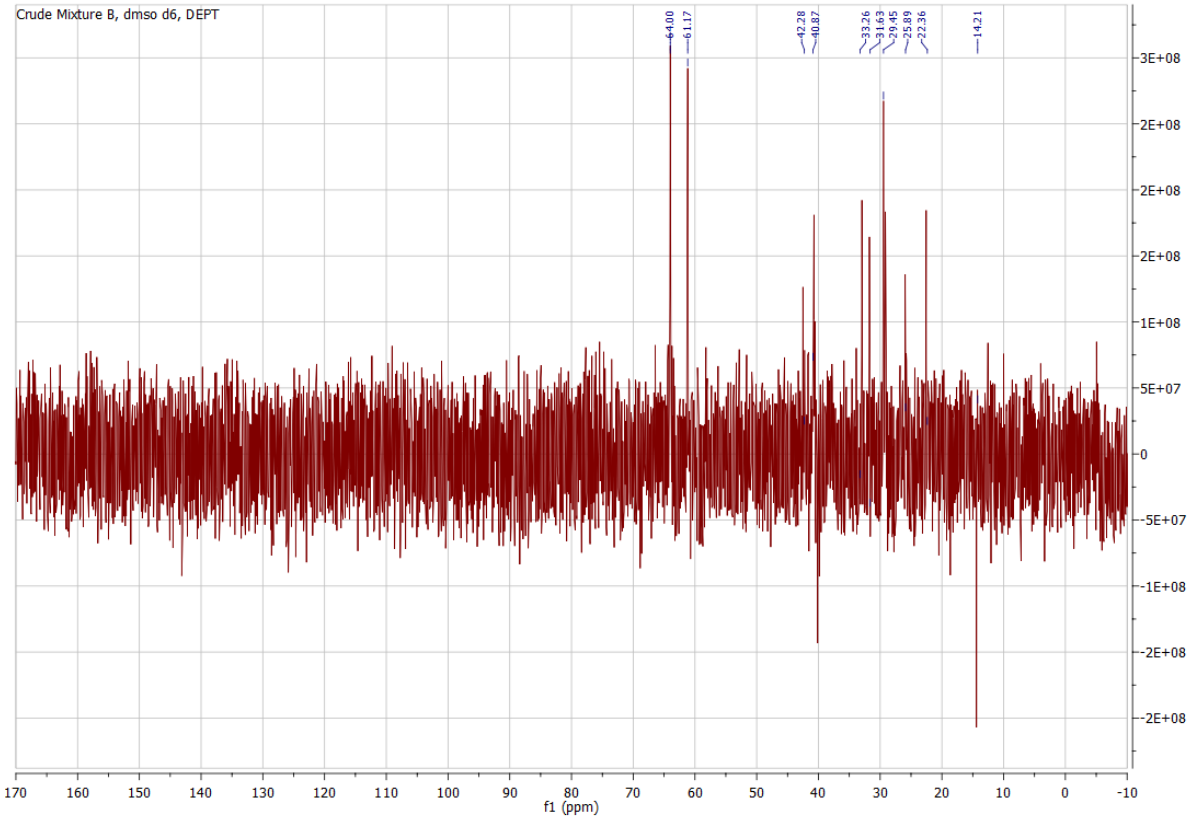


Figure S43: DEPT (DMSO-d_6, 75MHz) of crude Mixture B. DEPT was registered to show the presence of mainly –CH_2– of the aliphatic chains and the overlap of the –CH_3 of the species present in the crude mixture B.

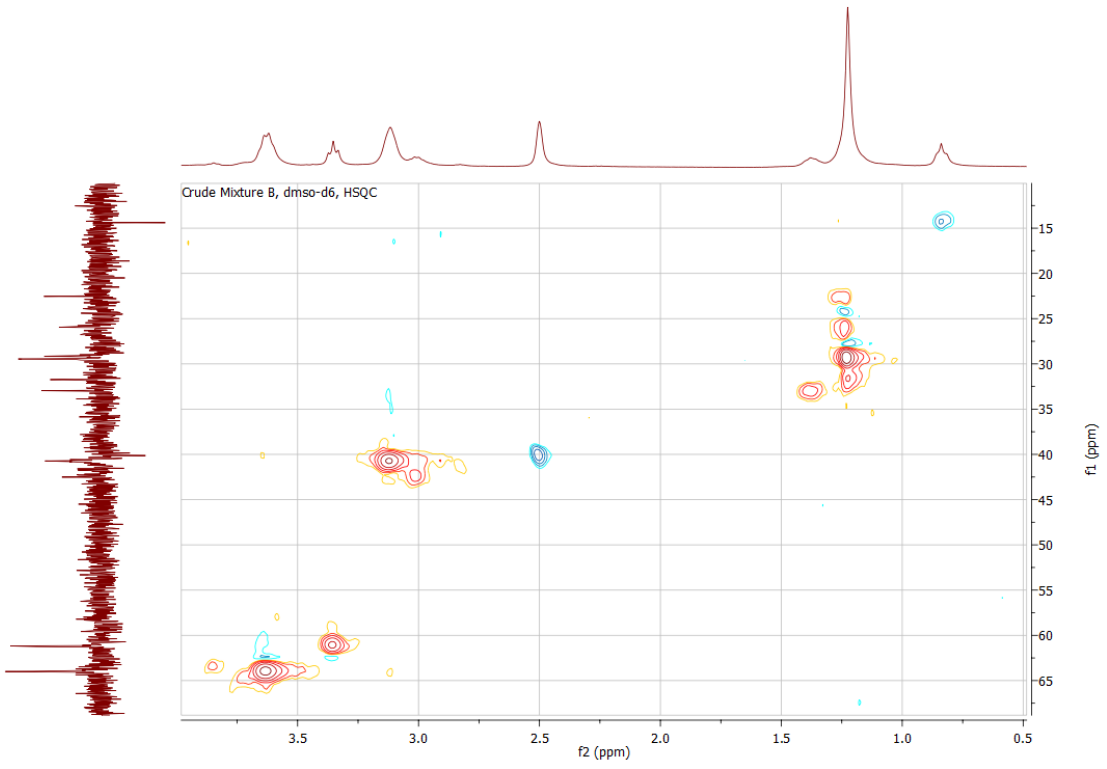


Figure S44: HSQC (DMSO-d_6, 75MHz) of crude Mixture B. DEPT spectra were reported on y axes, to show the -CH_3 at 0.88/14 ppm. Signal at 2.5 ppm from residual DMSO-d_5.

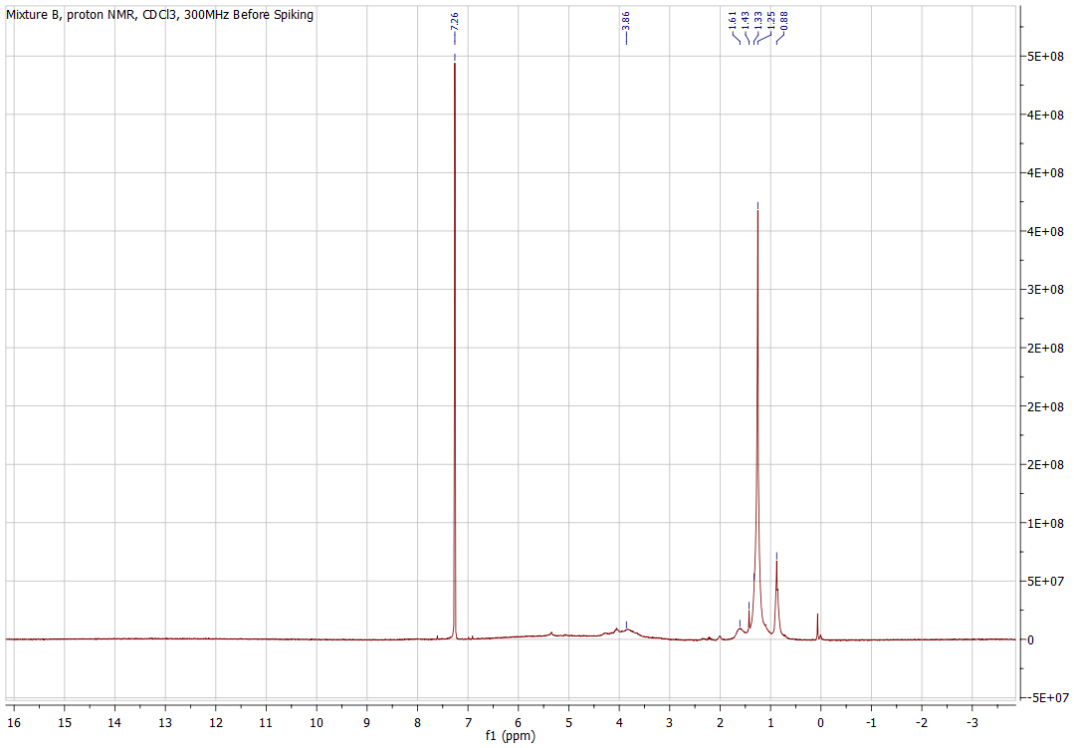


Figure S45. ^{1}H NMR ($CDCl_3$, 300MHz) of crude Mixture B.

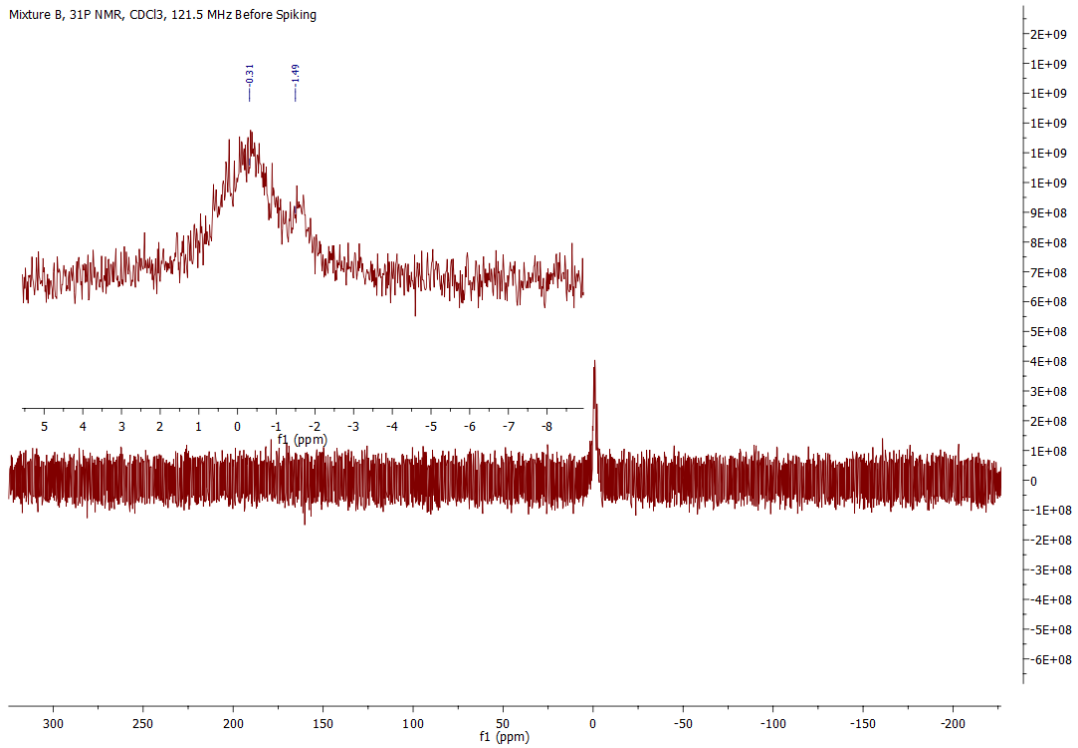


Figure S46. ^{31}P NMR ($CDCl_3$, 121.5 MHz) of crude Mixture B. The spectrum was recorded before spiking with compound **7s**.

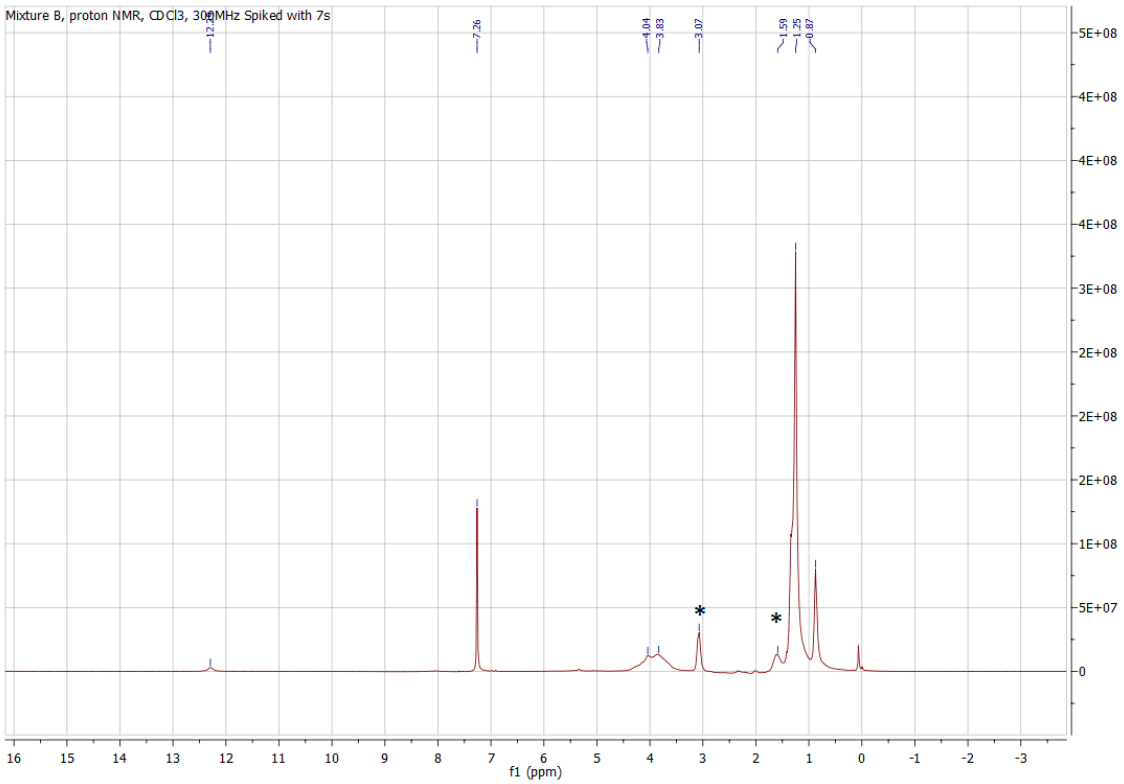


Figure S47: ^{1}H NMR ($CDCl_3$, 300MHz) of crude Mixture B spiked with compound **7s**. The characteristic signals of **7s** (*) emerged from the signals of crude mixture B.

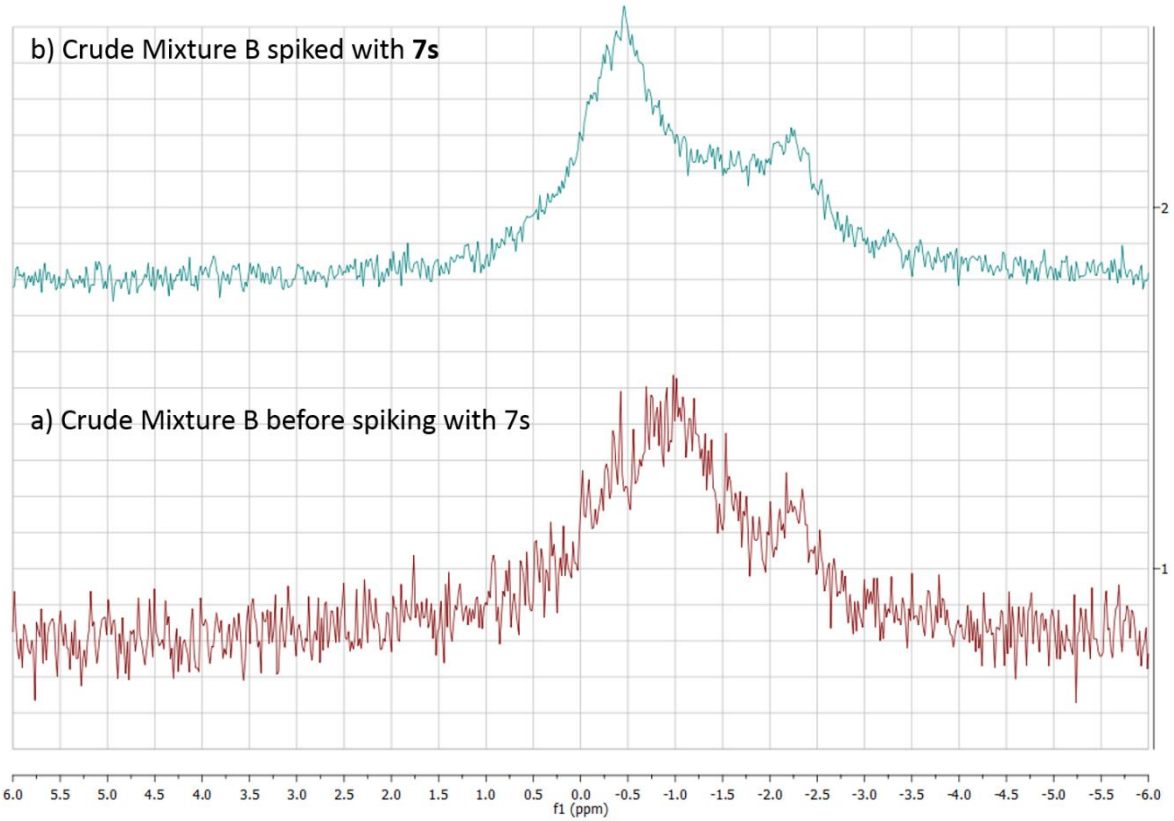


Figure S48. ^{31}P NMR ($CDCl_3$, 121.5 MHz) of crude Mixture B (in red, bottom) and Mixture B spiked with **7s** (in green, top) The characteristic signal of **7s** (–0.31 ppm) emerged from the signals of crude mixture B.

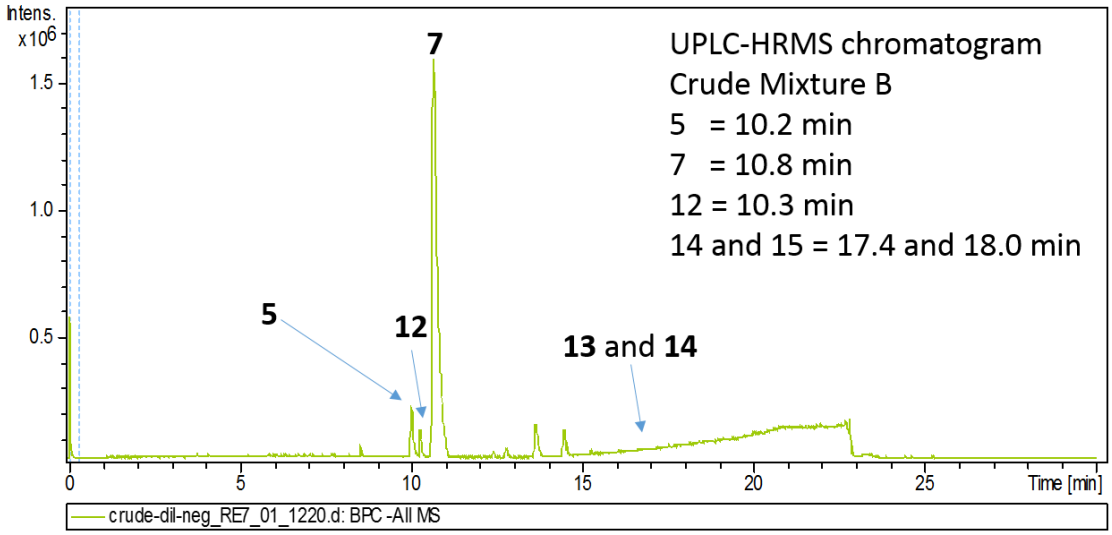


Figure S49. UPLC-HRMS total ion chromatogram (TIC) in the negative ion mode of untreated Mixture B.

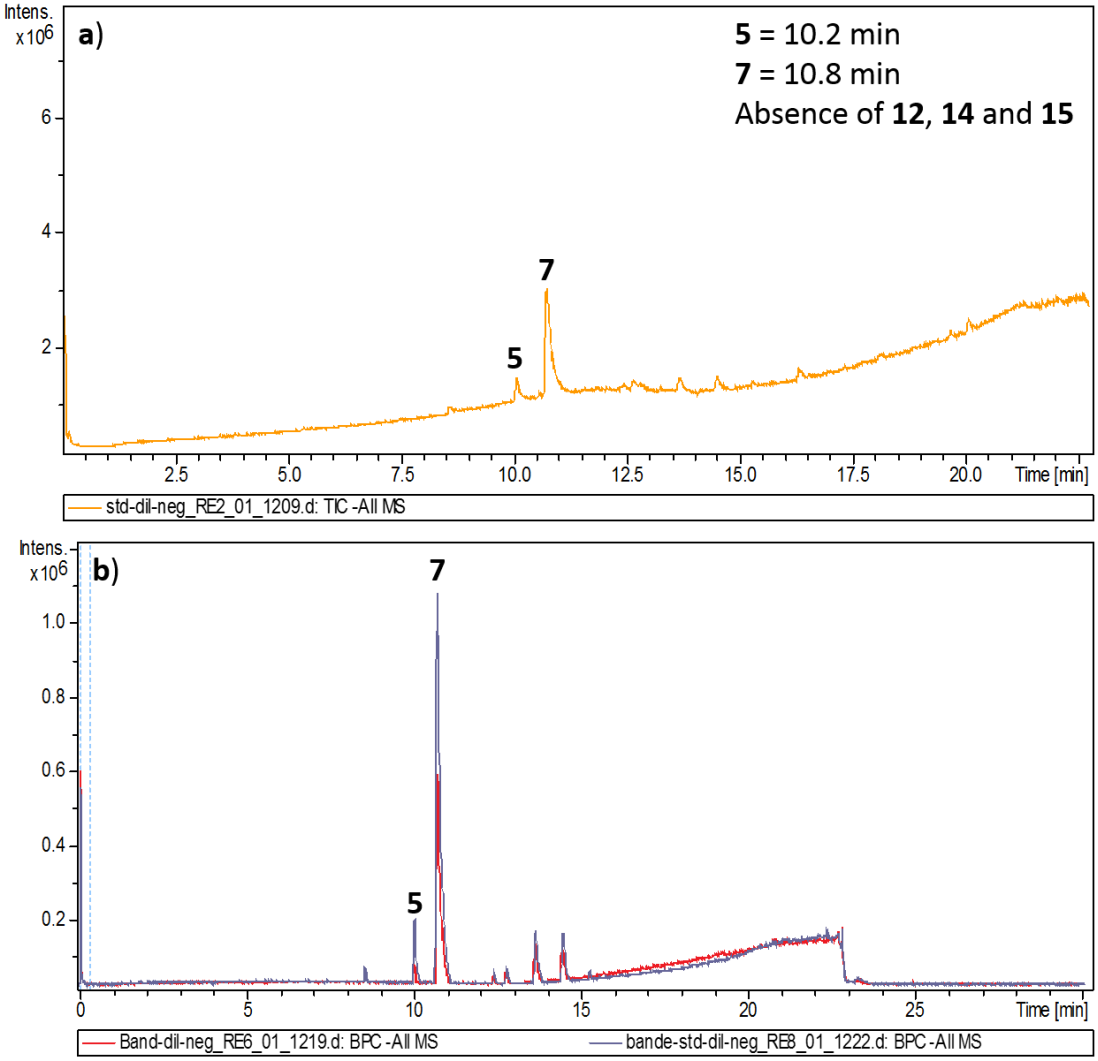


Figure S50. UPLC-HRMS total ion chromatogram (TIC) in the negative ion mode of: **a**) prep-TLC purified Mixture B, the extracted band contained mainly products **5** and **7**. **b**) UPLC-HRMS spiking of purified mixture (red) with standard mixture containing **7s**:**5s** (9:1 mol:mol, blue). Only the two peaks corresponding to **5** and **7** increase.

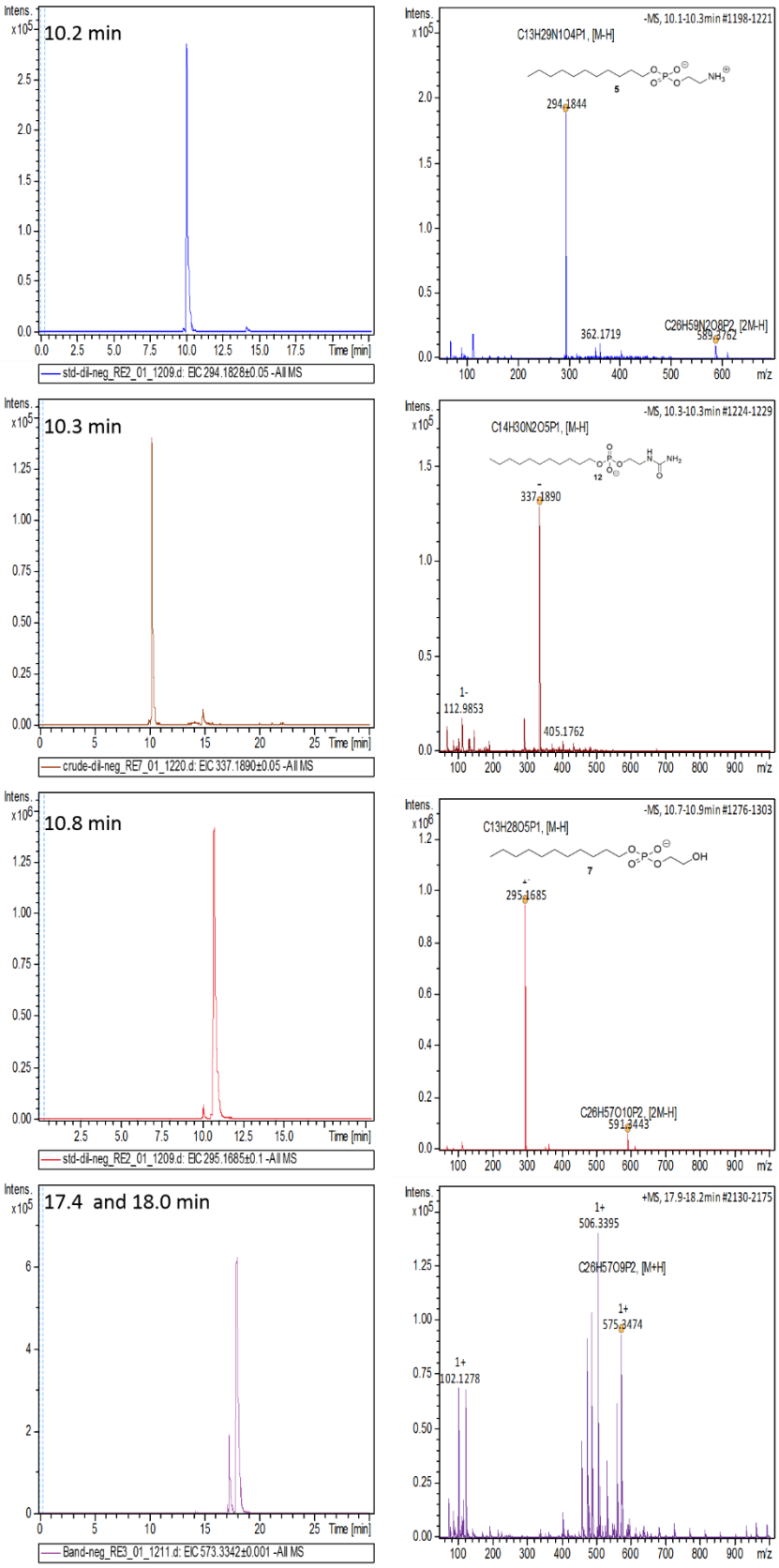


Figure S51. Mass extracted ion chromatograms of the TIC shown above of the major components of crude Mixture B. Left: UPLC profile, right: *m/z* and chemical formula and corresponding amphiphiles structures.

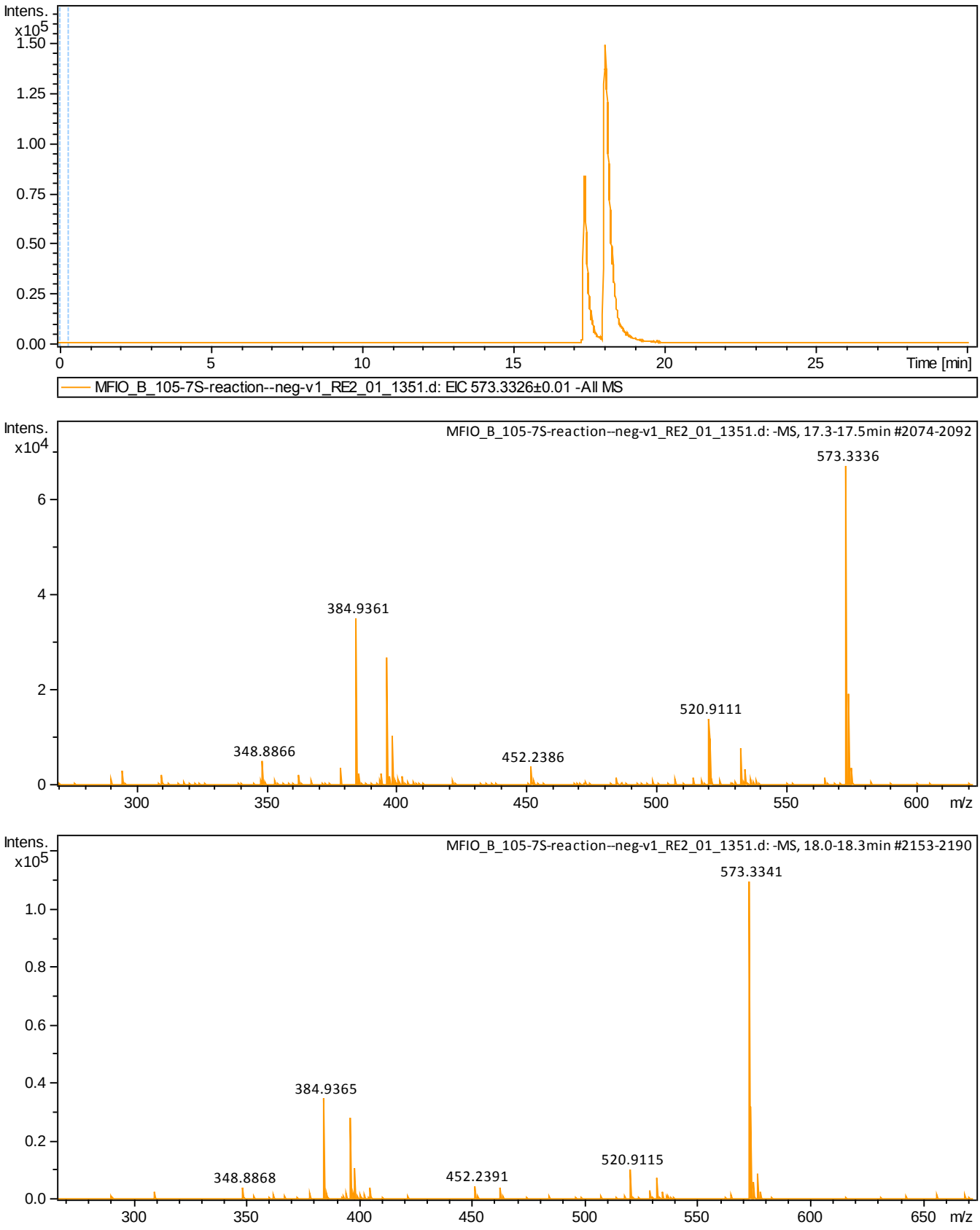


Figure S52. UPLC-HRMS of the condensation products of **7s** under prebiotic conditions. The two components of the non purified mixture containing only the more polar products already found in the prebiotic Mixture B with retention times 17.4 and 18 minutes and same *m/z* and chemical formula as 575.374 and $C_{26}H_{57}O_9P_2$ both corresponding to structures **13** and **14**.

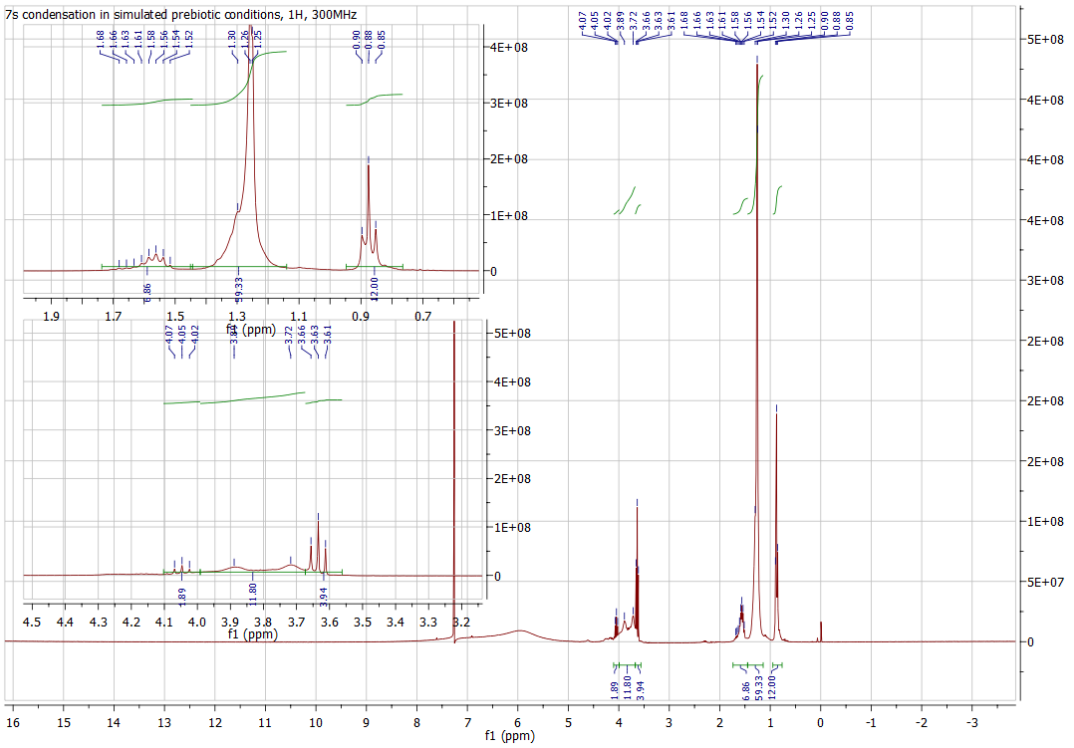


Figure S53. ^{1}H NMR ($CDCl_3$, 300MHz) of crude mixture obtained by condensation of **7s** (0.1 mmol) in the presence of urea (**2b**, 1 mmol) for 24 hrs at 80°C. Before analysis residual **2b** was washed out by using $CHCl_3$ (3 x 2mL washes).

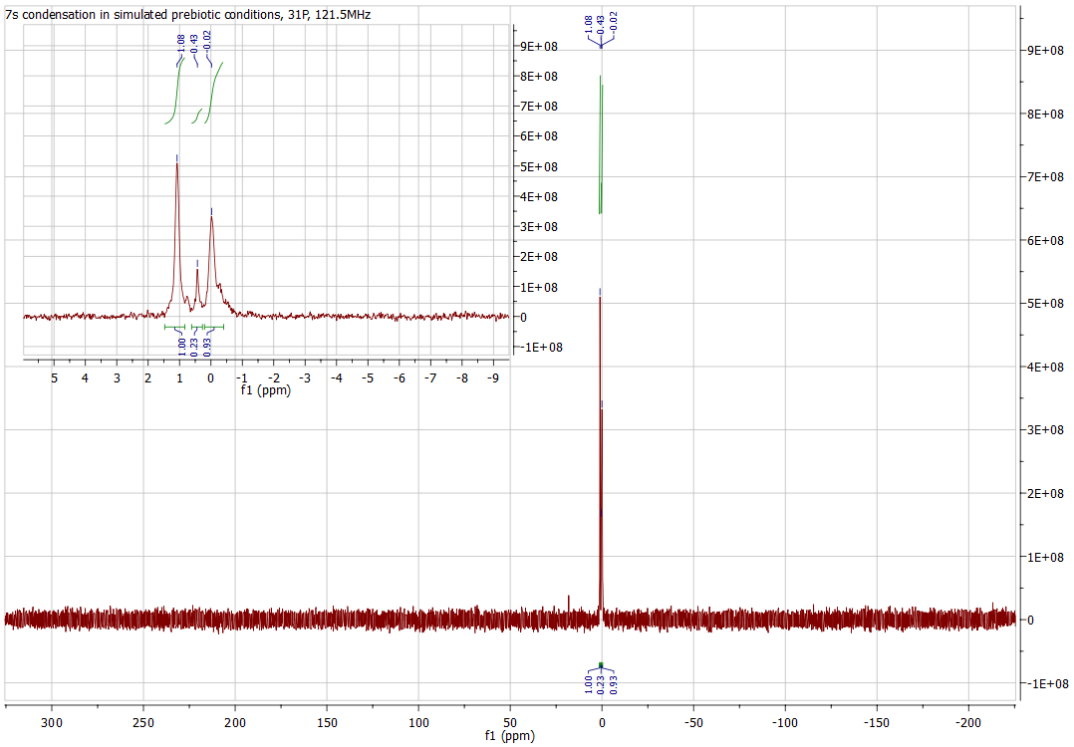


Figure S54. ^{31}P NMR ($CDCl_3$, 121.5 MHz) of crude mixture obtained by condensation of **7s** (0.1 mmol) in the presence of urea (**2b**, 1 mmol) for 24 hrs at 80°C.

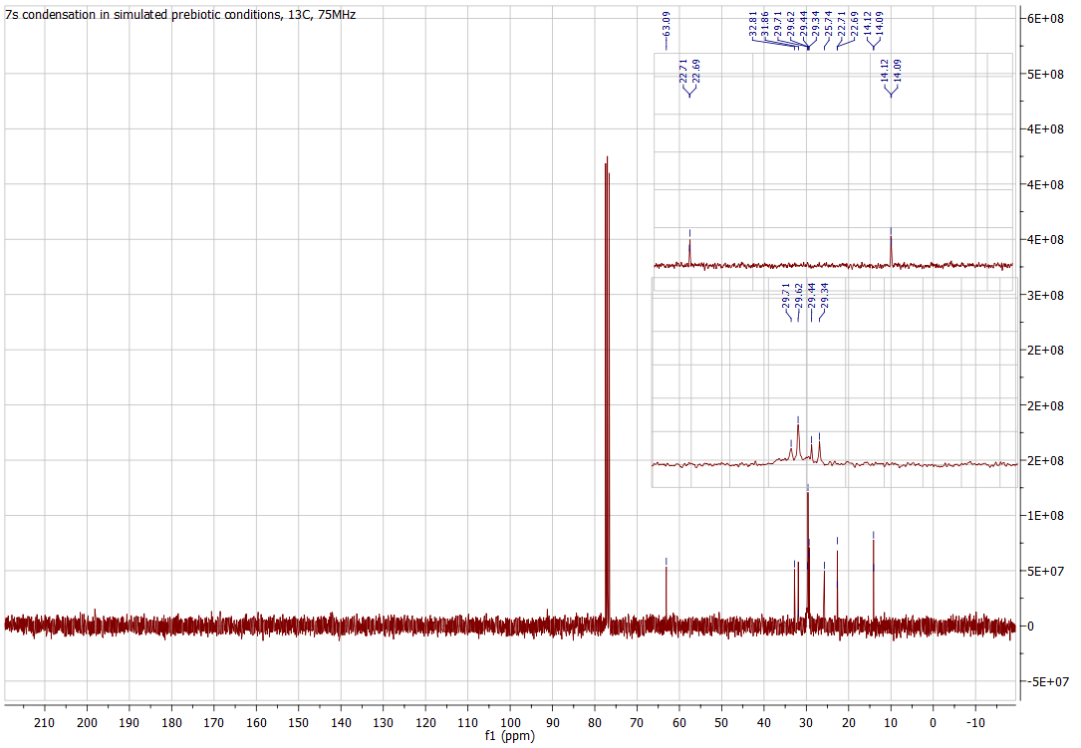


Figure S55: ^{13}C NMR ($CDCl_3$, 75 MHz) of crude mixture obtained by condensation of **7s** (0.1 mmol) in the presence of urea (**2b**, 1 mmol) for 24 hrs at 80°C. The urea signal is not present due to the washes perfomed before the analysis.

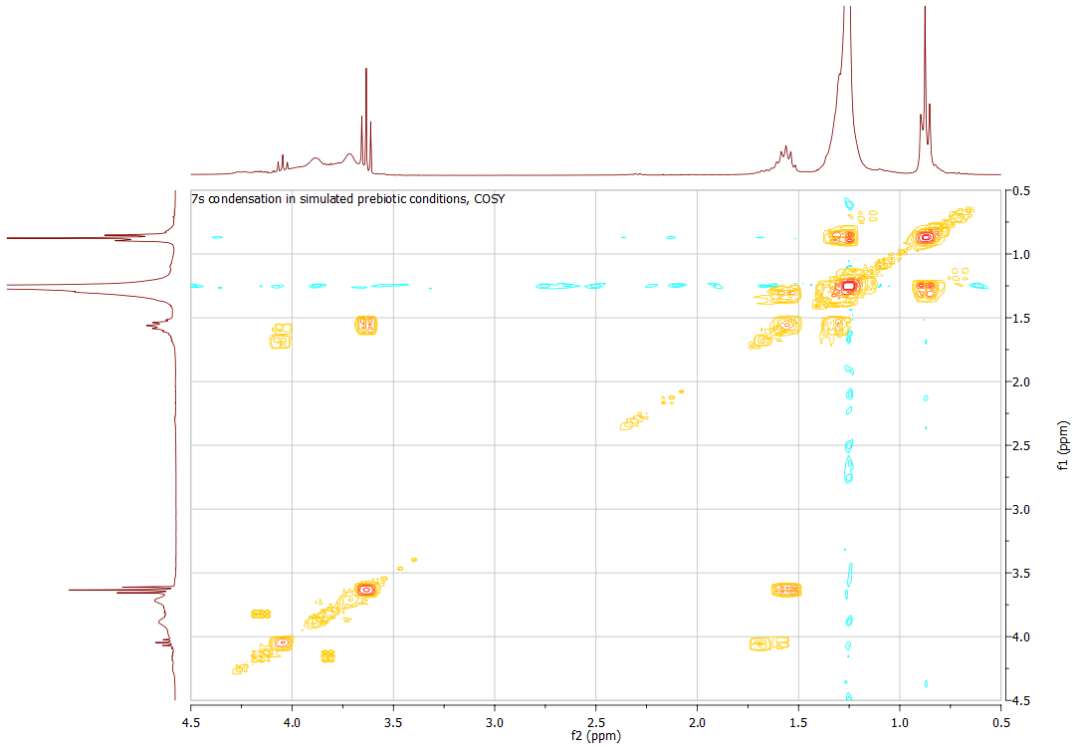


Figure S56: COSY ($CDCl_3$, 300 MHz) of crude mixture obtained by condensation of **7s** (0.1 mmol) in the presence of urea (**2b**, 1 mmol) for 24 hrs at 80°C.

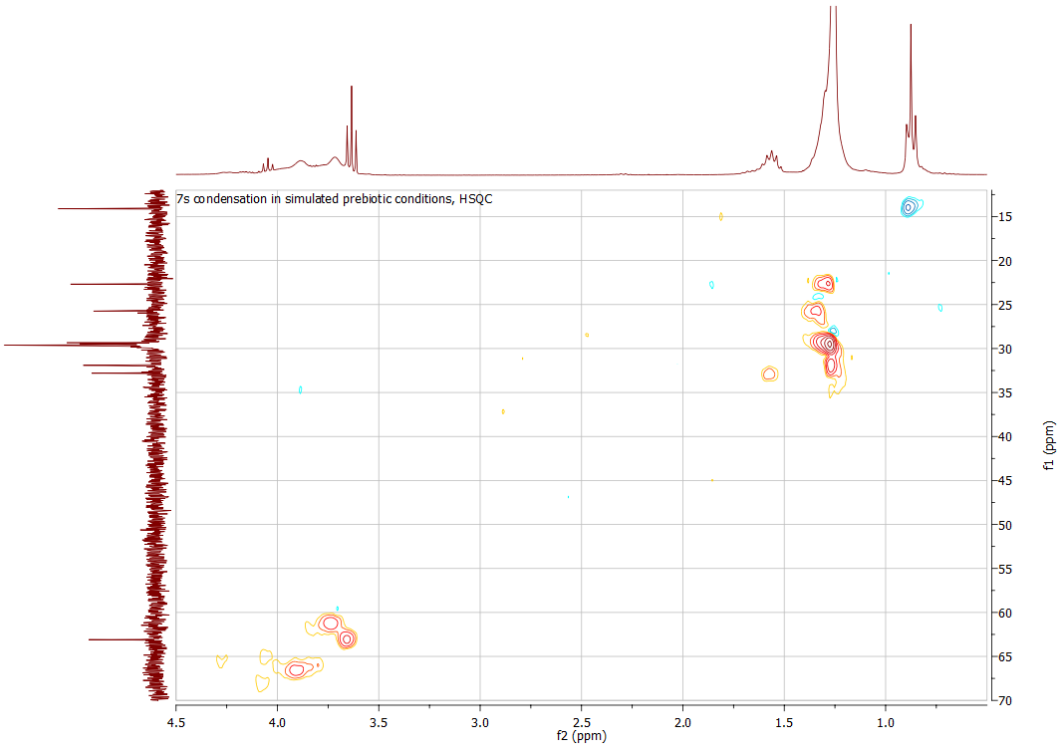


Figure S57: HSQC ($CDCl_3$, 75 MHz) of crude mixture obtained by condensation of **7s** (0.1 mmol) in the presence of urea (**2b**, 1 mmol) for 24 hrs at 80°C. The urea signal is not present due to the washes perfomed before the analysis.

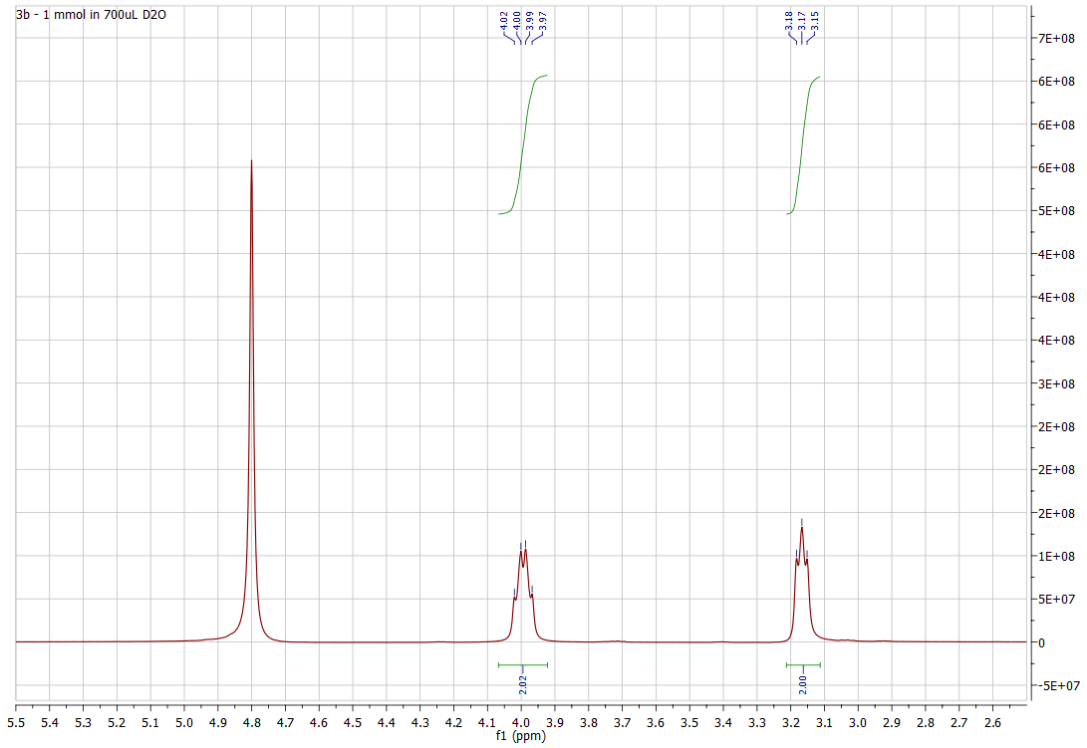


Figure S58. ^{1}H NMR (D_2O, 300MHz) of pure **3b** (1 mmol).

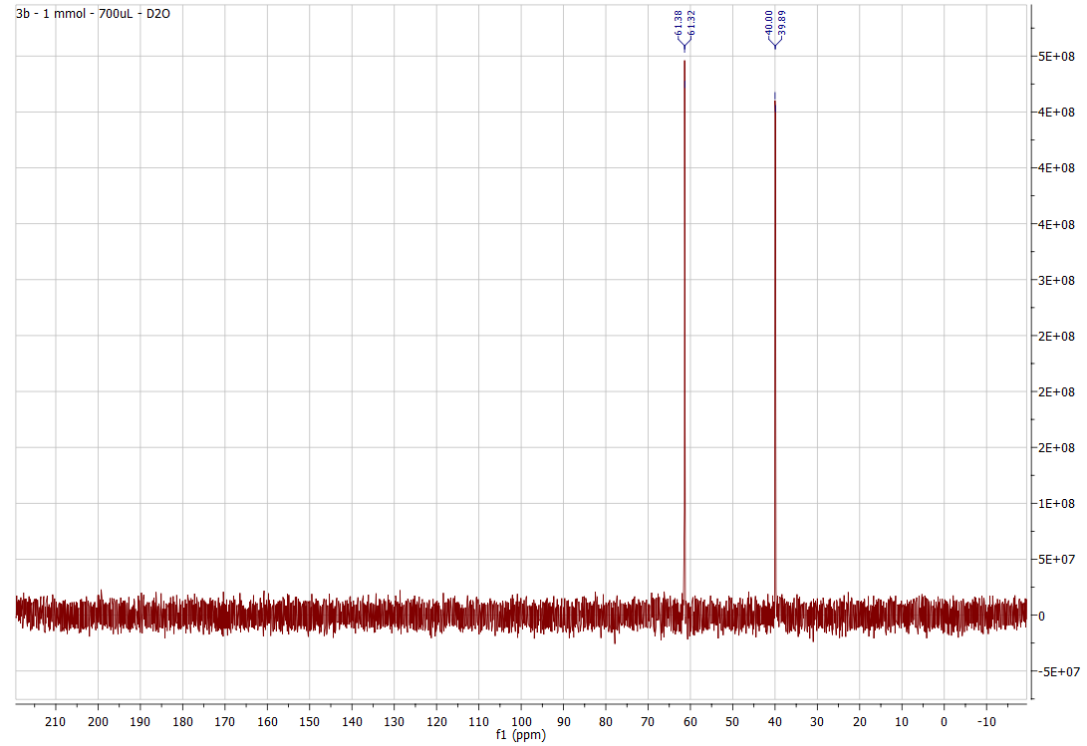


Figure S59. ^{13}C NMR (D_2O, 75MHz) of pure **3b** (1 mmol).

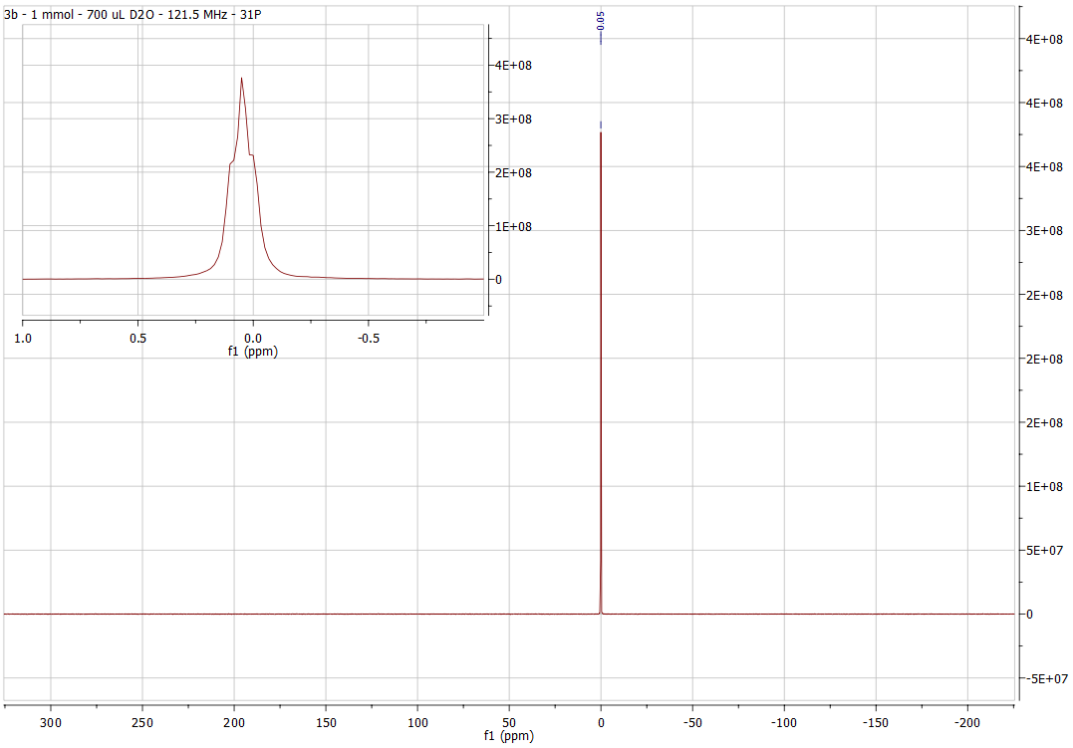


Figure S60. ^{31}P NMR (D_2O, 121.5 MHz) of pure **3b** (1 mmol).

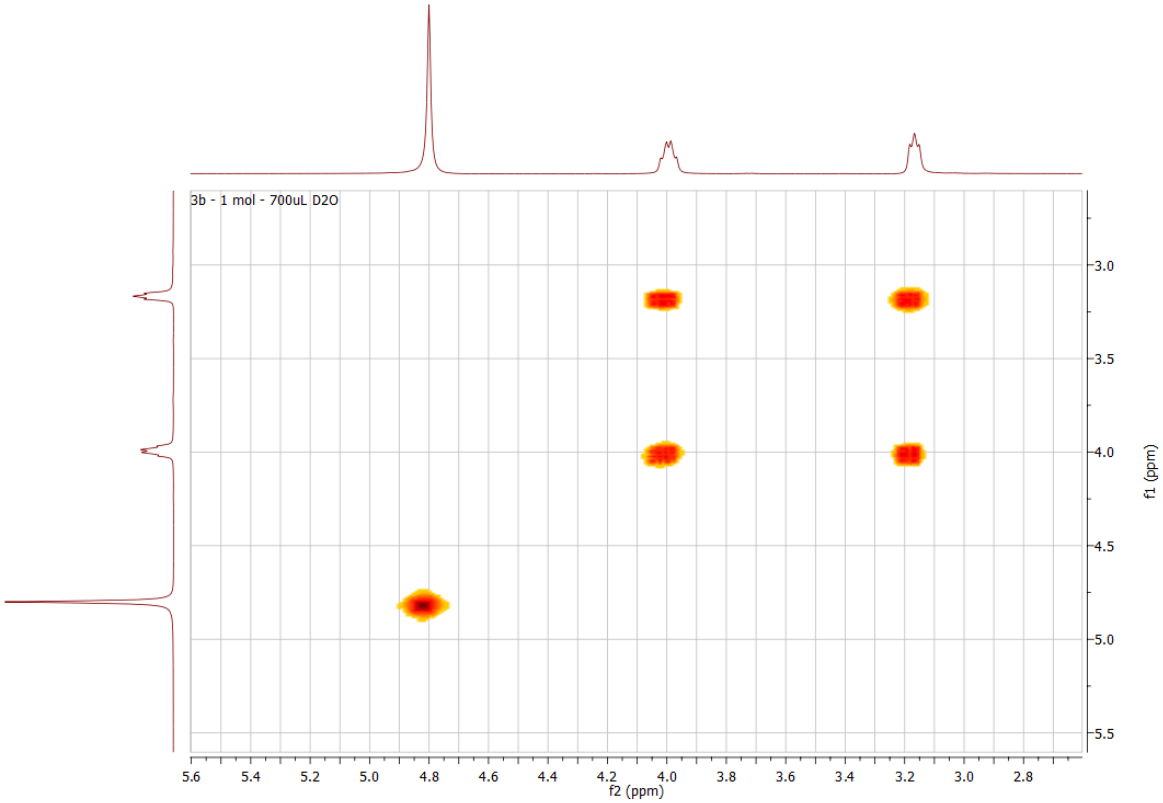


Figure S61. COSY (D_2O, 300 MHz) of pure **3b** (1 mmol).

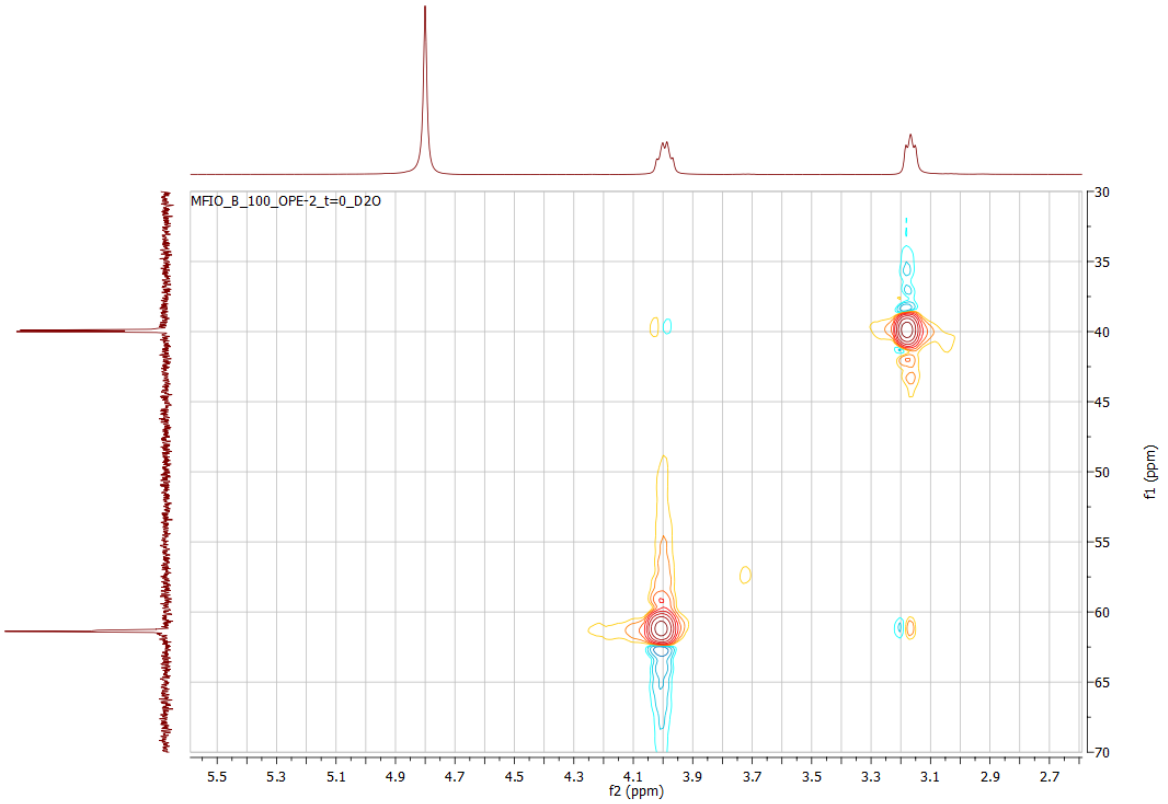


Figure S62: HSQC (D_2O, 75 MHz) of pure **3b** (1 mmol).

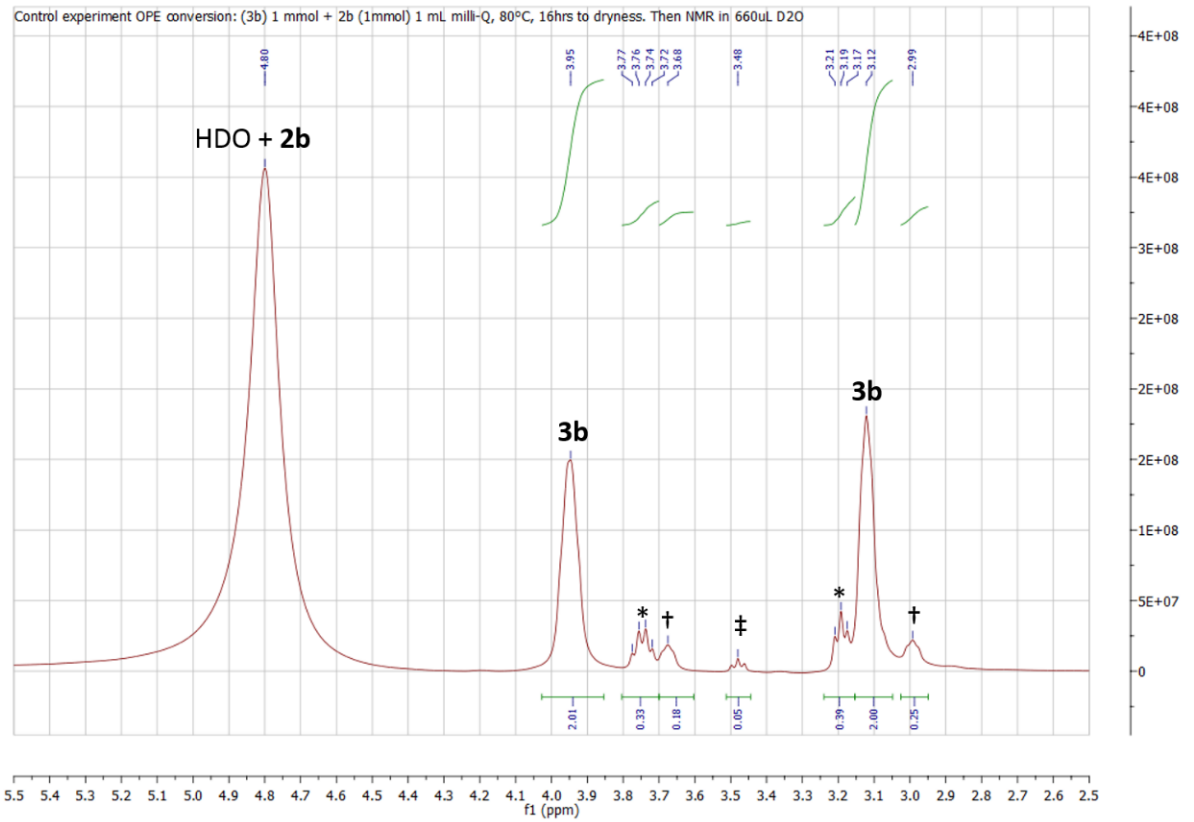


Figure S63. ^{1}H NMR (D_2O, 300MHz) of crude mixture obtained by reaction of pure **3b** (1 mmol) in the presence of urea (**2b**, 1 mmol) for 16 hrs at 80°C. The attributions deduced from the COSY (Fig. S64).

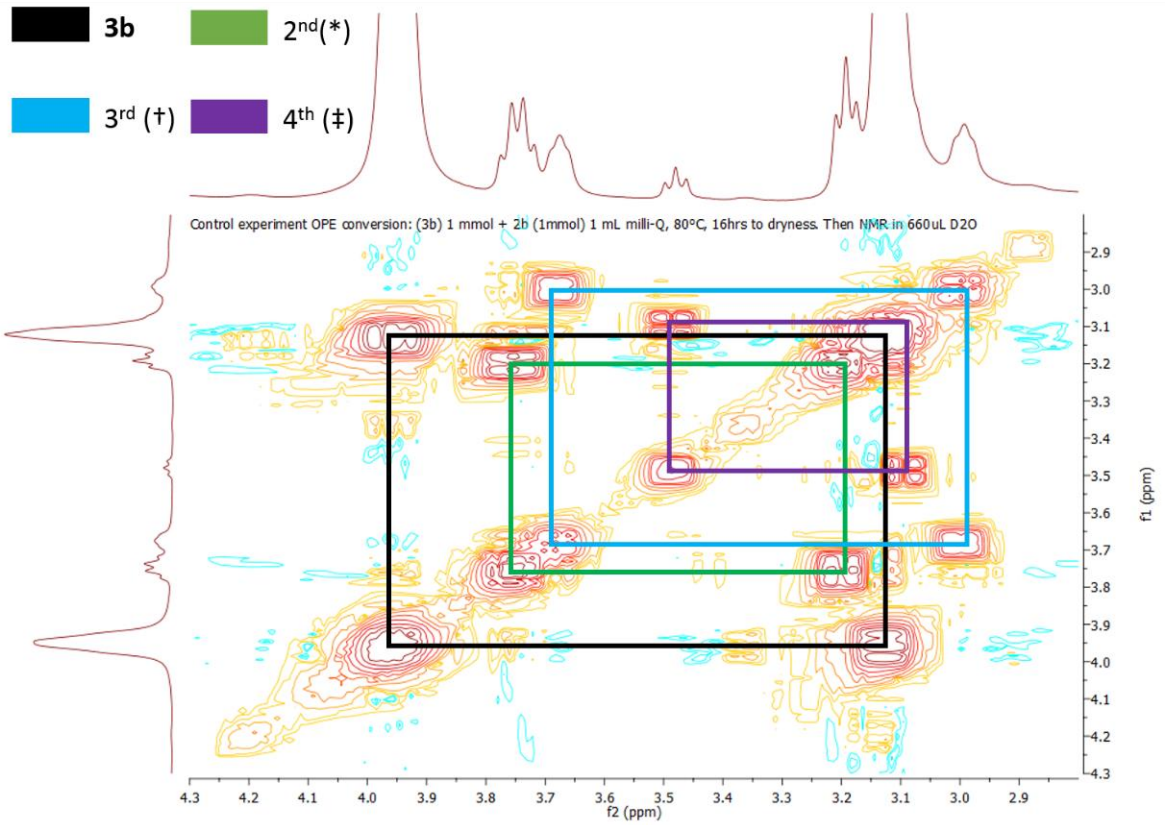


Figure S64. COSY (D_2O, 300MHz) of crude mixture obtained by reaction of pure **3b** (1 mmol) in the presence of urea (**2b**, 1 mmol) for 16 hrs at 80°C.

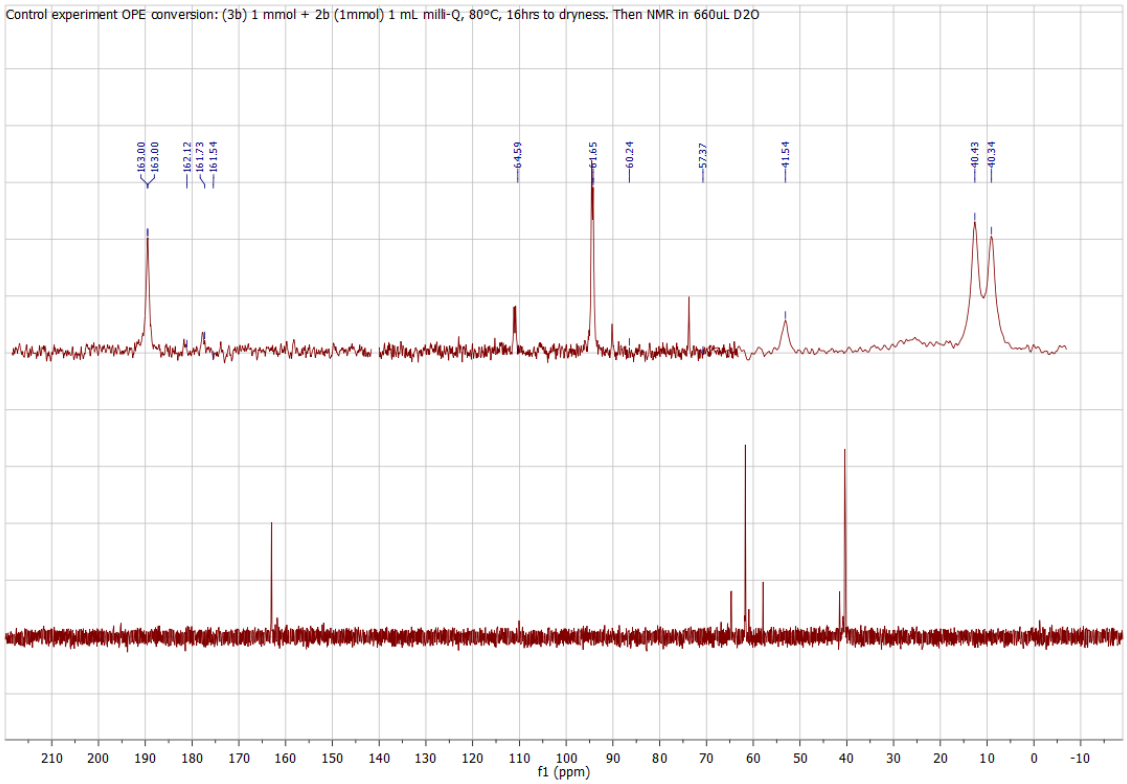


Figure S65: ^{13}C NMR (D_2O, 75MHz) of crude mixture obtained by reaction of pure **3b** (1 mmol) in the presence of urea (**2b**, 1 mmol) for 16 hrs at 80°C. Urea (**2b**) signal at 163.00 ppm is taken as internal reference. Bottom image: whole spectra; Top image: enlargements of the areas of interest (166.0 – 158.0 ppm, 70.0 – 56.0 ppm and 42.0 – 39.0 ppm).

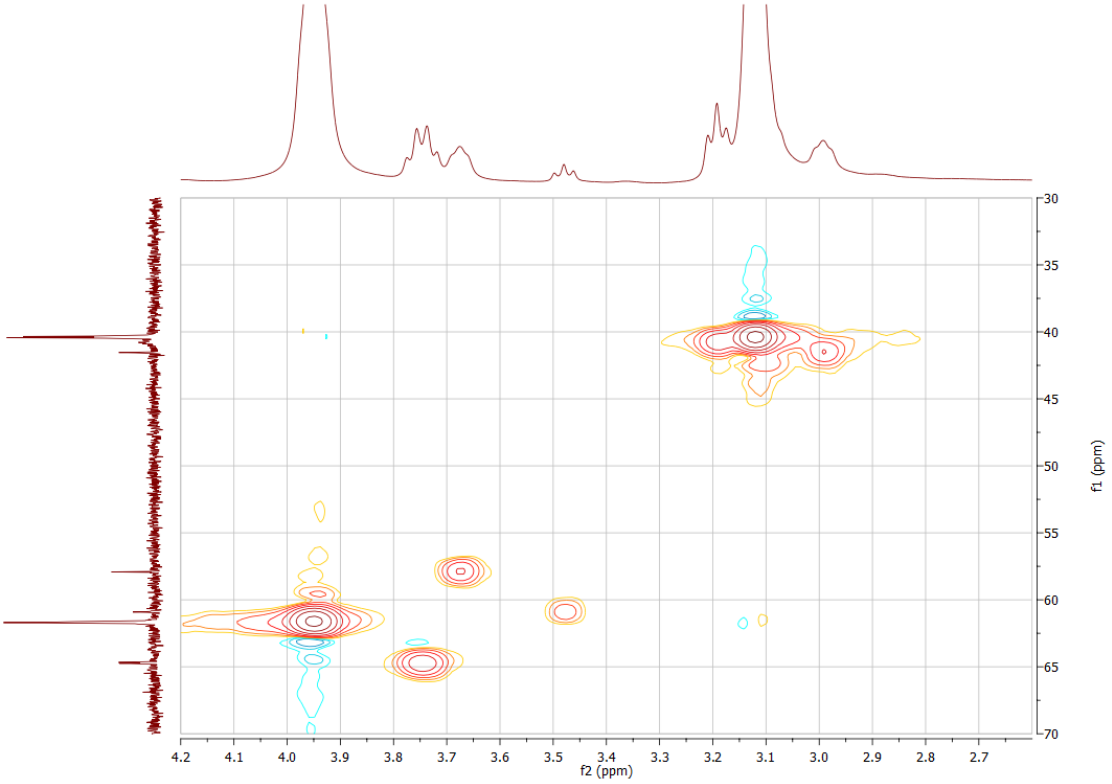


Figure S66. HSQC (D_2O, 75 MHz) of crude mixture obtained by reaction of pure **3b** (1 mmol) in the presence of urea (**2b**, 1 mmol) for 16 hrs at 80°C.

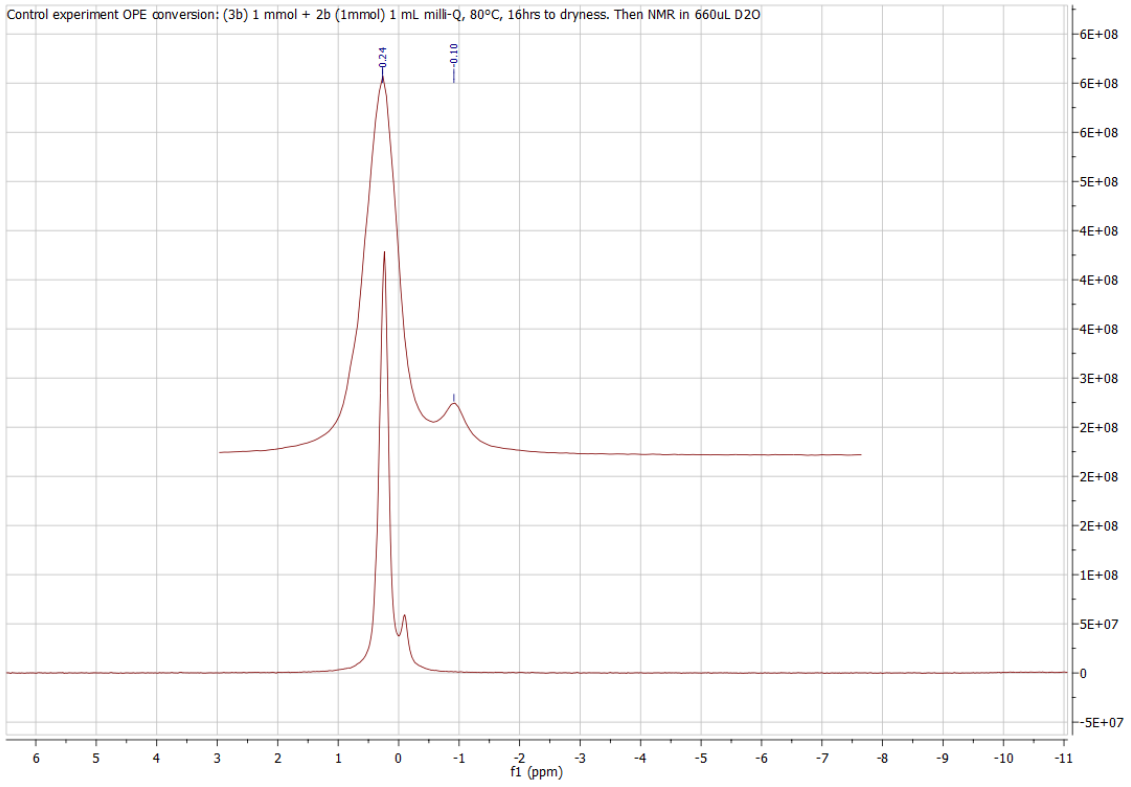


Figure S67: ^{31}P NMR (D_2O, 121.5 MHz) of crude mixture obtained by reaction of pure **3b** (1 mmol) in the presence of urea (**2b**, 1 mmol) for 16 hrs at 80°C. Top lane: enlarged zone.

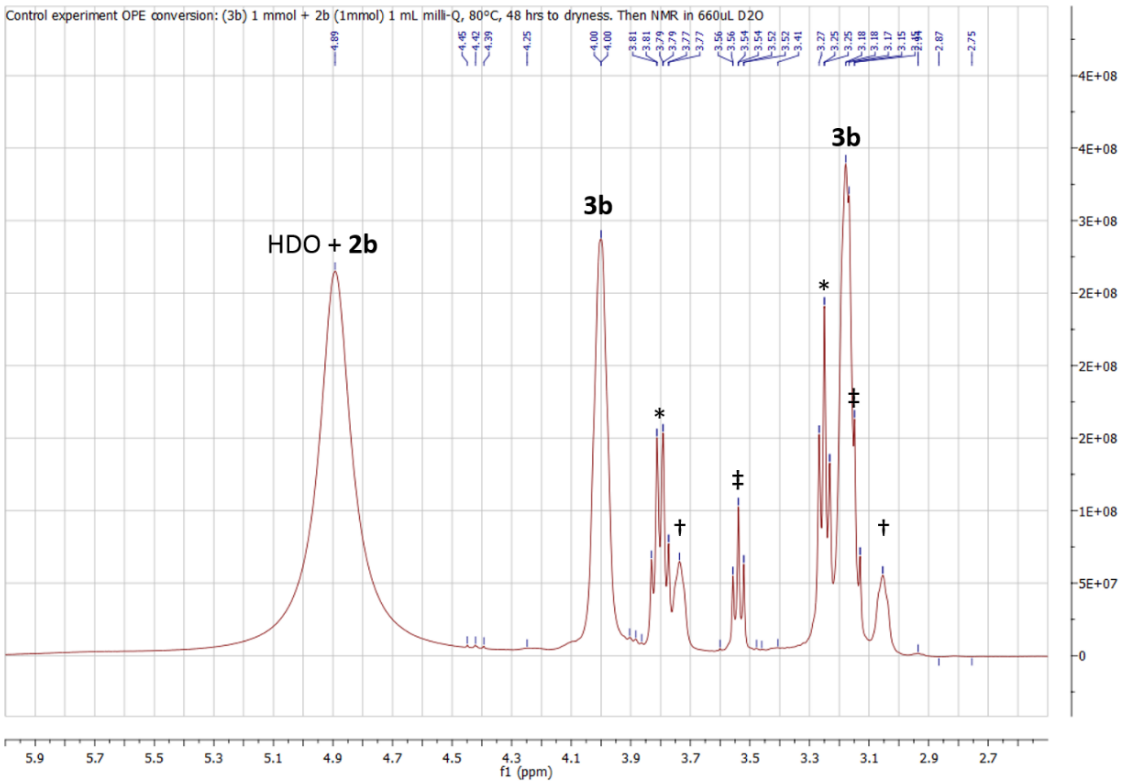


Figure S68. ^{1}H NMR (D_2O, 300MHz) of crude mixture obtained by reaction of pure **3b** (1 mmol) in the presence of urea (**2b**, 1 mmol) for 48 hrs at 80°C. The attributions deduced from the COSY (Fig. S69).

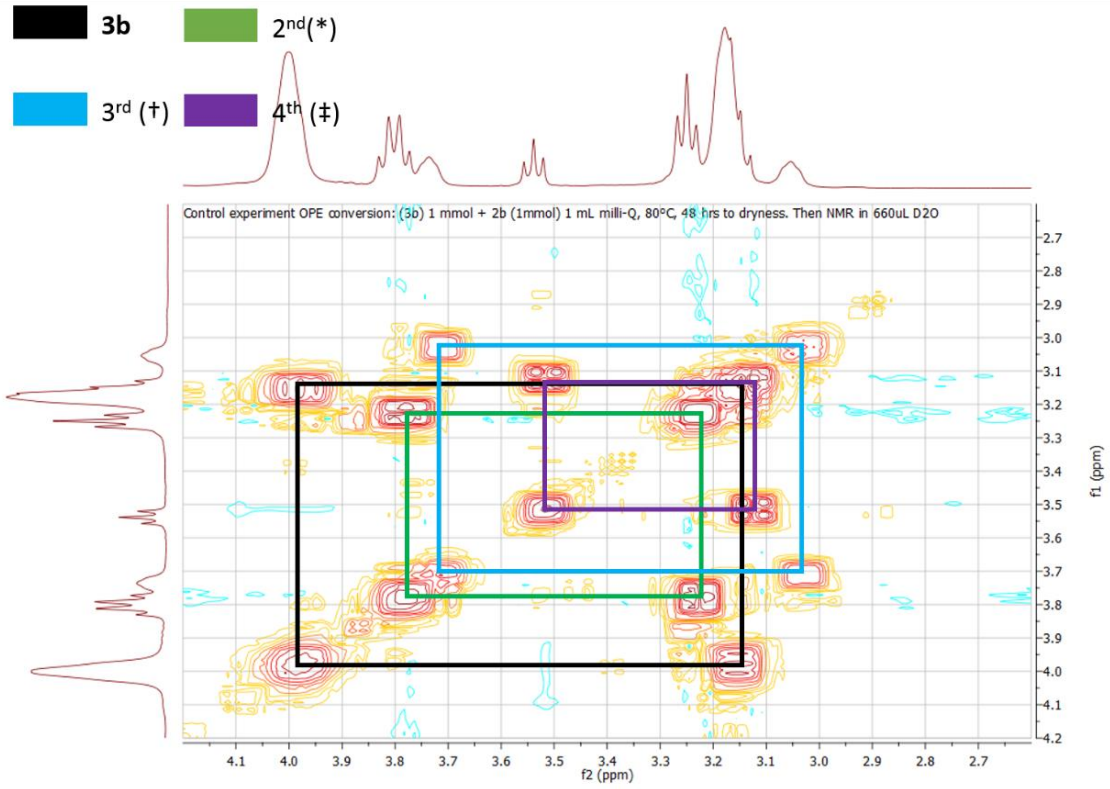


Figure S69. COSY (D_2O, 300MHz) of crude mixture obtained by reaction of pure **3b** (1 mmol) in presence of urea (**2b**, 1 mmol) for 48 hrs at 80°C.

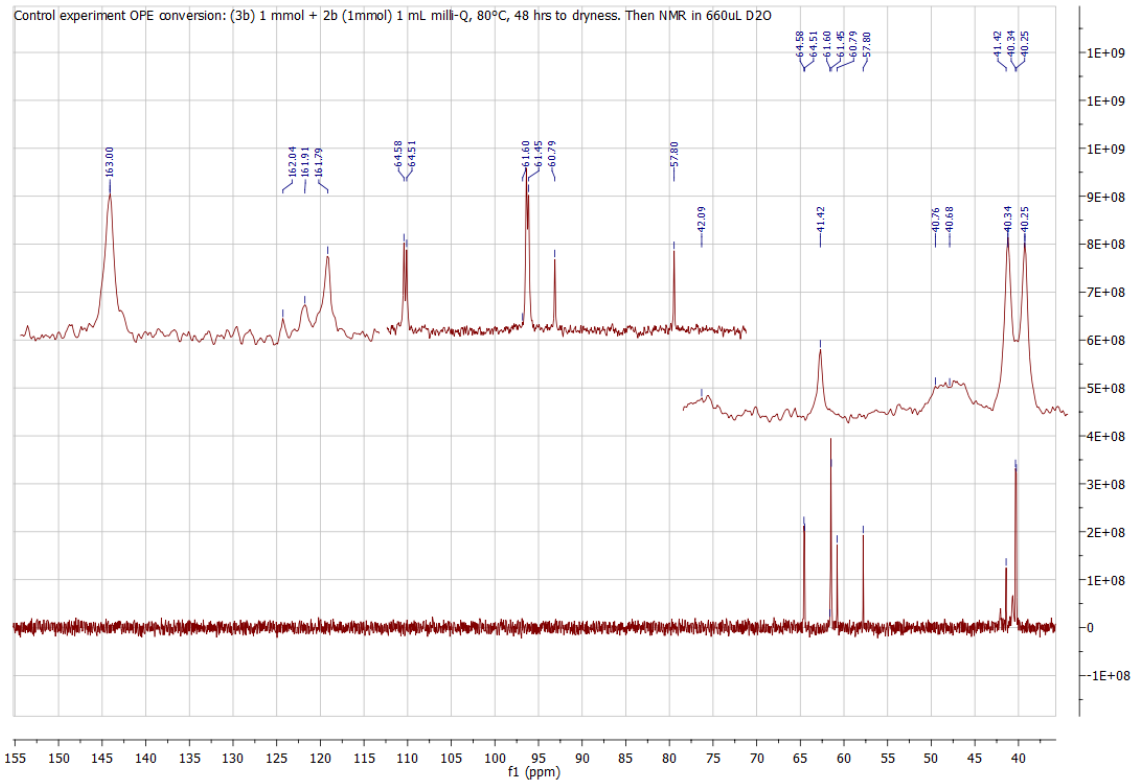


Figure S70. ^{13}C NMR (D_2O, 75MHz) of crude mixture obtained by reaction of pure **3b** (1 mmol) in the presence of urea (**2b**, 1 mmol) for 48 hrs at 80°C. The urea (**2b**) signal at 163.00 ppm is taken as internal reference. Bottom image: whole spectrum; Top image: enlargements of the areas of interest (163.5 – 161.5 ppm, 65.0 – 56.0 ppm and 42.5 – 40.0 ppm).

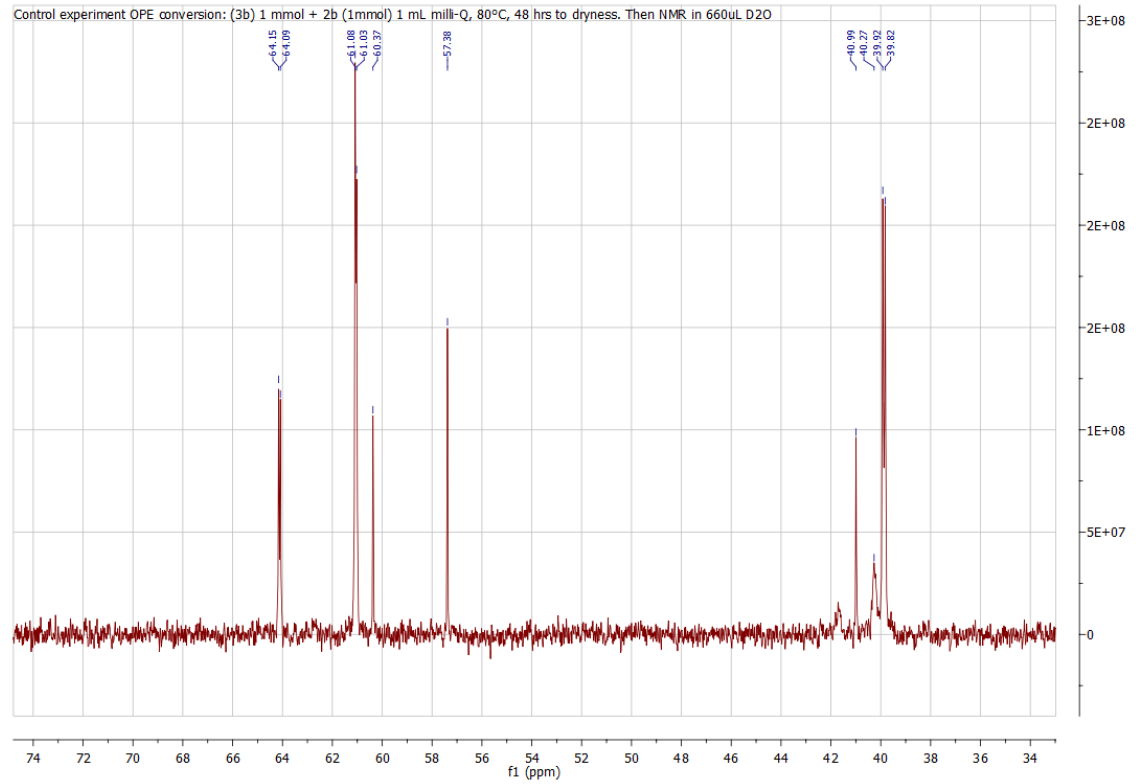


Figure S71. DEPT (D_2O, 75MHz) of crude mixture obtained by reaction of pure **3b** (1 mmol) in the presence of urea (**2b**, 1 mmol) for 48 hrs at 80°C. All carbons are -CH_2- except those of the area between 163.5 and 161.5 ppm (quaternary carbons of urea, 163.0 ppm or ureyl ones).

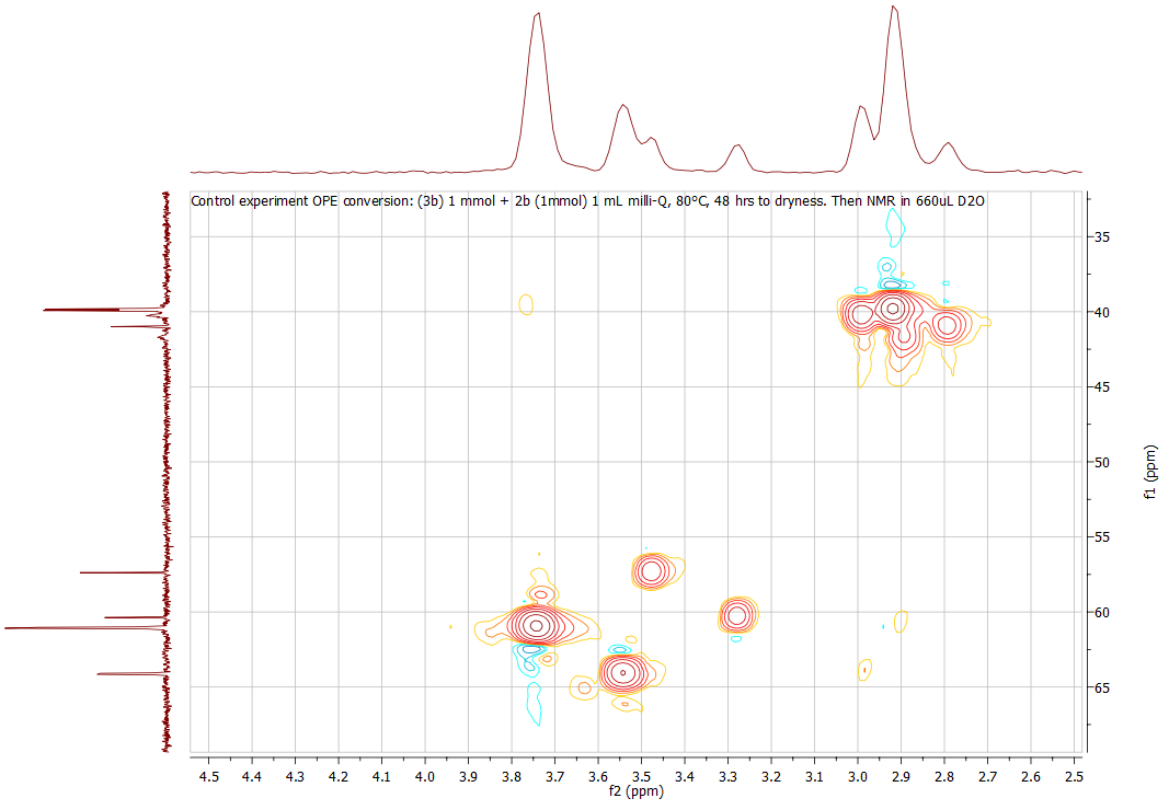


Figure S72. HSQC (D_2O, 75 MHz) of crude mixture obtained by reaction of pure **3b** (1 mmol) in the presence of urea (**2b**, 1 mmol) for 48 hrs at 80°C.

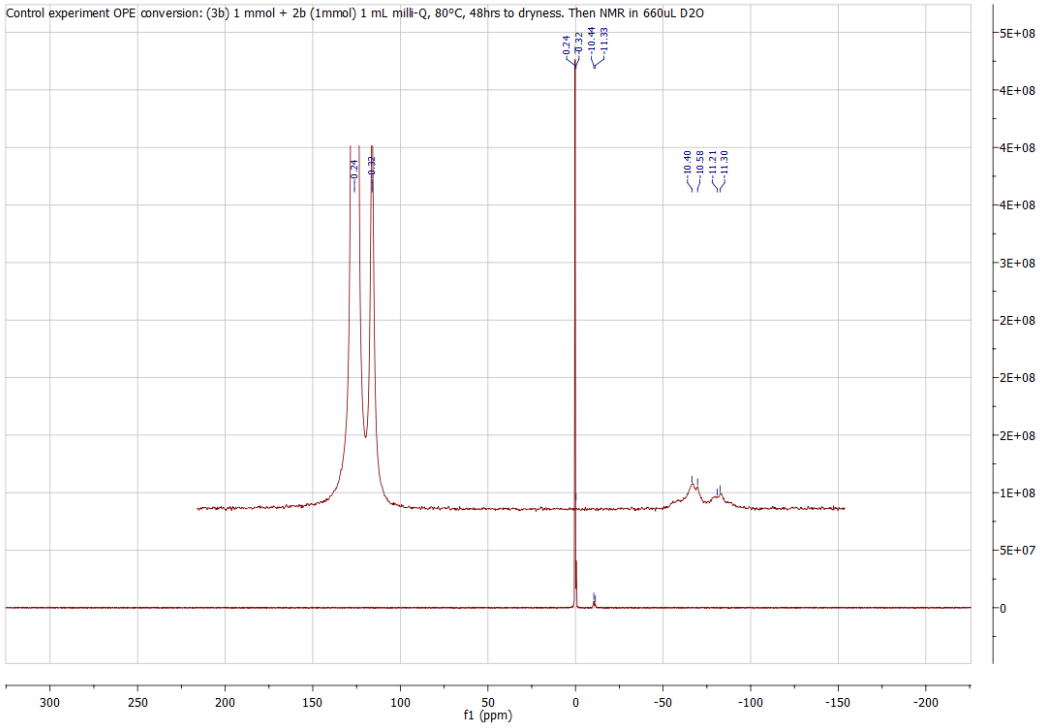


Figure S73. ^{31}P NMR (D_2O, 121.5 MHz) of crude mixture obtained by reaction of pure **3b** (1 mmol) in the presence of urea (**2b**, 1 mmol) for 48 hrs at 80°C. Small signals at –10 to –11 ppm are consistent with the presence of pyrophosphates.

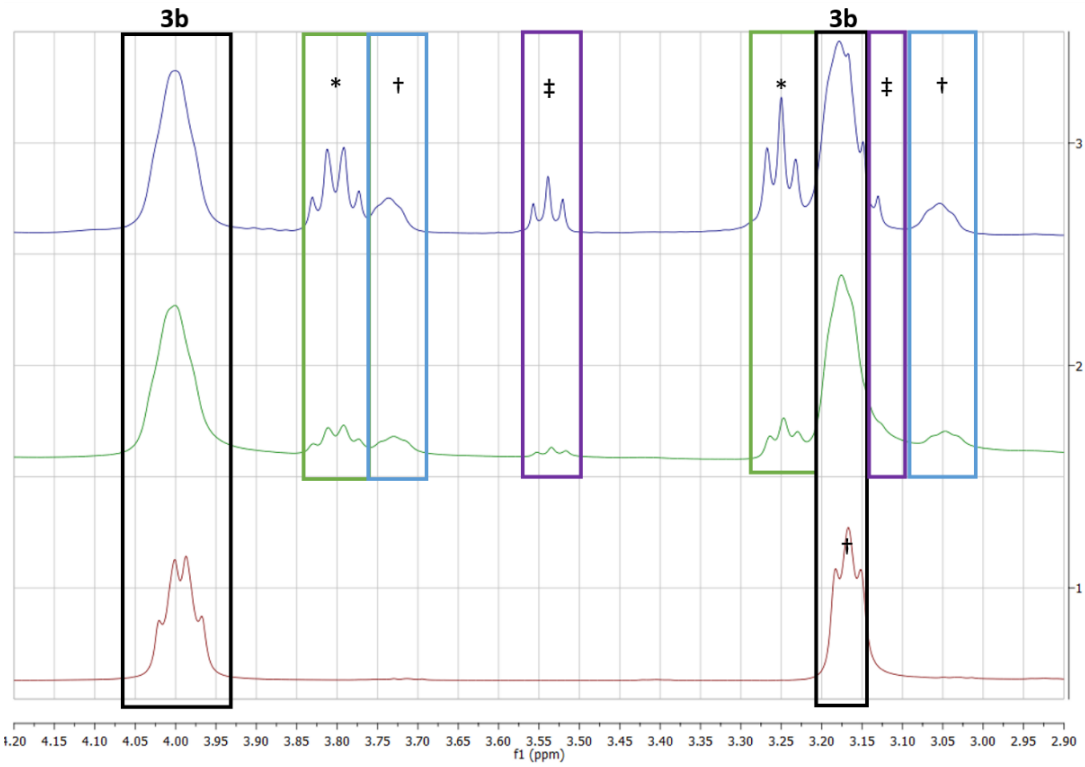


Figure S74. Stacked ^{1}H NMR (D_2O, 300 MHz) of crude mixture obtained by reaction of pure **3b** (1 mmol) in presence of urea (**2b**, 1 mmol) after 16 and 48 hrs at 80°C. Bottom: t=0; center t=16 hrs; top: 48 hrs. Downfield (correlated with upfield) quartets originate from O-phosphorylated compounds ($^{3}J_{PH}$ and $^{3}J_{HH}$ couplings of similar strength), whereas downfield (correlated with upfield) triplets are consistent with the lack of a phosphate group (no $^{3}J_{PH}$ coupling, only $^{3}J_{HH}$).

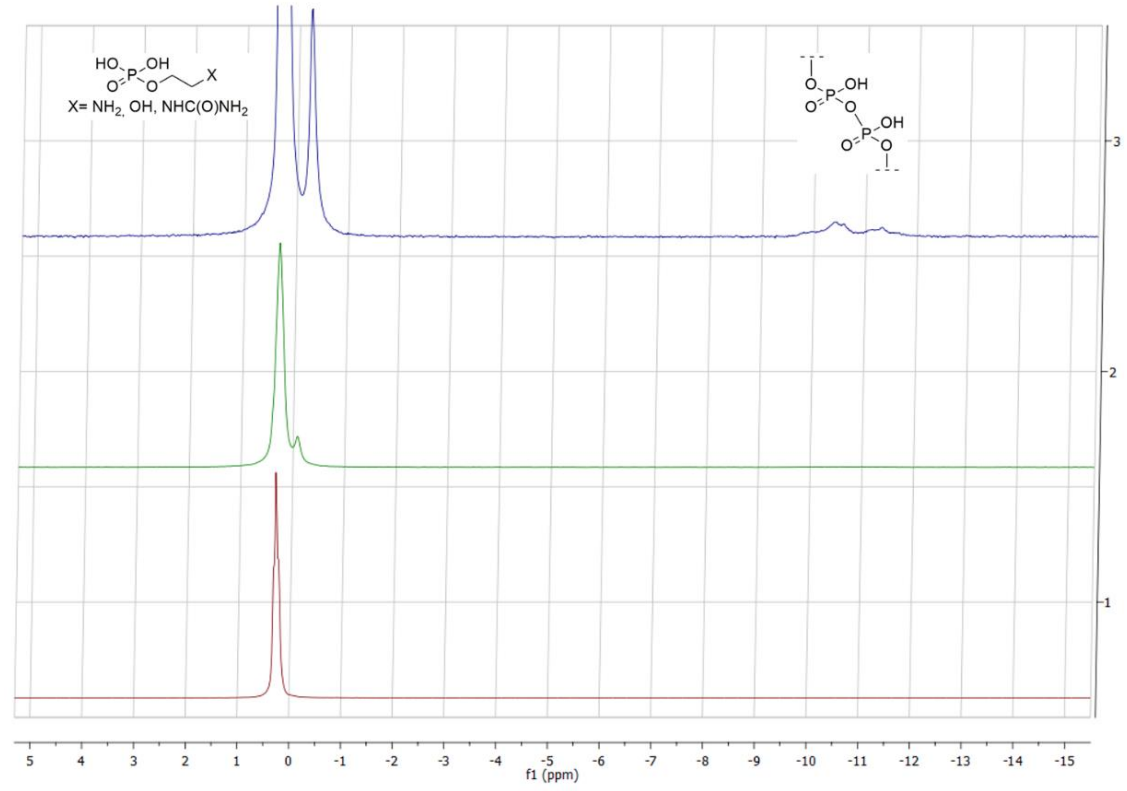


Figure S75. Stacked ^{31}P NMR spectra (D_2O, 121.5 MHz) of crude mixture obtained by reaction of pure **3b** (1 mmol) in the presence of urea (**2b**, 1 mmol) after 16 and 48 hrs at 80°C. Bottom: t=0; center t=16 hrs; top: 48 hrs.

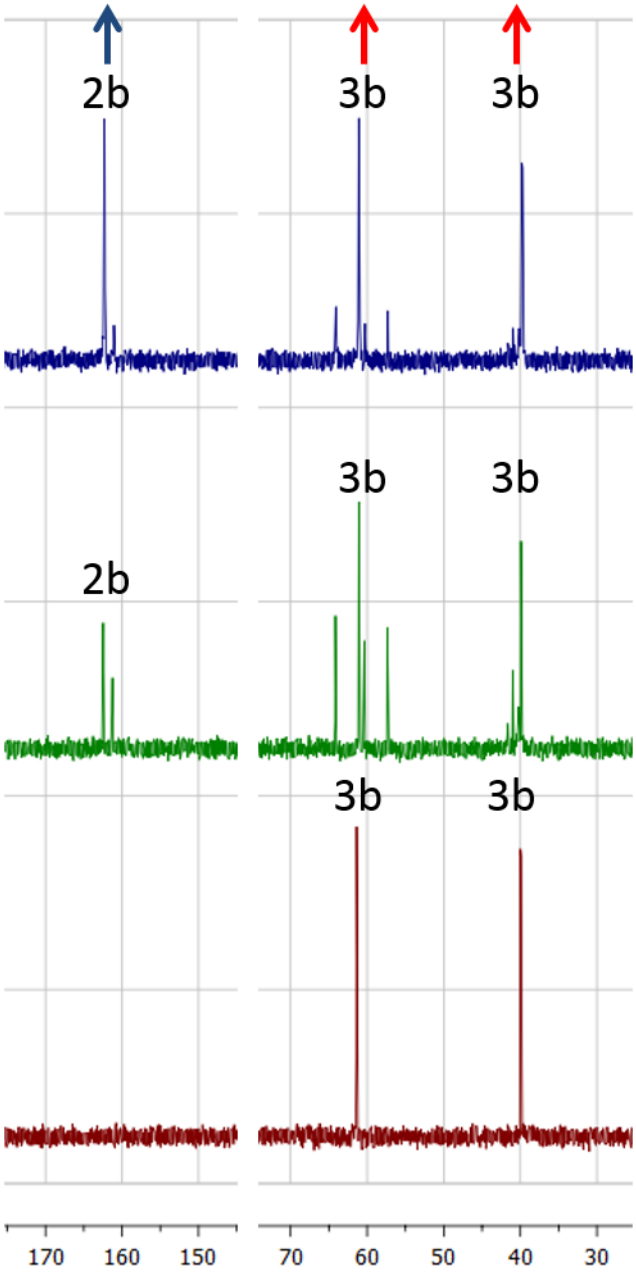


Figure S76. ^{13}C NMR (D_2O, 75 MHz) spiking of crude mixture obtained by reaction of pure **3b** (1 mmol) in the presence of urea (**2b**, 1 mmol) after 48 hrs at 80°C by adding 0.5 mmol of **3b**. Bottom: pure **3b** (1mmol); center t=48 hrs; top: after 48h, spiked with 0.5 mmol of **2b** and 0.5 mmol of **3b**.

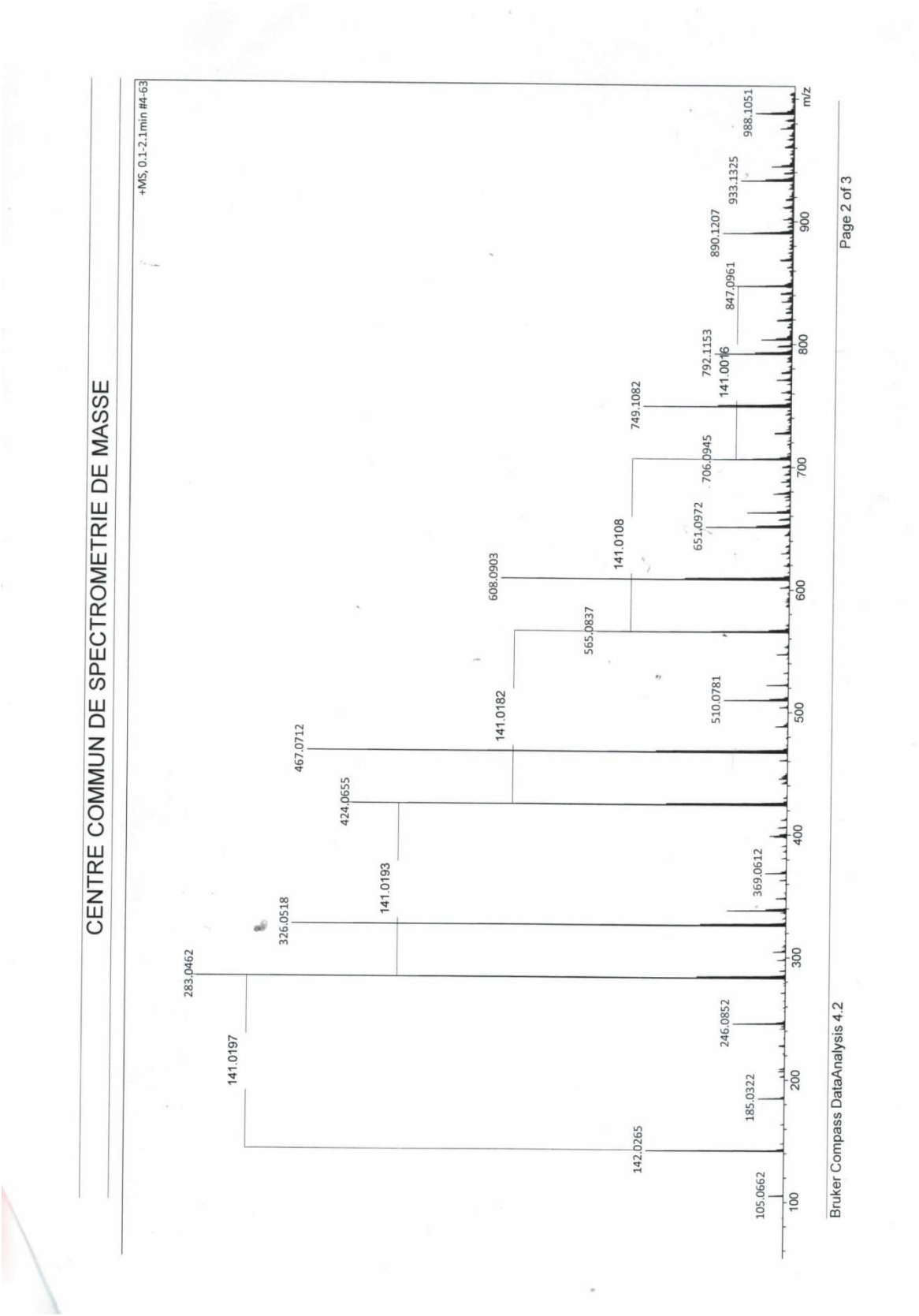


Figure S77. HR-MS (positive ion mode) of the crude mixture obtained by reaction of pure **3b** (1 mmol) in the presence of urea (**2b**, 1 mmol) after 48 hrs at 80°C.

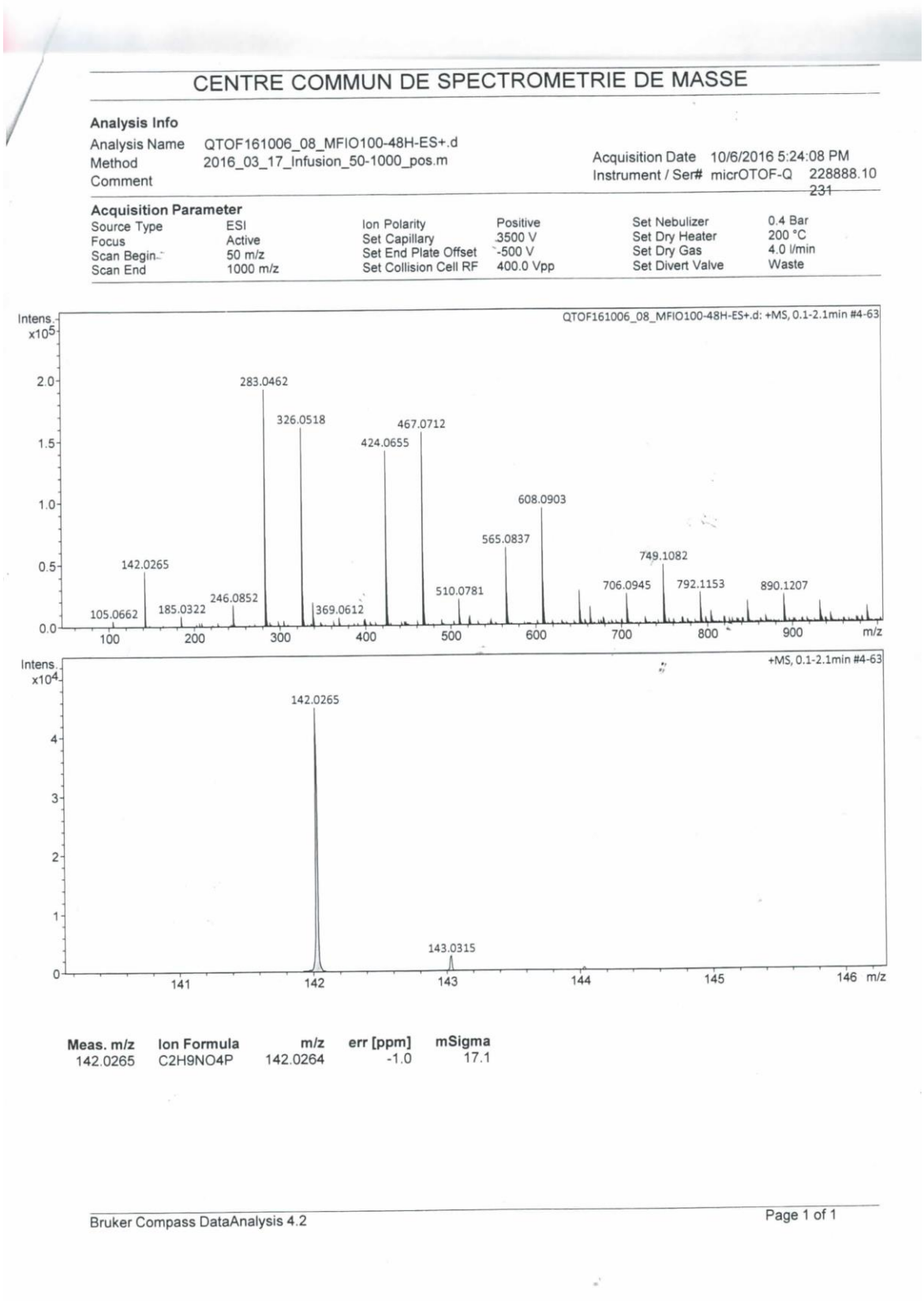


Figure S78. HR-MS (positive ion mode) of the crude mixture obtained by reaction of pure **3b** (1 mmol) in the presence of urea (**2b**, 1 mmol) after 48 hrs at 80°C.

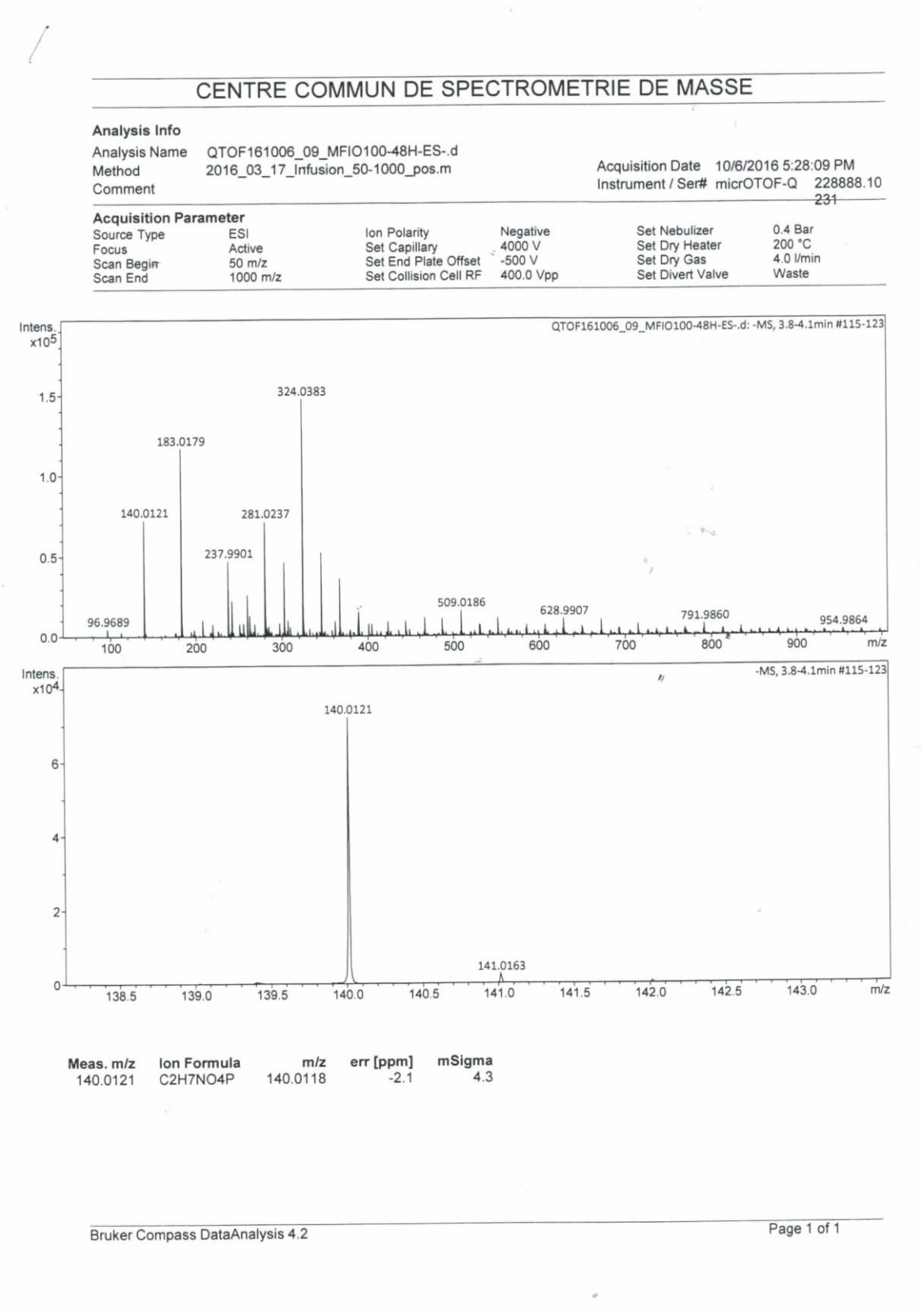


Figure S79. HR-MS (negative ion mode) of the crude mixture obtained by reaction of pure **3b** (1 mmol) in the presence of urea (**2b**, 1 mmol) after 48 hrs at 80°C.

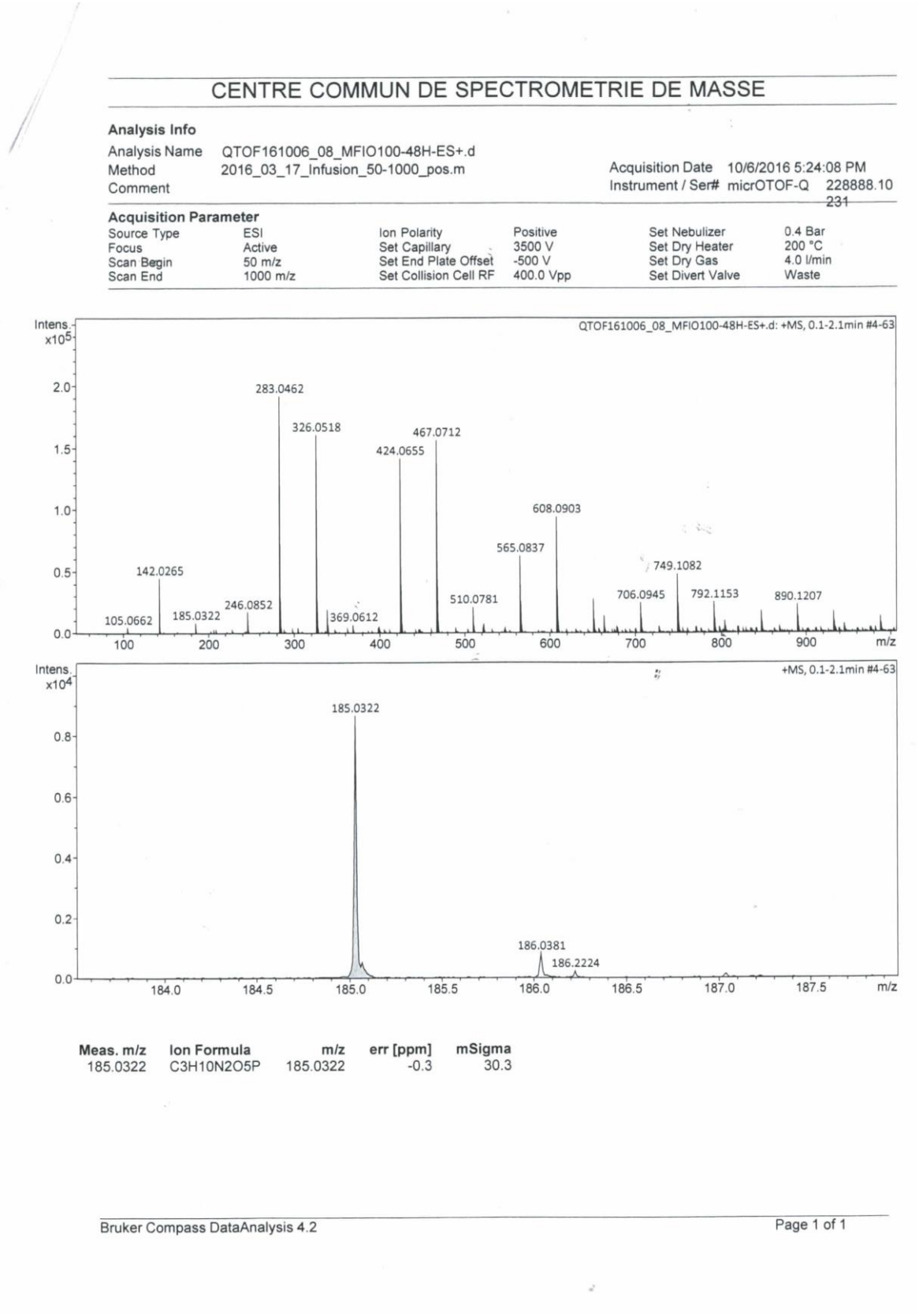


Figure S80. HR-MS (positive ion mode) of the crude mixture obtained by reaction of pure **3b** (1 mmol) in the presence of urea (**2b**, 1 mmol) after 48 hrs at 80°C.

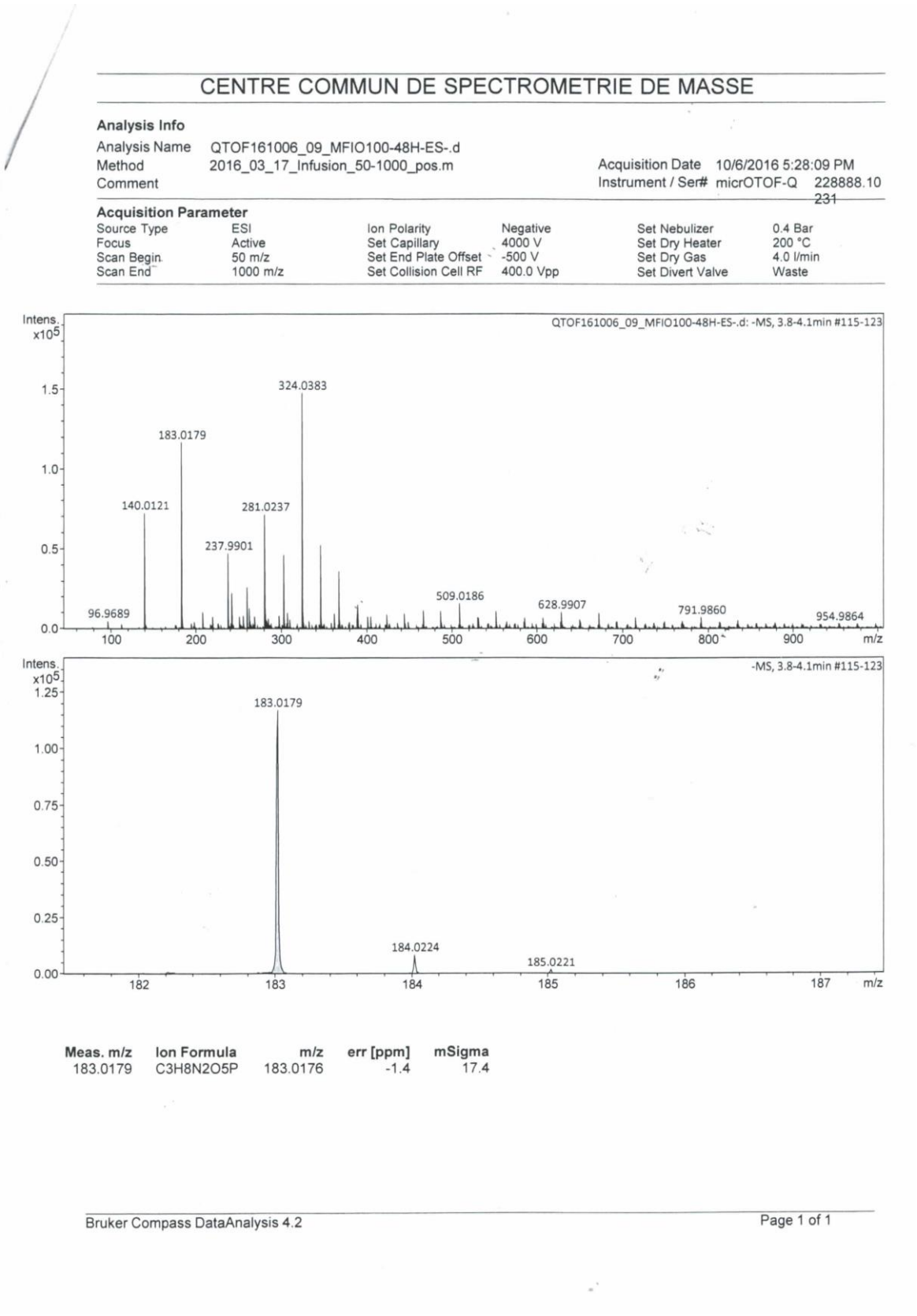


Figure S81. HR-MS (negative ion mode) of the crude mixture obtained by reaction of pure **3b** (1 mmol) in the presence of urea (**2b**, 1 mmol) after 48 hrs at 80°C.

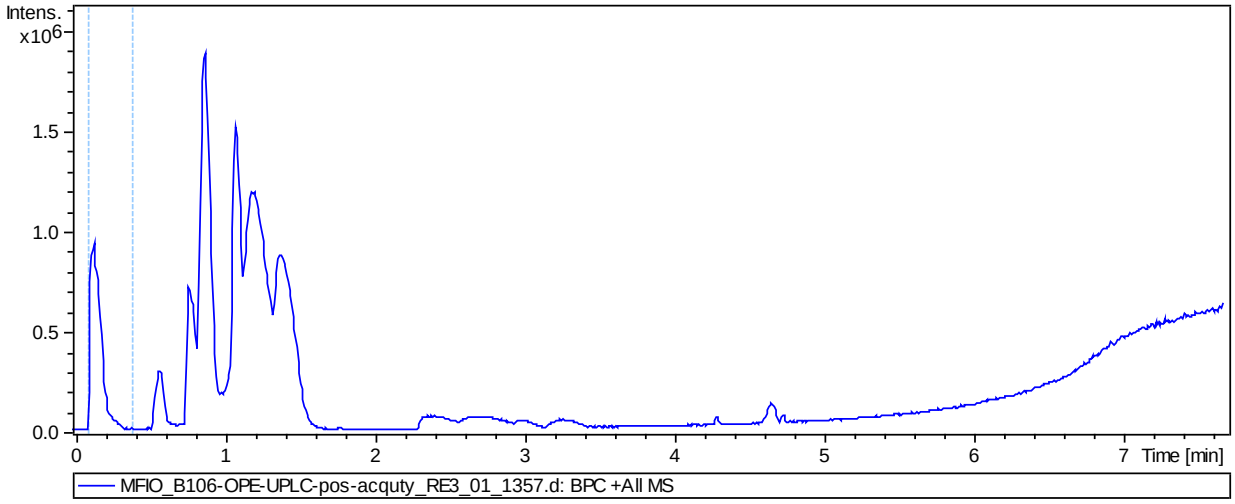


Figure S82. UPLC HR-MS total (positive) ion chromatogram of the crude mixture obtained by reaction of pure **3b** (1 mmol) in the presence of urea (**2b**, 1 mmol) after 48 hrs at 80°C.

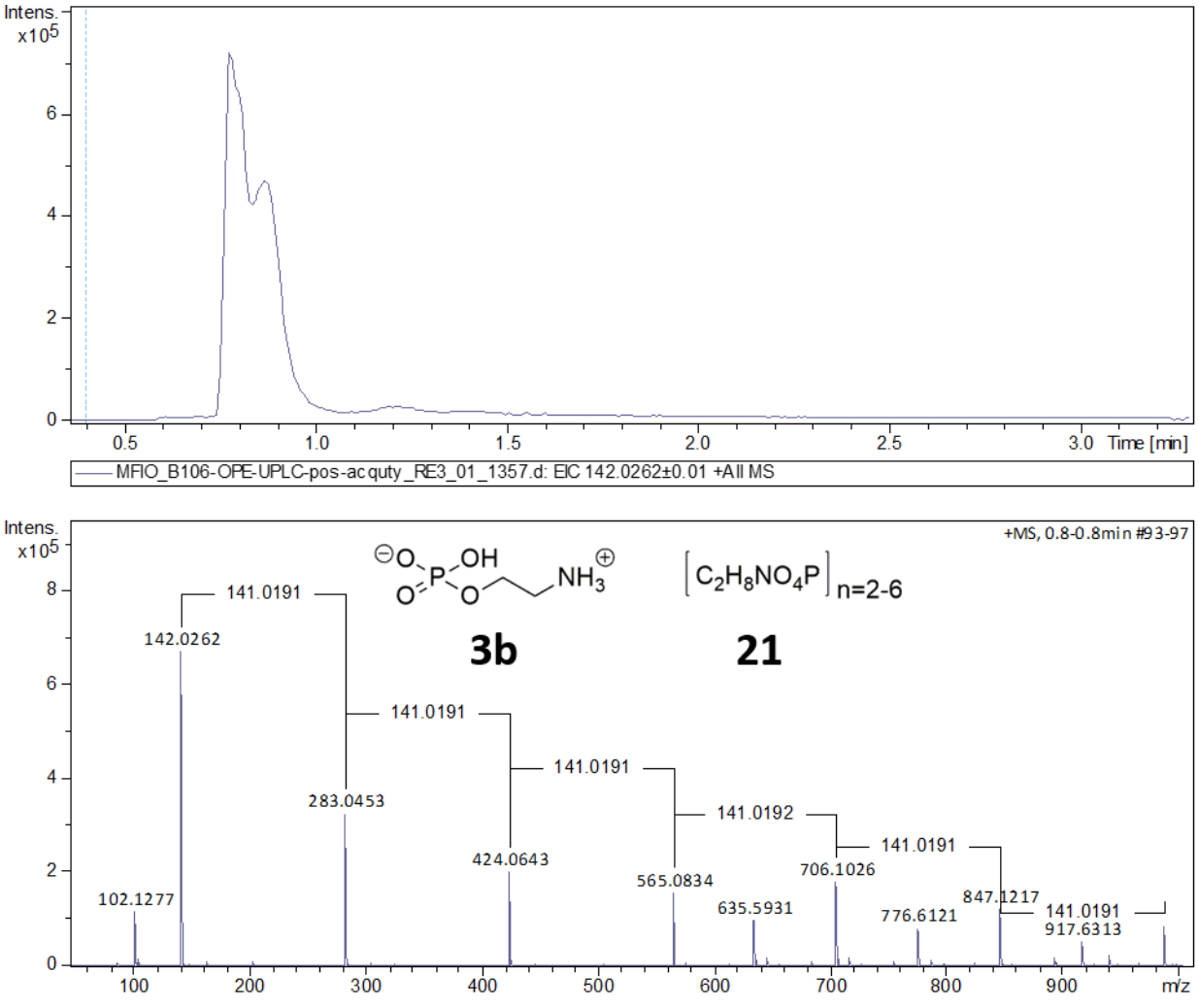


Figure S83. UPLC HR-MS extracted (positive) ion mode chromatogram on *m/z* 142.0262 of the crude mixture obtained by reaction of pure **3b** (1 mmol) in the presence of urea (**2b**, 1 mmol) after 48 hrs at 80°C. The MS profile shows the presence of **3b** (*m/z* 141.0190) and the associated polymer **21** $(C_2H_8N_2O_4P)_n$.

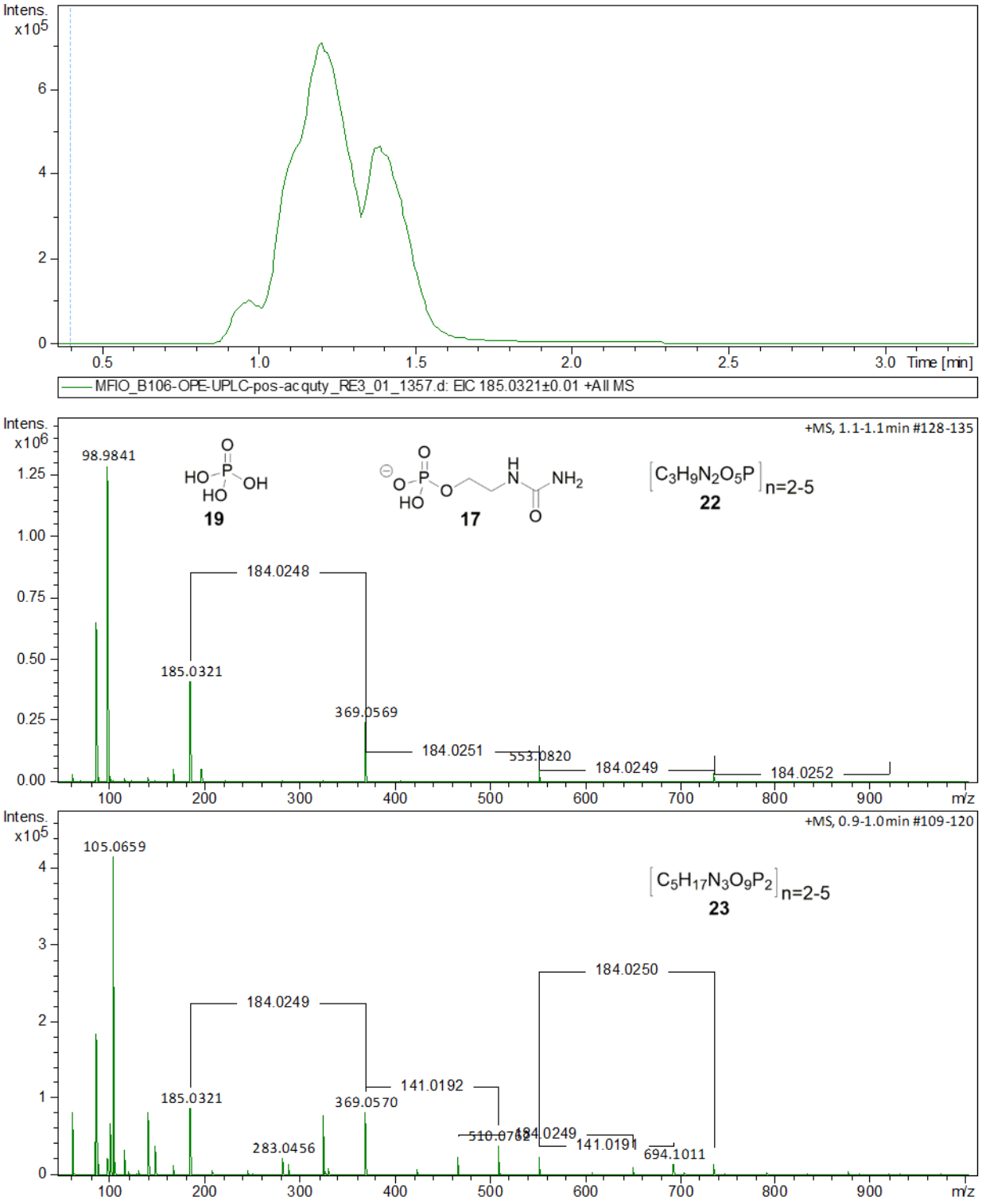


Figure S84. UPLC HR-MS extracted (positive) ion mode chromatogram on *m/z* 185.0321 of the crude mixture obtained by reaction of pure **3b** (1 mmol) in the presence of urea (**2b**, 1 mmol) after 48 hrs at 80°C. The MS profile show the presence of **17** (*m/z* 184.0248) and the associated co-polymer **23** $(C_2H_8N_2O_4P)_n$ between **3b** and **17**. Phosphate **19** was detected with *m/z* 98.9841 as $[M+H]^+$.

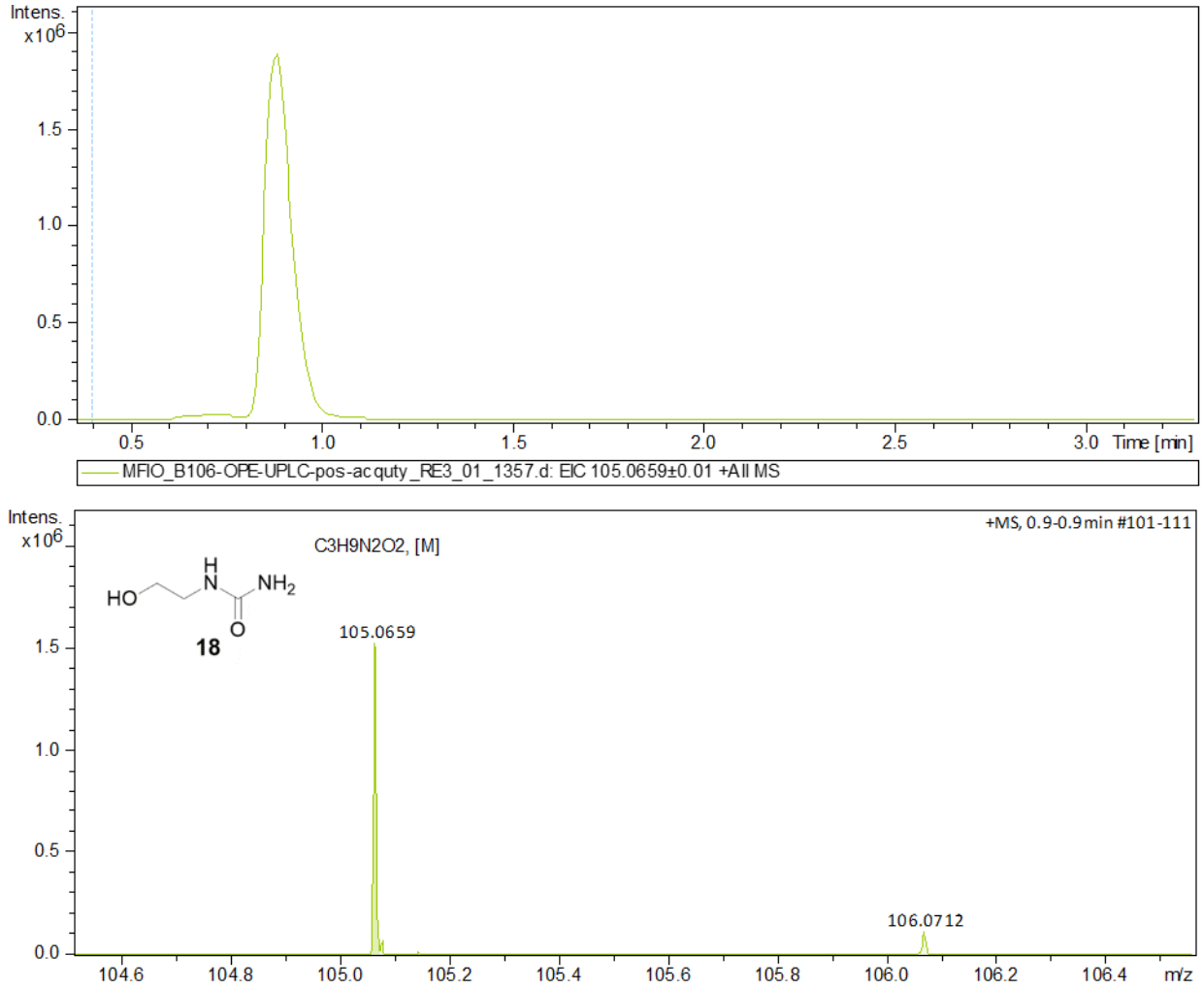


Figure S85. UPLC HR-MS (positive mode) of the crude mixture obtained by reaction of pure **3b** (1 mmol) in the presence of urea (**2b**, 1 mmol) after 48 hrs at 80°C. The MS profile show the presence of **18** (*m/z* 105.0659)