Investigation of Reductive Amination in the Synthesis of T-0632

Julie Kwon
jkwon2@wellesley.edu

Follow this and additional works at: https://repository.wellesley.edu/thesiscollection

Recommended Citation
https://repository.wellesley.edu/thesiscollection/238
Investigation of Reductive Amination in the Synthesis of T-0632

Julie Kwon
Dr. David R. Haines, Advisor

Department of Chemistry
A Thesis Submitted in Partial Fulfillment of the Requirement for the Bachelor of Arts Degree with Honors in Chemistry at Wellesley College

Spring 2014 © Julie Kwon
Acknowledgements

To Professor Haines, thank you for your endless anecdotes, advice, and most importantly your encouragements. Without you I would not be the chemist that I am. Thank you for being an amazing mentor in lab and in life. Thank you for pushing me to try new things outside of my comfort zone.

To Professor Moyer, Professor Elmore and Professor Geller, thank you for your supportive and constructive feedback. I could not have asked for a better team in writing this thesis.

To the Haines Lab, thank you for making lab such a fun place to be. Thank you for listening to my complaints when reactions go awry, and celebrating with me when something goes right!

To my parents and my sister, thank you for always being a solid place of support. Thank you for trusting me to try new things and fail sometimes. Mom and dad, I cannot express how grateful I am for all that you’ve sacrificed to get me here. And to my sister, thanks for taking care of me and being the best sibling anyone could ask for.

To my friends, thank you for all of the memories. I will forever remember the Thursday pub nights, our spring break adventures, and the nights where we would just cuddle in bed and talk. I cannot wait to see where life takes us and I’m grateful for all of you to be a part of my life. Thank you for putting up with all of my nerdy chemistry jokes for the past four years (and beyond). Thank you for making my college experience infinitely exciting and unforgettable.
Abstract

Type II diabetes is a highly prevalent disease in the world that is associated with many complications such as heart disease, stroke, hypertension, and blindness. It is a disease caused by an insufficient production of insulin or insensitivity to insulin, leading to higher blood glucose levels. One of the ways in which secretion is regulated is through the action of an incretin hormone, glucagon-like peptide-1 (GLP-1), which binds to the glucagon-like peptide-1 receptor (GLP-1R) in the pancreatic beta cells, causing the release of insulin into the bloodstream.

Common methods to combat type II diabetes include direct insulin injection, GLP-1 mimetics, and dipeptidyl peptidase IV (DPP IV) inhibitors. Current treatments are mostly peptide based and are highly susceptible to degradation, thus lowering the efficacy of the drug. Likewise, direct administration of GLP-1 is inefficient since its half-life is around two minutes in vivo.

In order to extend the effectiveness of the drug, small non-peptidic agonists of GLP-1R have been investigated. However, the development of such agonists requires characterization information about GLP-1R such as the important residues that help small molecules bind to the receptor as well as the residues that are important for the initiation of the production of insulin. A small molecule inverse agonist called T-0632 has affinity to GLP-1R and inhibits GLP-1 induced cAMP production in a concentration-dependent manner. Thus, our goal is to synthesize photolabile analogs of T-0632 that will allow for characterization of the important residues in the GLP-1R binding pocket.

Synthesis of a photolabile analog of T-0632 begins with iodination of the molecules leading up to T-0632. Iodine is then displaced by an azide, and when this azide group interacts with UV light, it is converted into a highly reactive nitrene group that covalently binds to the nearest residue in the binding pocket. This thesis focuses on the first and third steps of the overall synthesis of photolabile T-0632.
## Table of Contents

Acknowledgements .................................................. 1

Abstract ................................................................. 2

1 Introduction .................................................................. 5
   1.1 Background on type II diabetes .................................
   1.2 Glucagon-like peptide 1 and glucagon-like peptide 1 receptor
   1.3 Peptide GLP-1R agonists .................................
   1.4 Small molecule GLP-1R agonists .................................
   1.5 T-0632, a small molecule inverse agonist of GLP-1R  ....
   1.6 Photoaffinity labeling ..........................................
   1.7 Synthetic goal of this project .................................

2 Results and Discussion .............................................. 17
   2.1 Diphenylamine (1) synthesis ................................
   2.2 Synthesis of isatin (2) ........................................
   2.3 Synthesis of amine-HCl (3) ....................................
      2.3.1 Reduction of isatin (2) to amine-HCl (3) using TiCl₃
      2.3.2 Protected imines: the use of sulfinimine (2.1) as an intermediate
      2.3.3 Formation of the C-N bond in amine-HCl (3) via S_N2 mechanism

3 Conclusion ............................................................... 31

4 Experimental .......................................................... 32
   4.1 Diphenylamine (1) ..............................................
   4.2 Isatin (2) ..........................................................
4.3 Reduction of Oxime using TiCl₃

4.4 Synthesis of unprotected imine

4.5 Synthesis of t-butylimine

4.6 Sulfinimine (2.1)

4.7 Sulfinamide (2.2)

4.8 Amine-HCl (3) from sulfinamide (2.2)

4.9 T-07 (4)

4.10 Hydroxyoxindole (2.3)

4.11 Chlorination of hydroxyoxindole (2.3) and subsequent amination via S_N2
Introduction

1.1 Background on Type II Diabetes

Diabetes mellitus is a prevalent disease in the world, affecting as many as 25.8 million Americans of all ages as of 2011 and 382 million people in the world. It is estimated that the number of people in the world living with diabetes will increase to 592 million by 2035. Of the three main types of diabetes, type II diabetes is the most common and makes up about 90 to 95 percent of diabetic patients. Diabetes is associated with many complications such as heart disease, stroke, hypertension, blindness, kidney disease, and nervous system disease.

Type II diabetes mellitus is a disease that is characterized by insulin resistance, even in the case where the pancreas is producing a normal amount of insulin. The inefficiency of the body in its use of insulin gradually puts stress on the pancreas and causes the organ to stop producing the needed amount after several years.

As a result of this, patients with type II diabetes have higher blood glucose levels as compared to that of healthy individuals. Type II diabetes is often correlated with factors such as family history of diabetes, older age, obesity, and race/ethnicity. It is also of note that higher prevalence of obesity and diabetes among adults living in high-poverty areas have been observed, even after standardizing for individual education, income, and occupation.

Despite its prevalence and associations with many other diseases, there are limited treatment options for type II diabetes, which fall under two categories: insulin injection or oral medication. Insulin therapy can induce weight gain and increase the patient’s risk of hypoglycemic episodes. Some oral medication types such as sulfonylureas or metiglinides are often associated with unwanted weight gain and increased hypoglycemic occurrences. Additionally, these therapies do not change or hinder the progression of type II diabetes; they
only act as quick insulin boosters.\textsuperscript{5} Needless to say, there is still a lot of research to be done on the current treatment options for type II diabetes.

1.2 Glucagon-like Peptide 1 and Glucagon-like Peptide 1 Receptor

Investigation of a better treatment method for type II diabetes begins with understanding the mechanism of insulin production. There are many pathways in the body that control for glucose homeostasis, and one of the pathways involves the actions by incretin hormones, GIP and GLP-1R. In fact, the insulinotropic effect by the incretin hormones account for 60\% of the secreted insulin after a meal.\textsuperscript{6} GLP-1 is further divided into two active forms, 80\% of which is GLP-1 (7-36) amide and the remaining 20\% being GLP-1 (7-37).\textsuperscript{6} After a meal, the K-cells and the L-cells of the intestine secrete GIP and GLP-1 respectively, which then stimulate the GLP-1 receptor (GLP-1R) of the pancreatic \( \beta \) cells to secrete insulin (figure 1).\textsuperscript{6-7}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{incretins.png}
\caption{Production and the action of incretins in glucose homeostasis. Image taken from Ross and Ekoé.\textsuperscript{7}}
\end{figure}

In type II diabetic patients there is a decrease in stimulation of insulin secretion by exogenous GIP and GLP-1 by 54\% and 29\% respectively as compared to healthy subjects.
However, the glucose lowering effect by GLP-1 is relatively preserved unlike that of GIP, in which the glucose lowering effect is absent.\textsuperscript{6}

The relatively high efficacy of GLP-1 to lower glucose even in type II diabetic patients makes it an attractive therapeutic strategy. In addition, the ability of GLP-1 to amplify insulin release in a glucose-dependent manner makes it a promising target for clinical research.\textsuperscript{8} It should be noted, however, that hormone treatment with GLP-1 itself is not ideal since its half-life \textit{in vivo} is around 2 minutes. GLP-1 and GIP are both rapidly degraded at the N-terminus by the enzyme dipeptidyl peptidase IV (DPP IV). After incretins undergo cleavage, the remaining pieces of the hormone no longer have insulinotropic activity.\textsuperscript{9} The only effective way to use GLP-1 hormone treatment would be a direct and continuous administration of GLP-1 into the veins whenever a patient is having a meal, which is considered to be a highly impractical solution to most patients.

\section*{1.3 Peptide GLP-1R agonists}

The impracticality of direct hormone therapy opens up two new research areas: the development of GLP-1 mimetics and enhancers. Figure 2 shows the classification of GLP-1 related therapies for type II diabetes currently in clinical use or in development. Generally, GLP-1R agonists are divided into two categories: short-acting or long-acting. These compounds differ in their pharmacokinetics, implying that there are fundamental differences in the mechanism of action, efficacy, and viability.\textsuperscript{10} Short-acting compounds are characterized by the sharp rise in plasma peptide levels when administered\textsuperscript{11} whereas long-acting compounds are identified by their consistent and gradual activation of the GLP-1R.\textsuperscript{12}
The above figure shows that there are many ways to modify the GLP-1 pathway by improving in vivo half-life of GLP-1. One way to extend the half-life is to reduce the cleavage done by DPP IV. Exendin-4 based agonists, exenatide and lixisenatide, have molecular modifications in which the second and the third amino acids of the peptide at the N-terminal positions are exchanged, which results in resistance to cleavage by DPP IV. However, exendin-4 based agonists are still subjected to renal degradation; consequently a limit is put on the half-life.

To avoid degradation by both DPP IV and renal elimination, a plasma albumin can be used to bind to the peptide using two different binding types. Liraglutide is an example of a long-acting, noncovalent attachment to albumin, in which a 16-carbon fatty acid chain is
conjugated to the peptide. Albiglutide and dulaglutide, also long-acting agonists, are covalently associated with albumin or immunoglobulin G, respectively, to avoid renal elimination.\textsuperscript{10}

Another type of GLP-1R agonist is exenatide-LAR (long-acting release), which has a prolonged \textit{in vivo} half-life and extended release of hormone due to its coupling to polymer microspheres. Coupling of GLP-1 with chemicals like zinc has also shown a delay in absorption by subcutaneous tissue.\textsuperscript{10} These various peptide GLP-1R agonists are summarized in figure 3 below.

![Figure 3. Types of peptide GLP-1R agonists and their mechanism of action. Various strategies were used in order to prolong \textit{in vivo} half-lifes. a) exendin-4 based agonists, b) taspoglutide, c) albiglutide and dulaglutide, d) liraglutide, and e) exenatide-LAR. Figure adapted from Meier.\textsuperscript{10}](image-url)
With any treatment there are side effects and drawbacks. These short-acting and long-acting agonists are known to exhibit side effects such as induced nausea, vomiting, and local skin reactions around the injection site. Another crucial drawback is that these agonists are protein based, meaning they require a syringe to deliver into the body as opposed to oral administration.

1.4 Small molecule GLP-1R agonists

Small molecule GLP-1R agonists under the category of GLP-1 mimetics in figure 2 are interesting but difficult compounds to research. Developing a small molecule agonist, preferably in pill form, requires the knowledge of the active site of the receptor as well as the important functional groups for binding and activation. In terms of GLP-1R, a class B G-protein coupled receptor (GPCR), a three-dimensional structure of intact receptors is unavailable, leading to uncertainty in the basis of ligand binding and what it takes to activate the receptor.

Previous research has been done to mimic the secondary structures such as α-helices and β-turns of proteins, due to their likely role in the inhibition/facilitation of the protein-protein interaction. For example, a study done by Han et al. looked at an α-helix mimic of GLP-1 in hopes to develop a small molecule agonist of GLP-1R (figure 4).

![Figure 4. Tris-benzamides as an α-helix mimic of GLP-1.](image)

Adapted from Han et al.
When three of the synthesized tris-benzamides were tested with human GLP-1R over-expressed on COS-7 cells, stimulation of cAMP production was observed, confirming the agonistic nature of these small molecules.\textsuperscript{16}

Further investigation of such small molecule agonists of GLP-1R is valuable due to their practicality as a drug. Unlike hormonal treatment, these agonists can potentially be administered orally, increasing their feasibility as a treatment method.

1.5 T-0632, a small molecule inverse agonist of GLP-1R

A small molecule non-peptide ligand called T-0632 (T-06) was originally synthesized by a Japanese pharmaceutical company for the treatment of pancreatitis as a cholecystokinin-1 (CCK1) antagonist (figure 5).\textsuperscript{17} T-06 binds to the CCK1 receptor (CCK1R, a class A GPCR) in a concentration dependent, competitive manner for this inhibitory effect.\textsuperscript{18}

\begin{center}
\includegraphics[width=0.5\textwidth]{T-0632.png}
\end{center}

\textit{Figure 5. T-0632}

Like any drug, there were side effects to T-06, the biggest and the most surprising of which was the inhibition of GLP-1 induced cAMP production in a concentration-dependent manner as Tibaduiza \textit{et al.} reported in 2001.\textsuperscript{19}

Comparative binding studies of T-06 with human and rat GLP-1R showed that T-06 has almost 100-fold selectivity to human GLP-1R over rat GLP-1R despite a 91\% amino acid homology. In order to understand why human GLP-1R has greater selectivity, radioligand
competition binding experiments were done, which determined that the amino terminus is important for such selectivity. When the amino terminus of the rat GLP-1R was partially substituted with the comparable amino acid sequence from human GLP-1R, a higher T-06 affinity to rat GLP-1R was observed. Tryptophan 33 in human GLP-1R was found to be an important residue in additional studies done to identify a single residue that determines species selectivity to non-peptide binding. When tryptophan 33 was substituted in place for serine 33 in rat GLP-1R, there was a 100-fold increase in T-06 affinity. The reciprocal 100-fold loss of T-06 affinity was observed for the converse mutation of human GLP-1R.19

The work done by Tibaduiza et al. provided important initial insights into what determines non-peptide affinity to a class B receptor, which they suggest it to be in the extracellular amino-terminal domain.19 Looking ahead, additional binding studies with T-06 may provide valuable information in the development of a small molecule agonist of GLP-1R.

1.6 Photoaffinity labeling

As mentioned earlier, knowing the important residues in the binding site of GLP-1R is crucial when developing a small molecule agonist. In order to map out the binding pocket, some type of a visualization technique is needed. The schematic diagram of photoaffinity labeling is shown in figure 6 below. A natural or chemically modified substrate binds to the enzyme in a reversible manner. When the enzyme-substrate complex is exposed to light, a covalent bond forms between the enzyme and the substrate. The photolabeled enzyme is then digested with enzymes to give the labeled fragment.20
A study done by Dong et al. utilizes photoaffinity labeling using a known radioiodinated version of a small molecule pentapeptide NRTFD (Asn-Arg-Thr-Phe-Asp) in order to identify the active site in GLP-1R. Similarly, photoaffinity labeling studies using T-06 could yield valuable information to discern the important residues in the binding pocket of GLP-1R.

The labeling method that is of interest in the Haines lab is the use of an azide. We take iodinated T-06, replace I with an azide, and expose the azide group to UV light. Once exposed to UV light, the azide group degrades into a highly reactive nitrene intermediate, which then inserts into the closest amino acid residue in the receptor to form a covalent bond to it. The labeled receptor is then digested with selective proteases into several fragments to be examined in determining the amino acid residue that has been bound by the photoaffinity label.

1.7 Synthetic Goal of this Project

The goal of this project is to synthesize many photolabile analogs of T-06 to map out the binding site of GLP-1R. As mentioned before, the photoaffinity labeling method used in the Haines lab is the use of an azide, prepared via an iodine substituent. Figure 7 shows all of the possible placements of iodine in T-06.
In vitro testing, done at the Mayo Clinic in Arizona, is currently done with CCK1R rather than with GLP-1R due to the higher binding affinity of T-06 to CCK1R than to GLP-1R. Though at first glance it may seem illogical to test azido T-06 on CCK1R in order to study the binding interaction between T-06 and GLP-1R, there are several reasons to justify this cross referencing between two different classes of GPCRs.

CCK1R and GLP-1R are both GPCRs and have seven transmembrane domains, with N-terminal domains in the extracellular space. Additionally, compensatory mechanisms involving these two receptors have been observed in genetically altered mice with deletions of the glucagon receptor (Gcgr) and GLP-1. These mice actually exhibited improved oral glucose tolerance as well as an increase in β cell activity in the pancreas. This was a surprising result, since two of the major insulinotropic β cell receptors have been deleted. One of the reasons for this improved function was due to a compensatory expression and activity of the CCK1R, which portrays remarkable adaptability of the mechanism in which these receptors manage glucose homeostasis. Such similarity and plasticity between GLP-1R and CCK1R allows for initial binding studies to be done on CCK1R. Additionally, since T-06 binds to GLP-1R in the micromolar range and to CCK1R in the nanomolar range, binding studies with CCK1R can
be done with lower concentrations of T-06. Thus CCK1R binding studies are not only economically advantageous but also beneficial in terms of obtaining greater certainty of specificity of the binding site. Eventually, when T-06 is made in greater yield, binding studies will be done on GLP-1R as well.

T-06 analogs with varying iodine placements will give information about different parts of the binding pocket of the receptor. These pieces of information can then be assembled together to gain an understanding of the three dimensional picture of the receptor. Figure 8 shows the current overall synthesis of T-06.

**Figure 8. Overall synthetic scheme of T-06 (6).**
My role in this project is in two parts. First, improve the first few steps of the overall scheme in the synthesis of diphenylamine (1). In the past this was done via nucleophilic aromatic substitution, which resulted in many side reactions and low yield. My goal was to develop a new method that will increase yield and convenience.

My second goal was to find another method to accomplish the synthesis of Amine-HCl (3). In the past this reductive amination was done using oxime as an intermediate and catalytic hydrogenation (5% Pd on C, 50 psig of H₂) as a reducing method. While this method was successful in reducing oxime to amine, it additionally cleaved the iodine (figure 9).

![Figure 9. Cleavage of iodine due to catalytic hydrogenation of oxime.](image)

The iodine plays a crucial role in synthesizing azido T-06 and must be preserved. My goal was to find other reductive amination methods that keep the iodine intact. Such a method would then allow for early iodination of the precursors leading to T-06 in locations that are unavailable for iodination later on in the synthesis. This greatly improves the flexibility of the synthetic scheme and also opens up a possibility of having multiple iodinated places in one analog.

Investigation of the use of sulfimines, t-butyl protected imines, and hydroxyoxindoles as intermediates of amine-HCl will be discussed in this thesis.
Results and Discussion

2.1 2-Fluoro-N-(3-methoxyphenyl)aniline (1) synthesis

Previous efforts to synthesize 2-fluoro-N-(3-methoxyphenyl)aniline (1) (referred to as diphenylamine for short) were done via nucleophilic aromatic substitution. In this method, a basic reagent was used to deprotonate the amine group of m-anisidine to create a nucleophile, which then reacted with the electrophilic carbon of difluorobenzene to displace one of the fluorides. Unfortunately, due to the highly reactive nucleophile and excess difluorobenzene, some side reactions occurred with additional nucleophilic aromatic substitution reactions to yield triphenylamine and a black tar-like substance. The best yield obtained from this reaction was 16% after a 72-hour reaction time.22

Investigation of another method to synthesize diphenylamine (1) involved a copper catalyzed coupling reaction of phenylboronic acids and 2-fluoroaniline using a modified Chan-Lam coupling mechanism (figure 10).23

Figure 10. Mechanism of Chan-Lam coupling reaction.

The reaction first starts with the deprotonation of the amine in 2-fluoroaniline, which is facilitated by a mild base 2,6-lutidine, followed by the complexing of the lone pairs of the amine
to the copper (II) acetate. Myristic acid helps to increase the solubility of the copper catalyst by coordinating to the copper center.\textsuperscript{23} Transmetallation of the copper (II) complex and the boron complex follows. During the transmetallation process, the copper metal needs to oxidize from \( \text{Cu}^{2+} \) to \( \text{Cu}^{3+} \), which is accomplished by utilizing \( \text{O}_2 \) more efficiently through a vigorous stirring experimental condition. This tri-phenyl \( \text{Cu}^{3+} \) complex then undergoes nucleophilic aromatic addition with one of the amine groups as the nucleophile and the meta position of the methoxybenzene ring as the electrophile, effectively displacing copper (III). \( \text{Cu}^{3+} \) reduces to \( \text{Cu}^{1+} \) via reductive elimination.

This Chan-Lam method to synthesize diphenylamine (1) proved to be extremely efficient, with an 82\% yield after purification by flash chromatography. In addition to high yield, this mechanism allows for many iodination places. When this experiment was carried out with 2-fluoro-4-iodoaniline, we obtained iodinated diphenylamine (1) in 66\% yield. One could imagine that starting with an iodinated phenylboronic acid could yield many different placements of iodine in the synthesis of a photolabile T-06 analog.

\[
\begin{align*}
&\text{F} & &\text{H} & &\text{N} & &\text{O} \\
&* & &* & &* & &*
\end{align*}
\]

66\%

\textbf{Figure 11. Possible iodinated diphenylamines (1).} The asterisks indicate places of iodination. The blue asterisk indicates 2-fluoro-4-ido-N-(3-methoxyphenyl)aniline, which has been synthesized in 66\% yield.

Figure 11 above shows all of the possible iodinated diphenylamines (1) that could be synthesized from the Chan-Lam mechanism, if the appropriate starting material could be obtained. Notice that iodination at the carbon para to methoxy must be avoided because of the ring closure in the next step of the synthesis.
2.2 Synthesis of 1-(2-fluorophenyl)-6-methoxyisatin (1-(2-fluorophenyl)-6-methoxyindoline-2,3-dione) (2)

The synthesis of 1-(2-fluorophenyl)-6-methoxyisatin (2) (referred to as isatin) was done using the procedure from Leslie Kim ’08. This is a two-part reaction involving a Friedel-Crafts acylation. The mechanism of this reaction is shown in figure 12.

![Figure 12. Friedel-Crafts mechanism in the synthesis of isatin (2).](image)

The nucleophilic nitrogen of diphenylamine attacks the acyl chloride carbon in oxalyl chloride, and successfully makes the C-N bond. The AlCl₃ catalyst then coordinates with the chloride on the other acyl chloride, essentially making the attached carbon slightly positively charged. This electrophilic carbon can then react with the carbons of the two benzene rings, and there is selectivity for the methoxy benzene ring over the fluoro benzene ring due to the activating nature of the methoxy group to the ortho and para positions of the ring. The fluoro substituent neither activates nor deactivates, thus giving selectivity to the methoxy benzene ring for the ring closure. The para-position carbon of the methoxy ring then interacts with the electrophilic carbon to form isatin (2).

2.3 Synthesis of 3-amino-1-(2-fluorophenyl)-6-methoxyindolin-2-one hydrochloride (3)

There were many attempts to synthesize 3-amino-1-(2-fluorophenyl)-6-methoxyindolin-2-one hydrochloride (3) (referred to as amine-HCl) without the use of catalytic hydrogenation.
This is a crucial step that determines whether early iodination of the compounds before this step is useful. Figure 13 shows the various intermediates and attempted steps taken in the synthesis of amine-HCl (3).

![Diagram](image)

**Figure 13. Steps taken to synthesize Amine-HCl (3) from Isatin (2).** Red text indicates the previously investigated method of synthesizing Amine-HCl, which resulted in deiodination. Black and grey text represents the various attempted methods in order to avoid deiodination. Methods shown in grey indicate reactions that resulted in complete recovery of the starting material.

Below is a discussion of the various methods as mentioned in figure 13. Due to the complete recovery of starting material, use of unprotected imine and t-butyl protected imine methods will not be discussed. However, the experimental section will include the specific reagents and molar ratios of each reaction.

### 2.3.1 Reduction of (E)-1-(2-fluorophenyl)-3-(hydroxyimino)-6-methoxyindolin-2-one (2) to 3-amino-1-(2-fluorophenyl)-6-methoxyindolin-2-one hydrochloride (3) using TiCl₃

![Chemical Structures]

**Figure 14. Proposed synthesis of amine-HCl (3) via reduction of oxime using TiCl₃**
Figure 14 shows the proposed steps in this reduction method. The synthesis of \((E)-1-(2\text{-fluorophenyl})-3\text{-}(\text{hydroxyimino})-6\text{-methoxyindolin}-2\text{-one}\) (referred to as oxime) was achieved using a procedure provided by Leslie Kim.\(^{22}\) The oxime was conveniently synthesized by reacting isatin \((2)\), hydroxylamine hydrochloride, and sodium carbonate in a solution of 95% ethanol. Direct reduction of oxime to amine was done following a mild procedure by Leeds and Kirst.\(^{24}\) This involved the reduction of the carbon nitrogen double bond using a mild sodium cyanoborohydride reagent and titanium (III) chloride as a catalyst in a methanol solvent system. The \(^1\text{H}\text{-NMR}\) spectrum of the crude product showed a change from the starting material \(^1\text{H}\text{-NMR}\), indicating that some kind of reduction did occur in this reaction. However, the crude \(^1\text{H}\text{-NMR}\) resulted in two new different peaks at 5.3-5.4ppm, which lead us to believe that there were two different products in the crude mixture (figure 15).
We suspect that the two different peaks we see at 5.3-5.4ppm arose from two rotational conformers. At the single bond between N and C of the fluorobenzene ring, there is a chance for rotation of the benzene ring. If they were true conformational isomers, the energy barrier to rotate freely would be relatively low and thus there should be unhindered rotation. In our case however, the two peaks in the $^1$H-NMR suggest that there is not free rotation. There is also a new
chiral center at the five membered ring, which means that the proton at that chiral carbon is facing a certain direction. Thus, the fluoride of the fluorobenzene ring can either be facing towards or away from the proton, with both fluorobenzene rings being perpendicular to the rest of the molecule (figure 16).

![Diagram showing two positions of the fluorobenzene ring as compared to the rest of the molecule. The fluoride substituent is perpendicular and either facing out or into the molecule. This rotation is distinguishable, as shown by the two peaks at 5.3-5.4 ppm in the $^1$H-NMR in figure 15.]

**Figure 16.** Two positions of the fluorobenzene ring as compared to the rest of the molecule. The fluoride substituent is perpendicular and either facing out or into the molecule. This rotation is distinguishable, as shown by the two peaks at 5.3-5.4 ppm in the $^1$H-NMR in figure 15.

The orientation of the fluoride in terms of the proton in the chiral carbon is important because it affects proton shifts, as fluoride is a highly electronegative substituent. In the case where the fluoride and the chiral proton are facing the same direction, we would expect the peak on the $^1$H-NMR to be shifted slightly downfield as compared to the case where the fluoride and the chiral proton are facing opposite directions. Additionally, the five membered ring is affected by the position of the fluoride, causing it to be torqued in such a way that results in a proton shift.

Due to the low yield of product, high recovery of starting material, and difficulty in discerning these two rotational conformers, another method of synthesizing amine-HCl (3) was investigated.
2.3.2 Protected imines: the use of (Z)-N-(1-(2-fluoro-4-iodophenyl)-6-methoxy-2-oxoindolin-3-ylidene)-2-methylpropane-2-sulfinamide (2.1) as an intermediate

![Chemical Structures](image)

Figure 17. Schematic diagram of sulfinimine as an intermediate.

After the formation of isatin using a procedure implemented by Kim\textsuperscript{22}, the use of (Z)-N-(1-(2-fluoro-4-iodophenyl)-6-methoxy-2-oxoindolin-3-ylidene)-2-methylpropane-2-sulfinamide (2.1) (sulfinimine for short) as an intermediate in synthesizing amine-HCl was investigated. The overall process is as follows: synthesis of sulfinimine (2.1), reduction to N-(1-(2-fluoro-4-iodophenyl)-6-methoxy-2-oxoindolin-3-yl)-2-methylpropane-2-sulfinamide (2.2) (referred to as sulfinamide), then conversion to amine-HCl (3) (figure 17).

The reason we chose N-sulfanyl group as a particular auxiliary to imine was due to its highly activating feature of the C=N bond for addition reactions as well as its ease of cleavage under mild acid conditions. In the synthesis of sulfinimine (2.1) using a one-pot procedure from Davis \textit{et al.}\textsuperscript{25}, titanium (IV) ethoxide is used as a catalyst that acts as a Lewis acid to activate the ketone of isatin. Activation of the ketone in isatin makes that carbon electrophilic, and the
electrons on the amine group of 2-methylpropane-2-sulfinamide attack and replace the ketone. In order to avoid possible unwanted oxidation of the product, the crude product was not purified, though $^1$H-NMR of the crude product was taken for verification purposes. There was a noticeable peak at 1.4 ppm that was integrating for nine protons, which confirmed the addition of the t-butyl group.

Reduction of sulfinimine (2.1) was done following the procedure from Colyer et al.\textsuperscript{26} using sodium borohydride and a mixture of THF and water as the solvent. Again, purification of the product was not done to avoid possible oxidation. However, there was a noticeable difference in the $^1$H-NMR of crude sulfinamide (2.2) product as compared to the $^1$H-NMR of crude sulfinimine (2.1), which gave us the motivation to go on further in the synthesis.

Using methanol and HCl, the sulfinyl group was cleaved off of the amine group to synthesize amine-HCl (3).\textsuperscript{27} The product was almost immediately taken to the next reaction, which was the EDC-coupling reaction to synthesize T-07 (4), the precursor to T-06.\textsuperscript{22} The $^1$H-NMR of the aromatic region of T-07 (4) showed an integration of 14 protons (figure 18), while theoretically the expected integration of fluoro-iodo-T-07 is 13 protons.
Figure 18. $^1$H-NMR of hydroxy T-07. The spectrum above is only showing the aromatic region of this $^1$H-NMR. Integration in regions from 6.5ppm-9.0ppm shows that there are 14 protons, while the expected integration is 13 protons.

The extra peak that was seen suggested the presence of a hydroxyl group in place of the fluoride. The relatively clean coupling pattern in this aromatic region also supported the idea of the cleavage of fluoride, since fluoride couples to protons and makes the $^1$H-NMR data look far more complicated. In order to verify the presence of a hydroxyl group, an $^1$H-NMR of T-07 with D$_2$O was run, as shown in figure 19.
Figure 19. $^1$H-NMR of hydroxy T-07 with D$_2$O. Integration in regions from 6.5ppm-9.0ppm shows that there are 12 protons, indicating a loss of two hydrogen-bonding protons as compared to figure 18. One of the H-bonding proton is the N-H proton, and the other proton was deduced to be a proton from an -OH bond.

The disappearance of the peaks at 8.9ppm and 7.7ppm is shown in the above figure. In order to be certain that replacement of fluoride is reproducible, this procedure was repeated again with the same result. Suspecting that the titanium catalyst might be the culprit, the synthesis of sulfinimine (2.1) was done with a different catalyst, pyridinium $p$-toluenesulfonate (PPTS), using a procedure provided by Liu et al.\(^28\) Unfortunately the procedure involving PPTS resulted in a complete recovery of the starting material and PPTS proved to be an ineffective catalyst.

Because this hydroxy T-07 was a novel compound to the Haines lab, it was unclear whether this would be effective in biological testing. However, past research has highlighted the importance of fluorine in pharmaceuticals. This is evident in the abundance of fluorinated drugs, which make up roughly 20% of all pharmaceuticals as of 2007. In a lead compound, organofluorine plays a role in virtually all of these properties: physical, distribution, metabolism,
and excretion.\(^2\) Additionally, Meng et al.\(^3\) designed and synthesized fluorinated analogs of GLP-1 to test for their stability and efficacy. Overall reports showed that fluorinated GLP-1 analogs had higher proteolytic stability as well as higher efficacy, or biological activity.

Knowing these pieces of information about fluorinated compounds, it is very probable that the fluoride on T-06 also plays an important role in binding and activity. Of course, the exact role that fluoride plays in T-06 when binding to GLP-1R is unclear until biological testing is done.

The furthest compound that was synthesized using this method of reductive amination was T-07. This was due to several reasons, the biggest of which was percent yield. With each successive reaction, the yield decreased significantly. Another reason is the high probability of the loss in biological activity due to the loss of fluoride.

2.3.3 Formation of the C-N bond in amine-HCl (3) via S\(_{N2}\) mechanism

This is a method that uses an S\(_{N2}\) mechanism to synthesize amine-HCl (3). Two of the most crucial components of an S\(_{N2}\) mechanism are the presence of a good leaving group and a good nucleophile. In order to make the ketone of isatin (2) a good leaving group, we chose to reduce it to a hydroxyl group, and then displace the hydroxyl with a chloride. Figure 20 shows the schematic diagram of the proposed synthesis via S\(_{N2}\).

![Figure 20. Proposed synthesis of amine HCl (3) via S\(_{N2}\) mechanism.](image)
The t-butylamine group was chosen to be the amine-protecting group because of its relative ease of cleavage. Leclerc et al.\textsuperscript{31} reported that a simple treatment with hydrochloric acid and methanol in reflux for short period of time would deprotect the amine. In addition, the t-butyl group is slightly electron donating to the nitrogen, making it a decent nucleophile in this $\text{S}_\text{N}2$ reaction.

The first step in this method is a reduction of isatin to 1-(2-fluorophenyl)-3-hydroxy-6-methoxyindolin-2-one (2.3) (referred to as hydroxyoxindole). Sodium borohydride was used as a reducing agent, and the procedure was modeled after Tamami and Mahdavi.\textsuperscript{32} When this reaction was carried out for the first time, the presence of the hydroxyoxindole (2.3) was confirmed with GC-MS and the product was purified with flash chromatography. Purification of hydroxyoxindole (2.3) was followed by displacement of hydroxy with chloride and $\text{S}_\text{N}2$ reaction with t-butylamine. Purification of the crude product resulted in very little pure t-butylamine (2.4) and thus the subsequent deprotection to Amine-HCl (3) could not be done.

In order to obtain more t-butylamine (2.4), the reduction reaction to hydroxyoxindole (2.3) was done for the second time. This time, the GC-MS of the crude product did not show the presence of hydroxyoxindole (2.3). The same reaction was repeated twice with comparable results. Since all of the reaction conditions were kept the same, we suspected a possible defect in the starting reagents. $^1$H-NMR was taken of isatin (2), which proved the compound to be intact. Since the only other reagent in this reaction was NaBH\textsubscript{4}, we decided to test for possible decomposition in a simpler reduction reaction with cyclohexanone (figure 21).

![Figure 21](image_url)

\textit{Figure 21. Reduction of cyclohexanone to cyclohexanol using NaBH\textsubscript{4}.}
GC-MS was taken of the crude product of the reduction reaction to cyclohexanol. The data showed no peaks that could account for cyclohexanol, leading us to believe that NaBH₄ has fallen apart over the years that it has been opened. NaBH₄ is air and moisture sensitive and the suggested storing condition for NaBH₄ is in a tightly closed container in a dry place.³³ Since the storage conditions were not strictly regulated especially during the humid summers, it was reasonable to suspect that water has gotten in contact with NaBH₄, which resulted in decomposition.
Conclusion

In the investigation of a novel method of synthesizing amine-HCl (3), significant progress was made in the first step of the overall synthesis of T-0632. The Chan-Lam coupling reaction to synthesize diphenylamine (1) proved to be extremely convenient and efficient. Once diphenylamine (1) was converted to isatin (2), several attempts were made in the reduction of isatin (2) to amine-HCl (3). Such methods involved intermediates like oxime, imine, protected imine, and hydroxyoxindole. Some methods produced surprising results, such as the replacement of the fluoride with a hydroxyl in the case of using sulfinimine as an intermediate.

As of now, further research needs to be done in the reductive amination step to preserve the iodine. Iodinating early on in the synthesis is practical once a reductive amination method is found, and such early iodination would lead to different analogs of azido-T-06 that will help in the characterization of the GLP-1R.
Experimental

General

Unless stated otherwise, all reagents were obtained from Sigma-Aldrich or Alfa Aesar and used without further purification. Thin-layer chromatography (TLC) was done on Merck 60-F254 pre-coated silica gel plates, and visualized using exposure to UV light (254nm). Column chromatography was done using Sigma Aldrich silica gel (60 Å, 70-230 mesh) or Biotage flash silica cartridges for the SP1 system. $^1$H-NMR and $^{13}$C-NMR spectra were obtained using a Bruker 300 MHz WIN-NMR spectrometer and TMS was used as an internal standard. Unless otherwise noted, deuterated chloroform was the solvent used in NMR spectroscopy. Gas chromatography mass spectrometry was obtained using an Agilent 6890N Network GC System coupled with an electron impact Agilent 5937 Network Mass Selective Detector. Unless otherwise noted, the parameters used in GCMS is as follows: $T_{\text{initial}} = 120^\circ$C, $T_{\text{final}} = 325^\circ$C, rate $= 20^\circ$C/sec; split mode (inlet); mobile phase = He$_{(g)}$; constant flow mode, flow rate 1.0mL/min; injection volume 1.0µL, pressure 11.6 psi.

4.1 Synthesis of 2-fluoro-N-(3-methoxyphenyl)aniline (1)$^{23}$

All of the glassware and stir bar used in this reaction were stored in an oven (100°C) for more than 24 hours before the start of the experiment. In a 500mL round bottom flask, 3-methoxyphenylboronic acid (0.962g, 6.33mmol), Cu(OAc)$_2$ (0.077g, 0.422mmol), and myristic acid (0.193g, 0.844mmol) were added. A rubber septum cap was attached and the mixture was stirred vigorously. Anhydrous toluene (10ml), 2,6-lutidine (0.489mL, 4.22mmol)
and 2-fluoroaniline (0.408mL, 4.23mmol) were added by syringe in this order. The mixture was stirred at a high rate for 24 hours, diluted with ethyl acetate, and vacuum filtered through a silica gel plug to remove copper and salts. The mixture was purified by flash chromatography (1% to 3% ethyl acetate in hexanes) to yield 82% of 2-fluoro-N-(3-methoxyphenyl)aniline. The identity of diphenylamine (1) was confirmed using $^1$H-NMR in CDCl$_3$ and GC-MS.

GC-MS: m/e = 217 (M+); $^1$H-NMR (CDCl$_3$): $\delta$7.0ppm (td, 1H, H$_6$), $\delta$7.2ppm (t, 1H, H$_5$), $\delta$7.1ppm (dd, 1H, H$_3$), $\delta$7.0ppm (t, 1H, H$_5$), $\delta$6.82ppm (m, 1H, H$_4$), $\delta$6.66ppm (d, 1H, H$_4$), $\delta$6.64ppm (d, 1H, H$_2$), $\delta$6.52ppm (dd, 1H, H$_6$), $\delta$5.8ppm (s, 1H, NH), $\delta$3.75ppm (s, 3H, –OCH$_3$).

4.2 Synthesis of 1-(2-fluorophenyl)-6-methoxyisatin (2)

The following procedure is adapted from Kim’s thesis. Glassware and syringes were oven-dried overnight for the preparation of this reaction. Oxalyl chloride (6.2mmol) and amylene stabilized chloroform (4.5ml) were added to a reaction flask over an ice and salt bath. In a separate flask, diphenylamine (2.9mmol) and amylene stabilized chloroform (4.5ml) were mixed and the solution was added dropwise to the reaction flask over a 5-10 minute period. After all of the mixture has been added, the reaction flask was kept on ice for 10 minutes and then taken off of the ice/salt bath. The mixture was then stirred at room temperature for two hours. At the end of two hours, the chloroform was evaporated using a rotovap. Chloroform (5ml) was added and evaporated twice to remove unreacted oxalyl chloride. To this dark green oily mixture, 10ml of dichloromethane was quickly added and cooled in an ice saltwater bath. Aluminum chloride (2.9mmol) was added to the reaction flask and the mixture was warmed to room temperature. The reaction was stirred for 48 hours at room temperature before being poured over 10 grams of ice in 2.1ml of 2M HCl. The isatin was extracted with dichloromethane, and washed twice with
10% NaHCO₃. The organic layer was dried with anhydrous MgSO₄, filtered through phase paper, and the solvent was evaporated. The crude product was purified by flash chromatography (15-25% EtAc in hexanes). GC-MS: m/e = 271 (M+); ¹H-NMR (CDCl₃): δ7.7 ppm (d, 1H, H meta to –OCH₃), δ7.2-7.6 ppm (m, 4H of the fluorobenzene ring), δ6.62 ppm (dd, 1H, H para to nitrogen), δ6.16 ppm (d, 1H, H between –OCH₃ and nitrogen), δ3.85 ppm (s, 3, H of –OCH₃).

4.3 Reduction of (E)-1-(2-fluorophenyl)-3-(hydroxyimino)-6-methoxyindolin-2-one (oxime) using TiCl₃

Oxime (0.14g, 0.49mmol), NaCNBH₄ (0.09g, 1.46mmol), ammonium acetate (0.415g) and methanol (9.5ml) were added to the reaction flask. TiCl₃ (0.68ml, 2.2 eq) was measured in a graduated cylinder and added dropwise to the reaction flask over a 10-minute period. After the addition of TiCl₃, the flask was sealed with a septum cap and flushed with (+) N₂ gas for 10 minutes. The mixture was stirred overnight at room temperature. The mixture was diluted with water and extracted with dichloromethane. The aqueous layer was taken and base was added until the pH was raised to around 9.5, which was checked using a pH strip. The product was extracted with dichloromethane, dried with anhydrous MgSO₄, filtered through phase paper, and the solvent was evaporated. ¹H-NMR showed two different conformers, as explained in the results section. ¹H-NMR (CDCl₃): δ9.2 ppm (s, 1H, -OH of oxime), δ8.07 ppm (d, 1H, H meta to –OCH₃), δ 7.0-7.6 ppm (m, 4H of the fluorobenzene ring), δ6.6 ppm (dd, 1H, H para to nitrogen), δ6.15 ppm (d, 1H, H between –OCH₃ and nitrogen), δ5.3-5.4 ppm (s, two different H, chiral H), δ3.8 ppm (m, 3H, H of –OCH₃).
4.4 Synthesis of 1-(2-fluorophenyl)-3-imino-6-methoxyindolin-2-one (unprotected imine)

This procedure was following the synthesis provided by Kumar et al.\textsuperscript{34} Isatin (0.1g, 0.369mmol) was dissolved in 10ml of glacial acetic acid in a beaker. In a separate beaker, ammonium chloride (0.02g, 0.369mmol) was dissolved in 10ml of glacial acetic acid. These two solutions were combined into a roundbottom flask, and refluxed for 3-4 hours using a heating mantle and a variac set to 40V. After the reaction the solution was cooled to room temperature, diluted with water, and the product was extracted several times with dichloromethane. The organic layer was washed several times with water in order to wash out the remaining acetic acid and ammonium chloride. The organic layer was dried with anhydrous MgSO\textsubscript{4}, filtered through phase paper, and evaporated. \textsuperscript{1}H-NMR was taken of the crude product, which confirmed a complete recovery of the starting material. This procedure was also done with ammonium acetate instead of ammonium chloride with the same result. Additionally, the molar equivalence of isatin:ammonium acetate was increased from 1:1 to 1:5 with the same result.

4.5 Synthesis of t-butylimine

Procedure from Matesic et al.\textsuperscript{35} was referenced in this reaction. Molecular sieves (3Å, 1g) were added to a roundbottom flask. The flask was stored in an oven overnight at 120°C. The flask was taken out of the oven and cooled to room temperature. Iodoisatin (0.1g, 0.25mmol) and EtOAc (7.5ml) was added to the reaction flask and stirred for 10 minutes. T-butylamine (0.018g, 0.25mmol) was added and the mixture was further stirred for five minutes before the addition of glacial acetic acid (0.2ml per 0.1g of isatin). The mixture was refluxed for 1.5 hours, the sieves were filtered through phase paper, and the solution was evaporated. \textsuperscript{1}H-NMR of the crude product showed the recovery of the starting material.
4.6 Synthesis of Sulfinimine (2.1)\textsuperscript{25}

The reaction flask was flushed with nitrogen gas for 20 minutes before any reagent was added. Isatin (0.256g, 0.645mmol), 2-methylpropane-2-sulfinamide (0.0782g, 0.645 mmol), and titanium(IV) ethoxide (0.676ml, 3.23 mmol) were added to the flask and refluxed in 10 ml of dichloromethane for five hours. The progress of the reaction was monitored by TLC. Ice water (10ml) was added to the mixture, vacuum filtered through celite, and the solids were washed with dichloromethane. The organic layer was taken after separation using a separatory funnel, dried with anhydrous MgSO\textsubscript{4}, and the solvent was evaporated. \textsuperscript{1}H-NMR was run. In order to prevent unwanted oxidation the crude sulfinimine mixture was not purified.

4.7 Synthesis of Sulfinamide (2.2)\textsuperscript{26}

In a round bottom flask, sulfinimine (0.30g, 0.6 mmol) and 30ml of THF:water (98:2) were added and placed in a bath of THF and dry ice. Sodium borohydride (0.068g, 1.8 mmol) was added to the flask and stirred for three hours. The dry ice was added to the bath intermittently for two hours. The reaction flask was brought to room temperature for the remaining hour. The mixture was evaporated and 20ml of dichloromethane was added. No extraction was done using a separatory funnel. The solution was dried with anhydrous MgSO\textsubscript{4}, filtered through phase paper, and the solvent was evaporated.

4.8 Synthesis of Amine-HCl (3) from Sulfinamide (2.2)\textsuperscript{27}

In a reaction flask, sulfinamide (0.30g, 0.6 mmol) and 20 ml of 0.4M anhydrous HCl (4M HCl diluted in methanol) was combined and stirred at room temperature for five hours. After the
reaction, the solvent in the mixture was evaporated. $^1$H-NMR was taken of the crude product but no peaks were discernable.

4.9 Synthesis of T-07 (4) (procedure taken from Kim$^{22}$)

From the previous amine-HCl reaction, the yield was assumed to be 60% for the purposes of molar ratios. In the roundbottom containing amine-HCl (0.156g, 0.36mmol), isoquinoline-3-carboxylic acid (0.076g, 0.396 mmol) and dichloromethane (5ml). The mixture was cooled in an ice bath. The following reagents were added to the roundbottom flask in this order: diisopropylethylamine (0.063ml, 0.36mmol), hydroxybenzotriazole (0.054g, 0.396mmol), and N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride (0.076g, 0.396mmol). The mixture was then warmed to room temperature and stirred overnight. The mixture was quenched with water and the product was extracted with dichloromethane. The product was dried with anhydrous MgSO$_4$ and the solvent was evaporated. The crude product was purified by flash chromatography (20-60% EtOAc/Hex). $^1$H-NMR (CDCl$_3$): $\delta$8.95ppm (s, 1H, -NH), $\delta$8.9ppm (d, 1H, isoquinoline ortho to C=O), $\delta$8.5ppm (s, 1H, isoquinoline meta to C=O), $\delta$7.9-7.95ppm (2d, 2H, H para and meta to -OH), $\delta$6.85-7.75ppm (m, 5H, isoquinoline and H ortho to -OH), $\delta$7.7ppm (s, 1H, -OH), $\delta$7.15ppm (d, 1H, H meta to -OCH$_3$), $\delta$6.75ppm (d, 1H, H ortho to N), $\delta$6.6ppm (dd, 1H, H para to N), $\delta$5.5ppm (m, 1H, chiral H), $\delta$3.65ppm (s, 3H, -OCH$_3$).

4.10 Synthesis of hydroxyoxindole (2.3)

This procedure was following the procedure provided by Tamami and Mahdavi.$^{32}$ Isatin (0.27g, 1mmol), sodium borohydride (0.019g, 0.5mmol), and 15-20ml of wet dioxane were placed in a roundbottom flask and stirred at room temperature for 24 hours. During this time, the
roundbottom flask was covered with a septum cap and an escape needle was placed. A slight
color change in the mixture was observed from orange to a lighter shade of orange. When the
reaction ended, the mixture was vacuum filtered through a short column of silica and washed
thoroughly with dichloromethane. The solution was then dried with anhydrous MgSO₄, filtered
through fluted filter paper, and the solvent was evaporated. The crude product was purified by
flash chromatography (50-100% DCM/Hex followed by 2-3% MeOH/DCM). GC/MS: m/e =
273 (M+).

4.11 Chlorination of hydroxyoxindole³² and subsequent amination via S_N²³⁶

Hydroxyoxindole (0.16g, 0.59mmol), pyridine (0.05ml, 0.64mmol), and non-anhydrous
chloroform (3ml) were combined in a flask. In a separate reaction flask, thionyl chloride
(0.046ml, 0.64mmol) and chloroform (1ml) were combined over an ice bath. While the mixture
over ice was stirring, the hydroxyoxindole solution was added to the reaction flask dropwise over
a five-minute period. After all of the solution was added to the reaction flask, it was taken off the
ice bath and heated to 60°C using a variac set to 25V. The mixture was stirred for three hours,
and the color of the solution changed from yellow to a darker shade of yellow. The mixture was
cooled to room temperature and then t-butylamine (1.76mmol) was added to the reaction flask
and stirred at room temperature for 24 hours. The mixture turned into a reddish, brownish liquid.
The solution was poured into a separatory funnel and NaHCO₃ was added. The organic layer was
collected with several washes using dichloromethane, dried with anhydrous MgSO₄, filtered
through phase paper, and the solvent was evaporated. The crude product was purified by flash
chromatography (50-100% EtOAc/Hex followed by 10% MeOH/DCM). The isolated product
had absorbances on ¹H-NMR (CDCl₃) that indicated successful addition of the t-butyl group,
though replication of the procedure and further analysis is needed.
Index of Appendices

Appendix 1. $^1$H-NMR of non-iodinated diphenylamine (1)
Appendix 2. GC-MS of non-iodinated diphenylamine (1)
Appendix 3. $^1$H-NMR of iodinated diphenylamine (1)
Appendix 4. GC-MS of iodinated diphenylamine (1)
Appendix 5. $^1$H-NMR of non-iodinated isatin (2)
Appendix 6. GC-MS of non-iodinated isatin (2)
Appendix 7. $^1$H-NMR of iodinated isatin (2)
Appendix 8. GC-MS of iodinated isatin (2)
Appendix 9. $^1$H-NMR of hydroxy T-07 from sulfinimine reaction
Appendix 10. $^1$H-NMR of hydroxy T-07 from sulfinimine reaction w/ D$_2$O
Appendix 1: $^1$H-NMR of non-iodinated diphenylamine (1)
Appendix 2. GC-MS of non-iodinated diphenylamine (1)
Appendix 3. $^1$H-NMR of iodinated diphenylamine (1)
Appendix 4. GC-MS of iodinated diphenylamine (1)
Appendix 5. $^1$H-NMR of non-iodinated isatin (2)
Appendix 6. GC-MS of non-iodinated isatin (2)
Appendix 7. $^1$H-NMR of iodinated isatin (2)
Appendix 8. GC-MS of iodinated isatin (2)
Appendix 9. $^1$H-NMR of hydroxy T-07 from sulfinimine reaction
Appendix 10. $^1$H-NMR of hydroxy T-07 from sulfinimine reaction w/ D$_2$O
References


32. Tamami, B.; Mahdavi, H., Quaternized amino functionalized cross-linked polyacrylamide as a new solid–liquid phase transfer catalyst in reduction of carbonyl compounds with NaBH4. Tetrahedron 2003, 59 (6), 821-826.

