Studies Toward the Synthesis of Radioactive Tyrosine Kinase Inhibitors, Steroidal Antiestrogens, and Indoles as Probes of Biological Systems

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This dissertation is dedicated to my parents. Without their love and sacrifice, none of this would have been possible.
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Abstract of Dissertation

Nuclear medicine, specifically positron emission tomography (PET) and single-photon emission computed tomography (SPECT), has garnered significant interest in recent decades. The reliance of PET and SPECT on the late-stage introduction of radionuclides often requires the employment of synthetic transformations uncommon in the synthesis of stable compounds. This dissertation outlines three projects focusing on different aspects of radiotracer development: 1) the initial design of target molecules; 2) the development of new chemical methodology to synthesize target molecules; 3) the late-stage radiolabeling of the target molecules; and 4) the \textit{in vivo} evaluation of target molecules. First, this dissertation details the design and synthesis of potential radiotracers targeting receptor tyrosine kinases EGFR and HER2, two known cancer biomarkers. With the goal of enhancing the \textit{in vivo} properties the radiotracers for EGFR and HER2, elaborations of both the side chains and the aniline head group of a 4-anilinoquinazoline core were undertaken. Second, an $^{18}$F-labeled antiestrogen was synthesized as a PET imaging agent for estrogen-receptor positive tumors. The stable-isotope analog of the $^{18}$F-labeled antiestrogen was successfully synthesized, and the synthesis was translated to radiochemical conditions. The results of preliminary biodistribution and PET imaging studies of the $^{18}$F-labeled antiestrogen in normal mice and MCF-7 murine xenograft models are also described. Third, the development of new methodology for the late-stage installation of halogens at the benzo positions of indoles is discussed. This chapter details the optimization of a Pd-catalyzed stannation benzo positions of indole scaffolds for their further use in obtaining benzo-radiohalogenated indoles.
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<th>Description</th>
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<tbody>
<tr>
<td>AES</td>
<td>antiestrogen</td>
</tr>
<tr>
<td>AF-1</td>
<td>activation function 1</td>
</tr>
<tr>
<td>AF-2</td>
<td>activation function 2</td>
</tr>
<tr>
<td>AP1</td>
<td>activating protein 1</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>cAMP</td>
<td>cyclic adenosine monophosphate</td>
</tr>
<tr>
<td>CISH</td>
<td>chromogenic in situ hybridization</td>
</tr>
<tr>
<td>CREB</td>
<td>cAMP response element-binding protein</td>
</tr>
<tr>
<td>Cys</td>
<td>cysteine</td>
</tr>
<tr>
<td>DCIS</td>
<td>ductal carcinoma in situ</td>
</tr>
<tr>
<td>DCM</td>
<td>dichloromethane</td>
</tr>
<tr>
<td>DFG</td>
<td>aspartic acid – phenylalanine - glutamic acid</td>
</tr>
<tr>
<td>DIAD</td>
<td>diisopropyl azodicarboxylate</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-Dimethylformamide</td>
</tr>
<tr>
<td>DOTA</td>
<td>1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid</td>
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<tr>
<td>EGF</td>
<td>epidermal growth factor</td>
</tr>
<tr>
<td>EGFR</td>
<td>epidermal growth factor receptor</td>
</tr>
<tr>
<td>eNOS</td>
<td>endothelial nitrogen oxide synthase</td>
</tr>
<tr>
<td>ER</td>
<td>estrogen receptor</td>
</tr>
<tr>
<td>ErbB</td>
<td>avian erythroblastosis oncogene B</td>
</tr>
<tr>
<td>ERE</td>
<td>estrogen response element</td>
</tr>
<tr>
<td>Fac</td>
<td>facial</td>
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<tr>
<td>FISH</td>
<td>fluorescence in situ hybridization</td>
</tr>
<tr>
<td>Acronym</td>
<td>Definition</td>
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<tr>
<td>---------</td>
<td>------------</td>
</tr>
<tr>
<td>Glu</td>
<td>glutamic acid</td>
</tr>
<tr>
<td>h</td>
<td>hour</td>
</tr>
<tr>
<td>HER</td>
<td>human epidermal growth factor</td>
</tr>
<tr>
<td>HNSCC</td>
<td>head and neck squamous cell carcinoma</td>
</tr>
<tr>
<td>HSP</td>
<td>heat shock protein</td>
</tr>
<tr>
<td>IGIFR</td>
<td>insulin-like growth factor 1 receptor</td>
</tr>
<tr>
<td>LCMS</td>
<td>liquid chromatography mass spectrometry</td>
</tr>
<tr>
<td>Lys</td>
<td>lysine</td>
</tr>
<tr>
<td>MAPK</td>
<td>mitogen-activated protein kinase</td>
</tr>
<tr>
<td>Min</td>
<td>minute(s)</td>
</tr>
<tr>
<td>MW</td>
<td>molecular weight</td>
</tr>
<tr>
<td>NF-κB</td>
<td>nuclear factor κB</td>
</tr>
<tr>
<td>NR</td>
<td>nuclear receptor</td>
</tr>
<tr>
<td>NSCLC</td>
<td>non-small cell lung cancer</td>
</tr>
<tr>
<td>PEG</td>
<td>polyethylene glycol</td>
</tr>
<tr>
<td>PET</td>
<td>positron emission tomography</td>
</tr>
<tr>
<td>p.i.</td>
<td>post injection</td>
</tr>
<tr>
<td>PI3K</td>
<td>phosphoinositide 3-kinase</td>
</tr>
<tr>
<td>PTB</td>
<td>phosphotyrosine-binding</td>
</tr>
<tr>
<td>RUNX1</td>
<td>runt-related transcription factor 1</td>
</tr>
<tr>
<td>SH2</td>
<td>Src homology 2</td>
</tr>
<tr>
<td>SISH</td>
<td>silver-enhanced in situ hybridization</td>
</tr>
<tr>
<td>smTKI</td>
<td>small-molecule tyrosine kinase inhibitor</td>
</tr>
<tr>
<td>SP1</td>
<td>specificity protein 1</td>
</tr>
<tr>
<td>SPECT</td>
<td>single photon emission computed tomography</td>
</tr>
<tr>
<td>STAT5</td>
<td>signal transduction and activator of transcription 5</td>
</tr>
</tbody>
</table>
TBS  
*tern*-Butyl dimethylsilyl

Thr  
threonine

VEGFR  
vascular endothelial growth factor receptor
1.1 The role of nuclide-based imaging in personalized medicine

For several decades, the holy grail of medicine has been the idea of personalized medicine, in which the individual therapeutic needs of a patient can be assessed objectively and addressed in a manner that is uniquely tailored to each particular patient. One important aspect of personalized medicine would the determination, preferably via non-invasive procedures, of the exact biochemical nature of a patient’s illness, ultimately discerning the most effective therapeutic regimen for that individual. Molecular imaging, whether by ultrasound, optical methods, or radionuclide-based methods, provides a way to non-invasively visualize the biochemical processes thought by the clinician to be aberrant in the patient’s illness.

While the field of nuclear medicine has advanced significantly over the past several decades, the ideal of having a radiotracer for the early detection of each of the major, life-threatening diseases is far from being realized. Currently, the most utilized PET radiotracer is $[^{18}\text{F}]-\text{fluorodeoxyglucose (FDG)}$, which aids the visualization of most tumors and other sites of inflammation, yet provides no information as to which particular biochemical processes have gone awry in a particular tumor. This, in turn, leaves clinicians to rely on invasive procedures such as biopsies and subsequent immunohistochemical evaluation of the recovered tissue, to provide direction as to the appropriate therapeutic regimens for patients. Unfortunately, these invasive procedures are fraught with a high percentage of misdiagnoses. Development of imaging agents specific for particular subtypes of disease, e.g. various forms of cancer, would improve the standard of care for patients and ultimately give access to individualized treatment plans.
1.2 Brief overview of PET and SPECT imaging

1.2.1 PET imaging

Positron emission tomography, commonly referred to as PET, is a radionuclide-based imaging modality that utilizes positron-emitting radioisotopes. Positron-emitting radioisotopes are commonly produced via irradiation of a target in a cyclotron with protons. This irradiation produces radioisotopes with an overabundance of positive charge, leading to the conversion of a proton to a neutron while concomitantly releasing a positron and an electron neutrino. The positron ($\beta^+$), which is a positively charged beta particle, then travels through the tissue until it decelerates sufficiently to productively collide with an electron ($\beta^-$) in an annihilation event. This collision of the positron with the electron causes the release of two $\gamma$-rays, each with an energy of 511 keV, and traveling in opposite (~180°) paths from one another. This pair of $\gamma$-rays is then detected by the scanner. The sum of all coincident pairs of $\gamma$-rays counted by the detector is subsequently used to pinpoint, by computational algorithms, the location of the radioactive species generating these coincident $\gamma$-rays. This data is then further reconstructed to produce a visual representation of the loci of radioactivity within the patient.

The two most commonly used PET radioisotopes are carbon-11 and fluorine-18, as they are easily produced and their stable-isotope analogs are ubiquitous in biologically relevant compounds. $^{18}$F is most commonly produced in a cyclotron by the irradiation of $[^{18}$O]-water via the $^{18}$O(p,n)$^{18}$F reaction and decays 97% by positron emission and 3% by electron capture. Both decay pathways yield stable oxygen-18 as the daughter isotope. Other common PET radionuclides include copper-64, gallium-68, and zirconium-89. Their production, however, is more difficult, and the use of these radionuclides requires the incorporation of a chelating group into the targeting agent, which, for smaller-sized compounds, can greatly alter the biological properties of the targeting agent.
1.2.2 SPECT imaging

Single-photon emission computed tomography (SPECT) is a radionuclide-based imaging modality that utilizes radionuclides which decay directly to $\gamma$-rays, either by electron capture or isomeric transition. A small percentage of these emitted $\gamma$-rays pass through collimators and are then detected by gamma cameras. In order to ensure that the detected photons are coming directly from the radiation source, rather than, after deflection events, the collimators prevent the detection of all photons except those hitting the collimator at a very specific angle. While this ameliorates the amount of background radiation detected, it also prevents the detection of a high percentage of the emitted photons, leading to the poor sensitivity experienced with SPECT imaging.

The most commonly used SPECT radioisotopes in imaging are iodine-123 and technetium-99m. Iodine-123 has a half-life of 13h and a principal photon emission of 0.16 MeV. It is produced in a cyclotron by the following reaction: $^{124}\text{Xe} (p,2n) \rightarrow ^{123}\text{Cs} \rightarrow ^{123}\text{Xe} \rightarrow ^{123}\text{I}$. $^{123}\text{I}$ can be distributed over long distances due to its longer half-life. Radioactive iodine isotopes are commonly produced as NaI in NaOH. Radioiodination can be accomplished, in the presence of an oxidizing agent such as Chloramine-T, Iodogen, or peracetic acid, by direct iodination for arenes that are activated for electrophilic substitution. Side reactions, however, are common for this radiolabeling method. For other substrates, radioiodination requires the presence of a leaving group that is easily displaced by iodine via either nucleophilic or electrophilic exchange. These exchange reactions are largely based on iodo-demetalation reactions, often using organosilyl, organoboryl, or organostannyl compounds as precursors, which can be difficult to prepare. While the organosilyl and organoboryl precursors are more stable than the corresponding organostannyl compounds, they are less reactive, which at times leads to radioiodination at positions other than the ipso carbon. While very effective for radioiodinations, the iodo-demetalation approach also raises concerns about toxicity issues over the removal of metal residues after the radiolabeling reaction.

Technetium-99m, currently the most frequently used radioisotope for diagnostic scans, has a half-life of 6h. It is commercially available as a $^{99}\text{Mo}/^{99m}\text{Tc}$ generator, in which the longer-lived parent
isotope ($^{99}$Mo) slowly decays to the shorter-lived daughter isotope ($^{99m}$Tc), which can be collected by elution with saline as the carrier-free [$^{99m}$Tc]-TcO$_4$. As these generators need only be replenished monthly, $^{99m}$Tc has become the SPECT radioisotope of choice. As with other radiometals, the use of $^{99m}$Tc necessitates the presence of a chelating moiety on the targeting molecule, which often negatively impacts the biological properties of the targeting agent. The use of bifunctional chelating agents, however, allow for the binding of $^{99m}$Tc in a fashion that is stable \textit{in vivo} and also places the chelated radiometal a sufficient distance from the targeting agent as to minimize effects on the biological activity.\textsuperscript{8}

1.2.3 \textbf{Comparison between PET and SPECT modalities}

One important difference between PET and SPECT imaging modalities is that PET has sensitivity approximately two- to three-fold higher than that of SPECT, which leads to higher signal-to-noise ratios in PET and, ultimately, higher quality images with greater resolution. While PET is able to detect approximately 1\% of all emission events, SPECT is currently able to detect only 0.01 – 0.04\% of all emission events. The foundation of this difference in sensitivities lies in the method by which SPECT and PET determine which photons should be counted or recorded, that is, which photons are traveling from the loci of the injected radioactive species as opposed to photons which underwent deflection or arose from interaction of the original photon with other matter. In order to exclude photons not traveling directly from the loci of the radiotracer, SPECT relies on physical collimators, which prevent the photons from striking the detector unless they approach within a small angular range, as was mentioned in the previous section. PET, however, performs electronic collimation using the coincidence-detection method, in which only photons reaching the detector at the same time and at \textasciitilde$180^\circ$ from each other are recorded. The resulting large number of coincidence events is then reconstructed into an image showing the spatial distribution of radioactivity as a function of time.\textsuperscript{8} Electronic collimation allows a much larger angle of acceptance at each detector position, which grants PET its greater sensitivity compared to SPECT.\textsuperscript{9}
1.3 Elements of radiotracer design

Most often, the development of a radiotracer as a clinical diagnostic tool will center on the exploitation of a biological pathway known to be aberrant in the particular disease of interest. This aberrant biological pathway often contains a mutated or overexpressed receptor, which can serve as a biomarker to provide valuable diagnostic and prognostic information to clinicians.\textsuperscript{10}

The design and preparation of radiotracers requires the careful consideration of several parameters. As in medicinal chemistry, a radiochemist must design a molecule, whether a small-molecule or a larger substrate, such as a peptide, affibody, or antibody, that exhibits high affinity for the biological target of interest and demonstrates good pharmacokinetic properties, with optimal logP values $\leq 3$. Additionally, one must select the location and method for the introduction of the radioisotope. The location of the radioisotope should be such as to be easily, cleanly, and quickly installed, while not adversely affecting the \textit{in vivo} biological function of the molecule.

In addition to the usual considerations taken into account in synthetic chemistry, the synthesis of radiolabeled compounds imposes several unique constraints. For syntheses involving the incorporation of short-lived radioisotopes, it is imperative that the introduction of the radioisotope occur as near as possible to the last transformation of the synthetic route. This helps minimize the amount of radioactive decay that occurs between the installation of the radioactive isotope and the use of the radioactive probe for the imaging of a biochemical pathway. The percentage of injected mass that is the desired radioactive compound, otherwise referred to as the specific activity of a compound, is desired to be as high as possible. Each non-radioactive molecule present is capable of binding to the biological target but is unable to contribute to the imaging of that target. Therefore, having a high specific activity for a radiotracer is often important due to the small concentrations of the biological target present in the living organism. Therefore it is imperative to have as many targeted compounds be radioactive at the time of imaging as possible.
This need to install a radioactive isotope in the last steps of a synthetic sequence dictates the synthetic route to be taken. Oftentimes the optimal synthetic route to synthesize the stable-isotope analog of the desired radiotracer will not be amenable to the radiochemical synthesis and therefore an alternate, perhaps less efficient, synthetic route must be employed. The most important consideration is that the introduction of the radioisotope be accomplished in a rapid, highly effective manner, and that the purification of the radioactive compound can be accomplished quickly. If these two conditions are met, a lower efficiency in the other synthetic transformations can be accepted.

Throughout the following three chapters, these aforementioned principles of radiotracer design have been implemented. The design and synthesis of radiotracers based on tyrosine kinase inhibitors (Chapter 2) involved the defining of an appropriate disease biomarker, as well as the incorporation of functional groups that can be easily transformed into a radioactive moiety at a late synthetic stage. The synthesis of an $^{18}$F-labeled antiestrogen and its preliminary testing in vivo (Chapter 3) explores some of the challenges in the translation of stable-isotope chemistry to radiochemical conditions and highlights difficulties that can be encountered during the biological evaluation of radiotracers. The project focusing on the development of a Pd-catalyzed stannation of indoles (Chapter 4) highlights, through a discussion of the shortcomings of common halogenation methods for the radiohlabeling of indoles, that the requisite late-stage introduction of radioisotopes often imposes constraints that necessitate the development of new synthetic methodologies.
Chapter 2

Synthesis and Evaluation of Potential Radiotracers Targeting EGFR and HER2

2.1 The biology of the ErbB family of receptors

The ErbB (avian erythroblastosis oncogene B) family of growth factor receptors, also known as the HER (human epidermal growth factor receptor) family of growth factor receptors are expressed in cells of mesodermal and ectodermal origins and their basolateral location permits them to mediate signals between the mesenchyme and the epithelium for cell growth. The dysregulation of EGFR and HER2, two members of the ErbB family of receptor tyrosine kinases, plays a major role in various cancers. This dysregulation comes about via overexpression, in the case of HER2, and a combination of overexpression and mutation, in the case of EGFR. The overexpression of HER2 is known to occur in approximately 20% of all breast cancers including high-grade diseases such as extensive ductal carcinoma in situ (DCIS) and grade 2 and 3 invasive ductal carcinomas. HER2/neu amplification causes the number of receptors to increase from 20,000 receptors present in a normal cell to as many as 2,000,000 receptors present in a tumor cell. The overexpression of EGFR is known to play a role in non-small cell lung cancer (NSCLC), head and neck squamous cell carcinoma (HNSCC), and breast, bladder, prostate, colorectal, kidney, and glioma carcinomas. In fact, EGFR overexpression is known to occur in 80% of all HNSCC cases, 40 - 50% of glioblastomas, and approximately 70% of colorectal cancers. EGFR mutation is associated with gliomas and lung, ovarian, and breast cancers with EGFR mutations occurring in more than 10% of Caucasian and Asian NSCLC patients.

The ErbB family of receptor tyrosine kinases consists of four members: (1) EGFR/ErbB1/HER1; (2) HER2/ErbB2/Neu; (3) HER3/ErbB3; and (4) HER4/ErbB4. Each of these enzyme-linked cell surface
receptors has three domains. The ligand-binding extracellular domain contains ~620 amino acids and is responsible for binding the EGF-family of peptide ligands. An α-helix spanning the membrane constitutes the transmembrane domain. The third, cytoplasmic domain is made of three distinct regions: a juxtamembrane region of ~45 amino acids, a tyrosine kinase domain of ~270 amino acids, and carboxy-terminal regulatory sequences of ~230 amino acids.\textsuperscript{21}

The traditional, simplistic view of ErbB receptor activation and signaling has been that the binding of a ligand to the extracellular domain initiates receptor homo-/heterodimerization through a conformational change in the transmembrane domain. This conformational change triggers the phosphorylation of tyrosine residues in the cytoplasmic kinase domain responsible for the subsequent recruitment of myriad signaling proteins which contain either phosphotyrosine-binding (PTB) or Src homology 2 (SH2) domains.\textsuperscript{22,23} The nature and duration of signaling by ErbB receptors are thought to be influenced by several factors, including the identity of the ligand, the composition of the receptor dimer, and the specific structural determinants of the receptors.\textsuperscript{24} The kinase domain of an ErbB receptor is composed of a C-lobe and an N-lobe which are connected via a hinge region, which serves as the binding site for adenosine triphosphate (ATP). The cytoplasmic kinase domain of EGFR is most often found in its autoinhibited, inactive conformation until activated upon extracellular ligand binding. In the inactive conformation of EGFR, an α-helix, known as the Cα-helix, is rotated outward in comparison to the rest of the N-lobe of the kinase domain. This orientation of the Cα-helix moves the Glu738 residue out of the active site, disrupting a catalytically important salt bridge in which the side chain of Glu738 interacts with Lys721 on strand β3. This interaction is also impeded sterically by a short helix which immediately follows the aspartic acid-phenylalanine-glycine (DFG) motif, which bears two leucine residues that interact with hydrophobic residues from the Cα-helix. This interaction stabilizes the inactive conformation of EGFR.\textsuperscript{23} In the active conformation of EGFR, these hydrophobic residues are exposed, which is energetically unfavorable. It is proposed that the ligand-induced dimerization of the receptor is the driving force in overcoming the free energy penalty associated with the conversion of EGFR to its active conformation.\textsuperscript{23} HER2 (ErbB2) has no known ligand and its kinase domain is always
in an extended, active conformation. As it is constitutively in an active conformation which is capable of undergoing dimerization, HER2 is the preferred dimerization partner for the other members of this family of receptors.

2.2 The inhibition of EGFR and HER2 as therapeutic targets

The inhibition of EGFR and HER2 has been the target of numerous anticancer therapies. Three of these therapies are small-molecule tyrosine kinase inhibitors which inhibit the ATP binding site of the cytoplasmic tyrosine kinase domain: gefitinib (Iressa™), erlotinib (Tarceva™), and lapatinib (Tykerb™). Four other clinically used therapies are monoclonal antibodies: trastuzumab (Herceptin™), pertuzumab (Perjeta™), cetuximab (Erbitux™), and panitumumab (Vectibix™). The most recently approved therapy is a trastuzumab conjugate known as ado-trastuzumab emtansine (Kadcyla™). These five antibody-based treatments work by inhibiting the extracellular ligand binding domains of the receptors.

2.2.1 4-Anilinoquinazolines as anti-EGFR/HER2 small-molecule tyrosine kinase inhibitors

The clinically used small-molecule tyrosine kinase inhibitors gefitinib (Iressa™), erlotinib (Tarceva™), and lapatinib (Tykerb™) (Figure 2.2) possess a common scaffold, based on the 4-anilinoquinazoline class of molecules. This 4-anilinoquinazoline class of compounds consists of a fused bicyclic ring system featuring a fused benzene and pyrimidine ring, and has additionally been functionalized at the C4 position with an aniline derivative. (Figure 2.1) The 4-anilinoquinazoline

Figure 2.1 A representative 4-anilinoquinazoline
Figure 2.2 Clinically used anti-EGFR/HER2 smTKIs bearing 4-anilinoquinazoline scaffolds

derivatives of erlotinib, gefitinib, and lapatinib are type I inhibitors, meaning that they bind to the ATP binding pocket of the receptor kinase. Type I inhibitors typically form between one and three hydrogen bonds with residues in the kinase hinge region (Figure 2.3), thereby mimicking the hydrogen bonds formed by the exocyclic amino group of adenine.\(^\text{25}\) The C6 and C7 positions of 4-anilinoquinazolines are oriented toward solvent exposed space when bound to the ATP binding site of EGFR and HER2, while the C5 position points to the ribose binding pocket and the 4-anilino head group is positioned within a pocket of hydrophobic residues at the back of the ATP binding site.

It was first reported by Wood, et al. in 2004 that lapatinib had a mechanism of action that is distinct from that of gefitinib and erlotinib.\(^\text{26}\) It was observed that the administration of lapatinib resulted in a prolonged inhibition of EGFR phosphorylation and that the dissociation rate of the EGFR-lapatinib complex was also significantly slower than the rates for the EGFR-erlotinib and EGFR-gefitinib complexes. While the half-lives of the EGFR-erlotinib and EGFR-gefitinib complexes were less than 10 min., the half-life of the EGFR-lapatinib complex was approximately 5h.\(^\text{26}\) Lapatinib was shown to have
Figure 2.3 Binding of a simple 4-anilinoquinazoline core to the EGFR tyrosine kinase domain.

a very slow off-rate from HER2 as well.\textsuperscript{26} The crystal structure of lapatinib bound to EGFR gives insight into these differences. The binding of lapatinib to the tyrosine kinase domain of EGFR induces an inactive conformation of the receptor in which the Cα-helix is pointed outward but the DFG loop is not.\textsuperscript{27} This is known as a DFG-in, Cα-helix out conformation. The Cα-helix is rotated outward approximately 45° to assume the inactive conformation.\textsuperscript{28} Thus, the extended aniline headgroup of lapatinib is able to participate in additional interactions with the hydrophobic region adjacent to the ATP pocket, as well as, with an extra hydrophobic pocket created by the outwardly displaced α-C helix.\textsuperscript{23,25,26} It is likely that lapatinib binds preferentially to the inactive structure because binding to the active conformation of EGFR would result in steric clash. These factors give rise to the enhanced resident time in EGFR observed for lapatinib, as compared with gefitinib and erlotinib, which bind to the active conformation of EGFR.

2.2.2 Challenges with the clinical use of anti-EGFR/HER2 small-molecule tyrosine kinase inhibitors

The use of EGFR- and HER2-targeted therapies has been hampered severely by the clinical difficulty of stratifying patients according to their sensitivities to the small-molecule anti-EGFR and anti-HER2 inhibitors. This inability to demonstrate a broad, general efficacy of these treatments has severely
limited the indications for which they have been approved by regulatory agencies, such as the U.S. Food and Drug Administration. In the case of gefitinib, the inability to establish a concrete patient population that responds to the treatment led to its regulatory approval being revoked, with the exception of individuals who were already responding positively to the treatment.

The current clinical standard for assessing the sensitivity of patients to anti-HER2 therapies relies on immunohistochemical staining and fluorescence in situ hybridization (FISH), while chromogenic in situ hybridization (CISH) and silver-enhanced in situ hybridization (SISH) are used with less frequency. HER2 testing has been problematic, with error rates having been reported to be as high as 20%. The accurate evaluation of HER2 status in a patient is very complex, with its accuracy being affected not only by the choice of antibody, but also by tissue fixation, fixation time, and the determination of thresholds for reporting positive results. The development of imaging agents specific for EGFR and HER2 could ameliorate the clinical difficulty of stratifying patients based on their sensitivities to anti-EGFR and anti-HER2 treatments.

2.3 Previous work toward imaging EGFR and HER2 with small-molecules using PET and SPECT
(Based on: Corcoran, E. B.; Hanson, R. N. Med. Res. Rev. 2014, 34, 596-643)

2.3.1 Reversible Small-Molecule Inhibitors

Over the past few decades, a substantial amount of effort has been dedicated to the development of an imaging agent for EGFR based on small-molecule tyrosine kinase inhibitors. These proposed imaging agents have been based on the 4-anilinoquinazoline scaffold. While no attempts have been made to radiolabel lapatinib, both gefitinib and erlotinib were radiolabeled (Figure 2.4). [11C]-gefitinib II-4 suffered from high nonspecific binding, which, coupled with the short half-life of the radioisotope, precluded the visualization of tumors in PET images. To address this, [18F]-gefitinib II-5 was prepared via a three-step radiosynthesis, but also failed to show any tumor-specific uptake. [11C]-erlotinib II-6 (Figure 2.4) was also studied using PET, and it was found that the uptake of [11C]-erlotinib II-6 correlates
well with the level of EGFR expression in cell lines of varying EGFR expression and the tumors were able to be visualized in the PET scan.

Another simple 4-anilinoquinazoline, PD 153035 **II-7** (Figure 2.5), was radiolabeled with $^{11}$C and its uptake correlated strongly with EGFR expression *in vivo* and gave high tumor/blood ratios at only 30 min. p.i. These tumor/blood ratios fell, however, to approximately 30-40% of maximal values by 1h p.i.

A derivative of PD153035, *m*-IPQ **II-9**, which features a radioiodinated aniline headgroup, was studied (Figure 2.5). **II-9** underwent rapid deiodination *in vivo*, leading to high blood radioactivity and low tumor/blood and tumor/muscle ratios of approximately unity. Despite this, $[^{125}]$-*m*-IPQ showed higher uptake in A431 (EGFR+) tumors than was observed with $[^{11}]$-PD153035. The synthesis of a third PD153035 analog **II-8**, bearing a $[^{18}]$-fluoroethoxy sidechain at C7 (Figure 2.5) was reported, but no biological data has been reported for this compound to our knowledge.
Figure 2.6 Small-molecule tyrosine kinase inhibitors with radiohalogenated sidechains

Although most of the radiohalogenated small-molecule tyrosine kinase inhibitors (smTKIs) feature the radiohalogen in the aniline headgroup, another common approach has been to radiohalogenate the C6 or C7 side chain of the radiotracer. One such radiotracer is **II-10** (Figure 2.6), which features a radioiodinated aliphatic chain at its C6 position. As one would predict, the instability of the sp<sup>3</sup> carbon-iodine bond caused high levels of deiodination in vivo. The incorporation of 125I into a benzamide side chain readily yielded more stable compounds **II-11** and **II-12** (Figure 2.6), but these radiotracers suffered from significant hepatobiliary uptake, which deterred their use in imaging studies. The corresponding para-<sup>18</sup>F-benzamide analog **II-13** was prepared by a multi-step fluoro denitration reaction, although with much difficulty and low radiochemical yields (4%, decay-corrected). Attempts to streamline this radiosynthesis failed, precluding it from evaluation in vivo.

Fernandes, et al. have explored the complexation of 99mTc to a 4-anilinoquinazoline core through the functionalization of the C6 position of the quinazoline scaffold with either a “4+1” mixed ligand chelate or a fac-tricarbonyl chelate. (Figure 2.7) In vitro studies established that the presence of a metal complex did not prohibit the cellular uptake of the inhibitors and did not impede the binding of the 4-anilinoquinazoline to EGFR. These compounds, however, were never studied by imaging studies in vivo. More polar radiometal chelates were employed by Garcia, et al. to introduce 67Ga and 111In into 4-anilinoquinazoline derivatives **II-19** and **II-20** (Figure 2.7). A DOTA-type chelate was used to incorporate 67Ga into derivative **II-20**. The resulting Ga(III) complex demonstrated thermodynamic stability in vitro, but failed to be taken up by A431 (EGFR+) cells. The authors hypothesized that the free
carboxylic acid moiety, together with the overall hydrophilicity of the molecule, may have prohibited the entrance of the radiotracer into the cell. Thus, $^{111}$In was complexed to a more hydrophobic DOTA-type chelate and, opposed to gallium, indium would coordinate all of the carboxylic acid moieties present in the chelate. The resulting $^{111}$In-labeled radiotracer II-19 was less hydrophilic than the corresponding $^{67}$Ga-labeled radiotracer II-20, but the cellular uptake remained very low.

### 2.3.2 Irreversible small-molecule inhibitors

As discussed above, the radiolabeled, reversible small-molecule inhibitors which target EGFR have often suffered from rapid in vivo metabolism, high non-specific binding, and rapid dissociation from the receptor. This resulted in poor image quality during in vivo studies employing these radiotracers. It
was suggested that irreversible small-molecule tyrosine kinase inhibitors may circumvent these issues by ensuring the binding of the radiotracer to the receptor for at least 8h. This irreversible binding results from the functionalization of the C6 position of the 4-anilinoquinazoline scaffold with a reactive group, typically a Michael acceptor. This placement of the reactive group allows for the irreversible modification of cysteine-773 in the kinase domain of the receptor, keeping the small-molecule inhibitor covalently bound to the receptor.

The earliest irreversible anti-EGFR small-molecule inhibitor to be investigated as a potential radiotracer was $[^{11}\text{C}]-\text{ML03 II-21 (Figure 2.8)}$. *In vitro* studies showed subnanomolar inhibition of EGFR autophosphorylation, but the *in vivo* performance of $[^{11}\text{C}]-\text{ML03}$ was poor. The uptake of II-21 in the kidneys and liver was four- to six-fold higher than the uptake in the tumor cells, resulting in tumor/background ratios of approximately unity. This low accumulation of the radiotracer in the tumor was attributed to the rapid clearance of the radiotracer, its low bioavailability and *in vivo* degradation to several hydrophilic metabolites. Due to these shortcomings, the tumor failed to be visualized in PET images.

It was proposed that the reactivity of the Michael acceptor moiety at the C6 position of the scaffold may contribute to the *in vivo* degradation and nonspecific binding of $[^{11}\text{C}]-\text{ML03 II-21}$. Therefore, Mishani, *et al.* prepared three $[^{11}\text{C}]-$labeled, irreversible inhibitors II-22,23,24 featuring less reactive Michael acceptor moieties. (Figure 2.9) All three of these inhibitors were subnanomolar inhibitors of EGFR autophosphorylation and low nanomolar inhibitors of cell growth. Of the three
inhibitors, \([^{11}\text{C}]-\text{ML04 II-22}\) was the most potent inhibitor. Based on the promise shown by \([^{11}\text{C}]-\text{ML04}\), the radiofluorination of the aniline headgroup of ML04 to give \([^{18}\text{F}]-\text{ML04 II-25}\) was undertaken by Dissoki, et al. in hope of overcoming the limitations encountered with short-lived radioisotopes such as \(^{11}\text{C}\). (Figure 2.9)

![Figure 2.9 Analogs of the irreversible inhibitor ML04](image)

\([^{18}\text{F}]-\text{ML04}\) demonstrated low nanomolar, irreversible inhibition of EGFR in a variety of cancer cell lines. The uptake of \(\text{II-25}\) in U87MG.wt (EGFR+) tumors peaked at 30 min. p.i., but the tumor/tissue ratios continued to improve until 3 h p.i., suggesting that the distribution of this radiotracer was limited by blood flow due to its very high binding potential. Thus, the uptake of \([^{18}\text{F}]-\text{ML04}\) is most likely a measure of blood flow rather than receptor concentration. One suggested remedy was the further deactivation of the Michael acceptor group to decelerate the interaction of the radiotracer with EGFR, thus lowering the association constant and reducing the binding potential.

Shaul, et al. developed additional irreversible inhibitor derivatives with less reactive groups at the C6 position (Figure 2.10). The chloro and methoxy amide derivatives \(\text{II-27 and II-28}\) were hypothesized to perform better \textit{in vivo} due to their less reactive nature compared with the \(\alpha, \beta\) – unsaturated amide present in ML04. The \(\alpha,\beta\)-unsaturated amide derivatives, however, were found to be more stable and more selective in their inhibition. Therefore, the \(\alpha,\beta\)-unsaturated amide class of molecules was further investigated. Optimization of properties in an effort to decrease the lipophilicity and increase the water solubility of these compounds resulted in the synthesis of morpholino-\([^{124}\text{I}]-\text{IPQA II-29}\) (Figure 2.11) by
Figure 2.10 Less reactive, irreversible inhibitors investigated to optimize pharmacokinetics

Pal, et al. Morpholino-[\textsuperscript{124}I]-IPQA II-29 demonstrated a subnanomolar \textit{in vitro} inhibition of EGFR autophosphorylation. Despite the lower lipophilicity of this compound as compared with previous similar compounds, a significant amount of the radiotracer continued to be rapidly degraded, extracted, and excreted by the liver within 60 min. p.i. These events precluded the use of II-29 for the imaging of tumors in the liver and suggested that further optimization of pharmacokinetic properties, \textit{i.e.} decreased extraction by the liver, increased renal clearance, improved plasma half-life, increased accumulation and retention in tumor tissue, remains necessary.

Figure 2.11 The structure of morpholino-[\textsuperscript{124}I]-IPQA.

Figure 2.12 \textsuperscript{18}F-click labeled smTKI by Kobus, et al.
Kobus, et al. were the first to employ “click” chemistry, i.e. a Huisgen 1,3-dipolar alkyne-azide cycloaddition, to install a radiolabel into a small-molecule EGFR imaging agent. (Figure 2.12) In vivo imaging studies showed that II-30 was retained in high levels in non-target tissues, although moderate radioactivity was taken up by the tumor. The retention of the radiotracer in non-target tissues was caused by its moderate solubility, rapid washout from blood, and low metabolic stability. The authors concluded that, although the presence of the triazole ring enhanced the solubility of the compound and decreased its lipophilicity, further modifications to the molecule, such as the introduction of a polyethylene glycol chain at the C7-position, might prove beneficial.

Figure 2.13 Radiotracers featuring a radiolabeled C7 alkyl or oligoethylene glycol chain

In 2007, Dissoki, et al. investigated this addition of a polyethylene glycol chain at the C7 position of 4-anilinoquinazoline PET radiotracers and its effect on the pharmacokinetic properties of the molecules. To study these effects, four inhibitors II-31 to II-34 were synthesized, each with an acryloyl moiety attached to the C6 position and a PEG chain of varying length attached to the C7 position of the 4-
anilinoquinazoline scaffold (Figure 2.13). As predicted, the introduction of a PEG group into the ML04 derivative II-34 caused a slight decrease in log P and substantially increased its water solubility. Similar results were not observed for the chloro derivatives II-31 – II-33, however.

The synthesis of $[^{18}F]$-F-PEG$_6$-IPQA II-35 (Figure 2.13), an acrylamide derivative featuring a $[^{18}F]$-fluoroPEG chain, was achieved by Pal, et al. Interestingly, the in vitro uptake of II-35 into cells showed that the uptake was ten-fold higher for cell lines that had the mutated L858R variant of EGFR than for cells lines with wild-type EGFR or dually mutated L858R/T790M EGFR. These results are particularly interesting when one considers that the L858R mutation is known to confer sensitivity to anti-EGFR small-molecule tyrosine kinase inhibitors and the T790M mutation confers resistance to these same inhibitors. Therefore, a radiotracer such as II-35 which binds preferentially to this particular mutated form of EGFR may allow for a noninvasive way using PET imaging to stratify patients by their sensitivity to anti-EGFR small-molecule tyrosine kinase inhibitors. $[^{18}F]$-F-PEG$_6$-IPQA II-35 showed markedly better pharmacokinetics as compared to morpholino-[$^{124}$I]-IPQA II-29, with better water solubility and increased renal clearance. The metabolism of II-35 resulted in the cleavage of the radiolabeled PEG$_6$ sidechain from the quinazoline scaffold, and the $^{18}$F-PEG$_6$ chain subsequently underwent rapid renal clearance and was unable to cross the cell membrane. Thus, despite in vivo metabolism, the only radioactivity seen in tumor cells was known to originate from intact $[^{18}F]$-F-PEG$_6$-IPQA.

Three additional 4-anilinoquinazoline derivatives were labeled with $^{11}$C, $^{124}$I, and $^{18}$F (II-36 – II-38) by Pantaleo, et. al. Despite II-36 showing strong inhibition of EGFR autophosphorylation in vitro, no significant retention in tumors was seen for any of the three derivatives and no significant difference in tumor uptake was seen between the EGFR+ and EGFR- cell lines. The authors reasoned that the lack of specific uptake of the radiotracer may be the result of the status of the target biomarker in vivo, nonselective binding, and metabolic instability.
2.4 Our hypothesis and rationale for improved small-molecule imaging agents

As discussed in the previous sections, attempts to develop small-molecule radiotracers for EGFR based on the 4-arylloquinazoline scaffold have been plagued by several shortcomings, namely rapid in vivo metabolism, quick off-rates, and high non-specific binding due to the lipophilicity of the molecules. I hypothesized that I could improve upon these attempts in several ways. First, the use of an oligoethylene glycol chain to link the radionuclide of choice to the scaffold would lower the lipophilicity of the molecule while placing the radionuclide in solvent-exposed space as not to impede binding. Second, a convergent synthetic approach would allow diverse radionuclides to be introduced into the probe via similar chemical methodology. Third, the presence of a basic N-heterocycle three methylene units from the 4-arylloquinazoline scaffold would improve binding affinity and specificity, as well as lower the lipophilicity of the molecule. Fourth, I hypothesized that the presence of an extended aniline headgroup would ameliorate the quick off-rates seen with the previous radiotracers based on reversible, small-molecule inhibitors. This “lapatinib-like” type inhibitor is able to inhibit the inactive conformation of EGFR, which extends the half-life of the binding event from <10 min. for inhibitors like gefitinib and erlotinib to a half-life of 300 min. for lapatinib.26 Fifth, a lapatinib-like inhibitor would also enable the also able to inhibit HER2, thus allowing us to develop the first small-molecule PET or SPECT radiotracer for HER2.

To address these five points, I chose to synthesize a small series of potential radiotracers. These radiotracers were based on four 4-arylloquinazoline scaffolds, two of which inhibit the active conformation of EGFR and two of which inhibit both HER2 and the inactive conformation of EGFR. These four scaffolds were to then be further derivatized by ligation to three linkers, bearing either $^{18}$F, $^{123}$I, or $^{99m}$Tc.
2.5 Original design and retrosynthetic strategy

My initial synthetic strategy toward the target series of radiotracers for EGFR and HER2 featured a series of four C7-hydroxy 4-anilinoquinazoline derivatives which were to be functionlized with a propargylated piperazine linker via either a Mitsunobu reaction or a base-mediated Williamson ether synthesis. The resultant alkynyl 4-anilinoquinazoline derivative would then be subjected to a Huisgen [3+2]-cycloaddition “click” reaction with a halogenated azide glycol linker, to yield the final radioactive compounds, as well as, the stable isotope imaging agent precursors.

![Diagram of synthetic strategy](image.png)

**Figure 2.14 Design and original retrosynthetic strategy for small-molecule radiotracers**

2.5.1 Choice of 4-anilinoquinazoline derivatives

I wished to test my hypothesis that lengthening the resident time of a 4-anilinoquinazoline radiotracer within the targeted receptor would enhance the quality and resolution of PET and SPECT images. To this aim, I chose to prepare both 4-anilinoquinazolines with simple aniline head groups,
which are known to have a shorter resident time in the receptor, and with extended aniline headgroups, which are known to have slower off-rates. I chose two simple aniline headgroups - one based on the substitution seen on gefitinib and another bearing a simple 4-fluoroaniline headgroup. Other derivatives based on these 4-anilinoquinazoline analogs have been shown to bind to and inhibit the active conformation of EGFR. For the analogs bearing an extended aniline headgroup, I chose to base one on lapatinib, while the second analog was based on the work of Waterson, et al.\textsuperscript{1} which detailed the incorporation of several extended aniline head groups into a series of alkynyl thienopyrimidines known to inhibit EGFR and HER2. The correlation between aniline headgroups and selective inhibition of EGFR or HER2 was studied (Figure 2.14). I chose to synthesize the pyridyl aniline headgroup as the corresponding derivative strongly inhibits both EGFR and HER2 and has the lowest clogP value among the potent derivatives. While selectivity for one receptor is favorable in most situations, I wanted to choose a headgroup that targeted both EGFR and HER2, the aim of this project was to explore the

![Figure 2.15 Comparison of select extended aniline headgroups by Waterson, et al.\textsuperscript{1}](image-url)
potential advantages of using an extended aniline headgroup. With this 2-picolly analog, I would be able to establish the validity of using a small-molecule radiotracer for the imaging of HER2, as well as, the ability of extended aniline headgroups to enhance the image quality for imaging EGFR by lengthening the resident time of the radiotracer in the receptor.

2.5.2 Choice of N-heterocycle-containing chain

Research by Hennequin, et al.\textsuperscript{32} showed that the presence of a basic N-heterocycle appended off the C7 position of 4-anilinoquinazoline derivatives enhanced the physicochemical properties and binding specificity of this class of compounds for another family of tyrosine kinases, the vascular endothelial growth factor receptor (VEGFR) family. The optimal placement of the N-heterocycle was three methylene units away from the quinazoline scaffold. I chose to adapt this idea of N-heterocycle incorporation for my project, to enhance the physicochemical properties of the target 4-anilinoquinazoline derivatives, as well as to lower the off-target binding of these derivatives. My original intent was to establish the chemistry using a propargyl piperazine derivative as the N-heterocycle and subsequently investigate other N-heterocycles to determine which one provided optimal properties for binding to EGFR and HER2. However, the choice of introducing a piperazinyl side chain featuring the alkynyl group necessary for the click reaction proved problematic, as will be discussed in the following sections.

2.5.3 Choice of radionuclides of interest

We were interested in synthesizing a series of potential radiotracers that could be tailored to meet a clinician’s need, and thus decided to develop radiotracers employing $^{18}\text{F}$ for PET imaging, as well as employing $^{123}\text{I}$ and $^{99m}\text{Tc}$ for SPECT imaging. The rationale for this decision was that, depending on the location and financial situation of a clinic, either the more expensive technology of PET could be used in an area with ready access to cyclotron-produced $^{18}\text{F}$ or the more affordable technology of SPECT for which longer-lived radioisotopes, such as $^{123}\text{I}$ or $^{99m}\text{Tc}$, could be delivered from a distant production site.
2.6 Synthesis of proposed 4-anilinoquinazoline derivatives

2.6.1 Synthesis of quinazoline core

The synthesis of quinazolines and 4-anilinoquinazolines is well-represented in the chemical literature. Using an adaptation of the already established synthetic procedure, commercially available methyl vanillate II-39 was protected as benzyl ether II-40, and subsequently nitrated using concentrated nitric acid and glacial acetic acid, in refluxing DCM to give II-41. Reduction of the nitro derivative to the corresponding aniline was accomplished with nickel borohydride to give II-42, and subsequent subjection to a Niementowski type quinazoline reaction using formamide and ammonium formate gave quinazolinone derivative II-43. Heating 2.5 in thionyl chloride and DMF at reflux for 4 h gave the key 4-chloroquinazoline intermediate II-44. This key intermediate was reacted with the desired simple aniline derivatives and deprotected using trifluoroacetic acid to provide the c7-hydroxy 4-anilinoquinazoline derivatives.

Figure 2.16 Synthesis of C7-hydroxy quinazoline derivatives
The introduction of extended aniline headgroups necessitated an alternate protecting group at the C7 position of the quinazoline substrate due to its incompatibility with the benzyl deprotection. We therefore investigated ways to deprotect the C7 phenol of II-44 prior to installation of the aniline headgroup. Deprotection of 4-chloroquinazoline II-44 was successful, but attempts to install the aniline headgroup in the presence of the free C7 phenol were unsuccessful, with neither the desired 4-anilinoquinazoline nor side product being observed. While attempts to protect the 4-chloro-7-hydroxy quinazoline as TBS ether II-45 were successful, no subsequent conversion to the 4-anilinoquinazoline was observed. Thus, we decided that we should revise our approach and install the side chain at C7 prior to the introduction of the aniline headgroup. As discussed later, this second approach proved successful.

Figure 2.17 Lessons learned regarding C7 functionalization

2.6.2 Difficulties with the introduction of piperazinyl side chain

My original desire was to functionalize the C7 position of the quinazoline scaffold with a basic N-heterocyclic linker that featured a terminal alkyne, which would provide a handle for further functionalization via “click” chemistry. Due to its two secondary amine moieties available for functionalization, I chose to synthesize a piperazine alkyne linker. I undertook the synthesis of a
propargylated piperazinyl linker (Figure 2.18). The propargyl piperazinyl alcohol derivative II-51, however, did not undergo a Mitsunobu reaction with free phenol II-54. Attempts to access the corresponding alkyl halide from II-51 via an Appel reaction were unsuccessful. Similarly, attempts to use an N-Boc derivative II-53 in both of these reactions also failed (Figure 2.20).
We proposed the functionalization of the C7 position first as a halogenated propyl ether II-57. To achieve this, the deprotected C7 phenol II-54 was reacted with 1-bromo-3-chloropropane in the presence of CsCO$_3$ in MeCN. The formation of two species was observed, however, upon analysis by LCMS, in which both the chloropropyl ether and the bromopentyl ether were observed. To circumvent this, I undertook the formation of the 3-chloropropyl ether via a Mitsunobu reaction employing DIAD (Figure 2.21). Although the desired compound II-57 was observed in low yield (~30%), this was not acceptable as the transformation was a key step of the synthetic sequence. Additional attempts to promote the Mitsunobu reaction by use of more reactive carbodiimide derivatives, such as 1,1’-(azdicarbonyl)-dipiperidine, failed to show any improvement.

Therefore, I re-examined the choice of the piperazinyl linker as the N-heterocycle, and decided that the placement of a triazole at a comparable position would be an adequate replacement, based on studies conducted by Hennequin, et al.$^{32}$ Subjection of the C7 phenolic 4-chloroquinazoline derivative with 5-chloro-1-pentyne under Williamson ether conditions did not yield the expected product; but rather, an undesired dialkylated derivative. We hypothesized that, due to small amounts of moisture in the reaction, pentynyl alcohol may have been formed in situ, and subsequently displaced the chlorine at the C4 position of the quinazoline scaffold. Attempts to control the side reaction by making the pentynyl chloride the limiting reagent, however, led to incomplete consumption of the phenol starting material, with only the undesired byproduct being observed.
2.7 Revised Synthetic Strategy

In light of these difficulties, I decided to take a more direct, simpler approach in which I would install the alkynyl ether, the precursor to the triazole, at the early stages of the synthetic route. Previous literature had shown that the presence of a triazole ring off the C7 position of the quinazoline scaffold was comparable to having a more basic N-heterocycle such as piperazine or piperidine at that position.\textsuperscript{32} The only downfall was that a triazole ring would lend less to the hydrophilicity of the final compounds as compared with a piperazine or piperidine derivative.\textsuperscript{32} I proposed a series of twelve final stable-isotope analogs to be synthesized (Figures 2.22, 2.23), which would contain either a stable-isotope fluoride, iodide, or \textit{fac}-Re(CO)\textsubscript{3} moiety, which would stand in lieu of \textit{fac}-Tc(CO)\textsubscript{3}, as no stable-isotope of Tc(CO)\textsubscript{3} is known.

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure2.png}
\caption{Stable isotope analogs of proposed radiotracers for the “Ca-helix in” conformation}
\end{figure}

2.7.1 Synthesis of alkynyl quinazoline derivatives

I decided to simplify my synthetic approach to a series of 4-anilinoquinazoline derivatives
Figure 2.23 Stable isotope analogs of proposed radiotracers for the “Ca-helix out” conformation

featuring an N-heterocycle at the C7 position of the scaffold. I chose to do this by synthesizing 4-anilinoquinazoline alkyne derivatives which would form, upon reaction with an azide linker, a triazole in the last step of the reaction sequence, thus providing an appropriate N-heterocycle. This would eliminate the difficulty I experienced with synthesizing piperazinyl linkers derivatives of the desired length and functionality. The synthesis of these derivatives, as outlined in Figure 2.24, began with a Williamson ether synthesis of II-39 with 5-chloropent-1-yn in the presence of Cs₂CO₃ to provide the alkynyl vanillate derivative II-70 in good yield. This alkyne derivative was incompatible with the strongly acidic nitration conditions (conc. HNO₃, glacial AcOH) that had been used in the previously outlined synthesis, and thus milder reaction conditions including SnCl₄ and fuming HNO₃ were used to obtain II-71 in good yield. Reaction conditions were also modified for the reduction of nitro derivative II-71 to the corresponding aniline derivative II-72. As the presence of hydride would introduce the competing deprotonation of the terminal alkyne, and the presence of acid was known to cause degradation, a milder set of reaction conditions was selected. Subjection of II-71 to Na₂S₂O₄ in (1:1) MeOH/H₂O gave the
desired aniline II-72 with no degradation of the alkyne seen. A Niementowski-type quinazoline formation reaction was performed on aniline II-72, in which the starting aniline and ammonium formate were refluxed in DMF. The quinazolinone product II-73 was obtained after crystallization from water. The chlorination of the quinazolinone scaffold proved problematic due to the presence of a terminal alkyne. Treatment of II-73 with the traditional reaction conditions of thionyl chloride in DMF caused severe degradation of the starting material, most likely due to the generation of stoichiometric amounts of HCl over the course of the reaction. Based on this reasoning, chlorination through the use of similar reagents, such as POCl₃ was not investigated. Instead, I employed Appel reaction conditions (PPh₃, CCl₄)

![Chemical structures](image)

**Figure 2.24 Revised synthesis of 4-anilinoquinazoline derivatives**

to furnish the key 4-chloroquinazoline intermediate II-74. From this key intermediate, the four target 4-anilinoquinazoline derivatives were easily obtained through heating of II-74 with the corresponding
aniline derivatives \textbf{II-75} to \textbf{II-78} in 2-propanol for 4-20 h, to give the desired 4-anilinoquinazoline derivatives \textbf{II-79} to \textbf{II-82}, which precipitated from the reaction solution upon their formation, allowing for easy purification. Thus, by employing mild reaction conditions, I was able to obtain the four desired quinazoline derivatives without much difficulty, owing to the early-stage alkylation of the C7 phenol.

\textbf{2.7.2 Synthesis of the iodinated stable-isotope analogs}

To incorporate an iodide into the desired imaging agents, I chose to employ an \textit{m}-iodobenzamide synthon. This was selected instead of a vinyl iodinated moiety due to the enhanced \textit{in vivo} stability of the aromatic carbon-iodine bond as compared to the sp\textsuperscript{2} vinylic carbon-iodine bond. To install a radioiodine into an aromatic ring, one can use three different methods: 1) direct electrophilic iodination, which can be directed with good regioselectivity by neighboring group participation; 2) displacement of a halogen or pseudohalogen with electrophilic radioiodine; or 3) displacement of a boronate or stannane with electrophilic iodine. As I hypothesized that the first two methods would not provide the desired radioiodinated linker in consistently high specific activities and regioselectivities, I chose to employ the latter method for the

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.25.png}
\caption{Synthesis of stable isotope iodine azide linker}
\end{figure}

synthesis of the \textit{m}-iodobenzamide tetraethyleneglycol azide linker \textbf{II-86}. The amino azide tetraethylene glycol derivative \textbf{II-84} was reacted with \textit{m}-tributylstannane benzoyl NHS ester \textbf{II-83} to give the \textit{m}-tributylstannane benzamidyl tetraethylene glycol linker \textbf{II-85} in 71\% yield. The tributylstannyl linker \textbf{II}-
was then subjected to electrophilic iodination conditions using N-iodosuccinimide in dry THF to give the final iodinated product III-86 in 81% yield after only 15 min. at room temperature.

The $m$-iodobenzamide derivative III-86 was then reacted with the alkynyl quinazoline derivatives III-79 to III-82 in a Cu(I)-catalyzed Huisgen [3+2] cycloaddition to yield the desired stable-isotope iodinated derivatives III-60, III-61, III-66, and III-67.

2.7.3 Synthesis fluorinated stable-isotope analogs of imaging agents

To incorporate fluorine into my target compounds, I synthesized a triethylene glycol linker III-89 bearing both a fluorine and a terminal azide. The commercially available ditosyl triethylene glycol III-87 was reacted with NaN$_3$ to yield the mono azide-substituted triethyleneglycol monotosyl derivative III-88. A nucleophilic displacement using TBAF was then accomplished to give the fluoro azido glycol derivative III-89. In contrast to those linkers seen previously bearing iodine or rhenium, the fluoro linker

Figure 2.26 Synthesis of the fluorinated 4-anilinoquinazoline imaging agents

is a triethylene glycol, rather than a tetraethylene glycol, derivative. I chose the triethylene glycol for the fluoride linker based on the fact that my collaborators at Massachusetts General Hospital have an already established protocol for the automated synthesis of $[^{18}\text{F}]-$III-89. To be consistent with this established
protocol, I synthesized the triethylene glycol fluoro derivative, instead of the tetaethylene glycol fluoro derivative.

### 2.7.4 Synthesis of rhenium analogs as stable-isotope analogs of technetium compounds

As no stable isotope of technetium exists, it is common practice to utilize the corresponding rhenium compounds as surrogates for technetium in the characterization and *in vitro* evaluation of technetium-containing compounds. There are several precursors which are used for the incorporation of $[\text{Re} \ (\text{CO})_3]^+$ into molecules, including the ammonium salt $[\text{NEt}_4]_2\text{Re(CO)}_3\text{Br}_3$, which is the most commonly used precursor. I wished to avoid using this salt as it forms NEt$_4$Br as a byproduct, which is often removed either by repeated washing with ethanol, or by crystallization of the product. Seeing as the solubility properties of my target compounds might complicate purification, I chose to use an alternative rhenium precursor (Figure 2.27). Lazarova, *et al.* reported the direct synthesis of $[\text{Re(CO)}_3(\text{H}_2\text{O})_3]\text{Br}$ upon heating $\text{Re(CO)}_5\text{Br}$ at reflux in water for 24h.$^{33}$ I, however, observed that $\text{Re(CO)}_5\text{Br}$ failed to dissolve readily in the refluxing water, and thus chose to investigate another means of obtaining $\text{fac-}[\text{Re(CO)}_3(\text{H}_2\text{O})_3]^+$. I ultimately chose to first convert $\text{Re(CO)}_5\text{Br}$ to $\text{Re(CO)}_5\text{OTf}$, as performed by He, *et al.*$^{34}$ The substitution of bromide by triflate allows for the easier displacement of the CO ligands with water due to the non-coordinating nature of the triflate anion. Recently, this transformation of $\text{Re(CO)}_5\text{Br}$ to $[\text{Re(CO)}_3(\text{H}_2\text{O})_3]\text{OTf}$ has been accomplished in a one-pot synthesis by Kunz, *et al.*$^{35}$ In this procedure $\text{Re(CO)}_5\text{Br}$ was refluxed together with AgOTf in methanol. The byproduct AgBr was filtered off, and the addition of the desired ligand and further refluxing in methanol provided the desired product in a streamlined fashion.$^{35}$

I chose to use a tridentate chelate, as tridentate chelates for Re/Tc were shown by Schibli, *et al.* to be more stable *in vivo* than bidentate chelates for Re/Tc.$^{36}$ This study showed that $^{99m}$Tc-complexes with bidentate ligands became almost entirely protein bound after 1h and were retained in high levels.
within the liver, kidneys, and blood pool. The $^{99m}$Tc-complexes with tridentate ligands, however, demonstrated no binding with serum proteins and cleared quickly from all organs and tissues. Thus, tridentate chelates are preferred over bidentate chelates for labeling molecules with $^{99m}$Tc. I chose the 2,2'-dipicolyl amine chelate II-91 specifically due to its high affinity for binding $[\text{Re(CO)}_3]^+$ and $[^{99m}\text{Tc(CO)}_3]^+$ and its positive charge, which will reduce its lipophilicity in vivo. In fact, Banerjee, et al. found that pyridyl nitrogen was the preferred coordinating ligand for $[\text{Re(CO)}_3]^+$ and $[^{99m}\text{Tc(CO)}_3]^+$ and established the preference of coordination as pyridyl nitrogen > carboxylate > halide > thiophene sulfur.

I conducted preliminary rhenium-labeling studies in which I optimized conditions for the introduction of rhenium (I) tricarbonyl into the simplified model system of the 2,2'-dipicolyl amine
chelate linker II-91. To make the desired 2,2’-dipicolyl amine azide linker II-91, the amino azide tetraethylene glycol II-84 underwent reductive amination with 2-picolylcarboxaldehyde II-90, in the present of NaBH(OAc)₃ in 1,2-DCE, at room temperature, overnight. The resulting 2,2’-dipicolyl amine ligand II-91 was coordinated to fac-[Re(CO)$_3$]$^+$ by reacting with an 0.028 M aqueous solution of fac[Re(CO)$_3$(H$_2$O)$_3$]OTf in H$_2$O, 1h, at room temperature. $^1$H NMR (in CDCl$_3$) showed that the characteristic peaks of the methylene groups α to the amine had changed from a singlet at 3.89 ppm to two doublets ($J = 16$ Hz) at δ = 4.61(2H) and 5.40 (2H). This suggests the chelation of Re(CO)$_3$, as the geminal hydrogens on each methylene carbon become diastereotopic upon chelation of rhenium. Low resolution MS confirmed the desired mass. Similar results were obtained when (1:1) H$_2$O/MeOH was used as the solvent and the reaction was stirred overnight, although after 1 h at room temperature, only 50% chelation was observed by $^1$H NMR for the (1:1) H$_2$O/MeOH solvent system.

Having optimized the chelation chemistry on the simplified chelate, I proceeded to synthesize the final 4-anilinoquinazoline ligand derivatives II-93 to II-96 (Figure 2.29). Due to the proclivity of the 2,2’-dipicolylamine moiety to chelate copper, it was necessary to use greater than stoichiometric amounts of [Cu(MeCN)$_4$]PF$_6$ in order to obtain the desired click products. Failure to wash the crude reaction mixture with NaEDTA resulted in the loss of the desired product on the silica column. This was remedied by the washing of the crude reaction mixture with a saturated, aqueous solution of NaEDTA. The crude reaction mixture was then purified by silica chromatography and the desired product eluted with (1:9) methanol/chloroform to give isolated yields of 28-42%.

Due to the insolubility of these final click ligands in water, the conditions which I had previously employed to chelate fac-[Re(CO)$_3$]$^+$ into the 2,2’-dipicolylamine linker were not optimal. The chelation took several days at room temperature. Changing the solvent system to (1:1) H$_2$O/MeOH did little to speed up the reaction, with the reaction still requiring several days at room temperature. Use of only methanol as the solvent allowed for the solvation of the ligand in the reaction medium and sped up the reaction significantly. After 15 h at room temperature, significant chelation of rhenium by the 4-anilinoquinazoline ligand was seen. The crude reaction mixture was purified by silica chromatography,
and the desired product eluted with (1:9) MeOH/DCM. The presence of unchelated ligand was seen by $^1$H NMR. The unchelated ligand was removed upon the partitioning of the impure compound between EtOAc and 0.5 M aqueous CuSO$_4$ to yield what was believed to be rhenium-labeled II-63. The $^1$H NMR spectrum showed splitting of the methylene protons α to the amine had into two doublets ($J = 17$ Hz) at $\delta = 4.46$ ppm, 4.941 ppm. Subsequent attempts to reproduce these results on a larger scale yielded multiple new species, as seen by TLC. Upon isolation of each species by silica chromatography, each showed the presence of diastereotopic methylene protons, suggesting that each new species contained the fac-Re(CO)$_3$ species. Similar results had been obtained by Benny, et al. in which chelation of fac-Re(CO)$_3$ following the click ligation led to the generation of two Re-chelated species which demonstrated different
retention times on HPLC, yet had the same mass. They did not characterize these two species further; but rather, adopted a “chelate then click” approach in which the 2,2’-dipicolylamine moiety was chelated to fac-Re(CO)₃ and then subjected to a copper-catalyzed click reaction. This approach yielded only one Re-chelated species in the work of Benny, et al. We chose to pursue a “chelate then click” approach, and first chelated fac-Re(CO)₃ to the 2,2’-dipicolylamine tetraethylene glycol azide linker II-91. This initial chelation appeared successful, as assessed by ¹H NMR, and we attempted to use the previously optimized “click” conditions for the unchelated II-91 to the 4-anilinoquinazoline alkyne derivatives. The previous reaction conditions of [Cu(MeCN)₄]PF₆ in (1:1) THF/H₂O did not promote the cycloaddition, and use of an added reducing agent such as BPDS (bathophenanthroline disulfonic acid, disodium salt hydrate) was unable to promote the progression of the reaction. The optimization of these final transformations is still ongoing.

2.8 Summary and future directions

This project has focused on the design and synthesis of stable-isotope analogs of potential PET and SPECT imaging agents which target the EGFR and HER2 proteins. I successfully synthesized four 4-anilinoquinazoline scaffolds bearing a pentynyloxy side chain, two of which featured simple aniline head groups for the inhibition of the active site of EGFR and two of which featured extended aniline head groups for the inhibition of HER2, as well as, the inactive conformation of EGFR. These four 4-anilinoquinazoline scaffolds were then subsequently functionalized with stable-isotope analogs of common PET and SPECT radionuclides, utilizing “click” chemistry conditions.

The final four fac-Re(CO)₃ chelated imaging agent analogs remain to be synthesized. The “click” reaction of the prechelated fac-Re(CO)₃ azide derivative with four 4-anilinoquinazoline alkyne derivatives is currently not optimized. Once these remaining four analogs are synthesized, the resulting twelve potential imaging agents remain to be tested in vitro and in vivo for their ability to selectively bind
EGFR and/or HER2, as well as to inhibit the phosphorylation of the receptors. We expect these further studies to support our hypothesis that the tethering of an imaging group to the C7 position of the 4-anilinoquinazoline scaffolds via an oligoethylene glycol chain will not adversely affect the binding and potency, as compared to those of the parent 4-anilinoquinazolines. Provided that this initial biological evaluation shows the promising activities of these derivatives as inhibitors of EGFR and/or HER2, there would be interest in translating this chemistry into radiochemical conditions and evaluating their performance as PET and SPECT imaging agents in murine xenograft models.

These twelve analogs represent the first investigation into using a small-molecule inhibitor to image the HER2 protein. Additionally, they also represent the initial attempt to use a small-molecule inhibitor specifically targeting the inactive conformation of EGFR for the purpose of imaging this receptor. Were they to demonstrate promising biological activities, these twelve analogs would constitute an interesting new approach toward the imaging of this very integral family of receptor tyrosine kinases.
Chapter 3

Development of radiofluorinated estrogen derivative for imaging the estrogen receptor

3.1 The biological importance of estrogen receptors

3.1.1 Role in normal cells

Estrogen receptors (ER) α and β (MW = 66kDa) are members of the nuclear receptor (NR) superfamily of proteins and members of the class I steroid receptor family. Estrogens, the general class of ligand bound by these receptors, play myriad roles in human physiology. These roles include the development and maintenance of the cardiovascular, musculoskeletal, immune, and central nervous systems, as well as, of sexual and reproductive function.\textsuperscript{39}

Estrogens play a pivotal role in initiating morphogenesis, but they depend upon growth factors and their receptors for communication between the epithelium and the mesenchyme. These growth factors include the epidermal growth factor (EGF) pathway, the FGF receptor pathway, the transforming growth factor beta family of peptides (BMPs), and the Notch signaling pathway.\textsuperscript{39} A decrease in estrogen levels has been linked to conditions such as osteoporosis, atherosclerosis, and Alzheimer’s disease. The most potent estrogenic ligand produced by the human body is 17β-estradiol (ES) \textbf{III-1 (Figure 3.1)}. ES \textbf{III-1} evokes a strong proliferative response and also causes very rapid, non-genomic effects such as the activation of kinases and phosphatases, including MAPK, PI3K, endothelial nitrogen oxide synthase (eNOS), HER2, caveolin 1, EGFR, insulin-like growth factor 1 receptor (IGFIR), SRC, and G proteins.\textsuperscript{40}

\textbf{Figure 3.1} Structure of 17β-estradiol (ES)
3.1.2 Structural features of ERα and ERβ

The two estrogen receptors ERα and ERβ share significant homology, although ERα and ERβ are products of separate genes (ESR1 and ESR2, respectively) located on different chromosomes. Both of these receptors bind to the same DNA response elements and have similar affinities for 17β-estradiol. ERα is expressed in major female organs (e.g., ovary, uterus, mammary glands, and vagina) and certain areas of the central nervous system. Experimental evidence suggests that the overall proliferative response to estradiol (ES) is the result of a balance between ERα and ERβ signaling.

Figure 3.2 Schematic diagram of the human estrogen receptors (ERs), denoting the six functional domains present in both ERα and ERβ.

Both ERα and ERβ have six functional domains, as shown in Figure 3.2. The most conserved domain is the DNA-binding domain (DBD), shown as the “C domain” in Figure 3.2. It is responsible for the ability of the receptor to recognize and bind DNA. The DBD houses two type-II zinc fingers, each of which features a zinc atom coordinated by four conserved cysteine residues in a tetrahedral geometry. This DNA-binding domain is also responsible for supplying the weak dimerization properties of the receptor. The ligand-binding domain (LBD), shown as the “E domain” in Figure 3.2, is located at the C-terminus of the receptor. The LBD consists of twelve α-helices and two short β-helices arranged in an α-helical sandwich secondary structural motif. The ligand binding domains of ERα and ERβ share a medium degree of homology (Figure 3.2), which accounts for their specificity in binding ligands.
ligand binding site of the estrogen receptors is quite accommodating, being able to bind substrates much larger than ES III-1. The ability of the receptors to bind a diverse array of compounds allows for environmental contaminants, such as polycyclic aromatic hydrocarbons, phthalates, pesticides, xenoestrogens, and phytoestrogens, to interfere with endocrine signaling in estrogen-responsive tissues. The binding of certain contaminants is thought to alter reproductive capacity and cause breast and endometrial cancers. The LBD is also home to the ligand-dependent activation function 2 (AF-2), which plays an important role in the transcriptional activity of the receptor. The N-terminal domain, denoted as the “A/B domain” in Figure 3.2, is the least conserved of the six functional domains, being highly varied in sequence and length. This domain contains a constitutively active activation function, known as activation function 1 (AF-1). The interaction of AF-1 with AF-2 causes the receptor to attain its maximal transcriptional activity and recruit a range of coregulatory protein complexes to the DNA-bound estrogen receptor. AF-1 activates target genes by interacting with components of the core transcription machinery. The low homology between the AF-1 regions of ERα and ERβ gives rise to subtype specificity for coactivator/corepressor recruitment and accounts for the functional differences observed between these ER subtypes.

3.1.3 Role of ERs in normal cells

In the absence of ligands, ERα is present in its monomeric form, often complexed with heat shock proteins (HSPs) and is distributed between the nucleus and the cytoplasm of the cell. Upon ligand binding, ER dissociates from HSPs, dimerizes, and relocates to the nucleus of the cell where it binds to DNA at sequence-specific response elements known as estrogen response elements (EREs) and recruits coactivator or corepressor multiprotein complexes. The identity of the recruited coactivators and corepressors is determined by the shape of the ligand-receptor complex. The nature of the coactivators and corepressors influences the activity of the ERs, ultimately activating or repressing gene transcription.

Ligand-bound estrogen receptors can also interact with other transcription factor complexes like Fos/Jun or SP-1 and thus influence transcription of genes whose promoters do not harbor EREs.
the initiation of transcription, post-translational modifications such as methylation and acetylation occur causing the dissociation of the complex. Simultaneously, the ERs are ubiquitinated, leading either to the further activation of the ERs or to their degradation. ERs are also known to elicit transcriptional responses in the absence of ligand due to their phosphorylation by protein kinase cascades, which are stimulated by hyperactive growth factor receptors, such as EGFR and IGFIR.  

3.1.4 The role of ERα in cancers

The alteration of ER expression is an important step in the development and progression of hormone-related cancers and also influences response to therapy. Several variables are altered during cancer transformation: 1) the combination of transcription factors bound to the chromatin regulatory sites of a gene, 2) the ratio and cellular localization of ERα and ERβ, 3) the expression levels of various co-regulatory proteins and signal transduction components, and 4) the nature of extracellular stimuli. These four variables also vary across different cancer cells. An ERα-mediated increase in proliferation has been correlated with inflammation and tumor development and, therefore, it has become routine diagnostic practice to check the level of ERα expression in a tumor. If ERα is highly expressed, the patient is prescribed anti-estrogen therapies, such as tamoxifen.

ERα has been shown to influence mammary carcinogenesis, as well as, prostate tumorigenesis. It appears likely that role of ERα in tumorigenesis stems from its promotion of increased cell division, which results in the accumulation of random, occasionally carcinogenic, DNA mutations. Specifically, ERα promotes cell cycle progression and induces G1/S cell cycle transition, by causing the increased expression of MYC and cyclin D1, respectively. As previously mentioned, ERα transcriptional activity can also be enhanced by kinase-mediated phosphorylation, thus inducing the proliferation of breast cancer cells. ERα protein expression correlates with low tumor grade and negative lymph node status. These ER+ tumors are usually less invasive and have a more favorable prognosis than ER- tumors and respond well to tamoxifen and other antiestrogen treatments. When ERα is expressed it mediates the adverse
effects of estrogens in most types of cancers. This forms the basis for the therapeutic use of antiestrogens to specifically target and block ERα signaling for the prevention and treatment of cancer.

### 3.1.4 Antiestrogens as a therapeutic approach to ER+ cancers

The ability of ERs to bind diverse substrates permits the binding of both agonists and antagonists. The binding of agonists and antagonists induces distinct conformations of the receptor which differ in the positioning of an important helix, known as helix H-12, in the AF-2 portion of the N-terminus. The positioning of helix H-12 determines the ability of the receptor to bind coactivators or corepressors. Upon the binding of an agonist in the ligand-binding domain, a major conformational change occurs in AF-2, in which helix H-12 is folded back over the ligand binding pocket, forming a shallow, hydrophobic binding site for consensus LXXLL motifs of coactivator proteins. The binding of an antagonist, however, prevents the positioning of H-12 over the ligand binding domain and instead reorients helix H-12 along the AF-2 groove, occluding AF-2.³⁹ This disrupts the interface between AF-1 and AF-2 which is responsible for transcriptional activity and renders the interaction unproductive. It is thought that helix H-12 blocks the coactivator interaction groove by mimicking the LXXLL motifs of the coactivator proteins with its own intrinsic related sequence. Other research suggests that H12 contains an extended corepressor box sequence that occludes the AF-2 site and prevents unwanted interaction with traditional nuclear receptor corepressors.³⁹

Antagonists of the estrogen receptors, also known as antiestrogens (AES), have been utilized as means of treating estrogen-dependent cancers. While tamoxifen is the most widely used antiestrogen for treatment of ER+ cancers, there are several other antiestrogens (III-2 to III-5) which have been investigated, some of which have been based upon the ES III-1 scaffold (Figure 3.3). A common feature of these steroidal antiestrogens is the presence of a long chain at either the 7α or 11β position. While at first examination it would appear that the 7α-substituted estradiol derivatives would bind differently than the 11β-substituted estradiol derivatives due to the different placement of the sidechains, that is not the case. The estradiol derivatives with a 7α chain rotate 180° around the C3 hydroxyl and C17 hydroxyl axis...
of the molecule, positioning the 7α chain in the channel occupied by 11β substituents. Due to the placement of hydroxyl moieties at either end of the molecule, this rotation of the antiestrogen leaves its characteristic hydrogen bonds with the receptor binding pocket essentially unchanged.\textsuperscript{41,42} Not every estradiol derivatives featuring a substituent at the 7α or 11β positions are antagonists, however. Zhang, \textit{et al.}\textsuperscript{43} found that the transformation of an estradiol derivative from an agonist to an antagonist occurs when the substituent at either the 7α or 11β position is lengthened to five non-hydrogen atoms. ES derivatives that comply with this “n ≥ 5 rule” act either as partial antagonists or pure antagonists of ER.

3.2 \textbf{Previous work in the development of radiotracers targeting ER}

An extensive body of literature exists on the radiolabeling of ES derivatives in search of a small-molecule radiotracer capable of imaging ER expression in tumors. Radiolabeled ES \textbf{III-1} derivatives have suffered from low ligand affinity for the ER and high non-specific binding. Many of the previous radiotracers were plagued by low specific activity due to the lack of methods for introducing high specific
activity radiohalogens. The *in vivo* instability of these radiotracers continues to complicate the development of a useful radiotracer for imaging ER.

![Chemical structures](image)

**Figure 3.4** 11β-substituted radiohalogenated estrogen derivatives

16α-[77Br]Bromo-11β-methoxyestradiol derivative **III-8** (Figure 3.4) was prepared by Katzenellenbogen, *et al.*[^44] In rats, **III-8** showed a more selective uptake and longer retention in target tissues than did desmethoxy derivative **III-6**. While the introduction of bromine at the 16α position enhanced the affinity of the compound for ER, the 11β-OMe group decreased receptor affinity significantly. However, **III-8** had significantly lower nonspecific binding than did **III-6** and ES **III-1**[^44].

For the radioiodinated derivatives, however, Hanson, *et al.* observed that the introduction of the 17α-vinyliodo moiety (**III-10, III-11**) enhanced the retention of the estradiol derivative in the uterus. The introduction of a methoxy group (**III-11**) at the 11β position of the steroid scaffold was also advantageous, leading to a substantial increase in uterine uptake of the tracer, as well as a significant enhancement of the uterus to blood ratio.[^45,^46]

The uptake of 16α-[18F]fluoro-17β-estradiol **III-12** in tumor, as measured by PET imaging, was shown to correlate strongly with the concentration in ER in tumor.[^47] [18F]-FES **III-12** has been used in the clinic to assess the effect of antiestrogen therapy in patients by permitting the visualization of the shrinkage and disappearance of estrogen-dependent tumors. Van Brocklin, *et al.* prepared other 16α-[18F]fluoro-17β-estradiol derivatives with the aim of improving the uptake characteristics of 16α-[18F]fluoro-17β-estradiol (FES) **III-12**, which was limited by blood flow and the tissue permeability.
characteristics of ER-rich tumors. This suggests that the uptake of FES may not directly reflect its binding affinity in these tissues nor its potential for selective uptake by tissues and tumors that are less ER-rich. The 16β-fluoro epimers have a binding affinity approximately 4.5 times higher than that of the 16α-fluoro epimers. Of these derivatives, the compound 16β-fluoromoxestrol demonstrated the most favorable target tissue uptake efficiencies and selectivity. The uptake of was nearly twice that of 16α-fluoroestradiol, which may be due to its reduced rate of metabolism. also demonstrated very selective uptake by tissues with relatively low concentrations of ER, e.g. kidney, thymus, and muscle.

![Figure 3.5](Image)

Figure 3.5 $^{18}$F-fluoroestradiol derivatives

The introduction of lengthy hydrocarbon chains at the 7α position of ES was investigated by DaSilva, et al. (Figure 3.6) This was based on the work of Bucourt, et al. in which a long spacer chain at the 7α position did not hinder the selective binding of the ES to the estrogen receptors. DaSilva, et al. conjectured that an alkyl spacer chain would allow installation of radionuclides into estradiol without
negatively impacting binding. The lipophilicity and bulk of the side chains decreased the binding affinity, resulting in substantial off-target, non-specific binding.  

\[
\begin{align*}
\text{III-16} & : M = 99\text{mTc}; n = 4 \\
\text{III-17} & : M = 99\text{mTc}; n = 6 \\
\text{III-18} & : M = 99\text{mTc}; n = 8
\end{align*}
\]

**Figure 3.7** 16α-substituted 99mTc-chelated estradiol derivatives  

16α-substituted 99mTc-chelated ES derivatives III-16 to III-18 synthesized by Huang, et al. (Figure 3.7) showed relatively high binding affinity to the ER in vitro, with preferential binding to ERα being observed. The hexyl chain demonstrated the highest affinity for ERα and thus was chosen for radiolabeling with 99mTc and in vivo studies.  

\[
\begin{align*}
\text{III-19}
\end{align*}
\]

**Figure 3.8** 17α-substituted 99mTc-chelated estradiol derivative  

Nayak, et al. synthesized a neutral tridentate 99mTc(I)-estradiol-pyridin-2-yl hydrazine derivative III-19 and evaluated its ability to image ER (Figure 3.8). In normal mice, this derivative showed significant uptake in ER-expressing organs, with tissue/muscle ratios of approximately 10:1. The tissue/blood ratio, however, was unity, and high liver uptake (>7 % ID/g) was seen even at 24h p.i.. This is likely due to the high lipophilicity of the steroidal compound. The tissue/muscle and tissue/blood ratios
in murine MCF-7 xenograft models were slightly lower, ranging from 3.5 to 7 and 0.3 to 1.0, respectively, over the first 24 h p.i. High uptake was seen in the liver and intestines (>80 % ID/g, combined), while uptake in tumor was less than 0.1 % ID/g.\textsuperscript{52}

### 3.3 Rationale for an 11β-aryl substituted radiofluorinated estradiol derivative

As previously discussed, the primary shortcomings of steroid-based radiotracers have been their lipophilicities and rapid metabolism. We chose to base our estradiol-based radiotracer on the antagonist RU 39411 \textit{III-20 (Figure 3.9)}. 11β-amidoalkoxyphenyl estradiols have been shown to have a high binding affinity for the ER. RU 39411, specifically is known to be a partial antagonist of ER\textsuperscript{53} and thus would minimize the agonist effects possible with other radiotracers that have been based on ER agonists. Additionally, the amine sidechain of \textit{III-20} would provide a functional handle for further elaboration of the side chain of the radiotracer. We proposed that the introduction of a polar, oligoethylene glycol (OEG) chain at the 11β position would result in a radiotracer that would have a significantly lower logP value and increased metabolic stability. As the ER binding pocket is not tolerant of polar substituents, it is important that the introduction of polar groups occur at either the C7α or C11β position as they can be oriented toward the solvent-exposed channel of the estrogen receptor. The functionalization of C7α is limited in the variety of substituents which can be appended. The C11β position, however, is more conducive to the introduction of varied substituents, although requiring a longer, more challenging

![Figure 3.9 Structure of antiestrogen RU39411](image-url)
synthetic route. Thus, based on our desire to append a long polar side chain at one of these sites, the 11β position was deemed a more appropriate choice. My role in this ongoing project in our lab has been two-fold: 1) the optimization of the synthetic route to the desired radiotracer III-21 (Figure 3.10) and 2) the preliminary radiochemistry and biological evaluation of the fluorinated antiestrogen that was previously prepared by a former group member, Lenny Dao.

![III-21](image)

**Figure 3.10** Proposed $[^{18}F]$-fluoroantiestrogen (FAES)

### 3.4 Synthesis of the target stable isotope 11β-fluorinated antiestrogen analog

Much work has been conducted toward the efficient synthesis of 11β-substituted steroid derivatives. Two general routes to these compounds have been 1) through the functionalization of an 11-

![Alkylation of keto steroid](image)

![Conjugate addition to allylic epoxide](image)

**Figure 3.11** Two select routes for the preparation of 11β-substituted steroids$^{54}$
The use of an 11-keto steroid as the key intermediate toward the synthesis of 11β-functionalized steroidal derivatives has numerous challenges, mainly due to the instability of several intermediates and substantial byproduct formation from competing side reactions. The synthetic strategy employing conjugate addition to a 5α,10α allylic epoxide has proven to be a more convenient route to obtaining 11β-substituted steroidal derivatives. The 5α,10α allylic epoxide can be synthesized with high regioselectivity and moderate to high stereoselectivity depending on the oxidation conditions employed (Figure 3.12). Formation of the 5,10-allyl epoxides is preferred under most oxidation conditions, with the exception of the use of H$_2$O$_2$ and benzonitrile. Under these reaction conditions, the 9α,11α-epoxide is formed exclusively. Diastereoselectivity of the 5,10-epoxidation is low, with ratios often moderately favoring the α-diastereomer. The most diastereoselective epoxidations reported have been obtained using Fe(II)-phthalocyanines with iodosobenzene, in which the 5α,10α-epoxide was obtained with an α:β ratio of 11:1. We chose to use a more common method employing hexafluoroacetone and hydrogen peroxide for the epoxidation of the estradiene derivative.

The choice of organometallic reagent determines the regioselectivity of the functionalization of the allylic epoxide moiety. The use of lithium aluminates or Grignard reagents leads to functionalization of the 10β position of the steroid (Figure 3.13). Lithium aurates are able to promote 11β substitution, but
a significant amount of the diene byproduct is obtained. Selective 11β-substitution is promoted by organocuprates, as well as, Cu(I)-catalyzed Grignard additions, with no 10β-substitution being observed under these reaction conditions. Due to the limited stability of preformed organocuprates, we chose to employ a Cu(I)-catalyzed Grignard reaction to introduce the desired aryl group at the 11β position.

Figure 3.13 Regioselective control via choice of organometallic alkylation reagent

Figure 3.14 Synthesis of estradiene derivative
Previous work conducted in our lab involving the synthesis of 11β-substituted estrogen derivatives involved a linear synthetic approach to install elaborate side chains at the 11β position. This linear approach imparted flexibility in the choice of substituents to append at the 11β position. As we had chosen to base our potential radiotracer on the antiestrogen RU39411 III-20, we devised a more convergent synthetic route in which a dimethylaminoethoxyphenyl Grignard reagent III-24 was utilized (Figure 3.14). While this shortened the synthetic route by several steps, it also gave rise to several new challenges.

The commercially available ketal protected diene steroid derivative III-22 was reacted with 50% hydrogen peroxide in the presence of hexafluoroacetone trihydrate, pyridine, and dichloromethane. This gave the desired α-epoxide derivative III-23 in a 60% yield, with a diastereoselectivity of 3:1 (α:β). The α-epoxide was then subjected to a conjugate addition of the para-dimethylaminoethoxy phenyl magnesium bromide III-24 using catalytic CuI.

The formation of such Grignard reagents has been widely reported to be problematic in the literature. Our group had traditionally used iodine as the initiator of magnesium for the formation of aryl Grignard reagents, which had worked well for other aryl Grignard reagents. The initial, unoptimized synthesis of the fluorinated antiestrogen accomplished by a former colleague, Lenny Dao, also employed this mode of Mg activation. However, this method of Grignard reagent formation was not reliably reproducible and required heating at reflux for extended periods of time. This irreproducibility led me to investigate other methods for the activation of magnesium. Grinding and clipping of the magnesium turnings to expose the Mg(0) surface failed to initiate any formation of the Grignard reagent, with full recovery of the aryl bromide being achieved following chromatography. The use of catalytic 1,2-dibromoethane, often used to activate magnesium by the entrainment method, proved equally unsuccessful even after prolonged heating at reflux.

Reports of the use of DIBALH to activate magnesium to form similar Grignard reagents at 20 °C on a process scale led me to investigate its use as the activator in this reaction. The initiation of Grignard
reagent formation was achieved at room temperature through the stirring of magnesium turnings with catalytic DIBALH and a small portion of ArBr (1 mol% and 5 mol%, respectively, with respect to magnesium turnings) for 30 min. The remaining portion of ArBr was added slowly, and complete consumption of ArBr was observed by $^1$H NMR after one hour. This activation method using DIBALH proved to be significantly more reproducible in our hands than the method employing catalytic iodine. In

![Chemical diagram](image)

**Figure 3.15 Optimization of the Grignard reagent formation**

contrast to the previously used procedure which required molecular iodine as an activator and several hours under reflux to form the desired Grignard reagent, the use of 1 mol % DIBALH consistently gave full conversion of the aryl bromide to the corresponding Grignard reagent within 60-90 min. at room temperature.

Under the previous conditions which used iodine as the initiator, the conjugate addition of the Grignard reagent to the allylic epoxide in the presence of catalytic CuI was completed after 16h at room temperature. Ultimately, the reaction of the allylic epoxide with 4-5 equivalents of the Grignard reagent gave full conversion of the allylic epoxide to the allylic alcohol after only 2.5 h at room temperature.

Overall, the development of a DIBALH activated procedure for the quick, reliable formation of the electron-rich Grignard reagent greatly improved the consistency of the Grignard reaction. The reaction time was shortened from 24 h with several hours of heating at reflux, to 5 h at room temperature and gave moderately high yields (87%). This was a significant improvement, as the Grignard addition
had been a key, yet highly unreliable, reaction requiring high heat and long reaction times. Reaction of the dieneone III-25 with acetyl bromide and acetic anhydride provided the aromatized compound III-27. A reduction of the C7 ketone and subsequent deprotection of the phenol in a one-pot fashion was accomplished with NaBH₄ and 10N aqueous NaOH to yield RU39411 III-20 (Figure 3.16). The

monodemethylation of III-20 was accomplished through the use of 1-chloroethylchloroformate and DIPEA in 1,2-dichloroethane, and subsequent heating in MeOH.

The monodemethylation of the amine side chain was problematic. While previous use of 1-chloroethylchloroformate in our lab to effect the monodemethylation of tertiary amines on other substrates was successful, the presence of the phenol prevented the conversion of RU39411 to its desmethyl analog III-28. This is most likely due to the protonation of the tertiary amine by the acidic phenol.

My original strategy was to circumvent the protonation of the amine side chain by protection of both the phenol and the secondary alcohol. (Figure 3.17) As future transformations would introduce a
terminal alkyne into the molecule, the protecting group must be removed without the use of strong acid or strong base. Protection as pivaloyl esters III-29 permitted the formation of the desired intermediate, but ester cleavage occurred as a result of refluxing in MeOH. I protected RU39411 as the diTBS ether III-30, which are stable to alcohols. The accumulation of chloride ion during the first step of the demethylation reaction, however, caused the cleavage of the TBS ethers. Attempts to obtain the diPMB ether derivative were unsuccessful.

I hypothesized that addition of an appropriate base (pKa > 11) would disfavor the zwitterionic form of RU39411. (Figure 3.18) Triethylamine promoted the formation of the desired quaternary ammonium salt, but triethylamine sequestered the chloride ion and thus prevented the demethylation of quaternary amine. The addition of an external chloride source, such as CuCl, failed to promote the demethylation of the quaternary ammonium salt. The use of DBU failed to show any significant reaction progression, but the use of Proton Sponge successfully promoted the demethylation of RU39411.

Figure 3.17 Investigation of protecting groups in the demethylation reaction

Figure 3.18 Use of base to restore the nucleophilicity of the amine side chain
Although the desmethyl product III-28 was formed, I was unable to isolate it, due to the formation of an adduct with the Proton Sponge, which I was unable to disrupt. Hünig’s base, however, promoted the demethylation of the amine sidechain without sequestering the chloride ion or forming an adduct with the product, giving a facile conversion of RU39411 to its corresponding desmethyl derivative III-28.

III-28 was protected as the diTBS ether III-33 and was, without further purification, alkylated with a propargyl tetraethylene glycol tosylate to give the key alkynyl intermediate III-34. The optimization of this step is still ongoing. Once isolated and characterized, this alkynyl steroid derivative will be subjected to a Cu(I)-catalyzed alkyne-azide click reaction with fluorotriethyleneglycol azide II-85 derivative to provide the target stable isotope analog of the 11β-fluorinated antiestrogen. (Figure 3.19)

![Figure 3.19 Proposed synthetic route to FAES.](image)

3.5 Translation to radiochemical conditions

I also undertook the translation of the synthesis to radiochemical conditions, using a small amount of alkynyl steroid intermediate that had been synthesized by Lenny Dao via the unoptimized synthetic route. I performed this translation to radiochemical conditions in conjunction with the
laboratory of Ralph Weisledder, M.D., Ph.D. at the Center for Systems Biology at the Massachusetts General Hospital.

A $\text{[}^{18}\text{F}\text{]}$-fluorinated triethylene glycol azide derivative was synthesized and subsequently ligated to the alkynyl antiestrogen derivative via a Cu(I)-catalyzed Huisgen [3+2] cycloaddition. Aqueous K$^{18}\text{F}$ was obtained from a commercial source and was dried by passing it through an alumina cartridge, and formulated with Kryptofix 2.2.2. The K$_{2.2.2}\text{[}^{18}\text{F}\text{]}$ was then reacted with a tosylated triethylene glycol azide derivative, in MeCN, to give the desired $^{18}\text{F}$-synthon, $^{[18}\text{F]}$-fluorotriethylene glycol azide. The synthesis of $^{[18}\text{F]}$-fluorotriethylene glycol azide was conducted using an automated synthesizer, and gave consistently high (>95%) decay-corrected radiochemical yields. This azide derivative was then subjected to a Cu(I)-catalyzed alkyne-azide click reaction. Attempts to use CuSO$_4$$\cdot$5H$_2$O and sodium ascorbate as the catalytic system for the [3+2] cycloaddition failed to show any formation of the triazole derivative, which may be due to the poor solubility of the steroidal alkyne. Switching to the preformed Cu(I) complex [Cu(MeCN)$_4$]PF$_6$ as the catalyst permitted the use of organic solvents, which better solubilized the steroidal alkyne. The use of [Cu(MeCN)$_4$]PF$_6$ in the presence of the reductant BPDS (bathophenanthroline disulfonic acid disodium salt hydrate) gave consistently high yields, although increasing the amount of radioactivity caused a slight decrease in yield. (Table 3.1)

Table 3.1 The optimization of the $^{18}$F-labeling of estrogen derivative via “click” chemistry

<table>
<thead>
<tr>
<th>Cu$^{1+}$ (60 mM)</th>
<th>ASC (200 mM)</th>
<th>BPD S (80 mM)</th>
<th>Cu$^{+}$ (80 mM)</th>
<th>PEG$_3$N$_3$ (1 mM)</th>
<th>$^{18}$F-PEG$_3$N$_3$ in DMF/H$_2$O</th>
<th>Add’l Solvent</th>
<th>Temp °C</th>
<th>MW 30W</th>
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The $^{18}$F-FAES compound was purified by reverse phase HPLC using an isocratic (35:65) MeCN/H$_2$O gradient with 0.1% formic acid. Coinjection of stable isotope FAES showed that the stable fluorinated antiestrogen had the same retention time as the $^{18}$F-FAES, giving us a high level of confidence that the radiofluorinated species we synthesized was indeed $^{18}$F-FAES. (Figure 3.20)

![HPLC analysis of reaction progress](image)

**Figure 3.20** Comparison of retention times for $^{19}$F-FAES and $^{18}$F-FAES

### 3.6 In vivo biological evaluation of $^{18}$F-FAES

#### 3.6.1 Blood half-life, biodistribution, and PET imaging studies in wild-type mice

The purified $^{18}$F-FAES was then formulated for injection into mice models for *in vivo* studies and PET imaging studies. The properties of $^{18}$F-FAES made it difficult to formulate. Previously published imaging studies of similar compounds employed 5-10% ethanol in water as the formulation, however, in our hands, this was not optimal due to the toxicity of high levels of ethanol. Ultimately, we settled on the use of Solutol HS15 (polyethylene glycol (15)-hydroxystearate) to aid the formulation of $^{18}$F-FAES for injection as a (1:1) mixture of Solutol HS15/DMA in saline.

We conducted a blood half-life study in female C57Bl/6 wild-type mice (n=3) (Figure 3.21). A blood half-life study establishes the rate at which the radioactive compound is exiting the blood stream into tissue (fast half-life) and the rate at which the remaining radioactivity is then cleared from the plasma.
by metabolism and excretion (slow half-life). Three female C57B1/6 wild-type mice were injected with $^{18}$F-FAES and aliquots of blood were withdrawn for each time point for the duration of 120 min.

The radioactivity of each aliquot was then measured in a gamma counter. The formulation of $^{18}$F-FAES used for the injection impacted the blood half-life of the compound. While using 5% DMSO in saline as the formulation gave the shortest half-life values, the radioactivity level in blood at 2 h was significantly higher than for those studies using (1:1) Solutol HS15/dimethylacetamide or ethanol as the cosolvents. As previously mentioned, the formulation of 4% (1:1) Solutol:dimethylacetamide in saline gave the best results out of the three formulations tested.

![Graph](image)

**Figure 3.21** Blood half-life studies of $^{18}$F-FAES in C57B1/6 mice

The biodistribution of $^{18}$F-FAES in female C57B1/6 wild-type mice was also measured at 120 min p.i. (**Figure 3.22**) At this time point, each organ was harvested and its radioactivity level was measured by a gamma-ray counter. The results show that the uptake of $^{18}$F-FAES in the wild-type mice was highest in the uterus, kidney, and liver at this time point, ~ 2 % ID/g of tissue, with a portion of $^{18}$F-FAES having been cleared from the body, as seen by the radioactivity level of ~ 5 % ID/g in the feces.

<table>
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<th>Co-solvent in Saline (0.9% NaCl)</th>
<th>Half-life (min)</th>
<th>Percent Fast (%)</th>
<th>Half-life Weighted (min)</th>
<th>Terminal % at 2 h</th>
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<td>DMSO (5%)</td>
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<td>49.6</td>
<td>3.2</td>
</tr>
<tr>
<td>1.1 Solutol:DMA (4%)</td>
<td>10.1</td>
<td>1.9</td>
<td>54.6</td>
<td>5.6</td>
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<td>Ethanol (8%)</td>
<td>25.1</td>
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</tbody>
</table>

The biodistribution of $^{18}$F-FAES in C57B1/6 mice was also visualized by a 2h dynamic PET scan (**Figure 3.23**) At 30s p.i., radioactivity is seen redistributing from the injection site in the tail of the mouse and beginning to appear in the liver. Uptake of radioactivity in the liver dominates the PET image at 3 min.
Figure 3.22 $^{18}$F-FAES biodistribution in female C57BL/6 wild type mice

Figure 3.23 Selected time points from a dynamic PET scan of the biodistribution of $^{18}$F-FAES in normal C57BL/6 mice.

p.i. At 15 min. p.i., significant uptake can be seen in the liver, intestines, gall bladder and the bladder. Uptake of radioactivity can also begin to be seen in the kidneys. By 1 h p.i., the visualization of uptake in
the liver has decreased, but high levels of radioactivity continue to be seen in the intestines, kidneys, gall bladder, and bladder. No further significant changes in uptake of radioactivity were observed in the PET scan after 1h post injection.

3.6.2 Biodistribution and PET imaging studies in MCF-7 xenograft mouse models

To evaluate the uptake of $^{18}$F-FAES in ER-positive tumors, we decided to study the distribution of $^{18}$F-FAES in an MCF-7 xenograft Nu/nu (nude) mouse model. While MCF-7 cells grow well in cultures, the growing of MCF-7 cells in a xenograft model is problematic. They grow slowly, and additionally require the subcutaneous implantation of an estrogen pellet in order for the cells to grow. It was noted during these studies that the morphologies of several organs in the xenograft mouse were abnormal.

We injected $^{18}$F-FAES into the femal MCF-7 xenograft Nu/nu mouse, and followed the uptake of radioactivity in tissue by a 2 h dynamic PET scan (Figure 3.24). At 30 sec. p.i., no accumulation of

![Figure 3.24 Dynamic PET scan and CT scan of MCF7 xenograft nu/nu mouse](image-url)
radioactivity is seen in tissue, but at 3 min p.i., high uptake of radioactivity is seen in the liver. The radioactivity had largely redistributed to the kidneys, intestine, and bladder by 1h p.i., and continued to pass from the kidney to the bladder, as seen at 1.5 h p.i. The tumor was not visualized clearly by PET, although a very slight increase in uptake can be seen from 1 h to 1.5 h p.i. A time-activity curve plot offers a quantitative explanation for the failure of PET to visualize the tumor (Figure 3.25).

![Figure 3.25 Time activity curve for ¹⁸F-FAES in MCF7 xenograft nu/nu model.](image)

Over the first hour following injection the uptake of radioactivity in the tumor increases. Although the radioactivity level present in blood continues to fall steadily over the course of the study, the level of radioactivity present in the tumor tissue never surpasses that seen in the blood. However, the ratio of radioactivity in the tumor to radioactivity in muscle increases over time. This increase accounts for the slight increase in definition of the tumor region seen in the PET image. The high radioactivity in the blood, however, hinders the clear visualization of the tumor against the background.

After the 2 h dynamic PET scan was complete, the radioactivity level of each organ was measured in a biodistribution study (Figure 3.26). The highest levels of radioactivity accumulation were
Figure 3.26 Biodistribution of $^{18}$F-AES in murine MCF7 xenograft model

seen in kidney, bone, and blood. Significantly lower uptake in the liver was seen for the MCF-7 xenograft model than for the wild-type mouse, which may merely represent a difference between the two species of mice. The biodistribution data also shows that the uptake in the MCF-7 tumor did not reach the level of uptake in several other organs. While disappointing, this is not entirely unexpected as steroids are known to demonstrate high levels of non-specific, off-target binding.

3.7 Summary and future directions

In this project, we optimized the synthesis of the target fluorinated antiestrogen derivative (FAES) based on antiestrogen RU39411. We also translated this chemistry to radiochemical conditions. We were able to establish preliminary in vivo properties of $^{18}$F-FAES in both wild-type mice and MCF-7 xenograft models. We hypothesized that the addition of an oligoethylene glycol sidechain would retard the in vivo metabolism of FAES, as well as, lower the levels of off-target binding and provide a higher contrast PET image than previous radiolabeled steroid derivatives. Unfortunately, the oligoethylene glycol sidechain did not significantly ameliorate the difficulty of formulating the steroid derivative, nor
did it provide a high contrast PET image. While uptake is seen in the tumor tissue, the high levels of radioactivity in the blood preclude the visualization of the tumor. Further optimization of the pharmacokinetic properties of the radiotracer is necessary. We also encountered difficulty in working with the MCF-7 xenograft murine model. The necessity of subcutaneously implanting an estrogen pellet, may affect the ability of our radiotracer to selectively bind to ER-overexpressing tissues like the tumor. Therefore, our understanding of how to work with this xenograft model must be improved.

Based on my observations of the in vivo distribution of $^{18}$F-FAES, I would propose several changes for future work in this area. First, I propose that instead of a tertiary amine group being present in the side chain of $^{18}$F-FAES, that one synthesize the corresponding quaternary ammonium salt, as it should not significantly alter the binding of the steroid scaffold to the estrogen receptor, while improving the water-solubility and pharmacokinetic properties of the compound. Second, I propose that the replacement of the 11β-phenyl group with a heterocycle, such as a pyridine derivative, could be advantageous, as the basic heterocycle would lower the log P of the molecule.
Chapter 4

Methodology development toward the synthesis of benzo-radiohalogenated indole derivatives

4.1 Introduction

The indole scaffold is ubiquitous in nature, being found in natural products, psychostimulants, hormones, and amino acids. Indoles are known to possess a wide variety of biological properties, including anti-cancer,\textsuperscript{55,56} anti-inflammatory,\textsuperscript{57-59} anti-malarial,\textsuperscript{60} and anti-diabetic\textsuperscript{61} properties. Additionally, several plant auxin hormones are based on an indole core. Due to the plethora of indole derivatives possessing biological activities, the synthesis of radioisotopically-labeled analogs of these indoles for use as imaging agents would be of great utility to the biomedical community.

Several indole-based radiotracers have featured radiohalogens on the benzo positions of the scaffold. Among these radiotracers have been benzoindole derivatives,\textsuperscript{62} ergoline alkaloid derivatives,\textsuperscript{63} naltrindole derivatives,\textsuperscript{64} β-carboline derivatives,\textsuperscript{65} and tryptophan derivatives (Figure 4.1).\textsuperscript{66} Many

![Figure 4.1 Select benzo-radiohalogenated indole derivatives](image)

Figure 4.1 Select benzo-radiohalogenated indole derivatives
indole derivatives feature substituents at the C2 or C3 positions which are essential for biological activity. Thus, the ability to install a radioactive isotope at the benzo positions for imaging purposes would be particularly useful as modifications at the C2 or C3 may jeopardize or alter biological activity. To date, however, such late stage transformations on the benzo positions of indoles remain more challenging than the corresponding transformations at the C2 and C3 positions of the indole scaffold as the benzo positions. Unlike the C2 and C3 positions, the benzo positions are not preferred positions for electrophilic substitution.

Nonradioactive halogens can be introduced easily into these benzo positions in the early stages of a synthetic route. Halogens can be introduced through use of either haloaniline or halophenylhydrazine precursors via palladium-catalyzed cyclization or Fischer indole synthesis. Alternatively, diazonium salts can also be used for the early-stage introduction of halogens. These strategies, however, are incompatible with radiohalogens which possess short half-lives and must be introduced very late in the synthetic route, most desirably in the last synthetic transformation. Additionally, high specific activity is often required for the resultant radiolabeled compound to be of use in the probing of biological systems. These criteria, unique to the synthesis of radiolabeled compounds for use as SPECT and PET imaging probes, focused our efforts on the development of new methods for labeling this important class of compounds.

The C2 and C3 positions of indoles can be radioiodinated via direct electrophilic addition using nucleophilic radioiodide in the presence of an oxidant, such as peracetic acid, Iodo-Gen, or Chloramine-T. These reactions are possible due to the rapidity with which these sites undergo electrophilic addition reactions. The benzo positions are less likely to undergo regioselective electrophilic additions, and therefore require the use of site-directing groups, often at the ipso position. Currently, the introduction of radiohalogens at the benzo positions of indole scaffolds is accomplished primarily by the following three methods: (1) acid or copper-catalyzed halogen radiohalogen exchange, (2) decomposition of diazonium fluoroborates, or (3) halodemetalation reactions in which an electrophilic halogen displaces an alkylmetal moiety. Of these methods, the indirect halogen-radiohalogen exchange is preferable as its mild reaction conditions provide radiohalogenated products in high specific
activity and allow for a wider range of functional group tolerance. The two most common metalated precursors for radiohalogenation reactions are boronates and trialkylstannanes. While boronate precursors have the advantage of being less toxic than the corresponding stannane derivatives, the halodemetalation reaction to furnish the radiohalogenated substrate occurs at a much slower rate for the boronate precursors than for the stannane precursors. In some cases, the slower rate of halodemetalation for the boronate derivatives favors the radiolabeling of other possible sites for electrophilic substitution.

We originally gained an interest in the radiohalogenation of the benzo positions of indoles through the preparation of $[^{123}\text{I}]-\text{AM630 IV-1}$, an inverse agonist of the cannabinoid receptor CB$_2$, by a former colleague, Anna Williams. For the radiiodination of IV-1, it was decided to employ a halodemetalation reaction as the final synthetic transformation in the synthetic route toward $[^{123}\text{I}]-\text{AM630 IV-1}$. The use of a boronate precursor was investigated, as the boronate was more easily accessed, but proved unsuitable for the desired radiiodination. In the case of AM630, the halodemetalation reaction of the boronate ester did not furnish the desired C6 iodinated product. Instead, it was concluded that competition from other possible sites of electrophilic addition was precluding the halodemetalation from occurring. It was hypothesized that this competing side reaction could be suppressed by employing the corresponding tributyltin precursor, as electrophilic substitutions of stannanes proceed at a faster rate than

![Figure 4.2 Structure of AM630](image-url)
do those of boronates. There is, however, a severe gap in the literature regarding the metalation, particularly the stannation, of the benzo positions of indoles.

4.2 Literature precedent for the stannation of the benzo positions of indoles.

While the stannation of the C2 position of the indole scaffold has been accomplished on multigram scale in excellent yield via transmetalation of an α-lithiated intermediate,78 the stannation of the benzo positions of the indole scaffold has proved more challenging. The stannation of the benzo positions of indoles has been traditionally accomplished via lithium-halogen exchange, followed by transmetalation to give the corresponding trialkyltin derivative.2,79 Cherry, et al. were able to accomplish the stannation of the C5 and C6 positions of an unsubstituted indole scaffold IV-4, IV-5 in moderate yields (Figure 4.3) through the formation of a lithiated intermediate after abstraction of the indolic hydrogen by potassium hydride to form the potassium indole salt.2 While effective, the use of harsh reagents such as hydrides and alkyl lithiums severely limits the functional group tolerance of these stannation reactions.

Although rare, several palladium-catalyzed stannations at the benzo positions of indoles have been reported.3,4,77,80 Such palladium-catalyzed stannations have largely suffered from poor yields (≤ 50%),4,5,76,77 high catalyst loading, and the need to protect or alkylate the indolic nitrogen.76,77 While some N-acyl indole derivatives IV-7 were successfully stannated in moderate (80%) yields under these
conditions (Figure 4.4), there is only one report of a 1H-indole being stannated in good yield (Figure 4.5). However, only one of the 1H-indole substrates reported was obtained in good yield (89%), while the others suffered from poor yields (<30%). No explanation of this discrepancy was given in the account.

**Figure 4.4** Successful Pd-catalyzed stannation of N-acylated indole.

![Chemical structure](image)

**Figure 4.5** Palladium-catalyzed stannation of C2-substituted indoles.

![Chemical structure](image)

as to the nature of this discrepancy between seemingly similar substrates. (Figure 4.5) Mentzel, et al. attempted the stannation of 7-Bromoindole IV-12 using palladium catalysis (Figure 4.6), but were unable to form any of the desired product using several palladium catalysts in the presence of hexa-n-butylditin.
The development of a generalized palladium-catalyzed method for the installation of a trialkyltin moiety at the benzo positions of the indole scaffold would constitute a significant improvement over more common methods which progress through a lithiated intermediate, the synthesis of which requires harsh reagents. Transition-metal catalysis would tolerate a much larger range of functional groups. This is especially important in the development of radiotracers, as the metalation and radiohalogenation steps must be carried out at a later synthetic stage when much of the functionality of the biologically active compound has already been installed.

4.3 Development of palladium-catalyzed stannation procedure for the benzo positions of indoles*

(Based on Corcoran, E. B., Williams, A. B., Hanson, R. N. Org. Lett. 2012, 14, 4630-4633.)

Initial studies were conducted by Anna Williams toward the stannation of the C5 and C6 benzo positions of indole-3-carbaldehyde, as this was similar to the structure of AM630 IV-1. I chose to extend these initial studies to include more electron-rich indole derivatives with the aim of developing a general method for the preparation of stannated indoles, as these have posed significant synthetic challenges. I began these studies by expanding on work previously done by Anna Williams regarding the stannation of the C5-/C6- benzo positions of the indole-3-carbaldehyde scaffold (Table 4.1). In her studies toward the Pd-catalyzed synthesis of the C5-/C6-tributyltin derivatives of these scaffolds, Anna Williams found that
common transmetalation conditions ([Table 1, entry 1), in which 6-bromoindole-3-carbaldehyde IV-15 was refluxed in toluene with hexabutylditin in the presence of Pd(PPh$_3$)$_4$, showed no evidence of any

**Table 4.1 Optimization of stannation of C6 position of indole carbaldehydes**

<table>
<thead>
<tr>
<th>entry</th>
<th>Pd</th>
<th>ligand</th>
<th>solvent</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pd(PPh$_3$)$_4$</td>
<td>n/a</td>
<td>toluene</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>PdCl$_2$dppf</td>
<td>n/a</td>
<td>toluene</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>PdCl$_2$dppf</td>
<td>n/a</td>
<td>1,4-dioxane</td>
<td>24</td>
</tr>
<tr>
<td>4</td>
<td>PdCl$_2$(PhCN)$_2$</td>
<td>PCy$_3$</td>
<td>1,4-dioxane</td>
<td>82</td>
</tr>
<tr>
<td>5</td>
<td>PdCl$_2$(PhCN)$_2$</td>
<td>dppe</td>
<td>1,4-dioxane</td>
<td>79</td>
</tr>
</tbody>
</table>

reaction progress over a period of 24 h. Similar results were obtained with 1,1’-bis(diphenylphosphino)ferrocene-palladium(II) dichloride (PdCl$_2$dppf) in toluene; however, the same conditions in 1,4-dioxane gave the desired product in an isolated yield of 24% (Table 4.1, entry 3). Use of more basic ligands, PCy$_3$ and dppe, with PdCl$_2$(PhCN)$_2$ as the precatalyst (Table 4.1, entries 4 and 5) gave 3 in increased yields of 82% and 79%, respectively.

Unlike the C6 regioisomer IV-15, the C5 regioisomer IV-17 failed to react favorably with the PdCl$_2$(PhCN)$_2$/PCy$_3$ catalytic system, giving the stannated product in only 22% yield (Table 4.2, entry 1). Increasing the basicity of the ligand by use of P(t-Bu)$_3$ (Table 4.2, entry 2) did not promote any formation of the desired stannane, while the use of less basic ligands dppe and 2-(dicyclohexylphosphino)biphenyl (PCy$_2$bph) (Table 4.2, entries 3 and 4) gave product in moderate to good yields (60% and 79%, respectively). Based on this and the finding that use of a more basic ligand than PCy$_3$, such as P(t-Bu)$_3$, failed to yield any of the desired C5-stannated indole-3-carbaldehyde derivative IV-18, I was interested in drawing a generalization about the nature of ligands needed to effect stannation at the C5 and C6
positions. I was also interested in investigating the effects of C3 substitution on the nature of the catalyst needed to accomplish the transmetalation of the C5 and C6 positions of the scaffold. Finally, I wished to explore how this effect might vary between the C5 and C6 regioisomers. I chose three indole scaffolds to investigate, which bore C3 substituents (CHO, H, and NMe₂) that represent electron-withdrawing, electron-neutral, and electron-donating substituents.

I began these studies by expanding the work begun by Anna Williams on the indole-3-carbaldehyde scaffold. From her preliminary observations, I hypothesized that transmetalation at the C5 position required the use of less basic ligands, while the C6 transmetalation of the carbaldehyde scaffold required a basic and bulky ligand. To test this hypothesis, I decided to screen additional, less basic phosphine ligands and assess their abilities to catalyze the stannation of the C5 position. The phosphine ligand PCy₂bph, used in conjunction with PdCl₂(PhCN)₂ as the precatalyst, promoted the stannation of the C5 position in an isolated yield of 79%. This was slightly higher than the yield (60%) obtained with the ligand dppe. I hypothesized that the PdCl₂(PhCN)₂/dppe catalytic system would give significantly lower yields of the C6-stannated product IV-16 due to the lower basicity of the ligand. This, however, proved to be incorrect, as an isolated yield of 79% was obtained for the C6-indole-3-carbaldehyde derivative, comparable to that obtained with the use of PCy₃ ligand. So, it appears for the indole-3-carbaldehyde scaffold, that less basic ligands are required for the C5-stannation, while both less basic and highly basic

<table>
<thead>
<tr>
<th>entry</th>
<th>Pd</th>
<th>ligand</th>
<th>solvent</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PdCl₂(PhCN)₂</td>
<td>PCy₃</td>
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</tr>
<tr>
<td>2</td>
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<td>Ph(t-Bu)₂</td>
<td>1,4-dioxane</td>
<td>0</td>
</tr>
<tr>
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<td>PdCl₂(PhCN)₂</td>
<td>dppe</td>
<td>1,4-dioxane</td>
<td>60</td>
</tr>
<tr>
<td>4</td>
<td>PdCl₂(PhCN)₂</td>
<td>PCy₂bph</td>
<td>1,4-dioxane</td>
<td>79</td>
</tr>
</tbody>
</table>

Table 4.2 Optimization of stannation of C5 position of indole-carbaldehydes
phosphine ligands were able to promote the stannation of the C6 position.

I then began optimizing the stannation of the C3-unsubstituted indoles. The use of the basic ligand dppe gave low to moderate yields. When used with PdCl₂(PhCN)₂ as the precatalyst, both the C5 (Table 4.4, entry 1) and C6 positions (Table 4.3, entry 1) could be stannated in moderate yields of 57% and 58%, respectively. These yields dropped dramatically, however, upon the use of Pd₂(dba)₃ as the precatalyst. The stannation at the C6 position IV-20 occurred in an isolated yield of 43%, while the C5 stannation IV-22 was more strongly affected, with isolated yields of only 29% being obtained. However, a similar disparity in isolated yields between the use of PdCl₂(PhCN)₂ and Pd₂(dba)₃ as precatalysts was not seen when the PCy₃ ligand was used. Both precatalysts gave C5 and (Table 4.4, entries 3 and 4) C6 (Table 4.3, entries 3 and 4) stannated products IV-22 and IV-20 in isolated yields >90%. These limited studies show that for the C3-unsubstituted indole derivatives, a more basic ligand is necessary to obtain the desired compounds in high isolated yields.

While the C5-/C6- stannation of these C3-unsubstituted indoles proceeded smoothly with the appropriate choice of precatalyst and ligand, the purification and isolation of these stannane derivatives was quite challenging. Whereas the indole-3-carbaldehyde derivatives could be purified using silica
Table 4.4 Optimization of the C5-stannation of C2-unsubstituted indoles

<table>
<thead>
<tr>
<th>entry</th>
<th>Pd</th>
<th>ligand</th>
<th>solvent</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>PdCl₂(PhCN)₂</td>
<td>dppe</td>
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<tr>
<td>2</td>
<td>Pd₂dba₃</td>
<td>dppe</td>
<td>1,4-dioxane</td>
<td>29</td>
</tr>
<tr>
<td>3</td>
<td>PdCl₂(PhCN)₂</td>
<td>PCy₃</td>
<td>1,4-dioxane</td>
<td>92</td>
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<tr>
<td>4</td>
<td>Pd₂dba₃</td>
<td>PCy₃</td>
<td>1,4-dioxane</td>
<td>90</td>
</tr>
</tbody>
</table>

which had been neutralized with (1:99) triethylamine/hexanes, similar chromatographic methods caused the degradation of 50% of the desired stannane product to the corresponding protodestannylated side product. Attempts to further neutralize the silica gel by use of (5:95) triethylamine/hexanes and (1:9) triethylamine/hexanes gave similarly disappointing results. Another common method for purifying stannanes, especially those which are more electron-rich and acid-sensitive has been through the use of basic alumina. I also attempted to purify the stannated C3-unsubstituted indoles using basic alumina, but saw >50% cleavage of the desired stannane to the protodestannylated product. I then investigated the use of reverse phase C18 chromatography for the isolation of these very acid-labile stannanes. In order to separate the stannane from the remaining ligand and Pd-species, I ran a water/acetonitrile gradient, which I believed would provide good separation, with the elution of the ligand and Pd-species occurring prior to the elution of the desired stannane. Unfortunately, the presence of water in the mobile phase of the column caused complete degradation of the stannane to the protodestannylated side product. The use of a larger C18 column (~10g column per 50mg scale of stannane) allowed for the separation of the stannane product from the ligand and Pd-species using an isocratic acetonitrile mobile phase. The avoidance of water allowed for the successful isolation of the desired stannated C3-unsubstituted indole derivatives with minimal cleavage of the tributyltin group. Additional precautions were necessary with regard to choice of NMR solvent. The trace amount of HCl present in CDCl₃ caused cleavage of the pure stannane compound to a 1:1 mixture of stannane/protodestannylated compound during the course of a short ¹H NMR scan. Prior neutralization of CDCl₃ with K₂CO₃ circumvented the cleavage of the stannane, with
the stannane being stable in CDCl₃ even after several hours at room temperature or several days at 0°C. Storage of these compounds in cyclohexane or benzene at 0°C was found to be preferable, as storage of the stannanes as neat oils at 0°C led to significant degradation of the compound after several days.

The third indole scaffold I investigated was that of gramine, which features a methyl(dimethylamine) group at the C3 position. I was unsure if the tertiary amine substituent present on the gramine substrate would prevent the transmetallation of the C5-/C6- positions by coordinating to the palladium catalyst. I decided to first investigate the feasibility of the palladium-catalyzed boronation of the gramine scaffold as a control reaction, as the boronation of indoles is more easily accomplished than the stannation. If boronation occurred, I would know that any subsequent difficulties encountered during stannation could be attributed to the inherent difficulties associated with stannation reactions as opposed to complications due to the presence of the tertiary amine. Thus, I undertook the Miyaura-type boronation of the C5-gramine derivative IV-23.

I began by employing the standard Miyaura conditions of PdCl₂dppf, KOAc, and bis(pinacolato)diboron in THF (Table 4.5, entry 1). No reaction progression was observed by TLC. Similarly, no reaction progression was observed upon changing the solvent to 1,4-dioxane (Table 4.5, entry 2). Substituting 1.5 M aqueous NaOH for KOAc as the base (Table 4.5, entry 3) was favorable, giving the desired C5-boronated gramine IV-24 was obtained in 12% yield. Decreasing the stoichiometry of NaOH from 3 equivalents to 1.5 equivalents had a positive effect, raising the yield to 34% (Table 4.5, entry 4). I thought this increase in yield might suggest that the reaction was sensitive to the presence of water in the reaction. To test this, I repeated the reaction conditions, now using a (1:1) water/dioxane mixture as the solvent (Table 4.5, entry 6). No reaction progression was seen. Use of an organic base, triethylamine, also failed to promote any boronation (Table 4.5, entry 5). I thought that a bulkier ligand such as PCy₂bph might be beneficial, but the catalytic system of Pd₂(dba)₃ / PCy₂bph (Table 4.5, entries 7 and 8) failed to catalyze the boronation of the gramine substrate in the presence of both KOAc and aqueous NaOH. In light of other observations we made later on, Pd₂(dba)₃ was a poor choice of
Table 4.5 Palladium-catalyzed boronation of 5-bromogramine

<table>
<thead>
<tr>
<th>entry</th>
<th>Pd</th>
<th>ligand</th>
<th>base</th>
<th>solvent</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>n/a</td>
<td>KOAc</td>
<td>THF</td>
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<td>PdCl₂dpff</td>
<td>n/a</td>
<td>KOAc</td>
<td>1,4-dioxane</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>PdCl₂dpff</td>
<td>n/a</td>
<td>NaOH (aq)</td>
<td>1,4-dioxane</td>
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<td>PdCl₂dpff</td>
<td>n/a</td>
<td>NaOH (aq)</td>
<td>1,4-dioxane</td>
<td>34</td>
</tr>
<tr>
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<td>PdCl₂dpff</td>
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<td>Et₃N</td>
<td>1,4-dioxane</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>PdCl₂dpff</td>
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<td>NaOH (aq)</td>
<td>(1:1) H₂O/1,4-dioxane</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Pd₂dba₃</td>
<td>PCy₂bph</td>
<td>KOAc</td>
<td>1,4-dioxane</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>Pd₂dba₃</td>
<td>PCy₂bph</td>
<td>NaOH</td>
<td>1,4-dioxane</td>
<td>0</td>
</tr>
</tbody>
</table>

* 3.0 equiv. of 1.5M NaOH(aq) used.  
* 1.5 equiv. of 1.5M NaOH(aq) used.

precatalyst for use with the gramine substrate. Most likely, if we had screened additional precatalysts such as Pd(OAc)₂ or PdCl₂(PhCN)₂, and used a more basic ligand, we would have had better success in effecting the boronation of 5-bromogramine. Instead of optimizing the boronation of this substrate, we decided to focus instead on the stannation of this substrate now that we knew that the tertiary amine would not prevent the transmetalation of the C5 position.

Adaptation of the Miyaura conditions to the stannation of 5-bromogramine IV-23, in which we used PdCl₂dpff with LiCl as an additive, gave the desired stannated gramine IV-25 in 24% (Table 4.6, entry 1). The use of a more basic ligand, PCy₃, with precatalyst Pd₂(dba)₃, yielded the desired stannane in 28% yield (Table 4.6, entry 3). Changing the precatalyst to PdCl₂(PhCN)₂ vastly improved the yield to 82% (Table 4.6, entry 4). Use of less basic ligands, such as dppe and PCy₂bph, gave only small amounts of the desired stannane (Table 4.6, entries 4 and 5), with 5-bromogramine being recovered from the reaction.

We then undertook the stannation of the 6-bromogramine derivative. As this derivative was not commercially available, it was synthesized from 6-bromo-1H-indole IV-19 in one step through
electrophilic addition of Eschenmoser’s salt to the C3 position. (Figure 4.7) This was accomplished in an unoptimized yield of 37%. The PdCl₂(PhCN)₂ / PCy₃ catalyst system was effective in stannating the C6

Table 4.6 Optimization of C5 stannation of the gramine scaffold

<table>
<thead>
<tr>
<th>entry</th>
<th>Pd</th>
<th>ligand</th>
<th>solvent</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
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<td>1,4-dioxane</td>
<td>82</td>
</tr>
<tr>
<td>2</td>
<td>PdCl₂(PhCN)₂</td>
<td>PCy₃</td>
<td>1,4-dioxane</td>
<td>82</td>
</tr>
<tr>
<td>3</td>
<td>Pd₂dba₃</td>
<td>PCy₃</td>
<td>1,4-dioxane</td>
<td>26</td>
</tr>
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<tr>
<td>5</td>
<td>Pd₂dba₃</td>
<td>PCy₂bph</td>
<td>1,4-dioxane</td>
<td>5</td>
</tr>
</tbody>
</table>

ᵃ LiCl used as an additive.

of the desired stannane (Table 4.6, entries 4 and 5), with 5-bromogramine being recovered from the reaction.

We then undertook the stannation of the 6-bromogramine derivative. As this derivative was not commercially available, it was synthesized from 6-bromo-1H-indole IV-19 in one step through electrophilic addition of Eschenmoser’s salt to the C3 position. (Figure 4.7) This was accomplished in an unoptimized yield of 37%. The PdCl₂(PhCN)₂ / PCy₃ catalyst system was effective in stannating the C6

Figure 4.7 Synthesis of 6-bromogramine
regioisomer to give the C6 stannated gramine derivative IV-27 in 70% yield (Table 4.7, entry 1). The use of Pd$_2$(dba)$_3$ as the precatalyst led to a significant drop in yield (Table 4.7, entry 2). Less basic ligands, such as dppe and PCy$_2$bph, were less effective at furnishing the stannated product, regardless of the precatalyst used. (Table 4.7, entries 3 and 4). Therefore, we can conclude that for the gramine derivative, basic ligands such as PCy$_3$ are more effective at catalyzing the stannation of both the C5 and C6 positions. This is likely due to the reticent oxidative addition of these electron-rich indole derivatives, which can be overcome by the use of a more basic palladium complex. It has also been observed that the use of Pd$_2$(dba)$_3$ as a precatalyst for this class of indole derivatives has a detrimental effect. This is peculiar, as the choice of precatalyst was not important for the stannation of the two other classes of indoles examined. The poor performance of the reaction in the presence of dba ligand may be due to the interaction of the tertiary amine side chain with the Pd$_2$(dba)$_3$ precatalyst. Perhaps the tertiary amine stabilizes the precatalyst, thus disfavoring the dissociation of the dba ligand and, consequently, the formation of the active catalyst.

Table 4.7 Optimization of C6 stannation of the gramine scaffold

<table>
<thead>
<tr>
<th>entry</th>
<th>Pd</th>
<th>ligand</th>
<th>solvent</th>
<th>yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>1,4-dioxane</td>
<td>70</td>
</tr>
<tr>
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<td>Pd$_2$(dba)$_3$</td>
<td>PCy$_3$</td>
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<td>15</td>
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<tr>
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<tr>
<td>4</td>
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<td>PCy$_2$bph</td>
<td>1,4-dioxane</td>
<td>13</td>
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</table>
4.4 Preliminary electrophilic halodestannylation reactions

We envisioned the use of these stannated indoles as key intermediates for the installation of radioactive iodine and radioactive fluorine. While iododestannylation reactions are often used in radiochemistry, fluorodestannylation are less common. This is partly due to the longstanding problem of obtaining electrophilic $^{18}$F in sufficiently high specific activity. There had been unpublished reports of electrophilic $^{18}$F with enhanced specific activity which largely inspired our idea to employ a fluorodestannylation for $^{18}$F labeling of these substrates. These initial findings, however, were not reproducible. The stannation procedure we developed could also be used in conjunction with the Pd-catalyzed C-$^{18}$F bond formation via reductive elimination from a Pd(IV) complexes described by Ritter, et al. This, however, may not be optimal as the less toxic boronates are also be compatible with the Pd-catalyzed method, and thus would present a desirable alternative to the stannanes. Additionally, one can envision these stannane intermediates as precursors to the corresponding iodonium salts, which could then be subsequently employed to provide the $^{18}$F-labeled substrates.

The stannanes will also be highly useful for the late-stage radioiodination of indole derivatives. To demonstrate this, we undertook the iododestannylation of the indole-3-carbaldehyde derivatives (Figure 4.8). The desired 5- and 6-iodo indole-3-carbaldehydes IV-28 and IV-29 were synthesized in good yields, but the iododestannylation of the $1H$-indole and gramine derivatives remain to be optimized.

![Figure 4.8 Iododestannylation of indole-3-carbaldehyde scaffolds](image)

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4.5 Summary and future directions

In summary, we have developed a palladium-catalyzed stannation for the C5 and C6 benzo positions of indole scaffolds bearing varied substituents at the C3 position. The optimal conditions for the iododestannylation of the 1H-indole and gramine substrates are still to be investigated. Additionally, the fluorodestannylation of each of these substrates will also be investigated. Ultimately, we envision the application of this stannation methodology to the preparation of a series of radiolabeled auxin plant hormones based on the indole scaffold, namely indole-3-acetic acid and 4-chloroindole-3-acetic acid. The study of these plant hormones is of longstanding interest to the plant physiology community. We propose that the stannation methodology described herein would provide access to longer-lived and more useful radiotracers for probing the biological actions of these hormones than those that have been prepared and investigated to date. 81
Chapter 5

Experimental Procedures

5.1 General methods and materials

$^1$H and $^{13}$C NMR spectra were obtained on 400 MHz and 500 MHz Varian spectrometers. Chemical shifts were reported relative to trimethylsilane (0.00 ppm) for $^1$H and chloroform-$d$ (77.2 ppm), methanol-$d_4$ (49.0 ppm), dimethyl sulfoxide-$d_6$ (39.5 ppm), and acetone-$d_6$ (29.8 ppm, 206.3 ppm) for $^{13}$C. Reactions were monitored by analytical thin layer chromatography (TLC) plates pre-coated with silica gel 60 F254 or silica gel C18 F254 and visualized by fluorescence quenching under UV light. Additionally, TLC plates were stained using p-anisaldehyde, iodine, phosphomolybdic acid, or potassium permanganate. Normal phase flash chromatography was performed using 60-200 mesh silica gel from Davisil; reverse phase flash chromatography was performed using C18 silica cartridges from Biotage on an Argonaut FlashMaster Solo automated chromatography system. All commercial reagents were used as purchased, without further purification, unless otherwise noted. All high resolution mass spectrometry data was obtained on a Waters Qtof (hybrid quadrupolar/time-of-flight) API US system by electrospray (ESI) in the positive mode. Mobile phases were water and acetonitrile with 0.1% formic acid. The optimized conditions were: capillary voltage = 3,000 kV, cone voltage = 35 or 10, source temperature = 120 °C and dissolvation temperature = 350 °C or 300 °C. Mass correction was done by an external reference using a Waters Lockspray accessory.
5.2 Experimental for chapter 2

Methyl 4-(benzyloxy)-3-methoxybenzoate (II-40)

To a solution of methyl vanillate II-39 (7.15g, 39.3 mmol) in acetone (100mL) was added K$_2$CO$_3$ (9.56g, 69.2 mmol) and stirred at room temperature, 35 min. Benzyl bromide (9.4 mL) then added to the reaction solution. The reaction was then heated at reflux for 16.5h. K$_2$CO$_3$ was removed by filtration, and volatiles were removed by rotary evaporation. Crude product was purified by silica column chromatography. Product eluted with 20:80 ethyl acetate/hexanes. White solid, 9.97g (93%). $^1$H NMR (400MHz, CDCl$_3$), δ 3.88 (s, 3H), 3.94 (s, 3H), 5.21 (s, 2H), 6.88 (d, $J = 8.1$Hz, 1H ), 7.29-7.44 (m, 5H), 7.56 (d, $J = 4$Hz, 1H), 7.59-7.62 (dd, $J = 12$ Hz, 1H); $^{13}$C NMR (100MHz, CDCl$_3$), δ 52.0, 56.0, 70.7, 112.3, 122. 9, 123.3, 127.2, 128.0,128.6, 131.2, 133.6, 134.3, 136.3.

Methyl 4-(benzyloxy)-5-methoxy-2-nitrobenzoate (II-41)

A solution of II-40 (10.08g, 37.02 mmol) in DCM (90mL) was cooled to -10°C. Glacial acetic acid (90mL) and concentrated nitric acid (25mL), respectively, were added dropwise to the reaction solution. Reaction brought to room temperature and then heated at reflux overnight. Reaction was neutralized with sat. NaHCO$_3$ and poured over ice. Organic material was then extracted with EtOAc and dried over MgSO$_4$. MgSO$_4$ was removed by filtration and volatiles were removed by rotary evaporation. Crude product was purified with silica column chromatography, product eluted with (30:70) ethyl acetate/hexanes. White solid, 10.8g (92%). $^1$H NMR (400MHz, CDCl$_3$), δ 3.84 (s, 3H), 3.87 (s, 3H), 5.13
(s, 2H), 6.84 (d, J = 8.1 Hz, 1H), 7.25-7.41 (m, 2H), 7.39 (d, J = 8.1 Hz, 2H), 7.55 (d, J = 2.2Hz, 1H), 7.58 (dd, J = 2.2Hz, 1H); \(^{13}\)C NMR (100MHz, CDCl\(_3\)), δ 53.3, 56.7, 71.5, 108.9, 111.1, 122.0, 127.6, 128.6, 128.7, 128.9, 135.2, 141.0, 149.4, 153.1, 166.4.

**Methyl 2-amino-4-(benzyl)-5-methoxybenzoate (II-42)**

![Methyl 2-amino-4-(benzyl)-5-methoxybenzoate](image)

To a solution of II-41 (2.14 g, 6.74mmol) in (2:1) dichloromethane/methanol was added NiCl\(_2\)-6H\(_2\)O (395 mg, 1.66 mmol). The reaction solution was cooled to -15°C, and NaBH\(_4\) (666 mg, 17.6 mmol) was then added in portions over 30 min. Reaction mixture was then concentrated to dryness and purified by silica column chromatography. Product eluted with (40:60) ethyl acetate/hexanes. Off-white solid, 1.611g (83%). \(^1\)H NMR (400MHz, CDCl\(_3\)), δ 3.78 (s, 3H), 3.80 (s, 3H), 5.03 (s, 2H), 5.55 (br s, 2H), 6.13 (s, 1H), 7.27 (m, 6H); \(^{13}\)C NMR (100MHz, CDCl\(_3\)), δ 51.3, 56.6, 70.3, 101.0, 102.2, 113.3, 127.2, 128.0, 128.6, 136.2, 140.782, 147.1, 154.0, 168.1.

**7-(benzyl)-6-methoxyquinazolin-4(3H)-one (II-43)**

![7-(benzyl)-6-methoxyquinazolin-4(3H)-one](image)

II-42 (2.587g, 9.000 mmol) was dissolved in formamide (200mL) upon heating to 130°C. Ammonium formate (1.811g, 28.72 mmol) was then added to the reaction solution and heated at 180°C overnight. Reaction cooled slightly, poured over water. Product precipitated and was collected by filtration as a tan solid in quantitative yield. \(^1\)H NMR (400 MHz, CDCl\(_3\)), δ 4.02 (s, 3H), 5.29 (s, 2H), 7.19 (s, 1H), 7.32 (t,
$J=8.1\text{Hz, 1H}$, 7.38 (t, $J=8.1\text{Hz, 2H}$), 7.47 (d, $J=8.1\text{Hz, 2H}$), 7.63 (s, 1H), 7.98 (br s, 1H), 12.14 (br s, 1H).

**7-(benzyloxy)-4-chloro-6-methoxyquinazoline (II-44)**

II-43 (349 mg, 1.23 mmol) was dissolved in thionyl chloride (1.5 mL) upon heating to 80°C. DMF (0.1 mL) was then added to the reaction solution. The reaction was heated at reflux for 5h. Volatiles were then removed by azeotroping with toluene by rotary evaporation. Resulting solid taken up in DCM and washed with 5% NaHCO$_3$, water, and brine. Crude product was purified by silica chromatography. Product eluted with (20:80) ethyl acetate/hexanes. Pale yellow solid, 275 mg (74%). $^1$H NMR (400 MHz, CDCl$_3$), δ 4.07 (s, 3H), 5.33 (s, 2H), 7.33 (m, 5H), 7.48 (s, 1H), 7.50 (s, 1H), 8.85 (s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$), δ 56.4, 71.2, 102.7, 108.2, 119.6, 127.4, 128.5, 128.8, 135.1, 148.8, 151.7, 152.4, 155.7, 159.0.

**7-((tert-butyldimethylsilyloxy)-4-chloro-6-methoxyquinazoline (II-45)**

To II-44 (259 mg, 0.914 mmol) was added TFA (4 mL). The reaction was heated under reflux, 4h. The reaction was cooled and evaporated to dryness. The product was purified by silica column chromatography to yield the product as an off-white solid in quantitative yield. Used without further purification. To a solution of the deprotected 4-chloroquinazoline derivative in DCM at 0°C, were added TBSCI (77 mg, 0.51 mmol) and DMAP (7 mg, 0.06 mmol) in DCM (2 mL), followed by the addition of
DBU (0.12 mL, 0.80 mmol). The reaction was slowly warmed to room temperature and stirred for 3 h.

The reaction was concentrated and the crude mixture was purified by silica column chromatography. The product was eluted with (1:1) EtOAc/Hex as a colorless oil, 9 mg (10%). \(^1\)H NMR (400 MHz, CDCl\(_3\)), \(\delta\) 0.24 (s, 6H), 1.03 (s, 9H), 3.96 (s, 3H), 7.19 (s, 1H), 7.61 (s, 1H), 8.03 (s, 1H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)), \(\delta\) -4.4, 18.7, 25.8, 56.0, 105.9, 117.9, 142.2, 145.2, 152.0, 152.5. (Some of the carbon peaks may be overlapping, accounting for the two carbons which appear to be missing.)

tert-butyl 4-(prop-2-yn-1-yl)piperazine-1-carboxylate (II-47)

To a solution of N-Boc piperazine II-46 (1.392 g, 7.474 mmol) in MeCN (25 mL), was added Cs\(_2\)CO\(_3\) (7.208 g, 22.12 mmol). The reaction was stirred at room temperature, 30 min. Propargyl bromide solution (80% w/w in toluene) (1.303 g, 8.763 mmol) was then added to the reaction and the reaction was heated under reflux overnight. The reaction was cooled and filtered. The filtrate was concentrated by rotary evaporation and purified by silica column chromatography. The product eluted with (1:9) EtOAc/Hex to give an orange oil, 1.676 g (92%). \(^1\)H NMR (400 MHz, CDCl\(_3\)), \(\delta\) 1.46 (s, 9H), 2.30 (t, \(J = 2\) Hz, 1H), 2.49 (t, \(J = 5.2\) Hz, 4H), 3.31 (d, \(J = 2.4\) Hz, 2H), 3.45 (t, \(J = 5.2\) Hz, 4H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)), \(\delta\) 28.5, 47.3, 51.7, 73.7, 78.5, 79.6, 154.7. (Two carbons may be overlapping to account for the missing carbon peak.)
(3-bromopropoxy)(tert-butyl)dimethylsilane (II-50)

To a flame-dried flask was added imidazole, (3.54 g, 52.0 mmol) followed by dry DCM (100 mL). A solution of TBSCl (7.61 g, 45.3 mmol) in dry DCM (100 mL) was added to the flask by cannula. The reaction was cooled to 0°C and a solution of 3-bromopropanol (2.4 mL, 26 mmol) in dry DCM (40 mL) was added by cannula. The reaction was slowly warmed to ambient temperature and stirred for 5 h. Reaction concentrated by rotary evaporation and was purified by silica column chromatography to give the product as a colorless oil in quantitative yield. $^1$H NMR (400 MHz, CDCl$_3$), δ 0.02 (s, 6H), 0.81 (s, 9H), 1.90 (p, $J$ = 6 Hz, 2H), 3.40 (t, $J$ = 5.6 Hz, 2H), 3.63 (t, $J$ = 6 Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$), δ -5.2, 18.5, 26.1, 30.8, 35.6, 60.6.

1-(3-((tert-butyldimethylsilyl)oxy)propyl)-4-(prop-2-yn-1-yl)piperazine (II-49)

To a solution of II-47 in DCM was added TFA. The reaction was stirred at room temperature for one hour. The reaction was then concentrated by rotary evaporation, redissolved in EtOAc and passed through a plug of sodium bicarbonate. Filtrate was concentrated by rotary evaporation and used without further purification. To a solution of the deprotected piperazine derivative (218 mg, 1.17 mmol) in MeCN (13 mL) was added Cs$_2$CO$_3$ (1.40 g, 4.30 mmol). The reaction was stirred at ambient temperature for 45 min. TBS ether II-50 was then added and the reaction was heated at reflux, 12 h. The reaction was cooled and filtered. The filtrate was concentrated by rotary evaporation and the crude product mixture was purified by silica column chromatography to give the pure desired product as a colorless oil, 298 mg (71%). $^1$H NMR (400 MHz, CDCl$_3$), δ 0.05 (s, 6H), 0.90 (s, 9H), 1.67 (p, $J$ = 7.2 Hz, 2H), 2.43 (m, 7H),
3.52 (m, 4H), 3.64 (t, $J = 6.8$ Hz, 2H), 4.70 (d, $J = 2$ Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$), $\delta$ -5.1, 18.5, 26.2, 30.0, 44.0, 53.1, 53.2, 55.4, 61.4, 74.7, 154.6.

3-(4-(prop-2-yn-1-yl)piperazin-1-yl)propan-1-ol (II-51)

To a solution of piperazine derivative II-49 (121 mg, 0.408 mmol) in freshly distilled THF (7.1 mL) at 0°C was added a 1M solution of TBAF in THF (1.0 mL). The reaction was slowly warmed to ambient temperature and stirred for 15h. The reaction was concentrated by rotary evaporation and purified by silica column chromatography to give the desired product as a colorless oil, 25 mg (34%). $^1$H NMR (400 MHz, CDCl$_3$), $\delta$ 1.69 (p, $J = 6$ Hz, 2H), 2.25 (t, $J = 2$ Hz, 1H), 2.61 (br t, $J = 6$ Hz, 8H), 3.28 (d, $J = 2.4$ Hz, 1H), 3.78 (t, $J = 5.2$ Hz, 2H), 4.36 (br s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$), $\delta$ 27.3, 46.8, 52.0, 53.3, 58.8, 64.6, 73.5, 78.7.

Tert-butyl 4-(3-((tert-butyldimethylsilyl)oxy)propyl)piperazine-1-carboxylate (II-52)

To a solution of N-Boc piperazine (218 mg, 1.17 mmol) in MeCN (13.3 mL) was added Cs$_2$CO$_3$ (1.398 g, 4.291 mmol). The reaction was stirred at ambient temperature for 45 min. TBS ether (275 mg, 1.09 mmol) was then added and the reaction was heated under reflux for 12h. The reaction was then cooled and filtered. The filtrate was concentrated by rotary evaporation and the crude mixture was purified by silica column chromatography to give the product as a colorless oil, 298 mg (71%). $^1$H NMR (400 MHz, CDCl$_3$), $\delta$ 0.00 (s, 6H), 0.84 (s, 9H), 1.41 (s, 9H), 1.62 (p, $J = 6.4$ Hz, 2H), 2.32 (t, $J = 4.4$ Hz, 4H), 2.35
(t, J = 7.2 Hz, 2H), 3.37 (t, J = 4.8 Hz, 4H), 3.60 (t, J = 6.8 Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$), δ -5.2, 18.4, 26.0, 28.5, 30.0, 53.1, 55.3, 61.3, 79.6, 154.8. (Two carbons may be overlapping to account for the missing carbon peak.)

**Tert-butyl 4-(3-hydroxypropyl)piperazine-1-carboxylate (II-53)**

![Chemical Structure](image)

To a solution of II-52 (298 mg, 0.831 mmol) in freshly distilled THF (15 mL) at 0°C was added a 1M solution of TBAF in THF (2.1 mL). The reaction was slowly warmed to ambient temperature and stirred for 15h. The reaction was concentrated by rotary evaporation and purified by silica column chromatography to give the desired product as a colorless oil, 220 mg (59%). $^1$H NMR (400 MHz, CDCl$_3$), δ 1.41 (s, 9H), 1.66 (p, J = 6 Hz, 2H), 2.41 (t, J = 4.4 Hz, 4H), 2.55 (t, J = 6 Hz, 2H), 3.37 (t, J = 5.2 Hz, 4H), 3.73 (t, J = 5.2 Hz, 2H), 4.45 (b s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$), δ 26.0, 27.3, 28.5, 53.2, 58.6, 64.2, 79.8, 154.7.

**Methyl 3-methoxy-4-(pent-4-yn-1-yl oxy)benzoate (II-70)**

![Chemical Structure](image)

To a solution of methyl vanillate (420 mg, 2.303 mmol) in MeCN (20 mL) was added Cs$_2$CO$_3$ (1.322 g, 4.068 mmol). After stirring at r.t., 40 min., 5-chloropent-1-yne (0.26 mL, 2.4 mmol) and NaI (116 mg, 0.773 mmol) were added. Reaction was heated at reflux, 14h. Reaction was cooled to r.t. and filtered. Volatiles were removed by rotary evaporation and the crude product was purified by silica chromatography. Product eluted with (15:85) ethyl acetate: hexanes, to yield a white solid, 525 mg.
(92%). $^1$H (500MHz, CDCl$_3$), δ 2.01 (t, $J=3$Hz, 1H), 2.04 (m, $J=7$Hz, 2H), 2.41 (td, $J=6.5$Hz, 2.5Hz, 2H), 3.88 (s, 3H), 3.90 (s, 3H), 4.15 (t, $J=6.5$Hz, 2H), 6.89 (d, $J=8.5$Hz, 1H), 7.54 (d, $J=2$Hz, 1H), 7.64 (dd, $J=8.5$Hz, 1.5Hz, 1H); $^{13}$C (125MHz, CDCl$_3$), δ 15.1, 27.9, 52.0, 56.0, 67.2, 69.2, 83.2, 111.6, 112.4, 122.7, 123.5, 148.9, 152.4, 166.8.

**Methyl 5-methoxy-2-nitro-4-(pent-4-yn-1-yl)oxy)benzoate (II-71)**

![Methyl 5-methoxy-2-nitro-4-(pent-4-yn-1-yl)oxy)benzoate (II-71)](image)

To a solution of II-70 (52 mg, 0.210 mmol) in DCM (1.4 mL) at -41°C was added a pre-mixed solution of SnCl$_4$ (0.3mL, 1.0M in dichloromethane) and fuming nitric acid (0.01mL), dropwise. Reaction stirred 10 min, at -41°C; the reaction was then warmed to 0°C and stirred for 5 min. Reaction diluted with water, extracted with dichloromethane and washed with 10% sodium bicarbonate solution. Organic layers dried over MgSO$_4$, filtered. Filtrate was concentrated by rotary evaporation and purified by silica chromatography. Product eluted with (15:85) EtOAc/Hex and recrystallized from hexanes to yield a yellow solid, 51mg (83%), m.p. 63-64°C. $^1$H NMR (400 MHz, CDCl$_3$), δ 2.01 (t, $J=5.2$ Hz, 1H), 2.08 (m, $J=6.8$ Hz, 2H), 2.43 (td, $J=6.4$ Hz, 2.8 Hz, 2H), 3.91 (s, 3H), 3.97 (s, 3H), 4.20 (t, $J=6$ Hz, 2H), 7.08 (s, 1H), 7.49 (s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$), δ 15.2, 27.8, 53.5, 56.8, 68.0, 69.7, 83.0, 108.2, 111.1, 121.8, 141.3, 149.9, 153.0, 166.6.
Methyl 2-amino-5-methoxy-4-(pent-4-yloxy)benzoate (II-72)

\[
\text{\includegraphics[width=0.3\textwidth]{methyl_2-amino-5-methoxy-4-(pent-4-yloxy)benzoate.png}}
\]

To a solution of II-71 (414 mg, 1.41 mmol) in warm MeOH (45 mL) were added Na$_2$S$_2$O$_4$ (1.424 g, 8.3 mmol) and water (7.2 mL). The reaction was then heated at 65°C for 1h. After being cooled to r.t., passed through a bed of Celite, and concentrated by rotary evaporation, the reaction was partitioned between EtOAc and brine, dried over MgSO$_4$ and filtered. The filtrate was then concentrated by rotary evaporation and purified by silica chromatography to yield a yellow solid, 291 mg (78%), m.p. 94-97°C. $^1$H NMR (500 MHz, CDCl$_3$), δ 1.98 (t, J = 3 Hz, 1H), 2.04 (m, J = 6.5 Hz, 2H), 2.40 (td, J = 7 Hz, 2.5 Hz, 2H), 3.80 (s, 3H), 3.85 (s, 3H), 4.09 (t, J = 6 Hz, 2H), 5.59 (br s, 2H), 6.17 (s, 1H), 7.31 (s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$), δ 15.3, 27.8, 51.6, 56.7, 66.9, 69.3, 83.4, 100.4, 102.2, 113.1, 140.8, 147.2, 154.3, 168.3.

6-methoxy-7-(pent-4-yloxy)quinazolin-4(3H)-one (II-73)

\[
\text{\includegraphics[width=0.3\textwidth]{6-methoxy-7-(pent-4-yloxy)quinazolin-4(3H)-one.png}}
\]

To a solution of II-72 (3.066 g, 11.64 mmol) in hot formamide (27 mL) was added ammonium formate (2.684 g, 42.56 mmol). The reaction was heated at 125°C, 5h. The reaction was then poured over water (30 mL) and allowed to cool to r.t. The reaction was then filtered and washed with cold water to give the product as a tan solid, 3.007 g (89%), m.p. 232-235 °C. $^1$H NMR (400 MHz, CDCl$_3$), δ 1.93 (m, J = 6.8 Hz, 2H), 2.35 (td, J = 7.6 Hz, 2.4 Hz, 2H), 2.87 (t, J = 1.6 Hz, 1H), 3.89 (s, 3H), 4.17 (t, J = 6 Hz, 2H), 7.14 (s, 1H), 7.46 (s, 1H), 8.00 (s, 1H), 12.11 (br s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$), δ 15.2, 28.2, 56.5, 67.6, 72.4, 84.2, 105.7, 109.3, 116.3, 144.5, 145.5, 149.5, 154.2, 160.7.
4-chloro-6-methoxy-7-(pent-4-yn-1-yloxy)quinazoline (II-74)

![Chemical Structure]

To a solution of 4 (82.5 mg, 0.319 mmol) and triphenylphosphine (177 mg, 0.673 mmol) in 1,2-dichloroethane (7.5 mL) was added carbon tetrachloride (0.10 mL). The reaction was heated at 70°C for 4h, and then cooled to r.t. The reaction was then partitioned between DCM and water, and the organic layers were washed with brine and dried over MgSO\(_4\). After filtration, the filtrate was concentrated by rotary evaporation and purified by silica chromatography to yield the product as a white solid, 65 mg (74%), m.p. 134-135°C. \(^1\)H NMR (500 MHz, CDCl\(_3\)), δ 2.01 (t, \(J = 2.5\) Hz, 1H), 2.14 (m, \(J = 7\) Hz, 2H), 2.47 (td, \(J = 7\) Hz, 3 Hz, 2H), 4.05 (s, 3H), 4.32 (t, \(J = 6.5\) Hz, 2H), 7.35 (s, 1H), 7.38 (s, 1H), 8.86 (s, 1H); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)), δ 15.3, 27.7, 56.5, 67.8, 69.6, 83.0, 102.8, 107.7, 119.6, 149.2, 151.8, 152.6, 156.3, 159.1.

3-chloro-4-((3-fluorobenzyl)oxy)aniline (II-77)

![Chemical Structure]

To a solution of 2-chloro-4-nitrophenol (315 mg, 1.82 mmol) in MeCN (30 mL) was added Cs\(_2\)CO\(_3\) (1.232 g, 3.780 mmol). The reaction was stirred at room temperature, 30 min. 3-fluorobenzyl chloride (0.50 mL, 4.2 mmol) was added to the reaction, followed by NaI (136 mg, 0.904 mmol). The reaction was heated at reflux for 3.5 h, then cooled and filtered. The crude product was purified by silica chromatography to give the product as an off-white solid, 976 mg (95%). This nitro derivative was then reduced to the aniline as follows: To a solution of the nitro derivative (886 mg, 3.15 mmol) in (2:1) glacial acetic acid/ water was added Fe(s) (3.695 g, 15.47 mmol). The reaction was heated at reflux for 3.5 h, then cooled and filtered. The crude product was purified by silica column chromatography and was
eluted as a light brown solid, 328 mg (37%). $^1$H NMR (500 MHz, CDCl$_3$), δ 3.50 (br s, 2H), 5.20 (s, 2H), 6.49 (dd, $J = 8.5$ Hz, 2.5 Hz, 1H), 6.75 (t, $J = 2$ Hz, 1H), 6.78 (s, 1H), 6.98 (td, $J = 8$ Hz, 2.5 Hz, 1H), 7.18 (m, 2H), 7.31 (td, $J = 8$ Hz, 6 Hz, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$), δ 114.2, 114.4, 114.5, 114.8, 115.0, 117.1, 117.4, 122.82, 122.84, 124.7, 130.2, 130.3, 139.8, 139.9, 141.7, 147.0, 161.9, 164.4.

3-chloro-4-(pyridin-2-ylmethoxy)aniline (II-78)

![Chemical Structure]

To a solution of 2-chloro-4-nitrophenol (551 mg, 3.18 mmol) in MeCN (50 mL) was added Cs$_2$CO$_3$ (2.082 g, 6.39 mmol). The reaction was stirred at room temperature for 30 min. Picolyl chloride HCl (606 mg, 6.78 mmol) was then added, followed by NaI (275 mg, 1.84 mmol). The reaction was heated under reflux for 3 h, cooled and filtered. The filtrate was concentrated by rotary evaporation and purified by silica column chromatography, to give the nitrated product as an off-white solid, 752 mg (89%). The nitro derivative was reduced to the aniline as follows: To a solution of the nitro derivative (750 mg, 2.83 mmol) in (2:1) glacial acetic acid/water (15 mL) was added Fe(s) (2.915 g, 12.42 mmol). The reaction was heated at reflux for 3 h, then cooled and filtrated. The filtrate was concentrated and purified by silica column chromatography to give the product as a pink solid, 388 mg (58%). $^1$H NMR (500 MHz, CDCl$_3$), δ 3.58 (br s, 2H), 5.19 (s, 2H), 6.50 (dd, $J = 11$ Hz, 3.5 Hz, 1H), 6.75 (s, 1H), 6.80 (d, $J = 11$ Hz, 1H), 7.21 (t, $J = 8.5$ Hz, 1H), 7.65 (d, $J = 9.5$ Hz, 1H), 7.72 (td, $J = 10$ Hz, 1.5 Hz, 1H), 8.57 (d, $J = 6.5$ Hz, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$), δ 72.0, 114.6, 116.5, 117.0, 122.1, 123.2, 123.8, 137.8, 143.2, 146.5, 148.4, 157.3.
**N-(3-fluorophenyl)-6-methoxy-7-(pent-4-yn-1-yloxy)quinazolin-4-amine (II-79)**

![Chemical Structure](image)

To a solution of **II-74** (106 mg, 0.384 mmol) in 2-propanol (5 mL) was added 4-fluoroaniline (0.1 mL, 1.0 mmol). The reaction was heated at reflux, 4h. Reaction cooled to r.t. and filtered to give the desired product as a white solid, 134 mg (84%), m.p. 239-240 °C. \(^1\)H NMR (500 MHz, DMSO-\(d_6\)), 2.00 (m, \(J = 6\) Hz, 2H), 2.37 (td, \(J = 7\) Hz, 2.5 Hz, 2H), 2.88 (t, \(J = 2.5\) Hz, 1H), 4.31 (s, 3H), 4.23 (t, \(J = 6\) Hz, 2H), 7.13 (td, \(J = 8.5\) Hz, 3 Hz, 1H), 7.40 (s, 1H), 7.50 (m, \(J = 8\) Hz, 1 Hz, 1H), 7.60 (dd, \(J = 7.5\) Hz, 1 Hz, 1H), 7.71 (dt, \(J = 11\) Hz, 2.5 Hz, 1H), 8.40 (s, 1H), 8.88 (s, 1H), 11.53 (s, 1H); \(^{13}\)C NMR (100 MHz, DMSO-\(d_6\)), \(\delta\) 15.1, 27.9, 57.8, 68.3, 72.7, 83.9, 101.0, 104.8, 108.1, 112.2, 112.5, 113.3, 113.5, 121.1, 130.9, 131.0, 136.5, 139.4, 149.3, 150.9, 156.2, 158.8, 161.2, 163.7.

**N-(3-chloro-4-fluorophenyl)-6-methoxy-7-(pent-4-yn-1-yloxy)quinazolin-4-amine (II-80)**

![Chemical Structure](image)

To a solution of **II-74** (200 mg, 0.723 mmol) in 2-propanol (9.5 mL) was added aniline derivative (155 mg, 1.06 mmol). The reaction was heated at reflux, 4h. Reaction cooled to r.t. and product collected by filtration as a white solid, 283 mg (93%), m.p. 256 °C. \(^1\)H NMR (500 MHz, DMSO-\(d_6\)), \(\delta\) 2.00 (m, \(J = 6.5\) Hz, 2H), 2.34 (td, \(J = 7\) Hz, 2.5 Hz, 2H), 2.88 (t, \(J = 3\) Hz, 1H), 4.02 (s, 3H), 4.25 (t, \(J = 6.5\) Hz, 2H), 7.33 (s, 1H), 7.55 (t, \(J = 9\) Hz, 1H), 7.71 (dddd, \(J = 4.5\) Hz, 3 Hz, 1H), 8.02 (dd, \(J = 7\) Hz, 2.5 Hz, 1H), 8.25 (s, 1H), 8.87 (s, 1H), 11.31 (br s, 1H); \(^{13}\)C NMR (125 MHz, DMSO-\(d_6\)), \(\delta\) 15.1, 27.9, 57.8, 68.3, 72.7, 83.9,
To a solution of \textbf{II-74} (205 mg, 0.739 mmol) in 2-propanol (10 mL) was added aniline \textbf{II-77} (192 mg, 0.761 mmol). The reaction was heated at reflux, 12h. Reaction cooled to r.t. and filtered to give the desired product as a white solid, 376 mg (96%), m.p. 250-251°C. $^1$H NMR (500 MHz, DMSO-$d_6$), $\delta$ 1.99 (m, $J = 6.5$ Hz, 2H), 2.37 (td, $J = 7.5$ Hz, 2.5 Hz, 2H), 2.88 (t, $J = 3$ Hz, 1H), 4.02 (s, 3H), 4.22 (t, $J = 6$ Hz, 2H), 5.30 (s, 2H), 7.17 (td, $J = 8$ Hz, 3 Hz, 1H), 7.31 (m, 2H), 7.34 (s, 1H), 7.37 (s, 1H), 7.46 (m, 1H), 7.63 (dd, $J = 9$ Hz, 2.5 Hz, 1H), 7.87 (dd, $J = 2.5$ Hz, 1 Hz, 1H), 8.34 (s, 1H), 8.83 (s, 1H), 11.46 (s, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$), $\delta$ 15.1, 27.9, 57.7, 68.3, 70.0, 72.7, 83.9, 100.9, 104.8, 107.9, 114.6, 114.7, 114.8, 115.4, 115.6, 121.8, 124.0, 125.4, 127.1, 131.2, 131.3, 136.0, 140.0, 140.1, 149.4, 150.8, 152.2, 156.0, 158.7, 161.6, 164.1.
N-(3-chloro-4-(pyridin-2-ylmethoxy)phenyl)-6-methoxy-7-(pent-4-yn-1-yloxy)quinazolin-4-amine (II-85)

To a solution of II-74 (205 mg, 0.742 mmol) in 2-propanol (10 mL) was added aniline II-78 (199 mg, 0.849 mmol). The reaction was heated at reflux, 12h. Reaction cooled to r.t. and filtered to give the desired product as a white solid, 362 mg (96 %), m.p. 253°C. \(^1\)H NMR (500 MHz, DMSO-d\(_6\)), \(\delta\) 1.99 (m, \(J = 6.5\) Hz, 2H), 2.37 (td, \(J = 7\) Hz, 2.5 Hz, 2H), 2.88 (t, \(J = 2.5\) Hz, 1H), 4.02 (s, 3H), 4.22 (t, \(J = 5.5\) Hz, 2H), 5.34 (s, 2H), 7.33 (d, \(J = 9.5\) Hz, 1H), 7.37 (s, 1H), 7.38 (t, \(J = 7\) Hz, 1H), 7.60 (d, \(J = 7.5\) Hz, 1H), 7.63 (dd, \(J = 9\) Hz, 2.5 Hz, 1H), 7.89 (m, 2H), 8.34 (s, 1H), 8.61 (d, \(J = 4.5\) Hz, 1H), 8.83 (s, 1H), 11.46 (br s, 1H); \(^{13}\)C NMR (100 MHz, DMSO-d\(_6\)), \(\delta\) 15.1, 27.9, 57.7, 68.3, 71.7, 72.7, 83.9, 101.0, 104.8, 107.9, 114.6, 121.7, 122.2, 123.9, 125.4, 127.1, 131.3, 136.2, 138.0, 149.5, 149.8, 150.8, 152.2, 156.0, 156.7, 158.7.

N-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-3-(tributylstannyl)benzamide (II-89)

To a solution of NHS ester II-83 (390 mg, 0.767 mmol) in dichloroethane (20mL) was added azide derivative II-84 (169 mg, 0.776 mmol), followed by triethylamine (0.51 mL, 3.6 mmol). The reaction was stirred 12h at room temperature, followed by removal of volatiles by rotary evaporation. The crude product was then purified by silica chromatography, which had been pre-neutralized with (1:99) triethylamine: hexane, to give the product as a colorless oil, 335 mg (71%). IR(cm\(^{-1}\)): 1639, 2100, 3339;
$^1$H NMR (400MHz, CDCl$_3$), δ 0.87 (t, $J = 7.2$ Hz, 9H), 1.06 (t, $J = 8$ Hz, 6H), 1.30 (m, $J = 6.4$ Hz, 6H), 1.50 (m, $J = 8$ Hz, 6H), 3.33 (t, $J = 4.4$ Hz, 2H), 3.61 (m, 14H), 6.36 (br s, 1H), 7.34 (t, $J = 7.2$ Hz, 1H), 7.57 (d, $J = 7.2$ Hz, 1H), 7.64 (d, $J = 8$ Hz, 1H), 7.90 (s, 1H); $^{13}$C NMR (100MHz, CDCl$_3$), δ 9.8, 13.8, 27.5, 29.2, 39.9, 50.8, 70.1, 70.2, 20.4, 70.76, 70.80, 70.83, 126.4, 127.9, 134.1, 135.2, 139.7, 143.1, 168.4.

$N$-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)ethyl)-3-iodobenzamide (II-90)

To a solution of stannane derivative II-85 (335 mg, 0.586 mmol) in dry THF (19 mL), at 0°C and shielded from light, was added $N$-iodosuccinimide (140 mg, 0.621 mmol). The reaction was stirred at 0°C, 30 min. and was then warmed to r.t. The volatiles were removed by rotary evaporation and the crude product was purified by silica chromatography. The product eluted with (7:3) ethyl acetate:hexanes, to give a colorless oil, 212 mg (81%). IR (cm$^{-1}$): 1645, 2101, 3335; $^1$H NMR (400MHz, CDCl$_3$), δ 3.34 (t, $J = 4.4$ Hz, 2H), 3.62 (m, 14H), 6.88 (br s, 1H), 7.15 (t, $J = 10$ Hz, 1H), 7.76 (d, $J = 8$ Hz, 1H), 7.81 (d, $J = 7.6$ Hz, 1H), 8.14 (s, 1H); $^{13}$C NMR (100MHz, CDCl$_3$), δ 40.1, 50.8, 69.8, 70.2, 70.4, 70.7, 70.79, 70.84, 94.3, 126.5, 130.3, 136.3, 136.8, 140.4, 166.1.
To a solution of quinazoline derivative II-79 (28 mg, 0.067 mmol) and azide derivative II-86 (29 mg, 0.065 mmol) in (1:1) THF/H$_2$O (20 mL) was added [Cu(MeCN)$_4$]PF$_6$ (25 mg, 0.067 mmol). The reaction was stirred at 90°C, 2.5 h. Reaction was cooled to r.t. and partitioned between EtOAc and brine. Organics were then dried over MgSO$_4$, filtered, and concentrated by rotary evaporation. The product was purified by silica chromatography, eluting with (1:9) MeOH/EtOAc. Product was precipitated from DCM to give a colorless, amorphous solid, 17 mg (36%). $^1$H NMR (500 MHz, CDCl$_3$), δ 2.26 (p, $J = 7$ Hz, 2H), 2.92 (t, $J = 7$ Hz, 2H), 3.56-3.60 (m, 12H), 3.79 (t, $J = 5$ Hz, 2H), 3.92 (s, 3H), 4.15 (t, $J = 6.5$ Hz, 2H), 4.44 (t, $J = 5$ Hz, 2H), 6.79 (td, $J = 8.5$ Hz, 2.5 Hz, 1H), 7.00 (br s, 1H), 7.10 (t, $J = 8$ Hz, 1H), 7.19 (s, 1H), 7.21 (s, 1H), 7.27 (q, $J = 8.5$ Hz, 1H), 7.40 (d, $J = 8$ Hz, 1H), 7.48 (s, 1H), 7.69 (d, $J = 11.5$ Hz, 1H), 7.72 (d, $J = 8$ Hz, 1H), 7.78 (d, $J = 8$ Hz, 1H), 7.97 (br s, 1H), 8.10 (s, 1H), 8.64 (s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$), δ 22.3, 28.5, 40.1, 50.4, 56.5, 68.1, 69.7, 69.8, 70.4, 70.58, 70.65, 70.73, 94.4, 100.3, 108.5, 109.0, 109.3, 109.4, 110.6, 110.9, 117.1, 122.5, 126.6, 130.1, 130.2, 130.4, 136.2, 136.7, 140.5, 140.7, 140.8, 147.0, 147.4, 149.9, 153.4, 154.4, 156.4, 162.0, 164.4, 166.4.
To a solution of quinazoline derivative II-80 (53 mg, 0.12 mmol) and azide derivative II-86 (53 mg, 0.12 mmol) in (1:1) THF/H\textsubscript{2}O (25 mL) was added [Cu(MeCN)]\textsubscript{4}PF\textsubscript{6} (47 mg, 0.13 mmol). The reaction was stirred at 90°C, 15h. Reaction was cooled to r.t. and partitioned between EtOAc and brine. Organics were then dried over MgSO\textsubscript{4}, filtered, and concentrated by rotary evaporation. The product was purified by silica chromatography, eluting with (1:9) MeOH/EtOAc. Product was precipitated from DCM to give a colorless, amorphous solid, 89 mg (90%). \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}), δ 2.21 (m, \textit{J} = 7 Hz, 2H), 2.90 (t, \textit{J} = 7.5 Hz, 2H), 3.56 (m, 12H), 3.79 (m, 4H), 4.11 (m, 3H), 4.43 (t, \textit{J} = 5 Hz, 2H), 7.07 (m, 4H), 7.34 (br s, 1H), 7.49 (s, 1H), 7.538 (m, 1H), 7.72 (d, \textit{J} = 7.5 Hz, 1H), 7.76 (d, \textit{J} = 7.5 Hz, 1H), 7.80 (dd, \textit{J} = 6 Hz, 2 Hz, 1H), 8.09 (s, 1H), 8.38 (br s, 1H), 8.60 (br s, 1H); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}), δ 22.2, 28.5, 40.1, 50.3, 56.3, 60.6, 67.9, 69.6, 69.7, 70.3, 70.5, 70.6, 70.7, 94.3, 100.6, 108.4, 109.3, 116.5, 116.7, 120.7, 120.9, 122.1, 122.2, 122.5, 124.4, 126.5, 130.4, 135.8, 136.2, 136.5, 140.5, 147.0, 149.8, 153.5, 154.2, 156.0, 156.6, 166.4.
To a solution of quinazoline derivative II-81 (50 mg, 0.095 mmol) and azide derivative II-86 (53 mg, 0.12 mmol) in (1:1) THF/H₂O (25 mL) was added [Cu(MeCN)]₄PF₆ (41 mg, 0.11 mmol). The reaction was stirred at 90°C, 6h. Reaction was cooled to r.t. and partitioned between EtOAc and brine. Organics were then dried over MgSO₄, filtered, and concentrated by rotary evaporation. The product was purified by silica chromatography, eluting with (1:9) MeOH/EtOAc. Product was precipitated from DCM to give a colorless, amorphous solid, 74 mg (83%). ¹H NMR (500 MHz, CDCl₃), δ 2.21 (m, J = 7 Hz, 2H), 2.90 (t, J = 6 Hz, 2H), 3.54 (m, 12H), 3.78 (t, J = 5.5 Hz, 2H), 3.82 (s, 3H), 4.10 (m, 3H), 4.42 (t, J = 5 Hz, 2H), 5.09 (s, 2H), 6.89 (d, J = 9 Hz, 1H), 6.98 (td, J = 8 Hz, 2 Hz, 1H), 7.08 (m, 3H), 7.18 (m, 2H), 7.31 (m, 2H), 7.47 (s, 1H), 7.49 (dd, J = 8.5 Hz, 2.5 Hz, 1H), 7.68 (d, J = 2.5 Hz, 1H), 7.72 (dd, J = 8 Hz, 2H), 8.10 (t, J = 1.5 Hz, 1H), 8.24 (br s, 1H), 8.57 (s, 1H); ¹³C NMR (100 MHz, CDCl₃), δ 22.2, 28.5, 40.1, 50.3, 56.3, 60.6, 67.9, 69.6, 69.7, 70.3, 70.5, 70.56, 70.63, 94.3, 100.6, 108.3, 109.2, 114.0, 114.2, 114.4, 114.9, 115.1, 122.3, 122.5, 122.6, 122.6, 123.3, 125.0, 126.5, 130.27, 130.33, 133.1, 136.2, 136.6, 139.27, 139.34, 140.41, 146.9, 147.2, 149.6, 150.8, 153.6, 154.1, 156.8, 161.9, 164.3, 166.4.
To a solution of quinazoline derivative II-83 (50 mg, 0.098 mmol) and azide derivative II-86 (54 mg, 0.12 mmol) in (1:1) THF/H₂O (25 mL) was added [Cu(MeCN)]₄PF₆ (46 mg, 0.12 mmol). The reaction was stirred at 90°C, 15h. Reaction was cooled to r.t. and partitioned between EtOAc and brine. Organics were then dried over MgSO₄, filtered, and concentrated by rotary evaporation. The product was purified by silica chromatography, eluting with (1:9) MeOH/EtOAc. Product was precipitated from DCM to give a colorless, amorphous solid, 60 mg (66%). ¹H NMR (500 MHz, CDCl₃), δ 2.23 (m, J = 6 Hz, 2H), 2.90 (t, J = 6.5 Hz, 2H), 3.55 (m, 12H), 3.79 (t, J = 5.5 Hz, 2H), 3.83 (s, 3H), 4.11 (m, 2H), 4.43 (t, J = 5.5 Hz, 2H), 5.24 (s, 2H), 6.93 (d, J = 9 Hz, 1H), 7.09 (t, J = 8 Hz, 1H), 7.15 (br s, 1H), 7.23 (dd, J = 7 Hz, 5 Hz, 1H), 7.29 (s, 1H), 7.47 (m, 2H), 7.63 (d, J = 7.5 Hz, 1H), 7.72 (m, 4H), 8.10 (s, 1H), 8.20 (br s, 1H), 8.56 (m, 2H); ¹³C NMR (100 MHz, CDCl₃), δ 22.2, 28.5, 40.1, 50.3, 56.4, 60.6, 68.0, 69.6, 69.8, 70.3, 70.5, 70.6, 70.7, 71.7, 94.3, 100.6, 108.2, 109.1, 114.0, 121.5, 122.3, 122.5, 123.0, 123.1, 125.1, 126.5, 130.3, 133.0, 136.2, 136.6, 137.2, 140.4, 146.9, 149.2, 149.7, 150.8, 153.5, 154.2, 156.8, 156.9, 166.4.

2-(2-azidoethoxy)ethyl 4-methylbenzenesulfonate (II-88)

To a solution of triethylene glycol ditosylate II-87 (1.980 g, 4.778 mmol) in EtOH (50 mL) was added NaN₃ (142 mg, 2.18 mmol). The reaction was heated under reflux for two hours. The reaction was
cooled and concentrated by rotary evaporation. The crude reaction mixture was purified by silica column chromatography to give a colorless oil, 366 mg (27%). $^1$H NMR (500 MHz, CDCl$_3$), $\delta$ 2.45 (s, 3H), 3.36 (t, $J = 5.5$ Hz, 2H), 3.60 (m, 3H), 3.63 (t, $J = 6.5$ Hz, 2H), 3.69 (t, $J = 5.5$ Hz, 2H), 4.15 (t, $J = 5.5$ Hz, 2H), 7.34 (d, $J = 10.5$ Hz, 2H), 7.79 (d, $J = 10$ Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$), $\delta$ 21.7, 50.7, 68.8, 69.4, 70.1, 70.6, 70.8, 128.08, 129.9, 132.9, 145.0.

1-azido-2-(2-fluoroethoxy)ethane (II-89)

To a solution of azide (153 mg, 0.471 mmol) in tert-amyl alcohol (1.9 mL) was added TBAF$\cdot$3H$_2$O. The reaction was heated under reflux for 1.5 h, cooled, and concentrated by rotary evaporation. The reaction mixture was then diluted with EtOAc and extracted with saturated sodium bicarbonate (40 mL x 5). The organic layers were dried over MgSO$_4$. The MgSO$_4$ was removed by filtration and the filtrate was concentrated by rotary evaporation. The product was obtained as a colorless oil and used without further purification, 78 mg (55%) $^1$H NMR (500 MHz, CDCl$_3$), $\delta$ 3.39 (t, $J = 5$ Hz, 2H), 3.67 (m, 6H), 3.72 (t, $J = 4.5$ Hz, 1H), 3.78 (t, $J = 4$ Hz, 1H), 4.51 (t, $J = 4$ Hz, 1H), 4.61 (t, $J = 4$ Hz, 1H).
7-(3-(1-(2-(fluoroethoxy)ethyl)-1H-1,2,3-triazol-4-yl)propoxy)-N-(3-fluorophenyl)-6-methoxyquinazolin-4-amine (II-58)

To a solution of quinazoline derivative II-79 (26 mg, 0.063 mmol) in (1:1) THF/H₂O (50mL) was added azide derivative II-89, followed by [Cu(MeCN)₄]PF₆. The reaction was heated at reflux, 12h. The reaction was extracted with EtOAc and washed with brine. The organic layers were dried over MgSO₄. MgSO₄ was removed by filtrated and the filtrate was concentrated by rotary evaporation. The crude reaction mixture was purified by silica column chromatography to give the product as a colorless oil, 19 mg (86%). ¹H NMR (500 MHz, CDCl₃), δ 2.22 (p, J = 5.6 Hz, 2H), 2.90 (t, J = 6.4 Hz, 2H), 3.58 (m, 4H), 3.64 (m, 1H), 3.70 (m, 1H), 3.82 (t, J = 3.6 Hz, 2H), 3.86 (s, 3H), 4.12 (t, J = 4.8 Hz, 2H), 4.47 (m, 3H), 4.57 (t, J = 3.2, 1H), 6.77 (tdd, J = 6.8 Hz, 2 Hz, 0.8 Hz, 1H), 7.15 (s, 1H), 7.18 (s, 1H), 7.25 (m, 1H), 7.36 (dd, J = 6.4 Hz, 0.8 Hz, 1H), 7.54 (s, 1H), 7.63 (dt, J = 9.2 Hz, 1.6 Hz, 1H), 7.94 (br s, 1H), 8.62 (s, 1H); ¹³C NMR (100 MHz, CDCl₃), δ 22.2, 28.5, 50.3, 56.4, 68.0, 69.7, 70.4, 70.6, 70.8, 82.4, 84.1, 100.0, 108.4, 109.1, 109.3, 110.6, 117.1, 122.6, 130.0, 130.1, 140.6, 140.7, 147.1, 147.4, 149.9, 153.3, 154.3, 156.3, 161.9, 164.3.
7-(3-(1-(2-(fluoroethoxy)ethyl)-1H-1,2,3-triazol-4-yl)propoxy)-N-(3-fluorophenyl)-6-methoxyquinazolin-4-amine (II-59)

Prepared according to procedure for II-58, 10 mg (16%). $^1$H NMR (500 MHz, CDCl$_3$), $\delta$ 2.22 (p, $J = 6.5$ Hz, 2H), 2.92 (t, $J = 7.5$ Hz, 2H), 3.60 (m, 4H), 3.57 (t, $J = 4$ Hz, 1H), 3.72 (t, $J = 4$ Hz, 1H), 3.81 (s, 3H), 3.83 (t, $J = 5$ Hz, 2H), 4.12 (t, $J = 6$ Hz, 2H), 4.49 (m, 3H), 4.58 (t, $J = 4$ Hz, 1H), 7.08 (t, $J = 9$ Hz, 1H), 7.13 (s, 1H), 7.24 (s, 1H), 7.52 (dd, $J = 9.5$ Hz, 4 Hz, 3 Hz, 1H), 7.58 (s, 1H), 7.77 (dd, $J = 6.5$ Hz, 2.5 Hz, 1H), 8.16 (br s, 1H), 8.59 (s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$), $\delta$ 14.4, 22.1, 28.5, 50.4, 56.3, 60.6, 67.9, 69.7, 70.4, 70.6, 70.8, 82.4, 84.1, 100.2, 108.4, 109.1, 116.5, 116.7, 120.8, 121.0, 122.3, 122.6, 124.6, 135.7, 147.1, 147.4, 149.9, 153.4, 153.6, 156.0, 156.5.

$N$-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-7-(3-(1-(2-(fluoroethoxy)ethyl)-1H-1,2,3-triazol-4-yl)propoxy)-6-methoxyquinazolin-4-amine (II-60)

Prepared according to procedure for II-58, 24 mg (84 %). $^1$H NMR (500 MHz, CDCl$_3$), $\delta$ 2.29 (m, 3H), 2.96 (t, $J = 8$ Hz, 2H), 3.60 (m, 4H), 3.851 (t, $J = 5$ Hz, 2H), 4.00 (s, 3H), 4.21 (t, $J = 6$ Hz, 2H), 4.51 (t, $J = 5$ Hz, 2H), 5.17 (s, 2H), 6.98 (s, 1H), 6.99 (s, 1H), 7.01 (td, $J = 8.5$ Hz, 2.5 Hz, 1H), 7.08 (br s, 1H),
7.21 (m, 6H), 7.34 (dddd, $J = 13.5$ Hz, 7.5 Hz, 5.5 Hz, 1H), 7.51 (dd, $J = 9$ Hz, 3 Hz, 1H), 7.55 (s, 1H), 7.76 (d, $J = 2.5$ Hz, 1H), 8.616 (s, 1H).

$N$-(3-chloro-4-(pyridin-2-ylmethoxy)phenyl)-7-(3-(1-(2-(2-fluoroethoxy)ethyl)-1H-1,2,3-triazol-4-yl)propoxy)-6-methoxyquinazolin-4-amine (II-65)

Prepared according to procedure for II-58, 8 mg (14 %). $^1$H NMR (500 MHz, CDCl$_3$), $\delta$ 2.26 (p, $J = 7$ Hz, 2H), 2.94 (t, $J = 7.5$ Hz, 2H), 3.59 (m, 4H), 3.65 (m, 1H), 3.71 (m, 1H), 3.84(t, $J = 5$ Hz, 2H), 3.91 (s, 3H), 4.17 (t, $J = 6$ Hz, 2H), 4.49 (m, 3H), 4.58 (t, $J = 4$ Hz, 1H), 5.27 (s, 2H), 6.97 (d, $J = 9$ Hz, 1H), 7.11 (s, 1H), 7.18 (s, 1H), 7.24 (ddd, $J = 7$ Hz, 4.5 Hz, 1 Hz, 1H), 7.46 (dd, $J = 9$ Hz, 2.5 Hz, 1H), 7.55 (s, 1H), 7.58 (br s, 1H), 7.65 (d, $J = 8$ Hz, 1H), 7.74 (m, 1H), 8.59 (m, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$), $\delta$ 22.3, 28.6, 50.3, 56.4, 68.1, 69.7, 70.4, 70.6, 70.8, 71.7, 82.4, 84.1, 99.9, 108.6, 109.0, 114.2, 121.5, 122.3, 122.5, 123.0, 123.2, 125.1, 132.8, 137.3, 147.1, 147.3, 149.9, 150.9, 153.6, 154.3, 156.6, 157.0.
2-(2-(2-azidoethoxy)ethoxy)ethoxy)-N,N-bis(pyridin-2-ylmethyl)ethanamine (II-91)

![Chemical Structure](image)

To a solution of 11-azido-3,6,9-trioxaundecan-1-amine II-84 (556 mg, 2.55 mmol) in DCE (5 mL) at room temperature was added 2-pyridine carboxaldehyde (503 mg, 4.69 mmol), followed by sodium triacetoxyborohydride (734 mg, 3.47 mmol). The reaction was stirred at r.t. for 3 h, and then all volatiles were removed by rotary evaporation. The crude product was purified by silica chromatography, eluting with (1:9) methanol:ethyl acetate to give the product as an amber oil, 851 mg (91%). IR (cm⁻¹): 604.76, 756.80, 1002.55, 1054.50, 1217.07, 1434.74, 1595.36, 3228.39. ¹H NMR (500 MHz, CDCl₃), δ 2.81 (t, J = 6 Hz, 2H), 3.35 (t, J = 4.5 Hz, 2H), 3.55 (m, 2H), 3.62-3.66 (m, 10H), 3.89 (s, 4H), 7.14 (ddd, J = 7.5 Hz, 5 Hz, 1 Hz, 2H), 7.54 (d, J = 8 Hz, 2H), 7.64 (td, J = 8 Hz, 1.5 Hz, 2H), 8.54 (dd, J = 4.5 Hz, 0.5 Hz, 2H); ¹³C NMR (100 MHz, CDCl₃), δ 50.9, 53.8, 60.9, 69.7, 70.2, 70.6, 70.87, 70.91, 122.2, 123.2, 136.8, 149.2, 159.9. (Two carbons may be overlapping to account for the missing carbon peak.)

fac[Re(CO)₃]⁻ 2-(2-(2-azidoethoxy)ethoxy)ethoxy)-N,N-bis(pyridin-2-ylmethyl)ethanamine (II-92)

![Chemical Structure](image)

To a solution of II-91 in (29 mg, 0.072 mmol) in water was added 0.029 M aqueous fac-[Re(CO)₃(H₂O)₃]OTf (2.5 mL). The reaction was stirred at room temperature for 1 h. Complete conversion of II-91 seen by ¹H NMR, no further purification. ¹H NMR (400 MHz, CDCl₃), δ 3.36 (t, J = 5.2 Hz, 2H), 3.66 (m, 6H), 3.75 (m, 4H), 4.02 (d, J = 6.8 Hz, 4H), 4.61 (d, J = 16 Hz, 2H), 5.4 (d, J =
16Hz, 2H), 7.21 (t, J = 6.8 Hz, 2H), 7.77 (d, J = 8 Hz, 2H), 7.82 (td, J = 6.8 Hz, 1.2 Hz, 2H), 8.65 (d, J = 4.8 Hz, 2H).

\[ N-(3\text{-fluorophenyl})-6\text{-methoxy}-7-(3-(1-(pyridin-2-yl)-2-(pyridin-2-ylmethyl)-5,8,11\text{-trioxo-2-azatridecan-13-yl})-1H-1,2,3\text{-triazol-4-yl})propoxy)quinazolin-4-amine (II-93) \]

To a solution of quinazoline derivative II-79 (25 mg, 0.069 mmol) in (1:1) THF/H\textsubscript{2}O (25 mL) were added azide derivative II-91 (27 mg, 0.067 mmol) and [Cu(MeCN)\textsubscript{4}]PF\textsubscript{6} (32 mg, 0.086 mmol). The reaction was heated under reflux, 12h. The reaction was cooled, partitioned between EtOAc and 4M aqueous NaEDTA solution, and washed with brine. The organic layers were dried over MgSO\textsubscript{4}. MgSO\textsubscript{4} was removed by filtrated and the filtrate was concentrated by rotary evaporation. The crude reaction mixture was purified by silica column chromatography eluting with (15:85) MeOH/CHCl\textsubscript{3}, to give the product as a colorless oil, 11 mg (24%). \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}), δ 2.26 (p, J = 7 Hz, 2H), 2.77 (t, J = 6.5 Hz, 2H), 2.94 (t, J = 7.5 Hz, 2H), 3.45 (m, 8 H), 3.55 (t, J = 6 Hz, 2H), 3.77 (t, J = 4.5 Hz, 2H), 3.83 (s, 3H), 3.86 (s, 3H), 4.16 (t, J = 5.5 Hz, 2H), 4.46 (t, J = 5.5 Hz, 2H), 6.79 (td, J = 8.5 Hz, 2 Hz, 1H), 7.12 (ddd, J = 7.5 Hz, 5 Hz, 1 Hz, 2H), 7.20 (s, 1H), 7.26 (s, 1H), 7.274 (m, 1H), 7.37 (dd, J = 7.5 Hz, 1 Hz, 1H), 7.50 (s, 1H), 7.52 (s, 2H), 7.61 (td, J = 8 Hz, 1.5 Hz, 2H), 7.65 (dt, J = 11.5 Hz, 2.5 Hz, 1H), 8.24 (br s, 1H), 8.50 (dd, J = 5 Hz, 1 Hz, 2H), 8.66 (s, 1H); \textsuperscript{13}C NMR (100 MHz, CDCl\textsubscript{3}), δ 22.2, 28.4, 50.3, 53.6, 56.4, 60.8, 68.0, 69.6, 69.7, 70.4, 70.6, 70.7, 100.3, 108.6, 109.1, 109.4, 109.4, 110.6, 110.8, 117.1, 117.2, 122.3, 122.6, 123.3, 130.0, 130.1, 136.8, 140.8, 140.9, 146.8, 147.7, 149.1, 149.9, 153.6, 154.3, 156.5, 159.8, 161.9, 164.4.
**Formula:**

\[
N-(3\text{-chloro-4-fluorophenyl})-6\text{-methoxy}-7-(3\text{-}(1\text{-}(1\text{-}(pyridin-2\text{-yl})-2\text{-}(pyridin-2\text{-ylmethyl})-5,8,11\text{-trioxa-2-azatridecan-13-yl})-1H-1,2,3\text{-triazol-4-yl})propoxy)quinazolin-4\text{-amine (II-94)}
\]

Prepared according to procedure for II-93. 82 mg (82%). \(^1\)H NMR (500 MHz, CDCl\(_3\)), \(\delta\) 2.27 (br s, 2H), 2.78 (br s, 2H), 2.92 (t, \(J = 6.5\) Hz, 2H), 3.46 (m, 12H), 3.77 (t, \(J = 5.5\) Hz, 2H), 3.82 (s, 3H), 3.86 (s, 4H), 4.15 (br s, 2H), 4.44 (t, \(J = 5\) Hz, 2H), 5.10 (s, 2H), 6.88 (d, \(J = 9\) Hz, 1H), 6.99 (td, \(J = 8.5\) Hz, 2.5 Hz, 1H), 7.12 (m, 3H), 7.19 (m, 2H), 7.32 (m, 2H), 7.50 (br s, 4H), 7.60 (td, \(J = 7.5\) Hz, 1.5 Hz, 2H), 7.71 (br s, 1H), 8.30 (br s, 1H), 8.50 (d, \(J = 4.5\) Hz, 2H), 8.59 (s, 1H); \(^1\)C NMR (100 MHz, CDCl\(_3\)), \(\delta\) 22.2, 28.3, 28.4, 29.5, 50.3, 53.6, 56.5, 60.9, 67.9, 69.8, 70.4, 70.6, 70.7, 108.6, 109.3, 114.1, 114.3, 114.5, 115.0, 115.173, 122.3, 122.4, 122.7, 123.2, 123.4, 125.15, 130.3, 130.4, 133.3 136.8, 139.4, 139.5, 146.9, 147.5, 149.2, 149.8, 150.8, 153.8, 154.2, 156.9, 159.9, 162.0, 164.4.
$N$-(3-chloro-4-((3-fluorobenzyl)oxy)phenyl)-6-methoxy-7-(3-(1-(1-(pyridin-2-yl)-2-(pyridin-2-ylmethyl)-5,8,11-trioxa-2-azatridecan-13-yl)-1H-1,2,3-triazol-4-yl)propoxy)quinazolin-4-amine (II-95)

Prepared according to procedure for II-93, 37 mg (42%). $^1$H NMR (500 MHz, CDCl$_3$), δ 2.29 (t, $J = 6.5$ Hz, 2H), 2.79 (t, $J = 5.5$ Hz, 2H), 2.95 (t, $J = 7.5$ Hz, 2H), 3.46 (m, 8H), 3.55 (br s, 2H), 3.77 (t, $J = 5$ Hz, 2H), 3.82 (s, 3H), 3.86 (s, 4H), 4.18 (br s, 2H), 4.46 (t, $J = 5$ Hz, 2H), 7.09 (t, $J = 9$ Hz, 1H), 7.14 (ddd, $J = 7.5$ Hz, 5 Hz, 1 Hz, 2H), 7.19 (s, 1H), 7.24 (s, 1H), 7.52 (br s, 3H), 7.62 (td, $J = 8$ Hz, 1.5 Hz, 2H), 7.82 (br s, 1H), 8.20 (br s, 1H), 8.52 (d, $J = 4.5$ Hz, 2H), 8.63 (s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$), δ 22.2, 28.4, 50.3, 53.5, 56.4, 60.8, 67.9, 69.8, 70.4, 70.5, 70.7, 100.2, 108.8, 109.2, 116.6, 116.8, 122.1, 122.2, 122.3, 122.7, 123.3, 124.5, 135.8, 136.8, 146.8, 147.7, 149.1, 149.9, 153.6, 154.4, 156.6, 159.9.
7-(3-(1-(2-(2-(bis(pyridin-2-ylmethyl)amino)ethoxy)ethyl)-1H-1,2,3-triazol-4-yl)propoxy)-N-(3-chloro-4-(pyridin-2-ylmethoxy)phenyl)-6-methoxyquinazolin-4-amine (II-96)

Prepared according to the procedure for II-93. 14 mg (33%) \(^{1}H\) NMR (500 MHz, CDCl\(_3\)), \(\delta\) 2.23 (m, 2H), 2.79 (br s, 2H), 2.91 (m, 2H), 3.51 (m, 2H), 3.61 (m, 2H), 3.67 (m, 2H), 3.86 (m, 4H), 3.98 (s, 3H), 4.44 (t, \(J = 5\) Hz, 2H), 5.25 (s, 2H), 6.94 (d, \(J = 8.5\) Hz, 1H), 7.14 (m, 3H), 7.22 (t, \(J = 7\) Hz, 2H), 7.32 (br s, 1H), 7.48 (m, 3H), 7.61 (m, 3H), 7.72 (td, \(J = 8\) Hz, 2 Hz, 1H), 7.79 (m, 1H), 8.49 (d, \(J = 4.5\) Hz, 2H), 8.54 (s, 1H), 8.57 (d, \(J = 4\) Hz, 1H); \(^{13}C\) NMR (100 MHz, CDCl\(_3\)), \(\delta\) 1.2, 21.8, 27.9, 29.9, 49.8, 55.3, 56.6, 61.8, 67.2, 68.0, 68.9, 69.9, 70.1, 70.2, 70.6, 71.7, 100.7, 107.7, 109.2, 114.1, 121.5, 122.4, 122.96, 123.02, 123.1, 123.5, 125.1, 132.9, 137.3, 137.5, 146.1, 149.3, 150.0, 150.9, 154.5, 156.9, 157.0, 158.5. (Four carbons may be overlapping to account for the two missing carbon peaks.)
5.3 Experimental for chapter 3


To a solution of III-22 (2.003 g, 6.37 mmol) in dry DCM (2 mL) were added trifluoroacetone trihydrate (163 mg, 0.74 mmol) and pyridine (0.4 mL), respectively. The solution was cooled to 0°C, and 50% aqueous hydrogen peroxide (0.647 g, 19.0 mmol) was added dropwise. The reaction was warmed to room temperature and stirred overnight. The reaction was diluted with DCM and quenched with aqueous sodium thiosulfate solution (0.25 M, 50 mL). The reaction was partitioned between sat. ammonium chloride solution and DCM. The organic layers were dried over MgSO₄. MgSO₄ was removed by filtration and the filtrate was concentrated by rotary evaporation. The concentrate was triturated with diethyl ether to yield the pure α-diastereomer, which was collected by filtration as a fluffy white solid, 1.0085 g (48%); ¹H NMR (500 MHz, CDCl₃), δ 0.88 (s, 3H), 1.20 (ddd, J = 25.5 Hz, 12.5 Hz, 4 Hz, 1H), 1.50 (m, 4H), 1.68 (m, 2H), 1.87 (m, 3H), 2.02 (m, 1H), 2.09 (m, 5H), 2.43 (m, 2H), 3.87 (m, 4H), 6.05 (q, J = 3 Hz, 1H); ¹³C NMR (100 MHz, CDCl₃), δ 14.9, 22.1, 22.3, 25.3, 28.2, 31.8, 33.8, 36.1, 37.3, 40.4, 46.5, 46.8, 60.2, 61.8, 64.3, 64.5, 107.1, 125.9, 136.8, 221.5.
Preparation of Grignard reagent: To magnesium clippings (3.20 g, 125.6 mmol) in a flame-dried round bottom flask was added freshly distilled THF (54 mL). To this flask was added a solution of aryl bromide III-26 in dry THF (8 mL, 0.714 M). DIBALH solution (0.53 mL, 1.0 M in hexanes) was added in a dropwise manner to the reaction flask. The reaction was stirred at r.t., 1 h. To the reaction was added slowly the remaining solution of aryl bromide III-26 in dry THF (15 mL, 0.714 M). The reaction was stirred for 2 h at room temperature. The resultant Grignard reagent III-24 was used without any purification.

Copper-catalyzed Grignard addition reaction: To a room temperature solution of epoxide III-23 (1.088 g, 3.292 mmol) in freshly distilled THF (8 mL) was added copper(I) iodide (95 mg, 0.50 mmol). The reaction was cooled to 0°C, and the Grignard reagent was transferred slowly to the reaction flask via cannulation. The reaction was allowed to warm to room temperature. After 4.5 h, the reaction was cooled to 0°C, diluted with EtOAc (40 mL), and quenched by the slow addition of aqueous ammonium chloride solution (40 mL, 0.3 M). The reaction was partitioned and the organic layers were washed with brine and dried over MgSO₄. MgSO₄ was removed by filtration, and the filtrate was evaporated to dryness by rotary evaporation to give the crude allylic alcohol product. This crude product was used without further purification in the next transformation.
Acid dehydration and deketalization: The crude product residue was dissolved in glacial acetic acid (8.28 mL) and deionized water (3.52 mL) and heated at 60-65°C for 2.5h. The reaction was then cooled and slowly quenched with saturated aqueous sodium bicarbonate. The quenched reaction was then extracted with EtOAc and washed with brine. The organic layers were dried over MgSO₄. MgSO₄ was removed by filtration and the filtrate was evaporated to drying by rotary evaporation. The crude product was purified by silica chromatography, eluting with (5:95) MeOH/EtOAc to give an amber-colored oil. Precipitation of the product from hexanes gave an off-white solid, 1.243 g (87%). m.p. = 195-198°C. ¹H NMR (500MHz, CDCl₃), δ 0.56 (s, 3H), 1.51 (m, 3H), 1.89 (dd, J = 13.5 Hz, 6.5 Hz, 1H), 1.99 (m, 2H), 2.09 (m, 2H), 2.23 (s, 6H), 2.35 (m, 4H), 2.63 (m, 3H), 2.71 (t, J = 5.5 Hz, 2H), 2.74 (m, 1H), 4.02 (t, J = 6 Hz, 2H), 4.38 (d, J = 6 Hz, 1H), 5.79 (s, 1H), 6.83 (d, J = 8 Hz, 2H), 7.07 (d, J = 8.5 Hz, 2H); ¹³C NMR (125 MHz, CDCl₃), δ 14.5, 21.9, 25.9, 26.8, 30.9, 35.5, 36.9, 37.8, 38.0, 39.6, 46.0, 47.7, 50.7, 58.4, 65.9, 114.7, 123.4, 127.9, 130.1, 136.0, 145.1, 156.1, 157.1, 199.4, 219.0.

(11S)-11-(4-(2-(dimethylamino)ethoxy)phenyl)-17-oxo-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-3-yl acetate (III-27)

To a solution of dienone derivative III-25 (503 mg, 1.16 mmol) and acetic anhydride (145 mg, 1.42 mmol) in dry DCM (25 mL) at 0°C was added acetyl bromide (360 mg, 2.93 mmol). The reaction was slowly warmed to ambient temperature and allowed to stir for 12h. The reaction was concentrated by rotary evaporation and purified by silica column chromatography. The product eluted as a colorless oil, and was precipitated from hexanes to give a white solid, 230 mg (43%) ¹H NMR (500 MHz, CDCl₃), δ
0.45 (s, 3H), 1.47 (m, 3H), 1.92 (dd, J = 5.5 Hz, 1H), 2.00 (m, 3H), 2.14 (d, J = 12.5 Hz, 1H), 2.21 (s, 3H), 2.28 (s, 6H), 2.30 (m, 2H), 2.41 (m, 2H), 2.63 (m, 2H), 2.80 (m, 3H), 2.90 (m, 3H), 3.04 (td, J = 12 Hz, 4.5 Hz, 1H), 6.63 (d, J = 8.5 Hz, 3H), 6.86 (s, 1H), 6.94 (m, 3H);

$^{13}$C NMR (100MHz, CDCl$_3$), δ 15.1, 21.1, 21.3, 27.1, 29.9, 34.8, 35.2, 37.9, 39.9, 45.9, 47.6, 48.0, 52.1, 58.3, 65.7, 113.9, 119.1, 121.5, 127.5, 130.3, 134.9, 135.5, 137.3, 148.1, 156.0, 169.5, 219.0.

(11S,17S)-11-(4-(2-(dimethylamino)ethoxy)phenyl)-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthrene-3,17-diol (III-20)

To a solution of III-27 (501 mg, 1.05 mmol) in dry MeOH (13 mL) was added NaBH$_4$ (75 mg, 1.6 mmol). The reaction was stirred at room temperature for 1.5h, then 10M aqueous NaOH (0.07 mL) added. Reaction stirred at room temperature 12h. The reaction was then concentrated and the crude mixture was purified by silica column chromatography, eluting with (1:9) MeOH/EtOAc with 2% triethylamine. The product was precipitated from hexanes to give a light brown solid, 373 mg (81%). $^1$H NMR (500 MHz, CDCl$_3$), δ 0.35 (s, 3H), 1.18 (m, 6H), 1.67 (q, J = 8.5 Hz, 1H), 1.75 (dd, J = 12.5 Hz, 5.5 Hz, 1H), 1.91 (d, J = 12.5 Hz, 1H), 2.05 (m, 2H), 2.34 (m, 6H), 2.49 (d, J = 12.5 Hz, 1H), 2.62 (m, 2H), 2.78 (m, 3H), 3.66 (t, J = 8 Hz, 1H), 3.91 (m, 3H), 6.36 (dd, J = 8 Hz, 2 Hz, 1H), 6.46 (s, 1H), 6.49 (d, J = 8.5 Hz, 2H), 6.74 (d, J = 8.5 Hz, 1H), 6.94 (d, J = 8.5 Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$), δ 13.1, 14.3, 22.8, 23.3, 28.4, 31.7, 35.7, 38.4, 43.8, 45.5, 47.5, 51.9, 58.2, 65.0, 82.6, 113.5, 113.9, 115.9, 127.6, 129.5, 130.6, 136.3, 137.4, 154.2, 155.5.

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(11S,17S)-11-(4-(2-(methylamino)ethoxy)phenyl)-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthrene-3,17-diol (III-28)

To a solution of RU39411 III-20 (104 mg, 0.239 mmol) in dry 1,2-DCE (4 mL) was added Hunig’s base (300 µL). Reaction was cooled to 0°C and 1-chloroethyl chloroformate (400 µL) was added. The reaction was slowly warmed to ambient temperature and then heated under reflux, 3 h. The reaction was concentrated, redissolved in MeOH (4 mL) and heated again under reflux, 3 h. The reaction was concentrated and the crude reaction mixture was purified by reverse phase C18 column chromatography. The product eluted with (1:3) MeCN/H$_2$O as an amorphous solid. Upon precipitation from hexanes an off-white solid was obtained, 42 mg (42%). 1H NMR (500 MHz, CDCl$_3$), δ 0.35 (s, 3H), 1.20 (m, 2H), 1.30 (m, 3H), 1.67 (q, $J = 8.5$ Hz, 1H), 1.77 (dd, $J = 13.5$ Hz, 5.5 Hz, 1H), 2.06 (m, 2H), 2.49 (m, 4H), 2.71 (dd, $J = 3.5$ Hz, 1H), 2.79 (td, $J = 13.5$ Hz, 5 Hz, 2H), 2.87 (m, 3H), 3.67 (t, $J = 9$ Hz, 1H), 3.87 (m, 2H), 3.96 (m, 1H), 6.37 (dd, $J = 8.5$ Hz, 2.5 Hz, 1H), 6.51 (d, $J = 2.5$ Hz, 1H), 6.57 (d, $J = 8.5$ Hz, 2H), 6.78 (d, $J = 8$ Hz, 1H), 6.96 (d, $J = 8.5$ Hz, 2H); 13C NMR (100 MHz, CDCl$_3$), δ 13.1, 23.4, 28.1, 30.4, 30.6, 35.6, 36.2, 38.4, 43.9, 45.6, 47.6, 51.0, 52.0, 66.6, 82.7, 113.6, 113.9, 115.6, 127.7, 129.9, 130.7, 136.4, 137.7, 154.1, 155.8.
2-(4-((11S,17S)-3,17-bis((tert-butyldimethylsilyl)oxy)-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-11-yl)phenoxy)-N,N-dimethylethan-1-amine (III-30)

To a solution of RU39411 III-20 (373 mg, 0.856 mmol), DBU (0.76 mL, 5.1 mmol) and DMAP (32 mg, 0.26 mmol) in dry DCM (10 mL) at 0°C was added TBSCI (846 mg, 5.63 mmol). The reaction was slowly warmed to room temperature and stirred for 15h. The reaction was concentrated by rotary evaporation and purified by silica column chromatography to provide the desired product as a colorless oil, 271 mg (48%). $^1$H NMR (500 MHz, CDCl$_3$), δ 0.01 (s, 3H), 0.04 (s, 3H), 0.14 (s, 6H), 0.28 (s, 3H), 0.86 (s, 9H), 0.94 (s, 9H), 1.09 (m, 3H), 1.84 (m, 2H), 1.98 (d, $J = 15$ Hz, 2H), 2.08 (q, $J = 13.5$ Hz, 2H), 2.30 (s, 6H), 2.40 (d, $J = 16.5$ Hz, 1H), 2.66 (t, $J = 7$ Hz, 2H), 2.81 (m, 2H), 2.91 (m, 1H), 3.57 (t, $J = 8$ Hz, 1H), 3.90 (m, 1H), 3.94 (t, $J = 7.5$ Hz, 2H), 6.39 (d, $J = 11$ Hz, 1H), 6.57 (s, 1H), 6.61 (d, $J = 10$ Hz, 2H), 6.78 (d, $J = 10$ Hz, 1H), 6.92 (d, $J = 10$ Hz, 2H); $^{13}$C NMR (100MHz, CDCl$_3$), δ -4.5, -4.2, 13.4, 18.3, 18.4, 23.5, 25.9, 26.1, 28.2, 29.9, 30.4, 31.0, 35.6, 38.6, 44.2, 46.1, 46.2, 47.7, 51.8, 58.5, 65.8, 82.6, 113.6, 117.9, 120.2, 127.6, 130.7, 131.8, 136.4, 137.7, 152.9, 155.8.
(11S,17S)-11-(4-(2-(dimethylamino)ethoxy)phenyl)-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthrene-3,17-diyl bis(2,2-dimethylpropanoate) (III-29)

To a solution of RU39411III-20 (338 mg, 0.775 mmol) in dry DCM (25 mL) were added triethylamine (0.7 mL) and DMAP (16 mg, 0.13 mmol), followed by pivaloyl chloride (0.6 mL). The reaction was stirred at ambient temperature, 15h. The reaction was partitioned between DCM and brine. The organic layers were dried over MgSO₄. MgSO₄ was removed by filtration, and the filtrate was concentrated. The crude reaction mixture was purified by silica column chromatography, to give the product as a colorless oil, 177 mg (38%). ¹H NMR (500 MHz, CDCl₃), δ 0.40 (s, 3H), 0.87 (m, 1H), 1.23 (m, 14H), 1.38 (m, 4H), 1.74 (m, 1H), 1.89 (dd, J = 13 Hz, 6 Hz, 1H), 2.03 (m, 2H), 2.15 (m, 3H), 2.30 (s, 6H), 2.33 (m, 2H), 2.66 (t, J = 6 Hz, 2H), 2.89 (m, 2H), 2.99 (td, J = 12 Hz, 5 Hz, 1H), 3.92 (m, 3H), 4.60 (t, J = 7 Hz, 1H), 6.59 (dd, J = 8.5 Hz, 2 Hz, 1H), 6.63 (d, J = 8.5 Hz, 2H), 6.80 (d, J = 2 Hz, 1H), 6.92 (m, 3H); ¹³C NMR (100 MHz, CDCl₃), δ 14.3, 23.5, 27.3, 27.4, 27.6, 28.0, 30.2, 35.2, 38.4, 39.0, 39.2, 43.7, 46.0, 46.1, 47.6, 51.8, 58.5, 65.7, 83.1, 113.7, 119.1, 121.6, 127.7, 130.6, 135.7, 135.9, 137.7, 148.5, 156.0, 177.5, 178.9.
2-(4-((11S,17S)-3,17-bis((tert-butyldimethylsilyl)oxy)-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-11-yl)phenoxy)-N-methylethan-1-amine (III-33)

To a solution of III-28 (300 mg, 0.71 mmol), DMAP (26 mg, 0.22 mmol) and Hunig’s base (725 µL, 4.25 mmol) in DMF (7 mL) at 0°C was added TBSCl (2.375 g, 15.76 mmol). The reaction was slowly warmed to room temperature and stirred for 4.5 h. The reaction was concentrated and used without further purification. $^1$H NMR (500 MHz, CDCl$_3$), δ 0.01 (s, 3H), 0.04 (s, 3H), 0.13 (s, 3H), 0.14 (s, 3H), 0.28 (s, 3H), 0.86 (s, 9H), 0.94 (s, 9H), 1.14 (m, 1H), 1.28 (m, 2H), 1.39 (m, 2H), 1.63 (m, 2H), 1.85 (m, 1H), 1.98 (dt, $J = 13$ Hz, 2.5 Hz, 1H), 2.08 (m, 1H), 2.39 (dd, $J = 2$ Hz, 1H), 2.50 (s, 3H), 2.80 (m, 2H), 2.94 (m, 2H), 3.54 (m, 2H), 3.88 (t, $J = 4$ Hz, 1H), 3.90 (m, 2H), 6.38 (dd, $J = 8.5$ Hz, 2.5 Hz, 1H), 6.59 (d, $J = 3$ Hz, 1H), 6.62 (d, $J = 9$ Hz, 2H), 6.78 (d, $J = 8.5$ Hz, 1H), 6.93 (d, $J = 8$ Hz, 2H); $^{13}$C NMR (100 MHz, CDCl$_3$), δ -4.5, -4.2, 8.3, 13.4, 18.3, 18.4, 23.5, 25.9, 26.1, 28.2, 29.9, 30.4, 31.0, 35.6, 36.2, 38.6, 44.2, 46.2, 47.7, 51.7, 53.0, 66.4, 82.6, 113.6, 117.9, 120.2, 127.6, 130.8, 131.8, 136.6, 137.7, 152.9, 155.7.

3,6,9,12-tetraoxapentadec-14-yn-1-yl 4-methylbenzenesulfonate (III-34)

To a solution of tetraethylene glycol (612 mg, 3.15 mmol) in MeCN (30 mL) was added Cs$_2$CO$_3$ (3.085 g, 9.468 mmol). The reaction was stirred at ambient temperature for 40 min. Propargyl bromide (0.28 mL, 3.0 mmol) was then added to the reaction. The reaction was stirred at ambient temperature for 16h. The reaction was filtered, and the filtrate was concentrated by rotary evaporation. The crude mixture was
purified by silica column chromatography. To a solution of this glycol derivative (49 mg, 0.21 mmol) and dry triethylamine in dry DCM (2 mL) was added tosyl chloride (63 mg, 0.33 mmol). The reaction was stirred at room temperature, 15h. The reaction was concentrated and the crude mixture was purified by silica column chromatography to yield the desired product as a colorless oil, 24 mg (30%, 2 steps). \[^1\text{H} \text{NMR} (400 \text{ MHz, CDCl}_3), \delta 2.45 \text{ (s, 3H), 2.49 (t, } J = 2.2 \text{ Hz, 1H), 3.59-3.64 (m, 12H), 3.66 (m, 2H), 4.14 (m, 2H), 4.18 (d, } J = 2.2 \text{ Hz, 2H), 7.37 (d, } J = 8 \text{ Hz, 2H), 7.80 (d, } J = 8 \text{ Hz, 2H).} \]
5.4 Experimental for chapter 4

6-(tributylstannyl)-1H-indol-3-carbaldehyde (IV-16)

A reaction tube under argon atmosphere was charged with 6-bromoindole-3-carbaldehyde 1 (20 mg, 0.0893 mmol), PdCl$_2$(PhCN)$_2$ (2.6 mg, 0.0045 mmol), and PCy$_3$ (2.5 mg, 0.0089 mmol). Dioxane (2 mL) and hexa-$n$-butylditin (0.13 mL, 0.27 mmol) were added via syringe to the reaction tube. The reaction was heated at 110°C overnight, then cooled and filtered through celite and activated carbon. The filtrate was then concentrated by rotary evaporation and purified on a silica gel column which had been neutralized with 1% triethylamine. Product was recovered as a light pink solid, 32 mg (82%). m.p. = 80-82°C; $^1$H NMR (400MHz, CDCl$_3$), $\delta$ 0.85 (t, $J = 7.2$ Hz, 9H), 1.06 (t, $J = 8.4$ Hz, 6H), 1.29 (m, $J = 8$ Hz, 6H), 1.52 (m, $J = 8$ Hz, 6H), 7.38 (d, $J = 8$ Hz, 1H), 7.60 (s, 1H), 7.85 (d, $J = 2.8$ Hz, 1H), 8.23 (d, $J = 7.2$ Hz, 1H), 9.75 (br s, 1H), 10.05 (s, 1H); $^{13}$C NMR (100MHz, CDCl$_3$), $\delta$ 9.9, 13.7, 27.5, 29.3, 111.9, 119.1, 124.8, 129.8, 132.0, 135.6, 136.5, 137.4, 185.7. HRMS, ESI (+), [M+H] calculated, 436.1666; found 436.1668.

5-(tributylstannyl)-1H-indole-3-carbaldehyde (IV-18)

Prepared using the procedure for IV-16; PdCl$_2$(PhCN)$_2$ and PCy$_2$bph were used (79% yield). Product was isolated as a light pink solid, m.p. = 84-87°C. $^1$H NMR (500MHz, CDCl$_3$), $\delta$ 0.85 (t, $J = 7.6$ Hz, 9H), 1.05 (t, $J = 7.2$ Hz, 6H), 1.29 (m, $J = 7.6$ Hz, 6H), 1.51 (m, $J = 7.6$ Hz, 6H), 7.39 (d, $J = 8$ Hz, 1H), 7.45 (d, $J = 8.4$ Hz, 1H), 7.83 (d, $J = 2.8$ Hz, 1H), 8.44 (s, 1H), 9.75 (br s, 1H), 10.05 (s, 1H); $^{13}$C NMR (100MHz,
CDCl₃, δ 9.8, 13.8, 27.5, 29.2, 111.7, 119.1, 124.7, 129.8, 132.0, 135.5, 136.1, 137.2, 185.7. HRMS, ESI (+), [M+H] calculated 436.1666; found 436.1668.

6-(tributylstannyl)-1H-indole (IV-20)

Prepared by the procedure for IV-22; Pd₂dba₃ and PCy₃ were used (97% yield). Product isolated as a clear, pale yellow oil. ¹H NMR (400 MHz, CDCl₃), δ 0.87 (t, J = 7.2 Hz, 9H), 1.05 (t, J = 8.8 Hz, 6H), 1.31 (m, J = 7.6 Hz, 6H), 1.52 (m, J = 7.6 Hz, 6H), 6.51 (s, 1H), 7.08 (t, J = 2.8 Hz, 1H), 7.24 (d, J = 8.0 Hz, 1H), 7.33 (d, J = 8.4 Hz, 1H), 7.75 (s, 1H), 7.96 (br s, 1H); ¹³C NMR (100MHz, CDCl₃), δ 9.9, 13.9, 27.7, 29.4, 102.2, 111.1, 123.8, 128.4, 129.1, 129.6, 130.7, 136.1.

5-(tributylstannyl)-1H-indole (IV-22)

To a reaction tube under argon atmosphere were added 5-bromo-1H-indole 5 (44 mg, 0.22 mmol) in dioxane (2mL), Pd₂dba₃ (10 mg, 0.010 mmol) in dioxane (4mL), and PCy₃ (6 mg, 0.022 mmol) in dioxane (2mL). To the reaction was added hexa-n-butylditin (0.30 mL, 0.60 mmol) via syringe. The reaction was heated at 110°C for 3h, then cooled and filtered through celite and activated carbon. The filtrate was then concentrated by rotary evaporation and purified by reverse phase C18 chromatography to yield the product as a clear, faint yellow oil, 90.3 mg (99%). ¹H NMR (400 MHz, CDCl₃), δ 0.87 (t, J = 7.2 Hz, 9H), 1.05 (t, J = 8.4 Hz, 6H), 1.31 (m, J = 7.6 Hz, 6H), 1.55 (m, J =5.2 Hz, 6H), 6.53 ( t, J = 2 Hz, 1H), 7.17 ( t, J = 2.8 Hz, 1H), 7.25 (dd, J = 5.2 Hz, 2.8 Hz, 1H), 7.39 (d, J = 7.2 Hz, 1H), 7.75 (s, 1H), 8.10 (br s, 1H); ¹³C NMR (100MHz, CDCl₃), δ 9.9, 13.9, 27.7, 29.4, 102.4, 111.1, 123.8, 128.5, 129.2, 129.7, 130.8, 136.1.
N,N-dimethyl-1-(5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indol-3-yl)methanamine  (IV-24)

A reaction tube was charged with 5-bromogramine IV-23 (52 mg, 0.21 mmol), bis(pinacolato)diboron (110 mg, 0.434 mmol), and PdCl$_2$dpf$_2$DCM (11 mg, 0.013 mmol). After the reaction tube was evacuated and flushed with argon, 1,4-dioxane was added, followed by 1.5M aqueous NaOH (0.2 mL). The reaction was heated at 110°C, 12h. The reaction was cooled and 1,4-dioxane removed by rotary evaporation. The residue was then partitioned between DCM and saturated aqueous ammonium chloride. The organic layers were dried over MgSO$_4$. MgSO$_4$ was removed by filtration, and the filtrate was concentrated to dryness by rotary evaporation. The crude product was purified by silica chromatography, as an off-white solid, 21 mg (34%). $^1$H NMR (400 MHz, CDCl$_3$), δ 1.30 (s, 12H), 2.23 (s, 6H), 3.60 (s, 2H), 7.08 (s, 1H), 7.20 (s, 1H), 7.28 (d, $J$ = 8 Hz, 1H), 7.57 (d, $J$ = 8 Hz, 1H), 8.12 (br s, 1H); $^{13}$C NMR (100 MHz, CDCl$_3$), δ 25.10, 25.14, 30.0, 43.8, 53.1, 75.3, 83.8, 111.3, 126.2, 127.6, 128.6, 138.4.

N,N-dimethyl-1-(5-(tributylstannyl)-1H-indol-3-yl)methanamine (IV-25)

A reaction tube was charged with 5-bromogramine 9 (44 mg, 0.18 mmol). Then, under argon atmosphere, solutions of PdCl$_2$(PhCN)$_2$ (4 mg, 0.01 mmol) in dioxane (4 mL) and PCy$_3$ (5 mg, 0.02 mmol) in dioxane (4 mL) were added, followed by the addition of hexa-n-butylditin (0.30 mL, 0.60 mmol) by syringe. The reaction was heated at 110°C for 10-12 h, cooled, and filtered through a plug of celite and activated charcoal. The filtrate was concentrated by rotary evaporation and then purified by
reverse phase C18 chromatography. The product was isolated as a faint yellow, clear oil, 73 mg (82%).

\[ ^1H \text{ NMR (CDCl}_3, 500 \text{ MHz), } \delta 0.87 \text{ (t, } J = 7.6 \text{ Hz, 9H), } 1.05 \text{ (t, } J = 8 \text{ Hz, 6H), } 1.32 \text{ (m, } J = 7.2 \text{ Hz, 6H), } 1.55 \text{ (m, } J = 8 \text{ Hz, 6H), } 2.31 \text{ (s, 6H), } 3.67 \text{ (s, 2H), } 7.09 \text{ (s, } J = 2 \text{ Hz, 1H), } 7.24 \text{ (d, } J = 6.8 \text{ Hz, 1H), } 7.35 \text{ (d, } J = 8 \text{ Hz, 1H), } 7.77 \text{ (s, 1H), } 8.23 \text{ (br s, 1H); } ^{13}C \text{ NMR (CDCl}_3, 100 \text{ MHz), } \delta 9.9, 14.0, 27.7, 29.4, 45.6, 54.4, 111.1, 112.9, 123.3, 127.5, 128.5, 129.7, 130.3, 136.5. \text{ HRMS, ESI (+), } [M+H] \text{ calculated, 465.2296; found 465.2292.} \]

1-(6-bromo-1H-indol-3-yl)-N,N-dimethylmethanamine (IV-26)

To a solution of IV-20 (511 mg, 2.61 mmol) in dry DCM (8 mL) at 0°C was added Eschenmoser’s salt (614 mg, 3.32 mmol). The reaction was slowly warmed to room temperature and stirred, 20 h. The reaction was partitioned between DCM and water, and the organic layers were dried over MgSO\(_4\). MgSO\(_4\) was removed by filtration and the filtrate was concentrated by rotary evaporation. The crude reaction mixture was purified by silica column chromatography to yield the product as an amber solid, 242 mg (37%). \[ ^1H \text{ NMR (500 MHz, CDCl}_3), \delta 2.29 \text{ (s, 6H), } 3.61 \text{ (s, 2H), } 6.96 \text{ (s, 1H), } 7.15 \text{ (dd, } J = 2 \text{ Hz, 8.5 Hz, 1H), } 7.37 \text{ (d, } J = 2 \text{ Hz, } 1H), 7.5 \text{ (d, } J = 8 \text{ Hz, 1H).} \]
**N,N-dimethyl-1-(6-(tributylstanny)-1H-indol-3-yl)methanamine (IV-27)**

![Chemical Structure](image)

Prepared according procedure used for **IV-25**: PdCl$_2$(PhCN)$_2$ and PCy$_3$ were used (70% yield). Product isolated as a clear, light yellow oil. $^1$H NMR (CDCl$_3$, 500 MHz), δ 0.87 (t, $J$ = 5.6 Hz, 9H), 1.05 (t, $J$ = 6.4 Hz, 6H), 1.32 (m, $J$ = 6.4 Hz, 6H), 1.53 (m, $J$ = 5.2 Hz, 6H), 2.29 (s, 6H), 3.63 (s, 2H), 7.08 (d, $J$ = 2 Hz, 1H), 7.18 (d, $J$ = 6.4 Hz, 1H), 7.467 (s, 1H), 7.67 (d, $J$ = 6 Hz, 1H), 8.38 (br s, 1H); $^{13}$C NMR (CDCl$_3$, 100MHz), δ 9.9, 13.9, 27.7, 29.3, 29.4, 45.5, 54.6, 113.3, 118.9, 119.1, 123.4, 127.2, 128.0, 133.8, 136.8. HRMS, ESI (+), [M+H] calculated, 465.2296; found 465.2292.

**5-ido-1H-indole-3-carbaldehyde (IV-28)**

![Chemical Structure](image)

To a solution of stannane **IV-18** (27 mg, 0.062 mmol) in dry THF (2.1 mL) in a dark reaction tube was added N-iodosuccinimide (17 mg, 0.075 mmol). The reaction was stirred at room temperature for 30 min. The reaction was partitioned between DCM and water and the organic layers were dried over MgSO$_4$. The MgSO$_4$ was removed by filtration and the filtrate was concentrated to dryness by rotary evaporation. Product purified by silica chromatography, eluting with (45:55) EtOAc/Hex, as an off-white solid, quantitative yield. $^1$H NMR (400 MHz, acetone-d$_6$), δ 7.41 (d, $J$ = 8.8 Hz, 1H), 7.57 (dd, $J$ = 1.6 Hz, 8.8 Hz, 1H), 8.23 (d, $J$ = 2.8 Hz, 1H), 8.61 (d, $J$ = 1.6 Hz, 1H), 10.01 (s, 1H), 11.31 (br s, 1H).
6-iodo-1H-indole-3-carbaldehyde (IV-29)

To a solution of stannane IV-16 (32 mg, 0.073 mmol) in dry THF (2 mL) in a dark reaction tube was added N-iodosuccinimide (22 mg, 0.096 mmol). The reaction was stirred at room temperature for 30 min. The reaction was partitioned between DCM and water and the organic layers were dried over MgSO₄. The MgSO₄ was removed by filtration and the filtrate was concentrated to dryness by rotary evaporation. Product purified by silica chromatography, eluting with (45:55) EtOAc/Hex, as a yellow solid, 18 mg (91%). ¹H NMR (400 MHz, acetone-d₆), δ 7.53 (dd, J = 8.8 Hz, 1.6 Hz, 1H), 7.64 (s, 1H), 7.87 (d, J = 1.6 Hz, 1H), 7.99 (d, J = 8.8 Hz, 1H), 10.02 (s, 1H), 11.09 (br s, 1H).
References


