UNIVERSITY OF CAPE TOWN

“Synthesis of side-chain-modified mycothiol analogues incorporating carbazole quinones, and evaluation as inhibitors of enzymes in the *Mycobacteria*”

By

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DEDICATION

I would like to dedicate this work to my beloved wife ***Yollande Ngalula Tshilumba*** *and mother* ***Mrs Marie Jeanne Ngwanda Diansobo.***

DECLARATION

I declare that **"Synthesis of side-chain-modified mycothiol analogues incorporating carbazole quinones, and evaluation as inhibitors of enzymes in the *Mycobacteria*"** is my own work and that all sources that I have used or quoted have been indicated and acknowledged by means of complete references.

DENIS NGUMBU MUHUNGA

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ABSTRACT

Mycothiol is found only in *Actinobacteria* including *M. tuberculosis*, and appears to play an important role in the bacterium's defence against xenobiotics and oxidative stress. The biosynthetic pathway to this pseudo-disaccharide has been elucidated and a range of mycothiol-dependent enzymes have been identified. Compounds **A.3**, which have naphthoquinonyl units tethered to the pseudodisaccharide core, have significant inhibitory activity against mshB and mca in the biosynthetic pathway. These findings, together with the independent discovery of anti-TB activity associated with carbazole quinones such as **B**, led to the design and synthesis of a new class of hybrid molecules **C** which have the carbazole quinone *motif* tethered to the pseudodisaccharide core. The synthesis of these hybrid molecules involved initial preparation of phenylthio- or methyl-glucosides having either an amine or a suitably-functionalized carbon side-chain at C-2. Carbazole-quinones **37** and **39**, bearing carboxyalkyl substituents, were prepared using Knölker methodology, and coupled to phenylthio-2-amino-glucoside **36** to generate, after deprotection, the desired hybrid structures **38** and **40.** The preferred strategy for preparing 2-*C*-alkylglucosides was via initial preparation of a 2-C-carboxymethyl glucoside **44** from D-glucal, and conversion of this to the Weinreb amide **45** for intended coupling with Grignard and other organometallic reagents to form ketones. The Weinreb amide was shown to only react with simple metal-aryl reagents, and but not with those derived from the required tri-substituted benzenes. In the exploration of alternative strategies, the side-chain in **44** was successfully converted to either a 2-C-vinyl or the corresponding *(E)*-vinyl bromide side-chain, but both proved unreactive in attempts at Pd-mediated cross-coupling reactions to further develop the side-chain. Compound **44** could also be readily converted to either a 2-*C*-haloethyl or 2-*C*-aminoethyl derivative: the former resisted conversion to Wittig reagents, while the latter was unreactive in attempted Buchwald-Hartwig amination but successfully underwent reductive amination with a substituted benzaldehyde to give an advanced precursor of the target hybrid molecules. Compounds **38** and **40** together with similar analogues prepared in parallel with this study showed significant inhibitory activity against mshB, allowing for a preliminary SAR conclusion that the inhibitory activity is dependent on the covalent association of the glycoside and the carbazole-quinone, the point of attachment of the sugar unit to the carbazole quinone, and the length of the tether.

ABBREVIATIONS

LIST OF ABBREVIATIONS

Ac	Acetyl
AIDS	Acquired Immunodeficiency Syndrome
aq.	Aqueous
Ar	Aromatic
Asp	Asparagine
br	Broad
CAN	Ceric ammonium nitrate
cat.	Catalytic
d	doublet (s)
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	1,3-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DIEA	*N*,*N*-Diisopropylethylamine
DMAP	*N,N*-Dimethylaminopyridine
DMSO	Dimethylsulfoxide
DTNB	5,5'-dithiobis-(2-nitrobenzoic acid)
EDC	Ethylene dichloride
EI	Electron impact
ES	Electro-spray
eq.	Equivalent
g	Grams
His	Histidine
HIV	Human Immunodeficiency Virus
HOBt	1-hydroxybenzotriazole

Hr	Hour
HRMS	High-resolution mass spectrometry
Hz	Hertz
IR	Infrared
J	Coupling constant
LAH	Lithium aluminium hydride
Lit	Literature
m	Meta
m	Multiplet
M^+	Molecular ion
mg	Milligram(s)
MHz	Megahertz
MIC	Minimum Inhibition concentration
min	Minute(s)
mL	Millilitre(s)
mmol	Millimole(s)
Mp	Melting point
m/z	Mass to charge ratio
NMR	Nuclear magnetic resonance
O	Ortho
p	Para
PIDA	Phenyliodine(III) Diacetate
Pet-ether	Petroleum ether
t-RNA	Transfer ribonucleic acid
rt	Room temperature
s	Singlet (s)
S_N2	Bimolecular nucleophilic substitution
t	Triplet (S)

TBAF	Tetrabutyl ammonium fluoride
TBAI	Tetrabutyl ammonium iodide
td	Triplet of doublets
TEA	Triethyl amine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TMED	Tetramethylethylenediamine
TIPSCl	Triisopropylsilyl chloride
TLC	Thin layer chromatography
q	Quartet (s)
UV	Ultra violet
WHO	World Health Organisation
X	Halide(s)
δ	Chemical shift in ppm

CONFERENCE PRESENTATIONS

Denis Ngumbu Muhunga, Gammon, D.W., "Stereoselective synthesis of 2-C-alkylglucosides, potential inhibitors of mycobacterial MshB and related enzymes", *Bio-National Organic Chemistry Conference*, 10th Frank Warren Conference, September 14-19, 2008, Berg-en-Dal, Kruger National Park, South Africa. Poster presentation.

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CHAPTER 1

INTRODUCTION

1.1. Tuberculosis

Tuberculosis (TB) is a common and often deadly infectious disease caused by the bacillus *Mycobacterium tuberculosis* (*Mtb*), it threatens the lives of millions of people, and has been ranked as the second leading cause of death after the human immunodeficiency virus (HIV) worldwide.[1] In many cases, TB attacks the lungs (pulmonary TB), but can also affect other parts of the body.[1] The infection is spread *via* air droplets released when people who have active TB (pulmonary TB) cough, sneeze, spit or talk. TB infection can be either active or latent and the following symptoms are often present in actively infected people: fever, chest pain, weight loss, fatigue, night sweats, cough, shortness of breath and a prolonged cough producing sputum. These symptoms are potentially contagious because they are caused by actively replicating tubercle bacilli in the lungs. However, no symptoms or evidence of disease can be seen in people with latent infection. It remains risky to everyone since the infection can be reactivated during their lifetime. There is a probability that *Mtb* infected people will develop TB disease but this probability is much higher with people co-infected with HIV.[1] *Mtb* is an aerobic, non-motile bacillus, and has a slow rate of division (16 to 20 hours) compared to other bacteria such as *Escherichia coli* that can divide roughly every 20 minutes.[2] A characteristic of the cell surface of *Mtb*, is the waxy coating (mycolic acid) which makes the cells impervious to Gram staining because of the high lipid content in its cells.[3] Hence, Ziehl-Neelsen staining or acid-fast staining techniques detections are used. Due to the lack of outer cell membrane, the *Mtb* is classified as acid-fast Gram positive bacteria, when the mycobacteria do not retain the crystal violet stain.[3]

1.1.1. Global incidence

TB is one of the world's deadliest diseases and more than 2 billion people (are estimated to be infected with *Mtb*.[4] According to World Health Organization (WHO) studies in 2012, there were an estimated 8.6 million TB cases and ~1.3 million people died from the disease, including among the latter 940 000 who were HIV-negative and 320 000 who were HIV-positive.[1]

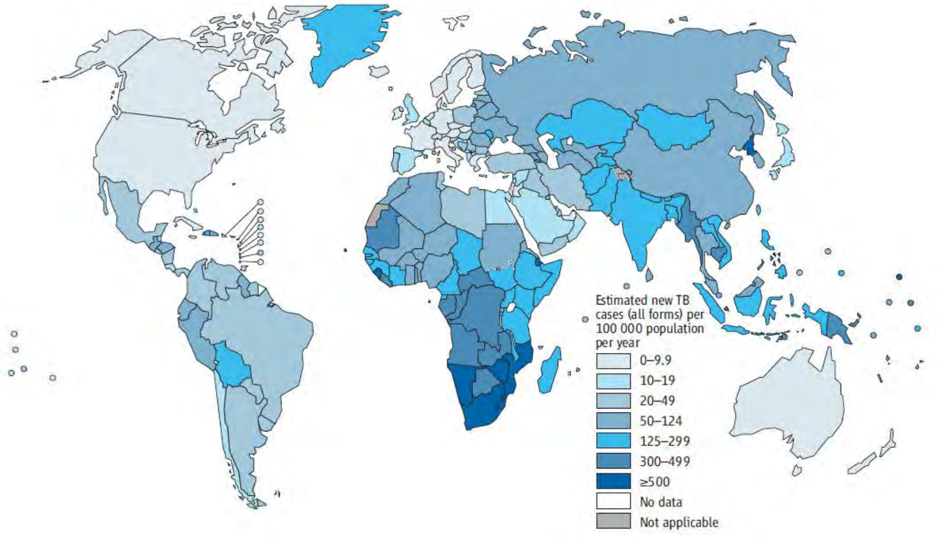


Figure 1.1. Estimate TB incidence rate, 2012.[1]

1.1.2. HIV and Tuberculosis

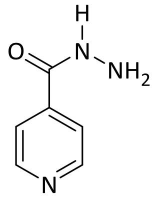
HIV is the most important risk factor for developing TB in patients already infected with *M. tuberculosis*.[4,5,6] People living with HIV are more exposed than others to be infected with TB. In 2012, it was observed that among TB patients with HIV test results 13% were HIV positive worldwide.[6] Consequently, HIV and TB can work together and shorten lifespan if TB is found untreated. Moreover, the development of the TB disease in a person living with both HIV infection and untreated latent TB infection is higher than someone without HIV infection. On the other hand, a person who has both HIV infection and TB disease has an AIDS-defining condition,[7,8] but latent TB infection or TB disease can be effectively treated. In order to prevent TB disease and reduce the rate of death in

HIV infected patients with TB, the use of combination antiretroviral therapy (CART)[9,10] has been recommended. One of the strategies in developing countries to decrease the mortality of TB in HIV infected, is to ensure that people living with HIV are tested for TB infection, and treatments are recommended if TB test is positive because it can quickly progress to TB disease in people infected with HIV since the immune system is already weakened.[11] After knowing health status of HIV infected person with regard to TB, the next challenge would be to treat the infection. It has been discovered that TB disease has developed some resistance to most of the available drugs.[1]

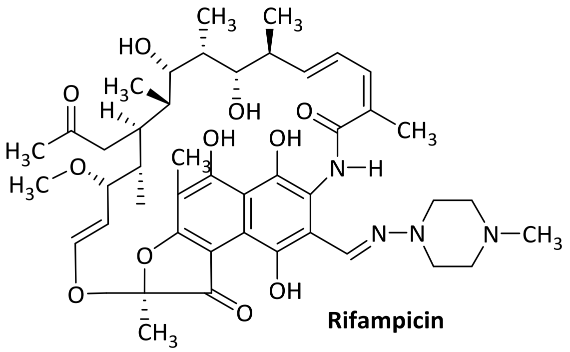
1.1.3. Drug-resistant TB

The emergence of multidrug-resistant TB (MDR-TB) to at least rifampicin (RMP) and isoniazid (INH) associated with treatment failure, complications and mortality, presents additional challenges in TB control. Globally, the WHO estimated about 3.6% of new cases and 20.2 % of previously treated cases have MDR-TB.[1] There is an increasing recognition of the importance of drug-resistant (DR-TB) at younger ages, which has led to the usage of second-line anti-TB drugs, which are less potent, more toxic, more expensive and requires a long treatment (**Figure 1.2**).[12-15] The generation of MDR-TB is a result of poor clinical and control practice of new patients with TB. The WHO revealed that a poor management and erratic use of second line drugs may lead to extensively drug-resistant (XDR-TB).[1] It was discovered that 9.6% of more than 3 million cases of MDR-TB were developing XDR-TB as a result of poor management of drug sensitive as well as drug resistant TB.[1] Recently, there has been growing concern of XDR-TB, which appears also to be threatening the global effort to control TB.[16] XRD-TB is a form of drug resistance showing resistance against rifampicin, isoniazid, a fluoroquinolone, and any of the second-line injectable drugs like Kanamycin, amikacin or capreomycin.

a) First-line anti-tuberculosis drugs

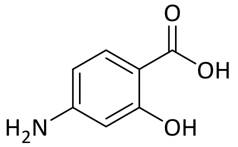


Isoniazid

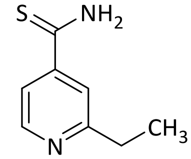


Rifampicin

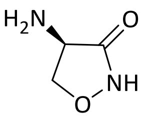
b) Second-line anti-tuberculosis drugs



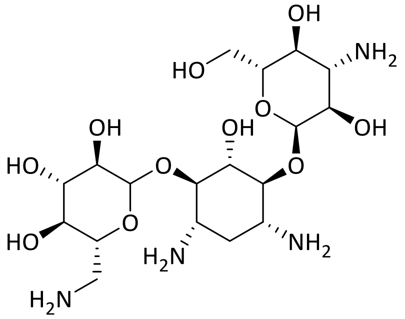
p-Aminosalicyclic acid
(Oral drugs)



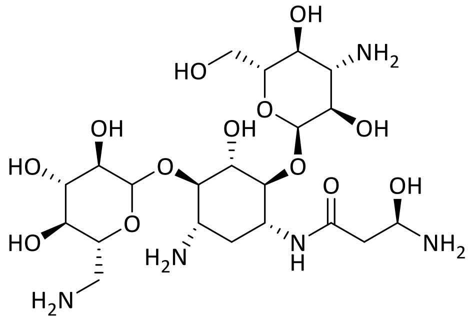
Ethionamide
(Oral drugs)



Cycloserine
(Oral drugs)



Kanamycin
(Injectable drugs)



Amikacin
(Injectable drugs)

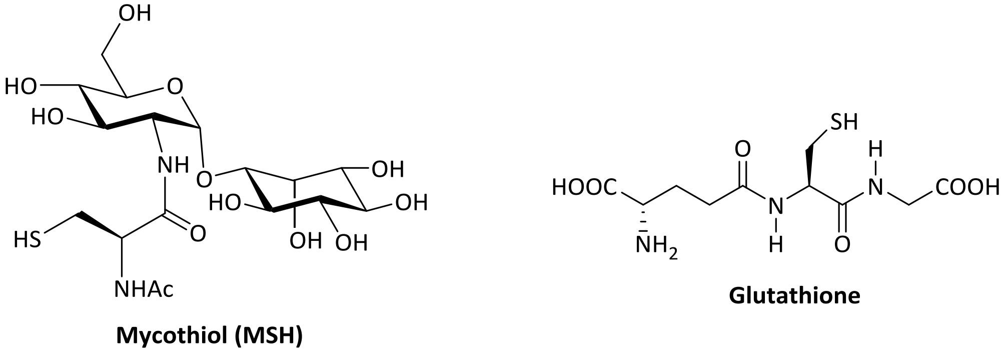
Figure 1.2. Drugs used for TB treatment.

Due to a growing resistance of the bacteria to conventional antibiotics, there is a need for new therapeutic strategies which will tackle both TB and the instances of drug resistance arising. One of the approaches has been to try to develop potential therapeutic agents that inhibit the biosynthesis of the unique defensive agents in *Mycobacterium tuberculosis*, typically low molecular weight thiols; one such target is mycothiol (MSH), an important cellular anti-oxidant.

1.2. Mycothiol

1.2.1. Introduction

Mycothiol (**MSH**) is a low molecular-mass thiol and has been identified as the major thiol in number of most actinomycetes including *Mtb*.[13] MSH is produced at significant levels in *mycobacteria* and *streptomycetes* such as *Micromonospora*, *Actinomadura*, and *Nocardiopsis spp*.[13] MSH plays a significant role in the detoxification of thiol-reactive substances, including formaldehyde in *Amycolatopsis methanolica*,[14] various electrophiles, and antibiotics. The alkylating agents are detoxified by their conversion to an S-conjugate by MSH. MSH has functional similarities with glutathione (GSH), a major low-molecular-mass thiol present in nearly all eukaryotes and in many bacteria.[17] During respiration, bacteria and the host cell internally generate toxic oxidants and reactive oxygen species (ROS) as anti-microbial response. GSH plays a role of protecting cells against these reactive species. However, actinomycetes do not have GSH but instead have MSH which appears to perform the same role. It was proven that MSH influences the susceptibility of *M. smegmatis* to antibiotics such as rifampin and isoniazid by providing growth in an oxygen-rich environment [17-20].



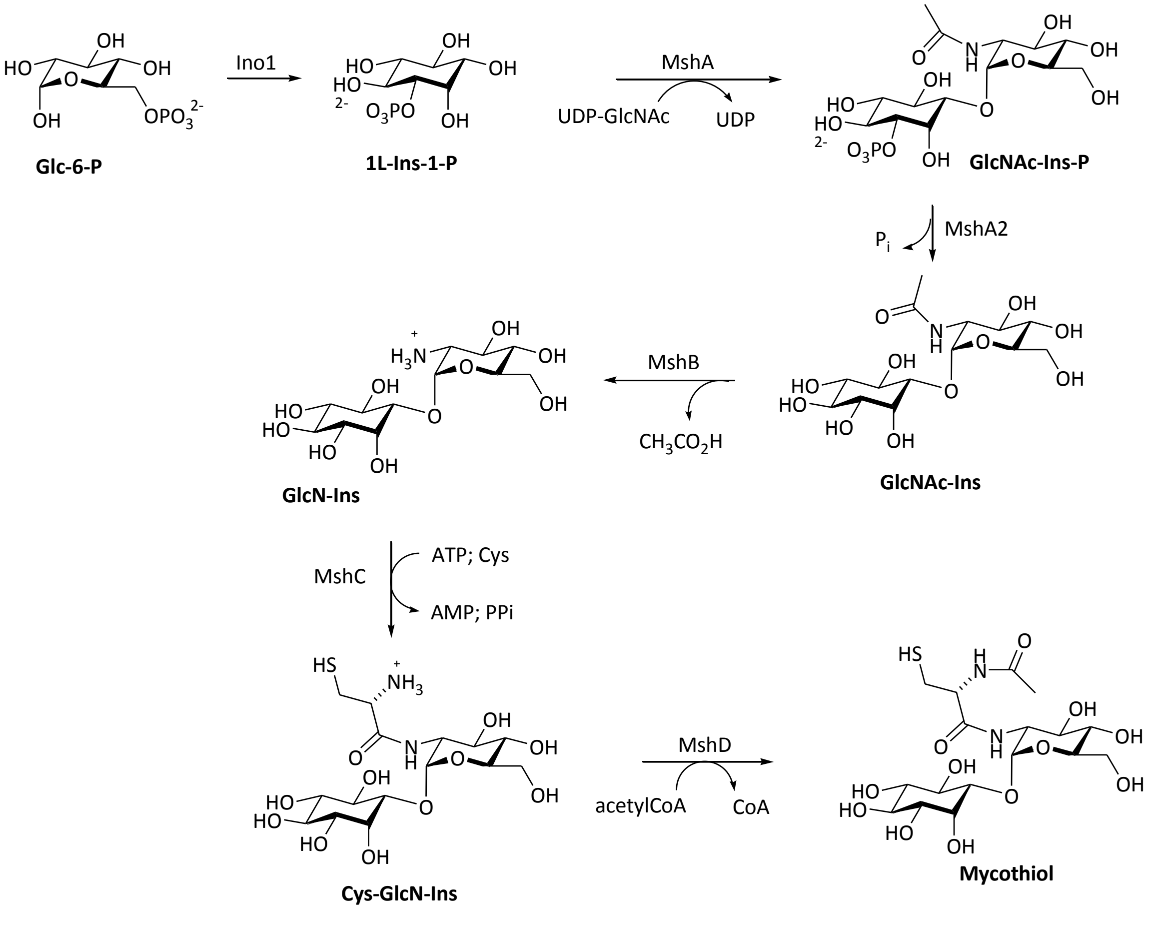
Mycothiol (MSH)

Glutathione

Figure 1.3. Chemical structure of mycothiol and glutathione.

1.2.2. Biosynthesis of Mycothiol

The biosynthesis of MSH proceeds through a five step pathway (Scheme 1.1)[13] and the initial substrates 1L-inositol-1-phosphate combines with UDP-N-acetylglucosamine to form N-acetylglucosaminylinositol phosphate, a reaction catalysed by N-acetylglucosamine transferase, MshA.[21,22] The removal of the phosphate group by the phosphatase MshA2 gives N-acetylglucosaminylinositol,[22] which is then N-deacetylated in a reaction catalysed by MshB deacetylase.[23,24] The resulting glucosaminylinositol (GlcN-Ins) is then ligated with L-cysteine in a reaction catalyzed by a ligase MshC to form cysteinylglucosaminylinositol (Cys-GlcN-Ins).[25] Acetylation of Cys-GlcN-Ins by an acetyltransferase (MshD) produces mycothiol .[26]



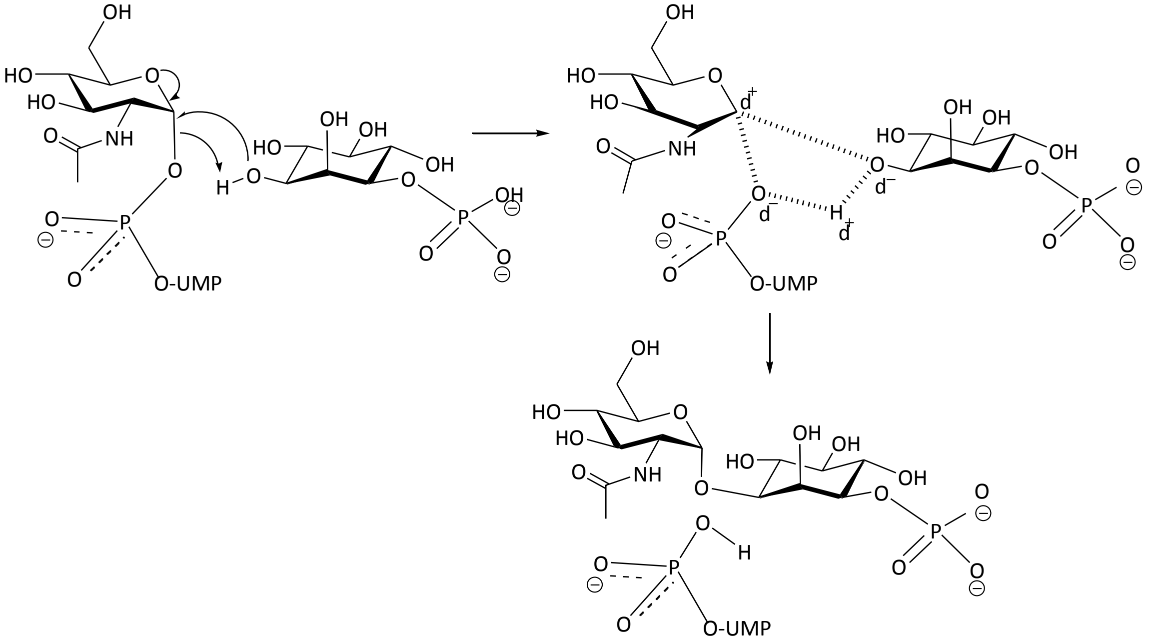
Scheme 1.1. Biosynthetic pathway for synthesis of MSH.

In *M. smegmatis*, the lack of mycothiol ligase activity affects the sensitivity of mycothiol to many alkylating agents, antibiotics and oxidative stress.[27] Thus, the enzymes involved in MSH biosynthesis appear to have potential for the development of new drugs. MshB,[23] MshC [25] and MShD[26] were identified using a variety of approaches and was found that their enzymatic activities are required for MSH production in the *M. smegmatis*; homologues of these genes were identified in the *Mtb* genomes.

A glycosyltransferase MshA (*Rv0486*) was also identified by transposon mutagenesis,[21] but the biochemical reaction catalysed was not defined. In the mycothiol biosynthesis, the MshB was the first gene to be identified, and the mycothiol *S*-conjugate amidase (Mca) was discovered and shown to have two homologs in the *M. smegmatis*. The *Rv1082* encodes for an amidase that cleaves mycothiol *S*-conjugates was found in the *M. tuberculisis* genome, while the Rv1170 was efficient catalyst for the deacetylation of GlcNAc-Ins.[23]

1.2.2.1. Mycothiol Glycosyltransferase, MshA, and Phosphatase MshA2.

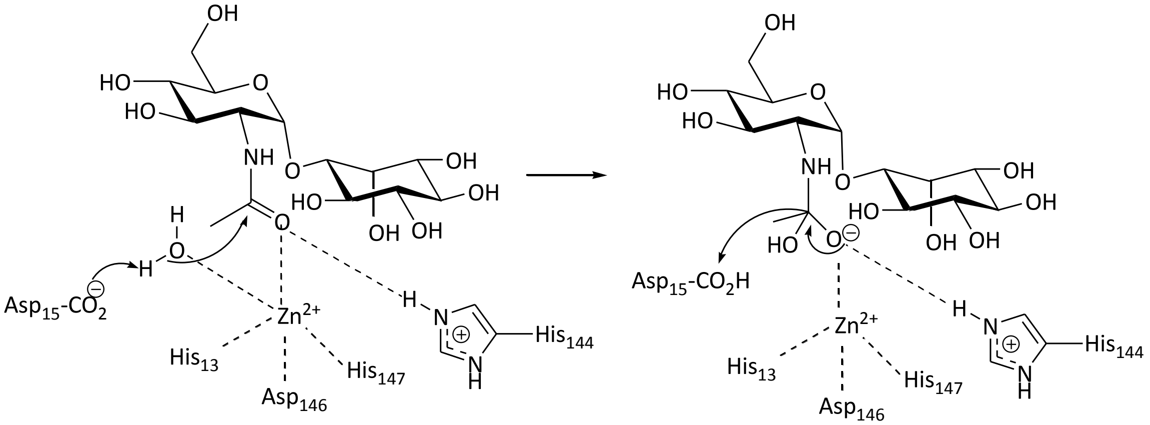
The transfer of *N*-acetylglucosamine (GlcNAc) from UDP-GlcNAc to 1-L-*myo*-inositol-1-phosphate (L-I1P) is catalysed by MshA, and its mechanism involves the formation of substantial oxocarbenium character in the transition state, which is similar to glycosidase enzymes.[28] Campell *et al*[29] identified for the first time the mshA gene Rv0486, which is required for the growth of *Mtb*[21], and essential for the production of GlcNAc-Ins in the *M. smegmatis*.[30] After the establishment of GlcN-Ins as intermediate in MSH biosynthesis, the most definitive test for substrate identity production using a cell-free extract as catalyst was provided by the assay based upon GlcN-Ins production. 1DL-Ins-1-P was identified as a strong substrate, whereas 1D-Ins-1-P as a poor substrate. This led to suggest that 1L-Ins-1-P is the GlcNAc-acceptor for MshA. Therefore, UDP-GlcNAc is an effective donor substrate for MshA, which is involved in the biosynthesis of glycolipids 1 and 2, precursors of the linkage region of the cell wall.[30] An additional enzyme, the GlcNAc-Ins-P phosphatase designated MshA2, is required to complete the mycothiol biosynthetic pathway.[31] The existence of an epimerase activity was demonstrated by Bewley *et al*[32] when the 1D-Ins-1P was served directly as an acceptor. This could lead to the wrong stereoisomer because the dephosphorylation will produce GlcNAc-(α1,1)-1L-Ins, which is inactive to MshB, whereas GlcNAc-(α1,1)-1D-Ins is fully active. The proposed mechanism suggests being the attack nucleophilic by the hydroxyl of 1-L-Ins-1-P and the departure of UDP occurs on the same face, resulting in the retention of anomeric configuration (**Scheme 1.2**).[33]



Scheme 1.2: Proposed mechanism: substrate-assisted catalysis mechanism, featuring oxycarbenium-ion like transition state, with asymmetric phosphor bond breakage, and glycosidic bond formation.

1.2.2.2. Mycothiol Deacetylase, MshB

A deacetylase (MshB, TB gene Rv1170) an enzyme involved in the biosynthesis pathway of mycothiol, moves the acetyl group from the precursor GlcNAc-Ins to yield GlcN-Ins. Steenkamp and Spies[34] were the first to identify the substrate as well as the products involved in the fourth and the fifth step of MSH biosynthesis and which was later confirmed by Anderberg *et al.*[35] MshB is a Zn^{2+} metalloprotein, and its activity is completely dependent on the presence of a divalent metal cation. The X-ray crystallographic reveals a protein that folds in a manner resembling a C-terminal domain consisting of two β-sheets and two helices and lactate dehydrogenase in the N-terminal domain.[36] The zinc binding site is in the N-terminal domain occupying a position equivalent to that of the NAD^+ co-factor of lactate dehydrogenase, and the cation Zn^{2+} is 5 coordinate with two water molecules and three residues from MshB (His-13, Asp-16, His-147) (**Scheme 1.3**).



Scheme 1.3: Proposed catalytic mechanism for MshB.

One water molecule would be displaced upon binding of substrate (GlcNAc-Ins), while the other water molecule acted as nucleophile assisted by the general base carboxylate of Asp-15. In addition to the Zn^{2+} acting an electrophilic centre in the hydrolysis, His-144 imidazole could form a hydrogen bond to the oxyanion of the tetrahedral intermediate.[36,20] Although the MshB is not essential for MSH synthesis or growth of *Mtb*, the inactivation of the mshB gene does not fully block MSH biosynthesis because the mycothiol *S*-conjugate amidase (Mca) has been shown to have a low GlcNAc-Ins deacetylase activity that enable the production of MSH in the absence of MshB. The Mca may assist in the removal of an antibiotics from the infectious bacteria by cleaving the S-conjugate formed by the reaction of MSH and an antibiotic.[13,17]

1.2.2.3. Mycothiol Ligase, MshC

The Mshc catalyses the ATP-dependent condensation of cysteine and GlcN-Ins. It became the second MSH biosynthesis gene to be identified from *M. smegmatis* protein. The mshC gene identified from the N-terminal protein sequence, and the ligase activity was demonstrated from the crude extract of *M.smegmati*.[37] The mshC gene and MshC have been shown to be essential for *the* growth[38,39] and production of MSH in *M. smegmatis*.[19] An ortholog (Rv 2130c) was found in the *Mtb* genome with 78% identity, which has been previously annoted as Cys*S*2, a second cysteinyl-tRNA synthetase gene. It was shown that the Cys*S* is the functional cysteinyl-tRNA synthetase gene of *Mtb* when its sequence was compared to that homologous CysS of the *E.coli*. The mshc (Cys*S*2) gene probably occurs from the duplication of Cys*S* gene early in the evolution of actinomycetes.[38,39] The kinetic mechanism of MshC, based on steady state kinetic as well as positional isotope exchange (PIX) studies demonstrated that enzymatic process occurs in two steps: cysteine adenylation and the subsequent ligation of cysteine to GlN-Ins. Therefore, MshC is a Bi Uni Uni Bi Ping Pong mechanism (**Figure 1.4**), with ATP binding followed by cysteine binding, release of pyrophosphate (PP_i), binding of GlcN-Ins, followed by the release of Cys-GlcN-Ins and AMP.[40,41] A stable bisubstrate analog of the adenylate (CSA), which is 5'-O-[N-(L-cysteinyl)sulfamonyl]adenosine exhibits competitive inhibition versus ATP (K_i ~ 300 nM) and non-competitive inhibition versus cysteine.[40]

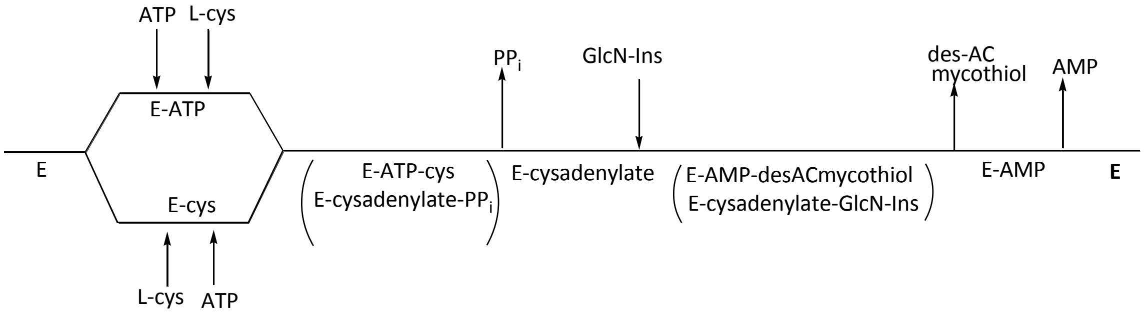


Figure 1.4. The proposed Bi Uni Uni Bi Ping Pong kinetic mechanism of MshC.

1.2.2.4. Mycothiol Acetylase, MshD.

The GCN5 acetyl-transferase, MshD,[14] catalyses the transfer of an acetyl group from acetyl-CoA to the amino group of Cys-GlcN-Ins to yield MSH. The MshD is the third MSH biosynthesis gene found and was identified in the *Mtb* as ORF Rv0819, coding for a protein of 315 amino acids.[14] The MshD has been shown to have activity in the cell extracts of *E. coli* when cloning the Rv0819 gene. In a binary complex was determined the structure of *Mtb* MshD with AcCoA and ternary complex with CoA and desacetylmycothiol (DAM) to a resolution of 1.7 and 1.8 Å, respectively.[42,43]

1.2.2.5. Mycothiol –*S*-Conjugate Amidase, Mca.

Newton *et al*[17] characterised the enzyme mycothiol-*S*-conjugate amidase (Mca). Mca cleaves the amide bond linking cysteine and glucosamine in MSH-S-conjugates to form D-glucosamine-α-(1-1)myo-D-inositol, having specificity for S-conjugates. It can be then recycled into MSH biosynthesis.[23] The Mca is key enzyme in the detoxification pathway of MSH and is unique, and has been purified from *M. smegmatis*. The *Mtb* homologue Rv1082 has been cloned and expressed in *E. coli*. As glutathione GSH, the MSH reacts with electrophile and alkylating reagents to form *S*-conjugates, which latter serve as a substrate for Mca. The process involved the hydrolysis of the cysteinyl-glucosamine bond of MSH *S*-conjugates (MSR) to produce the cysteine-*S*-conjugates (mercapturic acid) for excretion from the cell into the medium and GlcN-Ins for the intracellular regeneration of MSH (**Figure 1.5**).[17]

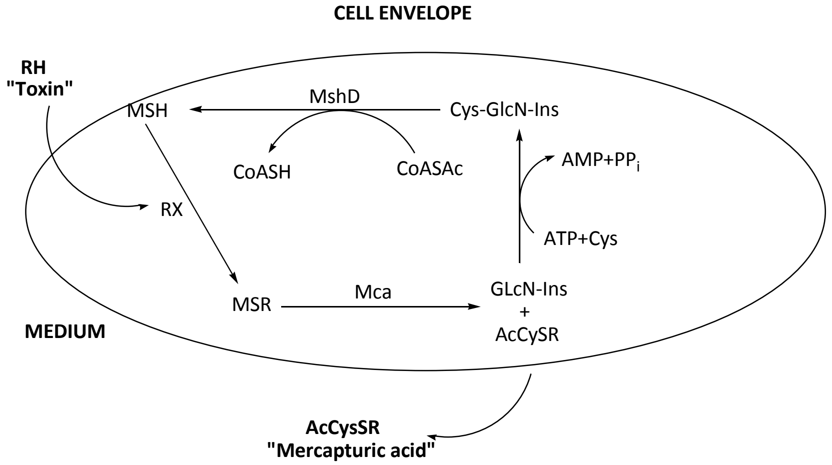
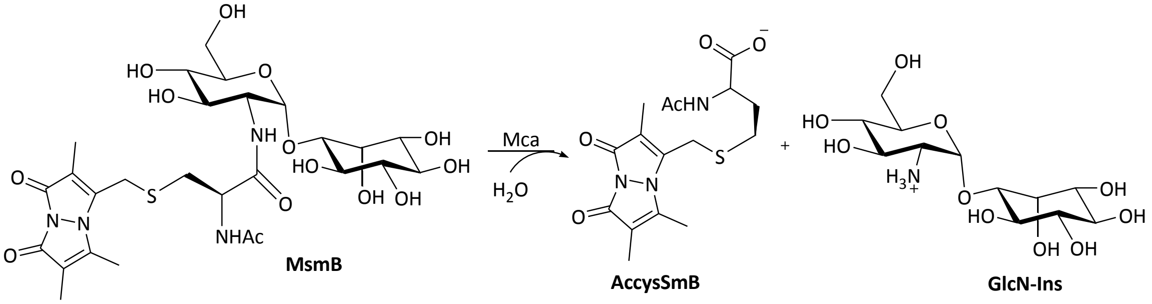


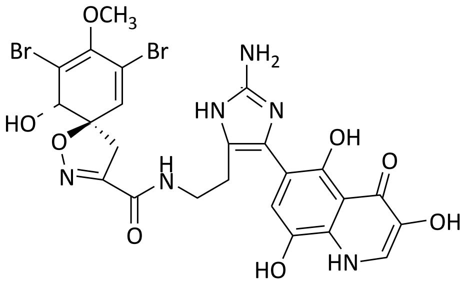
Figure 1.5. Scheme for cellular detoxification of thiol-reactive.

The pure enzyme of Mca contains Zn^{2+} and Ca^{2+} as the only divalents metals. This led to suggestion that the Mca is strongly zinc metalloenzyme since the Zn^{2+} is the metal ion associated with the activity, the common catalytic component in amidases. Fahey *et al*[44] demonstrated that the Mca exhibits high activity with 14 substrates (mycothiol-S-conjugates) while its deacetylase activity is very low and significant with GlcNAc-Ins for the production of GlcN-Ins when GlcNAc-Ins levels are high. Under physiologically relevant conditions, the mycothiol-*S*-conjugate of rifamycin showed to be a substrate for Mca in oxidized and reduced form. In addition, the mycothiol *S*-conjugate of the antibiotic cerulenin as a substrate for Mca displayed significant activity. Bewley and co-worker elucidated the conformation of mycothiol-*S*-bimane in aqueous solution by nuclear magnetic resonance.[45] Mca can be important target for the TB drugs and its activity with MSmB as substrate has been used to screen marine extracts and synthetic libraries to identify potential inhibitors. In the *M.smegmatis*, the conversion of the cellular mycothiol to its biname derivative (MsmB) was possible in the presence of the alkylating agent monobromobimane (mBBr). The cleavage of amide bond of the stable fluorescent MsmB gives GlCN-Ins and the biname derivative of N-acetylcysteine (AcCySmB), a mercuric acid will be exported from the cell into the medium.[46,47]

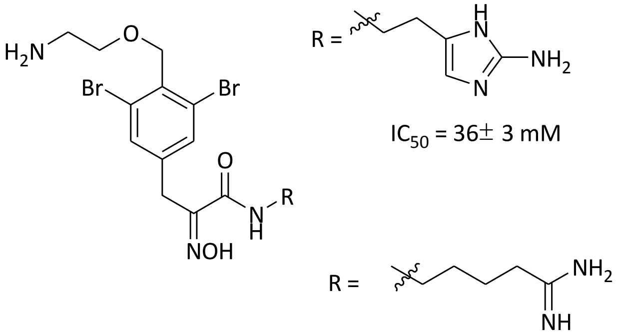


Scheme 1.4: Mca (amidase) catalyses MsmB.

A series of bromotyrosine-derived compounds, including marine natural products and members of a psammaplin A-inspired combinatorial synthetic library, were screened for their ability to inhibit the *Mtb* detoxification enzyme mycothiol-S-conjugate amidase (Mca).[46,48]

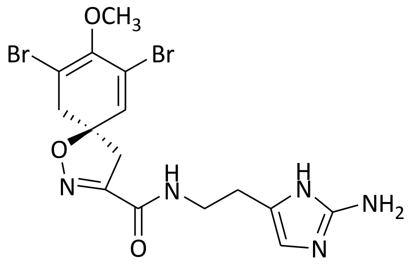


$IC_{50} = 2.2 \pm 0.2$ mM

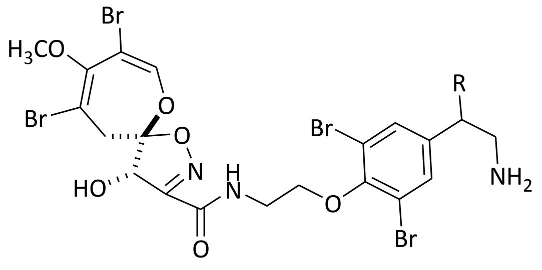


$IC_{50} = 36 \pm 3$ mM

$IC_{50} = 2.8 + 0.5$ mM

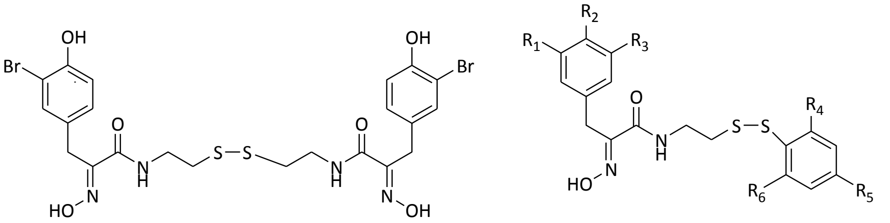


$IC_{50} = 100 \pm 21$ mM



R = H $IC_{50} = 20 \pm 11$ mM

R = OH $IC_{50} = 26 \pm 12$ mM



IC_{50} = 2.8 +0.5 mM

R_1	R_2	R_3	R_4	R_5	R_6	
Br	H	H	H	F	H	IC_{50} = 90 ± 4 mM
Br	H	H	CH_3	H	CH_3	IC_{50} = 65 ± 18 mM
Br	H	H	H	NH_2	H	IC_{50} = 185 ± 62 mM
F	H	F	H	F	H	IC_{50} = 2720 ± 640 mM
F	H	F	H	NH_2	H	IC_{50} = 450 ± 173 mM
Cl	OH	H	H	F	H	IC_{50} = 37 ± 10 mM
Cl	OH	H	H	NH_2	H	IC_{50} = 35 ± 11 mM

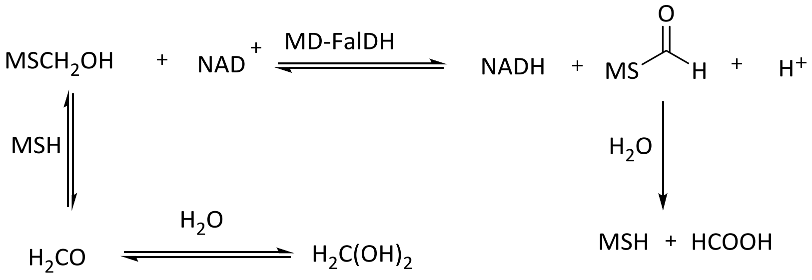
Figure 1.6. Inhibition of mycothiol-S-conjugate amidase from *Mtb* by synthetic and natural bromotyrosine-derived compounds.

1.2.2.6. Mycothiol Disulfide Reductase, Mtr

The oxidation of MSH to mycothiol disulphide (MSSM) can be achieved using another route in the mycobacteria. The disulphide reductase Mtr is the enzyme responsible for maintaining it in the reduced state. *Cole et al*[2] identified in the genome the sequence similarity of the disulphide reductase gene (mtr) to the gene encoding glutathione reductase, and was designated gor (glutathione oxido-reductase). Cloning mtr for mycothione reductase, Rv2855, and expressing in *M. smegmatis*, it has been shown to reduce the MSH of AcCys-GlcN.[49] In the *Mtb*, the pure Mtr demonstrated to be closely related to glutathione, trypanothione disulphide reductases, and it has been also shown that the enzyme is selective for MSSM and removal of the Ins residue does not affect the activity.[50,51]

1.2.2.7. Mycothiol-dependent detoxification

The MSH-dependent formaldehyde dehydrogenase, MD-FalDH, catalyses the oxidation of the thiohemiacetal of MSH with formaldehyde. The enzyme is similar to the zinc-containing medium chain alcohol dehydrogenase and was the first enzyme of metabolism to be identified.[14,52] As expected, the *S*-formylmycothiol was formed and subsequently hydrolysed by a hydrolase to produce formic acid and MSH (**Scheme 1.5**). This pathway is accepted by analogy with the GSH-dependent pathway. In the higher organism, the enzyme GSH-dependent formaldehyde dehydrogenase is responsible for the detoxification of small amounts of formaldehyde produced during metabolism of various C_1 donors.[53,54] Duine proposed an alternative path involving oxidation to the carbonate ester followed by hydrolysis to release MSH and CO_2.[54]



Scheme 1.5: Proposed scheme for the action of mycothiol-dependent formaldehyde dehydrogenase (MD-FalDH).

1.3. A potent antitubercular target.

Mycothiol has anti-oxidant activity to protect the *M.tuberculosis* against various harmful compounds as well as the ability to detoxify a variety of toxic compounds. The sensitivity of *M.smegmatis* to antibiotics such as rifampin and isoniazid is also influenced by MSH. Because of these, MSH is a candidate for protecting *M.tuberculosis* from inactivation by the host during infection as well as for resisting anti-tubercular drugs. There is a strong need for the establishment of new chemo preventives and the discovery of new drugs. So, targeting MSH can achieve *M.tuberculosis* inhibitory activity constituting mycothiol as an attractive and effective antitubercular target.

1.4. Aims and Objectives

The aim of this project concern the design and synthesis of a range of "hybrid" molecules as potential inhibitors of enzymes implicated in the biosynthesis of mycothiol in the *Mycobacteria*, including *M.tuberculosis*, the causative agent for TB. We will attempt the construction of molecules which combine features of the structure of mycothiol itself, and in particular its glucosamine unit, with heterocyclic compounds in the class of carbazole quinones, which have some demonstrated anti-TB activity. The particular challenge is how to connect these two structures through an appropriate linker, which provides a metabolically stable mimic of the amide bond which links the cysteine unit to glucosamine in mycothiol. The activity of these against a range of enzymes associated with the biosynthesis and role of mycothiol will be determined. Then, the structure–activity relationship of compounds will be evaluated.

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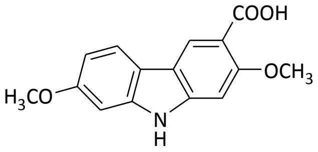
CHAPTER 2

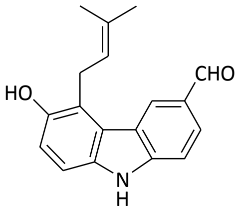
RESULTS AND DISCUSSION

2.1. Target molecules.

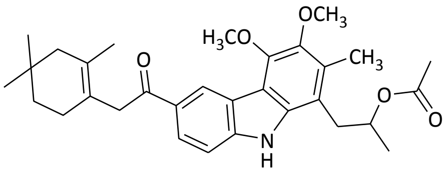
This study was prompted by reports of a series of carbazoles isolated independently from several sources and found to have antitubercular activity.[1-3] **A.1.1** exhibited weak activity (MIC of 100 μg.mL^{-1}) against *M. tuberculosis* strain $H_{37}R_v$[1-7] while **A.1.2** isolated from the stem bark of *Micromelum hirsutum* was found to have an MIC of 31.5 μg.mL^{-1} against *M. tuberculosis* $H_{37}Rv$ and the **A.1.3** (**Figure 2.1**) was most active with an MIC values of 4.0 μg.mL^{-1} (8 μM) against *M. tuberculosis* and was relatively nontoxic for the mammalian cell line.[8,9]



A.1.1



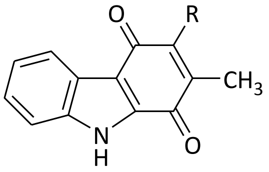
A.1.2



A.1.3

Figure 2.1. Natural occurring carbazole alkaloids with anti-TB activity.

Our research was informed by two independent observations. Firstly, in a study of some synthetic carbazole-quinones, the carbazole-1,4-quinone **A.2.2** showed poor anti-TB activity with an MIC of 117 μM, while carbazole-1,4-quinone **A.2.1** exhibited the highest activity among all carbazole quinones evaluated with an MIC of 2.2 μg.mL^{-1} (9.0 μM) against *M. tuberculosis* and was also relativeely nontoxic for the mammalian cell line.[8]

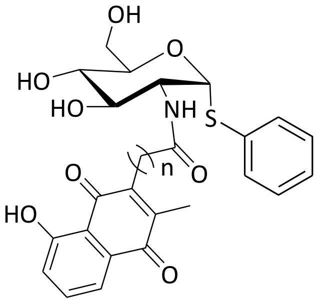


R= C_7H_{15} **(A.2.1)**

R= OCH_3 **(A.2.2)**

Figure 2.2. Chemical structure of carbazole-1,4-quinone with anti-tubercular activity.

The second observation related to the fact that several synthetic routes to mycothiol and its analogues have been developed,[10,11] and recent work[12] suggested that compounds **A.3**, which have naphthoquinonyl units tethered to the glycoside core, have significant inhibitory activity against mshB and mca in the biosynthetic pathway (**See table 1**).



n = 2 -5

A.3

Figure 2.3. Naphtoquinones having anti-TB activity.

It was found that the inhibition of this series of naphthoquinones was competitive and varies with the length of the spacer, with the highest inhibitory activity associated with the naphthoquinone bearing the longest spacer (n=5). A later computational study postulated that these compounds adopt preferred conformations where the side-chain naphthoquinonyl units are not extended but rather curled towards, and in hydrophobic interactions with the phenyl group in the aglycone.[12] In the light of these findings, it seemed reasonable to investigate replacement of the naphthoquinonyl units with the carbazole-1,4-quinone analogues and to explore the extent to which this modifies the anti-TB effect of carbazole quinones such as **B**[13,14] and enzyme-inhibitory activity of the conjugates **C.**

Table 1. Inhibition of Mca and MshB by substituted naphthoquinones analogues of mycothiol.

Substrate	Mca	MshB
1a	**28.8**	**57.4**
1b	**37.8**	**81.6**
1c	**23.2**	**81.4**
1d	**44.5**	**94.8**

A related objective was to develop synthetic routes to analogues of **C** such as **D** (**Figure 2.4**) in which the amide NH in the acylglucosamine unit has been replaced by a methylene group, or **E** in which the amide has been replaced by a simple alkyl unit, or **F** in which the 2-amino group of the glucose unit has been replaced by an amino-alkyl substituent incorporated into the tether, with **D**, **E** and **F** having non-hydrolysable tethers. These compounds could potentially serve as inhibitors of MshB and their anti-mycobacterial activity could be compared to the parent carbazole quinones.

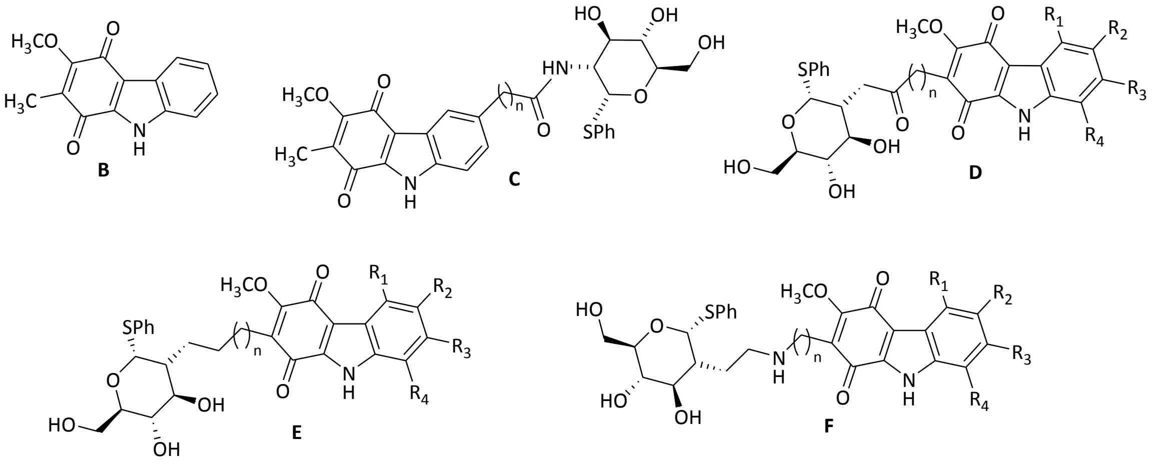
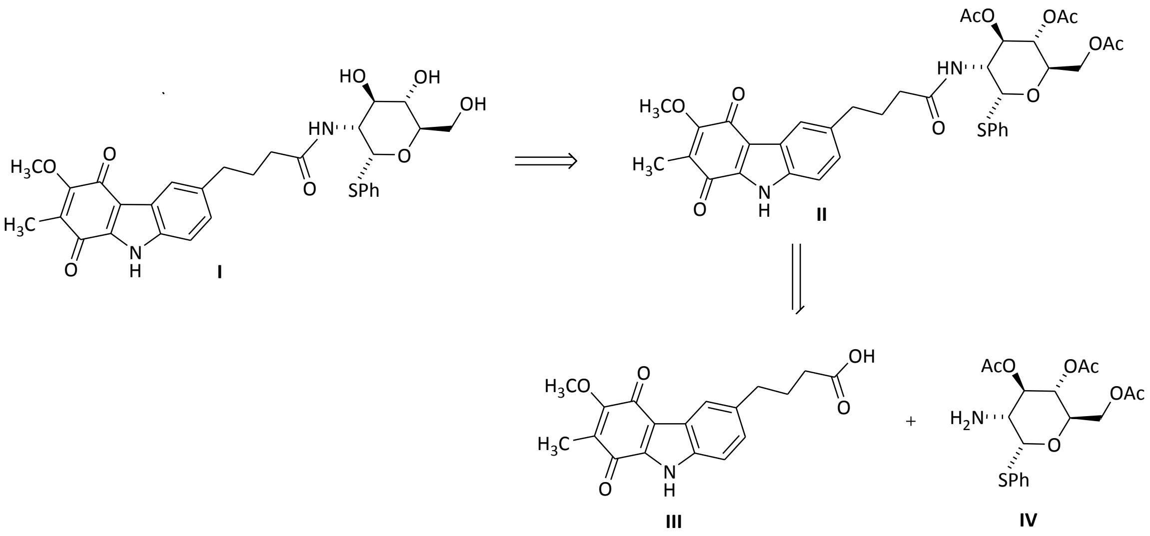


Figure 2.4. Proposed target molecules.

2.2. Results and discussion.

There were a number of important considerations with regard to the synthetic approach to the target hybrid compounds. Firstly, it was noted that the mode of action of the anti-TB carbazole quinones is not known, and there is therefore little information on what the optimum point of attachment to the tether might be. Secondly, the first generation of naphthoquinone-containing hybrids was also not studied with respect to optimizing the point of attachment of the tether. The key synthetic challenges were therefore to (a) prepare a series of carbazole quinones with tethers linked at various positions, and (b) to establish methodology for coupling the tethered carbazole quinones to an appropriate glycoside. The strategy is illustrated for hybrid structure **C** in **Scheme 2.1**, which could be obtained by amide coupling of the glucoside **IV** having a free amine at the 2-position and the carbazole-1,4-quinone **III** to form hybrid structure **II**, in this case with the alkyl tether attached at the 6-position. Simple deacetylation then allows the formation of the desired product **I**.

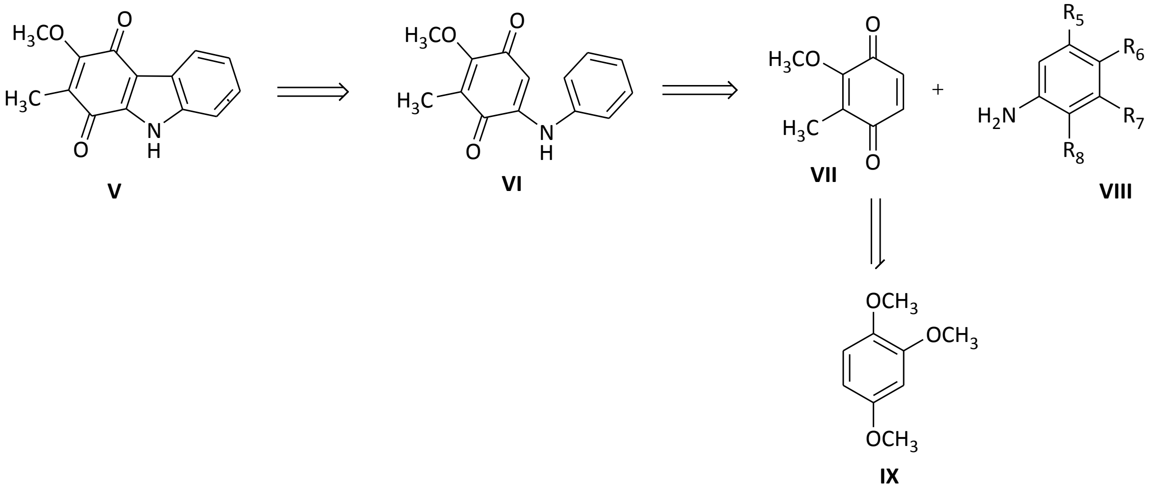


Scheme 2.1. Retrosynthetic pathway of analogues **C.**

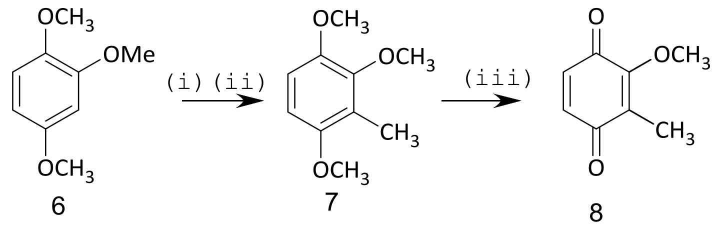
2.2.1. Synthesis of carbazole-1,4-quinone derivatives.

It was envisaged that carbazole-1,4-quinones having a range of different substituents would be synthesized, including those having a carboxyalkyl chain of variable length at either the 6 or 7 position. This would serve as a tether *via* an amide link to the glucosamine unit. It was recognized that the use of an amide link in the tether is potentially problematic, in that this may be susceptible to the deacetylase activity of MshB and other enzymes. For this reason, the plan was to use the amide linkage initially due to its ease of formation, but to then investigate alternative linkage chemistry which provides a metabolically stable mimic of the amide bond which links the cysteine unit to glucosamine in mycothiol.

The selected approach for the synthesis of the carbazole-1,4-quinone **V** was based on the Knölker methodology, and is shown retrosynthetically in **Scheme 2.2.**[13] It involves a catalytic oxidative cyclization of quinone derivative **VI**, prepared by conjugate addition of amine **VIII**, obtained commercially or by synthesis, to quinone **VII**. The latter is obtained by methylation of trimethoxybenzene **IX** followed by oxidative demethylation.

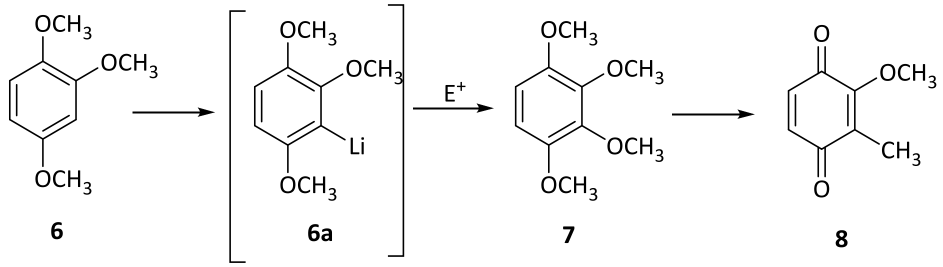


Scheme 2.2. Synthetic pathway of carbazole-1,4-quinone derivatives **V**.



Scheme 2.3. Reagents and condition**:** (i) 2.0 eq. *n*-BuLi (10.0 M in ether), THF, -78 ^{o}C, 1 h, Ar: (ii) 4.0 eq. CH_3I, rt, overnight; (iii) 1.5 eq. PIDA, $MeOH/H_2O$ (3:1), 19 h, rt, Ar.

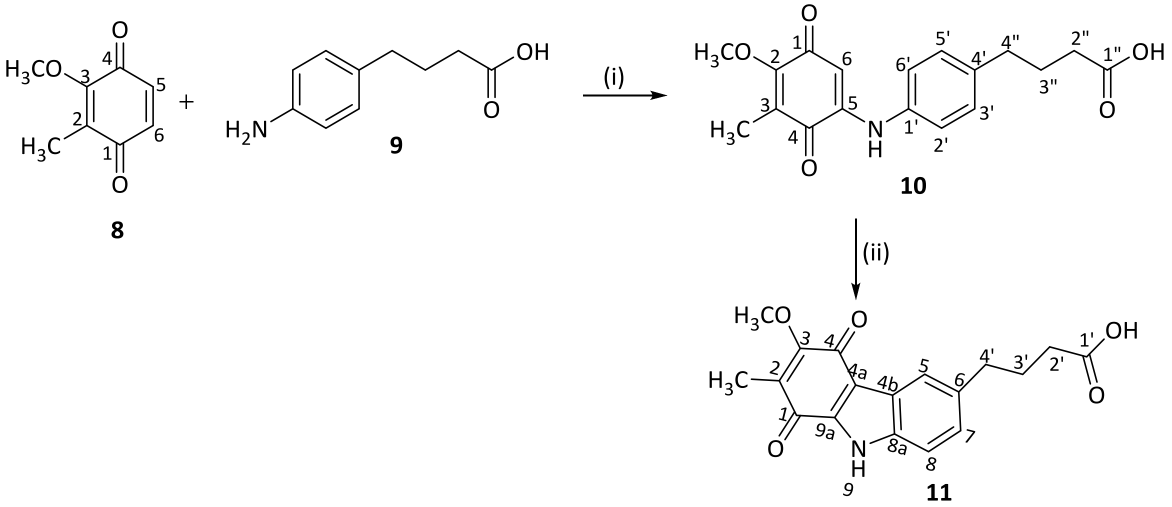
Following this plan, the first synthesis was initiated from the starting trimethoxybenzene **6** which was sequentially treated with *n*-butyl lithium and methyl iodide at -78 ^{o}C to afford the 3-methyl derivative **7** in 79 % yield. In this first step, the regioselectivity is understood (**Scheme 2.4**) to arise from the two *meta*-oriented directed metallation groups (DMG's) on the aromatic ring interacting with the *n*-butyl lithium reagent in its specific aggregation state $(R\text{-}Li)_n$, *via* the Lewis-basic oxygen atoms on the DMG and the Lewis-acidic lithium. The very basic *n*-butyl lithium then deprotonates the ring at the nearest ortho-position to give the lithiated intermediate **6a.** The reactivity of the organolithiated species **6a** can be explained by the low degree of solvation in THF and formation of the aggregated state.[15-18] Treatment of the lithiated intermediate **6a** with 4.0 eq of the electrophilic reagent methyl iodide achieves the selective methylation (**Scheme 2.4**).



Scheme 2.4. Metallation of the 1,2,4-trimethoxybenzene.

The oxidative demethylation of the 3-methyl derivative **7** was attempted with phenyliodine(III) diacetate (PIDA) in the presence of ethanol and water as a co-solvents to afford quinone derivative **8** (**Scheme 2.4**). The reaction proceeded smoothly in the presence of water due to its activation of the hypervalent iodine: the omission of water is known to lead to unwanted side reactions.[15] The structure was confirmed by NMR spectroscopy: the ^{1}H NMR spectrum of the product showed two doublet of doublet signals at δ 6.69 and 6.60 ppm respectively with large coupling *J* = 10.1 Hz confirming the presence of *ortho*-protons on the quinone nucleus, while two singlets at δ 4.02 and 1.95 ppm were assigned to the methoxy and methyl groups, respectively. The proton-decoupled ^{13}C NMR spectrum included two signals at δ 188.3 ppm and 183.2 ppm for the two carbonyl carbons.

The successfully prepared quinone **8** was thus now available for 1,4-addition reactions with different substituted aryl amines.



Scheme 2.5. Reagents and conditions: (i) quinone **8** (2 eq.) and amine **9** (1 eq.), MeOH, rt, Ar, overnight; (ii) Pd(OAc)$_2$ (5 mol%), 0.1 eq. K$_2$CO$_3$, 1 g PivOH, 120 ^{o}C, Air, overnight.

In the first instance, the formation of the quinone **10** was achieved by 1,4-addition reaction of commercially available *para*-substituted amine **9** to the quinone **8** (**Scheme 2.5**). A long reaction time and an excess of quinone **8** were necessary to ensure complete reoxidation of intermediate **C** (**Scheme 2.6**), the tautomer of initially formed enol **B**, and this yielded the product in 82% yield, together with a small amount of the hydroquinone **D.** In the ^{1}H NMR spectrum of **10** (**Figure 2.5**), the quinone proton appeared as a singlet at δ 5.93 ppm, slightly upfield from the corresponding signal in the ^{1}H NMR spectrum of starting quinone **8**. This can be accounted for by the nitrogen atom acting as an electron donor to the π system, counteracting the role of the ketone as electron-withdrawing group. The singlet at δ 7.50 ppm was assigned to the amine proton. The ^{13}C NMR spectrum also confirmed the proposed structure, with 3 signals at δ 184.4 ppm, 182.5 ppm and 178.8 ppm consistent with the presence of the two quinone carbonyl groups and carboxyl carbon.

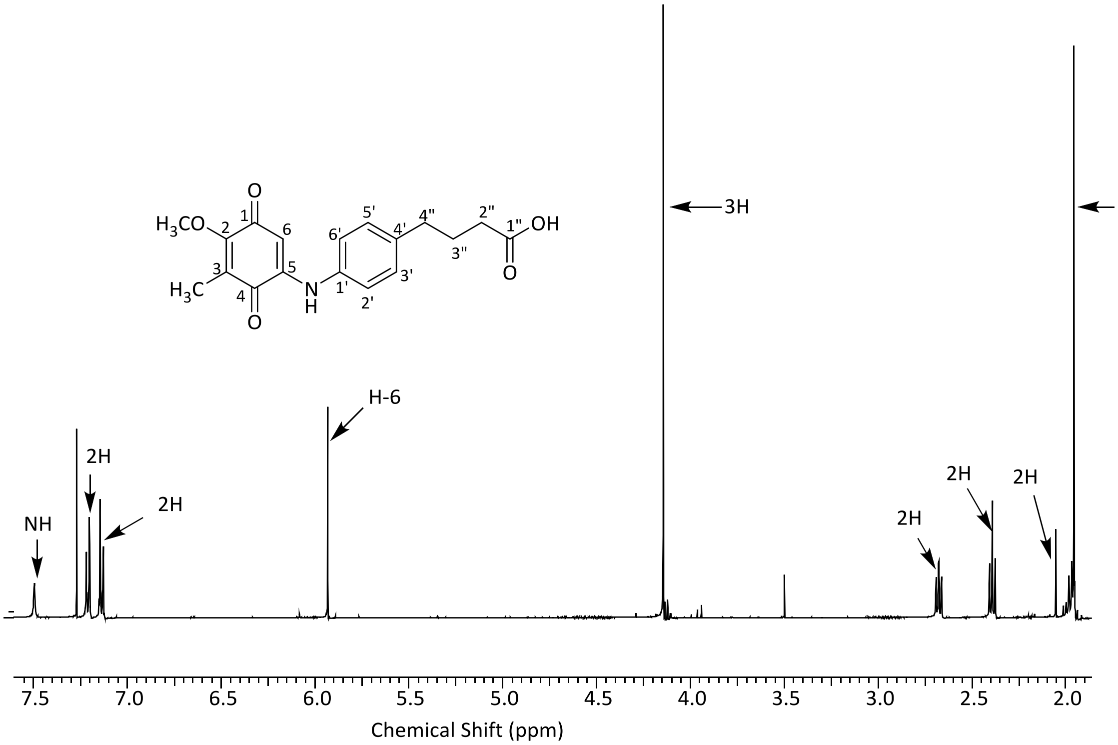
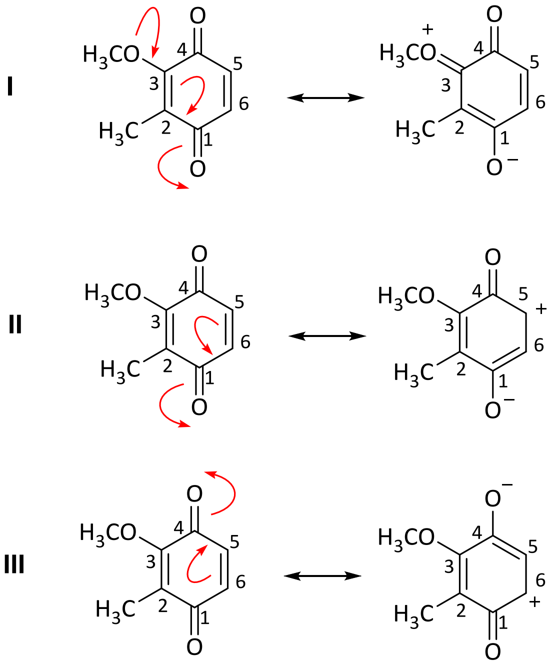


Figure 2.5. ^{1}H NMR spectrum of the quinone **10** in $CDCl_3$.

The regioselectivity of the reaction could be explained by the presence of the methoxy group at C-3, which releases electrons into the adjacent enone (pathway **I**, Scheme below), thus favouring development of electrophilic character (or a low-energy LUMO) at C-6 (pathway **III** in Scheme below) rather than at C-5 (pathway **II** in Scheme below).

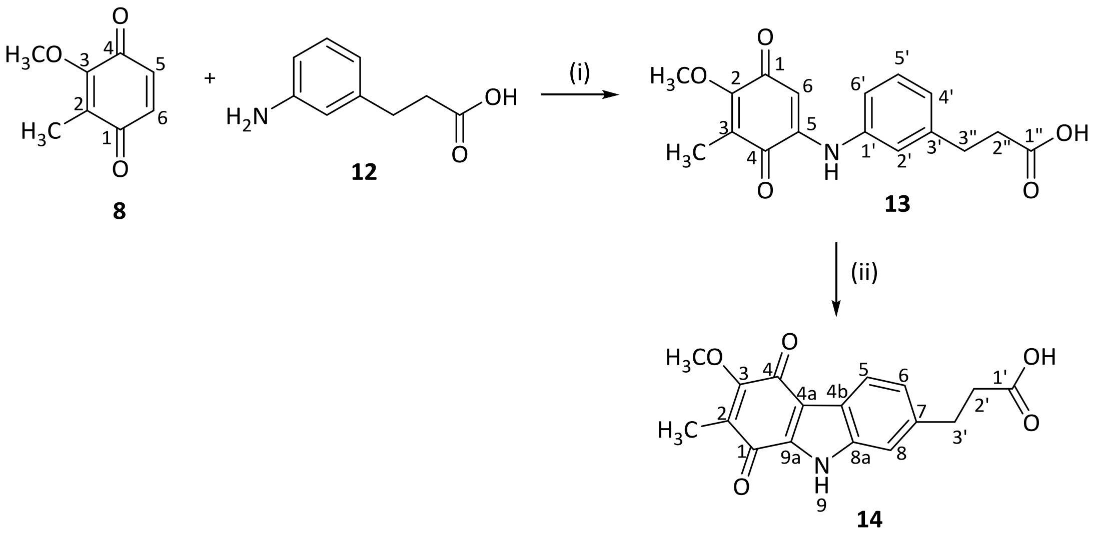
The 1,4-addition of the amine at position C-6 gives rise to the species **A**, which undergoes proton transfer to form enol **B**. Tautomerisation to **C** followed by oxidation in the presence of the starting material quinone leads exclusively to the quinone **10** (**Scheme 2.6**).



Scheme 2.6. Proposed mechanism of the formation of 1,4-addition product.

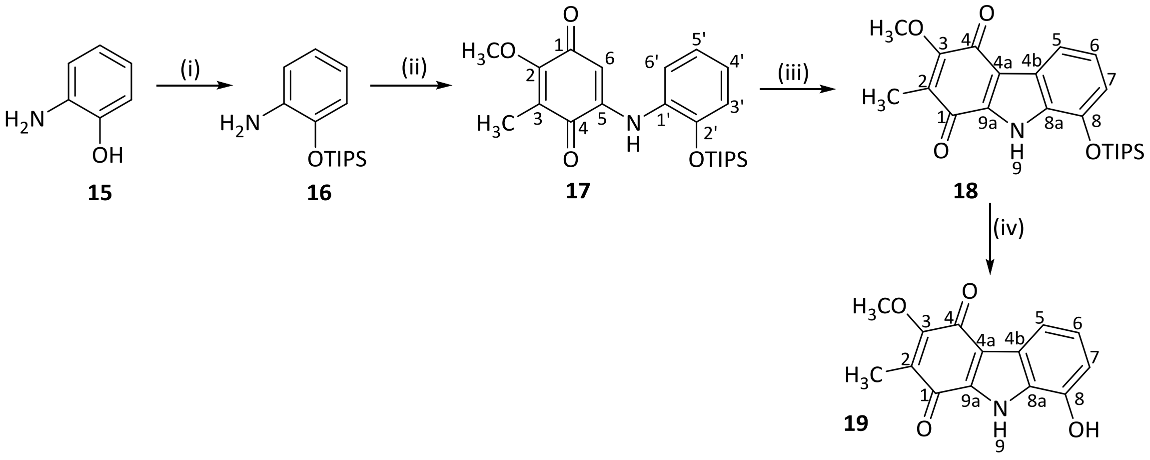
The palladium (II)-catalysed oxidative cyclization of diarylamines is one of the transformations described by Akermark et *al*[19] to create the C-C bond in the formation of carbazole alkaloids. Moreover, the double C-H activation in the presence of stoichiometric amount of Palladium (II) and copper (II) salts described by Knölker et *al*[13,14] also resulted in the formation of the bi-aryl C-C bond and confirmed the approach. In this study the palladium (II)-mediated cyclization of **10** was conducted using 5 mol% of the catalyst and 0.1 eq. K_2CO_3 at 120 ^{0}C in pivalic acid to afford carbazole quinone **11** **(Scheme 2.5)** in 85% yield. During the reaction, the flask was left open to ensure the reoxidation of Pd(0) to Pd(II) by atmospheric oxygen. The addition of a further equivalent of catalyst did not improve either reaction time or yield. The ^{1}H NMR spectrum of **11** showed the expected disappearance of the singlet at δ 5.93 ppm in the ^{1}H NMR spectrum of **10** for the proton attached to the quinone moiety. In addition, no *ortho*-coupling was observed between H-5 and H-4b since the ^{1}H NMR of **11** displayed a singlet at δ 7.80 ppm for H-5.

This confirmed the effective cyclisation of the quinone **10** with palladium acetate to give the carbazole quinone **11**. The ESI mass spectrum also displayed the expected molecular ion at *m/z* = 350.2 corresponding to [M+Na]$^{+}$.



Scheme 2.7. Reagents and conditions: 2 eq. of quinone **8**, 1 eq. of Amine **10**, MeOH, rt, overnight, Ar; (ii) Pd(OAc)$_2$ (10 mol%), 0.1 eq. K_2CO_3, 2 g PivOH, 120 °C, Air, overnight.

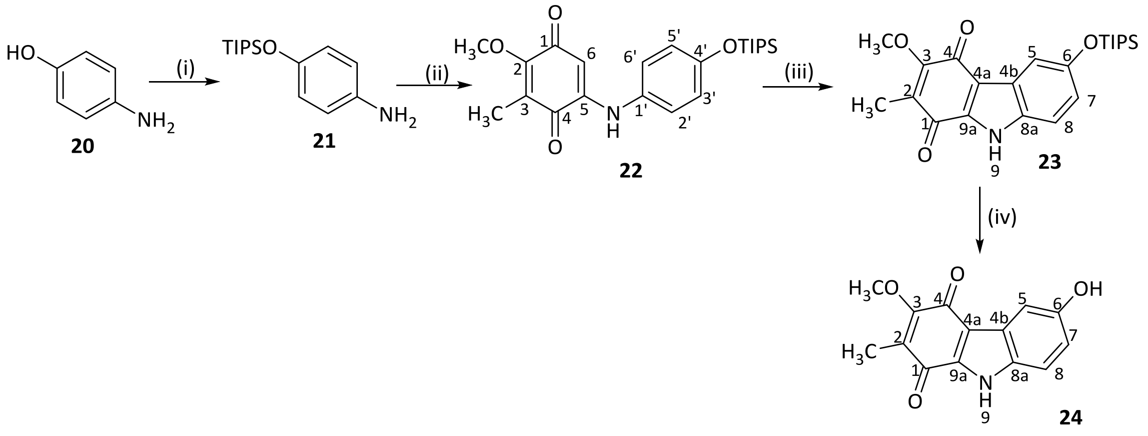
In a repeat of the approach described above, the quinone **8** and the readily available *meta*-substituted amine **12** were combined in methanol at room temperature in order to achieve a further 1,4-addition reaction (**Scheme 2.7**). The reaction proceeded to completion overnight, and yielded a violet solid in 76% yield after filtration. The ^{1}H NMR spectrum of **13** confirmed the structure with; in particular, two singlets at δ 7.53 and 5.97 ppm corresponding to the NH proton and the proton attached to the quinone moiety (H-6), while the ^{13}C NMR spectrum gave, *inter alia,* signals corresponding to the three carbonyl carbons. In the ESI mass spectrum, the ion with *m/z* = 316.1 corresponded to the ion $[M+H]^+$. The palladium-catalysed cyclization of **13** was then attempted using 10 mol% of catalyst over 24 h. Carbazole quinone **14** was obtained in a moderate yield of 51% as sole product and increasing the amount of catalyst to 15 mol% or shortening of the reaction time did not improve the yield. More importantly, prolonged reaction time resulted in the decomposition of starting material. The disappearance of the proton at 4a in the ^{1}HNMR spectrum of **14**, as well as the appearance of two doublets (δ 7.89 and 7.14 ppm), which were assigned to protons at positions 5 and 6, and a singlet at δ 7.31 ppm for H-8, confirmed the structural assignment of the carbazole quinone **14.** The HSQC spectrum confirmed that seven carbon atoms have no attached protons. In addition, ESI-MS gave expected molecular ions at *m/z* = 314.1 $[M+H]^+$ and 336.1 $[M+Na]^+$.



Scheme 2.8. Reagents and conditions: (i) 1.3 eq. TIPSCl, 1.5 eq. imidazole, DMF, 14 h, rt, Ar; (ii) 1 eq. amine **15**, 1.6 eq. quinone **8**, MeOH, 2 h, rt, Ar; (iii) $Pd(OAc)_2$ (10 mol%), 0.1 eq. K_2CO_3, PivOH, 120 ^oC, air, 3 h; (iv) 1.0 eq. of **18**, 1.2 eq. TBAF, DCM, 30 min, rt.

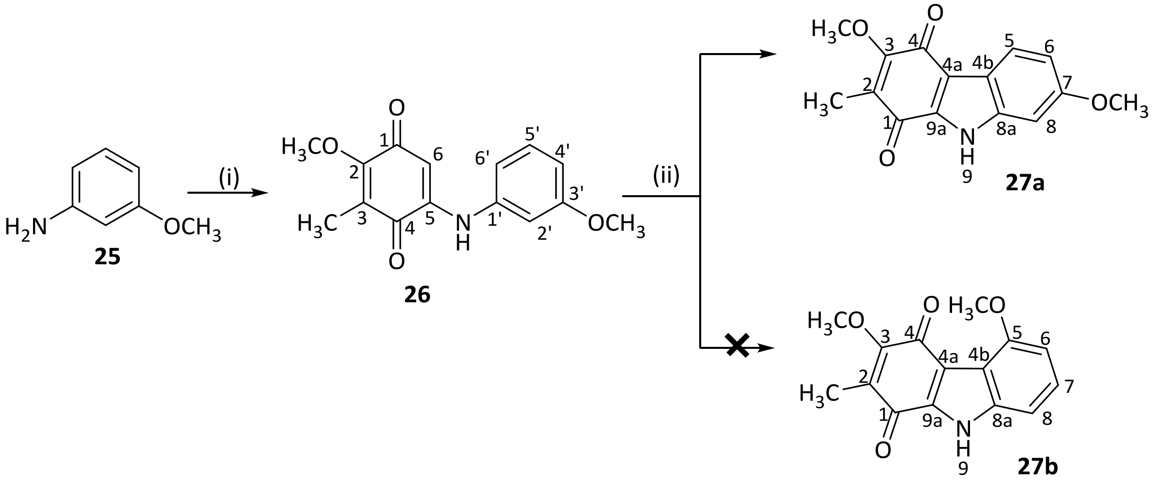
A key consideration in devising a strategy for the synthesis of the 8-hydroxy analogue of the carbazole quinone was the possibility that a free hydroxyl group in a starting material such as 2-aminophenol **15 (Scheme 2.8)** would be incompatible with the sequence established above, *viz.* conjugate addition of amine to the quinone followed by oxidative cyclization. The availability of TIPSCl at low cost, together with its thermal stability and resistance to oxidation led to it being preferred for the protection of hydroxyl group. The hydroxyl group in **15** was therefore first protected as the TIPS ether **16** in quantitative yield by treating **15** with TIPSCl in THF at room temperature for 14 h in the presence of imidazole.[20-22] The ^{1}H NMR spectrum of **16** showed a doublet at δ 1.14 ppm with *J* = 7.6 Hz, integrating for 18 protons of the six methyl groups attached to the methine carbon of isopropyl group, and a multiplet at δ 1.28-1.36 ppm corresponding to the three CH groups. The *O*-protected amine **16** and the quinone **8** were then combined for the 1,4-addition reaction in methanol at room temperature. The reaction proceeded to completion in two hours, yielding the quinone **17** in 69% yield. The ^{1}H NMR spectrum of **17** displayed a broad peak at δ 8.05 ppm for the amine proton and a one-proton singlet at δ 6.11 ppm corresponding to H-6 in the attached quinone moiety. The mass spectrum gave an ion at *m/z* = 416.3, corresponding to $[M+H]^+$. The oxidative cyclization of **17** using a catalytic amount of the palladium acetate was conducted in an open flask as before, giving rise to the carbazole quinone **18** in 82 % yield after 3 h. The ^{1}H NMR of **18** showed the absence of signals for the characteristic protons 6 and 6′ in its precursor **17**. The signal at δ 7.16 ppm which appears as a triplet with *J* = 7.9 Hz corresponded to the proton H-6, since it has two neighbouring protons. Two doublets were also visible at δ 7.74 and 6.81 ppm and were assigned to either H-5 or H-7. These protons did not show doublets of doublets because the constant couplings of the meta protons are usually small. These observations confirmed the formation of the carbon-carbon bond between C-4a and C-4b, respectively. The carbazole quinone **18** was then deprotected in order to prepare hydroxylated carbazole quinone **19** in order to assay its anti-TB activity in relation to the known compound **B**. Removal of the TIPS group was achieved following the conventional approach by the addition of TBAF (1.2 eq.) in DCM, which yielded **19** as a brown solid in 53% yield. The moderate yield of reaction was possibly due to the water-solubility of the carbazole quinone **19** which could be lost during aqueous work-up. The attempt to optimize using THF was unsuccessful and gave 24% yield. In the ^{1}H

NMR spectrum of **19**, two broad singlets were observed at δ 12.67 and 10.02 ppm corresponding respectively to the protons in the NH and OH groups. The absence of isopropyl signals and the appearance of signal for OH in the ^{1}H NMR of **19** confirmed the structure of carbazole quinone **19**, and further corroborated mass spectroscopy with an ion at m/z = 258.1 consistent with [M+H]$^+$.



Scheme 2.9. Reagents and conditions: (i) 1.5 eq. TEA, 0.5 eq. DMAP, 1.5 eq.TIPSCl, 2 h, rt; (ii) 1.0 eq. amine **21**, 2.0 eq. quinone **8**, MeOH, 4 h, rt; (iii) Pd(OAc)$_2$ (5 mol%), 0.1 eq. K$_2$CO$_3$, PivOH, 120 ^{o}C, air, 5 h; (iv) 1 eq. of **23**, 1.2 eq. TBAF, DCM, 30 min, rt, Ar.

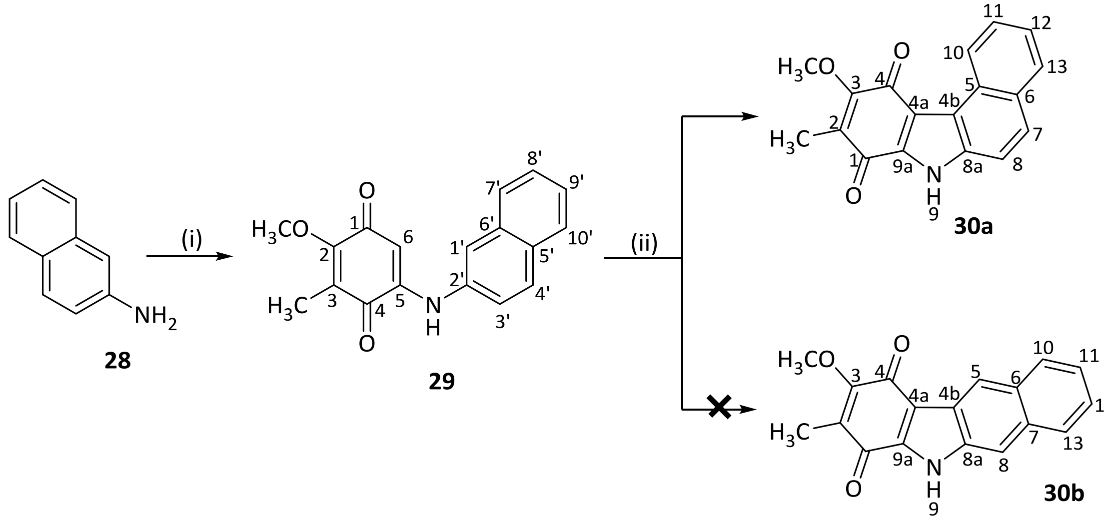
Following a similar approach to that for preparation of **19** the protection of the hydroxyl group of the commercially available *p*-aminophenol **20** was achieved at room temperature in 2 hours using 0.5 eq DMAP as a catalyst and 1.5 eq. TIPSCl, giving protected amine **21** in a moderate yield of 64 % (**Scheme 2.9**). The ^{1}H NMR spectrum of **21** showed a doublet at δ 1.10 ppm with *J* = 6.9 Hz, integrating for 18 protons, which are attributed to six methyl groups attached to the methine carbon of isopropyl group, and a multiplet at δ 1.26 - 1.18 ppm corresponding to the three C*H* protons. The TEA as auxiliary base was used to deprotonate the protonated DMAP and regenerates the catalyst. The 1,4-addition reaction of the protected amine **21** with quinone **8** in methanol was conducted at room temperature. After 4 h, the reaction was complete to give, after purification, the quinone **22** as a red solid in 96% yield. In the ^{1}H NMR spectrum of **22**, the appearance of the signal at δ 5.80 ppm for the H-6 as well as a singlet at δ 7.38 ppm integrating for one proton (NH) confirmed its structural assignment. After applying the same conditions as before for the cyclisation of the quinone **22** using a catalytic amount of the palladium, a brown solid was isolated in 55% yield and shown to be carbazole quinone **23**. This moderate yield was possibly due to the presence of the bulky TIPS ether near the reaction site. No signal for proton 4a was visible in the ^{1}HNMR spectrum of **23** compare to the ^{1}H NMR spectrum of **22**. This confirmed the structural assignment of the carbazole quinone **23**. The triisopropylsilyl ether was removed by treatment with 0.1 M TBAF to give, after, purification, the desired carbazole quinone **24** as brown red solid in 63% yield. Its structural assignment showed the disappearance of triisopropyl proton signals in the ^{1}H NMR of **24,** while the ^{13}C NMR confirmed the absence of the triisopropyl carbon signals.



Scheme 2.10. Reagents and conditions: (i) 1.0 eq. amine **25**, 2.0 eq. quinone **8**, MeOH, rt, overnight; (ii) Pd(OAc)$_2$ (5 mol%), 0.1 eq. K$_2$CO$_3$, PivOH, 120 ^{o}C, air, 5 h.

Reaction of the commercially available amine **25** with the quinone **8** in order to achieve the 1,4-addition reaction gave, after purification, a brown solid in 96% yield, which was revealed to be the quinone **26**. The ^{1}H NMR spectrum confirmed the structural assignment of **26** by displaying a singlet at δ 6.00 ppm for proton-6, with the NH resonating as a broad peak at δ 7.49 ppm and two singlets at δ 4.15 and 3.82 ppm attributed to the two methoxy protons. In addition, ESI-MS gave molecular ions at *m/z* = 274.1 $[M+H]^+$. Oxidative cyclization of **26** with 5 mol% of $Pd(OAC)_2$ gave, after purification, the desired carbazole quinone **27a** as red solid in 60% yield, with no evidence for the alternative, less likely carbazole quinone **27b** obtained from ring-closing to the carbon between the amine and methoxy groups. This could be due to steric demands of the methoxy group in the transition state leading to product **27b**. The evidence of the structure of **27a** included the appearance in the ^{1}H NMR spectrum of a doublet of doublets at δ 6.93 (*J* = 2.2, 8.5 Hz), formed by coupling of the H-6 to both an ortho-proton (H-5) and a meta-proton (H-8). The evidence for the structure of **27a** included the appearance in the ^{1}H NMR spectrum of a doublet of doublets at δ 6.93 (*J* = 2.2, 8.5 Hz), resulting from coupling of the H-6 to both an ortho-proton (H-5) and a meta-proton (H-8). The doublet at 6.89 ppm for H-8 also confirms the structure and the cyclization is also confirmed by the disappearance of the characteristic signals for H-6 and H-6' that are present in the ^{1}H NMR spectrum of starting material **26.** This suggested the successful cyclisation of the quinone **26** and, the ESI-MS gave molecular ions at *m/z* = 272.0 $[M+H]^+$.

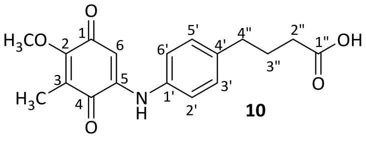
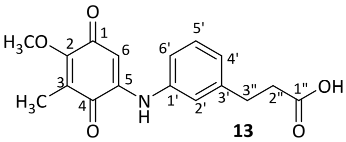
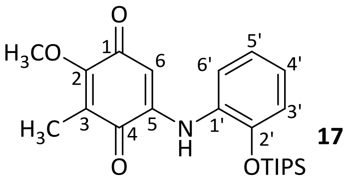
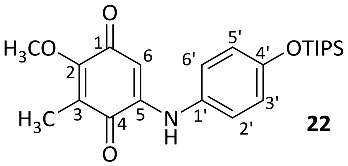
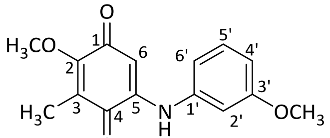
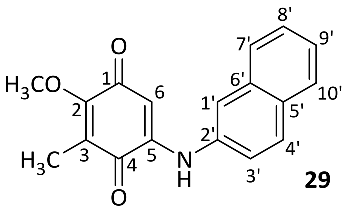
In the context of exploring the structure-activity profile of the carbazole quinones, it was of interest to prepare an analogue with a bulky lipophilic group such as an aromatic ring attached to the carbazole-1,4-quinone. The methodology described above was therefore applied to β-naphthylamine, in order to attempt formation of an extended benzo-fused carbazole quinone.



Scheme 2.11. Reagents and conditions: (i) 1.0 eq. amine **28**, 2.0 eq. quinone **8**, MeOH, overnight, rt; (ii) $Pd(OAc)_2$ (10 mol%), 0.1 eq. K_2CO_3, PivOH, 120 ^oC, air, overnight.

The readily available β-naphthylamine **28** and quinone **8** in methanol were stirred overnight at room temperature to achieve the 1,4-addition reaction. This gave a darkish brown solid in 74% yield, which was shown to be quinone **29**. The ^1H NMR confirmed the structural assignment of **29** with the presence of the characteristic singlet at δ 6.16 ppm for H-6 and a broad peak at δ 7.71 ppm integrating for one proton for the amine proton. In addition, ESI-MS gave the expected molecular ions of *m/z* = 294.1 $[M+H]^+$. The oxidative cyclization of **29** could give rise to two compounds **30a** and **30b** due to the fact that coupling can occur at two possible positions on the aromatic ring. The attempted oxidative cyclization of **29** using 5 mol% of palladium did not proceed to completion over 24h, and prolonged reaction time led to the decomposition of the product. However, on repeating the reaction with 10 mol% catalyst, a black solid was obtained in a low 8% yield. The ^1H NMR of the product revealed the absence of singlets expected for H-5 and H-8 in structure 3**0b.** This instead confirms the isolation of the carbazole quinone **30a**, and the ^1H NMR displayed signals as doublets with ortho-coupling (*J* = 8.2 or 9.1 Hz) or multiplets. The difficulty of insertion of palladium near the bulky aromatic group might explain the poor yield of the reaction, and the starting material was recovered.

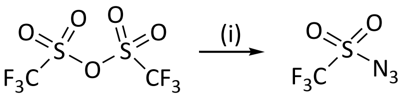
Table 2. Comparison of selected ^{1}H NMR and ^{13}C NMR signals for the series of aryl-quinonyl amines, precursors of the carbazole quinones.

Structure	**H-6 (ppm)**	**NH (ppm)**	**-OCH$_3$ (ppm)**	**-CH$_3$ (ppm)**	**C=O (ppm)**	**C=O (ppm)**
 **10**	5.93	7.50	4.14	1.96	184.4	182.5
 **13**	5.97	7.53	4.14	1.96	184.3	182.8
 **17**	6.11	8.05	4.15	1.97	184.3	182.6
 **22**	5.80	7.38	4.14	1.95	184.3	182.3
 **26**	6.00	7.49	4.15	1.96	184.3	182.5
 **29**	6.16	7.71	4.17	1.99	184.3	182.7

With a series of aryl-quinonyl amines now in hand, where the quinonyl unit was the same in all compounds, it was of interest to compare the ^{1}H NMR data to investigate any diagnostic shifts. The comparison is shown in **Table 2**. The proton NMR chemical shifts (δ_H) of the methoxy and methyl singlets remain virtually the same throughout the series, as do the δ_C values for the quinone carbonyl and methyl groups. However, small differences in δ_H are seen for the amine-protons and H-6. Signals for the latter appear furthest downfield in compounds **13**, **17**, **26** and **29**, which have either *ortho*- or *meta*-substituted aromatic rings.

2.2.2. Synthesis of the key glucosamine derivative 36.

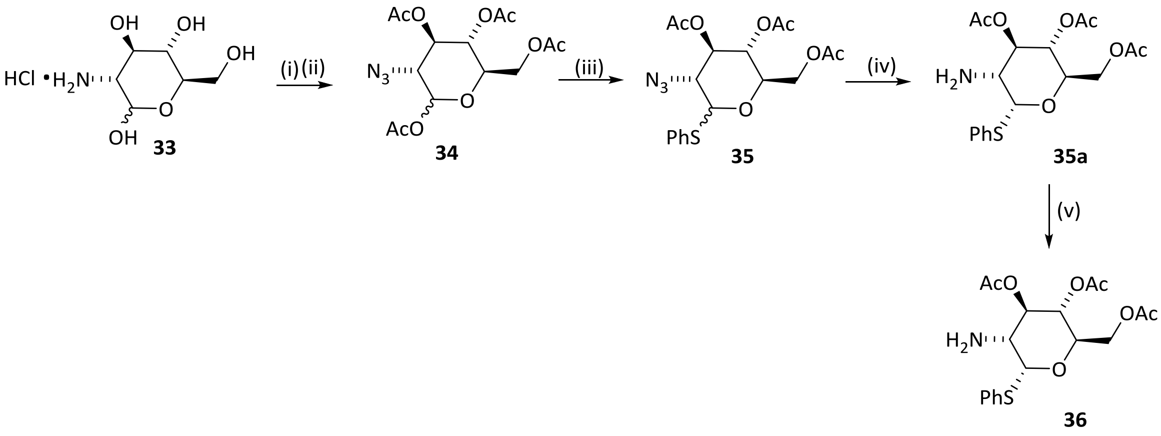
In order to achieve one of our goals as described in the **Figure 2.4**, the synthesis of the target molecule **C** was required, in which the sugar and carbazole quinone are linked by an amide bond. This would require a 2-aminoglucoside for coupling with the carboxy-terminated alkyl chain at the 6- or 7-position of the carbazole-quinone. Using Ernst's approach,[23] we successfully synthesized the target selectively protected glucosamine **36** from glucosamine using the diazo transfer reaction.



31 **32**

Scheme 2.12. NaN_3, toluene/H_2O, 0-10 ^oC, 2.5 h

Triflic azide was freshly prepared by treating triflic anhydride **31** with sodium azide in an emulsion of H_2O/toluene at 0 ^oC (**Scheme 2.12**), to provide a 1 M stock solution. (The reagent needs to be handled with care because it is shock-sensitive.)

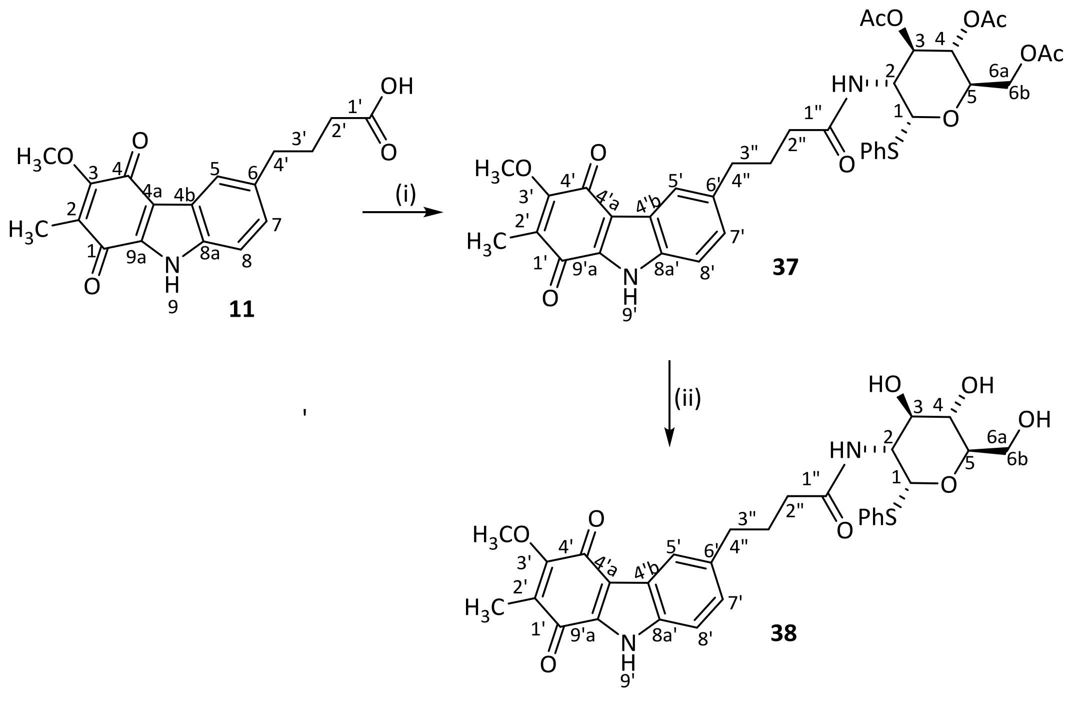


Scheme 2.13. (i) **32**, $NaHCO_3$, $CuSO_4.5H_2O$, H_2O/MeOH/toluene,15 h, r.t; (ii) Ac_2O, pyridine, 0 ^oC - r.t, 18 h(iii) $BF_3.OEt_2$, dry CH_2Cl_2, PhSH, reflux; (iv) Recristallisation from EtOH; (v)H_2, Pd/C, EtOAc, 24 h.

The triflic azide **32** (stock solution) was then added to glucosamine hydrochloride **33** in the presence of $CuSO_4.5H_2O$ as catalyst to provide the 2-azido derivative, which was further acetylated to give an anomeric mixture (α/β isomers) of acetylated 2-azido glucose **34** as a white precipitate in 89% yield. Glucosyl acetate **34** was then treated with thiophenol (PhSH) in the presence of Lewis acid $BF_3.Et_2O$ to give a mixture of anomers (α/β: 3/1), which on recrystallization from EtOH gave the α-thioglycoside **35a** in 46% yield as white needles. The NMR spectra were in agreement with the literature.[23] The hydrogenation of the α-thioglycoside **35a** was conducted in EtOAc with Pd/C (10 wt %) under H_2 atmosphere to give, after purification, the 2-amino α-thioglycoside **36** as colourless needles in 75 % yields. Its structure was confirmed by analysis of the ^1H NMR spectrum, which showed a doublet of doublets for H-2, shifted upfield from its position at δ 3.99 ppm in **35a** to 3.32 ppm in **36**. It is important to note that we observed spontaneous *O-N* migration of one acetyl group to form the 2-acetamido-glucoside, even when the α-thioglycoside **36** was stored at low temperatures. It was therefore freshly prepared for each subsequent coupling reaction with substituted carbazole quinones.

2.2.3. Amide bond formation

Formation of the target hybrid structures required efficient formation of the amide bond. While there are many methods for achieving this, we found EDC.HCl to be an effective reagent for activation of the carboxylic acid in the presence of amine group. Its water-solubility as well as of the urea by-product formed during the reaction with the amine meant it was favoured over DCC, which gives a by-product difficult to remove from the reaction mixture.[24] To promote coupling to form the peptide, additives such as HOBt are commonly used, and were used in this work.



Scheme 2.14. Reagents and conditions: (i) 1.0 eq. **11**, 1.5 eq. EDC.HCl, 1.0 eq. HOBt, 4.5 eq DIEA, THF, 0 ^{o}C, Ar; (ii) 1.25 eq. **36**, rt, overnight, Ar; (iii) MeONa, MeOH, rt, Ar, overnight.

In the first attempt at formation of peptide bond, acid **11** was combined with amine **36** in THF at 0 ^{o}C under Ar and the amide **37** was isolated after filtration in a yield of 52%. The ^{1}H NMR spectrum of **37** showed the appearance of a doublet at δ 5.92 ppm (*J* = 8.8 Hz) integrating for one proton assignable to the amide-proton (N*H*CO) (**Figure 2.6**). In addition, the ^{1}H NMR spectrum displayed three singlets at δ 2.08, 2.07 and 2.06 ppm for the acetyl methyl groups, while the ^{13}C NMR revealed six signals at δ 181.0, 179.1, 172.7, 171.7, 170.7 and 169.3 ppm corresponding to the six carbonyl carbons present in the molecule. The ESI mass spectrum showed the molecular ions at *m/z* = 707.5, 724.5 and 729.5 consistent with $[M+H]^+$, $[M+NH_4]^+$ and $[M+Na]^+$ ions, thus confirming the molecular structure of compound **37**.

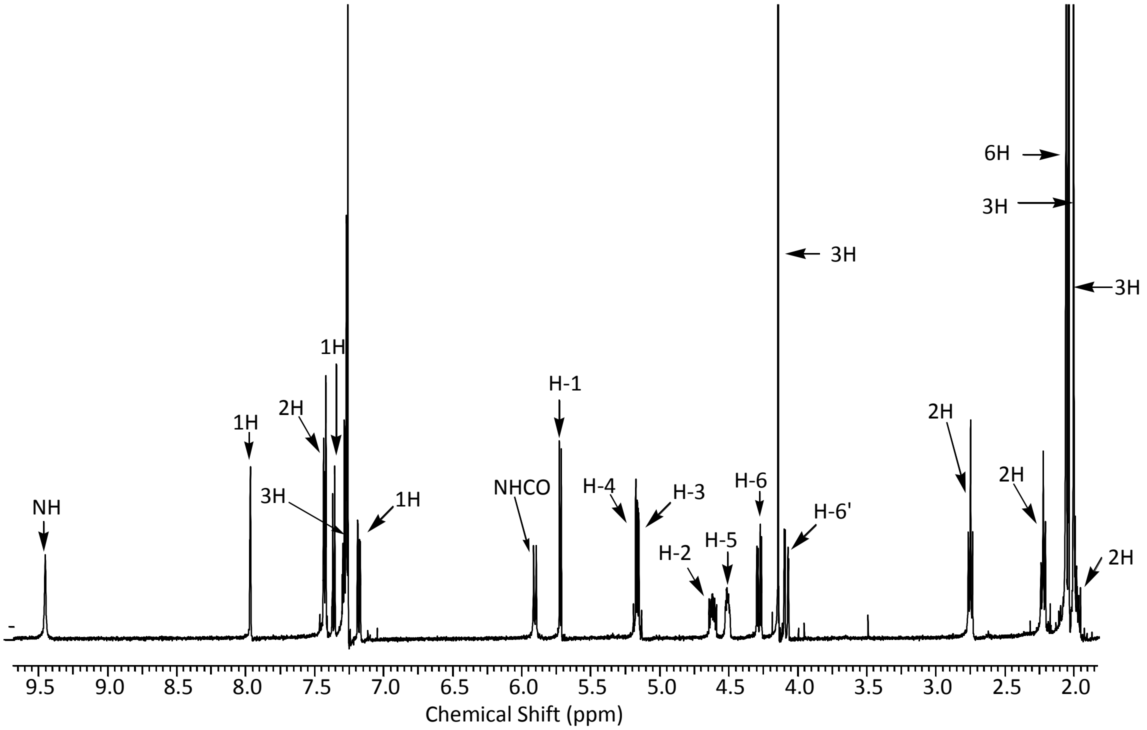
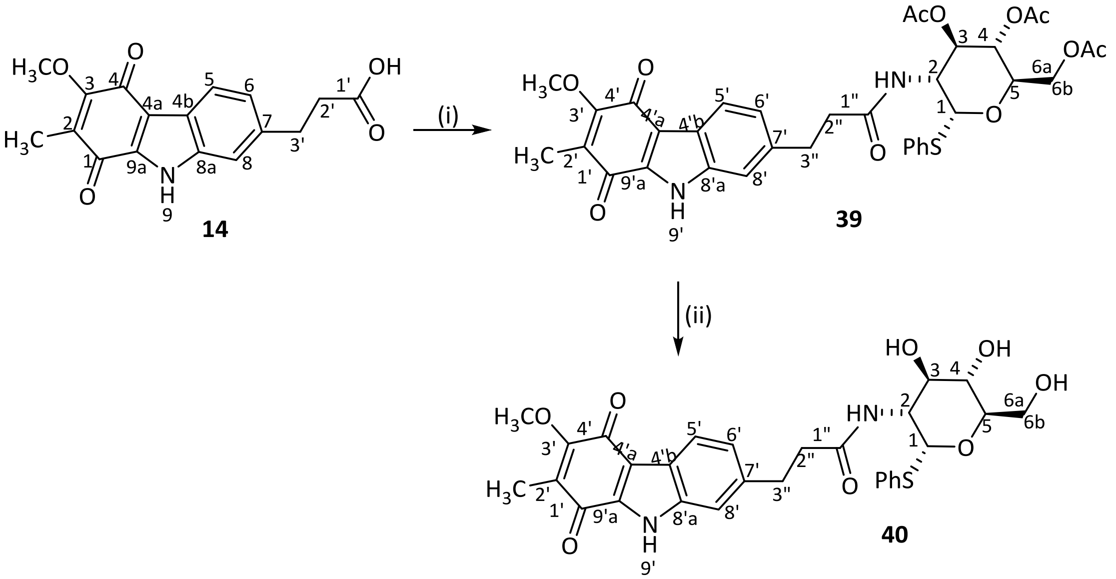


Figure 2.6. ^{1}H NMR spectrum of compound **37** in $CDCl_3$.

Deacetylation of **37** with catalytic sodium methoxide in methanol afforded **38** in a very good yield (95%).[25] The absence of acetyl signals in ^{1}H NMR and ^{13}C NMR spectrum of **38** supported the successful deacetylation of compound **37**. In addition, the IR spectrum showed an absorption band at ν (cm^{-1}) = 3398, confirming the presence of OH functional groups and ESI-HRMS gave a molecular ion at m/z 603.1789, corresponding to $[M+Na]^+$.



Scheme 2.15. Reagents and conditions: (i) 1.0 eq. **14**, 1.5 eq. EDC.HCl, 1.0 eq. HOBt, 4.5 eq. DIEA, THF, 0 ^{o}C, Ar; (ii) 1.25 eq. **36**, rt, overnight, Ar; (iii) MeONa, MeOH, rt, Ar, overnight.

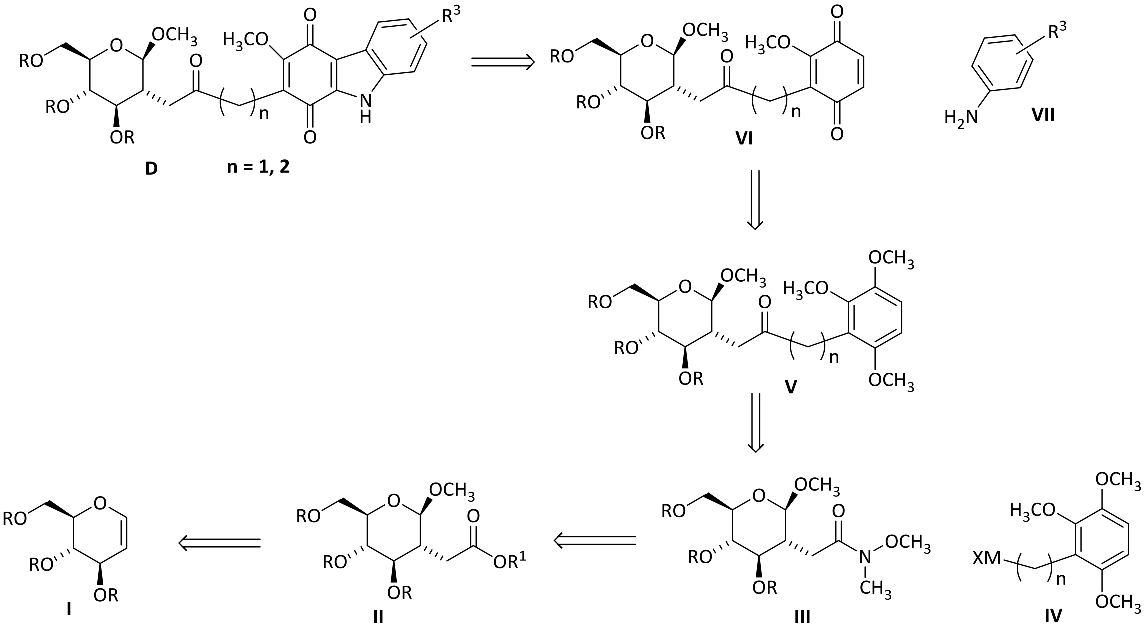
Coupling of the carboxylic acid **14** and the amine **36** under the conditions described above (**Scheme 2.15**) resulted in the formation of the amide **39** in a moderate yield (60%) after filtration. In the ^{1}H NMR spectrum, the singlet at δ 5.93 ppm corresponded to the proton attached to the nitrogen atom, while the ^{13}C NMR spectrum revealed six signals for carbonyl carbons at δ 180.9, 179.0, 171.8, 171.8, 170.6, 169.3 ppm. In addition, ESI-MS gave molecular ions with *m/z* = 693.4 and 715.4, which were are attributed to the $[M+H]^+$ and $[M+Na]^+$ ions, respectively. The deacetylation of **39** was conducted with a catalytic amount of sodium methoxide in methanol to give, after filtration, the unprotected sugar derivative **40** in high yield (96%). The disappearance of the three acetyl groups was evident from the ^{1}H NMR spectrum of **40**. In addition, the doublet for H-1 of the sugar moiety appeared slightly upfield from δ 5.66 ppm in the ^{1}HNMR spectrum of **39** to 5.60 ppm in the ^{1}HNMR spectrum of **40.** The ^{13}C NMR spectrum of **40** displayed three signals at δ = 180.7, 177.2, 172.1 ppm for the two carbonyl groups of the quinone and one of the amide. In addition, ESI-HRMS gave an ion at m/z = 589.1632 corresponding to $[M+Na]^+$.

The successful completion of the synthesis of hybrid structures **38** and **40** thus demonstrated a viable synthetic route to these compounds, and these were combined with similar structures prepared by other researchers in the research group and submitted for separate assays of their anti-TB activity and activity as inhibitors of mshB. The results of these assays are presented and discussed later in this thesis.

2.2.4. Attempted synthesis of analogues D and E, involving a C–C coupling step in the formation of the tethered hybrid molecule (see Figure 2.4).

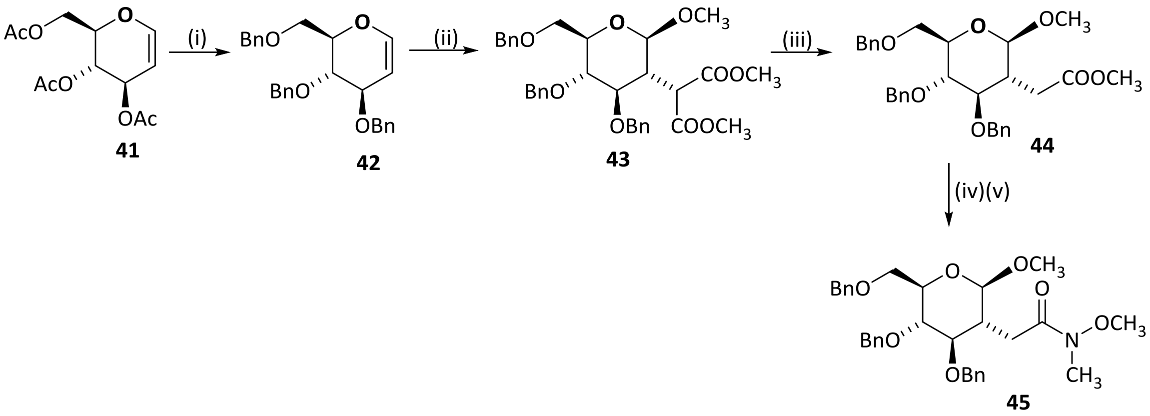
2.2.4.1. Use of Grignard strategy

Many approaches have been used for carbon-carbon bond formation resulting in the preparation of ketone. A classic example is the reaction of a Grignard reagent with a carboxylic acid derivative, but this often leads to low yield of ketones and can be accompanied by over-alkylation to give the tertiary alcohols. This has led to variations such as the acylation of organometallic reagents with carboxylic acid derivatives such as Weinreb amides (*N*-methoxy-*N*-methylamides), where the second nucleophilic addition of Grignard reagent to form a ketone is suppressed.[26,27] In addition, the Weinreb amide displays good versatility and reacts with both organolithiums and Grignard reagents to form various ketones.[28]



Scheme 2.16. Retrosynthetic pathway to ketone formation.

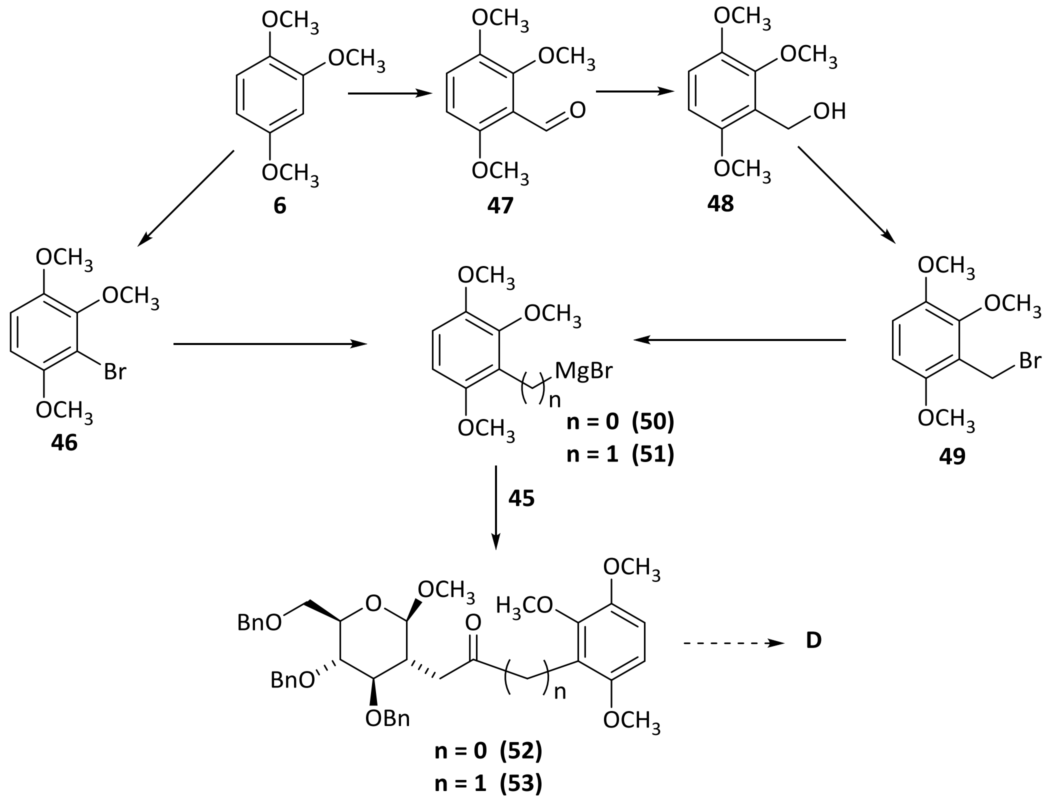
The proposed synthetic route to derivatives **D** therefore attempted to combine the strategies of assembling the carbazole-quinone unit using the Knölker methodology, with preparation of a 2-*C*-carboxyalkyl glycoside and final coupling using the Weinreb approach. The Weinreb amide **III** (**Scheme 2.16**) was therefore identified as a key intermediate for preparation of the ketone **VIII**, which would be obtained by 1,4 addition of the amine **VII** to the quinone **VI**, followed by oxidative cyclisation. The quinone **VI** would be obtained by oxidation of **V**, which in turn was obtained by the reaction of the key intermediate **III** with the organomettalic **IV**. The key intermediate Weinreb amide **III** could be prepared from the hydrolysis of the ester **II**, and subsequently conversion of the corresponding acid to the Weinreb amide **III** in a one pot reaction. The selective addition of malonate to the protected glucal **I**, followed by decarboxylation could give access to the ester **II** (**Scheme 2.16**).



Scheme 2.17. Reagents and conditions: (i) NaOH, TBAI, THF, BnBr, 5 h, rt; (ii) Dimethyl malonate, $NaHCO_3$, CAN, MeOH, 0 °C, 7 h, Ar; (iii) LiI, DMSO, 180 °C, reflux, 2.5 h; (iv) $LiOH.H_2O$, Amberlite-IR120, MeOH-H_2O, rt; (v) 2-chloro-4,6-dimethoxy-[1,3,5]-triazine, *N*-methylmorpholine, *N*,*O*-dimethylhydroxylamine hydrochloride, THF, rt.

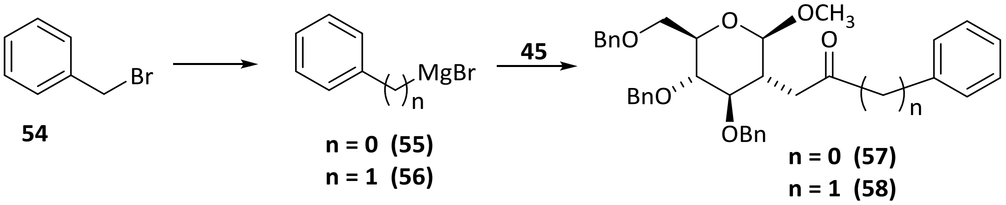
In order to implement this strategy, the first goal was to prepare the key Weinreb amide **45** starting from readily available D-glucal **41**. Misra's protocol[29] allowed the direct one-pot conversion of acetylated glucal **41** to the corresponding benzylated glucal **42** in very good yield 92%. Based on the Linker procedure,[30] we then constructed the side chain at C-2 by treating the benzylated glucal **42** with 10.0 eq. of dimethyl malonate in the presence of CAN and $NaHCO_3$. The malonate **43** was isolated in good yield (91%) and decarboxylation using 1.5 eq. of LiI in DMSO afforded the ester **44** in 95 % yield. To access the key intermediate Weinreb amide **45**, the ester **44** was hydrolysed with 2.5 eq. of $LiOH.H_2O$ to give the carboxylic acid. Without purification, the carboxylic acid was then converted to the Weinreb amide **45** in the presence of *N*,*O*-dimethylhydroxylamine hydrochloride[31] to give, after purification, the desired product in 73% yield as a colourless oil. The ^1H NMR analysis revealed three singlets δ 3.53, 3.41 and 3.03 ppm, which were assigned to the methyl protons of the OCH_3, $NOCH_3$ and NCH_3 groups respectively. This was corroborated by the ^{13}C NMR spectrum which showed three carbon signals at δ 61.1 ppm (OCH_3), 56.7 ppm (OCH_3) and 32.4 ppm (NCH_3). In addition, the IR spectrum showed absorption bands at ν (cm^{-1}) = 1646 (C=O) and 1265

(C-N), respectively. EI-HRMS confirmed the assigned structure, giving an ion with *m/z* = 550.2799 corresponding to $[M+H]^+$.



Scheme 2.18. Synthetic pathway to analogue **D**.

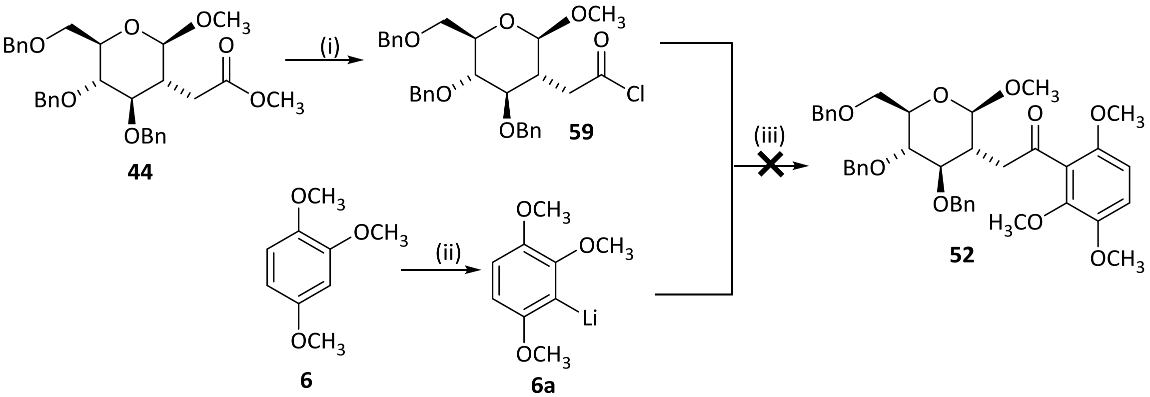
The direct reaction of magnesium metal with organic halides is the most common method used to prepare Grignard reagents **50** and **51**. Starting with the commercially available **6**, the aryl bromide **46** was prepared in 85% yield in Et_2O at low temperature after sequential addition of *n*-BuLi and CBr_4 (**Scheme 2.14**). The NMR analysis data was in agreement with that reported by Lopes *et al.*[15] However, reaction of the aryl bromide **46** with Mg in either Et_2O or THF failed to give the desired Grignard reagent **50**, with only the starting aryl bromide **46** recovered after purification. It is probable that the situation of the C–Br bond between two *m*-oriented methoxyl groups makes it inaccessible to the metal. Even the addition of small amount of the initiator (iodine) did not improve the reaction. To counter this steric effect of the methoxy groups, we investigated the use of the alkyl bromide **49** as an alternative. It was found that the most efficient way of accessing this was to treat the available starting material **6** sequentially with *n*-BuLi and DMF to give the aldehyde **47** in 72% yield, which on reduction with $NaBH_4$ afforded the alcohol **48** in 92% yield. Subsequent treatment with PBr_3 in pyridine gave the desired alkyl bromide **49** in 91% yield. The appearance in its ^1H NMR spectrum of a singlet at δ 4.68 ppm integrating for two protons provided evidence for the benzylic protons in the CH_2Br group. The ^{13}C NMR spectrum displayed an additional carbon peak at δ 60.9 ppm (for $\mathit{C}H_2Br$) compared with the ^{13}C NMR of starting material **6**.[32-34] However, the alkyl bromide **49** also proved to be unreactive towards Mg in either Et_2O or THF. The methodology was, however, successful when applied to benzyl bromide **54** to give Grignard reagent **56** (**scheme 2.19**).



Scheme 2.19. Synthesis pathway to the hybrid molecules **57** and **58**.

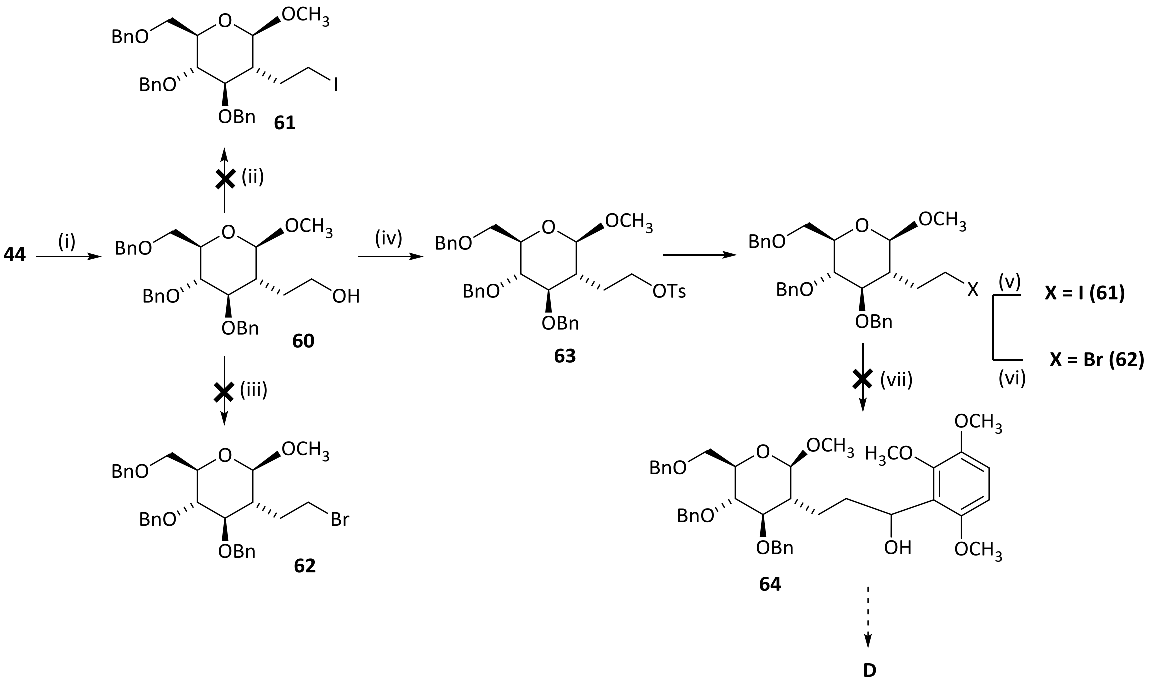
At this stage the possibility of forming the desired ketones was investigated using the simplified Grignard reagents. In the first attempt, Weinreib amide **45** was reacted with commercially available Grignard reagent **55** in THF at 0 °C. The ^1H NMR spectrum of **57** confirmed the absence of the Weinreb amide, and multiplets integrating for the 20 protons in four phenyl groups were apparent in the aromatic region. The absence of an absorption band in the IR for the amide C-N and the presence of band C=O at 1690 cm^{-1} confirmed the structure of **57**. The formation of the Grignard reagent from benzyl bromide **54** and Mg in Et_2O was then carried out, and after cooling to 0 °C, the Weinreb amide **45** was added to the reaction mixture and stirred at room temperature overnight. Ketone **58** was obtained in 67% yield with the ^1H NMR confirming the structural assignment of **58** by displaying 20 protons for the four phenyl groups, and a multiplet at δ 3.65-3.59 ppm corresponding to the two protons H-9 and H-9'. In addition the corresponding signal for C-9 was visible at δ 49.5 ppm in the ^{13}C NMR spectrum. The ESI-HRMS mass spectrum confirmed the structure of **58**, with the ion at *m/z* = 598.3184 corresponding to $[M+NH_4]^+$ ($C_{36}H_{42}NO_6$ requires 598.3169). We thus demonstrated that Weinreb amide **45** reacted efficiently with Grignard reagents **55** and **56** to form corresponding ketones.

Since the synthesis of ketone **52** *via* Weinreb amide **45** was unsuccessful, an attempt was made to prepare it via the route described below (**Scheme 2.20)** involving reaction of an acid chloride with the *ortho*-lithiated species **6a**.



Scheme 2.20. Reagents and conditions: (i) (a) $LiOH.H_2O$, Amberlite IR-120, $MeOH-H_2O$, rt; (b) Oxalyl chloride, 3 drops of DMF, rt to reflux, 30 min; (ii) 2.0 eq. *n*-BuLi (10.0 M in ether), THF, -78 °C, 1 h, Ar; (iii) -18 °C to rt, 3 h.

To investigate the route, the first step was the preparation of acid chloride **59**. This was achieved by hydrolysis of the ester **44** at room temperature, followed by addition of oxalyl chloride and a catalytic amount of DMF.[35] However, when the crude preparation of **59** was added to lithiated derivative **6a** it failed to give expected ketone **52** and trimethoxybenzene **6** was recovered after the work-up process. With this failure to generate the desired ketone **V** in **Scheme 2.16** where the aryl group was suitably functionalized for conversion to the quinone, alternative strategies, which still avoided the formation of an amide, were explored for linking the aryl and sugar units. One alternative envisaged exploiting the possibility of generating alcohol **60** from ester **44** (**Scheme 2.21**)**,** converting this to an iodide or bromide and then attempting formation of a Grignard reagent from this for coupling to a formyl derivative **47** of the trimethoxybenzene.



Scheme 2.21. Reagents and conditions: (i) $LiAlH_4$, 1,4-dioxane, 0 ^oC to rt, 3 h; (ii) Imidazol, PPh_3, I_2, THF, 0 ^oC to reflux, 4 h; (iii) Pyridine, PBr_3, Et_2O, 5 min, rt, Ar; (iv) Pyridine, *p*-TsCl, 0 ^oC to rt, 4 h; (v) NaI, DMF, 60 ^oC, 4 h; (Vi) LiBr, DMF, 60 ^oC, overnight; (vii) Mg, reflux to 0 ^oC, Et_2O, aldehyde **47**, rt.

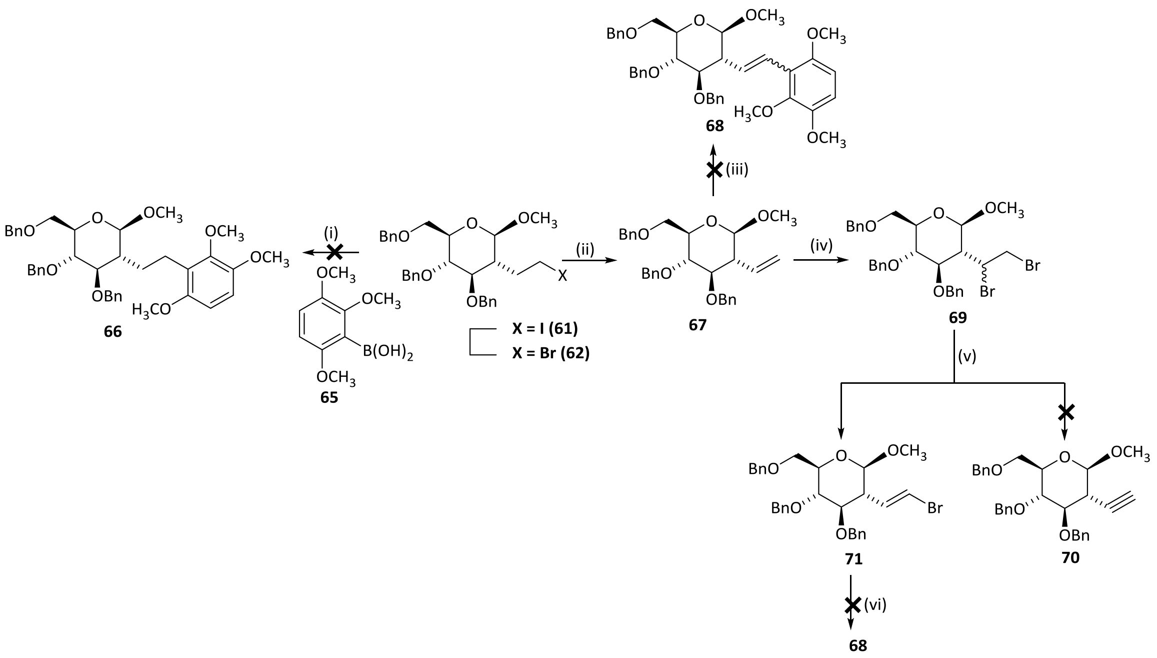
Linker *et al*[30] have reported the reduction of the ester **44** to the alcohol **60** and the primary alcohol was prepared in this way in 79 % yield. However, the direct conversion of the alcohol **60** to the glycosyl iodide **61** or bromide **62** failed.

The alcohol was therefore treated with tosyl chloride in pyridine at 0 ^oC to room temperature[36] to give tosylate **63** in moderate yield (60%). The NMR analysis of the product showed a singlet at δ 2.42 ppm corresponding to three protons of the methyl substituent on the aromatic ring, and also the integration of peaks showed clearly 19 protons attached to the aromatic rings, while the carbon of methyl group appeared at δ 21.5 ppm in its ^{13}C NMR spectrum. In addition, the IR spectrum showed an absorption band at ν (cm^{-1}) = 1092 (S=O) and 901 (S-O).

Treatment of the tosylate **63** with 5.0 eq of NaI in DMF at 60 ^{o}C afforded the iodide **61** in 66% yield.[37] In the ^{1}H NMR of glycosyl iodide **61**, the anomeric proton (H-1) had shifted slightly downfield from its position in the spectrum of tosylate **63** and the disappearance of the signal for the tosyl methyl protons was evident. In addition, the IR spectrum showed an absorption band at ν (cm^{-1}) = 463 (C-I). Treatment of the tosylate with 1.5 eq. of LiBr under the same conditions gave bromide **62** in 69% yield. However, the attempted synthesis of alcohol **64** was unsuccessful, apparently due to the failure to prepare the Grignard reagent from either the iodide or bromide with both the iodide **61** and bromide **62** recovered unchanged after treatment with Mg turnings in Et_2O or THF followed by the aldehyde. Following this series of failures of the Grignard approach, attention turned to other strategies based on metal-mediated sp^2-sp^3 and sp^2-sp^2 cross-coupling.

2.2.4.2. Use of Pd-mediated cross-coupling

Aryl halides are widely used as precursors in organic synthesis for carbon-carbon (**derivative E**) and carbon-heteroatom (**derivative F**) bond formation in transition metal catalysed cross-coupling processes such as the Heck, Suzuki, Sonogashira, Stille, and Ullmann-type coupling reactions.



Scheme 2.22. Reagents and conditions: (i) boronic acid **65**, *t*-BuOK, tricyclohexylphosphine (10%), dioxane, $Pd(OAc)_2$, 100 ^oC, 24 h; (ii) DBU, DMF, 80 ^oC, 3 h; (iii) Aryl bromide **46**, PPh_3, $Pd(OAc)_2$, Et_3N, alkene **67**, dioxane, 100 ^oC, 24 h, Ar; (iv) Br_2, DCM, 0 ^oC to rt; (v) *t*-BuOK, THF, 60 ^oC, 24 h or $NaNH_2$, toluene, 120 ^oC, 24 h; (vi) *E*-vinyl bromide **71**, boronic acid **65**, $(PPh_3)_2PdCl_2$, K_2CO_3, 1,4-dioxahe/H_2O, 100 ^oC, 24, Ar.

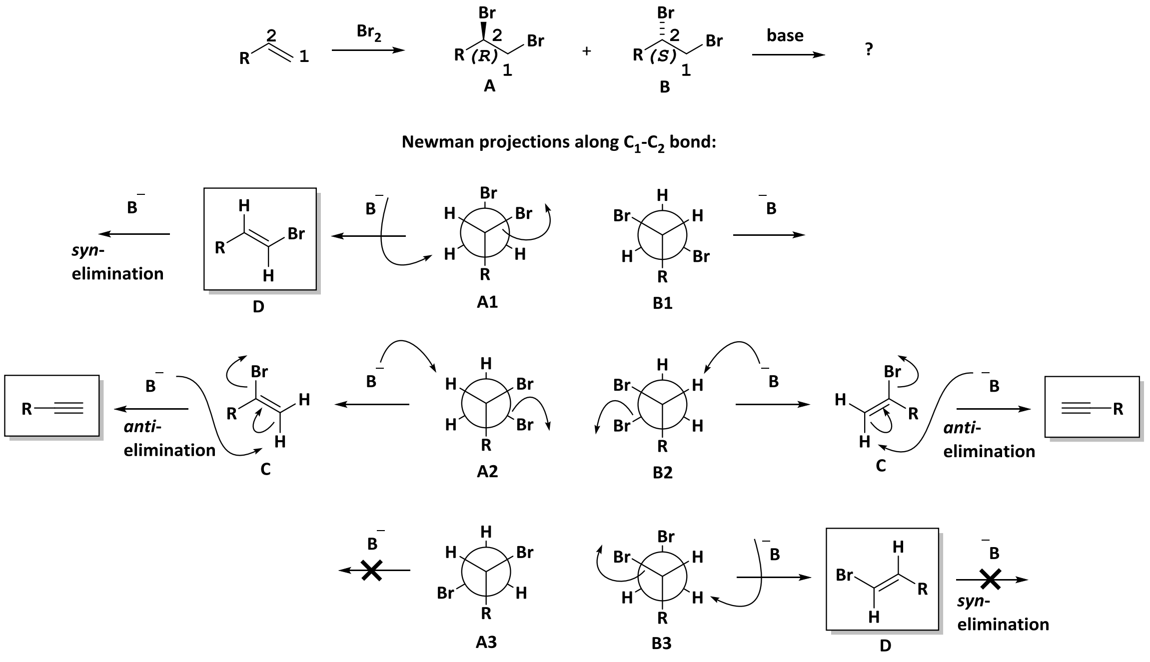
Few successes have been achieved for reactions of alkyl electrophiles, specially unactivated (i.e., not allylic or benzylic) substrates that possess β-hydrogens.[38-42] Fu *et al*[43] established the first palladium or nickel cross-coupling of boronic acid and unactivated alkyl bromide (electrophile) that possess β-hydrogens. He describes a catalyst system that achieves this objective at room temperature. The choice of the less bulky triphenylphosphine catalyst was important for the feasibility of the reaction, and the catalyst system tolerated a wide variety of functional groups such as esters, ethers, thioesters, amides, acetals and nitriles. Aryl, vinyl and unhindered alkyl boronic acids are suitable partners in the processs.[43] The challenge in the present study was to attempt to couple alkyl iodide **61** or bromide **62** with the boronic acid **65** having three methoxy groups, with a view to preparing compounds **66** and **68**, for further transformation to molecule **E** using the Knölker methodology,[13] as described in the scheme 2.2.

The palladium cross-coupling of boronic acid **65** with the unactivated bromide **62** was conducted under conditions established by Fu *et al*.[43] The boronic acid **65** was first prepared in 60% yield from the protocol described by Lopes *et al*.[15] The NMR and MS spectra were in agreement with that reported in the literature.[15] Two equivalents of boronic acid **65** were then combined with iodide **61** or bromide **62** together with three equivalents of *t*-BuOK in dioxane and $Pd(OAc)_2$. However no reaction was observed and starting materials **61** or **62** were recovered, with evidence that the starting material boronic acid had decomposed. Attention therefore turned to the more well-established sp^2-sp^2 cross-coupling reactions.

Over past several decades the Heck reaction has received intensive study,[44] especially the palladium-catalysed arylation and vinylation which have been shown to be one of the most powerful means for the formation of carbon-carbon bond in organic synthesis.[45-49] The phosphine ligands stabilize the active palladium intermediate and assist the initial oxidative-addition to C-X bonds.[50-53] In order to explore the application of the Heck reaction, a strategy was adopted involving preparation of sugar derivatives with vinyl halides in the side chain for attempted cross-coupling with the aryl bromide. Alkene **67** was successfully prepared in 68% yield by treating iodide **61** or bromide **62** with DBU.[54] The ^1H NMR spectrum of **67** displayed a doublet for H-1 at δ 4.21 ppm, while the presence of vinyl group attached at C-2 was deduced by the presence of a doublet of doublets of doublets (ddd) at δ 5.68 ppm for H-7, a triplet of doublets at δ 5.30 ppm for H-8, and doublet of doublets at δ 5.25 ppm for H-8'. In the mass spectrum of **67**, the presence of an ion with *m/z* = 492.2737 was consistent with the ion $[M+NH_4]^+$. The IR spectrum showed an absorption band at ν (cm^{-1}) = 3100 (=C-H), 1643 (C=C).

A Heck reaction was then attempted between aryl bromide **46** and alkene **67**, using 5% mol $Pd(OAc)_2$ as a catalyst, in the presence of Et_3N and dioxane as a solvent at 100 ^oC for 24 h. However, no reaction was observed, and only the starting material was recovered. Increasing the catalyst to 10 mol% yielded no coupling product to form the product **68**.

A Sonogashira reaction was then considered as an alternative. The Sonogashira cross-coupling reaction requires a terminal alkyne and aryl bromide as coupling partners, using a palladium (0) complex in the presence of base, with copper iodide as a co-catalyst. It was envisaged that the required alkyne **70** could be prepared from the alkene **67** *via* a bromination-dehydrobromination sequence. Alkene **67** was therefore treated first with 1.1 eq of bromine in DCM at 0 ^{o}C to give a mixture of unseparable diastereoisomeric 1,2 dibromides **69** in 91% yield in a ratio of 2:1 according to the ^{1}H NMR data. The ^{1}H NMR spectra of the two diasteroisomers **69** were similar, and two doublets were identified as H_{α}-1 and H_{β}-1 at δ 4.47 ppm (*J* = 7.9 Hz) and 4.24 ppm (*J* = 8.1 Hz), corresponding to two anomeric protons of two diasteroisomers. The presence of an ion m/z = 652.1100 corresponding to the ion $[M+NH_4]^+$ was visible in the mass spectrum of the 1,2 dibromide **69**. The 1,2 dibromoalkane **69** was then treated with excess potassium *tert*-butoxide (3.0 eq.) in refluxing THF for 24 h. Somewhat unexpectedly the vinylbromide **71** was isolated as the only product (66%) instead of expected alkyne **70**. The identity of **71** was established by NMR spectroscopy: the ^{1}H NMR spectrum showed a deshielded doublet at δ 6.21 ppm with a large coupling *J* = 13.6 Hz, which was identified as proton H-8 and a doublet of doublet at δ 5.90 ppm with couplings *J* = 9.0, 13.6 Hz corresponding to the proton H-7. The large value of $J_{7,8}$ provided confirmation of the *E*-vinylbromide configuration of the isolated compound **71**. When the $NaNH_2$ was used as base in refluxing toluene, the *E*-vinyl bromide **71** was formed at 61% yield.[55-57]



Scheme 2.23. Analysis of the bromination-dehydrobromination sequence starting with a generalized terminal alkene.

Bromination of the terminal alkene results in a mixture of two 1,2-dibromo diastereomers **A** and **B**. Examination of the three possible conformers of each of these reveals that only two of each (**A1**, **A2**, **B2** and **B3**) have the required *anti* peri-planar orientation of H and Br on adjacent carbons, required for concerted elimination to take place. Both **A2** and **B2** allow for elimination of the Br from C-1, and each leads to the same 2-bromoalkene **C** which is set up for the *anti*-elimination to form the terminal alkyne. However, both of these conformations (**A2** and **B2**) are the highest energy ones on account of the two unfavourable *gauche* interactions (Br↔Br, and Br↔R) and in any case approach of the hindered base to the H on C-2 would be sterically demanding. In contrast, conformations **A1** and **B3** have lower energy on account of just one unfavourable *gauche* interaction (Br↔Br) and are set up for elimination of the more accessible H on C-1 and the Br on C-2. In both cases, irrespective of whether the starting material dibromide has *R* or *S* configuration, the same *E*-vinyl bromide **D** is produced. This elimination is kinetically favoured and predominates, and the *E*-vinyl bromide does not react further with base, as it does not have the configuration allowing for the required *anti*-elimination to give the alkyne.

If the general argument presented above is now applied to the specific substrates in question, the significance of the stereoelectronic factors becomes apparent (**Figure 2.7**). If the most favourable conformations **A1** and **B3** of the dibromide are now placed in the context of the sugar derivative, the favourable conformation around the C_2-$C_{1'}$ bond also needs to be taken into account. This is illustrated in **Figure 2.7** for both the 1′(*R*) and 1′(S*)* dibromo derivatives. In the 1′(*R*) isomer the favourable conformations are illustrated by Newman diagrams, and shown in structure **E**, which gives rise to the (*E*)-vinyl bromide **G** when treated with base as shown. Similarly, the favoured conformations of the 1′(S*)* derivative give rise to structure **F**, which, as shown, also leads to the (*E*)-vinyl bromide **G** when treated with base.

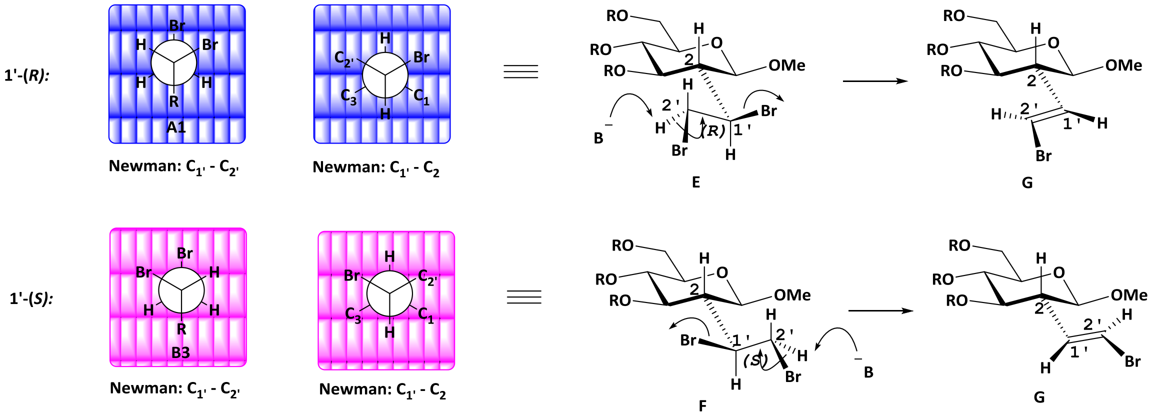
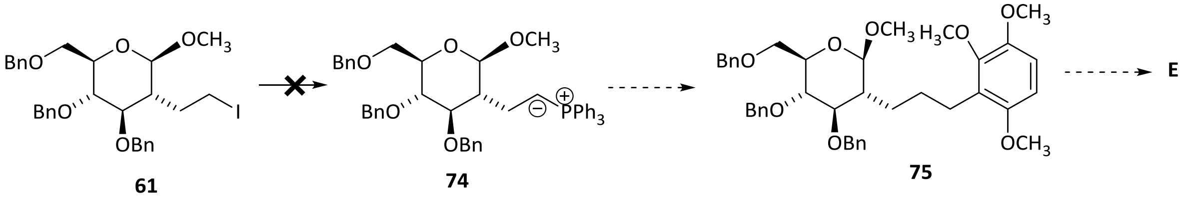


Figure 2.7. Newman projections of the diastereomeric dibromides **69** and illustration of their conversion to the same E-vinylbromide **71.**

Although the intended alkyne was not prepared, the E-vinyl bromide **71** was identified as a useful starting material for attempted Suzuki-Miyaura cross-coupling with the boronic acid **65** to potentially give the compound **68**.[58-59] However, when the *E*-vinyl bromide **71** was treated with boronic acid **65** in the presence of $(PPh_3)_2PdCl_2$ (5 mol%) and K_2CO_3 in 1,4-dioxane/H_2O at 100 ^oC for 24 h, no coupling product **68** was observed, even though TLC of the reaction mixture showed the consumption of starting materials, and products were difficult to identify.

With the continuing failure of metal-mediated cross-coupling methods, alternatives were considered. One of these is the use of the classical Wittig and related reactions in which aldehydes or ketones are reacted with an ylide to give an alkene, with *E*/*Z* selectivity dependent *inter alia* on the nature of the ylide.

2.2.4.3. Use of Wittig strategy

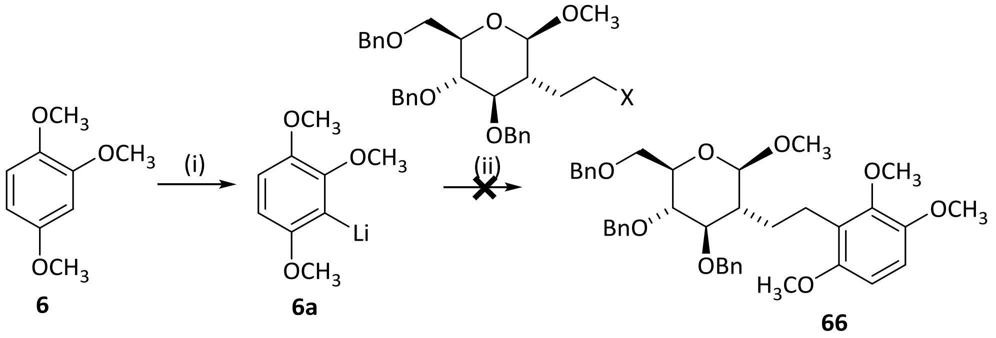


Scheme 2.24. Synthetic pathway to the Wittig product **75**.

The formation of the desired phosphonium salt **74** failed when the glycosyl iodide **61** was sequentially treated with PPh_3 and *n*-BuLi. The cause may be the lack of reactivity of the glycosyl iodide to involve in the S_N2 reaction in the presence of PPh_3.

2.2.4.4. Use of lithiated derivative 6a.

Another method to the target molecule **E** was to couple the glycosyl iodide **61** and the lithiated derivative **6a** (**Scheme 2.25**). Then, the resulting product **66** will be used to construct the target molecule **E** using Knölker methodology, as described in the **scheme 2.2**.



Scheme 2.25. Reagents and conditions: (i) 2.0 eq. *n*-BuLi (10.0 M in ether), THF, -78 ^{o}C, 1 h, Ar: (ii) 4.0 eq. of iodide **61** or bromide **62**, rt, overnight.

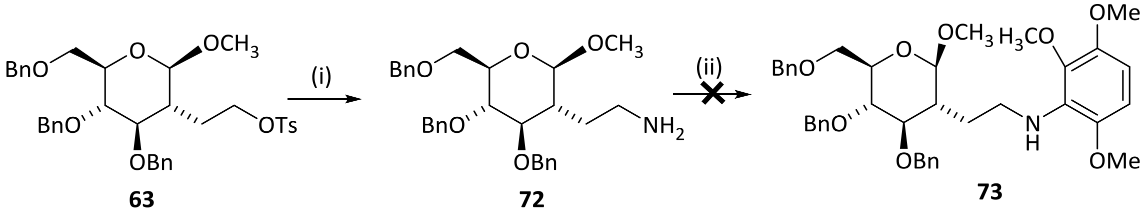
The commercially available starting material **6** was treated with *n*-BuLi at -78 ^{o}C in THF to form the lithiated derivative **6a** (**See scheme 2.25**). In this solution, 4.0 eq. of the glycosyl iodide **61** or bromide **62** was added, and the reaction failed to give the desired compound **66**, Iodide **61** or bromide **62** were recovered, and the starting material **6** which was obtained by protonation of **6a** during work-up process. Presumably, the presence of methoxy groups impedes the reaction.

After many attempts to form carbon-carbon bond, we then turned our attention to forming a carbon-nitrogen bond in order to synthesize hybrid molecule **F** (**Figure 2.4**).

2.2.5. Attempted synthesis of analogues F using C–N bond formation in the key step to form the tethered hybrid molecule.

2.2.5.1. The Buchwald-Hartwig amination.

The Buchwald-Hartwig amination is an organic reaction used to make carbon-nitrogen bonds. This is essentially a cross-coupling reaction of an aryl halide with an amine using palladium as a catalyst (**Scheme 2.26**).

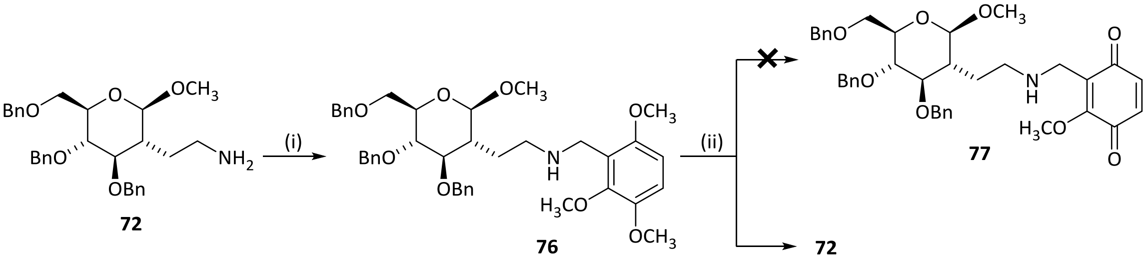


Scheme 2.26. (i) (a) NaN_3, DMF, 80 °C, 3 h; (b) PPh_3/H_2O, THF, 65 °C, 3 h; (ii) Pd_2db_3, (±) BINAP, toluene, Aryl bromide **46**, reflux.

Our initial attempt to the amine **72** involved the displacement of the tosyl group employing the sodium azide as a source of amine, and reduction of the azide under Staudinger conditions.[60] Treatment of tosyl derivative **63** with 2.0 eq. of NaN_3 in DMF at 80 °C for 3 h afforded the azide, which was then immediately reduced using 2.0 eq. of PPh_3 under Staudinger conditions to give one product, the amine **72** in 68% yield. In the ^{1} HNMR spectrum of **72**, the triplet corresponding to the H-8 and H-8' had shifted from δ 2.02 - 1.91 ppm (H-8) and δ 1.81 - 1.71 ppm (H-8') in the spectrum of **63** to δ 2.69 ppm in the spectrum of **72**, supported its structure as well as the absence of the methyl peaks attached of tosyl group in the ^{13}C NMR. In addition, the IR spectrum showed an absorption band at ν_{max}/cm^{-1} 3006, confirming the presence of NH group. The Buchwald-Hartwig amination[61] reaction was attempted using 1.0 eq. of amine **72**, 2.0 eq of the aryl bromide **46,** Pd_2dba_3 as catalyst in the presence of $CsCO_3$, and BINAP as ligands in toluene. No reaction took place after refluxing the reaction mixture for 24 h. Replacing Pd_2dba_3 and toluene by $Pd(OAc)_2$ and dioxane respectively, the reaction also failed to give the desired product **73** after heating at 100 °C, and only the starting materials were recovered. Presumably, the large electrophile is problematic for all Pd-mediated cross-coupling reactions involving the aryl bromide **64** or boronic acid **65,** and the steric effect of the methoxy group might be the cause of failure of the reaction.

2.2.5.2. *Via* condensation of amine and aldehyde (Reductive amination reaction).

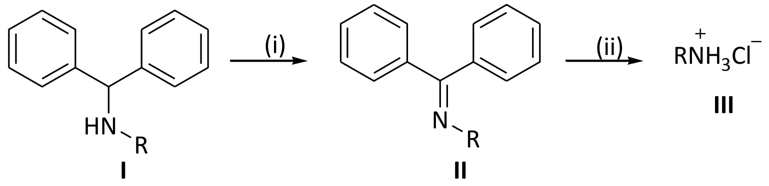
In this approach, the aldehyde **47** and amine **72** are mixed with a reducing agent such as $NaBH_4$.



Scheme 2.27. Reagents and conditions: (i) (a) aldehyde **47**, MeOH, rt, overnight; (b) NaBH$_4$, 5 h, r; (ii) PIDA, MeOH/H$_2$O, overnight, rt.

We conducted a one pot reaction between the amine **72** and aldehyde **47** at room temperature. After stirring overnight, the reducing agent NaBH$_4$ (1.5 eq.) was added to the reaction mixture.[62] The reaction was complete within 5 h, and yielded a brown oil (72%) after filtration, shown to be the amine **76**. The ^{1}H NMR spectrum of **76** showed two doublet of doublet peaks characteristic of two *ortho* protons at δ 6.73 ppm with *J* = 8.8 Hz and 6.50 ppm with *J* = 9.0 Hz and three singlets at 3.79, 3.78 and 3.71 ppm relative to three methyl groups of the trimethoxybenzene unit. The ^{13}C NMR confirmed the structure of **76** by displaying four methoxy peaks at δ 61.0, 56.7, 56.3 and 55.8 ppm. From the mass spectrum, we detected the ion mass *m/z* = 672.3536, which was related to the ion $[M+H]^+$. In addition, the IR spectrum showed an absorption band at ν_{max}/cm^{-1} 3059, confirming the presence of NH group.

We then attempted an oxidation reaction on compound **76** using 1.5 eq of PIDA at room temperature, and stirred for 19 h. In the ^{1}H NMR spectrum of compound **77**, the expected singlet peak characteristic of the methyl protons of the quinone moiety were not visible, and two signal peaks relative to two *ortho* protons could not be seen. The mass spectrum showed the ion mass m/z = 492.2748, which matches with the ion $[M+H]^+$ of the amine **72**. The NMR data (^{1}H NMR and ^{13}C NMR) was the same with the one reported for the amine **72**. The mechanism proceed through the formation of imine, followed by the hydrolysis to give amine **72**. In this reaction, we therefore simply achieved an oxidative deprotection of a benzylated amine. This had been demonstrated by Honek *et al*,[63] who described the oxidative deprotection of diphenylmethylamines **I** by the addition of DDQ to form imine **II**, which was hydrolysed under mildy acidic conditions to give the desired product as the hydrochloride salts **III** (**Scheme 2.28**).



Scheme 2.28. Reagents and conditions: (i) DDQ, benzene, crushed 4Å molecular sieves, 60 °C, 1 h.; (ii) 0.1 N HCl, diethyl ether, 6 h.

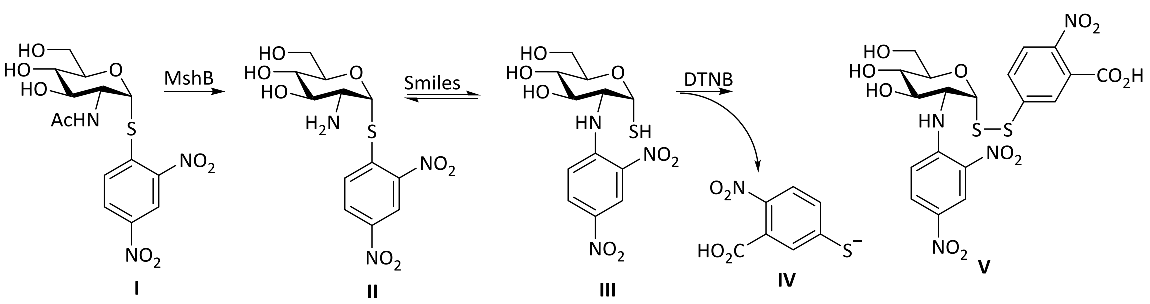
The failure of a number of approaches to formation of hybrid structures through C–C, C–N, or C–O bond forming strategies suggests that the entire approach needs to be re-evaluated. Some commentary on this problem is given in the conclusion to this chapter.

2.3. Biological evaluation and SAR interpretation.

Several of the compounds prepared in this study, together with other related ones prepared by co-workers, were assessed for inhibitory activity against MshB, using the method established by Lamprecht *et al*.[65]

2.3.1. Description of the assay.

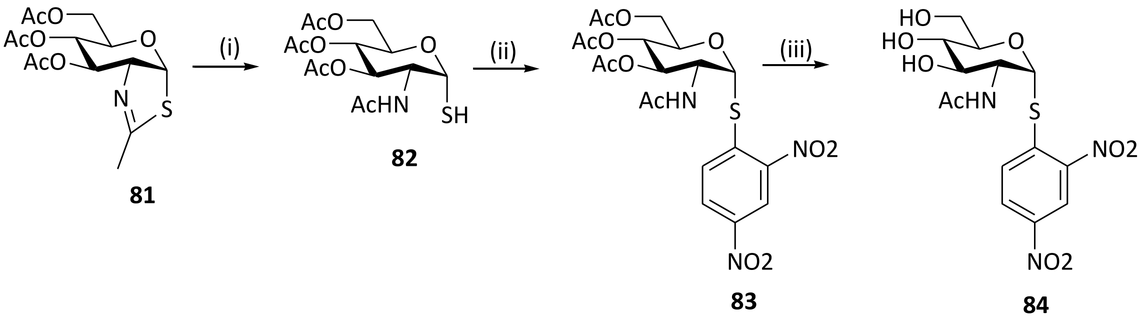
The determination of biological activity of compounds was based on the sequence described in the **scheme 2.29**.



Scheme 2.29. Proposed formation of the chromophore **IV** and the mixed disulphide **V**.

The assay is based on the observation that the dinitrophenyl thioglycoside **I** is a competitive substrate for MshB. It therefore undergoes *N*-deacetylation in the presence of MshB to give the free amine **II**, and this product undergoes subsequent Smiles rearrangement to give the anomeric thiol **III**. This can then be reacted with DTNB to form the chromophore **IV** and the disulphide **V (Scheme 2.29)**. The inhibitory activity of compounds can therefore be calculated on the basis of the extent to which production of disulphide **V** and the chromophore is diminished. This means that a good inhibitor will block the action of MshB, and no production of the disulphide **V** will be observed.

The substrate for the assay was selectively prepared (**Scheme 2.30**)[65-68] from the protected starting material GlcNAc-thiazoline triacetate **81** obtained from Dr Anwar Jardine (University of Cape Town). Acetal hydrolysis of the thiazoline **81** gave the mercaptan **82**, and this was followed by triethylamine-mediated *S*-alkylation with 1-fluoro-2,4-dinitrobenzene to afford **83**, which upon *O*-deacetylation gave the unprotected 2,4-dinitrophenyl thioglucoside **84**.[65-68] The NMR data were in agreement with the literature.



Scheme 2.30. Reagents and conditions: (i) 2 drops TFA, 2 drops H_2O, MeOH; (ii) 1-fluoro-2,4-nitrobenzene, Et_3N, DCM, 25 ^oC; (iii) MeOH, H_2O, acetone, Amberlite IR-400 (OH^-).

The compounds prepared in this study (**B**, **27**, **38**, **40** in Figure 2.8) were assayed for inhibitory activity against MshB as part of a series of compounds prepared by others in parallel studies. Compound **B** is the carbazole quinone with the highest anti-TB activity among a set of carbazole quinone analogues on which this study was based, and compounds **B1** and **27** represents variations of these. **GlcNAcSPh** is an analogue of the natural substrate of MshB, known to also have substrate activity less than the natural substrate.[64] Compounds **38**, **40**, **78**, **79** and **80** are the hybrid compounds which have in common the carbazole quinone and phenyl-2-acylamino-1-thio-α-D-glucopyranoside units, tethered via an alkyl chain attached at various points to the carbazole quinone unit.

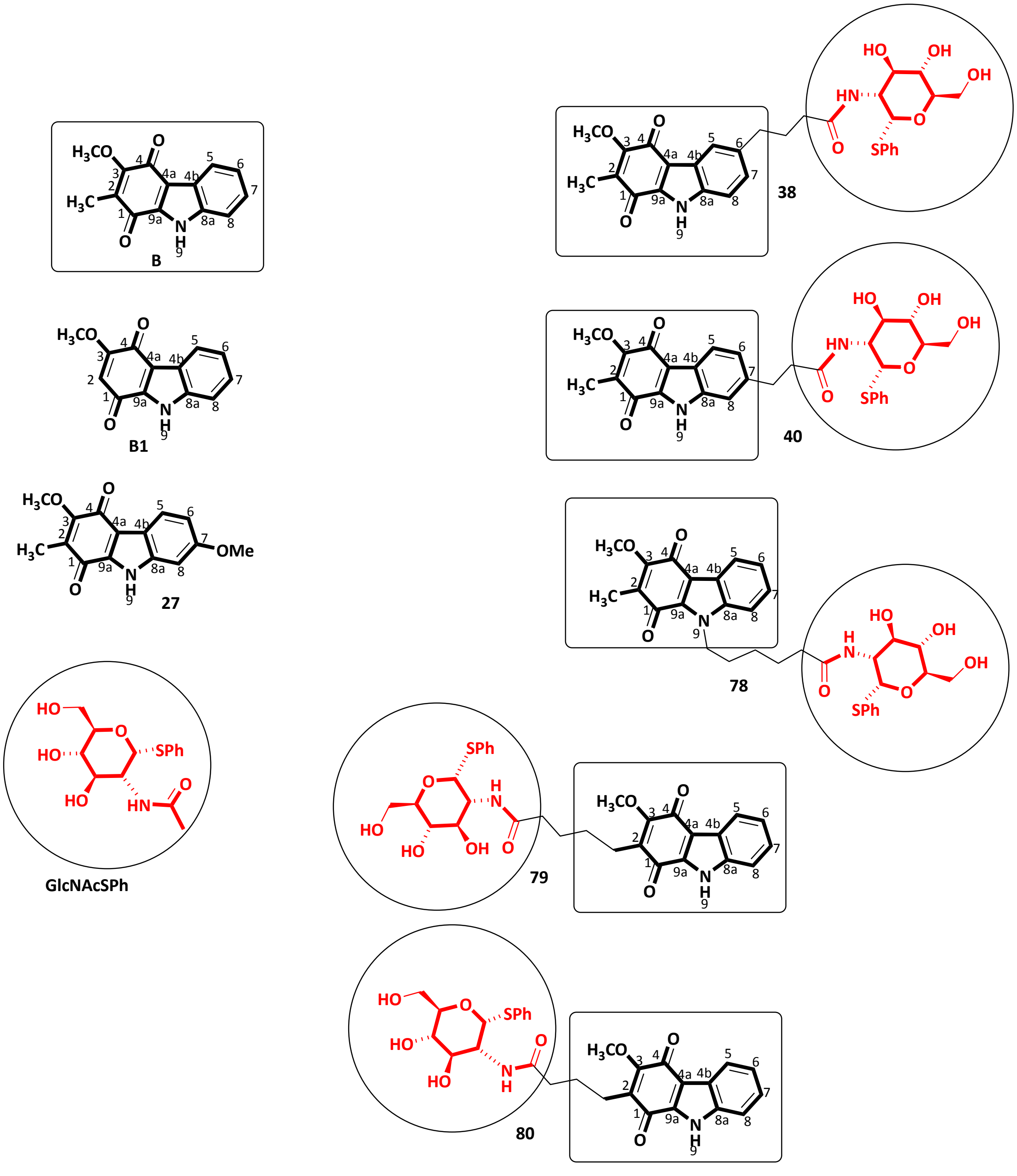


Figure 2.8. Structures of compounds submitted for inhibitory activity against MshB.

2.3.2 Assay results.

The assay results are shown below in **Figure 2.9**. This data represents the activity of the enzyme in the presence of both the substrate and the respective potential inhibitor from the list of compounds: the best inhibitor is therefore the one showing lowest activity of the enzyme. Firstly it is important to note that, as expected, no activity is observed in the absence of MshB. Furthermore, the absence of inhibitory activity for **GlcNAcSPh** is important since this demonstrates that this does not compete with the 2,4-dinitrophenyl thioglucoside **84** as a substrate in the assay.

In addition, it was noted that the compounds to be assayed were prepared in DMSO solution before dilution with buffer and combining with the enzyme. Therefore, the activity of the enzyme against the 2,4-dinitrophenyl thioglucoside (**GlcNAcSDNP**) as substrate with only DMSO present was set at 100% (bar labelled “DMSO” in **Figure 2.9**), and other activities measured relative to this, noting that these represent % inhibition at a given concentration of substrate (150µM). Therefore, compounds showing <100% activity have inhibitory activity.

Taking these considerations into account, the rest of the data therefore shows that only three compounds show significant inhibitory activity, with **78** exhibiting about 70% inhibition, followed by **79** with about 50% and **80** with 40% inhibition. Compound **38**, prepared in this study, shows about 20% inhibition, while the C-7-substituted analogue **40** does not show any inhibitory activity. From an SAR point of view, this can be interpreted as follows: the absence of inhibitory activity in either the sugar unit or the carbazole quinone units on their own, and the presence of activity in some of the hybrid, tethered molecules suggest that the combination of the two is indeed important for inhibitory activity. Furthermore, it is clear that the point of attachment of the sugar unit, and to an extent the length of the tether, do contribute to inhibitory activity.

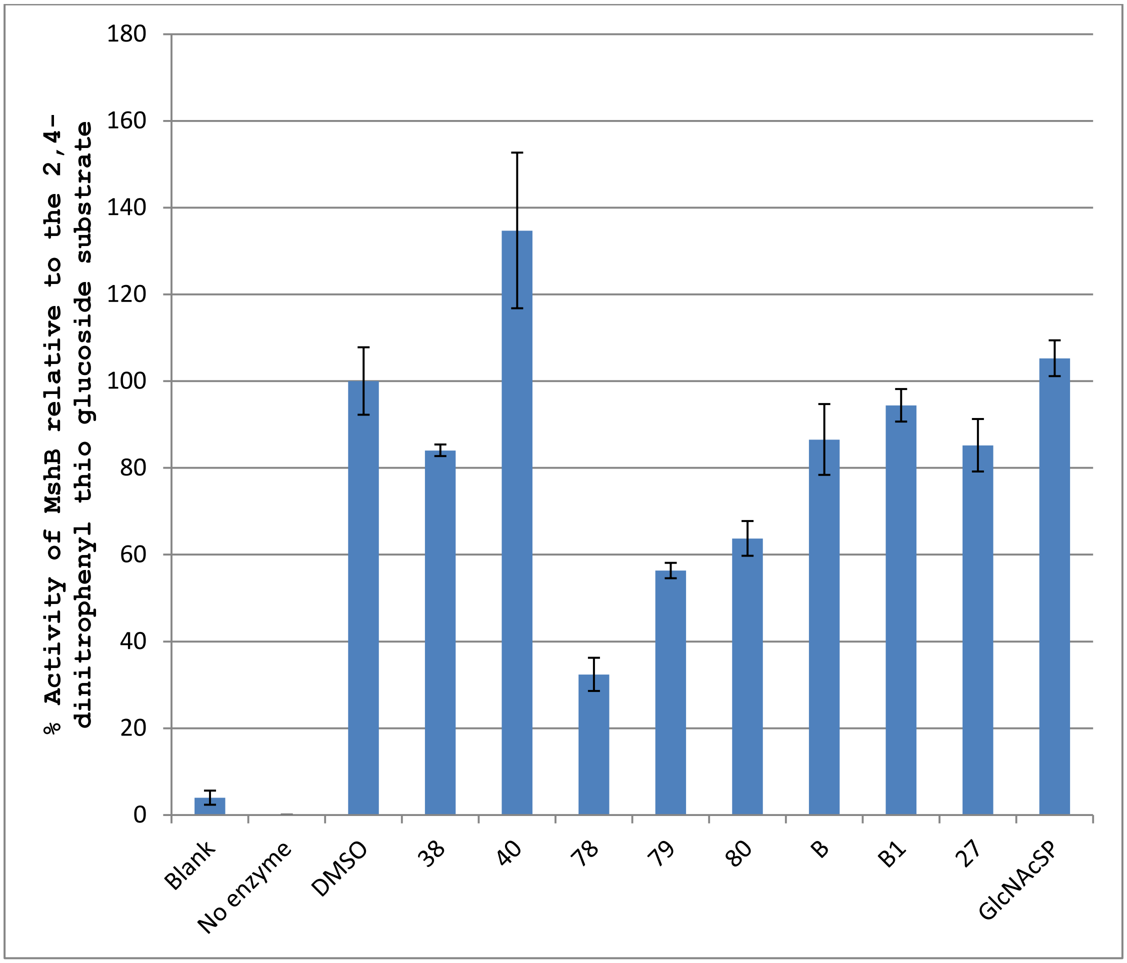


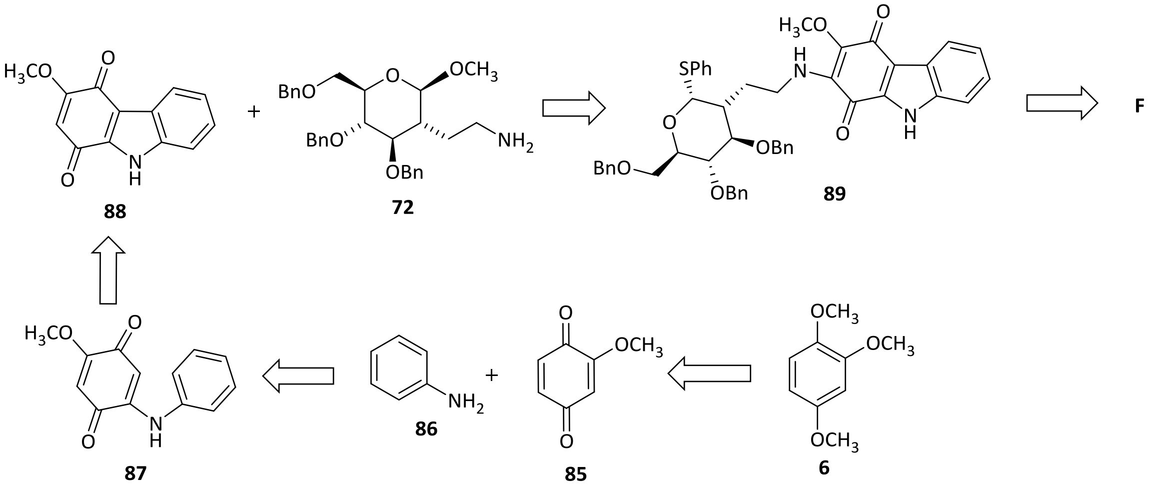
Figure 2.9. Inhibition of MshB by mycothiol analogues incorporating carbazole quinones.

2.4. Conclusion.

This project aimed to synthesize and evaluate analogues of mycothiol incorporating carbazole quinones as inhibitors of enzymes in the *Mycobacteria.* Some works suggest that molecules, which have naphthoquinonyl unit **A.3** tethered to the pseudodissacharide core, have a significant inhibitory activity against mshB and mca in the biosynthesis pathway. The phenyl-2-acetamido-1-thio-α-D-glucoside has been shown to be substrate for MshB. These findings, together with the independent discovery of anti-TB activity associated with carbazole quinone led to establish a methodology for the construction of the hybrid molecule **38** and **40**, which involves the

Pd-mediated coupling for the formation of carbazole quinone using Knölker's strategy. We also studied the reactivity of Weinreb amide **45** in the presence of Grignard reagents to provide ketone **57** and **58**. We were also able to link the amine **74** and the aldehyde **47** in one pot reaction. Unfortunately, the attempt to oxidize failed to provide the quinone **77**. Again, the attempt to create carbon-carbon bond with Pd failed to provide the desired hybrid **66** and **68**. Biological results proved that the combination of carbazole quinone unit and the sugar unit is very important for the inhibitory activity. The compound **78** has the highest inhibitory activity against MshB among a set of series of carbazole quinone analogues assayed. This demonstrated that the structure-activity relationship do contribute to inhibitory activity, which depend on the point of attachment as long as the length of tether.

In terms of ongoing work, it is suggested that the preparation of the target hybrid molecule **F** could be achieved via the 1,4-addition of a nucleophile such as amine **72** (or possibly the corresponding alcohol) to the carbazole quinone **88** to provide the hybrid molecule **89** (or the corresponding ether). The carbazole quinone **88** would be prepared by the standard Knölker methodology (**scheme 2.31**).



Scheme 2.31. Retrosythetic pathway to compound **F.**

2.5. References

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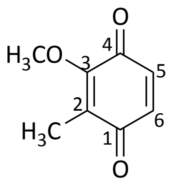
CHAPTER 3

EXPERIMENTAL

3.1. General

All solvents were dried and distilled before use. Commercially available reagents were used without purification. Reactions were performed under an inert atmosphere of nitrogen or argon in flame dried glassware. Reactions were monitored by thin-layer chromatography (TLC) carried out on Merck silica gel 60-F_{254} pre-coated plates and products visualized under ultraviolet light or by wetting the plate with either a solution of anisaldehyde in ethanolic sulphuric acid, ceric ammonium sulphate or ninhydrin followed by heating. Column chromatography was performed on silica gel 60, eluting with the mixtures of light petroleum and ethyl acetate, or on a Biotage Isolera system, using prepacked silica columns. Nuclear magnetic resonance spectra (^{1}H, ^{13}C and 2D NMR) were recorded either on a Varian Mercury 300 MHz, a Varian Unity 400 MHz or a Bruker Advance III with Ultra Shield 400 Plus magnet, at 25 ^{0}C in deuterated $CDCl_3$ or DMSO as the solvent. The ^{1}H, ^{13}C and 2D NMR spectra of carbazole quinone derivatives were recorded on a Bruker 300 or 400 MHz, or DRX-500 spectrometer. Infrared spectra were recorded on a Perkin-Elmer Spectrum 100 FT-IR spectrometer in the range 4000 – 600 cm^{-1} using thin films of compound on NaCl disks. Microanalyses were determined using a Fisons EA1108 CHNS-O instrument. Chemical shifts are given in ppm relative to tetramethylsilane (TMS, $\delta = 0.00$ ppm), which is used as internal standard. Assignments were confirmed by COSY and HSQC analysis. Coupling constants (*J*) are reported in Hertz (Hz). The spin multiplicities are indicated by the symbol s (singlet), d (doublet), dd (doublet of doublet), t (triplet), m (multiplet), and br (broad). Melting points were determined using a Reichert-Jung Thermovar hot-plate microscope and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR spectrometer and absorption frequencies given in cm^{-1}. Mass spectra were recorded on a JEOL GC MATE II magnetic sector mass spectrometer and the base peaks are given. Mass Spectra results for the carbazole quinone derivatives were obtained using an Agilent 6890N GC/5973N MSD gas chromatography/mass spectrometry system. HRMS data were obtained using a Waters Synapt G2 with direct injection (1 µL) into a stream into a stream of 80% acetonitrile, 0.1% formic acid using a Waters UPLC at flow rate of 0.1mL/min. Source: Electrospray positive/negative, Capillary voltage 3 kV, Cone Voltage 25 V.

2-Methyl-3-methoxy-1,4-benzoquinone (8)



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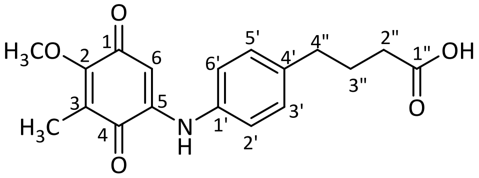
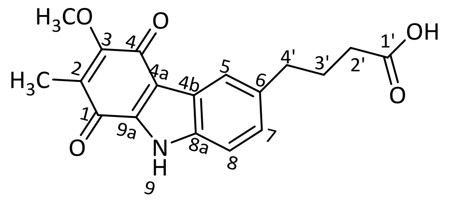
A solution of 1,2,4-trimethoxybenzene **6** (4.50 g, 26.8 mmol) in dry THF (50 mL) was cooled to -78 °C. *n*-BuLi (10.0 M, 5.34 mL, 53.4 mmol) was added dropwise and the resulting mixture was stirred at the same temperature for 1 h. Methyl iodide (30.37 g, 214 mmol) was then added dropwise and the reaction allowed to warm to room temperature gradually overnight. The mixture was quenched with saturated aqueous NH_4Cl solution and extracted with ether (50 mL x 3). The combined organic phase was washed with water and brine, dried over $MgSO_4$, filtered and the filtrate evaporated under reduced vacuum to afford compound **7** as a yellow oil. Without further purification, the crude product **7** was taken up in a mixture of H_2O (61 mL) and MeOH (20 mL) and iodobenzene diacetate (13.14 g, 40.79 mmol) added with stirring at room temperature. The mixture was stirred for 19 h at room temperature and then extracted with ether (50 mL x 3). The combined organic layer was washed with saturated $NaHCO_3$, water and brine, dried $MgSO_4$, filtered and the filtrate evaporated under reduced vacuum to afford an orange oil. The product was purified by column chromatography on silica gel using pentane-ethyl acetate (8:1) as eluent to give **8** as an orange oil (3.21 g, 79 %, over two steps, lit.[13]). ^{1}H NMR (500 MHz, chloroform-d) δ = 6.69 (dd, *J* = 1.0, 10.1 Hz, 1 H), 6.60 (dd, *J* = 1.0, 10.1 Hz, 1 H), 4.02 (s, 3 H, OC*H₃*), 1.95 (s, 3 H, C*H₃*). ^{13}C NMR (125 MHz, chloroform-d) δ = 188.3 (*C*=O), 183.2 (*C*=O), 155.6 (*C*), 136.3 (*C*H), 134.7 (*C*H), 129.0 (*C*), 60.8 (O*C*H$_3$), 8.6 (*C*H$_3$). IR (ATR): ν (cm^{-1}) = 1699, 1643. (UV) (MeOH): λ (nm) = 96, 203, 251, 366. ESI-MS (+10 V): *m/z* = 153.2 [M+H]$^+$.

2-Methoxy-3-methyl-5-[(4'-(3"-carboxypropyl(anilino)]-1-4-benzoquinone (10)



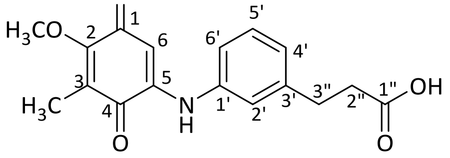
Quinone **8** (276 mg, 1.81 mmnol) and amine **9** (152 mg, 0.921 mmol) were mixed in methanol (5 mL). The solution was stirred at room temperature overnight under argon. A precipitate formed and was isolated by filtration, then washed with methanol and dried under reduced vacuum to afford the quinone **10** (223 mg, 82%) as a violet solid, mp 129-131 °C (decomp). ^{1}H NMR (500 MHz, chloroform-d) δ = 7.50 (s, 1 H, N*H*), 7.23 - 7.19 (m, 2 H), 7.15 - 7.12 (m, 2 H), 5.93 (s, 1 H, H-6), 4.14 (s, 3 H, OC*H₃*), 2.68 (t, *J* = 7.3, 8.2 Hz, 2 H), 2.39 (t, *J* = 7.4, 7.4 Hz, 2 H), 2.02 - 1.96 (m, 2 H), 1.96 (s, 3 H, C*H₃*). ^{13}C NMR (125 MHz, chloroform-d) δ = 184.4 (*C*=O), 182.5 (*C*=O), 178.8 (*C*=O), 157.9 (*C*), 143.2 (*C*), 138.7 (*C*), 135.5(*C*), 129.6 (2 x *C*H), 122.9 (*C*), 122.4 (2 x *C*H), 98.4 (*C*H), 61.5 (*C*H$_3$), 34.4 (*C*H$_2$), 33.1 (*C*H$_2$), 26.1 (*C*H$_2$), 8.5 (*C*H$_3$). IR (ATR): ν (cm^{-1}) = 3303, 2939, 1711, 1633, 1588, 1527, 1445, 14.17, 1346, 1272, 1214, 1158, 1001, 811, 686, 635. (UV) (MeOH): λ (nm) = 196, 202, 207, 254 (sh), 279 (sh), 321 (sh). ESI-MS (+50 V): *m/z* = 330.2 [M+H]$^+$, 352.1 [M+Na]$^+$, 681.3 [2M+Na]$^+$. ESI-MS (-25 V): *m/z* = 327 [M-H]$^+$, 657 [2M+H]$^+$. Calcd for $C_{18}H_{19}NO_5$: C, 65.64; H, 5.81; N, 4.25. Found: C, 65.75; H, 5.89: N, 4.28.

3-Methoxy-2-methyl-6-(carboxypropyl)-9*H*-carbazole-1,4-quinone (11)



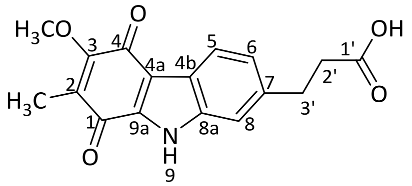
A solution of **10** (100 mg, 0.304 mmol), Pd(OAc)$_2$ (5 mol%), K_2CO_3 (0.4 mg, 0.029 mmol), and pivalic acid (1000 mg) was weighed and transferred into a test tube. The uncapped test tube was placed in an oil bath and the mixture stirred under air at 120 °C overnight. The mixture was then cooled to room temperature, diluted with EtOAc, washed with water, dried over $MgSO_4$, filtered, and the filtrate evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (DCM / 0.1% AcOH in EtOAc in 1:1 ratio) to afford carbazole quinone **11** (84.9 mg, 85%) as a red solid, mp 214-217 °C (decomp). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 7.80 (s, 1 H, H-5), 7.44 (d, *J* = 8.2 Hz, 1 H, H-8), 7.24 - 7.19 (m, 1 H, H-7), 4.02 (s, 3 H, OCH_3), 2.70 (t, *J* = 7.6 Hz, 2 H), 2.19 (t, *J* = 7.4 Hz, 2 H), 1.90 (s, 3 H, CH_3), 1.86 - 1.79 (m, 2 H). ^{13}C NMR (125 MHz, DMSO-d_6) δ = 180.6 (*C*=O), 178.5 (*C*=O), 157.8 (*C*=O), 137.7 (*C*), 136.6 (*C*), 136.1(*C*), 127.2 (*C*), 126.4 (*C*), 123.9 (*C*), 123.4 (*C*), 120.4 (*C*H), 113.8 (*C*H), 113.0 (*C*H), 61.1 (OCH_3), 34.8 (CH_2), 33.7 (CH_2), 27.0 (CH_2), 8.6 (CH_3). IR (ATR): ν (cm^{-1}) = 3220, 2949, 2915, 2854, 2048, 2030, 2009, 1696, 1628, 1592, 1536, 1430, 1399, 1377, 1289, 1245, 1206, 1103, 1044, 1001, 951, 906, 818, 761, 745, 687, 640. (UV) (MeOH): λ (nm) = 197, 229, 260, 265 (sh), 282 (sh), 311, 409. ESI-MS (+50 V): *m/z* = 350.2 [M+Na]$^+$, 677.3 [2M+Na]$^+$. ESI-MS (-50 V): *m/z* = 325.8 [M-H]$^+$. Calcd for $C_{18}H_{17}NO_5$: C, 66.05; H, 5.23; N, 4.28. Found: C, 66.11; H, 5.26: N, 4.31.

2-Methoxy-3-methyl-5-[(3’-(2”-carboxyethyl(anilino)]-1-4-benzoquinone (13)



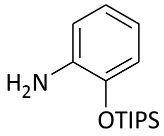
Quinone **8** (95 mg, 0.624 mmnol) and amine **12** (59 mg, 0.357 mmol) were combined in methanol (4 mL). The solution was stirred at room temperature overnight under argon. A precipitate formed and was isolated by filtration, then washed with methanol and dried under vacuum to afford the quinone **13** (84.1 mg, 75%) as a violet solid, mp 141-143 °C (decomp). ^{1}H NMR (500 MHz, chloroform-d) δ = 7.53 (s, 1 H, N*H*), 7.32 (t, *J* = 7.9 Hz, 1 H, H-5'), 7.10 (s, 1 H, H-2'), 7.04 (d, *J* = 5.7 Hz, 2 H, H-6', H-4'), 5.97 (s, 1 H, H-6), 4.14 (s, 3 H, OCH_3), 2.97 (t, *J* = 7.6 Hz, 2 H), 2.70 (t, *J* = 7.4 Hz, 2 H), 1.96 (s, 3 H, CH_3). ^{13}C NMR (125 MHz, chloroform-d) δ = 184.3 (*C*=O), 182.8 (*C*=O), 176.5 (*C*=O), 157.9 (*C*), 143.1 (*C*), 142.1 (*C*), 137.6 (*C*), 129.8 (*C*H), 125.5 (*C*H), 123.1 (*C*), 122.2 (*C*H), 120.2 (*C*H), 98.6 (*C*H), 61.5 (OCH_3), 35.1 (CH_2), 31.9 (CH_2), 8.5 (CH_3). IR (ATR): ν (cm^{-1}) = 3297, 3031, 2961, 2921, 2757, 2583, 2056, 1726, 1648, 1568, 1525, 1452, 1376, 1346, 1215, 1158, 995, 975, 858, 836, 800, 757, 687, 661, 586, 548. (UV) (MeOH): λ (nm) = 209, 262, 282 (sh), 325, 514. ESI-MS (+25 V): *m/z* = 316.1 [M+H]$^+$, 653.3 [2M+Na]$^+$. ESI-MS (-10 V): *m/z* = 628.9 [2M-H]$^-$. Calcd for $C_{17}H_{17}NO_5$: C, 64.75; H, 5.43; N, 4.44. Found: C, 64.90; H, 5.39: N, 4.45.

3-Methoxy-2-methyl-7-(carboxyethyl)-9*H*-carbazole-1,4-quinone (14)



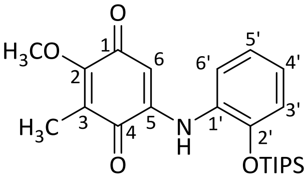
A solution of **13** (440 mg, 1.40 mmol), $Pd(OAc)_2$ (20 mol%), K_2CO_3 (19.2 mg, 0.507 mmol), and pivalic acid (3000 mg) was weighed in air and transferred into a 25 mL round bottom flask. The uncapped flask was placed in an oil bath and the mixture was stirred under air at 120 °C overnight. The solution was then cooled to room temperature, diluted with EtOAc, washed with water, dried over $MgSO_4$, filtered, and the filtrate evaporated under reduced pressure. The crude product was purified by silica gel column chromatography (DCM / 0.1% AcOH in EtOAc in 1:1 ratio) to afford carbazole quinone **14** (255 mg, 51%) as a pale orange solid, mp 213-215 °C (decomp). ^{1}H NMR (500 MHz, DMSO-d$_6$) δ = 7.89 (d, *J* = 8.2 Hz, 1 H, H-5), 7.31 (s, 1 H, H-8), 7.14 (d, *J* = 9.5 Hz, 1 H, H-6), 4.02 (s, 3 H, OC*H$_3$*), 2.93 (t, *J* = 7.4 Hz, 2 H), 2.55 (t, *J* = 7.6 Hz, 2 H), 1.90 (s, 3 H, C*H$_3$*). ^{13}C NMR (125 MHz, DMSO-d$_6$) δ = 180.7 (*C*=O), 177.2 (*C*=O), 158.6 (*C*=O), 138.2 (*C*), 136.9 (*C*), 126.4 (2 x *C*), 126.1 (*C*), 122.9 (*C*), 121.9 (*C*), 114.1 (*C*H), 112.8 (*C*H), 111.8 (*C*H), 61.1 (O*C*H$_3$), 36.1 (*C*H$_2$), 30.5 (*C*H$_2$), 8.3 (*C*H$_3$). IR (ATR): ν (cm^{-1}) = 3232, 3021, 2928, 2860, 1708, 1654, 1625, 1535, 1457, 1431, 1376, 13001, 1255, 1200, 1107, 995, 824, 754, 674, 627, 594. (UV) (MeOH): λ (nm) = 228, 261, 271 (sh), 280 (sh), 313, 392. ESI-MS (+100 V): *m/z* = 314.1 [M+H]$^+$, 336.1 [M+Na]$^+$, 627.1 [2M+H]$^+$, 643.9 [2M+NH$_4$]$^+$, 649.2 [2M+Na]$^+$. ESI-MS (-10 V): *m/z* = 624.9 [2M-H]$^-$. Calcd for $C_{17}H_{15}NO_5$: C, 65.17; H, 4.83; N, 4.47. Found: C, 65.15; H, 4.84: N, 4.50.

2-Triisopropylsilyloxyaniline (16)



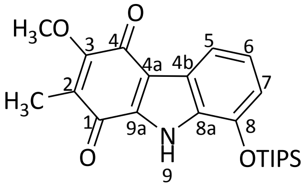
A solution of 2-aminophenol **15** (0.514 g, 4.71 mmol), TIPSCl (1.28 mL, 5.99 mmol), and imidazole (0.478 g, 7.02 mmol) in THF (5 mL) was stirred at room temperature for 14 h. Water was added to quench the reaction, and the organic layer was separated. The aqueous layer was extracted twice with ethyl acetate. The combined organic layers were washed with brine, and dried over Na_2SO_4, then concentrated under reduced pressure. The resulting crude was purified by silica gel column chromatography (eluent: Hexane/EtOAc: 20/1) to afford the TIPS ether **16** as a brown oil in almost quantitative yield (1.23 g, 98%, lit.[20]). ^{1}H NMR (500 MHz, chloroform-d) δ = 6.77 - 6.79 (m, 2 H), 6.74 (dd, *J* = 1.6 Hz, 8.1 Hz, 1H), 6.58-6.64 (m, 1H), 1.28 - 1.36 (m, 3 H, Si(C*H*)CH$_3$CH$_3$), 1.14 (d, *J* = 7.6 Hz, 18 H, Si(CH)C*H$_3$*C*H$_3$*). ^{13}C NMR (126 MHz, chloroform-d) δ = 143.3, 137.8 (Aromatic *C*), 121.5, 118.3, 117.9, 115.5 (Aromatic *C*H), 18.0 (6 x *C*H$_3$, 3 x Si(CH)*C*H$_3$*C*H$_3$), 17.7 (*C*H), 12.9 (*C*H), 12.2 (*C*H). IR (ATR): ν (cm^{-1}) = 3416, 2942, 2892, 2865, 1609, 1593, 1570, 1544, 1542, 1503, 1462, 1393, 1367, 1333, 1275, 1224, 1137, 1069, 1034, 1012, 995, 919, 881, 804, 741, 675. ESI-MS (+25 V): *m/z* = 266.1 $[M+H]^+$, 531.3 $[2M+H]^+$. Calcd for $C_{15}H_{27}NOSi$: C, 67.87; H, 10.25; N, 5.28. Found: C, 67.77; H, 10.23; N, 5.24.

2-Methoxy-3-methyl-5-[2'-(triisopropylsilyloxy)anilino]-1,4-benzoquinone (17)



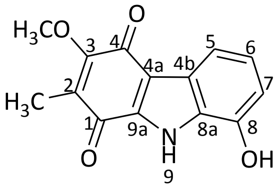
Amine **16** (1.97 g, 7.42 mmnol) and quinone **8** (1.48 g, 1.87 mmol) were combined in methanol (10 mL). The mixture was stirred at room temperature overnight under argon. The resulting mixture was purified by silica gel column chromatography (eluent: Pentane/EtOAc: 20/1) to afford the quinone **17** (2.31 g, 99 %) as a violet oil. ^1H NMR (500 MHz, chloroform-d) δ = 8.05 (br. s., 1 H, N*H*), 7.36 (dd, *J* = 1.4, 7.7 Hz, 1 H), 7.02 - 6.95 (m, 2 H), 6.92 (dd, *J* = 1.4, 7.7 Hz, 1 H), 6.11 (s, 1 H, H-6), 4.15 (s, 3 H, OCH_3), 1.97 (s, 3 H, CH_3), 1.38 - 1.29 (m, 3 H, $Si(CH)CH_3CH_3$), 1.13 (d, *J* = 7.6 Hz, 18 H, 3 x $Si(CH)CH_3CH_3$). ^{13}C NMR (126 MHz, chloroform-d) δ = 184.3 (*C*=O), 182.6 (*C*=O), 157.7 (*C*), 147.6 (*C*), 142.0 (*C*), 129.0 (*C*), 124.9 (*C*H), 123.3 (*C*), 121.3 (*C*H), 120.4 (*C*H), 118.5 (*C*H), 99.0 (*C*H), 61.4 (OCH_3), 17.7 (6 x CH_3, 3 x $Si(CH)CH_3CH_3$), 12.2 (3 x *C*H, $Si(CH)CH_3CH_3$), 8.6 (CH_3). IR (ATR): ν (cm^{-1}) = 3476, 3378, 2943, 2892, 2866, 1611, 1558, 1588, 1542, 1502, 1461, 1391, 1334, 1275, 1223, 1156, 1137, 1114, 1069, 1033, 997, 917, 881, 814, 740, 702, 679. ESI-MS (+25 V): *m/z* = 416.3 $[M+H]^+$, 849.6 $[2M+NH_4]^+$. Calcd for $C_{23}H_{33}NO_4Si$: C, 66.47; H, 8.00; N, 3.37. Found: C, 66.52; H, 8.03; N, 3.39.

3-Methoxy-2-methyl-8-triisopropylsilyloxy-9*H*-carbazole-1,4-quinone (18)



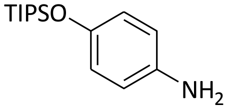
A solution of **17** (221 mg, 0.530 mmol), Pd (OAc) $_2$, (5 mol %), K_2CO_3 (7.36 mg, 0.0530 mmol), and pivalic acid (2000 mg) were weighed in air and transferred into a 10 mL bottom flask. The uncapped flask was placed in an oil bath and the mixture stirred under air at 120 °C for 3 h. The solution was then cooled to room temperature, diluted with EtOAc, washed successively with saturated K_2CO_3, water, brine and dried over $MgSO_4$. The filtrate was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (eluent: Pentane/EtOAc: 3/1) to afford carbazole quinone **18** (180 mg, 82 %) as a brown darkish solid, mp 274-276 °C (Decomp). ^{1}H NMR (500 MHz, chloroform-d) δ = 9.09 (br. s., 1 H, N*H*), 7.74 (d, *J* = 7.9 Hz, 1 H), 7.16 (t, *J* = 7.9 Hz, 1 H, H-6), 6.81 (d, *J* = 7.6 Hz, 1 H), 4.15 (s, 3 H, OCH_3), 2.02 (s, 3 H, CH_3), 1.43 - 1.36 (m, 3 H, Si(C*H*)CH_3CH_3), 1.15 (d, *J* = 7.6 Hz, 18 H, 3 x Si(CH)CH_3CH_3). ^{13}C NMR (126 MHz, chloroform-d) δ = 181.2, 179.4, 158.6, 143.4, 135.2, 130.4, 127.0, 126.0, 125.4, 115.7, 115.3, 113.8, 61.7 (OCH_3), 18.2, 13.1, 8.8 (CH_3). ESI-MS (+10 V): *m/z* = 414.3 $[M+H]^+$, 849.5 $[2M+Na]^+$. Calcd for $C_{23}H_{31}NO_4Si$: C, 66.79; H, 7.56; N, 3.39. Found: C, 66.82; H, 7.60; N, 3.44.

8-Hydroxy-3-methoxy-2-methyl-9*H*-carbazole-1,4-quinone (19)



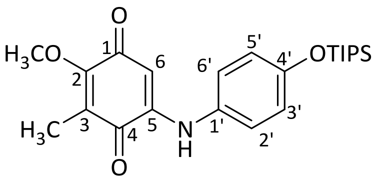
To a solution of **18** (1.21 g, 2.93 mmol) in dry DCM (5 mL) was added 0.1 M tetrabutylammonium fluoride in THF (35.1 mL, 3.51 mmmol) at 0 °C. After being stirred at the same temperature for 30 minutes, the reaction was poured into brine. The aqueous layer was extracted 3 times with portions of DCM. The combined extract was washed with brine, dried over Na_2SO_4, filtered and the filtrate concentrated in *vacuo*. The residue was purified by silica gel chromatography (eluent: isohexane/ethylacetate: 3/1) to give the carbazole quinone **19** as a brown solid (396 mg, 53%), mp 266-269 °C (decomp). ^{1}H NMR (500 MHz, DMSO-d$_6$) δ = 12.67 (br. s., 1 H, N*H*), 10.02 (br. s., 1 H, O*H*), 7.45 (d, *J* = 7.9 Hz, 1 H), 7.10 (t, *J* = 7.7 Hz, 1 H, H-6), 6.73 (d, *J* = 7.6 Hz, 1 H), 4.01 (s, 3 H, OC*H$_3$*), 1.90 (s, 3 H, C*H$_3$*). ^{13}C NMR (126 MHz, DMSO-d$_6$) δ = 180.3 (*C*=O), 178.5 (*C*=O), 157.6, 145.3, 135.7, 128.2, 126.5, 125.5, 125.1, 114.1, 112.0, 109.7, 61.1 (O*C*H$_3$), 8.6 (*C*H$_3$). IR (ATR): ν (cm^{-1}) = 3056, 2916, 2012, 1976, 1940, 1905, 1794, 1738, 1633, 1572, 1519, 1458, 1430, 1369, 1314, 1250, 1212, 1176, 1147, 1112, 1074, 1023, 976, 934, 890, 840, 812, 756, 738, 665, 644. ESI-MS (+10 V): *m/z* = 258.1 [M+H]$^+$, 532.3 [2M+NH$_4$]$^+$. Calcd for $C_{14}H_{11}NO_4$: C, 65.37; H, 4.31; N, 5.44. Found: C, 65.48; H, 4.35; N, 5.46.

4-Triisopropylsilyloxyaniline (21)



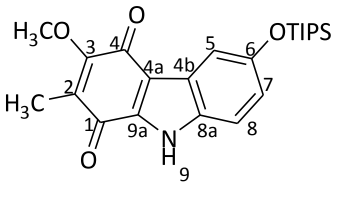
p-Aminophenol **20** (507 mg, 4.64 mmol) in DMF (6 mL) was treated with TEA (0.970 mL, 6.96 mmol) and DMAP (283 mg, 2.32 mmol). TIPSCl (1.49 mL, 6.96 mmol) was slowly added and the reaction mixture was stirred at room temperature for 2 h under nitrogen atmosphere. The reaction was diluted with EtOAC (50 mL) and quenched with water (25 mL). The layers were separated and the aqueous layer was extracted with EtOAc (50 mL). The combined organic layers were washed twice with aqueous LiCl, citric acid (5% solution) and brine then dried over $NaSO_4$, filtrated and the filtrate concentrated in vacuo. Purification by column chromatography (eluent: Pentane/EtOAc: 7/3) to afford the amine **21** as a brown oil (0.788 mg, 64%). ^{1}H NMR (500 MHz, chloroform) δ = 6.74 - 6.69 (m, 2 H), 6.60 - 6.56 (m, 2 H), 1.26 - 1.18 (m, 3 H, Si(C*H*)CH$_3$CH$_3$), 1.10 (d, *J* = 6.9 Hz, 18 H, Si(CH)C*H$_3$*C*H$_3$*). ^{13}C NMR (126 MHz, chloroform) δ = 148.9 (C), 140.1 (C), 120.7 (2 x *C*H), 116.5 (2 x *C*H), 18.2 (6 x *C*H$_3$, Si(CH)*C*H$_3$*C*H$_3$), 12.8 (3 x *C*H, Si(*C*H)C*H$_3$*C*H$_3$*). IR (ATR): ν (cm^{-1}) = 3348, 3213, 3035, 2943, 2892, 2865, 1607, 1570, 1558, 1542, 1505, 1463, 1392, 1245, 1120, 1071, 996, 908, 881, 828, 676. ESI-MS (+10 V): *m/z* = 266.1 $[M+H]^+$. Calcd for $C_{15}H_{27}NOSi$: C, 67.87; H, 10.25; N, 5.28. Found: C, 67.91; H, 10.31; N, 5.29.

2-Methoxy-3-methyl-5-[4'-(triisopropylsilyloxy)anilino]-1,4-benzoquinone (22)



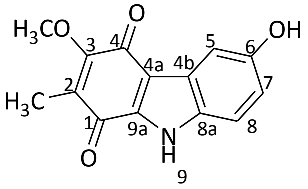
Amine **21** (213 mg, 0.803 mg) and quinone **8** (244mg, 1.60 mmol) were combined in methanol (3 mL). The solution was stirred at room temperature for 4 h under argon. The resulting mixture was purified by silica gel column chromatography (eluent: Pentane/EtOAc: 8/1) to afford the quinone **22** (344 mg, 96 %) as a red solid, mp 143-147 ^{o}C. ^{1}H NMR (500 MHz, chloroform-d) δ = 7.38 (s, 1 H, N*H*), 7.09 - 7.05 (m, 2 H), 6.92 - 6.87 (m, 2 H), 5.80 (s, 1 H, H-6), 4.14 (s, 3 H, OCH_3), 1.95 (s, 3 H, CH_3), 1.30 - 1.22 (m, 3 H, $Si(CH)CH_3CH_3$), 1.11 (d, *J* = 7.3 Hz, 18 H, $Si(CH)CH_3CH_3$). ^{13}C NMR (126 MHz, chloroform-d) δ = 184.4 (*C*=O), 182.3 (*C*=O), 158.1 (*C*), 154.0 (*C*), 143.9 (*C*), 130.4 (*C*), 124.2 (2 x *C*H), 122.8 (*C*), 120.8 (2 x *C*H), 97.7 (*C*H), 61.5 (OCH_3), 17.9 (6 x CH_3, $Si(CH)CH_3CH_3$), 12.6 (3 x *C*H, $Si(CH)CH_3CH_3$), 8.4 (CH_3). IR (ATR): ν (cm^{-1}) = 3256, 2945, 2865, 2009, 1658, 1581, 1540, 1500, 1416, 1376, 1342, 1281, 1266, 1210, 1156, 1137, 1107, 1065, 998, 983, 911, 880, 843, 822, 741, 709, 618, 660. ESI-MS (+10 V): *m/z* = 416.3 $[M+H]^+$, 853.6 $[2M+Na]^+$. Calcd for $C_{23}H_{33}NO_4Si$: C, 66.47; H, 8.00; N, 3.37. Found: C, 66.41; H, 8.03; N, 3.39.

3-Methoxy-2-methyl-6-triisopropylsiloxy-9*H*-carbazole-1,4-quinone (23)



A solution of **22** (241 mg, 0.579 mmol), Pd (OAc) $_2$ (5 mol %), K_2CO_3 (7.99 mg, 0.0579 mmol), and pivalic acid (2000 mg) were weighed in air and transferred into a test tube. The uncapped test tube was placed in an oil bath and the mixture stirred under air at 120 $^{\circ}$C overnight. The solution was then cooled to room temperature, diluted with EtOAc, successively washed with saturated K_2CO_3, water, dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (eluent: Pentane/EtOAc: 3/1) to afford carbazole quinone **23** (131 mg, 55 %) as a brown solid, mp 251-253 $^{\circ}$C (Decomp). ^{1}H NMR (500 MHz, DMSO-d$_6$) δ = 12.80 (br. s., 1 H), 7.44 - 7.38 (m, 2 H), 6.94 (dd, *J* = 2.5 Hz, 8.8 Hz, 1 H), 4.01 (s, 3 H, OC*H$_3$*), 1.89 (s, 3 H, C*H$_3$*), 1.30 - 1.20 (m, 3 H, Si(C*H*)CH_3CH_3), 1.08 (d, *J* = 7.3 Hz, 18 H, Si(CH)C*H$_3$*C*H$_3$*). ^{13}C NMR (126 MHz, DMSO-d$_6$) δ = 180.3 (*C*=O), 178.3 (*C*=O), 157.8, 152.6, 136.5, 133.1, 126.4, 124.6, 120.3, 115.0, 113.2, 109.4, 61.1 (O*C*H$_3$), 17.8, 12.0, 8.5 (*C*H$_3$). IR (ATR): ν (cm^{-1}) = 3205, 2943, 2864, 2723, 2449, 1655, 1619, 1584, 1530, 1508, 1478, 1459, 14361389, 1373, 1304, 1284, 1250, 1206, 1133, 1105, 1074, 1002, 962, 916, 871, 832, 817, 763, 743, 725, 681, 643. ESI-MS (+10 V): *m/z* = 414.3 [M+H]$^+$, 849.5 [2M+Na]$^+$. Calcd for $C_{23}H_{31}NO_4Si$: C, 66.79; H, 7.56; N, 3.39. Found: C, 66.54; H, 7.47; N, 3.31.

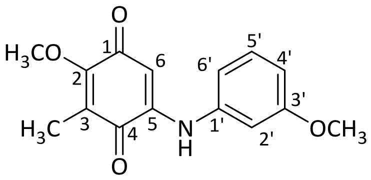
6-Hydroxy-3-methyoxy-2-methyl-9*H*-carbazole-1,4-quinone (24)



To a solution of **23** (160 mg, 0.387 mmol) in dry DCM (4 mL) was added 0.1 M tetrabutylammonium fluoride in THF (4.64 mL, 0.464 mmol) at 0 $^{\circ}$C. After being stirred at the same temperature for 30 minutes, the reaction was poured into brine. The aqueous layer was extracted 3 times with portions of DCM. The combined extract was washed with brine, dried over Na_2SO_4, filtered and the filtrated concentrated in *vacuo.*

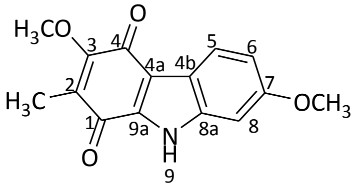
The residue was purified by silica gel chromatography (eluent: Isohexane/EtOAc: 3/1) to give the carbazole quinone **24** as a brown red solid (63 mg, 63%), mp 282-284 °C (decomp). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 12.66 (s, 1 H, N*H*), 9.49 (s, 1 H, O*H*), 7.35 - 7.31 (m, 2 H), 6.86 (dd, *J* = 2.2, 9.1 Hz, 1 H), 4.00 (s, 3 H, OC*H₃*), 1.88 (s, 3 H, C*H₃*). ^{13}C NMR (126 MHz, DMSO-d_6) δ = 180.0 (C=O), 178.0 (*C*=O), 157.6 (*C*), 154.7 (*C*), 135.6 (*C*), 131.8(*C*), 126.2 (*C*), 124.6 (*C*), 116.8 (*C*H), 114.5 (*C*H), 112.8 (*C*), 104.2 (*C*H), 60.8 (O*C*H$_3$), 8.3 (*C*H$_3$). IR (ATR): ν (cm^{-1}) = 3221, 1699, 1684, 1624. Calcd for $C_{14}H_{11}NO_4$: C, 65.37; H, 4.31; N, 5.44. Found: C, 65.53; H, 4.27; N, 5.52.

2-Methoxy-3-methyl-5-[3’-(methoxy)anilino]-1,4-benzoquinone (26)



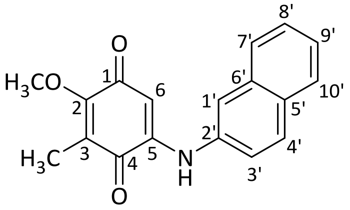
Amine **25** (161 mg, 1.31 mmnol) and quinone **8** (400 mg, 2.63 mmol) were combined in methanol (5 mL). The solution was stirred at room temperature overnight under argon. The resulting mixture was purified by silica gel column chromatography (eluent: Pentane/EtOAc: 8/1) to afford quinone **26** as a brown solid (344 mg, 96 %), mp 175-175 °C. ^{1}H NMR (500 MHz, chloroform-d) δ = 7.49 (br. s., 1 H, NH), 7.29 (t, *J* = 8.0 Hz, 1 H), 6.82 - 6.79 (m, 1 H), 6.75 - 6.71 (m, 2 H), 6.00 (s, 1 H, H-6), 4.15 (s, 3 H, OC*H₃*), 3.82 (s, 3 H, OC*H₃*), 1.96 (s, 3 H, C*H₃*). ^{13}C NMR (125 MHz, chloroform-d) δ = 184.3 (*C*=O), 182.5 (*C*=O), 160.5 (*C*), 157.7 (*C*), 142.38 (*C*), 138.7(*C*), 130.4 (*C*H), 123.0 (*C*), 114.4 (*C*H), 110.8 (*C*H), 108.0 (*C*H), 99.1 (*C*H), 61.5 (O*C*H$_3$), 55.4 (O*C*H$_3$), 8.5 (*C*H$_3$). IR (ATR): ν (cm^{-1}) = 3312, 2997, 2948, 2031, 1917, 1716, 1640, 1581, 1527, 1436, 1378, 1340, 1284, 1213, 1187, 1148, 1088, 980, 862, 848, 825, 757, 716, 680, 634. ESI-MS (+25 V): *m/z* = 274.1 [M+H]$^+$, 569.1 [2M+Na]$^+$. ESI-MS (+50 V): *m/z* = 291.2 [M+NH$_4$]$^+$. Calcd for $C_{15}H_{15}NO_4$: C, 65.92; H, 5.53; N, 5.13. Found: C, 65.81; H, 5.50; N, 5.09.

3,7-Dimethoxy-2-methyl-9*H*-carbazole-1,4-quinone (27a)



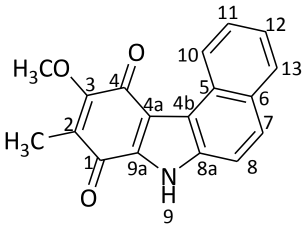
A solution of **26** (209 mg, 0.765 mmol), Pd (OAc)$_2$ (5 mol %), K_2CO_3 (10.6 mg, 0.0765 mmol), and pivalic acid (2000 mg) were weighed in air and transferred into a 10 mL bottom flask. The uncapped flask was placed in an oil bath and the mixture stirred under air at 120 °C for 5 h. The solution was then cooled to room temperature, diluted with EtOAc, washed successively with saturated K_2CO_3, water, dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (eluent: Pentane/EtOAc: 3/1) to afford carbazole quinone **27a** (125.5 mg, 60 %) as a green solid, mp 245-247 °C (Decomp). ^{1}H NMR (500 MHz, DMSO-d$_6$) δ = 12.65 (br. s., 1 H, N*H*), 7.83 (d, *J* = 8.8 Hz, 1 H, H-5), 6.93 (dd, *J* = 2.2, 8.5 Hz, 1 H, H-6), 6.89 (d, *J* = 1.9 Hz, 1 H, H-8), 3.98 (s, 3 H, OC*H*$_3$), 3.80 (s, 3 H, OC*H*$_3$), 1.88 (s, 3 H, C*H*$_3$). ^{13}C NMR (126 MHz, DMSO-d$_6$) δ = 179.9 (*C*=O), 178.5 (*C*=O), 158.4 (*C*), 157.0 (*C*), 138.8 (*C*), 135.0 (*C*), 126.2 (*C*), 122.1 (*C*H), 117.5 (*C*H), 114.9 (*C*), 114.0 (*C*), 95.0 (*C*H), 60.8 (O*C*H$_3$), 55.1 (O*C*H$_3$), 8.4 (*C*H$_3$). IR (ATR): ν (cm^{-1}) = 3241, 3064, 2998, 2947, 2847, 2056, 2030, 1734, 1699, 1639, 1604, 1527, 1440, 1403, 1369, 1291, 1266, 1228, 1191, 1159, 1099, 1025, 994, 922, 841, 815, 752, 713, 628. ESI-MS (+10 V): *m/z* = 272.0 [M+H]$^+$, 565.1 [2M+Na]$^+$. Calcd for $C_{15}H_{13}NO_4$: C, 66.41; H, 4.83; N, 5.16. Found: C, 66.35; H, 4.81; N, 5.13.

2-Methoxy-3-methyl-5-[2′-naphthylamino]-1,4-benzoquinone (29)



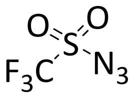
Amine **28** (51.4 mg, 0.359 mg) and quinone **8** (99.8 mg, 0.656 mmol) were combined in methanol (3 mL). The solution was stirred at room temperature overnight under argon. The resulting mixture was purified by silica gel column chromatography (eluent: Pentane/EtOAc: 8/1) to afford quinone **29** (77.4 mg, 74 %) as a brown darkish solid, mp 189-191 °C. ^{1}H NMR (500 MHz, chloroform-d) δ = 7.86 (d, *J* = 8.8 Hz, 1 H), 7.83 (d, *J* = 8.2 Hz, 1 H), 7.79 (d, *J* = 8.2 Hz, 1 H), 7.71 (br. s., 1 H, NH), 7.66 (d, *J* = 1.9 Hz, 1 H), 7.52 (dt, *J* = 1.3, 7.6, 7.6 Hz, 1 H), 7.49 - 7.45 (m, 1 H), 7.32 (dd, *J* = 2.2, 8.8 Hz, 1 H), 6.16 (s, 1 H, H-6), 4.17 (s, 3 H, OCH_3), 1.99 (s, 3 H, CH_3). ^{13}C NMR (126 MHz, chloroform-d) δ = 184.3 (*C*=O), 182.7 (*C*=O), 157.8 (*C*), 142.8 (*C*), 135.0 (*C*), 133.7 (C), 130.9 (*C*), 129.7 (*C*H), 127.8 (*C*H), 127.4 (*C*H), 127.1 (*C*H), 125.8 (*C*H), 123.1 (*C*), 121.4 (*C*H), 118.7 (*C*H), 99.1 (*C*H), 61.5 (OCH_3), 8.5 (CH_3). IR (ATR): ν (cm^{-1}) = 3206, 3061, 3006, 2951, 2853, 2057, 1698, 1642, 1563, 1484, 1452, 1360, 1328, 1267, 1215, 1158, 979, 897, 843, 817, 785, 718, 745, 664. ESI-MS (+10 V): *m/z* = 294.1 [M+H]$^+$, 609.1 [2M+Na]$^+$. Calcd for $C_{18}H_{15}NO_3$: C, 73.71; H, 5.15; N, 4.78. Found: C, 73.76; H, 5.16; N, 4.82.

10-methoxy-9-methyl-7*H*-benzo[c]carbazole-8,11-dione (30a)



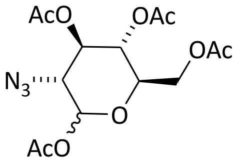
A solution of amine **29** (447 mg, 1.52 mmol), Pd (OAc)$_2$ (10 mol %), K_2CO_3 (21.1 mg, 0.152 mmol), and pivalic acid (3500 mg) was weighed in air and transferred into a 10 mL bottom flask. The uncapped flask was placed in an oil bath and the mixture stirred under air at 120 °C overnight. The solution was then cooled to room temperature, diluted with EtOAc, successively washed with saturated K_2CO_3, water, dried over $MgSO_4$ and filtered. The filtrate was evaporated under reduced pressure and the crude product was purified by silica gel column chromatography (eluent: Pentane/EtOAc: 3/1) to afford carbazole quinone **30a** (33.6 mg, 8 %) as a black solid mp 292-294 °C. ^{1}H NMR (500 MHz ,DMSO-d_6) δ = 13.33 (br. s., 1 H), 9.64 (d, *J* = 8.2 Hz, 1 H), 7.99 (d, *J* = 8.2 Hz, 1 H), 7.85 (d, *J* = 9.1 Hz, 1 H), 7.63 (d, *J* = 9.1 Hz, 1 H), 7.58 - 7.53 (m,1 H), 7.48 - 7.37 (m, 1 H), 4.05 (s, 3 H, OCH_3), 1.95 (s, 3 H, CH_3). ^{13}C NMR (126 MHz, DMSO-d_6) δ = 180.0, 178.0, 158.4, 135.7, 135.0, 130.7, 128.9, 128.2, 128.0, 126.7, 126.6, 126.4, 125.5, 119.9, 116.4, 114.0, 61.2 (OCH_3), 8.5 (CH_3). IR (ATR): ν (cm^{-1}) = 3226, 3134, 305, 951, 2850, 1917, 1717, 1623, 1542, 1508, 1457, 1421, 1395, 1367, 1322, 1281, 1249, 1207, 1104, 997, 978, 932, 863, 806, 743, 679, 662, 636, 618. ESI-MS (+10 V): *m/z* = 292.0 $[M+H]^+$, 600.1 $[2M+NH_4]^+$, 605.2 $[2M+Na]^+$. Calcd for $C_{18}H_{13}NO_3$: C, 74.22; H, 4.50; N, 4.81. Found: C, 74.29; H, 4.46; N, 4.80.

Trifluoromethanesulfonyl azide (32)



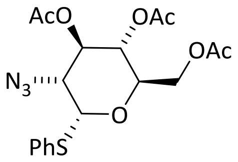
Sodium azide (39.0 g, 600 mmol) was dissolved in water (100 mL) before toluene (100 mL) was added and the mixture stirred at 0 °C. Triflic anhydride (40 mL, 238 mmol) was added drop-wise and stirring continued for 30 min, after which the temperature was raised to 10 °C and the biphasic mixture stirred for a further 2 h. Saturated $NaHCO_3$ was added until effervescence ceased. The organic phase was separated and the aqueous phase extracted with toluene (2 x 50 mL). The extracts were combined to form the stock solution of triflic azide (maximum possible concentration ~ 1.15 M). Note: this reagent is shock-sensitive and should be handled with care.

1,3,4,6-Tetra-*O*-acetyl-2-azido-2-deoxy-β-D-glucopyranoside (34)



Glucosamine hydrochloride **33** (30g, 139 mmol), $NaHCO_3$ (46.7 g, 556 mmol) and $CuSO_4.5H_2O$ (1.40 g, 5.60 mmol) were dissolved in H_2O (92.4 mL). A portion of triflic azide **32** stock solutions (157 mL) was added, followed by the addition of MeOH (610 mL) to yield a homogeneous mixture that was stirred at room temperature for 15 h. Over the course of the reaction, the colour of the solution changed from blue to green. The solvents were removed under reduced pressure, and the residue left to dry on a high vacuum pump for 3 days. The residue was then dissolved in dry pyridine (100 mL) and cooled to 0 ^{o}C. Acetic anhydride (100 mL) was added drop-wise to the solution, which was stirred for 30 min at 0 ^{o}C before being warmed to room temperature and left to stir for a further 17 h until complete consumption of the starting material. The solvents were removed under reduced pressure, and the crude residue dissolved in EtOAc (1 L) and washed with H_2O (2 x 500 mL). The aqueous fractions were extracted with EtOAc (2 x 500 mL), and the combined organic fractions were dried (Na_2SO_4) and reduced under reduced pressure. The syrupy residue was purified by column chromatography (silica gel, eluting in petroleum ether /EtOAc: 7/3) to obtain the product as a white precipitate of α/β isomers (46.3 g, 89 %). The β isomer was obtained by recrystallisation of the purified product mixture in EtOAc/Pet Ether and used for analysis, m.p. 93-94 ^{o}C (β-isomer), lit.[23] m.p. 96-97 ^{o}C. ^{1}H NMR (300 MHz, $CDCl_3$) β-isomer: δ 5.55 (d, *J* = 8.6, 1H, H-1), 5.06 (m, 2H, H-3, H-4), 4.29 (dd, *J* = 4.6, 12.5, 1H, H-6), 4.08 (dd, *J* = 2.2, 12.5, 1H, H-6’), 3.83 – 3.77 (m, 1H, H-5), 3.70 –3.61 (m, 1H, H-2), 2.18 (s, 3H, CH_3, OAc), 2.08 (s, 3H, CH_3, OAc), 2.06 (s, 3H, CH_3, OAc), 2.01 (s, 3H, CH_3, OAc). ^{13}C NMR (75 MHz, $CDCl_3$) β-isomer: δ 170.5 (*C*=O), 169.7 (*C*=O), 169.6 (*C*=O), 168.5 (*C*=O), 92.6, 72.8, 72.7, 67.8, 62.6, 61.4, 20.8 (*C*H_3, OAc), 20.6 (*C*H_3, OAc), 20.6 (*C*H_3, OAc), 20.5 (CH_3, OAc). IR (NaCl, dry film) vmax (cm-1): 2115 (N_3), 1756 (C=O). Anal. Calcd for $C_{14}H_{19}N_3O_9$: C, 45.04; H, 5.13; N, 11.26. Found: C, 45.33; H, 5.26; N, 10.91.

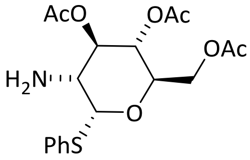
Phenyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-1-thio-α-D-glucopyranoside (35a)



The azide **34** (10.0 g, 26.8 mmol) was dissolved in dry CH_2Cl_2 (40 mL) and cooled to 0 °C. To this solution, $BF_3.OEt_2$ (15.3 mL, 121 mmol) was added drop-wise and the solution left stirring for 30 minutes to form the oxocarbenium ion intermediate. Thiophenol (4.13 mL, 40.2 mmol) was then added dropwise and the solution allowed to warm to room temperature, before the temperature was increased and the solution refluxed for 14 hours until the reaction was complete (determined by TLC). After cooling to room temperature, crushed ice was added and the reaction mixture extracted with CH_2Cl_2 (2 x 150 mL). The CH_2Cl_2 extract was washed with 150 mL water, dried over Na_2SO_4, filtered and the filtrate concentrated *in vacuo*. Purification by column chromatography (Pet Ether/EtOAc: 8/2) to yield the product as a mixture of isomers (α/β:3/1, 9.03 g, 80%, lit.[23]). Pure crops of the α-thioglycoside were isolated by crystallisation from EtOH and obtained as white needles (5.19 g, 46 %), however, further separation of the isomers was not possible and the mother liquor remained as a mixture, m.p. 93-95 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.45 – 7.40 (m, 2 H, Aromatic C*H*), 7.28 – 7.21 (m, 3 H, Aromatic C*H*), 5.56 (d, *J* = 5.6 Hz, 1 H, H-1), 5.26 (dd, *J* = 9.2, 10.6 Hz, 1 H, H-3), 4.96 (dd, *J* = 9.2, 10.3 Hz, 1 H, H-4), 4.52 (ddd, *J* = 2.2, 5.0, 10.3 Hz, 1 H, H-5), 4.21 (dd, *J* = 5.2, 12.4 Hz, 1 H, H-6’), 3.99 (dd, *J* = 5.6, 10.6 Hz, 1 H, H-2), 3.96 (dd, *J* = 2.3, 12.3 Hz, 1 H, H-6), 2.02 (s, 3 H, CH_3, OAc), 1.98 (s, 3 H, CH_3, OAc), 1.95 (s, 3 H, CH_3, OAc). ^{13}C NMR (101 MHz, $CDCl_3$) δ = 170.4 (C=O), 169.7 (2 x C=O), 132.5 (Aromatic *C*), 132.2 (2 C, Aromatic *C*H), 129.2 (2 C, Aromatic *C*H), 128.1 (Aromatic *C*H), 86.6 (*C*-1), 72.1 (*C*-3), 68.9 (*C*-4), 68.6 (*C*-5), 62.0 (*C*-

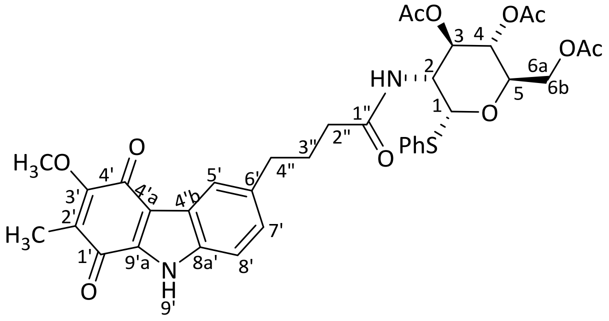
6), 61.7 (C-2), 20.6 (2 x *C*H_3, 2 OAc), 20.6 (*C*H_3, OAc). IR (NaCl, dry film) vmax (cm-1): 2111 (N_3), 1750 (C=O). EI-MS *m/z*: $[M]^+$ Calcd for $C_{18}H_{21}N_3O_7S$: 423.11; Found: 423.06.

Phenyl 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-1-thio-α-D-glucopyranoside (36)



Pd/C (10 wt %, 125 mg) was added to a round-bottomed flask before the sides of the flask were washed down with Et_2O (5 mL). **35** (500 mg, 1.18 mmol) was added to the suspension, and the atmosphere of the flask flushed with H_2 gas. The solution was stirred at room temperature under a H_2 atmosphere (provided by a balloon) for 24 h. The mixture was filtered through a plug of Celite and the solvent removed at room temperature under reduced pressure. The product (482 mg, 1.21 mmol) was obtained in quantitative yield as syrup, which was then crystallised from Pet Ether/EtOAc and obtained as a colourless needles, mp 84-87 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ = 7.60 – 7.43 (m, 2H, Aromatic C*H*), 7.38 – 7.20 (m, 3 H, Aromatic C*H*), 5.56 (d, *J* = 5.2 Hz, 1 H, H-1), 5.10 – 4.95 (m, 2 H, H-3, H-4), 4.56 (ddd, *J* = 2.2, 5.0, 9.7 Hz, 1 H, H-5), 4.32 (dd, *J* = 5.1, 12.3 Hz, 1 H, H-6), 4.07 (dd, *J* = 2.3, 12.3 Hz, 1 H, H-6'), 3.32 (dd, *J* = 5.2, 10.2 Hz, 1 H, H-2), 2.09 (s, 3 H, C*H*$_3$, OAc), 2.04 (s, 6 H, 2 x C*H*$_3$, OAc). ^{13}C NMR (75 MHz, $CDCl_3$) δ = 170.7 (*C*=O), 170.6 (*C*=O), 169.8 (*C*=O), 133.5 (Aromatic *C*), 131.9 (2 C, Aromatic *C*H), 129.1 (2 C, Aromatic *C*H), 127.8 (Aromatic *C*H), 91.3 (*C*-1), 74.7 (*C*-3), 69.0 (2 C, *C*-4,*C*-5), 62.3 (*C*-6), 55.2 (*C*-2), 20.8 (*C*H_3, OAc), 20.7 (2 x *C*H_3, OAc). EI-MS *m/z*: $[M]^+$ Calcd for $C_{18}H_{23}NO_7S$: 397.12; Found 397.14. HRMS (ESI-TOF): Calcd for $C_{18}H_{23}NO_7S$, 397.1195; Found 398.1265 $[M+H]^+$, 420.1087 $[M+Na]^+$.

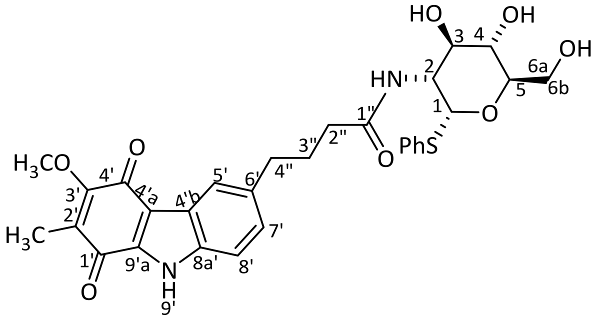
Phenyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-[4"-(4',9'-dihydro-3'-methoxy-2'-methyl-1',4'-dioxo-1*H*-carbazol-6'-yl)butanamido]-1-thio-α-D-glucopyranoside (37)



A solution of **11** (40.4 mg, 0.123 mmol), EDC.HCl (35.4 mg, 0.185 mmol), HOBt (16.6 mg, 0.123 mmol) and DIEA (94 µL, 0.55 mmol) in THF (4 mL) at 0 °C under argon was stirred for 1 h. A freshly prepared solution of **36** (93.9 mg, 0.236 mmol) in THF (1.5 mL) was added to the mixture and left overnight at room temperature. A precipitate formed and was isolated by filtration, then washed with methanol and dried under reduced vacuum to afford **37** (44.9 mg, 52%) as a pale orange solid, mp 238-240 °C (decomp). ^{1}H NMR (500 MHz, chloroform-d) δ = 9.47 (s, 1 H, N*H*), 7.98 (s, 1 H), 7.46 - 7.42 (m, 2 H), 7.37 (d, *J* = 8.5 Hz, 1 H), 7.32 - 7.28 (m, 3 H), 7.19 (dd, *J* = 1.6, 8.5 Hz, 1 H), 5.92 (d, *J* = 8.8 Hz, 1 H, N*H*CO), 5.73 (d, *J* = 5.4 Hz, 1 H, H-1), 5.21 - 5.13 (m, 2 H, H-3, H-4), 4.67 - 4.59 (m, 1 H, H-2), 4.55 - 4.49 (m, 1 H, H-5), 4.29 (dd, *J* = 4.9, 12.5 Hz, 1 H, H-6a), 4.16 (s, 3 H, OCH_3), 4.09 (dd, *J* = 2.2, 12.3 Hz, 1 H, H-6b), 2.76 (t, *J* = 7.4 Hz, 2 H, CH_2), 2.26 - 2.21 (m, 2 H, CH_2), 2.07 (s, 3 H, OAc), 2.05 (s, 3 H, OAc), 2.01 (s, 3 H, OAc), 1.99 (CH_3), 2.01 - 1.94 (m, 2 H, CH_2). ^{13}C NMR (125 MHz, chloroform-d) δ = 181.0 (*C*=O), 179.1 (*C*=O), 172.7 (*C*=O), 171.7 (*C*=O), 170.7 (*C*=O), 169.3 (*C*=O), 158.3 (*C*), 137.7 (*C*), 135.8 (*C*), 135.7 (*C*), 132.8 (*C*), 131.6 (2 x *C*H), 129.2 (2 x *C*H), 127.9 (*C*H), 127.7 (*C*H), 126.6 (*C*H), 124.5 (*C*H), 121.7 (*C*H), 114.5 (*C*H), 112.8 (*C*H), 87.7 (*C*-1), 71.3 (*C*-3), 68.9 (*C*-5), 68.1 (*C*-4), 62.0 (*C*-6), 61.5 (OCH_3), 52.6 (*C*-2), 35.9 (CH_2), 35.2 (CH_2), 27.4 (CH_2), 20.8 (CH_3, OAc), 20.7 (CH_3, OAc), 20.6 (CH_3, OAc), 8.5 (CH_3). IR (ATR): ν (cm^{-1}) = 3313, 3217, 3071, 3004, 2948, 2851, 1741, 1647, 1626, 1590, 1536, 1481, 1434, 1375, 1296, 1223, 1090, 1037, 1007, 956, 905, 819, 763, 744, 687, 602, 578. (UV) (MeOH): λ (nm) = 202, 229, 251 (sh), 259, 269 (sh), 284 (sh), 311, 404. ESI-MS (+50 V): *m/z* = 707.5 $[M+H]^+$, 724.5 $[M+NH_4]^+$, 729.5 $[M+Na]^+$. ESI-MS (-50 V): m/z = 705.1 $[M-H]^-$. Calcd for $C_{36}H_{38}N_2O_{11}S$: C, 61.18; H, 5.42; N, 3.96; S, 4.54. Found: C, 61.37; H, 5.55;

N, 4.11; S, 5.01.

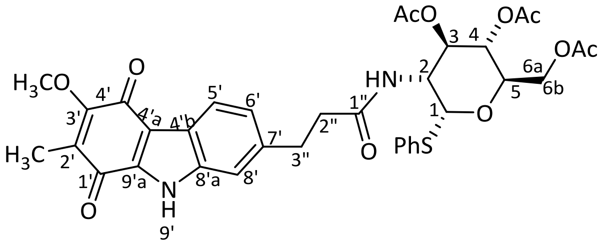
Phenyl 2-deoxy-2-[4”-(4’,9’-dihydro-3’-methoxy-2’-methyl-1’,4’-dioxo-1*H*-carbazol-6’-yl)butanamido]-1-thio-α-D-glucopyranoside (38)



Methanolic solution of sodium methoxide (0.5M, 72 µL, 0.036 mmol) was added to a stirred solution of **37** (51 mg, 0.072 mmol) in dry methanol (2 mL) and the reaction mixture was stirred overnight at room temperature. A precipitate formed and was isolated by filtration, then washed with methanol and dried under reduced vacuum to afford 3**8** (44.9 mg, 95%) as an orange solid, mp 267-269 °C (decomp). ^{1}H NMR (500MHz, chloroform-d) δ = 8.06 (d, *J* = 6.6 Hz, 1 H, N*H*), 7.79 (s, 1 H), 7.45 - 7.38 (m, 3 H), 7.32 - 7.20 (m, 3 H), 7.16 (d, *J* = 8.5 Hz, 1 H), 5.65 (d, *J* = 5.0 Hz, 1 H, H-1), 5.19 (br. s., 1 H, O*H*-3), 4.94 (br. s., 1 H, O*H*-4), 4.58 (br. s., 1 H, O*H*-6), 4.01 (s, 3 H, OCH_3), 3.89 - 3.82 (m, 2 H, H-2, H-5), 3.65 - 3.59 (m, 1 H, H-6a), 3.58 - 3.52 (m, 1 H, H-6b), 3.48 - 3.42 (m, 1 H, H-4), 3.26 (d, *J* = 9.8 Hz, 1 H, H-3), 2.68 (t, *J* = 6.8 Hz, 2 H, CH_2), 2.18 (td, *J* = 7.3, 11.8 Hz, 2 H, CH_2), 1.89 (s, 3 H, CH_3), 1.87 - 1.78 (m, 2 H, CH_2). ^{13}C NMR (125 MHz, DMSO-d$_6$) δ = 180.9 (*C*=O), 178.2 (*C*=O), 172.6 (*C*=O), 157.8 (*C*), 137.7 (*C*), 135.1 (*C*), 131.2 (3 x *C*H), 129.0 (4 x *C*H), 127.0 (*C*H), 126.3 (*C*H), 124.1 (*C*H), 120.3 (*C*H), 113.4 (*C*H), 87.7 (*C*-1), 74.3 (*C*-5), 70.7 (*C*-4), 70.5 (*C*-3), 61.0 (O*C*H$_3$), 60.1 (*C*-6), 60.4 (*C*-2), 54.8 (*C*H), 34.9 (*C*H$_2$), 34.8 (*C*H$_2$), 27.6 (*C*H$_2$), 8.5 (*C*H$_3$). IR (ATR): ν (cm^{-1}) = 3398, 3284, 3220, 2948, 2923, 2048, 2030, 1629, 1591, 1537, 1479, 1450, 1433, 1377, 1296, 1278, 1224, 1207, 1106, 1071, 1046, 1007, 957, 920, 819, 763, 741, 686, 613, 549. (UV) (MeOH): λ (nm) = 202, 228, 250 (sh), 259, 267 (sh), 280 (sh), 311, 407. ESI-MS (+50 V): *m/z* = 581.3 [M+H]$^+$, 603.3

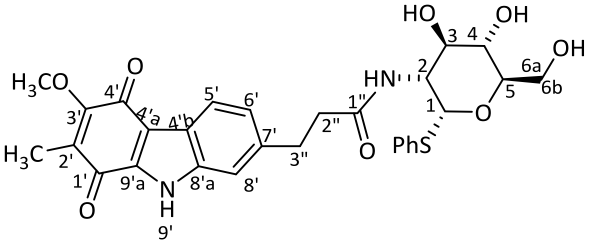
$[M+Na]^+$. ESI-MS (-50 V): *m/z* = 579.0 $[M-H]^-$. Calcd for $C_{30}H_{32}N_2O_8S$: C, 62.05; H, 5.55; N, 4.82; S, 5.52. Found: C, 61.89; H, 6.07; N, 4.70; S, 5.70. (ESI-HRMS): $[M+Na]^+$ calcd for $C_{30}H_{32}N_2NaO_8S$: 603.1777; found: 603.1789.

Phenyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-[3”-(4’,9’-dihydro-3’-methoxy-2’-methyl-1’,4’-dioxo-1*H*-carbazol-7’-yl)propanamido]-1-thio-α-D-glucopyranoside (39)



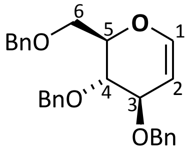
A solution of **14** (40.4 mg, 0.160 mmol), EDC.HCl (46 mg, 0.24 mmol), HOBt (21mg, 0.16 mmol) and DIEA (123 µL, 0.720 mmol) in THF (4 mL) at 0 °C under argon was stirred for 1 h. A freshly prepared **36** (93.9 mg, 0.236 mmol) in 1.5 mL was added to the mixture and left overnight at room temperature. A precipitate formed and was isolated by filtration, then washed with methanol and dried under reduced vacuum to afford **39** (67 mg, 60%) as a red dark solid, mp 240-241 °C (decomp). ^{1}H NMR (500MHz, chloroform-d) δ = 8.04 (d, *J* = 8.5 Hz, 1 H, N*H*), 7.36 - 7.30 (m, 3 H), 7.29 (m, 2 H), 7.27 - 7.25 (m, 2 H), 7.17 (dd, *J* = 1.4, 8.4 Hz, 1 H), 5.93 (d, *J* = 8.5 Hz, 1 H, N*H*CO), 5.66 (d, *J* = 5.4 Hz, 1 H, H-1), 5.17 - 5.06 (m, 2 H, H-3, H-4), 4.62 - 4.56 (m, 1 H, H-2), 4.47 (dt, *J* = 2.8, 4.9 Hz, 1 H, H-5), 4.26 (dd, *J* = 5.0, 12.3 Hz, 1 H, H-6a), 4.15 (s, 3 H, OC*H₃*), 4.05 (dd, *J* = 2.2, 12.3 Hz, 1 H, H-6b), 3.06 (q, *J* = 7.4 Hz, 2 H, C*H₂*), 2.63 - 2.56 (m, 2 H, C*H₂*), 2.05 (s, 3 H, OAc), 2.03 (s, 3 H, OAc), 2.01 (s, 3 H, OAc), 1.93 (s, 3 H, C*H₃*). ^{13}C NMR (125 MHz, chloroform-d) δ = 180.9 (*C*=O), 179.0 (*C*=O), 171.8 (*C*=O), 171.8 (*C*=O), 170.6 (*C*=O), 169.3 (*C*=O), 158.1 (*C*), 139.2 (*C*), 137.3 (*C*), 135.6 (*C*), 132.6 (*C*), 131.1 (*C*H), 131.1 (*C*H), 129.3 (*C*H), 129.2 (*C*H), 129.2 (*C*H), 127.7 (*C*H), 126.7 (*C*H), 125.1 (*C*H), 122.6 (C), 114.7 (C), 112.4 (*C*H), 87.2 (*C*H), 71.2 (*C*H), 68.8 (*C*H), 68.0 (*C*H), 61.9 (*C*H), 61.5 (*C*H$_3$), 52.6 (*C*H), 37.9 (*C*H$_2$), 31.6 (*C*H$_2$), 20.7 (*C*H$_3$), 20.6 (*C*H$_3$), 20.6 (*C*H$_3$), 8.5 (*C*H$_3$). IR (ATR): ν (cm^{-1}) = 3255, 3061, 2935, 2850, 1741, 1627, 1538, 1436, 1370, 1219, 1161, 1095, 1035, 997, 913, 825, 742, 689, 596. (UV) (MeOH): λ (nm) = 202, 229, 253 (sh), 260, 269 (sh), 281 (sh), 314, 392. ESI-MS (+50 V): *m/z* = 693.4 $[M+H]^+$, 715.4 $[M+Na]^+$. ESI-MS (-25 V): *m/z* = 691.1 $[M-H]^-$. Calcd for $C_{35}H_{36}N_2O_{11}S$: C, 60.68; H, 5.24; N, 4.04; S, 4.63. Found: C, 61.02; H, 5.32; N, 3.91; S, 4.74.

Phenyl 2-deoxy-2-[3-(4,9-dihydro-3-methoxy-2-methyl-1,4-dioxo-1*H*-carbazol-7-yl)propanamido]-1-thio-α-D-glucopyranoside (40)



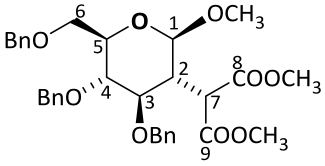
Methanolic solution of sodium methoxide (0.5M, 172 µL, 0.0867 mmol) was added to a stirred solution of **39** (30 mg, 0.0433 mmol) in dry methanol (2 mL) and the reaction mixture was stirred overnight at room temperature. A precipitate formed and was isolated by filtration, then washed with methanol and dried under vacuum to afford **40** (19.9 mg, 96%) as a red solid, mp 276-280 °C (decomp). ^1H NMR (500 MHz, DMSO-d_6) δ = 7.89 (d, *J* = 6.9 Hz, 1 H, N*H*), 7.41 (d, *J* = 7.3 Hz, 2 H), 7.37 - 7.29 (m, 3 H), 7.27 (d, *J* = 7.3 Hz, 1 H), 7.14 (d, *J* = 7.9 Hz, 1 H), 7.11 - 7.06 (m, 1 H), 5.60 (d, *J* = 5.4 Hz, 1 H, H-1), 5.18 (d, *J* = 5.7 Hz, 1 H, O*H*), 4.89 (d, *J* = 6.0 Hz, 1 H, O*H*), 4.58 (t, *J* = 5.8 Hz, 1 H, O*H*), 4.00 (s, 3 H, OCH_3), 3.91 - 3.84 (m, 2 H), 3.61 - 3.37 (m, 5 H), 3.29 - 3.23 (m, 2 H), 2.46 - 2.40 (m, 2 H), 1.91 (s, 3 H, CH_3). ^{13}C NMR (125 MHz, DMSO-d_6) δ = 180.7 (*C*=O), 177.2 (*C*=O), 172.1 (*C*=O), 158.7 (*C*), 138.2 (*C*), 136.8 (*C*), 136.4 (*C*), 135.1 (*C*), 131.3 (*C*H), 131.3 (*C*H), 129.0 (*C*), 128.9 (*C*), 128.9 (*C*H), 127.0 (*C*H), 126.1 (*C*H), 125.1 (*C*H), 123.0 (*C*H), 114.3 (*C*), 111.6 (*C*H), 87.8 (*C*H), 74.3 (*C*H), 70.8 (*C*H), 70.6 (*C*H), 61.3 (OCH_3), 60.4 (*C*-6), 54.7 (*C*H), 37.3 (CH_2), 30.8 (CH_2), 8.3 (CH_3). IR (ATR): ν (cm^{-1}) = 3249, 3061, 2925, 2048, 2030, 2009, 1658, 1629, 1577, 1532, 1475, 1446, 1377, 1337, 1299, 1261, 1110, 1063, 1026, 997, 931, 855, 786, 741, 687, 629, 609, 554. (UV) (MeOH): λ (nm) = 202, 228, 254 (sh), 262, 271 (sh), 282 (sh), 315, 390. ESI-MS (+25 V): *m/z* = 567.3 [M+H]$^+$, 589.3 [M+Na]$^+$. ESI-MS (-25 V): *m/z* = 656.0 [M-H]$^-$. Calcd for $C_{29}H_{30}N_2O_8S$: C, 61.47; H, 5.34; N, 4.94; S, 5.66. Found: C, 61.60; H, 5.40; N, 4.87; S, 5.51. (ESI-HRMS): [M+Na]$^+$ calcd for $C_{29}H_{30}N_2NaO_8S$: 589.1621; found: 589.1632.

3,4,5-tri-*O*-benzyl-D-glucal (42)



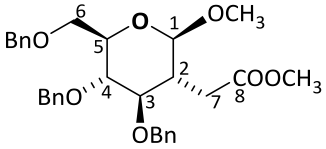
To a solution of 3,4,5-tri-*O*-acetyl-D-glucal **40** (10.0 g, 36.7 mmol) in THF (60 mL) were added powdered NaOH (8.92 g, 22.3 mmol), TBAI (360 mg, 0.975 mmol), and benzyl bromide (19.8 mL, 167 mmol) successively and the reaction mixture was allowed to stir briskly for 5 h at room temperature. After the TLC showed completed conversion, the reaction mixture was poured into water and extracted with CH_2Cl_2. The organic layer was washed with water, dried over Na_2SO_4, and concentrated under reduced vacuum. The resulting crude mixture was purified by silica gel column chromatography (eluent: Hexane/EtOAc: 10/1) to afford benzylated glycal **42** as a colourless oil (14.1 mg, 92%, lit.[29]). ^{1}H NMR (400 MHz, chloroform-d) δ = 7.35 - 7.23 (m, 15 H, Aromatic C*H*), 6.43 (d, *J* = 6.0 Hz, 1 H, H-1), 4.88 (dd, *J* = 2.7, 6.1 Hz, 1 H, H-2), 4.84 (d, *J* = 11.5 Hz, 1 H, C*H$_2$*-Ph), 4.68 - 4.53 (m, 5 H, C*H$_2$*-Ph), 4.24 - 4.19 (m, 1 H, H-3), 4.10 - 4.04 (m, 1 H, H-4), 3.87 (dd, *J* = 6.2, 8.6 Hz, 1 H, H-5), 3.84 - 3.74 (m, 2 H, H-6, H-6’). ^{13}C NMR (101 MHz, chloroform-d) δ = 144.7 (*C*-1), 138.4, 138.3, 138.1 (3 x Aromatic *C*), 128.4, 127.9, 127.8, 127.7, 127.6 (Aromatic *C*H), 100.0 (*C*-2), 76.8 (*C*-4), 75.8 (*C*-3), 74.5 (C-5), 73.7, 73.5, 70.5 (3 x *C*H$_2$-Ph), 68.6 (C-6). (ESI-HRMS): [M+Na]$^+$ calcd for $C_{27}H_{28}NaO_4$: 439.1885; found: 439,1832.

Methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-C-[bis-(methoxycarbonyl)methyl]-β-D-glucopyranoside (43)



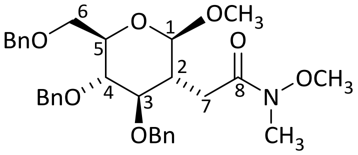
A solution of benzyl-protected glycal **42**(4.02 g, 10.0 mmol), dimethyl malonate (11.5 mL, 100 mmol) and $NaHCO_3$ (3.36 g, 40.0 mmol) in dry methanol (35 mL) was cooled to 0 °C under argon atmosphere. At this temperature, a solution of CAN (22.1 g, 40.3 mmol), which was dried in a desiccator under high vaccum overnight in methanol (50 mL) was added dropwise over a period of 7 h until the TLC showed completed conversion of the starting material. After stirring for 3 min at 0 °C, an ice-cold diluted solution of sodium thiosulfate (350 mL) was added, and the mixture was extracted with CH_2Cl_2 (3 x 100 mL). The combined organic phases were dried Na_2SO_4, filtered and the filtrate concentrated *in vacuo*, and the excess of malonate was removed at 0.01 mbar in a kugelrohr. The resulting crude mixture was purified by silica gel column chromatography (eluent: Hexane/EtOAc: 7/3) to afford malonate **43** as a colourless oil (5.08 g, 91%, lit.[30]). ^{1}H NMR (400 MHz, chloroform-d) δ = 7.32 - 7.17 (m, 13 H, Aromatic C*H*), 7.13 - 7.06 (m, 2 H, Aromatic C*H*), 4.83 (d, *J* = 11.0 Hz, 1 H, C*H₂*-Ph), 4.74 (d, *J* = 11.0 Hz, 1 H, C*H₂*-Ph), 4.67 (d, *J* = 8.8 Hz, 1 H, H-1), 4.61 - 4.46 (m, 4 H, C*H₂*-Ph), 3.85 (d, *J* = 3.8 Hz, 1 H, H-7), 3.75 (d, *J* = 8.4 Hz, 1 H, H-4), 3.69 (br.s., 2 H, H-6, H-6'), 3.62 (s, 3 H, COOC*H₃*), 3.60 (br. s., 1 H, H-3), 3.51 (s, 3 H, COOC*H₃*), 3.45 (dd, *J* = 2.1, 5.4 Hz, 1 H, H-5), 3.39 (s, 3 H, OC*H₃*), 2.48 - 2.39 (m, 1 H, H-2). IR (KBr, dry film) vmax (cm^{-1}) = 1734 (C=O), 1607 (C=C), 1052 (C-O)**.** (ESI-HRMS): $[M+NH_4]^+$ calcd for $C_{33}H_{38}NO_9$: 596.2860; found: 596.286.

Methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-C-[(methoxycarbonyl)methyl]-β-D-glucopyranoside (44)



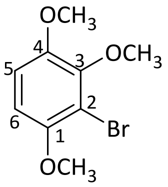
A solution of the malonate **43** (4.29 g, 7.41 mmol) and lithium iodide (1.98 g, 14.8 mmol) in DMSO (20 mL) was heated at 180 °C for 2.5 h. DMSO was removed at 0.01 mbar and the crude product was purified by silica gel column chromatography (eluent: Hexane/EtOAc: 4/1) to afford the ester **44** as a colourless oil (3.67 g, 95%, lit.[30]). ^{1}H NMR (400 MHz, chloroform-d) δ = 7.35 - 7.21 (m, 13 H, Aromatic C*H*), 7.18 - 7.13 (m, 2 H, Aromatic C*H*), 4.88 (d, *J* = 11.2 Hz, 1 H, C*H₂*-Ph), 4.75 (d, *J* = 11.0 Hz, 1 H, C*H₂*-Ph), 4.64 - 4.52 (m, 4 H, C*H₂*-Ph), 4.27 (d, *J* = 8.6 Hz, 1 H, H-1), 3.74 - 3.71 (m, 2 H, H-6, H-6'), 3.60 (d, *J* = 9.3 Hz, 1 H, H-4), 3.53 (s, 3 H, COOC*H₃*), 3.51 (d, *J* = 2.2 Hz, 1 H, H-3), 3.48 (d, *J* = 9.2 Hz, 1 H, H-5), 3.45 (s, 3 H, OC*H₃*), 2.47 (dq, *J* = 5.6, 15.4 Hz, 2 H, H-7, H-7'), 2.17 - 2.08 (m, 1 H, H-2). IR (KBr, dry film) ν max (cm^{-1}) = 1756 (C=O), 1600 (C=C), 1057 (C-O). (ESI-HRMS): $[M+NH_4]^+$ calcd for $C_{31}H_{40}NO_7$: 538.2805; found: 538.2806.

Methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-C-[(*N*-methoxy-*N*-methylcarbonyl)methyl]-β-D-glucopyranoside (45)



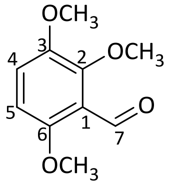
A solution of the ester **44** (1.42 g, 2.73 mmol) and $LiOH.H_2O$ (1.15 g, 27.3 mmol) in $MeOH/H_2O$ (4/1: 15 mL) was heated under reflux until TLC showed complete conversion of the starting material. After cooling to room temperature, the pH value was adjusted to 3-4 with Amberlite IR-120, and the solvent was removed. The crude product was treated with 2-chloro-4,6-dimethoxy-1,3,5-triazine (612 mg, 3.49 mmol) and *N*-methylmorpholine (881 µL, 8.01 mmol) in THF (20 mL). A white precipitate was formed during stirring for 1 h, and *N*,*O*-dimethylhydroxylamine hydrochloride (300 mg, 3.08 mmol) was added. The mixture was stirred overnight and then quenched with H_2O (10 mL) and extracted with diethyl ether (3 X 5 mL). The combined organic phases were washed with saturated aqueous Na_2CO_3 (5 mL) followed by 1N HCl (6 mL) and brine (6 mL). The organic layer was dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated *in vacuo*. The resulting crude mixture was purified by silica gel column chromatography (eluent: Hexane/EtOAc: 4/1) to afford **45** as a colourless oil (1.14 g, 76%). ^{1}H NMR (400 MHz, chloroform-d) δ = 7.32 - 7.17 (m, 13 H, Aromatic C*H*), 7.15 - 7.09 (m, 2 H, Aromatic C*H*), 4.84 (d, *J* = 11.2 Hz, 1 H, CH_2-Ph), 4.72 (d, *J* = 11.0 Hz, 1 H, CH_2-Ph), 4.61 - 4.48 (m, 4 H, CH_2-Ph), 4.45 (d, *J* = 8.6 Hz, 1 H, H-1), 3.82 - 3.77 (m, 1 H, H-3), 3.73 (d, *J* = 3.7 Hz, 1 H, H-6), 3.69 - 3.64 (m, 1 H, H-6'), 3.59 - 3.55 (m, 1H, H-4), 3.53 (s, 3 H, OCH_3), 3.46 (td, *J* = 3.1, 9.8 Hz, 1 H, H-5), 3.41 (s, 3 H, NOCH_3), 3.03 (s, 3 H, NCH_3), 2.60 (t, *J* = 5.4 Hz, 2 H, H-7, H-7'), 2.17 - 2.08 (m, 1 H, H-2). ^{13}C NMR (101 MHz, chloroform-d) δ = 172.54 (*C*=O), 138.7, 138.3, 138.3, 138.3 (Aromatic *C*), 128.4, 128.3, 127.8, 127.7, 127.6, 127.5 (Aromatic *C*H), 103.3 (*C*-1), 82.0 (*C*-3), 80.0 (*C*-4), 75.1 (*C*-5), 74.5, 74.5, 73.5 (3 X *C*H_2-Ph), 69.2 (*C*-6), 61.1 (O*C*H_3), 56.7 (O*C*H_3), 44.5 (*C*-2), 32.4 (N*C*H_3), 28.7 (*C*-7). IR (KBr, dry film) ν_{max} (cm^{-1}) = 1646 (C=O), 1265 (C-N). (ESI-HRMS): $[M+H]^+$ calcd for $C_{32}H_{40}O_7$: 550.2805; found 550.2799.

2-Bromo-1,3,4-trimethoxybenzene (46)



n-Butyl lithium in hexanes (2.5 M, 4.04 mL, 10.1 mmol) was added dropwise to a solution of 1,2,4–trimethoxybenzene **6** (1.54 g, 9.16 mmol) in anhydrous ether (25 mL) at -10 °C under an argonium atmosphere. A white precipitate was formed. The reaction mixture was stirred additionally for 1.5 h at room temperature. Then CBr_4 (3.35 g, 10.1 mmol) dissolved in anhydrous ether (10 mL) was added dropwise at -10 °C under an argonium atmosphere, and the mixture was allowed to stir overnight. The reaction was extracted with EtOAc (3 x 20 mL) and saturated NH_4Cl until complete neutralization. The total organic layer was washed with brine (3 x 15 mL), dried over anhydrous Na_2SO_4, filtered, and the filtrate concentrated under reduced pressure to obtain a crude residue, which was subjected to silica-gel chromatography (eluent: Hexane/EtOAc: 4/1) to give **46** (1.92 g, 85%, lit.[15]) as a yellow solid, mp 68-70 °C. ^{1}H NMR (400 MHz, chloroform-d) δ = 6.84 (d, *J* = 9.0 Hz, 1 H), 6.63 (d, *J* = 9.0 Hz, 1 H), 3.88 (s, 3 H, OC*H₃*), 3.85 (s, 3 H, OC*H₃*), 3.84 (s, 3 H, OC*H₃*). ^{13}C NMR (101 MHz, chloroform-d) δ = 151.2 (*C*), 148.0 (*C*), 147.9 (*C*), 111.8 (*C*H), 108.3 (*C*Br), 106.7 (*C*H), 60.5 (O*C*H₃), 56.7 (O*C*H₃), 56.7 (O*C*H₃). (ESI-HRMS): $[M+H]^+$ calcd for $C_9H_{12}BrO_3$: 246.9970; found 246.9972.

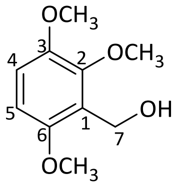
2,3,6-trimethoxybenzaldehyde (47)



A solution of 1,2,4-trimethoxybenzene **6** (5.0 g, 29.7 mmol), TMEDA (3.80 g, 32.7 mmol) in 30 mL THF at -78 °C under argon atmosphere, was added dropwise *n*-Buthyllithium (2.5 M in hexane, 17.8 mL, 44.6 mmol). The mixture was stirred at this temperature for 1 h and then DMF (4.58 mL, 59.4 mmol) was added. The reaction was allowed to warm at room temperature and stirred overnight. The mixture was quenched with 1 N hydrochloride solution (10 mL). Et_2O (50 mL) and saturated aqueous $NaHCO_3$ (20 mL) were added, and the layers were separated. The organic layer was washed with saturated aqueous NH_4Cl (20 mL) and dried over Mg_2SO_4. The sulphate magnesium was removed by filtration, and the filtrate concentrated *in vacuo*. Column chromatography (eluent: Petroleum ether/EtOAc: 7/3) gave the product **47** as an orange oil (4.22 g, 72%, lit.[15]). ^{1}H NMR (400 MHz, chloroform-d) δ = 10.44 (d, *J* = 0.5 Hz, 1 H, C*H*O), 7.09 (d, *J* = 9.2 Hz, 1 H, C*H*), 6.64 (d, *J* = 9.2 Hz, 1 H, C*H*), 3.92 (s, 3 H, OC*H₃*), 3.85 (s, 3 H, OC*H₃*), 3.84

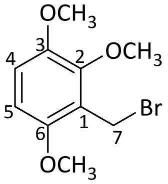
(s, 3 H, OC*H3*). ^{13}C NMR (101 MHz, chloroform-d) δ = 189.3 (*C*=O), 154.6 (*C*), 151.8 (*C*), 146.5 (*C*), 119.5 (*C*), 118.9 (*C*H), 106.1 (*C*H), 61.6 (O*C*H$_3$), 56.5 (O*C*H$_3$), 55.9 (O*C*H$_3$). IR (KBr, dry film) ν$_{max}$ (cm^{-1}) = 2840 (H-C=O), 1692 (C=O), 1487 (C=C), 1099 (C-O). (ESI-HRMS): [M+H]$^+$ calcd for $C_{10}H_{13}O_4$: 197.0814; found 197.0810.

2,3,6-trimethoxybenzyl alcohol (48)



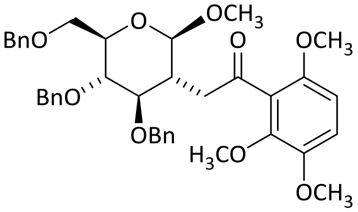
The aldehyde **47** (302 mg, 1.54 mmol) was dissolved in DCM (4 mL) and MeOH (4 mL). Sodium borohydride (58.2 mg, 1.54 mmol) was added to the reaction mixture, the solution was stirred for 30 min at room temperature. Saturated aqueous NH_4Cl (5 mL) was added, and the reaction mixture was diluted with DCM. The layers were separated, and the organic layer was washed with saturated aqueous NH_4Cl (1 X 10 mL) and saturated NaCl (1 X 10 mL). The organic layer was dried over Na_2SO_4, filtered and the filtrate concentrated *in vacuo*. Column chromatography (eluent: Petroleum ether/EtOAc: 7/3) gave the product **48** as a solid (208 mg, 92%, lit.[28]). ^{1}H NMR (400 MHz, chloroform-d) δ = 6.82 (d, *J* = 9.0 Hz, 1 H, C*H*), 6.60 (d, *J* = 9.0 Hz, 1 H, C*H*), 4.78 (d, *J* = 6.8 Hz, 2 H, C*H2*), 3.89 (s, 3 H, OC*H3*), 3.84 (s, 3 H, OC*H3*), 3.83 (s, 3 H, OC*H3*), 2.54 (t, *J* = 6.8 Hz, 1 H, O*H*). ^{13}C NMR (101 MHz, chloroform-d) δ = 152.4(*C*), 148.2(*C*), 147.2(*C*), 123.5(*C*), 112.2 (*C*H), 105.6 (*C*H), 61.5 (*C*H$_2$), 56.4 (O*C*H$_3$), 55.9 (O*C*H$_3$), 55.6 (O*C*H$_3$). IR (KBr, dry film) ν$_{max}$ (cm^{-1}) = 3297 (O-H), 1486 (C=C), 1076 (C-O). (ESI-HRMS): [M+Na]$^+$ calcd for $C_{10}H_{14}NaO_4$: 221.0790; found 221.0813.

2,3,6-trimethoxybenzylbromide (49)



To a stirred solution of alcohol **48** (249 mg, 1.26 mmol) in dry Et_2O (4 mL) at room temperature under argon atmosphere, was added dry pyridine (0.002 mL, 0.0252 mmol) followed by dropwise addition of PBr_3 (0.002 mL, 0.503 mmol) over 5 min. The resulting light yellow solution, with visible off white precipitate, was stirred for 1 h and then diluted with 5 mL Et_2O and quenched with 7 mL H_2O. The solution was allowed to stir further for 20 min and then the layers were separated. The aqueous layer was extracted further with Et_2O (3 X 7 mL). The combined organic layers were washed with 20 mL of saturated $NaHCO_3$ solution, dried over anhydrous $MgSO_4$, filtered and the filtrate concentrated in *vacuo*. Column chromatography (eluent: Petroleum ether/EtOAc: 7/3) gave **49** as a solid (297 mg, 91%) mp 39-41°C. ^{1}H NMR (400 MHz, chloroform-d) δ = 6.85 (d, *J* = 9.0 Hz, 1 H, C*H*), 6.58 (d, *J* = 9.0 Hz, 1H, C*H*), 4.68 (s, 2 H, C*H₂*), 3.99 (s, 3 H, OC*H₃*), 3.86 (s, 3 H, OC*H₃*), 3.83 (s, 3H, OCH3). ^{13}C NMR (101 MHz, chloroform-d) δ = 152.2 (*C*), 148.6 (*C*), 147.0 (*C*), 121.2 (*C*), 113.5 (*C*H), 105.8 (*C*H), 60.9 (*C*H_2), 56.5 (O*C*H_3), 56.2 (O*C*H_3), 56.1 (O*C*H_3). IR (KBr, dry film) ν_{max} (cm^{-1}) = 1484 (C=C), 1073 (C-O), 530 (C-Br). (ESI-HRMS): $[M+H]^+$ calcd for $C_{10}H_{14}BrO_3$: 263.0106; found: 263.0126.

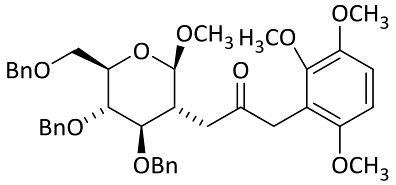
Attempted preparation of methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-C-[(2,3,6-trimethoxyphenylcarbonyl)methyl]-β-D-glucopyranoside (52)



(a) Magnesium turnings (37.2 mg, 1.53 mmol) were treated with 2-bromo-1,3,4-trimethoxybenzene **46** (135 mg, 0.546 mmol) in dry E_2tO (1.5 mL). The reaction mixture was heated under reflux under Ar for 2 h and then cooled to 0 °C and a solution of Weinreb amide **45** (300 mg, 0.546 mmol) in dry E_2tO (3 mL) was added. The reaction was stirred at 0 °C and then allowed to warm to room temperature overnight. The mixture was quenched with saturated NH_4Cl (2 mL) and extracted with E_2tO (3 x 5 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated under vacuum, and we re-isolated all reactants instead of product **52**.

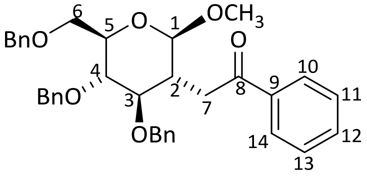
(B) A solution of the ester **44** (500 mg, 0.960 mmol) and $LiOH.H_2O$ (403 mg, 9.60 mmol) in $MeOH/H_2O$ (4/1: 5 mL) was heated under reflux until TLC showed complete conversion of the starting material. After cooling to room temperature, the pH value was adjusted to 3-4 with Amberlite IR-120, and, after filtration, the solvent was removed. The residue (acid) was diluted with dry DCM (10 mL), and oxalyl chloride (1.08 g, 8.49 mmol) and dry DMF (3 drops) were added. After 15 min at room temperature, the mixture was heated under reflux for 30 min. The solvent was evaporated under reduced vacuum, the residue **59** dissolved in dry THF, and immediately used without further purification. Separately, a solution of 1,2,4-trimethoxybenzene **6** (250 mg, 1.49 mmol) in dry THF (8 mL) was treated with n-BuLi (2.5 mL, 0.7 mL) at -18 °C. After 15 min, a solution of freshly prepared **59** in dry THF was added, and the mixture was allowed to warm up at room temperature over 5 h. Saturated NH_4Cl solution and Et_2O were added, and the organic layer was washed with H_2O and filtered through Na_2SO_4. The filtrate was evaporated under reduced pressure and the residue purified by silica gel (eluent: petroleum ether/EtOAc: 2/1), but no product **52** was formed. We did not have evidence of the preparation of the compound **59** but it was recovered the compound **6**, which was obtained after protonation of **6a** during work-up process.

Attempted preparation of methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-C-[-3-(1,2,4-trimethoxyphenyl)-2-oxo-propyl]-β-D-glucopyranoside (53)



Magnesium turnings (39.0 mg, 1.61 mmol) were treated with 1,2,3-trimethoxybenzylbromide **49** (150 mg, 0.575 mmol) in Et_2O (1.5 mL). The reaction mixture was heated under reflux for 2 h and then cooled to 0 °C. Then a solution of Weinreb amide **45** (316 mg, 0.575 mmol) in dry Et_2O (3.5 mL) was added. The reaction mixture was stirred at this temperature and allowed to warm to room temperature overnight. The mixture was quenched with saturated NH_4Cl (2.5 mL) and extracted with Et_2O (3 x 5 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated under vacuum, and we re-isolated starting materials instead of the desired product **53**.

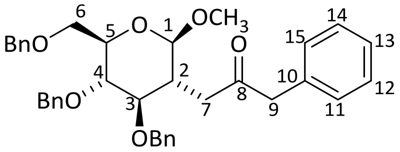
Methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-C-[(phenylcarbonyl)methyl]-β-D-glucopyranoside (57)



To a solution of the Weinreb amide **45** (533 mg, 0.970 mmol) in 10 mL of anhydrous Et_2O was added via a syringe the phenyl magnesium bromide **55** (3.0 M in Et_2O, 0.65 mL, 1.94 mmol) at 0 °C. The mixture was allowed to reach the room temperature and stirred until the completion of starting material. The reaction mixture was quenched with 3 mL of saturated NH_4Cl and extracted with DCM (3 X 5 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and the filtrate concentrated under reduced vacuum. Purification by Column chromatography (eluent: Petroleum ether/EtOAc: 7/3) to afford **57** (473 mg, 86%), mp 94-96 °C as a white solid. ^1H NMR (400

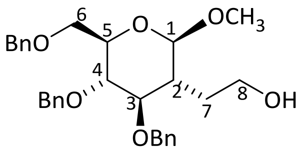
MHz, chloroform-d) δ = 7.87 - 7.83 (m, 2 H, Aromatic), 7.55 - 7.50 (m, 1 H, Aromatic), 7.44 - 7.27 (m, 11 H, Aromatic), 7.22 - 7.18 (m, 6 H, Aromatic), 4.90 (d, *J* = 11.4 Hz, 1 H, C*H₂*-Ph), 4.80 (d, *J* = 10.8 Hz, 1 H, C*H₂*-Ph), 4.68 - 4.55 (m, 4 H, C*H₂*-Ph), 4.45 (d, *J* = 8.6 Hz, 1 H, H-1), 3.79 - 3.76 (m, 2 H, H-6, H-6'), 3.76 - 3.72 (m, 1 H, H-3), 3.65 (t, *J* = 9.2 Hz, 1 H, H-5), 3.57 - 3.51 (m, 1 H, H-4), 3.40 (s, 3 H, OC*H₃*), 3.11 (dd, *J* = 2.7, 5.4 Hz, 2 H, H-7, H-7'), 2.37-2.28 (m, 1 H, H-2). ^{13}C NMR (101 MHz, chloroform-d) δ = 199.2 (*C*=O), 138.3, 138.3, 137.3, 132.6 (Aromatic *C*), 128.4, 128.3, 128.2, 127.8, 127.7, 127.5 (Aromatic *C*H), 103.5 (*C*-1), 82.1 (*C*-3), 80.2 (*C*-5), 75.2 (*C*-4), 74.7, 74.6, 73.5 (3 X *C*H$_2$-Ph), 69.1 (*C*-6), 56.7 (O*C*H$_3$), 45.2 (*C*-2), 36.1 (*C*-7). IR (KBr, dry film) ν_{max} (cm^{-1}) = 1690 (C=O), 1596 (C=C), 1114 (C-O). (ESI-HRMS): $[M+NH_4]^+$ calcd for $C_{36}H_{42}NO_6$: 584.3012; found: 584.3162.

Methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-C-[(benzylcarbonyl)methyl]-β-D-glucopyranoside (58)



Magnesium turnings (33.0 mg, 1.36 mmol) were treated with benzyl bromide (82.1 mg, 0.480 mmol) in Et$_2$O (1.5 mL). A vigorous reaction ensued which was cooled in an ice bath. Once the reaction had dissipated, the reaction mixture was heated under reflux for 2 h yielding the Grignard reagent. The reaction mixture was cooled to 0 °C and a solution of Weinreb amide **45** (264 mg, 0.480 mmol) in dry Et$_2$O (3 mL) was added. The reaction was stirred briefly at this temperature and then allowed to warm to room temperature overnight. The mixture was quenched with saturated NH$_4$Cl (2 mL) and extracted with Et$_2$O (3 X 5 mL). The combined organic layers were dried over anhydrous Na$_2$SO$_4$, filtered and the filtrate concentrated under reduced vacuum. Purification by Column chromatography (eluent: Petroleum ether/EtOAc: 7/3) to afford **58** (58.1 mg, 67%) as a colorless syrup. ^{1}H NMR (400 MHz, chloroform-d) δ = 7.42 - 7.15 (m, 18 H, Aromatic), 7.15 - 7.10 (m, 2 H, Aromatic), 4.86 (d, *J* = 11.2 Hz, 1 H, CH_2-Ph), 4.77 (d, *J* = 10.8 Hz, 1 H, CH_2-Ph), 4.68 - 4.54 (m, 3 H, CH_2-Ph), 4.47 (d, *J* = 11.2 Hz, 1 H, CH_2-Ph), 4.28 (d, *J* = 8.6 Hz, 1 H, H-1), 3.78 - 3.73 (m, 2 H, H-6, H-6'), 3.70 - 3.66 (m, 1 H, H-4), 3.65 - 3.59 (m, 2 H, H-8, H-8'), 3.59 - 3.55 (m, 1 H, H-3), 3.47 (td, *J* = 3.2, 9.5 Hz, 1 H-5), 3.38 (s, 3 H, OCH_3), 2.69 - 2.60 (m, 1 H, H-7), 2.54 - 2.45 (m, 1 H, H-7'), 2.25 - 2.15 (m, 1 H, H-2). ^{13}C NMR (101MHz, chloroform-d) δ = 207.0 (*C*=O), 138.2, 138.1, 134.4, 130.4 (Aromatic *C*), 129.5, 128.5, 128.4, 128.3, 127.8, 127.8, 127.7, 127.7, 127.6, 126.8 (Aromatic *C*H), 103.5 (*C*-1), 82.1 (*C*-3), 79.9 (*C*-4), 75.2 (*C*-5), 74.7, 74.6, 73.5 (3 X *C*H$_2$-Ph), 69.0 (*C*-6), 56.7 (O*C*H$_3$), 49.5 (*C*-8), 44.8 (*C*-2), 39.8 (*C*-7). IR (KBr, dry film) ν_{max} (cm^{-1}) = 1712 (C=O), 1551 (C=C), 1108 (C-O). (ESI-HRMS): $[M+NH_4]^+$ calcd for $C_{36}H_{42}NO_6$: 598.3169; found: 598.3184.

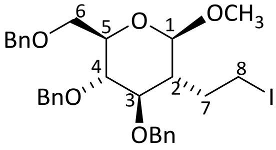
Methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-C-(ethanol)-β-D-glucopyranoside (60)



A suspension of the ester **44** (2.11 mg, 4.05 mmol) and LiAlH$_4$ (461 mg, 12.2 mmol) in 1,4-dioxane (12 mL) was stirred at reflux for 3 h until TLC showed complete conversion of the starting material. Ethyl acetate (8 mL) and H$_2$O (20 mL) were added sequentially. The mixture was filtered over celite and was extracted with DCM (4 X 20 mL). The combined organic layers were dried over Na$_2$SO$_4$, filtered and the filtrate concentrated under reduced vacuum. The resulting crude mixture was purified by silica gel column chromatography (eluent: Hexane/EtOAc: 4/1) to afford **60** as a colorless oil (1.58g, 79%, lit.[30]). ^{1}H NMR (400 MHz, chloroform-d) δ = 7.37 - 7.25 (m, 13 H, Aromatic), 7.23 - 7.18 (m, 2 H, Aromatic), 4.95 (d, *J* = 11.4 Hz, 1 H, CH_2-Ph), 4.78 (d, *J* = 11.0 Hz, 1 H, CH_2-Ph), 4.69 -

4.55 (m, 4 H, C*H₂*-Ph), 4.15 (d, *J* = 8.4 Hz, 1 H, H-1), 3.76 (br. s., 2 H, H-6, H-6'), 3.68 - 3.63 (m, 3 H, H-8, H-8', H-4), 3.53 (s, 3 H), 3.49 (d, *J* = 6.8 Hz, 1 H, H-5), 3.37 (d, *J* = 9.3 Hz, 1 H, H-3), 1.88 - 1.76 (m, 2 H, H-7, H-2), 1.61 (dd, *J* = 6.9, 14.4 Hz, 1 H, H-7'), 0.94 (t, *J* = 7.3 Hz, 1 H, O*H*). ^{13}C NMR (101 MHz, chloroform-d) δ = 138.2, 138.1, 138.0 (Aromatic *C*), 128.4, 128.3, 127.8, 127.6 (Aromatic *C*H), 104.7 (*C*-1), 83.2 (*C*-3), 79.7 (*C*-4), 75.2 (*C*-5), 75.0, 74.6, 73.5 (3 X *C*H$_2$-Ph), 69.0 (*C*-6), 61.8 (*C*-8), 56.7 (O*C*H$_3$), 45.9 (*C*-2), 30.7 (*C*-7). (ESI-HRMS): [M+NH$_4$]$^+$ calcd for $C_{30}H_{40}NO_6$: 510.2856; found: 510.2987.

Methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-C-(iodoethyl)-β-D-glucopyranoside (61)

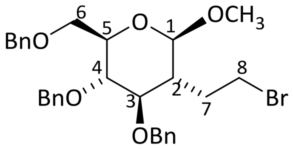


(A) A solution of alcohol **60** (450 mg, 0.914 mmol) in THF (8 mL) containing imidazole (93.3 mg, 1.37 mmol) and triphenylphosphine (289 mg, 1.10 mmol) was cooled to 0 °C then iodine (310 mg, 1.23 mmol) was added. The mixture was stirred under reflux for 4 h, then evaporated and co-evaporated with toluene under reduced vacuum. The residue was purified by column chromatography (eluent: petroleum ether/EtOAc: 1/1), and the alcohol **60** was recovered instead of our desired product **61**.

(B) A mixture of tosylate **63** (210 mg, 0.325 mmol), sodium iodide (243 mg, 1.62 mmol), and DMF (4 mL) was heated at 60 °C for 4 h. Water (5 mL) was added and the resulting mixture was extracted with pentane (3 X 8 mL). The combined organic layers were dried over Na$_2$SO$_4$, filtered and the filtrate concentrated under vacuum. The resulting crude mixture was purified by silica gel column chromatography (eluent: Hexane/EtOAc: 4/1) to afford **61** as a white solid (129 mg, 66%), mp 81-83 °C. ^{1}H NMR (400 MHz, chloroform-d) δ = 7.41 - 7.28 (m, 13 H, Aromatic C*H*), 7.25 - 7.19 (m, 2 H, Aromatic C*H*), 4.94 (d, *J* = 11.2 Hz, 1 H, C*H₂*-Ph), 4.80 (d, *J* = 11.0 Hz, 1 H, C*H₂*-Ph), 4.69 - 4.55 (m, 4 H, C*H₂*-Ph), 4.12 (d, *J* =

8.6 Hz, 1 H, H-1), 3.78 - 3.74 (m, 2 H, H-6, H-6’), 3.68 - 3.60 (m, 1 H, H-4), 3.51 (s, 3 H, O*CH*$_3$), 3.48 - 3.41 (m, 1 H, H-5), 3.38 - 3.28 (m, 2 H, H-3, H-8), 3.28 - 3.19 (m, 1 H, H-8’), 2.24 - 2.13 (m, 1 H, H-7), 2.04 - 1.93 (m, 1 H, H-7’), 1.77 - 1.68 (m, 1 H, H-2). ^{13}C NMR (101 MHz, chloroform-d) δ = 138.2, 138.0, 138.0 (Aromatic *C*), 128.5, 128.4, 128.3, 127.9, 127.9, 127.8, 127.8, 127.8, 127.6 (Aromatic *C*H), 104.5 (*C*-1), 82.8 (*C*-3), 79.7 (*C*-4), 75.2, 75.1 (2 X *C*H$_2$-Ph), 74.7 (*C*-5), 73.5 (*C*H$_2$-Ph), 69.0 (*C*-6), 56.7 (O*C*H$_3$), 48.6 (*C*-2), 33.3 (*C*-7), 4.3 (*C*-8). IR (KBr, dry film) ν_{max} (cm^{-1}) = 1450 (C=C), 1110 (C-O), 463 (C-I). (ESI-HRMS): $[M+NH_4]^+$ calcd for $C_{30}H_{39}INO_5$: 621.1906; found 621.2006.

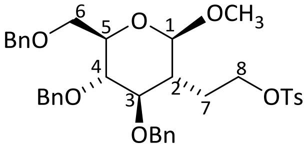
Methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-C-(bromoethyl)-β-D-glucopyranoside (62)



(A) To a stirred solution of alcohol **60** (260 mg, 0.528 mmol) in dry Et$_2$O (6 mL) at room temperature under argon atmosphere, was added dry pyridine (0.850 µL, 0.0106 mmol) followed by dropwise addition of PBr$_3$ (0.020 mL, 0.211 mmol) over 5 min. The resulting light yellow solution, with visible off white precipitate, was stirred for 1 h and then diluted with 4 mL Et$_2$O and quenched with 6 mL H$_2$O. The solution was allowed to stir further for 20 min and then the layers were separated. The aqueous layer was extracted further with Et$_2$O (3 x 5 mL). The combined organic layers were washed with 15 mL of saturated NaHCO$_3$ solution, dried over anhydrous MgSO$_4$, filtered and the filtrate concentrated under reduced vacuum. The residue was purified by column chromatography (Petroleum ether/ Ethyl acetate: 7/3), and the alcohol **60** was recovered instead of our desired product **62**.

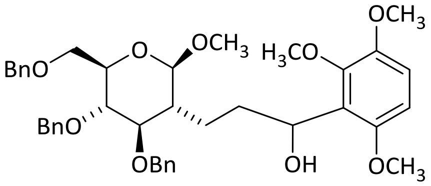
(B) A mixture of tosylate **63** (340 mg, 0.526 mmol), lithium bromide (68.5 mg, 0.789 mmol), and DMF (8 mL) was heated at 60°C overnight. Water (7 mL) was added and the resulting mixture was extracted with ether (3 X 8 mL). The combined organic layers were dried over Na_2SO_4, filtered and the filtrate concentrated under reduced vacuum. The resulting crude mixture was purified by silica gel column chromatography (eluent: Hexane/EtOAc: 4/1) to afford **62** as a white solid (181 mg, 62%, mp 69-71 °C). ^{1}H NMR (400 MHz, chloroform-d) δ = 7.38 - 7.25 (m, 13 H, Aromatic C*H*), 7.22 - 7.17 (m, 2 H, Aromatic C*H*), 4.93 (d, *J* = 11.2 Hz, 1 H, *CH₂*-Ph), 4.78 (d, *J* = 11.0 Hz, 1 H, *CH₂*-Ph), 4.68 - 4.54 (m, 4 H, *CH₂*-Ph), 4.11 (d, *J* = 8.6 Hz, 1 H, H-1), 3.74 (d, *J* = 4.0 Hz, 2 H, H-6, H-6'), 3.63 (t, *J* = 9.2 Hz, 1 H, H-4), 3.56 - 3.51 (m, 1 H, H-5), 3.50 (s, 3 H, OC*H₃*), 3.48 - 3.43 (m, 2 H, H-8, H-8'), 3.37 - 3.31 (m, 1 H, H-3), 2.18 - 2.09 (m, 1 H, H-7), 2.00 - 1.89 (m, 1 H, H-7'), 1.77 (ddd, *J* = 3.9, 10.8, 15.0 Hz, 1 H, H-2). ^{13}C NMR (101 MHz, chloroform-d) δ = 138.4, 138.2, 138.2 (Aromatic *C*), 138.0, 128.5 128.4, 128.3, 127.9, 127.8, 127.8, 127.8, 127.6 (Aromatic *C*H), 104.7 (*C*-1), 82.9 (*C*-3), 79.7 (*C*-4), 75.2 (*C*-8), 75.0, 74.7, 73.5 (3 X *C*H₂-Ph), 69.0 (*C*-6), 56.7 (O*C*H₃), 46.7 (*C*-2), 32.0 (*C*-8), 31.8 (*C*-7). IR (KBr, dry film) ν_{max} (cm^{-1}) = 1498 (C=C), 1001 (C-O), 696 (C-Br). (ESI-HRMS): $[M+NH_4]^+$ calcd for $C_{30}H_{39}BrNO_5$: 572.2012; found: 572.2023.

Methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-C-(tosylethyl)-β-D-glucopyranoside (63)



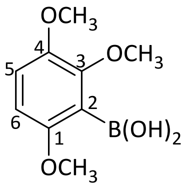
p-Toluenesulfonyl chloride (847 mg, 4.40 mmol) was added to a stirred solution of pyridine (7 mL) and alcohol **60** (1.99 g, 4.04 mmol) at 0 °C. The reaction was allowed to warm at room temperature, stirred for 4 h, and quenched with H_2O (15 mL) extracted with DCM (3 x 12 mL), and the combined organic layers were washed with 3 M HCl (3 x 7 mL) followed by 10% $NaHCO_3$ (1 x 10 mL). The organic layer was dried over Na_2SO_4, filtered and the filtrate concentrated under reduced vacuum. The resulting crude mixture was purified by silica gel column chromatography (eluent: Hexane/EtOAc: 4/1) to afford **63** as colourless oil (1.57 g, 60%). ^{1}H NMR (400 MHz, chloroform-d) δ = 7.74 (d, *J* = 8.2 Hz, 2 H, Aromatic C*H*), 7.38 - 7.24 (m, 15 H, Aromatic C*H*), 7.21 - 7.15 (m, 2 H, Aromatic C*H*), 4.88 (d, *J* = 11.2 Hz, 1 H, CH_2-Ph), 4.74 (d, *J* = 10.8 Hz, 1 H, CH_2-Ph), 4.66 - 4.53 (m, 4 H, CH_2-Ph), 4.20 - 4.11 (m, 1 H, H-7), 4.09 - 4.05 (m, 1 H, H-1), 4.06 - 4.00 (m, 1 H, H-7'), 3.73 (d, *J* = 2.4 Hz, 2 H, H-6, H-6'), 3.62 - 3.55 (m, 1 H, H-4), 3.43 (s, 3 H, OCH_3), 3.40 (d, *J* = 3.1 Hz, 1 H, H-5), 3.31 (dd, *J* = 8.8, 10.6 Hz, 1 H, H-3), 2.42 (s, 3 H, Ar-CH_3), 2.02 - 1.91 (m, 1 H, H-8), 1.81 - 1.71 (m, 1 H, H-8'), 1.69 - 1.62 (m, 1 H, H-2). ^{13}C NMR (101 MHz, chloroform-d) δ = 144.5, 138.2, 138.0, 138.0, 133.3 (Aromatic *C*), 129.7, 128.5, 128.4, 128.3, 127.9, 127.7, 127.6 (Aromatic *C*H), 104.3 (*C*-1), 82.5 (*C*-3), 79.7 (*C*-4), 75.1 (*C*-5), 74.8, 74.6, 73.5 (3 X *C*H_2-Ph), 69.3 (*C*-7), 68.9 (*C*-6), 56.6 (O*C*H_3), 44.3 (*C*-2), 27.1 (*C*-8), 21.5 (Ar -*C*H_3). IR (KBr, dry film) ν_{max} (cm^{-1}) = 1457 (C=C), 1092 (S=O), 901 (S-O). (ESI-HRMS): $[M+NH_4]^+$ calcd for $C_{37}H_{46}NO_8S$: 664.2944; found 664.2972.

Methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-C-[(2,3,6-trimethoxyphenyl)-8-ol-etthyl]-β-D-glucopyranoside (64)



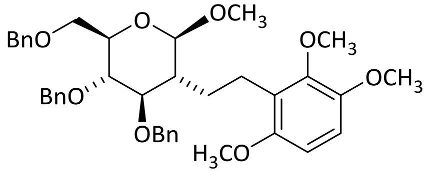
Magnesium turnings (12.6 mg, 0.52 mmol) were treated with iodide **61** (209 mg, 0.480 mmol) in Et_2O (5 mL).No vigorous reaction took place after 5 h. The reaction failed to give the Grignard, and the aldehyde **47** (102 mg, 0.521 mmol) could not be added to the reaction mixture.

(2,3,6-trimethoxy)boronic acid (65)



n-Butyl lithium in hexanes (2.5 M, 2.92 mL, 7.30 mmol) was added dropwise to a solution of 1,2,4–trimethoxybenzene **6** (1.12 g, 6.63 mmol) in anhydrous ether (30 mL) at -10 °C under an argon atmosphere. A white precipitate was formed. The reaction mixture was stirred additionally for 1.5 h at room temperature. Then trimethylborate (0.367 mL, 7.30 mmol) dissolved in anhydrous ether (10 mL) was added dropwise at -10 °C under an argon atmosphere, and the mixture was allowed to stir overnight. The reaction was extracted with EtOAc (3 x 15 mL) and saturated NH_4Cl until complete neutralization. The total organic layer was washed with brine (3 X 10 mL), dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated under reduced pressure to obtain a crude residue, which was subjected to silica-gel chromatography (eluent: Hexane/EtOAc: 4/1) to give **65** (847 mg, 60%, lit.[15]) as an oil. ^{1}H NMR (400 MHz, chloroform-d) δ = 6.99 (d, *J* = 9.0 Hz, 1 H), 6.67 (d, *J* = 9.0 Hz, 1 H), 3.93 (s, 3 H, OCH_3), 3.87 (s, 3 H, OCH_3), 3.84 (s, 3 H, OCH_3). (ESI-HRMS): $[M+H]^+$ calcd for $C_9H_{14}BO_5$: 213.0934; found: 213.0939.

Attempted preparation of methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-C-[(2,3,6-trimethoxyphenyl)ethyl)]-β-D-glucopyranoside (66)

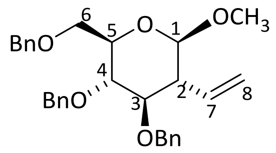


(A). A mixture of iodide **61** (189 mg, 0.314 mmol), boronic acid **64** (100 mg, 0.471 mmol), KO*t*-Bu (105 mg, 0.942 mmol) and tricyclohexylphosphine (10%) in dioxane (6 mL) were degassed for 30 min under argon atmosphere. To the mixture was added $Pd(OAc)_2$ (10 mol%, 26.2 mg) , and the resulting suspension was stirred at 100 °C for 24 h. The reaction mixture was then poured into Et_2O (6 mL), filtered through a short pad of silica gel copious washings EtOAc (12 mL), the filtrate concentrated under reduced vacuum and purified by column chromatography (eluent: Petroleum Ether/EtOAc: 4/1), and we re-isolated the glycosyl iodide **61** and the boronic acid **64** decomposed.

(B). A mixture of bromide **62** (93.0 mg, 0.167 mmol), boronic acid **64** (77.0 mg, 0.335 mmol), KO*t*-Bu (56.0 mg, 0.501 mmol) and tricyclohexylphosphine (10%) in dioxane (5 mL) were degassed for 30 min under argon atmosphere. To the mixture was added and $Pd(OAc)_2$ (10 mol%, 11.2 mg) , and the resulting suspension was stirred at 100 °C for 24 h. The reaction mixture was then poured into Et_2O (5 mL), filtered through a short pad of silica gel copious washings EtOAc (10 mL), the filtrate concentrated under reduced vacuum and purified by column chromatography (Pet Ether/EtOAc: 4/1), and we isolated the glycosyl bromide **62** and the boronic acid **64** decomposed.

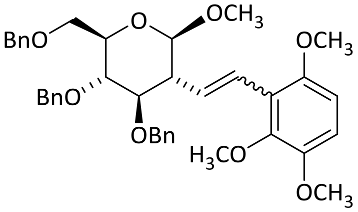
(C). A solution of 1,2,4-trimethoxylbenzene **6** (92.2 mg, 0.548 mmol) in dry THF (10 mL) was cooled to -78 °C. *n*-BuLi (2.5 M, 0.438 mL, 1.096 mmol) was added dropwise. The resulting mixture was stirred at the same temperature for 1 h. The electrophile (glycosyl iodide **61)** (600 mg, 0.996 mmol) was then added dropwise and the reaction was allowed to warm to room temperature gradually overnight. The mixture was quenched with saturated aqueous NH_4Cl solution (5 mL) and extracted with ether (3 x 8 mL). The combined organic phase was washed with water and brine, dried over $MgSO_4$, filtered and the filtrate evaporated under reduced vacuum. Purified by column chromatography (eluent: Petroleum Ether/EtOAc: 4/1), and we isolated the glycosyl iodide **61** and the compound **6** after work-up process.

Methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-C-(vinyl)-β-D-glucopyranoside (67)



To a solution of iodide **61** (115 mg, 0.191 mmol) in DMF (4 mL) was added DBU (43.6 mg, 0.286 mmol). The resulting mixture was heated at 80 °C for 3 h. The mixture was cooled in ice and water, and extracted with DCM (3 x 3 mL). The combined organic extracts were washed with water, dilute HCl, water, saturated $NaHCO_3$ solution, brine and dried over Na_2SO_4, filtered and the filtrate concentrated under reduced vacuum. The resulting crude mixture was purified by silica gel column chromatography (eluent: Hexane/EtOAc: 4/1) to afford **67** as a white solid (61.6 mg, 68%), mp 52-55 °C. ^{1}H NMR (400 MHz, chloroform-d) δ = 7.37 - 7.23 (m, 13 H, Aromatic C*H*), 7.21 - 7.17 (m, 2 H, Aromatic C*H*), 5.68 (ddd, *J* = 8.6, 10.3, 17.1 Hz, 1 H, H-7), 5.30 (td, *J* = 0.8, 17.2 Hz, 1 H, H-8), 5.25 (dd, *J* = 1.7, 10.3 Hz, 1 H, H-8'), 4.81 (d, *J* = 10.8 Hz, 1 H, *CH$_2$*-Ph), 4.74 - 4.64 (m, 2 H, *CH$_2$*-Ph), 4.63 - 4.53 (m, 3 H, *CH$_2$*-Ph), 4.21 (d, *J* = 8.4 Hz, 1 H, H-1), 3.80 - 3.69 (m, 2 H, H-6, H-6'), 3.63 - 3.57 (m, 1 H, H-4), 3.49 (s, 3 H, OC*H$_3$*), 3.47 (d, *J* = 1.8 Hz, 1 H, H-5), 3.46 - 3.43 (m, 1 H, H-3), 2.51 - 2.42 (m, 1 H, H-2). ^{13}C NMR (101 MHz, chloroform-d) δ = 138.3, 138.3, 138.3 (Aromatic *C*), 134.1 (*C*-7), 128.4, 128.3, 128.0, 127.9, 127.7, 127.7, 127.7, 127.5 (Aromatic *C*H), 119.8 (*C*-8), 103.2 (*C*-1), 83.1 (*C*-3), 78.7 (*C*-4), 75.2 (*C*-5), 74.9 (2 x *C*H$_2$-Ph), 73.5 (*C*H$_2$-Ph), 69.2 (*C*-6), 56.7 (O*C*H$_3$), 53.1 (*C*-2). IR (KBr, dry film) ν_{max} (cm^{-1}) = 3100 (=C-H), 1643 (C=C), 1494 (C=C, Ar), 1119 (C-O). (ESI-HRMS): $[M+NH_4]^+$ calcd for $C_{30}H_{38}NO_5$: 492.2750; found: 492.2737.

Attempted synthesis of methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-C-[2-(1,3,4-trimethoxyphenyl)vinyl]-β-D-glucopyranoside (68)

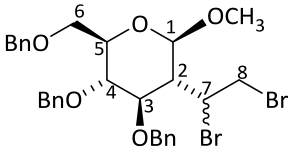


(**1. Heck**). Aryl bromide **46** (20.0 mg, 0.0809 mmol) were added under nitrogen atmosphere to a stirred solution containing PPh_3 (0.654 mg, 0.00249 mmol), $Pd(OAc)_2$

(5mol%, 0.699 mg), Et_3N (0.8 µL, 0.0632 mmol) and alkene **67** (33.4 mg, 0.0632 mmol) in dioxane (2 mL). The resulting solution was allowed to stir at 100 °C for 24 h. The homogeneous reaction mixture was then diluted with EtOAc (4 mL) and filtered through a bed of silica gel layered over Celite, and the volatiles were removed in vacuo. But starting materials were recovered instead of product **68**.

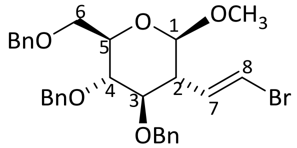
(2. Suzuki). $(PPh_3)_2PdCl_2$ (3.54 mg, 0.00505 mmol), boronic acid **64** (23.0 mg, 0.111 mmol), and K_2CO_3 (41.8 mg, 0.303 mmol) were weighed into a round-bottom flask and stir under nitrogen at room temperature. The alkenyl bromide **71** (56.0 mg, 0.101 mmol) and H_2O (0.1 mL) in 1,4-dioxane (1.5 mL) were then added by syringe. The resulting solution was allowed to stir at 100 °C for 24 h. The homogeneous reaction mixture was then diluted with EtOAc (4 mL) and filtered through a bed of silica gel layered over Celite, and the volatiles were removed in vacuo. It was observed the consumption of starting materials on TLC but we were unable to identify by NMR spectroscopic.

Methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-C-(1,2-dibromoethyl)-β-D-glucopyranoside (69)



A solution of alkene **67** (76.6 mg, 0.155 mmol) in DCM (3 mL) was cooled in an ice bath at 0 °C. After a slow addition of bromine (27.3 mg, 0.171 mmol), the reaction mixture was stirred for 30 min at room temperature. Saturated aqueous sodium thiosulphate (0.5 mL) was added to the reaction mixture. After stirring for 5 min, water (1 mL) and DCM (2 ml) were added to the reaction mixture. The organic layer was separated and washed with water (1.5 mL) and brine (1.5 mL), dried with $MgSO_4$, filtered and the filtrate concentrated *in vacuo.* The resulting crude mixture was purified by silica gel column chromatography (eluent: Hexane/EtOAc: 4/1) to afford **69** as diastereoisomers (colourless oil, 91%, ratio: 2/1). Isomer A: ^{1}H NMR (400 MHz, chloroform-d) δ = 7.29 - 7.19 (m, 13 H, Aromatic C*H*), 7.14 - 7.11 (m, 2 H, Aromatic C*H*), 4.91 (d, *J* = 11.4 Hz, 1 H, CH_2-Ph), 4.69 (d, *J* = 11.2 Hz, 1 H, CH_2-Ph), 4.60 (d, *J* = 8.1 Hz, 1 H, H-7), 4.55 - 4.48 (m, 4 H, CH_2-Ph), 4.47 (d, *J* = 7.9 Hz, 1 H, H-1), 4.06 (t, *J* = 9.8 Hz, 1 H, H-8), 3.79 (dd, *J* = 6.2, 10.1 Hz, 1 H, H-8'), 3.72 - 3.67 (m, 3 H, H-5, H-6, H-6'), 3.67 - 3.64 (m, 1 H, H-3), 3.47 (s, 3 H, OCH_3), 3.45 - 3.42 (m, 1 H, H-4), 2.21 - 2.15 (m, 1 H, H-2). ^{13}C NMR (101 MHz, chloroform-d) δ = 138.0 (2*C*), 137.8 (*C*), 128.5 (*C*H), 128.4 (*C*H) , 128.4 (*C*H), 127.8 (*C*H), 127.7 (*C*H), 103.7 (*C*-1), 80.5 (*C*-3), 79.5 (*C*-5), 75.3 (*C*-4), 75.2, 74.8, 73.6 (3 X CH_2-Ph), 68.9 (*C*-6), 56.6 (OCH_3), 51.1 (*C*-7), 48.3 (*C*-2), 35.8 (*C*-8). Isomer B: ^{1}H NMR (400 MHz, chloroform-d) δ = 7.32 - 7.22 (m, 13 H, Aromatic C*H*), 7.05 (dd, *J* = 2.9, 6.6 Hz, 2 H, Aromatic C*H*), 5.00 (d, *J* = 11.5 Hz, 1 H, CH_2-Ph), 4.80 (d, *J* = 11.4 Hz, 1 H, CH_2-Ph), 4.72 (d, *J* = 10.8 Hz, 1 H, CH_2-Ph), 4.67 - 4.64 (m, 1 H, H-7), 4.64 - 4.59 (m, 3 H, CH_2-Ph), 4.24 (d, *J* = 8.1 Hz, 1 H, H-1), 4.03 - 3.97 (m, 1 H, H-8), 3.75 - 3.72 (m, 1 H, H-8'), 3.72 - 3.70 (m, 3 H, H-5, H-6, H-6'), 3.67 - 3.66 (m, 1 H, H-3), 3.48 (s, 3 H, OCH_3), 3.42 - 3.39 (m, 1 H, H-4), 2.28 - 2.22 (m, 1 H, H-2). ^{13}C NMR (101 MHz, chloroform -d) δ = 138.1 (2 *C*), 137.8 (*C*), 128.5 (*C*H), 128.4 (*C*H) , 127.8 (*C*H) , 127.8 (*C*H), 127.6 (*C*H), 127.6 (*C*H), 127.5 (*C*H), 127.0 (*C*H), 102.2 (*C*-1), 81.3 (*C*-3), 80.4 (*C*-5), 75.2 (*C*-4), 75.2, 74.8, 74.3 (3 X CH_2-Ph), 68.7 (*C*-6), 57.0 (OCH_3), 53.0 (*C*-7), 48.5 (*C*-2), 35.9 (*C*-8). IR (KBr, dry film) ν_{max} (cm^{-1}) = 1458 (C=C), 1091 (C-O), 540 (C-Br). (ESI-HRMS): $[M+NH_4]^+$ calcd for $C_{30}H_{38}Br_2NO_5$: 652.1096; found 652.1100.

Methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-C-(1-bromovinyl)-β-D-glucopyroside (71)

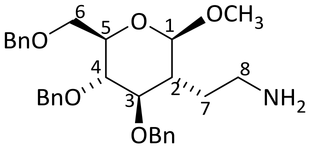


(A). To a stirred solution of 1,2 dibromoalkane **69** (295 mg, 0.455 mmol) in dry THF (5 mL) was added *t*-BuOK (153 mg, 1.36 mmol). The resulting mixture was stirred and refluxed at 60 °C for 24 h. After cooling to room temperature, the solvent was evaporated, the mixture diluted with H_2O, and the aqueous solution extracted with DCM (3 X 5 mL). The organic layers were combined, washed with water, dried over Na_2SO_4, filtered and the filtrate was concentrated under reduced vacuum. The residue was purified by column chromatography (Pet ether/ EtOAc: 4/1) to give **71** as a colorless syrup (146 mg, 66%).

(B). To a stirred solution of 1,2 dibromoalkane **69** (160 mg, 0.246 mmol) in dry toluene (3 mL) was added $NaNH_2$ (52.0 mg, 1.33 mmol). The resulting mixture was stirred and refluxed at 120 °C for 24 h. A saturated solution of NH_4Cl (5 mL) was added portion wise to the mixture with vigorous stirring at -78 °C. After the mixture was concentrated at reduced pressure, water and ether were added to the residue. The organic layer was separated. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and the filtrate concentrated *in vacuo*. The residue was purified by column chromatography (Pet ether/ EtOAc: 4/1) to give **71** as colorless syrup (73.2 mg, 61%).

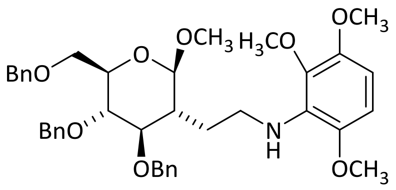
^{1}H NMR (400 MHz, chloroform-d) δ = 7.29 - 7.23 (m, 13 H, Aromatic C*H*), 7.16 (dd, *J* = 1.8, 7.3 Hz, 2 H, Aromatic C*H*), 6.21 (d, *J* = 13.6 Hz, 1 H, H-8), 5.90 (dd, *J* = 9.0, 13.6 Hz, 1 H, H-7), 4.78 (d, *J* = 11.0 Hz, 1 H, CH_2-Ph), 4.70 (d, *J* = 10.6 Hz, 1 H, CH_2-Ph), 4.60 - 4.51 (m, 4 H, CH_2-Ph), 4.13 (d, *J* = 8.4 Hz, 1 H, H-1), 3.71 - 3.67 (m, 2 H, H-6, H-6’), 3.59 - 3.52 (m, 1 H, H-4), 3.43 (s, 3 H, OCH_3), 3.43 - 3.40 (m, 1 H, H-5), 3.39 - 3.34 (m, 1 H, H-3), 2.43 - 2.35 (m, 1 H, H-2). ^{13}C NMR (101 MHz, chloroform-d) δ = 138.2, 138.1, 137.7 (Aromatic *C*), 133.2 (*C*-7), 128.4, 128.3, 127.9, 127.8, 127.8, 127.7, 127.6 (Aromatic *C*H), 108.9 (*C*-8), 102.4 (*C*-1), 82.2 (*C*-3), 78.6 (*C*-4), 75.2 (*C*-5), 75.1, 74.9, 73.4 (3 X *C*H_2-Ph), 69.0 (*C*-6), 56.6 (O*C*H_3), 52.7 (*C*-2). IR (KBr, dry film) ν_{max} (cm^{-1}) = 3026 (=C-H), 1731 (C=C), 1453 (C=C, Ar), 1110 (C-O), 695 (C-Br). (ESI-HRMS): $[M+NH_4]^+$ calcd for $C_{30}H_{37}BrNO_5$: 570.1855; found: 570.1851.

Methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-C-(1-aminoethyl)-β-D-glucopyranoside (72)



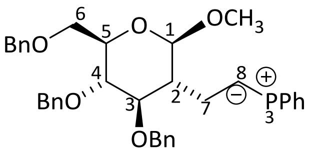
A mixture of tosylate **63** (80 mg, 0.124 mmol) and NaN_3 (16.19 mg, 0.249 mmol) was stirred in DMF (1.5 mL) at 80 °C for 3 h. Then, the mixture was extracted with DCM and water (H_2O). The organic layer was concentrated, and the residue was dissolved in THF (2 mL), and then the Ph_3P (65.3 mg, 0.249 mmol) and H_2O (0.01 mL) were added. After stirring at 65 °C for 3 h, the mixture was diluted with ethyl acetate (8 mL). The organic layer was washed with brine, dried over Na_2SO_4, filtered and the filtrate was concentrated. The residue was purified by column chromatography (eluent: DCM/MeOH: 9/1) to give the amine **72** as a white solid (41.4 mg, 68%, mp 151-152 °C). ^{1}H NMR (400 MHz, chloroform-d) δ = 7.33 - 7.19 (m, 13 H, Aromatic C*H*), 7.17 - 7.11 (m, 2 H, Aromatic C*H*), 4.88 (d, *J* = 11.2 Hz, 1 H, *CH₂*-Ph), 4.72 (d, *J* = 10.8 Hz, 1 H, *CH₂*-Ph), 4.64 - 4.49 (m, 4 H, *CH₂*-Ph), 4.06 (d, *J* = 8.6 Hz, 1 H, H-1), 3.71 - 3.67 (m, 2 H, H-6, H-6'), 3.59 - 3.52 (m, 1 H, H-4), 3.44 (s, 3 H, OC*H₃*), 3.40 (ddd, *J* = 2.5, 4.2, 9.7 Hz, 1 H, H-5), 3.31 (dd, *J* = 8.7, 10.7 Hz, 1 H, H-3), 2.69 (t, *J* = 7.2 Hz, 2 H, H-8, H-8'), 1.67 (dt, *J* = 2.2, 4.1 Hz, 1 H, H-2), 1.59 (dd, *J* = 4.2, 7.5 Hz, 1 H, H-7), 1.52 - 1.44 (m, 1 H, H-7'). ^{13}C NMR (101 MHz, chloroform-d) δ = 138.4, 138.3, 138.1 (Aromatic *C*), 128.4, 128.3, 127.8, 127.7, 127.6 (Aromatic *C*H), 104.9 (*C*-1), 83.4 (*C*-3), 79.9 (*C*-4), 75.2 (*C*-5), 75.0, 74.7, 73.5 (3 X *C*H₂-Ph), 69.2 (*C*-6), 56.7 (O*C*H₃), 45.2 (*C*-2), 40.5 (*C*-8), 32.0 (*C*-7). IR (KBr, dry film) ν_{max} (cm^{-1}) = 3006 (N-H), 1463 (C=C, Ar), 1093 (C-O). (ESI-HRMS): $[M+H]^+$ calcd for $C_{30}H_{38}NO_5$: 492.2750; found: 492.2760.

Attempted preparation of methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-C-[(2,3,6-trimethoxyphenylamino) ethyl]-β-D-glucopyranoside (73)



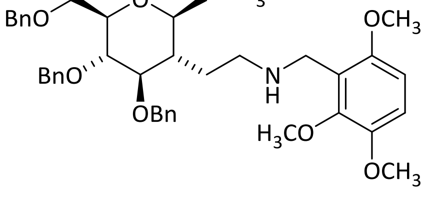
Under argon atmosphere, a suspension of Pd_2db_3 (12.77 mg, 0.0140 mmol) and (±) BINAP (18.5 mg, 0.0279 mmol) in toluene (1.5 mL) was stirred for 1 h at rt. To the mixture were added aryl bromide **46** (138 mg. 0.558 mmol) and $CsCO_3$ (272 mg, 0.837 mmol), and the mixture was stirred for 0.5 h at rt. Then amine **72** (137 mg, 0.279 mmol) was added and the mixture was stirred under reflux for 6 h. After cooling to rt, the mixture was filtered through a plug of celite and concentrated. Column chromatography (Eluent: Pet Ether/EtOAc: 1/1). It was recovered starting materials.

Attempted preparation of methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-C-[(triphenylphosphonium iodide) ethyl]-β-D-glucopyranoside (74)



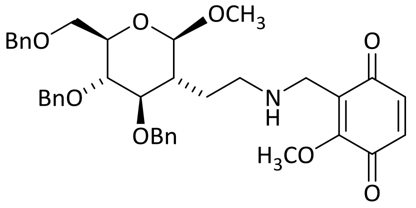
A mixture of iodide **61** (42.2 mg, 0.070 mmol), PPh_3 (27.3 mg, 0.105 mmol), and toluene (2.5 mL) was heated at 105 °C for 3 days. The reaction mixture was then cooled to room temperature and filtered. The filtrate was washed with tert-butyl methyl ether (5 mL) and dried at room temperature, and we recovered starting materials.

Methyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-C-[(2,3,6-trimethoxybenzylamino)ethyl]-β-D-glucopyranoside (76)



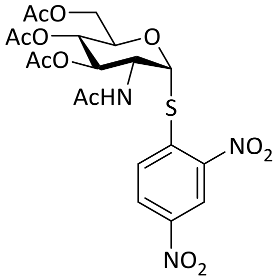
A mixture of amine **72** (294 mg, 0.598 mmol) and aldehyde **47** (129 mg, 0.658 mmol) in methanol (2.5 mL) was stirred overnight. Then $NaBH_4$ was added in small portion and the resulting solution was stirred for 5 h. The mixture was quenched with saturated aqueous $NaHCO_3$ (3 mL) and extracted with DCM (3 x 5 mL). The combined organic phase was washed with water and brine, dried over $MgSO_4$, filtered and the filtrate evaporated *in vacuo* to afford **76** as a brown oil (284 mg, 72%).^{1}H NMR (400 MHz, chloroform-d) δ = 7.35 - 7.22 (m, 13 H, Aromatic C*H*), 7.18 - 7.13 (m, 2 H, Aromatic C*H*), 6.73 (d, *J* = 8.8 Hz, 1 H, Aromatic C*H*), 6.50 (d, *J* = 9.0 Hz, 1 H, Aromatic C*H*), 4.85 (d, *J* = 11.0 Hz, 1 H, CH_2-Ph), 4.74 (d, *J* = 10.8 Hz, 1 H, CH_2-Ph), 4.63 - 4.51 (m, 4 H, CH_2-Ph), 4.12 (d, *J* = 8.2 Hz, 1 H, H-1), 3.82 (s, 2 H, H-9, H-9’), 3.79 (s, 3 H, OCH_3), 3.78 (s, 3 H, OCH_3), 3.71 (s, 3 H, OCH_3), 3.71 - 3.64 (m, 2 H, H-6, H-6’), 3.59 - 3.52 (m, 1 H, H-4), 3.44 (s, 3 H, OCH_3), 3.43 - 3.38 (m, 1 H, H-5), 3.35 (dd, *J* = 8.8, 10.3 Hz, 1 H, H-3), 2.74 - 2.63 (m, 2 H, H-8, H-8’), 1.82 - 1.73 (m, 1 H, H-7), 1.72 - 1.61 (m, 2 H, H-2, H-7’). ^{13}C NMR (101 MHz, chloroform-d) δ = 152.6, 148.6, 147.0, 138.4, 138.3, 138.2 (Aromatic *C*), 128.4, 128.3, 127.8, 127.8, 127.7, 127.7, 127.6, 127.5 (Aromatic *CH*) , 111.6 (C-1), 105.4, 104.7 (Aromatic *CH*), 83.3 (*C*-3), 79.8 (*C*-4), 75.1 (*C*-5), 75.0, 74.6, 73.5 (3 X CH_2-Ph), 69.2 (*C*-6), 61.0, 56.7, 56.3, 55.8 (4 x OCH_3), 47.1 (*C*-8), 45.8 (*C*-2), 42.2 (*C*-9), 27.9 (*C*-7). IR (KBr, dry film) ν_{max} (cm^{-1}) = 3059 (NH), 1419 (C=C), 1152 (C-O). (ESI-HRMS): $[M+H]^+$ calcd for $C_{40}H_{50}NO_8$: 672.3536; found: 672.3558.

Attempted preparation of methyl-3,4,6-tri-*O*-benzyl-2-deoxy-2-C-[(3-methoxy-1,4-benzoquinone amino)ethyl]-β-D-glucopyranoside (77)



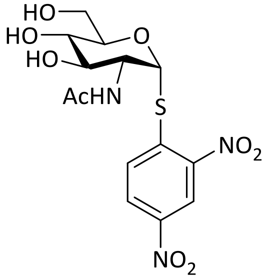
In a stirred solution of hybrid **76** (136 mg, 0.202 mmol) in a mixture of H_2O (1mL) and MeOH (3 mL) was added iodobenzene diacetate (100 mg, 0.304 mmol) at room temperature. The mixture was stirred for 19 h and then extracted with ether (4 mL x 3). The combined organic layer was washed with saturated $NaHCO_3$, water and brine, dried over $MgSO_4$, filtered and the filtrate evaporated *in vacuo*. The crude mixture was purified by column chromatography on silica gel using pentane-ethyl acetate (8:1). The expected product **77** was not isolated but the amine **72** was isolated. $[M+H]^+$ calcd for $C_{30}H_{38}NO_5$: 492.2750; found: 492.2748.

2,4-Dinitrophenyl 2-acetamido-2-deoxy-1-thio-3,4,6-tri-*O*-acetyl-α-D-glucopyranose (83)



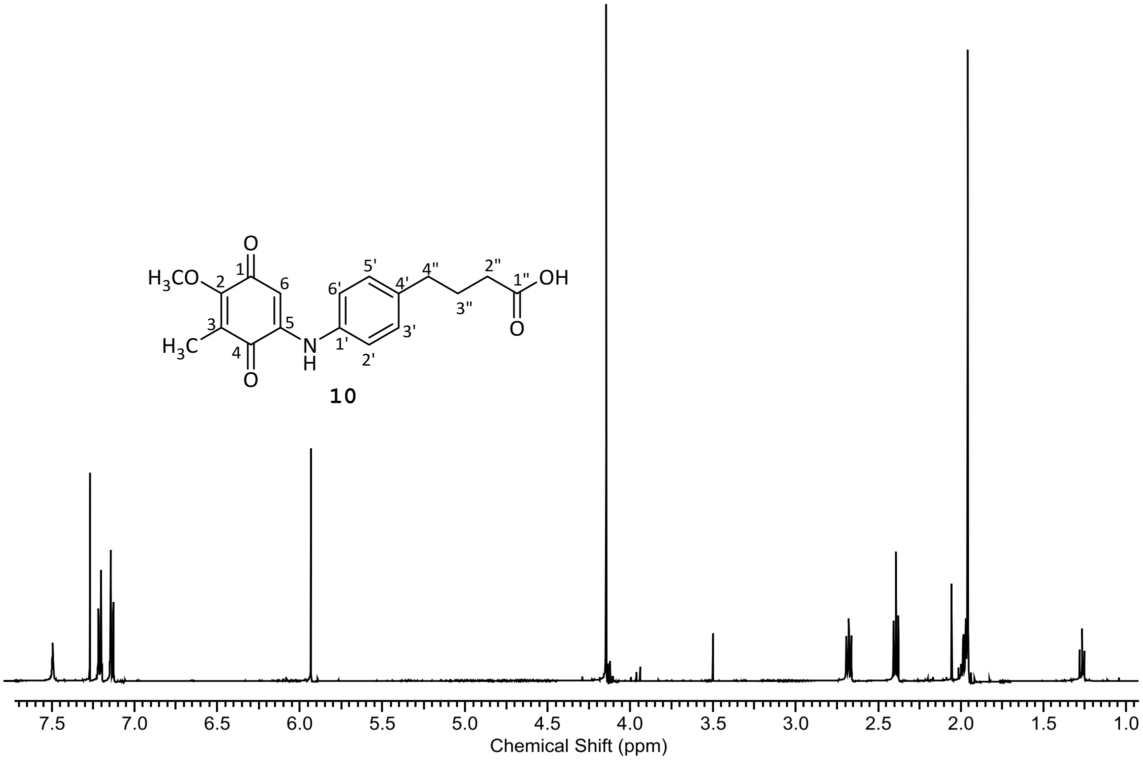
A solution of the thiazoline **81** (479 mg, 2.9 mmol) in MeOH (5 mL) was cooled to 0 °C, before addition of 2 drops of TFA and 2 drops H_2O. The reaction mixture was allowed to warm to room temperature over a period of 2 h. The reaction mixture was concentrated under reduced pressure to give of the mercaptan **82** as a colorless syrup. Without further purification, a solution of mercaptan **81** in 10 mL DCM stirred under a N_2 atmosphere was added 1-fluoro-2,4-dinitrobenzene (89.1 µL, 1.32 mmol) and triethylamine (220 µL, 1.58 mmol). The mixture was allowed to stir under a N_2 atmosphere for 24 h. The reaction mixture was then concentrated and the resulting product was purified using flash chromatography (eluent: 2% $MeOH/CH_2Cl_2$) to give the compound **83** (75%, 523 mg, lit.[65]) as a yellow solid. ^1H NMR (400 MHz, chloroform-d) δ = 9.03 (d, 1 H), 8.39(dd, 1 H), 8.05 (d, 1 H), 6.11 (d, 1 H), 5.22 (m, 2 H), 4.64 (m, 2 H), 4.23 (m, 2 H), 4.03 (dd, 1 H), 2.07 (s, 3 H), 2.03 (s, 3 H), 1.99 (s, 3 H), 1.94 (s, 3 H). (ESI-HRMS): $[M+Na]^+$ calcd for $C_{20}H_{23}NaO_{12}S$: 552.09; found: 552.10.

2,4-Dinitrophenyl 2-acetamido-2-deoxy-1-thio-α-D-gluco-pyranose (84)

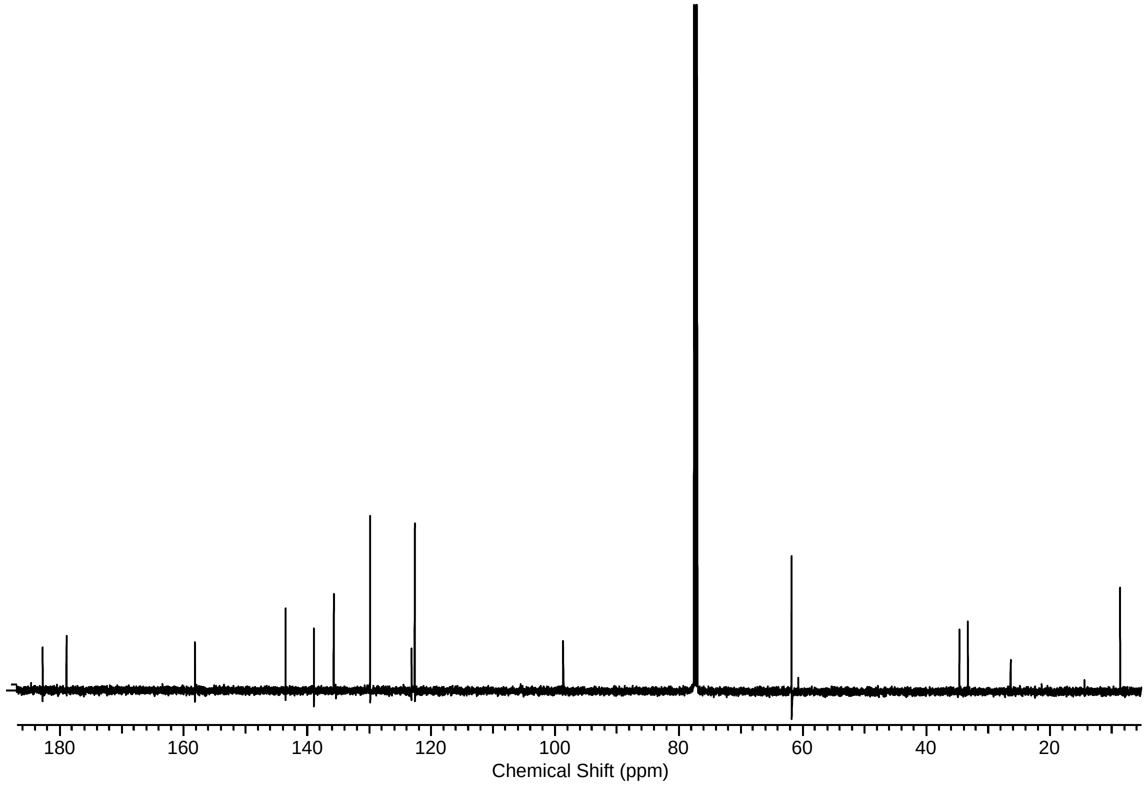


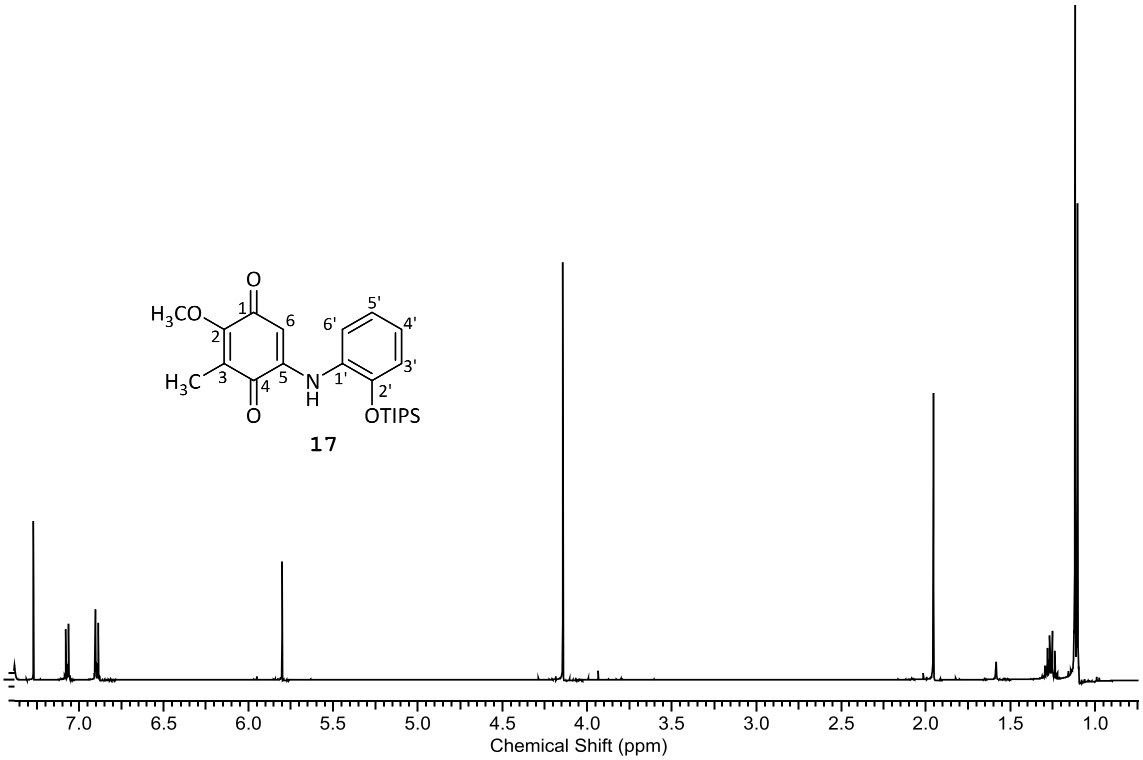
To a solution of **83** (182 mg, mmol) in 2.8 mL of H_2O (19%), 10.0 mL of MeOH (50%) and 6.2 mL of acetone (31%), was added 2 g of Amberlite IRA-400 (OH^-). The reaction mixture was allowed to stir for 1 h at ambient temperature. The volatile solvents were removed in vacuo and the residual aqueous product mixture was subjected to C-18 flash chromatography (eluent: 50% MeCN/H_2O). Aqueous fractions containing the compound were collected and dried by lyophilization, resulting in 50 mg (25.1 mg, 18% yield, lit.[65]) of pure **84** as an amorphous yellow solid. ^1H NMR (500 MHz, DMSO-d_6) δ = 8.82 (d, 1 H), 8.42 (dd, 1 H), 8.26 (d, 1 H), 8.15 (d, 1 H), 6.07 (d, 1 H), 5.27 (d, 1 H), 5.11 (d, 1 H), 4.57(t, 1 H), 3.97 (m,1 H), 3.69 (m, 1 H), 3.60 (m, 2 H), 3.50 (q, 1 H), 3.29 (m, 1 H), 1.80 (s, 3 H). (ESI-HRMS): $[M+H]^+$ calcd for $C_{33}H_{38}NO_9$: 404.0764; found: 404.0772.

3.2. Appendix



10

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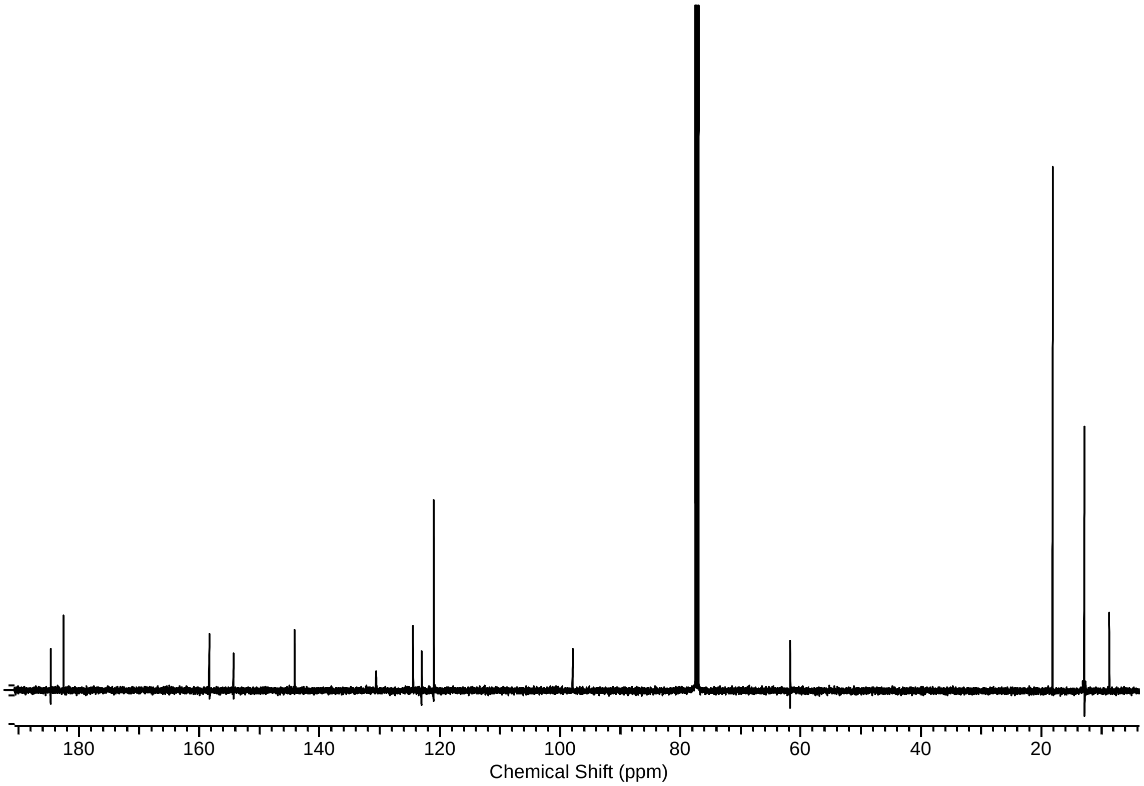
Chemical Shift (ppm)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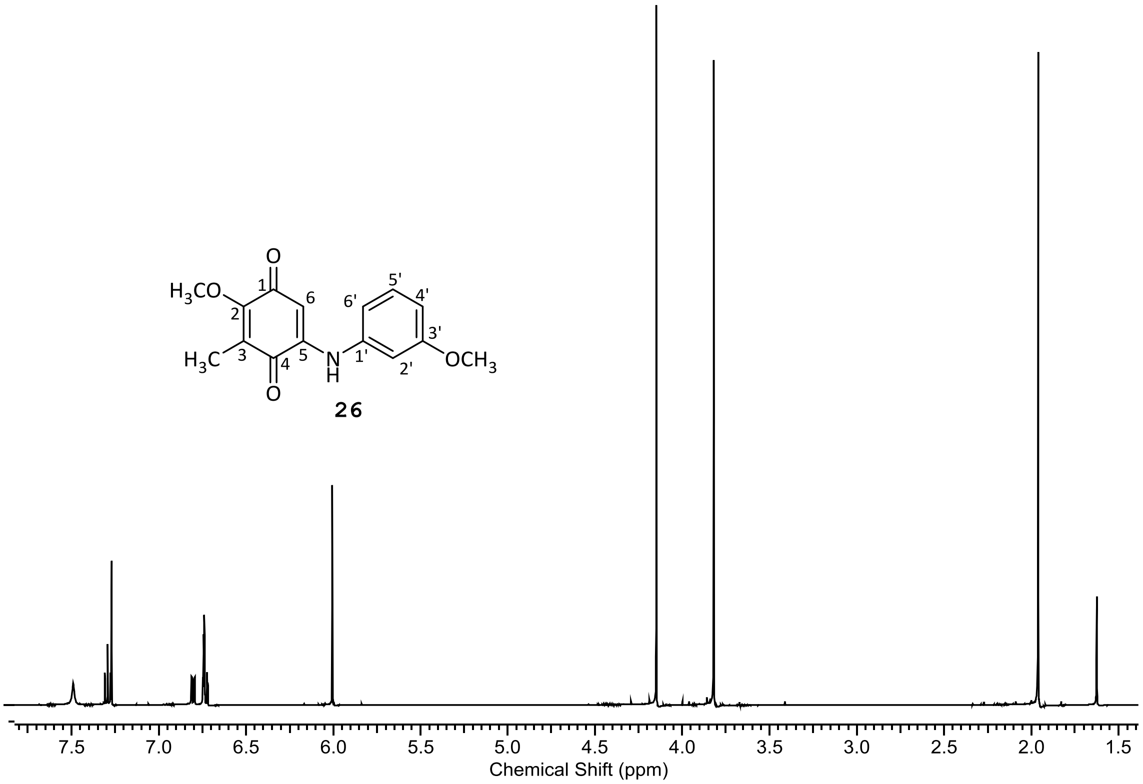
Chemical Shift (ppm)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6'
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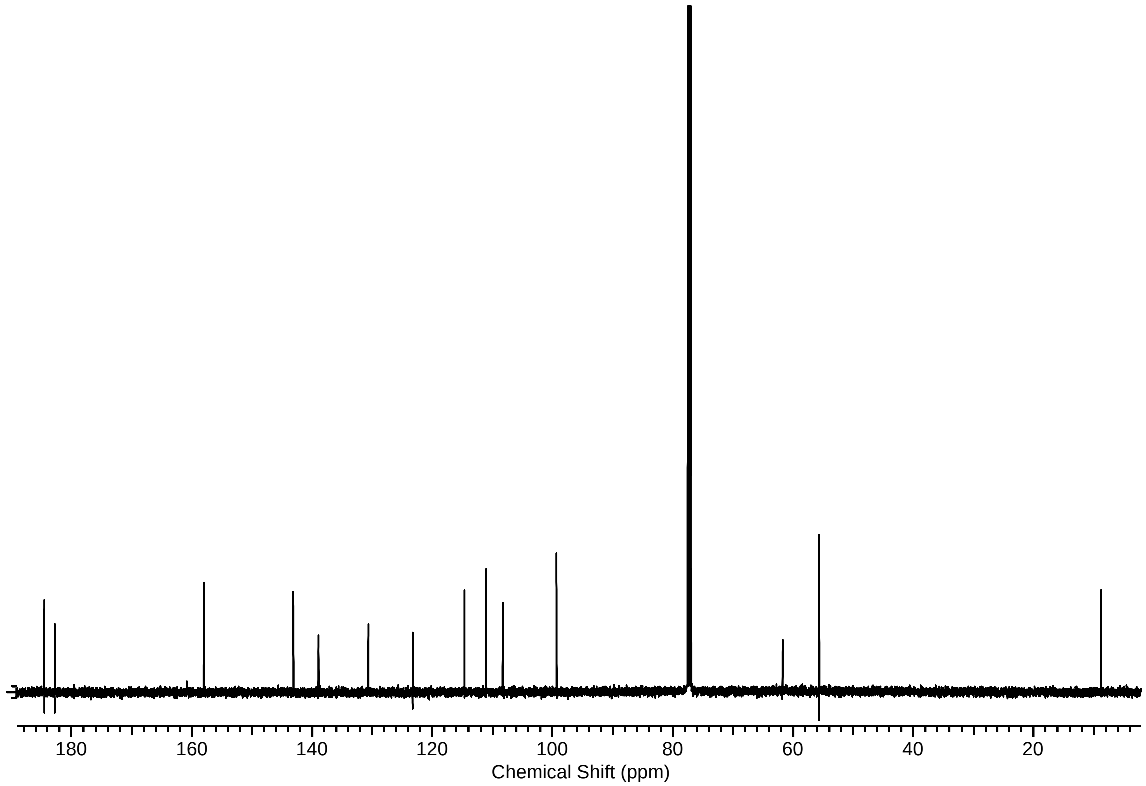
Chemical Shift (ppm)

26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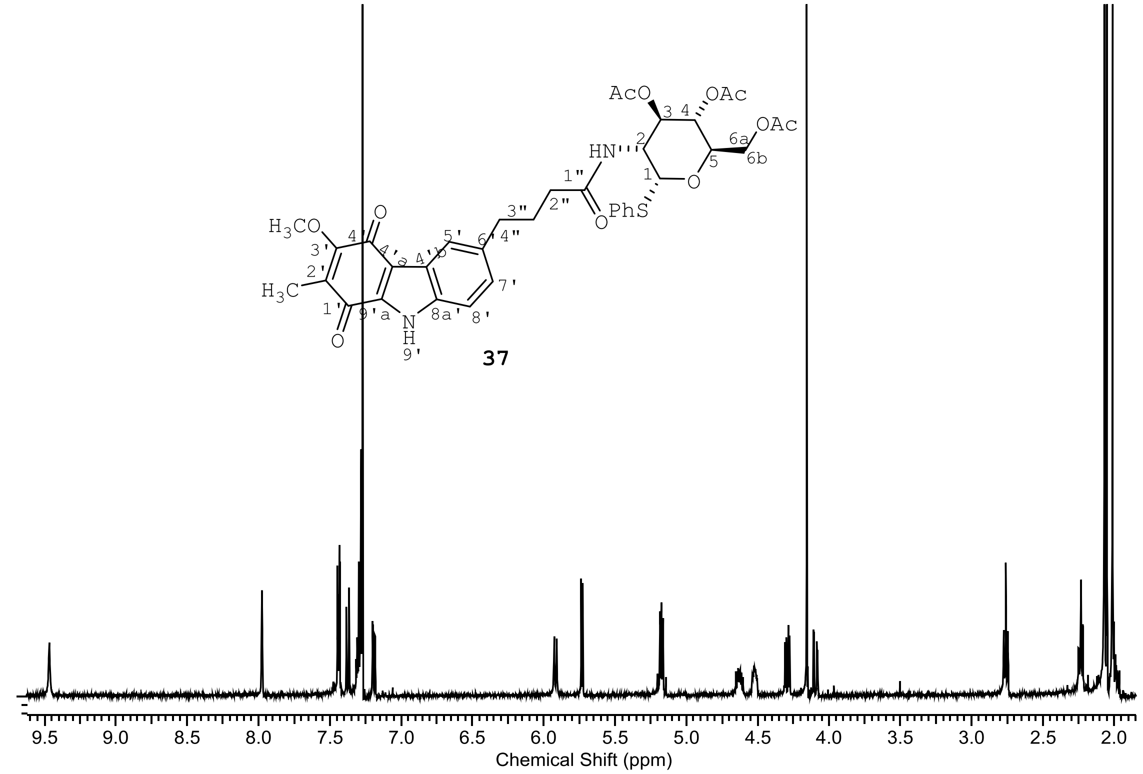
Chemical Shift (ppm)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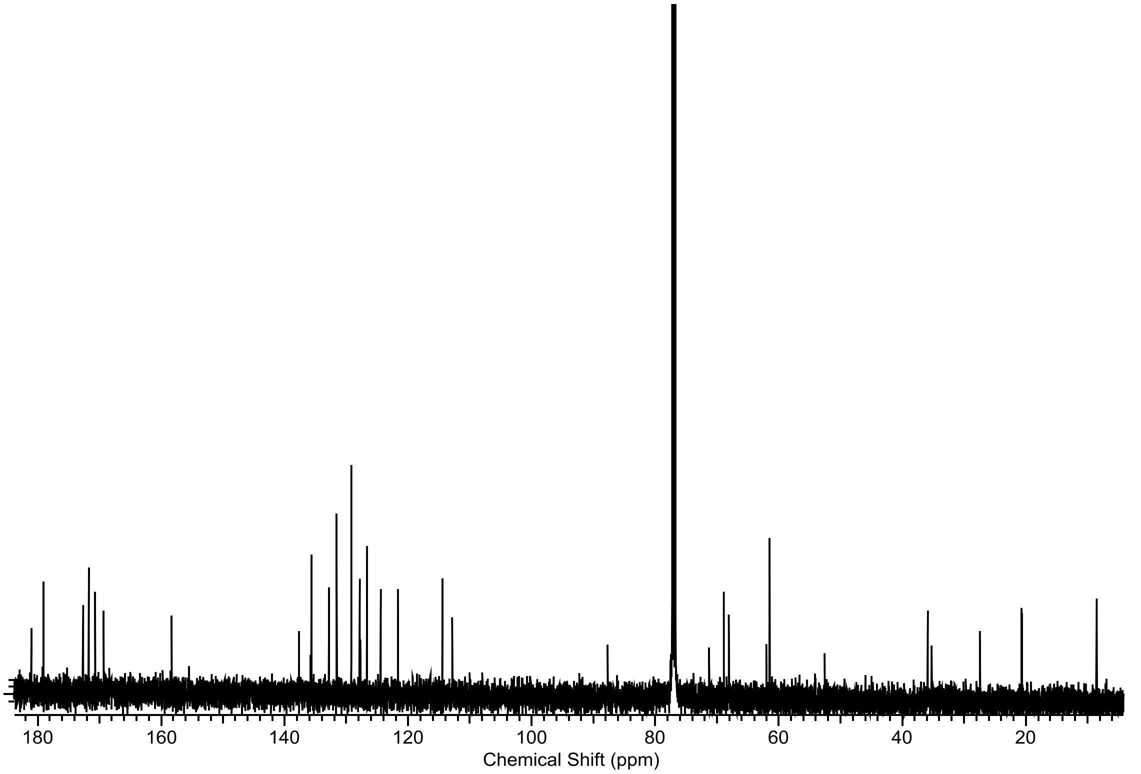
Chemical Shift (ppm)

37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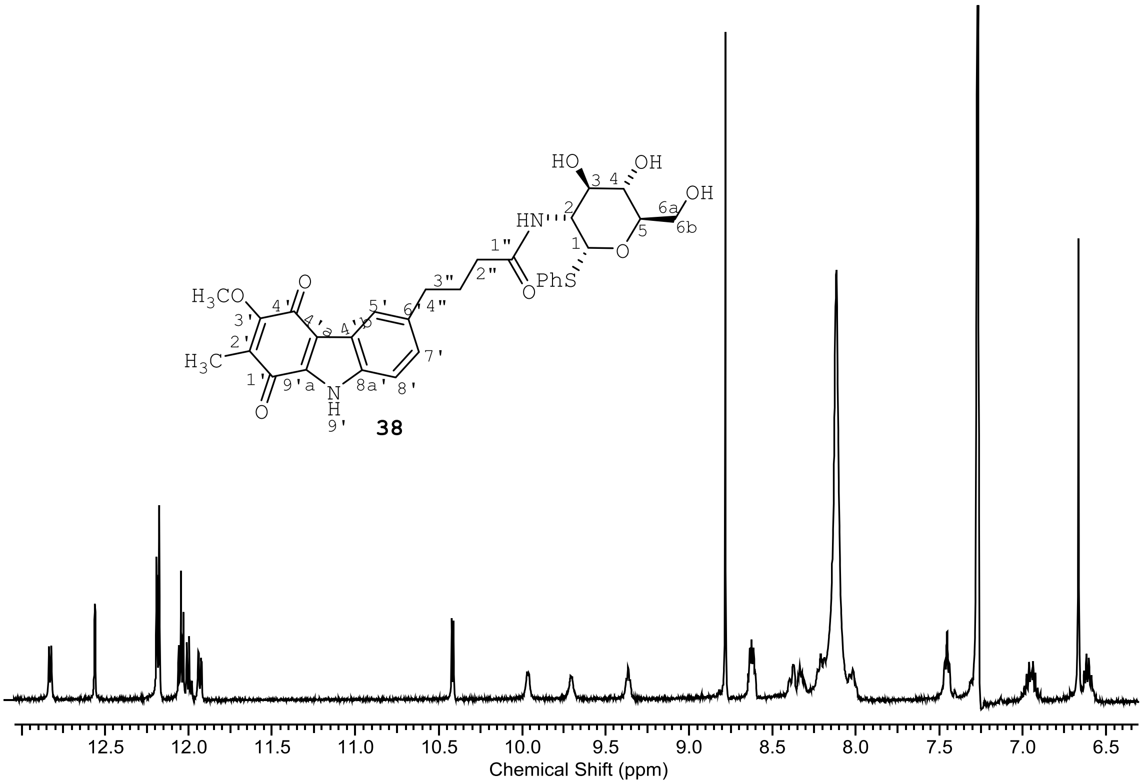
Chemical Shift (ppm)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7'
8'
8a'
N
H
9'
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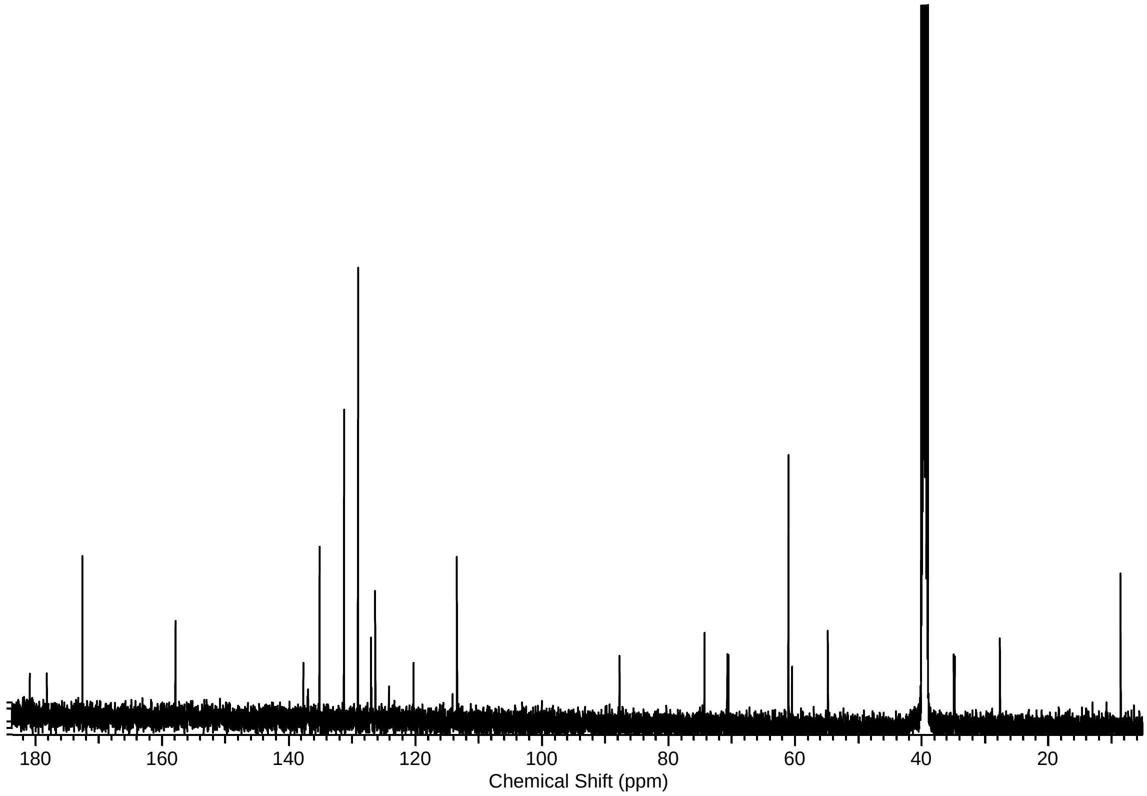
Chemical Shift (ppm)

38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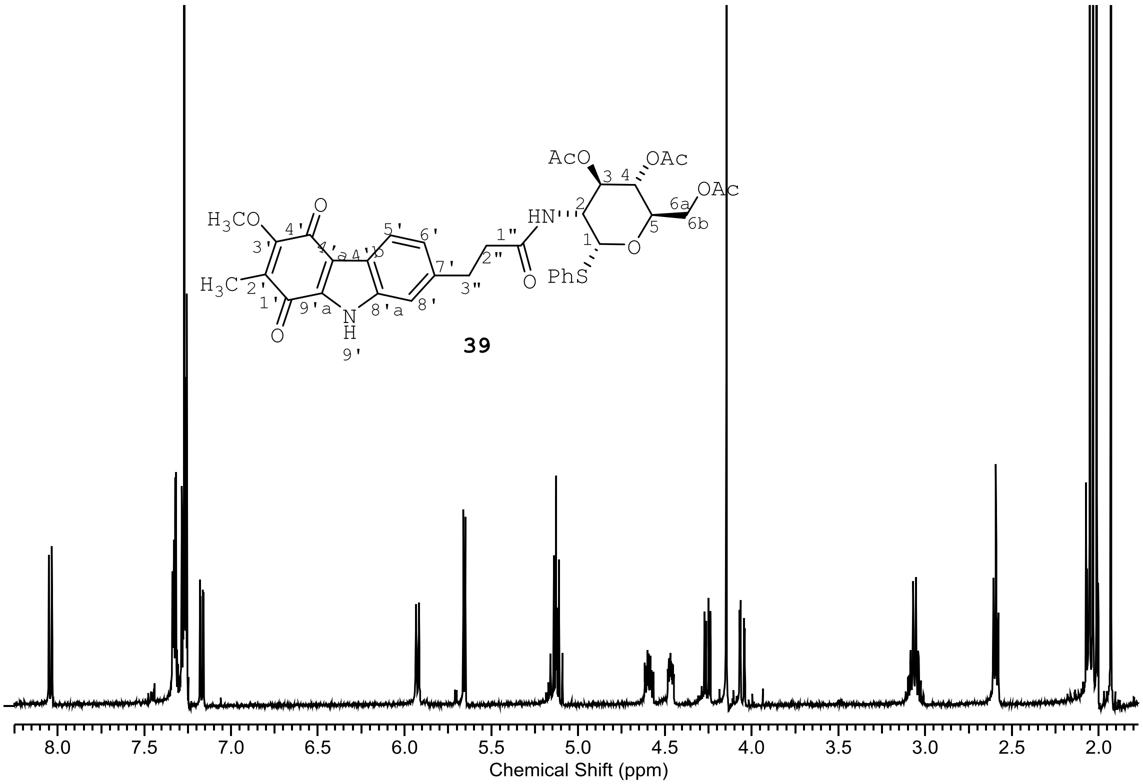
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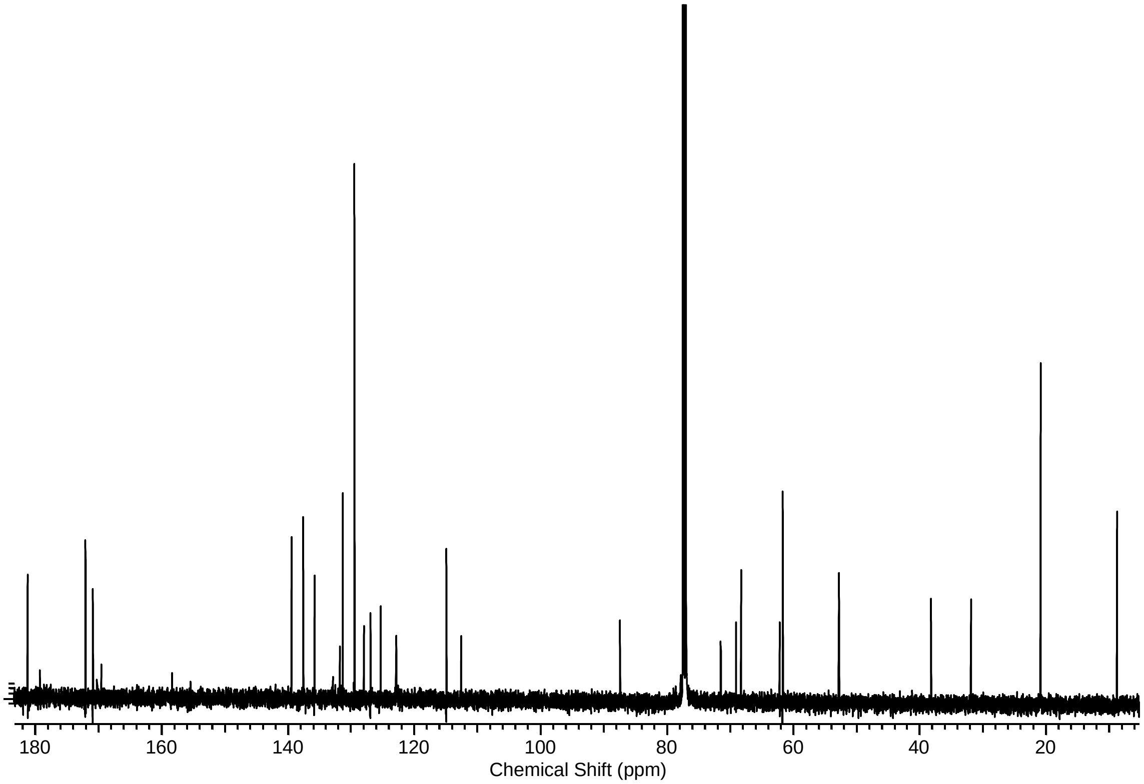
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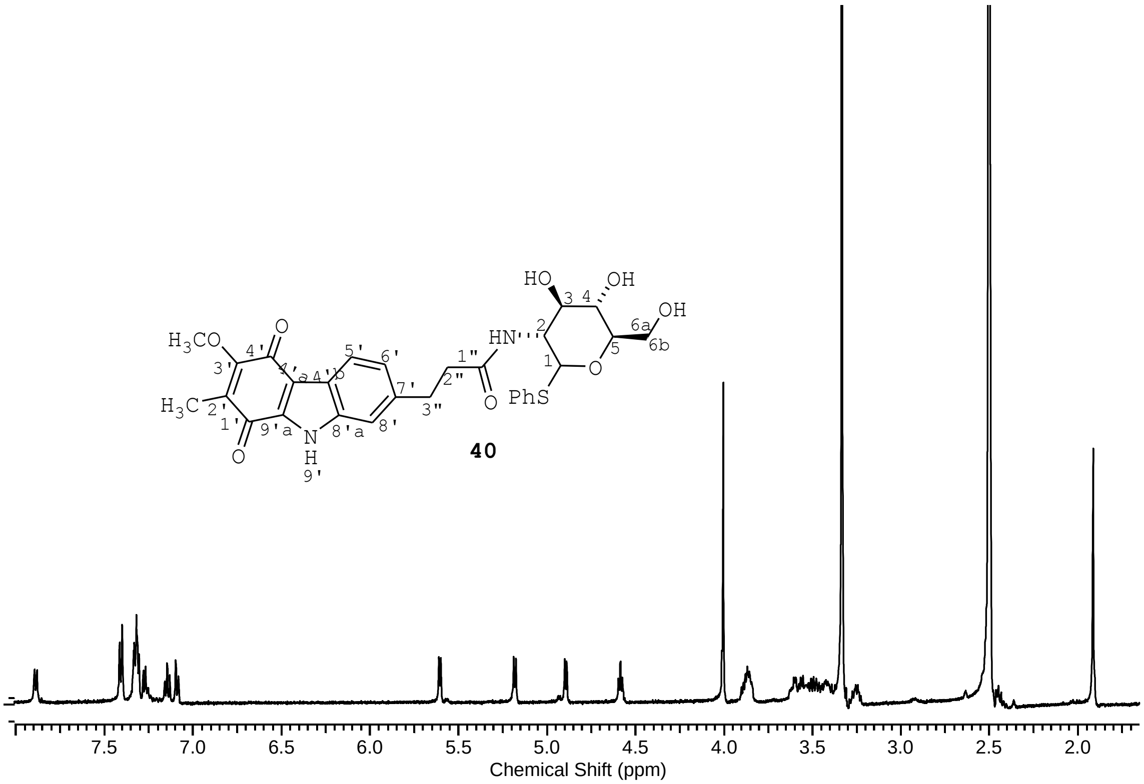
Chemical Shift (ppm)



40

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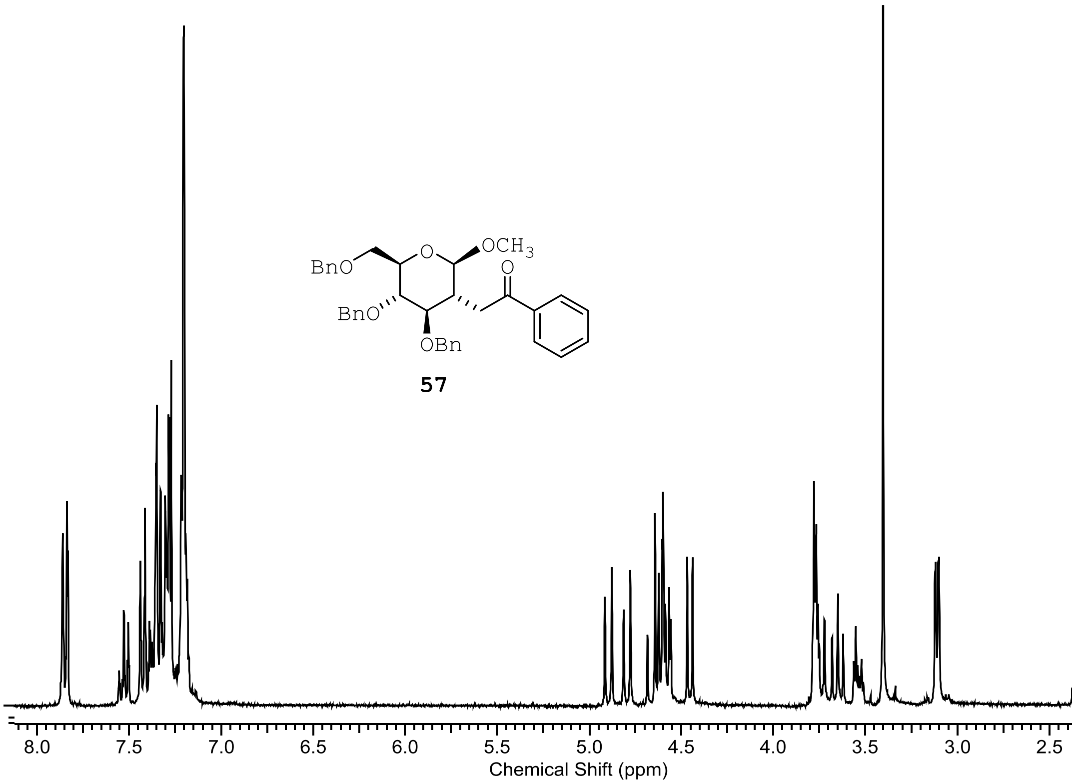
Chemical Shift (ppm)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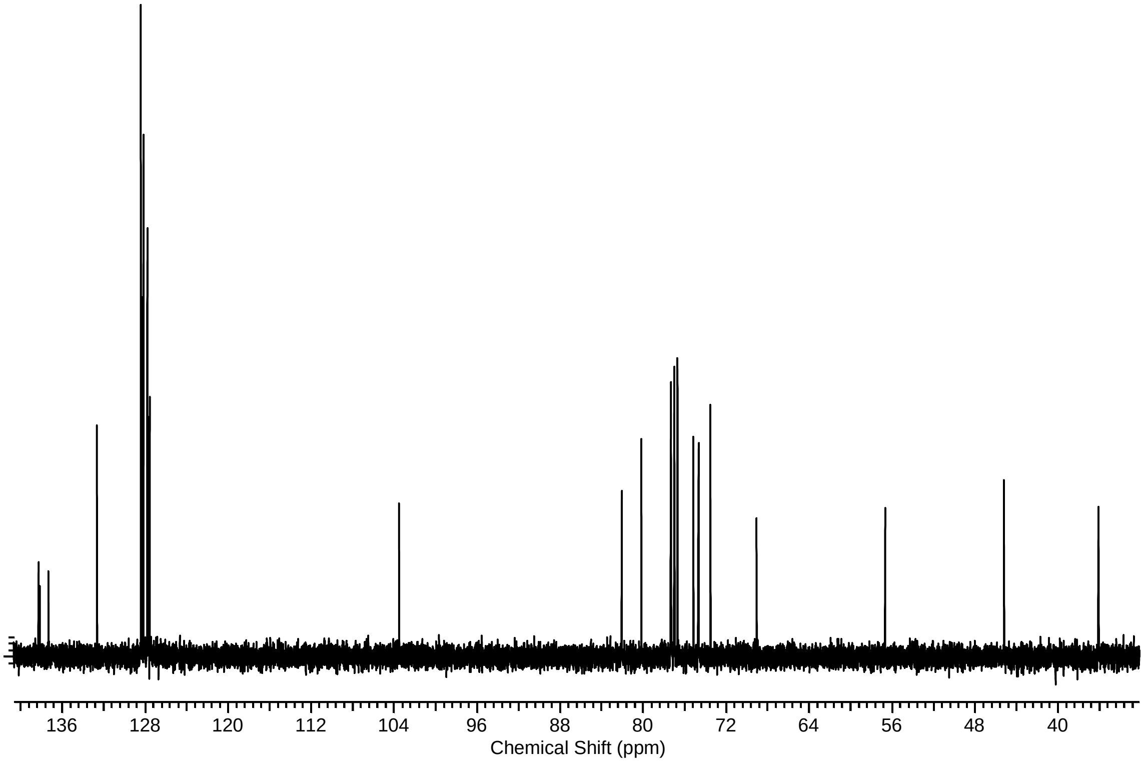
Chemical Shift (ppm)

57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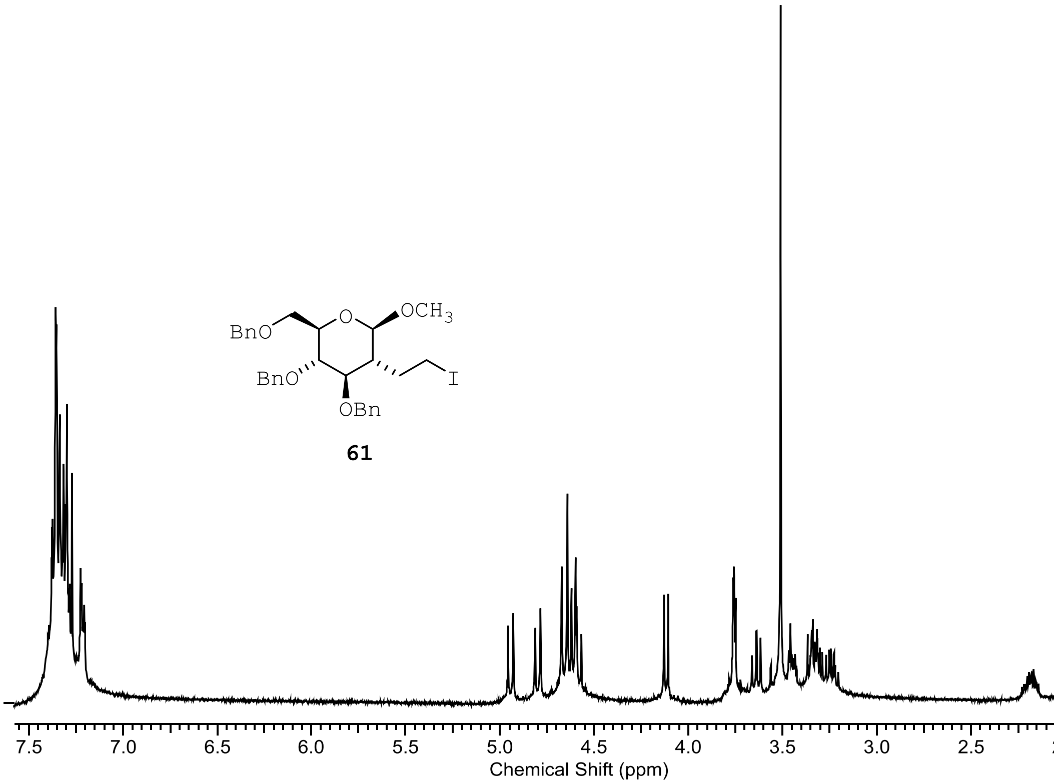
Chemical Shift (ppm)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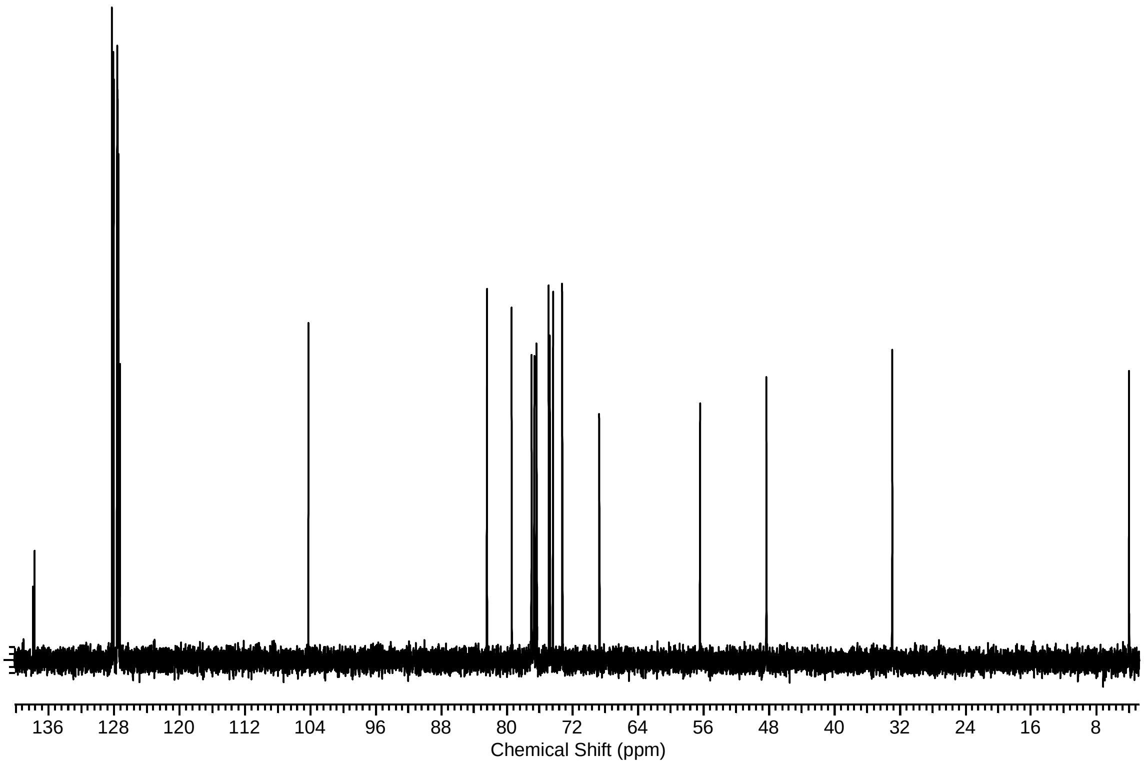
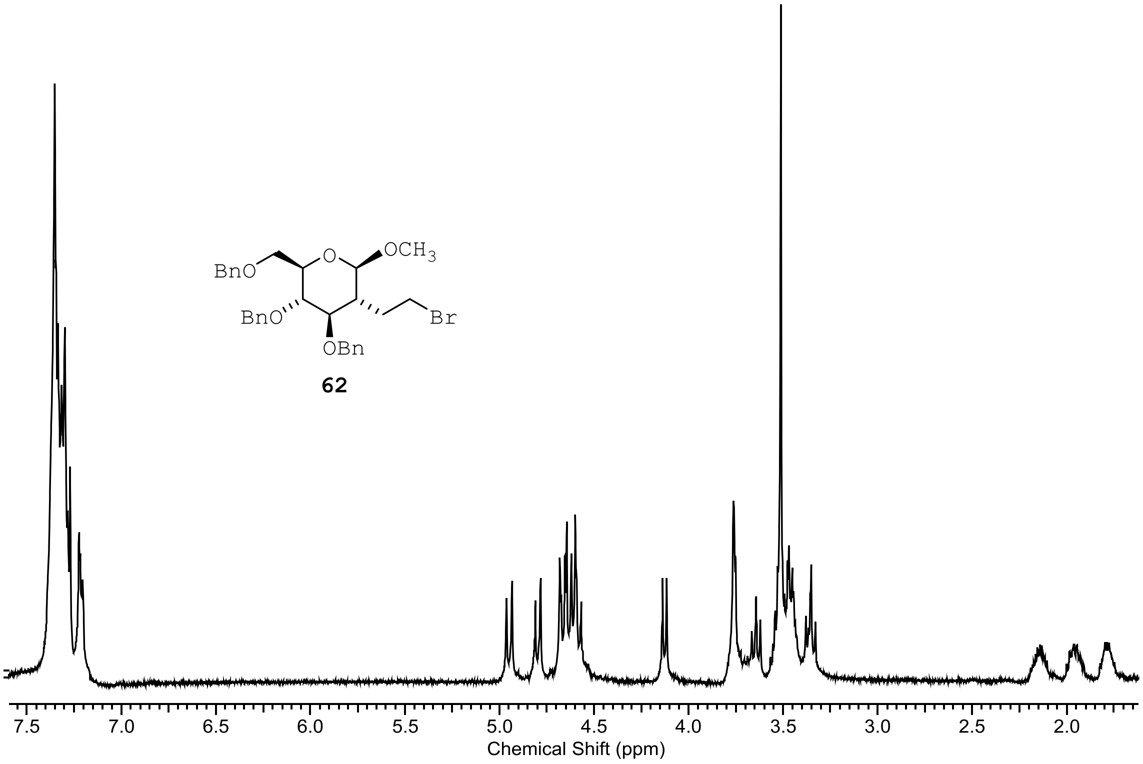
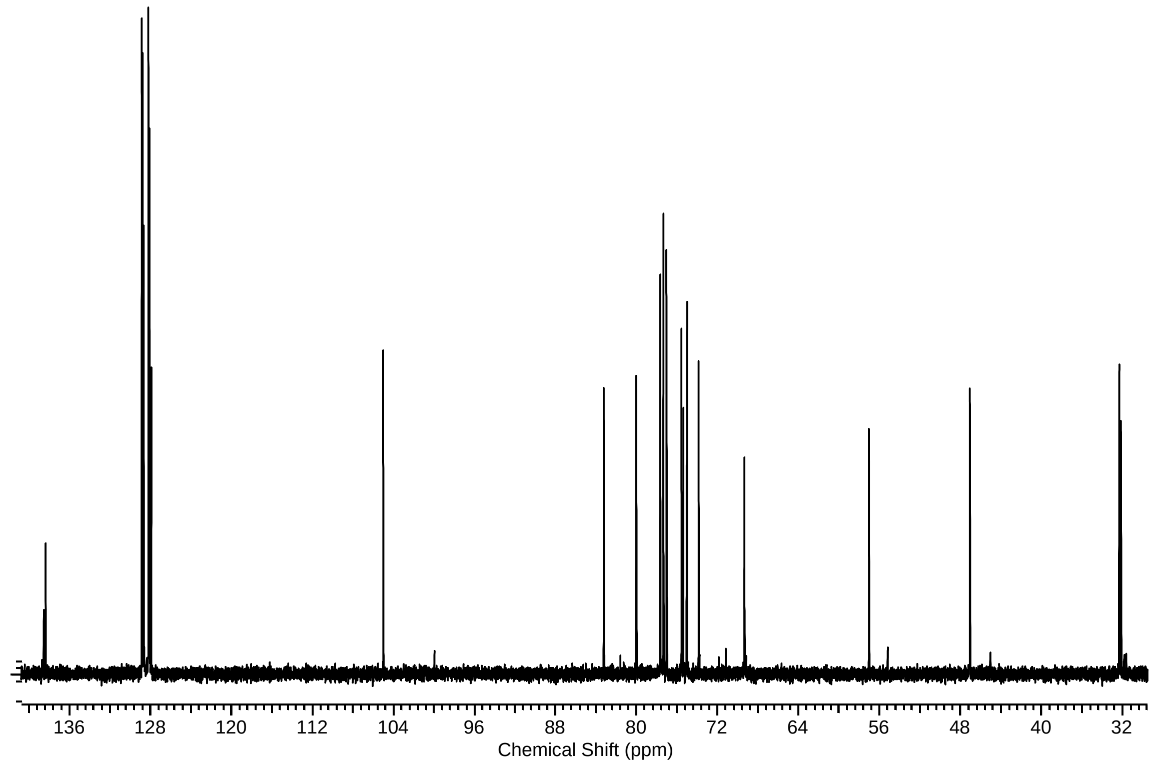
2.5
Chemical Shift (ppm)

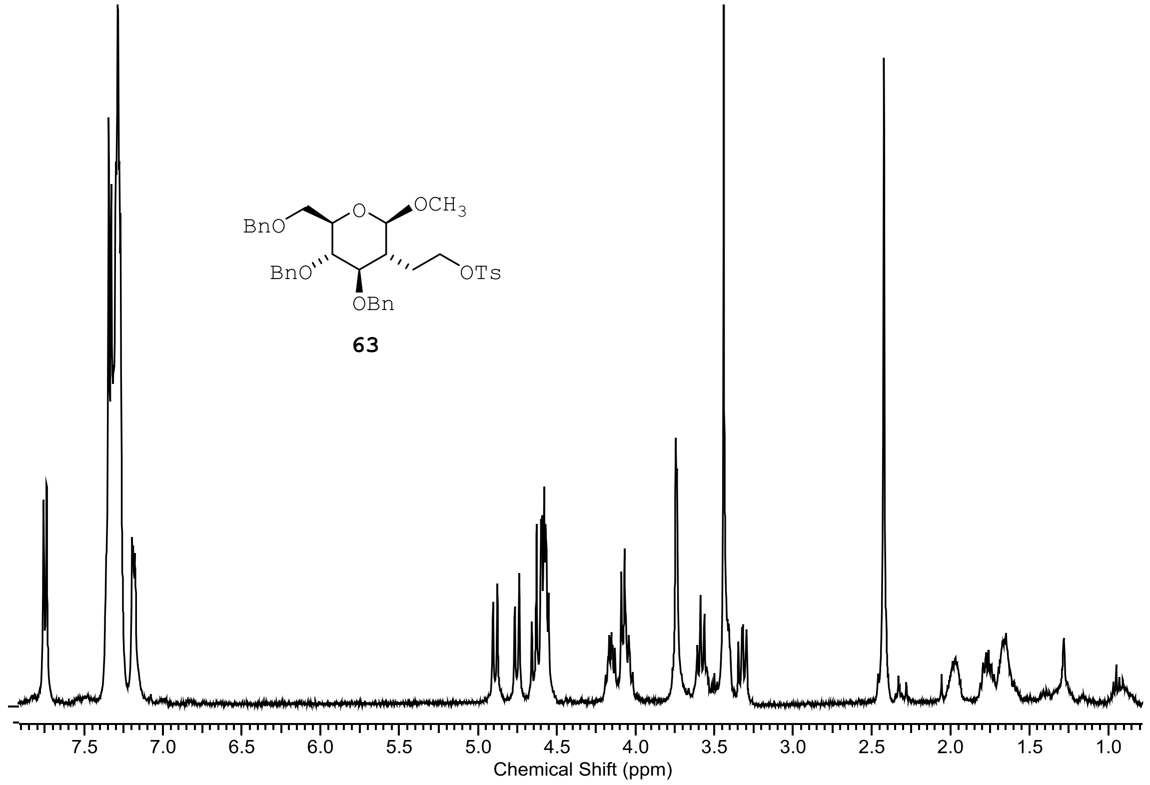
61


136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
Chemical Shift (ppm)

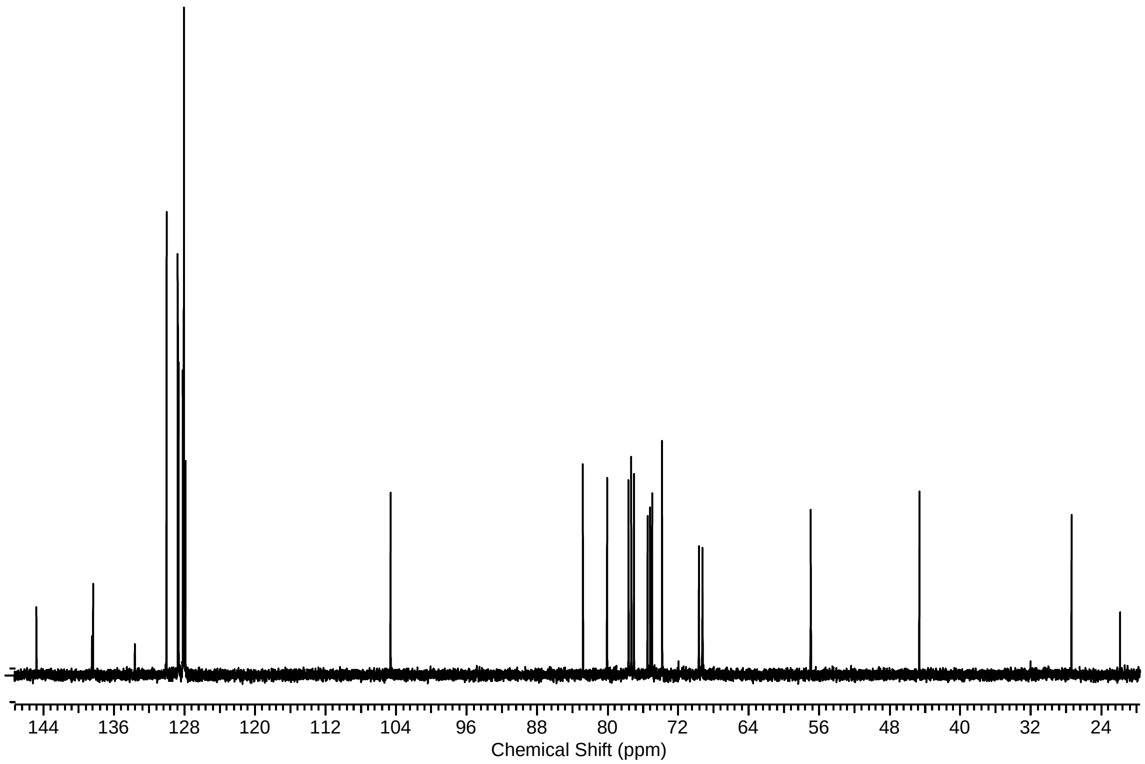

BnO
O
OCH3
BnO
Br
OBn
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
Chemical Shift (ppm)

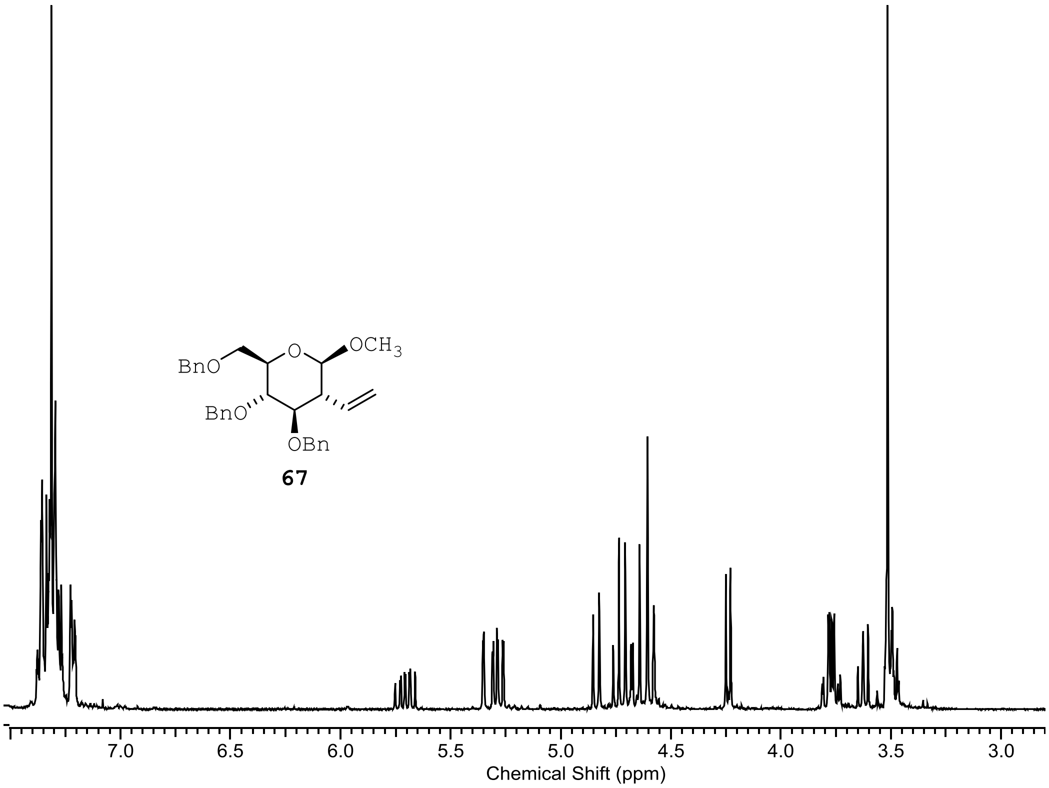
62


136
128
120
112
104
96
88
80
72
64
56
48
40
32
Chemical Shift (ppm)

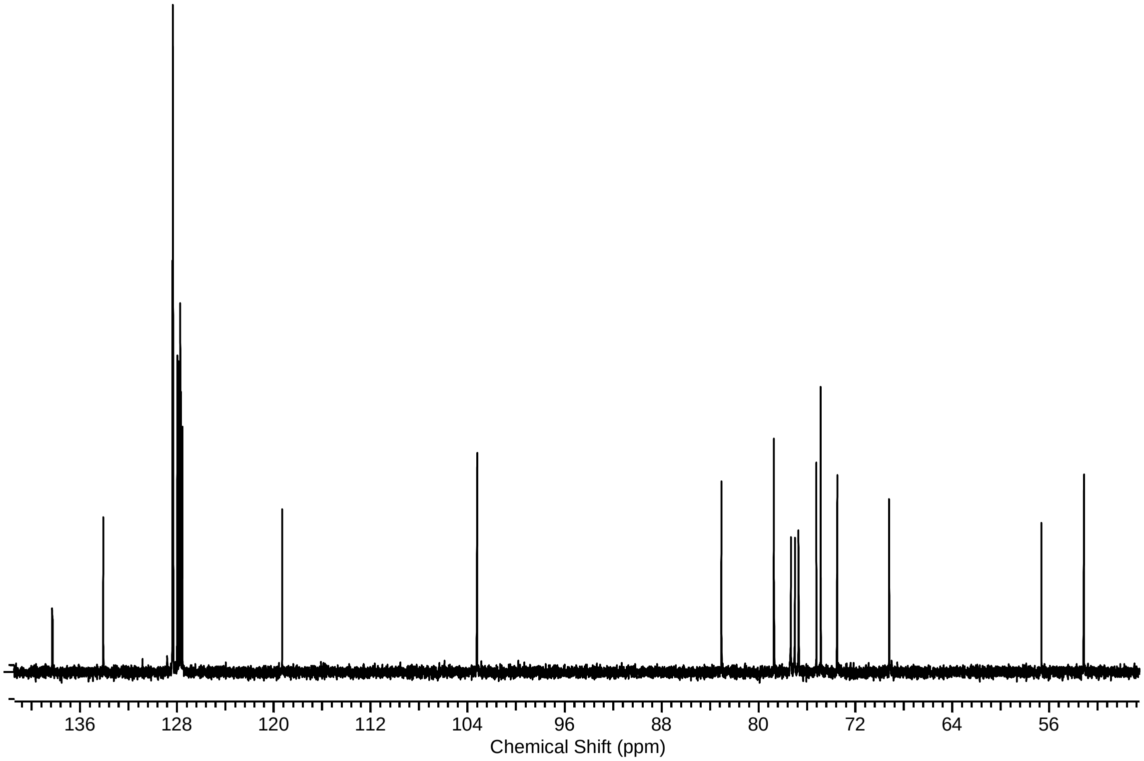

BnO
O
OCH$_3$
BnO
OTs
OBn
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
Chemical Shift (ppm)

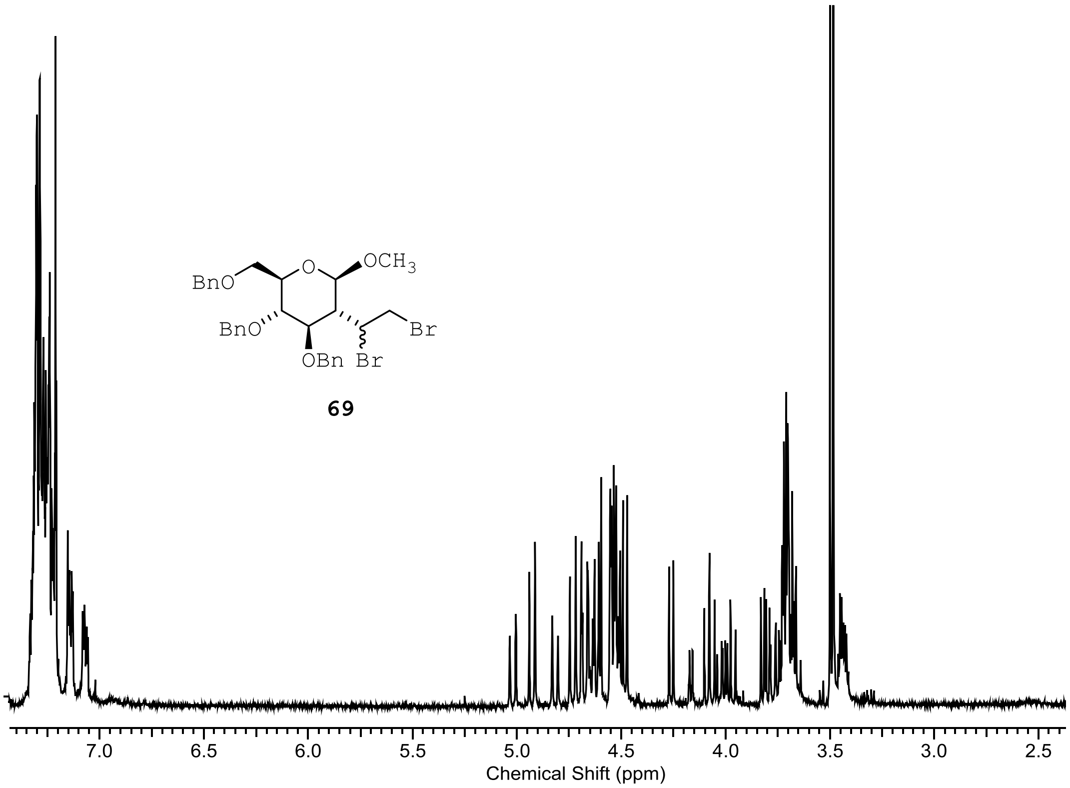
63


144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
Chemical Shift (ppm)

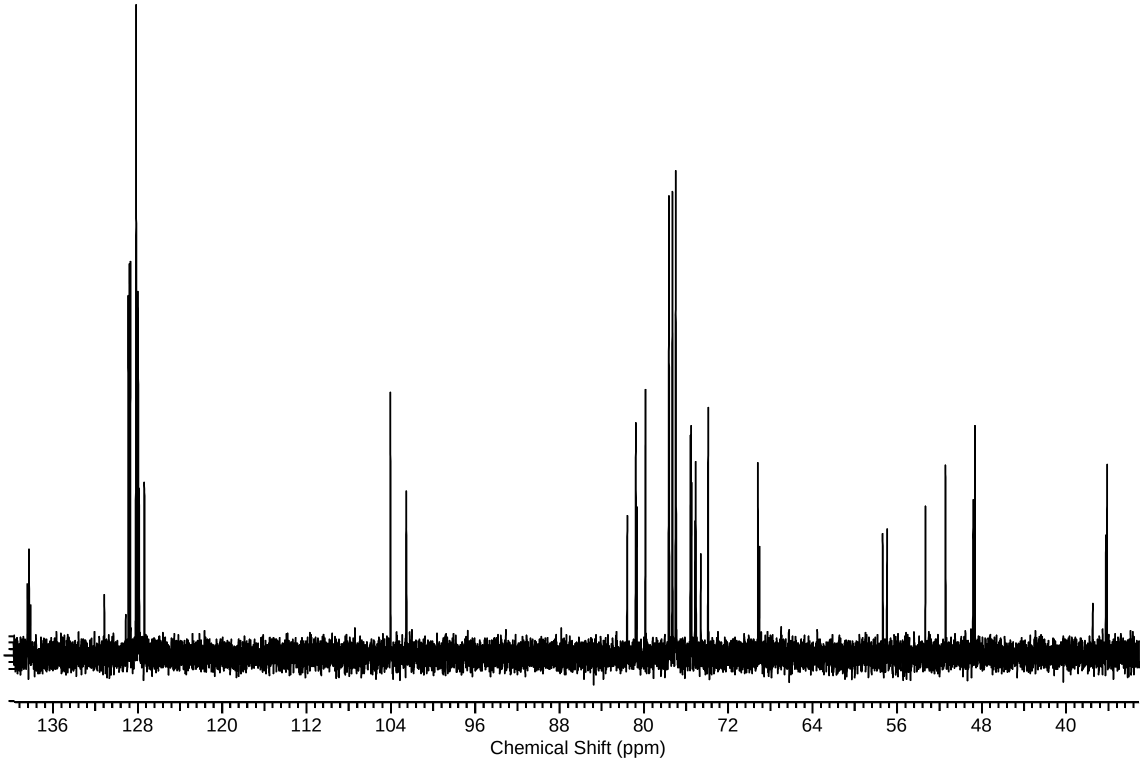

BnO
OCH3
O
BnO
OBn
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
Chemical Shift (ppm)

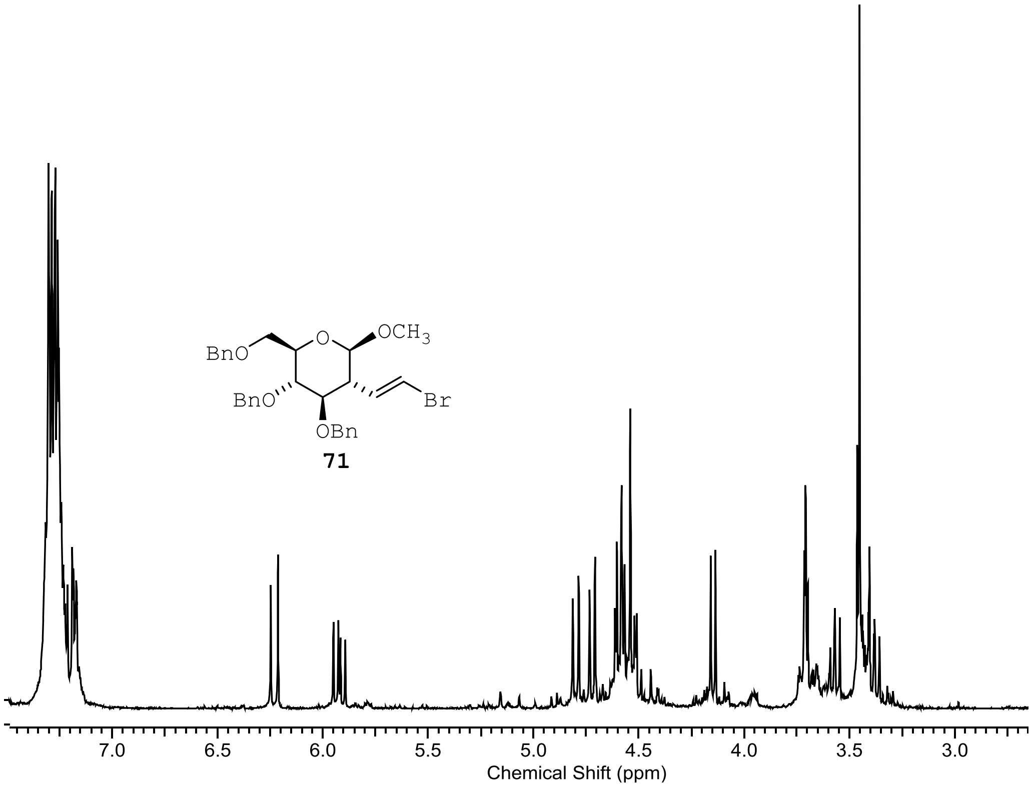
67

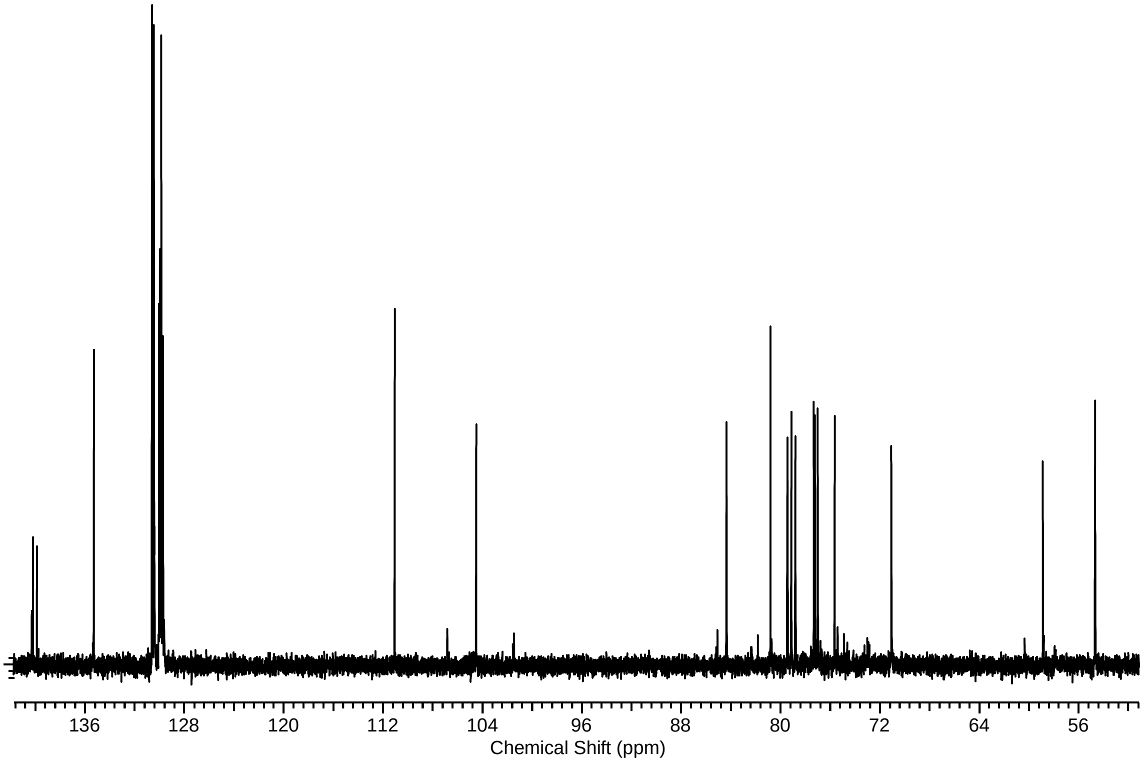

136
128
120
112
104
96
88
80
72
64
56
Chemical Shift (ppm)

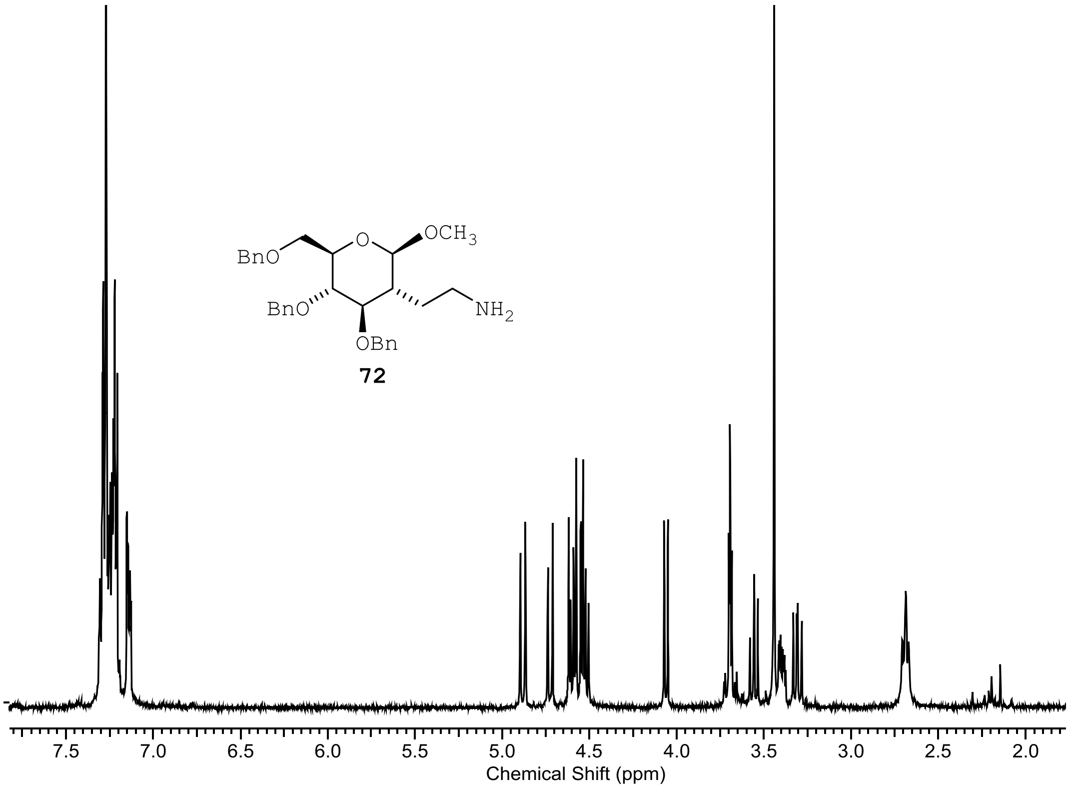

BnO
O
OCH3
BnO
Br
OBn
Br
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
Chemical Shift (ppm)

69

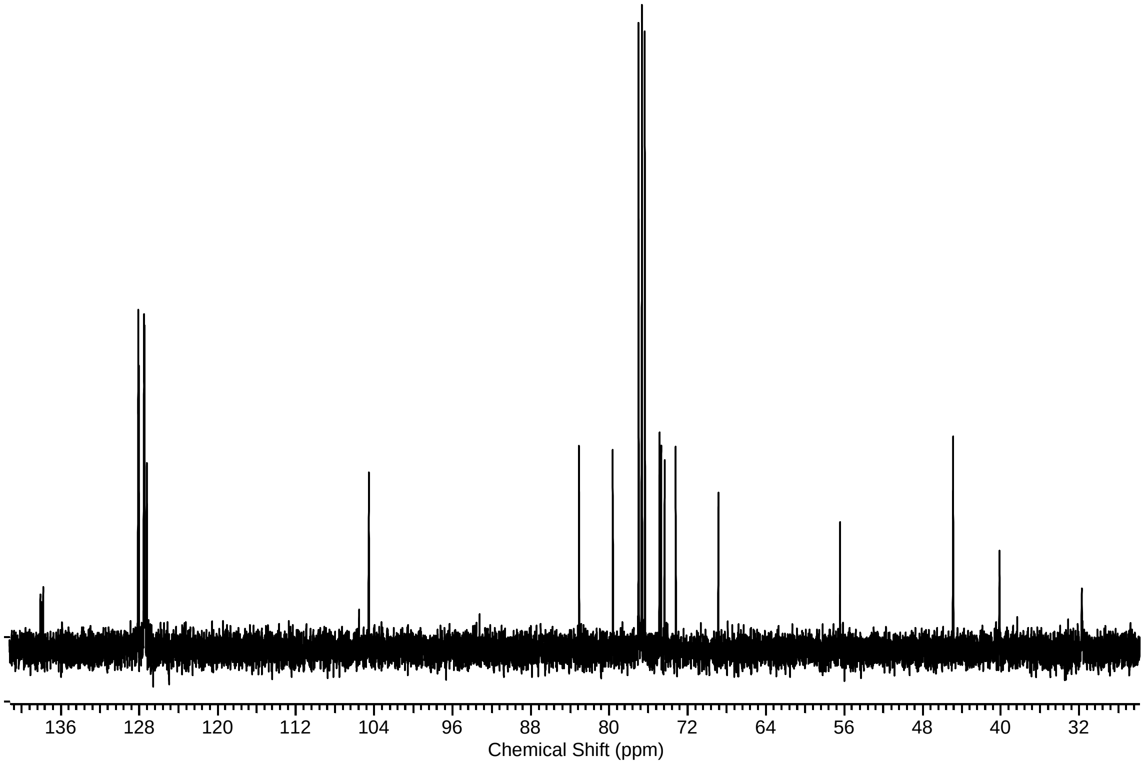

136
128
120
112
104
96
88
80
72
64
56
48
40
Chemical Shift (ppm)

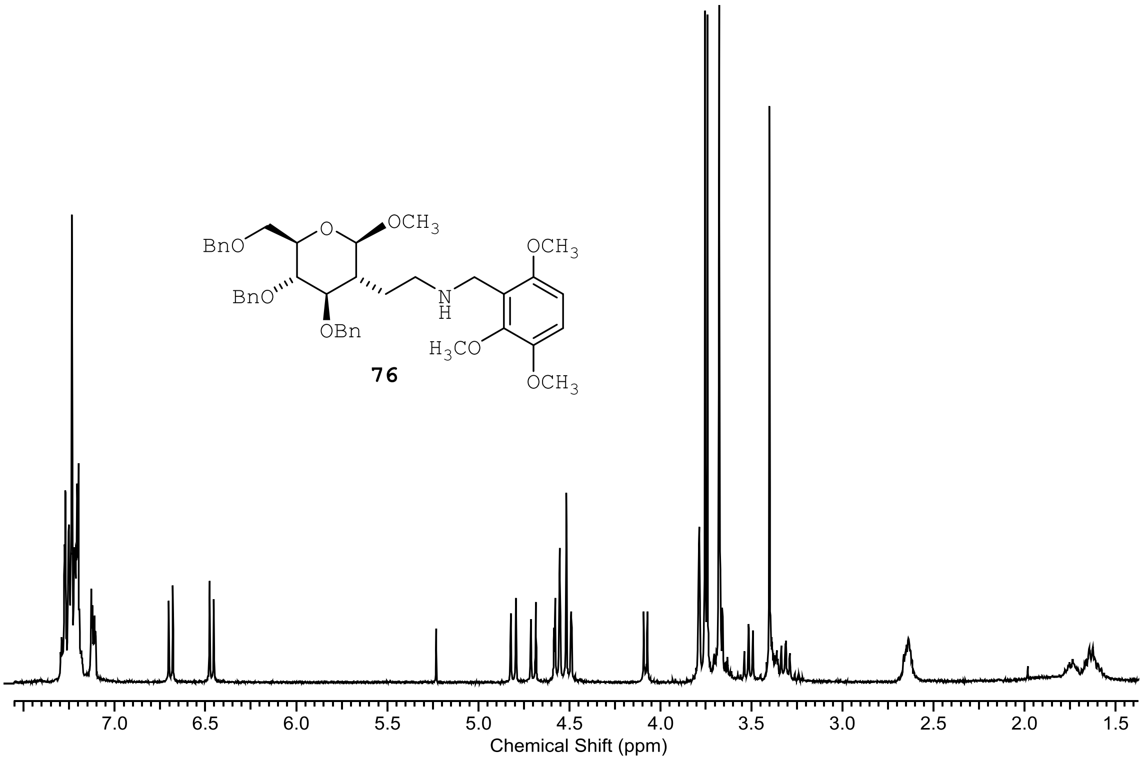

71
BnO
O
OCH3
BnO
Br
OBn
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
Chemical Shift (ppm)

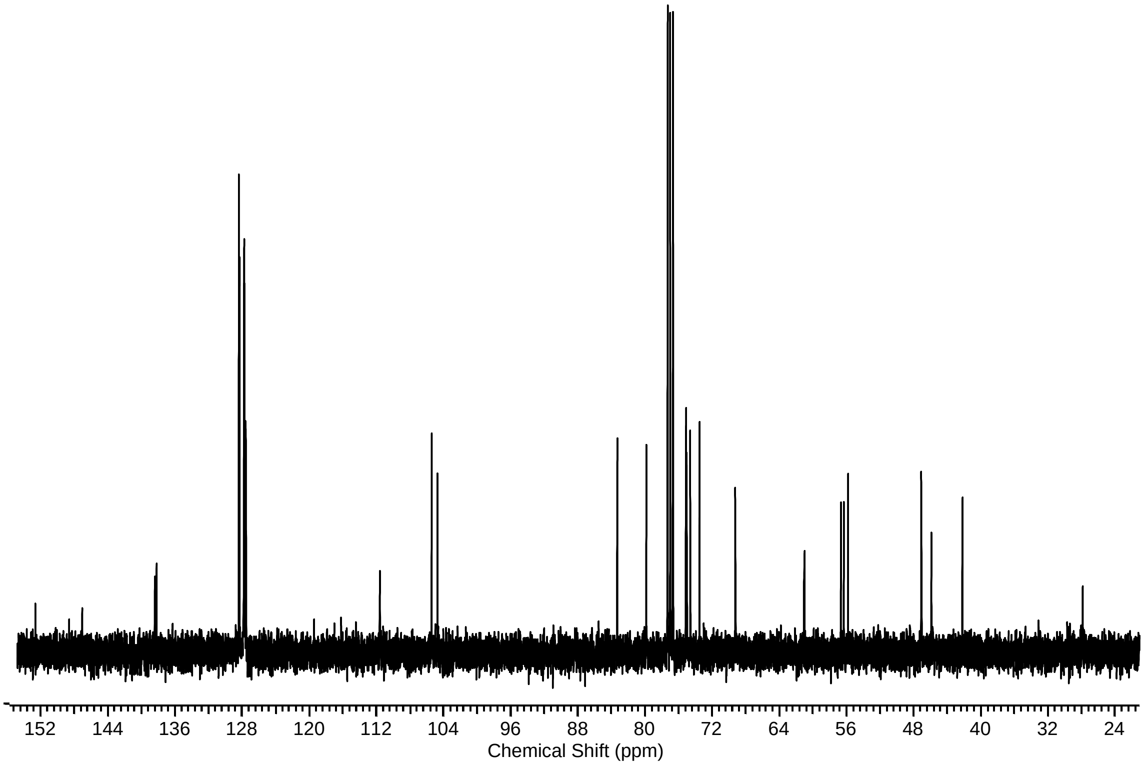

136
128
120
112
104
96
88
80
72
64
56
Chemical Shift (ppm)


BnO
O
OCH$_3$
BnO
NH$_2$
OBn
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
Chemical Shift (ppm)

72


136
128
120
112
104
96
88
80
72
64
56
48
40
32
Chemical Shift (ppm)


BnO
BnO
OBn
O
OCH3
N
H
OCH3
H3CO
OCH3
76
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
Chemical Shift (ppm)


152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
Chemical Shift (ppm)