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Synthesis and characterization of new biocompatible amino amphiphilic compounds derived from oleic acid as nanovectors for drug delivery†

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Abstract: Amphiphilic molecules have been actively explored as promising materials in the field of bio and nanotechnology. These molecules are constituted by a polar head and a lipophilic tail and in an aqueous medium are self-assemble to form different types of macromolecular structures such as micelles, monolayer vesicles, bars, sheets and tubes. In this work, a convergent synthetic approach for the synthesis of two new amphiphilic compounds based on a versatile amino polar head, a tetraethylene glycol spacer and a lipophilic tail derived from oleic acid has been developed. Subsequently, after a self-assembly process in aqueous medium, nanostructures as micelles have been obtained and characterized. Finally, a procedure for the inclusion of the highly lipophilic drug Dexamethasone has been carried out in order to study the ability of these micelles to act as nanovectors for drug delivery.

Keywords: Amphiphilic compounds, self-assembly, nanovectors, drug delivery

1. Introduction

In the last years, amphiphilic molecules have been highly used in the development of nanostructures, representing a great promise for targeted delivery, improved bioavailability, and drugs controlled release[1], [2], [3], [4], [5]. These molecules consist of a polar head and a lipophilic tail that are distributed in an aqueous medium to form different types of structures such as micelles, monolayer vesicles (also known as liposomes), bars, sheets and tubes[6]. The cell membrane of living cells, formed by a bilayer self-organization is the most illustrative example of a complex nanosystem formed from units of phospholipid. Among the different types of structures formed by amphiphilic compounds, micelles have received growing scientific attention[7]. Micelles, in general, are self-assembled particles composed of amphiphilic compounds[8]. In an aqueous environment, these compounds are distributed with the hydrophobic tails in the centre of the micelle, and the polar heads towards the aqueous medium. In this way, they auto-assemble to form spheroidal structures with a hydrophilic shell and a hydrophobic core to minimize the contact of the hydrophobic segments with the aqueous environment by allowing a good grade of stability[9]. CMC, critical micelle concentration, is a relatively small range of concentrations separating the limit below which virtually no micelles are detected and the limit above which virtually all additional amphiphilic molecules form micelles. Generally, polymeric micelles show very low CMC values in a range from 1 μg/ml to 10 μg/ml [10], [11], [12].

Micelles have a particle size between 10 to 100 nm that is important to allow a high stability and a high bioavailability. This size makes it possible to inject these micelles into systemic circulation without risk of blood vessel blockage. The fate *in vivo* of micelles depends on their sizes, particles

under 200 nm are less phagocytosed by macrophages after the opsonization, compared to those with larger dimensions [13].

One of the advantages of using micelles in drug delivery is their ability to transport drugs with different degrees of polarity thanks to their structure consisting in a hydrophilic shell and a hydrophobic core.

Drugs will be distributed differently by chemical conjugation, physical entrapment or ionic interactions depending on the nature of the drug and the amphiphilic compound properties: Hydrophilic drugs will bind to the surface (case 1), those with different hydrophilicity and lipophilicity ratios will be between the polar part and the lipophilic part of the nanosystem (case 2-3-4), and finally very lipophilic drugs will be distributed inside the micelle core (case 5) (Figure 1) [14].

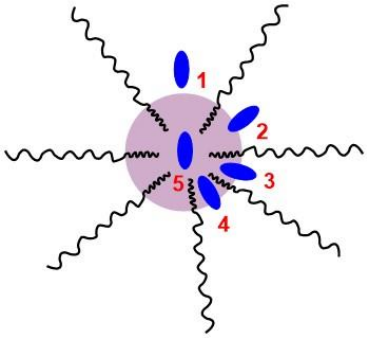


Figure 1. Possible pattern of drug association with a micelle. [14]

It is known that about 90% of drugs are lipophilic and are characterized by a low solubility in water this causes a difficult distribution and accumulation in fatty tissues leading to a delayed release of the drug and an increase of side effects. Micelles are therefore used for the transport of highly lipophilic drugs, increasing the solubility of drugs from 10 to 8400 times and consequently their bioavailability [15], [16].

One of the most successful examples of micellar formulation as alternative solubilizing agents is the formulation of paclitaxel (PTX) in a poly (*D, L*-lactide) MePEG diblock copolymer which increases the solubilized PTX levels in water around 5000 times [17].

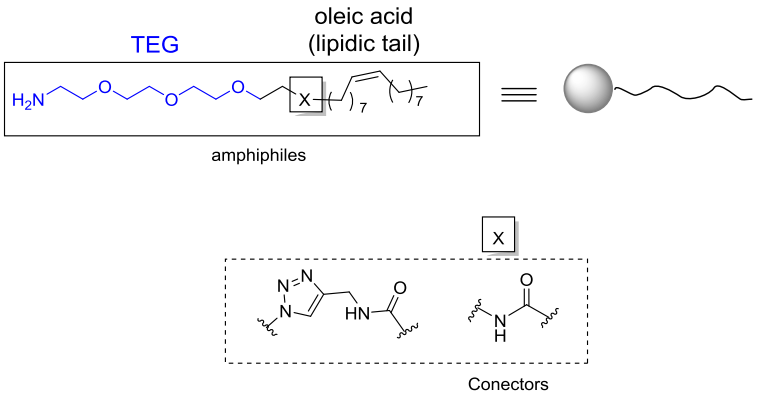
Micelles can also be used in active targeting, directing the drug towards the specific cell-tissue-organ. Ligands such as carbohydrates [18], folic acid [19], antibodies [20], proteins [21], peptides [22] and aptamers [23] have been used.

In summary, the main advantages of micelles in drug delivery are the following:

i) high dynamic stability that permits their application *in vivo*
ii) the hydrophobic core of micelles confers them the ability to transport highly lipophilic drugs
iii) the hydrophilic shell of micelles increases their solubility in water, resulting in greater bioavailability and lower toxicity for poorly water-soluble drugs.
iv) the possibility of modifying their surface with specific ligands confers the ability to direct drugs to specific targets.

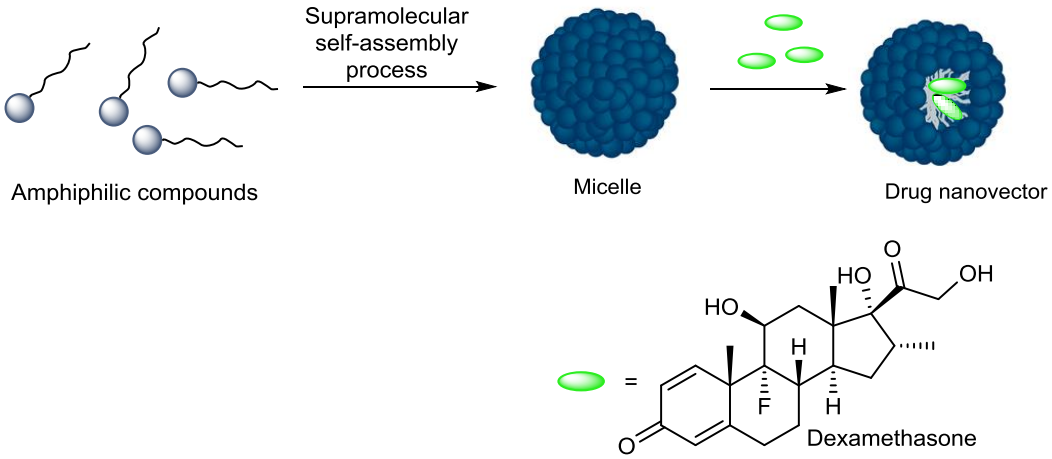
This work is placed in the field of nanotechnologies applied to drug delivery and specifically focused on the synthesis of amphiphilic compounds in order to obtain a new family of micelles as drug nanovectors within the organism.

Both of these amphiphilic compounds synthesized present a versatile amino polar head, a spacer and a lipophilic tail. The spacer in all cases is tetraethylene glycol, a polymer derived from polyethylene glycol which presents two important advantages: i) an adequate hydrophilic-hydrophobic balance for the optimal formation of the micelle. ii) the ability to avoid the activation of the immune system. It has been discovered that PEG derivatives are biocompatible they are not attacked by macrophages escaping the opsonization. Furthermore, the amphiphilic compounds synthesized present oleic acid as the lipophilic tail (Scheme 1).



Scheme 1. General structure of the amphiphilic compounds.

Micelles have been obtained in water by a self-assembly process of the amphiphilic compounds synthesized. After the characterization of micelles, the internalization of ***Dexamethasone***, a synthetic anti-inflammatory corticosteroid highly lipophilic drug [24], has been studied in order to verify the advantages of the use of our micelles in drug delivery (Scheme 2).



Scheme 2. Formation of micelles by supramolecular self-assembly of amphiphilic compounds and internalization of Dexamethasone.

2. Method

2.1. Materials and techniques

Unless otherwise stated, the starting materials, reagents, and solvents were purchased as high-grade commercial products from Sigma-Aldrich. THF, CH_2Cl_2, DMF and Toluene were dried using molecular sieves, and highest quality solvents were used. All non-aqueous reactions were performed under an argon atmosphere in oven-dried glassware.

Analytical TLC was run on silica gel plates supported by alluminio Alufram® Sil.G / V245 Merck di 0,25 nm. Plates eluted and dried with 5% of phosphomolybdic acid in ethanol.

Flash chromatography was performed on glass column using silica gel type 60 (particle size 230-400 mesh, Merck). The composition of the eluent used is different for for each compound.

1H- and 13C- spectra were recorded on a Bruker AMX-500 e Bruker Advance DRX-500 (500 MHz) instrument at rt at the centre of Research, Technology and Innovation of the University of Seville's NMR core facility. Chemical shifts (δ) are expressed in parts per million relative to the residual solvent peak for 1H and 13C nucleus (acetone-d6: δH = 2.05, δC = 29.84; CDCl3: δH = 7.26, δC = 77.16; DMSO-d6: δH = 2.50, δC = 39.52; methanol-d4: δH = 3.31, δC = 49.00); coupling constants (J) are in hertz (Hz). The following abbreviations are used to describe peak patterns when appropriate: s (singlet), d (doublet), t (triplet), q (quartet), sext (sextuplet), m (multiplet), app (apparent), and br (broad).

High resolution mass spectrometry (HRMS) was carried out on a Kratos MS-80-RFA spectrometer and in a AutoSpec micro-mass spectrometer at the centre of Research, Technology and Innovation of the University of Seville.

2.2. Synthesis of the amphiphilic derivatives

2.2.1. Synthesis of the first amphiphilic derivative.

1,11-mesyl-3,6,9-trioxaundecane (1)

To a solution of tetraethylene glycol (8.90 mL, 15.49 mmol) in dry THF (200 mL) under argon atmosphere, and Et_3N (17.9 mL, 128.71 mmol) was added MsCl (9.96 mL, 128.71 mmol) drop by drop at 0°C and was stirred for 1 h. After this time the mixture was allowed to warm slowly at room temperature. Then the solvent was evaporated and the mixture was dissolved in CH_2Cl_2 (50 mL) and washed 3 times with NH_4Cl (3x15 mL), afterwards was neutralized with $NaHCO_3$ (15 mL) and washed with brine (15mL). The organic extract was dried over anhydrous Na_2SO_4, filtered, and evaporated to obtain the product **1** as a yellowish oil (5.42 g, 15.48 mmol, 99.97%).

***Rf* (CH$_2$Cl$_2$/MeOH 9:1):** 0.62

^{1}H NMR (500 MHz, CDCl$_3$): δ 4.383-4.365 (m, 4H, $CH_2C\underline{H}_2OMs$), 3.776-3.677 (m, 4H, $C\underline{H}_2CH_2OMs$), 3.671-3.633 (m, 8H, $OC\underline{H}_2C\underline{H}_2O$), 3.066 (s, 6H, $C\underline{H}_3$).

^{13}C NMR (125.7 MHz, CDCl$_3$): δ 70.752, 70.620 ($\underline{C}H_2O$), 69.331, 69.128 ($\underline{C}H_2CH_2OSO_2$), 52.689 ($\underline{C}H_2OSO_2$), 37.769 ($OSO_2\underline{C}H_3$).

HRMS calcd for C$_{10}$H$_{22}$O$_9$S$_2$ [M+H]$^+$: 351.0778; found 351.0778.

1,11-diazido-3,6,9-trioxaundecane (2)

To a solution of **1** (18,80 g, 53.66 mmol) in dry EtOH (35,8 mL) was added sodium azide (8.72 g, 134.15 mmol). The mixture was allowed to reflux during 24h, under argon atmosphere and after this time, was added NaCl (50 mL) to deactivate the sodium azide. Then the solvent (EtOH) was removed by rotary evaporation. Successively the mixture was extracted with CH_2Cl_2 in order to obtain the product in the organic phase, and after all, was evaporated the solvent. Then the crude product was purified by flash chromatography column on silica gel with AcOEt: Hexan (1:2), to yield **2** as a yellowish oil (11,9 g, 48,71 mmol, 91%).

***Rf* (AcOEt/Hexan 3:1):** 0.58

^{1}H NMR (500 MHz, CDCl$_3$): δ 3.694-3.654 (m, 12H, $OC\underline{H}_2C\underline{H}_2O$ and $C\underline{H}_2CH_2N_3$), 3.386 (t, *J*=5 Hz, 4H, $C\underline{H}_2N_3$).

^{13}C NMR (125.7 MHz, CDCl$_3$): δ 70.856 ($\underline{C}H_2O$), 70.162 ($\underline{C}H_2CH_2N_3$), 50.846 ($\underline{C}H_2N_3$).

HRMS calcd for C$_8$H$_{16}$N$_6$O$_3$Na [M+Na]$^+$: 267.1176; found 267.1179

11-azido-3,6,9-trioxaundecan-1-amine (3)

To a solution of **2** (7,83 g, 32,04 mmol) in HCl 1M (96,10 mL) and ethyl acetate (56,91 mL) at 0 °C, was added dropwise a solution of triphenylphosphine (9,24 g, 35,24 mmol) in ethyl acetate (85,36 ml). Afterwards, the temperature was allowed to reach room temperature and stirred over 7 hours. The mixture was separated in a separatory funnel, and in the acqueous phase was added NaOH until PH >14 (5 mL). Successively was added CH_2Cl_2 (30 mL) and separated the organic phase which was dried over anhydrous Na_2SO_4. Later the solvent was removed by rotary evaporation and the product **3** (4,70 g, 21,52 mmol, 72%) was isolated by a flash chromatography column on silica gel eluting with AcOEt: Hexan (1:1).

***Rf* (AcOEt/Hexan 1:1):** 0

^{1}H NMR (500 MHz, CDCl$_3$): δ 3.687-3.619 (m, 10H, $OC\underline{H}_2C\underline{H}_2O$ and $C\underline{H}_2CH_2N_3$), 3.515 (t, *J*=5 Hz, 2H, $C\underline{H}_2CH_2NH_2$), 3.388 (t, *J*=5 Hz, 2H, $C\underline{H}_2N_3$), 2.881-2.861 (m, 2H, $C\underline{H}_2NH_2$), 1.806 (s, 2H, $N\underline{H}_2$).

^{13}C NMR (125.7 MHz, CDCl$_3$): δ 73.387 ($\underline{C}H_2CH_2NH_2$), 70.851, 70.801, 70.772, 70.422, 70.162 ($\underline{C}H_2O$) 50.841 ($\underline{C}H_2N_3$), 41.857 ($\underline{C}H_2NH_2$).

HRMS calcd for C$_8$H$_{18}$N$_4$O$_3$ [M+H]$^+$: 219.1452; found 219.1447

N-(2-propin-1-yl) oleamide (4)

A solution of propargylamine (0.15 mL, 2.34 mmol) and dimethylaminopyridine (0.066 g, 0.54 mmol) in dry CH_2Cl_2 (4 mL) was added to a solution of oleoyl chloride (0.50 g, 1.8 mmol) and diisopropylcarbodiimide (0.4 mL, 2.7 mmol) in dry CH_2Cl_2 (4 mL), under argon atmosphere and was stirred overnight. Afterwards the reaction was dissolved in CH_2Cl_2 then was treated with HCl 4N (2x7 mL), neutralized with $NaHCO_3$ (7 mL) and finally washed with brine (7 mL). The organic phase was dried over anhydrous Na_2SO_4, and the solvent evaporated to afford the crude product. To obtain the pure product **5** (0.505g, 1.34 mmol, 57%), as a white solid, the crude was purified with chromatography column on silica gel using AcOEt: Hexan (1:5).

***Rf* (Hexan/AcOEt 3:1):** 0.55

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.579 (bs, 1H, NHCO), 5.390-5.353 (m, 2H, CH=CH), 4.079 (m, 2H, HCCCH_2NHCO), 2.249-2.230 (m, 1H, HCCCH_2NHCO), 2.225-2.194 (m, 2H, HNCOCH_2), 2.051-2.011 (m, 4H, CH_2CH=CHCH_2), 1.673-1.644 (m, 2H, HNCOCH_2CH_2), 1.326-1.292 (m, 20H, CH_2), 0.905 (t, 3H, *J*=7 Hz, CH_3).

^{13}C NMR (125.7 MHz, $CDCl_3$): δ 170.37 (COO), 155,67 (CONH), 79.967 (*t*-BuC), 70,729, 70.683, 70.662, 70.049, 68.914, 64.361, 60.365, 53.401, 50.712, 42.429 (BocNHCH_2COO), 28.313.

HRMS calcd for $C_{21}H_{37}NONa$ $[M+Na]^+$: 342.2753, found 342.2767.

[(Z)-4-Octadec-9-enoic-amidomethyl-1H(1,2,3-Triazol-1-yl)] -3,6,9-trioxaundecan-amine (5)

A solution of **3** (0.25 g, 0.71 mmol) and **4** (0.23 g, 0.71 mmol), in CH_2Cl_2 (6 mL), was added to a solution of $CuSO_4$ (0,018 g, 0.11 mmol) and sodium ascorbate (0,059 g, 0.30 mmol) in water (8 mL). Afterwards, the reaction mixture was stirred vigorously over three days. Then the mixture was separated and the organic phase was dried over anhydrous Na_2SO_4. The CH_2Cl_2 was removed by rotary evaporation to yield the crude product. The triazole derived, was isolated by flash chromatography column on silica gel, eluting with a mixture of CH_2Cl_2:MeOH (9:1). The final product **5** was a white solid (0,34 g, 0.51 mmol, 30%).

***Rf* (CH_2Cl_2/MeOH 9:1):** 0.08

^{1}H NMR (500 MHz, MeOD): δ 7.802 (s, 1H, H-triazol), 5.388-5.232 (m, 2H, CH=CH), 4.473 (t, J=6, 2H, CH_2CH_2triazole), 4.254 (s, 2H, NHCH_2-triazol), 3.796 (t, *J*=5 Hz, 2H, CH_2-triazol), 3.606-3.596 (m, 2H, CH_2CH_2NH_2), 3.550-3.503 (m, 8H, OCH_2CH_2O), 3.032 (s, 2H, CH_2NH_2), 2.121 (t, *J*= 7.5 Hz, 2H, CH_2CO), 1.934-1.922 (m, 4H, CH_2CH=CHCH_2), 1.7914-1.733 (m ,2H, NH_2), 1.513 (m, 2H, CH_2), 1.220-1.194 (m, 20H, CH_2 oleic acid), 0.799 (t, *J*=7 Hz, 3H, CH_3).

^{13}C NMR (125.7 MHz, MeOD): δ 176.191 (C=O), 130.871, 130.772 (C=C), 124.903, 71.496, 71.354, 71.342, 71.224, 70.373, 68.152, 51.376, 36.965, 35.543, 33.026, 30.807, 30.5272, 30.406, 30.330, 30.309, 30.298, 30.216, 28.123, 28.104, 26.916, 23.702, 14.438.

HRMS calcd for $C_{29}H_{55}N_5O_4$ $[M+H]^+$: 538.43; found 538.43.

(Z)-(1-azido-3,6,9-trioxaundecan) -oleamide (6)

To a solution of **3** (0.10 g, 0.46 mmol) in dry CH_2Cl_2 (0.58 mL), under argon atmosphere, was added Et_3N (0.042 mL, 0.56 mmol) and the oleoyl chloride (0.066 mL, 0.46 mmol). Then the reaction was stirred vigorously for 1 day at room temperature. To the reaction mixture was added CH_2Cl_2 (10 mL) and extracted with HCl 1N (3x5 mL). Afterwards the organic phase was washed with a saturated acqueous $NaHCO_3$ solution (5 mL). The organic phase was dried over anhydrous Na_2SO_4. The solvent was evaporated and the product was purified by flash chromatography column on silica gel with CH_2Cl_2: MeOH (15:1) to yield the compuond **6** (0.123g, 57 %).

***Rf* (CH_2Cl_2/MeOH 9:1):** 0.60

^{1}H NMR (500 MHz, $CDCl_3$): δ 5.986 (s, 1H, NHCO), 5.353-5.328 (m, 2H, CH=CH), 3.690-3.60 (m, 10H, OCH_2CH_2O and CH_2CH_2N$_3$), 3.459 (t, *J*=5 Hz, 2H, OCH_2CH_2NHCO), 3.470-3.449 (m, 2H, CH_2CH_2NHCO), 3.390 (t, J= 5, 2H, CH_2N$_3$), 2.168 (t, *J*=7.5 Hz, 2H, CH_2CO), 2.024-1.986 (m, 4H, CH_2CH=CHCH_2), 1.638-1.609 (m, 2H, CH_2CH_2CO), 1.300-1.253 (m, 20H, CH_2 oleic acid), 0.892 (t, J=6.5 Hz, 3H, CH_3).

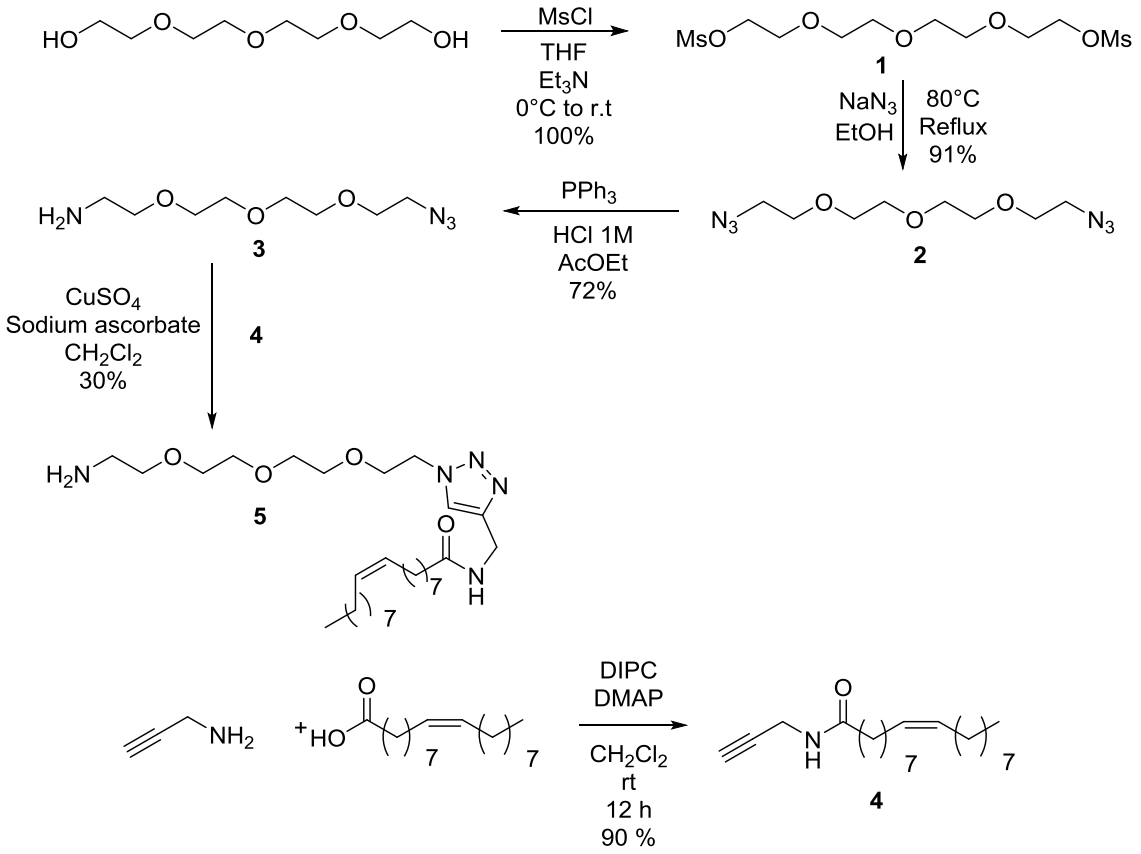
^{13}C NMR (125.7 MHz, $CDCl_3$): 173.380 (C=O), 130.132, 129.905 (C=C), 70.886, 70.787, 70.738, 70.399, 70.226, 70.121, 50.836, 39.294, 36.889, 32.044, 29.913, 29.878, 29.837, 29.665, 29.457, 29.314, 27.365, 27.336, 25.886, 22.820, 14.249.
HRMS calcd for $C_{26}H_{50}N_4O_4Na$ $[M+Na]^+$: 505.3724; found 505.3718

1-amino-3,6,9-trioxaundecan-(Z) -9-Octadecenamide (7)
The azide **9** (0.423 g, 0.88 mmol) was dissolved in dry THF (3.83 mL), under argon atmosphere, and cooled to 0°C. To this solution was added 1.75 mL (1.75 mmol) of a 1M solution of LAH in THF. After stirring at 0°C for 1 h, the reaction mixture was quenched 0.62 mL of saturated acqueous anhydrous Na_2SO_4 solution, and stirred for 30 min, at room temperature. The white precipitate (aluminium salts) formed, was filtered with celite and washed with ether (5 x 10 mL) and then with CH_2Cl_2 (10 mL). The organic phase was combined, dried over anhydrous Na_2SO_4, filtered and concentrate. Then the crud product was purified by flash chromatography column on silica gel with CH_2Cl_2: MeO (15:1) to yield the product **7** (0.261 g, 0.57 mmol, 65%).
***Rf* (CH_2Cl_2/MeOH 15:1) =** 0
^{1}H NMR (500 MHz, $CDCl_3$): δ 6.632 (s, 1H, NHCO), 5.350-5.325 (m, 2H, CH=CH), 3.657-3.557 (m, 10H, OCH_2CH_2O and $OCH_2CH_2NH_2$), 3.482-3.436 (m, 2H, CH_2CH_2NHCO), 3.036-3.031 (m, 2H, CH_2NHCO), 2.985-2.957 (m, 2H, CH_2NH_2), 2.937-2.924 (m, 2H, NH_2), 2.204-2.174 (m, 2H, CH_2CO), 2.022-1.984 (m, 4H, $CH_2CH=CHCH_2$), 1.634-1.605 (m, 2H, CH_2CH_2CONH), 1.298-1.266 (m, 20H, CH_2 oleic acid), 0.878 (t, *J*=7 Hz, 3H, CH_3).
^{13}C NMR (125.7 MHz, $CDCl_3$): δ 173.682 (C=O), 130.118, 129.906 (C=C), 70.603, 70.560, 70.303, 70.248, 39.308, 36.808, 32.036, 29.908, 29.888, 29.834, 29.791, 29.657, 29.484, 29.467, 29.444, 29.333, 27.363, 27.340, 25.930, 22.811, 14.237
HRMS calcd for $C_{26}H_{52}N_2O_4$ $[M+H]^+$: 457.71; found 457.40

3. Results and discussion Section

3.1. Synthesis of the amphiphilic compounds

The first amphiphilic compound synthesized **5** has tetraethylene glycol as a spacer, the versatile amine group as a polar head and the oleic acid as a lipophilic tail. It was obtained through a five steps sequence (Scheme 3).

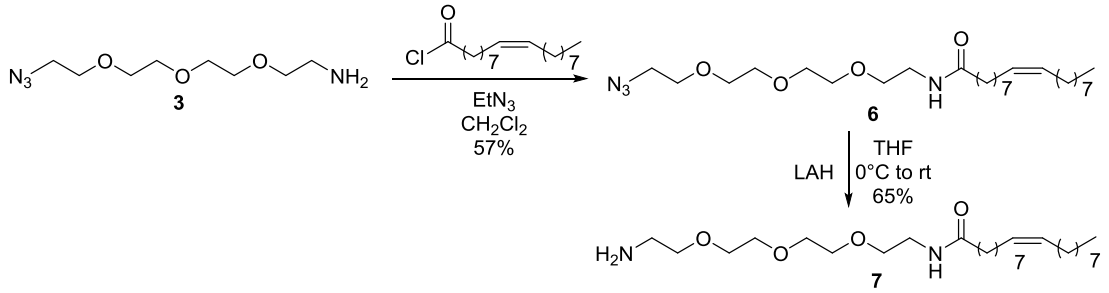


Scheme 3. Synthesis of the amphiphilic compound **5**

The amphiphilic compound **5** was obtained through a sequence of reactions starting from the mesylation of tetraethylene glycol with mesyl chloride. The dimesylated derivative **1** was transformed into diazide **2** by substitution with sodium azide. Subsequently, a monoreduction of diazide **2** with triphenylphosphine as a reducing agent yielded compound **3**. The linking of **3** to the lipidic part of the amphiphilic compound was carried out through a Cu (I) catalyzed Huisgen reaction, whith this purpous, the corresponding alkynic derivative of oleic acid was previously prepared by amidation of oleic acid with propargylamine. Once intermediate **4** was obtained, the Huisgen reaction was carried out with the corresponding azide **3**, obtaining the final compound **5**.

In this way, we have been able to obtain the amphiphilic compound **5** with the amine group as a versatile polar head. In fact, the presence of this group may constitute a binding site to other specific functional groups as it can be a drug, a recognition ligand or an anionic group like phosphonate.

The second amphiphilic compound synthesized, compound **7**, was very similar to compound **5**, in this case, the connection between the polar head and the lipophilic chain is an amide group instead of a triazole group. (Scheme 4)



Scheme 4. Synthesis of the amphiphilic compound **7**

A reaction between compound **3** and oleoyl chloride yielded the amide **6**. Finally, the reduction of the azide group of **6** with LAH carried out the compound **7**. The use of LAH as a reducing reagent

was supposed to reduce also the amide to amine. However, the amide remained unchanged and LAH only reduced the azide group.

3.3. Preparation and characterization of micelles

In all cases, the range of CMC obtained was [0.01 mM-0.08 mM] using the pyrene method [25]. Micelles were formed in water solutions at a concentration of 1.25 mg/ml (0,02 M in the case of compound **7**) much greater than CMC. Micelle formation process was previously optimized and consists on the dispersion of the amphiphilic compound in MilliQ water. Then, the sample was ultrasonicated by a sonic tip (Digital ultrasonic sonicator Q500 of 500 watts), for 30 min. After sonication, a microfiltration process was carried out with a 30 mm membrane filter (Interlab Ltd. Customables syringe filters) in order to eliminate suspended particles.

Micelles were then characterised by DLS and TEM. Figure 2 represents two electronic transmission microscope pictures with different magnification of micelles obtained from amphiphilic compound **5 (M5)**. As it can be seen, micelles are monodisperse, therefore they are not present aggregates, and they present sizes in a range of 50-89 nm.

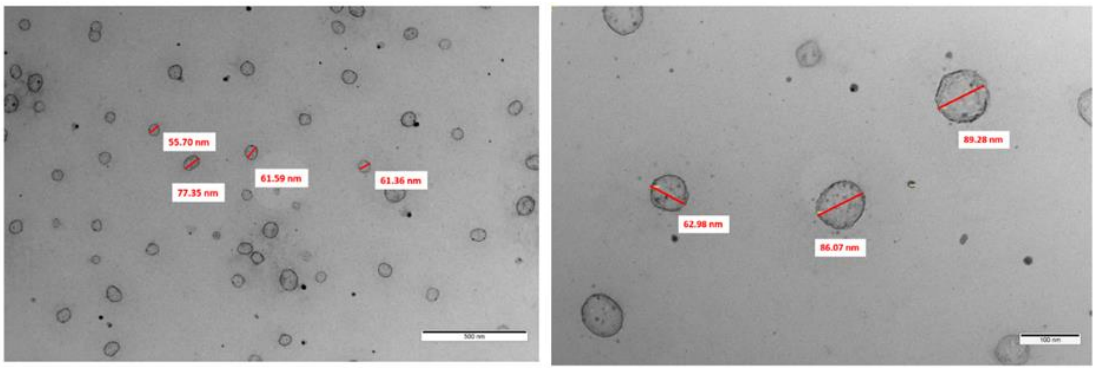


Figure 2. TEM images of **M5**.

In addition to microscopic analysis, the sample was analysed by DLS (Dynamic light scattering) in order to determine their hydrodynamic size. In the case of M5, it was of 99.80 nm (Figure 3).

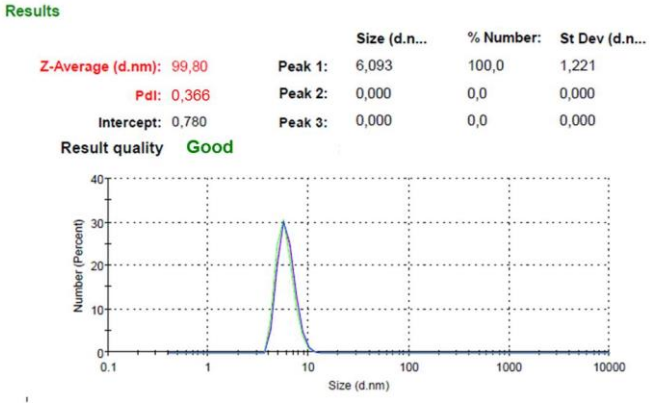


Figure 3. DLS Analysis of **M5**.

It can be seen that both techniques determined an equivalent micelles size.

Figure 4 represents a TEM photograph of micelles from compound **7 (M7)** with diameters between 70-120 nm and DLS results with hydrodynamic size of 40 nm.

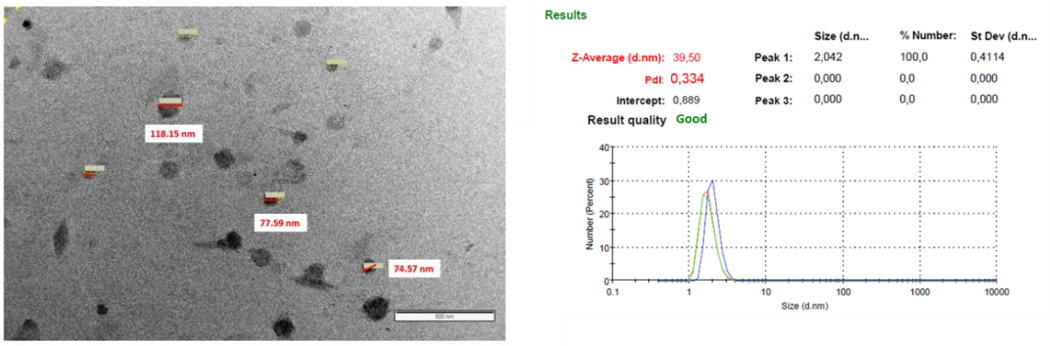


Figure 4. TEM and DLS Analysis of **M7**.

In this case, one more time, both techniques confirm the presence of micelles.

3.4. Inclusion of dexamethasone in micelles

Dexamethasone (Dexa) was introduced in the synthesized micelles, in order to verify the ability of these nanocarriers to contain a highly insoluble drug. This test was performed using synthesized micelles of compound **5 (M5)** and **7 (M7).**

A procedure in 3 steps developed by the laboratory has been carried out:

i) Addition of the solid drug (5.9 mg) directly to the previously prepared water solution of micelles and stirring 72 hours at 50 ° C. During this time, the sample was covered with an aluminum foil to prevent the degradation of the photosensitive drug Dexamethasone.

ii) Centrifugation at 2000 rpm during 15 min, obtaining a precipitate, which represents the drug not included, and a solution containing the micelles with the drug inside.

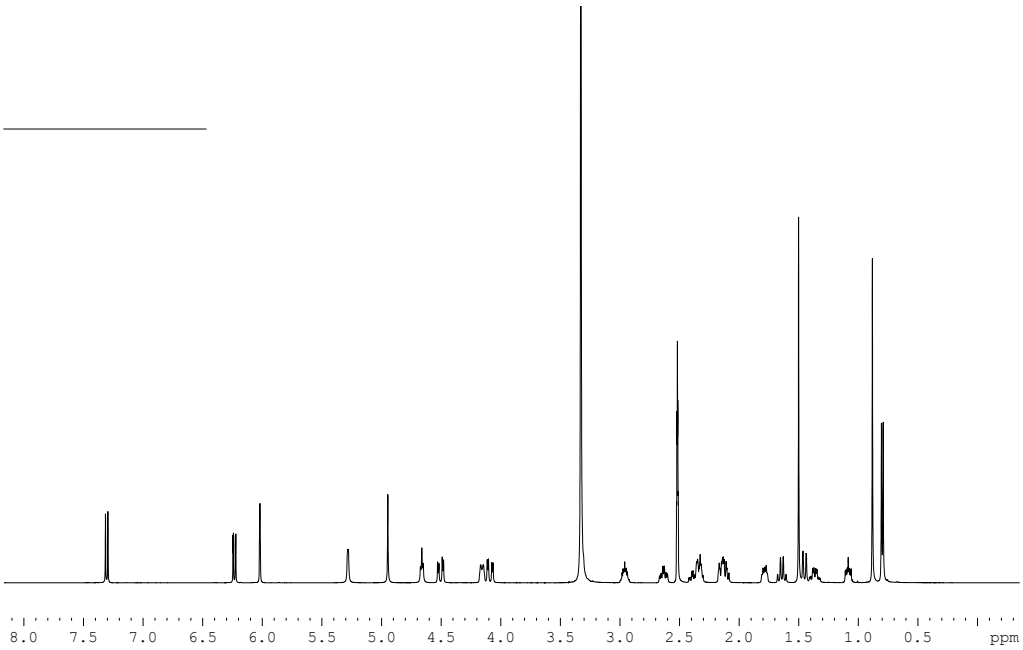
iii) Lyophilization for the elimination of water of the samples.

Table 1. Quantity in mg of drug and micelles before and after the inclusion process.

Initial mg of Dexa	**Initial mg of amphiphile**	**mg of Dexa as a precipitate**	**mg of Micelle + Dexa**	**mg of Dexa included in micelles**	**% of included drug (partition coefficient)**
5.9 mg	10 mg of **5** (in 8 mL H_2O milliQ)	2.7 mg	13.2 mg	3.2 mg	54.2 %
6.2 mg	10 mg of **7** (in 10 mL H_2O miliQ)	1.6 mg	14.6 mg	4.6 mg	74.2 %

To confirm the presence of the drug inside the micelles, the analysis of the samples by ^{1}H-NMR into different deuterated solvents has been carried out and the results obtained are shown in figure 5

a)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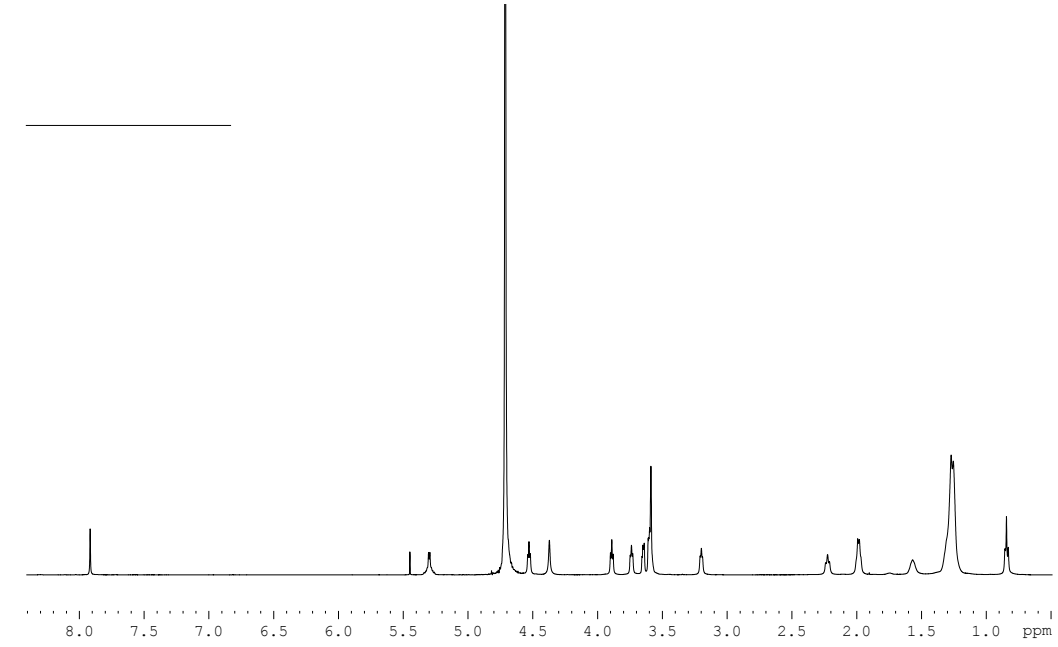
b)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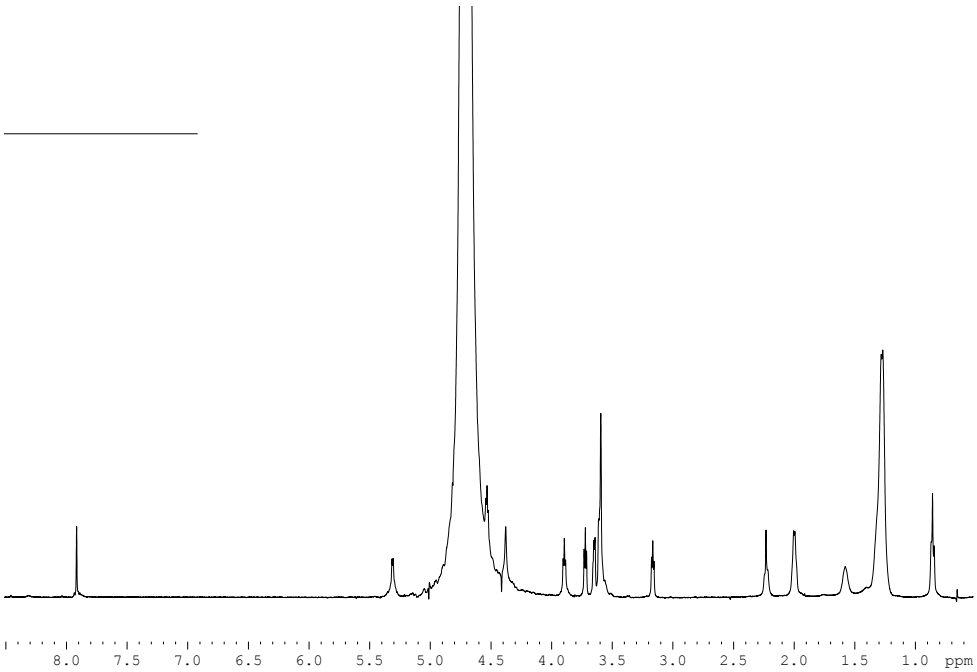
c)


8.0
7.5
7.0
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6.0
5.5
5.0
4.5
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3.5
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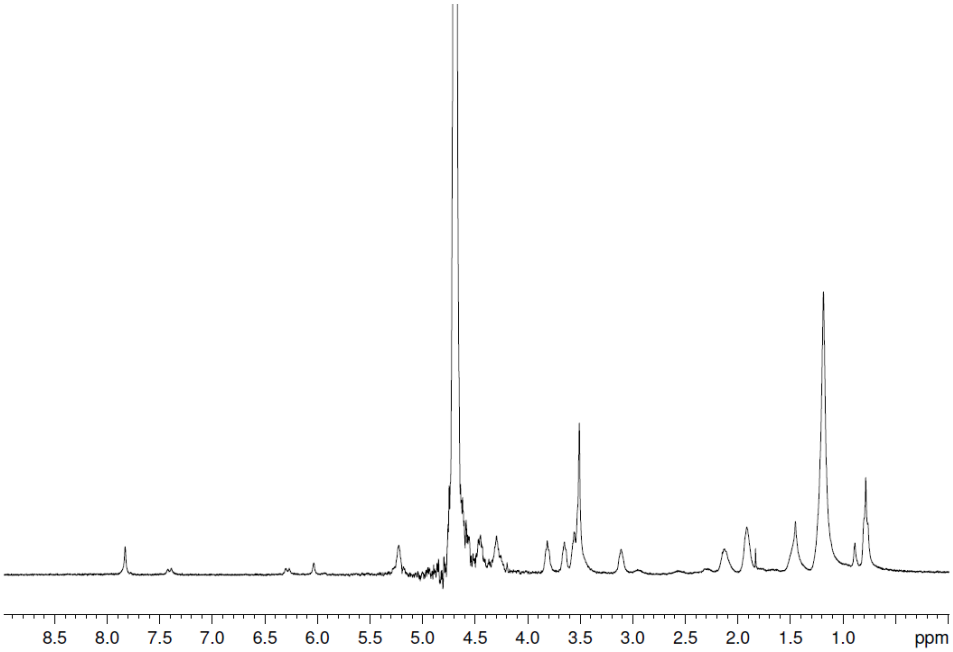
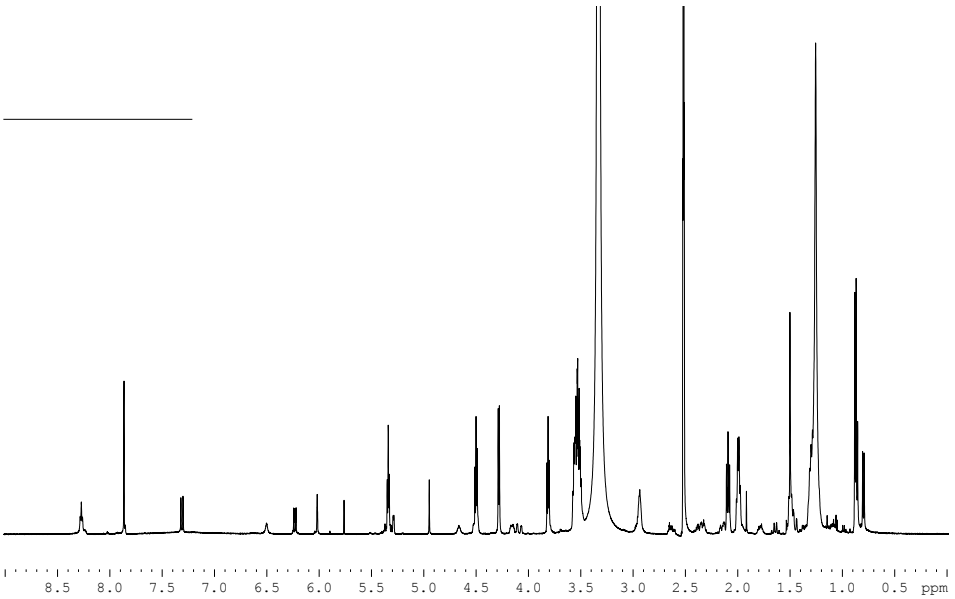
1.5
1.0
ppm

d)


8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

e)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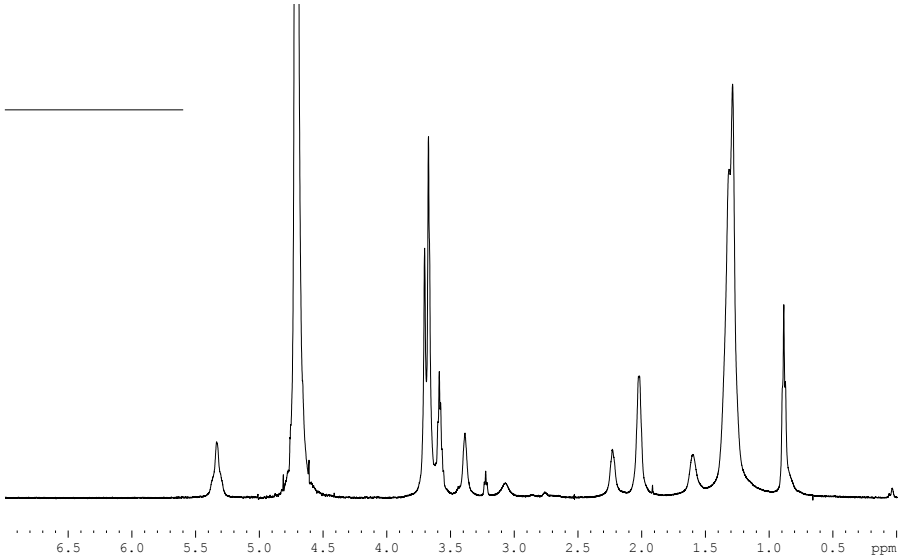
f)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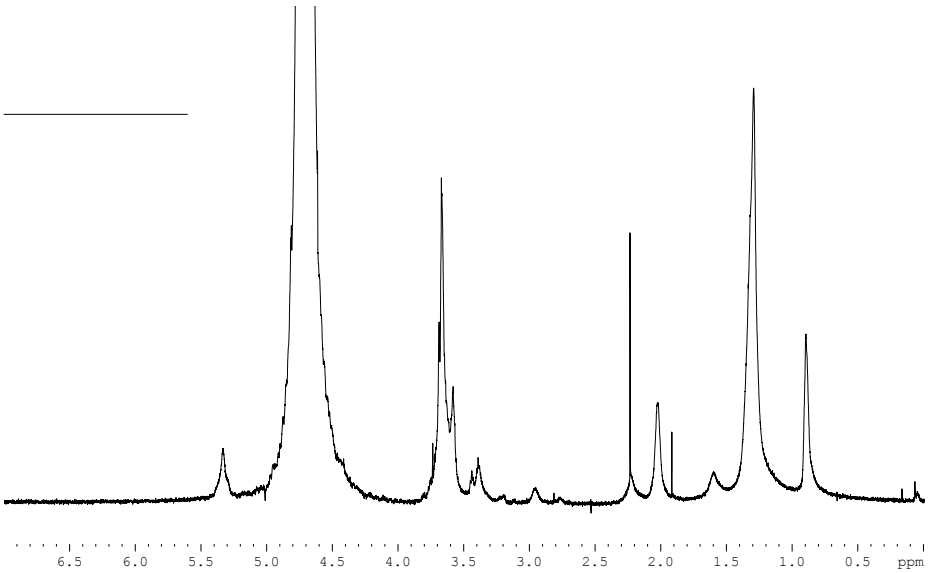
g)


6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm

h)

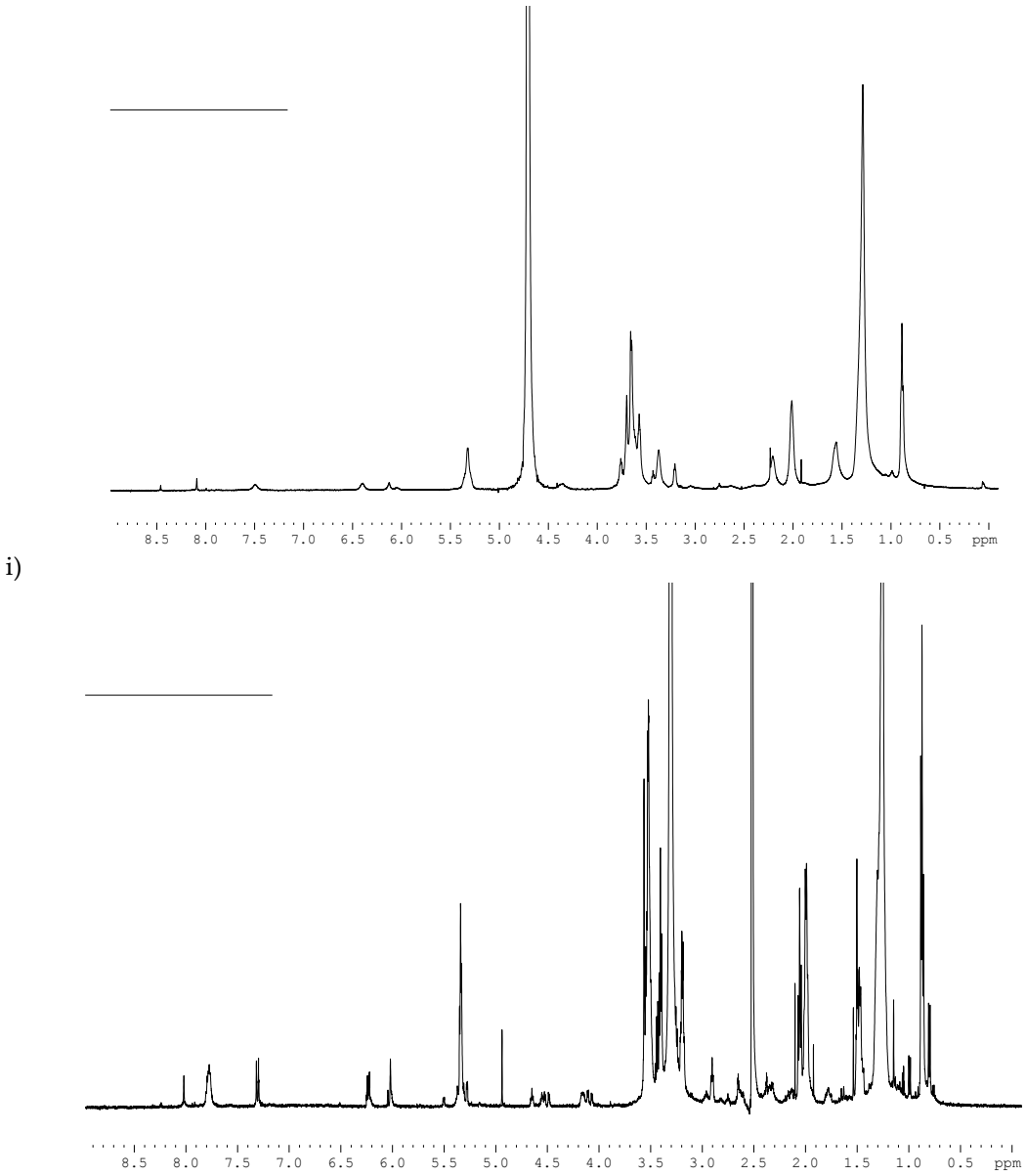
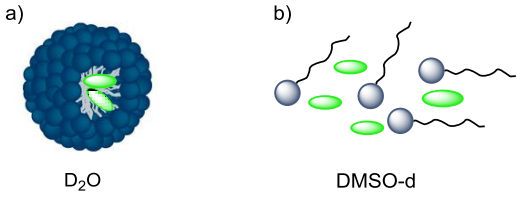


Figure 5. a) *^{1}H-NMR* of dexamethasone in deuterated DMSO. *^{1}H-NMR* of **5** in D_2O. c) *^{1}H-NMR* of **(M5)** in D_2O. d) *^{1}H-NMR* of **M5** + dexamethasone in D_2O. e) *^{1}H-NMR* of **M5** + Dexamethasone in deuterated DMSO. f) *^{1}H-NMR* of **7** in D_2O. g) *^{1}H-NMR* of **(M7)** in D_2O. h) *^{1}H-NMR* of **M7** + dexamethasone in D_2O. i) *^{1}H-NMR* of **M7 +** Dexamethasone deuterated DMSO.

^{1}H-RMN of micelles + Dexa in D_2O highlights only the signals corresponding to the protons of the amphiphilic compound, whereas ^{1}H-NMR of micelles + Dexa in DMSO which is an organic solvent and causes the leakage of the drug, show the proton signals corresponding to both, the amphiphilic compound and Dexamethasone. This analysis represents a further indication of the internalization of the drug (Scheme 5).

In conclusion, it is possible to considerate that the drug is located inside the hydrophobic cavity of the micelles. This is evidenced not only by a gravimetric method but also by NMR which shows all the signals corresponding to the drug.



Scheme 5. Representation of micelles + Dexa in D_2O a) and amphiphilic compounds + Dexa in DMSO-d b)

4. Conclusions

The goals of this experimental work were to synthesize a new family of amphiphilic compounds in order to obtain micelles as drug nanocarriers.

We obtained two final amphiphilic compounds **5** and **7** characterized by an amine as a versatile polar head, from compounds **5** and **7** two different micelles were obtained, characterized by DLS and TEM, which have different sizes and distribution in water.

In order to verify the ability of these micelles to contain drugs, the inclusion of the highly lipophilic drug Dexamethasone into micelles derived from compound **5 (M5) and 7 (M7)** was performed, characterized by an average size of about 100 nm, contain about 54 % and 74% respectively of the previously added drug that represents a good percentage and demonstrates their ability to encapsulate a highly lipophilic drug in their core.

In the future, **M5** and **M7** micelles can be functionalized by exploiting the free amine groups of their amphiphilic monomers, in order to address them to specific target.

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Conflicts of Interest: The authors declare no conflict of interest.

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