Synthesis of Benzoquinone Antioxidants and a Bleomycin Disaccharide Library

by

Manikandadas Mathilakathu Madathil

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Graduate Supervisory Committee:

Sidney M. Hecht, Chair
Seth Rose
Neal Woodbury

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ABSTRACT

Healthy mitochondria are essential for cell survival. Described herein is the synthesis of a family of novel aminoquinone antioxidants designed to alleviate oxidative stress and prevent the impairment of cellular function. In addition, a library of bleomycin disaccharide analogues has also been synthesized to better probe the tumor targeting properties of bleomycin.

The first study involves the synthesis of a benzoquinone natural product and analogues that closely resemble the redox core of the natural product geldanamycin. The synthesized 5-amino-3-tridecyl-1,4-benzoquinone antioxidants were tested for their ability to protect Friedreich's ataxia (FRDA) lymphocytes from induced oxidative stress. Some of the analogues synthesized conferred cytoprotection in a dose-dependent manner in FRDA lymphocytes at micromolar concentrations. The biological assays suggest that the modification of the 2-hydroxyl and *N*-(3-carboxypropyl) groups in the natural product can improve its antioxidant activity and significantly enhance its ability to protect mitochondrial function under conditions of oxidative stress.

The second project focused on the synthesis of a library of bleomycin disaccharide-dye conjugates and monitored their cellular uptake by fluorescence microscopy. The studies reveal that the position of the carbamoyl group plays an important role in modulating the cellular uptake of the disaccharide. It also led to the discovery of novel disaccharides with improved tumor selectivity.

ACKNOWLEDGEMENTS

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Last but not the least; I am eternally indebted to my parents and my sister for always letting me pursue my dreams and for their constant love, support and encouragement. To them, I dedicate this thesis.

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LIST OF ABBREVIATIONS

Å	angstrom
anh	anhydrous
aq	aqueous
atm	atmosphere
BLM	bleomycin
Bn	benzyl
Boc	t-butoxycarbonyl
br s	broad singlet
Bu_2BOTf	dibutylborontriflate
BSO	buthionine sulfoximine
CAN	ceric ammonium nitrate
d	doublet
dd	doublet of doublets
DEM	diethyl maleate
DIBAL	diisobutylaluminium hydride
DIPEA	diisopropyl ethylamine
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dt	doublet of triplets
EtOAc	ethyl acetate

Fmoc	*N*-(9-fluorenylmethoxy-carbonyloxy)
FRDA	Friedreich's Ataxia
g	grams
GSH	glutathione
h	hours
^{1}H	NMR proton nuclear magnetic resonance spectroscopy
HOBt	1-hydroxybenzotriazole
HPLC	high performance liquid chromatography
Hz	hertz
J	coupling constant
JC-1	5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolocarbocyanine iodide
m	multiplet
MALDI-TOF	matrix assisted laser desorption ionization - time of flight
MHz	mega-hertz
min	minutes
mL	milliliter(s)
mmol	millimole(s)
MW	molecular weight
μmol	micromole(s)
NADH	nicotinamide adenine dinucleotide
nm	nanometer
NMR	nuclear magnetic resonance

N	normal
q	quartet
quin	quintet
R_f	ratio of fronts
ROS	reactive oxygen species
RNA	ribonucleic acid
rt	room temperature
s	singlet
satd	saturated
SMP	submitochondrial particles
t	triplet
TEA	triethylamine
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TMRM	tetramethylrhodamine methyl ester
TMSOTf	trimethylsilyl triflate
TSTU	2-succinimide-1,1,3,3-tetramethyluronium tetrafluoroborate
v	volume

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CHAPTER 1

1. INTRODUCTION

The mitochondria are cellular organelles that play a vital role in maintaining cellular function and are essential for cell survival.[1,2] In addition to the generation of ATP, mitochondria play a key role in many cellular processes such as ion homeostasis,[3] innate immune response[4] and programmed cell death.[5] Impaired mitochondrial function is associated with several pathological conditions such as Alzheimer's disease,[6] Parkinson's disease,[7] cancer,[8] diabetes,[9] epilepsy,[10] Huntington's disease[11] and obesity.[12]

Mitochondrial dysfunction can arise from either a primary or secondary mitochondrial disorder.[13] A primary disorder is caused by a mutation of any one of the genes encoding mitochondrial proteins while a secondary disorder is attributed to external effects like viral infections[14] and off-target drug effects.[15,16] Mitochondrial DNA (mtDNA) is particularly susceptible to damage by reactive oxygen species (ROS) as the mitochondrion is the main source of ROS in cells.[17]

In healthy mitochondria ROS is generated by tightly regulated cellular enzymes like NADPH oxidase and NO synthase.[18] As a result in normal cells ROS is always present in low concentrations and plays a crucial role in physiological processes like cell signaling and immune response.[19] In diseased cells, however, disruptions in the mitochondrial electron transport chain can cause overproduction of ROS leading to oxidative stress, exposing cellular components to oxidative damage.[20-25] In neuronal degenerative diseases such as Alzheimer's

disease,[26] Parkinson disease[27-29] and amyotrophic lateral sclerosis (ALS),[30] this exposure to oxidative stress has been found to cause mutations and deletions in the mitochondrial DNA (mtDNA) causing mtDNA damage.

Mitochondrial DNA encodes for 13 of the approximately 100 proteins that make up the electron transport chain machinery located in the inner membrane of mitochondria.[31] The machinery works by electron transport, driven by the generation of an electrochemical gradient across the mitochondrial inner membrane. In a dysfunctional mitochondria, the flow of electrons through complex I is interrupted and electrons are redirected to oxygen, generating superoxide ($O_2^{-\bullet}$). Superoxide by itself is relatively inert towards biological molecules like lipid membranes, proteins and DNA; however, it can undergo a spontaneous or enzyme catalyzed (superoxide dismutase) disproportionation reaction with itself to form hydrogen peroxide (H_2O_2) and molecular oxygen (O_2). The peroxide can undergo the Fenton reaction (Figure 1.1) in presence of Fe^{2+} ions to produce a hydroxyl radical and a hydroxide ion.

$$2O_2^{-\cdot} + 2H^+ \longrightarrow H_2O_2 + O_2$$
$$H_2O_2 + Fe^{2+} \longrightarrow Fe^{3+} + OH^{\cdot} + OH^-$$

Figure 1.1. Formation of hydroxyl radical by the Fenton reaction.

The hydroxyl radicals diffuse through cells readily and are capable of reacting with virtually any biological molecule like DNA, proteins and lipids. It can also react with superoxide to generate the highly reactive singlet oxygen ($^1O_2$).

A particularly deleterious and damaging reaction mediated by hydroxyl radicals involves the abstraction of hydrogen atoms (H•) from lipid membranes to form a carbon centered radical (R•).[32] This radical reacts readily with oxygen to form the highly reactive peroxyl radical (ROO•) which is capable of abstracting a hydrogen atom from the lipid membrane to further generate R•. This ultimately leads to a chain reaction producing multiple oxidative lesions from a single hydroxyl radical.[32]

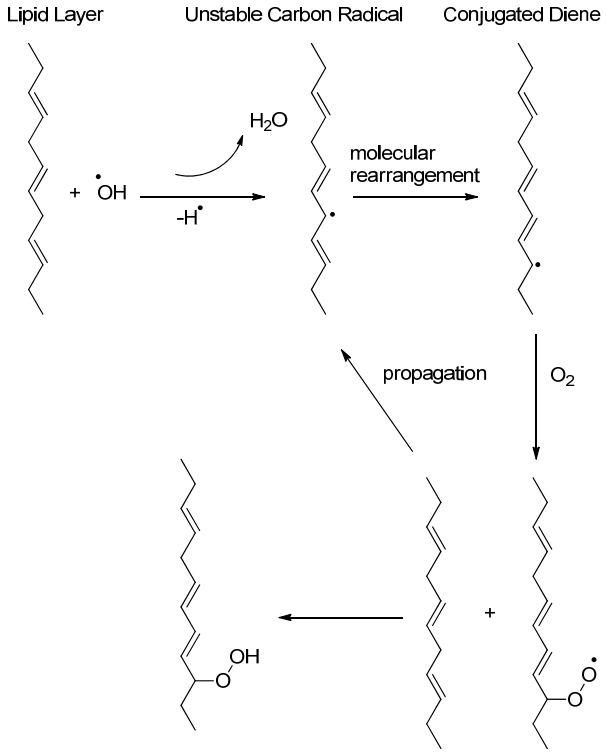


Figure 1.2. Reaction sequence of lipid peroxidation. (modified from ref. 32)

1.1 Mitochondrial diseases

Friedreich's ataxia is a neurodegenerative disease characterized by the progressive ataxia of the limbs, muscle weakness, skeletal deformities and

cardiomyopathy. The biochemical basis of the disease is attributed to a deficiency in frataxin, a mitochondrial protein[33] essential for the assembly of Fe-S clusters, resulting in a compromised mitochondrial respiratory chain. Frataxin deficiency, therefore, leads to higher levels of H_2O_2 and Fe^{2+}, resulting in increased generation of ROS. Oxidative stress has been found to play an important role in the progression of the disease;[34] therefore, strategies to overcome oxidative stress may have therapeutic potential.

Huntington's disease is a neurodegenerative genetic disorder clinically characterized by chorea, ataxia and dementia. The biochemical basis of the disease is attributed to an abnormally expanded cytidine-adenosine-guanosine (CAG) repeat in the huntington gene on chromosome 4.[35] Biochemical analyses have shown a deficiency of complexes II, III and IV coupled with a decrease in aconitase acitivity.[36-38] These findings suggest that antioxidant therapy may be helpful in treating patients with Huntington's disease.

Alzheimer's disease is another neurodegenerative disease linked to mitochondrial dysfunction. Several complementary studies have shown that mitochondrial function is severely compromised in Alzheimer's disease cells. It has been observed that complex IV activity is significantly reduced in the brain of patients with Alzheimer's disease.[39-41] Another distinct pathological feature of Alzheimer's disease cells is the decrease in the expression of nuclear and mtDNA encoded COX subunits.[42]

Amyotrophic lateral sclerosis (ALS) is a motor neuron disease arising from a dysfunctional mitochondrion. Mutations in the gene for superoxide

dismutase 1 (SOD-1) is observed in about 20% of patients suffering from the disease, causing neuronal damage by enhanced peroxidation.[43,44] Increased concentration of 8-hydroxy-2-deoxyguanosine, an indicator of oxidative DNA damage, has also been observed in the plasma and urine of ALS patients, and its amount has been found to increase progressively with time.[45]

The mitochondrion plays a central role in diverse cellular functions. It is therefore hardly surprising that mitochondrial damage cripples cellular function. Evidence is emerging that mitochondrial dysfunction is a common pathogenic feature in several neurodegenerative diseases. The development of therapeutic strategies to alleviate mitochondrial defects may provide novel drugs for the treatment of neurodegenerative disorders

1.2 Cancer

Cancer is a deadly disease causing one out of every eight deaths worldwide.[46] It is actually a collection of more than 100 distinct diseases originating from most of the cell types and organs of the human body.[47] A common characteristic of cancerous cells is their ability to proliferate in an unrestrained manner and to invade beyond normal tissue boundaries and metastasize to other organs.[47]

Chemotherapeutic agents capable of inflicting damage specifically to cancer cells are potent tools in fighting unregulated cell growth. They restrict cell proliferation by inhibiting crucial cellular functions like cell division,[48-50] protein synthesis[51] and deoxyribonucleic acid (DNA) replication and transcription.[52] The discovery of DNA as the hereditary material[53] and the subsequent finding that

chemical agents capable of causing DNA damage and mutations can cause cancer,[54] led to the increased scrutiny of cancer cell chromosomes.[55,56] The identification and the isolation of the first oncogene further validated the role of DNA mutations in enabling cells to proliferate and metastasize.[57,58] These findings, which implicated DNA damage as a key cause for the development of human cancer, also underlined its importance as a therapeutic target.

The two major categories of drug-DNA interactions are intercalation and groove binding.[59] Intercalation requires the insertion of a planar molecule between DNA base pairs resulting in a decrease of DNA helical twist and lengthening of DNA.[60] Groove binders do not induce conformational changes in the DNA. They bind to the minor groove of the DNA and are stabilized by intermolecular interactions.[61]

Therapeutic agents that affect DNA function by modulating its interaction with DNA processing enzymes like endonucleases, topoisomerases or polymerases are subjects of intense study.[62,63] Disruptions in the function of DNA processing enzymes have a profound effect on cancer cells as compared to normal cells owing to their rapid division. For left uncorrected, this would lead to the accumulation of DNA mutations, causing cell death.[64-68]

The design and synthesis of small molecules capable of targeting DNA in cancer cells is an active field of research in the pharmaceutical industry. The anticancer effects of radiation therapy and many chemotherapeutic agents can be attributed to the cytotoxicities arising from DNA damage, which cripples vital cellular processes such as transcription and replication.[69] Targeting particular

components of DNA repair pathways in cancer cells like DNA double-strand break repair, base excision repair and nucleotide excision repair would improve the efficacy of anticancer treatments.[69]

The bleomycins (BLMs), first discovered by Umezawa et al.,[70,71] are a family of water soluble glycopeptidic antibiotics used in anticancer chemotherapy owing to their cytotoxicity towards cancer cells. Different structural variants of naturally occurring BLMs, differing primarily at the C-terminus of the glycopeptide, have been identified from fermentation broths. The initially proposed structure of BLM was revised in 1978[72] and confirmed by total synthesis in 1982.[73,74] Many natural products like phleomycins (PLMs),[75-78] tallysomycins (TLMs)[79,80] and zorbamycin (ZBM),[81] which are structurally and biosynthetically related to the BLMs, have been isolated (Figure 1.3).[82]

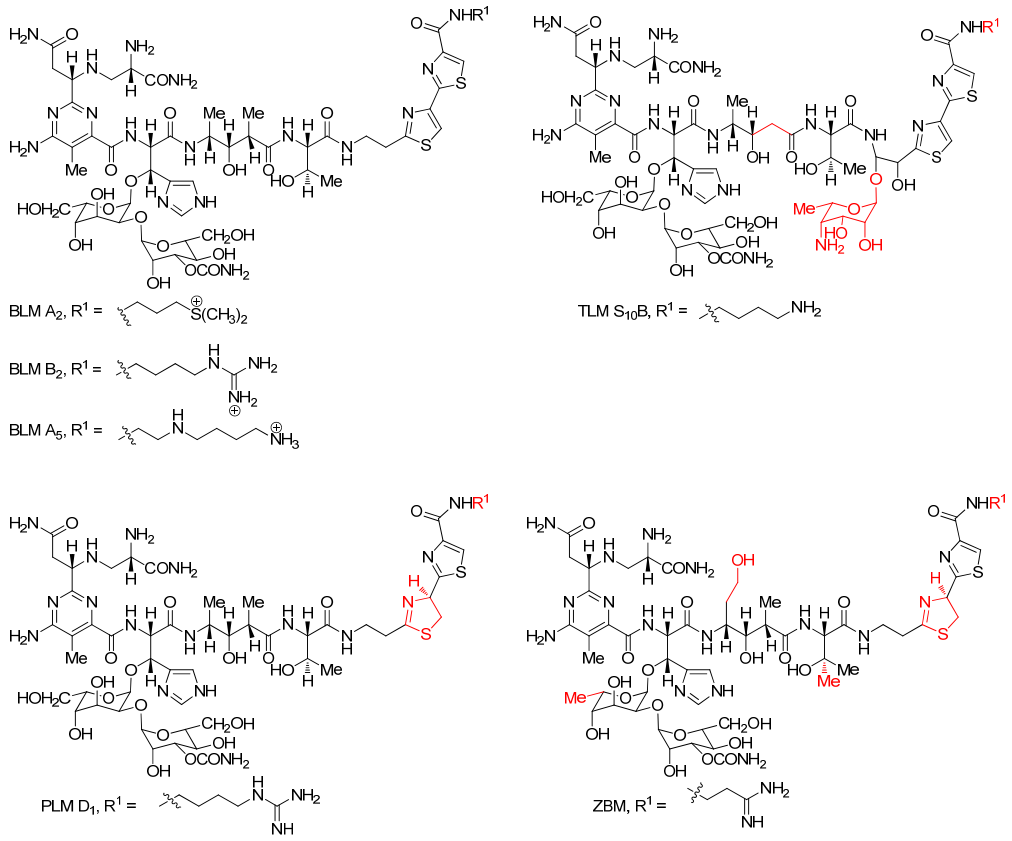


Figure 1.3. Structures of bleomycins (BLMs) and structurally related antitumor antibiotics: tallysomycin (TLM), phleomycin (PLM) and zorbamycin (ZBM). Structural differences between the different natural products and BLMs are highlighted in red. (adapted from ref. 82)

The structure of BLMs can be dissected into four functional domains (Figure 1.4).[83] The metal binding domains comprised of the pyrimidoblamic acid subumit along with the adjacent β–hydroxyhistidine moiety. This domain provides the coordination sites required for Fe^{2+} complexation and molecular oxygen activation responsible for DNA cleavage.[84] The bithiazole and C-terminal domain is responsible for the affinity of BLM towards DNA. In addition, it is also

believed to play a crucial role in polynucleotide recognition and DNA cleavage selectivity.[84] The (2*S*, 3*S*, 4*R*)-4-amino-3-hydroxy-2-methylpentanoic acid (AHM) subunit acts as a linker between the DNA and metal binding sites of bleomycin and is essential for efficient cleavage of DNA by BLMs.[84]

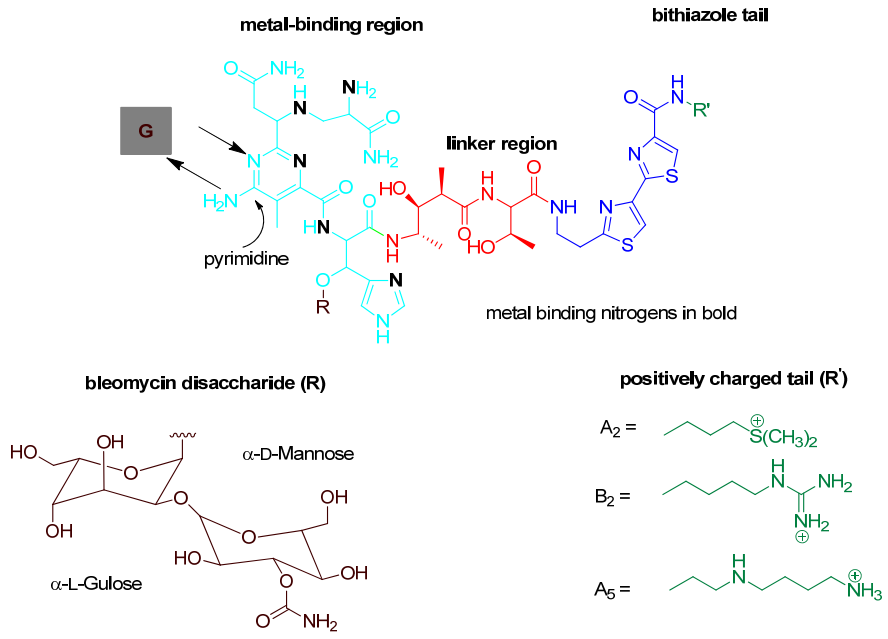


Figure 1.4. Core structure of BLMs. The nitrogen atoms involved in metal-complexation appear in bold. (adapted from ref. 83)

The biological activities of BLMs arise from their ability to cleave nucleic acids in a sequence selective, metal-dependent manner in presence of oxygen.[83,85-88] Bleomycins sold under the trade name Blenoxane are used clinically in combination with etoposide and cisplatin for the treatment of testicular cancer and certain types of lymphomas.[89,90] The low myelosuppression, and immunosuppression of BLM promotes its widespread use in combination chemotherapy;[83-86] however, BLM-induced pneumonitis causing extensive

damage of lung vasculature is a major dose-limiting side effect.[83] The favourable features of BLM have prompted continued efforts targeted at the synthesis of analogues with improved clinical efficacy and lower toxicity.

The disaccharide moiety of BLM has remained largely unexplored mainly because of the lack of BLM analogues containing different sugars. Preliminary studies however indicate that the carbohydrate domain plays a crucial role in BLM activity.[91-93] Modification of the disaccharides in BLM has resulted in analogues with modified selectivity and improved DNA cleavage activity.[94] A major portion of this thesis describes efforts directed towards the modification of the disaccharide moiety in BLM. These studies have led to the identification of novel disaccharides with better cellular targeting profiles.

CHAPTER 2

2. SYNTHESIS OF AMINOQUINONE ANTIOXIDANTS

2.1 Introduction

Mitochondria are cellular organelles essential for the normal functioning of eukaryotic cells. The primary function of the mitochondria is to support aerobic respiration and generate enough ATP to support cellular metabolism.[1,2] In addition to being the powerhouse of the cell, the mitochondria also play an important role in immune response,[4] production of reactive oxygen species (ROS)[95,96] and apoptosis.[5] Owing to their fundamental role in several cellular processes, mitochondrial dysfunction can endanger cell survival. Unsurprisingly, mitochondrial defects have been linked to the pathogenesis of a number of human diseases.[13,97]

Mitochondrial density varies from one tissue to another and depends on the dependence of that tissue on oxidative phosphorylation for its energy needs. Consequently, neuronal, cardiac and skeletal muscle cells, which have a high density of mitochondria, have been found to be the most sensitive to energy-linked defects arising from defective mitochondria.[97]

Mitochondrial proteins are encoded by two distinct genetic systems: mitochondrial DNA (mtDNA) and nuclear DNA (nDNA). Mitochondrial DNA is a circular, double stranded DNA that codes for 13 proteins and 24 nucleic acids (two ribosomal RNAs (rRNAs) and 22 transfer RNAs (tRNAs), that are essential for intramitochondrial protein synthesis.[13] The majority of the mitochondrial

respiratory chain polypeptides are encoded by nuclear DNA.[98,99] These peptides, synthesized in the cytoplasm with a mitochondrial targeting sequence, are translocated into the mitochondria. The targeting sequence is cleaved before the protein is assembled on the inner mitochondrial membrane.[100] The replication, repair, transcription and translation of mtDNA remain entirely dependent on proteins encoded by nDNA.[98,99] This dependency ensures that damage to nuclear or mitochondrial genes can cause mitochondrial dysfunction and human disease.[97]

New strategies need to be developed and implemented to tackle the effects of mitochondrial dysfunction. Novel drugs capable of restoring mitochondrial electron transport chain and ATP production might be effective in preventing the progression of organelle degradation. As the underlying biochemistries of a number of mitochondrial diseases are similar and can be alleviated by lowering oxidative stress,[101] studies leading to the development of potent antioxidants offer great potential.

Natural electron carriers like α-tocopherol[102] and coenzyme Q_{10}[103] (Figure 2.1) have recently received attention as potential therapeutic agents to prevent mitochondrial damage. The utility of coenzyme Q_{10} has been limited by its poor water solubility; however, given its favorable safety profile efforts have been directed at improving its pharmacokinetic properties. Idebenone is an analogue of coenzyme Q_{10} that can restore respiration in ubiquinone-deficient and rotenone-blocked mitochondria. It is not specifically targeted to the mitochondria, but is capable of accepting electrons from complex I and reducing oxidative stress.

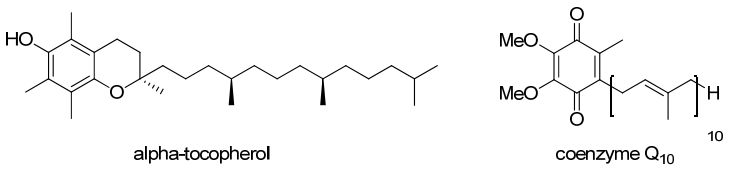


Figure 2.1. Structures of α-tocopherol and coenzyme Q_{10}.

Our research efforts were directed towards the synthesis of coenzyme Q analogues capable of transporting single electrons. As the reductive stress encountered in mitochondrial dysfunction is initially a one electron process, molecules in which the one-electron reduced intermediate is stabilized by dipole interactions, substituent effects, resonance or captodative effects should be better equipped to deal with cellular reductive stress. These molecules are denoted as multifunctional radical quenchers (MRQs) and should be capable of accepting electrons from superoxide, donating electrons to complex III and quench carbon-centered radicals as a consequence of trafficking single electrons.

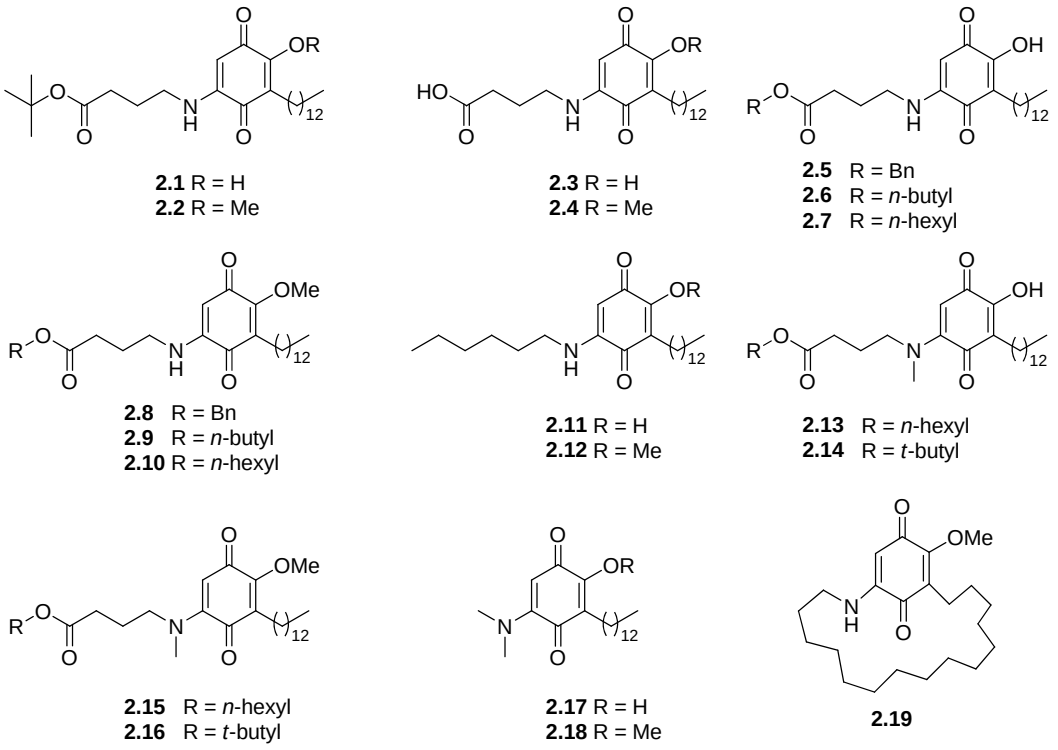


Figure 2.2. Structures of benzoquinone antioxidants.prepared for evaluation.

Compound **2.3** is a natural product, first isolated from *Embelia ribes* Burm. (Myrsinaceae)–a species used in traditional Chinese medicine.[104] The synthesis of compound **2.3**, which closely resembles the redox active core of the natural product geldanamycin, has been reported.[105] Geldanamycin is a benzoquinone ansamycin which exhibits antiproliferative activities against a broad range of human tumor cell lines.[106] It has been reported that an analogue of geldanamycin (17-AAG) possessing the same redox core undergoes reduction in normal epithelial cells under physiological conditions.[107] The reduced hydroquinone is formed *in situ* and binds to its target protein Hsp 90 with greater affinity than the quinone. Considering the structural similarities between the

redox cores of **2.3** and geldanamycin, it seemed likely that **2.3** would also undergo reduction under physiological conditions to the corresponding hydroquinone, potentially enabling it to protect cells from oxidative stress.

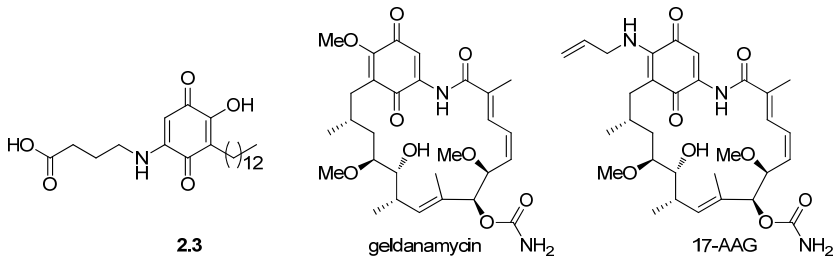


Figure 2.3. Structures of **2.3**, geldanamycin and 17-AAG.

2.2 Results

2.2.1 Synthesis of natural product 2.3

A retrosynthetic analysis of aminoquinones is shown in Figure 2.4.

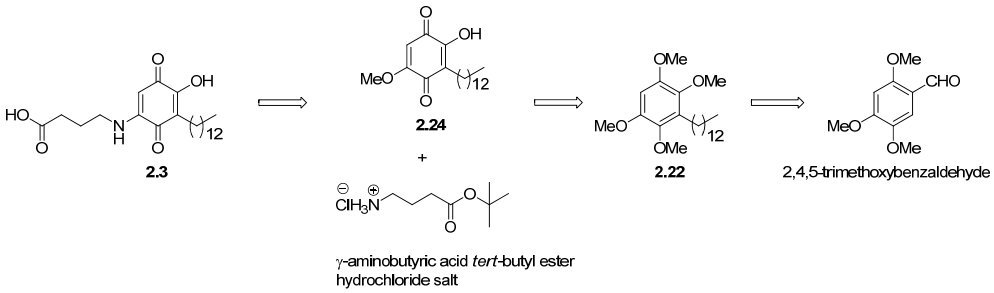
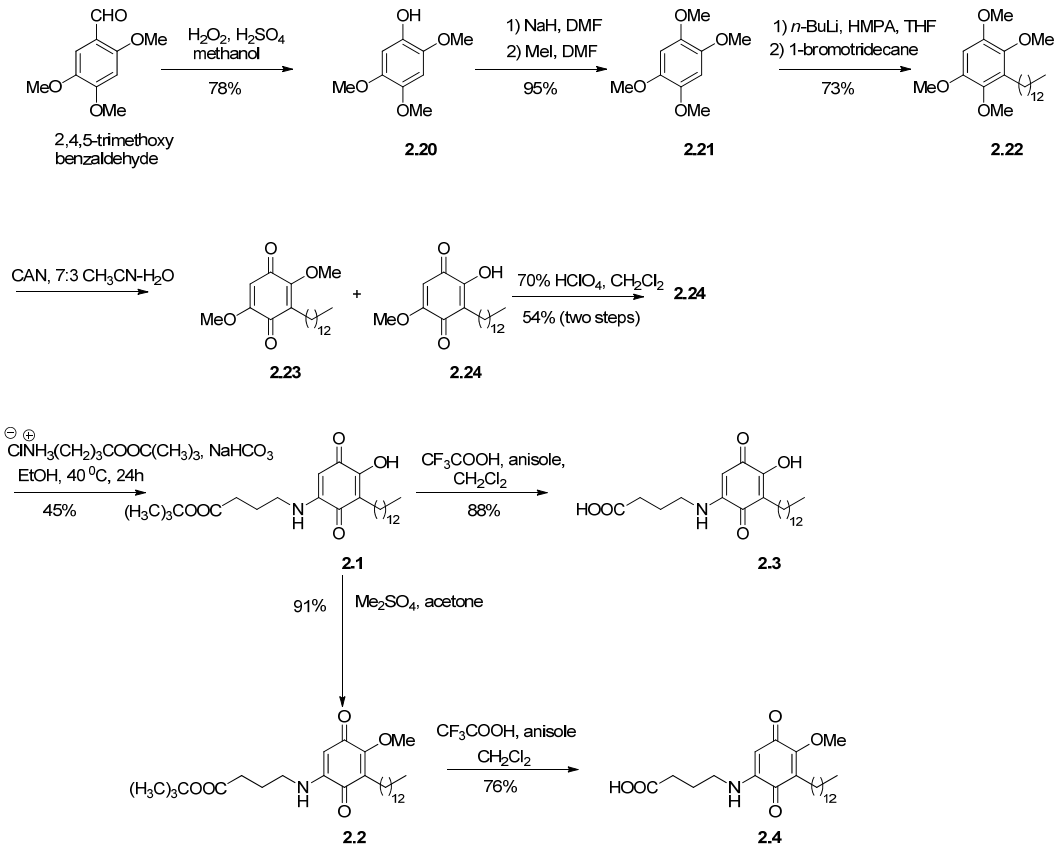


Figure 2.4. Retrosynthetic analysis of natural product **2.3**.

Natural product **2.3** and its analogues could be synthesized from 2,4,5-trimethoxybenzaldehyde by appropriate functional group transformations. As outlined in Scheme 2.1, the synthesis of compound **2.3** began with the H_2O_2-mediated oxidation of commercially available 2,4,5-trimethoxybenzaldehyde to yield 2,4,5-trimethoxyphenol (**2.20**) in 78% yield.[108] Deprotonation of phenol

2.20 with sodium hydride and subsequent alkylation with methyl iodide proceeded smoothly to afford 1,2,4,5-tetramethoxybenzene (**2.21**) in 95% yield.[109] The *n*-butyllithium-mediated alkylation of compound **2.21** with purified 1-bromotridecane yielded compound **2.22** in 73% yield.[109] The alkylated tetramethoxybenzene **2.22** was then subjected to cerium(IV) ammonium nitrate oxidation to give a crude mixture containing quinones **2.23** and **2.24**, which underwent perchloric acid-catalyzed selective demethylation to afford hydroxyquinone **2.24** exclusively in 54% yield over two steps.[109] The selective demethylation has been reported to take place regioselectively with the removal of the more hindered methoxy group.[109] The aminocarboxypropyl group was introduced by treating hydroxyquinone **2.24** with γ-aminobutyric acid *tert*-butyl ester hydrochloride salt in the presence of a large excess of sodium bicarbonate to yield the *tert*-butyl ester **2.1** in 45% yield. The *tert*-butyl ester was cleaved upon treatment with trifluoroacetic acid in the presence of anisole,[110] which on precipitation from methanol afforded natural product **2.3** in 88% yield. The *tert*-butyl ester **2.1** was further methylated with dimethyl sulfate in dry acetone to yield methoxyquinone **2.2** in 91% yield which, upon treatment with trifluoroacetic acid in the presence of a catalytic amount of anisole, gave the acid **2.4** in 76% yield.

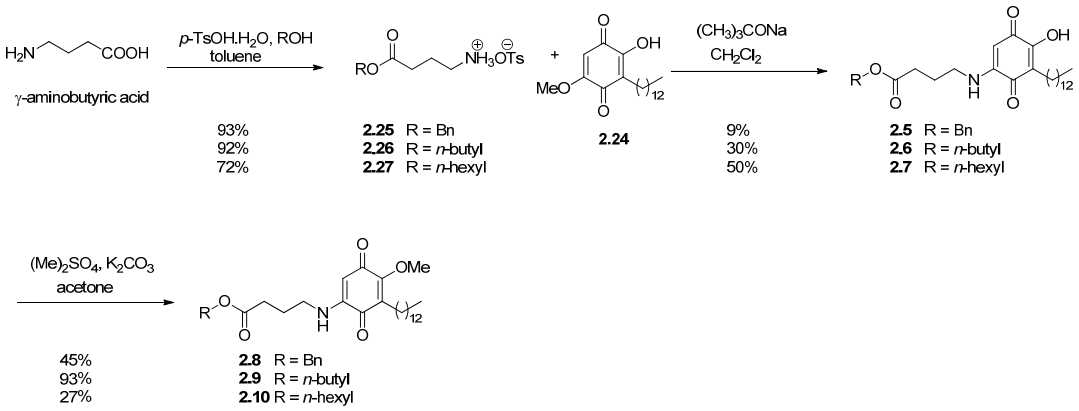


Scheme 2.1. Synthesis of aminoquinone **2.3** and analogues.

2.2.2 Synthesis of *N*-carboxypropyl esters of 2.3.

As shown in Scheme 2.2, the key step in the synthesis involved the conjugate addition of the different esters of γ-aminobutyric acid to the methoxyquinone **2.24**. The benzyl ester of γ-aminobutyric acid was synthesized according to a reported procedure[111] to yield the ester **2.25** in 93% yield. The butyl and hexyl esters **2.26** and **2.27** were synthesized as their tosylate salts in 92% and 72% yields, respectively, by the same procedure. The esters were then coupled to the hydroxyquinone **2.24** in presence of potassium *tert*-butoxide to obtain hydroxy quinone esters **2.5**, **2.6** and **2.7** in 9%, 30% and 50% yields

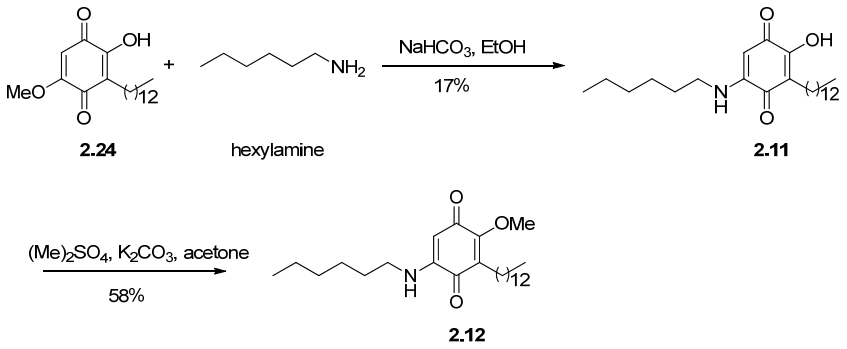
respectively. The quinone esters thus obtained were methylated with dimethyl sulfate in dry acetone to yield methoxyquinones **2.8**, **2.9** and **2.10** in 45%, 93% and 27% yields respectively.



Scheme 2.2. Synthesis of *N*-carboxypropyl esters of quinone **2.3**.

2.2.3 Synthesis of *N*-alkylaminoquinones

The synthesis of the *N*-alkylamine analogues of compound **2.3** was carried out to better understand the importance of the ester moiety to the overall antioxidant activity. As outlined in Scheme 2.3, methoxyquinone **2.24** was coupled to hexylamine to yield the corresponding hydroxyquinone **2.11** in 17% yield. The hydroxyquinone **2.11** thus obtained was methylated with dimethyl sulfate in dry acetone to yield methoxyquinone **2.12** in 58% yield.

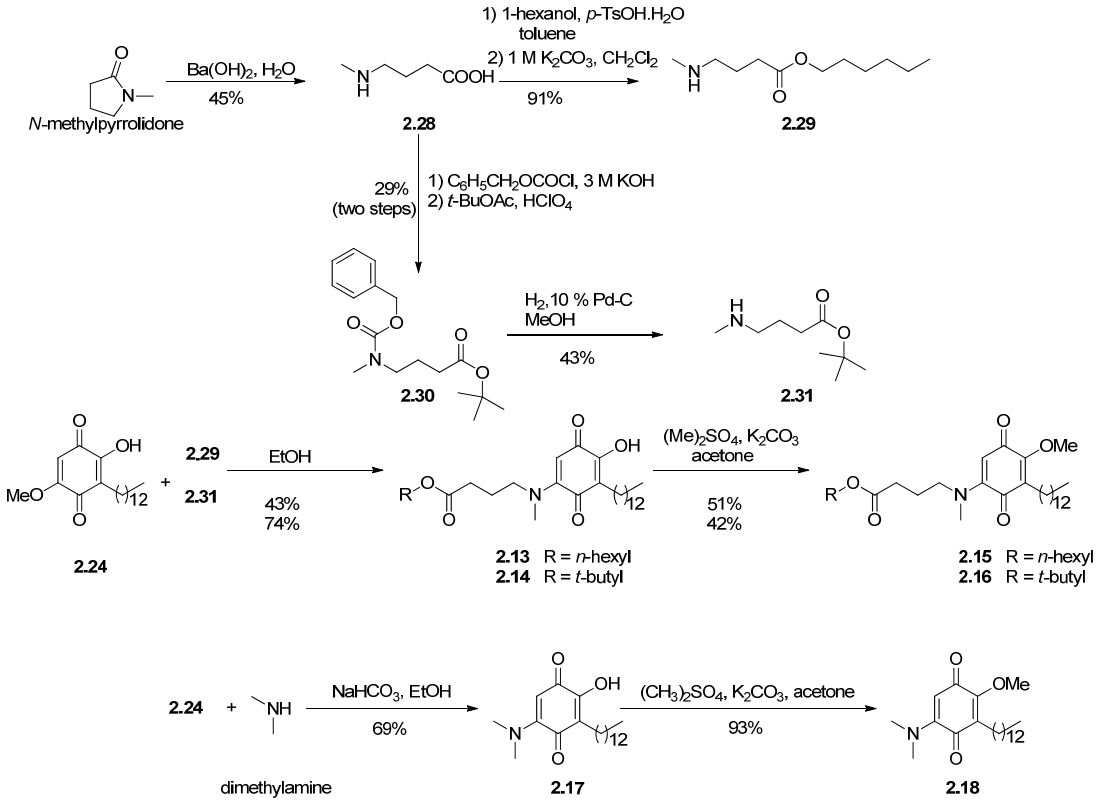


Scheme 2.3. Synthesis of *N*-alkylaminoquinone analogues.

2.2.4 Synthesis of *N,N*-dialkylaminoquinones

As a part of the structure-activity relationship (SAR) study, *N,N*-dialkylated analogues were synthesized to ascertain the importance of the –NH moiety to the antioxidant activity of the quinones. The synthesis of the *N*-methylated analogues, shown in Scheme 2.4, began with the hydrolysis of *N*-methyl-2-pyrrolidone to yield 4-(methylamino)butanoic acid (**2.28**) in 45% yield according to a reported procedure.[112] The acid **2.28** was converted to hexyl ester **2.29** which was then coupled to benzoquinone **2.24** to afford hydroxyquinone **2.13** in 43% yield. The hydroxyquinone was then methylated to afford methoxyquinone **2.15** in 51% yield. The synthesis of quinone **2.16** began with the *N*-CBz protection of the acid **2.28** followed by its esterification to afford the *tert*-butyl ester **2.30** in 29% yield over two steps. The CBz group was then deprotected by catalytic hydrogenation to yield ester **2.31** in 43% yield, which when coupled to benzoquinone **2.24** afforded quinone **2.14** in 74% yield. The hydroxyquinone **2.14** was then subjected to dimethyl sulfate-mediated methylation to yield

methoxyquinone **2.16** in 42% yield. The synthesis of **2.17** was achieved by coupling of the benzoquinone **2.24** with dimethylamine, which proceeded in 69% yield. The methylation of the hydroxyquinone **2.17** provided the methoxyquinone **2.18** in 93% yield.

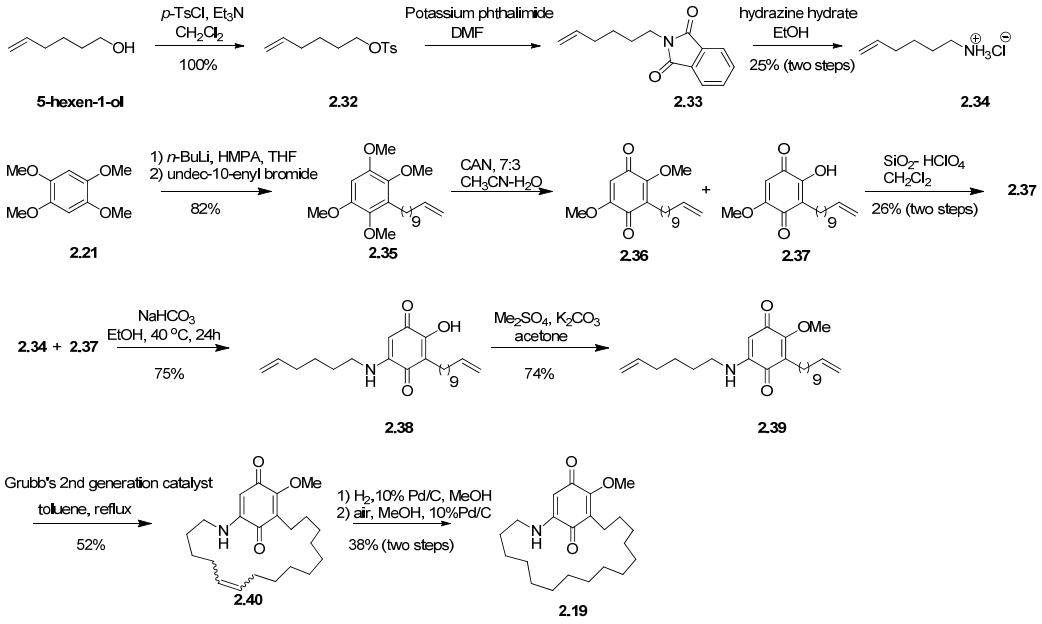


Scheme 2.4. Synthesis of *N*,*N*-dialkylaminoquinone analogues.

2.2.5 Synthesis of a cyclic geldanamycin analogue 2.19

The synthesis of cyclic geldanamycin analogue **2.19** is outlined in Scheme 2.5. Hex-5-en-1-amine hydrochloride (**2.34**) was synthesized according to a reported procedure.[113] The synthesis of compound **2.19** began with the alkylation of tetramethoxybenzene (**2.21**) with purified 11-bromo-1-undecene to yield **2.35**

in 82% yield. The oxidation of compound **2.35** with cerium(IV) ammonium nitrate provided a crude mixture of quinones **2.36** and **2.37**, respectively, which upon treatment with $HClO_4$-SiO_2[114] led to regioselective demethylation to form hydroxyquinone **2.37** in 26% yield over two steps. Attempts to carry out $HClO_4$-mediated demethylation to generate quinone **2.37**, as in the synthesis of hydroxyquinone **2.24** (Scheme 2.1) led to the formation of an inseparable mixture of products. The quinone **2.37** was coupled with hex-5-en-1-amine hydrochloride (**2.34**) to form hydroxyquinone **2.38** in 75% yield. Quinone **2.38** was methylated to protect the phenolic hydroxyl group to yield methoxyquinone **2.39** in 74% yield. Compound **2.39** was subjected to ring closing metathesis in presence of Grubb's catalyst to yield alkene **2.40** as a mixture of diastereomers in 52% yield. The reduction of alkene by catalytic hydrogenation followed by air oxidation provided **2.19** in 38% yield over two steps.[115,116]



Scheme 2.5. Synthesis of cyclic analogue **2.19**.

2.2.6 Biochemical results

2.2.6.1 Cytoprotection

The synthesized analogues were tested for their ability to confer cytoprotection to cultured cells as shown in Table 2.1. Cell viability was determined by trypan blue exclusion assay in Friedreich's ataxia lymphoblast cell line GM15850 (Coriell Institute). This technique was used to assess the cytoprotective effects of the compounds in cultured cells treated with diethyl maleate (DEM) to induce cell death by glutathione (GSH) depletion.[117] The viability of DEM-treated FRDA cells was determined by their ability to exclude the dye trypan blue. Viable cells exclude trypan blue, whereas non-viable cells take up the dye and stain blue. As outlined in Table 2.1, compound **2.2** was the most efficient, exhibiting 80% cytoprotection at 0.5 μM concentration. Benzoquinone analogue **2.4** afforded greater cyoprotection to FRDA lymphocytes at 5 μM concentration than did the *tert*-butyl ester **2.1** (74 vs 50%). The natural product **2.3** afforded the least protection when tested at this concentration.

As shown below, the methoxyquinones **2.2**, **2.4**, **2.8**, **2.9**, **2.10**, **2.12**, **2.16** and **2.18** offered greater cytoprotection when compared to their corresponding hydroxyquinones **2.1**, **2.3**, **2.5**, **2.6**, **2.7**, **2.11**, **2.14** and **2.17**. The *N*-methylated compound **2.16** exhibited similar activity to unmethylated **2.2** at a concentration of 2.5 μM. The alkyl esters **2.9** and **2.10** also exhibited similar activities at tested concentrations. The cyclic analogue **2.19** offered concentration-dependent cytoprotection, affording 83% protection at 2.5 μM concentration.

Table 2.1. Cytoprotection of cultured FRDA lymphocytes from the effects of oxidative stress[a]

Compounds	Concentration of test compounds				
	5 μM	2.5 μM	1 μM	0.5 μM	0.1 μM
2.1	50 ± 2.9				
2.2	93 ± 4.0	84 ± 5.0	80 ± 4.0	80 ± 2.0	
2.3	36 ± 7.3				
2.4	74 ± 5.5				
2.5	48 ± 5.8				
2.6	58 ± 9.0				
2.7	49 ± 9.9				
2.8	71 ± 6.4				
2.9	82 ± 2.9				
2.10	90 ± 2.0				
2.11	70 ± 4.8				
2.12	80 ± 3.2				
2.14		74 ± 4.0	21 ± 6.0		
2.16		82 ± 5.0	64 ± 15		
2.17		24 ± 3.0		18 ± 4.0	21 ± 3.0
2.18		90 ± 3.0		66 ± 3.0	53 ± 9.0
2.19		83 ± 5.4		69 ± 2.3	36 ± 4.3

[a] The viability of untreated cells was defined as 100%; cells treated with DEM alone had 18 ± 10% viability.

This experiment was performed by Jennifer Jaruvangsanti.

2.2.6.2 Inhibition of lipid peroxidation

The ability of the synthesized analogues to quench lipid peroxidation was evaluated in FRDA lymphocytes. These cells were placed under oxidative stress by depleting them of glutathione (GSH) using diethyl maleate (DEM).[117-119] The extent of lipid peroxidation was quantified using a fatty acid sensitive fluorescent reporter C_{11}-BODIPY$^{581/591}$ (Molecular Probes).[120,121] Upon oxidation of the phenylbutadiene moiety of the fluorophore, the red emitting form of the dye (595 nm) is converted into a green emitting form (520 nm). Increased C_{11}-BODIPY$^{581/591}$-green (oxidized) fluorescence, a measure of peroxyl radical production, was determined by flow cytometric analysis, which is expressed as % scavenging activity. The results in Table 2.2 show that analogue **2.16** was very effective in suppressing lipid peroxidation at 5 and 10 μM concentrations (97 and 100% suppression of lipid peroxidation), while the natural product **2.3** was much less active (24% suppression at 10 μM concentration). Methoxyquinones **2.2**, **2.10** and **2.12** also exhibited concentration-dependent suppression of lipid peroxidation, affording 86, 98 and 94% suppression, respectively, at 10 μM concentration.

Table 2.2. Suppression of lipid peroxidation by 3-alkyl-1,4-benzoquinone derivatives of *N*-(3-carboxylpropyl)-5-amino-2-hydroxy-3-tridecyl-1,4-benzoquinone (**2.3**) antioxidants in cultured FRDA lymphocytes treated with diethyl maleate (DEM)[a]

Compound	Scavenging activity (%)	
	5 μM	10 μM
untreated control[b]	100	100
treated control[c]	0	0
2.1	26 ± 6.7	37 ± 1.4
2.2	72 ± 1.8	86 ± 1.8
2.3	8.0 ± 6.6	24 ± 7.4
2.4	41 ± 7.2	51 ± 5.0
2.7	9.0 ± 2.5	40 ± 9.9
2.10	81 ± 1.6	98 ± 1.2
2.11	27 ± 11	50 ± 1.2
2.12	79 ± 1.9	94 ± 1.4
2.16	97 ± 2.1	100 ±1.60
2.19	66 ± 5.1	81 ± 4.6

[a] Values have been calculated as [(100 - % mean) / (100-% mean of the untreated control)] × 100.

[b] No DEM treatment.

[c] DEM treatment.

The experiment was carried out by Dr. Omar Khdour.

2.2.6.3 Inhibition of mitochondrial complex I and NADH oxidase activity

As shown in Tables 2.3 and 2.4, the methoxy hydroquinones were generally found to be much less inhibitory than the corresponding hydroxyquinones. The hydroxyquinones **2.1**, **2.5**, **2.6**, **2.7** and **2.11** exhibits significantly higher inhibitory activities as compared to their corresponding methoxyquinones **2.2**, **2.8**, **2.9**, **2.10** and **2.12**. Compounds **2.2** (540 μM), **2.10** (513 μM) and **2.12** (482 μM) had the least inhibitory effect on complex I activity. The effect of varying the length of the ester side chains on the ability of methoxyquinones **2.8**, **2.9** and **2.10** to inhibit complex I, is not well understood. *N*-methylation significantly increases the inhibitory activity of methoxyquinones. *N*-methylated methoxyquinone **2.15** is a potent inhibitor of complex I while unmethylated **2.10** is not (1.9 μM vs 513 μM)

Methoxyquinone **2.2** exhibited the least inhibition of NADH oxidase activity (77% at 5 μM). Methoxyquinones **2.8** (27% vs >85% at 5 μM) and **2.10** (47% vs >85% at 5 μM) were more potent inhibitors of NADH oxidase activity as compared to complex I. The effect of *O*-methylation and *N*-methylation on the NADH inhibitory activities of compounds needs to be studied further.

Table 2.3. Complex I inhibition by test compounds.

Compound	Complex I inhibition	
	IC_{50} (μM)	I_{max} (%)
2.1	10 ± 0.6	64 ± 13

2.2	540 ± 17.0	>85 ± 2.3
2.3	2.0 ± 0.1	84 ± 1.3
2.4	1.9 ± 0.1	98 ± 2.8
2.5	1.7 ± 0.1	70 ± 0.3
2.6	11 ± 0.6	58 ± 4.0
2.7	2.0 ± 0.4	51 ± 1.4
2.8	34 ± 2.5	≥53 ± 1.5
2.9	98 ± 8.0	≥ 60 ± 4.7
2.10	513 ± 38.0	>85 ± 3.2
2.11	20 ± 1.7	77 ± 6.2
2.12	482 ± 24.0	>85 ± 3.6
2.13	3.4 ± 0.1	78 ± 0.8
2.15	1.9 ± 0.1	90 ± 0.7
2.17	1.5 ± 0.1	90 ± 3.4
2.18	1.60 ± 0.03	90 ± 1.1

Table 2.4: NADH oxidase activity (complexes I, III and IV)

Compound	**NADH oxidase activity (Complex I, III, IV) %**		
	10 μM	5 μM	1 μM
2.2		77 ± 4.0	84 ± 1.0
2.3		62 ± 3.0	77 ± 4.0
2.8		48 ± 3.0	67 ± 6.0

2.10		27 ± 3.0	54 ± 5.0
2.11		39 ± 1.0	67 ± 6.0
2.12		47 ± 3.0	74 ± 16
2.13	18 ± 0.5	36 ± 1.0	82 ± 2.4
2.15	17 ± 0.4	33 ± 0.9	81 ± 1.5
2.17	5.0 ± 0.1	6.6 ± 0.1	18 ± 0.5
2.18	15 ± 0.4	34 ± 0.6	70 ± 2.1

The experiment was carried out by Dr. Valerie C. Collin and Sriloy Dey.

2.2.6.4 Preservation of mitochondrial membrane potential ($\Delta\psi_m$)

The ability of the test compounds to preserve mitochondrial membrane potential under conditions of oxidative stress was studied. Assessment of $\Delta\psi_m$ is an important indicator of cellular function during stress-induced cell death. Changes in mitochondrial membrane potential ($\Delta\psi_m$) were measured using two different fluorescent dyes, tetramethylrhodamine methyl ester (TMRM) and 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolocarbocyanine iodide (JC-1). TMRM is a potentiometric, cell-permeable fluorescent indicator that accumulates in the highly negatively charged interior of mitochondria inner membrane in a Nernstian manner. The fluorescence signal of TMRM can be directly co-related to $\Delta\psi_m$ across the inner mitochondrial membrane. Therefore the accumulation of dye into mitochondria and the intensity of signal is a direct function of mitochondrial potential. Loss of mitochondrial membrane potential is indicated by a reduction in

TMRM red fluorescence. The detection of mitochondrial depolarization using TMRM was accomplished by flow cytometry. Figure 2.5 illustrates representative two-dimensional density dot plots of TMRM-stained lymphocyte cells showing the percentage of cells with intact $\Delta\psi_m$ (TMRM fluorescence in top right quadrant) vs. the percentage of cells with reduced $\Delta\psi_m$ (TMRM fluorescence in bottom left and right quadrants). The results show that DEM treatment decreased the percentage of cells with TMRM fluorescence in the top right quadrant, indicating that DEM treatment caused depolarization of $\Delta\psi_m$. Compound **2.2** preserved mitochondrial membrane potential as compared to the natural product **2.3**. The methoxy hydroquinone esters **2.9**, **2.10** and the cyclic analogue **2.19** prevented the loss of $\Delta\psi_m$, consistent with the cytoprotection results.

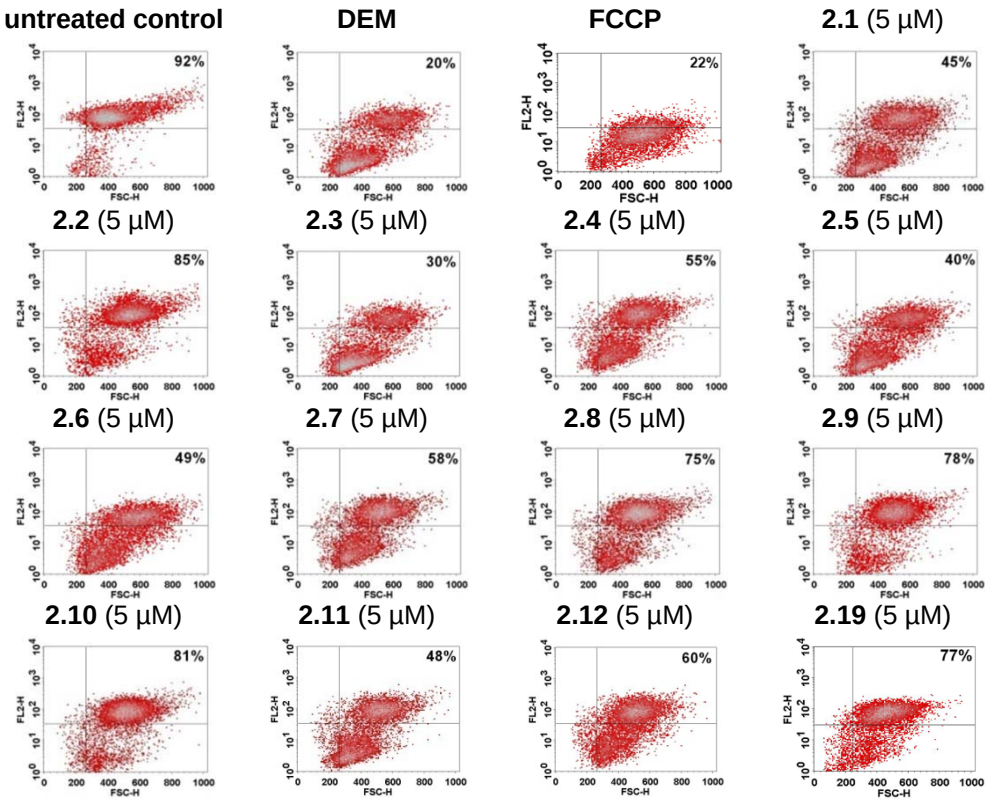


Figure 2.5 Effect of nitrogen-containing 1,4-benzoquinone derivatives on mitochondrial membrane potential of cultured FRDA cells. Representative flow cytometric two dimensional color density dot plot analyses of mitochondrial membrane potential $\Delta\psi_m$ in FRDA lymphocytes stained with TMRM and analyzed using the FL2-H channel. The cells were washed twice in phosphate buffered saline, and suspended in phosphate buffered saline containing 20 mM glucose. The percentage of cells with intact $\Delta\psi_m$ is indicated in the top right quadrant of captions. In each analysis, 10,000 events were recorded. Data are expressed as means ± SEM of three independent experiments run in duplicate. The experiment was carried out by Dr. Omar Khdour.

These results were further confirmed with JC-1 dye in primary FRDA fibroblasts treated with buthionine sulfoximine (BSO) (Figure 2.6). BSO was used in this cellular model to induce an oxidative insult by inhibiting *de novo* glutathione synthesis.[122] JC-1 is a lipophilic, cationic dye that can selectively enter into mitochondria and reversibly change color from green to red as the membrane potential increases by forming aggregates.[123] The dye fluoresces red when it aggregates in the matrix of healthy energized mitochondria, whereas it fluoresces green in cells with depolarized $\Delta\psi_m$. In untreated FRDA cells and cells treated with compounds **2.2** and **2.19**, JC-1 probe was mainly in the aggregated state (red–orange), suggesting that compound **2.2**, and to a lesser extent **2.19**, preserved mitochondrial membrane potential in BSO–treated primary FRDA fibroblasts. Treatment with 1 mM BSO prevented JC-1 mitochondrial

accumulation, resulting in a pronounced green fluorescence due to complete loss of mitochondrial membrane potential. A significant mitochondrial membrane depolarization was observed with natural product **2.3** in BSO–treated cells. Carbonyl cyanide 4-(trifluoromethoxy) phenylhydrazone (FCCP), a commonly used uncoupler of oxidative phosphorylation in mitochondria, was employed to dissipate the chemiosmotic proton gradient ($\Delta\mu H^+$). The pronounced green fluorescence resulting from FCCP treatment reflects the depolarization of mitochondrial inner membrane potential. These data indicate that compound **2.2** is able to prevent oxidative–stress induced collapse of $\Delta\psi_m$, an event indicating mitochondrial function disruption that occurs prior to cell death. The results show that compound **2.2** is able to prevent ROS–induced damage of intracellular lipids, and is able to maintain mitochondrial function and confer cytoprotection in FRDA lymphocytes despite severe oxidative stress.

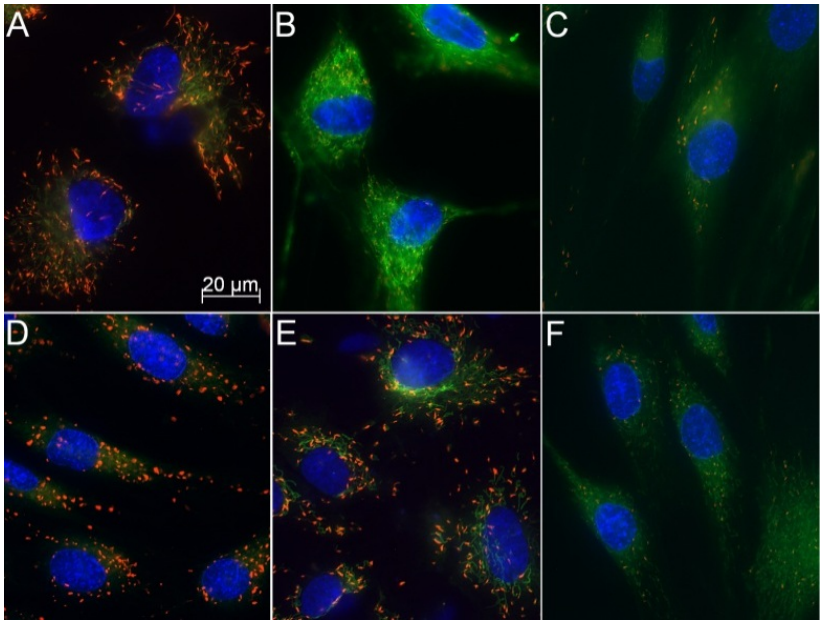


Figure 2.6. Representative fluorescence microscopy images of JC-1-stained primary FRDA fibroblasts were examined under Zeiss fluorescent microscope in control FRDA fibroblasts. Red indicates JC-1 aggregates, which are formed in the mitochondria when a sufficiently high membrane potential is reached. When the $\Delta\psi_m$ collapses as a result of glutathione depletion, the reagent (JC-1) no longer accumulates inside the mitochondria. Instead, it is distributed throughout the cell in the monomeric form which fluoresces green. Hoechst 33342 was used to identify all nuclei. (A) untreated primary FRDA fibroblasts (B) FRDA fibroblasts treated for 2 hours with 25 µM of the uncoupler FCCP (C) FRDA fibroblasts treated for 24 hours with 1 mM BSO (D) compound **2.2** (5 µM) (E) compound **2.19** (5 µM) (F) compound **2.3** (5 µM). The experiment was carried out by Dr. Omar Khdour.

2.3 Discussion

2.3.1 Synthesis of natural product 2.3 and its analogues

The alkylation of tetramethoxybenzene (**2.21**) carried out according to a published procedure[109] initially failed to give any product of interest. To ascertain the reason for the failure of the reaction, the reaction mixture was quenched with D_2O after treatment with *n*-BuLi. The NMR spectrum of the purified product confirmed the incorporation of deuterium in **2.21**. It was therefore concluded that the reaction was being quenched by the addition of 1-bromotridecane. Consequently the commercial sample of 1-bromotridecane was purified by flash column chromatography before use in the reaction, which enhanced the yield of the reaction significantly.

The penultimate step in the synthesis of the natural product **2.3** was based on an $NaHCO_3$-mediated conversion of vinylogous ester into the corresponding vinylogous amide[124] involving conjugate addition of α-amino acid to the ester. The reaction did not proceed well in the presence of the unprotected

γ-aminobutyric acid, possibly due to γ-butyrolactam formation. This problem was solved by using γ-aminobutyric acid *t*-butyl ester hydrochloride salt for conjugate addition to the hydroxyquinone **2.24**.

The deprotection of the Boc ester **2.1** to afford **2.3** could not be carried out with trifluroacetic acid alone and required the addition of an equivalent amount of anisole to the reaction mixture. Anisole is believed to act as a carbonium ion scavenger, thereby facilitating the deprotection of the *t*-Boc group.

The ROS scavenging activity of natural antioxidants like α-tocopherol and coenzyme Q_{10} depend not only on its redox core but also on the length of its lipophilic side chain. For α-TOH, the lipophilic side chain facilitates the insertion of the redox core into the liposomes and suppresses its migration between liposomal membranes. The design of the different *N*-carboxypropyl esters **2.5**, **2.6**, **2.7**, **2.8**, **2.9** and **2.10** was directed towards optimizing the lipophilic character, and thereby increasing the antioxidant activities of the synthesized aminoquinones.

A key step in the synthesis of the cyclic analogue **2.19** involved the oxidation of **2.35** with cerium(IV) ammonium nitrate to provide a crude mixture of quinones **2.36** and **2.37**. The demethylation of **2.36** to yield **2.37** in presence of 70% $HClO_4$ led to the formation of a significant amount of byproducts which could not be separated from the product of interest. The formation of the byproducts was attributed to the oxidation of the alkene functional group by perchloric acid. This led to the use of a milder reagent $HClO_4$-SiO_2[114] to carry out the regioselective demethylation of **2.36** to form hydroquinone **2.37**.

The successful synthesis of **2.19** involved a key ring closing metathesis reaction. Repeated attempts to subject **2.38** to a ring closing metathesis reaction with Grubb's catalyst were met with failure. Amino groups have been reported to deactivate the Grubb's catalyst by substituting the ligands on the catalyst .We hypothesized that the phenolic group, albeit less nucleophilic, was deactivating the catalyst by a similar mechanism. To circumvent this problem the hydroxyl

group was methylated and the corresponding methoxyquinone **2.39** was subjected to a ring closing metathesis reaction which proceeded smoothly as anticipated.

2.3.2 Discussion of biochemical results

2.3.2.1 Cytoprotection

The ability of the synthesized quinones to protect cultured Friedreich's ataxia lymphocyte from cell death by oxidative stress was measured (Table 2.1). For all the analogues synthesized the conversion of the hydroxyl group to the methoxy group was found to increase their ability to confer cytoprotection in a dose dependent manner. The improved activity of the methoxyquinones over the hydroxyquinones could be attributed to their greater stability under physiological conditions. The effect of *N*-methylation on the cytoprotective ability is not well understood and needs to be studied further. Hydroxyquinone **2.14** and the corresponding methoxyquinone **2.16** exhibited similar activities. The slight increase in activity of methoxyquinone **2.10** containing a hexyl side chain as compared to quinone **2.9** bearing a butyl side chain at 5 μM concentration suggested that synthesizing analogues with longer lipophilic ester side chains might improve activity. Initial biological results suggest that the ester moiety in the amine side chain might not be essential for activity. This conclusion is supported by the similar cytoprotective activities of methoxyquinone **2.9** and hexyl analogue **2.12** and needs to be studied in greater detail. The cyclic analogue **2.19** with a lipophilic chain exhibits similar cytoprotection to the *tert*-butyl ester **2.2**.

2.3.2.2 Inhibition of lipid peroxidation

The ability of the synthesized quinones to suppress lipid peroxidation in cultured Friedreich's ataxia lymphocytes treated with diethyl maleate (DEM) was measured (Table 2.2). Consistent with the results observed for cytoprotection, the methoxyquinones offered greater protection against lipid peroxidation as compared to the corresponding hydroxyquinones. The presence of the ester moiety in the amine side chain might not be essential to quench lipid peroxidation. This conclusion, which is in agreement with the results for cytoprotection, is based on the result that **2.12**, which lacks an ester group, exhibits similar activities as esters **2.2** and **2.10** in quenching lipid peroxidation. Preliminary results suggest that *N*-methylation improves the ability of the compounds to quench lipid peroxidation, as suggested by the improved activity of compound **2.16** as compared to **2.2** (97 vs 72%) at 5 μM concentration.

2.3.2.3 Mitochondrial complex I and NADH oxidase Activity

As shown in Tables 2.3 and 2.4, methoxy hydroquinones were generally found to be much less inhibitory than the corresponding hydroxyquinones. *O*-methylation was found to have a profound impact on complex I inhibitory activities of the synthesized analogues. The inhibitory concentrations of methoxyquinones **2.8**, **2.9**, **2.10** and **2.12** are much higher than those of the corresponding hydroxyquinones **2.5**, **2.6**, **2.7** and **2.11**, respectively. The lack of the ester moiety in the amine side chain decreases the ability of the compound to inhibit complex I, as observed for compound **2.12** which has a higher IC_{50} value as compared to most synthesized methoxyquinones studied. The presence of the

-NH moiety is crucial for preventing complex I inhibition as all the tested *N*,*N*-dialkylamino quinones (**2.13**, **2.15**, **2.17** and **2.18**) exhibited low inhibitory concentrations for complex I. Compound **2.2** exhibited the highest IC_{50} value for complex I and NADH oxidase activity, in agreement with its ability to protect FRDA lymphocytes from oxidative stress.

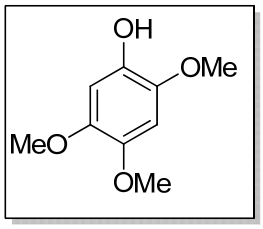
2.3.2.4 Preservation of mitochondrial membrane potential

The ability of the synthesized quinones to preserve mitochondrial membrane potential in cultured Friedreich's ataxia lymphocytes was measured (Figures 2.5 and 2.6). The methoxyquinones **2.8**, **2.9**, **2.10**, **2.12** and **2.19** were more effective at preserving loss of $\Delta\psi_m$ than the corresponding hydroxyquinones **2.5**, **2.6**, **2.7** and **2.11**. Compound **2.2** was most effective at preserving mitochondrial membrane potential. These results were further confirmed with JC-1 dye in primary FRDA fibroblasts treated with buthionine sulfoximine (BSO) which showed that compound **2.2** and **2.19** are able to prevent ROS-induced damage of intracellular lipids, and maintain mitochondrial function in FRDA lymphocytes despite severe oxidative stress.

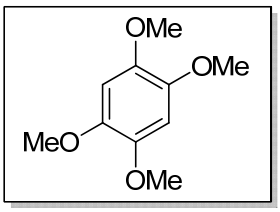
2.4 Experimental

General Methods. The chemicals were all ACS reagent grade and were used without further purification, except for 1-bromotridecane and undecyl bromide which were purified by silica gel flash column chromatography prior to use. The reactions were carried out under an atmosphere of argon. Flash column chromatography was carried out using silica gel (Silicycle R10030B, 60 particle size, 230-400 mesh), applying a low pressure stream of nitrogen. Analytical thin

layer chromatographic separations were carried out on glass plates coated with silica gel (60 particle size F254, SiliCycle TLG-R10011B-323). The TLC chromatograms were developed by immersing the plates in 2.5% potassium permanganate in ethanol or 2% anisaldehyde + 5% sulfuric acid + 1.5% glacial acetic acid in ethanol, followed by heating, or else visualized by UV irradiation (254 nm). Melting points were recorded on a MelTemp apparatus and are uncorrected. Tetrahydrofuran was distilled from sodium/benzophenone ketyl and dichloromethane from calcium hydride. ^{1}H and ^{13}C NMR spectra were recorded on a Gemini 300 or Varian Inova 400, or on a Varian Inova 500 spectrometer, using $CDCl_3$ as solvent and internal standard, unless otherwise indicated. ^{1}H NMR chemical shifts were reported relative to residual $CHCl_3$ at 7.26 ppm, or to residual DMSO-d_5 at 2.50 ppm; ^{13}C NMR shifts were reported relative to the central line of $CDCl_3$ at 77.16 ppm, or to ^{13}C DMSO-d_6 at 39.51 ppm. Splitting patterns are designated as s, singlet; br s, broad singlet; d, doublet; dd, doublet of doublets; dt, doublet of triplets; m, multiplet; q, quartet; quin, quintet. High resolution mass spectrometric data were obtained at the Michigan State Mass Spectrometry Facility or at the Arizona State University CLAS High Resolution Mass Spectrometry Facility.

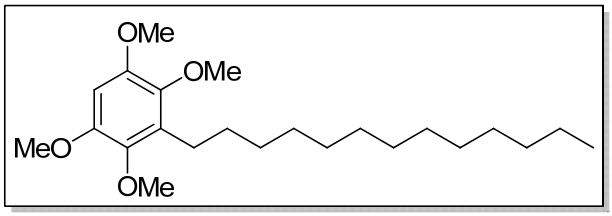


2,4,5-Trimethoxyphenol (2.20).[108,109] To a solution containing 10 g (51 mmol) of 2,4,5-trimethoxybenzaldehyde and 6.4 mL of H_2O_2 (35% wt solution in H_2O) in 102 mL of methanol was added 1.0 mL (18 mmol) of concentrated H_2SO_4 dropwise under an atmosphere of argon at room temperature. The reaction mixture was heated to reflux for 2 h, diluted with water and extracted with three 100-mL portions of dichloromethane. The combined organic layer was washed with brine, dried ($MgSO_4$) and concentrated under diminished pressure. The crude residue was applied to a silica gel column (12 × 4 cm). Step gradient elution with 1:4→1:2 ethyl acetate–hexanes afforded compound **2.20** as a yellow solid: yield 7.34 g (78%); silica gel TLC R_f 0.45 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 3.48 (s, 6H), 3.52 (s, 3H), 6.08 (br s, 1H), 6.33 (s, 1H) and 6.36 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 56.4, 57.0, 57.2, 99.6, 100.9, 139.6, 142.1 and 143.8.



1,2,4,5-Tetramethoxybenzene (2.21).[109] To a solution of 1.38 g (60% oil dispersion, 57.5 mmol) of sodium hydride washed with several portions of hexane in 32 mL of anh *N*,*N*-dimethylformamide was added a solution of 7.06 g (38.3 mmol) of phenol **2.20** in 32 mL of anh *N*,*N*-dimethylformamide. The reaction mixture was stirred at 0 °C for 30 min under an argon atmosphere and 4.78 mL (10.9 g, 76.6 mmol) of methyl iodide was added dropwise. The reaction mixture

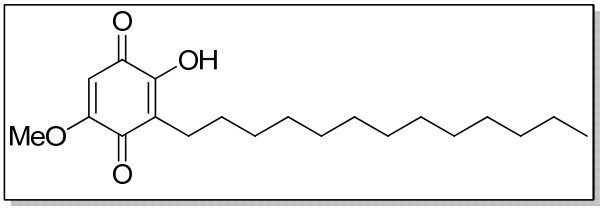
was then stirred at room temperature for 13 h and quenched by addition of 10 mL of methanol. The solvent was concentrated under diminished pressure to afford a crude residue. The residue was extracted with five 10-mL portions of dichloromethane, was washed successively with 50 mL of 3% aq HCl, distilled water and brine, and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (8 × 4 cm). Elution with 1:4 ethyl acetate–hexanes gave compound **2.21** as a colorless solid: yield 7.21 g (95%); mp 102-103 °C, lit[109] mp 101-102 °C; silica gel TLC R_f 0.32 (1:2 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 3.70 (s, 12H) and 6.47 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 57.1, 100.7 and 143.2.



1,2,4,5-Tetramethoxy-3-tridecylbenzene (2.22).[109]

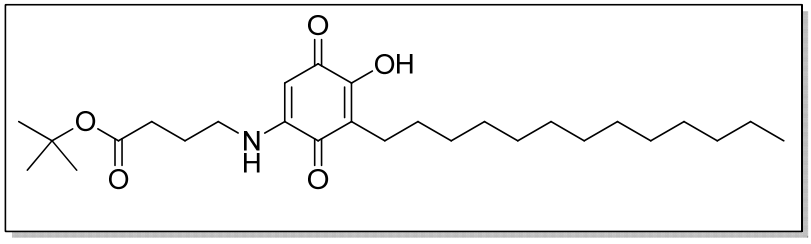
To a solution containing 1.0 g (5.0 mmol) of 1,2,4,5-tetramethoxybenzene (**2.21**) and 87 μL (90 mg, 0.5 mmol) of hexamethylphosphoramide in 25 mL of anh THF was added 3.4 mL (1.6 M in hexanes, 5.5 mmol) of *n*-butyllithium dropwise at –40 ºC over a period of 5 min. The reaction mixture was warmed to 0 °C over a period of 2 h, then 1.4 mL (1.4 g, 5.5 mmol) of purified 1-bromotridecane was added and the reaction mixture was stirred at room temperature under an argon atmosphere for 15 h. The reaction mixture was quenched by the addition of 20

mL of saturated NH_4Cl and extracted with five 10-mL portions of ether. The combined organic layer was washed with distilled water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (6 × 3 cm). Elution with 1:9 ethyl acetate–hexanes afforded compound **2.22** as a colorless solid: yield 1.4 g (73%); silica gel TLC R_f 0.45 (1:1 ethyl ether–hexanes); mp 31-32 ^oC, lit[109] mp 31-32 ^oC; 0.2 g (20%) of unreacted 1,2,4,5-tetramethoxybenzene (**2.21**) was recovered; ^1H NMR ($CDCl_3$) δ 0.87 (t, 3H, J = 6.8 Hz), 1.14-1.46 (m, 20H), 1.47-1.58 (m, 2H), 2.61 (dd, 2H, J = 8.8 and 6.9 Hz), 3.76 (s, 6H), 3.82 (s, 6H) and 6.40 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 22.7, 24.7, 29.4, 29.5, 29.6, 29.70, 29.75, 29.76, 30.0, 30.8, 32.0, 56.2, 60.4, 60.9, 96.7, 131.1, 141.1 and 148.8.



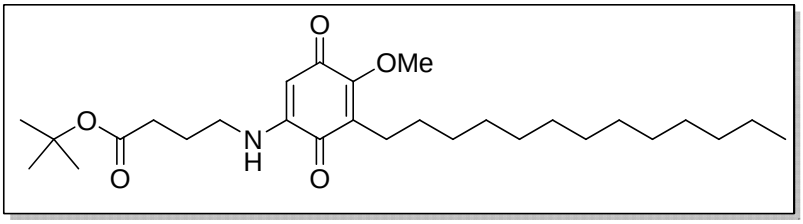
2-Hydroxy-5-methoxy-3-tridecylcyclohexa-2,5-diene-1,4-dione (2.24).[109] To a solution containing 0.10 g (0.26 mmol) of 1,2,4,5-tetramethoxy-3-tridecylbenzene (**2.22**) in 2.60 mL of acetonitrile was added dropwise a solution containing 0.28 g (0.52 mmol) of cerium(IV) ammonium nitrate in 2.6 mL of 7:3 acetonitrile–water at −7 °C (salt–ice bath) over a period of 30 min. The reaction mixture was stirred at room temperature for 3 h and then diluted with 10 mL of ether. The organic layer was washed successively with distilled water and brine and then dried

($MgSO_4$). The solvent was concentrated under diminished pressure to afford a mixture of quinones **2.23** and **2.24**. To a solution of this mixture in 2.60 mL of dichloromethane was added 1.10 mL (13.0 mmol) of 70% perchloric acid dropwise at 0 °C. The reaction mixture was then stirred at 0 °C for 9 h and diluted with 10 mL of dichloromethane. The organic layer was washed successively with distilled water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (7 × 2 cm). Elution with 1:4 ethyl acetate–hexanes gave compound **2.24** as a yellow–orange solid: yield 48 mg (54%); mp 90-92 °C, lit[125] mp 90-91°C; silica gel TLC R_f 0.58 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3H, J = 6.8 Hz), 1.17-1.33 (m, 20H), 1.39-1.49 (m, 2H), 2.41 (t, 2H, J = 8.0 Hz), 3.84 (s, 3H), 5.82 (s, 1H) and 7.32 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.2, 22.7, 22.8, 28.1, 29.48, 29.54, 29.68, 29.69, 29.77, 29.78, 29.79, 29.80, 32.0, 56.9, 102.3, 119.4, 151.7, 161.2, 181.8, and 183.0.



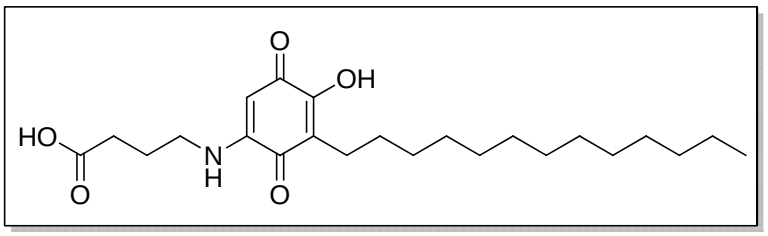
***tert*-Butyl 4-(4-hydroxy-3,6-dioxo-5-tridecylcyclohexa-1,4-dienylamino)butanoate (2.1).**[105] To a solution of 42.0 mg (0.13 mmol) of 2-hydroxy-5-methoxy-3-tridecyl-(1,4)-benzoquinone (**2.24**) and 1.00 g (13.0 mmol) of sodium bicarbonate in 9.70 mL of ethanol was added 39.0 mg (0.19 mmol) of

γ-aminobutyric acid *tert*-butyl ester hydrochloride salt. The reaction mixture was stirred at 45 °C for 27 h under an argon atmosphere and quenched by the addition of 5 mL of water. The aqueous layer was extracted with seven 2-mL portions of dichloromethane. The combined organic layer was washed with distilled water and brine and then dried (Na_2SO_4). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (5 × 2 cm). Elution with dichloromethane gave compound **2.1** as a dark red solid: yield 27 mg (45%); mp 96-97 °C, lit[105] mp 82-85 °C; silica gel TLC R_f 0.38 (dichloromethane); ^{1}H NMR ($CDCl_3$) δ 0.86 (t, 3H, J = 6.5 Hz), 1.20-1.32 (m, 20H), 1.38-1.46 (m, 11H), 1.94 (quin, 2H, J = 6.9 Hz), 2.31 (t, 2H, J = 7.0 Hz), 2.34-2.40 (m, 2H), 3.21 (dd, 2H, J = 12.9 and 6.6 Hz), 5.35 (s, 1H) and 6.58 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.3, 22.79, 22.84, 23.5, 28.23, 28.24, 29.5, 29.6, 29.73, 29.75, 29.81, 29.83, 29.84, 32.1, 32.8, 42.4, 81.2, 91.9, 115.9, 149.9, 155.1, 172.1, 179.0 and 182.6; mass spectrum (LCT electrospray), *m/z* 486.3181 $(M + Na)^+$ ($C_{27}H_{45}NO_5Na$ requires *m/z* 486.3195).



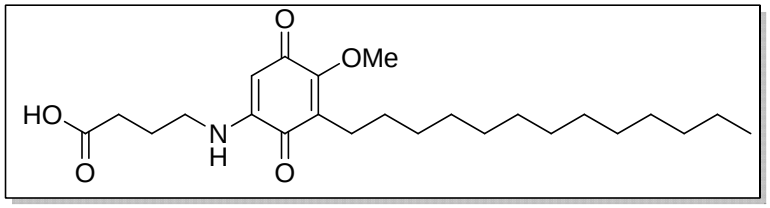
***tert*-Butyl 4-(4-methoxy-3,6-dioxo-5-tridecylcyclohexa-1,4-dienylamino)butanoate (2.2).** To a solution containing 22.0 mg (47.0 μmol) of quinone **2.1** and 0.25 g (1.80 mmol) of potassium carbonate in 1.2 mL of anh

acetone was added 23.0 μL (31.0 mg, 0.23 mmol) of dimethyl sulfate. The reaction mixture was heated to reflux overnight, cooled to room temperature and concentrated under diminished pressure. The crude reaction mixture was redissolved in 10 mL of dichloromethane and washed with 5 mL of 1 N HCl. The aqueous layer was extracted with three 10-mL portions of dichloromethane. The combined organic layer was dried ($MgSO_4$) and concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (24 × 2 cm). Elution with 1:5 ethyl acetate–hexanes gave compound **2.2** as a bright red amorphous solid: yield 21 mg (91%); silica gel TLC R_f 0.60 (1:2 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3H, J = 6.8 Hz), 1.16-1.42 (m, 22H), 1.45 (s, 9H), 1.82-2.03 (quin, 2H, J = 9.0 Hz), 2.31 (t, 2H, J = 7.2 Hz), 2.35-2.39 (m, 2H), 3.14 (dd, 2H, J = 13.0 and 6.8 Hz), 4.10 (s, 3H), 5.28 (s, 1H) and 5.94 (t, 1H, J = 5.6 Hz); ^{13}C NMR ($CDCl_3$) δ 14.3, 22.8, 23.1, 23.6, 28.20, 28.24, 28.8, 29.5, 29.6, 29.7, 29.81, 29.83, 32.1, 32.9, 42.1, 61.8, 81.1, 96.1, 127.6, 146.9, 158.5, 172.18, 172.20, 181.8 and 183.9; mass spectrum (APCI), *m/z* 478.3532 $(M + H)^+$ ($C_{28}H_{48}NO_5$ requires *m/z* 478.3532).



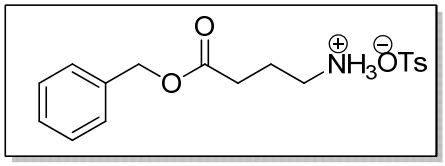
4-(4-Hydroxy-3,6-dioxo-5-tridecylcyclohexa-1,4-dienylamino)butanoic Acid (2.3).[105] To a solution containing 28 mg (60 μmol) of ester **2.1** in 0.4 mL of

dichloromethane were added 6.5 µL (6.5 mg, 60 µmol) of anisole and 0.4 mL (0.6 g, 5.4 mmol) of trifluoroacetic acid. The reaction mixture was stirred at room temperature for 24 h under an argon atmosphere. The reaction mixture was concentrated under diminished pressure and the excess trifluoroacetic acid was removed by co-evaporation three times with cyclohexane to afford a crude residue. The residue was precipitated from methanol to give compound **2.3** as a red amorphous solid: yield 21 mg (88%); mp 194-195 °C, lit[105]mp 177-180 °C; ^{1}H NMR (DMSO-d_6) δ 0.85 (t, 3H, J = 6.8 Hz), 1.15-1.42 (m, 22H), 1.74 (quin, 2H, J = 14.4 and 7.2 Hz), 2.26 (q, 4H, J = 6.9Hz), 3.14 (dd, 2H, J = 13.8 and 6.7 Hz), 5.32 (s, 1H), 7.78 (t, 1H, J = 6.2 Hz), 10.5 (br s, 1H), and 12.2 (br s, 1H); ^{13}C NMR (DMSO-d_6) δ 14.0, 22.1, 22.2, 22.8, 27.6, 28.8, 28.9, 29.0, 29.02, 29.06, 29.08, 29.1, 30.9, 31.3, 41.4, 91.8, 115.6, 149.3, 156.7, 174.2, 178.5 and 182.5; mass spectrum (LCT electrospray), *m/z* 430.2564 $(M + Na)^+$ ($C_{23}H_{37}NO_5Na$ requires *m/z* 430.2569).



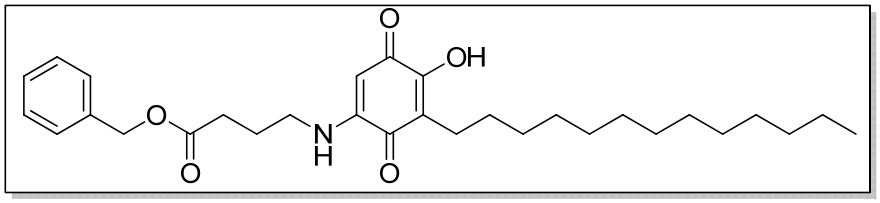
4-(4-Methoxy-3,6-dioxo-5-tridecylcyclohexa-1,4-dienylamino)butanoic Acid (2.4). To a solution containing 9.0 mg (19 µmol) of ester **2.2** in 120 µL of dichloromethane was added 2.0 µL (2.0 mg, 19 µmol) of anisole, and 130 µL (0.2 g, 1.7 mmol) of trifluoroacetic acid. The reaction mixture was stirred at room temperature for 24 h under an argon atmosphere. The reaction mixture was co-

evaporated with six 5-mL portions of cyclohexane and the solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (22 × 2 cm). Elution with 100:1 chloroform–methanol gave compound **2.4** as a red amorphous solid: yield 6.0 mg (76%); silica gel TLC R_f 0.32 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.9 Hz), 1.22-1.41 (m, 22H), 1.98 (quin, 2H, J = 6.9 Hz), 2.33-2.40 (m, 2H), 2.47 (t, 2H, J = 6.9 Hz), 3.20 (q, 2H, J = 6.6 Hz), 4.11 (s, 3H), 5.29 (s, 1H), and 5.97 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.3, 18.5, 22.8, 23.1, 23.2, 28.8, 29.5, 29.6, 29.7, 29.81, 29.84, 31.3, 32.1, 42.0, 51.0, 58.6, 61.8, 96.2, 127.7, 146.9, 158.5, 176.6, 181.8, and 184.0; mass spectrum (APCI), *m/z* 422.2898 $(M + H)^+$ ($C_{24}H_{40}NO_5$ requires 422.2906).



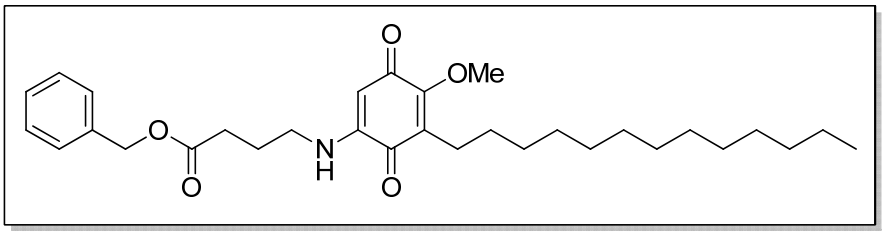
4-(Benzyloxy)-4-oxobutan-1-aminium 4-Methylbenzenesulfonate (2.25).[111] A solution of 1.00g (9.70 mmol) of 4-aminobutanoic acid, 2.02 g (1.08 mmol) of *p*-toluenesulfonic acid monohydrate and 1.24 mL (1.29 g, 1.24 mmol) of benzyl alcohol in 20 mL of toluene was heated to reflux for 24 h, using a Dean-Stark distilling receiver. The reaction mixture was cooled to room temperature and diluted with 20 mL of anh diethyl ether to afford *p*-toluenesulfonate **2.25** as a crystalline, colorless solid: yield 3.30 g (93%); silica gel TLC R_f 0.47 (9:1 chloroform–methanol); ^{1}H NMR ($CDCl_3$) δ 1.89 (quin, 2H, J = 7.3 Hz), 2.28-2.40

(m, 5H), 2.87 (dt, 2H, J = 12.8 and 6.3 Hz), 5.04 (s, 2H), 7.11 (d, 2H, J = 7.9 Hz), 7.27-7.37 (m, 5H) and 7.76-7.85 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 21.4, 22.6, 30.9, 39.3, 66.5, 126, 128.30, 128.35, 128.6, 129.2, 135.9, 140.9, 141.2 and 172.3.



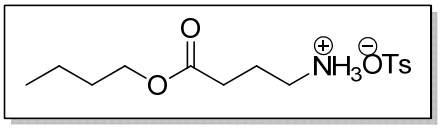
Benzyl 4-((4-Hydroxy-3,6-dioxo-5-tridecylcyclohexa-1,4-dien-1-yl)amino)butanoate (2.5). To a solution containing 57.0 mg (0.17 mmol) of 2-hydroxy-5-methoxy-3-tridecyl-(1,4)-benzoquinone (**2.24**) in 8 mL of dichloromethane was added a solution containing 185 mg (0.51 mmol) of *p*-tolunesulfonate salt **2.25** and 60.0 mg (97%, 0.51 mmol) of potassium *tert*-butoxide in 8 mL of dichloromethane dropwise over a period of 10 min. The reaction mixture was stirred at room temperature for 20 h under an argon atmosphere, then washed with 5 mL of 1 N HCl. The aqueous layer was extracted with seven 2-mL portions of dichloromethane. The combined organic layer was washed successively with water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (24 × 3 cm). Elution with diethyl ether gave compound **2.5** as a dark red solid: yield 11.0 mg (9%); silica gel TLC R_f 0.25 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.90 (t, 3H, J = 6.8 Hz), 1.21-1.36 (m, 20H), 1.38-1.52 (m, 2H), 1.96-2.09 (m, 2H), 2.31-2.45 (m, 3H), 2.44-2.61 (m,

2H), 3.15-3.34 (m, 2H), 5.15 (s, 2H), 5.37 (s, 1H), 6.56 (s, 1H) and 7.13-7.46 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.3, 21.6, 22.8, 23.4, 28.2, 29.5, 29.6, 29.7, 29.80, 29.83, 31.6, 32.1, 42.2, 66.8, 92.0, 125.4, 128.3, 128.5, 128.6, 128.8, 129.2, 135.68, 135.72, 138.00, 138.02, 149.8, 155.1, 172.6, 179 and 182.5; mass spectrum (APCI), *m/z* 498.3206 $(M + H)^+$ ($C_{30}H_{44}NO_5$ requires 498.3219).

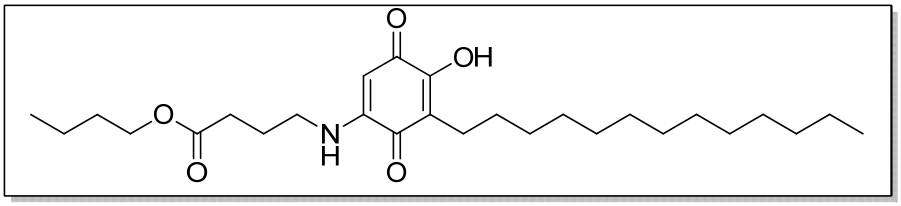


Benzyl 4-((4-Methoxy-3,6-dioxo-5-tridecylcyclohexa-1,4-dien-1-yl)amino)butanoate (2.8). To a solution containing 12.0 mg (24.0 μmol) of quinone **2.5** and 125 mg (0.91 mmol) of potassium carbonate in 0.6 mL of anh acetone was added 45.0 μL (60.0 mg, 0.48 mmol) of dimethyl sulfate. The reaction mixture was heated to reflux overnight, then allowed to cool to room temperature and concentrated under diminished pressure. The crude mixture was redissolved in 10 mL of dichloromethane and washed with 5 mL of 1 N HCl. The aqueous layer was extracted with three 10-mL portions of dichloromethane. The combined organic layer was dried ($MgSO_4$) and concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (23 × 2 cm). Elution with 20% diethyl ether in hexane gave compound **2.8** as a bright red solid: yield 8 mg (45%); silica gel TLC R_f 0.40 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.81-0.97 (m, 3H), 1.15-1.34 (m, 20H),

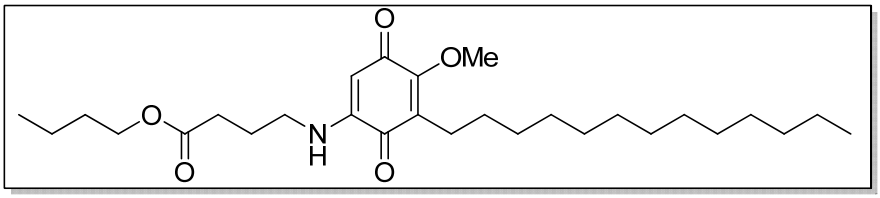
1.32-1.45 (m, 2H), 1.98 (quin, 2H, J = 7.0 Hz), 2.29-2.41 (m, 2H), 2.45 (t, 2H, J = 7.1 Hz), 3.15 (q, 2H, J = 6.7 Hz), 4.11 (s, 3H), 5.14 (s, 2H), 5.25 (s, 1H), 5.92 (t, 1H, J = 5.5 Hz) and 7.19-7.44 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.3, 22.8, 23.1, 23.5, 28.8, 29.5, 29.6, 29.7, 29.81, 29.84, 31.7, 32.1, 42.0, 61.8, 66.8, 96.2, 127.7, 128.50, 128.55, 128.8, 135.8, 146.8, 158.5, 172.7, 181.8 and 183.9; mass spectrum (APCI), *m/z* 512.3379 $(M + H)^+$ ($C_{31}H_{46}NO_5$ requires 512.3376).



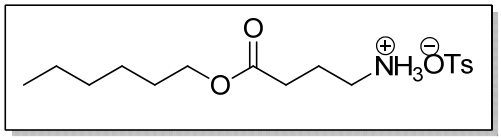
4-Butoxy-4-oxobutan-1-aminium 4-Methylbenzenesulfonate (2.26).[126] A solution of 1.00g (9.70 mmol) of 4-aminobutanoic acid, 2.02 g (1.08 mmol) of *p*-toluenesulfonic acid monohydrate and 1.10 mL (891 mg, 1.24 mmol) of 1-butanol in 20 mL of toluene was heated to reflux for 24 h, using a Dean-Stark distilling receiver. The reaction mixture was allowed to cool to room temperature and diluted with 20 mL of anh diethyl ether to afford the *p*-toluenesulfonate salt **2.26** as a crystalline, colorless solid: yield 2.96 g (92%); silica gel TLC R_f 0.25 (9:1 chloroform–methanol); ^{1}H NMR (CD_3OD) δ 0.94 (t, 3H, J = 7.4 Hz), 1.33-1.46 (m, 2H), 1.55-1.67 (m, 2H), 1.88-1.96 (m, 2H), 2.37 (s, 3H), 2.44 (t, 2H, J = 7.2 Hz), 2.92-3.02 (m, 2H), 4.09 (t, 2H, J = 6.6 Hz), 4.86 (s, 3H), 7.24 (d, 2H, J= 10.5 Hz) and 7.71 (d, 2H, J = 10.0 Hz); ^{13}C NMR (CD_3OD) δ 14.0, 20.1, 21.4, 23.7, 31.6, 31.8, 40.1, 65.6, 126.8, 129.5, 141.6, 143.3 and 174.1.



Butyl 4-((4-Hydroxy-3,6-dioxo-5-tridecylcyclohexa-1,4-dien-1-yl)amino)butanoate (2.6). To a solution containing 82.0 mg (0.24 mmol) of 2-hydroxy-5-methoxy-3-tridecyl-(1,4)-benzoquinone (**2.24**) in 11.5 mL of dichloromethane was added a solution containing 241 mg (0.73 mmol) of *p*-tolunesulfonate salt **2.26** and 72.0 mg (97%, 0.73 mmol) of potassium *tert*-butoxide in 11.5 mL of dichloromethane dropwise over a period of 10 min. The reaction mixture was stirred at room temperature for 20 h under an argon atmosphere. The reaction mixture was then washed with 5 mL of 1 N HCl and the aqueous layer was extracted with seven 2-mL portions of dichloromethane. The combined organic layer was washed with water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (24 × 3 cm). Elution with diethyl ether gave compound **2.6** as a dark red solid: yield 34 mg (30%); silica gel TLC R_f 0.16 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3H, J = 6.8 Hz), 0.93 (t, 3H, J = 7.4 Hz), 1.11-1.52 (m, 24H), 1.53-1.68 (m, 2H), 1.99 (quin, 2H, J = 6.9 Hz), 2.32-2.54 (m, 4H), 3.23 (q, 2H, J = 6.6 Hz), 4.10 (t, 2H, J = 6.7 Hz), 5.36 (s, 1H), 6.58 (s, 1H) and 8.09 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 13.8, 14.3, 19.3, 22.79, 22.84, 23.3, 28.2, 29.5, 29.6, 29.7, 29.80, 29.82, 30.7, 31.6, 32.1, 42.3, 64.9, 91.9, 116, 149.8, 155.1, 172.9, 179 and 182.6; mass spectrum (APCI), *m/z* 464.3374 $(M + H)^+$ ($C_{27}H_{46}NO_5$ requires 464.3376).

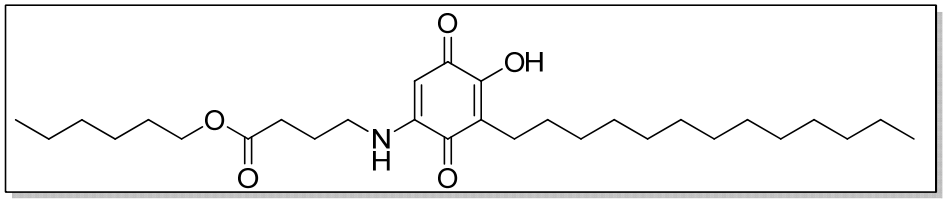


Butyl 4-((4-Methoxy-3,6-dioxo-5-tridecylcyclohexa-1,4-dien-1-yl)amino)butanoate (2.9). To a solution containing 8.0 mg (16 μmol) of hydroxyquinone **2.6** and 84 mg (0.6 mmol) of potassium carbonate in 1.0 mL of anh acetone was added dropwise 30 μL (0.3 mmol) of dimethyl sulfate. The reaction mixture was heated to reflux overnight and allowed to cool to room temperature. The crude reaction mixture was concentrated under diminished pressure and redissolved in 10 mL of dichloromethane. The organic layer was washed with 5 mL of 1 N HCl and the aqueous layer was extracted with three 10-mL portions of dichloromethane. The combined organic layer was dried ($MgSO_4$) and concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (24 × 2 cm). Step gradient elution with 20% diethyl ether→30% diethyl ether in hexane gave compound **2.9** as a bright red solid: yield 7.7 mg (93%); silica gel TLC R_f 0.67 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, J = 6.8 Hz), 0.93 (t, 3H, J = 7.4 Hz), 1.16-1.46 (m, 23H), 1.51-1.71 (m, 3H), 1.96 (quin, 2H, J = 7.0 Hz), 2.31-2.49 (m, 4H), 3.16 (dd, 2H, J = 13.0 and 6.7 Hz), 4.02-4.15 (m, 5H), 5.28 (s, 1H) and 5.95 (t, 1H, J = 5.6 Hz); ^{13}C NMR ($CDCl_3$) δ 13.9, 14.3, 19.3, 22.8, 23.1, 23.5, 28.8, 29.5, 29.6, 29.73, 29.81, 29.84, 30.8, 31.7, 32.1, 42.1, 61.8, 64.9, 96.2, 127.7, 146.9, 158.5, 173, 181.8 and 184; mass spectrum (APCI), *m/z* 478.3516 $(M + H)^+$ ($C_{28}H_{48}NO_5$ requires 478.3532).



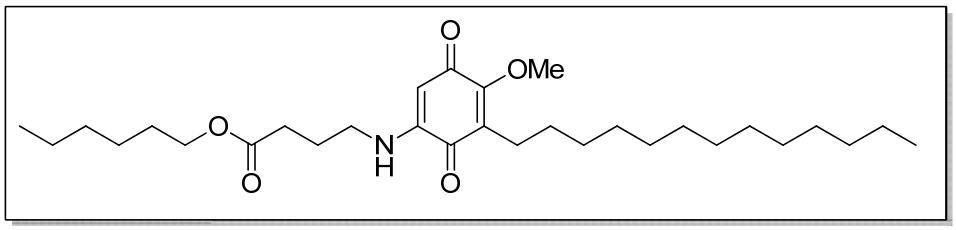
4-(Hexyloxy)-4-oxobutan-1-ammonium 4-Methylbenzenesulfonate (2.27).[126]

A solution of 1.00g (9.70 mmol) of 4-aminobutanoic acid, 2.02 g (1.08 mmol) of *p*-toluenesulfonic acid monohydrate and 1.51 mL (1.23 g, 1.24 mmol) of 1-hexanol in 20 mL of toluene was heated to reflux for 24 h, using a Dean-Stark distilling receiver. The reaction mixture was allowed to cool to room temperature and diluted with 20 mL of anh diethyl ether to afford *p*-toluenesulfonate salt **2.27** as a crystalline, colorless solid: yield 2.50 g (72%); silica gel TLC R_f 0.22 (9:1 chloroform–methanol); ^{1}H NMR ($CDCl_3$) δ 0.88 (t, 3H, *J* = 7.2 Hz), 1.21-1.35 (m, 6H), 1.55 (quin, 2H, *J* = 14.0 and 7.2 Hz), 1.85 (quin, 2H, *J* = 14.8 and 7.3 Hz), 2.27 (t, 2H, *J* = 7.3 Hz), 2.36 (s, 3H), 2.80-2.92 (m, 2H), 3.98 (t, 2H, *J* = 6.9 Hz), 7.18 (d, 2H, *J* = 7.9 Hz) and 7.72-7.83 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.2, 21.5, 22.67, 22.71, 25.7, 28.6, 31.0, 31.6, 39.4, 65.0, 126.1, 129.2, 140.9, 141.2 and 172.6.



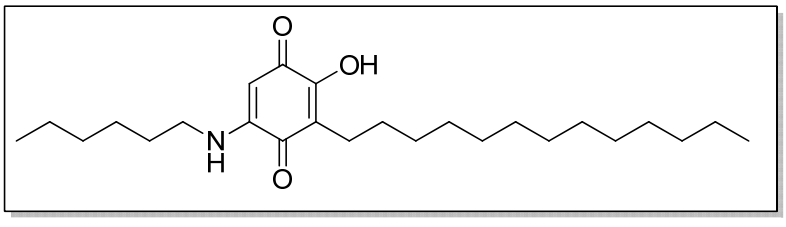
Hexyl 4-((4-Hydroxy-3,6-dioxo-5-tridecylcyclohexa-1,4-dien-1-yl)amino)butanoate (2.7). To a solution containing 37.0 mg (0.11 mmol) of 2-hydroxy-5-methoxy-3-tridecyl-(1,4)-benzoquinone (**2.24**) in 5.2 mL of

dichloromethane was added a solution containing 119 mg (0.33 mmol) of *p*-tolunesulfonate salt **2.27** and 33.0 mg (97%, 0.33 mmol) of potassium *tert*-butoxide dropwise in 5.2 mL of dichloromethane. The reaction mixture was stirred at room temperature for 20 h under an argon atmosphere. The reaction mixture was then washed with 5 mL of 1 N HCl and the aqueous layer was extracted with seven 2-mL portions of dichloromethane. The combined organic layer was washed with water and brine and then dried (Na_2SO_4). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (24 × 3 cm). Elution with diethyl ether gave compound **2.7** as a dark red solid: yield 27 mg (50%); silica gel TLC R_f 0.40 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.85-0.90 (m, 6H), 1.18-1.51 (m, 28H), 1.58-1.65 (m, 2H), 1.99 (quin, 2H, *J* = 14.0 and 7.2 Hz), 2.35-2.43 (m, 4H), 3.23 (q, 2H, *J* = 6.7 Hz), 4.09 (t, 2H, *J* = 6.8 Hz), 5.36 (s, 1H), 6.58 (s, 1H) and 8.08 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 14.3, 22.70, 22.79, 22.83, 23.3, 25.7, 28.2, 28.7, 29.5, 29.6, 29.7, 29.80, 29.82, 31.5, 31.6, 32.1, 42.3, 65.2, 91.9, 116, 149.8, 155.1, 172.9, 179 and 182.6; mass spectrum (APCI), *m/z* 492.3684 $(M + H)^+$ ($C_{29}H_{50}NO_5$ requires 492.3689).

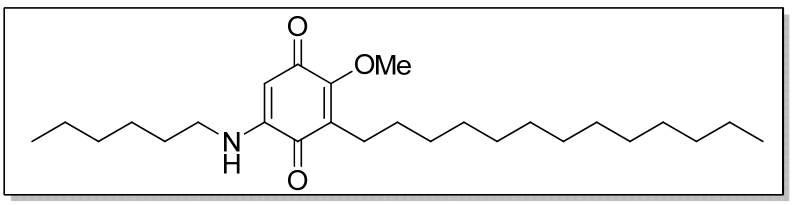


Hexyl 4-((4-Methoxy-3,6-dioxo-5-tridecylcyclohexa-1,4-dien-1-yl)amino)butanoate (2.10). To a solution containing 29 mg (59 μmol) of

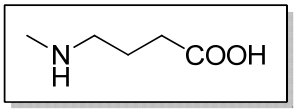
hydroxyquinone **2.7** and 0.3 g (2.2 mmol) of potassium carbonate in 1.5 mL of anh acetone was added dropwise 28 μL (37 mg, 0.3 mmol) of dimethyl sulfate. The reaction mixture was heated to reflux overnight, allowed to cool to room temperature and concentrated under diminished pressure to afford a crude residue. The residue was redissolved in 10 mL of dichloromethane and washed with 5 mL of 1 N HCl. The aqueous layer was then extracted with three 10-mL portions of dichloromethane. The combined organic layer was dried ($MgSO_4$) and concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (23 × 2 cm). Step gradient elution with 20% diethyl ether→30% diethyl ether in hexane gave compound **2.10** as a bright red solid: yield 8 mg (27%); silica gel TLC R_f 0.40 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.81-0.97 (m, 6H), 1.15-1.45 (m, 28H), 1.54-1.70 (m, 2H), 1.96 (quin, 2H, J = 11.2 and 5.6 Hz), 2.31-2.48 (m, 4H), 3.16 (q, 2H, J = 6.6 Hz), 4.02-4.21 (m, 5H), 5.26 (s, 1H) and 5.87-6.06 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 14.3, 15.4, 22.7, 22.8, 23.1, 23.5, 25.7, 28.7, 28.8, 29.5, 29.6, 29.7, 29.80, 29.84, 31.6, 31.8, 32.1, 42.1, 61.7, 65.2, 66.0, 96.2, 127.6, 146.9, 158.5, 173.0, 181.7 and 183.9; mass spectrum (APCI), *m/z* 506.3836 $(M + H)^+$ ($C_{30}H_{52}NO_5$ requires 506.3845).



5-(Hexylamino)-2-hydroxy-3-tridecylcyclohexa-2,5-diene-1,4-dione (2.11). To a solution containing 49.0 mg (0.15 mmol) of 2-hydroxy-5-methoxy-3-tridecyl-(1,4)-benzoquinone (**2.24**) in 12 mL of EtOH was added 97.0 μL (74.0 mg, 0.73 mmol) of hexylamine dropwise followed by 1.20 g (14.6 mmol) of $NaHCO_3$. The reaction mixture was stirred at room temperature for 20 h under an argon atmosphere and then washed with 5 mL of 1 N HCl. The aqueous layer was extracted with seven 2-mL portions of dichloromethane. The combined organic layer was washed with water and brine and then dried (Na_2SO_4). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (24 × 3 cm). Elution with 10% diethyl ether in hexane gave compound **2.11** as a dark red solid: yield 10.0 mg (17%); silica gel TLC R_f 0.53 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.81-0.96 (m, 6H), 1.16-1.50 (m, 28H), 1.65 (quin, 2H, J = 14.4 and 6.8 Hz), 2.30-2.43 (m, 2H), 3.15 (dd, 2H, J = 12.8 and 6.4 Hz), 3.22-3.34 (br s, 1H), 5.32 (s, 1H) and 6.41 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 14.3, 22.6, 22.79, 22.84, 26.8, 28.2, 28.3, 29.51, 29.56, 29.6, 29.7, 29.81, 29.83, 31.4, 31.5, 32.0, 32.1, 43.0, 91.6, 115.8, 149.8, 155.3, 178.8 and 182.7; mass spectrum (APCI), *m/z* 406.3313 $(M + H)^+$ ($C_{25}H_{44}NO_3$ requires 406.3321).

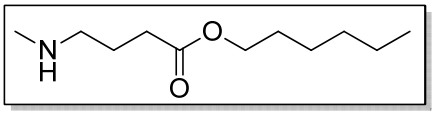


5-(Hexylamino)-2-methoxy-3-tridecylcyclohexa-2,5-diene-1,4-dione (2.12). To a solution containing 15 mg (40 μmol) of quinone **2.11** and 84 mg (1.4 mmol) of potassium carbonate in 1.0 mL of anh acetone was added 20 μL (27 mg, 0.2 mmol) of dimethyl sulfate. The reaction mixture was heated to reflux for 3h and stirred at room temperature overnight. The reaction mixture was then concentrated under diminished pressure and the crude residue was redissolved in 10 mL of dichloromethane and washed with 5 mL of 1 N HCl. The aqueous layer was extracted with three 10-mL portions of dichloromethane. The combined organic layer was dried ($MgSO_4$) and concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (24 × 2 cm). Elution with 10% diethyl ether in hexane gave compound **2.12** as a bright red solid: yield 9.0 mg (58%); silica gel TLC R_f 0.76 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.82-0.98 (m, 6H), 1.18-1.46 (m, 27H), 1.51-1.73 (m, 3H), 2.27-2.46 (m, 2H), 3.07 (dd, 2H, J = 13.2 and 6.4 Hz), 4.11 (s, 3H), 5.25 (s, 1H) and 5.81 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.1, 14.3, 22.7, 22.8, 23.1, 26.8, 28.3, 28.8, 29.5, 29.6, 29.7, 29.81, 29.83, 31.5, 32.1, 42.7, 61.8, 95.9, 127.5, 146.9, 158.7, 181.7 and 184.1; mass spectrum (APCI), *m/z* 420.3470 $(M + H)^+$ ($C_{26}H_{46}NO_3$ requires 420.3478).



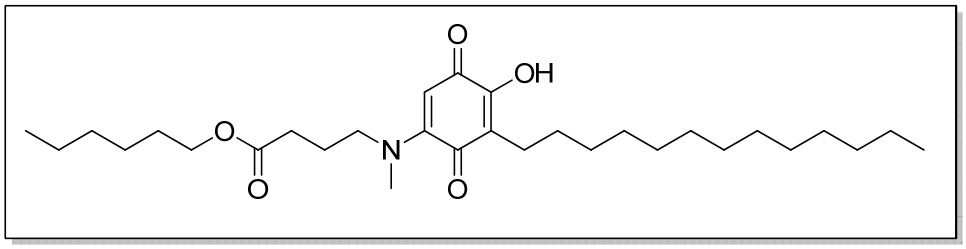
4-(*N*-Methylamino)butanoic Acid (2.28).[112] To a solution containing 9.70 g (104 mmol) of *N*-methyl-2-pyrrolidone in 111 mL of distilled water was added 10.9 g

(63.5 mmol) of $Ba(OH)_2$. The heterogeneous mixture was heated to reflux for 5 h and then cooled to 0 °C and saturated with CO_2 gas (dry ice). The resulting white precipitate was collected by filtration and washed with cold water. The clear filtrate was concentrated under diminished pressure and the resulting moist residue was triturated with acetonitrile, filtered and washed with ether. The crude residue thus obtained was further dried by co-evaporating three times with toluene and triturated with methanol to yield *N*-methyl butyric acid (**2.28**) as a colorless solid: yield 5.45 g (45%); ^{1}H NMR (DMSO-d_6) δ 1.09 (quin, 2H, J = 13.6 and 6.8 Hz), 1.41-1.59 (m, 2H), 1.86 (d, 3H, J = 0.9 Hz), 2.20 (t, 2H, J = 6.9 Hz), 2.50-2.57 (m, 1H) and 4.67 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 14.0, 23.2, 27.0, 41.0 and 171.3.



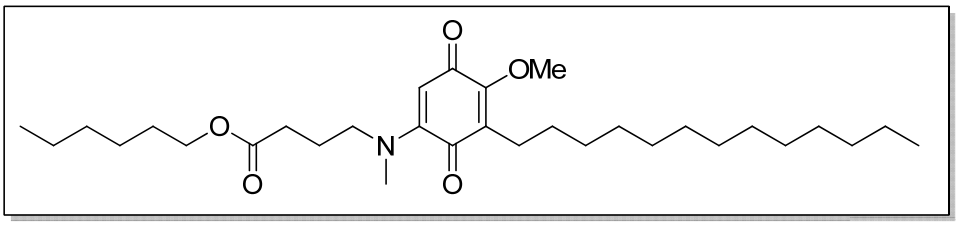
Hexyl 4-(*N*-Methylamino)butanoate (2.29). A solution containing 3.52 g (30.0 mmol) of 4-(*N*-methylamino)butanoic acid (**2.28**), 6.25 g (32.4 mmol) of *p*-toluenesulfonic acid hydrate and 4.70 mL (3.82 g, 37.2 mmol) of 1-hexanol in 62 mL of toluene was heated to reflux for 12 h using a Dean-Stark distilling receiver. The cooled reaction mixture was concentrated under diminished pressure to afford a crude residue. The residue was dissolved in 10 mL of hexane and the resulting solution cooled to −72 °C for 40 min and filtered to yield the amine **2.29** as its tosylate salt. The tosylate salt obtained was dissolved in 100 mL of dichloromethane and washed with 1 M K_2CO_3. The organic layer was dried

($MgSO_4$) and concentrated under diminished pressure to generate the free amine **2.29** as a colorless oil: yield 5.50 g (91%); ^{1}H NMR ($CDCl_3$) δ 0.73-0.87 (m, 3H), 1.15-1.34 (m, 6H), 1.48-1.60 (m, 2H), 1.73 (quin, 2H, *J* = 14.4 and 7.2 Hz), 2.22-2.32 (m, 2H), 2.35 (d, 3H, *J* = 10.7 Hz), 2.52 (t, 2H, *J* = 7.1 Hz) and 3.88-4.07 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 14.0, 22.5, 25.1, 25.6, 28.6, 31.4, 32.1, 36.3, 50.9, 64.6 and 173.6.



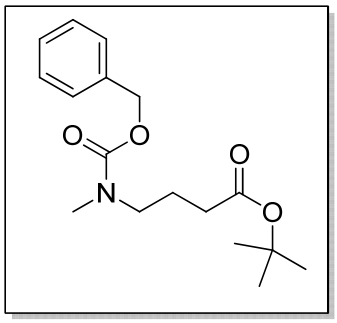
Hexyl 4-((4-Hydroxy-3,6-dioxo-5-tridecylcyclohexa-1,4-dien-1-yl)(methyl)amino)butanoate (2.13). To a solution containing 60.0 mg (0.18 mmol) of 2-hydroxy-5-methoxy-3-tridecyl-(1,4)-benzoquinone (**2.24**) in ethanol was added 360 mg (1.79 mmol) of amine **2.29**. The reaction mixture was stirred at room temperature for 12 h and then washed with brine and dried ($MgSO_4$). The organic layer was concentrated under diminished pressure to afford the crude residue. The residue was applied to a silica gel column (24 × 2 cm). Elution with 60:1 dichloromethane–methanol gave compound **2.13** as a red solid: yield 39.0 mg (43%); silica gel TLC R_f 0.32 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.81-0.95 (m, 6H), 1.19-1.28 (m, 14H), 1.27-1.37 (m, 12H), 1.36-1.48 (m, 2H), 1.51-1.68 (m, 2H), 2.00 (quin, 2H, *J* = 15.0 and 7.5 Hz), 2.38 (t, 4H, *J* = 7.5 Hz), 3.14 (s, 3H), 3.63 (t, 3H, *J* = 7.0 Hz), 4.07 (t, 2H, *J* = 7.0 Hz) and 5.49 (s, 1H);

^{13}C NMR ($CDCl_3$) δ 14.1, 14.3, 22.7, 22.8, 23.2, 25.6, 25.7, 28.5, 28.7, 29.5, 29.6, 29.78, 29.79, 29.81, 29.83, 29.87, 31.2, 31.6, 31.8, 32.1, 32.9, 54.4, 63.2, 65.0, 98.0, 117.5, 153.0, 172.9, 178.7 and 184.6; mass spectrum (APCI), *m/z* 506.3848 $(M + H)^+$ ($C_{30}H_{51}NO_5$ requires 506.3845).



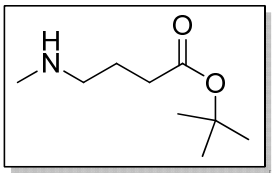
Hexyl 4-((4-Methoxy-3,6-dioxo-5-tridecylcyclohexa-1,4-dien-1-yl)(methyl)amino)butanoate (2.15). To a solution containing 19 mg (40 μmol) of hydroxyquinone **2.13** in anh acetone was added 0.2 g (1.4 mmol) of potassium carbonate and 20 μL (27 mg, 0.2 mmol) of dimethyl sulfate dropwise. The reaction mixture was heated to reflux for 1.5 h and cooled to room temperature and stirred at 23 °C for 12 h. The reaction mixture was concentrated under diminished pressure and redissolved in 50 mL of dichloromethane. The organic layer was washed with brine, dried ($MgSO_4$) and concentrated under diminished pressure to afford a crude red residue. The residue was applied to a silica gel column (24 × 2 cm). Elution with 60:1 dichloromethane–methanol gave compound **2.15** as a red solid: yield 10 mg (51%); silica gel TLC R_f 0.61 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.84-0.91 (m, 6H), 1.20-1.40 (m, 26H), 1.55-1.68 (m, 4H), 1.97 (2H, quin, J = 14.5 and 7.0 Hz), 2.31-2.39 (m, 4H), 2.99 (s, 3H), 3.50-3.59 (m, 2H), 4.00-4.19 (m, 5H) and 5.40 (s, 1H); ^{13}C NMR

($CDCl_3$) δ 22.7, 22.8, 23.3, 23.6, 25.7, 28.7, 29.1, 29.5, 29.6, 29.72, 29.76, 29.81, 29.83, 29.92, 29.98, 31.3, 31.6, 32.1, 40.6, 53.6, 61.3, 65.0, 95.8, 102.4, 129.6, 150.9, 156.6, 173.1, 181.5 and 185.7; mass spectrum (APCI), *m/z* 520.4002 $(M + H)^+$ ($C_{31}H_{54}NO_5$ requires 520.4002).

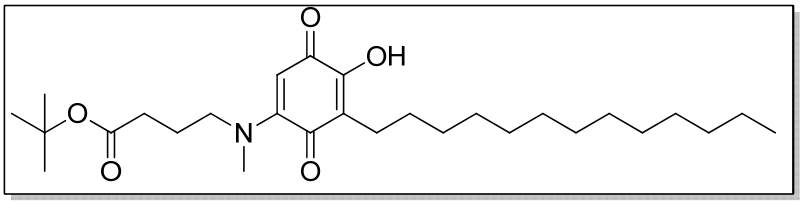


***tert*-Butyl 4-(((Benzyloxy)carbonyl)(methyl)amino)butanoate (2.30).**[127-129] To a solution containing 900 mg (7.68 mmol) of acid **2.28** in 10.3 mL of 3 M aq KOH was added 1.14 mL (1.36 g, 7.68 mmol) of 95% benzyl chloroformate dropwise over a period of 10 min under an argon atmosphere. The reaction mixture was stirred at room temperature for 2 h and quenched by the addition of 7.9 mL of 5 M aq HCl solution dropwise. The aqueous layer was extracted with three 30-mL portions of ethyl acetate. The combined organic extract was washed with water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was dissolved in 8.5 mL of *tert*-butylacetate and 130 μL (1.48 mmol) of 70% perchloric acid was added dropwise. The reaction mixture was stirred at room temperature for 18 h and quenched by the addition of 20 mL of satd aq $NaHCO_3$. The aqueous layer was extracted with three 30-mL portions of dichloromethane. The combined

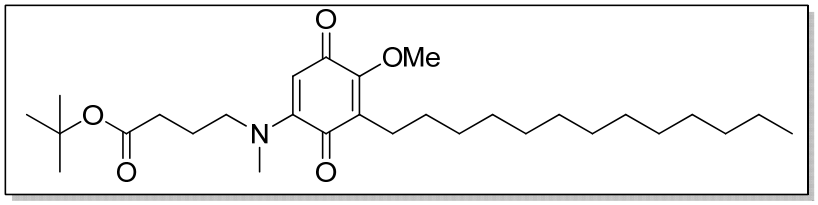
organic layer was washed with water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (20 × 3 cm). Elution with 1:5 ethyl acetate–hexanes afforded compound **2.30** as a colorless oil: yield 372 mg (29% over two steps); silica gel TLC R_f 0.52 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H), 1.81 (dd, 2H, J = 15.6 and 6.8 Hz), 2.15-2.26 (m, 2H), 2.91 (s, 3H), 3.20 (br s, 2H), 5.11 (s, 2H) and 7.25-7.38 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 22.8, 27.8, 32.2, 34.0, 47.9, 66.7, 79.9, 127.5, 127.6, 128.2, 136.7, 155.9 and 171.9.



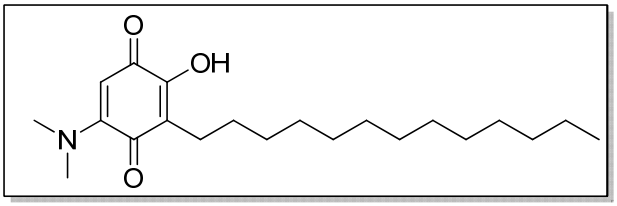
***tert*-Butyl 4-(*N*-methylamino)butanoate (2.31).**[129] To a solution containing 372 mg (1.21 mmol) of ester **2.30** in 4.4 mL of methanol was added 40.0 mg of 10% Pd/C. Hydrogen gas was bubbled through the solution for 2 h under atmospheric pressure. The catalyst was removed by filtration through a pad of Celite and the filtrate was concentrated under diminished pressure carefully (as the product is volatile) to afford compound **2.31** as a colorless oil: yield 91 mg (43%); ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 9H), 2.01-2.11 (m, 2H), 2.31 (t, 2H, J = 7.1 Hz), 2.64 (s, 3H), 2.95 (dd, 2H, J = 13.0 and 5.1 Hz) and 8.48 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 21.7, 28.2, 32.4, 33.2, 48.9, 81.0 and 171.7.



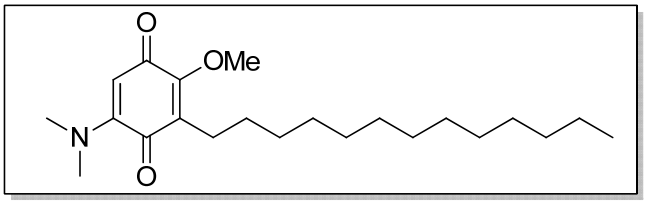
***tert*-Butyl 4-((4-Hydroxy-3,6-dioxo-5-tridecylcyclohexa-1,4-dien-1-yl)(methyl)amino)butanoate (2.14).** To a solution containing 71.0 mg (0.21 mmol) of 2-hydroxy-5-methoxy-3-tridecyl-(1,4)-benzoquinone (**2.24**) in ethanol was added 730 mg (4.21 mmol) of the amine **2.31**. The reaction mixture was stirred at room temperature for 12 h, concentrated under diminished pressure and diluted by the addition of 20 mL of dichloromethane. The organic layer was washed with brine and dried ($MgSO_4$), then concentrated under diminished pressure to afford the crude residue as a red solid. The residue applied to a silica gel column (20 × 3 cm). Elution with 9:1 hexane–ethyl acetate gave compound **2.14** as a red solid: yield 75 mg (74%); silica gel TLC R_f 0.45 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.85 (t, 3H, J = 6.7 Hz), 1.18-1.31 (m, 22H), 1.43 (s, 9H), 1.94 (dt, 2H, J = 14.0 and 6.9 Hz), 2.27 (t, 2H, J = 7.0 Hz), 2.32-2.39 (m, 2H), 3.08 (br s, 3H), 3.59 (br s, 2H) and 5.48 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.2, 22.8, 23.1, 28.2, 28.4, 29.4, 29.6, 29.73, 29.75, 29.77, 29.78, 29.8, 32.0, 32.4, 41.4, 54.5, 80.8, 97.6, 117.4, 153.0, 153.3, 172.1, 178.6 and 184.8; mass spectrum (APCI), *m/z* 478.3533 $(M + H)^+$ ($C_{28}H_{48}NO_5$ requires 478.3532).



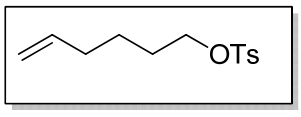
***tert*-Butyl 4-((4-Methoxy-3,6-dioxo-5-tridecylcyclohexa-1,4-dien-1-yl)(methyl)amino)butanoate (2.16).** To a solution containing 43.0 mg (0.09 mmol) of hydroxyquinone **2.14** in 2.5 mL of anh acetone was added 473 mg (3.42 mmol) of potassium carbonate and 50.0 μL (66.0 mg, 0.45 mmol) of dimethyl sulfate dropwise. The reaction mixture was heated to reflux for 3h and allowed to cool to room temperature, then concentrated under diminished pressure to afford a crude residue. The residue was dissolved in 50 mL of dichloromethane, washed with brine and then dried ($MgSO_4$). The organic layer was concentrated under diminished pressure to afford a crude red residue. The residue was applied to a silica gel column (20 × 3 cm). Elution with 60:1 dichloromethane–methanol gave compound **2.16** as a red solid: yield 30 mg (42%); silica gel TLC R_f 0.58 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3H, J = 6.9 Hz), 1.22-1.32 (m, 20H), 1.33-1.39 (m, 2H), 1.44 (s, 9H), 1.92 (dt, 2H, J = 14.8 and 7.3 Hz), 2.26 (t, 2H, J = 7.2 Hz), 2.33-2.39 (m, 2H), 2.99 (s, 3H), 3.48-3.55 (m, 2H), 4.05 (s, 3H) and 5.40 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.2, 22.8, 23.4, 23.6, 28.20, 28.24, 29.0, 29.5, 29.6, 29.74, 29.79, 29.81, 29.83, 30.0, 32.1, 32.5, 40.6, 53.7, 61.3, 80.7, 102.3, 129.6, 150.9, 156.6, 172.3, 181.4 and 185.7; mass spectrum (APCI), *m/z* 492.3695 $(M + H)^+$ ($C_{29}H_{50}NO_5$ requires 492.3689).



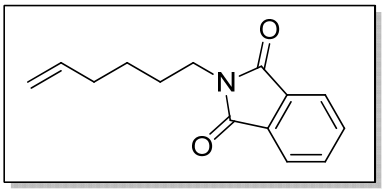
5-(*N,N*-Dimethylamino)-2-hydroxy-3-tridecylcyclohexa-2,5-diene-1,4-dione (2.17). To a solution containing 38.0 mg (0.11 mmol) of 2-hydroxy-5-methoxy-3-tridecyl-(1,4)-benzoquinone (**2.24**) in 12 mL of ethanol was added 470 mg (5.65 mmol) of $NaHCO_3$ and 140 μL (126 mg, 1.12 mmol) of a 40% by wt solution of dimethylamine in water dropwise. The reaction mixture was stirred at room temperature for 20 h and then concentrated under diminished pressure to afford a crude residue. The residue was diluted with 50 mL of dichloromethane. The organic layer was washed with two 10-mL portions of 1 N HCl, dried ($MgSO_4$) and then concentrated under diminished pressure to afford a red solid. The crude residue was applied to a silica gel column (20 × 2 cm). Elution with 60:1 dichloromethane–methanol gave compound **2.17** as a red solid: yield 27 mg (69%); silica gel TLC R_f 0.36 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3H, J = 7.2 Hz), 1.20-1.35 (m, 16H), 1.36-1.48 (m, 4H), 2.34-2.47 (m, 4H), 3.23 (br s, 6H), 3.85 (s, 1H) and 5.48 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.3, 22.8, 23.2, 28.4, 29.51, 29.56, 29.6, 29.71, 29.78, 29.81, 29.82, 29.83, 29.85, 32.1, 43.7, 56.9, 97.6, 102.3, 117.2, 153.7 and 185.0; mass spectrum (APCI), *m/z* 350.2692 $(M + H)^+$ ($C_{21}H_{36}NO_3$ requires 350.2695).



5-(*N*,*N*-Dimethylamino)-2-methoxy-3-tridecylcyclohexa-2,5-diene-1,4-dione (2.18). To a solution containing 26.0 mg (74.0 μmol) of hydroxyquinone **2.17** in 7.4 mL of anh acetone was added 388 mg (2.81 mmol) of potassium carbonate and 35.0 μL (47.0 mg, 0.37 mmol) of dimethyl sulfate dropwise. The reaction mixture was heated to reflux for 1.5 h and allowed to cool to room temperature and then stirred for another 12 h. The reaction mixture was concentrated under diminished pressure and then diluted with 50 mL of dichloromethane. The organic layer was washed with 10 mL brine and dried (Na_2SO_4), then concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (20 × 2 cm). Elution with dichloromethane gave compound **2.18** as a red solid: yield 25 mg (93%); silica gel TLC R_f 0.50 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 0.87 (t, 3H, J = 6.8 Hz), 1.20-1.32 (m, 20H), 1.33-1.45 (m, 2H), 2.29-2.44 (m, 2H), 3.12 (s, 6H), 4.06 (s, 3H) and 5.38 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 14.3, 22.8, 23.6, 29.0, 29.5, 29.6, 29.7, 29.80, 29.82, 29.83, 29.9, 32.1, 42.8, 61.3, 102.3, 129.5, 151.4, 156.8, 181.4 and 185.9; mass spectrum (APCI), *m/z* 364.2859 $(M + H)^+$ ($C_{22}H_{38}NO_3$ requires 364.2852).

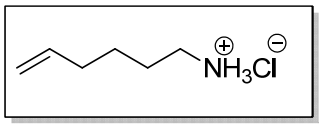


Hex-5-en-1-yl 4-Methylbenzenesulfonate (2.32).[130] To a solution containing 2.0 g (20 mmol) of 5-hexen-1-ol and 3.1 mL (2.2 g, 5.5 mmol) of triethylamine in 60 mL of anh dichloromethane was added 4.2 g (22 mmol) of *p*-toluenesulfonyl chloride at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 12 h. The reaction mixture was then diluted with 100 mL of dichloromethane and washed with two 30-mL portions of 10% aq $NaHCO_3$ and brine. The organic layer was dried ($MgSO_4$) and then concentrated under diminished pressure to afford a crude residue. The residue was purified by flash column chromatography on a silica gel column (24 × 3 cm). Elution with 4:1 hexanes–ethyl acetate gave compound **2.32** as a colorless oil: yield 5.07 g (100%); silica gel TLC R_f 0.65 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.41 (quin, 2H, *J* = 15.2 and 7.6 Hz), 1.60-1.71 (m, 2H), 1.97 (q, 2H, *J* = 14.4 and 7.2 Hz), 2.45 (s, 3H), 4.03 (t, 2H, *J* = 6.4 Hz), 4.89-4.95 (m, 2H), 5.65-5.78 (m, 1H), 7.34 (d, 2H, *J* = 8.4 Hz) and 7.79 (d, 2H, *J* = 8.4 Hz); ^{13}C NMR ($CDCl_3$) δ 21.8, 24.7, 28.3, 33.0, 70.6, 115.2, 128.0, 129.9, 133.3, 138.0 and 144.8.

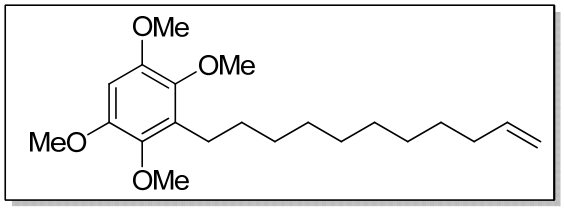


2-(Hex-5-en-1-yl)isoindoline-1,3-dione (2.33).[113] To a solution containing 5.1 g (20 mmol) of tosylate **2.32** in 40 mL of DMF was added 4.4 g (24 mmol) of potassium phthalimide and the mixture heated at 60 °C for 24h. The reaction mixture was allowed to cool to room temperature and then the solution was

filtered. The filtrate was then washed with brine and extracted with three 30-mL portions of ether. The combined organic layer was washed with brine and dried ($MgSO_4$), then concentrated under diminished pressure to afford **2.33** as colorless oil. The crude residue was used for the next reaction.

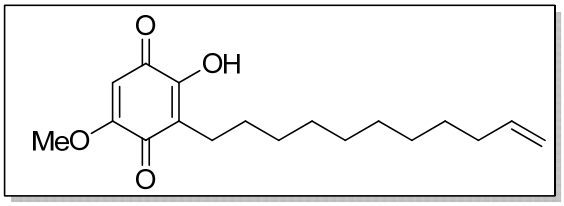


Hex-5-en-1-ammonium Chloride (2.34).[113] To a solution containing 3.10 g (13.3 mmol) of the crude phthalimide **2.33** in 16 mL of ethanol was added 400 μL (13.3 mmol) of hydrazine hydrate. The reaction mixture was heated at 60 °C for 12 h. The cooled reaction mixture was treated dropwise with 4.7 mL of conc HCl and then again heated to reflux for an additional 2 h. The cooled reaction mixture was filtered to remove a white precipitate. The filtrate was concentrated under diminished pressure to afford a crude residue. The residue was triturated successively with chloroform and ether to afford amine hydrochloride **2.34** as a yellow solid: yield 686 mg (25% over two steps); ^{1}H NMR ($CDCl_3$) δ 1.50 (quin, 2H, *J* = 15.2 and 7.6 Hz), 1.79 (quin, 2H, *J* = 15.2 and 7.2 Hz), 2.09 (dd, 2H, *J* = 14.4 and 7.2 Hz), 3.00 (br s, 2H), 4.93-5.07 (m, 2H), 5.70-5.85 (m, 1H) and 8.25 (br s, 3H); ^{13}C NMR ($CDCl_3$) δ 25.8, 27.1, 33.1, 40.0, 115.5 and 137.7.



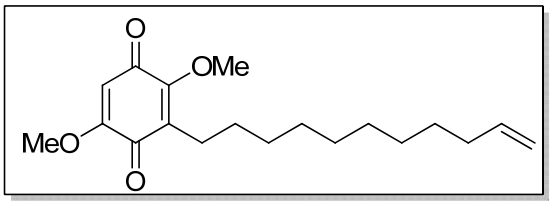
1,2,4,5-Tetramethoxy-3-(undec-10-en-1-yl)benzene (2.35). To a solution containing 630 mg (3.18 mmol) of 1,2,4,5-tetramethoxybenzene (**2.21**) and 56.0 μL (58.0 mg, 0.32 mmol) of hexamethyl phosphoramide in 16 mL of anh THF was added 1.40 mL (2.5 M in hexanes, 3.50 mmol) of *n*-butyllithium dropwise at –40 °C over a period of 1 h. The reaction mixture was allowed to warm to –10 °C over a period of 2 h and 770 μL (0.82 g, 3.50 mmol) of purified 11-bromoundec-1-ene was added. The reaction mixture was stirred at room temperature under an argon atmosphere for 15 h and quenched by the addition of 20 mL of satd aq NH_4Cl solution. The aqueous layer was extracted with five 10-mL portions of diethyl ether. The combined organic layer was washed with water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (6 × 3 cm). Step gradient elution with hexane→2:1 hexane–ethyl acetate afforded **2.35** as a colorless oil: yield 0.91 g (82%); silica gel TLC R_f 0.83 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.23-1.43 (m, 12H), 1.49-1.56 (m, 2H), 2.03 (q, 2H, J = 14.4 and 6.8 Hz), 2.59-2.63 (m, 2H), 3.77 (s, 6H), 3.84 (s, 6H), 4.90-5.00 (m, 2H), 5.76-5.86 (m, 1H) and 6.41 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 24.8, 29.1, 29.3, 29.59, 29.64, 29.65, 30.1, 30.9, 33.9, 56.3, 61.0, 96.7, 114.2, 131.2,

139.4, 141.2 and 148.9; mass spectrum (EI), *m/z* 350.2451 $(M)^+$ ($C_{21}H_{34}O_4$ requires 350.2457).

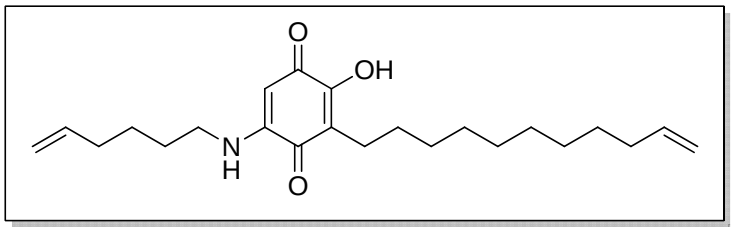


2-Hydroxy-5-methoxy-3-(undec-10-en-1-yl)cyclohexa-2,5-diene-1,4-dione (2.37). To a solution containing 3.33 g (9.50 mmol) of alkenyltetramethoxy benzene **2.35** in 95 mL of acetonitrile was added dropwise a solution containing 10.4 g (19.0 mmol) of cerium(IV) ammonium nitrate in 95 mL of 7:3 acetonitrile–water at −7 °C (salt–ice bath) over a period of 30 min. The reaction mixture was allowed to warm to room temperature and stirred for 3 h and was then quenched by the addition of 300 mL of ether. The organic layer was washed with distilled water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude mixture of quinones **2.36** and **2.37**. To the solution of the crude residue dissolved in 95 mL of dichloromethane was added 9.50 g (4.75 mmol) of $HClO_4$-SiO_2 and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture was filtered and then concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (23 × 3 cm). Elution with 9:1 hexane–ethyl acetate gave compound **2.37** as a yellow–orange solid; silica gel TLC R_f 0.46 (1:1 ethyl acetate–hexanes): yield 745 mg (26% over two steps); ^1H

NMR ($CDCl_3$) δ 1.06-1.24 (m, 12H), 1.24-1.35 (m, 2H), 1.86 (q, 2H, *J* = 14.4 and 7.6 Hz), 2.23-2.33 (m, 2H), 3.71 (s, 3H), 4.72-4.88 (m, 2H) and 5.58-5.72 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 22.6, 28.0, 28.9, 29.1, 29.40, 29.48, 29.50, 29.57, 33.8, 56.8, 102.2, 114.1, 119.3, 139.2, 151.6, 161.1, 181.7 and 182.9; mass spectrum (APCI), *m/z* 306.1836 $(M)^+$ ($C_{18}H_{26}O_4$ requires 306.1831).

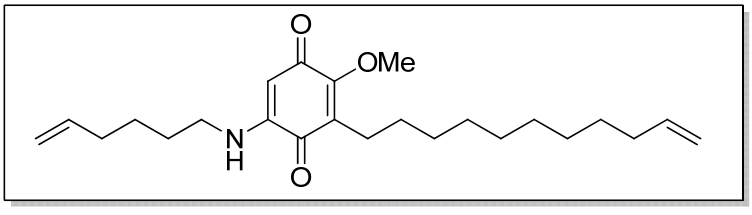


2,5-Dimethoxy-3-(undec-10-en-1-yl)cyclohexa-2,5-diene-1,4-dione (2.36). Yellow solid; silica gel TLC R_f 0.61 (1:1 ethyl acetate–hexanes); ^1H NMR ($CDCl_3$) δ 1.20-1.44 (m, 14H), 1.97-2.05 (m, 2H), 2.41 (dd, 2H, *J* = 13.4 and 6.2 Hz), 3.79 (s, 3H), 4.03 (s, 3H), 4.87-5.01 (m, 2H), 5.71 (s, 1H) and 5.74-5.84 (m, 1H) ; ^{13}C NMR ($CDCl_3$) δ 23.2, 28.8, 29.0, 29.2, 29.46, 29.55, 29.57, 29.7, 33.9, 56.5, 61.4, 105.5, 114.2, 130.8, 139.3, 156.0, 158.9, 182.5 and 183.7; mass spectrum (APCI), *m/z* 320.1977 $(M)^+$ ($C_{19}H_{28}O_4$ requires 320.1988).

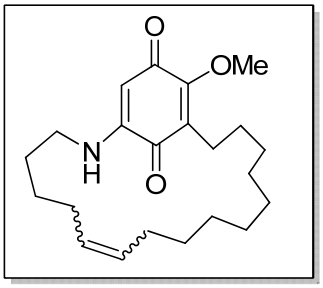


5-(Hex-5-en-1-ylamino)-2-hydroxy-3-(undec-10-en-1-yl)cyclohexa-2,5-diene-1,4-dione (2.38). To a solution containing 141 mg (1.04 mmol) of amine

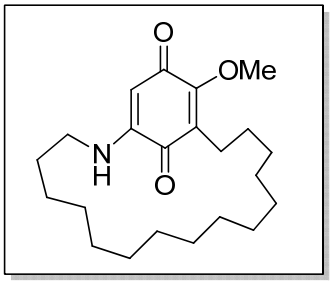
hydrochloride **2.34** in 35 mL of ethanol was added 123 mg (96%, 1.04 mmol) of potassium *t*-butoxide and the reaction mixture stirred at room temperature for 30 min. To the reaction mixture was added a solution of 107 mg (0.35 mmol) of hydroxyquinone **2.37** in 35 mL of ethanol dropwise over a period of 15 min. The reaction mixture was stirred for 12 h. The reaction mixture was concentrated under diminished pressure to afford a crude residue. The resulting residue was dissolved in 30 mL of dichloromethane and washed with 10 mL of 1 N HCl. The organic layer was dried ($MgSO_4$) and then concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (24 × 2 cm). Elution with 50:1 dichloromethane–methanol gave compound **2.38** as a bright red solid: yield 126 mg (75%); silica gel TLC R_f 0.13 (chloroform); ^{1}H NMR ($CDCl_3$) δ 1.17-1.38 (m, 12H), 1.39-1.53 (m, 4H), 1.60-1.76 (m, 2H), 2.01 (dd, 2H, *J* = 14.1 and 6.9 Hz), 2.08 (dd, 2H, *J* = 13.6 and 6.8 Hz), 2.32-2.41 (m, 2H), 3.15 (d, 2H, *J* = 4.5 Hz), 4.86-5.07 (m, 4H), 5.33 (s, 1H), 5.68-5.86 (m, 2H), 6.46 (s, 1H) and 8.25 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 22.7, 26.2, 27.6, 28.2, 29.02, 29.22, 29.53, 29.57, 29.62, 29.67, 33.3, 33.9, 42.8, 91.7, 114.2, 115.4, 115.8, 137.9, 139.3, 149.8, 155.4, 178.8 and 182.6; mass spectrum (APCI), *m/z* 374.2694 $(M + H)^+$ ($C_{23}H_{36}NO_3$ requires 374.2695).



5-(Hex-5-en-1-ylamino)-2-methoxy-3-(undec-10-en-1-yl)cyclohexa-2,5-diene-1,4-dione (2.39). To a solution containing 144 mg (0.39 mmol) of quinone **2.38** and 2.00 g (38.0 mmol) of potassium carbonate in 9.6 mL of anh acetone was added 190 μL (253 mg, 1.93 mmol) of dimethyl sulfate. The reaction mixture was heated to reflux for 3 h and allowed to cool to room temperature and stirred overnight. The solvent was concentrated under diminished pressure to afford a crude product. The crude product was dissolved in 20 mL of dichloromethane and washed with 5 mL of 1 N HCl. The aqueous layer was extracted with three 10-mL portions of dichloromethane. The combined organic layer was dried ($MgSO_4$) and concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (24 × 2 cm). Elution with 20% diethyl ether in hexane gave compound **2.39** as a bright red solid: yield 110 mg (74%); silica gel TLC R_f 0.36 (dichloromethane); ^{1}H NMR ($CDCl_3$) δ 1.18-1.39 (m, 14H), 1.45 (quin, 2H, J = 15.2 and 7.6 Hz), 1.63 (quin, 2H, J = 14.8 and 7.2 Hz), 1.97-2.11 (m, 4H), 2.34 (t, 2H, J = 7.6 Hz), 3.08 (dd, 2H, J = 13.2 and 6.0 Hz), 4.09 (s, 3H), 4.86-5.05 (m, 4H), 5.23 (s, 1H) and 5.69-5.87 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 23.0, 26.3, 27.7, 28.7, 29.0, 29.2, 29.5, 29.6, 29.7, 33.3, 33.9, 42.5, 61.7, 95.8, 114.2, 115.3, 127.4, 138.0, 139.3, 146.8, 158.5, 181.6 and 184.0; mass spectrum (APCI), *m/z* 388.2858 $(M + H)^+$ ($C_{24}H_{38}NO_3$ requires 388.2852).



19-Methoxy-2-azabicyclo[16.3.1]docosa-1(21),7,18-triene-20,22-dione (2.40). To a solution containing 31 mg (80 μmol) of quinone **2.39** in toluene was added 7.0 mg (8.0 μmol) of Grubb's 2nd generation catalyst. The reaction mixture was heated at 80 °C for 12 h and then allowed to cool to room temperature. The solvent was concentrated under diminished pressure to afford crude residue. The residue was applied to a silica gel column (30 × 3 cm). Elution with 1:9 ethyl acetate–hexane afforded compound **2.40** as a purple-red solid (mixture of diastereomers): yield 15 mg (52%); silica gel TLC R_f 0.23 (dichloromethane); major diastereomer ^{1}H NMR ($CDCl_3$) δ 1.08-1.35 (m, 12H), 1.35-1.53 (m, 4H), 1.57-1.70 (m, 2H), 1.92-2.04 (m, 4H), 2.42-2.52 (m, 2H), 3.08-3.21 (m, 3H), 4.08-4.14 (m, 2H), 5.24-5.31 (m, 2H), 5.31-5.43 (m, 1H) and 5.82-5.92 (m,1H); mixture of diastereomers ^{13}C NMR ($CDCl_3$) δ 22.2, 26.6, 26.85, 26.97, 26.98, 27.11, 27.15, 27.2, 27.38, 27.44, 27.7, 28.2, 28.3, 28.4, 28.5, 28.6, 28.8, 28.9, 29.1, 29.3, 29.8, 30.0, 31.6, 32.3, 42.1, 53.6, 61.7, 62.9, 95.7, 95.9, 127.5, 128.6, 129.5, 131.5, 132.3, 147.0, 158.8, 158.9, 181.6 and 184.2; mass spectrum (APCI), *m/z* 360.2546 $(M + H)^+$ ($C_{22}H_{34}NO_3$ requires 360.2539).



19-Methoxy-2-azabicyclo[16.3.1]docosa-1(21),18-diene-20,22-dione (2.19). To a solution containing15.5 mg (0.04 mmol) of quinone **2.40** in 5 mL of ethyl acetate was added 23 mg of 10% Pd/C and H_2 gas was bubbled through the solution at room temperature for 4h. The reaction mixture was then diluted with 1 mL of methanol and stirred at room temperature overnight. The reaction mixture was purged by bubbling air and then concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (20 × 3 cm). Step gradient elution with dichloromethane→100: 1 dichloromethane–methanol afforded compound **2.19** as a purple-red solid: yield 6 mg (38% over two steps); silica gel TLC R_f 0.3 (dichloromethane); ^{1}H NMR ($CDCl_3$) δ 1.06-1.39 (m, 22H), 1.43-1.53 (m, 2H), 1.60-1.69 (m, 2H), 2.43-2.53 (m, 2H), 3.12-3.22 (m, 2H), 4.12 (s, 3H), 5.28 (s, 1H) and 5.89 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 22.2, 26.3, 27.4, 27.69, 27.75, 27.81, 27.87, 28.0, 28.2, 28.49, 28.53, 28.55, 28.63, 29.1, 42.2, 61.8, 95.8, 127.3, 146.9, 158.9, 181.6 and 184.1; mass spectrum (APCI), *m/z* 362.2702 $(M + H)^+$ ($C_{22}H_{36}NO_3$ requires 362.2695).

Cytoprotection

FRDA lymphocytes were grown in RPMI 1640 medium (Gibco) supplemented with 15% fetal calf serum, 2 mM glutamine (HyClone) and 1% penicillin-streptomycin mix (Cellgro). Cells were seeded at a density of 5×10^5 cells/mL and treated with different concentrations of the indicated compounds. Cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air for 17 h. After pre-incubation, the cells were treated with 5 mM DEM. Cell viability was determined by staining cells with 0.4% trypan blue and counting the cells using a hemacytometer. At least 500 cells were counted in each experimental group. At the time of assay, < 20% of DEM treated cells were viable (trypan blue negative), whereas in non DEM-treated controls, > 90% of the cells were viable. Cell viability was expressed as the percentage of control. Data are expressed as means ± S.E.M. (n = 3).

Inhibition of lipid peroxidation

Lipid peroxidation was determined in FRDA lymphocyte cells depleted of glutathione. Following pretreatment with the indicated compounds at 5 μM or 10 μM concentration for 16 h, the cells were treated with 500 nM C_{11}-BODIPY$^{581/591}$ in the dark at 37 °C for 30 min before inducing lipid peroxidation with 5 mM diethyl maleate (DEM) for 140 min, and then subjected to flow cytometry analysis using the FL1-H channel for C_{11}-BODIPY$^{581/591}$ – green (oxidized form). In each analysis, 10,000 events were recorded. Increased C_{11}-BODIPY$^{581/591}$ – green fluorescence, a measure of intracellular lipid peroxidation, was determined by a shift in BODIPY$^{581/591}$ – green fluorescence to the right on the *x*-axis of the FACS histogram relative to the untreated control.

Inhibition of mitochondrial complex I and NADH oxidase activity

Beef heart mitochondria were obtained by a large-scale procedure.[131] Inverted submitochondrial particles (SMP) were prepared by the method of Matsuno-Yagi and Hatefi[132] and stored in a buffer containing 0.25 M sucrose and 10 mM Tris–HCl, pH 7.4, at –80 °C. Inhibitory effects of quinone analogues on bovine heart mitochondrial complex I (NADH: ubiquinone oxidoreductase) were evaluated by modification of a method described previously.[133] Stock solutions (2 mg/mL in ethanol) of quinone analogues were prepared and kept in the dark at –80 °C. Maximal ethanol concentration never exceeded 2% and had no influence on the control enzymatic activity. The enzymatic activities were assayed at 30 °C and monitored spectrophotometrically with a Molecular Devices SPECTRA Max-M5 (340 nm, $\varepsilon = 6.22\ mM^{-1}\ cm^{-1}$). NADH oxidase activity was determined in a reaction medium (2.5 mL) containing 50 mM Hepes, pH 7.5, containing 5 mM $MgCl_2$. The final amount of mitochondrial protein was 30 μg. The reaction was initiated by adding 50 μM NADH after the pre-equilibration of SMP with inhibitor for 5 min. The initial rates were calculated from the linear portion of the traces. The inhibition of NADH-Q_1 oxidoreductase (complex I) activity was also determined under the same experimental conditions except that the reaction medium (2.5 mL) contained 0.25 M sucrose, 1 mM $MgCl_2$, 2 μM antimycin A, 2 mM KCN, 50 μM ubiquinone Q_1 and 50 mM phosphate buffer, pH 7.4. IC_{50} values were taken as the final compound concentrations in the assay cuvette that yielded 50% inhibition of the enzymatic activity.

CHAPTER 3

3. SYNTHESIS OF A BLEOMYCIN DISACCHARIDE LIBRARY

3.1 Introduction

Carbohydrates are an important class of biomolecules that play a pivotal role in mediating various biological processes. Glycopeptides, glycolipids and other glycoconjugates are known to participate in signal transduction,[134] inflammation,[135] cell-cell interactions,[136] fertility and development.[137]

Extracellular communication is crucial to a wide range of cellular processes that are essential for cell viability including growth, reproduction and motility. Such communication involves the interaction of cell surface receptors and their respective substrates, a process first described by Emil Fischer with his "lock-and-key" hypothesis.[138] A comprehensive understanding of cell surface interactions would enable the manipulation of these communications and the processes they control. Cell surface glycoconjugates have been found to play an important role in cellular communication.

Carbohydrate residues are recognized by specific cell surface carbohydrate-binding proteins called lectins and are then internalized into the cell by receptor-mediated endocytosis.[139] This mechanism is believed to play a crucial role in mediating the cellular uptake of many glycosylated natural products and controls their biological activity. Glycotargeting is a strategy that utilizes the

highly specific carbohydrate–lectin interactions to improve the bioavailability of biologically active molecules by conjugation to sugar residues.[140,141]

The bleomycins, originally isolated from *Streptomyces verticillus* in 1966 by Umezawa and colleagues[142] are a family of glycopeptides antibiotics that are used clinically in combination with other agents to treat several tumors.[143-148] The antitumor activity of bleomycin is attributed to its ability to cleave DNA specifically at 5'-GC-3' and 5'-GT-3' sequences.[149] The specific cytotoxicity of bleomycin towards tumor cells has led to their clinical use in the treatment of squamous cell carcinomas and malignant lymphomas.[86,150] All the members of the bleomycin family share essentially the same core structure which can be dissected into several functional domains as shown in Figure 1.3.[83]

The therapeutic utility of bleomycin is enhanced by its low myelosuppression and immunosuppression properties and underscored by its low therapeutic dose. The major hindrance to its widespread use, however, is the appearance of lung fibrosis in a significant percentage of patients treated with the drug.[83,89,151] This limitation highlights the need to understand the molecular mechanisms of bleomycin activity which could lead to the development of more highly selective and potent analogues with improved pharmocological properties.

Synthetic methods to evaluate the functional role of the bleomycin domains have shown that altering any position within the metal binding domain, the linker region or bithiazole moiety severely reduced the ability of the drug to mediate dsDNA cleavage, putatively its main source of cytotoxicity.[83,85,86,152-154] In terms of function the carbohydrate domain is the least well understood. The

role of the sugars in bleomycin activity is still unclear although *in vitro* and *in vivo* studies have shown reduced efficacy of deglycobleomycin in mediating dsDNA cleavage.[91-93]

The natural disaccharide moeity in bleomycin consists of the monosaccharide subunits D-mannose and L-gulose. The 3-position of the D-mannose moiety has a carbamoyl functional group which has been shown to play an important role in coordination of a variety of metal ions.[155-158] Although bleomycin and deglycobleomycin show analogous DNA cleavage properties *in vitro*,[159] differences in the behaviour of BLM and deglyco BLM have become more evident when *in vivo* assays were performed.[91-93] The importance of the carbohydrate residue to the tumor selectivity of BLM was further validated by the imaging studies carried out with ^{111}In-BLEDTA complex (Figure 3.1).[91,160,161] These studies included the findings that radionuclide derivatives of deglyco BLM failed to produce images comparable to those observed for the corresponding BLM derivative.[91,160,161] In summary, these results strongly suggest that the carbohydrate moiety in bleomycin may play an important role in cellular recognition and possible uptake.

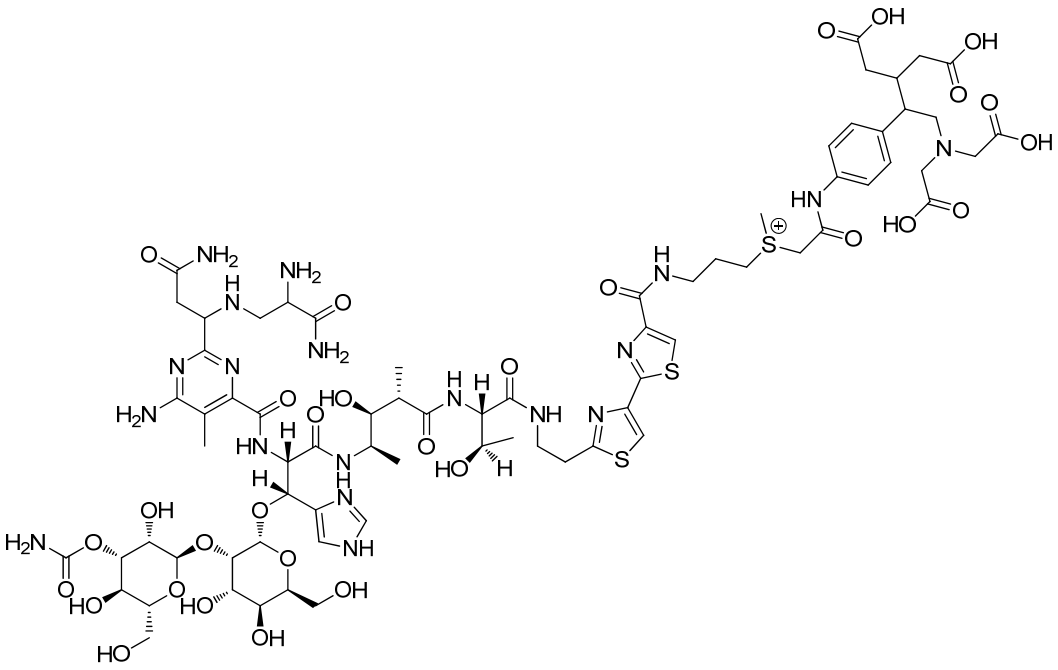


Figure 3.1. Chemical structure of BLEDTA.

To fully understand the importance of the carbohydrate domain in bleomycin to its overall potency, a library of bleomycin disaccharides which are structural analogues of the natural bleomycin disaccharide were synthesized. Our preliminary studies had shown that the carbamoyl functional group, which plays a crucial role in metal chelation (Figure 3.2), was essential for cellular recognition and uptake.[162] Thus the carbamoyl group was modified and its position was altered systematically to afford a library of disaccharides which were then conjugated to a fluorophore to monitor its cellular distribution and uptake by fluorescence microscopy (Figure 3.3). These studies would potentially provide conclusive evidence regarding the role of the carbohydrate moiety in tumor cell

binding and targeting by BLM and also help in identifying tumor targeting disaccharides capable of improving the therapeutic index of BLM.

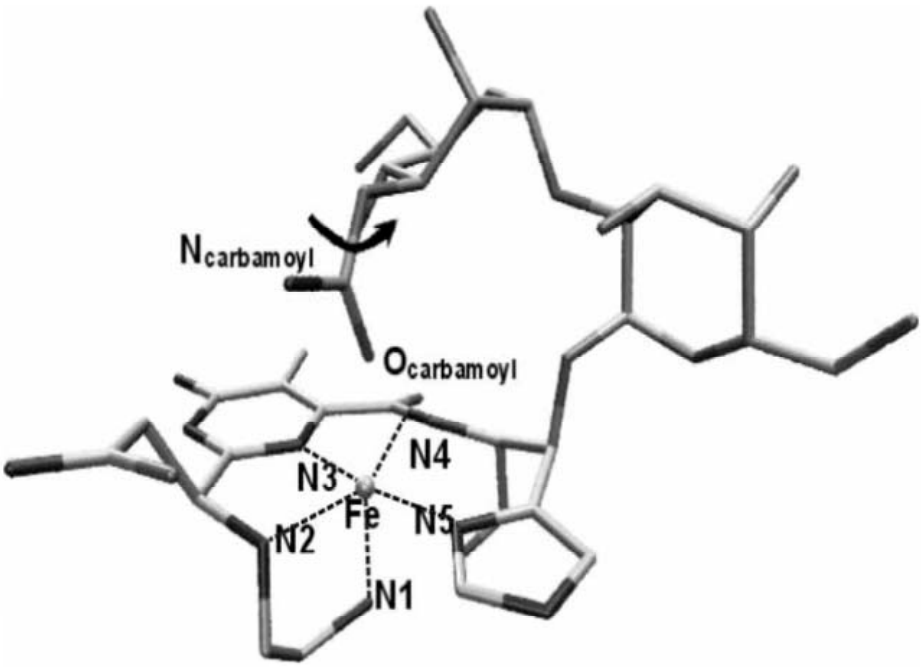


Figure 3.2. Proposed mode of coordination of Fe^{2+} with BLM.[156]

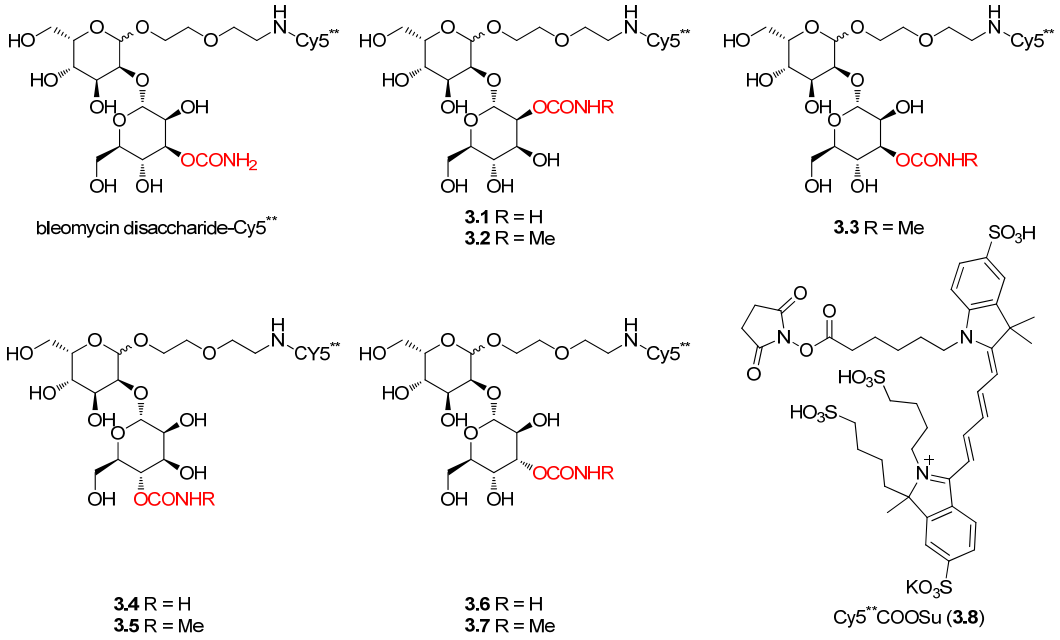


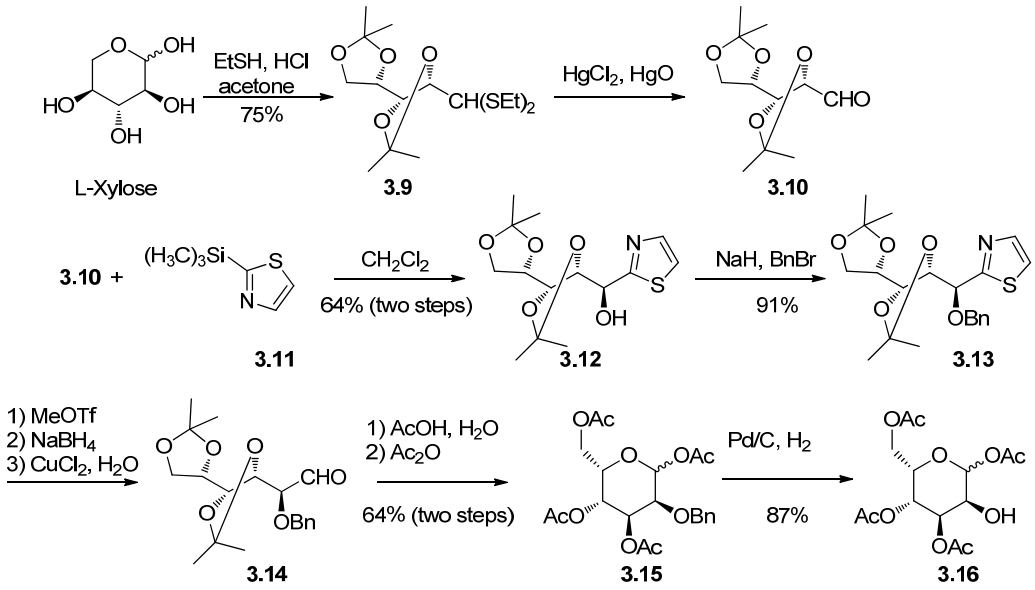
Figure 3.3. Structures of disaccharide-dye conjugates prepared for evaluation.

3.2 Results

3.2.1 Synthesis of gulose acceptor

The synthesis of gulose acceptor **3.16** was carried out according to a published procedure.[163] As outlined in Scheme 3.1, the synthesis began with the commercially available L-xylose which was first converted to the corresponding fully protected dithioacetal **3.9** in 75% yield. The Hg(II) promoted hydrolysis of the dithioacetal **3.9** afforded the aldehyde **3.10** which was then coupled to 2-(trimethylsilyl)thiazole[164] (**3.11**) followed by a desilylative workup to afford the alcohol **3.12** in 64% yield over two steps. The hydroxyl group of **3.12** was then benzylated to yield ether **3.13** in 91% yield. Ether **3.13** was converted to the corresponding aldehyde **3.14** through a one-pot sequence of transformations involving the cleavage of the thiazole ring to afford the formyl group. Aldehyde

3.14 was then subjected to acid hydrolysis followed by exhaustive acetylation to yield tetra-*O*-acetyl-2-*O*-benzyl-L-gulopyranoside (**3.15**) in 64% yield over two steps. The benzylated gulose **3.15** was then subjected to debenzylation by hydrogenolysis over palladium-on-carbon to yield the gulose acceptor **3.16** in 87% yield.



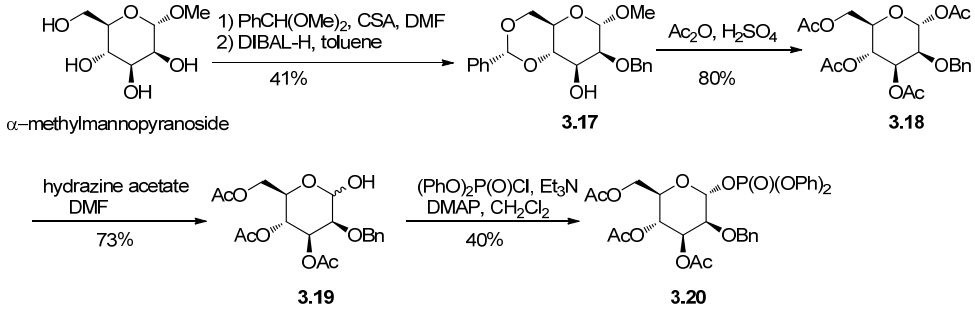
Scheme 3.1 Synthesis of gulose acceptor **3.16**.

3.2.2 Synthesis of mannose and altrose donors

The synthetic strategy adopted for the synthesis of the library of disaccharides required the generation of mannose and altrose monosaccharides with benzyl group at C2, C3 or C4 positions respectively. The benzyl groups were used to mask the positions at which the carbamoyl or methylcarbamoyl groups were later introduced into the disaccharide.

3.2.2.1 Synthesis of mannose donor 3.20

As shown in Scheme 3.2, the synthesis of the mannose donor **3.20** with a C2 benzyl group, began with the commercially available α-D-methylmannopyranoside, which was converted to 2-benzyl benzylidene acetal **3.17** in 41% yield over two steps.[165] The benzylidene acetal was then cleaved and subjected to exhaustive acetylation to yield tetra-*O*-acetyl-2-*O*-benzyl-D-mannopyranoside (**3.18**) in 80% yield. Mannopyranoside **3.18** was converted to **3.19** by the selective hydrazine acetate-mediated deacetylation of the anomeric acetate in 73% yield. The activation of pyranoside **3.19** as a glycosyl donor was accomplished through treatment of **3.19** with diphenyl chlorophosphate in presence of DMAP and Et_3N to yield the α-glycosyl diphenyl phosphate **3.20** in 40% yield.[166]

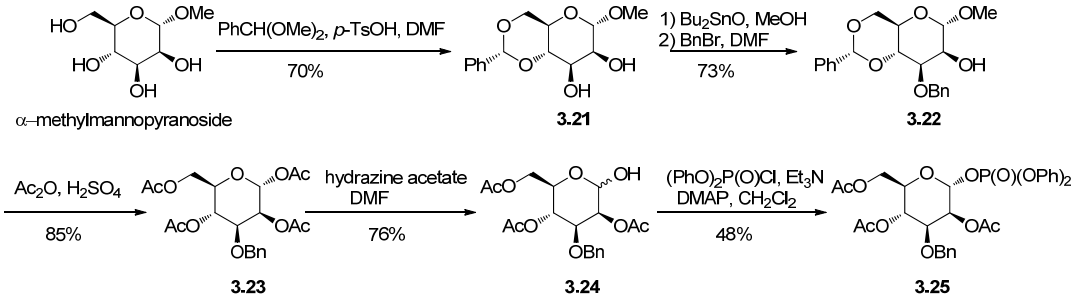


Scheme 3.2 Synthesis of C2 benzyl mannose donor **3.20**.

3.2.2.2 Synthesis of mannose donor 3.25

The synthesis of the mannose donor **3.25** with a C3 benzyl group is outlined in Scheme 3.3. The synthesis began with the conversion of commercially available α-D-methylmannopyranoside to the diol **3.21** in 70% yield.[167] The

alkylation of the 2,3-*O*-dibutylstannylene derived from the reaction of the diol **3.21** and Bu_2SnO with benzyl bromide resulted in exclusive benzylation of the equatorial C3 alcohol to provide acetal **3.22** in 73% yield.[167] The benzylidene acetal **3.22** was then cleaved and subjected to exhaustive acetylation to yield tetra-*O*-acetyl-3-*O*-benzyl-D-mannopyranoside (**3.23**) in 85% yield. The mannopyranoside **3.23** was converted to **3.24** by the selective hydrazine acetate-mediated deacetylation of the anomeric acetate in 76% yield. The activation of pyranoside **3.24** as a donor was accomplished through treatment of **3.24** with diphenyl chlorophosphate in presence of DMAP and Et_3N to yield the α-glycosyl diphenyl phosphate **3.25** in 48% yield.[166]

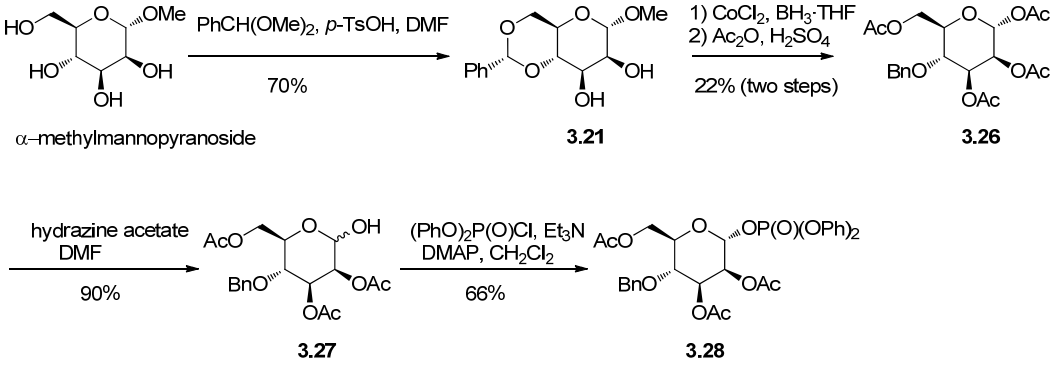


Scheme 3.3 Synthesis of C3 benzyl mannose donor **3.25**.

3.2.2.3 Synthesis of mannose donor 3.28

The synthesis of the mannose donor **3.28** with a C4 benzyl group, began with the conversion of commercially available α-D-methylmannopyranoside to the diol **3.21**[167] in 70% yield as shown in Scheme 3.4. The diol was then subjected to a regioselective reductive ring-opening in presence of $CoCl_2$ and BH_3·THF to yield the 4-benzyl pyranoside. The latter was subjected to exhaustive acetylation,

affording tetra-*O*-acetyl-4-*O*-benzyl-D-mannopyranoside (**3.26**) in 22% yield over two steps.[168] Mannopyranoside **3.26** was converted to pyranoside **3.27** by the selective hydrazine acetate-mediated deacetylation of the anomeric acetate in 90% yield. The activation of pyranoside **3.27** as a glycosyl donor was accomplished through treatment of **3.27** with diphenyl chlorophosphate in presence of DMAP and Et_3N to yield the α-glycosyl diphenyl phosphate **3.28** in 66% yield.[166]

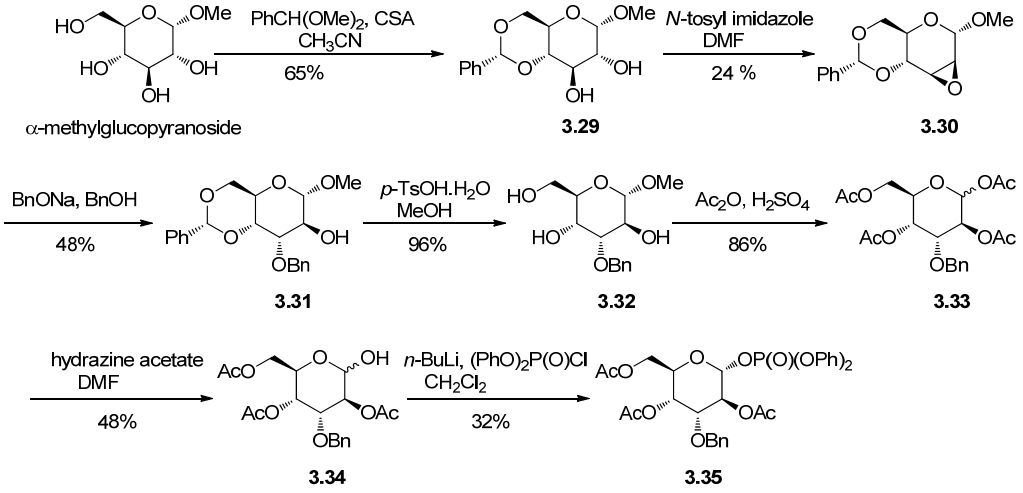


Scheme 3.4 Synthesis of C4 benzyl mannose donor **3.28**.

3.2.2.4 Synthesis of altrose donor 3.35

The synthesis of the altrose donor **3.35** with a C3 benzyl group, began with the conversion of commercially available α-D-methylglucopyranoside to the methyl 4,6-di-*O*-benzylidene-D-glucopyranoside (**3.29**)[169] in 65% yield as outlined in Scheme 3.5. A two-step protocol involved the conversion of **3.29** to the corresponding 2,3-anhydromannopyranoside **3.30**[170] in 24% yield, followed by the regioselective opening of the oxirane at C3 with sodium benzyloxide was adopted to obtain the altropyranoside **3.31**[171] in 48% yield. The benzylidene acetal of **3.31** was then cleaved in 96% yield and the product was subjected to

exhaustive acetylation to yield tetra-*O*-acetyl-3-*O*-benzyl-D-altropyranoside (**3.33**) as a mixture of anomers in 86% yield. Altropyranoside **3.33** was converted to pyranoside **3.34** by the selective hydrazine acetate-mediated deacetylation of the anomeric acetate in 48% yield. The activation of **3.34** as a glycosyl donor was accomplished through treatment of **3.34** with diphenyl chlorophosphate in presence of *n*-BuLi to yield the α-glycosyl diphenyl phosphate **3.35** in 32% yield.



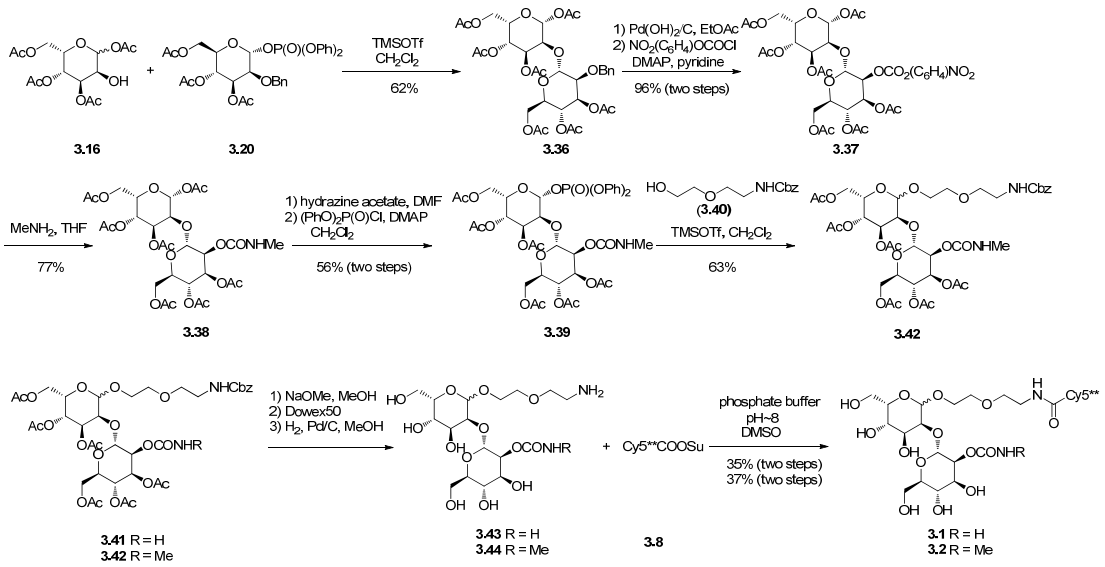
Scheme 3.5 Synthesis of C3 benzyl altrose donor **3.35**.

3.2.3 Synthesis of disaccharides

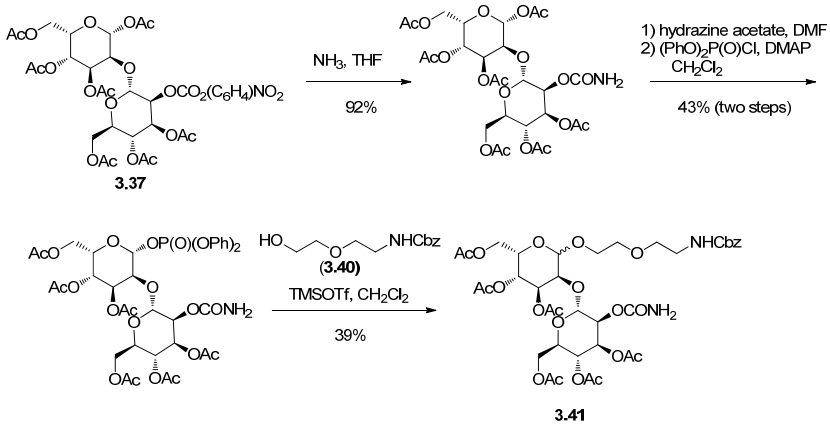
3.2.3.1 Syntheses of C2 modified mannose disaccharide-dye conjugates

The syntheses of the C2 modified mannose disaccharide-dye conjugates **3.1** and **3.2** (Scheme 3.6) began with the coupling of gulose acceptor **3.16** and mannose donor **3.20** to yield the C2 benzylated disaccharide **3.36** in 62% yield.[163] Disaccharide **3.36** was subjected to hydrogenolysis over palladium-on-carbon and then converted to the *p*-nitrophenyl carbonate **3.37** in 96% yield over two steps.[167] The *p*-nitrophenyl carbonate **3.37** was then subjected to aminolysis with

methylamine to yield **3.38** in 77% yield. Disaccharide **3.38** was converted to glycosyl donor **3.39** by a selective hydrazine acetate-mediated deacetylation of the anomeric acetate, followed by activation with diphenyl chlorophosphate in presence of DMAP and Et_3N to yield the α-glycosyl diphenyl phosphate **3.39** in 56% yield over two steps.[166] The glycosyl donor **3.39** was then coupled with CBz-protected linker **3.40**[172] to yield linker coupled disaccharide **3.42** in 63% yield. The linker–disaccharide conjugate **3.41** was synthesized in an analogous fashion by Chandrabali Bhattacharya from α-D-methylmannopyranoside and L-xylose monosaccharides (Scheme 3.1 and 3.2). The key step was the ammonolysis of nitrophenyl ester **3.37** to introduce the carbamoyl group at C2 position of the D-mannose moiety. The linker coupled disaccharide **3.41** was synthesized as shown in Scheme 3.7. The disaccharides **3.41**[173] and **3.42** were then subjected to a one-pot deacetylation and hydrogenolysis to afford the fully deprotected linker disaccharide conjugates **3.43** and **3.44**, which were then coupled to Cy5**COOSu (**3.8**)[174] (Figure 3.3) to afford the dye-disaccharide conjugates **3.1** and **3.2** in 35% and 37% yields over two steps respectively.



Scheme 3.6 Synthesis of C2 modified mannose disaccharide-dye conjugates.

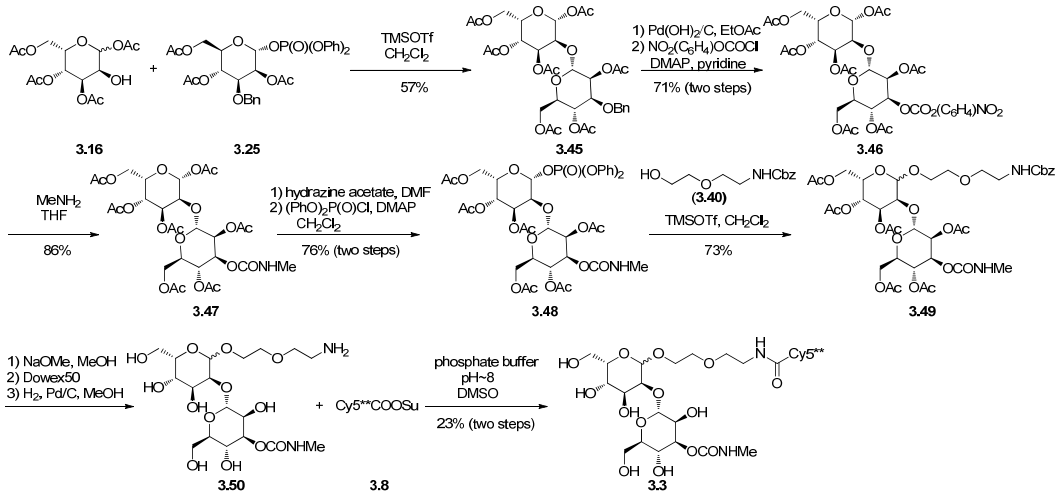


Scheme 3.7 Synthesis of C2 modified mannose disaccharide-linker conjugate **3.41**. The synthesis was carried out by Chandrabali Bhattacharya.

3.2.3.2 Synthesis of C3 modified mannose disaccharide-dye conjugate

The synthesis of the C3 modified mannose disaccharide-dye conjugate **3.3** is outlined in Scheme 3.8. It began with the coupling of gulose acceptor **3.16** and

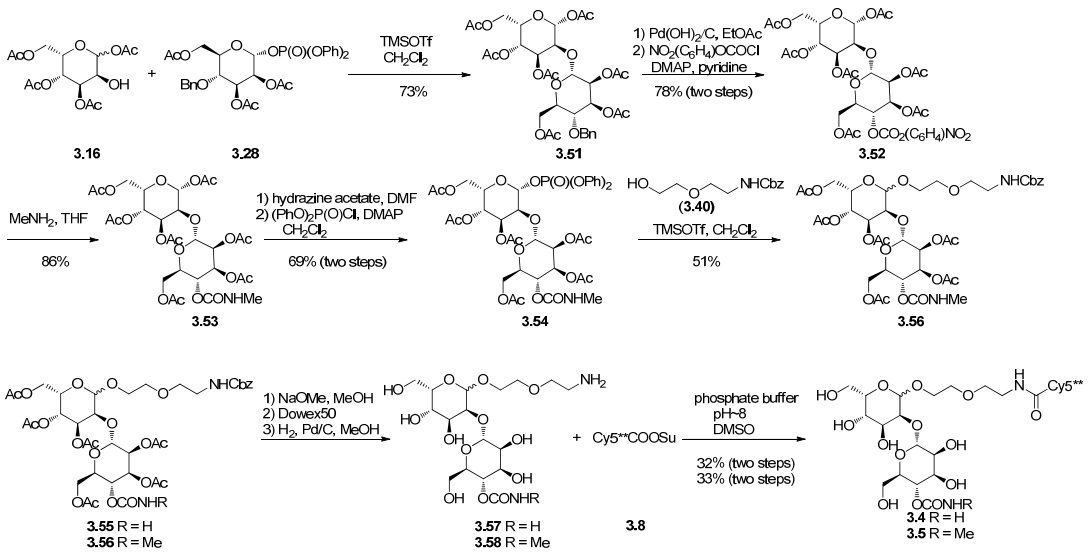
mannose donor **3.25** to yield the C3 benzylated disaccharide **3.45** in 57% yield.[163] Disaccharide **3.45** was subjected to hydrogenolysis over palladium-on-carbon and then converted to the *p*-nitrophenyl carbonate **3.46** in 71% yield over two steps.[167] The *p*-nitrophenyl carbonate **3.46** was then subjected to aminolysis with methylamine to yield **3.47** in 86% yield. Disaccharide **3.47** was converted to glycosyl donor **3.48** by selective hydrazine acetate-mediated deacetylation of the anomeric acetate, followed by activation with diphenyl chlorophosphate in presence of DMAP and Et_3N to yield the α-glycosyl diphenyl phosphate **3.48** in 76% yield over two steps.[166] Glycosyl donor **3.48** was then coupled with CBz-protected linker **3.40**[172] to yield linker coupled disaccharide **3.49** in 73% yield. The disaccharide was then subjected to a one-pot deacetylation and hydrogenolysis to afford the fully deprotected linker disaccharide conjugate **3.50** which was then coupled to Cy5**COOSu (**3.8**)[174] (Figure 3.3) to afford the dye-disaccharide conjugate **3.3** in 23% yield over two steps.



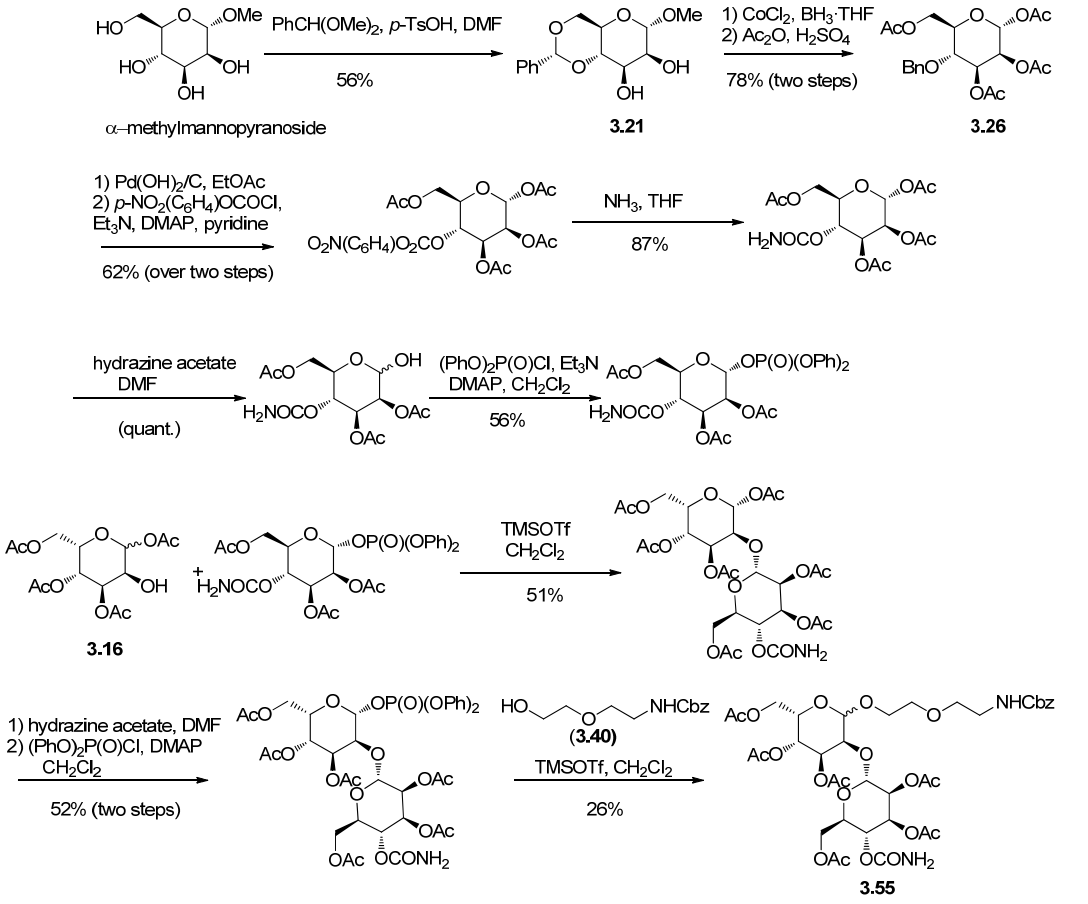
Scheme 3.8 Synthesis of C3 modified mannose disaccharide-dye conjugate

3.2.3.3 Syntheses of C4 modified mannose disaccharide-dye conjugates

The syntheses of the C4 modified mannose disaccharide-dye conjugates **3.4** and **3.5** are outlined in Scheme 3.9. They began with the coupling of gulose acceptor **3.16** and mannose donor **3.28** to yield the C4 benzylated disaccharide **3.51** in 73% yield.[163] Disaccharide **3.51** was subjected to hydrogenolysis over palladium-on-carbon and then converted to the *p*-nitrophenyl carbonate **3.52** in 78% yield over two steps.[167] The *p*-nitrophenyl carbonate **3.52** was then subjected to aminolysis with methylamine to yield **3.53** in 86% yield. Disaccharide **3.53** was converted to glycosyl donor **3.54** by selective hydrazine acetate-mediated deacetylation of the anomeric acetate, followed by activation with diphenyl chlorophosphate in presence of DMAP and Et_3N to yield the α-glycosyl diphenyl phosphate **3.54** in 69% yield over two steps.[166] The glycosyl donor **3.54** was then coupled with CBz-protected linker **3.40**[172] to yield linker coupled disaccharide **3.56** in 51% yield. The linker–disaccharide conjugate **3.55** was synthesized by Chandrabali Bhattacharya from α-D-methylmannopyranoside and L-xylose monosaccharides (Scheme 3.1 and Scheme 3.10). The disaccharides **3.55**[173] and **3.56** were then subjected to a one-pot deacetylation and hydrogenolysis to afford the fully deprotected linker disaccharide conjugates **3.57** and **3.58** respectively, which were then coupled to Cy5**COOSu (**3.8**)[174] (Figure 3.3) to afford the dye-disaccharide conjugates **3.4** and **3.5** in 32% and 33% yields, over two steps respectively.



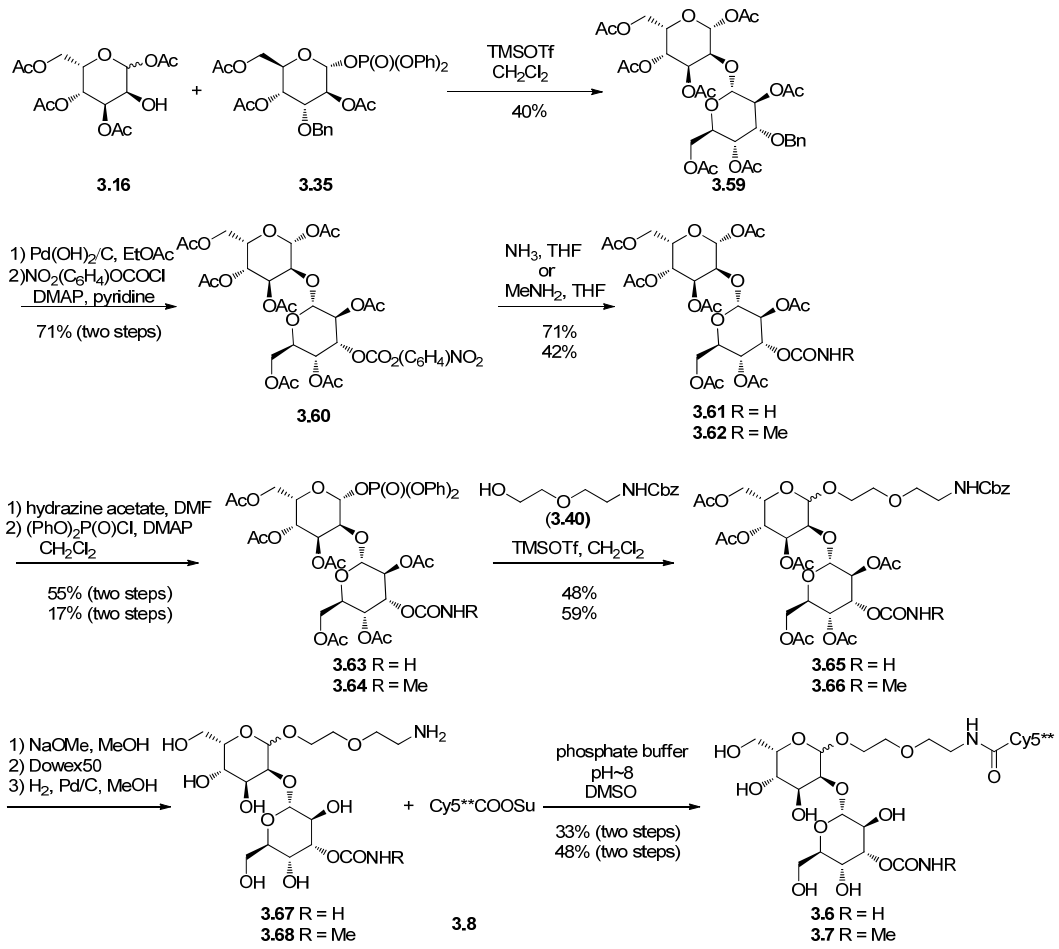
Scheme 3.9 Synthesis of C4 modified mannose disaccharide-dye conjugates.



Scheme 3.10 Synthesis of C4 modified mannose disaccharide-linker conjugate **3.55**. The synthesis was carried out by Chandrabali Bhattacharya.

3.2.3.4 Syntheses of C3 modified altrose disaccharide-dye conjugates

The syntheses of the C3 modified altrose disaccharide-dye conjugates **3.6** and **3.7** are shown in Scheme 3.11. They began with the coupling of gulose acceptor **3.16** and altrose donor **3.35** to yield the C3 benzylated disaccharide **3.59** in 40% yield.[163] Disaccharide **3.59** was subjected to hydrogenolysis over palladium-on-carbon and then converted to the *p*-nitrophenyl carbonate **3.60** in 71% yield over two steps.[167] The *p*-nitrophenyl carbonate **3.60** was then treated with ammonia or methylamine to yield **3.61** in 71% and **3.62** in 42% yields respectively. Disaccharides **3.61** and **3.62** were converted to the glycosyl donors **3.63** and **3.64**, respectively, by a selective hydrazine acetate-mediated deacetylation of the anomeric acetate, followed by activation with diphenyl chlorophosphate in presence of DMAP and Et_3N to yield the α-glycosyl diphenyl phosphate **3.63** in 55% yield and **3.64** in 17% yield over two steps.[166] Glycosyl donors **3.63** and **3.64** were then coupled with CBz-protected linker **3.40**[172] to yield linker coupled disaccharides **3.65** and **3.66** in 48% and 59% yields, respectively. The linker coupled disaccharides were then subjected to a one-pot deacetylation and hydrogenolysis to afford the fully deprotected linker disaccharide conjugates **3.67** and **3.68**, which were then coupled to Cy5**COOSu (**3.8**)[174] (Figure 3.3) to afford the dye-disccharide conjugates **3.6** and **3.7** in 33% and 48% yields, over two steps, respectively.



Scheme 3.11 Synthesis of C3 modified altrose disaccharide-dye conjugates.

3.2.4 Biological evaluation of fluorescent carbohydrate analogues

Cells were cultured on 16-well glass chamber slides for 48 h, and incubated with a 25 μM solution of the appropriate disaccharide-dye conjugate at 37 ^{o}C for 1 h. The cells were then fixed with a 4% solution of paraformaldehyde and washed twice with PBS buffer. Fluorescence microscopy imaging was carried out with a Zeiss Axiovert 200M inverted microscope with 40x oil objective.

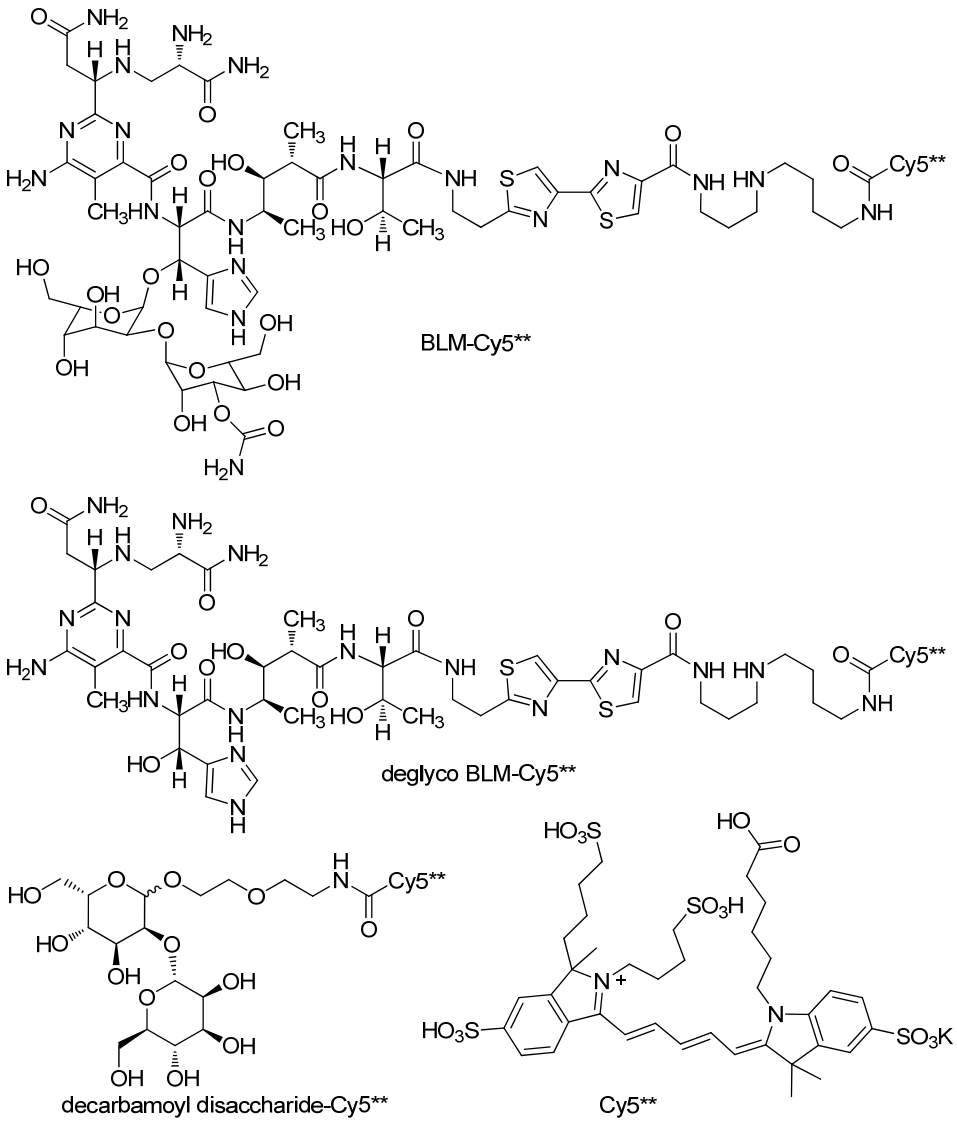


Figure 3.4. Chemical structures of Cy5** conjugates and Cy5** dye.

Fluorescent probe Cy5** was chosen to circumvent the problems of autofluorescence and non-specific cell surface binding. It is a member of the cyanine dye family and has emission wavelengths in the red or near-infrared region. The cell binding/uptake of the dye (Cy5**), BLM disaccharide-Cy5** conjugate, BLM-Cy5** conjugate and deglyco BLM-Cy5** conjugate in human

prostate cancer cell (DU-145) was compared to its uptake in normal human prostate cells (PZ-HPV-7). As shown in Figure 3.5, the free dye exhibits very low uptake by either human cancer or normal prostate cell lines. The high uptake of the BLM disaccharide-Cy5** conjugate and BLM-Cy5** conjugate in the tumor cell line highlights the importance of the disaccharide moiety to tumor cell uptake. This is further validated by the low binding and uptake of deglyco BLM-Cy5** conjugate in the human prostate cancer cell line studied.

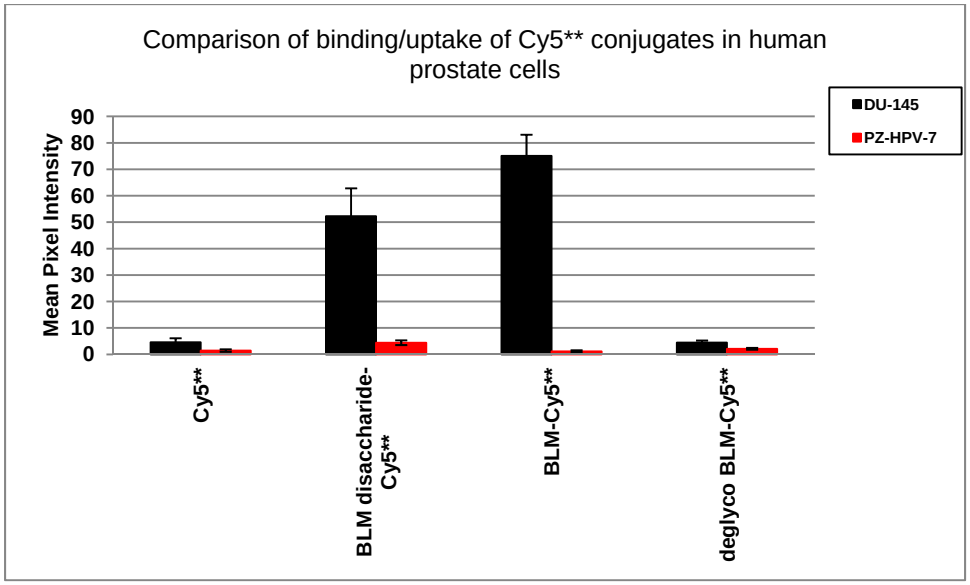


Figure 3.5. Quantification of the binding/uptake of BLM-Cy5**, deglycoBLM-Cy5**, BLM disaccharide-Cy5** and Cy5** by DU-145 prostate carcinoma cells and PZ-HPV-7 normal prostate cells (Figures 3.3 and 3.4). The DU-145 and PZ-HPV-7 cells were treated with 25 μM dye conjugates and exposed for 1 sec prior to imaging. The experiment was carried out by Dr. Zhiqiang Yu.

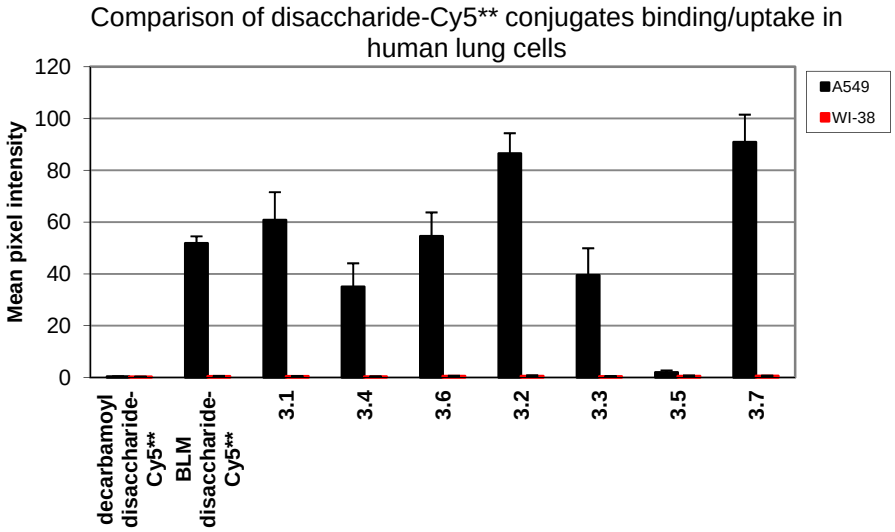


Figure 3.6. Quantification of the binding/uptake of BLM disaccharide-Cy5**, decarbamoyl disaccharide-Cy5** and disaccharide-Cy5** conjugates (Figures 3.3 and 3.4) by A549 lung carcinoma cells and WI-38 normal lung cells. The A549 and WI-38 cells were treated with 25 μM dye conjugates and exposed for 1 sec prior to imaging. The experiment was carried out by Dr. Zhiqiang Yu.

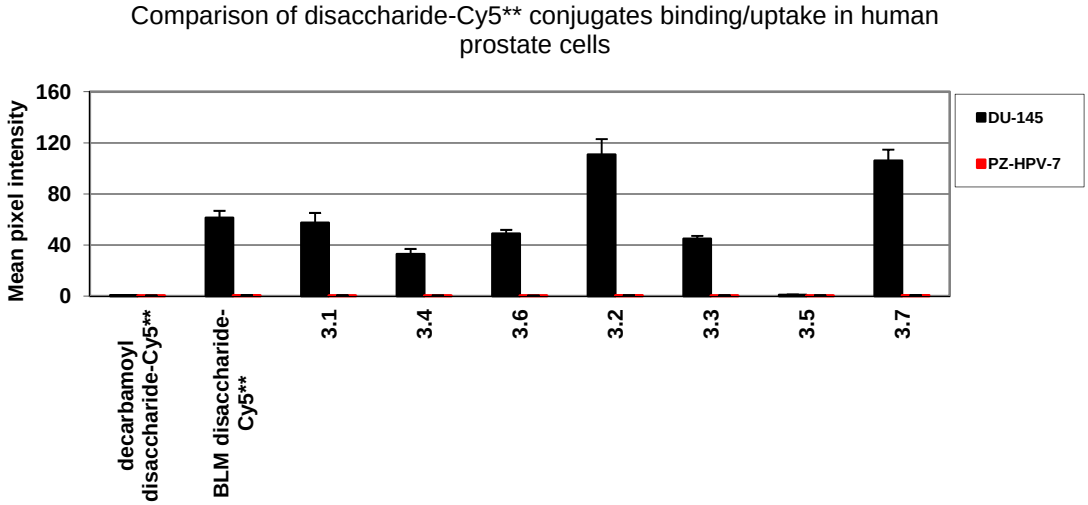


Figure 3.7. Quantification of the binding/uptake of BLM disaccharide-Cy5**,

decarbamoyl disaccharide-Cy5** and disaccharide-Cy5** conjugates (Figures 3.3 and 3.4) by DU-145 prostate carcinoma cells and PZ-HPV-7 normal prostate cells. The DU-145 and PZ-HPV-7 cells were treated with 25 μM dye conjugates and exposed for 1 sec prior to imaging. The experiment was carried out by Dr. Zhiqiang Yu.

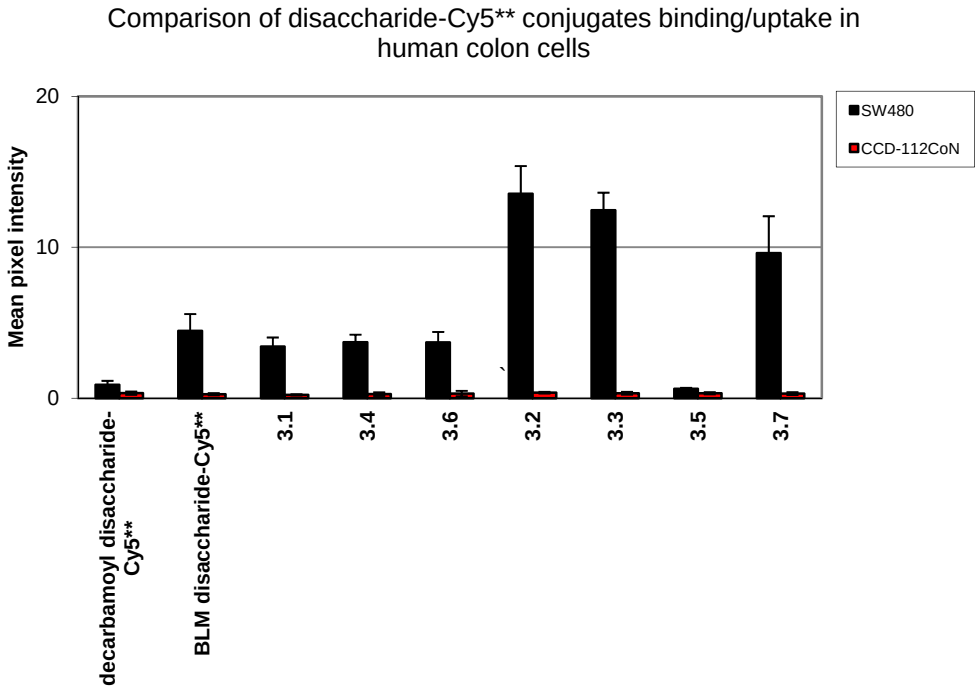


Figure 3.8. Quantification of the binding/uptake of BLM disaccharide-Cy5**, decarbamoyl disaccharide-Cy5** and disaccharide-Cy5** conjugates (Figures 3.3 and 3.4) by SW480 colon carcinoma cells and CCD-112CoN normal colon cells. The SW480 and CCD-112CoN cells were treated with 25 μM dye conjugates and exposed for 1 sec prior to imaging. The experiment was carried out by Dr. Zhiqiang Yu.

The binding uptake of decarbamoyl disaccharide-Cy5** conjugate, BLM disaccharide-Cy5** conjugate and the different synthesized disaccharide-Cy5** conjugates by A549 lung carcinoma cells and WI-38 normal lung cells (Figure 3.6) or by DU-145 prostate carcinoma cells and PZ-HPV-7 normal prostate cells (Figure 3.7) or by SW480 colon carcinoma cells and CCD-112CoN normal colon cells (Figure 3.8) were quantified by fluorescence imaging. As shown (Figures 3.6-3.8), the decarbamoylated BLM disaccharide exhibits very low binding and uptake in all tumor and normal cell lines tested highlighting the importance of the carbamoyl moiety to effective cell binding and uptake. The binding and uptake of the disaccharide-dye conjugates in human colon cells was lower relative to that in human prostate and lung cancer cells; however, the binding and uptake profiles were similar. Disaccharides having a carbamoyl group modified with the methylamino group (**3.2**, **3.3** and **3.7**) exhibited greater binding and uptake as compared to the disaccharides with unmodified carbamoyl group (**3.1** and **3.6**). Disaccharides with carbamoyl and methylcarbamoyl group at C4 position of the D-mannose subunit (**3.4** and **3.5**) exhibited low binding and uptake in all the tumor cell lines tested. The binding and uptake was generally higher for disaccharides **3.2** and **3.7** containing the modified carbamoyl group at C2 and C3 position respectively. All the disaccharide-dye conjugates exhibited very low uptake in normal cells, highlighting the role of the disaccharide moiety in the cancer cell specificity of BLM

3.3 Discussion

3.3.1 Synthesis of the disaccharide dye conjugates

The natural disaccharide moiety in bleomycin made up of L-gulose and D-mannose monosaccharide subunits has been found to play an important role in its tumor selectivity. Preliminary studies carried out by our group had highlighted the importance of the carbamoyl group present in the BLM disaccharide, towards the tumor cell binding and uptake of BLM.[175] To better study the importance of the carbamoyl moiety, a library of disaccharide-dye conjugates were synthesized (Schemes 3.6-3.11).

All the synthesized disaccharides had a L-gulose monosaccharide subunit, however in comparison to the natural BLM disaccharide which has a carbamoyl group at the 3-position of the D-mannose moiety, the synthesized disaccharides had

1) A carbamoyl group at C-2 or C-4 positions of the D-mannose moiety.

or

2) A methylcarbamoyl group at C-2, C-3 or C-4 positions of the D-mannose moiety

or

3) A carbamoyl or methylcarbamoyl group at C-3 positions of the D-altrose moiety, which is a C3 epimer of D-mannose.

The L-gulose pyranoside **3.16** was synthesized according to a published procedure from L-xylose in 24% yield over seven steps. The diethyl dithioacetal **3.9** prepared from L-xylose was subjected to a Hg(II)-promoted hydrolysis to

afford crude aldehyde **3.10**. The aldehyde was then coupled to thiazole **3.11**, which on treatment with tetrabutylammonium fluoride underwent desilylation to afford the alcohol **3.12**. The alcohol was converted to the benzyl ether **3.13** and subjected to the standard thiazole-to-formyl deblocking protocol to yield crude aldehyde **3.14**. The removal of isopropyldiene protecting groups and subsequent cyclization followed by exhaustive acetylation afforded benzylated pyranoside **3.15**. Compound **3.15** was then subjected to debenzylation by hydrogenolysis over palladium to afford the tetraacetylated gulose subunit **3.16**.

The synthetic strategy adopted also required the syntheses of C2, C3, C4 benzylated mannose and C3 benzylated altrose pyranosides, respectively. The benzylated mannose and altrose pyranosides **3.20**, **3.25**, **3.28** and **3.35** were coupled to gulose monosaccharide **3.16** to yield the corresponding benzylated disaccharides **3.36**, **3.45**, **3.51** and **3.59**, respectively. The latter was then debenzylated and activated as the nitrophenyl ester to facilitate the incorporation of carbamoyl or methylcarbamoyl moiety.

The synthesis of phosphate ester **3.20** (Scheme 3.2) was accomplished from commercially available α-D-methylmannopyranoside in 10% overall yield over four steps. Methylmannopyranoside was converted to benzylidene acetal **3.17**, via a two step reaction involving a dibenzyldiene intermediate. The reaction of α-D-methylmannopyranoside with benzaldehyde dimethyl acetal gave a mixture of endo and exo dibenzyldiene derivatives which underwent cleavage in presence of di-*iso*-butyl-aluminium hydride to afford acetal **3.17**. Compound **3.17** was then peracetylated to afford pyranoside **3.18**. Selective anomeric

deacetylation of **3.18** gave **3.19**, which was subsequently converted to phosphate ester **3.20**. The latter was then coupled to gulose acceptor **3.16** to yield the benzylated disaccharide **3.36** (Scheme 3.6). Disaccharide **3.36** was debenzylated and converted to nitrophenylester **3.37**. Ester **3.37** on treatment with methylamine afforded disaccharide **3.38** with a methylcarbamoyl group at C2 position of the D-mannose subunit. Disaccharide **3.38** was converted to phosphate ester **3.39** via a hydrazine acetate-mediated anomeric deacetylation and subsequent activation with diphenyl chlorophosphate in presence of DMAP and Et_3N. Attempts to convert **3.38** to **3.39** in presence of diphenyl chlorophosphate and *n*-BuLi as described in the literature led to significant loss of material due to competing phosphorylation of the methylcarbamoyl moiety. Phosphate ester **3.39** was coupled to CBz-protected linker **3.40** to afford linker coupled disaccharide **3.42**. Disaccharide-linker conjugates **3.41** (synthesized by Chandrabali Bhattacharya, Scheme 3.7) and **3.42** were then subjected to sodium methoxide-catalyzed deacetylation and subsequent debenzylation to afford the fully deprotected disaccharide-linker conjugates **3.43** and **3.44**. Disaccharides **3.43** and **3.44** were then coupled with the dye succinimidyl ester **3.8** (Figure 3.3) to afford the disaccharide-dye conjugates **3.1** and **3.2**.

The synthesis of phosphate ester **3.25** (Scheme 3.3) was also accomplished from commercially available α-D-methylmannopyranoside in 13% overall yield over five steps. Methylmannopyranoside was converted to the acetal **3.22** by regioselective benzylation of the diol **3.21** according to a published procedure. The regioselectivity is attributed to the exclusive benzylation of the equatorial C3

alcohol of 2,3-*O*-dibutylstannylene intermediate, derived from the reaction of diol **3.21** with Bu_2SnO. The acid-mediated cleavage and subsequent acetylation of **3.22** afforded pyranoside **3.23**. Selective anomeric deacetylation of **3.23** followed by treatment with diphenyl chlorophosphate gave the phosphate ester **3.25**. Mannose donor **3.25** obtained was then coupled with gulose acceptor **3.16** to afford the C3 benzylated disaccharide **3.45** (Scheme 3.8). Compound **3.45** was subjected to debenzylation by hydrogenolysis over palladium and converted to the nitrophenyl ester **3.46**. Incorporation of the methylcarbamoyl group at C-3 position of the D-mannose moiety was accomplished by treatment of ester **3.46** with methylamine to afford disaccharide **3.47**. Disaccharide **3.47** was converted to phosphate ester **3.48** via a hydrazine acetate-mediated anomeric deacetylation and subsequent activation with diphenyl chlorophosphate in presence of DMAP and Et_3N. The latter was coupled with CBz-protected linker **3.40** to afford the linker coupled disaccharide **3.49**. The linker coupled disaccharide **3.49** was subjected to sodium methoxide-catalyzed deacetylation and subsequent debenzylation to afford the fully deprotected disaccharide-linker conjugates **3.50** which when coupled with the dye succinimidyl ester **3.8** (Figure 3.3) afforded the disaccharide-dye conjugates **3.3**.

Phosphate ester **3.28** (Scheme 3.4) was synthesized from commercially available α-D-methylmannopyranoside in 9% overall yield over five steps. Methylmannopyranoside was first converted to the diol **3.21**. The 4,6-*O*-benzylidene acetal of the diol **3.21** was subjected to a regioselective borane-mediated ring-opening reduction and subsequently peracetylated to afford the

benzyl pyranoside **3.26**. The anomeric acetate of pyranoside **3.26** was selectively deacetylated by hydrazine acetate to afford **3.27** which was then converted to the phosphate ester **3.28** by treatment with diphenyl chlorophosphate. The mannose donor **3.28** with a C4 benzyl group was coupled with gulose acceptor **3.16** to afford the disaccharide **3.51** (Scheme 3.9). The latter was then subjected to debenzylation by hydrogenolysis over palladium and converted to the nitrophenyl ester **3.52**. Treatment of the nitrophenyl ester **3.52** with methylamine in THF afforded the disaccharide **3.53** containing a methylcarbamoyl group at C-4 postion of the D-mannose moiety. Hydrazine acetate-mediated selective anomeric deacetylation and subsequent activation with diphenyl chlorophosphate in presence of DMAP and Et_3N afforded the phosphate ester **3.54**. The phosphate ester was coupled with CBz-protected linker **3.40** to afford linker-disaccharide conjugate **3.56**. Disaccharide-linker conjugates **3.55** (synthesized by Chandrabali Bhattacharya, Scheme 3.10) and **3.56** were then subjected to sodium methoxide-catalyzed deacetylation and subsequent debenzylation to afford the fully deprotected disaccharide-linker conjugates **3.57** and **3.58**. Disaccharides **3.57** and **3.58** were then coupled with the dye succinimidyl ester **3.8** (Figure 3.3) to afford the disaccharide-dye conjugates **3.4** and **3.5**.

Disaccharides **3.61** and **3.62** have a carbamoyl group and a methylcarbamoyl group respectively at the C-3 position of the D-altrose subunit. D-altrose is a C3 epimer of D-mannose subunit present in the natural BLM disaccharide. Disaccharides **3.61** and **3.62** would help us to better understand the importance of the stereochemistry of the carbamoyl group. Altrose phosphate

ester **3.35** was synthesized from commercially available α-D-methylglucopyranoside in 1% overall yield over seven steps (Scheme 3.5). Methylglucopyranoside was first converted to benzyldiene diol **3.29**. The reaction was carried out in acetonitrile instead of *N*,*N*-dimethylformamide as in the case of mannose analogues. The change of solvent improved the ease of purification and enabled the isolation of the product by recrystallization. The diol **3.29** was then converted to oxirane **3.30** according to a published procedure. Regioselective opening of the epoxide **3.30** at C3 with sodium benzyloxide afforded the altropyranoside **3.31**. The regioselective ring opening has been attributed to the trans-diaxial effect. Cleavage of the benzyldiene acetal of **3.31** and subsequent peracetylation afforded altropyranoside **3.33**. Selective hydrazine acetate-mediated anomeric deacetylation of **3.33** followed by treatment with diphenyl chlorophosphate gave the phosphate ester **3.35**. The synthesis of the altrose phosphate **3.35** was found to proceed in satisfactory yields only in presence of *n*-BuLi. The need for a stronger activation conditions required for the synthesis of altrose donor as compared to the mannose phosphates is not well understood. The altrose donor was coupled with gulose acceptor **3.16** to afford the disaccharide **3.59** (Scheme 3.11). Disaccharide **3.59** was debenzylated by hydrogenolysis over palladium and converted to the nitrophenyl ester **3.60**. The nitrophenyl ester **3.60** was then treated with ammonia or methylamine to incorporate the carbamoyl or methylcarbamoyl group at C-3 postion of the D-altrose subunit to afford disaccharides **3.61** and **3.62**. The anomeric acetate of **3.61** and **3.62** was deprotected selectively by hydrazine acetate and treated with diphenyl

chlorophosphate in presence of DMAP and Et_3N to afford the phosphate esters **3.63** and **3.64**. The use of DMAP/triethylamine mixture for synthesis of phosphate ester was essential to suppress the phosphorylation of the amine moiety in the methylcarbamoyl group. The phosphate esters **3.63** and **3.64** were coupled with CBz-protected linker **3.40** to afford linker-disaccharide conjugate **3.65** and **3.66**. Disaccharide-linker conjugates **3.65** and **3.66** were then deacetylated and debenzylated to afford the fully deprotected disaccharide-linker conjugates **3.67** and **3.68**. The latter were then coupled with the dye succinimidyl ester **3.8** (Figure 3.3) to afford the disaccharide-dye conjugates **3.6** and **3.7**.

3.3.2 Biological evaluation of fluorescent carbohydrate analogues

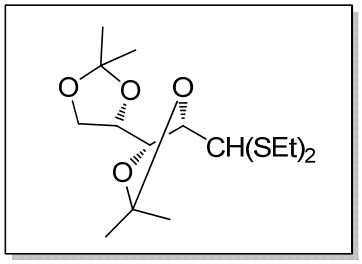
Bleomycin exhibits selective targeting of cancer cells. The initial studies (Figure 3.5) monitoring the binding and uptake clearly showed that the disaccharide was essential for the selective uptake of the BLM in tumor cells. The uptake of the dye itself was extremely low in the cancer as well as normal cells. Although the uptake efficiency varied for the different cancer cell lines monitored (Figures 3.6–3.8), the uptake profiles of the synthesized disaccharide–dye conjugates were similar implying broad specificity of the synthesized disaccharides. In all the cell lines tested, decarbamoyl BLM disaccharide exhibited low uptake, clearly indicating the importance of the carbamoyl moiety. In general, except for **3.5** the disaccharides having a methylcarbamoyl group exhibited greater uptake as compared to the disaccharides with the unmodified carbamoyl group. The disaccharide–dye conjugates did were not bound to any of

the normal human cells tested, further substantiating its role in selective targeting of cancer cells by BLM. The disaccharides **3.2** and **3.7**, having a modified carbamoyl group at C2 and C3 positions, respectively, exhibited the best binding and uptake profiles. The fluorescence studies indicate that the modified disaccharides if incorporated could potentially enhance the tumor selective binding and uptake of BLM.

3.4. Experimental

General Methods. The chemicals were all ACS reagent grade and were used without further purification. The reactions were carried out under an argon atmosphere unless specified. Flash column chromatography was carried out using silica gel (Silicycle R10030B, 60 particle size, 230-400 mesh), applying a low pressure stream of nitrogen. Analytical thin layer chromatographic separations were carried out on glass plates coated with silica gel (60 particle size F254, SiliCycle TLG-R10011B-323). The TLC chromatograms were developed by immersing the plates in 2.5% potassium permanganate in ethanol or 2% anisaldehyde + 5% sulfuric acid + 1.5% glacial acetic acid in ethanol, followed by heating, or else visualized by UV irradiation (254 nm). Melting points were recorded on a MelTemp apparatus and are uncorrected. Tetrahydrofuran was distilled from sodium/benzophenone ketyl and dichloromethane from calcium hydride. ^{1}H and ^{13}C NMR spectra were recorded on a Gemini 300 or Varian Inova 400, or on a Varian Inova 500 spectrometer, using $CDCl_3$ as solvent and internal standard, unless otherwise indicated. ^{1}H NMR chemical shifts were reported relative to residual $CHCl_3$ at 7.26 ppm, or to residual DMSO-d_5 at 2.50 ppm; ^{13}C

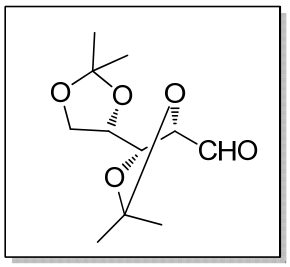
NMR shifts were reported relative to the central line of $CDCl_3$ at 77.16 ppm, or to ^{13}C DMSO-d_6 at 39.51 ppm. Splitting patterns are designated as s, singlet; br s, broad singlet; d, doublet; dd, doublet of doublets; dt, doublet of triplets; m, multiplet; q, quartet; quin, quintet. Cyanine dyes were obtained from our collaborators at General Electric. High resolution mass spectrometric data was obtained at the Michigan State Mass Spectrometry Facility or at the Arizona State University CLAS High Resolution Mass Spectrometry Facility.



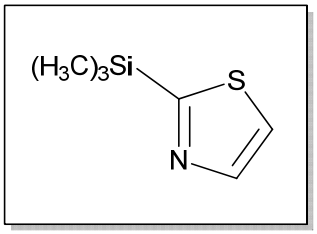
2,3,4,5-Di-*O*-isopropylidene-L-xylose Diethyl Dithioacetal (3.9).[163]

To a suspension of 8.00 g (53.3 mmol) of L-xylose in 3.2 mL of conc HCl was added, with vigorous magnetic stirring, 11.8 mL (10.1 g, 160 mmol) of ethanethiol. Stirring was continued at room temperature until the two layer mixture gave a homogenous solution (usually after 15-20 min) which was then diluted with 160 mL of acetone. After stirring for 5 h, the solution was neutralized with satd aq NH_4OH solution and co-evaporated with six 20-mL portions of toluene several times to afford a crude residue. The residue was applied to a silica gel column (28 × 5 cm). Elution with 1:1 ethyl acetate–hexanes gave **3.9** as a colorless syrup: yield 13.4 g (75%); $[\alpha]_D$ + 57.2 (*c* 1.8, C_6H_6), lit.[163] $[\alpha]_D$ + 51.3 (*c* 1.8, C_6H_6); silica gel TLC R_f 0.59 (3:1 ethyl acetate–hexanes); ^1H NMR

($CDCl_3$) δ 1.23-1.28 (m, 6H), 1.36 (s, 3H), 1.41 (s, 6H), 1.45 (s, 3H), 2.68-2.77 (m, 4H), 3.91 (dd, 2H, *J* = 9.8 and 4.5 Hz), 4.02-4.06 (m, 1H), 4.13 (dd, 1H, *J* = 5.3 and 2.1 Hz) and 4.31-4.34 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 14.26, 14.34, 24.9, 25.3, 25.6, 26.1, 27.1, 27.3, 53.0, 65.9, 75.2, 78.7, 80.1, 109.5 and 110.0.

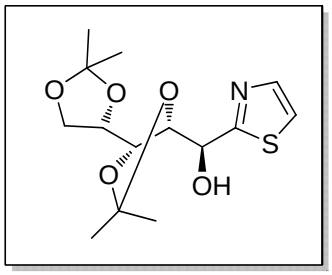


2,3,4,5-Di-*O*-isopropylidene-*aldehydo*-L-xylose (3.10).[163] To a stirred solution containing 2.60 g (7.70 mmol) of thioacetal **3.9** in 26 mL of acetone diluted with 2.6 mL of water was added 3.80 g (17.7 mmol) of yellow mercury(II) oxide and 3.80 g (13.9 mmol) of mercuric(II) chloride. The reaction mixture was stirred at 55 °C for 2 h and then allowed to cool to room temperature. The solvent was filtered through a pad of Celite 545® and concentrated under diminished pressure to afford a crude residue. The residue was suspended in three 30-mL portions of dichloromethane and filtered through a pad of Celite 545®. The organic layer was washed with 40 mL of 1 M aq KI, dried ($MgSO_4$) and then concentrated under diminished pressure to afford the crude aldehyde **3.10**. The aldehyde was used for the next reaction immediately.



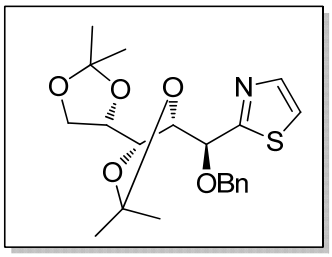
2-(Trimethylsilyl)thiazole (3.11).[164] A 500-mL, four-necked, round-bottomed flask, containing a magnetic stirring bar, was equipped with two 100-mL, pressure-equalizing dropping funnels and a low-temperature thermometer. The anh apparatus was filled with argon and kept under a slightly positive pressure during the entire reaction. The flask was charged with 80 mL of freshly distilled Et_2O and 42 mL (67 mmol) of a 1.6 M solution of *n*-BuLi in hexane. One of the two dropping funnels was charged with 5.5 mL (10 g, 61 mmol) of 2-bromothiazole in 20 mL of Et_2O and the other with 7.7 mL (6.6 g, 61 mmol) of chlorotrimethylsilane in 20 mL of Et_2O. The reaction flask was cooled to −78 ºC in an anh acetone bath. While the solution in the flask was stirred, 2-bromothiazole was added dropwise over a period of 1 h. After 20 min of additional stirring, chlorotrimethylsilane was added dropwise over 30 min and the stirring was continued for a period of 1 h at −78 ºC. The resulting mixture was then allowed to warm up to room temperature. A satd aq $NaHCO_3$ was added and the mixture was transferred into a 1 L separatory funnel. The organic layer was recovered and the aqueous layer was extracted with two 200-mL portions of Et_2O. The combined organic layer was dried (Na_2SO_4), filtered, and concentrated under diminished pressure with the external bath temperature not exceeding 40 °C. The residue was distilled from a 100-mL flask at diminished pressure in a Claisen

apparatus. The distillation was carried out under diminished pressure at 45 °C after a forerun at 25 °C consisting mainly of bromobutane was collected. The pure product **3.11** was isolated as a colorless oil: yield 7.3 g (76%); ^{1}H NMR ($CDCl_3$) δ 0.39 (s, 12H), 7.50 (1H, d, J = 3.0 Hz) and 8.09 (1H, d, J = 2.9 Hz); ^{13}C NMR ($CDCl_3$) δ 1.03, 127.3, 145.6 and 174.2.



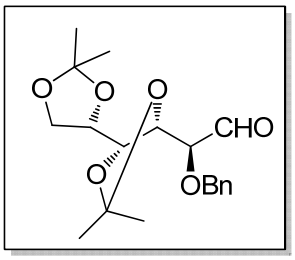
1,2,3,4-bis-*O*-(1-Methylethylidene)-5-*C*-2-thiazolyl-(5S)-D-xylitol (3.12).[163] To a stirred solution containing 2.22 g (9.65 mmol) of crude aldehyde **3.10** in 38 mL of anh dichloromethane cooled to –20 °C was added 2.00 mL (1.97 g, 12.5 mmol) of 2-(trimethylsilyl)thiazole (**3.11**) dropwise over a period of 15 min. The solution was stirred at 0 °C for 1 h and then concentrated under dimished pressure to afford a crude residue. The residue was dissolved in 38 mL of anh THF and treated with 3.00 g (9.65 mmol) of *n*-$Bu_4NF•3H_2O$ at 20 °C for 30 min and then concentrated under dimished pressure. The residue was diluted by the addition of 250 mL of dichloromethane. The organic layer was washed with three 50-mL portions of water, dried (Na_2SO_4) and then concentrated under diminished pressure to yield compound **3.12** as a crude residue. Recrystallization of the residue from cyclohexane afforded alcohol **3.12** as a colorless crystalline solid: yield 1.94 g (64% over two steps); $[\alpha]_D$ + 18.2 (*c* 1.1, $CHCl_3$), lit.[163] $[\alpha]_D$ + 18.5

(*c* 1.1, $CHCl_3$); silica gel TLC R_f 0.49 (1:1 ethyl acetate–hexanes); ^1H NMR ($CDCl_3$) δ 1.28 (s, 3H), 1.36 (s, 6H), 1.40 (s, 3H), 3.67 (t, 1H, *J* = 6.6 Hz), 3.79-3.84 (m, 2H), 4.12 (dd, 1H, *J* = 7.2 and 3.6 Hz), 4.31-4.34 (m, 1H), 4.56 (br s, 1H), 5.10 (d, 1H, *J* = 5.5 Hz), 7.30 (d, 1H, *J* = 3.2 Hz) and 7.71 (d, 1H, *J* = 3.2 Hz); ^{13}C NMR ($CDCl_3$) δ 25.6, 26.1, 27.07, 27.13, 65.7, 71.7, 75.5, 77.4, 79.8, 109.5, 110.2, 119.7, 142.1 and 170.9.



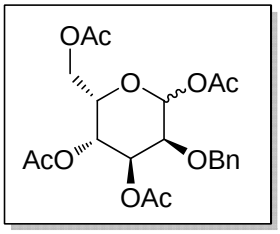
1,2,3,4-bis-*O*-(1-Methylethylidene)-5-*O*-(phenylmethyl)-5-*C*-2-thiazolyl-(5*S*)-D-xylitol (3.13).[163] To a solution containing 1.94 g (6.15 mmol) of alcohol **3.12** in anh DMF cooled to 0 °C was added 0.49 g (60% dispersion in oil, 12.3 mmol) of NaH portionwise and the reaction mixture was stirred at 0 °C for 0.5 h. To this solution was then added 1.10 mL (1.58 g, 9.20 mmol) of benzyl bromide and the reaction mixture was stirred at room temperature for 0.5 h. The reaction mixture was quenched by the addition of 1.2 mL of methanol, stirred for 10 min and then diluted with 40 mL of distilled water. The aqueous layer was extracted with three 100-mL portions of ether. The combined organic layer was dried ($MgSO_4$) and concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 4 cm). Elution with 6:1 ethyl acetate–hexanes gave ether **3.13** as a colorless solid: yield 2.26 g (91%); $[\alpha]_D$ −32.2 (*c* 1.1,

$CHCl_3$), lit.[163] $[\alpha]_D$ −32.3 (*c* 1.1, $CHCl_3$); silica gel TLC R_f 0.36 (9:1 toluene–methanol); ^1H NMR ($CDCl_3$) δ 1.20 (s, 3H), 1.25 (s, 3H), 1.29 (s, 3H), 1.33(s, 3H), 3.62-3.68 (m, 1H), 3.75-3.80 (m, 1H), 3.89-3.93 (m, 1H), 3.96-3.99 (m, 1H), 4.35 (dd, 1H, *J* = 7.3 and 2.5 Hz), 4.44 (d, 1H, *J* = 12.1 Hz), 4.63 (d, 1H, *J* = 12.1 Hz), 4.80 (d, 1H, *J* = 4.8 Hz), 7.21-7.28 (m, 5H), 7.32 (d, 1H, *J* = 3.2 Hz) and 7.78 (d, 1H, *J* = 3.2 Hz); ^{13}C NMR ($CDCl_3$) δ 14.0, 25.5, 26.03, 26.05, 26.7, 27.0, 65.5, 72.2, 75.5, 77.7, 78.5, 79.4, 109.4, 110.3, 120.1, 127.9, 128.1, 128.3, 136.8, 142.4 and 168.9.



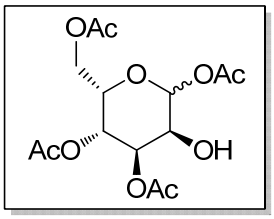
2-*O*-Benzyl-3,4,5,6-di-*O*-isopropylidene-*aldehydo*-L-gulose (3.14).[163] A solution containing 0.61 g (1.50 mmol) of *O*-benzyl ether **3.13** and 2.80 g of activated 4Å molecular sieves dissolved in 15 mL of anh acetonitrile was stirred at 20 °C for 10 min and then 0.22 mL (329 mg, 1.95 mmol) of methyl triflate was added dropwise. The suspension was stirred at room temperature for 15 min and then concentrated under diminished pressure to afford the crude *N*-methylthiazolium salt. To a stirred solution of the crude *N*-methylthiazolium salt in 15 mL of methanol cooled to 0 °C was added 0.12 g (3.30 mmol) of sodium borohydride. The reaction mixture was stirred at room temperature for 5 min and diluted with 5 mL of acetone. The solvent was filtered through a pad of Celite

545® and concentrated under diminished pressure to afford a crude mixture of thiazolidines. This was dissolved in 14 mL of acetonitrile and 1.4 mL of water and treated under vigorous stirring with 0.96 g (12.0 mmol) of CuO and 0.26 g (1.50 mmol) of $CuCl_2 \bullet 2H_2O$. The reaction mixture was stirred at 20 °C for 15 min, filtered through a pad of Celite 545® and then concentrated under diminished pressure to remove acetonitrile and most of the water (bath temperature not exceeding 40 °C) to afford a crude residue. The brown residue was triturated with four 50-mL portions of ether and the liquid phase was pipetted and filtered through a pad of Florisil® (60–100 mesh) to afford a colorless solution. After a further washing of Florisil® with 50 mL of ethyl acetate, the combined organic layer was concentrated under diminished pressure to yield the crude aldehyde **3.14** as a brown syrup, which was used immediately for the next reaction.



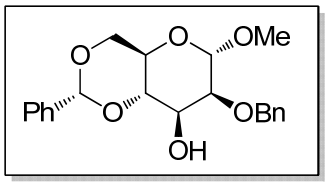
1,3,4,6-Tetra-*O*-acetyl-2-*O*-benzyl-L-gulopyranose (3.15).[163] A solution containing 470 mg (1.34 mmol) of the crude aldehyde **3.14** was dissolved in 7.4 mL of glacial acetic acid and 1.9 mL of distilled water and stirred at 100 °C for 40 min. The reaction mixture was then concentrated by co-evaporation three times with toluene to afford the crude 2-*O*-benzyl-L-gulose as a mixture of β-pyranose, α-pyranose and furanose forms. A solution of the crude residue and 0.16 g (1.34

mmol) of DMAP in 3.4 mL of pyridine and 3.4 mL of acetic anhydride was stirred at 20 °C for 12 h and concentrated under diminished pressure to yield a brown syrup. The crude residue was applied to a silica gel column (38 × 3 cm). Elution with 3:1 ethyl acetate–hexanes gave **3.15** as a yellow oil: yield 1.56 g (64% over two steps); silica gel TLC R_f 0.44 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ2.01 (s, 3H), 2.05 (s, 3H), 2.08 (s, 3H), 2.11 (s, 3H), 3.64 (dd, 1H, J = 8.3 and 4.9 Hz), 3.98-4.13 (m, 2H), 4.24-4.32 (m, 1H), 4.49 (d, 1H, J = 11.9 Hz), 4.63 (d, 1H, J = 11.9 Hz), 4.95 (dd, 1H, J = 3.9 and 2.5 Hz), 5.43-5.45 (m, 1H), 5.89 (d, 1H, J =8.3 Hz) and 7.23-7.34 (m, 5H).



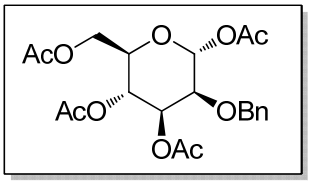
1,3,4,6-Tetra-*O*-acetyl-L-gulopyranose (3.16).[163] [176]To a solution containing 1.47 g (3.35 mmol) of **3.15** in 23 mL of ethyl acetate was added 0.73 g of 10% Pd/C and the reaction mixture was stirred overnight under 1 atm of H_2. The solvent was filtered through a pad of Celite 545® and concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (15 × 4 cm). Elution with 1:1 ethyl acetate–hexanes afforded **3.16** as a 77:20:3 mixture of α-pyranose, β-pyranose and furanose forms as determined by ^{1}H NMR: yield 1.02 g (87%); silica gel TLC R_f 0.52 (ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 1.91 (s, 3H), 2.00 (s, 3H), 2.03 (s, 6H), 3.22-3.52 (br s, 1H), 3.80 (dd,

1H, J = 8.4 and 3.5 Hz), 3.91-3.97 (m, 1H), 3.99-4.04 (m, 1H), 4.14-4.19 (m, 1H), 4.82-4.88 (m, 1H), 5.19 (t, 1H, J = 3.6 Hz) and 5.70 (d, 1H, J = 8.4 Hz); ^{13}C NMR ($CDCl_3$) δ 20.4, 20.5, 20.6, 20.8, 61.6, 66.2, 67.5, 69.5, 70.9, 92.1, 169.4, 169.6, 169.7 and 170.5.

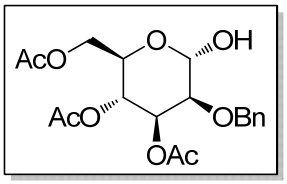


Methyl 4,6-*O*-Benzylidene-2-*O*-benzyl-α-D-mannopyranoside (3.17).[165] To a solution containing 5.00 g (26.0 mmol) of methyl α-D-mannopyranoside and 60.0 mg (0.26 mmol) of camphor sulfonic acid in 75 mL of DMF was added dropwise 9.7 mL (9.8 g, 65 mmol) of benzaldehyde dimethyl acetal. The resulting solution was heated to 60 °C on a rotary evaporator under a pressure of 250 mbar. After 3 h, silica gel TLC (1:3 ethyl acetate–hexanes) indicated complete conversion of starting material (R_f 0.0) to two products (R_f 0.50 and 0.80). To the reaction mixture was then added 4.90 mL (4.90 g, 32.4 mmol) of benzaldehyde dimethyl acetal and 30.0 mg (0.13 mmol) of camphor sulfonic acid. The reaction mixture was stirred under diminished pressure. After 2 h, silica gel TLC (1:3 ethyl acetate–hexanes) indicated the formation of a single product (R_f 0.80). The solvent was concentrated under diminished pressure, the residue was co-evaporated with 50 mL of toluene and then dissolved in 100 mL of dichloromethane. The organic layer was washed with 50 mL of satd aq $NaHCO_3$ and brine. The organic phase was then dried ($MgSO_4$), filtered and concentrated

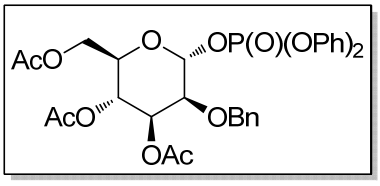
under diminished pressure. The resulting crude mixture of *endo* and *exo* dibenzylidene derivatives was dissolved in 150 mL of freshly distilled toluene and cooled to −40 °C under an argon atmosphere. Then 65 mL of DIBAL (1 M solution in toluene, 64.9 mmol) was added slowly to the reaction mixture. The reaction mixture was allowed to warm to room temperature slowly. After 2 h, silica gel TLC analysis (1:3 ethyl acetate–hexanes) indicated complete consumption of starting material (R_f 0.80) and formation of two products (R_f 0.40 and R_f 0.30). The reaction mixture was quenched by the dropwise addition of 50 mL of methanol and the mixture was diluted with 250 mL of dichloromethane. The organic layer was washed with 200 mL of 10% solution of Rochelle's salt and brine and then dried ($MgSO_4$). The organic layer was filtered and the filtrate was concentrated under diminished pressure. The resulting residue was purified by flash column chromatography (1:3 ethyl acetate–hexanes) to afford the undesired compound methyl 4,6-*O*-benzylidene-3-*O*-benzyl-α-D-mannopyranoside (**3.22**) (R_f 0.30) and the desired methyl 4,6-*O*-benzylidene-2-*O*-benzyl-α-D-mannopyranoside (**3.17**) as a colorless crystalline solid: yield 3.0 g (41%); silica gel TLC R_f 0.40 (1:3 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 3.34 (s, 3H), 3.79-3.82 (m, 3H), 3.96 (t, 1H, J = 8.0 Hz), 4.10-4.12 (m, 1H), 4.26-4.27 (m, 1H), 4.72-4.75 (m, 3H), 5.53 (s, 1H), 7.33-7.41 (m, 8H) and 7.42-7.55 (m, 2H).



1,3,4,6-Tetra-*O*-acetyl-2-*O*-benzyl-α-D-mannopyranoside (3.18).[177] To a solution containing 3.57 g (9.59 mmol) of acetal **3.17** in 70 mL of Ac_2O was added a catalytic amount of H_2SO_4 and the reaction mixture was stirred at 25 °C for 40 min. The reaction mixture was poured into a stirring mixture of 100 mL of ethyl acetate and 80 mL of satd aq $NaHCO_3$. The organic and aqueous layers were separated and the organic layer was washed with 60 mL of brine and dried ($MgSO_4$). The organic layer was filtered and concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (17 × 5 cm). Elution with 2:1 ethyl acetate–hexanes afforded **3.18** as a yellow oil: yield 3.35 g (80%); silica gel TLC R_f 0.66 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.98 (s, 3H), 2.04 (s, 3H), 2.08 (s, 3H), 2.12 (s, 3H), 3.82 (dd, 1H, J = 3.2 and 2.2 Hz), 4.01 (ddd, 1H, J = 10.0, 4.8 and 2.3 Hz), 4.08-4.15 (m, 1H), 4.23-4.28 (m, 1H), 4.56-4.76 (m, 2H), 5.19 (dd, 1H, J = 10.0 and 3.3 Hz), 5.43-5.52 (m, 1H), 6.18 (d, 1H, J = 1.9 Hz) and 7.27-7.38 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 20.8, 20.90, 20.93, 21.1, 62.4, 66.0, 70.7, 71.1, 73.0, 74.0, 91.3, 128.1, 128.2, 128.6, 137.3, 168.8, 169.6, 170.4 and 170.9.

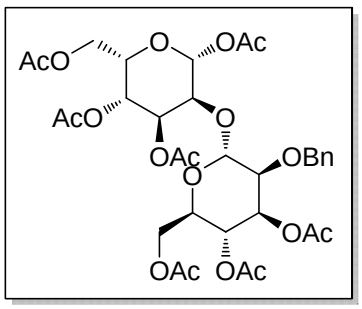


3,4,6-Tri-*O*-acetyl-2-*O*-benzyl-α-D-mannopyranoside (3.19). To a solution containing 1.13 g (2.58 mmol) of compound **3.18** in 21 mL of anh DMF was added 286 mg (3.10 mmol) of hydrazine acetate. The reaction mixture was stirred at room temperature for 1.5 h and quenched by the addition of 100 mL of ethyl acetate. The organic layer was washed with three 50-mL portions of brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (20 × 3 cm). Elution with 1:2 ethyl acetate–hexanes afforded pyranoside **3.19** as a colorless oil: yield 793 mg (73%); silica gel TLC R_f 0.23 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.97 (s, 3H), 2.00 (s, 3H), 2.02 (s, 3H), 3.81-3.87 (m, 1H), 4.05-4.17 (m, 2H), 4.20 (dt, 1H, J = 9.3 and 4.7 Hz), 4.56-4.63 (m, 3H), 5.21-5.33 (m, 2H), 5.40 (t, 1H, J = 9.9 Hz) and 7.21-7.36 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 20.57, 20.58, 20.7, 62.7, 66.6, 68.2, 70.9, 72.8, 75.6, 92.2, 127.70, 127.72, 128.2, 137.6, 169.8, 170.2 and 171.1; mass spectrum (APCI), *m/z* 397.1498 $(M + H)^+$ ($C_{19}H_{25}O_9$ requires 397.1498).



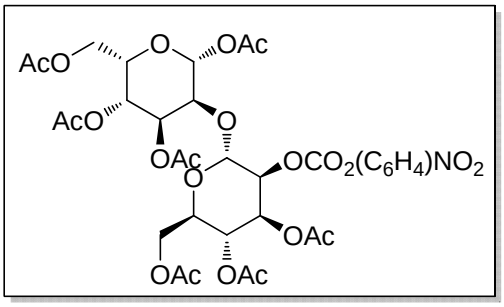
3,4,6-Tri-*O*-acetyl-2-*O*-benzyl-α-D-mannopyranosyl Diphenyl Phosphate (3.20). To a stirred solution containing 793 mg (2.00 mmol) of **3.19** in 120 mL of anh dichloromethane was added 305 mg (2.50 mmol) of DMAP, 3.00 mL (2.17 g, 21.6 mmol) of Et_3N and 4.00 mL (5.20 g, 19.2 mmol) of diphenyl

chlorophosphate. The reaction mixture was stirred at 0 °C for 2 h and poured into a stirring mixture of 300 mL of ethyl acetate and 150 mL of satd aq $NaHCO_3$. The aqueous and organic layers were separated and the organic layer was washed with three 50-mL portions of water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (20 × 3cm). Elution with 1:2 ethyl acetate–hexanes afforded **3.20** as a colorless oil: yield 508 mg (40%); silica gel TLC R_f 0.44 (1:1 ethyl acetate–hexanes);^{1}H NMR ($CDCl_3$) δ 2.17 (s, 3H), 2.20 (s, 3H), 2.23 (s, 3H), 4.10-4.25 (m, 3H), 4.42 (dd, 1H, *J* = 12.2 and 3.9 Hz), 4.76-4.88 (m, 2H), 5.49 (d, 1H, *J* = 8.0 Hz), 5.73 (t, 1H, *J* = 10.1 Hz), 6.21 (d, 1H, *J* = 5.7 Hz) and 7.33-7.62 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 20.39, 20.46, 20.53, 61.7, 65.3, 69.8, 70.8, 73.1, 74.4, 96.6, 119.9, 120.05, 120.09, 120.14, 124.59, 125.63, 127.8, 127.9, 128.3, 129.3, 129.8, 136.8, 149.9, 150.1, 150.8, 169.3, 169.8 and 170.53; mass spectrum (APCI), *m/z* 629.1788 $(M + H)^+$ ($C_{31}H_{34}O_{12}P$ requires 629.1788).



1,3,4,6-Tetra-*O*-acetyl-2-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-benzyl-α-D-mannopyranosyl)-β-L-gulopyranose (3.36). To a stirred solution containing 234 mg (0.67 mmol) of glycosyl acceptor **3.16** and 508 mg (1.17 mmol) of glycosyl

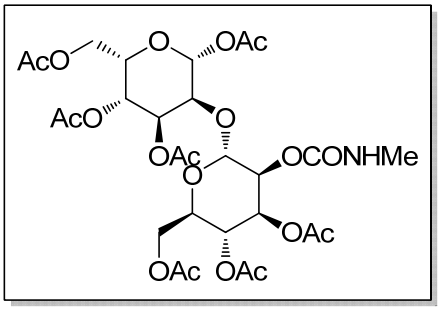
donor **3.20** in 4.8 mL of anh dichloromethane at 0 °C, was added 244 μL (300 mg, 1.35 mmol) of TMSOTf. The reaction mixture was stirred at 0 °C for 10 min at which time it was poured into a two phase mixture of 30 mL of ethyl acetate and 30 mL of satd aq $NaHCO_3$. The organic and aqueous layers were separated and the organic layer was washed with two 20-mL portions of brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (30 × 3 cm). Elution with 2:1 ethyl acetate–hexanes afforded compound **3.36** as a colorless oil: yield 302 mg (62%); silica gel TLC R_f 0.2 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.84 (s, 3H), 1.94 (s, 3H), 1.99 (s, 3H), 2.04 (s, 3H), 2.08 (s, 3H), 2.09 (m, 6H), 3.51-3.61 (m, 1H), 3.87-4.23 (m, 5H), 4.31 (t, 1H, J = 6.3 Hz,), 4.44-4.47 (m, 1H), 4.56-4.69 (m, 1H), 4.80-4.97 (m, 2H), 5.02-5.07 (m, 2H), 5.27-5.47 (m, 2H), 5.78 (d, 1H, J = 8.5 Hz,) and 7.16-7.36 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 20.61, 20.63, 20.66, 20.67, 20.69, 20.72, 61.3, 62.2, 65.3, 65.7, 66.0, 67.7, 68.8, 69.2, 70.4, 71.3, 72.2, 73.9, 90.6, 94.2, 127.7, 128.1, 128.2, 137.6, 168.7, 169.36, 169.37, 169.4, 170.0, 170.3 and 170.6; mass spectrum (APCI), *m/z* 727.2453 (M + H)$^+$ ($C_{33}H_{43}O_{18}$ requires 727.2450).



1,3,4,6-Tetra-*O*-acetyl-2-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-((*p*-nitrophenyl)carbamoyl)-α-D-mannopyranosyl)-β-L-gulopyranose (3.37). To a solution containing 200 mg (0.27 mmol) of disaccharide **3.36** in 38 mL of ethyl acetate was added a catalytic amount of $Pd(OH)_2/C$ and the reaction mixture was stirred overnight under 1 atm of H_2. The solvent was filtered through a pad of Celite 545® and the filtrate was concentrated under diminished pressure to afford a crude residue. The residue was used for the next reaction; silica gel TLC R_f 0.08 (1:1 ethyl acetate–hexanes).

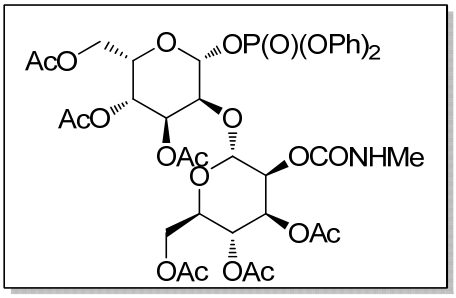
To a solution containing 198 mg (0.31 mmol) of the crude residue in 1.2 mL of anh pyridine was added 151 mg (1.24 mmol) of DMAP and 276 mg (1.24 mmol) of *p*-nitrophenyl chloroformate. The reaction mixture was stirred at 40 °C overnight at which time it was poured into a mixture of 30 mL ethyl acetate and 10 mL of H_2O. The organic and aqueous layers were separated and the organic layer was washed with three 10-mL portions of 1 N HCl and 10 mL of satd aq $NaHCO_3$ and then brine. The solution was dried ($MgSO_4$) and filtered and the filtrate was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 3 cm). Elution with 1:1 ethyl acetate–hexanes afforded **3.37** as a colorless foam: yield 211 mg (96% over two steps); silica gel TLC R_f 0.30 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ1.98 (m, 3H), 2.03 (s, 6H), 2.10 (s, 3H), 2.12 (s, 3H), 2.14 (s, 3H), 2.17 (s, 3H), 3.96-4.18 (m, 2H), 4.19-4.29 (m, 2H), 4.35 (t, 1H, J = 6.5 Hz), 4.96-5.03 (m, 2H), 5.06-5.23 (m, 3H), 5.27-5.40 (m, 2H), 5.44 (t, 1H, J = 3.0 Hz), 5.88 (d, 1H, J = 8.4 Hz), 7.39 (d, 2H, J = 8.0 Hz) and 8.26 (d, 2H, J = 9.1 Hz); ^{13}C NMR ($CDCl_3$)

δ 20.70, 20.72, 20.75, 20.76, 20.9, 61.3, 62.0, 65.5, 65.7, 67.8, 68.8, 69.4, 70.1, 71.4, 73.5, 90.6, 94.5, 121.7, 125.4, 145.6, 149.8, 151.6, 155.3, 168.7, 169.3, 169.5, 169.7, 169.7, 170.5 and 170.6; HRMS (APCI), *m/z* 802.2053 $(M + H)^+$ ($C_{33}H_{40}NO_{22}$ requires *m/z* 802.2042).



1,3,4,6-Tetra-*O*-acetyl-2-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-(methylcarbamoyl)-α-D-mannopyranosyl)-β-L-gulopyranose (3.38). To a solution containing 201 mg (0.25 mmol) of nitrophenyl ester **3.37** in 6 mL of anh THF was added dropwise at 0 °C 125 μL (2 M solution in THF, 0.25 mmol) of CH_3NH_2. The reaction mixture was stirred at room temperature for 15 h at which time silica gel TLC analysis indicated that the reaction was complete. The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 3 cm). Elution with 1:1 ethyl acetate–hexanes afforded disaccharide **3.38** as a colorless oil: yield 134 mg (77%); silica gel TLC R_f 0.14 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.94 (s, 3H), 1.98-2.15 (m, 18H), 2.75 (d, 3H, *J* = 3.7 Hz), 3.93-4.13 (m, 4H), 4.18-4.22 (m, 2H), 4.30-4.33 (m, 1H), 4.87-5.10 (m, 4H), 5.17-5.21 (m, 2H) and 5.33 (m, 2H); ^{13}C NMR ($CDCl_3$) δ

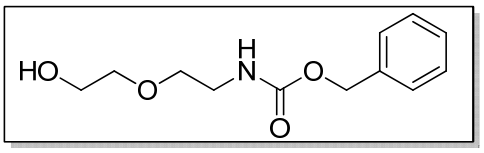
20.62, 20.63, 20.68, 20.72, 20.75, 20.77, 20.85, 27.6, 61.4, 62.0, 65.9, 67.6, 68.0, 70.5, 71.4, 90.7, 93.2, 155.38, 155.40, 155.49, 169.24, 169.27, 169.30, 170.50, 170.51, 170.6 and 170.9; HRMS (APCI), *m/z* 694.2169 $(M + H)^+$ ($C_{28}H_{40}NO_{19}$ requires *m/z* 694.2195).



3,4,6-Tri-*O*-acetyl-2-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-(methylcarbamoyl)-α-D-mannopyranosyl)-β-L-gulopyranosyl Diphenyl Phosphate (3.39). To a solution containing 108 mg (0.16 mmol) of disaccharide **3.38** in 1.2 mL of anh DMF was added 17.0 mg (0.19 mmol) of hydrazine acetate. The reaction mixture was stirred at room temperature for 1.5 h and quenched by the addition of 20 mL of ethyl acetate. The organic solution was washed with three 10-mL portions of brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was used for next reaction.

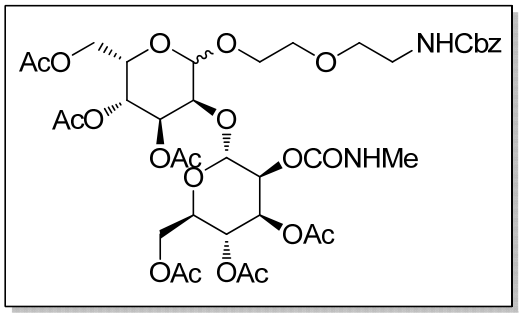
To a stirred solution containing 90.0 mg (0.14 mmol) of the crude residue in 8.2 mL of anh dichloromethane was added 21.0 mg (0.17 mmol) of DMAP, 210 μL (152 mg, 1.49 mmol) of Et_3N and 270 μL (351 mg, 1.32 mmol) of diphenyl chlorophosphate. The reaction mixture was stirred at 0 °C for 2 h and poured into a mixture of 40 mL of ethyl acetate and 20 mL of satd aq $NaHCO_3$. The organic layer was washed with three 10-mL portions of water and brine and

then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 3 cm). Elution with 2:1 ethyl acetate–hexanes afforded phosphate ester **3.39** as a colorless oil: yield 82 mg (56% over two steps); silica gel TLC R_f 0.18 (2:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.67 (s, 3H), 1.94 (d, 6H, J = 7.4 Hz), 2.01 (s, 3H), 2.11 (s, 3H), 2.16 (s, 3H), 2.76 (s, 3H), 3.89-4.39 (m, 7H), 4.75-5.05(m, 4H), 5.10-5.30 (m, 2H), 5.44 (s, 1H), 5.68 (s, 1H) and 7.11-7.39 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 20.4, 20.70, 20.76, 20.8, 20.9, 27.7, 61.2, 62.0, 65.5, 65.8, 67.5, 69.1, 69.3, 69.4, 71.4, 71.5, 71.7, 95.9, 96.34, 120.31, 120.33, 125.6, 125.72, 125.78, 125.83, 129.7, 130.0, 155.4, 169.3, 169.7, 169.8, 170.4, 170.67 and 170.68; HRMS (APCI), *m/z* 884.2371 $(M + H)^+$ ($C_{38}H_{47}NO_{19}$ requires *m/z* 884.2378).



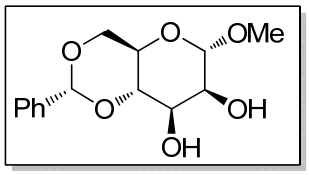
Benzyl 2-(2-Hydroxyethoxy)ethylcarbamate (3.40).[172] To a solution containing 1.01 g (9.61 mmol) of 2-(2-aminoethoxy)ethanol in 100 mL of THF at room temperature was added 1.34 mL (9.61 mmol) of Et_3N and 1.49 mL (1.78 g, 10.6 mmol) of CBzCl. The reaction mixture was stirred for 1 h and was then diluted with 250 mL of ethyl acetate. The organic layer was washed with two 250-mL portions of H_2O, two 250-mL portions of brine, and was then dried ($MgSO_4$) and concentrated under diminished pressure. The residue was purified by flash

chromatography on a silica gel column (30 × 4 cm). Elution with 9:1 ethyl acetate–hexanes afforded alcohol **3.40** as a colorless oil: yield 2.21 g (96%); silica gel TLC R_f 0.30 (9:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) 3.30 (m, 2H), 3.45 (m, 4H), 3.52 (s, 1H), 3.62 (m, 2H), 5.03 (s, 2H), 5.86 (m, 1H) and 7.27 (m, 5H); ^{13}C NMR ($CDCl_3$) 40.5, 61.1, 66.3, 69.7, 72.0, 127.72, 127.75, 128.1, 136.3 and 156.5.



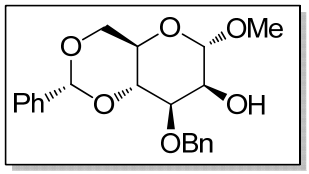
3,4,6-Tri-*O*-acetyl-2-*O*-(3,4,6-tri-*O*-acetyl-2-*O*-(methylcarbamoyl)-α-D-mannopyranosyl)-α,β-L-gulopyranosyl Benzyl 2-(2-Ethoxy)ethylcarbamate (3.42). To a stirred solution containing 90.0 mg (0.10 mmol) of phosphate ester **3.39** in 1.1 mL of anh dichloromethane was added a solution of 22.0 mg (0.09 mmol) of CBz linker **3.40** in 1.1 mL of anh dichloromethane at 0 °C. To the cooled reaction mixture was then added 33.0 μL (41.0 mg, 0.18 mmol) of TMSOTf and the reaction mixture was stirred at 0 °C for 15 min at which time it was poured into a mixture of 20 mL of ethyl acetate and 20 mL of satd aq $NaHCO_3$. The aqueous and organic layers were separated and the organic layer was washed with three 10-mL portions of water and brine, then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue.

The residue was applied to a silica gel column (25 × 3 cm). Elution with 12:12:1 ethyl acetate–hexanes–methanol afforded disaccharide–linker conjugate **3.42** as a colorless oil: yield 56 mg (63%); silica gel TLC R_f 0.20 (12:12:1 ethyl acetate–hexanes–methanol); ^{1}H NMR ($CDCl_3$) δ 1.96 (s, 3H), 2.00 (s, 3H), 2.01 (s, 3H), 2.05-2.08 (m, 6H), 2.10 (s, 3H), 2.78 (d, 3H, *J* = 4.6 Hz), 3.38 (d, 2H, *J* = 4.4 Hz), 3.51-3.70 (m, 4H), 3.78-3.87 (m, 1H), 3.95 (d, 1H, *J* = 3.5 Hz), 4.00-4.15 (m, 4H), 4.20-4.30 (m, 2H), 4.45 (t, 1H, *J* = 6.1 Hz), 4.89-5.12 (m, 6H), 5.20-5.30 (m, 3H), 5.42-5.49 (m, 1H), 5.46 (s, 1H) and 7.27-7.38 (m, 5H); ^{13}C NMR ($CDCl_3$) δ20.71, 20.73, 20.77, 20.80, 20.84, 20.88, 27.7, 62.3, 62.7, 63.9, 66.0, 66.3, 66.7, 68.7, 68.9, 69.2, 70.1, 70.2, 70.4, 97.2, 97.9, 128.21, 128.23, 128.28, 128.59, 128.61, 136.7, 155.5, 169.4, 169.80, 169.84, 170.0, 170.66 and 170.69; HRMS (APCI), *m/z* 873.3166 $(M + H)^+$ ($C_{38}H_{53}N_2O_{21}$ requires *m/z* 873.3141).



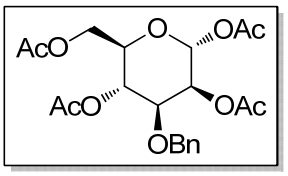
Methyl-4,6-*O*-benzylidene-α-D-mannopyranoside (3.21).[167] To a solution containing 7.00 g (36.0 mmol) of α-D-mannopyranoside in 85 mL of DMF was added 5.60 mL (5.68 g, 37.3 mmol) of benzaldehyde dimethyl acetal and a catalytic amount of *p*-TsOH. The reaction mixture was stirred at 60 °C under diminished pressure for 1 h, allowed to cool to room temperature and then poured into a stirring mixture of 120 mL of ethyl acetate and 100 mL satd aq $NaHCO_3$. The organic layer was washed with three 50-mL portions of brine and dried

($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (30 × 5 cm). Elution with 4:1 ethyl acetate–hexanes afforded acetal **3.21** as a colorless solid: yield 7.13 g (70%); silica gel TLC R_f 0.31 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 3.38 (s, 3H), 3.78 (m, 2H), 3.87 (m, 1H), 3.98 (m, 2H), 4.25 (m, 1H), 4.72 (d, 1H), 5.55 (s, 1H), 7.36 (m, 3H) and 7.47 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 55.2, 63.3, 68.8, 69.0, 71.1, 79.0, 101.6, 102.4, 126.5, 128.6, 129.5 and 137.4.



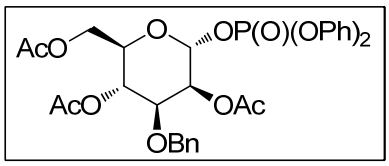
Methyl 4,6-*O*-Benzylidene-3-*O*-benzyl-α-D-mannopyranoside (3.22).[167] To a solution containing 2.00 g (7.10 mmol) of acetal **3.21** in 60 mL of methanol was added 1.94 g (7.79 mmol) of Bu_2SnO. The solution was heated to reflux for 1.5 h affording a clear solution. The solvent was concentrated under diminished pressure and the resulting solid was dried under vacuum overnight. The white residue was dissolved in 60 mL of DMF and treated with 1.69 mL (2.43 g, 14.2 mmol) of benzyl bromide and then warmed to 100 °C for 30 min. The cooled reaction mixture was poured into a stirred mixture of 90 mL ethyl acetate and 60 mL satd aq $NaHCO_3$. The organic layer was separated and washed with 60 mL of brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (30 × 5 cm). Elution with 3:7 ethyl acetate–hexanes afforded acetal **3.22** as a

colorless oil: yield 1.93 g (73%); silica gel TLC R_f 0.30 (3:7 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 3.38 (s, 3H), 3.77 (m, 3H), 4.05 (m, 2H), 4.27 (m, 1H), 4.70 (m, 2H), 4.84 (m, 1H), 5.62 (s, 1H) and 7.28-7.52 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 55.2, 60.7, 63.5, 65.4, 69.1, 70.1, 73.2, 75.8, 79.0, 101.3, 101.8, 126.3, 127.2, 127.8, 128.11, 128.16, 128.5, 128.7, 129.2, 137.8 and 138.2.



1,2,4,6-Tetra-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranoside (3.23).[167] To a solution containing 1.93 g (4.40 mmol) of acetal **3.22** in 30 mL of Ac_2O was added a catalytic amount of H_2SO_4 and the solution was stirred at room temperature for 40 min. The reaction mixture was quenched by the addition of 120 mL of ethyl acetate and 80 mL of satd aq $NaHCO_3$. The organic and aqueous layers were separated and the organic layer was washed with brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (30 × 5 cm). Elution with 2:1 ethyl acetate–hexanes afforded pyranoside **3.23** as a yellow oil: yield 1.94 g (85%); silica gel TLC R_f 0.34 (3:7 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 2.02 (s, 3H), 2.07 (s, 3H), 2.11 (s, 3H), 2.15 (s, 3H), 3.83 (dd, 1H, J = 9.7 and 3.4 Hz), 3.90 (m, 1H), 4.04 (m, 1H), 4.19 (m, 1H), 4.41 (m, 1H), 4.64 (m, 1H), 5.24 (m, 1H), 5.34 (dd, 1H, J = 3.4 and 2.1 Hz), 6.09 (d, 1H, J = 2.0 Hz) and 7.24-7.37 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.4, 20.98, 21.08, 21.13, 62.6, 67.0,

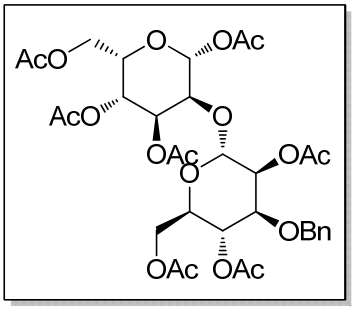
67.2, 71.0, 71.7, 74.3, 91.2, 128.0, 128.2, 128.6, 137.6, 168.3, 169.8, 170.2 and 171.0.



2,4,6-Tri-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl Diphenyl Phosphate (3.25). To a solution containing 1.40 g (3.19 mmol) of acetate **3.23** in 25 mL of DMF was added 353 mg (3.83 mmol) of hydrazine acetate. The solution was stirred at room temperature for 1.5 h and quenched by the addition of 100 mL of ethyl acetate. The organic phase was washed with three 50-mL portions of brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (20 × 4 cm). Elution with 1:2 ethyl acetate–hexanes afforded monosaccharide **3.24** as a colorless oil. This material was used for the next reaction immediately: yield 968 mg (76%); ^{1}H NMR ($CDCl_3$) δ 1.95 (s, 3H), 2.02 (s, 3H), 2.10 (s, 3H), 3.90 (dd, 1H, *J* = 9.7 and 3.3 Hz), 4.00-4.11 (m, 2H), 4.16 (ddd, 1H, *J* = 12.3, 7.7 and 4.6 Hz), 4.33 (s, 1H), 4.38 (dd, 1H, *J* = 12.3 and 4.3 Hz), 4.60 (d, 1H, *J* = 12.2 Hz), 5.13-5.23 (m, 2H), 5.28-5.33 (m, 1H) and 7.18-7.31 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 14.2, 20.78, 20.85, 21.0, 60.6, 62.9, 67.5, 68.5, 68.8, 71.4, 74.0, 92.3, 127.78, 127.83, 128.4, 137.7, 169.9, 170.6 and 171.1.

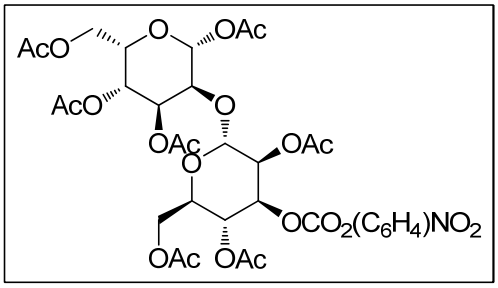
To a stirred solution containing 968 mg (2.44 mmol) of pyranoside **3.24** in 144 mL of anh dichloromethane was added 372 mg (3.05 mmol) of DMAP, 3.67

mL (2.66 g, 26.3 mmol) of Et_3N and 4.83 mL (6.26 g, 23.4 mmol) of diphenyl chlorophosphate. The reaction mixture was stirred at 0 °C for 2h and poured into a mixture of 300 mL of ethyl acetate and 150 mL of satd aq $NaHCO_3$. The organic layer was washed with three 50-mL portions of water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (20 × 4 cm). Elution with 1:2 ethyl acetate–hexanes afforded **3.25** as a colorless oil: yield 737 mg (48%); silica gel TLC R_f 0.38 (1:1 ethyl acetate–hexanes); ^1H NMR ($CDCl_3$) δ 1.93 (s, 3H), 1.97 (s, 3H), 2.10 (s, 3H), 3.84 (dd, 1H, *J* = 9.7 and 3.3 Hz,), 3.89-4.03 (m, 2H), 4.10-4.20 (m, 1H), 4.33 (d, 1H, *J* = 12.1 Hz), 4.57 (d, 1H, *J* = 12.1 Hz), 5.27 (t, 1H, *J* = 10.0 Hz), 5.38 (dd, 1H, *J* = 8.6 and 6.2 Hz), 5.91 (dd, 1H, *J* = 6.4 and 1.6 Hz) and 7.16-7.38 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 20.5, 20.62, 20.67, 61.8, 66.2, 67.2, 67.3, 70.9, 71.5, 73.4, 77.4, 96.5, 119.90, 119.95, 125.67, 125.71, 127.9, 128.3, 129.85, 137.2, 150.08, 150.15, 169.3, 169.6 and 170.4; mass spectrum (APCI), *m/z* 629.1770 $(M + H)^+$ ($C_{31}H_{34}O_{12}P$ requires 629.1788).



1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-benzyl-α-D-mannopyranosyl)-β-L-gulopyranose (3.45). To a stirred solution containing 340

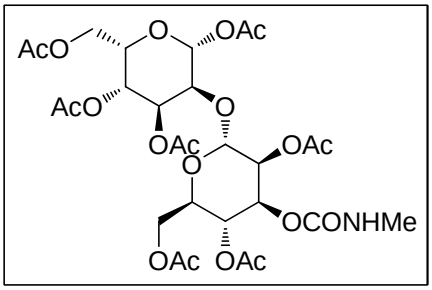
mg (0.98 mmol) of gulose acceptor **3.16** and 737 mg (1.17 mmol) of mannose donor **3.25** in 7.0 mL of anh dichloromethane cooled to 0 °C was added 352 μL (526 mg, 1.95 mmol) of TMSOTf at 0 °C. The reaction mixture was stirred for 10 min at which time it was poured into a mixture of 30 mL of ethyl acetate and 30 mL of satd aq $NaHCO_3$. The organic and aqueous layers were separated and the organic layer was washed with two 20-mL portions of brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (30 × 3 cm). Elution with 2:1 ethyl acetate–hexanes afforded disaccharide **3.45** as a colorless oil: yield 407 mg (57%); silica gel TLC R_f 0.31 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.92 (s, 3H), 2.00-2.01 (m, 6H, *J* = 2.8 Hz,), 2.04 (s, 3H, *J* = 5.3 Hz), 2.08 (d, 6H, *J* = 1.9 Hz), 2.12 (s, 3H), 3.61 (ddd, 1H, *J* = 12.7, 9.6 and 3.3 Hz), 3.84-3.95 (m, 2H), 3.96-4.20 (m, 4H), 4.26-4.37 (m, 2H), 4.59 (t, 1H, *J* = 10.4 Hz,), 4.90-5.18 (m, 4H), 5.39 (dd, 1H, *J* = 11.1 and 3.3 Hz), 5.86 (d, 1H, *J* = 8.3 Hz) and 7.24 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 20.56, 20.59, 20.61, 20.64, 20.65, 20.75, 20.78, 61.4, 62.3, 65.5, 66.9, 67.2, 67.5, 69.4, 71.3, 73.8, 90.5, 95.1, 127.6, 127.7, 127.9, 128.3, 137.4, 168.7, 168.8, 168.9, 169.1, 169.4, 169.6, 170.3 and 170.4; mass spectrum (APCI), *m/z* 727.2444 $(M + H)^+$ ($C_{33}H_{43}O_{18}$ requires 727.2450).



1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-((*p*-nitrophenyl)carbamoyl)-α-D-mannopyranosyl)-β-L-gulopyranose (3.46). To a solution containing 470 mg (0.56 mmol) of disaccharide **3.45** in 40 mL of ethyl acetate was added a catalytic amount of $Pd(OH)_2/C$ and the reaction mixture was stirred overnight under 1 atm of H_2. The solvent was filtered through a pad of Celite 545® and the filtrate was concentrated under diminished pressure to afford a crude residue. The residue was used for the next reaction; silica gel TLC R_f 0.16 (1:2 ethyl acetate–hexanes); mass spectrum (APCI), *m/z* 637.1993 $(M + H)^+$ ($C_{26}H_{37}O_{18}$ requires 637.1980).

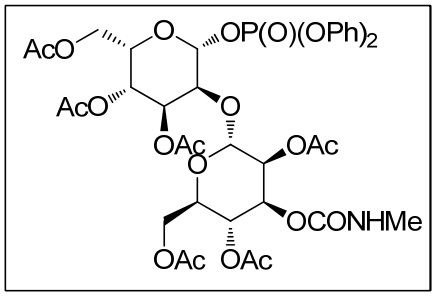
To a solution containing 338 mg (0.53 mmol) of the crude residue in 2 mL of pyridine was added 259 mg (2.12 mmol) of DMAP and 471 mg (2.12 mmol) of *p*-nitrophenyl chloroformate. The reaction mixture was stirred at 40 °C overnight at which time it was poured into a mixture of 30 mL of ethyl acetate and 10 mL of distilled water. The organic and aqueous layers were separated and the organic layer was washed with three 10-mL portions of 1 N HCl and 10 mL of satd aq $NaHCO_3$. The organic layer was then washed with brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue applied to a silica gel column (25 × 3 cm). Elution with 1:1 ethyl

acetate–hexanes afforded the ester **3.46** as a colorless foam: yield 320 mg (71% over two steps); silica gel TLC R_f 0.24 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 3H), 2.05 (s, 3H), 2.06-2.14 (m, 15H), 3.95 (dd, 1H, *J* = 8.4 and 3.0 Hz), 3.99-4.16 (m, 4H), 4.16-4.27 (m, 2H), 4.30 (dd, 1H, *J* = 15.0 and 8.7 Hz,), 5.21-5.35 (m, 2H), 5.39 (dd, 1H, *J* = 14.8 and 11.5 Hz), 4.91-5.08 (m, 2H), 5.84 (d, 1H, *J* = 8.4 Hz), 7.33 (d, 2H, *J* = 9.0 Hz) and 8.21 (d, 2H, *J* = 9.0 Hz); ^{13}C NMR ($CDCl_3$) δ 20.57, 20.63, 20.64, 20.70, 20.71, 20.8, 61.3, 61.9, 65.3, 65.5, 67.6, 67.7, 69.2, 69.8, 71.3, 74.3, 90.5, 94.9, 122.0, 125.3, 145.6, 151.4, 155.2, 168.6, 169.2, 169.37, 169.41, 169.7, 170.36 and 170.43; mass spectrum (APCI), *m/z* 742.1841 $(M\text{-}AcOH)^+$ ($C_{31}H_{36}NO_{20}$ requires 742.1831).



1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-(methylcarbamoyl)-α-D-mannopyranosyl)-β-L-gulopyranose (3.47). To a solution containing 320 mg (0.40 mmol) of disaccharide **3.46** in 12 mL of THF was added 200 μL (0.4 mmol) of 2 M methylamine in THF at 0 °C. The reaction mixture was stirred at room temperature for 15 h at which time silica gel TLC analysis indicated that the reaction was complete. The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 3

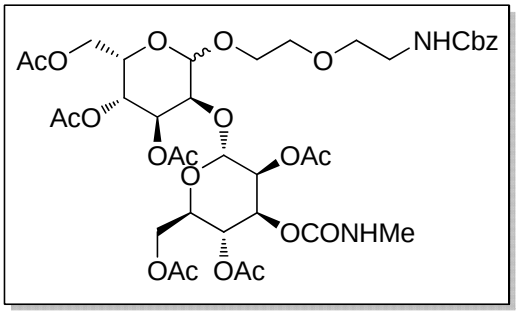
cm). Elution with 1:1 ethyl acetate–hexanes afforded disaccharide **3.47** as a colorless oil: yield 239 mg (86%); silica gel TLC R_f 0.17 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.98 (d, 6H, J = 7.5 Hz), 2.03-2.11 (m, 12H), 2.13 (d, 3H, J = 8.8 Hz), 2.69 (d, 3H, J = 4.2 Hz), 3.88-4.22 (m, 6H), 4.31 (t, 1H, J = 6.0 Hz), 4.67 (d, 1H, J = 4.1 Hz), 4.89-5.01 (m, 2H), 5.00-5.10 (m, 2H), 5.12-5.20 (m, 1H), 5.38 (s, 1H) and 5.82 (d, 1H, J = 8.3 Hz,); ^{13}C NMR ($CDCl_3$) δ 20.66, 20.69, 20.71, 20.79, 27. 6, 61.4, 62.1, 65.4, 66.0, 67.7, 69.17, 69.27, 69.33, 69.38, 71.31, 77.36, 90.6, 94.8, 155.4, 168.6, 169.2, 169.4, 169.8, 170.42 and 170.49; mass spectrum (APCI), *m/z* 694.2206 $(M + H)^+$ ($C_{28}H_{40}NO_{19}$ requires 694.2195).



3,4,6-Tri-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-(methylcarbamoyl)-α-D-mannopyranosyl)-β-L-gulopyranosyl Diphenyl Phosphate (3.48). To a solution containing 65.0 mg (0.09 mmol) of disaccharide **3.47** in 0.8 mL of anh DMF was added 11.0 mg (0.11 mmol) of hydrazine acetate. The reaction mixture was stirred at room temperature for 1.5 h and quenched by the addition of 20 mL of ethyl acetate. The organic layer was washed with three 10-mL portions of brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford

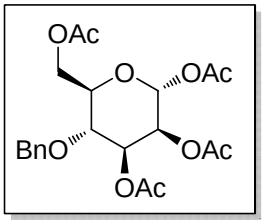
a crude residue which was used for next reaction; mass spectrum (APCI), *m/z* 652.2086 $(M + H)^+$ ($C_{26}H_{38}NO_{18}$ requires 652.2089).

To a stirred solution containing 43.0 mg (0.07 mmol) of the crude residue in 4.0 mL of anh dichloromethane was added 10.0 mg (0.08 mmol) of DMAP and 100 μL (72.0 mg, 0.71 mmol) of Et_3N and 131 μL (170 mg, 0.06 mmol) of diphenyl chlorophosphate. The reaction mixture was stirred at 0 °C for 2 h and then poured into a mixture of 40 mL of ethyl acetate and 20 mL of satd aq $NaHCO_3$. The organic and aqueous layers were separated and the organic layer was washed with three 10-mL portions of water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 3 cm). Elution with 2:1 ethyl acetate–hexanes afforded phosphate ester **3.48** as a colorless oil: yield 44 mg (76% over two steps); silica gel TLC R_f 0.25 (3:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.70 (s, 3H), 1.98 (s, 3H), 2.06 (s, 3H), 2.12 (d, 6H, J = 11.4 Hz), 2.21 (s, 3H), 2.75 (d, 3H, J = 4.5 Hz), 3.93-4.22 (m, 5H), 4.25-4.40 (m, 2H), 4.56 (d, 1H, J = 4.6 Hz), 4.93-5.05 (m, 2H), 5.12-5.24 (m, 2H), 5.29 (s, 1H), 5.44 (s, 1H), 5.65-5.73 (m, 1H) and 7.13-7.40 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 20.5, 20.9, 27.7, 36.7, 61.3, 62.0, 65.7, 67.5, 69.2, 69.4, 69.7, 71.2, 71.3, 71.7, 95.6, 96.29, 96.34, 120.36, 120.41, 125.7, 125.8, 129.7, 130.0, 150.2, 150.3, 150.4, 150.5, 155.3, 169.36, 169.42, 169.49, 169.9, 170.5 and 170.7; mass spectrum (APCI), *m/z* 884.2369 $(M + H)^+$ ($C_{38}H_{47}O_{21}PN$ requires 884.2378).



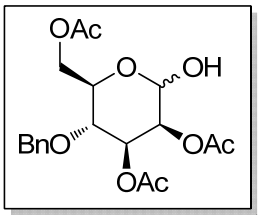
3,4,6-Tri-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-(methylcarbamoyl)-α-D-mannopyranosyl)-α,β-L-gulopyranosyl Benzyl 2-(2-Ethoxy)ethylcarbamate (3.49). To a stirred solution containing 44 mg (50 μmol) of the phosphate ester **3.48** in 0.6 mL of anh dichloromethane was added a solution of 11 mg (40 μmol) of the CBz-protected linker **3.40** in 0.6 mL of anh dichloromethane at 0 °C. To the cooled reaction mixture was added 16 μL (20 mg, 90 μmol) of TMSOTf and the reaction mixture was stirred at 0 °C for 15 min. The reaction mixture was poured into a mixture of 10 mL ethyl acetate and 10 mL satd aq $NaHCO_3$. The organic and aqueous layers were separated and the organic layer was washed with three 10-mL portions of water and brine and then dried ($MgSO_4$). The organic layer was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 3 cm). Elution with 12:12:1 ethyl acetate–hexanes–methanol afforded linker conjugate **3.49** as a colorless oil. The product was isolated as a (5:3) mixture of anomers: yield 32 mg (73%); silica gel TLC R_f 0.11 (12:12:1 ethyl acetate–hexanes–methanol); ^{1}H NMR ($CDCl_3$) (major anomer) δ 2.03 (s, 3H), 2.05 (s, 3H), 2.06-2.15 (m, 12H), 2.71 (d, 3H, J = 4.8 Hz), 3.40 (s, 1H), 3.51-3.74 (m, 6H), 3.79-3.89 (m, 1H), 3.92-4.01 (m, 1H), 3.99-4.21 (m, 4H), 4.21-4.41 (m, 2H), 4.55-4.63 (m, 2H), 4.89-5.04 (m, 2H), 5.09 (d, 2H, J

= 5.6 Hz), 5.12-5.30 (m, 3H), 5.32-5.41 (m, 1H), 5.65-5.73 (m, 1H) and 7.27-7.39 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 20.78, 20.83, 20.87, 20.91, 20.93, 20.98, 21.0, 27.67, 27.69, 40.9, 41.1, 53.6, 61.8, 61.9, 62.3, 62.7, 63.9, 65.6, 65.7, 66.1, 66.4, 66.7, 67.9, 68.0, 68.6, 68.8, 69.0, 69.3, 69.5, 69.72, 69.76, 70.0, 70.1, 70.3, 70.4, 70.52, 70.55, 70.7, 72.3, 97.1, 97.2, 120.38, 120.43, 128.2, 128.3, 128.60, 128.65, 129.8, 130.0, 136.8, 155.7, 156.7, 169.33, 169.37, 169.39, 169.47, 169.54, 169.6, 170.0, 170.5, 170.6, 170.7, 170.8 and 170.9; mass spectrum (APCI), *m/z* 873.3150 $(M + H)^+$ ($C_{38}H_{53}N_2O_{21}$ requires 873.3141).



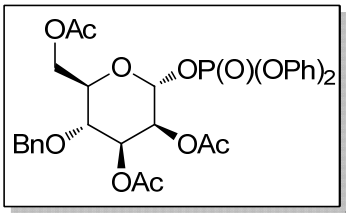
1,2,3,6-Tetra-*O*-acetyl-4-*O*-benzyl-α-D-mannopyranoside (3.26).[168,178] To a stirred solution containing 5.43 g (19.2 mmol) of acetal **3.21** in 50 mL of anh THF was added 58.0 mL (57.6 mmol) of a 1 M solution of BH_3 in THF and 7.48 g (57.6 mmol) of anh $CoCl_2$ at room temperature. The reaction mixture was stirred for 15 min at room temperature and quenched by the addition of 100 mL of ethyl acetate. The organic phase was filtered and the filtrate was treated with 20 mL of a 20% aq solution of $NaBH_4$. The solution was again filtered and washed successively with sat aq $NaHCO_3$ and water, and then dried ($MgSO_4$). The solution was concentrated under diminished pressure to afford a crude residue. To a solution containing 3.44 g (12.1 mmol) of the crude residue in 85 mL of Ac_2O

was added a catalytic amount of H_2SO_4. The solution was stirred at room temperature for 12 h. The reaction mixture was quenched by the addition of 120 mL of ethyl acetate and 80 mL of satd aq $NaHCO_3$. The organic and aqueous layers were separated and the organic layer was washed with brine and dried ($MgSO_4$). The solution was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (30 × 5 cm). Elution with 2:1 ethyl acetate–hexanes afforded pyranoside **3.26** as a yellow oil: yield 1.17 g (22% over two steps); silica gel TLC R_f 0.26 (2:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 3H), 2.08 (s, 3H), 2.13 (s, 3H), 2.15 (s, 3H), 3.87 (t, 1H, J = 9.7), 3.99 (dt, 1H, J = 9.9 and 3.4 Hz), 4.32 (d, 2H, J = 3.5 Hz), 4.59 (d, 1H, J = 11.2 Hz), 4.70 (d, 1H, J = 10.8 Hz), 5.26 (dd, 1H, J = 3.3 and 2.1 Hz), 5.37 (dd, 1H, J = 9.5 and 3.4 Hz), 6.04 (t, 1H, J = 6.1 Hz), and 7.24-7.38 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 20.92, 20.97, 20.99, 21.04, 62.9, 68.9, 71.6, 71.8, 72.6, 75.2, 90.8, 127.9, 128.3, 128.7, 137.5, 168.4, 169.8, 169.9 and 170.8.



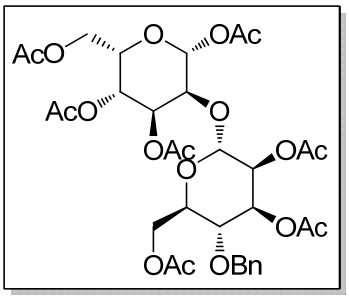
2,3,6-Tri-*O*-acetyl-4-*O*-benzyl-α,β-D-mannopyranose (3.27). To a stirred solution containing 1.09 g (2.49 mmol) of acetate **3.26** in 20 mL of anh DMF was added 274 mg (2.98 mmol) of hydrazine acetate. The reaction mixture was stirred at room temperature for 1.5 h and quenched by the addition of 100 mL of ethyl

acetate. The organic layer was washed with three 50-mL portions of brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (20 × 3 cm). Elution with 1:2 ethyl acetate–hexanes afforded pyranoside **3.27** as a colorless oil: yield 884 mg (90%); silica gel TLC R_f 0.36 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.92 (s, 3H), 2.01 (s, 3H), 2.08 (s, 3H), 3.77 (t, 1H, J = 10.0 Hz), 4.11 (ddd, 1H, J = 9.7, 4.1 and 2.1 Hz), 4.17-4.34 (m, 2H), 4.69-4.48 (m, 3H), 5.09 (s, 1H), 5.17-5.23 (m, 1H), 5.33-5.38 (m, 1H) and 7.18-7.32 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 20.69, 20.73, 63.1, 69.2, 70.5, 71.5, 72.8, 74.6, 77.4, 91.8, 127.6, 127.8, 128.3, 137.5, 170.0, 170.2 and 171.0; HRMS (APCI), *m/z* 397.1483 (M + H)$^+$ ($C_{19}H_{25}O_9$ requires *m/z* 397.1498).



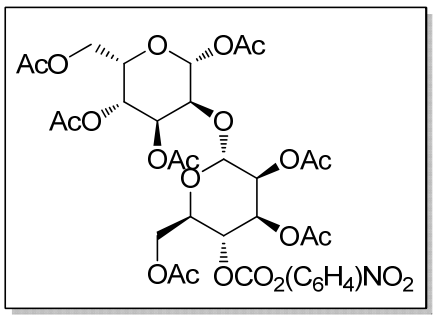
2,3,6-Tri-*O*-acetyl-4-*O*-benzyl-α-D-mannopyranosyl Diphenyl Phosphate (3.28). To a stirred solution containing 812 mg (2.05 mmol) of **3.27** in 80 mL of anh dichloromethane was added 313 mg (2.56 mmol) of DMAP and 3.10 mL (2.25 g, 22.1 mmol) of Et_3N, 4.10 mL (5.33 g, 19.7 mmol) of diphenyl chlorophosphate. The reaction mixture was stirred at 0 °C for 2 h and then poured into a mixture of 300 mL of ethyl acetate and 150 mL of satd aq $NaHCO_3$. The aqueous and organic layers were separated and the organic layer was washed with

three 50-mL portions of distilled water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (20 × 4 cm). Elution with 1:2 ethyl acetate–hexanes afforded **3.28** as a colorless oil: yield 857 mg (66%); silica gel TLC R_f 0.29 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.93 (s, 3H), 1.96 (s, 3H), 2.09 (s, 3H), 3.80 (t, 1H, J = 9.6 Hz), 3.91-4.12 (m, 2H), 4.18 (dd, 1H, J = 12.2 and 4.2 Hz), 4.50-4.68 (m, 2H), 5.27-5.38 (m, 2H), 5.80 (d, 1H, J = 6.1 Hz) and 7.11-7.38 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 20.74, 20.9, 62.4, 69.1, 70.9, 71.8, 72.1, 75.0, 77.4, 96.3, 120.1, 120.4, 125.7, 125.9, 127.9, 128.2, 128.6, 129.9, 130.0, 137.3, 150.1, 150.3, 169.5, 169.6 and 170.5; HRMS (APCI), *m/z* 629.1794 $(M + H)^+$ ($C_{31}H_{34}O_{12}P$ requires *m/z* 629.1788).



1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-benzyl-α-D-mannopyranosyl)-β-L-gulopyranose (3.51). To a stirred solution containing 217 mg (0.62 mmol) of gulose acceptor **3.16** and 471 mg (0.75 mmol) of mannose donor **3.28** in 4.5 mL of anh dichloromethane cooled to 0 °C was added 230 µL (283 mg, 1.25 mmol) of TMSOTf. The reaction mixture was stirred at 0 °C for 10 min and then poured into a mixture of 30 mL of ethyl acetate and 30 mL of satd

aq $NaHCO_3$. The aqueous and organic layers were separated and the organic layer was washed with two 20-mL portions of brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (30 × 3 cm). Elution with 2:1 ethyl acetate–hexanes afforded **3.51** as a colorless oil: yield 330 mg (73%); silica gel TLC R_f 0.25 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.92 (s, 3H), 2.02 (s, 3H), 2.07 (t, 6H, *J* = 3.2 Hz), 2.08-2.11 (m, 6H), 2.15 (d, 3H, *J* = 3.7 Hz), 3.70-3.83 (m, 1H), 3.92-4.18 (m, 4H), 4.23-4.40 (m, 2H), 4.50-4.71 (m, 2H), 4.89 (dd,1H, *J* = 7.2 and 1.7 Hz), 4.96-4.99 (m, 1H), 5.01-5.10 (m, 2H), 5.10-5.16 (m, 1H), 5.35-5.45 (m, 1H), 5.85 (d, 1H, *J* = 8.4 Hz) and 7.18-7.34 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 20.68, 20.71, 20.73, 20.79, 20.84, 20.88, 20.9, 61.4, 65.6, 67.7, 69.1, 69.5, 70.3, 71.3, 71.7, 72.4, 74.8, 90.7, 95.0, 127.6, 127.89, 127.99, 128.46, 128.49, 137.6, 168.8, 169.32, 169.36, 169.4, 169.7, 170.5 and 170.6; HRMS (APCI), *m/z* 727.2439 $(M + H)^+$ ($C_{33}H_{43}O_{18}$ requires *m/z* 727.2450).

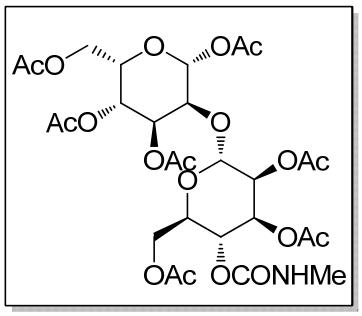


1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-((*p*-nitrophenyl)carbamoyl)-α-D-mannopyranosyl)-β-L-gulopyranose (3.52). To a

solution containing 140 mg (0.19 mmol) of disaccharide **3.51** in 13 mL of ethyl acetate was added a catalytic amount of $Pd(OH)_2/C$ and the reaction mixture was stirred overnight under 1 atm of H_2. The solvent was filtered through a pad of Celite 545® and the filtrate was concentrated under diminished pressure to afford a crude residue. The residue was used for the next reaction; silica gel TLC R_f 0.08 (1:1 ethyl acetate–hexanes).

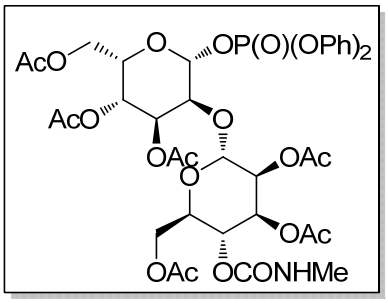
To a solution containing 120 mg (0.19 mmol) of the crude residue in 2.0 mL of anh pyridine was added 92.0 mg (0.76 mmol) of DMAP and 168 mg (0.76 mmol) of *p*-nitrophenyl chloroformate. The reaction mixture was stirred at 40 °C overnight and then poured into a mixture of 30 mL of ethyl acetate and 10 mL of H_2O. The aqueous and organic layers were separated and the organic layer was washed with three 10-mL portions of 1 N HCl and 10 mL of satd aq $NaHCO_3$ and brine. The organic solution was dried ($MgSO_4$) and concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 3 cm). Elution with 1:1 ethyl acetate–hexanes afforded ester **3.52** as colorless foam: yield 121 mg (78% over two steps); silica gel TLC R_f 0.30 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.98 (s, 3H), 2.03 (s, 3H), 2.11 (d, 6H, J = 5.0 Hz), 2.14 (s, 3H), 2.19 (d, 3H, J = 5.4 Hz), 3.99 (dd, 1H, J = 8.4 and 3.3 Hz), 4.02-4.25 (m, 4H), 4.27 (d, 1H, J = 2.4 Hz), 4.35 (t, 1H, J = 6.0 Hz), 4.46-4.55 (m, 2H), 4.93-5.01 (m, 2H), 5.11-5.18 (m, 2H), 5.24 (dd, 1H, J = 10.1 and 3.3 Hz), 5.32 (dd, 1H, J = 7.7 and 4.3 Hz), 5.43 (t, 1H, J = 3.5 Hz), 5.89 (d, 1H, J = 8.5 Hz), 7.29-7.39 (m, 2H) and 8.25 (t, 2H, J = 6.0 Hz); ^{13}C NMR ($CDCl_3$) δ 20.69, 20.71, 21.0, 61.3, 61.7, 65.6, 67.7, 68.6, 68.8, 70.0, 71.3, 71.4,

90.6, 95.1, 121.7, 125.4, 145.7, 151.8, 155.2, 168.7, 169.29, 169.33, 169.38, 169.58, 169.65, 169.7, 169.8, 170.44, 170.46 and 170.58; HRMS (APCI), *m/z* 802.2035 $(M + H)^+$ ($C_{33}H_{40}NO_{22}$ requires *m/z* 802.2042).



1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(methylcarbamoyl)-α-D-mannopyranosyl)-β-L-gulopyranose (3.53). To a solution containing 121 mg (0.15 mmol) of **3.52** in 3.2 mL of anh THF was added 76.0 μL (0.15 mmol) of a 2 M solution of CH_3NH_2 in THF at 0 °C. The reaction mixture was stirred at room temperature for 15 h at which time silica gel TLC analysis indicated that the reaction was complete. The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 3 cm). Elution with 1:1 ethyl acetate–hexanes afforded disaccharide **3.53** as a colorless oil: yield 90 mg (86%); silica gel TLC R_f 0.14 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.96 (t, 3H, *J* = 3.4 Hz), 2.04 (d, 3H, *J* = 6.4 Hz), 2.11 (dd, 12H, *J* = 5.4 and 2.8 Hz), 2.17 (d, 3H, *J* = 2.5 Hz), 2.76 (d, 3H, *J* = 4.8 Hz), 3.97 (dd, 1H, *J* = 8.4 and 3.2 Hz), 4.00-4.39 (m, 3H), 4.48-4.80 (m, 1H), 4.93 (d, 1H, *J* = 7.2 Hz), 4.99 (dd, 1H, *J* = 7.0 and 4.4 Hz), 5.04-5.10 (m, 2H), 5.08-5.17 (m, 2H), 5.29 (dd, 1H, *J* = 13.2 and 9.8 Hz), 5.42 (t, 1H, *J* = 3.5

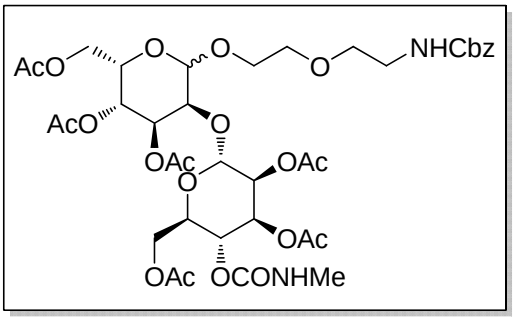
Hz), 5.87 (d, 1H, J = 8.4 Hz) and 6.28 (d, 1H, J = 4.2 Hz); ^{13}C NMR ($CDCl_3$) δ 20.68, 20.75, 20.76, 20.80, 20.82, 20.84, 27.8, 61.5, 61.8, 62.5, 62.7, 65.6, 66.0, 66.3, 66.8, 67.8, 68.9, 69.75, 69.79, 71.4, 90.7, 169.3, 169.59, 169.61, 169.65, 170.53, 170.55 and 170.7; HRMS (APCI), *m/z* 694.2199 $(M + H)^+$ ($C_{28}H_{40}NO_{19}$ requires *m/z* 694.2195).



3,4,6-Tri-*O*-acetyl-2-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(methylcarbamoyl)-α-D-mannopyranosyl)-β-L-gulopyranosyl Diphenyl Phosphate (3.54). To a solution containing 44.0 mg (0.06 mmol) of disaccharide **3.53** in 0.5 mL of anh DMF was added 7.00 mg (0.08 mmol) of hydrazine acetate. The reaction mixture was stirred at room temperature for 1.5 h and quenched by the addition of 20 mL of ethyl acetate. The organic solution was washed with three 10-mL portions of brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was used for the next reaction.

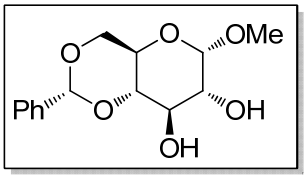
To a stirred solution containing 43.0 mg (0.07 mmol) of the crude residue in 4 mL of anh dichloromethane was added 10.0 mg (0.08 mmol) of DMAP, 100 μL (72.0 mg, 0.71 mmol) of Et_3N and 130 μL (160 mg, 0.63 mmol) of diphenyl chlorophosphate. The reaction mixture was stirred at 0 °C for 2 h and then poured

into a mixture of 40 mL of ethyl acetate and 20 mL of satd aq $NaHCO_3$. The aqueous and organic layers were separated and the organic layer was washed with three 10-mL portions of water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 3 cm). Elution with 2:1 ethyl acetate–hexanes afforded the phosphate ester **3.54** as a colorless oil: yield 38 mg (69% over two steps); silica gel TLC R_f 0.48 (2:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.95 (s, 3H), 2.00 (s, 3H), 2.09 (s, 3H), 2.12 (s, 3H), 2.15 (s, 3H), 2.21 (s, 3H), 2.57 (d, 3H, J = 4.0 Hz), 3.70 (s, 1H), 4.03 (s, 2H), 4.15 (d, 2H, J = 9.6 Hz), 4.24 (d, 2H, J = 12.2 Hz), 4.32-4.38 (m, 1H), 4.99 (d, 2H, J = 12.6 Hz), 5.05-5.25 (m, 2H), 5.30 (s, 1H), 5.45 (s, 1H), 5.71 (d, 1H, J = 7.4 Hz) and 7.19-7.41 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 20.77, 20.83, 20.89, 20.93, 27.6, 61.3, 62.3, 65.6, 66.3, 67.5, 68.8, 69.2, 69.5, 70.7, 70.8, 71.7, 95.1, 96.4, 120.4, 125.7, 129.8, 130.0, 150.4, 155.4, 169.37, 169.39, 169.6, 169.9, 170.5 and 170.73, 170.76; HRMS (APCI), *m/z* 884.2381 $(M + H)^+$ ($C_{38}H_{47}NO_{21}P$ requires *m/z* 884.2378).

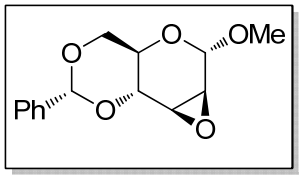


3,4,6-Tri-*O*-acetyl-2-*O*-(2,3,6-tri-*O*-acetyl-4-*O*-(methylcarbamoyl)-α-D-mannopyranosyl)-α,β-L-gulopyranosyl Benzyl 2-(2-Ethoxy)ethylcarbamate

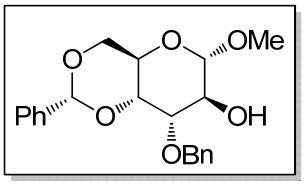
(3.56). To a stirred solution containing 38.0 mg (0.04 mmol) of phosphate ester **3.54** in 0.5 mL of anh dichloromethane was added a solution of 10.0 mg (0.04 mmol) of CBz-protected linker **3.40** in 0.5 mL of anh dichloromethane at 0 °C. To the cooled reaction mixture was then added 14.0 μL (17.0 mg, 0.08 mmol) of TMSOTf. The reaction mixture was stirred at 0 °C for 15 min and then poured into a mixture of 20 mL of ethyl acetate and 20 mL of satd aq $NaHCO_3$. The aqueous and organic layers were separated and the organic layer was washed with three 10-mL portions of water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 3 cm). Elution with 12:12:1 ethyl acetate–hexanes–methanol afforded **3.56** as a colorless oil. The product isolated as a mixture of anomers: yield 19 mg (51%); silica gel TLC R_f 0.14 (12:12:1 ethyl acetate–hexanes–methanol); ^{1}H NMR ($CDCl_3$) δ 1.92-2.14 (m, 18H), 2.71 (t, 3H, J = 4.1 Hz), 3.40 (d, 3H, J = 4.9 Hz), 3.52-3.77 (m, 8H), 3.85 (dd,1H, J = 8.4 and 3.2 Hz), 3.95 (t, 1H, J = 3.9 Hz), 4.27 (dd, 2H, J = 13.4 and 7.3 Hz), 4.40 (t, 1H, J = 6.4 Hz), 4.88-5.04 (m, 3H), 5.05-5.22 (m, 6H), 5.25 (dd, 1H, J = 7.3 and 3.6 Hz) and 7.28-7.40 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 20.78, 20.83 20.85, 20.87, 20.92, 20.95, 27.7, 61.9, 62.3, 63.1, 63.8, 65.7, 66.8, 66.9, 68.1, 68.7, 68.8, 69.6, 69.8, 70.2, 71.0, 72.3, 97.2, 97.5, 128.27, 128.33, 128.65, 128.67, 169.5, 169.7, 169.8, 169.9, 170.57, 170.63 and 170.7; HRMS (APCI), *m/z* 873.3142 $(M + H)^+$ ($C_{38}H_{53}N_2O_{21}$ requires *m/z* 873.3141).



Methyl-4,6-*O*-benzylidene-α-D-glucopyranoside (3.29).[169] To a solution containing 10.0 g (51.5 mmol) of α-D-methyl glucopyranoside in 200 mL of acetonitrile was added 14.0 mL (14.2 g, 92.7 mmol) of benzaldehyde dimethyl acetal and 600 mg (2.57 mmol) of camphor sulfonic acid. The reaction mixture was heated to reflux for 20 min and then allowed to cool to room temperature and neutralized by the addition of 400 μL of triethylamine. The reaction mixture was diluted with 800 mL of ethyl acetate. The organic layer was washed with three 250-mL portions of water and dried ($MgSO_4$). The organic layer was concentrated under diminished pressure to afford a crude residue. The residue was recrystallized from 1:7 dichloromethane–hexanes to afford acetal **3.29** as a colorless solid: yield 9.48 g (65%); silica gel TLC R_f 0.17 (2:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 3.45-3.47 (m, 4H), 3.63 (dd, 1H, J = 9.1 and 3.9 Hz), 3.71-3.85 (m, 2H), 3.93 (t, 1H, J = 9.2 Hz), 4.29 (dd, 1H, J = 9.7 and 4.3 Hz), 4.80 (d, 1H, J = 3.9 Hz), 5.53 (s, 1H) and 7.33-7.53 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 55.7, 62.5, 69.1, 72.0, 73.0, 81.0, 99.9, 102.1, 126.4, 128.4, 129.4 and 137.2.

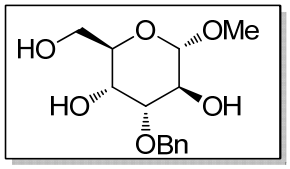


Methyl 2,3-Anhydro-4,6-*O*-benzyl-α-D-mannopyranoside (3.30).[170,179] To a solution containing 2.44 g (60% in oil dispersion, 60.9 mmol) of NaH in 290 mL of anh DMF at 0 ºC was added 8.20 g (29.0 mmol) of acetal **3.29** under an argon atmosphere. The reaction mixture was stirred at room temperature for 0.5 h. To the above stirred solution at 0 ºC was then added 7.10 g (31.9 mmol) of *N*-tosylimidazole. The suspension was stirred at room temperature for 1 h. The reaction mixture was poured with stirring into 2.5 L of ice–cold water and the resulting solid was filtered and washed with water to afford a crude residue. The residue so obtained was triturated with methanol to obtain the epoxide **3.30** as a colorless solid: yield 1.83 g (24%); silica gel TLC R_f 0.68 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 3.17 (d, 1H, J = 3.6 Hz), 3.45-3.49 (m, 4H), 3.64-3.79 (m, 3H), 4.21-4.32 (m, 1H), 4.91 (s, 1H), 5.57 (s, 1H), 7.35-7.53 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 50.7, 54.0, 55.9, 61.8, 69.6, 75.0, 97.0, 102.6, 126.3, 128.5, 129.4 and 137.2.



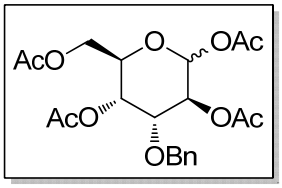
Methyl 4,6-*O*-Benzylidene-3-*O*-benzyl-α-D-altropyranoside (3.31).[171] A solution containing 214 mg (9.32 mmol) of sodium metal in 2.9 mL of anh benzyl alcohol was heated (~ 100 ºC) until all of the sodium metal had dissolved. The cooled solution was treated with 1.07 g (4.05 mmol) of anhydromannopyranoside **3.30**. The reaction mixture was then heated to reflux for 15 min, cooled and

diluted by the addition of 20 mL of ether. The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (20 × 5 cm). Elution with 1:4 ethyl acetate–hexanes afforded acetal **3.31** as a colorless solid: yield 723 mg (48%); silica gel TLC R_f 0.55 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 1H), 3.42 (s, 3H), 3.77 (t, 1H, J = 10.3 Hz), 3.84 (t, 1H, J = 2.8 Hz), 3.93 (d, 1H, J = 2.8 Hz), 3.98 (dt,1H, J = 9.3 and 4.6 Hz), 4.28-4.45 (m, 2H), 4.55 (d, 1H, J = 6.0 Hz), 4.70-4.90 (m, 2H), 5.56 (s, 1H) and 7.23-7.53 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 55.8, 58.7, 69.4, 70.2, 72.9, 74.9, 77.2, 102.0, 102.4, 126.3, 127.5, 127.7, 128.30, 128.36, 129.1, 137.7 and 138.7.



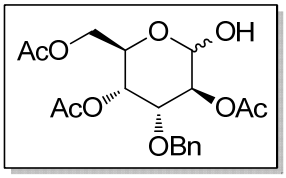
Methyl-3-*O*-benzyl-α-D-altropyranoside (3.32).[171] To a solution containing 1.67 g (4.48 mmol) of acetal **3.31** in 4.2 mL of methanol was added 43.0 mg (0.22 mmol) of *p*-toluenesulfonic acid monohydrate at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred for 4 h. The reaction mixture was quenched by the addition of 1.90 mL (1.38 g, 13.4 mmol) of triethylamine and concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (10 × 3 cm). Elution with 5:1 ethyl acetate–hexanes afforded methyl pyranoside **3.32** as a colorless oil: yield 1.22 g (96%); silica gel TLC R_f 0.17 (ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 3.01 (d, 1H, J =

9.3 Hz), 3.33 (s, 3H), 3.53 (d, 1H, *J* = 15.3 Hz), 3.70-3.77 (m, 2H), 3.80 (dt, 2H, *J* = 8.8 and 4.3 Hz), 3.96 (s, 2H), 4.40-4.78 (m, 4H) and 7.21-7.35 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 55.5, 61.9, 63.4, 67.3, 69.2, 72.0, 77.4, 101.5, 127.9, 128.0, 128.5 and 138.0.



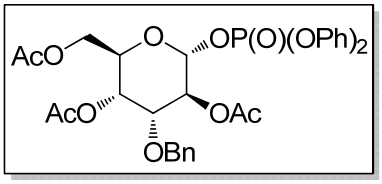
1,2,4,6-Tetra-*O*-acetyl-3-*O*-benzyl-α,β-D-altropyranoside (3.33). To a solution containing 532 mg (1.87 mmol) of methyl pyranoside **3.32** in 13 mL of Ac_2O was added a catalytic amount of H_2SO_4. The solution was stirred overnight at room temperature. The reaction mixture was then poured into a stirred mixture of 120 mL of ethyl acetate and 80 mL of satd aq $NaHCO_3$. The organic and aqueous layers were separated and the organic layer was washed with brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (30 × 3 cm). Elution with 1:2 ethyl acetate–hexanes afforded the product **3.33** as a 3:2 mixture of α and β anomers as determined by ^{1}H NMR; yield 705 mg (86%); silica gel TLC R_f 0.55 (1:1 ethyl acetate–hexanes); α anomer ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 3H), 2.06-2.09 (m, 6H), 2.14 (s, 3H), 3.96 (t, 1H, *J* = 3.2 Hz), 4.11-4.16 (m, 1H), 4.24-4.37 (m, 2H), 4.55-4.75 (m, 2H), 5.03-5.09 (m, 1H), 5.29 (s, 1H), 5.99 (d, 1H, *J* = 11.3 Hz) and 7.27-7.38 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 20.91, 20.92, 21.04, 21.05, 62.6, 66.3, 66.6, 68.0, 72.46, 72.49, 91.4, 127.8, 128.1, 128.5, 137.5, 169.0, 169.7,

169.8 and 170.9; HRMS (APCI), *m/z* 379.1387 $(M - CH_3COO)^+$ ($C_{19}H_{23}O_8$ requires *m/z* 379.1393).



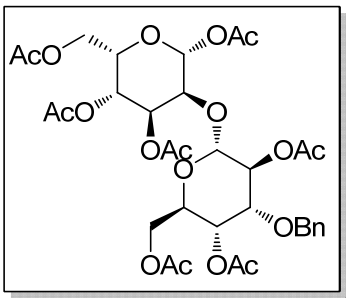
2,4,6-Tri-*O*-acetyl-3-*O*-benzyl-α,β-D-altropyranoside (3.34). To a solution containing 1.93 g (4.40 mmol) of monosaccharide **3.33** in 35 mL of anh DMF was added 486 mg (5.28 mmol) of hydrazine acetate. The reaction mixture was stirred at room temperature for 1.5 h and quenched by the addition of 100 mL of ethyl acetate. The organic layer was then washed with three 50-mL portions of brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (20 × 4 cm). Elution with 1:2 ethyl acetate–hexanes afforded **3.34** as a colorless oil. The product isolated as a mixture of anomers as analyzed by ^{1}H NMR: yield 837 mg (48%); silica gel TLC R_f 0.31 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.95 (s, 3H), 1.96 (s, 3H), 2.05 (s, 3H), 2.07 (s, 3H), 2.11 (s, 3H), 2.15 (s, 3H), 3.73-3.95 (br s, 1H), 3.98-4.05 (m, 1H), 4.09 (d, 1H, *J* = 8.6 Hz), 4.12-4.27 (m, 4H), 4.32 (dt, 1H, *J* = 14.2 and 7.1 Hz), 4.36-4.46 (m, 1H), 4.54-4.75 (m, 4H), 4.89-4.94 (m, 2H), 4.96-5.08 (m, 4H), 5.24 (t, 1H, *J* = 12.1 Hz) and 7.41-7.27 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 20.80, 20.82, 20.86, 20.98, 21.02, 62.9, 63.2, 64.1, 66.2, 66.9, 68.3, 70.0, 70.3, 72.9, 73.3, 73.8, 74.2, 91.6, 92.8, 128.1, 128.2, 128.4, 128.5, 128.7, 128.8, 136.2, 137.3, 169.73, 169.78, 169.83, 170.4, 170.95 and

170.96; HRMS (APCI), *m/z* 379.1394 $(M - OH)^+$ ($C_{19}H_{23}O_8$ requires *m/z* 379.1393).



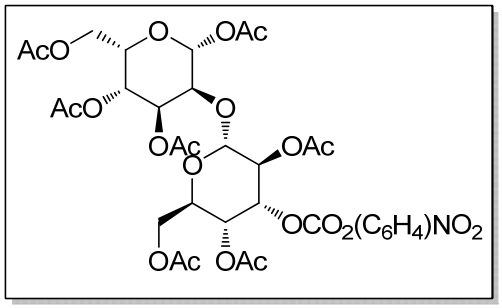
2,4,6-Tri-*O*-acetyl-3-*O*-benzyl-α-D-altropyranosyl Diphenyl Phosphate (3.35). To a stirred solution containing 637 mg (1.61 mmol) of pyranoside **3.34** in 2.7 mL of anh dichloromethane was added 1.21 mL (1.6 M, 1.93 mmol) of *n*-BuLi solution at −78 °C. The reaction mixture was stirred at this temperature for 10 min and 400 μL (520 mg, 1.93 mmol) of diphenyl chlorophosphate was added dropwise. The reaction mixture was stirred at −78 °C for an additional 10 min and poured into a mixture of 20 mL of ethyl acetate and 10 mL of satd aq $NaHCO_3$. The organic and aqueous layers were separated and the organic layer was washed with three 10-mL portions of water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (20 × 3 cm). Elution with 1:2 ethyl acetate–hexanes afforded phosphate ester **3.35** as a colorless oil: yield 324 mg (32%); 121 mg of unreacted starting material was also recovered; silica gel TLC R_f 0.40 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.97 (s, 3H), 1.98 (s, 3H), 2.00 (d, 3H, *J* = 2.1 Hz), 3.99 (dd, 1H, *J* = 6.3 and 3.1 Hz), 4.05-4.28 (m, 3H), 4.50-4.62 (m, 2H), 5.13 (dd, 1H, *J* = 7.0 and 3.2 Hz), 5.19 (dd, 1H, *J* = 6.4 and

2.2 Hz), 5.96 (dd, 1H, J = 7.1 and 2.2 Hz) and 7.12-7.36 (m, 15H); ^{13}C NMR ($CDCl_3$) δ 20.74, 20.76, 20.9, 62.8, 66.9, 68.20, 68.28, 71.6, 72.94, 72.97, 95.5, 120.30, 120.35, 125.7, 128.0, 128.2, 128.5, 129.8, 129.9, 137.1, 150.2, 150.4, 169.9 and 170.6; HRMS (APCI), *m/z* 569.1598 $(M - CH_3COO)^+$ ($C_{29}H_{30}O_{10}P$ requires *m/z* 569.1576).



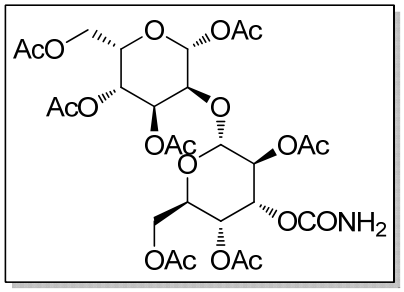
1,3,4,6-Tetra-O-acetyl-2-O-(2,4,6-tri-O-acetyl-3-O-benzyl-α-D-altropyranosyl)-β-L-gulopyranose (3.59). To a stirred solution containing 180 mg (0.52 mmol) of gulose acceptor **3.16** and 324 mg (0.52 mmol) of altrose donor **3.35** in 3.7 mL of anh dichloromethane at 0 °C was added 190 μL (234 mg, 1.03 mmol) of TMSOTf. The reaction mixture was stirred at 0 °C for 10 min at which time it was poured into a mixture of 30 mL of ethyl acetate and 30 mL of satd aq $NaHCO_3$. The aqueous and organic layers were separated and the organic layer was washed with two 20-mL portions of brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (30 × 3 cm). Elution with 1:2 ethyl acetate–hexanes afforded disaccharide **3.59** as a colorless oil: yield 149 mg (40%); silica gel TLC R_f 0.24 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ

1.93 (s, 3H), 1.97 (s, 3H), 2.00 (s, 3H), 2.02 (s, 3H), 2.04-2.06 (m, 6H), 2.08 (s, 3H), 3.72-3.83 (m, 1H), 3.94-4.16 (m, 2H), 4.16-4.35 (m, 3H), 4.35-4.62 (m, 3H), 4.79-5.01 (m, 4H), 5.24 (d, 1H, J = 0.4 Hz), 5.35-5.42 (m, 1H), 5.90 (d, 1H, J = 8.4 Hz) and 7.15-7.30 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 20.7, 20.80, 20.81, 20.86, 20.89, 21.0, 61.6, 62.6, 65.3, 65.5, 66.4, 67.8, 68.4, 68.8, 72.0, 72.7, 90.6, 95.4, 127.4, 127.6, 127.9, 128.5, 137.7, 169.0, 169.2, 169.4, 169.5, 169.9, 170.5, 170.7; HRMS (APCI), *m/z* 667.2230 $(M - CH_3COO)^+$ ($C_{31}H_{39}O_{16}$ requires *m/z* 667.2238).

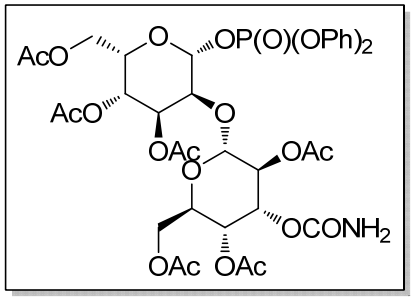


1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-((*p*-nitrophenyl)carbamoyl)-α-D-altropyranosyl)-β-L-gulopyranose (3.60). To a solution containing 190 mg (0.26 mmol) of disaccharide **3.59** in 18 mL of ethyl acetate was added a catalytic amount of $Pd(OH)_2/C$ and the reaction mixture was stirred overnight under 1 atm of H_2. The solvent was filtered through a pad of Celite 545® and the filtrate was concentrated under diminished pressure to afford a crude residue. The crude product was used for the next reaction; silica gel TLC R_f 0.12 (1:1 ethyl acetate–hexanes).

To a solution containing 198 mg (0.31 mmol) of the crude residue in 1.1 mL of anh pyridine was added 151 mg (1.24 mmol) of DMAP and 280 mg (1.24 mmol) of *p*-nitrophenyl chloroformate. The reaction mixture was stirred at 40 °C overnight and then poured into a mixture of 30 mL ethyl acetate and 10 mL of H_2O. The aqueous and organic layers were separated and the organic layer was washed with three10-mL portions of 1 N HCl and10 mL of satd aq $NaHCO_3$ and brine. The solvent was dried ($MgSO_4$) and then concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 3 cm). Elution with 1:1 ethyl acetate–hexanes afforded ester **3.60** as a colorless foam: yield 177 mg (71% over two steps); silica gel TLC R_f 0.28 (1:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 2.02 (s, 3H), 2.04 (s, 3H), 2.09 (s, 3H), 2.10 (s, 3H), 2.12 (s, 3H), 2.13 (s, 3H), 2.14 (s, 3H), 3.99-4.17 (m, 3H), 4.23-4.38 (m, 2H), 4.41-4.50 (m, 1H), 4.89-5.02 (m, 2H), 5.02-5.13 (m, 2H), 5.20 (dt, 1H, J = 10.4 and 5.2 Hz), 5.25-5.34 (m, 1H), 5.43 (t, 1H, J = 3.5 Hz), 5.94 (d, 1H, J = 8.4 Hz), 7.42 (t, 2H, J = 7.1 Hz) and 8.22-8.30 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 20.66, 20.71, 20.72, 20.76, 20.9, 61.5, 62.2, 64.7, 65.1, 65.4, 67.6, 68.1, 68.6, 71.3, 72.1, 90.5, 94.5, 121.4, 125.4, 136.0, 145.6, 149.8, 151.6, 155.2, 168.8, 168.9, 169.1, 169.3, 169.5, 170.4 and 170.6; HRMS (APCI), *m/z* 742.1851 (M – $CH_3COO)^+$ ($C_{31}H_{36}NO_{20}$ requires *m/z* 742.1831).



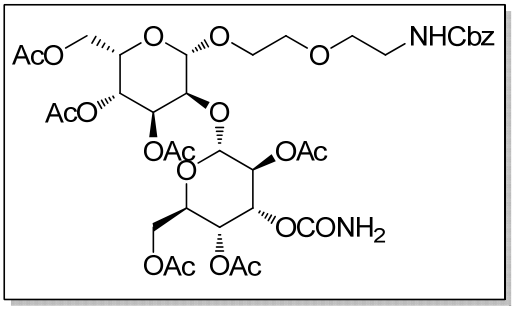
1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-carbamoyl-α-D-altropyranosyl)-β-L-gulopyranoside (3.61). To a solution containing 73.0 mg (0.09 mmol) of ester **3.60** in 2 mL of anh THF was added a solution of 0.7 mL of anh THF saturated with NH_3 at 0 °C. The reaction mixture was allowed to warm to room temperature and then stirred for 2.5 h at which time silica gel TLC analysis indicated that the reaction was complete. The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (20 × 3 cm). Elution with 3:1 ethyl acetate–hexanes afforded disaccharide **3.61** as a colorless oil: yield 44 mg (71%); silica gel TLC R_f 0.38 (ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 2.00 (s, 3H), 2.05 (s, 3H), 2.11 (s, 6H), 2.13 (s, 3H), 2.16 (s, 3H), 2.17 (s, 3H), 3.98 (dd, 1H, *J* = 8.1 and 3.3 Hz), 4.02-4.38 (m, 7H), 4.75 (d, 1H, *J* = 3.3 Hz), 4.82-4.96 (m, 2H), 4.99-5.12 (m, 2H), 5.13 (dd, 1H, *J* = 7.8 and 4.4 Hz), 5.44 (t, 1H, *J* = 3.7 Hz) and 6.11 (d, 1H, *J* = 8.1 Hz); ^{13}C NMR ($CDCl_3$) δ 20.72, 20.75, 20.79, 20.82, 20.83, 20.87, 21.2, 61.8, 62.4, 64.6, 64.9, 65.5, 66.8, 67.6, 69.0, 69.5, 71.7, 91.0, 94.4, 155.6, 168.9, 169.3, 169.4, 169.6, 170.2, 170.5 and 170.7; HRMS (APCI), *m/z* 680.2039 $(M + H)^+$ ($C_{27}H_{38}NO_{19}$ requires *m/z* 680.2038).



3,4,6-Tri-*O*-acetyl-2-*O*-(2,4,6-Tri-*O*-acetyl-3-*O*-carbamoyl-α-D-altropyranosyl)-β-L-gulopyranosyl Diphenyl Phosphate (3.63). To a solution containing 44.0 mg (60.0 μmol) of disaccharide **3.61** in 0.5 mL of anh DMF was added 7.00 mg (80.0 μmol) of hydrazine acetate. The reaction mixture was stirred at room temperature for 1.5 h and then quenched by the addition of 20 mL of ethyl acetate. The organic layer was washed with three 10-mL portions of brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was used for the next reaction.

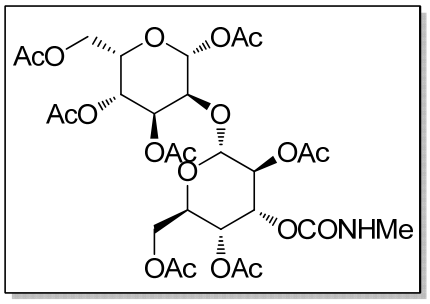
To a stirred solution containing 41.0 mg (60.0 μmol) of the crude residue in 4 mL of anh dichloromethane was added 10.0 mg (80.0 μmol) of DMAP, 100 μL (72.0 mg, 0.68 mmol) of Et_3N and 125 μL (162 mg, 0.61 mmol) of diphenyl chlorophosphate at 0 °C. The reaction mixture was stirred at 0 °C for 2 h and then poured into a mixture of 40 mL of ethyl acetate and 20 mL of satd aq $NaHCO_3$. The aqueous and organic layers were separated and the organic layer was washed with three 10-mL portions of distilled water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 2 cm). Elution with 2:1 ethyl acetate–hexanes afforded phosphate ester **3.63** as a colorless oil: yield 31 mg

(55% over two steps); silica gel TLC R_f 0.30 (2:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.83 (s, 3H), 1.98 (s, 3H), 2.04 (s, 3H), 2.12 (d, 3H, J = 2.8 Hz), 2.15 (d, 6H, J = 3.9 Hz), 3.98-4.09 (m, 2H), 4.09-4.25 (m, 4H), 4.26-4.36 (m, 2H), 4.66 (d, 1H, J = 9.8 Hz), 4.83 (d, 1H, J = 2.1 Hz), 4.91 (d, 1H, J = 6.4 Hz), 5.03 (t, 1H, J = 5.7 Hz), 5.09-5.19 (m, 2H), 5.45 (d, 1H, J = 3.2 Hz), 5.74 (t, 1H, J = 8.0 Hz) and 7.09-7.41 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 20.62, 20.66, 20.77, 20.83, 20.88, 61.6, 62.2, 64.5, 64.7, 65.1, 67.1, 67.3, 68.9, 71.7, 94.1, 120.28, 120.32, 120.37, 125.98, 125.99, 126.23, 126.24, 129.93, 129.94, 130.1, 155.9, 168.8, 169.0, 169.3, 169.5, 170.4, and 170.8; HRMS (APCI), *m/z* 870.2230 (M + H)$^+$ ($C_{37}H_{45}NO_{21}P$ requires *m/z* 870.2222).

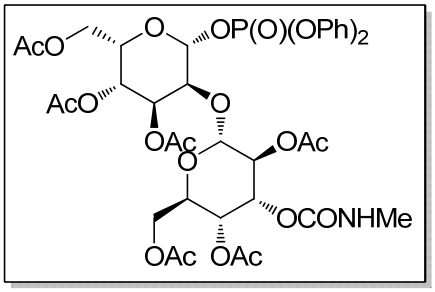


3,4,6-Tri-*O*-acetyl-2-*O*-(2,4,6-Tri-*O*-acetyl-3-*O*-carbamoyl-α-D-altropyranosyl)-β-L-gulopyranosyl Benzyl 2-(2-Ethoxy)ethylcarbamate (3.65). To a stirred solution containing 31 mg (40 μmol) of phosphate ester **3.63** in 0.45 mL of anh dichloromethane was added a solution of 8.0 mg (30 μmol) of CBz-protected linker **3.40** in 0.45 mL of anh dichloromethane at 0 °C. To the reaction mixture was added 12 μL (15 mg, 80 μmol) of TMSOTf and the reaction mixture was stirred at 0 °C for 15 min. The reaction mixture was poured into a

mixture of 10 mL of ethyl acetate and 10 mL satd aq $NaHCO_3$. The aqueous and organic layers were separated and the organic layer was washed with three 10-mL portions of water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 2 cm). Elution with 12:12:1 ethyl acetate–hexanes–methanol afforded **3.65** as a colorless oil: yield 15 mg (48%); silica gel TLC R_f 0.17 (11:11:1 ethyl acetate–hexanes–methanol); ^{1}H NMR ($CDCl_3$) δ ^{1}H NMR ($CDCl_3$) δ 1.95-2.07 (m, 6H), 2.07-2.15 (m, 12H), 3.41 (t, 2H, *J* = 9.5 Hz), 3.59 (d, 2H, *J* = 5.0 Hz), 3.61-3.71 (m, 3H), 3.87 (dt, 1H, *J* = 12.8 and 6.5 Hz), 3.94-4.04 (m, 1H), 4.04-4.20 (m, 3H), 4.21-4.26 (m, 1H), 4.36-4.48 (m, 1H), 4.49-4.60 (m, 1H), 4.75 (d, 1H, *J* = 7.5 Hz), 4.84-5.05 (m, 4H), 5.05-5.20 (m, 4H), 5.21-5.29 (m, 1H), 5.32-5.49 (m, 2H) and 7.27-7.38 (m, 5H) ; ^{13}C NMR ($CDCl_3$) δ 20.75, 20.77, 20.82, 20.85, 20.88, 20.92, 40.9, 62.1, 62.3, 62.6, 65.1, 65.2, 66.9, 67.8, 68.1, 68.5, 68.6, 69.2, 70.37, 70.45, 99.5, 128.3, 128.4, 128.5, 128.7, 136.6, 155.7, 169.0, 169.4, 169.61, 169.65, 170.6, 170.82 and 170.89; HRMS (APCI), *m/z* 859.2973 $(M + H)^+$ ($C_{37}H_{51}N_2O_{21}$ requires *m/z* 859.2984).



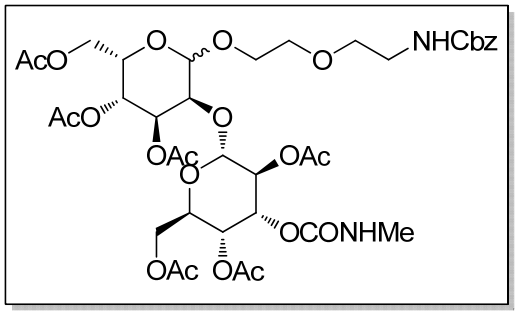
1,3,4,6-Tetra-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-(methylcarbamoyl)-α-D-altropyranosyl)-β-L-gulopyranose (3.62). To a solution containing 86.0 mg (0.11 mmol) of ester **3.60** in 2.4 mL of anh THF was added 54.0 μL (0.11 mmol) of a 2 M solution of CH_3NH_2 in THF at 0 °C. The reaction mixture was stirred at room temperature for 15 h at which time analysis by silica gel TLC indicated that the reaction was complete. The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (35 × 2 cm). Elution with 2:1 ethyl acetate–hexanes afforded disaccharide **3.62** as a colorless oil: yield 31 mg (42%); silica gel TLC R_f 0.13 (3:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 3H), 2.05 (s, 3H), 2.11 (s, 6H), 2.13 (s, 3H), 2.15 (s, 3H), 2.16 (s, 3H), 2.79 (d, 3H, J = 4.7 Hz), 3.98 (dd, 1H, J = 8.0 and 3.3 Hz), 4.04-4.30 (m, 4H), 4.33 (dt, 1H, J = 12.1 and 6.1 Hz), 4.71-4.77(m, 1H), 4.84-4.95(m, 1H), 5.06 (dd, 2H, J = 10.1 and 6.6 Hz), 5.11-5.19 (m, 1H), 5.21-5.41 (m, 2H), 5.43 (dd, 1H, J = 10.0 and 6.3 Hz) and 6.10 (d, 1H, J = 8.0 Hz); ^{13}C NMR ($CDCl_3$) δ 20.77, 20.81, 20.82, 20.85, 20.88, 20.9, 21.3, 27.8, 61.8, 62.5, 64.8, 65.0, 65.5, 66.4, 66.7, 67.6, 69.2, 71.6, 91.1, 94.7, 155.9, 169.0, 169.3, 169.4, 169.6, 170.1, 170.5 and 170.8; HRMS (APCI), *m/z* 694.2204 (M + H)$^+$ ($C_{28}H_{40}NO_{19}$ requires *m/z* 694.2195).



3,4,6-Tri-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-(methylcarbamoyl)-α-D-altropyranosyl)-β-L-gulopyranosyl Diphenyl Phosphate (3.64). To a solution containing 31.0 mg (40.0 μmol) of disaccharide **3.62** in 0.5 mL of anh DMF was added 5.00 mg (50.0 μmol) of hydrazine acetate. The reaction mixture was stirred at room temperature for 1.5 h and then quenched by the addition of 20 mL of ethyl acetate. The organic solution was washed with three 10-mL portions of brine and dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was used for the next reaction.

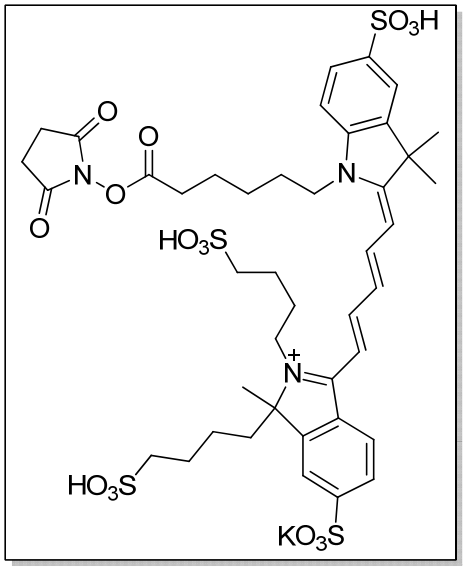
To a stirred solution containing 22.0 mg (30.0 μmol) of the residue in 2 mL of anh dichloromethane was added 6.00 mg (40.0 μmol) of DMAP, 52.0 μL (38.0 mg, 370 μmol) of Et_3N and 70.0 μL (91.0 mg, 330 μmol) of diphenyl chlorophosphate at 0 °C. The reaction mixture was stirred at 0 °C for 2 h and then poured into a mixture of 40 mL of ethyl acetate and 20 mL of satd aq $NaHCO_3$. The aqueous and organic layers were separated and the organic layer was washed with three 10-mL portions of distilled water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 2 cm). Elution with 2:1 ethyl acetate–hexanes afforded phosphate ester **3.64** as a colorless oil: yield 7.0 mg

(17% over two steps); silica gel TLC R_f 0.28 (3:1 ethyl acetate–hexanes); ^{1}H NMR ($CDCl_3$) δ 1.85 (s, 3H), 1.98 (s, 3H), 2.04 (s, 3H), 2.12 (s, 3H), 2.15 (d, 6H, *J* = 2.5 Hz), 2.63 (d, 3H, *J* = 4.7 Hz), 3.98-4.08 (m, 2H), 4.09-4.26 (m, 3H), 4.30 (t, 1H, *J* = 6.1 Hz), 4.63 (d, 1H, *J* = 10.5 Hz), 4.80 (d, 1H, *J* = 3.0 Hz), 4.89 (s, 1H), 5.00-5.06 (m, 1H), 5.13 (dd, 1H, *J* = 10.5 and 3.1 Hz), 5.18 (d, 1H, *J* = 3.0 Hz), 5.45 (d,1H, *J* = 2.9 Hz), 5.73 (t, 1H, *J* = 8.0 Hz), 6.46 (d, 1H, *J* = 4.8 Hz) and 7.12-7.40 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 20.67, 20.72, 20.77, 20.8, 20.9, 27.4, 61.6, 62.3, 64.67, 64.72, 65.1, 66.7, 67.2, 69.1, 71.7, 94.2, 96.52, 96.56, 120.1, 120.2, 120.32, 120.37, 126.0, 126.1, 129.9, 130.1, 156.1, 168.8, 169.0, 169.4, 169.5, 170.5 and 170.8; HRMS (APCI), *m/z* 884.2403 $(M + H)^+$ ($C_{38}H_{47}NO_{21}P$ requires *m/z* 884.2378).

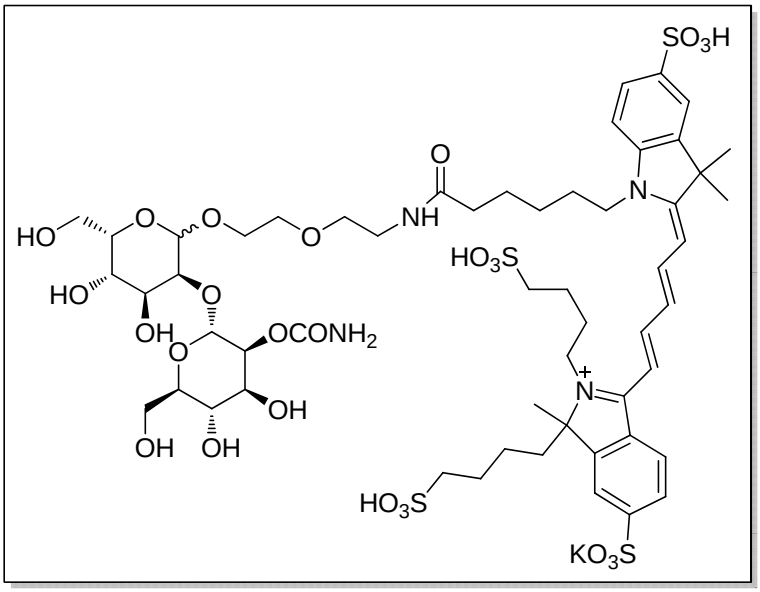


3,4,6-Tri-*O*-acetyl-2-*O*-(2,4,6-tri-*O*-acetyl-3-*O*-(methylcarbamoyl)-α-D-altropyranosyl)-α,β-L-gulopyranosyl Benzyl 2-(2-Ethoxy)ethylcarbamate (3.66). To a stirred solution containing 17 mg (19 μmole) of phosphate ester **3.64** in 0.25 mL of anh dichloromethane was added a solution of 5.0 mg (17 μmole) of CBz-protected linker **3.40** in 0.25 mL of anh dichloromethane at 0 °C. To the reaction mixture was added 7.0 μL (8.6 mg, 34 μmol) of TMSOTf. The reaction

mixture was stirred at 0 °C for 15 min and then poured into a mixture of 10 mL ethyl acetate and 10 mL satd aq $NaHCO_3$. The aqueous and organic layers were separated and the organic layer was washed with three 10-mL portions of distilled water and brine and then dried ($MgSO_4$). The solvent was concentrated under diminished pressure to afford a crude residue. The residue was applied to a silica gel column (25 × 2 cm). Elution with 12:12:1 ethyl acetate–hexanes–methanol afforded **3.66** as a colorless oil: yield 10 mg (59%); silica gel TLC R_f 0.14 (11:11:1 ethyl acetate–hexanes–methanol); ^{1}H NMR ($CDCl_3$) δ 1.97 (d, 3H, J = 8.6 Hz), 2.04 (d, 3H, J = 4.2 Hz), 2.07-2.15 (m, 12H), 2.75 (d, 3H, J = 4.7 Hz), 3.34-3.44 (m, 2H), 3.51-3.70 (m, 8H), 3.72 (dd, 1H, J = 10.3 and 5.6 Hz), 3.82-3.93 (m, 1H), 3.95-4.25 (m, 3H), 4.26-4.56 (m, 1H), 4.63 (d, 1H, J = 7.2 Hz), 4.86-5.02 (m, 1H), 4.96-5.28 (m, 6H), 5.33-5.51 (m, 1H), 5.83 (d, 1H, J = 4.7 Hz) and 7.27-7.39 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 20.79, 20.84, 20.86, 20.89, 20.93, 21.0, 29.8, 41.0, 61.9, 62.2, 62.3, 62.7, 62.9, 65.26, 65.33, 66.9, 67.1, 70.2, 70.4, 70.5, 72.3, 128.3, 128.4, 128.66, 128.67, 136.6, 169.61, 169.65, 169.68, 170.6, 170.7, 170.8 and 170.9; HRMS (APCI), *m/z* 873.3150 $(M + H)^+$ ($C_{38}H_{53}N_2O_{21}$ requires *m/z* 873.3141).



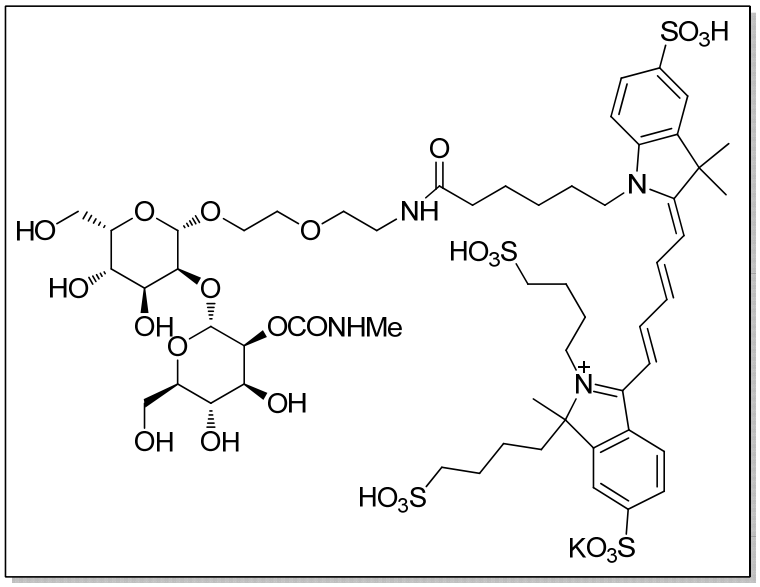
Cy5**succinimidyl ester (3.8).[174] To a solution containing 0.5 mg (0.6 μmol) of Cy5**COOH was added 5.0 mg (16 μmol) of TSTU dissolved in 100 μL of anh DMF, followed by 7.5 μL (5.6 mg, 43 μmol) of anh DIPEA dissolved in 75 μL of anh DMF. The reaction mixture was stirred at room temperature for 3 h and then diluted with 3 mL of ethyl acetate. The solution was then centrifuged at 12000 rpm for 10 min. The supernatant solution was discarded and the residue washed with 1 mL of ethyl acetate. The residue was then dried under vacuum in the dark for 30 min to afford the product **3.8** as a dark blue solid: yield 480 μg (86%); mass spectrum (MALDI-TOF), *m/z* 1023.5 $(M + H)^+$ (theoretical *m/z* 1023.2).



Disaccharide-dye Conjugate 3.1. To a solution of 2.20 mg (2.60 mmol) of compound **3.41**[173] in 1 mL of anh methanol was added a freshly prepared solution of 0.4 M sodium methoxide in methanol. The reaction mixture was allowed to stir at room temperature for 3 h, and the complete consumption of starting material was confirmed by MALDI-TOF mass spectral analysis. The reaction mixture was then quenched by the addition of 500 mg of Dowex 50x resin, shaken for 15 min and filtered. To the solution of the crude product in methanol was added Pd/C and H_2 gas was bubbled through for 1 h. The complete consumption of starting material was confirmed by MALDI-TOF mass spectral analysis. The reaction mixture was filtered through Celite 545® and then concentrated under diminished pressure to afford **3.43**, which was used for the next reaction; HRMS (APCI), *m/z* 473.1986 $(M + H)^+$ ($C_{17}H_{33}N_2O_{13}$ requires *m/z* 473.1983).

To 101 μg (0.21 μmol) of **3.43** was added a solution of 106 μg (0.11 μmol) of Cy5**COOSu (Figure 3.3) in 100 μL of 0.2 M phosphate buffer and the

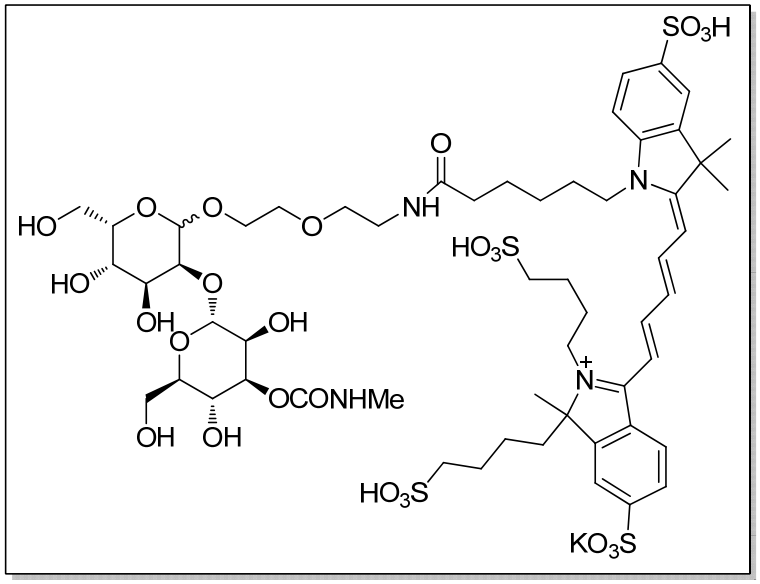
reaction mixture was stirred overnight in the dark. The reaction mixture was purified on an Alltech Alltima C_{18} reversed phase semi-preparative (250 × 10 mm, 5 μm) HPLC column using aq 0.1% TFA and CH_3CN mobile phases. A linear gradient was employed (99:1 0.1% aq TFA–CH_3CN→69:31 0.1% aq TFA–CH_3CN) over a period of 35 min at a flow rate of 4 mL/min. The fractions containing the desired product eluted at 23.5 min and were collected, frozen and lyophilized to give **3.1** as a blue solid: yield 48 μg (35% over two steps); HRMS (APCI), *m/z* 669.1883 $(M - K - 2H)^{2-}$ ($C_{55}H_{78}N_4O_{26}S_4^{2-}$ requires *m/z* 669.1899).



Disaccharide-dye Conjugate 3.2. To a solution of 4.40 mg (5.00 mmol) of compound **3.42** in 2 mL of anh methanol was added a freshly prepared solution of 0.4 M sodium methoxide in methanol. The reaction mixture was allowed to stir at room temperature for 3 h, and the complete consumption of starting material was confirmed by MALDI-TOF mass spectral analysis. The reaction mixture was then

quenched by the addition of 500 mg of Dowex 50x resin, shaken for 15 min and filtered. To the solution of the crude product in methanol was then added Pd/C and H_2 gas was bubbled through for 1 h. The complete consumption of starting material was confirmed by MALDI-TOF mass spectral analysis. The reaction mixture was filtered through Celite 545® and concentrated under diminished pressure to afford **3.44**, which was used for the next reaction; HRMS (APCI), *m/z* 487.2140 $(M + H)^+$ ($C_{18}H_{35}N_2O_{13}$ requires *m/z* 487.2139).

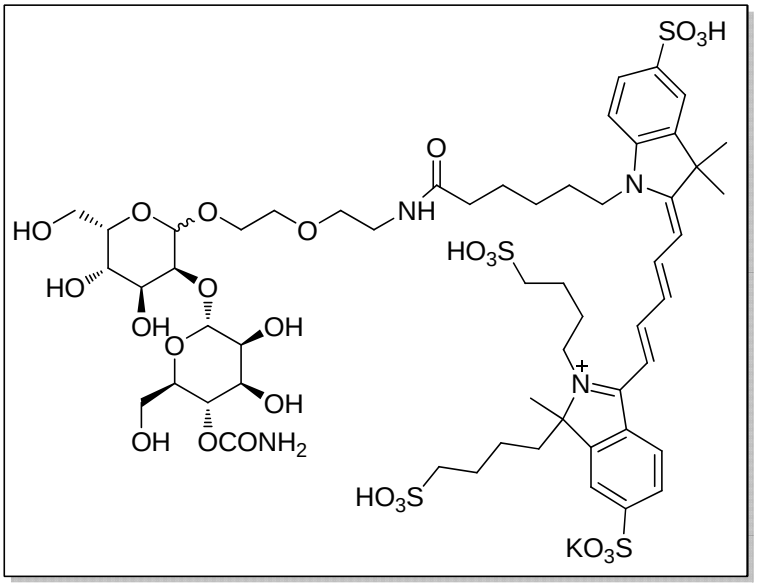
To 101 μg (0.21 μmol) of **3.44** was added a solution of 106 μg (0.11 μmol) of Cy5**COOSu (Figure 3.3) in 100 μL of 0.2 M phosphate buffer and the reaction mixture was stirred overnight in the dark. The reaction mixture was purified on an Alltech Alltima C_{18} reversed phase semi-preparative (250 × 10 mm, 5 μm) HPLC column using aq 0.1% TFA and CH_3CN mobile phases. A linear gradient was employed (99:1 0.1% aq TFA–CH_3CN→69:31 0.1% aq TFA–CH_3CN) over a period of 35 min at a flow rate of 4 mL/min. The fractions containing the desired product eluted at 23.5 min and were collected, frozen and lyophilized to give **3.2** as a blue solid: yield 53 μg (37% over two steps); HRMS (APCI), *m/z* 676.1996 $(M - K - 2H)^{2-}$ ($C_{56}H_{80}N_4O_{26}S_4^{2-}$ requires *m/z* 676.1977).



Disaccharide-dye Conjugate 3.3. To a solution of 5.80 mg (6.60 mmol) of compound **3.49** in 2 mL of anh methanol was added a freshly prepared solution of 0.4 M sodium methoxide in methanol. The reaction mixture was allowed to stir at room temperature for 3 h, and the complete consumption of starting material was confirmed by MALDI-TOF mass spectral analysis. The reaction mixture was then quenched by the addition of 500 mg of Dowex 50x resin, shaken for 15 min and filtered. To the solution of the crude product in methanol was added Pd/C and H_2 gas was bubbled through for 1 h. The complete consumption of starting material was confirmed by MALDI-TOF mass spectral analysis. The reaction mixture was filtered through Celite 545® and then concentrated under diminished pressure to afford **3.50**, which was used for the next reaction. HRMS (APCI), *m/z* 487.2133 $(M + H)^+$ ($C_{18}H_{35}N_2O_{13}$ requires *m/z* 487.2139).

To 87.0 μg (0.18 μmol) of **3.50** was added a solution of 90.0 μg (0.09 μmol) of Cy5**COOSu (Figure 3.3) in 150 μL of 0.2 M phosphate buffer and the

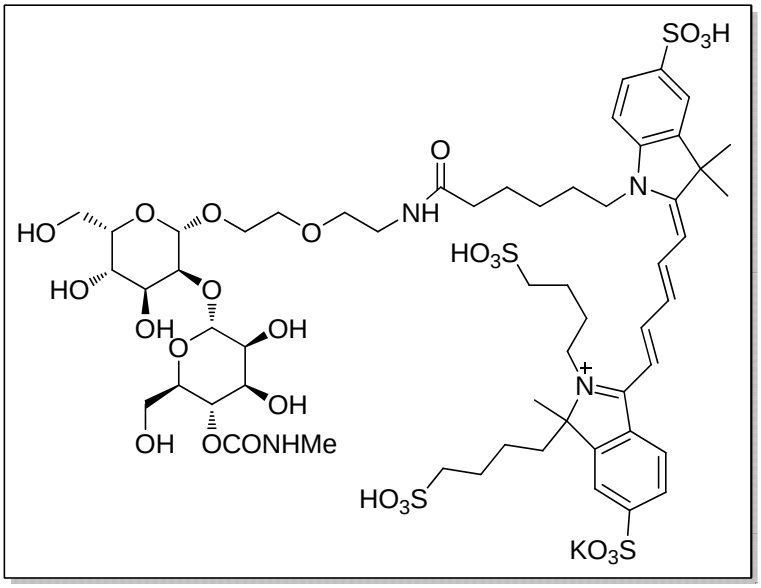
reaction mixture was stirred overnight in the dark. The reaction mixture was purified on an Alltech Alltima C_{18} reversed phase semi-preparative (250 × 10 mm, 5 μm) HPLC column using aq 0.1% TFA and CH_3CN mobile phases. A linear gradient was employed (99:1 0.1% aq TFA–CH_3CN→69:31 0.1% aq TFA–CH_3CN) over a period of 35 min at a flow rate of 4 mL/min. The fractions containing the desired product eluted at 23.9 min and were collected, frozen and lyophilized to give **3.3** as a blue solid: yield 27 μg (23% over two steps); HRMS (APCI), *m/z* 676.1984 $(M - K - 2H)^{2-}$ ($C_{56}H_{80}N_4O_{26}S_4^{2-}$ requires *m/z* 676.1977).



Disaccharide-dye Conjugate 3.4. To a solution containing 2.20 mg (2.56 mmol) of compound **3.55**[173] in 1 mL of anh methanol was added a freshly prepared solution of 0.4 M sodium methoxide in methanol. The reaction mixture was allowed to stir at room temperature for 3 h, and the complete consumption of starting material was confirmed by MALDI-TOF mass spectral analysis. The

reaction mixture was then quenched by the addition of 500 mg of Dowex 50x resin, shaken for 15 min and filtered. To the solution of the crude product in methanol was then added Pd/C and H_2 gas was bubbled through for 1 h. The complete consumption of starting material was confirmed by MALDI-TOF mass spectral analysis. The reaction mixture was filtered through Celite 545® and then concentrated under diminished pressure to afford **3.57**, which was used for the next reaction; HRMS (APCI), *m/z* 473.1972 $(M + H)^+$ ($C_{17}H_{33}N_2O_{13}$ requires *m/z* 473.1983).

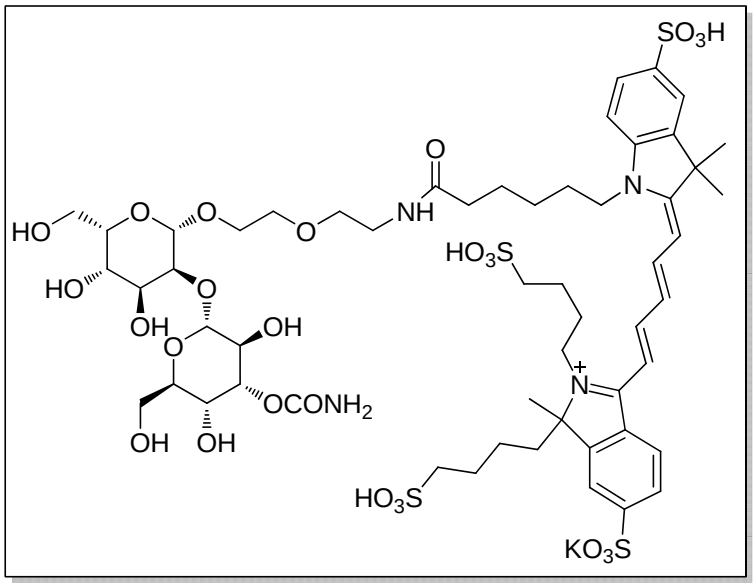
To 101 μg (0.21 μmol) of **3.57** was added a solution of 106 μg (0.11 μmol) of Cy5**COOSu (Figure 3.3) in 100 μL of 0.2 M phosphate buffer and the reaction mixture was stirred overnight in the dark. The reaction mixture was purified on an Alltech Alltima C_{18} reversed phase semi-preparative (250 × 10 mm, 5 μm) HPLC column using aq 0.1% TFA and CH_3CN mobile phases. A linear gradient was employed (99:1 0.1% aq TFA–CH_3CN→69:31 0.1% aq TFA–CH_3CN) over a period of 35 min at a flow rate of 4 mL/min. The fractions containing the desired product eluted at 23.5 min and were collected, frozen and lyophilized to give **3.4** as a blue solid: yield 44 μg (32% over two steps); HRMS (APCI), *m/z* 669.1880 $(M - K - 2H)^{2-}$ ($C_{55}H_{78}N_4O_{26}S_4^{2-}$ requires *m/z* 669.1899).



Disaccharide-Dye Conjugate 3.5. To a solution containing 2.70 mg (3.10 mmol) of **3.56** in 2 mL of anh methanol was added a freshly prepared solution of 0.4 M sodium methoxide in methanol. The reaction mixture was allowed to stir at room temperature for 3 h, and the complete consumption of starting material was confirmed by MALDI-TOF mass spectral analysis. The reaction mixture was then quenched by the addition of 500 mg of Dowex 50x resin, shaken for 15 min and filtered. To the solution of the crude product in methanol was added Pd/C and H_2 gas was bubbled through for 1 h. The complete consumption of starting material was confirmed by MALDI-TOF mass spectral analysis. The reaction mixture was filtered through Celite 545® and concentrated under diminished pressure to afford **3.58**, which was used for the next reaction; HRMS (APCI), *m/z* 487.2153 $(M + H)^+$ ($C_{18}H_{35}N_2O_{13}$ requires *m/z* 487.2139).

To 134 μg (0.27 μmol) of **3.58** was added a solution of 90.0 μg (0.09 μmol) of Cy5**COOSu (Figure 3.3) in 150 μL of 0.2 M phosphate buffer and the

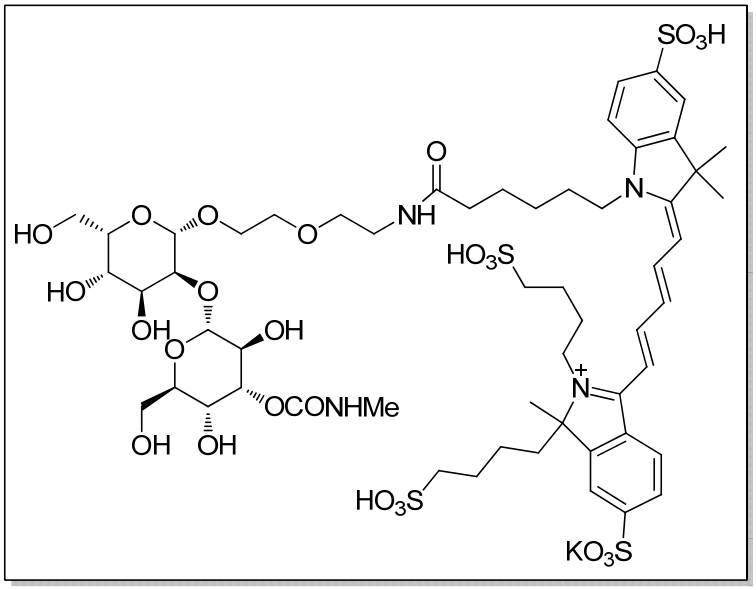
reaction mixture was stirred overnight in the dark. The reaction mixture was purified on an Alltech Alltima C_{18} reversed phase semi-preparative (250 × 10 mm, 5 μm) HPLC column using aq 0.1% TFA and CH_3CN mobile phases. A linear gradient was employed (99:1 0.1% aq TFA–CH_3CN→69:31 0.1% aq TFA–CH_3CN) over a period of 35 min at a flow rate of 4 mL/min. The fractions containing the desired product eluted at 24.8 min and were collected, frozen and lyophilized to give **3.5** as a blue solid: yield 60 μg (33% over two steps); HRMS (APCI), *m/z* 676.1995 $(M - K - 2H)^{2-}$ ($C_{56}H_{80}N_4O_{26}S_4^{2-}$ requires *m/z* 676.1977).



Disaccharide-Dye Conjugate 3.6. To a solution containing 2.40 mg (2.80 mmol) of compound **3.65** in 2 mL of anh methanol was added a freshly prepared solution of 0.4 M sodium methoxide in methanol. The reaction mixture was allowed to stir at room temperature for 3 h, and the complete consumption of starting material was confirmed by MALDI-TOF mass spectral analysis. The reaction mixture was

then quenched by the addition of 500 mg of Dowex 50x resin, shaken for 15 min and filtered. To the solution of the crude product in methanol was then added Pd/C and H_2 gas was bubbled through for 1 h. The complete consumption of starting material was confirmed by MALDI-TOF mass spectral analysis. The reaction was filtered through Celite 545® and then concentrated under diminished pressure to afford **3.67**, which was used for the next reaction. HRMS (APCI), *m/z* 473.1978 $(M + H)^+$ ($C_{17}H_{33}N_2O_{13}$ requires *m/z* 473.1983).

To 87.0 μg (0.18 μmol) of **3.67** was added a solution of 90.0 μg (0.09 μmol) of Cy5**COOSu (Figure 3.3) in 150 μL of 0.2 M phosphate buffer and the reaction mixture was stirred overnight in the dark. The reaction mixture was purified on an Alltech Alltima C_{18} reversed phase semi-preparative (250 × 10 mm, 5 μm) HPLC column using aq 0.1% TFA and CH_3CN mobile phases. A linear gradient was employed (99:1 0.1% aq TFA–CH_3CN→69:31 0.1% aq TFA–CH_3CN) over a period of 35 min at a flow rate of 4 mL/min. The fractions containing the desired product eluted at 23.5 min and were collected, frozen and lyophilized to give **3.6** as a blue solid: yield 39 μg (33% over two steps); HRMS (APCI), *m/z* 669.1916 $(M - K - 2H)^{2-}$ ($C_{55}H_{78}N_4O_{26}S_4^{2-}$ requires *m/z* 669.1899).



Disaccharide-Dye Conjugate 3.7. To a solution containing 1.00 mg (1.10 mmol) of compound **3.66** in 2 mL of anh methanol was added a freshly prepared solution of 0.4 M sodium methoxide in methanol. The reaction mixture was allowed to stir at room temperature for 3 h, and the complete consumption of starting material was confirmed by MALDI-TOF mass spectral analysis. The reaction mixture was then quenched by the addition of 300 mg of Dowex 50x resin, shaken for 15 min and filtered. To the solution of the crude product in methanol was added Pd/C and H_2 gas was bubbled through for 1 h. The complete consumption of starting material was confirmed by MALDI-TOF mass spectral analysis. The reaction mixture was filtered through Celite 545® and then concentrated under diminished pressure to afford **3.68**, which was used for the next reaction. HRMS (APCI), *m/z* 487.2143 $(M + H)^+$ ($C_{18}H_{35}N_2O_{13}$ requires *m/z* 487.2139).

To 87.0 μg (0.18 μmol) of **3.68** was added a solution of 90.0 μg (0.09 μmol) of Cy5**COOSu (Figure 3.3) in 150 μL of 0.2 M phosphate buffer and the

reaction mixture was stirred overnight in the dark. The reaction mixture was purified on an Alltech Alltima C_{18} reversed phase semi-preparative (250 × 10 mm, 5 μm) HPLC column using aq 0.1% TFA and CH_3CN mobile phases. A linear gradient was employed (99:1 0.1% aq TFA–CH_3CN→69:31 0.1% aq TFA–CH_3CN) over a period of 35 min at a flow rate of 4 mL/min. The fractions containing the desired product eluted at 24.7 and were collected, frozen and lyophilized to give **3.7** as a blue solid: yield 57 μg (48% over two steps); HRMS (APCI), *m/z* 676.1967 $(M - K - 2H)^{2-}$ ($C_{56}H_{80}N_4O_{26}S_4^{2-}$ requires *m/z* 676.1977).

Quantification of the binding/uptake by fluorescence microscopy

A549 lung carcinoma cells and WI-38 normal lung cells were grown in RPMI 1640 (Gibco) supplemented with 10% fetal bovine serum (Hyclone) and 1% pencillin-streptomycin mix antibiotic supplements (Cellgro). SW480 colon carcinoma cells and CCD-112CoN normal colon cells were grown in MEDM (Gibco) supplemented with 10% fetal bovine serum (Hyclone) and 1% pencillin-streptomycin mix antibiotic supplements (Cellgro). Fluorescence images were obtained using a Zeiss Axiovert 200M inverted microscope fitted with an AxioCam MRm camera equipped with a 300-w xenon lamp, ET-CY5 and CY7 cyanine filter. Adherent cancer cells were grown on 16-well glass chamber slide. Cells were rinsed with phosphate buffered saline when the cell confluence was about 70%, then the media was replaced with RPMI 1640 (no phenol red). The reporter molecules were subsequently added to afford the final desired concentration (25 μM). The cells were incubated at 37 °C for 1 h, washed with

phosphate buffered saline and then fixed with 4% paraformaldehyde at 37 °C for 5 min. The slide was then mounted with Prolong Antifade Gold reagent (Invitrogen) and covered with glass coverslip and then dried for 1 h before microscope imaging analysis. For comparative studies, the exposure time and laser intensity were kept identical for accurate intensity measurements. Pixel intensity was quantified using AxioVision Release 4.7 version software, and the mean pixel intensity was generated as gray level. Cell lines were maintained at 37 °C under a humidified atmosphere of 5% CO_2 and 95% air.

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