Synthetic Approaches Towards a Radiofluorinated Agent for Imaging Alzheimer’s Disease

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A thesis submitted to

The Faculty of
the College of Science of
Northeastern University
in partial fulfillment of the requirements
for the degree of Master of Science

April 9, 2015

Thesis directed by

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ACKNOWLEDGMENTS

Firstly, I would like to extend my deepest gratitude to Dr. Robert Hanson for not only being my thesis advisor, but also for allowing me to do research in his lab and guiding me through each process. He took me in as an undergraduate student three years ago and provided me with the training and resources I needed to succeed as a chemist. Without his advisement throughout my project, I would not have had the encouragement and guts to plow through the multitude of problems that I constantly ran into.

Next, I would like to thank my lab mates Nisl Gajadeera, Dr. Emily Corocoran, and James Teh. They were always approachable and open to listening to my ramblings about graduate school or classes and never complained once. Special thanks to James who took me in under his wing as an undergraduate and taught me the techniques I needed to know to do well in research. He made me feel welcome in the Hanson group and always had good insight, advice, and tips in chemistry and in life.

I am also grateful for Dr. George O’Doherty for not only serving on my committee, but also for being my organic chemistry professor both in undergraduate and graduate studies. Dr. O’Doherty never forgot my face throughout the last five years, and was always available to talk. Without his commitment to being a great teacher and mentor, I would not have had the knowledge to succeed as an organic chemist.

I would also like to thank Dr. Jim Aggen for serving on my thesis committee as well as teaching me some novel synthetic chemistry in my Organic Synthesis 1 course last semester. His dedication to teaching organic chemistry in an organized and thoughtful way proved to be useful during my research in the Hanson lab.
I want to extend a huge thank you to Neil Vasdev and his group at Massachusetts General Hospital. Without them, this project would have never existed in the first place. Thank you for providing the tools and methods necessary to reach my goal.

I would like to thank my family for supporting me in my academics whether that is physically, emotionally, or monetarily. Without them, I don’t know if I could be where I am right now.

Finally, I would like to thank my boyfriend Shawn Gelsinger for not only keeping me in check emotionally, but also for supporting me through my studies. Thank you for listening to my frustrations, trying your hardest to understand my scientific nonsense, and reading through all my thesis drafts, even when playing video games is probably a better use of your time.
ABSTRACT OF THESIS

Alzheimer’s Disease (AD) is a neurodegenerative disease in which the patient’s memory slowly declines, accompanied by general physical degeneration and death. There are several hypotheses about the cause of AD, one of them being the aggregation of amyloid beta (Aβ) protein. Currently, the only way to definitively identify AD is by postmortem autopsy, in which the Aβ plaque is clearly identified. A potential noninvasive method for early diagnosis is a radiotracer for imaging Aβ deposits in the brain using positron emission tomography (PET) or single photon emission computer tomography (SPECT).

The Vasdev group has proposed alternative radio-fluorinated tracers that bind to Aβ plaque more effectively. In their initial studies, 8-hydroxyquinolines labeled with fluorine-18 at the 4- or 2- positions were evaluated for their potential clinical utility. Due to the synthetic inaccessibility of the 3- position, that analog was not pursued. As part of our ongoing collaboration with the Vasdev group, we undertook the preparation of the corresponding 3-fluoro and 3-iodo 8-hydroxyquinolines and their precursors.

Several synthetic strategies were devised and evaluated to obtain precursors and nonlabeled standards for in vitro/in vivo studies. Substantial prior art exists for aromatic radiofluorination precursors, including diaryl iodonium salts and aryl stannanes. During this project, many synthetic issues were encountered, which lead to the development of an alternative strategy proceeding via a stannylated intermediate that could be used for either radiofluorinated (PET) or radioiodinated (SPECT) tracers. The results of these synthetic studies are discussed, as well as the potential for applications for therapeutic areas.
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<td>8HQ</td>
<td>8-hydroxyquinoline</td>
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<tr>
<td>AD</td>
<td>Alzheimer’s Disease</td>
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<tr>
<td>Aβ</td>
<td>Amyloid beta</td>
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<tr>
<td>APOE</td>
<td>Apolipoprotein</td>
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<tr>
<td>BBB</td>
<td>Blood brain barrier</td>
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<tr>
<td>CQ</td>
<td>Clioquinol</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>HTIB</td>
<td>[Hydroxy(tosyloxy)iodo]benzene – Koser’s Reagent</td>
</tr>
<tr>
<td>H$_2$SO$_4$</td>
<td>Sulfuric Acid</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Liquid chromatography mass spectrometry</td>
</tr>
<tr>
<td>NBS</td>
<td>N-bromosuccinimide</td>
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<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
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<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>PiB</td>
<td>Pittsburgh Compound B</td>
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<tr>
<td>SPECT</td>
<td>Single photon emission computer tomography</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
<tr>
<td>WT</td>
<td>Wild type</td>
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<tr>
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Chapter 1: Background and Diagnosis of Alzheimer’s Disease

1.1 Alzheimer’s Disease

Alzheimer’s Disease (AD) is variation of dementia in which patients slowly lose their memory as they age. What begins as misplacing keys can eventually lead to forgetting peoples’ names and faces. It affects almost 5 million Americans age 65 and older. Although the direct cause of AD is unknown, there is a strong genetic correlation between the genotype apolipoprotein (APOE) and the development of AD. Dubbed as the “amyloid cascade hypothesis”, many believe that the APOE gene causes the production of the protein amyloid beta (Aβ) in the brain. When the Aβ proteins begin to aggregate in the brain, inflammation occurs around the site of Aβ plaque accumulation, which leads to the degradation and loss of function in the neurons. Many studies suggest that the APOE gene is not solely responsible for AD, as there are many patients diagnosed with AD who do not possess the APOE gene. Instead, there are other environmental and lifestyle factors to consider as well which are not entirely known. However, in spite of all the controversy regarding what the cause of AD is, all AD patients have two things in common: the existence of Aβ deposits in the brain and neurofibrillary tangles containing tau protein.

There is still no pharmaceutical intervention that is known to stop or retard the development of AD, although some medications can temporarily reduce the symptoms of AD. The most promising strategy is to detect AD as early as possible using imaging techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT), because such radioactive compounds can be detected in the picomolar range. This sensitivity opened the possibility of developing a noninvasive
procedure to detect Aβ protein within the brains of patients prior to the onset of symptoms, at a point when intervention may be effective.

Prior to the development of Aβ radiotracers, fluorescent dyes were used during autopsy to stain for Aβ plaque. This observation later became the starting point for the development of amyloid imaging probes. Radioactive derivatives of Congo red and Thioflavin T which were administered to AD patients in vivo led to the analogs such as $[^{18}\text{F}]$FDDNP and $[^{11}\text{C}]$PIB—both of which are widely used today and will be discussed later in the paper.\textsuperscript{5}

The high sensitivity of PET and SPECT is a double-edged sword. Although this property may be useful in detecting early signs of AD, it also results in significant non-specific binding of the probe to white matter in the brain. Therefore, a good imaging probe for Aβ plaque must have the following characteristics: (1) high selectivity/binding affinity for Aβ plaque ($K_d < 20\text{nM}$); (2) high in vivo metabolic stability. If the probe were to undergo metabolism in vivo, the PET/SPECT would not be able to differentiate between the parent compound and the labeled metabolites; (3) Facile radiosynthesis with high radiochemical yield ($>20\%$); (4) successful in vitro labeling of post mortem AD brains in order to verify the affinity of the probes; (5) ability to cross the blood brain barrier without being a p-glycoprotein substrate (LogP between 2 and 3.5); (6) successful ex vivo labeling of Aβ plaque in mice and primates.\textsuperscript{5}
1.2 Positron Emission Tomography

Positron Emission Tomography (PET) is the imaging modality that is most effective for noninvasively detecting AD in patients. Due to the defined energy and short half-life associated with clinically useful positron emitting radionucleotides, this technique generates a highly resolved image that reflects the distribution of the tracer. A patient is injected intravenously with a radiolabeled analogue of a molecule of interest, which will bind to specific substructures of the brain. As the radionucleotide decays, the positron is emitted which combines with an electron, and undergoes annihilation. This event results in two antiparallel coincident gamma ray photons (511keV). The PET scanner detects these gamma rays and then reconstructs this information using appropriate algorithms to generate an image of the brain disposition (Figure 1.1). An artificial color scale then “paints” the image to indicate how much radiotracer is bound to the individual tissue volumes in the brain.⁶
Figure 1.1: A graphical depiction of how a PET scanner translates photon emissions into images used for diagnosis (figure from Gambhir, used with permission).\textsuperscript{8}

Depending on which radionucleotide is used, the radiotracer will have different half-lives or imaging characteristics. As shown in Figure 1.2, there are several isotopes available for clinical use.\textsuperscript{7} Both $^{18}$F and $^{11}$C are the isotopes most frequently used in medical clinics, which are readily available by medical cyclotrons.
1.3 Imaging Agents

The objectives of a noninvasive imaging agent for AD are to detect the disease at an early stage and to provide a prognosis for the patient and doctor. As such, its localization should be associated with the disease process. None of the currently approved radiopharmaceuticals available for nuclear medicine can provide that information. $^{18}$F-fluorodeoxyglucose, the most widely used radiopharmaceutical, is an analog of glucose and its localization tracks with metabolic activity. Although it has been studied in patients with AD, decreased glucose utilization follows pathology in this case, rather than preceding it. Radiotracers that indicate cerebral blood flow provide some correlation with AD progression, but are neither diagnostic nor prognostic. Other imaging agents which target intracellular biomarkers, but which do not display affinity for Aβ or tau tangles also had low correlation with disease progress. Therefore, it was clear that a new class of radiotracer that provides a clean association with disease progression was necessary.

Over the past several years, radiotracers that are capable of providing external visualization of Aβ aggregation have been approved for clinical use. Several reviews have recently been published, describing their development from the Aβ stain, thioflavin-T. The following section will briefly describe several of the more prominent agents.
1.3.1 Pittsburgh Compound B

Pittsburgh Compound B (PiB) is an Aβ binding radiotracer that was developed at the University of Pittsburgh in 2002. PiB was synthesized in a project that examined various thioflavin-T derivatives and their binding affinities to Aβ (Figure 1.3). With a $K_D$ of 4.7nM to Aβ protein, it was one of the first and most Aβ selective radiotracers developed. It remains the standard against which AD imaging agents are compared.

![Figure 1.3: PET scans of various patients that have been injected with PiB, where NC normal control group, MCI moderate cognitive impairment group, and AD Alzheimer’s Disease group (figure from Jia et. al., used with permission).](image)

PiB remains the most widely studied and used radiotracer for AD to date. Due to its high sensitivity for Aβ, it has been used extensively in *in vivo* and post-mortem studies. In addition, its relatively short half-life (20 minutes) is advantageous for researchers looking to compare different radiotracers to one another sequentially. There is such a breadth of knowledge about PiB that almost every radiotracer developed is always compared to the sensitivity of PiB. It has become the gold standard of radiotracers today. However, the short half-life of $^{11}$C still remains a problem in the clinic. Less than 10% of the PET scanners available in the United States have the capability to produce $^{11}$C on-site.
The other 90% rely on the availability of $^{18}$F radiotracers.\textsuperscript{10} Other analogues of PiB have been developed—notably one that uses $^{18}$F in place of an aromatic hydrogen—a radiotracer called flutametamol (Figure 1.4). Studies show that it is successful in binding to A$\beta$, but less effective than PiB due to the non-specific binding nature of the $^{18}$F isotope.\textsuperscript{10} Since Flutametamol has only recently completed clinical trials, not much has been published on its effectiveness when compared to the research on PiB.

![Chemical structures of Pittsburgh Compound B and Flutametamol](image-url)

**Figure 1.4:** Chemical structures of Pittsburgh Compound B and Flutametamol.

### 1.3.2 Florbetapir and Florbetaben

Recently, more work has been done in developing radiofluorinated probes with appropriate binding affinities to A$\beta$ as well as enhanced *in vitro* and *in vivo* properties. Stilbene derivatives were discovered to have high affinity to A$\beta$ and proved to be a promising lead in developing a $^{18}$F probe for AD. After conducting various SAR studies on these stilbene derivatives, Florbetaben was developed (Figure 1.5).\textsuperscript{22} It showed great brain penetration and binding affinity to A$\beta$ ($K_i=6.5$nM). In an effort to improve the brain uptake and increase washout, the aza-analog, florbetapir, was developed. Both florbetapir and florbetaben have been recently approved by the FDA for use in clinics in 2012 and 2014.\textsuperscript{5}
1.3.3 Metal Ion Association

Exposure to metals has long been considered one of the potential initiating factors for AD. Although there has not been any conclusive proof for this hypothesis, several studies identified metal binding sites on Aβ. Investigators observed an increased concentration of iron, copper, and zinc ions in the postmortem brains of patients diagnosed with AD. Serendipitously, clinical trials of an oral anti-amebic, Clioquinol (CQ) (Figure 1.6), found that it displayed an avidity towards metals and was able to bind to the Aβ deposits in mouse brain tissue in an AD model. One of the leading hypotheses of why CQ has such a high affinity for Aβ was due to its ability to chelate ions. However, further investigation showed it was not due to chelation as copper and zinc ion concentration increased in the brains of mice that were dosed with CQ, which suggested
otherwise. The conclusion was that localization was due to binding to Aβ directly. The suggested mechanism by Vaira et. al. (Figure 1.7) shows a 2:1 ligand to metal stoichiometry of CQ to the metal ions in the brain.

\[
\text{Figure 1.6: Chemical structure of Clioquinol, a first generation anti-amebic drug that successfully binds to Aβ in the brain.}
\]

\[
\text{Figure 1.7: Cartoon sketch of the observed binding mechanism of Clioquinol to Cu(II) (left) and Fe(II) (right) in a ML}_2\text{ form (figure from Vaira et. al., used with permission).}
\]

Based on its high affinity to iron and zinc ions in Aβ, as well as its facile passage across the blood brain barrier, this compound was considered a promising lead for radiotracer analogs. This led to the development of a radioiodinated version of CQ to be used for SPECT imaging of Aβ. However, [\(^{125}\)I]CQ and [\(^{123}\)I]CQ showed low brain uptake in preclinical and clinical studies.
Chapter 2: Rationale, Design, and Synthesis of 8-hydroxyquinolin Derivative

2.1 Rationale and Design of $^{18}$F Imaging Derivative

The Vasdev Radiochemistry Group at Massachusetts General Hospital undertook the development of radiofluorinated 8-hydroxyquinoline derivatives (8HQ) (Scheme 2.1) as imaging agents for AD with good retention of the radiotracer. They have initially prepared both the 2-fluoroquinolin-8-ol and the 4-fluoroquinolin-8-ol isomers. However, the 8-(benzyloxy)-4-fluoroquinoline was unstable and further studies with that isomer were discontinued. The 2-fluoroquinolin-8-ol, [${}^{18}$F]CABS13, on the other hand, showed great promise as a radiotracer. With its high lipophilicity being similar to that of Clioquinol with a measured logD of 2.07, it was anticipated that crossing the blood brain barrier (BBB) would not constitute a problem. The compound even showed great binding affinity to $\alpha$β-Zn aggregates in vitro with a $K_d$ of 1.5 nM. Scheme 2.1 shows the reaction scheme that the Vasdev group followed in order to reach the corresponding radiotracer. In radiochemistry, the benzyl group is typically not utilized due to the extra step and purification required to reach the final product. However, the Vasdev group was able to use the benzyl protecting group by finding a relatively simple way to get to the final product in acceptable yields (30%) and well within the half-life limitation of Fluorine-18 (110 min).

![Scheme 2.1](image)

**Scheme 2.1:** The reaction scheme used by the Vasdev group in order to reach to the 2-fluoroquinolin-8-ol. Although the 4-fluoroquinolin-8-ol was pursued, the benzyloxy intermediate was not stable.
To demonstrate in vivo avidity and selectivity, this imaging agent was then injected into the tail-vein of transgenic mice with double mutation (APP/Ps1) for Alzheimer’s disease, as well as controlled wild type (WT) mice. Scans were conducted within a period of 30 minutes, and the concentration of drug in the blood was tracked as well. The injected dose per gram of tissue was plotted against time for both transgenic mice and wild type mice (Figure 2.1). The AD mice showed much higher uptake and retention of the radiotracer, whereas the WT mice showed quick but low uptake and faster clearance from the brain. $[^{18}F]$CABS13 showed better retention in the brain compared to other existing radiotracers e.g., $[^{11}C]$PiB and $[^{125}I]$CQ. However, there were no publications in which the quality of the scans from these radiotracers were compared.

**Figure 2.1:** Intravenous dosage per gram of tissue plotted against time with the AD mice in the white dots, and control mice in the black dots. AD mice showed higher uptake of the radiotracer with better retention compared to the control mice, which had low uptake and rapid clearance (figure from Vasdev et. al., used with permission).
When comparing the brains of the AD mice versus the WT mice, there was a noticeable difference in the uptake of $[^{18}\text{F}]{\text{CABS13}}$. As seen in Figure 2.2, the transgenic mice showed a greater affinity of the probe to $\text{A}\beta$ when compared to the control.

![Brain section AD brain Control](image)

**Figure 2.2**: Radiotracer uptake on transgenic mice (AD brain) and WT mice (Control) with an intensity gradient shown on the right. There is an indication of 2-fluoroquinolin-8-ol present in the cerebral cortex (CX), cerebellum (CB), and hippocampus (HC) (figure from Vasdev et. al., used with permission).¹

With what is known about $[^{18}\text{F}]{\text{CABS13}}$, i.e., a $K_d$ of 2.5 nM, good metabolic stability, yield of 30%, successful *in vivo* and *ex vivo* labeling of transgenic mice, and LogD of 2.07, this molecule fulfilled most of the requirements needed for a desirable radiotracer.¹ ⁵ In order to ensure that $[^{18}\text{F}]{\text{CABS13}}$ really was a promising lead, the Vasdev group pursued a longitudinal study in mice, looking at the uptake of the tracer in 3, 7, and 10 month old transgenic and wild type mice with well-developed plaque formation. What they found was consistent with their *in vivo* studies — there was a higher uptake in transgenic mice over WT mice for all 3, 7, and 10 month old mice (Figure 2.3).
Figure 2.3: The probe uptake in the brains of mice with developed plaque-formation at 3, 7, and 10 months. The noticeably higher area under the curve for transgenic mice suggests a promising lead for $[^{18}\text{F}]\text{CABS13}$ as a PET tracer (Reprinted with permission from Vasdev et. al. Copyright 2015 American Chemical Society).

With that in mind, the Vasdev group went on to evaluate $[^{18}\text{F}]\text{CABS13}$ in baboons to determine whether there was a similar uptake of the probe in higher animals. As they tracked the radioactivity in the cranium, they noticed a maximum uptake in the torcula with little to no penetration in any other part of the brain. At five minutes, they observed the distribution of activity expand to the superior sagittal, occipital, and transverse sinuses. However, this activity could easily be accounted for as blood pool uptake in the brain. As they tracked the activity over time in the plasma, it was determined that the radiotracer was rapidly metabolized within 20 minutes to a polar, but unidentified, metabolite (Figure 2.4). That metabolite was not free fluoride as determined by the lack of radioactivity associated with bone. Due to the low retention and rapid metabolism of
[\textsuperscript{18}F]CABS13, the 8-hydroxyquinoline was no longer pursued. No possible alternatives were suggested in the publication.

\textbf{Figure 2.4}: High radioactivity was found in the torcula portion of the brain. However, there was minimal penetration in any other part of the brain over a time span of 5 minutes. The time-activity curve shows rapid metabolism of [\textsuperscript{18}F]CABS13 after 20 minutes (Reprinted with permission from Vasdev et. al. Copyright 2015 American Chemical Society).\textsuperscript{15}

Although the animal studies conducted on [\textsuperscript{18}F]CABS13 were unsuccessful due to the metabolism and poor retention in higher animals, the Hanson group, in collaboration
with the Vasdev group, devised an alternate approach to labeled 8-hydroxyquinoline derivatives. Their strategy was to prepare a 3-fluoro-quinolin-8-ol precursor based on the hypothesis that introduction of the fluoro group at the 3- position would help mitigate the problems observed with $[^{18}\text{F}]\text{CABS13}$.

### 2.2 Synthetic Strategy 1: Diaryl Iodonium Tosylates

Conventional methods of preparing $^{18}\text{F}$ labeled aryl compounds are often not only low yielding, but also require the leaving group to be in the 2- and 4- positions. Initially proposed by Pike in 1995, iodonium salts became one of the most versatile and promising methods for radiofluorination of PET probes. With the yields ranging from 50-75% at activated position, its versatility also allows for reasonable yields with meta-substituted $^{18}\text{F}$ aryl radiotracers. Some of the suggested synthetic methods of generating the diaryliodonium precursors required the reaction of [hydroxy(tosyloxy)iodo]benzene (HTIB-Koser’s reagent) with either an electron-rich arene, an arylboronic acid, or a tri-n-butylstannane. Due to some toxicity concerns associated with stannyl compounds, the most common route to obtain the diaryliodonium tosylate was through an arylboronic acid (Scheme 2.2).

![Scheme 2.2](image)

**Scheme 2.2:** If our parent compound can be synthesized into a boronic acid, a simple coupling to HTIB can result in a stable and efficient diaryliodonium tosylate precursor to be used for radiopharmaceutical use.
2.2.1 Synthetic Design

Synthetic strategies for 2/4/5/7-substituted 8-hydroxyquinolines are well established. Introduction of halogens or their equivalents at the 5-/7- positions can be achieved via electrophilic substitution of the appropriate 8-HQ, as that ring is electron rich. Substitution at the 2-/4- positions is accessible via synthesis of the corresponding 2-/4-oxo derivative followed by conversion to the 2-/4-chloro derivative with phosphorus oxychloride. The 3- and 6- position, however, correspond to meta-substitution which is unfavorable via electrophilic substitution on the parent 8-HQ or via total synthesis from monocyclic precursors. A review of the literature provided few examples of 3-halogenated 8-HQs, and none derived directly from 8-HQ. In order for the addition of a leaving group to be added to the 3-position of an aromatic ring, a strong electron-withdrawing group, the nitro group, can be used to direct meta addition. The redistribution of electron density within the quinoline now makes the pyridine ring more electron rich, particularly at the 3-position. Starting with the commercially available 8-nitroquinoline, the synthetically useful bromine moiety can be selectively introduced. Reduction of the 8-nitro group to the 8-amino group reverses the electron density distribution between the two rings and permits nucleophilic hydrolysis to the desired 8-hydroxyl group. The 8-hydroxyl group can be protected as the benzyl ether, similar to the Vasdev group strategy, followed by transmetallation with triisopropylborate to generate the requisite boronic acid intermediate. Coupling with HTIB would provide the proposed precursor (Scheme 2.3). The synthesis of compounds 1-5 had been previously described by Gershon et. al., and their activity as antifungal agents reported. Therefore, it
appeared that the initial steps in this sequence should proceed without difficulty.

Unfortunately, as will be described, reproducing the literature procedures was not trivial.

Scheme 2.3: Proposed scheme in preparation of the diaryl iodonium precursor.

2.2.2 Synthetic Challenges

The initial steps in the synthesis of the precursor were based on the literature procedure described by Gerhson et al. Starting with the commercially available 8-nitroquinoline (1) (1.0 equiv), N-bromosuccinimide (NBS x 0.5 equiv.) were reacted under electrophilic bromination conditions to give substitution in the 3-position.

However, in addition to the 3-substitution, there was significant conversion of the product
to the 3,6-dibromo-byproduct. Presumably, as the starting material is depleted, bromination at the slightly less active 6-position becomes more competitive. Fortunately, this dibrominated byproduct was readily separated from the desired product (2) by column chromatography in 59% yield. Although this reaction seemed like a simple substitution, it was not possible to obtain only the 3-bromo-8-nitroquinoline. Reducing the equivalent to avoid bis-substitution lowered the overall yield and still generated some di-brominated material (plus starting material) that required chromatographic separation. Increasing the ratio of NBS to 1 consumed all of the starting material but generated more of the di-bromo byproduct (Scheme 2.4).

![Scheme 2.4](image)

**Scheme 2.4**: Electrophilic bromination of 8-nitroquinoline yielded both the monobrominated and the dibrominated compounds. Therefore, reaction times had to be carefully monitored.

Typical reductions of nitro groups to primary amines employ hydrogen and palladium catalysts. However, we were concerned that the use of palladium may also cause hydrogenolysis of the 3-bromo group. Instead, we used an alternate reducing agent that is more tolerant of aryl halides, i.e., iron powder in acid. In this scheme, a 33% acetic acid solution and an excess of iron powder, successfully reduced the 8-nitro group to the corresponding 8-amino-3-bromoquinoline (3). The resultant product was unstable to oxygen and tended to slowly degrade (oxidize) even under refrigerant conditions (-20°C).
Therefore, purification of the product and its subsequent transformation needed to be done in rapid succession.

The conversion of the 8-amino quinoline to the corresponding 8-hydroxyquinoline was based on the aromatic nucleophilic displacement reaction described by Gershon et al. They had reported the successful preparation of similar halo-8-hydroxyquinolines using strong acid (70% sulfuric acid), high temperature (>230 °C), sealed tube and prolonged heating (1-3 days). However, they reported that the attempted synthesis of their 3-fluorinated compound gave unidentified products. Based on their procedures, we adapted their reaction conditions (70% H$_2$SO$_4$, 220 °C, 8 h) by using a Q-Tube™ pressure reactor (purchased from Sigma-Aldrich). This pressure reactor contained a thick walled tube with a safe pressure-release system to avoid any accidental explosions. The Q-Tube was placed in a beaker of sand and heated to 220 °C. After 8 hours, the reaction was quenched by neutralization with concentrated ammonium hydroxide and the products were then extracted with ethyl acetate. Analysis of the reaction mixture using TLC and NMR indicated the presence of only starting material (3). Due to reaction conditions (acid, temperature, and pressure), tracking the progress of the reaction was difficult and time-consuming. If the acidic solution was spotted on the TLC, the silica gel would melt. If the pressure were released in the Q-Tube, the reaction would no longer be as effective with lost pressure. As a result, it was necessary to employ multiple reaction conditions to find the optimal route for the 8-amino hydrolysis. After each reaction was quenched, extraction gave only the starting material and product in the organic layer. Higher temperatures and/or prolonged heating tended to degrade the starting material and product to water-soluble compounds (noted by poor mass balance in
the organic layer). Lower temperatures and shorter reaction times gave less degradation but lower conversion yields. Ultimately, conditions were identified that gave only the starting material and product with acceptable conversion to the desired 3-bromo-8-hydroxyquinoline. Fortunately, there were no byproducts formed, which made separating the starting and final material straightforward.

The specific experimental reaction conditions evaluated are shown in Table 2.1. When optimizing this reaction, the reaction times, temperature, and acid concentration were varied. With lower acidic concentrations, the mixture was left to stir for as long as seven days, only to yield 10-25% of the material. Increasing the acidity of the reaction caused decomposition and low yields while higher temperatures resulted in a mixture of products, primarily decomposition materials. Finally, the condition which produced the most consistent yield of 30-40% with recovered starting material was a three day reaction at 60% H₂SO₄, and 230 °C. It was apparent that there would not be enough material to reach our final product using these small-scale methods. Therefore, a 10 gram batch of the 3-bromo-8-hydroxyquinoline, utilizing our procedure, was prepared by an outside vendor, V-Pharma.
Table 2.1: The hydrolysis reaction was optimized by means of varying the acidity, reaction time, and temperature.

Most of the 3-bromo-8-hydroxyquinoline received from VPharma was converted to the 8-(benzyloxy)-3-bromoquinoline. Benzylation was achieved by simple deprotonation of the phenol and its reaction with benzyl bromide. Potassium carbonate was used for deprotonation and the reaction was conducted under anhydrous conditions. When non-anhydrous DMF was used as the solvent, double addition of the benzyl group to both the phenol and the “pyridyl” nitrogen was observed (Scheme 2.5). Presumably, the use of the more reactive bromide rather than chloride decreased the discrimination between the two competing nucleophiles. In the future, benzyl chloride may be more appropriate.
Scheme 2.5: With potassium carbonate as a mild base, the alcohol can be deprotonated, then reacted with benzyl bromide. If the reaction were not done in anhydrous conditions, double addition of the benzyl group would result.

Following protection of the 8-phenolic group, the next step was to convert the 3-bromo substituent to the corresponding boronic acid. This specific boronic acid had not been reported in literature, so generic boronation reactions were pursued. For typical (hetero)aryl bromides, reaction with n-butyllithium and triisopropylborate at -78 °C yielded clean boronate esters. The isopropylborate would be hydrolyzed to form the free acid upon contact with water. What was identified instead, based upon LC-MS and \textsuperscript{1}H NMR data, was the formation of an O-Li salt, along with some other free boronic acid. Other literature methods using similar conditions mentioned the use of pH to remove any free lithium ions from the n-butyllithium. However, the pH changes did not remove the O-Li salt in our case. The crude reaction mixture was then purified on silica gel with DCM using a 1-5% gradient of methanol. NMR (\textsuperscript{1}H) analysis determined one component to be the O-methyl ester, dimethyl (8-(benzyloxy)quinolin-3-yl)boronate, and another to be the O-lithium salt, lithium (8-(benzyloxy)quinolin-3-yl)boronate (Scheme 2.6). The methanol had reacted with the free acid to form the ester in situ. The only way to get a clean boronic acid was through means of crystallization. However, crystallization requires both larger quantities of material than we had available and significant
experimentation to get the right conditions. Thus, the generation of the diaryliodonium precursor via use of a boronic acid was abandoned.

Scheme 2.6: Upon reacting 5 with $n$-BuLi and triisopropylborate in THF at -78 °C, the formation of the free boronic acid resulted in many byproducts—none of which were the free acid.

2.3 Synthetic Strategy 2: Stannylation

Electrophilic destannylation is a strategy used to prepare radiohalogenated tracers from specific stannylated precursors. However, due to the toxicity associated with organotin reagents, many groups have tended to avoid them for pharmaceutical or radiopharmaceutical research. However, its synthetic versatility for introduction of electrophiles has been described for many useful transformations, ranging from radiohalogenation to diaryliodonium salt formation (Figure 2.5). 23
Figure 2.5: Stannylation’s versatility allows for numerous transformations and additions of functional groups (figure from Pickett et. al., used with permission).²³

The Stille coupling of bistrichlorotin to the 8-(benzoxoxy)-3-bromoquinoline was carried out under anhydrous conditions with a catalytic amount (5 mol%) of tetrakis(triphenylphosphine)-Pd(0). After all the starting material had been consumed, as determined by TLC, the crude material was worked up, leading to isolation of the 3-stannylated material in an acceptable yield (48%). When this intermediate was reacted HTIB (Koser’s reagent) to obtain the desired diaryliodonium product, the brominated material was found instead.

2.4 Synthetic Strategy 3: Iodonium Auxiliary

Consultation with the Vasdev group generated alternative approaches for preparing the requisite precursors. In the course of their work with [¹⁸F]CABS13, they had developed a labeling method that proceeded via an iodonium ylide, which would avoid the potential isolation issues associated with diaryl iodonium salts and the
bidirectional fluorination of such salts. The key intermediate for the iodonium ylide is the aryl iodide that could be obtained via the aryl stannane, which we had already prepared. Interestingly, this aryl stannane could also be used directly as the precursor for a potential radioiodinated 8-HQ for SPECT imaging. They had also developed an alternate method for protection-deprotection of the 8-hydroxy group that avoided catalytic hydrogenation. This specifically used the benzyloxymethyl ester, which could be removed using mild acid.

After realizing that diaryliodonium salts may be precursors for radiofluorination, it was appreciated that two products could form if the precursor contained electron-deficient substituents. Such occurrence would complicate the isolation process without which the radiofluorinated byproducts would produce confounding PET images (Figure 2.6). Satyamurthy and Barrio developed a precursor for no-carrier added nucleophilic fluorination by employing iodonium ylide auxiliaries.\textsuperscript{24}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{diagram.png}
\caption{If a diaryliodonium tosylate contained one $R_1$ as an electron withdrawing substituent (CHO, NO$_2$) and $R_2$ as an electron donating substituent (CH$_3$, OCH$_3$) reaction with $^{18}$F resulted in the desired product and an unwanted product with the electron-withdrawing aryl group radiofluorinated in higher yields.\textsuperscript{24}}
\end{figure}

Iodonium ylides provide stable precursors that can be made under open-flask conditions, and give regiospecific nucleophilic substitution of F-18.\textsuperscript{24} It is suitable for use in a wide array of substrates including non-activated arenes, where diaryliodonium tosylates lack.\textsuperscript{25} When Meldrum’s acid, the original auxiliary used by Satyamurthy and
Barrio, was attached to an iodinated electron-neutral biphenyl substrate, an unstable thick oil was made as a result. Instead, Rottstein et. al. proposed other spiroiodinium ylides as other more stable and higher yielding precursors.\(^{25}\) A typical one-pot reaction yielded stable, crystalline material that could be used on a wide array of molecular scaffolds, from hindered heterocyclic compounds to aromatics with electron-withdrawing groups in the ortho-, meta-, and para- positions. The spirocyclic ylide functions as an excellent leaving group because of the reduced electron density at the \textit{ipso} carbon due to resonance in comparison to the \textit{alpha} carbon (Figure 2.7).\(^{25}\) This leads to a greater leaning towards the \(^{18}\)F reductive elimination.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure2.7.png}
\caption{The \textit{ipso} carbon’s resonance due to the spiroiodine (III) auxiliary allows this compound to be better suited for reductive elimination (figure from Vasdev et. al., used with permission).\(^{25}\)}
\end{figure}

\textbf{2.4.1 Synthetic Strategy and Design}

Due to the reactivity of the stannylated precursor, a 3-iodo-8(benzyloxy)quinoline was easily obtained by electrophilic substitution of the stannyl intermediate with \(N\)-iodosuccinimide in DCM in high yields (91\%). The iodinated intermediate precursor was
taken on to a simple one-pot reaction under open-container conditions as reported by Rottstein et. al. In this procedure the iodoquinoline is initially oxidized with oxone to form an iodoso intermediate. This is followed by addition of the spirocyclic malonic reagent to form the ylide precursor, suitable for radiofluorination (Scheme 2.7).

![Scheme 2.7: Proposed method in preparing iodonium ylide precursor.](image)

The first part of the synthesis proceeded as expected in the reaction of the iodoquinoline and oxone to form the iodoso intermediate. However, after adding the spirocyclic malonic reagent and adjusting pH to basic conditions, LC-MS indicated that the iodoso intermediate had converted back to the iodinated starting material. The reason for this hydrolytic instability is not obvious. This reaction is currently undergoing evaluation to improve yields and optimization of isolation methods.

Historically, hydrogen/palladium-on-carbon is used for rapid cleavage of the benzyl ethers. We were concerned that this reagent could also cause catalytic dehalogenation, particularly of the 3-iodo/bromo-quinolines. Therefore, mild alternative methods were examined. Previously we had used TFA-toluene-thioanisole deprotection of O-benzyl ethers in heterocyclic compounds, but this would usually be most effective when the compound has a substituent ortho to the benzyl ether. We hypothesized that in
In our case the nitrogen in the quinoline would constitute an acceptable ortho- substituent to successfully deprotect the group. However, after stirring the reagent with the intermediate at room temperature for 18 hours, there was no evidence of debenzylation reaction.

Another reagent that is used in cleavage of benzyl ethers is boron trichloride/tribromide. When this was tested on the 3-bromo-8-(benzyloxy)quinoline, complete conversion to the 8-hydroxyquinoline product was achieved within 30 minutes at ambient temperature. This would be a wholly acceptable deprotection procedure due to its short reaction time (within an acceptable range in the 110 min half-life of Fluorine-18).

### 2.5 Future Plans

There exists a need for a greater number of highly sensitive radiotracers for Aβ with a high signal-to-noise ratio for non-invasive diagnosis for AD. The development of these radiopharmaceuticals can help dramatically in the early clinical diagnosis of patients with AD and to increase their quality of life. The Hanson group has expended considerable effort in producing a 3-fluoroquinolin-8-ol precursor. We are looking forward to collaborating on the biological testing of these compounds to determine their potential as Aβ radiotracers. The stannylated precursor will be sent to the Vasdev group at MGH for *in vitro* and *in vivo* testing. Our hope is that agents substituted at the synthetically challenging (but no longer inaccessible) 3-position will have better *in vivo* properties than the previously prepared [^{18}F]CABS13.
As noted previously, the synthesis of the iodonium ylide still needs to be optimized. Due to time constraints, this reaction has only been tried once and will benefit in checking to see if the degradation can be avoided.

The 3-iodoquinolin-8-ol also shows great promise in a potential SPECT imaging compound as there is only one SPECT imaging device used in humans, \(^{123}\text{I}]\text{IMPY}. This situation is largely due to the difficulties in balancing Iodine-123 properties with brain pharmacokinetics.\(^5\)

The 3-bromoquinolin-8-ol can be utilized in a variety of ways, for example it has potential use in developing a therapeutic agent for AD. By taking advantage of the reactivity of the 3-bromo group in Pd(0) catalyzed coupling reactions, commercially available boronic acids can be coupled onto that position. This would allow for SAR studies to be conducted to determine its efficacy in metal coordinating effects, similar to Clioquinol. The flexibility of this molecule shows great promise in not only creating a radiopharmaceutical to diagnose AD, but also to develop drugs to treat AD.
Chapter 3: Experimental Procedures

3.1 Reagents and Instrumentation

All reagents were purchased and used as received, without purification. Silica gel chromatography was performed on 35-70 mesh silica gel (60Å) using glass columns. All $^1$H NMR was obtained on a Varian spectrometer (400 MHz) at room temperature. Reported chemical shifts (δ) are given in parts per million (ppm) and are referenced to chloroform-đ (δ=7.26) or tetramethylsilane (δ=0.00). Melting points were determined in a Thermo Scientific manual melting point apparatus. LC-MS analysis was conducted on a HPLC SunFire™C18 column (4.6 mm x 50 mm, 3.5 µm column; 10 µL injection; 30% to 100% acetonitrile/water and 0.1% formic acid gradient; 150 mL/min flow rate) with 254 nm UV single wave length detection and Waters Micromass ZQ time-of-flight (TOF) mass spectrometer (electrospray ionization). Analytical thin-layer chromatography (TLC) was performed on polyester sheets precoated with silica gel matrix 60 F254 obtained from Sigma-Aldrich and visualized under UV light or stained with iodine.

3.2 Experimental Procedures

3-bromo-8-nitroquinoline (2)

\[
\begin{align*}
\text{NO}_2 & \quad \text{N} & \quad \text{Br} \\
\text{N} & \quad \text{NO}_2 & \quad \text{Br} \\
\text{HOAc} & \quad 100^\circ\text{C} & \quad \text{NBS}
\end{align*}
\]

To 5 mL of acetic acid was added 8-nitroquinoline (0.2 g, 1.15 mmol) and N-bromosuccinimide (0.102 g, 0.575 mmol). The solution was heated to 100 °C and stirred
under reflux for 2 hours. The reaction solution was cooled to ambient temperature, poured over 20 mL of H₂O, and stirred for 30 minutes. The crude product was removed by filtration and air dried. The crude product (a mixture of mono-brominated, dibrominated, and unbrominated material) was purified by column chromatography (9 g) using 3:1 hexane/ethyl acetate as the eluent. Chromatography gave 2 as a white solid 0.121 g (42% yield, mp 122 °C, lit mp 123 °C) as well as the 3,6-dibromo-8-nitroquinoline (0.054 g, mp: 179 °C).

1H NMR (400MHz, CDCl₃) δ 9.05 (1H, s), 8.43 (1H, s), 8.05-8.07 (1H, d, J = 7.2 Hz), 7.97 (1H, d, J = 8.8 Hz), 7.7 (1H, t, J = 8.0 Hz) for 2.

1H NMR (400MHz, CDCl₃) δ 9.04 (1H, d, J = 1.2 Hz), 8.34 (1H, d, J = 2.4 Hz), 8.12 (2H, d, J = 1.2 Hz) for 3,6-dibromo-8-nitroquinoline.

**3-bromoquinolin-8-amine (3)**

![Chemical structure diagram]

Intermediate 2 (0.847 g, 3.3 mmol) was dissolved in 30 mL of acetic acid-water (2:1), followed by the addition of iron powder (0.935 g, 16.7 mmol). The reaction mixture was stirred at room temperature for 18 hours. The solvent was removed by rotary evaporation, the product mixture was suspended in dichloromethane and filtered through Celite to remove iron salts. The resultant solid was purified by column chromatography on silica gel (35 g) with dichloromethane as the eluent. Fractions containing the product were
combined, evaporated to dryness to yield 3 as a yellow solid 0.45 g (60.2% yield, mp 104 °C, lit mp = 106-107 °C).

\(^1\)H NMR (400MHz, CDCl\(_3\)) \(\delta\) 8.72 (1H, d, \(J = 2.4\) Hz), 8.21 (1H, d, \(J = 2.0\) Hz), 7.32-7.38 (1H, t, \(J = 8.0\) Hz), 7.06 (1H, d, \(J = 8.0\) Hz), 6.92 (1H, d, \(J = 7.2\) Hz), 4.8-5.2 (3H, s).

8-hydroxy-3-bromoquinoline (4)-Optimized method

8-amino-3-bromoquinoline (3) (0.3 g, 1.3 mmol) was dissolved in 10 mL of 70% sulfuric acid and placed in a Q-Tube. The tube was sealed and heated with stirring at 220°C for 3 days. After cooling to ambient temperature, the reaction solution was brought to pH 8 by addition of NH\(_4\)OH (16 M), followed by extraction with ethyl acetate (4 x 25 mL). The organic phase was combined, dried over Na\(_2\)SO\(_4\), filtered, evaporated to dryness. The residue was purified by column chromatography on silica gel (7 g) with 3:1 dichloromethane/hexane as the eluent. Fractions containing the product were combined, evaporated to dryness to give 4 as a white solid 0.184 g (34% yield, mp 99-101 °C, lit mp 111.5-112.5 °C\(^1\). In the process of optimizing this reaction to yield 4, acidic conditions, temperature, and stir time were varied. Under each of the conditions, the starting material (3) was either found in excess or completely decomposed. Details of the yields and optimization process can be found in Table 2.1.
1H NMR (400 MHz, CDCl₃) δ 8.77 (1H, d, J = 2.0 Hz), 8.32 (1H, d, J = 2.0 Hz), 7.99 (1H, s), 7.48 (1H, t, J = 8.0 Hz), 7.18 (1H, dd, J = 23.2 Hz, J = 9.6 Hz). LCMS found 225.90, [M+2H]⁺

8-(benzyloxy)-3-bromoquinoline (5)

3-bromo-8-hydroxyquinoline 4 (0.105 g, 0.48 mmol) was dissolved in 3mL of anhydrous DMF containing K₂CO₃ (0.071 g, 0.515 mmol), followed by addition of benzylbromide (0.088 g, 0.515 mmol). The reaction mixture was warmed to 60 °C for 2 hours with stirring. Formation of a light yellow solution was observed. The reaction mixture was poured over water and extracted with ethyl acetate (3 x 25 mL). The combined organic phases were washed with brine; then dried over Na₂SO₄ (anhydrous). The mixture was filtered, evaporated to dryness, and the residue was purified by column chromatography silica gel (6 g) using a 1:1 dichloromethane/hexane to 100% dichloromethane gradient. Combining fractions containing product followed by removal of solvent gave 5 as a pale yellow solid (0.114g, 77.5% yield, mp 120 °C).

1H NMR (400 MHz, CDCl₃) δ 8.94 (1H, d, J = 2.4), 8.28 (1H, d, J = 2.0 Hz), 7.49 (2H, d, J = 7.2 Hz), 7.27-7.4 (5H, m), 7.03 (1H, d, J = 8.0 Hz), 5.41 (2H, s). MP: 120°C. LCMS found 313.89, [M+H]⁺
Attempted synthesis of (8-(benzyloxy)(quinolin-3-yl)boronic acid

8-(benzyloxy)-3-bromoquinoline (5) (0.166 g, 0.528 mmol) was dissolved in 9 mL of anhydrous tetrahydrofuran. The temperature of the solution was brought down to -78 °C while stirring under argon. n-butyllithium (1.3 M in hexanes, 0.447 mL, 0.581 mmol) was added dropwise and the mixture was stirred for 30 minutes. Triisopropylborate was added to the solution and formation of a light amber solution was observed. The mixture was stirred for 30 minutes at -78 °C then brought to ambient temperature. The reaction was quenched with the addition of 10% hydrochloric acid and extracted with dichloromethane (5 mL x 3). The organic phases were collected, dried over MgSO₄ (anhydrous), filtered, evaporated to dryness, and purified by chromatography on silica gel (5 g) using a 100% dichloromethane to 5% methanol in dichloromethane elution gradient. The fractions containing the dimethyl (8-benzyloxy)quinolin-3-yl)boronate (14.3 mg) and lithium (8-(benzyloxy)quinolin-3-yl)boronate (79 mg) were collected and evaporated down to give amber oils. LCMS found 308.06 and 292.06 respectively, [M+H]⁺. Isolation of each individual byproduct proved to be difficult, so NMR spectra were not obtained.
8-(benzyloxy)-3-(tributylstannyl)quinoline (6)

8-(benzyloxy)-3-bromoquinoline (5) (0.250 g, 0.796 mmol) was added to 8mL of dry dioxane containing 5 % mole equivalents of Tetrakis (0.046 g, 0.04 mmol) and bis(tributyltin) (0.508 g, 0.875 mmol). The mixture was heated to 120 °C, stirred under argon gas and refluxed for 16 hours. After cooling to ambient temperature, the reaction mixture was filtered through celite to remove stannyl salts. The solvent was removed by rotary evaporation, then purified by chromatography on silica gel (7.5 g) with 100% hexanes followed by 20% ethyl acetate in hexanes as the eluent. The fractions containing the product were combined and evaporated to dryness to yield 6 as a pale yellow oil (0.200 g, 48% yield).

$^1$H NMR (400MHz, CDCl$_3$) δ 9.00 (1H, d, $J = 1.2$ Hz), 8.2 (1H, s), 7.53 (2H, d, $J = 7.2$ Hz), 7.2-7.4 (5H, m), 6.9 (1H, t, $J = 4.4$ Hz), 5.42 (2H, s), 1.52-1.62 (6H, m), 1.3-1.4 (7H, m), 1.14-1.2 (5H, t, $J = 8.0$ Hz), 0.84-0.94 (9H, t, $J = 7.2$ Hz). LCMS found 525.97, [M+H]$^+$
Synthesis of diaryliodonium tosylate

8-(benzyloxy)-3-(tributylstannyl)quinoline (6) (0.097 g, 0.185 mmol) was dissolved in 4 mL of anhydrous dichloromethane. Kosers reagent (0.08 g, 0.203 mmol) was added to the solution and the mixture was stirred for 4 days under argon gas. The solvent was removed by rotary evaporation and purified by chromatography on silica gel (4 g) with a 100% dichloromethane to 10% isopropanol in dichloromethane elution gradient to yield 5 as a light orange solid (7.1 mg) and HTIB (31.3 mg) as a light orange oil. NMR spectra matched shifts found in the previous reaction to obtain 5.

8-(benzyloxy)-3-iodoquinoline (7)

8-(benzyloxy)-3-(tributylstannyl)quinoline (6) (0.257 g, 0.490 mmol) was dissolved in 5 mL of anhydrous THF. The solution was degassed with argon for 10 minutes before the addition of N-iodosuccinimide (0.121 g, 0.539 mmol). The mixture was stirred at room temperature for 2 hours under argon gas. Consumption of starting material was determined by thin layer chromatography. The mixture was evaporated down to dryness, redissolved in dichloromethane, and washed with saturated sodium bicarbonate (10 mL x
3) followed by brine (10 mL x 1). The organic phases were combined, dried over MgSO$_4$ (anhydrous), filtered, and evaporated to dryness. The residue was purified by chromatography on silica gel (9 g) with a 10%-30% ethyl acetate in hexanes elution gradient. The fractions containing the product were combined and evaporated to dryness to give 7 as a white solid (0.128 g, 72.5% yield).

$^1$H NMR (400MHz, CDCl$_3$) δ 9.08 (1H, s), 8.51 (1H, s), 7.5 (2H, d, $J = 7.2$ Hz), 7.2-7.4 (5H, m), 7.05 (1H, d, $J = 8.0$ Hz), 5.43 (2H, s). LCMS found 361.83, [M+H]$^+$

**Attempted synthesis of spirocyclic ylide**

8-(benzyloxy)-3-idoquinoline (6) (0.050 g, 0.138 mmol) was dissolved in 0.2 mL of chloroform followed by the addition of trifluoro acetic acid (1 mL) and oxone (0.068 g, 0.221 mmol). The mixture was stirred at room temperature for 2 hours until consumption of starting material was determined by thin layer chromatography. All volatile contents were removed by rotary evaporation. The dried residue was resuspended in ethanol (1 mL) and 6,10-dioxaspiro[4.5]decane-7,9-dione (23.5 mg, 0.138 mmol) was added. The pH of the solution was adjusted with 10% Na$_2$CO$_3$(aq) (w/v, 0.33 M solution) until the reaction pH > 10. A light purple mixture with precipitate was observed. The mixture was stirred at room temperature for 18 hours. The reaction mixture was diluted with water (10 mL), and extracted with chloroform (10 mL). The chloroform extract was washed with
water (3 x 10 mL) and brine (1 x 10 mL). The organic phase was dried over MgSO₄ (anhydrous), filtered, and evaporated to dryness. Rₓ from thin layer chromatography and LC-MS indicated that all the iodoso intermediate had converted back to the iodinated starting material (7).

**Mild deprotection to 3-bromoquinolin-8-ol (4)**

8-(benzyloxy)-3-bromoquinolin (5) (100 mg, 0.318 mmol) was dissolved in 2.5 mL of anhydrous DCM. The solution was degassed with argon and stirred at -15 °C. A solution of BBr₃ (1.0 M solution, 0.637 mmol) was injected drop wise into the solution over 10 min while the reaction mixture was cooled to -78 °C. The solution was stirred at -78 °C for 30 minutes until the full conversion of 5 was determined by LC-MS. The reaction was quenched with the addition of 3 mL of a 10:1 chloroform/methanol solution. The crude solution was warmed to ambient temperature, evaporated to dryness, and purified by chromatography on silica gel (9 g) using a 100% dichloromethane to 10% methanol in dichloromethane elution gradient. The fractions containing the product were combined and evaporated to dryness to yield 4 as a white solid (0.069 g, 89% yield). NMR spectra matched shifts in original hydroxy substitution reaction. LCMS found 225.83, [M+H]+
REFERENCES


Appendices – NMR and LC-MS spectra
INDEX | FREQUENCY | PPM | HEIGHT
--- | --- | --- | ---
1 | 3807.445 | 8.038 | 83.9
2 | 3805.880 | 8.025 | 79.8
3 | 3327.766 | 8.343 | 96.3
4 | 3327.548 | 8.337 | 91.2
5 | 3240.828 | 6.122 | 141.5
6 | 3240.355 | 6.119 | 141.0
7 | 2898.712 | 7.258 | 61.8

![Chemical Structure](image)
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![Chemical Structure Diagram](image_url)
Exact Mass: 313.01
Exact Mass: 307.14
Exact Mass: 291.12
Exact Mass: 525.21
Exact Mass: 361.00