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SÍNTESIS Y BIOACTIVIDAD DE ÉTERES LIPÍDICOS ANTITUMORALES ANÁLOGOS DE EDELFOSINA

SYNTHESIS AND BIOACTIVITY OF ANTITUMOR ETHER LIPIDS ANALOGS OF EDELFOSINE

ALEJANDRO MARTÍN RONCERO

SEPTIEMBRE 2021

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SYNTHESIS AND BIOLOGICAL EVALUATION OF ALKYL ETHER PHOSPHOLIPIDS (AEPS) ANALOGS OF EDELFOSINE

Trabajo para optar al grado de Doctor en Ciencias Químicas

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Fdo. Isidro Sánchez Marcos

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Este trabajo ha sido realizado en el Departamento de Química Orgánica de la Facultad de Ciencias Químicas de la Universidad de Salamanca, bajo la dirección del Dr. **D. Isidro Sánchez Marcos**, al que quiero expresar mi más sincero agradecimiento por la confianza que ha depositado en mí ofreciéndome la oportunidad de realizar esta tesis, la ayuda que me ha prestado y todo lo que he aprendido durante este tiempo. Muchas gracias por todo.

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Me gustaría agradecer a mis compañeros de laboratorio, a los que ya considero amigos sin duda alguna, todos los momentos que hemos compartido juntos. A aquellos que me enseñaron a desenvolverme en el laboratorio y de los que he aprendido muchísimo: Aitor, Ana y Mateo, siempre llevaré un pedacito de vosotros adonde vaya. A los que comenzaron la andadura del doctorado conmigo: Ignacio y Alejandro por todo lo bueno y malo que hemos pasado y que nos ha hecho crecer como investigadores. A los que vienen pisando fuerte: Alberto, Nieves, Rocío, Carmen y Laura por aceptar de buen grado mis consejos y hacerme dudar de todo. Tampoco me olvido de nuestro querido técnico Juan, para que sigas teniendo esa curiosidad y ganas de aprender Química. Muchas gracias a todos por vuestra ayuda para que este trabajo haya llegado a buen puerto.

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“O sol che sani ogne vista turbata,
Tu mi contenti sì quando tu solvi,
Che, non men che saver, dubbiar m'aggrata”

¡Oh Sol que curas la vista turbada,
Tú me contentas tanto cuando así esclareces,
Que no menos que el saber, dudar me agrada!

Dante Alighieri, *La Divina Comedia*

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Abbreviations and acronyms

Abbreviations and acronyms used within this work are shown below:

AEPs: Alkyl-Ethers Phospholipids.

APAF1: Apoptotic Protease-Activating Factor-1.

APCs: Alkyl Phosphocholines.

APCI: Atmospheric Presure Chemical Ionization.

APLs: Alkyl Phospholipids.

ATLs: Antitumoral Lipids.

BID: BH3 interacting-domain death agonist

t-BuOH: *tert*-butanol.

calc.: calculated.

CASMER: Cluster of Apoptotic Signaling Molecule-Enriched Rafts.

CC: column chromatography.

CuTC: copper(I) thiophene-2-carboxylate.

d: doblet (in NMR).

DCM: dichloromehtane.

DEPT: Distorsionless Enhancement by Polarization Transfer.

DISC: Death-Inducing Signaling Complex.

ESI-HRMS: ElectroSpray Ionization High Resolution Mass Spectrometry.

EtOAc: ethyl acetate.

EtOH: ethanol.

FADD: Fas-Associated Death Domain.

Hex: hexanes.

HMBC: Heteronuclear Multiple Bond Coherence.

HMQC: Heteronuclear Multiple-Quantum Coherence.

IR: infrared spectrum

J: coupling constant.

JNK: c-Jun N-terminal Kinase.

LTA: Lead (IV) TetraAcetate.

MeOH: methanol.

mTOR: mammalian target of Rapamycin.

NMO: *N*-methylmorpholine *N*-oxide.

NMR: Nuclear Magnetic Resonance.

obs.: observed.

PC: *PhosphoCholine*.

PDK: 3-Phosphoinositide-Dependent protein Kinase.

PI3K: Phophatidyllnositol-3-Kinasa.

PIP_2: phosphatidylinositol-4,5-bisphosphate.

PIP_3: phosphatidylinositol-3,4,5-trisphosphate.

Pyr: pyridine.

$PhCH_3$: toluene.

PTEN: Phosphatase and Tensin homologue.

q: quartet (in NMR).

quin: quintuplet (in NMR).

rt: room temperature.

RTK: ligand-activated Receptor Tyrosine Kinases.

s: singlet (in NMR).

t: triplet (in NMR).

THF: tetrahydrofuran.

TLC: Thin Layer Chromatography.

TrCl: tritil chloride.

TsCl: *p*-toluensulfonyl chloride.

p-TsOH: *p*-toluensulfonic acid.

TPSCl: 2,4,6-triisopropylbenzenesulfonyl chloride.

Introduction

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Introduction

Cancer is one of the deathliest diseases worldwide. According to the WHO (World Health Organization), around 10 million deaths have been estimated in 2020 due to this illness, which made of it the second cause of morbidity and mortality worldwide.[1] WHO definition of cancer is "a generic term for a large group of diseases that can affect any part of the body, characterized by the rapid creation of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs".[2] Due to the severity and lack of knowledge about this disease, there is currently a large amount of research aimed at its treatment and prevention.

Nowadays, there are several types of cancer treatments.[3] Usually, different combinations of them are used, such as surgery with chemotherapy and/or radiotherapy. Among the most used are:

- Surgery, in which all or part of the tumor is removed.
- Radiotherapy, in which high doses of radiation are used to kill cancer cells or shrink some tumors.
- Chemotherapy, in which drugs, or a combination of drugs, are used to kill cancer cells.
- Immunotherapy, in which drugs that increase the ability of the immune system are used to fight against the disease.
- Targeted therapy, in which drugs target proteins responsible of processes related to cell growth, survival, division and motility.

However, the great variability of cancer types makes it difficult to establish a general mechanism of action of this disease and, therefore, to choose between the different treatments. For this reason, the so called Precision Medicine is currently being studied, in which a genetic study of the patient is carried out by means of a tumor biopsy. Once analyzed, the oncologist can choose to follow one of the aforementioned therapies that best fits the patient's need. It seems that this kind of approach towards the cure of cancer is very promising, but the problem is that is necessary to know all the genetic

causes of each cancer, something that has not been discovered yet, and requires a large amount of both human and material resources to be able to investigate them.[4]

The present work is part of the research line recently started in our work group aimed at the study of Synthetic Antitumor Lipids (ATLs) as suitable agents for cancer chemotherapy.[5]

1. Synthetic Antitumor Lipids

Antitumor Lipids (ATLs) are a family of structurally related synthetic compounds that display antitumor activity. Their structure is composed of a polar head formed by a phosphocholine unit or some derivative and a hydrophobic tail formed by a long hydrocarbon chain (C16-C22).[6]

These compounds are structurally based on the endogenous ether lipids, such as the plasmalogens (Figure 1), with diverse functions within the cell. However, the most important functions are membrane related ones, such as organization and stabilization of membrane domains, or help in membrane-fusion processes. Moreover, plasmalogens exhibit antioxidant properties.[7]

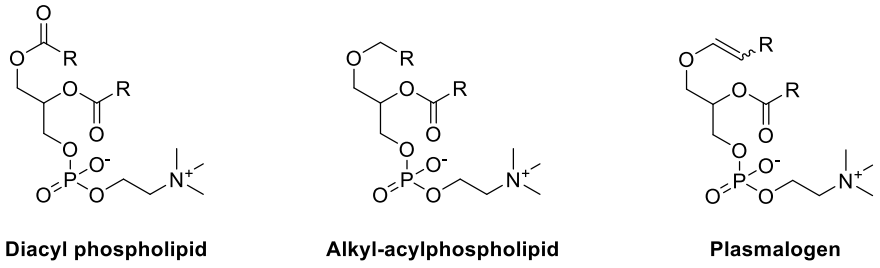


Figure 1. Structures of some endogenous lipids

1.1. Antitumor Synthetic Lipids background

ATLs arise as metabolically stable analogs of the endogenous lipid lysophosphatidylcholine **I** (Figure 2). This substance is involved in antiinflammatory processes, but its medicinal use is not possible due to its metabolic instability, as it is rapidly degraded by lipases and acetyltransferases. So, it was necessary the modification of positions C1 and C2, changing the ester bond (easily degraded by lipases) for an ether bond. In the decade of 1960, professor Eibl and coworkers synthesized Edelfosine **II** (1-*O*-octadecyl-2-*O*-methyl-*rac*-glycero-3-phosphocholine) while looking for substances with immunomodulatory properties (Figure 2).[8, 9] This new phospholipid lacked ester bonds in favor of ether bonds at the glycerol backbone.

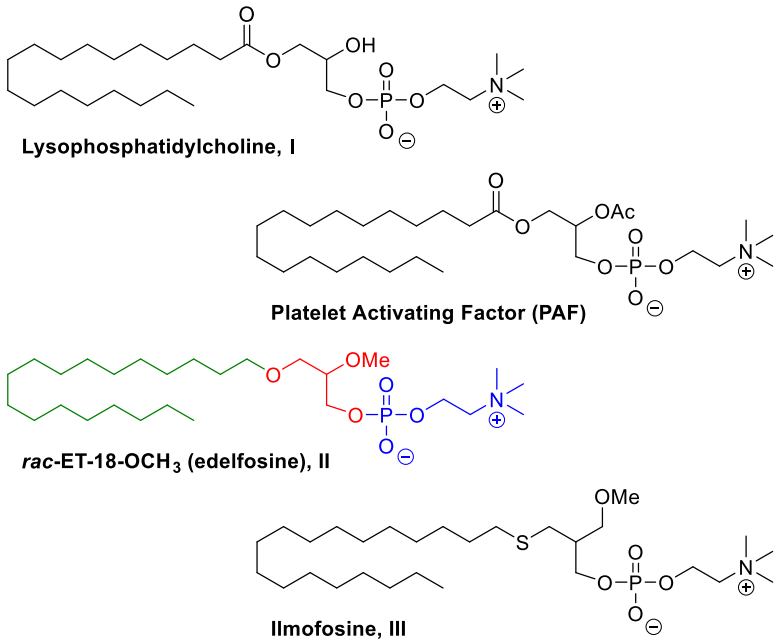


Figure 2. Structure of endogenous lipids (lysophosphatidylcholine, **I**, and PAF) and alkylphospholipids (edelfosine, **II**, and ilmofosine, **III**)

Later, it will be discovered that this type of lipids also showed antitumor activity with very promising results, since it selectively caused death in tumor cells *in vitro* while sparing normal cells.[10-12] This new family of compounds was called Alkyl Ether

Phospholipids (AEPs) and edelfosine, **II**, was considered the prototype of this kind of compounds. In order to improve the pharmacological properties of edelfosine, different analogs began to be synthesized, such as ilmofosine **III** in which the glycerol fragment is replaced by 1-thio-2-(methoxymethyl)glycerol.[13]

In the late 90's, professor Eibl and coworkers synthesized miltefosine, **IV**, (hexadecylphosphocholine),[14] which lacks glycerol core and shows a C16-alcohol directly attached to the phosphocholine (Figure 3). This new compound started a subfamily called alkyl phosphocholines (APCs) and was identified as the minimum unit with antitumor activity of this kind of lipids. In 1998, miltefosine was approved, in many Europe countries, as palliative treatment for skin metastasis of breast cancer under the name Miltex®.[15]

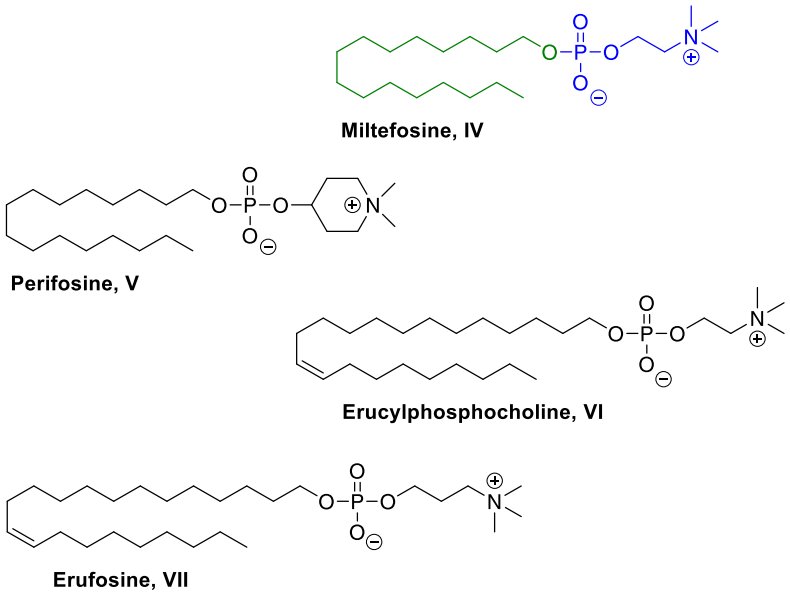


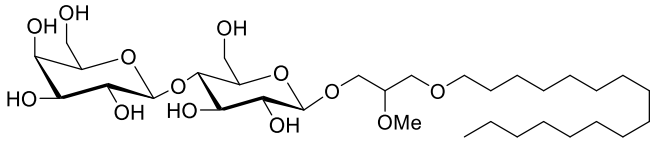
Figure 3. Alkylphosphocholines (APCs) structure

Within the APCs family, other variations of miltefosine with promising results have been obtained (Figure 3). Among them, the highlighted ones are: perifosine **V**,[16] in which choline is substituted for a unit of *N*,*N*-dimethylpiperidin-4-ol;

erucylphosphocholine **VI**,[17] which possesses a C22 hydrocarbon chain with a *cis* Δ^{13} double bond, and its analog erufosine **VII**,[18] with a homocholine in the polar head.

As perifosine lacks phosphocholine in its structure, its metabolic stability against phospholipases is improved, which makes it plausible for oral treatment.[19] In the case of the long-chain analogs, it has been found that the *in vitro* hemolytic toxicity inherent in other ATLs is greatly reduced, so they could be administered intravenously.[17, 20]

In the early 2010, it was performed a structural modification of the polar head of edelfosine in which the fosfocholine unit was changed by a series of mono- and disaccharides. The compound with the best results was ohmline **VIII**, having a lactose attached to the glycerol core (Figure 4). This glycolipid has proved to be able to inhibit metastasis in tumor cells *in vitro*.[21-23]



Ohmline, VIII

Figure 4. Ohmline structure

Last works performed about synthesis of ATLs are aimed at preparing prodrugs that preserve the antitumor properties of parent drugs but improving their hemolytic toxicity.[24, 25]

Regarding the mechanism of action of the ATLs, there have been a lot of experiments in that area, and, despite a clear mechanism has not been attributed to them yet, it has been observed that ATLs interact with some signaling pathways within the cell, among which some survival and pro-apoptotic pathways stand out, in addition to their direct interaction with the endogenous *de novo* lipid synthesis.[6, 26-28] Thus, it was observed that this family of antitumor compounds does not target cellular DNA itself,

but acts by incorporating themselves into the plasma membrane in specific domains called *Lipid Rafts*.[29]

Recently, some research is being carried out on the role of the immune system in cancer, specifically neutrophils. A high neutrophil/lymphocyte ratio is usually present in cancer cases with low survival prognosis. That is why some proteins secreted by neutrophils are being considered as tumor biomarkers. In general, neutrophils interact with the so-called *circulating tumor cells* (CTCs) which are precursors of metastasis and stimulate angiogenesis, in addition to other physiological actions that favor tumor progression.[30, 31]

However, some evidence has also been found to show that tumor-associated neutrophils have antitumor properties in the early stages of the disease.[32] A recent work indicates that arginase-1 production by neutrophils generates apoptosis in tumor cells through an endoplasmic reticulum stress-mediated mechanism.[33] These results also indicate a synergistic effect between arginase-1 production (L-Arg depletion) and the antitumor activity of ether lipid edelfosine.

2. Lipid rafts

Since the 1980's, many evidences have been accumulated that have changed the classical view of the cell membrane, both its organization and the signaling processes it regulates. It has been discovered that membrane lipids can organize themselves into domains in different ways depending of their function, and one of those domains are the so-called lipid rafts.[34-38]

Lipid rafts are defined as membrane microdomains enriched in sphingolipids and cholesterol (Figure 5), which gives them a special rigidity and allows them to be compared with a raft floating in a sea of membrane phospholipids (hence their name).[39] With the corresponding stimuli, signaling proteins can be recruited to these domains. This makes them suitable to act as platforms where plenty of signaling processes can be carried out, among which some cell survival and pro-apoptotic routes stand out.[38, 40-42] Furthermore, these domains are suitable as targets in the fight against coronaviruses, since the main receptor for SARS-CoV-2 accumulates in lipid rafts.[43]

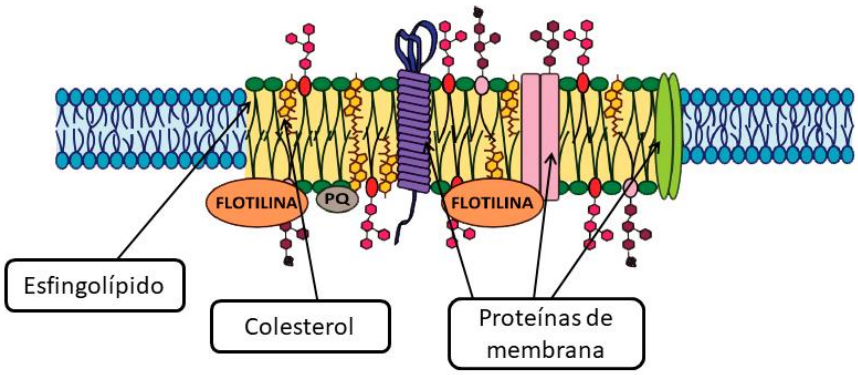


Figure 5. Lipid rafts composition

Nowadays, one of the signs by which malignancy in a tumor can be recognized is because the lipid metabolism of the affected cells is altered, for example, increased

lipid absorption, storage or synthesis can contribute to the tumor growth.[44] That said, cholesterol is the main regulator of the lipid organization in the cell membrane and, therefore, of the formation of lipid rafts.

All this, added to the fact that cancer cells have been observed to have high levels of lipid rafts and cholesterol,[45] makes these microdomains an excellent therapeutic target for cancer treatment.[38]

PI3K/Akt survival pathway

In this survival pathway, the PI3K protein kinase (Phosphatidylinositol-3-kinase) is responsible for initiating the signaling cascade that leads to proliferation and cell growth processes (Figure 6).

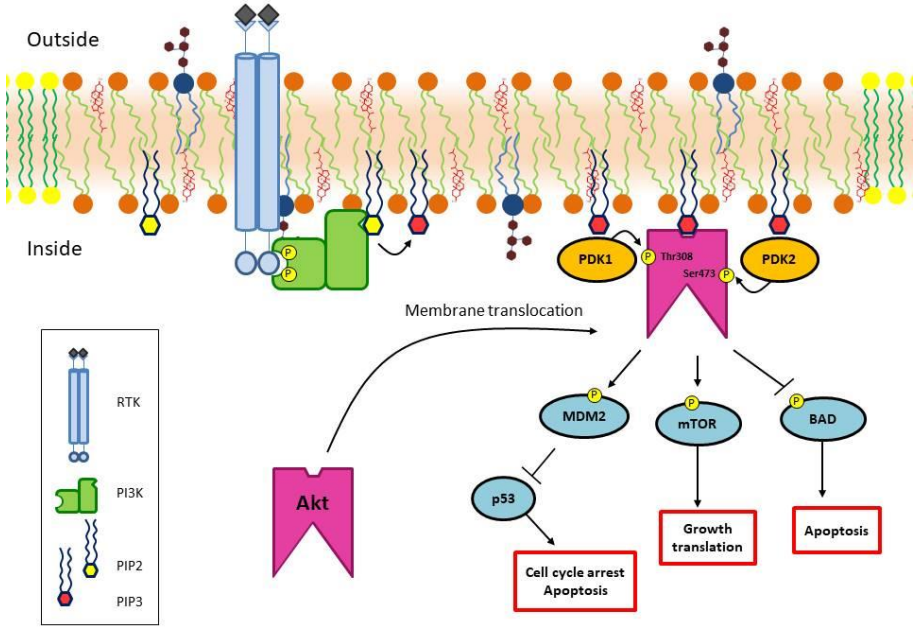


Figure 6. PI3K/Akt signaling pathway (Adapted from Ref [46])

PI3K is a cytoplasmic protein whose function is to phosphorylate phosphatidylinositol-4,5-bisphosphate (PIP2) to phosphatidylinositol-3,4,5-trisphosphate (PIP3), which are

membrane glycolipids, so it is necessary for this kinase to bind the internal face of the membrane in order to perform its function. This occurs when an endogenous ligand binds to the receptor tyrosine kinase (RTK). Then, PI3K is recruited to lipid rafts where its phosphorylation takes place and can access its substrate (PIP2). Once PIP3 is produced, the serine/threonine kinase Akt is recruited and binds PIP3, where it is activated by phosphorylation at Thr308 and Ser473 by other kinases, PDK1 and PDK2 (Phosphoinositide-Dependent Kinase), thus being prepared to phosphorylate their substrates; among which stand out the activation of mTOR (mammalian target of rapamycin), protein involved in cell growth regulation, or the inactivation of the tumor suppressor p53, which is a promoter of apoptosis in response to cellular stress.[46, 47]

This route is highly regulated in healthy cells, while in many cancers mutations have occurred in the genes that encode the proteins involved in this process. A table with the different mutations producing the deregulation of the survival pathway can be found in the work published by Vivanco and coworkers.[46]

Fas/CD95 pro-apoptotic pathway

It has been proved that Fas/CD95 death receptor is recruited to the membrane in response of a cellular stimulus, specifically to lipid rafts (Figure 7).[48, 49] The binding of Fas with its endogenous ligand FasL produces its trimerization and the recruitment of Fas associated death domain protein (FADD) and procaspase-8, thus forming the Death Inductor Signaling Complex (DISC).[50] Once the DISC is formed, two types of apoptotic pathways can occur: type I or extrinsic, and type II or intrinsic.[49, 51]

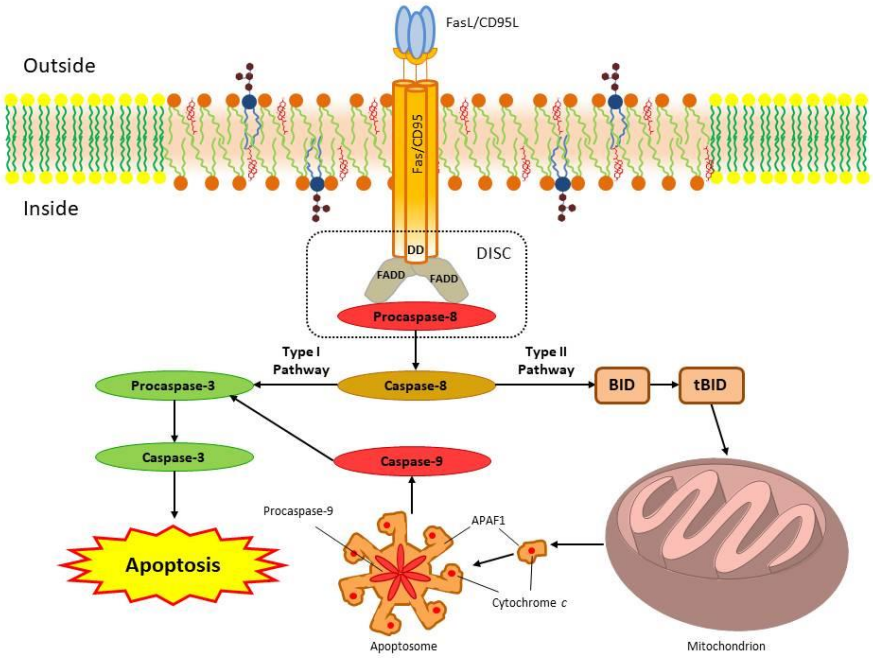


Figure 7. Fas/CD95 pro-apoptotic pathway (adapted from Ref [51])

In type I pathway, large amounts of caspase-8 are activated by proteolysis of procaspase-8. This leads directly to the breakdown of procaspase-3, activating caspasa-3 and thus starting the caspasa-mediated apoptosis signaling pathway (Figure 7).

In type II pathway, not enough caspase-8 is produced to continue with apoptosis signaling, so it is necessary to amplify this signal by breaking down the BID protein

(BH3 interacting-domain death agonist). The truncated BID protein (tBID) is transferred to the mitochondrion.[52] Once there, it interacts with the mitochondrial proteins BAD and BAX, resulting in the opening of mitochondrial pores with loss of membrane potential, release of cytochrome c and APAF1 (Apoptotic Protease-Activating Factor-1). Finally, APAF1 interacts with cytochrome c and procaspase-9, thus forming the apoptosome. The formation of this complex leads to the activation of caspase-9 and, with it, the breakdown of procaspase-3, thus starting the caspasa route.

In a same manner that the survival pathway previously described, these latter pro-apoptotic pathways are deregulated in many types of cancers, blocking them at some point in the signaling cascade preventing apoptosis.[53]

3. ATLs and cancer

Given their amphipathic nature, ATLs behave like surfactants. This property makes cell lysis possible at concentrations above its critical micellar concentration (CMC). A low CMC indicates that a substance has a preference for interacting with itself forming micelles, rather than mixed mixtures with other compounds. Edelfosine has a low CMC (5-10 μM)[54] so it has preference to interact with itself, which also indicates a low detergent power. This fact suggests that the apoptotic mechanism of this lipid must follow other routes.

At clinical concentrations, ATLs accumulate in the lipid bilayer due to their similarities to membrane lipids (Figure 8). Specifically, they accumulate in lipid rafts, as previously discussed. Once ATLs have been incorporated into the cell membrane, or internalized in the cell to the membrane of the endoplasmic reticulum (ER), they cause biophysical changes that interfere with phospholipid metabolism, cell survival and growth, as well as activating mechanisms of cellular stress that lead to apoptosis (Figure 8).[55] ATLs are capable of entering the cell through endocytosis or through translocases.[55-57]

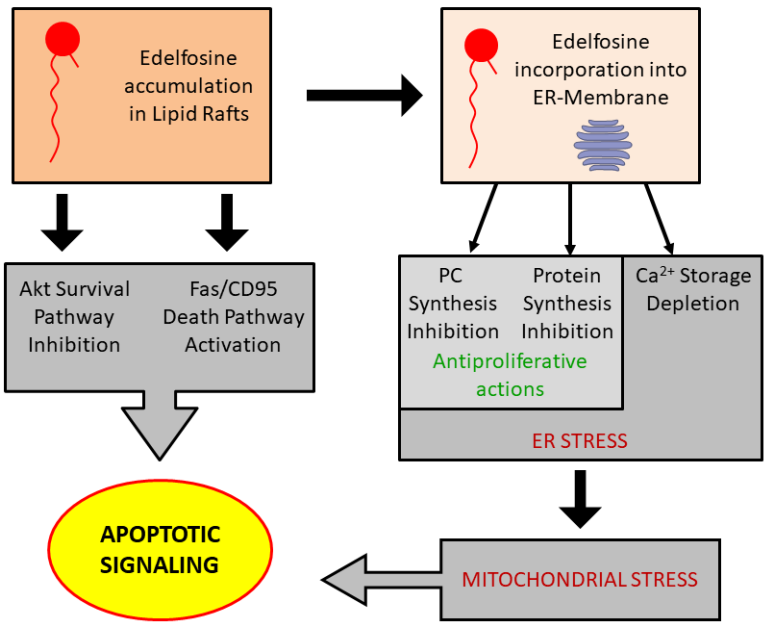


Figure 8. Some of the biophysical and biochemical changes triggered by ATLs

In addition, edelfosine and other ATLs have proven to be selectively incorporated into tumor cells while sparing normal cells.[12, 58-61] This may be due to the fact that tumor cells present higher levels of cholesterol and lipid rafts than healthy cells.

In the following paragraphs, some of the cellular processes in which ATLs intervene will be briefly described.

Phospholipid metabolism inhibition

One of the first observed activities of ATLs in general was that they inhibited phosphatidylcholine (PC) biosynthesis by inhibiting the enzyme cytidine-5'-triphosphate:phosphocholine cytidyltransferase (CCT) in the Kennedy pathway of PC synthesis (Figure 9).[62-65]

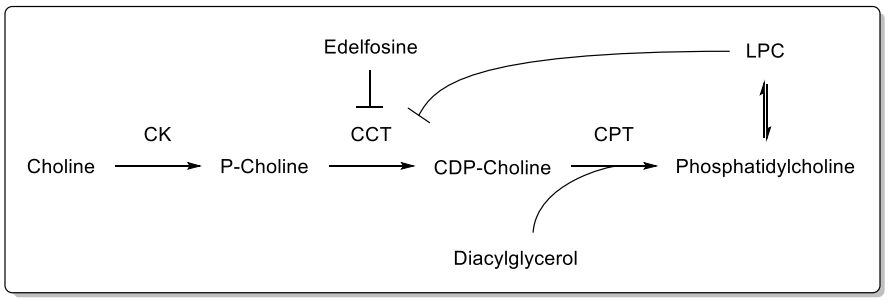


Figura 9. Membrane phospholipids biosynthesis (Kennedy pathway). LPC: Lysophosphatidylcholine; CK: Choline kinase; CCT: cytidine-5'-triphosphate:phosphocholine cytidyltransferase; CPT: Choline phosphotransferase; CDP-Choline: choline cytidine-5'-diphosphate.

This enzyme, CCT, is found in the cell nucleus as well as in other cytoplasmic compartments, but, when it is activated, CCT moves to the endoplasmic reticulum (ER). There is where ATLs in general, and particularly edelfosine, bind to the enzyme inhibiting it, thus interrupting PC synthesis. As a consequence, the cell does not have a sufficient amount of PC to be able to generate another membrane at the moment of replication. Therefore, the cell cycle is truncated, thus preventing cell proliferation.[66]

Recently, an *in silico* molecular docking study has been carried out where the interaction of several ATLs with the active site of CCT is studied. In this work, the possible binding of these lipids with CCT is theoretically corroborated and some modifications are suggested for a better binding.[67]

Cell survival pathway inhibition

One of the apoptotic mechanisms of action of ATLs occurs through the inhibition of the PI3K/Akt survival route, previously described.

This pathway is over-activated in many types of cancer, making it a good target for Targeted Therapy. ATLs, once they have been incorporated into the lipid rafts (Figure 10), prevent the activation of Akt either by inhibiting the binding of PI3K to the rafts, or by displacement of the glycolipids PIP2 and PIP3.[68] Thus, Akt cannot be phosphorylated and therefore the signaling cascade that leads to cell survival cannot take place.[69]

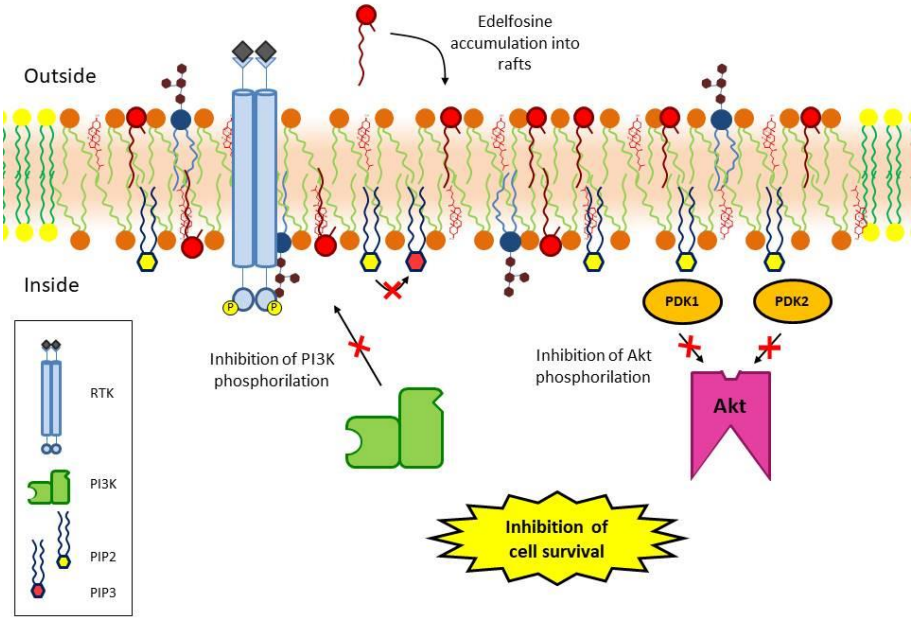


Figure 10. Edelfosine action in the survival pathway

Pro-apoptotic signaling pathways activation

Another of the most studied mechanism of ATLs is their ability to recruit various pro-apoptotic proteins to lipid rafts through the Fas/CD95 apoptotic pathway.

As previously stated, the binding of FasL to Fas causes its trimerization and the formation of the DISC. However, in the treatment with ATLs (Figure 11), the incorporation of the alkyl phospholipid to the plasma membrane is capable of causing a reorganization of the lipid rafts and, consequently, stimulates the trimerization of Fas without the need for its binding to the endogenous ligand FasL.[59, 60] This initiates the apoptosis signaling pathway and causes cell death.

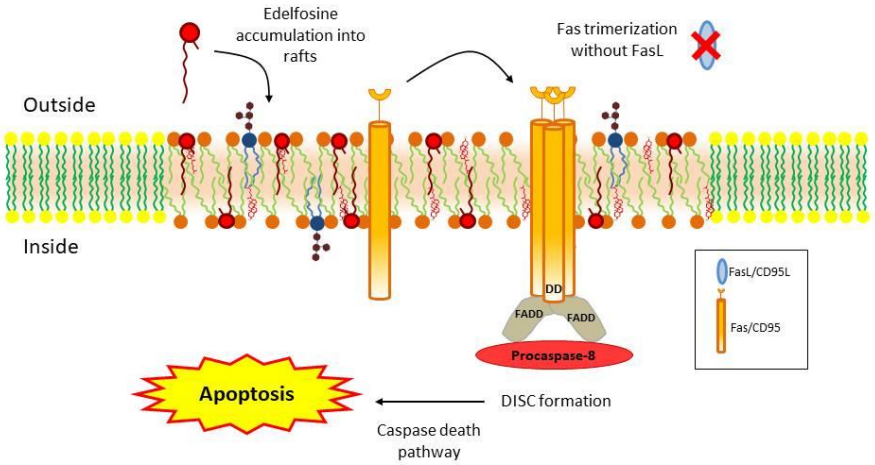


Figure 11. Action of edelfosine on the pro-apoptotic Fas/CD95 pathway

Although the ability of ATLs to cause activation of Fas receptor is common in most tumor cell lines tested, there are differences in the way apoptosis occurs between hematological cancers (leukemia, myeloma…) and solid tumors.[70] While in the former this mechanism is sufficient to trigger a rapid apoptotic response, in solid tumors longer incubation times are necessary and edelfosine is internalized and preferentially accumulated in the endoplasmic reticulum (Figure 12). This is where edelfosine inhibits the synthesis of PC (as mentioned above), in addition to protein synthesis and disrupts

other functions of the reticulum, such as the reduction of calcium stored in it.[71] All these processes generate stress in the reticulum and send a pro-apoptotic signal to the mitochondria, thus generating cellular apoptosis by the intrinsic pathway.

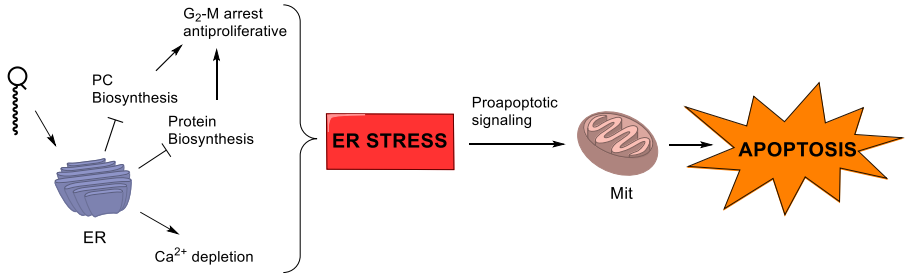


Figure 12. Action of edelfosine at the endoplasmic reticulum (ER) (Adapted from Ref [71])

Role of ATLs in metastasis

During the ATL-mediated reorganization phase of lipid rafts, the behavior of a great variety of membrane proteins is modified. One of them is the calcium-activated potassium channel SK3 (Figure 13). The SK3 channel has been found to be involved in many calcium-related signaling pathways, including cell migration of tumor cells in some types of cancer, such as bone cancer. It has been shown that in tumor cells this channel is found to form a complex with the calcium channel Orai1 in lipid rafts, whereas this complex was not detected in healthy cells. The incorporation of ATLs, specifically ohmline **VIII**, in lipid rafts promotes the breakdown of the SK3-Orai1 complex, which inhibits the entry of calcium into the cell and, therefore, the cell migration that is a precursor to metastasis in some cancers.[21-23, 72, 73]

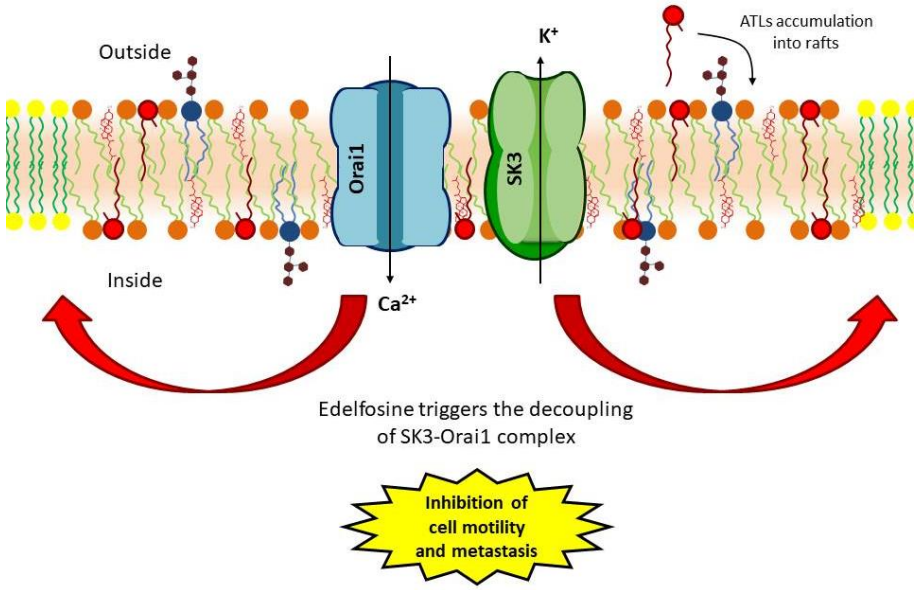


Figure 13. Antimetastasic activity of ATLs

Clinical and preclinical studies

Up to date, due to their high hemolytic toxicity, only miltefosine and edelfosine have been approved as drugs for very specific use. Miltefosine has been approved for palliative treatment in cutaneous metastases of breast cancer under the name of Miltex®.[15] It has also been approved for antiparasitic agent against visceral infections of parasites of genus *Leishmania* under the name of Impavido®.[74, 75] While edelfosine is used as purgative treatment of bone marrow in patients with acute leukemia.[76]

However, preclinical and clinical studies are being carried out both of these two ATLs and of the other mentioned previously.[77] In 2010, professor Mollinedo and coworkers demonstrated that edelfosine was capable of selectively inducing apoptosis in mantle lymphoma and chronic lymphocytic leukemia tumor cells both *in vivo* and *in vitro*.[61]

Regarding the most recent analogs, perifosine is in phase II and III clinical studies for some types of both hematological and solid tumors;[78-83] whereas erufosine and erucylphosphocholine only *in vitro* studies have been carried out.[6]

The most recent works refer to combined therapy between ATLs and other antitumor agents or even radiotherapy. It has been tested in phase II clinical studies, among others, perifosine with dexamethasone and bortezomib[84] or lenalidomide[85] in multiple myeloma, as well as perifosine with sorafenib[86] in patient with lymphoproliferative diseases; getting promising results. The possibility of combined therapy between various cationic analogs of APCs, which can be transformed into the antitumor lipid trough the action of endogenous enzymes, and a DNA plasmid encoding TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) –a factor needed in apoptosis of tumor cells– is also being studied; managing to reduce hematological toxicity and achieving promising results for its use as a medicine.[25, 87]

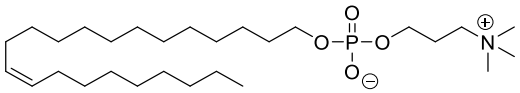
Other studies with edelfosine are aimed at finding a way to introduce it in multitarget combination therapies with other antitumor drugs. Given its high hemolytic and gastrointestinal toxicity, its incorporation into the body encapsulated in lipid nanoparticles has been studied, which reduce or even eliminate this toxicity.[87, 88] Among these studies, those that combine edelfosine and doxorubicin[89-91] or the prodrug squalene-gemcitabine stand out,[92-94] both showing promising advances in edelfosine therapies due to their better pharmacokinetics and the synergistic effect with these drugs.

4. Alkylphospholipids analogs

As the aim of this Thesis work is the synthesis of new AELs, in this section the different attempts to achieve alkylphospholipid analogues with different types of modifications are discussed.

4.1. Polar head modifications

Some attempts have been made to modify the polar head of edelfosine, among which erufosine molecule stands out. The modification of this molecule consists in the introduction of a homocholine unit, substituting the characteristic choline moiety of edelfosine, among other changes as the esterification of the polar head to a C22 alkyl chain with an unsaturation at the ω-9 position.



Erufosine

Within the performed modifications, the highlighted ones are: a) phosphorousless alkyllipids; b) glycosilated glycerolipids and c) slight modifications of the phosphocholine unit.

a) Phosphorousless alkyllipids

Daniel and coworkers[95] synthesized edelfosine derivatives (**IX-XIV**) substituting the entire fragment of phosphoric ester by N-alkylated aminoalcohols (Figure 14). All the compounds were evaluated *in vitro* against several tumor cell lines, showing cytotoxicity equivalent to that of edelfosine.

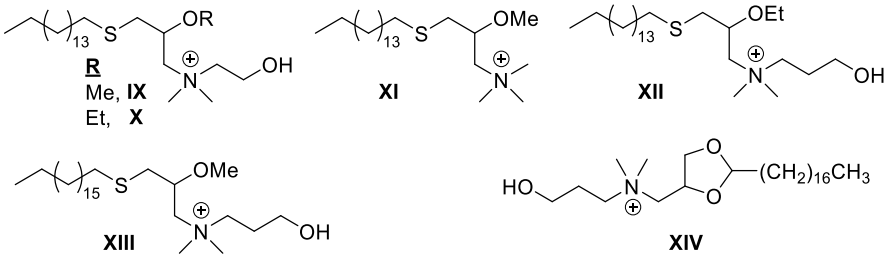


Figure 14

Romanova and coworkers obtained some edelfosine analogues (**XV-XXII**)[96, 97] in which the polar head consisted of an ester with a carboxylic acid which contains a quaternary ammonium salt in its structure (Figure 15). The cytotoxic activity of these compounds was also studied. It was observed that **XV-XVI** and **XIX-XXII** were active at micromolar level, for the K562 cell line (leukemia), while **XVII** and **XVIII** were inactive against the evaluated cell lines.

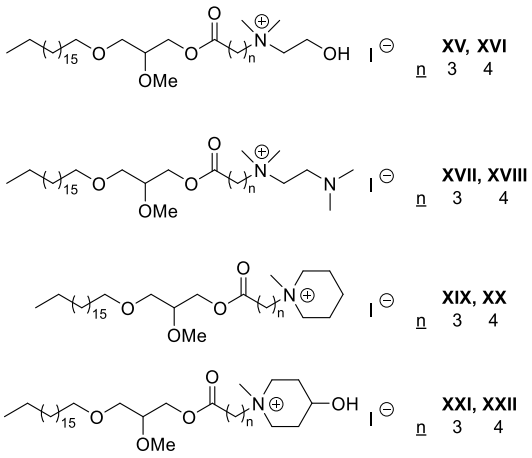
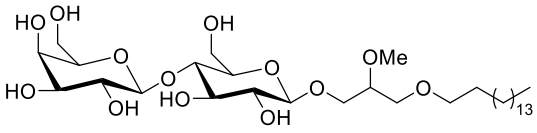


Figure 15

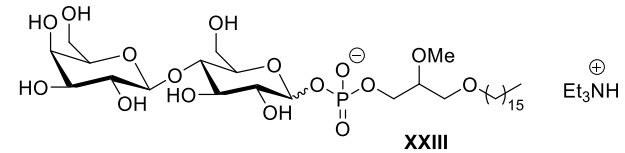
b) Glycolipids

Another common functionalization among edelfosine analogs is the formation of the polar head with a sugar, thus obtaining glycolipids. The best example of this group is ohmline, which is active as calcium SK3 channel inhibitor, protein involved in metastasis processes in tumor cells.

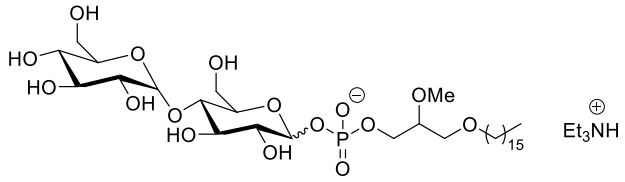


Ohmline

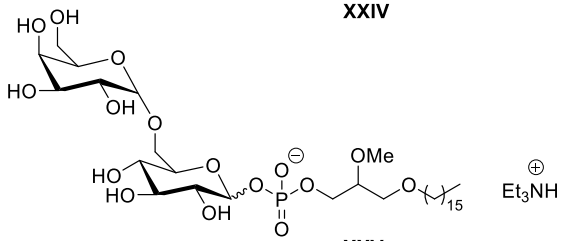
Hence, some ohmline analogues were synthesized in order to improve the antimetastasic activity inherent in this kind of lipids. Jaffrès and coworkers[98] obtained glycol-phospho-glycero-ether-lipids (GPGEL) **XXIII-XXV**. Those compounds were tested for inhibition of SK3 channel, displaying comparable potency to that of the ohmline (Figure 16).



XXIII



XXIV



XXV

Figure 16

In other work published by Arthur and coworkers,[99] a comparative study between compounds with different 2-NH_2-monosacharides as polar heads (**XXVI-XXXII**) is performed (Figure 17).

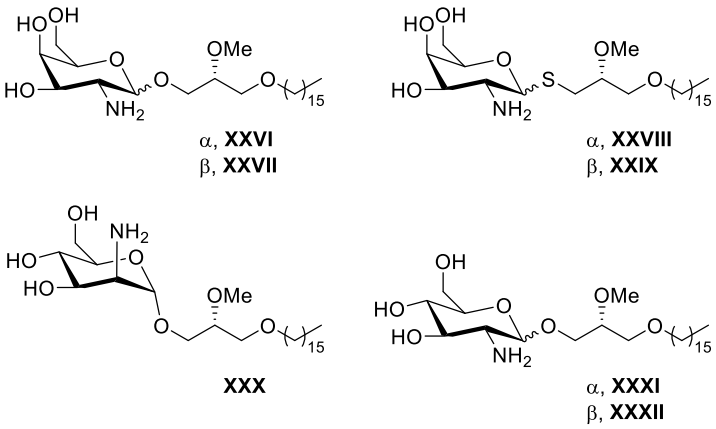
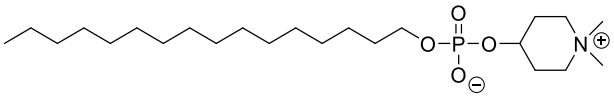


Figure 17

Antitumor tests for all these compounds were carried out in solid tumor cell lines. Some of them resulted active in inducing apoptosis to cancer stem cells (CSC).

c) Slight modifications in the polar head

In this section are mentioned those synthesized compounds that differ from edelfosine in some modification of the phosphocholine unit, but maintaining the elements of the phosphoric ester and the quaternary ammonium. The best known example of this type of structures is perifosine, in which choline is replaced by an unit of *N*,*N*-dimethyl-4-hydroxypiperidine.



Perifosine

In a work published by Makriyannis and coworkers,[100] the synthesis and biological evaluation of a number of edelfosine derivatives (**XXXIII**) was reported (Figure 18). These compounds were characterized by the change of the choline moiety for 1- or 2-methylcholine, or the substitution of the trimethylammonio group for *N*,*N*-dimethylamino, *N*-methylpyrrolidino or *N*-methylmorpholino groups. All of them exerted cytotoxicity at micromolar levels for the three leukemic cell lines tested and most of them spare normal peripheral lymphocytes.

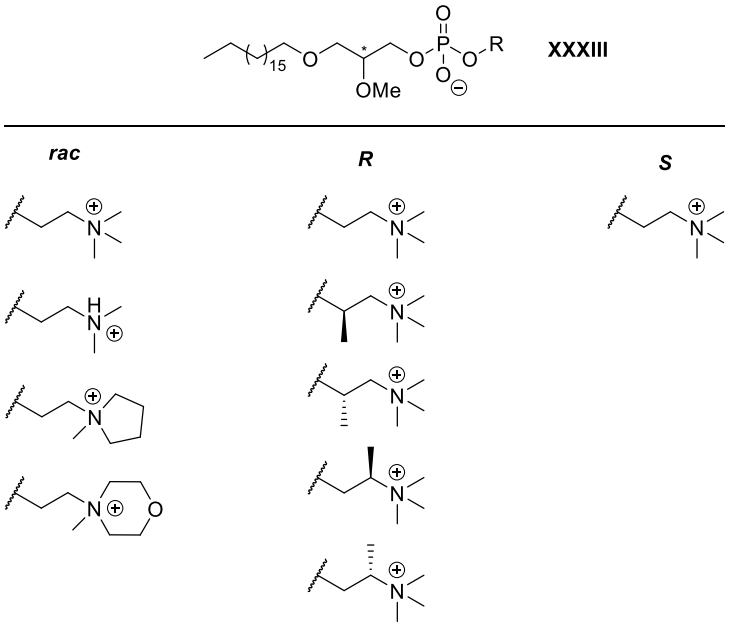


Figure 18

Another type of modification was carried out by Sharma and coworkers[101] in which they substitute the choline unit for dithiocarbamates (**XXXIV**), losing the characteristic zwitterion of edelfosine (Figure 19). Despite of that, the cytotoxicity of all compounds was tested for five solid tumors cell lines. Some of them obtained promising results.

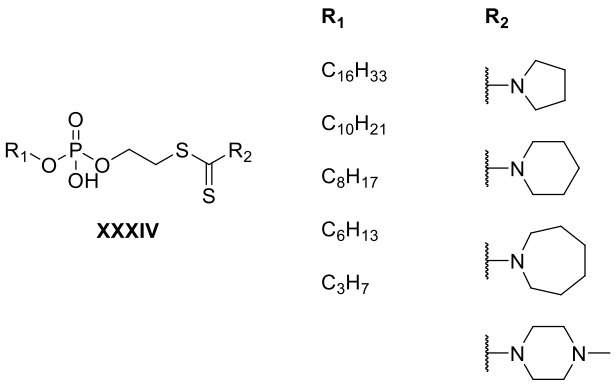
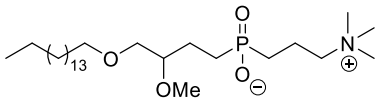


Figure 19

First synthesis of a phosphinate analog **XXXV** was reported by Regan and coworker (Figure 20).[102] This modification was made in order to improve the metabolic stability of edelfosine due to the enzymatically inert phosphorous-carbon bond.

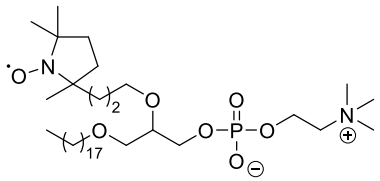


XXXV

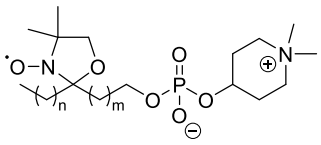
Figure 20

4.2. Hydrophobic tail modifications

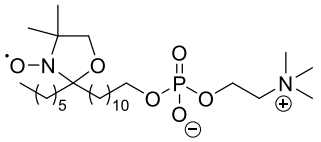
Spin-labeled alkylphosphocholines (**XXXVII**-**XLII**, Figure 21) were synthesized by Mravljak and coworkers[103] as electron paramagnetic resonance probes, following the previous works of Lai and coworkers in the field,[104] who synthesized an analogue of the lipid edelfosine **XXXVI** which displayed cytotoxicity against leukemic cells *in vitro*. This is a very interesting approach, as these compounds could be used to study drug and tumor distribution *in vivo*, due to the inherent selectivity of edelfosine towards tumor cells. In addition, compound **XXXVII** was found to be moderately active against three breast cancer cell lines and exerted low hemolytic activity.



XXXVI



n	m	
3	12	**XXXVII**
5	10	**XXXVIII**
7	8	**XXXIX**
10	5	**XL**
12	3	**XLI**



XLII

Figure 21

Glycerophosphonocholine and glycerophosphocholine alkylphospholipids containing a carbamate (**XLIII**, **XLIV** and **XLVII**) or dicarbamate (**XLV** and **XLVI**) at C2 were synthesized and evaluated for their cytotoxic activity against solid tumor cancer cells *in vitro*, showing promising results (Figure 22).[105]

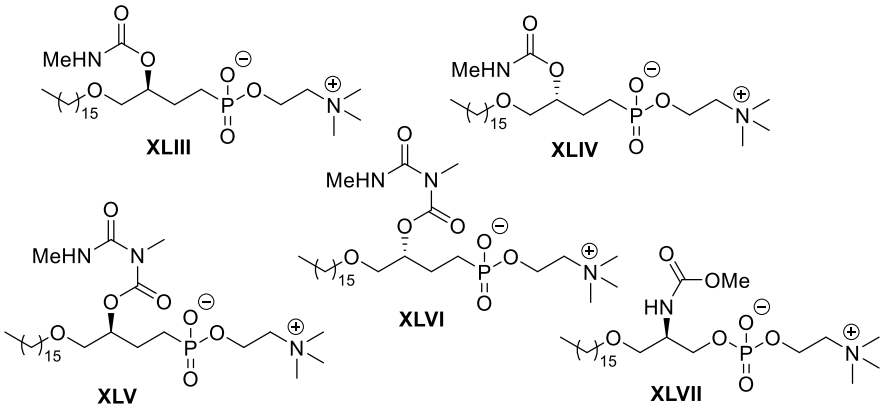


Figure 22

Jolliffe and coworkers[106] synthesized a variety of alkylphospholipid derivatives in order to evaluate their antifungal, antibacterial and hemolytic activity (**XLVIII-LIII**). Some of them were modifications in the length and saturation of the alkyl chain, but others contain diverse kind of carbon skeletons. An example of the latter derivatives is shown in figure 23, despite being inactive as antifungal.

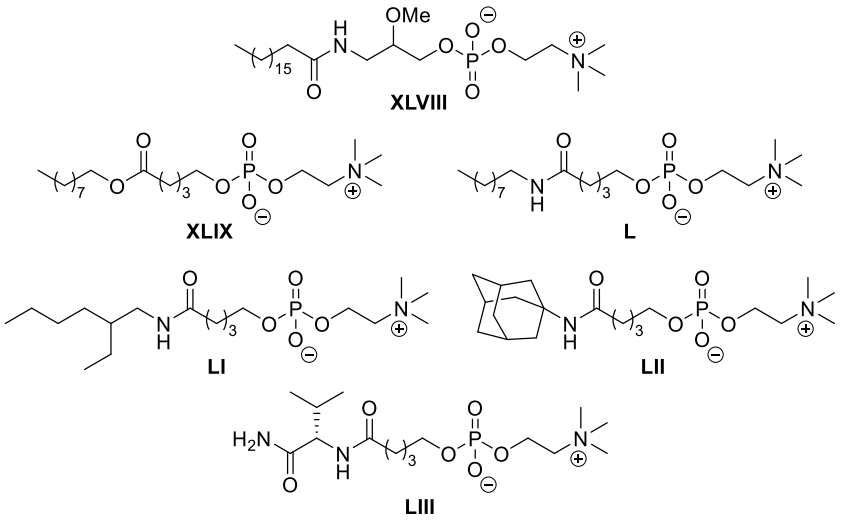
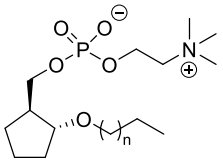
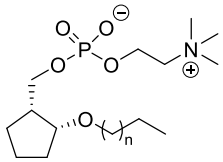


Figure 23

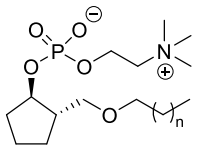
Lee and coworkers, searching for new analogues of alkylphospholipids (Figure 24), obtained a series of compounds with a cyclopentane (**LIV-LVI**)[107] or cyclopentanecarboxylate (**LVII** and **LVIII**)[108] moiety near the polar head of the phospholipid. Akt inhibition activity and cytotoxicity were evaluated for the synthesized compounds obtaining moderate to good results.



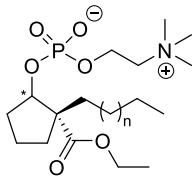
LIV, n=10, 11, 16, 18, 20



LV, n=10, 11, 16, 18, 20



LVI, n=10, 11, 16, 18, 20



LVII, *R,* n=10, 13, 15, 17, 19

LVIII, *S,* n=10, 13, 15, 17, 19

Figure 24

Fluorescent analogs

Herein, a record of some works performed by Prof. Acuña and coworkers about the synthesis of fluorescent analogues of alkylphospholipids is commented (Figure 25). The first attempt to obtain fluorescent analogs of these antitumor lipids consisted in the synthesis of edelfosine and miltefosine derivatives with a tethered phenylpolyene at the alkyl chain, yielding the compounds **LIX**,[109, 110] **LXI and LXII.**[111] Compound **LIX** exerted high cytotoxicity, comparable to that of the parent drug, and **LXI** and **LXII** showed high activity against the *Leishmania donovani* parasite.

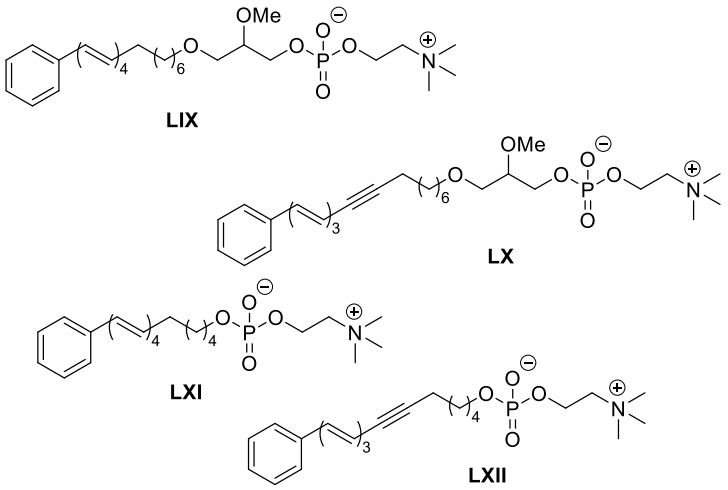


Figure 25

Due to their low emission yield and UV excitation, the next attempt was to synthesize BODIPY-labeled analogs of alkylphospholipids at the end of the long alkyl chain, resulting in the compounds **LXIII-LXVII**[112-114] (Figure 26). Edelfosine analogs **LXVI** and **LXVII** were very useful in unveiling some of the actions that these antitumor lipids perform inside cell, as the dysfunction effect that takes place in the mitochondria during the culture with edelfosine.[114] Also, the new compounds **LXIII-LXV** retained the antiparasite activity of miltefosine with the photophysical properties of the attached BODIPY derivative, improving the understanding of the alkylphospholipid activity inside the parasite.[112]

As a result of the latter experiment, a fluorescent miltefosine linked to a cell-penetrating peptide, **LXVIII**,[115] was synthesized (Figure 26). The culture of a miltefosine-invulnerable *Leishmania* strain with **LXVIII** resulted in fast killing of the parasite, avoiding the resistance.

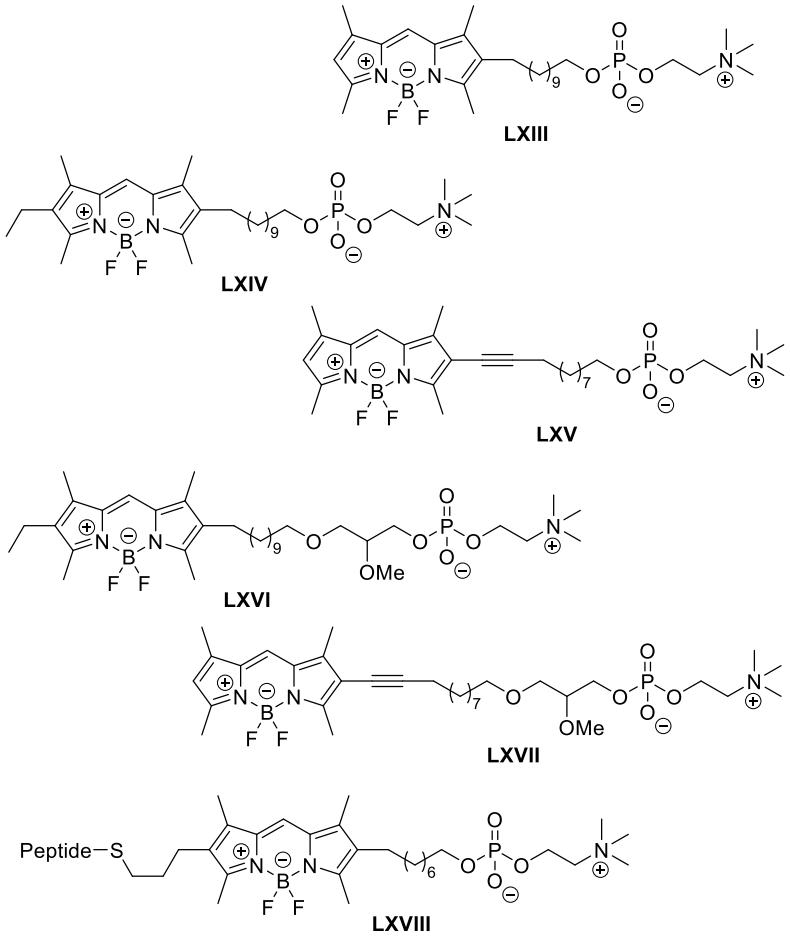


Figure 26

Bioconjugates

Nowadays, there is a growing interest on bioconjugates in fields of biotechnology, nanotechnology and medicine.[116-120] Szoka and coworker[121] synthesized bioconjugates of cholesterol with glycerophospholipids (SML, sterol-modified glycerophospholipids, **LXIX-LXXIV**) to explore the effect of the lipid attachment in cholesterol behavior in a bilayer (Figure 27). Moreover, they discovered that SMLs form liposomes upon hydration, so can be used as drug delivery systems in organs predisposed to the extraction of free cholesterol from the membrane, as they prove using SML-doxorrubicin liposomes.

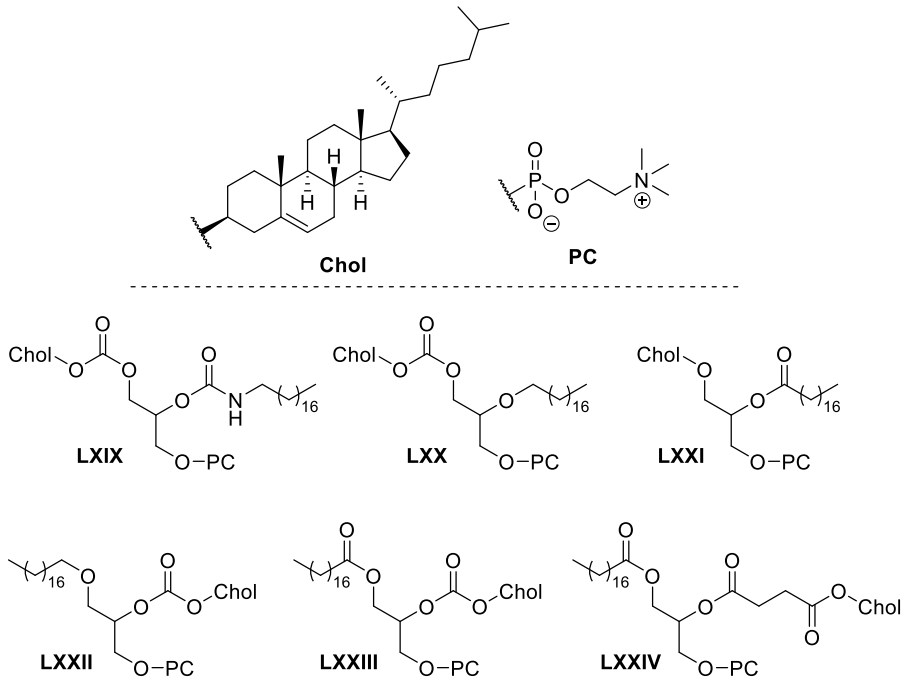


Figure 27

Antecedents

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In the work line directed to the preparation of edelfosine analogs and antitumor bioconjugates, our group has carried out the synthesis of a series of compounds of this type (Figure 28).

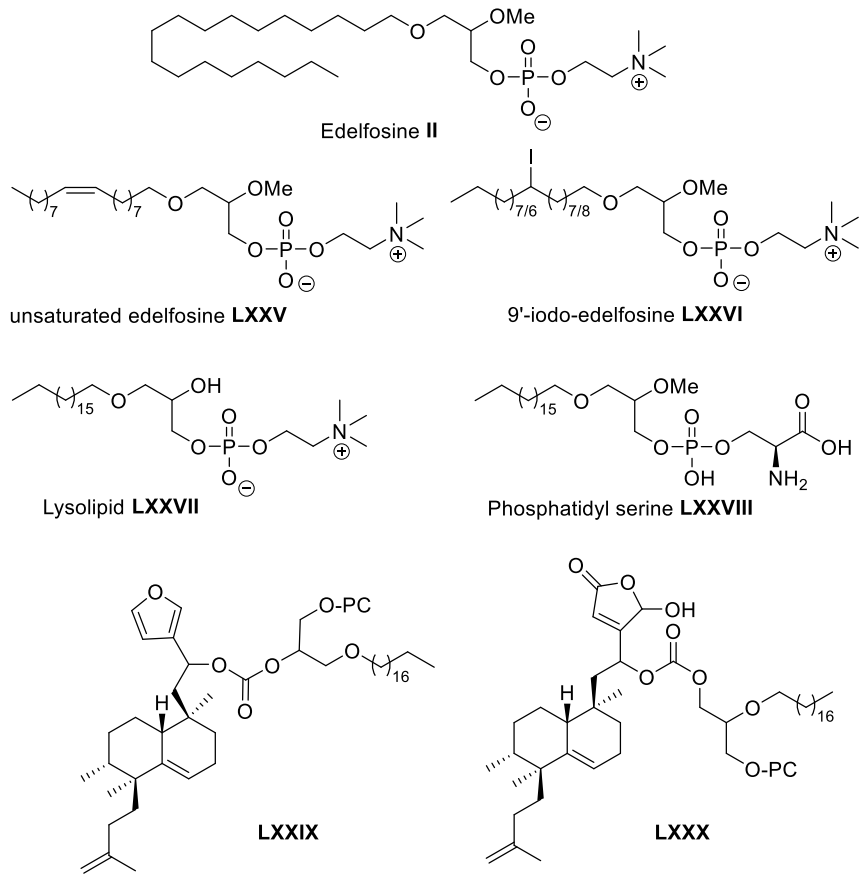
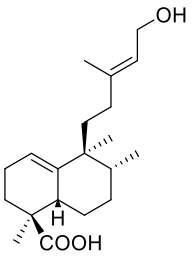


Figure 28

Among the edelfosine analogs can be found: unsaturated and iodinated edelfosine **LXXV** and **LXXVI**, lysolipid **LXXVII** and phosphatidylserine **LXXVIII**; as well as bioconjugates of ATLs with antitumor sesterterpenes and sesterterpenolides **LXXIX** and **LXXX** (Figure 28). These compounds were tested against some tumor cell lines, resulting in: compounds **II**, **LXXV** and **LXXVI** exert apoptotic activity, in addition that

iodinated derivative **LXXVI** is better incorporated into the cell. Edelfosine **II** was the most potent molecule as cytotoxic. Regarding the bioconjugates **LXXIX** and **LXXX**, it was found that their antitumor activity increased slightly compared to the sesterterpenes alone.[5, 122]

Since the furoterpenes with which the bioconjugates were made were synthesized from the natural product *ent*-halimic acid ***1***, a bibliographic study on the diterpenes of the family of natural halimanes has been carried out,[123] published in 2018 in Natural Product Reports, in which a classification and a biosynthetic proposal of said compounds was made.[124, 125] All natural sources, their biological activities[124, 125] and syntheses of halimanes known up to that time are also detailed.



ent-halimic acid, ***1***

In the next section of this work, a summary of the review is presented, and an update of new natural halimanes is also made.

5. Halimane diterpenoids

Until the time of publication of the review carried out by our group,[123] there was no known study regarding halimane-type diterpenes. In this Thesis, we take the opportunity to present a short summary of it and update this topic, adding the new natural compounds of this kind.

Terpenes are one group of natural products with a large number of diverse carbon skeletons. They are inherent to nearly all life forms, and they show many different types of functional activities.

Within terpenes, diterpenes are one of the largest families of natural molecules, with more than 18000 compounds derived from GGPP (*E,E,E*-geranylgeranyl diphosphate). Classification of these compounds is made according to their biogenesis leading to 126 different carbon skeletons known until now.[126]

Diterpenes can be grouped in two big groups; those in which pyrophosphate is involved in the generation of a carbocation in the first step of the biogenesis, and those generated from previous cyclizations and subsequent loss of the pyrophosphate group. Labdanes and related diterpenes (like clerodanes) are included in the latter. Within them the small group of halimanes (Figure 29) is framed as indicated in the general biogenetic scheme (Scheme 1).

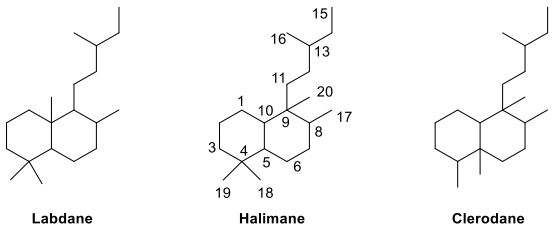
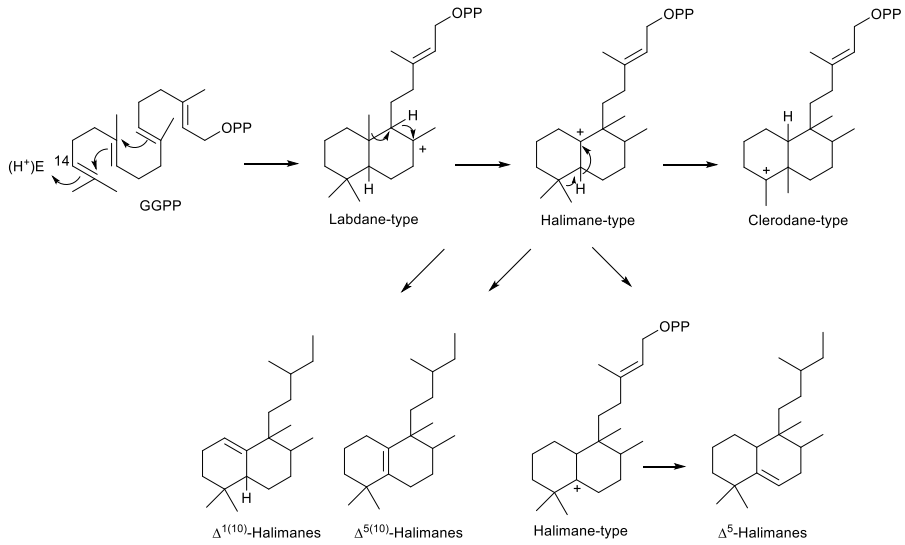


Figure 29

The halimane-type carbocation can be in position 10 leading to $\Delta^{1(10)}$ or $\Delta^{5(10)}$ halimanes or in position 5, due to a hydride 1,2-shift, leading to Δ^{5} halimanes. Other

halimanes can be understood as biogenetic transformations of these three core skeletons mentioned above, as we report in the review.



Scheme 1

Due to the high number of rearranged *ent*-labdane derivatives isolated in *Halimium viscosum* and in order to simplify their nomenclature and to facilitate their classification, the name of *ent*-halimane was proposed for that carbon skeleton.

Nowadays, biological activities of these compounds (such as antitumour, anti-inflammatory, antimicrobial, antifungal, germination inhibitors, etc.) are being studied from different points of view. Compounds such as tuberculosinol, isotuberculosinol and analogues (Figure 30) can be considered as Virulence Factor (VF) in *Mycobacterium tuberculosis,* microorganism which causes tuberculosis illness, major source of morbidity and mortality worldwide. These compounds could be targets in a new route to the anti-infective therapy.[127] Recently, tuberculosinyl-adenosine derivatives are being tested as biomarkers for an early diagnosis of tuberculosis. Moreover, 1-TbAd has been shown to be a naturally evolved phagolysosome disruptor.[128] Recently, crystal structure of halimadienyl diphosphate synthase,

Rv3377c, which catalyzes biosynthesis of 1-TbAd, has been obtained from *Mycobacterium tuberculosis*.[129]

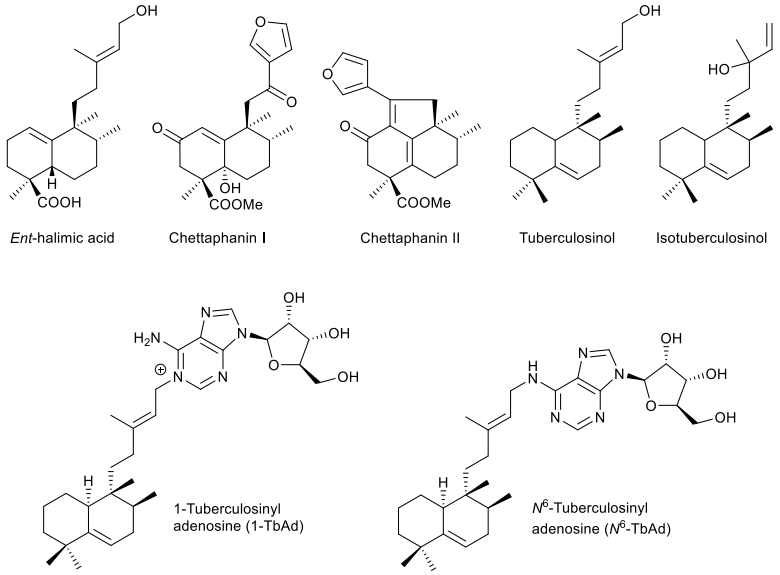


Figure 30

Halimane diterpenoids form a group of secondary metabolites that can be found in different plant species of several families and in other taxonomic groups such as marine organisms or microorganisms.

5.1. Classification

The classification of the halimanes diterpenoids is made according to the endocyclic double bond position. Dihydro-, seco-, nor- and rearranged halimanes have also been considered (Figure 31), resulting in six different groups.

In this manner, we have divided halimanes in six different groups: **1.** Halim-1(10)-enes, **2.** Halim-5(10)-enes, **3.** Halim-5-enes, **4.** Dihydrohalimenes, **5.** Seco- and norhalimenes

and **6.** Rearranged halimanes. As can be seen in figure 31, first four groups could be divided in four series: 'normal', 'antipode or enantio', 8-epi and 8-epi-enantio. According to the biogenesis of each group, those derivatives in which methyls Me-17 and Me-20 are *cis* to each other are denominated halimanes or *ent*-halimanes, and those in which methyls Me-17 and Me-20 are *trans* to each other are called 8-epi-halimanes or 8-epi-*ent*-halimanes (Figure 31). Derivatives from all these groups of compounds are known, except for 8-epi-*ent*-halim-5-ene.

To the best of our knowledge, most of seco-, nor-, and rearranged halimanes (groups 5 and 6) known nowadays belong to the 'enantio' series, except four tetranorhalimenes and three rearranged halimanes.

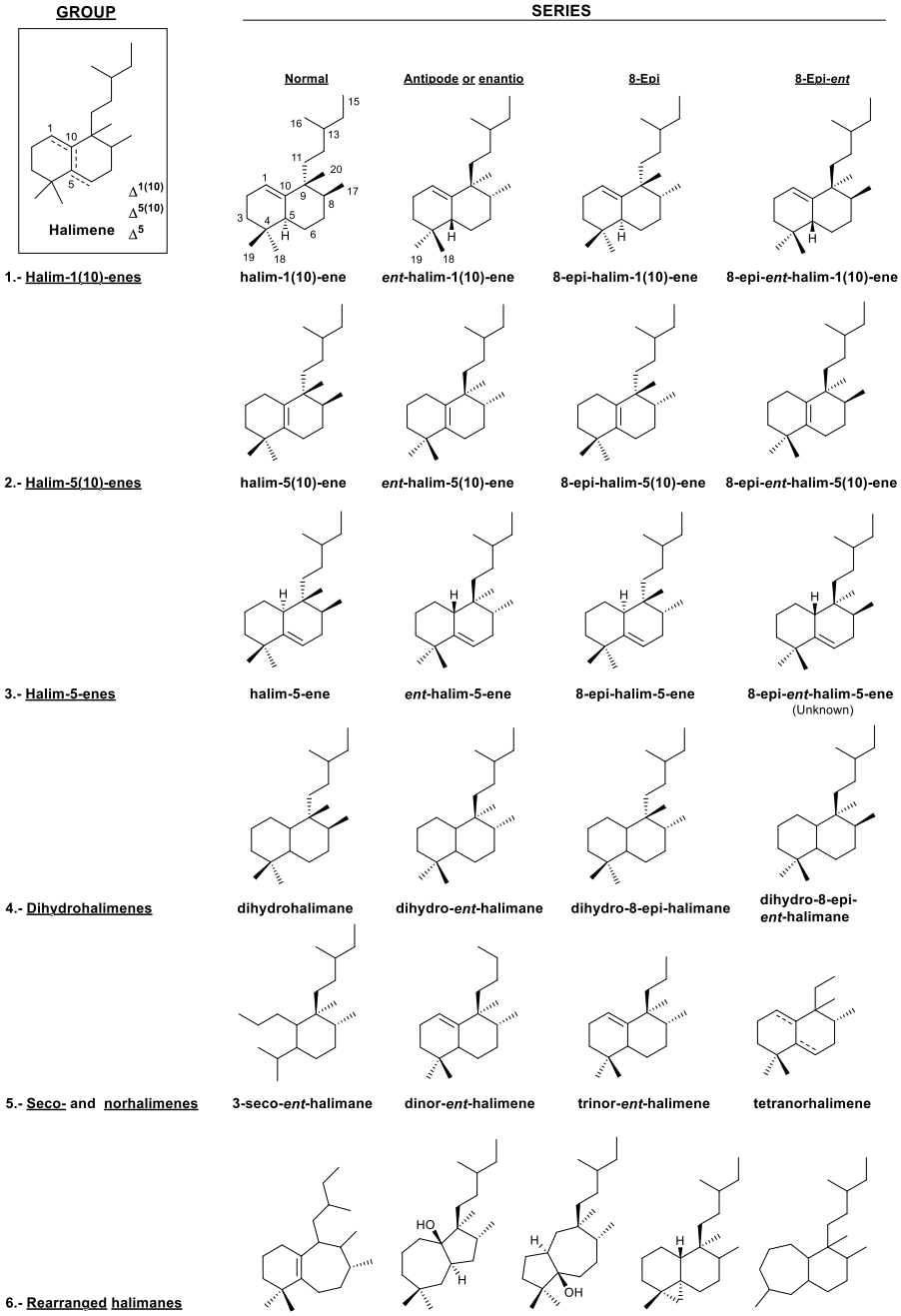


Figure 31

5.2. Natural halimanes

From now on, the structures, properties and trivial name of the most important halimanes of every group will be shown. For the complete information about this natural products, in the review made by our group can be found the structures, classification, bioactivities, and natural source of all known halimanes until date, along with some tables summarizing all the information (tables were published as S.I.).

Halim-1(10)-enes group

The first known halimanes are found in the group of halim-1(10)-enes and *ent*-halim-1(10)-enes is the most numerous class, that is why we start the classification from them.

***Ent*-halim-1(10)-enes**

In this group, the Euphorbiaceae, Cistaceae, Leguminoseae, Compositae, Jungermanniaceae, Velloziaceae and Annonaceae plant families have been studied together with marine organisms of genus *Spurilla* and *Agelas*. Forty nine halimanes have been found which belong to this group. In this summary, we only represent the most characteristic compounds of this series (Figure 32).

Ent-halimic acid ***1*** was used as starting material in the synthesis of chettaphanin I ***2***, establishing, in this manner, the absolute configuration of the first halimane discovered (chettaphanin I, ***2***).

Crassin D ***3***, isolated from *Croton crassifolius*, have a tricyclic system formed by cyclization of C1 with C12 of the halimane side chain, and compound ***4*** displays an ether bond between C5 and C12.

8'-Oxo-agelasine C ***5*** is a novel purine diterpene recently isolated from *Agelas nakamurai* and it is the only halimane-purine presenting a carbonyl group at adenine C8.

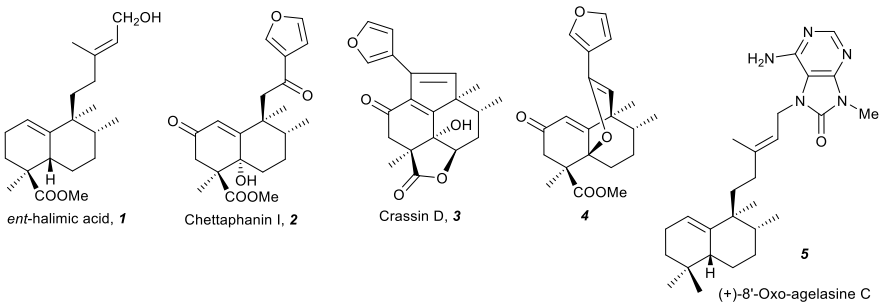


Figure 32

Halim-1(10)-enes

Only three compounds of this kind are known ***6***, ***7*** and ***8*** (Figure 33).

The structure of the natural product agelasine C ***8*** was established by its enantiomer synthesis correcting, in this manner, the original structure proposed by Nakamura and co-workers.[130] Agelasine C ***8*** was isolated from Okinawan sea sponge *Agelas sp*. and *Agelas citrina* and it exerts antifungal activity, along with Na,K-ATPase enzyme inhibition activity.

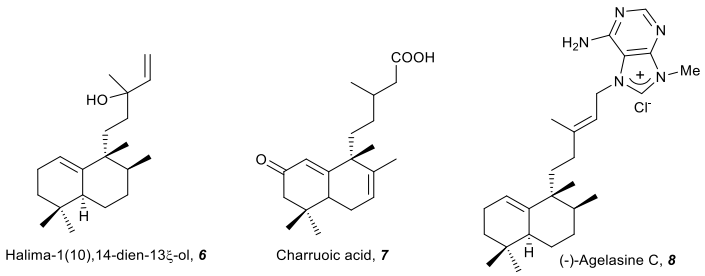
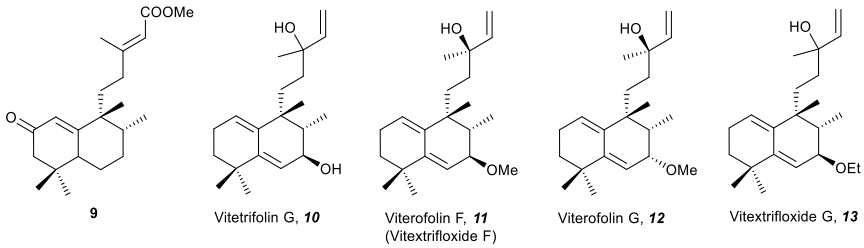


Figure 33

8-Epi-halim-1(10)-enes

Until now, only five natural products ***9-13*** have been described within 8-epi-halim-1(10)-ene series (Figure 34). Compounds ***10-13*** show a trans-annular diene system $\Delta^{1(10),5}$ and vitextrifloxide G ***13*** exhibits moderate cytotoxicity and topoisomerase I inhibition activity.



9 Vitetrifolin G, ***10*** Viterofolin F, ***11*** (Vitextrifloxide F) Viterofolin G, ***12*** Vitextrifloxide G, ***13***

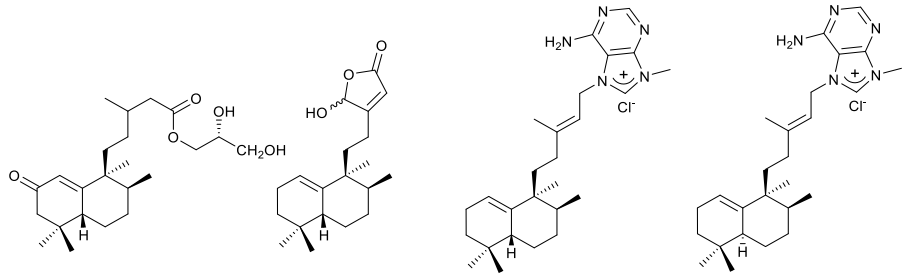
Figure 34

8-Epi-*ent*-halim-1(10)-enes

8-Epi-*ent*-halim-1(10)-ene skeleton compounds are not too numerous (Figure 35). There are only seven compounds within this series, all of them isolated from marine organisms.

Palmadorin S ***14*** is characterized by being esterified at C15 with a glycerol unit. Echinohalimane A ***15***, was found to exhibit cytotoxicity towards various tumor cell lines and displays an inhibitory effect on the release of elastase by human neutrophils.

Two more agelasines, ***16*** and ***17***, are found in this group, both possessing bioactivities such as antifouling (epi-agelasine C ***16***) and antifungal and antibacterial (Isoagelasine C ***17***) activities.



Palmadorin S, ***14*** Echinohalimane A, ***15*** Epi-agelasine C, ***16*** Isoagelasine C, ***17***

Figure 35

Halim-5(10)-enes group

Together with the halim-1(10)-ene, these compounds form the two most numerous groups of halimanes.

Ent-halim-5(10)-enes

It is the largest class within the halim-5(10)-enes group (Figure 36), with forty seven different compounds.

Chettaphanin II ***18***, which along with chettaphanin I ***2*** is one of the first known halimanes, was isolated from *Adenochlaena siamensis*. Chettaphanin II ***18*** synthesis permitted to confirm its structure and to establish its absolute configuration. Chettaphanin II ***18*** shows a tricyclic system resulted of a bond between C1-C12.

As chettaphanin II **18**, the major part of the *ent*-halim-5(10)-enes known are furo-*ent*-halimanes. In some of them the furan fragment appears oxidized in the form of 15,16-butenolide.

Crotohalimaneic acid ***19*** and crotohalimoneic acid ***20*** show activity against several human cancer cell lines.

Crassifolin A ***21***, crassifolin B ***22***, and penduliflaworosin ***24*** show anti-angiogenic activity using a wild-type zebrafish *in vivo* model, and crassifolin D ***23*** shows antiviral and anti-angiogenic activities.

Crolaevinoid A **25** displays a highly oxygenated functionalization, including a δ-lact-17,12-olide.

3α-hydroxy-5(10)-didehydrochiliolide ***26*** is highly active against a human pancreatic adenocarcinoma cell line at micromolar concentrations and ***27*** shows antitumour activity.

In this group, the halimane-purine agelasine J ***28*** is included. Agelasine J was isolated from the Solomon Islands marine sponge *Agelas* cf. *mauritiana.* It shows antimalarial and antimicrobial activity and MCF7 cells cytotoxicity.

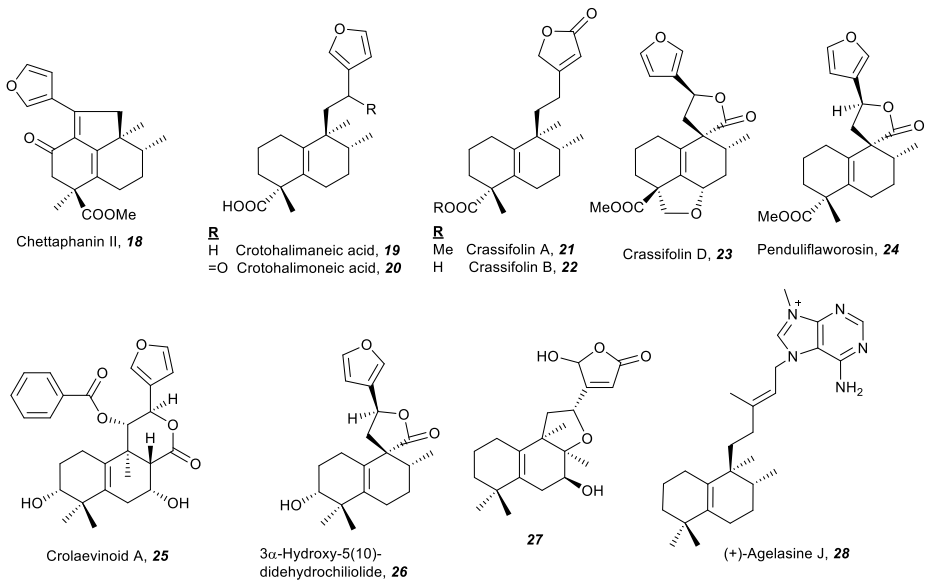


Figure 36

Halim-5(10)-enes

In this class, only nine natural halimanes are known. Within the most significant compounds (figure 37), two halimane-purines isolated from an Okinawan marine sponge *Agelas sp*. can be found: agelasine O ***29*** and agelasine S ***30***. Agelasine O ***29,*** in which C18 is esterified with 2-carboxy-4-bromopyrrole, results biologically active as antibacterial and antifungal. Agelasine S ***30*** exerts antibacterial and antifungal activities and is the only derivative of this type difunctionalized at C1 and C3. Moreover, amoenolide M ***31*** is esterified at C19 with *p*-hydroxyphenylpropionic acid and crassifolius A ***32*** displays cytotoxic activity.

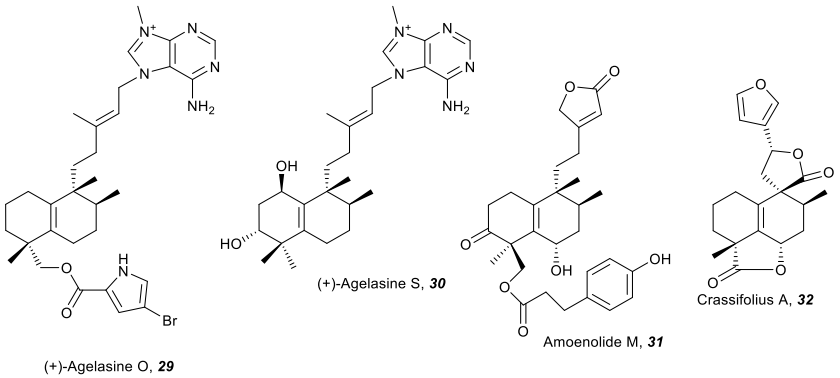


Figure 37

8-Epi-halim-5(10)-enes

Twelve natural products belong to this series. The most usual derivatives in this series are those which present oxygenated functions on ring B, normally on carbons C6 and C7 (Figure 38). In the side chain, only isoleojaponin ***37*** shows a furan ring and all the rest possess allylic groups. Compound ***33*** exerts moderate anti-hyperlipidemic activity and compounds ***34*** and **35** result cytotoxic against HCT 116 human colon carcinoma cell line.

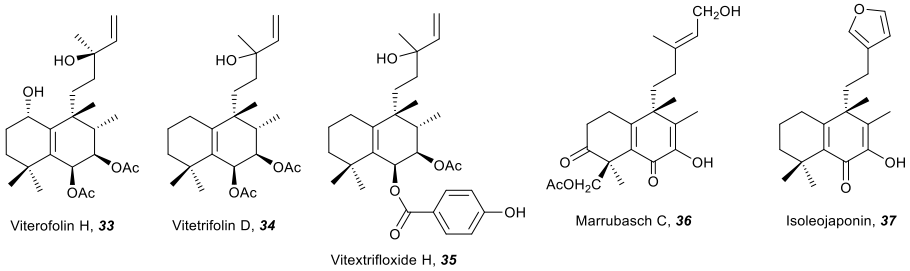


Figure 38

8-Epi-*ent*-halim-5(10)-enes

The first halim-5(10)-enes known appear within this series, salmantic acid ***38***, its methyl ester ***39*** and salmantidiol ***40*** isolated from *Cistus laurifolius* (Figure 39).

The five compounds of this series are functionalized at C3. Leucasperone A ***41*** shows inhibition of prostaglandin induced contractions.

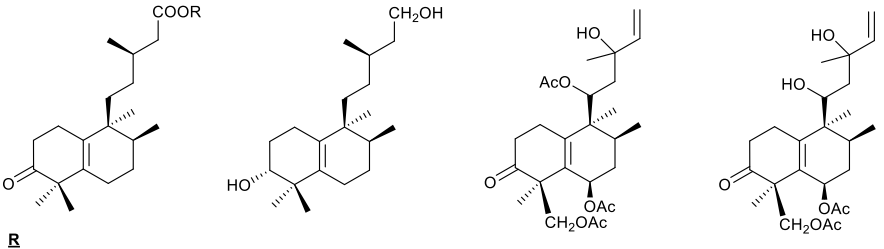


Figure 39

Halim-5-enes group

This group of halimanes, having a double bond in ring B, is the most interesting due to the biological activities that some of them show.

***Ent*-halim-5-enes**

The *ent*-halim-5-ene class is not very numerous; only eight compounds are known (Figure 40). Most of them show oxygenated functions in ring A.

3α-Hydroxy-5,6-didehydrochiliolide ***46*** displays a 12,20-butanolide and is highly active against a human pancreatic adenocarcinoma cell line at micromolar concentrations.

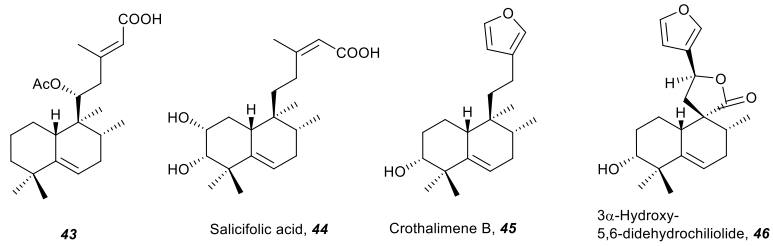


Figure 40

Halim-5-enes

This is the most numerous halimenes group with a double bond at C5 (Figure 41) and perhaps the most interesting group considering the bioactivity of its derivatives,

because tuberculosinol ***47*** and isotuberculosinol (also known as nosyberkol) ***48*** are found among them. Twenty natural halimanes have been isolated which match this series.

Some studies proved that the bacteria *Mycobacterium tuberculosis* does produce the halim-5-enes tuberculosinol ***47*** and isotuberculosinol ***48***. These two compounds inhibit phagolysosome maduration and macrophage phagocytosis in human-like cells. In addition to this, two new natural products derived from tuberculosinol have been isolated, 1-TbAd ***49*** and N^6-TbAd ***50***. These are characterized by possessing a unit of adenosine attached at C15. Moreover, these diterpene-nucleoside compounds are being tested as biomarkers for tuberculosis.

In the halim-5-enes series the side chain can be saturated or unsaturated and furans or functionalized butanolides can be found on it.

Micromonohalimanes A and B ***52*** and ***53,*** which present antibacterial activity, have been characterized. Micromonohalimane B ***53*** is the only halimane which includes a chlorine atom in its structure.

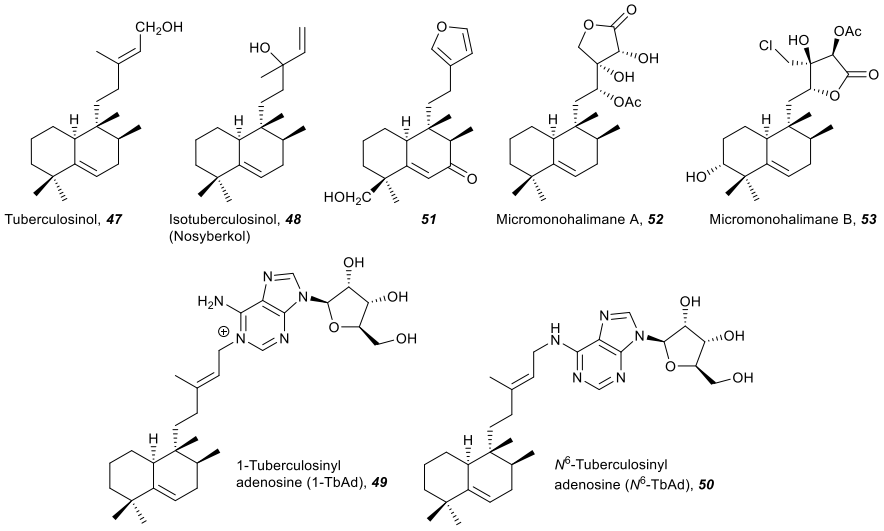


Figure 41

8-Epi-halim-5-enes

Only six compounds of this class are known ***54-55*** (Figure 42). All of them display allylic functions at the side chain except ***59***, which shows a butadiene moiety.

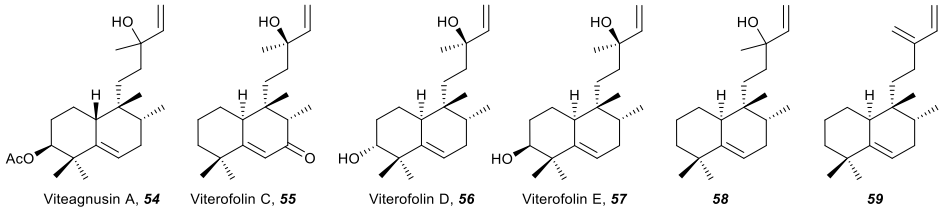


Figure 42

Dihydrohalimenes group

A structural characteristic of this group of twelve compounds is that all of them show oxygenated functions at C5, except diasin ***67***. In figure 43, the most representative compounds of this group have been represented. Ambliol B and C, ***60*** and ***61***, were isolated from *Dysidea amblia* being the only occasion that halimanes and 8-epi-halimanes coexist in the same organism.

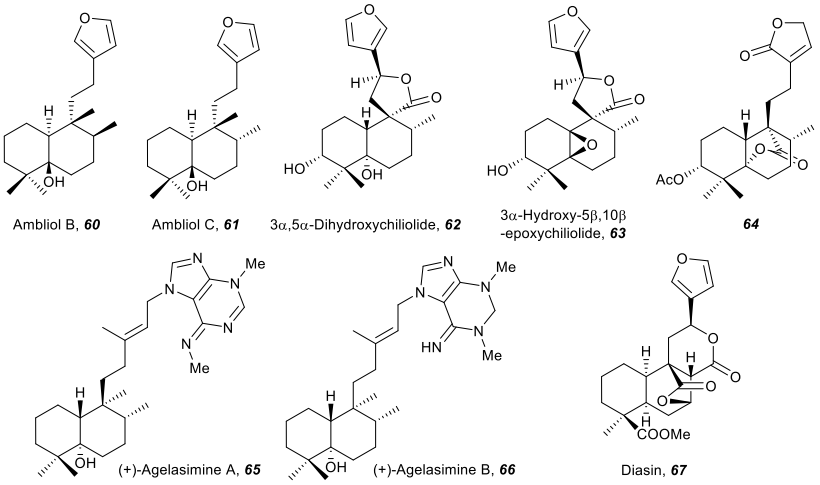


Figure 43

In this group halimane-purines as agelasimines A and B (***65*** and ***66*** respectively), isolated from *Agelas mauritiana*, are included. Both compounds show a wide range of

interesting biological activities, such as cytotoxicity, inhibition of adenosine transfer into rabbit erythrocytes, Ca^{2+}-channel antagonistic action and α_1 adrenergic blockade.

Secohalimenes and norhalimenes group

All secohalimenes known, twenty three compounds, are included in the *ent*-halimenes series, and are formed by cleavage of the C3-C4 bond. In figure 44 the most representative compound of this group are shown. Tessmannic acid ***68*** exhibit antibacterial and antifungal activity. Butanolides ***69***, ***70*** and ***71*** are biologically actives as antitumour agents.

The norhalimenes known can be mono-, di-, tri- and tetranorhalimenes derivatives ***74***-***77*** (Figure 44).

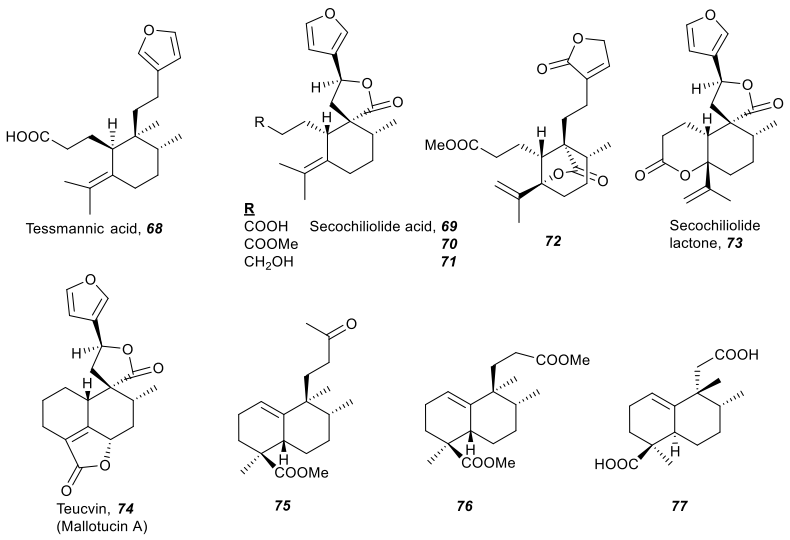
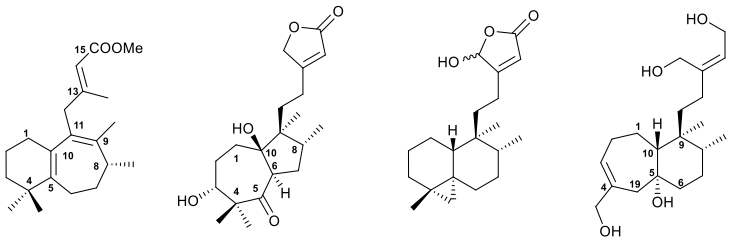


Figure 44

Rearranged halimane group

The different rearranged halimanes known are shown in figure 45. They can be classified in four different groups. Herein, we only report one halimane of each skeleton; scopariusin A ***78***, randainin A ***79***, dytesinin A ***80***, jewenol B ***81***.



Scopariusin A, ***78*** Randainin A, ***79*** Dytesinin A, ***80*** Jewenol B, ***81***

Figure 45

5.3. New natural halimanes

Since the publication of the halimane diterpenoids review,[123] isolation and characterization of 51 new halimanes have been published. Over the following paragraphs, a classification and a brief description of the structures and bioactivity of these new halimanes are shown. In addition, a table with all the natural sources, bioactivity (if any) and references of the new published halimanes is shown below (Table 1).

Table 1. Natural source (including the part of the plant), activity and references of the new natural halimanes

	NATURAL SOURCES		ACTIVITY	REF.
***Ent*-halim-5(10)-enes**				
EBC-204, ***82***	*Croton insularis*	Aerial parts	Weak cytotoxicity	131
EBC-205, ***83***	*C. insularis*	A. P.	Weak cytotoxicity	131
Crohalifurane D, ***84***	*C. crassifolius*	Roots		132
Crohalifurane E, ***85***	*C. crassifolius*	Roots		132
Crohalifurane F, ***86***	*C. crassifolius*	Roots		132
Crohalifurane G, ***87***	*C. crassifolius*	Roots		132
Crohalifurane H, ***88***	*C. crassifolius*	Roots		132
Crohalifurane I, ***89***	*C. crassifolius*	Roots		132
Crohalifurane J, ***90***	*C. crassifolius*	Roots		132

Crohalifurane K, ***91***	*C. crassifolius*	Roots		132
Crohalifurane L, ***92***	*C. crassifolius*	Roots		132
Crohalifurane M, ***93***	*C. crassifolius*	Roots		132
Crohalifurane N, ***94***	*C. crassifolius*	Roots		132
Crohalifurane O, ***95***	*C. crassifolius*	Roots		132
Crohalifurane P, ***96***	*C. crassifolius*	Roots		132
Crapenoid A, ***97***	*C. crassifolius*	Roots	Moderate anti-inflammatory activity	133
Crapenoid B, ***98***	*C. crassifolius*	Roots		133
3*S*-Acetoxy-mallotucin D, ***99***	*C. laui*	Leaves		134
Mallotucin C, ***100***	*Mallotus repandus*	Bark		135
Mallotucin D, ***101***	*M. repandus*	Bark		135
Halim-5(10)-enes				
102	*Leonurus japonicus*	A. P.		136
103	*L. japonicus*	A. P.		136
104	*L. japonicus*	A. P.		136
Leojaponin G, ***105***	*L. japonicus*	A. P.	Weak anti-melanogenesis	137
Leojaponin H, ***106***	*L. japonicus*	A. P.		137
Leojaponin K, ***107***	*L. japonicus*	A. P.		136, 137
8-Epi-*ent*-halim-5(10)-enes				
(-)-EBC-232, ***108***	*Croton insularis*	A. P.	Moderate cytotoxicity	138
EBC-323, ***109***	*C. insularis*	A. P.	Moderate cytotoxicity	138
***Ent*-halim-5-enes**				
Crotonolide K, ***110***	*Croton poomae* Esser	Leaves, stem	Potent inhibitor of NO production (anti-inflammatory)	139
Norhalimanes				
6-Epi-crotoeurin C, ***111***	*Croton laui*	Leaves	Inhibitor of NO production (anti-inflammatory)	133, 134
3*S*-Methoxy-teucvin, ***112***	*C. crassifolius*	Roots	Moderate anti-inflammatory	140

			activity	
3*R*-Methoxy-teucvin, ***113***	*C. crassifolius*	Roots	Moderate anti-inflammatory activity	140
Crocleropene A, ***114***	*C. caudatus*	Leaves, twing	Weak cytotoxicity	141
Crocleropene B, ***115***	*C. caudatus*	L. and t.	Weak cytotoxicity	141
Scordidesin A, ***116***	*Teucrium scordium* L. subsp. *scordioides*	A. P.	Moderate antimicrobial	142
Crohalifurane B, ***117***	*C. crassifolius*	Roots	Moderate inhibition of NO production (anti-inflammatory)	132
Crotonpenoid A, ***118***	*C. yanhuii*	L. and t.	Moderate PXR agonist	143
Crotonpenoid B, ***119***	*C. yanhuii*	L. and t.	Moderate PXR agonist	143
Gratissihalimanoic ester, ***120***	*C. gratissimus*	Leaves		144
Crohalifurane C, ***121***	*C. crassifolius*	Roots		132
Crohalifurane A, ***122***	*C. crassifolius*	Roots		132
Norcrocrassinone, ***123***	*C. crassifolius*	Roots	Anti-Alzheimer's disease	145
Rearranged halimanes				
Dodovisin C, ***124***	*Dodonaea viscosa*	A. P.	-	146
Dodovisin D, ***125***	*D. viscosa*	A. P.	-	146
Dodovisin E, ***126***	*D. viscosa*	A. P.	Moderate ATP citrate lyase inhibition	146
Dodovisnoid G, ***127***	*D. viscosa*	A. P.	Anti HSV-1	147
Dodovisin F, ***128***	*D. viscosa*	A. P.	-	146
Dodovisnoid B, ***129***	*D. viscosa*	A. P.	-	147
Dodovisnoid C, ***130***	*D. viscosa*	A. P.	-	147
Dodovisnoid D, ***131***	*D. viscosa*	A. P.	Anti HSV-1	147
Dodovisnoid E, ***132***	*D. viscosa*	A. P.	Anti HSV-1	147

Ent-halim-5(10)-enes

Within *ent*-halim-5(10)-enes group (Figure 46), twenty new compounds were found. All of them exhibit a carboxylic function at C18, except for ***86*** and ***96***, which possess the mentioned function at C19.

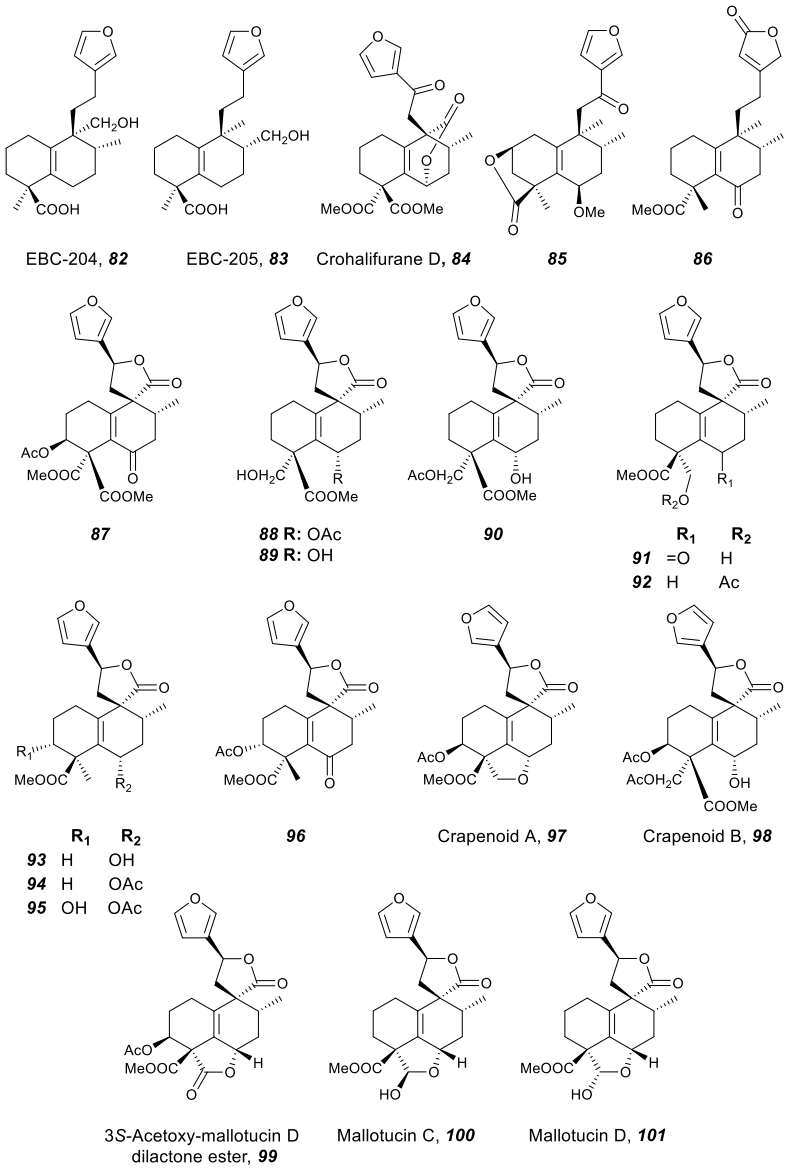


Figure 46

EBC-204 and EBC-205, ***82*** and ***83***,[131] were isolated from the plant *Croton insularis* and they show the unusual functionalization at C20 or C17 respectively. Following with the genus *Croton* (Euphorbiaceae), the furo-halimane 3*S*-acetoxy-mallotucin D dilactone ester ***99*** was isolated from the leaves of *Croton laui***.** Mallotucin C ***100*** and mallotucin D ***101***[135] were isolated from the chloroform extract of the bark of *Mallotus repandus*. In terms of activity, only crapenoid A ***97*** displayed moderate anti-inflammatory activity and ***82-83*** showed weak cytotoxicity. (Figure 46)

Crohalifuranes D-P (***84-96***)[132] and crapenoids A and B, ***97*** and ***98***,[133] were isolated from the roots of *Croton crassifolius*. Their name refers to the existence of a furan fragment in the side chain of the diterpene, except for crohalifurane F ***86***, which shows a γ-butenolide, and most of them display oxygenated functionalization at C6 in the form of hydroxyl, ether, lactol or ester/lactone groups.

Halim-5(10)-enes

Six new compounds were found inside halim-5(10)-enes group (Figure 47), all of them containing oxygenated functions at ring B. Compounds ***102-104***,[136] were isolated from the aerial parts of *Leonurus japonicas* (Lamiaceae), and anti-inflammatory activity tests were carried out showing no activity on either the halimane compounds. Also from this plant, leojaponins G, H and K ***105-107*** were obtained.[137]

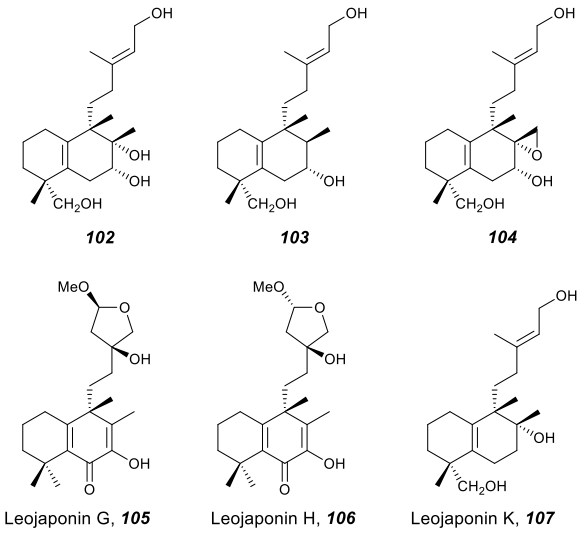


Figure 47

8-Epi-*ent*-halim-5(10)-enes

In the 8-epi-*ent*-halim-5(10)-enes group (Figure 48), only two new halimanes were included, EBC-232, ***108***, and EBC-323, ***109***, isolated from *Croton insularis***.**[138] In order to elucidate the structure and configuration of both diterpenes, five *in silico* methods were tested. The proposed structures contain an unusual oxo-6,7-spiro ring system fused to a dihydrofuran. However, absolute configuration of ***109*** could not be determined.

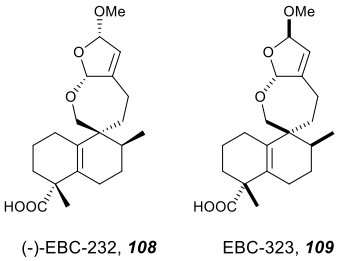
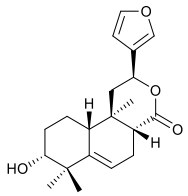


Figure 48

***Ent*-halim-5-enes**

Within the *ent*-halim-5-enes group, crotonolide K ***110*** (Figure 49) has been isolated from the leaves and stems of *Croton poomae* Esser. This compound has been evaluated as inhibitor of NO production and it showed potent inhibitory effects.



Crotonolide K, ***110***

Figure 49

Norhalimanes

Thirteen new natural norhalimanes (Figure 50) have been isolated since the publication of the review, indeed, nine 19-norhalimanes,[132-134, 140-143] two 20-norhalimanes,[132, 144] and two tetranorhalimanes.[132, 145] Most of them exhibit furan fragments at the side chains, except tetranor-derivatives ***122*** and ***123***. Moreover, all of them show a carboxylic function at C18, except ***117***, and eight of them also have oxygenated function at C6 (***111-116***, ***118*** and ***119***). Almost all of these compounds have been isolated from plants of genus *Croton* (Table X) except for scordidesin A ***116***, which was separated from the aerial parts of *Teucrium scordium* L. subsp. *scordioides*.

Among them, compounds ***120*** and ***121*** represent the first halimanes lacking Me-20, and crotonpenoid A and B, ***118*** and ***119***, show a tricyclo[7.2.1.$0^{2,7}$]dodecane core unknown until now in nature within the diterpene family.

Most of them exhibit some kind of bioactivity. Crotonpenoids A and B, ***118*** and ***119***, exert agonistic activity on the human pregnane X receptor (PXR), which is a promising

therapeutic target in cholestatic disorders. Norcrocrassinone ***123*** exhibits low anti-Alzheimer disease activity.

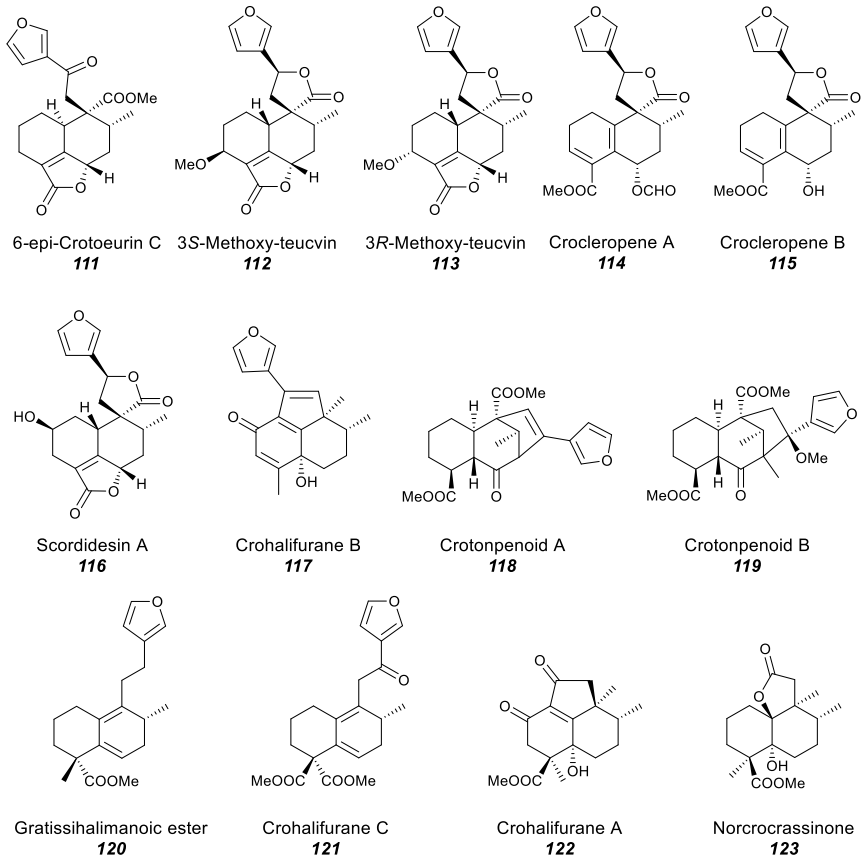


Figure 50

Rearranged halimanes

All the new rearranged halimanes (Figure 51), dodovisins C-F (***124-126, 128***)[146] and dodovisnoids B-E and G (***129-132, 127***),[147] have been isolated from the aerial parts of *Dodonaea viscosa*. Five of them, ***128-132***, show a tricyclo[5.4.0.0^{1,3}]undecane core, where C19 is attached both to C4 and C5 forming a cyclopropane ring. In the other four, ***124-127***, methyl C19 has been included in ring A as consequence of a ring expansion.

Dodovisnoids D, E and G (***131***, ***132*** and **127**) were found active against the herpes simplex virus type-1 (HSV-1) in micromolar concentrations.

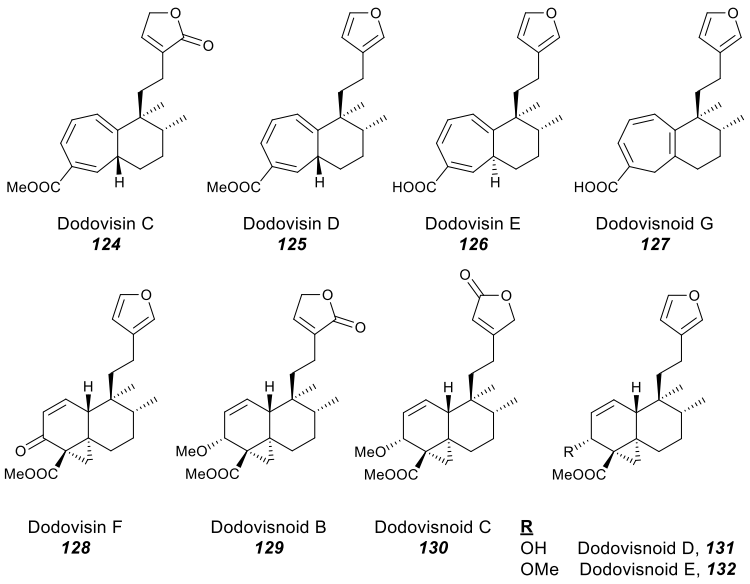


Figure 51

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FIRMADO POR	FECHA FIRMA
SELLO UNIVERSIDAD DE SALAMANCA	20-07-2021 18:11:01
SÁNCHEZ MARCOS ISIDRO	20-07-2021 10:20:59

Objectives

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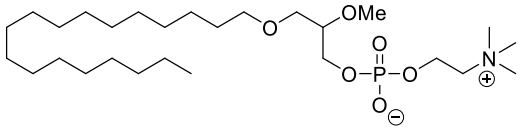
Antitumor ether lipids form a group of compounds known collectively as APLs (alkylphospholipids) that exhibit anticancer activity by acting at cell membrane level. Edelfosine can be considered as the prototype of this kind of compounds and has the ability to induce apoptosis preferentially in tumor cells, sparing healthy cells, exerting its action at the level of lipid rafts.



Edelfosine

The general objective of this work is to carry out the synthesis of a series of analogous alkylphospholipids of edelfosine that contain different heteroatoms or fluorophores in the C1 alkyl chain.

The introduction of heteroatoms or fluorophores in the C1 chain will allow, on the one hand, to study and evaluate the activity of said molecules with respect to edelfosine and, on the other hand, the presence of a fluorophore will allow a better observation at the subcellular level, making possible to visualize the pathway followed and facilitating the location and spread of the tumor.

The concrete objectives of this work are described below:

Objectives

1.- Synthesis of edelfosine analogs **1** and **2** that contains heteroatoms or fluorophores in C9', in the middle of the alkyl chain (Figure 52).

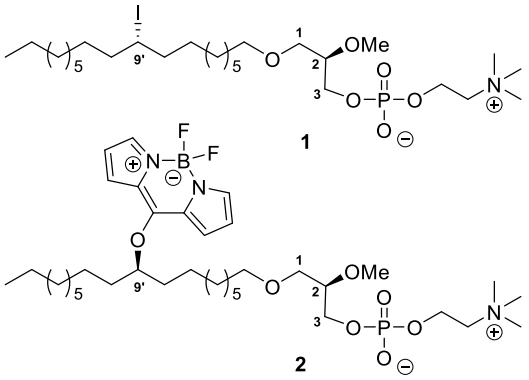


Figure 52

The first modification proposed in the structure of edelfosine is related to the work previously carried out in our research group. These were based on functionalization with different groups at positions in the middle of edelfosine alkyl chain (Figure 53). In this way, an analog of edelfosine unsaturated at C9', **I**, was obtained, by which it was possible to access, through addition of hydrogen iodide to **I**, to a mixture of analogs with iodide at C9' or C10', **II**, which gave promising biological results.[1]

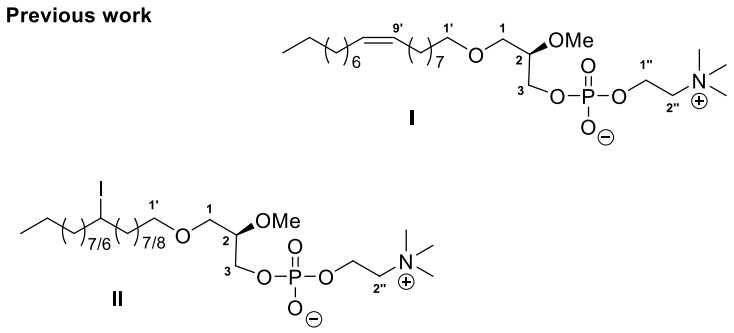


Figure 53

Objectives

Thus, we propose the stereoselective synthesis of a iodinated edelfosine analog, **1**, following a totally different synthetic route from that of **II** and which will be the basis for the synthesis of other edelfosine analogs. Furthermore, the synthesis of edelfosine derivatives linked to fluorescent compounds with the BODIPY structure (Figure 52), **2**, is proposed; since they would be good compounds for the traceability of this drug *in vivo*.

2.- Synthesis of edelfosine analogs **3**, **4**, **5**, **6** and **7** containing heteroatoms or fluorophores in C16', at the end of the alkyl chain (Figure 54).

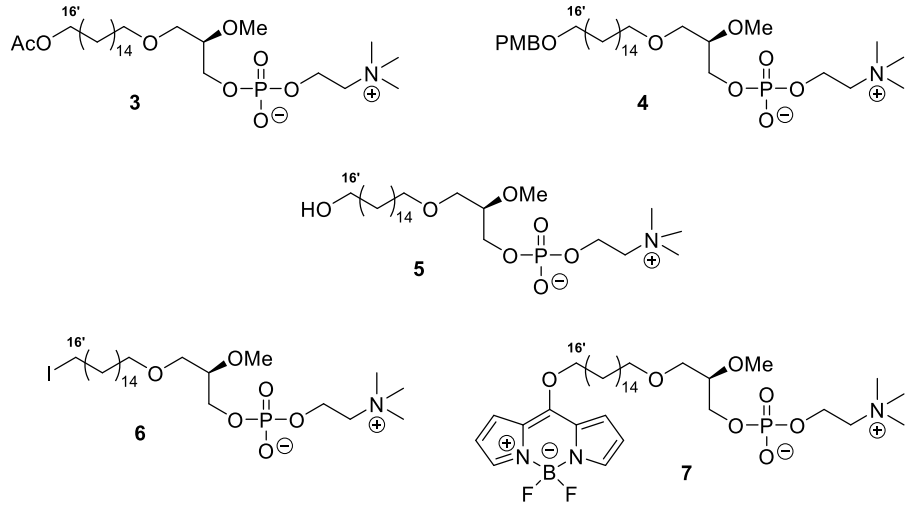


Figure 54

3.- Synthesis of edelfosine analogs **8**, **9** and **10** containing heteroatoms or fluorophores in C2', at the beginning of the alkyl chain (Figure 55).

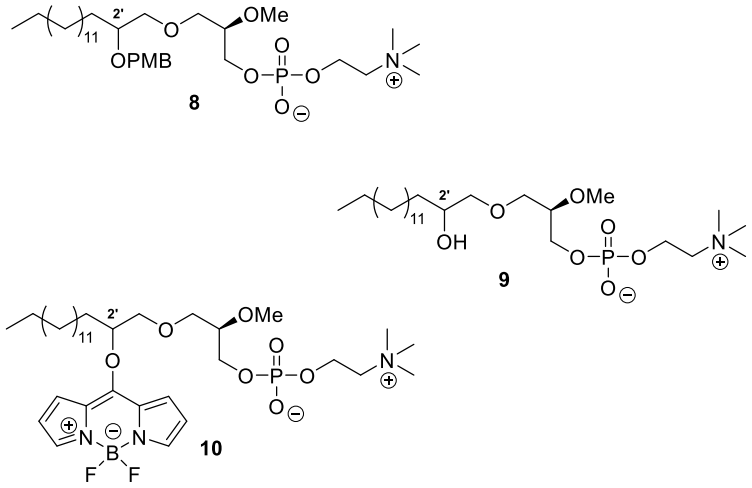


Figure 55

To the best of our knowledge, there is no studies of this kind of compounds in bibliography.

4.- Brief descriptive study of the photophysic characteristics of absorption and fluorescence of the edelfosine analogs **2**, **7** and **10**.

5.- Study of the biological activity of the synthesized analogs. This study will be carried out at the "Centro de Investigaciones Biológicas (CIB), departamento de Biomedicina Molecular, CSIC, Madrid" (Prof. Dr. Faustino Mollinedo).

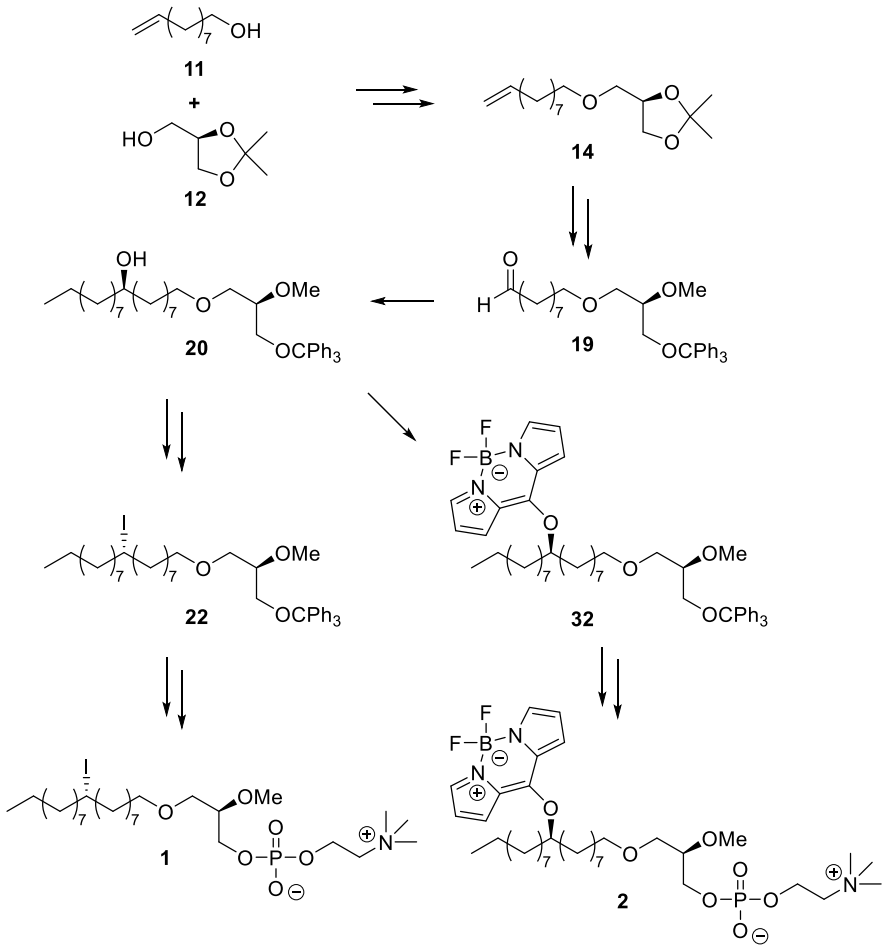
Results and discussion

FIRMADO POR	FECHA FIRMA
SELLO UNIVERSIDAD DE SALAMANCA	20-07-2021 18:11:01
SÁNCHEZ MARCOS ISIDRO	20-07-2021 10:20:59

7. Synthesis of C9' edelfosine analogs.

Scheme 1 shows the synthetic route followed for the obtaining of C9'-functionalized edelfosine analogs, **1** and **2**.

A proper choice of commercial starting materials will facilitate the synthesis of these analogs, such as 9-decen-1-ol **11**, nonylmagnesium bromide and *R*-solketal **12** (Scheme 2).



Scheme 2

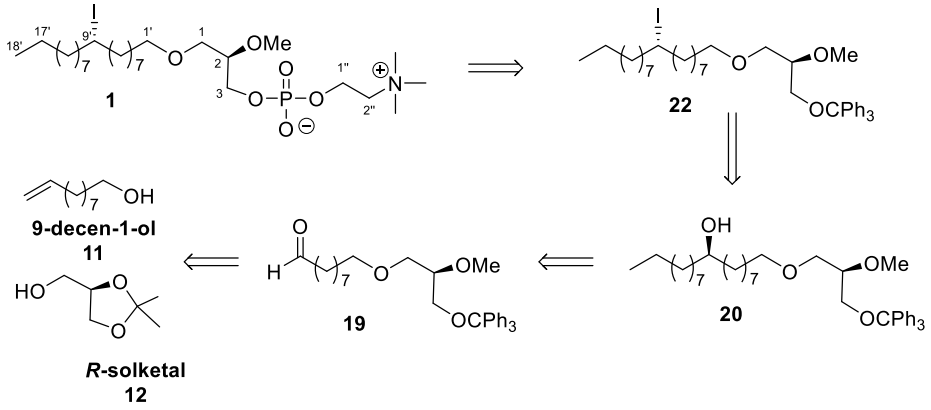
In the following paragraphs, the synthesis of compounds **1** and **2** will be discussed.

1.1. Synthesis of iodinated analog **1**.

1.2. Synthesis of fluorescent analog **2**.

7.1. Synthesis of iodinated analog 1

Synthesis of edelfosine analog **1** will proceed according to the following retrosynthetic scheme (Scheme 3):



Scheme 3

Access to phosphatidylcholine **1** from the intermediate **22** requires deprotection at C3 followed by the incorporation of the phosphocholine unit.

It is possible to obtain iododerivative **22** from aldehyde **19** passing through hydroxyderivative **20**. We use (-)-TADDOL as chiral auxiliary to control the formation of the C9' stereogenic center. Then, in order to transform **20** into **22** it is required to use a tosylate or analog as a synthetic intermediate. Aldehyde **19** can be achieved from the commercial starting materials **11** and **12**.

Synthesis of **1** will be divided in three sections to facilitate its explanation:

1.1.1. Synthesis of aldehyde **19**

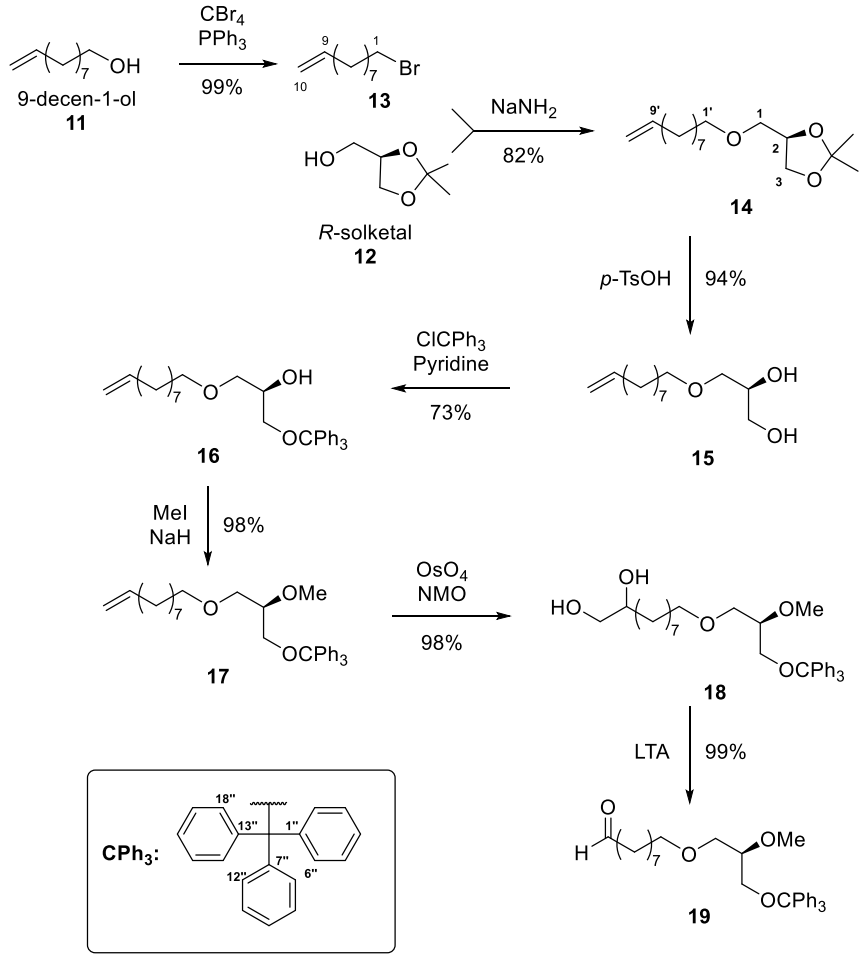
1.1.2. Synthesis of hydroxyderivative **20**

1.1.3. Obtaining of iodinated analog **1**

Synthesis of aldehyde 19

Aldehyde **19** is obtained following the seven-step synthesis proposed in scheme 4, in which 9-decen-1-ol **11** is used as starting material. This compound displays a terminal double bond which will be useful in the formation of the aldehyde functional group of **19**, as it can be oxidized in different ways.

Reaction of **11** with carbon tetrabromide in presence of triphenylphosphine afforded bromoderivative **13** in quantitative yield (Scheme 4).



Scheme 4

By heating of **12** with sodium amide and subsequent reaction with **13**, the alkylsolketal derivative **14** is obtained, which by treatment with *p*-toluensulfonic acid provides the diol **15**.

The necessary regioselective protection of the primary hydroxyl group of **15** is achieved by treatment with trityl chloride (triphenylmethane chloride) in pyridine,[2] obtaining **16**. Indeed, regioselective monoprotection of the primary hydroxyl group is achieved, which can be corroborated by observing the ^{1}H NMR spectra of **15** and **16**. In the spectrum of **15**, the C3 hydrogens appear at 3.68-3.55 ppm as a multiplet, while in the spectrum of **16** they appear shielded as an AB system at 3.21 and 3.19 ppm.

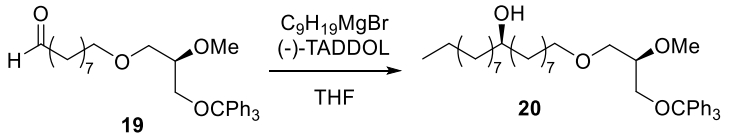
Methylation of **16** is carried out by reaction of sodium hydride, followed by treatment with methyl iodide, obtaining **17**. In the ^{1}H NMR spectrum of **17** a signal is observed at 3.42 ppm (3H, s, OMe) corresponding to the methoxyl group.

Cis-hydroxilation of the double bond is achieved by treatment of **17** with osmium tetroxide and *N*-methylmorpholine *N*-oxide, obtaining **18**. In the ^{1}H NMR spectrum of **18**, signals are observed between 3.6-3.2 ppm corresponding to 10 geminal hydrogens to oxygen functions.

Oxidation of the glycol is performed by reaction of **18** with lead tetraacetate (LTA), achieving aldehyde **19**. In the ^{1}H NMR spectrum of **19** can be observed a signal at 9.76 ppm (1H, t, *J* = 1.8 Hz, H-9') characteristic of a formyl group.

Preparation of the hydroxiderivative 20

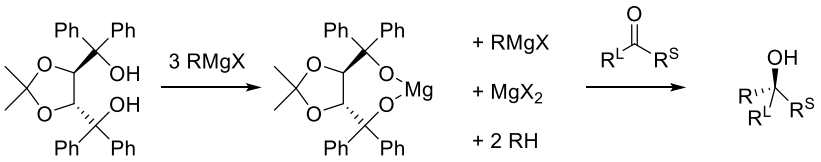
Reaction of **19** with nonylmagnesium bromide in presence of an asymmetric inductor as (-)-TADDOL is produced estereoselectively and leads to **20** with configuration 9'*R* (Scheme 5).



Scheme 5

In the ^{13}C NMR of **20,** a signal at 72.2 ppm can be observed corresponding to the geminal carbon to the secondary hydroxyl group (C9'). In the mass spectrum of **20** appears a molecular ion of 639.4394 *m/z* corresponding to the formula $C_{41}H_{60}O_4Na$ ($M+Na^+$), which indicates the incorporation of 9 new carbons, forming, in this way, the C18 alkyl chain. The 9'*R* assignment of **20** is made considering that the chiral auxiliary used was (-)-TADDOL.

In reactions involving TADDOL as chiral auxiliary, the asymmetric addition is produced by a magnesium TADDOLate. This adduct is obtained following the procedure optimized by Seebach and coworkers.[3] In scheme 6, (-)-TADDOL is used to explain the addition process. R^L and R^S correspond to the larger and the smaller substituent respectively.



(-)-TADDOL

Scheme 6

When TADDOL is mixed with three equivalents of alkylmagnesium bromide, the two hydroxyls are first deprotonated with two of the three equivalents, thus forming the

chiral (-)-Mg-TADDOLate. Onces the carbonyk is added, asymmetric addition occurs. For said addition, Seebach and coworkers proposed three different mechanisms by which the addition of the alkyl group would take place through the Re side of the carbonyl in an asymmetric environment of a Mg-TADDOLate obtained from (-)-TADDOL (Figure 56).

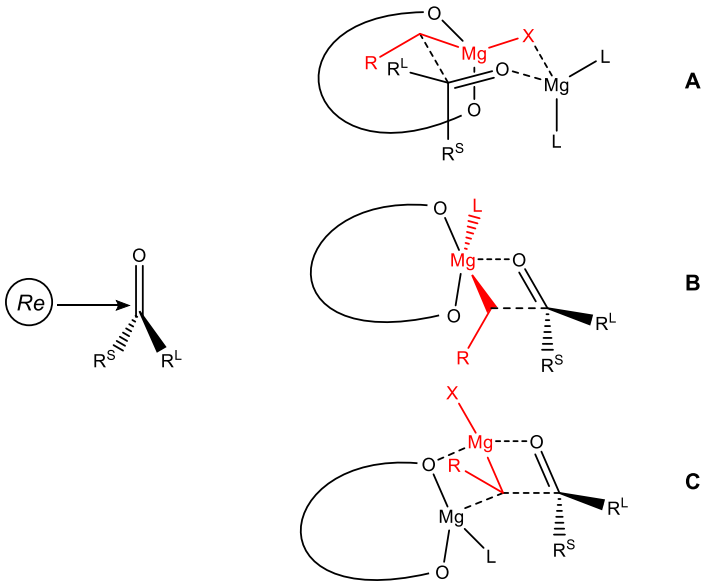


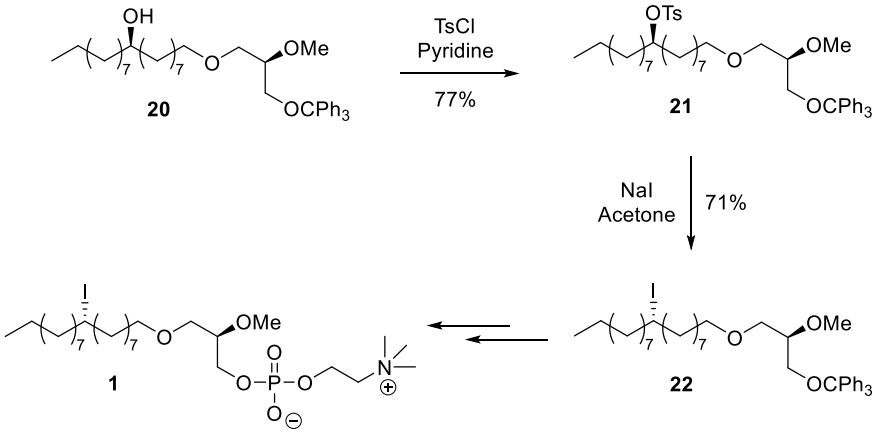
Figura 56

Mechanism A proposes the addition of the organometallic through an intermediate forming a 6-membered ring with two tetracoordinated magnesium centers. In mechanism B, it would pass through an adduct consisting of a 4-membered ring with the pentacoordinated magnesium atom forming a trigonal bipyramid. Finally, in mechanism C, the carbonyl would be attacked by an aggregate of Mg-TADDOLate with the Grignard reagent.

In all cases (Figure 56) it is observed that, when (-)-TADDOL is used, the carbonyl is attacked by the chiral alkylagnesium on the least hindered side, that is, the Re side.

Obtaining of the iodinated analog 1

Tosyl derivative **21** is formed by reaction of **20** with p-toluenesulfonyl chloride (Scheme 7), which without further purification is treated with sodium iodide, obtaining iododerivative **22**.

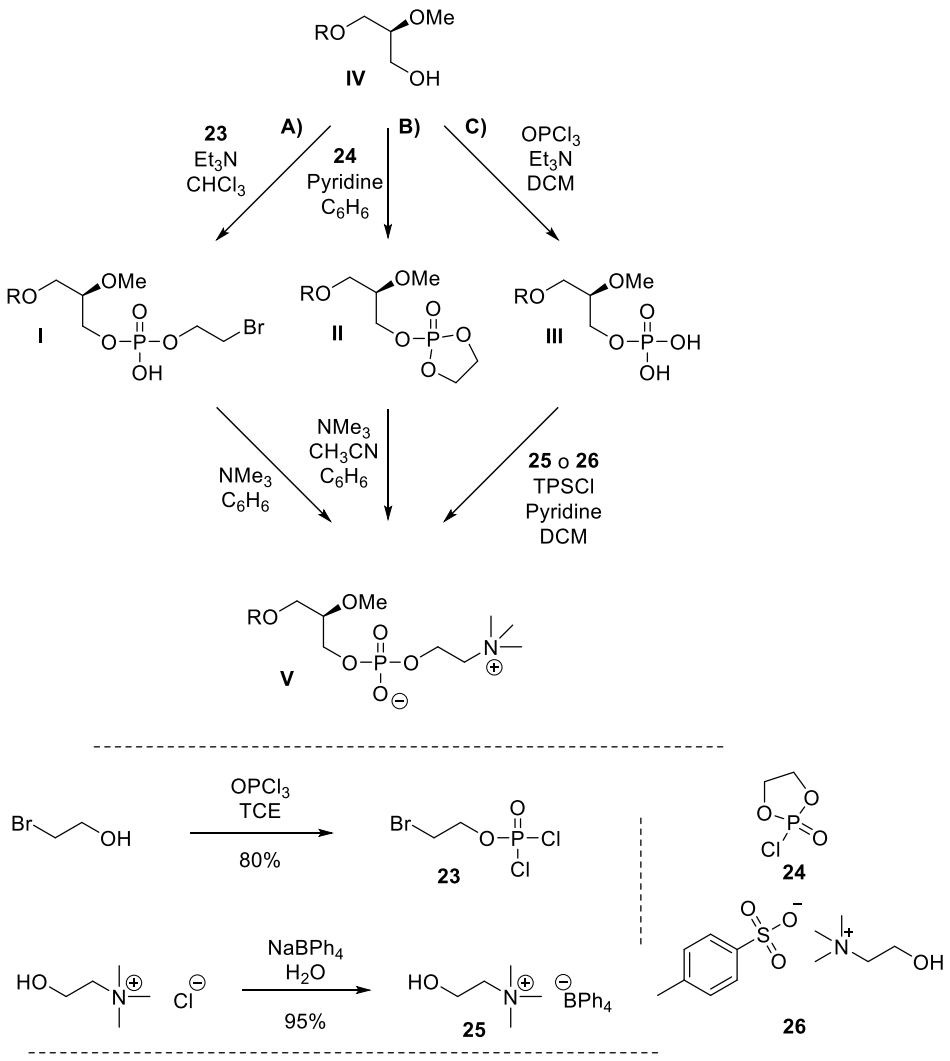


Scheme 7

In the ^{13}C NMR spectrum of **22** the signals 40.6 ppm (C-9 ') and 40.7 ppm (C-8' and 10 ') are observed corresponding to the geminal carbon to the iodine and the carbons of the neighboring methylenes respectively, which corroborates the structure.

To obtain the iodinated analog **1** from **22** it is necessary to deprotect the trityl group and then incorporate the phosphocholine unit at C3.

Phosphocholine can be introduced by a two-step sequence. Scheme 8 shows three procedures that allow this transformation to be carried out and that use 2-bromoethyl phosphate derivative **I** (Route A), phospholane **II** (Route B) or phosphatidic acid **III** (Route C) as intermediates.



Scheme 8

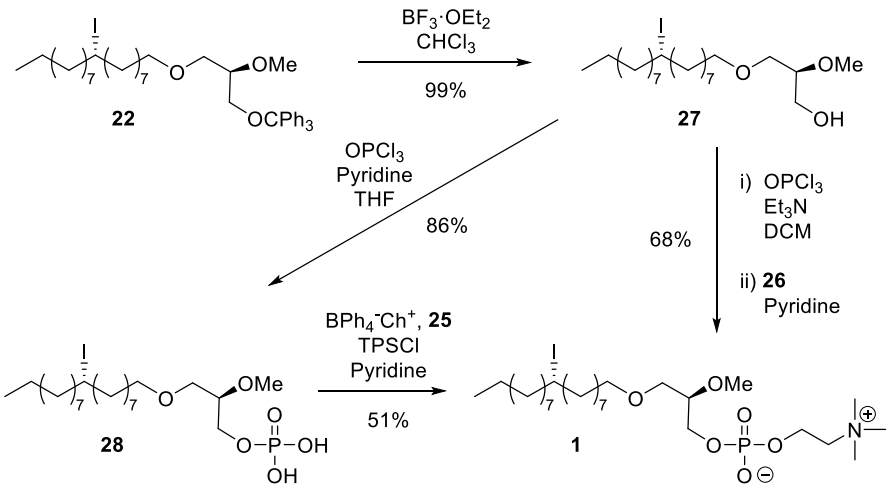
The formation of phosphatidylcholines by route A (Scheme 8) corresponds to the classical Berchtold methodology,[4] also used by Eibl and coworkers.[5] The 2-bromoethyl dichloro phosphate **23** needed is obtained *in situ* by reaction of 2-bromoethanol with freshly distilled phosphorus oxychloride ($OPCl_3$) using trichlorethylene (TCE) as solvent. Post-treatment of the hydroxyderivative **IV** with **23** provides intermediate **I**, which is mixed with trimethylamine to obtain phospholipid **V**.

Route B (Scheme 8) is similar to the previous one with the difference that **IV** reacts with commercial 2-chloro-2-oxa-1,3-dioxaphospholane **24**,[6, 7] obtaining intermediate **II**, which is treated with trimethylamine to obtain **V**.

Route C (Scheme 8) consists in the formation of phosphatidic acid **III** by reaction of **IV** with phosphorus oxychloride in the presence of triethylamine. Then, by treatment of **III** with choline tetraphenylborate ($BPh_4^-Ch^+$, **25**) in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl), the desired compound **V** is obtained.[8] The choline salt **25** is prepared by ion exchange, in which choline chloride reacts with sodium tetraphenylborate and then is purified by crystallization.

There is also a variant of this methodology, optimized by Pedersen and coworkers,[9] using triethylamine as a base and choline tosylate as the choline source (TsO^-Ch^+, **26**). So, in this manner, the reaction could be carried out in a one-pot procedure, with no need to isolate neither intermediate **III** nor the use of TPSCl.

By reaction of **22** with boron trifluoride etherate, (Scheme 9) C3 deprotection is achieved; obtaining **27**. In the mass spectrum, a molecular ion of 749.3391 *m/z* appears corresponding to the molecular formula $C_{41}H_{59}O_3INa$ ($M+Na^+$).



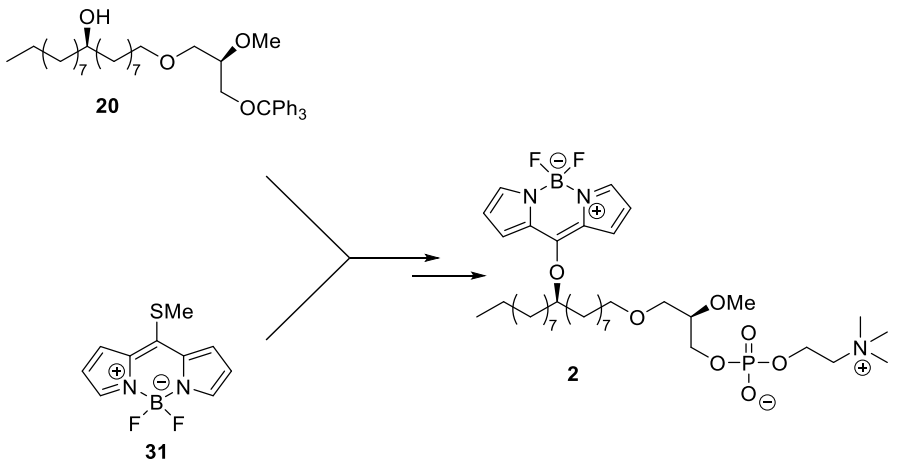
Scheme 9

By treating **27** with phosphorous oxychloride, phosphatidic acid **28** is obtained. By heating **28** with freshly prepared choline tetraphenylborate **25**, the iodinated analog of edelfosine **1** is obtained (44% from **27**).

A similar methodology allows direct access from **27** to **1**, without the need to isolate **28** (Scheme 9). The treatment of **27** with phosphorus oxychloride in the presence of triethylamine, and the subsequent addition of choline tosylate in pyridine, provides **1**. In this way, an improvement of the yield is achieved (68%).

7.2. Synthesis of fluorescent edelfosine 2

To carry out the synthesis of **2** (Scheme 10), first BODIPY **31** will be prepared and then its incorporation into the intermediate **20,** synthesized above, will be described.



Scheme 10

In this way, it is intended to obtain an edelfosine analog with a fluorescent tag that confers chemiluminescent properties that can be useful for the traceability of the drug *in vivo*.

In order to a better understanding of the synthesis of **2**, it will be divided in two sections:

1.2.1. Synthesis of BODIPY **31**.

1.2.2. Reaction of BODIPY **31** with hydroxyderivative **20**. Synthesis of **2**.

Synthesis of BODIPY 31

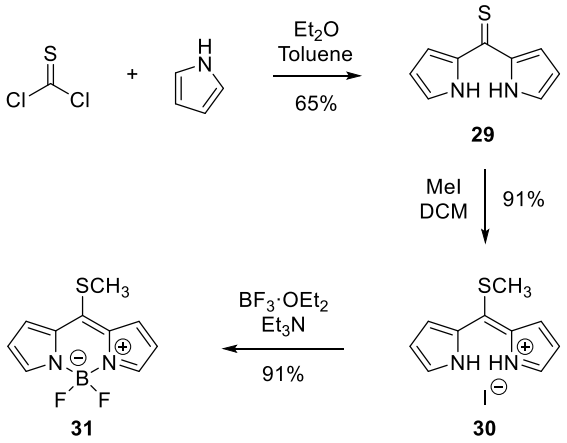
Fluorescence technologies such as imaging fluorescence and detection fluorescence represent powerful tools in different scientific fields, especially in Medicine or Biochemistry.[10]

Fluorescence-based intravital imaging methods have been essential for studying the function and distribution of small biologically active molecules and their molecular targets *in vivo*. Now "tags", fluorophores, can be attached to practically any biomolecule of interest, such as proteins, for example to antibodies, which accumulate in specific organs, obtaining images in animals and humans.

BODIPY (4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene) represents a class of small fluorescent molecules that has recently been widely recognized for its extraordinary versatility as a fluorescent marker in biological imaging applications. An advantage of BODIPY over other commonly used fluorescent agents is the neutral nature of its skeleton, which makes its penetration into the cell membrane excellent. Thus, BODIPYs are one of the few types of fluorophores with which intracellular images can be achieved in living cells.

Photodynamic therapy (PDT) is an alternative method for treatment of cancer. That technique needs photosensitizers that are able to generate ROS (Reactive Oxygen Species), which can destroy selectively and efficiently tumour cells. BODIPY dyes have been proven to show excellent results as photosensitizers in PDT both *in vitro* and *in vivo*, so they are a great group of compounds to be applicable in this new therapy.[11-13]

Among the many known derivatives of BODIPY,[14] in this first synthesis that we carried out of edelfosine analogs labeled with fluorophores, 8-(methyl-thio)-BODIPY **31** has been chosen due to its photophysical properties (good quantum yield and adequate emission λ_{max}).[15] The synthesis of **31** was carried out starting from pyrrole and thiophosgene[16] (Scheme 11).



Scheme 11

Thioketone **29** is obtained by reaction of pyrrole with thiophosgene. By treatment of **29** with methyl iodide in DCM, sulfur methylation is achieved, obtaining **30**. In the ^{1}H NMR spectrum of **30**, a signal is observed at 2.89 ppm corresponding to the hydrogens of methyl attached to sulfur.

By reaction of **30** with boron trifluoride etherate, BODIPY **31** is obtained. In the mass spectrum of **31**, a molecular ion of 239.0620 *m/z* appears, corresponding to the molecular formula $C_{10}H_{10}BN_2F_2S$ ($M+H^+$).

Reaction of BODIPY 31 with the hydroxyderivative 20. Synthesis of 2

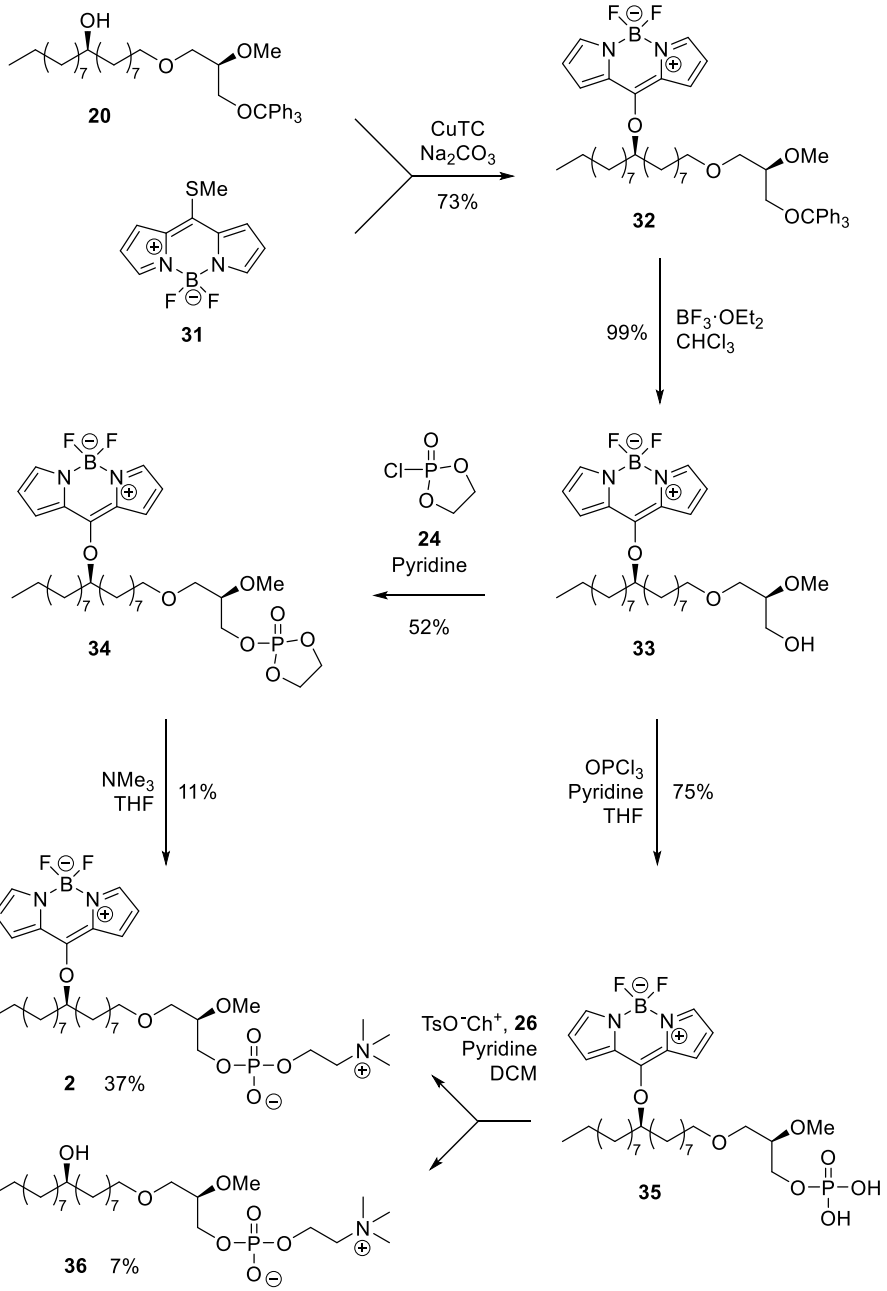
By reaction of the hydroxyderivative **20** with **31** in the presence of CuTC (copper (I) thiophene-2-carboxylate),[17] binding of the BODIPY to the alkyl chain is achieved, obtaining **32** (Scheme 12). In its mass spectrum, a molecular ion of 829.4898 *m/z* corresponding to the formula: $C_{50}H_{65}BN_2O_4F_2Na$ ($M+Na^+$) is observed, which corroborates the incorporation of the BODIPY unit into the structure.

The deprotection of **32** is carried out by reaction with $BF_3{\cdot}Et_2O$, obtaining **33** in good yield. In this case, two tests were carried out: the synthesis of **2** through the formation of an intermediate dioxaphospholane **34**, and the synthesis of **2** through the formation of phosphatidic acid **35**.

Reaction of **33** with commercial compound **24** in the presence of pyridine gives intermediate **34**, which was purified and mixed with trimethylamine in a sealed tube. Thus, analog **2** was obtained in low yield (6% from **33**). In the ^{1}H NMR spectrum of **2**, the quintuplet signal corresponding to H9' appears deshielded at 5.14 ppm, which, together with the signals of the hydrogens of the BODIPY fragment 7.70 (H-3'' and 5''), 7.27 (H-1'' and 7'') and 6.51 ppm (H-2'' and 6'') and those of the choline fragment 4.31 (H-1'''), 3.92-3.84 (H-3 and 2''') and 3.37 ppm (NMe_3), corroborate its structure as an edelfosine analog.

The reaction of **33** with recently distilled phosphorus oxychloride in pyridine leads to phosphatidic acid **35**. The introduction of the choline unit was carried out by reaction of **35** with **26** for 2 days, obtaining **2** with better yield (27% from **33**) than with the previous methodology. In addition, a secondary product **36** (7% yield) was obtained, corresponding to an edelfosine analog with hydroxyl at C9'.

In the ^{1}H NMR spectrum of **36**, hydrogen geminal to the hydroxyl group appears at 3.57 ppm, which is corroborated in ^{13}C NMR with a CH signal at 71.7 ppm. The mass spectrum shows a molecular ion at 540.4017 *m/z* corresponding to the formula $C_{27}H_{59}O_7NP$ ($M+H^+$).



Scheme 12

8. Synthesis of C16' substituted edelfosine analogs

In this section, the synthesis of edelfosine analogs **3**, **4**, **5** and **7** with oxygen function at the end of the alkyl chain and a synthetic approach to **6** with iodine in said position will be described (Figure 57).

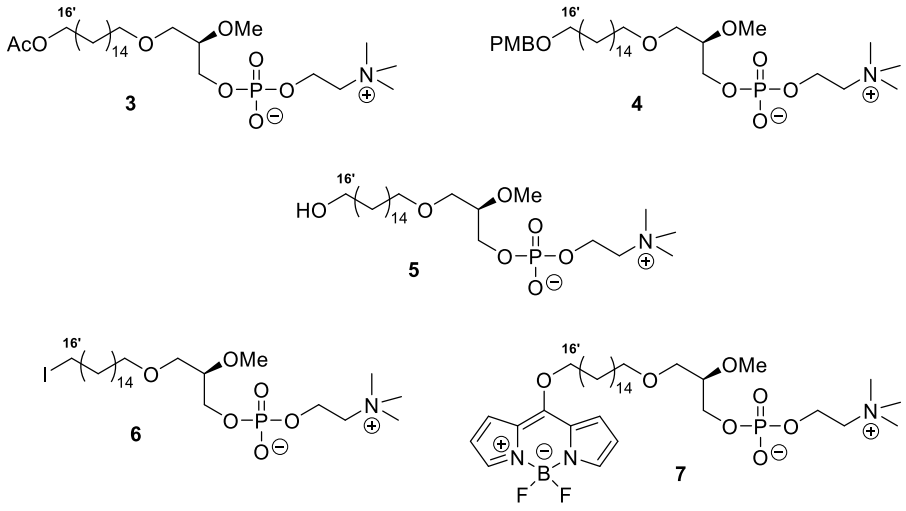
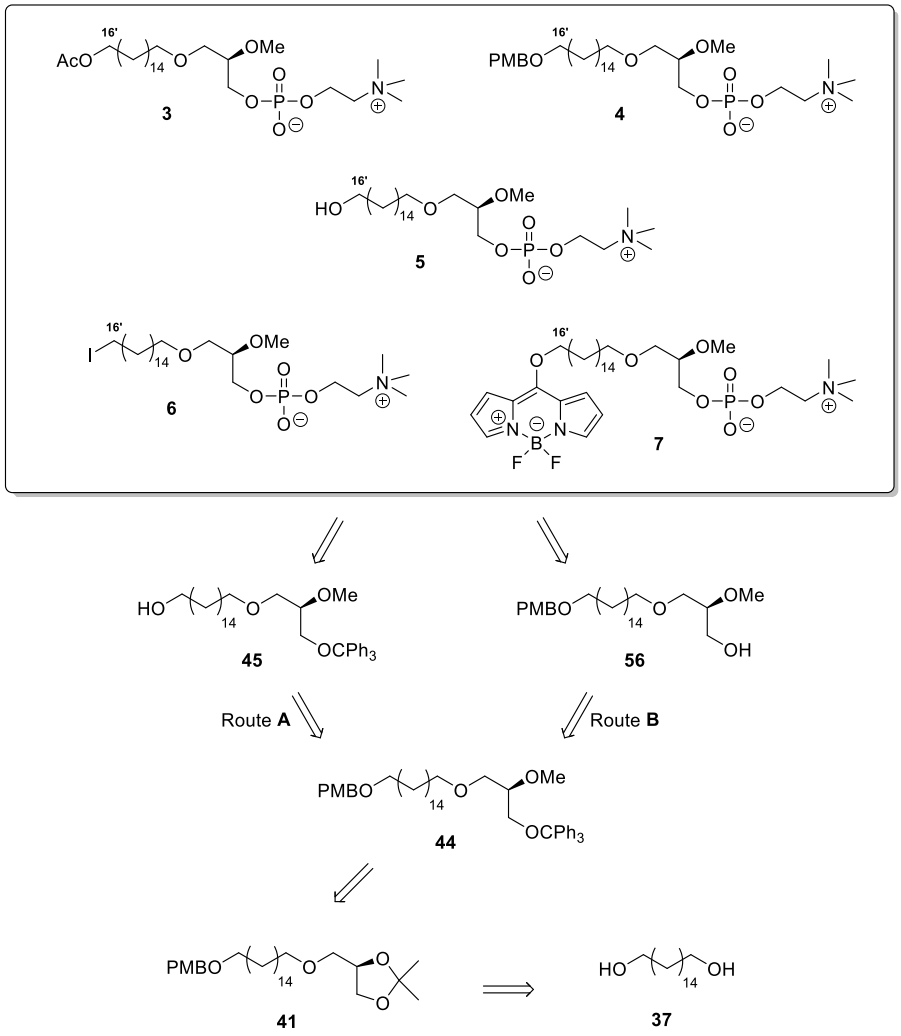


Figure 57

The general synthetic route proposed for obtaining edelfosine analogs with different functions at C16´ (**3**, **4**, **5**, **6** and **7**) is developed according to the following retrosynthetic scheme (Scheme 13).



Scheme 13

The final compounds can be accessed by two routes, both of which pass through key intermediate **44**.

From **44**, the synthesis of the proposed edelfosine analogs can be approached by developing two different synthetic strategies. Said strategies consist of: first elaborating the functionalization required at the end of the chain, at C16', to finally introduce the polar head (Route A); or else, first incorporate the phosphocholine unit and then properly functionalize C16' (Route B).

From **44**, **45** and **56** can be accessed by selective deprotections at C16' or C3 respectively (Scheme 13). Compound **44** can be reached from intermediate **41** by hydrolysis of the acetonide in an acidic medium and subsequent selective protection of each of the hydroxyl groups. Since no difference has been found in the activity of edelfosine and that of its analog that has a 16-carbon alkyl chain in C1, the commercial compound 1,16-hexadecanediol **37** will be used as starting material.

Obtaining the desired edelfosine analogs (**3**, **4**, **5**, **6** and **7**) will be approached taking into account the following sections:

2.1. Synthesis of key intermediate **44**

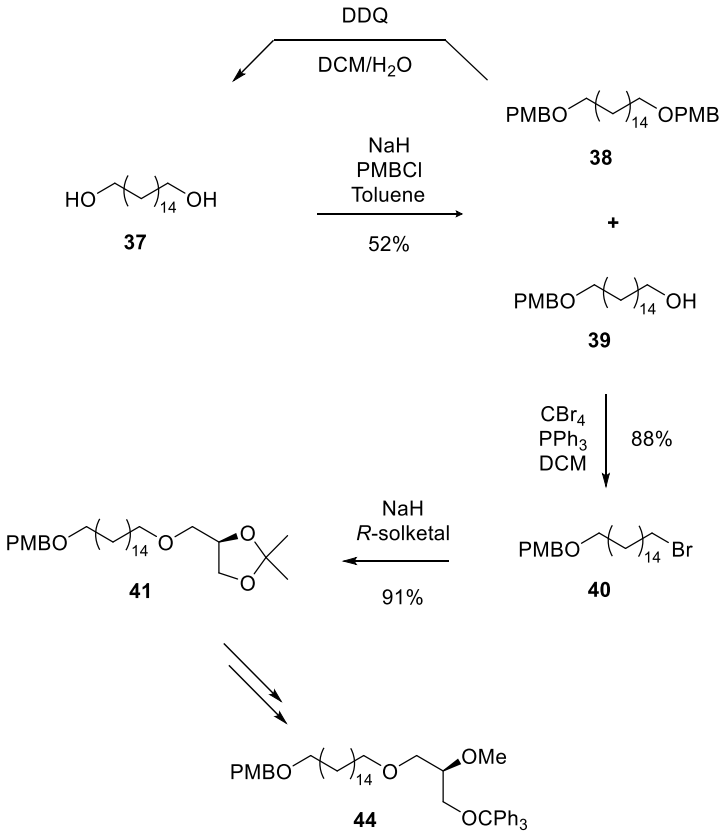
2.2. Route A: edelfosine analogs obtaining by using **45** as intermediate

2.3. Route B: edelfosine analogs obtaining by using **56** as intermediate

In the following paragraphs, the development of each of the aforementioned sections is described.

8.1. Synthesis of key intermediate 44

For the synthesis of **44** (Scheme 14), the commercial product 1,16-hexadecanediol **37** is used as starting material.



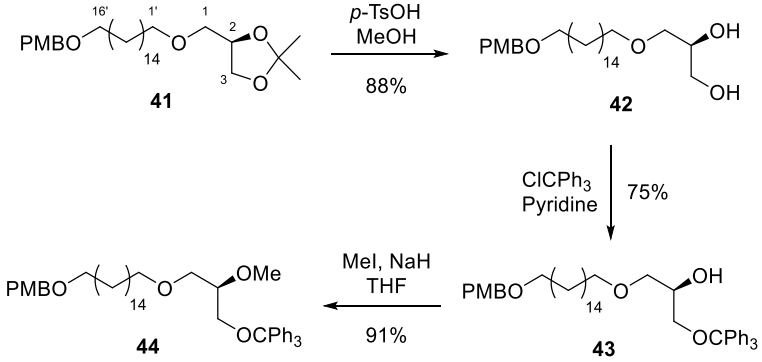
Scheme 14

The equimolar reaction of **37** with 4-methoxybenzyl chloride (PMBCl) in the presence of an excess of a strong base such as NaH, provides a mixture of **38**, **39** and the untransformed product **37**. Major compound **39** is identified as the desired monoprotection product, since in its mass spectrum it presents a molecular ion of 401.3010 *m/z* corresponding to a formula $C_{24}H_{42}O_3Na$, and that in its ^1H NMR spectrum

it presents at 3.64 and 3.43 two triplet signals corresponding to geminal hydrogens to a hydroxyl group and ether respectively. Compound **38** was identified as the diprotected product, which can be recovered by reaction with DDQ.

Bromoderivative **40** is obtained by reaction of **39** with carbon tetrabromide in the presence of triphenylphosphine (Scheme 14). In the ^{13}C NMR spectrum a signal appears at 34.1 ppm corresponding to the geminal carbon to the bromine. Incorporation of the glycerol fragment into **40** is achieved by reaction with *R*-solketal in the presence of NaH, obtaining **41** with good yield (Scheme 14).

To obtain **44** from **41**, selective deprotection of the acetonide group is necessary in order to gain access to the C2 and C3 hydroxyls (Scheme 15).



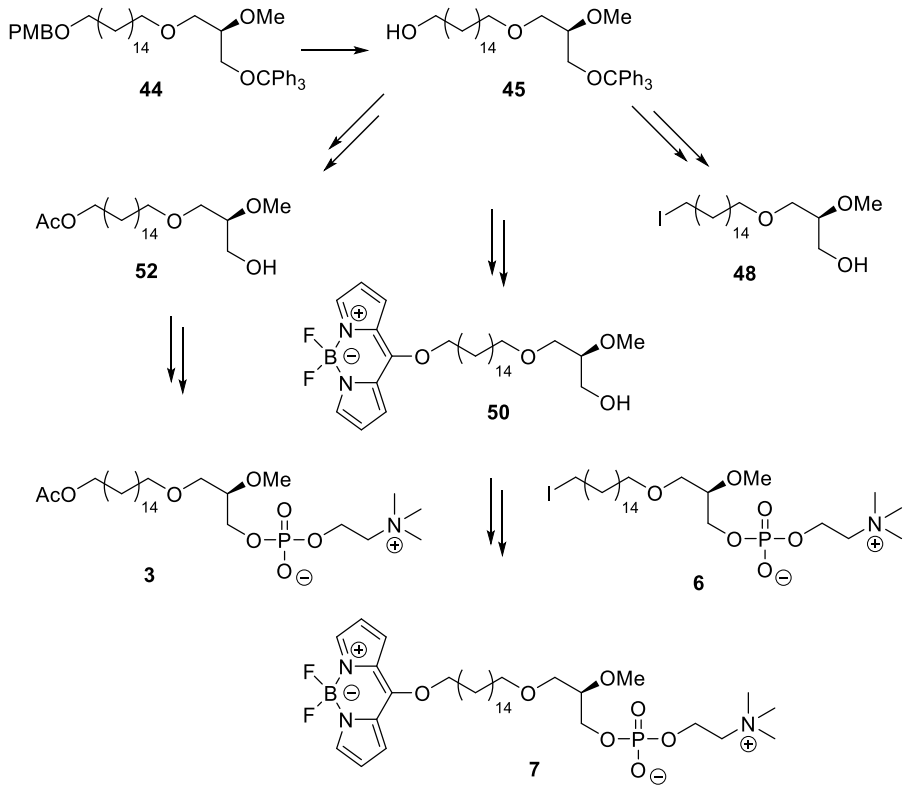
Scheme 15

The diol **42** is obtained with excellent yield by reaction of **41** with *p*-toluenesulfonic acid (*p*-TsOH) in methanol at room temperature and monitoring the progress of the reaction by TLC.

The installation of the methoxyl group on the C2 present in the final product derived from edelphosine, makes necessary the previous selective protection on the hydroxyl of C3. Reaction of **42** with trityl chloride in pyridine heating to 100 ^{o}C leads to compound **43** in good yield. Subsequently, the methyl group is introduced at C2 by reaction of **43** with sodium hydride and posterior addition of methyl iodide. Thus, the key intermediate **44** is obtained with excellent yield.

8.2. Route A: edelfosine analogs obtaining by using 45 as intermediate

In this route, from **44,** and using **45** as an intermediate, a procedure is used in which the alkyl chain at C16' is functionalized first, giving access to derivatives **48**, **50** and **52**, to later incorporate the polar head of phosphocholine and obtain the aforementioned analogs **3**, **6** and **7** (Scheme 16).



Scheme 16

In order to develop it correctly, the following sections will be described below:

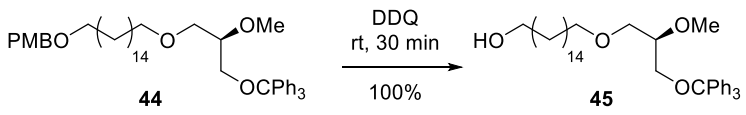
2.2.1. Selective deprotection of PMB group. Synthesis of **45**.

2.2.2. Formation of iodinated **48**, fluorescent **50** and acetylated **52** intermediates.

2.2.3. Polar head incorporation.

Selective deprotection of PMB group. Synthesis of 45.

In order to access the C16' hydroxyl group, it is necessary to carry out a selective deprotection at **44**. By reaction of **44** with DDQ (Scheme 17), compound **45** is obtained.



Scheme 17

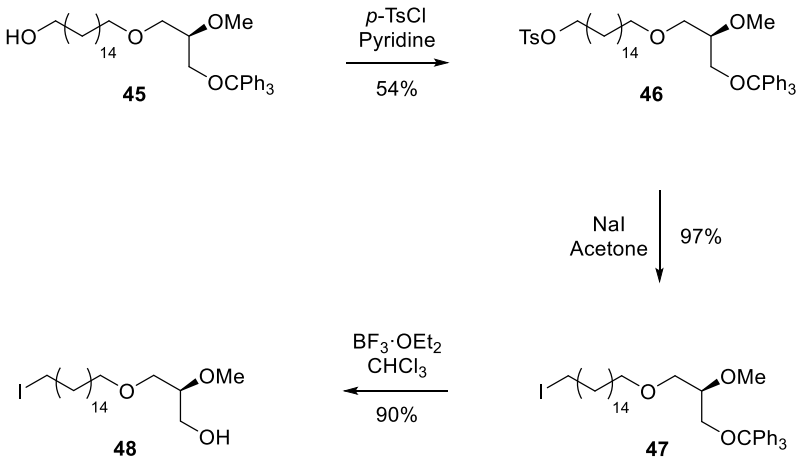
The reaction was carried out under different conditions as can be seen in table 2. Performing the reaction with 1 equivalent of DDQ, at room temperature for 30 minutes, controlling by TLC (Entry 4, Table 2), a quantitative yield is achieved.

Tabla 2. Conditions for selective deprotection of compound **44**

Entry	Tª	Time	DDQ Equivalents	45
1	t.a.	7 h	0.25	49%
2	t.a.	12 h	1.0	10%
3	t.a.	1.5 h	0.5	52%
4	t.a.	30 min	1.0	100%

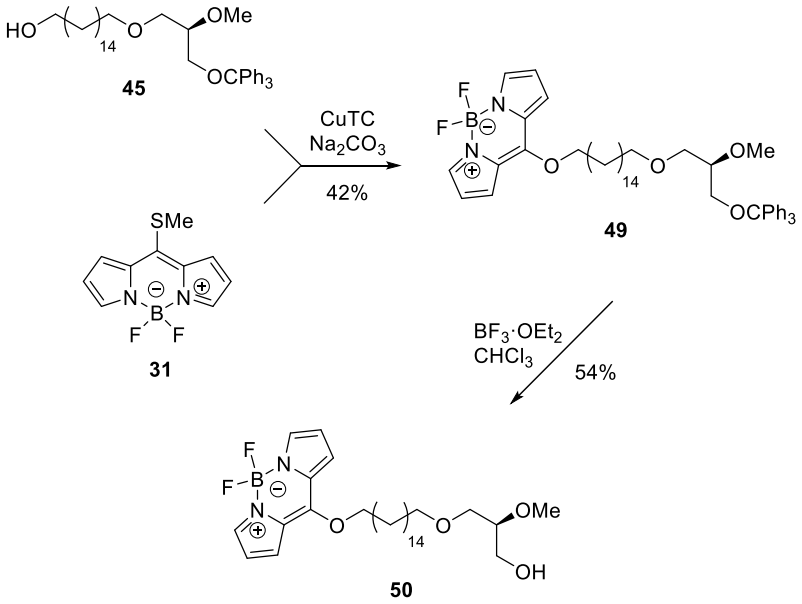
Formation of the intermediates 48, 50 and 52

To obtain the iodinated intermediate **48** (Scheme 18), **45** is treated with tosyl chloride (*p*-TsCl) in pyridine, thus obtaining **46**. By mixing **46** with sodium iodide in acetone, intermediate **47** is obtained with quantitative yield. In the ^{13}C NMR spectrum of **47** a signal appears at 7.3 ppm corresponding to C16' hydrogens. The deprotection of the trityl group of **47** is carried out with boron trifluoride in chloroform and low temperature, obtaining **48** with excellent yield.



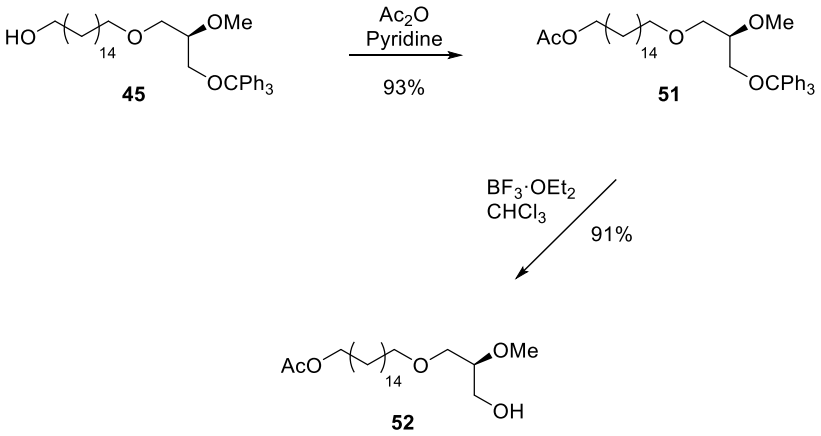
Scheme 18

Preparation of **50** (Scheme 19) is achieved using the same methodology as in the formation of **2**, edelfosine analog with the fluorophore in the middle of the chain. By reaction of intermediate **45** with BODIPY **31** in the presence of copper (I) thiophene-2-carboxylate and in a basic medium, **49** is obtained. In the ^{1}H NMR spectrum of **49**, the hydrogens on C16' appear deshielded at 4.66 ppm (2H, t) with respect to those of **45**, which appear at 3.60 ppm. By reaction of **49** with boron trifluoride the C3 position is deprotected, yielding **50**. In the ^{1}H NMR spectrum the presence of an ABX system (3.76 ppm, dd; 3.65 ppm, dd) corresponding to the geminal hydrogens to the primary hydroxyl in C3 is observed.



Scheme 19

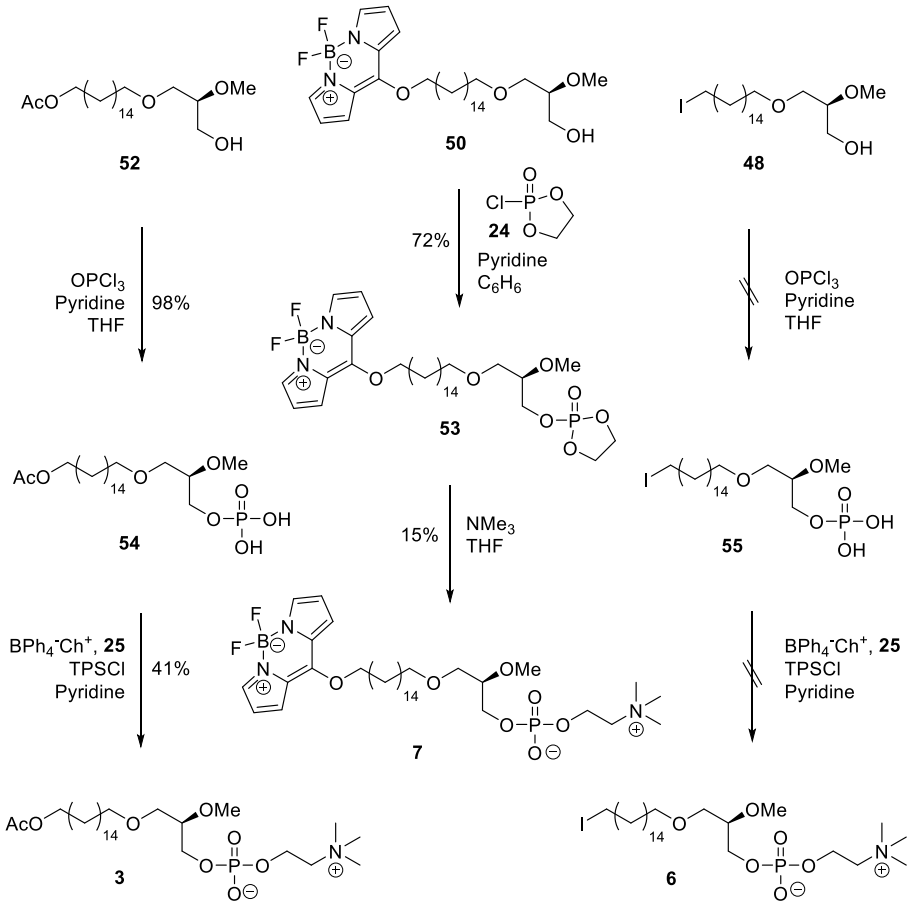
To obtain **52** (Scheme 20), **45** is treated with Ac_2O in pyridine, quantitatively obtaining the acetyl derivative **51**, which by treatment with boron trifluoride leads to **52** in good yield.



Scheme 20

Polar head incorporation

Finally, a phosphocholine unit needs to be incorporated into each of the synthesized intermediates, **48**, **50** and **52** (Scheme 21).



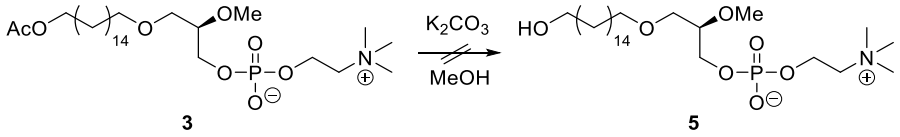
Scheme 21

By reaction of **50** with 2-chloro-2-oxa-1,3-dioxaphospholane **24** in the presence of pyridine (Scheme 21), intermediate **53** is obtained in good yield. By subsequent reaction of **53** with trimethylamine heating in a closed tube, the fluorescent derivative of edelfosine **7** is obtained, with low yield.

For the synthesis of **3** (Scheme 21), phosphorylation of **52** was first carried out with recently distilled phosphorus oxychloride, obtaining phosphatidic acid **54**. The reaction of **54** with $BPh_4^-Ch^+$ **25** in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) leads to **3** in good yield.

Following with **48**, a procedure similar to that carried out with **52** (Scheme 21) was performed. That is, by reaction of **48** with phosphorus oxychloride to obtain phosphatidic acid **55**, followed by treatment with $BPh_4^-Ch^+$ **25**, the expected analog, iododerivative **6,** was not achieved. In the future it will be necessary to prepare more quantity of **48** to test other methodologies of incorporation of the phosphocholine unit.

Once the acetylated edelfosine **3** had been obtained, an attempt was made to deprotect the C16' hydroxyl by treatment in a basic medium of potassium carbonate to achieve compound **5** (Scheme 22). However, satisfactory reaction conditions were not achieved, so that **5** could not be obtained by this route.



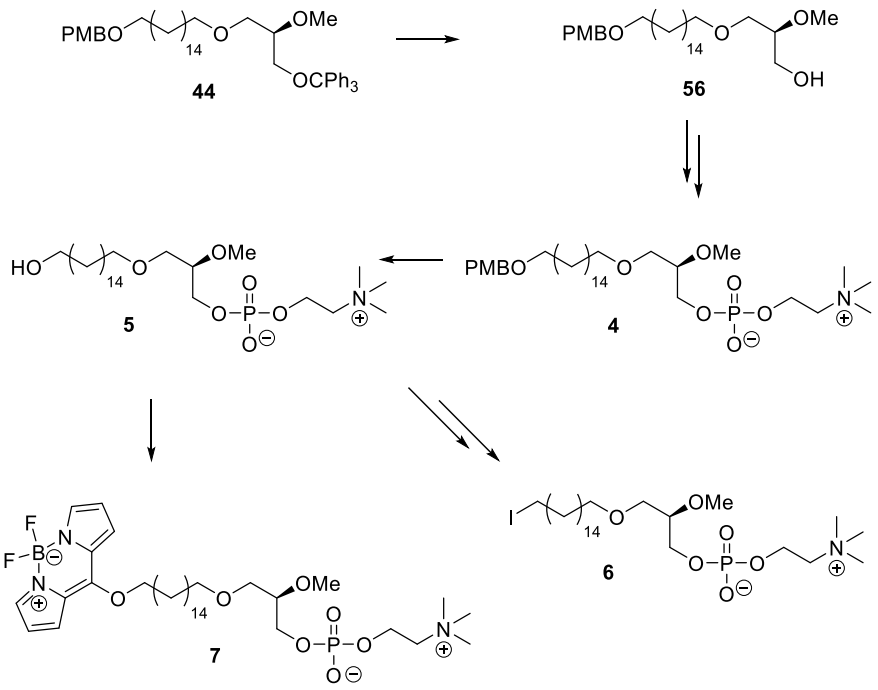
3 **5**

Scheme 22

Since this route did not give the expected results, it was thought to test the alternative route (Route B) of formation of edelfosine analogs, which was already mentioned above.

8.3. Route B: edelfosine analogs obtaining by using 56 as intermediate

In this route, a new way of obtaining edelfosine analogs is studied in which, starting at **44**, the hydroxyl group of C3 is deprotected first (Scheme 23) to incorporate the phosphocholine unit and then the function of C16' is modified, using **56** as an intermediate.



Scheme 23

This section will be developed dividing it into two parts.

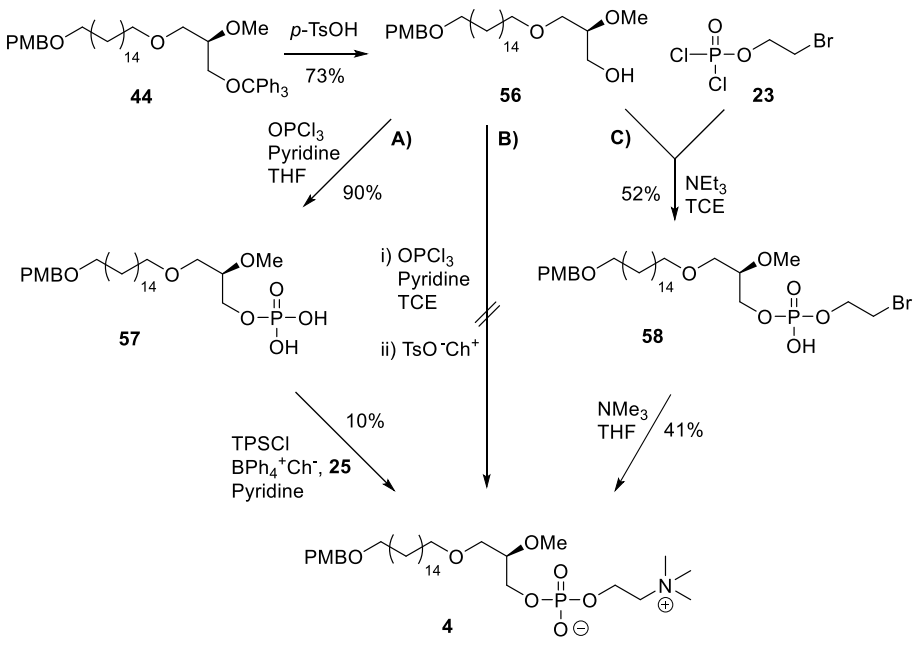
2.3.1. Synthesis of edelfosine analog **4**

2.3.2. C16' function modification

Synthesis of edelfosine analog 4

In order to access analog **4** from **44** it is necessary to selectively deprotect the C3 hydroxyl group of the glycerol fragment, in the presence of the 4-methoxybenzyl group.

In this case, the deprotection reaction of the trityl group of **44** was carried out with *p*-TsOH,[18] obtaining **56** with excellent yield (Scheme 24). In the mass spectrum of **56**, the molecular ion appears at 489.3538 *m/z* corresponding to a formula $C_{28}H_{50}O_5Na$, which corroborates the loss of the trityl group.



Scheme 24

Reaction of **56** (Route A, Scheme 24) with freshly distilled phosphorus oxychloride in pyridine leads to phosphatidic acid **57** in very good yield. However, subsequent reaction of **57** with choline tetraphenylborate in the presence of TPSCl provides **4** in only 10% yield. The reaction was tested under different conditions and using different choline salts, failing to improve the yield.

When the reaction is carried out from **56** with $OPCl_3$ in pyridine, followed by treatment with choline tosylate (Route B, Scheme 24), that is, without isolating the intermediate phosphatidic acid, the desired result was not achieved.

The route that gave the most satisfactory and reproducible result was route C (Scheme 24). This route consists of the reaction of 2-bromoethyl dichloro phosphate, **23**, with the hydroxyderivative **56**, forming compound **58**.

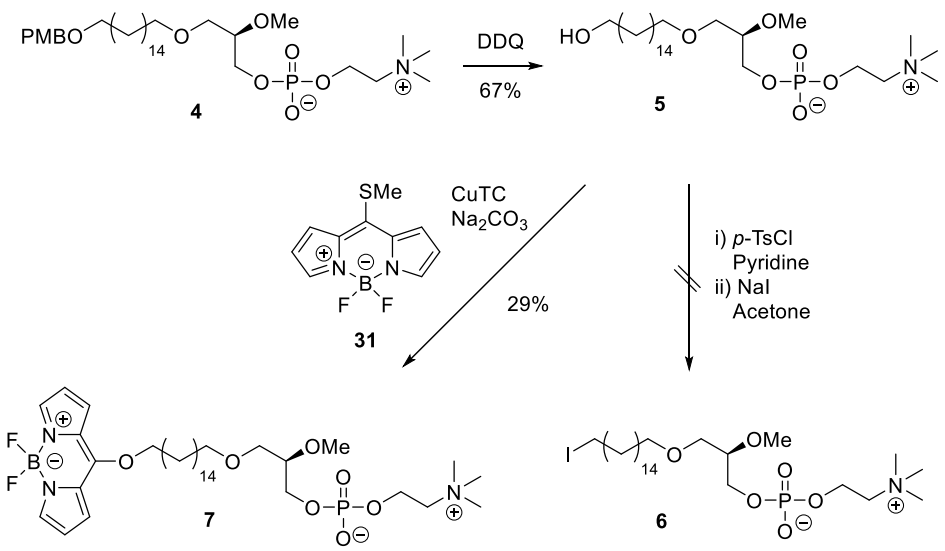
Reaction of bromoderivative **58** with trimethylamine dissolved in THF leads to the desired phosphatidylcholine **4** in good yield. In the ^{13}C NMR spectrum of **4,** the doublet signals, due to a coupling with phosphorus, of the carbons corresponding to C2, C2''', C3 and C1''' appear at 79.4, 66.2, 65.0 and 59.3 ppm.

C16' function modification

Once the edelfosine analog **4** has been made, the C16' function can be modified in a number of ways.

The first thing to consider is the solubility of phosphatidylcholine. In our experience working with these substrates, it was necessary to use a suitable solvent mixture ($CHCl_3$/MeOH/H_2O 65:30:5) to achieve solubilization of this type of compounds with a surfactant character. However, in this case, having the C16' hydroxyl protected with PMB, makes this compound quite lipophilic and can be dissolved in relatively nonpolar solvents, such as $CHCl_3$.

For the deprotection of **4**, DDQ was used as in previous cases, obtaining the hydroxyderivative analog **5** (Scheme 25).



Scheme 25

Compound **5**, with the free hydroxyl group, was problematic to handle due to its solubility as it almost totally loses lipophilic character. In the first reaction carried out, the work-up was performed as described in the literature; that is, adding an aqueous sodium bicarbonate solution to the reaction mixture and extracting with DCM. However, the desired compound does not appear in the ^{1}H NMR spectrum of the reaction crude. This shows that the objective compound **5** was much more hydrophilic than originally expected and, therefore, the extraction mother liquor was chromatographed on silica gel. Eluting with $CHCl_3$/MeOH/H_2O 60:40:5, a small percentage of **5** is isolated.

When the reaction is carried out in the same way, also controlling with TLC, and, once the reaction is over, the reaction mixture is chromatographed directly on silica gel packed with $CHCl_3$/MeOH 9:1, it is possible to obtain **5** in a good yield (67%).

From **5**, it was proposed to synthesize compounds by incorporating an iodine atom, **6**, at the end of the chain, or with the fluorescent label BODIPY, **7**.

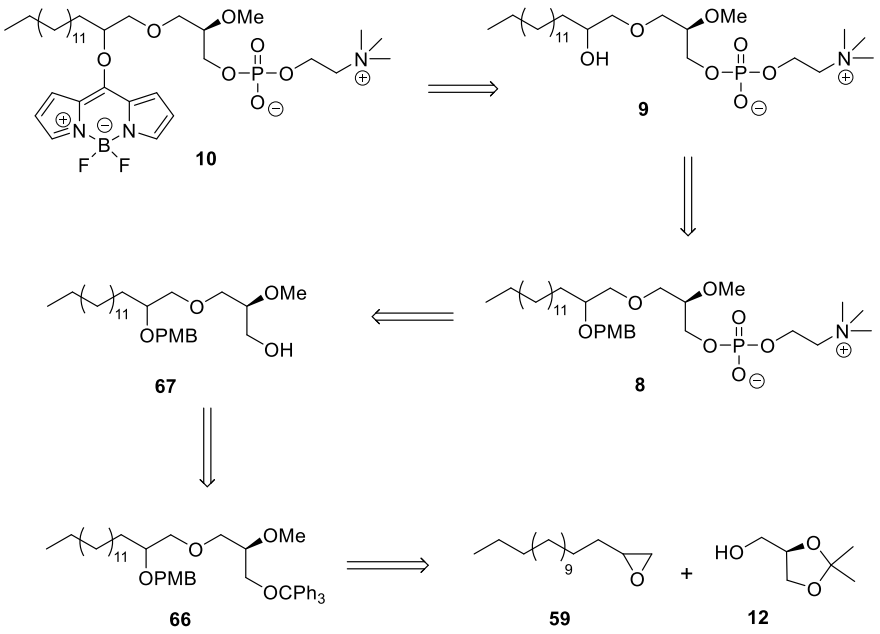
First, the synthesis of **6** was attempted. For this, it is necessary to form the tosylate, and then replace it with iodine. As the solubility of this type of compound gives problems, it was decided to try the reaction without isolating the intermediate tosylate (Scheme 25), with unsatisfactory results. The lack of starting material prevented further testing. Therefore, this compound will be tried to obtain in the future.

Synthesis of edelfosine analog **7** with the fluorophore at the end of the chain is carried out by reacting **5** with BODIPY **31** in a CH_3CN/Toluene 2: 1 solution. In this way it is possible to obtain **7** with a yield of 29%. In the mass spectrum of **7**, a molecular ion of 701.4257 *m/z* appears corresponding to a formula $C_{34}H_{60}O_7N_3BF_2P$.

9. Synthesis of C2' substituted edelfosine analogs

The proposed route to obtain C2' functionalized edelfosine analogs **8**, **9** and **10** is similar to the previous one and is developed according to the retrosynthetic scheme (Scheme 26).

In a similar way to C16' functionalized derivatives, the edelfosine analogs at C2' could be reached in two ways, taking into account the versatility of intermediate **66**. However, given that in the previous section better results were achieved by route B, that is, first incorporating the polar head and then completing the functionalization in C2', in this case this methodology was only used by transforming compound **66** into the hydroxylated intermediate **67**.



Esquema 26

From **67**, the edelfosine derivative **8** can be accessed, which can be used as an intermediate in the synthesis of analogs **9** and **10**.

Compound **66** is available from 1,2-epoxyhexadecane **59** and R-solketal **12** starting materials.

In the following paragraphs, the obtaining of these edelfosine analogs will be addressed taking into account the following sections:

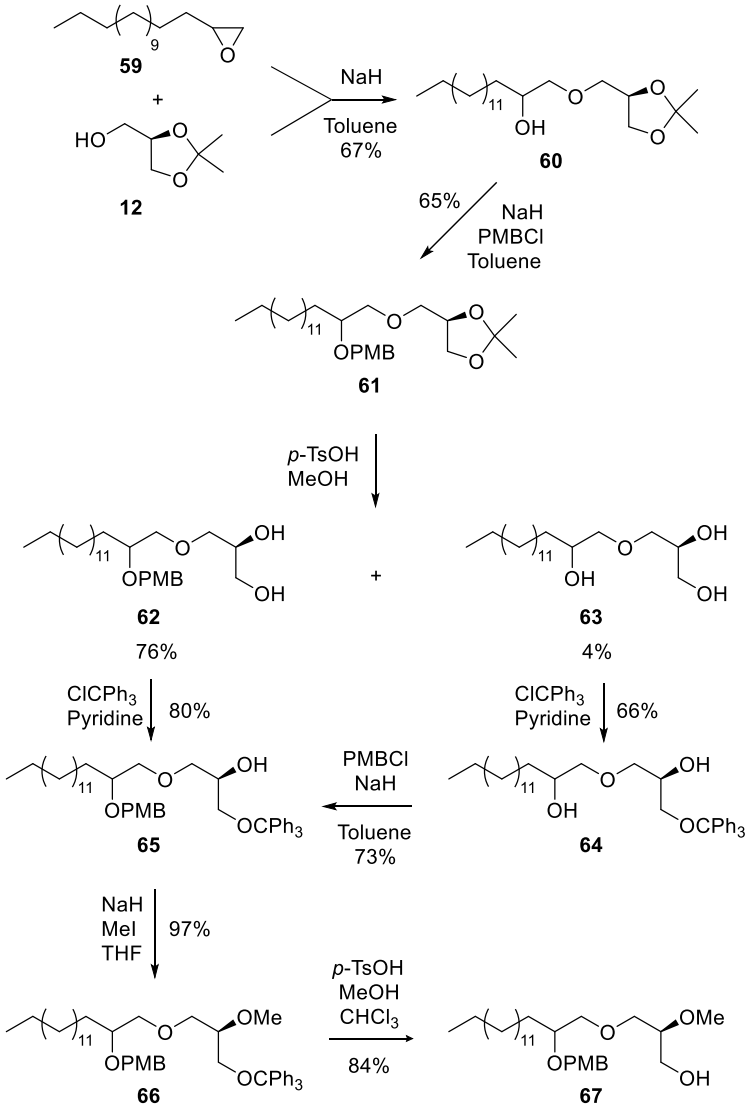
3.1 Intermediate **67** synthesis

3.2 Key analog **8** synthesis

3.3 Preparation of the edelfosine analogs **9** y **10**

9.1. Intermediate 67 synthesis

The synthesis of intermediate **67** is carried out in 6 steps, as can be seen in the following scheme (Scheme 27).



Scheme 27

In order to carry out an initial test of the activity of these new edelfosine analogs, their synthesis is carried out using commercial 1,2-epoxyhexadecane **59** as a starting material. To date, all the studies performed show that the use of an enantiomerically pure compound does not significantly improve the efficacy of edelfosine, therefore, the choice of this compound should not lead to negative results in terms of biological tests. In any case, the synthesis of each of the enantiomers can be carried out later, if their bioactivity is as good as expected.

Thus, by reaction of **59** with *R*-solketal **12** in the presence of sodium hydride, compound **60** can be accessed, which contains C2' function. Next, the hydroxyl is protected with PMB chloride, obtaining **61**. In the ^{1}H NMR spectrum of **61**, two signals are observed at 4.59 and 4.49 ppm, forming an AB system corresponding to the hydrogens of the benzyl carbon, due to the steric hindrance that prevents the free rotation of the CO bond, which makes benzylic hydrogens spectroscopically distinguishable.

To obtain diol **62**, different conditions were tested as shown in table 3.

Table 3. Optimization of the transformation of **61** into **62** with *p*-toluensulfonic acid in Methanol

Entry	Tª	Time	62	63
1	t.a.	12 h	12%	57%
2	40 ^{o}C	40 min	33%	25%
3	t.a.	1.5 h	26%	67%
4	t.a.	30 min	76%	4%

The best results (Entry 4) are achieved by performing the hydrolysis reaction with *p*-toluenesulfonic acid, in methanol at room temperature for 30 minutes. In this way, a good yield of **62** and a very low percentage of diprotected compound **63** are achieved.

Selective protection of the primary hydroxyl is achieved by reaction of **62** with trityl chloride in pyridine, obtaining **65** in good yield. Compound **65** can also be accessed

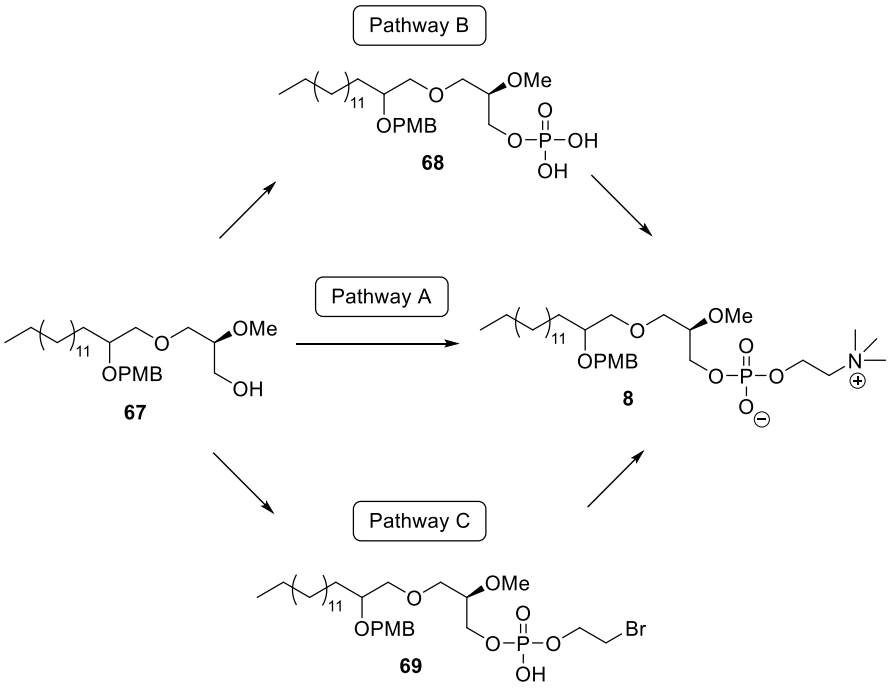
from triol **63** by regioselective protection with trityl chloride to obtain **64**, followed by reaction with PMBCl.

The alkylation of **65** is carried out by reaction with methyl iodide in the presence of a strong base, sodium hydride, obtaining the methoxy derivative **66** quantitatively.

The selective deprotection of the trityl group of **66** against the 4-methoxybenzyl group to obtain the hydroxy derivative **67** is carried out by a controlled reaction with *p*-toluenesulfonic acid using a MeOH/$CHCl_3$ 4:1 mixture as solvent to achieve solubilization of the starting compound **66**, due to the higher lipophilia of this compound compared to **61**.

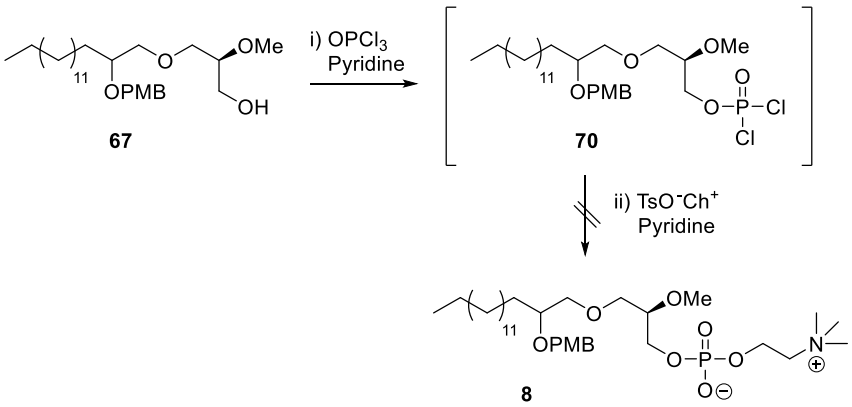
9.2. Key analog 8 synthesis

Once intermediate **67** has been obtained, it is necessary to introduce the phosphocholine unit to obtain edelfosine analogs. To achieve this objective, different routes were tested (Routes A-C, Scheme 28).



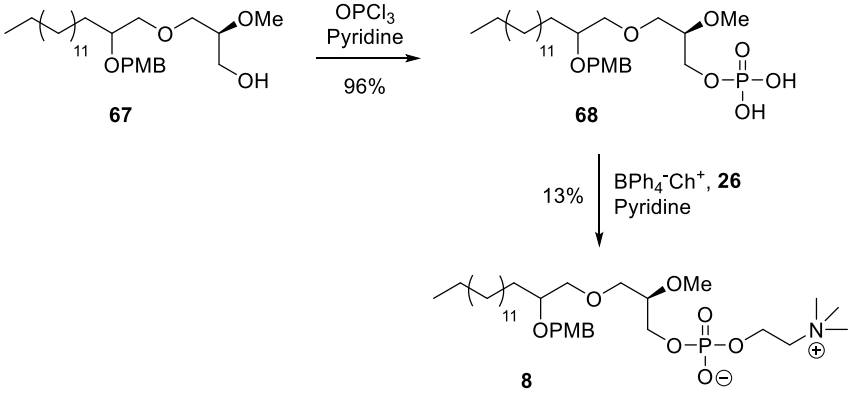
Scheme 28

The first one used was route A (Scheme 29), in which an attempt is made to incorporate the phosphocholine unit in a one-pot process. Thus, intermediate **67** is reacted first with phosphorous oxychloride, forming dichlorophosphate **70** which is not isolated, and then choline tosylate is added. Unfortunately, the desired compound **8** could not be obtained.



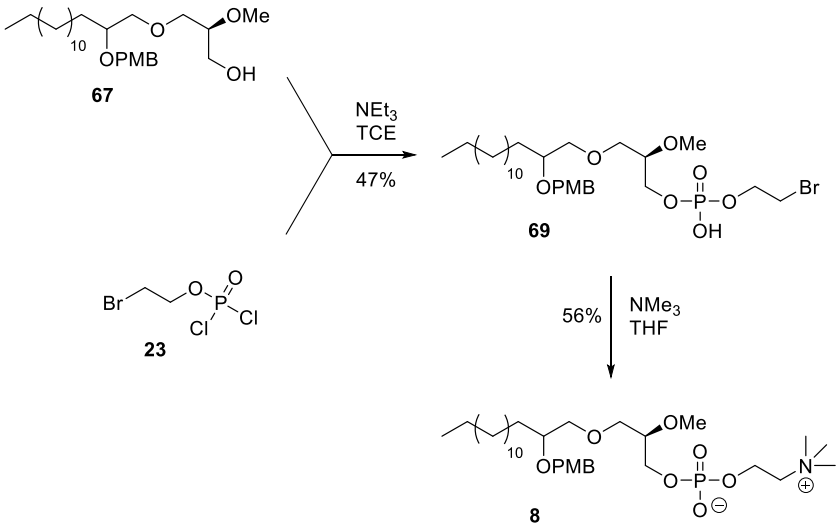
Scheme 29

Route B is then tested, in which phosphatidic acid **68** is first obtained, in good yield, by reaction of **67** with phosphorus oxychloride in pyridine (Scheme 30). Once this intermediate is obtained, it is mixed with 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) and choline tetraphenylborate **25**, obtaining analog **8** with 13% yield. Previously with other edelfosine analogs synthesized with this reaction, better yields have been achieved, for this reason it was decided to explore another methodology.



Scheme 30

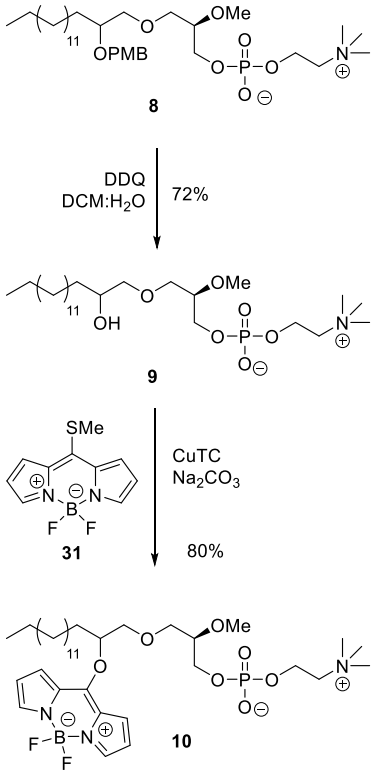
In route C (Scheme 31), hydroxyderivative **67** is mixed with 2-bromoethyl dichloro phosphate **23**, yielding compound **69**. Said compound is stirred with a solution of trimethylamine in THF for two days. After that time, purification by column chromatography is carried out and the edelosine analog **8** is separated with good yield.



Scheme 31

9.3. Preparation of the edelfosine analogs 9 and 10

Starting from **8**, it is intended to perform the synthesis of edelfosine analogs functionalized with heteroatoms **9** or fluorophores **10** at C2' (Scheme 32).



Scheme 32

By reaction of **8** with DDQ, the hydroxyderivative edelphosine analog **9** is obtained. The solubility problems mentioned above made it advisable to carry out the purification process by introducing the reaction mixture directly into the chromatography column packed in silica gel. In this way **9** is separated with a very good yield of 72%.

The reaction of **9** with BODIPY **31** in the presence of CuTC and basic medium leads to the fluorescent analog **10**. In the mass spectrum of **10** a molecular ion is observed at 701.4274 *m/z* corresponding to a molecular formula $C_{34}H_{60}O_7N_3BF_2P$ ($M+H^+$).

10.Fluorescence study

Due to the fact that some compounds with suitable optical properties have been prepared, a qualitative study of BODIPY **31** and the edelfosine analogs **2**, **7** and **10**, which contain BODIPY in different positions of the alkyl chain, has been carried out (Figure 58).

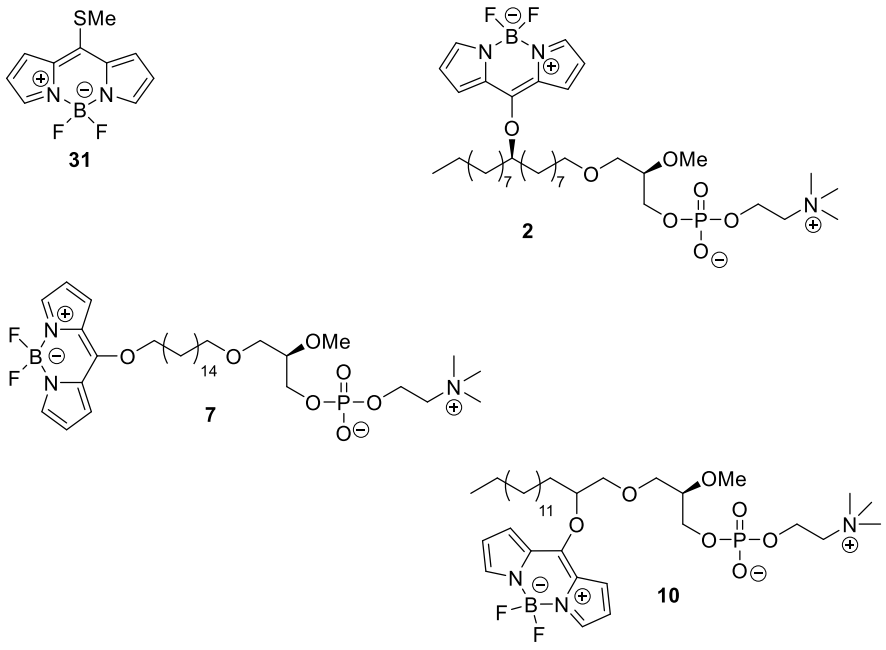


Figure 58

In figure 59 the absorption spectra maxima of the edelfosine analogs **2**, **7** and **10** and of BODIPY **31** can be observed to make a comparative study of the different molecules.

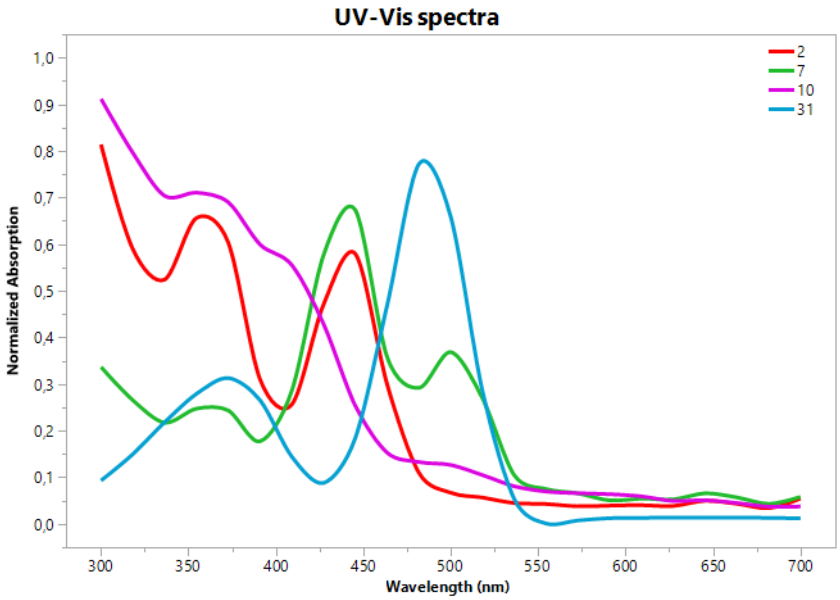


Figure 59. Absorption spectra of compounds **2, 7, 10** y **31**

As can be seen (Figure 59, Table 4), when the BODIPY fragment is incorporated into the edelfosine structure, its optical properties are not lost, although they do appear modified, according to the data described in the bibliography.[17] A hypsochromic effect is produced by exchanging the sulfide bond for the ether function.

Table 4. Absorption maximums of the studied compounds

	2		**10**		**7**			**31**	
$\lambda_{máx}$ (nm)	363	445	365	403	365	445	501	380	488
Abs	0.030	0.029	0.065	0.054	0.014	0.027	0.013	0.071	0.215
C (M)	4.11E-05	4.11E-05	2.85E-05	2.85E-05	2.85E-05	2.85E-05	2.85E-05	1.37E-05	1.37E-05
ε ($M^{-1}cm^{-1}$)	732	701	2293	1893	486	945	471	5182	15730

Figure 60 shows a comparative graph of the fluorescence emission spectra of compounds **2**, **7**, **10** and **31**. As can be seen, when excited at 440 nm, compounds **2** and **7** present a strong emission band, with a maximum at 486 nm, while compound **10** shows a much less intense band at the same wavelength. However, the emission increases considerably for **10** when the sample is excited at 400 nm (position of an absorption maximum of this compound).

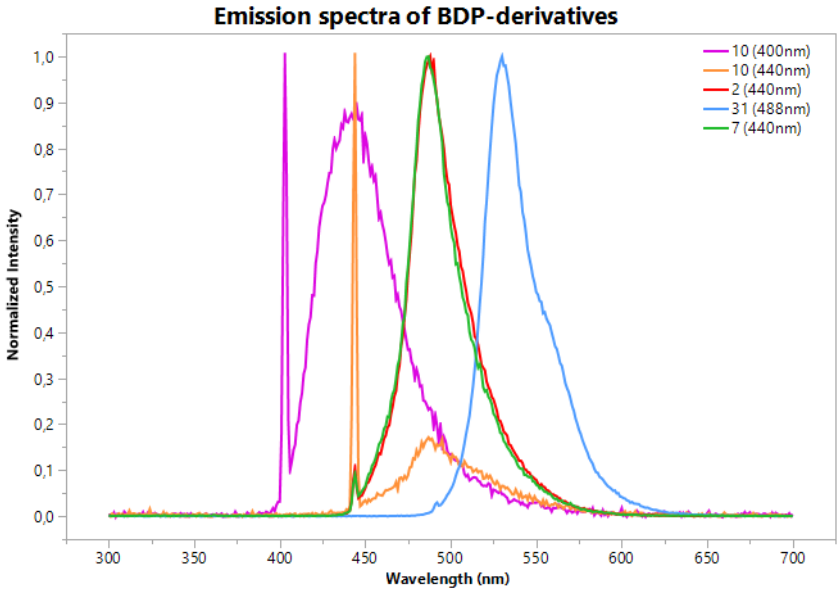


Figure 60. Fluorescence spectra of the studied compounds, **2**, **7**, **10** and **31**

11. Biological evaluation

In this section, the biological evaluations made with the synthesized edelfosine analogs during the development of this work are collected and the results obtained will be discussed.

The biological tests have been carried out at the "Centro de Investigaciones Biológicas del CSIC (CIB-CSIC)" in Madrid by Professor Dr. D. Faustino Mollinedo. The apoptosis induction tests were carried out following the procedure described below:

Cell culture

HL-60 (human acute myeloid leukemia) and Jurkat (human acute T-cell leukemia) cell lines were grown in RPMI-1640 culture medium containing 10% (v/v) heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin at 37 °C in a humidified atmosphere of 5% CO2 and 95% air. HeLa (human cervical carcinoma) cell line was grown in Dulbecco's Modified Eagle Medium (DMEM) culture medium containing 10% (v/v) heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin at 37 °C in a humidified atmosphere of 5% CO2 and 95% air. HL-60, Jurkat and HeLa cells were from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were periodically tested for Mycoplasma infection and found to be negative.

Cell Cycle Analysis

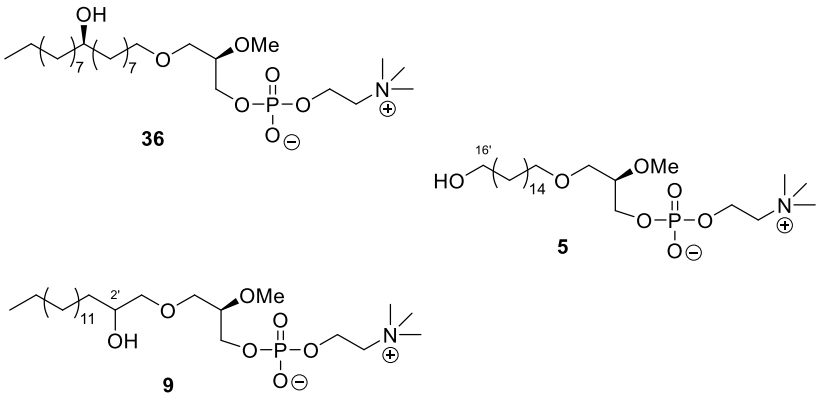
For cell cycle analysis, untreated and drug-treated cells (3-5 x 10^5) were centrifuged and fixed overnight in 70% ethanol at 4ºC. Then cells were washed three times with PBS, incubated for 1 h with 1 mg/mL RNase A and 20 µg/mL propidium iodide at room temperature, and analyzed with a Becton Dickinson FACSCalibur flow cytometer (San Jose, CA) as described previously.[19, 20] Quantification of apoptotic cells was calculated as the percentage of cells in the sub-G0/G1 peak in cell cycle analysis. The results given are the mean ± S.D. of at least three independent determinations (Tables 5 and 6).

Some structure-function relationship studies on HL-60 cells highlight the importance of the hydrophilic-lipophilic (amphipathic) character of the edelfosine molecule to promote apoptosis.

In order to explain the results shown in tables 5 and 6, the analyzed compounds have been grouped in three categories according to the different functionalization in their long hydrocarbon chain.

Hydroxyderivatives

Phospholipids **36**, **5** and **9** contain a hydroxyl group in different positions of the alkyl chain (C9', C16' and C2' respectively).

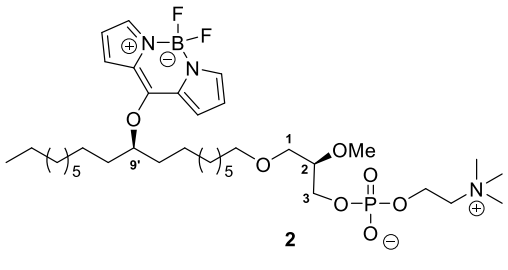


Depending on where the hydroxyl group is located, its activity as an apoptosis inducer changes dramatically (Tables 5 and 6), with analog **9** being the most active against the three cell lines tested, with an activity comparable to that of edelfosin (**EDLF**). However, as the hydroxyl group moves away from the polar head, its antitumor capacity is lost. Thus, analog **36** shows some antitumor activity against HL-60, especially at high concentrations, while analog **5** totally loses its cytotoxic capacity (Figure 61). These results make us think that the amphiphilic nature of this type of

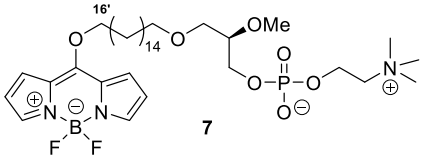
antitumor is crucial when it comes to being incorporated into lipid rafts and exercising their activity.

Fluorescent analogs

Compounds **2**, **7** and **10** have fluorescent labels at different positions of the alkyl chain (positions C9', C16' and C2' respectively). All three analogs are active against HL-60 at low concentrations, 10-30 μM, especially at long times (Tables 5 and 6). These compounds are interesting due to their possible use as tumor tracers *in vivo* because of their fluorescence.



2



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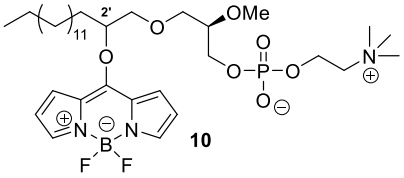
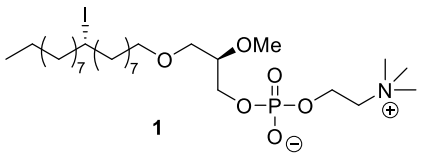
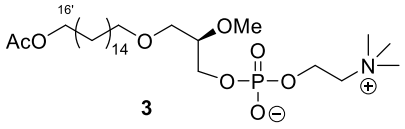


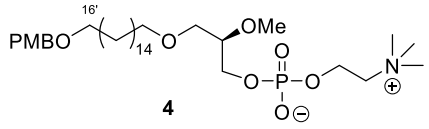
10

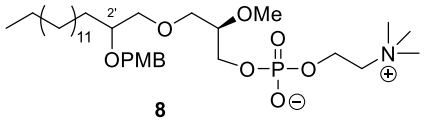
Other analogs

The latest analogs tested against tumor cell lines possess heteroatoms, such as iodine **1** or protected hydroxyl groups in the form of acetoxy, **3**, or ether, **4** and **8**.









None of these compounds have been found to be active for any of the cell lines tested (Tables 5 and 6).

Higher concentrations

At very high concentrations (50 and 70 μM) death percentage increases in general in HL-60 cells (Table 6). However, this effect is not observed in Jurkat or HeLa cells (except for **8** for HeLa cells).

Conclusion

The distinct apoptotic responses exerted by the above edelfosine analogs on the different cell types indicate that the response is cell type specific, and this could be due to a different lipid composition of the respective cell membranes. HL-60 cells behave as the most sensitive cells to this kind of compounds. Compound **9** is the only analog that is active at 10 μM against the three cell lines assayed in this study, suggesting that this compound could act similarly to and shares close biophysical properties with edelfosine.

The pro-apoptotic activity of the BODIPY analogs, albeit at higher concentrations than edelfosine, suggests that these fluorescent derivatives could be used to unveil the subcellular localization and the mechanism of action of edelfosine.

Because edelfosine should be taken up by the cancer cell to induce an apoptotic response from within the cell,[21, 22] the lack of activity of several edelfosine analogs could be explained either for their inability to be taken up by the cell or to trigger apoptosis.

Edelfosine acts through its interaction with cell membranes and particularly with lipid rafts.[23-27] In this regard, alterations in the long *O*-alkyl chain at C1 of the glycerol backbone C1 aliphatic seem to have dramatic consequences in the initial interaction with cell membranes and subsequent downstream processes to trigger apoptosis. These data pinpoint the importance of maintaining certain structural motifs and biophysical features in the structure of a newly synthesized edelfosine analog to preserve its pro-apoptotic activity.

Table 5. Time-course and dose-response of induction of apoptosis in human cancer cells by several edelfosine analogs. Apoptosis was determined as the percentage of cells in sub-G0/G1 as assessed by flow cytometry of propidium iodide-stained cells. The results given are the mean ± S.D. of at least three independent determinations. EDLF, edelfosine. Hydroxyderivatives shown below.

		HL-60			Jurkat			HeLa		
Compound	**μM**	**24 h**	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**
Untreated (Control)	--	1.8 ± 0.2	1.8 ± 0.3	2.1 ± 0.4	1.5 ± 0.2	1.6 ± 0.2	1.8 ± 0.3	1.0 ± 0.2	1.0 ± 0.2	1.2 ± 0.3
EDLF	10	40.8 ± 3.9	65.8 ± 6.3	71.6 ± 6.9	22.5 ± 2.3	27.8 ± 2.5	51.3 ± 4.9	10.5 ± 1.2	23.7 ± 2.2	35.3 ± 2.9
	20	47.2 ± 4.6	77.8 ± 7.5	84.4 ± 7.9	41.4 ± 3.9	45.5 ± 4.2	69.4 ± 6.0	28.7 ± 2.5	51.3 ± 4.9	67.2 ± 6.3
	30	53.4 ± 5.2	80.8 ± 7.7	87.1 ± 7.2	52.2 ± 5.1	58.8 ± 5.5	75.5 ± 6.2	32.6 ± 3.1	57.4 ± 5.6	73.5 ± 6.1
9	10	46.2 ± 4.3	53.5 ± 5.1	69.2 ± 6.7	7.9 ± 1.0	8.1 ± 1.1	8.3 ± 1.2	1.5 ± 0.6	20.8 ± 1.9	26.8 ± 2.3
	20	50.7 ± 5.7	59.1 ± 5.8	73.7 ± 6.5	16.1 ± 1.5	21.2 ± 1.8	27.1 ± 1.9	6.5 ± 0.8	26.2 ± 2.1	29.6 ± 2.3
	30	55.2 ± 6.1	60.2 ± 5.9	77.1 ± 6.7	24.7 ± 1.9	27.8 ± 2.5	29.5 ± 2.8	13.4 ± 1.1	36.6 ± 2.9	47.9 ± 3.5
36	10	2.5 ±0.2	2.1 ± 0.2	3.0 ± 0.2	2.5 ± 0.2	2.3 ± 0.2	2.4 ± 0.2	1.6 ± 0.2	1.4 ± 0.2	1.5 ± 0.2
	20	8.3 ± 0.8	11.3 ± 1.0	39.8 ± 3.7	2.7 ± 0.2	3.7 ± 0.5	2.9 ± 0.4	1.0 ± 0.2	1.4 ± 0.2	1.6 ± 0.2
	30	27.4 ± 3.3	34.3 ± 3.8	44.2 ± 4.2	2.6 ± 0.3	3.7 ± 1.0	3.8 ± 1.1	1.1 ± 0.2	1.9 ± 0.2	1.4 ± 0.2
5	10	1.8 ± 0.2	1.7 ± 0.2	1.9 ± 0.2	2.1 ± 0.2	2.2 ± 0.2	2.1 ± 0.2	1.0 ± 0.2	2.1 ± 0.3	1.1 ± 0.2
	20	1.4 ± 0.2	1.9 ± 0.2	1.6 ± 0.2	3.5 ± 0.5	2.6 ± 0.3	2.5 ± 0.3	1.0 ± 0.2	2.7 ± 0.4	1.1 ± 0.2
	30	1.2 ± 0.2	1.4 ± 0.3	3.5 ± 0.5	2.9 ± 0.3	2.7 ± 0.3	2.5 ± 0.4	1.0 ± 0.2	2.5 ± 0.4	1.6 ± 0.3

Table 5 cont. Fluorescent analogs shown below.

Results and discussion

		HL-60			Jurkat			HeLa		
Compound	**μM**	**24 h**	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**
Untreated (Control)	--	1.8 ± 0.2	1.8 ± 0.3	2.1 ± 0.4	1.5 ± 0.2	1.6 ± 0.2	1.8 ± 0.3	1.0 ± 0.2	1.0 ± 0.2	1.2 ± 0.3
EDLF	10	40.8 ± 3.9	65.8 ± 6.3	71.6 ± 6.9	22.5 ± 2.3	27.8 ± 2.5	51.3 ± 4.9	10.5 ± 1.2	23.7 ± 2.2	35.3 ± 2.9
	20	47.2 ± 4.6	77.8 ± 7.5	84.4 ± 7.9	41.4 ± 3.9	45.5 ± 4.2	69.4 ± 6.0	28.7 ± 2.5	51.3 ± 4.9	67.2 ± 6.3
	30	53.4 ± 5.2	80.8 ± 7.7	87.1 ± 7.2	52.2 ± 5.1	58.8 ± 5.5	75.5 ± 6.2	32.6 ± 3.1	57.4 ± 5.6	73.5 ± 6.1
10	10	3.7 ± 0.3	6.7 ± 0.5	5.4 ± 0.5	-	4.3 ± 0.5	2.1 ± 0.2	3.7 ± 0.3	5.7 ± 0.6	5.8 ± 0.4
	20	13.7 ± 1.2	28.7 ± 2.5	30.3 ± 2.8	-	6.5 ± 0.7	7.2 ± 0.7	4.7 ± 0.5	7.2 ± 0.6	7.3 ± 0.5
	30	15.8 ± 1.2	30.5 ± 2.7	52.5 ± 5.0	-	12.7 ± 1.1	19.2 ± 1.7	5.1 ± 0.6	8.9 ± 0.7	10.0 ± 0.9
2	10	4.9 ± 0.4	8.3 ± 0.5	6.2 ± 0.5	2.5 ± 0.4	2.1 ± 0.3	1.9 ± 0.3	2.1 ± 0.4	4.8 ± 0.5	4.2 ± 0.4
	20	20.9 ± 2.1	41.7 ± 3.9	43.6 ± 3.8	3.2 ± 0.4	6.9 ± 0.6	5.8 ± 0.6	6.5 ± 0.6	8.6 ± 0.7	7.5 ± 0.7
	30	45.1 ± 4.3	47.5 ± 4.2	63.7 ± 6.0	3.7 ± 0.5	7.3 ± 0.7	6.8 ± 0.7	10.1 ± 0.9	11.9 ± 1.2	12.1 ± 0.7
7	10	20.3 ± 1.9	24.2 ± 2.5	25.7 ± 2.2	-	2.1 ± 0.4	1.9 ± 0.3	3.0 ± 0.4	5.9 ± 0.5	3.9 ± 0.3
	20	25.6 ± 2.2	32.3 ± 3.1	38.7 ± 3.5	-	7.9 ± 0.5	5.8 ± 0.3	4.5 ± 0.3	9.6 ± 0.7	7.6 ± 0.6
	30	28.7 ± 2.5	45.2 ± 4.1	49.3 ± 4.7	-	9.3 ± 1.0	11.2 ± 1.1	12.3 ± 1.1	9.8 ± 0.8	17.7 ± 1.5

Table 5 cont. Other analogs shown below.

Results and discussion

		HL-60			Jurkat			HeLa		
Compound	**μM**	**24 h**	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**	**24 h**	**48 h**	**72 h**
Untreated (Control)	--	1.8 ± 0.2	1.8 ± 0.3	2.1 ± 0.4	1.5 ± 0.2	1.6 ± 0.2	1.8 ± 0.3	1.0 ± 0.2	1.0 ± 0.2	1.2 ± 0.3
EDLF	10	40.8 ± 3.9	65.8 ± 6.3	71.6 ± 6.9	22.5 ± 2.3	27.8 ± 2.5	51.3 ± 4.9	10.5 ± 1.2	23.7 ± 2.2	35.3 ± 2.9
	20	47.2 ± 4.6	77.8 ± 7.5	84.4 ± 7.9	41.4 ± 3.9	45.5 ± 4.2	69.4 ± 6.0	28.7 ± 2.5	51.3 ± 4.9	67.2 ± 6.3
	30	53.4 ± 5.2	80.8 ± 7.7	87.1 ± 7.2	52.2 ± 5.1	58.8 ± 5.5	75.5 ± 6.2	32.6 ± 3.1	57.4 ± 5.6	73.5 ± 6.1
8	10	2.3 ± 0.3	1.7 ± 0.2	1.9 ± 0.2	2.1 ± 0.2	2.2 ± 0.2	2.3 ± 0.2	1.0 ± 0.2	1.5 ± 0.2	1.0 ± 0.2
	20	3.0 ± 0.3	3.7 ± 0.3	4.7 ± 0.3	2.1 ± 0.2	2.1 ± 0.2	2.8 ± 0.3	1.0 ± 0.2	1.5 ± 0.2	1.0 ± 0.2
	30	4.6 ± 0.4	6.1 ± 0.5	13.8 ± 1.3	2.0 ± 0.2	2.7 ± 0.2	2.9 ± 0.4	1.0 ± 0.2	2.5 ± 0.2	2.1 ± 0.6
3	10	3.7 ± 0.4	1.2 ± 0.2	1.0 ± 0.2	2.0 ± 0.4	1.9 ± 0.2	2.0 ± 0.3	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2
	20	4.5 ± 0.4	3.4 ±0.4	2.6 ± 0.4	2.9 ± 0.4	2.3 ± 0.4	2.0 ± 0.3	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2
	30	5.8 ± 0.5	6.0 ± 0.5	9.2 ± 0.9	3.7 ± 0.4	3.0 ± 0.4	1.9 ± 0.2	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.2
4	10	1.9 ± 0.2	1.4 ± 0.2	1.2 ± 0.3	2.0 ± 0.4	2.3 ± 0.4	1.9 ± 0.2	1.0 ± 0.2	1.7 ± 0.2	1.0 ± 0.2
	20	2.2 ± 0.4	1.0 ± 0.2	2.4 ± 0.4	2.7 ± 0.4	2.5 ± 0.4	2.1 ± 0.4	1.0 ± 0.2	1.1 ± 0.2	1.3 ± 0.3
	30	2.6 ± 0.4	2.7 ± 0.4	10.5 ± 1.0	2.5 ± 0.4	2.7 ± 0.4	2.1 ± 0.4	1.0 ± 0.2	1.0 ± 0.2	2.2 ± 0.4
1	10	3.3 ± 0.4	2.7 ± 0.3	1.6 ± 0.3	1.7 ± 0.3	2.7 ± 0.4	2.5 ± 0.4	1.0 ± 0.2	1.1 ± 0.2	1.0 ± 0.2
	20	3.7 ± 0.4	3.6 ± 0.4	1.7 ± 0.3	1.7 ± 0.3	2.8 ± 0.4	3.5 ± 0.5	1.3 ± 0.2	1.2 ± 0.2	1.3 ± 0.3
	30	3.8 ± 0.5	4.3 ±0.5	8.6 ± 0.8	2.0 ± 0.4	2.8 ± 0.4	9.4 ± 0.9	2.8 ± 0.4	3.1 ± 0.4	4.8 ± 0.5

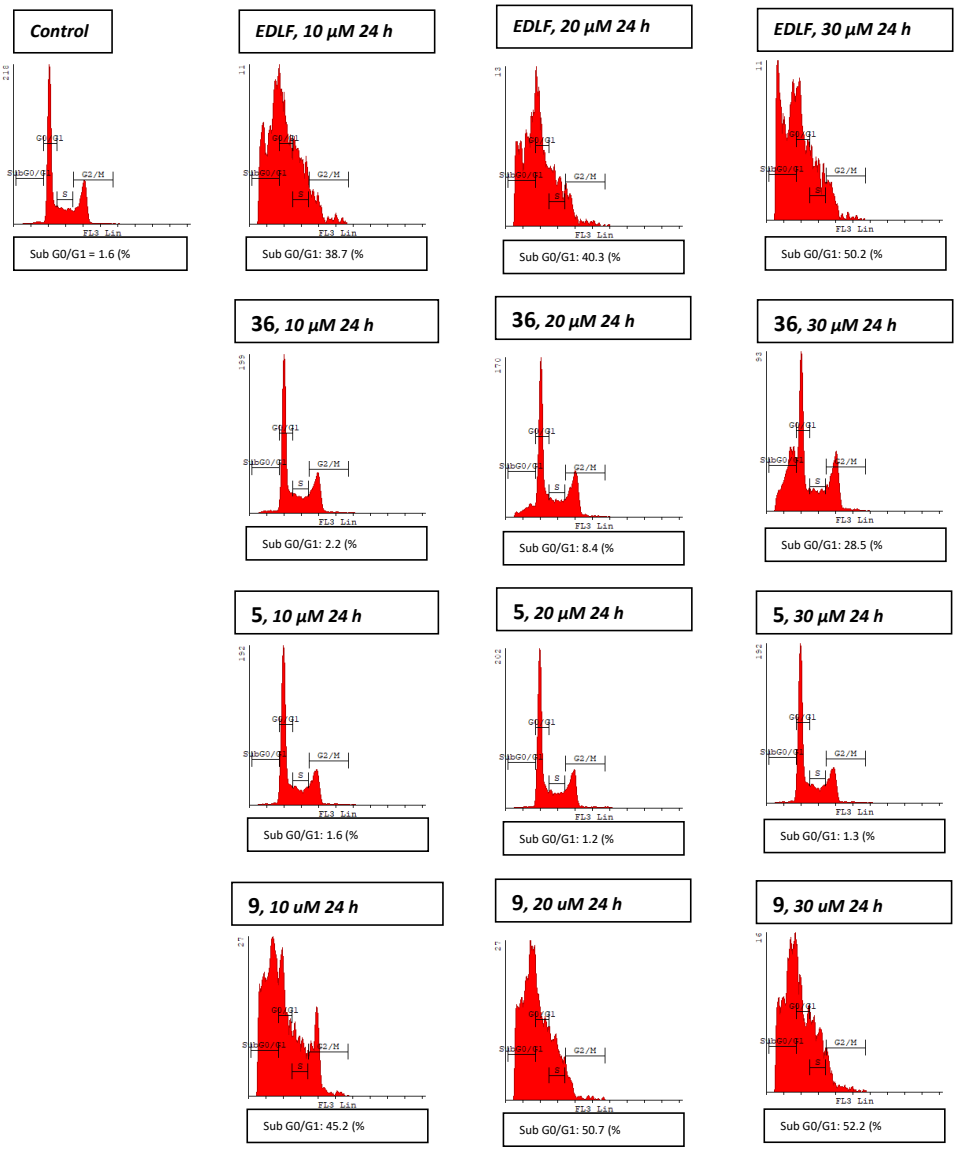


Figure 61. Dose-response of the effects of edelfosine (**EDLF**), **36**, **5** and **9** in HL-60 cells. Cells were incubated with different concentrations of the above compounds for 24 h, and their DNA content was analyzed by fluorescence flow cytometry. The positions of the sub-G0/G1, G0/G1, S and G2/M regions are indicated in the respective histograms, and the proportion of cells in each phase of the cell cycle was quantified by flow cytometry. The cell population in the sub-G0/G1 region represents cells with hypodiploid DNA content, an indicator of apoptosis. Untreated control cells were run in parallel. Data shown are representative of three independent experiments.

Table 6. Dose-response of induction of apoptosis in human cancer cells treated for 72 h with several edelfosine analogs. Apoptosis was determined as the percentage of cells in sub-G0/G1 as assessed by flow cytometry of propidium iodide-stained cells. The results given are the mean ± S.D. of at least three independent determinations. EDLF: edelfosine.

			HL-60	Jurkat	HeLa
	Compound	**μM**	**72 h**	**72 h**	**72 h**
	Untreated (Control)	--	2.1 ± 0.4	1.8 ± 0.3	1.2 ± 0.3
	EDLF	30	88.3 ± 7.1	76.4 ± 6.3	72.8 ± 5.6
		50	>90	>90	76.6 ± 6.3
		70	>90	>90	80.3 ± 6.2
Hidroxiderivatives	**9**	30	74.5 ± 6.5	27.5 ± 2.3	48.9 ± 3.4
		50	75.7 ± 6.2	35.3 ± 2.1	58.2 ± 4.3
		70	77.3 ± 6.8	40.3 ± 3.5	64.5 ± 5.7
	36	30	40.3 ± 3.9	3.3 ± 0.9	1.4 ± 0.5
		50	42.5 ± 3.8	23.2 ± 2.0	1.7 ± 0.7
		70	43.6 ± 4.0	28.1 ± 2.3	--
	5	30	2.8 ± 0.8	2.2 ± 0.6	1.7 ± 0.5
		50	2.9 ± 0.7	2.3 ± 0.7	1.7 ± 0.5
		70	2.9 ± 0.7	2.4 ± 0.7	1.8 ± 0.6
Other analogs	**8**	30	14.3 ± 1.2	2.3 ± 0.7	2.5 ± 0.9
		50	17.4 ± 1.5	2.3 ± 0.8	17.5 ± 1.8
		70	39.2 ± 3.4	2.4 ± 1.0	39.3 ± 3.2
	3	30	9.1 ± 1.0	1.9 ± 0.8	1.0 ± 0.3
		50	9.0 ± 1.3	2.1 ± 0.8	1.1 ± 0.3
		70	30.2 ± 3.1	2.0 ± 0.9	1.1 ± 0.4
	4	30	12.5 ± 1.3	2.0 ± 0.7	2.2 ± 0.8
		50	26.7 ± 2.5	2.0 ± 0.7	2.1 ± 0.7
		70	55.2 ± 4.7	2.1 ± 0.8	2.1 ± 0.7
	1	30	8.8 ± 0.9	8.7 ± 0.9	4.1 ± 0.5
		50	8.9 ± 1.0	8.8 ± 0.9	5.2 ± 0.7
		70	38.7 ± 3.9	9.2 ± 0.7	5.0 ± 0.5
Fluorescent analogs	**10**	30	55.6 ± 5.5	21.4 ± 2.0	10.7 ± 1.2
		50	65.3 ± 6.2	34.3 ± 3.3	21.2 ± 1.0
		70	68.2 ± 6.5	48.7 ± 4.5	30.9 ± 2.9
	2	30	66.8 ± 6.1	6.9 ± 0.8	12.3 ± 1.1
		50	73.5 ± 6.9	23.1 ± 2.1	12.5 ± 1.2
		70	82.7 ± 7.3	28.1 ± 2.8	14.1 ± 1.2
	7	30	45.1 ± 4.3	11.8 ± 1.2	19.3 ± 1.5
		50	59.9 ± 6.1	20.3 ± 2.0	19.8 ± 1.7
		70	66.5 ± 6.3	51.7 ± 5.0	35.7 ± 3.1

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General Methodology

FIRMADO POR	FECHA FIRMA
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Rotaciones específicas ($[\alpha]_D^{20}$)

Se midieron en un polarímetro digital Perkin-Elmer 241, en cubetas de 1 dm de paso óptico a una temperatura de 20 ^{o}C y en la línea de emisión del sodio (λ= 589 nm). La concentración a la que se realizó la medida y el disolvente se especifican en cada caso.

Espectrometría de masas

Las medidas se llevaron a cabo en un espectrómetro de cuadrupolo-tiempo de vuelo Applied Biosystems QSTAR XL. Consta de sondas de electrospray, fotospray y APCI, pudiendo trabajar en modo positivo y negativo. Es capaz de determinar la masa exacta de un compuesto, con un margen de error de 0.0005 %.

Espectroscopia de IR

Las medidas se realizaron utilizando un espectrofotómetro Shimadzu IR Affinity-1, en película sobre cristales de NaCl.

Espectroscopia de RMN

Experimentos unidimensionales de ^{1}H y ^{13}C

Se ha utilizado un espectrómetro Espectrómetro Varian Mercury 200 MHz (Servicio General de RMN, Nucleus, Universidad de Salamanca) equipado con una sonda tetranuclear ^{1}H, ^{19}F, ^{13}C, ^{31}P; y también un Espectrómetro Bruker Avance Neo 400 MHz (Servicio General de RMN, Nucleus, Universidad de Salamanca) equipado con una criosonda Prodigy CPPBBO BB-H&F con gradiente en z y un cambiador de muestras de 24 posiciones.

El disolvente empleado para realizar los espectros fue $CDCl_3$, cuyas referencias respecto al disolvente residual $CHCl_3$ son: 7.26 ppm en ^{1}H y 77.0 ppm en ^{13}C; o piridina deuterada (Pyr-d5), cuyas referencias respecto al disolvente residual C_5H_5N son: 8.74, 7.58 y 7.22 ppm en ^{1}H y 150.4, 135.9 y 123.9 ppm en ^{13}C. Los desplazamientos químicos (δ) se expresan en ppm y las constantes de acoplamiento (*J*), en Hz.

La multiplicidad de los carbonos se obtiene al aplicar una secuencia de pulsos DEPT (Distosionless Enhancement by Polarization Transfer). Esta secuencia distingue los carbonos protonados CH, CH_2 y CH_3 usando pulsos de protón a través del desacoplador de 90^o y 135^o.

Experimento bidimensional $^1H/^{13}C$: HMQC

Estos experimentos nos proporcionan información acerca de la correlación heteronuclear1H/13C a un enlace y se obtienen utilizando la secuencia rutinaria Bruker inv4gs.

Experimento bidimensional $^1H/^{13}C$: HMBC

Para conocer las correlaciones a larga distancia, 2 ó 3 enlaces, se viene usando la secuencia de pulsos inv4gslplrnd.

Técnicas generales cromatográficas

Cromatografía en capa fina (CCF)

Se realizan sobre placas de gel de sílice Merck (60 F254) de 0.2 mm de espesor. Para revelarlas se sumergen en una disolución de molibdato amónico en H_2SO_4/H_2O al 0.05:1 p/v, y después se someten a un calentamiento a 120 ^oC durante unos segundos.

Las sustancias que presentan fluorescencia son visualizadas con luz ultravioleta de λ = 254 nm y λ = 336 nm antes de ser reveladas.

Cromatografía en columna (CC)

Se lleva a cabo utilizando una columna de vidrio, empaquetando con gel de sílice Merck-60. Se disponen de dos tipos de sílice que, dependiendo del tamaño de partícula, se tiene gel de sílice normal con un tamaño de partícula de 0.200-0.063 nm, y gel de sílice flash con un tamaño de partícula de 0.040-0.063 nm, necesitando aplicar presión adicional.

La elución se realiza utilizando disolventes y mezclas de disolventes de polaridad creciente, elución por gradiente, siendo mezclas *n*-Hex/AcOEt, Tol/$CHCl_3$ o $CHCl_3$/MeOH. La composición de cada una de las fracciones se sigue usando CCF.

Purificación de reactivos y disolventes

Acetato de etilo (AcOEt): Se compra y se utiliza sin purificar.

Acetonitrilo (CH_3CN): Se destila sobre CaH_2 bajo atmósfera de Ar.

Cloroformo ($CHCl_3$): Se destila.

Diclorometano (CH_2Cl_2): Se destila sobre CaH_2 bajo atmósfera de Ar.

N,*N*-Dimetilformamida (DMF): Se compra y se utiliza sin purificar.

Éter (Et_2O): Se somete a ebullición sobre Na y se destila sobre Na y benzofenona.

n-Hexano (C_6H_{14}): Se destila.

Metanol (MeOH): Se destila.

Oxicloruro de fósforo ($OPCl_3$): Se destila a vacío justo antes de cada uso (65 torr, 35 ^oC).

Piridina (C_5H_5N): Se destila sobre BaO y se almacena con KOH.

Tetrahidrofurano (THF): Se somete a ebullición sobre Na y se destila sobre Na y benzofenona.

Tolueno ($PhCH_3$): Se almacena con Na.

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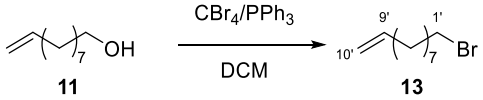
Experimental Section

FIRMADO POR	FECHA FIRMA
SELLO UNIVERSIDAD DE SALAMANCA	20-07-2021 18:11:01
SÁNCHEZ MARCOS ISIDRO	20-07-2021 10:20:59

Reaction of 9-decen-1-ol with CBr_4 and PPh_3: 13



11 **13**

To a solution of **11** (1.53 g, 9.8 mmol) in DCM (16 mL) at 0 °C was added CBr_4 (3.25 g, 9.8 mmol) and PPh_3 (3.86 g, 14.7 mmol) under argon atmosphere. The mixture was stirred for 2 h and then warmed up to room temperature for 18 h. The solution was filtered through a pad of Celite® and the solvent was removed *in vacuo*. The residue was purified by column chromatography (Hexanes) to afford **13** (2.11 g, 99%).

10-Bromodec-1-ene:[*] **13**

IR (liquid film) ν_{max} cm^{-1} 3076, 2928, 2854, 1458, 1244, 993, 910, 723, 646, 563.

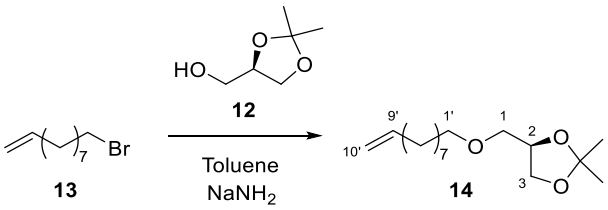
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 5.80 (1H, ddt, *J* = 17.2, 10.2, 6.9 Hz, H-9'), 4.98 (1H, d, *J* = 17.2 Hz, H_A-10'), 4.93 (1H, d, *J* = 10.2 Hz, H_B-10'), 3.39 (2H, t, *J* = 6.9 Hz, H-1'), 2.03 (2H, q, *J* = 6.9 Hz, H-8'), 1.84 (2H, quin, *J* = 6.9 Hz, H-2'), 1.45-1.34 (4H, m, H-3' and 7'), 1.30 (6H, s, H-4', 5' and 6').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 139.0 (C-9'), 114.2 (C-10'), 33.9 (C-1'), 33.7 (C-8'), 32.8 (C-2'), 29.2-28.1 (C-3', 4', 5', 6' and 7').

HMQC and HMBC see 2D tables.

[*] Systematic numbering of the nomenclature does not correspond to that of the spectroscopic assignment.

Reaction of 13 with *R*-solketal: 14



To a solution of **12** (858 mg, 6.49 mmol) in toluene (12.8 mL) was slowly added $NaNH_2$ (253 mg, 6.49 mmol) under argon atmosphere. The mixture was stirred at 120 °C for 1 h. A solution of **13** (1.42 g, 6.49 mmol) in toluene (18 mL) was then added dropwise. After 3 h, the reaction was cooled to room temperature and quenched by addition of ice and NH_4Cl (sat.). The mixture was extracted with EtOAc and washed with H_2O and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and solvent removed *in vacuo*. The residue was purified by column chromatography (Hexanes/EtOAc 95:5) to yield **14** (1.43 g, 82%).

(*R*)-4-((dec-9-en-1-yloxy)methyl)-2,2-dimethyl-1,3-dioxolane: **14**

$[\alpha]_D^{20}$ = -4.2 (c = 0.96, $CHCl_3$).

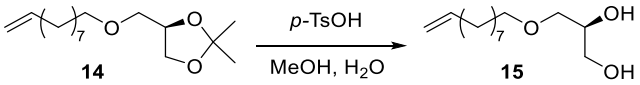
IR (liquid film) ν_{max} cm^{-1} 3076, 2986, 2927, 2856, 1641, 1369, 1213, 1119, 910, 847.

^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) 5.78 (1H, ddt, *J* = 16.8, 10.2, 6.2 Hz, H-9'), 5.01-4.86 (2H, m, H-10'), 4.23 (1H, quin., *J* = 6.2 Hz, H-2), 4.03 (1H, dd, *J* = 8.2, 6.2 Hz, H_A-3), 3.70 (1H, dd, *J* = 8.2, 6.2 Hz, H_B-3), 3.53-3.34 (4H, m, H-1 and 1'), 2.01 (2H, q, *J* = 6.2 Hz, H-8'), 1.55 (2H, quin., *J* = 6.0 Hz, H-2'), 1.28 (10H, m, H-3'-7'), 1.40 and 1.34 (3H, s each, CMe_2).

^{13}C NMR (50 MHz, $CDCl_3$, δ ppm) 139.3 (C-9'), 114.3 (C-10'), 109.5 (CMe_2), 74.9 (C-2), 72.05 (C-1'), 72.03 (C-1), 67.1 (C-3), 34.0 (C-8'), 29.8 (C-2'), 29.6-26.2 (C-3'-7'), 27.0 and 25.6 (CMe_2).

ESI-HRMS (*m/z*) $C_{16}H_{31}O_3$ ($M+H^+$): calc.: 271.2273, obs.: 271.2266, Δ = -2.58 ppm.

Reaction of 14 with *p*-TsOH: 15



To a solution of **14** (1.55 g, 5.74 mmol) in MeOH (16.5 mL) was added *p*-TsOH (1.08 g, 5.74 mmol) and 0.1 mL of H_2O. The reaction mixture was stirred at 40 ^oC for 8 h. The reaction was quenched by addition of H_2O and the mixture extracted with EtOAc (three times). The organic layers were washed with $NaHCO_3$ 6% and H_2O, dried over anhydrous Na_2SO_4, filtered and solvent removed *in vacuo* to give **15** (1.25 g, 94%).

(*S*)-3-(dec-9-en-1-yloxy)propane-1,2-diol: **15**

$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$= +1.59 (c = 1.3, $CHCl_3$).

IR (liquid film) ν_{max} cm^{-1} 3396, 3076, 2926, 2855, 1641, 1463, 1119, 1047, 993, 908.

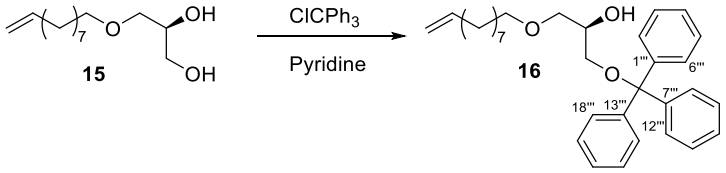
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 5.78 (1H, ddt, *J* = 17.0, 10.2, 6.8 Hz, H-9'), 4.96 (1H, d, *J* = 17.0 Hz, H_A-10'), 4.90 (1H, d, *J* = 10.2 Hz, H_B-10'), 3.83 (1H, quin, *J* = 5.8 Hz, H-2), 3.66 (1H, dd, *J* =11.2, 3.6 Hz, H_A-3), 3.58 (1H, dd, *J* =11.2, 5.8 Hz, H_B-3), 3.46-3.41 (4H, m, H-1 and 1'), 2.01 (2H, q, *J* = 6.8 Hz, H-8'), 1.54 (2H, quin, *J* = 7.0 Hz, H-2'), 1.39-1.26 (10H, m, H-3'-7').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 139.1 (C-9'), 114.1 (C-10'), 72.2 (C-1'), 71.7 (C-1), 70.6 (C-2), 64.1 (C-3), 33.7 (C-8'), 29.5 (C-2'), 29.3-26.0 (C-3'-7').

ESI-HRMS (*m/z*) $C_{13}H_{26}O_3Na$ ($M+Na^+$): calc.: 253.1779, obs.: 253.1778, Δ = -0.39 ppm.

HMQC and HMBC see 2D tables.

Reaction of 15 with trityl chloride: 16



To a solution of **15** (1.01 g, 4.36 mmol) in pyridine (8.6 mL) was added trityl chloride ($ClCPh_3$) (1.36 g, 4.8 mmol) under argon atmosphere. The mixture was then stirred at 100 ^{o}C for 3 h under argon. The reaction was cooled down and then quenched by addition of H_2O. The resulting mixture was extracted with EtOAc (three times) and washed with HCl 2M, $NaHCO_3$ 6% and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and solvent removed *in vacuo*. The residue was purified by column chromatography (Hexanes/EtOAc 95:5) to afford **16** (1.51 g, 73%).

(*R*)-1-(dec-9-enyloxy)-3-(trityloxy)propan-2-ol: **16**

$[\alpha]_D^{20}$ = +1.85 (c = 1.6, $CHCl_3$).

IR ν_{max} cm^{-1} 3445, 3059, 3024, 2926, 2854, 1958, 1639, 1597, 1491, 1449, 1117, 1076, 991, 901, 764, 704, 633.

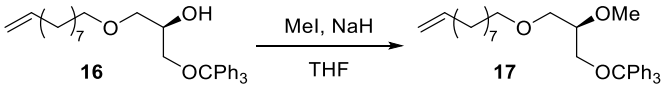
^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) 7.44-7.21 (15H, m, CPh_3), 5.80 (1H, ddt, *J* = 16.8, 10.0, 6.8 Hz, H-9'), 5.05-4.87 (2H, m, H-10'), 3.93 (1H, m, H-2), 3.51 (1H, dd, *J* = 9.8, 4.4 Hz, H_A-1), 3.45 (1H, dd, *J* = 9.8, 4.4 Hz, H_B-1), 3.41 (2H, t, *J* =6.8 Hz, H-1'), 3.19 (1H, dd, *J* = 9.4, 5.6 Hz, H_A-3), 3.16 (1H, dd, *J* = 9.4, 5.6 Hz, H_B-3), 2.40 (1H, d, *J* = 4.8 Hz, OH), 2.04 (2H, q, *J* = 6.8 Hz, H-8'), 1.52 (2H, m, H-2'), 1.26 (10H, br s, H-3'-7').

^{13}C NMR (50 MHz, $CDCl_3$, δ ppm)[*] 144.1 (C-1''', 7''' and 13'''), 139.5 (C-9'), 128.9 (C-3''', 5''', 9''', 11''', 15''' and 17'''), 128.1 (C-2''', 6''', 8''', 12''', 14''' and 18'''), 127.3 (C-4''', 10''' and 16'''), 114.4 (C-10'), 86.8 (CPh_3), 72.3 (C-1'), 71.9 (C-1), 70.1 (C-2), 64.8 (C-3), 34.1 (C-8'), 29.9 (C-2'), 29.7-26.3 (C-3'-7').

ESI-HRMS (*m/z*) $C_{32}H_{40}O_3Na$ ($M+Na^+$): calc.: 495.2875, obs.: 495.2873, Δ = -0.40 ppm.

[*] From now on, the assignment of CPh_3 carbons will only displays the ones of a phenyl.

Reaction of 16 with MeI: 17



To a solution of **16** (1.62 g, 3.43 mmol) in THF (7.1 mL) was added NaH 60% (205 mg, 5.14 mmol) and the mixture was stirred at 75 ^{o}C for 2 h under argon atmosphere. After that time, MeI (2.43 g, 17.14 mmol) was added dropwise and the mixture was stirred for 4 h. The reaction was then cooled down and quenched by addition of ice and NH_4Cl (sat.). The resulting mixture was extracted with EtOAc (three times) and washed with HCl 2M, H_2O and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and solvent removed *in vacuo*. The residue was purified by column chromatography (Hexanes/EtOAc 97:3 and 9:1) to give **17** (1.63 g, 98%).

1-*O*-(dec-9-enyl)-2-*O*-methyl-3-*O*-trityl-*sn*-glycerol: **17**

$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ = +8.97 (c = 0.97, $CHCl_3$).

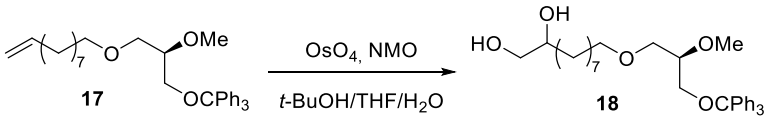
IR ν_{max} cm^{-1} 3059, 3032, 2926, 2855, 1958, 1728, 1639, 1491,1449, 1358, 1317, 1221, 1090, 908, 764, 706, 633.

^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) 7.49-7.16 (15H, m, C$\underline{Ph_3}$), 5.82 (1H, ddt, *J* = 16.8, 10.2, 6.8 Hz, H-9'), 5.06-4.90 (2H, m, H-10'), 3.61-3.38 (5H, m, H-1, 1' and 2), 3.42 (3H, s, O$\underline{Me}$), 3.20 (2H, m, H-3), 2.05 (2H, q, *J* = 6.8 Hz, H-8'), 1.54 (2H, m, H-2'), 1.28 (10H, br s, H-3'-7').

^{13}C NMR (50 MHz, $CDCl_3$, δ ppm) 144.4 (C-1'''), 139.4 (C-9'), 129.0 (C-3''' and 5'''), 128.0 (C-2''' and 6'''), 127.2 (C-4'''), 114.4 (C-10'), 86.9 ($\underline{C}Ph_3$), 80.2 (C-2), 72.0 (C-1'), 71.2 (C-1), 63.2 (C-3), 58.4 (O$\underline{Me}$), 34.1 (C-8'), 29.9 (C-2'), 29.7-26.3 (C-3'-7').

ESI-HRMS (*m/z*) $C_{33}H_{42}O_3Na$ ($M+Na^+$): calc.: 509.3032, obs.: 509.3022, Δ = -1.96 ppm.

Reaction of 17 with OsO_4: 18



To a solution of **17** (766 mg, 1.56 mmol) in *t*-BuOH/THF/H_2O 7:2:1 (23.3 mL) was added NMO (846 mg, 6.26 mmol) and OsO_4 2.5% in *t*-BuOH (160 µL, 0.015 mmol) and the mixture was stirred at room temperature for 24 h under argon atmosphere. The reaction was then quenched by addition of Na_2SO_3 (sat.) and stirred for 30 min. The resulting mixture was extracted with EtOAc (three times) and then washed with HCl 2M, H_2O and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and solvent removed *in vacuo*. The residue was purified by column chromatography (Hexanes/EtOAc 9:1) to obtain **18** (800 mg, 98%).

10-((*R*)-2-methoxy-3-(trityloxy)propoxy)decane-1,2-diol: **18**

$[\alpha]_D^{20}$ = +5.8 (c = 0.62, $CHCl_3$).

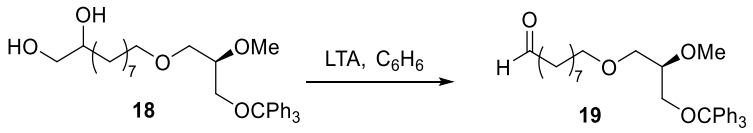
IR (liquid film) ν_{max} cm^{-1} 3404, 3057, 2928, 2855, 1960, 1728, 1597, 1489, 1449, 1090, 899, 744, 706, 633.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 7.46-7.22 (15H, m, C$\underline{Ph_3}$), 3.58-3.39 (8H, m, H-1, 2, 1', 9' and 10'), 3.41 (3H, s, O$\underline{Me}$), 3.20 (2H, m, H-3), 1.55 (2H, br s, H-8'), 1.43 (2H, br s, H-2'), 1.26 (10H, br s, H-3'-7').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 144.3 (C-1'''), 129.0 (C-3''' and 5'''), 128.0 (C-2''' and 6'''), 127.2 (C-4'''), 86.9 (C$\underline{Ph_3}$), 80.1 (C-2), 72.5 (C-9'), 72.0 (C-1'), 71.2 (C-1), 67.0 (C-10'), 63.1 (C-3), 58.3 (O$\underline{Me}$), 33.4 (C-8'), 29.9 (C-2'), 29.9-25.9 (C-3'-7').

ESI-HRMS (*m/z*) $C_{33}H_{44}O_5Na$ ($M+Na^+$): calc.: 543.3086, obs.: 543.3084, Δ = -0.37 ppm.

Reaction of 18 with LTA: 19

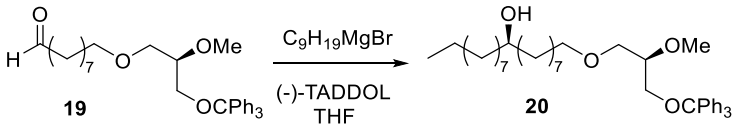


To a solution of **18** (556 mg, 1.07 mmol) in C_6H_6 (10.2 mL) was added lead(IV) tetraacetate (LTA) (1.04 g, 2.35 mmol) and the mixture was stirred at room temperature under argon atmosphere for 30 min. The reaction was then filtered through a pad of Celite® and the solution was washed with $NaHCO_3$ 6%, H_2O and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and solvent removed *in vacuo* to yield **19** (520 mg, 99%).

(*R*)-9-(2-methoxy-3-(trityloxy)propoxy)nonanal: **19**

^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) 9.76 (1H, t, *J* = 1.8 Hz, H-9'), 7.48-7.19 (15H, m, C$\underline{Ph_3}$), 3.60-3.35 (5H, m, H-1, 2 and 1'), 3.41 (3H, s, O$\underline{Me}$), 3.21 (2H, m, H-3), 2.42 (2H, dt, *J* = 6.7, 1.8 Hz, H-8'), 1.70-1.50 (2H, m, H-2'), 1.26 (10H, m, H-3'-7').

Reaction of 19 with $C_9H_{19}MgBr$: 20



In a 100 mL Schlenk tube equipped with magnetic stirring bar, (-)-TADDOL (1.34 g, 2.87 mmol) was solved in THF (11 mL) under argon atmosphere and a nonylmagnesium bromide 1M solution in Et_2O (5.7 mL, 5.7 mmol) was added and the mixture was cooled to -50 ^oC. In that moment, nonylmagnesium bromide (2.9 mL, 2.9 mmol) was added again and the solution was warmed to room temperature by immersing the flask in a 20 ^oC water bath. The resulting mixture was then cooled to -90 ^oC and a solution of **19** (359 mg, 0.74 mmol) in THF (15 mL) was added. The mixture was stirred

for 17 h. The reaction was quenched by addition of Et_2O (50 mL) and NH_4Cl (sat.). The organic layer was washed with brine, dried over anhydrous Na_2SO_4, filtered and solvent removed under reduced pressure. Flash chromatography (Hexanes/EtOAc 95:5, 9:1 and 8:2) gave **20** (270 mg, 59%).

(*R*)-1-((*R*)-2-methoxy-3-(trityloxy)propoxy)octadecan-9-ol: **20**

$[\alpha]_D^{20}$ = +1.4 (c = 0.1, $CHCl_3$).

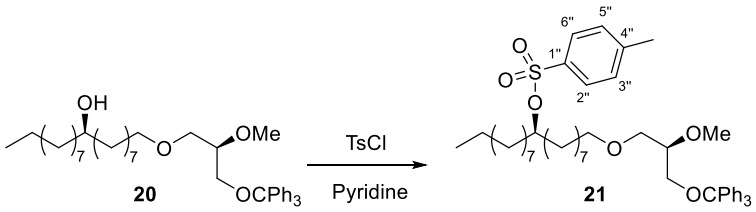
IR (liquid film) ν_{max} cm^{-1} 3422, 2926, 2855, 1448, 1117, 704.

^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) 7.48-7.22 (15H, m, C$\underline{Ph_3}$), 3.57-3.37 (6H, m, H-1, 2, 1' and 9'), 3.41 (3H, s, O$\underline{Me}$), 3.20-3.18 (2H, m, H-3), 1.53-1.49 (4H, m, H-8' and 10'), 1.41-1.21 (26H, m, H-2'-7' and 11'-17'), 0.88 (3H, t, *J* = 6.4 Hz, H-18').

^{13}C NMR (50 MHz, $CDCl_3$, δ ppm) 144.3 (C-1'''), 129.0 (C-3''' and 5'''), 128.0 (C-2''' and 6'''), 127.2 (C-4'''), 86.8 (C$\underline{Ph_3}$), 80.1 (C-2), 72.2 (C-9'), 71.9 (C-1'), 71.2 (C-1), 63.1 (C-3), 58.3 (O$\underline{Me}$), 37.8 (C-8' and 10'), 32.1-22.9 (C-2'-7' and 11'-17'), 14.4 (C-18').

ESI-HRMS (*m/z*) $C_{41}H_{60}O_4Na$ ($M+Na^+$): calc.: 639.4389, obs.: 639.4394, Δ = 0.78 ppm.

Reaction of 20 with TsCl: 21



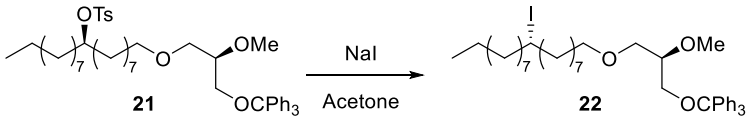
To a solution of **20** (270 mg, 0.45 mmol) in pyridine (3.5 mL) was added TsCl (205 mg, 1.1 mmol) and the mixture was stirred under argon at 0 °C for 24 h, controlling the progression by TLC. Then, the reaction was quenched by addition of ice and the resulted mixture was extracted with EtOAc and washed with HCl 2M, $NaHCO_3$ 6% and

H_2O. The organic layer was dried over anhydrous Na_2SO_4, filtered and solvent removed *in vacuo* to obtain **21** (265 mg, 77%).

1-*O*-((*R*)-9-(tosyloxy)octadecanyl)-2-*O*-methyl-3-*O*-trityl-*sn*-glycerol: **21**

^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) 7.79 (2H, d, *J* = 8.2 Hz, H-2'' and 6''), 7.48-7.22 (15H, m, C$\underline{Ph_3}$), 7.48-7.43 (2H, m, H-3'' and 5''), 4.57 (1H, m, H-9'), 3.56-3.37 (5H, m, H-1, 2 and 1'), 3.41 (3H, s, O$\underline{Me}$), 3.20 (2H, d, *J* = 4.0 Hz, H-3), 2.43 (3H, s, Ph-$\underline{Me}$), 1.56-1.54 (4H, m, H-8' and 10'), 1.26-1.17 (26H, m, H-2'-7' and 11'-17'), 0.90 (3H, t, *J* = 6.2 Hz, H-18').

Reaction of 21 with NaI: 22



To a solution of **21** (200 mg, 0.24 mmol) in acetone (4.8 mL) was added NaI (353 mg, 2.40 mmol) and the reaction mixture was stirred under argon atmosphere at 50 ^oC for 24 h. The reaction was then quenched by addition of H_2O and extracted with EtOAc. The mixture was washed with Na_2SO_3 10%, $NaHCO_3$ 6% and brine. The organic layer was dried over anhydrous Na_2SO_4, filtered and solvent removed *in vacuo*. The residue was purified by column chromatography (Hexanes/EtOAc 98:2) to afford **22** (128 mg, 71%).

1-*O*-((*S*)-9-iodooctadecanyl)-2-*O*-methyl-3-*O*-trityl-*sn*-glycerol: **22**

$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ = +5.0 (c = 0.54, $CHCl_3$).

IR (liquid film) ν_{max} cm^{-1} 2926, 2855, 1728, 1448, 1120, 1090, 1078, 706.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 7.45-7.18 (15H, m, C$\underline{Ph_3}$), 4.09 (1H, tt, *J* = 8.8, 4.5 Hz, H-9'), 3.55 (1H, dd, *J* = 10.0, 4.0 Hz, H_A-1), 3.52 (1H, m, H_B-1), 3.46 (1H, m, H-2), 3.39 (2H, t, *J* = 6.5 Hz, H-1'), 3.39 (3H, s, O$\underline{Me}$), 3.20 (1H, dd, *J* = 10.0, 4.8 Hz, H_A-3), 3.17 (1H,

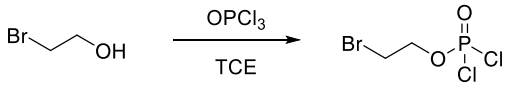
dd, *J* = 10.0, 4.8 Hz, H_B-3), 1.83 and 1.66 (2H, m each, H-8' and 10'), 1.51-1.26 (26H, m, H-2'-7' and 11'-17'), 0.86 (3H, t, *J* = 6.5 Hz, H-18').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 144.1 (C-1'''), 128.7 (C-3'''and 5'''), 127.7 (C-2''' and 6'''), 126.9 (C-4'''), 86.6 (<u>C</u>Ph_3), 79.9 (C-2), 71.6 (C-1'), 70.9 (C-1), 62.9 (C-3), 58.1 (O<u>Me</u>), 40.7 (C-8' and 10'), 40.6 (C-9'), 31.9 (C-16'), 29.5-26.0 (C-2'-7' and 11'-15'), 22.7 (C-17'), 14.1 (C-18').

ESI-HRMS (*m/z*) $C_{41}H_{59}O_3INa$ ($M+Na^+$): calc.: 749.3407, obs.: 749.3391, Δ = -2.13 ppm.

HMQC and HMBC see 2D tables.

Synthesis of 2-bromoethyl dichlorophosphate: 23



23

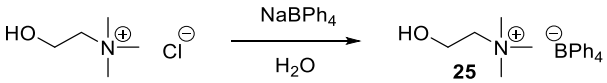
To a solution of $OPCl_3$ (500 mg, 3.8 mmol) in trichloroethylene (TCE) (0.25 mL) under argon atmosphere was slowly added 2-bromoethanol (1.00 g, 6.5 mmol) and the reaction was stirred at room temperature for 12 h. After that time, toluene (5 mL) was added and the solution was concentrated at 40 ^{o}C *in vacuo* to afford **23** (775 mg, 80 %).

<u>2-Bromoethyl</u> dichlorophosphate: **23**

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 4.58 (2H, dt, *J* =10.4 and 6.3 Hz, PO-<u>CH_2</u>), 3.61 (2H, dt, *J* =6.3 and 1.0 Hz, <u>CH_2</u>Br).

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 69.8 (PO-<u>CH_2</u>, d, *J* = 8.0 Hz), 27.2 (<u>CH_2</u>Br, d, *J* = 11.0 Hz).

Reaction of choline chloride with sodium tetraphenylborate: 25

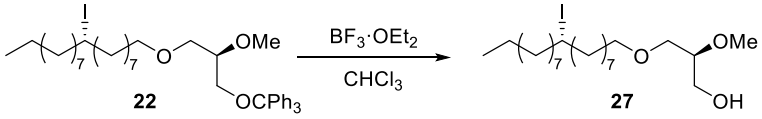


To a solution of choline chloride (1.0 g, 7.16 mmol) in H_2O (25 mL) at room temperature was added a solution of sodium tetraphenylborate (1.7 g, 4.97 mmol) in H_2O (25 mL) and the mixture was stirred at that temperature for 30 min, in which a white solid appeared. That solid was filtered, dried and dissolved in toluene/EtOH 1:1. Solvent was removed *in vacuo* and the white solid was crystallized in CH_3CN to yield **25** (2.0 g, 95%).

Choline tetraphenylborate: **25**

^{1}H NMR (200 MHz, DMSO-d6, δ ppm) 7.17-6.76 (20H, m, B$\underline{Ph_4}$), 5.26 (1H, t, *J* = 4.1 Hz, OH), 3.81-3.79 (2H, m, $\underline{CH_2}$OH), 3.40-3.30 (2H, m, $\underline{CH_2}$NMe$_3$), 3.08 (9H, s, N$\underline{Me_3}$).

Reaction of 22 with $BF_3{\cdot}OEt_2$: 27



To a solution of **22** (105 mg, 0.14 mmol) in $CHCl_3$ (1.4 mL) at 0 °C under argon atmosphere was added $BF_3{\cdot}OEt_2$ (72 µL, 0.57 mmol) and the reaction mixture was stirred at that temperature for 12 h. The reaction was then quenched by addition of a $CHCl_3$/H_2O/MeOH 2:2:1 mixture and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and solvent removed *in vacuo*. The residue was purified by column chromatography (Hexanes/EtOAc 9:1) to give **27** (67 mg, 99%).

(*S*)-3-((*S*)-9-iodooctadecyloxy)-2-methoxypropan-1-ol: **27**

$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ = -7.6 (c = 0.75, $CHCl_3$).

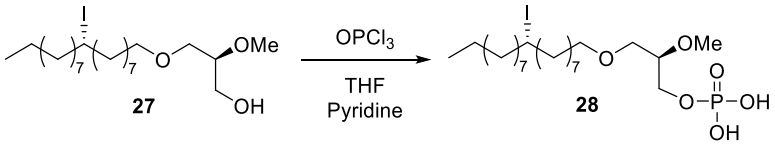
IR (liquid film) ν_{max} cm^{-1} 3460, 2926, 2855, 1732, 1464, 1123.

^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) 4.11 (1H, m, H-9'), 3.76 (1H, dd, *J* = 11.6, 4.4 Hz, H_A-3), 3.64 (1H, dd, *J* = 11.6, 4.4 Hz, H_B-3), 3.56-3.44 (2H, m, H-1), 3.46 (3H, s, OMe), 3.46-3.41 (3H, m, H-2 and 1'), 1.93-1.66 (4H, m, H-8' and 10'), 1.56 (2H, m, H-2'), 1.27 (24H, m, H-3'-7' and 11'-17'), 0.88 (3H, t, *J* = 6.6 Hz, H-18').

^{13}C NMR (50 MHz, $CDCl_3$, δ ppm) 80.0 (C-2), 72.1 (C-1'), 70.9 (C-1), 62.9 (C-3), 58.0 (OMe), 41.2 (C-9'), 40.9 (C-8' and 10'), 32.1-22.9 (C-2'-7' and 11'-17'), 14.4 (C-18').

ESI-HRMS (*m/z*) $C_{22}H_{45}O_3INa$ ($M+Na^+$): calc.: 507.2311, obs.: 507.2306, Δ = -0.98 ppm.

Reaction of 27 with $OPCl_3$: 28



To a solution of **27** (67 mg, 0.14 mmol) in THF (0.7 mL) at 0 °C under argon atmosphere was added pyridine (23 µL) and $OPCl_3$ (14 µL, 0.15 mmol) and the reaction mixture was stirred at that temperature for 5 h. Then, $NaHCO_3$ 6% was slowly added and stirred for 15 min. The reaction was quenched by addition of ice and HCl 2M until pH = 2 was achieved. The mixture was then extracted with EtOAc and washed with H_2O. The organic layer was then dried over anhydrous Na_2SO_4, filtered and solvent removed *in vacuo* to afford **28** (68 mg, 86%).

(*R*)-3-((*S*)-9-iodooctadecyloxy)-2-methoxypropyl dihydrogen phosphate: **28**

$[\alpha]_D^{20}$ = -0.6 (c = 0.14, $CHCl_3$).

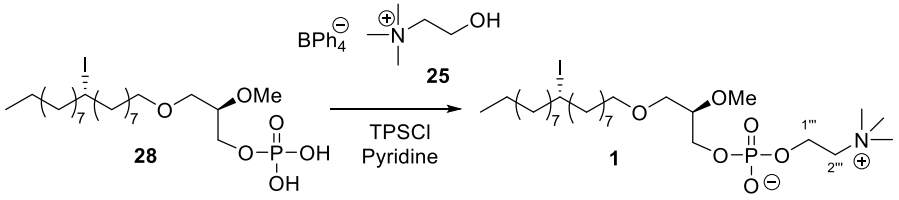
IR (liquid film) ν_{max} cm^{-1} 2924, 2855, 1110.

^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) 4.17-4.04 (3H, m, H-3 and 9'), 3.60-3.42 (5H, m, H-1, 2 and 1'), 3.49 (3H, s, OMe), 1.87-1.66 (4H, m, H-8' and 10'), 1.56 (2H, m, H-2'), 1.27 (24H, m, H-3'-7' and 11'-17'), 0.87 (3H, t, *J* = 6.6 Hz, H-18').

^{13}C NMR (50 MHz, $CDCl_3$, δ ppm) 79.3 (C-2), 72.2 (C-1'), 69.9 (C-1), 66.1 (C-3), 58.3 (OMe), 40.9 (C-9'), 40.9 (C-8' and 10'), 32.1-22.9 (C-2'-7' and 11'-17'), 14.3 (C-18').

ESI-HRMS (*m/z*) $C_{22}H_{47}O_6PI$ ($M+H^+$): calc.: 565.2155, obs. 565.2152, Δ = -0.53 ppm.

Reaction of 28 with 25: 1



To a solution of **28** (87 mg, 0.15 mmol) in pyridine (1.2 mL) was added 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) (55 mg, 0.18 mmol) and **25** (65 mg, 0.15 mmol) under argon atmosphere and the reaction mixture was stirred at 70 °C for 1 h. Then, the reaction was cooled to room temperature and stirred for other 3 h. After that time, the reaction was quenched by addition of H_2O and the solvent was removed under reduced pressure. The residue was purified by flash chromatography, eluting from $CHCl_3$/MeOH 9:1 to $CHCl_3$/MeOH/NH_4OH 65:30:5, to afford **1** (51 mg, 51%)

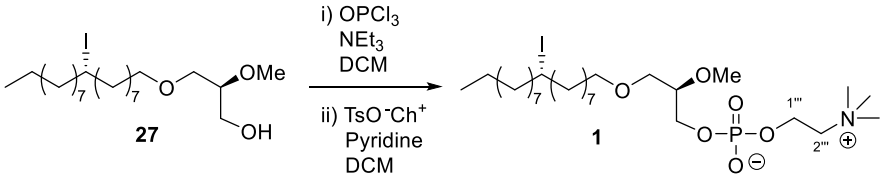
1-*O*-((*S*)-9-iodooctadecyl)-2-*O*-methyl-*sn*-glycero-3-phosphocholine: **1**

^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) 4.31 (2H, m, H-1'''), 4.17-4.04 (1H, m, H-9'), 3.84 (4H, m, H-3 and 2'''), 3.62-3.39 (5H, m, H-1, 2 and 1'), 3.43 (3H, s, OMe), 3.39 (9H, s, Me_3N), 1.92-1.61 (4H, m, H-8' and 10'), 1.53 (2H, m, H-2'), 1.27 (24H, m, H-3'-7' and 11'-17'), 0.87 (3H, t, *J* = 6.6 Hz, H-18').

^{13}C NMR (50 MHz, $CDCl_3$, δ ppm) 79.9 (C-2), 72.0 (C-1'), 70.5 (C-1), 66.6 (C-2'''), 65.2 (C-3), 59.5 (C-1'''), 58.0 (O<u>Me</u>), 54.6 (N<u>Me$_3$</u>), 41.1 (C-9'), 40.9 (C-8' and 10'), 32.1-22.9 (C-2'-7' and 11'-17'), 14.3 (C-18').

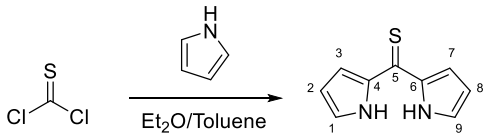
ESI-HRMS (*m/z*) $C_{27}H_{57}NO_6PINa$ ($M+Na^+$): calc.: 672.2866, obs.: 672.2854, Δ = -1.78 ppm.

Reaction of 27 with $OPCl_3$ and TsO^-Ch^+: 1



To a solution of $OPCl_3$ (50 µL, 0.53 mmol) and NEt_3 (0.1 mL) in DCM (4 mL) at 0 °C under argon atmosphere was added a solution of **27** (260 mg, 0.53 mmol) in DCM (2.5 mL) dropwise over 20 min and the reaction mixture was stirred at room temperature for 40 min. After that time, a solution of TsO^-Ch^+ (30 mg, 1.07 mmol) and pyridine (1 mL) in DCM (1 mL) was added and the mixture left to stir for 19 h. Then, H_2O (1 mL) was added. After 40 min, the mixture is concentrated with toluene/EtOH 1:1 (3 x 15 mL) at 40 °C under reduced pressure. The obtained residue was purified by flash chromatography ($CHCl_3$/MeOH/H_2O 65:30:5) to afford **1** (236 mg, 68%).

Reaction of thiophosgene with pyrrole: 29



29

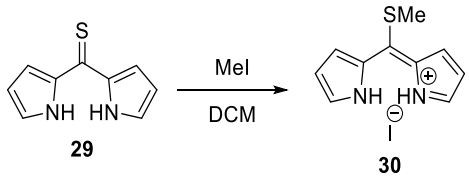
To a solution of thiophosgene (327 mg, 2.85 mmol) in toluene (8.6 mL) at 0 °C under argon atmosphere was slowly added a solution of pyrrole (382 mg, 5.69 mmol) in Et_2O (8.5 mL) and the reaction was stirred at that temperature for 30 min. Then, an aqueous solution of MeOH 10% (5.7 mL) was added and left to stir for 30 min. Solvent was then removed under reduced pressure and the residue was chromatographed over neutral alumina. Eluting with toluene/$CHCl_3$ 9:1 gave **29** (328 mg, 65%).

Bis(1*H*-pyrrol-2-yl)methanethione: **29**

^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) 9.81 (2H, s, N-H), 7.20 (2H, H-1 and 9), 7.04 (2H, br s, H-3 and 7), 6.41 (2H, br s, H-2 and 8).

ESI-HRMS (*m/z*) $C_9H_9N_2S$ ($M+H^+$): calc.: 177.0486, obs.: 177.0481, Δ = -2.82 ppm.

Reaction of 29 with MeI: 30



29 **30**

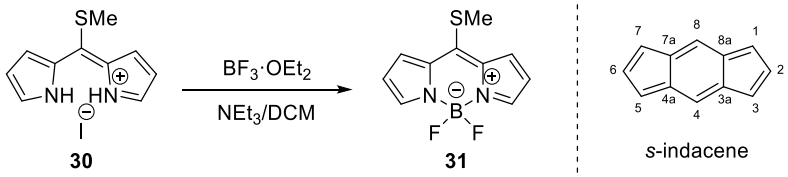
To a solution of **29** (295 mg, 1.66 mmol) in DCM (4.9 mL) at room temperature under argon atmosphere was added MeI (0.47 mL, 7.53 mmol) and the reaction mixture was stirred for 24 h, controlling by TLC. After that time, solvent was removed under reduced pressure to obtain **30** (465 mg, 91%).

2-((Methylthio)(1*H*-pyrrol-2-yl)methylene)-2*H*-pyrrolium iodide: **30**

^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) 11.93 (2H, s, N-H), 7.87 (2H, s, H-1 and 9), 7.26 (2H, s, H-3 and 7), 6.65 (2H, s, H-2 and 8), 2.89 (3H, s, SMe).

ESI-HRMS (*m/z*) $C_{10}H_{11}N_2S$ ($M+H^+$): calc.: 191.0643, obs.: 191.0637, Δ = -3.14 ppm.

Reaction of 30 with BF_3: 31



To a solution of **30** (465 mg, 1.52 mmol) in DCM (10.8 mL) under argon atmosphere was added NEt_3 (0.34 mL, 2.4 mmol) and the reaction was stirred at room temperature for 30 min. After that time, $BF_3{\cdot}OEt_2$ (0.29 mL, 2.28 mmol) was added and the mixture was stirred for 30 more minutes. Then, solvent was removed in *vacuo*. The residue was purified by flash chromatography (Hexanes/EtOAc 9:1 and 7:3) to yield **31** (362 mg, 91%).

8-(Methylthio)-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene: **31**

UV (EtOH) (λ_{max} nm) 488, 380.

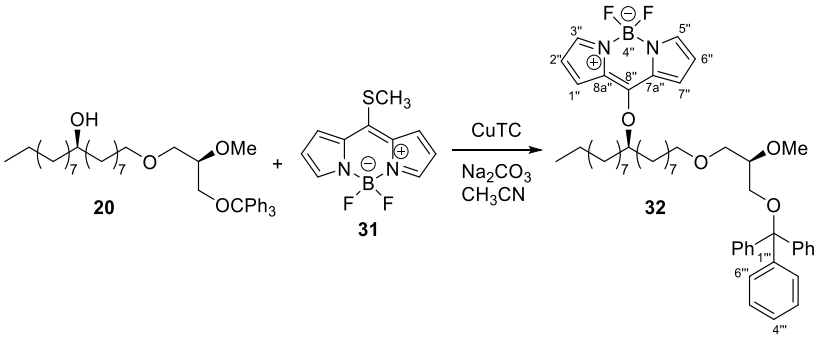
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 7.79 (2H, s, H-3 and 5), 7.41 (2H, d, *J* = 4.2 Hz, H-1 and 7), 6.53 (2H, d, *J* = 4.2 Hz, H-2 and 6), 2.91 (3H, s, SMe).

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 153.9 (C-8), 141.3 (C-3 and 5), 133.8 (C-7a and 8a), 127.6 (C-1 and 7), 117.8 (C-2 and 6), 20.4 (SMe).

ESI-HRMS (*m/z*) $C_{10}H_{10}BN_2F_2S$ ($M+H^+$): calc.: 239.0626, obs.: 239.0620, Δ = -2.51 ppm.

HMQC and HMBC see 2D tables.

Reaction of 20 with 31: 32



To a solution of **31** (138 mg, 0.58 mmol) in CH_3CN (1.9 mL) under argon atmosphere was added copper(I) thiophene-2-carboxylate (CuTC) (92 mg, 0.48 mmol) and a solution of **20** (120 mg, 0.19 mmol) in CH_3CN (0.4 mL) and the mixture was stirred at room temperature for 10 minutes. After that time, Na_2CO_3 (50 mg, 0.47 mmol) was added and the reaction was stirred at 55 ^oC for 12 h. Then, solvent was removed *in vacuo* and the resulting residue was purified by flash chromatography (Hexanes/EtOAc 98:2) to obtain **32** (115 mg, 73%).

1-*O*-((*R*)-9-(4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacen-8-yloxy)octadecanyl)-2-*O*-methyl-3-*O*-trityl-*sn*-glycerol: **32**

$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\mathbf{20}}$ = -0.60 (c = 0.65, $CHCl_3$).

IR (liquid film) ν_{max} cm^{-1} 2956, 2927, 2856, 1728, 1552, 1400, 1286, 1126, 966.

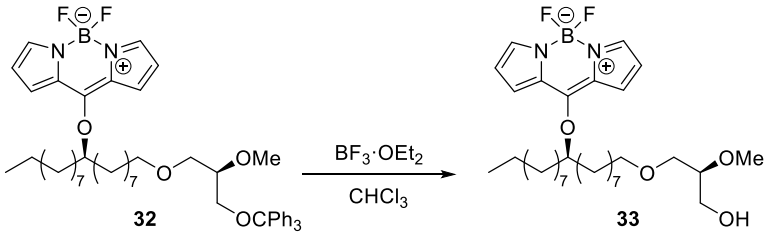
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 7.71 (2H, d, *J* = 1.0 Hz, H-3'' and 5''), 7.47-7.23 (15H, m, C$\underline{Ph_3}$), 7.27 (2H, m, H-1'' and 7''), 6.51 (2H, dd, *J* = 4.0, 1.0 Hz, H-2'' and 6''), 5.14 (1H, quin, *J* = 6.0 Hz, H-9'), 3.57-3.39 (5H, m, H-1, 2 and 1'), 3.41 (3H, s, O$\underline{Me}$), 3.21 (1H, dd, *J* = 10.2, 4.8 Hz, H_A-3), 3.19 (1H, dd, *J* = 10.2, 4.8 Hz, H_B-3), 1.88-1.84 (4H, m, H-8' and 10'), 1.54-1.51 (2H, m, H-2'), 1.43-1.26 (24H, m, H-3'-7' and 11'-17'), 0.88 (3H, t, *J* = 6.8 Hz. H-18').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 161.7 (C-8''), 144.3 (C-1'''), 139.0 (C-3'' and 5''), 129.0 (C-3''' and 5'''), 128.0 (C-2''' and 6'''), 127.1 (C-4'''), 126.8 (C-7a'' and 8a''), 124.8

(C-1'' and 7''), 116.4 (C-2'' and 6''), 86.8 (C-9'), 86.7 (C$\underline{\text{P}}$h$_3$), 80.1 (C-2), 71.9 (C-1'), 71.2 (C-1), 63.1 (C-3), 58.3 (O$\underline{\text{Me}}$), 34.1 (C-8' and 10'), 32.1-22.9 (C-2'-7' and 11'-17'), 14.3 (C-18').

ESI-HRMS (*m/z*) $C_{50}H_{65}BN_2O_4F_2Na$ ($M+Na^+$): calc.: 829.4903, obs.: 829.4898, Δ = -0.60 ppm.

Reaction of 32 with $BF_3{\cdot}OEt_2$: 33



To a solution of **32** (115 mg, 0.19 mmol) in $CHCl_3$ (1.9 mL) at 0 °C under argon atmosphere was added $BF_3{\cdot}OEt_2$ (98 µL, 0.77 mmol) and the reaction mixture was stirred at that temperature for 12 h. After that time, 1 mL of a $CHCl_3/H_2O$/MeOH 2:2:1 mixture was added to the reaction flask and then extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4, filtered and solvent removed *in vacuo*. The residue was purified by column chromatography (Hexanes/EtOAc 9:1) to give **33** (106 mg, 99%).

1-*O*-((*R*)-9-(4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacen-8-yloxy)octadecanyl)-2-*O*-methyl-*sn*-glycerol: **33**

$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ = -6.1 (c = 0.99, $CHCl_3$).

IR (liquid film) ν_{max} cm^{-1} 3452, 2926, 2854, 1552, 1400, 1255, 966.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 7.71 (2H, br s, H-3'' and 5''), 7.27 (2H, br s, H-1'' and 7''), 6.51 (2H, br s, H-2'' and 6''), 5.14 (1H, quin., *J* = 6.0 Hz, H-9'), 3.75 (1H, m, H_A-3), 3.64 (1H, m, H_B-3), 3.55 (1H, dd, *J* = 10.0, 5.0 Hz, H_A-1), 3.51 (1H, dd, *J* = 10.0, 5.0 Hz,

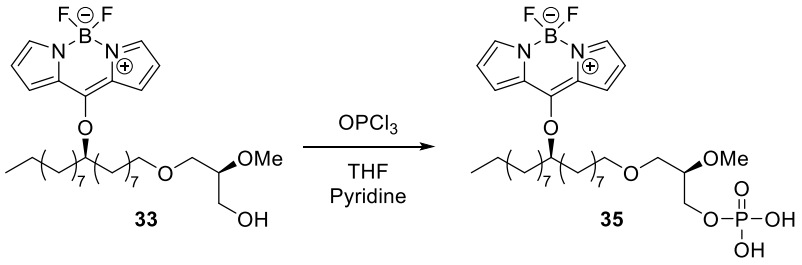
H_B-1), 3.46 (3H, s, OMe), 3.44-3.41 (3H, m, H-2 and 1'), 2.17 (1H, br s, OH), 1.85 (4H, m, H-8' and 10'), 1.55 (2H, m, H-2'), 1.43 (4H, m, H-7' and 11'), 1.29 and 1.25 (20H, br s, H-3'-6' and 12'-17'), 0.87 (3H, t, *J* = 6.8 Hz, H-18').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 161.4 (C-8''), 138.8 (C-3'' and 5''), 126.6 (C-7a'' and 8a''), 124.6 (C-1'' and 7''), 116.1 (C-2'' and 6''), 86.5 (C-9'), 79.8 (C-2), 71.8 (C-1'), 70.6 (C-1), 62.7 (C-3), 57.7 (OMe), 33.9 (C-8' and 10'), 31.8 (C-16'), 29.4-29.2 (C-3'-6' and 12'-15'), 26.0 (C-2'), 25.1 (C-7' and 11'), 22.6 (C-17'), 14.1 (C-18').

ESI-HRMS (*m/z*) $C_{31}H_{51}BN_2O_4F_2Na$ ($M+Na^+$): calc.: 587.3808, obs.: 587.3802, Δ = -1.02 ppm.

HMQC and HMBC see 2D Tables.

Reaction of 33 with $OPCl_3$: 35



To a solution of **33** (106 mg, 0.19 mmol) in THF (1.0 mL) at 0 °C under argon atmosphere was added pyridine (31 µL) and $OPCl_3$ (19 µL, 0.20 mmol) and the reaction mixture stirred at that temperature for 5 h. Then, $NaHCO_3$ 6% was slowly added and stirred for 15 min. The reaction was quenched by addition of ice and HCl 2M until pH = 2 was achieved. The mixture was then extracted with EtOAc and washed with H_2O. The organic layer was then dried over anhydrous Na_2SO_4, filtered and solvent removed *in vacuo* to afford **35** (92 mg, 75%).

(*R*)-3-((*R*)-9-(4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacen-8-yloxy)octadecyloxy)-2-methoxypropyl dihydrogen phosphate: **35**

$[\alpha]_D^{20}$ = -3.4 (c = 0.25, $CHCl_3$).

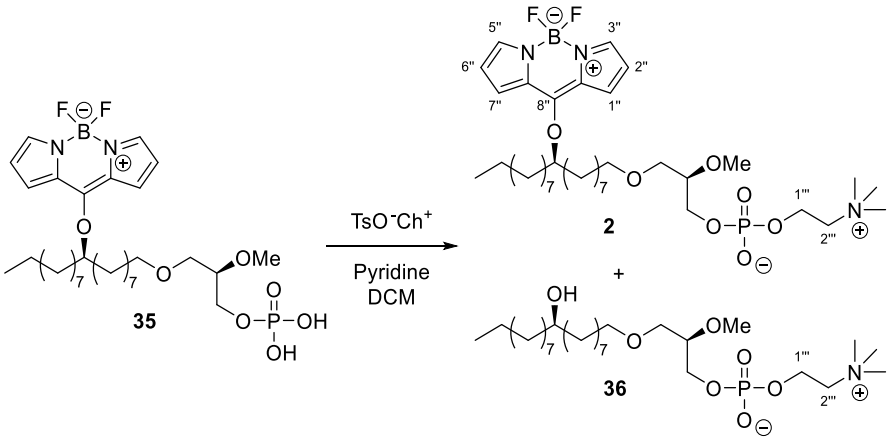
IR (liquid film) ν_{max} cm^{-1} 2926, 2854, 1552, 1400, 1128, 966.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 7.70 (2H, br s, H-3'' and 5''), 7.27 (2H, m, H-1'' and 7''), 6.51 (2H, dd, *J* = 4.1, 1.8 Hz, H-2'' and 6''), 5.13 (1H, quin, *J* = 5.8 Hz, H-9'), 4.17-4.04 (2H, m, H-3), 3.60-3.42 (5H, m, H-1, 2 and 1'), 3.49 (3H, s, OMe), 1.87-1.66 (4H, m, H-8' and 10'), 1.56 (2H, m, H-2'), 1.27 (24H, m, H-3'-7' and 11'-17'), 0.87 (3H, t, *J* = 6.6 Hz, H-18').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 161.3 (C-8''), 138.7 (C-3'' and 5''), 126.5 (C-7a'' and 8a''), 124.5 (C-1'' and 7''), 116.1 (C-2'' and 6''), 86.5 (C-9'), 79.0 (C-2), 71.8 (C-1'), 69.4 (C-1), 65.9 (C-3), 58.0 (OMe), 33.9 (C-8' and 10'), 31.8-22.6 (C-2'-7' and 11'-17'), 14.0 (C-18').

ESI-HRMS (*m/z*) $C_{31}H_{51}BN_2O_7F_2PNa_2$ ($M\text{-}H^+\text{+}2Na^+$): calc.: 689.3290, obs.: 689.3284, Δ = -0.87 ppm.

Reaction of 35 with choline tosylate: 2



To a solution of **35** (48 mg, 0.074 mmol) in DCM (0.5 mL) was added pyridine (0.2 mL, 2.46 mmol) and TsO^-Ch^+ (40 mg, 0.148 mmol) at room temperature under argon

atmosphere and the reaction was allowed to stir for 2 days. After that time, the mixture was concentrated with toluene/EtOH 1:1 (3 x 10 mL) at 40 ^{o}C under reduced pressure. The obtained residue was purified by flash chromatography eluting with $CHCl_3$/MeOH/H_2O 65:30:5 to afford a mixture of BODIPY **31**, **2** and **36** (26 mg). Size exclusion chromatography over Sephadex® LH-20 using $CHCl_3$/MeOH 1:1 as eluent yielded **2** (20 mg, 37%) and **36** (3 mg, 7%).

(*R*)-1-*O*-((*R*)-9-(4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacen-8-yloxy)octadecyl)-2-*O*-methyl-*sn*-glycero-3-phosphocholine: **2**

$[\alpha]_D^{20}$ = -4.6 (c = 0.50, $CHCl_3$).

UV (EtOH) (λ_{max} nm) 443, 356, 296.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 7.70 (2H, br s , H-3'' and 5''), 7.27 (2H, m, H-1'' and 7''), 6.51 (2H, d, *J* = 4.2, 2.0 Hz, H-2'' and 6''), 5.14 (1H, quin., *J* = 5.8 Hz, H-9'), 4.31 (2H, br s, H-1''') 3.92-3.84 (2H, m, H-3 and 2'''), 3.51-3.50 (2H, m, H-1_A and 2), 3.45-3.36 (3H, m, H-1_B and 1'), 3.41 (3H, br s, OMe), 3.37 (9H, br s, NMe_3), 1.89-1.68 (4H, m, H-8' and 10'), 1.53-1.37 (6H, m, H-2', 7' and 11'), 1.25 (20H, br s, H-3'-6' and 12'-17'), 0.87 (3H, t, *J* = 6.6 Hz, H-18').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 161.4 (C-8''), 138.7 (C-3'' and 5''), 126.5 (C-7a'' and 8a''), 124.6 (C-1'' and 7''), 116.2 (C-2'' and 6''), 86.5 (C-9'), 79.5 (C-2), 71.6 (C-1'), 70.1 (C-1), 66.2 (C-2'''), 65.0 (C-3), 59.2 (C-1'''), 57.8 (OMe), 54.4 (NMe_3), 33.9 (C-8' and 10'), 31.8-22.7 (C-2'-7' and 11'-17'), 14.1 (C-18').

ESI-HRMS (*m/z*) $C_{36}H_{63}BN_3O_7F_2PNa$ ($M+Na^+$): calc.: 752.4357, obs.: 752.4356, Δ = -0.13 ppm.

(*R*)-1-*O*-((*R*)-9-hydroxyoctadecyl)-2-*O*-methyl-*sn*-glycero-3-phosphocholine: **36**

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 4.32 (2H, br s, H-1'''), 3.93 (1H, m, H_A-3), 3.88 (1H, m, H_B-3), 3.81 (2H, br s, H-2'''), 3.57-3.52 (3H, m, H-1_A, 2 and 9'), 3.46 (1H, dd, *J* = 10.8, 6.8 Hz, H_B-1), 3.44 (3H, s, OMe), 3.41 (2H, m, H-1'), 3.38 (9H, br s, NMe_3), 1.54 (2H, m, H-

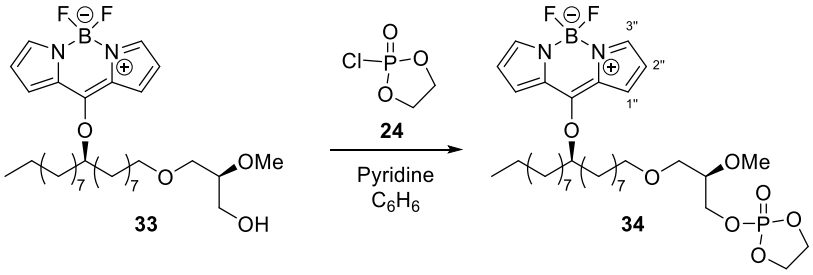
2'), 1.41 (4H, m, H-8' and 10'), 1.28 and 1.26 (24H, br s each, H-3'-7' and 11'-17'), 0.88 (3H, t, *J* = 6.8 Hz, H-18').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 79.6 (C-2), 71.7 (C-9'), 71.6 (C-1'), 70.0 (C-1), 66.4 (C-2'''), 65.0 (C-3), 59.2 (C-1'''), 57.7 (O<u>Me</u>), 54.4 (N<u>Me_3</u>), 37.5 and 37.4 (C-8' and 10'), 31.9 (C-16'), 29.8 (C-2'), 29.6-25.6 (C-3'-7' and 11'-15'), 22.6 (C-17'), 14.1 (C-18').

ESI-HRMS (*m/z*) $C_{27}H_{59}O_7NP$ ($M+H^+$): calc.: 540.4024, obs.: 540.4017, Δ = -1.28 ppm.

HSQC see Spectroscopy.

Reaction of 33 with 24: 34



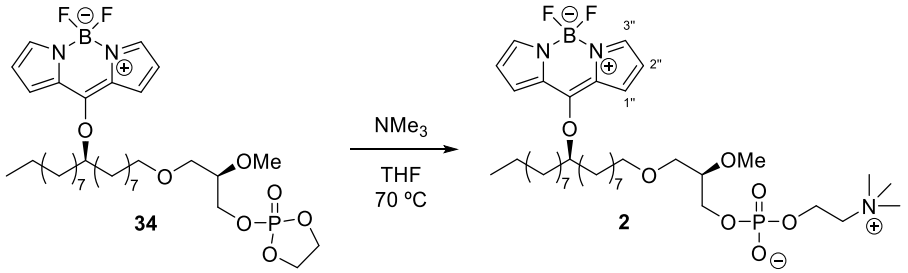
To a solution of **33** (57 mg, 0.10 mmol) in C_6H_6 (1.5 mL) at 0 °C under argon atmosphere was added pyridine (0.1 mL) and 2-chloro-2-oxa-1,3,2-dioxaphospholane **24** (55 µL, 0.61 mmol) and the reaction mixture was stirred at that temperature for 12 h. After that time, solvent was removed *in vacuo* and the resulting residue was purified by flash chromatography ($CHCl_3$/MeOH 98:2) to obtain **34** (35 mg, 52%).

<u>2-((*R*)-3-((*R*)-9-(4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacen-8-yloxy)octadecyl)-2-methoxypropoxy)-1,3,2-dioxaphospholane 2-oxide:</u> **34**

^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) 7.71 (2H, br s, H-3'' and 5''), 7.25 (2H, m, H-1'' and 7''), 6.51 (2H, dd, *J* = 4.2, 2.0 Hz, H-2'' and 6''), 5.14 (1H, quin., *J* = 5.8 Hz, H-9'), 4.48-4.11 (6H, m, H-3 and PO-<u>CH_2</u>-<u>CH_2</u>-O), 3.55-3.39 (5H, m, H-1, 2 and 1'), 3.49 (3H, s,

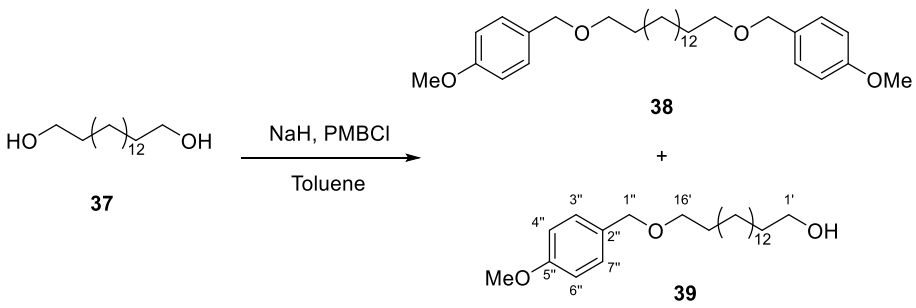
OMe), 1.88-1.80 (4H, m, H-8' and 10'), 1.65 (6H, m, H-2', 7' and 11'), 1.25 (20H, br s, H-3'-6' and 12'-17'), 0.87 (3H, t, *J* = 5.8 Hz, H-18').

Reaction of 34 with NMe_3: 2



A 1M solution of NMe_3 in THF (1.0 mL, 1.0 mmol) was added to a sealed tube containing a solution of **34** (35 mg, 0.05 mmol) in THF (1.0 mL) under argon atmosphere and the reaction was stirred at 70 ^{o}C for 24 h. The mixture was then cooled down and solvent was removed under reduced pressure. The obtained residue was purified by flash chromatography ($CHCl_3$/MeOH/H_2O 65:30:5) to afford **2** (4 mg, 11%).

Reaction of 1,16-hexadecanediol with PMBCl: **38** and **39**



To a solution of **37** (3.11 g, 12.08 mmol) in toluene (92.7 mL) was added NaH 60% (0.71 g, 17.90 mmol) and PMBCl (2.0 mL, 14.46 mmol) under argon atmosphere. The mixture was stirred at a 120 ^{o}C for 12 h. After that time, the reaction was quenched by addition of ice and NH_4Cl (sat.). The mixture was then extracted with EtOAc and the organic layer was washed with HCl 2M, $NaHCO_3$ 6% and NaCl (sat.), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by column chromatography. Eluting with hexanes/EtOAc (98:2), hexanes/EtOAc (9:1 and 8:2) and MeOH afforded **38** (714 mg, 28%), **39** (991 mg, 52%) and 1.80 g of **37** respectively.

1,16-Bis(4-methoxybenzyloxy)hexadecane: **38**

^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) 7.26 (4H, d, *J* = 8.7 Hz, H-3'' and H-7''), 6.87 (4H, d, *J* = 8.7 Hz, H-4'' and H-6''), 4.43 (4H, s, H-1''), 3.80 (6H, s, OMe), 3.43 (4H, t, *J* = 6.6 Hz, H-1' and 16'), 1.67-1.46 (4H, m, H-2' and H-15'), 1.25 (24H, br s, H-3'-14').

16-(4-methoxybenzyloxy)hexadecan-1-ol: **39**

IR (KBr) ν_{max} cm^{-1} 3315, 2918, 2849, 2792, 2360, 1617, 1589, 1518, 1463, 1362, 1304, 1257, 1176, 1102, 1065, 1030, 1004, 936, 822.

^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) 7.26 (2H, d, *J* = 8.7 Hz, H-3'' and H-7''), 6.87 (2H, d, *J* = 8.7 Hz, H-4'' and H-6''), 4.43 (2H, s, H-1''), 3.80 (3H, s, OMe), 3.64 (2H, t, *J*= 6.5 Hz, H-1'), 3.43 (2H, t, *J* = 6.6 Hz, H-16'), 1.67-1.46 (4H, m, H-2' and H-15'), 1.25 (24H, br s, H-3'-14').

^{13}C NMR(50 MHz, $CDCl_3$, δ ppm) 159.3 (C-5''), 130.9 (C-2''), 129.5 (C-3'' and 7''), 113.9 (C-4'' and 6''), 72.7 (C-1''), 70.4 (C-16'), 63.1 (C-1'), 55.4 (OMe), 33.0-26.0 (C-2'-15').

ESI-HRMS (*m/z*) $C_{24}H_{42}O_3Na$ ($M+Na^+$): calc.: 401.3026, obs.: 401.3010, Δ = -4.02 ppm.

Reaction of 38 with DDQ: 37

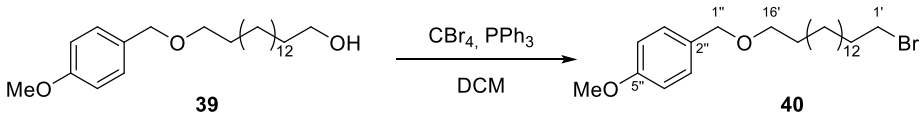
38 **37**

To a solution of **38** (714 mg, 1.43 mmol) in DCM/H_2O 18:1 (15 mL) was added DDQ (650 mg, 2.86 mmol) and the reaction was stirred at room temperature for 30 minutes, controlling the progression by TLC. The reaction was quenched by addition of $NaHCO_3$ and the mixture was extracted with DCM. The organic layer was washed with $NaHCO_3$ 6% and NaCl (sat.), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to yield **37** (365 mg, 99%).

1,16-hexadecanediol: **37**

^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) 3.64 (4H, t, *J*= 6.4 Hz, H-1 and 16), 1.60-1.51 (4H, m, H-2 and H-15), 1.25 (24H, br s, H-3-14).

Reaction of 39 with CBr_4: 40



39 **40**

To a solution of **39** (3.40 g, 8.98 mmol) in DCM (18.0 mL) was slowly added CBr_4 (2.98 g, 8.98 mmol) and PPh_3 (3.53 g, 13.47 mmol) at 0 ^{o}C and the reaction was stirred at room temperature for 12 h. After that time, solvent was removed *in vacuo* and the

residue was purified by column chromatography (Hexanes/EtOAc 98:2) to obtain **40** (3.48 g, 88 %).

1-((16-bromohexadecyloxy)methyl)-4-methoxybenzene: **40**

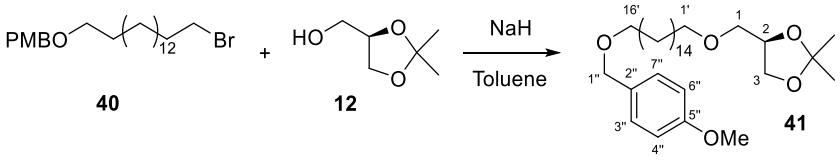
IR (liquid film) ν_{max} cm^{-1} 2922, 2851, 1614, 1514, 1466, 1364, 1302, 1248, 1173, 1101, 1038, 822, 812.

^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) 7.26 (2H, d, *J* = 8.7 Hz, H-3'' and 7''), 6.87 (2H, d, *J*= 8.7 Hz, H-4'' and 6''), 4.43 (2H, s, H-1''), 3.80 (3H, s, OMe), 3.43 (2H, t, *J* = 6.5 Hz, H-16'), 3.41 (2H, t, *J* = 6.8 Hz, H-1'), 1.99–1.71 (2H, m, H-2'), 1.60-1.54 (2H, m, H-15'), 1.26 (24H, br s, H-3'-14').

^{13}C NMR (50 MHz, $CDCl_3$, δ ppm) 159.0 (C-5''), 130.9 (C-2''), 129.2 (C-3'' and 7''), 113.7 (C-4'' and 6''), 72.5 (C-1''), 70.2 (C-16'), 55.3 (OMe), 34.1 (C-1'), 32.8-26.2 (C-2'-15').

ESI-HRMS (*m/z*) $C_{24}H_{41}O_2NaBr$ ($M+Na^+$): calc.: 463.2182, obs.: 463.2188, Δ = 1.27 ppm.

Reaction of 40 with *R*-solketal: 41



To a solution of *R*-solketal **12** (0.60 g, 4.69 mmol) in toluene (4.0 mL) was added NaH 60% (0.64 g, 16.40 mmol) and the reaction was stirred under argon atmosphere for 1 h. After that, a solution of **40** (1.98 g, 4.68 mmol) in toluene (14.2 mL) was slowly added and the reaction was allowed to stir for 12 h. Then, the reaction was quenched by addition of ice and NH_4Cl (sat.). The mixture was extracted with EtOAc and washed with H_2O y NaCl (sat.), dried over anhydrous Na_2SO_4, filtered and solvent removed under reduced pressure. The residue was purified by column chromatography (Hexanes/EtOAc 9:1) to afford **41** (1.52 g, 91 %) and recover 500 mg of s. m. **40** (Hexanes/EtOAc 98:2).

(*R*)-4-((16-(4-methoxybenzyloxy)hexadecyloxy)methyl)-2,2-dimethyl-1,3-dioxolane: **41**

$[\alpha]_D^{20}$ = -6.28 (c = 1.03, $CHCl_3$)

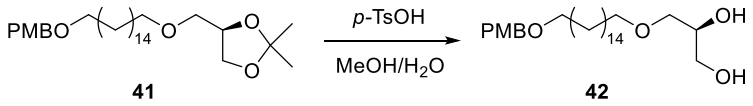
IR (liquid film) ν_{max} cm^{-1} 2985, 2926, 2852, 1614, 1514, 1463, 1369, 1247, 1099, 844.

^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) 7.24 (2H, d, *J* = 8.6 Hz, H-3'' and 7''), 6.86 (2H, d, *J* = 8.6 Hz, H-4'' and 6''), 4.41 (2H, s, H-1''), 4.25 (1H, quin, *J* = 6.2 Hz, H-2), 4.04 (1H, dd, J = 8.2 and 6.4 Hz, H_A-3), 3.78 (3H, s, Ph-OMe), 3.71 (1H, dd, *J* = 8.2 and 6.4 Hz, H_B-3), 3.49-3.36 (6H, m, H-1, 1' and 16'), 1.58-1.53 (4H, m, H-2' and 15'), 1.41 and 1.35 (3H, s each., CMe_2), 1.24 (24H, br s, H-3'-14').

^{13}C NMR (50 MHz, $CDCl_3$, δ ppm) 159.3 (C-5''), 131.0 (C-2''), 129.4 (C-3'' and 7''), 113.9 (C-4'' and 6''), 109.6 (CMe_2), 75.0 (C-2), 72.7 (C-1''), 72.1 (C-1'), 72.0 (C-1), 70.4 (C-16'), 67.1 (C-3), 55.4 (Ph-OMe), 30.0-29.7 (C-3'-14'), 27.0 (Me), 26.4 and 26.3 (C-2' and 15'), 25.6 (Me).

ESI-HRMS (*m/z*) $C_{30}H_{52}O_5Na$ ($M+Na^+$): calc.: 515.370696, obs.: 515.3716, Δ = 1.75 ppm.

Reaction of 41 with *p*-TsOH: 42



41 **42**

To a solution of **41** (3.0 g, 6.09 mmol) in MeOH (18.5 mL) under argon atmosphere was added *p*-TsOH (1.15 g, 6.09 mmol) and 0.1 mL of H_2O and the reaction was stirred at room temperature for 30 min, controlling the progression by TLC. Then, the reaction mixture was extracted with EtOAc and the organic layer was washed with $NaHCO_3$ (6%), H_2O and NaCl (sat.), dried over anhydrous Na_2SO_4, filtered and solvent removed *in vacuo* to give **42** (2.44 g, 88%).

(*S*)-3-(16-(4-methoxybenzyloxy)hexadecyloxy)propane-1,2-diol: **42**

$[\alpha]_D^{20}$ = -0.42 (c = 0.99, $CHCl_3$).

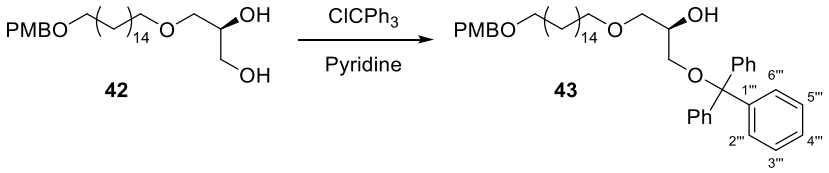
IR (liquid film) ν_{max} cm^{-1} 3343, 2918, 2848, 1612, 1463, 1246, 1103, 1031, 821.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 7.26 (2H, d, *J* = 8.7 Hz, H-3'' and 7''), 6.88 (2H, d, *J* = 8.7 Hz, H-4'' and 6''), 4.43 (2H, s, H-1''), 3.86 (1H, quin, *J* = 6.0 Hz, H-2), 3.80 (3H, s, Ph-OMe), 3.68 (2H, m, H-3), 3.58-3.35 (6H, m, H-1, 1' and 16'), 1.78-1.43 (4H, m, H-2' and 15'), 1.24 (24H, br s, H-3'-14').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 159.1 (C-5''), 130.8 (C-2''), 129.2 (C-3'' and 7''), 113.7 (C-4'' and 6''), 72.5 (C-1''), 72.5 (C-1'), 71.8 (C-1), 70.4 (C-2), 70.2 (C-16'), 64.3 (C-3), 55.3 (Ph-OMe), 29.8-29.4 (C-3'-14'), 26.2 and 26.1 (C-2' and 15').

ESI-HRMS (*m/z*) $C_{27}H_{48}O_5Na$ ($M+Na^+$), calc.: 475.339396, obs.: 475.3401, Δ = 1.48 ppm.

Reaction of 42 with $ClCPh_3$: 43



To a solution of **42** (2.00 g, 4.41 mmol) in pyridine (8.8 mL) was added $ClCPh_3$ (1.42 g, 5.07 mmol) under argon atmosphere and the reaction was stirred at 100 °C for 4 h, controlling the progression by TLC. After that time, the reaction was cooled and quenched by addition of H_2O. Then, the mixture was extracted with EtOAc and the organic layer was washed with HCl 2M, $NaHCO_3$ 6% and NaCl (sat.), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by columns chromatography (Hexanes/EtOAc 9:1 and 85:15) to separate **43** (1.62 g, 75%) and recover 600 mg of s. m. **42** (EtOAc).

(*R*)-1-(16-(4-methoxybenzyloxy)hexadecyloxy)-3-(trityloxy)propan-2-ol: **43**

$[\alpha]_D^{20}$ = -0.32 (c = 1.6, $CHCl_3$)

IR (liquid film) ν_{max} cm^{-1} 3464, 3059, 2926, 2852, 1612, 1514, 1448, 1247, 1093, 1035, 705.

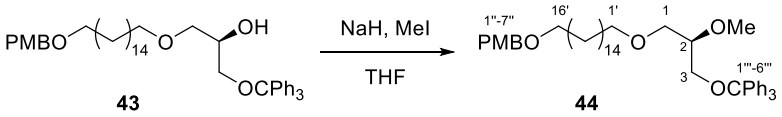
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 7.45-7.42 (6H, m, C$\underline{Ph_3}$), 7.32-7.21 (11H, m, H-3'', 7'' and C$\underline{Ph_3}$), 6.87 (2H, d, *J* = 8.5 Hz, H-4'' and 6''), 4.43 (2H, s, H-1''), 3.95 (1H, m, H-2), 3.80 (3H, s, Ph-O$\underline{Me}$), 3.53 (1H, dd, *J* = 9.7, 4.4 Hz, H_A-1), 3.47 (1H, dd, *J* = 9.7, 6.6 Hz, H_B-1), 3.45-3.40 (4H, m, H-1' and 16'), 3.21 (1H, dd, *J* = 9.4, 5.6 Hz, H_A-3), 3.18 (1H, dd, *J* = 9.4, 5.6 Hz, H_B-3), 2.41 (1H, d, *J* = 4.7 Hz, OH), 1.63-1.52 (4H, m, H-2' and 15'), 1.24 (24H, br s, H-3'-14').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 159.1 (C-5''), 143.9 (C-1'''), 130.9 (C-2''), 129.2 (C-3'' and 7''), 128.7 (C-3''' and 5'''), 127.8 (C-2''' and 6'''), 127.0 (C-4'''), 113.7 (C-4'' and 6''), 86.6 (C$\underline{Ph_3}$), 72.5 (C-1''), 72.0 (C-1), 71.6 (C-1'), 70.2 (C-16'), 69.8 (C-2), 64.6 (C-3), 55.3 (Ph-O$\underline{Me}$), 29.8-29.5 (C-3'-14'), 26.2 y 26.1 (C-2' and 15').

ESI-HRMS (*m/z*) $C_{46}H_{62}O_5Na$ ($M+Na^+$), calc.: 717.448947, obs.: 717.4503, Δ = 1.89 ppm.

HMQC and HMBC see 2D tables.

Reaction of 43 with MeI: 44



43 **44**

To a solution of **43** (1.62 g, 2.33 mmol) in THF (5.4 mL) was added NaH 60% (149 mg, 3.73 mmol). The reaction was stirred at 75 ^{o}C for 10 minutes when MeI (0.88 mL, 12.3 mmol) was slowly added and left to stir for 6 h. After that time, the reaction was quenched by addition of ice. The mixture was extracted with EtOAc and the organic layer was washed with HCl 2M, H_2O and NaCl (sat.), dried over anhydrous Na_2SO_4, filtered and solvent removed under reduced pressure to afford **44** (1.51 g, 91%).

1-*O*-(16-(4-methoxybenzyloxy)hexadecyl)-2-*O*-methyl-3-*O*-trityl-*sn*-glycerol: **44**

$[\alpha]_D^{20}$ = -0.70 (c = 1.6, $CHCl_3$)

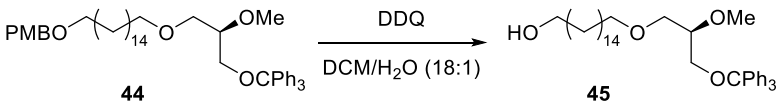
IR (liquid film) ν_{max} cm^{-1} 3059, 2926, 2852, 1612, 1512, 1448, 1247, 1099, 1035, 705.

^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) 7.50-7.43 (6H, m, C<u>Ph$_3$</u>), 7.33-7.21 (11H, m, H-3'', 7'' and C<u>Ph$_3$</u>), 6.87 (2H, d, *J* = 6.8 Hz, H-4'' and 6''), 4.43 (2H, s, H-1''), 3.78 (3H, s, Ph-O<u>Me</u>), 3.54 (1H, m, H_A-1), 3.49 (1H, m, H-2), 3.46 (1H, m, H_B-1), 3.43-3.36 (4H, m, H-1' and 16'), 3.42 (3H, s, O<u>Me</u>), 3.20 (2H, m, H-3), 1.56 (2H, m, H-2' and 15'), 1.26 (24H, br s, H-3'-14').

^{13}C NMR (50 MHz, $CDCl_3$, δ ppm) 159.1 (C-5''), 144.1 (C-1'''), 130.8 (C-2''), 129.2 (C-3'' and 7''), 128.8 (C-3''' and 5'''), 127.9 (C-2''' and 6'''), 127.0 (C-4'''), 113.8 (C-4'' and 6''), 86.6 (<u>C</u>Ph$_3$), 79.9 (C-2), 72.5 (C-1''), 71.7 (C-1'), 70.9 (C-1), 70.3 (C-16'), 62.9 (C-3), 58.1 (O<u>Me</u>), 55.2 (Ph-O<u>Me</u>), 29.8-29.6 (C-3'-14'), 26.2 and 26.1 (C-2' and 15').

ESI-HRMS (*m/z*) $C_{47}H_{64}O_5Na$ ($M+Na^+$): calc.: 731.4646, obs.: 731.4654, Δ = 1.10 ppm.

Reaction of 44 with DDQ: 45



44 **45**

To a solution of **44** (800 mg, 1.13 mmol) in DCM/H_2O 18:1 (11.4 mL) was added DDQ (255 mg, 1.13 mmol) and the reaction was stirred at room temperature for 30 minutes, controlling the progression by TLC. The reaction was quenched by addition of $NaHCO_3$ and the mixture was extracted with DCM. The organic layer was washed with $NaHCO_3$ 6% and NaCl (sat.), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to yield **45** (660 mg, 100%).

<u>(*R*)-16-(2-methoxy-3-(trityloxy)propoxy)hexadecan-1-ol</u>: **45**

$[\alpha]_D^{20}$ = -0.14 (c = 1.02, $CHCl_3$)

IR (liquid film) ν_{max} cm^{-1} 3441, 2926, 2854, 1490, 1448, 1089, 705, 632.

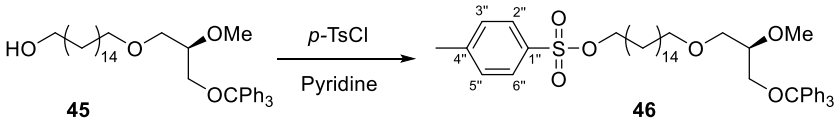
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 7.44-7.41 (6H, m, C<u>Ph$_3$</u>), 7.28-7.17 (9H, m, C<u>Ph$_3$</u>), 3.60 (2H, t, *J* = 6.7 Hz, H-16'), 3.54 (1H, dd, *J* = 9.6, 4.0 Hz, H_A-1), 3.49 (1H, dd, *J* = 9.6, 6.0 Hz, H_B-1), 3.47-3.43 (1H, m, H-2), 3.38 (3H, s, O<u>Me</u>), 3.37 (2H, t, *J* = 6.7 Hz, H-1'), 3.18 (1H, dd, *J* = 9.4, 4.6 Hz, H_A-3), 3.16 (1H, dd, *J* = 9.4, 4.6 Hz, H_B-3), 1.57-1.43 (4H, m, H-2' and 15'), 1.22 (24H, br s, H-3'-14').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 144.1 (C-1'''), 128.7 (C-3''' and 5'''), 127.7 (C-2''' and 6'''), 126.9 (C-4'''), 86.6 (<u>C</u>Ph$_3$), 79.9 (C-2), 71.7 (C-1'), 70.9 (C-1), 63.1 (C-16'), 62.9 (C-3), 58.1 (O<u>Me</u>), 32.8-25.7 (C-2'-15').

ESI-HRMS (*m/z*) $C_{39}H_{56}O_4Na$ ($M+Na^+$), calc.: 611.407082, obs.: 611.4080, Δ = 1.50 ppm.

HMQC and HMBC see 2D tables.

Reaction of 45 with TsCl: 46



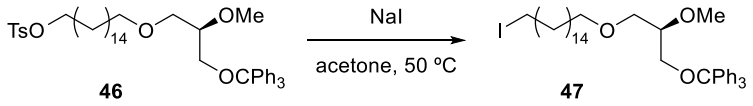
To a solution of **45** (217 mg, 0.37 mmol) in pyridine (3.2 mL) at 0 °C was added *p*-TsCl (176 mg, 0.92 mmol) and the reaction was stirred at 0 °C for 24 h. After that time, the reaction was quenched by addition of ice and the mixture was extracted with EtOAc. The organic layer was washed with HCl 2M, $NaHCO_3$ 6% and H_2O, dried over anhydrous Na_2SO_4, filtered and solvent removed *in vacuo*. The residue was purified by column chromatography (Hexanes/EtOAc 92:8) to yield **46** (110 mg, 54%) and recover 54 mg of s. m. **45** (Hexanes/EtOAc 7:3)

<u>1-*O*-(16-tosyloxyhexadecyl)-2-*O*-methyl-3-*O*-trityl-*sn*-glycerol</u>: **46**

IR (liquid film) ν_{max} cm^{-1} 305, 2926, 2854, 1732, 1699, 1598, 1448, 1361, 1178, 1097, 958, 705, 555.

^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) 7.80 (2H, d, *J* = 8.6 Hz, H-2'' and 6''), 7.50-7.23 (17H, m, H-3'', 5'' and C<u>Ph$_3$</u>), 4.03 (2H, t, *J* = 6.2 Hz, H-16'), 3.57-3.37 (5H, m, H-1, 2 and 1'), 3.43 (3H, s, O<u>Me</u>), 3.23-3.20 (2H, m, H-3), 2.45 (3H, s, Ph-<u>Me</u>), 1.63-1.55 (4H, m, H-2' and 15'), 1.26 (24H, br s, H-3'-14').

Reaction of 46 with NaI: 47



46 **47**

To a solution of **46** (110 mg, 0.148 mmol) in acetone (5.0 mL) was added NaI (222 mg, 1.48 mmol) and the reaction was stirred at 50 ^{o}C for 18 h. After that time, the reaction was quenched by addition of H_2O and the mixture was extracted with EtOAc. The organic layer was washed with Na_2SO_3 10 %, $NaHCO_3$ 6% and NaCl (sat.), dried over anhydrous Na_2SO_4, filtered and solvent removed under reduced pressure to afford **47** (100 mg, 97 %).

<u>1-*O*-(16-iodohexadecyl)-2-*O*-methyl-3-*O*-trityl-*sn*-glycerol</u>: **47**

$[\alpha]_D^{20}$ = -1.0 (c = 0.25, $CHCl_3$)

IR (liquid film) ν_{max} cm^{-1} 3059, 2924, 2852, 1732, 1699, 1598, 1448, 1118, 705.

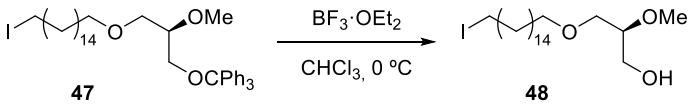
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 7.47-7.44 (6H, m, C<u>Ph$_3$</u>), 7.31-7.20 (9H, m, C<u>Ph$_3$</u>), 3.57 (1H, dd, *J* = 9.6, 4.0 Hz, H_A-1), 3.52 (1H, dd, *J* = 9.6, 5.6 Hz, H_B-1), 3.50-3.45 (1H, m, H-2), 3.41 (2H, t, *J* = 6.8 Hz, H-1'), 3.41 (3H, s, O<u>Me</u>), 3.21 (1H, dd, *J* = 10.0, 4.6 Hz, H_A-3), 3.20 (1H, dd, *J* = 10.0, 4.6 Hz, H_B-3), 3.19 (2H, t, *J* = 6.8 Hz, H-16'), 1.82 (2H, quin, *J* = 6.8 Hz, H-15'), 1.56-1.49 (4H, m, H-2' and 14'), 1.25 (22H, br s, H-3'-13').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 144.1 (C-1'''), 128.7 (C-3''' and 5'''), 127.7 (C-2''' and 6'''), 126.9 (C-4'''), 86.6 (<u>C</u>Ph_3), 79.9 (C-2), 71.7 (C-1'), 70.9 (C-1), 62.9 (C-3), 58.1 (O<u>Me</u>), 33.6 (C-15'), 30.5-26.1 (C-2'-14'), 7.4 (C-16').

ESI-HRMS (*m/z*) $C_{39}H_{55}O_3INa$ ($M+Na^+$), calc.: 721.3088, obs.: 721.3081, Δ = -0.99 ppm.

HMQC and HMBC see 2D tables.

Reaction of 47 with $BF_3{\cdot}OEt_2$: 48



47 **48**

To a solution of **47** (80 mg, 0.114 mmol) in $CHCl_3$ (1.4 mL) was slowly added $BF_3{\cdot}OEt_2$ (72 µL, 0.57 mmol) at 0 °C under argon atmosphere and the reaction was stirred for 1 h, controlling the progression by TLC. After that time, the reaction was quenched by addition of $CHCl_3/H_2O$/MeOH 2:2:1 and extracted with $CHCl_3$. The organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by column chromatography (Hexanes/EtOAc 8:2) to give **48** (47 mg, 90 %).

(*S*)-3-(16-iodohexadecyloxy)-2-methoxypropan-1-ol: **48**

$[\alpha]_D^{20}$ = -1.05 (c = 0.65, $CHCl_3$)

IR (liquid film) ν_{max} cm^{-1} 3437, 2924, 2852, 1463, 1118, 719

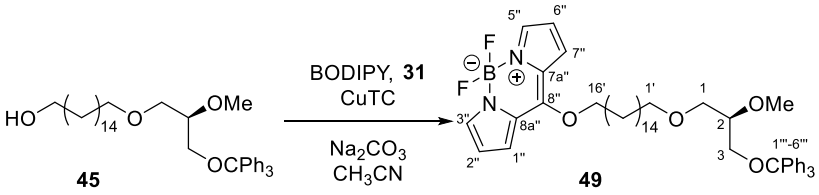
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 3.76 (1H, dd, *J* = 11.4, 2.4 Hz, H_A-3), 3.65 (1H, dd, *J* = 11.4, 5.2 Hz, H_B-3), 3.56 (1H, dd, *J* = 10.0, 5.0 Hz, H_A-1), 3.52 (1H, dd, *J* = 10.0, 5.0 Hz, H_B-1), 3.47 (3H, s, OMe), 3.45-3.37 (3H, m, H-2 and 1'), 3.19 (2H, t, *J* =7.2 Hz, H-16'), 2.17 (1H, br s, OH), 1.82 (2H, quin, *J* =7.2 Hz, H-15'), 1.57 (2H, quin, *J* =7.2 Hz, H-2'), 1.25 (24H, br s, H-3'-14').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 79.8 (C-2), 71.9 (C-1'), 70.6 (C-1), 62.7 (C-3), 57.8 (OMe), 33.6 (C-15'), 30.5 (C-14'), 29.6-26.1 (C-2'-13'), 7.4 (C-16').

ESI-HRMS (*m/z*) $C_{20}H_{42}IO_3$ ($M+H^+$), calc.: 457.2173, obs.: 457.2164, Δ = -1.97 ppm.

HMQC and HMBC see 2D tables.

Reaction of 45 with BODIPY: 49



To a solution of **31** (160 mg, 0.68 mmol) in CH_3CN (2.8 mL) under argon atmosphere was added CuTC (142 mg, 0.68 mmol) and a solution of **45** (132 mg, 0.22 mmol) in CH_3CN (1 mL) and the mixture was stirred at room temperature for 10 minutes. After that time, Na_2CO_3 (60 mg, 0.56 mmol) was added and the reaction was stirred at 55 ^oC for 12 h. Then, solvent was removed *in vacuo* and the resulting residue was purified by flash chromatography (hexanes/EtOAc 9:1) to give **49** (74 mg, 42 %).

1-*O*-(16-(4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacen-8-yloxy)hexadecanyl)-2-*O*-methyl-3-*O*-trityl-*sn*-glycerol: **49**

$[\alpha]_D^{20}$ = 0.40 (c = 1.01, $CHCl_3$).

IR (liquid film) ν_{max} cm^{-1} 3059, 2926, 2854, 1724, 1556, 1400, 1259, 1128, 765.

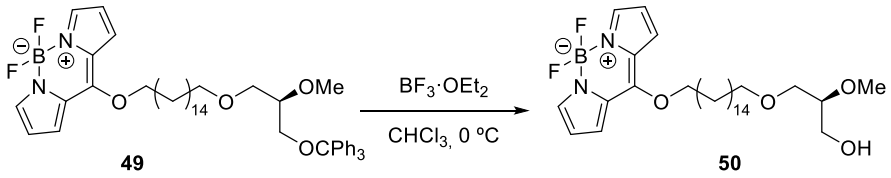
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 7.70 (2H, s, H-3'' and 5''), 7.44-7.41 (6H, m, CPh_3), 7.30 (2H, d, *J* = 4.1 Hz, H-1'' and 7''), 7.28-7.17 (9H, m, CPh_3), 6.49-6.48 (2H, m, H-2'' and 6''), 4.66 (2H, t, *J* = 6.2 Hz, H-16'), 3.54 (1H, dd, *J* = 10.0, 4.0 Hz, H_A-1), 3.50 (1H, dd, *J* = 10.0, 5.0 Hz, H_B-1), 3.47-3.42 (1H, m, H-2), 3.38 (2H, t, *J* = 6.7 Hz, H-1'), 3.38 (3H, s, OMe), 3.18 (1H, dd, *J* = 9.9, 4.8 Hz, H_A-3), 3.16 (1H, dd, *J* = 9.9, 4.8 Hz, H_B-3), 1.96-1.89 (2H, m, H-15'), 1.53-1.46 (4H, m, H-2' and 14'), 1.23 (22H, br s, H-3'-13').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 161.5 (C-8''), 144.0 (C-1'''), 139.0 (C-3'' and 5''), 128.7 (C-3''' and 5'''), 127.7 (C-2''' and 6'''), 126.9 (C-4'''), 126.2 (C-7a'' and 8a''), 125.1 (C-1'' and 7''), 116.2 (C-2'' and 6''), 86.6 (CPh_3), 79.8 (C-2), 75.4 (C-16'), 71.7 (C-1'), 70.9 (C-1), 62.8 (C-3), 58.1 (OMe), 29.7-25.8 (C-2'-15').

ESI-HRMS (*m/z*) $C_{48}H_{61}O_4BN_2F_2Na$ ($M+Na^+$), calc.: 801.458467, obs.: 801.4595, Δ = 1.29 ppm.

HMQC and HMBC see 2D tables.

Reaction of 49 with BF_3: 50



49 **50**

To a solution of **49** (54 mg, 0.068 mmol) in $CHCl_3$ (1.4 mL) at 0 ^oC and under argon atmosphere was slowly added $BF_3{\cdot}OEt_2$ (34 µL, 0.138 mmol) and the reaction was stirred at 0 ^oC for 1.5 h, controlling the progression by TLC. After that time, the reaction was quenched by addition of $CHCl_3/H_2O$/MeOH 2:2:1. The mixture was extracted with $CHCl_3$ and the organic layer was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (Hexanes/EtOAc 8:2) to afford **50** (20 mg, 54 %).

(*S*)-3-(16-(4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacen-8-yloxy)hexadecyloxy)-2-methoxypropan-1-ol: **50**

$[\alpha]_D^{20}$ = -3.0 (c = 0.05, $CHCl_3$)

Espectro IR ν_{max} cm^{-1} 3454, 2926, 2854, 1726, 1556, 1408, 1259, 1114, 968, 767.

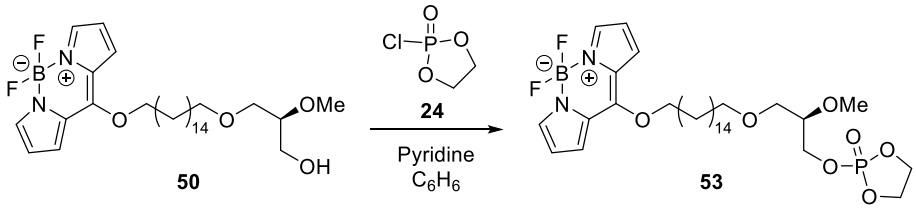
Espectro RMN ^{1}H (400 MHz, $CDCl_3$, δ ppm) 7.73 (2H, s , H-3'' and 5''), 7.34 (2H, d, *J* = 4.2 Hz, H-1'' and 7''), 6.52 (2H, dd, *J* = 4.2, 2.0 Hz, H-2'' and 6''), 4.70 (1H, t, *J* = 6.4 Hz, H-16'), 3.76 (1H, dd, *J* =11.8, 4.6 Hz, H_A-3), 3.65 (1H, dd, *J* =11.8, 4.6 Hz, H_B-3), 3.56 (1H, dd, *J* =10.0, 5.0 Hz, H_A-1), 3.53 (1H, dd, *J* =10.0, 5.0 Hz, H_B-1), 3.47 (3H, s, OMe), 3.45-3.41 (3H, m, H-2 and 1'), 1.96 (2H, m, H-15'), 1.60-1.50 (4H, m, H-2' and 14'), 1.26 (22H, br s, H-3'-13').

Espectro RMN ^{13}C (100 MHz, $CDCl_3$, δ ppm) 161.5 (C-8''), 139.0 (C-3'' and 5''), 126.2 (C-7a'' and 8a''), 125.1 (C-1'' and 7''), 116.2 (C-2'' and 6''), 79.8 (C-2), 75.4 (C-16'), 71.9 (C-1'), 70.6 (C-1), 62.7 (C-3), 57.8 (OMe), 29.6-25.8 (C-2'-15').

ESI-HRMS (*m/z*) $C_{29}H_{51}O_4BN_3F_2$ ($M+NH_4^+$), calc.: 553.3972, obs.: 553.3965, Δ = -1.10 ppm.

HMQC and HMBC see 2D tables.

Reaction of 50 with 24 and NMe_3: 53

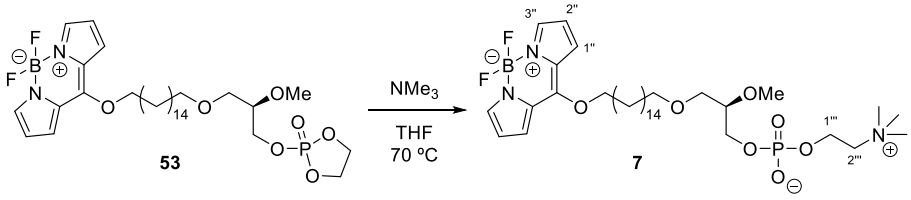


To a solution of **50** (14 mg, 0.026 mmol) in C_6H_6 (1 mL) at 0 °C under argon atmosphere, pyridine (0.1 mL) and 2-chloro-2-oxa-1,3,2-dioxaphospholane **24** (20 µL, 0.208 mmol) were added and the reaction mixture was stirred at that temperature for 12 h. After that time, solvent was removed *in vacuo* and the resulting residue was purified by flash chromatography eluting with $CHCl_3$/MeOH 98:2 to obtain **53** (12 mg, 72%).

2-((*R*)-3-(16-(4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacen-8-yloxy)hexadecyl)-2-methoxypropoxy)-1,3,2-dioxaphospholane 2-oxide: **53**

^{1}H NMR (200 MHz, $CDCl_3$, δ ppm) 7.73 (2H, br s, H-3'' and 5''), 7.34 (2H, d, *J* = 4.1 Hz, H-1'' and 7''), 6.52 (2H, dd, *J* = 4.1, 2.0 Hz, H-2'' and 6''), 4.70 (2H, t, *J* = 6.2 Hz, H-16'), 4.46-4.16 (6H, m, H-3 and PO-CH_2-CH_2-O), 3.55-3.39 (5H, m, H-1, 2 and 1'), 3.47 (3H, s, OMe), 2.00-1.89 (2H, m, H-15'), 1.55 (4H, m, H-2' and 14'), 1.26 (22H, br s, H-3'-13').

Reaction of 53 with NMe_3: 7



A 1M solution of NMe_3 in THF (1 mL, 1 mmol) was added to a sealed tube containing a solution of **53** (12 mg, 0.018 mmol) in THF (1 mL) under argon atmosphere and the reaction was stirred at 70 ^{o}C for 24 h. The mixture was then cooled down and solvent was removed under reduced pressure. The obtained residue was purified by flash chromatography using $CHCl_3$/MeOH/H_2O 65:30:5 as eluent to afford **7** (2 mg, 15%).

1-*O*-(16-(4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacen-8-yloxy)hexadecanyl)-2-*O*-methyl-*sn*-glycero-3-phosphocholine: **7**

UV (EtOH) (λ_{max} nm) 502, 442, 297.

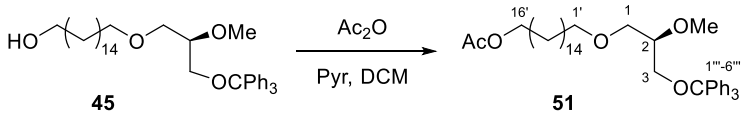
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 7.72 (2H, s, H-3'' and 5''), 7.34 (2H, s, H-1'' and 7''), 6.51 (2H, s, H-2'' and 6''), 4.69 (2H, t, *J* = 6.1 Hz, H-16'), 4.32-4.26 (2H, m, H-1''') 3.96-3.81 (2H, m, H-3), 3.70 (2H, br s, H-2'''), 3.48-3.42 (3H, m, H-1 and 2), 3.39 (3H, s, OMe), 3.36 (2H, t, *J* = 6.8 Hz, H-1'), 3.23 (9H, br s, NMe_3), 1.96-1.93 (2H, m, H-15'), 1.52-1.48 (4H, m, H-2' and 14'), 1.25 (22H, br s, H-3'-13').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 161.5 (C-8''), 139.0 (C-3'' and 5''), 126.2 (C-7a'' and 8a''), 125.2 (C-1'' and 7''), 116.2 (C-2'' and 6''), 79.5 (C-2), 75.4 (C-16'), 71.7 (C-1'), 70.1 (C-1), 66.4 (C-2'''), 65.0 (C-3), 59.2 (C-1'''), 57.7 (OMe), 54.4 (NMe_3), 29.7-25.8 (C-2'-15').

ESI-HRMS (*m/z*) $C_{34}H_{60}O_7N_3BF_2P$ ($M+H^+$), calc.: 701.4261, obs.: 701.4257, Δ = -0.60 ppm.

HSQC see Spectroscopy.

Reaction of 45 with Ac_2O: 51



45 **51**

To a solution of **45** (190 mg, 0.32 mmol) in DCM (4 mL) was added Ac_2O (0.5 mL) and piridina (0.5 mL) and the reaction was stirred at room temperature for 12 h. The mixture was then extracted with DCM and the organic layer was washed with HCl 2M, $NaHCO_3$ 6% y NaCl (sat.), dried over anhydrous Na_2SO_4, filtered and solvent removed under reduced pressure. The residue was purified by column chromatography (Hexanes/EtOAc 95:5) to give **51** (190 mg, 93 %).

(*R*)-16-(2-methoxy-3-(trityloxy)propoxy)hexadecyl acetate: **51**

$[\alpha]_D^{20}$ = 0.41 (c = 1.04, $CHCl_3$).

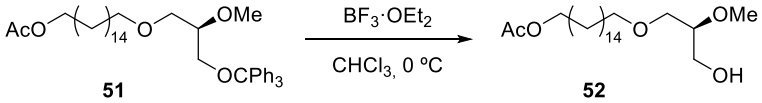
IR (liquid film) ν_{max} cm^{-1} 3059, 2926, 2854, 1741, 1448, 1365, 1238, 1118, 705.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 7.44-7.41 (6H, m, C$\underline{Ph_3}$), 7.28-7.17 (9H, m, C$\underline{Ph_3}$), 4.02 (2H, t, *J* = 6.7 Hz, H-16'), 3.54 (1H, dd, *J* = 9.6, 4.0 Hz, H_A-1), 3.49 (1H, dd, *J* = 9.6, 6.0 Hz, H_B-1), 3.47-3.42 (1H, m, H-2), 3.38 (3H, s, O$\underline{Me}$), 3.37 (2H, t, *J* = 6.7 Hz, H-1'), 3.18 (1H, dd, *J* = 9.4, 4.6 Hz, H_A-3), 3.16 (1H, dd, *J* = 9.4, 4.6 Hz, H_B-3), 2.04 (3H, s, O$\underline{Ac}$), 1.62-1.46 (4H, m, H-2' and 15'), 1.22 (24H, br s, H-3'-14').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 171.1 (OO$\underline{C}CH_3$) 144.1 (C-1'''), 128.7 (C-3''' and 5'''), 127.7 (C-2''' and 6'''), 126.9 (C-4'''), 86.6 ($\underline{C}Ph_3$), 79.7 (C-2), 71.7 (C-1'), 70.9 (C-1), 64.7 (C-16'), 62.9 (C-3), 58.1 (O$\underline{Me}$), 29.7-25.9 (C-2'-15'), 21.0 (OOC$\underline{CH_3}$).

ESI-HRMS (*m/z*) $C_{41}H_{62}O_5N$ ($M+NH_4^+$): calc.: 648.4623, obs.: 648.4610, Δ =-1.93 ppm.

Reaction of 51 with $BF_3{\cdot}OEt_2$: 52



51 **52**

To a solution of **51** (190 mg, 0.30 mmol) in $CHCl_3$ (3 mL) at 0 ^oC was slowly added $BF_3{\cdot}OEt_2$ (152 µL, 1.2 mmol) under argon atmosphere. The reaction was stirred at 0 ^oC for 90 minutes, controlling the progression by TLC. After that time, the reaction was quenched by addition of $H_2O/CHCl_3$/MeOH 2:2:1. The mixture was then extracted with $CHCl_3$ and the organic layer was dried over anhydrous Na_2SO_4, filtered and solvent removed *in vacuo*. The residue was purified by column chromatography (Hexanes/EtOAc 8:2) to afford **52** (106 mg, 91%).

1-*O*-(16-acetoxyhexadecyl)-2-*O*-methyl-*sn*-glycerol: **52**

$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ = -1.96 (c = 0.25, $CHCl_3$)

IR (liquid film) ν_{max} cm^{-1} 3466, 2926, 2854, 1741, 1465, 1365, 1240, 1118, 1043, 669.

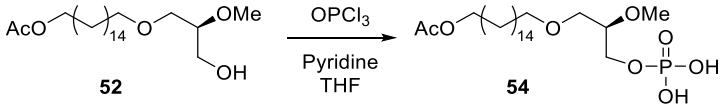
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 4.04 (2H, t, *J* = 6.7 Hz, H-16'), 3.77-3.73 (1H, m, H_A-3), 3.66-3.61 (1H, m, H_B-3), 3.55 (1H, dd, *J* = 10.0, 5.0 Hz, H_A-1), 3.52 (1H, dd, *J* = 10.0, 5.0 Hz, H_B-1), 3.46 (3H, s, OMe), 3.46-3.42 (3H, m, H-2 and 1'), 2.04 (3H, s, OAc), 1.64-1.53 (4H, m, H-2' and 15'), 1.25 (24H, br s, H-3'-14').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 171.2 ($OO\underline{C}CH_3$), 79.8 (C-2), 71.9 (C-1'), 70.6 (C-1), 64.7 (C-16'), 62.7 (C-3), 57.8 (OMe), 29.6-25.9 (C-2'-15'), 21.0 ($OOC\underline{CH_3}$).

ESI-HRMS (*m/z*) $C_{22}H_{45}O_5$ ($M+H^+$), calc.: 389.326151, obs.: 389.3259, Δ = -0.64 ppm.

HMQC and HMBC see 2D tables.

Reaction of 52 with $OPCl_3$: 54



To a solution of **52** (100 mg, 0.26 mmol) in THF (1.3 mL) at 0 °C was added pyridine (44 µL, 0.54 mmol) and $OPCl_3$ (27 µL, 0.28 mmol) under argon atmosphere and the reaction was stirred at0 °C for 3.5 h, plus other 1.5 h at room temperature. After that time, the reaction was quenched by addition of $NaHCO_3$ (6%) and stirring for 15 minutes. Subsequently, HCl 2M was added dropwise until reaching pH = 2. The mixture was then extracted with plenty EtOAc (3 x 15 mL) and the organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give **54** (120 mg, 98%).

1-*O*-(16-acetoxyhexadecyl)-2-*O*-methyl-*sn*-glycero-3-phosphate: **54**

IR (liquid film) ν_{max} cm^{-1} 2916, 2850, 1740, 1465, 1114, 1053, 1020, 966.

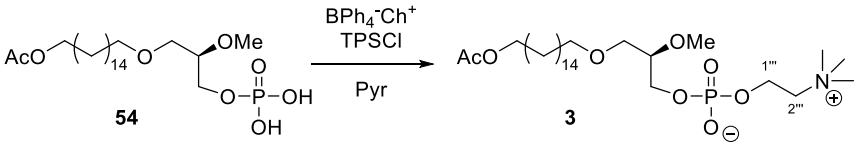
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 4.16-4.03 (2H, m, H-3), 4.05 (2H, t, *J* = 6.7 Hz, H-16'), 3.63-3.57 (1H, m, H-2), 3.56-3.52 (2H, m, H-1), 3.49 (3H, s, OMe), 3.45 (3H, t, *J* = 6.4 Hz, H-1'), 2.04 (3H, s, OAc), 1.65-1.53 (4H, m, H-2' and 15'), 1.25 (24H, br s, H-3'-14').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 171.4 (OO<u>C</u>CH_3), 79.1 (C-2), 72.0 (C-1'), 69.5 (C-1), 65.8 (C-3), 64.7 (C-16'), 57.8 (OMe), 29.7-25.9 (C-2'-15'), 21.0 (OOC<u>C</u>H_3).

ESI-HRMS (*m/z*) $C_{22}H_{45}O_8PNa$ ($M+Na^+$), calc.: 491.274428, obs.: 419.2744, Δ = -0.06 ppm.

HMQC and HMBC see 2D tables.

Reaction of 54 with choline tetraphenylborate: 3



To a solution of **54** (120 mg, 0.26 mmol) in pyridine (2 mL) was added $BPh_4^-Ch^+$ (108 mg, 0.26 mmol) and TPSCl (97 mg, 0.32 mmol) under argon atmosphere and the reaction was stirred at 70 ^oC for 2 h. After that time, the reaction was cooled to room temperature and left to stir for 12 h. Then, H_2O was added and the mixture was stirred for 10 minutes. The solvent was then removed *in vacuo* and the obtained residue was purified by column chromatography ($CHCl_3$/MeOH/H_2O 65:30:5) to yield **3** (58 mg, 41%).

1-*O*-(16-acetoxyhexadecyl)-2-*O*-methyl-*sn*-glycero-3-phosphocholine: **3**

$[\alpha]_D^{20}$ = -0.32 (c = 0.70, $CHCl_3$)

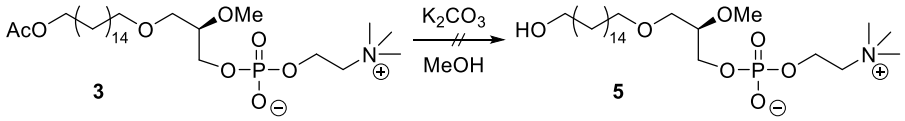
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 4.41 (2H, br s, H-1'''), 4.04 (2H, t, *J* = 6.7 Hz, H-16'), 4.02-3.95 (2H, m, H-3), 3.94-3.88 (2H, m, H-2'''), 3.54-3.36 (5H, m, H-1, 2 and 1'), 3.43 (12H, br s, OMe y NMe_3), 2.03 (3H, s, OAc), 1.64-1.50 (4H, m, H-2' and 15'), 1.24 (24H, br s, H-3'-14').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 171.2 ($OOCCH_3$), 79.3 (C-2), 71.8 (C-1'), 69.8 (C-1), 66.0 (C-3), 65.7 (C-2'''), 64.6 (C-16'), 59.8 (C-1'''), 57.9 (OMe), 54.4 (NMe_3), 29.7-25.9 (C-2'-15'), 21.0 ($OOCCH_3$).

ESI-HRMS (*m/z*) $C_{27}H_{57}O_8P$ ($M+H^+$), calc.: 554.3816, obs.: 554.3818, Δ = 0.30 ppm.

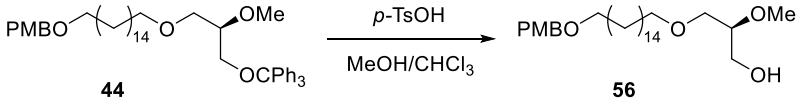
HMQC see Spectroscopy.

Reaction of 3 with K_2CO_3: 5



To a solution of **3** (17 mg, 0.03 mmol) in MeOH (1 mL) was added K_2CO_3 (14 mg, 0.11 mmol) and the reaction was stirred at room temperature for 12 h, controlling the progression with TLC. After that time, the reaction was diluted with $CHCl_3$ (1.5 mL) until reaching the proportion $CHCl_3$/MeOH 6:4, and the mixture was purified by column chromatography using $CHCl_3$/MeOH (8:2) and $CHCl_3$/MeOH/H_2O (65:30:5) as eluent. Desired product was not obtained.

Reaction of 44 with *p*-TsOH: 56



To a solution of **44** (700 mg, 0.99 mmol) in MeOH/$CHCl_3$ 4:1 (12.3 mL) was added *p*-TsOH (171 mg, 0.90 mmol) and 0.1 mL of H_2O under argon atmosphere and the reaction was stirred at room temperature for 1 h, controlling the progression by TLC. Then, the mixture was extracted with EtOAc, washed with con $NaHCO_3$ 6% and NaCl (sat.), dried over anhydrous Na_2SO_4, filtered and solvent removed *in vacuo*. The residue was purified by column chromatography (Hexanes /EtOAc 8:2) to obtain **56** (340 mg, 73%).

(*S*)-2-methoxy-3-(16-(4-methoxybenzyloxy)hexadecyloxy)propan-1-ol: **56**

$[\boldsymbol{\alpha}]_{\boldsymbol{D}}^{\boldsymbol{20}}$ = -0.93 (c = 0.90, $CHCl_3$)

IR (liquid film) ν_{max} cm^{-1} 3446, 2924, 2852, 1716, 1608, 1514, 1465, 1247, 1114, 821.

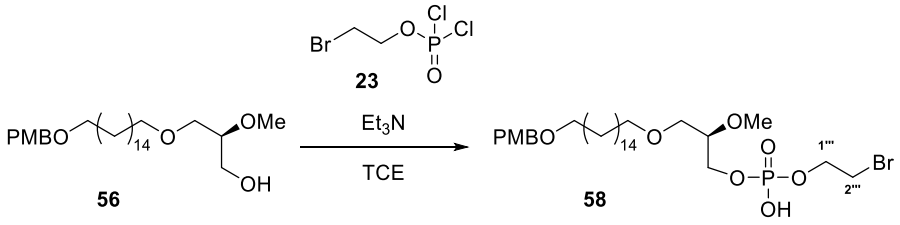
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 7.26 (2H, d, *J* = 8.5 Hz, H-3'' and 7''), 6.87 (2H, d, *J* = 8.5 Hz, H-4'' and 6''), 4.43 (2H, s, H-1''), 3.80 (3H, s, Ph-OMe), 3.78-3.73 (1H, m, H_A-3), 3.67-3.62 (1H, m, H_B-3), 3.55 (1H, dd, *J* = 9.9, 5.0 Hz, H_A-1), 3.52 (1H, dd, *J* = 9.9, 5.0 Hz, H_B-1), 3.47 (3H, s, OMe), 3.46-3.41 (5H, m, H-2, 1' and 16'), 2.18 (1H, t, *J* =5.2 Hz, OH) 1.63-1.53 (4H, m, H-2' and 15'), 1.25 (24H, br s, H-3'-14').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 159.1 (C-5''), 130.8 (C-2''), 129.2 (C-3'' and 7''), 113.7 (C-4'' and 6''), 79.8 (C-2), 72.5 (C-1''), 71.9 (C-1'), 70.6 (C-1), 70.2 (C-16'), 62.7 (C-3), 57.8 (OMe), 55.2 (Ph-OMe), 29.8-29.4 (C-3'-14'), 26.2 and 26.1 (C-2' and 15').

ESI-HRMS (*m/z*) $C_{28}H_{50}O_5Na$ ($M+Na^+$), calc.: 489.3551, obs.: 489.3538, Δ = -2.63 ppm.

HMQC and HMBC see 2D tables.

Reaction of 56 with 43: 58



To a solution of **23** (245 mg, 0.5 mmol) in TCE (0.6 mL) at 0 °C under argon atmosphere was added a solution of **56** (238 mg, 0.5 mmol) and Et_3N (106 µL, 0.75 mmol) in TCE (0.7 mL) and the reaction was stirred for 12 h, removing generated HCl (g) every 3 minutes in the first hour by passing a stream of argon. After that time, the reaction was quenched by addition of ice and pyridine (3 eq) and left to stir for 1 h. Then, HCl 2M was slowly added until reaching pH = 2, when the organic layer is separated and neutralized by slowly addition of a 10% aqueous solution of $Ba(OAc)_2$. After that, the organic layer was concentrated *in vacuo* and the residue was crystalized in acetone at 0 °C, affording a white solid. The obtained residue was solved in $CHCl_3$ and was added

HCl 2M until reaching pH = 2. The organic layer was separated and concentrated *in vacuo* to give **58** (173 mg, 52 %).

2-Bromoethyl ((*R*)-2-methoxy-3-(16-(4-methoxybenzyloxy)hexadecyloxy)propyl) hydrogen phosphate: **58**

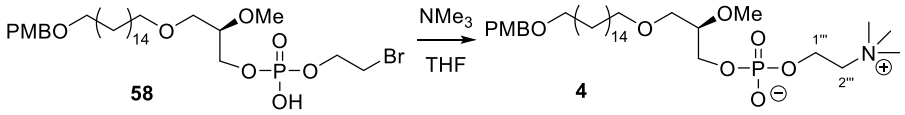
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 7.26 (2H, d, *J* = 8.7 Hz, H-3'' and 7''), 6.87 (2H, d, *J* = 8.7 Hz, H-4'' and 6''), 4.43 (2H, s, H-1''), 4.29 (2H, dt, *J* =7.6, 6.5 Hz, H-1'''), 4.21-4.16 (1H, m, H_A-3), 4.12-4.06 (1H, m, H_B-3), 3.80 (3H, s, Ph-OMe), 3.59-3.50 (5H, m, H-1, 2 and 2'''), 3.47 (3H, s, OMe), 3.45-3.41 (4H, m, H-1' and 16'), 1.63-1.53 (4H, m, H-2' and 15'), 1.25 (24H, br s, H-3'-14').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 159.0 (C-5''), 130.8 (C-2''), 129.2 (C-3'' and 7''), 113.7 (C-4'' and 6''), 78.7 (C-2, d, *J* = 7 Hz), 72.5 (C-1''), 71.9 (C-1'), 70.2 (C-16'), 69.1 (C-1), 66.9 (C-3, d, *J* = 5 Hz), 66.5 (C-1''', d, *J* = 5 Hz) 58.1 (OMe), 55.2 (Ph-OMe), 29.7-29.5 (C-3'-14'), 29.1 (C-2''', d, *J* = 8 Hz), 26.2 and 26.0 (C-2' and 15').

ESI-HRMS (*m/z*) $C_{30}H_{54}O_8{}^{79}BrPNa$ ($M+Na^+$), calc.: 675.2632, obs.: 675.2623, Δ = -1.27 ppm.

HMQC and HMBC see 2D tables.

Reaction of 58 with trimethylamine: 4



In a pear-shaped flask containing **58** (60 mg, 0.09 mmol) was added a 1 M solution of NMe_3 in THF (1.00 mL, 1.00 mmol) under argon atmosphere and the reaction was stirred at room temperature for 2 days. After that time, the mixture was concentrated *in vacuo* and the residue was purified by flash chromatography ($CHCl_3$/MeOH/H_2O 65:30:5) to yield **4** (24 mg, 41 %).

1-*O*-(16-((4-methoxybenzyl)oxy)hexadecyl)-2-*O*-methyl-*sn*-glycero-3-phosphocholine: **4**

$[\alpha]_D^{20}$ = +1.3 (c = 0.40, $CHCl_3$)

IR (liquid film) ν_{max} cm^{-1} 2922, 2852, 1722, 1265, 1246, 1097, 727.

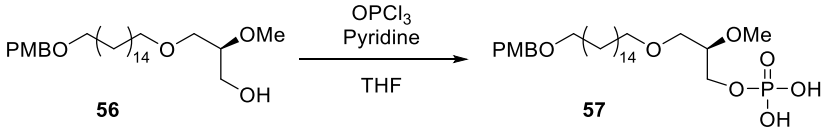
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 7.24 (2H, d, *J* = 8.4 Hz, H-3'' and 7''), 6.86 (2H, d, *J* = 8.4 Hz, H-4'' and 6''), 4.41 (2H, s, H-1''), 4.28 (2H, br s, H-1'''), 3.94-3.88 (1H, m, H_A-3), 3.84-3.78 (1H, m, H_B-3), 3.78 (3H, s, Ph-OMe), 3.70 (2H, br s, H-2'''), 3.52-3.48 (2H, m, H-1_A and 2), 3.46-3.38 (5H, m, H-1_B, 1' and 16'), 3.42 (3H, s, OMe), 3.28 (9H, br s, NMe_3), 1.61-1.51 (4H, m, H-2' and 15'), 1.24 (24H, br s, H-3'-14').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 159.0 (C-5''), 130.8 (C-2''), 129.2 (C-3'' and 7''), 113.7 (C-4'' and 6''), 79.4 (C-2, d, *J* = 8 Hz), 72.4 (C-1''), 71.8 (C-1'), 70.2 (C-16'), 70.1 (C-1), 66.2 (C-2''', d, *J* = 6 Hz), 65.0 (C-3, d, *J* = 5 Hz), 59.3 (C-1''', d, *J* = 4 Hz), 57.7 (OMe), 55.2 (Ph-OMe), 54.3 (NMe_3), 29.8-29.5 (C-3'-14'), 26.2 and 26.1 (C-2' and 15').

ESI-HRMS (*m/z*) $C_{33}H_{63}O_8NP$ ($M+H^+$), calc.: 632.42858, obs.: 632.4279, Δ = -0.66 ppm.

HMQC and HMBC see 2D tables.

Reaction of 56 with $OPCl_3$: 57



56 **57**

To a solution of **56** (82 mg, 0.176 mmol) in THF (0.8 mL) at 0 °C was added pyridine (0.30 mL, 3.71 mmol) and $OPCl_3$ (18 µL, 0.196 mmol) under argon atmosphere and the reaction was stirred at 0 °C for 3.5 h, plus other 1.5 h at room temperature. After that time, the reaction was quenched by addition of $NaHCO_3$ (6%) and stirring for 15 minutes. Subsequently, HCl 2M was added dropwise until reaching pH = 2. The mixture was then extracted with plenty EtOAc (3 x 10 mL) and the organic layer was washed

with H_2O, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to give **57** (85 mg, 88%).

(2*R*)-2-methoxy-3-(16-(4-methoxybenzyloxy)hexadecyloxy)propyl dihydrogen phosphate: **57**

IR (liquid film) ν_{max} cm^{-1} 2918, 2850, 1614, 1516, 1467, 1249, 1101, 819.

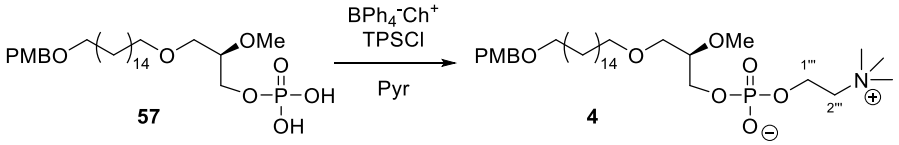
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 7.25 (2H, d, *J* = 8.6 Hz, H-3'' and 7''), 6.86 (2H, d, *J* = 8.6 Hz, H-4'' and 6''), 4.42 (2H, s, H-1''), 4.02 (2H, m, H-3), 3.79 (3H, s, Ph-OMe), 3.56-3.40 (7H, m, H-1, 2, 1' and 16'), 3.44 (3H, s, OMe), 1.61-1.52 (4H, m, H-2' and 15'), 1.24 (24H, br s, H-3'-14').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 159.0 (C-5''), 130.8 (C-2''), 129.2 (C-3'' and 7''), 113.7 (C-4'' and 6''), 79.0 (C-2), 72.5 (C-1''), 71.8 (C-1'), 70.2 (C-16'), 69.9 (C-1), 65.5 (C-3), 57.9 (OMe), 55.2 (Ph-OMe), 29.7-29.5 (C-3'-14'), 26.1 and 26.0 (C-2' and 15').

ESI-HRMS (*m/z*) $C_{28}H_{50}O_8P$ ($M\text{-}H^+$): calc.: 545.32378, obs.: 545.3247, Δ = 1.7 ppm.

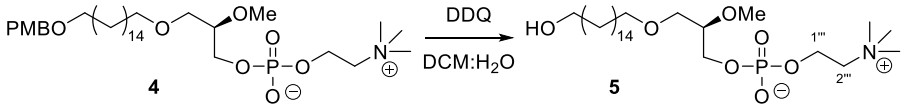
HMQC and HMBC see 2D tables.

Reaction of 57 with choline tetraphenylborate: 4



To a solution of **57** (80 mg, 0.15 mmol) in pyridine (1.1 mL) under argon atmosphere was added $BPh_4^-Ch^+$ (62 mg, 0.15 mmol) and TPSCl (55 mg, 0.18 mmol) and the reaction was stirred at 70 °C for 2 h. Then, the reaction was cooled to room temperature and left to stir for 12 h. After that time, the reaction was quenched by addition of H_2O, then, the solvent was removed *in vacuo*. The resulting residue was purified by column chromatography ($CHCl_3$/MeOH/H_2O 65:30:5) to afford **4** (10 mg, 11%).

Reaction of 4 with DDQ: 5



To a solution of **4** (34 mg, 0.52 mmol) in DCM/H_2O 18:1 (1 mL) was added DDQ (14 mg, 0.062 mmol) under argon atmosphere and the reaction was stirred at room temperature for 6.5 h, controlling the progression by TLC. After that time, the mixture was purified by column chromatography ($CHCl_3$/MeOH/H_2O 65:30:5) to give **5** (18 mg, 67 %).

1-*O*-(16-hydroxyhexadecyl)-2-*O*-methyl-*sn*-glycero-3-phosphocholine: **5**

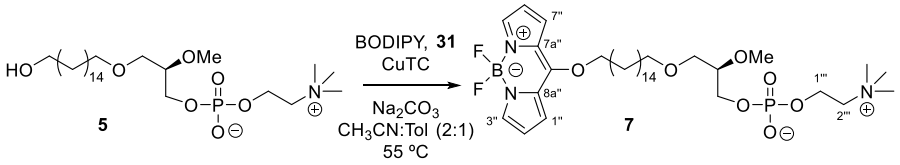
^{1}H NMR (400 MHz, Pyr-d5, δ ppm) 4.80 (2H, br s, H-1'''), 4.42 (2H, br s, H-3), 4.25-4.09 (2H, m, H-2'''), 3.89 (2H, t, *J* = 6.5 Hz, H-16'), 3.86-3.84 (1H, m, H-2), 3.79-3.75 (1H, m, H_A-1), 3.72-3.67 (1H, m, H_B-1), 3.61 (9H, s, NMe_3), 3.54 (3H, s, OMe), 3.45 (2H, t, *J* = 5.8 Hz, H-1'), 1.80-1.71 (2H, m, H-15'), 1.61-1.47 (4H, m, H-2' and 14'), 1.27 (22H, br s, H-3'-13').

^{13}C NMR (100 MHz, Pyr-d5, δ ppm) 80.1 (C-2), 71.5 (C-1'), 71.0 (C-1), 66.7 (C-2'''), 65.1 (C-3), 61.9 (C-16'), 59.6 (C-1'''), 57.6 (OMe), 54.1 (NMe_3), 33.5 (C-14'), 30.0-29.6 (C-3'-13'), 26.3 and 26.3 (C-2' and 15').

ESI-HRMS (*m/z*) $C_{25}H_{54}O_7NPNa$ ($M+Na^+$), calc.: 534.3530, obs.: 534.3530, Δ = 0.03 ppm.

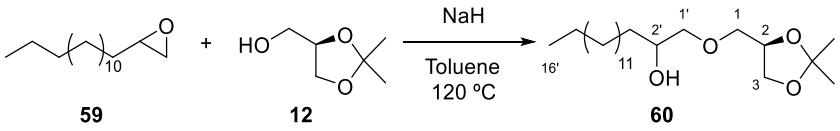
HMQC see Spectroscopy.

Reaction of 5 with BODIPY 31: 7



To a solution of **5** (15 mg, 0.03 mmol) in CH_3CN/toluene 2:1 (0.9 mL) under argon atmosphere was added **31** (37 mg, 0.15 mmol), CuTC (25 mg, 0.135 mmol) and Na_2CO_3 (10 mg, 0.105 mmol) and the reaction was stirred at 55 ^oC for 18 h. After that time, solvent was removed under reduced pressure and the residue was purified by column chromatography ($CHCl_3$/MeOH/H_2O 65:30:5) to yield **7** (6 mg, 29%).

Reaction of 1,2-epoxyhexadecane 59 with *R*-solketal: 60



To a solution of *R*-solketal **12** (1.49 g, 11.25 mmol) in toluene (35 mL) was added **59** (1.78 g, 7.39 mmol) and NaH 60% (588 mg, 14.7 mmol) under argon atmosphere and the reaction was stirred at 120 ^oC for 12 h. After that time, the reaction was quenched by addition of ice and the mixture was extracted with EtOAc. The organic layer was washed with HCl 2M y NaCl (sat.), dried over anhydrous Na_2SO_4, filtered and solvent removed *in vacuo*. The residue was purified by column chromatography (Hexanes/EtOAc 8:2) to obtain **60** (1.57 g, 67%) as a colourless oil.

1-(((*R*)-2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)hexadecan-2-ol: **60**

^1H and ^{13}C NMR spectra from the mixture of epimers of **60** are described below using **60a** and **60b** to refer each one, but no assignment of the absolute configuration could be done without further experiments.

IR (liquid film) ν_{max} cm^{-1} 3462, 2924, 2852, 1458, 1369, 1255, 1213, 1082, 844, 721.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm)

- **60a.** 4.30 (1H, dd, *J* = 11.0, 6.4 Hz, H-2), 4.06 (1H, dd, *J* = 8.3, 6.4 Hz, H_A-3), 3.79 (1H, m, H-2'), 3.74 (1H, dd, *J* = 8.3, 6.4 Hz, H_B-3), 3.56 (2H, m, H-1), 3.52 (1H, m, H_A-1'), 3.33 (1H, m, H_B-1'), 1.43 (2H, m, H-3'), 1.43 and 1.37 (3H, s each, C$\underline{Me_2}$), 1.25 (24H, br s, H-4'-15'), 0.88 (3H, t, *J* = 6.8 Hz, H-16').
- **60b.** 4.28 (1H, dd, *J* = 11.0, 6.4 Hz, H-2), 4.05 (1H, dd, *J* = 8.3, 6.4 Hz, H_A-3), 3.79 (1H, m, H-2'), 3.72 (1H, dd, *J* = 8.3, 6.4 Hz, H_B-3), 3.56 (2H, m, H-1), 3.52 (1H, m, H_A-1'), 3.33 (1H, m, H_B-1'), 1.43 (2H, m, H-3'), 1.43 and 1.37 (3H, s each, C$\underline{Me_2}$), 1.25 (24H, br s, H-4'-15'), 0.88 (3H, t, *J* = 6.8 Hz, H-16').

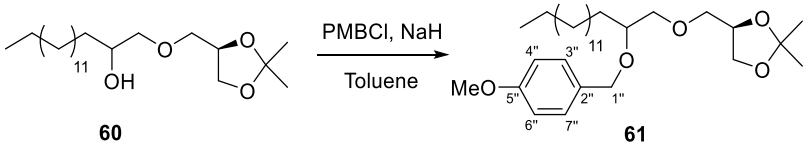
^{13}C NMR (100 MHz, $CDCl_3$, δ ppm)

- **60a.** 109.6 (C$\underline{Me_2}$), 76.2 (C-1'), 74.8 (C-2), 72.3 (C-1), 70.3 (C-2'), 66.6 (C-3), 33.0 (C-3'), 31.9 (C-14'), 29.7-29.4 (C-5'-13'), 26.7 (Me), 25.5 (C-4'), 25.4 (Me), 22.7 (C-15'), 14.1 (C-16').
- **60b.** 109.5 (C$\underline{Me_2}$), 76.2 (C-1'), 74.7 (C-2), 72.2 (C-1), 70.2 (C-2'), 66.5 (C-3), 32.9 (C-3'), 31.9 (C-14'), 29.7-29.4 (C-5'-13'), 26.7 (Me), 25.5 (C-4'), 25.4 (Me), 22.7 (C-15'), 14.1 (C-16')

ESI-HRMS (*m/z*) $C_{22}H_{44}O_4Na$ ($M+Na^+$): calc.: 395.3132, obs.: 395.3129, Δ = -0.8 ppm.

HMQC and HMBC see 2D tables.

Reaction of 60 with PMBCl: 61



60 **61**

To a solution of **60** (3.54 g, 9.5 mmol) in toluene (71 mL) was added NaH 60% (570 mg, 14.25 mmol) and PMBCl (1.64 g, 10.5 mmol) under argon atmosphere and the reaction was stirred at 120 ^{o}C for 12 h. After that time, the reaction was quenched by addition of ice and the mixture was extracted with EtOAc. The organic layer was washed with HCl 2M y NaCl (sat.), dried over anhydrous Na_2SO_4, filtered and solvent removed *in vacuo*. The residue was purified by column chromatography (Hexanes/EtOAc 95:5) to afford **61** (3.05 g, 65%) as a colourless oil.

(4*R*)-4-((2-(4-methoxybenzyloxy)hexadecyloxy)methyl)-2,2-dimethyl-1,3-dioxolane: **61**

IR (liquid film) ν_{max} cm^{-1} 2924, 2854, 1314, 1514, 1463, 1247, 1039, 844.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm)[*] 7.27 (2H, d, *J* = 8.0 Hz, H-3'' and 7''), 6.87 (2H, d, *J* = 8.0 Hz, H-4'' and 6''), 4.59 (1H, d, *J* = 12.0 Hz, H_A-1'', *R* or *S*), 4.58 (1H, d, *J* = 12.0 Hz, H_A-1'', *R* or *S*), 4.49 (1H, d, *J* = 12.0 Hz, H_B-1'', *R* and *S*), 4.26 (1H, quin, *J* = 8.0 Hz, H-2), 4.06 (1H, dd, *J* = 8.0, 6.0 Hz, H_A-3, *R* or *S*), 4.05 (1H, dd, *J* = 8.0, 6.0 Hz, H_A-3, *R* or *S*), 3.80 (3H, s, OMe), 3.77 (1H, dd, *J* = 8.0, 6.0 Hz, H_B-3, *R* or *S*), 3.75 (1H, dd, *J* = 8.0, 6.0 Hz, H_B-3, *R* or *S*), 3.58-3.45 (5H, m, H-1, 1' and 2'), 1.50-1.47 (2H, m, H-3'), 1.42 and 1.39 (3H, s each, CMe_2), 1.26 (24H, br s, H-4'-15'), 0.88 (3H, t, *J* = 6.8 Hz, H-16').

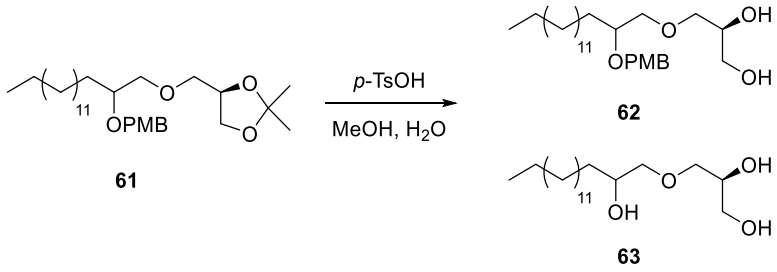
^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 159.1 (C-5''), 131.1 (C-2''), 129.4 (C-3'' and 7''), 113.7 (C-4'' and 6''), 109.3 (CMe_2), 77.7 (C-2'), 74.7 (C-2), 74.5 (C-1'), 72.4 (C-1), 71.7 (C-1''), 66.9 (C-3), 55.3 (OMe), 31.9 (C-3'), 29.7-29.4 (C-5'-14'), 26.8 and 25.4 (CMe_2), 25.4 (C-4'), 22.7 (C-15'), 14.1 (C-16').

ESI-HRMS (*m/z*) $C_{30}H_{52}O_5Na$ ($M+Na^+$): calc.: 515.3707, obs.: 515.3704, Δ = -0.5 ppm.

HMQC and HMBC see 2D tables.

[*] All those compounds whose C2' epimers could be distinguished by H^1 NMR will be marked with an asterisk (*) and "*R* or *S*" will be written beside each signal susceptible to be assigned.

Reaction of 61 with *p*-TsOH: 62 and 63.



To a solution of **61** (1.97 g, 4.00 mmol) in MeOH (14.5 mL) under argon atmosphere was added *p*-TsOH (680 mg, 4.00 mmol) and 0.1 mL of H_2O and the reaction was stirred at room temperature for 30 minutes, controlling the progression by TLC. The reaction was then quenched by addition of ice and the resulting mixture was extracted with plenty EtOAc (3 x 50 mL). The organic layer was washed with $NaHCO_3$ 6% and NaCl (sat.), dried over anhydrous Na_2SO_4, filtered and solvent removed *in vacuo*. The residue was purified by column chromatography. Eluting with hexanes/EtOAc 1:1 yielded **62** (1.39 g, 76%) and, with EtOAc, **63** (51 mg, 4%).

(2*S*)-3-(2-(4-methoxybenzyloxy)hexadecyloxy)propane-1,2-diol: **62**

IR (liquid film) ν_{max} cm^{-1} 3417, 2924, 2852, 1612, 1514, 1463, 1247, 1111, 1039, 821.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 7.27 (2H, d, *J* = 8.8 Hz, H-3'' and 7''), 6.87 (2H, d, *J* = 8.8 Hz, H-4'' and 6''), 4.53 (1H, d, *J* = 11.2 Hz, H_A-1''), 4.50 (1H, d, *J* = 11.2 Hz, H_B-1''), 3.85 (1H, m, H-2), 3.80 (3H, s, OMe), 3.69 (1H, dd, *J* = 11.4, 4.0 Hz, H_A-3), 3.62 (1H, dd, *J* = 11.4, 5.2 Hz, H_B-3), 3.59-3.51 (5H, m, H-1, 1' and 2'), 1.52 (2H, m, H-3'), 1.25 (24H, br s, H-4'-15'), 0.88 (3H, t, *J* = 6.8 Hz, H-16').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 159.2 (C-5''), 130.6 (C-2''), 129.4 (C-3'' and 7''), 113.8 (C-4'' and 6''), 77.8 (C-2'), 74.1 (C-1'), 73.2 (C-1), 71.5 (C-1''), 70.5 (C-2), 64.0 (C-3), 55.2 (OMe), 31.9 (C-14'), 31.5 (C-3'), 29.7-29.4 (C-5'-13'), 25.5 (C-4'), 22.7 (C-15'), 14.1 (C-16').

ESI-HRMS (*m/z*) $C_{27}H_{48}O_5Na$ ($M+Na^+$): calc.: 475.3394, obs.: 475.3389, Δ = -1.0 ppm.

HMQC and HMBC see 2D tables.

(2*S*)-3-(2-hydroxyhexadecyloxy)propane-1,2-diol: **63**

IR (liquid film) ν_{max} cm^{-1} 3375, 2920, 2850, 1467, 1263, 1124, 1060, 740.

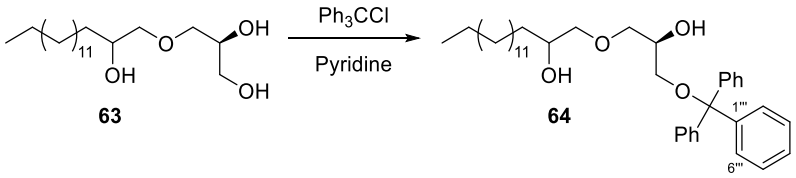
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 3.90 (1H, m, H-2), 3.80 (1H, m, H-2'), 3.71 (1H, d, *J* = 12.0 Hz, H_A-3), 3.65 (1H, m, H_B-3), 3.57 (2H, m, H-1), 3.53 (1H, m, H_A-1'), 3.35 (2H, m, H_B-1'), 1.42 (2H, m, H-3'), 1.25 (24H, br s, H-4'-15'), 0.88 (3H, t, *J* = 6.8 Hz, H-16').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 76.0 (C-1'), 72.9 (C-1), 70.6 (C-2), 70.5 (C-2'), 63.9 (C-3), 33.2 (C-3'), 31.9 (C-14'), 29.7–29.4 (C-5'-13'), 25.5 (C-4'), 22.7 (C-15'), 14.1 (C-16').

ESI-HRMS (*m/z*) $C_{19}H_{40}O_4Na$ ($M+Na^+$): calc.: 355.2819, obs.: 355.2816, Δ = -0.83 ppm.

HMQC and HMBC see 2D tables.

Reaction of 63 with $ClCPh_3$: 64



To a solution of **63** (94 mg, 0.28 mmol) in pyridine (1.2 mL) was added $ClPPh_3$ (79 mg, 0.29 mmol) under argon atmosphere and the reaction was stirred at 100 ^{o}C for 6 h. After that time, the reaction was quenched by addition of H_2O and the mixture was extracted with EtOAc. The organic layer was washed with HCl 2M, $NaHCO_3$ 6% and NaCl (sat.), dried over anhydrous Na_2SO_4, filtered and solvent removed *in vacuo*. The residue was purified by column chromatography (Hexanes/EtOAc 8:2) to give **64** (43 mg, 66%) and recover 57 mg of s. m. **63** (EtOAc).

1-((R)-2-hydroxy-3-(trityloxy)propoxy)hexadecan-2-ol: **64**

IR (liquid film) ν_{max} cm^{-1} 3390, 2922, 2850, 1448, 1263, 1076, 1033, 740, 702, 632.

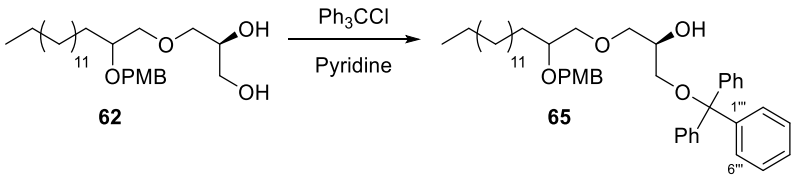
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 7.44-7.41 (6H, m, C<u>Ph$_3$</u>), 7.32-7.24 (9H, m, C<u>Ph$_3$</u>), 3.96 (1H, m, H-2), 3.74 (1H, m, H-2'), 3.62 (1H, m, H_A-1), 3.56 (1H, m, H_B-1), 3.48 (1H, m, H_A-1'), 3.30 (1H, m, H_B-1'), 3.21 (2H, m, H-3), 1.41 (2H, m, H-3'), 1.26 (24H, br s, H-4'-15'), 0.88 (3H, t, *J* = 6.8 Hz, H-16').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 143.7 (C-1'''), 128.6 (C-3''' and 5'''), 127.9 (C-2''' and 6'''), 127.1 (C-4'''), 86.7 (<u>C</u>Ph$_3$), 76.0 (C-1'), 72.6 (C-1), 70.4 (C-2'), 69.9 (C-2), 64.4 (C-3), 33.0 (C-3'), 31.9 (C-14'), 29.7-29.4 (C-5'-13'), 25.5 (C-4'), 22.7 (C-15'), 14.1 (C-16').

ESI-HRMS (*m/z*) $C_{38}H_{54}O_4Na$ ($M+Na^+$): calc.: 597.3914, obs.: 597.3909, Δ = -0.94 ppm.

HMQC and HMBC see 2D tables.

Reaction of 62 with $ClCPh_3$: 65



To a solution of **62** (1.28 g, 2.83 mmol) in pyridine (5.8 mL) was added $ClCPh_3$ (908 mg, 3.11 mmol) under argon atmosphere and the reaction was stirred at 100 ^{o}C for 4 h, controlling the progression by TLC. After that time, the reaction was cooled to room temperature and quenched by addition of ice and the mixture was extracted with EtOAc. The organic layer was washed with HCl 2M, $NaHCO_3$ 6% y NaCl (sat.), dried over anhydrous Na_2SO_4, filtered and solvent removed *in vacuo*. The residue was purified by column chromatography (Hexanes/EtOAc 9:1 and 85:15) to afford **65** (1.58 g, 80%).

<u>(2*R*)-1-((2-((4-methoxybenzyl)oxy)hexadecyl)oxy)-3-(trityloxy)propan-2-ol</u>: **65**

IR (liquid film) ν_{max} cm^{-1} 3435, 2924, 2852, 1312, 1514, 1448, 1247, 1076, 705.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm)* 7.46-7.42 (6H, m, C<u>Ph$_3$</u>), 7.32-7.22 (11H, m, H-3'', 7'' and C<u>Ph$_3$</u>), 6.84 (2H, d, *J* = 8.6 Hz, H-4'' and 6''), 4.53 (1H, d, *J* = 11.4 Hz, H_A-1''), 4.47

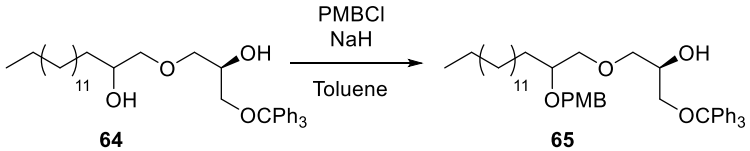
(1H, d, *J* = 11.4 Hz, H_B-1'', *R* or *S*), 4.46 (1H, d, *J* = 11.4 Hz, H_B-1'', *R* or *S*), 4.00-3.96 (1H, m, H-2), 3.79 (3H, s, OMe), 3.63-3.48 (5H, m, H-1, 1' and 2'), 3.26-3.18 (2H, m, H-3), 1.50-1.42 (2H, m, H-3'), 1.27 (24H, br s, H-4'-15'), 0.89 (3H, t, *J* = 6.8 Hz, H-16').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 159.1 (C-5''), 143.8 (C-1'''), 130.9 (C-2''), 129.3 (C-3'' and 7''), 128.6 (C-3''' and 5'''), 127.6 (C-2''' and 6'''), 127.0 (C-4'''), 113.7 (C-4'' and 6''), 86.6 (CPh_3), 77.7 (C-2'), 74.4 (C-1'), 72.9 (C-1), 71.6 (C-1''), 69.9 (C-2), 64.7 (C-3), 55.2 (OMe), 31.9 (C-14'), 31.7 (C-3'), 29.6-29.3 (C-5'-13'), 25.4 (C-4'), 22.7 (C-15'), 14.1 (C-16').

ESI-HRMS (*m/z*) $C_{46}H_{62}O_5Na$ ($M+Na^+$): calc.: 717.44895, obs.: 717.4477, Δ = -1.75 ppm.

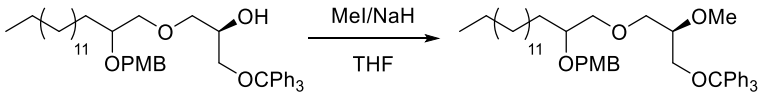
HMQC and HMBC see 2D tables.

Reaction of 64 with PMBCl: 65



To a solution of **64** (43 mg, 0.075mmol) in toluene (1 mL) was added NaH 60% (8 mg, 0.19 mmol) and PMBCl (10 µL, 0.075 mmol) under argon atmosphere and the reaction was stirred at 120 °C for 4 h, controlling the progression by TLC. After that time, the reaction was cooled, quenched by addition of ice and extracted with EtOAc. The organic layer was then washed with HCl 2M, $NaHCO_3$ 6% and NaCl (sat.), dried over anhydrous Na_2SO_4, filtered and solvent removed *in vacuo*. The residue was purified by column chromatography (Hexanes/EtOAc 9:1) to obtain **65** (28 mg, 73%) and recover 11 mg of s. m. **64** (Hexanes/EtOAc 8:2).

Reaction of 65 with MeI: 66



65 **66**

To a solution of **65** (1.58 g, 2.27 mmol) in THF (6 mL) was added MeI (1.64 g, 11.5 mmol) and NaH 60% (198 mg, 4.95 mmol) under argon atmosphere and the reaction was stirred at 75 ^{o}C for 5 h. After that time, the mixture was cooled, quenched by addition of ice and extracted with EtOAc. The organic layer was washed with HCl 2M, $NaHCO_3$ 6% y NaCl (sat.), dried over anhydrous Na_2SO_4, filtered and solvent removed *in vacuo* to give **66** (1.56 g, 97%).

1-*O*-(2-(4-methoxybenzyloxy)hexadecyl)-2-*O*-methyl-3-*O*-trityl-*sn*-glycerol: **66**

IR (liquid film) ν_{max} cm^{-1} 2924, 2852, 1714, 1606, 1514, 1448, 1249, 1087, 705.

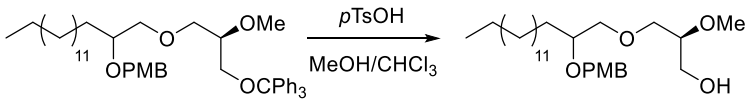
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm)* 7.47-7.20 (17H, m, H-3'', 7'' and C$\underline{Ph_3}$), 6.86-6.82 (2H, m, H-4'' and 6''), 4.55 (1H, dd, *J* = 11.3 Hz, H_A-1'', *R* or *S*), 4.54 (1H, dd, *J* = 11.3 Hz, H_A-1'', *R* or *S*), 4.44 (1H, dd, *J* = 11.3 Hz, H_B-1'', *R* or *S*), 4.44 (1H, dd, *J* = 11.3 H_B-1'', *R* or *S*), 3.79 (3H, s, Ph-O$\underline{Me}$), 3.64-3.54 (3H, m, H-1 and 1$_A$'), 3.52-3.47 (3H, m, H-1$_B$', 2 and 2'), 3.45 (3H, s, O$\underline{Me}$), 3.23-3.20 (2H, m, H-3), 1.47-1.42 (2H, m, H-3'), 1.27 (24H, m, H-4'-15'), 0.89 (3H, t, *J* = 6.6 Hz, H-16').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 159.0 (C-5''), 144.0 (C-1'''), 131.2 (C-2''), 129.4 (C-3'' and 7''), 128.7 (C-3''' and 5'''), 127.8 (C-2''' and 6'''), 126.9 (C-4'''), 113.6 (C-4'' and 6''), 86.6 (C$\underline{Ph_3}$), 79.9 (C-2), 77.6 (C-2'), 74.7 (C-1'), 71.8 (C-1), 71.7 (C-1''), 63.2 (C-3), 58.3 (O$\underline{Me}$), 55.2 (Ph-O$\underline{Me}$), 32.0 (C-14'), 31.9 (C-3'), 29.7-29.4 (C-5'-13'), 25.4 (C-4'), 22.7 (C-15'), 14.1 (C-16').

ESI-HRMS (*m/z*) $C_{47}H_{68}O_5N$ ($M+NH_4^+$): calc.: 726.5092, obs.: 726.5084, Δ = -0.84 ppm.

HMQC and HMBC see 2D tables.

Reaction of 66 with *p*-TsOH: 67



66 **67**

To a solution of **66** (868 mg, 1.22 mmol) in MeOH/$CHCl_3$ 4:1 (15 mL) under argon atmosphere was added *p*-TsOH (208 mg, 1.08 mmol) and 0.1 mL of H_2O and the reaction was stirred at room temperature for 6 h, controlling the progression by TLC. After that time, the reaction mixture was extracted with plenty EtOAc (3 x 30 mL). The organic layer was washed with $NaHCO_3$ 6% y NaCl (sat.), dried over anhydrous Na_2SO_4, filtered and solvent removed *in vacuo*. The residue was purified by column chromatography (Hexanes/EtOAc 7:3) to afford **67** (480 mg, 84%).

(2*S*)-2-methoxy-3-(2-(4-methoxybenzyloxy)hexadecyloxy)propan-1-ol: **67**

IR (liquid film) ν_{max} cm^{-1} 3440, 2924, 2852, 1714, 1606, 1514, 1462, 1249, 1118, 1037, 821.

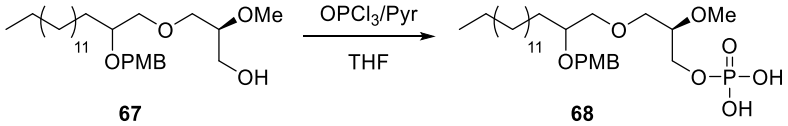
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 7.28-7.25 (2H, m, H-3'' and 7''), 6.89-6.85 (2H, m, H-4'' and 6''), 4.58 (1H, d, *J* = 11.3 Hz, H_A-1''), 4.49 (1H, d, *J* = 11.3 Hz, H_B-1''), 3.80 (3H, s, Ph-OMe), 3.75 (1H, dd, *J* = 11.2, 4.0 Hz, H_A-3), 3.64 (1H, dd, *J* = 11.2, 4.0 Hz, H_B-3), 3.63-3.49 (5H, m, H-1, 1' and 2'), 3.46 (3H, s, OMe), 3.45-3.42 (1H, m, H-2), 1.53-1.48 (2H, m, H-3'), 1.26 (24H, br s, H-4'-15'), 0.88 (3H, s, H-16').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 159.1 (C-5''), 130.9 (C-2''), 129.4 (C-3'' and 7''), 113.7 (C-4'' and 6''), 80.0 (C-2), 77.7 (C-2'), 74.5 (C-1'), 71.6 (C-1''), 71.1 (C-1), 62.5 (C-3), 57.8 (OMe), 55.2 (Ph-OMe), 31.9 (C-14'), 31.7 (C-3'), 29.7-29.3 (C-5'-13'), 25.4 (C-4'), 22.7 (C-15') 14.1 (C-16').

ESI-HRMS (*m/z*) $C_{28}H_{50}O_5Na$ ($M+Na^+$): calc.: 489.3550, obs.: 489.3546, Δ = -0.82 ppm.

HMQC and HMBC see 2D tables.

Reaction of 67 with $OPCl_3$: 68



To a solution of **67** (100 mg, 0.214 mmol) in THF (1 mL) at 0 °C under argon atmosphere was added pyridine (0.36 mL, 4.45 mmol) and $OPCl_3$ (22 µL, 0.236 mmol) and the reaction was stirred at 0 °C for 3.5 h, plus other 1.5 h at room temperature. After that time, the reaction was quenched by addition of $NaHCO_3$ (6%) and stirring for 15 minutes. Subsequently, HCl 2M was added dropwise until reaching pH = 2. The mixture was then extracted with EtOAc and the organic layer was washed with H_2O, dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo* to yield **68** (113 mg, 96%).

(2*R*)-2-methoxy-3-(2-(4-methoxybenzyloxy)hexadecyloxy)propyl dihydrogen phosphate: **68**

IR (liquid film) ν_{max} cm^{-1} 3200, 2924, 2852, 1612, 1514, 1463, 1247, 1041.

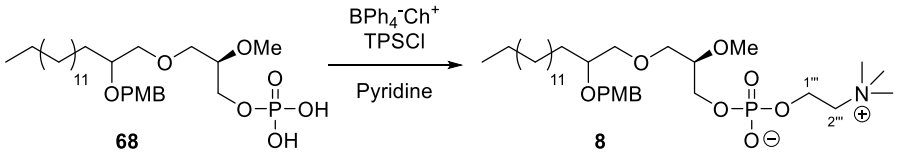
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm)* 7.27 (2H, d, *J* = 8.5 Hz, H-3'' and 7''), 6.86 (2H, d, *J* = 8.5 Hz, H-4'' and 6''), 4.61 (1H, dd, *J* =11.4 Hz, H_A-1'', *R* or *S*), 4.60 (1H, dd, *J* =11.4 Hz, H_A-1'', *R* or *S*), 4.50 (1H, d, *J* =11.4 Hz, H_B-1''), 4.13-4.06 (2H, m, H-3), 3.78 (3H, s, Ph-OMe), 3.59–3.48 (6H, m, H-1, 2, 1' and 2'), 3.45 (3H, s, OMe), 1.47-1.45 (2H, m, H-3'), 1.26 (24H, br s, H-4'-15'), 0.89 (3H, t, *J* = 6.4 Hz, H-16').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 159.1 (C-5''), 130.5 (C-2''), 129.6 (C-3'' and 7''), 113.7 (C-4'' and 6''), 78.8 (C-2), 77.6 (C-2'), 74.2 (C-1'), 71.5 (C-1''), 70.1 (C-1), 65.5 (C-3), 58.0 (OMe), 55.2 (Ph-OMe), 31.9 (C-14'), 31.6 (C-3'), 29.7–29.3 (C-5'-13'), 25.4 (C-4'), 22.7 (C-15'), 14.1 (C-16').

ESI-HRMS (*m/z*) $C_{28}H_{50}O_8P$ ($M\text{-}H^+$): calc.: 545.32378, obs.: 545.3245, Δ = 1.36 ppm.

HMQC and HMBC see 2D tables.

Reaction of 68 with choline tetraphenylborate: 8



To a solution of **68** (65 mg, 0.118 mmol) in pyridine (1 mL) under argon atmosphere was added 2,4,6-triisopropylbenzenesulfonyl chloride (TPSCl) (89 mg, 0.295 mmol) and $BPh_4^-Ch^+$ (99 mg, 0.236 mmol), recently prepared, and the reaction was stirred at 70 ^oC for 13 h. Then, the reaction mixture was cooled, quenched by addition of H_2O and extracted with $CHCl_3$. The organic layer was washed with NaCl (sat.), dried over anhydrous Na_2SO_4, filtered and solvent removed *in vacuo*. The residue was purified by column chromatography ($CHCl_3$/MeOH/H_2O 65:30:5) to give **8** (10 mg, 13%).

1-*O*-(2-(4-methoxybenzyloxy)hexadecyl)-2-*O*-methyl-*sn*-glycero-3-phosphocholine: **8**

IR (liquid film) ν_{max} cm^{-1} 3414, 2922, 2852, 1712, 1606, 1512, 1465, 1253, 1089, 968.

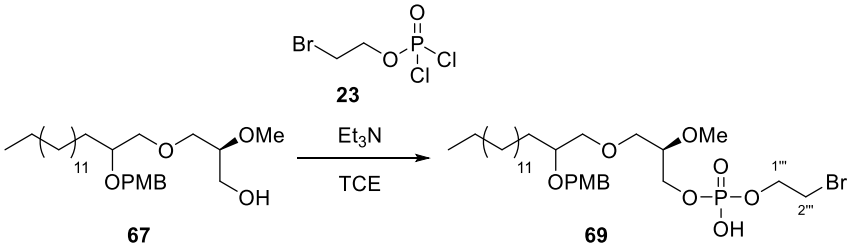
^{1}H NMR (400 MHz, $CDCl_3$, δ ppm)* 7.25 (2H, d, *J* = 8.0 Hz, H-3'' and 7''), 6.85 (2H, d, *J* = 8.0 Hz, H-4'' and 6''), 4.57 (1H, d, *J* = 11.2 Hz, H_A-1''), 4.46 (1H, dd, *J* = 11.2 Hz, H_B-1'', *R* or *S*), 4.45 (1H, dd, *J* = 11.2 Hz, H_B-1'', *R* or *S*), 4.25 (2H, br s, H-1'''), 3.91-3.86 (2H, m, H-3), 3.77 (3H, s, Ph-OMe), 3.69 (2H, br s, H-2'''), 3.59-3.46 (6H, m, H-1, 2, 1' and 2'), 3.42 (3H, s, OMe), 3.27 (9H, br s, NMe_3), 1.47-1.43 (2H, m, H-3'), 1.24 (24H, br s, H-4'-15'), 0.87 (3H, t, *J* = 6.8 Hz, H-16').

^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 159.1 (C-5''), 131.1 (C-2''), 129.4 (C-3'' and 7''), 113.7 (C-4'' and 6''), 79.7 (C-2), 77.8 (C-2'), 74.5 (C-1'), 71.5 (C-1''), 71.0 (C-1), 66.2 (C-2'''), 64.7 (C-3), 59.2 (C-1'''), 57.9 (OMe), 55.3 (Ph-OMe), 54.3 (NMe_3), 31.9 (C-14'), 31.8 (C-3'), 29.7–29.3 (C-5'-13'), 25.5 (C-4'), 22.7 (C-15'), 14.1 (C-16').

ESI-HRMS (*m/z*) $C_{33}H_{62}O_8NPNa$ ($M+Na^+$): calc.: 654.41053, obs.: 654.4098, Δ = -1.12 ppm.

HMQC and HMBC see 2D tables.

Reaction of 67 with 23: 69



To a solution of **23** (210 mg, 0.92 mmol) in TCE (0.5 mL) at 0 °C under argon atmosphere was added a solution of **67** (169 mg, 0.36 mmol) and Et_3N (200 µL, 1.43 mmol) in TCE (0.9 mL) and the reaction was stirred for 12 h, removing generated HCl (g) every 3 minutes in the first hour by passing a stream of argon. After that time, the reaction was quenched by addition of ice and pyridine (3 eq) and left to stir for 1 h. Then, HCl 2M was slowly added until reaching pH = 2, when the organic layer is separated and neutralized by slowly addition of a 10% aqueous solution of $Ba(OAc)_2$. After that, the organic layer was concentrated *in vacuo* and the residue was crystalized in acetone at 0 °C, affording a white solid. The obtained residue was solved in $CHCl_3$ and was added HCl 2M until reaching pH = 2. The organic layer was separated and concentrated *in vacuo* to give **69** (111 mg, 47%).

2-Bromoethyl (2*R*)-2-methoxy-3-(2-(4-methoxybenzyloxy)hexadecyloxy)propyl hydrogen phosphate: **69**

IR (liquid film) ν_{max} cm^{-1} 2924, 2852, 1718, 1612, 1514, 1247, 1020.

^{1}H NMR (400 MHz, $CDCl_3$, δ ppm) 7.26 (2H, d, *J* = 8.0 Hz, H-3'' and 7''), 6.86 (2H, d, *J* = 8.0 Hz, H-4'' and 6''), 4.59 (1H, d, *J* = 12.0 Hz, H_A-1''), 4.49 (1H, d, *J* = 12.0 Hz, H_B-1''), 4.24 (2H, br s, H-1'''), 4.11-4.05 (2H, m, H-3), 3.79 (3H, s, Ph-OMe), 3.56-3.50 (8H, m, H-1, 2, 1', 2' and 2'''), 3.46 (3H, s, OMe), 1.47 (2H, m, H-3'), 1.25 (24H, m, H-4'-15'), 0.87 (3H, t, *J* = 6.6 Hz, H-16').

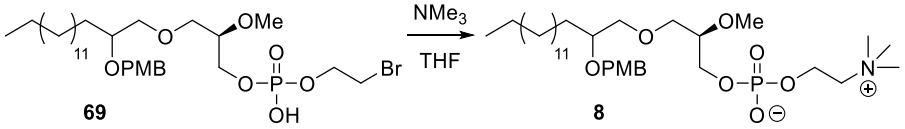
^{13}C NMR (100 MHz, $CDCl_3$, δ ppm) 159.1 (C-5''), 130.9 (C-2''), 129.4 (C-3'' and 7''), 113.7 (C-4'' and 6''), 78.9 (C-2), 77.6 (C-2'), 74.5 (C-1'), 71.5 (C-1''), 70.2 (C-1), 66.1 (C-

1'''), 66.0 (C-3), 58.1 (OMe), 55.2 (Ph-OMe), 31.9 (C-14'), 31.8 (C-3'), 31.7 (C-2'''), 29.7-29.3 (C-5'-13'), 25.4 (C-4'), 22.7 (C-15'), 14.1 (C-16').

ESI-HRMS (*m/z*) $C_{30}H_{54}O_8P^{79}BrNa$ ($M+Na^+$): calc.: 675.26319, obs.: 6752632, Δ = -0.01 ppm.

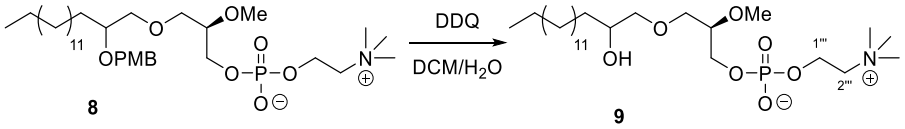
HMQC and HMBC see 2D tables.

Reaction of 69 with trimethylamine: 8



To a solution of **69** (102 mg, 0.156 mmol) under argon atmosphere was added a 1M solution of NMe_3 in THF (1.5 mL, 1.5 mmol) and the reaction was stirred at room temperature for 2 days. After that time, the mixture was concentrated under reduced pressure and the residue was purified by column chromatography ($CHCl_3$/MeOH/H_2O 65:30:5) to yield **8** (55 mg, 56%).

Reaction of 8 with DDQ: 9



To a solution of **8** (48 mg, 0.074 mmol) in DCM/H_2O 18:1 (1 mL) was added DDQ (20 mg, 0.088 mmol) and the reaction was stirred at room temperature for 6.5 h, controlling the progression by TLC. After that time, the mixture was purified by column chromatography ($CHCl_3$/MeOH/H_2O 65:30:5) to afford **9** (24 mg, 72 %) and recover 10 mg of the s. m. **8**.

1-*O*-(2-hydroxyhexadecyl)-2-methyl-*sn*-glycero-3-phosphocholine: **9**

IR (liquid film) ν_{max} cm^{-1} 3369, 2924, 2850, 1722, 1467, 1242, 1091, 727.

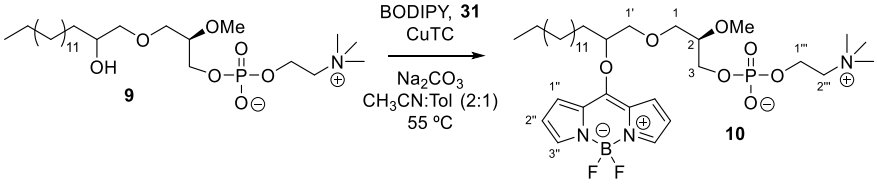
^{1}H NMR (400 MHz, Pyr-d5, δ ppm) 4.77 (2H, br s, H-1'''), 4.45-4.37 (2H, m, H-3), 4.15 (2H, br s, H-2'''), 4.07 (1H, m, H-2'), 3.95-3.83 (3H, m, H-1 and 2), 3.66-3.63 (2H, m, H-1'), 3.63 (9H, br s, NMe_3), 3.52 (3H, s, OMe), 1.65-1.50 (4H, m, H-3' and 4') 1.28 (22H, br s, H-5'-15'), 0.87 (3H, t, *J* = 6.9 Hz, H-16').

^{13}C NMR (100 MHz, Pyr-d5, δ ppm) 79.9 (C-2), 76.9 (C-1'), 70.7 (C-1), 70.0 (C-2'), 66.6 (C-2'''), 64.6 (C-3), 59.6 (C-1'''), 57.4 (OMe), 54.2 (NMe_3), 34.3 (C-3'), 31.9 (C-14'), 30.1-29.4 (C-5'-13'), 26.1 (C-4'), 22.7 (C-15'), 14.1 (C-16').

ESI-HRMS (*m/z*) $C_{25}H_{55}O_7NP$ ($M+H^+$): calc.: 512.37107, obs.: 512.3704, Δ = -1.26 ppm.

HMQC and HMBC see 2D tables

Reaction of 9 with BODIPY 31: 10



To a solution of **9** (8 mg, 0.015 mmol) in CH_3CN/toluene 2:1 (1.0 mL) under argon atmosphere was added **31** (11 mg, 0.05 mmol), CuTC (10 mg, 0.05 mmol) and Na_2CO_3 (4 mg, 0.04 mmol) and the reaction was stirred at 55 ^{o}C for 18 h. After that time, solvent was removed *in vacuo* and the residue was purified by column chromatography ($CHCl_3$/MeOH/H_2O 65:30:5) to obtain **10** (9 mg, 80%).

1-*O*-(2-(4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacen-8-yloxy)hexadecanyl)-2-*O*-methyl-*sn*-glycero-3-phosphocholine: **10**

UV (EtOH) (λ_{max} nm) 415, 359, 269, 251.

^{1}H NMR (400 MHz, Pyr-d5, δ ppm) 7.78 (2H, br s, 3'' and '''), 7.55 (2H, br s, H-1'' and 7''), 6.48 (2H, br s, H-2'' and 6''), 5.00 (1H, m, H-2'), 4.72 (2H, br s, H-1'''), 4.45 (2H, m, H-3), 4.05 (2H, br s, H-2'''), 3.88-3.84 (3H, m, H-1 and 2), 3.63 (2H, m, H-1'), 3.44 (12H, br s, O<u>Me</u> and N<u>Me$_3$</u>), 1.61 (2H, m, H-3'), 1.25 (24H, br s, H-4'-15'), 0.85 (3H, t, *J* = 6.8 Hz, H-16').

^{13}C NMR (100 MHz, Pyr-d5, δ ppm) 159.4 (C-8''), 132.1 (C-3'' and 5''), 131.8 (C-7a'' and 8a''), 126.4 (C-1'' and 7''), 110.8 (C-2'' and 6''), 80.0 (C-2'), 79.9 (C-2), 77.0 (C-1'), 70.2 (C-1), 66.8 (C-2'''), 64.2 (C-3), 59.5 (C-1'''), 57.2 (O<u>Me</u>), 54.0 (N<u>Me$_3$</u>), 34.3 (C-3'), 31.9 (C-14'), 30.0-29.4 (C-5'-13'), 26.1 (C-4'), 22.7 (C-15'), 14.1 (C-16').

ESI-HRMS (*m/z*) $C_{34}H_{60}O_7N_3BF_2P$ ($M+H^+$), calc.: 701.4261, obs.: 701.4274, Δ = 1.92 ppm.

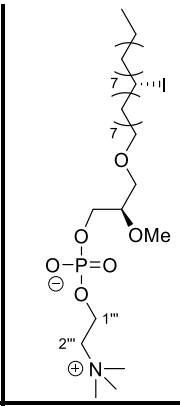
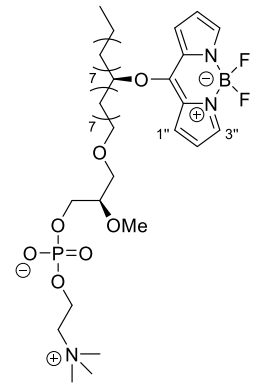
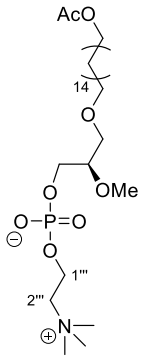
HMQC see Spectroscopy.

HMQC was necessary to assign H-2' as the signal is overlapped by the big signal of H_2O.

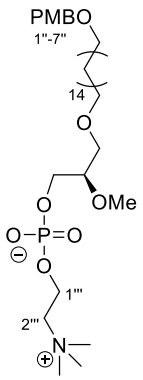
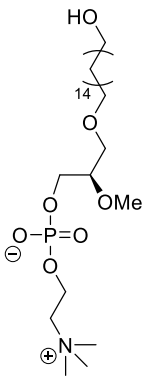
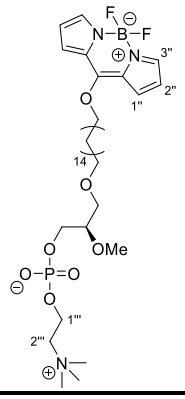
C^{13} NMR Tables

FIRMADO POR	FECHA FIRMA
SELLO UNIVERSIDAD DE SALAMANCA	20-07-2021 18:11:01
SÁNCHEZ MARCOS ISIDRO	20-07-2021 10:20:59

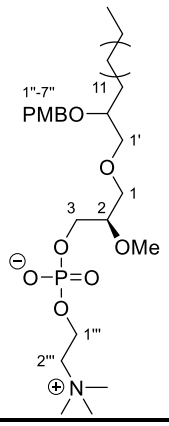
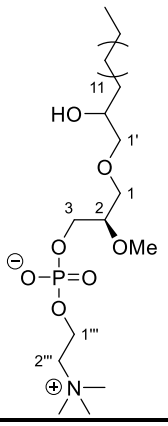
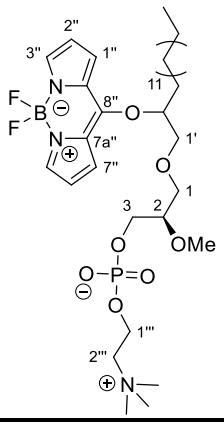
C^{13} NMR Tables

			
C/n°	**1**	**2**	**3**
1	70.5	70.1	69.8
2	79.9	79.5	79.3
3	65.2	65.0	66.0
OMe	58.0	57.8	57.9
1'	72.0	71.6	71.8
2'-7'	32.1-22.9	31.8-22.7	29.7-25.9
8' and 10'	40.9	33.9	
9'	41.1	86.5	
11'-15'	32.1-22.9	31.8-22.7	
16'			64.6
17'			-
18'	14.3	14.1	-
OO$\underline{C}$CH$_3$	-	-	171.2
OOC$\underline{CH_3}$	-	-	21.0
1'' and 7''	-	124.6	-
2'' and 6''	-	116.2	-
3'' and 5''	-	138.7	-
7a'' and 8a''	-	126.5	-
8''	-	161.4	-
1'''	59.5	59.2	59.8
2'''	66.6	66.2	65.7
N$\underline{Me_3}$	54.6	54.4	54.4

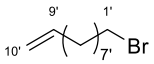
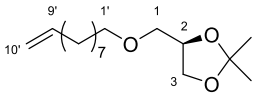
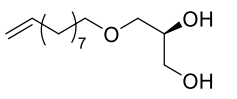
C^{13} NMR Tables

				
C/n°		**4**	**5** (Pyr-d5)	**7**
1		70.1	71.0	70.1
2		79.4, d, *J* = 8 Hz	80.1	79.5
3		65.0, d, *J* = 5 Hz	65.1	65.0
OMe		57.7	57.6	57.7
1'		71.8	71.5	71.7
2'		26.2	26.3	29.7-25.8
3'-13'		29.8-29.5	30.0-29.6	
14'			33.5	
15'		26.1	26.3	
16'		70.2	61.9	75.4
1''	**PMB**	72.4	-	-
2''		130.8	-	-
3'' and 7''		129.2	-	-
4'' and 6''		113.7	-	-
5''		159.0	-	-
Ph-OMe		55.2	-	-
1'' and 7''	**BDP**	-	-	125.2
2'' and 6''		-	-	116.2
3'' and 5''		-	-	139.0
7a'' and 8a''		-	-	126.2
8''		-	-	161.5
1'''		59.3, d, *J* = 4 Hz	59.6	59.2
2'''		66.2, d, *J* = 6 Hz	66.7	66.4
NMe_3		54.3	54.1	54.4

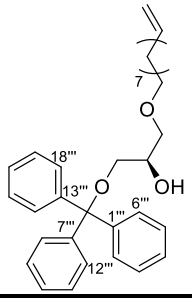
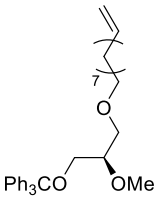
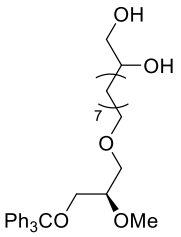
C^{13} NMR Tables

				
C/n°		**8**	**9** (Pyr-d5)	**10** (Pyr-d5)
1		71.0	70.7	70.2
2		79.7	79.9	79.9
3		64.7	64.6	64.2
OMe		57.9	57.4	57.2
1'		74.5	76.9	77.0
2'		77.8	70.0	80.0
3'		31.8	34.3	34.3
4'		25.5	26.1	26.1
5'-13'		29.7-29.3	30.1-29.4	30.0-29.4
14'		31.9	31.9	31.9
15'		22.7	22.7	22.7
16'		14.1	14.1	14.1
1''	**PMB**	71.5	-	-
2''		131.1	-	-
3'' and 7''		129.4	-	-
4'' and 6''		113.7	-	-
5''		159.1	-	-
Ph-OMe		55.3	-	-
1'' and 7''	**BDP**	-	-	126.4
2'' and 6''		-	-	110.8
3'' and 5''		-	-	132.1
7a'' and 8a''		-	-	131.8
8''		-	-	159.4
1'''		59.2	59.6	59.5
2'''		66.2	66.6	66.8
NMe_3		54.3	54.2	54.0

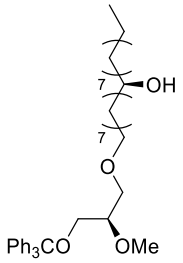
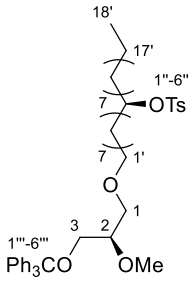
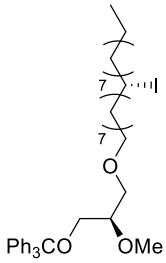
C^{13} NMR Tables

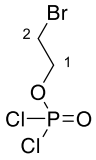
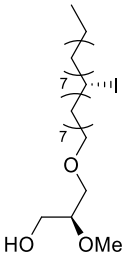
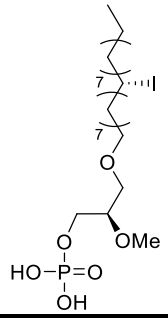
			
C/n°	**13**	**14**	**15**
1	-	72.03	71.7
2	-	74.9	70.6
3	-	67.1	64.1
$\underline{C}Me_2$	-	109.5	-
$C\underline{Me_2}$	-	27.0 and 25.6	-
1'	33.9	72.05	72.2
2'	32.8	29.8	29.5
3'-7'	29.2-28.1	29.6-26.2	29.3-26.0
8'	33.7	34.0	33.7
9'	139.0	139.3	139.1
10'	114.2	114.3	114.1

C^{13} NMR Tables

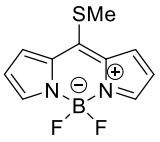
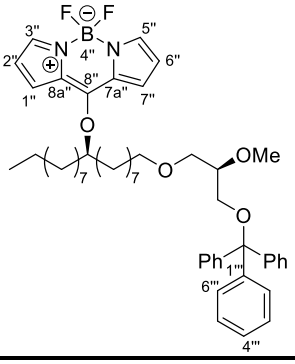
			
C/n°	**16**	**17**	**18**
1	71.9	71.2	71.2
2	70.1	80.2	80.1
3	64.8	63.2	63.1
OMe	-	58.4	58.3
1'	72.3	72.0	72.0
2'	29.9	29.9	29.9
3'-7'	29.7-26.3	29.7-26.3	29.9-25.9
8'	34.1	34.1	33.4
9'	139.5	139.4	72.5
10'	114.4	114.4	67.0
1'''	144.1	144.4	144.3
2''' and 6'''	128.1	128.0	128.0
3''' and 5'''	128.9	129.0	129.0
4'''	127.3	127.2	127.2
$\underline{C}Ph_3$	86.8	86.9	86.9

C^{13} NMR Tables

			
C/n°	**20**	**21**	**22**
1	71.2	71.2	70.9
2	80.1	80.1	79.9
3	63.1	63.1	62.9
OMe	58.3	58.4	58.1
1'	71.9	71.9	71.6
2'-7'	32.1-22.9	32.1-22.8	31.9-22.6
8' and 10'	37.8	34.4	40.7
9'	72.2	84.9	40.6
11'-17'	32.1-22.9	32.1-22.8	31.9-22.6
18'	14.4	14.4	14.1
1''	-	144.3	-
2'' and 6''	-	128.0	-
3'' and 5''	-	129.0	-
4''	-	127.2	-
Ph-Me	-	21.8	-
1'''	144.3	144.5	144.1
2''' and 6'''	128.0	128.2	127.7
3''' and 5'''	129.0	129.8	128.7
4'''	127.2	135.1	126.9
CPh$_3$	86.8	86.8	86.6

			
C/n°	**23**	**27**	**28**
1	69.8, d, *J* = 8.0Hz	70.9	69.9
2	27.2, d, *J* = 11.0Hz	80.0	79.3
3	-	62.9	66.1
OMe	-	58.0	58.3
1'	-	72.1	72.2
2'-7'	-	32.1-22.9	32.1-22.9
8' and 10'	-	40.9	40.9
9'	-	41.2	40.9
11'-17'	-	32.1-22.9	32.1-22.9
18'	-	14.4	14.3

C^{13} NMR Tables

		
C/n°	**31**	**32**
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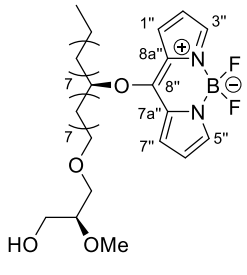
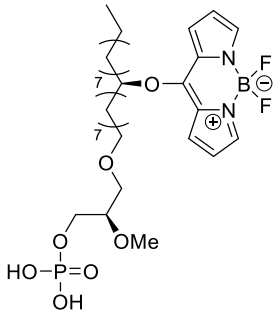
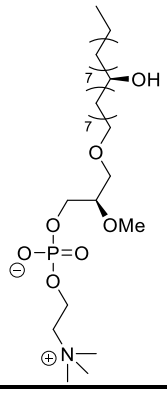
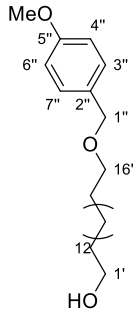
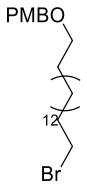
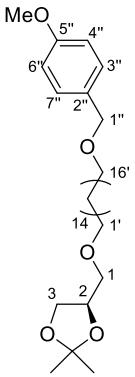
2	-	80.1
3	-	63.1
OMe	-	58.3
1'	-	71.9
2'-7'	-	32.1-22.9
8' and 10'	-	34.1
9'	-	86.8
11'-17'	-	32.1-22.9
18'	-	14.3
1'' and 7''	127.6	124.8
2'' and 6''	117.8	116.4
3'' and 5''	141.3	139.0
8''	153.9	161.7
7a'' and 8a''	133.8	126.8
SMe	20.4	-
1'''	-	144.3
2''' and 6'''	-	128.0
3''' and 5'''	-	129.0
4'''	-	127.1
$\underline{C}Ph_3$	-	86.7

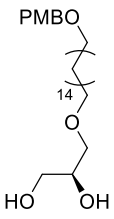
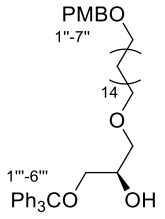
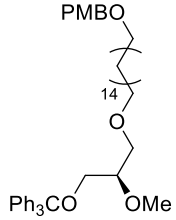
C^{13} NMR Tables

			
C/n°	**33**	**35**	**36**
1	70.6	69.4	70.0
2	79.8	79.0	79.6
3	62.7	65.9	65.0
OMe	57.7	58.0	57.7
1'	71.8	71.8	71.6
2'	26.0	31.8-22.6	29.8
3'-6'	29.4-29.2		29.6-25.6
7'	25.1		
8' and 10'	33.9	33.9	37.5 and 37.4
9'	86.5	86.5	71.7
11'	25.1	31.8-22.6	29.6-25.6
12-15'	29.4-29.2		
16'	31.8		31.9
17'	22.6		22.6
18'	14.1	14.0	14.1
1'' and 7''	124.6	124.5	-
2'' and 6''	116.1	116.1	-
3'' and 5''	138.8	138.7	-
7a'', 8a''	126.6	126.5	-
8''	161.4	161.3	-
1'''	-	-	59.2
2'''	-	-	66.4
NMe_3	-	-	54.4

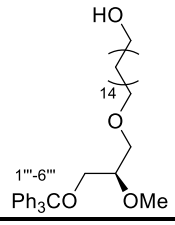
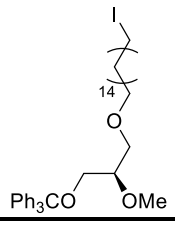
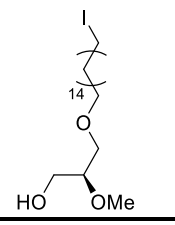
C^{13} NMR Tables

			
C/n°	**39**	**40**	**41**
1	-	-	72.0
2	-	-	75.0
3	-	-	67.1
OMe	55.4	55.3	55.4
$\underline{C}Me_2$	-	-	109.6
$C\underline{Me_2}$	-	-	27.0 and 25.6
1'	63.1	34.1	72.1
2' and 15'	33.0-26.0	32.8-26.2	26.4 and 26.3
3'-14'			30.0-29.7
16'	70.4	70.2	70.4
1''	72.7	72.5	72.1
2''	130.9	130.9	131.0
3'' and 7''	129.55	129.2	129.4
4'' and 6''	113.9	113.7	113.9
5''	159.3	159.0	159.3

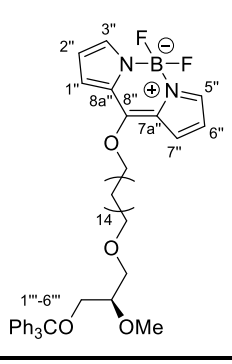
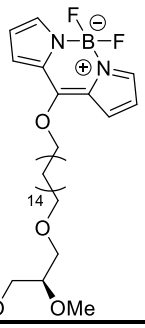
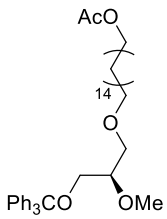
C^{13} NMR Tables

			
C/n°	**42**	**43**	**44**
1	71.8	72.0	70.9
2	70.4	69.8	79.9
3	64.3	64.6	62.9
OMe	-	-	58.1
1'	72.5	71.6	71.7
2' and 15'	26.2 and 26.1	26.2 and 26.1	26.1
3'-14'	29.8-29.4	29.8-29.5	29.8-29.6
16'	70.2	70.2	70.3
1''	72.5	72.5	72.5
2''	130.8	130.9	130.8
3'' and 7''	129.2	129.2	129.2
4'' and 6''	113.7	113.7	113.8
5''	159.1	159.1	159.1
Ph-OMe	55.3	55.3	55.2
1'''	-	143.9	144.1
2''' and 6'''	-	127.8	127.9
3''' and 5'''	-	128.7	128.8
4'''	-	127.0	127.0
$\underline{C}Ph_3$	-	86.6	86.8

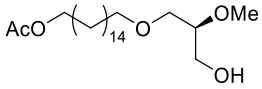
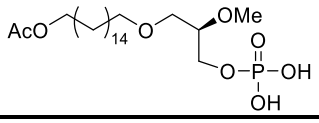
C^{13} NMR Tables

			
C/n°	**45**	**47**	**48**
1	70.9	70.9	70.6
2	79.9	79.9	79.8
3	62.9	62.9	62.7
OMe	58.1	58.1	57.8
1'	71.7	71.7	71.9
2'-14'	32.8-25.7	30.5-26.1	33.6-26.1
15'		33.6	
16'	63.1	7.4	7.4
OO<u>C</u>CH$_3$	-	-	-
OOC<u>CH$_3$</u>	-	-	-
1'''	144.1	144.1	-
2''' and 6'''	127.7	127.7	-
3''' and 5'''	128.7	128.7	-
4'''	126.9	126.9	-
<u>C</u>Ph$_3$	86.8	86.6	-

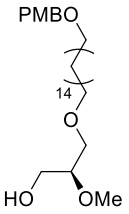
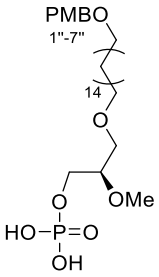
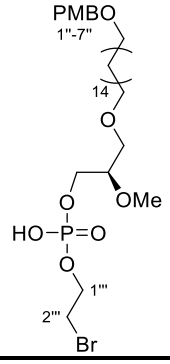
C^{13} NMR Tables

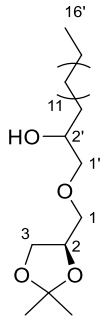
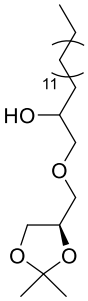
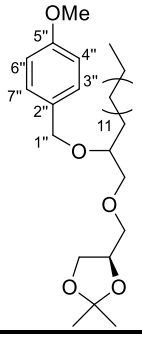
			
C/n°	**49**	**50**	**51**
1	70.9	70.6	70.9
2	79.8	79.8	79.7
3	62.8	62.7	62.9
OMe	58.1	57.8	58.1
1'	71.7	71.9	71.7
2' and 15'	29.7-25.8	29.6-25.8	29.7-25.9
3'-14'			
16'	75.4	75.4	64.7
1'' and 7''	125.1	125.1	-
2'' and 6''	116.2	116.2	-
3'' and 5''	139.0	139.0	-
7a'' and 8a''	126.2	126.2	-
8''	161.5	161.5	-
OO<u>C</u>CH$_3$	-	-	171.1
OOC<u>CH$_3$</u>	-	-	21.0
1'''	144.0	-	144.1
2''' and 6'''	126.9	-	127.7
3''' and 5'''	127.7	-	128.7
4'''	126.2	-	126.9
<u>C</u>Ph$_3$	86.6	-	86.6

C^{13} NMR Tables

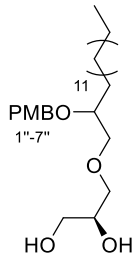
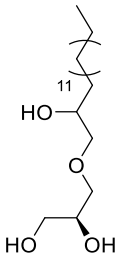
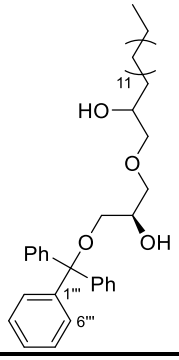
		
C/n°	**52**	**54**
1	70.6	69.5
2	79.8	79.1
3	62.7	65.8
OMe	57.8	57.8
1'	71.9	72.0
2'-15'	29.6-25.9	29.7-25.9
16'	64.7	64.7
OO<u>C</u>CH$_3$	171.2	171.4
OOC<u>CH$_3$</u>	21.0	21.0

C^{13} NMR Tables

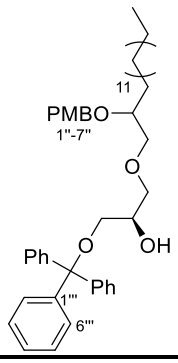
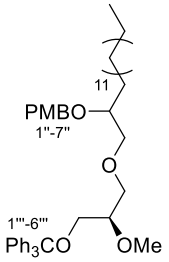
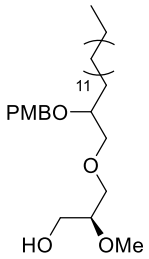
			
C/n°	**56**	**57**	**58**
1	70.6	69.9	69.1
2	79.8	79.0	78.7, d, *J* = 7 Hz
3	62.7	65.5	66.9, d, *J* = 5 Hz
OMe	57.8	57.9	58.1
1'	71.9	71.8	71.9
2' and 15'	26.2 and 26.1	26.1 and 26.0	26.2 and 26.0
3'-14'	29.8-29.4	29.7-29.5	29.7-29.5
16'	70.2	70.2	70.2
1''	72.5	72.5	72.5
2''	130.8	130.8	130.8
3'' and 7''	129.2	129.2	129.2
4'' and 6''	113.7	113.7	113.7
5''	159.1	159.0	159.0
Ph-OMe	55.2	55.2	55.2
1'''	-	-	66.5, d, *J* = 5 Hz
2'''	-	-	29.1, d, *J* = 9 Hz

			
C/n°	**60a (2'*R* or *S*)**	**60b (2'*R* or *S*)**	**61**
1	72.3	72.2	72.4
2	74.8	74.7	74.7
3	66.6	66.5	66.9
$\underline{C}Me_2$	109.6	109.5	109.3
$C\underline{Me}_2$	26.7 and 25.4	26.7 and 25.4	26.8 and 25.4
1'	76.2	76.2	74.5
2'	70.3	70.2	77.7
3'	33.0	32.9	31.8
4'	25.5	25.5	25.4
5'-13'	29.7-29.3	29.7-29.4	29.7-29.4
14'	31.9	31.9	31.9
15'	22.7	22.7	22.7
16'	14.1	14.1	14.1
1''	-	-	71.7
2''	-	-	131.1
3'' and 7''	-	-	129.4
4'' and 6''	-	-	113.7
5''	-	-	159.1
Ph-OMe	-	-	55.3

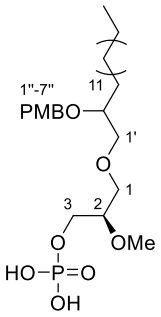
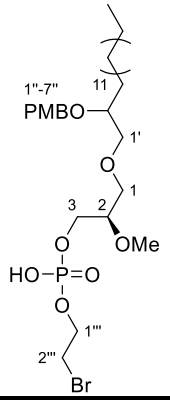
C^{13} NMR Tables

			
C/n°	**62**	**63**	**64**
1	73.2	72.9	72.6
2	70.5	70.6	69.9
3	64.0	63.9	64.4
1'	74.1	76.0	76.0
2'	77.8	70.5	70.4
3'	31.5	33.2	33.0
4'	25.5	25.5	25.5
5'-13'	29.7-29.4	29.7-29.4	29.7-29.4
14'	31.9	31.9	31.9
15'	22.7	22.7	22.7
16'	14.1	14.1	14.1
1''	71.5	-	-
2''	130.6	-	-
3'' and 7''	129.4	-	-
4'' and 6''	113.8	-	-
5''	159.2	-	-
Ph-OMe	55.2	-	-
1'''	-	-	143.7
2'''-6'''	-	-	127.9
3'''-5'''	-	-	128.6
4'''	-	-	127.1
$\underline{C}Ph_3$	-	-	86.7

C^{13} NMR Tables

			
C/n°	**65**	**66**	**67**
1	72.9	71.8	71.1
2	69.9	79.9	80.0
3	64.7	63.2	62.5
OMe	-	58.3	57.8
1'	74.4	74.7	74.5
2'	77.7	77.6	77.7
3'	31.7	31.9	31.7
4'	25.4	25.4	25.4
5'-13'	29.6-29.3	29.7-29.4	29.7-29.3
14'	31.9	32.0	31.9
15'	22.7	22.7	22.7
16'	14.1	14.1	14.1
1''	71.6	71.7	71.6
2''	130.9	131.2	130.9
3'' and 7''	129.3	129.4	129.4
4'' and 6''	113.7	113.6	113.7
5''	159.1	159.0	159.1
Ph-OMe	55.2	55.2	55.2
1'''	143.8	144.0	-
2''' and 6'''	127.6	127.8	-
3''' and 5'''	128.6	128.7	-
4'''	127.0	126.9	-
$\underline{C}Ph_3$	86.6	86.6	-

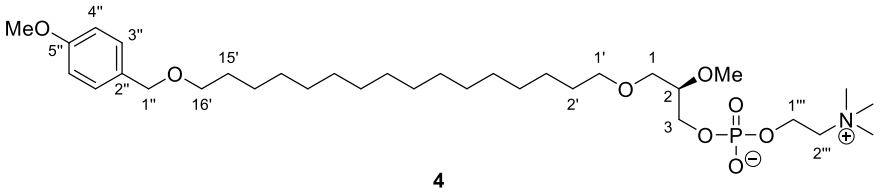
C^{13} NMR Tables

		
C/n°	**68**	**69**
1	70.1	70.2
2	78.8	78.9
3	65.5	66.0
OMe	58.0	58.1
1'	74.2	74.5
2'	77.6	77.6
3'	31.6	31.8
4'	25.4	25.4
5'-13'	29.7-29.3	29.7-29.3
14'	31.9	31.9
15'	22.7	22.7
16'	14.1	14.1
1''	71.5	71.5
2''	130.5	130.9
3'' and 7''	129.6	129.4
4'' and 6''	113.7	113.7
5''	159.1	159.1
Ph-OMe	55.2	55.2
1'''	-	66.1
2'''	-	31.7

FIRMADO POR	FECHA FIRMA
SELLO UNIVERSIDAD DE SALAMANCA	20-07-2021 18:11:01
SÁNCHEZ MARCOS ISIDRO	20-07-2021 10:20:59

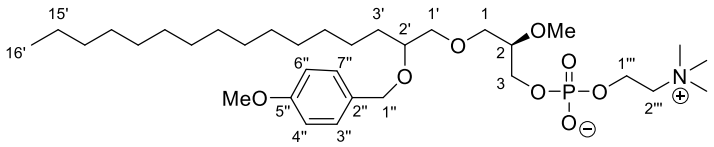
2D NMR Tables

FIRMADO POR	FECHA FIRMA
SELLO UNIVERSIDAD DE SALAMANCA	20-07-2021 18:11:01
SÁNCHEZ MARCOS ISIDRO	20-07-2021 10:20:59



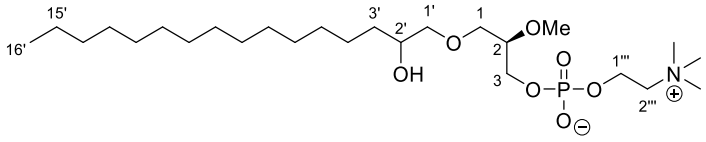
4

C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	70.1	CH_2	3.52-3.48 (1H, m, H_A) 3.46-3.38 (5H, m, H_B)	
2	79.4 (d, *J* = 8 Hz)	CH	3.52-3.48 (2H, m)	1
3	65.0 (d, *J* = 5 Hz)	CH_2	3.94-3.88 (1H, m, H_A) 3.84-3.78 (1H, m, H_B)	1, 2
OMe	57.7	CH_3	3.42 (3H, s)	
1'	71.8	CH_2	3.46-3.38 (5H, m)	1
2'	26.2	CH_2	1.61-1.51 (4H, m)	1'
3'-14'	29.8-29.5	CH_2	1.24 (24H, br s)	1', 2', 15', 16'
15'	26.1	CH_2	1.61-1.51 (4H, m)	16'
16'	70.2	CH_2	3.46-3.38 (5H, m)	15', 1''
1''	72.4	CH_2	4.41 (2H, s)	16', 3'', 7''
2''	130.8	C_4	-	1'', 4'', 6''
3'', 7''	129.2	CH	7.24 (2H, d, *J* = 8.4 Hz)	1''
4'', 6''	113.7	CH	6.86 (2H, d, *J* = 8.4 Hz)	3'', 7''
5''	159.0	C_4	-	1'', 3'', 4'', 6'', 7'' Ph-OMe
Ph-OMe	55.2	CH_3	3.78 (3H, s)	
1'''	59.3 (d, *J* =4 Hz)	CH_2	4.28 (2H, br s)	
2'''	66.2 (d, *J* = 6 Hz)	CH_2	3.70 (2H, br s)	
NMe_3	54.3	CH_3	3.28 (9H, br s)	



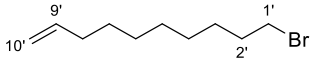
8

C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	71.0	CH_2	3.59-3.46 (6H, m)	
2	79.7	CH		OMe
3	64.7	CH_2	3.91-3.86 (2H, m)	
OMe	57.9	CH_3	3.42 (3H, s)	
1'	74.5	CH_2	3.59-3.46 (6H, m)	
2'	77.8	CH		1''
3'	31.8	CH_2	1.47-1.43 (2H, m)	
4'	25.5	CH_2	1.24 (24H, br s)	
5'-13'	29.7-29.3	CH_2		
14'	31.9	CH_2		16'
15'	22.7	CH_2		16'
16'	14.1	CH_3	0.87 (3H, t, *J* = 6.8 Hz)	
1''	71.5	CH_2	4.57 (1H, d, *J* =11.2 Hz, H_A) 4.46 (1H, d, *J* =11.2 Hz, H_B)	2'', 3'', 7''
2''	131.1	C_4	-	1'', 4'', 6''
3'', 7''	129.4	CH	7.25 (2H, d, *J* = 8.0 Hz)	1''
4'', 6''	113.7	CH	6.85 (2H, d, *J* = 8.0 Hz)	3'', 7''
5''	159.1	C_4	-	3'', 4'', 6'', 7'', Ph-OMe
Ph-OMe	55.3	CH_3	3.77 (3H, s)	
1'''	59.2	CH_2	4.25 (2H, br s)	
2'''	66.2	CH_2	3.69 (2H, br s)	
NMe_3	54.3	CH_3	3.27 (9H, br s)	



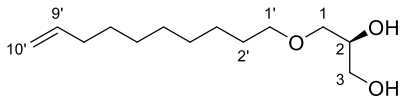
9

C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	70.7	CH_2	3.95-3.83 (3H, m)	3, 1’
2	79.9	CH		1, 3, OMe
3	64.6	CH_2	4.45-4.37 (2H, m)	1
OMe	57.4	CH_3	3.52 (3H, s)	2
1’	76.9	CH_2	3.66-3.63 (2H, m)	1
2’	70.0	CH	4.07 (1H, m)	1’, 3’
3’	34.3	CH_2	1.65-1.50 (4H, m)	1’
4’	26.1	CH_2		
5’-13’	30.1-29.4	CH_2	1.28 (22H, br s)	
14’	31.9	CH_2		16’
15’	22.7	CH_2		16’
16’	14.1	CH_3	0.87 (3H, t, *J* = 6.9 Hz)	
1’’’	59.6	CH_2	4.77 (2H, br s)	
2’’’	66.6	CH_2	4.15 (2H, br s)	NMe_3
NMe_3	54.2	CH_3	3.63 (9H, br s)	NMe_3



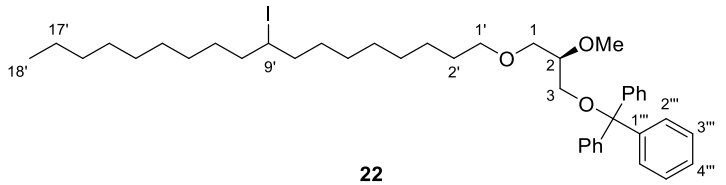
13

C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1'	33.9	CH_2	3.39 (2H, t, *J* = 6.9 Hz)	2'
2'	32.8	CH_2	1.84 (2H, quin, *J* = 6.9 Hz)	1'
3', 7'	29.2-28.1	CH_2	1.45-1.34 (4H, m)	
4'-6'		CH_2	1.30 (6H, br s)	
8'	33.7	CH_2	2.03 (2H, q, *J* = 6.9 Hz)	9', 10'
9'	139.0	CH	5.80 (1H, ddt, *J* = 17.2, 10.2, 6.9 Hz)	7', 8', 10'
10'	114.2	CH_2	4.98 (1H, d, *J* = 17.2 Hz, H_A) 4.93 (1H, d, *J* = 10.2 Hz, H_B)	8'



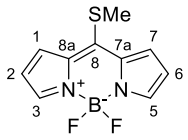
15

C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	72.2	CH_2	3.46-3.41 (4H, m)	3, 1’
2	70.6	CH	3.83 (1H, quin, *J* = 5.8 Hz)	1, 3
3	64.1	CH_2	3.66 (1H, dd, *J* =11.2, 3.6 Hz, H_A) 3.58 (1H, dd, *J* =11.2, 5.8 Hz, H_B)	1, 2
1’	71.7	CH_2	3.46-3.41 (4H, m)	1, 2’
2’	29.5	CH_2	1.54 (2H, quin, *J* = 7.0 Hz	
3’-7’	29.3-26.0	CH_2	1.39-1.26 (10H, m)	
8’	33.7	CH_2	2.01 (2H, q, *J* = 6.8 Hz)	7’, 9’, 10’
9’	139.1	CH	5.78 (1H, ddt, *J* = 17.0, 10.2, 6.8 Hz)	7’, 8’, 10’
10’	114.1	CH_2	4.96 (1H, d, *J* = 17.0 Hz, H_A) 4.90 (1H, d, *J* = 10.2 Hz, H_B)	8’



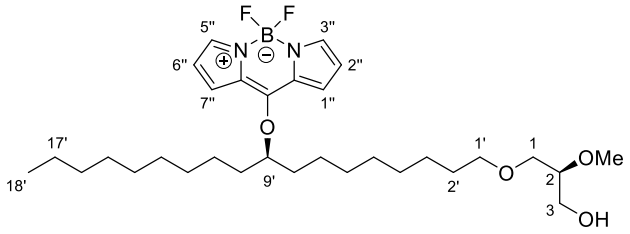
22

C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	70.9	CH_2	3.55 (1H, dd, *J* = 10.0, 4.0 Hz, H_A) 3.52 (1H, m, H_B)	3, 1’
2	79.9	CH	3.46 (1H, m)	1, 3, OMe
3	62.9	CH_2	3.20 (1H, dd, *J* = 10.0, 4.8 Hz, H_A) 3.17 (1H, dd, *J* = 10.0, 4.8 Hz, H_B)	1, 2
OMe	58.1	CH_3	3.39 (3H, s)	
1’	71.6	CH_2	3.39 (2H, t, *J* = 6.5 Hz)	1, 2’
2’-7’	29.4	CH_2	1.51-1.26 (26H, m)	
8’, 10’	40.7	CH_2	1.83 (2H, m) 1.66 (2H, m)	OMe, 2’
9’	40.6	CH	4.09 (1H, tt, *J* =8.8, 4.5 Hz)	8’, 10’
11’-15’	29.5-26.0	CH_2	1.51-1.26 (26H, m)	
16’	31.9	CH_2		18’
17’	22.7	CH_2		18’
18’	14.1	CH_3	0.86 (3H, t, *J* = 6.5 Hz)	
1’’’	144.1	C_4	-	2’’’, 3’’’, 5’’’, 6’’’
2’’’, 6’’’	127.7	CH	7.45-7.18 (15H, m)	3’’’, 5’’’
3’’’, 5’’’	128.7	CH		4’’’
4’’’	126.9	CH		3’’’, 5’’’
C$\mathbf{Ph_3}$	86.6	C_4	-	2’’’, 3’’’, 5’’’, 6’’’



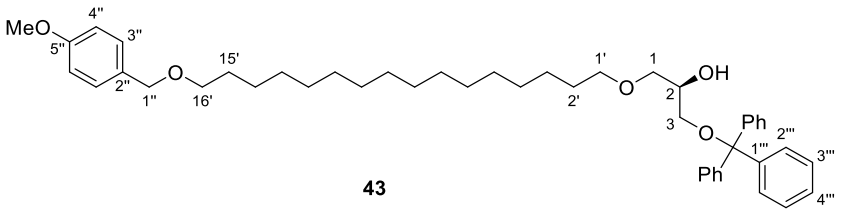
31

C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1, 7	127.6	CH	7.41 (2H, d, *J* = 4.2 Hz)	3, 5
2, 6	117.8	CH	6.53 (2H, d, *J* = 4.2 Hz)	1, 3, 5, 7
3, 5	141.3	CH	7.79 (2H, s)	2, 6
7a, 8a	133.8	C_4	-	1, 2, 6, 7
8	153.9	C_4	-	2, 3, 5, 6
OMe	20.4	CH_3	2.91 (3H, s)	



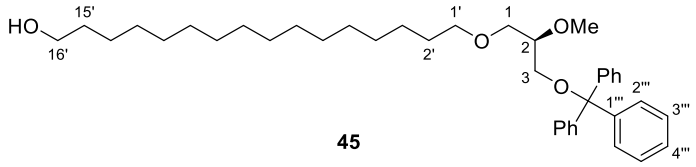
33

C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	70.6	CH_2	3.55 (1H, dd, *J* = 10.0, 5.0 Hz, H_A) 3.51 (1H, dd, *J* = 10.0, 5.0 Hz, H_B)	2, 1’
2	79.8	CH	3.44-3.41 (3H, m)	1, OMe
3	62.7	CH_2	3.75 (1H, m, H_A) 3.64 (1H, m, H_B)	1
OMe	57.7	CH_3	3.46 (3H, s)	2
1’	71.8	CH_2	3.44-3.41 (3H, m)	1, 2’
2’	26.0	CH_2	1.55 (2H, m)	1’
3’-6’	29.4-29.2	CH_2	1.29 and 1.25 (20H, br s)	
7’	25.1	CH_2	1.43 (4H, m)	8’, 9’
8’, 10’	33.9	CH_2	1.85 (4H, m)	9’
9’	86.5	CH	5.14 (1H, quin., *J* = 6.0 Hz)	8’, 10’
11’	25.1	CH_2	1.43 (4H, m)	9’, 10’
12’-15’	29.4-29.2	CH_2	1.29 and 1.25 (20H, br s)	
16’	31.8	CH_2		18’
17’	22.6	CH_2		18’
18’	14.1	CH_3	0.87 (3H, t, *J* = 6.8 Hz)	
1’’, 7’’	124.6	CH	7.27 (2H, br s)	2’’, 3’’, 5’’, 6’’
2’’, 6’’	116.1	CH	6.51 (2H, br s)	1’’, 3’’, 5’’, 7’’
3’’, 5’’	138.8	CH	7.71 (2H, br s)	1’’, 2’’, 6’’, 7’’
7a’’, 8a’’	126.6	C_4	-	1’’, 2’’, 3’’, 5’’, 6’’, 7’’
8’’	161.4	C_4	-	16’



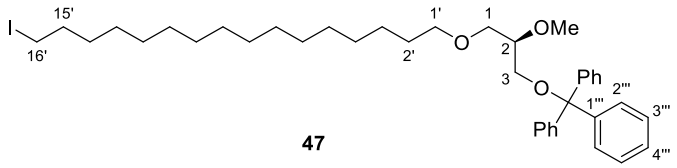
43

C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	72.2	CH_2	3.53 (1H, dd, *J* = 9.7, 4.4 Hz, H_A) 3.47 (1H, dd, *J* = 9.7, 6.6 Hz, H_B)	1', OH
2	69.8	CH	3.95 (1H, m)	1, 3, OH
3	64.6	CH_2	3.21 (1H, dd, *J* = 9.4, 5.6 Hz, H_A) 3.18 (1H, dd, *J* = 9.4, 5.6 Hz, H_B)	1, OH
OH	-	-	2.41 (1H, d, *J* = 4.7 Hz)	
1'	71.6	CH_2	3.45-3.40 (4H, m)	1
3'-14'	29.8-29.5	CH_2	1.24 (24H, br s)	
2', 15'	26.2 and 26.1	CH_2	1.63-1.52 (4H, m)	1', 16'
16'	70.2	CH_2	3.45-3.40 (4H, m)	1''
1''	72.5	CH_2	4.43 (2H, s)	16'
2''	130.9	C_4	-	1'', 3'', 4'', 6'', 7''
3'', 7''	129.2	CH	7.32-7.21 (11H, m)	4'', 6''
4'', 6''	113.7	CH	6.87 (2H, d, *J* = 8.5 Hz)	3'', 7''
5''	159.1	C_4	-	4'', 6'', Ph-OMe
Ph-OMe	55.3	CH_3	3.80 (3H, s)	
1'''	143.9	C_4	-	2'''-6'''
2''', 6'''	127.8	CH	7.45-7.42 (6H, m) 7.32-7.21 (11H, m)	3''', 4''', 5'''
3''', 5'''	128.7	CH		2''', 4''', 6'''
4'''	127.0	CH		2''', 3''', 5''', 6'''
C$\mathbf{Ph_3}$	86.6	C_4	-	3, 3''', 5'''



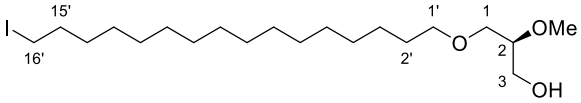
45

C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	70.9	CH_2	3.54 (1H, dd, *J* = 9.6, 4.0 Hz, H_A) 3.49 (1H, dd, *J* = 9.6, 6.0 Hz, H_B)	3, 1’
2	79.9	CH	3.47-3.43 (1H, m)	1, 3, OMe
3	62.9	CH_2	3.18 (1H, dd, *J* = 9.4, 4.6 Hz, H_A) 3.16 (1H, dd, *J* = 9.4, 4.6 Hz, H_B)	1, 2
OMe	58.1	CH_3	3.38 (3H, s)	
1’	71.7	CH_2	3.37 (2H, t, *J* = 6.7 Hz)	1
3’-14’	32.8-25.7	CH_2	1.22 (24H, br s)	
2’, 15’		CH_2	1.57-1.43 (4H, m)	
16’	63.1	CH_2	3.60 (2H, t, *J* = 6.7 Hz)	
1’’’	144.1	C_4	-	2’’’-6’’’
2’’’, 6’’’	127.7	CH	7.44-7.41 (6H, m) 7.28-7.17 (9H, m)	3’’’, 4’’’, 5’’’
3’’’, 5’’’	128.7	CH		2’’’, 4’’’, 6’’’
4’’’	126.9	CH		2’’’, 3’’’, 5’’’, 6’’’
$\underline{C}Ph_3$	86.6	C_4	-	3, 3’’’, 5’’’



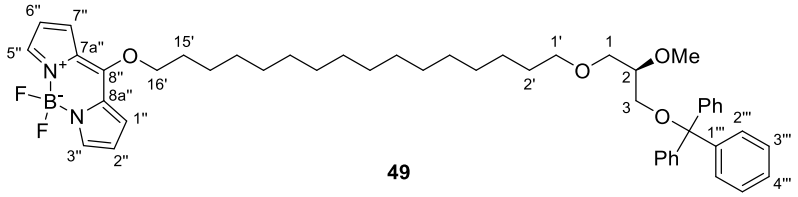
47

C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	70.9	CH_2	3.57 (1H, dd, *J* = 9.6, 4.0 Hz) A 3.52 (1H, dd, *J* = 9.6, 5.6 Hz) B	3, 1’
2	79.9	CH	3.50-3.45 (1H, m)	1, 3
3	62.9	CH_2	3.21 (1H, dd, *J* = 10.0, 4.6 Hz) A 3.20 (1H, dd, *J* = 10.0, 4.6 Hz) B	1, 2
1’	71.1	CH_2	3.41 (2H, t, *J* =6.8 Hz)	1
2’	26.1	CH_2	1.56-1.49 (4H, m)	
3’-14’	30.5-28.5	CH_2	1.56-1.49 (4H, m) 1.25 (22H, br s)	
15’	33.6	CH_2	1.82 (2H, quin, *J* = 6.8 Hz)	16’
16’	7.4	CH_3	3.19 (2H, t, *J* = 6.8 Hz)	
1’’’	144.1	C_4	-	2’’’, 3’’’, 4’’’, 5’’’, 6’’’
3’’’ and 5’’’	128.7	CH	7.47-7.20 (15H, m)	2’’’, 4’’’, 6’’’
2’’’ and 6’’’	127.7	CH		3’’’, 4’’’, 5’’’
4’’’	126.9	CH		2’’’, 3’’’, 5’’’, 6’’’
$\underline{C}Ph_3$	86.6	C_4	-	3, 3’’’, 5’’’
OMe	58.1	CH_3	3.41 (3H, s)	



48

C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	70.6	CH_2	3.56 (1H, dd, *J* = 5.0, 10.0 Hz, H_A) 3.52 (1H, dd, *J* =5.0, 10.0 Hz, H_B)	2, 1’
2	79.8	CH	3.45-3.37 (3H, m)	1, OMe
3	62.7	CH_2	3.76 (1H, dd, *J* = 2.4, 11.4 Hz, H_A) 3.65 (1H, dd, *J* = 5.2, 11.4 Hz, H_B)	1, 2
OMe	57.8	CH_3	3.47 (3H, s)	2
1’	71.9	CH_2	3.45-3.37 (3H, m)	1, 2’
2’	29.6-26.1	CH_2	1.57 (2H, quin, *J* = 7.2 Hz)	1’
3’-13’		CH_2	1.25 (24H, br s)	
14’	30.5	CH_2	1.25 (24H, br s)	15’, 16’
15’	33.6	CH_2	1.82 (2H, quin, *J* = 7.2 Hz)	16’
16’	7.4	CH_2	3.19 (2H, t, *J* = 7.2 Hz)	15’



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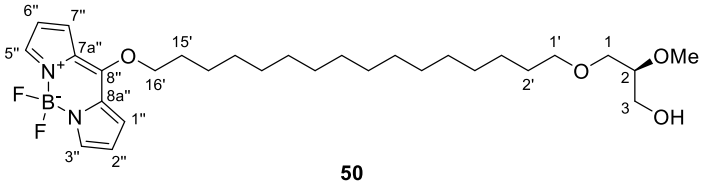
C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	70.9	CH_2	3.54 (1H, dd, *J* = 10.0, 4.0 Hz, H_A) 3.50 (1H, dd, *J* = 10.0, 5.0 Hz, H_B)	3, 1’
2	79.8	CH	3.47-3.42 (1H, m)	1, 3, OMe
3	62.8	CH_2	3.18 (1H, dd, *J* = 9.9, 4.8 Hz, H_A) 3.16 (1H, dd, *J* = 9.9, 4.8 Hz, H_B)	1, 2
OMe	58.1	CH_3	3.38 (3H, s)	
1’	71.7	CH_2	3.38 (2H, t, *J* = 6.7 Hz)	1
2’	29.7-25.8	CH_2	1.53-1.46 (4H, m)	1’
3’-13’		CH_2	1.23 (22H, br s)	
14’		CH_2	1.53-1.46 (4H, m)	
15’		CH_2	1.96-1.89 (2H, m)	14’, 16’
16’	75.4	CH_2	4.66 (2H, t, *J* = 6.2 Hz)	15’
1’’, 7’’	125.1	CH	7.30 (2H, d, *J* = 4.1 Hz)	16’, 2’’, 3’’, 5’’, 6’’
2’’, 6’’	116.2	CH	6.49-6.48 (2H, m)	1’’, 7’’
3’’, 5’’	139.0	CH	7.70 (2H, s)	1’’, 2’’, 6’’, 7’’
7a’’, 8a’’	126.2	C_4	-	2’’, 6’’
8’’	161.5	C_4	-	16’, 2’’, 3’’, 5’’, 6’’
1’’’	143.9	C_4	-	2’’’-6’’’
2’’’, 6’’’	127.8	CH	7.44-7.41 (6H, m) 7.28-7.17 (9H, m)	3’’’, 4’’’, 5’’’
3’’’, 5’’’	128.7	CH		2’’’, 4’’’, 6’’’
4’’’	127.0	CH		2’’’, 3’’’, 5’’’, 6’’’
C$\mathbf{Ph_3}$	86.6	C_4	-	3, 3’’’, 5’’’



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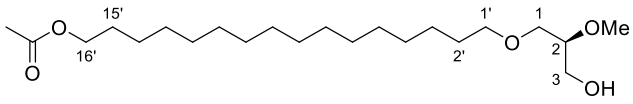
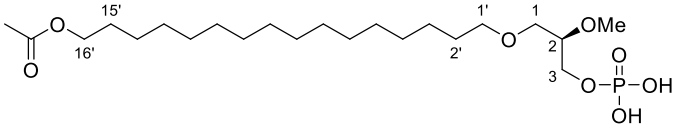
C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	70.6	CH_2	3.56 (1H, dd, *J* =10.0, 5.0 Hz, H_A) 3.53 (1H, dd, *J* =10.0, 5.0 Hz, H_B)	2, 3, 1’
2	79.8	CH	3.45-3.41 (3H, m)	1, OMe
3	62.7	CH_2	3.76 (1H, dd, *J* =11.8, 4.6 Hz, H_A) 3.65 (1H, dd, *J* =11.8, 4.6 Hz, H_B)	1
OMe	57.8	CH_3	3.47 (3H, s)	
1’	71.9	CH_2	3.45-3.41 (3H, m)	1, 2’
2’	29.7-25.8	CH_2	1.60-1.50 (4H, m)	1’
3’-13’		CH_2	1.26 (22H, br s)	
14’		CH_2	1.60-1.50 (4H, m)	
15’		CH_2	1.96 (2H, m)	14’, 16’
16’	75.4	CH_2	4.70 (1H, t, *J* = 6.4 Hz)	15’
1’’, 7’’	125.1	CH	7.34 (2H, d, *J* = 4.2 Hz)	2’’, 3’’, 5’’, 6’’
2’’, 6’’	116.2	CH	6.52 (2H, dd, *J* = 4.2, 2.0 Hz)	1’’, 3’’, 5’’, 7’’
3’’, 5’’	139.0	CH	7.73 (2H, s)	1’’, 2’’, 6’’, 7’’
7a’’, 8a’’	126.2	C_4	-	1’’, 2’’, 6’’, 7’’
8’’	161.5	C_4	-	16’



52

C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	70.6	CH_2	3.55 (1H, dd, *J* = 10.0, 5.0 Hz, H_A) 3.52 (1H, dd, *J* = 10.0, 5.0 Hz, H_B)	2, 1’
2	79.8	CH	3.46-3.42 (3H, m)	1, OMe
3	62.7	CH_2	3.77-3.73 (1H, m, H_A) 3.66-3.61 (1H, m, H_B)	1
OMe	57.8	CH_3	3.46 (3H, s)	
1’	71.9	CH_2	3.46-3.42 (3H, m)	1
3’-14’	29.6-25.9	CH_2	1.25 (24H, br s)	
2’, 15’		CH_2	1.64-1.53 (4H, m)	
16’	64.7	CH_2	4.04 (2H, t, *J* = 6.7 Hz)	OOC$\underline{CH_3}$
OO$\underline{C}CH_3$	171.2	C_4	-	16’, OOC$\underline{CH_3}$
OOC$\underline{CH_3}$	21.0	CH_3	2.04 (3H, s)	



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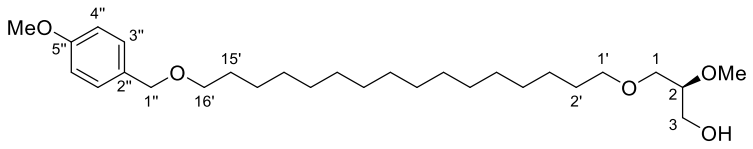
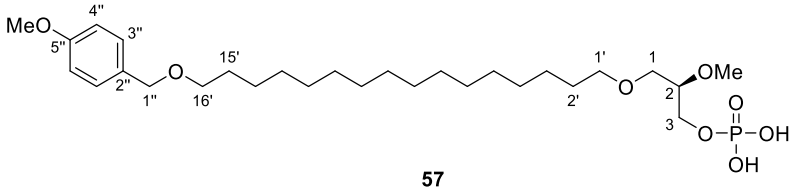
C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	69.5	CH_2	3.56-3.52 (2H, m)	
2	79.1	CH	3.63-3.57 (1H, m)	OMe
3	65.8	CH_2	4.16-4.03 (2H, m)	
OMe	57.8	CH_3	3.49 (3H, s)	
1'	72.0	CH_2	3.45 (3H, t, *J* = 6.4 Hz)	
3'-14'	29.7-25.9	CH_2	1.25 (24H, br s)	
2', 15'		CH_2	1.65-1.53 (4H, m)	
16'	64.7	CH_2	4.05 (2H, t, *J* = 6.7 Hz)	
OO$\underline{C}$CH$_3$	171.4	C_4	-	16', OOC$\underline{CH_3}$
OOC$\underline{CH_3}$	21.0	CH_3	2.04 (3H, s)	



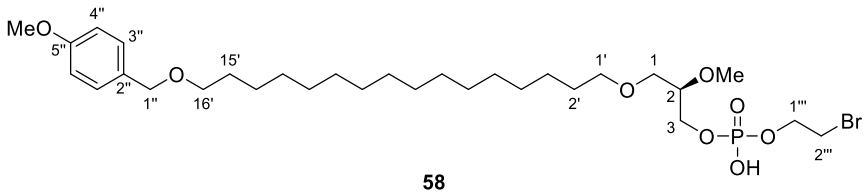
56

C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	70.6	CH_2	3.55 (1H, dd, *J* = 9.9, 5.0 Hz) A 3.52 (1H, dd, *J* = 9.9, 5.0 Hz) B	1’
2	79.8	CH	3.46-3.41 (5H, m)	1, OMe
3	62.7	CH_2	3.78-3.73 (1H, m) A 3.67-3.62 (1H, m) B	1
1’	71.9	CH_2	3.46-3.41 (5H, m)	1, 2’
3’-14’	29.8-29.4	CH_2	1.25 (24H, br s)	
2’	26.2	CH_2	1.63-1.53 (4H, m)	1’
15’	26.1	CH_2		16’
16’	70.2	CH_2	3.46-3.41 (5H, m)	15’, 1’’
1’’	72.5	CH_2	4.43 (2H, s)	16’, 3’’, 7’’
2’’	130.8	C_4	-	1’’, 4’’, 6’’
3’’, 7’’	129.2	CH	7.26 (2H, d, *J* = 8.5 Hz)	1’’, 4’’, 6’’
4’’, 6’’	113.7	CH	6.87 (2H, d, *J* = 8.5 Hz)	3’’, 7’’
5’’	159.1	C_4	-	1’’, 3’’, 4’’, 6’’, 7’’ Ph-OMe
Ph-OMe	55.2	CH_3	3.80 (3H, s)	
OMe	57.8	CH_3	3.47 (3H, s)	2



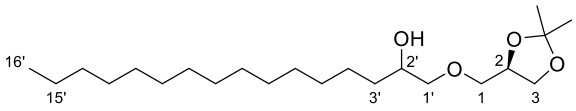
57

C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	69.9	CH_2	3.56-3.40 (7H, m)	
2	79.0	CH		1, OMe
3	65.5	CH_2	4.02 (2H, m)	1
OMe	57.9	CH_3	3.44 (3H, s)	
1'	71.8	CH_2	3.56-3.40 (7H, m)	1
2', 15'	26.1 and 26.0	CH_2	1.61-1.52 (4H, m)	1', 16'
3'-14'	29.7-29.5	CH_2	1.24 (24H, br s)	
16'	70.2	CH_2	3.56-3.40 (7H, m)	15', 1''
1''	72.5	CH_2	4.42 (2H, s)	16', 3'', 7''
2''	130.8	C_4	-	1'', 4'', 6''
3'', 7''	129.2	CH	7.25 (2H, d, *J* = 8.6 Hz)	1''
4'', 6''	113.7	CH	6.86 (2H, d, *J* = 8.6 Hz)	3'', 7'', Ph-OMe
5''	159.0	C_4	-	1'', 3'', 4'', 6'', 7'' Ph-OMe
Ph-OMe	55.2	CH_3	3.79 (3H, s)	



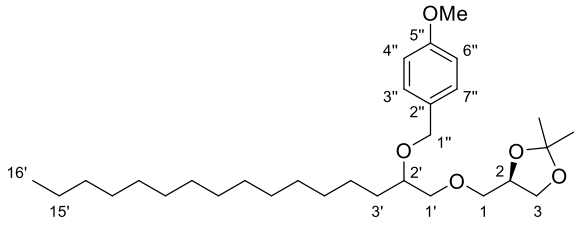
58

C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	69.1	CH_2	3.59-3.50 (5H, m)	2, 3
2	78.7 (d, *J* = 7 Hz)	CH		1, 3, OMe
3	66.9 (d, *J* = 5 Hz)	CH_2	4.21-4.16 (1H, m, H_A) 4.12-4.06 (1H, m, H_B)	1, 2
OMe	58.1	CH_3	3.47 (3H, s)	2
1'	71.9	CH_2	3.45-3.41 (4H, m)	1, 2'
2', 15'	26.2 and 26.0	CH_2	1.63-1.53 (4H, m)	1', 16'
3'-14'	29.7-29.5	CH_2	1.25 (24H, br s)	
16'	70.2	CH_2	3.45-3.41 (4H, m)	15', 1''
1''	72.5	CH_2	4.43 (2H, s)	16', 3'', 7''
2''	130.8	C_4	-	1'', 4'', 6''
3'', 7''	129.2	CH	7.26 (2H, d, *J* = 8.7 Hz)	1''
4'', 6''	113.7	CH	6.87 (2H, d, *J* = 8.7 Hz)	3'', 7''
5''	159.0	C_4	-	1'', 3'', 4'', 6'', 7'' Ph-OMe
Ph-OMe	55.2	CH_3	3.80 (3H, s)	
1'''	66.5 (d, *J* = 5 Hz)	CH_2	4.29 (2H, dt, *J* =7.6, 6.5 Hz)	2'''
2'''	29.1 (d, *J* = 9 Hz)	CH_2	3.59-3.50 (5H, m)	1'''



60a

C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	72.3	CH_2	3.56 (2H, m)	3, 1'
2	74.8	CH	4.30 (1H, dd, *J* = 11.0, 6.4 Hz)	1
3	66.6	CH_2	4.06 (1H, dd, *J* = 8.3, 6.4 Hz, H_A) 3.74 (1H, dd, *J* = 8.3, 6.4 Hz, H_B)	1
CMe$_2$	109.6	C_4	-	3, C$\underline{\text{Me}}_2$
CMe$_2$	26.7	CH_3	1.43 and 1.37 (3H, s each)	Me
	25.4	CH_3		Me
1'	76.2	CH_2	3.52 (1H, m, H_A) 3.33 (1H, m, H_B)	1
2'	70.3	CH	3.79 (1H, m)	1'
3'	33.0	CH_2	1.43 (2H, m)	1’
4'	25.5	CH_2	1.25 (24H, br s)	2’
5'-13'	29.7-29.3	CH_2		
14’	31.9	CH_2		16’
15'	22.7	CH_2		16'
16'	14.1	CH_3	0.88 (3H, t, *J* = 6.8 Hz)	



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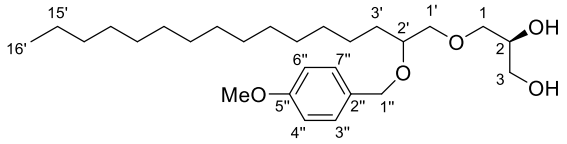
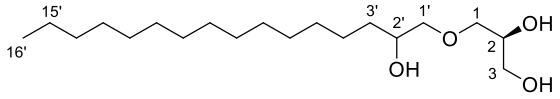
C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	72.4	CH_2	3.58-3.45 (5H, m)	3, 1'
2	74.7	CH	4.26 (1H, quin, *J* = 8.0 Hz)	1, 3
3	66.9	CH_2	4.06 (1H, dd, *J* = 8.0, 6.0 Hz, H_A) 3.77 (1H, dd, *J* = 8.0, 6.0 Hz, H_B)	1
C$\underline{\mathbf{Me}}_2$	109.3	C_4	-	3, C$\underline{\mathrm{Me}}_2$
C$\underline{\mathbf{Me}}_2$	26.8	CH_3	1.42 and 1.39 (3H, s each)	Me
	25.4	CH_3		Me
1'	74.5	CH_2	3.58-3.45 (5H, m)	1
2'	77.7	CH	3.58-3.45 (5H, m)	1', 1''
3'	31.8	CH_2	1.50-1.47 (2H, m)	2'
4'	25.4	CH_2	1.26 (24H, br s)	2'
5'-13'	29.7-29.4	CH_2		
14'	31.9	CH_2		16'
15'	22.7	CH_2		16'
16'	14.1	CH_3	0.88 (3H, t, *J* = 6.8 Hz)	
1''	71.7	CH_2	4.59 (1H, d, *J* = 12.0 Hz, H_A) 4.49 (1H, d, *J* = 12.0 Hz, H_B)	3'', 7''
2''	131.1	C_4	-	1'', 4'', 6''
3'', 7''	129.4	CH	7.27 (2H, d, *J* = 8.0 Hz)	1''
4'', 6''	113.7	CH	6.87 (2H, d, *J* = 8.0 Hz)	
5''	159.1	C_4	-	4'', 6'', OMe
Ph-OMe	55.3	CH_3	3.80 (3H, s)	



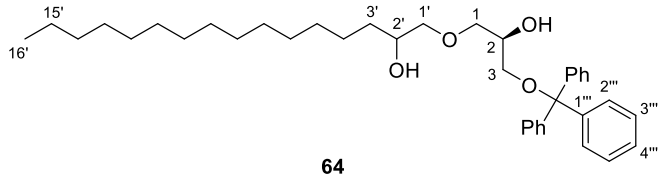
62

C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	73.2	CH_2	3.59-3.51 (5H, m)	2, 3, 1’
2	70.5	CH	3.85 (1H, m)	1, 3
3	64.0	CH_2	3.69 (1H, dd, *J* = 11.4, 4.0 Hz, H_A) 3.62 (1H, dd, *J* = 11.4, 5.2 Hz, H_B)	1, 2
1’	74.1	CH_2	3.59-3.51 (5H, m)	1, 2’, 3’
2’	77.8	CH		1’, 3’, 1’’
3’	31.5	CH_2	1.52 (2H, m)	1’, 2’
4’	25.5	CH_2	1.25 (24H, br s)	3’
5’-13’	29.7-29.4	CH_2		
14’	31.9	CH_2		16’
15’	22.7	CH_2		16’
16’	14.1	CH_3	0.88 (3H, t, *J* = 6.8 Hz)	
1’’	71.5	CH_2	4.53 (1H, d, *J* = 11.2 Hz, H_A) 4.50 (1H, d, *J* = 11.2 Hz, H_B)	2’, 3’’, 7’’
2’’	130.6	C_4	-	1’’, 4’’, 6’’
3’’, 7’’	129.4	CH	7.27 (2H, d, *J* = 8.8 Hz)	1’’
4’’, 6’’	113.8	CH	6.87 (2H, d, *J* = 8.8 Hz)	3’’, 7’’
5’’	159.2	C_4	-	3’’, 4’’, 6’’, 7’’, Ph-OMe
Ph-OMe	55.2	CH_3	3.80 (3H, s)	



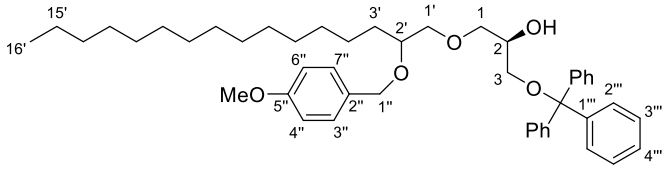
63

C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	72.9	CH_2	3.57 (2H, m)	1’
2	70.6	CH	3.90 (1H, m)	1
3	63.9	CH_2	3.71 (1H, d, *J* = 12.0 Hz, H_A) 3.65 (1H, m, H_B)	1
1’	76.0	CH_2	3.53 (1H, m, H_A) 3.35 (1H, m, H_B)	1
2’	70.5	CH	3.80 (1H, m)	1’
3’	33.2	CH_2	1.42 (2H, m)	1’
4’	25.5	CH_2	1.25 (24H, br s)	
5’-13’	29.7-29.4	CH_2		
14’	31.9	CH_2		16’
15’	22.7	CH_2		16’
16’	14.1	CH_3	0.88 (3H, t, *J* = 6.8 Hz)	



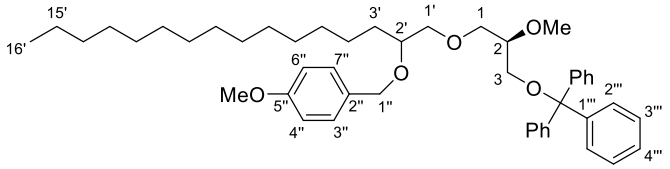
64

C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	72.6	CH_2	3.62 (1H, m, H_A) 3.56 (1H, m, H_B)	3
2	69.9	CH	3.96 (1H, m)	1, 3
3	64.4	CH_2	3.21 (2H, m)	1
1'	76.0	CH_2	3.48 (1H, m, H_A) 3.30 (1H, m, H_B)	
2'	70.4	CH	3.74 (1H, m)	
3'	33.0	CH_2	1.41 (2H, m)	
4'	25.5	CH_2	1.26 (24H, br s)	
5'-13'	29.7-29.4	CH_2		
14'	31.9	CH_2		16'
15'	22.7	CH_2		16'
16'	14.1	CH_3	0.88 (3H, t, *J* = 6.8 Hz)	
1'''	143.7	C_4	-	2'''-6'''
2''', 6'''	127.9	CH	7.44-7.41 (6H, m) 7.32-7.24 (9H, m)	3''', 4''', 5'''
3''', 5'''	128.6	CH		2''', 4''', 6'''
4'''	127.1	CH		2''', 3''', 5''', 6'''
$\underline{C}Ph_3$	86.7	C_4	-	2''', 6'''



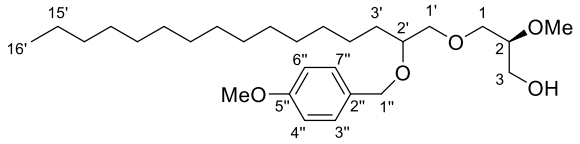
65

C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	72.9	CH_2	3.63-3.48 (5H, m)	3, 1’
2	69.9	CH	4.00-3.96 (1H, m)	1, 3
3	64.7	CH_2	3.26-3.18 (2H, m)	1
1'	74.4	CH_2	3.63-3.48 (5H, m)	1, 2’
2’	77.7	CH		1’, 1’’
3'	31.7	CH_2	1.50-1.42 (2H, m)	1’, 2’
4’	25.4	CH_2	1.27 (24H, br s)	2’
5'-13'	29.6-29.3	CH_2		
14’	31.9	CH_2		16’
15'	22.7	CH_2		16’
16'	14.1	CH_3	0.89 (3H, t, *J* = 6.8 Hz)	
1’’	71.6	CH_2	4.53 (1H, d, *J* = 11.4 Hz, H_A) 4.47 (1H, d, *J* = 11.4 Hz, H_B)	3’’, 7’’
2''	130.9	C_4	-	1’’, 4’’, 6’’
3'', 7''	129.3	CH	7.32-7.22 (11H, m)	1’’
4'', 6''	113.7	CH	6.84 (2H, d, *J* = 8.6 Hz)	3’’, 7’’
5''	159.1	C_4	-	3’’, 4’’, 6’’, 7’’, Ph-OMe
Ph-O<u>Me</u>	55.2	CH_3	3.79 (3H, s)	
1’’’	143.8	C_4	-	2’’’, 3’’’, 4’’’, 5’’’, 6’’’
2’’’, 6’’’	127.6	CH	7.46-7.42 (6H, m) 7.32-7.22 (11H, m)	3’’’, 4’’’, 5’’’
3’’’, 5’’’	128.6	CH		2’’’, 4’’’, 6’’’
4’’’	127.0	CH		2’’’, 3’’’, 5’’’, 6’’’
<u>C</u>Ph$_3$	86.6	C_4	-	3, 3’’’, 7’’’



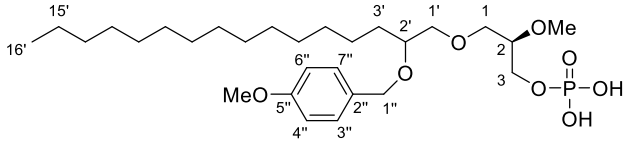
66

C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	71.8	CH_2	3.64-3.54 (3H, m)	3, 1’
2	79.9	CH	3.52-3.47 (3H, m)	1, 3, OMe
3	63.2	CH_2	3.23-3.20 (2H, m)	1, 2
OMe	58.3	CH_3	3.45 (3H, s)	2
1'	74.7	CH_2	3.64-3.54 (3H, m, H_A) 3.52-3.47 (3H, m, H_B)	1
2’	77.6	CH	3.52-3.47 (3H, m)	1’, 1’’
3'	31.9	CH_2	1.47-1.42 (2H, m)	1’, 2’
4’	25.4	CH_2	1.27 (24H, m)	2’
5'-13'	29.7-29.4	CH_2		
14’	32.0	CH_2		16’
15'	22.7	CH_2		16’
16'	14.1	CH_3	0.89 (3H, t, *J* = 6.6 Hz)	
1’’	71.7	CH_2	4.55 (1H, dd, *J* = 11.3 Hz, H_A) 4.44 (1H, dd, *J* = 11.3 Hz, H_B)	3’’, 7’’
2''	131.2	C_4	-	1’’, 4’’, 6’’
3'', 7''	129.4	CH	7.47-7.20 (17H, m)	1’’
4'', 6''	113.6	CH	6.86-6.82 (2H, m)	
5''	159.0	C_4	-	4’’, 6’’, Ph-OMe
Ph-O<u>Me</u>	55.2	CH_3	3.79 (3H, s)	
1’’’	144.0	C_4	-	2’’’, 3’’’, 4’’’, 5’’’, 6’’’
2’’’, 6’’’	127.8	CH	7.47-7.20 (17H, m)	3’’’, 4’’’, 5’’’
3’’’, 5’’’	128.7	CH		2’’’, 4’’’, 6’’’
4’’’	126.9	CH		2’’’, 3’’’, 5’’’, 6’’’
<u>C</u>Ph$_3$	86.6	C_4	-	3, 3’’’, 7’’’



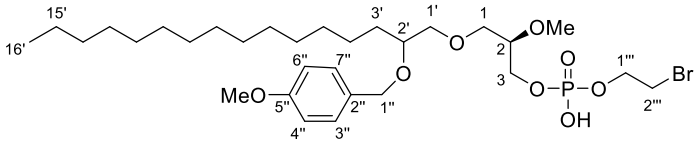
67

C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	71.1	CH_2	3.63-3.49 (5H, m)	2, 3, 1’
2	80.0	CH	3.45-3.42 (1H, m)	1, 3, OMe
3	62.5	CH_2	3.75 (1H, dd, *J* = 11.2, 4.0 Hz, H_A) 3.64 (1H, dd, *J* = 11.2, 4.0 Hz, H_B)	1, 2
OMe	57.8	CH_3	3.46 (3H, s)	2
1'	74.5	CH_2	3.63-3.49 (5H, m)	1, 2’
2’	77.7	CH		1’, 1’’
3'	31.7	CH_2	1.53-1.48 (2H, m)	1’, 2’
4’	25.4	CH_2	1.26 (24H, br s)	2’
5'-13'	29.7-29.3	CH_2		
14’	31.9	CH_2		16’
15'	22.7	CH_2		16’
16'	14.1	CH_3	0.88 (3H, s)	
1’’	71.6	CH_2	4.58 (1H, d, *J* = 11.3 Hz, H_A) 4.49 (1H, d, *J* = 11.3 Hz, H_B)	3’’, 7’’
2''	130.9	C_4	-	1’’, 4’’, 6’’
3'', 7''	129.4	CH	7.28-7.25 (2H, m)	1’’
4'', 6''	113.7	CH	6.89-6.85 (2H, m)	3’’, 7’’
5''	159.1	C_4	-	3’’, 4’’, 6’’, 7’’, Ph-OMe
Ph-OMe	55.2	CH_3	3.80 (3H, s)	



68

C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	70.1	CH_2	3.59-3.48 (6H, m)	2, 1’
2	78.8	CH		OMe
3	65.5	CH_2	4.13-4.06 (2H, m)	2
OMe	58.0	CH_3	3.45 (3H, s)	
1’	74.2	CH_2	3.59-3.48 (6H, m)	2'
2’	77.6	CH		1’’
3’	31.6	CH_2	1.47-1.45 (2H, m)	2’
4’-13’	29.7-25.4	CH_2	1.26 (24H, br s)	
14’	31.9	CH_2		16’
15’	22.7	CH_2		16'
16’	14.1	CH_3	0.89 (3H, t, *J* = 6.4 Hz)	
1’’	71.5	CH_2	4.61 (1H, dd, *J* = 2.0, 11.4 Hz, H_A) 4.50 (1H, d, *J* = 11.4 Hz, H_B)	3’’,4’’, 6’’, 7’’
2’’	130.5	C_4	-	1’’, 4’’, 6’’
3’’, 7’’	129.4	CH	7.27 (2H, d, *J* = 8.5 Hz)	4’’, 6’’
4’’, 6’’	113.7	CH	6.86 (2H, d, *J* = 8.5 Hz)	3’’, 7’’
5’’	159.1	C_4	-	3’’, 4’’, 6’’, 7’’, Ph-OMe
Ph-OMe	55.2	CH_3	3.78 (3H, s)	



69

C/nº	δ ^{13}C	multiplicidad	δ ^{1}H	HMBC
1	70.2	CH_2	3.56-3.50 (8H, m)	1’
2	78.9	CH		1, OMe
3	66.0	CH_2	4.11-4.05 (2H, m)	1, 2
OMe	58.1	CH_3	3.46 (3H, s)	
1’	74.5	CH_2	3.56-3.50 (8H, m)	1
2’	77.6	CH		1’, 1’’
3’	31.8	CH_2	1.47 (2H, m)	2’
4’	25.4	CH_2	1.25 (24H, m)	2’
5’-13’	29.7-29.3	CH_2		
14’	31.9	CH_2		16’
15’	22.7	CH_2		16’
16’	14.1	CH_3	0.87 (3H, t, *J* = 6.6 Hz)	
1’’	71.5	CH_2	4.59 (1H, d, *J* = 12.0 Hz, H_A) 4.49 (1H, d, *J* = 12.0 Hz, H_B)	2’, 3’’, 7’’
2’’	130.9	C_4	-	1’’, 4'', 6''
3’’, 7’’	129.4	CH	7.26 (2H, d, *J* = 8.0 Hz)	1’’
4’’, 6’’	113.7	CH	6.86 (2H, d, *J* = 8.0 Hz)	3’’, 7’’
5’’	159.1	C_4	-	1’’, 3’’, 4’’, 6’’, 7’’, Ph-OMe
Ph-OMe	55.2	CH_3	3.79 (3H, s)	
1’’’	66.1	CH_2	4.24 (2H, br s)	2’’’
2’’’	31.7	CH_2	3.56-3.50 (8H, m)	1’’’

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SELLO UNIVERSIDAD DE SALAMANCA	20-07-2021 18:11:01
SÁNCHEZ MARCOS ISIDRO	20-07-2021 10:20:59

Spectroscopy

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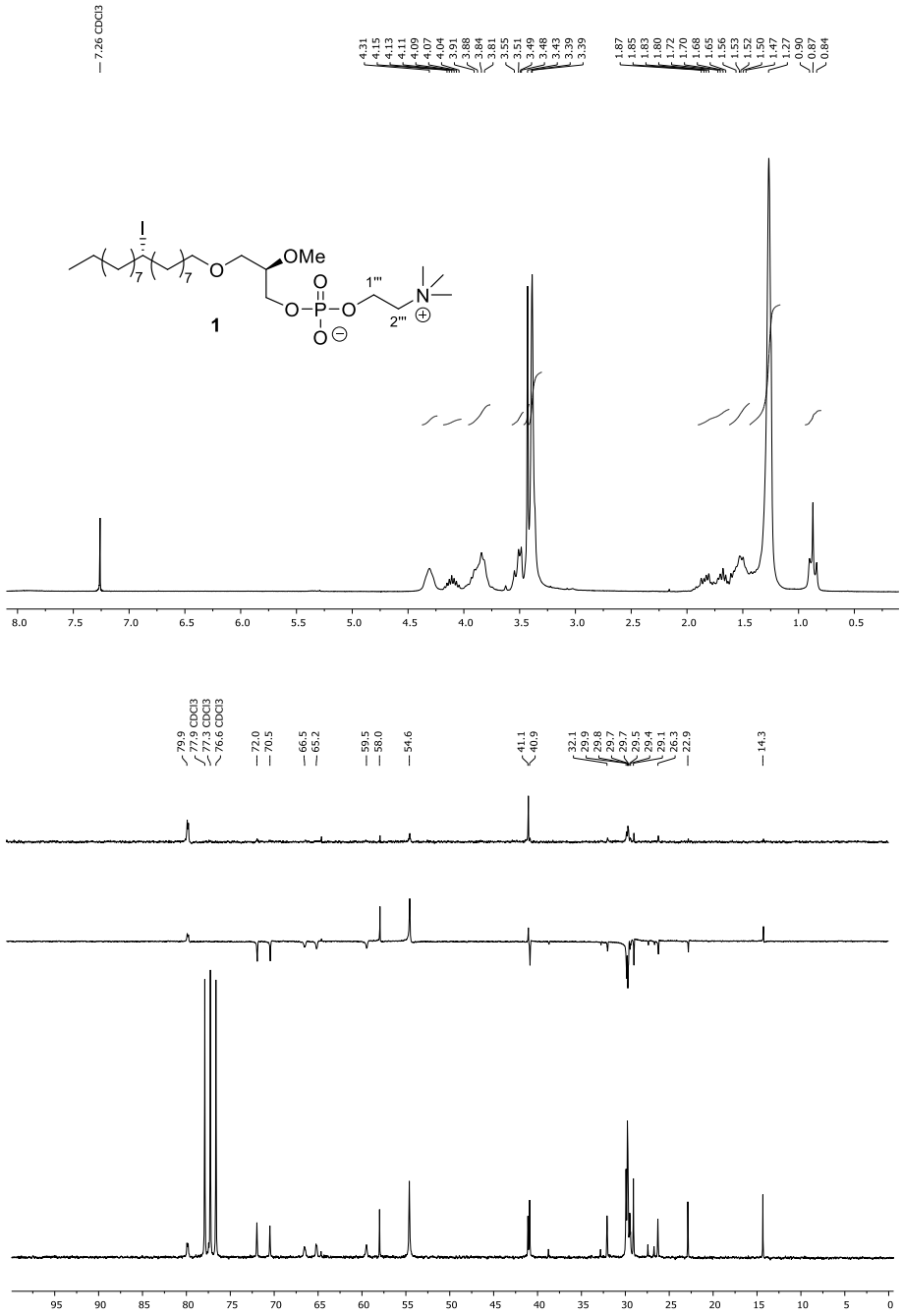

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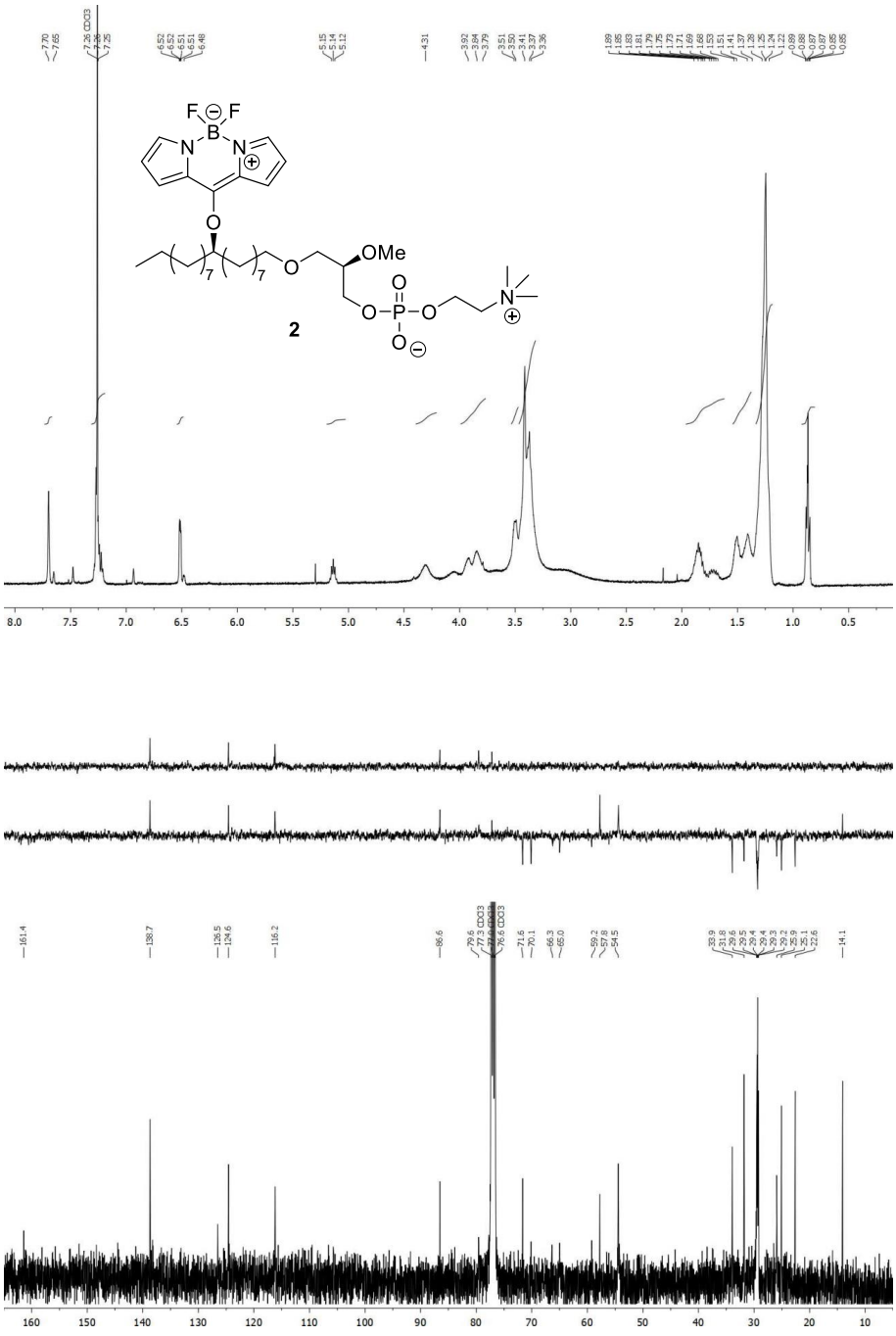

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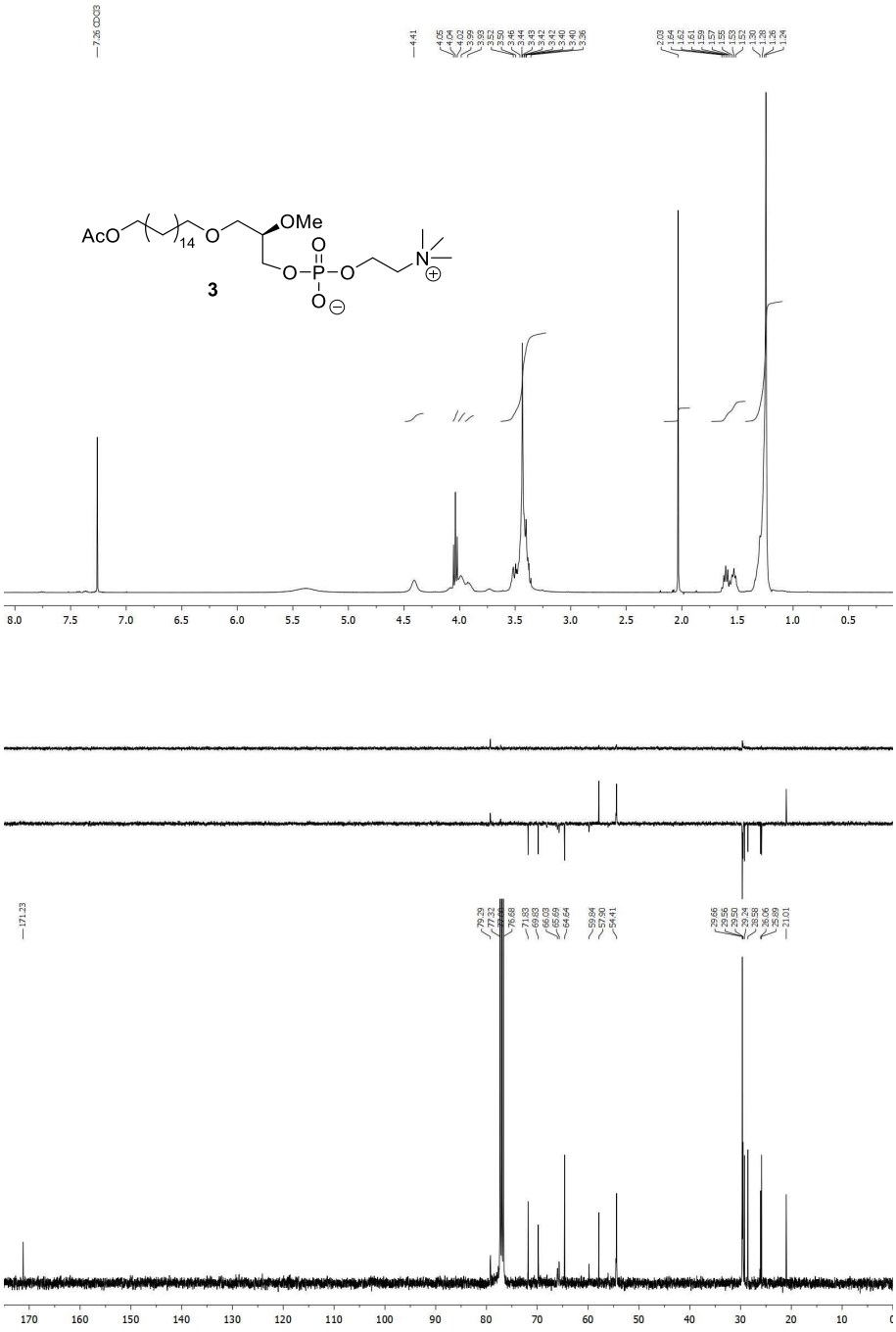

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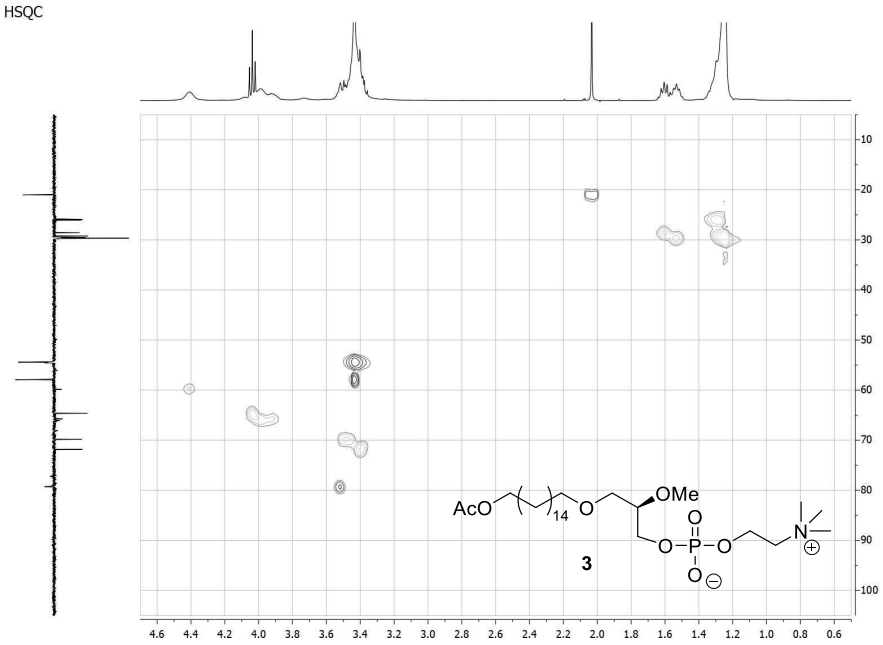

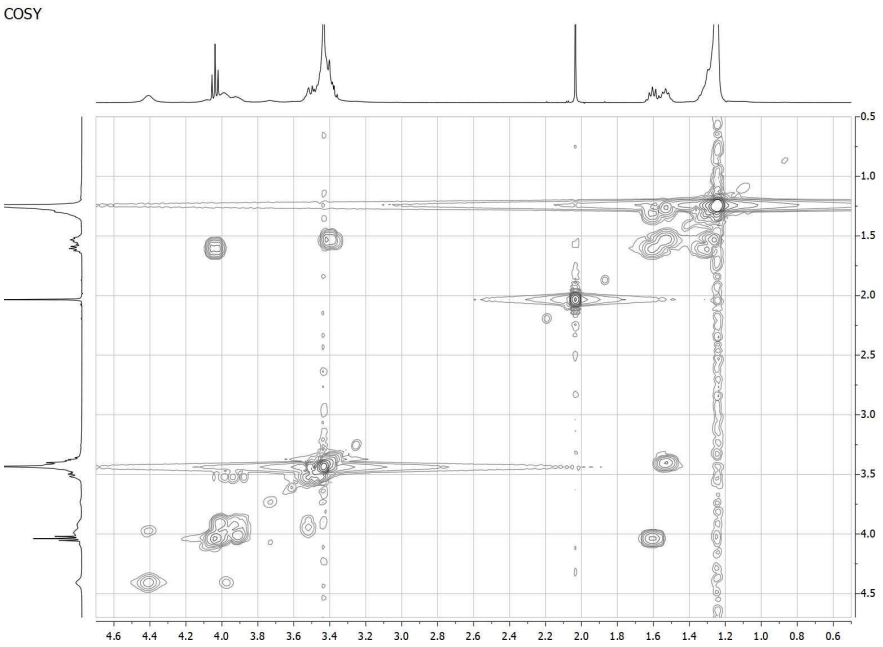

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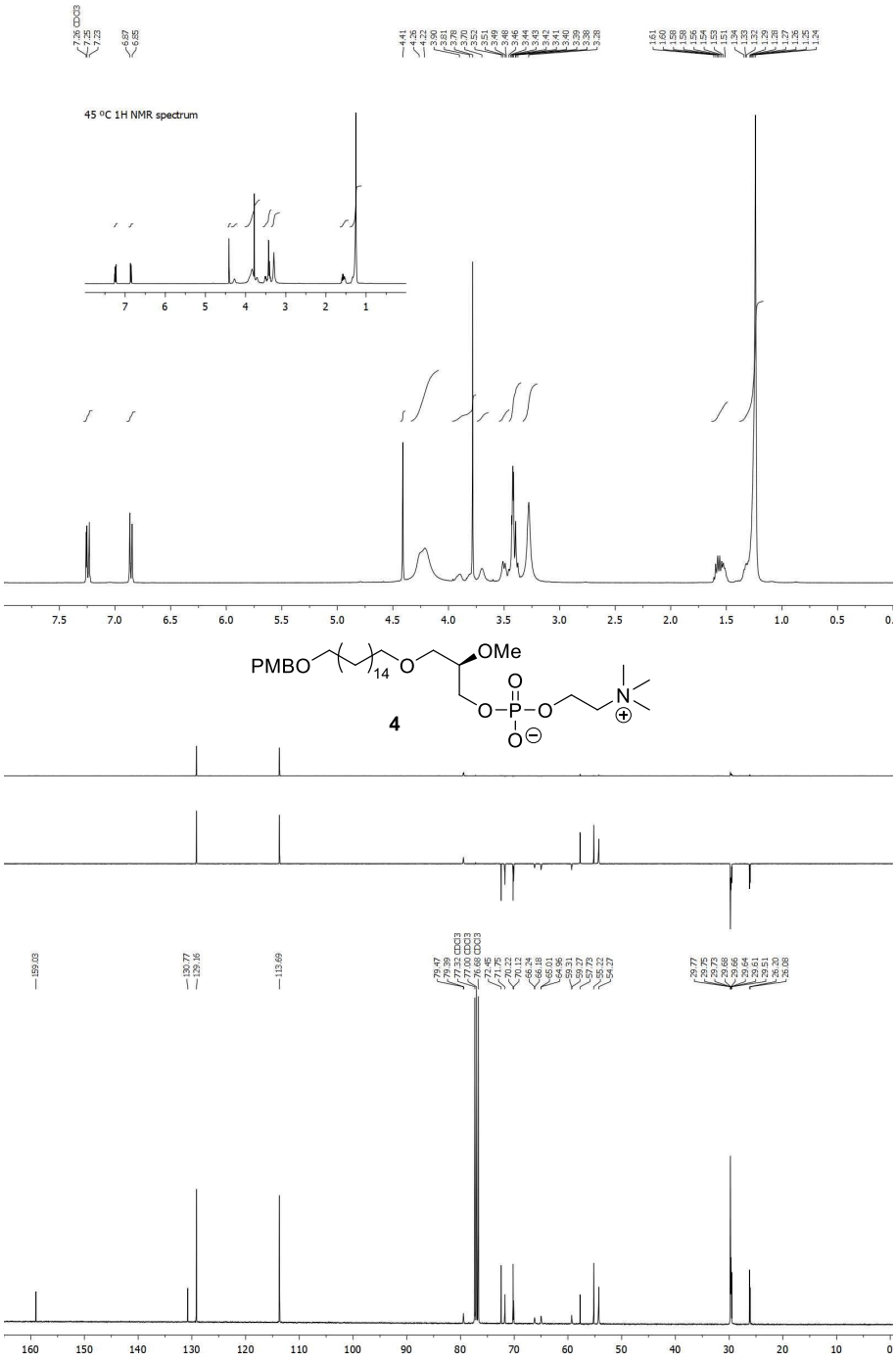

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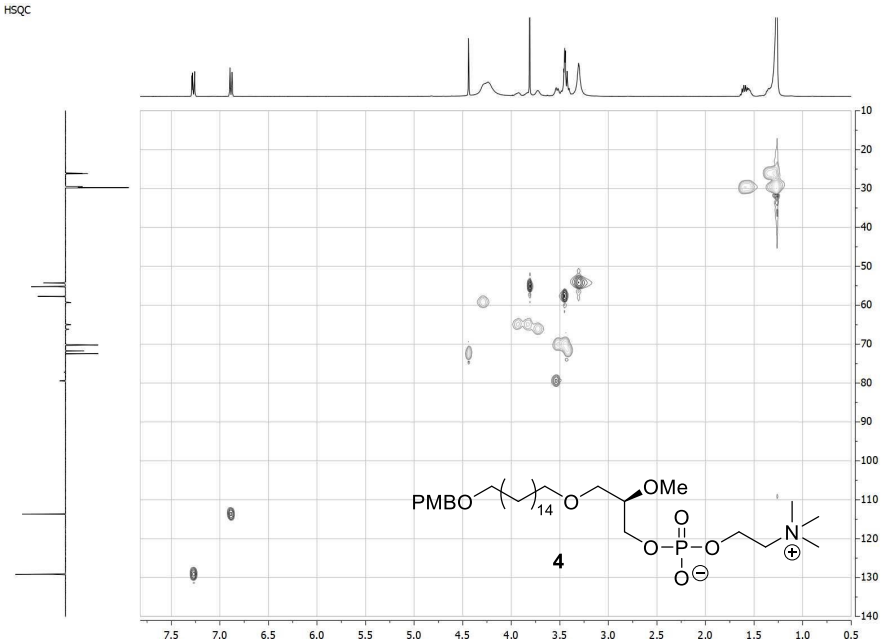

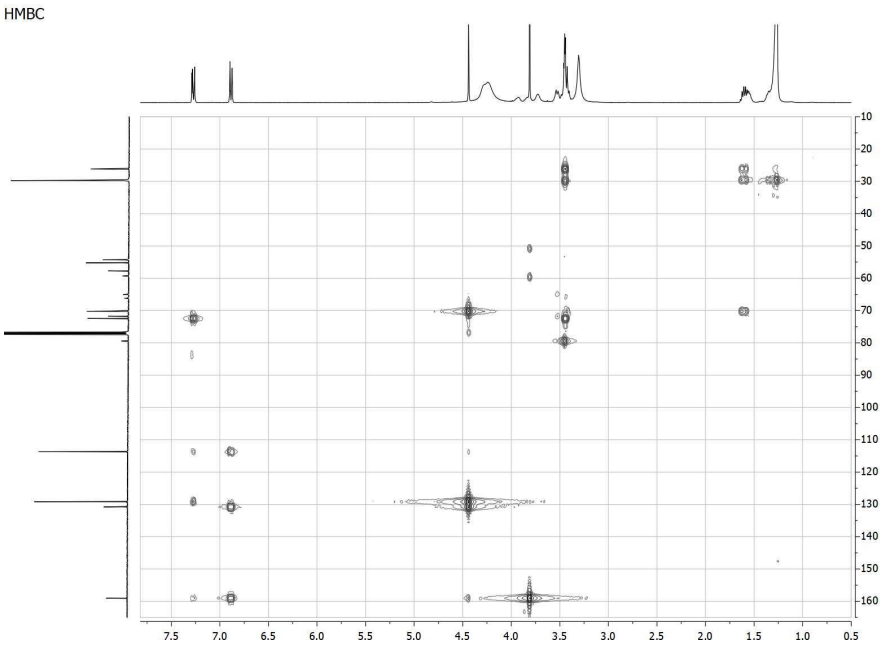

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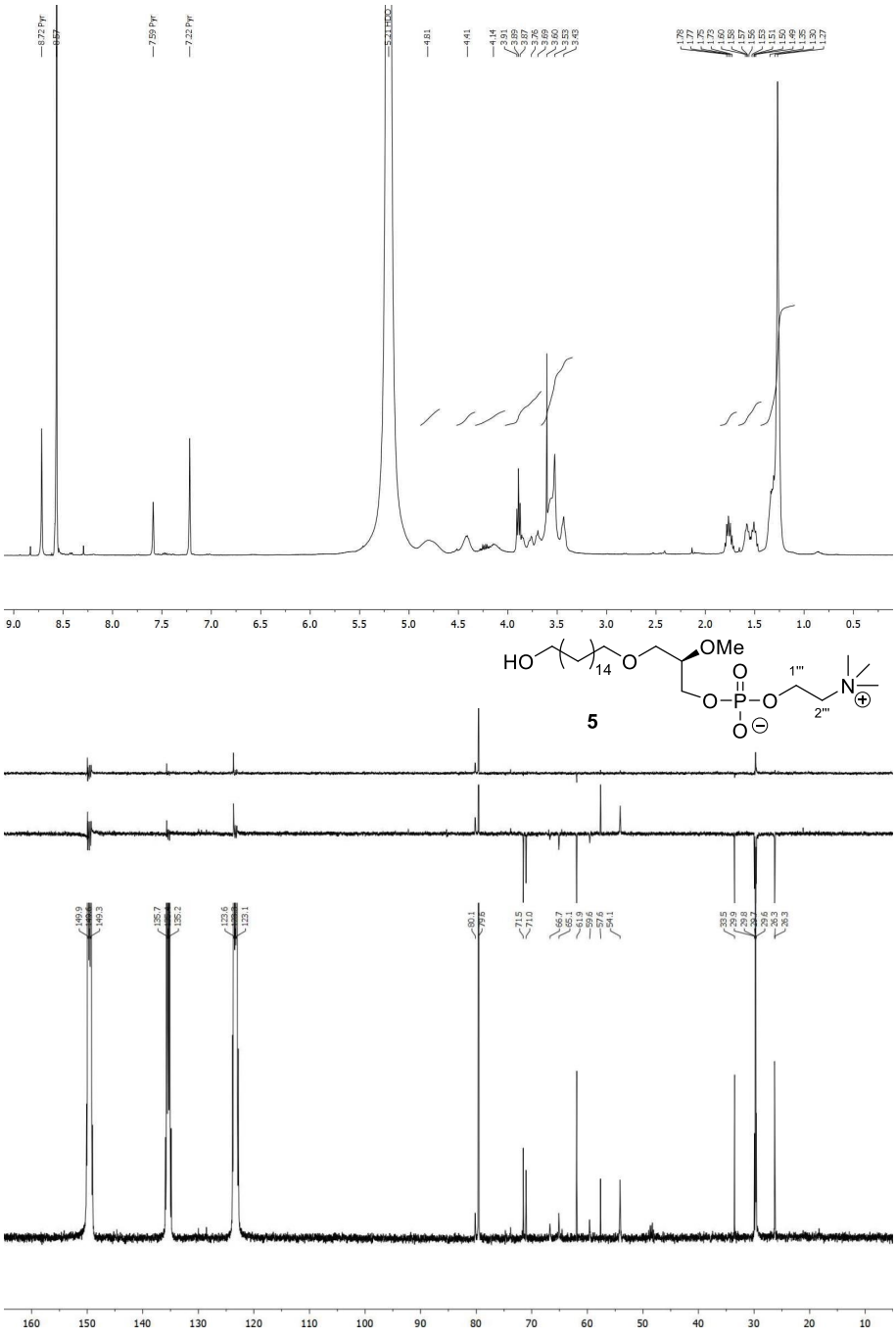

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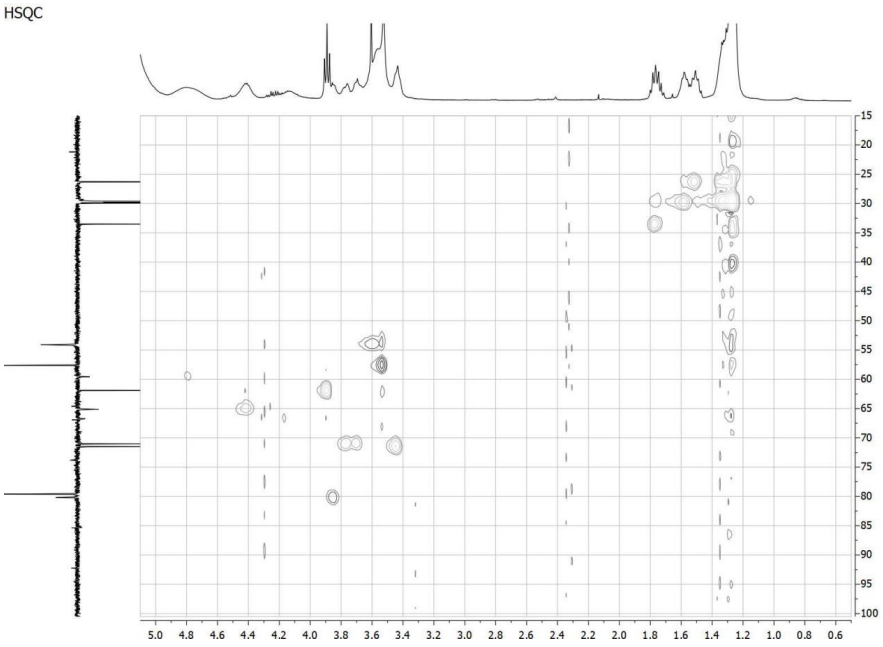

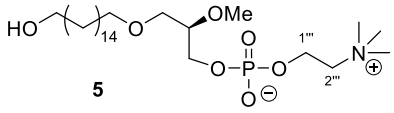

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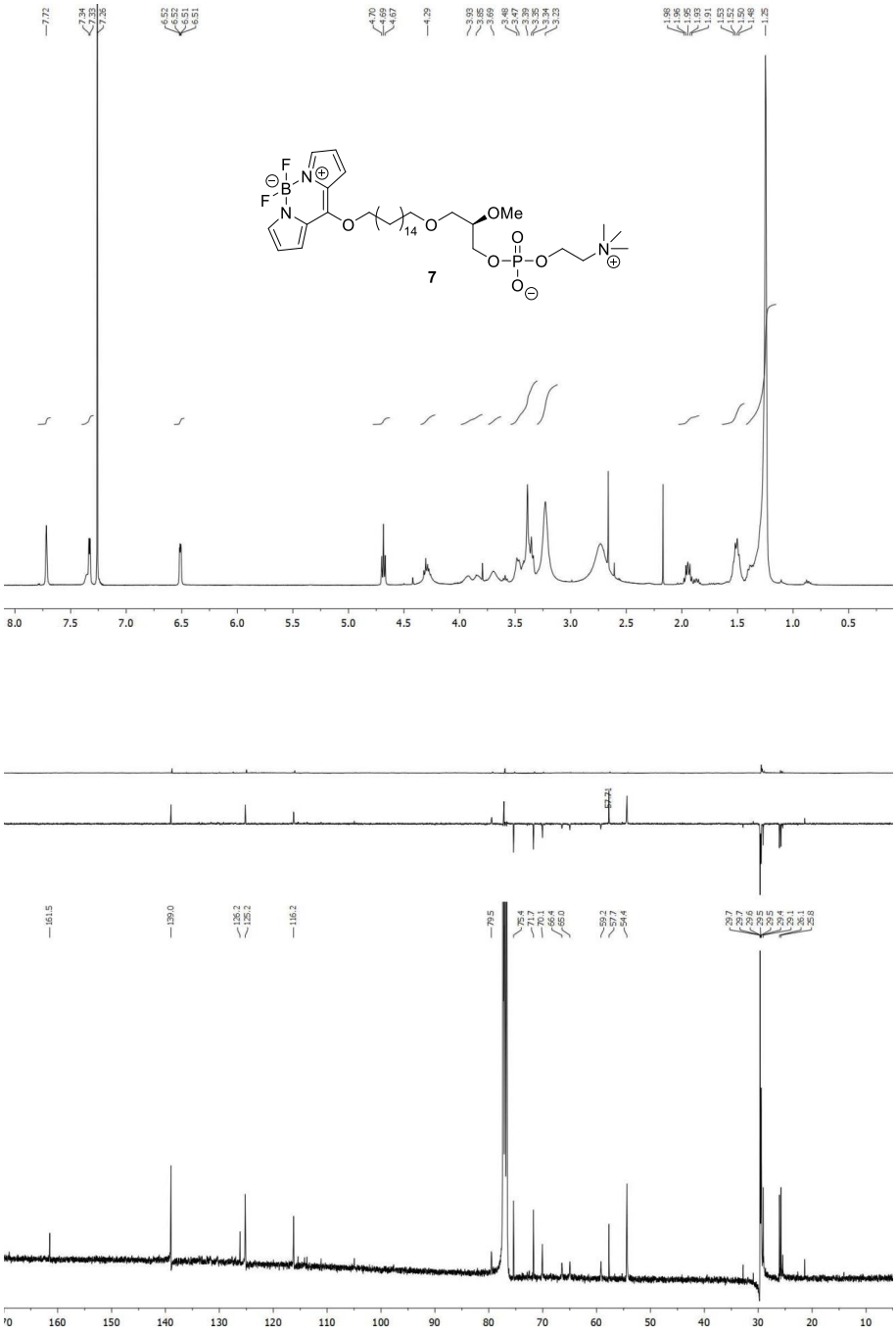

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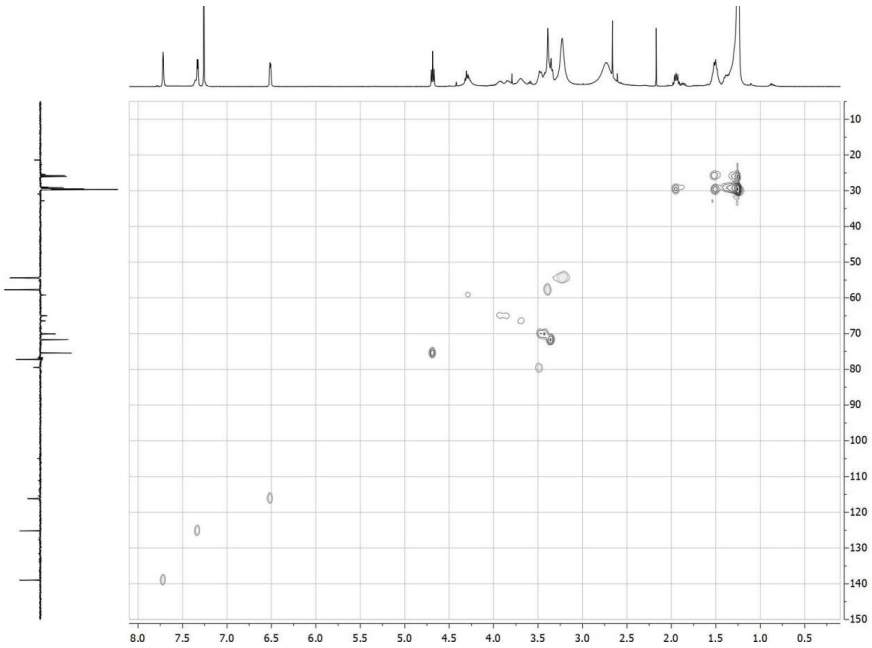

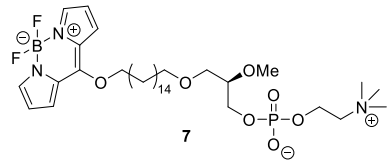

7

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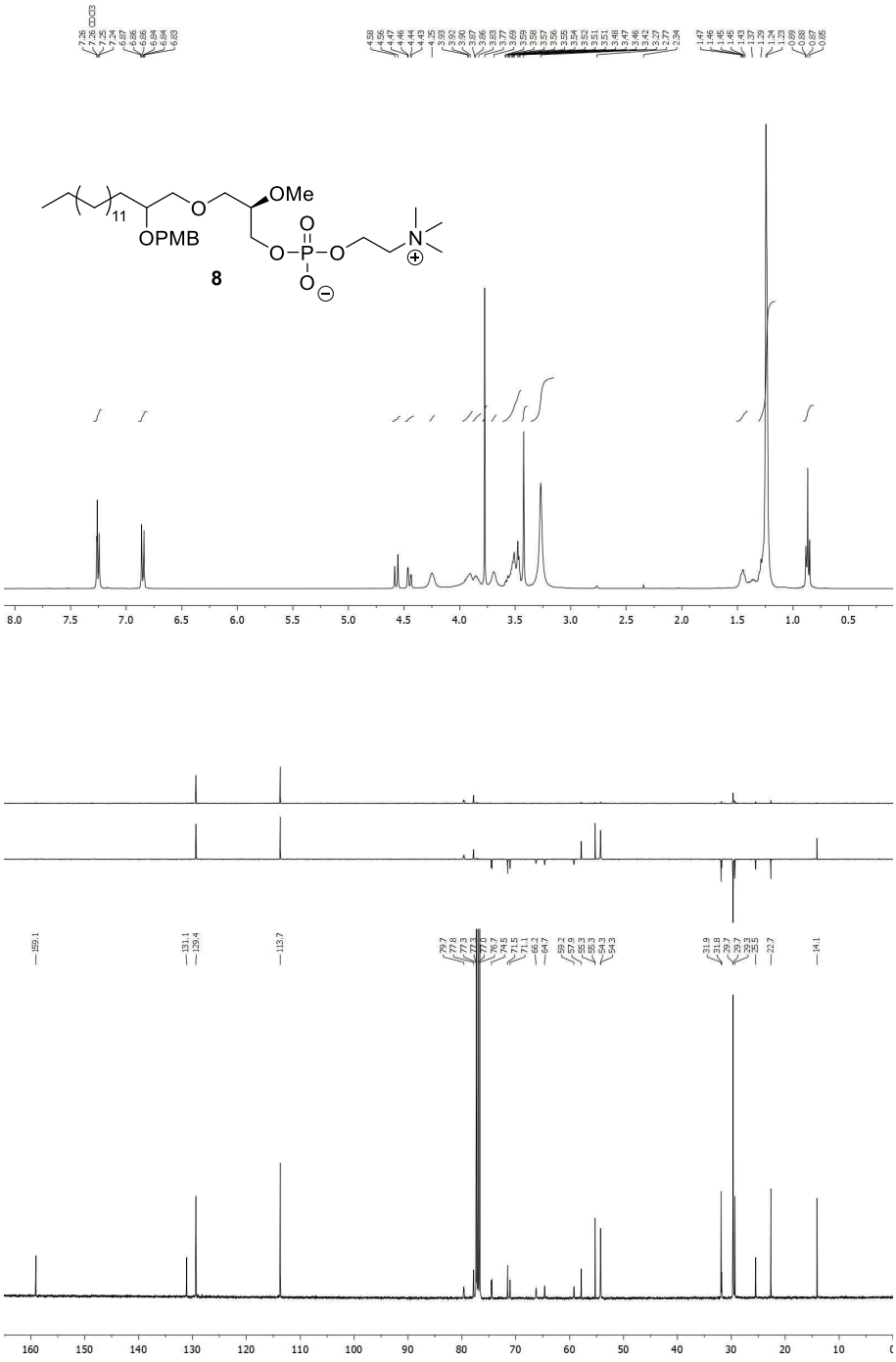

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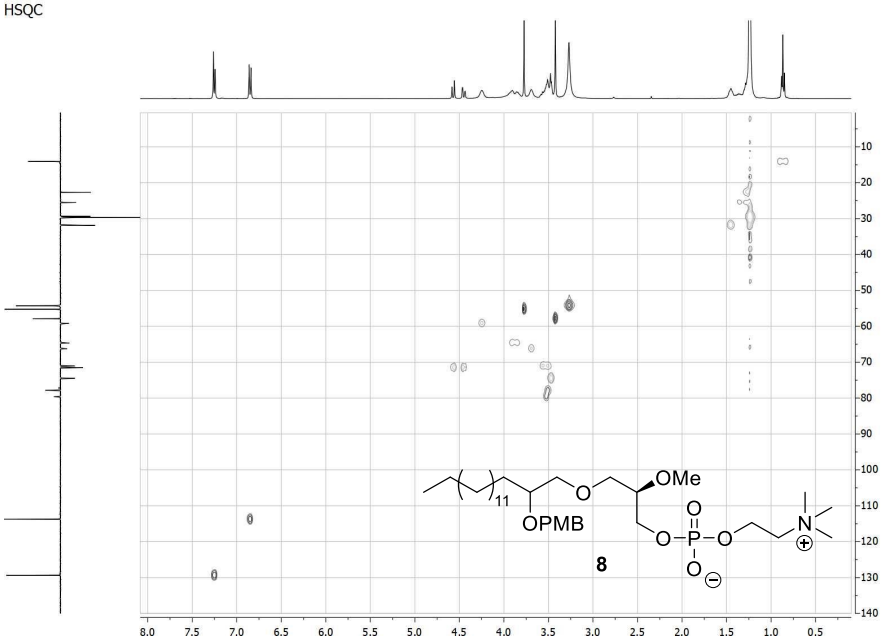

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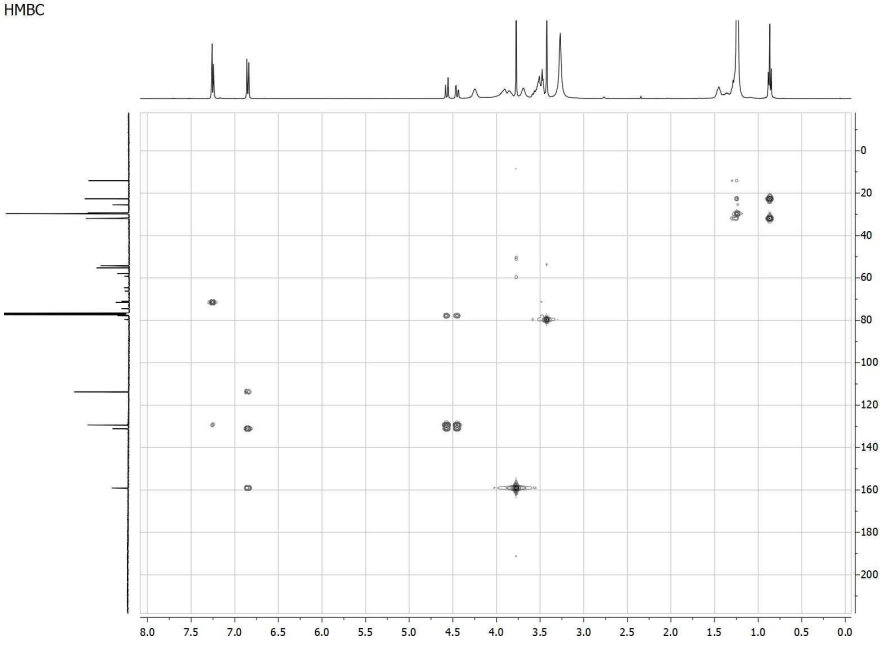

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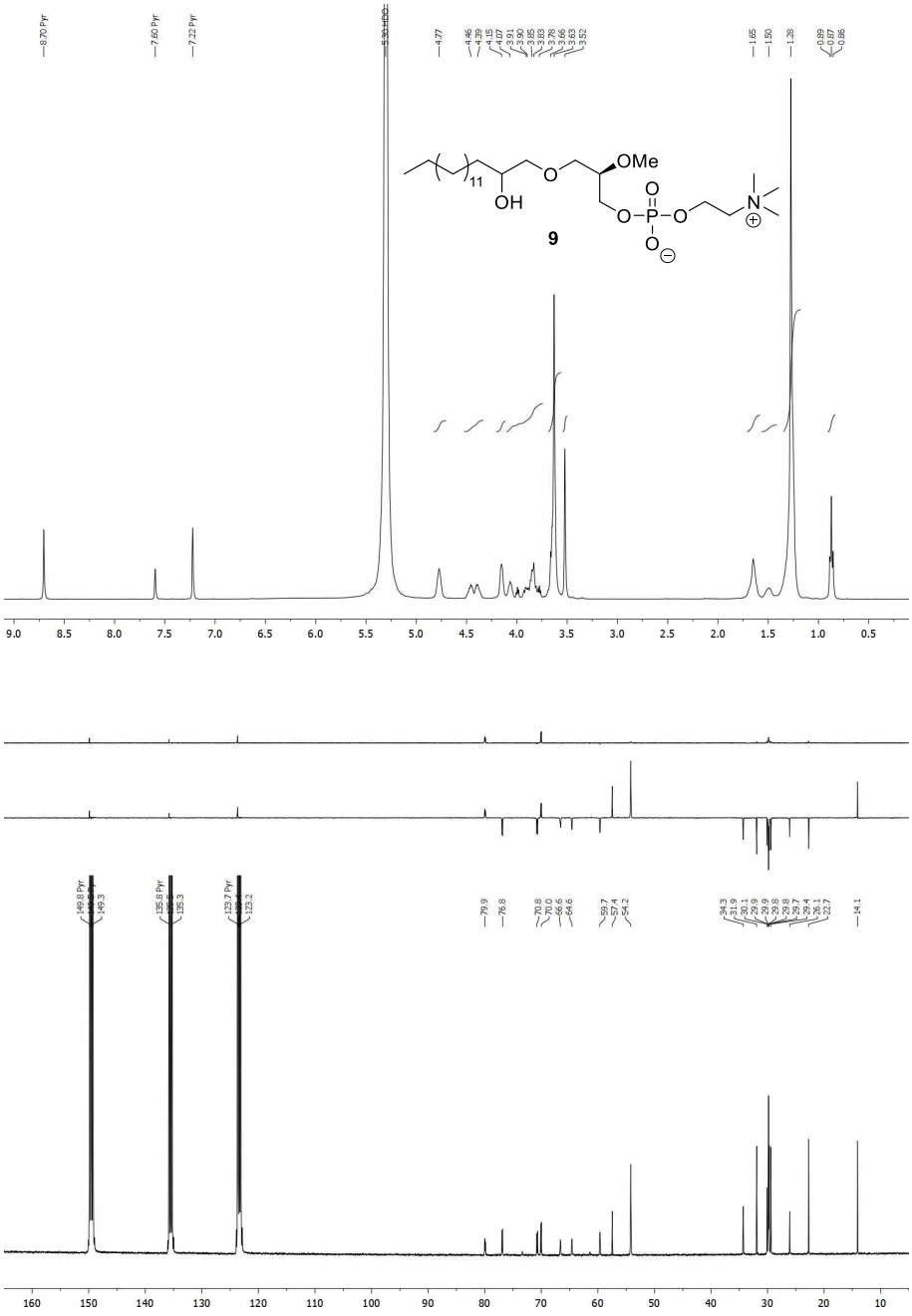

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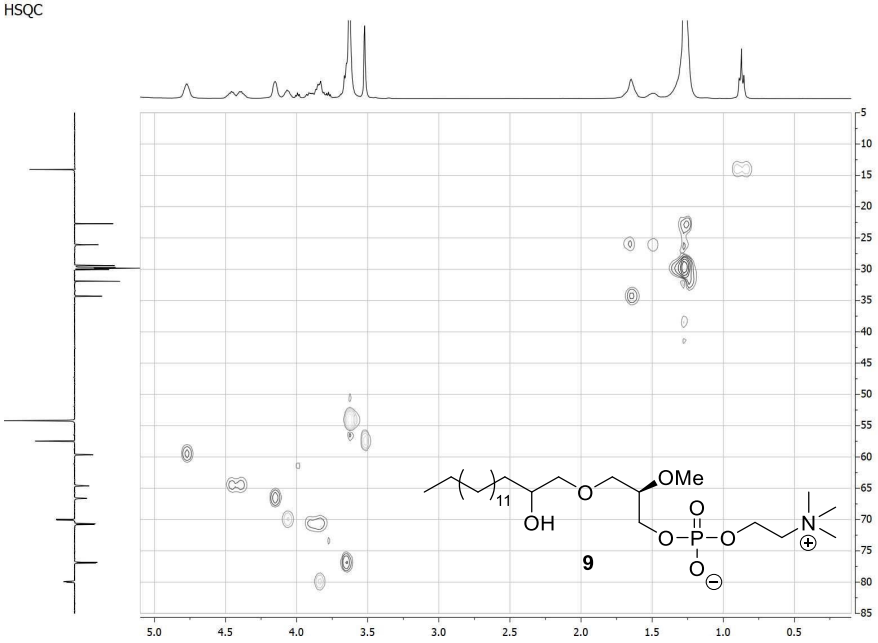

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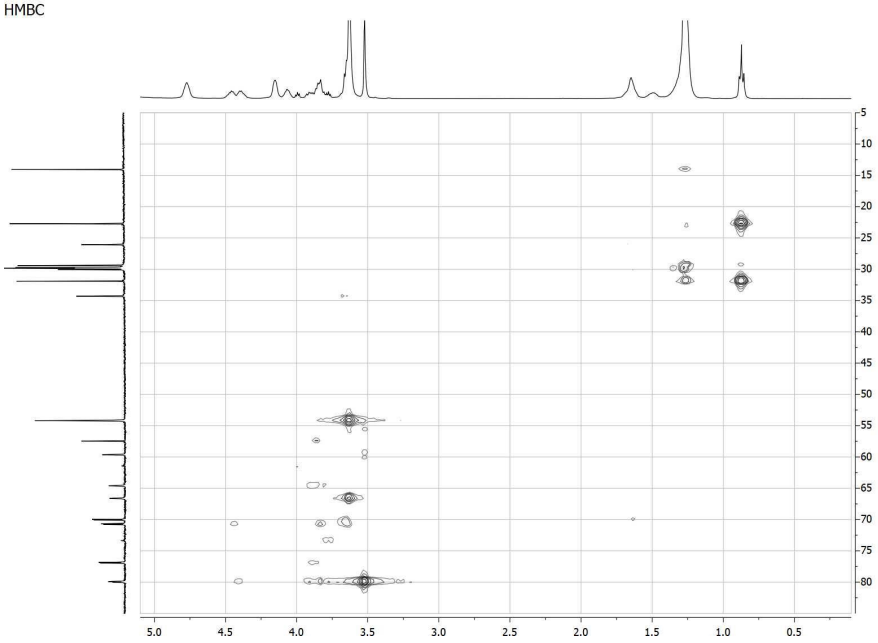

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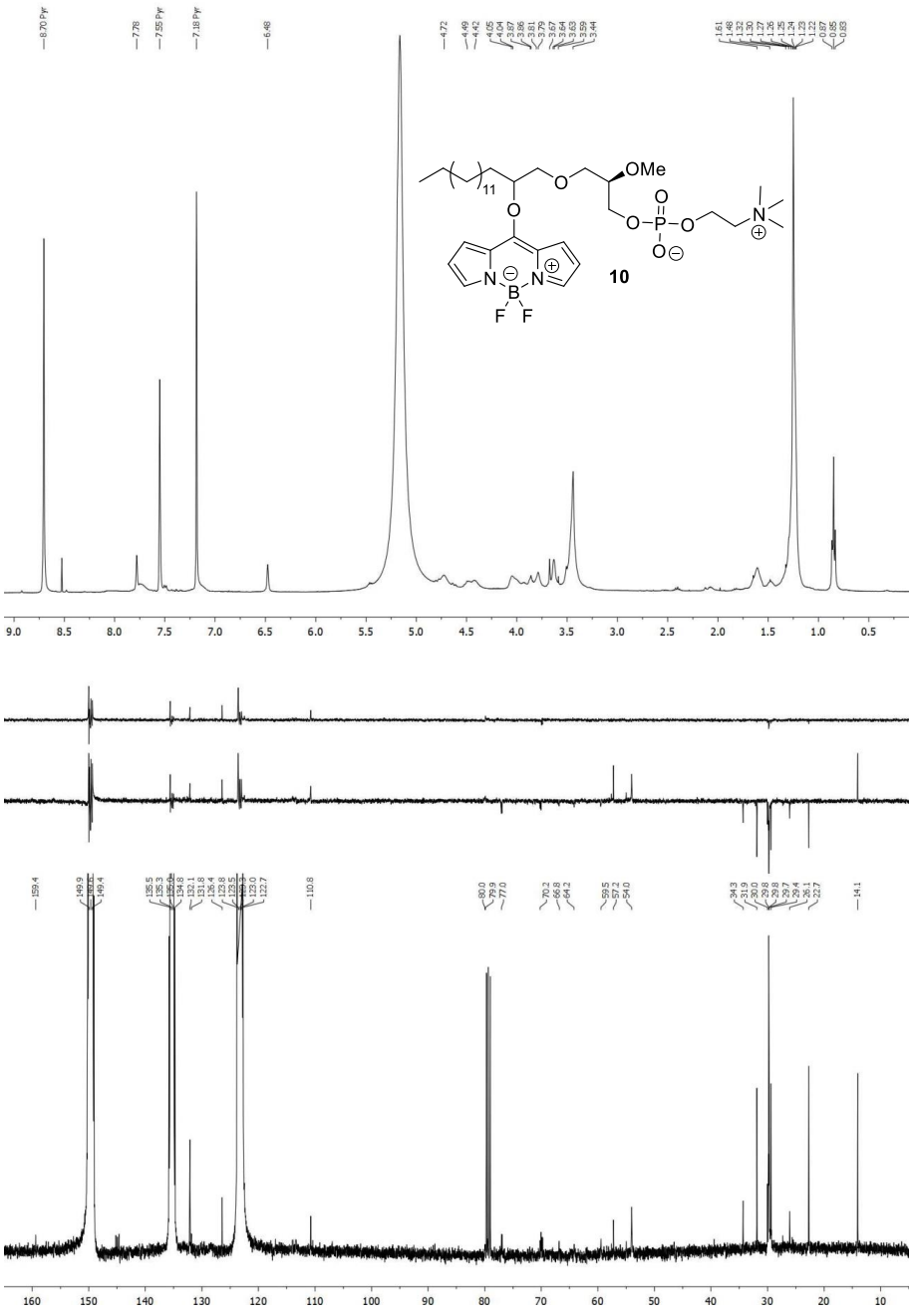

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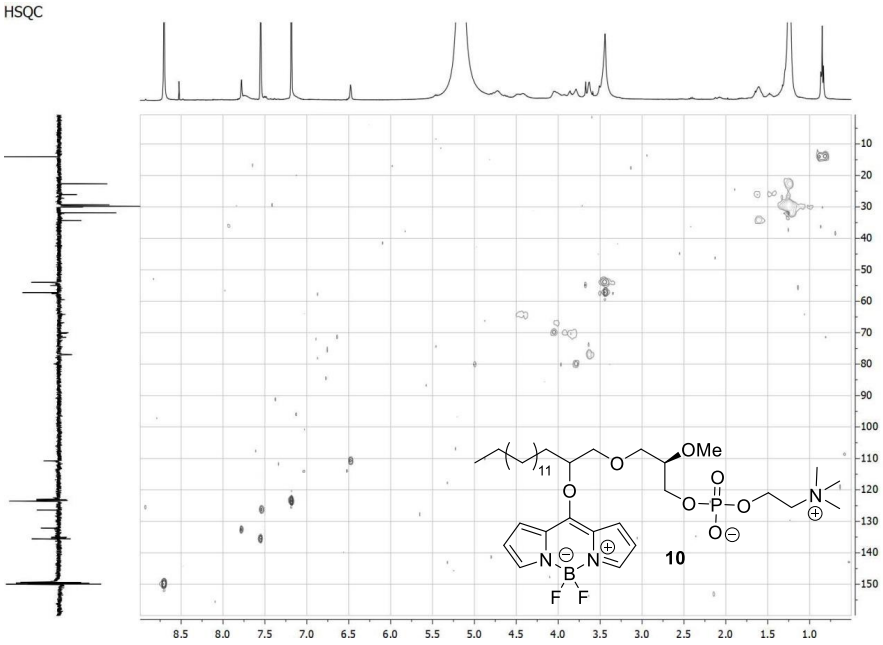

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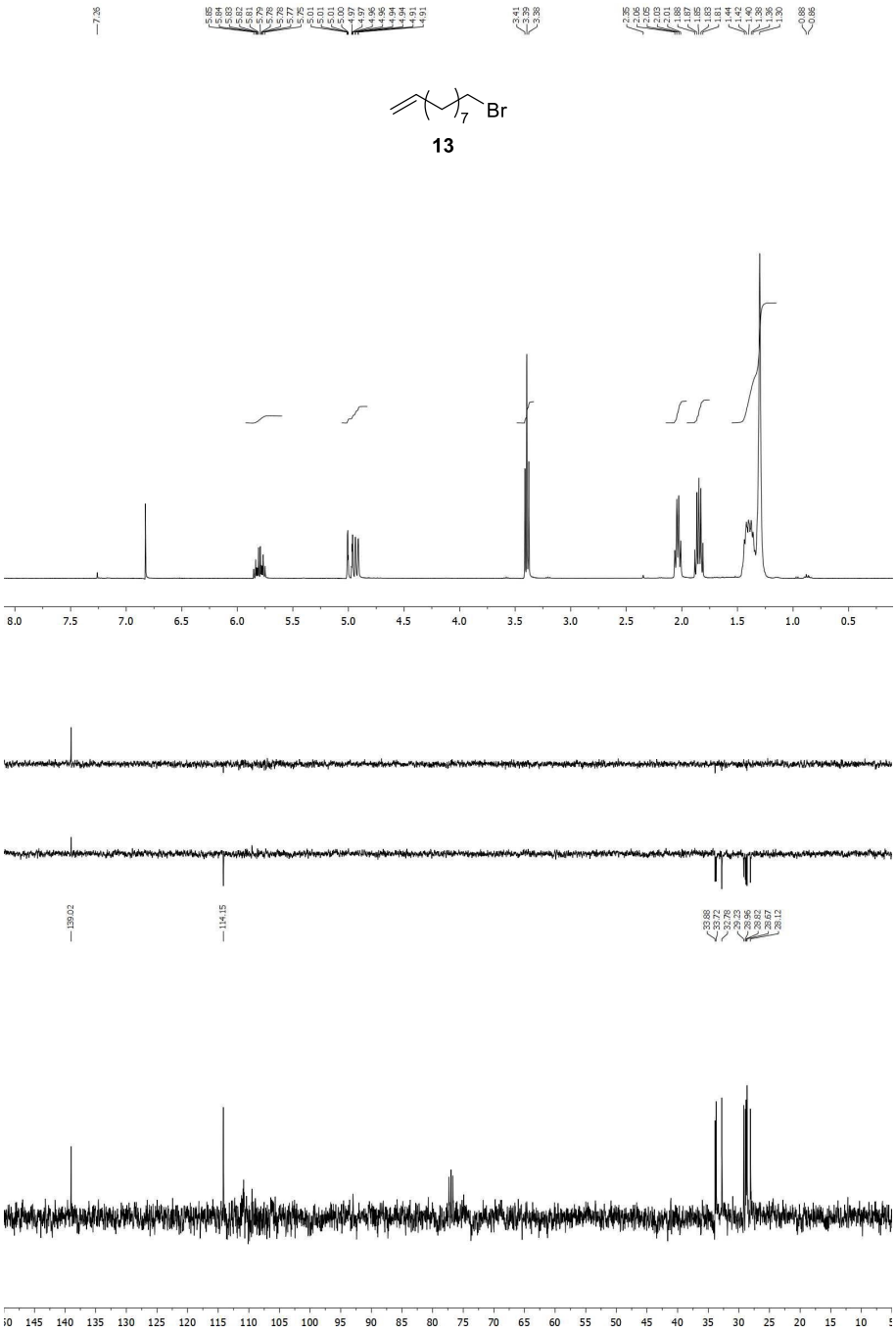

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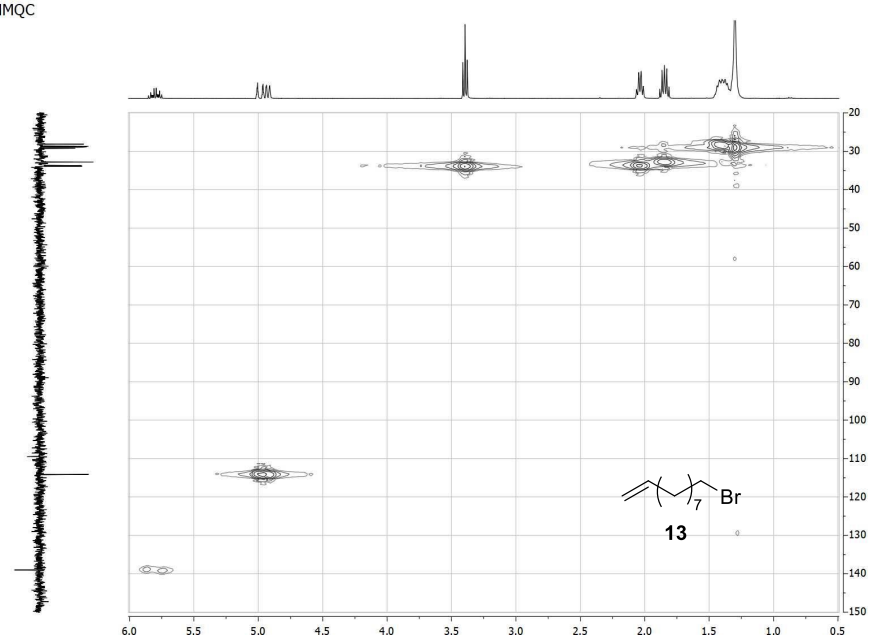

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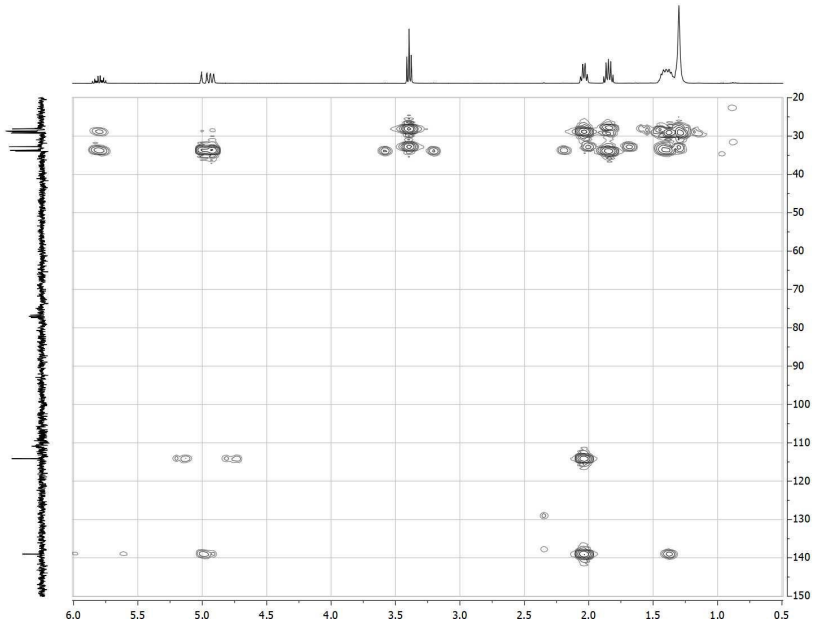

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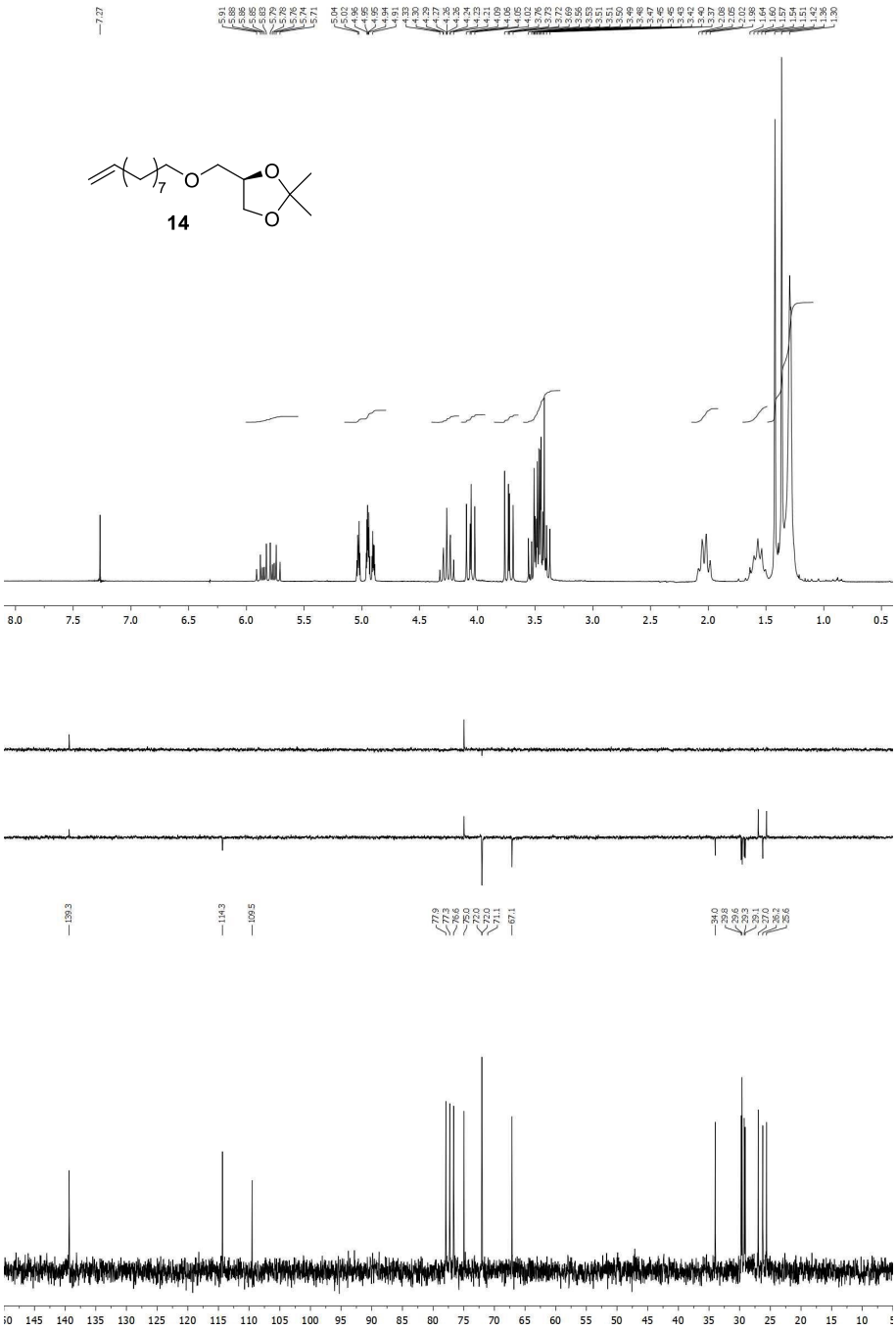

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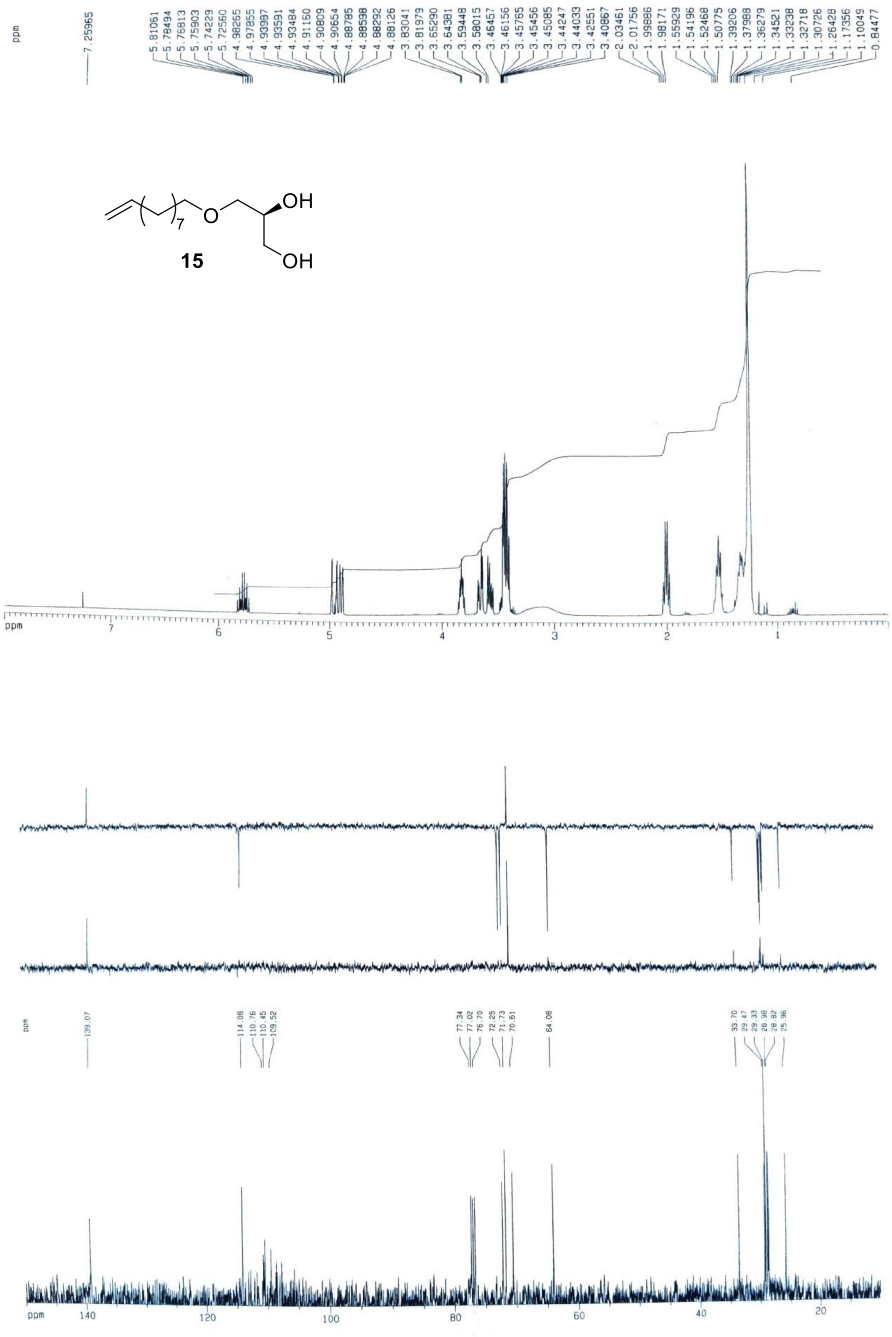

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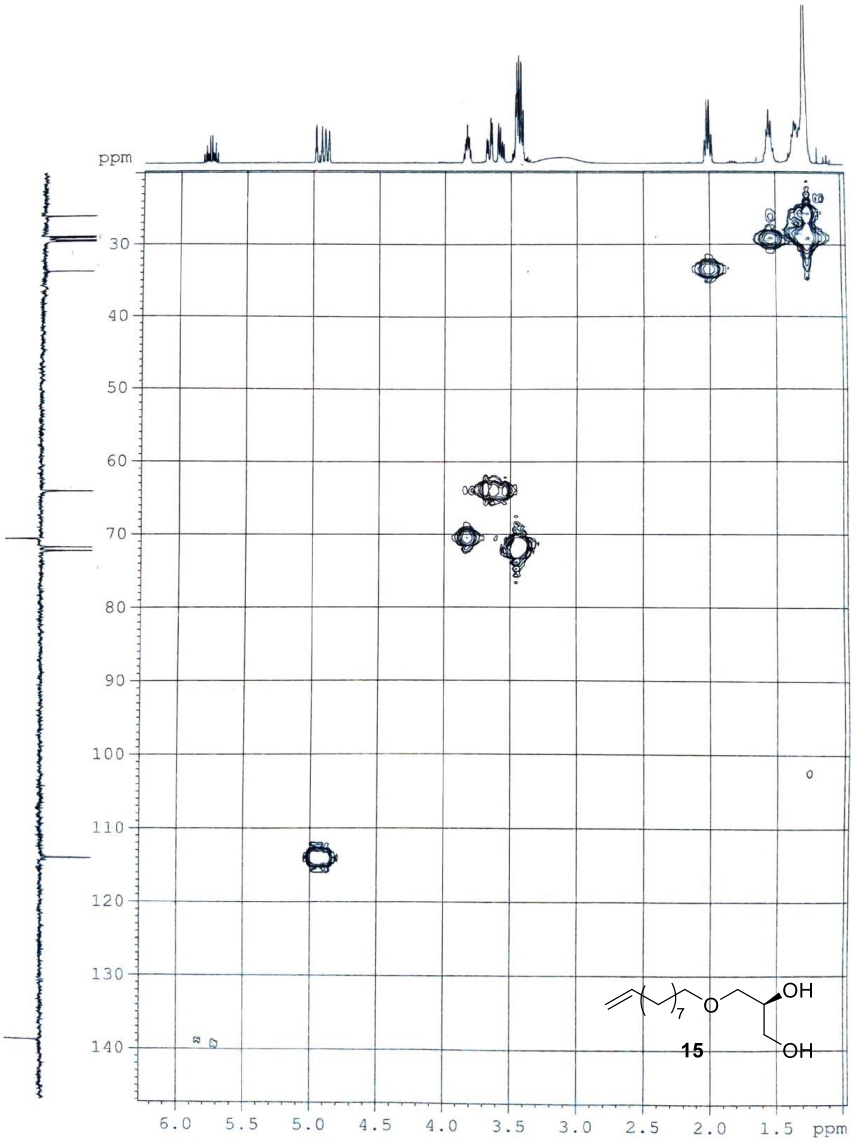

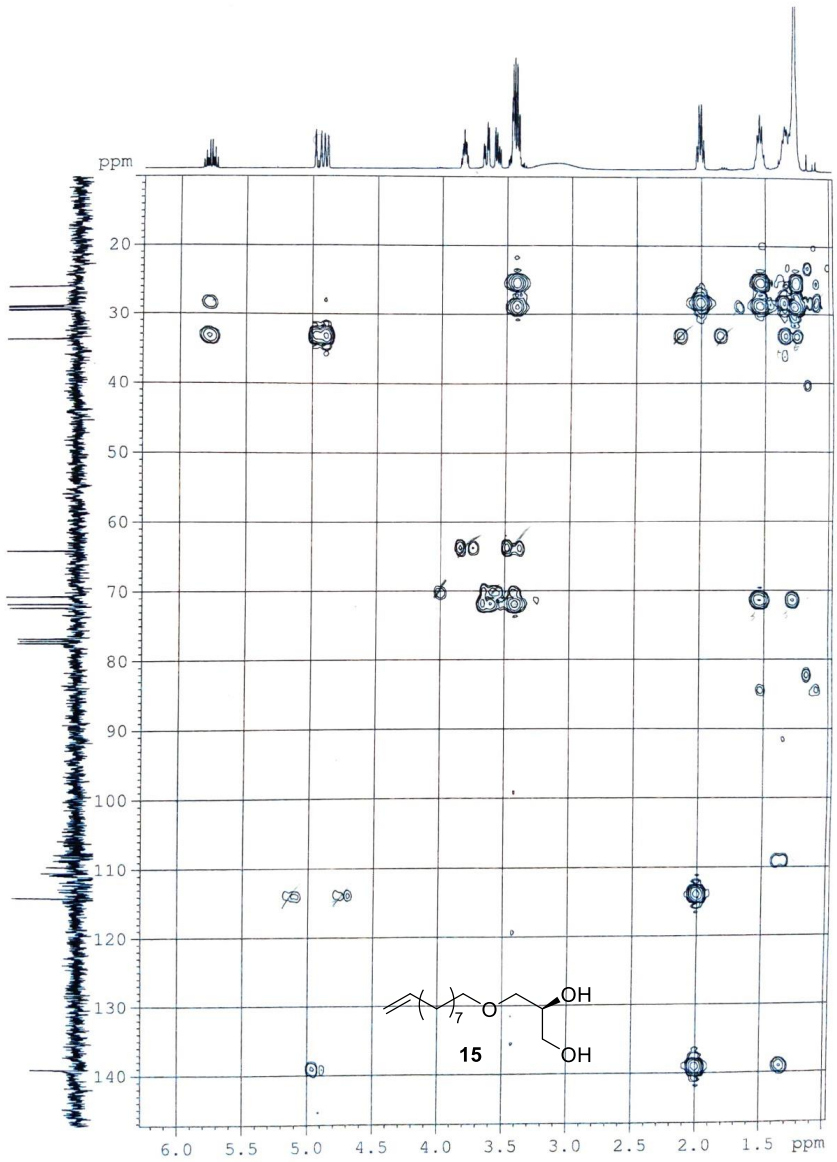

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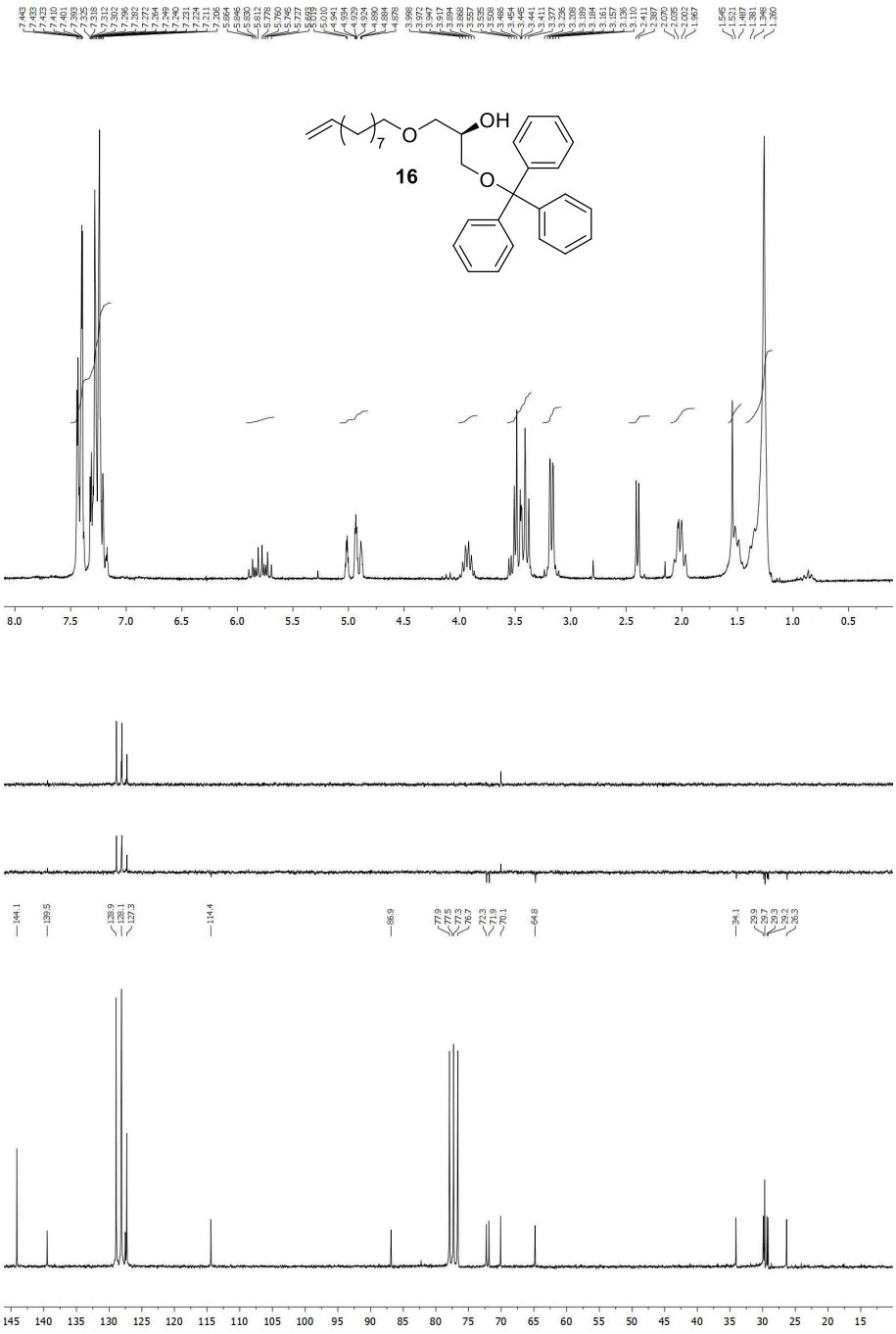

FIRMADO POR	FECHA FIRMA
SELLO UNIVERSIDAD DE SALAMANCA	20-07-2021 18:11:01
SÁNCHEZ MARCOS ISIDRO	20-07-2021 10:20:59

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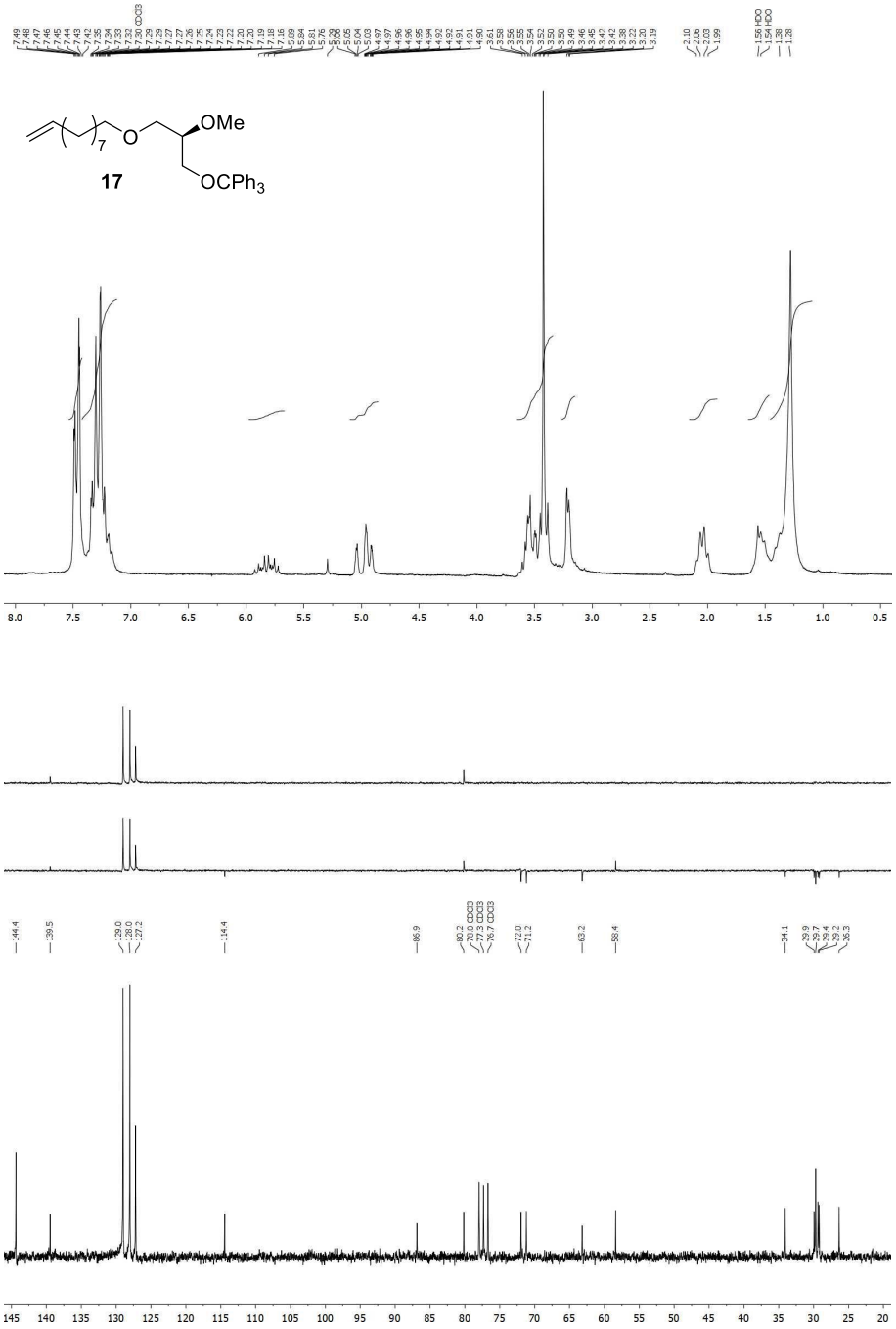

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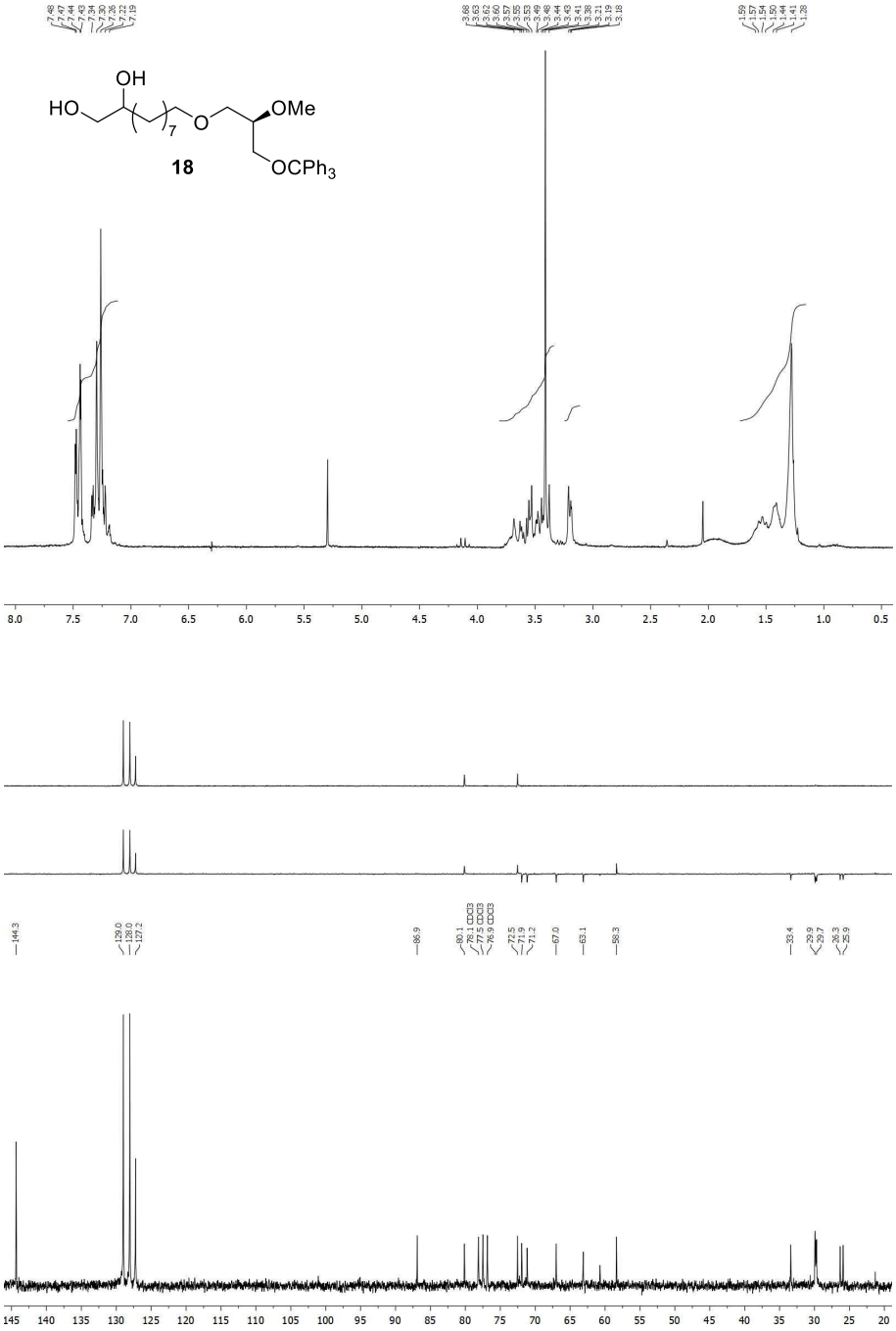

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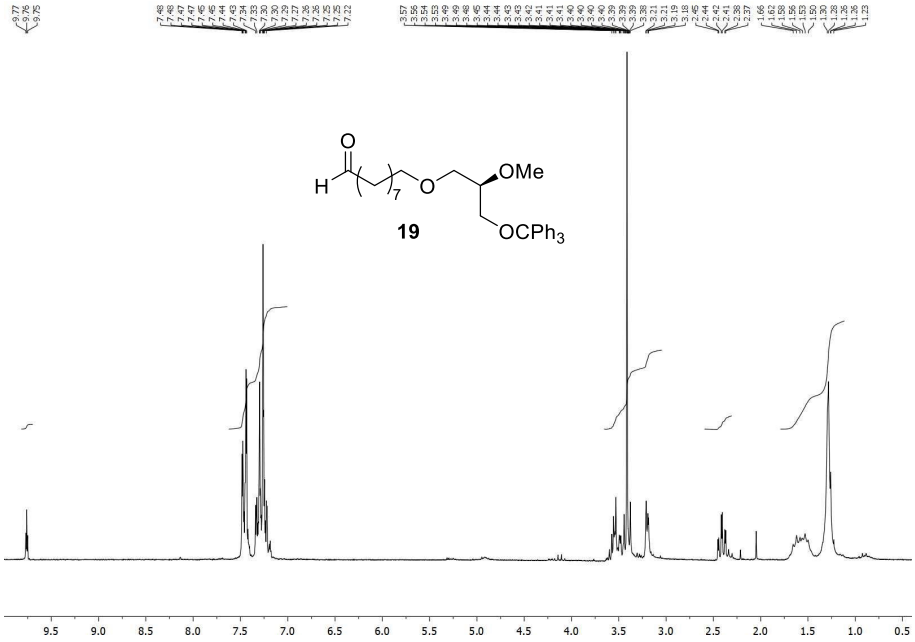

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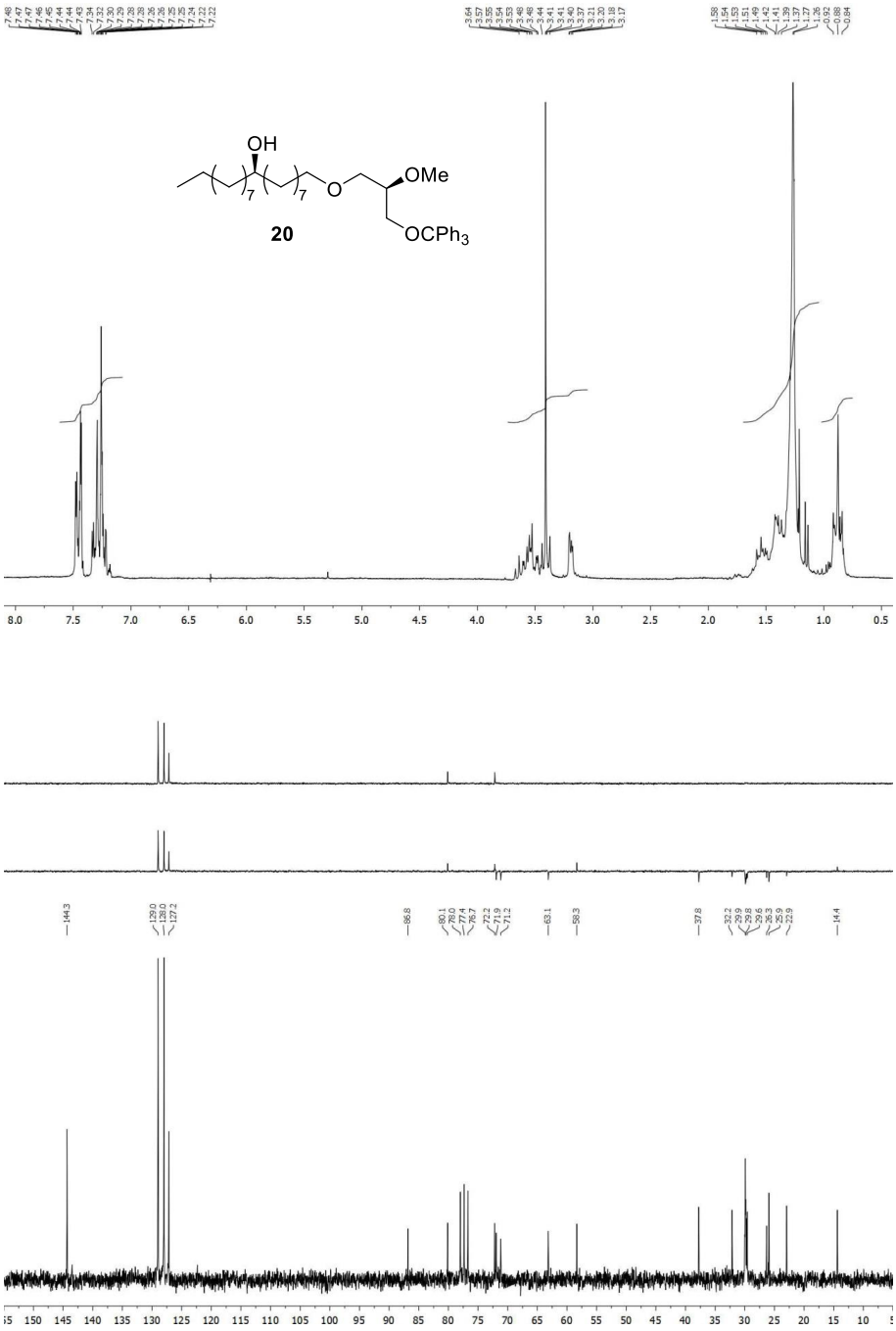

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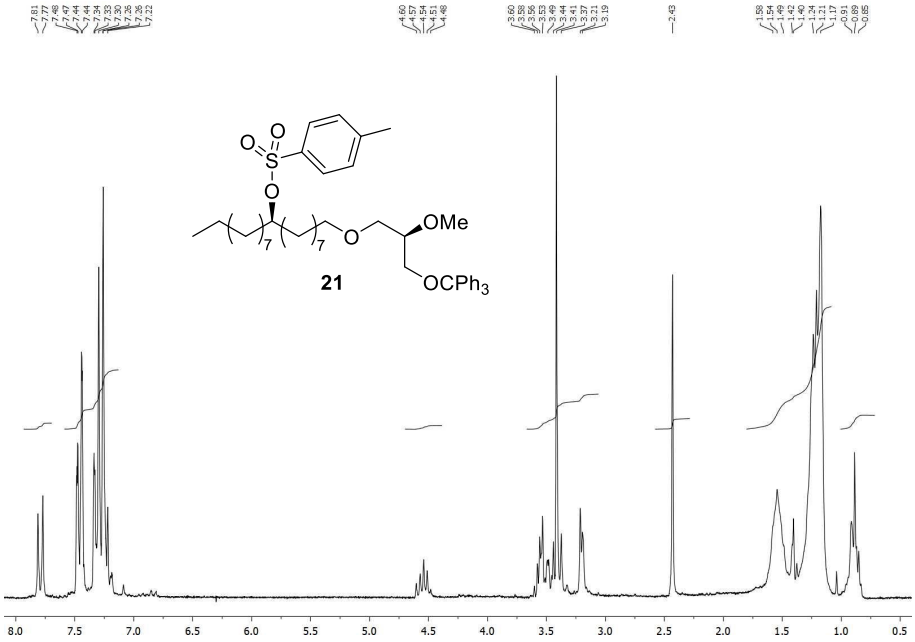

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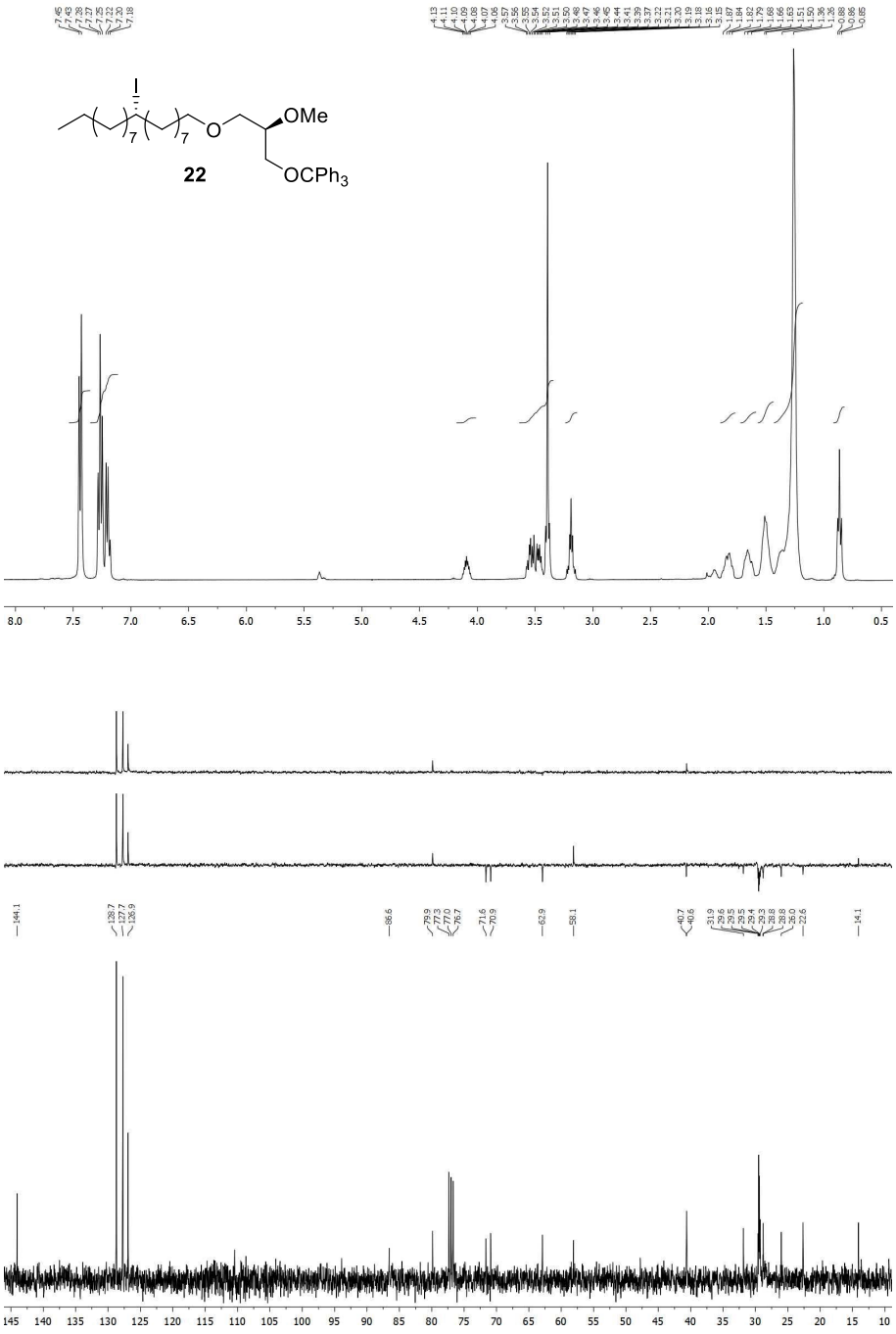

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SELLO UNIVERSIDAD DE SALAMANCA	20-07-2021 18:11:01
SÁNCHEZ MARCOS ISIDRO	20-07-2021 10:20:59

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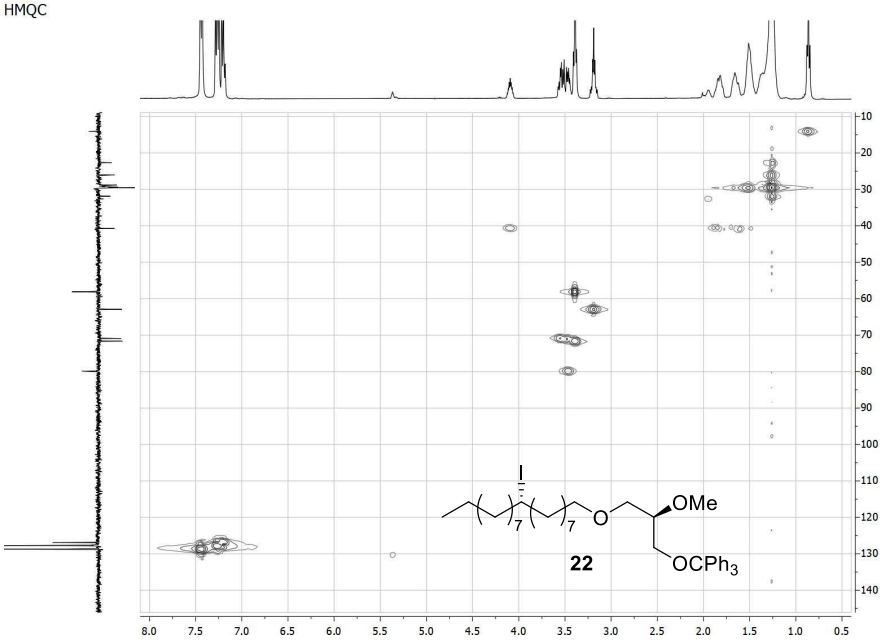

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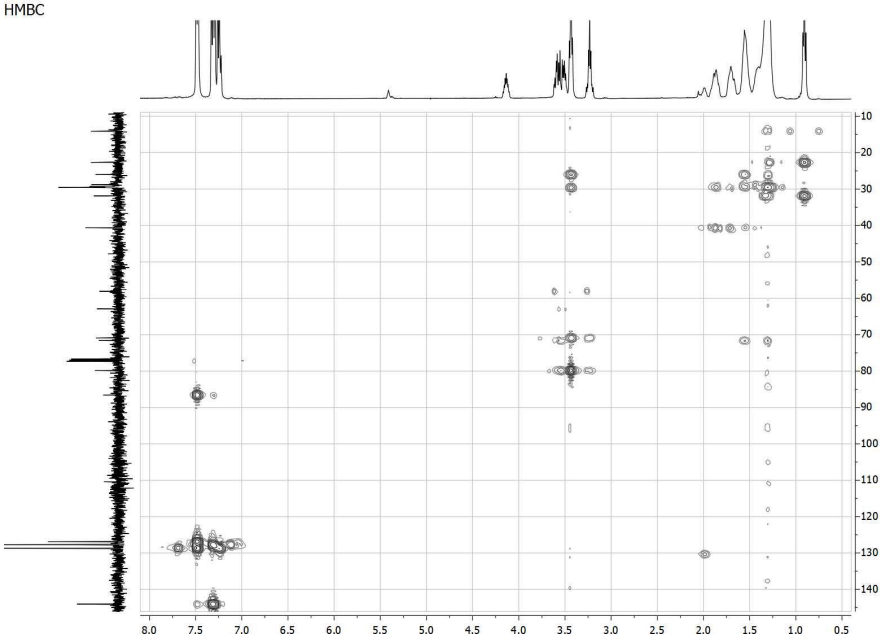

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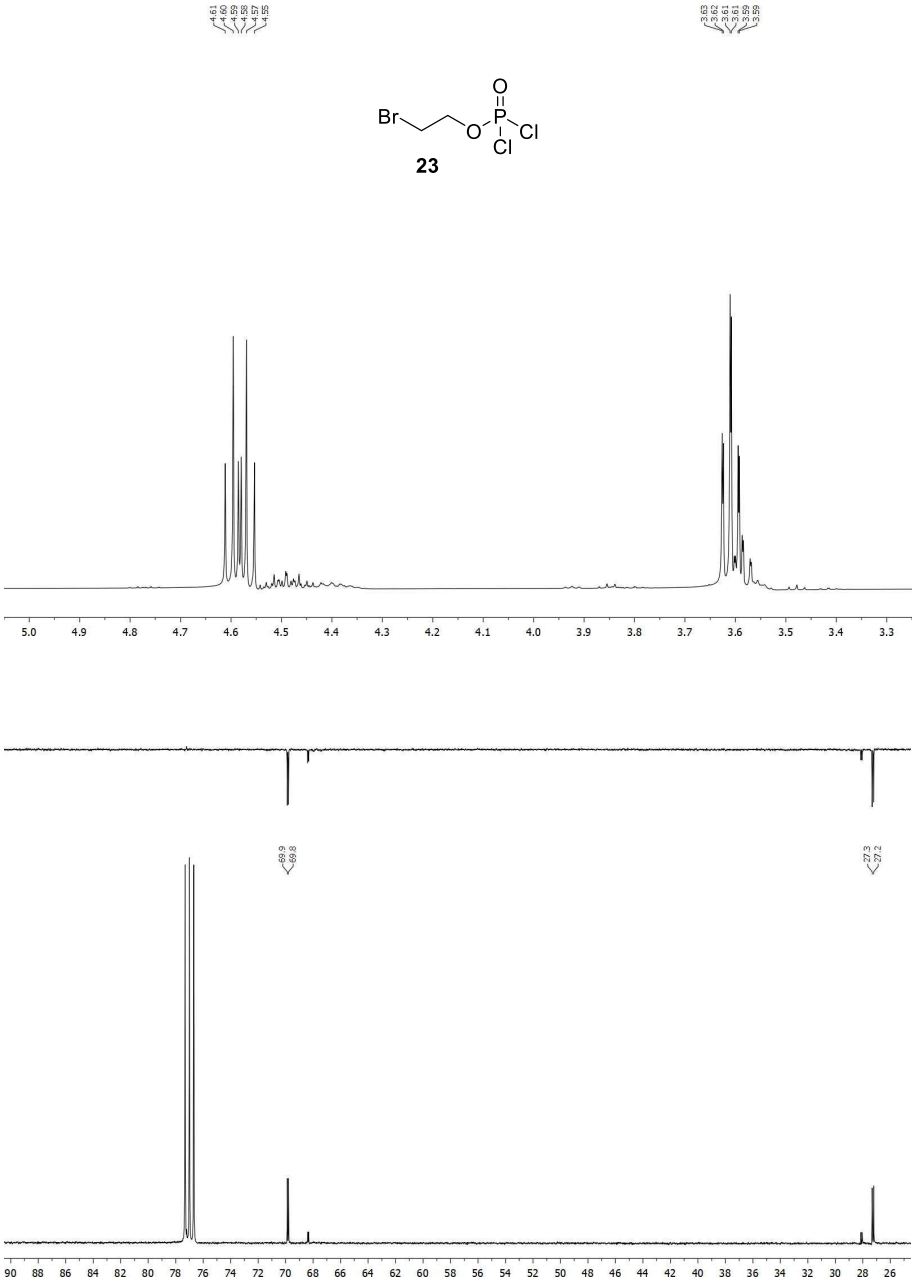

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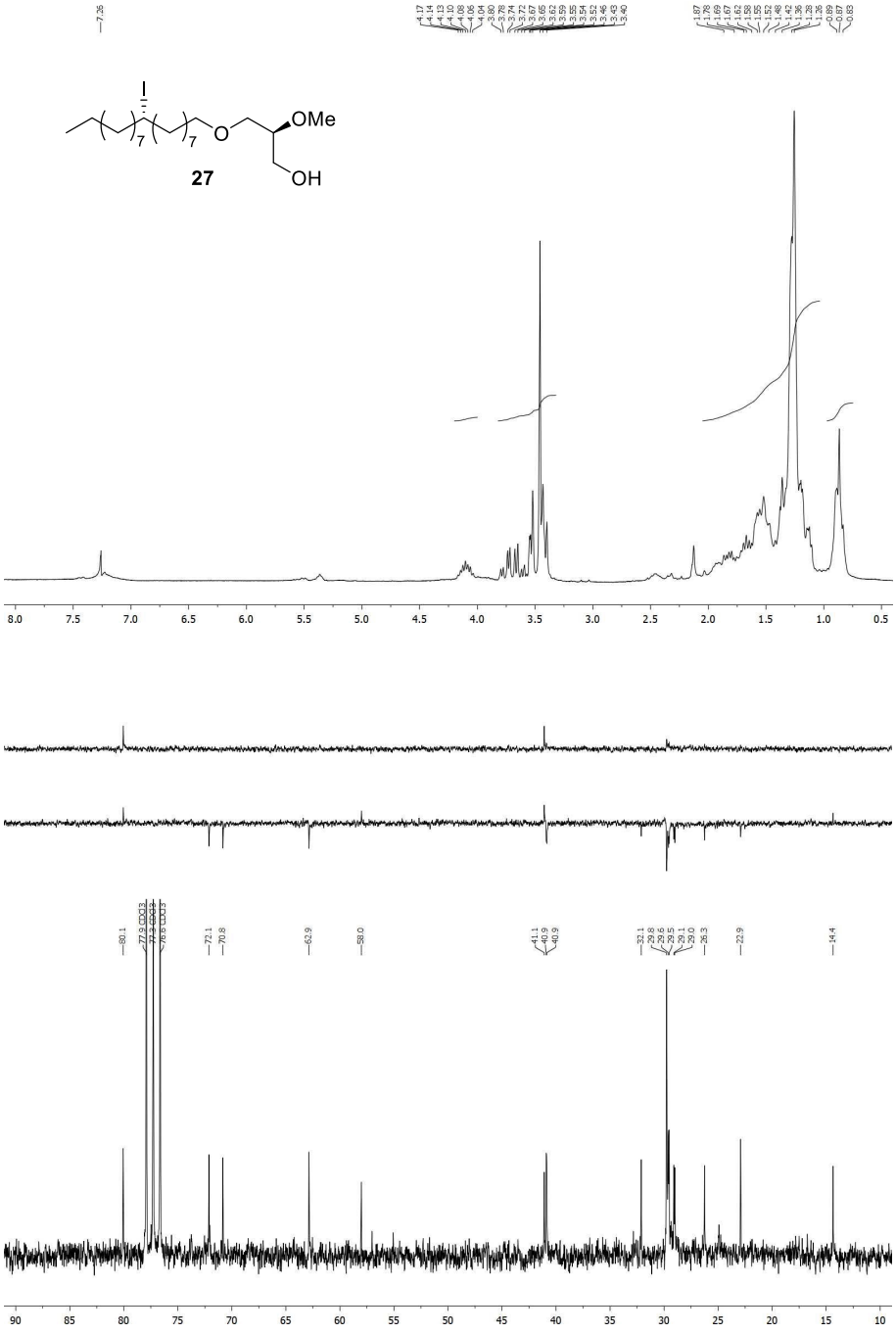

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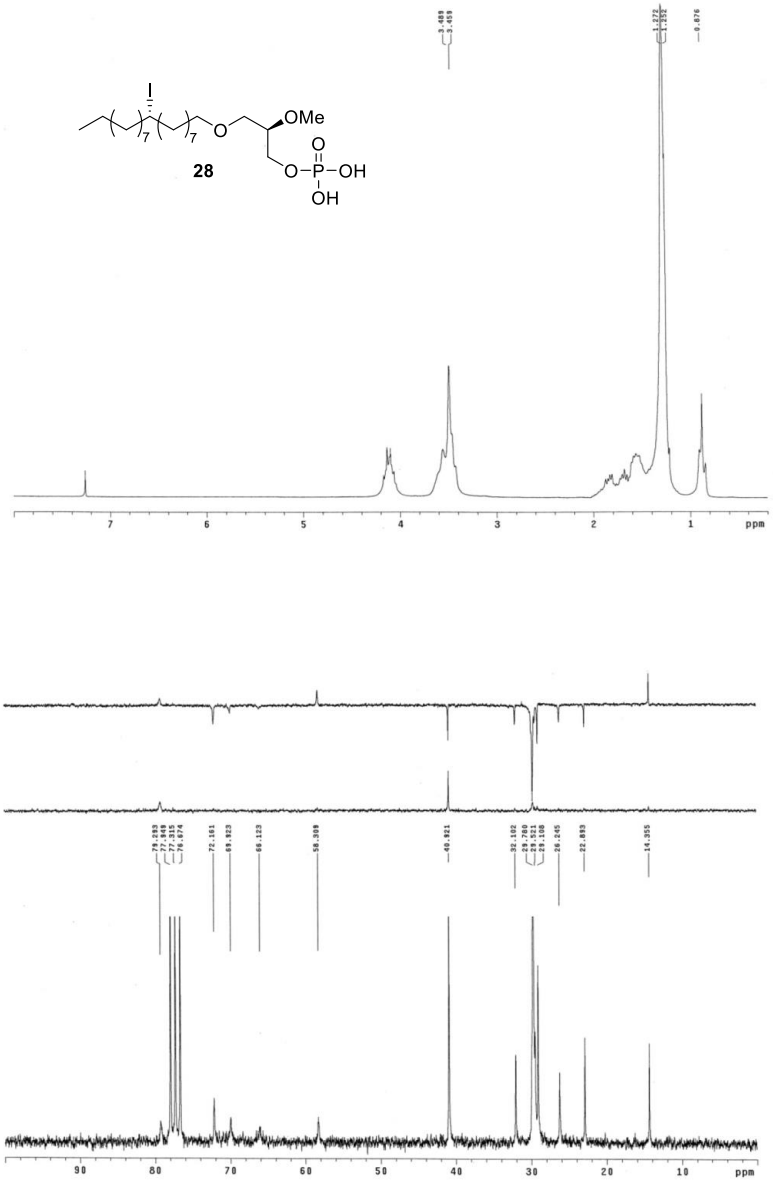

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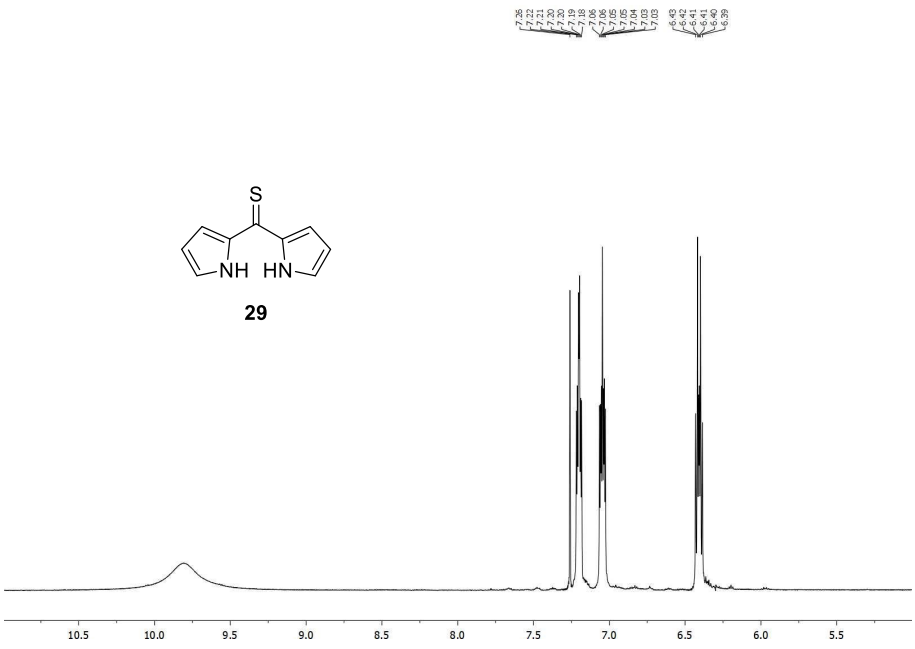

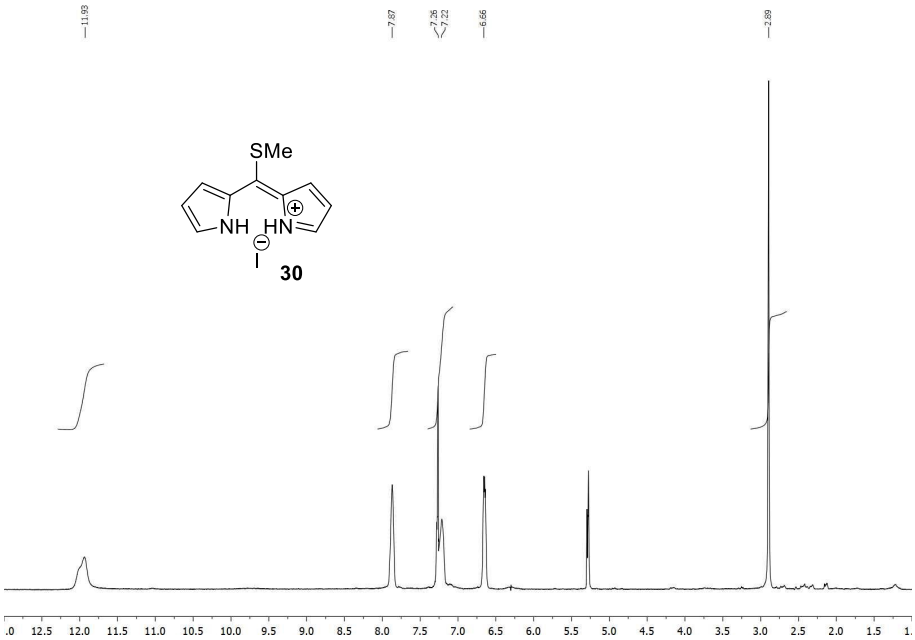

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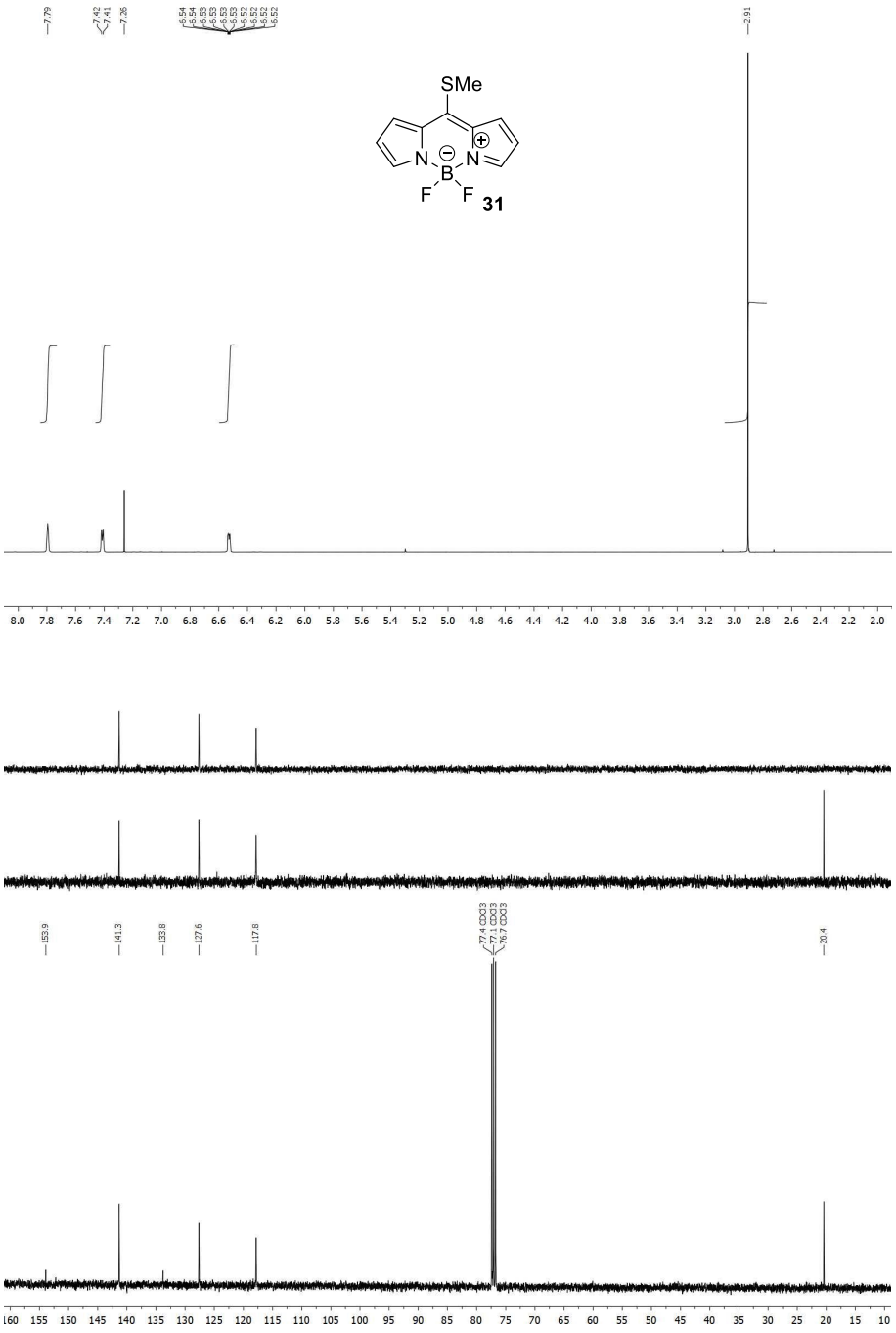

FIRMADO POR	FECHA FIRMA
SELLO UNIVERSIDAD DE SALAMANCA	20-07-2021 18:11:01
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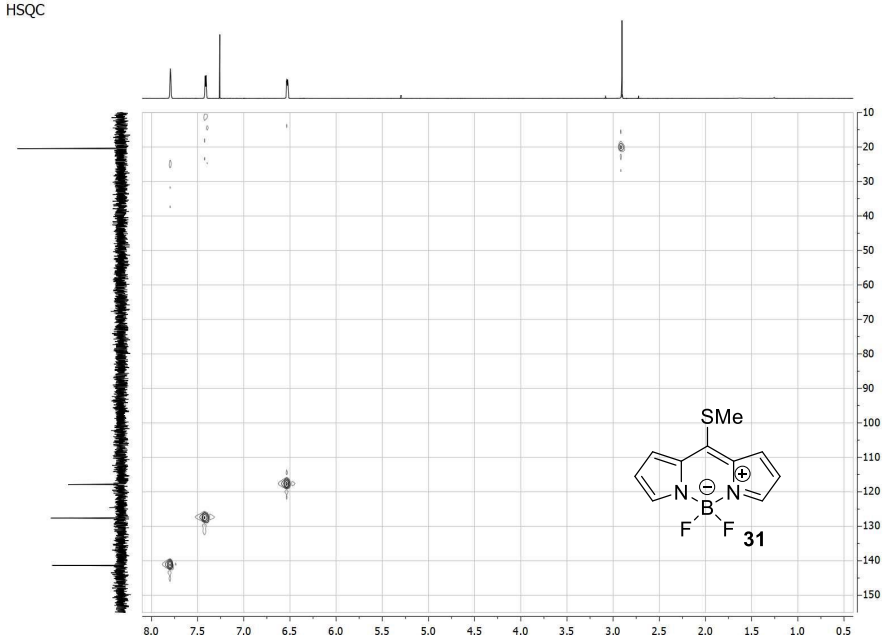

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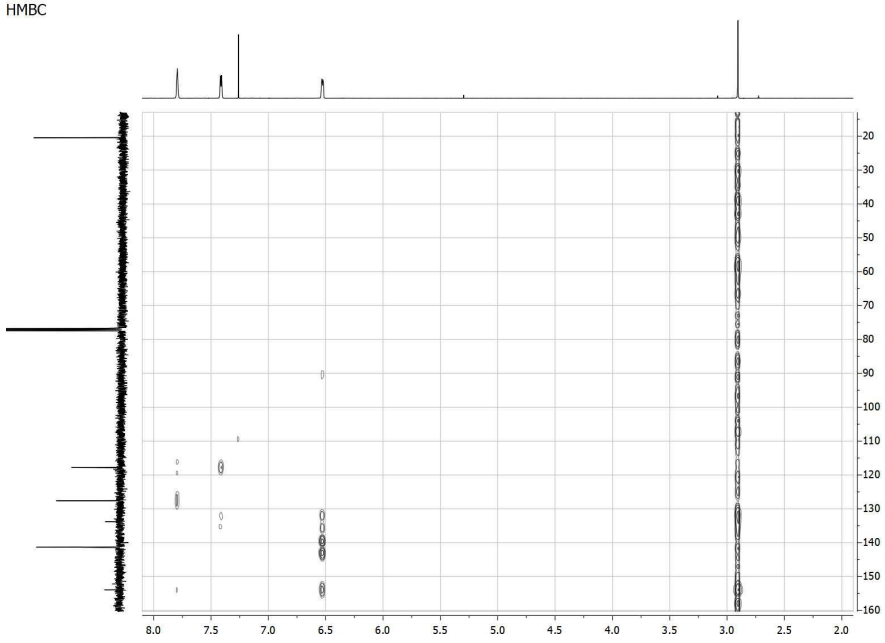

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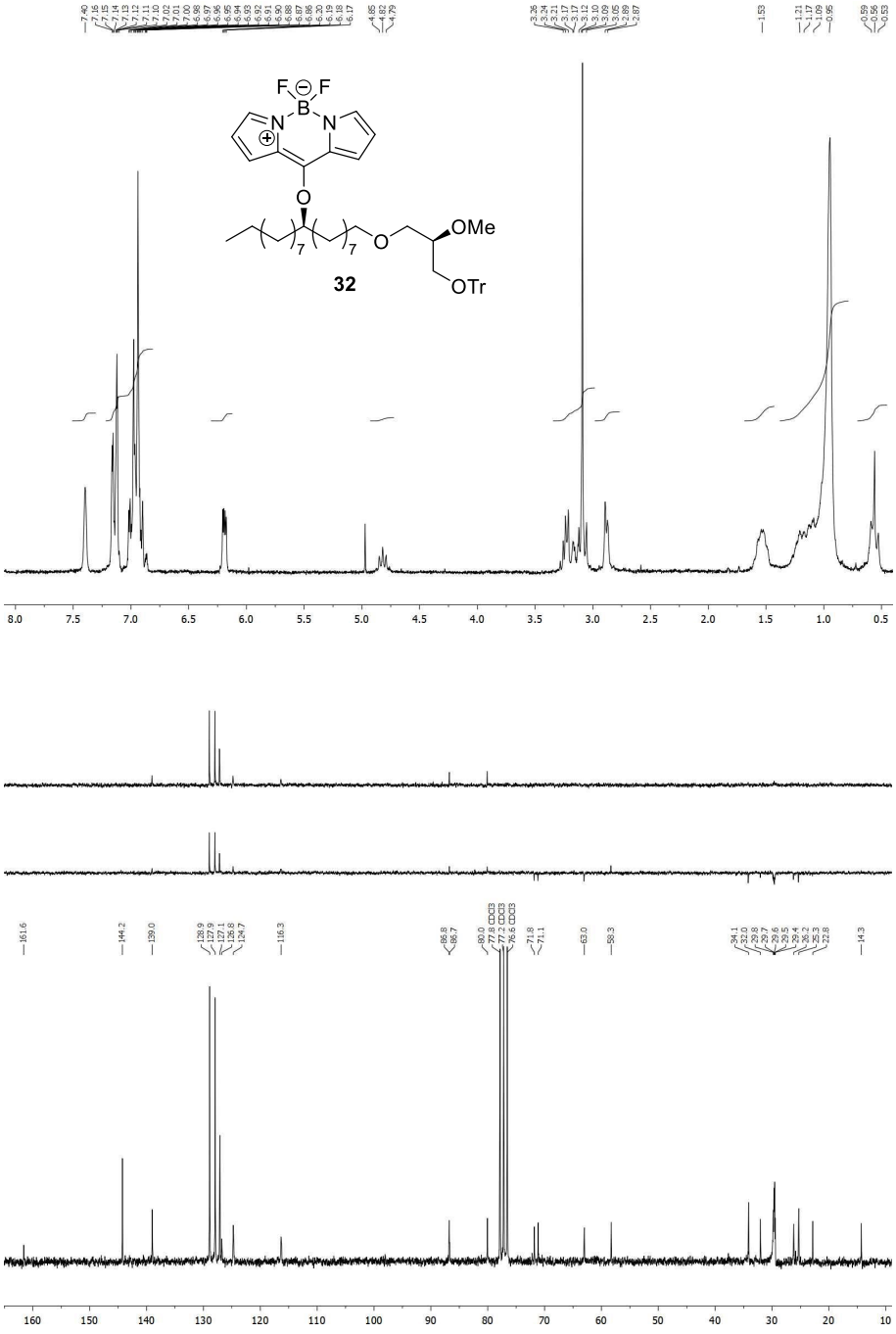

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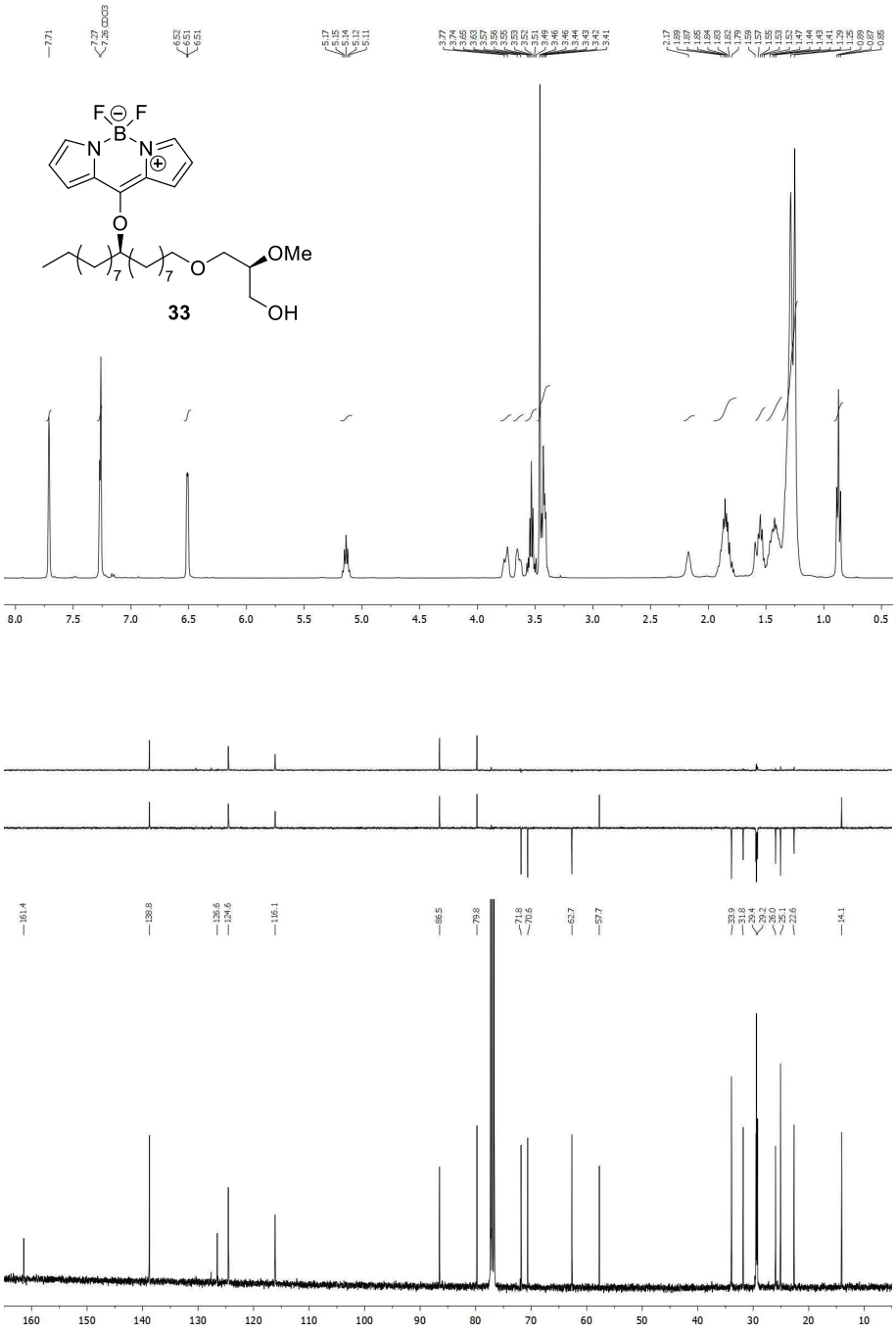

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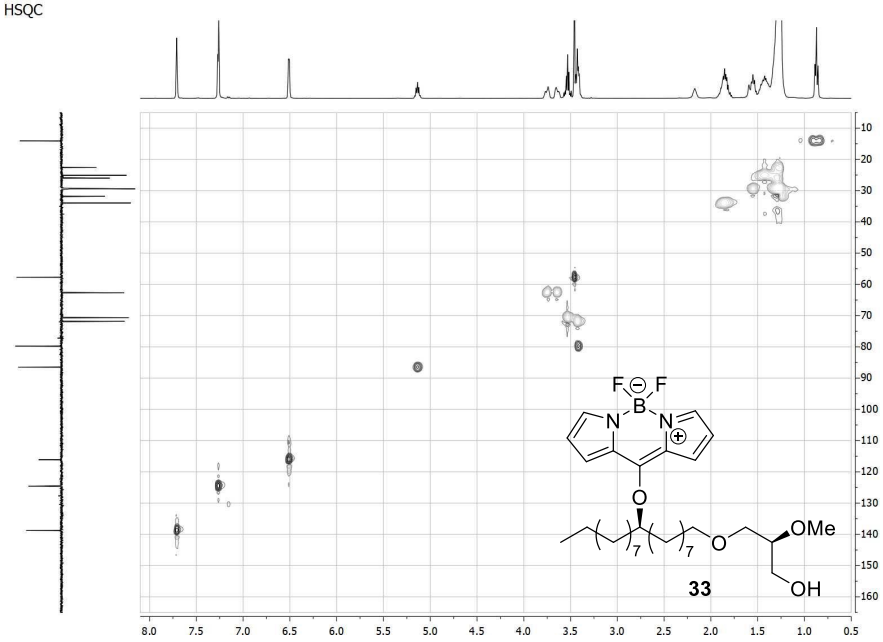

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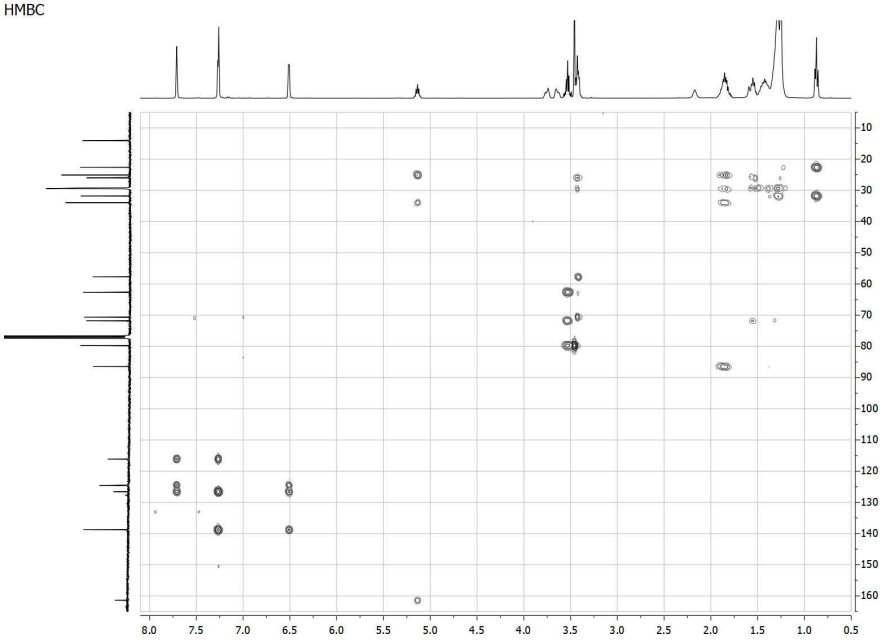

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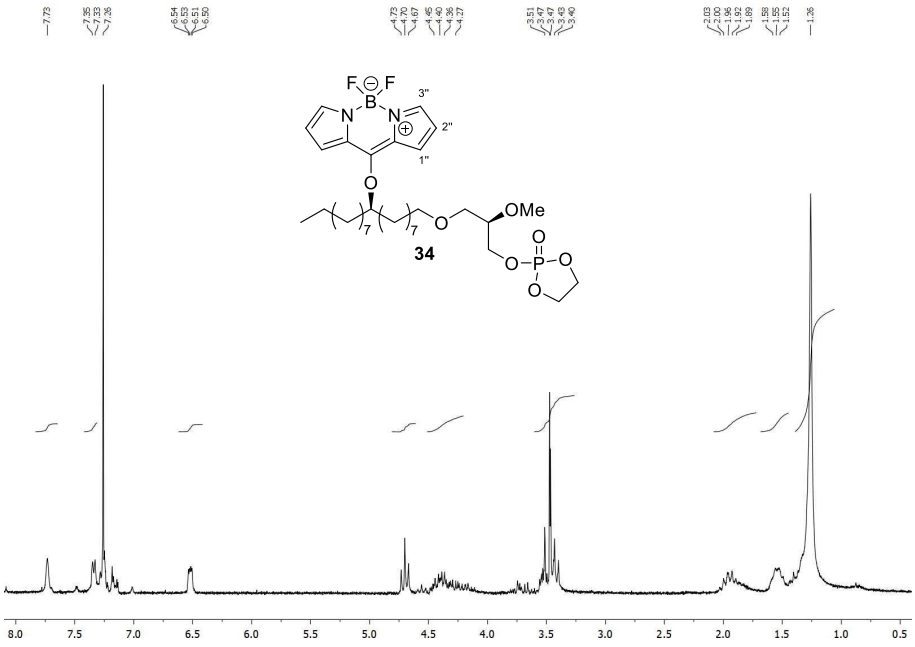

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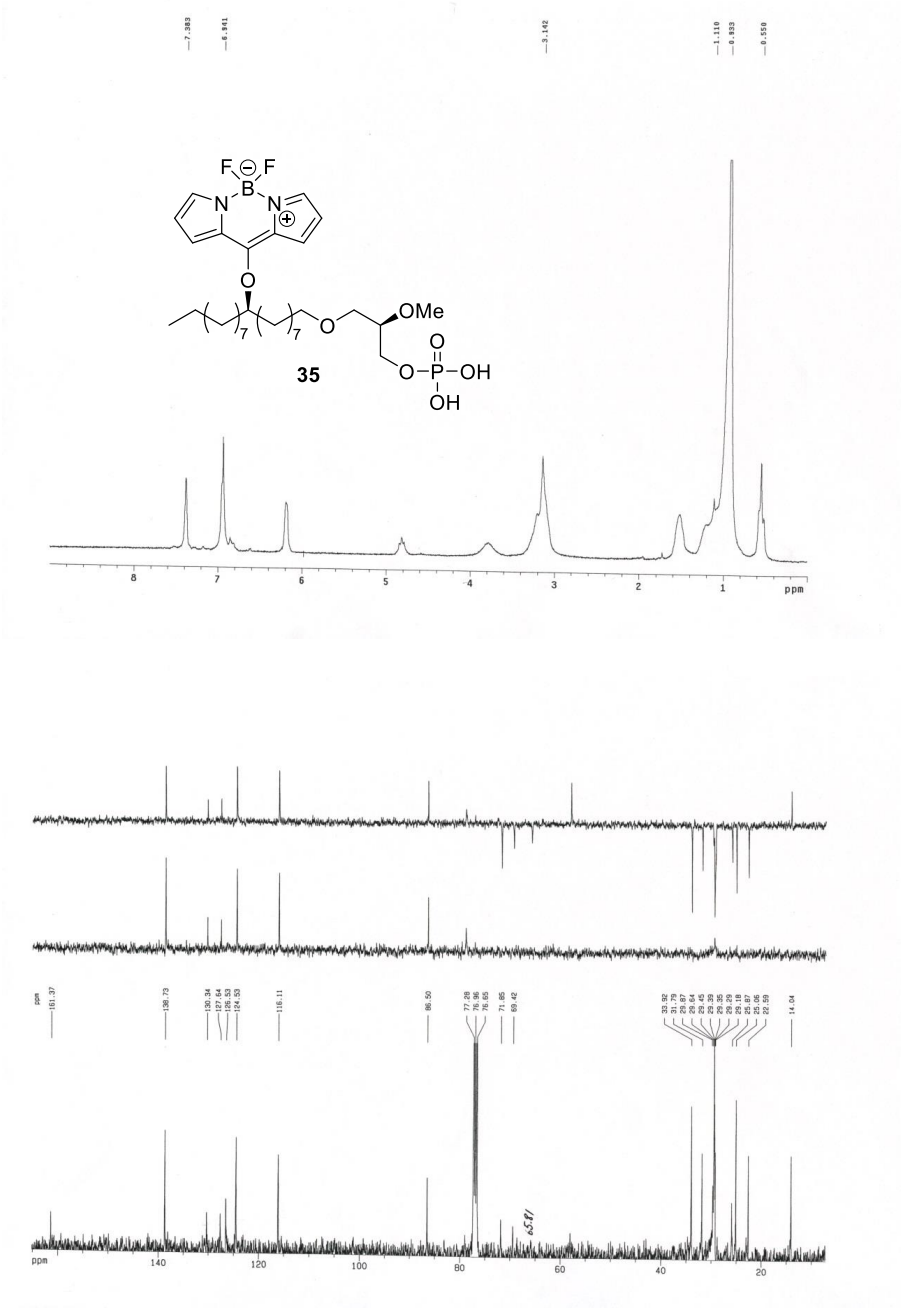

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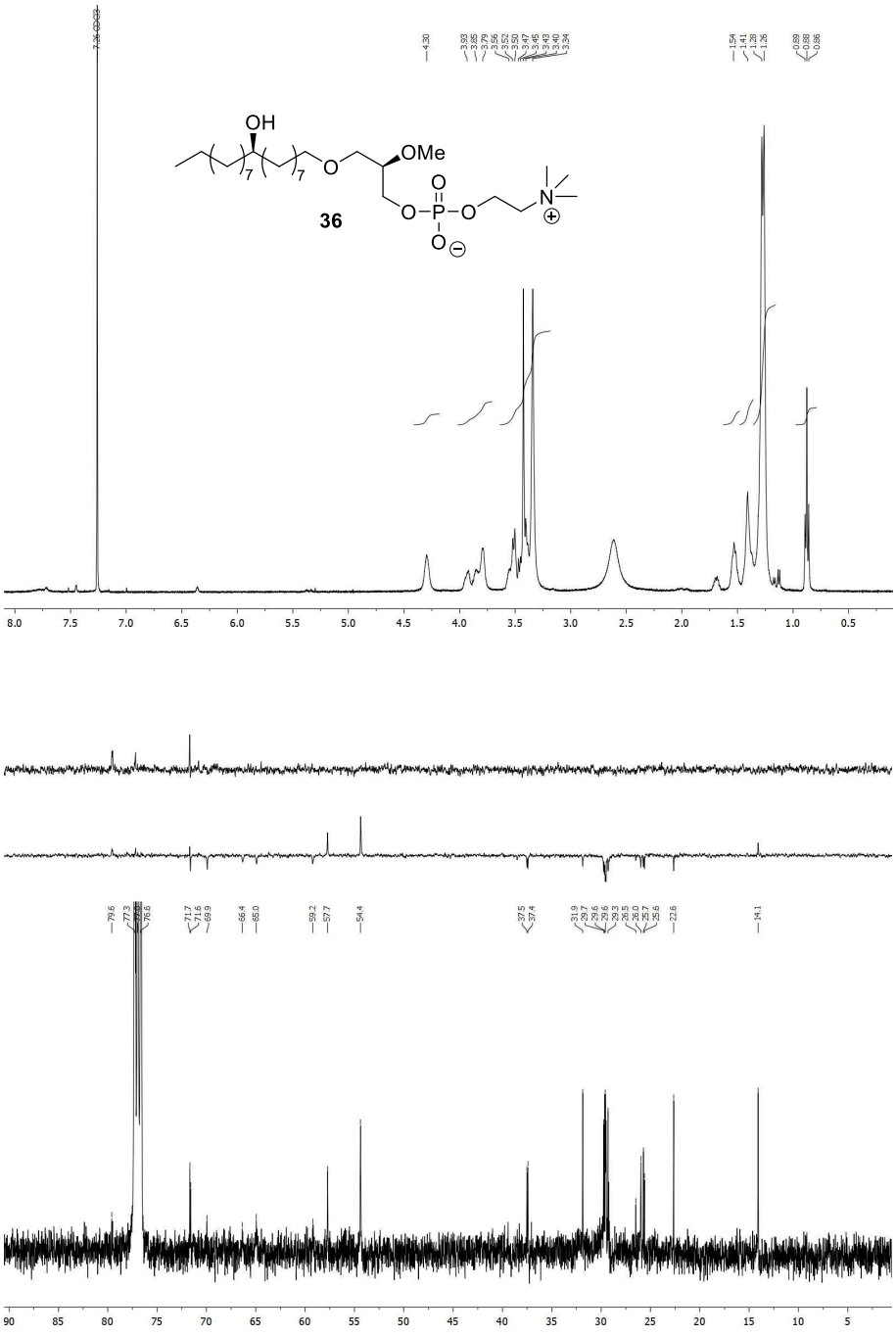

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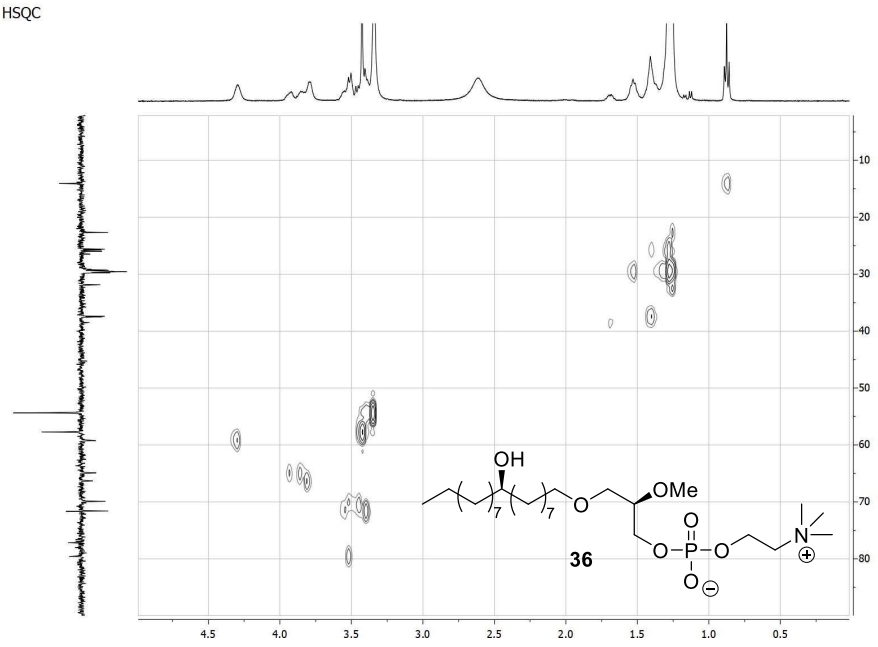

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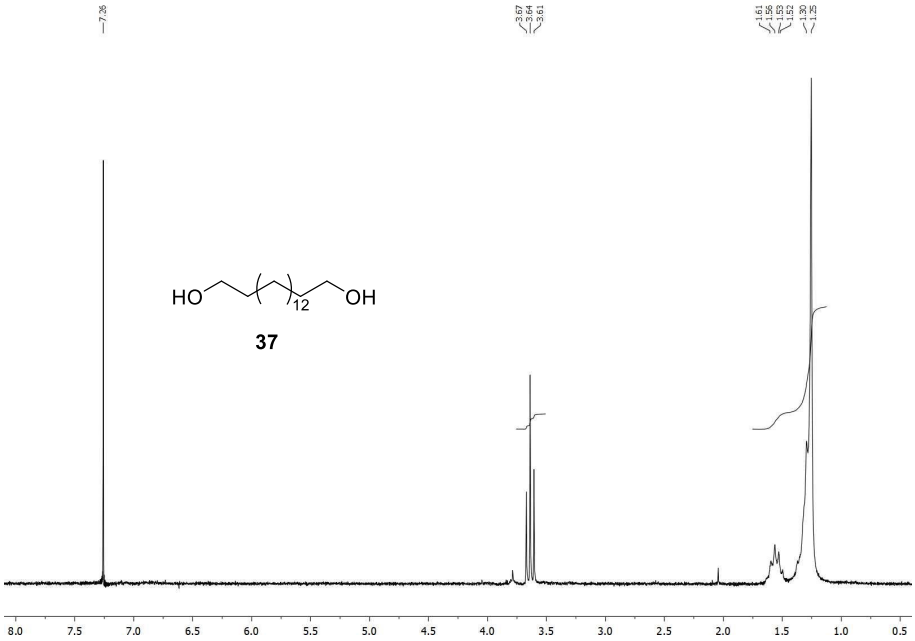

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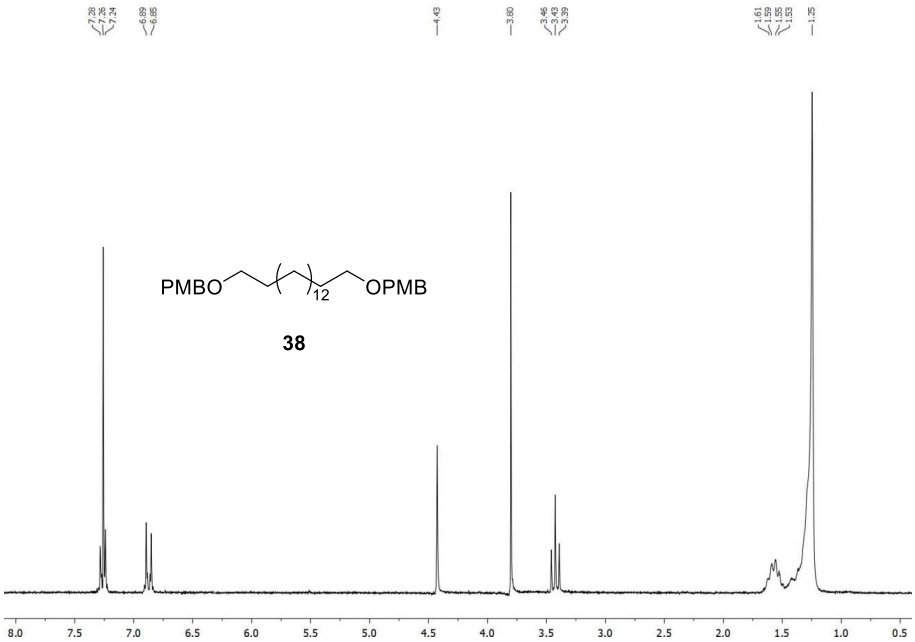

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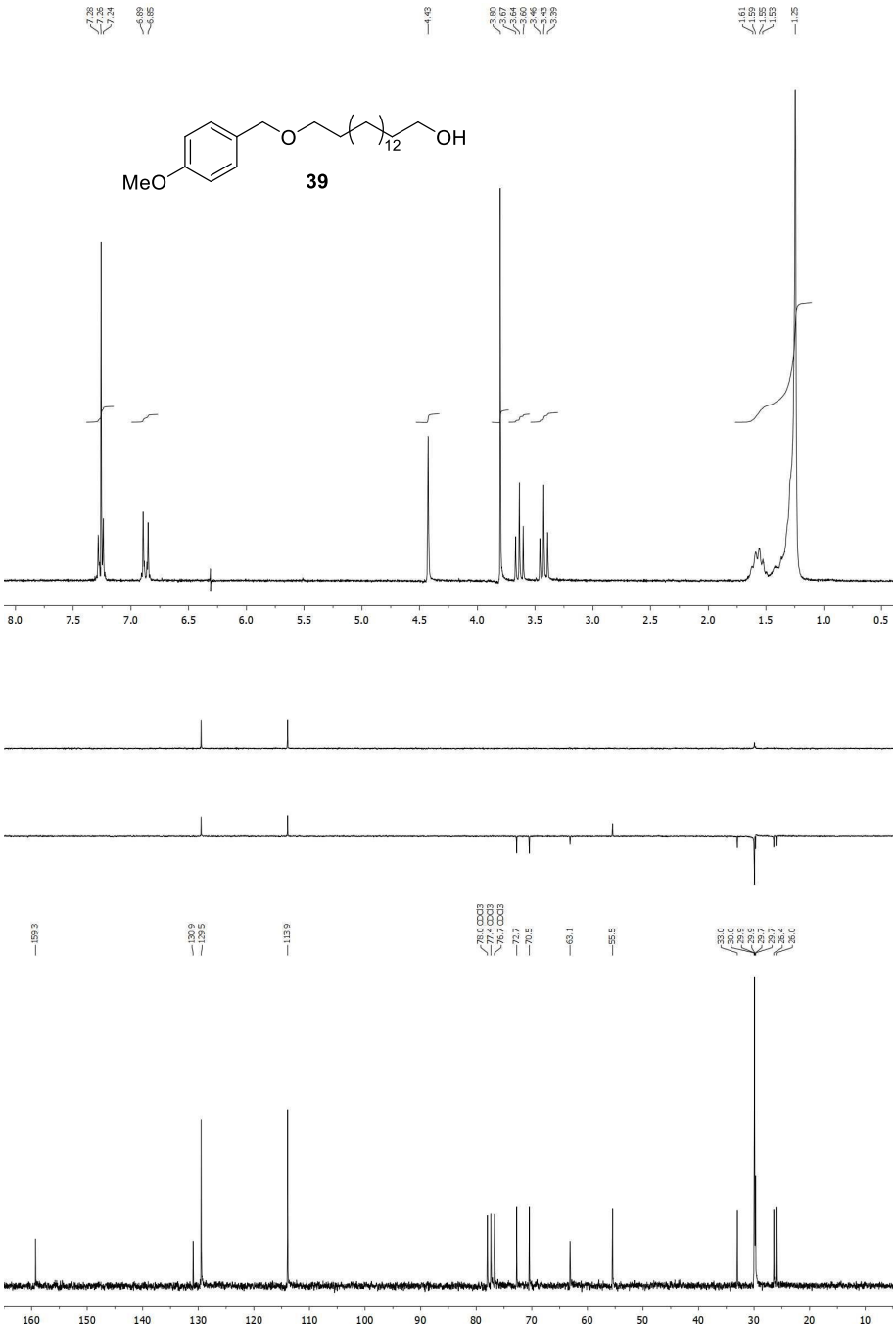

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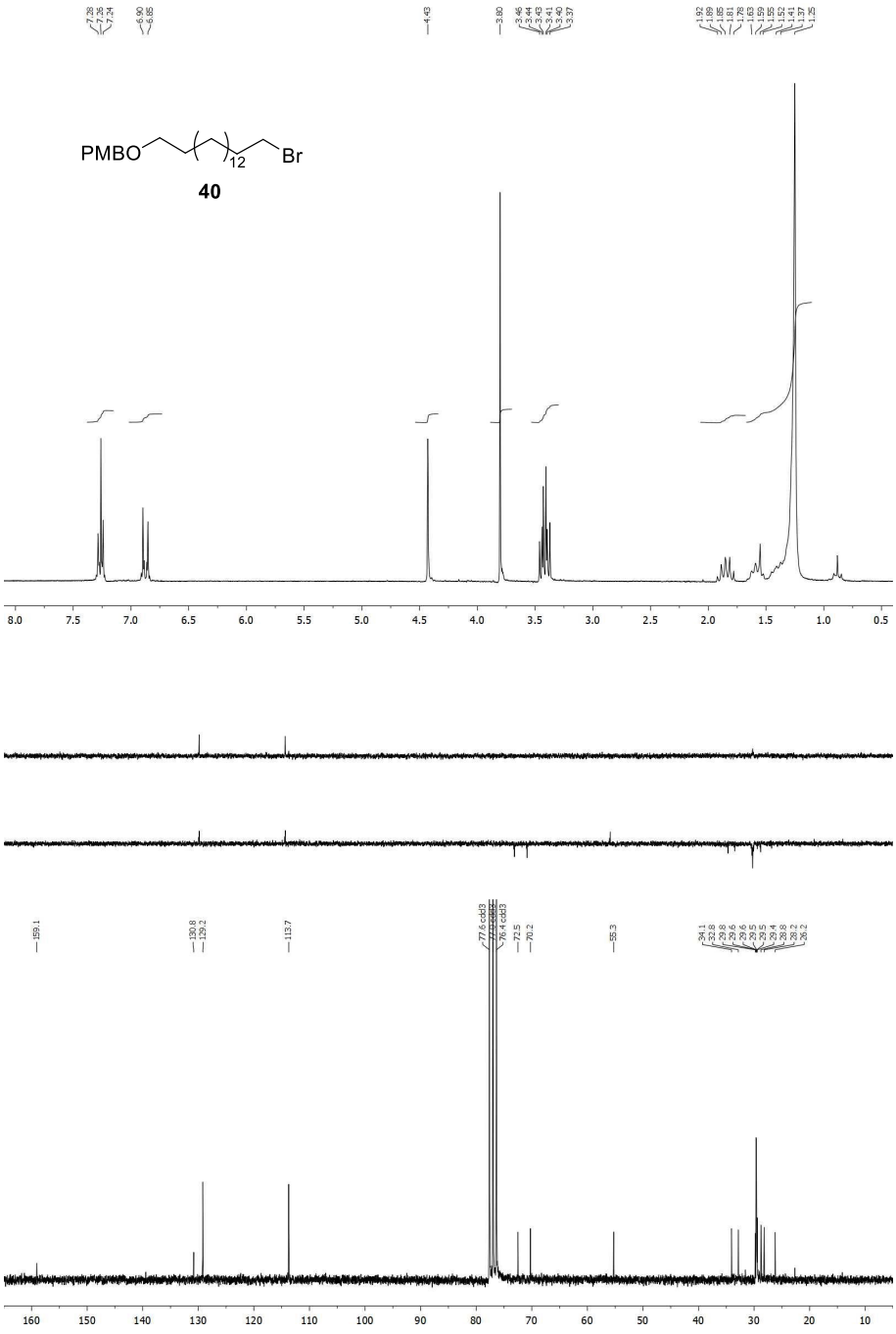

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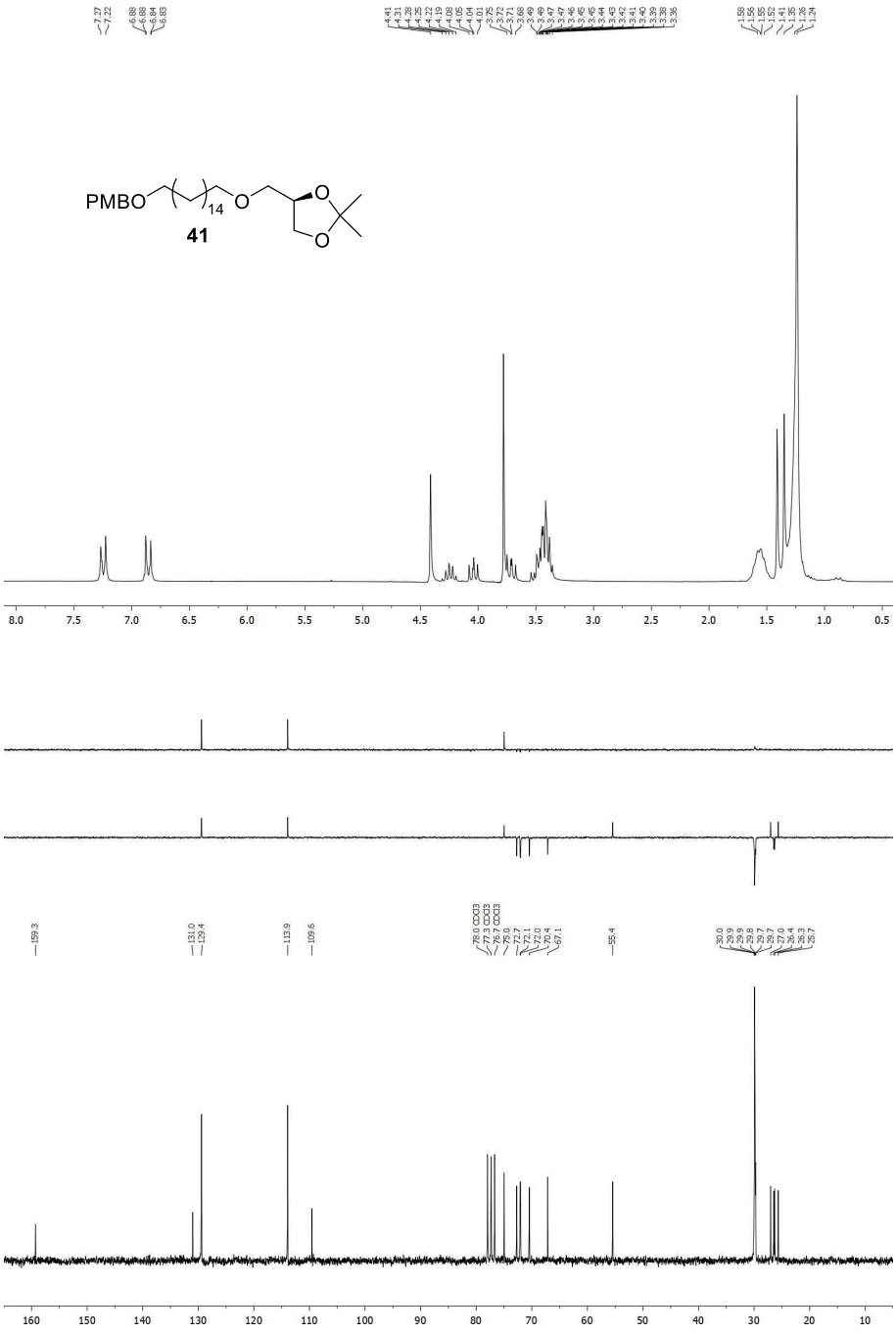

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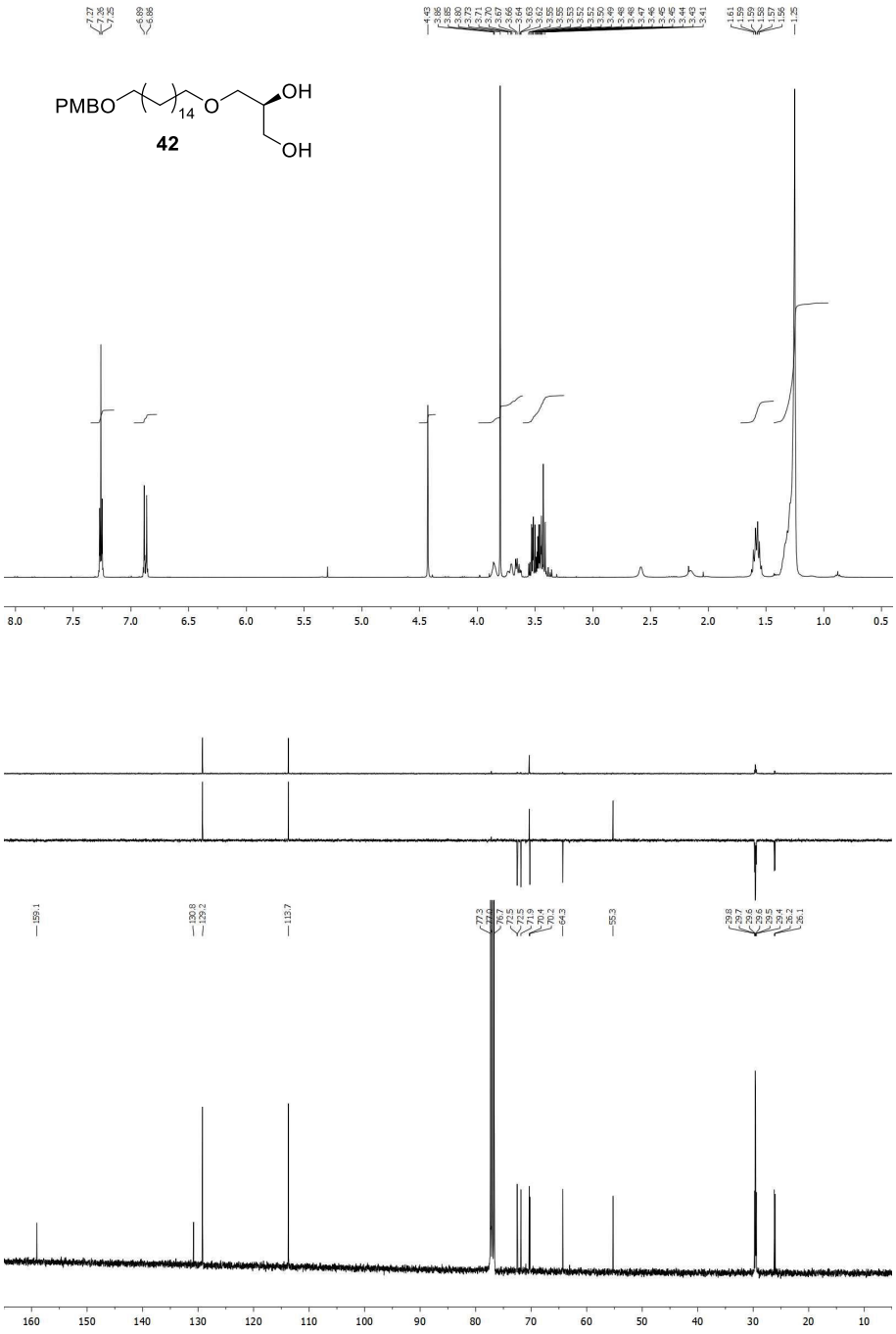

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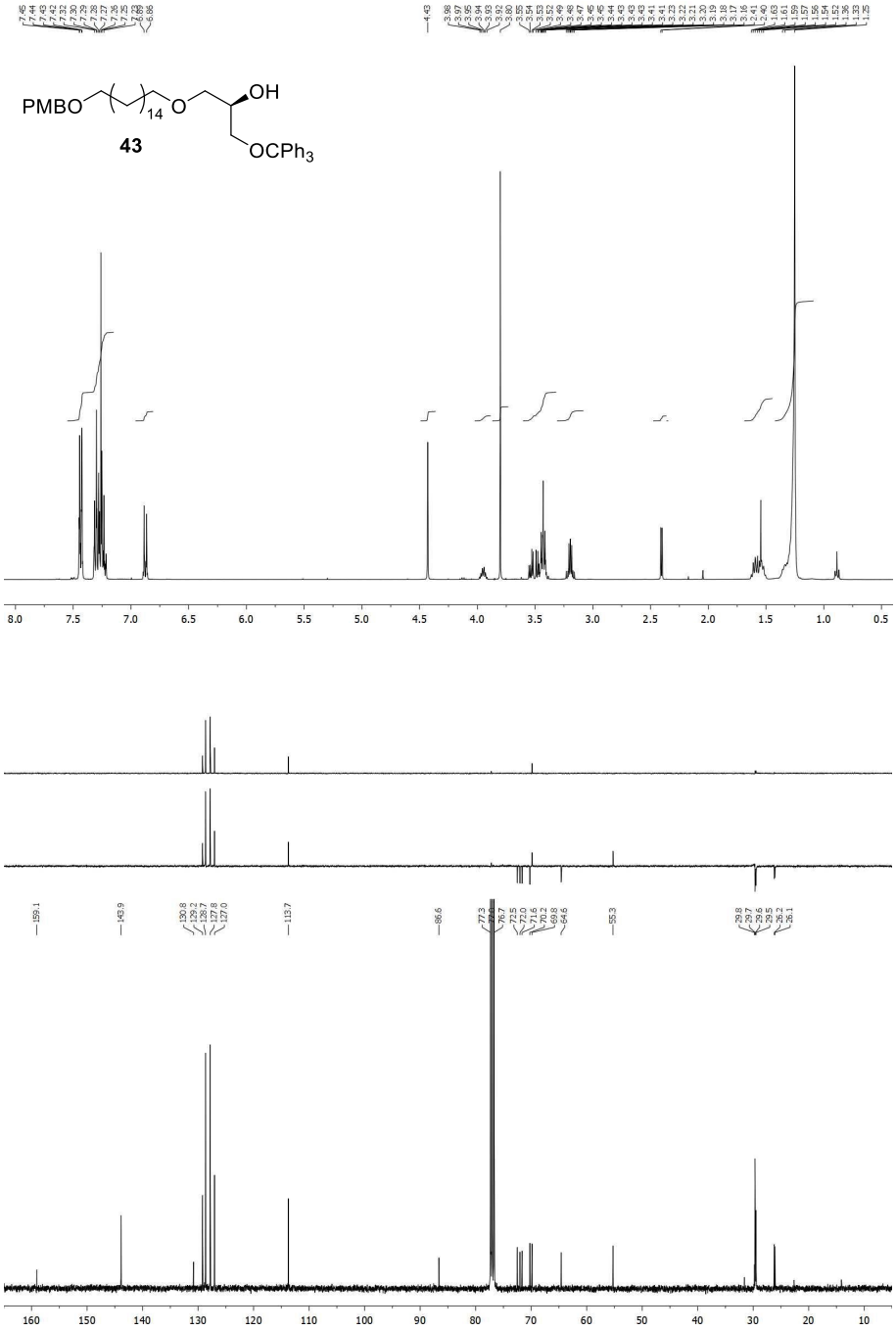

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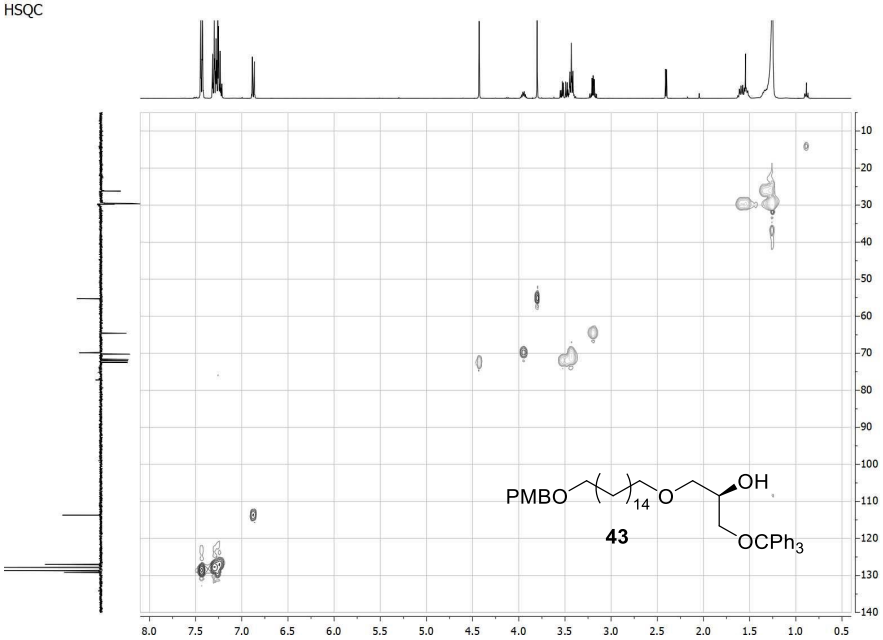

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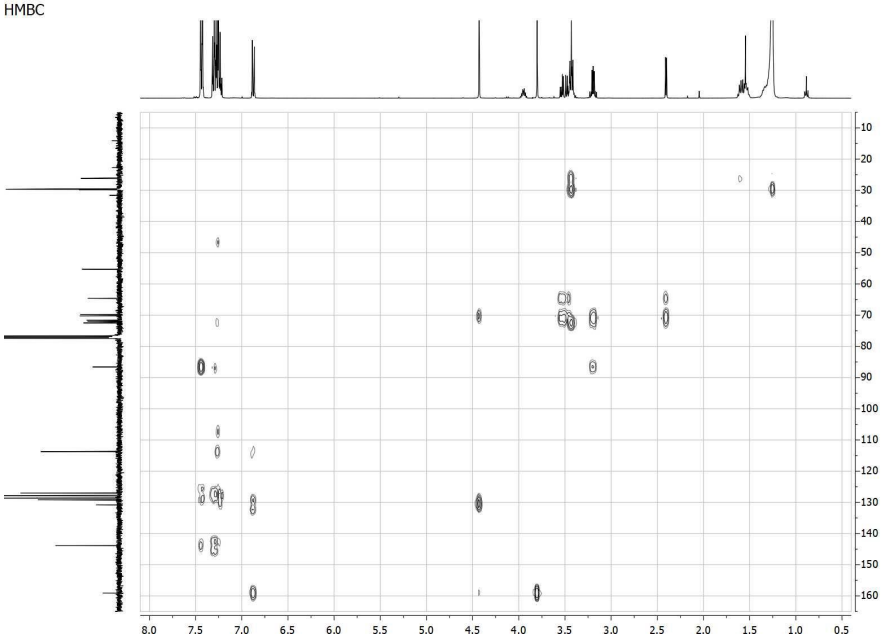

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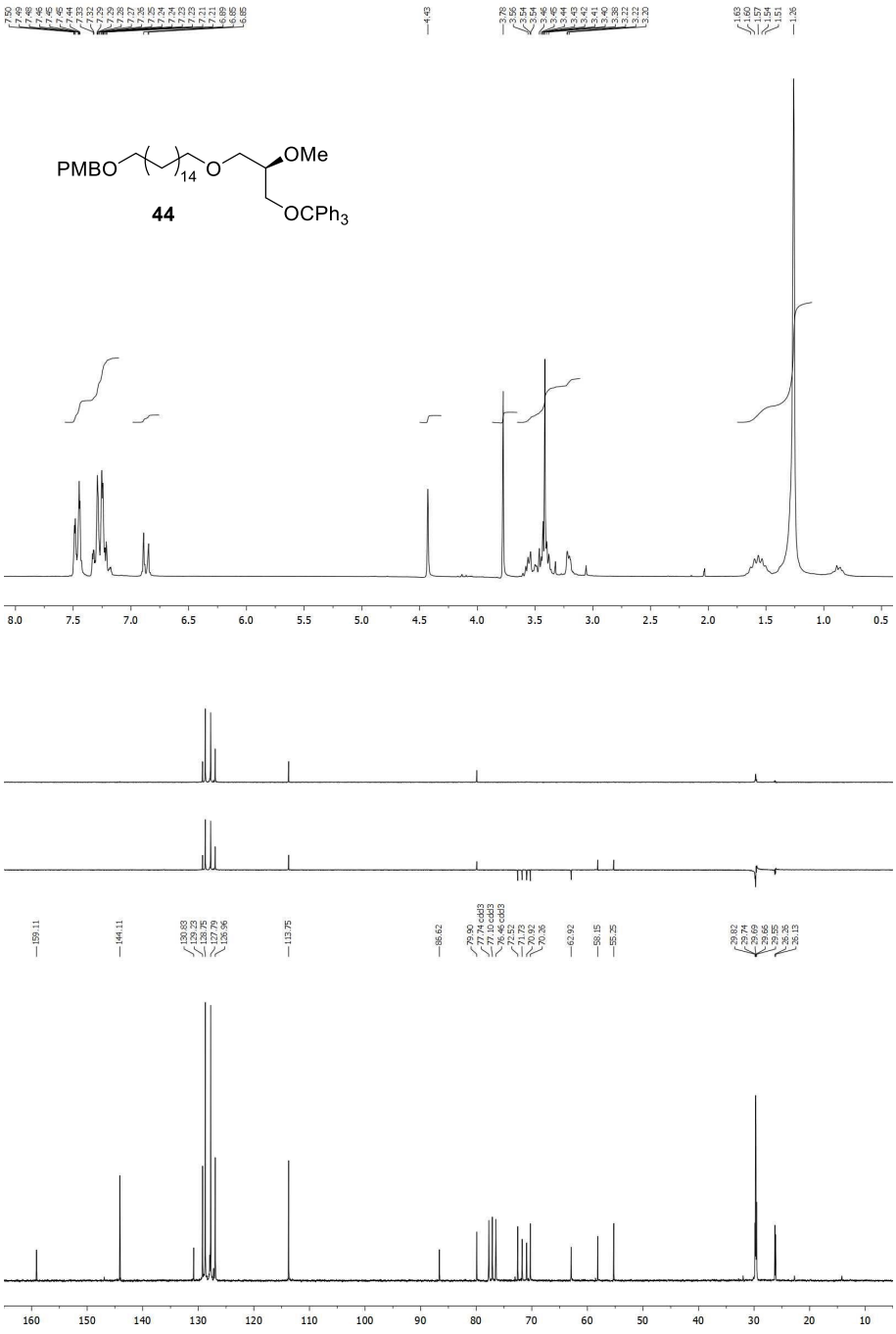

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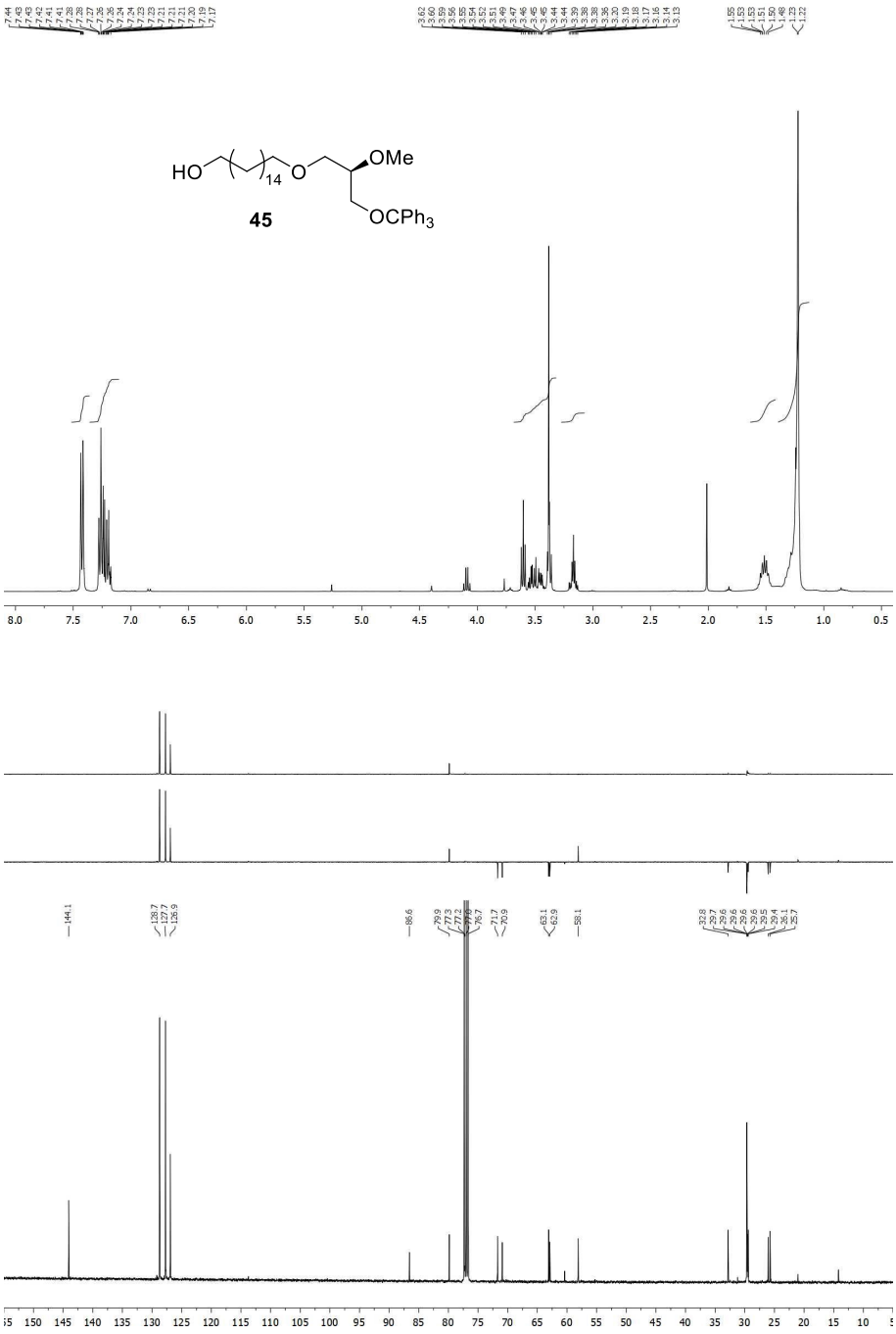

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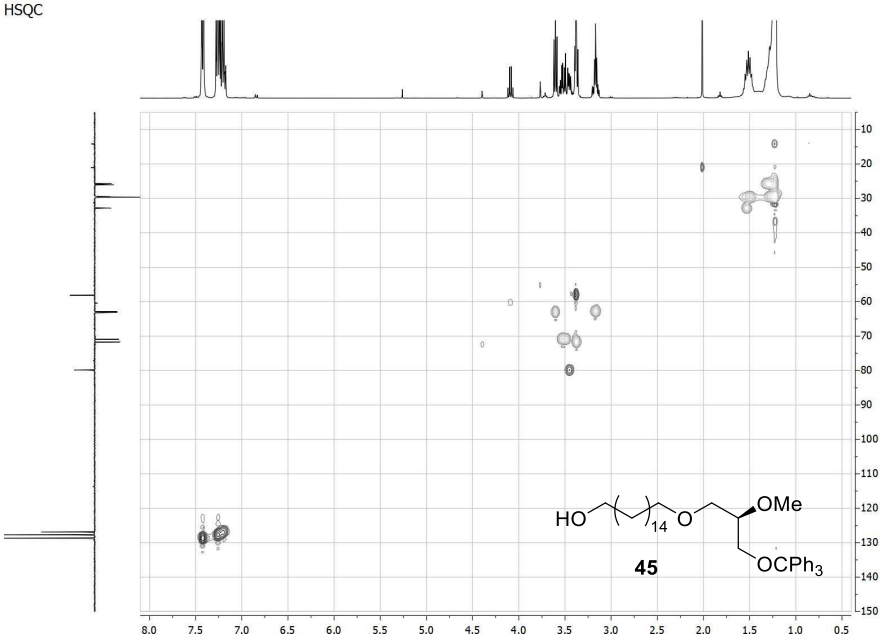

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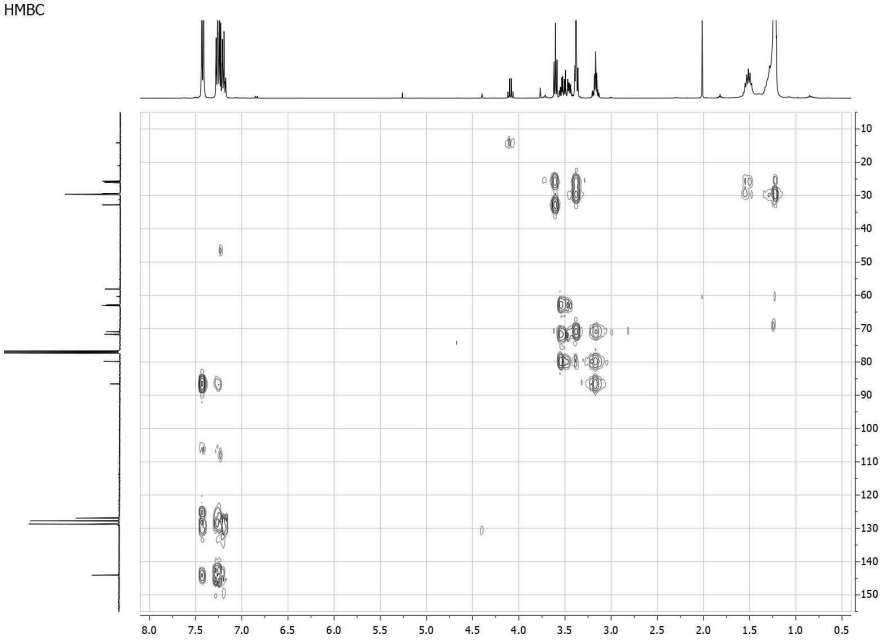

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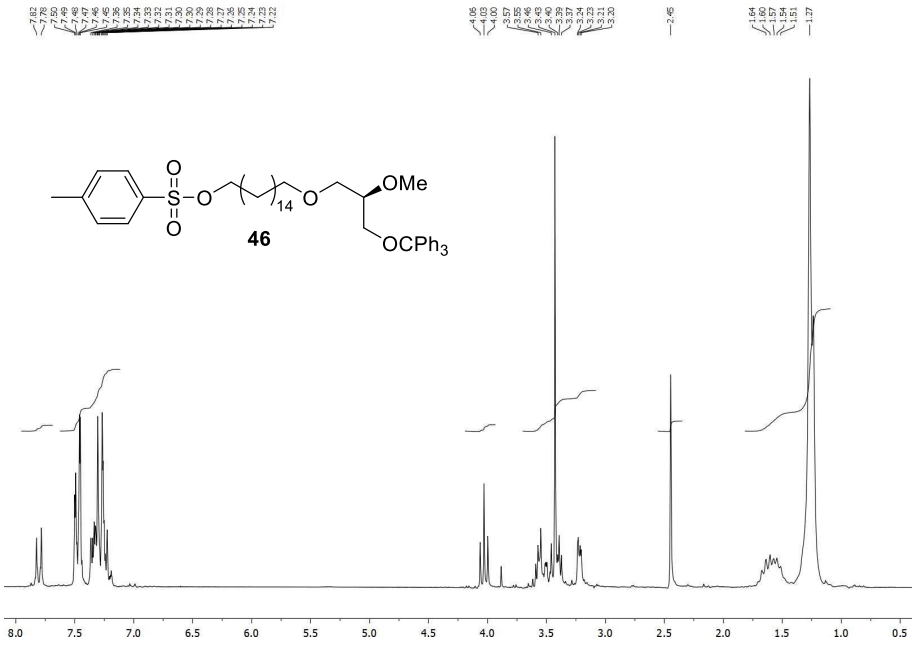

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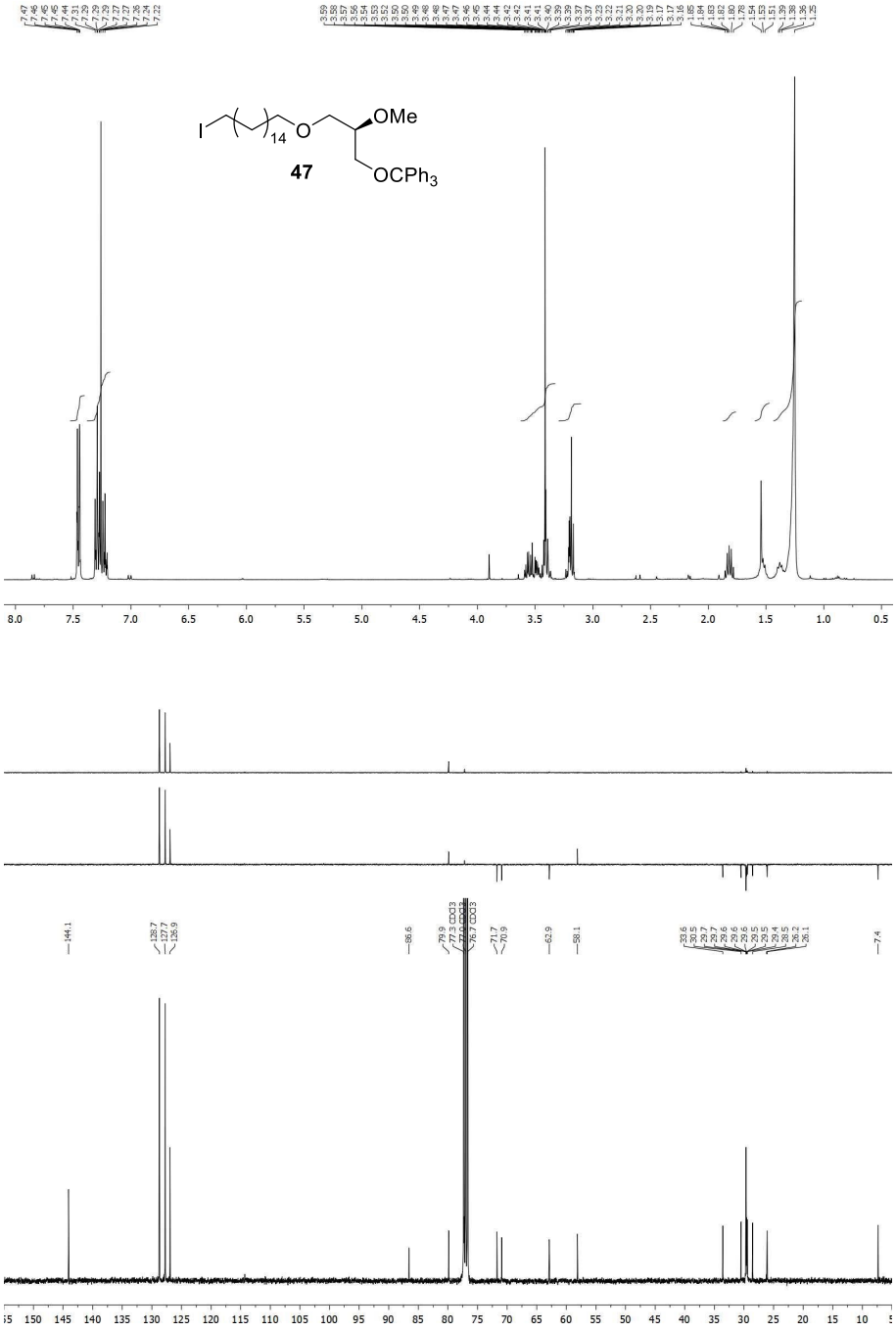

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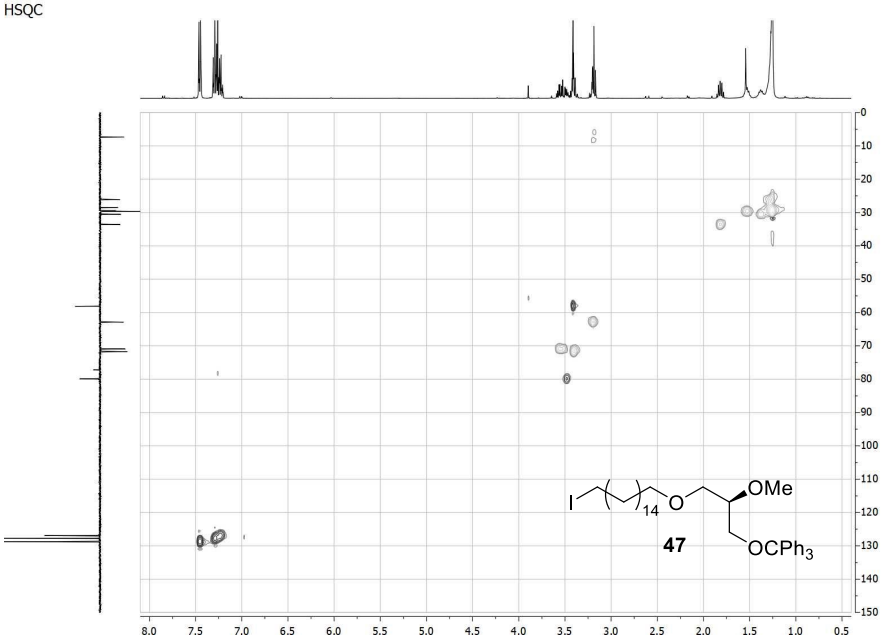

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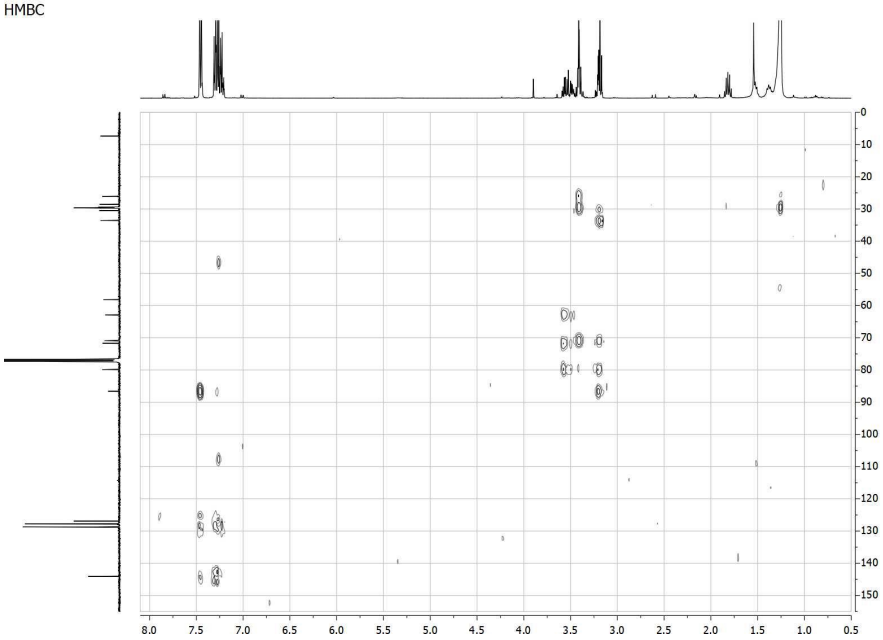

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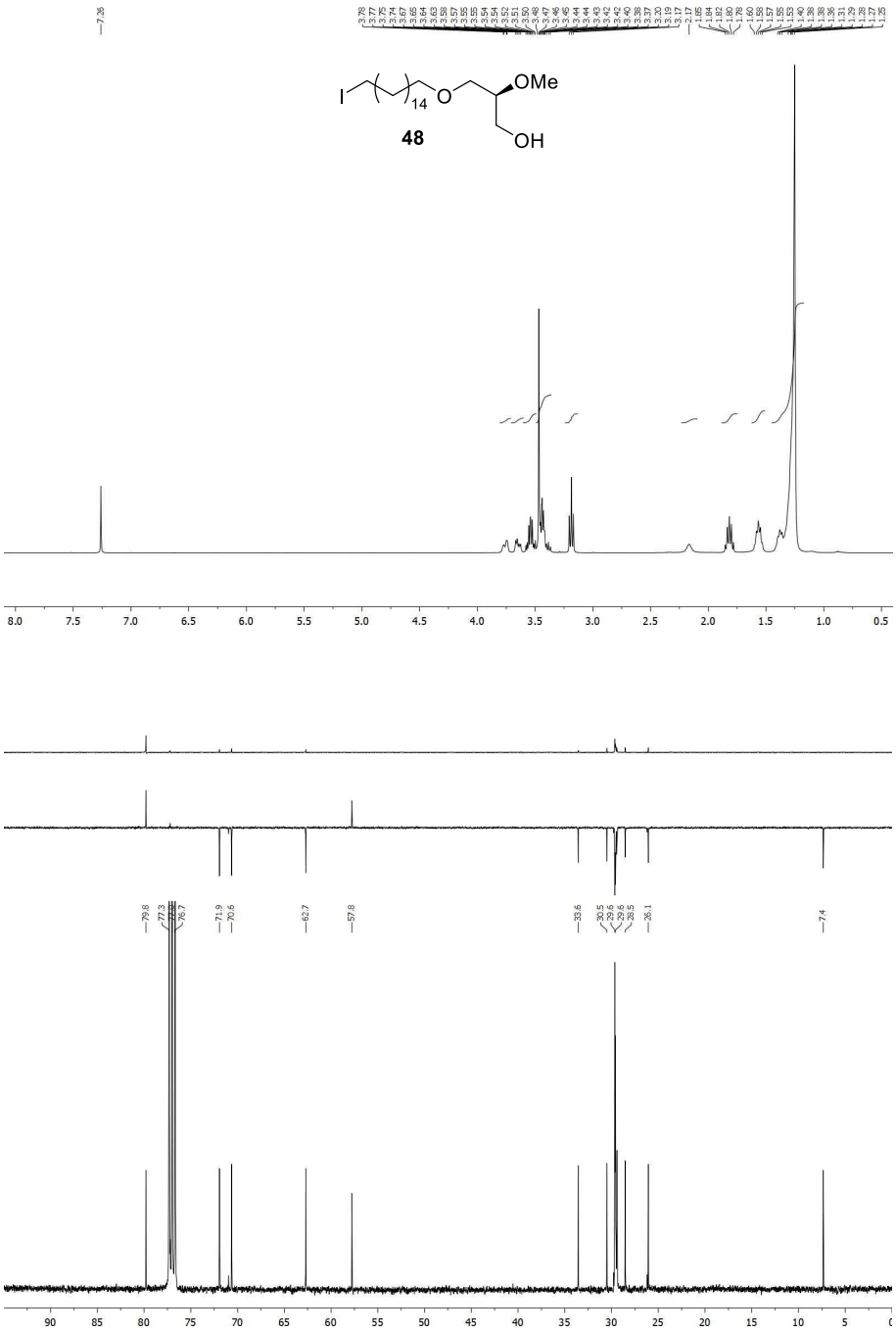

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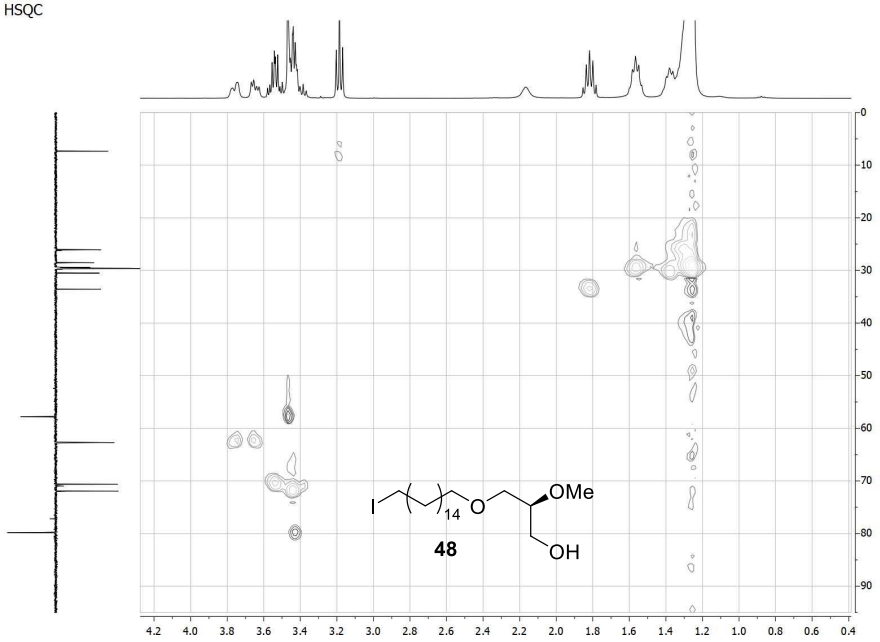

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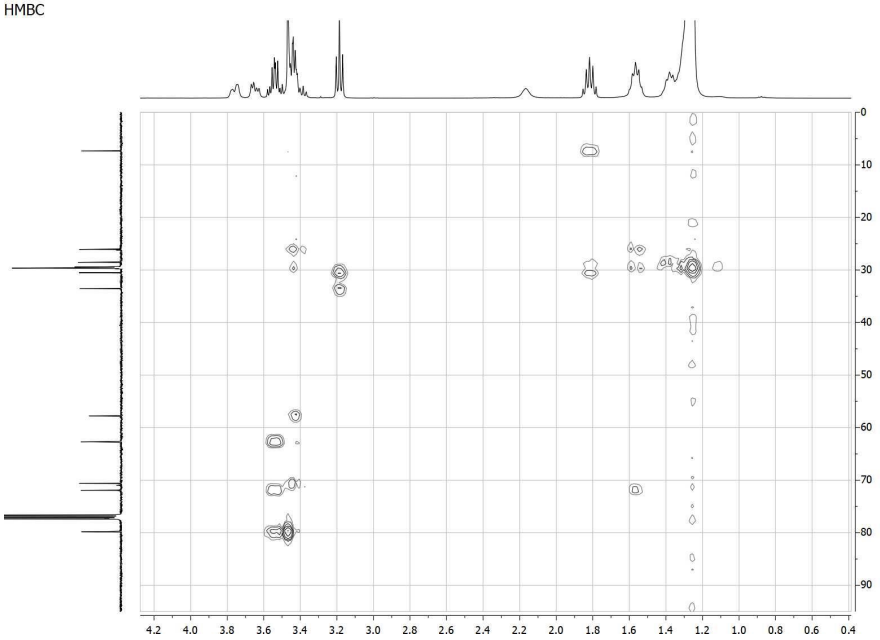

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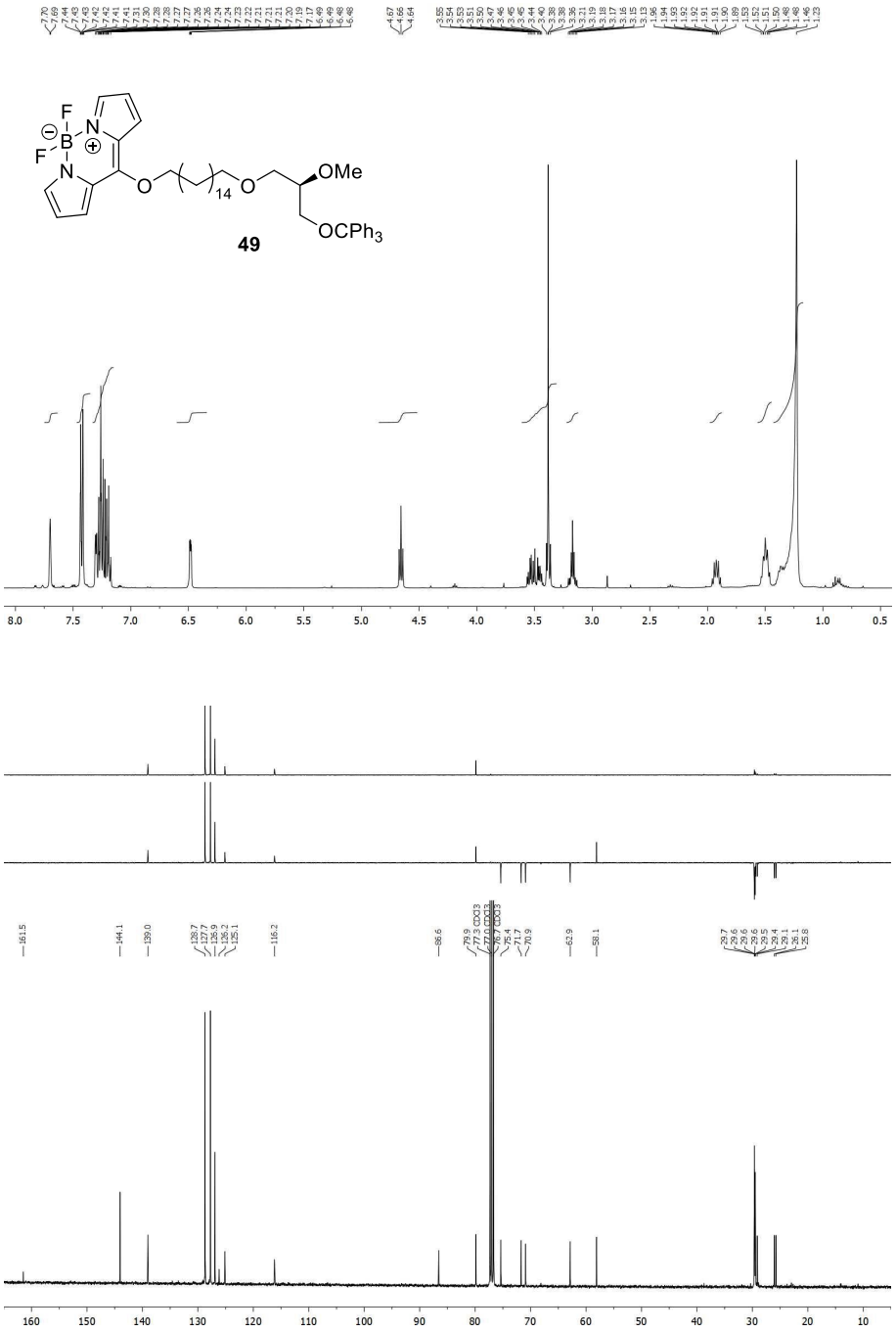

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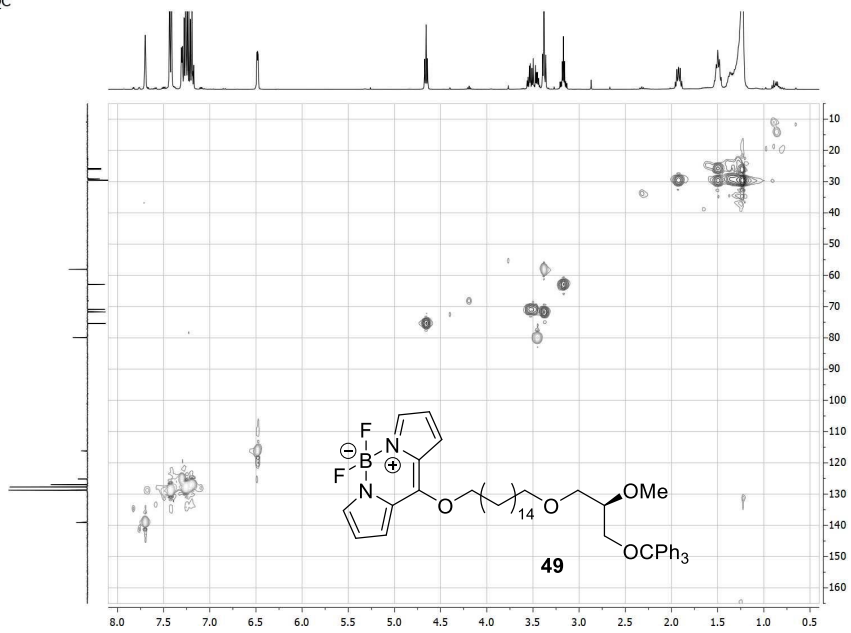

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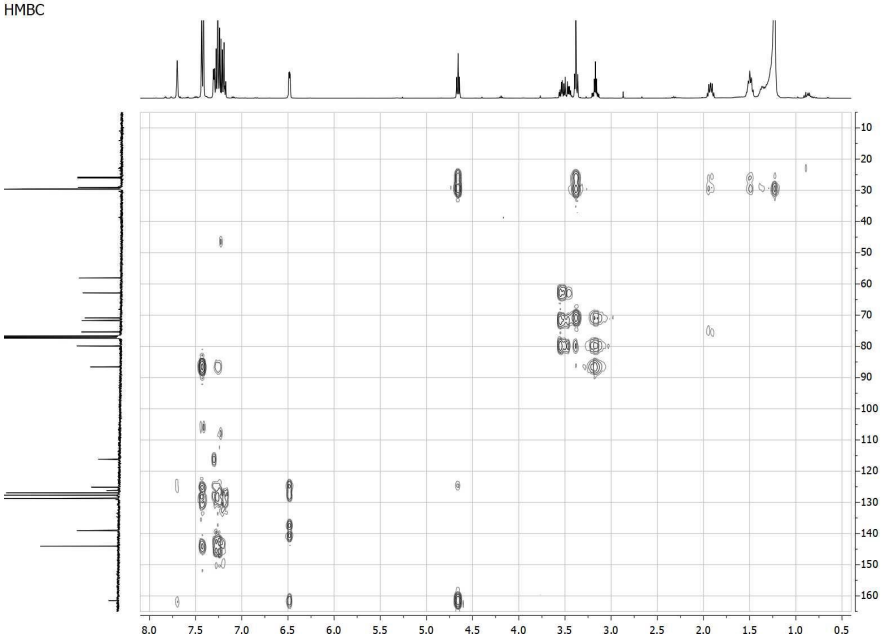

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SELLO UNIVERSIDAD DE SALAMANCA	20-07-2021 18:11:01
SÁNCHEZ MARCOS ISIDRO	20-07-2021 10:20:59

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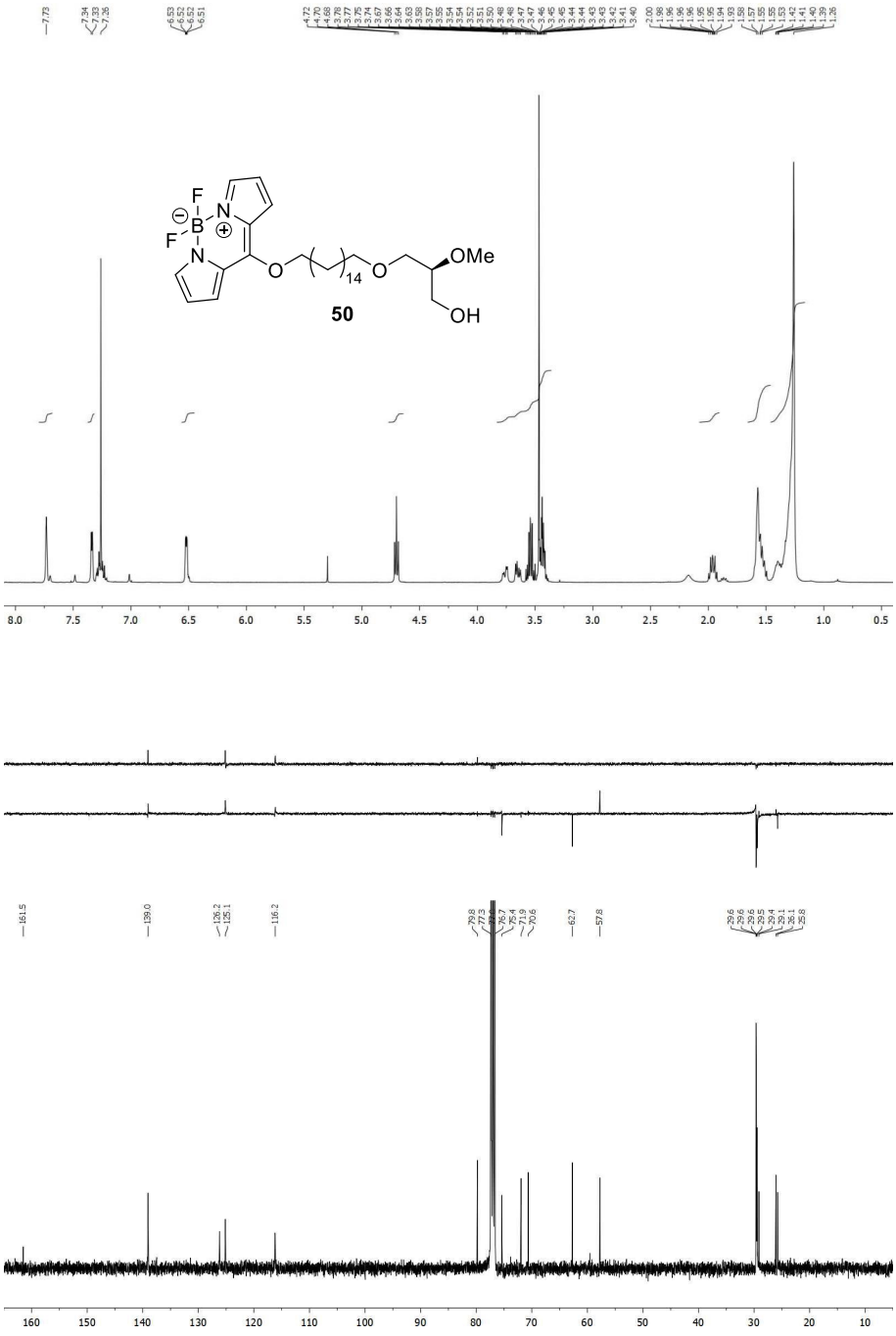

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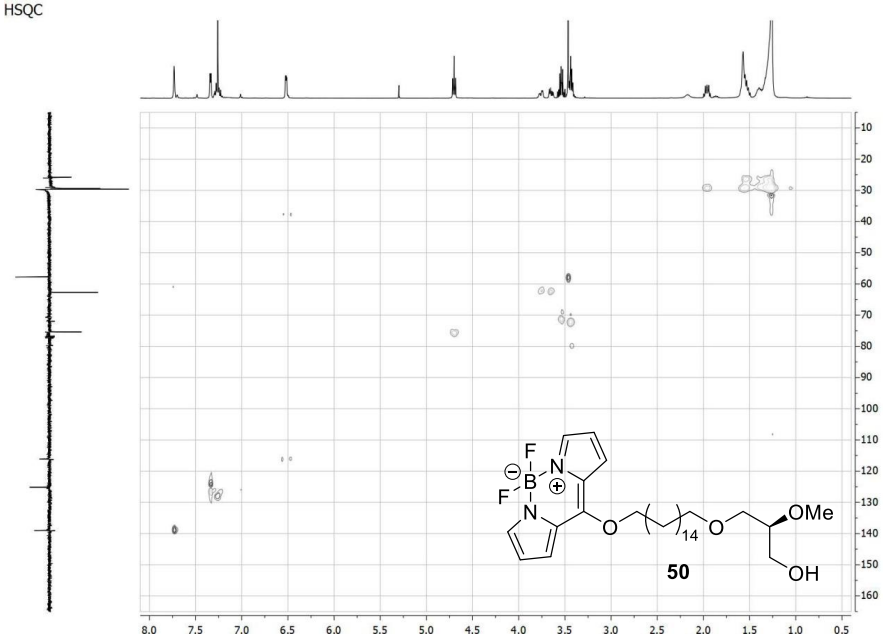

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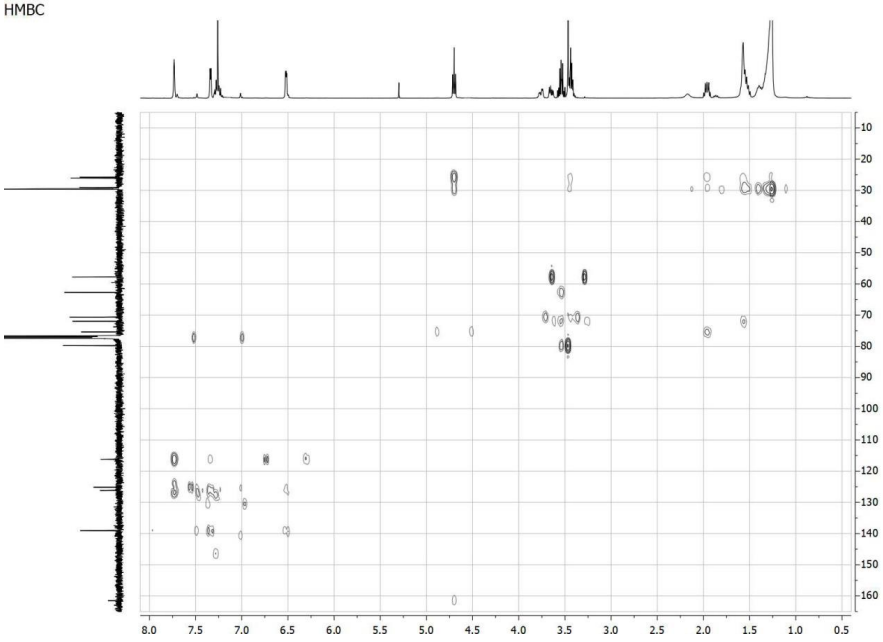

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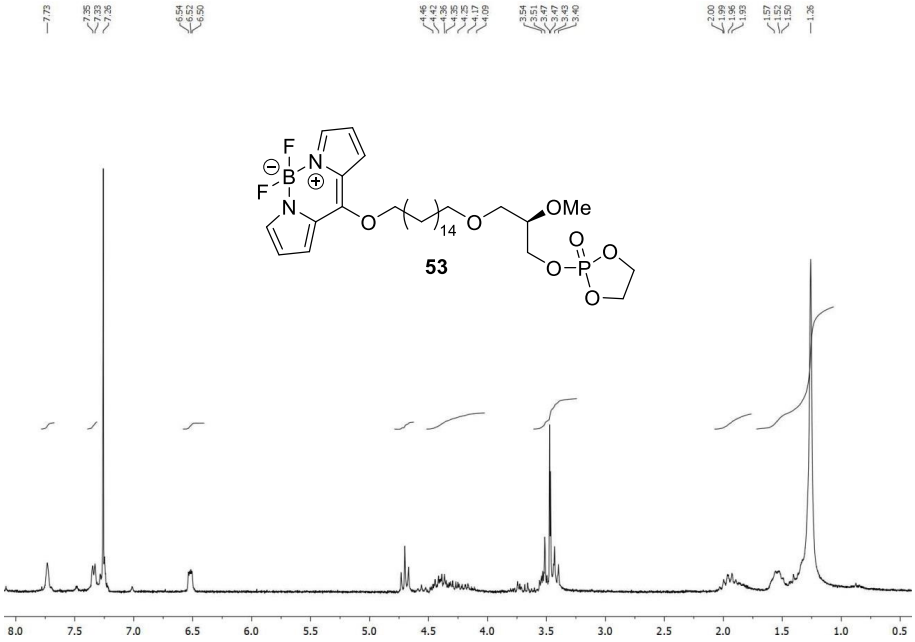

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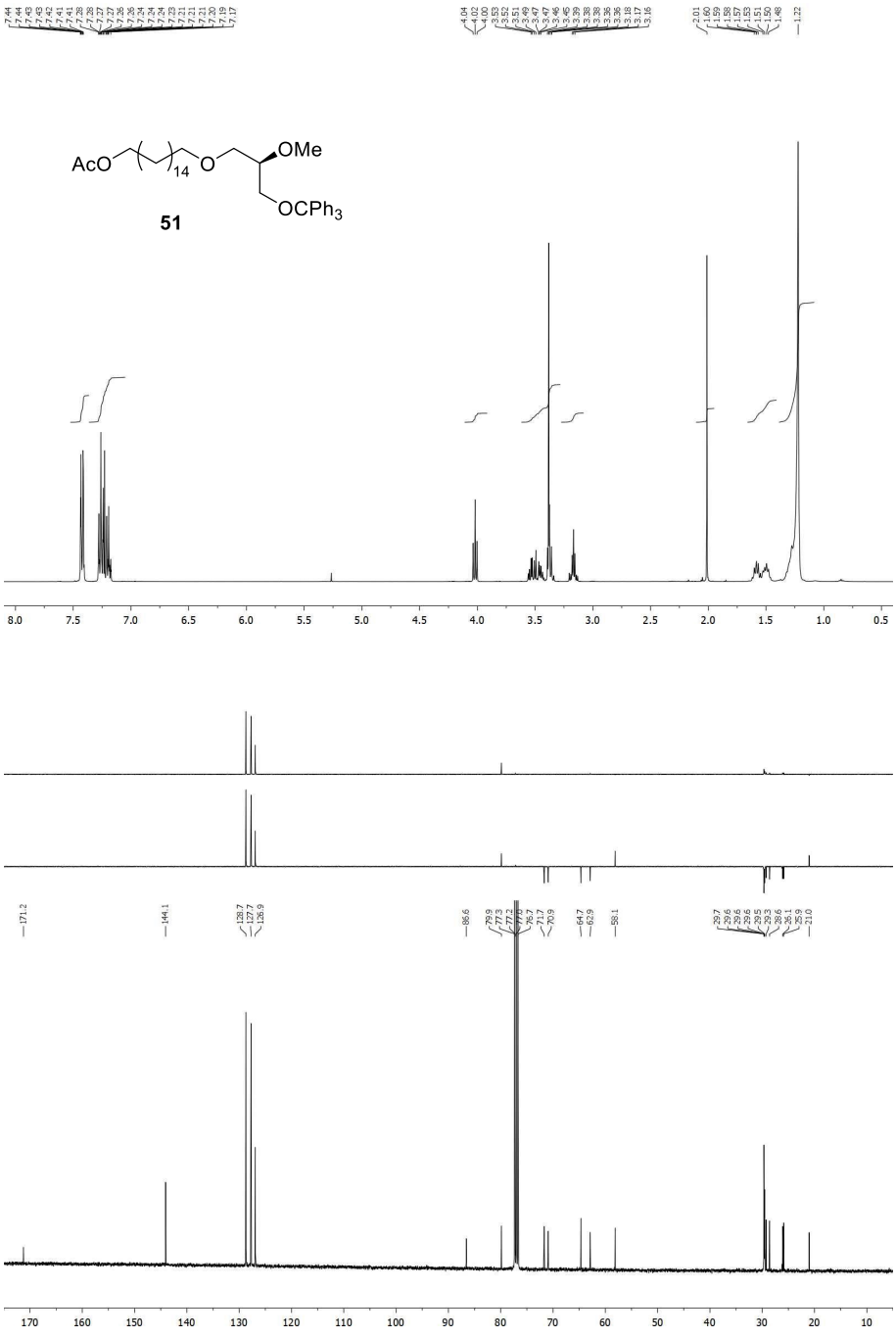

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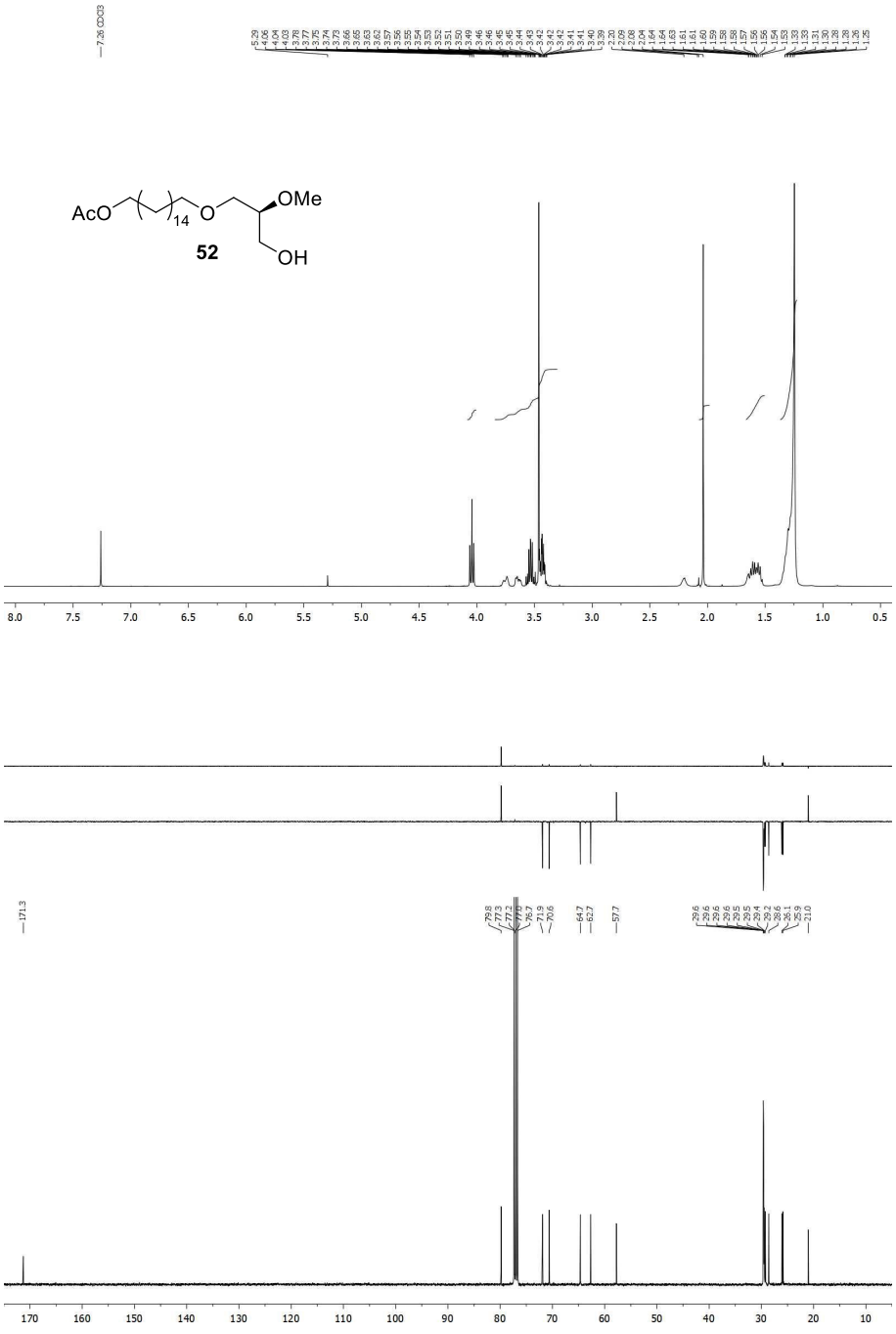

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SELLO UNIVERSIDAD DE SALAMANCA	20-07-2021 18:11:01
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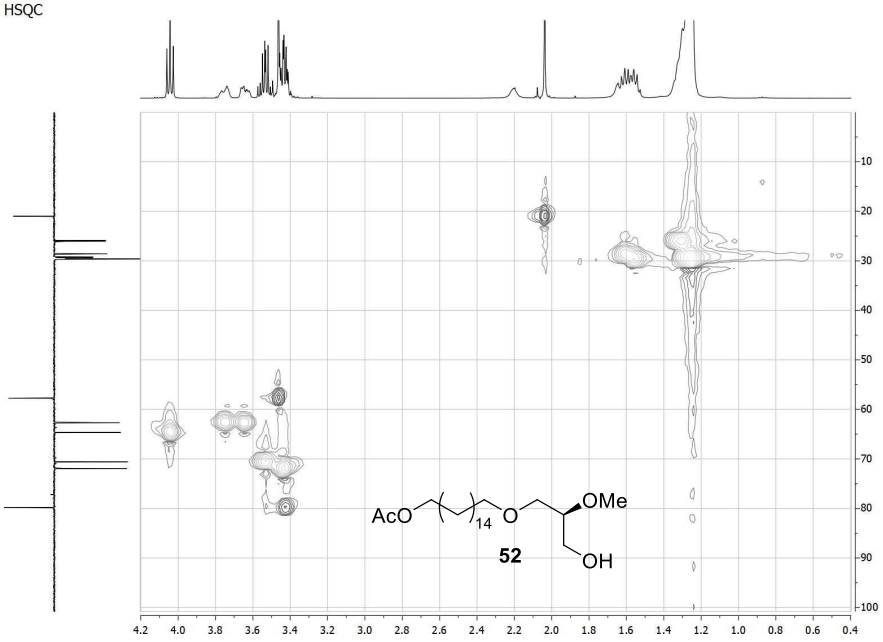

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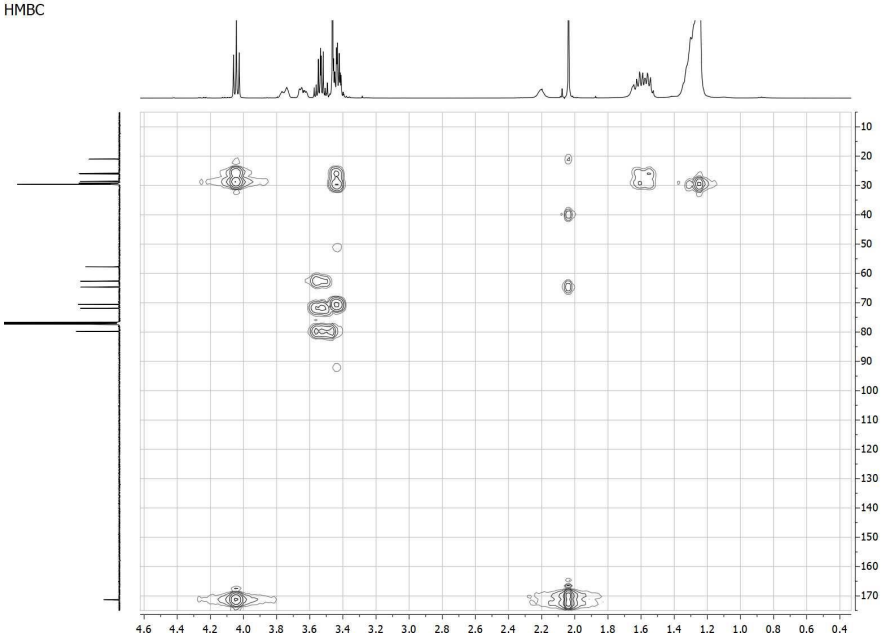

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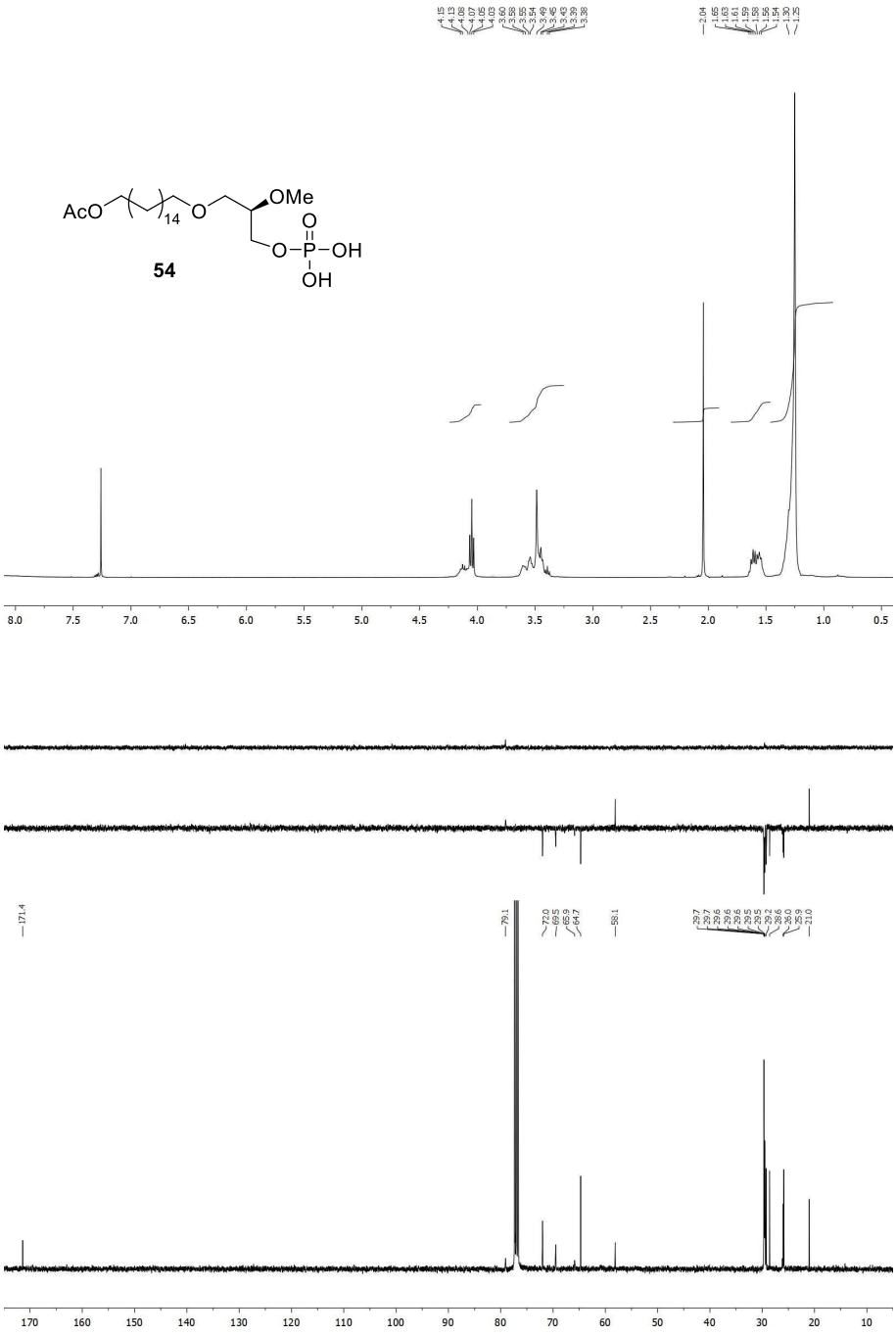

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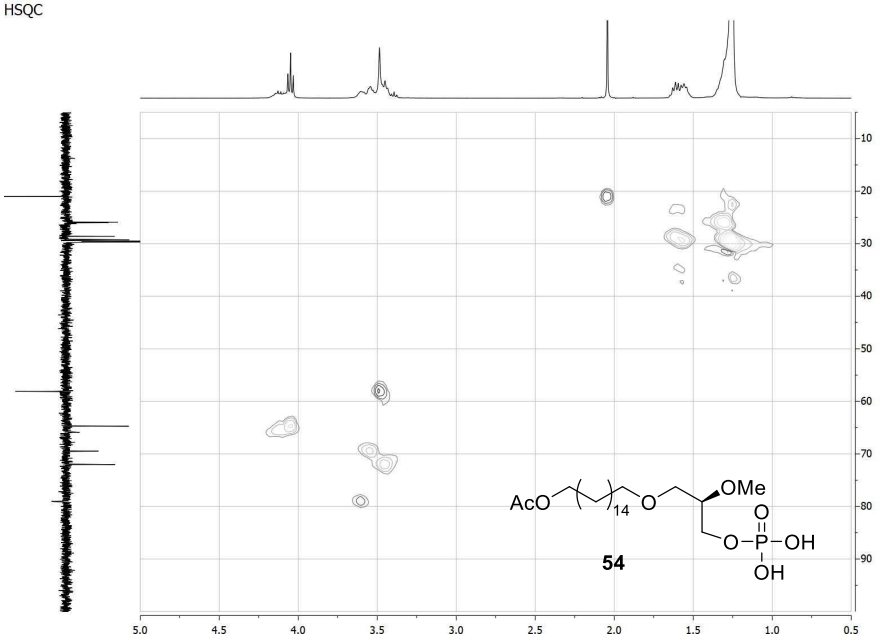

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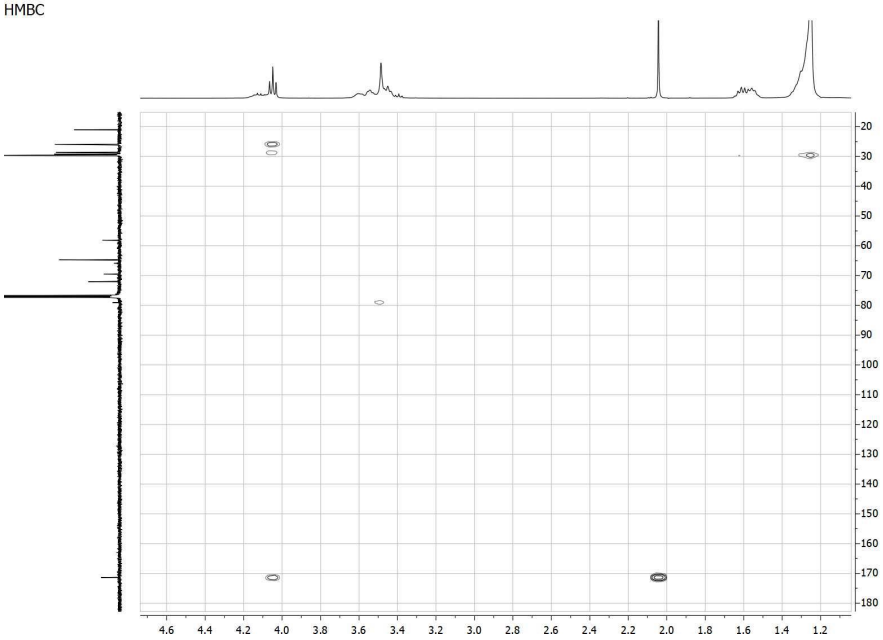

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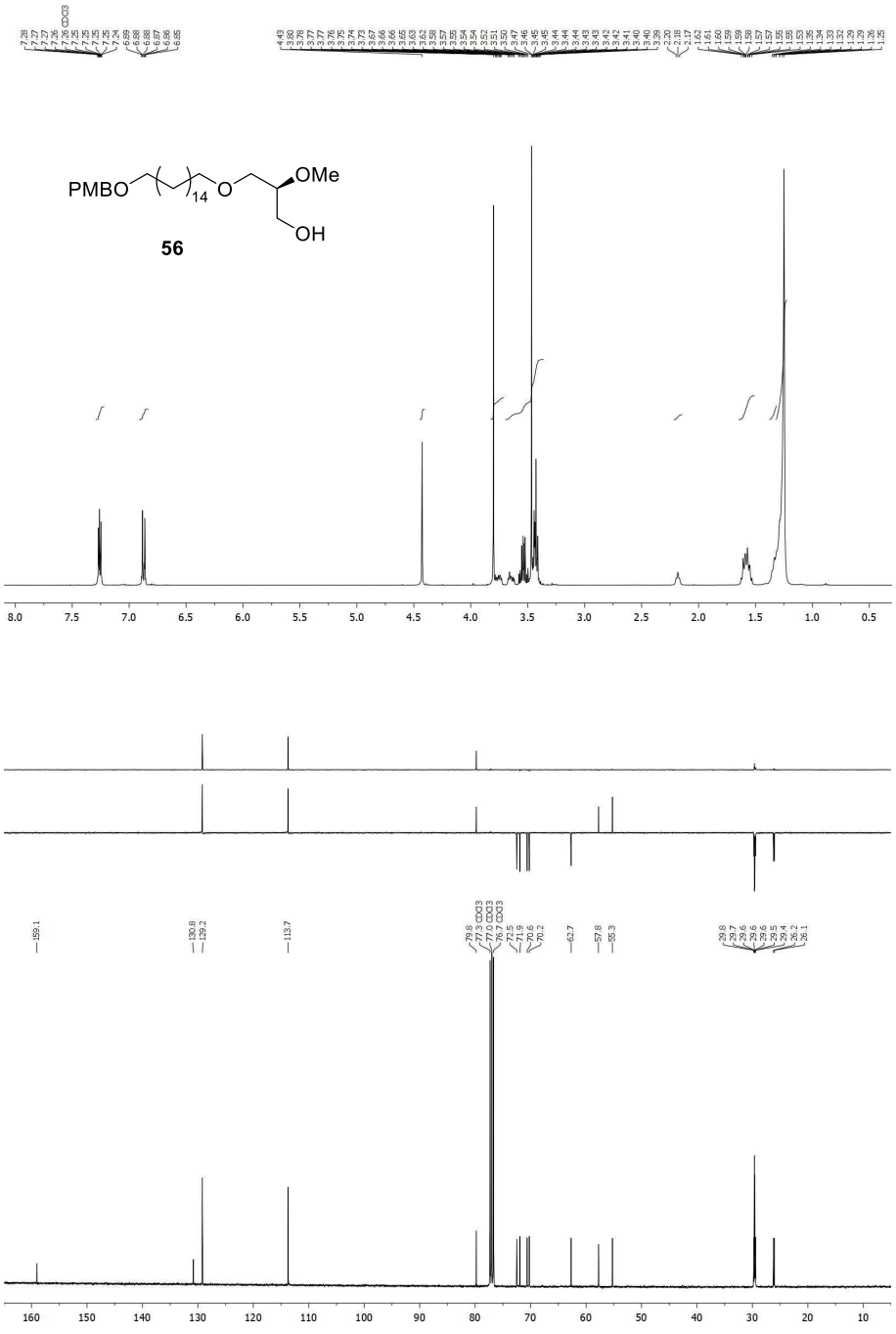

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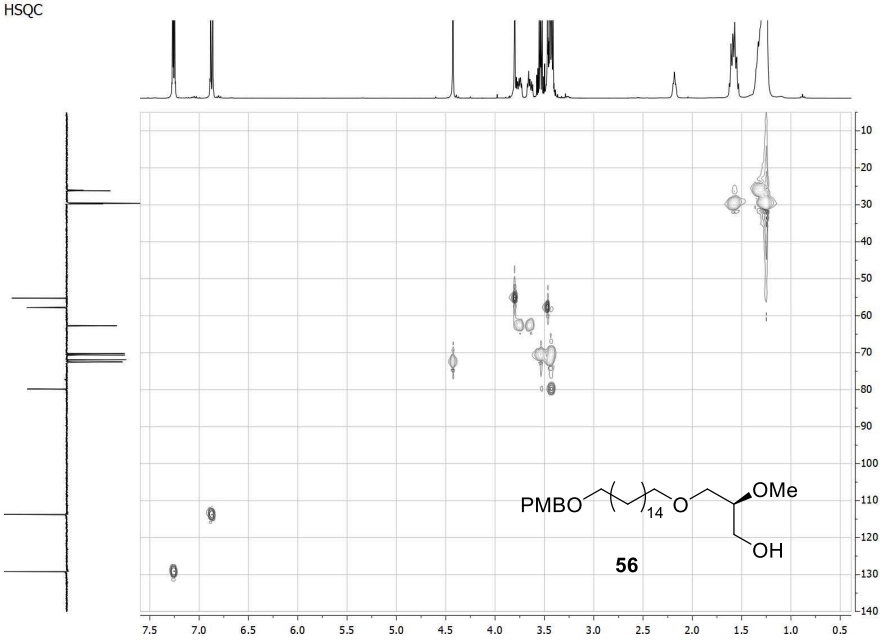

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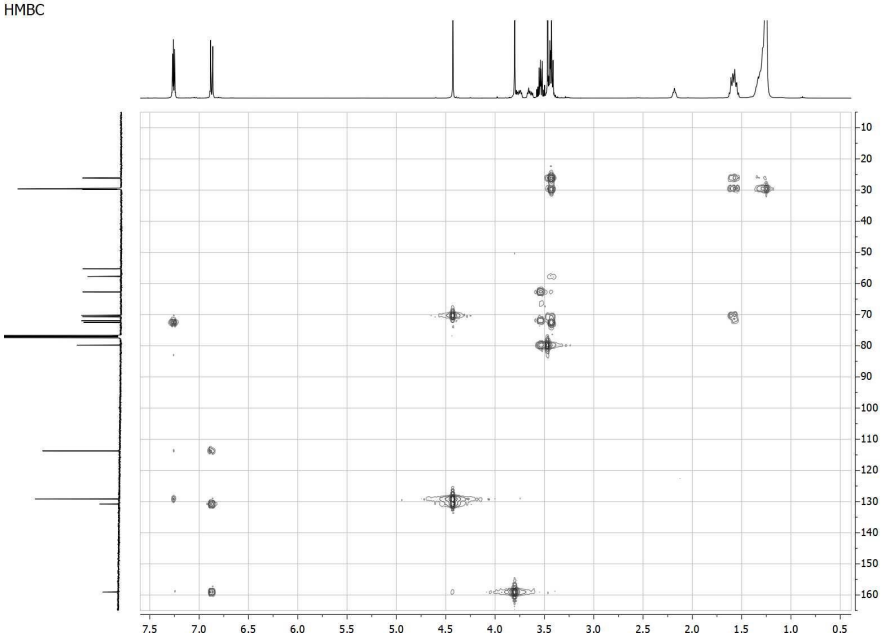

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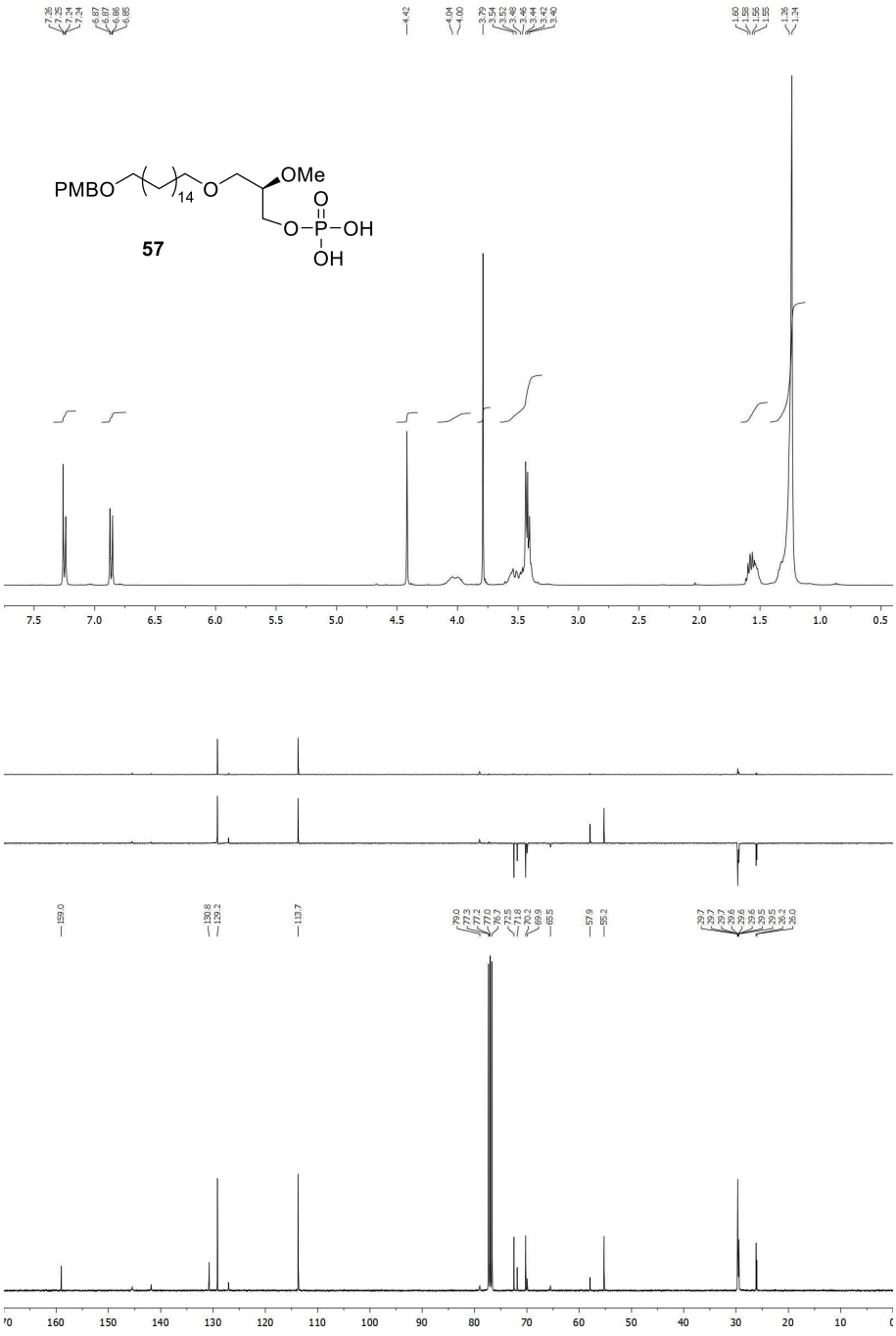

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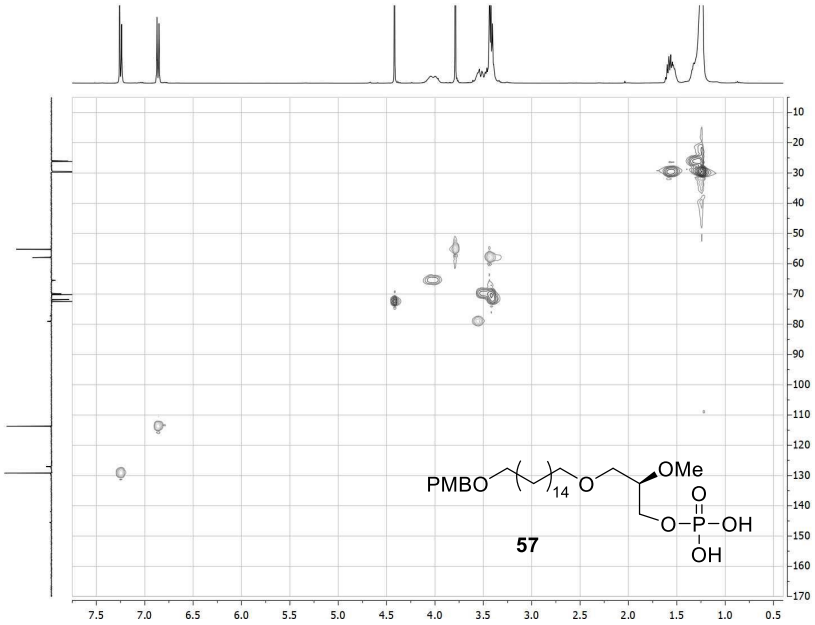

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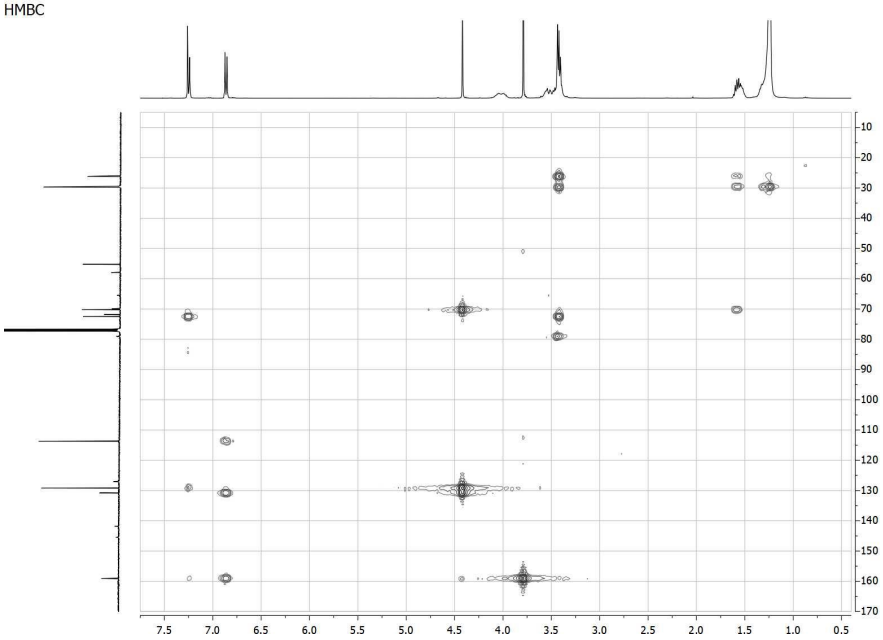

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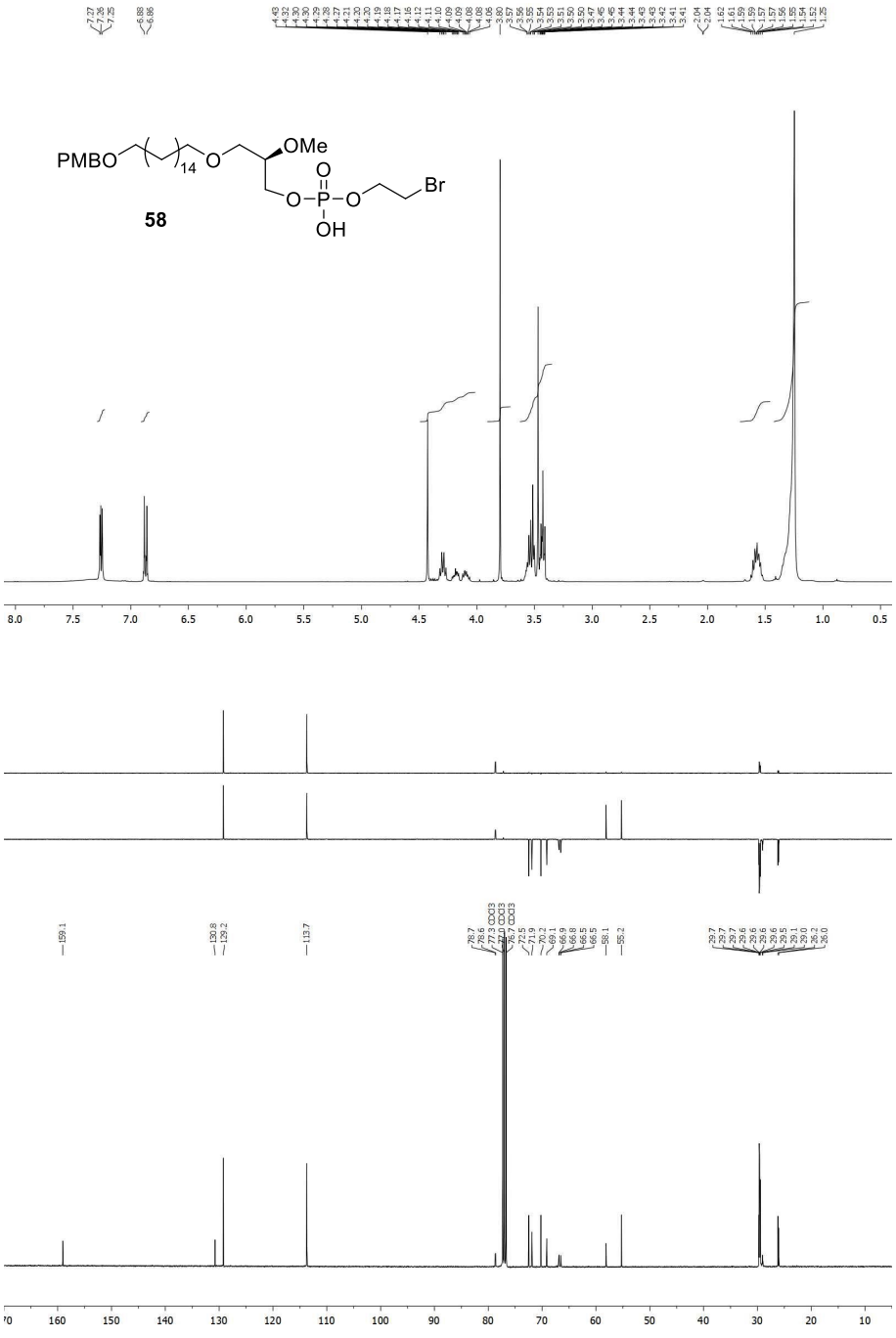

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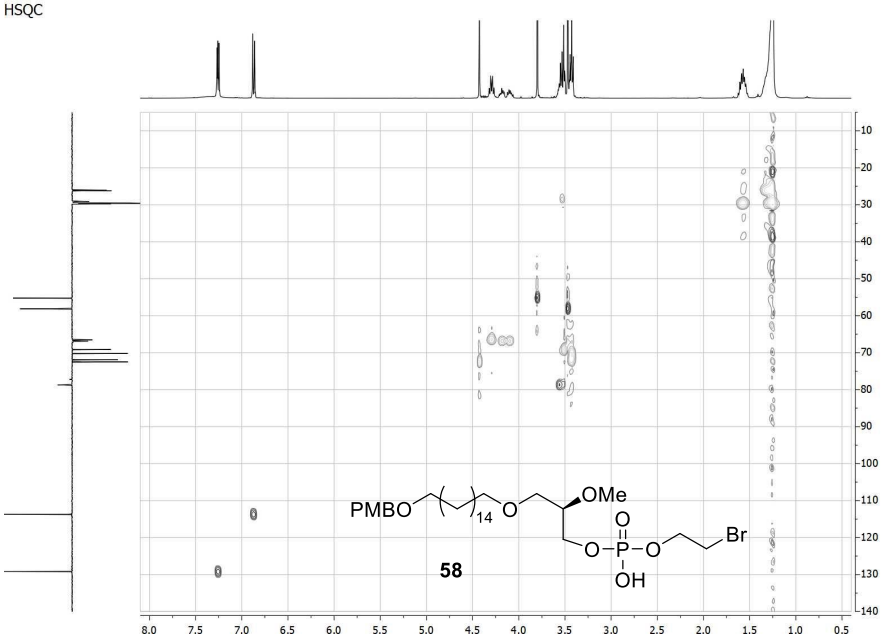

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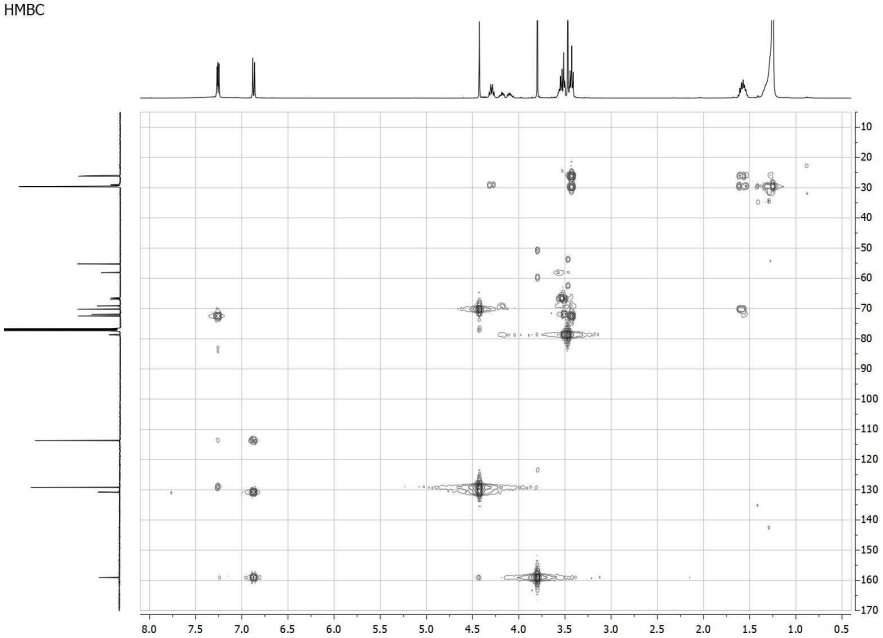

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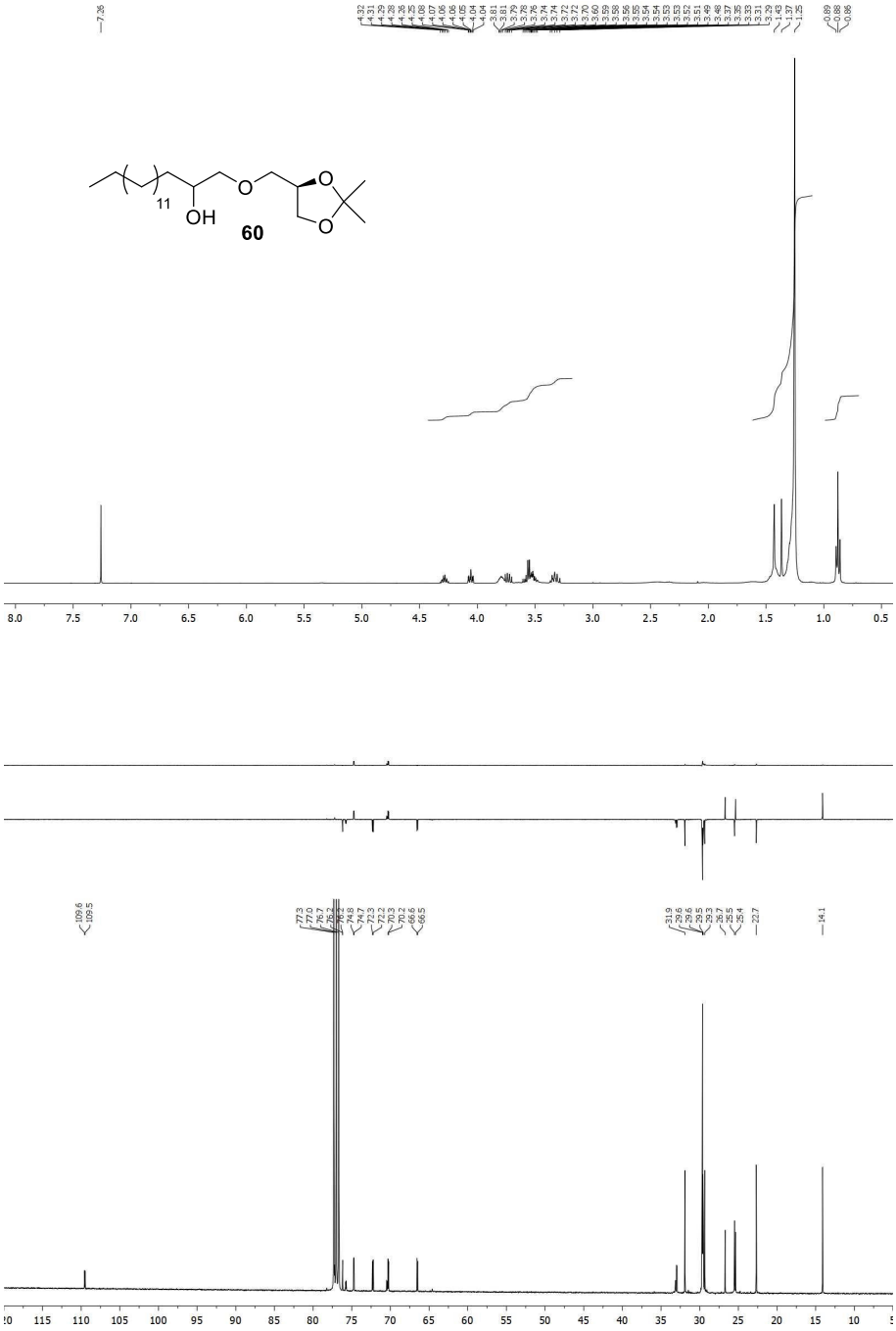

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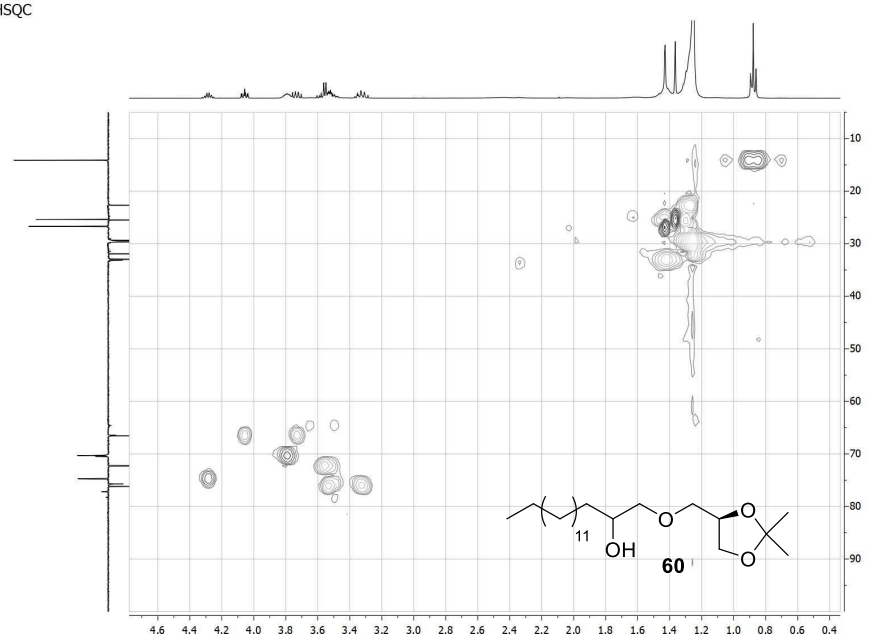

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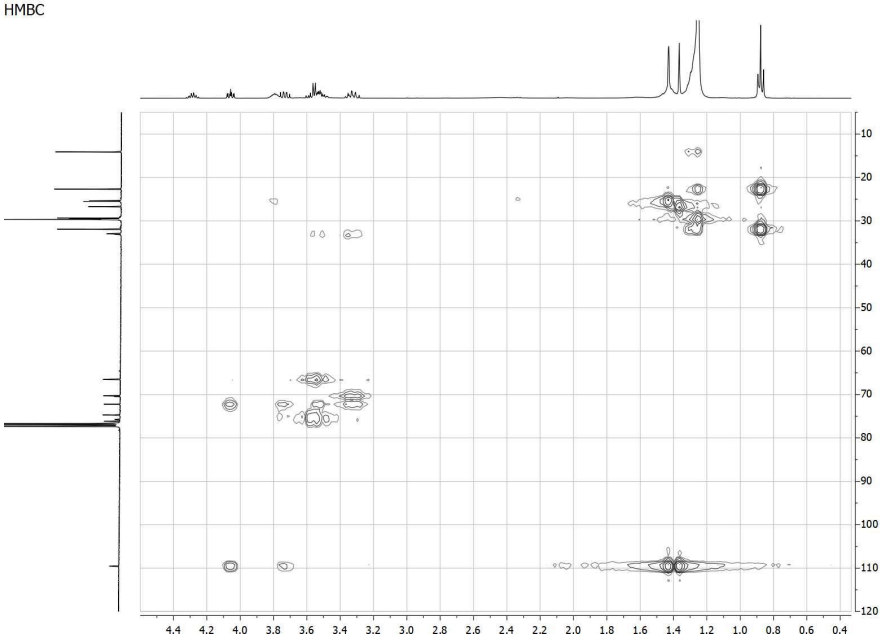

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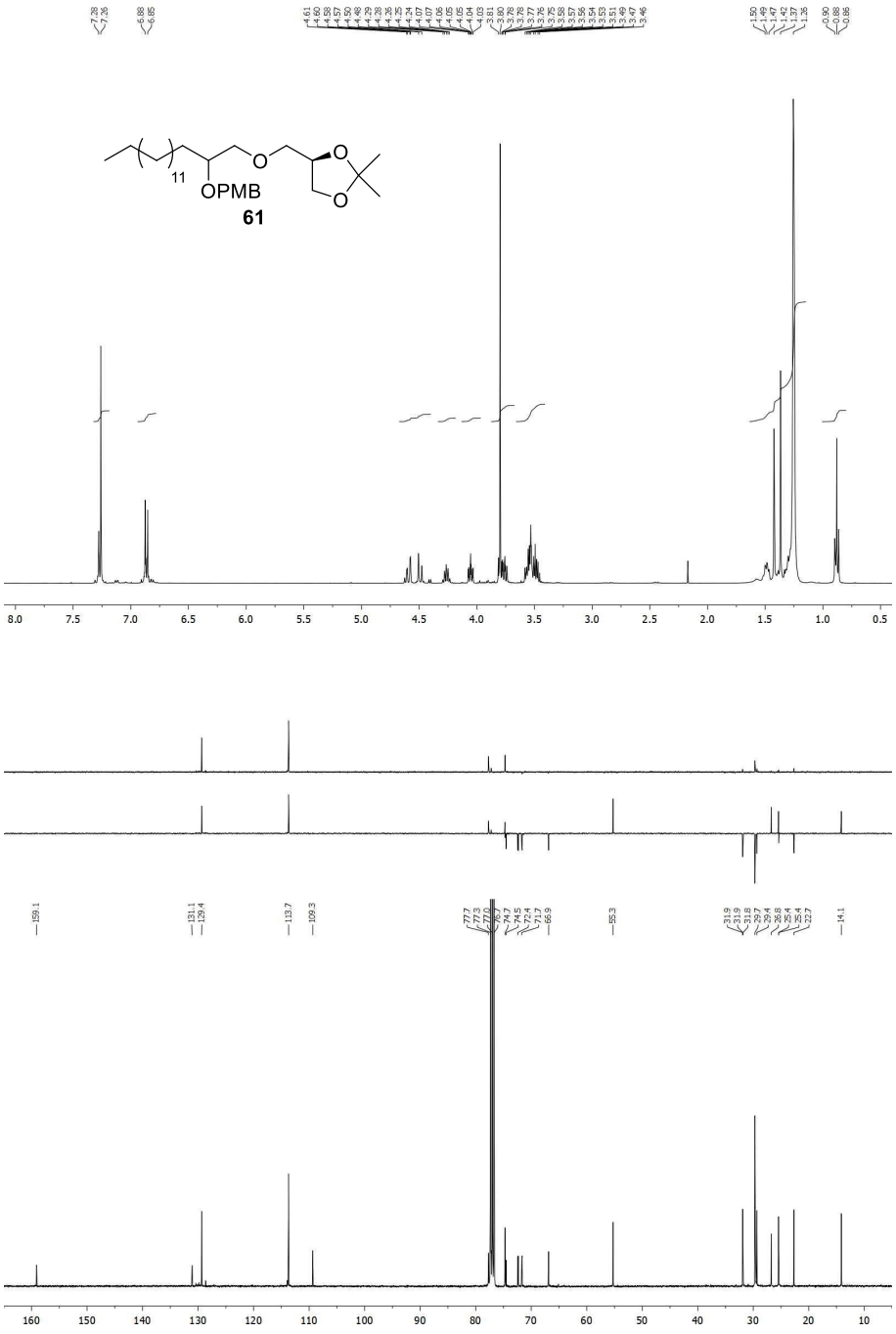

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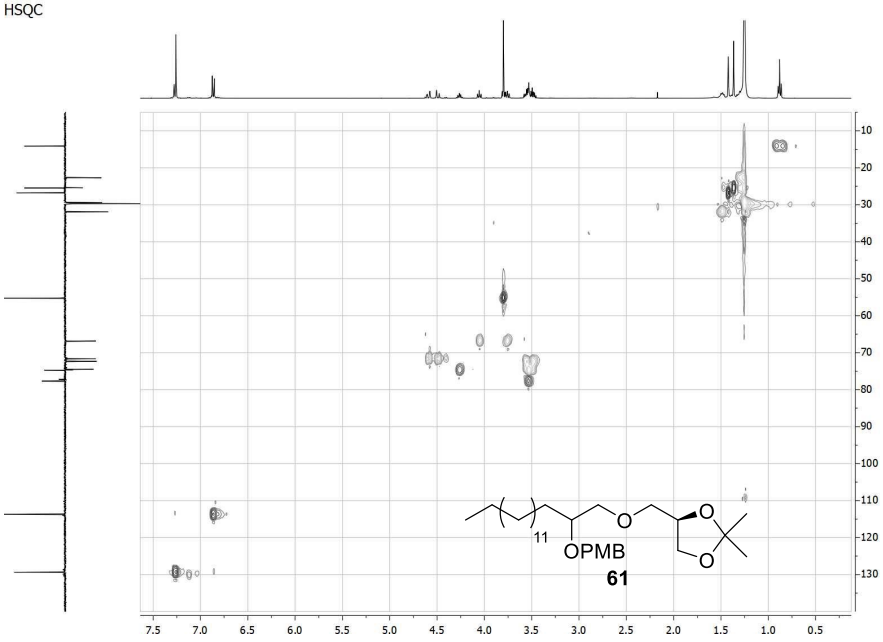

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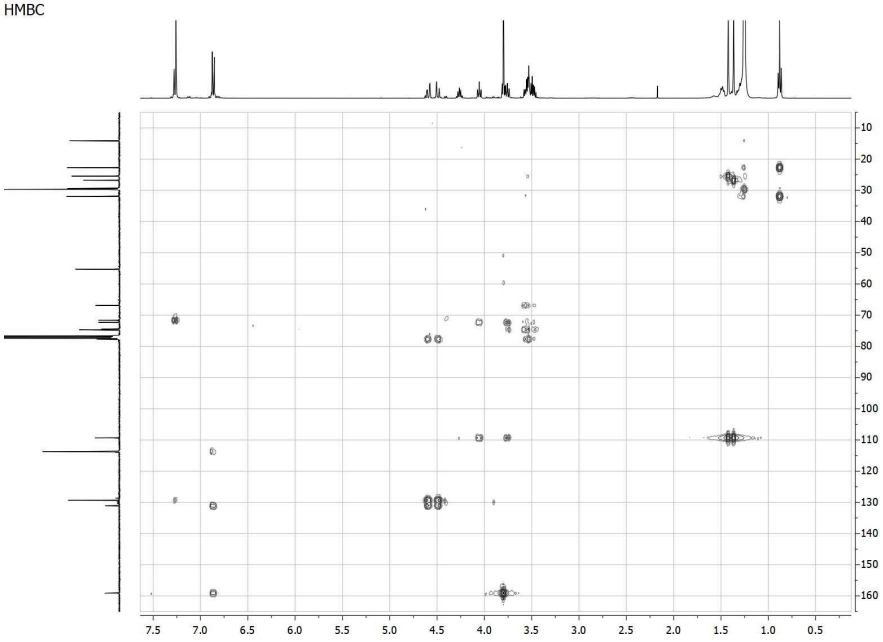

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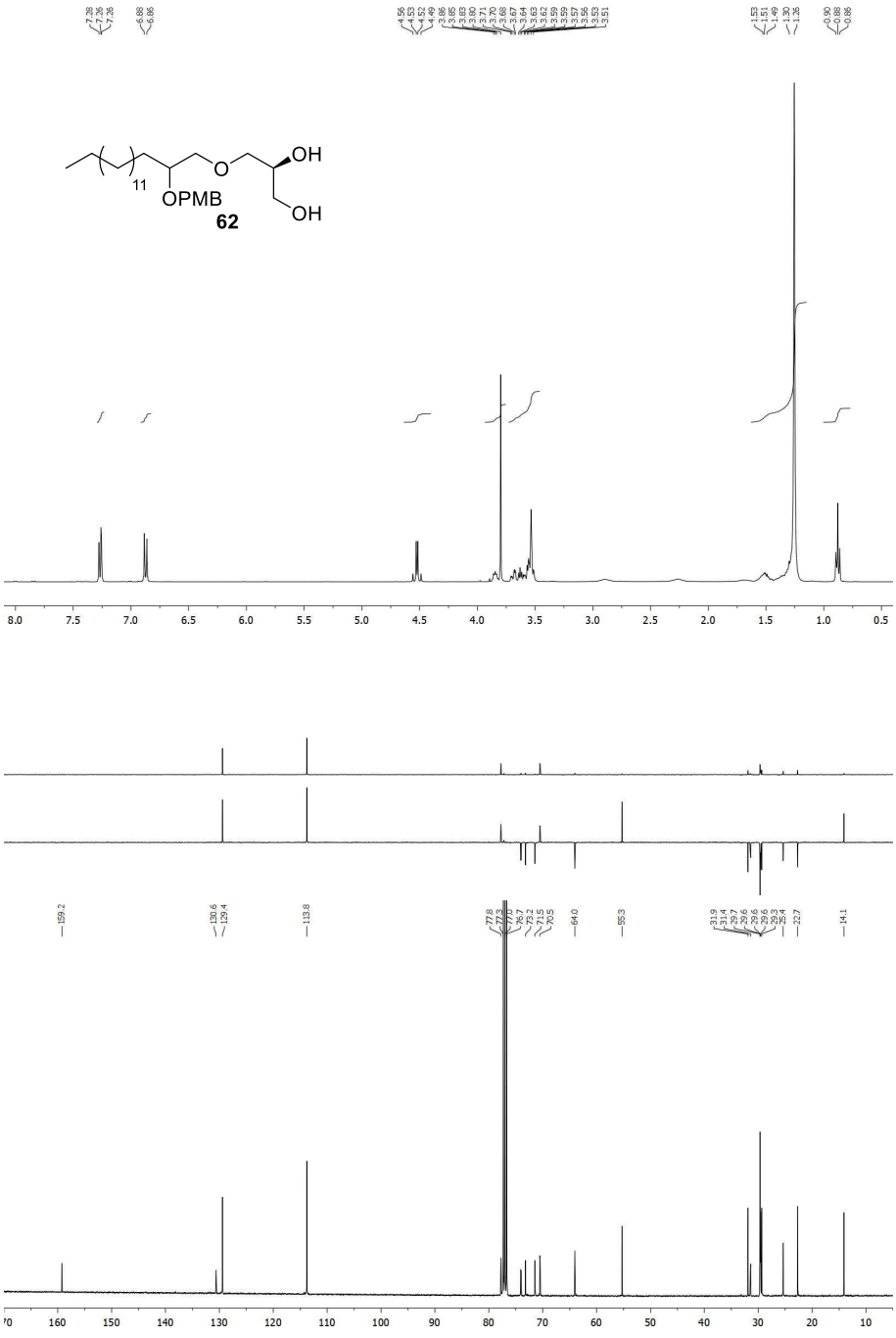

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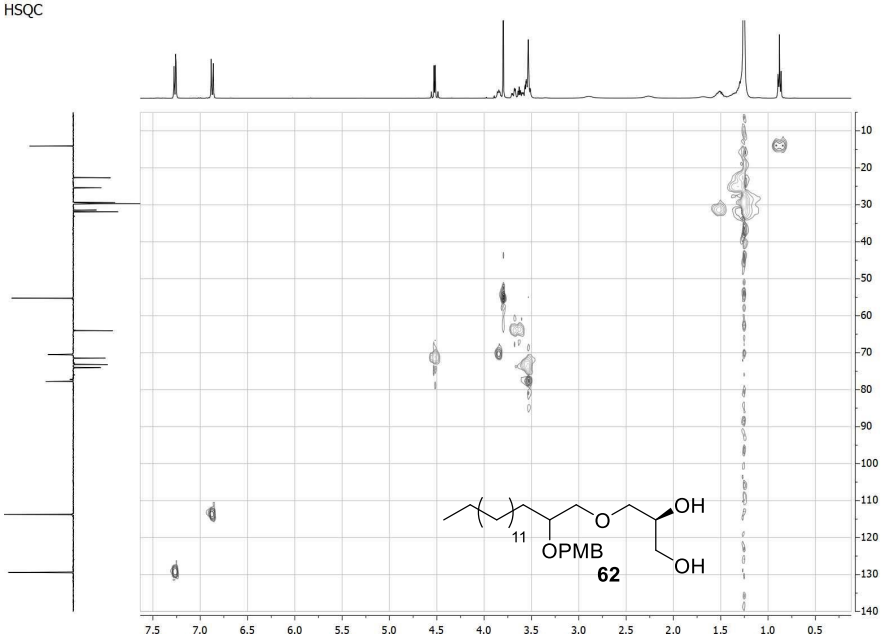

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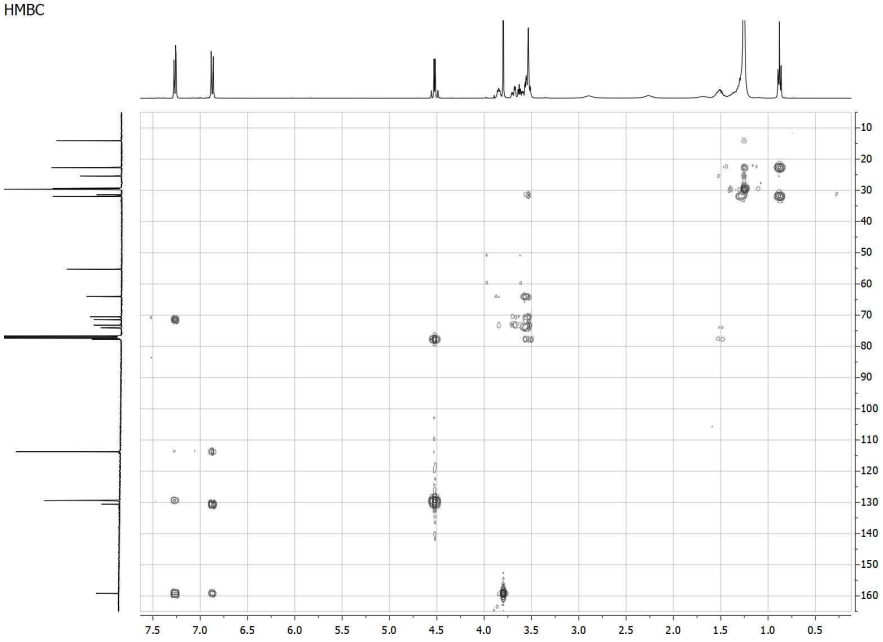

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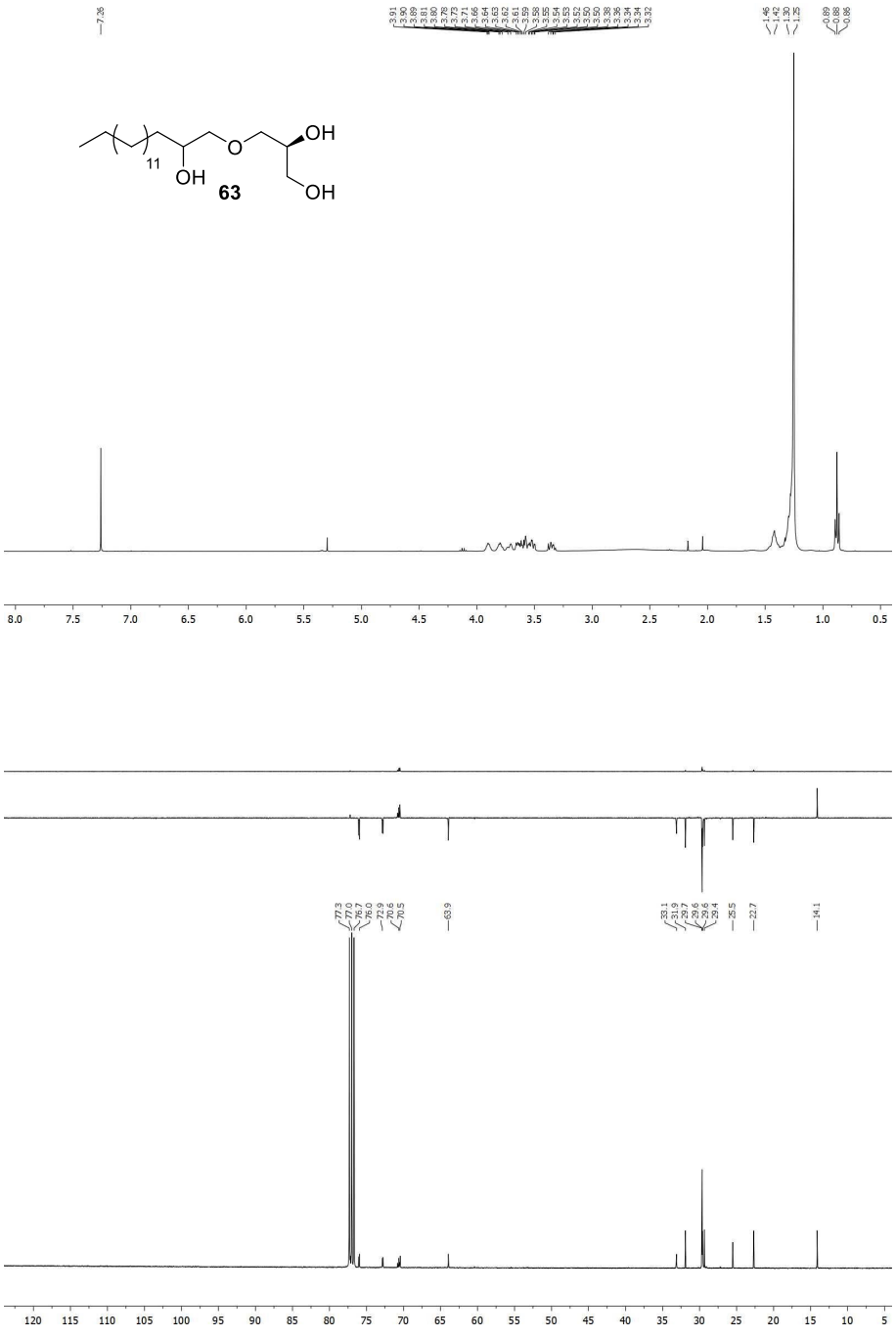

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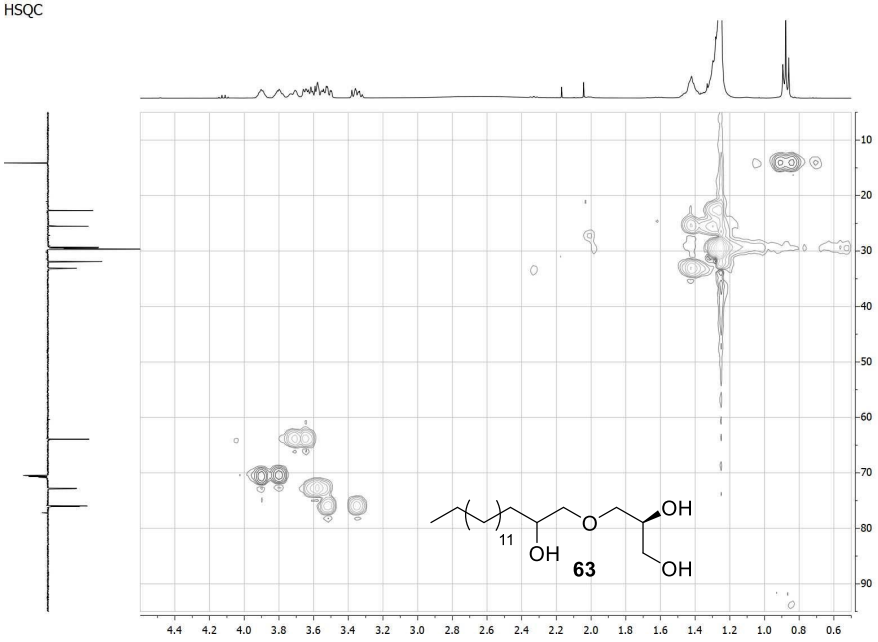

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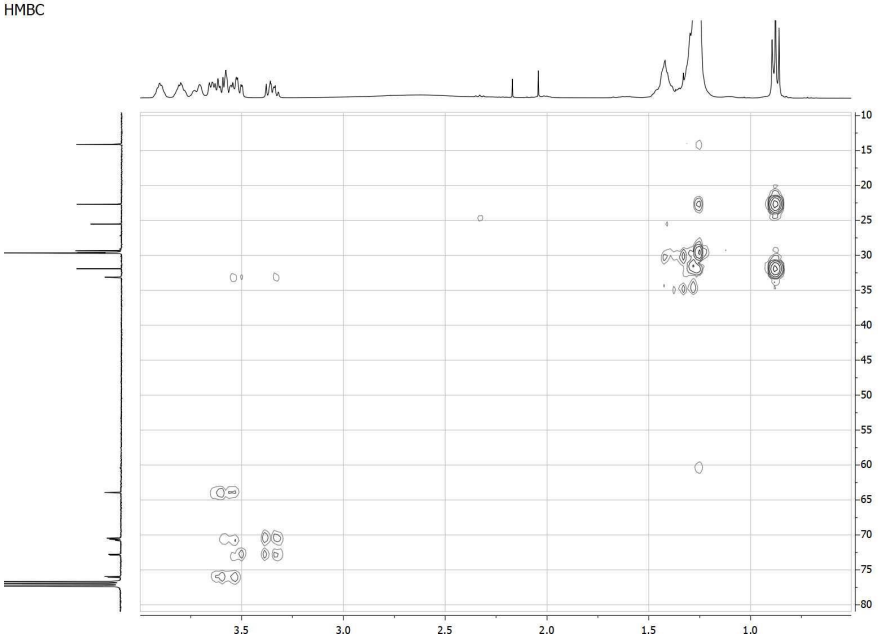

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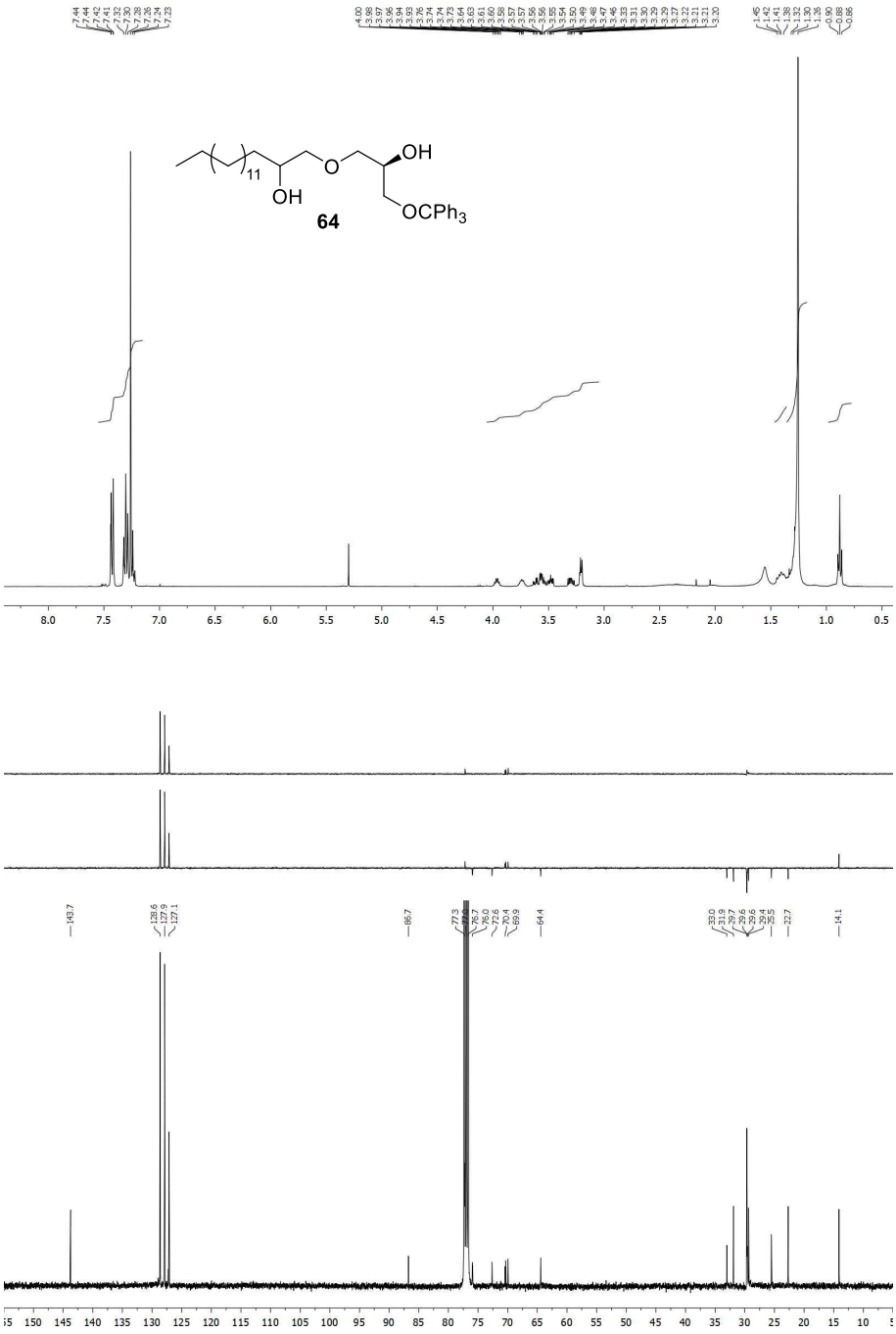

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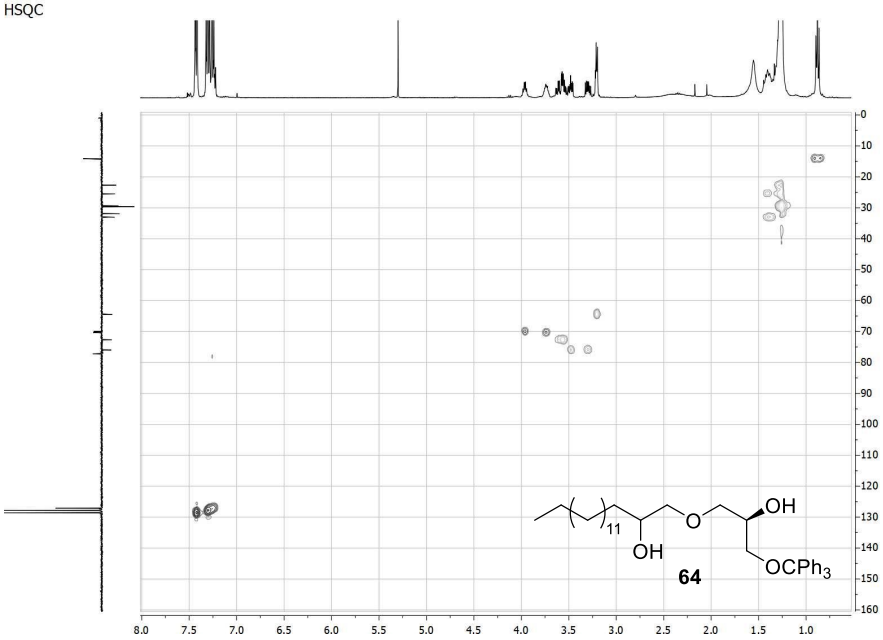

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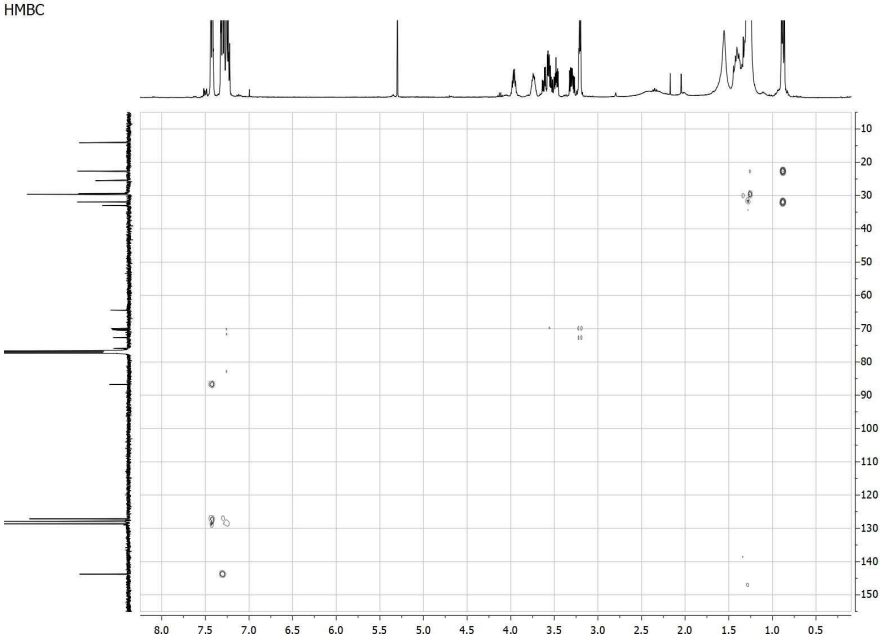

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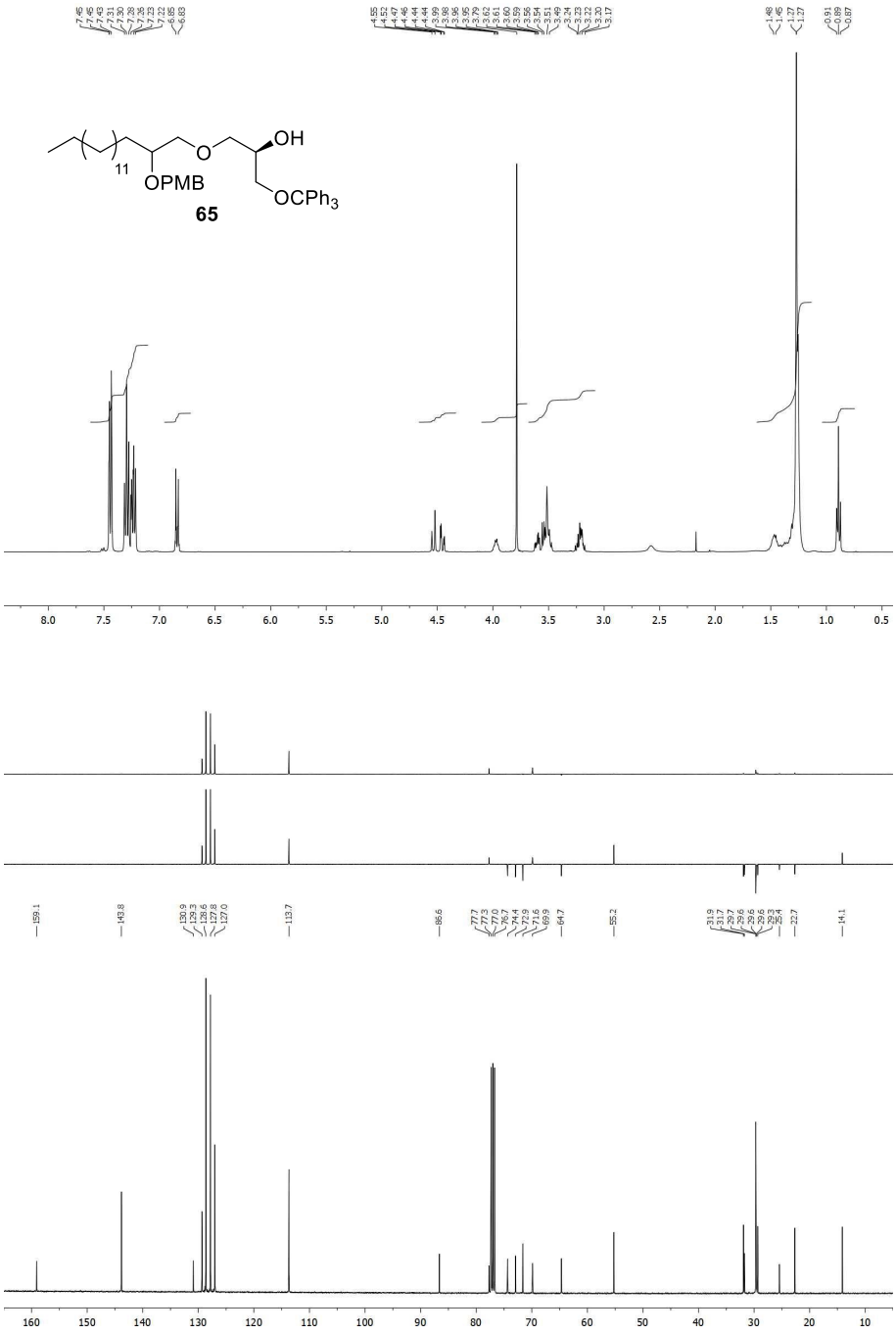

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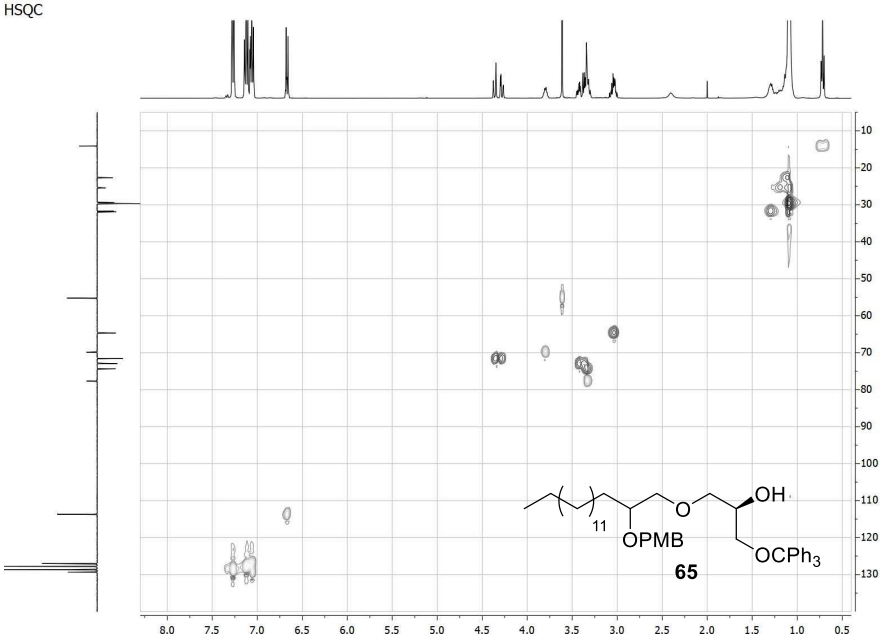

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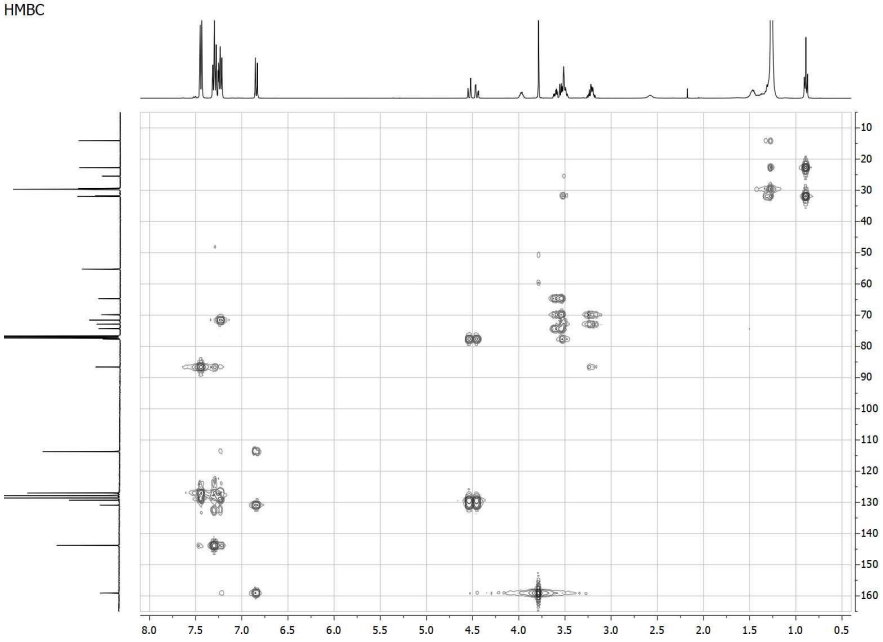

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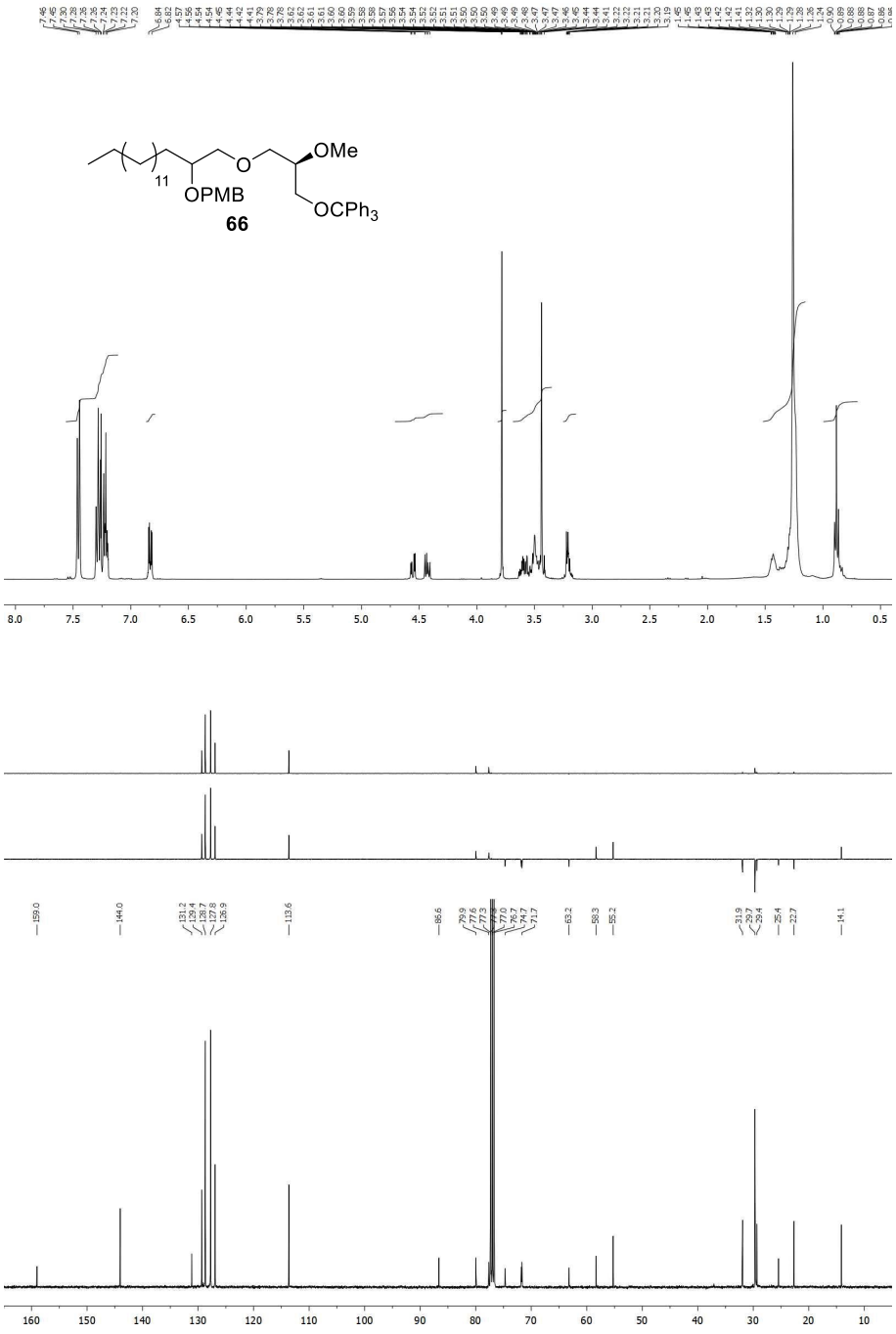

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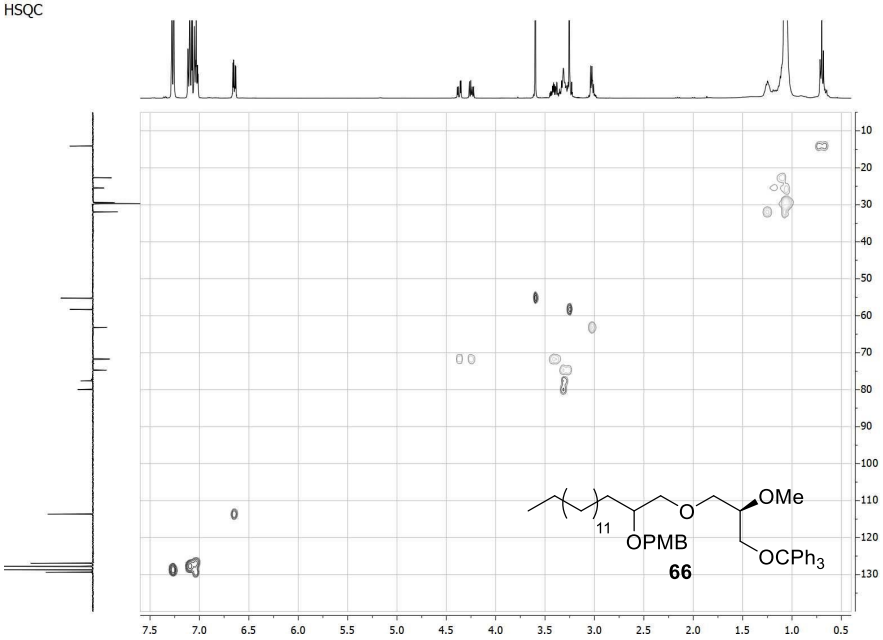

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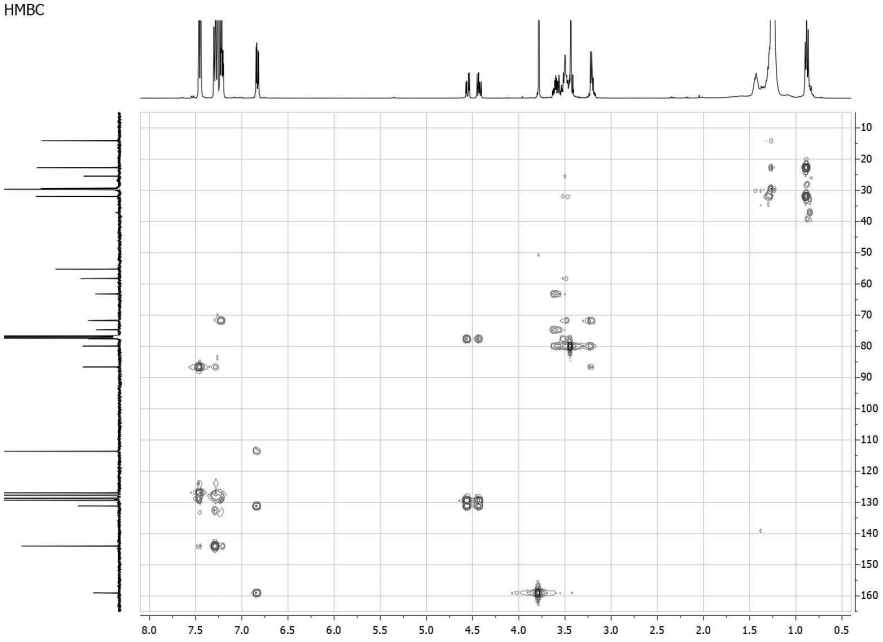

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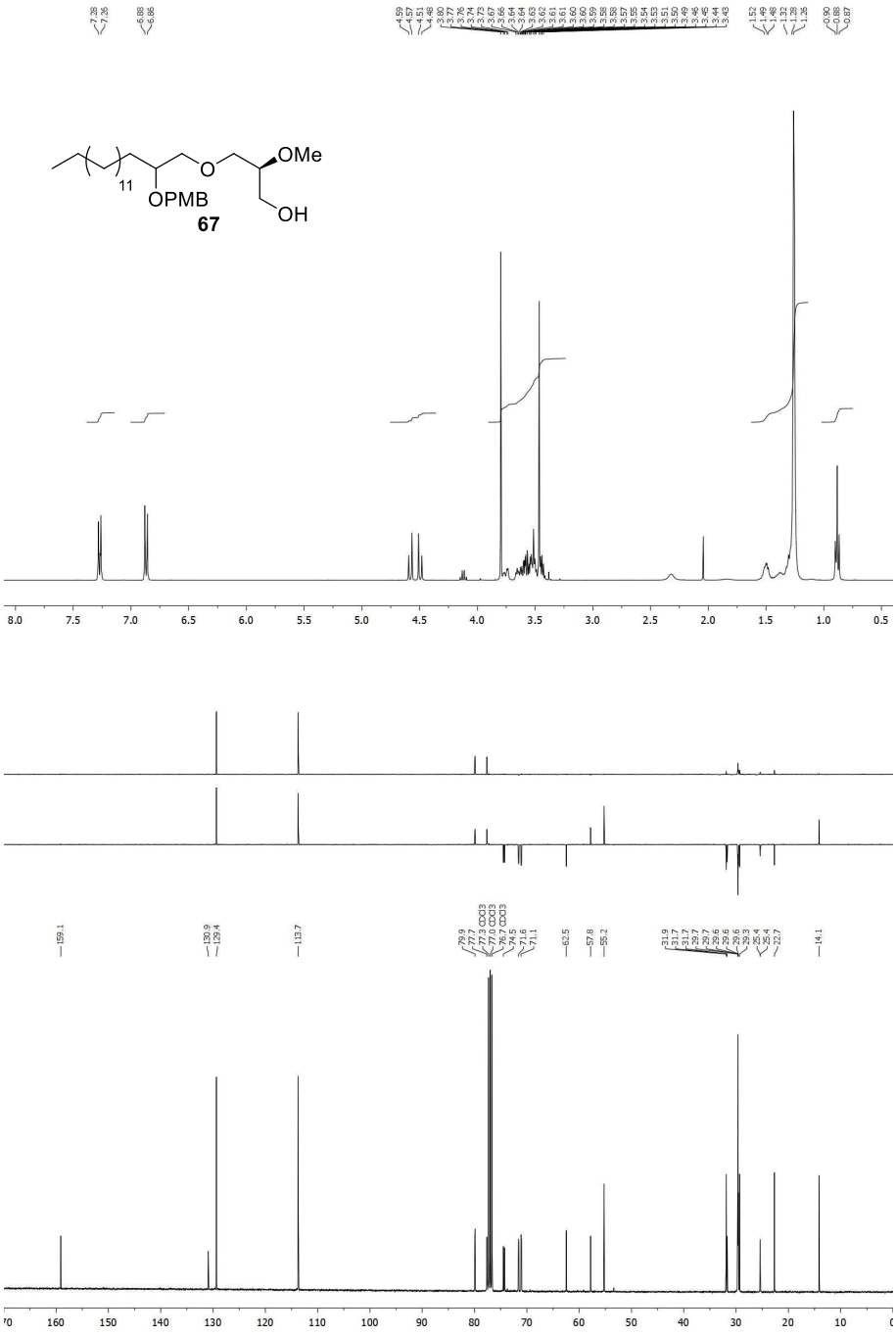

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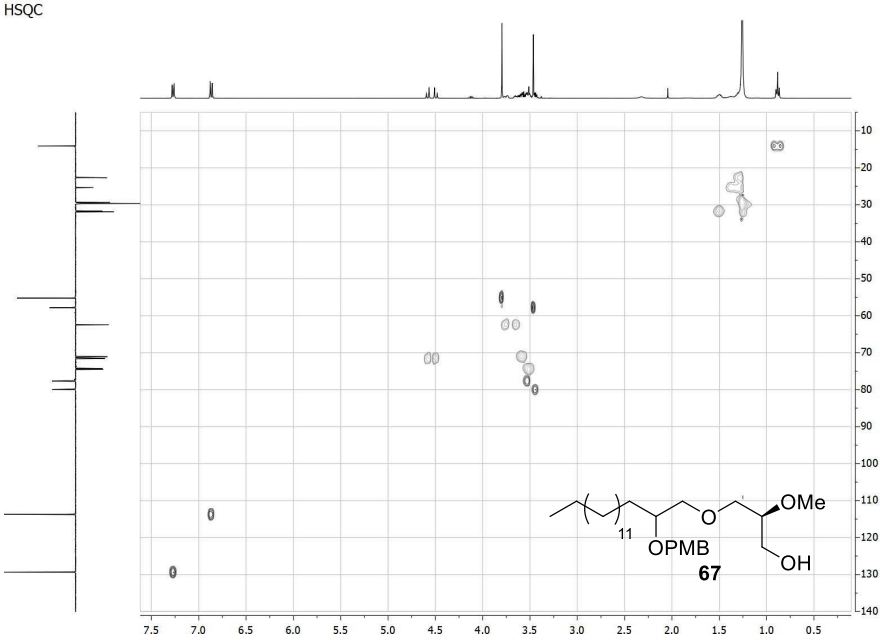

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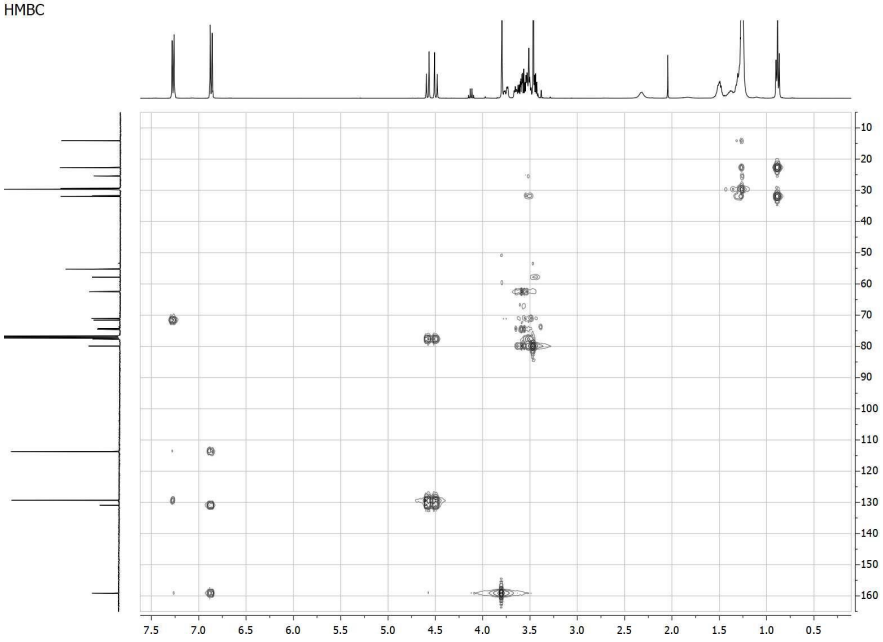

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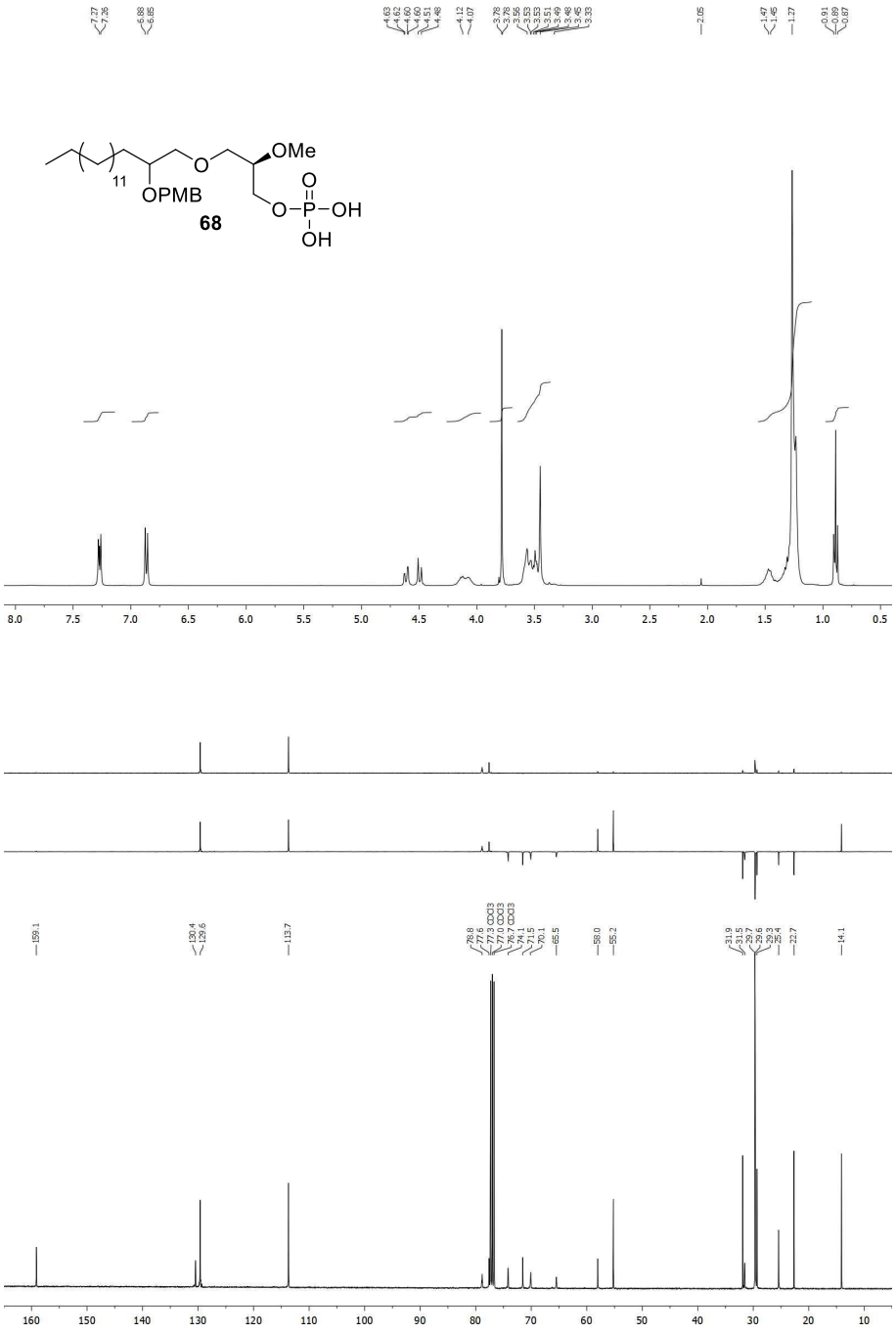

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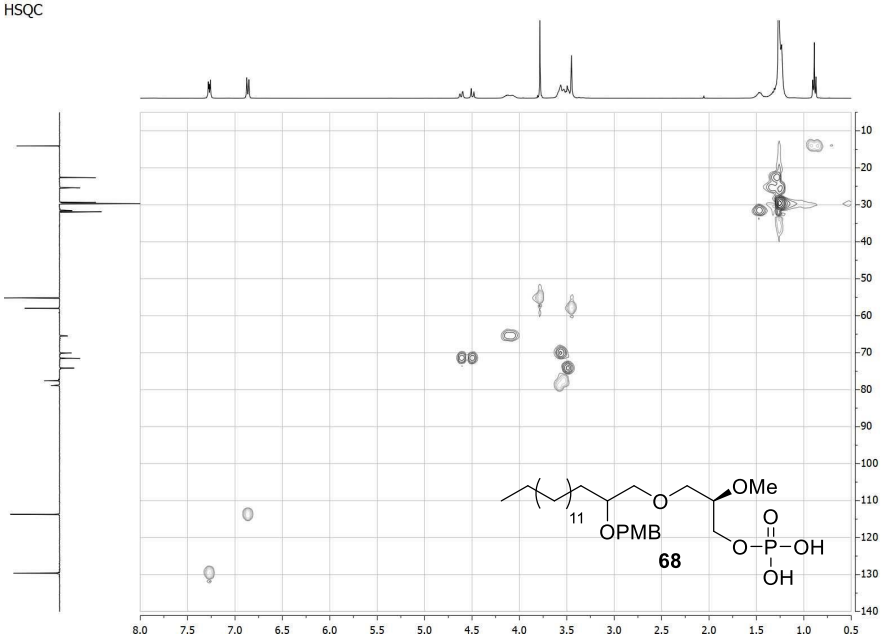

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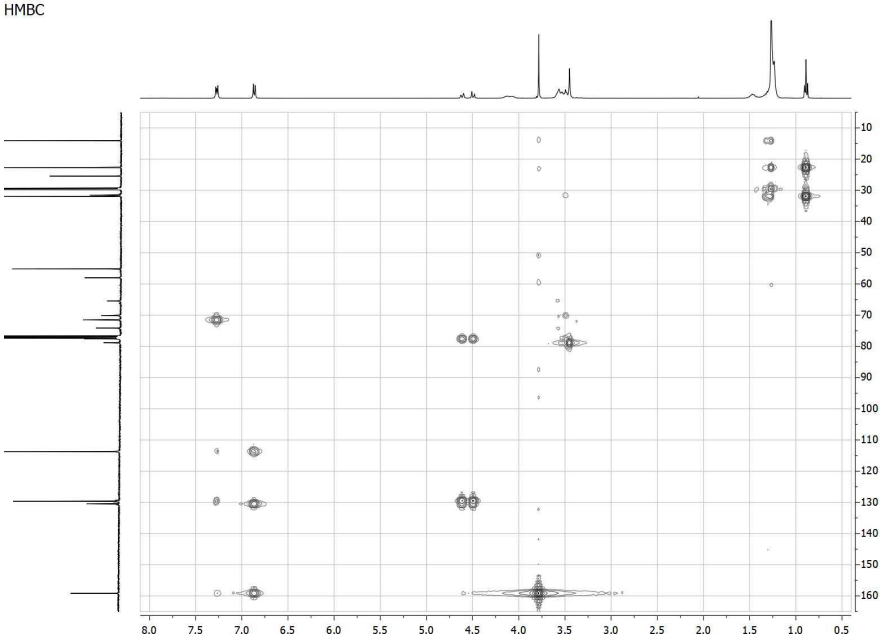

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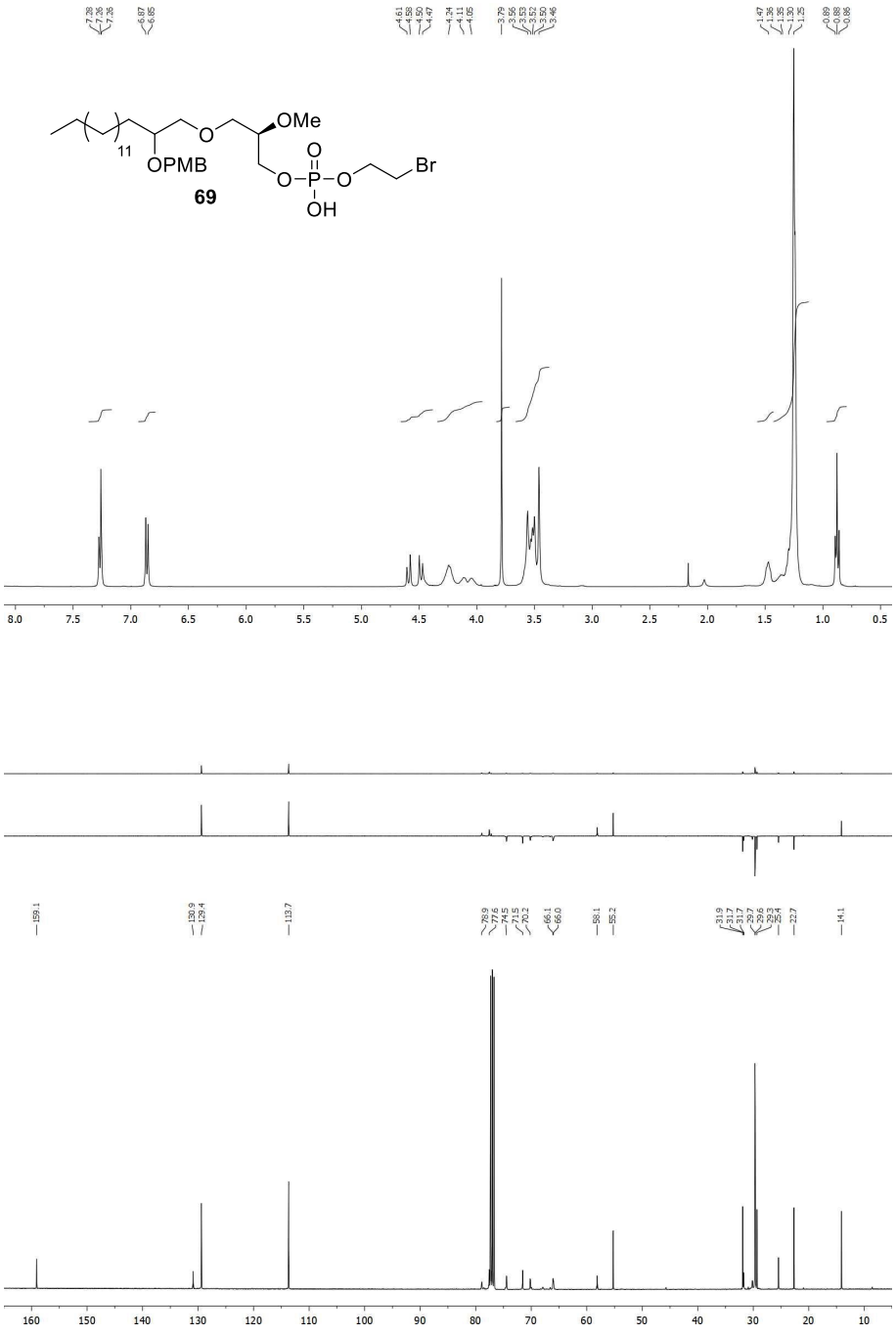

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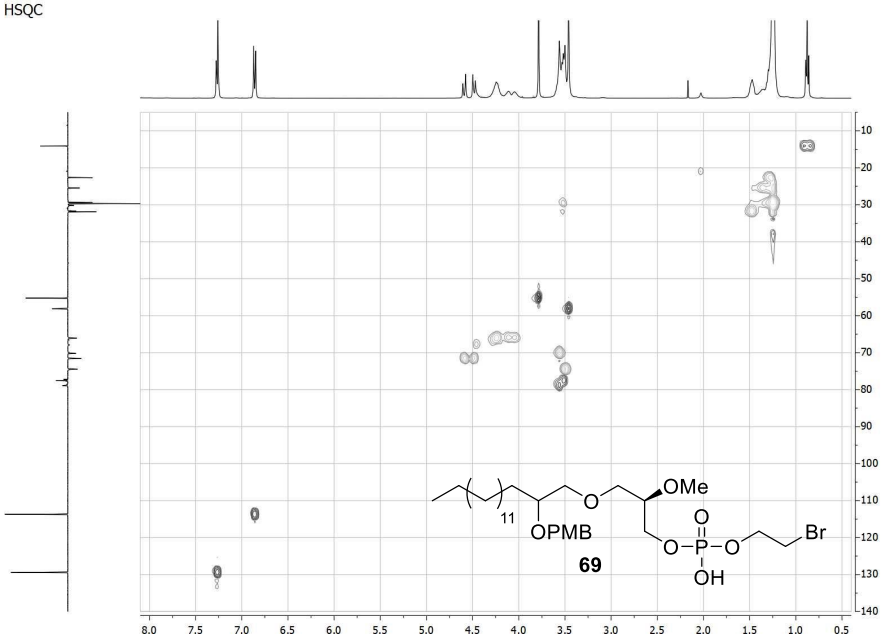

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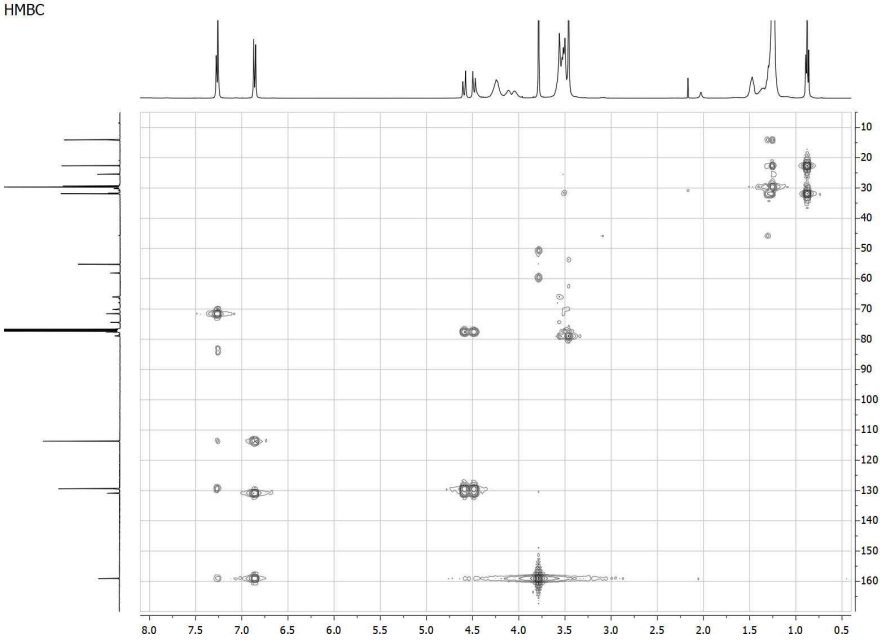

Conclusions

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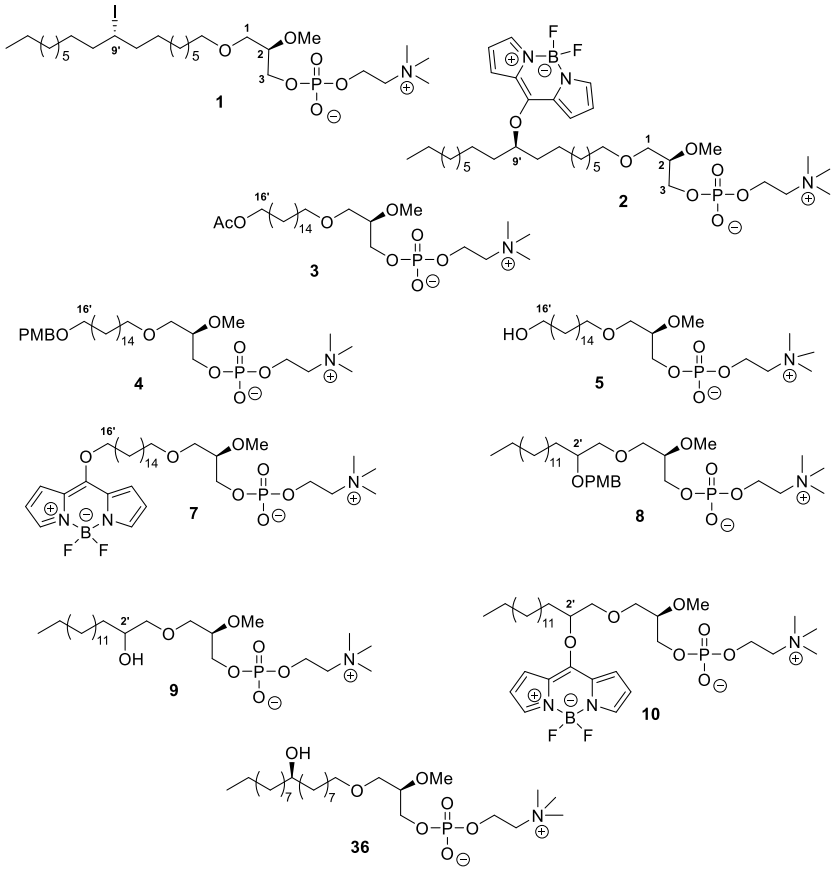
Conclusions

1. In this work, the synthesis of a series of antitumor ether lipids (AELs) analogues of edelfosine, **1-5**, **7-10** and **36**, functionalized with heteroatoms or fluorophores at the C2', C9' and C16' positions of the alkyl chain, has been carried out.



2. A bibliographic review has been performed on antitumor alkyl lipids and bioconjugate analogs.

3. The bibliographic review carried out on natural halimane skeleton diterpenes published by our group in 2018 in the journal Natural Product Reports has been updated, finding 51 new natural halimanes.

4. In fluorescent edelfosine analogs, 8-alkyloxy-BODIPY has been used as a fluorophore, which appears attached at different positions in the alkyl chain.

5. Analogs **1**, **2** and **36** synthesized contain iodine, BODIPY and hydroxyl group respectively in position C9' of the alkyl chain. All of them were synthesized using a similar sequence, in which the functionalization of the alkyl chain is first elaborated and finally the polar head is incorporated.

6. Different procedures have been tried for the incorporation of the phosphocholine fragment. The procedure of generating the intermediate phosphatidic acid, followed by reaction with choline tetraphenylborate or tosylate, gave acceptable results for analogs **1**, **2** and **3**. The methodology of preparing a 2-bromoethyl dichloro phosphate intermediate, followed by reaction with trimethylamine, produced reproducible results and with good yields in general.

7. Analogs **5** and **7**, with hydroxyl and BODIPY groups at the end of the chain (C16') respectively, turned out to be poorly lipophilic, so the synthetic sequence was inverted to carry out their synthesis. In this strategy, first the polar head of phosphocholine with C16', protected in the form of 4-methoxybenzyloxy, is incorporated to obtain **4**, then the ether is hydrolyzed and the BODIPY unit is incorporated, obtaining **5** and **7** respectively.

8. The C2' analogs (**8**, **9** and **10**) were prepared, in good yields, following the sequence of first incorporating the polar head and finally making the C2' functionalization.

9. A descriptive study of the fluorescent characteristics of the compounds derived from BODIPY (**2**, **7**, **10** and **31**) has been carried out. In phospholipids **2**, **7** and **10** there is a hypsochromic shift in their absorption maxima with respect to 8-methylthio-BODIPY **31**, but they maintain good molar extinction coefficients, in addition to fluorescence.

10. Biological tests carried out at the CIB in Madrid indicate that the response of the edelfosine analogs evaluated is specific for each cell type, since they have been more potent in the HL-60 line. In general, the hydroxyl analogue at C2', **9**, has turned out to be the most potent compared to the three lines evaluated, whose results are comparable to edelfosine itself.

11. The hydroxyderivatives **36**, **5** and **9** are found to be more active the closer the hydroxyl is to the phosphocholine unit. This is due to the loss of the amphiphilic character as the hydroxyl is in more distant positions.

12. Fluorescent analogs **2**, **7**, and **10** achieved promising results for *in vivo* tumor traceability, as they show some activity at low concentrations for the HL-60 cell line.

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Compound index

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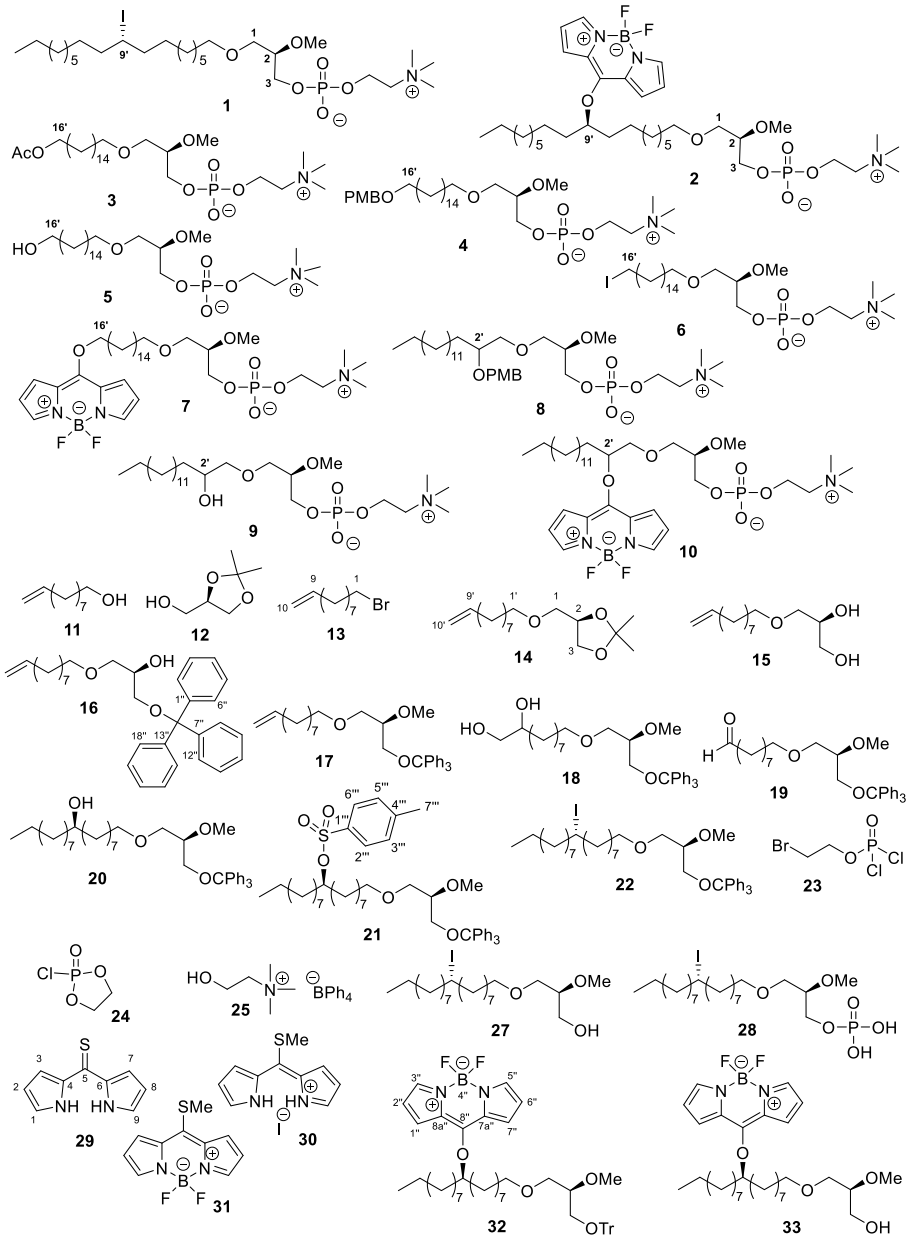

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