SYNTHESIS AND EVALUATION OF AMPHIPHILIC SCORPION-LIKE AND STAR MACROMOLECULES FOR BIOMEDICAL APPLICATIONS

by

BAHAR DEMIRDIREK

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ABSTRACT OF THE THESIS

SYNTHESIS AND EVALUATION OF AMPHIPHILIC SCORPION-LIKE AND STAR MACROMOLECULES FOR BIOMEDICAL APPLICATIONS

By BAHAR DEMIRDIREK

THESIS DIRECTOR:

Professor Kathryn E. Uhrich

Self-assembled and unimolecular amphiphilic macromolecules with pseudo-double branched and single tails were synthesized. Degradation behavior, drug loading efficiency, drug release rate and stability of macromolecules were investigated. The anti-tumor drug, Camptothecin and the anti-inflammatory drug, Indomethacin were used to evaluate drug loading and release efficiency. Unimolecular amphiphilic macromolecules were more stable in the presence of the enzyme, lipase and human serum albumin (HSA) compared to self-assembled macromolecules. Pseudo-branched macromolecules drug release rate was slower compared to linear macromolecules.

Furthermore, self-assembled single tail amphiphilic macromolecule (AScMs) were modified to achieve active tumor targeting and to achieve high concentration of drug with cyclo(RGDfk) and Doxorubicin, respectively. Cyclo(RGDfk) conjugated AScMs showed higher uptake in tumor cells compared to AScMs alone. Furthermore, AScMs and

Cyclo(RGDfk)-AScMs showed similar uptake profile with healthy cells. Higher loading efficiency was determined in Doxorubicin conjugated AScMs. On the other hand, lower IC50 value (high toxicity) was determined by AScMs-Dox conjugation.

Self-assembled pseudo branched macromolecules were modified with two carboxylate acids to achieve higher Low Density Lipoprotein (LDL) uptake. LDL uptake of branched macromolecules was compared with linear self-assembled macromolecules which are modified with carboxylic acid and sulfuric acid by our group members. Two carboxylate acid conjugated-AScMs (2CM branched) achieved the highest LDL uptake compared to other macromolecules.

DEDICATION

This thesis is dedicated to my lovely mother Zeliha Demirdirek, my father Haluk Demirdirek and my sister Basak Demirdirek for all the beauty and greatness they give to me.

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CHAPTER ONE: OVERVIEW

1.1 Polymeric Micelles as Drug Carriers

1.1.1 Drug Delivery System

Drug delivery methods have a significant effect on drug efficiency. Most drugs are toxic or less therapeutic at concentrations above or below, respectively, their therapeutic window [1-4]. The most common approach for delivery of pharmaceuticals is oral administration [5, 6], however, it is not easy to maintain drug release rate for 24 hours. Therefore, various drug delivery systems are currently under development to increase drug efficiency, prevent harmful side effects, to minimize drug degradation and loss, and to increase drug bioavailability. Drug carriers or delivery systems can be soluble polymers, microcapsules, lipoprotein liposomes and micelles (Scheme 1-1). Drug carriers can be made with different characteristics such as slowly degradable, pH and temperature sensitive [7, 8] or targeted [9-11].

Among drug carriers, polymeric micelles have received much attention because of their small particle size, low toxicity and ease of modification (Scheme 1-2). Polymeric micelles are colloidal dispersions that have a diameter between 5 to 100 nm [12-17]. Polymeric micelles self-assemble to form core-shell nanoparticles in aqueous media above their critical micelle concentration (CMC) and critical micellization temperature (CMT). Lower CMC values are more stable and very important in pharmacological terms because micelles with high CMC values may dissociate into unimers with a large volume

of the blood [17]. The inner core of micelle systems typically contains the hydrophobic compound, while the outer shell of micelle system helps to solubilize micelle system.

1.1.2 Cancer Treatment with Use of Polymeric Micelles and Active Tumor Targeting

Cancer is a serious disease and a leading cause of death. According to the American Cancer Society, 13 % of all deaths are caused by cancer [18]. Cancer treatments are usually a combination of surgery, radiotherapy and chemotherapy. The main problems of chemotherapy drugs are low solubility, rapid phagocytic and renal clearances and systematic toxicity [19].

Polymer micelles can be used to increase the efficiency of chemotherapy drugs. Polymeric micelles gets trapped in the tumor because the lymphatic system is absent in tumors [20-23]. Although nanosized micelles spontaneously accumulate in tumor by the enhanced permeability and retention effect (EPR), micelles are also observed to accumulate quite significantly in reticuloundothelial sites such as liver, spleen and kidney [24-27]. Insufficient uptake in tumor site will decrease the therapeutic effect of the administered drug dose, and nonspecific diffusion healthy tissues limits the dosage. Drug delivery to tumors can be enhanced by attaching targeting ligands to the hydrophilic part of the polymeric micelle. These ligands can be antibodies, peptides, sugar moieties and folate residues [28-30].

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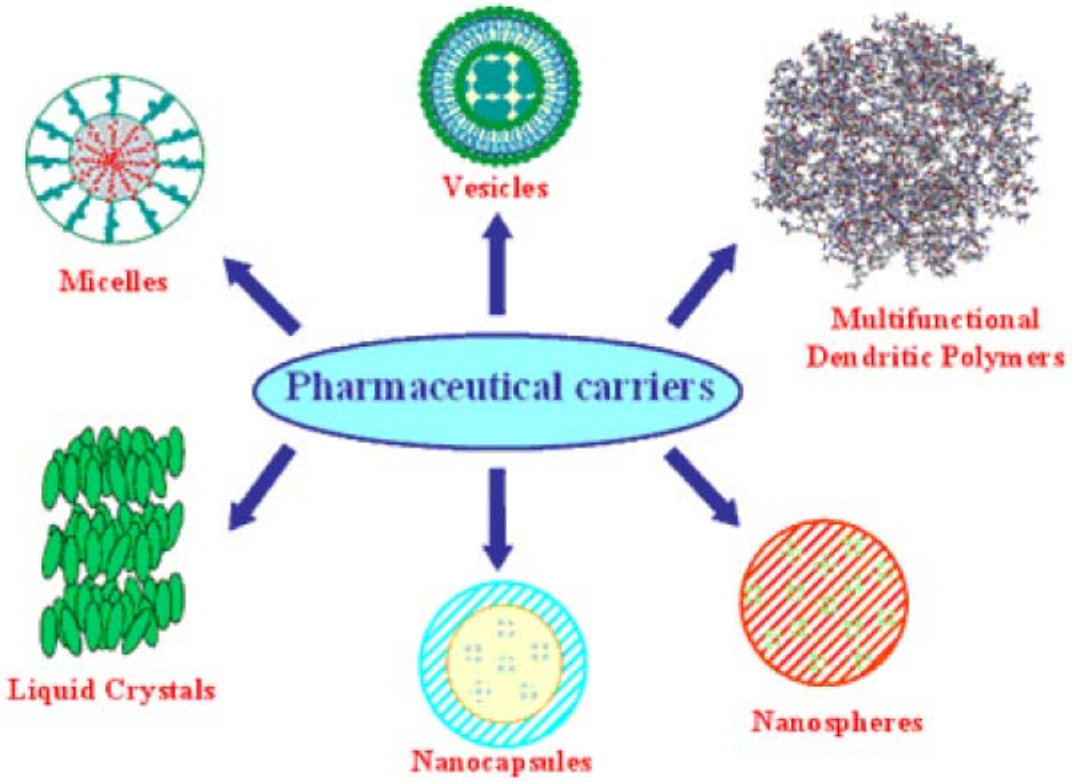
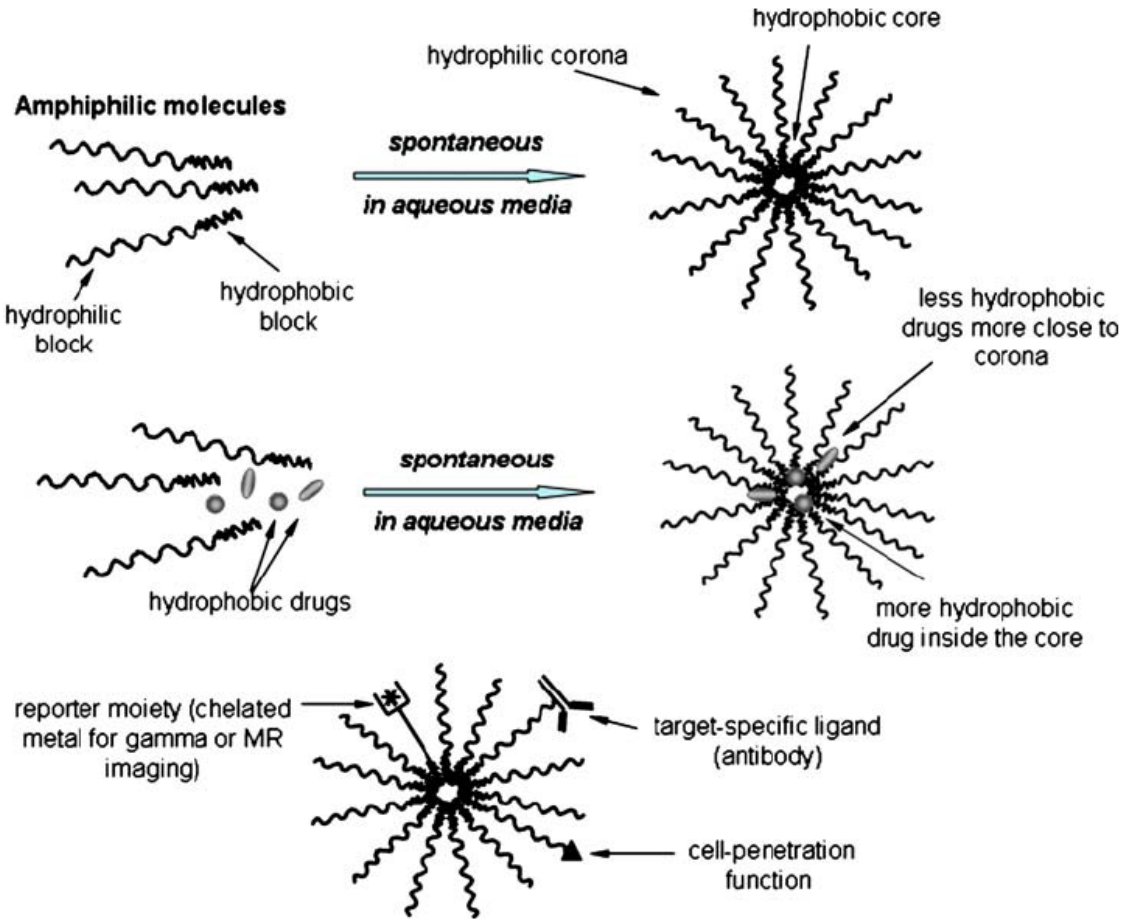


Scheme 1-1 Representative pharmaceutical carriers [31]



Scheme 1-2 Polymeric micelles [14]

CHAPTER TWO: HYDROLYTIC AND ENZYMATIC DEGRADATION OF BIODEGRADABLE AMPHIPHILIC MACROMOLECULES: SELF-ASSEMBLED AND UNIMOLECULAR MICELLES

2.1 Introduction

Biocompatible polymers are currently used in drug delivery, particularly for hydrophobic drugs which can be solubilized within the hydrophobic core of micelles [1-7]. Biodegradable macromolecules have attracted particular attention because they degrade in *vivo* following interaction with cells, enzymes and body fluids [8, 9]. The biodegradation products are particularly low-molecular weight molecules that can be absorbed in the body or metabolized [10]. Micelles as drug delivery systems are desirable because of their ability to solubilize drug, as well as maintain their small particle sizes (10-100 nm), slow accumulation in leaky vascular tumor systems and inflammed areas via the enhanced permeability and retention effect (EPR) [11]. For micelles, many parameters that influence drug delivery such as drug loading efficiency and drug release characteristics can be affected by degradation [12-19]. Therefore, evaluation of biodegradation kinetics is critical for biomedical applications of macromolecules.

Two classes of amphiphilic macromolecules are studied in this chapter: macromolecules **1** and **2** self-assemble in aqueous solutions to form micelles (Scheme 2-1) [16], whereas macromolecules **3** and **4** are unimolecular micelles [16]. These macromolecules have enhanced drug loading capacity, slower drug release rate and faster resolution rate [20] compared to Cremophor EL and Pluronic micelles that are currently used in patients [21,

22]. These macromolecules also shower great potential as low density lipoprotein targeting [23], gene delivery [24] and drug delivery [20] agents. These macromolecules consist of ester bonds located in two different locations: in the hydrophobic part of macromolecules, that contains multiple acyl chains and the hydrophobic domain with PEG.

In this study, for possible drug and gene delivery applications, stability and degradation properties of macromolecules are examined in aqueous solution. Experiments are performed in physiological conditions and in the presence of lipase, an enzymes that cleaves ester bonds [25, 26].

2.2 Experimental Section

2.2.1 Materials

Lipase from porcine pancreas, camptothecin, human serum albumin (HSA), NaN_3, DMSO, phosphate citrate buffer (PCB) and phosphate buffered saline (PBS) tablets were purchased from Sigma-Aldrich (St. Louis, MO). Tetrahydrofuran (THF), regenerated cellulose membranes (Spectra/Por Mw 3500 Da) and PTFE (polytetrafluoroethylene) filters were purchased from Fisher Scientific (Atlanta, GA). All amhiphilic macromolecules were synthesized as previously described [16].

2.2.2 Characterization Methods

Molecular weight was determined by gel permeation chromatography (GPC). Measurements were performed on Waters Breeze GPC system equipped with Styragel®

HR3 column (ID 7.8 mm, and length 300 mm), 1515 isocratic HPLC pump and Waters 717 plus Autosampler and with a Water 2414 refractive index detector. THF was used as the mobile phase. The average molecular weight of the sample was calibrated against narrow molecular weight poly(ethylene glycol) (PEG) standards (Polysciences, Warrington, PA). Samples were dissolved into THF and filtered through a 0.45 μm PTFE syringe filter (Whatman, Clifton, NJ) before injection into the column at a flow rate of 0.8 ml/min.

The volume-wt particle size distribution of the macromolecules in aqueous solution was measured by dynamic light scattering (DLS) at different time intervals using a Malvern Instruments Zetasizer Nano ZS-90 instrument (Southboro, MA) at a 90° scattering angle at 25°C. Macromolecules were dissolved in deionized water (1 mg/ml) and filtered with 0.45 μm PTFE filters prior to analysis.

2.2.3 Hydrolytic Degradation

Hydrolytic degradation experiments of macromolecules was performed at 37 °C (Isotemp Incubator 655D, Fisher Scientific, USA) in phosphate buffer solutions (Sigma Aldrich). At different time intervals, 1 mL samples were withdrawn, frozen at -20 ºC and lyophilized (FreeZone Benchtop and Console Freeze Dry System, Kansas City) at < 133 x 10^{-3} mBar (condenser T = -50ºC) for 48-72 h. Then samples were analyzed by GPC.

2.2.4 Enzymatic Degradation

Enzymatic degradation experiments of macromolecules were performed in presence of lipase. Macromolecules (2.5 mg/ml) were incubated in PBS (pH 7.4) containing the enzyme (10 units/ml) and NaN_3 (0.02 %) to prevent to prevent microbial growth at 37 °C (Isotemp Incubator 655D, Fisher Scientific, USA). At different time intervals, samples were withdrawn and extracted with methylene chloride (5 ml), which is then removed under vacuum. The samples were redissolved in THF prior to GPC analysis.

2.2.5 Camptothecin (CPT) Loading

Camptothecin (0.6 mg) was dissolved with 4:1 chloroform:methanol mixture (2 ml). Macromolecules (8 mg) were also dissolved in 4:1 chloroform:methanol (2 ml). The drug solution was added dropwise at room temperature within 5 minutes to the macromolecule solution. Solvents were evaporated under vacuum until completely dried. The solids were redissolved with PBS (4.0 ml) and the mixture sonicated (FS60, Fisher Scientific) at room temperature for 2 min for complete dissolution. The solutions were incubated for 2 h at 37 °C (Isotemp Incubator 655D, Fisher Scientific, USA). The final solutions were syringe filtered (0.45 μm PTFE) to remove precipitated drug.

Camptothecin concentration was detected by UV-visible spectrophotometry at 375 nm as described elsewhere [27]. CPT-loaded macromolecules were diluted with DMSO (10x dilution) to disrupt the micelles. CPT standards were prepared with DMSO: PBS (10:1). Calculation of weight percentage loading and encapsulation efficiency were determined as follows:

$$\text{Weight \% Loading} = \frac{\text{Concentration of drug detected}}{\text{Concentration of macromolecules}} \times 100$$

$$\text{Encapsulation Efficiency (\%)} = \frac{\text{Concentration of drug detected}}{\text{Initial concentration of drug}} \times 100$$

2.2.6 Camptothecin (CPT) Release

The lyophilized macromolecule-drug samples were dissolved in PBS pH 7.4 or 4% w/v HSA in PBS solution to simulate physiological conditions. The release system consisted of a regerated cellulose flat membrane sheet (MW 3500 Da) placed between the donor and receptor compartment of acrylic equilibrium dialysis cells (Bel-Art Products, NJ). Macromolecule-drug solutions in PBS (5 ml) were placed in the donor cells and fresh PBS (5 ml) solutions were placed in the receptor cells. At certain time intervals, receptor solutions (5 ml) were retrieved for analysis and the receptor cells were replaced with fresh PBS (5 ml). CPT concentrations were detected by UV-visible spectrophometry at 375 nm.

2.2.7 pH and Temperature Stability

Lyophilized CPT-loaded macromolecules were resolubilized in PBS pH 7.4 or phosphate citrate buffer (PCB) pH 5.0 to obtain 1 mg/mL final macromolecular concentration. The samples were placed in 2 ml cuvettes equipped with outer water reservoirs that were connected to a chemical transfer pump to allow circulation of heated water through the cuvette reservoirs (Uvonic Instruments, NJ) At specific temperatures within the 25 ºC – 50 ºC range, samples were analyzed at relevant wavelengths (λmax values): for drug

detection (365 nm) and macromolecule detection (285 nm). The buffered drug-macromolecular solutions were allowed to equilibrate for 5 min at each temperature before UV analysis (% transmittance).

2.2.8 Storage Stability

The micellar sizes from CPT-loaded macromolecules were determined by dynamic light scattering (DLS). Macromolecular solutions in PBS were filtered with 0.45 μm PTFE filters before measurement. The macromolecular solutions were kept at 0 °C, 25 °C, and 37 °C for three weeks. The particle size distribution of the macromolecules in aqueous solution was measured at different time intervals by DLS using a Malvern Instruments Zetasizer Nano ZS-90 instrument (Southboro, MA) at a 90° scattering angle at 25°C.

2.3 Results and Discussion

2.3.1 Hydrolytic Degradation

Degradation of macromolecules was studied in physiological pH and temperature. Polymer degradation was monitored for molecular weight changes (GPC) and size in solution (DLS). Hydrolytic degradation experiments showed no changes in the molecular weight for four weeks as determined by GPC. Particle size of macromolecules was monitored by DLS; no higher aggregation was observed. The micellar size remained constant: 20.5 nm for **1** and 11.1 nm for **2**, and 43.1 nm for **3** and 18.5 nm for **4**.

2.3.2 Enzymatic Degradation

Enzymatic degradation was measured relative to the initial molecular weight. The data is shown in Scheme 2-2. Overall, rapid enzymatic degradation was observed in the self-assembled macromolecules (**1** and **2**) relative to **3** and **4**. This observation is reasonable because macromolecules **1** and **2** may more effectively interact with the enzyme as unimers. The enzymatic degradation was monitored by GPC to measure changes in molecular weight. The ester bonds in the hydrophobic part were expected to first cleave upon incubation with lipase enzyme, which is soluble in water and hydrolyzes ester groups in the hydrophobic domains of proteins [28, 29]. As an example, macromolecule **2** consists of four ester bonds in the hydrophobic region and two ester bonds in the hydrophilic region. The GPC spectrum of macromolecule **2** showed that the alkyl chains (MW 296, Tr 11.6 min) in the hydrophobic region are detected before PEG (MW 2000, Tr 9.35 min). Only in the later stage of degradation (14 days) does the PEG peak became visible, the intensity of PEG then increased with longer degradation times. After 42 days, the only visible peak for macromolecule **2** was the peak corresponding to PEG alone. In contrast, for the macromolecule **1** was the PEG peak (Mw = 5000, Tr 7.93 min) the only visible peak. Upon analyzing the unimolecular micelles (**3** and **4**), a 40% decrease in molecular weight was observed after five weeks. These macromolecules degrade slower because they are covalently attached to form unimolecular micelles, making it difficult for the enzymes to interact with the hydrophobic chains of the micellar core.

2.3.3 Camptothecin (CPT) Loading and Release

CPT loading and encapsulation efficiencies of the macromolecule-based micelles were evaluated (Table 2-1). On average, CPT loadings were below 5 weight percent.

CPT release from the macromolecule-based micelles were evaluated with and without human serum albumin (HSA). CPT release from both macromolecules 1 and 2, was slowed in the presence of HSA (Scheme 2-3). In contrast, CPT release from macromolecules **3** and **4** only slightly changed in the presence of HSA. The contrast between the release of CPT from self-assembled micelles (**1** and **2**) and unimolecular micelles (**3** and **4**) reflects the polymers stability. In particular, CPT-HSA binding is significant in the self-assembled micelles due to the dynamic nature of the macromolecule-micelle equilibrium such that CPT is exposed to HSA. In contrast, the covalently-bound unimolecular micelles (**3** and **4**) offer CPT "protection" from HSA binding.

2.3. 4 pH and Temperature Stability

CPT-loaded macromolecule-based micellar solutions were analyzed for micellar thermal stability from 25 ºC to 50ºC. As possible injectables, the drug-macromolecule solutions will be introduced into the body and subjected to a sudden temperature change from room temperature (25ºC) to body temperature (37ºC). Thus, any detected phase transition or aggregation at this temperature shift will show micellar instability. Good thermal stability was observed for all CPT-loaded macromolecules within the temperature range (25 ºC - 50ºC) (Scheme 2-4).

As potential drug carriers, the CPT-loaded micelles were also evaluated for micellar stability at physiological pH (7.4) and lysosomal pH (5.0). Good pH micellar stability at both pH conditions for all CPT-loaded micelle as noted neither phase transition nor aggregations were observed.

2.3. 5. Storage Stability

CPT-loaded macromolecule-based micellar solutions (1 mg/ml) were prepared and stored at 23 ºC, 37 ºC and 0 ºC for three weeks. Particle sizes of macromolecules were measured using dynamic light scattering (DLS). The size was relatively constant for three weeks, indicating that the polymer solutions are stable.

2.4 Conclusion

In conclusion, these macromolecules show great promise for biomedical applications because of their drug loading efficiency, drug release and degradation rate. The macromolecules are stable upon storage in aqueous media, then degrade in presence of enzymes.

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Scheme 2-1 Chemical structure of amphiphilic macromolecules [21]

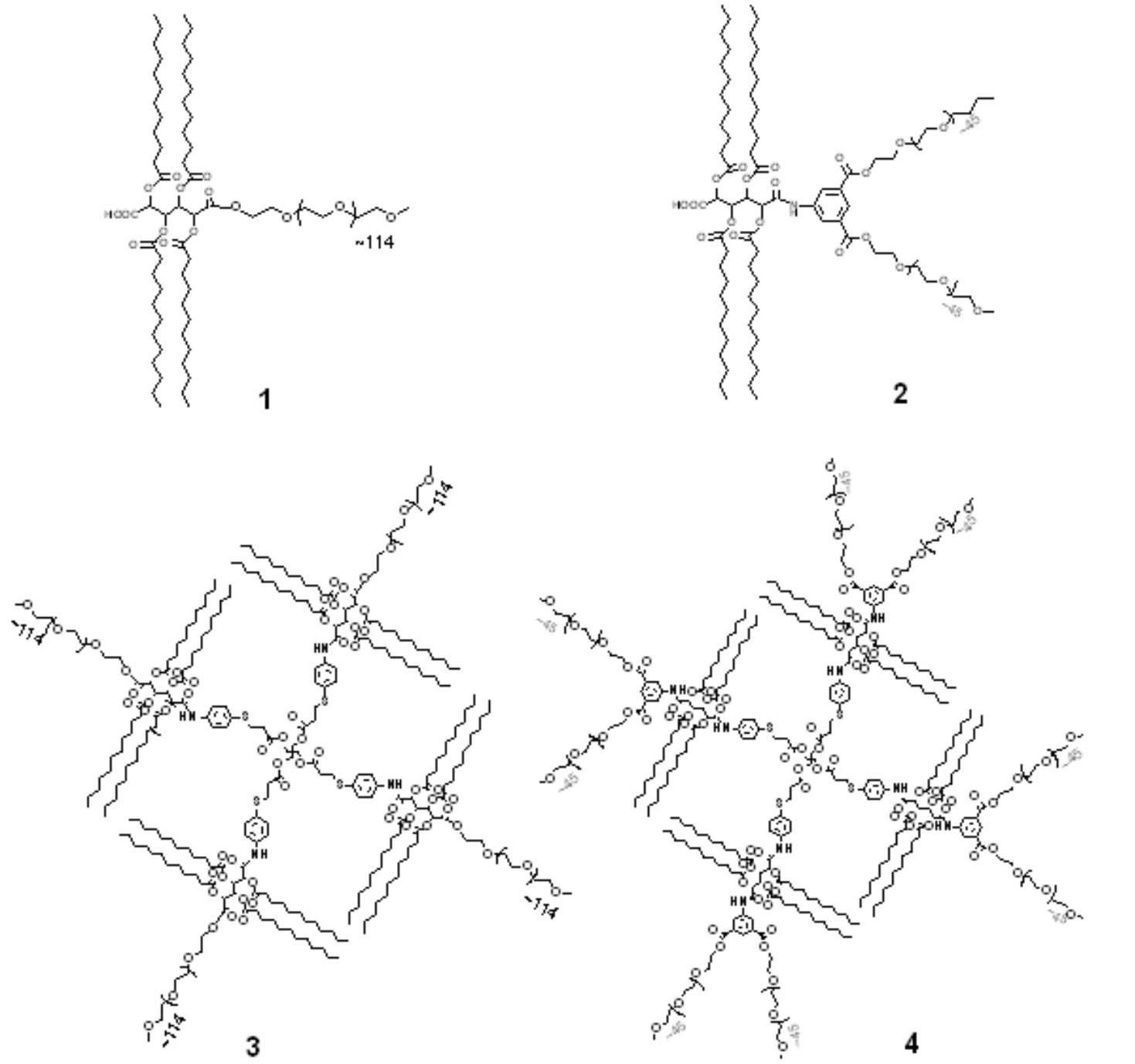
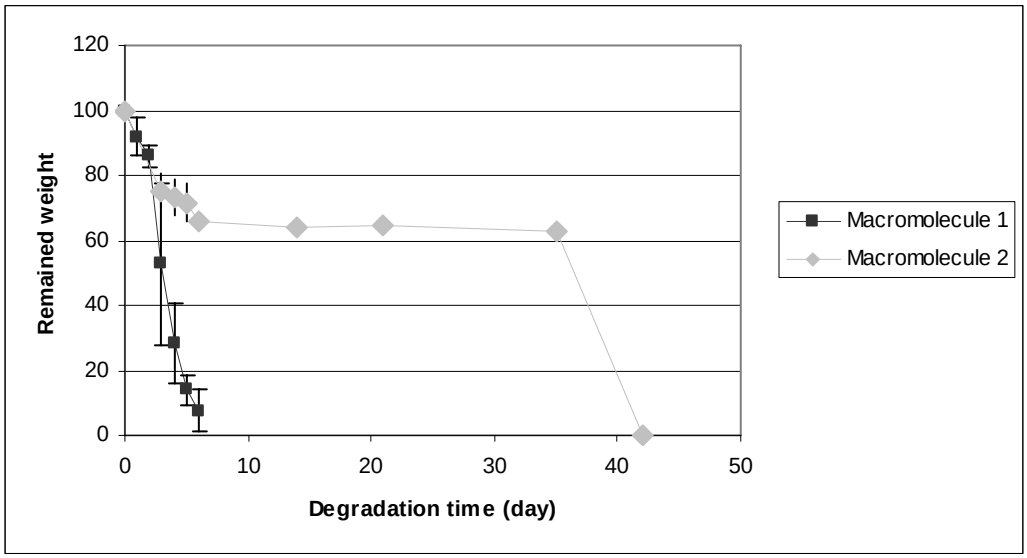
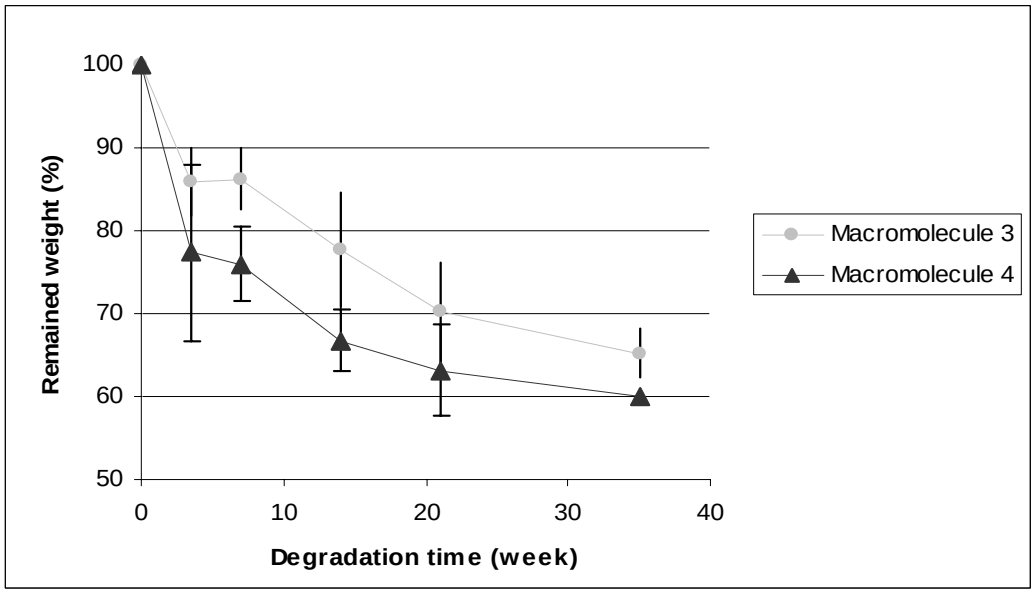


Table 2-1 Encapsulation of CPT- loaded macromolecules

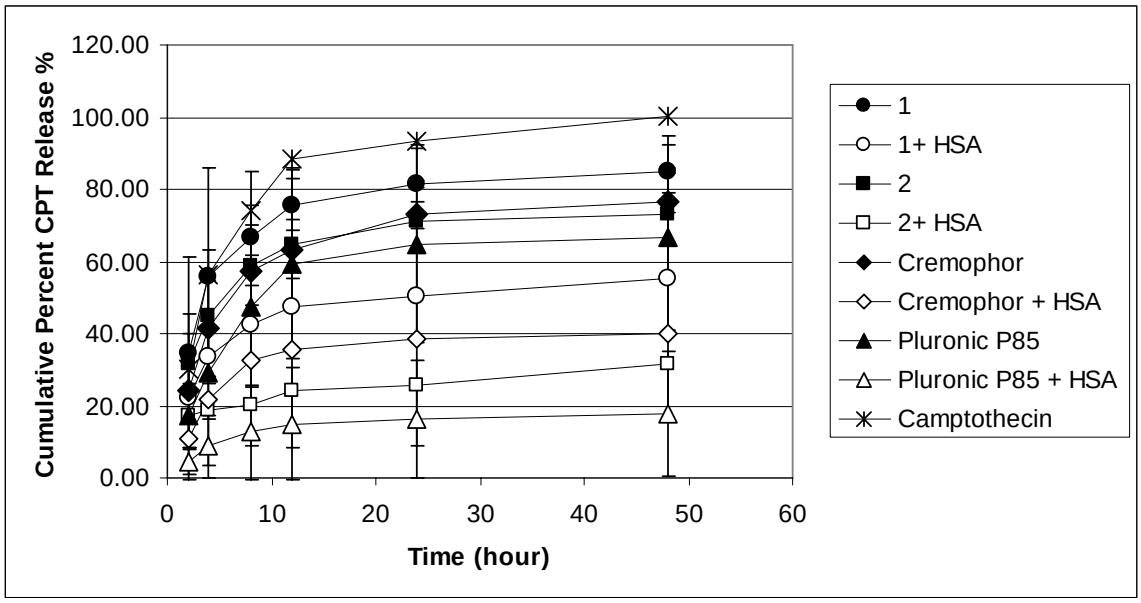
Solution	Encapsulation Efficiency (%)	Weight % Loading
CPT-loaded 1	30.04 ± 14.2	2.35 ± 1.1
CPT-loaded 2	32.89 ± 5.1	2.55 ± 0.8
CPT-loaded 3	17.32 ± 7.0	1.39 ± 0.7
CPT-loaded 4	44.96 ± 10.4	3.60 ± 2.0
CPT-loaded Cremophor EL	57.44 ± 3.0	4.29 ± 1.6
CPT-loaded Pluronic –P85	47.66 ± 23.4	3.36 ± 2.7

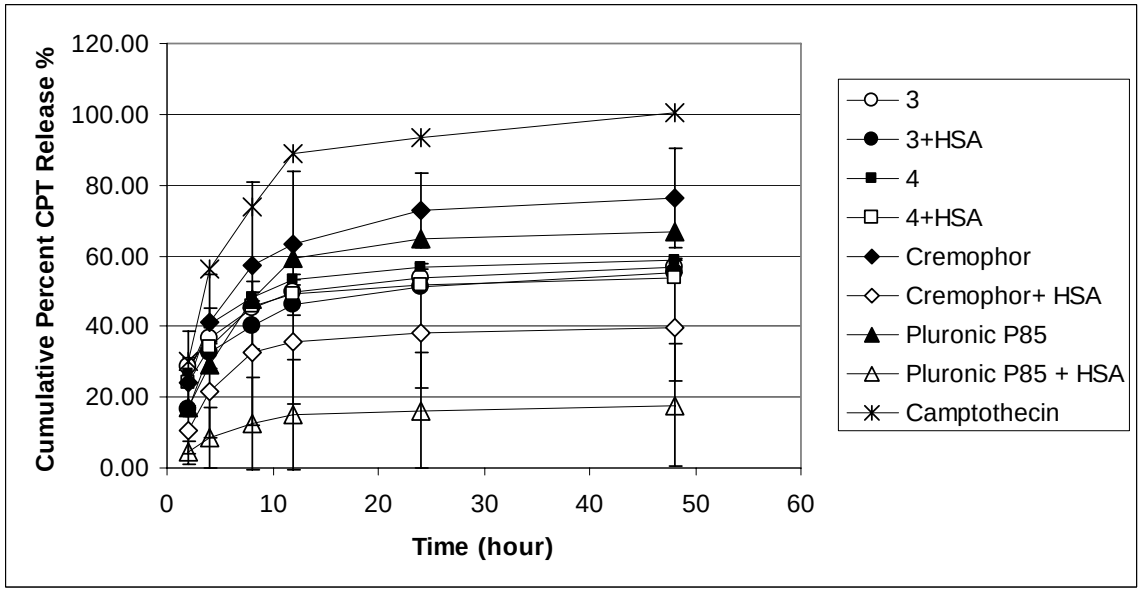
Scheme 2-2 Enzymatic degradation as measured by GPC in aqueous solution (2.5 mg/ ml) using lipase at 10 units/ml (37 °C, pH 7.4)



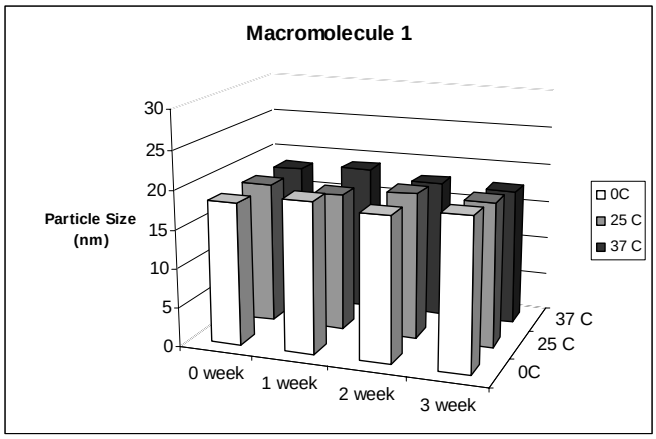


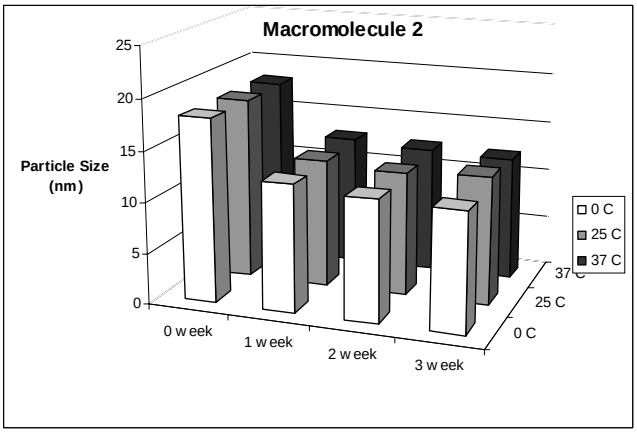
Scheme 2-3 CPT release following encapsulation into macromolecules

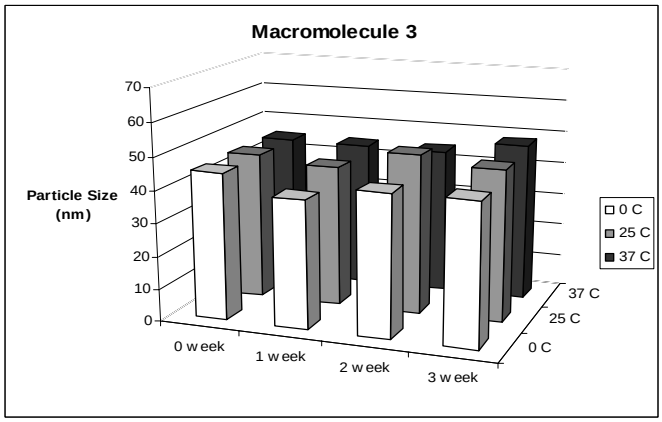


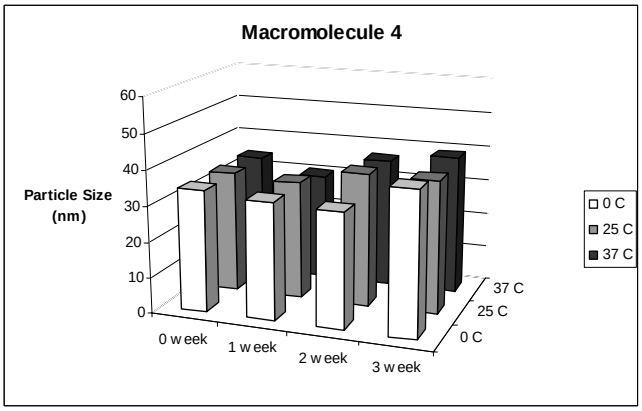


Scheme 2- 4 Solution storage stability as a function of temperature

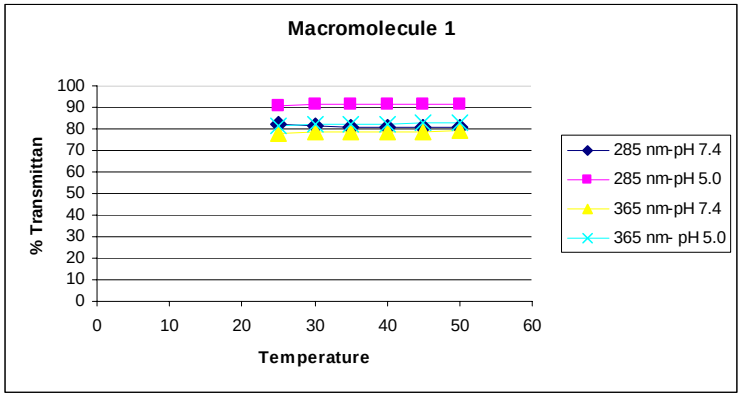


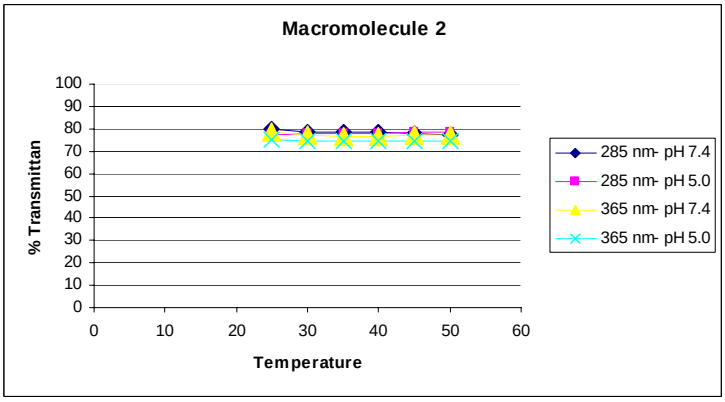


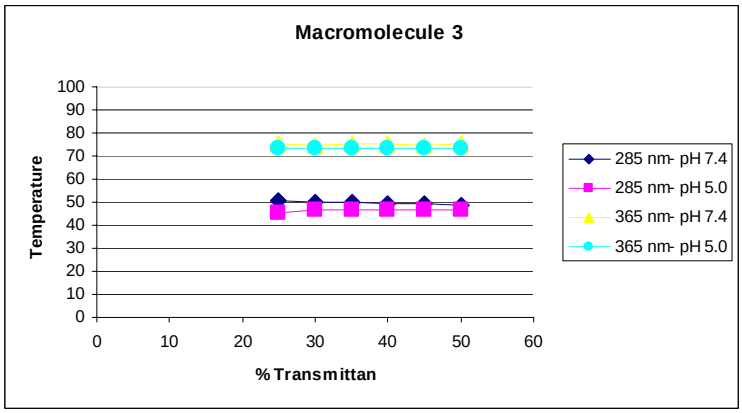


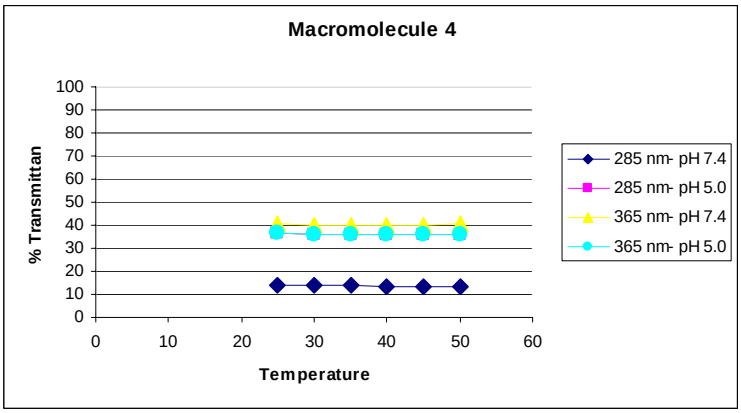


Scheme 2-5 pH and temperature stability in aqueous solution (1 mg/ ml)









CHAPTER THREE: EVALUATION OF DRUG DELIVERY POTENTIAL OF AMPHIPHILIC SCORPION-LIKE AND STAR-LIKE MACROMOLECULES

3.1 Introduction

Various drug delivery systems such as synthetic polymers, liposomes, micelles, lipid particles are currently used to solubilize hydrophobic pharmaceuticals, and increase their bioavailability [1-5]. Polymeric micelles receive particular attention because of their small particle size (10-100nm) [6, 7]. Micelle systems typically consists of a hydrophobic domain, that forms the inner core and a hydrophilic domain that the forms outer shell.

Poly(ethylene glycol) (PEG) is often used in the hydrophilic domain of micelle system [8-10] because it forms a water-bound barrier and it increases the circulation time of nanoparticles [11]. PEG has the capability to repel opsonins and avoid macrophages depending on its density, conformation, chain flexibility, molecular weight and charge [12, 13]. PEG conjugation to drugs and proteins is known to improve their pharmacokinetics and biodistribution properties [14, 15]. Compared to linear PEG, branched PEG provides better coverage of protein surface [16].

Critical micelle concentration (CMC) is an important parameter for micelle systems. Micelles with high critical micelle concentration are unstable upon strong dilution and may dissociate into unimers upon injection due to a large volume of the blood [17]. In this chapter, four type of polymeric micelles (Scheme 4-1), were compared to Cremaphor

EL and Pluronic P85, which are widely used in pharmaceutical applications. Drug delivery potential was compared upon their drug loading ability, drug release kinetics and resolubility.

3.2 Experimental Section

3.2.1 Materials

Indomethacin, DMA, HPLC grade water and phosphate buffered saline (PBS) tablets were purchased from Sigma-Aldrich (St. Louis, MO). Regenerated cellulose membranes (Spectra/Por Mw 3500 Da) and PTFE (polytetrafluoroethylene) filters were purchased from Fisher Scientific (Atlanta, GA). All amhiphilic macromolecules were synthesized as previously described [18].

3.2.2 Indomethacin (IMC) Loading

Indomethacin was dissolved in methylene chloride to make a final solution 2.5 mg/ml. The polymers were dissolved in HPLC-grade water to make final solution (0.5 mg/ml). Indomethacin solution (1.0 mL) was added dropwise into polymer solution (50 ml) at room temperature with stirring. The final mixtures were capped and covered with aluminum foil and stirred at room temperature. After 24 hours, the solution was uncapped and the methylene chloride evaporated overnight. The solutions were filtered under vacuum. Drug-loaded micelles were disrupted with addition of N'N-dimethylacetamide (DMA) (1:1) and detected by UV-visible spectrophotometry at 318 nm. Experiments were performed in triplicate.

Calculation of weight percentage loading and encapsulation efficiency were determined as follows:

$$\text{Weight \% Loading} = \frac{\text{Concentration of drug detected}}{\text{Concentration of macromolecules}} \times 100$$

$$\text{Encapsulation Efficiency (\%)} = \frac{\text{Concentration of drug detected}}{\text{Initial concentration of drug}} \times 100$$

3.2.3 Indomethacin Release

The lyophilized polymer-drug samples were dissolved in phosphate buffered saline (PBS) at pH 7.4 and room temperature. The release equilibrium dialysis cells consisted of cellulose membrane (MW 3500 Da) placed between the donor cell and receptor cell of equilibrium dialysis cells (Bel-Art Products, NJ) and incubated at 37 °C. Polymer-drug solutions were added into donor cells and PBS solutions were added into receptor cells. At certain time points, receptor solutions (5 mL) were retrieved and replaced with fresh PBS (5 mL). The drug concentrations were detected by UV-visible spectrophometry at 318 nm.

3.2.4 Resolubilization of Indomethacin-Loaded Polymeric Micelles

The indomethacin-loaded polymer solutions were lyophilized at 133 x 10^{-3} mBar (condenser T = - 50 °C) for 48 hours. Lyophilized solids were dissolved with deionized water in UV-visible cuvette to obtain indomethacin concentrations 1.0 mg/ml. The cuvettes were shaken using Roto-Mix 50800 (Dubuque, IA) at speed 2.2, visually assessed and analyzed by UV spectrophotometery. The solubilization rate was

determined using a timer (Sunbeam 90952, Boca Raton, FL), from the time of addition of water to complete dissolution of all particles. Experiments were performed triplicate.

3.3 Results and Discussion

Macromolecules were loaded with indomethacin by a oil/water emulsion method using 1:10 (IMC: polymer) feed ratio by weight. Loading efficiency was compared with polymers widely used in pharmaceutical applications, Pluronic P85 and Cremophor EL. Higher encapsulation efficiencies and drug loadings were achieved in amphiphilic macromolecules (38-51%) compared the controls, Pluronic (6%) and Cremophor (31%) (Table 3-1).

IMC release from the macromolecules was also compared with Pluronic P85, Cremophor EL and indomethacin alone. Amphiphilic macromolecules (compounds **1**-**4**) achieved sustained release behavior over 48 hours relative to the free indomethacin control (Scheme 3-2). Slower IMC release was observed from amphiphilic macromolecules (compounds **1**-**4**) relative to the control polymers within the first 48 hours. Overall, the IMC release from pseudo double-chained PEG amphiphilic macromolecules is the slowest (Scheme 3-2).

Resolubilization of IMC-loaded amphiphilic macromolecules was also studied. Resolubilization times of pseudo-branched PEG amphiphilic macromolecules (3.7- 4.5 min) were faster than linear macromolecules (4.6-5.4 min) (Table 3-2). Overall,

resolubilization rate of macromolecules **1**-**4** were faster than the controls, Pluronic P85 (7.5 min) and Cremophor EL (8.4 min).

3.4 Conclusion

Drug loading potential, release and resolubilization rate of macromolecules were evaluated with indomethacin, anti-inflammatory drug. Macromolecules achieved higher drug loading efficiency, slower drug release rate and faster resolubilization rate than control polymers.

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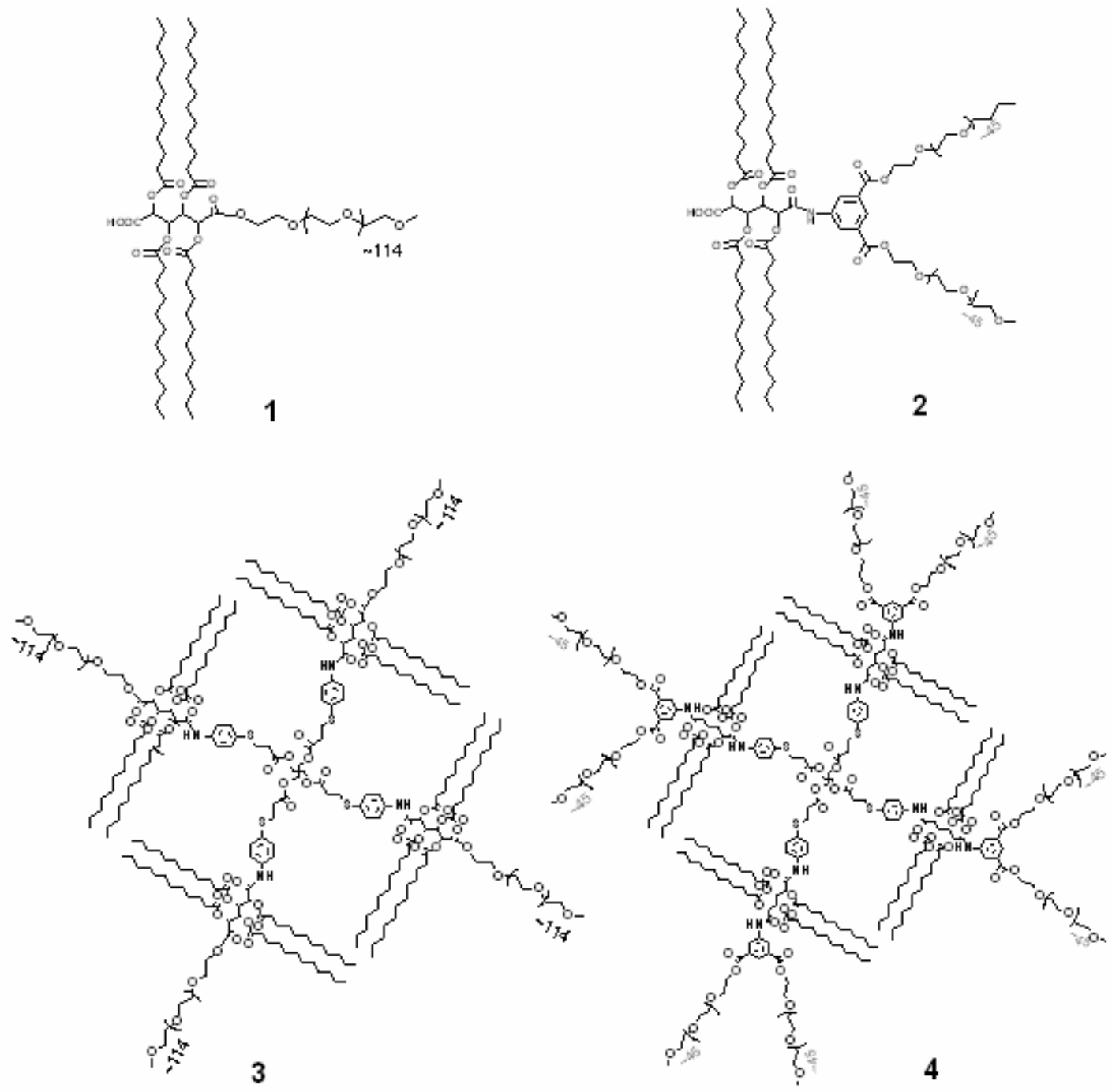
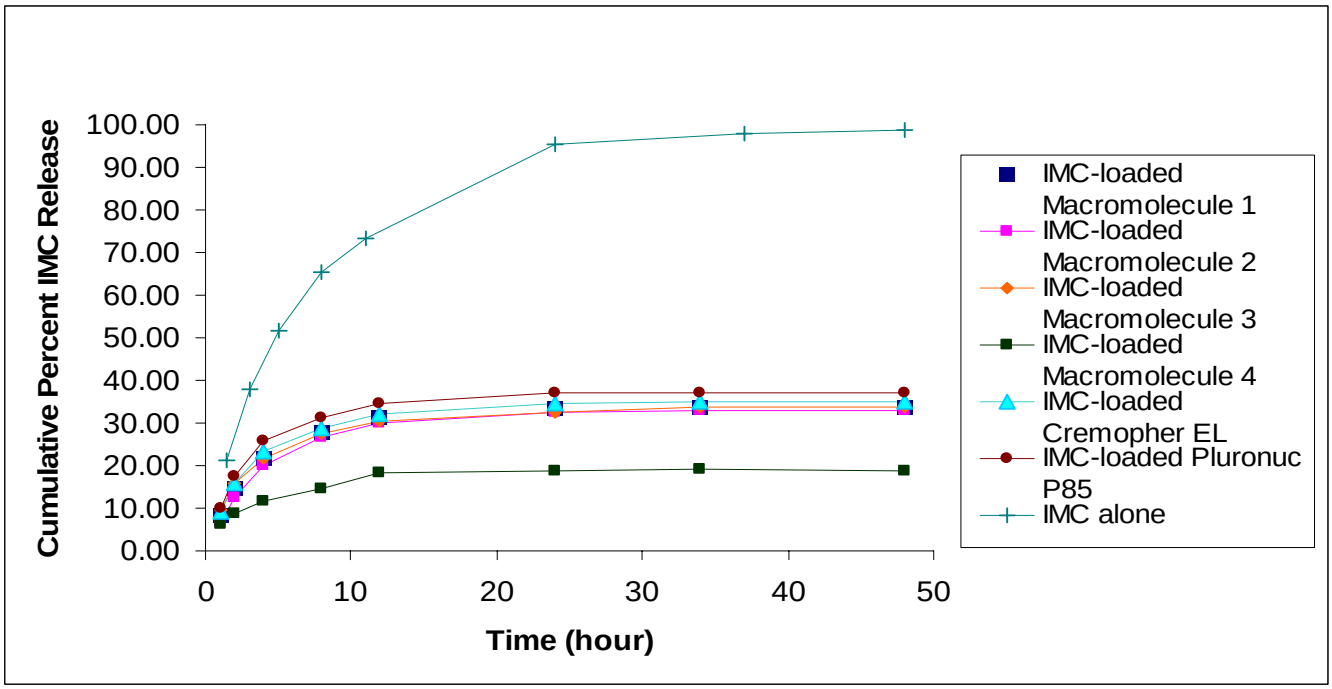
Scheme 3-1 Chemical structure of macromolecules [18]



Sample	Encapsulation Efficiency (%)	Weight % Loading
IMC- Macromolecule 1	41.38 ± 3.2	4.14 ± 0.3
IMC- Macromolecule 2	51.36 ± 18.3	5.14 ± 1.8
IMC- Macromolecule 3	38.56 ± 4.9	3.86 ± 0.5
IMC- Macromolecule 4	46.55 ± 4.9	4.66 ± 0.5
IMC- Pluronic P85	6.32 ± 0.8	0.63 ± 0.1
IMC- Cremophor EL	30.6 ± 3.3	3.06 ± 0.3

Table 3-1 Indomethacin loading



Scheme 3-2. Indomethacin release from macromolecules

Lyophilized sample	**Time (min)**
IMC-loaded Macromolecule 1	4.6 ± 0.7
IMC-loaded Macromolecule 2	3.7 ± 0.8
IMC-loaded Macromolecule 3	5.4 ± 0.6
IMC-loaded Macromolecule 4	3.7 ± 0.3
IMC-loaded Pluronic P85	7.5 ± 0.7
IMC-loaded Cremophor EL	8.4 ± 1.5

Table 3-2 Resolubilization of IMC-loaded polymers

CHAPTER FOUR: CONJUGATION OF PEPTIDES TO AMPHIPHILIC SCORPION-LIKE POLYMERIC MICELLES FOR TARGETING DRUG DELIVERY

4.1 Introduction:

In recent years, polymeric micelle systems have been modified to incorporate tumor targeting ligands to facilitate tumor-targeted delivery of hydrophobic drug molecules [1]. To enhance cellular internalization of macromolecules at desired tissue, cell-specific ligands can be attached on the surface of carriers [2, 3] (Scheme 4-1). Hence, the tumor-specific drug delivery potential of polymeric micelles can be enhanced by covalently attaching targeting ligands; peptides, antibodies, sugars to the hydrophilic part of the micelle surface [4-6].

In this chapter, polymeric micelles system was decorated with peptide, Cyclo(RGDfk). It is well-known that the RGD (Arg-Gly-Asp) motif binds selectively to $a_v\beta_3$ and $a_v\beta_5$ integrin receptors[7]. These $a_v\beta_3$ integrins are essential to tumor growth and metastasis and are highly expressed on many tumor cells such as osteosarcomas, neuroblastomas, carcinomas of the breast, the prostate, the lung, and the bladder [8]. In its linear tripeptide form, RGD demonstrates inefficient binding to integrin receptors. [9] However, the cyclic form of the peptide, Cyclo(RGDfk) , has a rigid skeleton with a much higher binding affinity to integrins [8-11] .

A radiolabeled form of Cyclo(RGDfk) was used to study tumor targeting potential *in vivo*, and specific accumulation to the tumor site [7, 12]. This demonstrates the usefulness of this cyclic RGD peptide sequence for tumor-targeting applications.

In this work, amphiphilic scorpion like-macromolecules (AScMs) were modified by the incorporation of a cyclic RGD targeting peptide. The ability of RGD-modified AScMs to encapsulate 1,1'-Dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) and to deliver the hydrophobic molecule to malignant glioma tumor cells was demonstrated and negative control was performed with Chinese Hamster Ovary (CHOs) cells.

4.2 Experimental Section

4.2.1 Materials

O-bis amino poly(ethylene glycol) and 1'1-carbodiimidazole were purchased from Sigma-Aldrich (St. Louis, MO). Cyclo(RGDfk) peptide was purchased from Ana-Spec, CA. Regenerated cellulose (RC) membranes (Spectra/ Por MWCO 3500 Da), acrylic equilibrium dialysis cells (Scienceware, Bel-Art Products NJ) were purchased from Fisher Scientific. 1,1'-Dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate (DiI) was purchased from Invitrogen. All other reagents and solvents were purchased from Sigma-Aldrich.

4.2.2 Characterization Methods

Molecular weight was determined by gel permeation chromatography (GPC). Measurements were performed on Waters Breeze GPC system equipped with Styragel® HR3 column (ID 7.8 mm, and length 300 mm), 1515 isocratic HPLC pump and Waters

717 plus Autosampler and with a Water 2414 refractive index detector. THF was used as the mobile phase in GPC system. The average molecular weight of the sample was calibrated against narrow molecular weight poly(ethylene glycol) (PEG) standards (Polysciences, Warrington, PA). Samples were dissolved into THF and filtered through a 0.45 μm PTFE syringe filter (Whatman, Clifton, NJ) before injection into the column at a flow rate of 0.8 ml/min.

The volume-wt particle size distribution of the macromolecules in aqueous solution was measured by dynamic light scattering (DLS) at different time intervals using a Malvern Instruments Zetasizer Nano ZS-90 instrument (Southboro, MA) at a 90° scattering angle at 25°C. Macromolecules were dissolved in deionized water (1 mg/ml) and filtered with 0.45 μm PTFE filters prior to DLS analysis.

Chemical structure was confirmed by ^{1}H spectroscopy with samples (~ 5 mg/ml) dissolved in $CDCl_3$-*d* solvent on Varian 300 MHz and 400 MHz spectrometers, using tetramethylsilane as the reference signal.

4.2.3 Synthesis of Molecule 2

To a solution of di-tertbutyl dicarbonate (0.019 g, 0.087 mmol) in dioxane (1 ml), O-bis(amino poly(ethylene glycol) (0.23 g, 0.077 mmol) **1** in aqueous solution of NaOH (2 ml) was added dropwise and maintained at 0°C. After 12 hours of reaction, the organic solvent was removed by rotary evaporation, redissolved with CH_2Cl_2 (10 ml) and washed with HCl (10 ml) and brine (10 ml). The organic layer was concentrated and precipitated by 10-fold diethyl ether. After filtering, product was collected as white powder: 0.20 g,

80% yield. ^{1}H NMR ($CDCl_3$): δ 3.68 (m, 240 H, CH_2O), 1.4 (s, 9H, CH_3). IR (KBr, cm^{-1}): 2876 (C-H), 1106 (C-O). T_m=62-63 °C.

4.2.4 Synthesis of Molecule 3

Molecule **2** (0.23 g, 0.074 mmol) was twice distilled with toluene. Then molecule **1**, MA12 (0.41 g, 0.44 mmol) [15] were dissolved in methylene chloride (10 ml) and DMF (3 ml). DCC (1 ml) was added dropwise to the reaction mixture under argon. After 2 days stirring under room temperature, by product was removed by vacuum filtration. Then, the reaction mixture was acidified by 0.1 N HCl solutions (10 ml x 2) and washed by brine (10 ml x 2). The organic portion was dried over magnesium sulfate ($MgSO_4$). The solution was concentrated to 1 ml and precipitated by diethyl ether (50 ml). After filtration, light yellow product was collected: 92% yield. ^{1}H NMR ($CDCl_3$): δ 5.75 (d, 2H, C*H*), 5.51 (m, 1H, C*H*), 5.14 (d, 1H, C*H*), 4.43 (t, 4H, CH_2), 3.68 (m, ~240 H, CH_2O), 2.37 (m, 4H, CH_2), 2.25 (m, 4H, CH_2), 1.59 (m, 4H, CH_2), 1.24 (m, 48H, CH_2), 0.84 (t, 12H, CH_3).

4.2.5 Synthesis of Molecule 4

Molecule **3** was dissolved in methylene chloride (5 ml) in an ice-bath. TFA (4 ml) was added dropwise to the solution. After 2 hours stirring at 0 °C, the solvent was removed by rotary evaporation. The product was dissolved in methylene chloride (10 ml) and washed with 10 % Na_2CO_3 (5 ml), water (5ml) and brine (5 ml). The organic part was dried with $MgSO_4$ and precipitate by diethyl ether. NH_2 peak was confirmed by TNBSA assay and compared with heterobifunctional poly(ethylene glycol). 80% NH_2 was calculated. ^{1}H

NMR ($CDCl_3$): δ 5.75 (d, 2H, C<u>*H*</u>), 5.51 (m, 1H, C<u>*H*</u>), 5.14 (d, 1H, C<u>*H*</u>), 4.43 (t, 4H, C<u>H_2</u>), 3.68 (m, ~240 H, C<u>H_2</u>O), 2.37 (m, 4H, C<u>H_2</u>), 2.25 (m, 4H, C<u>H_2</u>), 1.59 (m, 4H, C<u>H_2</u>), 1.24 (m, 48H, C<u>H_2</u>), 0.84 (t, 12H, C<u>H_3</u>). GPC: MW: 3900

4.2.6 Synthesis Molecule 5

Molecule **4** (32.63 mg, 8.29 μmol) was dissolved with CH_2Cl_2 (1 ml) and DMSO (1 ml) solution and added to peptide (5 mg, 8.29 μmol) solution in DMSO (1 ml). Then 1,1′-carbonyldiimidazole (CDI) (4.03 mg, 24.8 μmol) was added to mixture. After 4 hours stirring under argon, methylene chloride was removed by rotary-evaporation. After 4 hours dialysis against water (2L), mixture was lyophilized at 133 x 10^{-3} mBar (condenser T = -50 °C) for 48 hours. The product was obtained as white powder: 81 % yield. ^{1}H NMR (D_2O): δ 7.2 (s, 3H, ArH), 3.68 (m, ~240 H, C<u>H_2</u>O), 2.37 (m, 4H, C<u>H_2</u>), 2.25 (m, 4H, C<u>H_2</u>), 1.59 (m, 4H, C<u>H_2</u>), 1.24 (m, 48H, C<u>H_2</u>), 0.84 (t, 12H, C<u>H_3</u>). T_m = 52-53 °C GPC: 4800

4.2.7 DiI Encapsulation

Macromolecules (10 mg/ml) and DiI (10 μg/ml) were dissolved in 0.5 ml acetone. The mixture was added dropwise to 3 ml water at room temperature. Acetone was evaporated under vacuum without heat with stirring. Then the solution was centrifuged (EBA 12, Hettich Zentrifugen, 3000 RPM for 10 min) to precipitate unloaded dye. Solutions were diluted with DMSO, 10;1, DMSO; polymer ratio. DiI was detected by UV-visible spectrophotometry at 550 nm. Calibration was performed using DiI standards in DMSO analyzed by UV-visible spectrophotometry.

$$\text{Weight \% Loading} = \frac{\text{Concentration of drug detected}}{\text{Concentration of polymer}} \times 100$$

$$\text{Encapsulation Efficiency (\%)} = \frac{\text{Concentration of drug detected}}{\text{Initial concentration of drug}} \times 100$$

4.2.8 Cell Uptake Study

Cell uptake studies were performed by Carolyn Waite, PhD student in Prof. Charlie Roth's research group.

4.3 Results and Discussion

Amphiphilic scorpion-like macromolecule was successfully modified for active tumor targeting as shown in Scheme 4-1. Cyclo(RGDfk) was conjugated to amphiphilic macromolecule in four steps. The amino group of the heterobifunctional PEG was first protected with tert-butyl bicarbonate then to attached hydroxyl group of MA12 with DCC. The amino of molecule **3** is deprotected by TFA to form compound **3**, then coupled with Cyclo(RGDfk) to form compound **4**.

The micellar size plays an important role in cellular internalization process. The size of macromolecule was studied by dynamic light scattering (DLS) and showed that c(RGDfk)-macromolecules are approximately 15 nm in size. Larger scale aggregation was not observed.

DiI was physically encapsulated in using solvent evaporation method using 1:100 (DiI: macromolecule) feed ratio by weight. Encapsulation efficiency for the Cyclo(RGDfk)-AScMs (19.6 %) were larger than non-conjugated AScMs (13.2 %).

Carolyn Waite (Chemical Engineering, Rutgers) performed cell uptake studies. A172 glioma cells treated with the molecule ***5*** *exhibited a four-fold increase in DiI fluorescence compared to cells treated with the native, non-conjugated AScMs polymer at the same concentration (Scheme 4-2). In contrast, no fluorescence increase was observed with CHOs cells.*

4.4 Conclusion

Cyclo(RGDfk) was successfully conjugated to AScMs and characterized by ^{1}H NMR, GPC and DLS. Macromolecules formed stable micelles in solutions. Cyclo(RGDfk)-AScMs achieved higher uptake compare to AScMs in tumor cells (A172)

4.5 Future Work

Cyclo(RGDfk) conjugated scorpion-like macromolecules can be modified to determine gene delivery activity. Previously, amine-terminated scorpion-like macromolecules on the hydrophobic core were synthesized in our group for gene delivery studies [14]. AScMs-Cyclo(RGDfk) can be modified with amine-terminated hydrophobic core for targeting gene delivery systems.

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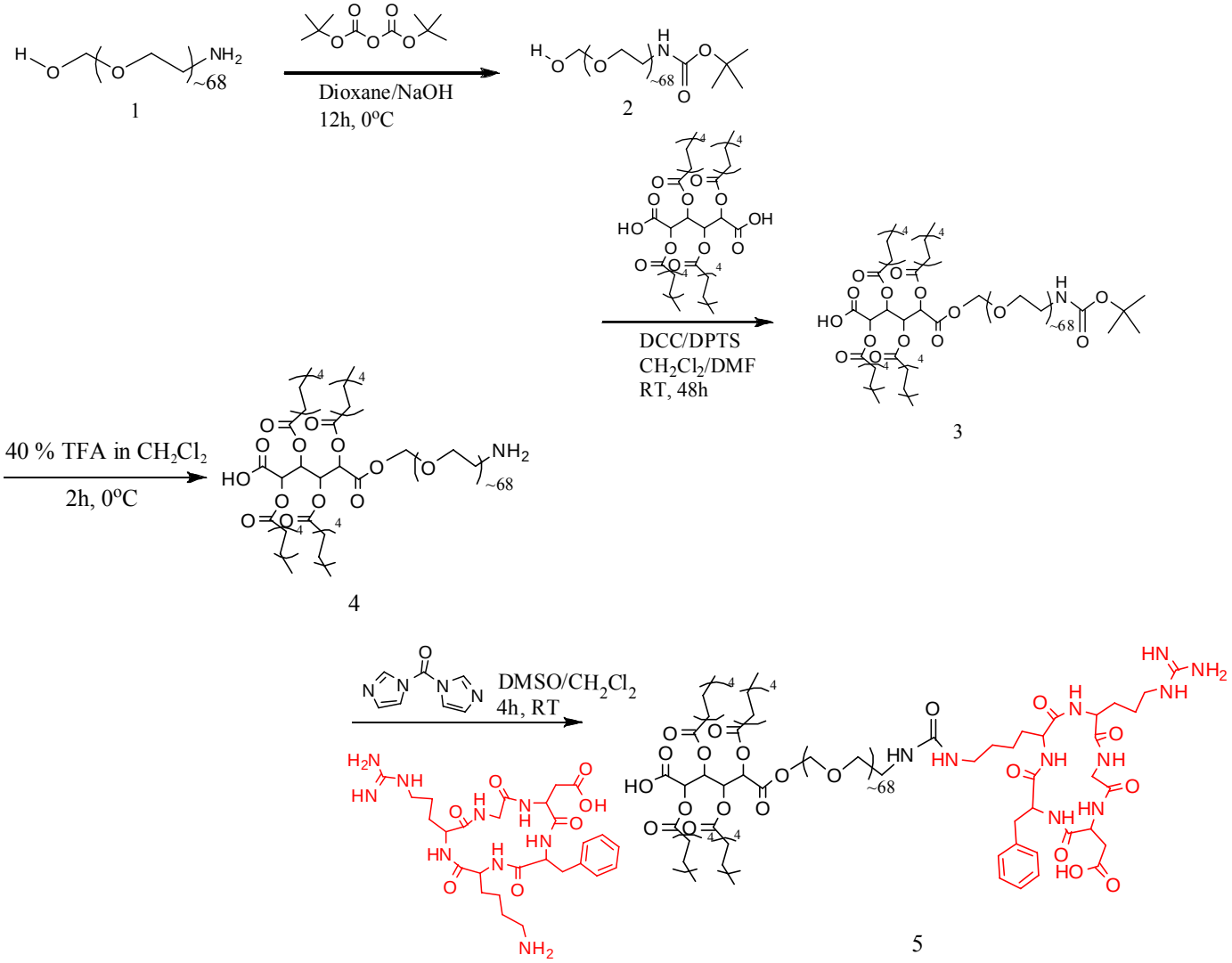
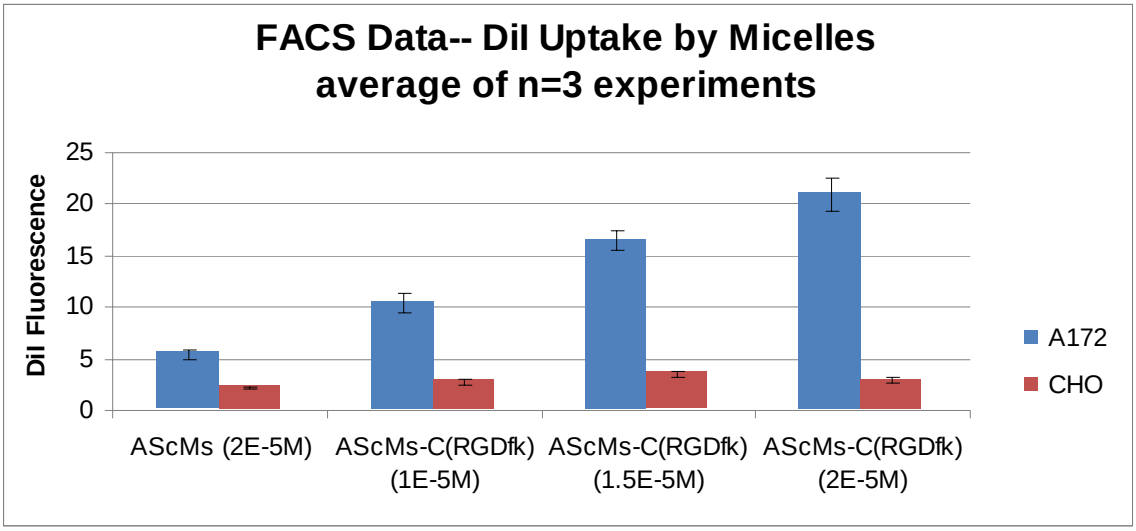


Scheme 4-1 Synthesis of AScMs-Cyclo(RGDfk)



Scheme 4-2 Cell uptake in A172 cells and CHOs cells

----*Results obtained from our collaborator, Prof. Roth's research group*

APPENDICES

5.1 In V*itro* Loading, Release and Cytotoxicity Studies of Doxorubicin

5.1.1 Introduction

Doxorubicin is an antitumor drug and belongs to the class of anthracycline antibiotics. The main problem of doxorubicin is dose limitations resulting from non-specific cardiotoxicity and bone narrow toxicity [1]. Several drug carriers has been used to minimize side effects such as liposomes [2] and micelles [3, 4]. Besides physical encapsulation, doxorubicin can be chemically conjugated to polymers either in the backbone or side chains [1, 5]. Polymer-drug complexes have several advantages compared to free drug such as protection against deactivation, increased water solubility, lower immunogenecity and antigenicity, higher stability and higher drug encapsulation efficiency [6, 7].

In this chapter doxorubicin was physically encapsulated into scorpion-like macromolecules (AScMs) that are already chemically conjugated to doxorubicin. Furthermore, the release profile of doxorubicin was studied at two different pH values: pH 7.4 and pH 5.0. Cytotoxicity studies (MTT Assay) of micelle system was studied with HepG2 ovarian cancer cell line.

5.1.2 Experimental Section

5.1.2.1 Materials

Phosphate buffer saline tablets, phosphate citrate buffer tablets, Doxorubicin HCl were purchased from Sigma-Aldrich (St. Louis, MO). Regenerated cellulose (RC) membranes (Spectra/Por MWCO 3500 Da), acrylic equilibrium dialysis cells (Scienceware, Bel-Art Products NJ), Corning cell culture flasks were purchased from Fisher Scientific. Hepatocellular carcinoma (HepG2) cells, fetal bovine serum, penicillin-streptomycin solutions, trypsin-EDTA (1x) solution, MTT cell proliferation assay and Modified Eagle's minimum essential media were purchased from ATCC (Manassa, VA). Doxorubicin conjugated scorpion-like macromolecules (DNM) was synthesized as previously described [8]. All other solvents and reagents were purchased from Sigma-Aldrich.

5.1.2.2 Doxorubicin Encapsulation

Polymers (AScMs, DNM, Pluronic P85, Cremophor EL (CRE)) (1 mg) was dissolved in dimethylformamide (3.8 ml) and added to doxorubicin (DOX) solution (0.2 mL of 10 mg/mL DMF). Triethylamine (1.0 μL) was added to the solution to allow encapsulation of doxorubicin into hydrophobic micellar core. The solution was covered with aluminum foil and parafilm, and then was shaken using vortex mixer. Polymer-drug solutions and the control were dialyzed for 24 h against 1 L distilled water using cellulose membranes (MW 3500). Doxorubicin was detected by UV-Visible Spectrophotometry at 480 nm, and calibrated using standards in DMF (1-60 μg/mL).

Calculation of weight percentage loading and encapsulation were determined as follows:

$$\text{Weight \% Loading} = \frac{\text{Concentration of drug detected}}{\text{Concentration of polymer}} \times 100$$

$$\text{Encapsulation Efficiency (\%)} = \frac{\text{Concentration of drug detected}}{\text{Initial concentration of drug}} \times 100$$

5.1.2.3 Doxorubicin Release

Chemically and physically doxorubicin-loaded polymers were resolubilized in phosphate buffered saline (PBS) pH 7.4 and phosphate citrate buffer pH 5.0. The cellulose membrane (MWCO 3500 kDa) was pre-soaked in PBS for 12 hours and placed between the donor cell and receptor cell of equilibrium dialysis cells. The doxorubicin-polymer solutions (5 mL) were added into donor cells and fresh buffer solutions added into receptor cells. At specific time intervals receptor solutions were retrieved and replaced with fresh buffer solutions (5 mL). Doxorubicin concentration was determined by UV-vis spectrophotometry at 480 nm.

5.1.2.4 Cytotoxicity Assay

This study was performed with Leilani Del Rosario.

Cell Media was prepared with 10 % v/v fetal bovine serum and 1 % v/v penicillin-streptomycin presence of media, HepG2 cells were grown at 37 ℃ with 5 % CO_2. Polymers cytotoxicity was determined with MTT cell proliferation assy. 5000 cells/well were plated in 96-well plates and incubated for 24 hours at 37 ℃ with 5 % CO_2. Micelle solutions (DNM-DOX, AScMs-DOX, CRE-DOX) and Doxorubicin in PBS and DMSO

were diluted in range between 10^{-1} to 10^{-4} M and added to the wells. Then cells were incubated at 37 ℃ with 5 % CO_2. After 72 hours, MTT reagent (10 μl) was added to cells and incubated at 37 ℃ with 5 % CO_2. MTT detergent (100 μl) was added and covered with aluminum foil, incubation was for 2 hours. Measurements were taken on the Absorbance Microplate Reader at 570 nm.

5.1.3 Discussion

Doxorubicin was physically encapsulated into different micelle systems using dialysis method. The highest encapsulation efficiency was achieved in DNM (Table 5-1), this effect is likely due to the hydrophobic interaction in the micellar core. In AScMs alone, encapsulation efficiency was higher than control polymers; Pluronic P85 and Cremophor EL.

In vitro release of DNM was performed at pH values; 7.4 and 5.0 (Scheme 5-1). Release of doxorubicin was slightly faster at lysosomal pH 5 due to pH sensitive hydrazone bond between the drug and polymer.

The IC50 value of DNM was 10-times lower (higher toxicity) than doxorubicin encapsulated AScMs. AScMs IC50 value is comparable to Cremophor EL and doxorubicin in PBS. This shows us advantages of drug conjugation.

5.1.4 Conclusion

DNM loading, release profile and cytotoxicity assay was studied. Higher loading efficiency was measured in DNM compared to non-conjugated AScMs. DNM released faster in lysosomal pH 5.0. Higher toxicity was achieved in DNM in HepG2, ovarian cancer cells.

5.2 Synthesis of 2CM and 0CM Branched Amphiphilic Scorpion-like Macromolecules

5.2.1 Introduction

Previously, several different amphiphilic macromolecules showed promising results for inhibition of LDL uptake [9, 10]. LDL uptake inhibition studies were performed with linear amphiphilic macromolecules; their particle size are around 18 nm and critical micelle concentration (CMC) is 10^{-7} M [11]. In this study, we synthesized branched amphiphilic macromolecules which consist of one and two carboxylic acid in the hydrophobic region.

5.2.2 Experimental Section

5.2.2.1 Materials

Monomethoxy-poly(ethylene glycol) (mPEG) with molecular weights of 2000 DA and 1,3-dicyclohexylcarbodiimide (DCC) in 1 M methylene chloride was purchased from Sigma-Aldrich. 4-(dimethylamino)pyridinium p-toluenesulfonate (DPTS) was synthesized as previously described [12]. 5-aminoisophthalic acid, N-hydroxyl

succinimide (NHS), thionyl chloride and all other reagents and solvents were purchased from Aldrich.

5.2.2.2 Methods

Structure confirmation was done by ^{1}H NMR spectroscopy with samples (~ 5 mg/ml) dissolved in $CDCl_3$-*d* solvent on Varian 300 MHz and 400 MHz spectrometers, using tetramethylsilane as the reference signal.

The volume-wt particle size distribution of the macromolecules in aqueous solution was measured by dynamic light scattering (DLS) at different time intervals using a Malvern Instruments Zetasizer Nano ZS-90 instrument (Southboro, MA) at a 90° scattering angle at 25°C. Macromolecules were dissolved in deionized water (1 mg/ml) and filtered with 0.45 μm PTFE filters prior to DLS analysis.

Molecular weight was determined by gel permeation chromatography (GPC). Measurements were performed on Waters Breeze GPC system equipped with Styragel® HR3 column (ID 7.8 mm, and length 300 mm), 1515 isocratic HPLC pump and Waters 717 plus Autosampler and with a Water 2414 refractive index detector. THF was used as the mobile phase in GPC system. The average molecular weight of the sample was calibrated against narrow molecular weight poly(ethylene glycol) (PEG) standards (Polysciences, Warrington, PA). Samples were dissolved into THF and filtered through a 0.45 μm PTFE syringe filter (Whatman, Clifton, NJ) before injection into the column at a flow rate of 0.8 ml/min.

5.2.2.3 Synthesis of 2CM Branched AScMs

Molecule **1** (0.18 g, 0.035 mmol) was dissolved with thionyl chloride (20 mL) and heated up to 70°C and heated to reflux for 4 hours. The excess was removed by rotary evaporation after cooling to room temperature. The product was dried under vacuum over night. The acyl chloride product was dissolved in 5 mL methylene chloride, then was added to a solution of 5-aminoisophtalic acid in 10 ml THF and 2 ml pyridine. After 6 hours stirring under room temperature, THF and pyridine was removed by rotary evaporation. The product was redissolved with methylene chloride. Then, the reaction mixture was acidified by 0.1 N HCl solutions (10 ml x 2) and washed by brine. The organic portion was dried over magnesium sulfate ($MgSO_4$) filtered and the solution concentrated to 1 ml and was precipitated by diethyl ether (50 ml).

2CM branched was obtained as a slightly yellow solid. 0.12 g, 67 % yield. ^{1}H NMR ($CDCl_3$): δ 8.42 (s, 2H, Ar*H*), 8.38 (s, 4H, Ar*H*), 5.75 (d, 2H, C*H*), 5.51 (m, 1H, C*H*), 5.14 (d, 1H, C*H*), 4.43 (t, 4H, C*H*$_2$), 3.68 (m, ~360 H, C*H*$_2$O), 2.37 (m, 4H, C*H*$_2$), 2.25 (m, 4H, C*H*$_2$), 1.59 (m, 4H, C*H*$_2$), 1.24 (m, 48H, C*H*$_2$), 0.84 (t, 12H, C*H*$_3$). IR (KBr, cm^{-1}) 3436 (N-H), 2885 (C-H), 1719 (C=O), 1280, (C-O), 960 (Ar-H). T_m = 50-51 °C.

5.2.2.4 Synthesis of 0CM branched AScMs

Molecule **1** (0.18 g, 0.035 mmol) was distilled with toluene (3x 20 ml) to remove water impurity. Then molecule 1 and N-hydroxysuccinimide (0.04 g, 0.34 mmol) was dissolved in methylene chloride (20 ml) and DMF (10 ml). DCC (1.0 ml) was added dropwise to

the reaction mixture under argon. After 12 hours stirring under room temperature, the DCU by product was removed by vacuum filtration. Then, the reaction mixture was acidified by 0.1 N HCl solution (10 ml x 2) and washed by brine. The organic portion was dried over magnesium sulfate ($MgSO_4$) filtered and the solution was concentrated to 1 ml and was precipitated by diethyl ether (50 ml).

0 CM branched was obtained as a white solid. 0.11 g, 0.67 % yield. ^{1}H NMR ($CDCl_3$): δ 8.42 (s, 2H, Ar*H*), 8.38 (s, 4H, Ar*H*), 5.75 (d, 2H, C*H*), 5.51 (m, 1H, C*H*), 5.14 (d, 1H, C*H*), 4.43 (t, 4H, C*H*$_2$), 3.68 (m, ~360 H, C*H*$_2$O**),** 2,81 (t, 4H, CH_2)2.37 (m, 4H, CH_2), 2.25 (m, 4H, CH_2), 1.59 (m, 4H, CH_2), 1.24 (m, 48H, CH_2), 0.84 (t, 12H, CH_3) T_m = 52-53 °C.

5.2.3 Discussion

Several linear amphiphilic macromolecules were synthesized and modified for high-oxidized LDL inhibition uptake in our research group. In this study, pseudo-branched amphiphilic macromolecules were designed to study how size and branched PEG affect LDL uptake. Instead of 5000 molecular weight, 2000 molecular weight of poly(ethylene glycol) was used in branched form. 0 CM branched was synthesized as a control. 1CM branched was prepared as described previously [18]. 2CM branched were prepared to investigate how two carboxylates in the hydrophobic domain influence LDL uptake. 2CM coupled to branched macromolecules with 5-amino carboylic acid linker.

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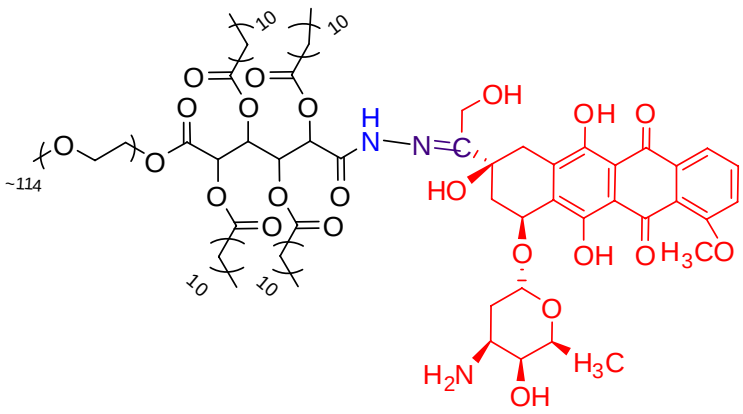
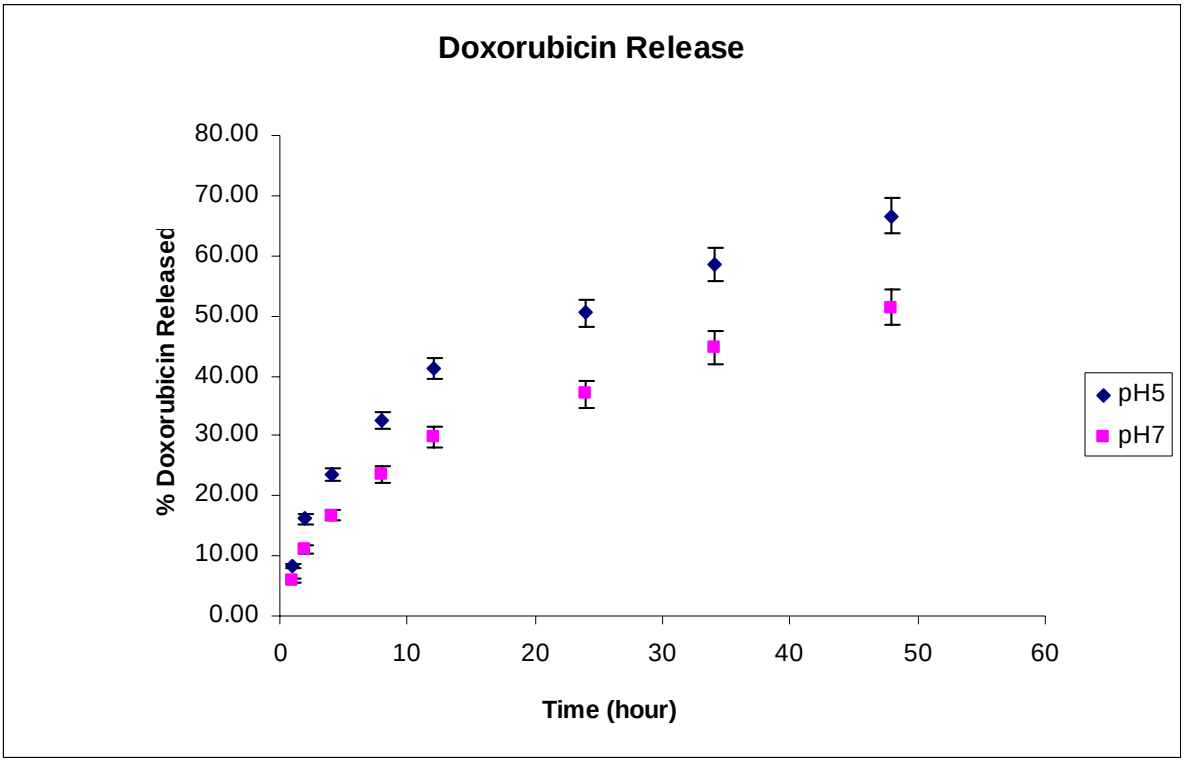


Scheme 5-1 Chemical structure of AScMs-doxorubicin (DNM) [8]

Polymer + Doxorubicin	**Encapsulation Efficiency (%)**	**Weight % Loading**
Doxorubicin-AScM (DNM)	29.70±7.06	59.39 ±4.00
AScM	20.76 ±6.77	41.55 ±3.39
Cremophor EL	19.82 ±6.86	39.64 ±3.43
Pluronic P85	18.27 ±3.51	36.53 ±1.76

Table 5-1 Results of doxorubicin encapsulation

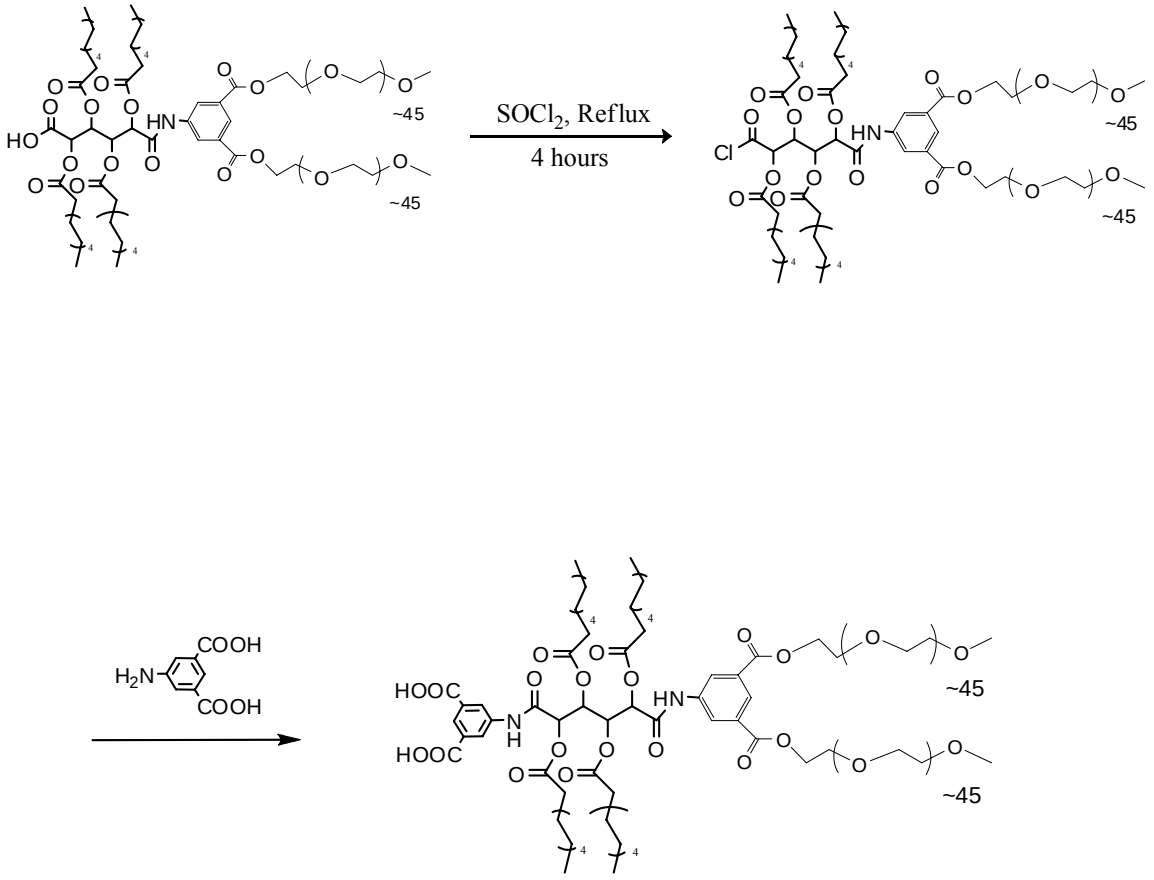


Scheme 5-2 Doxorubicin release from DNM

SAMPLE	**IC50 (M)**
DOX in PBS	8.4×10^{-5}
DOX-loaded AScMs	2.3×10^{-4}
DOX-loaded Cremophor EL	6.0×10^{-5}
DOX-loaded DNM	2.4×10^{-5}
DOX in DMSO	2.7×10^{-5}

Table 5-2 IC50 values for HepG2 cells

Scheme 5-3 Synthesis of 2CM branched AScMs



Scheme 5-4 Synthesis of 0CM branched AScMs