Synthesis of molecular probes for one-electron reduction

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

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ABSTRACT

Controlled drug release plays an important role in medicine because it can be a factor in human health care. It is defined as the ability to release a drug inside the body at a specific time. Radiation-sensitive functionalized materials can be controlled by outside triggers such as radiation and have great potential for application in controlled and targeted drug delivery. Radiation-sensitive functional groups are known and studied, however, there are very few examples. Previous studies have revealed that 2-oxoalkyl group can successfully undergo radiolytic cleavage, but under relatively high doses of radiation. It is desirable that the radiolysis efficiency of 2-oxoalkyl group be improved in order to gain applications in clinical medicine. Thus, one objective of this thesis was to study the effect of a few substituents on the radiolysis efficiency of substituted 2-oxoalkyl groups. Therefore, a few molecular probes that contain an aryl 2-oxoalkyl group with substituted acetophenone of monoesters of adipic acid, and coumarin scaffold of monoesters of adipic acid have been successfully synthesized. These compounds were studied under X-ray radiation at various doses to assess the potential of selective cleavage of the aryl oxo-methyl ester linkage. The radiolysis studies showed that less than 10% of the ester linkages in these compounds were degraded under a dose of up to 20 Gy of radiation. Compounds were also studied for their hydrolytic rate for the aryl oxo-methyl ester linkage using TLC method. Hydrolysis at around neutral pH happened at a slower rate for compounds that are sterically more hindered at the oxo-methyl position.
Poly-L-glutamic acid (PGA) is a natural polypeptide that is biodegradable and biocompatible. Therefore, it has been exploited as drug carrier system. In this thesis, PGA has been modified with a phenacyl group. The lipophilic phenacyl group is assumed to assist the formation of nanoparticles for the modified PGA. Radiolysis studies of such modified PGA showed that the grafted phenacyl group can be selectively cleaved upon radiation with a clinically relevant dose.

Lawsone is a commercially available natural product. It has been used as starting material for the synthesis of different biologically active compounds. Lawsone also has various biological effects including anticancer activity. One anticancer mechanism of lawsone is associated with its ability to undergo one-electron reduction. Thus, another aspect of this thesis was to synthesize lawsone derivatives as potential anticancer agents. The preparation of glycosylated lawsone derivatives was of particularly interest. Direct glycosylation of lawsone using a number of methods was unsuccessful in providing fully deprotected lawsone glycosides. Then the Mannich reaction was employed to produce a group of lawsone derivatives including one compound bearing a glucose residue. The synthesized lawsone derivatives will be evaluated for anticancer activity in the future work.
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<td>UV</td>
<td>ultraviolet</td>
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1. Introduction

1.1. Cancer

The American Cancer Society reports that more than 1,500 people die each day due to cancer. Currently, radiotherapy, chemotherapy and surgery are the possible treatment options for cancer. Chemotherapy treatment involves drugs to treat cancer or relieve cancer symptoms. Often the combination of chemotherapy, radiotherapy and surgery is necessary and useful to overcome cancer. Radiotherapy and surgery are the localized therapies that work only when malignant cells are confined to the treated area. Thus, chemotherapy is crucial for the systemic treatment of metastases relating to the local and regional growth of tumors.¹

The anticancer drugs used in chemotherapy are systemic anti-proliferative agents that prefer to kill the dividing cells. However, the prolonged use of chemotherapy results in lethal damage to proliferating normal cells along with the tumor cells.² Therefore, most chemotherapeutic agents suffer from low selectivity towards tumor cells, and low therapeutic index. The side effects diminish the extent of the dose that can be administered to treat tumors.³

1.2. Anti-cancer drug delivery

Novel drug delivery systems aim at increasing the benefit of drugs and decreasing side effects,⁴ with a goal of increasing their pharmacokinetics and their biodistribution.⁵ Anticancer drugs in conventional chemotherapy are not specific or targeted to the cancer cells; therefore,
targeted delivery of anticancer drugs to cancer tissues is being developed to meet the challenge.\textsuperscript{6}

Drug delivery systems can increase the bioavailability of drugs by entrapment of the drugs within a suitable carrier such as liposomes and micro/nanoparticles of natural or synthetic polymers.\textsuperscript{7} Therefore, the objective of all drug delivery system is to distribute medications to specific body parts through an agent that can control drug bioavailability by a physiological or chemical trigger.\textsuperscript{8} To achieve this goal polymeric microspheres, polymer micelles and hydrogel-type materials have been demonstrated to improve drug targeting specificity, decreasing the drug toxicity, decreasing dosing frequency, and offering protection against biochemical degradation, which results in increasing treatment success rates.\textsuperscript{7}

1.3. Prodrug approach

To improve the therapeutic activity and reduce the side effects of current anticancer drugs, anticancer prodrugs have to be developed. Prodrugs are advantageous in affecting tumor cells and safe to normal cells.\textsuperscript{3} The design of a prodrug aims to make the active drug work on the specific spot, to offer the necessary pharmacological impact, and to limit side effects.\textsuperscript{9}

Consequently, the goal of prodrug design is to temporarily change the physicochemical properties of drugs in order to modify drug pharmacokinetics, prolong action, decrease toxicities and side effects, enhance selectivity, and solve challenges related to formulation.\textsuperscript{10} This can be achieved by a variety of mechanisms that include hypoxic reduction in solid tumors, activation by enzymes overexpressed in tumor tissues, and targeting of antigens or receptors specifically expressed on tumor cell surface.\textsuperscript{3} Therefore, using targeted anticancer
prodrugs for tumor spot selective activation is an effective approach for improving chemotherapy.\textsuperscript{11}

1.4. Stimuli-responsive polymers in drug delivery

New polymers are now being developed that are able to act in response to their environment by changing their physical and chemical properties.\textsuperscript{12} These polymers have been designed to respond to various types of stimuli, such as pH, temperature, mechanical force, various small molecules and biomolecules that are present, as well as electric/magnetic fields. As a result, the polymers are called “stimuli-responsive polymers” or “smart/ intelligent polymers”.\textsuperscript{12} For example, pH-responsive polymers can be reversibly manipulated by changes in external pH. This approach can be used for anticancer drug delivery since for most tumors’ the extracellular pH is more acidic (5.8-7.2). Thus, the release of a drug can be initiated via manipulating the pH.\textsuperscript{13} Another example of stimuli-responsive systems is a photo-responsive system that uses light. This can be utilized in polymer chemistry as an energy source for polymer synthesis, as well as an information source or a trigger, controlling reversibly the physical and chemical properties of polymers.\textsuperscript{14}

1.5. Radiation-sensitive functional groups

Very few radiation-sensitive functional groups are known and have been studied. Consequently, a radiation-responsive drug delivery system is rarely studied. One functional group that has been reported to undergo radiolytic cleavage is the 2-oxoalkyl group. Some studies reveal that the carbonyl group positively affected the release of the leaving group X (Scheme 1) through
one-electron reductive pathway.\textsuperscript{15} Antitumor drugs such as, fluorodeoxyuridine (5-FdUrd) that are toxic to normal cells as well as to tumor cells have been connected to 2-oxoalkyl groups to make prodrugs that can be activated through radiation.\textsuperscript{16} Exposure to radiation can be controlled and so, much like a prodrug, the radiolytic drugs can become active in a specific area, during a particular time, and with a specific dosage.\textsuperscript{15}

An activation mechanism (Scheme 1) has been proposed, in which one-electron is absorbed, and can enter to the $\pi^*$ of the double bond (b). Moreover, by thermal activation the electron in the $\pi^*$ can enter into the $\sigma^*$ (c) that leads to a weakened $\sigma$ bond that can cleave readily (d). Without the 2-oxoalkyl groups, the reaction does not continue. Furthermore, under aerobic conditions, this reaction does not happen\textsuperscript{17} because the oxygen can prevent radiolytic reduction and make a superoxide anion radical.

![Scheme 1](image)

**Scheme 1:** Proposed mechanism for one-electron reduction.

Another molecule, indolequinone was studied for its radiolytic cleavage as well as bio-reductive cleavage. Activation of the indolequinone (e) under reductive conditions results in the formation of hydroquinone (f) or the semiquinone radical (g). This reaction may occur under hypoxic condition resulting in the reduction by one-electron or by two-electron reducing enzymes, and these reactions, along with the effect of oxygen on leaving group to produce h and i, were studied (Scheme 2).\textsuperscript{18}
There are several other functional groups in which the reductive cleavage of a leaving group has been observed/assumed to occur in biological systems through enzymatic processes. The functional groups studied include nitrobenzyl (j), nitroimidazolyl (k) and benzoquinone (l) as shown in Figure 1. The nitrobenzyl can cleave upon one-electron reduction in under hypoxic conditions. This reactivity has been reported in biological systems.
and enzyme-catalyzed processes. Nitroimidazolyl showed that it can increase its cytotoxicity and hypoxia selectivity in radiotherapeutic outcome \textit{in vivo}. In addition, benzoquinone activation has the ability to release a drug. \textsuperscript{11}

Figure 1. Structures of nitrobenzyl group (j), nitroimidazolyl group (k), and benzoquinone group (l)

1.6. Poly-L-glutamic acid (PGA)

In recent years biocompatible polymers gained much attention in the research community. These macromolecules have specific pharmacokinetic characteristics, such as prolong blood circulate, strengthening retention of tissues; along with creating advantageous accumulation in lesions that have leaky vasculature.\textsuperscript{19} Consequently, polymers have become useful drug delivery tools. Moreover, the fusion of small molecular weight anticancer agents to biocompatible polymers can enhance drug deposition at the tumor site and decrease dangerous outcomes of the drug on normal tissues.

For instance, poly-L-glutamic acid is a useful carrier of cancer therapeutics and imaging agents.\textsuperscript{20} Poly-L-glutamic acid polymers can be conjugated to anti-oxidative agents without
impacting the pharmacological properties of the drugs. Due to such characteristics, these polymers have potential as renal targeting drug carriers.\textsuperscript{21}

Poly-L-glutamic acid is a polypeptide made up of L-glutamic acid. It has two forms, poly-\(\alpha\)-L-glutamic acid and poly-\(\gamma\)-L-glutamic acid according to whether the \(\alpha\) or \(\gamma\) carboxy group is involved in the formation of the peptide bond. Their structures are shown in Figure 2.\textsuperscript{22} Poly-\(\gamma\)-L-glutamic acid is a naturally-occurring polymer which can be isolated from bacteria, especially those of \textit{Bacillus} species, or from a traditional food in Japan called Natto.\textsuperscript{23} The application of poly-\(\gamma\)-L-glutamic acid has been increasing rapidly due to its ability of being biodegradable, edible and non-immunogenic.\textsuperscript{22}

Poly-\(\alpha\)-L-glutamic acid, which will be referred to in this thesis later on as poly-L-glutamic acid (PGA), is made up of naturally occurring L-glutamic acid monomers bonded to each other with amide bonds. In this polymer, L-glutamic acid monomers are connected between the \(\alpha\) -carboxyl and the \(\alpha\) -amino groups of adjacent monomers.\textsuperscript{24} The pendant free \(\gamma\) carboxyl group in each repeating unit of L-glutamic acid carries a negative charge at a neutral pH. As a result, the polymer becomes slightly water-soluble. The carboxyl groups facilitate chemical modification of the polymer or drug attachment.\textsuperscript{20} PGA is also biodegradable and biocompatible, which can be degraded by lysosomal enzymes.\textsuperscript{25}
These characteristics make PGA the right factor for polymer–drug conjugates that can be used for selective delivery of chemotherapeutic agents. A number of antitumor agents including doxorubicin, daunorubicin, 1-β-D-araninofuranosylcytosine (Ara-C), uracil and uradine, cyclophosphamide, L-phenylalanine mustard, as well as mitomycin C, have been conjugated to PGA.²⁰ Virtually all of these PGA conjugates have led to better antitumor activity than the parent drug.²⁰ We are interested in the modification of PGA with functional groups that could be cleaved by a dosage of radiation.

1.7. Quinones

Many drugs currently contain the quinone functional group.²⁶ Quinones are very important in chemistry and biochemistry.²⁷

There are two major mechanisms that are behind the cytotoxic and anticancer characteristics of the natural quinone structure. Several enzymes can reduce quinone to hydroquinone, which results in the formation of a fully aromatic system.¹¹ The first pathway, through the one-electron reduction, provides the semiquinone radicals via reductive enzymes such as cytochrome P450 reductase and NADH dehydrogenase. The other pathway occurs through a two-electron
reduction pathway to produce hydroquinone via reductive enzymes such as DT-diaphorase (Scheme 3).28

**Scheme 3:** Pathway of quinone reduction

![Scheme 3](image)

One of the active compounds in biology is 1,4-naphthoquinone. Naphthoquinones are widely available in nature; they are often found in bacteria, fungi, and in several plants.29 1,4-Naphthoquinone is often found in a genus of *Diospyros* L., which is the largest genus in the Ebenaceae family of plants that includes around 500 species. This genus appears in both tropical and temperate regions.28

**Figure 3.** Naphthoquinone structure
The efficacies of these compounds against tumor cells have been shown by various studies that have demonstrated various modes of action. For example, a study indicated that a natural benzoquinone derivative had enhanced anti-proliferative and apoptotic activities against human hormone-refractory prostate cancer cells (PC-3 and DU-145). These compounds need reductive activation in order to form electrophilic species that are toxic to cells. Furthermore, the drugs act as substrates for the reductases found in most of the cells. These drugs can address solid tumors with defined hypoxic fractions and tumor tissues with the activating enzymes.

The bioreduction of naphthoquinones is affected by their redox properties; the ability of these compounds to accept electrons can be changed by adding substituents. Thus, the substituents become electron acceptors or donors to the naphthoquinone rings.

1.7.1 2-Hydroxy-1,4-naphthoquinone (lawsone)

One of the naturally occurring naphthoquinones is lawsone, also called 2-hydroxy-1,4-naphthoquinone (Figure 4). Lawsone is responsible for the red dye of henna. In some countries, henna is used for coloring hair, as well as dying nails and skin. In addition, it has some medicinal benefits. For example, it can be used to treat burns. Henna also have a positive effects on treating microorganisms, headaches, lumbago, bronchitis, ophthalmic, syphilitic sores and amenorrhoea. They also have antibacterial properties. The most important benefit of lawsone is its anticancer property. Lawsone prevents the development of cancer and inhibits cancer cell growth. Thus, this compound can be helpful in developing various drugs for fighting cancer or targeting other types of conditions as mentioned above.
The majority of lawsone’s biological activity arises from and can be explained by the redox cycling and alkylating properties lawsone possesses.\textsuperscript{33} Through oxidation or alkylation of cellular structures, lawsone targets and damages the cells, potentially causing their death. This element is exploited during the use of lawsone in cancer therapy. In this case, cancer cells are more susceptible to oxidative damage as compared to their non-cancerous counterparts. Thus, lawsone can be used to kill the cancerous cells while sparing the non-cancerous ones.\textsuperscript{33}

### 1.7.2 Redox properties of lawsone

In the treatment of cancer, the redox cycling is important to the metabolism of lawsone.\textsuperscript{34} The quinone function of lawsone can be reduced to the semiquinone and thus to the hydroquinone through a chain of two one-electron reductions (Scheme 4). These species are reoxidized by molecular oxygen (O\textsubscript{2}) and create reactive oxygen species (ROS), such as superoxide, hydroxyl radical and hydrogen peroxide.\textsuperscript{29} The superoxide radical is a very reactive species and it is unstable. This instability results in the formation of hydrogen peroxide, which is toxic to cells because it can permeate membranes. ROS are dynamic-oxidizing agents and they
can most likely damage macromolecules such as DNA, proteins and lipids. Thus, ROS can lead to oxidative stress and apoptosis in the cells.\textsuperscript{29}

\begin{center}
\includegraphics[width=0.8\textwidth]{scheme4.png}
\end{center}

\textbf{Scheme 4:} Redox properties of lawsone\textsuperscript{29}

\subsection*{1.7.3 Preparation methods for lawsone}

The synthesis 2-hydroxy-1,4-naphthoquinone has been derived through two main methods: 1) by ring construction or 2) by the use of naphthalene derivatives as starting materials.\textsuperscript{32}

The first method involved the compound 3-methyl-1H-indane, which was oxidized by sodium dichromate, converted to phenylacetic acid and cyclized to produce lawsone as shown in Scheme 5.\textsuperscript{35}

\begin{center}
\includegraphics[width=0.8\textwidth]{scheme5.png}
\end{center}

\textbf{Scheme 5.} Preparation of lawsone \textsuperscript{35}
The other method was achieved by using the economical naphthalene. Some examples of the most useful starting materials for making lawsone (Scheme 6) are examined below. 1-Amino-2-naphthol which is one of the starting materials, has been used with sodium bisulfite in aqueous 6N sodium hydroxide to produces 1-amino-2-naphthol-4-sulfonic acid, which upon hydrolysis with concentrated sulfuric acid in methanol, leads to 2-methoxylawsones.\textsuperscript{32} \(\beta\)-naphthol is another example of the starting material that is converted into \(\beta\)-naphthoquinone which reacts with acetic anhydride-sulfuric acid to produce 1,2,4-trihydroxynaphthalene triacetate, which is then hydrolyzed and oxidized into the desired lawsone.\textsuperscript{36}

\begin{center}
\textbf{Scheme 6.} Other methods used to prepare lawsone\textsuperscript{32}
\end{center}

1.8. The benefits of using sugars in anticancer drugs
The anticancer agents conjugating with sugar residues have received a lot of attention in recent years. Unlike normal cells, cancer cells have the ability to accept larger amount of glucose as explained by the Warburg effect.\textsuperscript{37} Glycoconjugation means linking a drug to glucose or other sugar in order to improve cancer targeting and selectivity.\textsuperscript{38}

Some anticancer drugs contain glycosylated natural products. For example, Digoxin is a well-known glycoside drug that has anticancer activity.\textsuperscript{39} The most important features of the sugar moieties present are increased water solubility, and thus the enhanced bioavailability of such compounds.

\textbf{Figure 5.} Digoxin structure \textsuperscript{39}

Over-expression of glycolytic enzymes and insulin-independent glucose transporter GLUT-1 mediates the effect, which has been recognized as a hallmark of cancer development.\textsuperscript{37} The idea of using glucose as cancer targeting molecular tool has been exploited. Several studies have shown that antitumor drugs bearing glucose residues display improved cancer selectivity compared to normal cells as well as decreasing toxicity of the drugs.\textsuperscript{38}
1.9. Objectives

Currently, the aim of cancer research is to design a drug that is made up of cytotoxic compounds that selectively interact with molecules and target tumor cells with minimal toxicity to normal cells.

In this thesis we are going to study two separate yet related research topics: one is the radiolytic cleavage of aryl-substituted 2-oxoalkyl groups through one-electron reduction process induced by radiation; and the other is the synthesis of naphthoquinone derivatives that are potential anticancer agents after activation through one electron biochemical reduction. Thus, the following specific objectives of the thesis are identified. First, a group of molecular probes containing an aryl-substituted 2-oxo group will be synthesized for radiolytic studies aiming to improve radiolytic efficiency. Second, the biocompatible poly-L-glutamic acid (PGA) will be modified with a radiation-labile functional group to produce radiation-sensitive nano-materials. Third, a group of lawsone derivatives will be synthesized as potential anticancer agents.
2. Results and discussion

2.1. Radiation-sensitive molecular probes

2.1.1. Synthesis of molecular probes for radiolysis study

Radiation–sensitive functional groups can respond to X-ray or gamma ray radiation and undergo selective cleavage of certain bond. The 2-oxoalkyl group has been reported to undergo radiolytic cleavage. In addition, this group is removed by X-ray radiation under hypoxic conditions in an aqueous solution. Under hypoxic conditions, X-ray radiation produces hydrated electrons, as well as hydrogen radicals and hydroxyl radicals in an aqueous medium.

\[
\text{H}_2\text{O} \xrightarrow{\text{X-radiation}} \text{H}^+ + \text{OH}^- + e_{(eq)}
\]

One problem associated with the radiolytic cleavage of 2-oxo-alkyl group is that it needs a relatively large dose of radiation; however, we are hoping to achieve the cleavage of such a group under clinically relevant radiation doses (< 30 Gy).

According to the proposed radiolysis mechanism of 2-oxo-alkyl group (Scheme 7), this radiolysis rate may be affected by the following factors:

a) The ease of the compound in absorbing electron(s)

b) The leaving ability of the leaving group

c) The stability of the free radical generated after the cleavage of the bond.
Previous studies have shown that an aromatic functionality in the neighboring environment of the 2-oxo-alkyl group can accelerate its radiolytic cleavage. Here we anticipate that an aromatic ring directly linked to the 2-oxo-alkyl group may be able to enhance its radiolytic rate. In order to improve the radiolytic efficiency of 2-oxo-alkyl group, we plan to attach an aromatic ring to the 2-oxo-alkyl group. According to the proposed activation mechanism (Scheme 7), structure m can absorb one electron which can enter into the $\pi^*$ of the C=O double bond. By thermal activation the electron in $\pi^*$ can enter into the $\sigma^*$, which leads to a weakened $\sigma$ bond that can cleave readily. The aryl functionality in the aryl 2-oxo-alkyl group is assumed to facilitate the fast capture of electron(s) and the stabilization of free radical species (n - p). These features are expected to result in a faster radiolytic cleavage of the aryl 2-oxo-alkyl group than the simple 2-oxo-alkyl group. The relative stability of the initial free radical (p).
generated from the radiolytic cleavage probably plays an important role in the final step of the cleavage. Since free radicals display the following trend of stability: $3^\circ > 2^\circ > 1^\circ$, alkyl substitutes at the carbon atom next to the oxo-group will stabilize the free radical and consequently accelerate the cleavage of the C-X bond in structure (p).

A group of molecular probes (1 - 6, Figure 6) have been designed to study the effect of these structure features on the radiolysis rate of aryl 2-oxo-alkyl group. Structures 1 - 5 are derivatives of simple acetophenone or substituted acetophenone and structure 6 contains a coumarin scaffold. All these molecules are monoesters of adipic acid. We are going to study poly-L-glutamic acid (PGA) modification, which has multiple carboxylic acid groups. Therefore, we have designed those molecular probes having one carboxylic acid functional group to resemble L-glutamic acid residues of the polymer. The carboxylic acid group in these probes will also make these molecules more hydrophilic and increase their water solubility.

![Figure 6. Designed molecular probes](image-url)
For the preparation of probes 1 – 4, adipic acid was reacted with one equivalent of a bromoacetophenone derivative (7a, 7b, 8a, and 8b) in triethylamine (Et₃N) and EtOAc at room temperature to provide monoester (1 - 4) in 20% to 60% yield (Scheme 8). The corresponding diesters (9 – 12) were also obtained as the minor product for each of these reactions. The structures of these products were confirmed by ¹H NMR and ¹³C NMR spectral data.

Scheme 8. Synthesis of designed compounds (1 - 4)

For the preparation of probe 5, we did not use the reagent 2-bromo-2-methylpropiophenone to react with adipic acid. It is likely that the bromide would react with the acid via an S₆2 mechanism, which is anticipated to be very slow for this 3° bromide. Therefore, we tried to form this ester through an acid and alcohol as shown in Scheme 9. This reaction happens through a mechanism of Steglich esterification reaction. The synthesis of 5 and 14...
was started with 2-hydroxy-2-methylpropiophenone that was combined with adipic acid, 4-N, N-dimethylaminopyrdine (DMAP) and N, N-diisopropylcarbodiimide. The reaction was first tried in dimethylformamide (DMF) as solvent at either room temperature or 50℃, but it gave mixed products that was difficult to purify. Changing the solvent to dichloromethane (DCM) at room temperature provides the desired product (5) in 19% yield and the corresponding diester (14) in 21% yield. Their structures were also confirmed by ¹H NMR, and ¹³C NMR data.

![Chemical structures](image)

**Scheme 9. Synthesis of probe 5**

Probe 6 contains a coumarin scaffold which can be viewed as an extended aryl system with fluorescent property. For the preparation of 6, adipic acid was reacted with 3-(bromoacetyl)-coumarin in the presence of triethylamine as the base at room temperature. However, the reaction was not efficient and the product was an inseparable mixture as TLC showed. Changing the base to sodium bicarbonate was attempted; TLC investigations revealed that the reaction did not occur either at room temperature or under reflux. However,
using N,N-diisopropylethylamine (DIPEA) in EtOAc as the solvent under reflux temperature gave successfully the desired product 6 in 17% yield. The diester (16) was also formed in small quantity (2% yield) in this reaction. The structures of these compounds were confirmed by $^1$H NMR, and $^{13}$C NMR.

Scheme 10. Synthesis of molecular probe 6

2.1.2. Synthesis of tyrosine derivatives
The 2-oxoalkyl group has been reported in the literature to undergo radiolytic cleavage when it is linked to a leaving group. One particular example is a tyrosine derivative in which the 2-oxoalkyl group is connected to the phenolic hydroxyl group (19, Scheme 11). Under hypoxic conditions, the 2-oxopropyl group in 19 can be released by radiolytic one-electron reduction. Here we repeated the synthesis of 19 according to the published procedure. Compound 19 is to be used as a positive control for the radiolytic studies of the synthesized molecular probes (1 – 6, Figure 6).

Scheme 11. Synthesis of tyrosine derivatives

2.1.3. Synthesis of cholesterol derivatives

A cholesterol fragment is a large hydrophobic group, and not toxic. It has been widely used as a lipophilic tag to modify biologically interesting molecules. When cholesterol is incorporated with radiation sensitive groups, large structural changes due to large increase in lipophilicity can be expected for the molecule being modified.

Here we prepared cholesterol derivative 21 with an acid functionality (Scheme 12). The
synthesis of 20 was started with solution of sodium hydride in tetrahydrofuran stirred at room
temperature then followed by adding cholesterol and tert-butyl bromoacetate. The mixture was
stirred at 80°C to provide 20 in 67% yield. Then, 20 was dissolved in DCM and treated with
trifluoroacetic acid-water (95:5) at 0°C to provide 21.

Compound 21 can, in theory, be linked to a radiation sensitive group, such as the group
containing an aryl 2-oxo-alkyl moiety. However, since the synthesized probes (1 - 6) did not
show favorable radiolytic properties (see section 2.1.5), the idea of incorporating the
cholesterol moiety into the radiolytic group was not further investigated.
2.1.4. Synthesis of modified poly-L-glutamic acid (PGA)

Poly-L-glutamic acid (PGA) is a biocompatible polymer that shows great potential as a drug carrier. Each side chain of the amino acid residues in PGA has one free carboxylic acid.
group which is the basis for chemical modification. Modification of PGA with a lipophilic group can produce modified PGA (m-PGA), which may facilitate the formation of nanoparticles suitable for drug delivery. In addition, when the lipophilic group contains radiolytically labile chemical linkage(s), such m-PGA will be responsive to radiation. Drug carriers comprising of such radiation-sensitive material can achieve controlled drug releases upon activation by radiation. Towards this end, we have prepared a couple of m-PGA products modified with phenacyl group.

The commercially available PGA sodium salt was treated with HCl (pH = 1) to give the acid form of PGA (Scheme 13). PGA was then treated with phenacyl bromide and triethylamine (Et₃N) in dry DMF at 40°C to provide modified PGA. By using different amounts of reagents, one could achieve different degrees of modification. Table 1 summarizes two reaction conditions and the corresponding product details. Upon the completion of the reaction, the product was precipitated out by adding hexane-ethyl acetate (1:1, v/v), washed, and collected after centrifuge. The recovered yield of the material is typically over 80% according to this procedure (entry 1 in Table 1). However, ¹H NMR spectral data of the product m-PGA-Ph-24 indicates the presence of triethylamine, presumably in the form of HBr salt and/or carboxylic acid salt of the glutamic acid residues. In order to remove triethylammonium salt, the work-up procedure of product m-PGA-Ph-32 (entry 2, Table 1) was modified. The initial product obtained from the procedure described earlier was re-dissolved in DMF and then re-precipitated out from water. ¹H NMR spectral data of m-PGA-Ph-32 indicates that the triethylammonium salt is no longer present.

The structures of m-PGA were all confirmed by ¹H NMR spectral data. Typically, a proton signal at around δ 5.5 ppm corresponds to the CH₂ residue of the phenacetyl group and
the signal at around $\delta$ 4.8 ppm resulted from the proton at the $\alpha$-carbon of the glutamic acid residue. The integration ratio of these two signals can be used to calculate the % of side chain residues that have been modified.

**Scheme 13.** Preparation of modified poly-L-glutamic acid (m-PGA).

**Table 1.** Reaction conditions and product details of PGA modification.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Phenacyl bromide (equiv.)</th>
<th>Et$_3$N (equiv.)</th>
<th>Reaction Time (h)</th>
<th>Product</th>
<th>Degree of modification (%)</th>
<th>Recovery yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.3</td>
<td>0.3</td>
<td>16</td>
<td>m-PGA-Ph-24</td>
<td>24</td>
<td>85</td>
</tr>
<tr>
<td>2</td>
<td>0.4</td>
<td>0.4</td>
<td>16</td>
<td>m-PGA-Ph-32</td>
<td>32</td>
<td>61</td>
</tr>
</tbody>
</table>

**2.1.5. Radiolytic studies of synthesized probes**

The radiolysis data for sections 2.1.5 and 2.1.6 were provided by our collaborator Dr. Wilson Roa at Cross Cancer Institute & the University of Alberta, Edmonton, Alberta, Canada.

The synthesized probes (1, 2, 4 and, 5) were subjected to X-ray radiation at different doses to evaluate the potential of selective cleavage of the aryl oxo-methyl ester linkage.$^{15,16}$
while compounds 3 and 6 were not determined because these two compounds were prepared after the evaluation of other compounds had been completed. After irradiation, the concentration of the original compound in each sample was measured by HPLC after 24 h and the results are summarized in Table 2. The data indicates that less than 10% of the sample was degraded when each of the probes was treated with X-ray radiation at a dose up to 20 Gy. The value in parenthesis indicates the % of decrease (–) or increase (+) of the concentration after irradiation relative to the control. Although compound 1 (having 8.5% degradation at 5 Gy) appears to have a higher degree of degradation than other compounds, the relatively low % of cleavage does not make such comparison meaningful. Thus, the structural influence on the radiolysis rate cannot be concluded based on these preliminary data.

Table 2. Change of sample concentration of probes (1, 2, 4, and 5) after X-ray irradiation

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (mg/mL) (±%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose 0 Gy</td>
</tr>
<tr>
<td>1</td>
<td>0.684</td>
</tr>
<tr>
<td>2</td>
<td>0.621</td>
</tr>
<tr>
<td>3</td>
<td>Nd</td>
</tr>
<tr>
<td>4</td>
<td>0.657</td>
</tr>
<tr>
<td>5</td>
<td>0.530</td>
</tr>
<tr>
<td>6</td>
<td>Nd</td>
</tr>
</tbody>
</table>

nd: not determined.
Compound 1 was also subjected to radiation under a much higher dose. Table 3 shows the sample concentration changes as a result of X-ray radiation under different doses. Again, the value in parenthesis indicates the % of decrease (−) or increase (+) of the concentration after the irradiation relative to the control. Radiation at a dose up to 200 Gy led to only about 6% of the sample being degraded. The data indicates that radiolytic cleavage is not significant even at a radiation dose that is much higher than the clinically relevant dose. For comparison purpose, compound 19 (Scheme 11) was also subjected to radiolysis study along with compound 1. The results indicated that only a small percentage (< 10%) of compound 19 underwent radiolytic cleavage under the radiation dose of up to 200 Gy (data not shown). This is in contrast to the report earlier that the same compound 19 underwent about 30% selective radiolytic cleavage at an radiation dose of 150 Gy.\textsuperscript{16} It is speculated that the experimental condition for radiolysis studies in our collaborator’s lab has not been optimized. Radiolysis studies need to be repeated with these probes when the experimental conditions have been modified.

Table 3. Concentration change of compound 1 after X-ray radiation at different doses

<table>
<thead>
<tr>
<th>X-ray dose (Gy)</th>
<th>C1 (µg/mL)</th>
<th>C2 (µg/mL)</th>
<th>C (average, µg/mL) (±%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>10.33</td>
<td>10.18</td>
<td>10.26</td>
</tr>
<tr>
<td>20</td>
<td>10.52</td>
<td>10.40</td>
<td>10.46 (+ 1.9)</td>
</tr>
<tr>
<td>100</td>
<td>9.87</td>
<td>9.69</td>
<td>9.78 (− 4.7)</td>
</tr>
<tr>
<td>200</td>
<td>9.96</td>
<td>9.79</td>
<td>9.88 (− 3.7)</td>
</tr>
</tbody>
</table>
At this point the radiolysis data for these synthetic probes are still preliminary results. It is still under investigation to determine the optimal experimental conditions for radiolysis studies. More experiments are needed in order to optimize the experimental conditions and fully characterize the degradation products.

2.1.6. Radiolytic studies of modified PGA

The m-PGA samples were subjected to X-ray radiation to study their radiolytic properties. Typically, an m-PGA sample was first dissolved in dimethylsulfoxide (DMSO) and then dd-H$_2$O was added. The resulting suspension was centrifuged, washed with dd-H$_2$O and then re-suspended in dd-H$_2$O for radiolysis study$^{15}$. The sample was radiated with X-ray radiation (200 kV, 15 mA) at a dose up to 30 Gy. The phenacyl group cleaved from m-PGA resulted in some aromatic compounds that were extracted with EtOAc and analyzed with UV spectroscopy. The UV absorbance was used to show the radiolysis effect. Figure 6 shows the radiolysis effect of one specific m-PGA under the dose of 5 Gy, 10 Gy or 30 Gy. The data shows that X-ray radiation significantly increases the cleavage of the phenacyl group in m-PGA. The fact that the control sample without radiation (0 Gy) also produces UV-absorbance suggests that auto-hydrolysis of the phenacyl ester group might have occurred to some extent, which requires further study to confirm.
2.1.7. Hydrolysis studies of synthesized probes

Radiolysis studies with m-PGA samples suggest that the phenacyl ester bond might undergo auto-hydrolysis. The substitution at the \( \alpha \)-position of the carbonyl group \((R_1 \text{ and } R_2)\) may increase the rate of radiolytic process but slow down the auto-hydrolysis rate of the ester linkage under physiological condition. In order to understand the effect of different substituents on the relative stability of phenacyl esters, we have qualitatively investigated the hydrolysis rate of molecular probes \((1, 2, 4 \text{ and } 5, \text{ Figure 8})\).
The hydrolysis rate of molecular probes (1, 2, 4 and, 5) has been studied under 0.5 M NaH₂PO₄/Na₂HPO₄ buffer solution (pH 7.0) at room temperature (22°C) using qualitative TLC (thin layer chromatography) analysis. The chemical reaction is shown in Scheme 14 and TLC was used to monitor the progress of the reaction. Results are presented in Figure 9. The hydrolysis of 1, 2, 4 and 5 yields the corresponding aromatic alcohol 1a, 2a, 4a and 5a as the hydrolyzed product (Scheme 14), which can be readily detected by UV absorbance or staining. The relative intensity of the product spot to the starting material spot indicates the degree of hydrolysis in each case. The TLC data indicate that compounds 1 and 2 are accompanied with significant hydrolysis after four to five days under the above conditions. On the other hand, hydrolysis is not significant for compounds 4 and 5 after stirring for two weeks under these conditions. The results indicate that steric hindrance at α-position of the carbonyl group can slow down the rate of auto-hydrolysis.
Scheme 14. The potential hydrolysis of molecular probes 1, 2, 4 and 5

1 R= R₁=R₂= H
2 R= R₁=H, R₂=CH₃
4 R = R₁= H, R₂= Ph
5 R=H, R₁=R₂=CH₃

1a R= R₁=R₂= H
2a R= R₁=H, R₂=CH₃
4a R = R₁= H, R₂= Ph
5a R=H, R₁=R₂=CH₃
Figure 9. TLC results of the hydrolysis reaction of molecular probes 1, 2, 4 and 5 under pH 7 at 22°C with (hexane / ethyl acetate/ methanol = 1:1:0.2). SM: starting material; P: hydrolysis product (1a, 2a, 4a and 5a); Salt of SM: the sodium salt form of the starting material; on each TLC plate, the first column represents the pure starting material dissolved in ethyl acetate. The second column represents the mixture of the starting material and the reaction medium. The third column represents the reaction medium.
2.2. Synthesis of lawsone derivatives

Lawsone (2-hydroxy-1,4-naphthoquinone) has the orange-red colour artefact that makes through the extraction or preparation of the dye from henna leaves. Lawsone is structurally related to naphthalene and characterized by its two carbonyl groups at the 1,4-position, and as such, are named 1,4-naphthoquinone. Carbonyl groups may also be present at the 1,2-position, with minor incidence.

Lawsone has various biological activities such as, antioxidant, anti-inflammatory, antibacterial and anticancer activities. This is the reason why this type of natural organic compounds is attractive in different areas of research. Lawsone has been used as a starting material in the synthesis of many clinically useful anti-cancer compounds such as atovaquone, lapachol and dichloroallyl lawsone.

We are interested in the preparation of glycosylated lawsone derivatives since conjugation of a sugar residue may affect the physical, chemical and
biological properties of lawsone. As discussed earlier in Section 1.8, cytotoxic drugs carrying a glucose residue may target cancer cells more selectively than normal cells. Therefore, glycosylated lawsone derivatives may be useful antitumor agents that have low toxicity to normal cells/tissue.37

2.2.1. Attempts to synthesize glycosylated lawsone

2.2.1.1. Synthesis of peracetylated glucosyl lawsone

The synthesis began with the protection of all OH groups in glucose with acetic anhydride in the presence of pyridine at room temperature. This reaction resulted in product 22, which was found in one particular spot on the TLC. Furthermore, removal of the acetyl group in the anomeric position was done in one step. In this case, ammonium carbonate was utilized in anhydrous dimethylformamide (DMF) to provide 23; this compound needed to be purified by chromatographic separation. 23 was then treated by trichloroacetonitrile with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as catalytic base in anhydrous dichloromethane to give 24 in 90% yield. This synthesis was carried out according to a reported procedure with some modification.45 Direct coupling of glycosyl imidate 24 with lawsone 25 was achieved in anhydrous tetrahydrofuran and dichloromethane with boron trifluoride diethyl etherate as the catalyst at room temperature, to provide 26 in 65% yield. In addition, 27 was yielded in 18%. Both compounds were confirmed by $^1$H NMR and $^{13}$C NMR data.
Scheme 15. Synthesis of peracetylated glucosyl lawsone derivatives

2.2.1.2 Attempts of deacetylation
To deprotect acetyl groups on the glucose residue of 26, we used various basic conditions (Table 4), which led to an unexpected and undesired result of the cleavage of glycoside linkage (Scheme 16). First the removal of acetyl group in compound 26 by tetrahydrofuran (THF) and water in triethylamine (Et₃N) was unsuccessful and TLC showed the formation of 25. A different base, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) was also tried. This was done with toluene solvent or methanol solvent, which showed a similar result. Furthermore, guanidine-guanidinium nitrate (G/GHNO₃) has been reported to be a very mild and efficient method to remove acetyl groups. Therefore G/GHNO₃ solution was prepared by using guanidinium nitrate in anhydrous MeOH/DCM and adding sodium methoxide (MeONa). Treatment of 26 with G/GHNO₃ resulted in the formation of 2-methyl-1,4-naphthoquinone 29 which was confirmed by ¹HNMR data.

Table 4. Conditions used in deacetylation reaction

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Temperature</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Et₃N</td>
<td>rt.</td>
</tr>
<tr>
<td>ii</td>
<td>DBU</td>
<td>rt.</td>
</tr>
<tr>
<td>iii</td>
<td>DBU</td>
<td>60 °C</td>
</tr>
<tr>
<td>iv</td>
<td>Guanidinium + guanidinium nitrate + MeONa</td>
<td>rt.</td>
</tr>
</tbody>
</table>
This unexpected result can be explained by the possible mechanism shown in Scheme 17. Using the formation of 29 as an example, methanol functions as a nucleophile under weakly basic condition. First, a lone pair in methanol attacks through a simple nucleophilic addition to the α,β-unsaturated carbonyl system (a 1,4-addition reaction). The intermediate (r) then regenerates the carbonyl system with the OR group (sugar residue) functioning as a leaving group, leading to the formation of 29. The formation of lawsone 25 in these deacetylation reactions would follow a similar pathway wherein H₂O is the initial nucleophile attacking the α,β-unsaturated carbonyl system followed by removal of the sugar residue.
2.2.1.3. TMS protection strategy

Glycosylated lawsone with tetramethysilyl (TMS) as the protecting group for the sugar OH groups was synthesized (Scheme 18). According to the known literature procedure, glucose was first protected via trimethysilyl chloride with triethylamine.

---

Scheme 17. Proposed mechanism for the cleavage of glycosic bond
(Et$_3$N) in dimethylformamide (DMF) to provide the desired product 30 with 88% yield. The protected compound 30 was treated by trimethylsilyl iodide in anhydrous DCM, which gave 31 in 74% yield. Simultaneously, 1,4-hydroxy-naphthoquinone 25 was reacted with sodium bis (trimethylsilyl) amide as base in THF at room temperature to provide a mixture of 32 and 33, which was further reacted with 31 in the presence of 15-crown-5. The reaction produced a mixture of products as indicated by multiple spots on TLC, presumably both 34 and 35, each of which as a β/α mixture. The mixture was not purified for structure characterization since TMS ethers are pretty unstable.

Then, the product mixture (34/35) was subjected directly to deprotection in order to obtain lawsone glycosides with free sugar (36/37) under various conditions (Table 5). Unfortunately, all the trials were unsuccessful (Scheme 19). The first method involved methanol under reflux. This condition led to the decomposition of 34/35 to form lawsone 25, which was noted by TLC. Moreover, amberlite IRC-50, a weakly acidic resin, in MeOH, was tried to achieve deprotection. This process gave a similar result as the one mentioned above. In addition, amberlite IRC-120 (H$^+$ form) in MeOH provided 2-methoxy-1,4-naphthoquinone 29 which was confirmed by $^1$HNMR data. The formation of 25/29 under these conditions is probably via the same mechanism as described in Scheme 17. TMS deprotection can generally be achieved under weak acid or base condition; however, in this case it was not successful.
Scheme 18. Attempted synthesis of glucosyl lawsone through TMS protection strategy
Table 5. Conditions used in deprotecting TMS groups

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Temperature</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>i)</td>
<td>Reflux</td>
<td>MeOH</td>
</tr>
<tr>
<td>ii) amberlite IRC-50 resin</td>
<td>rt.</td>
<td>MeOH</td>
</tr>
<tr>
<td>iii) amberlite IRC-120 resin</td>
<td>rt.</td>
<td>MeOH</td>
</tr>
</tbody>
</table>

Scheme 19. The result of deprotection of TMS group
2.2.1.4. Attempted synthesis of C-glycosides of lawsone via aldol condensation reaction

When a ketone enolate or enol reacts with an aldehyde, the reaction is called an aldol condensation and forms a new C-C bond. Lawsone is a typical enol (Scheme 20). In fact, lawsone has been used to couple with an aldehyde to form similar aldol condensation type of product. Through an aldol condensation lawsone can react with an aldehyde under mild conditions to give condensation product in very good yield.

![Scheme 20. Lawsone as an enol](image)

A sugar (aldose) is an aldehyde in its open chain form. It can potentially react with lawsone to yield a C-glycoside of lawsone (Scheme 21). The reaction mechanism of this reaction is shown in Scheme 22. The reaction is expected to be catalyzed by a weak acid or base. Therefore, direct coupling of lawsone with glucose to give C-glycoside of lawsone has been tried under different conditions as shown in Table 6. Unfortunately, none of the conditions tried yields the desired product. Some reagents appeared to give new spots on TLC, but when purified only starting material lawsone was obtained. With beta-alanine conditions, a polar spot on TLC was detected, which might be alanine attached to
lawsone.

Scheme 21. Attempted synthesis of C-glycosides of lawsone

Scheme 22. Proposed mechanism for the formation of lawsone C-glycosides
Table 6. Reaction conditions tested for the formation of lawsone C-glycoside

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaHCO₃ / H₂O</td>
<td>rt. / 60 °C / 100 °C</td>
</tr>
<tr>
<td>NaOH / H₂O</td>
<td>rt. / 60 °C</td>
</tr>
<tr>
<td>H₂SO₄ / H₂O</td>
<td>rt. / 60 °C / 100 °C</td>
</tr>
<tr>
<td>AcOH / HCl / H₂O</td>
<td>80 °C</td>
</tr>
<tr>
<td>AcOH</td>
<td>80 °C / 100 °C</td>
</tr>
<tr>
<td>AcOH / H₂O (1:1)</td>
<td>80 °C / 100 °C</td>
</tr>
<tr>
<td>AcOH /Beta-alanine/ H₂O (3:1)</td>
<td>50 °C / 80 °C</td>
</tr>
<tr>
<td>AcOH + Beta-alanine</td>
<td>50 °C</td>
</tr>
<tr>
<td>Et₃N / THF</td>
<td>rt. / 69 °C</td>
</tr>
<tr>
<td>Aniline / AcOH / Absolute ethanol</td>
<td>rt. / 50 °C</td>
</tr>
<tr>
<td>Aniline/ AcOH/ imidazole / ethanol (1:1)</td>
<td>rt. / 50 °C</td>
</tr>
<tr>
<td>EtOH / H₂O (1:1)</td>
<td>rt. / 50 °C / 80 °C / 100 °C</td>
</tr>
<tr>
<td>AcOH / DMF</td>
<td>100 °C</td>
</tr>
<tr>
<td>N, N-diisopropyl -ethylamine /DMF</td>
<td>rt. / 50 °C / 100 °C</td>
</tr>
<tr>
<td>AcOH+H₂SO₄</td>
<td>125 °C</td>
</tr>
</tbody>
</table>

2.2.2. Synthesis of lawsone derivatives via Mannich reaction

The Mannich reaction is one of the most useful reactions in organic chemistry, which has been utilized to form carbon-carbon bonds. This reaction is based on three components: an amine, an aldehyde, and an enolizable ketone,
which can produce β-amino carbonyl compounds. These compounds are very valuable intermediates for the synthesis of many pharmaceutical and natural products containing nitrogen in their structures. Lawsone derivatives have been reported to be prepared through Mannich reaction.

In this thesis, a group of lawsone derivatives (45 – 49) was produced by the Mannich reaction between lawsone, primary or secondary amine (ethanolamine, 3-amino-1-propanol, or diethanolamine), and an aldehyde (formaldehyde or benzaldehyde) in ethanol at room temperature in very good yield (Scheme 23, Table 7). The yields obtained were in the range of 82% to 98%. When the same reaction was tried with hexylamine and formaldehyde, TLC analysis indicated the formation of additional nonpolar spots, which complicated the isolation of the usual product. The structures of 45 – 49 were confirmed by \(^1\)HNMR and \(^{13}\)CNMR data.

\[ \text{Scheme 23. Synthesis of lawsone derivatives using Mannich reaction} \]
Table 7. The reagents used to synthesize lawsone derivatives via Mannich reaction

<table>
<thead>
<tr>
<th>Aldehyde</th>
<th>Amines</th>
<th>Solvent</th>
<th>Product</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>H₂N₃OH</td>
<td>ethanol</td>
<td><img src="45_46.png" alt="Chemical Structure" /></td>
<td>73%</td>
</tr>
<tr>
<td>46</td>
<td>HO⁻CH₂CH₂NH₂</td>
<td>ethanol</td>
<td><img src="46_47.png" alt="Chemical Structure" /></td>
<td>94%</td>
</tr>
<tr>
<td>47</td>
<td>HO⁻CH₂CH₂NH₂</td>
<td>ethanol</td>
<td><img src="47_48.png" alt="Chemical Structure" /></td>
<td>98%</td>
</tr>
<tr>
<td>48</td>
<td>H₂N₃OH</td>
<td>ethanol</td>
<td><img src="48_49.png" alt="Chemical Structure" /></td>
<td>86%</td>
</tr>
<tr>
<td>49</td>
<td>HO⁻CH₂CH₂NH₂</td>
<td>ethanol</td>
<td><img src="49_50.png" alt="Chemical Structure" /></td>
<td>97%</td>
</tr>
</tbody>
</table>
Hence, the Mannich reaction worked successfully to attach lawsone to an aldehyde and an amine. Next, we tried to use Mannich reaction to attach lawsone with glucose (Scheme 24). First D-glucose was converted to the imidate 24 as described earlier (Scheme 15). Then, tert-butoxycarbonyl (Boc) group was used to protect the amine group in 3-amino-1-propanol by reacting with di-tert-butyl dicarbonate in presence of triethylamine in CHCl₃ to afford 50. Compound 50 was ready to react with 24 in the presence of boron trifluoride diethyl etherate BF₃O(Et)₂ as the catalyst in dichloromethane to provide the desired product (51) in 14% yield. After that 51 was treated with sodium methoxide in anhydrous methanol to give the de-acetylated intermediate, which was then treated with trifluoracetic acid (TFA) - water (95:5) to remove the Boc group, affording 52. The glycosylated amine derivative 52 was reacted with lawsone in the presence of formaldehyde in ethanol at room temperature. The formation of 53 was not a very clean reaction as TLC showed multiple spots that was in contrast to those listed in Table 7. Flash chromatography had to be used to purify 53, which was obtained in 15% yield. The structure of 53 was confirmed by ¹H NMR data. Signals found in both aromatic and sugar regions confirm that lawsone and glucose are connected.
3. Conclusion

An important element of health care is controlled drug release. In this case, a drug is released inside a person. This is a process that occurs at a specific time in a specific location. Outside triggers can control radiation-sensitive functionalized material, such as radiation. These materials can be utilized during controlled and targeted delivery of drugs. Radiation-sensitive functional groups are known and studied. However, very few examples of such groups exist. Earlier studies show that 2-oxoalkyl group has the capacity to go through a radiolytic cleavage. Here, we synthesized a group of molecular probes (1 – 6) that encompass aryl substituted 2-oxoalkyl groups. These molecular probes were
studied under X-ray radiation at various doses. This was done in order to
determine the possibility of selective cleavage of the aryl oxo-methyl ester
linkage. The hydrolytic rate of the ester linkage in molecular probes (1, 2, 4 and
5, Figure 6) was studied. TLC analysis of the hydrolysis reaction mixture
indicated that hydrolysis occurred at a faster rate for 1 and 2 than for 4 and 5.

Poly-L-glutamic acid (PGA) is a natural polypeptide. It is both
biodegradable and biocompatible. As a result, it is being exploited for utilization
as a drug carrier system. In this research, we modified PGA with the lipophilic
phenacyl group in order to make radiation-sensitive nanomaterials. Preliminary
experimental data show that the phenacyl groups grafted on the PGA can be
selectively cleaved upon radiation with a clinically relevant dose (up to 20 Gy).

Lawsone is commercially available and it can be obtained from natural
sources. Lawsone as starting material has been used for the synthesis of
different biologically active compounds. Lawsone also has biological effects such
as anticancer and antibacterial activity. In this study lawsone was used as a
starting material to synthesize lawsone derivatives as potential anticancer
agents. We are particularly interested in the preparation of glycosylated lawsone
derivatives. Direct connection of lawsone to glucose through either O-glycosidic
linkage or C-glycosidic linkage was unsuccessful using various conditions in this
study. Through a Mannich reaction lawsone was successfully linked to various
polar amines including a glycosylated amine to provide lawsone derivatives as
potential anticancer agents. The structures of these lawsone derivatives were
classified by $^1$H and $^{13}$C NMR spectral data.
4. Future work

The first part of the research focuses on the synthesis of probes 1 - 6 to study the structure effects on radiolysis efficiency. However, the result of radiation study was not clear, therefore further studies are needed. For the modification of poly-L-glutamic acid, the phenacyl-modified polymer showed some indication of responsiveness to radiation. Therefore, more modifications of PGA can be done with different modifying groups. In the second part, the Mannich reaction works in providing glycosylated lawsone derivative 53. The synthesis of 53 could be repeated to get more material and obtain other analytical data including $^{13}$C NMR and mass spectrum data- due to time restrain, I was not able to complete this part of the work. All the synthesized lawsone derivatives (45 – 49 and 53) will be evaluated for anticancer activity. Furthermore, other glycosylated lawsone derivatives could be prepared using the Mannich reaction. A few examples are shown in Figure 10 for future research.
Figure 10. Glycosylated lawsone derivatives for future research
5. Experimental

5.1. General Methods

Thin Layer Chromatography (TLC) was performed on Silica Gel 60-F_{254} (Silicycle) with detection by quenching of fluorescence (254 nm), by dipping into 15% solution of H_{2}SO_{4} and/or Mostaine reagent [ammonium molybdate (NH_{4})_{6}Mo_{7}O_{24}.4H_{2}O (20 mg) and Cerium (IV) sulphate (Ce(SO_{4})_{2}, 0.4 g) in 10 % sulphuric acid (400 ml)] followed by charring on hot heating plate. superscript 1H NMR spectra were measured at 499.9 MHz (Varian Unity Inova 500 MHz). superscript 13C NMR spectra were recorded at 125 MHz on the same instrument. TMS (Me_{4}Si, 0 ppm) or solvent peaks were used as reference standard. Column chromatography was done on Silica Gel 60 (Silicycle 40-63 m). Elemental analyses were carried out on a CEC (SCP) 240-XAAnalyzer instrument by Lakehead University Instrumentation Laboratory (LUIL). All commercial reagents were used as supplied. Solvents for anhydrous reactions were dried according to literature procedures. Dichloromethane was distilled over calcium hydride, methanol was distilled over magnesium, and tetrahydrofuran was distilled over sodium.
5.2. Synthetic Procedures and Structure Characteristics

6-Oxo-6-(2-oxo-2-phenylethoxy)hexanoic acid (1) and bis(2-oxo-2-phenylethyl) adipate (9)

Et₃N (1.91 mL, 13.7 mmol, 1 eq.) was added to a solution of 2-bromoacetophenone (2.7 g, 13.7 mmol, 1 eq.) and adipic acid (2.0 g, 13.7 mmol, 1 eq.) in EtOAc (20 mL). The mixture was stirred at room temperature overnight. The solid was then filtered and washed using EtOAc. The filtrate was concentrated under vacuum and the residue was purified by column chromatography on silica gel (hexane/ethyl acetate = 1.5:1, 1:1) to provide 1 (0.73 g, 20%) as a white powder and 9 (0.47 g, 9%).

For 1: Rᵣ 0.34 (hexane / ethyl acetate 1:1); ¹H NMR: (500 MHz, chloroform-d): δ 7.98 – 7.89 (m, 1H), 7.63 (m, 1H), 7.56 – 7.47 (m, 2H), 5.38 (s, 2H), 2.56 (m, 2H), 2.48 – 2.39 (m, 2H), 1.79 (br. s, 2H). ¹³C NMR: (125 MHz, chloroform-d) δ 192.22, 179.54, 172.74, 134.14, 133.91, 128.86, 127.76,
For 9: \(^1\)H NMR (500 MHz, chloroform-\(d\)): \(\delta\) 7.92 (dt, \(J = 8.4, 1.4\) Hz, 4H), 7.66 – 7.57 (m, 2H), 7.50 (td, 4H), 5.36 (s, 4H), 2.56 (m, 4H), 1.88 – 1.78 (m, 4H). \(^13\)C NMR (125 MHz, chloroform-\(d\)): \(\delta\) 192.20, 172.73, 134.19, 133.86, 128.85, 127.75, 65.93, 33.50, 24.26.

6-Oxo-6-(1-oxo-1-phenylpropan-2-yl)oxy)hexanoic acid (2) and bis (1-oxo-1-phenylpropan-2-yl)adipate (10)

Adipic acid (1 g, 6.85 mmol, 1 eq.) was dissolved in EtOAc (20 mL) and Et\(_3\)N (0.95 mL, 6.85 mmol, 1 eq.) and bromopropiaphenone (1.46 g, 6.85 mmol, 1 eq.) was added. The reaction mixture was stirred at room temperature overnight. Usual work-up as described for the preparation of compound 1 followed by column chromatography on silica gel (hexane/ethyl acetate = 1.5:1, 1:1) provided 2 (0.92 g, 48%) as syrup and 10 (0.85 g, 30%). Compound 10 was not fully purified. A clean \(^1\)H or \(^13\)C NMR spectrum for 10 was not obtained.

For 2: \(R_f\) 0.6 (hexane/ethyl acetate 1:1); \(^1\)H NMR (500 MHz, chloroform-\(d\)) \(\delta\) 10.5 (s, OH), 8.12 (s, 1H), 7.94 (dt, \(J = 8.6, 1.4\) Hz, 2H), 7.59 (td, \(J = 7.3, 1.5\) Hz, 2H).
Hz, 1H), 7.48 (td, J = 7.8, 1.9 Hz, 2H), 5.97 (q, J = 7.0 Hz, 1H), 2.52 – 2.34 (m, 4H), 1.71 (m, 4H), 1.53 (d, J = 7.1 Hz, 3H). $^{13}$C NMR: (125 MHz, chloroform-d) δ 197.02, 179.18, 172.75, 134.34, 133.60, 128.77, 128.44, 71.39, 33.52, 24.14, 23.95, 17.10. Anal. Calcd for C$_{15}$H$_{18}$O$_{5}$: C, 64; H, 6.51. Found: C, 64; H, 6.54.

6-{2-[(1, 1'-biphenyl)-4-yl]-2-oxoethoxy}-6-oxohexanoic acid (3)

and bis (2-[(1,1’biphenyl]-4-yl)-2-oxoethyl) adipate (11)

Adipic acid (1 g, 6.84 mmol, 1 eq.) was dissolved in EtOAc (20 mL) followed by the addition of Et$_3$N (0.90 mL, 6.84 mmol, 1 eq.). Then 2- bromo-4-phenylacetophenone (1.88 g, 6.84 mmol, 1 eq.) was added. The reaction mixture was stirred at room temperature overnight. The solid was filtered, the filtrate was concentrated, and the residue was purified by column chromatography on silica gel (hexane/ethyl acetate = 1:1.25 to give 3 (0.45 g,
19%) and 11 (0.27 g, 7%). A clean $^1$H or $^{13}$C NMR spectrum for 11 was not obtained.

For 3: Rf 0.3 (hexane/ethylacetate = 1.5:1), $^1$H NMR (500 MHz, chloroform-d): δ 11.1 (s, OH), 7.99 (m, 2H), 7.80 (m, 2H), 7.75 – 7.66 (m, 2H), 7.63 (m, 2H), 7.52 – 7.39 (m, 1H), 5.39 (s, 2H), 2.55 (m, 2H), 2.43 (m, 2H), 1.78 (m, 4H). $^{13}$C NMR (125 MHz, chloroform-d) δ 191.87, 179.62, 172.74, 146.59, 139.59, 129.00, 128.43, 128.35, 127.47, 127.27, 65.95, 44.97, 33.59, 33.49, 24.24, 24.05.

6-Oxo-6-(2-oxo-1,2-diphenyl ethoxy) hexanoic acid (4) and bis(2-oxo-1, 2-diphenylethyl)adipate (12)

Adipic acid (0.283 g, 1.94 mmol, 1 eq.) was dissolved in EtOAc (10 mL) followed by the addition of Et$_3$N (0.27 mL, 1.94 mmol, 1 eq.). Then 2-bromo-2-phenylacetophenone (0.5 g, 1.94 mmol, 1 eq.) was added. The mixture was stirred at room temperature overnight and diluted with EtOAc (50 mL), was washed with cooled HCl [1M, 20 mL], washed again with H$_2$O (320 mL), dried
over Na₂SO₄, and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/ethyl acetate = 2/1) to provide 4 (0.75 g, 60%) as a white powder and 12 (0.1 g, 10%).

For 4: Rₓ 0.37 (hexane/ethyl acetate = 1:1); ¹H NMR (500 MHz, chloroform-d) δ 7.96 – 7.90 (m, 2H), 7.54 – 7.30 (m, 8H), 6.86 (s, 1H), 2.59 – 2.49 (m, 2H), 2.47 – 2.41 (m, 2H), 1.74 (br s, 4H). ¹³C NMR: (125 MHz, chloroform-d) δ 193.82, 179.34, 172.80, 134.56, 133.49, 129.32, 129.13, 128.78, 128.65, 128.63, 77.59, 33.61, 33.54, 29.69, 24.15, 23.95.

For 12: ¹H NMR (500 MHz, chloroform-d) δ 8.00 – 7.88 (m, 4H), 7.52 – 7.41 (m, 6H), 7.44 – 7.27 (m, 10H), 6.86 (m, 2H), 2.57 – 2.48 (m, 2H), 2.50 – 2.41 (m, 2H), 1.81 – 1.70 (m, 4H). ¹³C NMR: (125 MHz, chloroform-d) δ 193.78, 172.75, 134.56, 133.52, 133.41, 129.23, 129.06, 128.73, 128.59, 128.58, 77.52, 33.49, 33.49, 24.11, 24.11.

6-((2-methyl-1-oxo-1-phenylpropan-2-yl)oxy)-6-oxohexanoic acid (5) and bis(2-methyl-1-oxo-1-phenylpropan-2-yl)adipate (14)
Adipic acid (0.3 g, 2.05 mmol, 1 eq.) was dissolved in DMF (3 ml). 2-Hydroxy-2-methylpropiophenone (0.312 mL, 2.05 mmol, 1 eq.) was added followed by the addition of 4-\(N,N\)-dimethylaminopyridine (DMAP) (0.205 g, 0.205 mmol, 0.1 eq.) and \(N,N\)-diisopropylcarbodiimide (DIPCI) (0.48 mL, 3.70 mmol, 1.5 eq.). The mixture was stirred at room temperature for three days. The solid was filtered and washed with hexane/ethyl acetate (1/1). The filtrate was concentrated in vacuo and the residue purified by column chromatography on silica gel (hexane/ethylacetate = 3/1) to provide 5 (0.15 g, 19%) as a white powder and 14 (0.17 g, 21%).

For 5: R\(_f\) 0.34 (hexane/ethyl acetate = 2:1); \(^1\)H NMR (500 MHz, chloroform-\(d\)) \(\delta\) 11.57 (s, 1H), 8.02 – 7.95 (m, 2H), 7.53 – 7.46 (m, 1H), 7.41 (t, \(J = 7.9\) Hz, 2H), 2.25 (m, 4H), 1.73 (s, 6H), 1.48 (m, 4H). \(^1\)C NMR: (125 MHz, chloroform-\(d\)) \(\delta\) 199.38, 179.58, 172.28, 134.64, 132.45, 128.45, 128.33, 84.28, 45.00, 34.02, 33.57, 25.37, 23.76, 23.75.

For 14: \(^1\)H NMR (500 MHz, chloroform-\(d\)) \(\delta\) 7.96 (m, 4H), 7.41 – 7.33 (m, 6H), 2.14 – 2.06 (m, 4H), 1.69 (s, 12H), 1.26 (m, 4H). \(^1\)C NMR: (125 MHz, chloroform-\(d\)) \(\delta\) 199.19, 172.17, 134.60, 132.42, 128.45, 128.32, 84.20, 33.96, 25.37, 23.58.

6-oxo-6-(2-oxo-2-(2-oxo-2H-chromen-3-yl)ethoxy)hexanoic acid (6) and bis(2-oxo-2-(2-oxo-2H-chromen-3-yl)ethyl)adipate (16)
Adipic acid (0.27 g, 1.872 mmole, 1 eq.) was dissolved in EtOAc (7 mL), then 
N,N-diisopropylethylamine (0.32 mL, 1.872 mmole, 1 eq.) was added. The 
mixture was stirred for 5 min at room temperature. 3-(Bromoacetyl)-coumarin 
(0.5 g, 1.872 mmole, 1 eq.) was added, and the mixture was stirred at 80 °C for 
overnight. TLC checked until complete reaction followed by a work up: by 
filtration of the solid and the concentration of the filtrate under vacuo. The residue 
was purified by column chromatography on silica gel (hexane/ethyl acetate = 1:4) 
to provide compound 6 (0.11 g, 18%) and 16 (0.02 g, 2%).

For 6: $^1$H NMR (500 MHz, chloroform-d) δ 8.65 (d, $J = 0.8$ Hz, 1H), 7.70 (m, 2H), 
7.44 – 7.34 (m, 2H), 5.39 (s, 2H), 2.56 – 2.49 (m, 2H), 2.43 (t, $J = 6.7$ Hz, 2H), 
1.77 (m, 4H). $^{13}$C NMR: (125 MHz, chloroform-d) δ 190.16, 178.32, 172.70, 
159.19, 155.31, 149.10, 135.13, 130.57, 125.31, 121.92, 118.07, 116.87, 69.15, 
33.44, 24.26, 24.01.

Anal. Calcd for C$_{17}$H$_{16}$O$_7$: C, 61.38; H, 4.81. Found: C, 61.65; H, 4.60.
For 16: $^1$H NMR (500 MHz, chloroform-$d$) $\delta$ 8.65 (s, 2H), 7.74 – 7.63 (m, 4H), 7.44 – 7.25 (m, 4H), 5.42 (s, 4H), 2.55 (m, 4H), 1.83 (m, 4H). $^{13}$C NMR (125 MHz, chloroform-$d$) $\delta$ 190.15, 172.76, 159.18, 155.30, 149.05, 135.10, 130.57, 125.29, 125.24, 124.84, 121.96, 118.08, 116.85, 69.12, 33.48, 33.23, 29.70, 24.29, 24.19.

**Methyl (S)-2-((tert-butoxycarbonyl) amino)-3-(4-(2-oxopropoxy)phenyl) propanoate (18)**

![Chemical structure](image)

Compound 17 (0.5 g, 1.69 mmole, 1 eq.) was dissolved in anhydrous acetone (3 mL) and then potassium carbonate (0.5 g, 3.38 mmol, 2 eq.), and chloroacetone (0.134 mL, 1.69 mmol, 1 eq.) were added. The mixture was stirred at 75 °C and left for overnight. TLC analysis of the reaction mixture showed no new spot; therefore, potassium iodide (0.56 g, 1.69 mmol, 1 eq.) added. The mixture was refluxed for 6 hours until TLC indicated completion of the reaction. The solid was then filtered and the filtrate concentrated under vacuum. The
residue was purified by column chromatography on silica gel (hexane/ethyl acetate= 1.5:1) to provide compound 18 (0.24 g, 45%).

R_f 0.38 (hexane/ethyl acetate=1.5:1); ^1^H NMR (500 MHz, chloroform-d) δ 7.07 (d, J = 8.3 Hz, 2H), 6.85 – 6.77 (m, 2H), 5.21 (d, J = 8.0 Hz, 1H), 4.57 – 4.49 (m, 2H), 3.74 – 3.67 (s, 3H), 3.00 (m, 1H), 2.30 (m, 2H), 2.03 (s, 3H), 1.42 (s, 9H).

^13^C NMR: (125 MHz, chloroform-d) δ 205.55, 172.38, 171.03, 156.86, 155.16, 130.53, 129.34, 114.59, 79.74, 77.57, 73.02, 60.34, 54.63, 52.17, 37.37, 28.29, 26.53, 20.98, 14.20.

Methyl (S)-2-amino-3-(4-(2-oxopropoxy)phenyl)propanoate hydrochloride (19)

To a solution of 18 (0.1 g, 0.321 mmol, 1 eq.) in EtOAc (1 mL) was added hydrogen chloride solution in diethyl ether (HCl-Et₂O) (3 mL). The mixture was stirred at room temperature for 2 h. The mixture was then concentrated vacuo and EtOAc (3X, 5 mL) was added, concentrated in vacuo, and dried under high
vacuum to provide compound 19 (0.09 g, 97%). $^1$H NMR (500 MHz, DMSO-$d_6$) δ 8.65 (s, 2H), 7.14 (d, $J = 8.2$ Hz, 2H), 6.86 (d, $J = 8.2$ Hz, 2H), 4.80 (s, 2H), 4.22 (t, $J = 6.4$ Hz, 1H), 3.68 (s, 3H), 3.09 (m, 2H), 2.16 (s, 3H). $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ 204.58, 169.90, 157.48, 130.97, 127.25, 115.01, 72.48, 53.75, 53.06, 35.42, 26.70. The $^1$H and $^{13}$C NMR data were in agreement with those reported in the literature. $^{16}$

3-0-(tert-butyloxycarbonylmethyl)cholesterol (20)

Sodium hydride (0.827 g, 20.68 mmol, 4 eq.) was added to tetrahydrofuran (20 mL) and then cholesterol (2.0 g, 5.17 mmol, 1 eq.) was added. Tert-butyl bromoacetate (4.032 g, 20.68 mmol, 4 eq.) was then added slowly. The mixture was left overnight at 80°C. Water was then added dropwise to the reaction mixture until bubbling stopped, and the solvent was removed in vacuo. H$_2$O (50 mL) was added and the mixture extracted with DCM (3 x 50 mL). The combined organic layer was dried over Na$_2$SO$_4$ and concentrated in vacuo. The residue was purified by column chromatography on silica gel (hexane/ethyl
acetate = 5:0.3) to provide compound 20 (0.13 g, 67%). R$_f$ 0.34 (hexane /ethyl acetate = 1:1). $^1$H NMR (500 MHz, chloroform-$d$) δ 5.38 – 5.33 (m, 1H), 4.01 (s, 2H), 3.29 – 3.18 (m, 1H), 2.39 (ddd, J = 13.3, 4.8, 2.2 Hz, 1H), 2.31 – 2.22 (m, 1H), 2.05 – 1.89 (m, 3H), 1.90 – 1.77 (m, 2H), 1.62 – 1.50 (m, 3H), 1.50 (s, 9H, 3 CH$_3$), 1.46 – 1.36 (m, 1H), 1.35 (m, 3H), 1.25 (m, 1H), 1.20 – 1.06 (m, 5H), 1.09 – 1.00 (m, 1H), 1.00 (s, 3H, CH$_3$), 0.91 (d, J = 6.4 Hz, 6H, 2CH$_3$), 0.87 (d, 6H, 2 CH$_3$) 0.67 (s, 3H, CH$_3$). $^{13}$C NMR (125 MHz, chloroform-$d$) δ 170.10, 140.62, 121.81, 81.34, 79.74, 66.04, 56.75, 56.13, 50.12, 42.30, 39.76, 39.51, 38.74, 37.12, 36.82, 36.18, 35.78, 31.93, 31.86, 28.23, 28.11, 28.01, 24.28, 23.82, 22.83, 22.57, 21.05, 19.35, 18.71, 11.85.

3-O-carboxymethyl-cholesterol (21)

To a solution of 20 (1.04 g, 1.86 mmol) dissolved in DCM (15 mL) was added trifluoroacetic acid (15 mL) and H$_2$O (1.5 mL) at 0°C. The mixture was stirred at 0°C for 1 h and then concentrated in vacuo to provide compound 21
Preparation of poly-L-glutamic acid (H\textsuperscript{+} form)

The commercially available sodium salt of poly-L-glutamic acid (MW 50,000 – 100,000, 300 mg, 1.98 mmol) was dissolved in double distilled water (dd-H\textsubscript{2}O) (3 mL) and cooled in an ice bath. Hydrogen chloride solution (1 M, 3 mL) was added drop-wise till pH 1 and the mixture was left to stir for 2 hours. The mixture was then centrifuged for 15 min and the supernatant was removed. The solid was washed with dd-H\textsubscript{2}O water until a neutral pH was achieved. The solid was dried in the presence of P\textsubscript{2}O\textsubscript{5} under high vacuum overnight to give PGA (H\textsuperscript{+} form, 210 mg, 82%).

Preparation of m-PGA-Ph-24

Poly-L-glutamic acid (H\textsuperscript{+} form, 125 mg, 0.97 mmol) was dissolved in dry dimethylformamide (10 mL) and heated in an oil bath at 40\degree C. Triethylamine (29.4 mg, 40.5 \mu L, 0.29 mmol) was added and stirred for 5 min, and then bromoacetophenone (58 mg, 0.29 mmol) was added. The mixture was stirred at 40\degree C
for 16 h and then cooled to room temperature. A mixture of hexane-ethyl acetate (1:1, v/v, 30 mL) was added slowly to the above reaction mixture to precipitate out the product. The solid was collected after centrifugation (5000 rpm, 10 min) and washed with hexane-ethyl acetate (1:1, v/v, 15 mL×2). The solid was then suspended in ethyl acetate (10 mL) and the mixture stirred for 4 h. After centrifugation and washing with hexane-ethyl acetate (1:1, v/v, 10 mL), the solid was further dried under high vacuum to provide m-PGA-Ph-24 (135 mg) with 24% of the side chains being modified.

$^1$H NMR (500 MHz, trifluoroacetic acid-d): $\delta$7.80 (br s, 0.48H, Ar-H), 7.60 (br s, 0.24H, Ar-H), 7.40 (br s, 0.48H, Ar-H), 5.50 (br s, 0.48 H, OCH$_2$CO), 4.80 (br s, 1H, H-α), 2.50 – 2.80 (2 br s, 2H, H-γ), 1.90 – 2.40 (m, 2H, H-β).

**Preparation of m-PGA-Ph-32**

Poly-L-glutamic acid (H$^+$ form, 120 mg, 0.93 mmol) was dissolved in dry dimethylformamide (DMF, 9 mL) and heated in an oil bath at 40°C. Triethylamine (37.4 mg, 51.5 $\mu$L, 0.37 mmol) was added and stirred for 5 min, and then 2-bromoacetophenone (74 mg, 0.37 mmol) was added. The mixture was stirred at 40°C for 16 h and then cooled to room temperature. A mixture of hexane-ethyl acetate (3:1, v/v, 35 mL) was added slowly to the reaction mixture to precipitate out the product. The solid was collected after centrifugation (5000 rpm, 10 min) and then washed with hexane-ethyl acetate (1:1, v/v, 10 mL, 3X). The solid was then suspended in ethyl acetate and the mixture stirred at room temperature for 16 h. The solid was collected after centrifugation and dried under high vacuum to
provide the crude product (133 mg). The crude product (128 mg) was re-
dissolved in dimethylformamide (DMF, 6 mL) and ddH₂O (30 ml) was added to
give a clear solution. Sodium chloride (3.0 g) was added to the clear solution to
facilitate precipitation. The solid was collected after centrifugation and then
washed with ddH₂O (3X, 10 mL). The fine powder was further dried under high
vacuum in the presence of P₂O₅ for 16 h to provide m-PGA-Ph-32 (100 mg) with
32% of the side chains being modified.

¹H NMR (500 MHz, trifluoroacetic acid-d): δ 7.80 (br s, 0.64H, Ar-H), 7.60 (br s,
0.32H, Ar-H), 7.40 (br s, 0.64, Ar-H), 5.50 (br s, 0.64 H, OCH₂CO), 4.80 (br s, 1H,
H-α), 2.50 – 2.70 (2 br s, 2H, H-γ), 2.05 – 2.30 (m, 2H, H-β).

2,3,4,6-Tetra-O-acetyl-α/β-D-glucopyranose (23)

Glucose (10 g, 55.55 mmol, 1 eq.) was dissolved in pyridine (39 mL) and acetic
anhydride (28.35 g, 277.77 mmol, 5 eq.) was added. The mixture was stirred at
room temperature. Then H₂O (30 mL) was added in the mixture and stirred for 30
- 40 min. Cold H₂O (500 mL) was added, extracted with EtOAc (3X, 200 mL), and
washed with HCl [6M, 100 ml] checked pH= 1-2, washed again with saturated
NaHCO₃ (100 mL) checked pH= 8, dried over Na₂SO₄, and concentrated under
vacuo to provide compound 22 (23 g, 94%). 22 (10 g, 23.89 mmol, 1 eq.) was dissolved in DMF, and ammonium carbonate (5 g, 47.79 mmol, 2 eq.) was added. The mixture was stirred at 30 ℃ for 20 h. After that was added CHCl₃ (50 mL) and cooled HCl [1M, 50 mL], extracted with CHCl₃ (2X 50 mL). The organic layer was washed with saturated NaHCO₃ (20 mL) and then with H₂O (40 mL), dried over Na₂SO₄, concentrated under vacuo. The residue was purified by column chromatography on silica gel (hexane/ethyl acetate = 1:1) to provide compound 23 (3.16 g, 43%).

2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl trichloroacetimidate (24)

23 (1.3 g, 3.72 mmol, 1 eq.) was dissolved in anhydrous DCM (10mL) and trichloroacetonitrile (CCl₃CN) (1.86 mL, 18.6 mmol, 5 eq.) and 1,8-diazabicyclo [5.4.0] undec-7-ene (DBU) (0.1 mL, 0.744 mmol, 0.2 eq.) were added. The mixture was stirred at room temperature for 3 h. The mixture was concentrated under vacuo and the residue was purified by column chromatography on silica
gel (hexane/ethyl acetate/ Et₃N = 2:1:0.5%) to provide compound 24 (1.4 g, 90%).

2-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyloxy)-naphthalene-1,4-dione (26) and 4-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyloxy)-naphthalene-1,2-dione (27)

To a solution of 24 (0.81 g, 1.79 mmol, 1.2 eq.) in anhydrous THF (7 mL) and 2-hydroxy-1,4-naphthoquinone (lawsone) (0.32 g, 1.49 mmol, 1 eq.) in anhydrous DCM (5 mL) at room temperature was added a solution of boron trifluoride – diethyl etherate (BF₃.O(Et)₂) in DCM (0.1 M, 1.25 mL, 0.537 mmol, 0.3 eq.). The mixture was stirred for 4 h. The reaction was checked by TLC until completion and followed by a work-up: quenched with saturated aqueous NaCO₃H (20 mL), extracted with EtOAc (1X, 50 ml) then (3X, 10 mL), dried with Na₂SO₄, concentrated, purified by column chromatography on silica gel (toluene/acetone = 9:1, then 6:1), and concentrated to provide compound 26
(0.59 g, 65%) and 27 (0.27 g, impure). Compound 27 was not clean and was re-purified by column chromatography on silica gel (toluene/acetone = 5:1) to provide 0.16 g (18%) of pure material.

For 26: $^1$H NMR (500 MHz, chloroform-$d$) δ 8.19 – 8.03 (m, 1H), 7.82 – 7.69 (m, 1H), 6.43 (s, 1H), 5.43 – 5.31 (m, 2H), 5.23 (d, $J = 7.5$, 1H), 5.17 (dd, $J = 10.1$, 8.8Hz, 1H), 4.27 (m, 1H), 4.21 (m, 1H), 3.97 (m, 1H), 2.19 – 2.00 (m, 12H, 4 CH$_3$). $^{13}$C NMR: (125 MHz, chloroform-$d$) δ 207.03, 184.91, 178.78, 170.55, 170.16, 169.35, 169.09, 157.29, 135.17, 134.32, 133.74, 133.12, 131.60, 130.99, 126.72, 126.61, 126.46, 126.15, 114.74, 97.65, 77.36, 77.10, 76.85, 75.79, 72.80, 72.18, 70.46, 70.35, 67.90, 66.45, 61.69, 61.31, 30.94, 29.69, 20.67, 20.60, 20.58, 20.35.

For 27: $^1$H NMR (500 MHz, chloroform-$d$) δ 8.10 (d, $J = 7.5$ Hz, 1H), 7.73 (m, 2H), 7.64 (m, 1H), 6.10 (s, 1H), 5.52 (m, 2H), 5.19 (d, $J = 8.2$ Hz, 1H), 4.34 (dd, $J = 12.5$, 6.0 Hz, 1H), 4.27 – 4.16 (m, 1H), 4.11 – 4.01 (m, 1H), 2.16 – 2.01 (m, 12H, 4 CH$_3$). $^{13}$C NMR: (125 MHz, chloroform-$d$) δ 207.10, 192.44, 179.62, 178.59, 170.54, 169.98, 169.42, 169.41, 165.38, 135.53, 134.48, 131.89, 131.18, 130.34, 129.75, 129.54, 129.00, 128.54, 127.61, 126.97, 124.87, 106.33, 97.17, 72.90, 71.99, 70.31, 68.48, 67.89, 67.15, 61.50, 44.99, 30.95, 29.71, 20.77, 20.64, 20.63, 20.58, 20.57.

$^{1,2,3,4,6}$-Penta-O-trimethylsilyl-$\alpha$/\$\beta$-D-glucopyranose (30)
To a dissolved solution of glucose (1.033 g, 5.742 mmol, 2 eq.) in DMF (25 mL) was added Et₃N (3.18 g, 31.58 mmol, 5.5 eq.) and trimethylsilyl chloride (TMS-Cl) (4.76 g, 31.58 mmol, 5.5 eq.). The mixture was stirred at room temperature. Pentane (100 mL) and ice (100 mL) was added to the reaction mixture. The organic layer was separated and the aqueous layer extracted with pentane (3x 50 mL). The combined organic layer was washed with H₂O (2X, 100 mL) and then with saturated aqueous NaCl solution (2X, 100 mL), dried over Na₂SO₄, and concentrated to provide 30 (3.5 g, 88%).

2,3,4,6-Tetra-O-trimethylsilyl-α-D-glucopyranosyl iodide (31)

To a solution of 30 (3.5 g, 5.74 mmol, 1 eq.) in anhydrous DCM (15 mL) was added trimethylsilyl iodide (TMS-I) (6.3 mL, 6.31 mmol, 1.1 eq.). The reaction mixture was stirred at room temperature for 10 min. The mixture was concentrated under vacuo at less than 30°C and the remaining liquid was co-
distilled with benzene (3x), dried under high vacuo to give 31 (4.5 g, 74%).

2-(2,3,4,6-Tetra-O-trimethylsilyl-α/β-D-glucopyranosyloxy)-naphthalen-1,4-dione (34) and 4-(2,3,4,6-tetra-O-trimethylsilyl-α/β-D-glucopyranosyloxy)-naphthalen-1, 2-dione (35)

![Chemical Structures 34 and 35]

2-Hydroxy-1,4-naphthaquinone (0.9 g, 5.22 mmol, 1 eq.) was dissolved in THF (10 mL) and sodium bis(trimethylsilyl)amide (NaN(SiMe₃)₂) (5.2 mL, 28.46 mmol, 5.5 eq.) was added. The mixture was stirred at room temperature for 10 min to generate the corresponding enolates of lawsone.

15-Crown-5 (1.14 g, 5.22 mmol, 1 eq.) was added and the mixture was stirred at room temperature for 4 h. The mixture was concentrated under vacuo and DCM (60 mL) was added. The organic layer was washed with half saturated aqueous NaCl solution (2x 20 mL), dried over Na₂SO₄, and concentrated, to provide crude glycosylated product 34 and 35 (3.7 g). The product was not purified but used directly for the trials of deprotection reaction.
2-Hydroxy-3-\{[(2-hydroxyethyl)amino]methyl\}naphthalene-1,4-dione (45)

![Chemical structure of 2-Hydroxy-3-\{[(2-hydroxyethyl)amino]methyl\}naphthalene-1,4-dione (45)](image)

2-Hydroxy-1,4-naphthoquinone (0.1 g, 0.57 mmol, 1.0 eq.) was dissolved in anhydrous ethanol and then ethanolamine (0.035 g, 0.57 mmol, 1.0 eq.) was added. The mixture was stirred for 5 min at 45°C. Formaldehyde (0.017 g, 0.57 mmol 1.0 eq.) was added and the mixture was stirred for 6 h at 45°C. The solid was filtered to provide compound 45 (0.109 g, 73%). $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 8.32 – 8.28 (br s, 1H), 7.95 (dd, $J = 7.7$, 1.2 Hz, 1H), 7.82 (dd, $J = 7.6$, 1.3 Hz, 1H), 7.72 (td, $J = 7.5$, 1.3 Hz, 1H), 7.58 (td, $J = 7.5$, 1.3 Hz, 1H), 4.02 (s, 2H), 3.67 (t, $J = 5.4$ Hz, 2H), 2.96 (t, $J = 5.4$ Hz, 2H). $^{13}$C NMR: (125 MHz, DMSO-$d_6$) $\delta$ 184.96, 182.29, 179.11, 172.14, 135.41, 134.04, 133.97, 133.65, 132.11, 131.98, 131.23, 131.04, 125.75, 125.72, 125.60, 125.48, 125.45, 107.76, 107.09, 56.66, 48.28, 42.08.

2-Hydroxy-3-\{[(3-hydroxypropyl)amino]methyl\}naphthalene-1,4-dione (46)
2-Hydroxy-1,4-naphthoquinone (0.1 g, 0.57 mmol, 1.0 eq.) was dissolved in anhydrous ethanol and then 3-amine-1-propanol (0.1 mL, 0.55 mmol, 1.1 eq.) was added. The mixture was stirred for 20 min at room temperature. Formaldehyde (0.1 mL, 0.55 mmol, 1.1 eq.) was added and the mixture was stirred at room temperature overnight. The product was filtered out to provide orange solid compound 46 (0.15 g, 94%).

\(^1\)H NMR (500 MHz, DMSO-\(d_6\)) \(\delta\) 8.17 (br s, 1H), 7.94 (dd, \(J = 7.7, 1.3\) Hz, 1H), 7.82 (dd, \(J = 7.6, 1.3\) Hz, 1H), 7.70 (td, \(J = 7.5, 1.4\) Hz, 1H), 7.57 (td, \(J = 7.5, 1.3\) Hz, 1H), 4.089 (s, 1 H), 3.94 (s, 2H), 3.47 (t, \(J = 6.0\) Hz, 2H), 2.93 (t, \(J = 7.5\) Hz, 2H), 1.82 – 1.72 (m, 2H).\(^{13}\)C NMR: (125 MHz, DMSO-\(d_6\)) \(\delta\) 185.11, 178.91, 172.15, 135.58, 134.08, 132.08, 131.04, 125.80, 125.55, 108.01, 58.78, 44.70, 42.20, 28.94.
2-Hydroxy-3-\{[(bis(2-hydroxyethyl)amino)methyl]naphthalene-1,4-dione (47)

2-Hydroxy-1,4-naphthaquinone (0.1 g, 0.57 mmol, 1.0 eq.) was dissolved in anhydrous ethanol and then diethanol amine (0.05 mL, 0.57 mmol, 1.0 eq.) was added to react for 20 min at room temperature. Formaldehyde (0.1 mL, 0.57 mmol, 1.0 eq.) was added and the mixture was stirred at 45 °C overnight. The product was filtered out to provide the orange solid compound 47 (0.113 g, 98%).

2-Hydroxy-3-\{[(2-hydroxyethyl)amino]phenylmethyl\}naphthalene-1, 4-dione (48)
2-Hydroxy-1,4-naphthoquinone (0.1 g, 0.57 mmol, 1.0 eq.) was dissolved in anhydrous ethanol and ethanolamine (0.038 mL, 0.6 mmol, 1.1 eq.) was added to react for 20 min at room temperature. Benzaldehyde (0.064 mL, 0.6 mmol, 1.1 eq.) was added and the mixture was stirred at room temperature. The product was filtered out to provide the orange solid compound 48 (0.16 g, 86%).

\[ ^1\text{H NMR: } (500 \text{ MHz, DMSO-d}_6) \delta 9.82 \text{ (br s, 1H), 9.18 (br s, 1H), 7.93 (dd, } J = 7.7, 1.3 \text{ Hz, 1H), 7.83 (dd, } J = 7.6, 1.3 \text{ Hz, 1H), 7.72 (td, } J = 7.6, 1.4 \text{ Hz, 1H), 7.64 - 7.56 (m, 3H), 7.37 (m, 2H), 7.35 - 7.28 (m, 1H), 5.59 (s, 1H), 5.13 (s, 1H), 3.68 - 3.58 (m, 2H), 3.00 (m, 2H). ^13\text{C NMR: } (125 \text{ MHz, DMSO): } \delta 184.80, 178.78, 171.03, 138.96, 135.10, 134.24, 131.93, 131.35, 128.88, 128.42, 128.29, 125.83, 125.57, 111.86, 59.39, 56.99, 56.51, 48.29. \]

2-Hydroxy-3-[[2-hydroxypropyl]amino]phenylmethyl)naphthalene-1,4-dione (49)

2-Hydroxy-1,4-naphthoquinone (0.1 g, 0.57 mmol, 1.0 eq.) was dissolved in anhydrous ethanol and then 3-amine-1-propanol (0.041 mL, 0.63 mmol, 1.1
eq.) was added to reacting for 20 min at room temperature. Benzaldehyde (0.07 mL, 0.63 mmol, 1.1 eq.) was added and the mixture was stirred at room temperature overnight. The product was filtered out to provide orange solid compound 49 (0.198 g, 97%). $^1$H NMR (500 MHz, DMSO-d$_6$) δ 9.78 (br s, 1H), 9.00 (br s, 1H), 7.93 (d, $J = 7.7$ Hz, 1H), 7.83 (d, $J = 7.6$ Hz, 1H), 7.72 (t, $J = 7.6$ Hz, 1H), 7.65 – 7.56 (m, 3H), 7.37 (t, $J = 7.5$ Hz, 2H), 7.31 (t, $J = 7.4$ Hz, 1H), 5.53 (s, 1H), 4.78 (s, 1H), 3.47 (m, 2H), 3.00 (m, 1H), 2.93 (m, 1H), 1.80 (m, 2H). $^{13}$C NMR: (125 MHz, DMSO-d$_6$) δ 184.83, 178.72, 171.03, 139.14, 135.17, 134.21, 131.97, 131.30, 128.82, 128.32, 128.16, 125.81, 125.56, 111.67, 59.29, 58.96, 44.62, 29.14.

Tert-butyl-(3-hydroxypropyl)carbamate (50)

3-Amino-1-propanol (2.1 g, 29.29 mmol, 1.0 eq.) and triethylamine (3.25 g, 32.21 mmol, 1.1 eq.) were dissolved in CHCl$_3$ (20 mL). Di-tert-butyldicarbonate (7.0 g, 32.21 mmol, 1.1 eq.) dissolved in 10 ml CHCl$_3$ was added dropwise to the mixture. The mixture was stirred at room temperature overnight. The mixture was then extracted with water (3x 10mL) and the organic layer dried over Na$_2$SO$_4$, and concentrated under vacuo. The residue was purified by column
chromatography on silica gel (hexane/ethyl acetate= 2:1) and concentrated to provide compound \(50\) (3.69 g, 71%).

**3-tert-Butoxycarbonylamino-prop-1-yl 2,3,4,6-tetra-O-acetyl-\(\beta\)-D-glucopyranoside (51)**

\[\text{H}\]
\[
\text{BocN} \\
\text{O} \\
\text{AcO} \\
\text{O} \\
\text{OAc} \\
\text{OAc} \\
\text{OAc}
\]

\(50\) (1.49 g, 3.28 mmol, 1.0 eq.) and \(24\) (0.574 g, 3.28 mmol, 1.0 eq.) were dissolved in DCM (3 mL). BF\(_3\)OEt\(_2\) in DCM solution (0.1 M, 0.9 mL) was added to the reaction mixture under argon. This reaction mixture was stirred at room temperature overnight. Sodium bicarbonate solution (20 mL) was added and the mixture was extracted with dichloromethane (3 x 20 mL). The organic layer was dried with Na\(_2\)SO\(_4\), concentrated, and the residue purified by column chromatography on silica gel (hexane /ethyl acetate= 1:1) to provide compound \(51\) (0.49 g, 29 %) which was obtained as slightly impure material. \(^1\)H NMR (500 MHz, chloroform-\(d\)): \(\delta\) 5.71 (m, 2H), 5.30 (m, 1H), 5.17 (m, 1H), 5.11 (m, 1H), 4.34 (m, 1H), 4.15 (m, 2H), 3.95 – 3.84 (m, 1H), 3.78 – 3.70 (m, 1H), 3.72 – 3.65 (m, 1H), 3.45 – 3.34 (m, 1H), 3.37 – 3.23 (m, 1H), 2.16 – 2.04 (m, 12H), 1.88 – 1.75 (m, 2H).

**3-Amino-prop-1-yl \(\beta\)-D-glucopyranoside (52)**
51 (0.16 g, 0.316 mmol, 1 eq.) was deprotected to remove the acetyl groups by dissolving in anhydrous methanol (3 mL), followed by adding catalytic amount of sodium methoxide solution to adjust the reaction mixture to have pH = 9. The progress of the reaction was monitored by TLC until the reaction was complete and followed by a work up: Resin-IR-69 was added until the pH = 7, followed by filtration and concentration in vacuo, purified by column chromatography on silica gel (chloroform/methanol = 3:0.5) to provide the deacetylated intermediate (0.1 g, 94%). The deacetylated intermediate was dissolved in trifluoroacetic acid (TFA) (3 mL) and water (0.2 mL) and stirred at rt for 30 min when TLC showed the completion of reaction. The reaction was worked up by concentration under vacuo to give free amine 52 (0.045 g, 71%) as TFA salt.

2-Hydroxy-3-(1-β-D-glucopyranosyloxy-prop-3-yl)-aminomethyl-naphthalene-1,4-dione (53)
52 (0.45 g, 0.189 mmol, 1.0 eq.) was dissolved in ethanol (3 mL), followed by the addition of diisopropylethylamine (42.51 mg, 0.189 mmol, 1.0 eq.). The mixture was stirred at room temperature for 5 min under argon, and 2-hydroxy-1,4-naphthoquinone (32.88 g, 0.189 mmol, 1.0 eq.) was added. The mixture was stirred at room temperature for 10 min under argon. After that formaldehyde (5.6 mg, 0.189 mmol, 1.0 eq.) was added, and the reaction mixture was stirred at room temperature overnight. Solvent was then removed and the residue was purified by repeated column chromatography (chloroform/methanol= 3:2) and (chloroform/methanol/ H₂O = 3:2:0.1) to give 53 (0.01 g, 14%) which was slightly impure. ¹H NMR (500 MHz, chloroform-d) δ 7.97 (d, J = 7.6 Hz, 1H), 7.88 (m, 1H), 7.63 (m, 1H), 7.49 (m, 1H), 4.33 (d, J = 7.0 Hz, 1H), 4.11 (m, 2H), 3.90 – 3.83 (m, 3H), 3.77 (m, 2H), 3.52 – 3.41 (m, 4H), 2.60 (m, 1H), 1.31 – 1.23 (m, 2H).
6. References


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7. Appendix

7.1. $^1$H and $^{13}$C Spectra of Corresponding Compounds

1. $^1$H NMR spectrum of 1
2. $^{13}$C NMR spectrum of 1
3. $^1$H NMR spectrum of 9
4. $^{13}$C NMR spectrum of 9
5. $^1$H NMR spectrum of 2
6. $^{13}$C NMR spectrum of 2
7. $^1$H NMR spectrum of 3
8. $^{13}$C NMR spectrum of 3
9. $^1$H NMR spectrum of 4
10. $^{13}$C NMR spectrum of 4
11. $^1$H NMR spectrum of 12
12. $^{13}$C NMR spectrum of 12
13. $^1$H NMR spectrum of 5
14. $^{13}$C NMR spectrum of 5
15. $^1$H NMR spectrum of 14
16. $^{13}$C NMR spectrum of 14
17. $^1$H NMR spectrum of 6
18. $^{13}$C NMR spectrum of 6
19. $^1$H NMR spectrum of 16
20. $^{13}$C NMR spectrum of 16
21 $^1$H NMR spectrum of 18
22 $^{13}$C NMR spectrum of 18
23 $^1$H NMR spectrum of 19
24 $^{13}$C NMR spectrum of 19
25 $^1$H NMR spectrum of 20
26 $^{13}$C NMR spectrum of 20
27 $^1$H NMR spectrum of 21
28 $^1$H NMR spectrum of m-PGA-Ph-24
29 $^1$H NMR spectrum of m-PGA-32
30 $^1$H NMR spectrum of 26
31 $^{13}$C NMR spectrum of 26
32 $^1$H NMR spectrum of 27
33 $^{13}$C NMR spectrum of 27
34 $^1$H NMR spectrum of 45
35 $^{13}$C NMR spectrum of 45
36 $^1$H NMR spectrum of 46
37 $^{13}$C NMR spectrum of 46
38 $^1$H NMR spectrum of 48
39 $^{13}$C NMR spectrum of 48
40 $^1$H NMR spectrum of 49
41 $^{13}$C NMR spectrum of 49
42 $^1$H NMR spectrum of 53
$^1$H NMR spectrum of 1
$^{13}$C NMR spectrum of 1
$^1$H NMR spectrum of 9
$^{13}$C NMR spectrum of 9
$^1$H NMR spectrum of 2
$^{13}\text{C} \text{ NMR spectrum of 2}$
$^1$H NMR spectrum of 3
$^{13}$C NMR spectrum of 3
$^1$H NMR spectrum of 4
$^{13}$C NMR spectrum of 4
$^1$H NMR spectrum of 12
$^{13}$C NMR spectrum of 12
$^1$H NMR spectrum of 5
$^{13}$C NMR spectrum of 5
$^1$H NMR spectrum of 14
$^{13}$C NMR spectrum of 14
$^1$H NMR spectrum of 6
$^{13}$C NMR spectrum of 6
$^1$H NMR spectrum of 16
$^{13}$C NMR spectrum of 16
\(^1\text{H NMR spectrum of 18}\)
$^{13}$C NMR spectrum of 18
$^1$H NMR spectrum of 19
$^{13}$C NMR spectrum of 19
$^1$H NMR spectrum of 20
$^{13}$C NMR spectrum of 20
$^1$H NMR spectrum of 21
$^1$H NMR spectrum of m-PGA-Ph-24
$^1$H NMR spectrum of m-PGA-32
$^1$H NMR spectrum of 26
$^{13}$C NMR spectrum of 26
$^1$H NMR spectrum of 27
$^{13}$C NMR spectrum of 27
$^1$H NMR spectrum of 45
$^{13}$C NMR spectrum of 45
$^1$H NMR spectrum of 46
$^{13}$C NMR spectrum of 46
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$^{13}$C NMR spectrum of 48
$^1$H NMR spectrum of 49
$^{13}$C NMR spectrum of 49
$^1$H NMR spectrum of 53