SYNTHESIS OF SITE SPECIFIC DNA METHYLATING COMPOUNDS
TARGETING PANCREATIC β-CELLS

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This project involves synthesizing compounds that are capable of producing cytotoxic N3-methyladenine DNA adducts in pancreatic β-cells. The compounds will consist of three units: 1) A cell targeting ligand, glucose, which is specific to the insulin producing pancreatic β-cells, 2) a DNA methylating unit, Me-lex, which has been shown to selectively make N3-methyladenine adducts, and 3) linker component which will connect the two units previously mentioned. The linker portion is critical in the binding of the compounds to DNA as well as the ability of the compounds to be transported into the pancreatic β-cells. Several stable analog compounds were synthesized to test the ability of the compounds to be transported selectively into pancreatic β-cells and test their binding to DNA.
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CHAPTER 1: INTRODUCTION
DNA-alkylating drugs are the earliest and most common treatment for cancer chemotherapy. DNA-alkylating drugs work by damaging (alkylating) DNA, thereby killing cells. Normal cells are able to overcome the damage caused by DNA-alkylating agents by going into cell cycle arrest until the damage gets repaired. Cancer cells on the other hand divide rapidly and so the DNA damage in these cells does not get repaired. Therefore cancer cells succumb to the treatment while normal cells do not. But, some normal cells like hair cells, and cells of the gastric lining normally divide rapidly (like cancer cells) and therefore get destroyed as a result of this treatment. This is the reason why cancer patients receiving the DNA alkylating drugs for treatment often lose their hair and develop gastric problems. These side effects result because DNA-alkylating drugs cannot be targeted only to cancer cells.

A more serious problem with DNA-alkylating drugs is that they can cause mutations. Mutations are a result of the wide spread damage inflicted on DNA. These mutations can develop into a secondary cancer, which is a serious side effect for cancer chemotherapy patients. It would be very advantageous to develop new drugs that do not cause mutations and lead only to cell death (cytotoxicity).

Me-lex, a molecule well described in literature is a DNA-alkylating (methylating) compound that produces only one kind of damage to DNA. This particular damage leads to cell death and not to mutations, due to the formation of a DNA adduct known as N3-methyladenine (3-MeA). Therefore, Me-lex could be used to destroy cancer cells. However, Me-lex has no cell specificity. If Me-lex could be targeted to a particular cell (cancer cells) it would be possible to destroy those cells without mutations. The ability to target a specific cell requires a cell-specific ligand to be attached to Me-lex.
In order to target a cell, a cell-specific ligand has to be identified. The cell-targeting ligand has to be easy to synthesize, and should be able to maintain its cell-targeting ability upon modification. Streptozotocin (STZ), Figure 1, is a potent DNA-alkylating drug that targets the insulin producing pancreatic β-cells because of its specific transport through the GLUT-2 glucose transporter.\textsuperscript{9-10} The glucose unit in STZ is believed to be responsible for its selective transport. The high levels of the GLUT-2 transporter on the surface of pancreatic β-cells makes STZ very toxic to these cells.\textsuperscript{11} Streptozotocin was developed to study diabetes in mice by analyzing the sequence of events in pancreatic β-cell destruction (type-1 diabetes).\textsuperscript{12-15} There is evidence that large molecules, such as porphoryin rings connected to glucose can be transported by glucose transporters into the pancreatic β-cell.\textsuperscript{16} Therefore it is likely that Me-lex attached to glucose would be transported into the pancreatic β-cells too.
Figure 1.1: Structure of Streptozotocin (STZ).
The ability to target Me-lex to pancreatic β-cells by attaching it to glucose will provide proof that this strategy can be used to destroy unwanted cells by targeting Me-lex to those cells. An added advantage of targeting Me-lex to the pancreatic β-cells is that it could be used as a new model for studying type-1 diabetes.\textsuperscript{11-13} STZ is currently used to induce diabetes in animals. However, due to the extensive DNA damage, and consequent mutations caused by STZ, animals treated with STZ develop tumors. DNA damage caused by Me-lex would not cause such tumors.

The goal of this thesis is to synthesize new compounds that incorporate the pancreatic β-cells-targeting abilities of glucose and the selective DNA-methylating abilities of Me-lex into a single molecule. Such molecules would have the ability to selectively deliver cytotoxic damage only to pancreatic β-cells.
CHAPTER 2: BACKGROUND
2.1. Background:

The molecules designed and synthesized for this project should be able to target the pancreatic β-cells via the GLUT-2 cells, bind to the minor groove of DNA at A/T rich regions within those cells, and methylate the N3 position of adenine on DNA. Therefore designing compounds that have these three properties requires a good understanding of DNA structure and reactivity, and of the characteristics that enable transport through the GLUT-2 glucose transporter.

2.1.1. DNA Structure and Reactivity:

DNA (deoxyribose nucleic acid) is composed of two strands held together through hydrogen bonding. Each strand has a phosphate, sugar, and base. The phosphate and sugar form the backbone. The bases are adenine (A), thymine (T), guanine (G) and cytosine (C). Hydrogen bonding between the bases brings the two complementary strands together to form a double helix; A and T are paired together by two hydrogen bonds and G and C are paired together by three hydrogen bonds. Figure 2.1 shows the structure of B-DNA. This double helix is a right-handed spiral which results in two distinct grooves, the minor groove and the major groove. The minor groove is deep and narrow and therefore sites within the minor groove are more difficult to access. The major groove is wide and shallow making the sites within the major groove easier to access. Typically, most proteins and enzymes interact with DNA in the major groove.
Figure 2.1: Structure of B-DNA.
Figure 2.2 represents the hydrogen bonding interactions between the G/C base pairs and the A/T base pairs. There are sites on each base pair that lie in the major groove and sites that lie in the minor groove as shown. The most accessible sites in the major groove at a G/C base pair are the N7 of guanine, O\(^6\) of guanine, and N\(^4\) of cytosine. The sites on the G/C base pair that are in the minor groove and usually difficult to access are the N3 of guanine, N\(^2\) of guanine, and O\(^2\) of cytosine. The sites on the A/T base pair that are easily accessible in the major groove are the N7 of adenine, O\(^4\) of thymine, and N\(^6\) of adenine. The sites on the A/T base pair that are less accessible are the minor groove sites O\(^2\) of thymine and N3 of adenine. The site of particular interest for this project is the N3 position of adenine located, within the minor groove.
Figure 2.2: Hydrogen bonding interactions between the DNA base pairs.
2.1.2. Reactivity of DNA towards alkylating agents:

There are many sites that can be alkylated on DNA. Some of these sites lie within the minor groove, some lie within the major groove, and some are located on the sugar-phosphate backbone. In Figure 2.3, the arrows represent all the sites that can be alkylated by alkylating agents.\textsuperscript{1-2, 7, 17-18} Alkylation at some of these sites can lead to the death of a cell. Alkylation at other sites can lead to mutations, which is usually an undesired effect. There are some sites at which alkylation has no consequence and there are some sites at where alkylation leads to both cell death mutations.

In the major groove, methylation at the N7 of guanine is benign; however, methylation/alkylation at the O\textsuperscript{6} of guanine is known to cause both mutations and cell death.\textsuperscript{1-2, 7, 17-19} A general trend appears to be that alkylation at sites involved in the hydrogen bonding of the base pairs results in mutations while alkylation at other sites usually does not lead to mutations. There is evidence that methylation at the N3 position of adenine in the minor groove causes only cell death and not mutations.\textsuperscript{7, 17, 19-2} Therefore the N3 position of adenine would be an attractive site on DNA to methylate in order to bring about cell death.

There are multiple nucleophilic sites on DNA that can react with alkylating agents. The most nucleophilic and accessible site on DNA and therefore the most commonly alkylated site is the N7 of guanine in the major groove. Alkylation in the minor groove is less uncommon do to the sites being less accessible. Alkylation at each of these sites can lead to different biological consequences.
Figure 2.3: Methylation/alkylation sites in the major and minor groove of DNA are indicated by arrows.
2.1.3. Biological consequences of DNA methylation:

The biological consequences of DNA alkylation depend upon the site of alkylation and the alkylating unit. Methylation at the N7 of guanine in the major groove appears to have no biological consequence.\textsuperscript{1, 7, 17-18} The O\textsuperscript{6} of guanine, also in the major groove, and involved in hydrogen bonding interactions with cytosine is another commonly methylated site. Methylation at this site can result in both mutations and cell-toxicity.\textsuperscript{1, 7, 17-18}

Methylation at other sites that can result in mutations are the N\textsuperscript{2} of guanine, the O\textsuperscript{2} and O\textsuperscript{4} of thymine, and O\textsuperscript{2} of cytosine.\textsuperscript{1, 7, 17} However, methylation at the N3 site of adenine, in the minor groove, has shown to result in cytotoxicity, without leading to mutations.\textsuperscript{7, 17, 19-21} Thus compounds that can exclusively make 3-MeA adducts can be used to kill cells without any risk of mutations.
2.1.4. Structure and Properties of Me-lex:

Me-lex is known to bind to the minor groove of DNA at A/T rich regions and methylate the N3 position of adenine. Me-lex (Figure 2.4a) is a crescent shaped, neutral compound that consists of an N-methylpyrrolecarboxamide dipeptide (lex), with a propyl group at one end (C-terminus), and a reactive methyl sulfonate at the other end (N-terminus). Me-lex binds selectively to A/T rich regions in the minor groove due to specific interactions with bases in these regions. The crescent shape allows Me-lex to fit perfectly within the minor groove as illustrated in Figure 2.4b. Me-lex establishes favorable hydrogen bonding interactions between the N-H of the amide hydrogens of Me-lex and the N3 position of adenine and the O² of thymine in these regions (Figure 2.5a). Me-lex also establishes numerous van der Waal contacts that aid in its binding with DNA. Typically, Me-lex requires three to four A/T base pairs in a sequence, without any interfering guanine bases for it to bind to these regions. The reason it does not bind to the G/C base pair regions in the minor groove is because the exocyclic amine on the C2 position of guanine is located in the minor groove and causes steric hindrance with the C-H on the pyrrole of Me-lex (Figure 2.5b).
Figure 2.4: a) Structure of Me-lex. b) Computational model showing Me-lex bound within the minor groove of DNA.
Figure 2.5: a) Me-lex’s hydrogen bonding interactions with adenine in the minor groove.

b) Steric hindrance between Me-lex and guanine in the minor groove.
When Me-lex binds selectively to the A/T rich regions in the minor groove; the molecule places the reactive methyl sulfonate group at these A/T rich regions as shown in Figure 2.6. In these regions the N3 position of adenine is the most nucleophilic site. Therefore, Me-lex exclusively methylates DNA at this site; resulting in > 95 % of the methylated DNA-adducts formed by Me-lex being 3-MeA adducts. Once the reactive methyl group is transferred to the N3 position of adenine, the resulting sulfonate anion with a negative charge, gets eliminated from DNA due to electrostatic repulsion with the negatively charged phosphate backbone of DNA. Thus, this molecule functions as an efficient delivery agent for the methyl group and does not have any further biological consequences of its own by staying associated with the DNA.
Figure 2.6: DNA-methylation at A/T rich regions in the minor groove of DNA.
2.1.5. Biological consequences of 3-MeA formation by Me-lex:

The 3-MeA adducts formed by Me-lex have been shown to be cytotoxic in yeast, E. coli, and mammalian cells.\textsuperscript{19-23} It has also been shown that the extent of cell death correlates to the levels of 3-MeA adducts formed.\textsuperscript{19-23} The 3-MeA adducts formed by Me-lex have also been shown to be non-mutagenic. While Me-lex has attractive properties in terms of cell toxicity, its development into a useful drug is limited by the fact that it has no cell specificity. The ability to deliver Me-lex to a particular cell would aid in the development of drugs for cancer chemotherapy. The goal of this project is to develop new compounds which can use the selective DNA-methylating property of Me-lex and deliver it to a cell of interest.
2.1.6. DNA methylation of Streptozocin:

Streptozocin (STZ, Figure 2.7) is a potent DNA-methylating agent that is known to methylate DNA at multiple sites and has been shown to have selective pancreatic β-cell toxicity.\textsuperscript{9-11, 13-15} STZ has a glucose unit to which it is attached to a N-methyl-N-nitrosurea unit. The nitrosurea unit methylates the DNA and it is believed that the glucose unit is responsible for the ability of STZ to target pancreatic β-cells due to selective transport through the low affinity glucose transporter, GLUT-2, that is present on the surface of the pancreatic β-cells.\textsuperscript{24, 25, 27}

Methylation by STZ occurs at multiple sites on DNA. The sites that can be methylated by STZ include: N7 of guanine, O\textsuperscript{6} of guanine, N\textsuperscript{7} of adenine, and N\textsuperscript{3} of adenine.\textsuperscript{9} Over 70 % of the DNA adducts formed by STZ is N7-methylguanine, which is a begin adduct.\textsuperscript{1,7, 9, 17, 18} STZ also forms the O\textsuperscript{6}–methylguanine adduct in the major groove, which is known to cause both mutations and cell death.\textsuperscript{1, 7, 17, 18} The formation of O\textsuperscript{6}-MeG adduct by STZ is believed to result in tumors. It is likely that STZ kills pancreatic β-cells due to the formation of 3-MeA adducts and O\textsuperscript{6} - MeG.\textsuperscript{7, 14}

STZ has been used for studying type-1 diabetes by killing pancreatic β-cells in rodent (mice) models.\textsuperscript{12, 14} However, in these cases the study of type-1 diabetes is complicated by the fact that STZ causes extensive damage to DNA at multiple sites resulting in mutations which cause tumors.
Figure 2.7: Structure of Streptozotocin (STZ).
The glucose unit of STZ is responsible for targeting it specifically to pancreatic β-cells. The glucose unit has also been known to target other molecules to pancreatic β-cells through the GLUT-2 glucose transporter. Figure 2.8a shows another small compound containing the glucose unit that has been known to selectively target the pancreatic β-cells. There is a recent patent\textsuperscript{16} that describes porphoryin-glucose conjugates in photodynamic therapy for cancer (Figure 2.8b). Their uptake in these cancer cells was believed to involve glucose transporters on the surface of the cells. All the evidence indicates that it should be possible to deliver a compound like Me-lex to the pancreatic β-cells by attaching glucose to Me-lex at an appropriate location.
Figure 2.8: a) Chlorozotocin is a small molecule that can be transported by glucose transporters. 

b) Structure of Pyro-2DG.
2.2. Design of molecules:

The goal of this project is to develop new compounds which can produce 3-MeA adducts selectively in pancreatic β-cells. New molecules have been designed (Figure 2.9) that include glucose as the cell-targeting component, and Me-lex as the DNA-methylating component. The design of the new molecules must be such that the glucose unit maintains its ability to be transported by the GLUT-2 glucose transporter, and that the bis-pyrrole unit maintains its ability to selectively bind to the minor groove of DNA at A/T rich regions, and methylate the N3 position of adenine. As seen in the figure 2.9, the new compounds have the glucose unit connected to the C-terminus of the DNA-binding unit (bis-pyrrole dipeptide) while the DNA-methylating unit is connected to the N-terminus at the other end.

The bis-pyrrole and the glucose unit will be connected by a linker. The linker is the only part of the molecule that can be varied in this design. The linker has to be such that it optimizes the DNA-methylating and cell targeting functions of the molecules. The linker can also be modified, if necessary, to improve DNA-binding of the compounds, as well as increase water solubility. For this project the linker was varied only in length (R = (CH₂)ₙ were n = 1, 2, 3).
Figure 2.9: Design of molecules for this project.
CHAPTER 3: SYNTHESIS OF FINAL COMPOUNDS
3.1. Synthetic Strategy:

The synthesis of compounds that can specifically target pancreatic β-cells, selectively bind to the minor groove of DNA at A/T rich regions within those cells, and methylate adenines within those regions, can be divided into four components. These components include a cell-targeting unit (glucose), a DNA-binding unit (bis-pyrrole dipeptide), a linker unit (R), and a DNA-methylating unit (methyl sulfonate). Each of these components was synthesized/obtained separately and functionalized appropriately so that they could be assembled together to form the desired compounds described below.

Figure 3.1 shows the overall synthetic strategy for assembling the four components. DNA-binding unit consists of a bis-pyrrole dipeptide to which other components will be added on either side to form amide linkages. Therefore the DNA-binding unit was constructed with an amine at one end (N-terminus) of the molecule and a carboxylic acid at the other end (C-terminus). Linker units were obtained commercially as amino esters. The amines of the linkers were connected to the C-terminus of the bis-pyrrole dipeptide to form an amide linkage. Subsequently, the ester at the other end of the linker was hydrolyzed into a carboxylic acid and condensed with the amine of cell-targeting unit. The N-terminus of the bis-pyrrole was then derivatized and converted into the reactive methyl sulfonate.

Certain considerations were taken into account while constructing and assembling these components. The DNA-methylating methyl sulfonate is a reactive group and therefore has to be added at the last step in the synthesis. The cell-targeting unit (glucose) has several hydroxyl (OH) groups that have to be masked so that they do not interfere with the various reactions and manipulations that are required for synthesizing and assembling the final compounds. The
hydroxyl groups would be regenerated just before the introduction of the reactive methyl sulfonate.
Figure 3.1: Synthetic strategy.
3.2. Synthesis of cell targeting ligand:

The cell-targeting ligand, glucose, has four nucleophilic OH groups and one nucleophilic amine group. The amine of glucose has to be condensed with the carboxylic acid at the C-terminus of the DNA-binding unit. In order to prevent interference from the OH groups during the condensation they have to be protected first. Two different protecting groups were considered. Protection with acetyl groups can be easily accomplished in high yields using acetic anhydride, and the resulting compounds are easily purified and characterized. Subsequently, deprotection can be achieved using basic conditions, but it is also possible to deprotect the acetyl groups using strong acid. Therefore strongly acidic or basic conditions used during chemical manipulations may result in premature deprotection of the OH groups. Protection with benzyl groups resulted in lower yields than the acetyl protection, and resulted in compounds that are more difficult to characterize by NMR due to the overwhelming number of aromatic hydrogens in the spectrum. However, the benzyl groups are stable to acidic and basic conditions, can be removed in a single hydrogenation step, and the only byproduct formed upon deprotection is toluene which can be easily removed by rotary evaporation.

The protection of oxygens on glucose by both protecting groups is well described in literature,\textsuperscript{28,35} and outlined in Schemes 3.2.1 and 3.2.2. In both cases the synthesis begins with commercially available D(+) glucosamine hydrochloride. The amine is first masked as the imine (10) and then the OH groups are protected with either the acetyl (11) or benzyl groups (40). Subsequently the imine is converted back to the amine (12, 41). The protected glucosamines 12 and 41 were synthesized in large quantities and stored as their hydrochloride salts until required.
3.3 Synthesis of DNA-binding unit:

The DNA-binding unit was synthesized as described in literature with certain modifications. This modified procedure has been optimized in our laboratory earlier. The synthetic sequence that was used is outlined in Scheme 3.3.1.
Commercially available N-methylpyrrole was converted into the trichloroacetyl compound 1 and then nitrated to give 2, which was then reacted with the amines of various linkers to give compound 3 in excellent yields. The nitro compound 3 was reduced to an amine and condensed with 1 to afford the bis-pyrrole unit 5 in excellent yields. The nitro group on compound 5 was then reduced to the amine and condensed with acryloyl chloride to afford alkene 14. Compound 14 now contains the bis-pyrrole triamide core required for selective recognition of the minor groove of DNA at A/T rich regions, and also incorporates the linker unit that will connect this molecule to the cell targeting ligand. The ester on compound 14 was hydrolyzed into the carboxylic acid in nearly quantitative yields for subsequent condensation with the amine on the targeting ligand to give 15 in good yields. The overall yield of 15 starting from N-methyl pyrrole was 34 %.
3.4. Assembly of DNA-binding unit and cell targeting unit:

The DNA-binding unit in the form of compound 15 (Figure 3.2a), has an alkene at the N-terminus that can be converted into the methyl sulfonate, and has a carboxylic acid at the C-terminus which can be combined with the amines of 12 and 41 (Figure 3.2 b & c, respectively) by forming amide linkages.
Figure 3.2: a) DNA-recognition unit 15 functionalized as an alkene-carboxylic acid. b) Acetyl protected glucosamine hydrochloride, 12. c) Benzyl protected glucosamine hydrochloride, 41.
The DNA-binding unit 15 with varying linker length (R = (CH$_2$)$_n$ were n = 1, 2, 3) was condensed with compound 12 to yield 16 as shown in Scheme 3.4.1.

Scheme 3.4.1

This condensation was achieved using standard carbodiimide coupling methods. The mechanism for this reaction using EDCI as the carbodiimide, is illustrated in Scheme 3.4.2a. EDCI is a water soluble carbodiimide that gets protonated at the imide nitrogens by the carboxylic acid 15 and then reacts with the carboxylat anion to form the activated O-acylurea intermediate. This activated carboxylic acid can react to form the desired product. However, this O-acylisourea intermediate can undergo rearrangement to form an unreactive N-acylurea which cannot react with an amine.$^{36}$

a) EDCI (1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride)

Scheme 3.4.2a
Therefore, HOBT is often used in these reactions to trap the intermediate, minimize the side reaction, and increase yields. The intermediate formed by HOBT can react with an amine to form the amide product and regenerate HOBT as illustrated in scheme 3.4.2b. It has been reported in literature and reaction conditions that the use of CuCl$_2$ and DMAP in these reactions can lower the formation of the N-acylurea intermediate and increase product yields. Therefore using a combination of these reagents to synthesize 16 resulted in yields greater than 85%.

Scheme 3.4.2b

The alkene, 16, now has to be converted into the sulfonic acid. This conversion was attempted using sodium bisulfite at pH 8 as shown in scheme 3.4.3.

Scheme 3.4.3
The reaction was monitored by TLC for the disappearance of the alkene. Once TLC indicated complete conversion of the alkene (only a baseline spot was observed) the solution was acidified with HCl and the solvent removed by rotary evaporation. However, NMR analysis of the crude product indicated that while the sulfonic acid had been successfully formed (indicated by the characteristic shifts of the two methylenes next to the sulfonic acid), the glucose unit had undergone some transformation, possibly deprotection, as indicated by the presence of multiple acetyl peaks integrating to less than what was expected, and by the presence of a number of glucose ring hydrogens. All attempts to isolate and characterize individual components from this mixture was unsuccessful.

If the glucose unit was indeed getting deprotected, it would not be a problem since the next step planned after the formation of the sulfonic acid was the deprotection of the glucose. In fact, it would be advantageous to accomplish both the conversion of the alkene into the sulfonic acid and deprotection of the glucose in a single step. However, it was unclear whether the deprotection was taking place during the reaction (since basic conditions are known to cause the deprotection) or during the subsequent acidification.

In order to address these questions and to identify the optimum conditions under which deprotection of the glucose unit could be achieved, investigations were carried out using the model compound shown in Figure 3.3. The use of the model compound was necessary to investigate the effects of various conditions on the glucose unit without any interference from other functional groups (present on 16) and also because this model compound was more easily obtained than compound 16.
Figure 3.3: Structure of model compound.
Model compound 26 was synthesized as shown in Scheme 3.4.4 and the subjected to the same reaction conditions that were used for the conversion of compound 16 to the sulfonic acid (see Scheme 3.4.3). Refluxing 26 under these mildly basic conditions for prolonged periods resulted in no alteration to compound 26, as verified by TLC and NMR. However, when compound 26 was subjected to acidic conditions (concentrated HCl) the glucose unit was clearly affected, as evidence by numerous peaks in the H$^1$ NMR spectrum in the regions where the acetyl hydrogens and the glucose ring hydrogens show up. However, acidification with 1M HCl did not affect the protected glucose unit.

Model compound 26 was also used to determine the optimum conditions for the deprotection reaction. The use of several different conditions are reported in literature for this purpose.$^{40-43}$ Some of these conditions were investigated with compound 26. The investigated conditions and the observed results are shown in Table 3.1.
Table 3.1: Various reaction conditions used for removal of acetyl groups from glucose.

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Temperature (0 °C)</th>
<th>Time</th>
<th>Acidification with</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>7N NH₃/MeOH</td>
<td>25</td>
<td>3 hrs.</td>
<td>-</td>
<td>Partial Deprotection</td>
</tr>
<tr>
<td>K₂CO₃/H₂O</td>
<td>25</td>
<td>4 hrs.</td>
<td>1M HCl</td>
<td>Partial Deprotection</td>
</tr>
<tr>
<td>6M HCl</td>
<td>85</td>
<td>1 hr</td>
<td>-</td>
<td>Partial Deprotection</td>
</tr>
<tr>
<td>1M HCl</td>
<td>25</td>
<td>24 hrs.</td>
<td>-</td>
<td>No Deprotection</td>
</tr>
<tr>
<td>NaOCH₃/MeOH</td>
<td>25</td>
<td>3 hrs.</td>
<td>Dowex 50 WX-4</td>
<td>Complete Deprotection</td>
</tr>
<tr>
<td>NaOCH₃/MeOH</td>
<td>-10</td>
<td>15 mins.</td>
<td>Dowex 50 WX-4</td>
<td>Complete Deprotection</td>
</tr>
<tr>
<td>NaOCH₃/MeOH</td>
<td>-78</td>
<td>1 hour</td>
<td>Dowex 50 WX-4</td>
<td>Partial Deprotection</td>
</tr>
</tbody>
</table>
The removal of the acetyl groups was monitored by NMR. Based upon these studies, the use of NaOCH₃/MeOH at -10 °C, followed by acidification with Dowex 50 WX-4 resin was adopted as the most suitable method for deprotection reactions as shown in Scheme 3.4.5. However, the NaOCH₃ used had to be freshly prepared; the use of NaOCH₃ prepared earlier resulted in incomplete deprotection.

![Diagram](image)

**Scheme 3.4.5**

While complete deprotection was achieved using these conditions as indicated by the complete disappearance of the acetyl CH₃ peaks in the NMR spectrum, two sets of peaks were observered for the glucose hydrogens. Based upon literature evidence,⁴⁴ and upon further NMR experiments (HMQC and COSY), it was determined that two anomeric forms of the glucose unit were present as shown in Figure 3.4.
Figure 3.4: a) Anomeric forms of 27. b) $^1$H NMR of both anomers in d$_6$-DMSO.
Based upon these studies with the model 26, compound 16 was first treated with NaHSO$_3$ at pH 8 in order to convert the alkene into the sulfonate, but the reaction was not acidified. This reaction requires the presence of oxygen for efficient conversion since it is believe to proceed through a radical mechanism$^{26,38}$ as shown in Scheme 3.4.6. The crude product obtained from the reaction was examined by NMR to confirm the disappearance of the alkene and the presence of the acetyl groups. Acidification of this product with 1M HCl and Dowex 50 WX-4 resin is currently being investigated.

Scheme 3.4.6

Since the conversion of alkene 16 to the desired sulfonic acid has proved to be difficult due to the instability of the acetyl protecting groups on the glucose to the reaction conditions, the acetyl protected glucose unit of 16 was replaced with benzyl protected glucose, since the benzyl groups is stable to both acidic and basic conditions. Thus, compound 41 was condensed with 15b using carbodiimide coupling as shown in Scheme 3.4.7 to yield 42.
Scheme 3.4.7

Compound 42 was converted into the sulfonate using NaHSO₃ at pH 8 as shown in Scheme 3.4.8. Protonation of the sulfonate with concentrated HCl successfully produced the sulfonic acid while leaving the benzyl groups intact. Compound 43 was purified by flash column chromatography.

Scheme 3.4.8

An attempts to remove the benzyl groups on the glucose of compound 43 are currently in progress. An initial attempt using H₂ (75 psi) and 10 % Pd on carbon as a catalyst was unsuccessful. However, evidence in literature indicates that other researchers have had similar problems in the removal of the benzyl groups using 10 % Pd/C. It has been reported that the use of palladium black as a catalyst will result in quantitative yields for debenzylation. The use of this new catalyst is currently being explored in the laboratory.
Once the benzyl groups can be successfully removed the next step is the methylation of the sulfonic acid to the methyl sulfonate as shown in Scheme 3.4.9. This methylation will be attempted using either trimethyl ortho formate or methyl-p-tolytriazine as the methylating agent. It is known that these methylating agents can methylate sulfonic acids of similar compounds, and it has been verified that they do not methylate the hydroxyl groups on the glucose. Once the sulfonic acid is methylated the compounds will be used in experiments with DNA to characterize its ability to methylate DNA.
Scheme 3.4.9

Deprotection of Benzyl groups

Methylation

Scheme 3.4.9
4.1. Significance:

The desired compounds for this project have to achieve two main functions in order to succeed in selectively destroying pancreatic β-cells. They should be efficiently transported by the GLUT-2 glucose transporter on the pancreatic β-cells, and they should exhibit DNA-binding properties comparable to Me-lex (and consequently similar DNA-methylation characteristics). Failure to perform either of these two functions efficiently will render the molecules incapable of destroying the pancreatic β-cells.

The linker is the component that is being varied in the design of the compounds in order to optimize both DNA-binding and transport via the GLUT-2 transporter. However the features of the linker that favor efficient DNA-binding may not be the same features that favor efficient transport through the transporter. Therefore, it is essential to determine the features influencing each of the two functions independently.

Since the target compounds are reactive methyl sulfonates that can react with various nucleophiles, stable analogs have to be synthesized for use in experiments to determine DNA-binding and cell transport properties. Therefore, for the DNA-binding studies model compounds were designed in which the reactive methyl sulfonate (Figure 4.1a) was replaced by a stable methyl sulfone (Figure 4.1b). In order to investigate cell transport, model compounds were designed in which the reactive methyl sulfonate was replaced by a fluorescent tag, NBD or coumarin (Figure 4.1c and d, respectively).
Figure 4.1: Compounds designed for a) DNA-methylation studies. b) DNA-binding studies.

c) Cell transport studies with NBD d) Cell transport studies with coumarin.
4.2. Synthesis of stable analogs for DNA-binding experiments:

A general synthetic route was first designed (scheme 4.2.1) that would result in a molecule having the methyl sulfone unit attached to the bis-pyrrole triamide with the linker attached at the other end and functionalized as a carboxylic acid. This molecule could then be attached to various targeting ligands for different projects.

Scheme 4.2.1

Compound 5b that was synthesized earlier (Scheme 3.3.1) was used as the starting point for determining the feasibility of this synthetic scheme. As shown in Scheme 4.2.2a the nitro group on compound 5b was reduced to an amine and condensed with 3-(methylsulfonyl)propanoic acid using carbodiimide coupling, to give compound 25b. The ethyl ester of compound 25b was then hydrolyzed using basic conditions. Hydrolysis of the ethyl ester was achieved, but the product that was isolated was the alkene 15b and not the desired product. Presumably, the basic conditions resulted in an elimination reaction as shown in Scheme 4.2.2b.
Various reaction conditions were explored such as altering temperature, solvents, reaction time, and the base used, but none of them resulted in the hydrolysis of the ester while leaving the sulfone intact. Therefore, an alternative synthetic procedure was adopted where in the ester was hydrolyzed and attached to the cell targeting ligand before the methyl sulfone was introduced. This alternative synthetic procedure is shown in Scheme 4.2.3. The ethyl ester of compound 5 (synthesized earlier, see Scheme 3.1.1) was converted to the carboxylic acid using base hydrolysis to give quantitative yields of 9. Compound 9 was then condensed with the targeting
ligand 12 using carbodiimide coupling to form compound 13. This nitro group on compound 13 was reduced to the amine and condensed with 3-(methylsulfonyl)propanoyl chloride to give 18.

Scheme 4.2.3

The acetyl groups of compound 18b were removed using freshly prepared NaOCH₃/MeOH at -10 °C in anhydrous THF. Dowex 50 WX-4 resin was subsequently used to acidify the reaction mixture. The optimization of this procedure has been described earlier in Section 3.4. This procedure resulted in the formation of compound 44b the desired product for DNA-binding experiments in quantitative yields as shown Scheme 4.2.4. The synthesis and isolation of compounds 44a and c are currently in progress. Compound 44b is soluble in water, which makes it convenient for binding studies which are conducted in an aqueous medium.
The methylsulfone compounds, with the targeting ligand glucose attached will be used to determine the strength of their binding interactions with the A/T rich binding site on DNA. The strength of their binding will be compared to the strength of binding of the methyl sulfone corresponding to Me-lex (compound 17, Scheme 4.2.5). If the DNA-binding of the new compounds is comparable to that of compound 17, then it is likely that the DNA-methylating characteristics of the newly synthesized methyl sulfonates with the glucose ligand attached will be similar to that of Me-lex. Compound 17 was synthesized as shown in Scheme 4.2.5 based on published procedures.²³
4.3. Synthesis of model compounds cell transport studies:

Model compounds containing fluorophores will be used to determine if the glucose unit is capable of transporting the DNA-binding bis-pyrrole unit into cells that contain the GLUT-2 transports. A comparison of the transport of fluorescently tagged compounds into cells expressing the transporter and cells lacking the transporter (available with collaborators) will provide the desired information. Therefore, the synthesis of compounds containing the fluorophores NBD or coumarin was attempted (Figure 4.2).
Figure 4.2: Compounds for cell transport studies a) NBD. b) Coumarin.
As shown in Scheme 4.3.1 commercially available NBD-Cl (4-chloro-7-nitro-2,1,3-benzoxadiazole) was condensed with β-alanine ethyl ester hydrochloride using triethylamine (TEA) to yield 33.

Scheme 4.3.1

However, the conversion of ester 33 into the carboxylic acid using base hydrolysis was unsuccessful. The proton NMR spectrum after the hydrolysis reaction indicated the presence of three aromatic hydrogens. Therefore an alternative procedure, described in literature, was used to make compound 19 by reacting β-alanine with NBD-Cl as shown in Scheme 4.3.2.

Scheme 4.3.2

Compound 19 was then condensed with 13 using carbodiimide coupling to give 21 as shown in Scheme 4.3.3.
Scheme 4.3.3

The final step in this synthesis is deacetylation of 21, which is yet to be accomplished. The acetyl groups will be removed using NaOCH₃ as outlined in Scheme 4.3.4.

Scheme 4.3.4

Once compound 21 is successfully deacetylated these compounds will be tested by collaborators to determine if they are transported by the GLUT-2 glucose transporter.
For the preparation of the model compound containing coumarin as the fluorescent tag (see Figure 4.2b). Compound 15b (synthesized earlier scheme 4.2.3) was reduced to an amine and condensed with a N-Boc-β-Alanine to form compound 29 as shown in Scheme 4.3.5.

Scheme 4.3.5

The Boc protecting group on 29 must be removed with strong acid in order to condense it with coumarin-3-carboxylic acid. Tetrafluoroacetic acid (TFA) was used to remove the Boc group, however, it also caused partial deprotection of the acetyl groups as determined by NMR. Therefore an alternative approach was adopted in which β-alanine ethyl ester hydrochloride was first attached coumarin-3-carboxylic acid as shown in Scheme 4.3.6.
Scheme 4.3.6

Commercially available β-alanine ethyl ester hydrochloride was condensed with the coumarin compound using carbodiimide coupling to yield 31. The ester of 31 was then hydrolyzed by using base hydrolysis at room temperature to give the carboxylic acid 32. The condensation of 32 with compound 15 is currently ongoing in the laboratory.
CHAPTER 5: EXPERIMENTAL
5.1. General:

All solvents and reagents were purchased with the highest grade available from VWR International (West Chester, Pennsylvania) or Sigma-Aldrich (Atlanta, Georgia). Flash chromatography was performed with silica gel 60 Geduran® (40-63 µm mesh, Merck). TLC was performed on aluminum plates coated with silica gel 60 (F₂₅₄, Merck) that had a fluorescence indicator and were detected by UV visualization. All rotary evaporations were carried out using a Buchi R-3000 or a Buchi R-114 rotary evaporator equipped with a Brinkman model B-16 vacuum aspirator. Hydrogenations were performed using a Parr Hydrogenation Apparatus in a 500 mL Parr jar. Melting points were determined using a Mel-Temp II.

All anhydrous reactions were carried out under positive pressure argon or nitrogen. Glassware for anhydrous reactions were dried overnight in the oven set at 150 °C, assembled while still hot, and cooled to room temperature under argon or nitrogen. Solvent and reagents for anhydrous reactions were purchased in sure-seal capped bottles and transferred to reactions by oven dried needles and glass syringes.

All ¹H-NMR and ¹³C-NMR spectra were obtained with a Bruker 400MHz NMR spectrometer, using deuterated D6-DMSO, deuterated chloroform, or deuterated methanol as the solvent. The deuterated D6-DMSO was obtained in sealed ampoules and 100 mL bottles from Sigma-Aldrich. The deuterated chloroform was obtained from Alfa Aesar. The deuterated methanol was obtained from Sigma-Aldrich. The spectra were reported in ppm and referenced to deuterated D6-DMSO (2.49 ppm for ¹H, 39.5 ppm for ¹³C) or referenced to chloroform (7.26 ppm for ¹H, 77 ppm for ¹³C). The NMR tubes were 5 mm Pyrex glass obtained from Wilmad-LabGlass, Buena, New Jersey.
5.2. Synthesis:

**2,2,2-Trichloro-1-(1-methyl-1\textsubscript{H}-pyrrol-2-yl)ethanone (1).** Trichloro acetyl chloride (50 mL) was added to 250 mL of dry dichloromethane in a 1000 mL flask flushed with argon. In a dropping funnel, N-methyl pyrrole (50 mL) was dissolved 100 mL of dry dichloromethane. This mixture was then added drop wise to the trichloro acetyl chloride and stirred overnight. To quench the reaction a solution of potassium carbonate (32 g in 250 mL of DI water) was added to the empty drop funnel and added over a two hour period. The mixture was then extracted with DI water (2 x 300 mL) and dichloromethane (1 x 300mL). The organic layer was dried over MgSO\textsubscript{4}, filtered, and the filtrate concentrated under rotary evaporation to produce an oily substance. A pure seed was added and the flask placed under vacuum until dry to yield a dark brown solid \textbf{1} (80.35 g, 80%): mp 51-57 °C. TLC (1:1 EtOAc/Hexane) R\textsubscript{f} = 0.56. \textsuperscript{1}H NMR data (CDCl\textsubscript{3}): δ 7.45 (dd, J = 1.6, 6 Hz, 1H), 6.94 (s, 1H), 6.17 (dd, J = 2.4, 6 Hz), 3.93 (s, 3H). \textsuperscript{13}C NMR data: δ 172.34, 135.80, 124.19, 121.18, 109.60, 96.55, 38.45.

**2,2,2-Trichloro-1-(1-methyl-4-nitro-1\textsubscript{H}-pyrrol-2-yl)ethanone (2).** Compound \textbf{1} (25 g) was dissolved in acetic anhydride (175 mL) in a 500 mL round bottom flask and cooled to -40 °C in a dry ice/acetone bath. After maintaining the temperature for 20 minutes fuming nitric acid (12.3 mL) was added slowly in a addition funnel for one hour with constant stirring. Stirring continued for an additional 45 minutes while keeping the temperature at -40 °C. The solution was then allowed to warm to room temperature over two hours. The flask was then immersed in an ice water bath as cold DI water (101 mL) was added slowly in portions over one hour. This mixture was then allowed to stir overnight at which point a brown-orange precipitate was formed and filtered under vacuum until dryness to give \textbf{2} (23.48 g, 80%): mp 112-120°C. TLC (1:1
EtOAc/Hexane) \( R_f = 0.84 \). \(^1\)H NMR data (CDCl\(_3\)): \( \delta \) 7.94 (d, \( J = 1.6 \) Hz, 1H), 7.75 (d, \( J = 1.6 \) Hz, 4.05 (s, 3H). \(^{13}\)C NMR data: \( \delta \) 173.31, 134.74, 133.08, 121.10, 116.81, 95.03,

**Ethyl 2-(1-methyl-4-nitro-1\(H\)-pyrrole-2-carboxamido)acetate (3a).** The nitro pyrrole 2 (30.01 g, 11.1 mmol) was added to a 500 mL round bottom flask followed by glycine ethyl ester hydrochloride (23.55 g, 25.5 mmol) and took up in EtOAc dried over sieves (100 mL). A drop funnel was fitted to reaction flask and filled with TEA (35.5 mL) dissolved in EtOAc dried over sieves (50 mL). Argon was bubbled through this solution, and this mixture was added drop wise over a period of 15 hours under argon. Once all of the TEA mixture was added, the reaction was allowed to stir for and additional 48 hours under argon. The white precipitate formed was removed by filtration and the filtrate was extracted with 1M HCl (2 x 100 mL) and DI water (1 x 100 mL). The organic layer was dried over MgSO\(_4\), filtered and the solution concentrated by rotary evaporation to give a yellow solid 3a (28.38 g, 100%): mp 98-103 °C. TLC (1:1 EtOAc /Hexane) \( R_f = 0.52 \). \(^1\)H NMR data (CDCl\(_3\)): \( \delta \) 7.51 (d, \( J = 1.6 \) Hz, 1H), 7.12 (d, \( J = 2 \) Hz, 1H), 6.48 (s, 1H), 4.20 (q, \( J = 6.8, 7.2 \) Hz, 2H), 4.08 (d, \( J = 5.2 \), 2H), 3.90 (s, 3H), 1.26 (t, \( J = 7.2 \) Hz, 3H). \(^{13}\)C NMR data: \( \delta \) 170.12, 160.71, 134.32, 128.67, 126.11, 108.33, 60.99, 41.16, 37.89, 14.55.

**Ethyl 3-(1-methyl-4-nitro-1\(H\)-pyrrole-2-carboxamido)propanoate (3b).** Compound 3b was synthesized by a procedure similar to the one described above for 3a using (30.02 g, 11.1 mmol) of 2, (17.05 g, 25.5 mmol) of β-alanine ethyl ester hydrochloride and (8.5 mL) of TEA to obtain 3b (27.85 g, 95%): mp 120-124 °C. TLC (1:1 EtOAc/Hexane) \( R_f = 0.42 \). \(^1\)H NMR data (CDCl\(_3\)): \( \delta \) 7.47 (d, \( J = 1.2 \) Hz, 1H), 6.99 (d, \( J = 1.6 \), 1H), 6.62 (s, 1H), 4.12 (q, \( J = 7.2 \), 2H), 3.91 (s, 3H), 3.58 (q, \( J = 6 \) Hz, 2H), 2.54 (t, \( J = 7.2 \) Hz, 2H), 1.22 (t, \( J = 7.2 \) Hz, 3H). \(^{13}\)C NMR data (CDCl\(_3\)): \( \delta \) 172.64, 160.21, 134.98, 126.64, 126.24, 106.98, 61.00, 37.86, 34.82, 33.81, 14.19.
**Ethyl 4-(1-methyl-4-nitro-1H-pyrrole-2-carboxamido)butanoate (3c).** Compound 3c was synthesized by a procedure similar to the one described above for 3a using (30.02 g, 11.1 mmol) of 2, (28.05 g, 25.5 mmol) of aminobutyrate hydrochloride and (35.5 mL) of TEA to obtain 3c (30.56 g, 98%): mp 54-59 °C. TLC (1:1 EtOAc/Hexane) \( R_f = 0.36 \). \(^1\)H NMR data (CDCl\(_3\)): δ 7.54 (d, \( J = 1.6 \) Hz, 1H), 7.13 (d, \( J = 1.6 \) Hz, 1H), 6.81 (s, 1H), 4.11 (q, \( J = 3.6 \) Hz, 7.2 Hz, 2H), 3.96 (s, 3H), 3.41 (q, \( J = 6.8 \) Hz, 6 Hz, 2H), 2.41 (t, \( J = 6.8 \) Hz, 2H), 1.92 (pentet, \( J = 6.8 \) Hz, 2H), 1.23 (t, \( J = 7.2 \) Hz, 3H). \(^{13}\)C NMR data (CDCl\(_3\)): δ 173.88, 160.53, 134.84, 126.69, 126.42, 107.03, 60.84, 39.25, 37.91, 31.98, 24.22, 21.07, 14.17.

**1-Methyl-4-nitro-N-propyl-1H-pyrrole-2-carboxamide (4).** In a flask flushed with Ar, the nitro pyrrole 2 (0.642 g, 2.37 mmol) was dissolved in EtOAc dried over sieves (5 mL), propylamine (870 µL) was added while stirring at room temperature for two days to yield 4 (0.455 g, 91%): mp 124-130 °C. TLC (1:1 Hexane/EtOAc) \( R_f = 0.53 \). \(^1\)H NMR data (CDCl\(_3\)): δ 7.54 (d, \( J = 1.6 \) Hz, 1H), 7.06 (d, \( J = 1.6 \) Hz, 1H), 6.09 (s, 1H), 3.98 (s, 3H), 3.35 (q, \( J = 6.8 \) Hz, 7.2 Hz, 2H), 1.61 (m, 2H), 0.97 (t, \( J = 7.6 \) Hz, 3H). \(^{13}\)C NMR data (CDCl\(_3\)): δ 160.37, 134.91, 126.54, 106.59, 41.28, 37.81, 22.85, 11.36.

**Ethyl 2-(1-methyl-4-(1-methyl-4-nitro-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamido acetate (5a).** The nitro pyrrole ester 3a (13.61 g, 53 mmol) was dissolved in ethanol (50 mL) in a 500 mL Parr jar, 10% water wet Pd/C (2.50 g) was added to this and the mixture was shaken on a hydrogenator under pressurized hydrogen (70 psi) until reaction was complete by TLC (100% EtOAc). The Pd/C was filtered through celite and the filtrate concentrated by rotary evaporation and kept under vacuum overnight to yield a pale yellow solid. The amine was dissolved in EtOAc (50 mL) dried over sieves and stirred with a mechanical stirrer. Once dissolved, compound 1 (14.47 g, 53 mmol) was added and allowed to stir for four
days. The mixture was concentrated until minimal amount of EtOAc was present. At which time the flask was placed in refrigerator over night to give yellow solid 5a (12.84 g, 79 %): mp 222-226 °C. TLC (100% EtOAc) Rf = 0.63. 1H NMR data (D6-DMSO): δ 10.26, 8.45 (t, J = 5.6 Hz, 1H), 8.18 (s, 1H), 7.58 (d, J = 2 Hz, 1H), 7.27 (s, 1H), 6.92 (s, 1H), 4.11 (q, J = 6.8 Hz, 7.2 Hz, 2H), 3.96 (s, 3H), 3.88 (d, J = 5.6 Hz, 2H), 3.81 (s, 3H), 1.21 (t, J = 7.2 Hz, 3H). 13C NMR data (D6-DMSO): δ 170.65, 161.84, 157.36, 134.24, 128.69, 126.73, 122.80, 121.97, 119.06, 108.07, 104.96, 60.81, 41.15, 37.93 36.60, 14.59.

**Ethyl 3-(1-methyl-4-(1-methyl-4-nitro-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamido) propanoate (5b).** The nitro pyrrole ester 3b (13.0 g, 48.3 mmol) was dissolved in ethanol (60 mL) in a 500 mL Parr jar, 10 % water wet Pd/C (1.0 g) was added to this and the mixture was shaken on a hydrogenator under pressurized hydrogen (70 psi) until reaction was complete by TLC (EtOAc). The mixture was then acidified with concentrated HCl (4.10 mL). The Pd/C was filtered through celite and the filtrate concentrated by rotary evaporation and kept under vacuum overnight to yield a yellow solid. In a 500 mL round bottom flask, this solid and nitro pyrrole 1 was dissolved in EtOAc (250 mL) dried over sieves. TEA (17.00 mL) dissolved in EtOAc was added drop wise using a drop funnel. The mixture was then allowed to stir 48 hours at which time a yellow precipitate formed. After the reaction was complete by TLC (EtOAc), the yellow precipitate was filtered then stirred in DI water for 1 hour and filtered again to give pure 5b (28.18 g 94%): mp 187-190 °C. TLC (EtOAc) Rf = 0.55. 1H NMR data (D6-DMSO): δ 10.22, 8.17 (s, 1H), 8.08 (t, J = 6 Hz, 1H), 7.57 (d, J = 2 Hz, 1H), 7.21 (s, 1H), 6.83 (d, J = 1.6 Hz, 1H), 4.06 (q, J = 6.8 Hz, 7.2 Hz, 2H), 3.95 (s, 3H), 3.81 (s, 3H), 3.40 (m, 2H), 1.18 (t, J = 6.8 Hz, 3H). 13C NMR data (D6-DMSO): δ 171.84, 161.64, 157.31, 134.23, 128.68, 126.74, 123.42, 121.81, 118.57, 108.01, 104.58, 60.36, 37.93, 36.50, 35.28, 34.46, 14.56.
Ethyl 4-(1-methyl-4-(1-methyl-4-nitro-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamido) butanoate (5c). Compound 5c was synthesized by a procedure similar to the one described above for 5b using (31.26 g, 0.11 mol) of pyrrole 3c, (29.25 g, 0.11 mol) of 1, and (37.6 mL) of TEA to obtain 5c (31.15 g, 80%): mp 134-137 °C. TLC (EtOAc) Rf = 0.54. 1H NMR data (CDCl3): δ 7.82 (s, 1H), 7.60 (d, J = 1.6 Hz, 1H), 7.22 (d, J = 2 Hz, 1H), 7.18 (d, J = 1.6 Hz, 1H), 6.54 (d, J = 1.6 Hz, 1H), 6.26 (t, J = 8 Hz, 1H), 4.13 (q, J = 7.2 Hz, 2H), 4.03 (s, 3H), 3.92 (s, 3H), 3.42 (q, J = 6.4 Hz, 2H), 1.92 (pentet, 6.8 Hz, 2H), 1.25 (t, J = 6.8 Hz, 3H).

13C NMR data (CDCl3): δ 173.19, 161.65, 157.31, 134.24, 128.68, 126.76, 123.63, 121.78, 118.45, 108.00, 104.49, 60.23, 38.19, 37.94, 36.49, 31.51, 25.15, 14.58.

1-Methyl-4-(1-methyl-4-nitro-1H-pyrrole-2-carboxamido)-N-propyl-1H-pyrrole-2-carboxamide (6). Compound 4 (9.75 g 46.2 mmol) was dissolved in ethanol (60 mL) in a 500 mL Parr jar, 10 % water wet Pd/C (5.00 g) was added to this and the mixture was shaken on a hydrogenator under pressurized hydrogen (70 psi) until reaction was complete by TLC (6:1 CHCl3/MeOH). The Pd/C was filtered through celite and the filtrate concentrated by rotary evaporation and kept under vacuum overnight. This amine and nitro pyrrole 1 (12.67 g, 46.2 mmol) was dissolved in EtOAc (20 mL) dried over sieves. The reaction was stirred for 48 hours at room temperature at which point a yellow precipitate formed. After reaction was complete by TLC (6:1 CHCl3/MeOH) the yellow precipitate was filtered to give pure 6 (8.78 g, 56 %): mp 220-222 °C. TLC (6:1 CHCl3/MeOH) Rf = 0.51. 1H NMR data (CDCl3): δ 10.21 (s, 1H), 8.16 (s, 1H), 8.03 (s, 1H), 7.56 (s, 1H), 7.19 (s, 1H), 6.83 (s, 1H), 3.94 (s, 3H), 3.79 (s, 3H), 3.11 (m, 2H), 1.48 (m, 2H), 0.86 (t, J = 3.2 Hz, 3H). 13C NMR data (CDCl3): δ 161.57, 157.11, 134.26, 128.64, 126.79, 123.86, 121.75, 118.33, 107.99, 104.41, 40.61, 37.92, 36.44, 23.03, 11.89 (2C).
Methyl 1-methyl-4-(1-methyl-4-nitro-1H-pyrrolo-2-carboxamido)-1H-pyrrole-2-carboxylate (7). Argon was bubbled through 100 mL of EtOAc containing 8.6 mL of DIEA in a 250 mL round bottom flask. Methyl 4-amino-1-methyl-1H-pyrrole-2-carboxylate, HCl (4.98 g, 0.03 mol) was then added to the solution followed by nitro compound 1 (7.10 g, 0.026 mol). The solution was allowed to stir under Ar for 48 hours. The yellow precipitate that was formed was filtered and washed with cold DI H2O and dried under vacuum to yield yellow solid 7 (4.67 g, 58 %): mp 235-239 °C. TLC (1:1 Hexane/EtOAc) Rf = 0.31. 1H NMR data (D6-DMSO): δ 10.26 (s, 1H), 8.18 (d, J = 2Hz, 1H), 7.54 (d, J = 2 Hz, 1H), 7.45 (s, 1H), 6.88 (d, J = 2 Hz, 1H), 3.94 (s, 3H), 3.84 (s, 3H), 3.74 (s, 3H).


1-Methyl-4-(1-methyl-4-nitro-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxylic acid (8). The nitro ester 7 (1.00 g, 3.27 mmol) was suspended in EtOH (6 mL), and a solution of NaOH (0.654g, 5eq.) in H2O was added. This suspension was allowed to reflux at 70 °C until the disappearance of 7 was indicated by TLC (EtOAc). This solution was concentrated by rotary evaporation to dryness to produce a yellow solid. The yellow solid was then dissolved in 2 mL of H2O. This solution was then cooled to 0 °C and acidified with concentrated HCl until the pH was 1. At this point a yellow precipitate fell out of solution and was filtered to yield 8 (0.789 g, 83%): mp 215-218 °C. TLC (6:1 CHCl3:MeOH) Rf = 0.42. 1H NMR data (D6-DMSO): δ 10.23 (s, 1H), 8.18 (d, J = 1.6 Hz, 1H), 7.54 (d, J = 2 Hz, 1H), 7.41 (d, J = 2 Hz, 1H), 6.82 (d, J = 2 Hz, 1H), 3.94 (s, 3H), 3.82 (s, 3H). 13C NMR data (D6-DMSO): δ 162.34, 157.41, 134.25, 128.73, 126.61, 122.36, 120.87, 120.26, 108.82, 108.19, 37.94, 36.69.

2-(1-Methyl-4-(1-methyl-4-nitro-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamido)acetic acid (9a). Compound 5a (3.03 g, 7.95 mmol) was dissolved in EtOH (20
mL) in a 100 mL round bottom flask to this NaOH (1.27 g, 4 eq.) and H₂O (5 mL) solution was added and allowed to reflux until the disappearance of 5a by TLC (6:1 CHCl₃/MeOH). This solution was then concentrated by the removal of the EtOH. The aqueous solution was then cooled in an ice/water bath and the medium was acidified by concentrated HCl until the pH was 1. A yellow solid was produced and filtered to give yellow solid 9a (2.61g, 94%): mp 262-264 °C. TLC (1:1 CHCl₃/MeOH) \( R_f = 0.27 \). \(^1\)H NMR data (D₆-DMSO): \( \delta \) 12.5 (s, 1H), 10.30 (s, 1H), 8.20 (d, \( J = 2 \) Hz, 1H), 7.60 (d, \( J = 2 \) Hz, 1H), 7.29 (d, \( J = 2 \) Hz, 1H), 6.92 (d, \( J = 1.6 \) Hz, 1H), 3.97 (s, 3H), 3.82 (d, \( J = 3.6 \) Hz, 5H). \(^{13}\)C NMR data (D₆-DMSO): \( \delta \) 172.10, 161.77, 157.35, 134.23, 128.69, 126.74, 122.95, 121.95, 118.94, 108.10, 104.87, 37.94, 36.60.

3-(1-Methyl-4-(1-methyl-4-nitro-1\(^\text{H}\)-pyrrole-2-carboxamido)-1\(^\text{H}\)-pyrrole-2-carboxamido)propanoic acid (9b). Compound 9b was synthesized using a procedure similar to the ones described above for 9a using (2.01 g, 5.7 mmol) of 5b and NaOH (0.92 g, 4 eq.) to give yellow solid 9b (1.81 g, 98%): mp 226-230 °C. TLC (5:2 CHCl₃/MeOH) \( R_f = 0.38 \). \(^1\)H NMR data (D₆-DMSO): \( \delta \) 12.2 (s, 1H), 8.47, (s, 1H), 8.07 (s, 1H), 7.57 (s, 1H), 7.20 (s, 1H), 6.84 (s, 1H), 3.95 (s, 3H), 3.80 (s, 3H). \(^{13}\)C NMR data (D₆-DMSO): \( \delta \) 173.69, 161.61, 157.32, 134.21, 128.66, 126.73, 123.44, 121.87, 118.56, 108.19, 104.62, 37.97, 36.52, 35.41, 34.72.

4-(1-Methyl-4-(1-methyl-4-nitro-1\(^\text{H}\)-pyrrole-2-carboxamido)-1\(^\text{H}\)-pyrrole-2-carboxamido)butanoic acid (9c). Compound 9c was synthesized using a procedure similar to the ones described above for 9a using (2.00 g, 4.9 mmol) of 5c and NaOH (0.789 g, 4 eq.) to give yellow solid 9c (1.78 g, 96%): mp 240-245 °C. TLC (5:2 CHCl₃/MeOH) \( R_f = 0.39 \). \(^1\)H NMR data (D₆-DMSO): \( \delta \) 10.29 (s, 1H), 8.37 (s, 1H), 8.15 (d, \( J = 2 \) Hz, 1H), 7.57 (d, \( J = 2 \) Hz, 1H), 7.19 (d, \( J = 1.6 \) Hz, 1H), 6.83 (d, \( J = 1.6 \) Hz, 1H), 3.93 (s, 3H), 3.80 (s, 3H), 3.13 (q, \( J = 5.6 \) Hz, 6.4 Hz, 2H), 2.46 (t, \( J = 7.2 \) Hz, 2H), 1.66 (t, \( J = 7.2 \) Hz, 3H). \(^{13}\)C NMR data (D₆-DMSO): \( \delta \)
2-Deoxy-2-(4-methoxybenzylidene)amino-β-D-glucopyranose. (IUPAC: (E)-6-(hydroxymethyl)-3-(3-methoxybenzylideneamino)tetrahydro-2H-pyran-2,4,5-triol) (10). Synthesized from D-glucosamine hydrochloride as described in literature. In a 500 mL round bottom flask cooled to 0 °C sodium hydroxide (11.18 g, 0.279 mols) was dissolved in 234 mL of DI water while stirring. D-glucosamine (50.00 g, 0.232 mols) was then added and in 5 minutes the solution turned clear. 4-methoxybenzaldehyde (31.0 mL, 0.254 mols) was added to form a two layer mixture which was stirred for 30 minutes at 0 °C. The mixture was then allowed to sit in the refrigerator over night to yield a white precipitate that was filtered under vacuum until dryness to give 9 (42.83 g, 62%): mp 153-154 °C. $^1$H NMR data (D6-DMSO): δ 8.10 (s, 1H), 7.67 (d, J = 8.4 Hz, 2H), 6.97 (d, J = 8.8 Hz, 2H), 6.52 (s, 1H), 4.93 (d, J = 5.2 Hz, 1H), 4.91 (d, J = 5.2 Hz, 1H), 4.67 (d, J = 8.8 Hz, 1H), 4.55 (t, J = 5.6 Hz, 1H), 3.79 (s, 3H), 3.71 (dd, J = 3 Hz, 7.7 Hz, 1H), 3.42 (m, 2H), 3.21 (m, 1H), 3.13 (m, 1H), 2.77 (t, J = 8 Hz, 1H). $^{13}$C NMR data (D6-DMSO): δ 161.66, 161.49, 130.07, 129.56 (2C), 114.35, 96.08, 79.64, 78.65, 77.32, 75.04, 70.80, 61.72, 55.72.

1,3,4,6-Tetra-O-actyl-2-deoxy-2-(4-methoxybenzylidene)amino-β-D-glucopyranose. (IUPAC: (E)-6-(acetoxymethyl)-3-(3-methoxybenzylideneamino)tetrahydro-2H-pyran-2,4,5-triyl triacetate) (11). Compound 11 was synthesized as described in literature. Pyridine (250 mL) was dried over sieves. The imine 10 (48.02 g, 0.142 mmol) was dissolved in pyridine (236 mL) at 0°C in an ice water bath. Once dissolved acetic anhydride (142 mL) was added and the flask was immediately removed from ice water bath. The temperature was allowed to reach room temperature overnight while stirring. The volume of the solution was reduced to half by
distillation under vacuum. The solution was then poured into ice water (~2000 mL) forming a white precipitate. The solution was allowed to stir for 1 hour and then placed in refrigerator overnight. The white precipitate was then filtered under vacuum to give 11 (54.47 g, 83%): mp 169-171°C. $^1$H NMR data (D6-DMSO): $\delta$ 8.29 (s, 1H), 7.64 (d, $J = 8.8$ Hz, 2H), 6.97 (d, $J = 8.4$ Hz, 2H), 6.05 (d, $J = 8.4$, 1H), 5.43 (t, $J = 9.6$ Hz, 1H), 4.96 (t, $J = 9.6$ Hz, 1H), 4.26 (m, 2H), 4.00 (d, $J = 10.81$, 1H), 3.78 (s, 3H), 3.44 (t, $J = 8.4$ Hz, 2H), 2.06 (s, 3H), 1.97 (s, 3H), 1.96 (s, 3H), 1.81 (s, 3H). $^{13}$C NMR data (D6-DMSO): $\delta$ 170.50, 169.89, 169.43, 169.05, 164.90, 162.27, 130.37 (2C), 128.71, 114.65 (2C), 92.97, 72.78, 72.68, 71.96, 68.24, 62.10, 55.82, 20.99, 20.91, 20.90, 20.65.


Compound 12 was synthesized as described in literature.$^{28}$ Acetyl chloride (3.95 mL) was added to anhydrous methanol (27.38 mL) in a 100 mL round bottom flask at 0 °C with constant stirring for 1 hour. This solution was then added to a stirred solution of imine 10 (25.04 g, 50.7 mmol) in acetone (770.6 mL) at room temperature. This mixture was stirred for 1 hour and then cooled to 0 °C. To the cooled solution ether (273.8 mL) was added and stirred for an additional 45 min. at 0 °C. A white precipitate was filtered under vacuum to give 11 (18.92 g, 97%): mp 120 °C-decomposition. $^1$H NMR data (D6-DMSO): $\delta$ 8.42 (s, 3H), 5.85 (d, $J = 8.8$, 1H), 5.31 (t, $J = 9.6$ Hz, 1H), 4.96 (t, $J = 9.6$ Hz, 1H), 4.26 (m, 2H), 4.00 (d, $J = 10.8$ Hz, 1H), 3.78 (s, 3H), 3.44 (t, $J = 8.4$ Hz, 2H), 2.15 (s, 3H), 2.02 (s,3H), 1.99 (s, 3H), 1.97 (s, 3H). $^{13}$C NMR data (D6-DMSO): $\delta$ 170.41, 170.26, 169.76, 169.09, 90.56, 72.06, 70.78, 68.23, 61.70, 52.55, 21.37, 21.29, 20.94, 20.80.
(2S,3R,4R,5S,6R)-6-(Acetoxymethyl-3-(2-(1-methyl-4-(1-methyl-4-nitro-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamido)acetamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate (13a). In a flask flushed with Ar, 12 (2.03g, 53 mmol) was dissolved in 8 mL anhydrous DMF along with EDCI (1.57 g, 1.5 eq.), DMAP (1.62 g, 2.5 eq.), HOBT (1.27 g, 1.57 eq.), and CuCl₂ (71 mg, 0.01 eq.). Once in solution, the carboxylic acid 9a (2.00 g, 53 mmol) was then added and allowed to stir at room temperature over 48 hours until the disappearance of starting material by TLC (3:1 EtOAc/MeOH). This solution was diluted with DCM (100 mL) and extracted with H₂O (150 mL, 2x), sat. NaHCO₃ (100 mL, 2x), and 1M HCl (100mL, 2x). The organic layer was dried over MgSO₄. The solution was filtered and concentrated by rotary evaporation until a solid began to fall out of solution. The solution was warmed up again until the solid redissolved and then allowed to slowly reach room temperature overnight at which point an orange-brown solid fell out of solution. The flask was then placed in the refrigerator to cool further for 24 hours. The crystals were then filtered and dried to give 13a (3.18 g, 87%): mp 118-121 °C. TLC (6:1 CHCl₃/MeOH) Rₜ = 0.52. ¹H NMR data (D₆-DMSO): δ 10.27 (s, 1H), 8.4 (t, J = 8.4 Hz, J = 10 Hz, 1H), 8.18 (d, J = 2 Hz, 1H), 7.83 (d, J = 9.6, 1H), 7.59 (d, J = 1.6, 1H), 7.26 (d, J = 2 Hz, 1H), 6.88 (d, J = 2 Hz, 1H), 5.73 (d, J = 8.8 Hz, 1H), 5.24 (t, J = 9.6, 1H), 4.86 (t, J = 9.6 Hz, 1H), 4.18 (dd, J = 4.4 Hz, 4Hz, 8.4 Hz, 1H), 4.03 (m, 2H), 3.98 (s, 3H), 3.77 (3H), 3.62 (d, J = 6 Hz, 2H), 2.05 (s, 3H), 1.99 (s, 3H), 1.94 (s, 3H), 1.94 (s, 3H). ¹³C NMR data (D₆-DMSO): δ 171.25, 170.47, 170.05, 169.71, 169.31, 161.31, 161.59, 157.32, 154.24, 128.66, 126.75, 123.48, 121.81, 118.58, 108.02, 104.43, 92.15, 72.62, 71.99, 68.52, 61.96, 52.35, 37.92, 36.48, 36.01, 35.84, 20.95 (2C), 20.85, 20.68.
**triacetate (13b).** Compound 13b was synthesized using a procedure similar to the one described for 13a using (4.44 g, 12.2 mmol) of 9b and (4.69 g, 12.2 mmol) of 12 to give a yellow solid 13b (6.62 g, 77 %): mp 175-179 °C. TLC (6:1 CHCl₃/MeOH) Rₓ = 0.46. ¹H NMR data (D₆-DMSO):  δ 10.23 (s, 1H), 8.10 (s, 1H), 8.10 (d, J = 5.2 Hz, 1H), 7.57 (d, J = 4 Hz, 1H), 7.22 (d, J = 1.6 Hz, 1H), 6.81 (d, J = 1.6 Hz, 1H), 5.72 (d, J = 8.8 Hz, 1H), 5.18 (t, J = 9.6 Hz, 1H), 4.88 (t, J = 4.4 Hz, 4.8 Hz, J = 7.6 Hz, 1H), 3.98 (m, J; 3H), 3.96 (s, 3H), 3.80 (s, 3H), 2.31 (t, J = 7.2 Hz, 2H), 2.0 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H), 1.88 (s, 2H). ¹³C NMR data (D₆-DMSO): δ 171.25, 170.47, 170.05, 169.71, 169.31, 161.59, 157.32, 134.24, 128.66, 126.75, 123.48, 121.81, 118.58, 104.43, 92.15, 72.62, 71.97, 68.52, 61.96, 52.35, 37.92, 36.48, 36.01, 35.84, 20.95 (2C), 20.85, 20.68.

**Ethyl 2-(4-(4-acrylamido-1-methyl-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamido)butanamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate (13c).** Compound 13c was synthesized using a procedure similar to the one described for 13a using (3.25 g, 8.6 mmol) of 9c and of 12 (3.00 g, 7.82 mmol) to give a yellow solid 13c (4.88 g, 86 %): mp 171-176 °C. TLC (6:1 CHCl₃/MeOH) Rₓ = 0.44. ¹H NMR data (D₆-DMSO): δ 10.23 (s, 1H), 8.17 (s, 1H), 8.05 (m, 2H), 7.19 (s, 1H), 6.84 (s, 1H), 5.70 (d, J = 8Hz, 1H), 5.17 (t, J = 9.6 Hz, 1H), 4.88 (t, J = 9.6 Hz, 1H), 4.17 (dd, J = 4.4 Hz, J = 8.4 Hz, 1H), 4.0 (m, 3H), 3.96 (s, 3H), 3.79 (s, 3H), 3.10 (s, 3H), 2.06 (s, 3H), 2.03 (s, 3H), 2.01 (s, 3H), 1.89 (s, 3H), 1.64 (m, 2H). ¹³C NMR data (D₆-DMSO): δ 172.67, 170.48, 170.04, 169.34, 161.56, 157.30, 134.23, 128.67, 126.75, 123.64, 121.77, 118.43, 108.00, 104.43, 92.18, 72.62, 71.97, 68.52, 61.93, 52.23, 38.42, 37.93, 36.48, 33.71, 26.13, 20.96 (2C), 20.86, 20.77.

**Ethyl 2-(4-(4-acrylamido-1-methyl-1H-pyrrole-2-carboxamido)-1-methyl-1H-pyrrole-2-carboxamido)acetate (14a).** Compound 5a (2.04 g, 5.4 mmol) was dissolved in 50
mL of ethanol and 1.72 g 10% water wet Pd/C was added in a Parr jar. The mixture was shaken under pressurized hydrogen (70 psi) until 5a had disappeared by TLC (EtOAc). The Pd/C was filtered through celite and the filtrate was concentrated by rotary evaporation. The residue was placed under high vacuum overnight. The flask was flushed with N₂ and the residue was dissolved in anhydrous THF (20 mL) and DIEA (3.13 mL, 24 mmol). The solution was bubbled with N₂ and cooled to -40 °C for 1 hour. Acryloyl chloride (489 µL, 0.5 mmol) was added to the cooled solution drop wise. The reaction temperature was maintained between -40 °C to -20 °C and protected from light. The reaction continued until it was complete by TLC (6:1 CHCl₃/MeOH). The solution was concentrated by rotary evaporation, re-dissolved in DCM (50 mL) and extracted with DI water (2 x 60 mL). The organic layer was dried over MgSO₄. The solution was concentrated by rotary evaporation until dry to give pure 14a (2.11 g, 97%): mp 101-106 °C. TLC (6:1 CHCl₃/MeOH) Rᵣ = 0.66. ¹H NMR data (D₆-DMSO): δ 10.12 (s, 1H), 9.94 (s, 1H), 8.40 (t, J = 5.6 Hz, 1H), 7.24 (d, J = 9.6 Hz, 2H), 6.9 (s, 2H), 6.35 (m, 1H), 6.17 (dd, J = 1.6 Hz, 1H), 5.66 (dd, J = 1.6 Hz, 1H), 4.10 (q, J = 6.8 Hz, 2H), 3.87 (d, J = 5.7 Hz, 2H), 3.83 (s, 3H), 3.78 (s, 3H), 1.18 (t, J = 8 Hz, 3H). ¹³C NMR data (D₆-DMSO): δ 170.68, 162.14, 161.95, 158.81, 131.92, 126.11, 123.45, 122.62, 122.53, 122.14, 118.95, 105.14, 104.51, 60.82, 36.63, 36.52, 14.59.

Ethyl 3-(4-(4-acrylamido-1-methyl-1H-pyrrole-2-carboxamido)1-methyl-1H-pyrrole-2-carboxamido)propanoate (14b). Compound 14b was synthesized using a procedure similar to the one described for 14a using 351 mg, (0.90 mmol) of 5b, 81 µL (0.98 mmol) of acryloyl chloride and 515 µL (3.1 mmol) of DIEA to yield 14b as a brown solid (0.329 g, 88%): mp 66-71 °C. TLC (6:1 CHCl₃/MeOH) Rᵣ = 0.39. ¹H NMR data (D₆-DMSO): δ 10.10 (s, 1H), 9.88 (s, 1H), 8.03 (t, J = 4.9 Hz, 1H), 7.23 (d, J = 1.6 Hz, 2H), 7.16 (d, J = 1.6 Hz, 1H), 6.88
(d, J = 1.6 Hz, 1H), 6.81 (d, J = 1.6 Hz, 1H), 6.35 (m, 1H), 6.15 (dd, J = 2 Hz, 1H), 5.63 (dd, J = 5 Hz, 1H), 4.03 (q, J = 6.5 Hz, 7.2 Hz 2H), 3.81 (s, 3H), 3.76 (s, 3H), 1.15 (t, J = 7.2 Hz, 3H). 

$^{13}$C NMR (D6-DMSO): $\delta$ 171.86, 162.10, 161.75, 158.76, 131.97, 123.45, 123.12, 122.50, 122.15, 118.91, 118.45, 104.74, 104.45, 60.36, 36.64, 36.43, 35.26, 34.48, 31.17, 14.56.

Ethyl 4-(4-(4-acrylamido-1-methyl-1H-pyrrole-2-carboxamido)-1-methyl-1H-pyrrole-2-carboxamido)butanoate (14c). Compound 14c was synthesized using a procedure similar to the one described for 14a using 421 mg, (1.04 mmol) of 5c, 94 µL (1.14 mmol) of acryloyl chloride and 603 µL (3.6 mmol) of DIEA to yield 14c as a brown solid (0.350 g, 79%): mp 75-80 °C. TLC (6:1 CHCl₃/MeOH) $R_f$ = 0.63. $^1$H NMR data (D6-DMSO): $\delta$ 10.11 (s, 1H), 9.89 (s, 1H), 8.03 (t, J = 5.5 Hz, 1H), 7.26 (d, J = 1.6 Hz, 1H), 7.17 (d, J = 1.6 Hz, 1H), 6.90 (d, J = 1.6 Hz, 1H), 6.84 (d, J =1.6 Hz, 1H), 6.35 (m, 1H), 6.17 (dd, J = 2 Hz, 14.8 Hz, 1H), 5.65 (dd, J = 2 Hz, 8 Hz, 1H), 4.03 (q, J = 6.8 Hz, 7.2 Hz), 3.83 (s, 3H), 3.78 (s, 3H), 3.17 (q, J = 6.4 Hz, 6 Hz, 2H), 2.31 (t, J = 7.6 Hz, 2H), 1.73 (m 2H), 1.17 (t, J = 6.8 Hz, 3H). $^{13}$C NMR (D6-DMSO): $\delta$ 173.18, 162.09, 161.75, 158.74, 131.95, 126.06, 123.48, 123.33, 122.45, 122.14, 118.89, 118.32, 104.63, 104.41, 60.22, 38.16, 36.65, 36.42, 31.50, 25.17, 14.58.

2-(4-(4-Acrylamido-1-methyl-1H-pyrrole-2-carboxamido)-1-methyl-1H-pyrrole-2-carboxamido)acetic acid (15a). Compound 14a (1.28 g, 3.18 mmol) was dissolved in reagent acetone (16 mL). NaOH (0.501 g, 1.2 mmol) dissolved in 4 mL of DI H₂O was added to the reaction flask. After 1 hour no starting material was left as indicated by TLC (6:1 CHCl₃:MeOH). The acetone was removed by rotary evaporation completely leaving the DI H₂O. The flask was then cooled in an ice/water bath at which point the pH of the medium was adjusted to 1 with concentrated HCl producing a brown precipitate. The brown precipitate was filtered by vacuum filtration to give pure 15a (0.894, 72 %): mp 130-133 °C. TLC 2:1
(CHCl₃:MeOH) R_f = 0.52. ¹H NMR data (D₆-DMSO): δ 10.15 (s, 1H), 9.94 (s, 1H), 8.312 (t, J = 5 Hz, 1H), 7.26 (s, 1H), 7.23 (s, 3H), 6.91 (d, J = 5.8 Hz, 2H), 6.37 (m, 1H), 6.17 (dd, J = 2 Hz, 1H), 5.65 (dd, 2.4 Hz, 8 Hz, 1H), 3.91 (d, J = 6 Hz, 2H), 3.84 (s, 3H), 3.83 (s, 3H). ¹³C NMR (D₆-DMSO): δ 172.21, 162.12, 161.88, 131.98, 126.02, 123.49, 122.73, 122.17, 118.82, 105.07, 104.52, 65.38, 36.62, 36.50, 15.63.

3-(4-(4-Acrylamido-1-methyl-1H-pyrrole-2-carboxamido)-1-methyl-1H-pyrrole-2-carboxamido)propanoic acid (15b). Compound 15b was synthesized using a procedure similar to the one described for 15a using (250 mg, 0.602 mmol) of 14b and 0.096 g of NaOH (2 mmol) to yield 15b as a brown solid (0.163 g, 70 %): mp 107-110 °C. TLC 5:2 (CHCl₃:MeOH) R_f = 0.30. ¹H NMR data (D₆-DMSO): δ 10.08 (s, 1H), 9.85 (s, 1H), 7.98 (t, J = 5 Hz, 1H), 7.21 (s, 1H), 7.13 (s, 1H), 6.85 (s, 1H), 6.78 (s, 1H), 6.31 (m, 1H), 6.12 (d, J = 1.6 Hz, 1H), 3.82 (s, 3H), 3.78 (s, 3H), 3.31 (t, J = 6.4 Hz, 2H). ¹³C NMR (D₆-DMSO): δ 173.48, 162.11, 161.72, 158.75, 131.98, 123.46, 123.16, 122.49, 122.15, 118.91, 118.42, 104.72, 104.45, 36.65, 36.44, 35.30, 34.49, 21.54.

4-(4-(4-Acrylamido-1-methyl-1H-pyrrole-2-carboxamido)-1-methyl-1H-pyrrole-2-carboxamido)butanoic acid (15c). Compound 15c was synthesized using a procedure similar to the one described for 15a using (285 mg, 0.66 mmol) of 14c and 0.106 g of NaOH (2.6 mmol) to yield 15c as a brown solid (0.208 g, 78 %): mp 71-76 °C. TLC 5:2 (CHCl₃:MeOH) R_f = 0.63. ¹H NMR data (D₆-DMSO): δ 10.16 (s, 1H), 9.90 (s, 1H), 8.04 (t, J = 4.8 Hz, 1H), 7.25 (s, 1H), 7.17 (s, 1H), 6.90 (s, 1H), 6.84 (s, 1H), 6.37 (m, 1H), 6.18 (dd, J = 1.3 Hz, 1H), 5.66 (dd, J = 1.2 Hz, 8.8 Hz, 1H), 3.82 (s, 3H), 3.80 (s, 3H), 3.17 (t, J = 6.2 Hz, 2H), 2.23 (t, J = 7.2 Hz, 2H), 1.70 (pentet, J = 6.5 Hz, 6.8 Hz, 7.32 Hz, 2H). ¹³C NMR (D₆-DMSO): δ 175.23, 174.80, 162.16,
6-(Acetoxymethyl)-3-(2-(4-(4-acrylamido-1-methyl-1H-pyrrole-2-carboxamido)-1-methyl-1H-pyrrole-2-carboxamido)acetamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate (16a). In a flask flushed with N\textsubscript{2}, 15a (0.849 g, 2.27 mmol) was dissolved in 2 mL of anhydrous DMF along with EDCI (0.657 g, 1.5 eq.), DMAP (0.701 g, 2.5 eq.), HOBT (0.926 g, 3.0 eq.), CuCl\textsubscript{2} (0.031 g, 0.1 eq.), and the amine hydrochloride 12 (0.960 g, 2.5 mmol) was added and the solution was allowed to stir at room temperature over a period of two days. The solution was diluted with 100 mL DCM and the organic layer extracted with H\textsubscript{2}O (2 x, 150 mL), sat. NaHCO\textsubscript{3} (2 x, 150 mL), and 1M HCl (2 x, 150 mL). The organic layer was then dried over MgSO\textsubscript{4}. The resulting solution was rotary evaporated until dry to yield the product, 16a as brown-orange solid. (1.36 g, 85 %): mp 135-138 °C. TLC (6:1 CHCl\textsubscript{3}/MeOH) R\textsubscript{f} = 0.74. \textsuperscript{1}H NMR (D6-DMSO): \(\delta\) 10.09 (s, 1H), 9.93 (s, 1H), 8.31 (t, J = 2.8 Hz, 1H), 7.81 (d, J = 9.2 Hz, 1H), 7.24 (d, J = 10 Hz, 2H), 6.91 (d, J = 14 Hz, 2H), 6.37 (m, 1H), 6.17 (dd, J = 2.8 Hz, 15.6 Hz, 5.74 (d, 8.8 Hz, 1H), 5.65 (dd, J = 3.2 Hz, 8 Hz, 1H), 4.18 (dd, 8.8 Hz, 7.6 Hz, 1H), 3.93 (s, 3H), 3.82 (s, 3H), 3.62 (d, J = 5.6 Hz, 2H), 2.06 (s, 3H), 2.05 (s, 3H), 1.99 (s, 3H), 1.95 (s, 3H). \textsuperscript{13}C NMR (D6-DMSO): \(\delta\) 170.49, 170.26, 170.09, 169.72, 169.42, 162.12, 162.02, 158.81, 131.97, 123.48, 122.87, 122.15, 118.91, 118.75, 105.03, 104.55, 92.20, 72.31, 72.01, 68.77, 61.96, 52.07, 43.08, 36.62, 36.42, 21.02, 20.95, 20.86, 20.84.

6-(Acetoxymethyl)-3-(3-(4-(4-acrylamido-1-methyl-1H-pyrrole-2-carboxamido)-1-methyl-1H-pyrrole-2-carboxamido)propanamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate (16b). Compound 16b was synthesized similar to the procedure described above for 16a using 0.147 g (0.379 mmol) of 15b, 2 mL anhydrous DMF along with, EDCI (0.108 g, 1.5
eq.), DMAP (0.112 g, 2.5 eq.), HOBT (0.151 g, 3.0 eq.), and CuCl₂ (0.007 g, 0.1 eq.) and 0.149 g (0.388 mmol) of 12 to yield 16b as a brown-orange solid (0.260 g, 95 %): mp 131-136 °C. TLC (6:1 CHCl₃/MeOH) Rₕ = 0.50. ¹H NMR (D₆-DMSO): δ 10.09 (s, 1H), 9.93 (s, 1H), 8.31 (t, J = 4.8 Hz, 1H), 7.81 (d, J = 9.2 Hz, 1H), 7.24 (d, J = 10 Hz, 2H), 6.91 (d, J = 14 Hz, 2H), 6.80 (d, J = 1.6 Hz, 1H), 6.36 (m, 1H), 6.18 (dd, J = 2 Hz, 15 Hz, 1H), 5.65 (dd, J = 2 Hz, 8 Hz, 1H), 5.17 (t, J = 10 Hz, 1H), 4.88 (t, J = 9.9 Hz, 1H), 4.18 (dd, J = 5.2 Hz, 8Hz, 1H), 3.97 (m, 2H), 3.82 (s, 3H), 3.78 (s, 3H), 2.54 (t, J = 1.6 Hz, 2H), 2.30 (q, J = 1.6 Hz, 7.2 Hz, 2H), 2.00 (s, 3H), 1.99 (s, 3H), 1.96 (s, 3H), 1.87 (s, 3H). ¹³C NMR (D₆-DMSO): δ 171.26, 170.50, 170.07, 169.73, 169.34, 162.09, 161.68, 158.74, 131.96, 126.05, 123.45, 123.15, 122.48, 122.12, 118.86, 118.41, 104.58, 104.32, 92.12, 71.96, 68.46, 61.93, 52.29, 36.46, 36.42, 36.26, 36.03, 35.83, 20.97, 20.87 (2C), 20.70.

6-(Acetoxymethyl)-3-(4-(4-acrylamido-1-methyl-1H-pyrrole-2-carboxamido)-1-methyl-1H-pyrrole-2-carboxamido)butanamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate (16c). Compound 16c was synthesized similarly to the procedure described above for 16a using 0.240 g (0.598 mmol) of 15c, 2 mL anhydrous DMF along with, EDCI (0.174 g, 1.5 eq.), DMAP (0.183 g, 2.5 eq.), HOBT (0.243 g, 3.0 eq.), and CuCl₂ (0.010 g, 0.1 eq.) and 0.230 g (0.599 mmol) of 12 to yield 16c as a brown-orange solid (0.432 g, 98 %): mp 136-139 °C. TLC (6:1 CHCl₃/MeOH) Rₕ = 0.40. ¹H NMR (D₆-DMSO): δ 10.09 (s, 1H), 9.89 (s, 1H), 8.02 (t, J = 9.2 Hz, 1H), 7.26 (d, J = 1.2 Hz, 1H), 7.17 (d, J = 1.2 Hz, 1H), 6.90 (d, J = 1.6 Hz, 2H), 6.83 (d, J = 1.6 Hz, 1H), 6.33 (m, 1H), 6.15 (dd, J = 1.6 Hz, 15.2 Hz, 1H), 5.70 (d, J = 8.8 Hz, 1H), 5.65 (dd, J = 1.6 Hz, 8.4 Hz, 1H), 5.16 (t, J = 10 Hz, 1H), 4.88 (t, J = 9.6 Hz, 1H), 4.17 (dd, J = 4.4 Hz, 8 Hz, 1H), 4.00 (m, 4H), 3.82 (s, 3H), 3.78 (s, 3H), 3.11 (q, J = 5.2 Hz, 6 Hz, 2H), 2.04 (s, 3H), 2.00 (s, 3H), 1.97 (s, 3H), 1.91 (s, 3H), 1.64 (pentet, J = 7.5 Hz, 6.9 Hz, 14 Hz, 2H). ¹³C NMR
(D6-DMSO): δ 172.70, 170.52, 170.07, 169.74, 169.38, 162.10, 161.69, 158.75, 131.93, 126.10, 123.47, 123.34, 122.43, 122.13, 118.32, 105.03, 104.60, 92.17, 72.61, 71.96, 68.49, 61.91, 52.24, 49.06, 36.65, 36.42, 33.72, 26.17, 20.97, 20.87 (2C), 20.78.

1-Methyl-4-(1-methyl-4-(3-methylsulfonyl)propanamido)-1H-pyrrole-2-carboxamido)-N-propyl-1H-pyrrole-2-carboxamide (17). Compound 6 (0.166 g, 0.410 mmol) was dissolved in 50 mL of ethanol and 0.120 g 10% water wet Pd/C was added in a Parr jar. The mixture was shaken under pressurized hydrogen (70 psi) until 6 had disappeared by TLC (6:1 CHCl₃/MeOH). The Pd/C was filtered through celite and the filtrate was concentrated by rotary evaporation. The residue was placed under high vacuum overnight. The flask containing the reduced compound 6 was flushed with N₂ and dissolved with 4 mL of anhydrous DMF and anhydrous DIEA (289 µL, 1.7 mmol). The mixture was cooled to -40 °C for 45 minutes. The 3-(methylsulfonyl) propanoyl chloride (0.094 g, 0.55 mmol) was then added to the cooled mixture and the temperature was allowed to slowly reach RT until TLC (6:1 CHCl₃/MeOH) indicated the disappearance of the reduced compound 6. The solvent was then removed by rotary evaporation and the solid was took up in 100 mL of DCM and extracted with HCl (2x, 100 mL) and DI H₂O (2x, 100 mL). The organic layer was then dried over MgSO₄. The resulting solution was concentrated by rotary evaporation to yield the crude product and purified with flash column chromatography (9:1 EtOAc/MeOH) to give pure 17 (0.055 g, 25 %): mp > 150 °C. TLC (9:1 EtOAc/MeOH) Rₛ = 0.36. ¹H NMR (D6-DMSO): δ 10.0 (s, 1H), 9.83 (s, 1H), 7.96 (t, J = 5.2 Hz, 1H), 7.15 (d, J = 1.6 Hz, 2H), 6.83 (d, J = 9.6 Hz, 2H), 3.81 (s, 3H), 3.77 (s, 3H), 3.10 (m, 2H), 2.99 (s, 3H), 2.72 (t, J = 7.6 Hz, 2H), 1.47 (q, J = 7.2 Hz, 2H), 0.85 (t, J = 7.2 Hz, 3H). ¹³C NMR (D6-DMSO): δ 166.57, 161.70, 158.78, 123.55, 123.33, 122.39, 122.12, 118.65, 118.22, 104.59, 104.33, 50.22, 36.59, 36.36, 28.68, 23.03, 11.89.
6-(Acetoxymethyl)-3-(2-(1-methyl-4-(1-methyl-4-(3-(methylsulfonyl)propanamido)-1H-pyrrole-2-carboxamido)acetamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate (18a).

Compound 16a (0.317 g, 0.467 mmol) was dissolved in 52 mL of ethanol and 0.412 g 50 % water wet Pd/C was added in a Parr jar. The mixture was shaken under pressurized hydrogen (70 psi) until 16a had disappeared by TLC (6:1 CHCl₃/MeOH). The Pd/C was filtered through celite and the filtrate was concentrated by rotary evaporation. The residue was placed under high vacuum overnight. The flask with flushed with N₂ and the reduced compound 16a was dissolved in 2.5 mL of anhydrous DMF along anhydrous DIEA (193 µL, 1.1 mmol) and cooled to -40 °C in a dry ice acetone bath for 45 minutes. 3-(methylsulfonyl)propanoyl chloride (0.081 g, 0.467 mmol) was then added and temperature was allowed to reach room temperature over night. The reaction stirred for two days at room temperature until the disappearance of the reduced compound 16a was indicated by TLC (6:1 CHCl₃/MeOH). The solution was diluted with 50 mL DCM and the organic solution was extracted with H₂O (2x, 60 mL), 1 M HCl (2x, 50 mL), and sat. NaHCO₃ (2x, 50 mL). The organic layer was then dried over MgSO₄. The resulting solution was rotary evaporated until dry to yield the product, 18a as an orange solid (0.170 g, 46 %): mp 94-97 °C. TLC (6:1 CHCl₃/MeOH) Rₚ = 0.64. ¹H NMR (D6-DMSO): δ 10.06 (s, 1H), 9.92 (s, 1H), 8.33 (t, J = 12 Hz, 1H), 7.23 (d, J = 8 Hz, 2H), 6.87 (dd, J = 1.6 Hz, 2 Hz, 2H), 5.73 (d, J = 8.8 Hz, 1H), 5.24 (t, J = 10 Hz, 1H), 4.85 (t, J = 9.2 Hz, 1H), 4.17 (dd, J = 4.4 Hz, 8.3 Hz, 1H), 3.95 (m, 3 H), 3.63 (d, J = 5.6 Hz, 2H), 3.39 (t, J = 11.2 Hz, 2H), 3.00 (s, 3H), 2.058 (s, 3H), 1.99 (s, 3H), 1.95 (s, 3H), 1.94 (s, 3H). ¹³C NMR (D6-DMSO): δ 170.52, 170.28, 170.11, 169.75, 169.46, 166.56, 162.02, 132.17, 123.27, 122.83, 122.61, 122.24, 118.65, 104.42, 118.73, 105.01, 104.42, 92.18, 72.28, 71.98, 68.71, 61.93, 52.02, 20.21, 43.06, 36.62, 36.44, 28.68, 20.96, 20.88 (2C), 20.85
6-(Acetoxymethyl)-3-(3-(1-methyl-4-(1-methyl-4-(3-(methylsulfonyl)propanamido)-1H-pyrrole-2-carboxamido)-1H-pyrrole carboxamido)propanamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate (18b). Compound 18b (0.383 g, 0.540 mmol) was dissolved in 50 mL of ethanol and 0.333 g 10 % water wet Pd/C was added in a Parr jar. The mixture was shaken under pressurized hydrogen (70 psi) until 16b had disappeared by TLC (6:1 CHCl$_3$/MeOH). The Pd/C was filtered through celite and the filtrate was concentrated by rotary evaporation. The residue was placed under high vacuum overnight. The flask was flushed with N$_2$ and the reduced compound 6 along with 3-(methylsulfonyl) propanoic acid (0.0824 g, 0.542 mmol), EDCI (0.160 g, 1.5 eq.), HOBT (0.218 g, 3.0 eq.), DMAP (0.166 g, 2.5 eq.) and CuCl$_2$ (0.008 g, 0.1 eq.) were dissolved in 3 mL of anhydrous DMF and allowed to stir at room temperature for two days until the disappearance of starting material as indicated by TLC (6:1 CHCl$_3$/MeOH). The solution was diluted with 50 mL DCM and the organic solution was extracted with H$_2$O (2 x, 60 mL), 1 M HCl (2 x, 50 mL), and sat. NaHCO$_3$ (2 x, 50 mL). The organic layer was then dried over MgSO$_4$. The resulting solution was rotary evaporated until dry to yield the crude product. The crude product was purified by flash column chromatography (6:1 CHCl$_3$/MeOH) yield 18b as a brown solid (0.168 g, 40 %): mp 94-97 °C. TLC (6:1 CHCl$_3$/MeOH) R$_f$ = 0.40.

$^1$H NMR (D6-DMSO): δ 10.06 (s, 1H), 9.87 (s, 1H), 8.09 (d, J = 9.2 Hz, 1H), 7.95 (t, J = 8.4 Hz, 1H), 7.16 (d, J = 1.2 Hz, 2H), 6.85 (d, J = 1.6 Hz, 1H), 6.79 (d, J = 1.6 Hz, 2H), 5.72 (d, J = 8.8 Hz, 1H), 5.17 (t, J = 10.4 Hz, 1H), 4.88 (t, J = 10 Hz, 1H), 4.16 (dd, J = 8.4 Hz, 4 Hz, 1 H), 4.08 (m, 3H), 3.80 (s, 3H), 3.77 (s, 3H), 3.01 (s, 3H), 2.30 (t, J = 7.2 Hz, 2H), 2.00 (s, 3H), 1.99 (s, 3H), 1.96 (s, 3H), 1.87 (s, 3H). $^{13}$C NMR (D6-DMSO): δ 171.28, 170.76, 170.49, 170.06, 169.73, 169.33, 166.55, 162.78, 161.69, 158.76, 123.29, 123.17, 122.49, 122.14, 118.63, 118.44, 104.58, 104.34, 92.14, 72.62, 71.99, 61.95, 61.04, 52.33, 50.22, 49.63, 20.95, 20.86 (2C), 20.69.
6-(Acetoxymethyl)-3-(4-(1-methyl-4-(1-methyl-4-(3-(methylsulfonyl)propanamido)-1H-pyrrole-2-carboxamido)-1H-pyrrole carboxamido)butanamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate (18c). Using the procedure described for 18a, 0.737 g, (1.0 mmol) of 16c, 603 µL (3.6 mmol) of DIEA and 0.195 g of 3-(methylsulfonyl)propanoyl chloride to yield 18c as a brown solid (0.385 g, 46 %): mp 154-160 °C. TLC (4:1 EtOAc/MeOH) R$_f$ = 0.63. $^1$H NMR data (D6-DMSO): δ 10.02 (s, 1H), 9.83 (s, 1H), 7.97 (t, J = 9.2 Hz, 1H), 7.14 (dd, J = 1.6 Hz, 2 Hz, 2H), 6.83 (dd, J = 1.6 Hz, 6.4 Hz, 2H), 5.70 (d, J = 8.8 Hz, 1H), 5.15 (t, J = 9.6 Hz, 1H), 4.87 (t, J = 9.6 Hz, 1H), 4.16 (dd, J = 8 Hz, 4Hz, 1H), 3.97 (m, 3H), 3.80 (s, 3H), 3.76 (s, 3H), 3.10 (q, J = 6.4 Hz, 2H), 2.99 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.94 (s, 3H). $^{13}$C NMR data (D6-DMSO): δ 172.69, 170.50, 170.06, 169.73, 169.36, 166.54, 161.69, 158.76, 123.35, 123.29, 122.44, 122.14, 118.62, 118.31, 104.62, 104.31, 92.19, 72.62, 71.97, 68.52, 52.25, 50.21, 36.61, 36.40, 36.25, 33.72, 28.66, 26.16, 20.96, 20.87 (2C), 20.78.

3-[N-(7'-Nitrobenz-2'-oxa-1', 3’diazol-4’yl)amino]propanoic. (IUPAC: 2-(7-nitrobenzo[c][1,2,5]oxadiazol-4-ylamino)acetic acid) (19). Compound 19 was synthesized from 4-chloro-7-nitro-benzofurazane as described in literature. β-Alanine (6.24 g, 70 mmol, 7eq.) and NaHCO$_3$ (5.88 g, 70 mmol, 7eq) were dissolved in 20 mL of D.I water (20 mL). After addition of 4-chloro-7-nitro-benzofurazane (2.0 g, 10 mmol) in methanol (80 mL) the mixture was stirred for 1.5 hours at 50 °C until TLC (4:1 CHCl$_3$/MeOH) indicated disappearance of starting material. The methanol was then removed by rotary evaporation leaving behind the water. The mixture was then cooled in an ice water bath while adjusting the pH to 1 with 1M HCl producing a brown precipitate. The brown precipitate was then filtered to give pure 19 (2.14 g, 90 %): mp > 200 °C. TLC 4:1 (CHCl$_3$/MeOH) R$_f$ = 0.20. $^1$H NMR data (D6-DMSO): δ
12.43 (s, 1H), 9.45 (s, 1H), 8.51 (d, J = 8 Hz, 1H), 6.44 (d, J = 8.8 Hz, 1H), 2.71 (t, J = 2.4 Hz, 2H). $^{13}$C NMR (D6-DMSO): δ 172.87, 145.33, 144.84, 144.62, 138.38, 121.49, 99.86, 32.79.

1-Methyl-4-(1-methyl-4-(2-(7-nitrobenzo[c][1,2,5]oxadiazol-4-ylamino)acetamido-1H-pyrrole-2-carboxamido N-propyl-1H-pyrrole-2-carboxamide (20). Compound 6 (0.140 g, 0.419 mmol) was dissolved in 50 mL of ethanol and 0.1184 g 10 % water wet Pd/C was added in a Parr jar. The mixture was shaken under pressurized hydrogen (70 psi) until 6 had disappeared by TLC (6:1 CHCl$_3$/MeOH). The Pd/C was filtered through celite and the filtrate was concentrated by rotary evaporation. The residue was placed under high vacuum overnight. The flask was flushed with N$_2$, the reduced compound 20 was dissolved in anhydrous DMF (3.5 mL) along with carboxylic acid 19 (0.111 g, 0.466 mmol), EDCI (0.123 g, 1.5 eq.), DMAP (0.130 g, 2.5 eq.), HOBT (0.1750 g, 3eq), and CuCl$_2$ (0.008g, 0.1 eq.). The mixture was allowed to stir for two days until the disappearance of the starting material was indicated by TLC (6:1 CHCl$_3$/MeOH). The solution was diluted with 100 mL DCM and the organic solution was extracted with H$_2$O (2x, 150 mL), 1 M HCl (2x, 100 mL), and sat. NaHCO$_3$ (2x, 100 mL). The organic layer was then dried over MgSO$_4$. The resulting solution was rotary evaporated until dry to yield the crude product. Flash column chromatography (6:1 CHCl$_3$/MeOH) yielded pure 20 as an orange solid (0.067 g, 37%): mp 152-157 °C. TLC (6:1 CHCl$_3$/MeOH) $R_f$ = 0.36. $^1$H NMR data (D6-DMSO): δ 9.99 (s, 1H), 9.83 (s, 1H), 9.52 (s, 1H), 8.54 (t, J = 8 Hz, 1H), 7.98 (t, J = 4 Hz, 1H), 7.17 (d, J = 8 Hz, 2H), 6.84 (d, J = 8.4 Hz, 2H), 6.48 (d, J = 8.8 Hz, 2H), 3.82 (s, 3H), 3.78 (s, 3H), 3.10 (d, J = 8 Hz, 2H), 2.72 (d, J = 6.8 Hz, 2H), 1.46 (m, 2H), 0.85 (t, J = 7.2 Hz, 3H). $^{13}$C NMR (D6-DMSO): δ 167.45, 161.66, 158.76, 123.52, 123.25, 122.39, 122.13, 118.61, 118.20, 104.54, 104.26, 41.67, 40.77, 36.61, 36.37, 23.04, 11.90.
6-(Acetoxymethyl)-3-(2-(1-methyl-4-(1-methyl-4-(2-(7-nitrobenzo[c][1,2,5]oxadiazol-4-ylamino)acetamido)-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamido)acetamido)tetrahydro-2H-pyran-2,4,5-triyi triacetate (21a). Compound 13a (0.344 g, 0.495 mmol) was dissolved in 30 mL of ethanol and of 0.278 g 50 % water wet Pd/C was added in a Parr jar. The mixture was shaken under pressurized hydrogen (60 psi) until 6 had disappeared by TLC (6:1 CHCl₃/MeOH). The Pd/C was filtered through celite and the filtrate was concentrated by rotary evaporation then further dried under high vacuum overnight. The flask was flushed with N₂, the reduced compound 13a was dissolved in anhydrous DMF (3mL) along with carboxylic acid 19 (0.118 g, 0.495 mmol), EDCI (0.147 g, 1.5 eq.), DMAP (0.151 g, 2.5 eq.), HOBT (0.200 g, 3eq), and CuCl₂ (0.009 g, 0.1 eq.). The mixture was allowed to stir for two days until the disappearance of the starting material was indicated by TLC (6:1 CHCl₃/MeOH). The solution was diluted with 150 mL DCM and the organic solution was extracted with H₂O (2x, 200 mL), 1 M HCl (2x, 200 mL), and sat. NaHCO₃ (2x, 200 mL). The organic layer was then dried over MgSO₄. The resulting solution was rotary evaporated until dry to yield the crude product. Flash column chromatography (6:1 CHCl₃/MeOH) yielded pure 21a as an orange solid (0.200 g, 48%): mp 144 °C to decomp. TLC (6:1 CHCl₃/MeOH) Rₐ = 0.36.

¹H NMR data (D₆-DMSO): δ 9.92 (s, 1H), 9.90 (s, 1H), 9.52 (s, 1H), 8.52 (t, J = 5.2 Hz, 1H), 8.32 (t, J = 5.2 Hz, 1H), 7.82 (d, J = 9.6 Hz, 1H), 7.22 (s, 1H), 7.17 (d, J = 1.6 Hz, 1H), 6.87 (s, 2H), 6.46 (d, J = 9.2 Hz, 1H), 5.74 (d, J = 8.8 Hz, 1H), 5.24 (t, J = 9.6 Hz, 1H), 4.85 (t, J = 9.6 Hz, 1H), 4.18 (dd, J = 4.8 Hz, 8 Hz, 2H), 3.40 (m, 3H), 3.81 (s, 3H), 3.76 (s, 3H), 2.05 (s, 3H), 1.99 (s, 3H), 1.95 (s, 3H), 1.94 (s, 3H). ¹³C NMR (D₆-DMSO): δ 170.51, 170.27, 170.11, 169.74, 169.45, 167.46, 162.01, 158.80, 132.16, 132.08, 129.1, 123.19, 122.82, 122.55, 122.24,
6-(Acetoxymethyl)-3-(3-(1-methyl-4-(1-methyl-4-(2-(7-nitrobenzo[c][1,2,5]oxadiazol-4-ylamino)acetamido)-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamido)propanamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate (21b). Compound 21b was synthesized similar to the procedure described above for 20a, reducing 0.4067 g (0.573 mmol) of 15b to the amino and dissolving in 4 mL anhydrous DMF along with, compound 20 (0.152 g, 0.64 mmol), EDCI (0.164 g, 1.5 eq.), DMAP (0.179 g, 2.5 eq.), HOBT (0.234 g, 3.0 eq.), and CuCl₂ (0.008 g, 0.1 eq.) to yield 21b as a dark brown solid (0.349 g, 68%): mp TLC (6:1 CHCl₃/MeOH) Rᶠ = 0.27. ¹H NMR data (D₆-DMSO): δ 10.01 (s, 1H), 9.84 (s, 1H), 9.50 (s, 1H), 8.50 (s, 1H), 8.07 (d, J = 8.8 Hz, 1H), 7.93 (t, J = 6 Hz, 1H), 7.13 (s, 1H), 6.83 (s, 1H), 6.75 (s, 1H), 6.46 (d, J = 8.4 Hz, 1H), 5.68 (d, J = 8.8 Hz, 1H), 5.15 (t, J = 9.6 Hz, 1H), 4.85 (t, J = 10 Hz, 1H), 4.15 (dd, J = 4.4 Hz, 9.2 Hz, 2H), 3.96 (m, 3H), 3.76 (s, 3H), 3.73 (s, 3H), 2.71 (q, J = 6.8 Hz, 2H), 2.27 (t, J = 6.8 Hz, 2H), 1.95 (s, 6H), 1.92 (s, 3H), 1.83 (s, 3H). ¹³C NMR (D₆-DMSO): δ 171.21, 170.50, 170.06, 169.74, 169.34, 167.45, 161.68, 158.77, 145.44, 144.92, 138.43, 123.20, 123.13, 12..49, 122.26, 118.62, 118.44, 104.58, 104.32, 99.93, 92.13, 72.61, 71.96, 68.47, 61.93, 52.30, 36.62, 36.42, 36.03, 35.82, 20.97, 20.87, 20.70.

6-(Acetoxymethyl)-3-(4-(1-methyl-4-(1-methyl-4-(2-(7-nitrobenzo[c][1,2,5]oxadiazol-4-ylamino)acetamido)-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamido)butanamido)tetrahydro-2H-pyran-2,4,5-triyl triacetate (21c). Compound 21c was synthesized similar to the procedure described above for 20a, reducing 0.5424 g (0.751 mmol) of 15c to the amino and dissolving in 4 mL anhydrous DMF along with, compound 20 (0.197 g, 0.8 mmol), EDCI (0.213 g, 1.5 eq.), DMAP (0.243 g, 2.5 eq.), HOBT (0.307 g, 3.0...
eq.), and CuCl₂ (0.015 g, 0.1 eq.) to yield \(21c\) as a dark brown solid (0.403 g, 60 %): mp TLC (6:1 CHCl₃/MeOH) \(R_f = 0.38\). \(^{1}H\) NMR data (D₆-DMSO): \(\delta = 10.05\ (s, 1H), 9.86\ (s, 1H), 9.52\ (s, 1H), 8.55\ (t, J = 5.6 Hz, 1H), 8.05\ (t, J = 5.6 Hz, 1H), 7.16\ (s, 2H), 6.86\ (d, J = 6.6 Hz, 2H), 6.49\ (m, 1H), 5.72\ (d, J = 8.45 Hz, 1H), 5.18\ (t, J = 6 Hz, 1H), 4.88\ (t, J = 6.4 Hz, 1H), 4.20\ (dd, J = 3.2 Hz, 6 Hz, 2H), 4.18\ (t, J = 2 Hz, 3H), 3.83\ (s, 3H), 3.79\ (s, 3H), 3.12\ (t, J = 2 Hz, 2H), 2.06\ (s, 3H), 2.02\ (s, 3H), 1.98\ (s, 3H), 1.93\ (s, 3H), 1.65\ (pentet, J = 1.2 Hz, 4.8 Hz, 2H). \(^{13}C\) NMR (D₆-DMSO): \(\delta = 172.70, 170.50, 170.05, 169.74, 169.36, 167.45, 162.78, 161.67, 158.76, 144.84, 144.55, 138.38, 123.20, 122.42, 122.24, 118.61, 118.29, 104.60, 104.25, 99.88, 92.17, 72.61, 71.95, 68.48, 61.90, 60.68, 52.22, 38.35, 36.61, 36.40, 36.24, 33.71, 31.22, 26.16, 20.95, 20.86, 20.77.

**Methyl 4-(4-acrylamido-1-methyl-1\(^{1}H\)-pyrrole-2-carboxamido)-1-methyl-1\(^{1}H\)-pyrrole-2-carboxylate (22).** Compound 7 (4.10 g, 19.6 mmol) was dissolved in 50 mL of EtOH and 0.50 g 10 % water wet Pd/C was added in a parr jar. The mixture was shaken under pressurized hydrogen (70 psi) until 7 had disappeared as indicated by TLC (EtOAc). The Pd/C was filtered through celite and the filtrate was concentrated by rotary evaporation and the residue was kept under high vacuum overnight. The flask with the residue was flushed with Ar and the residue was dissolved in anhydrous THF (8 mL) and anhydrous DIEA (3.32 mL) was added to the solution. The solution was bubbled with Ar and cooled to -40 °C. Acryloyl chloride (1.20 mL) was added drop wise to the solution which was then allowed to stir at -20 °C, protected from light, until the reaction was complete as indicated by TLC (6:1 CHCl₃/MeOH). The solvent was removed by rotary evaporation and the resulting solid dissolved in 50 mL of EtOAc and extracted with DI H₂O (2x, 60 mL). The organic layer was then dried over MgSO₄. The resulting solution was rotary evaporated until dry to yield the product, 22 as an orange solid.
(4.18 g, 96 %): mp 179-184 °C. TLC (6:1 CHCl₃/MeOH) Rₜ = 0.58. ¹H NMR data (D6-DMSO): δ 10.11 (s, 1H), 9.93 (s, 1H), 7.44 (d, J = 1.6 Hz, 1H), 7.25 (d, J = 1.6 Hz, 1H), 6.90 (d, J = 2.0 Hz, 1H), 6.87 (d, J = 2.4 Hz, 1H), 6.33 (m, 1H), 6.16 (dd, J = 2.0 Hz, 15.2 Hz, 1H), 5.65 (dd, J = 2.0 Hz, 8 Hz, 1H), 3.82 (s, 3H), 3.81 (s, 3H), 3.72 (s, 3H). ¹³C NMR (D6-DMSO): δ 162.10, 161.26, 158.77, 131.91, 126.10, 125.70, 123.29, 122.21, 121.22, 119.03, 118.97, 108.77, 104.54, 51.44, 36.62 (2C).

3-(5-(5-(Methoxycarbonyl)-1-methyl-1H-pyrrol-3-ylcarbamoyl)-1-methyl-1H-pyrrol-3-ylamino)-3-oxopropane-1-sulfonic acid (23). To a solution of alkene 22 (1.68 g, 5.1 mmol) dissolved in 4 mL EtOH, NaHSO₃ (1.06 g, 10 mmol) dissolved in 1mL of DI H₂O was added all at once. The pH was adjusted to 8 with 5 % NaOH and the mixture was refluxed until 22 had disappeared and a baseline spot appeared by TLC (6:1 CHCl₃/MeOH). The solution was then rotovaped until a minimal amount of water remained. This solution was then cooled in an ice/water bath and the pH adjusted to 1 with concentrated HCl. A pale yellow precipitate fell out of solution, which was vacuum filtered to give pure 23 as a pale yellow solid (2.10 g, 81 %): mp decomposition 197 °C. TLC (5:2 CHCl₃/MeOH) Rₜ = 0.27. ¹H NMR data (D6-DMSO): δ 9.97 (s, 1H), 9.89 (s, 1H), 7.44 (s, 1H), 7.14 (s, 1H), 6.88 (s, 1H), 6.84 (s, 1H), 3.82 (s, 3H), 3.80 (s, 3H), 3.72 (s, 3H), 2.64 (d, J = 8.4 Hz, 2H), 2.54 (d, J = 8.8 Hz, 2H). ¹³C NMR (D6-DMSO): δ 169.02, 161.28, 158.89, 123.40, 122.89, 121.21, 118.97, 118.74, 51.41, 48.03, 36.62, 36.50, 33.00, 31.16.

1-Methyl-4-(1-methyl-4-(3-sulfopropanamido)-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxylic acid (24). To a solution of sulfonic acid 23 (33 mg, 79 µmol) suspended in 4 mL of EtOH, NaOH (19 mg, 0.48 mmol) dissolved in 1 mL of DI H₂O was added all at once. The mixture was refluxed until 23 had disappeared and a baseline spot appeared by TLC (6:1
The solution was then rotovaped until a minimal amount of water remained. This solution was then cooled in an ice/water bath and the pH adjusted to 1 with concentrated HCl. A pale yellow precipitate fell out of solution, which was vacuum filtered to give pure 24 as a pale yellow solid (25 mg, 72%): mp decomposition 169 °C. TLC 3:1 CHCl₃/MeOH Rᵣ = 0.20. ¹H NMR data (D6-DMSO): δ 9.95 (s, 1H), 9.70 (s, 1H), 7.11 (d, J = 2.0 Hz, 1H), 7.07 (d, J = 1.6 Hz, 1H), 6.80 (d, J = 1.6 Hz, 1H), 6.50 (d, J = 1.6 Hz, 1H), 6.04 (s, 1H), 3.79 (s, 3H), 3.77 (s, 3H), 2.66 (d, J = 8.8 Hz, 2H), 2.52 (d, J = 7.2 Hz, 2H). ¹³C NMR (D6-DMSO): δ 168.57, 158.42, 123.89, 123.47, 122.40, 119.63, 118.24, 112.25, 104.02, 101.01, 47.95, 36.47, 36.30, 32.55.

**Ethyl 3-(1-methyl-4-(1-methyl-4-(3-(methylsulfonyl)propanamido)-1H-pyrrole-2-carboxamido)1H-pyrrole-2-carboxamido)propanoate (25).** Compound 5b (1.03 g, 2.65 mmol) was dissolved in 50 mL of ethanol and 0.500 g 50% water wet Pd/C was added in a Parr jar. The mixture was shaken under pressurized hydrogen (70 psi) until 5b had disappeared by TLC (EtOAc). The Pd/C was filtered through celite and the filtrate was concentrated by rotary evaporation. The residue was placed under high vacuum overnight. The 3-(methylsulfonyl) propanoic acid (0.500 g, 3.3 mmol) was dissolved in 4 mL of anhydrous DMF along with, EDCI (0.785 g, 1.5 eq.), DMAP (0.818 g, 2.5 eq.), HOBT (1.11 g, 3.0 eq.), and CuCl₂ (0.042 g, 0.1 eq.). After stirring for 1 hour the reduced compound 5b was cannulated into the mixture and stirred at room temperature for two days until the disappearance of starting material as indicated by TLC (6:1 CHCl₃/MeOH). The solution was diluted with 100 mL of DCM and the organic solution was extracted with H₂O (2x, 200 mL), sat. NaHCO₃ (2x, 200 mL), and 1 M HCl (2x, 200 mL). The organic layer was then dried over MgSO₄. The resulting solution was rotary evaporated until dry to yield a red-brown solid 17 (0.052 g, 40%): mp 75-80 °C. TLC (6:1
CHCl$_3$/MeOH) R$_f$ = 0.64. $^1$H NMR data (D6-DMSO): $\delta$ 10.06 (s, 1H), 9.87 (s, 1H), 8.05 (t, J = Hz, 1H), 7.16 (d, J = 8 Hz, 2H), 6.85 (d, J = Hz, 2H), 4.04 (q, J = 6.8 Hz, 7.2 Hz, 3H), 3.81 (s, 3H), 3.78 (s, 3H), 3.00 (s, 3H), 2.72 (t, J = 8 Hz, 2H), 1.17 (t, J = 7.2 Hz, 3H). $^{13}$C NMR data (D6-DMSO): $\delta$ 171.84, 170.76, 161.74, 158.76, 123.14, 122.14, 122.14, 118.63, 118.44, 104.73, 104.33, 61.03, 60.34, 49.63, 36.40, 35.26, 34.49, 27.58, 14.55, 14.47.

(2S,3R,4R,5S,6R)-6-(Acetoxymethyl)-3-benzamidotetrahydro-2H-pyran-2,4,5-triyll triacetate (26). Compound 12 (4.12 g, 11 mmol) was suspended in 100 mL of DCM. While vigorously stirring the solution was cooled to 0 °C in an ice cold water bath. Saturated NaHCO$_3$ (100 mL) was added to the cooled solution making a heterogeneous system. Benzoyl chloride (2 mL, 17 mmol) mixed with DCM (10 mL) was added to the heterogeneous solution drop wise in a drop funnel. After 45 minutes of stirring at 0 °C a white precipitate formed, the reaction was allowed to reach room temperature before filtering the pure white solid 26 (2.78 g, 56 %): mp > 200 °C. TLC (6:1 CHCl$_3$/MeOH) R$_f$ = 0.67. $^1$H NMR data (D6-DMSO): $\delta$ 8.56 (d, J = 9.2 Hz, 1H), 7.71 (dd, J = 1.2 Hz, 8 Hz, 2H), 7.50 (m, 3H), 5.88 (d, J = 8.8 Hz, 1H), 5.34 (t, J = 9.6 Hz, 1H), 4.97 (t, J = 9.6 Hz, 1H), 4.22 (m, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.85 (s,3H).

$^{13}$C NMR data (D6-DMSO) $\delta$: 170.54, 170.13, 169.76, 169.35, 167.06, 134.52, 132.01, 128.92, 127.54, 92.32, 72.83, 72.14, 68.39, 61.93, 53.01, 20.99, 20.95, 20.89, 20.72.

N-((2R,3R,4R,5S,6R)-2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)benzamide (27). Compound 27 was deacetylated using a combo of procedures from literature.$^{30,31}$ Compound 26 (0.052 g, 0.12 mmol) was dissolved in 1.1 mL of dry THF and cooled to -10 °C. Freshly prepared 1 M NaOCH$_3$ (416 μL, 0.461 mmol) was added to the cooled solution and deprotection was monitored by TLC (6:1CHCl$_3$/MeOH) until reaction was complete in 20 minutes. The mixture was treated with Dowex 50WA resin, filtered, and the solvent
removed by rotary evaporation to give 27 as a white solid (0.029 g, 91%, 4:1 diasteromers): mp 159-163 °C. TLC (3:1 CHCl₃/MeOH) Rₜ = 0.34. ¹H NMR data (D₆-DMSO): δ 8.17 (d, J = 8.9 Hz, 1H), 8.03 (d, J = 7.2 Hz, 1H), 7.87 (m, 4H), 7.49 (m, 6H), 6.55 (d, J = 6 Hz, 1H), 6.45 (d, J = 4.4 Hz, 1H), 5.07 (t, J = 4 Hz, 1H), 4.96 (d, J = 5.2 Hz, 1H), 4.70 (d, J = 5.2 Hz, 1H), 4.46 (t, J = 5.9 Hz, 1H), 3.74 (m, 3H), 3.62 (m, 3H), 3.51 (m, 1H). ¹³C NMR data (D₆-DMSO) δ: 166.88, 134.95, 131.57, 128.59, 127.92, 127.78, 90.87, 72.61, 71.46, 70.49, 61.58, 55.83.

1-Methyl-4-(1-methyl-4-(1-methyl-4-nitro-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamido)-N-propyl-1H-pyrrole-2-carboxamide (28). Compound 28 was synthesized as described in literature. Compound 6 (0.359 g, 1.1 mmol) was dissolved in 50 mL of EtOH and 0.340 g 50% water wet Pd/C was added in a Parr jar. The mixture was shaken under pressurized hydrogen (70 psi) until 6 had disappeared as indicated by TLC (6:1 CHCl₃/MeOH). The Pd/C was filtered through celite and the filtrate was concentrated by rotary evaporation to yield 0.330 g (0.99 mmol) of reduced compound 6 and stored under high vacuum overnight. Compound 2 was added to a solution of reduced compound 6 (0.330 g, 0.99 mmol) dissolved in anhydrous acetonitrile at 0 °C. The mixture was then allowed to reach room temperature and stir for two days or until TLC (EtOAc) indicated the disappearance of both starting compounds. The solvent was evaporated under rotary evaporation and the brown residue was recrystallized from methanol to afford 0.138 g (28%) of 28 as a yellow powder: mp > 200 °C. TLC (EtOAc) Rₜ = 0.36. ¹H NMR data (D₆-DMSO): δ 10.29 (s, 1H), 9.94 (s, 1H), 8.19 (d, J = 1.6 Hz, 1H), 8.00 (t, J = 5.6, 1H), 7.58 (d, J = 1.2 Hz, 1H), 7.26 (d, J = 1.6 Hz, 1H), 7.17 (d, J = 1.6 Hz, 1H), 7.02 (d, J = 1.6 Hz, 1H), 6.84 (d, J = 2.0 Hz, 1H), 3.95 (s, 3H), 3.85 (s, 3H), 3.78 (s, 3H), 3.10 (m, 2H), 1.47 (m, 2H), 0.85 (t, J = 7.2 Hz, 3H). ¹³C NMR data (D₆-DMSO): δ 161.67, 158.77, 157.36,
6-(Acetoxymethyl)-2-(3-(4-(4-(3-(tert-butoxycarbonylamino)propanamido)-1-methyl-1H-pyrrole-2-carboxamido)-1-methyl-1H-pyrrole-2-carboxamido)propanamido)tetrahydro-2H-pyran-2,4,5-tryl triacetate (29). Compound 13b (0.313 g, 0.44 mmol) was dissolved in 30 mL of EtOH and 0.200 g 50 % water wet Pd/C was added in a parr jar. The mixture was shaken under pressurized hydrogen (70 psi) until 13b had disappeared as indicated by TLC (6:1CHCl₃/MeOH). The Pd/C was filtered through celite and the filtrate was concentrated by rotary evaporation and the reduced compound 13b was stored under high vacuum overnight. The reduced compound 13b and N-Boc-beta-alanine (0.170 g, 0.89 mmol) was dissolved in 3 mL of anhydrous DMF along with, EDCI (0.143 g, 1.5 eq.), DMAP (0.140 g, 2.5 eq.), HOBT (0.179 g, 3.0 eq.), and CuCl₂ (0.005 g, 0.1 eq.). The mixture was allowed to stir for two days until the disappearance of starting material as indicated by TLC (6:1 CHCl₃/MeOH). The solution was diluted with 100 mL of DCM and the organic solution was extracted with H₂O (2x, 100 mL), sat. NaHCO₃ (2x, 100 mL), and 1 M HCl (2x, 100 mL). The organic layer was then dried over MgSO₄. The resulting solution was rotary evaporated until dry to yield a red-brown solid 29 (0.211 g, 57%): mp 106-110 °C. TLC (6:1 CHCl₃/MeOH) Rᵣ = 0.65. ¹H NMR data (D6-DMSO): δ 9.87 (s, 1H), 8.10 (d, J = 8.8 Hz, 2H), 7.19 (dd, J = 1.6 Hz, 10.4 Hz, 2H), 6.83 (dd, J = 1.6 Hz, 9.2 Hz, 2H), 5.74 (d, J = 9.2 Hz, 1H), 5.20 (t, J = 9.2 Hz, 1H), 4.90 (t, J = 9.2 Hz, 1H), 4.19 (dd, J = 4.4 Hz, 8 Hz, 2H), 4.01 (m, 3H), 3.83 (s, 3H), 3.80 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.89 (s, 3H), 1.39 (s, 9H). ¹³C NMR data (D6-DMSO): δ 171.28, 171.02, 170.47, 170.05, 169.71, 169.30, 161.65, 158.52, 124.90, 123.33,
Tert-butyl 3-(1-methyl-5-(1-methyl-5-(propylcarbamoyl)-1H-pyrrole-3-ylcarbamoyl)-1H-pyrrole-3-ylamino)3-oxopropylcarbamate (30). Compound 30 was synthesized using a procedure similar to the one described for 29 using 0.2110 g (0.633 mmol) of 6, 3 mL anhydrous DMF along with, EDCI (0.190 g, 1.5 eq.), DMAP (0.194 g, 2.5 eq.), HOBT (0.258 g, 3.0 eq,), CuCl$_2$ (0.01 g, 0.1 eq.) and 0.121 g (0.633 mmol) N-Boc-beta-alanine to yield 30 as a red-brown solid (0.227 g, 74 %): mp 171-176 °C. TLC (6:1 CHCl$_3$/MeOH) $R_f$ = 0.53. $^1$H NMR (D$_6$-DMSO): $\delta$ 9.84 (d, J = 4.8 Hz, 1H), 7.97 (t, J = 5.6 Hz, 1H), 7.15 (t, J = 1.6 Hz, 2H), 6.83 (dd, J = 1.6 Hz, 4 Hz, 3H), 3.81 (s, 3H), 3.78 (s, 3H), 3.14 (m, 4H), 2.49 (q, J = 6 Hz, 6.8 Hz, 2H), 1.47 (m, 2H), 1.36 (s, 10 H), 0.86 (t, J = 7.2 Hz, 3H). $^{13}$C NMR (D$_6$-DMSO) $\delta$: 171.62, 168.05, 161.69, 158.82, 123.55, 123.21, 122.46, 122.38, 18.58, 118.18, 204.57, 104.34, 78.08, 60.31, 36.54, 36.35, 28.71, 23.04, 14.52, 11.89.

Ethyl 3-(2-oxo-2H-3-carboxamido)propanoate (31). In a flask flushed with Ar, coumarin-3-carboxylic acid (0.503 g, 0.26 mmol) was dissolved in 4 mL of DMF along with EDCI (0.774 g, 1.5 eq.), DMAP (0.799 g, 2.5 eq.), HOBT (1.06 g, 3.0 eq,), CuCl$_2$ (0.037 g, 0.1 eq.), and $\beta$-Alanine ethyl ester hydrochloride (0.413 g, 0.27 mmol). The reaction was allowed to stir for two days until TLC (3:1 EtOAc/MeOH) indicated the disappearance of starting carboxylic acid. The mixture was diluted with 100 mL of DCM and the organic solution was extracted with H$_2$O (2x, 100 mL), sat. NaHCO$_3$ (2x, 100 mL), and 1 M HCl (2x, 100 mL). The organic layer was then dried over MgSO$_4$. The resulting solution was rotary evaporated until dry to yield a white solid 29 (0.607 g, 80%): mp 116-118 °C. TLC (3:1 EtOAc/MeOH) $R_f$ = 0.67. $^1$H NMR (D$_6$-DMSO): $\delta$ 8.88 (s, 2H), 7.98 (d, J = 8 Hz, 1H), 7.74 (t, J = 7.6 Hz, 1H), 7.50 (d, J
= 8 Hz, 1H), 7.44 (t, J = 7.6 Hz, 1H), 4.08 (q, J = 6.8 Hz, 7.2 Hz, 2H), 3.56 (q, J = 6.4 Hz, 6 Hz, 2H), 2.58 (t, J = 2.4 Hz, 3H), 1.19 (t, J = 7.2 Hz, 3H). $^{13}$C NMR (D6-DMSO): δ 171.92, 161.54, 160.82, 154.37, 148.16, 134.61, 130.78, 125.60, 119.90, 118.91, 116.59, 60.56, 35.54, 34.13, 14.52.

3-(2-Oxo-2H-chromene-3-carboxamido)propanoic acid (32). To a solution of ester 31 (0.493 g, 1.7 mmol) suspended in 12 mL of acetonitrile, NaOH (0.277 g, 6.9 mmol) dissolved in 3 mL of DI H$_2$O was added all at once. The mixture was stirred at room temperature until 31 had disappeared and a baseline spot appeared by TLC (3:1 EtOAc/MeOH). The solution was then rotary evaporated until a minimal amount of water remained. This solution was then cooled in an ice/water bath and the pH adjusted to 1 with concentrated HCl. A white precipitate fell out of solution, which was vacuum filtered to give pure 32 as a white solid (0.397 g, 88 %): mp 204-206 °C. TLC (3:1 EtOAc/MeOH) R$_f$ = 0.73. $^1$H NMR data (D6-DMSO): δ 12.36 (s, 1H), 8.88 (s, 2H), 7.98 (d, J = 8 Hz, 1H), 7.74 (t, J = 7.6 Hz, 1H), 7.49 (d, J = 8.4 Hz, 1H), 7.43 (t, J = 7.6 Hz, 1H), 3.52 (d, J = 6.4 Hz, 2H). $^{13}$C NMR (D6-DMSO): δ 173.58, 161.44, 160.85, 154.34, 148.12, 134.56, 130.75, 125.57, 119.06, 118.90, 116.57, 35.56, 34.14.

Ethyl 3-(7-nitrobenzo[c][1,2,5]oxadiazol-4-ylamino)propanoate (33). In a flask flushed with Ar, 4-chloro-7-nitro-2,1,3-benzoxadiazole (0.102 g, 0.50 mmol) and β-Alanine ethyl ester hydrochloride (0.085 g, 0.51 mmol) were dissolved in 2 mL of EtOAc dried over sieves. To this mixture TEA (175 μL, 0.13 mmol, 2.5 eq.) dried over sieves was added causing the color of the reaction to change from yellow to orange. The reaction was monitored by TLC (3:1 EtOAc/MeOH) until complete disappearance of 4-chloro-7-nitro-2,1,3-benzoxadiazole. The solution was rotary evaporated to dryness and then dissolved in 50 mL of DCM and the organic
solution was extracted with 1M HCl (2x, 60 mL). The organic layer was then dried over MgSO₄.

The resulting solution was rotary evaporated until dry to yield a red-brown solid 33 (0.163 g, 87 %): mp decomposition at 170 °C. TLC (3:1 EtOAc/MeOH) Rₜ = 0.65. ¹H NMR data (D6-DMSO): δ 9.43 (s, 1H), 8.52 (d, J = 8.4 Hz, 1H), 6.43 (d, J = 8.8 Hz, 1H), 4.05 (q, J = 7.2 Hz, 2H), 3.67 (s, 2H), 2.75 (t, 6.8 Hz, 2H), 1.15 (t, J = 7.2 Hz, 3H). ¹³C NMR (D6-DMSO): δ 171.29, 145.23, 144.76, 144.20, 138.27, 121.46, 99.85, 60.67, 14.50.

N-(2-bromoethyl)-1-methyl-4-nitro-1H-pyrrole-2-carboxamide (34). Compound 2 (4.92 g, 18 mmol) was dissolved in 40 mL of EtOAc dried over sieves. 2-bromoethylamine hydrobromide (9.28 g, 45 mmol) was then added to solution resulting in a suspension. TEA (7.53 mL, 54 mmol) dissolved in 10 mL of EtOAc dried over sieves was added drop wise to the mixture via a drop funnel. The mixture stirred for two days at which time a brown precipitate formed. The precipitate was filtered and the filtrate extracted with 1M HCl (2x, 60 mL) and DI H₂O (2x, 60 mL). The organic layer was then dried over MgSO₄. The resulting solution was rotary evaporated until dry to yield a pale orange solid 34 (3.23 g, 64 %): mp 130-135 °C. TLC (6:1 CHCl₃/MeOH) Rₜ = 0.52. ¹H NMR data (D6-DMSO): δ 8.69 (s, 1H), 8.14 (d, J = 1.6 Hz, 1H), 7.44 (d, J = 2 Hz, 1H), 3.89 (s, 3H). ¹³C NMR (D6-DMSO): δ 160.38, 134.24, 128.60, 162.40, 108.10, 41.15, 37.90, 32.46. ¹H NMR data (CDCl₃): δ 7.58 (d, J = 1.6 Hz, 1H), 7.14 (d, J = 1.6 Hz, 1H), 6.40 (s, 1H), 6.40 (s, 3H), 3.80 (q, J = 6 Hz, 5.6 Hz, 2H), 3.56 (t, J = 5.6 Hz, 2H). ¹³C NMR (D6-DMSO): δ 160.32, 135.00, 126.95, 125.80, 107.36, 41.03, 37.95, 32.13.

6-(Acetoxymethyl)-3-(3-(1-methyl-4-propionamido-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamido)propanamido)tetrahydro-2H-pyran-2,4,5-tryl triacetate (35a). Compound 16b (0.110 g, 0.15 mmol) was dissolved in 20 mL of 200 proof EtOH and 0.200 g 50 % water wet Pd/C was added in a parr jar. The mixture was shaken under pressurized hydrogen
(80 psi) until 16b had disappeared as indicated by TLC (6:1CHCl₃/MeOH). The Pd/C was filtered through celite and the filtrate was concentrated by rotary evaporation to yield pure 35a as a white solid (0.108g, 98 %): mp 141-148 °C. TLC (6:1CHCl₃/MeOH) Rf = 0.47. ¹H NMR data (D6-DMSO): δ 9.85 (s, 1H), 9.75 (s, 1H), 8.09 (d, J = 9.2 Hz, 1H), 7.96 (t, J = 6.0 Hz, 1H), 7.17 (dd, J = 1.2 Hz, 13.2 Hz, 2H), 6.82 (dd, J = 1.6 Hz, 14.8 Hz, 2H), 5.72 (d, J = 1.2 Hz, 1H), 5.18 (t, J = 10 Hz, 1H), 4.88 (t, J = 10 Hz, 1H), 4.20 (dd, J = 4.4 Hz, 8.0 Hz, 1H), 3.98 (m, 3H), 3.81 (s, 3H), 3.78 (s, 3H), 2.30 (t, J = 7.2 Hz, 2H), 2.24 (q, J = 7.6 Hz, 2H), 2.001 (s, 3H), 1.99 (s, 3H), 1.97 (s, 3H), 1.88 (s, 3H), 1.05 (t, J = 7.2 Hz, 3H). ¹³C NMR (D6-DMSO): δ 171.27, 170.72, 170.50, 170.07, 169.74, 169.34, 161.69, 158.83, 123.12, 122.54, 118.52, 118.42, 104.56, 104.38, 92.13, 72.61, 71.96, 68.47, 61.93, 52.31, 36.54, 36.41, 36.04, 35.81, 29.21, 20.97, 20.87, 20.70, 10.48.

6-(Acetoxymethyl)-3-(4-(1-methyl-4-propionamido-1H-pyrrole-2-carboxamido)-1H-pyrrole-2-carboxamido)butanamido)tetrahydro-2H-pyran-2,4,5-tryl triacetate (35b).

Compound 35b was synthesized similar to the procedure described above for 35a, using 0.100 g (0.13 mmol) of 16c and 0.100 g of 50 % water wet Pd/C to yield pure 35b as a white solid (0.087 g, 87 %): mp 141-147 °C. TLC (6:1CHCl₃/MeOH) Rf = 0.44. ¹H NMR data (D6-DMSO): δ 9.84 (s, 1H), 9.75 (s, 1H), 8.01 (m, 2H), 7.16 (d, J = 8.8 Hz, 2H), 6.84 (s, 2H), 5.71 (d, J = 8.8 Hz, 1H), 5.17 (t, J = 10 Hz, 1H), 4.90 (t, J = 10 Hz, 1H), 4.18 (dd, J = 4.8 Hz, 8 Hz, 1H), 3.99 (m, 3H), 3.81 (s, 3H), 3.78 (s, 3H), 3.43 (m, 2H), 3.11 (q, J = 6 Hz, 2H), 2.22 (q, J = 7.6 Hz, 2H), 2.05 (s, 3H), 2.00 (s, 3H), 1.97 (s, 3H), 1.92 (s, 3H), 1.65 (t, J = 7.2 Hz, 2H), 1.06 (t, J = 7.2 Hz, 3H). ¹³C NMR (D6-DMSO): δ 172.71, 170.76, 170.48, 170.04, 169.72, 169.33, 161.72, 158.88, 123.37, 123.18, 122.57, 118.54, 118.31, 104.66, 104.43, 92.22, 72.66, 72.00, 68.57, 52.30, 38.41, 36.52, 36.38, 33.74, 29.22, 26.18, 20.95, 20.85, 20.77, 10.46.
1-Methyl-4-propionamido-1H-pyrrole-2-carboxamido)-N-(3-oxo-3-(2,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-ylamino)propyl)-1H-pyrrole-2-carboxamide (36). Compound 35a (0.055 g, 0.077 mmol) was deacetylated with anhydrous 7N methanolic ammonia (3mL). The reaction was monitored by TLC (6:1CHCl₃/MeOH) until compound 35a condensed to a baseline spot. Once a baseline spot, the solvent system was changed to 100 % MeOH to ensure complete deprotection if the acetyl groups. When complete by TLC (MeOH) the mixture was rotovaped to dryness and placed under vacuum to yield crude 35a. The crude compound was then recrystallized in EtOH to yield 36 as a white solid (0.10 g, 24 %, mixture of diasteromers 2:1): mp 215-219 °C. TLC (3:2 CHCl₃/MeOH) Rᵣ = 0.57. ¹H NMR data (D₆-DMSO): δ 9.85 (s, 1H), 9.75 (s, 1H), 8.09 (d, J = 9.2 Hz, 1H), 7.96 (t, J = 5.2 Hz, 1H), 7.79 (d, J = 7.8 Hz, 1H, minor) 7.75 (d, J = 8.8 Hz, 1H, major), 7.18 (d, J = 16 Hz, 2H), 6.83 (d, J = 14 Hz, 2H), 6.51 (d, J = 6 Hz, 1H, major), 6.42 (d, J = 4.4 Hz, 1H, minor), 4.89 (m, 2H), 4.84 (d, J = 4 Hz, 1H, minor), 4.62 (d, J = 5.2 Hz, 1H, major). ¹³C NMR (D₆-DMSO): δ 171.3, 170.9, 161.6, 158.7, 123.29, 123.12, 122.58, 121.75, 118.54, 118.36, 104.54, 104.37, 91.04, 72.57, 71.60, 70.93, 61.60, 54.73, 36.57, 36.42, 35.81, 29.21, 10.29.

Ethyl 4-(4-(4-(3-bromopropanamido)1-methyl-1H-pyrrole-2-carboxamido)-1-methyl-1H-pyrrole-2-carboxamido)butanoate (37). Compound 5c (0.203 g, 0.501 mmol) was dissolved in 50 mL of EtOH and 0.250 g 50 % water wet Pd/C was added in a parr jar. The mixture was shaken under pressurized hydrogen (70 psi) until 5c had disappeared as indicated by TLC (6:1CHCl₃/MeOH). The Pd/C was filtered through celite and the filtrate was concentrated by rotary evaporation and the reduced compound 5c was stored under high vacuum overnight. The reduced compound 5c was dissolved in DCM (15 mL) and cooled to 0 °C in an ice/water bath. Saturated NaHCO₃ (15 mL) was added to forma two-phase system, while stirring
vigorously. 3-Bromopropionyl chloride (38 μL, 0.37 mmol) was dissolved in DCM (5 mL) and added drop wise via a drop funnel for over 5 minutes. The mixture stirred vigorously for 30 minutes at 0 °C then allowed to stir at room temperature for 2 hours. When the reaction was complete by TLC (6:1CHCl₃/MeOH) the organic layer was removed by transferring the solution to a separatory funnel. The organic layer was then dried with MgSO₄, filtered, and rotary evaporated to dryness to yield a pure brown solid 37 (0.221 g, 84 %): mp 75-79 °C. TLC (6:1CHCl₃/MeOH) Rₐ = 0.64. ¹H NMR data (D6-DMSO): δ 10.09 (s, 1H), 9.89 (s, 1H), 8.03 (t, J = 6 Hz, 1H), 7.18 (s, 2H), 6.86 (d, J = 11.2 Hz, 2H), 4.06 (q, J = 6.8 Hz, 7.2 Hz, 2H), 3.83 (s, 3H), 3.79 (s, 3H), 3.74 (t, J = 6.4 Hz, 2H), 3.18 (t, J = 5.6 Hz, 2H), 2.88 (t, J = 6.4 Hz, 1H), 2.32 (t, J = 7.2 Hz, 2H), 1.73 (t, J = 6.8 Hz, 3H), 1.20 (m, 2H). ¹³C NMR (D6-DMSO): δ 173.18, 166.84, 166.62, 161.76, 158.78, 12.32, 123.27, 122.48, 122.15, 118.65, 118.30, 104.64, 104.37, 60.22, 38.16, 36.58, 36.41, 31.50, 30.14, 25.17, 14.56.

3-(5-(5-(4-Ethoxy-4-oxobutylcarbamoyl)-1-methyl-1H-pyrrol-3-ylcarbamoyl)-1-methyl-1H-pyrrol-3-ylamino)-3-oxopropane-1-sulfonic acid (38). To a solution of compound 37 (0.093 g, 0.18 mmol) dissolved in 4 mL EtOH, 15 % aqueous Na₂SO₃ (4mL) was added and the mixture refluxed until 37 disappeared and a baseline spot appeared by TLC (6:1 CHCl₃/MeOH). The solvent was then removed by rotary evaporation until a minimal amount of water remained. This solution was then cooled in an ice/water bath and the pH adjusted to 1 with concentrated HCl. A brown precipitate fell out of solution, which was vacuum filtered to give pure 38 as a brown solid (0.087 g, 94 %): mp 165-170 °C. TLC (5:2 CHCl₃/MeOH) Rₐ = 0.32. ¹H NMR data (D6-DMSO): δ 9.94 (s, 1H0, 9.82 (s, 1H), 8.00 (t, J = 5.6 Hz, 1H), 7.15 (d, J = 10 Hz, 2H), 6.82 (s, 2H), 4.02 (q, J = 7.2 Hz, 2H), 3.78 (s, 3H), 3.76 (s, 3H), 2.69 (t, J = 7.2 Hz, 2H), 2.58 (t, J = 8.4 Hz, 2H), 2.22 (t, J = 7.2 Hz, 2H), 1.69 (pentet, J = 3.6 Hz, 7.2 Hz, 6.8
Hz, 2H), 1.17 (t, J = 7.2 Hz, 3H). $^{13}$C NMR (D6-DMSO): δ 173.28, 168.96, 161.76, 158.81, 123.28, 123.08, 122.54, 118.55, 118.25, 104.61, 104.32, 60.21, 48.03, 38.15, 36.54, 36.39, 32.98, 31.50, 25.16, 14.58

6-(Acetoxymethyl)-3-(3-(4-(4-(3-bromopropanamido)-1-methyl-1H-pyrrole-2-carboxamido)-1-methyl-1H-pyrrole-2-carboxamido)propanamido)tetrahydro-2H-pyran-2,4,5-triy1 triacetate (39). Compound 39 was synthesized using a procedure similar to compound 37, using 0.0536 g (0.077 mmol) of 13a and 10.7 μL (0.11 mmol) of 3-Bromopropionyl chloride to yield a brown precipitate that was vacuum filtered to give pure 39 as a brown solid (0.013 g, 22 %): mp 140-144 °C. TLC (6:1 CHCl₃/MeOH) RF = 0.67. $^1$H NMR data (D6-DMSO): δ 9.98 (s, 3H), 9.92 (s, 3H), 8.35 (t, J = 5.6 Hz, 1H), 7.83 (d, J = 9.2 Hz, 1H), 7.22 (s, 1H), 7.17 (s, 1H), 6.89 (s, 2H), 5.75 (d, J = 8.8 Hz, 1H), 5.24 (t, J = 9.6 Hz, 1H), 4.85 (t, J = 9.6 Hz, 1H), 4.17 (dd, J = 4.8 Hz, 8 Hz, 2H), 3.98 (m, 3H), 3.82 (s, 3H), 3.76 (s, 3H), 2.05 (s, 3H), 1.99 (s, 3H), 1.94 (s, 3H), 1.93 (s, 3H). $^{13}$C NMR (D6-DMSO): δ 170.50, 170.27, 170.10, 169.73, 169.44, 166.83, 166.61, 162.01, 158.81, 123.24, 122.83, 122.58, 122.13, 118.71, 118.64, 104.97, 104.47, 92.19, 72.29, 71.98, 68.73, 61.95, 52.02, 43.08, 36.59, 36.44, 30.17, 21.04, 20.96, 20.88, 20.86.

(E)-2,4,5-tris(benzyloxy)-6-(benzyloxymethyl)-N-(4-methoxybenzylidene)tetrahydro-2H-pyran-3-amine (40). Compound 40 was synthesized as described in literature. A mixture of 10 (0.787 g, 2.6 mmol) and benzyl bromide (2.53 mL, 21.1 mmol) in dry DMF (6 mL) was stirred vigorously in an ice/salt bath while NaH (57-63 % in oil, 0.887 g, 36.9 mmol) was added in four portions. The mixture was then allowed to reach room temperature overnight. EtOAc (50 mL) was added drop wise while stirring and then the solvent was removed by rotary evaporation. The residue was taken up in EtOAc (50 mL) and DI H₂O (50 mL), the organic
layer was separated, dried with MgSO₄, and the solvent removed by rotary evaporation. The compound was then dry loaded onto a column by dissolving the residue in EtOAc and adding about 5 g of SiO₂ gel, then removing the solvent by rotary evaporation to give a solid. Flash column chromatography of the solid (4:1 Hexane/EtOAc) afforded 0.870 g (50 %) of a yellow oil. TLC (4:1 EtOAc/Hexane) Rf = 0.29. The NMR obtained was not well resolved to identify each peak due to the large number of phenyl groups present on the molecule, the ranges of signals were: ¹H NMR data (D6-DMSO): δ 8.33 (s, 1H), 7.75 (d, J = 8.8 Hz, 2H), 7.42 – 7.14 (phenyl, 30H), 7.0 (d, J = 8.8 Hz, 2H), 4.92 – 4.59 (10H), 3.93 (s, 3H). ¹³C NMR (D6-DMSO): δ 186.03, 133.64, 133.38, 133.12, 127.24, 125.27, 123.61, 123.57, 123.50, 12.36, 123.23, 12.11, 123.01, 122.84, 122.72, 122.34, 109.58, 93.85, 73.50, 69.07, 67.63, 66.19, 64.10, 50.84.

**2-Amino-1,3,4,6-tetra-O-2-deoxy-β-D-glucose Hydrochloride (41).** Compound 40 (0.870 g, 1.32 mmol) was dissolved in acetone (10 mL), treated with HCl (5N, 1.5 mL), refluxed for 30 minutes, and then refrigerated overnight after having cooled to room temperature. The white mass was broken, filtered using vacuum filtration, washed with acetone, and recrystallized with 190 proof EtOH to yield 41 (0.52 g, 68 %) as white crystals: mp decomposition 200 °C. The NMR obtained was not well resolved to identify each peak due to the large number of phenyl groups present on the molecule, the ranges of signals were: ¹H NMR data (D6-DMSO): δ 8.20 (s, 3H), 7.47 -7.15 (phenyl groups), 4.85 - 4.51, 3.71 -3.04. ¹³C NMR (D6-DMSO): δ 138.61, 138.54, 138.19, 137.45, 128.76, 128.69, 128.64, 128.56, 128.28, 128.20, 128.15, 128.10, 127.99, 127.97, 98.99, 80.15, 78.72, 74.81, 74.40, 74.16, 72.84, 71.02, 68.73, 55.24.

**4-Acrylamido-1-methyl-N-(1-methyl-5-(3-oxo-3(2,4,5-tris(benzyloxy)-6-(benzyloxymethyl)tetrahydro-2H-pyran-3-ylamino)propylcarbamoyl)-1H-pyrrolo-2-carboxamide (42).** In a flask flushed with N₂, 15b (0.103 g, 0.26 mmol) was
dissolved in 4 mL of anhydrous DMF along with EDCI (0.105 g, 2.0 eq.), DMAP (0.106 g, 3.5 eq.), HOBT (0.105 g, 3.0 eq.), CuCl$_2$ (0.009 g, 0.3 eq.) and the amine hydrochloride 41 (0.152 g, 0.26 mmol) was added and the solution was allowed to stir at room temperature over a period of two days. The solution was diluted with 50 mL DCM and the organic layer extracted with H$_2$O (2 x, 50 mL), 5 % NaHCO$_3$ (2 x, 50 mL), and 1M HCl (2 x, 50 mL). The organic layer was then dried over MgSO$_4$. The resulting solution was rotary evaporated until dry to yield the crude product, 16a which was purified using flash column chromatography (6:1 CHCl$_3$/MeOH) as pale yellow solid (0.150 g, 62 %): mp 50-55 °C. TLC (6:1 CHCl$_3$/MeOH) $R_f$ = 0.67. The NMR obtained was not well resolved to identify each peak due to the large number of phenyl groups present on the molecule, the ranges of signals were: $^1$H NMR (D6-DMSO): δ 10.08 (s, 1H), 9.6 (s, 1H), 8.0 (m, 3H), 7.36- 7.17 (m), 6.9 (s, 1H), 6.83 (d, J = 1.2 Hz, 1H), 6.36 (m, 1H), 6.19 (dd, J = 1.6 Hz, 1H), 5.64 (dd, J = 2 Hz, 1H), 4.8-4.49 (m). $^{13}$C NMR (D6-DMSO): δ 142.32, 162.15, 161.79, 158.80, 138.80, 138.70, 138.37, 132.08, 128.73, 128.69, 128.66, 128.60, 128.33, 128.21, 128.04, 127.91, 127.75, 125.86, 123.50, 123.24, 122.22, 118.49, 104.56,

3-(1-Methyl-5-(1-methyl-5-(3-oxo-3(2,4,5-tris(benzyloxy)-6- (benzyloxymethyl)tetrahydro-2H-pyran-3-ylamino)propylcarbamoyl)-1H-pyrrol-3-ylcarbamoyl)-1H-pyrrol-3-ylamino)-3-oxopropane-1-sulfonic acid (43). To a solution of alkene 42 (47.7 mg, 0.052 mmol) dissolved in 12 mL EtOH, NaHSO$_3$ (0.105 g, 1 mmol) dissolved in 4 mL of DI H$_2$O was added all at once. The pH was adjusted to 8 with 5 % NaOH and the mixture was refluxed until 42 had disappeared and a baseline spot appeared by TLC (6:1 CHCl$_3$/MeOH). The EtOH was then removed by rotary evaporation until a minimal amount of water remained. This solution was then cooled in an ice/water bath and the pH adjusted to 1 with concentrated HCl. A pale yellow precipitate fell out of solution, which was vacuum filtered
to give pure 43 as a pale yellow solid (0.04 g, 77%): mp 116-120°C. TLC (2:1 EtOAc/MeOH) 
R_f = 0.48. The NMR obtained was not well resolved to identify each peak due to the large 
number of phenyl groups present on the molecule, the ranges of signals were: \(^1\)H NMR data (D6-
DMSO): δ 10.08 (s, 1H), 9.6 (s, 1H), 8.0 (m, 3H), 7.36- 7.17 (m).

1-Methyl-4-(1-methyl-4-(3-(methylsulfonyl)propanamide)-1H-pyrrole-2-
carboxamid)-N-(3-oxo-3-[(2R,3R,4R,5S,6R)-2,4,5-trihydroxy-6-(hydromethyl)tetrahydro-
2H-pyran-3-ylamino)propyl)-1H-pyrrole-2-carboxamide (44). Compound 18b (0.052 g, 
0.065 mmol) was dissolved in 1.5 mL of anhydrous THF and cooled to – 10 °C in a dry 
ice/acetone bath. Freshly prepared NaOCH\(_3\)/MeOH (1M, 261 μL, 0.26 mmol) was added to the 
cooled solution. The reaction was monitored by TLC (6:1 CHCl\(_3\)/MeOH) initially until all spots 
collapsed to a baseline spot then TLC solvent was switched to MeOH and reaction was 
monitored until one spot was indicated by TLC, approx. 15 minutes. Once complete by TLC 
Dowex 50-WA acidic resin was added to the cooled solution while stirring for 10 minutes. The 
Dowex was filtered and washed with 1:1 DI H\(_2\)O/MeOH. The solvent was removed by rotary 
evaporation to give pure 44 as a brown solid (0.04 g, 97%, mix of diastereomers): mp 172-180 
°C. TLC (MeOH) R_f = 0.62. \(^1\)H NMR data (D6-DMSO): δ 10.06 (s, 1H), 9.87 (s, 1H), 7.97 (tt, J 
= 4.8 Hz, 1H), 7.77 (d, J = 8.4 Hz, 1H), 7.74 (d, J = 9.6 Hz, 1H), 7.17 (dd, J = 4 Hz, 5.6 Hz, 2H), 
6.85 (d, J = 1.6 Hz, 2H), 6.42 (s, 1H), 4.93 (d, J = 3.2 Hz, 1H), 4.45 (d, J = 4 Hz, 1H), 3.82 (s, 
1H), 3.79 (s, 1H), 3.01 (s, 1H), 2.72 (t, J = 9.2 Hz, 2H). \(^13\)C NMR (D6-DMSO): δ 171.22, 
166.55, 161.65, 158.75, 123.26, 122.15, 118.63, 118.34, 104.54, 104.29, 91.04, 72.55, 71.58, 
70.92, 54.70, 50.20, 36.62, 36.43, 35.85, 28.65.
CHAPTER 6: RESULTS AND DISCUSSION
The goals of this project were to develop compounds that can deliver a specific DNA-methylating agent to pancreatic β-cells and that can be used to study the structural features that govern selective cell-targeting and DNA-damage. These compounds will be used to demonstrate that a molecule that can form cytotoxic N3-methyladenine DNA-adducts exclusively can be directed to a particular cell-type by attaching it to targeting ligand and that this strategy can be used to destroy the targeting cell.

This thesis describes the synthesis of three categories of compounds for this project (Figure 6.1). All three categories of compounds included a glucose unit at one end for achieving selective targeting of the pancreatic β-cells via the GLUT-2 glucose transporter. They also contain a bis-pyrrole triamide unit that can achieve site-specific DNA-binding in the minor groove of DNA at A/T rich regions. The difference between the three categories lies at the N-terminus of these peptide molecules. One set of compounds have fluorescent tags at this terminus and are designed for use in experiments to characterize cell-transport. Another set of compounds bear a methyl sulfone unit at the N-terminus and will be used to characterize the DNA-binding properties of this class of compounds. Finally, the third category of compounds have a reactive methyl-sulfonate at the N-terminus. These compounds will be used to form the cytotoxic N3-methyl adenine DNA-adducts in pancreatic β-cells.
Figure 6.1: Structure of compounds being synthesized for this project.

- for forming cytotoxic N3-methyladenine adducts in pancreatic beta-cells

- for DNA binding experiments

- for cell targeting experiments
The only variable in all of these compounds is the length of the linker unit. In each of the categories, compounds were synthesized with three linkers each differing by 1 CH\(_2\) unit. The compounds, differing in linker length, would help to explain how linker length influences DNA-binding and transport through the glucose transporter.

The synthetic schemes used for preparing the different compounds were similar and varied only in the sequence in which different components were added to compound 5 (Figure 6.2). Compound 5 has incorporated into it the bis-pyrrole unit that binds to DNA in the minor groove at A/T rich regions and the linker unit terminating as an ethyl ester. The nitro group in compound 5 was reduced to an amine and then further functionalized with either the methyl sulfonate, methyl sulfone or fluorescent molecules as desired. The terminal ester on compound 5 was hydrolyzed and attached to the targeting ligand glucose.
Figure 6.2: Structure of compound 5.

- $a$, $R = \text{CH}_2$
- $b$, $R = \text{CH}_2\text{CH}_2$
- $c$, $R = \text{CH}_2\text{CH}_2\text{CH}_2$
The synthesis of compound 44b has been accomplished by the following procedure shown in Scheme 6.1. Following this same scheme, compound 18a and 18c have also been successfully prepared and only require the deprotection of the hydroxyl groups on the glucose unit in order to convert them to the desired compounds. These compounds will be used in binding studies to characterize their DNA-binding properties.

Scheme 6.1
The synthesis of the compounds desired for the cell transport studies was carried out by the following steps outlined in Scheme 6.2. Using this procedure compound 21 has been synthesized. The deprotection of the glucose hydroxyl groups will result in the desired compounds containing fluorescent tags, which will be sent to collaborators in order to determine their selective transport through the GLUT-2 transporter.

Scheme 6.2
For the synthesis of the desired DNA-methylating methyl sulfonate, compound 5 was first converted to compound 15, Scheme 6.3. To this compound was attached the targeting ligand with the hydroxyl groups protected with benzyl groups or with acetyl groups. Compound 16 with the acetyl protected glucose was converted into the sulfonate salt, but the acidification of this sulfonate to the sulfonic acid resulted in partial deprotection of the glucose unit. Efforts are ongoing to successfully achieve this transformation. The benzyl protected compound has been successfully converted into the sulfonic acid, 43. The removal of the benzyl protecting groups by hydrogenation has proved to be very sensitive to the catalyst used for the reaction, and different catalysts are currently being explored to achieve efficient deprotection. Once the sulfonic acid compounds with the deprotected glucose unit are obtained, the final step required to make the desired methyl sulfonate compounds is the methylation of the sulfonic acid.

Scheme 6.3
The methyl sulfonate compounds, once prepared, will be first test for their ability to produce 3-MeA adducts upon reaction with genomic DNA by using HPLC methods. The ability of the compounds to form the desired cytotoxic DNA adducts will depend critically upon their ability to bind within the minor groove of DNA at A/T rich regions. Molecular dynamics simulations performed using one of the desired molecules (R = CH$_2$) indicates that this compound will indeed be able to bind within the minor groove at A/T rich regions (Figure 6.3) and that glucose unit itself contributes to the binding by forming favorable hydrogen bonding interactions within the groove. Furthermore, based upon the solubility properties of the methyl sulfone compound 44b, it is likely that these desired DNA-methylating compounds will be water soluble, which is a big advantage for use in biological systems.
Figure 6.3: Molecular Mechanics model of methyl sulfonate compound bound to minor groove of DNA at A/T rich regions.
Precursor compounds in all three categories have been synthesized with three different linkers each differing by 1 CH$_2$ group. The differences between the properties of these compounds varying in linker-length will be used to identify the optimum length required for DNA-binding, cell-transport, and overall cell-toxicity. If desired, the strength of DNA-binding can be improved by introducing a third pyrrole unit into the molecule. The decreased water solubility due to this third pyrrole is not expected to be critical since the glucose unit provides significant water solubility.

In transport experiments, the presence of the fluorescent tags may interfere with the passage of the compounds through the GLUT-2 transporter. This is one of the reasons why two different fluorescent tags are being explored. If the fluorescently tagged compounds are unable to get into cells via the transporter, it does not necessarily imply that the methyl sulfonate compounds will be unable to traverse through these transporters. However, if these compounds with the tags do selectively get into cells containing the GLUT-2 glucose transporter, then it is likely that the methyl sulfonate compounds too will be able to pass through these transporters.
References


APPENDIX

Appendix A. Compounds Synthesized.

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![Chemical structure of 18a](image)

Chemical Formula: C_{35}H_{45}N_{15}O_{15}S
Molecular Weight: 782.77

46%

18b

![Chemical structure of 18b](image)

C_{35}H_{45}N_{15}O_{15}S
Mol. Wt.: 782.77

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18c

![Chemical structure of 18c](image)

Chemical Formula: C_{35}H_{45}N_{15}O_{15}S
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Appendix B. List of Abbreviation

3-MeA - 3-methyladenine
3-MeG - 3-methylguanine
6-MeG - 6-methylguanine
A – adenine
Ac - acetyl
A/T – adenine-thymine
Bn - benzyl
C – cytosine
CDCl$_3$ – Deuterated Chloroform
DCM – dichloromethane
DIEA – diisopropylethylamine
DNA – deoxyribose nucleic acid
DMAP – 4-dimethylaminopyridine
DMF – dimethylformamide
d$_6$ -DMSO – Dimethylsulfoxide
EDCI – 1-(3-dimethylaminopropyl)-3-ethylcarbodiimidehydrochloride
EtOAc – ethyl acetate
EtOH – ethanol
G – guanine
HCl – hydrochloric acid
HOBT – hydroxybenotriazole
Me-lex – methyl 3-(1-methyl-5-(1-methyl-5-(propylcarbomyl)-1H-pyrrol-3-ylcarbamoyl)-1H-pyrrol-3-ylamino)-3-oxopropane-1-sulfonate

MeOH – methanol

Mp – melting point

NaOCH₃ – sodium methoxide

NaOH – sodium hydroxide

NMR – nuclear magnetic resonance

Pd/C – palladium on carbon

Rₚ – retention factor

TEA – triethylamine

THF – tetrahydrofuran

TLC – thin layer chromatography

T - thymine