CHEMISTRY AND BIOLOGY OF SOME TANNIN ANALOGUES AND
STUDIES TOWARD THE SYNTHESIS OF LIHOUIDINE

A Thesis in
Chemistry
by
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ABSTRACT

The syntheses of three fluorescent tannin analogues (two monomers and one dimer) was accomplished. The synthesis of the dimeric ellagitannin/gallotannin proved especially challenging. The onset of the synthesis involved a hetero Diels-Alder dimerization of an orthoquinone unit catalyzed by Sc(OTf)$_3$ to produce the core of the molecule, and a [3+2]-cycloaddition using a modification to the Sharpless Cu(I)-catalyzed protocol to attach the fluorescent tags. Several in vivo biological experiments were performed with these synthetic tannins and disappointingly the dimeric tannin was found inactive (unable to elicit TNF-α secretion from J744 cells). However, the two monomeric fluorescent gallotannins were found as active as the parent penta-$O$-galloyl-$\beta$-D-glucose that will allow further probing into the exact biological mechanism by which these tannins are able to achieve their documented tumoricidal action.

Furthermore, studies towards the synthesis of lihouidine, a marine natural product with moderate anticancer properties, were pursued. A Buchwald-Hartwig arylamination was used to join two early fragments. Numerous acidic aerobic conditions failed to cyclize this adduct through a proposed biomimetic electrocyclization. Gratifyingly, subjection of this adduct to fuming nitric acid led to an unprecedented nitration/Michael-like cyclization cascade to form a key advanced intermediate in the synthesis.
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Chapter 1 – Overview of the Immunomodulatory effects of ellagitannins

1 Immune function: LPS, lipid A and TNFα

Tannins are produced by a wide variety of plants such as oak, acer, tea, and sumach.\textsuperscript{1} Several important functions, such as stabilization of the cell wall and protection against herbivores, have been attributed to tannins.\textsuperscript{1} Ellagitannins, a subclass of hydrolyzable tannins, are composed of only glucose and biaryl-linked gallic acid, but despite their simplicity of design, over 500 natural products have been identified as belonging to this class. Interest in the active components of the polyphenol-rich medicinal plants used for years in China and Japan to treat a variety of inflammatory diseases has led to the isolation of numerous novel compounds. Among these species, several dimeric and macrocyclic members of the ellagitannin family of secondary plant metabolites have demonstrated the surprising ability to induce substantial tumor remission in mice inoculated with sarcoma-180, MM2 mammary carcinoma, MH134 hepatoma, and Meth-A fibrosarcoma tumor cells. A particularly interesting example is coriariin A (1), which was isolated from Coriaria japonica A. Gray (Figure 1.1). Coriariin A elicited 3 out of 6 tumor regressors and a 238% increase in life span in mice inoculated with sarcoma-180 tumor cells (5-10 mg/kg dose).\textsuperscript{2} Careful studies of the biological mechanism-of-action of these active ellagitannins have prompted speculation that these natural products act as immunomodulators through inducible cytokine mediators such as interleukin-1 beta (IL-1β) and tumor necrosis factor alpha (TNFα).
The antitumor chemotherapeutic window is small however, as the putative tumor-lethal cytokine TNFα is implicated as a causative agent in an assortment of diseases including most notably bacterial sepsis. Sepsis, which causes over 200,000 deaths per year, is characterized by a systemic pro-inflammatory response to the lipid A component of lipopolysaccharide (LPS) in Gram-negative bacteria and to lipoteichoic acid, peptidoglycan, and exotoxins of Gram-positive bacteria. In addition, chronic over-expression of TNFα has been linked to inflammatory diseases such as cachexia, diabetes, rheumatoid arthritis, and Crohn's disease.

The antitumor properties of TNFα include direct necrosis of solid tumors \textit{in vitro} and disruption of the tumor vasculature \textit{in vivo}. In fact, one of the earliest successful human cancer treatments involved administering \textit{E. coli} extracts containing LPS to patients, presumably a regimen that involved stimulation of the secretion of non-lethal doses of TNFα \textit{in vivo}. Similar TNFα secretion dose-response curves upon exposure of human peripheral blood mononuclear cells (hPBMC’s) to either some specific ellagitannins or to LPS have raised the question of whether the tannins may be achieving their documented tumoricidal action through the well-studied LPS/lipid A cellular receptor system. Consequently, the potential for ellagitannins to act as lipid A receptor
agonists (i.e., mimicking LPS/lipid A activity), and the prospects for redesigning the ellagitannin structure to develop a lipid A receptor antagonist (i.e., counteracting LPS/lipid A activity) are under study.

1.1 Lipid A

The outer surface of the outer membrane of the cell wall of Gram-negative bacteria is composed primarily of the amphipathic glycolipid lipopolysaccharide.\footnote{LPS} LPS (also known as endotoxin and enteric LPS) is the major surface-associated antigen for several Gram-negative bacteria and is involved in several pathological activities associated with the immune response of the human host.\footnote{A single \textit{E. coli} cell contains around 2 to 4 million LPS molecules.} A single \textit{E. coli} cell contains around 2 to 4 million LPS molecules.

The main constituent and the agent responsible for the biological response to LPS is a lipid component, lipid A. Besides lipid A, LPS also contains two other structural domains: the oligosaccharide core, and the O-antigen in enteric bacteria. The O-antigen is a long heteropolysaccharide chain that is strain-specific and is usually composed of identical repeating 20 to 40 oligosaccharide units of three sugars with a branching sugar at the first and third saccharide of each unit. The oligosaccharide core is further divided into the inner and outer core. The inner core is made up of two or more 2-keto-3-deoxyoctonic acid (KDO) units and two or three L-glycero-D-mannose-heptose saccharides. The outer core is usually composed of about three sugars with one or more appended as side chains. The lipid A region anchors LPS to the cell wall, and so lysis of the bacteria is necessary for exposure of lipid A. Lipid A, whose structure is highly
conserved among different species of Gram-negative bacteria, is made up of a β-D-glucosaminyl-(1-6)-D-glucosamine disaccharide (Figure 1.2). The 1 and 4’ positions are mono- or diphosphorylated, and the disaccharide can be acylated by up to four 3-hydroxy-containing or nonhydroxylated acyl residues as ester or amide linkages at specific positions (C2, C3, C2’, C3’). Additional fatty acids can be attached (secondary substitution) in cases where there is a hydroxyl group on the hydrocarbon moiety. Lipid A (2) (Figure 1.2) from Escherichia coli, which carries five fatty acids that are fourteen carbons in length and one which is twelve carbons in length, is exemplary of this structural class.

![Lipid A (2) and precursor Ia (3)](image)

Figure 1.2: Structures of lipid A (2) from *E. coli* and its biosynthetic precursor Ia (3).

The structural details of each lipid A isoform determines whether the compound will cause a host-mediated response or not. For example, lengthening the acyl chains leads to a reduction in cytotoxicity, as evidenced by the lipid A’s of *Chlamydia psittaci*, *Bacteroides fragilis*, and *Legionella pneumophilia*. Those lipid A structures featuring
either a monosaccharide core or a dimeric structure with only four acyl side chains are significantly less toxic.\(^9\) Pentacylated forms of lipid A are less active than the natural hexaacylated forms, whereas bisacylated and tetraacylated derivatives as seen in the antagonistic biosynthetic precursor Ia (3) (also known as lipid IVa or compd. 406) are completely inactive.\(^{10, 11}\) Interestingly, synthetic \textit{E. coli} lipid A is less potent than the natural material. The chirality of the 3-hydroxyacyl chains apparently plays no role on the biological activity of the substance as both \textit{R}- and \textit{S}- configurations induce similar responses. The incorporation of at least one phosphate group is a requirement for bioactivity. These residues apparently are needed for recognition by the cell-surface receptor(s) for LPS.\(^9\)

1.1.1 Role in disease

LPS via the lipid A component is one of the most potent immunostimulatory substances known (as low ng to pg/mL range).\(^{12}\) LPS released in a human host due to bacterial growth or bacterial lysis interacts with a pattern-recognition receptor complex present in many host cells including monocytes and macrophages. This recognition event activates these cells to release several pro-inflammatory cytokines, members of a large class of small intercellular signaling molecules consisting of more than 160 polypeptides that orchestrate immune responses to invading pathogens by allowing cells to communicate with one another. Among these cytokines are tumor necrosis factor alpha (TNF\(\alpha\)), interleukin-1 (IL-1), and interleukin-6 (IL-6). The cytokines then activate other phagocytic cells and up-regulate the innate immune system in an attempt to clear the
invading microbe. This systemic inflammatory response to LPS leads to sepsis. Sepsis is characterized by fever, mental confusion, diarrhea, and hypotension. Severe sepsis, commonly referred to as *septic shock syndrome*, is a very dangerous disease that can lead to organ damage and even failure, and eventually death. An LPS concentration of less than 1 ng/mL is sufficient to cause the symptoms of sepsis, but more than 100 ng/mL is required to produce septic shock.\(^\text{13}\) After septic shock onset, patients also can succumb to secondary infections caused by opportunistic pathogens. That is, following the initial period of hyperimmune response, several anti-inflammatory cytokines are released to maintain homeostasis and as a result patients may become temporarily immunodepressed and susceptible to other pathogens.

### 1.1.2 Impact on human health

Despite great efforts, sepsis continues to be a severe health problem with mortality rates ranging between 20-40% overall, and 40-60% for septic shock cases.\(^\text{14, 15}\) Although the mortality rate has been decreasing over the last few decades, it is still the tenth leading cause of death in the United States. There were 660,000 cases (240 per 100,000 population) of sepsis reported in 2000 according to a joint study by the Center for Disease Control and Prevention and Emory University School of Medicine.\(^\text{16}\) It has been an especially difficult problem in men (more common than in women by a factor of 1.28) and nonwhites (mean relative risk of 1.90 compared to whites). Sepsis is most dangerous in black men, the elderly, immuno-compromised individuals, and in critically ill patients.
Before 1987, Gram-negative bacterial infections were the principal cause of sepsis, but in more recent years, this role has been overtaken by Gram-positive bacteria. In 2000, Gram-positive bacteria accounted for 52.1 percent, Gram-negative bacteria 37.6 percent, polymicrobial origins 4.7 percent, fungi 4.6 percent, and anaerobes 1.0 percent of all cases. The organs that failed the most frequently were the lungs (18%), followed by the kidneys (15%), and then cardiovascular (7%), hematological (6%), metabolic (4%), and neurological failure (2%). At present, there is no effective treatment for severe sepsis.

1.2 TNFα

Tumor necrosis factor alpha (TNFα), also known as cachectin, is a pleiotropic cytokine with broad biological capabilities. Like other cytokines, TNFα has several advantageous properties including accelerating wound healing, cell growth modulation, cellular differentiation, and endogenous antimicrobial activity through activation of the immune system. TNFα was initially identified as an anticancer agent in 1975 when it was observed to destroy solid murine tumors \textit{in vivo} and was cytotoxic against several tumor cell lines. Many stimuli can activate the synthesis of TNFα including TNFα itself, other cytokines (such as IL-1, INF-γ, IL-2, platelet activating factor, and granulocyte macrophage-CSF), phorbol ester, IgE, reactive oxygen metabolites, X-rays, mycobacterial proteins, cyanobacteria, zymosan (an insoluble carbohydrate from the cell wall of yeast), malarial parasite antigens, viruses (Sendai, herpes simplex, and Epstein-Barr), and, most importantly, LPS. Several known agents are able to down-regulate
TNFα production. These species include cytokines (IL-4, IL-10, and transforming growth factor-β), prostaglandin E₂, Iloprost (a prostacycline analog), histamine, antioxidants (such as N-acetylcysteine and glutathione), glucocorticoids (including dexamethasone, cortisol, and danazol), lipoxygenase inhibitors, and certain drugs (such as pentoxifylline, thalidomide, ambroxol, and cyclosporin A).

1.2.1 TNFα generated from LPS exposure: Mediator of septic shock

The link between TNFα and LPS goes back more than 100 years. At the conclusion of the 19th century, the New York surgeon William Coley reported that inoperable tumors could be treated with Gram-negative and Gram-positive bacteria. Carswell and co-workers later coined the term “tumor necrosis factor” for the cytokine that caused hemorrhagic necrosis of tumors in animal models injected with LPS. LPS (via the lipid A component) is capable of activating a variety of cells including macrophages, monocytes, fibroblast, astrocytes, Kupffer cells, smooth muscle cells, keratinocytes, neutrophils, lymphocytes, platelets, microglial cells, intestinal paneth cells, endothelial, mast cells, natural killer cells, and tumor cells. At present, there are 13 different members in the TNF ligand family, including another factor called TNFβ (also known as lymphotoxin, LTα, or cytotoxin), which has similar cytotoxic activities to TNFα since it binds to the same receptors, but it is produced only by activated T-lymphocytes.

TNFα is first expressed as a 26 kDa (233 amino acid) transmembrane peptide that is cleaved by the metalloprotease TNF-alpha-converting-enzyme (TACE) in activated
cells to the soluble 17 kDa (157 amino acids) mature protein.\textsuperscript{22} The 26 kDa pro-peptide also is functional through cell-to-cell contact. The cleaved TNF\(\alpha\) generates its diverse biological effects through binding to two distinct cell surface receptors of 55-60 kDa (known as p55TNFR, TNFR1, or CD120a) and of 75-80 kDa (p75TNFR, TNFR2, or CD1120b). It is believed that p55TNFR is the principal receptor whereas p75TNFR is responsible for increasing the local concentration of TNF\(\alpha\) and passing it on to p55TNFR once the ligand has been bound.

### 1.2.2 Monocyte response pathway

Bacterial components such as LPS are usually first recognized by monocytes and macrophages through the lipid A receptor system, as will be described in the next section. The lipid A region of LPS is recognized by either a bound receptor cluster found on mononuclear phagocytes (monocytes and macrophages) or by a soluble binding molecule which activates cells that lack the lipid A receptor system. This receptor binding leads to the transcription of more than 120 genes, including those that code for several cytokines.\textsuperscript{23} Among the first expressed cytokines are TNF\(\alpha\), interleukin-1\(\alpha\), interleukin-1\(\beta\), and interleukin-6. These pleiotropic cytokines are first produced locally but also can be released by cells at sites remote from the infection. Circulating TNF\(\alpha\) levels peak at 1.5 h when the host is challenged with LPS and usually return to normal within 4 h of response.\textsuperscript{24} Use of TNF\(\alpha\) antibodies prevented the same reaction when mice and primates were exposed to otherwise lethal levels of LPS.\textsuperscript{25, 26} Interestingly, the levels of other cytokines such as IL-1\(\beta\) and IL-6, which are normally associated with sepsis, also were
attenuated, an observation that suggests an early role for TNFα in the development of septic shock progression.\textsuperscript{27}

1.2.3 Role of LPBP, CD14, Tlr4, and other proteins

There is a highly developed pathogen recognition immune system in humans. In particular, several bacterial products are recognized as xenobiotic including foreign DNA and RNA, lipoproteins, flagellin, zymosan, peptidoglycan, and, of course, LPS. The pathogen-associated molecular patterns are recognized by a group of at least ten membrane receptors called toll-like receptors (TLRs). Among these receptors is TLR4, a 92 kDa protein (841 amino acids), that is partly responsible for recognizing LPS via the lipid A binding site (Figure 1.3). TLR4-deficient mice (C3H/HeJ) are hypo-responsive to the effects of LPS, an observation that will be used to advantage in probing the role of ellagitannins in immunostimulation (see Section 4.2).\textsuperscript{28} Interestingly, TLR4 knockout mice showed an immunological response to whole \textit{E. coli} similar to that observed with normal mice, suggesting that there are other receptors involved.\textsuperscript{29} It is now known that there are other bacterial components including lipoprotein, peptidoglycan, bacterial DNA with a CpG sequence, flagellin, and fimbriae, which can act as antigens through other TLR members such as TLR2, TLR5, or TLR9.\textsuperscript{30-33}

LPS circulating in an infected host is first recognized by the serum protein lipopolysaccharide binding protein (LBP), a 60kDa carrier peptide with a very high affinity for the lipid A portion of LPS (K\textsubscript{d} varying between 1 to 58 nM).\textsuperscript{34-36} LBP, an acute-phase protein, functions as an opsonin (binding enhancer for receptors) that aids in
the recognition of LPS by macrophages through binding to the surface of bacteria or to the lipid A segment of LPS that is presented by erythrocytes.\textsuperscript{37}

Once bound, LPS is transported by LBP to either the soluble (sCD14) or the membrane-anchored CD14 receptor (mCD14), which is attached to the cell membrane by a glycosylphosphatidylinositol (GPI) group.\textsuperscript{38-40} The soluble CD14 glycoprotein mediates recognition by cells such as endothelial cells that lack the membrane bound form of the receptor. Membrane bound CD14 is expressed mainly on myeloid cells, including macrophages, monocytes, and granulocytes, and some other cells such as liver parenchymal cells, gingival fibroblasts, and B cells. Mice lacking the CD14 receptor molecule showed little-to-no response when injected with otherwise lethal doses of either LPS or whole \textit{E. coli}.\textsuperscript{41} In humans injected with LPS, CD14 antibodies partially reduced the clinical symptoms and cytokine response of sepsis.\textsuperscript{42} These data, and the fact that the CD14 protein lacks an intracellular domain necessary for signal transduction, suggested that there was another transmembrane protein that was part of the LPS receptor system. This elusive signal transmitting protein was, in fact, identified as TLR4 and through an as yet unidentified interaction, CD14 helps transfer LPS to this receptor/signaling molecule. In addition, TLR4 appears to require an accessory protein called MD-2 in order to engage LPS.\textsuperscript{43} MD-2, a secreted glycoprotein, forms a complex with the membrane bound TLR4 for effective recognition of this ligand. MD-2 knockout mice did not induce up-regulation of TNF\(\alpha\) release in response to LPS, demonstrating that MD-2 is a necessary adaptor component for signaling.\textsuperscript{44}

Malhotra also has suggested that cell-surface adhesion molecules known as selectins may act as low-affinity receptors for LPS, also through the lipid A recognition
site, when it is present in high concentrations. CD14 knockout mice have shown a CD14-dependence to macrophage activation at concentrations lower than 10 ng/mL of LPS, but not at greater than 100 ng/mL of LPS.\textsuperscript{45, 46} At the higher concentrations, the CD14-lacking mice exhibited a response similar to that of the wild-type mice. P-selectin and L-selectin have been reported to bind LPS.\textsuperscript{47, 48} In addition, the use of antibodies against both receptors significantly reduced their ability to bind fluorescently labeled LPS and decreases the levels of both TNF$\alpha$ mRNA and oxygen radicals formed in neutrophils. These observations have lead Malhotra’s group to suggest that the selectins act as LPS receptors at high LPS concentrations.

Moesin, a 78 kDa cell-surface protein with both a diverse biological activity profile and signal transduction capability, may also be involved in the recognition of LPS. An antimoesin monoclonal antibody completely blocked LPS induced TNF$\alpha$ release without impeding LPS-to-CD14 binding.\textsuperscript{49, 50} Van Dyke and co-workers have shown that moesin is able to bind LPS, CD14, and TLR4. At this time, it is not known whether moesin acts as an independent LPS receptor or in unison with CD14/TLR4, although the latter appears more likely.
Figure 1.3: Lipid A recognition leading to cytokine secretion.

Another receptor pathway that has been implicated in the recognition of LPS utilizes CD18 antigens, which are also called CD11/CD18 or \( \beta_2 \)-integrins. Due to their lower affinity for LPS, they have been posited to play only a minor role in its recognition.\(^{51}\) There is some evidence that CD11/CD18, much like CD14, signals through TLR4 and is necessary for optimal production of COX-2, IL-2, and IL-12, but it is not needed for the LPS-induced expression of cytokines such as TNF\(\alpha\).\(^{52, 53}\) Other unidentified molecules may be involved in the recognition of LPS. Triantifilou and colleagues have reported that LPS was able to bind several proteins like the heat shock proteins Hsp70 and Hsp90, chemokine receptor 4, and growth differentiation factor 5.\(^{54}\) \(^{55}\) Recent reviews that have focused on LPS/lipid A receptor molecules can be consulted for further details.\(^{56, 57}\) After LPS interacts with the TLR4/MD-2 complex, several intracellular signaling pathways are activated. A more thorough discussion of the TLR4 intracellular signaling pathways can be found in some current reviews.\(^{58, 59}\)
1.3.1 LPS receptor agonists

There are very few reports of LPS (lipid A) receptor agonists in the literature. One example is the microtubule stabilizer taxol ((4), paclitaxel) (Figure 1.4).\textsuperscript{60, 61} It has been reported to act by binding to TLR4. In addition, some 4-\(O\)-phosphono-D-glucosamine derivatives such as GLA-60 (5) and its analogues have shown agonistic properties. Synthetic acyclic lipid A-like analogues such as ER-112022 (6) also have shown agonistic activity through the TLR4 receptor without the need for CD14.\textsuperscript{62}

![Figure 1.4: Some LPS receptor agonists.](image-url)
1.3.2 LPS receptor antagonists

There are considerably more reports of LPS receptor antagonists than LPS receptor agonists in the literature.\textsuperscript{63-65} One of the earliest approaches to developing LPS-like antagonists relied on analogues of active lipid A itself. These analogues competitively inhibited LPS/lipid A binding to the TLR4/CD14 receptor complex. For example, the lipid A's of the non-pathogenic Gram-negative bacteria \textit{Rhodobacter capsulatus} (7) and \textit{Rhodobacter sphaeroides} ((8), RsDPLA) were used as lead compounds (Figure 1.5). An early derivative, E-5531 (9), has been replaced by the easier-to-synthesize, more robust, and more potent E-5564 (10) (also known as Eritoran).\textsuperscript{66, 67} Biosynthetic lipid A precursor lipid Ia ((3), lipid IVa, or compd. 406, Figure 1.2), and to a lesser extent Lipid X (11), also are antagonists. Several other small molecules have also been reported to inhibit TNF\(\alpha\) production and to antagonize LPS activity by targeting biological targets downstream from the LPS receptor(s).\textsuperscript{63-65}
Figure 1.5: Some antagonists that act through the TLR4/CD14 receptor.

2 Initial discovery of ellagitannins as immunostimulants

Polyphenol-rich folk medicines from China and Japan, including tannin-containing decoctions, had been used for some time to treat a variety of ailments. In
1987, Okuda, Miyamoto, and colleagues reported that several tannins displayed tumoricidal activity against a model cancer cell line, sarcoma-180. They found that the tannins’ anticancer activity was due to their ability to act as immunostimulants in a manner reminiscent of LPS.

2.1.1 Agrimoniin

Although agrimoniin (12) had been known for some time, since it had been isolated before from the Rosaceae plants Agrimonia pilosa Ledeb, Agrimonia japonica (MIQ) KOIDZ, and Potentilla kleiniana WIGHT et ARNOTT, its antitumor properties were not known before Miyamoto’s work. Agrimoniin, the first dimeric ellagitannin to be isolated and characterized, significantly extended the life span of mice inoculated with mammary carcinoma MM2 cells, and in some cases even lead to tumor regression. Agrimoniin was found to be effective upon either pre- or posttreatment and showed an IC$_{50}$ of 2.6 µg/mL. In some preliminary experiments, agrimoniin appeared to cause an enhancement in the growth rate of macrophages, lymphocytes, and adherent peritoneal exudate cells, as well as an enlargement of spleen size, observations which supported the idea that tannins act as immunostimulators. Agrimoniin (at a 5 mg/kg dose) also was found to be active in mice inoculated with sarcoma-180 by effecting a 75% increase in the life span (%ILS) of the mice, but no tumor regressors out of 6 mice were detected. An increase in dose to 10 mg/kg led to 3/6 cured mice and a 136 %ILS. This compound also has diverse biological activities including antileishmanial properties and HIV-1 reverse transcriptase inhibition.
2.1.2 Coriariin A

Among the 63 tannins initially surveyed by Okuda’s and Miyamoto’s groups, coriariin A (1) showed the strongest activity against sarcoma-180 tumor cells with a 238 \%ILS and tumor regression in 3 out 6 mice at a 5 mg/kg dose. Coriariin A, a dimer of tellimagrandin II (15), was isolated from the Japanese poisonous plant *Coriaria japonica* A. Gray. Coriariin A, along with several oligomeric ellagitannins, was found to be a potent inhibitor of both poly(ADP-ribose) glycohydrolase (IC$_{50}$ 8.5 µM) and of histamine release (IC$_{50}$ 2.97 µM). The chemical synthesis of coriariin A has been accomplished. Further examination of other related tannins (now totaling 108) at different doses and through different methods of administration has led to the identification of several other tumoricidal compounds. The best results were found when the tannins were administered to mice through intraperitoneal injection 4 days before treatment with cancer cells.
Figure 1.7: Structure of coriariin A (1) and its methylated derivative 13.

2.1.3 Other ellagitannins

Besides agrimoniin and coriariin A, the work of Miyamoto and colleagues showed that several other tannins that met some particular structural requirements were active against sarcoma-180 tumor cells. From their structural-activity relationship studies, some general trends emerged. Condensed tannins, caffeic acid derivatives, berganin derivatives, dehydroellagitannins, and gallotannins exhibited little-to-no anticancer activity. In general, monomeric ellagitannins were found to be less potent than dimeric ones. Exceptions to this generalization are the two particularly active monomers tellimagrandin II (15) with 3 out of 6 tumor regressors, although with only a 18 % ILS at a 10 mg/kg dose, and rugosin A (18) with one cured mouse out of six and a 110 % ILS at a 5 mg/kg dose (Figure 1.8). The gallotannin penta-\(O\)-galloyl-\(\beta\)-D-glucose (\(\beta\)-PGG, 16) was relatively inactive with no regressors and an 82 % ILS at 10 mg/kg dose. Compounds containing an open chain glucose unit also were ineffective.

Several macrocyclic ellagitannins such as the trimer oenothein A (19) (1 regressor/6 mice and 103 % ILS at 10 mg/kg) and the dimer oenothein B (17) (4
regressors/6 mice and 196 %ILS at 10 mg/kg) were very active. Exposed phenolic hydroxyls were necessary for activity as nocacosa-\(O\)-methylcoriariin A (13), the completely methylated derivative of 1, was ineffective, whereas coriariin A proved to be the most potent tannin tested at 5 mg/kg. Increases in the degree of oligomerization didn’t necessarily correlate to an increase in activity. The dimer (of tellimagrandin I (14)) oenothein B was more potent than both the trimer oenothein A and the tetramer woodfordin F, which had no regressors at 10 mg/kg. In some cases, adding more galloyl groups led to an increase in activity, but that trend was not general. Compounds possessing two galloyl groups in the place of the HHDP moiety appear to be more potent: rugosin F (22, no regressors and 35 %ILS) < rugosin D (21, one regressor/6 mice and 172 %ILS), and agrimonin < coriariin A. However, gemin A (20, one regressor/6 mice and 176 %ILS at 10 mg/kg dose) seemed to be less potent than agrimonin at 10 mg/kg dose.

As a comparison to the immunostimulatory effects of tannins, OK-432 (Picibanil), a whole bacterial streptococcal preparation that is a known immunopotentiator, cured 4 out of 12 mice and showed a 79 %ILS at a 10 mg/kg dose.
Figure 1.8: Structures of some anticancer ellagitannins and gallotannins.
2.2 Agrimoniin- mouse and hPBMC studies; IL-1β release

Miyamoto and Okuda examined some immune system components in their search for biological mediators of tannin-induced anti-tumor activity. Specifically, treatment of mice with agrimoniin (12) stimulated growth of peritoneal exudate cells (PECs). The adherent PECs (presumably macrophages) did appear to inhibit tumor cell growth (MM2 and MH134). Removal of serum from mice treated with both MM2 tumor cells and agrimoniin, and addition of that serum to a culture of only adherent PEC and MM2 cells, led to complete tumor lysis. The serum was assumed to contain immunostimulatory factors such as cytokines. The cell lytic activity of the serum peaked after 6 days following intraperitoneal injection.

Analysis with an enzyme-linked immunosorbent assay (ELISA) of the supernatant above hPBMC's treated with agrimoniin led to the identification of significant quantities (average of 1203 pg/mL) of the cytokine Interleukin-1 beta (IL-1β) compared to the untreated cells (300 pg/mL). Following the same experimental procedure, treatment of hPBMC's with *E. coli* LPS at 10 µg/mL led to the release of an average of 1350 pg/mL of IL-1β. IL-1β has been known to activate macrophages and lymphocytes and stimulate secretion of other cytokines such as IL-2, IL-3, IL-6, and interferon (IFN). Thus, the authors hypothesized that the generated IL-1β was the agent responsible for agrimoniin’s tumoricidal activities through stimulation of monocytes, macrophages, and NK cells.
3 Ellagitannins as TNFα secretion inducers

The ellagitannins’ potent antitumor activity through an apparent up-regulation of the immune system is well founded. Miyamoto’s group suggested that the secretion of IL-1β seen in murine PEC’s and hPBMC’s could explain the observed antitumor actions of hydrolyzable tannins. However, at the time that Miyamoto’s group was pursuing the immunostimulatory experiments, little was known about the “newly discovered” related cytokine TNFα, and no simple assays were available. Also given IL-1β’s late entry into the cytokine cascade, it was conceivable that a mediator different than IL-1β was responsible for the primary activity of the antitumor ellagitannins. Since much post-Miyamoto research established that TNFα was, indeed, a tumor-lethal substance, it seemed like a logical target for further tannin-related study. Additionally, TNFα is among the first cytokines released in response to immunostimulation and it is known to induce the later production and release of IL-1β. Finally, a readily available commercial ELISA-based TNFα assay had become available. Therefore, some known tumoricidal ellagitannins, an analogue of coriariin A, and the gallotannin 1,2,3,4,6-penta-O-galloyl-beta-D-glucose (β-PGG, 16) were tested for their ability to induce TNFα output from hPBMC’s.

3.1 β-PGG, agrimoniin, and a model dimer analog of coriariin A induce TNFα secretion from hPBMC’s

The initial experiments involved monitoring TNFα generation using a qualitative test with the L929 murine fibroblast lysis assay. This indirect analysis revealed that
significant amounts of TNFα were present in the supernatant of hPBMC’s after 4 hours of incubation with agrimoniin at 28 µM. Thus, this crude assay provided the first evidence that the cytokine TNFα was generated by cells treated with an ellagitannin. Using the same experimental procedure, the monomeric gallotannin β-PGG (16) and the dimer 23, an analogue of coriariin A, were assessed quantitatively for their TNFα inducing capabilities utilizing an ELISA kit. A time-course study revealed similarities between both β-PGG and 23, with low levels of TNFα generated at 4 hours and a maximum release at 24 hours (Figure 1.10). However, the two tannins were observed to have considerably different TNFα eliciting proficiency, as the dimeric species 23 was found to be significantly more potent than β-PGG at every time point examined.

Figure 1.9: Structures of an analogue of coriariin A.
3.2 Structure/activity profile of ellagitannins and gallotannins

A comparison between the dose-response curves of the monomeric gallotannin β-PGG (16) and the tannin dimers coriariin A (1) and 23 for TNFα output after 24 hours of incubation with hPBMC’s showed very different responses. Therefore, the secretion of TNFα appears to be responsive to the size of the tannin. The monomer β-PGG was less potent at generating TNFα from hPBMC’s than either of the dimers 1 or 23 at similar concentrations (Figure 1.11). This observation correlates well with the very different anticancer profiles between coriariin A (3 out of 6 regressors and a 238 %ILS) and β-PGG (zero regressors and 82 %ILS) reported by Miyamoto. Since a measure of IL-1β secretion amounts did not reveal a similar correlation, it remains unlikely that IL-1β is the primary mediator responsible for the tumoricidal activities of tannins. It is also noteworthy that the TNFα-inducing abilities of coriariin A and its model analogue 23 are similar (Figure 1.11). These data show that the HHDP group is not necessary for activity.
and that two unconnected galloyl groups can take its place, which is consistent with Miyamoto’s *in vivo* SAR studies of ellagitannins against sarcoma-180.²

![Graph](image.png)

Figure 1.11: Dose dependence profile of TNFα release (24 hours) upon hPBMC stimulation by several tannins and LPS.

4 Ellagitannins as lipid A-like agonists

The similarity in TNFα dose-response data between the immunostimulatory tannins and LPS raises the real possibility that the tannin constructs may be utilizing the previously discussed lipid A receptor system (LBP, CD14, TLR4, etc.) to transduce the ellagitannin binding event into eventual TNFα secretion. In order to test this premise, two types of experiments were pursued. Initially, the role that the LPS-sensitive cell surface receptor CD14 might play in tannin-mediated TNFα secretion from hPBMC’s was assayed using monoclonal antibodies for the CD14 receptor as a competitive blocker. Followup studies examined whether the tannin stimulus utilized TLR4 as the signal transduction molecule linking the cell surface binding event to gene transcription.
4.1 Use of CD-14 antibodies as a probe for the participation of CD14 in tannin-stimulated TNFα release

Commercially available monoclonal antibodies to the human CD14 receptor have been used previously to test for the participation of this receptor in various biological processes. This approach has proven effective when there is a competition for the CD14 binding site between a CD14 ligand of interest and the antibody. For the tannin series, use of a positive control (LPS) with hPBMC's and antibody MY4 led to the expected result; nearly complete suppression of TNFα production compared to LPS without antibody (Figure 1.12, columns 1 and 2). Similar experimental trials with the naturally occurring ellagitannin coriariin A (1) and its non-coupled analogue 23 provided the first glimpse that CD14 was indeed implicated in the tannin mediated upregulation of TNFα production. The antibody MY4 at 10 µg/mL diminished coriariin A's ability to induce TNFα secretion compared to control by about 70%, whereas the gallotannin/ellagitannin analogue 23 was affected a little less by the antibody (approximately 55% reduction in TNFα formation). These results clearly demand a role for CD14 in the tannin-mediated induction of TNFα secretion from hPBMC's, but the data are not as convincing as with the natural ligand LPS. The intervention of other, non-CD14-utilizing pathways may be indicated for the tannins, perhaps involving the selectin receptors discussed along with Figure 1.3. Nevertheless, the evidence is unequivocal that at least some of the TNFα-generating pathway stimulated by tannins follows the same receptor system as does the natural ligand LPS/lipid A.
Figure 1.12: The monoclonal antibody to CD14 suppresses TNFα release from hPBMC's stimulated by (a) LPS (2.5 µg/mL), (b) the model dimer 23 (27 µM), and (c) coriariin A (1) (27 µM).

4.2 Use of HeJ/OuJ mice as a probe for TLR-4 participation in tannin-stimulated TNFα release

A strain of otherwise healthy mice that was nonresponsive to LPS stimulation were discovered in the 1960s. Subsequent studies traced this condition to a mutation in the TLR4 gene, which rendered the receptor inactive. This difference in response to LPS is exemplified in Figure 1.13, where peritoneal exudate cells (PEC's) from both normal mice (C3H/OuJ) and TLR4-inactive mice (C3H/HeJ) were treated with LPS. The normal mouse PEC's responded as expected, with high levels of TNFα secretion observed at 24 hours within the context of a standard dose-response curve. However, the TLR4-defective HeJ mouse PEC's exhibited no discernable TNFα-generating capability upon LPS stimulation. The identical experiment with the gallotannin/ellagitannin hybrid 23 provided some insight into the role that the TLR4 receptor might play in tannin-mediated secretion of TNFα. There appeared to be a relatively small (compared to the LPS data, Figure 1.13) but consistent decrease of TNFα release with the TLR4-inactive
HeJ mice compared to the OuJ mice controls (Figure 1.14). The difference between these two mouse strains was much less dramatic than in the LPS challenge experiment, with TNFα concentrations hovering around 50 – 60% less in the HeJ series throughout the concentration range. Nevertheless, as with the CD14 competitive binding experiments, there is an unmistakably diminished response with the TLR4-inactive mice, suggesting that at least some of the tannins' effects require a functioning TLR4 receptor. Taken together, these mechanistic experiments provide preliminary data that is entirely consistent with a model whereby the tannin stimulant utilizes, at least in part, the same key receptor components CD14 and TLR4 that the native ligand LPS uses. Therefore, the active tannin species appear to operate, at least in part, as LPS (lipid A) receptor mechanistic agonists.

Figure 1.13: The TNFα secretion response upon treating normal OuJ mice and TLR4-inactive HeJ mice with LPS.
Figure 1.14: The TNF-α secretion response upon treating normal OuJ mice and TLR4-inactive HeJ mice with the dimeric tannin construct 23.

5 Conclusions

Ellagitannins such as agrimoniin, oenothein B, and coriariin A have documented tumoricidal activities against numerous cancer xenograft models. These tannin species appear to operate through up-regulation of the immune system. Preliminary evidence in hand is consistent with a model featuring key molecular-level interactions between the tannin and the lipid A receptor complex leading to the release of TNF-α by hPBMC’s. Much refinement of this preliminary and necessarily crude mechanistic picture will be a necessary first step toward realizing a better understanding of their exact mode of action. With this goal in mind, the next two chapters will discuss the synthesis and later the corresponding in vitro biological testing of three fluorescently labeled tannin analogues that were designed to probe the question of whether tannins are competitive binding agents with LPS.
Chapter 2 – Synthesis of Some Tannin Analogues

1 Purpose of Synthesis

The similarity between the dose response curves of tannins such as coriariin A and the known immunostimulator lipopolysaccharide (LPS, component of the wall of Gram-negative bacteria) for production of tumor necrosis factor alpha (TNF-α) from human peripheral blood mononuclear cells (hPBMC’s) raised the possibility that these two structurally unrelated molecules could in fact be acting through the same receptor and signaling pathway. Over the last few decades, several of the key molecules involved in the receptor system of LPS have been identified, including CD14 and TLR4 (Figure 1.3). As discussed in the previous chapter, the amount of TNF-α produced by hPBMC's in the presence of an ellagitannin (1 or 23, Figure 2.1) is attenuated by the use of an anti-CD14 antibody (MY4). In addition, an in-house investigation revealed that peritoneal exudate cells (PEC's) from TLR4-deficient mice (C3H/HeJ) produced less TNF-α in comparison to those from normal mice (C3H/OuJ) when each is treated with galloellagittannin hybrid 23. In order to further investigate the biological mechanism of action of tannins, three tannin analogues were designed (Figure 2.2). These analogues (one dimer and two monomers) all contained a tetraethylene glycol linker that will allow the attachment of the fluorescent marker, fluorescein. These species will be used as competitive binding probes for LPS to explore the question of whether tannins act at the same receptors as does LPS.
Figure 2.1: Structures of coriariin A (1) and model dimer 23.

Figure 2.2: Structure of model tannin analogues 24-26.

2 Retrosynthesis of Model Ellagitannin/Gallotannin Analogue Dimer 87

The initial approach taken towards the model ellagitannin/gallotannin analogue dimer 24 (seen retrosynthetically in Figure 2.3) is based on the coupling of the
pentabenzylated dehydrodigalloyl ether diacid 29, which can be envisioned as coming from the commercially available methyl gallate (30), and trichloroacetimidate 28 containing the glucose moiety. This earlier part of the synthesis was closely related to previously published work for the syntheses of coriariin A (1) and model dimer 23, although some modifications and improvements were implemented.\textsuperscript{81, 82, 90} A second key step in this retrosynthetic analysis is the addition of the appropriately protected linker-galloyl unit 27 to diol 26. This linker galloyl species would allow for the introduction of a fluorescent marker in a penultimate step before the final global removal of the benzyl protecting groups.

Figure 2.3: Retrosynthetic analysis of model analogue dimer 24.
3 Synthesis of Model Ellagitannin/Gallopinnin Analogue Dimer 24

One of the most challenging goals was the synthesis of diacid 29 in the quest to make analogue 24. Upon mild heating of bright-red orthoquinone 32 in the presence of B(OAc)_3 in chloroform, a mixture of regioisomeric benzodioxene products, 33a and 33b, are obtained (Figure 2.4). Treatment of this mixture with mild base leads to the β-elimination of phenoxide, which produces a new mixture of orthoquinones 34a and 34b. Reduction of these orthoquinones yields a set of regioisomeric digalloyl ethers 35a and 35b, respectably. Both of these ethers, including 35b, which contains the “undesired” para-C-O linkage, are converted to the protected dehydrodigalloyl ether 36. Regioisomer 35b is converted to the desired species 36 via a Smiles rearrangement.109 Hydrolysis of 36 yields the benzylated diacid 29 in quantitative yield.
Figure 2.4: Synthesis of diacid 29.

The difficulty in this route lies in the replication of the conversion of 32 into 36 reported by the first investigator in our lab at 44% yield. Despite numerous attempts, yields of only 15-22% have been obtained. Furthermore, the reaction suffers from a lack
of scalability and at times has produced the tribenzylated form of 31 as a major impurity, which cannot be separated by any method from dimer 36. The synthesis of orthoquinone 32 is achieved by the oxidation of catechol 31, which is easily obtained from the commercially available methyl gallate (30) in three steps (Figure 2.5).

Several conditions were examined for the Diels-Alder reaction of 32 in an attempt to develop a more reliable method to make 36 (Table 2.1). Simply heating quinone 32 in the absence of any catalyst led to similar yields (entry 1). Lowering the temperature to 45 °C also led to similar yields, but temperatures lower than 40 °C or higher than 60 °C furnished 36 in decreased yield. Treatment of quinone 32 with alumina at 50 °C (entry 3) led to similar yields, whereas use of high pressure conditions (entry 4) resulted in slightly lower yields. Several Lewis acids including some lanthanides and transition metals were also screened for their ability to promote this transformation at room temperature. In most cases the yields were higher (entries 5-14). The best results were obtained using either Sc(OTf)₃ or CeCl₃ (entries 8 and 10) to catalyze the reaction in 37 and 36% yields, respectively. Both sets of conditions also were scalable and did not yield any inseparable impurity.
The synthesis of trichloroacetimidate 28, involved six fairly high-yielding reactions except the first, starting from the commercially available tetra aceto α-bromoglucose (38) and ortho-nitro benzyl alcohol (39) (Figure 2.6). Coupling of 38 and 39 followed by methanolysis of the acetate groups affords tetraol 40. Alcohols 4 and 6 in tetraol 40 were selectively protected as a benzylidene acetal, whereas alcohols 2 and 3
were protected as TBS ethers. Next, the photolabile O(1)-ortho-nitro benzyl protecting group\textsuperscript{104} in 41 was replaced by a trichloroacetimidate group to make the glycosyl donor 28. It was necessary to use the silyl protecting groups in 28 even though it lengthens the synthesis. As predicted by the work of Fraser-Reid and others, the use of an electron releasing group at O (2) of the glucopyranose unit armed it for anomeric esterification, whereas the opposite is true for an electron-withdrawing group such as an ester\textsuperscript{105}. This hypothesis was confirmed by earlier work on the model dimer analogue 23 (Figure 2.7); attempts to couple the electron-depleted tetragallolated trichloroacetimidate 42 and diacid 29 were unsuccessful\textsuperscript{82}.

Figure 2.6: Synthesis of Trichloroacetimidate 29.

Figure 2.7: Attempted coupling of diacid 29 with trichloroacetimidate 42.

Two molecules of 28 were coupled to the diacid 29 through a Schmidt trichloroacetimidate acylation\textsuperscript{100} to afford 44, a compound that is beginning to resemble
the target molecule \textbf{24} (Figure \textbf{2.8}). As expected from the work of Schmidt \textit{et al}, dimer \textbf{44} was obtained mostly free of other anomeric stereoisomers (<5%). In CDCl$_3$, the major anomer showed a doublet at 5.90 ppm ($J = 6.0$ Hz, 1H) and a doublet at 5.85 ppm ($J = 6.0$ Hz, 1H), which correspond to a β,β-anomeric linkage based on the work of Khanbabaee.$^{110}$ Following this coupling, the four TBS protecting groups were removed with tetrabutylammonium fluoride (TBAF). The TBAF deprotection media was buffered with acetic acid$^{101}$ in this and later silicon-group deprotections to preserve the nucleophile-sensitive anomeric esters groups formed with the dehydrodigalloyl core. Other deprotection conditions such as TBAF alone, tris(dimethylamino)sulfur (trimethylsilyl)difluoride (TAS-F), hydrogen fluoride, and hydrogen fluoride-pyridine led to the hydrolysis of the dehydrodigalloyl ester bonds.$^{81}$ After taking off the silicon-based protecting groups, the resulting tetraol was combined with four equivalents of tribenzylated gallic acid \textbf{45} to form \textbf{46} using Keck’s modification$^{102}$ of the Steglich dicyclohexylcarbodiimide (DCC) esterification procedure.$^{103}$
Figure 2.8: Synthesis of diol 26.

Next, the hydroxyls on the 4 and 6 positions of the glucose substructures were liberated by iodine/methanol treatment, and subsequently the alcohol on the 6 position was protected selectively with another silyl protecting group (t-butyldiphenylsilyl), by
taking advantage of the much higher nucleophilicity of the primary alcohol on the 6 position over the secondary alcohol on the 4 position to deliver 48. With the use of another DCC mediated coupling, diol 48 was gallolated at the free 4 positions with two equivalents of the gallic acid derivative 45. The TBAF deprotection (buffered with acetic acid) that followed temporarily delivered a product with a free 6 position alcohol, but a galloyl shift occurred as the galloyl group from the 4 position is transferred to the free 6 position in yielding 26. This rearranged structure was determined from the $^1$H NMR spectrum of 26. Based on the work of Khanbabaee, the proton on C3 (~6.3-5.5 ppm) shows up significantly upfield from those on C6 (~5.0-4.5 ppm) when both alcohols are acylated with galloyl groups. As it is seen in the $^1$H NMR spectrum of 49, there are 8 protons between 6.06-5.72 ppm corresponding to those on C1, C2, C3, and C4. However, in diol 26 there are only 6 hydrogens between 5.94-5.53 ppm, suggesting that the galloyl group has been transferred to the alcohol on C6. Later, when diol 26 was acylated with the galloyl linker moiety, 8 protons are once again seen between 6.3-5.5 ppm in the $^1$H NMR spectrum (see 59, 62, 68, or 77). The gallic acid derivative 45 was obtained by benzylation of commercially available methyl gallate 30 followed by hydrolysis of the methyl ester 50 (Figure 2.9).

![Figure 2.9: Conversion of methyl gallate 30 into acid 45.](image-url)
3.1 Attempts at the Synthesis of Model Ellagitannin/Gallotannin Analogue Dimer 24 Utilizing a Teoc Protected Linker.

The 2-trimethylsilyl ethoxycarbonyl (Teoc) protected linker galloyl 27a was synthesized as shown in Figure 2.10. Selective benzylation\textsuperscript{94} of one phenol in catechol 31 followed by attachment of the linker 52, which was synthesized by displacement\textsuperscript{91} of one the tosyl groups from commercially available penta (ethylene glycol) di-\textit{p}-toluenesulfonate, afforded galloyl linker azide 53. Staudinger reduction\textsuperscript{92} of azide 53 gave amine 54, which was subsequently protected with a Teoc group.\textsuperscript{93} Hydrolysis of the methyl ester afforded acid 27a. Initially, removal of a protecting group from the linker amine was expected to afford a handle for attachment of fluorescein. For example, commercially available fluorescein isothiocyanate ((57) FITC) appeared to be a good coupling partner due to its successful reaction with model galloyl linker amine 54 to make thiourea 58 (Figure 2.11).
Figure 2.10: Synthesis of Teoc protected linker galloyl 27a.

Figure 2.11: Coupling of amine 54 and FITC (57).
The protected linker galloyl acid 27a was joined to diol 26 through another DCC mediated coupling to give 59 (Figure 2.12). Unfortunately, attempts to remove the Teoc groups in 59 with either trifluoroacetic acid (TFA) or tetrabutylammonium fluoride (TBAF) were unsuccessful (Table 2.2). When TBAF was used, the starting material (59) was recovered mostly unaffected after 48 hours of reaction (entry 4). Resubjection of this material (59) to TBAF and acetic acid, which was added as a buffer, only led to complete destruction of the compound. This disappointing result perhaps could be explained by the information that would be learned later with other similar systems (i.e., the deprotection of 62 and 68, Figure 2.14 and 2.18). Apparently, the deprotection of the Teoc groups may have yielded a highly reactive free amine that destroyed the tannin structure, possibly by amidation of a galloyl ester. A milder deprotection was needed. Thus, a carbonyl benzyloxy (Cbz) protecting group, which could be removed through hydrogenolysis, was chosen.
Figure 2.12: Synthesis of Teoc linker dimer 59.

<table>
<thead>
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<th>Entry</th>
<th>Reagent</th>
<th>Conditions</th>
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<tr>
<td>1</td>
<td>1000 equiv TFA</td>
<td>0 °C, 2 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>2</td>
<td>3000 equiv TFA</td>
<td>0 °C to rt, 15 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>3</td>
<td>2000 equiv TFA</td>
<td>rt, 100 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>4</td>
<td>6.5 equiv TBAF</td>
<td>rt, 48 h</td>
<td>Incomplete</td>
</tr>
<tr>
<td>5</td>
<td>6.5 equiv TBAF, 4.3 equiv AcOH</td>
<td>rt, 48 h</td>
<td>0%, decomp.</td>
</tr>
</tbody>
</table>

Table 2.2: Deprotection attempts of Teoc linker dimer 59.
3.2 Attempts at the Synthesis of Model Ellagitannin/Gallotannin Analogue Dimer 24 Utilizing a Cbz Protected Linker.

The Cbz protected linker was synthesized in good yield (Figure 2.13). Amine 54 was combined with benzyl chloroformate followed by hydrolysis of the methyl ester in 61 at room temperature to yield acid 27b. Hydrolysis of 61 at reflux led to the removal of the Cbz group as well. Coupling of acid 27b with diol 26 occurred uneventfully to give 62 (Figure 2.14).

![Chemical structure](image)

Figure 2.13: Synthesis of Cbz protected linker galloyl 27b.

Compound 62 was then submitted to hydrogenolysis in an attempt to perform a global deprotection of all benzyls and Cbz groups. Both Pd/C and Pd(II) oxide were unsuccessful in the hydrogenolysis reaction, perhaps because the free amines poisoned the catalysts. Both Pd(OH)$_2$/C and Pd(OAc)$_2$ were found to work well, but unfortunately because of the instability of the free amine polyphenol intermediate, 63 could not be isolated. Again, it is possible that once the primary amines were exposed by removal of the Cbz protecting groups, they attacked the nucleophile-sensitive ester groups on the tannin core. Even an attempted hydrogenolysis in the presence of 2 equivalents of the
fluorescent tag FITC (57) failed to capture the free amines (Figure 2.15). Next, protonation of the free amines to render them unreactive until they were in the presence of FITC was examined as a viable solution for adding the fluorescent tag. Thus, the acid sensitive t-butoxycarbonyl (Boc) protecting group was studied.

Figure 2.14: Synthesis and attempted deprotection of Cbz linker dimer 62.
Figure 2.15: Attempted deprotection of Cbz linker dimer 62 in the presence of FITC (57).

3.3 Attempts at the Synthesis of Model Ellagitannin/Gallotannin Analogue Dimer 24 Utilizing a Boc Protected Linker.

Treatment of amine 54 with t-butoxycarbonyl anhydride affords the Boc protected species 64, which was hydrolyzed to give acid 27c (Figure 2.16). Furthermore, galloyl Boc protected linker amine 64 was examined as a model system for the deprotection, which later would be used with the dimer tannin (Figure 2.17). Compound 64 was deprotected successfully with TFA in the presence of perbenzylated model dimer 65 (see Figure 2.28 for its synthesis). The perbenzylated model dimer 65 was stable to the reaction conditions at 0 °C and room temperature for a few hours, but was found to be susceptible to hydrolysis if the reaction was allowed to run for 12 h. Gratifyingly, model
dimer 65 was found to be stable in the presence of the ammonium TFA salt 66 for days. Addition of FITC (57) and base to this mixture led to successful addition of a fluorescein molecule to the linker amine to form 67 without affecting perbenzylated model dimer 65 (Figure 2.17). With this precedent in hand, the Boc protected version of dimer 25 was attempted next.

Figure 2.16: Synthesis of Boc protected linker galloyl 27c.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Equiv TFA</th>
<th>Conditions</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>0 °C, 2.5 h deprot.</td>
<td>deprot. 64 + tannin 65 OK</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>rt, 1 h</td>
<td>deprot. 64 + tannin 65 OK</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>rt, 12 h</td>
<td>deprot. 64 + decomp. Tannin 65</td>
</tr>
</tbody>
</table>

Table 2.3: Deprotection conditions of Boc linker amine 64 in the presence of model dimer 65.
Figure 2.17: Model reaction for coupling of linker amine 64 and FITC (57) in the presence of model dimer 65.

As before, diol 26 was coupled with the Boc protected linker acid 27c to give 68 (Figure 2.18). Subjection of the Boc protected dimer 68 to the already established deprotection conditions afforded the TFA ammonium salt 69. Unfortunately, addition of base to 69, in the presence of FITC (57), led to destruction of the tannin structure. Perhaps the fact that the linked amines are part of the tannin structure could explain the difference in reactivity between the ammonium salt 66 (in the presence of perbenzylated model dimer 65) and dimeric species 69. It is possible that the amines in 69 preferred to react in an intramolecular amidation reaction faster than in an intermolecular acylation with FITC (57).
Figure 2.18: Attempted deprotection of Boc linker dimer 68 in the presence of FITC (57).
3.4 Attempts at the Synthesis of Model Ellagitannin/Gallotannin Analogue Dimer Utilizing an Azide/Alkyne Cycloaddition.

An alternative approach to coupling tannin with fluorescent tag, which utilized Huisgen-type 1,3-dipolar cycloaddition of azides and alkynes, was explored next. Thus, the tannin core could have an azide or alkyne linker ready for coupling with a fluorescent molecule bearing a complementary alkyne or azide arm. Since linker azide 53 had already been synthesized, the fluorescent tag would carry the alkyne. The necessary alkyne 72 was made from a known procedure\textsuperscript{95, 96} by coupling commercially available fluoresceinamine 71 and 10-undecynoic acid (70) via 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) as seen in Figure 2.19. With fluorescein alkyne 72 in hand, coupling with linker azide 53 using the Sharpless Cu(I)-catalyzed modification\textsuperscript{97} to the Huisgen cycloaddition (Table 2.4) was examined. Use of the copper sulfate/sodium ascorbate system in catalytic amounts gave the desired cycloadduct 73. Interestingly, the reaction proceeded in good yields in several aqueous solvent combinations including t-butanol, dioxane, acetonitrile, and acetone. As shown in Figure 2.20, the fluorescein moiety also proved to be stable to hydrogenolysis, which would be required later in the synthesis. Cycloadduct 73 was debenzylated using Pearlman’s catalyst, (Pd(OH)$_2$), to produce a compound, probably 74, incorporating two extra hydrogen atoms. After a few days, 74 air oxidized to the desired species 75, which was found to have fluorescent properties similar to the parent fluorescein in methanol.
Figure 2.19: Synthesis of fluorescein alkyne 72.

Table 2.4: Cyclization of azide 53 with fluorescein alkyne 72 using various solvents.
The next step involved building the dimer tannin with azide linkers. Hydrolysis of methyl ester 53 afforded acid azide 76 (Figure 2.21). Two equivalents of acid 76 were then coupled to diol 26 to give 77. Surprisingly, bis azide 77 did not react with fluorescein alkyne 72 under the previously developed conditions (Table 2.5, entries 1-2). Even heating to 50 °C did not result in the desired reaction (entry 3). To test if this lack of reactivity was due to insolubility of tannin 77, CH₂Cl₂ and THF as solvents also were examined, but unfortunately without any success (entries 4-7). At this point, efforts towards a fluorescently labeled tannin were diverted to the simpler monomeric species.
Figure 2.21: Synthesis of bis azide dimer tannin 77.
<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Conditions</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:1 H₂O/t-BuOH</td>
<td>rt, 36 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>2</td>
<td>1:8 H₂O/dioxane</td>
<td>rt, 3 d</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>3</td>
<td>1:9 H₂O/dioxane</td>
<td>50 °C, 2 d</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>4</td>
<td>1:1:1 H₂O/dioxane/CH₂Cl₂</td>
<td>rt, 10 d</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>5</td>
<td>1:1:1 H₂O/dioxane/CH₂Cl₂</td>
<td>45 °C, 20 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>6</td>
<td>1:4 H₂O/THF</td>
<td>rt, 10 d</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>7</td>
<td>1:4 H₂O/THF</td>
<td>60 °C, 16 h</td>
<td>0%, SM recovered</td>
</tr>
</tbody>
</table>

Table 2.5: Some attempts at cyclizing bis azide dimer 77 and fluorescein alkyne 72.

4 Synthesis of Model Gallotannin Analogue Monomer Utilizing an Azide/Alkyne Cycloaddition.

The synthesis of fluorescent gallotannin monomer 85 began with the known tetragalloylated alcohol 82 (Figure 2.22). Tetragallolated alcohol 82 came from the per-gallolation of β-glucose followed by selective O(1) ammonolysis of pentakis-O-(tri-O-benzylgalloyl)-β-D-Glucose 81. Tetragallolated alcohol 82 was then coupled with acid 76 to give azide 83 as a ca. 75:25 inseparable mixture of β:α anomers. This determination
was based on the relative integrations of the anomeric protons in the $^1$H NMR of 83 for each anomer using the work of Khanbabaee as a guide. The $\beta$-anomer shows a doublet at 6.27 ppm ($J = 8.1$ Hz), whereas the $\alpha$-anomer has a doublet at 6.87 ppm ($J = 3.5$ Hz).

Similarly to the dimeric bis azide 77 (Table 2.5), azide 83 was found to be unreactive under the previously established conditions used for azide 53. Use of $t$-butanol, acetone, or tetrahydrofuran as solvents up to 60 °C under the Sharpless cycloaddition conditions only led to recovery of the starting material (Table 2.6, entry 1-4). Fortunately, heating azide 83 and alkyne 72 in an industrial microwave oven at 100 °C gave the desired cyclization product 84 (entries 5-6). Tetrahydrofuran was found to work best as a solvent since in $t$-butanol the reaction did not go to completion, possibly due to insolubility of the tannin starting compound in $t$-butanol. Hydrogenation of cycloadduct 84 removed all of its fourteen benzyl groups (Figure 2.23). As with compound 73 (Figure 2.20), the cycloaddition product was allowed to air oxidize for a few days to obtain the desired fluorescent polyphenolic gallotannin monomer 85, which was found to have similar fluorescent properties to fluorescein: $\lambda_{em} = 514$ nm ($\lambda_{ex} = 490$ nm) for 85 in a pH = 9 phosphate buffer versus $\lambda_{em} = 515$ nm ($\lambda_{ex} = 490$ nm) for FITC.
Figure 2.22: Synthesis of gallotannin monomer azide 83.
Table 2.6: Some attempts at cyclizing azide monomer 83 and fluorescein alkyne 72.
5 Synthesis of Model Ellagitannin/Gallotannin Analogue Dimer Utilizing an Azide/Alkyne Cycloaddition.

The success of the “Sharpless + microwave” chemistry suggested that a reinvestigation of the dimer synthesis was in order. Following the same procedure established with the monomeric gallotannin 83, two molecules of the alkyne fluorescein 72 were coupled to the dimer ellagitannin/gallotannin bis azide 77 to give the desired dimeric species 78 (Figure 2.24). Hydrogenolysis of all benzyl protecting groups followed by air oxidation of the resulting product gave finally the fluorescently labeled model dimer 86. Dimeric 86 was found to have similar fluorescent properties to fluorescein: $\lambda_{em} = 516$ nm ($\lambda_{ex} = 490$ nm) in a pH = 9 phosphate buffer.
6 Synthesis of a Second Model Gallotannin Analogue Monomer Utilizing an Azide/Alkyne Cycloaddition.

Earlier structure activity relationship studies had revealed that TNF-α secretion was less sensitive to the substitution pattern at alcohols 4 and 6 compared to the anomeric
alcohol.\textsuperscript{87, 99} Thus, another model monomer having the linker azide attached at position 4 was prepared. To this end, known\textsuperscript{98} 4,6-\textit{O}-benzylidene glucose (87) was gallolated to produce 88 as mostly (> 90\%) the \textit{β}-anomer based on the relative integrations of the anomeric protons of each anomer in the \textit{\textit{1}H} NMR spectrum of 88 (Figure 2.25). Removal of the benzylidene protecting group under mildly acidic conditions afforded diol 89. As with the dimer tetraol 47 (Figure 2.8), diol 89 was selectively protected as the TBDPS ether. Unfortunately, attempts to gallolate 90 at the free alcohol gave only the recovered starting material. Apparently in the monomer case, the TBDPS group proved to be too bulky for additional substitution at the 4 position. However, protecting diol 89 with TBSCl followed by gallolation did give the desired tetragallolated species 93. The TBS protecting group from 93 was then removed with TBAF treatment and similarly to the dimer case (Figure 2.8), a galloyl transfer occurred from position 4 to position 6 to give 94. The \textit{\textit{1}H} NMR spectrum of 94 shows only three protons between 6.18-5.65 ppm corresponding to those on C1, C2, and C3. This observation suggests that the galloyl group has been transferred to the alcohol at C6 (see discussion at the end of section 3). The newly formed alcohol 94 was then coupled using DCC with acid 76 to produce azide 95.
Azide 95 was then combined with one molecule of fluorescein alkyne 72 utilizing microwave heating to give triazole 96 (Figure 2.26). Hydrogenation of perbenzyloxy 96 gave the polyphenol 97. Monomeric 97 was found to have similar fluorescent properties to fluorescein: $\lambda_{em} = 513$ nm ($\lambda_{ex} = 490$ nm) in a pH = 9 phosphate buffer.

Figure 2.25: Synthesis of azide gallotannin monomer 95.
Figure 2.26: Synthesis of gallotannin monomer 97.

7 Synthesis of Model Ellagitannin/Gallotannin Analogue Dimer 23 and Penta-O-Galloyl-β-D-Glucose 16.
In order to validate the biological data of the fluorescent tannins 85, 86, and 97, their non-fluorescent counterparts (dimer 23 and monomer 16) were also synthesized. Two different syntheses of model dimer 23 have been reported.82, 90 The first route involves a biomimetic Diels-Alder dimerization of orthoquinone 100 catalyzed by B(OAc)₃ (Figure 2.27).90 Orthoquinone 100 was made from the coupling of tetrargallolated alcohol 82 (its synthesis is shown in Figure 2.22) and acyl chloride 98 followed by TBAF-mediated deprotection and ortho-chloranil oxidation of 99. Acyl chloride 98 was made in three steps from methyl 3-benzyloxy-4,5-dihydroxybenzoate (31). The cycloaddition dimer product of 100 was then subjected to base-induced elimination, reduction of the orthoquinones, and benzylation of the resulting catechols for purification purposes to give 65. Hydrogenation of 65 gave 23. Unfortunately, it was later found that the transformation of 100 to 65 usually occurs in unsatisfactory overall low yield (<10% overall) and the 65 obtained often was contaminated with a mixture of inseparable impurities.82
Figure 2.27: Synthesis of model dimer tannin 23 via hetero Diels-Alder dimerization of orthoquinone 100.

The second synthesis, which proved more reliable, involved the use of the advanced intermediate 47. Tetraol 47 was gallolated with four equivalents of the gallic acid derivative 45 and then fully hydrogenated to afford the gray solid polyphenolic model dimer 23 (Figure 2.28). This route was used for making 23 for the biological studies discussed in Chapter 3. Penta-O-galloyl-β-D-glucose 16 was synthesized by complete debenzylation of pentakis-O-(tri-O-benzylgalloyl)-β-D-Glucose 81 (Figure
Pentakis-\(O\)-(tri-\(O\)-benzylgalloyl)-\(\beta\)-D-Glucose 81 was made according to Armitage and co-workers\(^{108}\) (Figure 2.22).

![Figure 2.28: Synthesis of model dimer tannin 23 via advanced intermediate 47.](image)

Figure 2.28: Synthesis of model dimer tannin 23 via advanced intermediate 47.

![Figure 2.29: Synthesis of model \(\beta\)-PGG 16.](image)

Figure 2.29: Synthesis of model \(\beta\)-PGG 16.

8 Conclusions.

Three fluorescent tannin analogues (two monomers and one dimer) were synthesized in order to provide probes for the biological mechanism-of-action of tannins. The synthesis of the dimeric ellagitannin/gallotannin 86 proved especially challenging. Initially, three differently protected linker amines (Teoc (59), Cbz (62), Boc (68)) were tested in their coupling to fluorescein isothiocyanate (FITC) after removal of the protecting groups, but all attempts were unsuccessful. Compound 86 was eventually
made by coupling a dimeric tannin linked azide 77 with a fluorescein alkyne derivative (72) via a [3+2]-cycloaddition using a modification to the Sharpless Cu(I)-catalyzed protocol.97 The Huisgen cycloaddition was followed by hydrogenolysis for removal of several benzyl protecting groups to expose the molecule’s multiple phenolic groups. The monomers (85 and 97) were also synthesized following a similar route.
Chapter 3 – Biological Studies of Some Tannin Analogues.

1 Purpose of the biological tests

The current thinking now appears to be that tannins impose their anticancer activity through the up-regulation of the immune system, in particular the induction of TNF-α secretion by hPBMC’s (see Chapter 1). Based on some preliminary unpublished data with anti-CD14 antibodies (MY4) and TLR4-defective mice (C3H/HeJ), it has become apparent that perhaps tannins achieve their action through the well-known LPS receptor system. The use of synthetic fluorescently labeled tannin analogues will allow a better understanding of the specific location of the cell receptor that binds tannins and the mechanistic details by which tannins stimulate hPBMC’s.

2 Finding Responsive Cell Lines

Among the first goals for the biological studies was to establish cell lines from currently commercially available types that were responsive to the synthesized tannins. In addition, the development of a working protocol for these tests was needed. For all biological tests described here, a commercially available enzyme-linked immunosorbent assay (ELISA) kit for human or mouse TNF-α was used. Initial testing of the model tannin dimer 23 performed in cooperation with William J. Scheuchenzuber director of The Hybridoma and Cell Culture Laboratory showed in several tests that model dimer 23
was unable to elicit TNF-α from the human monocyte cell lines: THP-1, TUR, and U937 at either 3 or 24 hours (Figure 3.2). In fact, TUR and U937 were not activated even by LPS, whereas THP-1 was. THP-1 was also found unresponsive to coriariin A (1) at 24 h (Figure 3.3). This tannin was capriciously insoluble in the cell media and on occasions required stirring in warm water for long periods of time. Therefore the tannin was dissolved in a small amount of DMSO (<1% of total volume) before further diluting it in the cell media. With the human cell lines in general, no noticeable difference was noticed in blank runs with and without DMSO.

Figure 3.1: Structures of coriariin A (1) and model dimeric tannin 23.
Figure 3.2: TNF-α (pg/mL) excreted by human cell lines in response to tannin 23 at various concentrations (one run for each data point).

![Bar chart showing TNF-α excretion by human cell lines in response to tannin 23 at various concentrations.]

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Tannin 10 uM</th>
<th>Tannin 25 uM</th>
<th>Tannin 50 uM</th>
<th>Tannin 100 uM</th>
<th>LPS 10 ug/mL</th>
<th>Control w/ DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>THP-1</td>
<td>![Bars]</td>
<td>![Bars]</td>
<td>![Bars]</td>
<td>![Bars]</td>
<td>![Bars]</td>
<td>![Bars]</td>
</tr>
<tr>
<td>TUR</td>
<td>![Bars]</td>
<td>![Bars]</td>
<td>![Bars]</td>
<td>![Bars]</td>
<td>![Bars]</td>
<td>![Bars]</td>
</tr>
<tr>
<td>U937</td>
<td>![Bars]</td>
<td>![Bars]</td>
<td>![Bars]</td>
<td>![Bars]</td>
<td>![Bars]</td>
<td>![Bars]</td>
</tr>
</tbody>
</table>

Figure 3.3: TNF-α (pg/mL) excreted by THP-1 in response to coriariin A (1) at various concentrations after 24 h (one run for each data point).

![Bar chart showing TNF-α excretion by THP-1 in response to coriariin A (1) at various concentrations.]

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Tannin 10 uM</th>
<th>Tannin 25 uM</th>
<th>Tannin 50 uM</th>
<th>Tannin 100 uM</th>
<th>LPS 10 ug/mL</th>
<th>Control w/ DMSO</th>
</tr>
</thead>
<tbody>
<tr>
<td>THP-1</td>
<td>![Bars]</td>
<td>![Bars]</td>
<td>![Bars]</td>
<td>![Bars]</td>
<td>![Bars]</td>
<td>![Bars]</td>
</tr>
</tbody>
</table>

Gratifyingly, further studies found that 23 was able to elicit TNF-α from the mouse macrophage lines P388, RAW264, and J744A at 24 hours, whereas the mouse
monocyte cell line Wehi-3B was completely inactive (Figures 3.4 and 3.5). After 3 hours, the tannin did not significantly stimulate the P388, RAW264, and J774 cells. The Wehi-3B cells also were unresponsive to LPS. However, with the P388, RAW264, and J774 cell lines, LPS elicited TNF-α secretion starting from 3 h. Thus, based on the cell lines tested, it appears that monocytes, whether human or murine, are unresponsive to the tannin 23. Apparently, these monocyte cells, which are the cellular precursor of macrophages, lack a receptor or some protein component to bind 23 or induce a response to the binding of 23. Another interesting observation is that THP-1 was responsive to LPS but not 23. This observation may indicate that different receptors are utilized in the THP-1 cells for tannin 23 and LPS. Furthermore, DMSO seemed to only slightly increase the amount of TNF-α released, but in most cases this increase was not very significant. In all of the studies with the mouse macrophage cell lines, the control was found to be consistently high. It appears that these cells naturally secrete TNF-α without stimulation.

Figure 3.4: TNF-α (pg/mL) excreted by murine cell lines in response to tannin 23 at various concentrations (one run for each data point).
Further experiments with the J774 cell line produced the time course in Figure 3.6. The model gallotannin 23 (50 μM) elicited TNF-α release after only 6 hrs and the TNF-α amount continued to rise for the length of the experiment. At 48 hours, 23 even caused a greater discharge than LPS. Again, the control amount of TNF-α was fairly high during the experiment. A simple washing of the cells 1 h before the experiment with fresh media was found to be an easy solution for this problem (Figure 3.7). Repeating the experiment after the pre-wash led to a different result: the control was very low for the duration of the experiment. Moreover, at a concentration of 25 μM, the tannin elicited a good response with over 1000 pg/mL of TNF-α after 36 hours and this value continued to increase for the rest of the time course. The LPS caused a rapid increase in TNF-α production after 3 hours and leveled off at about 3300 pg/mL of TNF-α for the remainder
of the experiment. A concentration profile for tannin 23 at 36 hours showed a concentration dependent response for TNF-α production (Figure 3.8).

![Figure 3.6: Time course with J774 for model dimer 23 (50 µM) and LPS (1 µg/mL). Data points were collected in triplicates.](image)

![Figure 3.7: Time course with J774 for model dimer 23 (25 µM) and LPS (1 µg/mL) after a pre-wash of cells. Data points were collected in triplicates.](image)
According to a recent report, THP-1 cells can be incubated with phorbol 12-myristate 13-acetate (PMA) to promote the maturation of the monocyte cells into a more macrophage-like stage. Since it was preferable to perform biological tests on a human cell line and there wasn’t a human macrophage line available, this protocol presented a good opportunity to gain access to a responsive human cell line. Thus, the model dimer 23 was tested once again with THP-1 cells that had been pre-exposed to PMA. The morphology (flatter and more adherent) of these cells after a 48 hour incubation with PMA appeared to be more macrophage-like than before. A survey of possible harvesting methods found that the best results were obtained when cells were pre-treated with PMA 48 hours prior to the experiment, despite considerably high basal levels of TNF-α. Pretreatment 24 hours prior to the experiment or adding simultaneously the stimulant (LPS or tannin 23) and PMA was less satisfactory. However, after numerous attempts,
the THP cell line (pretreated with PMA) was found too unpredictable. Inexplicably, in some runs the control’s TNF-α value was often unreasonably high, while in others the model tannin 23 had no appreciable response.

3 Testing Model Fluorescent Dimer 86.

Figure 3.9: Structure of fluorescent dimer 86.

Having identified a suitable cell line and protocol for these experiments, the synthesized fluorescent tannins were next examined. Unfortunately, subjecting J744 cells to the model fluorescent ellagitannin/gallotannin dimer 86 gave some disappointing results. LPS was found to stimulate the cells greatly, whereas the control was very low (Figure 3.10). The model dimer 23 was very active with this cell line and almost as potent as LPS, stimulating around 2400 pg/mL of TNF-α at 24 and 48 hours. However, the fluorescent dimer 86 was only slightly above the control with around 300 pg/mL of TNF-α released. A second run of the experiment a few months later with a different batch of
the fluorescent dimer 86 delivered similar results (Figure 3.11). The tannin 86 again only stimulated the release of a very low amount of TNF-α, a result which was indistinguishable from the blank runs. Apparently, adding the linkers with attached fluorescein altered the structure enough that it was no longer active. Perhaps the critical peripheral galloyl groups are not accessible to the cell receptor by this alternation. Interestingly, this time the model dimer 23 was much less activate than LPS, although still above basal levels. This result may have occurred because this particular tannin batch was several months old at the time. In both experiments, tannin 23 stimulated the cells significantly after only 6 hours, which had not been noticed before.

![Graph](image)

Figure 3.10: Time course with J744 cells for fluorescent dimer 86. Concentrations of stimulants: tannins 23 and 86 (25 μM); LPS (1 μg/mL). Data points were collected in triplicates.
Figure 3.11: A second run of the time course experiment with J744 for fluorescent dimer 86. Concentrations of stimulants: tannins 23 and 86 (25 µM); LPS (1 µg/mL). Data points were collected in triplicates.
Testing Model Fluorescent Model Monomers 85 and 97.

Figure 3.12: Structures of fluorescent monomeric gallotannins 85 and 97 and β-PGG (16).

Treating J744 cells with fluorescent monomer 85 led to gratifying results. These cells when subjected to tannin 85 was not very active for the first 24 h, but excreted almost 850 pg/mL of TNF-α after 48 h (Figure 3.13). A freshly made batch of β-PGG (16) produced a very similar time course profile to that of 85. There was little TNF-α excreted before 48 h, but at 48 h there was an increased of TNF-α release to around 850 pg/mL. The cells that were incubated with LPS also appeared to be differentiated to a more macrophage-like stage as they were more spread out and more adherent. This observation was noticed in several other experiments for LPS treated cells.
Figure 3.13: Time course experiment with J744 for new batch of β-PGG (16). Concentrations of stimulants: tannins 16 (50 µM); LPS (1 µg/mL). Data points were collected in triplicates.

In a different experiment, J744 cells were treated independently with either fluorescent monomer 85, fluorescent monomer 97, β-PGG (16), LPS, or media (Figure 3.14). Once again the fluorescent gallotannin 85 was moderately active, leading to the production of close to 400 pg/mL of TNF-α at 48 h. β-PGG (16) was found to induce the secretion of similar amounts of TNF-α during the same time points. Although slightly lower, the fluorescent gallotannin 97 was also above the blank at 48 hours with around 225 pg/mL of the TNF-α secreted. LPS at a concentration of 1 µg/mL rapidly reached a plateau of 2500 pg/mL and remained there for the entirety of the experiment (data not shown). Lowering the concentration of the tannins to 25 µM also lowered the TNF-α output in an apparent dose dependent manner, albeit differently for each tannin (Figure 3.15). Tannin 85 experienced a decrease to about 275 pg/mL of TNF-α, and β-PGG (16)
decreased dramatically to just above 100 pg/mL, whereas tannin 97 remained about the same.

Figure 3.14: Time course experiment with J744 for fluorescent monomers 85 and 97. Concentrations of stimulants: tannins 85, 97, and 16 (50 µM). Data points were collected in triplicates.
Figure 3.15: Time course experiment with J744 for fluorescent monomers 85 and 97. Concentrations of stimulants: tannins 85, 97, and 16 (25 µM). Data points were collected in triplicates.

5 Conclusions.

In these *in vivo* experiments, the murine macrophage cell line J744 was found to be a viable experimental platform. In the process, an experimental protocol was developed for examining the secretion of TNF-α by these cells in response to tannin stimulation. A time course using the model dimer 23 revealed that the tannin elicited significant amounts of TNF-α after only 6 hours. Varying the concentration of 23 showed a dose dependency for the amount of TNF-α excreted. Unfortunately, the fluorescently dimeric tannin 86 was found to be completely inactive towards these cells. However, the fluorescent monomeric gallotannins 85 and to a lesser extent 97 were found to be capable of inducing TNF-α output from J744 cells. The tannin 85 was found to have a very similar time course to β-PGG (16) at a concentration of 50 µM.
Future work will involve the use of confocal microscopy using fluorescent monomer analogue 85 to identify the exact location of the cell receptor that binds tannins. Furthermore, competitive binding studies will be performed between tannin 85 and added LPS for the putative common receptor in J744 cells. These experiments perhaps will help validate the hypothesis that tannins exhibit their effect through the same receptor system as LPS.
Chapter 4 – Lihouidine: Background and Synthesis

1 Isolation and Biological Activity of Lihouidine

Lihouidine (102) was isolated from a new crimson marine sponge species of the genus *Suberea* and family Aplysinellidae at the Lihou reef in the Coral Sea near Australia.\textsuperscript{112} The novel structure of this marine alkaloid has been recognized elsewhere in the literature among other marine natural products from 2004.\textsuperscript{188, 189} It is composed of 2 aaptamine (103) units joined by a six-membered ring that contains a spiro center. Lihouidine is a blood-red, crystalline, optically inactive, and cytotoxic fused polyaromatic alkaloid pigment. Despite limited solubility in the cell media, lihouidine exhibited IC\textsubscript{50} = 3 \(\mu\)g/mL (5\(\mu\)M) against P388D1 mouse lymphoma cells. Aaptamine, a potent \(\alpha\)-adrenoceptor blocker, was first isolated from an Okinawan marine sponge *Aaptos aaptos* and has attracted the interest of several synthetic groups.\textsuperscript{113-115}

![Figure 4.1: Structure of lihouidine and aaptamine](image)

Figure 4.1: Structure of lihouidine and aaptamine
2 Retrosynthesis of Lihoudine

The retrosynthetic analysis of lihouidine can be seen in Figure 4.2. Lihoudine was envisioned as coming from bis orthoquinone 104 (or their equivalents). In the forward direction, two consecutive Michael additions of the β-ketoamide enolate of 104 with its orthoquinone units, followed by methylation, would afford 1. The β-ketoamide 104 could come from ester 106 and acetamide 105, which could in turn come from aniline 108. Compound 106 was initially seen as coming from uncyclized 107 via an electrocyclization, tautomerization, and air oxidation cascade. This transformation will be discussed in more detail bellow in section 3.3. Methyl ester 107 was envisioned as coming from the union of aniline 108 and known chloride 109. The aniline 108 could be derived from acid 112 through 4-quinolone 111, whereas chloride 109 could be made from commercially available isatin 110.
Figure 4.2: Retrosynthesis of lihouidine.

3 Synthesis of Dibenzyl Protected 106

Initially, use of a dibenzyl protected quinoline 108 was envisioned as the best option in the synthesis because of the ease of deprotection at a later stage in the synthesis. Thus, progress towards the synthesis of 102 began with commercially available catechol 115.
3.1 Attempt at the Synthesis of the Dibenzyl Protected 4-Quinolone 111a

In their synthesis of aaptamine, Kelly and co-workers reported that veratrole (113) could be aminated through an ortho-lithiation followed by addition of the resultant anion to trimethylsilylmethyl azide (Figure 4.3).\textsuperscript{117, 154} Summiting 2,3-dibenzyloxy catechol (116) to similar conditions failed to furnish the desired aniline 117 (Figure 4.4). As a workaround, a two step process was utilized instead (Figure 4.5).

![Figure 4.3: Kelly’s route to 2,3-dimethoxy aniline (114).](image)

![Figure 4.4: Attempt at using Kelly’s chemistry to make aniline 117.](image)

Acid 118 was first fully benzylated and then the intermediate benzyl ester was hydrolyzed to afford acid 120.\textsuperscript{148} Curtius rearrangement\textsuperscript{116} of acid 120 with diphenyl phosphoryl azide in ethanol, followed by hydrolysis of the resulting carbamate 121, led to 2,3-dibenzyloxy aniline (117). Michael addition of 117 to methyl propiolate, followed by heating the derived vinylogous carbamate 122 at 250 °C, did not lead to the desired cyclization product 111a.\textsuperscript{117, 118} Heating 122 in polyphosphoric acid at 120 °C also did not lead to the desired cyclization to give 111a.\textsuperscript{149}
3.2 Synthesis of the Cyclization Precursor 107

The cyclization step to obtain 4-quinolone 111 might be achieved by starting with a dimethoxy version of 122, based on the work of Kelly et al.\textsuperscript{117} Although the ortholithiation chemistry shown in Figure 4.3 could be reproduced, it was soon abandoned as a first step in the synthesis due to the high cost of the trimethylsilylmethyl azide reagent. Instead, treatment of commercially available 2,3-dimethoxy benzoic acid (123) with diphenyl phosphorphy azide in ethanol followed by hydrolysis of the intermediate carbamate 124 with potassium hydroxide was used to afford 2,3-dimethoxy aniline (114). Michael addition of aniline 114 to methyl propiolate\textsuperscript{117, 118} or the derivative of Meldrum’s acid 131\textsuperscript{119} and refluxing either of the vinylogous carbamates 126 or 127 at 250°C yielded quinolone 111b. Treatment of 4-quinolone 111b with POCl\textsubscript{3} afforded chloride 128.\textsuperscript{117} Although chloride 128 had previously been synthesized,\textsuperscript{117} this approach
represents a more efficient and inexpensive route. Since the removal of the methyl ether
protecting groups later in the synthesis was anticipated to be challenging, they were
exchanged\textsuperscript{7} for the easier-to-remove benzyl ethers to make chloride 129. Attempts to
remove the methyl ethers in 128 with BBr\textsubscript{3} at -78 °C led to lower yields (53%) for 129.

Figure 4.6: Synthesis of chloride 129.
Nucleophilic aromatic substitution of chloride 129 with n-methylamine in various solvents was next surveyed (Table 4.1). Heating chloride 129 in DMF and 40% NH₂CH₃ in H₂O led to low yields of the desired methyl aniline 108a due to an unexpected addition of dimethylamine contributed by the decomposition of DMF to yield N,N-dimethyl aniline 132 (entry 1). Heating chloride 129 in DMF using a microwave reactor led to increased yields of aniline 108a (57%), although dimethyl aniline 132 was still being isolated (entry 2). Ethanol as a solvent only led to recovery of starting material with conventional heating and proved to be a slow reaction media for this transformation in the microwave as it took 2 hours for the reaction to go to completion (entries 3-5). In phenol and toluene, no reaction occurred (entries 6 and 7). The best solvent was found to be n-methylformamide, as aniline 108a was obtained in 68% utilizing microwave heating (entry 8).

![Chemical structures](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Time</th>
<th>Conditions</th>
<th>Yield of 108a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DMF</td>
<td>16 h</td>
<td>160 °C, sealed tube</td>
<td>44% + 42% of 132</td>
</tr>
<tr>
<td>2</td>
<td>DMF</td>
<td>10 min</td>
<td>150 °C, MW</td>
<td>57% + ~10% of 132</td>
</tr>
<tr>
<td>3</td>
<td>ethanol</td>
<td>20 h</td>
<td>150 °C, sealed tube</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>4</td>
<td>ethanol</td>
<td>12 min</td>
<td>140 °C, MW</td>
<td>trace, SM recovered</td>
</tr>
<tr>
<td>5</td>
<td>ethanol</td>
<td>2 h</td>
<td>130 °C, MW</td>
<td>68%</td>
</tr>
<tr>
<td>6</td>
<td>phenol</td>
<td>12 min</td>
<td>150 °C, MW</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>7</td>
<td>toluene</td>
<td>36 h</td>
<td>160 °C, sealed tube</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>8</td>
<td>n-methylformamide</td>
<td>12 min</td>
<td>150 °C, MW</td>
<td>68%</td>
</tr>
</tbody>
</table>

Table 4.1: Conditions for displacement of the chloride in 129.
Quinoline 109 was made by modification of a known route from isatin as shown in Figure 4.7. Isatin (133) was heated with malonic acid in acetic acid to produce 2-hydroxycinchoninic acid (134). Heating 2-hydroxycinchoninic acid in POCl₃ followed by addition of methanol produced chloride 109a. Similarly, heating 134 in POBr₃ followed by methanol addition yielded bromide 109b.

![Synthesis of chloride 109a and bromide 109b.](image)

Next, the coupling of aniline 108a and the activated 2-chloroquinoline 109a or 2-bromoquinoline 109b was explored. The palladium-catalyzed tin-free aryl amination reaction (Buchwald-Hartwig reaction) is a powerful transformation that has been developed to include the construction of arylamines. Ever since its discovery, this field of research has been very active.

Simply heating aniline 108a and bromide 109b in refluxing ethanol led to the transesterification of the methyl ester in 109b via nucleophilic attack of the solvent (Table 4.2, entry 1). Thus, various Buchwald-Hartwig aryl aminations conditions with either Pd(OAc)₂ or dipalladium tris(benzylideneacetone) (Pd₂dba₃) as the palladium (0) source were screened under both conventional and microwave heating. The bulky and electron-rich 2-(dicyclohexylphosphino)biphenyl (135, DCPB) as a ligand was used
in all trials.\textsuperscript{156} It was found that using NaOtBu was incompatible with the labile methyl ester functionality of the quinoline halides (entry 4). Therefore, the more tolerant K$_3$PO$_4$ was used instead (entries 5-10).\textsuperscript{156} Most conditions failed to produce significant quantities of the desired adduct 107a, but eventually persistence paid off. Heating chloride 109a and aniline 108a in refluxing toluene for two days in the presence of Pd(OAc)$_2$ yielded the coupled product 107a in 10-32\% yield. Surprisingly, bromide 109b under similar conditions afforded only trace amounts of 107a (entry 8). Since bromides usually are known to be better coupling partners than chlorides, this unexpected result is not easy to explain. Switching from Pd(OAc)$_2$ to Pd$_2$dba$_3$ as a catalyst for this transformation greatly improved the yields (to 60\%) when using chloride 109a (entry 9). Irradiation in a microwave,\textsuperscript{157} did manage to lower reaction times greatly to 3 h, but the improvement was not enough to warrant a switch due to the mass throughout limitations of the microwave reactor (entry 10).
Table 4.2: Coupling conditions for aniline 108a and halides 109a and 109b.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Halide</th>
<th>Pd source</th>
<th>Base</th>
<th>Solvent</th>
<th>Conditions</th>
<th>Time</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>109b</td>
<td></td>
<td>ethanol</td>
<td>reflux</td>
<td>4 d</td>
<td></td>
<td>0%, transesterification</td>
</tr>
<tr>
<td>2</td>
<td>109b</td>
<td>Pd(OAc)₂</td>
<td>NaOtfBu</td>
<td>Toluene</td>
<td>MW, 100 °C</td>
<td>10 min</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>3</td>
<td>109b</td>
<td>Pd(OAc)₂</td>
<td>NaOtfBu</td>
<td>Toluene</td>
<td>MW, 150 °C</td>
<td>30 min</td>
<td>0%</td>
</tr>
<tr>
<td>4</td>
<td>109a</td>
<td>Pd₂dba₃</td>
<td>NaOtfBu</td>
<td>Toluene</td>
<td>80 °C</td>
<td>4 d</td>
<td>0%, only amine recovered</td>
</tr>
<tr>
<td>5</td>
<td>109a</td>
<td>Pd(OAc)₂</td>
<td>K₃PO₄</td>
<td>mesitylene</td>
<td>150 °C</td>
<td>36 h</td>
<td>3%</td>
</tr>
<tr>
<td>6</td>
<td>109a</td>
<td>Pd(OAc)₂</td>
<td>K₃PO₄</td>
<td>Toluene</td>
<td>MW, 150 °C</td>
<td>2 h</td>
<td>trace</td>
</tr>
<tr>
<td>7</td>
<td>109a</td>
<td>Pd(OAc)₂</td>
<td>K₃PO₄</td>
<td>Toluene</td>
<td>110 °C</td>
<td>2 d</td>
<td>10-32%</td>
</tr>
<tr>
<td>8</td>
<td>109b</td>
<td>Pd(OAc)₂</td>
<td>K₃PO₄</td>
<td>Toluene</td>
<td>110 °C</td>
<td>2 d</td>
<td>trace</td>
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<tr>
<td>9</td>
<td>109a</td>
<td>Pd₂dba₃</td>
<td>K₃PO₄</td>
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<td>110 °C</td>
<td>2 d</td>
<td>60%</td>
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<td>10</td>
<td>109a</td>
<td>Pd₂dba₃</td>
<td>K₃PO₄</td>
<td>Toluene</td>
<td>MW, 150 °C</td>
<td>3 h</td>
<td>40% + SM recovered</td>
</tr>
</tbody>
</table>

3.3 Electrocyclization Attempts on Precursor 107a

Initially a plausible pathway to lihoudine was speculated to involve the cyclization sequence shown in Figure 4.8. Protonation of 107 might be followed by an electrocyclization, tautomerization, and finally air oxidation cascade to make cyclization product 106. A sequence such as the one illustrated in Figure 4.8 has not been proposed before. However, Mullen and co-workers reported that a molecule such as 138 undergoes a mild base-promoted (either K₂CO₃/ethanolamine or sodium tert-butoxide/1,5-diazabicyclo[4.3.0]non-5-ene (DBN)) cyclization to give 140 (Figure 4.9). Although,
the authors did not speculate on the possible mechanism, under the basic conditions it is feasible that this reaction might go through intermediate 139. Indeed, intermediate 139 might yield 140 via an electrocyclization followed by an air oxidation similar to that proposed in Figure 4.8. However, an earlier report offers a different mechanistic perspective for the base-catalyzed dimerization of 141 to afford 142 using the NaOtBu/DBN system, which does not include an electrocyclization step. Nevertheless, compound 107a was subjected to a variety of acidic conditions under an aerobic atmosphere as shown in Tables 4.3 and 4.4.

![Proposed biomimetic cyclization sequence towards 106.](image)

Figure 4.8: Proposed biomimetic cyclization sequence towards 106.
Figure 4.9: Mild base-promoted cyclizations of 138 as a possible precedent for the electrocyclization step.

All attempts to cyclize compound 107a under thermal conditions (Table 4.3) mostly led to the recovery of starting material. In a few cases, removal of the benzyl protecting group (entries 9 and 14) or decomposition (entry 7) was observed. A palladium mediated cyclization (entry 7, DCPB = 2-(dicyclohexylphosphino)biphenyl) was also attempted, but this approach only led to decomposition. This entry suggests that the
Buchwald-Hartwig coupling reaction used to make 107a should be kept completely free of air to avoid product decomposition (Table 4.2). Disappointedly, photochemical conditions (Table 4.4) under all wavelengths tested (350, 300, and 254 nm) also led to recovery of mostly starting material.

\[
\begin{align*}
\text{BnO} & \quad \text{OBn} \\
\text{N} & \quad \text{N} \\
\text{CO}_2\text{Me} & \quad \text{air atmosphere} \\
\text{Me} & \quad \text{N} \\
\text{OBn} & \quad \text{BnO}
\end{align*}
\]

Table 4.3: Some thermal, acid-mediated electrocyclization attempts of compound 107a.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Solvent</th>
<th>Temperature</th>
<th>Time</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>SiO\textsubscript{2}</td>
<td>CH\textsubscript{2}Cl\textsubscript{2}</td>
<td>rt</td>
<td>16 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>2</td>
<td>TFA</td>
<td>CH\textsubscript{2}Cl\textsubscript{2}</td>
<td>rt</td>
<td>16 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>3</td>
<td>TFA</td>
<td>CH\textsubscript{2}CN</td>
<td>Reflux</td>
<td>2 d</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>4</td>
<td>AcOH</td>
<td>CH\textsubscript{2}CN</td>
<td>rt</td>
<td>20 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>5</td>
<td>AcOH</td>
<td></td>
<td>75 °C</td>
<td>24 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>6</td>
<td>AcOH</td>
<td></td>
<td>100 °C</td>
<td>3 d</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>7</td>
<td>Pd(OAc)\textsubscript{2}, DCPB</td>
<td>toluene</td>
<td>Reflux</td>
<td>3 d</td>
<td>0%, decomposition</td>
</tr>
<tr>
<td>8</td>
<td>TFA, sealed tube</td>
<td>CH\textsubscript{3}CN</td>
<td>150 °C</td>
<td>18 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>9</td>
<td>TFA, MW, 300W</td>
<td>CH\textsubscript{3}CN</td>
<td>150 °C</td>
<td>3 h</td>
<td>0%, loss of benzyls</td>
</tr>
<tr>
<td>10</td>
<td>AcOH, sealed tube</td>
<td>toluene</td>
<td>160 °C</td>
<td>16 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>11</td>
<td>AcOH</td>
<td>Ph\textsubscript{2}O</td>
<td>155 °C</td>
<td>16 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>12</td>
<td>AcOH, sealed tube</td>
<td>mesitylene</td>
<td>200 °C</td>
<td>16 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>13</td>
<td>Sc(OTf)\textsubscript{3}</td>
<td>CH\textsubscript{3}CN</td>
<td>rt</td>
<td>40 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>14</td>
<td>Sc(OTf)\textsubscript{3}, sealed tube</td>
<td>CH\textsubscript{3}CN</td>
<td>100 °C</td>
<td>20 h</td>
<td>0%, loss of benzyls</td>
</tr>
</tbody>
</table>

Table 4.4: Some photochemical, acid-mediated electrocyclization attempts of compound 107a.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Solvent</th>
<th>Wavelength</th>
<th>Time</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TFA</td>
<td>CH\textsubscript{3}CN</td>
<td>350nm</td>
<td>20 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>2</td>
<td>TFA</td>
<td>CH\textsubscript{3}CN</td>
<td>300nm</td>
<td>16 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>3</td>
<td>TFA</td>
<td>CH\textsubscript{3}CN</td>
<td>254nm</td>
<td>16 h</td>
<td>0%, SM recovered</td>
</tr>
</tbody>
</table>
4 Synthesis of the Dimethyl Protected 106

After the initial disappointing results for the cyclization of 107a, the dimethyl version of 107, which would allow an easier interpretation of spectral data when the time came to examine other more indirect methods to cyclize 107, was prepared next. This rerouting of the synthesis, therefore, would mean reassigning the potentially challenging task of deprotecting the methyl ethers to later step in the synthesis. Similarly to the dibenzyl protected chloride 129, nucleophilic aromatic substitution of chloride 128 with n-methylamine under microwave irradiation or conventional heating in a sealed tube afforded aniline 108a (Figure 4.10). As expected, coupling of aniline 108b and chloride 109a gave cyclization precursor 107b.

Figure 4.10: Synthesis of dimethoxy quinoline 107b.

4.1 Electrocyclization Attempts on Compound 107b

Similarly to compound 107a, compound 107b was also subjected to a variety of acidic conditions under an air atmosphere in an attempt to cyclize it to 106b, as seen in
Table 4.5. In most cases, the starting material was recovered unaltered, except when 107b was heated in the acid solution. In these cases, ester hydrolysis occurred without cyclization (entries 2-4).

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Solvent</th>
<th>Temp.</th>
<th>Time</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>H₂SO₄</td>
<td>rt</td>
<td>16 h</td>
<td>0%</td>
<td>SM recovered</td>
</tr>
<tr>
<td>2</td>
<td>H₂SO₄</td>
<td>70 °C</td>
<td>14 h</td>
<td>0%</td>
<td>ester hydrolysis</td>
</tr>
<tr>
<td>3</td>
<td>MW, 300W</td>
<td>H₂SO₄</td>
<td>100 °C</td>
<td>10 min</td>
<td>0%, ester hydrolysis</td>
</tr>
<tr>
<td>4</td>
<td>MW, 300W</td>
<td>CH₃SO₃H</td>
<td>100 °C</td>
<td>10 min</td>
<td>0%, ester hydrolysis</td>
</tr>
<tr>
<td>5</td>
<td>4 equiv AlCl₃</td>
<td>CH₃CN</td>
<td>rt</td>
<td>16 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>6</td>
<td>4 equiv ZnBr₂</td>
<td>CH₃CN</td>
<td>rt</td>
<td>16 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>7</td>
<td>4 equiv TiCl₄</td>
<td>CH₂Cl₂</td>
<td>rt</td>
<td>14 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>8</td>
<td>4 equiv TiCl₄</td>
<td>CH₂CN</td>
<td>70 °C</td>
<td>16 h</td>
<td>0%, SM recovered</td>
</tr>
</tbody>
</table>

Table 4.5: Some thermal, acid-mediated electrocyclization attempts of compound 107b.

4.2 Palladium-Mediated Cyclization Attempts on Compound 107b

Palladium mediated couplings on unfunctionalized arenes and olefins, the Fujiwara-Moritani oxidative Heck reaction, have been the subject of the research of several groups. For example, Ishii and co-workers reported that palladium acetate catalyzed an oxidative coupling reaction between arene 143 with ethyl acrylate in the presence of molybdovanadophosphoric acid under atmospheric dioxygen to give 145.¹²²
Figure 4.11: Precedent for the palladium-mediated cyclization.

Compound 107b was subjected to palladium acetate under microwave heating at temperatures ranging from 100-200°C in a variety of polar solvents. However, none of these conditions led to the desired cyclization (Table 4.6). Instead, starting material or non-cyclized products were obtained.
Table 4.6: Some palladium-mediated cyclizations attempts on compound 107b.

4.3 Bromination Attempts on Compound 107b

If compound 107b could be brominated at position 5 of the lower quinoline ring, the resulting bromo compound could serve to close the desired ring via a Heck reaction (Figure 4.12). Perhaps, the electron richness afforded by the two electron donating methoxy groups to the aryl ring in 107b would direct a regioselective bromination.
Trecourt et al. showed that treatment of 7,8-dimethoxyquinoline (147) with bromine and pyridine in refluxing CCl$_4$ leads to mostly bromination at position 5 (148c) (see Figure 4.13). The intramolecular Heck reaction has been well studied. An example was provided by Fagnou and colleagues in their synthesis of allocolchicinoid as chloride 149 was cyclized to 150 (Figure 4.14).

Figure 4.12: General strategy behind bromination reaction.

Figure 4.13: Precedent for bromination reaction.

Figure 4.14: Precedent for Heck reaction.

Subjection of compound 107b to similar brominating conditions also led to a regioselective bromination, not at position 5 (compound 146a), but at position 3 of the
quinoline ring (compound 146b) as shown in Table 4.7 (entries 1 and 2). Based on literature precedent, the use of acidic media should protonate all three nitrogens of 107b, making their respective rings less electron rich (less likely to brominate) and therefore enhance bromination of the desired position 5 (compound 146a).\textsuperscript{129-132} Instead, either recovery of the starting material (entries 3, 4, and 5) or hydrolysis of the methyl ester (entry 6) were observed.

![Chemical structures](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Solvent</th>
<th>Temperature</th>
<th>Time</th>
<th>Result (Yield of 33b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1 equiv Br\textsubscript{2}, 1 equiv pyridine</td>
<td>CCl\textsubscript{4}</td>
<td>reflux</td>
<td>13 h</td>
<td>39% + 60% SM</td>
</tr>
<tr>
<td>2</td>
<td>1 equiv Br\textsubscript{2}</td>
<td>CH\textsubscript{2}Cl\textsubscript{2}</td>
<td>-78 °C</td>
<td>1 h</td>
<td>45% + 52% SM</td>
</tr>
<tr>
<td>3</td>
<td>1.1 equiv NBS</td>
<td>H\textsubscript{2}SO\textsubscript{4}</td>
<td>0 °C to rt</td>
<td>20 h</td>
<td>0%, mostly SM</td>
</tr>
<tr>
<td>4</td>
<td>1.1 equiv Br\textsubscript{2}</td>
<td>H\textsubscript{2}SO\textsubscript{4}</td>
<td>0 °C to rt</td>
<td>12 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>5</td>
<td>1.1 equiv Br\textsubscript{2}</td>
<td>TFA</td>
<td>0 °C to rt</td>
<td>12 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>6</td>
<td>1.1 equiv Br\textsubscript{2}</td>
<td>H\textsubscript{2}SO\textsubscript{4}</td>
<td>60 °C</td>
<td>20 h</td>
<td>0%, ester hydrolysis</td>
</tr>
</tbody>
</table>

Table 4.7: Bromination conditions for compound 107b.

### 4.4 Nitration Attempts on Compound 107b

With the failure of the bromination route, nitration of 107b could offer an alternative if the nitration occurred regioselectively at position 5 of the quinoline ring of compound 107b. According to most literature reports, under the typical acidic conditions of nitrating reactions, the nitrogen is protonated (electron-deficient) and thus impedes nitration in the pyridine ring of quinolines.\textsuperscript{133} Instead, nitration occurs on the benzene
portion, usually at position 5, as shown by the work of Denny and colleagues in the nitration of quinoline 151 (Figure 4.15).134

\[
\text{OMe} \quad \begin{array}{c} \text{HN} \\ \text{N} \end{array} \quad \text{NO}_2 \quad \begin{array}{c} \text{HN} \\ \text{N} \end{array} \quad \text{OMe}
\]

151 \begin{array}{c} \text{1.1 equiv KNO}_3 \\ \text{conc. H}_2\text{SO}_4, 0\degree\text{C}, 15 \text{ min.}
\end{array} \quad 152

Figure 4.15: Precedent for nitration reaction.

However, treatment of the Buchwald-Hartwig adduct 107b with fuming nitric acid in concentrated sulfuric acid lead to an unprecedented nitration/Michael addition cyclization cascade to afford 153a and 153b (Table 4.8, entry 1).135 Apparently, compound 107b is first nitrated on the arene ring, which activates the quinoline ring for a Michael-type addition by the electron rich lower quinoline (Figure 4.16). Deprotonation followed by air oxidation would rearomatize the product to form 153a. Use of potassium nitrate in sulfuric acid to make nitric acid in situ also leads to similar products, but in lower yields (entry 2).134 Screening of other nitrating agents revealed that most conditions did not cause the same transformation, with nearly all attempts returning starting material.136-142 One exemption to this general observation was tetrabutylammonium nitrate in trifluoroacetic anhydride, which yielded compound 154 (entry 10).143, 144 Attempts to optimize reaction conditions led to the observation that methanesulfonic acid also could serve as a solvent (entry 16) with similar yields to the sulfuric acid runs. Gratifyingly, submiting compound 107b to only fuming nitric acid at 0\degree\text{C} for 1 h led to a substantial increase in yield (up to 52%) of 153a with only traces of 153b (entry 17). Stopping the reaction after 10 minutes, led to a decrease in yield (18%) of 153a (entry
18). Diluting the nitric acid completely halted any reaction (entry 19). Treatment of 107b with only concentrated sulfuric acid did not result in any reaction (Table 4.5, entry 1).
<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions</th>
<th>Solvent</th>
<th>Temp</th>
<th>Time</th>
<th>(Yield of 154+153a+153b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.3 equiv fuming HNO₃</td>
<td>H₂SO₄</td>
<td>0 °C</td>
<td>1 h</td>
<td>10%+31%+9%</td>
</tr>
<tr>
<td>2</td>
<td>1.3 equiv KNO₃</td>
<td>H₂SO₄</td>
<td>0 °C</td>
<td>1 h</td>
<td>8%+14%+5%</td>
</tr>
<tr>
<td>3</td>
<td>1.3 equiv fuming HNO₃, 2.6 equiv Ac₂O</td>
<td>CH₂Cl₂</td>
<td>-50 °C</td>
<td>0.5 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>4</td>
<td>2 equiv fuming HNO₃</td>
<td>Ac₂O</td>
<td>-50 °C to 10 °C</td>
<td>3 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>5</td>
<td>2 equiv fuming HNO₃</td>
<td>Ac₂O</td>
<td>0 °C to rt</td>
<td>14 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>6</td>
<td>1.3 equiv fuming HNO₃, 0.1 equiv Yb(OTf)₃</td>
<td>DCE</td>
<td>83 °C</td>
<td>14 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>7</td>
<td>1 equiv NO₂BF₄</td>
<td>sulfolane</td>
<td>rt</td>
<td>1 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>8</td>
<td>4 equiv NO₂BF₄</td>
<td>sulfolane</td>
<td>rt</td>
<td>12 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>9</td>
<td>1 equiv NO₂BF₄</td>
<td>sulfolane</td>
<td>55 °C</td>
<td>14 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>10</td>
<td>1.3 equiv Bu₄NNO₃, 10 equiv TFAA</td>
<td>CHCl₃</td>
<td>0 °C to rt</td>
<td>1 h</td>
<td>71%+0%+0%</td>
</tr>
<tr>
<td>11</td>
<td>1.3 equiv fuming HNO₃</td>
<td>CH₂Cl₂</td>
<td>-78 °C</td>
<td>1 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>12</td>
<td>1.3 equiv fuming HNO₃</td>
<td>CF₃SO₃H</td>
<td>0 °C</td>
<td>1 h</td>
<td>0%, decomp.</td>
</tr>
<tr>
<td>13</td>
<td>1.3 equiv fuming HNO₃, 10 equiv CF₃SO₃H</td>
<td>CH₂Cl₂</td>
<td>-60 °C</td>
<td>14 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>14</td>
<td>1.3 equiv fuming HNO₃, 10 equiv CF₃SO₃H</td>
<td>CH₂Cl₂</td>
<td>rt</td>
<td>14 h</td>
<td>0%, decomp.</td>
</tr>
<tr>
<td>15</td>
<td>1.3 equiv fuming HNO₃</td>
<td>AcOH</td>
<td>rt</td>
<td>4 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>16</td>
<td>1.3 equiv fuming HNO₃</td>
<td>CH₃SO₃H</td>
<td>rt</td>
<td>1 h</td>
<td>0%+22%+5%</td>
</tr>
<tr>
<td>17</td>
<td>fuming HNO₃</td>
<td>0 °C</td>
<td>1 h</td>
<td>12%+52%+0%</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>fuming HNO₃</td>
<td>0 °C</td>
<td>10 min</td>
<td>20%+18%+0%</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>35% HNO₃</td>
<td>rt</td>
<td>16 h</td>
<td>0%, SM recovered</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.8: Some nitrating conditions for compound 107b.
Figure 4.16: Proposed Mechanism for nitration cyclization reaction.

Compound 153a was determined to have the structure as shown based on a correlation in the HMBC of 153a between the singlet at 7.54 ppm in the $^1$H NMR spectrum (labeled A in Figure 4.17)) and two carbons at 146.7 and 150.3 ppm in the $^{13}$C NMR spectrum (labeled B). These carbons have methoxy groups attached, as they correlate with the two singlets (integrate for 3 protons each) at 4.22 and 3.95 ppm in the $^1$H NMR spectrum (labeled C). Both carbons at 146.7 and 150.3 ppm also were found to be quaternary based on a DEPT-135 experiment. Although it is inconsequential to the rest of the synthesis, the nitro group in 153a was assigned to the position shown based on a
NOESY experiment. The methoxy protons from the ester at 3.85 ppm did not exhibit a through space coupling with either the doublet at 9.47 or at 7.76 ppm. However, a NOESY experiment of 153b did show a correlation between the methoxy protons from the ester at 4.19 ppm and the doublet at 8.52 ppm (Figure 4.17). An HMBC of 153b similarly to 153a, showed a correlation of an aromatic singlet (at 7.58 ppm) to the two carbons containing the methoxy groups. Unfortunately, an X-ray crystal structure could not be obtained for either compound. The NMR spectra can be found in section 5.5.

Figure 4.17: HMBC correlation for 153a and NOESY correlation for 153b.

The next step involved the removal of the unwanted nitro groups in 153a and 153b (Figure 4.18). Reduction of the two nitro isomers 153a and 153b with iron metal\textsuperscript{145} followed by a Sandmeyer reaction of the resulting amines 155a and 155b utilizing hypophosphorous acid\textsuperscript{146,147} afforded 106b in 49% yield. Other Sandmeyer-type reagents used to remove the amino group, such as copper (I) oxide (45%) and sodium hydrosulfite as a substitute for hypophosphorous acid (20%), were less successful.\textsuperscript{158,159} The correct bond connection as shown in 106b was determined by HMBC NMR (see section 5.5). The singlet at 7.56 ppm in the $^1$H NMR (labeled A in Figure 4.19) couples to carbons at 143.4 and 152.3 ppm in the $^{13}$C NMR (labeled B). These carbons represent the two
carbons where the methoxy ethers are substituted (both are quaternary carbons based on a DEPT-135 experiment). These two carbons also coupled to the hydrogens on the methoxy groups, which are singlets at 4.14 and 4.03 ppm in the $^1$H NMR, respectively (labeled C). Submitting the dibenzyloxy protected compound 107a to the same conditions did not lead to a similar cyclization cascade as with the dimethoxy substrate 107b, and only decomposition was observed (Figure 4.20). With cyclized 106b in hand, the next task was to join it with acetamide 105b.

![Chemical structures and reactions]

**Figure 4.18**: Sequence for the removal of undesired nitro group.

![HMBC correlation diagram]

**Figure 4.19**: HMBC correlation for 106b.
Figure 4.20: Failed attempt at nitration/cyclization cascade for dibenzyloxy quinoline 107a.

5 Coupling of Dimethoxy Ester 106b and Dimethoxy Acetamide 105b

The acetamide moiety 105b was obtained in good yield from acetylating aniline 108b with acetic anhydride at 100 °C (Figure 4.21). Running this reaction at room temperature, did not lead to the acylated product. Disappointingly, treating acetamide 105b with LDA and adding this solution to methyl ester 106b at room temperature in an attempt to make β-ketoamide 157, only returned the recovered starting material (Figure 4.22). Even heating the enolate of 105b and ester 106b in refluxing THF led to no reaction. Thus, a long quest began to find an alternative solution. Simple addition of the lithium enolate of N,N-dimethylacetamide (158) to methyl ester 109a gave β-ketoamide 159 in quantitative yield. Addition of the enolate of acetamide 105b to methyl ester 109a also successfully made its corresponding β-ketoamide 160. This result suggested that perhaps the problem in coupling 105b and 106b was linked to a lack of reactivity by the ester of 106b.
Figure 4.21: Synthesis of acetamide 105b.

Figure 4.22: Attempted synthesis of β-ketoamide 157 via an LDA enolate.
5.1 Model System with Ester 107b

Deprotonating acetamide 105b with LHMDS and addition to methyl ester 107b yielded 57% of β-ketoamide 161, along with 23% of the ester 107b (Figure 4.23). Increasing the temperature to 60 °C led to a slight yield improvement of 161 (63%). Unfortunately, an attempted union between the LHMDS enolate of acetamide 105b and ester 106b under similar conditions failed once again to give β-ketoamide 157 (Figure 4.24). Even heating the two components together at 102 °C only led to recovered starting material after 3 days. Apparently, the aryl rings to the left of the ester in 106b (as drawn in Figure 4.24) prevent either the enolate from approaching the carbonyl or the carbonyl itself from going through the necessary tetrahedral intermediate.

Figure 4.23: Condensation of acetamide 105b and ester 107b.
Figure 4.24: Attempted condensation of acetamide 105b and ester 106b.

As an alternative strategy, the cyclization of β-ketoamide 161 into 157 was examined. Based on the cyclization of 107b (Table 4.8), β-ketoamide 161 was treated with Bronsted and Lewis acids with the expectation that the more electron-depleted nature of the β-ketoamide might force a cyclization without the need of nitration first. However, all conditions gave decomposition when they were pushed to the limit (Table 4.9). Even submitting β-ketoamide 161 to fuming nitric acid, as had been done with 107b, did not lead to a productive cyclization (entry 5).
Table 4.9: Attempts at cyclizing β-ketoamide 161 under acidic conditions.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagent</th>
<th>Solvent</th>
<th>Conditions</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TiCl₄</td>
<td>CH₂Cl₂</td>
<td>15 h, rt</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>2</td>
<td>TiCl₄</td>
<td>ClCH₂CH₂Cl</td>
<td>16 h, 80 °C</td>
<td>0%, decomp.</td>
</tr>
<tr>
<td>3</td>
<td>TFA</td>
<td>CH₃CN</td>
<td>20 h, rt</td>
<td>0%, decomp.</td>
</tr>
<tr>
<td>4</td>
<td>CH₃SO₃H</td>
<td>CH₃CN</td>
<td>100 °C, 10 min, MW</td>
<td>0%, decomp.</td>
</tr>
<tr>
<td>5</td>
<td>fuming HNO₃</td>
<td></td>
<td>0 °C, 1 h</td>
<td>0%, decomp.</td>
</tr>
</tbody>
</table>

5.2 Model System with a Weinreb Amide

Weinreb amide 164 was obtained by hydrolysis of methyl ester 107b followed by a DCC coupling of the resulting acid 163 with dimethylhydroxyl amine•HCl (Figure 4.25). In addition, Weinreb amide 164 was also obtained by conversion of methyl ester 109a to Weinreb amide 165 catalyzed by AlCl₃, and then coupling 165 to dimethoxy aniline 108b with the use of the already established Buchwald-Hartwig conditions. Weinreb amide 164 was then treated with the LDA enolate of acetamide 105b, but as with the earlier ester attempts, no desired product was obtained (Figure 4.26). In each
case, only the two starting compounds were recovered.

\[
\text{MeO} \quad \text{OMe} \quad \text{N} \quad \text{N} \quad \text{CO}_2 \text{Me} \quad \text{LiOH \cdot H}_2\text{O, THF, H}_2\text{O, 60 °C, 12 h, 55%}}
\]

\[
\text{N} \quad \text{Cl} \quad \text{O} \quad \text{O} \quad \text{MeO} \quad \text{OMe} \quad \text{N} \quad \text{N} \quad \text{CO}_2 \text{H} \quad \text{DCC, Et}_3\text{N, CH}_2\text{Cl}_2, \text{rt, 14 h, 100%}}
\]

\[
\text{107b} \quad \text{163} \quad \text{164}
\]

\[
\text{O} \quad \text{O} \quad \text{(CH}_3\text{)}_2\text{NOH \cdot HCl} \quad \text{N} \quad \text{NO O} \quad \text{Cl} \quad \text{MeO} \quad \text{OMe} \quad \text{N} \quad \text{N} \quad \text{CO} \quad \text{2} \quad \text{H}\text{MeO} \quad \text{OMe} \quad \text{N} \quad \text{NH} \quad \text{(CH}_3\text{)}_2\text{NOH} \quad \text{HCl}
\]

\[
\text{109a} \quad \text{165} \quad \text{108b} \quad \text{164}
\]

Figure 4.25: Synthesis of Weinreb amide 164.

\[
\text{LDA, Dioxane, 0 °C to 102 °C, dioxane, 12 h, 0%, SM recovered}
\]

\[
\text{MeO} \quad \text{OMe} \quad \text{164} \quad \text{105b} \quad \text{161}
\]

Figure 4.26: Attempts at coupling the enolate of 105b with Weinreb amide 164.
5.3 Model System with a Carboxylic Acid

Silyl enol ethers and ketene silyl acetals are well established as enolate surrogates in several reports. Although there are far fewer reports on the synthesis and the chemistry of ketene silyl aminals, the possibility of using such a species was considered as a source of the enolate for acetamide \textsuperscript{105b}.\textsuperscript{185-187} Tanabe and co-workers reported that pentafluorophenylammonium triflate (PFPAT) could catalyze C-acylations of ketene silyl acetal \textsuperscript{167} with acid chloride \textsuperscript{166} to obtain \(\beta\)-ketoester \textsuperscript{168} in good yield (Figure 4.27).\textsuperscript{172} Despite numerous attempts under several conditions, the required ketene silyl aminal \textsuperscript{169} could not be isolated (Table 4.10).\textsuperscript{185-187} In most cases, the acetamide starting material and aniline \textsuperscript{108b} (from the loss of the acetate group) were the only products isolated.

![Figure 4.27: C-acylation of ketene silyl acetals to acyl chlorides catalyzed with PFPAT.](image)

\[
\begin{align*}
\text{Cl}_R & \quad R' \quad \text{OTMS} \\
\text{166} & \quad \rightarrow \\
0.05 \text{ equiv PFPAT} & \quad \text{167} \\
\rightarrow & \quad \text{168} \\
60-92\% & \quad \text{R', R', R''}
\end{align*}
\]
Masamune and co-workers reported that carboxylic acids could be C-alkylated under virtually neutral conditions.\textsuperscript{161} Treatment of carboxylic acids such as 170 with carbonyldiimidazole (171) converted the acid into the corresponding imidazolide 172, which was subjected without isolation to the neutral magnesium salt of methyl malonate (173) to give \( \beta \)-ketoesters such as 175 (Figure 4.28). The reaction apparently goes through intermediate 174, which decarboxylates to furnish 175. Thus, this methodology might be useful towards making the desired \( \beta \)-ketoamide. Aniline 108b was acylated with mono benzyl malonate (176) to afford 177 (Figure 4.29). \( \beta \)-Ketoamide 177 then was debenzylated to furnish \( \beta \)-ketoacid 178, which upon stirring with magnesium ethoxide, was converted to 179. Acid 163 was treated with carbonyldiimidazole, and upon complete consumption of the acid, the mixture was treated with magnesium salt 179.
Unfortunately, the only identifiable compound isolated from the reaction mixture was the starting acid 163.

\[
\begin{align*}
&\text{R} = \text{c-hexyl, 85%} \\
\text{Figure 4.28: Masamune’s } C\text{-acylating procedure.}
\end{align*}
\]
Figure 4.29: Application of Masamune’s methodology towards β-ketoamide 161.

Conversion of acid 163 to its corresponding acyl chloride 182 could not be accomplished utilizing 1-chloro-N,N,2-trimethyl-1-propenylamine (181) in THF,
probably due to the insolubility of the acid 163 (Figure 4.30). However, the transformation occurred cleanly in CH₂Cl₂. Treating acyl chloride 182 with the lithium enolate of acetamide 105b led to only traces of the desired product 161 (<5%).

![Figure 4.30: Synthesis of acyl chloride 182 and subsequent treatment with the enolate of acetamide 105b.](image)

Hydrolysis of methyl ester 106b was somewhat problematic (Table 4.11). Heating methyl ester 106b with LiOH•H₂O in refluxing THF could not effect the desired hydrolysis (entry 1). Addition of hydrogen peroxide to this solution, which has previously been used to hydrolyze unreactive esters, was also inadequate (entry 2). It was only when ester 106b was heated to above 100 °C in DMF that it began to hydrolyze, albeit slowly (entry 3). That it took heating to 150 °C for ester 106b to be completely
hydrolyzed really speaks to the unreactivity of the carbonyl function and reaffirms what was learned with attempted enolate additions (entry 4). Acid 183 then was treated with 1-chloro-\(N,N,2\)-trimethyl-1-propenylamine (181),\(^{162}\) and when all the acid was consumed, without isolation of the acyl chloride 184, TMSCN and SnCl\(_4\) were added to the acyl chloride in an attempt to make acyl cyanide 185 (Figure 4.31).\(^{166}\) However, the acyl cyanide 185 could not be isolated.

\[
\begin{align*}
\text{MeO} & \quad \text{OMe} \\
\text{CO}_2\text{Me} & \quad \text{LiOH}H_2O
\end{align*}
\]

\[
\begin{align*}
\text{MeO} & \quad \text{OMe} \\
\text{CO}_2\text{H} & \quad \text{Me}
\end{align*}
\]

<table>
<thead>
<tr>
<th>Entry</th>
<th>Additive</th>
<th>Solvent</th>
<th>Conditions</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>THF/H(_2)O</td>
<td>60 °C, 14 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>2</td>
<td>(H_2)O(_2)</td>
<td>THF/H(_2)O</td>
<td>60 °C, 16 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>3</td>
<td>DMF</td>
<td></td>
<td>45 °C, 16 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>4</td>
<td>DMF</td>
<td></td>
<td>100 °C, 16 h</td>
<td>~20% + SM recovered</td>
</tr>
<tr>
<td>5</td>
<td>DMF</td>
<td></td>
<td>150 °C, 16 h</td>
<td>64%</td>
</tr>
</tbody>
</table>

Table 4.11: Attempts at hydrolyzing ester 106b.
Figure 4.31: Attempts at making acyl cyanide 185.

5.4 Model System with an Aldehyde

Deprotonation of acetamide 105b followed by the addition of its enolate to aldehyde 186 afforded coupled product 187 (Figure 4.32). The β-alcohol in 187 was then oxidized to β-ketoamide 160 with manganese dioxide. Aldehyde 186 was made by the partial reduction of ester 109a with Dibal at low temperatures (-78 °C). Similarly to aldehyde 186, subjection of acetamide 105b with LDA and addition of this enolate to aldehyde 188 gave addition product 189 in 33% yield. Aldehyde 188 was obtained by reduction of Weinreb amide 164 with a 1 M solution of Dibal in hexanes at -78 °C (Figure 4.33).160 The aldol strategy, although it is more likely to work than others, does add extra steps to the synthesis. Thus, it should be reserved as a later option.
Figure 4.32: Synthesis of aldehyde 186 and its coupling with acetamide 105b.
Figure 4.33: Synthesis of aldehyde 188 and its coupling with acetamide 105b.

Ester 106b was found to be unreactive towards several different hydride sources (Table 4.12). Adding Dibal at -78 °C to 106b in an attempt to isolate aldehyde 190 only led to 106b being recovered (entry 1). Even at room temperature, 106b treated with Dibal led to only the recovery of the starting compound (entry 2). Subjection to NaBH₄ and the more reactive LiBH₄ in methanol left 106b untouched (entries 3 and 4). However, heating 106b with LiBH₄ in refluxing THF led to its decomposition (entry 5). The reaction yielded several polar compounds that could not be characterized by the usual spectroscopic analysis. Perhaps reduction of the quinoline rings occurred. Surprisingly, even lithium aluminum hydride (LAH) did not react with the ester in 106b after 2 hours at room temperature (entry 6). However, increasing the reaction time (12 h) and number of equivalents of LAH (10 equiv) led to decomposition. Although the attempts at
reduction of ester **106b** have not been completely pushed to their limits, it does appear that it could present a challenge.

![Chemical structure](image)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagent</th>
<th>Solvent</th>
<th>Conditions</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dibal</td>
<td>toluene</td>
<td>-78 °C, 2 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>2</td>
<td>Dibal</td>
<td>THF</td>
<td>0 °C to rt, 2 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>3</td>
<td>NaBH₄</td>
<td>MeOH</td>
<td>rt, 14 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>4</td>
<td>LiBH₄</td>
<td>THF, MeOH</td>
<td>rt, 14 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>5</td>
<td>LiBH₄</td>
<td>THF</td>
<td>60 °C, 24 h</td>
<td>0%, decomp.</td>
</tr>
<tr>
<td>6</td>
<td>LiAlH₄</td>
<td>THF</td>
<td>0 °C to rt, 2 h</td>
<td>0%, mostly SM recovered</td>
</tr>
<tr>
<td>7</td>
<td>LiAlH₄</td>
<td>THF</td>
<td>rt, 12 h</td>
<td>0%, decomp.</td>
</tr>
</tbody>
</table>

Table 4.12: Reduction attempts for ester **106b**.

### 5.5 Model System with a Ketone

Versions of **106b** that could act as a nucleophile instead of an electrophile might overcome the steric problems encountered with **106b**. For this purpose, the model methyl ketone **192** was investigated. Ketone **192** was constructed from the methylation of Weinreb amide **164** with methylmagnesium bromide (Figure 4.34).³⁶⁰ Attempts to add methyl lithium to methyl ester **107b** at low temperatures were unsuccessful in yielding the desired ketone **192**. Ketone **192** was also made from the addition of the ethyl acetate enolate to methyl ester **107b**, followed by hydrolysis and decarboxylation of the resulting β-ketoester **193** to furnish **192**.
Figure 4.34: Synthesis of methyl ketone 192.

The addition of the enolate derived from ketone 192 to the electrophilic carbamoyl chloride 194 was investigated (Table 4.13). Trimethylamine as a base was fruitless at affecting the desired union between 192 and 194 (entries 1 and 2). At room temperature, lithium hexamethyldisilazane (LHMDS) was equally ineffective (entry 3). However, deprotonation of ketone 192 with LHMDS and heating this enolate with carbamoyl chloride 194 in refluxing THF, did give 22% of the desired β-ketoamide 161 along with a small amount of recovered ketone 192 (entry 4). Carbamoyl chloride 194, which was found surprisingly stable to water and column chromatography, was synthesized from aniline 108b and phosgene as shown in Figure 4.35. Carbamoyl chloride 194 was also treated with TMSCN and NaCN in an attempt to form the perhaps electronically better enolate coupling partner carbamoyl cyanide 195, but these attempts were unsuccessful (Figure 4.36).
<table>
<thead>
<tr>
<th>Entry</th>
<th>Base</th>
<th>Temperature</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Et₃N</td>
<td>0 °C to rt</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>2</td>
<td>Et₃N</td>
<td>reflux</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>3</td>
<td>LHMDS</td>
<td>-78 °C to rt</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>4</td>
<td>LHMDS</td>
<td>-78 °C to reflux</td>
<td>22% + ~10% 70</td>
</tr>
</tbody>
</table>

Table 4.13: Attempts at adding the enolate of methyl ketone 192 to carbamoyl chloride 194.

Figure 4.35: Synthesis of carbamoyl chloride 194.

Figure 4.36: Attempts at converting carbamoyl chloride 194 into carbamoyl cyanide 195.
The carbamate group might be another way of making the aniline 108b species electrophilic in nature. Apparently, due to the lack of reactivity of aniline 108b, initial attempts at making carbamate 197 were unsuccessful (Table 4.14). Heating aniline 108b and 4-nitrophenylchloroformate (196) as high as 150 °C with both conventional and microwave irradiation, did not lead to the production of 197. A possible solution could be to perhaps deprotonate aniline 108b with NaH instead of diisopropylethylamine or triethylamine, which had been used in the literature.

![Chemical structures](image)

**Table 4.14**: Attempts at making carbamate 197.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Solvent</th>
<th>Base</th>
<th>Conditions</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CH₂Cl₂</td>
<td>Et₃N</td>
<td>0 °C to rt, 15 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>2</td>
<td>CICH₂CH₃Cl</td>
<td>DIEA</td>
<td>0 °C to 80 °C, 15 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>3</td>
<td>CICH₂CH₃Cl</td>
<td>DIEA</td>
<td>145 °C, 15 h, sealed tube</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>4</td>
<td>CICH₂CH₃Cl</td>
<td>DIEA</td>
<td>150 °C, 45 min, MW</td>
<td>0%</td>
</tr>
<tr>
<td>5</td>
<td>DIEA</td>
<td></td>
<td>150 °C, 15 h, sealed tube</td>
<td>0%, SM recovered</td>
</tr>
</tbody>
</table>

Next, CO₂ (g) was bubbled through a solution of the enolate of methyl ketone 192 in attempt to make the corresponding β-ketoacid 198 and trap this presumably unstable β-ketoacid with aniline 108b to produce β-ketoamide 161 (Figure 4.37). However, all attempts to trap β-ketoacid 161 were unsuccessful. Ketone 192 was deprotonated at low temperatures with the bases Et₃N, LDA, and LHMDS, and after bubbling CO₂ (g) through the solution, was subsequently treated with aniline 108b under Steglich esterification conditions at low temperatures.
Figure 4.37: Attempts at trapping β-ketoacid 198 with aniline 108b to make β-ketoamide 161.

5.6 Model System with a β-Ketoester

Tang and others have reported that β-ketoesters can be aminolyzed to make the corresponding β-ketoamides with mild heating, usually about 100 °C. For example, heating aniline 200 with β-ketoester 199 at 130 °C afforded β-ketoamide 201 (Figure 4.38). Thus, β-ketoester 193 was heated with aniline 108b in the presence of DMAP as high as 320 °C without any reaction (Table 4.15, entries 4-6). Subjecting 193 and 108b to AlMe₃ in THF at room temperature and at reflux was also unsuccessful (entries 1 and 2).
Figure 4.38: Precedent for aminolysis of a β-ketoester.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Base/Acid</th>
<th>Solvent</th>
<th>Conditions</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AlMe₃</td>
<td>CH₂Cl₂</td>
<td>rt, 16 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>2</td>
<td>AlMe₃</td>
<td>THF</td>
<td>rt, 16 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>3</td>
<td>AlMe₃</td>
<td>THF</td>
<td>60 °C, 20 h</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>4</td>
<td>DMAP</td>
<td>toluene</td>
<td>150 °C, MW, 30 min</td>
<td>0%, SM recovered</td>
</tr>
<tr>
<td>5</td>
<td>DMAP</td>
<td>toluene</td>
<td>200 °C, MW, 30 min</td>
<td>0%, mostly SM recovered</td>
</tr>
<tr>
<td>6</td>
<td>DMAP</td>
<td>xylene</td>
<td>320 °C, sealed tube, 20 h</td>
<td>0%, SM recovered</td>
</tr>
</tbody>
</table>

Table 4.15: Attempts at aminolizing β-ketoester 193 with aniline 108b.

6 Future Steps

Once a solution is found for the coupling of 105 and 106, the final steps of the synthesis will involve the demethylation of a compound such as 157 followed by an oxidation of the resulting catechols to the corresponding orthoquinones to obtain 104. Based on several reports, the dimethylation and oxidation might be accomplished in one
transformation (Figure 4.39). Thus, in planning for such an event, dimethoxyquinoline 128 was subjected to a variety of oxidative-demethylating conditions in an attempt to establish a one-step protocol.\textsuperscript{173-180} Several oxidants were employed including cerium ammonium nitrate (CAN), cobalt (III) fluoride, manganese dioxide, two hypervalent iodine reagents (PIDA and Stang’s), nitric acid, and silver oxide (Table 4.16). All attempts, however, gave either decomposition or the recovery of starting material.

Figure 4.39: Proposed oxidative dimethylation of 157 to give 104.
Table 4.16: Attempts at a one-step oxidative demethylation to orthoquinone 202.

Therefore, a selective demethylation of dimethyl ether 128 in the presence of methyl ester 109a was also tested, which together could serve as a model system for ester 106b (Table 4.17).\textsuperscript{181-183} Dimethoxy ether 128 could be selectively deprotected in the presence of methyl ester 109a with both AlCl\textsubscript{3} and BBr\textsubscript{3} (entries 2 and 4). As expected, heating 128 and 109a with aqueous HBr led to both the demethylation of 128 and the hydrolysis of ester 109a (entry 3). This route obviously would require a separate oxidation step of the catechol product 203.
Entry | Reagent | Solvent | Conditions | Yield
--- | --- | --- | --- | ---
1 | AlCl₃ | CH₂Cl₂ | 0 °C, 1.5 h | Incomplete, ester 109a OK
2 | AlCl₃ | CH₂Cl₂ | 0 °C, 3 h | Clean deprotection, ester 109a OK
3 | 48% HBr (aq) | Ac₂O | 120 °C | Clean deprotection, ester 109a hydrol.
4 | 1M BBr₃ | CH₂Cl₂ | -78 °C to rt | Clean deprotection, ester 109a OK

Table 4.17: Selective demethylation of dimethoxy 128 in the presence of methyl ester 109a.

Once a molecule such as 104 is obtained, the final steps of the synthesis will involve the treatment of 104 with mild base to cyclize intramolecularly the β-ketoamide into each of the orthoquinone moieties in a Michael-like fashion as shown in Figure 4.40. Methylation of all four phenols in cyclized product 209 should afford lihoidine (1). The Michael-additions of 1,3-carbonyls to orthoquinones has been reported. 6-Bromo-1,2-naphthalenedione (210) was reacted with several cyclic and acyclic 1,3-dicarbonyls to form catechol addition products in respectable yields (Figures 4.41 and 4.42).
Figure 4.40: Proposed final steps in the synthesis of lihouidine (1).

Figure 4.41: Michael additions of cyclic 1,3-dicarboxyls into orthoquinone 210.
Figure 4.42: Michael additions of noncyclic 1,3-dicarbonyls into orthoquinone 210.

7 Conclusions

In this chapter, studies towards the synthesis of the marine natural product lihouidine (1) were described. The early part of the synthesis arrived at the dimethoxy aniline 108b, which was coupled to the 2-chloroquinoline 109a utilizing the arylamination chemistry developed by Buchwald and Hartwig. Attempts at an electrocyclization of the Buchwald-Hartwig adduct 107b under aerobic acidic conditions were unsuccessful. However, in an attempt to nitrate 107b, it was found that 107b underwent an unprecedented nitration/cyclization cascade when treated with fuming nitric acid. Removal of the undesired nitro group from the cyclization product produced the key compound 106. Unfortunately, addition of acetamide 105b to the ester group in 106 has presented a challenge. Several strategies for the union of these two species were explored in various model systems. Once a successful strategy is devised, the goal of the project will be arriving at the last key step in the synthesis. This transformation will
utilize two consecutive intramolecular Michael additions by a 1,3-ketoamide onto the
orthoquinones of 104 followed by methylation of the resulting spiro product 209 to finish
the first total synthesis of lihouidine (1).
Chapter 5 – Experimental Procedures

5.1 General Experimental

Flame-dried glassware under an argon or nitrogen atmosphere was employed for all reactions involving air and moisture sensitive reagents or solvents. All reactions were performed with magnetic stirring. A CEM discover instrument was used for microwave heated reactions. Reaction product solutions and chromatography fractions were concentrated using a rotary evaporator at approximately 20 mm Hg followed by concentration by vacuum pump at ca. 0.1 mm Hg. Commercial-grade reagents and solvents were used without further purification except as indicated below. Before July, 2004, all reaction solvents were distilled under argon from the appropriate reagent immediately before use. Tetrahydrofuran was distilled from sodium benzophenone ketyl or dianion. Dichloromethane, ether, acetonitrile, and benzene were distilled from calcium hydride. Dimethoxyethane was distilled from sodium and fluorenone. Methanol was distilled from magnesium. N,N-Dimethylformamide was dried over 3 Å molecular sieves. After July, 2004, reaction solvents (tetrahydrofuran, dichloromethane, dimethoxyethane, acetonitrile, methanol, toluene, benzene, and ether) were passed through a Glass Contour activated alumina column under nitrogen immediately before use. Flash column chromatography was performed using 32-63 μm silica gel with the indicated solvent systems. All melting points are uncorrected. Chemical shifts are reported in δ units using tetramethylsilane (TMS) or a deuterated solvent, usually CDCl₃, as an internal standard for ¹H NMR and ¹³C NMR. Low- and high-resolution mass spectra except low- and high-
resolution fast atom bombardment (FAB) were obtained according to the specified technique and were performed at the Huck Institute of the Life Sciences – Proteonomics and Mass Spectrometry Core Facility at the Pennsylvania State University, University Park, PA. Low- and high-resolution FAB mass spectra were performed at the Department of Chemistry and Biochemistry at the University of Texas at Austin, TX. Combustion analyses were performed by Midwest Microlab, IN or Galbraith Laboratories, Inc. at Knoxville, TN. Human TNF-α Enzyme Linked ImmunoSorbent Assay (ELISA) kits were purchased from R and D Systems, Minneapolis, MN.

5.2 Ellagitannin Synthesis

Diaryl Ether 36. Known diaryl ether 36 was made with modification of a published procedure. The orthoquinone 32 (482 mg, 1.77 mmol) was dissolved in CH₃CN (18 mL). Sc(OTf)³ (87.0 mg, 0.177 mmol) was added and the mixture was stirred at room temperature for 16 h. The reaction mixture was dissolved in CH₂Cl₂ (50 mL) and washed with water (8 mL). The organic layer was dried with Na₂SO₄, filtered, and concentrated in vacuo to produce a red foamy oil.

The mixture of regioisomeric benzodioxene products 33a and 33b were immediately stirred with sodium acetate (145 mg, 1.77 mmol) in acetic acid (10 mL) and
THF (5 mL) for 2 h. This reaction mixture was partitioned between CH$_2$Cl$_2$ (50 mL) and water (10 mL). The organic layer was dried with Na$_2$SO$_4$, filtered, and concentrated in vacuo. The residual acetic acid was removed as a hexanes azeotrope (3 x 20 mL) to give a foamy red oil.

The new regioisomeric mixture of dehydrodigalloyl quinones 34a and 34b was immediately stirred with Na$_2$S$_2$O$_4$ (490 mg, 2.81 mmol) in a mixture of THF (18 mL), ethyl acetate (18 mL), and water (18 mL) at 0 °C for 15 minutes. The dark red reaction mixture turned to a bright yellow solution over this time. The product was extracted with ethyl acetate (3 x 50 mL) and the combined organic layers were washed with brine (20 mL). The organic layer was dried with Na$_2$SO$_4$, filtered, and concentrated in vacuo to give a yellow oil.

The crude regioisomeric digalloyl ethers 35a and 35b were benzylated with benzyl bromide (383 µL, 3.22 mmol), K$_2$CO$_3$ (556 mg, 4.02 mmol), and KI (80 mg, 0.48 mmol) in refluxing acetone (18 mL) for 14 h. The reaction mixture was cooled and filtered through Celite with acetone washing (50 mL), and the filtrate was concentrated in vacuo to a brown oil. The crude product was purified by flash column chromatography with 25% ethyl acetate in hexanes as the eluent to afford 241 mg (37%) of diaryl ether 36 as a yellow oil. $^1$H NMR (CDCl$_3$, 300 MHz) δ 7.45-7.13 (m, 27 H), 6.90 (d, $J = 1.8$ Hz, 1H), 5.14 (s, 4 H), 5.12 (s, 4 H), 4.97 (s, 2 H), 3.79 (s, 3 H), 3.71 (s, 3 H). The spectral data match those reported in the literature.$^{90}$
**Diol 48.** To a solution of 4,6-tetraol 47\(^3\) (102 mg, 0.036 mmol) in CH\(_2\)Cl\(_2\) (2 mL) was added imidazole (12 mg, 0.18 mmol) and \(\tau\)-butyldiphenylsilyl chloride (38 µL, 0.15 mmol) at room temperature. The reaction mixture was stirred at room temperature for 17 h, and then it was diluted with ethyl acetate (25 mL) and washed with 1M aqueous H\(_3\)PO\(_4\) (5 mL), water (5 mL), and brine (5 mL). The organic layer was dried with Na\(_2\)SO\(_4\), filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography with 30% ethyl acetate in hexanes as the eluent to afford 113 mg (96%) of bis TBDPS ether 48 as a whitish semisolid. IR (CDCl\(_3\)) 3650-3300, 1728 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.64-7.60 (m, 8H), 7.49-6.97 (m, 108H), 5.89 (d, \(J = 8.2\) Hz, 1H), 5.84 (d, \(J = 8.1\) Hz, 1H), 5.63 (ddd, \(J = 10.1, 8.3, 2.0\) Hz, 2H), 5.52 (appar t, \(J = 9.4\) Hz, 1H), 5.50 (appar t, \(J = 9.5\) Hz, 1H), 5.14-4.79 (m, 34H), 4.14 (dd, \(J = 9.1, 2.5\) Hz, 1H), 4.10 (dd, \(J = 9.9, 2.8\) Hz, 1H), 4.00 (dd, \(J = 10.9, 3.8\) Hz, 2H), 3.91 (dd, \(J = 10.9, 4.7\) Hz, 2H), 3.71-3.68 (m, 2H), 3.43 (d, \(J = 2.4\) Hz, 2H), 1.02 (s, 18H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\), \(\delta\) 166.7, 166.6, 165.4, 165.2, 163.8, 161.1, 152.6 (2), 152.5, 149.8, 147.4, 146.3, 143.1, 142.9, 142.4, 137.6, 137.4, 137.3 (2), 136.7, 136.5 (2), 136.4 (2), 136.3, 136.2, 135.6, 135.5, 132.5 (2), 129.9, 128.7, 128.5 (2), 128.4, 128.3, 128.2, 128.1 (3), 128.0, 127.9 (2), 127.8 (2), 127.7 (2), 127.5 (3), 124.0, 123.7 (2), 123.3, 117.8, 110.5,
Bis TBDPS Ether 49. To a solution of diol 48 (102 mg, 0.031 mmol) in CH$_2$Cl$_2$ (1.5 mL) was added 3,4,5-tribenzyloxy benzoic acid (45) (27 mg, 0.062 mmol), 4-di(methylamino)pyridine (2.0 mg, 0.016 mmol), 4-di(methylamino)pyridine•HCl (2.5 mg, 0.016 mmol), and dicyclohexylcarbodiimide (16 mg, 0.078 mmol) at room temperature. The reaction mixture was stirred at room temperature for 18 h, and then this solution was cooled to -20 °C for 30 minutes and filtered through Celite with ether washing (50 mL). The filtrate was washed with 1 M aqueous H$_3$PO$_4$ (8 mL), water (8 mL), and brine (8 mL). The organic layer was dried with Na$_2$SO$_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography with 30% ethyl acetate in hexanes as the eluent to afford 117 mg (91%) of 49 as a white semisolid. IR (CDCl$_3$) 1732 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.64-7.01 (m, 150H), 6.06-600 (appar t, $J = 9.4$ Hz, 2H), 5.91 (t, $J = 9.7$ Hz, 1H), 5.90 (t, $J = 9.6$ Hz, 1H), 5.81 (t, $J = 9.6$ Hz, 1H), 5.80-5.72 (m, 3H), 5.23-4.88 (m, 46H), 3.97-3.86 (m, 4H), 3.77-3.72 (m, 2H), 0.97 (s, 9H), 0.96 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 165.7 (2), 165.3, 165.0, 164.5, 163.9, 161.0, 152.7, 152.6 (2), 152.4, 149.9, 147.5, 146.4, 143.8, 143.1,
143.0, 142.8, 142.7 (2), 142.6, 137.7, 137.4 (2), 137.3, 136.7, 136.6, 136.4 (2), 136.3, 135.6, 135.5, 132.8, 132.7 (2), 129.7, 129.6 (2), 128.7, 128.5 (4), 128.4 (2), 128.3 (2), 128.2 (2), 128.1 (2), 128.0, 127.9, 127.8, 127.7, 127.6, 127.5 (2), 127.4, 124.1, 123.9, 123.8 (3), 117.8, 110.7, 110.1, 109.8, 109.2, 109.1, 92.9, 92.6, 75.7, 75.6, 75.5, 75.1, 75.0, 73.6 (2), 71.8, 71.5, 71.2, 71.1, 71.0, 69.0, 68.8, 62.4, 62.2, 26.7, 19.1; -FAB m/z (relative intensity) 4032.3 (M - Bn, 59%); Anal. Calcd for C_{261}H_{228}O_{44}Si_{2}: C, 76.00; H, 5.57; Found: C, 75.98; H, 5.71.

**Diole 26.** To a solution of bis TBDPS ether 49 (49 mg, 0.012 mmol) in THF (1 mL) was added acetic acid (2 µL, 0.04 mmol) and a 1 M solution of tetrabutylammonium fluoride in THF (55 µL, 55 mmol) at room temperature. The reaction mixture was stirred at room temperature for 27 h. After this time period, the solution was diluted with ethyl acetate (20 mL) and washed with 1 M aqueous H_3PO_4 (5 mL), water (5 mL), and brine (5 mL). The organic layer was dried with Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography with 40% ethyl acetate in hexanes as the eluent to afford 32 mg (73%) of diole 26 as a white foamy oil. IR (thin film) 3650-3250, 1724 cm^{-1}; ^1H NMR (400 MHz, CDCl₃) δ 7.44-6.97 (m, 130H), 5.94 (d, J = 8.3 Hz, 1H), 5.91 (d, J = 8.4 Hz, 1H), 5.68 (appar t, J = 8.0 Hz, 1H), 5.66 (appar t, J = 7.8 Hz, 1H), 5.53 (appar t, J = 9.1 Hz, 2H), 5.10-4.80 (m, 46H), 4.42 (d, J = 11.5 Hz,
1H), 4.36 (d, J = 12.0 Hz, 1H), 3.80-3.76 (m, 6H); 13C NMR (100 MHz, CDCl3) δ 167.1, 166.5, 166.4, 165.4, 165.2, 163.9, 161.3, 152.6 (2), 152.5, 149.8, 147.4, 146.3, 143.2, 143.0, 142.9, 142.5, 137.6, 137.3 (3), 136.6, 136.5 (2), 136.4, 136.3 (2), 136.1, 128.7, 128.5, 128.4, 128.3 (3), 128.2, 128.1 (2), 128.0 (2), 127.9 (2), 127.6 (2), 127.5 (3), 124.1, 124.0, 123.9, 123.8, 123.6 (2), 123.3, 117.6, 110.3, 109.6, 109.5, 109.3, 109.1, 109.0, 93.0, 92.6, 75.7, 75.5, 75.4, 75.1 (2), 71.2, 71.1, 71.0, 70.9, 68.6, 68.5, 62.9, 62.9; FAB m/z (relative intensity) 3646.7 (M + H, 26%); HRMS calcd for C229H192O44 (M + H) 3646.2865, found 3646.6591.

Azide 52. To a solution of penta (ethylene glycol) di-p-toluenesulfonate (51) (1.04 g, 1.83 mmol) in 95% aqueous ethanol (17 mL) was added sodium azide (62 mg, 0.92 mmol) at room temperature. The reaction solution was refluxed for 4 h behind a blast shield. The reaction mixture was cooled to room temperature and was concentrated in vacuo to remove ethanol, giving a colorless oil. This residue was diluted with ether (60 mL) and washed with water (2 x 20 mL) and brine (20 mL). The combined organic layers were dried with Na2SO4, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography with 50% ethyl acetate in hexanes as the eluent to afford 181 mg (23%) of azide 52 as a colorless oil along with 395 mg (38%) of recovered starting material, also as a colorless oil. IR (thin film) 2107 cm⁻¹; 1H NMR (400 MHz, CDCl3) δ 7.80 (d, J = 8.4 Hz, 2H), 7.34 (d, J = 8.0 Hz, 2H), 4.16 (t, J = 4.9 Hz, 2H), 3.70-3.59 (m, 16H), 3.39 (t, J = 5.1 Hz, 2H), 2.45 (s, 3H); 13C NMR (100 MHz, CDCl3) δ 144.7, 132.8, 129.7, 127.8, 70.6, 70.5 (2), 70.4, 70.3, 69.9, 69.7, 69.1, 68.5,
50.5, 21.5; ESI m/z (relative intensity) 440.1 (M + Na⁺, 100%); Anal. Calcd for C₁₇H₂₇N₃SO₇: C, 48.91; H, 6.52; N, 10.07; Found: C, 48.98; H, 6.86; N, 10.11.

3,4-Bis-benzyloxy-5-hydroxy-benzoic Acid Methyl Ester (101). Known dibenzyloxy ether 101 was prepared by modification of a literature procedure.⁹⁴ Catechol 31 (1.50 g, 5.47 mmol), K₂CO₃ (907 mg, 6.56 mmol), and benzyl bromide (651 µL, 5.47 mmol) were mixed in acetone (28 mL) at room temperature. The cloudy light brown reaction mixture was stirred at room temperature for 24 h, and then the solution was filtered through Celite with acetone washing (50 mL). Concentration of the filtrate in vacuo gave a pink foam. The crude product was purified by flash column chromatography using 20% ethyl acetate in hexanes as the eluent to afford 1.38 g (69%) of phenol 101 as a white solid. ¹H NMR (300 MHz, CDCl₃) δ 7.50-7.35 (m, 6H) 7.33 (s, 5H), 7.27 (d, J = 1.8 Hz, 1H), 5.73 (s, 1H) 5.18 (s, 2H), 5.16 (s, 2H), 3.88 (s, 3H). The spectral data match those reported in the literature.⁹⁴

Galloyl Linker Azide 53. Azide 52 (187 mg, 0.448 mmol), phenol 101 (245 mg, 0.672 mmol), and K₂CO₃ (186 mg, 1.34 mmol) were mixed together in acetone (4 mL). The reaction mixture was refluxed for 14 h, and then this solution was cooled to room temperature and filtered through Celite with acetone washing (40 mL). The filtrate was
concentrated in vacuo to give a light yellow-brown oil. The crude product was purified by flash column chromatography with 5% methanol in 50:50 ethyl acetate/hexanes as the eluent to afford 238 mg (87%) of linker azide \textbf{53} as a colorless oil. IR (thin film) 2104, 1717 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.46-7.28 (m, 12 H), 5.12 (s, 4H), 4.20 (t, $J = 4.9$ Hz, 2H), 3.89 (s, 3H), 3.87 (t, $J = 4.9$ Hz, 2H), 3.73-3.71 (m, 2H), 3.67-3.62 (m, 12H), 3.36 (t, $J = 5.1$ Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 166.6, 152.6, 152.4, 142.1, 137.6, 136.6, 128.5, 128.4, 128.1, 128.0, 127.9, 127.5, 125.1, 108.9, 108.6, 74.9, 71.1, 70.8, 70.6 (3), 70.0, 69.6, 68.7, 52.2, 50.6; ESI $m/z$ (relative intensity) 627.3 (M + NH$_4^+$, 100%); Anal. Calcd for C$_{32}$H$_{39}$N$_3$O$_9$: C, 63.04; H, 6.45; N, 6.89; Found: C, 62.47; H, 6.63; N, 6.96.

![Galloyl Linker Amine 54](image)

**Galloyl Linker Amine 54.** Azide \textbf{53} (111 mg, 0.18 mmol), triphenylphosphine (96 mg, 0.36 mmol), and water (82 µL, 4.6 mmol) were mixed together in THF (4 mL) at room temperature. The clear solution was refluxed for 5 h, and then the reaction mixture was cooled to room temperature and concentrated in vacuo to yield a colorless oil. The crude product was purified by flash column chromatography with 5% triethylamine in 20:80 methanol/ethyl acetate as the eluent to afford 87 mg (82%) of linker amine \textbf{54} as a colorless oil. IR (thin film) 3309, 1716 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.46-7.27 (m, 12H), 5.12 (s, 2H), 5.11 (s, 2H), 4.19 (t, $J = 4.8$ Hz, 2H), 3.88 (s, 3H), 3.88-3.86 (m, 2H), 3.73-3.70 (m, 2H), 3.65-3.59 (m, 12H), 3.47 (t, $J = 5.2$ Hz, 2H), 2.84 (br s, 2H); $^{13}$C
NMR (100 MHz, CDCl$_3$) $\delta$ 166.4, 152.4, 152.3, 142.0, 137.4, 136.5, 128.4, 128.3, 128.0, 127.8 (2), 127.4, 125.0, 108.8, 108.5, 74.8, 71.0, 70.7 (2), 70.5 (2), 70.4 (2), 70.1, 69.5, 68.6, 52.0, 41.6; ESI $m/z$ (relative intensity) 584.2 (M + H, 100%); HRMS calcd for C$_{32}$H$_{41}$NO$_9$ (M + H) 584.2860, found 584.2860.

**Galloyl Linker Teoc Protected Amine 56.** To a solution of amine 54 (54 mg, 0.093 mmol) in CH$_2$Cl$_2$ (2 mL) was added 4-di(methylamino)pyridine (4.0 mg, 0.033 mmol) and 2-trimethylsilyl ethoxycarbonyl imidazole 55 (20 mg, 0.094 mmol) at room temperature. The reaction mixture was stirred at reflux for 16 h, and then the solution was cooled to room temperature and concentrated in vacuo. The crude orange oil was diluted with ethyl acetate (20 mL) and washed with 1 M aqueous H$_3$PO$_4$ (5 mL), water (5 mL), and brine (5 mL). The organic layer was dried with Na$_2$SO$_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography with 5% methanol in 50:50 ethyl acetate/hexanes as the eluent to afford 43 mg (64%) of Teoc protected amine 56 as a colorless oil. IR (thin film) 3358, 1719 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.46-7.27 (m, 12H), 5.20 (br s, 1H), 5.11 (s, 4H), 4.20 (t, $J = 4.9$ Hz, 2H), 4.13 (t, $J = 8.5$ Hz, 2H), 3.88 (s, 3H), 3.87 (t, $J = 4.7$ Hz, 2H), 3.73-3.71 (m, 2H), 3.65-3.56 (m, 10H), 3.52 (t, $J = 5.1$ Hz, 2H), 3.36-3.33 (m, 2H), 0.97 (t, $J = 8.5$ Hz, 2H), 0.03 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 166.5, 156.8, 152.5, 152.4, 142.1, 137.5, 136.6, 128.4 (2), 128.1, 127.9, 127.8, 127.4, 125.1, 108.9, 108.6, 74.9, 71.1, 70.8, 70.6, 70.5 (2), 70.4, 70.2, 70.1, 69.5, 68.7, 62.8, 52.1, 40.6, 17.7, -1.55; ESI $m/z$ (relative intensity)
745.2 (M + NH$_4$, 100%), 750.2 (M + Na, 86%); Anal. Calcd for C$_{38}$H$_{53}$NO$_{11}$Si: C, 62.70; H, 7.34; N, 1.92; Found: C, 62.21; H, 7.40; N, 1.96.

Galloyl Linker Teoc Protected Amine Acid 27a. To a solution of methyl ester 56 (64 mg, 0.088 mmol) in methanol (1.5 mL) and water (0.5 mL) was added LiOH•H$_2$O (37 mg, 0.88 mmol) at room temperature. The reaction mixture was stirred at reflux for 4 h, and then the solution was cooled to room temperature and concentrated in vacuo. The crude colorless product oil was diluted with ethyl acetate (25 mL) and washed with 1 M aqueous H$_3$PO$_4$ (5 mL), water (5 mL), and brine (5 mL). The organic layer was dried with Na$_2$SO$_4$, filtered, and concentrated in vacuo to afford 62 mg (98%) of acid 27a as a colorless oil. IR (thin film) 3400-2500, 3336, 1714 cm$^{-1}$; $^1$H NMR (360 MHz, CDCl$_3$) $\delta$ 10.3 (br s, 1H), 7.46-7.28 (m, 12H), 5.28 (br s, 1H), 5.14 (s, 2H), 5.12 (s, 2H), 4.21 (t, $J = 4.7$ Hz, 2H), 4.14 (t, $J = 8.4$ Hz, 2H), 3.87 (t, $J = 4.6$ Hz, 2H), 3.74-3.71 (m, 2H), 3.66-3.60 (m, 10H), 3.54 (t, $J = 5.1$ Hz, 2H), 3.37-3.35 (m, 2H), 0.97 (t, $J = 8.5$ Hz, 2H), 0.03 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 170.3, 156.9, 152.5, 152.4, 142.6, 137.5, 136.5, 128.4 (2), 128.1, 127.9 (2), 127.4, 124.5, 109.3 (2), 74.9, 71.0, 70.8, 70.6, 70.5, 70.4, 70.2, 70.1, 69.6, 68.7, 62.9, 40.6, 17.7, -1.55; ESI m/z (relative intensity) 736.2 (M + Na, 100%), 731.2 (M + NH$_4$, 91%); HRMS calcd for C$_{37}$H$_{51}$NO$_{11}$Si (M + Na) 736.3129, found 736.3145.
Dimer Teoc Protected Linker 59. To a solution of diol 26 (31 mg, 0.0085 mmol) in CH$_2$Cl$_2$ (1.0 mL) was added acid 27a (14 mg, 0.020 mmol) via cannula as a CH$_2$Cl$_2$ solution (0.5 + 0.5 + 0.5 mL). 4-Di(methylamino)pyridine (1.5 mg, 0.012 mmol), 4-di(methylamino)pyridine•HCl (1.5 mg, 0.009 mmol) and dicyclohexylcarbodiimide (6.0 mg, 0.029 mmol) were added to this solution at room temperature. The cloudy reaction mixture was stirred at room temperature for 15 h. Then the solution was cooled to -20 °C for 30 minutes and filtered through Celite with a 1:1 mixture of ether and ethyl acetate washing (50 mL). The filtrate was washed with 1 M aqueous H$_3$PO$_4$ (8 mL), water (8 mL), and brine (8 mL). The combined organic layers were dried with Na$_2$SO$_4$, filtered, and concentrated in vacuo to an oil (42 mg). The crude product was purified by flash column chromatography using 2.5% methanol in 50:50 ethyl acetate/hexanes as the eluent to afford 33 mg (77%) of dimer Teoc protected 59 as a whitish oil and 2 mg (5%) of a mixture of monoesterified/monoalcohol products as a whitish oil. IR (thin film) 1731, 1713 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.49-6.95 (m, 154H), 6.09 (appar t, $J = 8.7$ Hz, 2H), 5.98 (t, $J = 9.7$ Hz, 1H), 5.97 (t, $J = 9.6$ Hz, 1H), 5.82-5.73 (m, 4H), 5.12-4.88 (m, 56H), 4.83-4.69 (m, 4H), 4.33-4.26 (m, 4H), 4.12 (t, $J = 8.2$ Hz, 4H), 4.02-3.98
(m, 4H), 3.72-3.70 (m, 4H), 3.62-3.58 (m, 4H), 3.53 -3.47 (m, 2H), 3.32-3.30 (m, 4H),
0.97-0.92 (m, 4H), 0.01 (s, 18H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) δ 165.6, 165.4 (2), 165.1,
164.9, 164.8, 163.9, 161.2, 156.8, 152.6 (2), 152.5, 152.4 (2), 152.3, 149.7, 147.5, 146.2,
143.9, 143.1 (2), 143.0, 142.8, 142.7, 142.5 (2), 137.6, 137.5, 137.4, 137.3, 137.0, 136.7,
136.5, 136.4, 136.3 (2), 136.2, 130.9, 128.8, 128.7, 128.5 (2), 128.4 (2), 128.3 (2), 128.2,
128.1, 128.0 (2), 127.9 (2), 127.8, 127.7, 127.5, 124.5 (2), 124.3, 123.7, 123.6, 123.1, 117.4,
109.2, 109.1, 108.7, 92.9, 92.5, 75.5, 75.1, 75.0, 74.9, 73.3 (2), 72.8, 72.7, 72.6, 71.4,
71.3, 71.1 (2), 71.0, 70.9, 70.7, 70.5, 70.4, 70.2, 70.1, 69.4, 68.7, 68.1, 62.9, 40.6, 38.7,
17.7, -1.50; -FAB m/z (relative intensity) 5037.9 (M – H, 7%), 4947.4 (M – Bn, 100%);
HRMS calcd for C\(_{303}\)H\(_{290}\)N\(_2\)O\(_{64}\)Si\(_2\) (M – H) 5034.8960, found 5037.8875.

![Galloyl Linker Cbz Protected Amine 61](image)

**Galloyl Linker Cbz Protected Amine 61.** To a solution of amine 54 (480 mg, 0.82 mmol) in THF (8 mL) was added 4-di(methylamino)pyridine (127 mg, 1.04 mmol) at room temperature. After cooling to 0 °C, benzyl chloroformate (300 µL, 2.10 mmol) was added to the reaction mixture. This solution was stirred at room temperature for 16 h, and then the reaction mixture was diluted with ethyl acetate (40 mL) and washed with 1 M aqueous H\(_3\)PO\(_4\) (10 mL), water (10 mL), and brine (10 mL). The organic layer was dried with Na\(_2\)SO\(_4\), filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography using 5% methanol in 50:50 ethyl acetate/hexanes as the eluent to afford 445 mg (75%) of Cbz protected amine 61 as a colorless oil. IR (thin
film) 3354, 1715 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.46-7.25 (m, 17H), 5.41 (br s, 1H), 5.12-5.08 (m, 6H), 4.17 (t, \(J = 4.7\) Hz, 2H), 3.87 (s, 3H), 3.84 (t, \(J = 4.7\) Hz, 2H), 3.68 (t, \(J = 4.2\) Hz, 2H), 3.65-3.49 (m, 12H), 3.37 (t, \(J = 4.7\) Hz, 2H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 166.4, 156.3, 152.4, 152.2, 141.9, 137.4, 136.5, 128.3 (3), 128.1, 128.0, 127.9, 127.8 (2), 127.7, 127.3, 125.0, 108.7, 108.4, 74.7, 70.9, 70.6, 70.4 (2), 70.3 (2), 70.0, 69.8, 69.4, 68.5, 66.4, 52.0, 40.7; APCI m/z (relative intensity) 718.4 (M + H, 100%); HRMS calcd for C\(_{40}\)H\(_{47}\)NO\(_{11}\) (M + H) 718.3227, found 718.3165.

**Galloyl Linker Cbz Protected Amine Acid 27b.** To a solution of methyl ester 61 (165 mg, 0.230 mmol) in methanol (1.8 mL) and water (0.6 mL) was added LiOH•H\(_2\)O (48 mg, 1.2 mmol) at room temperature. The reaction mixture was stirred at room temperature for 13 h. The reaction contents were concentrated in vacuo to remove methanol, and then the crude colorless product oil was diluted with ethyl acetate (25 mL) and was washed with 1 M aqueous H\(_3\)PO\(_4\) (5 mL), water (5 mL), and brine (5 mL). The organic layer was dried with Na\(_2\)SO\(_4\), filtered, and concentrated in vacuo to afford 156.5 mg (97%) of acid 27b as a colorless oil. IR (thin film) 3700-2500, 3333, 1715 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.45 (br s, 1H), 7.48-7.22 (m, 17H), 5.62 (br s, 1H), 5.13 (s, 2H), 5.09 (s, 4H), 4.15 (t, \(J = 4.1\) Hz, 2H), 3.82 (t, \(J = 3.8\) Hz, 2H), 3.68 (t, \(J = 4.6\) Hz, 2H), 3.65-3.48 (m, 12H), 3.37 (t, \(J = 5.0\) Hz, 2H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 169.7, 156.5, 152.4, 152.2, 142.3, 137.4, 136.4, 128.3 (3), 128.0, 127.9 (2), 127.8, 127.7, 127.3, 124.6, 109.1, 109.0, 74.8, 70.9, 70.6, 70.4, 70.3 (2), 70.2, 70.0, 69.8, 69.4, 68.5, 66.4,
40.6; APCI m/z (relative intensity) 704.2 (M + H, 100%); HRMS calcd for C_{39}H_{45}NO_{11} (M + H) 704.3071, found 704.1857.

Dimer Cbz Protected Linker 62. To a solution of diol 26 (45.5 mg, 0.0125 mmol) in CH_{2}Cl_{2} (2 mL) was added acid 27c (22 mg, 0.031 mmol) via cannula as a CH_{2}Cl_{2} solution (0.5 + 0.5 + 0.5 mL). 4-Di(methylamino)pyridine (1.4 mg, 0.011 mmol), 4-di(methylamino)pyridine•HCl (1.4 mg, 0.0088 mmol) and dicyclohexylcarbodiimide (7.2 mg, 0.035 mmol) were added to this solution at room temperature. The yellowish cloudy reaction mixture was stirred at room temperature for 15 h, and then this solution was cooled to -20 °C for 30 minutes and filtered through Celite and washed with a 1:1 mixture of ether and CH_{2}Cl_{2} (50 mL). The filtrate was concentrated in vacuo, and then the crude product residue was diluted with ethyl acetate (25 mL) and washed with 1 M aqueous H_{3}PO_{4} (8 mL), water (8 mL), and brine (8 mL). The organic layer was dried with Na_{2}SO_{4}, filtered, and concentrated in vacuo to an oil. The crude product was purified by flash column chromatography with 2.5% methanol in 50:50 ethyl acetate/hexanes as the eluent to afford 48 mg (77%) of dimer Cbz protected 62 as a white foamy oil along with 4.9 mg (9%) of a mixture of monoesterified/monoalcohol products as a colorless oil. IR (thin film) 1727 cm^{-1}; ^{1}H NMR (400 MHz, CDCl_{3}) \delta 7.51-6.91 (m, 164H), 6.09 (t, J =
8.9 Hz, 2H), 5.98 (t, J = 9.7 Hz, 1H), 5.96 (t, J = 9.6 Hz, 1H), 5.78 (td, J = 9.1, 1.4 Hz, 4H), 5.33 (br s, 2H), 5.18-4.61 (m, 64H), 4.30 (m, 4H), 3.98 (m, 4H), 3.71-3.29 (m, 32H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta\) 165.6, 165.5, 165.4, 165.1, 164.9, 164.8, 163.9, 161.2, 156.4, 152.7 (2), 152.6 (2), 152.5, 152.4 (3), 152.3, 149.7, 147.6, 146.2 (2), 143.2, 143.1, 143.0, 142.9 (2), 142.7, 142.6 (3), 137.6 (3), 137.4 (2), 137.4 (2), 136.7 (2), 136.6, 136.5 (2), 136.4 (2), 136.3, 136.2, 134.5, 129.7, 129.0, 128.7, 128.5 (2), 128.4 (2), 128.3 (2), 128.2, 128.1 (3), 128.0, 127.9, 127.8, 127.7, 127.5, 124.5 (2), 123.7 (2), 123.6, 123.1, 109.4 (2), 109.3 (2), 109.2 (3), 109.1 (2), 109.0 (2), 108.8, 108.7, 92.9, 92.6, 75.5, 75.1 (2), 74.9, 73.4, 73.3 (3), 72.8, 72.7, 71.5, 71.3, 71.2, 71.1, 71.0, 70.9, 70.8 (2), 70.7, 70.6 (4), 70.5, 70.4, 70.2, 70.0 (3), 69.8, 69.5 (3), 69.4 (2), 68.7 (2), 66.6 (2), 40.9, 40.8; -FAB m/z (relative intensity) 5019.3 (M – H, 22%); HRMS calcd for C\(_{303}\)H\(_{278}\)N\(_2\)O\(_6\) (M – H) 5014.8482, found 5019.2972.

**Galloyl Linker Boc Protected Amine 64.** To a solution of amine 54 (551 mg, 0.944 mmol) in CH\(_2\)Cl\(_2\) (8 mL) was added 4-di(methylamino)pyridine (127 mg, 1.04 mmol) and di-\(\tau\)-butyl dicarbonate (227 mg, 1.04 mmol) at room temperature. The reaction mixture was stirred at room temperature for 16 h, and then the yellow solution was diluted with ether (40 mL) and washed with water (10 mL) and brine (10 mL). The organic layer was dried with Na\(_2\)SO\(_4\), filtered, and concentrated in vacuo. The crude yellow product oil was purified by flash column chromatography using 5% methanol in 50:50 ethyl acetate/hexanes as the eluent to afford 342.4 mg (53%) of Boc protected
amine 64 as a colorless oil along with 31.3 mg (4%) of the diBoc product. IR (thin film) 3365, 1714 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.49-7.23 (m, 12H), 5.13 (s, 2H), 5.11 (s, 2H), 4.20 (t, $J = 4.6$ Hz, 2H), 3.88 (s, 3H), 3.72 (t, $J = 5.1$ Hz, 2H), 3.68-3.53 (m, 12H), 3.51 (t, $J = 5.0$ Hz, 2H), 3.30 (t, $J = 4.9$ Hz, 2H), 1.43 (s, 9H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 166.4, 155.8, 152.4, 152.2, 141.9, 137.4, 136.4, 128.3 (2), 128.0, 127.8, 127.7, 127.3, 124.9, 108.7, 108.4, 74.7, 70.9, 70.7, 70.4 (2), 70.3 (2), 70.0, 69.6, 69.4, 68.5, 66.4, 52.0, 40.1, 28.2; ESCI m/z (relative intensity) 701.3 (M + NH$_4$), 100%), 706.3 (M + Na, 93%); HRMS calcd for C$_{37}$H$_{49}$NO$_{11}$ (M + H) 684.3384, found 684.3428.

Galloyl Linker Boc Protected Amine Acid 27c. To a solution of methyl ester 64 (134 mg, 0.196 mmol) in methanol (1.2 mL) and water (0.4 mL) was added LiOH•H$_2$O (41.0 mg, 0.980 mmol) at room temperature. The reaction mixture was stirred at room temperature for 16 h, and then this solution was concentrated in vacuo to remove methanol. The crude colorless product residue was diluted with ethyl acetate (25 mL) and washed with 1 M aqueous H$_3$PO$_4$ (5 mL), water (5 mL), and brine (5 mL). The organic layer was dried with Na$_2$SO$_4$, filtered, and concentrated in vacuo to afford 113.5 mg (86%) of acid 27c as a colorless oil. IR (thin film) 3700-2200, 3352, 1713 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 8.15 (br s, 1H), 7.48-7.25 (m, 12H), 5.12 (s, 2H), 5.11 (s, 2H), 4.21 (t, $J = 4.5$ Hz, 2H), 3.87 (t, $J = 4.3$ Hz, 2H), 3.75-3.49 (m, 14H), 3.31 (t, $J = 4.7$ Hz, 2H), 1.44 (s, 9H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 169.8, 156.0, 152.5, 152.3, 142.5, 137.5, 136.5, 128.4 (2), 128.1, 127.9, 127.8, 127.4, 124.7, 109.3, 109.2, 74.9, 71.0, 70.8, 70.5
ESCI m/z (relative intensity) 687.3 (M + NH₄, 100%), 692.3 (M + Na, 82%), 670.3 (M + H, 4%); HRMS calcd for C₃₆H₄₇NO₁₁ (M + H) 670.3227, found 670.3214.

Dimer Boc Protected Linker 68. To a solution of diol 26 (21.4 mg, 0.0059 mmol) in CH₂Cl₂ (2 mL) was added acid 27c (11.8 mg, 0.0176 mmol) via cannula as a CH₂Cl₂ solution (0.5 + 0.5 + 0.5 mL), and then 4-di(methy lamino)pyridine (0.6 mg, 0.005 mmol), 4-di(methylamino)pyridine•HCl (0.7 mg, 0.004 mmol) and dicyclohexylcarbodiimide (3.4 mg, 0.017 mmol) were added to this solution at room temperature. The yellowish cloudy reaction mixture was stirred at room temperature for 16 h, and then this solution was cooled to -20 °C for 30 minutes, filtered through Celite, and washed with a 1:1 mixture of ether and CH₂Cl₂ (30 mL). The filtrate was concentrated in vacuo. The crude product residue was diluted with ethyl acetate (25 mL) and washed with 1 M aqueous H₃PO₄ (8 mL), water (8 mL), and brine (8 mL). The organic layer was dried with Na₂SO₄, filtered, and concentrated in vacuo to an oil. The crude product was purified by flash column chromatography using 2.5% methanol in 50:50 ethyl acetate/hexanes as the eluent to afford 17.4 mg (60%) of dimer Boc protected
68 as a white foamy oil. IR (thin film) 1729 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.51-6.92 (m, 154H), 6.09 (t, $J = 8.7$ Hz, 2H), 5.98 (t, $J = 9.8$ Hz, 1H), 5.96 (t, $J = 9.6$ Hz, 1H), 5.78 (td, $J = 9.1$, 2.6 Hz, 4H), 5.12-4.61 (m, 60H), 4.30 (m, 4H) 4.02 (t, $J = 5.3$ Hz, 2H), 3.99 (t, $J = 5.3$ Hz, 1H), 3.76-3.40 (m, 28H), 3.32-3.21 (m, 4H), 1.41 (s, 18H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 165.6, 165.5, 164.9, 164.8, 163.9, 156.0, 152.7 (4), 152.6 (2), 152.5 (2), 152.4 (2), 152.3 (2), 149.7, 147.5, 143.0, 142.9, 142.7, 142.5, 137.6, 137.5, 137.4 (2), 136.8, 136.7 (3), 136.6, 136.5, 136.4 (4), 136.3, 136.2, 128.8, 128.7, 128.5 (2), 128.4 (2), 128.3 (2), 128.2, 128.1 (2), 127.9, 127.8 (2), 127.7, 127.5, 127.4, 124.5 (2), 123.7 (2), 123.6, 123.1, 109.6, 109.2 (2), 109.1 (3), 106.1, 92.6 (2), 79.3, 79.1 (3), 76.0, 75.5, 75.1 (2), 75.0 (2), 71.2 (3), 71.1 (2), 71.0 (2), 70.9 (2), 70.7, 70.6, 70.5 (3), 70.4, 70.2, 69.5 (4), 69.2, 68.7, 40.3 (2), 28.4. FAB $m/z$ (relative intensity) 4948.9 (M + H, 100%); HRMS calcd for C$_{301}$H$_{282}$N$_2$O$_6$4 (M + H) 4948.8952, found 4948.8940.

**Galloyl Linker Azide Acid 76.** To a solution of methyl ester 53 (570 mg, 0.934 mmol) in methanol (5.4 mL) and water (1.8 mL) was added LiOH$\cdot$H$_2$O (392 mg, 9.34 mmol) at room temperature. The reaction mixture was stirred at room temperature for 13 h, and then this solution was concentrated in vacuo to remove methanol. The crude colorless oil was diluted with ethyl acetate (50 mL) and washed with 1 M aqueous H$_3$PO$_4$ (10 mL), water (10 mL), and brine (10 mL). The organic layer was dried with Na$_2$SO$_4$, filtered, and concentrated in vacuo to afford 590 mg (100%) of acid 76 as a colorless oil. IR (thin film) 3500-2500, 2104, 1715, cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 11.60 (br s,
1H), 7.48-7.23 (m, 12H), 5.14 (s, 2H), 5.11 (s, 2H), 4.20 (t, \(J = 4.7 \text{ Hz}, 2\text{H}\)), 3.87 (t, \(J = 4.6 \text{ Hz}, 2\text{H}\)), 3.74-3.71 (m, 2H), 3.67-3.60 (m, 12H), 3.33 (t, \(J = 5.0 \text{ Hz}, 2\text{H}\)); \(^{13}\text{C} \text{NMR}\) (75 MHz, CDCl\(_3\)) \(\delta \) 170.9, 152.5, 152.3, 142.6, 137.4, 136.5, 128.4, 128.3, 128.0, 127.9, 127.8, 127.4, 124.5, 109.3, 109.1, 74.8, 71.0, 70.7, 70.5 (2), 70.4, 69.9, 69.5, 68.6, 50.5; APCI \(m/z\) (relative intensity) 613.4 (M + NH\(_4\), 34%), 568.3 (M – N\(_2\), 100%); HRMS calcd for C\(_{31}\)H\(_{37}\)N\(_3\)O\(_9\) (M + NH\(_4\)) 613.2874, found 613.2868; Anal. Calcd for C\(_{31}\)H\(_{37}\)N\(_3\)O\(_9\): C, 62.51; H, 6.26; N, 7.05; Found: C, 62.49; H, 6.26; N, 7.03.

**Dimer Azide Linker 77.** To a solution of diol 26 (79.2 mg, 0.022 mmol) in CH\(_2\)Cl\(_2\) (3 mL) was added acid 76 (38.8 mg, 0.065 mmol) via cannula as a CH\(_2\)Cl\(_2\) solution (0.5 + 0.5 + 0.5 mL), and then 4-di(methylamino)pyridine (2.4 mg, 0.020 mmol), 4-di(methylamino)pyridine•HCl (2.4 mg, 0.015 mmol) and dicyclohexylcarbodiimide (13.4 mg, 0.065 mmol) were added to this solution at room temperature. The yellowish cloudy reaction mixture was stirred at room temperature for 24 h, and then this solution was cooled to -20 °C for 30 minutes, filtered through Celite, and washed with a 1:1 mixture of ether and CH\(_2\)Cl\(_2\) (50 mL). The filtrate was concentrated in vacuo, and then the crude product was diluted with ethyl acetate (25 mL) and washed with 1 M aqueous H\(_3\)PO\(_4\) (8 mL), water (8 mL), and brine (8 mL). The organic layer was dried with
Na$_2$SO$_4$, filtered, and concentrated in vacuo to an oil. The crude product was purified by flash column chromatography using 1% methanol in 50:50 ethyl acetate/hexanes as the eluent to afford 72.2 mg (68%) of dimer azide 77 as a colorless oil along with 25 mg (27%) of a mixture of monoesterified/monoalcohol products as a colorless oil. IR (thin film) 2104, 1731 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.50-6.93 (m, 154H), 6.09 (t, $J = 8.8$ Hz, 2H), 5.98 (t, $J = 9.8$ Hz, 1H), 5.97 (t, $J = 9.6$ Hz, 1H), 5.78 (ddd, $J = 10.4$, 7.8, 2.7 Hz, 4H), 5.18-4.65 (m, 60H), 4.36-4.23 (m, 4H), 4.07-3.93 (m, 4H), 3.73 (t, $J = 4.9$ Hz, 2H), 3.69 (t, $J = 4.9$ Hz, 2H), 3.65-3.47 (m, 24H), 3.33-3.26 (m, 4H); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$ 165.6, 165.4 (2), 165.1, 164.9, 164.8, 163.9, 161.2, 152.7, 152.6 (2), 152.5, 152.4 (2), 152.3 (2), 149.7, 147.5, 146.2, 143.9, 143.2, 143.1, 143.0, 142.9 (2), 142.7, 142.5 (2), 137.6 (2), 137.5, 137.4, 137.3 (2), 136.7 (3), 136.5, 136.4 (3), 136.3, 136.2, 128.7, 128.5 (2), 128.4 (2), 128.3 (3), 128.2 (2), 128.1 (2), 128.0 (2), 127.9 (3), 127.8 (2), 127.7, 127.5 (2), 124.5 (2), 123.7 (2), 123.6, 123.1, 110.6, 110.4, 109.8, 109.2 (3), 109.1 (2), 108.7, 92.9, 92.5, 75.5, 75.1, 75.0, 74.9 (2), 73.3 (2), 72.8, 72.6, 71.2, 71.1 (2), 71.0, 70.9, 70.7 (2), 70.6, 70.5 (3), 69.9, 69.5, 69.4 (2), 68.7, 50.6, 49.2; FAB m/z (relative intensity) 4803.9 (M + H, 8%); HRMS calcd for C$_{291}$H$_{262}$N$_6$O$_{60}$ (M + H) 4800.7713, found 4803.9280.
**Perbenzylated Dimer with Attached Fluorescein 78.** In a microwave vessel, azide 77 (32.7 mg, 0.0068 mmol) was dissolved in THF (1.5 mL) and water (1.5 mL). To the reaction mixture was added CuSO$_4$·5H$_2$O (3.4 mg, 0.014 mmol), sodium ascorbate (5.4 mg, 0.027 mmol), and alkyne 72 (8.7 mg, 0.017 mmol). The yellow reaction mixture was heated in a microwave reactor (300 watts) at 100 °C for 20 minutes, and then this solution was cooled to room temperature and was diluted with ethyl acetate (40 mL) and washed with water (5 mL). The organic layer was dried with Na$_2$SO$_4$, filtered, and concentrated in vacuo to an oil. The crude product was purified by flash column chromatography using 3% acetic acid in ethyl acetate as the eluent. The residual acetic acid was removed as a hexanes azeotrope (3 x 20 mL) to give 23.5 mg (59%) of 78 as an orange oil. IR (thin film) 3400, 1731 cm$^{-1}$; $^1$H NMR (300 MHz, $d_8$-THF) $\delta$ 10.90 (br s, 2H), 9.44 (s, 2H), 8.95 (br s, 2H), 8.20 (s, 2H), 7.92 (d, $J = 8.3$ Hz, 2H), 7.60-6.92 (m, 158H), 6.64-6.56 (m, 8H), 6.45 (dd, $J = 8.6$, 1.9 Hz, 4H), 6.31 (t, $J = 6.7$ Hz, 2H), 6.20 (t, $J = 9.7$ Hz, 2H), 5.94-5.77 (m, 4H), 5.24-4.75 (m, 58H), 4.60-4.50 (m, 2H), 4.39-4.28 (m, 4H), 4.09-3.95 (m, 4H), 3.75-3.65 (m, 4H), 3.62-3.30 (m, 28H), 2.31 (t, $J = 7.6$ Hz, 4H), 2.10-2.00 (m, 4H), 1.67-1.55 (m, 8H), 1.40-1.20 (m, 16H); $^{13}$C NMR (75 MHz, $d_8$-THF)
δ 172.0, 165.8, 160.5, 153.6 (2), 153.5 (4), 148.4, 144.0, 143.9, 143.7, 142.2, 139.1, 139.0, 138.9 (2), 138.2 (2), 137.8 (3), 130.0, 129.4, 129.3, 129.2 (3), 129.0, 128.9 (3), 128.8, 128.7 (2), 128.6 (2), 128.4 (2), 128.3, 126.4, 125.0 (2), 124.9, 112.9, 111.9, 109.9 (2), 109.7 (3), 109.6, 103.1, 77.1, 76.2, 75.6 (2), 75.5, 71.8, 71.7, 71.6, 71.5 (2), 71.4, 71.3, 71.2, 70.9, 70.4 (2), 69.5, 50.5, 49.9, 37.7, 34.8, 32.9, 30.5, 30.4, 30.3 (2), 30.2, 30.1, 30.0, 28.0, 27.6, 26.8, 26.4, 26.3, 23.6; FAB m/z (relative intensity) 5824.2 (M + H, 100%); HRMS calcd for C_{353}H_{320}N_{8}O_{72} (M + H) 5823.1703, found 5824.1896.

**Dimer with Attached Fluorescein 86.** To a solution of perbenzylated 78 (23.5 mg, 0.0040 mmol) in THF (4 mL) was added 10% Pd/C (70 mg). The reaction mixture was purged with hydrogen and stirred under a balloon of hydrogen for 20 h at room temperature. The black reaction solution was filtered through Celite with THF washing (50 mL). Concentration in vacuo afforded 12.2 mg (90%) of a slightly yellow-gray solid. After allowing this compound to be exposed to air as a solid for one week, the hydrogenation product afforded 86 as an orange solid. IR (thin film) 3650-2800, 1731, 1622 cm\(^{-1}\); \(^1\)H NMR (400 MHz, \(d_8\)-THF) δ 9.49 (s, 2H), 9.04 (s, 2H), 8.90 (br s, 2H), 8.45-8.05 (m, 32H), 7.93 (d, J = 7.9 Hz, 2H), 7.62-7.50 (m, 4H), 7.30-6.80 (m, 32H),
6.63-6.58 (m, 6H), 6.46 (dd, J = 8.7 Hz, 2.2 Hz, 2H), 6.43 (d, J = 2.2 Hz, 2H), 6.33 (dd, J = 8.4 Hz, 2.2 Hz, 2H), 6.28 (s, 1H), 6.13 (d, J = 8.3 Hz, 2H), 5.94 (t, J = 9.7 Hz, 2H), 5.66-5.15 (m, 6H), 4.98-4.88 (m, 4H), 4.78-4.39 (m, 4H), 4.30-4.08 (m, 4H), 3.88-3.30 (m, 24H), 2.34 (appr t, J = 7.9 Hz, 4H), 2.24 (appr t, J = 7.6 Hz, 4H), 2.10-2.00 (m, 4H), 1.80-1.60 (m, 20H); \(^{13}\)C NMR (100 MHz, \(d_8\)-THF) \(\delta\) 169.1, 166.1, 166.0, 160.5, 158.1, 157.4, 153.5, 152.5, 148.5, 148.0, 147.5, 146.8, 146.2 (2), 146.1 (2), 146.0, 142.2, 141.4 (2), 132.6, 131.6, 131.5, 130.7, 130.0, 128.9, 126.4, 124.9, 123.7, 121.2, 120.3 (2), 117.1, 112.9, 112.3, 111.8, 111.7 (2), 110.1, 110.0 (2), 109.9 (2), 103.2 (2), 93.8, 93.7, 71.8, 71.4, 71.3, 71.2, 70.5, 70.3, 69.8, 69.6, 69.4, 68.2, 67.9, 50.6, 49.1, 37.7, 34.9, 32.9, 32.2 (2), 31.1, 31.0, 30.6, 30.4, 30.3, 30.1, 26.8, 26.4, 26.0, 25.8, 23.5; -FAB m/z (relative intensity) 3388.8 (M - H, 100%); HRMS calcd for C\(_{164}\)H\(_{158}\)N\(_8\)O\(_72\) (M - H) 3389.8870, found 3388.8381. Fluorescence: \(\lambda_{em} = 516\) nm (\(\lambda_{ex} = 490\) nm) in a pH = 9 phosphate buffer using a 1 cm path length quartz cuvette in a PTi MD-5020 fluorimeter.

![Monomer Azide Linker 83](image.png)

**Monomer Azide Linker 83.** To known alcohol 82\(^{1}\) (170 mg, 0.091 mmol) in CH\(_2\)Cl\(_2\) (7 mL) was added acid 76 (81.0 mg, 0.136 mmol), 4-di(methylamino)pyridine (5.6 mg, 0.046 mmol), 4-di(methylamino)pyridine•HCl (7.3 mg, 0.0.46 mmol) and dicyclohexylcarbodiimide (28.0 mg, 0.136 mmol) at room temperature. The yellowish cloudy reaction mixture was stirred at room temperature for 24 h and then this solution was cooled to -20 °C for 30 minutes, filtered through Celite, and washed with a 1:1
mixture of ether and CH_2Cl_2 (50 mL). The filtrate was concentrated in vacuo and then the crude product was diluted with ethyl acetate (40 mL) and washed with 1 M aqueous H_3PO_4 (10 mL), water (10 mL), and brine (10 mL). The organic layer was dried with Na_2SO_4, filtered, and concentrated in vacuo to an oil. The crude product was purified by flash column chromatography using 50% ethyl acetate in hexanes as the eluent to afford 196.9 mg (88%) of monomer linker azide 83 as a colorless foamy oil. Ester 83 was obtained as a ca. 75:25 inseparable mixture of β:α anomers. IR (thin film) 2104, 1731 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl_3, β-anomer) \(\delta\) 7.50-7.14 (m, 80H), 6.27 (d, \(J = 8.1\) Hz, 1H), 6.10 (t, \(J = 9.7\) Hz, 1H), 5.85 (dd, \(J = 9.6, 8.4\) Hz, 1H), 5.75 (t, \(J = 9.7\) Hz, 1H), 5.21-4.83 (m, 28H), 4.80 (dd, \(J = 11.9, 2.1\) Hz, 1H), 4.51-4.34 (m, 2H), 4.12-4.00 (m, 4H), 3.82 (t, \(J = 4.3\) Hz, 2H), 3.70-3.52 (m, 12H), 3.30 (t, \(J = 5.0\) Hz, 2H); \(^{13}\)C NMR (75 MHz, CDCl_3, both anomers) \(\delta\) 165.8, 165.6, 165.5 (2), 165.1, 164.9 (2), 164.8, 164.1, 163.9, 156.7, 152.9, 152.6 (2), 152.5 (3), 152.4 (2), 152.2, 143.3, 143.2, 143.1 (2), 143.0 (2), 142.8, 142.6, 142.5, 137.4, 137.3, 137.2 (2), 136.6 (2), 136.4 (2), 136.3 (2), 136.2 (2), 136.1, 128.5, 128.4, 128.3 (3), 128.2, 128.1 (2), 128.0 (2), 127.9, 127.8 (2), 127.6, 127.5 (3), 124.5 (2), 123.7, 123.6 (3), 123.5, 123.4, 123.2, 109.4, 109.2, 109.1, 109.0, 108.8, 92.9, 75.2, 75.0 (2), 74.8, 73.2, 71.3, 71.2, 71.1 (2), 71.0, 70.9 (2), 70.7 (2), 70.5, 69.9, 69.7, 69.5, 68.6, 63.1, 50.5; ESI m/z (relative intensity) 2469.2 (M + Na, 4%); HRMS calcd for C_{149}H_{135}N_3O_{30} (M + Na) 2468.9028, found 2469.8840. Fluorescence (MeOH, \(\lambda_{ex} = 468\) nm): 535 nm.
**Perbenzylated Monomer with Attached Fluorescein 84.** To a solution of azide 83 (50.6 mg, 0.0207 mmol) in THF (1.5 mL) and water (1.5 mL) was added alkyne 72 (13.7 mg, 0.0269 mmol), CuSO₄•H₂O (5.2 mg, 0.021 mmol), and sodium ascorbate (8.2 mg, 0.041 mmol). The reaction mixture was heated at 100 °C in a microwave reactor (300 watts) for 10 minutes. This orange solution was cooled to room temperature and was diluted with ethyl acetate (30 mL), and then the product was washed with water (10 mL) and brine (10 mL). The organic layer was dried with Na₂SO₄, filtered, and concentrated in vacuo to an orange solid. The crude product was purified via preparative plate silica gel chromatography with 3% acetic acid in 70% ethyl acetate in hexanes as the eluent. The residual acetic acid was removed as a hexanes azeotrope (3 x 20 mL) to afford 57 mg (93%) of triazole 84 as a yellow solid. Triazole 84 was obtained as a ca. 75:25 inseparable mixture of β:α anomers. IR (thin film) 3322, 1729 cm⁻¹; ¹H NMR (400 MHz, d₆-acetone, β-anomer) δ 9.51 (s, 1H), 9.10 (br s, 2H), 8.45 (s, 1H), 7.89 (d, J = 7.8 Hz, 1H), 7.69-7.08 (m, 82H), 6.78-6.52 (m, 6H), 6.57 (d, J = 7.9 Hz, 1H), 6.41 (t, J = 9.5 Hz, 1H), 6.01 (t, J = 9.7 Hz, 1H), 5.95 (t, J = 8.8 Hz, 1H), 5.25 (s, 1H), 5.21-4.84 (m, 29H),...
4.56 (dd, $J = 11.7$, 4.4 Hz, 1H), 4.39 (t, $J = 5.0$ Hz, 2H), 4.10 (t, $J = 4.0$ Hz, 2H), 3.81 (t, $J = 3.9$ Hz, 2H), 3.74 (t, $J = 4.3$ Hz, 2H), 3.65-3.39 (m, 12H), 2.60 (t, $J = 6.7$ Hz, 2H), 2.38 (t, $J = 6.9$ Hz, 2H), 1.66 (t, $J = 6.3$ Hz, 2H), 1.59 (t, $J = 6.1$ Hz, 2H), 1.36-1.22 (m, 8H); $^{13}$C NMR (100 MHz, $d_6$-acetone) $\delta$ 172.6, 169.5, 166.4, 166.0, 165.9, 164.8, 160.2, 153.7, 153.5 (2), 153.4 (2), 153.3 (2), 148.2, 143.9, 143.7 (2), 143.6, 143.2 (2), 141.9, 138.8, 138.7, 138.6, 138.5, 137.9, 137.8, 137.7 (2), 137.6, 137.5 (2), 137.4 (3), 130.1, 129.4, 129.3 (3), 129.2 (2), 129.1, 129.0 (2), 128.9 (3), 128.8 (3), 128.7 (2), 128.6 (2), 128.5 (2), 126.8, 125.6, 125.0 (2), 124.9, 124.7, 124.3, 114.6, 113.2, 111.8, 109.8 (3), 109.7, 109.5, 109.4, 103.3, 93.9, 75.5 (2), 75.4, 75.3, 74.4, 73.6, 72.8, 72.4, 72.0, 71.7 (2), 71.6, 71.5, 71.4 (2), 71.2 (2), 71.1, 71.0, 70.9, 70.2, 70.1, 69.5, 64.0, 50.4, 37.7, 29.9 (2), 26.2, 26.1; FAB $m/z$ (relative intensity) 2959.7 (M + H, 100%) 2982.0 (M + Na, 23%) 2869 (M – Bn, 33%); HRMS calcd for C$_{180}$H$_{164}$N$_4$O$_{36}$ (M + H) 2958.1204, found 2959.6986.

Monomer with Attached Fluorescein 85. To a solution of perbenzylated 84 (32.0 mg, 0.0108 mmol) in THF (4 mL) was added 10% Pd/C (45 mg). The reaction
mixture was purged with hydrogen and stirred under a balloon of hydrogen for 12 h at room temperature. This black solution was then filtered through Celite with THF washing (50 mL). Concentration of the filtrate in vacuo afforded 22.9 mg (100%) of a yellow solid. The product was not fluorescent green in methanol and LR-MS showed 2 extra hydrogens. After allowing this compound to be exposed as a solid to air for one week, the hydrogenation product became an orange solid, which produced a fluorescent green color solution in methanol. Triazole \textbf{85} was obtained as a ca. 75:25 inseparable mixture of \( \beta:\alpha \) anomers. IR (thin film) 3700-2700, 1726 cm\(^{-1}\); \(^1\)H NMR (400 MHz, \(d_6\)-acetone) \( \delta \) 9.20 (s, 1H), 9.13-7.80 (br s, 16H), 8.23 (s, 1H), 7.71 (s, 1H), 7.57 (dd, \( J = 8.6, 1.8 \) Hz, 1H), 7.21 (d, \( J = 1.8 \) Hz, 1H), 7.18 (s, 2H), 7.17 (d, \( J = 1.7 \) Hz, 1H), 7.06 (s, 2H), 7.04 (s, 2H), 6.99-6.90 (m, 4H), 6.57 (d, \( J = 2.2 \) Hz, 2H), 6.48 (dd, \( J = 8.4, 2.3 \) Hz, 2H), 6.29 (s, 1H), 6.27 (d, \( J = 8.5 \) Hz, 1H), 6.02 (t, \( J = 9.7 \) Hz, 1H), 5.66 (t, \( J = 9.7 \) Hz, 1H), 5.62 (t, \( J = 9.1 \) Hz, 1H), 4.59-4.52 (m, 2H), 4.49 (t, \( J = 4.9 \) Hz, 2H), 4.44-4.36 (m, 1H), 4.27-4.06 (m, 2H), 3.86-3.77 (m, 4H), 3.68-3.51 (m, 12H), 2.61 (t, \( J = 7.5 \) Hz, 2H), 2.32 (t, \( J = 7.4 \) Hz, 2H), 1.64-1.50 (m, 4H), 1.34-1.16 (m, 8H); \(^{13}\)C NMR (100 MHz, \(d_6\)-acetone) \( \delta \) 172.4, 170.5, 166.1 (2), 165.6, 165.3, 164.6, 157.5, 152.0, 147.9, 147.3, 146.1, 145.7 (3), 145.6, 141.0, 139.2, 139.1, 138.9, 138.7, 138.1, 137.6, 132.0, 131.3, 129.4, 128.7, 125.7, 123.5, 122.9, 121.0, 120.2 (3), 120.0, 119.4, 116.9, 111.7, 109.9 (2), 109.8 (2), 107.9, 103.1, 93.3, 73.6, 72.7, 71.5, 70.7 (2), 70.6 (2), 69.7, 69.6, 69.3, 69.0, 67.7, 62.4, 50.4, 37.3, 37.1, 29.8, 29.4, 25.9, 25.6, 21.0; ESCI \( m/z \) (relative intensity) 1699.4 (M + H, 100%), 1721.4 (M + Na, 45%); HRMS (-ESCI) calcd for C\(_{82}\)H\(_{82}\)N\(_4\)O\(_{36}\) (M - H) 1697.4631, found
Fluorescence: $\lambda_{em} = 514$ nm ($\lambda_{ex} = 490$ nm) in a pH = 9 phosphate buffer using a 1 cm path length quartz cuvette in a PTi MD-5020 fluorimeter.

4,6-O-Dibenzyldiene Protected Monomer 88. To a solution of known triol 87 (750 mg, 2.80 mmol) in CH$_2$Cl$_2$ (25 mL) was added 3,4,5-tribenzyloxy benzoic acid (45) (3.90 g, 8.95 mmol), 4-di(methylamino)pyridine (342 mg, 2.80 mmol), 4-di(methylamino)pyridine•HCl (444 mg, 2.80 mmol) and dicyclohexylcarbodiimide (1.80 g, 8.95 mmol). The reaction mixture was stirred at room temperature for 18 h, and then this solution was cooled to -20 °C for 30 minutes, filtered through Celite, and washed with ether (50 mL). The filtrate was washed with 1 M aqueous H$_3$PO$_4$ (8 mL), water (8 mL), and brine (8 mL). The organic layer was dried with Na$_2$SO$_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography with 25% ethyl acetate in hexanes as the eluent to afford 3.14 g (73%) of triester 88 as a white solid ($>90\%$ β-anomer). mp 171-174 ºC; IR (CH$_2$Cl$_2$) 1732 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) δ 7.60-7.00 (m, 56H), 6.12 (d, $J = 8.2$ Hz, 1H), 5.92 (t, $J = 9.5$ Hz, 1H), 5.76 (t, $J = 8.9$ Hz, 1H), 5.58 (s, 1H), 5.13-4.90 (m, 18H), 4.52 (d, $J = 6.0$ Hz, 1H), 4.05-3.86 (m, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 165.2 (2), 164.2, 157.0, 152.6, 152.5 (2), 152.4, 143.1, 143.0, 142.9, 137.3 (2), 136.7, 136.5 (2), 136.4, 136.3, 129.2, 128.6, 128.5, 128.4 (2), 128.3 (2), 128.2, 128.1 (3), 128.0 (2), 127.9 (2), 127.8, 127.6, 127.5 (2), 126.2,
124.2, 123.6, 123.3, 109.5, 109.4, 109.2, 101.7, 93.4, 78.5, 77.2, 75.1 (2), 72.0, 71.9, 71.2, 71.1, 71.0, 68.4, 67.5; ESCI m/z (relative intensity) 1557.5 (M + Na, 100%); HRMS calcd for C\textsubscript{97}H\textsubscript{82}O\textsubscript{18} (M + Na) 1557.5399, found 1557.5374.

![Diagram of Monomer Diol 89]

**Monomer Diol 89.** To a solution of 4,6-\textit{O}-dibenzylidene 88 (2.0 g, 1.3 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (20 mL) and methanol (20 mL) was added iodine (990 mg, 3.90 mmol). The red reaction mixture was stirred at 50 °C for 2 days, covered with aluminum foil, and then the reaction mixture was cooled to room temperature and concentrated in vacuo to remove methanol. The crude product was diluted with CH\textsubscript{2}Cl\textsubscript{2} (40 mL) and washed with 1 M aqueous Na\textsubscript{2}S\textsubscript{2}O\textsubscript{3} (15 mL). The organic layer was dried with Na\textsubscript{2}SO\textsubscript{4}, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography using 50% ethyl acetate in hexanes as the eluent to afford 1.43 g (76%) of diol 89 as a white foamy semisolid. IR (thin film) 3700-2900, 1720 cm\textsuperscript{-1}; \textsuperscript{1}H NMR (300 MHz, CDCl\textsubscript{3}) \textit{\delta} 7.44-7.12 (m, 51H), 6.10 (d, \textit{J} = 8.2 Hz, 1H), 5.71 (t, \textit{J} = 9.0 Hz, 1H), 5.51 (t, \textit{J} = 9.3 Hz, 1H), 5.08 (s, 4H), 5.07 (s, 2H), 5.04 (s, 2H), 5.03 (s, 4H), 4.97 (s, 2H), 4.83 (s, 4H), 4.14-3.78 (m, 4H), 3.74 (br s, 1H), 3.43 (br s, 1H); \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}) \textit{\delta} 167.1, 165.2, 164.3, 152.6 (2), 152.5, 143.1 (2), 137.2 (2), 136.4 (2), 136.2, 128.6, 128.5 (3), 128.4 (3), 128.3, 128.2, 128.1 (3), 127.9 (2), 127.8, 127.6, 127.5 (2), 123.6 (2), 123.4, 109.3 (2), 109.1, 92.8, 77.2, 76.9, 75.1 (2), 71.1, 71.0, 69.4, 61.8; ESCI m/z (relative
intensity) 1469.6 (M + Na, 23%); HRMS calcd for C_{90}H_{78}O_{18} (M + Na) 1469.5086, found 1469.4966.

**TBDPS Protected Monomer 90.** To a solution of 4,6-diol 89 (215 mg, 0.149 mmol) in CH_{2}Cl_{2} (5 mL) was added imidazole (51.0 mg, 0.745 mmol) and t-butyldiphenylsilyl chloride (43 µL, 0.16 mmol) at room temperature. The reaction mixture was stirred at room temperature for 18 h, and then this solution was diluted with CH_{2}Cl_{2} (15 mL) and washed with 1 M aqueous H_{3}PO_{4} (5 mL). The organic layer was dried with Na_{2}SO_{4}, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography with 30% ethyl acetate in hexanes as the eluent to afford 177.5 mg (71%) of alcohol 90 as a colorless foamy oil. IR (thin film) 3700-3100, 1718 cm^{-1}; \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}) \delta 7.71 (d, J = 6.6 Hz, 4H), 7.46-7.13 (m, 57H), 6.10 (d, J = 8.1 Hz, 1H), 5.69 (t, J = 9.0 Hz, 1H), 5.61 (t, J = 9.4 Hz, 1H), 5.09 (s, 6H), 5.06 (s, 6H), 4.98 (s, 2H), 4.84 (s, 4H), 4.19 (td, J = 9.2, 2.6 Hz, 1H), 4.12-4.01 (m, 2H), 3.84 (dt, J = 9.4, 3.9 Hz, 1H), 3.53 (d, J = 2.9 Hz, 1H), 1.08 (s, 9H); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}) \delta 166.9, 165.3, 164.2, 152.6, 143.1, 143.0, 137.3 (2), 136.5, 136.3, 135.7, 135.6, 132.8, 129.9 (2), 128.5 (2), 128.4, 128.3, 128.1 (2), 128.0 (2), 127.9, 127.8 (3), 127.6, 127.5, 123.9, 123.8, 123.6, 109.4 (2), 109.1, 92.8, 77.2, 76.1, 75.1 (2), 71.2 (2), 71.1 (2), 70.6, 63.9, 26.9, 19.3; ESI m/z (relative intensity) 1707.7 (M + Na, 100%); HRMS calcd for C_{106}H_{96}O_{18}Si (M + Na) 1707.6264, found 1707.6237.
TBS Protected Monomer Alcohol 92. To a solution of 4,6-diol 89 (1.02 g, 0.706 mmol) in CH₂Cl₂ (30 mL) was added 4-di(methylamino)pyridine (17.0 mg, 0.141 mmol), triethylamine (492 µL, 3.53 mmol), and t-butyldimethylsilyl trifluoromethanesulfonate (195 µL, 0.847 mmol) at room temperature. The reaction mixture was stirred at room temperature for 12 h, and then this solution was diluted with CH₂Cl₂ (15 mL) and washed with 1 M aqueous H₃PO₄ (5 mL) and brine (5 mL). The organic layer was dried with Na₂SO₄, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography with 40% ethyl acetate in hexanes as the eluent to afford 970 mg (88%) of alcohol 92 as a colorless foamy oil. IR (thin film) 3700-3200, 1722 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 7.38-6.98 (m, 51H), 5.93 (d, J = 7.8 Hz, 1H), 5.54 (t, J = 8.8 Hz, 1H), 5.48 (t, J = 9.3 Hz, 1H), 4.97 (s, 4H), 4.95 (s, 2H), 4.92 (s, 6H), 4.85 (s, 2H), 4.74 (s, 4H), 4.00-3.94 (m, 2H), 3.83 (dd, J = 10.7, 5.5 Hz, 1H), 3.70 (dt, J = 9.2, 4.6 Hz, 1H), 3.60 (br s, 1H), 0.76 (s, 9H), 0.01 (s, 3H), 0.00 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 166.6, 165.3, 164.3, 152.5 (2), 143.0 (2), 142.9, 137.3 (2), 136.5, 136.4, 136.3, 128.6, 128.5 (2), 128.4 (2), 128.3, 128.2, 128.1 (2), 128.0 (2), 127.9 (3), 127.8, 127.6, 127.5 (2), 124.0, 123.8, 123.5, 109.4 (2), 109.1, 92.8, 77.2, 76.5, 75.5, 75.1, 75.0, 71.4, 71.1 (2), 71.0, 64.0, 25.9, 18.3, -5.4, -5.5; ESI m/z (relative intensity) 1583.7 (M + Na, 100%); HRMS calcd for C₉₆H₉₂O₁₈Si (M + Na) 1583.5951, found 1583.6019.
**TBS Protected Monomer 93.** To a solution of alcohol 92 (885 mg, 0.567 mmol) in CH$_2$Cl$_2$ (20 mL) was added 3,4,5-tribenzyloxy benzoic acid (45) (299 mg, 0.680 mmol), 4-di(methylamino)pyridine (14.0 mg, 0.113 mmol), 4-di(methylamino)pyridine•HCl (18.0 mg, 0.113 mmol) and dicyclohexylcarbodiimide (140 mg, 0.680 mmol) at room temperature. The reaction mixture was stirred at room temperature for 16 h, and then this solution was cooled to -20 °C for 30 minutes, filtered through Celite, and washed with a 1:1 mixture of ether and CH$_2$Cl$_2$ (100 mL). The filtrate was concentrated in vacuo, and then the crude product was diluted with ethyl acetate (50 mL) and washed with 1 M aqueous H$_3$PO$_4$ (10 mL), water (10 mL), and brine (10 mL). The organic layer was dried with Na$_2$SO$_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography with 30% ethyl acetate in hexanes as the eluent to afford 1.15 g (100%) of tetraester 93 as a colorless foamy oil. IR (thin film) 1728 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) δ 7.43-6.95 (m, 68H), 6.09 (d, $J = 8.1$ Hz, 1H), 5.88 (t, $J = 9.8$ Hz, 1H), 5.67 (t, $J = 9.0$ Hz, 1H), 5.59 (t, $J = 9.8$ Hz, 1H), 5.07-4.96 (m, 12H), 4.94-4.85 (m, 12H), 4.02-3.96 (m, 1H), 3.84 (dd, $J = 11.8$, 2.4 Hz, 1H), 3.72 (dd, $J = 11.7$, 4.8 Hz, 1H), 0.75 (s, 9H), 0.00 (s, 6H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 165.7, 165.0, 164.7, 164.3, 152.6 (2), 152.5 (2), 143.1, 143.0, 142.9, 137.4, 137.3 (2), 136.5 (3), 136.4, 136.3 (2), 128.6, 128.5 (2), 128.4 (2), 128.3, 128.2, 128.1 (3), 128.0 (3), 127.9 (2), 127.8 (2), 127.6 (3), 127.5 (3), 124.1, 123.9 (2), 123.6, 109.5, 109.4, 109.3,
109.2 (2), 92.9, 77.2, 76.0, 75.1 (2), 73.5, 71.6, 71.2, 71.1 (3), 71.0, 69.4, 62.4, 25.8, 18.3, -5.4 (2); ESCI m/z (relative intensity) 2005.7 (M + Na, 7%); HRMS calcd for C\(_{124}H_{114}O_{22}Si\) (M + Na) 2005.7469, found 2005.7421.

**Monomer Alcohol 94.** To a solution of TBS ether 93 (62 mg, 0.031 mmol) in THF (2 mL) was added acetic acid (5 µL, 0.09 mmol) and a 1 M solution of tetrabutylammonium fluoride in THF (141 µL, 141 mmol) at room temperature. The reaction mixture was stirred at room temperature for 14 h, and then this solution was diluted with ethyl acetate (20 mL) and washed with 1 M aqueous H\(_3\)PO\(_4\) (5 mL), water (5 mL), and brine (5 mL). The organic layer was dried with Na\(_2\)SO\(_4\), filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography with 15% ethyl acetate in hexanes as the eluent to afford 40 mg (68%) of alcohol 94 as a colorless foamy oil. IR (thin film) 3650-3300, 1724 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\) 7.49-7.12 (m, 68H), 6.18 (d, \(J = 8.1\) Hz, 1H), 5.76 (t, \(J = 8.9\) Hz, 1H), 5.65 (t, \(J = 9.2\) Hz, 1H), 5.17-4.90 (m, 20H), 4.82 (s, 4H), 4.77-4.65 (m, 1H), 4.15-4.05 (m, 1H), 4.00-3.85 (m, 2H), 1.65 (br s, 1H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 166.8, 166.7, 165.2, 164.3, 152.6, 152.5, 143.1 (2), 142.8, 137.4, 137.2 (2), 136.6, 136.4, 136.2, 128.5 (3), 128.3 (3), 128.2 (2), 128.1 (3), 128.0 (2), 127.9 (3), 127.8, 127.6, 127.5, 127.4, 124.3, 123.7, 123.6, 123.4, 109.4, 109.3, 109.1, 92.9, 77.2, 76.5, 75.6, 75.1 (2), 75.0, 71.1, 71.0, 69.4, 63.4;
ESCI $m/z$ (relative intensity) 1887.7 ($M + NH_4$, 100%), 1891.7 ($M + Na$, 32%); HRMS calcd for $C_{118}H_{100}O_{22}$ ($M + Na$) 1891.6604, found 1891.6599.

**Monomer Linker Azide 95.** To a solution of alcohol 94 (94 mg, 0.050 mmol) in CH$_2$Cl$_2$ (8 mL) was added acid 76 (45 mg, 0.075 mmol), 4-di(methylamino)pyridine (3.1 mg, 0.025 mmol), 4-di(methylamino)pyridine•HCl (4.0 mg, 0.025 mmol), and dicyclohexylcarbodiimide (12.4 mg, 0.060 mmol) at room temperature. The yellowish cloudy reaction mixture was stirred at room temperature for 12 h, and then this solution was cooled to -20 °C for 30 minutes, filtered through Celite, and washed with a 1:1 mixture of ether and CH$_2$Cl$_2$ (50 mL). The filtrate was concentrated in vacuo, and then the crude product residue was diluted with ethyl acetate (25 mL) and washed with 1 M aqueous H$_3$PO$_4$ (8 mL), water (8 mL), and brine (8 mL). The combined organic layers were dried with Na$_2$SO$_4$, filtered, and concentrated in vacuo to an oil. The crude product was purified by flash column chromatography with 40% ethyl acetate in hexanes as the eluent to afford 73.1 mg (60%) of monomer linker azide 95 as a colorless oil. IR (thin film) 2103, 1729 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) δ 7.50-7.15 (m, 80H), 6.23 (d, $J = 8.1$ Hz, 1H), 6.05 (t, $J = 9.7$ Hz, 1H), 5.85 (t, $J = 9.0$ Hz, 1H), 5.76 (t, $J = 9.7$ Hz, 1H), 5.19-4.90 (m, 28H), 4.77 (d, $J = 10.8$ Hz, 1H), 4.49-4.34 (m, 2H), 4.10 (dd, $J = 9.2$, 4.5 Hz, 2H), 3.79 (t, $J = 4.6$ Hz, 2H), 3.65 (t, $J = 4.7$ Hz, 2H), 3.63-3.53 (m, 12H), 3.32 (t, $J = 5.1$ Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 165.6, 165.0, 164.2, 152.7, 152.6 (2),
152.5 (2), 143.2 (2), 143.1, 143.0, 142.6, 137.5 (2), 137.4, 137.3 (2), 136.8, 136.4, 136.3 (2), 128.6, 128.5 (3), 128.4 (2), 128.3, 128.2, 128.1 (4), 128.0, 127.9 (3), 127.8, 127.6 (3), 124.6, 123.7, 123.5, 123.4, 109.4, 109.3 (2), 109.2, 109.1, 108.9, 93.0, 77.3, 75.1, 75.0, 73.3, 73.2, 71.4, 71.2 (2), 71.1, 71.0, 70.8, 70.6 (3), 70.0, 50.6; ESCI m/z (relative intensity) 2465.0 (M + NH₄, 5%); HRMS calcd for C₁₄₉H₁₃₅N₃O₃₀ (M + Na) 2468.9028, found 2469.8887.

**Perbenzylated Monomer with Attached Fluorescein 96.** In a microwave vessel, azide 95 (56.0 mg, 0.0229 mmol) was dissolved in THF (2 mL) and water (2 mL). To the reaction mixture was added CuSO₄•5H₂O (5.7 mg, 0.023 mmol), sodium ascorbate (9.1 mg, 0.046 mmol), and alkyne 72 (15.2 mg, 0.0297 mmol). The yellow reaction mixture was heated using a microwave reactor (300 watts) at 100 °C for 20 minutes. This solution was cooled to room temperature and was diluted with ethyl acetate (40 mL) and washed with water (5 mL). The organic layer was dried with Na₂SO₄, filtered, and concentrated in vacuo to an orange oil. The crude product was purified by flash column chromatography with 3% acetic acid in ethyl acetate as the eluent. The residual acetic acid was removed
as a hexanes azeotrope (3 x 20 mL) to give 50 mg (74%) of triazole 96 as an orange oil. 
IR (thin film) 3700-3400, 1729 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 9.03 (br s, 2H), 8.18 (br s, 1H), 7.97 (br s, 1H), 7.50-7.12 (m, 92H), 6.93 (br s, 1H), 6.70 (br s, 2H), 6.56 (br s, 3H), 6.22 (d, \(J = 8.0\) Hz, 1H), 6.06 (t, \(J = 9.6\) Hz, 1H), 5.85 (t, \(J = 9.0\) Hz, 1H), 5.75 (t, \(J = 9.6\) Hz, 1H), 5.20-4.85 (m, 29H), 4.77 (d, \(J = 10.7\) Hz, 1H), 4.45 (d, \(J = 6.9\) Hz, 1H), 4.38 (t, \(J = 5.6\) Hz, 2H), 4.06 (d, \(J = 3.6\) Hz, 2H), 3.75 (s, 4H), 3.62 (s, 2H), 3.57-3.43 (m, 10H), 2.61 (br s, 2H), 2.31 (br s, 2H), 1.57 (br s, 4H), 1.34-1.07 (m, 8H); \(^{13}\)C NMR (100 MHz, d\(_8\)-THF) \(\delta\) 172.0, 169.0, 166.4, 165.9, 165.8, 164.8, 160.4, 153.8, 153.7, 153.6 (2), 153.5 (2), 148.5, 144.1 (2), 144.0, 143.9, 143.5, 142.2, 139.1, 139.0, 138.8, 138.3, 137.9, 137.8 (2), 130.0, 129.3, 129.2 (2), 129.0, 128.9 (3), 128.8, 128.7 (3), 128.6 (2), 128.4 (3), 128.3 (2), 126.4, 125.8, 125.1, 125.0, 124.9 (2), 124.6, 114.4, 112.9, 111.9, 110.0, 109.9 (2), 109.8, 109.7, 109.6, 103.2, 94.0, 83.2, 75.6 (3), 75.5, 74.4, 73.8, 72.9, 71.8 (2), 71.7 (2), 71.6, 71.5 (2), 71.4, 71.2, 71.0, 70.4, 70.2, 70.0, 68.0, 67.8, 67.6, 64.0, 37.8, 30.6, 30.3, 30.2, 30.1, 26.3. FAB m/z (relative intensity) 1479.6 (M/2 + H, 100%); HRMS calcd for C\(_{180}H_{164}N_4O_{36}\)\(^{2}\) (M + 2H) 1479.5641, found 1479.5650.
**Monomer with attached fluorescein 97.** To a solution of perbenzylated 96 (55.0 mg, 0.0186 mmol) in THF (4 mL) was added 10% Pd/C (80 mg). The reaction solution was purged with hydrogen and stirred under a balloon of hydrogen for 12 h. The reaction mixture was then filtered through Celite with THF washing (50 mL). Concentration of the filtrate in vacuo afforded 30 mg (95%) of polyphenol 97 as a yellow solid. IR (thin film) 3700-2500, 1726 cm\(^{-1}\); \(^1\)H NMR (400 MHz, d\(_6\)-acetone) δ 9.19 (s, 1H), 8.85-8.22 (br s, 16H), 7.72 (s, 1H), 7.57 (dd, \(J = 8.6, 2.2\) Hz, 1H), 7.19-7.14 (m, 3H), 7.12 (s, 2H), 7.11 (s, 1H), 7.02 (s, 1H), 7.01 (s, 1H), 6.97 (s, 1H), 6.96 (s, 1H), 6.95-6.90 (m, 2H), 6.56 (t, \(J = 2.3\) Hz, 2H), 6.48 (td, \(J = 8.4, 2.4\) Hz, 2H), 6.32 (dd, \(J = 8.3, 2.5\) Hz, 1H), 6.28 (s, 1H), 6.03 (dt, \(J = 9.7, 2.4\) Hz, 1H), 5.62 (t, \(J = 9.5\) Hz, 2H), 4.54 (d, \(J = 11.3\) Hz, 2H), 4.49 (t, \(J = 4.7\) Hz, 2H), 4.44-4.37 (m, 1H), 4.11 (t, \(J = 4.4\) Hz, 2H), 3.83 (t, \(J = 5.0\) Hz, 2H), 3.77 (t, \(J = 4.4\) Hz, 2H), 3.66-3.50 (m, 12H), 2.61 (t, \(J = 7.5\) Hz, 2H), 2.32 (t, \(J = 7.3\) Hz, 2H), 1.65-1.52 (m, 4H), 1.35-1.17 (m, 8H); \(^1^3\)C NMR (100 MHz, d\(_6\)-acetone) δ 172.5, 170.1, 166.4, 166.0, 165.7, 165.6, 164.9, 157.8, 152.3, 148.1, 147.5, 146.1 (2), 146.0, 145.9 (2), 144.7, 141.0, 139.8, 139.3, 139.2, 139.0, 138.1, 132.4, 131.5, 124.0, 123.8, 121.4, 120.6, 120.5, 120.4, 119.9, 117.2, 112.0 (2), 110.4, 110.3, 110.1, 103.4 (2), 103.3, 93.4, 73.9, 71.6, 71.1, 71.0, 70.9, 70.1, 69.8, 68.0, 63.0, 50.7, 37.7, 30.6, 30.5, 26.2, 26.0; ESCI \(m/z\) (relative intensity) 1699.4 (M + H, 45%), 1721.4 (M + Na, 30%); HRMS (-ESCI) calcd for C\(_{82}H\(_{80}\)N\(_4\)O\(_{36}\) (M - H) 1697.4631, found 1697.4655. Fluorescence: \(\lambda_{em} = 516\) nm (\(\lambda_{ex} = 490\) nm) in a pH = 9 phosphate buffer using a 1 cm path length quartz cuvette in a PTi MD-5020 fluorimeter.
5.3 Lihouidine Synthesis Studies

2,3-(Benzyloxy)benzoic Acid (120). Known compound 120 was made with modification of a published protocol. Benzyl bromide (9.9 mL, 83 mmol) was added to a mixture of K$_2$CO$_3$ (14.3 g, 104 mmol), KI (2.6 g, 16 mmol), and commercially available 2,3-dihydroxy benzoic acid (118) (4.0 g, 26 mmol) in acetone (50 mL) at room temperature. The reaction mixture was refluxed for 16 h. After cooling to room temperature, the reaction contents were filtered through Celite with acetone (75 mL) rinsing. This solution was concentrated in vacuo and the crude yellow oil was purified by flash column chromatography using 15% ethyl acetate in hexanes as the eluent to afford 9.84 g (89%) of 2,3-(benzyloxy)benzoic acid benzyl ester (119) as a yellow oil.

The 2,3-(benzyloxy)benzoic acid benzyl ester (9.84 g, 23.2 mmol) was added to a solution of LiOH•H$_2$O (9.73 g, 232 mmol) in methanol (80 mL) and water (26.4 mL) at room temperature. The reaction mixture was refluxed for 16 h. After cooling to room temperature, the reaction contents were concentrated in vacuo to get rid of methanol. Water (200 mL) was then added and the aqueous layer was washed with ether (3 x 50 mL). The aqueous layer was then acidified at 0 °C with concentrated HCl (~50 mL). The aqueous solution was extracted with ether (3 x 100 mL). The organic layer was washed with water (50 mL) and brine (50 mL). The combined organics were dried with Na$_2$SO$_4$, filtered, and concentrated in vacuo to afford 8.26 g (100%) of acid 120 as a white solid. mp 113-115 °C (lit. 113-114 °C) $^{38}$, $^1$H NMR (300 MHz, CDCl$_3$) δ 9.60 (br s, 1H), 7.72-
7.02 (m, 13H), 5.23 (s, 2H), 5.19 (s, 2H). The spectral data matches those reported in the literature.\textsuperscript{148}

![Compound Structure](image)

(2,3-Bis-benzyloxy-phenyl)-carbamic acid Ethyl Ester (121). Diphenyl phosphoryl azide (4.0 mL, 19 mmol), triethylamine (1.8 mL, 25 mmol), and ethanol (7.0 mL, 0.12 mol) were added to a solution of 2,3-bis-benzyloxy benzoic acid (120) (4.13 g, 12.4 mmol) in THF (40 mL) at room temperature.\textsuperscript{116} The reaction mixture was refluxed for 12 h. After cooling to room temperature, the contents were concentrated in vacuo. The crude product was dissolved in ethyl acetate (50 mL) and washed with saturated aqueous NaHCO\(_3\) (2 x 25 mL), water (25 mL), and brine (25 mL). The combined organics were dried with Na\(_2\)SO\(_4\), filtered, and concentrated in vacuo. The product was purified by flash column chromatography using 30% ether in hexanes as the eluent to yield 4.0 g (85%) of carbamate 121 as a yellow oil. IR (thin film) 3420, 1732 cm\(^{-1}\); \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.73 (d, \(J = 8.2\) Hz, 1H), 7.47 (d, \(J = 7.2\) Hz, 2H), 7.42-7.31 (m, 8H), 7.15 (br s, 1H), 7.02 (t, \(J = 8.3\) Hz, 1H), 6.72 (dd, \(J = 8.3, 1.2\) Hz, 1H), 5.15 (s, 2H), 5.07 (s, 2H), 4.17 (q, \(J = 7.1\) Hz, 2H), 1.28 (t, \(J = 7.1\) Hz, 3H); \(^{13}\)C NMR (100 MHz, \(d_6\)-acetone) \(\delta\) 153.9, 152.3, 138.3, 138.0, 137.4, 133.8, 129.3, 129.2, 129.1, 128.8, 128.6, 128.4, 124.6, 112.3, 109.1, 75.3, 71.2, 61.2, 14.7; ESI \(m/z\) (relative intensity) 378.2 (M + H, 100%), 400.1 (M + Na, 52%); HRMS calcd for C\(_{23}\)H\(_{23}\)NO\(_4\) (M + H) 378.1705, found 378.1700.
2,3-Bis-benzyloxy phenylamine (117). To a solution of carbamate 121 (7.9 g, 21 mmol) in ethanol (40 mL) was added KOH (5.8 g, 0.10 mol) at room temperature. The reaction mixture was refluxed for 16 h. After cooling to room temperature, the reaction mixture was concentrated in vacuo to get rid of ethanol. The crude product was dissolved in ether (50 mL) and washed with brine (25 mL). The combined organics were dried with Na$_2$SO$_4$, filtered, and concentrated in vacuo. The crude material was purified by flash column chromatography using 40% ether in hexanes as the eluent to afford 5.9 g (92%) of aniline 117 as an orange oil. IR (thin film) 3465, 3373 cm$^{-1}$; $^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 7.38-7.29 (m, 4H), 7.28-7.12 (m, 6H), 6.72 (t, $J = 8.1$ Hz, 1H), 6.30 (d, $J = 7.7$ Hz, 1H), 6.21 (d, $J = 7.9$ Hz, 1H), 4.94 (s, 2H), 4.93 (s, 2H), 3.65 (s, 2H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 151.8, 140.9, 137.6, 136.9, 134.9, 128.0, 128.0, 128.0, 127.5, 127.4, 127.0, 123.8, 108.8, 103.5, 73.9, 70.1; ESI m/z (relative intensity) 328.2 (M + Na, 100%), 306.2 (M + H, 79%); HRMS calcd for C$_{20}$H$_{19}$NO$_2$ (M + H) 306.1494, found 306.1508.

4-Chloro-7,8-dimethoxyquinoline (128). Known compound 128$^{117}$ was made by a different route than that published. Diphenyl phosphoryl azide (87.8 mL, 412 mmol), triethylamine (39.7 mL, 549 mmol), and ethanol (155.0 mL, 2745 mmol) were added to a solution of commercially available 2,3-dimethoxy benzoic acid (123) (50.0 g, 275 mmol)
in THF (300 mL) at room temperature. The reaction mixture was refluxed for 12 h. After cooling to room temperature, the contents were concentrated in vacuo and the (2,3-dimethoxy)-phenylcarbamic acid ethyl ester 124 was carried on crude.

To a solution of the crude carbamate 124 (61.8 g, 275 mmol) in ethanol (200 mL) was added KOH (154.0 g, 2745 mmol) and the mixture was refluxed for 16 h. After cooling to room temperature, the reaction solution was concentrated in vacuo to get rid of ethanol. The crude product was dissolved in ether (200 mL) and washed with water (100 mL). The product was extracted with 1M aqueous HCl (3 x 75 mL) and separated from the organic phase. Then the aqueous layer was made basic with cold 6M aqueous NaOH (75 mL) and this aqueous solution was extracted with ether (3 x 75 mL). The combined organic layers were washed with brine (75 mL), dried with Na₂SO₄, filtered, and concentrated in vacuo to afford 35.3 g (84%) of 2,3-dimethoxy phenylamine (114) as a brown oil. ¹H NMR (300 MHz, CDCl₃) δ 6.85 (t, J = 8.0 Hz, 1H), 6.39 (dd, J = 8.0, 1.0 Hz, 1H), 6.34 (dd, J = 8.0, 1.0 Hz, 1H), 3.89 (br s, 2H), 3.84 (s, 3H), 3.82 (s, 3H). The spectral data matches those reported in the literature.

Commercially available 2,2-dimethyl-[1,3]dioxane-4,6-dione (130) (20.2 g, 140 mmol) was heated at 100°C in trimethyl orthoformate (100 mL) for 1.5 h. To the resulting 5-methoxymethylene-2,2-dimethyl-[1,3]dioxane-4,6-dione (131) was added aniline 114 (14.0 g, 93.4 mmol) as a methyl orthoformate solution (25 + 15 + 10 mL) via cannula. The reaction mixture was heated at 100°C for 10 h. After cooling to room temperature, the crude reaction solution was concentrated in vacuo to yield the Michael adduct 5-[(2,3-Dimethoxy-phenylamino)-methylene]-2,2-dimethyl-[1,3]dioxane-4,6-dione (127) as a brown solid.
The crude product 127 was dissolved in diphenyl ether (100 mL) and heated at 250°C for 30 minutes. After cooling to room temperature, the reaction contents were poured over hexanes (500 mL) to precipitate the crude 7,8-dimethoxy-1H-quinolin-4-one (111b) as a brown solid.

The crude 4-quinolinone 111b was dissolved in POCl₃ (35 mL) and heated at 100°C for 12 h. Excess POCl₃ was distilled off (20 mm Hg) and the remaining crude product was dissolved in CH₂Cl₂ (100 mL). The remaining POCl₃ was destroyed by washing with cold 6 M NaOH (~50 mL) and the product was extracted with CH₂Cl₂ (3 x 100 mL). The combined organic phases were dried with Na₂SO₄, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography using 50% ethyl acetate as the eluent to afford 12.0 g (59% from aniline 114) of 4-chloro-7,8-dimethoxy-quinoline (128) as a yellow solid. mp 79-80°C (lit. 80-81.5°C); ¹H NMR (300 MHz, CDCl₃) δ 8.78 (d, J = 4.6 Hz, 1H), 7.94 (d, J = 9.3 Hz, 1H), 7.42 (d, J = 9.4 Hz, 1H), 7.35 (d, J = 4.7 Hz, 1H), 4.12 (s, 3H), 4.04 (s, 3H). The spectral data match those reported in the literature.¹¹⁷

7,8-Bis-benzyloxy-4-chloroquinoline (129). 48% Aqueous HBr was added to 4-chloro-7,8-dimethoxy quinoline (128) (2.0 g, 8.9 mmol) and the mixture was heated at reflux for 16 h. After cooling to room temperature, the reaction contents were poured through a Buchner funnel to remove the solid crude product diol, and this product was
washed with ethyl acetate (50 mL). The yellow solid was dried in vacuo to afford 2.3 g (99%) of the catechol•HBr salt.

This catechol•HBr salt was dissolved in acetone (50 mL). Potassium carbonate (9.5 g, 69 mmol), KI (572 mg, 3.45 mmol), and benzyl bromide (2.5 mL, 21 mmol) were added to this solution at room temperature. The reaction mixture was refluxed for 16 h. After cooling to room temperature, the reaction contents were filtered through Celite with acetone washing (75 mL) and the filtrate was concentrated in vacuo. The product was purified by flash column chromatography using 20% ethyl acetate in hexanes as the eluent to afford 2.2 g (73%) of chloride 129 as a yellow solid. mp 127-128 °C; \(^{1}\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.72 (d, \(J = 4.6\) Hz, 1H) 7.83 (d, \(J = 9.3\) Hz, 1H), 7.59-7.50 (m, 2H), 7.45-7.22 (m, 8H), 5.32 (s, 2H), 5.18 (s, 2H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 151.7, 150.0, 145.0, 143.0, 142.5, 137.7, 136.6, 128.5, 128.5, 128.1, 128.0, 127.8, 127.4, 122.6, 119.7, 119.5, 118.2, 76.2, 71.9; ESI m/z (relative intensity) 376.1 (M + H, 100%), 378.1 (M + H, 45%), 398.1 (M+ Na, 9%), 400.1 (M + Na, 4%); HRMS calcd for C\(_{23}\)H\(_{18}\)ClNO\(_2\) (M + H) 376.1104, found 376.1096.

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\text{(7,8-Bis-benzyloxy-quinolin-4-yl)-methylamine (108a).} \]

Chloride 129 (104 mg, 0.277 mmol), 40% methylamine in water (2.0 mL, 23 mmol), and \(n\)-methylformamide (2.5 mL) were heated in a microwave reactor (300 watts) at 150 °C for 12 minutes. After cooling to room temperature, the crude material was extracted with CH\(_2\)Cl\(_2\) (3 x 15 mL)
and the combined organic phases were washed with 1M aqueous Na$_2$CO$_3$ (10 mL). The combined organic layers were dried with Na$_2$SO$_4$, filtered, and concentrated in vacuo. The crude product was purified via flash column chromatography using 3% triethylamine in ethyl acetate as the eluent to afford 70 mg (68%) of aniline 108a as a yellow solid. mp 146-147 °C; IR (CH$_2$Cl$_2$) 3470 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.58 (d, $J = 5.2$ Hz, 1H), 7.54-7.46 (m, 2H), 7.45-7.20 (m, 9H), 7.08 (d, $J = 9.2$ Hz, 1H), 6.28 (d, $J = 5.2$ Hz, 1H), 5.38 (s, 2H), 5.30 (br s, 1H), 5.13 (s, 2H), 2.93 (d, $J = 3.7$ Hz, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 150.9 (2), 150.7, 144.1, 143.3, 138.1, 137.1, 128.5, 128.4, 127.9, 127.8, 127.5, 127.4, 115.2, 115.2, 114.9, 97.3, 75.8, 71.8, 29.9; ESI $m/z$ (relative intensity) 371.2 (M + H, 100%); HRMS calcd for C$_{24}$H$_{22}$N$_2$O$_2$ (M + H) 371.1760, found 371.1774.

2-Chloro-quinoline-4-carboxylic Acid Methyl Ester (109a). Known chloride 109a was made with modification of a published protocol.$^{121}$ Commercially available isatin 133 (30.0 g, 204 mmol) and malonic acid (21.2 g, 204 mmol) were mixed in acetic acid (40 mL) at room temperature and heated to reflux. As the reaction mixture was stirred at refluxed for 12 h, the product precipitated. After cooling to room temperature, the reaction solution was filtered and the solid product was washed with water (100 mL). The product was dried in vacuo and the residual water was removed via a benzene azeotrope. Further drying of the product gave 37.98 g (98%) of 2-oxo-1,2-dihydro-quinoline-4-carboxylic acid (134) as a red-brown solid.
2-Oxo-1,2-dihydro-quinoline-4-carboxylic acid (134) (15.2 g, 80.1 mmol) was dissolved in POCl₃ (30.0 mL, 322 mmol) and heated at 100 °C for 2 h. The black reaction mixture then was cooled to 0 °C and methanol (30 mL) was added dropwise over 30 minutes with stirring as HCl (g) evolved. The ice bath then was removed and the reaction solution was warmed to room temperature for 1 h and then was concentrated in vacuo to remove the methanol. The gummy black oil was dissolved in CH₂Cl₂ (100 mL) and cold 6 M aqueous NaOH (~50 mL) was added to make mixture pH = 10. The crude material then was extracted with CH₂Cl₂ (3 x 75 mL) and the combined organic layers were dried with Na₂SO₄, filtered, and concentrated in vacuo. The crude black gum was purified via flash column chromatography using 15% ethyl acetate in hexanes as the eluent to afford 6.44 g (32%) of 2-chloroquinoline 109a as a white solid. mp 86-88 °C (lit. 89-90 °C)

IR (thin film) 1731 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 8.73 (d, J = 8.5 Hz, 1H), 8.08 (d, J = 8.5 Hz, 1H), 7.90 (s, 1H), 7.70 (t, J = 7.6 Hz, 1H), 7.66 (t, J = 7.7 Hz, 1H), 4.05 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 165.1, 149.9, 148.7, 137.4, 130.7, 128.9, 128.2, 125.5, 123.7, 123.7, 52.8; ESI m/z (relative intensity) 222.0 (M + H, 80%), 224.0 (M + H, 15%); HRMS calcd for C₁₁H₈NO₂Cl (M + H) 222.0322, found 222.0317.

2-Bromo-quinoline-4-carboxylic Acid Methyl Ester (109b). Known bromide 109b was made with modification of a published protocol.¹²¹ To 2-oxo-1,2-dihydro-quinoline-4-carboxylic acid (134) (2.61g, 13.8 mmol) was added POBr₃ (11.9 g, 41.4 mmol) and the reaction mixture was heated at 130 °C for 3 h. The black reaction mixture
was cooled to 0 °C and CH₂Cl₂ (10 mL) was added, and then methanol (10 mL) was added dropwise over 30 minutes with stirring as HCl (g) evolved. The ice bath was removed and the reaction solution was warmed to room temperature for 1 h and then was concentrated in vacuo to remove the methanol. The gummy black oil was dissolved in CH₂Cl₂ (80 mL) and cold 6 M aqueous NaOH (~30 mL) was added to bring the mixture to pH = 10. The crude material then was extracted with CH₂Cl₂ (3 x 50 mL) and the combined organics were dried with Na₂SO₄, filtered, and concentrated in vacuo. The crude black gum was purified via flash column chromatography using 15% ethyl acetate in hexanes as the eluent to afford 813 mg (22%) of 2-bromoquinoline 109b as a brown solid. mp 91-93 °C (lit. 93-94 °C); IR (thin film) 1732 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 8.67 (d, J = 8.3 Hz, 1H), 8.03 (d, J = 8.2 Hz, 1H), 7.97 (s, 1H), 7.74 (t, J = 7.4 Hz, 1H), 7.62 (t, J = 7.5 Hz, 1H), 4.03 (s, 3H); ESI m/z (relative intensity) 266.0 (M + H, 100%), 268.0 (M + H, 95%); HRMS calcd for C₁₁H₉NO₂Br (M + H) 265.9817, found 265.9816.

2-[(7,8-Bis-benzyloxy-quinolin-4-yl)-methyl-amino]-quinoline-4-carboxylic Acid Methyl Ester (107a). Aniline 108a (300 mg, 0.81 mmol), chloride 109a (233 mg, 1.05 mmol), Pd₂dba₃ (77 mg, 0.08 mmol), 2-(dicyclohexylphosphino)biphenyl (60 mg, 0.17 mmol), and K₃PO₄ (516 mg, 2.40 mmol) were mixed in toluene (10 mL) at room
temperature. After removing all oxygen from the solution through the freeze-pump-thaw technique, it was refluxed for 2 days. The reaction mixture was cooled to room temperature and poured over water (15 mL). The mixture then was extracted with CH$_2$Cl$_2$ (3 x 60 mL). The combined organics were dried with Na$_2$SO$_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography using 3% triethylamine in 40% ethyl acetate in hexane as the eluent to give 269 mg (60%) of adduct 107a as an orange oil. IR (CH$_2$Cl$_2$) 1727 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 9.00 (d, $J$ = 4.0 Hz, 1H), 8.43 (d, $J$ = 8.3 Hz, 1H), 7.90 (d, $J$ = 8.3 Hz, 1H), 7.65-7.56 (m, 3H), 7.53 (d, $J$ = 9.2 Hz, 1H), 7.42-7.24 (m, 10H), 7.22 (d, $J$ = 4.3 Hz, 1H), 6.91 (s, 1H), 5.46 (s, 2H), 5.21 (s, 2H), 3.78 (s, 3H), 3.67 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 166.6, 156.0, 151.6, 151.5, 151.0, 148.6, 146.3, 143.6, 137.9, 136.8, 136.4, 130.1, 128.6, 128.5, 128.2, 128.0, 127.8, 127.4, 125.4, 124.2, 122.1, 120.3, 118.9, 118.0, 117.8, 113.6, 76.2, 72.0, 52.5, 39.0; ESI m/z (relative intensity) 556.1 (M + H, 100%), 578.2 (M + Na, 54%); HRMS calcd for C$_{35}$H$_{29}$N$_3$O$_4$ (M + H) 556.2236, found 556.2238.

(7,8-Dimethoxy-quinolin-4-yl)-methylamine (108b). Method A: Chloride 128 (8.83 g, 39.5 mmol), 40% methylamine in water (80.0 mL, 924 mmol), and $n$-methylformamide (40 mL) were heated in a sealed tube at 150 °C for 12 h. After cooling to room temperature, the reaction mixture was extracted with CH$_2$Cl$_2$ (3 x 100 mL) and the organic phases were washed with 1M aqueous Na$_2$CO$_3$ (50 mL). The combined organic layers were dried with Na$_2$SO$_4$, filtered, and concentrated in vacuo. Excess $n$-
methylformamide was distilled off (20 mm Hg) and the crude product was purified by flash column chromatography using 3% triethylamine in 20:80 methanol/ethyl acetate as the eluent to afford 6.67 g of aniline 108b as a brown solid. The brown solid contained some \( n \)-methylformamide that could not be separated from the desired product by any method except hydrolysis. To aniline 108b (6.67 g, 30.6 mmol) dissolved in water (12 mL) and ethanol (48 mL) was added KOH (17.2 g, 306 mmol). The orange mixture was stirred at reflux for 4 h. After cooling to room temperature, the mixture was concentrated in vacuo to remove ethanol. Water (50 mL) was added to the residue and the product was extracted with CH\(_2\)Cl\(_2\) (3 x 100 mL). The combined organic layers were dried with Na\(_2\)SO\(_4\), filtered, and concentrated in vacuo to yield 6.04 g (70%) of aniline 108b as an orange-brown solid.

Method B: Chloride 128 (114 mg, 0.510 mmol), 40% methylamine in water (2 mL), and \( n \)-methylformamide (2 mL) were heated in a microwave (300 watts) at 150 °C for 30 minutes. The reaction solution was worked-up and purified as in method A to yield 70 mg (63%) of aniline 108b. mp 208-210 °C; IR (KBr) 3350 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)) \( \delta \) 8.58 (d, \( J = 5.2 \) Hz, 1H), 7.52 (d, \( J = 9.2 \) Hz, 1H), 7.14 (d, \( J = 9.2 \) Hz, 1H), 6.31 (d, \( J = 5.3 \) Hz, 1H), 5.39 (br s, 1H), 4.08 (s, 3H), 3.97 (s, 3H), 3.00 (d, \( J = 4.2 \) Hz, 3H); \(^1\)3C NMR (100 MHz, CDCl\(_3\)) \( \delta \) 151.5, 151.2, 150.7, 143.9, 143.6, 115.1, 114.8, 112.4, 97.1, 61.5, 56.6, 29.8; ESI m/z (relative intensity) 219.1 (M + H, 100%); HRMS calcd for C\(_{12}\)H\(_{14}\)N\(_2\)O\(_2\) (M + H) 219.1134, found 219.1126.
N-(7, 8-Dimethoxy-quinolin-4-yl)-N-methyl-acetamide (105b). To aniline 108b (700 mg, 3.21 mmol) dissolved in pyridine (8 mL) was added acetic anhydride (3.0 mL, 32 mmol) at room temperature. The solution was heated at 100 °C for 12 h. After cooling to room temperature, the reaction mixture was concentrated in vacuo to remove pyridine. The reaction mixture was then dissolved in CH₂Cl₂ (50 mL) and washed with 1 M aqueous Na₂CO₃ (20 mL). The organic layer was dried with Na₂SO₄, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography using 3% triethylamine in ethyl acetate as the eluent to produce 802 mg (96%) of acetamide 105b as an orange solid. mp 115-117 °C; IR (CH₂Cl₂) 1660 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 9.00 (d, J = 4.5 Hz, 1H), 7.61 (d, J = 9.3 Hz, 1H), 7.47 (d, J = 9.3 Hz, 1H), 7.20 (d, J = 4.5 Hz, 1H), 4.05 (s, 3H), 3.97 (s, 3H), 3.26 (s, 3H), 1.73 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 170.0, 152.1, 151.1, 148.8, 144.7, 143.5, 120.9, 117.7, 116.3, 61.7, 56.6, 36.6, 21.8; ESI m/z (relative intensity) 261.1 (M + H, 100%), 283.1 (M + Na, 54%); HRMS calcd for C₁₄H₁₆N₂O₃ (M + H) 261.1239, found 261.1226.
2-[(7,8-Dimethoxy-quinolin-4-yl)-methyl-amino]-quinoline-4-carboxylic Acid

**Methyl Ester (107b).** Aniline 108b (4.0 g, 18 mmol), chloride 109a (6.0 g, 24 mmol), Pd$_2$dba$_3$ (1.3 g, 1.5 mmol), 2-(dicyclohexylphosphino)biphenyl (1.0 g, 2.9 mmol), and K$_3$PO$_4$ (11.7 g, 55.0 mmol) were mixed in toluene (40 mL) at room temperature. After removing all oxygen from the reaction mixture through the freeze-pump-thaw technique, the solution was refluxed for 2 days. The reaction mixture was then cooled to room temperature and added to water (30 mL). The product was extracted with CH$_2$Cl$_2$ (3 x 100 mL). The combined organic layers were dried with Na$_2$SO$_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography using 3% triethylamine in 60:40 ethyl acetate/hexane as the eluent to give 4.42 g (60%) of adduct 107b as an orange solid. mp 116-118 °C; IR (CH$_2$Cl$_2$) 1726 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) δ 9.01 (d, $J = 4.6$ Hz, 1H), 8.42 (dd, $J = 8.5$, 0.9 Hz, 1H), 7.91 (d, $J = 8.1$ Hz, 1H), 7.66 (ddd, $J = 8.3$, 7.0, 1.3 Hz, 1H), 7.61 (d, $J = 9.3$ Hz, 1H), 7.38 (ddd, $J = 8.3$, 7.0, 1.2 Hz, 1H), 7.32 (d, $J = 9.3$ Hz, 1H), 7.23 (d, $J = 4.6$ Hz, 1H), 6.92 (s, 1H), 4.22 (s, 3H), 4.05 (s, 3H), 3.82 (s, 3H), 3.75 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) δ 167.3, 156.7, 152.9, 152.4, 151.8, 149.3, 146.5, 144.4, 137.2, 130.8, 128.2, 126.1, 124.9, 122.4, 120.9, 119.7, 118.3, 116.5, 114.2, 62.6, 57.5, 53.2, 39.8; ESCI m/z (relative intensity)
404.2 (M + H, 100%), 426.1 (M + Na, 12%); HRMS calcd for C_{23}H_{21}N_{3}O_{4} (M + H) 404.1610, found 404.1596.

2-[(3-Bromo-7,8-dimethoxy-quinolin-4-yl)-methyl-amino]-quinoline-4-carboxylic Acid Methyl Ester 146b. Method A: Compound 107b (25.4 mg, 0.063 mmol) was dissolved in CH_{2}Cl_{2} (1 mL) and the solution was cooled to -78 °C. Bromine (3.2 µL, 0.063 mmol) as a prechilled (-78 °C) CH_{2}Cl_{2} (250 + 250 µL) solution was added to 107b dropwise. The reaction mixture was stirred at -78 °C for 1 h, at which time the solution was diluted with CH_{2}Cl_{2} (10 mL) and washed with 2 M aqueous NaOH (5 mL). The organic layer was dried with Na_{2}SO_{4}, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography using 3% triethylamine in 40:60 ethyl acetate/hexane as the eluent to give 13.6 mg (45%) of bromide 146b as a yellow oil and 13.2 mg of recovered starting material (52%).

Method B: Compound 107b (15.1 mg, 0.037 mmol) was dissolved in CCl_{4} (1 mL). Bromine (3 µL, 0.056 mmol) was added at room temperature and the reaction mixture was refluxed for 1 h. Pyridine (6 µL, 0.07 mmol) was added and the solution was stirred at reflux for an additional 10 h. After cooling to room temperature, the reaction mixture was worked-up and purified as in method A to give 7.0 mg (39%) of bromide 146b along with 9.0 mg (60%) of recovered starting material. IR (CH_{2}Cl_{2}) 1727 cm\(^{-1}\); \(^1\)H
NMR (300 MHz, CDCl$_3$) $\delta$ 9.11 (s, 1H), 8.42 (dd, $J = 8.5$, 1.1 Hz, 1H), 7.96-7.89 (m, 1H), 7.66 (t, $J = 7.3$ Hz, 1H), 7.57 (d, $J = 9.3$ Hz, 1H), 7.37 (t, $J = 8.2$ Hz, 1H), 7.33 (d, $J = 9.4$ Hz, 1H), 6.62 (br s, 1H), 4.18 (s, 3H), 4.02 (s, 3H), 3.83 (s, 3H), 3.63 (s, 3H); $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 166.7, 154.8, 153.6, 152.4, 148.8, 144.2, 143.9, 137.1, 130.1, 128.7, 128.0, 127.5, 125.5, 124.1, 123.7, 120.2, 118.9, 117.0, 116.9, 62.1, 56.8, 52.6, 36.7; ESI m/z (relative intensity) 482.1 (M + H, 100%), 484.1 (M + H, 93%), 504.1 (M + Na, 25%), 506.1 (M + Na, 23%); HRMS calcd for C$_{23}$H$_{20}$BrN$_3$O$_4$ (M + H) 482.0715, found 482.0710.

**General Methods for Nitration of Quinoline Methyl Ester 107b.** Method A: Quinoline 107b (700 mg, 1.74 mmol) was dissolved in ice cold fuming HNO$_3$ (5 mL) and the deep red solution was stirred at 0 $^\circ$C for 1 h. The reaction mixture was diluted with ice and made basic by addition of cold 6 M aqueous NaOH. The product was extracted with CH$_2$Cl$_2$ (3 x 75 mL). The combined organic layers were dried with Na$_2$SO$_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography starting with 3% triethylamine in 30:70 ethyl acetate/hexane as the eluent and increasing in polarity to 3% triethylamine in ethyl acetate to give 407.6 mg (52%) of cyclized nitro compound 153a as an orange solid along with 96.1 mg (12%) of uncyclized compound 154 as an orange solid.
Method B: Quinoline 107b (38.8 mg, 0.096 mmol) was dissolved in concentrated H₂SO₄ (3 mL) and the red solution was cooled to 0 °C. Ice cold fuming HNO₃ (5.7 µL, 0.125 mmol) was added and the deep red solution was stirred at 0 °C for 1 h. The same work-up and purification from Method A produced 13.9 mg (31%) of cyclized nitro compound 153a as an orange solid along with 3.7 mg (9%) of cyclized nitro compound 10b as an orange solid and 4.0 mg (10%) of uncyclized compound 154 as an orange solid.

Method C: To a solution of quinoline 107b (29.7 mg, 0.074 mmol) in chloroform (1 mL) was added tetrabutylammonium nitrate (29.3 mg, 0.0962 mmol). To the orange solution at 0 °C was added trifluoroacetic anhydride (103 µL, 0.74 mmol) to make a dark red solution. The reaction mixture was warmed to room temperature and stirred at room temperature for 1h. The solution was diluted with CH₂Cl₂ (25 mL) and washed with 2 M aqueous NaOH (5 mL). The organic layer was dried with Na₂SO₄, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography using 3% triethylamine in ethyl acetate as the eluent to give 23.5 mg (71%) of nitro product 154 as an orange solid.

2,3-Dimethoxy-7-methyl-12-nitro-7H-4,7,8-triaza-benzo[de]naphthacene-13-carboxylic Acid Methyl Ester (153a). mp 68-70 °C. IR (CH₂Cl₂) 1730 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 9.47 (d, J = 2.5 Hz, 1H), 9.03 (d, J = 4.5 Hz, 1H), 8.31 (dd, J = 9.2, 2.5 Hz, 1H), 7.76 (d, J = 8.9 Hz, 1H), 7.54 (s, 1H), 7.28 (d, J = 4.5 Hz, 1H), 4.22 (s, 3H), 3.95 (s, 3H), 3.85 (s, 3H), 3.63 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 165.4, 157.0, 152.7, 151.3, 150.3, 148.4, 146.7, 145.4, 143.8, 142.0, 137.5, 128.8, 123.9, 123.1, 121.2, 119.3, 114.9, 114.4, 112.6, 62.5, 57.3, 53.0, 39.4; ESI m/z (relative intensity) 447.1 (M +
H, 100%), 404.2 (M – CO₂, 34%); HRMS calcd for C₂₃H₁₈N₄O₆ (M + H) 447.1305, found 447.1291.

2,3-Dimethoxy-7-methyl-9-nitro-7H-4,7,8-triaza-benzo[de]naphthacene-13-carboxylic Acid Methyl Ester (153b). mp 162 °C (dec.); IR (thin film) 1726 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 8.72 (d, J = 5.2 Hz, 1H), 8.52 (d, J = 2.4 Hz, 1H), 8.41 (dd, J = 9.2, 2.4 Hz, 1H), 7.90 (d, J = 9.2 Hz, 1H), 7.58 (s, 1H), 6.69 (d, J = 5.3 Hz, 1H), 4.19 (s, 3H), 4.18 (s, 3H), 4.04 (s, 3H), 3.88 (s, 3H); ¹³C NMR (125 MHz, CDCl₃) δ 168.7, 152.5, 151.1, 148.9, 144.5, 144.3, 135.9, 130.0, 128.8, 127.7, 124.3, 121.5, 121.2, 121.1, 116.9, 115.5, 113.8, 109.8, 100.8, 61.7, 57.0, 53.7, 31.4; ESI m/z (relative intensity) 447.1 (M + H, 100%); HRMS calcd for C₂₃H₁₈N₄O₆ (M + H) 447.1305, found 447.1308.

2-[(7,8-Dimethoxy-5-nitro-quinolin-4-yl)-methyl-amino]-quinoline-4-carboxylic Acid Methyl Ester (154). mp 161-164 °C; IR (thin film) 1727 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 9.04 (d, J = 4.6 Hz, 1H), 8.50 (d, J = 8.2 Hz, 1H), 7.79 (d, J = 8.3 Hz, 1H), 7.61 (t, J = 7.7 Hz, 1H), 7.58 (s, 1H), 7.37 (t, J = 7.7 Hz, 1H), 7.30 (d, J = 4.6 Hz, 1H), 7.16 (s, 1H), 4.25 (s, 3H), 4.02 (s, 3H), 3.88 (s, 3H), 3.44 (s, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 166.4, 154.8, 152.5, 150.0, 149.9, 148.1, 146.3, 145.3, 142.3, 136.4, 129.8, 127.7, 125.3, 124.5, 120.7, 120.6, 114.8, 113.4, 112.1, 62.2, 57.1, 52.4, 39.2; ESI m/z (relative intensity) 449.2 (M + H, 100%), 471.2 (M + Na, 12%); HRMS calcd for C₂₃H₁₉N₃O₄ (M + H) 449.1461, found 449.1440.
2,3-Dimethoxy-7-methyl-7H-4,7,8-triaza-benzo[de]naphthacene-13-carboxylic Acid Methyl Ester (106b). To a solution of cyclized nitro regio-isomers 153a and 153b (451 mg, 1.01 mmol) in ethanol (6 mL) and 0.5 M aqueous HCl (2 mL) was added Fe (1.13 g, 20.2 mmol, -325 mesh) at room temperature. The orange reaction mixture was stirred at room temperature for 12 h. The solution then was concentrated in vacuo to remove ethanol. The orange residue was dissolved in CH$_2$Cl$_2$ (75 mL) and washed with saturated aqueous Na$_2$CO$_3$ (10 mL). The organic layer was dried with Na$_2$SO$_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography using 3% triethylamine in CH$_2$Cl$_2$ followed by 3% triethylamine in 10:90 MeOH/CH$_2$Cl$_2$ as the eluent to afford 360 mg (86%) of amine 155a and amine 155b as a red solid.

To a solution of amine 155a and 155b (360 mg, 0.864 mmol) in 4 M aqueous HCl (5 mL) at 0 °C was added NaNO$_2$ (179 mg, 2.59 mmol). After stirring at 0 °C for 5 minutes, 50% hypophosphorous acid (5.0 mL, 48 mmol) was added. The red reaction mixture was stirred at 0 °C for 12 h and then the solution was treated with cold 6 M aqueous NaOH. The product was extracted with CH$_2$Cl$_2$ (3 x 75 mL). The combined organic layers were dried with Na$_2$SO$_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography using 3% triethylamine in CH$_2$Cl$_2$ followed by 3% triethylamine in 10:90 MeOH/CH$_2$Cl$_2$ as the eluent to afford 171 mg (49% yield).
% of ester 106b as a yellow oil. IR (CH$_2$Cl$_2$) 1731 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$
8.67 (d, $J = 5.3$ Hz, 1H), 7.86 (d, $J = 8.4$ Hz, 1H), 7.66 (ddd, $J = 8.2$, 6.9, 1.2 Hz, 1H),
7.59 (d, $J = 8.3$ Hz, 1H), 7.56 (s, 1H), 7.41 (ddd, $J = 8.1$, 7.0, 1.1 Hz, 1H), 6.59 (d, $J = 5.3$
Hz, 1H), 4.14 (s, 3H), 4.13 (s, 3H), 4.03 (s, 3H), 3.87 (s, 3H); $^{13}$C NMR (125 MHz,
CDCl$_3$) $\delta$ 169.9, 152.3, 152.0, 148.5, 146.3, 146.2, 143.7, 143.4, 135.1, 130.6, 127.7,
125.6, 124.3, 122.4, 122.3, 115.4, 114.7, 108.4, 99.8, 61.5, 56.9, 53.2, 31.0; ESI m/z (relative intensity) 402.1 (M + H, 100%); HRMS calcd for C$_{23}$H$_{19}$N$_3$O$_4$ (M + H)
402.1454, found 402.1477.

2-[(7,8-Dimethoxy-quinolin-4-yl)-methyl-amino]-quinoline-4-carboxylic Acid (163). To methyl ester 107b (500 mg, 1.24 mmol) in THF (10 mL) and water (10 mL)
was added LiOH•H$_2$O (520 mg, 12.4 mmol) at room temperature. The mixture was
stirred at reflux for 12 h. After cooling to room temperature, ice water (5 mL) was added
to the solution, and then 1 M aqueous HCl was added until the pH of the solution was
neutral. The crude product was extracted with CH$_2$Cl$_2$ (3 x 50 mL). The combined organic
layers were dried with Na$_2$SO$_4$, filtered, and concentrated in vacuo to afford 267.5 mg
(55%) of acid 163 as a yellow solid. mp 155-156 $^\circ$C; IR (CH$_2$Cl$_2$) 3200-2300, 1704 cm$^{-1}$;
$^1$H NMR (300 MHz, CDCl$_3$) $\delta$ 9.00 (d, $J = 5.0$ Hz, 1H), 8.69 (br s, 1H), 8.57 (d, $J = 8.3$
Hz, 1H), 7.89 (d, $J = 8.3$ Hz, 1H), 7.61 (t, $J = 7.4$ Hz, 1H), 7.52 (d, $J = 9.4$ Hz, 1H), 7.33
(t, J = 7.5 Hz, 1H), 7.22 (d, J = 5.1 Hz, 1H), 7.18 (d, J = 9.5 Hz, 1H), 7.05 (s, 1H), 3.93 (s, 6H), 3.72 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 169.7, 156.1, 153.8, 152.7, 149.6, 148.1, 142.4, 141.8, 141.1, 129.9, 127.3, 126.3, 124.6, 121.5, 120.7, 120.0, 115.6, 113.8, 61.5, 56.6, 39.5; ESI m/z (relative intensity) 390.5 (M + H, 100%); HRMS calcd for C$_{22}$H$_{19}$N$_3$O$_4$ (M + H) 390.1454, found 390.1450.

![Chemical Structure](image.png)

2-[(7,8-Dimethoxy-quinolin-4-yl)-methyl-amino]-quinoline-4-carboxylic acid methoxy-methyl-amide (164). Method A: To a solution of acid 163 (177 mg, 0.450 mmol) in CH$_2$Cl$_2$ (5 mL) was added N,O-dimethylhydroxylamine hydrochloride (67 mg, 0.68 mmol), triethylamine (188 µL, 1.35 mmol), and dicyclohexylcarbodiimide (140 mg, 0.680 mmol) at room temperature. The solution was stirred at room temperature for 14 h. The reaction contents were concentrated in vacuo. The crude product was purified by flash column chromatography with 3% triethylamine in ethyl acetate as the eluent to give 203.1 mg (100%) of Weinreb amide 164 as an orange semisolid.

Method B: Aniline 108b (150 mg, 0.687 mmol), chloride 165 (224 mg, 0.893 mmol), Pd$_2$dba$_3$ (56 mg, 0.055 mmol), 2-(dicyclohexylphosphinobiphenyl (39 mg, 0.11 mmol), and K$_3$PO$_4$ (437 mg, 2.06 mmol) were mixed in toluene (5 mL) at room temperature. After removing all oxygen from the reaction mixture through the freeze-pump-thaw technique, the solution was refluxed for 2 days. The reaction mixture was
then cooled to room temperature and added to water (10 mL). The product was extracted with CH₂Cl₂ (3 x 50 mL). The combined organic layers were dried with Na₂SO₄, filtered, and concentrated in vacuo. The crude product was purified as in Method A to give 96.7 mg (33%) of adduct 164 as an orange semisolid. IR (CH₂Cl₂) 1657 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 8.90 (d, J = 4.6 Hz, 1H), 7.80 (d, J = 8.2 Hz, 1H), 7.57-7.51 (m, 3H), 7.24 (d, J = 7.2 Hz, 1H), 7.20 (d, J = 4.9 Hz, 1H), 7.16 (d, J = 4.6 Hz, 1H), 6.28 (s, 1H), 4.10 (s, 3H), 3.93 (s, 3H), 3.64 (s, 3H), 3.21 (br s, 3H), 3.13 (br s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 168.2, 155.8, 152.1, 151.6, 151.0, 147.7, 145.7, 143.7, 142.5, 130.1, 127.3, 124.6, 123.6, 121.7, 119.7, 119.0, 117.6, 115.7, 108.8, 61.8, 61.3, 56.7, 38.8, 31.9; ESI m/z (relative intensity) 433.2 (M + H, 100%), 455.2 (M + Na, 38%); HRMS calcd for C₂₄H₂₄N₄O₄ (M + H) 433.1876, found 433.1889.

1-{2-{[7,8-Dimethoxy-quinolin-4-yl]-methyl-amino}-quinolin-4-yl}-ethanone (192). Method A: To a solution of Weinreb amide 164 (70 mg, 0.16 mmol) in THF (3 mL) was added dropwise at 0 °C a 3 M solution of methylmagnesium bromide in ether (162 µL, 0.486 mmol). After 2 h at 0 °C, the reaction solution was poured into a saturated aqueous solution of NH₄Cl (1 mL). After the reaction contents were brought to neutral pH with a saturated aqueous Na₂CO₃ solution, the product was extracted with CH₂Cl₂ (3 x 25 mL). The combined organic layers were dried with Na₂SO₄, filtered, and
concentrated in vacuo. The crude product was purified by flash column chromatography using 3% triethylamine in ethyl acetate as the eluent to give 39.2 mg (62%) of methyl ketone 192 as a yellow oil.

Method B: To a solution of DMF (0.5 mL) and toluene (3 mL) was added NaH (60% dispersion in oil, 20.0 mg, 0.496 mmol). Methyl ester 107b (100 mg, 0.248 mmol) and ethyl acetate (2.0 mL, 21 mmol) were added at room temperature. The reaction mixture was heated at 85 °C for 12 h. After cooling to room temperature, the solution was poured into ice and the product was extracted with CH₂Cl₂ (3 x 25 mL). The combined organic layers were dried with Na₂SO₄, filtered, and concentrated in vacuo.

The crude β-keto ester 193 (114 mg, 0.248 mmol) was dissolved in 10% aqueous H₂SO₄ and heated at 95 °C for 12 h. After cooling to room temperature, the reaction mixture was diluted with ice water and neutralized with solid K₂CO₃ (~ 1g). The product was extracted with CH₂Cl₂ (3 x 30 mL). The combined organic layers were dried with Na₂SO₄, filtered, and concentrated in vacuo. The crude product was purified as in Method A to afford 45.0 mg (47% from methyl ester 107b) of methyl ketone 192 as a yellow oil.

IR (thin film) 1697 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) δ 9.01 (d, J = 4.6 Hz, 1H), 8.09 (d, J = 8.3 Hz, 1H), 7.90 (d, J = 8.4 Hz, 1H), 7.66 (t, J = 7.7 Hz, 1H), 7.61 (d, J = 9.3 Hz, 1H), 7.36 (t, J = 7.7 Hz, 1H), 7.32 (d, J = 9.4 Hz, 1H), 7.24 (d, J = 4.6 Hz, 1H), 6.58 (s, 1H), 4.19 (s, 3H), 4.03 (s, 3H), 3.73 (s, 3H), 2.35 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 201.5, 155.9, 152.2, 151.7, 151.0, 148.6, 145.7, 144.9, 143.7, 130.2, 127.5, 125.1, 124.3, 121.5, 119.0, 119.0, 117.4, 115.7, 110.8, 61.9, 56.8, 39.0, 30.1; ESI m/z (relative intensity) 388.2 (M + H, 100%), 410.2 (M + Na, 6%); HRMS calcd for C₂₃H₂₁N₃O₃ (M + H) 388.1661, found 388.1661.
To a solution of aniline 108b (195 mg, 0.893 mmol) in CH₂Cl₂ (12 mL) at 0 °C was added phosgene as a 20% solution in toluene (1.4 mL, 2.7 mmol). Triethylamine (141 µL, 1.01 mmol) was added and the reaction mixture was stirred at room temperature for 1h. The solution was poured into ice water (10 mL). The product was extracted with CH₂Cl₂ (3 x 40 mL). The combined organic layers were dried with Na₂SO₄, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography using ethyl acetate as the eluent to give 228.8 mg (91%) of carbamoyl chloride 194 as a gray solid. mp 96-98°C; IR (CH₂Cl₂) 1731 cm⁻¹; ¹H NMR (400 MHz, CDCl₃) δ 9.02 (d, J = 4.4 Hz, 1H), 7.62 (d, J = 9.2 Hz, 1H), 7.48 (d, J = 9.3 Hz, 1H), 7.22 (d, J = 4.5 Hz, 1H), 4.16 (s, 3H), 4.07 (s, 3H), 3.46 (s, 3H); ¹³C NMR (75 MHz, CDCl₃) δ 152.4, 151.1, 148.9, 147.4, 145.3, 143.8, 120.5, 118.2, 117.5, 116.7, 61.9, 56.9, 39.9; ESI m/z (relative intensity) 281.1 (M + H, 100%), 283.1 (M + H, 42%), 303.1 (M + Na, 29%), 305.1 (M + Na, 13%); HRMS calcd for C₁₃H₁₂ClN₂O₃ (M + H) 281.0693, found 281.0706.
**N-(7,8-Dimethoxy-quinolin-4-yl)-3-{2-[(7,8-dimethoxy-quinolin-4-yl)-methyl-amino]-quinolin-4-yl}-N-methyl-3-oxo-propionamide (161).** Method A: To a solution of acetamide 105b (100 mg, 0.248 mmol) in THF (3 mL) at 0 °C was added LHMDS as a 1M solution in THF (744 µL, 0.744 mmol). After 0.5h at 0 °C, methyl ester 107b (100 mg, 0.248 mmol) was added via cannula as a THF solution (1 + 0.5 + 0.5 mL). The reaction mixture was then heated at reflux for 16h. After cooling to room temperature, the solution was poured into ice water (10 mL). The product was extracted with CH$_2$Cl$_2$ (3 x 40 mL). The combined organic layers were dried with Na$_2$SO$_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography using ethyl acetate as the eluent to give 98.4 mg (63%) of β-keto amide 161 as a yellow foamy oil.

Method B: To a solution of ketone 192 (20 mg, 0.052 mmol) in THF (1 mL) at 0 °C was added dropwise LHMDS as a 1M THF solution (156 µL, 0.156 mmol). After 0.5 h at 0 °C, carbamoyl chloride 194 (15.9 mg, 0.057 mmol) was added to the reaction via cannula as THF solution (0.5 + 0.5 + 0.5 mL). The reaction mixture was then refluxed for 14h. After cooling to room temperature, the solution was poured into ice water. The work-up and purification was the same as in method A to yield 7.2 mg (22%) of β-keto
amide 161. IR (thin film) 3700-3000, 1661 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)) 14.32 (br s, 1H), 8.91 (appart t, \(J = 4.8\) Hz, 2H), 7.78 (d, \(J = 8.4\) Hz, 1H), 7.67 (d, \(J = 8.0\) Hz, 1H), 7.56-7.45 (m, 3H), 7.40 (d, \(J = 9.3\) Hz, 1H), 7.24 (t, \(J = 10.1\) Hz, 1H), 7.08 (d, \(J = 4.6\) Hz, 3H), 6.31 (s, 1H), 4.65 (s, 1H), 4.19 (s, 3H), 4.15 (s, 3H), 4.07 (s, 3H), 4.03 (s, 3H), 3.62 (s, 3H), 3.39 (s, 3H); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) 170.9, 169.8, 155.6, 152.1, 151.8, 151.2, 150.8, 150.7, 147.8, 147.1, 145.3, 144.9, 143.3, 143.2, 141.5, 129.5, 127.1, 124.3, 122.9, 121.3, 120.5, 119.7, 118.7, 117.8, 117.6, 117.1, 116.2, 115.3, 110.4, 92.7, 61.5, 60.0, 56.5, 56.4, 38.5, 35.9; ESCI \(m/z\) (relative intensity) 632.2, (M + H, 100%), 654.3 (M + Na, 19%); HRMS calcd for C\(_{36}\)H\(_{33}\)N\(_5\)O\(_6\) (M + H) 632.2509, found 632.2519.

![Chemical structure](image)

2-[(7,8-Dimethoxy-quinolin-4-yl)-methyl-amino]-quinoline-4-carbaldehyde (188). To a solution of Weinreb amide 164 (76.8 mg, 0.178 mmol) in toluene (5 mL) at -78 °C was added dropwise a 1 M solution of Dibal in hexane (355 µL, 0.355 mmol). After 1 h at -78 °C, the reaction mixture was warmed to 0 °C and poured into ice. The product was extracted with CH\(_2\)Cl\(_2\) (3 x 30 mL). The combined organic layers were dried with Na\(_2\)SO\(_4\), filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography using ethyl acetate as the eluent to give 48.7 mg (73%) of aldehyde 188 as a yellow oil. IR (thin film) 1703 cm\(^{-1}\); \(^1\)H NMR (300 MHz, CDCl\(_3\)) 9.93 (s, 1H), 8.94 (d, \(J = 4.5\) Hz, 1H), 8.69 (d, \(J = 8.3\) Hz, 1H), 7.87 (d, \(J = 8.4\) Hz, 1H), 7.61
(t, J = 7.7 Hz, 1H), 7.51 (d, J = 9.3 Hz, 1H), 7.34 (t, J = 7.6 Hz, 1H), 7.23 (d, J = 9.3 Hz, 1H), 7.18 (d, J = 4.6 Hz, 1H), 6.72 (s, 1H), 4.10 (s, 3H), 3.94 (s, 3H), 3.67 (s, 3H); \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}) 192.8, 156.4, 152.3, 151.8, 150.7, 148.8, 145.8, 143.8, 138.5, 130.5, 127.3, 125.1, 124.5, 121.6, 119.3, 118.8, 118.7, 117.7, 115.9, 62.0, 56.8, 39.0; ESI \textit{m}/\textit{z} (relative intensity) 374.1 (M +H, 41%); HRMS calcd for C\textsubscript{22}H\textsubscript{19}N\textsubscript{3}O\textsubscript{3} (M + H) 374.1505, found 374.1502.

2,3-Dimethoxy-7-methyl-7\textit{H}-4,7,8-triaza-benzo[de]naphthacene-13-carboxylic Acid (183). To methyl ester 106b (11 mg, 0.027 mmol) in DMF (2 mL) was added LiOH•H\textsubscript{2}O (23.0 mg, 0.548 mmol) at room temperature. The reaction mixture was stirred at 150 °C for 14 h. After cooling to room temperature, ice water was added to the solution, then 1 M aqueous HCl was added until the pH of the reaction contents was neutral. The crude product was extracted with CH\textsubscript{2}Cl\textsubscript{2} (3 x 25 mL). The combined organic layers were dried with Na\textsubscript{2}SO\textsubscript{4}, filtered, and concentrated in vacuo to afford 6.7 mg (64%) of acid 183 as a yellow oil. IR (CH\textsubscript{2}Cl\textsubscript{2}) 3700-2400, 1714 cm\textsuperscript{-1}; -ESI \textit{m}/\textit{z} (relative intensity) 386.1 (M – H, 20%).
N-(7,8-Dimethoxy-quinolin-4-yl)-N-methyl-malonamic Acid Benzyl Ester (177). To a solution of aniline 108b (60.0 mg, 0.275 mmol) in CH₂Cl₂ (5 mL) was added mono benzyl malonate (80.0 mg, 0.413 mmol), 4-di(methylamino)pyridine (6.7 mg, 0.055 mmol), 4-di(methylamino)pyridine•HCl (8.7 mg, 0.055 mmol), and dicyclohexylcarbodiimide (68.0 mg, 0.330 mmol) at room temperature. The reaction mixture was stirred at room temperature for 14 h. The reaction contents were diluted with CH₂Cl₂ (30 mL) and washed with water (8 mL). The combined organic layers were dried with Na₂SO₄, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography with 70% ethyl acetate in hexanes as the eluent to afford 77 mg (71%) of β-amide ester 177 as a yellow sticky solid. mp 193-196 °C; IR (thin film) 3319, 1738, 1666 cm⁻¹; ¹H NMR (300 MHz, CDCl₃) 8.92 (d, J = 4.5 Hz, 1H), 7.58 (d, J = 9.2 Hz, 1H), 7.39 (d, J = 9.3 Hz, 1H), 7.37-7.25 (m, 5H), 7.14 (d, J = 4.5 Hz, 1H), 5.05 (s, 2H), 4.11 (s, 3H), 4.04 (s, 3H), 3.38 (s, 3H), 3.20-3.10 (m, 2H); ¹³C NMR (100 MHz, CDCl₃) 166.9, 165.7, 157.6, 152.6, 147.9, 144.9, 143.6, 135.3, 135.1, 128.4, 128.4, 128.2, 120.7, 118.3, 118.1, 67.1, 67.0, 57.1, 56.5, 41.2; ESI m/z (relative intensity) 395.1 (M + H, 100%), 417.1 (M + Na, 27%); HRMS calcd for C₂₂H₂₂N₂O₅ (M + H) 395.1607, found 395.1597.

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\text{N-(7,8-Dimethoxy-quinolin-4-yl)-N-methyl-malonamic Acid (178). To a solution of benzyl ester 177 (77.0 mg, 0.195 mmol) in THF (5 mL) was added 10% Pd/C}
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(20 mg). The reaction mixture was purged with hydrogen and stirred under a balloon of hydrogen for 20 h. The reaction mixture was then filtered through Celite with THF washing (50 mL). Concentration of the filtrate in vacuo afforded 43.6 mg (73%) of β-amide acid 178 as a white solid. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.95 (d, $J = 3.9$ Hz, 1H), 8.23 (br s, 1H), 7.62 (d, $J = 9.2$ Hz, 1H), 7.43 (d, $J = 9.2$ Hz, 1H), 7.33 (d, $J = 3.8$ Hz, 1H), 4.13 (s, 3H), 4.05 (s, 3H), 3.41 (s, 3H), 3.19 (s, 2H).

2-Chloro-quinoline-4-carboxylic Acid Methoxy-methyl-amide (165). To a solution of $N,O$-dimethylhydroxylamine•HCl (132 mg, 1.35 mmol) in CH$_2$Cl$_2$ (3 mL) at 0 °C was added dropwise AlMe$_3$ as a 2 M solution in toluene (675 µL, 1.35 mmol). After addition of AlMe$_3$, some bubbles evolved from the solution. After stirring this mixture for 20 minutes at 0 °C, methyl ester 109a (100 mg, 0.451 mmol) was added as a solid. The reaction solution was stirred at room temperature for 14 h, and then the mixture was poured into ice water. The reaction contents were then acidified with 1 M aqueous HCl (10 mL) to pH = 2 and the product was extracted with CH$_2$Cl$_2$ (3 x 30 mL). The combined organic layers were dried with Na$_2$SO$_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography with 50% ethyl acetate in hexanes as the eluent to give 96.3 mg (85%) of Weinreb amide 165 as a white solid. mp 129-130 °C; IR (CH$_2$Cl$_2$) 1660 cm$^{-1}$; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.98 (d, $J = 8.5$ Hz, 1H), 7.77 (d, $J = 8.3$ Hz, 1H), 7.69 (t, $J = 7.7$ Hz, 1H), 7.52 (t, $J = 7.6$ Hz, 1H), 7.33
(s, 1H), 3.39 (br s, 3H), 3.32 (br s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 166.4, 149.5, 147.7, 144.5, 130.8, 128.7, 127.4, 124.8, 123.0, 118.9, 61.5, 32.0; ESCI $m/z$ (relative intensity) 251.1 (M + H, 100%), 253.1 (M + H, 44%); HRMS calcd for C$_{12}$H$_{11}$ClN$_2$O$_2$ (M + H) 251.0587, found 251.0587.

![2-Chloro-quinoline-4-carbaldehyde](image)

**2-Chloro-quinoline-4-carbaldehyde (186).** To a solution of ester 109a (52.0 mg, 0.235 mmol) in toluene (2 mL) at -78 °C was added a 1 M solution of Dibal in hexanes (235 µL, 0.235 mmol) dropwise. After stirring this solution for 1 h at -78 °C, the reaction mixture was poured over 1 M aqueous H$_3$PO$_4$ (5 mL). The product was extracted with ether (3 x 30 mL) and washed with brine (5 mL). The organic layer was dried with Na$_2$SO$_4$, filtered, and concentrated in vacuo. The crude product was purified by flash column chromatography with 30% ethyl acetate in hexanes as the eluent to give 32.9 mg (73%) of aldehyde 186 as a white solid. mp 99 °C; IR (CH$_2$Cl$_2$) 1698 cm$^{-1}$; $^1$H NMR (360 MHz, CDCl$_3$) $\delta$ 10.40 (s, 1H), 8.86 (d, $J$ = 8.4 Hz, 1H), 8.06 (d, $J$ = 8.4 Hz, 1H), 7.81 (t, $J$ = 7.7 Hz, 1H), 7.72 (s, 1H), 7.69 (t, $J$ = 7.2 Hz, 1H); $^{13}$C NMR (90 MHz, CDCl$_3$) $\delta$ 191.0, 150.5, 148.9, 139.2, 131.1, 129.3, 128.9, 127.1, 124.3, 122.5; ESCI $m/z$ (relative intensity) 192.0 (M + H, 100%), 194.0 (M + H, 42%); HRMS calcd for C$_{10}$H$_6$ClNO (M + H) 192.0216, found 192.0210.
5.4 Ellagitannin In Vivo Biological Experiments

**Dose-Response Data: General Procedure.** All cell lines were obtained from commercial sources. The cells were counted and the viability was determined by Trypan Blue exclusion (typically, viability exceeded 95%). The concentration of the cells in the 0.5 mL wells was adjusted to 1 x 10^5 cells/well by diluting with the required amount of DMEM-10 media one day before the experiment. One hour before the experiment, the cells were washed with fresh media and 0.5 mL of DMEM-10 media was added to each well. A 250 µM stock solution for each tannin was made by first dissolving the tannin in ~20 µL of DMSO and then the required amount of media. The appropriate amount of a tannin (or LPS) stock solution in Hanks Buffer Saline Solution was added to each well to furnish the concentration values reported in the Figures. Each concentration value was run in triplicate, and blank runs ensured that (bacterial) contamination did not complicate the experiments. The culture plates were incubated in a 5% CO₂, 37 °C humidified incubator for the indicated time. At the end of the time interval, 250 µL of the culture supernatant from each well was harvested, after centrifugation at 400 g, 25 °C, 10 min, with brake, and stored at -78 °C pending ELISA analysis for the cytokine. The ELISA assays were conducted per the manufacturer's instructions using standard calibration curves to calculate cytokine concentration from observed absorbance readings. The cytokine values reported are averages of three runs + SE.

**Time Course Data General Procedure:** Each well was prepared as with the dose-response experiments. A separate well was used for each time point and the appropriate time, a sample was collected and stored in the -78 °C pending ELISA analysis.
for the cytokine. The ELISA assays were conducted per the manufacturer's instructions using standard calibration curves to calculate cytokine concentration from observed absorbance readings. The cytokine values reported are averages of three runs + SE.

5.5 2D NMR Spectra
Figure 5.1: HMBC of 153a.
Figure 5.2: NOESY of 153a.
Figure 5.3: HMBC of 153b.
Figure 5.4: NOESY of 153b.
Figure 5.5: HMBC of 106b.
Bibliography


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