Nanocarriers based on orthogonal clickable block copolycarbonates

Final Master Project

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ABSTRACT: "NANOCARRIERS BASED ON ORTHOGONAL CLICKABLE BLOCK COPOLYMERS"

Amphiphilic block copolymers are promising materials for drug delivery due to their ability to form self-assemblies in aqueous solution, which are able to both encapsulate and transport drugs. In order to specifically aim the target tissue, drugs' release can be controlled if stimuli-responsive moieties are incorporated to the macromolecular structure. Polycarbonate-based copolymers have been studied as drug delivery systems because of their biocompatibility and biodegradability. The use of cyclic carbonates as monomers and their subsequent ring opening polymerization (ROP) provides a high control of molecular mass and dispersity. Moreover, those derived from 2,2-bis(hydroxymethyl)propionic acid (bis-MPA) enable the incorporation of functionality onto its carboxyl group and, through various post-polymerization stages, their modification with stimuli-responsive units can be developed.

The aim of this Final Master Project is the synthesis and characterization of amphiphilic and stimuli-responsive block copolycarbonates, as well as the study of their self-assembly in water to form polymeric aggregates. To this end, it has been developed a synthetic approach for orthogonal clickable block copolycarbonates, based on allylic and propargylic pendant groups, which are able to be selectively functionalized with hydrophilic/hydrophobic stimuli-responsive moieties by widely-reported click reactions.

For this purpose, ROP of MAC monomer has been studied in order to synthetize an allyl-based homopolymer, PMAC, which has been functionalized through radical thiol-ene reaction with hydrosoluble thiols. Consequently, hydrosoluble and thermoresponsive polymers that show LCST behavior have been prepared, proving that they can be useful as hydrophilic block in the preparation of block copolycarbonates. Moreover, copolymerization of PMAC homopolymer and MPC monomer and the subsequent orthogonal post-polymerization modifications have been carried out. The combination of CuAAC and radical thiol-ene reactions has leaded to the synthesis of thermoresponsive amphiphilic block copolycarbonates that show self-assembly in water, and to the preparation of amphiphilic and potentially thermo and light-responsive block copolycarbonates.

RESUMEN: “NANOTRANSPORTADORES BASADOS EN COPOLÍMEROS BLOQUE ORTOGONALMENTE FUNCIONALIZABLES MEDIANTE QUÍMICA “CLICK””

Los copolímeros bloque anfífilos son materiales muy prometedores para la liberación controlada de fármacos, debido a su capacidad de formar autoensamblados en agua que los encapsulen y transporten. Incorporándoles unidades sensibles a estímulos, se logra la liberación del fármaco en el tejido diana. Aquellos basados en policarbonatos son de especial interés por sus propiedades de biocompatibilidad y biodegradabilidad. Para su síntesis, el uso de carbonatos cíclicos como monómeros y su polimerización por apertura de anillo (ROP), presenta un gran control de la masa molecular y dispersidad. Además, aquellos monómeros derivados del ácido bis-MPA posibilitan la incorporación de cadenas laterales mediante las que anexionar unidades con repuesta a estímulos.

Este Trabajo Fin de Master tiene como objetivo la síntesis y caracterización de copolicarbonatos bloque anfífilos que respondan a estímulos, así como el estudio de su autoensamblado en agua. Para ello, se ha desarrollado una ruta sintética para copolímeros bloque basados en cadenas laterales alílicas y propargílicas que, por tanto, permitan su funcionalización ortogonal mediante reacciones “click”, con cadenas hidrófilas e hidrófobas sensibles a estímulos.

En primer lugar, se ha realizado un estudio de la ROP del monómero MAC obteniendo un homopolímero basado en grupos alilo, PMAC, el cual ha sido funcionalizado mediante reacciones tiol-eno. Como resultado, se han sintetizado polímeros solubles en agua y con termo-respuesta, útiles como bloque hidrófilo del futuro copolímero bloque. Se ha desarrollado además tanto la copolimerización del homopolímero PMAC y el monómero MPC, como la funcionalización ortogonal de sus bloques mediante la combinación de las reacciones CuAAC y tiol-eno. La ruta ha concluido, por un lado, en la síntesis de un copolicarbonato bloque anfífilo con termo-respuesta que forma autoensamblados en agua. Finalmente, también se ha logrado la preparación de un copolicarbonato bloque anfífilo y con unidades que responden tanto a la temperatura como a la luz.

Acronym list

AIBN: 2,2′-azobis(2-methylpropionitrile)

APC: aliphatic polycarbonate

BC: block copolymer

bis-MPA; 2,2-bis(hydroxymethyl)propionic acid

CuAAC: copper-catalyzed azide-alkyne cycloaddition

DBU: 1,8-diazabicyclo(5.4.0)undec-7-ene

DCM: dichloromethane

D_h: hydrodynamic diameter

DLS: dynamic light scattering

DMF: *N*, *N*-dimethylformamide

DMPA: 2,2-dimethoxy-2-phenylacetophenone

DP: degree of polymerization

DSH: 3-mercapto-1,2-propanediol

LCST: lower critical solution temperature

MAC: 5-methyl-5-allyloxycarbonyl-1,3-dioxan-2-one

MPC: 5-methyl-5-propargyloxycarbonyl-1,3-dioxan-2-one

OEG: oligo(ethylene glycol)

PEG: poly(ethylene glycol)

ROP: ring opening polymerization

SEC: size exclusion chromatography

T_{cp}: cloud point temperature

TEGSH: 2-{2-[2-(2-mercaptoethoxy)ethoxy]ethoxy}ethanol

TEM: transmission electron microscopy

THF: tetrahydrofuran

TU: 1-(3,5-bis(trifluoromethyl)-phenyl)-3-cyclohexylthiourea

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1. INTRODUCTION

1.1. Polymeric nanocarriers for drug delivery

One of the objectives in biomedical research is to find the least harmful therapies for those cases in which invasive medical treatments with severe secondary effects are performed. The development of new drug delivery systems pretends to maximize the therapeutic activity of drugs while adverse reactions are minimized.

Nanotechnology, which is defined as the understanding and control of matter at dimensions between 1 and 100 nanometers, has been the origin of several advances in medicine in the last years. Nanocarriers of drugs or molecules of biomedical interest show specific characteristics that make them able to overcome some problems associated to traditional therapies. Since the recognition of particles as foreign bodies is related to their size, nanometric structures are capable of infiltrating blood vessels with minimal immune response.[1] Moreover, nanocarriers can be used to improve the solubility and stability of hydrophobic drugs, allowing their transport in biological media and the use of lower doses to achieve the desired effect. By the incorporation of units sensitive to internal stimuli (pH, redox reactions, temperature) or external (light, temperature) into these materials, the specific release of the drug in the target tissue is achieved. Then, both efficiency and specificity of dosage can be improved.[2]

The drug transport capacity has been studied for many different materials, both inorganic and organic.[3] Among them, polymers have manifested various advantages over others as nanocarriers. The variety in their structures allows the formation of systems that enable the encapsulation of drugs and their transport through the bloodstream. This versatility also allows their functionalization with stimuli-responsive moieties that, as explained, facilitate a controlled release. In addition, the use of biocompatible polymers avoids the response of the immune system, whereas those that are biodegradable can experience the gradual destruction of their chain once their function has been carried out. Aliphatic polyesters are one of the most widely studied biodegradable materials. However, aliphatic polycarbonates (APCs) are of special interest due to the fact that they have a lower degradation rate than polyesters, and do not generate harmful acid by-products for the body such as the formers do.[4] Hence, this type of polymers can be used for the synthesis of alternative biodegradable materials with greater stability and less aggressiveness for the biological system.

1.2. Block copolymers and self-assembly properties in aqueous media

Polymers are macromolecules whose structure comprises multiple repetitions of low relative molecular mass-units known as repeating units. The term monomer refers to a molecule that, through a polymerization reaction, gives rise to the polymer chains. Moreover, the degree of polymerization (DP) indicates the average number of repeating units in a polymer. The number of different repeating units of which polymers are constituted divides them into two types: homopolymers, if they are all the same; or copolymers, if they have two or more types. Among copolymers, it is possible to obtain different distributions along their chain depending on the polymerization type and the relative fraction of the monomers. Thus, they are classified into statistical, alternating, graft or block copolymers (BCs), as the most common types, as shown in figure 1.1.

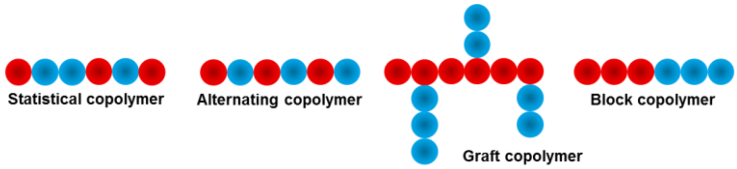

Figure 1.1. Types of copolymers.

BCs are formed by repeating units distributed in separate segments along the chain. Then, if the physicochemical properties of the polymeric building blocks are different enough, BCs tend to segregate at the nanoscale.[5] This is the case of amphiphilic BCs, which result from the combination of a hydrophilic and a hydrophobic block. In aqueous media, this type of materials have self-assembly properties such that, in order to avoid energetically unfavorable interactions between the hydrophobic block and water, form a variety of nanostructures as spherical, cylindrical micelles or vesicles, among others.[6] This variability in the morphology of aggregates depends on the hydrophobic/hydrophilic ratio of the chain, as schematized in figure 1.2, as well as the molar mass, the method of preparing the assemblies and more.[7]

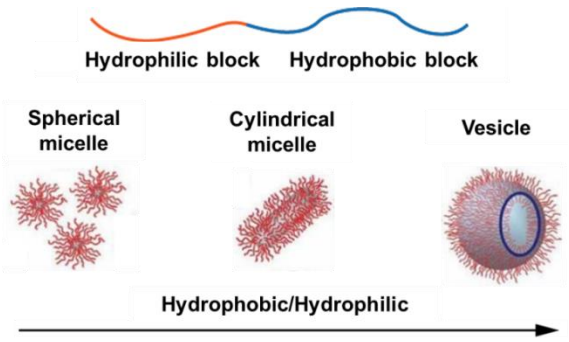

Figure 1.2. Self-assembling of amphiphilic BC in aqueous media depending on the hydrophobic/hydrophilic ratio.

1.3. Controlled polymerizations applied to block copolymers: ROP

Properties of amphiphilic BCs, such as dispersity (*Đ*) and DP of each block, drastically influences the size and morphology of self-assemblies, since they play a conspicuous role in controlling the interaction between the different polymeric segments with the solvent and within each other.[5] Thus, it is crucial to find polymerization techniques with good control over the polymer properties and, hence, self-assembly. The synthesis of BCs can be addressed by three methods: the couple of two homopolymers by exploiting their active chain-ends, sequential addition of monomers *via* controlled polymerization methods or polymerization of a monomer using a macroinitiator.[8]

Regarding controlled polymerization, the fundamental requirement is that, in order to allow adequate control of the polymer structure, the termination reaction is suppressed or highly minimized. In ring opening polymerization (ROP) chain elongation occurs through successive additions of the terminal group of the polymer to a reactive center of a cyclic monomer, that opens and joins the chain (figure 1.3).[9] This polymerization technique makes use of the enthalpy loss associated with ring stress relief to open cyclic monomers and carry out the polymerization.[10]

ROP of cyclic carbonate monomers is the most effective method for synthesizing APCs. When compared to others such as polycondensation, which has low control over molecular weight and dispersity; or the copolymerization of CO_2 with epoxides, which requires the use of metal catalysts, ROP shows good reproducibility and the possibility of synthesizing polycarbonates with high molecular weight and low dispersity.[11]

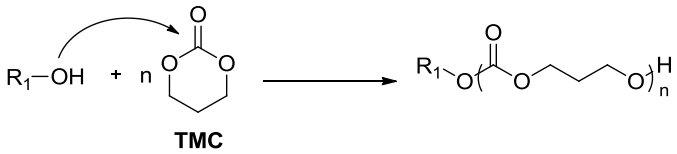

Figure 1.3. Synthesis of aliphatic polycarbonates by ROP of a cyclic carbonate, TMC.

Three main requirements are necessary to carry out a ROP: a cyclic monomer, a catalytic system and the initiator. Among cyclic carbonates, those derived from 2,2-bis(hydroxymethyl)propionic acid (bis-MPA) are of special interest to the synthesis of APCs because functionality can be incorporated onto its carboxyl group, resulting in cyclic monomers with different pendant groups (e.g., allyl, propargyl or azide groups) that can be used for post-polymerization modifications.[12] Moreover, ROP can be carried out through various catalytic mechanisms; however, given the growing biomedical

application of this type of polymers, the use of organocatalysts to replace the metallic ones avoids toxic waste. Whereas several organocatalytic systems have been studied for ROP of cyclic carbonates, for those derived from bis-MPA the use of TU-DBU has shown good control over both molecular weight and dispersity.[13] Finally, in terms of choosing initiator, it must contain a functional group that favors the opening of the ring.

Synthesis of BCs can be carried out by using a polymer as macroinitiator, in which one of its end groups enables the beginning of the polymerization. For instance, previous works have used TU-DBU as catalytic system and the terminal hydroxyl group of hydrophilic poly(ethylene glycol) (PEG) for the opening of a cyclic carbonate and subsequent synthesis of the hydrophobic block of an amphiphilic BC (figure 1.4).[14]

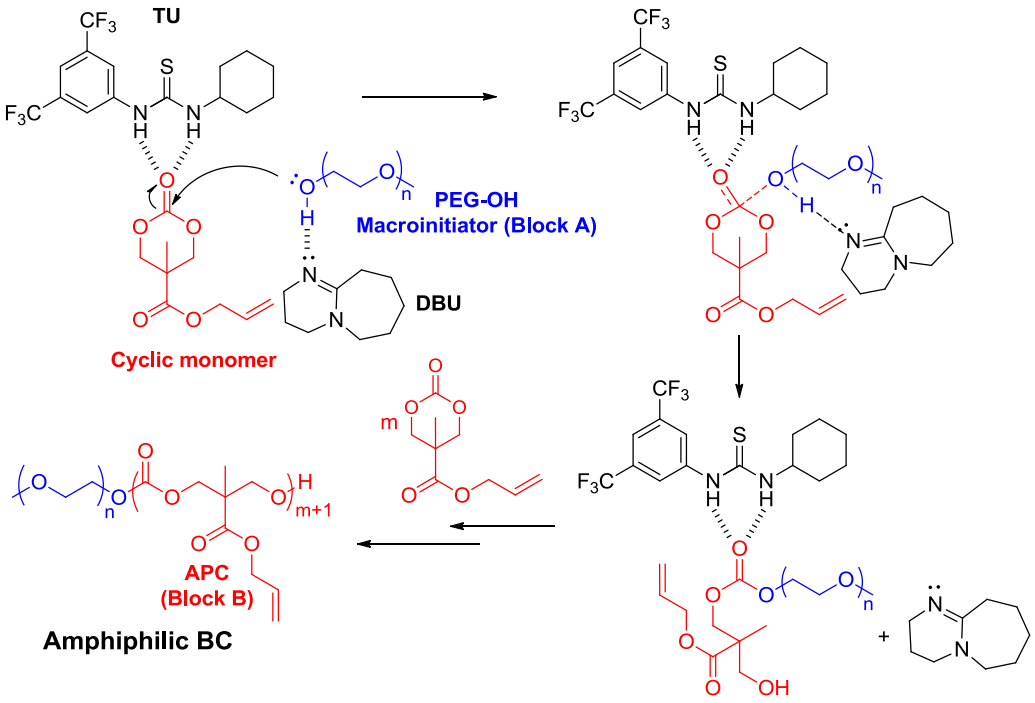

Figure 1.4. Synthesis of amphiphilic BCs based on the MAC monomer by ROP (Adapted from reference 15).[15]

1.4. Post-polymerization modifications

Functionalities, such as stimuli-responsive moieties, can be introduced in the side chain of a previously synthetized polymeric backbone through different post-polymerization modifications. "Click chemistry" was introduced by Sharpless in 2001 and comprises some highly specific reactions, with quantitative yields and simple to perform that, consequently, can be very useful for post-polymerization functionalization. Two of them are highlighted due to their great importance in polymers science: Cu(I)-catalyzed alkyne-azide cycloaddition (CuAAC) and thiol-ene reaction.

CuAAC between an organic azide and terminal alkyne was first reported in 2002 and it has become the most used click reaction.[16,17] The copper catalyzed mechanism is regioselective and yields only the 1,4 disubstituted 1,2,3-triazole (Figure 1.5a), while the non-catalyzed cycloaddition gives a mixture of 1,4 and 1,5 disubstituted ones.

The reaction of thiols and alkenes, which is gathered by the term thiol-ene reaction, is another widely-used click reaction in polymer chemistry. It is an anti-Markovnikov addition of the thiol to an alkene (Figure 1.5b) that, when performed with non-activated olefins, proceeds by a radical mechanism initiated by radical initiators activated either with light or heat.[18]

The combination of these reactions with controlled polymerization techniques constitutes a fundamental tool for the design of new and well defined amphiphilic and stimuli-responsive block copolycarbonates. When ROP of cyclic carbonates provided with propargyl or allyl pendant groups is performed, just as figure 1.4 exemplifies, CuAAC and thiol-ene reactions have been carried out, respectively, to modify the main chain and introduce functionality to each polymer.[19]

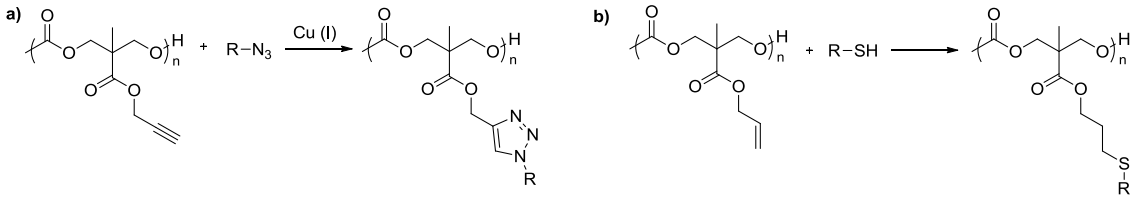

Figure 1.5. CuAAC (a) and thiol-ene (b) click reactions applied to post-polymerization modifications of APCs.

1.5. Thermoresponsive polymeric nanocarriers

Polymeric self-assemblies can be designed to respond to different stimuli, allowing for the on-demand release of any encapsulated cargoes, as figure 1.6 schematizes.

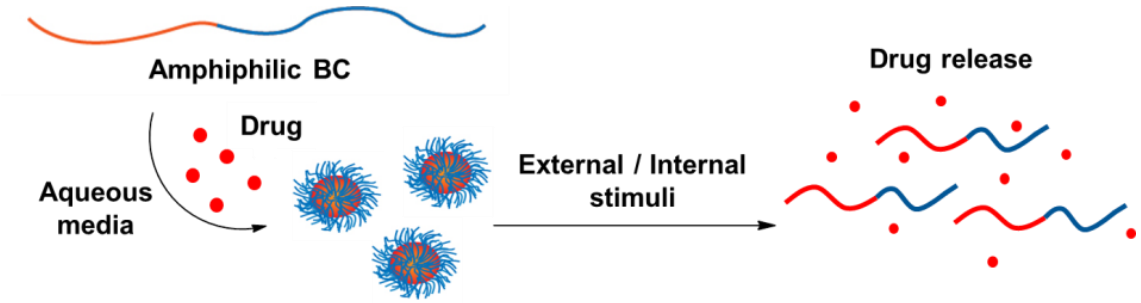

Figure 1.6. Drug delivery process of amphiphilic and stimuli-responsive BC based nanocarriers.

Amongst the various stimuli applicable, temperature is one of the most used for the design of nanocarriers. Since damaged tissues tend to have slightly higher temperatures than healthy ones,[20] and temperature can also be externally applied in a non-invasive manner, it can be both used as an internal and external stimulus. In this sense, solubility of certain polymers can be affected by the temperature of the medium so that they turn from soluble to insoluble, or vice versa. If the polymeric nanocarrier is hydrophilic at room temperature and becomes insoluble when body temperature is exceeded, it might be useful for drug delivery applications. Thus, since the polymer turns hydrophobic by heating, the temperature at which this occurs is called Low Critical Solution Temperature (LCST).[21]

Below LCST, the hydrophilic behavior is based on hydrogen bonds that are present between the polymer and surrounding water molecules. Above, hydrogen bonds are weakened and the polymer chains, dehydrated. Hence, they cannot be solubilized leading to polymer aggregation. This behavior is called "coil to globule transition" because polymers exhibit at first hydrated coiled conformation and, at high temperature, they minimize their contact with water by becoming larger globules (figure 1.7).

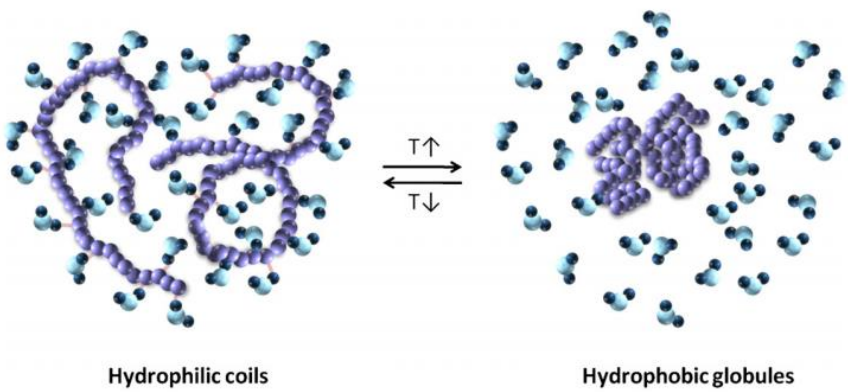

Figure 1.7. Coil to globule transition of a thermoresponsive polymer. Reused with permission from Elsevier.[22]

In particular, aliphatic polycarbonates bearing pendent allyl ether groups have been modified with oligo(ethylene glycol) (OEG) and PEG chains, enabling to generate thermoresponsive polycarbonates.[23] LCST behavior in water is shown due to the solubility of the OEG and PEG chains and the insolubility of the main chain. These polymers can be synthesized by ROP of an allyl-based cyclic carbonate and subsequent thiol-ene reaction to introduce a thiol provided with PEG chain. Moreover, if the thermoresponsive polymer is attached to a hydrophobic block, thermoresponsive amphiphilic BCs with self-assembly properties can be obtained.

1.6. Antecedents in the CLIP group

The Liquid Crystals and Polymers Group (CLIP) of the University of Zaragoza has been studying in recent years the synthesis and behavior of amphiphilic BCs with different structures, and functionalized with groups that confer stimuli-responsiveness for drug delivery.

The first system developed by CLIP group consisted on linear-dendritic amphiphilic BCs synthetized by coupling of two preformed blocks of PEG and bis-MPA based dendrons functionalized with azobenzene light-responsive units.[24] The study of their self-assembly properties verified the formation of nanofibers, micelles and vesicles, whose morphology varied depending of the hydrophobic/hydrophilic ratio of the blocks.[25] Moreover, encapsulation of both hydrophobic and hydrophilic molecules was achieved through the formation of vesicles from linear-dendritic polymers.[26] Lately, the incorporation of linear chains of oligo and diethylene glycol polymethacrylates as hydrophilic and thermoresponsive block in bis-MPA based dendrons functionalized with azobenzene groups, allowed to synthesize amphiphilic BCs with both thermo and photoresponse.[27]

In pursuit of more simple and biodegradable alternatives, amphiphilic linear-linear BCs composed by a hydrophilic PEG block linked to a bis-MPA based aliphatic polycarbonate hydrophobic block have been recently developed. Post-polymerization modifications through click chemistry reactions enabled to introduce light-responsive groups covalently bounded to the polymer,[28] as shown in figure 1.8, and also *via* supramolecular modifications.[19] Light responsive nanocarriers were hence successfully obtained. However, the wide variety in the possible structures of these cyclic carbonates, the ease of their functionalization and their biodegradability, lead the way on enhancing the versatility in the composition and response of the nanocarrier.

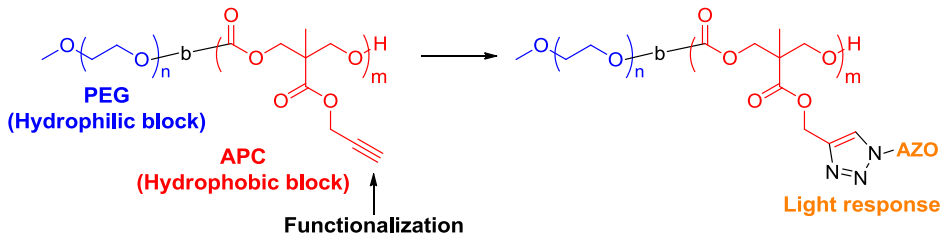

Figure 1.8. Amphiphilic linear-linear BC composed by PEG hydrophilic block and aliphatic polycarbonate hydrophobic block, functionalized with light-responsive azobenzene through CuAAC click reaction.

2. OBJECTIVES

The aim of this Final Master Project is the synthesis and characterization of amphiphilic and stimuli-responsive block copolycarbonates, as well as the study of their self-assembly in water to form polymeric aggregates. The proposed synthetic approach for BCs, which are based on 5-methyl-5-allyloxycarbonyl-1,3-dioxan-2-one (MAC) and 5-methyl-5-propargyloxycarbonyl-1,3-dioxane-2-one (MPC) monomers, is expected to provide high versatility in their functionalization. Allylic and propargylic pendant groups can be orthogonally functionalized by means of widely-reported click reactions to introduce functionality into the macromolecules. Specifically, hydrophilicity will be provided by groups attached to the allyl-based block by dint of thiol-ene reaction, whereas propargyl pendant groups will be functionalized with hydrophobic moieties by means of CuAAC reaction, as figure 2.1 schematizes. Furthermore, stimuli-responsive properties will be introduced either in one or two blocks, such as thermoresponsiveness in the hydrophilic block and light/pH-responsiveness in the hydrophobic one.

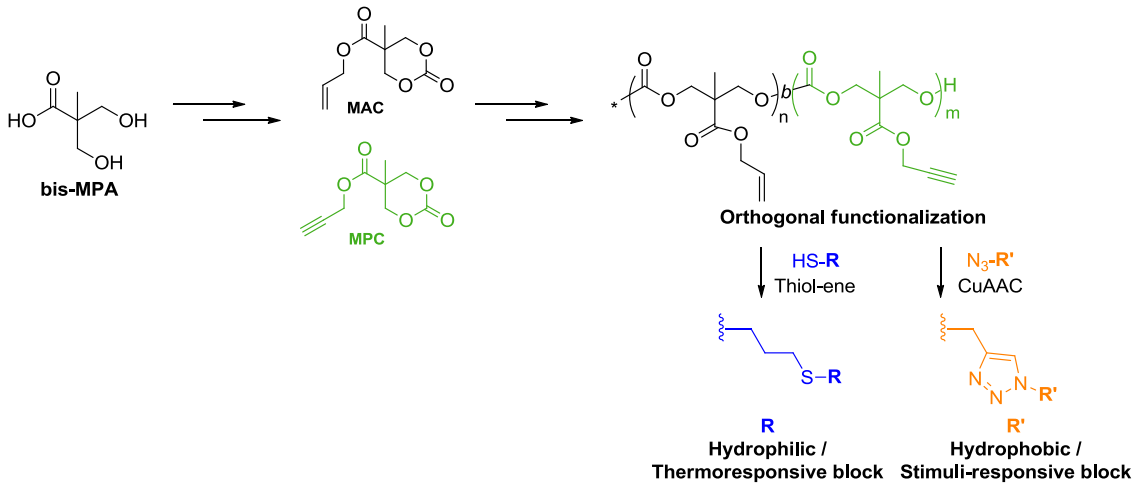

Figure 2.1. Basic scheme of the proposed synthetic approach for amphiphilic and stimuli-responsive BCs.

According to what has been said above, the specific tasks and proposed polymers series for this project are summarized in figure 2.2 and explained as follows:

Task 1. Synthesis, characterization and polymerization of MAC monomer. The first task is the synthesis of MAC monomer, using bis-MPA acid as precursor, and the subsequent study of its organocatalyzed ROP to obtain $PMAC_n$ homopolymer.

Task 2. Synthesis, characterization and study of hydrophilic and thermoresponsive properties of functionalized homopolymers (Series I). Study of $PMAC_n$ functionalization by thiol-ene reaction with different combinations of two hydrophilic

chains, 3-mercapto-1,2-propanediol (DSH) and 2-{2-[2-(2-mercaptoethoxy) ethoxy] ethoxy} ethanol (TEGSH), in order to optimize their properties as hydrophilic and thermoresponsive block for the future block copolymer. Thermoresponsive properties of the resultant polymers will be studied by means of turbidimetry method, dynamic light scattering (DLS) and transmission electron microscopy (TEM).

Task 3. Synthesis and characterization of amphiphilic and thermoresponsive block copolycarbonates by ROP and orthogonal click reactions (Series II). $PMAC_n$ is proposed to be used as macroinitiator for the polymerization of the second monomer, MPC, obtaining the $PMAC_n$-*b*-$PMPC_m$ BC. Highly selective 1,3-dipolar cycloaddition between alkyne pendant groups and organic azide must be first performed to introduce the hydrophobic block. Subsequent radical thiol-ene reaction, previously checked on the homopolymer, should provide hydrophilicity and thermoresponsiveness to the BC.

Task 4. Preparation, characterization and study of thermoresponse of polymeric aggregates in water. Polymeric aggregates will be prepared by employing co-solvent method and their characterization and study of thermoresponse will be approached by UV-vis spectroscopy, DLS and TEM.

Task 5. Synthesis and characterization of amphiphilic and stimuli-responsive block copolycarbonates by orthogonal click reactions (Series III and IV). In order to check the versatility of the orthogonal click reactions as strategy to incorporate more than one stimuli-responsive moiety, organic azides provided with pH-responsive (*Series III*) and light-responsive (*Series IV*) units are proposed to be introduced into the copolymer.

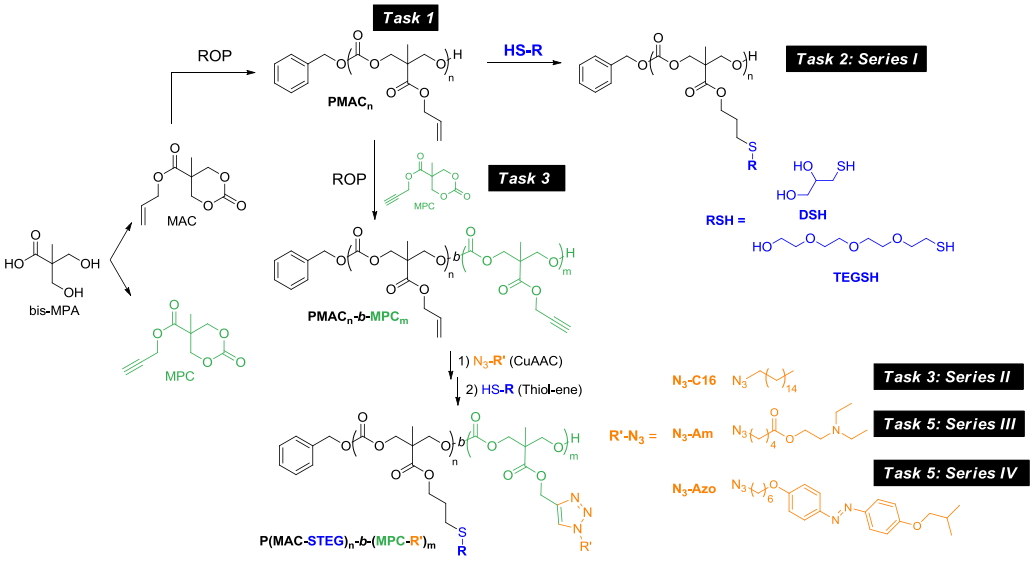

Figure 2.2. Summary of the synthetic pathway designed for this project.

3. RESULTS AND DISCUSSION

3.1. Synthesis, characterization and ROP of MAC monomer

Cyclic carbonates derived from bis-MPA are monomers of special interest because functionality can be easily incorporated onto its carboxyl group. Therefore, the first step is to synthesize the selected monomer, in this case 5-methyl-5-allyloxycarbonyl-1,3-dioxan-2-one (MAC), following the synthetic pathway schematized in figure 3.1. All experimental details are collected in section A.1 of annex A.

MAC synthesis begins with the protection of the diol group of bis-MPA, in which 2,2-dimethoxypropane was used as reactant to form the acetal (1), whereas *p*-toluenesulfonic acid worked as reaction catalyst. Then, esterification reaction of the carboxylic acid group with allyl alcohol was performed following the Moore and Stupp's procedure,[29] in which authors make use of 4-(dimethylamino)pyridinium 4-toluenesulfonate (DPTS) to accelerate the esterification of a carboxylic acid activated with *N, N'*-dicyclohexylcarbodiimide (DCC). The acetal of compound (2) is then eliminated to regenerate the diol, obtaining compound (3), which finally reacts with 1,1'-carbonyldiimidazole (CDI) in a sequential nucleophilic attack of both hydroxyl groups of (3) to the carbonyl carbon of CDI.[30] The cyclic carbonate MAC was obtained with an overall yield of 34 %. Its formation was corroborated in the FTIR spectrum by the appearance of bands above 3000 and at 1650 cm^{-1} corresponding to Csp^2-H and C=C bonds, respectively, and by the absence of the O-H band, which should be wide and intense and be located about 3600-3200 cm^{-1} (annex B, figure B.11). Allyl group was also identified by a doublet of doublet of triplets and two doublets of quartets located at 5.91, 5.36 and 5.31 ppm of the ^{1}H-NMR spectrum (annex B, figure B.9). All spectra are collected in annex B.

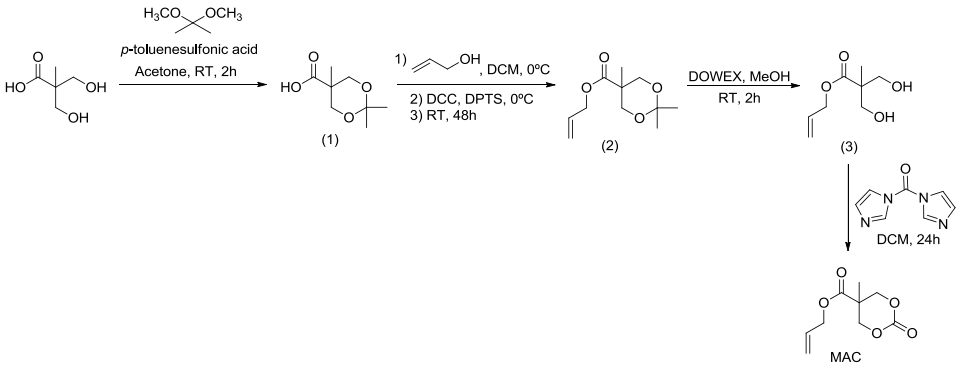

Figure 3.1. Synthesis pathway of 5-methyl-5-allyloxycarbonyl-1,3-dioxan-2-one (MAC).

The organocatalytic ROP of MAC has been investigated using DBU-TU as organocatalytic system and benzyl alcohol as initiator.[31] In the present work, the said reaction has been studied in order to find the best conditions for achieving a polymerization degree around 10 that, as it will be explained in the following sections, from this initial alkene functionalized polymer is possible to obtain thermoresponsive homopolymers by thiol-ene post-functionalization and PMAC-*b*-MPC block copolycarbonates through the subsequent copolymerization. The average theoretical degree of polymerization (DP_t) in a controlled polymerization is given by equation 1:

$$DP_t = \frac{[M]_0}{[I]_0} \cdot C \tag{1}$$

Where $[M]_0$ and $[I]_0$ are the initial concentration of monomer and initiator, respectively, and C the monomer conversion defined by equation 2:

$$Conversion\ (C) = \frac{[M]_0 - [M]_t}{[M]_0} \tag{2}$$

Where $[M]_t$ is the residual monomer concentration at a time *t*. Therefore, assuming a final conversion of 100 %, the study started by carrying the reaction out with a $[MAC]_0$:$[BnOH]_0$ relation of 1:0.1 according to previously reported conditions: dry dichloromethane, 35 °C, $[MAC]_0$=1.0 M and a [MAC]:[TU]:[DBU] relation of 1:0.05:0.01.[14] The evolution of both C and DP was studied by taking aliquots of the reaction every 15 min and studying each ^{1}H-NMR spectrum. Figure 3.2 shows ^{1}H-NMR spectrum after 15 min of starting the polymerization (above) and the evolution of the monomer and polymer signals along reaction time (below). As observed, the course of the reaction leads to a continuous decrease of the monomer signal *f'*, which corresponds to two of its diastereotopic protons, whereas the PMAC signal *i*, attributed to four protons per repetitive unit of the main chain, appears and increases as polymerization progresses. The evolution of experimental monomer conversion (C_{NMR}) was then estimated following equation 3, which makes use of the relative integration of *f'* and *i* signals at each reaction time:

$$C_{NMR}(\%) = \frac{I_{PMAC}(i)}{I_{PMAC}(i) + 2 \cdot I_{MAC}(f')} \cdot 100 \tag{3}$$

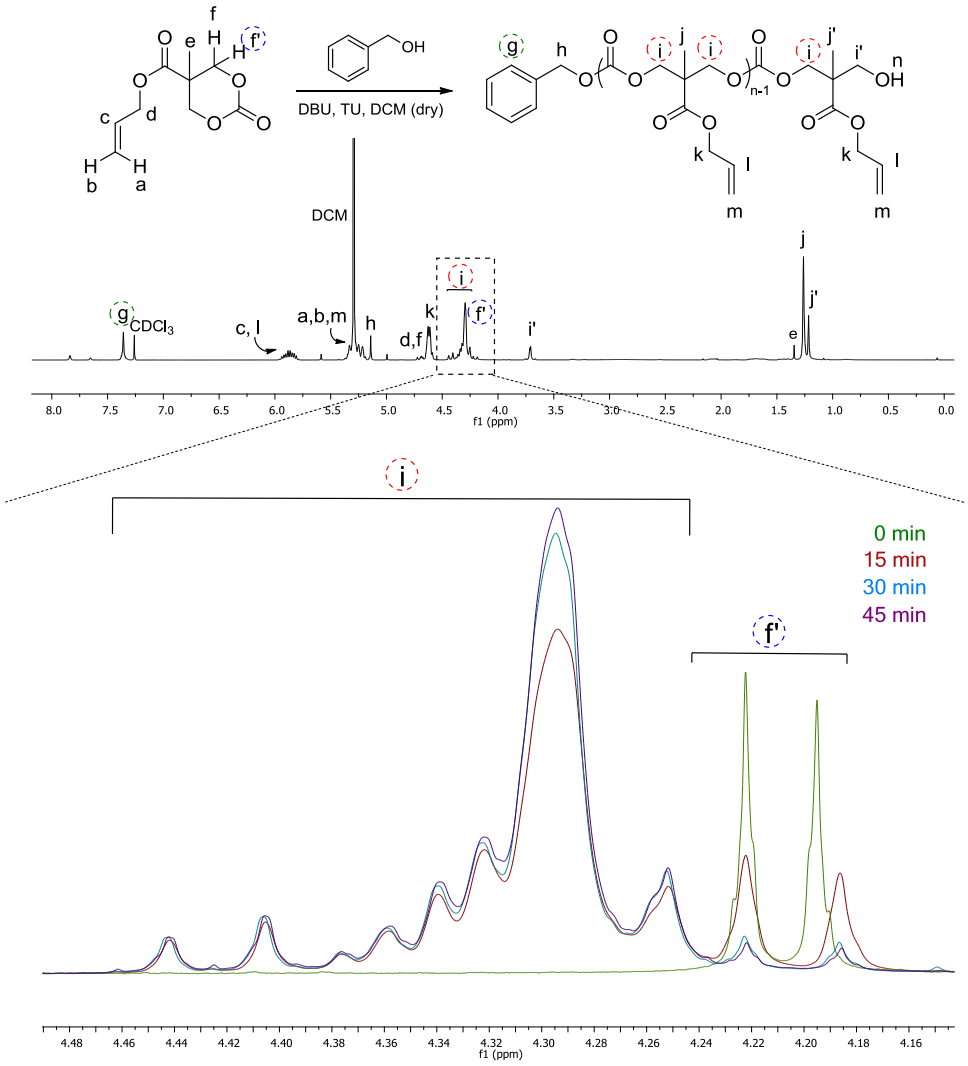

Figure 3.2. ^{1}H-NMR spectrum ($CDCl_3$, 300 MHz) registered after 15 min (above) and ^{1}H-NMR signals used in equation 3 to calculate experimental conversion registered at 0, 15, 30 and 45 min(below).

DP was also estimated experimentally from ^{1}H-NMR spectra (DP_{NMR}). Benzyl alcohol is broadly used as polymerization initiator because it provides easily-identifiable aromatic protons. Then, they allow studying the course of DP by relating the integration of terminal aromatic proton signals (*g* in figure 3.2 above) with those of the PMAC repetitive units (*i* in figure 3.2), or any other that appears in well-differentiated regions of the spectrum. This value can also be used to calculate the number-average molecular weight (M_n^{NMR}, equation 4), where $M_{initiator}$ is the molar mass of the benzyl alcohol (108.14 g/mol) and M_{MAC} that of each repetitive unit (200.19 g/mol):

$$M_n^{NMR} = M_{initiator} + DP_{NMR} \cdot M_{MAC} \qquad (4)$$

Some conclusions can be extracted from the results summarized in table 3.1. First, 100 % of monomer conversion was not achieved even after 4 h of reaction. Against, 93 % was found to be the maximum conversion for said reaction conditions, and it was reached after 1 h. Consequently, the final DP was approximately 7 after 1h of reaction.

Table 3.1. Data from ^{1}H-NMR study of MAC polymerization.

Time (min)	C_{NMR} (%)	DP_{NMR}	$DP_{theoretical}$	M_n^{NMR}	$M_n^{theoretical}$
0	0	0	0	-	-
15	73	5	7	1108	1508
30	90	6	9	1308	1908
45	92	6	9	1308	1908
60	**93**	**7**	**9**	**1508**	**1908**
90	93	7	9	1508	1908
240	93	7	9	1508	1908

To obtain a PMAC homopolymer with a higher DP, the reaction was carried out again, this time with a $[MAC]_0$:[BnOH] relation of 1:1/13. However, flash column chromatography performed during the purification process did not allow to properly separate the monomer from low-DP oligomers and, hence, the average-DP observed by ^{1}H-NMR was higher than expected (13 instead of 10) due to the removing of the low-DP fraction. Further analysis of PMAC polymer by MALDI-ToF MS revealed a major distribution with a spacing of 200 m/z, which is equal to that of a monomer unit, whose main peak (m/z = 1731) corresponds to a sodium charged polymer chain of DP=8 with a benzyl alcohol end group (annex C, figures C.1 and C.2). PolyTools software enabled to calculate M_n and average-DP, which resulted being 2036.6 g/mol and 10, respectively. Although it is usual to find divergences between NMR and MS results on polymers, differences in average-DP between MS and ^{1}H-NMR spectra may be partially explained by a minor distribution found at low molecular weight region of MS spectrum (figure C.2). As its main peak reveals (m/z = 997), it could correspond to a sodium charged polymer chain of DP=5 initiated with a water molecule instead of benzyl alcohol and, therefore, with one hydroxyl group at each end group; or maybe a result of a loss of a benzyl ester ascribed to midchain cleavage followed by loss of CO occurring during the ionization, as some authors propose.[32] However, since its presence is expected not to disturb final properties due to its low quantity with respect to the main distribution, DP=13 from ^{1}H-NMR spectrum will be considered as the DP of PMAC.

3.2. Synthesis, characterization and study of hydrophilic and thermoresponsive properties of functionalized homopolymers (*Series I*)

Self-assembly properties in aqueous media are often related to amphiphilic architectures, that is, polymers must have both hydrophilic and hydrophobic blocks. Since aliphatic polycarbonates constitute a hydrophobic chain, at least one of the moieties with which the polymer will be functionalized must provide hydrophilicity. Then, before copolymerization, it was proposed to study the functionalization of PMAC with two different chains, DSH and TEGSH, in order to optimize their properties as hydrophilic block. Additionally, thermoresponse was also studied according to the previous described thermoresponsiveness of OEG-based polymers. Temperature at which OEG-based polymers respond usually changes with DP and length of the OEG chain.[33] Ajiro *et al.* studied the thermoresponse of polycarbonates based on different chains, including 2-{2-(2-methoxyethoxy)ethoxy}ethyl tosylate, which is quite similar to TEGSH.[30] Cloud Point Temperature (T_{cp}, which refers to the temperature at which the phase transition of a polymer solution of certain concentration occurs from the soluble state to the aggregated state)[21] was obtained at 33 °C, close to body temperature, with DP=25. Then, it is expected that PMAC homopolymer, whose DP is lower but in the same order of magnitude, could show similar LCST values or even higher when functionalized with TEGSH, since the hydroxyl group located at the end of the chain will potentially provide higher hydrophilicity and, consequently, higher LCST values.[34]

Functionalization was performed through radical thiol-ene reaction using DMPA as radical UV-photoinitiator and a Hg UV-lamp (see annex A.3), by which quantitative conversion of the vinyl groups (according to ^{1}H-NMR spectra shown in annex B.3 and FTIR spectrum of figure 3.3b) was achieved with thiol:ene proportions of 2:1 after 4 h of reaction. Three polymers were obtained using different amounts of each hydrosoluble chain, as summarized in figure 3.3a. PMAC-SD was first synthetized; however, the resultant polymer turned out to be insoluble in water. PMAC was then functionalized with TEGSH, resulting in a hydrosoluble polymer that turned insoluble when heated (PMAC-STEG). Finally, it was hypothesized that the combination of both chains would affect the LCST and, consequently, the synthesis of one last statistic copolymer that combines both chains in a relation TEGSH:DSH of 75:25 was performed. As expected, a hydrosoluble polymer that, again, responded to temperature when heated was obtained (PMAC-STEG-*st*-SD).

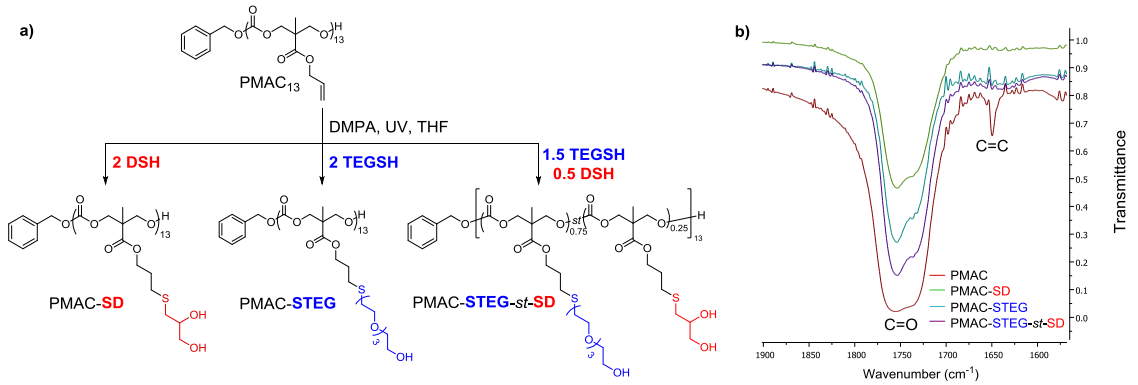

Figure 3.3. Functionalization of PMAC by thiol-ene reaction (*Series I)* (a) and 1900-1600 cm^{-1} region of all FTIR spectra, in which complete alkene functionalization is confirmed (b).

For the application of thermoresponsive polymers, several techniques can be employed to characterize them and, in fact, deviations of T_{cp} are usually noticed depending on the method used. Turbidimetry determines T_{cp} as the transition from a homogeneous solution into a heterogeneous cloudy phase with a concentrated polymer phase dispersed in a diluted polymer solution phase.[21] Then, the concentrated phase droplets scatter the incident light when performing an UV-vis spectroscopy experiment, leading to a decrease in transmittance. These phases re-mix upon cooling, which results in an increase in transmittance. In this case, transmittance was constantly measured during a temperature program, using a wavelength at which polymer solution does not absorb, and the resulting value was plotted (figure 3.4). T_{cp} was determined at the temperature showing a 10 % drop from the original transmittance due to the light scattering upon transition into a heterogeneous cloudy phase.

Measurements were performed by using two different scan rates: 1 and 0.5 ºC/min. Since T_{cp} results barely changed, 1 ºC/min was selected as scan rate (more information is detailed in annex E). T_{cp} of PMAC-STEG was 24 °C during the heating process with a polymer concentration of 1 mg/mL, and it decreased slightly (until 21 ºC) by cooling (figure 3.4.a, blue). Besides, PMAC-STEG-*st*-SD showed T_{cp} values at 17 °C that decreases to 14 ºC when cooled (figure 3.4a, red). Given that DSH is more hydrophobic than TEGSH (PMAC-SD was insoluble in water even at 0 ºC), decrease in T_{cp} should be ascribed to the increased hydrophobicity effect. As observed, a hysteresis curve appears when cooling. This could be a result of polymer-polymer interactions, such as H-bonds, that stabilize the aggregates and avoid a fast rehydratation of polymer chains.[33]

The concentration dependence of T_{cp} was also examined. As seen in figure 3.4b, the lowest concentrations, 0.5 and 0.1 mg/mL, broadened the transition behaviors in

PMAC-STEG and increased the LCST temperatures to 27 and 37 °C, respectively. For PMAC-STEG-*st*-SD, the lowest concentrations resulted in values of 16 and 24 °C (annex E, figure E.4). These results evidences the concentration-dependence of T_{cp}, whose shift as concentration decreases could be partially related to the formation of smaller hydrophobic aggregates at slightly higher temperature and, consequently, the solution turns cloudy later, in a more continuous way and scatters less light.[34]

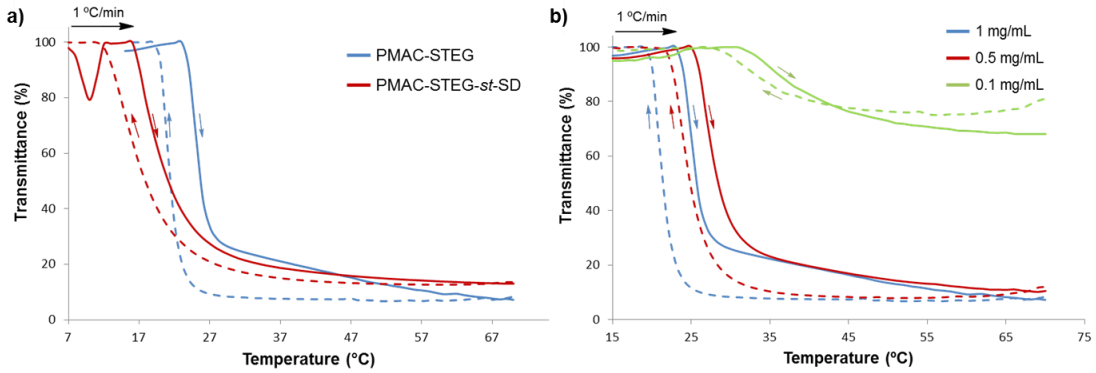

Figure 3.4. Transmittance of (a) aqueous solutions of PMAC-STEG and PMAC-STEG-*st*-SD ([polymer]=1 mg/mL) and (b) those of PMAC-STEG varying concentrations, at 450 nm upon heating from 7 ºC (PMAC-STEG-*st*-SD) or 15 ºC (PMAC-STEG) to 70 °C and cooling back.

T_{cp} was estimated by DLS at temperatures ranging from 15 to 50 ºC ([polymer]=0.1 mg/mL) in order to investigate thermoresponsive aggregation behavior. Although solutions were optically clear at the lowest temperature, DLS intensity curve (figure 3.5a) indicated the coexistence of small particles with hydrodynamic diameters (D_h) of approximately 30 and 5 nm for PMAC-STEG and PMAC-STEG-*st*-SD, respectively, and larger aggregates having D_h values about 190, 70 and 460 nm. However, as evidenced by number distribution shown in figure 3.5b, the latter are a minority. These results could be a consequence of the coiled conformation that polymers usually adopt below their LCST,[35] or of the formation of small micelles of single polymer chains.[36]

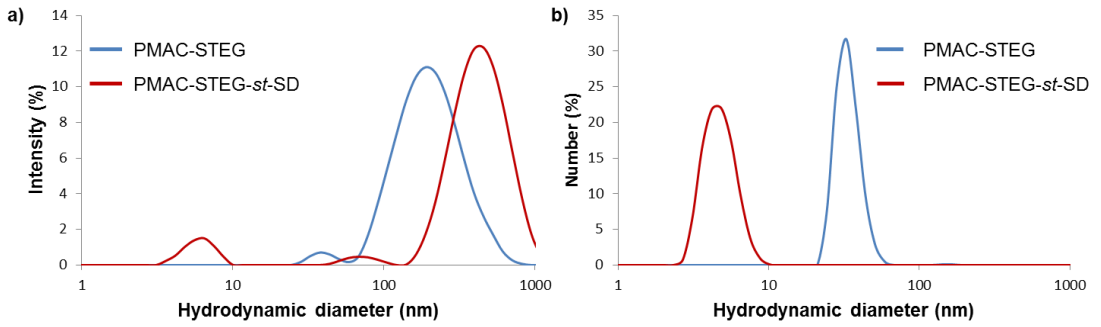

Figure 3.5. Intensity (a) and number size distribution (b) measured by DLS (logarithmic scale) at 15 ºC (below both LCSTs) for aqueous solution of PMAC-STEG (blue) and PMAC-SD (red).

Upon heating, the size of aggregates grew progressively, as shown in figure 3.6a for PMAC-STEG. Aggregation behavior is explained by the fact that, as temperature increases, OEG chains of the polymer are partially dehydrated leading to agglomeration and the formation of larger particles known as mesoglobules.[35] When D_h is represented as function of temperature, an abrupt transition is observed at almost the same T_{cp} determined by turbidimetry in said concentrations: around 35ºC for PMAC-STEG (figure 3.6b) and 20 ºC for PMAC-STEG-*st*-SD (figure 3.6c). After all these experiments, it was concluded that PMAC-STEG could be the best option to be used as hydrophilic and potentially thermoresponsive block of the amphiphilic copolymer, since its thermal response is closer to body temperature than that of PMAC-STEG-*st*-SD.

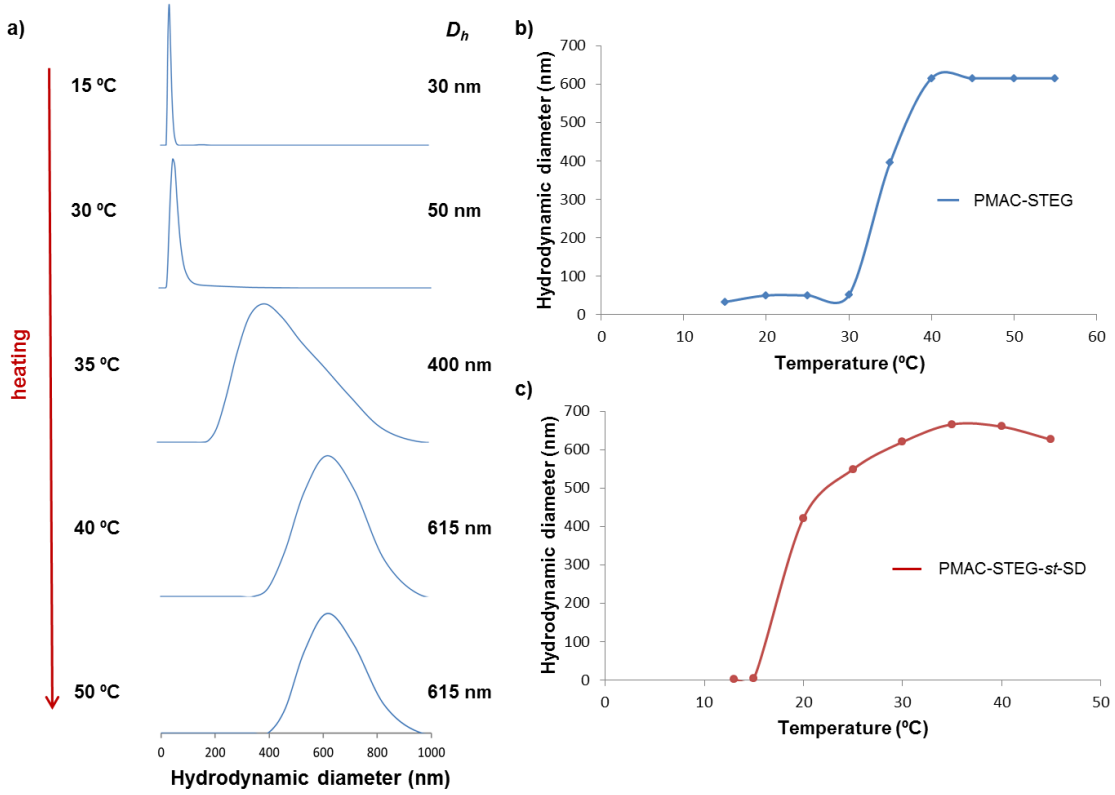

Figure 3.6. DLS number size distributions of PMAC-STEG in water at various temperatures (a) and plots of D_h as a function of temperature for PMAC-STEG (b) and PMAC-STEG-*st*-SD (c).

Changes in size and morphology after heating were studied by TEM. First, 0.5 mg/mL solutions of both polymers were prepared by solving each of them in water while temperature was kept below LCST. Samples were then deposited on a copper grid with carbon film and dyed using uranyl acetate. Moreover, both solutions were heated to 50 ºC and, again, deposited on different grids. Appreciable changes can be noticed in both cases when heating, as seen in figure 3.7.

Below LCST, both samples show high polydispersity but a major contribution of small and spherical micelles. Taking into account that the hydrodynamic diameter from DLS should be slightly larger than the diameter extracted by TEM, size of PMAC-STEG micelles (25 nm, figure 3.7a) matches that of DLS (30 nm). Small contribution of larger aggregates around 75 nm is also found (annex F, figure F.2). High polydisperse micelles with an average diameter of 35 nm are observed for PMAC-STEG-*st*-SD (figure 3.7b). These results agree with the previously commented of DLS, since intensity curve noticed the presence of more than one size distribution. With raising the temperature above the LCST, the diameter flagrantly increased to approx. 500 nm for PMAC-STEG and 180 nm for PMAC-STEG-*st*-SD (figure 3.7c and d, respectively). Then, as DLS showed, the dehydration of hydrosoluble TEGSH chains leads to an increase of hydrophobicity and, finally, to the formation of large aggregates in water. More TEM images are collected in annex F.

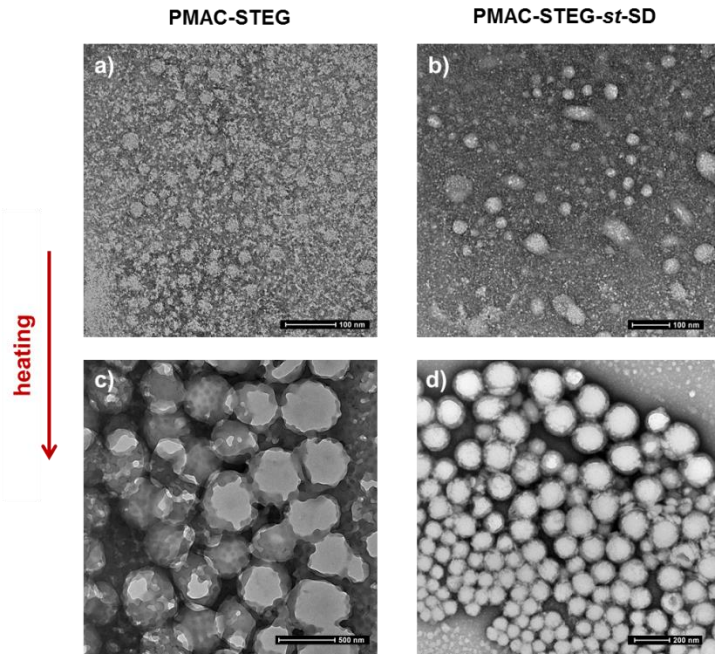

Figure 3.7. TEM images of PMAC-STEG and PMAC-STEG-*st*-SD aggregates below (a, b) and above (c, d) LCST.

3.3. Synthesis and characterization of amphiphilic and thermoresponsive block copolycarbonates by ROP and orthogonal click reactions (*Series II*)

In the work carried out by Pratt *et al.* the synthesis of block and statistical copolymers was studied through the sequential ROP of different cyclic carbonates including MPC;[13] however, the copolymerization with MAC has not been performed yet. Here, PMAC homopolymer was used as macroinitiator (although PMPC could be used as well) to study the copolymerization of the second monomer, MPC. ROP was performed following the same conditions used for the PMAC homopolymerization, using DBU-TU as organocatalytic system, DCM as solvent and a polymer concentration of 1.0 M. The macroinitiator:monomer relation was adjusted in order to reach a DP of the second block as similar as possible to the first one and, as a result, $PMAC_{13}$-*b*-MPC_{14} block copolymer was obtained according to ^{1}H-NMR and FTIR spectra (annex B.4, figures B.20 and B.21). Size Exclusion Chromatography (SEC) analysis also confirm the effectiveness of the copolymerization process, since block copolymer showed a monomodal distribution peak (*Đ* value of 1.15) shifted to lower retention time compared to PMAC due to the increase in the mass (figure 3.8).

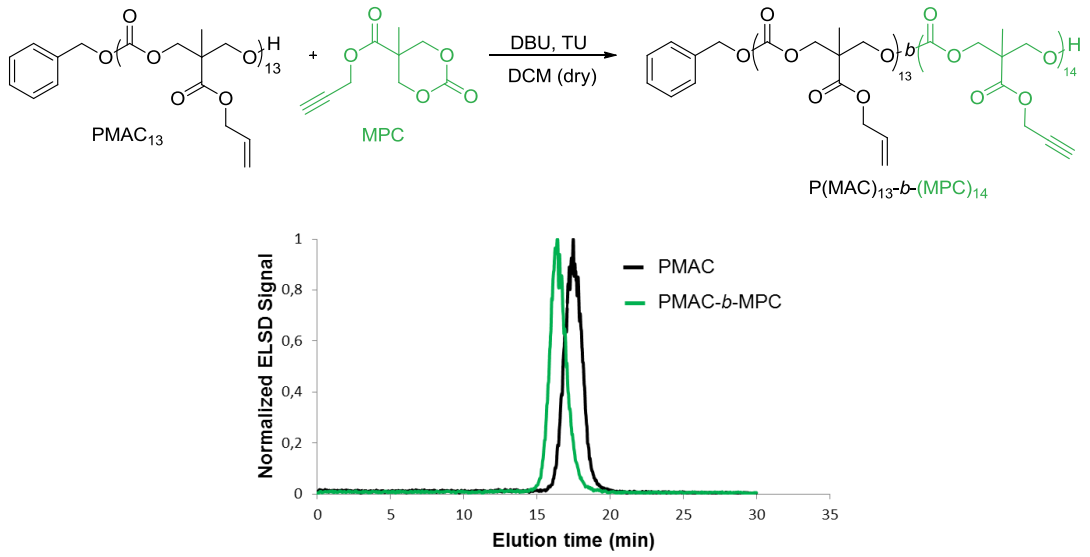

Figure 3.8. Copolymerization of PMAC and MPC (above) and SEC analysis of PMAC and PMAC-*b*-MPC (below).

The orthogonal functionalization of both blocks was then set. As said, propargylic pendant groups can be functionalized with azides attached to hydrophobic and stimuli-responsive moieties by CuAAC click reaction, whereas functionalization of the allylic block will provide water solubility and potential thermoresponse by means of thiol-ene

click reaction of TEGSH. Since thiols can react not only with alkene but also with alkyne groups,[37] the alkyne-selective 1,3-dipolar cycloaddition was first carried out. As seen in figure 3.9, the viability of both CuAAC and thiol-ene click reactions was first checked in *Series II*, in which an azide without any functional group was introduced to avoid interferences on subsequent reactions. N_3-C16 with a hydrophobic long alkyl chain was synthetized and incorporated to the copolymer for this purpose (synthesis procedure explained in annex A.5). Following the guidelines of aforementioned works,[14] the first reaction was be carried out with a [propargyl]:[azide]:[Cu(I)] relation of 1:2:0.3, and thiol-ene reaction was performed just as explained in section 3.2.

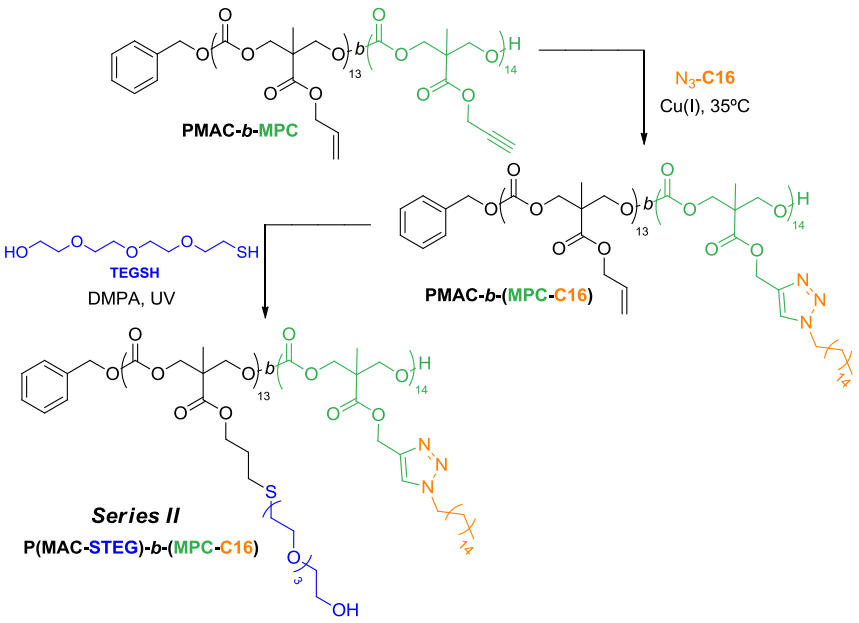

Figure 3.9. Synthetic pathway for the orthogonal functionalization of block copolycarbonates to get P(MAC-STEG)-*b*-(MPC-C16) amphiphilic block copolymer.

First, CuAAC quantitative modification of propargyl block was assessed by ^{1}H-NMR analysis (annex B, figure B.25) from the disappearance of the alkynyl proton (annex B, figure B.20, signal f), the shifting of the vicinal methylenic protons from 4.73 to 5.28 ppm (figure B.20 and B.25, signal e), and the appearance of the triazole proton at 7.59 ppm (figure B.25, signal f) in ^{1}H-NMR spectra. Then, thiol-ene reaction was conducted. Although almost all alkene groups were modified, as seen in ^{1}H-NMR spectrum collected figure B.26 of annex B, complete functionalization was not achieved since allyl protons are still observed when spectrum is highly magnified. Nevertheless, as shown in figure 3.10a, FTIR signal due to C=C bond is not detected when P(MAC-STEG)-*b*-(MPC-C16) spectrum is compared to that of PMAC-*b*-MPC. The presence of some residual alkene groups in contrast to the complete functionalization of PMAC can

be a consequence of steric impediment provided by the hydrophobic block. However, the residual alkene group should not have any significant influence on final properties. Besides, SEC of final copolymer showed a peak shifted to lower retention time compared to PMAC-*b*-MPC due to the increase in mass (figure 3.10b) and, again, proving that a very high functionalization was achieved. The small peak shown at higher elution time seems to coincide with some residual TEGSH (annex D, figure D.2).

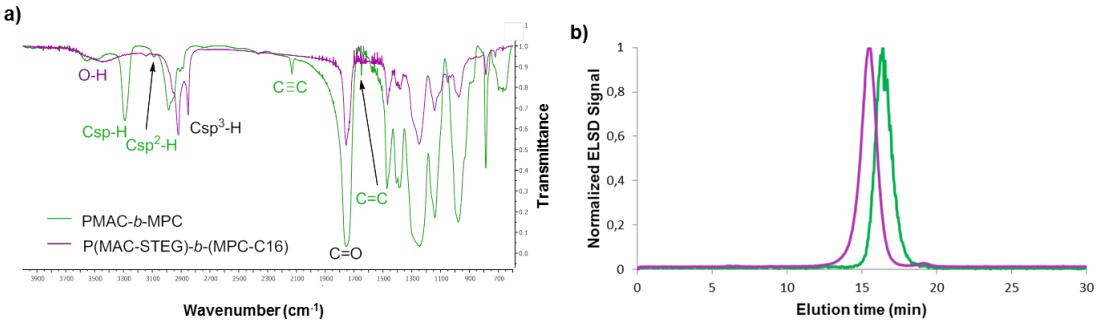

Figure 3.10. FTIR spectra (a) and SEC analysis (b) of PMAC-*b*-MPC (green) and P(MAC-STEG)-*b*-(MPC-C16) (purple).

3.4. Preparation, characterization and study of thermoresponsive behavior of polymeric aggregates in water

Self-assembly of P(MAC-STEG)-*b*-(MPC-C16) was carried out by co-solvent method. Water was gradually added (in 10 µL proportions) over a solution of the copolymer (5 mg) in THF (1 mL) until self-assembly occurs. The process was monitored by turbidimetry, recording the modification of the transmittance at 650 nm that happens when aggregates are formed (due to the light scattering by the aggregates). This measurement was carried out in absorbance mode as seen figure 3.11. Water was added until a constant value of turbidity was reached. The resulting aggregates were dialyzed against water for 2 days to remove THF, and finally filtered through a 1 µm cellulose acetate filter due to the presence of precipitate. Water suspension had a final concentration of aggregates of 0.9 mg/mL.

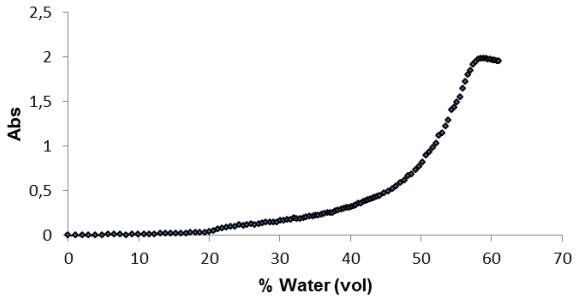

Figure 3.11. Turbidimetry curve recorded for P(MAC-STEG)-*b*-(MPC-C16) self-assembly.

DLS was used to determine the D_h of aggregates in water at room temperature. The experiment was performed three times to ensure the reproducibility of the measurements, so results collected in figure 3.12 shows an average. It is observed a single but broad distribution of aggregates with a mean D_h around 157 nm. Number size distribution curve is represented instead of intensity one because the latest show a higher contribution of the larger aggregates and, for highly polydisperse samples as this one is, it does not show a duly representation of the size of self-assemblies.

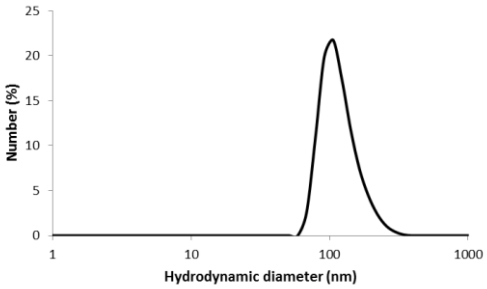

Figure 3.12. Number size distribution measured by DLS (logarithmic scale) of P(MAC-STEG)-*b*-(MPC-C16) self-assemblies after filtration at 25 ºC.

The morphology of the self-assemblies was inspected by TEM. It was observed that, although copolymer forms self-assemblies, it also tends to aggregate resulting in particles of large size (more than 1 µm) that finally precipitate. Therefore, three TEM samples were prepared: before dialysis, after dialysis and, finally, after filtration through 1 µm cellulose acetate filter. The first grid shows a highly polydisperse sample with some larger than 1 µm aggregates (annex F, figure F.13), but also others that range from 600 to 100 nm (figure 3.13a). The second sample shows, as consequence of precipitation, a great contribution of macroscopic aggregates (figure F.15). However, it is also observed some smaller self-assemblies with no defined structure (figure 3.13b). The latest are also seen after filtration and, again, they show diversity in morphology (figure 3.13c). Since no unique morphology is observed, further studies are required to get more information about self-assemblies structure.

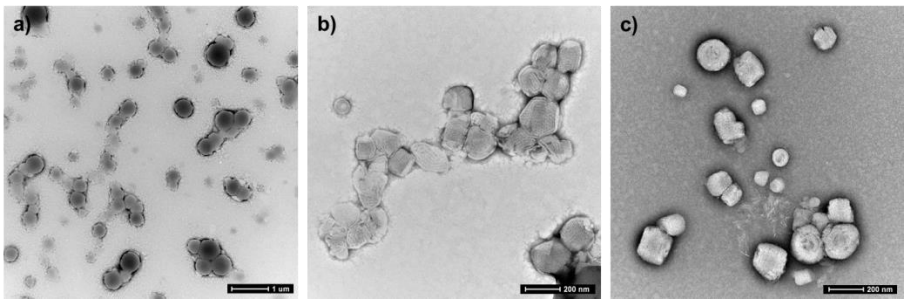

Figure 3.13. TEM images of P(MAC-STEG)-*b*-(MAC-C16) before dialysis (a), after dialysis (b) and after filtration (c).

In order to study the thermoresponsive behavior of P(MAC-STEG)-*b*-(MPC-C16), and since no change in turbidity was detected on heating, DLS measurements were performed at several temperatures following the same procedure explained in section 3.2. Aggregates have similar size distribution with an almost constant average D_h around 157 nm when heating from 15 to 30 ºC. However, a slight shift in the number size distribution peak occurs when temperature increases from 30 to 35 ºC. The resulting size distribution has a very similar mean D_h (159 nm) but a slightly broaden distribution. Upon heating to 70 ºC, the shape and size distribution is maintained, as seen in figure 3.14. Although the increase in size is not as large as in previous cases and clouding of the aggregates solution is not noticed, the temperature at which this occurs (35 ºC) is reasonably close to the T_{cp} of the already studied PMAC-STEG. Then, this could be a consequence of the thermoresponsive behavior of the hydrophilic block of the amphiphilic block copolymer and, hence, proving the thermoresponsiveness of the resulting aggregates.

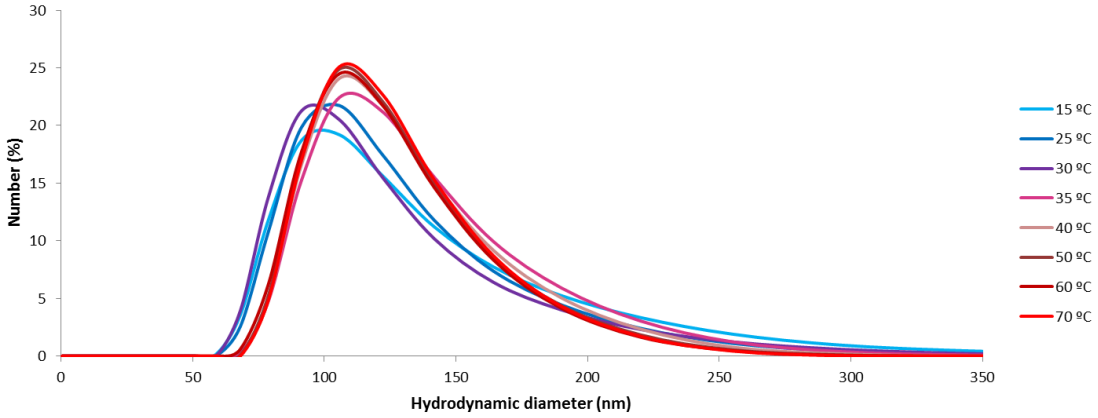

Figure 3.14. Number size distribution measured by DLS of P(MAC-STEG)-*b*-(MPC-C16) aggregates performed at from 15 to 70 ºC.

3.5. Synthesis and characterization of amphiphilic and stimuli-responsive block copolycarbonates by orthogonal click reactions (*Series III* and *IV*)

In order to check the versatility of the orthogonal click reactions as strategy to incorporate more than one stimuli-responsive moiety, two new polymer series in which the hydrophobic block was functionalized with a stimuli-responsive group were proposed to be synthetized. As seen in figure 3.15, propargyl pendant groups of *Series III* were functionalized with a pH-responsive amine and, subsequently, alkene block was modified with the thermoresponsive TEGSH chain. Furthermore, in the case of *Series IV*, a light-responsive azobenzene and TEGSH were introduced following the same strategy. Whereas in both series CuAAC leaded to quantitative functionalization of propargyl groups (according to ^{1}H-NMR spectra shown in figures B.32 and B.45 of annex B), the subsequent thiol-ene reactions did not show the expected results.

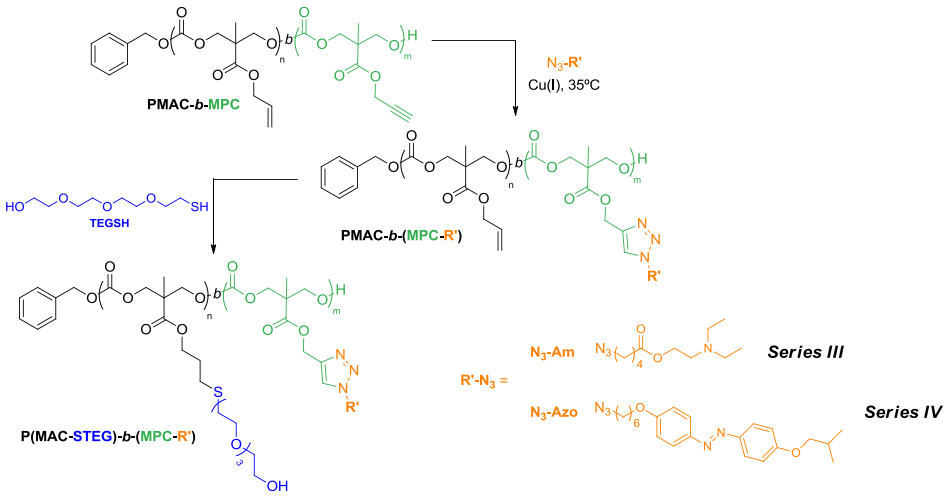

Figure 3.15. Synthetic pathway for the orthogonal functionalization of block copolycarbonates to get pH-responsive (*Series III*) and light-responsive (*Series IV*) amphiphilic block copolymers.

In the case of *Series III*, ^{1}H-NMR spectrum performed after thiol-ene functionalization shows three undesirable results. First, alkene groups are again not completely functionalized but 85 % of them are modified with TEGSH (figure 3.16 signals a and b). Secondly, signals of protons surrounding amine (figure 3.16, signals c, d, e and f) have suffered a chemical shift downfield. It has been reported that amines (such as DBU or trimethylamine, which it is more similar to the actual amine) can deprotonate thiols affording the thiolate anion and the formation of a bonded disulfide radical anion.[38] Consequently, if amine is basic enough, thiol deprotonation can retard thiol radical-mediated reaction. This would explain both the failure to reach complete conversions and the fact that signals are shifted, since the amine would be protonated.

A decrease in integration of c to f signals of the ^{1}H-NMR spectrum is also shown when compared to that of PMAC-*b*-(MPC-Am). Then, it is clear that the ester has suffered some kind of side reaction. When FTIR spectrum after thiol-ene reaction is observed, it is noticed that O-H band becomes broad, ranging from 3700 to 2500 cm^{-1} (annex B, figure B.34). This behavior is typically shown by carboxylic acid O-H bond and, hence, it is hypothesized that a hydrolysis process has occurred in the ester by which amine is pending, which could explain the decrease in integration of the ^{1}H-NMR signals.

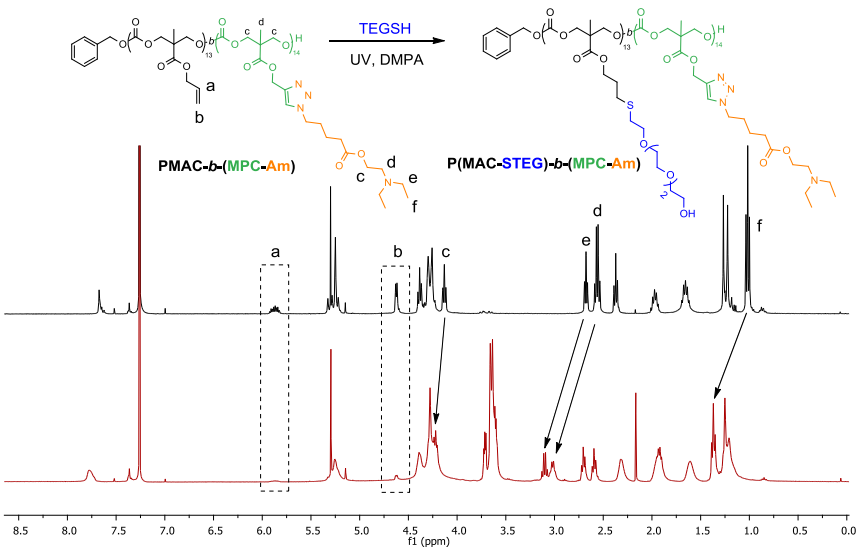

Figure 3.16. ^{1}H-NMR spectrum of pH-responsive copolymer before and after thiol-ene reaction

In the case of *Series IV*, both PMAC and the subsequent copolymer were synthetized again reaching a DP relation of PMAC$_{15}$-*b*-MPC$_{15}$ (experimental details are in annex A.7; and all spectra in annex B.7). While copolymer was easily functionalized with N_3-Azo giving PMAC-*b*-(MPC-Azo), problems were encountered when performing the thiol-ene reaction. DMPA, which absorbs from 310 to 390 nm, was used as photoinitiator just as for series *I, II* and *III;* however, alkene groups did not react since ^{1}H-NMR spectrum did not show any change in alkene protons integration. Hg-UV source with maximum emission at 365 nm was used for illuminating the sample. At this wavelength, the azobenzene attached to the polymer absorbs (figure 3.17b) and reversibly photoisomerize its N=N bond (figure 3.17a). Azobenzenes exist in a thermodynamically stable *trans (E)* isomer with a strong π-π* absorption band, in this case at 360 nm, and a weaker n-π* one around 450 nm.[39] By irradiating with UV light, they isomerize to *cis (Z)* (dashed line in figure 3.17b). Then, a possible explanation for the failure is that azobenzenes absorb and, consequently, isomerize when irradiating with 365 nm light and do not lead DMPA form radicals and start the reaction.

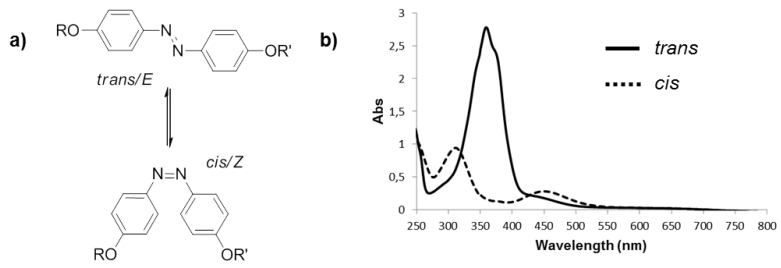

Figure 3.17. *Trans-cis* isomerization of azobenzene (a) and absorption spectrum of PMAC-*b*-(MPC-Azo) before (continuous line) and after irradiation under 365 nm light (dashed line) (b).

Taking into account these results, reaction was again carried out, this time using titanocene complex (Irgacure 784) as visible light photoinitiator and illuminating with a 530 nm LED as source. However, no functionalization was achieved, so the residual absorption of azobenzene at this wavelength seems to be enough to promote isomerization and still be an impediment for the advance of the reaction. Finally, thermal initiated thiol-ene reaction was tested using AIBN (2,2′-azobis(2-methylpropionitrile)) as thermal initiator. The reaction was performed at 90 ºC for three days and using thiol:ene and AIBN:ene proportions of 3:1 and 0.4:1, respectively. After ^{1}H-NMR spectrum analysis, it was observed that these conditions successfully leaded to the functionalization of aprox. 80 % of the allylic block (according to z and z' signals of figure 3.18). SEC analysis also noticed an increase of mass proving a high functionalization (annex D, table D.1 and figure D.3). Hence, synthesis of amphiphilic and potentially thermo and light-responsive block copolycarbonate P(MAC-STEG)-*b*-(MPC-Azo) has been achieved. Thermal initiation is then presented as an alternative to light-induced radical initiated thiol-ene reaction.

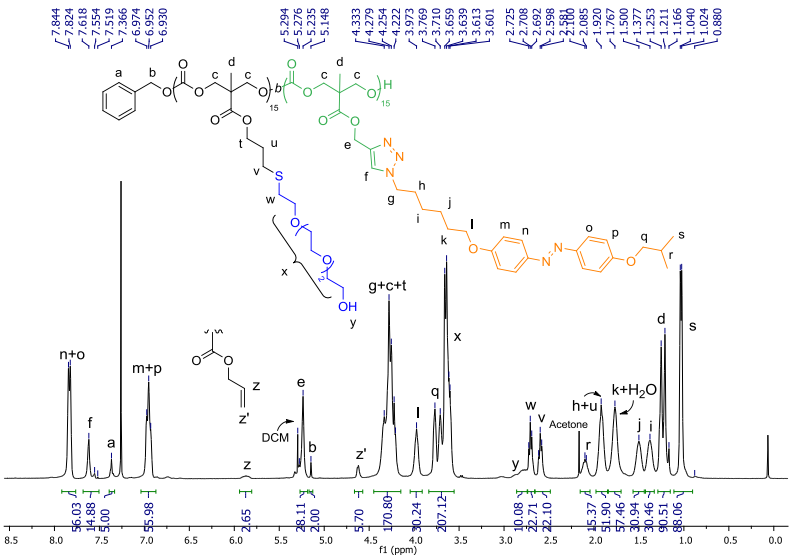

Figure 3.18. ^{1}H-NMR spectrum of P(MAC-STEG)-*b*-(MPC-Azo) ($CDCl_3$, 400 MHz)

4. SUMMARY AND CONCLUSIONS

Cyclic carbonates derived from bis-MPA are excellent monomers for the preparation by ROP of homopolymers, such as PMAC, that are able to be functionalized by post-polymerization modifications. In this way, radical thiol-ene reaction has been used to obtain thermoresponsive polycarbonates from PMAC, which are initially hydrosoluble but collapse above LCST. The resultant polymers, PMAC-STEG and PMAC-STEG-*st*-SD, show different T_{cp} depending on the composition. In particular, PMAC-STEG has a T_{cp} close to body temperature. According to the morphological changes observed at the LCST, from small micelles to larger aggregates, homopolymers could be conveniently exploited for the preparation of thermoresponsive nanocarriers whose T_{cp} can be adjusted by properly selecting the thiols used in the post-functionalization. Besides, they can be used as the hydrophilic block for obtaining amphiphilic block copolymers.

A synthetic route for orthogonal functionalization of block copolycarbonates has been developed. Copolymerization of PMAC with MPC by ROP is an effective route to obtain orthogonal clickable copolymers provided by allyl-based and a propargyl-based blocks and, therefore, with great versatility in their functionalization. The orthogonal modification of the blocks through CuAAC and thiol-ene reactions for the reaction of propargyl and allyl pendant groups, respectively, has leaded to the synthesis of three series of polymers. *Series II* gave rise to an amphiphilic and thermoresponsive block copolymer that forms self-assemblies in water and respond when heated above 35 ºC.

Following the same strategy, it has been tried to synthetize pH (*Series III*) and light-responsive (*Series IV*) amphiphilic block copolycarbonates. Although CuAAC reaction successfully introduced both stimuli-responsive moieties in each polymer series, UV light initiated thiol-ene reaction did not lead to the expected amphiphilic polymers. Nevertheless, the use of AIBN as thermal initiator in azobenzene containing block copolymer (*Series IV*) has finally leaded to the functionalization of allyl pendant groups to get the hydrophilic and thermoresponsive block. Therefore, an amphiphilic and potentially thermo- and light-responsive block copolycarbonate has been obtained.

The main conclusion of the project is that it is possible to prepare orthogonal clickable block copolymers able to be selectively functionalized with hydrophilic/hydrophobic stimuli-responsive moieties. In particular, thermoresponse close to physiological temperature can be easily introduced to modulate the properties of nanocarriers based on degradable polycarbonates.

5. REFERENCES

(1) Harashima, H.; Sakata, K.; Funato, K.; Kiwada, H. Enhanced Hepatic-Uptake of Liposomes Through Complement Activation Depending on the Size of Liposomes. *Pharm. Res.* **1994**, *11* (3), 402–406. https://doi.org/10.1023/A:1018965121222.

(2) Alexis, F.; Rhee, J.-W.; Richie, J. P.; Radovic-Moreno, A. F.; Langer, R.; Farokhzad, O. C. New Frontiers in Nanotechnology for Cancer Treatment. *Urol. Oncol.-Semin. Orig. Investig.* **2008**, *26* (1), 74–85. https://doi.org/10.1016/j.urolonc.2007.03.017.

(3) Din, F. U.; Aman, W.; Ullah, I.; Qureshi, O. S.; Mustapha, O.; Shafique, S.; Zeb, A. Effective Use of Nanocarriers as Drug Delivery Systems for the Treatment of Selected Tumors. *Int. J. Nanomedicine* **2017**, *12*, 7291–7309. https://doi.org/10.2147/IJN.S146315.

(4) Artham, T.; Doble, M. Biodegradation of Aliphatic and Aromatic Polycarbonates. *Macromol. Biosci.* **2008**, *8* (1), 14–24. https://doi.org/10.1002/mabi.200700106.

(5) Mai, Y.; Eisenberg, A. Self-Assembly of Block Copolymers. *Chem. Soc. Rev.* **2012**, *41* (18), 5969–5985. https://doi.org/10.1039/c2cs35115c.

(6) Letchford, K.; Burt, H. A Review of the Formation and Classification of Amphiphilic Block Copolymer Nanoparticulate Structures: Micelles, Nanospheres, Nanocapsules and Polymersomes. *Eur. J. Pharm. Biopharm.* **2007**, *65* (3), 259–269. https://doi.org/10.1016/j.ejpb.2006.11.009.

(7) Blanazs, A.; Armes, S. P.; Ryan, A. J. Self-Assembled Block Copolymer Aggregates: From Micelles to Vesicles and Their Biological Applications. *Macromol. Rapid Commun.* **2009**, *30* (4–5), 267–277. https://doi.org/10.1002/marc.200800713.

(8) Feng, H.; Lu, X.; Wang, W.; Kang, N.-G.; Mays, J. W. Block Copolymers: Synthesis, Self-Assembly, and Applications. *Polymers* **2017**, *9* (10), 494. https://doi.org/10.3390/polym9100494.

(9) Ravve, A. *Principles of Polymer Chemistry*, 3rd ed.; Springer-Verlag: New York, 2012. https://doi.org/10.1007/978-1-4614-2212-9.

(10) Nuyken, O.; Pask, S. D. Ring-Opening Polymerization-An Introductory Review. *Polymers* **2013**, *5* (2), 361–403. https://doi.org/10.3390/polym5020361.

(11) Xu, J.; Feng, E.; Song, J. Renaissance of Aliphatic Polycarbonates: New Techniques and Biomedical Applications. *J. Appl. Polym. Sci.* **2014**, *131* (5), 39822. https://doi.org/10.1002/app.39822.

(12) Tempelaar, S.; Mespouille, L.; Coulembier, O.; Dubois, P.; Dove, A. P. Synthesis and Post-Polymerisation Modifications of Aliphatic Poly(Carbonate)s Prepared by Ring-Opening Polymerisation. *Chem. Soc. Rev.* **2013**, *42* (3), 1312–1336. https://doi.org/10.1039/c2cs35268k.

(13) Pratt, R. C.; Nederberg, F.; Waymouth, R. M.; Hedrick, J. L. Tagging Alcohols with Cyclic Carbonate: A Versatile Equivalent of (Meth)Acrylate for Ring-Opening Polymerization. *Chem. Commun.* **2008**, No. 1, 114–116. https://doi.org/10.1039/b713925j.

(14) Roche Areta, J. A. Visible and NIR Responsive Nanocarriers from Amphiphilic Diblock Copolymers, Universidad de Zaragoza, Zaragoza, 2020.

(15) Chan, J. M. W.; Zhang, X.; Brennan, M. K.; Sardon, H.; Engler, A. C.; Fox, C. H.; Frank, C. W.; Waymouth, R. M.; Hedrick, J. L. Organocatalytic Ring-Opening Polymerization of Trimethylene Carbonate To Yield a Biodegradable

Polycarbonate. *J. Chem. Educ.* **2015**, *92* (4), 708–713. https://doi.org/10.1021/ed500595k.

(16) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective "Ligation" of Azides and Terminal Alkynes. *Angew. Chem. Int. Ed Engl.* **2002**, *41* (14), 2596–2599. https://doi.org/10.1002/1521-3773(20020715)41:14<2596::AID-ANIE2596>3.0.CO;2-4.

(17) Tornøe, C. W.; Christensen, C.; Meldal, M. Peptidotriazoles on Solid Phase: [1,2,3]-Triazoles by Regiospecific Copper(i)-Catalyzed 1,3-Dipolar Cycloadditions of Terminal Alkynes to Azides. *J. Org. Chem.* **2002**, *67* (9), 3057–3064. https://doi.org/10.1021/jo011148j.

(18) Hoyle, C. E.; Bowman, C. N. Thiol–Ene Click Chemistry. *Angew. Chem. Int. Ed.* **2010**, *49* (9), 1540–1573. https://doi.org/10.1002/anie.200903924.

(19) Roche, A.; Terriac, E.; Tejedor, R. M.; Oriol, L.; del Campo, A.; Pinol, M. Supramolecular Block Copolymers as Novel UV and NIR Responsive Nanocarriers Based on a Photolabile Coumarin Unit. *Eur. Polym. J.* **2020**, *126*, 109561. https://doi.org/10.1016/j.eurpolymj.2020.109561.

(20) Liu, R.; Fraylich, M.; Saunders, B. R. Thermoresponsive Copolymers: From Fundamental Studies to Applications. *Colloid Polym. Sci.* **2009**, *287* (6), 627–643. https://doi.org/10.1007/s00396-009-2028-x.

(21) Zhang, Q.; Weber, C.; Schubert, U. S.; Hoogenboom, R. Thermoresponsive Polymers with Lower Critical Solution Temperature: From Fundamental Aspects and Measuring Techniques to Recommended Turbidimetry Conditions. *Mater. Horiz.* **2017**, *4* (2), 109–116. https://doi.org/10.1039/c7mh00016b.

(22) Weber, C.; Hoogenboom, R.; Schubert, U. S. Temperature Responsive Bio-Compatible Polymers Based on Poly(Ethylene Oxide) and Poly(2-Oxazoline)s. *Prog. Polym. Sci.* **2012**, *37* (5), 686–714. https://doi.org/10.1016/j.progpolymsci.2011.10.002.

(23) Thomas, A. W.; Kuroishi, P. K.; Perez-Madrigal, M. M.; Whittaker, A. K.; Dove, A. P. Synthesis of Aliphatic Polycarbonates with a Tuneable Thermal Response. *Polym. Chem.* **2017**, *8* (34), 5082–5090. https://doi.org/10.1039/c7py00358g.

(24) del Barrio, J.; Oriol, L.; Alcala, R.; Sanchez, C. Azobenzene-Containing Linear-Dendritic Diblock Copolymers by Click Chemistry: Synthesis, Characterization, Morphological Study, and Photoinduction of Optical Anisotropy. *Macromolecules* **2009**, *42* (15), 5752–5760. https://doi.org/10.1021/ma9003133.

(25) del Barrio, J.; Oriol, L.; Sanchez, C.; Luis Serrano, J.; Di Cicco, A.; Keller, P.; Li, M.-H. Self-Assembly of Linear-Dendritic Diblock Copolymers: From Nanofibers to Polymersomes. *J. Am. Chem. Soc.* **2010**, *132* (11), 3762–3769. https://doi.org/10.1021/ja9083946.

(26) Blasco, E.; del Barrio, J.; Sanchez-Somolinos, C.; Pinol, M.; Oriol, L. Light Induced Molecular Release from Vesicles Based on Amphiphilic Linear-Dendritic Block Copolymers. *Polym. Chem.* **2013**, *4* (7), 2246–2254. https://doi.org/10.1039/c2py21025h.

(27) Garcia-Juan, H.; Nogales, A.; Blasco, E.; Carlos Martinez, J.; Sics, I.; Ezquerra, T. A.; Pinol, M.; Oriol, L. Self-Assembly of Thermo and Light Responsive Amphiphilic Linear Dendritic Block Copolymers. *Eur. Polym. J.* **2016**, *81*, 621–633. https://doi.org/10.1016/j.eurpolymj.2015.12.021.

(28) Roche, A.; Oriol, L.; Tejedor, R. M.; Pinol, M. Polymeric Self-Assemblies Based on Tetra-Ortho-Substituted Azobenzene as Visible Light Responsive

Nanocarriers. *Polymers* **2019**, *11* (12), 2060. https://doi.org/10.3390/polym11122060.
(29) Moore, J.; Stupp, S. Room-Temperature Polyesterification. *Macromolecules* **1990**, *23* (1), 65–70. https://doi.org/10.1021/ma00203a013.
(30) Ajiro, H.; Takahashi, Y.; Akashi, M. Thermosensitive Biodegradable Homopolymer of Trimethylene Carbonate Derivative at Body Temperature. *Macromolecules* **2012**, *45* (6), 2668–2674. https://doi.org/10.1021/ma300183t.
(31) Tempelaar, S.; Mespouille, L.; Dubois, P.; Dove, A. P. Organocatalytic Synthesis and Postpolymerization Functionalization of Allyl-Functional Poly(Carbonate)s. *Macromolecules* **2011**, *44* (7), 2084–2091. https://doi.org/10.1021/ma102882v.
(32) Tempelaar, S.; Barker, I. A.; Truong, V. X.; Hall, D. J.; Mespouille, L.; Dubois, P.; Dove, A. P. Organocatalytic Synthesis and Post-Polymerization Functionalization of Propargyl-Functional Poly(Carbonate)s. *Polym. Chem.* **2013**, *4* (1), 174–183. https://doi.org/10.1039/c2py20718d.
(33) Lutz, J.-F. Polymerization of Oligo(Ethylene Glycol) (Meth)Acrylates: Toward New Generations of Smart Biocompatible Materials. *J. Polym. Sci. Part -Polym. Chem.* **2008**, *46* (11), 3459–3470. https://doi.org/10.1002/pola.22706.
(34) Vancoillie, G.; Frank, D.; Hoogenboom, R. Thermoresponsive Poly(Oligo Ethylene Glycol Acrylates). *Prog. Polym. Sci.* **2014**, *39* (6), 1074–1095. https://doi.org/10.1016/j.progpolymsci.2014.02.005.
(35) Lutz, J.-F.; Weichenhan, K.; Akdemir, O.; Hoth, A. About the Phase Transitions in Aqueous Solutions of Thermoresponsive Copolymers and Hydrogels Based on 2-(2-Methoxyethoxy)Ethyl Methacrylate and Oligo(Ethylene Glycol) Methacrylate. *Macromolecules* **2007**, *40* (7), 2503–2508. https://doi.org/10.1021/ma062925q.
(36) Yu, L.; Zheng, Z.; Liu, Y.; Li, Z.; Wang, X. Synthesis and Properties of Tunable Thermoresponsive Aliphatic Polycarbonate Copolymers with Oligo Ethylene Glycol Containing Thioether and/or Sulphone Groups. *Rsc Adv.* **2015**, *5* (80), 64832–64840. https://doi.org/10.1039/c5ra10486f.
(37) Lowe, A. B. Thiol-Yne 'Click'/Coupling Chemistry and Recent Applications in Polymer and Materials Synthesis and Modification. *Polymer* **2014**, *55* (22), 5517–5549. https://doi.org/10.1016/j.polymer.2014.08.015.
(38) Love, D. M.; Kim, K.; Goodrich, J. T.; Fairbanks, B. D.; Worrell, B. T.; Stoykovich, M. P.; Musgrave, C. B.; Bowman, C. N. Amine Induced Retardation of the Radical-Mediated Thiol-Ene Reaction via the Formation of Metastable Disulfide Radical Anions. *J. Org. Chem.* **2018**, *83* (5), 2912–2919. https://doi.org/10.1021/acs.joc.8b00143.
(39) Beharry, A. A.; Woolley, G. A. Azobenzene Photoswitches for Biomolecules. *Chem. Soc. Rev.* **2011**, *40* (8), 4422–4437. https://doi.org/10.1039/c1cs15023e.