Sythesis of carporide Derivatives for Sodium-Proton Exchange Inhibition

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SYNTHESIS OF CARIPORIDE DERIVATIVES FOR
SODIUM-PROTON EXCHANGE INHIBITION

by

JACOB ADAM VERVYNCKT

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submitted in partial fulfillment of the requirements of
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SYNTHESIS OF CARIPORIDE DERIVATIVES FOR SODIUM-PROTON EXCHANGE INHIBITION

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Under the Direction of Hasan Palandoken

ABSTRACT

Over 200,000 people in the United States are diagnosed annually with malignant brain tumors. Current therapeutic methods prove ineffective at treating this type of cancer due to the inability to selectively target cancer cells. Research has shown that brain cancer cells are heavily dependent on the sodium-proton exchanger (NHE) and sodium-calcium exchanger (NCX) due to their increased metabolic activity. Inhibiting these proteins disrupts pH and ion balances within cancer cells while leaving healthy cells unaffected, leading to selective glial cell death. Therefore, NHE and NCX inhibition allow for selective targeting of brain cancer cells. The challenge that remains in using these inhibitors as a treatment is effectively delivering them to poorly vascularized tissues. As part of our target-specific approach to treating brain cancer, we have synthesized analogs of cariporide, a potent NHE inhibitor, to address these challenges. The present thesis will discuss our efforts towards the preparation of our analogs.

INDEX WORDS: Cariporide, Brain cancer, Sodium-proton exchanger, Glioma, Prodrug
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CHAPTER 1: INTRODUCTION

1.1 The Sodium-Proton Exchanger

The Sodium-Proton Exchanger (NHE) is a cell surface ion exchanger that allows sodium (Na\(^+\)) ions to be transferred into the cell and proton/hydrogen (H\(^+\)) ions to move out of the cell in a 1:1 ratio [1, 2]. In general, the NHE is a transport protein that works according to the concentration gradients of the ions, with the ions flowing from areas of higher concentration to areas of lower concentration. More specifically, NHE works as an antiporter by moving the Na\(^+\) and H\(^+\) ions in opposite directions across the cell membrane through the use of the thermodynamically favorable transfer of the Na\(^+\) ions from areas of high concentration outside of the cell to areas of low concentration inside the cell (Figure 1). Through this mechanism, NHE can move the H\(^+\) ions across the cell membrane without the consumption of energy in the form of adenose triphosphate (ATP), the form of energy used by cells [2]. In the majority of cases, NHEs are used to transport H\(^+\) ions out of the cell and Na\(^+\) ions into the cell. Because of this, NHE function is strongly dependent on the concentration of Na\(^+\) ions outside the cell [1]. However, NHE can also work in reverse, allowing H\(^+\) ions to enter the cell and Na\(^+\) ions to exit the cell [2].

Under anaerobic conditions (i.e., stroke, heart attack), there is a shift from oxidative to nonoxidative glycolysis to meet the cells’ energy needs [1]. Nonoxidative glycolysis leads to an intracellular accumulation of lactic acid (Figure 1). The pH decrease activates the NHE which decreases the excess intracellular H\(^+\) concentration. However, the concomitant Na\(^+\) ion concentration increase inside the cell is presumed to activate the sodium-calcium exchanger (NCX). NCX deals with the excess Na\(^+\) ions, but cause intracellular Ca\(^{2+}\) ion accumulation, which leads to irreversible cell damage. According to this ‘coupled
exchanger’ theory, NHE initiates the sequence of physiological events that lead to cell damage under anaerobic conditions.

Figure 1. Ion transporter activation during ischemia-reperfusion (I/R) injury

NHE proteins were first discovered in 1976 by Murer et al. [3] and can be found in virtually all vertebrates [4]. Currently, seven different isoforms (NHE1-NHE7), of the sodium-proton exchanger have been identified [2], occurring in every tissue type found in the human body [1, 2]. These seven isoforms share a general structure that contains about 800 amino acids with between 20 and 70% of the amino acid sequences being identical. The differentiation among the NHE isoforms allows them to accommodate the specific needs of their resident tissues [5]. Of the seven isoforms, NHE1 is the most common and is found in all tissue types, acting as a sort of “housekeeper,” maintaining the cell in its proper state [1].

NHEs perform a number of functions for the cell, including regulating both pH and volume and stimulating cell growth and proliferation. Through the transfer of H⁺ ions, NHEs can maintain the optimal intracellular pH [4]. Normal brain cells function best at physiological pH of about 6.99-7.05 [6]. NHEs, by working on a gradient, are some of the quickest and most energy efficient ways for a cell to maintain pH. Cells that lack NHEs are particularly susceptible to acidosis, which is a large increase in the intracellular acidity [5] and can be fatal to the cells.

NHEs are also important in the regulation of cell volume through intrusion of Na⁺ ions. Because the Na⁺ ion is so much larger than the H⁺ ion, the cell expands as sodium ions
enter and protons exit, allowing the cell to maintain normal volume when exposed to increases or decreases in extracellular water levels [5], helping the cell to maintain a healthy volume.

Finally, NHEs are important in the stimulation of cell growth and proliferation in cells, even to the point of a “proliferative advantage” [6]. Cell proliferation is activated by increasing intracellular pH through the stimulation of NHE by protein growth factors [4]. Furthermore, the increase in pH (thus basicity) of cells through NHE activation allows the cells to divide and grow at higher rates than cells lacking NHE, giving these cells a “proliferative advantage” [6].

In order for the NHEs to work, however, they must first be activated. This activation can come from a number of sources. Obviously, NHEs react to changes in the pH of the cell, responding quickly to any drops in the pH below a certain standard level that depends on cell type [1]. In this way, they are able to maintain the necessary physiological pH. Alternately, NHEs may be activated by a number of extrinsic factors including hormones and growth factors [5]. In doing so, the cell can initiate cell growth and reproduction in order to heal, maintain, or expand current tissue.

1.2 Glioma Cells

Malignant gliomas make up “65% of all primary brain tumors,” making them the most common and deadliest of all brain cancers [7]. Currently, there is no cure for malignant gliomas due to the ineffectiveness of current therapies, with the median survival time for patients being only 9 to 12 months after diagnosis [8].

In the human brain, normal brain cells (e.g., astrocytes), maintain their pH through a number of ion exchangers, such as the “Na\(^+\)/H\(^+\) exchanger (NHE1), the electrogenic Na\(^+\)-
HCO$_3^-$ cotransporter, the Na$^{+}$-dependent and Na$^{+}$ independent Cl$^-$/HCO$_3^-$ exchangers, H$^+$-lactate symporter, and the vacuolar type H$^+$-ATPase” [6]. However, glioma cells rely upon NHE1 isoform to a much greater extent than any other pH regulating mechanism [6, 9], causing the impact of the other mechanisms to be minimal. This increase in NHE1 activity in glioma cells is due to a number of factors, including the need for an increased pH to promote cell proliferation and an increased dependence upon nonoxidative glycolysis, leading to lactic acid production [7]. Thus, while normal brain cells display almost no use of the NHE1 at physiological pH [9], malignant glioma cells, which exist in hypoxic, acidotic environments, display an overactivation of the NHE1 isoform. With no oxygen present, glioma cells cannot perform normal glycolysis, and thus depend on nonoxidative glycolysis, which produces lactic acid (as a by-product) increasing the concentration of H$^+$ ions in the cell [7].

Under nonoxidative glycolysis conditions, the need to deplete this acid build-up leads to overactivation of the NHE in gliomas. This subsequent extrusion of hydrogen ions causes the exterior of the cell to become acidified quickly, causing the extracellular regions of glioma tumors to be much more acidic than the extracellular regions of normal tissues. Because of this, it has been shown that the extracellular pH is between 6.5-6.9 for glioma tumors, as opposed to 7.0-7.5 for normal cells [6]. Paradoxically, the internal pH of glioma cells has been measured to be between 7.12-7.24, which is more alkaline than that of normal cells, which have normal pH between 6.99-7.05 [6]. Through these studies, it has been determined that this difference is due to the need for a higher internal pH for cellular proliferation, although McLean et al. state that “there is increasing evidence that maintenance of the extracellular environment contributes to tumor growth and invasion as well” [6].
Therefore, by overactivating the NHE1 isoform, glioma cells are able to reproduce, grow, metastasize, and invade new tissues better than normal cells.

This proliferative advantage, however, is not the only reason that brain cancer is so difficult to cure. A number of other factors contribute to this challenge. First of all, the structure of glioma tumors makes them very hard to treat. As the tumor forms and its size increases, the peripheral regions of the tumor become highly vascularized and proliferative while the interior of the tumor becomes more and more anaerobic with proliferative cells scattered throughout the necrotic interior [7]. The outside cells of the tumor can be killed using traditional chemotherapy and radiotherapy. However, the lack of oxygen in malignant glioma tumor centers inhibits the formation of radicals during radiotherapy and chemotherapy, and thus there is no reactive disruption of the DNA to kill the cell [7] leaving the interior regions of the tumor unaffected by these treatments. Instead, the increase in nonoxidative glycolysis in these anaerobic areas allows the NHEs and other ion exchangers to keep the cells proliferating [10]. Therefore, while the highly vascularized outside portions of the tumor are easily killed through the conventional therapies [7], the interior cells are not affected, allowing the tumor to continue to grow even after the peripheral cells are killed.

Similarly, the location of the glioma tumors in the body presents further therapeutic challenges. Current chemo- and radiotherapies do not differentiate between healthy and cancerous cells, killing both equally. In many tissues, this practice is acceptable because the surrounding tissues can proliferate to heal that region. However, brain cells regenerate at a much slower rate, if at all, than normal body tissues. Therefore, key motor skills or other problems could occur through the death of astrocytes surrounding the glioma tumor, making the use of these therapies in brain cancer patients counterproductive.
1.3 NHE Inhibitors

In the brain, normal astrocytes display almost no change in pH while gliomas show a marked decrease in pH upon NHE inhibition [6]. This difference is due to the pH regulatory mechanism utilized by each type of cell. Since glioma cells are producing large amounts of acid and depend heavily on the NHE1 isoform, the inhibition of the NHE1 isoform greatly reduces the ability of glioma cells to extrude protons, which consequently decreases their intracellular pH (and thus disrupt nonoxidative glycolysis). Meanwhile, normal astrocytes do not produce large amounts of acid and do not rely on NHE. Thus, NHE inhibitors affect the glioma cells adversely while not harming the normal astrocytes [6]. Furthermore, it is “generally recognized that cancer cells cannot survive for long below certain low [intracellular pH] conditions, either because of triggering of the apoptotic pathway or because of the additional shutting down of glycolysis…” [9].

Currently, there are five classifications of NHE inhibitors including the aroylguanidines, heteroaroylguanidines, spacer-stretched aroylguanidines, the non-acyl guanidines, and the non-guanidine NHE inhibitors (Figure 2) [11].

Four of the five classifications of NHE inhibitors contain a guanidine residue (Figure 2). In 1969, Paolini discovered that sodium ions (Na⁺) are surrounded by three water molecules in aqueous media at physiological pH. Furthermore, he found that this trihydrated sodium ion is analogous to the guanidine functional group (which exists as the guanidinium ion at physiological pH), with the trihydrated sodium ion having approximately the same size, shape, and charge density as the guanidinium ion (Figure 3) [12]. Thus, the guandine/acyl-guanidine groups found in the first four classes of NHE inhibitors (Figure 2) exist in the protonated form at physiological pH.
Due to these properties guanidine is able to act as a “sodium mimic” in aqueous media at physiological pH, allowing it to inhibit the NHE inhibitor either by competing with
the sodium ion for the NHE binding sites that may overlap or by altering the sodium binding site [5].

Of the four guanidine based groups, the aroylguanidines and heteroaroylguanidines have been studied extensively, with most emphasis on the aroylguanidine cariporide (Figure 4) and the heteroaroylguanidine amiloride (Figure 4), along with their derivatives.

![Cariporide and Amiloride](image)

**Figure 4.** Cariporide and Amiloride

Amiloride (Figure 4) was synthesized before the discovery of NHE and has been used as a diuretic [11]. After the discovery of NHE, amiloride and its derivatives were among the only compounds used to study NHE inhibition. Amiloride decreases vascularization of glioma tumors and metastasis, and thus may be useful in treating glioma tumors by decreasing tumor growth [9]. Furthermore, amiloride and its derivatives have been shown to initiate cell death in glioma cells, presumably due to their NHE inhibitory activity [8].

However, amiloride and its derivatives also cause a number of negative side effects. First of all, amiloride and its derivate inhibit most forms of sodium movement across the cell membrane, and not NHE1 selectively [11, 13]. Inhibition concentration (i.e., IC$_{50}$ for 50% inhibition) levels for amiloride are in the micromolar range, which is too high for a pharmaceutical drug development. Finally, studies have shown that amiloride can permeate the cell, which prevents the cell from recovering completely to steady-state pH after treatment and causes subsequent problems with certain enzymes. In the end, this leads to
cytotoxicity and kill the cell [14]. As a result, amiloride and its derivatives are unlikely candidates for a new cancer therapy [11].

Cariporide (Figure 4) and its derivatives are more potent than amiloride and its derivatives. Whereas amiloride has an IC$_{50}$ in the micromolar range, cariporide has an IC$_{50}$ in the nanomolar range [11]. In addition, cariporide is very selective for NHE1 inhibition [14]. Finally, cariporide does not seem to permeate cells, thus does not cause cytotoxicity. Because of these reasons, cariporide is a much better pharmaceutical candidate to be developed for clinical use, and thus is the core structure used in the present thesis.

1.4 Current Problem to NHE Inhibition Therapy

Although NHE inhibitors have been shown to be detrimental to glioma cells in vitro, the challenge that remains is the delivery of these compounds to the non-vascularized tissues of a glioma tumor. With virtually no blood flow into the tissue (i.e., necrotic center of a brain tumor), it is impossible to deliver NHE inhibitors to where they need to be in order to combat brain cancer.
2.1 A Prodrug Approach

As a possible solution to the delivery problem of NHE inhibitors to non-vascularized tissues, we propose a prodrug approach (Figure 5). In this approach, the prodrug is constructed of an amino acid conjugate inhibitor attached to a peptide. The peptide will serve three main purposes:

1. It will render the amino acid conjugate inhibitor inactive as the peptide conjugate will be too large to fit into the NHE binding site;
2. It will allow for transportation across the blood-brain barrier (BBB) as various peptides are known to transport across BBB [15];
3. It will allow for selective cleavage of the inhibitor-peptide bond upon encountering glial regions (e.g., allow for site-specific release of the active inhibitor) as the peptide sequence will be designed to be a substrate for glioma-specific peptidases [16].

Figure 5. Our Prodrug Approach

Once the inactive prodrug has crossed the blood-brain barrier, it will remain inactive in the brain tissue. Upon the mutation of a normal astrocyte into a glioma cell, the mutated
cell will release peptidases that are specific to glioma cells, which will in turn cleave the peptide from the inhibitor, thus activating the inhibitor. Once activated, the inhibitor will inhibit NHE, with detrimental consequences (i.e., antiproliferation and cell death) to the glioma cells. The feasibility of such an approach recently has been demonstrated using amiloride peptide conjugates [10]. The present thesis focuses on the synthesis of an amino acid conjugate NHE inhibitor that can be incorporated into a peptide prodrug.

2.2 Our Inhibitor

In the design of our amino acid conjugate NHE inhibitor, we have chosen cariporide as our core structure, as it is a potent (e.g., nM IC$_{50}$) and selective inhibitor of NHE. As glycine is the simplest amino acid, we have chosen to incorporate it into our cariporide amino acid conjugate initially.

In order to determine the point of attachment of glycine to cariporide, we examined the existing NHE structure-activity relationship (SAR) data available for cariporide [17]. As the guanidine functionality is key to NHE inhibition, it could not be altered. SAR studies have also shown that substitution at C(2) and C(6) lead to loss of potency for cariporide, presumably due to increased steric hindrance impeding binding to NHE protein [17]. However, substitution of the isopropyl group with an amine at C(4) does not affect the activity of cariporide (Figure 6).

**Figure 6.** C(4)-analogs of cariporide [17]
Thus, we decided to attach glycine at the C(4)-position. Furthermore, our synthetic route allowed facile access to the C(3)-methylsulfonyl, and we decided to attach glycine at this position as well. Figure 7 depicts our proposed cariporide amino acid conjugates for the present thesis.

![Figure 7. Proposed amino acid conjugates of cariporide](image)

### 2.3 Synthesis of C(3)-Cari-Gly

The synthetic route for our C(3) derivative of cariporide is depicted in Figure 8. Using commercially available 4-chlorobenzoic acid (1), the chlorosulfonyl group of 2 was installed through an electrophilic aromatic substitution [17]. In the subsequent step, the t-butyl ester of glycine was reacted with the resultant sulfonyl chloride 2. This reaction also resulted in a side product in which the carboxylic acid in 2 formed an amide by-product with the t-butyl ester of glycine. To separate the two products, the reaction mixture was adjusted to a pH of 9-10, deprotonating the carboxylic acid in 3 (and thus rendering it water soluble) and allowing for selective extraction of the amide byproduct. The reaction mixture was then acidified, allowing for extraction of the desired product 3 in pure form. As we were
obtaining our desired product in pure form and were more interested in obtaining our C(3)-Cari-Gly derivative, we have not currently taken any additional steps towards improving the yield of this reaction. Guanidine was installed using a peptide coupling sequence. Finally, the t-butyl group was removed using methanesulfonic acid to afford the desired C(3)-Cari-Gly 4.

Figure 8. Synthesis of C(3)-Cari-Gly

2.4 Synthesis of C(4)-Cari-Gly

The synthesis of our C(4) derivative of cariporide (Figure 9) utilized a similar route as C(3)-Cari-Gly (Figure 8). 4-chlorobenzoic acid (1) was converted to the sulfonyl chloride 2. Reduction with sodium sulfite and subsequent methylation of the resultant sulfinic acid with methyl iodide provided 5 [17]. Unfortunately, substrate 5, which is well-poised for nucleophilic aromatic substitution, failed to react with the C-terminus protected glycine (t-BuO₂CCH₂NH₂). Subsequent studies with various nucleophiles (discussed in Section 2.6-2.8) showed that substrate 5 is capable of undergoing nucleophilic aromatic substitutions with nucleophiles stronger than t-BuO₂CCH₂NH₂.
2.5 Synthesis of C(4)-Cari-Gly: Fluoro Analog Approach

In an attempt to make 5 (Figure 9) a better substrate for nucleophilic aromatic substitution, we decided to substitute the chloro group in 5 with a fluoro group (Figure 10). Starting with commercially available 4-fluorobenzoic acid (8), a chlorosulfonyl group was attached at the C(3) position, and reduced to a sulfinic acid with sodium sulfite to afford 9. Basic conditions converted 9 into a bis-sodium salt, setting the compound up for methylation to obtain 10 [17]. The ester was selectively hydrolyzed to obtain 11 (fluoro analog of 5). Initial reactions with t-BuO₂CCH₂NH₂ were promising; however, unexpected purification problems arose. At the time, another approach we were examining simultaneously had more success. Thus, this synthetic route was put on hold.

Figure 9. Synthesis of C(4)-Cari-Gly
2.6 The Back-Building of Glycine: A New Approach

At the same time as we were attempting the fluoro analog approach (Figure 10), we began work on a new approach, in which we ‘back-built’ the t-butyl ester of glycine at the C(4) position. Using retrosynthetic analysis, we determined we could cleave the bond to create the two synthons in Figure 11. An amino group would be installed on the aromatic ring and subsequent SN2 alkylation reaction would ‘back-build’ glycine. The main advantage of this approach is that it allowed us to use nitrogen nucleophiles which are stronger than t-BuO₂CCH₂NH₂.
2.7 The Back-Building Approach: A Trial

To test our back-building strategy (Figure 11), commercially available 4-aminobenzoic acid (12) was alkylated with bromo-\textit{t}-butylester to obtain 13. The same peptide-coupling sequence as used in the synthesis of C\textsubscript{(3)}-\textit{Cari}-Gly (Figure 8) was used to install the guanidine residue, and the \textit{t}-butyl group was removed with methanesulfonic acid to afford 14 (Figure 12) [18].

2.8 Synthesis of C\textsubscript{(4)}-\textit{Cari}-Gly through the Back-building Approach

\textit{p}-Methoxybenzylamine (PMB-NH\textsubscript{2}), a better nucleophile than \textit{t}-BuO\textsubscript{2}CCH\textsubscript{2}NH\textsubscript{2}, substituted the C\textsubscript{(4)}-chloro with ease, installing the amine at C\textsubscript{(4)} and providing 15 (Figure 13). Once again, the amide byproduct was observed at the carboxylic acid of 15. The same acid-base extraction (as discussed in section 2.3) allowed for facile purification of 15.
Cleavage of the $p$-methoxybenzyl (PMB) group using trifluoroacetic acid (TFA) afforded 16, our synthetic equivalent. Alkylation of 16 with bromo-$t$-butylacetate installed the $t$-butyl ester of glycine at the C(4) position to obtain 6. Current efforts are focused on the installation of the guanidine residue to provide 7.

**Figure 13. Implementing the Back-Building Approach**
CHAPTER 3: CONCLUSION AND FUTURE WORK

After obtaining our C(3)-Cari-Gly and trial back-building analogs of cariporide, both compounds were tested for their NHE inhibition activity at the Center for Neuroscience, University of California, Davis. Both C(3)-Cari-Gly and C(4)-Cari-Gly-Trial had IC$_{50}$ values in the micromolar range (e.g., 100 μM). As both of these compounds lack the methylsulfonyl moiety at the C$_{(3)}$ position as found in the original cariporide compound, we believe that the methylsulfonyl moiety is necessary at the C$_{(3)}$ position for NHE inhibition (Figure 14). We will therefore include this moiety in all of our future inhibitors, including C(4)-Cari-Gly.

![Chemical Structures]

**Figure 14.** NHE inhibitory activity of cariporide amino acid conjugates

The C(4)-Cari-Gly compound (Figure 14) will soon be tested for its activity and will determine the path of future work. If the compound has an appropriate IC$_{50}$ value (i.e., nanomolar), it will be incorporated into a peptide matrix that is a substrate for glioma-specific peptidases. Also, the Fluoro Analog Approach (Figure 9) can be revisited to create an alternative synthetic route for the compound.
If compound 7 does not have an appropriate IC$_{50}$ value, work can begin on creating an amino acid conjugate inhibitor using other amino acids until a nanomolar-active inhibitor is found.
CHAPTER 4: EXPERIMENTAL PROCEDURES

All solvents and chemicals used in this these were reagent grade and were used as received unless otherwise indicated. Solvents were removed under reduced pressure (\textit{in vacuo}) by a combination of Buchi rotary evaporator operating at \textit{ca.} 25 mmHg and a Welch vacuum pump operating between 0.5 and 5 mmHg. Thin layer chromatography was performed on precoated silica plates (Kieselgel 60 F-254). UV active compounds were visualized on TLC plates by UV light (254 nm). The TLC plates were permanently stained with either p-anisaldehyde in ethanol/sulfuric acid solution or with phosphomolybdic acid in ethanol/sulfuric acid solution.

NMR spectra were recorded with a JEOL ECA-500 spectrometer (\textsuperscript{1}H at 500 MHz, \textsuperscript{13}C at 100 MHz). Chemical shifts are reported relative to residual undeuterated CHCl\textsubscript{3} (\textdelta{}7.26 ppm for \textsuperscript{1}H and \textdelta{}77.00 ppm for \textsuperscript{13}C NMR), undeuterated DMSO (\textdelta{}2.54 ppm for \textsuperscript{1}H and \textdelta{}40.45 ppm for \textsuperscript{13}C NMR) or tetramethylsilane (\textdelta{}0.00 ppm for \textsuperscript{1}H NMR and \textsuperscript{13}C NMR).

Melting points were determined by the use of an electrothermal digital melting point apparatus (Mel Temp II Laboratory Devices, USA) and are uncorrected.
Preparation of 4-chloro-3-(chlorosulfonyl)benzoic acid [17]

To chlorosulfonic acid (31.0 mL, 478.5 mmol) at 0 °C, 4-chlorobenzoic acid (1) (10.0 g, 63.8 mmol) was added portion wise over 30 min. The resulting reaction mixture was stirred at 140°C for 19 h under argon. Upon cooling to 0 °C, the reaction mixture was added dropwise to ice water (210 mL) and allowed to stir for 45 min. Filtration through a sintered glass funnel afforded 2 as a white solid, which was used directly in the next step (see Preparation of 3-(N-(2-t-butoxy-2-oxoethyl)sulfamoyl)-4-chlorobenzoic acid). \(^{1}\)H NMR (DMSO-d6) \(\delta 7.48\) (d, \(J = 8\) Hz, 1H), 7.81 (apparent dd, 1H), 8.38 (d, \(J = 1.7\) Hz, 1H).
Preparation of 3-(N-(2-t-butoxy-2-oxoethyl)sulfamoyl)-4-chlorobenzoic acid

To a solution of 4-chloro-3-(chlorosulfonyl)benzoic acid (2) (1.77 g (wet), 6.93 mmol) in tetrahydrofuran (THF) (80 mL) at room temperature was added t-butyl ester of glycine (H-Gly-OtBu·AcOH) (1.590 g, 8.312 mmol) and K$_2$CO$_3$ (3.063 g, 22.164 mmol). The reaction mixture stirred at room temperature for 1.5 h. At this time, the reaction had become a slurry, so more THF (20 mL) was added. After another 1.5 h of stirring, TLC (1:1 EtOAc/Hexane with 0.1% acetic acid) indicated complete consumption of 2. After removal of the solvent in vacuo, the residue was diluted with saturated NaHCO$_3$ (100 mL) and H$_2$O (30 mL) to obtain pH = 9-10. Impurities were extracted with EtOAc (2 x 100 mL). The pH was adjusted to 1-2 with 10% HCl (100 mL) in an ice bath, and the resultant mixture was extracted with EtOAc (2 x 100 mL). The organic layer was washed with H$_2$O (100 mL) and saturated NaCl (100 mL) and dried over Na$_2$SO$_4$. The solvent was removed in vacuo and the product dried under vacuum, resulting in 3 (0.75 g, 48% over two steps). $^1$H NMR (DMSO-d$_6$) δ 1.20 (s, 9H), 3.70 (d, J = 6.3, 2H), 7.75 (d, J = 8.55, 1H), 8.06 (apparent dd, 1H), 8.38 (d, J = 1.75, 1H), 8.50 (br m, 1H); $^{13}$C NMR (DMSO-d$_6$) δ 27.9, 45.1, 81.6, 130.5, 131.0, 132.8, 134.6, 136.1, 139.5, 166.1, 168.4; m.p. 148-151 °C.
Preparation of t-butyl 2-(2-chloro-5-(diaminomethylene)carbamoyl)phenylsulfonamido)acetate

To a solution of 3-(N-(2-t-butoxy-2-oxoethyl)sulfamoyl)-4-chlorobenzoic acid (3) (0.20 g, 0.572 mmol) in acetonitrile (CH₃CN) (5 mL) at 0 °C was added N-hydroxysuccinimide (HOSu) (0.079 g, 0.686 mmol) and N,N'-dicyclohexylcarbodiimide (0.141 g, 0.686 mmol). The reaction mixture stirred at 0 °C for 3.5 h. Remaining at 0 °C, the solution was filtered through steel wool and triethylamine (0.8 mL, 5.72 mmol) and guanidine hydrochloride (0.273 g, 5.86 mmol) were added to the resulting supernate. The reaction mixture was warmed to room temperature and stirred for 22.5 h, after which TLC (1:1 EtOAc/Hexane with 0.1% acetic acid) indicated complete consumption of 3. After removal of the solvent in vacuo, the residue was diluted with H₂O (50 mL) and extracted with EtOAc (100 mL). The organic layer was washed with H₂O (50 mL) and saturated NaCl (50 mL) and dried over Na₂SO₄. The solvent was removed in vacuo and dried under vacuum, resulting in 3a (crude product). ¹H NMR indicated purity ≥90 %. Crude product used in Preparation of 2-(2-chloro-5-(diaminomethylene)carbamoyl)phenylsulfonamido) acetic acid.
Preparation of 2-(2-chloro-5-(diaminomethylene-carbamoyl)phenylsulfonamido)acetic acid

To a solution of t-butyI 2-(2-chloro-5-(diaminomethylene-carbamoyl)phenylsulfonamido)acetate (3a) (0.140 g, 0.358 mmol) in chloroform (CHCl₃) (4 mL) at 0 °C was added methanesulfonic acid (MeSO₃H) (0.1 mL, 1.542 mmol). The reaction mixture stirred at 0 °C for 1.5 h and at room temperature for an additional 1.5 h, upon which TLC (1:1 EtOAc/Hexane with 0.1% acetic acid) indicated complete consumption of 3a. The solvent was removed in vacuo, resulting in an orange solid 4 (crude product). ¹H NMR indicated purity ≥ 90% which was deemed sufficient for biological testing.
Preparation of 3-(chlorosulfonyl)-4-fluorobenzoic acid [17]

To chlorosulfonic acid (20.0 mL, 299.5 mmol) at 0 °C, 4-fluorobenzoic acid (8) (5.00 g, 55.7 mmol) was added portion wise over 45 min. The resulting reaction mixture was stirred at 150°C for 20 h under argon. Upon cooling to 0 °C, the reaction mixture was added dropwise to ice water (100 mL) and allowed to stir for 45 min. Filtration through a sintered glass funnel afforded 8a as a white solid (6.37 g, 74.8%). $^1$H NMR (DMSO-d$_6$) δ 7.13 (t, J = 9.15 Hz, 1H), 7.82 (m, 1H), 8.16 (apparent dd, 1H); $^{13}$C NMR (DMSO-d$_6$) δ 117.0, 117.2, 126.7, 130.7, 133.3, 133.3, 134.9, 135.0, 160.6, 162.6, 166.5.
Preparation of 4-fluoro-3-sulfinobenzoic acid [17]

To a solution of sodium sulfite (Na$_2$SO$_3$) (31.48 g, 249.8 mmol) in H$_2$O (61.0 mL) at room temperature was added portion wise 3-(chlorosulfonyl)-4-fluorobenzoic acid (8a) (34.37 g (wet), 99.92 mmol) over 15 min at which the reaction mixture became a slurry. Approx. 25 mL 32% NaOH was added to attain pH = 9, creating a heterogeneous solution, which stirred at room temperature for 20 h. With TLC (1:1 EtOAc/Hexane with 0.1% acetic acid) indicating complete consumption of 8a, the reaction mixture was adjusted to pH = 1 with approx. 75 mL conc. HCl at 0 °C and extracted with EtOAc (3 x 250 mL). The organic layer was washed with saturated NaCl (2 x 250 mL) and dried over Na$_2$SO$_4$. The solvent was removed in vacuo and the product dried under vacuum, resulting in the white solid 9 (13.47 g, 66.0%). $^1$H NMR (DMSO-d$_6$) $\delta$ 7.44 (t, J = 8.6 Hz, 1H), 8.12 (m, 1H), 8.26 (apparent dd, 1H).
Preparation of sodium 4-fluoro-3-sulfinatobenzoate [17]

To a solution of 4-fluoro-3-sulfinobenzoic acid (9) (2.00 g, 9.80 mmol) in H₂O (10.0 mL) at room temperature was added NaOH (0.891 g, 22.28 mmol) to achieve pH = 10. Solvent was removed \textit{in vacuo} which provided a light yellow crystalline solid 9a (2.298 g, 99%), which was used directly in the next step (see Preparation of methyl 4-fluoro-3-(methylsulfonyl)benzoate).
Preparation of methyl 4-fluoro-3-(methylsulfonyl)benzoate [17]

To a solution of sodium 4-fluoro-3-sulfinatobenzoate (9a) (1.292 g, 5.21 mmol) in dimethylformamide (DMF) (26.0 mL) was added methyl iodide (CH$_3$I) (1.3 mL, 20.83 mmol) and K$_2$CO$_3$ (1.00 g, enough to maintain pH = 7). The reaction mixture stirred at 50-60 °C under argon for 5 h, upon which TLC (1:1 EtOAc/Hexane with 0.1% acetic acid) indicated complete consumption of 9a. CH$_3$I was distilled at 50-60 °C using a short path distillation apparatus. The remaining reaction mixture was poured into ice water. Filtration through a sintered glass funnel afforded 10 as a white solid (0.500 g, 41.3%). $^1$H NMR (DMSO-d$_6$) δ 3.36 (s, 3H), 3.86 (s, 3H), 7.64 (s, 1H), 8.30 (s, 2H); $^{13}$C NMR (DMSO-d$_6$) δ 44.0, 53.3, 118.8, 119.0, 127.2, 129.2, 129.3, 131.0 137.9, 138.0, 161.0, 163.1, 164.6; m.p. 108-110 °C.
Preparation of 4-fluoro-3-(methylsulfonyl)benzoic acid

To a solution of methyl 4-fluoro-3-(methylsulfonyl)benzoate (10) (0.500 g, 2.15 mmol) in THF (10.8 mL) at 0 °C was added lithium hydroxide monohydrate (LiOH·H₂O) (0.289 g, 6.89 mmol). The reaction mixture stirred at 0 °C for 23 h, upon which TLC (1:1 EtOAc/Hexane with 0.1% acetic acid) indicated complete consumption of 10. The solvent was removed in vacuo and the resulting residue was quenched with H₂O (15 mL) resulting in a pH = 9. Impurities were removed by extraction with EtOAc (3 x 15 mL). The pH of the reaction mixture was adjusted to 1-2 at 0 °C with approx. 3 mL 10% HCl and extracted with EtOAc (3 x 15 mL). The organic layer was washed with H₂O (15 mL) and saturated NaCl (15 mL) and dried over Na₂SO₄. The solvent was removed in vacuo and the product dried under vacuum, resulting in 11 (0.323 g, 68.9%). ¹H NMR (DMSO-d₆) δ 3.35 (s, 1H), 7.59 (t, J = 9.15 Hz, 1H), 8.26 (m, 1H), 8.32 (apparent dd, 1H); ¹³C NMR (DMSO-d₆) δ 44.0, 118.5, 118.7, 128.4, 129.0, 129.1, 131.1, 138.0, 138.1, 160.8, 162.9, 165.7; m.p. 207-210 °C.
Preparation of 4-(2-t-butoxy-2-oxoethylamino)benzoic acid [18]

To a solution of 4-aminobenzoic acid (12) (1.0 g, 7.29 mmol) in DMF (48.6 mL) at 0 °C was added t-butyl 2-bromoacetate (1.71 g, 8.75 mmol) and LiH (0.133 g, 16.77 mmol). The reaction mixture stirred at room temperature for 21 h under argon, upon which TLC (1:1 EtOAc/Hexane with 0.1% acetic acid) indicated complete consumption of 12. The reaction was quenched with H₂O (50 mL) and 10% HCl (5 mL) was added to pH = 1. The product was extracted with EtOAc (2 x 100 mL). The organic layer was washed with H₂O (2 x 100 mL) and saturated NaCl (100 mL) and dried over Na₂SO₄. The solvent was removed in vacuo and the product dried under vacuum, resulting in 13 as a white solid (0.88 g, 48%). ¹H NMR (DMSO-d6) δ 1.38 (s, 9H), 4.62 (s, 2H), 6.10 (br s, 2H), 6.62 (d, J = 8.6, 2H) 7.68 (d, J = 8.6, 2H); ¹³C NMR (DMSO-d6) δ 28.2, 61.3, 81.9, 113.8, 116.2, 131.9, 153.5, 165.8, 167.7; m.p. 63-68 °C.
Preparation of t-butyl 2-(4-(diaminomethylene carbamoyl)phenylamino)acetate [18]

To a solution of 4-(2-t-butoxy-2-oxoethylamino)benzoic acid (13) (0.20 g, 0.796 mmol) in acetonitrile (CH₃CN) (5.3 mL) at 0 °C was added N-hydroxysuccinimide (HOSu) (0.09 g, 0.793 mmol) and N,N'-dicyclohexylcarbodiimide (0.16 g, 0.793 mmol). The reaction mixture stirred at 0 °C for 4 h. Remaining at 0 °C, the solution was filtered through glass wool and triethylamine (1.1 mL, 7.93 mmol) and guanidine hydrochloride (0.38 g, 3.97 mmol) were added to the resulting supernate. The reaction mixture was warmed to room temperature and stirred for 21 h, after which TLC (1:1 EtOAc/Hexane with 0.1% acetic acid) indicated complete consumption of 13. After removal of the solvent in vacuo, the residue was diluted with H₂O (50 mL) and extracted with EtOAc (100 mL). The organic layer was washed with H₂O (50 mL) and saturated NaCl (50 mL) and dried over Na₂SO₄. The solvent was removed in vacuo and dried under vacuum, resulting in 13a (0.28 g, wet and slightly impure). The purity was deemed sufficient (≥ 90% by ¹H NMR) for use in the next step (see Preparation of 2-(4-(diaminomethylene carbamoyl)phenylamino)acetic acid). ¹H NMR (CDCl₃) δ 1.48 (s, 9H), 4.75 (s, 2H), 7.45 (d, J = 8.6, 2H), 8.19 (m, 2H).
Preparation of 2-(4-(diaminomethylene carbamoyl)phenylamino)acetic acid [18]

A solution of t-butyl 2-(4-(diaminomethylene carbamoyl)phenylamino)acetic acid (13a) (1.0 g, 3.97 mmol) in methanesulfonic acid (MeSO$_3$H) (10 mL) was stirred under argon at 150 °C for 16.5 h, upon which TLC (1:1 EtOAc/Hexane with 0.1% acetic acid) indicated complete consumption of 13a. The reaction mixture was quenched with H$_2$O (60 mL) and extracted with EtOAc (70 mL). The organic layer was washed with H$_2$O (35 mL) and saturated NaCl (30 mL) and dried over Na$_2$SO$_4$. The solvent was removed in vacuo. Chloroform (20 mL) was added to the resultant solid and removed in vacuo to azeotrope the remaining EtOAc, resulting in a solid 4 (crude product). $^1$H NMR indicated purity ≥ 90% which was deemed sufficient for biological testing.
Preparation of 4-chloro-3-sulfinobenzoic acid [17]

To a solution of 4-chloro-3-(chlorosulfonyl)benzoic acid (2) (73.55 g, 191.6 mmol) in H₂O (110 mL) at 0 °C was added sodium sulfite (Na₂SO₃) (60.37 g, 479 mmol). 32% NaOH was added to attain pH = 9, and the reaction was stirred at room temperature for 18.5 h after which a color change from dark brown to light brown had occurred. The reaction mixture was adjusted to pH = 1 with 10% HCl (225 mL) at 0 °C upon which a precipitate formed. The precipitate was filtered, resulting in the off-white solid 2a (30.0 g wet) which was used directly in the next step (see Preparation of 4-chloro-3-(methyl sulfonyl)benzoic acid).
Preparation of 4-chloro-3-(methylsulfonyl)benzoic acid [17]

To a solution of 4-chloro-3-sulfinobenzoic acid (2a) (9.0 g, 57.6 mmol) in H₂O (36 mL) and methanol (CH₃OH) (36 mL) at room temperature was added 32% NaOH to attain pH = 9 and iodomethane (CH₃I) (24 mL, 380.2 mmol). The reaction was stirred at 50 °C for 30 h, maintaining a pH = 9-10 with occasional addition of 32% NaOH. The reaction mixture was quenched with ice water (120 mL) and filtered. The supernatant was adjusted to pH = 1 with conc. HCl at 0 °C, upon which a white precipitate formed. The precipitate was filtered, resulting in the off-white solid 2a (1.6 g, 11.8%). $^1$H NMR (DMSO-d₆) δ 3.38 (s, 3H), 7.82 (d, J = 8 Hz, 1H), 8.16 (d, J = 7.45, 1H), 8.48 (s, 1H). $^{13}$C NMR (DMSO-d₆) δ 42.8, 131.1, 131.5, 133.1, 136.0, 136.3, 138.8, 165.8.
Preparation of 4-(4-methoxybenzylamino)-3-(methylsulfonyl)benzoic acid

To para-methoxybenzylamine (7.26 mL, 55.9 mmol) was added 4-chloro-3-(methylsulfonyl)benzoic acid (2) (0.88 g, 3.75 mmol) at room temperature. The reaction mixture was stirred at 150 °C for 2.75 h under argon, upon which TLC (EtOAc with 0.1% acetic acid) showed complete consumption of 2. At 0 °C, 1M NaOH (100 mL) was added to the reaction mixture and stirred for 15 min. Impurities were extracted with EtOAc (2 x 100 mL). The pH of the aqueous was adjusted to 1-2 with conc. HCl (approx. 16 mL) at 0 °C and extracted with EtOAc (3 x 100 mL). The organic layer was washed with H2O (75 mL) and saturated NaCl (75 mL) and dried over Na2SO4. The solvent was removed in vacuo and the product dried under vacuum, resulting in 16 (0.77 g, 61.3%). 1H NMR (DMSO-d6) δ 3.20 (s, 3H), 3.68, (s, 3H), 4.44 (d, J = 5.75, 2H), 6.81 (d, J = 9.2, 1H), 6.87 (d, 8.6, 2H), 7.16 (t, J = 5.75, 1H), 7.27 (d, J = 8.6, 2H), 7.86 (apparent dd, 1H), 8.19 (d, J = 1.7, 1H); 13C NMR (DMSO-d6) δ 42.7, 46.0, 55.5, 113.1, 114.5, 117.9, 121.4, 128.9, 130.5, 132.2, 136.3, 149.5, 159.0, 166.8; m.p. 212-218 °C.
Preparation of 4-amino-3-(methylsulfonyl)benzoic acid

To trifluoroacetic acid (12 mL, 134.63 mmol) was added 4-(4-methoxybenzylamino)-3-(methylsulfonyl)benzoic acid (15) (1.00 g, 2.99 mmol) at room temperature. The reaction mixture was stirred at 55 °C for 20 min under argon, upon which TLC (EtOAc with 0.1% acetic acid) showed complete consumption of 15. The solution was poured into 1M NaOH (150 mL) at 0 °C to obtain pH = 10 and extracted with EtOAc (3 x 150 mL) to remove impurities. The pH of the aqueous was adjusted to 1-2 with conc. HCl (approx. 8 mL) at 0 °C and extracted with EtOAc (3 x 150 mL). The organic layer was washed with H₂O (150 mL) and saturated NaCl (150 mL) and dried over Na₂SO₄. The solvent was removed in vacuo and the product dried under vacuum, resulting in 16 (0.649 g, quantitative). ¹H NMR (DMSO-d₆) δ 3.11 (s, 3H), 6.70 (br s, 2H), 6.88 (d, J = 8.6, 1H), 7.81 (t, J = 8 Hz, 1H), 8.11 (s, 1H); ¹³C NMR (DMSO-d₆) δ 42.5, 117.3, 118.0, 120.0, 132.1, 135.8, 151.1, 166.9; m.p. 246-250 °C.
Preparation of 4-(2-t-butoxy-2-oxoethylamino)-3-(methylsulfonyl)benzoic acid

![Chemical Structure](image)

To a solution of 4-amino-3-(methylsulfonyl)benzoic acid (16) (0.500 g, 2.32 mmol) in DMF (15.5 mL) was added lithium hydride (LiH) (0.051 g, 6.51 mmol) and bromo-t-butilacetate (0.589 g, 3.02 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 2 h under argon, upon which TLC (EtOAc with 0.1% acetic acid) showed complete consumption of 16. The solution was quenched with saturated NH₄Cl (50 mL) to obtain pH = 7 and extracted with CH₂Cl₂ (3 x 50 mL). The organic layer was washed with H₂O (75 mL) and saturated NaCl (75 mL) and dried over Na₂SO₄. The solvent was removed *in vacuo* and the product dried under vacuum, resulting in 6 (0.75 g, 98.2%). ¹H NMR (CDCl₃) δ 1.39 (s, 9H), 3.14 (s, 3H), 3.30 (s, 2H), 6.86 (br s, 2H) 6.91 (d, J = 8.55, 1H), 7.84 (t, J = 8.6, 1H), 8.16 (d, J = 1.7, 1H); ¹³C NMR (CDCl₃) δ 28.1, 42.8, 61.6, 82.7, 117.2, 118.8, 121.0, 132.8, 136.5, 150.1, 164.7, 167.1; m.p. 163-165 °C.
**APPENDIX OF NMR SPECTRA**

**Appendix A**  
$^1$H NMR Spectra of 4-chloro-3-(chlorosulfonyl)benzoic acid

**Appendix B**  
$^1$H NMR Spectra of 3-((2-t-butoxy-2-oxoethyl)sulfamoyl)-4-chlorobenzoic acid

**Appendix C**  
$^{13}$C NMR Spectra of 3-((2-t-butoxy-2-oxoethyl)sulfamoyl)-4-chlorobenzoic acid

**Appendix D**  
$^1$H NMR Spectra of $t$-butyl 2-(2-chloro-5-(diaminomethylene carbamoyl)phenylsulfonamido)acetate

**Appendix E**  
$^1$H NMR Spectra of 2-(2-chloro-5-(diaminomethylene carbamoyl)phenylsulfonamido)acetic acid

**Appendix F**  
$^1$H NMR Spectra of 3-(chlorosulfonyl)-4-fluorobenzoic acid

**Appendix G**  
$^{13}$C NMR Spectra of 3-(chlorosulfonyl)-4-fluorobenzoic acid

**Appendix H**  
$^1$H NMR Spectra of 4-fluoro-3-sulfinobenzoic acid

**Appendix I**  
$^1$H NMR Spectra of methyl 4-fluoro-3-(methylsulfonfyl)benzoate

**Appendix J**  
$^{13}$C NMR Spectra of methyl 4-fluoro-3-(methylsulfonfyl)benzoate

**Appendix K**  
$^1$H NMR Spectra of 4-fluoro-3-(methylsulfonfyl)benzoic acid

**Appendix L**  
$^{13}$C NMR Spectra of 4-fluoro-3-(methylsulfonfyl)benzoic acid

**Appendix M**  
$^1$H NMR Spectra of 4-(2-t-butoxy-2-oxoethylamino)benzoic acid

**Appendix N**  
$^{13}$C NMR Spectra of 4-(2-t-butoxy-2-oxoethylamino)benzoic acid

**Appendix O**  
$^1$H NMR Spectra of $t$-butyl 2-(4-(diaminomethylene carbamoyl)phenylamino)acetate

**Appendix P**  
$^1$H NMR Spectra of 2-(4-(diaminomethylene carbamoyl)phenylamino)acetic acid

**Appendix Q**  
$^1$H NMR Spectra of 4-chloro-3-(methylsulfonfyl)benzoic acid

**Appendix R**  
$^{13}$C NMR Spectra of 4-chloro-3-(methylsulfonfyl)benzoic acid

**Appendix S**  
$^1$H NMR Spectra of 4-(4-methoxybenzylamino)-3-(methylsulfonfyl)benzoic acid

**Appendix T**  
$^{13}$C NMR Spectra of 4-(4-methoxybenzylamino)-3-(methylsulfonfyl)benzoic acid

**Appendix U**  
$^1$H NMR Spectra of 4-amino-3-(methylsulfonfyl)benzoic acid

**Appendix V**  
$^{13}$C NMR Spectra of 4-amino-3-(methylsulfonfyl)benzoic acid

**Appendix W**  
$^1$H NMR Spectra of 4-(2-t-butoxy-2-oxoethylamino)-3-(methylsulfonfyl)benzoic acid

**Appendix X**  
$^{13}$C NMR Spectra of 4-(2-t-butoxy-2-oxoethylamino)-3-(methylsulfonfyl)benzoic acid
Appendix A. $^1$H NMR Spectra of 4-chloro-3-(chlorosulfonyl)benzoic acid
Appendix B. $^1$H NMR Spectra of 3-(N-(2-t-butoxy-2-oxoethyl)sulfamoyl)-4-chlorobenzoic acid
Appendix C. $^{13}$C NMR Spectra of 3-(N-(2-t-butoxy-2-oxoethyl)sulfamoyl)-4-chlorobenzoic acid
Appendix D. $^1$H NMR Spectra of $t$-butyl 2-(2-chloro-5-(diaminomethylene carbamoyl)phenylsulfonamido)acetate
Appendix E. $^1$H NMR Spectra of 2-(2-chloro-5-(diaminomethylene carbamoyl)phenylsulfonamido)acetic acid
Appendix F. $^1$H NMR Spectra of 3-(chlorosulfonyl)-4-fluorobenzoic acid
Appendix G. $^{13}$C NMR Spectra of 3-(chlorosulfonyl)-4-fluorobenzoic acid
Appendix H. $^1$H NMR Spectra of 4-fluoro-3-sulfinobenzoic acid
Appendix I. $^1$H NMR Spectra of methyl 4-fluoro-3-(methylsulfonyl)benzoate
Appendix J. $^{13}$C NMR Spectra of methyl 4-fluoro-3-(methylsulfonyl)benzoate
Appendix K. $^1$H NMR Spectra of 4-fluoro-3-(methylsulfonyl)benzoic acid
Appendix L. $^{13}$C NMR Spectra of 4-fluoro-3-(methylsulfonyl)benzoic acid
Appendix M. $^1$H NMR Spectra of 4-(2-tert-butoxy-2-oxoethylamino)benzoic acid
Appendix N. $^{13}$C NMR Spectra of 4-(2-tert-butoxy-2-oxoethylamino)benzoic acid
Appendix O. $^1$H NMR Spectra of $t$-butyl 2-(4-(diaminomethylene carbamoyl)phenylamino)acetate
Appendix P. $^1$H NMR Spectra of 2-(4-(diaminomethylene carbamoyl)phenylamino)acetic acid
Appendix Q. $^1$H NMR Spectra of 4-chloro-3-(methylsulfonyl)benzoic acid
Appendix R. $^{13}$C NMR Spectra of 4-chloro-3-(methylsulfonyl)benzoic acid
Appendix S. $^1$H NMR Spectra of 4-(4-methoxybenzylamino)-3-(methylsulfonyl)benzoic acid
Appendix T. $^{13}$C NMR Spectra of 4-(4-methoxybenzylamino)-3-(methylsulfonyl) benzoic acid
Appendix U. $^1$H NMR Spectra of 4-amino-3-(methylsulfonyl)benzoic acid
Appendix V. $^{13}$C NMR Spectra of 4-amino-3-(methylsulfonyl)benzoic acid
Appendix W. $^1$H NMR Spectra of 4-(2-t-butoxy-2-oxoethylamino)-3-(methylsulfonyl) benzoic acid
Appendix X. $^{13}$C NMR Spectra of 4-(2-t-butoxy-2-oxoethylamino)-3-(methylsulfonyl) benzoic acid
BIBLIOGRAPHY


[18] Results provided by Mark Graves, II.