Synthesis of Oleylphosphonates as Potential Inhibitors of DAGL and MAGL.

I PART

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Summary

Endocannabinoids are produced during the organ damage caused by different pathological situations. The most abundant endocannabinoid is 2-Arachidonoylglycerol (2-AG) present in large amounts within the brain. The endogenous levels of 2-AG are linked to the activities of two enzymes: diacylglycerol lipase (DAGL) and monoacylglycerol lipase (MAGL). In this paper we reported the synthesis of different oleylphosphonates substituted that should be specifically recognized from DAGL and MAGL of the endocannabinoid system.

Introduction

The Endocannabinoid System (SEC) is an endogenous system of signals, found in the nineties, thanks to research carried out on the main psychoactive component of Cannabis, the (-)-Δ9-tetrahydrocannabinol (THC) [1,2] (Fig. 1).

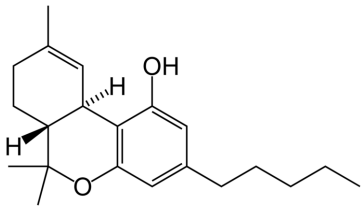
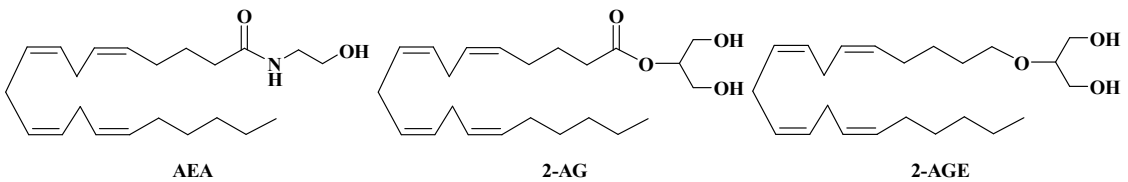


Figure 1

The assumption that the effects of THC were mediated by specific receptors (not by disruption of cell membranes) was confirmed by Howlett et al. [3,4], which demonstrated that cannabinoids inhibit the production of cAMP in neuroblastoma cells. The existence of cannabinoid receptors was confirmed by Matsuda et al. [5] only after molecular cloning of cannabinoid receptors in rat brain. The SEC, therefore, consists of cannabinoid receptors, their endogenous ligands (endocannabinoids) and proteins involved in their synthesis and inactivation. In human two cannabinoid receptors (GB1, GB2) have been characterized; they belong to a family of membrane metarbotropic receptors coupled to G proteins (G_i).

So far five endogenous agonists for these receptors have been discovered. These molecules, precisely defined endocannabinoids (Fig. 2), all seem to derive from arachidonic acid. In particular they are: *N*-Arachidonoylethanolamine (Anandamide, AEA), 2-Arachidonoylglycerol (2-AG), 2-Arachidonoylglicerol ether (Noladin, 2-AGE), O-Arachidonoylethanolamine (Virodhamine, OAE) and *N*-arachidonoyl dopamine (NADA), showing different activities towards the two cannabinoid receptors (Fig.2).



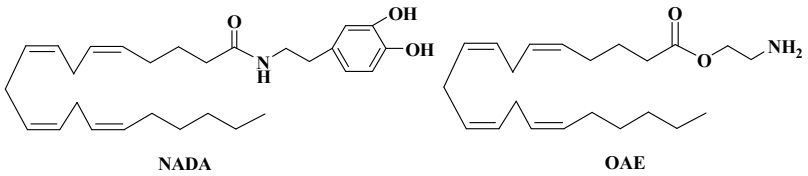


Figure 2

Endocannabinoids are produced during the organ damage caused by different pathological situations, in order to obtain an anti-oxidant, hypotensive, immunosuppressive, anti-inflammatory and analgesic action.

The most abundant endocannabinoid is 2-AG present in large amounts within the brain. [6-8]

This molecule acts as an endogenous neuromodulator of all signs of SEC (at both central and peripheral), with direct involvement in control of stimuli of neuronal and non-neuronal cells and of physio-pathological disorders of the central and peripheral organs. The 2-AG, intermediate of metabolism of glycerides and phosphoglycerides, behaves as endogenous ligand of CB1 and CB2 receptors, in contrast to anandamide (AEA), that has a predominant binding activity against the CB1 receptor; the power of 2-AG and its capillary action throughout the body can be attributed to its ability to act on both cannabinoid receptors.[9,10]

The endogenous levels of 2-AG are linked to the activities of two enzymes: diacylglycerol lipase (DAGL) and monoacylglycerol lipase (MAGL). MAGL and DAGL (both Ser-hydrolases), have high structural homology and operate in the opposite way: one catalyzing the biosynthesis of 2-AG and the other the metabolism of the endocannabinoid.[11-14].

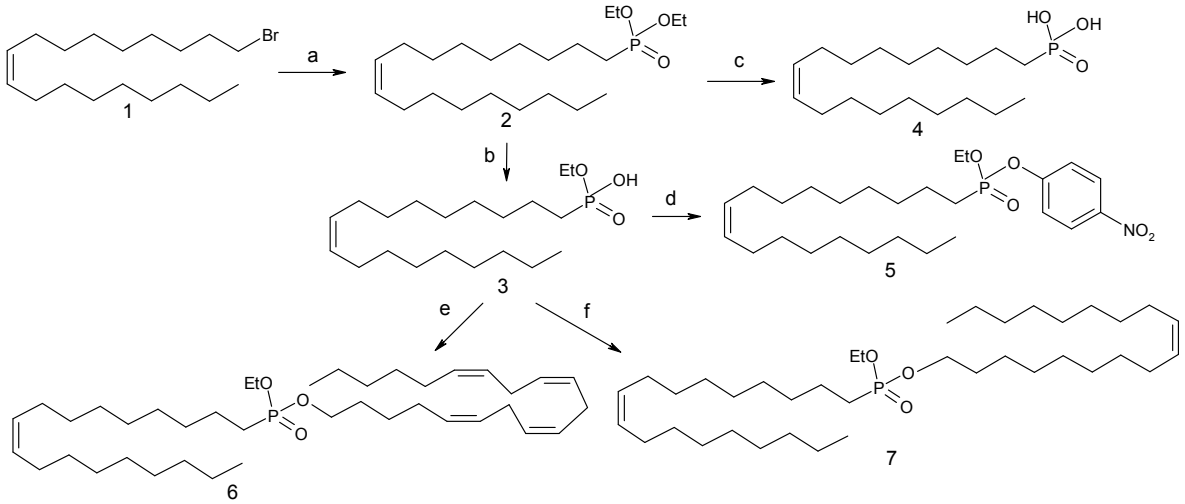
DAGL increases the levels of this endocannabinoid; two sn-1-DAG lipases (DAGLα and DAGLß) have been proposed as key enzymes in the biosynthetic pathway of 2-AG.

Instead MAGL that is inactive on anandamide, was cloned and proposed as the main catabolic enzyme of 2-AG. It has predominantly a pre-synaptic localization in the Central Nervous System.

Any potent and selective inhibitor for these proteins (MAGL and DAGL) was not yet discovered.

Results and discussion

In this paper we reported the synthesis of different oleylphosphonates substituted that should be specifically recognized from DAGL and MAGL of the endocannabinoid system. Thirteen oleylphosphonates were synthesized. They differ only in the phosphonic portion that is otherwise esterified. All molecules of this class show an oleic group (Scheme 1, Scheme 2).



a) TEP, NaI; b) H_2O/MeOH, KOH; c) BBr_3, Toluene; d) *p*-NF, DCC, DMAP, Toluene; e) (1) Oxalyl chloride, DMF, DCM; (2) arachidonic alcohol, DCM; f) (1) Oxalylchloride, DMF, DCM; (2) oleic alcohol, DCM.

Scheme 1

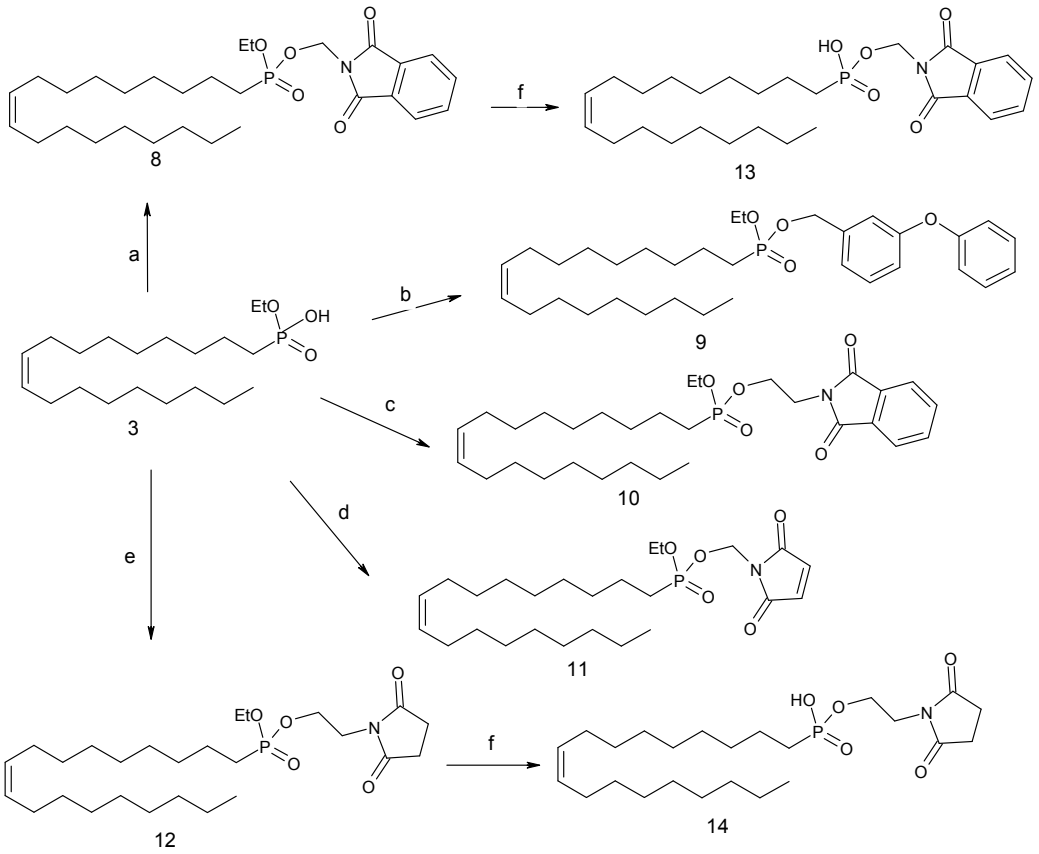
The compound diethyl-oleylphosphonate **2** was synthesized starting from oleic acid by reduction with $LiAlH_4$ to alcohol, subsequent bromination with *N*-bromosuccinimide and then Arbuzov reaction between triethyl phosphite and oleylbromide (Scheme 1). [15]

The phosphonate **2** was the starting material for the synthesis of all oleylphosphonates synthetized. The molecules **3** and **4** were prepared, starting from the phosphonic diester **2,** by partial hydrolysis with H_2O/MeOH and for reaction with BBr_3 respectively.

Ethyl-*p*-nitrophenyl-oleylphosphonate **5**, arachidonyl ethyl-oleylphosphonate **6** and oleyl ethyl-oleylphosphonate **7** were obtained through esterification reactions between ethyl-oleylphosphonate **3** and *p*-nitrophenol, arachidonic alcohol and oleic alcohol respectively (Scheme 1).

Ethyl-*N*-methylphthalimide-oleylphosphonate **8**, ethyl-3-phenoxybenzyl-oleylphosphonate **9**, ethyl-*N*-ethylphthalimide-oleylphosphonate **10**, ethyl-*N*-methylmaleimide-oleylphosphonate **11,** ethyl-*N*-ethylsuccinimide-oleylphosphonate **12** have been also prepared. They were obtained through an esterification of ethyl-oleylphosphonate **3** with the corresponding alcohols, in presence of BOP (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium-hexafluorophosphate) in DIEA (*N*,*N*-diisopropylethylamine) and DCM (dichloromethane).

The derivatives *N*-methylphthalimide-oleylphosphonate **13** and *N*-ethylsuccinimide-oleylphosphonate **14** were synthesized through a partial hydrolysis with H_2O/MeOH/KOH starting respectively from **8** and **12** (Scheme 2).



a) N-HMP, BOP, IEA, DCM; b) 3-PBA, BOP, DIEA, DCM; c) N-HEP, BOP, DIEA, DCM; d) N-IMM, BOP, DIEA, DCM; e) N-IES, BOP, DIEA, DCM; f) H_2O/MeOH, KOH.

Scheme 2

The inhibition of DAGL and MAGL enzymes of the potential ligands of SEC prepared in the present work will determine.

Experimental section

General methods

The synthesis were carried out in liquid phase and the final products were purified by chromatography (CC) using silica gel (Merk silica gel). Thin layer chromatography (TLC) was performed on silica gel 60F-264 (Merck) with fluorescent indicator. ^{1}H-NMR ^{13}C-NMR and ^{31}-P-NMR spectra were recorded in $CDCl_3$ on a Bruker 300 spectrometer. Chemical shifts are expressed in parts per million. Mass spectra (ESI-MS) were obtained by using a Finnigan LCQ Deca *ion trap* spectrometer.

Synthesis of octadec-9-enyl-phosphonic-acid-diethyl-ester (2)

A mixture of **1** (3.62 g, 10.94 mmol) and NaI (0.33 g, 2.19 mmol) was stirred under reflux for 30 minutes and triethylphosphite (TEP) (9.38 mL, d= 0.969 g/mL) was added. The reflux have been continued for 24 h and, after cooling of the mixture at room temperature, it was extracted with petroleum ether (PE). The organic layer was dried (Na_2SO_4), filtered and concentrated under reduced pressure. The obtained residue was purified by chromatography (eluent PE/EtOAc 6:4). The product was isolated as a yellow oil (71% yield). Mass [$M + H^+$]: 389.6; ^1H NMR ($CDCl_3$) δ 5.48-5.21 (m, 2H), 4.18-4.00 (m, 4H), 2.18-2.03 (m, 4H), 1.86-1.70 (t, 2H), 1.52-1.34 (m, 2H), 1.35-1.20 (m, 22H), 1.29-0.96 (t, 6H), 0.88-0.86 (t, 3H); ^{13}C NMR ($CDCl_3$) δ 130.6, 62.2, 31.9, 30.9, 29.9, 29.7, 29.3, 27.3, 16.3, 14.1; ^{31}P NMR ($CDCl_3$) δ 22.55.

Synthesis of octadec-9-enyl-phosphonic-acid monoethyl ester (3)

The compound **2** (3 g; 7.73 mmol) was dissolved in a solution H_2O/MeOH 1:1 (v/v) (30 mL) and later KOH (5.64 g; 100.52 mmol) was added. The mixture was stirred under reflux for 24 h. After cooling at room temperature, it was diluted with water and treated with H_2SO_4 (2N, 40 mL) and then was extracted with EtOAc. The organic layer was dried with (Na_2SO_4), filtered and concentrated under reduced pressure. The obtained residue was purified by chromatography (eluent EtOAc/MeOH 1:1). The product was isolated as a yellow oil (yield 65%). Mass [$M - H^+$]: 359.5. ^1H NMR ($CDCl_3$) δ 11.98 (s, 1H), 5.42-5.25 (m, 2H), 4.19-4.00 (m, 2H), 2.18-2.00 (m, 4H), 1.88-1.75 (m, 2H), 1.54-1.37 (m, 2H) 1.35-1.20 (m, 22H), 1.17-1.09 (t, 3H), 0.88-0.86 (t, 3H). ^{13}C NMR ($CDCl_3$) δ 130.6, 64.6, 34.1, 31.9, 30.3, 29.9, 29.7, 29.3, 27.7, 23.7, 16.0, 14.1. ^{31}P NMR ($CDCl_3$) δ 26.55.

Synthesis of octadec-9-enyl-dihydrogen phosphate (4)

To a solution of **2** (0.3 g, 0.8 mmol) in toluene (5 mL) was added BBr_3 (0.17 g, 0.7 mmol, d= 0.859 g/mL, solution 1M) at -20 °C. The mixture was warmed to 70 °C and stirred for 7 h. Methanol (5 mL) was then added and the solvent was evaporated in vacuo. The residue was purified by chromatography (eluent EtOAc/Methanol 2:8). The product was isolated as a colourless oil (yield 44%). Mass: [$M + H^+$]: 333.5. ^1H NMR ($CDCl_3$) δ 5.48-5.21 (m, 2 H), 2.10 (m, 4 H), 2.00 (s, 2 H), 1.80 (t, 2 H), 1.35-1.20 (m, 30 H), 0.96 (t, 3 H). ^{13}C NMR ($CDCl_3$) δ 130.7, 32.4, 30.3, 29.1, 27.7, 25.8, 23.7, 14.1. ^{31}P NMR ($CDCl_3$) δ 28.75.

Synthesis of Ethyl-*p*-nitrophenyl-oleylphosphonate (5)

To a solution of **3** (0.14 g, 0.4 mmol) in toluene (3 mL) was added DCC (N,N'-dicyclohexylcarbodiimide) (0.66 g, 3.2 mmol), DMAP (4-dimethylaminopyridine) (0.024 g, 0.2

mmol) and *p*-nitrophenol (0.05 g, 0.4 mmol). The mixture was stirred at 65 °C for 12 h. The mixture was treated with H_2SO_4 (2N, 20 mL) and extracted with EtOAc. The organic layer was dried (Na_2SO_4) and evaporated in vacuo. The residue was poured washed with toluene and removed under reduced pressure. The residue was purified by chromatography (eluent Hexane/EtOAc 9:1). The product was isolated as a colourless oil (yield 50%). Mass: [M + H^+]= 504.4. ^{1}H NMR ($CDCl_3$) δ 8.30-8.10 (m, 2 H), 6.90-7.20 (m, 2 H), 5.48-5.21 (m, 2 H), 4.18-4.00 (m, 2 H), 2.10 (m, 4 H), 2.00 (s, 1 H), 1.80 (t, 2 H), 1.35-1.20 (m, 30 H), 0.96 (t, 3 H). ^{13}C NMR ($CDCl_3$) δ 156.4, 141.2, 132.8, 129.6, 128.9, 127.6, 122.3, 117.8, 62.3, 29.9, 29.8, 29.6, 29.4, 29.2, 29.1, 27.3, 26.0, 14.8, 14.2. ^{31}P NMR ($CDCl_3$): δ 24.47.

Synthesis of Arachidonyl-ethyl-oleylphosphonate (6)

To a solution of **3** (0.25 g, 0.7 mmol) in DCM (5 mL) was added oxalyl chloride (0.27 g, 2.1 mmol, d= 1.455 g/mL). The mixture was cooled to 0-5 °C and DMF (dimethylformamide) was added dropwise. The mixture was warmed to room temperature and stirred for 4 h. The solvent was evaporated in vacuo to yield a red residue. To a solution of ethyl oleylphosphonyl chloride (0.27 g, 0.7 mmol) in DCM (5 mL) was added arachidonyl alcohol (0.4 g, 1.3 mmol). The mixture was stirred at room temperature over night. The solvent was removed under reduced pressure and the residue was purified by chromatography (eluent Hexane/EtOAc 9:1). The product was isolated as a colourless oil (yield 60%). Mass: [M + H^+]= 633.5. ^{1}H NMR ($CDCl_3$) δ 5.48-5.21 (m, 10 H), 4.18-4.00 (m, 4 H), 2.90-2.78 (m, 6 H), 2.18-1.96 (m, 8 H), 1.80 (t, 2 H), 1.35-1.20 (m, 37 H), 0.96 (t, 6 H). ^{13}C NMR ($CDCl_3$) δ 132.2, 130.7, 128.8, 127.3, 67.5, 62.0, 33.3, 32.4, 30.3, 29.1, 25.8, 23.7, 15.0, 14.1. ^{31}P NMR ($CDCl_3$) δ 22.95.

Synthesis of Ethyl-oleyl-oleylphosphonate (7)

To a solution of **3** (0.25 g, 0.7 mmol) in DCM (5 mL) was added oxalyl chloride (0.27 g, 2.1 mmol, d= 1.455 g/mL). The mixture was cooled to 0-5 °C and DMF was added dropwise. The mixture was warmed to room temperature and stirred for 4 h. The solvent was evaporated in vacuo to yield a red residue. To a solution of ethyl oleylphosphonyl chloride (0.27 g, 0.7 mmol) in DCM (5 mL) was added oleyl alcohol (0.35 g, 1.3 mmol). The mixture was stirred at room temperature over night. The solvent was removed under reduced pressure and the residue was purified by chromatography (eluent Hexane/EtOAc 8:2). The product was isolated as a colourless oil (yield 60%). Mass: [M + H^+]= 611.6. ^{1}H NMR ($CDCl_3$) δ 5.48-5.21 (m, 4 H), 4.23-3.90 (m, 4 H), 2.10 (m, 8 H), 1.80 (t, 2 H), 1.39-1.16 (m, 51 H), 0.96 (t, 6 H). ^{13}C NMR ($CDCl_3$) δ 130.7, 67.5, 62.0, 33.3, 32.4, 30.3, 29.1, 25.8, 23.7, 15.0, 14.1. ^{31}P NMR ($CDCl_3$): δ 22.85.

General procedure for the preparation of oleyphosphonate esters (8-12)

To a solution of the compound **3** (1.38 mmol, 1eq) in DCM dry (2.8 mL), at the temperature of 0-5 °C, DIEA (1.38 mmol; d=0.742 g/mL, 1eq) and BOP (2.07 mmol, 1.5 eq) were added. The mixture was stirred at room temperature and then *N*-(hydroxymethyl)phthalimide (*N*-HMP) (2.07 mmol; 1,5 eq) for **10** or 3-phenoxybenzyl alcohol (3-PBA) (2.07 mmol; d=1.15 g/mL; 1,5 eq) for **11** or *N*-(2-hydroxyethyl)phthalimide (*N*-HEP) (2.07 mmol; 1,5 eq) for **12** or *N*-(hydroxymethyl)-maleimide (*N*-IMM) (2.07 mmol; 1,5 eq) for **13** or *N*-(2-hydroxyethyl)-succinimide (*N*-IES) (2.77 mmol; 2 eq) for **14** was added respectively. The resulting mixture was stirred at room temperature for 12 h. After it was added of a saturated solution of NaCl (50 mL) and extracted with EtOAc (3 x 40 mL). The reunited organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure. The obtained residue oils were purified by chromatography.

***N*-Ethyl-methylphthalimide-oleylphosphonate (8)**

The obtained residue was purified by chromatography (eluent PE/EtOAc 1:1). The product was isolated as a white oil (yield 72%). Mass [M + Na^+]: 542.3, [M + H^+]: 520.3. ^{1}H NMR ($CDCl_3$) δ 7.88 (m, 2H), 7.85 (m, 2H), 6.37 (m, 2H), 5.42-5.33 (m, 2H), 4.19-4.15 (m, 2H), 2.18-2.00 (m, 4H), 1.77-1.75 (t, 2H), 1.54-1.21 (m, 27H), 0.88-0.86 (t, 3H). ^{13}C NMR ($CDCl_3$): δ 168.2, 133.7, 132.1, 130.7, 127.3, 70.8, 62.3, 31.9, 30.2, 29.4, 27.4, 22.8, 15.5, 14.1.

Ethyl-1,3-phenoxybenzyl-oleylphosphonate (9)

The obtained residue was purified by chromatography (eluent PE/EtOAc 1:1). The product was isolated as a colourless oil (yield 71%). Mass [M + H^+]: 543.7. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.33-7.25 (m, 3H), 7.13-6.98 (t, 2H), 6.92-6.88 (m, 4H), 5.42-5.33 (m, 2H), 5.29 (m, 2H), 4.19-3.88 (m, 2H), 2.18-1.90 (m, 4H), 1.77-1.63 (t, 2H), 1.32-1.11 (m, 27H), 0.88-0.86 (t, 3H). ^{13}C NMR ($CDCl_3$): δ 157.0, 140.9, 130.7, 128.7, 121.9, 117.5, 114.4, 73.5, 62.3, 31.9, 29.8, 27.7, 25.0, 22.8, 15.0, 14.1.

Ethyl-*N*-ethylphthalimide-oleylphosphonate (10)

The obtained residue was purified by chromatography (eluent PE/EtOAc 1:1). The product was isolated as a white oil (yield 69%). Mass [M + Na^+]: 556.3, [M + H^+]: 534.3. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.89 (m, 2H), 7.85 (m, 2H), 5.46-5.38 (m, 2H), 4.29 (t, 2H), 4.17-4.11 (m, 2H), 4.00 (t, 2H), 2.18-1.90 (m, 4H), 1.77-1.63 (t, 2H), 1.54-1.11 (m, 27H), 0.88-0.86 (t, 3H). ^{13}C NMR ($CDCl_3$,) δ 168.2, 133.4, 132.1, 130.7, 123.7, 65.8, 62.3, 38.8, 31.9, 30.2, 29.4, 27.4, 22.8, 15.5, 14.1.

Ethyl-*N*-methylmaleimide-oleylphosphonate (11)

The obtained residue was purified by chromatography (eluent PE/EtOAc 1:1). The product was isolated as a white solid (yield 56%) (m.p. 128-130°C). Mass [M + H^+]: 471.3. ^{1}H NMR ($CDCl_3$,) δ 6.94 (m, 2H), 6.22 (m, 2H), 5.42-5.38 (m, 2H), 4.19-4.00 (m, 2H), 2.18-2.00 (m, 4H), 1.77-1.37 (t, 2H), 1.54-1.11 (m, 27H), 0.88-0.86 (t, 3H). ^{13}C NMR ($CDCl_3$) δ 170.4, 135.7, 130.6, 65.7, 62.2, 37.8, 31.9, 30.9, 29.9, 29.7, 29.3, 27.7, 25.9, 22.8, 15.1, 14.8, 14.1.

Ethyl-*N*-ethylsuccinimide-oleylphosphonate (12)

The obtained residue was purified by chromatography (eluent EtOAc/MeOH 1:1). The product was isolated as a white solid (yield 52%) (m.p. 146-148°C). Mass [M + H^+]: 486.4. ^{1}H NMR ($CDCl_3$) δ 5.42-5.25 (m, 2H), 4.19-4.10 (m, 2H), 4.09-3.87 (t, 4H), 2.73-2.64 (m 4H), 2.18-2.00 (m, 4H), 1.77-1.37 (t, 2H), 1.54-1.11 (m, 27H), 0.88-0.86 (t, 3H). ^{13}C NMR ($CDCl_3$) δ 176.4, 130.7, 65.7, 62.2, 37.8, 31.9, 30.2, 29.9, 29.7, 29.3, 28.0, 27.7, 25.9, 22.8, 15.1, 14.8, 14.1.

General procedure for the preparation of oleylphosphonate (13-14)

The derivative **8** or the derivative **12** (0.77 mmol) was dissolved in a solution H_2O/MeOH 1:1 (v/v) (20 mL) and later KOH (11.55 mmol) was added. The mixture was stirred under reflux for 24 h. After cooling at room temperature, it was diluted with water, treated with H_2SO_4 (2N, 30 ml) and then it was extracted with EtOAc (3x40 mL). The reunited organic layers were dried (Na_2SO_4), filtered and concentrated under reduced pressure. The obtained residue was purified by chromatography (eluent EtOAc/MeOH 1:1).

Methylphthalimide-oleylphosphonate (13)

The product was isolated as white oil (yield 56%). Mass [M + H^+]: 492.3. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 11.98 (s, 1H), 7.88-7.26 (m, 2H), 7.85-7.26 (m, 2H), 6.37 (m, 2H), 5.42-5.25 (m, 2H), 2.18-2.00 (m, 4H), 1.77-1.75 (t, 2H), 1.54-1.37 (m, 2H), 1.31-1.29 (m, 22H), 0.88-0.86 (t, 3H). ^{13}C NMR ($CDCl_3$) δ 168.2, 133.7, 132.1, 130.7, 127.3, 70.8, 31.9, 30.2, 29.4, 27.4, 22.8, 15.5, 14.1.

***N*-Ethylsuccinimide-oleylphosphonate (14)**

The product was isolated as white solid (yield 83%) (m.p. 85°C). Mass [M + H^+]: 458.4. ^{1}H NMR ($CDCl_3$) δ 11.98 (s, 1H), 5.42-5.38 (m, 2H), 4.09-3.87 (m, 4H), 2.73-2.64 (m, 4H), 2.18-2.00 (m, 4H), 1.77-1.37 (t, 2H), 1.54-1.37 (m, 2H), 1.31-1.11 (m, 22H), 0.88-0.86 (t, 3H). ^{13}C NMR ($CDCl_3$) δ 176.4, 130.7, 65.7, 37.8, 31.9, 30.2, 29.9, 29.7, 29.3, 28.0, 27.7, 25.9, 22.8, 15.1, 14.1.

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