Chemical Probes for the Treatment of Castration-Resistant Prostate Cancer and Necrotizing Enterocolitis

by

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Submitted to the Graduate Faculty of

University of Pittsburgh in partial fulfillment

of the requirements for the degree of

Master of Science

University of Pittsburgh

2013

UNIVERSITY OF PITTSBURGH

Kenneth P. Dietrich School of Arts and Sciences

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Chemical Probes for the Treatment of Castration-Resistant Prostate Cancer and Necrotizing Enterocolitis

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This thesis describes the synthesis of several small molecules, structurally related to SID3712502, which have shown promise as inhibitors of castration-resistant prostate cancer by preventing nuclear translocation of the androgen receptor. This thesis also describes the preparation of a small library of α- and β-glycoslyated analogs of *N*-acetylglucosamine and *N*-acetylgalactosamine, which were prepared from an oxazoline intermediate or by an acid catalyzed isomerization with the desired glycosyl acceptors. The configuration of the lead compound, C34, was confirmed by synthesis and NMR studies. Four glucosamine analogs and two galactosamine analogs showed promising *in vitro* anti-inflammatory effects. These analogs were shown to be probes for reducing cytokine over-expression through inhibition of TLR4 and able to reduce the gross disease of necrotizing enterocolitis.

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LIST OF ABBREVIATIONS

Ac.....acetyl

AR……..androgen receptor

BF……...breast milk feed

BINAP…….. 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl

Boc.....tert-butyloxycarbonyl

CRPC…….castration-resistant prostate cancer

dba……. dibenzalacetone

DHT……....dihydrotestosterone

DMF.....*N*,*N*-dimethylformamide

ESI……..electrospray ionization

Et…….ethyl

FDA…….Food and Drug Administration

GFP…….green fluorescent protein

GnRH……..gonadotropin-releasing hormone

HRMS…….high-resolution mass spectroscopy

IL-6…….interleukin 6

iNOS……...nitric oxide synthases

i-Pr……..iso-propyl

IR……infrared spectroscopy

LC-HRMS….. liquid chromatography-high resolution mass spectroscopy

LPS……...lipopolysaccharides

Me…….methyl

NEC……..necrotizing enterocolitis

NF-κB……...nuclear factor kappa-light-chain-enhancer of activated B cells

NMR…….nuclear magnetic resonance

NSAIDs……nonsteroidal anti-inflammatory drugs

PAMPs……..pathogen-associated molecular patterns

PSA……...prostate-specific antigen

PAP……prostatic acid phosphatase

SAR……structure activity relationship

SIRS……...systemic inflammatory response syndrome

SPR……surface plasmon resonance

T3P…….propane phosphoric acid anhydride

t-Bu…….tert-butyl

THF…….....tetrahydrofuran

TLR…….toll-like receptor

TMSOTf……..trimethylsilyl trifluoromethanesulfonate

TNF-α……..tumor necrosis factor-alpha

UPDDI……University of Pittsburgh Drug Discovery Institute

ACKNOWLEDGEMENTS

The last two and a half years I have spent pursing my MS degree in Chemistry at the University of Pittsburgh have been some of the most challenging and rewarding of my life. While the path I set out on in September 2010 has diverged to a new destination, all the techniques, knowledge, and problem solving skills I have acquired here will serve me well as a winemaker in the future. I would first like to thank Professor Peter Wipf for not only providing me with a state-of-the-art laboratory space and the equipment to pursue my research goals, but for always being enthusiastic about chemistry and pushing me to be a better scientist. Professor Wipf is a man truly focused on and dedicated to pushing the limits of what we can accomplish through research and to finding solutions to science's unsolved problems. He is truly committed to teaching and seeing his students succeed. His example of a never tiring work ethic has pushed me to ask more of myself both now and in my future career.

I further need to acknowledge two of my lab mates, "my older brothers," who have been instrumental not only in my development as a scientist and as a person, but have become two of my closest friends. Dr. Igor Opsenica by chance became my bench mate at the beginning of my second year here and with him I had many hood discussions stretching beyond just the limits of chemistry. Dr. James Jaber and I were introduced around the same time and he has provided me with invaluable knowledge about chemistry and life and he always kept me motivated and thinking positive when it was most difficult. I also need to thank Pete Chambers and Dr. Erin

Skoda for always lending an ear to listen and helping me troubleshoot any problems I might have stumbled upon in lab. Thank you to the all of the Wipf group members for making my time here a rewarding and educational experience.

Finally I need to thank the other important people in my life outside of lab. My parents and my brothers for always supporting me in all that I do and understanding that I could not come home very often to visit. To my fiancée Stephanie, I could not have survived and prospered in graduate school as I have without your undying support and friendship through the good times and the bad and I am very thankful I met you when I moved to Pittsburgh.

1.0 SMALL MOLECULES CAN TARGET THE ANDROGEN RECEPTOR NUCLEAR TRANSLOCATION IN CASTRATION-RESISTANT PROSTATE CANCER

1.1 INTRODUCTION

1.1.1 The Androgen Receptor and Prostate Cancer

The androgen receptor (AR) is a 110-kDa ligand-inducible nuclear receptor of the steroid receptor family of transcription factors that regulates male developmental and physiological characteristics.[1] Like other steroid receptors the AR has four functional domains which were determined by X-ray crystallography: the NH_2-terminal domain with transcriptional activation function AF-1 (60% of the entire protein), a central DNA binding domain, a short hinge region, and a COOH-ligand/AF-2 cofactor binding domain.[1b] When androgens testosterone and dihydrotestosterone (Figure 1) are bound to the AR, they mediate male sexual differentiation and pubertal sexual maturation, the maintenance of spermogenesis, and male gonadoptropin regulation.[1a,1b] The complex can then either induce or repress expression of target genes.[2] Mutations of the AR can result in decreased androgen binding affinity, change its transcriptional activity, which leads to infertility, androgen insensitivity or prostate tumors.[1a]

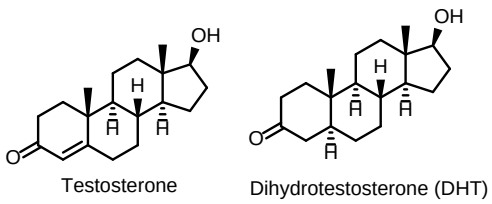


Figure 1. Androgens

Transcriptional activity of androgen-bound AR is altered by coregulatory proteins acting as coactivators or corepressors to hundreds of target genes, thus allowing for multiple mechanisms of transcriptional control (Figure 2).[1a,3] Unbound AR is found in the cytoplasm complexed with several heat shock protein chaperones and high molecular weight immunophilins, which help induce a high-affinity ligand binding conformation.[1b,1c] Androgen binding to the AR induces a conformational change and triggers dissociation from the cytoplasmic chaperone protein HSP90, self-dimerization and translocation into the nucleus where it binds specific genome sequences to activate target gene transcription (Figure 2).[1c,3-4] Dihydrotestosterone, synthesized by 5α-reductase enzymes from testosterone, is the main AR ligand in prostate tissue[1a,1b] and is bound 10-fold more tightly than testosterone.[4] The prostate is an androgen-dependent tissue in which androgens trigger development and also regulate cellular growth/function.[1b] It is this sensitivity to androgen levels and AR signaling that is important in controlling prostate cell homeostasis.[5] Any change in AR expression levels, androgens, or alterations in the AR mode of action effect the physiology of the prostate.[5]

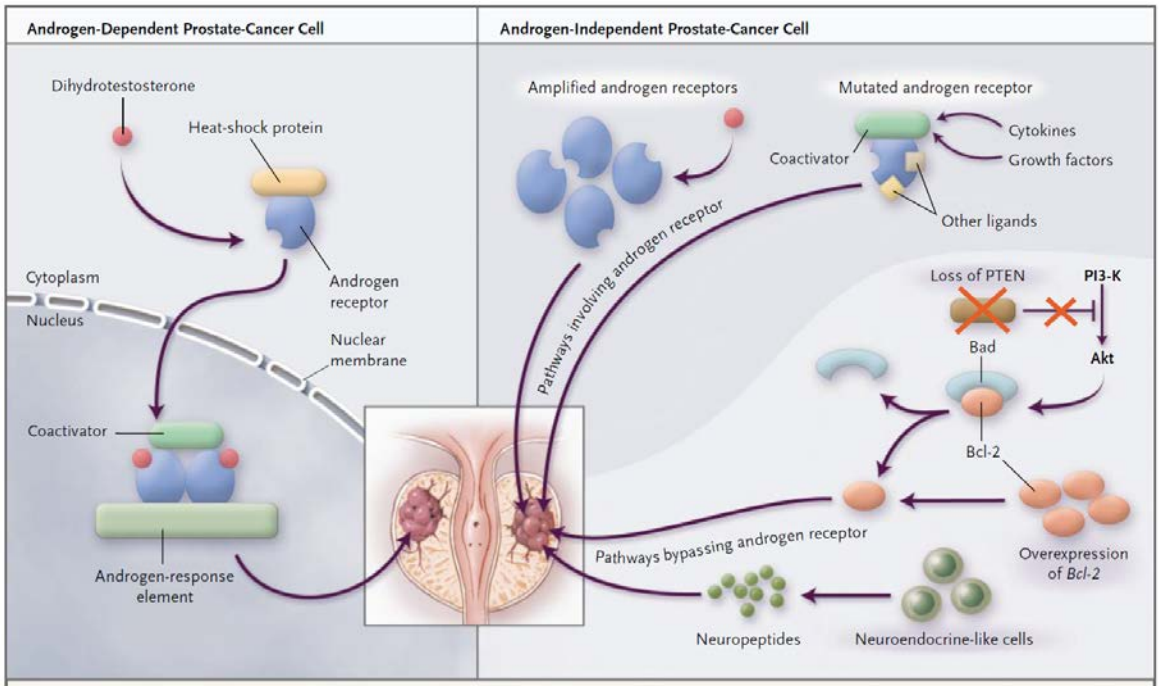


Figure 2. Regulation of the AR (reproduced with permission from Ref. 1c, Copyright Massachusetts Medical Society)

Androgens and the AR are essential in the occurrence and progression of two disease states that occur in elderly males: prostatic hyperplasia and prostate cancer.[1a,1b] The healthy human prostate is a tubular-alveolar gland with epithelial acini made up of basal and luminal cells that form a two-layered epithelium encapsulated in the stromal compartment.[6] The AR is contained within the cytoplasm of stromal and secretory epithelial cells and prostate cancer occurs when the cellular homeostasis is altered.[6] Prostatic hyperplasia, which can occur as men age, is an increase in epithelial cells that retain normal cellular function/size and are pathologically benign.[6] Prostate cancer among US males is the most commonly diagnosed cancer besides non-melanoma skin cancer and is the second leading cause of cancer deaths.[7] Around $11.9 billion is spent in the United States every year on prostate cancer treatment.[7] In 2012 alone, the NCI estimated that 241,740 men would be diagnosed with prostate cancer and

28,170 would die from it.[8] Prostate cancer incidence has a hereditary component but the major risk factor is increasing age[5] and the number of prostate cancer cases is expected to increase as the average age of the population rises.[6]

Cancer often occurs when the balance between proliferation and cell survival versus cell death is disrupted. As the AR is responsible for controlling proliferation, differentiation, apoptosis and secretion in the prostate, the connection between AR signaling and cancer has made it a target for prostate cancer treatment.[5-6] The first case of prostate cancer was described in 1853[9] and the discovery of the androgen dependency of the disease by Huggins and Hodges in the 1940's made androgen deprivation therapy the accepted treatment.[3] The cancer originates in the epithelial compartment of the prostate where overexpression of the AR and a change in function from pro-differentiation to pro-proliferation are required.[5]

The changes in AR function happen through one or more of the following mechanisms: receptor mutations, alternative splicing of AR mRNA, changes in the levels of coactivators and corepressors, and activation of different cell signaling pathways.[3,5] Somatic mutations are present in most prostate cancers and alter ligand affinity, coregulator interaction, dimerization, and stability of the AR.[5] Coregulators of the AR can either increase or decrease AR activity and their overexpression leads to increased proliferation and reduced apoptosis.[5] Inflammatory cytokines present in the body like IL-6, TNF-α, and IL-1β increase with age and IL-6, a target of NF-κB, has been linked to influencing AR function and thus prostate cancer.[5] Higher levels of NF-κB are common in prostate cancer and they help maintain nuclear AR, interfering with the cell cycle, which increases proliferation and decreases apoptosis.[5] The relationship between the AR, androgens, and prostate cancer progressions is more complicated than just depriving the cancer cells of androgen.

At diagnosis, 80-90% of prostate cancers are dependent on androgen-AR signaling as an activator of proliferation, as was first shown by Huggins and Hodges.[10] Androgen deprivation therapy was initially accomplished surgically by removal of the testes or by chemical castration with estrogen until the successful synthesis of synthetic gonadotropin-releasing hormone (GnRH) polypeptide.[9] Chemical castration with synthetic GNRH functions through a negative hormone feedback on the hypothalamus which in turn decreases levels of circulating testosterone.[4,9] Surgical removal of the testes has been shown to lower circulating testosterone levels to <20 ng/dL while chemical castration with synthetic GNRH generated levels <50 ng/dL.[4] Despite these castrate levels of androgens, almost all patients relapse in 18-24 months leading to the development of castration-resistant prostate cancer (CRPC)[9-10] which is currently incurable and responsible for all prostate cancer deaths.[11]

Prostate specific antigen (PSA) and prostate acid phosphate (PAP) are two accepted biomarkers of prostate cancer which become upregulated when the disease evolves to castration-resistant.[3] These biomakers are often used in the clinic to identify when the disease has relapsed and are a good indication of CRPC.[3,12] Amplification and overexpression of the AR is one of the most common changes in CRPC and the AR is able to localize to the nucleus independent of androgen levels.[3] The AR also is postulated to have different roles in the stroma versus the epithelium and the basal epithelial cells versus luminal epithelial cells, acting as a tumor suppressor in one and a tumor proliferator in the other.[10,13] This dual role of the AR in prostate tissues could help explain why androgen derivation therapy fails even in low-risk, early diagnosis patients.[12]

CRPC was initially thought to be androgen-independent after initial castration treatment; however recent studies show AR remains dependent on androgens.[3,11] The source of testosterone

and DHT at functional levels remains unknown and AR remains active in signaling pathways with lowered levels of androgens.[11] The higher than expected tissue levels of testosterone and DHT observed in CRPC patients are enough to activate the AR and it is proposed that intracrine metabolism of adrenal androgens or plasma membrane cholesterol may be responsible.[14] Upon castration in a mice model, AR exited the nucleus but re-entered and rebound the PSA enhancer, showing *in vivo* activation of the AR even at castrate levels of androgen.[15] AR overexpression[16] and knockdown[17] studies show that the AR is a key molecular determinant and is critical for proliferation and progression of the CRPC cancer. Increased AR cellular concentration is required for the progression of CRPC, can change antagonists into agonists, and can alter the coactivators interaction with the receptor.[16] Current prostate cancer therapy is focused on inhibiting androgen activation of the COOH-terminal domain of the androgen receptor through androgen deprivation therapy, antiandrogens, or disruption of steroid metabolic pathways.[4,18]

Antiandrogens are small molecules that compete with androgen for the binding of the AR.[18] Antiandrogens, while increasing patient survival initially, have not been able to prevent the recurrence and evolution of the cancer to the castration-resistant state.[18] It is hypothesized that the reason for this is the 50-500 fold higher AR binding affinity for the androgens over antiandrogens.[18] Cyproterone acetate (Figure 3) was the first antiandrogen used for prostate cancer treatment in the 1966, and it has a long half-life and good bioavailability *in vivo*.[9] The steroid core of the molecule allows it to function as an inhibitor of androgen-AR interaction and of gonadotropin secretion—an antiandrogen and a chemical castration agent in one.[9] In the 1970's, this was a good alterative to chemical castration by estrogen as cyproterone acetate had better efficacy and fewer side effects.[9]

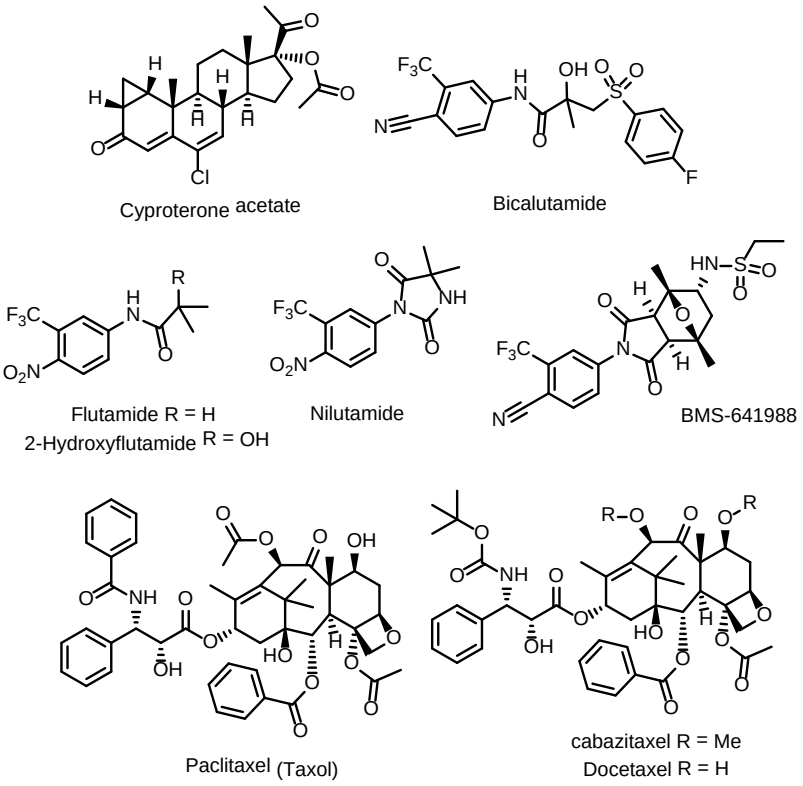


Figure 3. Traditional antiandrogens and Taxol-based inhibitors

Flutamide is an antiandrogen, acetanilide-derived compound that acts as a prodrug.[19] The active form is oxidized *in vivo* at the dimethylacetanilide functionality to 2-hydroxyflutamide (Figure 3), and it has been used in combination with GnRH agonists since 1989.[9,19] A short half-life *in vivo* requires dosing as high as 250 mg three times daily; however, the combo therapy is not effective in treating CRPC.[19] A structurally related nitrotriflurotoluene based antiandrogen, nilutamide (Figure 3), has a longer half-life than flutamide but has more side effects than the other FDA-approved non-steroidal antiandrogens.[20] It also does not increase patient survival in advanced prostate cancers.[20]

Bicalutamide, a non-steroidal trifluoromethylbenzonitrile antiandrogen, is used in conjunction with GnRH therapy and is considered the current standard-of-care for prostate

cancer (Figure 3).[21] Marketed as a racemic drug, the (*R*)-enantiomer has been found to be the principle active component, with the (*S*)-enantiomer being absorbed and cleared more rapidly.[21-22] The 60-fold difference in the effective *in vivo* dose of (*R*)- versus (*S*)-bicalutamide has been correlated to differing rates in drug metabolism.[22] The (*R*)-enantiomer has also been shown to have a better binding affinity for the AR.[21] Doses of 50 mg/day-150 mg/day are approved by the FDA for treatment of prostate cancer.[9] Higher doses have shown promise as a monotherapy, improving patient survival in locally advanced prostate cancer.[9]

BMS-641988 (Figure 3), is an oxabicyclo-imide-based antiandrogen that showed promise in Phase I clinical trials as a treatment for CRPC.[9] When compared to bicalutamide, a 20-fold higher binding affinity for the AR, a 3-7 fold higher antagonistic potency, and an improved anti-tumor activity in prostate tumor xenografts was observed.[23] Because of these initial positive results, a Phase I dose escalation clinical study was initiated with 61 CRPC patients. However, limited anti-tumor activity and an epileptic seizure in one patient resulted in termination of the trial.[9]

Taxol-based chemotherapeutic agents (Figure 3) have shown promise in improving the quality of life over the short term for CRPC patients.[24] Docetacel is currently seen as the standard-of-care in CRPC and has a survival benefit of 2-3 months.[25] Taxol derivatives reduce expression of PSA gene through inhibition of the AR by an unknown mechanism.[24] The current hypothesis is that taxol-based compounds increase the concentration of an AR suppressive nuclear factor, FOX01.[24a] Docetaxel, while FDA approved for treatment of CRPC, suffers from many of the common taxol toxicities issues.[25] Cabazitaxel is currently in Phase I clinical trials and may offer a better toxicity profile while prolonging survival in CRPC patients.

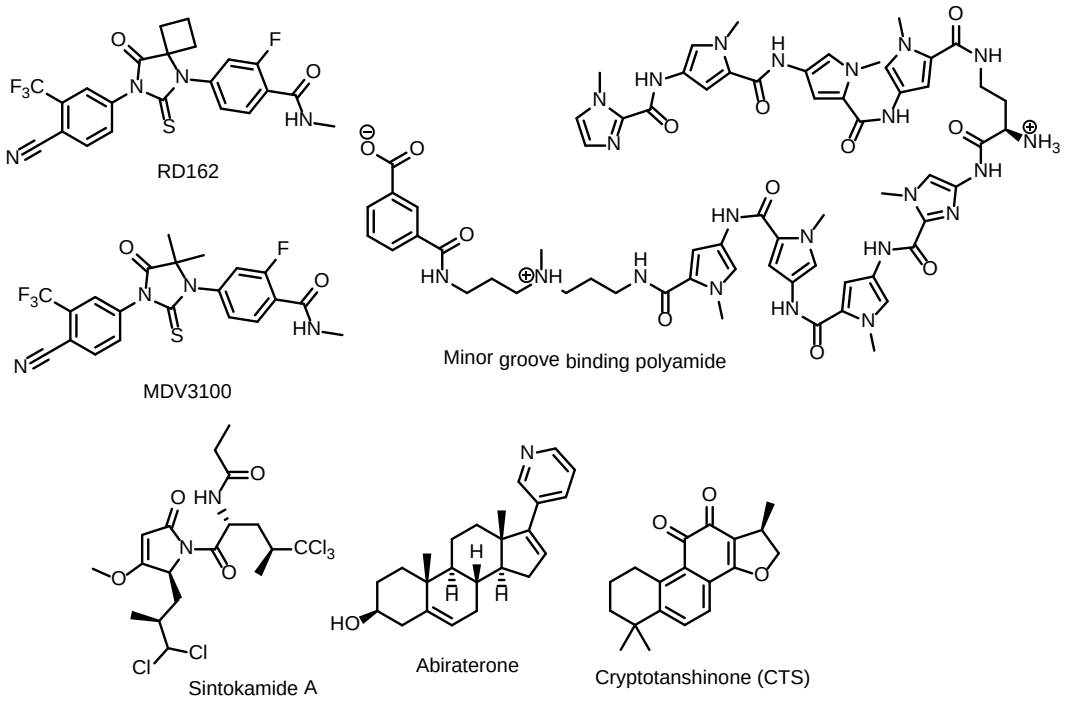


Figure 4. Modulators of AR activity

Besides the traditional antiandrogens and taxol-based compounds, researchers have tested other approaches to disrupt the androgen-AR interaction. MDV3100 and its structurally related analog RD162 (Figure 4) are diarylthiohydantoins based on the structure of nilutamide and are much more potent antiandrogens.[26] MDV3100 was chosen as the clinical candidate for the treatment of CRPC after an extensive SAR study because of its strong receptor antagonism and good pharmacokinetic properties.[26a] Unlike other antiandrogens, MDV3100 and RD162 were shown to be effective in the treatment of CRPC in a mouse model and in Phase I/II clinical trials.[9,26b] MDV3100 and RD162 display a 5-8 fold higher binding affinity than the traditional antiandrogens, reduce the efficiency of receptor nuclear translocation, and impair binding to DNA and the recruitment of coactivators.[26] However, MDV3100 can only increase survival by 3-5 months in patients with CRPC.

Abiraterone (Figure 4) or abiraterone acetate binds irreversibly and highly selectively to the CYP17 enzyme, blocking the *in situ* production of androgens and reducing their concentrations to 1 ng/dL level.[4] In CRPC patients, levels of PSA dropped >50% in 55% of the patients, and this treatment can be used to lower residual cellular androgen after androgen deprivation therapy.[4] The compound is currently in Phase III clinical trials which are expected to be completed in 2014. Sipuleucel-T (not shown), a cancer vaccine, was recently approved in 2010 for the treatment of CRPC and might provide a 4 month survival advantage based on a recent study.[27] Each vaccine is made specifically for the patient using autologous blood peripheral mononuclear cells, recombinant PAP–GM-CSF, T cells, B cells, natural killer cells and other cells from the hosts body.[27] The vaccine is designed to harness the cell-killing of the immune response and may one day be used as a conjunctive therapy.

Sintokamide A,[28] crytoanshinone,[29] and a DNA binding polyamide[30] (Figure 4) inhibit AR activity through three unique mechanisms. Sintokamide A was isolated from the marine sponge *Dysidea* sp. collected in Indonesia and was found to be the first inhibitor of the NH_2-terminus of the AR.[28] As all current therapies focus on the COOH-terminus of the AR, this compound provides a novel lead for androgen receptor modulation. Crytotanshinone is a known natural product/Chinese herbal medicine and it inhibits AR in normal prostate cancer cells and CRPC cells.[29] Cryptotanshinone repressed AR signaling by blocking complex formation with lysine-specific demethylase 1 function and blocks AR dimerization.[29] The structural similarity to DHT suggests that cryptotanshinone may act through a more common antiandrogen mechanism. A DNA minor groove binding polyamide was found to inhibit the AR-DNA interaction and PSA levels were reduced *in vitro* at similar levels to bicalutamide.[30] This polyamide represents another new approach to inhibit AR activity besides the traditional

androgen deprivation/antiandrogen treatments. However, an inhibitor of AR nuclear translocation, which is essential for cell proliferation, is currently not represented in the literature. Therefore, we chose to examine this as a possible treatment of CRPC.

1.1.2 SID3712502 and Initial Hit Development

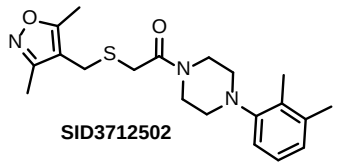
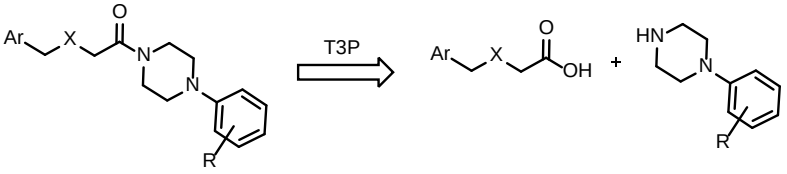
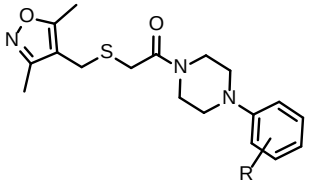


Figure 5. The Hit (SID3712502)

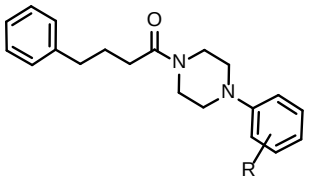
To examine our goal of identifying small molecules that block AR nuclear localization, we needed an assay that could test this hypothesis. Our collaborators in Professor Zhou Wang's lab at the University of Pittsburgh Medical Center were able to generate and stably transfect castration-resistant C4-2 cells with Green Fluorescence Protein (GFP) giving GFP-AR cells. These could then be used to study intracellular movement of the AR *in vitro*. The C4-2 cells were also transfected with PSA6.1-Luciferase (PSA6.1-Luc) and Renilla-Cytomegalovirus (pRL-CMV). As PSA is a known biomarker of prostate cancer and related to the progression of the disease, monitoring of its release is related to translocation of the AR into the nucleus.[3] The pRL-CMV releases PSA through a non-AR dependent pathway so this was monitored to demonstrate that the decrease in PSA was related to an AR dependent pathway. A high throughput screen of ~260,000 small molecules from the UPDDI library identified an initial group of compounds, which were active at a single dose of 10 μM.[31] These compounds were then dosed from 1 nM-50 μM, with the HSP-90 inhibitor 17AAG as a positive control and

DMSO as a negative control, and 35 compounds were selected based on their dose dependent response.[31] Further analysis narrowed this list to 3 novel lead structures, all of which inhibited GFP-AR nuclear localization but not GFP-GR or GFP-ER in the C4-2 cell model. SID3712502 (Figure 5) inhibited proliferation of AR-positive cancer cell lines LNCaP, C4-2, LAPC4, 22Rv1 and CWR-R1 at <1 μM and localized endogenous AR in C4-2 cells to the cytoplasm.[31] C4-2 xenograft tumor growth was inhibited in the mice model and no cell death at 10 mM was observed in any of the cell lines.[31] The body weight of mice was also not affected, suggesting this class of molecules is not cytotoxic.

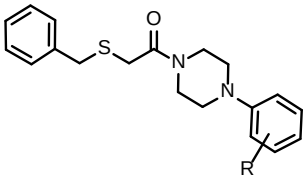




Structure	Estimated IC_{50}[a]
387-030, R = H	~50 μM
387-031, R = *o*-methyl	2-10 μM
387-032, R = *m*-methyl	~10 μM
387-033, R = *p*-methyl	~50 μM
387-034, R = *o*-methoxy	>50 μM



387-023, R = H	>50 μM
387-016, R = *o*-methyl	~10 μM
387-020, R = *m*-methyl	>50 μM
387-019, R = *p*-methyl	>50 μM
387-021, R = *o*-methoxy	>50 μM



387-024, R = H	>50 μM
387-025, R = *o*-methyl	~10 μM
387-026, R = *m*-methyl	>50 μM
387-027, R = *p*-methyl	10-50 μM
387-028, R = *o*-methoxy	>50 μM

[a]C4-2 cells transfected with PSA6.1-Luc, GFP-AR, and pRL-CMV and treated with 2, 10, 50 μM doses of compound for 24 h; lysed cells analyzed by Firefly and Renilla luciferase with a Dual-Luciferase Reporter on a LmaxII384 luminometer; Firefly luciferase values (AR dependent) were normalized to Renilla (pRL-CMV dependent and AR independent); Estimated IC_{50} values represent the point when the normalized Firefly luciferase values were reduced by half relative to DMSO (0 μM) control; performed in triplicate.

Figure 6. IC_{50} values for initial 15 compound screen[31]

To probe the SAR of SID3712502, an initial 15 compounds were synthesized by Dr. Erin Skoda in the Wipf group. Simple T3P coupling of several commercially available carboxylic acids and *N*-aryl piperazines gave 3 key structural variations: change in the substitution of the right aromatic ring, sulfur-carbon exchange in the 3 carbon linker, and variation of the isoxazole ring (Figure 6 and Figure 7). The effect of phenyl dimethyl substitution on biological activity was studied by changing the methyl substituent at the ring, adding a methoxy group, and completely omitting substitution. The *o*-methoxy was not tolerated in any of the analogs and the *o*-methyl was found to be essential to activity. This suggests that the orientation of the phenyl-piperazine is critical to activity but that the hydrogen bond acceptor was disfavored. The sulfur-carbon exchange in the 3 carbon linker was tolerated, and the phenyl substitution for the isoxazole did not affect activity. The importance of the piperazine core in SID3712502 was not investigated.

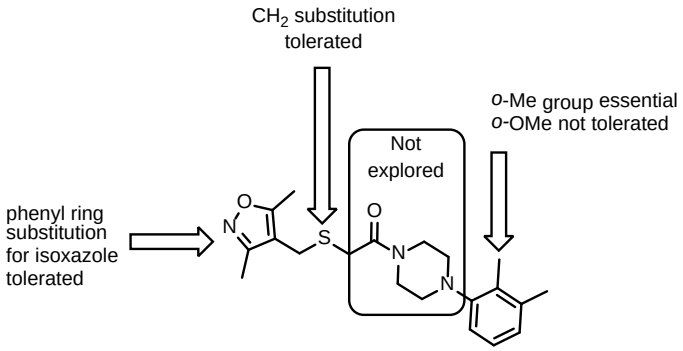
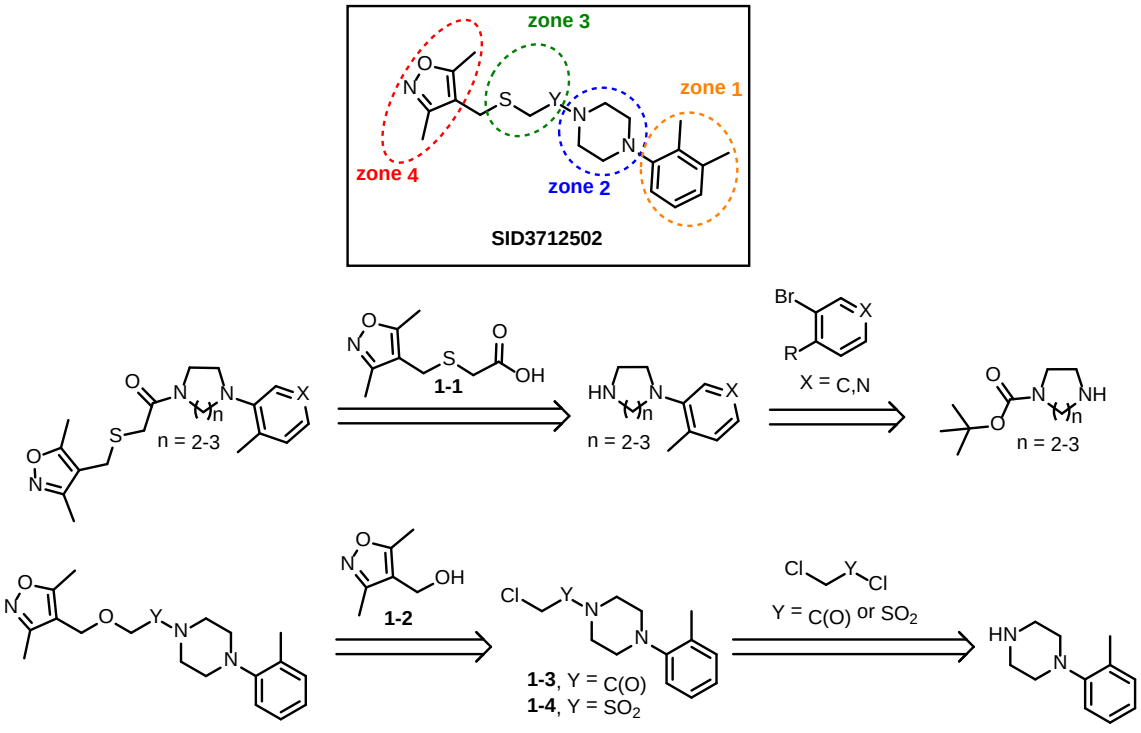


Figure 7. First round of SAR results for SID3712502

1.2 RESULTS AND DISCUSSION

1.2.1 Chemical Synthesis of SID3712502 Analogues

Following the first round of SAR, a retrosynthetic analysis led to two pathways to alter each of the four designated zones in SID3712502 (Scheme 1). Using Buchwald-Hartwig coupling conditions, we could probe the importance of the piperazine ring in zone 2 and the various substitutions and functionalities of the aromatic ring in zone 1. This would then be followed by coupling with the commercially available isoxazolethioacetic acid **(1-1)** to rapidly access a diverse set of analogs. The importance of the sulfur in the 3 carbon linker and the amide

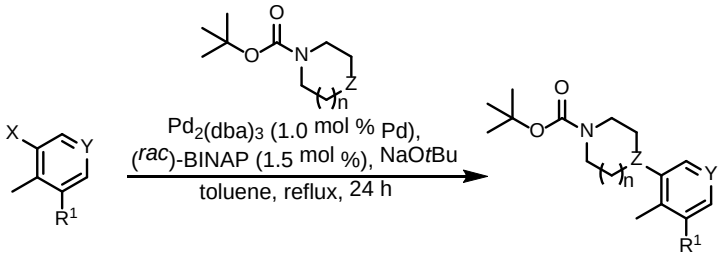


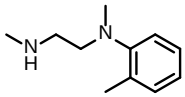
Scheme 1. Retrosynthetic analysis

functionality in zone 3 could be investigated by nucleophilic displacement of **1-3** and **1-4** with a variety of nucleophiles; this could be used to further probe the importance of the zone 4 isoxazole by replacement with a variety of other aromatic heterocycles.

Buchwald-Hartwig coupling with a variety of protected and unprotected heterocyclic diamines is precedented in the literature,[32] and we employed this reaction to rapidly access variations of both zone 1 and 2. The coupling of 3 different aryl halides with protected and unprotected diamines proceeded in moderate yields by heating in toluene in the presence of $Pd_2(dba)_3$ and racemic BINAP. This allowed access to a pyridine derivative **1-5**, a brominated tolyl derivative **1-6**, and three different alterations in the piperazine core **1-7** to **1-9** (Table 1). The low yield for **1-9** is hypothesized to be from its ability to favorably act as a bidentate ligand to Pd, thus limiting its reactivity.

Table 1. Buchwald-Hartwig coupling of diamines with aryl halides

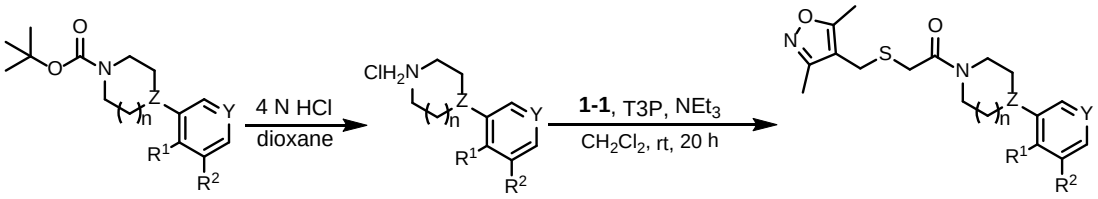


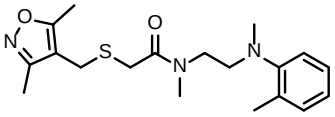
Entry	Compound	Structure					Yield
		X	Y	Z	R^1	n	
1[a]	**1-5**	Br	N	NH	H	1	54%
2[b]	**1-6**	I	CH	NH	Br	1	32%
3[b]	**1-7**	Br	CH	NH	H	2	48%
4	**1-8**	Br	CH	$CHNH_2$	H	1	73%
5	**1-9**						17%

[a] The reaction mixture was stirred for 40 h, [b] The reaction mixture was heated at 80 °C

Boc-deprotection by HCl in dioxane of the diamines **1-5** to **1-8** proceeded in near quantitative conversion to the corresponding HCl salts. T3P coupling with **1-1** gave the final compounds in good yield (Table 2, Entries 1-4). Two commercially available amines (Table 2, Entries 5 & 7) and **1-9** were also coupled to **1-1** in good yields. Replacement of the carbon opposite the essential *o*-methyl substitution with a nitrogen in **1-13** allowed examination of substituent effect beyond the initial methyl substitution variations. **1-10** similarly looked at this effect while adding a bromotolyl derivative that could be tritiated for analysis of where these compounds are actively binding the AR to prevent nuclear translocation. **1-11**, **1-12**, and **1-13** examined the effect of changing the piperazine ring to a diazapine, piperidine, and freely rotating

Table 2. Synthesis of zone 1 and 2 probes

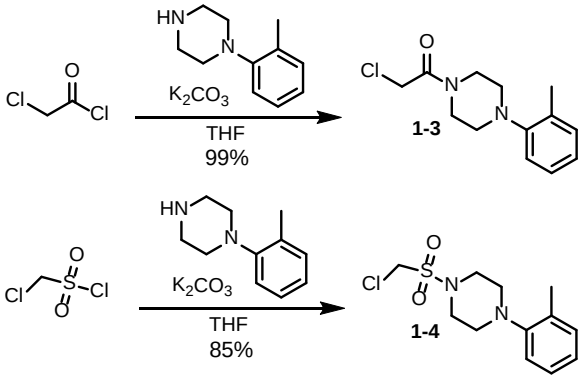


Entry	Boc-Protected Amine	Compound	Structure Y	Z	R^1	R^2	n	Yield
1	1-6	1-10	CH	N	CH_3	Br	1	quant.
2	1-7	1-11	CH	N	CH_3	H	2	quant.
3	1-8	1-12	CH	CHNH	CH_3	H	1	93%
4	1-5	1-13	N	N	CH_3	H	1	76%
5[a]	---	1-14	CCl	N	CH_3	H	1	quant.
6[a]	---	1-15						76%
7[a]	---	1-16	CH	N	F	H	1	98%

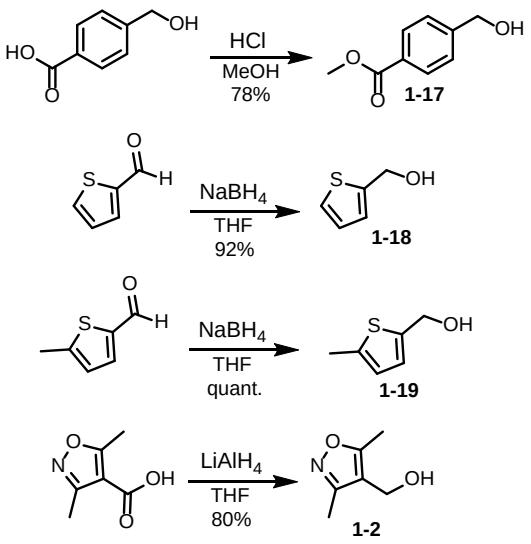
[a] Synthesis started from free amine

ethylenediamine linker. **1-14** and **1-16** examined the effect of chlorine substitution opposite the *o*-methyl and direct replacement of the *o*-methyl with fluorine, respectively.

For developing the SAR of zones 3 and 4, we chose to synthesize the precursors **1-3** and **1-4** by condensation of chloroacetyl chloride and chloromethanesulfonyl chloride, respectively, with *o*-tolylpiperazine, which resulted in high yields under basic conditions (Scheme 2). The alcohol nucleophiles **1-18** to **1-19** and **1-2** were prepared in good yields from the corresponding aldehydes and carboxylic acid by reduction with $NaBH_4$ and $LiAlH_4$, respectively (Scheme 3). The formation of methyl ester **1-17** under methanolic HCl conditions proceeded in moderate yield (Scheme 3). Combination of **1-3** and **1-4** with alcohols **1-17** to **1-19** and **1-2** would allow access to probes of zone 3 amide substitution, sulfur to oxygen replacement in zone 3, and variation of the aromatic heterocycle in zone 4.



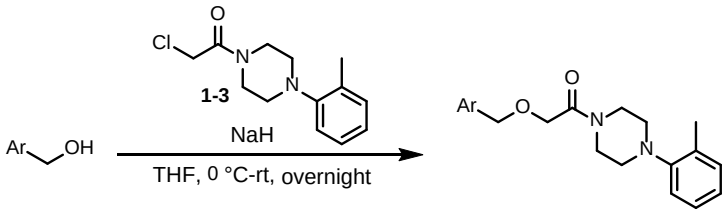
Scheme 2. Synthesis of alkylating agents

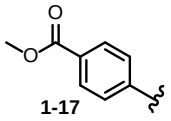
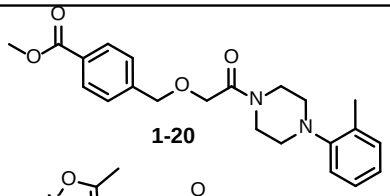
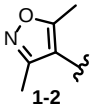
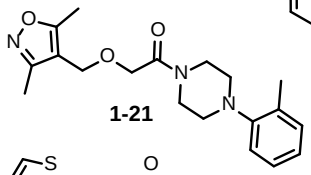
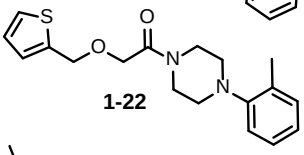
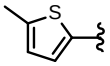
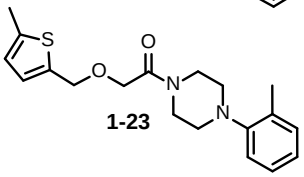


Scheme 3. Synthesis of heterocyclic alcohols (zone 4)

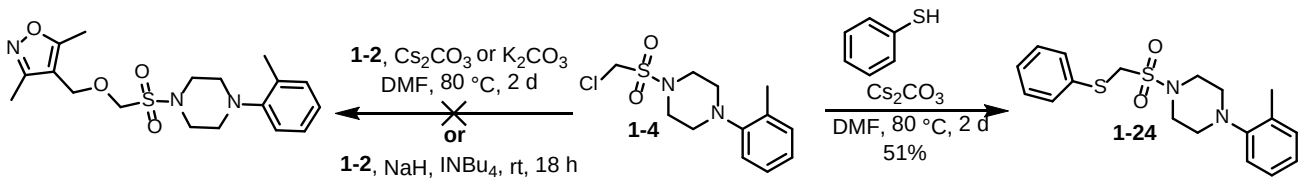
1-20 to **1-23** were accessed using literature conditions for the condensation of allyl alcohols with chloroacetamide.[33] The heterocyclic alcohols **1-17** to **1-19** and **1-2** were premixed with sodium hydride at 0 ºC followed by addition of **1-3** and warming to room temperature (Table 3). After stirring overnight, 4 variations of zone 3 and 4 were obtained where **1-17** allows attachment to a resin for surface plasmon resonance (SPR) protein affinity analysis, **1-18** and **1-19** probe the importance of the 5-methyl group in the thiophene for biological stability, and **1-2** examines the effect of oxygen substitution for sulfur. The two different thiophenes **1-18** and **1-19** were also added to probe the zone 4 heterocycle effect. All of these compounds were submitted to the Wang lab for *in vitro* testing in C4-2 cells.

Table 3. Synthesis of zone 3 and 4 probes



Entry	Alcohol	Product	Yield
1	 1-17	 1-20	34%
2	 1-2	 1-21	90%
3	 1-18	 1-22	70%
4	 1-19	 1-23	35%

Besides the analogs described above, which were submitted for testing in December 2012, we were more recently able to synthesize **1-24** (Scheme 4). Our original synthetic plan was to prepare an analog of SID3712502 by direct substitution of the amide functionality of zone 3 with a sulfonamide through nucleophilic displacement of the chloride of **1-4** as had been done previously with the analogous **1-3**. All attempted conditions however failed and only recovered starting material **1-4** was isolated in each case. **1-24** will be submitted in due course to probe not only the amide replacement with the sulfonamide but also the shortened zone 3 linker.

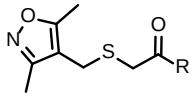


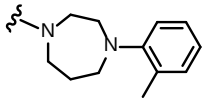
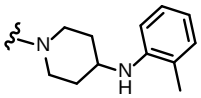
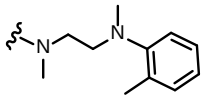
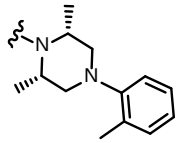
Scheme 4. Synthesis of sulfonamide analog **1-24**

1.2.2 Biological Evaluation

The Wang lab at the University of Pittsburgh Medical Center tested the synthetic analogs in their *in vitro* assay to see if they blocked AR nuclear localization similar to SID3712502. All compounds were first tested at 10 μM and 5 more active derivatives were then tested by dose dependent response, 0 μM to 12.8 μM.[31] The C4-2 cells were not transfected with GFP-AR as was done previously but instead used the endogenous AR found within the cells. This makes it difficult to directly compare this data directly to the first round of SAR. The previously unexplored piperazine ring in zone 2 was investigated by adding a carbon to the piperazine ring to give a diazepine derivative (Table 4, Entry 1), moving the one of the nitrogens outside the piperazine ring (Table 4, Entry 2), eliminating the ring for a more freely rotating 2 carbon linker (Table 4, Entry 3), and adding *cis*-dimethyl substitution to the piperazine core (Table 4, Entry 4). The *cis*-dimethyl derivative was found to be toxic to the cells at 10 μM, while the loss of the more restricted ring structure also decreases activity. The diazepine derivative and moving the nitrogen outside the piperazine ring however was effective in reducing the IC_{50} to ~1.0 μM for both compounds (Table 4, Entries 1 and 2).

Table 4. AR inhibition by zone 2 probes[31]



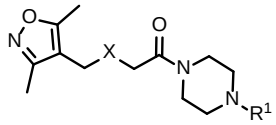
Entry	R	Estimated IC_{50}[b]	% Reduction[c]
1		~0.8 μM	91%
2		~1.0 μM	91%
3		---	66%
4[a]		Cells detached, toxicity	

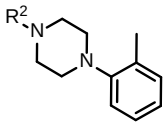
[a]Compound was synthesized by Mustafa Kazancioglu. [b]C4-2 (endogenous AR) cells transfected with PSA6.1-Luc and pRL-CMV and treated with 0.05, 0.2, 0.8, 3.2, 12.8 μM doses of compound for 24 h; lysed cells analyzed by Firefly and Renilla luciferase with a Dual-Luciferase Reporter on a LmaxII384 luminometer; Firefly luciferase values (AR dependent PSA release) were normalized to Renilla (AR independent PSA release); Estimated IC_{50} values represent the point when the normalized Firefly luciferase values were reduced by half relative to DMSO (0 μM) control; performed in triplicate. [c]Treated with a 10 μM dose of compound for 24 h and results analyzed same as above; performed once; Percent reduction values represent decrease of the normalized Firefly luciferase values relative to DMSO (0 μM) control.

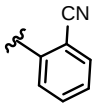
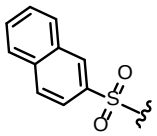
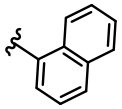
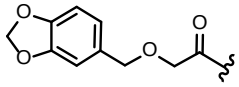
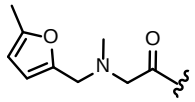
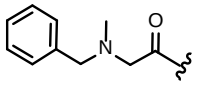
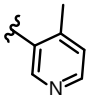
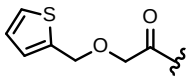
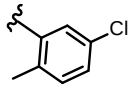
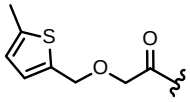
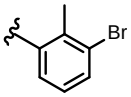
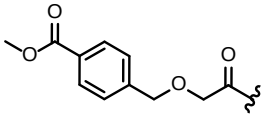
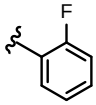
The synthetic probes of zones 1, 3, and 4 revealed less about the SAR for these analogues of SID3712502 than originally hoped (Table 5). None of the isoxazole zone 4 replacements were tolerated (Table 5, Entries 10-15) and with the exception of the sulfonamide derivative (Table 5, Entry 9). However, direct ether linker replacement for the thioether of SID3712502 was not tolerated (Table 5, Entry 4) suggesting that the poor activity of the heterocycle replacement may be from the zone 3 *N*-methyl or ether linker and not the zone 4 component. *O*-methyl replacement in zone 1 with a cyano group (Table 5, Entry 1) was tolerated and well as a naphthyl group in place of the *o*-tolyl zone 1 functionality (Table 5, Entry 2) from SID3712502.

Halogen substitution around the zone 1 aromatic ring (Table 5, Entry 6) was also tolerated as long as the essential *o*-methyl group was retained. Initial second round SAR results show promising trends (Figure 8) however, more complete dose dependent analysis of all the submitted analogs is need to confirm the trends and accurately calculate IC_{50} values. It appears

Table 5. AR inhibition by zone 1, 3, 4 probes[31]





Entry	X	R^1	Estimated IC_{50}[b]	% Reduction[c]	Entry	R^2	Estimated IC_{50}[b]	% Reduction[c]
1[a]	X = S		~0.6 μM	78%	**9**[a]		---	70%
2[a]	X = S		~1.5 μM	89%	**10**[a]		---	44%
3[a]	X = S		---	7%	**11**[a]		---	48%
4	X = O		---	36%	**12**[a]		---	28%
5	X = S		---	39%[e]	**13**		---	0%[d]
6	X = S		~0.4 μM	76%[e]	**14**		---	11%
7	X = S		---	44%[e]	**15**		---	38%
8	X = S		---	64%[e]				

[a]Compounds were synthesized by Mustafa Kazancioglu. [b]C4-2 (endogenous AR) cells transfected with PSA6.1-Luc and pRL-CMV and treated with 0.05, 0.2, 0.8, 3.2, 12.8 μM doses of compound for 24 h; lysed cells analyzed by Firefly and Renilla luciferase with a Dual-Luciferase Reporter on a LmaxII384 luminometer; Firefly luciferase values (AR dependent PSA release) were normalized to Renilla (AR independent PSA release); Estimated IC_{50} values represent the point when the normalized Firefly luciferase values were reduced by half relative to DMSO (0 μM) control; performed in triplicate. [c]Treated with a 10 μM dose of compound for 24 h and results analyzed same as above; performed once; Percent reduction values represent decrease of the normalized Firefly luciferase values relative to DMSO (0 μM) control. [d]Increase in PSA release observed versus DMSO control. [e]Non-AR dependent PSA decrease.

that no other zone 4 rings other than the isoxazole from the hit or phenyl ring from the first round of SAR are tolerated. The zone 3 sulfur linker cannot be substituted for the more metabolically stable ether or *N*-methyl linkers. Two different rings other than the zone 2 piperazine were tolerated and various substitutions around the zone 1 aromatic ring were tolerated as long as *ortho*-substitution was maintained. More importantly, the second round of SAR revealed several analogues with 4 exceptions (Table 5, Entries 5-8) that were active and selective for the AR dependent pathway of PSA release; this selectivity was not observed in the hit or in any of the compounds for the first round of SAR.

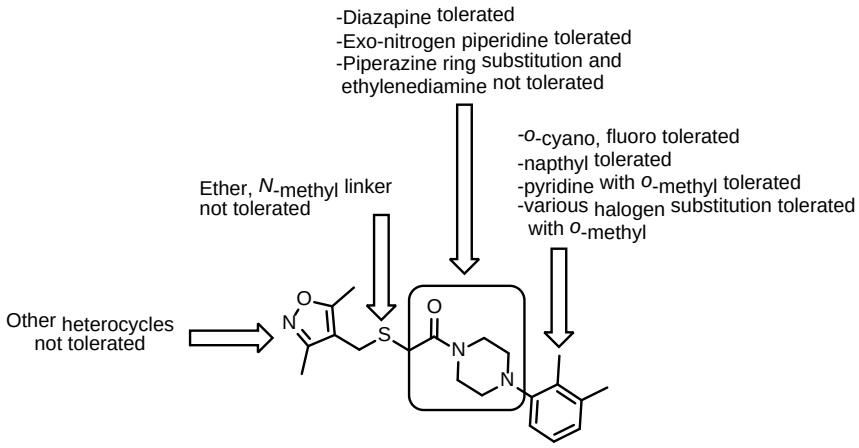


Figure 8. Second round of SAR results for SID3712502

1.3 CONCLUSIONS

The new synthetic analogues of SID3712502 have allowed us to expand our original hypothesis and to increase selectivity and potency. The second round of SAR has allowed further confirmation that zone 1 *ortho*-substitution is essential and repositioning of this functionality with different zone 2 ring systems, diazepine or by moving the nitrogen outside the

piperazine ring, allows a better binding fit. This exact binding interaction could be confirmed by a tritium labeling experiment or by synthesis of the zone 4 methyl ester derivative for a SPR experiment (Figure 9), as the originally synthesized ether analogue was not active (Table 5, Entry 15). The sulfur in the zone 3 linker could not be replaced by oxygen or methylated nitrogen as had been done in the round 1 SAR with carbon. More stable analogous forms of oxidized sulfur (Figure 9) could be synthesized to further probe what is important to activity in

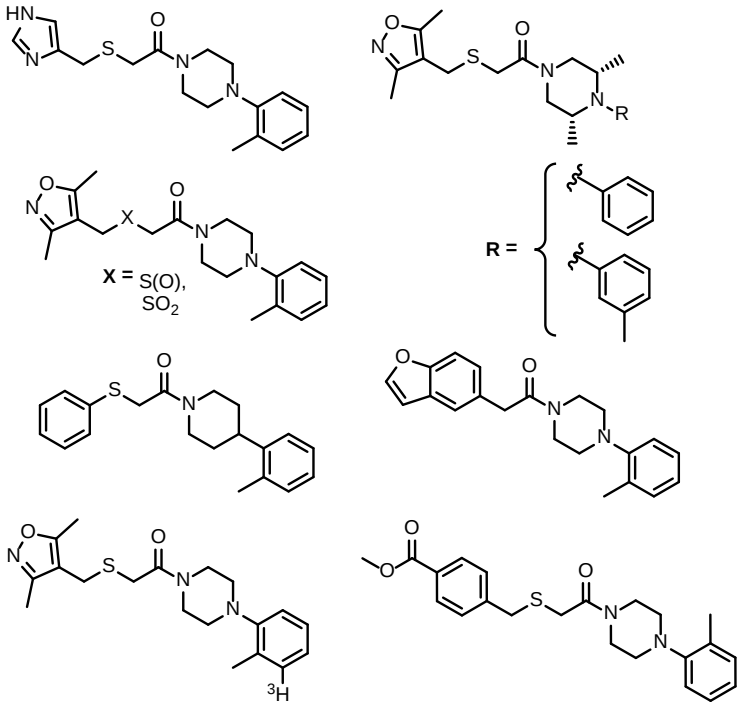


Figure 9. Planned analogs and ongoing work

zone 3. As it is currently not known if any other heterocycle besides the phenyl or isoxazole ring is tolerated in zone 4, the synthesis of a sulfur linked imidazole could probe this hypothesis. Tying back the ring with the benzofuran in zones 3 and 4 could determine if restriction across zone 3 while maintaining a zone 4 aromatic substituent could improve biological activity. *Cis*-dimethyl substitution on the opposite side of the piperazine ring (Figure 9) could test if two different analogs can act as *o*-methyl mimetics and determine if this piperazine substitution is

also toxic in the cell lines as was observed previously. The most important next step is the full biological characterization of the first two rounds of SAR to obtain IC_{50} values that can be directly compared.

2.0 SYNTHESIS OF *N*-ACETYLGLUCOSAMINE DERIVATIVES FOR MODULATING TLR4 IN NECROTIZING ENTEROCOLITIS

2.1 INTRODUCTION

2.1.1 Toll-like Receptor-4 and Necrotizing Enterocolitis

The human immune system protects itself from pathogens by avoidance, resistance and tolerance.[34] Avoidance reduces risk of exposure, resistance fights off the infection once established, and tolerance limits the negative impact of the immune upregulation on the host without decreasing the resistance.[34] The immune system can be subdivided into the innate immune system and the adaptive immune system. Adaptive immunity accounts for the generation of T-cells and B-cells with immunological memory to elicit a more effective and more rapid immune response in the case of re-infection by the same pathogen.[34-35] Innate immunity however acts as the body's first responder and determines the nature and level of the immune response based on the molecular signatures present.[35-36] Toll-like receptors (TLR) are members of a family of pattern-recognition receptors that are essential in the ability of the innate immune system to recognize pathogens and pathogen-associated molecular patterns (PAMPs) (Figure 10).[35-37] In mammals, 13 TLR are known (11 in humans) and they are responsible for differentiating self from non-self, which includes bacteria, fungi, viruses, and protozoans.[37a,37c]

TLR are type I transmembrane receptors responsible for detecting conserved metabolic products unique to microorganisms but absent from the host.[35-36]

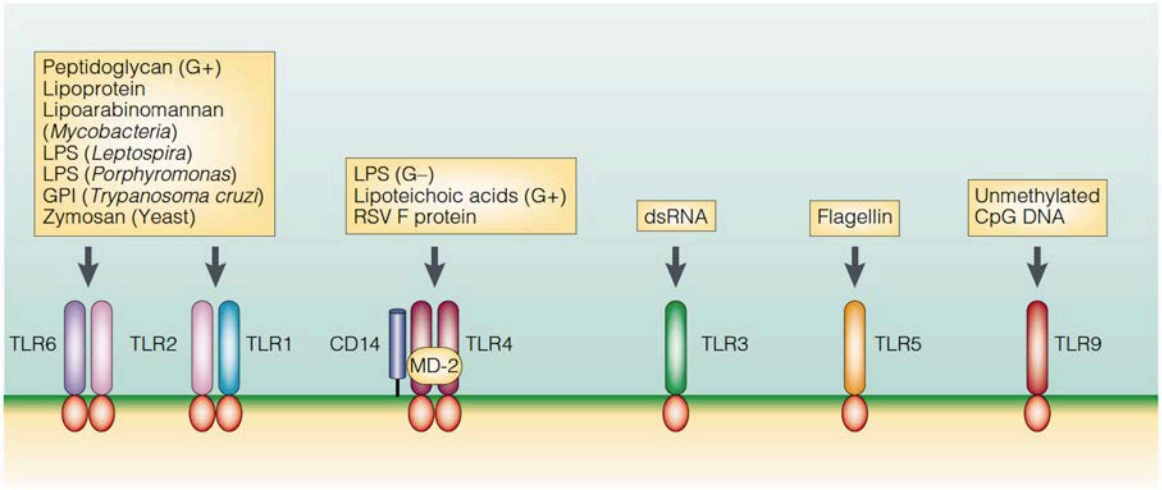


Figure 10. TLR and their ligands (reproduced with permission from Ref. 35)

Molecular signatures of microbial metabolism, such as bacterial lipopolysaccharide (LPS), lipoprotein, peptidoglycan, and lipoteichoic acid (Figure 11), are conserved but not exactly the same across a species of pathogens.[35,37b] The lipid-A portion of LPS (Figure 11) is an invariable pattern across all Gram-negative bacteria and thus triggers a response of the innate immune system while the rest of LPS is not conserved and elicits no response.[35-36,37b] Each of the 11 mammalian TLR, which can be aided by other proteins, recognizes specific PAMPs and more than one TLR can recognize certain ones.[35-36,37b] TLR4 was the first characterized mammalian Toll and it is expressed mostly in immune cells like macrophages and dendrites.[35,37b]

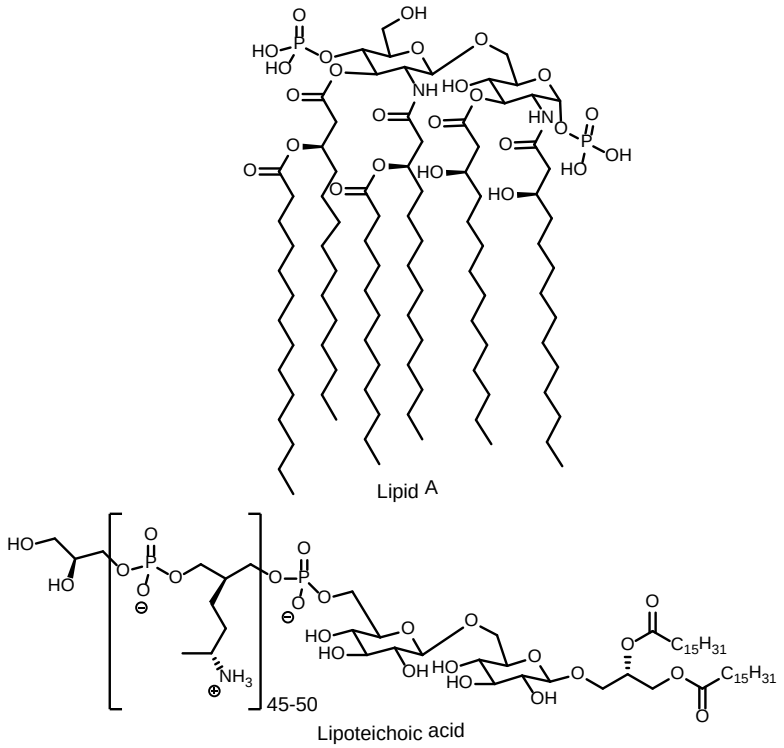


Figure 11. Lipid A portion of LPS and lipoteichoic acid

TLR4 recognizes LPS from Gram-negative bacteria, lipoteichoic acid from Gram-positive bacteria and RSV F protein.[35-36,37b] A complex between an extracellular protein MD-2 and TLR4 is required for receptor recognition of LPS, although its exact function is unknown.[35-36] Upon binding, LPS is delivered to the cytoplasm of the cell where further signaling is induced.[35-36,37b] TLR4 has the ability to recognize heat-shock proteins, specifically HSP60, produced by necrotic tissue and compounds like Taxol interact with the TLR4-MD2 complex in mice but not in humans.[35-36] Activation of TLR4 and other TLR leads to stimulation of intracellular transduction pathways and stimulates genes which trigger the release of inflammatory cytokines, chemokines, major histocompatibility complex (MHC), and co-stimulatory molecules.[35-36] Once the receptor is activated by an extracellular ligand, NFκB and mitogen activated protein (MAP) kinases induce transcription of immune response genes.[35] This

triggers the release of cytokines like IL-1β, TNF-α, IL-6, and IL-2 for immunological defense of the host.[35] The release of cytokines can have a negative effect on the host which leads tissue damage or other physiological costs caused by the infection or the immunological response to that pathogen.[34-35] TLRs have been recently linked to influencing the induction of cell autophagy as a mechanism of defense by the innate immune system to fight off infection.[37a]

Beyond the desired scope of the innate immune response, TLRs have been linked to human inflammatory and immune diseases. The broad expression of TLRs throughout the body and their ability to recognize a diverse set of PAMPs poises them to act as both a key to host immune defense and susceptible to react to local tissue damage.[38] TLR are implicated in severe sepsis and systematic human inflammatory response syndrome (SIRS),[39] Crohn's disease,[40] acute pancreatitis,[41] arthritis,[42] and atherosclerosis.[43] More recently, a connection has been proposed between LPS, TLR4, and necrotizing enterocolitis.[44]

Necrotizing enterocolitis (NEC) is one of the most prevalent and devastating diseases in premature infants occurring in 7% of all infants with a birth weight of 500-1500 g and a mortality rate of 20-30%.[45] The first cases were reported in the 1960's and the disease has proved very difficult to eradicate.[45] Premature birth results in high morbidity and mortality due to infections caused by immaturity of the innate immune system and the invasive medical operations these infants often undergo.[46] The more sensitive innate immune response of premature infants can result in an excessive inflammatory response causing necrotizing entercolitis, which affects the tissue of the small intestine and can even prevent neurological development.[45] The hypothesized cause(s) of the diseases are as follows: a genetic predisposition, intestinal mucosa immaturity, abnormal microbial colonization of the intestine compared to non-premature infants and a highly reactive innate immune system (Figure 12).[44-45]

Research in the last 5-10 years has pointed to TLR signaling playing a major role in the progression of NEC in neonatal infants.[44] In NEC murine models, a connection between the overexpression of TLR4 in enterocytes and intraluminal bacteria has been observed[47] and reciprocal signaling between TLR4 and TLR9 is important in the development of NEC.[48] TLR4 is known to disrupt the balance between intestinal mucosa injury and repair further establishing a connection between NEC and TLR4.[49]

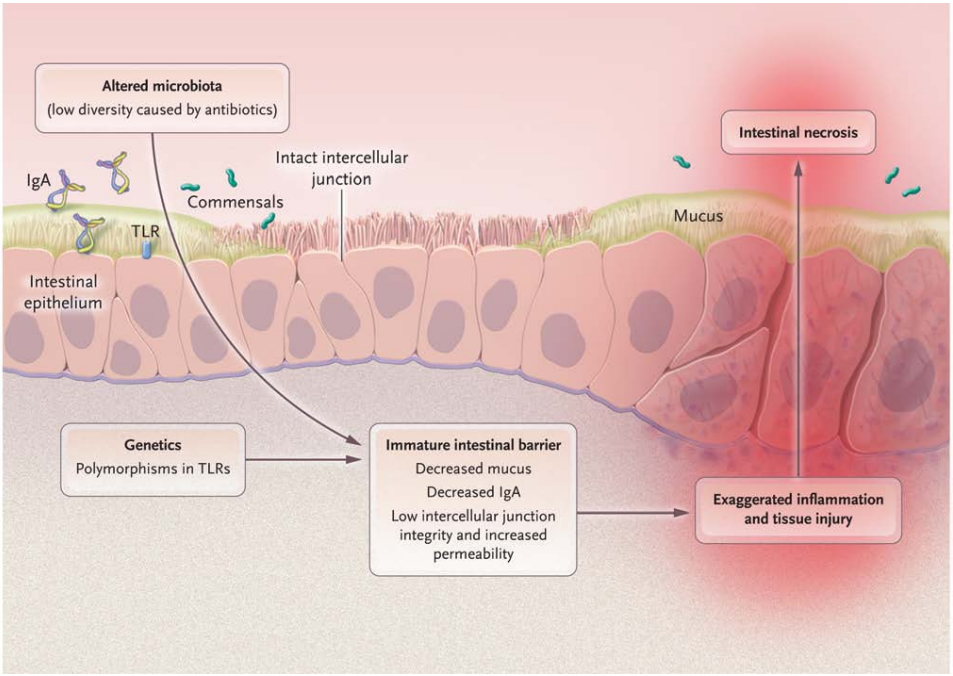


Figure 12. Necrotizing enterocolitis (reproduced with permission from Ref. 45, Copyright Massachusetts Medical Society)

Because toll-like receptors (TLRs) have been linked to massive upregulation of the immuno-inflammatory response and cancer they have become major targets for drug discovery.[50] To date there are 3 TLR4 antagonists in the developmental phase as targeted therapeutics: Eritoran (Figure 13) from Eisai pharmaceuticals (suspended in Phase III), AV411 from Avigen (Phase II), and NI0101 from NovImmune (Preclinical).[50] The crystal structure of the TLR4-MD-

2 complex with the Eritoran antagonist bound was recently reported in the literature, giving more information about the TLR4-MD-2 active site interaction.[51] Eritoran is a second generation synthetic structural analogue of Lipid A and has been evaluated thoroughly in the literature.[51-52] Synthetic TLR4 antagonists include mainly Lipid A mimetics, and are structurally similar to Eritoran in size and lipophilicity. Structural modifications that have been studied include changing both the nature of the lipid side chains or eliminating one of the pyranose rings of the core.[53] One of these Lipid A mimetics, CRX-526, had anti-inflammatory effects in two murine models for inflammatory bowl diseases like Crohn's disease and ulcerative colitis. There has been some evidence that some opioids may have TLR4-MD-2 modulation properties while have having no opioid receptor activity.[54] A 17 residue peptide mimetic of MD2-I was found to block association of the full length MD-2 with TLR4 which is needed for LPS mediated activation.[55] To date, there is no known treatment for NEC, but considerable research in the last 10 years has focused on its associated receptor, TLR4.

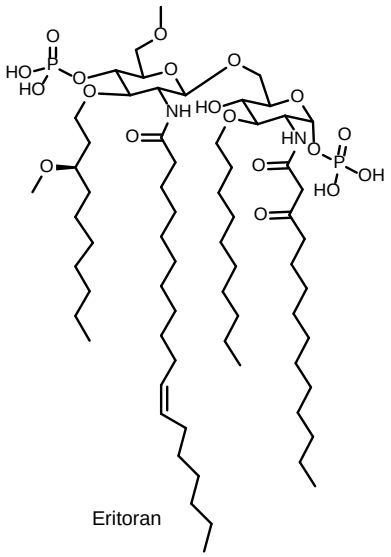


Figure 13. Structure of TLR4 modulator, eritoran

Carbohydrates are involved in a multitude of biological processes and are often called nature's third biopolymer.[56] Monosaccharides in nature can form oligo- and polysaccharides or can exist as glycone structures attached to peptides, lipids, or natural products.[56a,57] 2-Amino-2-deoxyglycosides, which mainly contain glycosides of *N*-acetylglucosamine, make up an important portions of glycoconjugates found in human milk, human blood, in bacterial lipopolysaccharide antigens and in plant root cells.[58] Human milk oligosaccharides are thought to have anti-inflammatory properties[59] which would explain why there is a lower incidence of inflammatory disease, like NEC, in breast-fed (BF) versus formula-fed infants.[60] Artificial oligosaccharides added to formula are structurally different than those naturally occurring in human breast milk.[60-61] While specific oligosaccharides are involved in the effect, the exact structure-function relationship is not clearly understood.[59a,61]

Glucosamine (Figure 14) is a known treatment for pain maintenance of osteoarthritis and has no adverse side effects when compared to NSAIDs nor does it affect glucose metabolism.[62] It has also been shown to have anti-inflammatory abilities to reduce the expression of cytokines by inhibiting NFκB activity in human retinal pigment epithelial cells[63] and in mouse arthritis models.[64] *N*-Acetylglucosamine has even been proposed as a replacement for glucose as an osmotic solute in peritoneal dialysis fluid due to its lack of pro-inflammatory effects and good toxicity profile.[65] Post-translational modification of nuclear and cytoplasmic proteins by *O*-glycosidic attachment of β-*N*-acetylglucosamine was shown to be necessary for cell viability and under cellular distress helped improve the tolerance of cells to a variety of stimuli.[66] Glucosamine and *N*-acetylglucosamine derived glycoconjugates have shown promise as central scaffolds for the development of glycosylated drugs.

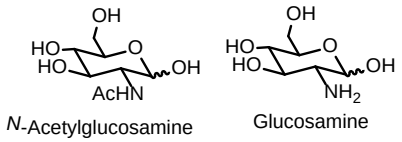


Figure 14. Nitrogen-containing monosaccharides common in nature

2.1.2 The Hit: C34

Lipid A mimetics structurally similar to Eritoran have become popular synthetic targets in the recent literature due to their biological activity.[53a,53b,67] An *in silico* similarity search based on the structure of Eritoran tetrasodium (E5564) was performed by Dr. Peter Wipf to identify lead compounds using the iResearch System Library of ChemNavigator®, the substance similarity Tanimoto scores implemented in SciFinder®'s substructure module, and ChemAxon®'s JChem® tools. An initial 700 hits were narrowed to approximately 10% and 68 individual compounds were chosen based on their commercial availability and chemical diversity. The 68 compounds were tested in mice for their ability to inhibit TLR4 signaling after LPS injection and *in vitro* for their ability to inhibit LPS induced TLR4 cytokine upregulation in both hematopoietic and epithelial cells. Compound C34 (Figure 15) was selected as the rapid synthesis of diverse analogs from common intermediates was straightforward and due to its potency and selectivity in the initial screen. However, the supplier, Enamine, could not provide the configuration of the 5 stereocenters in the C34 pyran ring, complicating the resynthesis. Our initial hypothesis was that C34 was either a derivative of glucosamine or galactosamine with α- or β-configuration at the anomeric carbon. Therefore, we initiated the synthesis of these compounds (Figure 15) as well as several other analogs for SAR purposes.

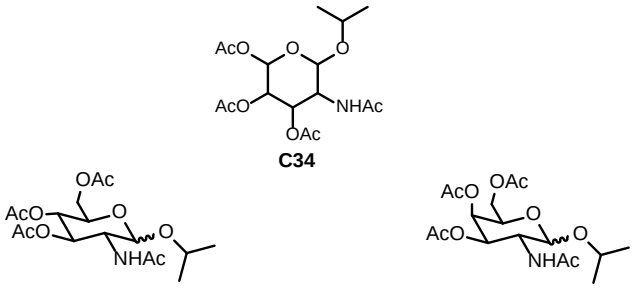


Figure 15. C34 and 4 possible stereoisomers

2.2 RESULTS AND DISCUSSION

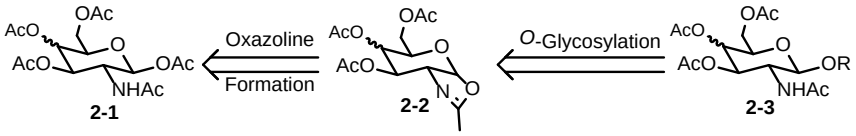
2.2.1 Analogue Chemical Synthesis and the Configuration of C34

As the glucosamine core of C34 makes up the central disaccharide scaffold of both Lipid A (Figure 11) and Eritoran (Figure 13), we sought to mimic their lipophilicity while reducing the molecular weight of our SAR analogs to make them more drug-like in character. Our strategy examined 3 areas of diversity: α- vs β-glycosidic linkage, C-4 configuration of the pyranose ring (glucosamine vs galactosamine), and varying the length, size, and lipophilicity of the glycosyl chain. The pyranose hydroxyl and amino groups were acetylated to mimic the longer chains in Lipid A and Eritoran.

Glycosylation methods for 2-amino-2-deoxyglycopyranosides are derived from the traditional literature protocols for carbohydrates but are distinctly different due to the C-2 nitrogen.[58a] Glycosylation of 2-acetamido-2-deoxy-D-glucosamine (*N*-acetylglucosamine, GlcNAc) under standard Koenigs-Knorr conditions, while proceeding with good stereoselectivity for the 1,2-*trans*-glycoside, resulted in a large amount of the oxazoline side product.[58a] The conditions were further limited to reactive glycosyl acceptors with a primary hydroxyl group.[58a]

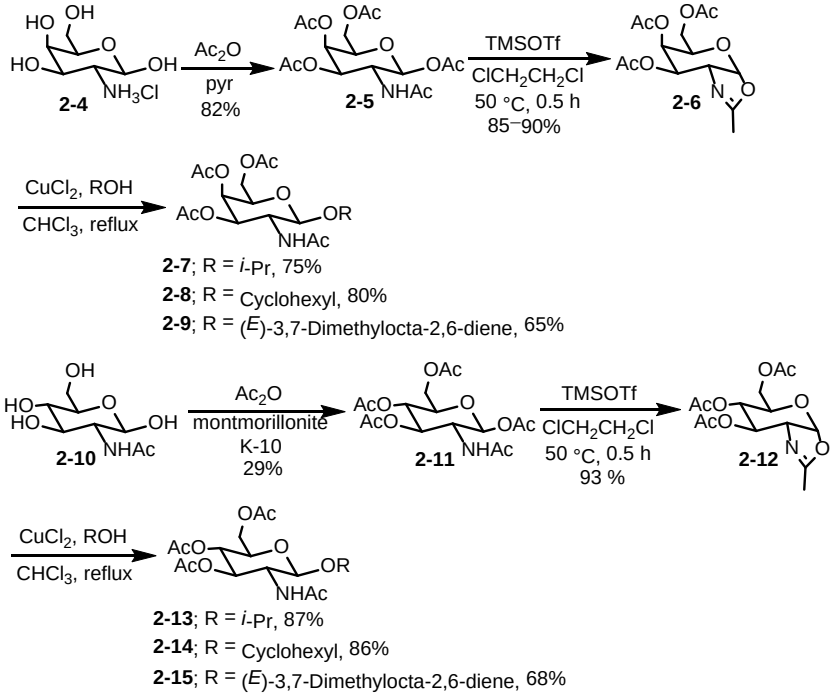
The *N*-acetyl group forms an oxazolinium intermediate which upon loss of a proton gives the oxazoline.[58a,68] Because of the tendency of GlcNAc glycosyl donors to form the oxazoline, various alternatives to the *N*-acetyl group have been examined in glycosylation reactions[69] including protection with phthaloyl,[70] tetrachlorophthaloyl,[71] 4,5-dichlorophthaloyl,[72] dithiasuccinoyl,[73] trichloro-[74] and trifluoroacetyl,[74a,75] trichloroethoxycarbonyl,[76] diacetyl,[77] dimethylmaleoyl,[78] thiodiglycol[79] groups or masking as the 2-azido group.[58b,80] While these groups provide an alterative to the oxazoline issue, each requires additional steps for the protection and subsequent substitution with the *N*-acetyl group of the desired compounds. Ring opening of aziridines such as those generated from iodosulfonamidation of glycals[81] or from the photolysis triazolines[82] present two other approaches for the formation of 2-amino-2-deoxyglycopyranosides. However, a more recent publication by Wittmann and co-worker showed promise for an opening of these oxazolines with a variety of glycosyl acceptors under mild copper (II) catalyzed conditions.[68]

We decided that opening of the oxazoline with a variety of alcohol nucleophiles would allow rapid access to the β-glycosylation analogs of 3,4,6-tri-acetyl-*N*-acetylglucosamine and 3,4,6-tri-acetyl-*N*-acetylgalactosamine all from the same key intermediate **2-1** (Scheme 5). The α-glycosides were imagined to be synthesized from an acid catalyzed isomerization of the corresponding β-glycosides.



Scheme 5. Retrosynthetic analysis for β-anomers of 2-acetamido-2-deoxy-D-pyranosides

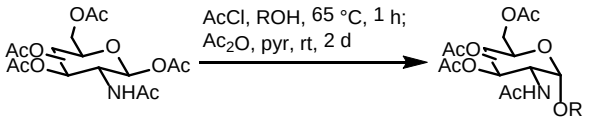
After synthesis of the fully protected β-pentaacetates of galactosamine **2-5**[83] and glucosamine **2-11**[84] using literature conditions, the corresponding oxazolines **2-6** and **2-12** were synthesized in high yields using the Jeanloz' protocol with TMSOTf in 1,2-dichloroethane.[85] Other literature methods to access the glyco-oxazolines using $SnCl_4$ in dichloromethane failed.[86] **2-6** and **2-12** were then heated at reflux in $CHCl_3$ with $CuCl_2$ and the desired glycosyl acceptors, giving the β-glycosides in good yields (Scheme 6) under the mild reactions conditions developed by Wittmann.[68] Using isopropanol, cyclohexanol, and geraniol as the glycosyl acceptors, we were able to synthesize 6 derivatives, with 3 pairs of different configurations at C-4. Glycosidic linkage with isopropanol gave compounds **2-7** and **2-13** with a small alkyl side chain whereas the cyclohexyl linked derivatives **2-8** and **2-14** where slightly larger in size. The geraniol glycosidic linked compounds **2-9** and **2-15** gave two more lipophilic derivatives, mimicking the longer acyl side chains of both Lipid A and Eritoran.



Scheme 6. Synthesis of 6 β-glycosides of 2-acetoamido-2-deoxy-D-glucose and galactose

The α-glycosides proved more difficult to access than we originally thought, and all acid catalyzed isomerization conditions with the corresponding β-glycosides were unsuccessful. Fortunately, literature conditions for the synthesis of the methyl α-D-glucosamine from D-glucosamine pentaacetate were found to give the desired products.[87] Heating the fully protected β-glucosamine and β-galactosamine pentaacetates (**2-11** and **2-5** respectively) in 5% HCl in alcohol solvent cleanly gave a 3:1 mixture of the α:β isomers, which after reprotection with acetic anhydride in pyridine were separated by chromatography on SiO_2 to give the α-glycosides **2-16** to **2-18** in moderate yields (Table 6). Compounds **2-16** and **2-17** allowed examination of the effect of the C-4 configuration on the α-glycosidic linkage with isopropanol (Table 6, Entries 1 and 2) as well as comparison to the corresponding β-glycosides **2-7** and **2-13**. The synthesis of α-glycoside **2-18** placed the cyclohexyl group opposite that of **2-14** allowing for comparison of the activity of α- and β-glycosidic linkage as well as the size of the α-glycosidic substitution compared to **2-16** and **2-17**.

Table 6. Acid mediated synthesis of the α-glycosides



Entry	Configuration	R	Yield	Product
1	α	*i*-Pr	61%	**2-16**
2	β	*i*-Pr	57%	**2-17**
3	α	Cyclohexyl	57%	**2-18**

As the configuration of C34 was unknown, we initially imagined that the commercial compound was a mixture of multiple compounds. However, we were delighted to discover by ^{1}H-NMR and ^{13}C-NMR that C34 was a single compound. We compared the NMR of the commercial sample to those of the four proposed stereoisomers we had synthesized, glucosamine

derivatives (**2-16** & **2-13**) and galactosamine derivatives (**2-17** & **2-7**). ^{1}H-NMR and ^{13}C-NMR spectra of the four possible stereoisomers when compared to the spectral data for C34 revealed that **2-13** closely resembled C34 but that of **2-16** matched exactly when the spectral data was overlaid (Figure 16 and Figure 17). To further support our claim, a co-NMR of C34 (2 mg) and **2-16** (2 mg) was also measured and the spectrum was identical to the previous NMRs (Figure 16 and Figure 17).

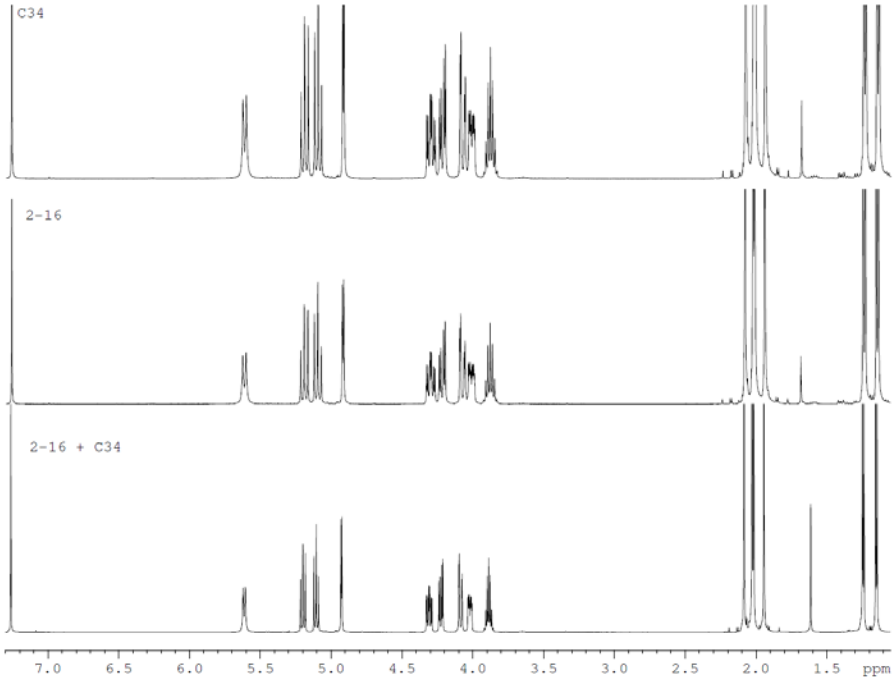


Figure 16. ^{1}H-NMR of C34, **2-16**, and a 1:1 mixture of both compounds

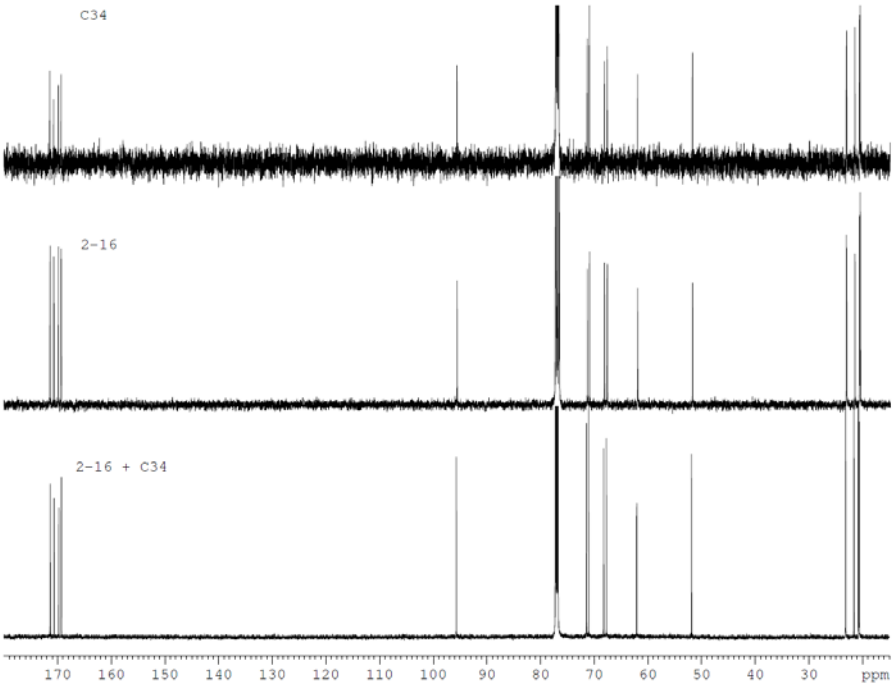
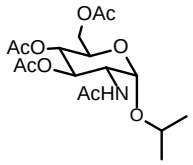


Figure 17. ^{13}C-NMR of C34, **2-16**, and a 1:1 mixture of both compounds

The coupling constants for H1-H2 (J = 3.7-3.8 Hz), correspond to α-configuration at the anomeric carbon, and the chemical shift of the anomeric carbon further supported our hypothesis that C34 was indeed equal to **2-16** (Table 7). The chemical shifts of all the protons and carbons within the three sets of data as well as the coupling constants for each proton were the same within the error of the instrumentation. With this data we felt confident in confirming that C34 was identical to **2-16** and had the structure and configuration as pictured below in Table 7.

Table 7. NMR studies of C34 vs **2-16**



a) ^{1}H-NMR comparison of C34 vs **2-16** in $CDCl_3$

Compound	NH	H1	H2	H3	H4	H5	H6	H6'
C34 400 MHz	5.62, d *J* = 9.6 Hz	4.93, d *J* = 3.8 Hz	4.35-4.27, m	5.20, app. t *J* = 10.4 Hz	5.11, app. t *J* = 10.0 Hz	4.05-3.99, m	4.23, dd *J* = 4.8, 12.2 Hz	4.09, dd *J* = 2.4, 12.4 Hz
2-16 400 MHz	5.62, d *J* = 9.6 Hz	4.92, d *J* = 3.8 Hz	4.34-4.26, m	5.19, app. t *J* = 10.4 Hz	5.10, app. t *J* = 10.0 Hz	4.05-3.98, m	4.22, dd *J* = 4.8, 12.4 Hz	4.08, dd *J* = 2.4, 12.4 Hz
2-16 + C34[a] 600 MHz	5.61, d *J* = 9.6 Hz	4.93, d *J* = 3.7 Hz	4.33-4.28, m	5.20, app. t *J* = 10.2 Hz	5.10, app. t *J* = 10.2 Hz	4.05-3.98, m	4.22, dd *J* = 4.8, 12.6 Hz	4.09, dd *J* = 2.4, 12.6 Hz

	$CH(CH_3)_2$	$C(O)CH_3$	$CH(CH_3)_2$
C34 400 MHz	3.89, sept *J* = 6.4 Hz	2.09, 2.03, 2.02, 1.95, 4 × s	1.25, d; 1.15, d *J* = 6.4 Hz; *J* = 6.0 Hz
2-16 400 MHz	3.88, sept *J* = 6.4 Hz	2.08, 2.02, 2.01, 1.94, 4 × s	1.24, d; 1.14, d *J* = 6.4 Hz; *J* = 6.0 Hz
2-16 + C34[a] 600 MHz	3.89, sept *J* = 6.0 Hz	2.08, 2.03, 2.02, 1.94, 4 × s	1.24, d; 1.15, d *J* = 6.0 Hz; *J* = 6.0 Hz

[a]**2-16** + **C34** were mixed in a 1:1 ratio in an NMR tube

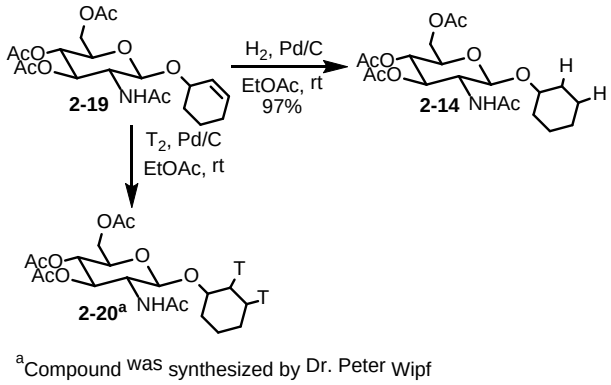
b) ^{13}C-NMR comparison of C34 vs **2-16** in $CDCl_3$

Compound	C1	C2	C3	C4	C5	C6	$C_{(O)CH_3}$	$C(O)CH_3$	$C_{H(CH_3)_2}$	$CH(CH_3)_2$
C34 150 MHz	95.7	51.9	68.2	71.0	67.7	62.1	171.4, 170.7, 169.8, 169.3	23.2, 23.1, 21.6, 20.8	71.4	20.7, 20.6
2-16 100 MHz	95.7	51.8	68.2	71.0	67.7	62.0	171.4, 170.7, 169.8, 169.3	23.2, 23.1, 21.6, 20.7	71.4	20.7, 20.6
2-16 + C34[a] 150 MHz	95.7	51.9	68.3	71.0	67.7	62.1	171.4, 170.7, 169.8, 169.3	23.2, 23.1, 21.6, 20.8	71.4	20.7, 20.6

[a]**2-16** + **C34** were mixed in a 1:1 ratio in an NMR tube

With the configuration of C34 confirmed by resynthesis and several analogs completed, we also prepared a tritium labeled version of **2-14** to probe the binding mode of action for these compounds. We wanted to know if C34 and its analogues inhibited TLR4 via direct binding or through another mechanism, such as allosteric interaction. Wild type and TLR4 knockout RAW macrophages could then be treated with this tritium labeled derivative and the labeled compound was detected in a scintillation counter. Hydrogenation of **2-19**, which was prepared by treatment

of **2-12** with cyclohexenol under Wittmann's conditions, with 10% Pd/C under an atmosphere of H_2 proceeded smoothly to give a near quantitative yield of the saturated cyclohexyl glycosylation product (Scheme 7).[88] With successful conditions for hydrogenation of **2-19**, we switched the gaseous atmosphere to tritium generated by a Trisorber system were able to access the tritium labeled compound **2-20** with a 57.6 Ci/mmol specific activity.



[a]Compound was synthesized by Dr. Peter Wipf

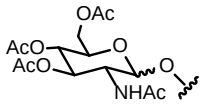
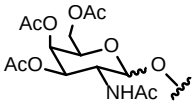
Scheme 7. Synthesis of the tritium-labeled analog

2.2.2 Biological Results

The Hackam group at Children's Hospital in Pittsburgh tested the synthetic analogs *in vitro* and *in vivo* against experimental NEC models. In the glucose series, **2-13**, **2-14**, **2-16** and **2-18** were shown in macrophages to reduce the levels of inflammatory cytokines triggered by LPS by 30-50% (Table 8). Only **2-8** and **2-9** in the galactose series showed a similar consistent activity across the cytokines examined (Table 8). This suggests that while the smaller isopropyl and cyclohexyl glycosyl groups were tolerated, the geraniol based analogs were too bulky to bind the receptor. There is also little difference between the active α- and β-analogs in both

series. While **2-7** was not able to reduce cytokine expression *in vitro*, overall there seemed to be little difference between the glucose and galactose series. This suggests that the configuration at C-4 of the *O*-acetyl group in the pyranose ring is not important for activity and that this group could possibly be eliminated in future analogs.

Table 8. *In vitro* percent reduction of cytokines in mouse RAW macrophages induced by LPS

Pyranosides	Percent Reduction in Cytokines[a]		
	**TNF-α**	**IL-6**	**iNOS**
2-13	24 ± 8%	32 ± 16%	---
2-14	38 ± 3%	48 ± 6%	65 ± 12%
2-15	7 ± 6%	24 ± 15%	---
2-16	42 ± 5%	55 ± 6%	62 ± 8%
2-18	48 ± 8%	39 ± 2%	53 ± 10%
			
2-7	3 ± 2%	2 ± 19%	---
2-8	30 ± 9%	36 ± 9%	---
2-9	18 ± 7%	50 ± 8%	---
2-17	46 ± 4%	33 ± 6%	51 ± 9%

[a]RAW 264.7 macrophages were treated with 10 μM of compound to 10 ng/mL of LPS and analyzed by qRT-PCR; N > 4 for each group; the percent reduction in cytokines is calculated by comparison to 10 ng/mL of LPS alone; Saline and the compounds alone showed no effect.

In vivo testing in an inflammatory mouse model with **2-14** and **2-16** showed that these two analogs significantly reduced the NFκB upregulation caused by LPS injection versus the control. Furthermore, **2-16** was shown to preserve the intestinal mucosa, reduce the gross disease and inflammatory cytokine expression, and lower the NEC severity score in both a mouse NEC model and in *ex vivo* isolated small intestine from infants with NEC. The tritium analog of **2-14** (Scheme 7) was shown by Professor Jeff Brodsky and co-workers at the University of Pittsburgh Department of Biology to directly bind to TLR4. In an *in vitro* labeling

assay with wild type and TLR4 knockout bone-marrow derived macrophages, the tritium labeled **2-20** bound wild type TLR4 expressing macrophages ~1.5 times more favorably than the TLR4 knockout macrophages. This data partially supports the initial hypothesis that the mechanism of action for all of these analogs might involve a direct binding interaction with TLR4. The *in vitro* reduction of cytokines, the *in vivo* reduction in the severity of NEC, and the direct binding supported by the interaction of **2-20** preferentially with wild type macrophages suggests that these analogs of C34 represent novel probes for modulating overactivation of TLR4 and diseases states like NEC.

2.3 CONCLUSIONS

β-Glycoslyated analogs of *N*-acetylglucosamine and *N*-acetylgalactosamine were rapidly access from their precursor oxazolines **2-12** and **2-6**, respectively, while the α-glycosides were synthesized through an acid catalyzed isomerization in 5% alcoholic HCl with the desired glycosyl acceptor. Glucose analogs **2-13**, **2-14**, **2-16** and **2-18** and galactose analogs **2-8** and **2-9** showed good reduction of cytokines *in vitro* and *in vivo*. **2-14** and **2-16** were shown to attenuate the severity of NEC in a mouse model by reducing cytokine expression. The originally unknown configuration of the commercial lead compound C34 was assigned by synthesis and NMR studies to be identical to **2-16**. The synthesis of the tritium-labeled analog **2-20** showed that this series of compounds may interact directly with TLR4, confirming our initial design strategy based on Eritoran. **2-16** was shown to preserve the intestinal mucosa, lower the levels of inflammatory cytokine expression and reduce the gross disease of NEC in both mouse intestines and *ex vivo* isolated human intestinal tissue. This series of analogs represents probes for

reducing inflammatory cytokine over-expression by inhibition of TLR4 and reducing the gross diseases of NEC. As it is currently unclear from the SAR presented herein if the β- or α-configuration at the anomeric carbon is preferred or if the *O*-acetyl function at C-4 is necessary, future synthetic analogues and biological studies should focus on the synthesis of more metabolically stable and possibly further simplified pyranosides.

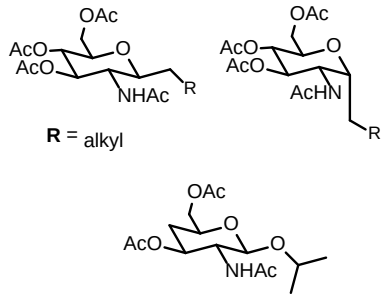


Figure 18. Proposed future synthetic analogs of C34

3.0 EXPERIMENTAL SECTION

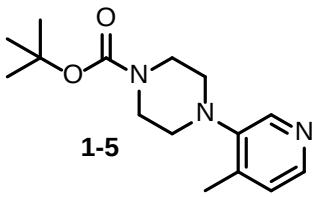
3.1 GENERAL

All moisture sensitive reactions were performed using syringe-septum techniques under an atmosphere of either dry N_2 or dry argon unless otherwise noted. All glassware used in moisture sensitive reactions was flame-dried or oven dried at 136 ºC overnight and cooled in a desiccator prior to use. All chemicals were purchased from commercial suppliers and unless otherwise noted were used as received. Tetrahydrofuran was dried by distillation over sodium/benzophenone under an argon atmosphere. Dry methylene chloride, dry dichloroethane, dry toluene, and dry ethyl acetate were purified by distillation over calcium hydride under an argon atmosphere. Deuterated chloroform was filtered through an alumina plug prior to use. Reactions were monitored by TLC analysis (pre-coated silica gel 60 F_{254} plates, 250 µm layer thickness) and visualized by using UV lamp (254 nm) and/or a potassium permanganate solution (3 g $KMnO_4$ and 4 g K_2CO_3 in 4 mL 5% NaOH solution and 200 mL H_2O). Flash column chromatography was performed with 40-63 µm silica gel (Silicycle). Infrared spectra were measured on a Smiths Detection IdentifyIR FT-IR spectrometer (ATR). Melting points were uncorrected and determined using a Laboratory Devices Mel-Temp II. Mass spectrometry data were recorded on a VG-70-70 HF instrument. All NMR data was collected at room temperature in $CDCl_3$ on a 400, 500, or 600 MHz Bruker instrument. Chemical shifts (δ) are reported in parts

per million (ppm) with internal $CHCl_3$ (δ 7.26 ppm for ^1H and 77.00 ppm for ^{13}C). ^1H NMR data are reported as follows: chemical shift, multiplicity (s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bm = broad multiplet, dd = doublet of doublets, dt = doublet of triplets, td = triplet of doublets, qd = quartet of doublets, sep = septet, app t = apparent triplet), integration, and coupling constant(s) (*J*) in Hertz (Hz). ^{13}C NMR spectra were run using a proton-decoupled pulse sequence.

3.2 EXPERIMENTAL PROCEDURES

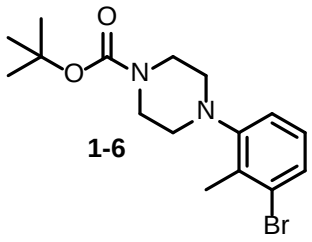
3.2.1 Chapter 1 Experimental



1-5

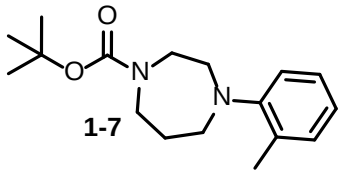
***tert*-Butyl 4-(4-methylpyridin-3-yl)piperazine-1-carboxylate (1-5)**. A microwave vial filled with argon was charged with *tert*-butyl 1-piperazinecarboxylate (252 mg, 1.35 mmol), NaO-*t*-Bu (130 mg, 1.35 mmol), (*rac*)-BINAP (10.7 mg, 0.017 mmol, 1.5% mol), $Pd_2(dba)_3$ (5.2 mg, 0.0056 mmol, 1% mol in Pd), and degassed toluene (6.8 mL). 3-Bromo-4-methylpyridine (200 mg, 1.13 mmol) was added, and the mixture was heated in sealed vial under argon at 110 °C for 40 h. After cooling to room temperature, the mixture was diluted with CH_2Cl_2, filtered over a Celite pad, and the volatiles were removed under reduced pressure. Chromatography on SiO_2 (70% EtOAc/hexanes) afforded **1-5** as a yellow oil (170 mg, 0.613 mmol, 54%): IR (ATR) 2976, 2861, 2819, 1693, 1591, 1454, 1416, 1365, 1250, 1235, 1169, 1124, 1037, 1001, 926, 830 cm^{-1};

^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.24 (s, 1 H), 8.21 (d, *J* = 4.8 Hz, 1 H), 7.09 (d, *J* = 4.8 Hz, 1 H), 3.58 (t, *J* = 4.8 Hz, 4 H), 2.93 (t, *J* = 4.8 Hz, 4 H), 2.31 (s, 3 H), 1.49 (s, 9 H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 154.7, 147.2, 144.8, 141.4 (2 C), 125.7, 79.9, 51.5, 28.4, 17.3; HR-LCMS (+ESI) *m/z* calcd for $C_{15}H_{24}N_3O_2$ (M+H) 278.1863, found 278.1857.



1-6

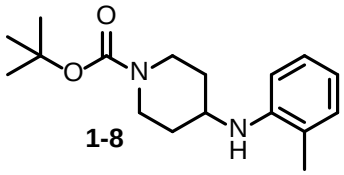
***tert*-Butyl 4-(3-bromo-2-methylphenyl)piperazine-1-carboxylate (1-6)**.[32b] A microwave vial filled with argon was charged with *tert*-butyl 1-piperazinecarboxylate (154 mg, 0.825 mmol), NaO-*t*-Bu (95.2 mg, 0.990 mmol), (*rac*)-BINAP (39.3 mg, 0.0619 mmol, 7.5% mol), $Pd_2(dba)_3$ (19.2 mg, 0.0206 mmol, 2.5% mol in Pd), and degassed toluene (2.1 mL). 2-Bromo-6-iodotoluene (121 uL, 0.825 mmol) was then added, and the mixture was heated in sealed vial under argon at 80 °C for 19 h. After cooling to room temperature, the mixture was diluted with CH_2Cl_2, filtered over a Celite pad, and volatiles were evaporated under reduced pressure. Chromatography on SiO_2 (10% EtOAc/hexanes) afforded **1-6** as a yellow oil (95 mg, 0.267 mmol, 32%): ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.30 (d, *J* = 8.0 Hz, 1 H), 7.02 (t, *J* = 8.0 Hz, 1 H), 6.95 (d, *J* = 7.5 Hz, 1 H), 3.57 (m, 4 H), 2.83 (t, *J* = 4.5 Hz, 4 H), 2.40 (s, 3 H), 1.49 (s, 9 H).



1-7

***tert*-Butyl 4-(*o*-tolyl)-1,4-diazepane-1-carboxylate (1-7)**. A microwave vial filled with argon was charged with 1-Boc-homopiperazine (223 mg, 1.10 mmol), NaO-*t*-Bu (116 mg, 1.20 mmol), (*rac*)-BINAP (47.8 mg, 0.0752 mmol, 7.5% mol), $Pd_2(dba)_3$ (23.3 mg, 0.0251 mmol, 2.5% mol

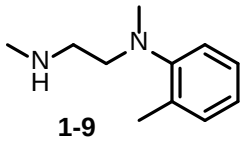
in Pd), and degassed toluene (2.8 mL). 2-Bromotoluene (175 mg, 1.00 mmol) was added, and the mixture was heated in sealed vial under argon at 80 °C for 19 h. After cooling to room temperature, the mixture was diluted with CH_2Cl_2, filtered over a Celite pad, and the volatiles were evaporated under reduced pressure. Chromatography on SiO_2 (10% EtOAc/hexanes) afforded **1-7** as a yellow oil (139 mg, 0.479 mmol, 48%): IR (ATR) 2973, 2828, 1689, 1598, 1491, 1457, 1411, 1364, 1233, 1215, 1156, 1122, 878, 761, 725 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$, rt, rotamers) δ 7.16 (d, *J* = 6.0 Hz, 1 H), 7.12 (d, *J* = 6.0 Hz, 1 H), 7.04 (d, *J* = 7.5 Hz, 1 H), 6.95 (t, *J* = 7.0 Hz, 1 H), 3.62-3.52 (m, 4 H), 3.12-3.04 (m, 4 H), 2.31 (s, 3 H), 2.00-1.88 (m, 2 H), 1.49 (s, 9 H); ^{13}C-NMR (100 MHz, $CDCl_3$, rt, rotamers) δ 155.6, 155.5, 153.9, 153.8, 132.9, 130.9, 126.5, 123.1, 120.8 (2 C), 79.3, 56.2, 56.0, 55.5, 55.2, 48.4, 48.0, 46.2, 45.4, 29.0, 28.9, 28.5, 18.5; HR-LCMS (+ESI) *m/z* calcd for $C_{17}H_{27}N_2O_2$ (M+H) 291.2067, found 291.2062.



1-8

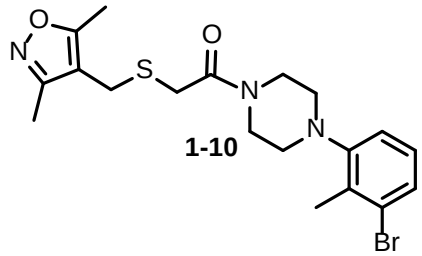
***tert*-Butyl 4-(*o*-tolylamino)piperidine-1-carboxylate (1-8)**. A microwave vial filled with nitrogen was charged with the 4-amino-1-Boc-piperidine (409 mg, 2.04 mmol), NaO-*t*-Bu (202 mg, 2.04 mmol), (*rac*)-BINAP (16.2 mg, 0.0255 mmol, 1.5% mol), $Pd_2(dba)_3$ (7.8 mg, 0.0085 mmol, 1% mol in Pd), and degassed toluene (3.0 mL). 2-Bromotoluene (297 mg, 1.70 mmol) was added, and the mixture was heated in sealed vial under argon at 110 °C for 24 h. After cooling to room temperature, the mixture was diluted with CH_2Cl_2, filtered over a Celite pad, and the volatiles were evaporated under reduced pressure. Chromatography on SiO_2 (20% EtOAc/hexanes) afforded **1-8** as an off-white solid (360 mg, 1.24 mmol, 73%): Mp 85.9-87.5 ºC; IR (ATR) 3388, 2926, 2858, 1632, 1604, 1586, 1513, 1448, 1313, 1267, 1239, 1267, 1195, 1129, 986, 911, 747 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.11 (app t, *J* = 7.6 Hz, 1 H), 7.06 (d, *J* = 7.2

Hz, 1 H), 6.67-6.61 (m, 2 H), 4.04 (bs, 2 H), 3.52-3.41 (m, 1 H), 3.37-3.32 (m, 1 H), 2.96 (app t, *J* = 11.6 Hz, 2 H), 2.12 (s, 3H), 2.10-2.02 (m, 2 H), 1.47 (s, 9 H), 1.42-1.31 (m, 2 H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 154.7, 144.6, 130.4, 127.1, 121.9, 116.9, 110.3, 79.5, 49.8, 42.6, 32.5, 28.4, 17.5; HR-LCMS (+ESI) *m/z* calcd for $C_{17}H_{27}N_2O_2$ (M+H) 291.2067, found 291.2062.



1-9

***N,N'*-Dimethyl-*N*-(*o*-tolyl)ethane-1,2-diamine (1-9)**.[89] A microwave vial filled with argon was charged with the *N,N'*-dimethylethylenediamine (180 mg, 2.04 mmol), NaO-*t*-Bu (202 mg, 2.04 mmol), (*rac*)-BINAP (16.2 mg, 0.026 mmol, 1.5% mol), $Pd_2(dba)_3$ (7.8 mg, 0.0085 mmol, 1% mol in Pd), and degassed toluene (10.2 mL). 2-Bromotoluene (297 mg, 1.70 mmol) was then added, and the mixture was heated in sealed vial under argon at 110 °C for 24 h. After cooling to room temperature, the mixture was diluted with CH_2Cl_2, filtered over a Celite pad, and the volatiles were evaporated under reduced pressure. Chromatography on basic Al_2O_3 (5% MeOH/CH_2Cl_2) afforded **1-9** as a brown oil (50.8 mg 0.285 mmol, 17%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.16 (t, *J* = 7.6 Hz, 2 H), 7.08 (d, *J* = 7.6 Hz, 1 H), 6.98 (d, *J* = 7.2 Hz, 1 H), 3.05 (t, *J* = 6.4 Hz, 2 H), 2.71 (t, *J* = 6.4 Hz, 2 H), 2.65 (s, 3 H), 2.43 (s, 3 H), 2.32 (s, 3 H), 1.36 (bs, 1 H); HR-LCMS (+ESI) *m/z* calcd for $C_{11}H_{19}N_2$ (M+H) 179.1543, found 179.1541.

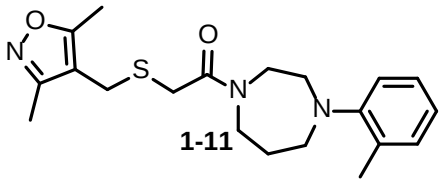


1-10

1-(4-(3-Bromo-2-methylphenyl)piperazin-1-yl)-2-(((3,5-dimethylisoxazol-4-yl)methyl)thio) ethan-1-one (1-10). A solution of **1-6** (77.0 mg, 0.217 mmol) in THF (0.3 mL) was cooled to 0

ºC, treated with 4N HCl in dioxanes (1.3 mL) and stirred at 0 ºC for 2 h. The yellow solid was filtered off from the solution, washed with ether, dried and carried crude on to the next step.

To a solution of ([(3,5-dimethylisoxazol-4-yl)methyl]thio)acetic acid (**1-1**, 35.0 mg, 0.174 mmol) in CH_2Cl_2 (1.7 mL) was added 4-(3-bromo-2-methylphenyl)piperazine HCl salt and triethylamine (121 µL, 0.870 mmol). The mixture was cooled to 0 °C and T3P (50% solution in EtOAc, 184 µL, 0.261 mmol) was added. The reaction mixture was allowed to warm to room temperature, stirred for 20 h, diluted with CH_2Cl_2, and washed with sat. NH_4Cl solution, sat. $NaHCO_3$ solution, and brine. The organic portion was dried (Na_2SO_4), filtered, and concentrated to give a brown oil. The crude material was purified by chromatography on SiO_2 (60% EtOAc/hexanes, base washed with 0.1% NEt_3 prior to use) to give **1-10** as a clear colorless oil (76.2 mg, 0.174 mmol, 100%, 100% pure by ELSD): IR (ATR) 2921, 2820, 1637, 1587, 1562, 1460, 1428, 1282, 1237, 1195, 1136, 1038, 994, 913, 780, 731, 714 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.32 (dd, *J* = 0.8, 7.6 Hz, 1 H), 7.03 (t, *J* = 8.0 Hz, 1 H), 6.94 (dd, *J* = 0.8, 8.0 Hz, 1 H), 3.77 (bs, 2 H), 3.63 (s, 2 H), 3.63-3.57 (m, 2 H), 3.23 (s, 2 H), 2.90 (t, *J* = 4.4 Hz, 2 H), 2.88-2.83 (m, 2 H), 2.43 (s, 3 H), 2.40 (s, 3 H), 2.31 (s, 3 H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 167.6, 166.8, 159.7, 152.2, 132.9, 128.1, 127.4, 126.6, 118.3, 109.7, 52.1, 51.8, 46.8, 42.2, 32.1, 23.8, 18.2, 11.1, 10.2; HR-LCMS (+ESI) *m/z* calcd for $C_{19}H_{25}N_3O_2BrS$ (M+H) 438.0845, found 438.0831.

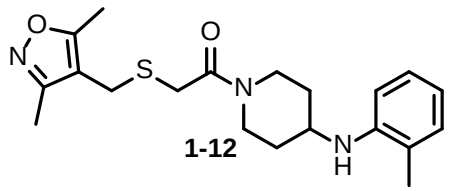


1-11

2-(((3,5-Dimethylisoxazol-4-yl)methyl)thio)-1-(4-(*o*-tolyl)-1,4-diazepan-1-yl)ethan-1-one (1-11). A solution of **1-7** (75.0 mg, 0.258 mmol) in THF (0.3 mL) was cooled to 0 ºC, treated with

4N HCl in dioxanes (1.6 mL) and stirred at 0 ºC for 2 h. The solution was concentrated *in vacuo* and the yellow solid was precipitated in ether, filtered off from the solution, washed with ether, dried and carried crude on to the next step.

To a solution of **1-1** (46.0 mg, 0.229 mmol) in CH_2Cl_2 (2.3 mL) was added 4-(*o*-tolyl)-1,4-diazepane HCl salt and triethylamine (159 μL, 1.14 mmol). The mixture was cooled to 0 °C and T3P (50% solution in EtOAc, 242 μL, 0.343 mmol) was added. The reaction mixture was allowed to warm to room temperature, stirred for 20 h, diluted with CH_2Cl_2, and washed with sat. NH_4Cl solution, sat. $NaHCO_3$ solution, and brine. The organic portion was dried (Na_2SO_4), filtered, and concentrated to give a brown oil. The crude material was purified by chromatography on SiO_2 (60% EtOAc/hexanes, base washed with 0.1% NEt_3 prior to use) to give **1-11** as a clear colorless oil (85.4 mg, 0.229 mmol, 100%, 100% pure by ELSD): IR (ATR) 2945, 2825, 1634, 1598, 1491, 1447, 1423, 1215, 1194, 1136, 915, 762, 726 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, rt, rotamers) δ 7.20 (app. d, *J* = 7.6 Hz, 1 H), 7.17 (app. t, *J* = 7.6 Hz, 1 H), 7.05 (app. d, *J* = 7.6 Hz, 1 H), 7.01 (app. td, *J* = 2.0, 7.2 Hz, 1 H), 3.82-3.78 (m, 2 H), 3.71-3.65 (m, 4 H), 3.24-3.20 (m, 3 H), 3.15 (t, *J* = 5.2 Hz, 1 H), 3.12-3.07 (m, 2 H), 2.46 (app s, 3 H), 2.32 (2 × s, 6 H), 2.04 (sept, *J* = 6.0 Hz, 2 H); ^{13}C-NMR (125 MHz, $CDCl_3$, rt, rotamers) δ 168.9, 168.8, 166.9, 166.8, 159.8 (2 C), 153.4, 153.3, 132.9 (2 C), 131.1 (2 C), 126.7, 126.6, 123.6, 123.4, 120.8, 120.7, 109.9, 56.4, 55.8, 55.5, 54.9, 50.1, 47.6, 47.2, 44.9, 32.2, 32.0, 29.5, 28.2, 23.7, 18.5 (2 C), 11.1, 10.2 (2 C); HR-LCMS (+ESI) *m/z* calcd for $C_{20}H_{28}N_3O_2S$ (M+H) 374.1897, found 374.1883.

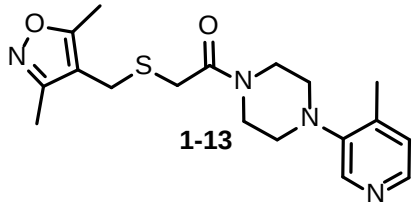


1-12

2-(((3,5-Dimethylisoxazol-4-yl)methyl)thio)-1-(4-(*o*-tolylamino)piperidin-1-yl)ethan-1-one (1-12). A solution of **1-8** in MeOH (0.5 mL) was cooled to 0 ºC, treated with 4N HCl in dioxanes (3.0 mL) and stirred at 0 ºC for 30 min and at room temperature for 30 min. The white solid was filtered off from the solution, washed with ether, dried and carried crude on to the next step.

To a solution of **1-1** (75.8 mg, 0.377 mmol) in CH_2Cl_2 (3.8 mL) was added 4-(*o*-tolylamino)piperidine HCl salt (103 mg, 0.452 mmol) and triethylamine (262 µL, 1.88 mmol). The mixture was cooled to 0 °C and T3P (50% solution in EtOAc, 399 µL, 0.565 mmol) was added. The reaction mixture was allowed to warm to room temperature, stirred for 20 h, diluted with CH_2Cl_2, and washed with sat. NH_4Cl solution, sat. $NaHCO_3$ solution, and brine. The organic portion was dried (Na_2SO_4), filtered, and concentrated to give a brown oil. The crude material was purified by chromatography on SiO_2 (60% EtOAc/hexanes, base washed with 0.1% NEt_3 prior to use) to give **1-12** as a clear colorless oil (131 mg, 0.351 mmol, 93%, 100% pure by ELSD): IR (ATR) 3388, 2926, 2858, 1632, 1604, 1586, 1513, 1448, 1313, 1267, 1195, 1129, 986, 911, 747 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.12 (td, *J* = 1.2, 7.6 Hz, 1 H), 7.07 (d, *J* = 7.2 Hz, 1 H), 6.67 (td, *J* = 1.2, 7.6 Hz, 1 H), 6.63 (d, *J* = 8.0 Hz, 1 H), 4.48-4.40 (m, 1 H), 3.81-3.73 (m, 1 H), 3.62 (s, 2 H), 3.62-3.53 (m, 1 H), 3.36 (bs, 1 H), 3.28-3.18 (m, 3 H), 2.98-2.90 (m, 1 H), 2.43 (s, 3 H), 2.30 (s, 3 H), 2.20-2.10 (m, 5 H), 1.52-1.32 (m, 2 H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 167.4, 166.8, 159.7, 144.4, 130.5, 127.1, 122.1, 117.2, 110.2, 109.9, 49.5, 45.3, 40.9,

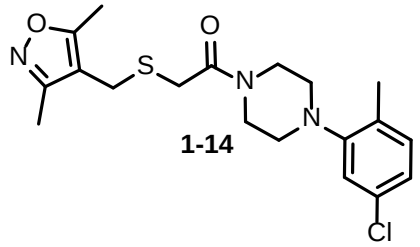
32.8, 32.2, 23.8, 17.5, 11.1, 10.2; HR-LCMS (+ESI) *m/z* calcd for $C_{20}H_{28}N_3O_2S$ (M+H) 374.1897, found 374.1891.



1-13

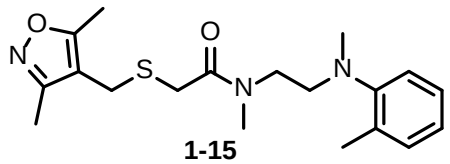
2-(((3,5-Dimethylisoxazol-4-yl)methyl)thio)-1-(4-(4-methylpyridin-3-yl)piperazin-1-yl) ethan-1-one (1-13). A solution of **1-5** in THF (0.3 mL) was cooled to 0 ºC, treated with 4N HCl in dioxanes (1.4 mL) and stirred at 0 ºC for 1 h and at room temperature for 2 h. The yellow solid was filtered off from the solution, washed with ether, dried and carried crude on to the next step. To a solution of **1-1** (45.0 mg, 0.224 mmol) in CH_2Cl_2 (2.2 mL) was added 1-(4-methylpyridin-3-yl)piperazine HCl salt and triethylamine (155 µL, 1.12 mmol). The mixture was cooled to 0 °C and T3P (50% solution in EtOAc, 237 µL, 0.335 mmol) was added. The reaction mixture was allowed to warm to room temperature, stirred for 20 h, diluted with CH_2Cl_2, and washed with sat. NH_4Cl solution, sat. $NaHCO_3$ solution, and brine. The organic portion was dried (Na_2SO_4), filtered, and concentrated to give a light brown oil. The crude material was purified by chromatography on SiO_2 (70% acetone/hexanes, base washed with 0.1% NEt_3 prior to use) to give **1-13** as an off white solid (61.0 mg, 0.169 mmol, 76%, 100% pure by ELSD): Mp 154.3-155.5 ºC; IR (ATR) 2918, 2821, 1638, 1591, 1438, 1279, 1235, 1202, 1139, 1139, 1033, 831, 736 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.24-8.21 (m, 2 H), 7.10 (d, *J* = 4.8 Hz, 1 H), 3.76 (t, *J* = 4.8 Hz, 2 H), 3.62 (s, 2 H), 3.60 (t, *J* = 4.8 Hz, 2 H), 3.23 (s, 2 H), 3.02 (t, *J* = 4.8 Hz, 2 H), 2.95 (t, *J* = 4.8 Hz, 2 H), 2.42 (s, 3 H), 2.32 (s, 3 H), 2.29 (s, 3 H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 167.6, 166.8 159.7, 146.7, 145.2, 141.5, 141.4, 125.8, 109.7, 51.5, 51.4, 46.8, 42.2, 32.0, 23.8,

17.3, 11.1, 10.2; HR-LCMS (+ESI) *m/z* calcd for $C_{18}H_{25}N_4O_2S$ (M+H) 361.1693, found 361.1680.



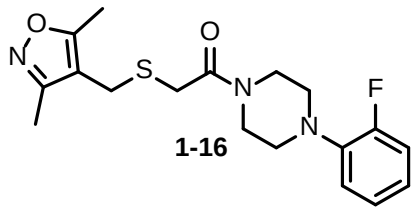
1-14

1-(4-(5-Chloro-2-methylphenyl)piperazin-1-yl)-2-(((3,5-dimethylisoxazol-4-yl)methyl)thio) ethan-1-one (1-14). To a solution of **1-1** (45.0 mg, 0.224 mmol) in CH_2Cl_2 (2.2 mL) was added 1-(5-chloro-2-methylphenyl)piperazine (56.5 mg, 0.268 mmol) and triethylamine (93.2 μL, 0.671 mmol). The mixture was cooled to 0 °C and T3P (50% solution in EtOAc, 237 μL, 0.335 mmol) was added. The reaction mixture was allowed to warm to room temperature, stirred for 20 h, diluted with CH_2Cl_2, and washed with sat. NH_4Cl solution, sat. $NaHCO_3$ solution, and brine. The organic portion was dried (Na_2SO_4), filtered, and concentrated to give a brown oil. The crude material was purified by chromatography on SiO_2 (50% EtOAc/hexanes, base washed with 0.1% NEt_3 prior to use) to give **1-14** as a clear colorless oil (88.1 mg, 0.224 mmol, 100%, 99.9% pure by ELSD): IR (ATR) 2921, 2818, 1635, 1592, 1489, 1438, 1270, 1224, 1195, 1148, 1039, 924, 910, 818, 728 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.11 (d, *J* = 8.0 Hz, 1 H), 6.99 (dd, *J* = 2.0, 8.0 Hz, 1 H), 6.94 (d, *J* = 2.4 Hz, 1 H), 3.76 (t, *J* = 4.4 Hz, 2 H), 3.63 (s, 2 H), 3.59 (t, *J* = 4.8 Hz, 2 H), 3.23 (s, 2 H), 2.91 (t, *J* = 4.8 Hz, 2 H), 2.86 (t, *J* = 4.8 Hz, 2 H), 2.43 (s, 3 H), 2.30 (s, 3 H), 2.27 (s, 3 H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 167.6, 166.8, 159.7, 151.7, 132.1, 131.8, 130.9, 123.7, 119.7, 109.7, 51.6, 51.5, 46.8, 42.2, 32.0, 23.7, 17.4, 11.1, 10.2; HR-LCMS (+ESI) *m/z* calcd for $C_{19}H_{25}N_3O_2ClS$ (M+H) 394.1351, found 394.1340.



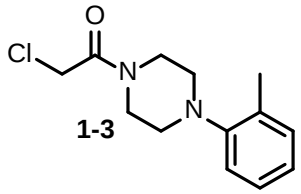
1-15

2-(((3,5-Dimethylisoxazol-4-yl)methyl)thio)-N-methyl-N-(2-(methyl(*o*-tolyl)amino)ethyl) acetamide (1-15). To a solution of **1-1** (60.8 mg, 0.302 mmol) in CH_2Cl_2 (3.0 mL) was added **1-9** (50.0 mg, 0.275 mmol) and triethylamine (115 μL, 0.825 mmol). The mixture was cooled to 0 °C and T3P (50% solution in EtOAc, 292 μL, 0.412 mmol) was added. The reaction was allowed to warm to room temperature, stirred for 20 h, diluted with CH_2Cl_2, and washed with sat. NH_4Cl solution, sat. $NaHCO_3$ solution, and brine. The organic portion was dried (Na_2SO_4), filtered, and concentrated to give a brown oil. The crude material was purified by chromatography on SiO_2 (60% EtOAc/hexanes, base washed with 0.1% NEt_3 prior to use) to give **1-15** as a light yellow oil (75.2 mg, 0.207 mmol, 75%, 99.6% pure by ELSD): IR (ATR) 2932, 2795, 1640, 1598, 1493, 1451, 1421, 1393, 1196, 1108, 1047, 766, 738 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$, rt, rotamers) δ 7.20-7.12 (m, 2 H), 7.08-6.94 (m, 2 H), 3.58 (d, *J* = 5.6 Hz, 2 H), 3.54 (t, *J* = 6.8 Hz, 1 H), 3.39 (t, *J* = 6.4 Hz, 1 H), 3.16-3.08 (m, 3 H), 2.97 and 2.95 (2 × s, 4 H), 2.71 and 2.67 (2 × s, 3 H), 2.38 (s, 3 H), 2.30 (s, 2 H), 2.28-2.25 (m, 4 H), (signals for these rotamers coalesced in DMSO-d6 @ 357 K); ^{13}C-NMR (125 MHz, $CDCl_3$, rt, rotamers) δ 169.2, 168.8, 166.7 (2 C), 159.7, 151.7, 150.8, 133.8, 132.9, 131.4, 131.2, 126.7, 126.5, 124.0, 123.2, 120.2, 119.9, 109.8, 53.9, 53.2, 48.4, 46.4, 43.3, 42.3, 36.7, 33.8, 32.4, 31.6, 23.7, 23.4, 18.2, 18.0, 11.0 (2 C), 10.1, (signals for these rotamers coalesced in DMSO-d6 @ 357 K); HR-LCMS (+ESI) *m/z* calcd for $C_{19}H_{28}N_3O_2S$ (M+H) 362.1897, found 362.1890.



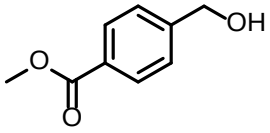
1-16

2-(((3,5-Dimethylisoxazol-4-yl)methyl)thio)-1-(4-(2-fluorophenyl)piperazin-1-yl)ethan-1-one (1-16). To a solution of **1-1** (75.8 mg, 0.377 mmol) in CH_2Cl_2 (3.8 mL) was added 1-(2-fluorophenyl)-piperazine (81.4 mg, 0.452 mmol) and triethylamine (262 μL, 1.88 mmol). The mixture was cooled to 0 °C and T3P (50% solution in EtOAc, 399 μL, 0.565 mmol) was added. The reaction was allowed to warm to room temperature, stirred for 20 h, diluted with CH_2Cl_2, and washed with sat. NH_4Cl solution, sat. $NaHCO_3$ solution, and brine. The organic portion was dried (Na_2SO_4), filtered, and concentrated to give a light brown oil. The crude material was purified by chromatography on SiO_2 (60% EtOAc/hexanes, base washed with 0.1% NEt_3 prior to use) to give **1-16** as a slight yellow oil (134 mg, 0.369 mmol, 98%, 100% pure by ELSD): IR (ATR) 2918, 2827, 1636, 1613, 1500, 1439, 1237, 1195, 1147, 1031, 909, 811, 753, 725 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.10-6.90 (m, 4 H), 3.79 (t, *J* = 5.2 Hz, 2 H), 3.63-3.59 (m, 4 H), 3.23 (s, 2 H), 3.10 (t, *J* = 4.8 Hz, 2 H), 3.05 (t, *J* = 5.2 Hz, 2 H), 2.28 (s, 3 H), 2.42 (s, 3 H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 167.5, 166.8, 159.7, 155.7 (d, *J* = 245.0 Hz), 139.4 (d, *J* = 8.8 Hz), 124.5 (d, *J* = 3.8 Hz), 123.3 (d, *J* = 8.8 Hz), 119.2 (d, *J* = 2.5 Hz), 116.3 (d, *J* = 20.0 Hz), 109.7, 50.7 (d, *J* = 2.5 Hz), 50.3 (d, *J* = 2.5 Hz), 46.6, 41.9, 32.1, 23.7, 11.1, 10.2; HR-LCMS (+ESI) *m/z* calcd for $C_{18}H_{23}N_3O_2FS$ (M+H) 364.1490, found 364.1474.



1-3

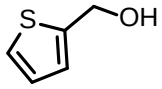
2-Chloro-1-(4-(*o*-tolyl)piperazin-1-yl)ethan-1-one (1-3).[90] To a solution of chloroacetyl chloride (0.698 g, 6.05 mmol) and potassium carbonate (1.14 g, 8.25 mmol) in THF (7.0 mL) was added 1-(2-methylphenyl)piperazine (1.00 g, 5.50 mmol) in THF (12.6 mL) at 0 °C. The reaction was gradually warmed to room temperature and stirred for 16 h. The crude was diluted with water, extracted with EtOAc (3 × 20 mL), and the combined organic extracts were washed sequentially with sat. $NaHCO_3$ solution, 0.1 N aqueous HCl, and brine, dried (Na_2SO_4), filtered and concentrated. The crude solid was filtered through a plug of SiO_2 (pretreated with 0.1% NEt_3 in 30% EtOAc/hexanes) and washed thoroughly with 30% EtOAc/hexanes to give **1-3** as an off white solid (1.37 g, 5.42 mmol, 99%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.22-7.16 (m, 2 H), 7.05-6.99 (m, 2 H), 4.12 (s, 2 H), 3.78 (t, *J* = 4.8 Hz, 2 H), 3.67 (t, *J* = 4.8 Hz, 2 H), 2.97 (t, *J* = 5.2 Hz, 2 H), 2.91 (t, *J* = 4.8 Hz, 2 H), 2.33 (s, 3 H).



1-17

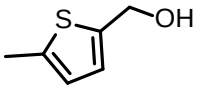
Methyl 4-(hydroxymethyl)benzoate (1-17).[91] 4-Hydroxymethylbenzoic acid (1.09 g, 6.57 mmol) was dissolved in MeOH (30 mL), acidified with conc. HCl (3 mL), and heated at reflux for 4 h. After cooling, the reaction mixture was neutralized with NEt_3 to pH 7 and evaporated. The crude residue was dissolved in CH_2Cl_2 (20 mL) and washed with a 5% $NaHCO_3$ solution (2 × 20 mL). The organic layer was separated, dried (Na_2SO_4), and evaporated to yield **1-17** as an off white solid (926 mg, 5.57 mmol, 78%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.04 (dd, *J* = 1.6, 8.4

Hz, 2 H), 7.44 (app. d, *J* = 8.4 Hz, 2 H), 4.78 (d, *J* = 6.0 Hz, 2 H), 3.92 (s, 3 H), 1.74 (t, *J* = 6.0 Hz, 1 H).



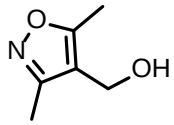
1-18

Thiophen-2-ylmethanol (1-18).[92] To a solution of 2-thiophenecarbaldehyde (0.500 g, 4.46 mmol) in MeOH (41 mL) at 0 ºC was added $NaBH_4$ (0.506 g, 13.4 mmol). The mixture was stirred for 3 h, after which water (50 mL) was added. After 30 min, the MeOH was evaporated and the reaction mixture was extracted with CH_2Cl_2 (3 × 50 mL), dried (Na_2SO_4), and concentrated. The crude brown oil, **1-18**, was used without further purification (0.468 g, 4.10 mmol, 92%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.29-7.26 (m, 1 H), 7.02-6.97 (m, 2 H), 4.83 (d, *J* = 4.4 Hz, 2 H), 1.81 (m, 1 H).



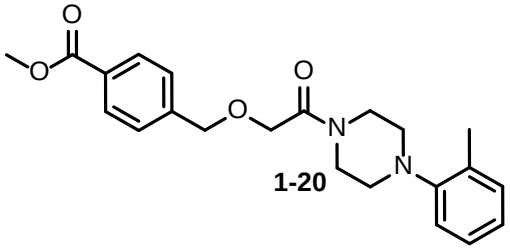
1-19

(5-Methylthiophen-2-yl)methanol (1-19).[93] To a solution of 5-methyl-2-thiophenecarbaldehyde (0.500 g, 3.96 mmol) in MeOH (36 mL) at 0 ºC was added $NaBH_4$ (0.450 g, 11.9 mmol). The reaction mixture was stirred for 3 h, water (50 mL) was added, and it was stirred for 30 min. The MeOH was evaporated and the crude residue was extracted with CH_2Cl_2 (3 × 50 mL), dried (Na_2SO_4), and concentrated. The brown oil, **1-19**, was used without further purification (508 mg, 3.96 mmol, 100%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.79 (app. d, *J* = 3.2 Hz, 1 H), 6.62-6.61 (m, 1 H), 4.73 (d, *J* = 6.0 Hz, 2 H), 2.47 (s, 3 H), 1.69 (bs, 1 H).



1-2

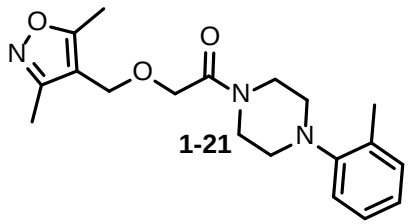
(3,5-Dimethylisoxazol-4-yl)methanol (1-2).[94] To a solution of 3,5-dimethylisoxazole-4-carboxylic acid (1.60 g, 11.3 mmol) in THF (69.4 mL) at 0 ºC was added a 2 M solution of $LiAlH_4$ in THF (5.60 mL) dropwise. The solution was allowed to warm to room temperature and stirred overnight. The resultant slurry was transferred to a 500 mL erlenmeyer flask and sodium sulfate decahydrate was added until the foaming subsided. Celite (2.3 g) was added and the slurry was filtered, washed with CH_2Cl_2 (75 mL), and the filtrate was concentrated to give **1-2** as a clear colorless oil (1.14 g, 8.97 mmol, 80%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 4.46 (s, 2 H), 2.38 (s, 3 H), 2.29 (s, 3 H), 1.63 (bs, 1 H).



1-20

Methyl 4-((2-oxo-2-(4-(*o*-tolyl)piperazin-1-yl)ethoxy)methyl)benzoate (1-20). A solution of **1-17** (69.0 mg, 0.415 mmol) in THF (0.50 mL) was cooled to 0 ºC and a 60% dispersion in mineral oil of NaH (17.4 mg, 0.435 mmol) was added. The resultant slurry was stirred at 0 ºC for 15 min, then room temperature for 15 min, cooled to 0 ºC and a solution of **1-3** (100 mg, 0.396 mmol) in THF (0.29 mL) was added dropwise. The mixture was allowed to warm to room temperature, stirred for 24 h, quenched with water (1 mL), diluted with EtOAc (15 mL) and water (5 mL), and extracted with EtOAc (2 × 15 mL). The combined organic layers were washed with brine, dried (Na_2SO_4) and concentrated. The residue was purified by chromatography on SiO_2 (45% EtOAc/hexanes, column base washed with 0.1% NEt_3 prior to use) to yield **1-20** as a

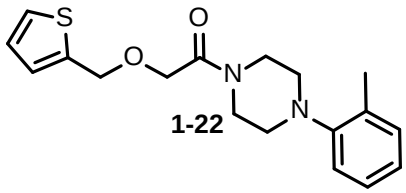
slight yellow oil (51.9 mg, 0.136 mmol, 34%, 100% pure by ELSD): IR (ATR) 2950, 1719, 1650, 1493, 1436, 1276, 1225, 1109, 1020, 974, 759, 724 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.04 (d, *J* = 8.0 Hz, 2 H), 7.45 (d, *J* = 8.0 Hz, 2 H), 7.21-7.14 (m, 2 H), 7.11 (t, *J* = 7.2 Hz, 1 H), 6.95 (d, *J* = 7.6 Hz, 1 H), 4.69 (s, 2 H), 4.27 (s, 2 H), 3.92 (s, 3 H), 3.77 (m, 2 H), 3.63 (app. t, *J* = 4.4 Hz, 2 H), 2.91-2.85 (m, 4 H), 2.32 (s, 3 H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 167.5, 166.9, 150.7, 142.5, 132.7, 131.2, 129.8, 129.7, 127.5, 126.7, 123.8, 119.2, 72.6, 69.7, 52.1, 52.0, 51.7, 45.6, 42.4, 17.8; HR-LCMS (+ESI) *m/z* calcd for $C_{22}H_{27}N_2O_4$ (M+H) 383.1965, found 383.1963.



1-21

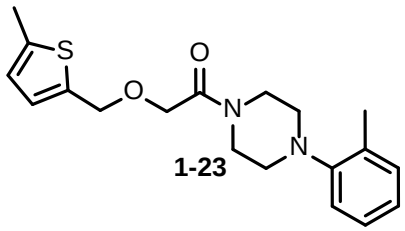
2-((3,5-Dimethylisoxazol-4-yl)methoxy)-1-(4-(*o*-tolyl)piperazin-1-yl)ethan-1-one (1-21). A solution of **1-2** (30.2 mg, 0.237 mmol) in THF (0.48 mL) was cooled to 0 ºC and a 60% dispersion in mineral oil of NaH (19.0 mg, 0.475 mmol) was added. The resultant slurry was stirred at 0 ºC for 30 min and **1-3** (60.0 mg, 0.237 mmol) was added. The mixture was allowed to warm to room temperature, stirred for 20 h, quenched with brine (1 mL), diluted with EtOAc (15 mL) and brine (5 mL), and extracted with EtOAc (2 × 15 mL). The combined organic layers were dried (Na_2SO_4), and concentrated. The residue was purified by chromatography on SiO_2 (60% EtOAc/hexanes) to yield **1-21** as a light yellow oil (73.5 mg, 0.214 mmol, 90%, 100% pure by ELSD): IR (ATR) 2918, 2817, 1645, 1599, 1493, 1443, 1369, 1273, 1225, 1116, 1030, 977, 764, 725 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.22-7.15 (m, 2 H), 7.02 (td, *J* = 1.2, 7.6 Hz, 1 H), 6.97 (app. d, *J* = 8.0 Hz, 1 H), 4.41 (s, 2 H), 4.17 (s, 2 H), 3.77 (m, 2 H), 3.59 (t, *J* = 4.8 Hz, 2 H), 2.89 (app. t, *J* = 3.6 Hz, 4 H), 2.41 (s, 3 H), 2.32 (s, 3 H), 2.30 (s, 3 H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 167.8, 167.5, 159.8, 150.7, 132.7, 131.2, 126.7, 123.9, 119.2, 110.5, 68.7, 61.7, 52.1,

51.7, 45.6, 42.3, 17.8, 11.1, 10.1; HR-LCMS (+ESI) *m/z* calcd for $C_{19}H_{26}N_3O_3$ (M+H) 344.1969, found 344.1960.



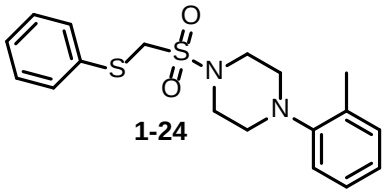
1-22

2-(Thiophen-2-ylmethoxy)-1-(4-(*o*-tolyl)piperazin-1-yl)ethan-1-one (1-22). A solution of **1-18** (32.5 mg, 0.285 mmol) in THF (0.48 mL) was cooled to 0 ºC and a 60% dispersion in mineral oil of NaH (19.0 mg, 0.475 mmol) was added. The resultant slurry was stirred at 0 ºC for 30 min and **1-3** (60.0 mg, 0.237 mmol) was added. The mixture was allowed to warm to room temperature, stirred for 18 h, quenched with brine (1 mL), diluted with EtOAc (15 mL) and brine (5 mL), and extracted with EtOAc (2 × 15 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified twice by chromatography on SiO_2 (50% EtOAc/hexanes then 24% acetone/hexanes) to yield **1-22** as a light yellow oil (54.9 mg, 0.166 mmol, 70%, 100% pure by ELSD): IR (ATR) 3067, 2911, 2857, 2817, 1646, 1599, 1493, 1441, 1274, 1224, 1150, 1113, 1030, 976, 763, 722, 705 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.32 (d, *J* = 5.2 Hz, 1 H), 7.22-7.14 (m, 2 H), 7.08-6.95 (m, 4 H), 4.80 (s, 2 H), 4.23 (s, 2 H), 3.76 (m, 2 H), 3.62 (t, *J* = 4.4 Hz, 2 H) 2.89 (t, *J* = 4.8 Hz, 4 H), 2.32 (s, 3 H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 167.5, 150.8, 139.6, 132.7, 131.1, 127.3, 126.8, 126.6, 126.4, 123.7, 119.2, 69.0, 67.3, 52.0, 51.7, 45.7, 42.3, 17.8; HR-LCMS (+ESI) *m/z* calcd for $C_{18}H_{23}N_2O_2S$ (M+H) 331.1475, found 331.1465.



1-23

2-((5-Methylthiophen-2-yl)methoxy)-1-(4-(*o*-tolyl)piperazin-1-yl)ethan-1-one (1-23). A solution of **1-19** (36.5 mg, 0.285 mmol) in THF (0.48 mL) was cooled to 0 ºC and was treated with a 60% dispersion in mineral oil of NaH (19.0 mg, 0.475 mmol). The resultant slurry was stirred at 0 ºC for 30 min and **1-3** (60.0 mg, 0.237 mmol) was added. The mixture was allowed to warm to room temperature, stirred for 12.5 h, quenched with brine (1 mL), diluted with EtOAc (15 mL) and brine (5 mL), and extracted with EtOAc (2 × 15 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by chromatography on SiO_2 (24% acetone/hexanes) to yield **1-23** as a light yellow oil (28.9 mg, 0.0839 mmol, 35%, 100% pure by ELSD): IR (ATR) 2916, 2857, 2817, 1648, 1599, 1493, 1443, 1274, 1224, 1152, 1113, 1030, 975, 802, 763, 724 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.22-7.14 (m, 2 H), 7.05-6.95 (m, 2 H), 6.83 (d, *J* = 3.2 Hz, 1 H), 6.64-6.61 (m, 1 H), 4.70 (s, 2 H), 4.21 (s, 2 H), 3.76 (m, 2 H), 3.63 (t, *J* = 4.8 Hz, 2 H), 2.89 (t, *J* = 5.2 Hz, 4 H), 2.47 (d, *J* = 0.8 Hz, 3 H), 2.32 (s, 3 H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 167.6, 150.8, 141.2, 137.2, 132.7, 131.1, 127.6, 126.6, 124.8, 123.7, 119.2, 68.8, 67.5, 52.0, 51.7, 45.7, 42.3, 17.8, 15.4; HR-LCMS (+ESI) *m/z* calcd for $C_{19}H_{25}N_2O_2S$ (M+H) 345.1631, found 345.1623.



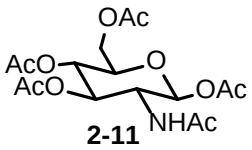
1-24

1-(((Phenylthio)methyl)sulfonyl)-4-(*o*-tolyl)piperazine (1-24). To a solution of 1-(2-methylphenyl)piperazine (0.500 g, 2.75 mmol) and triethylamine (0.390 mL, 2.75 mmol) in

CH_2Cl_2 (9.8 mL) at 0 ºC was added chloromethanesulfonyl chloride (0.460 g, 3.03 mmol). The reaction mixture was stirred at 0 ºC and was gradually warmed to room temperature. After 14 h, the reaction mixture was quenched with sat. NH_4Cl solution (3 mL), and extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed water (2 × 10 mL) and brine (10 mL), dried (Na_2SO_4), filtered, and concentrated. The crude solid was filtered through a plug of SiO_2 (pretreated with 0.1% NEt_3 in 30% EtOAc/hexanes) and washed thoroughly with 30% EtOAc/hexanes to give **1-4** (676 mg, 2.34 mmol, 85%) as an orange solid: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 7.21-7.17 (m, 2 H), 7.05-7.00 (m, 2 H), 4.57 (s, 2 H), 3.63 (t, *J* = 4.8 Hz, 4 H), 2.98 (t, *J* = 5.2 Hz, 4 H), 2.31 (s, 3 H).

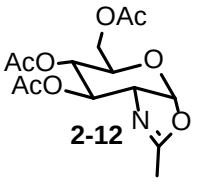
A solution of thiophenol (61.0 mg, 0.554 mmol), **1-4** (40.0 mg, 0.139 mmol) and Cs_2CO_3 (90.3 mg, 0.277 mmol) in DMF (0.28 mL) were stirred at 80 ºC for 2 d. The crude reaction mixture was diluted with brine (10 mL) and EtOAc (20 mL), and washed with brine (2 × 10 mL). The organic layer was dried (Na_2SO_4) and concentrated. The residue was purified by chromatography on SiO_2 (20% EtOAc/hexanes) yielded **1-24** as a clear colorless oil (25.7 mg, 0.0709 mmol, 51%): IR (ATR) 3054, 2918, 2823, 1598, 1581, 1493, 1440, 1342, 1324, 1262, 1225, 1153, 1112, 1070, 954, 765, 744, 725, 691 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 7.59 (d, *J* = 7.5 Hz, 2 H), 7.39-7.30 (m, 3 H), 7.21-7.14 (m, 2 H), 7.02 (t, *J* = 7.5 Hz, 1 H), 6.98 (d, *J* = 8.0 Hz, 1 H), 4.33 (s, 2 H), 3.51 (t, *J* = 4.5 Hz, 4 H), 2.92 (t, *J* = 4.5 Hz, 4 H), 2.28 (s, 3 H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 150.7, 133.4, 132.7, 131.2, 131.1, 129.4, 128.1, 126.7, 123.9, 119.4, 54.2, 51.8, 46.8, 17.7; HR-LCMS (+ESI) *m/z* calcd for $C_{18}H_{23}N_2O_2S_2$ (M+H) 363.1195, found 363.1190.

3.2.2 Chapter 2 Experimental



2-11

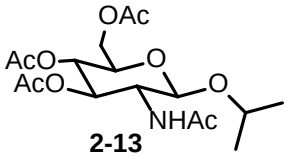
2-Acetamido-2-deoxy-1,3,4,6-tetra-O-acetyl-β-D-glucopyranose (2-11).[84] Acetic anhydride (7.76 mL, 82.3 mmol) was added to a 100 mL flask and cooled to 0 ºC. *N*-Acetyl-D-glucosamine (0.692 g, 3.13 mmol) and montmorillonite K-10 (2.40 g) were added sequentially over 15 min and the reaction mixture was stirred at room temperature for 24 h. The reaction mixture was filtered through a pad of Celite®, rinsed with methyl acetate (100 mL), and the filtrate was concentrated. The resulting orange residue was twice recrystallized from hot methanol, filtered, and the crystals were washed with ice-cold diethyl ether (3 × 2 mL) to afford **2-11** as a white crystalline solid (350 mg, 0.900 mmol, 29%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.69 (d, *J* = 8.8 Hz, 1 H), 5.41 (d, *J* = 9.6 Hz, 1 H), 5.17-5.09 (m, 2 H), 4.33-4.25 (m, 2 H), 4.13 (dd, *J* = 2.4, 12.6 Hz, 1 H), 3.78 (ddd, *J* = 2.4, 4.4, 9.6 Hz, 1 H), 2.12 (s, 3 H), 2.09 (s, 3 H), 2.05 (s, 3 H), 2.04 (s, 3 H). Note: This compound was commercially available through Alfa Aesar (Cat. No. = L09020) and was later purchased for the syntheses of **2-12**.



2-12

2,3-Dihydrooxazole-3,4,6-tri-O-acetyl-α-D-glucopyranoside (2-12).[85a,85b,86a] A solution of **2-11** (200 mg, 0.514 mmol) in dichloroethane (14.3 mL) in a 50 mL round bottom flask was treated with TMSOTf (0.100 mL, 0.539 mmol). The reaction mixture was stirred at 50 ºC for 35 min, cooled, and NEt_3 (0.220 mL, 1.54 mmol) was added. After stirring at room temperature for 10

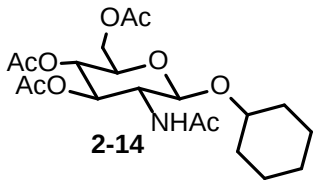
min, the solution was passed through a short plug of silica and washed with dichloromethane (25 mL) and ethyl acetate (15 mL). The solvent was removed under reduced pressure and the crude orange oil was purified by chromatography on SiO_2 (100% EtOAc, base washed with 1% NEt_3) giving **2-12** (158 mg, 0.478 mmol, 93%) as a colorless oil: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.97 (d, *J* = 7.6 Hz, 1 H), 5.27 (t, *J* = 2.4 Hz, 1 H), 4.93 (app. d, *J* = 9.2 Hz, 1 H), 4.18-4.13 (m, 3 H), 3.61 (quint., *J* = 4.4 Hz, 1 H), 2.12 (s, 3 H), 2.10 (s, 3 H), 2.09 (d, *J* = 1.6 Hz, 3 H), 2.08 (s, 3 H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 170.6, 169.5, 169.2, 166.7, 99.4, 70.4, 68.4, 67.5, 65.0, 63.3, 20.9, 20.8, 20.7, 14.0.



2-13

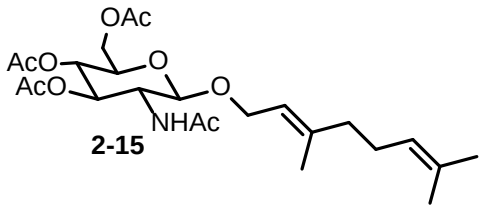
Isopropyl 3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranoside (2-13).[68] A solution of **2-12** (0.142 g, 0.424 mmol) and anhydrous $CuCl_2$ (58.0 mg, 0.431 mmol) in anhydrous $CHCl_3$ (0.82 mL) in a 2-5 mL conical sealed vessel under argon atmosphere was treated with anhydrous 2-propanol (0.134 mL, 1.75 mmol). The reaction mixture was heated at 62 ºC for 2 h. After cooling to room temperature, the mixture was diluted with acetone (15 mL) and sat. $NaHCO_3$ solution (7 mL), filtered through a short plug of Celite® with acetone (20 mL). The filtrate was concentrated, coevaporated with toluene and the crude residue was shaken with $CHCl_3$ and weakly acidic ion-exchange resin (Amberlite IRC-86, ca 1.5 g). The solution was filtered, the solvent was reduced under vacuum, and the residue was chromatographed on SiO_2 (75% EtOAc/hexanes) to give **2-13** (146 mg, 0.375 mmol, 87%) as a colorless solid: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.51 (d, *J* = 8.4 Hz, 1 H), 5.41 (dd, *J* = 9.2, 10.4 Hz, 1 H), 5.03, (app. t, *J* = 10.0 Hz, 1 H), 4.84 (d, *J* = 8.4 Hz, 1 H), 4.24 (dd, *J* = 5.2, 12.0 Hz, 1 H), 4.11 (dd, *J* = 2.4, 12.0 Hz, 1 H), 3.93 (sept, *J* = 6 Hz, 1 H), 3.73-3.60 (m, 2 H), 2.07 (s, 3 H), 2.02 (s, 3 H), 2.02 (s, 3 H),

1.94 (s, 3 H), 1.22 (d, J = 6.0 Hz, 3 H), 1.13 (d, J = 6.4 Hz, 3 H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 170.8, 170.7, 170.2, 169.5, 99.1, 72.6, 72.1, 71.5, 68.9, 62.3, 55.5, 23.3, 23.2, 21.9, 20.8, 20.7, 20.6; HRMS (+ESI-TOF) calcd for $C_{17}H_{28}NO_9$ (M+H) 390.1764, found 390.1742.



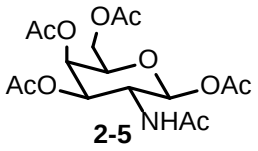
2-14

Cyclohexyl 3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranoside (2-14).[68,95] A solution of **2-12** (0.099 g, 0.301 mmol) and anhydrous $CuCl_2$ (41.0 mg, 0.301 mmol) in anhydrous $CHCl_3$ (0.68 mL) in a 2-5 mL conical sealed vessel under argon atmosphere was treated with cyclohexanol (0.130 mL, 1.22 mmol). The reaction mixture was heated at 62 ºC for 2.5 h, cooled to room temperature, diluted with ethyl acetate (15 mL), washed with 1 N HCl (2 × 9 mL), a sat. $NaHCO_3$ solution (1 × 10 mL), and brine (1 × 10 mL), dried ($MgSO_4$), evaporated, and purified by chromatography on SiO_2 (75% EtOAc/hexanes) to give **2-14** (112 mg, 0.260 mmol, 86%) as a colorless solid: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.53 (d, J = 8.4 Hz, 1 H), 5.40 (app. t, J = 8.9 Hz, 1 H), 5.04 (t, J = 9.6 Hz, 1 H), 4.86 (d, J = 8.4 Hz, 1 H), 4.26 (dd, J = 4.8 , 12.0 Hz, 1 H), 4.10 (dd, J = 2.4, 12.0 Hz, 1 H), 3.72-3.58 (m, 3 H), 2.07 (s, 3 H), 2.02 (s, 3 H), 2.01 (s, 3 H), 1.93 (s, 3 H), 1.92-1.18 (m, 10 H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.8, 170.7, 170.1, 169.5, 98.9, 77.7, 72.2, 71.6, 69.0, 62.3, 55.6, 33.3, 31.7, 25.5, 23.8, 23.7, 23.4, 20.8, 20.7 (2 C); HRMS (ESI) *m/z* calcd for $C_{20}H_{31}NO_9Na$ (M+Na) 452.1897, found 452.1898.



2-15

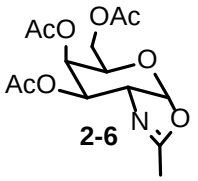
Geranoyl 3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranoside (2-15). A solution of **2-12** (0.120 g, 0.364 mmol) and anhydrous $CuCl_2$ (49.0 mg, 0.364 mmol) in anhydrous $CHCl_3$ (0.84 mL) in a 2-5 mL conical sealed vessel under argon atmosphere was treated with geraniol (0.270 mL, 1.47 mmol). The reaction mixture was heated at 62 ºC for 2 h, cooled to room temperature, diluted with ethyl acetate (15 mL), washed with 1 N HCl (2 × 9 mL), a sat. $NaHCO_3$ solution (1 × 10 mL), and brine (1 × 10 mL), dried ($MgSO_4$), evaporated, and purified by chromatography on SiO_2 (75% EtOAc/hexanes) to give **2-15** (120 mg, 0.248 mmol, 68%) as a colorless solid: $[\alpha]_D$ – 20.9 (*c* 1.0, CH_2Cl_2); Mp 104.8-105.8 ºC; IR (ATR) 3282, 2930, 1743, 1735, 1650, 1569, 1431, 1368, 1223, 1128, 1077, 1029, 975 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.40 (d, *J* = 8.4 Hz, 1 H), 5.30-5.26 (m, 2 H), 5.08-4.98 (m, 2 H), 4.70 (d, *J* = 8.4 Hz, 1 H), 4.32-4.12 (m, 4 H), 3.82 (app. q, *J* = 8.4 Hz, 1 H), 3.69-3.63 (m, 1 H), 2.11-2.02 (m, 4 H), 2.07 (s, 3 H), 2.02 (s, 3 H), 2.02 (s, 3 H), 1.94 (s, 3 H), 1.69 (s, 3 H), 1.66 (s, 3 H), 1.60 (s, 3 H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.9, 170.7, 170.1, 169.4, 142.2, 131.8, 123.8, 119.2, 98.8, 72.5, 71.9, 68.7, 65.1, 62.3, 54.9, 39.6, 26.3, 25.7, 23.4, 20.7 (2 C), 20.6, 17.7, 16.3; HRMS (ESI) *m/z* calcd for $C_{24}H_{37}NO_9Na$ (M+Na) 506.2366, found 506.2360.



2-5

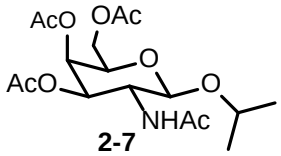
2-Acetamido-2-deoxy-1,3,4,6-tetra-O-acetyl-β-D-galactopyranose (2-5).[83,96] A solution of D-(+)-galactosamine hydrochloride (0.500 g, 2.32 mmol) in anhydrous pyridine (5 mL) was treated with acetic anhydride (2.36 mL, 27.8 mmol), stirred at room temperature for 2 d and poured onto

ice-cold water (75 mL). After a white solid precipitated, the solid was collected by vacuum filtration, washed with ice-cold water and co-evaporated with toluene (3 × 3 mL) to give **2-5** as a colorless powder (745 mg, 1.91 mmol, 83%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.71 (d, *J* = 8.8 Hz, 1 H), 5.41-5.37 (m, 2 H), 5.08 (dd, *J* = 3.2, 11.2 Hz, 1 H), 4.44 (app. q, *J* = 8.0 Hz, 1 H), 4.20-4.09 (m, 2 H), 4.02 (app t, *J* = 6.4 Hz, 1 H), 2.17 (s, 3 H), 2.13 (s, 3 H), 2.05 (s, 3 H), 2.02 (s, 3 H), 1.94 (s, 3 H).



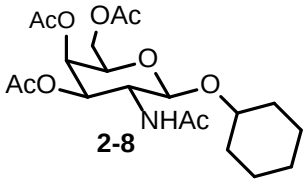
2-6

2,3-Dihydrooxazole-3,4,6-tri-O-acetyl-α-D-galactopyranoside (2-6).[97] A solution of **2-5** (400 mg, 1.03 mmol) in dichloroethane (28.6 mL) was treated with TMSOTf (0.200 mL, 1.08 mmol) at room temperature, heated at 50 ºC for 1 h, cooled, and treated with NEt_3 (0.440 mL, 3.08 mmol). The mixture was stirred at room temperature for 10 min, passed through a short plug of silica and washed with ethyl acetate (25 mL) and dichloromethane (30 mL). The solvent was evaporated under reduced pressure and the crude oil was purified by chromatography on SiO_2 (100% EtOAc, base washed with 1% NEt_3 prior to use) to yield **2-6** as a clear slight orange oil (304 mg, 0.923 mmol, 90%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.98 (d, *J* = 6.8 Hz, 1 H), 5.45 (d, *J* = 2.8 Hz, 1 H), 4.90 (dd, *J* = 3.2, 7.2 Hz, 1 H), 4.26-4.16 (m, 2 H), 4.10 (dd, *J* = 5.6, 11.2 Hz, 1 H), 3.98 (td, *J* = 1.2, 7.6 Hz, 1 H), 2.11 (s, 3 H), 2.06 (s, 6 H), 2.04 (d, *J* = 1.2 Hz, 3 H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.5, 170.2, 169.8, 166.4, 101.5, 71.8, 69.5, 65.3, 63.6, 61.6, 20.8, 20.7, 20.6, 14.4; HRMS (ESI) *m/z* calcd for $C_{14}H_{20}NO_8$ (M+H) 330.1189, found 330.1187.



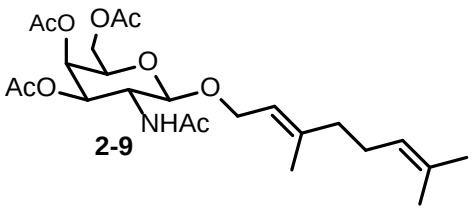
2-7

Isopropyl 3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-galactopyranoside (2-7). A solution of **2-6** (0.133 g, 0.404 mmol) and anhydrous $CuCl_2$ in anhydrous $CHCl_3$ (0.76 mL) in a 2-5 mL conical sealed vessel under argon atmosphere was treated with anhydrous 2-propanol (0.130 mL, 1.72 mmol). The reaction mixture was heated at 62 ºC for 2 h, cooled to room temperature, diluted with acetone (15 mL) and sat. $NaHCO_3$ solution (7 mL) filtered through a short plug of Celite®, and concentrated. The residue was coevaporated with toluene to remove residual water and shaken in $CHCl_3$ and weakly acidic ion-exchange resin (Amberlite IRC-86, ca 1.5 g). The solution was filtered, the solvent was reduced under vacuum, and the residue was chromatographed on SiO_2 (75% EtOAc/hexanes) to give **2-7** (119 mg, 0.305 mmol, 75%) as a colorless solid: $[\alpha]_D$ – 14.4 (*c* 1.0, CH_2Cl_2); Mp 188.5-189.5 ºC; IR (ATR) 3264, 2980, 1735, 1648, 1568, 1380, 1256, 1232, 1215, 1124, 1072, 1053, 1023 cm^{-1}; ^{1}H-NMR (600 MHz, $CDCl_3$) δ 5.57 (d, *J* = 7.8 Hz, 1 H), 5.42 (dd, *J* = 3.0, 11.1 Hz, 1 H), 5.35 (app. d, *J* = 3.6 Hz, 1 H), 4.86 (d, *J* = 8.4 Hz, 1 H), 4.16 (dd, *J* = 6.6, 11.4 Hz, 1 H), 4.10 (dd, *J* = 7.2, 11.4 Hz, 1 H), 3.95-3.90 (m, 2 H), 3.77-3.73 (m, 1 H), 2.12 (s, 3 H), 2.03 (s, 3 H), 1.99 (s, 3 H), 1.94 (s, 3 H), 1.23 (d, *J* = 6.6 Hz, 3 H), 1.13 (d, *J* = 6.6 Hz, 3 H); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 170.5, 170.3, 99.4, 72.8, 70.4, 69.6, 66.8, 61.5, 52.4, 23.5, 23.3, 22.0, 20.7 (2 C); HRMS (ESI) *m/z* calcd for $C_{17}H_{28}NO_9$ (M+H) 390.1764, found 390.1774.



2-8

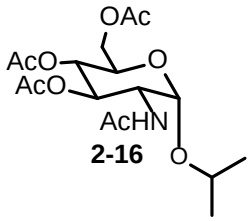
Cyclohexyl 3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-galactopyranoside (2-8). A solution of **2-6** (0.135 g, 0.411 mmol) and anhydrous $CuCl_2$ (55.0 mg, 0.411 mmol) in anhydrous $CHCl_3$ (0.68 mL) in a 2-5 mL conical sealed vessel under argon atmosphere was treated with cyclohexanol (0.170 mL, 1.66 mmol). The reaction mixture was heated at 62 ºC for 2 h, cooled to room temperature, diluted with ethyl acetate (15 mL), washed with 1 N HCl (2 × 9 mL), sat. $NaHCO_3$ solution (1 × 10 mL), and brine (1 × 10 mL), dried ($MgSO_4$), evaporated, and purified by chromatography on SiO_2 (75% EtOAc/hexanes) to give **2-8** (141 mg, 0.328 mmol, 80%) as a colorless solid: $[\alpha]_D$ – 14.6 (*c* 1.0, CH_2Cl_2); Mp 164.3-165.3 ºC; IR (ATR) 3331, 2936, 2857, 1735, 1661, 1541, 1364, 1251, 1219, 1079, 1031, 984 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 5.48 (d, *J* = 8.5 Hz, 1 H), 5.41 (dd, *J* = 3.5, 11.5 Hz, 1 H), 5.35 (app. d, *J* = 3.5 Hz, 1 H), 4.89 (d, *J* = 8.5 Hz, 1 H), 4.17 (dd, *J* = 6.5, 11.5 Hz, 1 H), 4.10 (dd, *J* = 7.0, 11.0 Hz, 1 H), 3.91 (app t, *J* = 10.5 Hz, 1 H), 3.80-3.74 (m, 1 H), 3.64-3.59 (m, 1 H), 2.13 (s, 3 H), 2.03 (s, 3 H), 1.99 (s, 3 H), 1.94 (s, 3 H), 1.93-1.17 (m, 10 H); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.4, 170.3 (3 C), 99.1, 78.0, 70.4, 69.6, 66.8, 61.4, 52.5, 33.3, 31.7, 25.5, 23.9, 23.8, 23.5, 20.7 (2 C); HRMS (ESI) *m/z* calcd for $C_{20}H_{32}NO_9$ (M+H) 430.2077, found 430.2084.



2-9

Geranoyl 3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-galactopyranoside (2-9). A solution of **2-6** (0.120 g, 0.364 mmol) and anhydrous $CuCl_2$ (49.0 mg, 0.364 mmol) in anhydrous

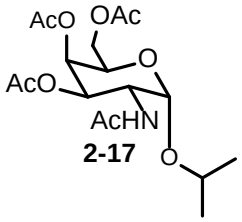
$CHCl_3$ (0.84 mL) in a 2-5 mL conical sealed vessel under argon atmosphere was treated with geraniol (0.270 mL, 1.47 mmol). The reaction mixture was heated at 62 ºC for 2 h, cooled to room temperature, diluted with ethyl acetate (15 mL), washed with 1 N HCl (2 × 9 mL), sat. $NaHCO_3$ solution (1 × 10 mL), and brine (1 × 10 mL), dried ($MgSO_4$), evaporated, and purified by chromatography on SiO_2 (75% EtOAc/hexanes) to give **2-9** (149 mg, 0.308 mmol, 65%) as a colorless solid: $[\alpha]_D$ – 17.0 (*c* 1.0, CH_2Cl_2); Mp 117.5-118.9 ºC; IR (ATR) 3279, 2982, 2939, 1737, 1659, 1555, 1536, 1431, 1374, 1241, 1226, 1131, 1064, 1053, 1036, 1012, 997, 958 cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 5.41 (d, *J* = 8.5 Hz, 1 H), 5.36-5.27 (m, 3 H), 5.09-5.06 (m, 1 H), 4.75 (d, *J* = 8.5 Hz, 1 H), 4.29 (dd, *J* = 6.5, 12.0 Hz, 1 H), 4.23-4.11 (m, 3 H), 4.94-3.88 (m, 2 H), 2.13 (s, 3 H), 2.12-2.00 (m, 4 H), 2.03 (s, 3 H), 1.99 (s, 3 H), 1.94 (s, 3 H), 1.68 (s, 3 H), 1.65 (s, 3 H), 1.60 (s, 3 H), ; ^{13}C-NMR (125 MHz, $CDCl_3$) δ 170.4 (2 C), 170.3, 170.2, 142.1, 131.8, 123.8, 119.3, 99.0, 70.6, 69.9, 66.9, 65.2, 61.6, 51.8, 39.6, 26.3, 25.7, 23.5, 20.7 (2 C), 17.7, 16.3; HRMS (ESI) *m/z* calcd for $C_{24}H_{37}NO_9Na$ (M+Na) 506.2366, found 506.2384.



2-16

Isopropyl 3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-α-D-glucopyranoside (2-16).[98] A solution of **2-11** (50.0 mg, 0.128 mmol) in a 5% HCl solution in 2-propanol (3.8 mL) was stirred at 65 ºC for 1 h, evaporated to dryness, dissolved in pyridine (0.48 mL) and treated with acetic anhydride (0.146 mL, 1.54 mmol). The reaction mixture was stirred at room temperature for 2 d and then coevaporated with toluene to give a brown oil which was a 3:1 mixture of α:β-isomers by crude NMR. Purification by chromatography on SiO_2 (75% EtOAc/hexanes) gave **2-16** (30.4 mg, 0.0781 mmol, 61%) as a foaming colorless solid: IR (ATR) 3359, 2975, 1750, 1730, 1676,

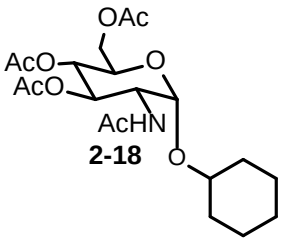
1534, 1437, 1376, 1364, 1243, 1225, 1150, 1122, 1038, 1031, 997 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.62 (d, *J* = 9.6 Hz, 1 H), 5.19 (app t, *J* = 9.6 Hz, 1 H), 5.10 (app t, *J* = 9.6 Hz, 1 H), 4.92 (d, *J* = 3.8 Hz, 1 H), 4.30 (td, *J* = 3.6, 9.6 Hz, 1 H), 4.22 (dd, *J* = 4.8, 12.4 Hz, 1 H), 4.08 (dd, *J* = 2.4, 12.4 Hz, 1 H), 4.03-3.99 (m, 1 H), 3.88 (sept, *J* = 6.0 Hz, 1 H), 2.07 (s, 3 H), 2.02 (s, 3 H), 2.01 (s, 3 H), 1.93 (s, 3 H), 1.23 (d, *J* = 6.4 Hz, 3 H), 1.14 (d, *J* = 6.4 Hz, 3 H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.4, 170.7, 169.8, 169.3, 95.7, 71.4, 71.0, 68.2, 67.7, 62.0, 51.8, 23.2, 23.1, 21.6, 20.7 (2 C), 20.6; HRMS (ESI) *m/z* calcd for $C_{17}H_{28}NO_9$ (M+H) 390.1764, found 390.1790.



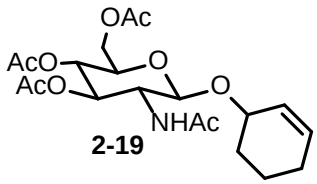
2-17

Isopropyl 3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-α-D-galactopyranoside (2-17).[98] A solution of **2-5** (50.0 mg, 0.128 mmol) in a 5% HCl solution in 2-propanol (3.8 mL) was stirred at 65 ºC for 1 h, evaporated to dryness, dissolved in pyridine (0.50 mL) and treated with acetic anhydride (0.146 mL, 1.54 mmol). The reaction mixture was stirred at room temperature for 2 d and then coevaporated with toluene to give a brown oil that was a 3:1 mixture of α:β-isomers by crude NMR. Purification by chromatography on SiO_2 (75% EtOAc/hexanes) gave **2-17** (28.6 mg, 0.0737 mmol, 57%) as a foaming colorless solid: IR (ATR) 3310, 2973, 2930, 1745, 1732, 1653, 1534, 1372, 1284, 1240, 1217, 1124, 1083, 1034, 992, 936, 878 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.54 (d, *J* = 9.6 Hz, 1 H), 5.35 (app. d, *J* = 2.8 Hz, 1 H), 5.13 (dd, *J* = 3.2, 11.2 Hz, 1 H), 4.95 (d, *J* = 4.0 Hz, 1 H), 4.56-4.50 (m, 1 H), 4.23 (app. t, *J* = 6.8 Hz, 1 H), 4.12-4.02 (m, 2 H), 3.88 (sept, *J* = 6.0 Hz, 1 H), 2.15 (s, 3 H), 2.03 (s, 3 H), 1.98 (s, 3 H), 1.95 (s, 3 H), 1.22 (d, *J* = 6.0 Hz, 3 H), 1.13 (d, *J* = 6.4 Hz, 3 H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.0, 170.4 (2 C),

169.9, 96.3, 71.0, 68.5, 67.5, 66.7, 62.0, 47.8, 23.3, 23.1, 21.7, 20.8, 20.7, 20.6; HRMS (ESI) *m/z* calcd for $C_{17}H_{28}NO_9$ (M+H) 390.1764, found 390.1776.

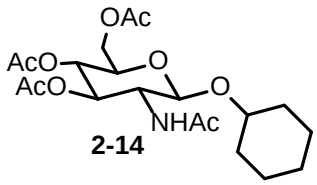


Cyclohexyl 3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-α-D-glucopyranoside (2-18).[99] A solution of **2-11** (50.0 mg, 0.128 mmol) in a 5% HCl solution in cyclohexanol (3.1 mL) was stirred at 65 ºC for 1 h, evaporated to dryness, dissolved in pyridine (0.50 mL, anhydrous) and treated with acetic anhydride (0.146 mL, 1.54 mmol). The reaction mixture was stirred at room temperature for 31 h and coevaporated with toluene to give a brown oil that was a 3:1 mixture of α:β-isomers by crude NMR. Purification by chromatography on SiO_2 (75% EtOAc/hexanes) gave **2-18** (31.7 mg, 0.0738 mmol, 57%) as a foaming colorless solid: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.63 (d, *J* = 9.6 Hz, 1 H), 5.19 (app. t, *J* = 10.2 Hz, 1 H), 5.08 (app. t, *J* = 10.8 Hz, 1 H), 4.96 (d, *J* = 3.6 Hz, 1 H), 4.32-4.26 (m, 1 H), 4.21 (dd, *J* = 4.8, 12.4 Hz, 1 H), 4.08 (dd, *J* = 2.4, 12.4 Hz, 1 H), 3.54 (sept, *J* = 4.0 Hz, 1 H), 2.07 (s, 3 H), 2.01 (s, 3 H), 2.00 (s, 3 H), 1.93 (s, 3 H), 1.91-1.20 (m, 10 H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 171.4, 170.7, 169.8, 169.3, 95.6, 71.4, 68.2, 67.7, 62.0, 51.9, 33.3, 31.5, 25.4, 24.1, 23.8, 23.2, 20.7 (2 C), 20.6; HRMS (ESI) *m/z* calcd for $C_{20}H_{32}NO_9$ (M+H) 430.2077, found 430.2086.



2-19

Cyclohexenyl 3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranoside (2-19). A solution of **2-12** (0.128 g, 0.388 mmol) and anhydrous $CuCl_2$ (52.0 mg, 0.388 mmol) in anhydrous $CHCl_3$ (0.88 mL) in a 2-5 mL conical sealed vessel under argon atmosphere was treated with cyclohexenol (0.16 mL, 1.57 mmol). The reaction mixture was heated at 62 ºC for 2 h, cooled to room temperature, diluted with ethyl acetate (25 mL), washed with 1 N HCl (2 × 9 mL), sat. $NaHCO_3$ solution (1 × 10 mL), and brine (1 × 10 mL), dried ($MgSO_4$), evaporated, and purified by chromatography on SiO_2 (75% EtOAc/hexanes) to give **2-19** (61.7 mg, 0.144 mmol, 37%, 1:1 mixture of diastereomers) as a colorless solid: $[\alpha]_D$ – 16.7 (*c* 1.0, CH_2Cl_2); Mp 177.8-178.9 ºC; IR (ATR) 3271, 3096, 2941, 1745, 1737, 1650, 1558, 1431, 1368, 1221, 1159, 1038, 733 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.90-5.84 (m, 2 H), 5.78-5.71 (m, 1H), 5.71-5.65 (m, 1 H), 5.58-5.45 (m, 3 H), 5.42-5.35 (m, 1 H), 5.07-5.00 (m, 2 H), 4.98 (d, *J* = 8.4 Hz, 1 H), 4.91 (d, *J* = 8.0 Hz, 1 H), 4.30-4.15 (m, 4 H), 4.12 (dd, *J* = 2.4, 12.0 Hz, 2 H), 3.75-3.65 (m, 3 H), 3.61-3.54 (m, 1 H), 2.08 (s, 6 H), 2.02 (2 x s, 12 H), 1.93 (2 x s, 6 H), 1.86-1.50 (m, 8 H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.8, 170.6, 170.2 (2 C), 169.5, 169.5, 132.3, 131.7, 127.5, 126.3, 99.1, 98.7, 73.1, 72.8, 71.9, 71.6, 69.0, 68.9, 62.3, 55.9, 55.5, 29.7, 28.3, 25.1, 25.0, 23.4, 20.8, 20.7 (2 C), 18.9, 18.6; HRMS (ESI) *m/z* calcd for $C_{20}H_{29}NO_9Na$ (M+Na) 450.1740, found 450.1745.

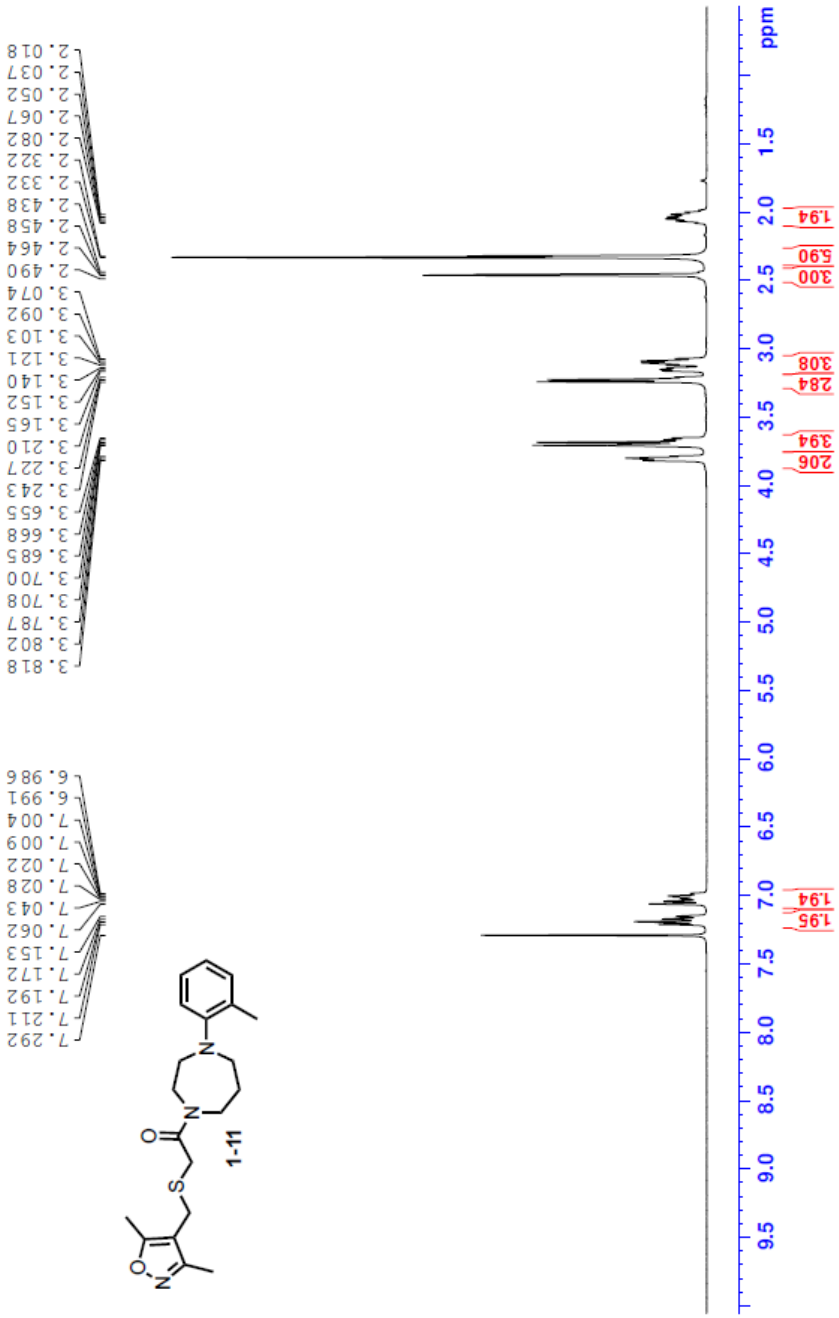


2-14

Cyclohexyl 3,4,6-tri-O-acetyl-2-(acetylamino)-2-deoxy-β-D-glucopyranoside.[95] A solution of **2-19** (20.0 mg, 0.047 mmol) in ethyl acetate (1.7 mL) was treated with 10% Pd/C (3.2 mg, 0.0030 mmol). The flask was evacuated, purged with H_2, stirred for 2 h under an atmosphere of H_2, filtered through Celite® and evaporated to give **2-14** as colorless powder (19.7 mg, 0.0454 mmol, 97%): ^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.57 (d, *J* = 8.4 Hz, 1 H), 5.40 (app. t, *J* = 9.2 Hz, 1 H), 5.04 (t, *J* = 9.6 Hz, 1 H), 4.86 (d, *J* = 8.4 Hz, 1 H), 4.26 (dd, *J* = 4.8 , 12.0 Hz, 1 H), 4.10 (dd, *J* = 2.4, 12.0 Hz, 1 H), 3.72-3.58 (m, 3 H), 2.07 (s, 3 H), 2.02 (s, 3 H), 2.01 (s, 3 H), 1.93 (s, 3 H), 1.91-1.18 (m, 10 H); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 170.8, 170.2, 169.5, 98.9, 77.7, 72.1, 71.5, 68.9, 62.3, 55.5, 33.2, 31.6, 25.5, 23.8, 23.7, 23.4, 20.8, 20.7 (2 C).

APPENDIX A

^{1}H-NMR AND ^{13}C-NMR OF SELECTED COMPOUNDS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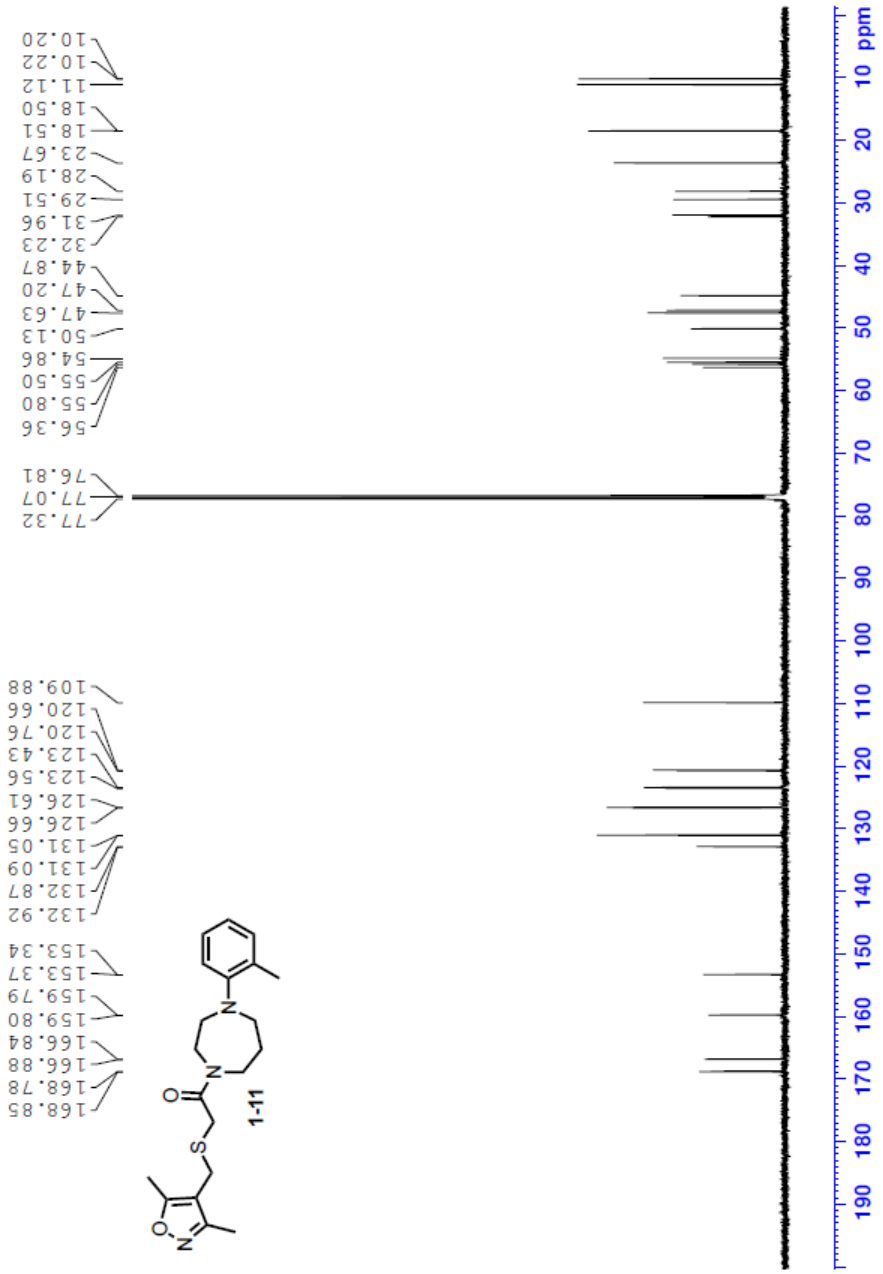
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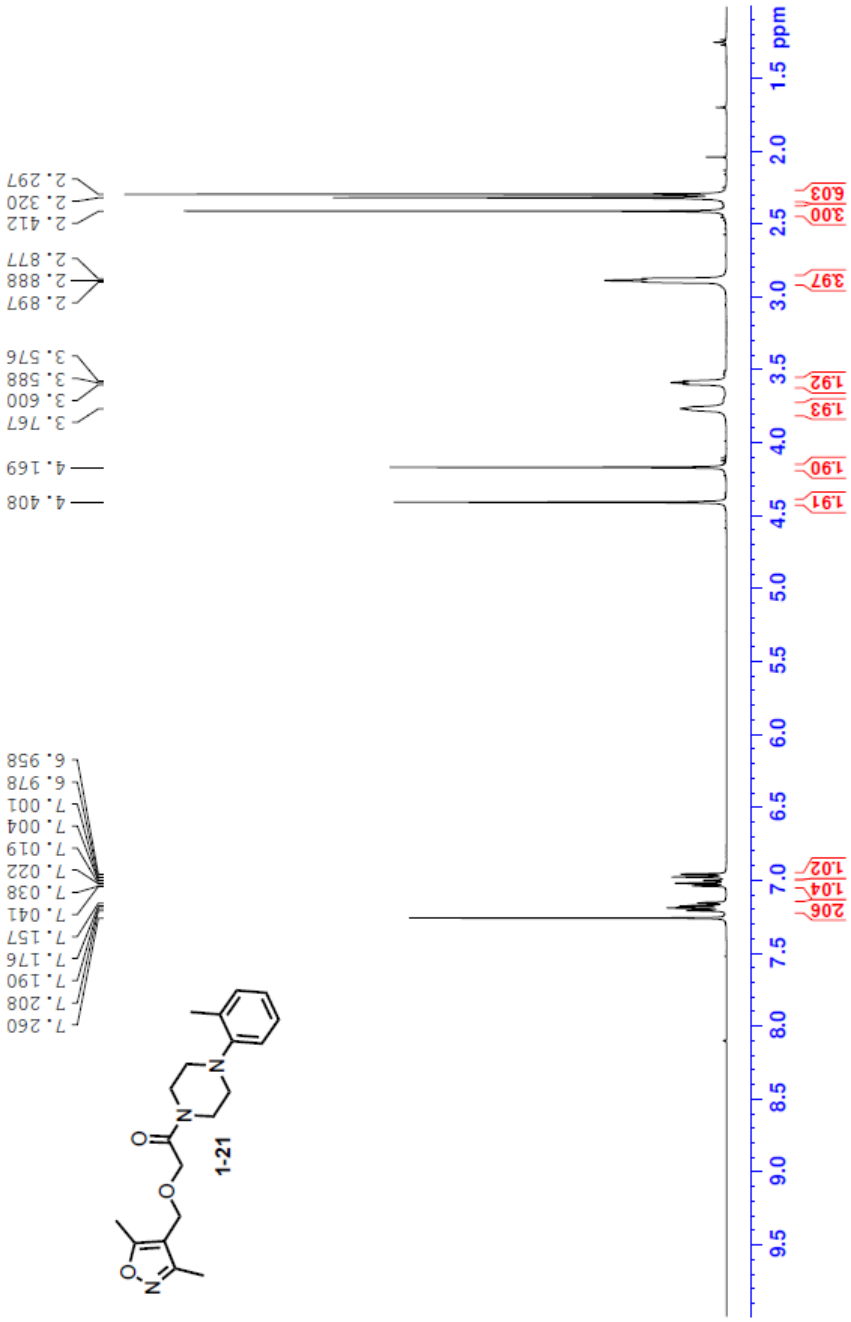
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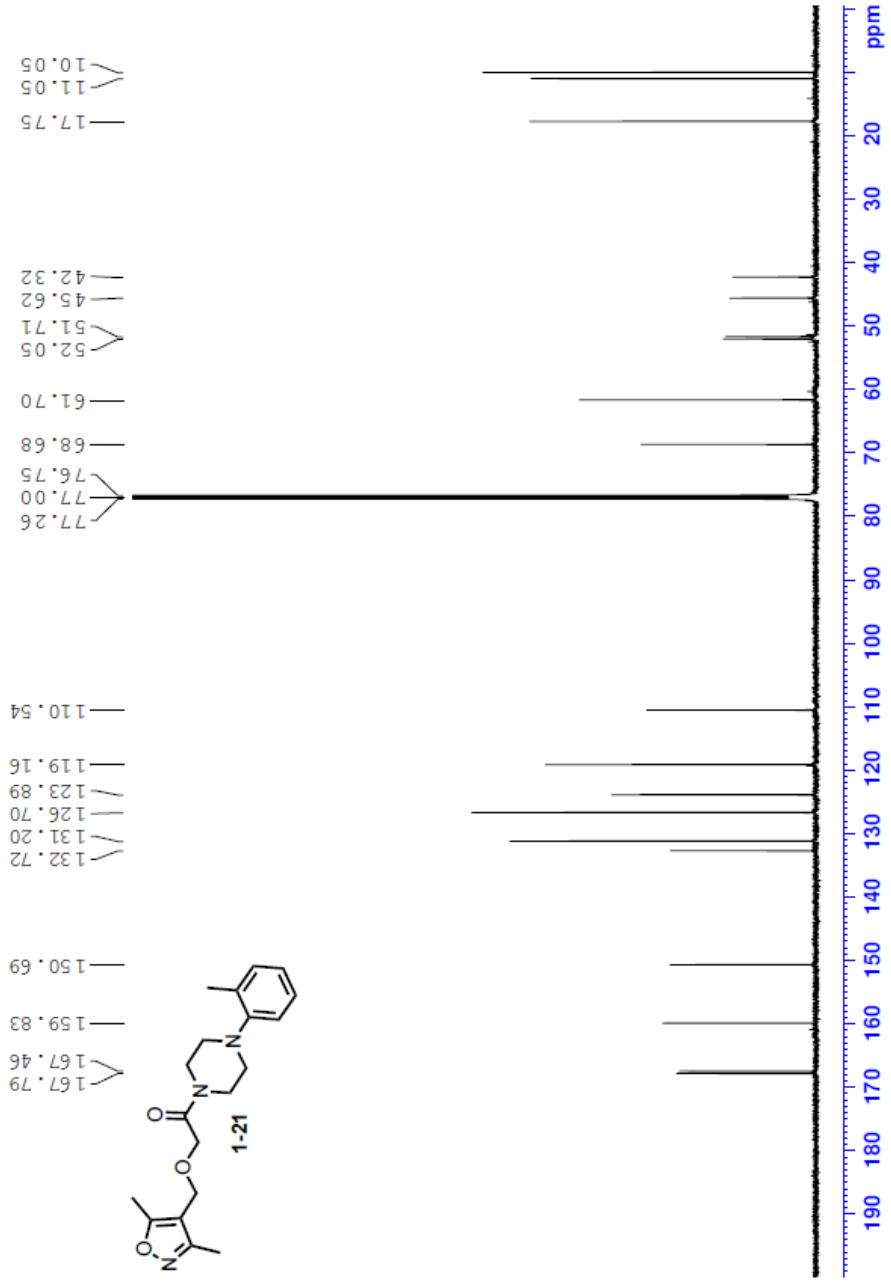
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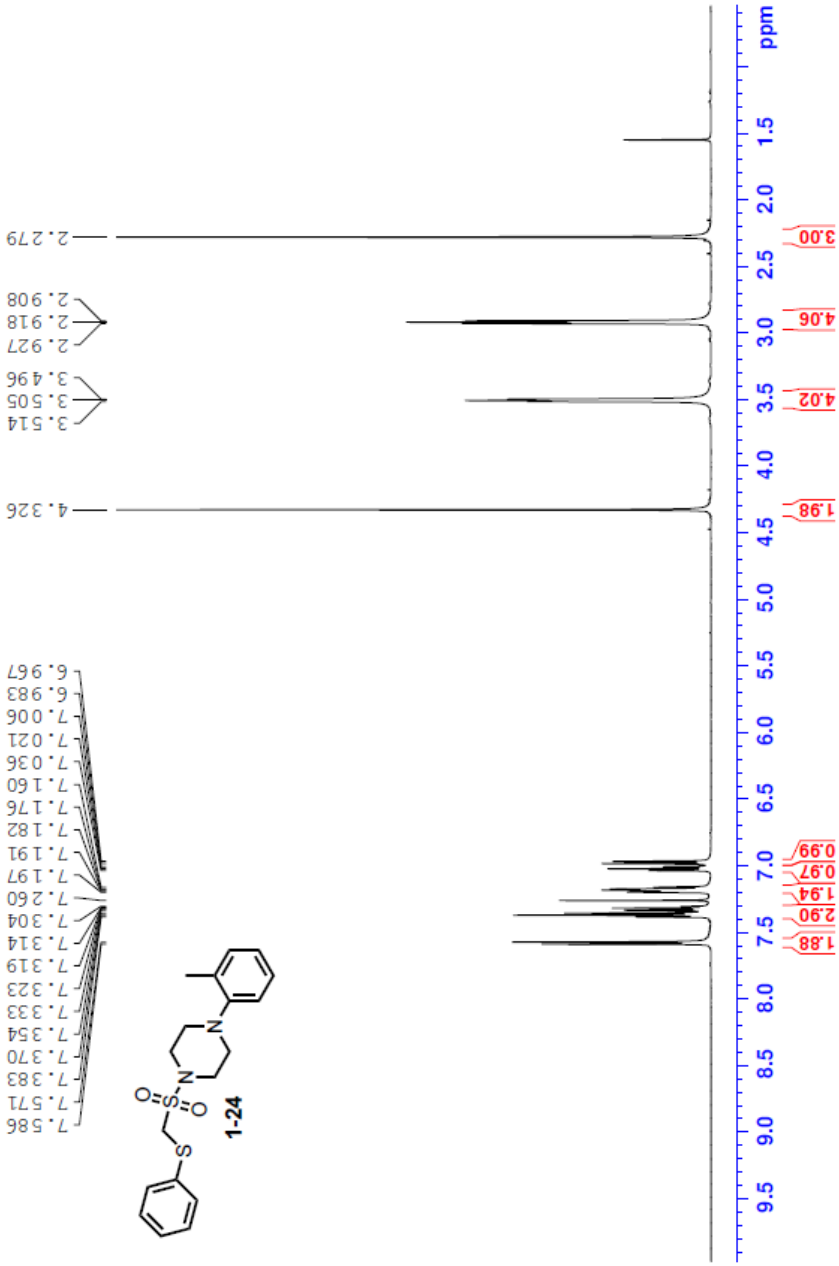
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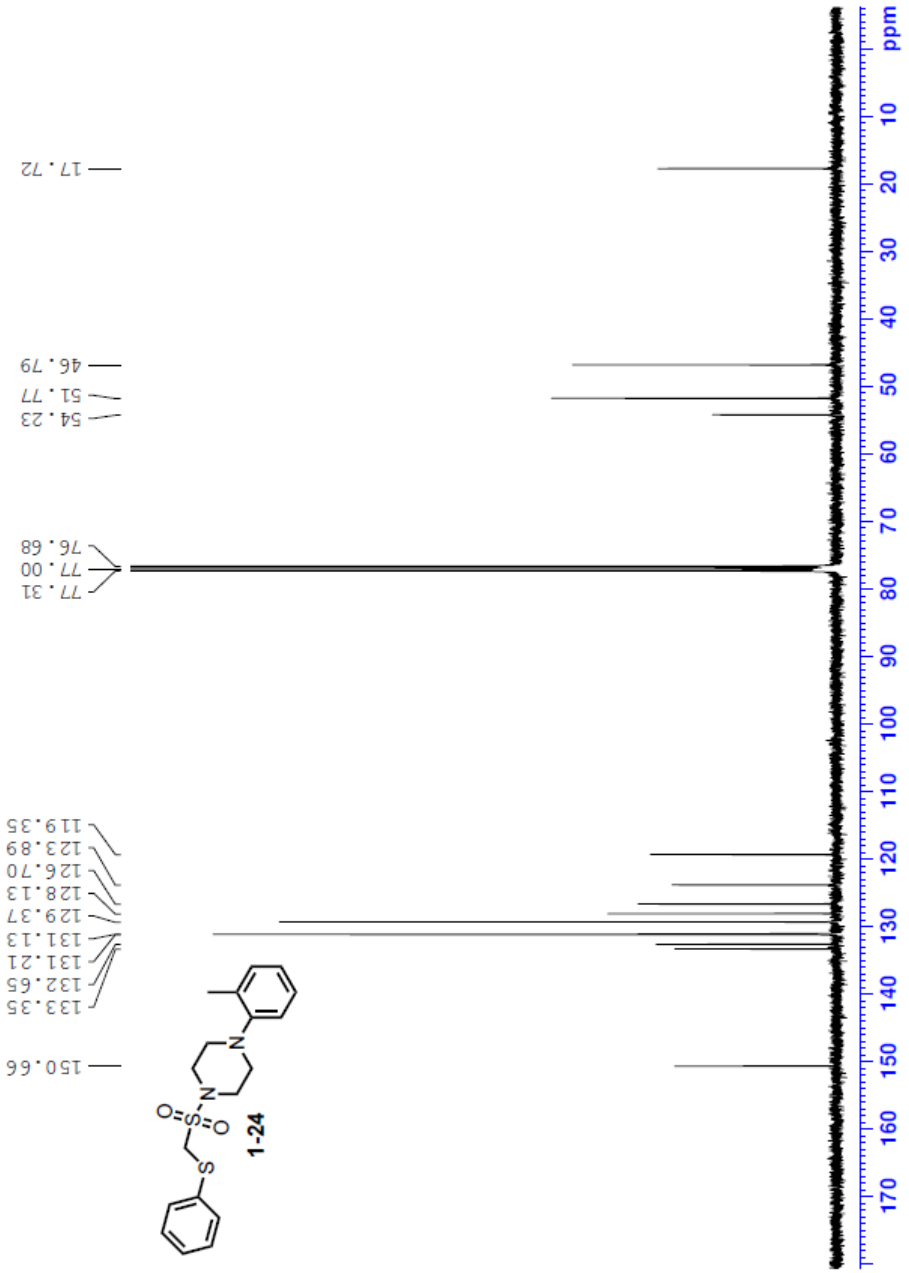
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1-24
ppm

BIBLIOGRAPHY

1. (a) Heinlein, C. A.; Chang, C. *Endocr. Rev.* **2002**, *23* (2), 175-200; (b) Dehm, S. M.; Tindall, D. J. *Mol. Endocrinol.* **2007**, *21* (12), 2855-63; (c) Debes, J. D.; Tindall, D. J. *N. Engl. J. Med.* **2004**, *351* (15), 1488-90.
2. Veldscholte, J.; Berrevoets, C. A.; Ris-Stalpers, C.; Kuiper, G. G. J. M.; Jenster, G.; Trapman, J.; Brinkmann, A. O.; Mulder, E. *J. Steroid Biochem. Mol. Biol.* **1992**, *41* (3–8), 665-69.
3. Waltering, K. K.; Urbanucci, A.; Visakorpi, T. *Mol. Cell Endocrinol.* **2012**, *360* (1–2), 38-43.
4. Godbole, A. M.; Njar, V. C. O. *Prostate Cancer* **2011**, *2011*, 1-13.
5. Hodgson, M. C.; Bowden, W. A.; Agoulnik, I. U. *World J. Urol.* **2012**, *30* (3), 279-85.
6. Nieto, M.; Finn, S.; Loda, M.; Hahn, W. C. *Int. J. Biochem. Cell Biol.* **2007**, *39* (9), 1562-68.
7. A Snapshot of Prostate Cancer 2012. National Cancer Institute. www.cancer.gov/aboutnci/servingpeople/snapshots/Prostate-Snapshot.pdf (accessed December 14, 2012).
8. SEER Stat Fact Sheets: Prostate. http://seer.cancer.gov/statfacts/html/prost.html (accessed December 14, 2012).
9. Haendler, B.; Cleve, A. *Mol. Cell Endocrinol.* **2012**, *352* (1–2), 79-91.

10. Wang, J.; Shang, Z.-q.; Niu, Y.-j. *Clin. Oncol. Cancer Res.* **2011**, *8* (2), 85-91.

11. Mohler, J. L., Castration-Recurrent Prostate Cancer Is Not Androgen-Independent Hormonal Carcinogenesis V. Li, J. J.; Li, S. A.; Mohla, S.; Rochefort, H.; Maudelonde, T., Eds. Springer New York: 2008; Vol. 617, pp 223-34.

12. Miyamoto, H.; Messing, E. M.; Chang, C. *Nat. Clin. Pract. Oncol.* **2005**, *2* (5), 236-37.

13. (a) Niu, Y.; Altuwaijri, S.; Lai, K.-P.; Wu, C.-T.; Ricke, W. A.; Messing, E. M.; Yao, J.; Yeh, S.; Chang, C. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105* (34), 12182-87; (b) Niu, Y.; Chang, T. M.; Yeh, S.; Ma, W. L.; Wang, Y. Z.; Chang, C. *Oncogene* **2010**, *29* (25), 3593-604.

14. Titus, M. A.; Schell, M. J.; Lih, F. B.; Tomer, K. B.; Mohler, J. L. *Clin. Cancer Res.* **2005**, *11* (13), 4653-57.

15. Zhang, L.; Johnson, M.; Le, K. H.; Sato, M.; Ilagan, R.; Iyer, M.; Gambhir, S. S.; Wu, L.; Carey, M. *Cancer Res.* **2003**, *63* (15), 4552-60.

16. Chen, C. D.; Welsbie, D. S.; Tran, C.; Baek, S. H.; Chen, R.; Vessella, R.; Rosenfeld, M. G.; Sawyers, C. L. *Nat. Med.* **2004**, *10* (1), 33-39.

17. Zegarra-Moro, O. L.; Schmidt, L. J.; Huang, H.; Tindall, D. J. *Cancer Res.* **2002**, *62* (4), 1008-13.

18. Wilson, E. M. *Ther. Adv. Urol.* **2010**, *2* (3), 105-17.

19. Brogden, R. N.; Chrisp, P. *Drugs Aging* **1991**, *1* (2), 104-15.

20. Dole, E. J.; Holdsworth, M. T. *Ann. Pharmacother.* **1997**, *31* (1), 65-75.

21. Cockshott, I. D. *Clin. Pharmacokinet.* **2004**, *43* (13), 855-78.

22. Mukherjee, A.; Kirkovsky, L.; Yao, X. T.; Yates, R. C.; Miller, D. D.; Dalton, J. T. *Xenobiotica* **1996**, *26* (2), 117-22.

23. Attar, R. M.; Jure-Kunkel, M.; Balog, A.; Cvijic, M. E.; Dell-John, J.; Rizzo, C. A.; Schweizer, L.; Spires, T. E.; Platero, J. S.; Obermeier, M.; Shan, W.; Salvati, M. E.; Foster, W. R.; Dinchuk, J.; Chen, S.-J.; Vite, G.; Kramer, R.; Gottardis, M. M. *Cancer Res.* **2009**, *69* (16), 6522-30.

24. (a) Gan, L.; Chen, S.; Wang, Y.; Watahiki, A.; Bohrer, L.; Sun, Z.; Wang, Y.; Huang, H. *Cancer Res* **2009**, *69* (21), 8386-94; (b) Jiang, J.; Huang, H. *Mol. Cell. Pharmacol.* **2010**, *2* (1), 1-5.

25. Schurko, B.; Oh, W. K. *Nat. Clin. Pract. Oncol.* **2008**, *5* (9), 506-07.

26. (a) Jung, M. E.; Ouk, S.; Yoo, D.; Sawyers, C. L.; Chen, C.; Tran, C.; Wongvipat, J. *J. Med. Chem.* **2010**, *53* (7), 2779-96; (b) Tran, C.; Ouk, S.; Clegg, N. J.; Chen, Y.; Watson, P. A.; Arora, V.; Wongvipat, J.; Smith-Jones, P. M.; Yoo, D.; Kwon, A.; Wasielewska, T.; Welsbie, D.; Chen, C. D.; Higano, C. S.; Beer, T. M.; Hung, D. T.; Scher, H. I.; Jung, M. E.; Sawyers, C. L. *Science* **2009**, *324* (5928), 787-90.

27. Higano, C. S.; Small, E. J.; Schellhammer, P.; Yasothan, U.; Gubernick, S.; Kirkpatrick, P.; Kantoff, P. W. *Nat. Rev. Drug Discovery* **2010**, *9* (7), 513-14.

28. Sadar, M. D.; Williams, D. E.; Mawji, N. R.; Patrick, B. O.; Wikanta, T.; Chasanah, E.; Irianto, H. E.; Soest, R. V.; Andersen, R. J. *Org. Lett.* **2008**, *10* (21), 4947-50.

29. (a) Wu, C.-Y.; Hsieh, C.-Y.; Huang, K.-E.; Chang, C.; Kang, H.-Y. *Int. J. Cancer* **2012**, *131* (6), 1423-34; (b) Xu, D.; Lin, T.-H.; Li, S.; Da, J.; Wen, X.-Q.; Ding, J.; Chang, C.; Yeh, S. *Cancer Lett.* **2012**, *316* (1), 11-22.

30. Nickols, N. G.; Dervan, P. B. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104* (25), 10418-23.

31. Wang, Z.; Eisermann, K.; Masood, K. Z. **2012**, *Unpublished results*.

32. (a) Cabello-Sanchez, N.; Jean, L.; Maddaluno, J.; Lasne, M.-C.; Rouden, J. *J. Org. Chem.* **2007**, *72* (6), 2030-39; (b) Larsen, S. B.; Bang-Andersen, B.; Johansen, T. N.; Jørgensen, M. *Tetrahedron* **2008**, *64* (13), 2938-50.

33. Yun, J. I.; Kim, H. R.; Kim, S. K.; Kim, D.; Lee, J. *Tetrahedron* **2012**, *68* (4), 1177-84.

34. Medzhitov, R.; Schneider, D. S.; Soares, M. P. *Science* **2012**, *335* (6071), 936-41.

35. Medzhitov, R. *Nat. Rev. Immunol.* **2001**, *1* (2), 135-45.

36. Takeda, K.; Kaisho, T.; Akira, S. *Annu. Rev. Immunol.* **2003**, *21*, 335-76.

37. (a) Bortoluci, K. R.; Medzhitov, R. *Cell. Mol. Life Sci.* **2010**, *67* (10), 1643-51; (b) Barton, G. M.; Medzhitov, R. *Current topics in microbiology and immunology* **2002**, *270*, 81-92; (c) Theofilopoulos, A. N. *J. Clin. Invest.* **2012**, *122* (10), 3464-66.

38. Keogh, B.; Parker, A. E. *Trends Pharmacol. Sci.* **2011**, *32* (7), 435-42.

39. Souza-Fonseca-Guimaraes, F.; Parlato, M.; Philippart, F.; Misset, B.; Cavaillon, J.-M.; Adib-Conquy, M.; group, t. C. s. *Crit. Care* **2012**, *16* (5), R206.

40. Baumgart, D. C.; Sandborn, W. J. *Lancet* **2012**, *380* (9853), 1590-605.

41. Awla, D.; Abdulla, A.; Regnér, S.; Thorlacius, H. *Inflamm. Res.* **2011**, *60* (12), 1093-98.

42. Pierer, M.; Wagner, U.; Rossol, M.; Ibrahim, S. *PLoS ONE* **2011**, *6* (8), e23539.

43. den Dekker, W. K.; Cheng, C.; Pasterkamp, G.; Duckers, H. J. *Atherosclerosis* **2010**, *209* (2), 314-20.

44. Afrazi, A.; Sodhi, C. P.; Richardson, W.; Neal, M.; Good, M.; Siggers, R.; Hackam, D. J. *Pediatr. Res.* **2011**, *69* (3), 183-88.

45. Neu, J.; Walker, W. A. *N. Engl. J. Med.* **2011**, *364* (3), 255-64.

46. Sharma, A. A.; Jen, R.; Butler, A.; Lavoie, P. M. *Clin. Immunol.* **2012**, *145* (1), 61-68.

47. Jilling, T.; Simon, D.; Lu, J.; Meng, F. J.; Li, D.; Schy, R.; Thomson, R. B.; Soliman, A.; Arditi, M.; Caplan, M. S. *J. Immunol.* **2006**, *177* (5), 3273-82.

48. Gribar, S. C.; Sodhi, C. P.; Richardson, W. M.; Anand, R. J.; Gittes, G. K.; Branca, M. F.; Jakub, A.; Shi, X. H.; Shah, S.; Ozolek, J. A.; Hackam, D. J. *J. Immunol.* **2009**, *182* (1), 636-46.

49. (a) Leaphart, C. L.; Cavallo, J.; Gribar, S. C.; Cetin, S.; Li, J.; Branca, M. F.; Dubowski, T. D.; Sodhi, C. P.; Hackam, D. J. *J. Immunol.* **2007**, *179* (7), 4808-20; (b) Richardson, W. M.; Sodhi, C. P.; Russo, A.; Siggers, R. H.; Afrazi, A.; Gribar, S. C.; Neal, M. D.; Dai, S.; Prindle, T., Jr.; Branca, M.; Ma, C.; Ozolek, J.; Hackam, D. J. *Gastroenterology* **2010**, *139* (3), 904-17, 17 e1-6; (c) Afrazi, A.; Sodhi, C. P.; Good, M.; Jia, H.; Siggers, R.; Yazji, I.; Ma, C.; Neal, M. D.; Prindle, T.; Grant, Z. S.; Branca, M. F.; Ozolek, J.; Chang, E. B.; Hackam, D. J. *J. Immunol.* **2012**, *188* (9), 4543-57.

50. Connolly, D. J.; O'Neill, L. A. J. *Curr. Opin. Pharmacol.* **2012**, *12* (4), 510-18.

51. Kim, H. M.; Park, B. S.; Kim, J.-I.; Kim, S. E.; Lee, J.; Oh, S. C.; Enkhbayar, P.; Matsushima, N.; Lee, H.; Yoo, O. J.; Lee, J.-O. *Cell* **2007**, *130* (5), 906-17.

52. Rossignol, D. P.; Wong, N.; Noveck, R.; Lynn, M. *Innate Immun.* **2008**, *14* (6), 383-94.

53. (a) Bazin, H. G.; Murray, T. J.; Bowen, W. S.; Mozaffarian, A.; Fling, S. P.; Bess, L. S.; Livesay, M. T.; Arnold, J. S.; Johnson, C. L.; Ryter, K. T.; Cluff, C. W.; Evans, J. T.; Johnson, D. A. *Bioorg. Med. Chem. Lett.* **2008**, *18* (20), 5350-54; (b) Dunn-Siegrist, I.; Tissières, P.; Drifte, G.; Bauer, J.; Moutel, S.; Pugin, J. *J. Biol. Chem.* **2012**, *287* (20), 16121-31; (c) Fort, M. M.; Mozaffarian, A.; Stöver, A. G.; Correia, J. d. S.; Johnson, D. A.; Crane, R. T.; Ulevitch, R. J.; Persing, D. H.; Bielefeldt-Ohmann, H.; Probst, P.; Jeffery, E.; Fling, S. P.; Hershberg, R. M. *J. Immunol.* **2005**, *174* (10), 6416-23.

54. Hutchinson, M. R.; Zhang, Y.; Shridhar, M.; Evans, J. H.; Buchanan, M. M.; Zhao, T. X.; Slivka, P. F.; Coats, B. D.; Rezvani, N.; Wieseler, J.; Hughes, T. S.; Landgraf, K. E.; Chan, S.; Fong, S.; Phipps, S.; Falke, J. J.; Leinwand, L. A.; Maier, S. F.; Yin, H.; Rice, K. C.; Watkins, L. R. *Brain, Behav., Immun.* **2010**, *24* (1), 83-95.

55. Slivka, P. F.; Shridhar, M.; Lee, G.-i.; Sammond, D. W.; Hutchinson, M. R.; Martinko, A. J.; Buchanan, M. M.; Sholar, P. W.; Kearney, J. J.; Harrison, J. A.; Watkins, L. R.; Yin, H. *ChemBioChem* **2009**, *10* (4), 645-49.

56. (a) Filice, M.; Palomo, J. M. *RSC Adv.* **2012**, *2* (5), 1729-42; (b) Wang, Z.; Du, J.; Che, P.-L.; Meledeo, M. A.; Yarema, K. J. *Curr. Opin. Chem. Biol.* **2009**, *13* (5–6), 565-72.

57. Weymouth-Wilson, A. C. *Nat. Prod. Rep.* **1997**, *14* (2), 99-110.

58. (a) Banoub, J.; Boullanger, P.; Lafont, D. *Chem. Rev.* **1992**, *92* (6), 1167-95; (b) Herzner, H.; Reipen, T.; Schultz, M.; Kunz, H. *Chem. Rev.* **2000**, *100* (12), 4495-538.

59. (a) Bode, L.; Kunz, C.; Muhly-Reinholz, M.; Mayer, K.; Seeger, W.; Rudloff, S. *Thromb. Haemost.* **2004**, *92* (6), 1402-10; (b) Kunz, C.; Rudloff, S. *Adv. Exp. Med. Biol.* **2008**, *606*, 455-65.

60. Bode, L.; Rudloff, S.; Kunz, C.; Strobel, S.; Klein, N. *J. Leukoc. Biol.* **2004**, *76* (4), 820-26.

61. Bode, L.; Jantscher-Krenn, E. *Adv. Nutr.* **2012**, *3* (3), 383S-91S.

62. Anderson, J. W.; Nicolosi, R. J.; Borzelleca, J. F. *Food Chem. Toxicol.* **2005**, *43* (2), 187-201.

63. Chen, C.-L.; Liang, C.-M.; Chen, Y.-H.; Tai, M.-C.; Lu, D.-W.; Chen, J.-T. *Invest. Ophthalmol. Visual Sci.* **2012**, *53* (4), 2281-91.

64. Sakai, S.; Sugawara, T.; Kishi, T.; Yanagimoto, K.; Hirata, T. *Life. Sci.* **2010**, *86* (9-10), 337-43.

65. Ciszewicz, M.; Wu, G.; Tam, P.; Polubinska, A.; Breborowicz, A. *Transl. Res.* **2007**, *150* (6), 337-42.

66. Darley-Usmar, V. M.; Ball, L. E.; Chatham, J. C. *J. Mol. Cell. Cardiol.* **2012**, *52* (3), 538-49.

67. (a) Stöver, A. G.; Da Silva Correia, J.; Evans, J. T.; Cluff, C. W.; Elliott, M. W.; Jeffery, E. W.; Johnson, D. A.; Lacy, M. J.; Baldridge, J. R.; Probst, P.; Ulevitch, R. J.; Persing, D. H.; Hershberg, R. M. *J. Biol. Chem.* **2004**, *279* (6), 4440-49; (b) Bowen, W. S.; Minns, L. A.; Johnson, D. A.; Mitchell, T. C.; Hutton, M. M.; Evans, J. T. *Sci. Signal.* **2012**, *5* (211), ra13.

68. Wittmann, V.; Lennartz, D. *Eur. J. Org. Chem.* **2002**, *2002* (8), 1363-67.

69. Debenham, J.; Rodebaugh, R.; Fraser-Reid, B. *Liebigs Ann.* **1997**, *1997* (5), 791-802.

70. Lemieux R, U.; Takeda, T.; Chung B, Y., Synthesis of 2-Amino-2-deoxy-β-D-glucopyranosides: Properties and Use of 2-Deoxy-2-phthalimidoglycosyl Halides. In *Synthetic Methods for Carbohydrates*, AMERICAN CHEMICAL SOCIETY: 1977; Vol. 39, pp 90-115.

71. (a) Debenham, J. S.; Madsen, R.; Roberts, C.; Fraser-Reid, B. *J. Am. Chem. Soc.* **1995**, *117* (11), 3302-03; (b) Castro-Palomino, J. C.; Schmidt, R. R. *Tetrahedron Lett.* **1995**, *36* (30), 5343-46.

72. Shimizu, H.; Ito, Y.; Matsuzaki, Y.; Iijima, H.; Ogawa, T. *Biosci., Biotechnol., Biochem.* **1996**, *60* (1), 73-76.

73. (a) Meinjohanns, E.; Meldal, M.; Paulsen, H.; Bock, K. *J. Chem. Soc., Perkin Trans. 1* **1995**, (4), 405-15; (b) Jensen, K. J.; Hansen, P. R.; Venugopal, D.; Barany, G. *J. Am. Chem. Soc.* **1996**, *118* (13), 3148-55.

74. (a) Wolfrom, M. L.; Bhat, H. B. *J. Org. Chem.* **1967**, *32* (6), 1821-23; (b) Blatter, G.; Beau, J.-M.; Jacquinet, J.-C. *Carbohydr. Res.* **1994**, *260* (2), 189-202.

75. zu Reckendorf, W. M.; Wassiliadou-Micheli, N. *Chem. Ber.* **1970**, *103* (6), 1792-96.

76. (a) Imoto, M.; Yoshimura, H.; Shimamoto, T.; Sakaguchi, N.; Kusumoto, S.; Shiba, T. *Bull. Chem. Soc. Jpn.* **1987**, *60* (6), 2205-14; (b) Paulsen, H.; Krogmann, C. *Liebigs Ann. Chem.* **1989**, *1989* (12), 1203-13; (c) Ellervik, U.; Magnusson, G. *Carbohydr. Res.* **1996**, *280* (2), 251-60; (d) Dullenkopf, W.; Castro-Palomino, J. C.; Manzoni, L.; Schmidt, R. R. *Carbohydr. Res.* **1996**, *296* (1–4), 135-47.

77. Castro-Palomino, J. C.; Schmidt, R. R. *Tetrahedron Lett.* **1995**, *36* (38), 6871-74.

78. E. Aly, M. R.; Castro-Palomino, J. C.; I. Ibrahim, E.-S.; El-Ashry, E.-S. H.; Schmidt, R. R. *Eur. J. Org. Chem.* **1998**, *1998* (11), 2305-16.

79. Castro-Palomino, J. C.; Schmidt, R. R. *Tetrahedron Lett.* **2000**, *41* (5), 629-32.

80. (a) Lemieux, R. U.; Ratcliffe, R. M. *Can. J. Chem.* **1979**, *57* (10), 1244-51; (b) Paulsen, H. *Angew. Chem., Int. Ed.* **1982**, *21* (3), 155-73; (c) Schmidt, R. R.; Kinzy, W., Anomeric-Oxygen Activation for Glycoside Synthesis: The Trichloroacetimidate Method. In *Advances in Carbohydrate Chemistry and Biochemistry*, Derek, H., Ed. Academic Press: 1994; Vol. Volume 50, pp 21-123.

81. Griffith, D. A.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1990**, *112* (15), 5811-19.

82. Dahl, R. S.; Finney, N. S. *J. Am. Chem. Soc.* **2004**, *126* (27), 8356-57.

83. Traar, P.; Belaj, F.; Francesconi, K. A. *Aust. J. Chem.* **2004**, *57* (11), 1051-53.

84. Knapp, S.; Huhn, R. A.; Amorelli, B. *Org. Synth.* **2007**, *84*, 68-76.

85. (a) Nakabayashi, S.; Warren, C. D.; Jeanloz, R. W. *Carbohydr. Res.* **1986**, *150* (1), c7-c10; (b) Norberg, O.; Deng, L.; Aastrup, T.; Yan, M.; Ramström, O. *Anal. Chem.* **2010**, *83* (3), 1000-07; (c) Love, K. R.; Andrade, R. B.; Seeberger, P. H. *J. Org. Chem.* **2001**, *66* (24), 8165-76.

86. (a) Srivastava, V. K. *Carbohydr. Res.* **1982**, *103* (2), 286-92; (b) Hesek, D.; Suvorov, M.; Morio, K.-i.; Lee, M.; Brown, S.; Vakulenko, S. B.; Mobashery, S. *J. Org. Chem.* **2004**, *69* (3), 778-84.

87. Takeda, R.; Yong Ryu, S.; Hill Park, J.; Nakanishi, K. *Tetrahedron* **1990**, *46* (16), 5533-42.

88. Lubineau, A.; Queneau, Y. *J. Org. Chem.* **1987**, *52* (6), 1001-07.

89. Gruseck, U.; Heuschmann, M. *Tetrahedron Lett.* **1987**, *28* (48), 6027-30.

90. (a) Jorand-Lebrun, C.; Pauwels, P. J.; Palmier, C.; Moret, C.; Chopin, P.; Perez, M.; Marien, M.; Halazy, S. *J. Med. Chem.* **1997**, *40* (24), 3974-78; (b) Glennon, R. A.; Slusher, R. M.; Lyon, R. A.; Titeler, M.; McKenney, J. D. *J. Med. Chem.* **1986**, *29* (11), 2375-80.

91. Grigorjeva, A.; Jirgensons, A.; Domracheva, I.; Yashchenko, E.; Shestakova, I.; Andrianov, V.; Kalvinsh, I. *Chem. Heterocycl. Comp.* **2009**, *45* (2), 161-68.

92. (a) Shaikh, N. S.; Junge, K.; Beller, M. *Org. Lett.* **2007**, *9* (26), 5429-32; (b) Punidha, S.; Agarwal, N.; Burai, R.; Ravikanth, M. *Eur. J. Org. Chem.* **2004**, *2004* (10), 2223-30.

93. Lozanova, A. V.; Moiseenkov, A. M.; Semenovskii, A. V. *Russ. Chem. Bull.* **1981**, *30* (4), 619-23.

94. Natale, N. R.; Quincy, D. A. *Synth. Commun.* **1983**, *13* (10), 817-22.

95. Iglesias-Guerra, F.; Romero, I.; Alcudia, F.; Vega-Pérez, J. M. *Carbohydr. Res.* **1998**, *308* (1–2), 57-62.

96. (a) Tarasiejska, Z.; Jeanloz, R. W. *J. Am. Chem. Soc.* **1958**, *80* (23), 6325-27; (b) Deng, S.; Gangadharmath, U.; Chang, C.-W. T. *J. Org. Chem.* **2006**, *71* (14), 5179-85.

97. Matta, K. L.; Johnson, E. A.; Barlow, J. J. *Carbohydr. Res.* **1973**, *26* (1), 215-18.

98. Lemieux, R. U.; James, K.; Nagabhushan, T. L.; Ito, Y. *Can. J. Chem.* **1973**, *51* (1), 33-41.

99. (a) Sauer, G.; Matsui, M.; Bloch, R.; Liang, J. S.; Fukushima, D. K. *J. Org. Chem.* **1969**, *34* (11), 3525-30; (b) Kadokawa, J.-i.; Nagaoka, T.; Ebana, J.; Tagaya, H.; Chiba, K. *Carbohydr. Res.* **2000**, *327* (3), 341-44.