Synthesis & Evaluation of Bipolar Biphenyl Proteomimetics as Nuclear Receptor CBIs
and
Applications of Palladium Chemistry to the Development of Radiotracers

A dissertation presented
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ABSTRACT OF DISSERTATION

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry in the Graduate School of Northeastern University August, 2011
Abstract

Protein-protein interactions (PPIs) are essential activation and communication mechanisms for countless biological processes. The ability to inhibit PPIs therefore exposes therapeutic pathways for ailments that may have otherwise been deemed untreatable. Recognition that a subset of PPIs are frequently mediated by the binding of short conserved alpha helices has resulted in the investigation of a number of approaches to mimicking alpha helices, including the use of substituted biphenyl and related poly-aryl scaffolds. Inhibition of nuclear receptor (NR) promoted gene transcription is of interest due to the role of NRs in promoting various human pathologies, including breast and prostate cancer. Because NR activity is in part mediated by the binding of coactivator proteins (CoA) through a short, conserved alpha helix, this NR-CoA interaction is an ideal system for evaluating alpha helix mimetics. Encouraged by recent success in the design of small molecules capable of disrupting NR-CoA interactions, and intrigued by the potential of substituted poly-aromatics as flexible mimics of alpha helices, our group has designed a bipolar biphenyl scaffold functionalized to mimic the key interactions that mediate NR-CoA binding. Chapter 1 introduces this and other relevant background information for this project.

Chapter 2 describes a small series of 3,3’-disubstituted derivatives of the bipolar biphenyl scaffold which were synthesized and subsequently evaluated as ERα and AR coactivator binding inhibitors (CBIs). This study was performed as an initial proof-of-concept to establish the capability of this scaffold, when substituted, to directly inhibit NR-CoA binding interactions.
In the interest of expanding the compound series, in terms of both the identity and the arrangement of substituents on the biphenyl scaffold, efficient synthetic methods were developed. *Chapter 3* outlines this methodology with particular focus on the optimization of Suzuki chemistry which was used to couple the biphenyls. Additionally, the synthesis resulted in a 2nd generation compound series, including mono- (2; 2’), and di- (2,3’; 3,2’; 2,2’) methylated and benzylated biphenyl derivatives which would be subject to further evaluation as NR-CBIs.

*Chapter 4* describes the evaluation of the 2nd generation biphenyls. Biological assays were again used to assess the effectiveness of these compounds as NR CBIs. Additionally, spectral methods were also used to assess the properties of the biphenyls themselves. The characterization of the 2,2’-dibenzylated biphenyls by $^1$H NMR revealed spectral anomalies which eventually led to the distinction of intramolecular probes by which biaryl atropisomerization could be physically observed. Once distinguished, methods were developed to exploit these resonances to evaluate the favored configurations and rotational energy barriers of the biphenyls.

Future directions for the development of biphenyl scaffolds as alpha helix mimetics are presented in *Chapter 5*. This discussion introduces both fundamental and applied objectives for invoking regio- and/or enantio- specificity to the biphenyls.

A distinct area of work is introduced in *Chapter 6*. Palladium catalysis was applied to a conceptually unrelated problem, to facilitate the conversion of halogenated indoles to their radiohalogenated analogues. 5- and 6- halogenated indoles were separately stannylated and boronated via palladium catalysis. The resultant metalated derivatives were then subjected to
site-specific radiolabeling via electrophilic aromatic halogenation. Particular facets of this work are discussed including the optimization of the palladium-catalyzed metalations and the apparent challenges of functionalizing 5- and 6- indoles.
Acknowledgments

I have benefitted greatly throughout graduate school from the support provided by the Department of Chemistry & Chemical Biology at Northeastern University. I very much appreciate the relationships that I have built with faculty members as well as peers, some of which been extremely influential to both my academic and my personal growth.

I owe enormous gratitude to my research advisor, Professor Robert Hanson. While always available to provide guidance, you continually resist supervising your students, leaving us with the choice of whether to struggle, survive, or thrive. Although I admittedly encountered my own struggles at times, this autonomy provided me the incentive to take command of my own research. Thanks to the freedom which you conceded, I have been able to explore seemingly obscure research tangents and I leave graduate school with confidence in my own potential as a scientist.

I am additionally grateful to members of the Hanson Research Group, both past and present, including Emmitt McCaskill, Adam Hendricks, Patrick Weiser, Lenny Dao, Emily Corcoran, Helen Pham, Anton Kozhushnyan, Kelton Barnsley, and James Teh. Emmitt and Adam were particularly helpful during my early years in teaching me the basic skills of organic synthesis. Emily, in addition to being a great friend, your approach to research has greatly influenced my own. Pat, we have paralleled each other through coursework, exams, and research and I very much appreciate the assistance and friendship along the way.
Beyond the Hanson Group, a number of faculty members must be acknowledged. I am much obliged to the members of my dissertation committee: Graham Jones, Alexandros Makriyannis, and Rein Kirss. Professor Kirss, I particularly appreciate the advice and assistance (and ligands!) you have provided over the years. Due to scheduling constraints, David Forsyth was unable to serve as a member of my committee, but I must acknowledge the guidance he has provided with particular regards to NMR. Additionally, Roger Kautz has been immensely helpful with performing complex NMR investigations and I have very much appreciated the infinite conversations along the way.

A number of collaborators have been invaluable in accomplishing my research objectives. Donald McDonnell (Duke University School of Medicine) and John Katzenellenbogen (University of Illinois, Urbana-Champaign) have performed biological studies on the biphenyl proteomimetics. Alexandros Makriyannis (Northeastern University) provided samples of AM630 which ultimately spurred my tangential work into the development of radiotracers. George Kabalka (University of Tennessee, Knoxville) continues to perform radioiodination experiments on our metalated indole derivatives, and similar radiofluorination studies will soon be performed by Henry Van Brocklin (University of California, San Francisco).

Finally, I must thank the various agencies that have provided funding for my graduate work. I was supported by the National Science Foundation as a GK-12 Fellow during 2007-2008, through which I worked with AP Chemistry classes at the John D. O’Bryant School of Math and Science in Roxbury, MA concurrently to performing graduate work. Additionally, I have been supported by a radiochemistry training award from the Department of Energy. The Department
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-Anna Williams
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<td>Absorption, distribution, metabolism, and excretion</td>
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<tr>
<td>AF-1</td>
<td>Activation Function-1</td>
</tr>
<tr>
<td>AF-2</td>
<td>Activation Function-2</td>
</tr>
<tr>
<td>aPP</td>
<td>Avian Pancreatic Polypeptide</td>
</tr>
<tr>
<td>AR</td>
<td>Androgen Receptor</td>
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<tr>
<td>CB₁</td>
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<td>Coactivator Binding Inhibitor</td>
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<td>CoA</td>
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<td>Dichloromethane</td>
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<tr>
<td>DFT</td>
<td>Density Functional Theory</td>
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<td>DMF</td>
<td>Dimethylformamide</td>
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<td>E₂</td>
<td>17β-estradiol</td>
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<td>ER</td>
<td>Estrogen Receptor</td>
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<td>ERE</td>
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<td>Estrogen Receptor-α</td>
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<tr>
<td>ERβ</td>
<td>Estrogen Receptor-β</td>
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<tr>
<td>FRET</td>
<td>Fluorescence Resonance Energy Transfer; Forster Resonance Energy Transfer</td>
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<tr>
<td>FxxLF</td>
<td>Phenylalanine-X-X-Leucine-Phenylalanine (NR Box variant)</td>
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<td>GPCR</td>
<td>G-Protein Coupled Receptor</td>
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<td>HepG2</td>
<td>Human Liver Carcinoma Cell Line (AR-negative)</td>
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<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>HMBC</td>
<td>Heteronuclear Multiple Bond Correlation</td>
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<tr>
<td>HPLC</td>
<td>High Pressure Liquid Chromatography</td>
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<tr>
<td>HRMS</td>
<td>High Resolution Mass Spectrometry</td>
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<tr>
<td>HSQC</td>
<td>Heteronuclear Single Quantum Correlation</td>
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<tr>
<td>HTS</td>
<td>High Through-put Screening</td>
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<td>LBD</td>
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<td>LCMS</td>
<td>Liquid Chromatography Mass Spectrometry</td>
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<td>LNCaP</td>
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<td>LxxLL</td>
<td>Leucine-X-X-Leucine-Leucine (NR Box)</td>
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<tr>
<td>MCF-7</td>
<td>Breast Cancer Cell Line (ER-positive)</td>
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<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
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<tr>
<td>NOE</td>
<td>Nuclear Overhauser Effect</td>
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<tr>
<td>NOESY</td>
<td>Nuclear Overhauser Effect Spectroscopy</td>
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<tr>
<td>NR</td>
<td>Nuclear Receptor</td>
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<td>NR Box</td>
<td>Nuclear Receptor Box (LxxLL)</td>
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<td>NRD</td>
<td>Nuclear Receptor Domain</td>
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<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<td>PET</td>
<td>Positron Emission Tomography</td>
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<td>PPAR</td>
<td>Peroxisome Proliferator-Activated Receptor</td>
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<td>PPI</td>
<td>Protein-Protein Interactions</td>
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<td>PR</td>
<td>Progesterone Receptor</td>
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<td>RBA</td>
<td>Radiometric Binding Assay</td>
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<td>RNA Pol II</td>
<td>RNA Polymerase II</td>
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<td>ROESY</td>
<td>Rotating Frame Nuclear Overhauser Effect Spectroscopy</td>
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<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
<td>SAR</td>
<td>Structure-Activity Relationship</td>
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<tr>
<td>SK-BR-7</td>
<td>Breast Cancer Cell Line (ER-negative)</td>
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<tr>
<td>SPECT</td>
<td>Single-Photon Emission Computed Tomography</td>
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<tr>
<td>SRC</td>
<td>Steroid Receptor Coactivator</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin-layer Chromatography</td>
</tr>
<tr>
<td>TR-FRET</td>
<td>Time Resolved- Fluorescence Resonance Energy Transfer</td>
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<td>TRβ</td>
<td>Thyroid Receptor-β</td>
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Chapter 1: Background

Part A: Proteomimetic Approach to the Development of NR-CBIs
1.1 Alpha Helical Mimetics as Designed Inhibitors of Protein-Protein Interactions

1.1.1 Inhibition of Protein-Protein Interactions by Rational Drug Design

The rapid growth in the pharmaceutical industry observed in recent decades should be largely attributed to an enhanced understanding of the structural properties, binding potential, and biological functions of proteins. Rational drug design is typically founded upon the ability to identify a protein target with a known endogenous ligand or substrate and design compounds capable of interacting with high potency to the receptor or enzyme’s small-molecule binding domain, out-competing the endogenous molecule. In the absence of a characterized binding domain, drug design approaches frequently mimic structural features of a known endogenous ligand. Fragment-based design as well as high-throughput screening (HTS) methods have provided further opportunities to identify lead compounds even in the absence of detailed structural data on the target protein of interest.

In a 2002 perspective, Hopkins and Groom, both of Pfizer, surmised that a finite, quantifiable number of “druggable” molecular targets exist, approximating this number to be between 600-1500.¹ This assessment was based on knowledge gleamed from the human genome project and two major intrinsic assumptions: that (1) a “druggable” protein target requires a small molecule binding cavity, and (2) successful drugs must generally comply with Lipinski’s Rule-of-Five which dictates restrictions in molecular size and solubility properties which typically coincide with effective pharmaceuticals.² While the authors acknowledge the existence of ~30,000 genes in the human genome, they attribute only about 3000 of these to be disease-modifying and, of
this group, approximately 600-1500 to belong to what they term the “druggable genome,” those targets known to naturally bind small molecules (Figure 1.1).¹

![Druggable Genome Diagram](image)

**Figure 1.1** A finite number of drug targets exist within the human genome, as dictated by pathological roles and modulation by endogenous small-molecules."¹

Recent work has instead revealed the potential for affecting biological pathways through the modulation of protein-protein interactions (PPIs). PPIs are essential activation and communication mechanisms which beget countless biological processes. Once considered indecipherable and therefore virtually uninhibitable, trends in protein binding modes are beginning to emerge and evidence is accumulating that the development of PPI inhibitors may in fact be feasible.³⁴⁵⁶ The potential to inhibit PPIs exposes therapeutic pathways for ailments that would have previously been deemed untreatable by such models as that presented by Hopkins and Groom, broadening the mechanisms at our disposal for interrogating and modulating known biological systems.
While interactions between proteins and small molecules generally cover contact surfaces ranging from ~300-1000Å², PPIs cover much larger surface areas of ~1500-3000Å² which do not tend to present the sort of compact binding cavities that have been exploited in the past by rational drug design. However, mounting interest in inhibition of PPIs is emerging from the recognition of ‘hot spots,’ or key residues that bind tightly, contributing most to the binding energy of the interaction. Efforts have since been underway to examine these ‘hot spots’ for commonalities that may emerge at the interfaces in order that they may be exploited in the development of novel strategies of PPI inhibition. ‘Hot spots’ generally comprise small, compact surface areas, frequently found toward the center of the contact surface, with flanking residues providing additional stability. The involved residues are sometimes adjacent, but frequently are discontiguous, increasing the challenge of mimicking these key interactions.5

Recognition of ‘hot spots’ initially gave rise to the hope that perhaps PPIs were indeed contingent on analogous binding cavities to those common to small-molecule binding and that these binding domains might then be amenable to standard drug design approaches such as computational docking and HTS. While such binding cavities have been found behind certain PPIs (e.g. p53/MDM2)7, there is a growing recognition that this is likely not the norm. Attempts to use HTS to identify lead compounds have proven largely ineffective, likely due to the fact that the libraries being screened are littered with chemotypes designed for ligand binding inhibition.3 However, a number of protein motifs (e.g. α-helices, β-turns) are emerging as recurrent for various subsets of PPIs, fostering the design of novel strategies of PPI inhibition.8
In a 2007 review, González-Muñiz and coworkers identified three strategies that are being explored to achieve PPI mimicry.4

(1) “the stabilization of protein secondary structures by means of different types of cyclic peptides…

(2) “the combination of peptide and non-peptide elements in the same molecule to fix either helical structures, β–pleated sheet or reverse turn conformation;

(3) “the development of organic non-peptide architectures able to bear the appropriate functional groups of a given protein in the right three-dimensional orientation.”

As alpha helix-mediated PPIs are of specific interest for my purposes (vide infra), further discussion of approaches to inhibit PPIs will focus on the development of alpha helix mimetics.

1.1.2 Alpha Helix Mimetics

The alpha helix is a common secondary structural motif in proteins and is generally defined by the φ and ψ angles of -57° and -47°, respectively, which dictate the inclusion of 3.6 residues and a 5.4Å rise per 360° turn. The structure is stabilized by the existence of hydrogen-bonding interactions between the backbone carbonyl of a given residue (i) and the backbone amide N-H of a residue four peptides removed (i+4)⁹. Alpha helices can play a variety of roles for proteins, for example, construing support to the overall tertiary structure of a given protein; contributing to
the formation of binding cavities; interacting with the major groove of DNA; constructing trans-
membrane channels (e.g. GPCRs, ion channels).\textsuperscript{10}

Amphipathic alpha helices, which display hydrophobic and hydrophilic side chains on opposing
faces of the helix, have been identified at polar/non-polar interfaces, such as the outer shell of a
protein surface or a membrane/water interface. In such helices, a “wet-edge” may form,
monofacially projecting side chains of similar polarity at the $i$, $i+3$, $i+4$, and $i+7$ positions (and
analogously, imparting opposite polarity to the opposing face of the helix). Alpha helices
involved in mediating PPIs commonly display such a configuration of substituents, with
lipophilic side chains available along one face of the helix to form hydrophobic contacts with the
protein-binding partner, and hydrophilic side chains on the opposite face that may interact with
the cellular environment.\textsuperscript{8,11,12}

The use of competitive peptide fragments may initially present as a seemingly obvious solution
to the desire to inhibit a given alpha helix-mediated PPI. However, peptide fragments have poor
ADME (absorption/distribution/metabolism/excretion) profiles, as they are high molecular
weight, highly hydrophilic molecules with peptide linkages that are easily subject to proteolytic
digestion. If administered orally they will have little chance at entering the bloodstream, and
even when administered intravenously, their ability to penetrate cellular membranes and
subsequently selectively bind target molecules is relatively poor. Additionally, efforts to use
peptide fragments to inhibit alpha helix-mediated PPIs will be further deterred by the poorly
structured nature of these peptides. Protein substructure comes at an entropic disadvantage and
therefore is an emergent property of the enthalpic advantages imparted on the macromolecule as
a whole. Even once formed, helices can be easily affected by changes in such variables as pH, ionic strength, and temperature. Without the support of larger protein substructure, the fragment is unlikely to adopt the appropriate conformation to induce binding affinity or target selectivity.\textsuperscript{9,13}

1.1.3 Peptidyl-Approaches toward Alpha Helix Mimetics

The potential of high affinity molecules capable of mimicking and, ultimately, inhibiting the protein-protein binding has led to the development of approaches to enhance the structural and conformational stability of peptide fragments. Recognizing the heightened propensity of certain amino acids (e.g. alanine) to adopt alpha-helical structures, non-conserved positions on peptide chains have been replaced by these residues.\textsuperscript{9,14,15} An innovative variant of this approach was achieved by Shepartz who grafted a peptide of interest onto aPP (avian pancreatic polypeptide), a 36 residue miniprotein with a remarkably high propensity (given its size) to form stable alpha helices in water.\textsuperscript{16}

Other groups have introduced non-covalent approaches to induce alpha helical stability, for example through the introduction of conscientiously spaced residues that form H-bonding, pi stacking, or cation-pi interactions (\textbf{Figure 1.2}).\textsuperscript{12,17}
Figure 1.2 Non-covalent approaches to stabilizing alpha helices.\textsuperscript{12}

Perhaps encouraged by the success of non-covalent approaches at inducing helix stability, a variety of covalently modified approaches are emerging, some of which are proving to be efficacious stabilizers of helical conformation (Figure 1.3).\textsuperscript{12,18} Amongst the most common of these are disulfide\textsuperscript{19} and amide\textsuperscript{20} linkers.  Verdi\’s hydrocarbon-linked “stapled peptides” are particularly noteworthy, demonstrating enormous potential for the stabilization of alpha-helical conformations and simultaneous the prevention of proteolytic degradation.\textsuperscript{21}

Figure 1.3 Covalent approaches to stabilizing alpha helices.\textsuperscript{12}

While the above approaches mainly incorporated linkers to stabilize natural peptides, an alternative approach uses non-natural residues, which alter the structure of the main peptide
chain, in an effort to induce helicity and reduce metabolic instability. Such approaches include the use of peptoids which project side chains from the amide nitrogen rather than the alpha-carbon, β-peptides which add an additional carbon atom to each residue from which the side chain is substituted, and azapeptides which replace the alpha-carbon with an additional nitrogen atom. Gellman has coined the term ‘foldamer’ to describe non-naturally occurring oligomers which adopt predictable, well-defined conformations based on non-covalent interactions. As one example, he’s introduce ααβααββ peptide foldamers as alpha helix mimetics. Another example of note is Kahne’s retroinverso approach in which he replaces the standard right-handed N→C α helix, composed of naturally occurring L-amino acids with a left-handed C→N α helix composed of unnatural D-amino acids. These retroinverso peptides are entirely superimposable on the natural peptide chain but are stable to proteolytic mechanisms.

The various peptidyl approaches here described are frequently successful at increasing the propensity for alpha-helical conformation and/or reducing proteolysis. However, their potential as effective pharmaceutical scaffolds remains a matter of heated debate due to their noncompliance with the standard notions of ‘drug-like’ molecules put forth in Lipinski’s rules, particularly with regards to molecular weight and quantity of heteroatoms, both of which contribute significantly to absorption. With current advances in biologics, proponents of peptidyl peptidomimetics argue that perhaps the pharmaceutical industry has become overly preoccupied with traditional notions of oral availability and development of these peptidyl approaches to PPI inhibition persists.
1.1.4 Non-Peptidyl Approaches toward Alpha Helix Mimetics

An alternative approach to the inhibition of alpha helix-mediated PPIs is the development of small organic ‘drug-like’ molecules that are appropriately substituted to mimic the relevant interactions of key ‘hot spot’ residues. While these approaches do not face the same obstacles with regard to bioavailability and metabolic stability, they present a formidable hurdle in terms of compound design as they attempt to employ compact, low molecular weight ligands to compete for complex binding interfaces.\textsuperscript{12,18,24,25}

Precedents for these non-peptidyl approaches actually predate many of the studies on stabilized helices. The ability of substituted 1,6-indanes to mimic the side-chains of consecutive peptides \((i, i+1)\) in an alpha-helical conformation was demonstrated in the mid-1990s.\textsuperscript{26} Subsequent work has demonstrated that an additional substituent at position 1 facilitates the mimicry of the \(i-1\) position as well (Figure 1.4).\textsuperscript{27}

The screening of chemical libraries has unearthed a handful of small-molecule leads (e.g. chalcones, benzodiazepines), but ultimately this strategy has not been particularly fruitful.\textsuperscript{24} More recent efforts to synthesize small-molecule alpha helix mimetics have recognized the role that amphipathic alpha helices often play in mediating protein-protein interactions and therefore have tended to focus on mimicking the side chains of the \(i, i+3\) or \(i+4\), and \(i+7\) residues. Common to these approaches is an attempt at rationally designing molecular scaffolds from

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{1_6-indanes.png}
\caption{1,6-indanes as \(i, i+1\) alpha helix mimetics}
\end{figure}
which substituents can be oriented in geometric configurations to mimic the projection of side chains from the alpha helices (Figure 1.5).

![Figure 1.5 Structural mimics of amphipathic alpha helices.][1]

Various small-molecule scaffolds have been employed, often centered upon rigid aromatic structures (Figure 1.6). The substituents may retain a fair degree of flexibility, but can be induced to penetrate the protein binding domain.

![Figure 1.6 Small-molecule alpha helix mimetics.][2]

Hamilton has innovated peptide-like flexibility into this small-molecule scaffold approach, initially introducing 2,3’,3’’-substituted terphenyls as mimetics of the $i$, $i+3/i+4$, and $i+7$ positions of a two turn alpha helix. The use of terphenyls takes advantage of the intrinsic [1]: https://example.com/figure1.5.png
[2]: https://example.com/figure1.6.png
rotational properties of bi- and poly-aromatic structures to engineer a flexible core from which substituents can be appropriately projected. Hamilton has termed such approaches ‘proteomimetics,’ defined as “non-peptide structure[s] that mimic larger areas of the protein surface.” Initial success with this approach has led to an assortment of variations, including the insertion of heteroatoms to improve solubility, the introduction of Rebek’s wet-edge to favor amphipathic conformations, and the terephthalamide derivatives which increase the overall polarity while encouraging the desired mono-facial arrangement of substituents through the support of hydrogen-bond networks. These and other related alpha helical proteomimetics are depicted in Figure 1.7.

Figure 1.7 Small-molecule scaffolds mimic various substitution patterns of alpha helices.
1.2 Biphenyls as Proteomimetics

The 1922 discovery that certain biphenyls can exhibit induced chirality along the biaryl axis, and particularly the observation that certain highly-hindered biphenyls can in fact exist as separable enantiomers, has given rise to a near century-long study of the physical properties of biphenyl and its derivatives. This induced chirality, termed atropisomerism, emerges from heightened energetic barriers at coplanarity which result in restricted rotation of the biphenyl core. An improved understanding of the nature of these compounds has resulted in their application across such diverse fields as materials chemistry, pharmaceuticals, natural product synthesis, and catalysis.

While free rotation might generally be expected about a biphenyl core, it is complicated by two opposing energetic forces. Energetic maxima due to steric effects emerge in the coplanar configuration (0°, 180°), while the orthogonal configuration (90°) eliminates the otherwise stabilizing effects of electronic conjugation due to a breach in pi-orbital overlap. Experimental observations of the gas-phase configuration of unsubstituted biphenyl measured a rotational angle of 44.4 ± 1.2° with measured energetic barriers to rotation of 6.0 ± 2.1 kJ/mol at 0° (coplanar) and 6.5 ± 2.0 kJ/mol at 90° (orthogonal). More recently, Johannson used theoretical studies to support these experiments, calculating a rotational angle of 45.8° which can be attributed to calculated energetic barriers of 8.0kJ/mol at 0° and 8.3kJ/mol at 90°.

This approximately 45° angle that is preferred in gaseous biphenyl illustrates a compromise between steric and electronic contributions. Simply depicted, an energy diagram of the rotation
of biphenyl about the biaryl axis reveals energy wells representing conformations which minimize steric interactions while preserving conjugation (Figure 1.8).

![Graph showing energy vs rotational angle]

**Figure 1.8** Energetic barriers at planarity and orthogonality constrain biphenyl conformation.

Addition of substituents to biphenyl complicates this depiction. Electronic contributions of the substituents are expected to have a fairly negligible effect on the system, however steric bulk, particularly when placed at close proximity to the biaryl linkage, significantly destabilizes coplanarity, encouraging rotational angles in the range of 50°-80° (as well as 100°-130°) with especially hindered systems approaching 90° (Figure 1.9). Thus, atropisomerization emerges in severely hindered biphenyls as a result of insuperable energy barriers at coplanarity.

![Graph showing energy vs rotational angle with arrows indicating barriers]

**Figure 1.9** Energetic barriers at co-planarity increase with increasing steric hindrance, favoring increasingly orthogonal conformations.
Biphenyl and related polyaromatics therefore present flexible organic scaffolds on which substituents can be conscientiously arranged with the purpose of being projected in favorable, geometrically appropriate configurations. This recognition clearly contributed to Hamilton’s selection of bi- and ter-phenyl scaffolds to mimic the side chain projection of single and double turn alpha helices.

Static crystal models have encouraged a general misapprehension of protein binding domains as rigid structures. Proteins are in fact dynamic systems, and binding interactions require significant energetic and conformational compromises. Therefore, structures that retain flexibility but simultaneously contain intrinsic preferences toward adopting configurations that are favorable for the desired binding interaction should have heightened inhibitory potential. While the goal of designing an alpha helix mimetic is to provide a pre-organized scaffold upon which functionalities can be appropriately arranged, retention of some flexibility allows a mutual energetically-minimized conformation to be achieved between the protein and the inhibitor.37

Significant experimental evidence exists to support the initial theoretical conception of this approach to alpha helix mimicry. The polyphenyl design recognizes that alpha helix-mediated PPIs frequently bind through a monofacial display of functionalities from positions $i$, $i+3/i+4$, and $i+7$. Computational models demonstrate similar disposition of side chains in the energetically minimized conformations of an alanine-rich alpha helix and the 2,3',3” tri-methylated terphenyl (Figure 1.10).38
Figure 1.10 A) polyalanine alpha helix; B) 3,2′,2″-trimethylterphenyl; C) Root mean square difference overlay of a polyalanine alpha helix and 3,2′,2″-trimethylterphenyl.\textsuperscript{38}

X-ray crystal structures offer further support for the energetic accessibility of the desired conformation with distance measurements of the 2,3′,3″ trimethylated terphenyl approaching that observed in the side chains of a theoretical alanine-rich alpha helix (Figure 1.11).\textsuperscript{28}

Figure 1.11 A) alpha-helical 12-mer peptide with \(i, i+3, i+7\) substituents, side view; B) top view; C) 3,2′,2″-trisubstituted terphenyl, top view; D) side view; E) X-ray structure of 3,2′,2″-trimethylterphenyl.\textsuperscript{28}
The ultimate test is whether or not these compounds can inhibit alpha helix-mediated PPIs. Indeed, terphenyl variants substituted 2,3′3″ have successfully inhibited interactions between Bcl-xL and an alpha-helical Bak-derived peptide,\textsuperscript{28,31,39} as well as the helical bundling of the fusion-active conformation of gp41.\textsuperscript{38} Additionally, heterocyclic biaryl derivatives containing 2,3′ substitution have been demonstrated as inhibitors of interactions between ERα and an alpha-helical SRC-1 derived peptide (\textit{vide infra}).\textsuperscript{40}

1.3 Nuclear Receptor Antagonists as Therapeutics

1.3.1 Nuclear Receptors: Structure and Transcriptional Activity

The nuclear receptor superfamily of transcription factors comprises the single largest family of transcription factors, consisting of 50 or more members. Induced by the binding of ligands, including steroids and other signaling hormones, the NR then undergoes a complex regulatory mechanism through interactions with DNA response elements and a host of further cofactors in order to ultimately promote transcription of NR cognate genes.\textsuperscript{41,42}

Structural homology is greatly apparent amongst NRs (\textbf{Figure 1.12}) and is representative of conserved mechanisms of eliciting transcription. Proximal to the N terminus is a poorly conserved A/B region which contains the AF-1 transactivation domain, responsible for non-ligand induced basal transcriptional activity. AF-1 is also cooperatively involved in the stronger NR transcriptional activity that occurs upon ligand binding. The C region contains the DNA binding domain (DBD). This region is highly conserved and binds DNA via zinc fingers at
particular NR-specific response elements which are located significantly upstream from the target gene that is to ultimately be promoted. The D region essentially is considered a hinge region which connects the DBD to the ligand binding domain. Region E is known as the ligand binding domain (LBD) but in reality plays a number of functions. This region is responsible not only for ligand binding, but also for association of the receptor with heat-shock proteins, receptor dimerization, and the recruitment of coactivator proteins (CoA) to the AF-2 transactivation domain.\textsuperscript{43}

![Figure 1.12](image)

**Figure 1.12** Structural homology of nuclear receptors.

Unbound NRs are mainly located in the cytoplasm and generally found in complex with heat shock proteins (e.g. Hsp90). Post-translational modifications, such as receptor phosphorylation, are potentially responsible for the transportation of the NR complex into the nucleus,\textsuperscript{44} an observation which encourages recognition that there potentially exist a number of other intracellular communication events responsible for transcriptional activity and subject to complex regulatory networks. An agonist-bound receptor will undergo conformational changes
that release the Hsp shield, allowing NR dimerization (homo- or hetero-). The binding of agonist is additionally responsible for initiating the formation of the coactivator binding pocket of the AF-2 domain which comprising residues from helices 3, 4, 5, and 11 and is formed by the ligand-induced rotation of helix 12. (Antagonist binding is thought to cause a slightly different conformational shift which blocks the AF2 binding domain, in some cases instead exposing an alternative groove capable of recruiting corepressor proteins.\textsuperscript{45} The initial binding of coactivator proteins does not instantly promote transcription. Instead, a complex assembly of proteins will provide a link between the bound NR response elements and the target gene and instigate chromatin remodeling via histone acetyltransferases and histone methyltransferases. This exposes the target gene and ultimately allows the binding of RNA polymerase II (RNA Pol II), thereby catalyzing gene transcription (Figure 1.13).\textsuperscript{46,47,48}

![Figure 1.13](image)

**Figure 1.13** Hormone-induced transcriptional pathway of nuclear hormone receptors.\textsuperscript{48}

1.3.2 Therapeutic Potential of NR Antagonism

A number of disease pathologies have been attributed to over-expression of NR-promoted genes. In particular, the steroid hormone receptors, particularly estrogen receptor (ER) and androgen
receptor (AR), are among the most frequently studied NRs due to the integral roles that they frequently play in the proliferation of hormone-responsive cancers.49,50

ER is a transcription factor that is naturally induced by the binding of estrogens, most notably the steroid 17β-estradiol. Two subtypes of ERs exist, estrogen receptor-alpha (ERα) and estrogen receptor-beta (ERβ), and additionally a number of alternative isoforms and splice variants have been identified (Figure 1.14).46

![17β-estradiol](A) and structural homology of ERα and ERβ.46

Figure 1.14 A) 17β-estradiol is a potent endogenous ER-agonist. B) Structural homology of ERα and ERβ.46

While ERα and ERβ display high sequence similarity and bind to identical response elements on DNA, their varied tissue distribution dictates unique receptor-specific biological functions. The morphological role of ERs has been repeatedly observed. Studies performed on ER knockout mice demonstrated that expression of ERα and ERβ are not essential for mouse survival but that their exclusion results in severe consequences to the reproductive system as well as stunted development in the cardiovascular, skeletal, immune, and nervous systems.51
The late 19th century recognition that oophorectomy was an effective treatment for a significant percentage of breast cancers, the subsequent identification of ER and resultant studies of ER structure and function, and the eventual recognition that ERα is overexpressed in approximately 60% of breast cancers have resulted in a wealth of research into the mechanism of ERα-promoted breast cancer proliferation. Studies performed in MCF-7 ERα-positive breast cancer cells have demonstrated estrogen-stimulated morphological changes with regard to the arrangement of actin filaments and the formation of cellular adhesion plaques, as well as contributions by estrogen to the migratory and invasive capabilities of these cells. The search for antagonists of ERα as breast cancer therapeutics which directly inhibit estrogen binding has proved fruitful resulting in such marketed pharmaceuticals as tamoxifen and raloxifene (and similarly the prostate cancer therapeutic bicalutamide, an AR antagonist which directly competes with the binding of androgens).

The investigation of ERα antagonists as breast cancer therapeutics might indeed represent the most developed example of NR antagonism, but is certainly not the only example. ERα as well as progesterone receptor (PR) antagonists are also potentially useful therapeutics for reproductive cancers. AR antagonists have been successfully developed for the treatment of prostate cancer. Fibrate antagonism of peroxisome proliferator activated receptor-alpha (PPARα) have been used to treat metabolic disorders, in particular high cholesterol, and the related PPARγ has been targeted by the thiazolidinediones for the treatment of diabetes and other metabolic disorders. A host of NR-affiliated pathologies rely on a conserved underlying mechanism, and methods to selectively modulate NR activity are therefore quite desirable.
1.3.3 Traditional Modes of NR Antagonism

Efforts at NR antagonism have been predominantly focused on inhibition of the initial ligand binding event. In most cases these compounds directly compete for the NR-LBD and cause distinctly different conformational shifts than the native ligand. This then prevents the formation of the coactivator binding domain (CBD, also referred to as AF2), thereby disrupting the recruitment of essential coactivator proteins and the subsequent assembly of the transcriptional apparatus responsible for promotion of NR cognate genes (Figure 1.15).45,57

![Figure 1.15](image_url) Inhibition of coactivator binding can effectively inhibit NR-protomoted gene transcription.58

To date, this strategy has experienced a great deal of therapeutic success. However complications have arisen, particularly in the development of side-effects and resistance mechanisms which develop from prolonged administration.59

Considering the case of ERα-LBD antagonists; tamoxifen has achieved significant clinical success as a breast cancer therapeutic. It has been observed to function as a selective estrogen
receptor modulator (SERM), exhibiting tissue-dependent agonist/antagonist activity. Tamoxifen’s success against breast cancer is indicative of its antagonistic activity in breast cancer cells, however it instead functions as an agonist in uterine tissue, the cardiovasculature, and bones. While beneficial side effects may be noted in the cardiac and bone tissue, this agonist activity threatens the patient by sharply increasing uterine cancer propensity. Additionally, as tamoxifen treatments are continued over prolonged time periods, it is important to note that resistance mechanisms to tamoxifen treatment frequently are observed to develop, potentially due to enhanced sensitivity of ERα through growth-factor induced activation.51,52,53

Alternative therapies have therefore been considered for modulating ERα-promoted transcription (Figure 1.16). Fulvestrant is an effective breast cancer therapy in post-menopausal women, antagonizing and promoting the degradation of ERs. However, estrogens are essential for a number of body functions as well as cognition, therefore, while survivable, this treatment option is highly disruptive. Aromatase inhibitors, such as exemestane, similarly function as full ER antagonists, but do so by preventing the natural formation of estrogens which are generated from the enzymatic aromatization of androgens. While this treatment method should not eliminate ERs, it universally prevents estrogenic activity.51,53

![Chemical structures of tamoxifen, fulvestrant, and exemestane.](image)

**Figure 1.16** Tamoxifen, fulvestrant, and exemestane target ER-promoted breast cancer through varied mechanisms.
Clearly alternative methods of ERα- and, more generally, NR-antagonism are desirable. Disruption of the binding of endogenous, small-molecule ligands has for decades been generally regarded as the most suitable option for therapeutic development. However, our improved understanding of protein-protein interactions and the development of methodologies towards the inhibition of such provides novel opportunities for biological intervention.

1.4 NR-CoA Interaction as Ideal System for Inhibition by Alpha Helix Mimetics

Although methods of NR modulation have been explored for decades, the vast majority of these have targeted the ligand binding event. Heightened understanding of the essential roles played by coregulator proteins in NR-promoted gene transcription underscores the breadth of other interactions that can be targeted. The tendency in pharmaceutical development has historically been to target interactions between endogenous ligands/substrates and their protein targets (vide supra), however the recent revelation that certain PPIs can be rationally inhibited through targeting of ‘hot spot’ interactions reveals a host of new therapeutic targets.

CoAs bind NRs through a conserved alpha-helical pentapeptide, termed the NR box, which has been deemed to be both necessary and sufficient for binding. The NR box consists of an LxxLL residue pattern (L = leucine, x = non-conserved residue) and is frequently contained in triplicate in coactivator proteins. When bound to the NR-CBD, the first and third leucine residues of the NR box penetrate downward into the hydrophobic groove of the CBD, while the second leucine residue makes less essential hydrophobic interactions within a nearby pocket. Flanking this groove are charged residues (in the ERα-CBD: K362, helix 3 and E542, helix 12) that align
with the intrinsic dipole of the alpha helical backbone of the NR box, inducing a ‘charge clamp’ interaction which orients and stabilizes the appropriate configuration for optimal binding affinity (Figure 1.17).\textsuperscript{62}

![Figure 1.17 Coactivator proteins bind the ER-CBD through a conserved LxxLL motif.\textsuperscript{63}]

Although the field of PPI inhibition is still in its relative infancy, this small amphipathic alpha helix offers an ideal system for inhibitory efforts by peptidyl and non-peptidyl alpha helix mimetics. Indeed, peptide fragments possessing NR box variants (LxxLL) have been demonstrated to successfully inhibit ERα-CoA binding via a ligand-independent mechanism.\textsuperscript{64} While unconstrained peptides are impractical as therapeutics, this study has motivated ongoing efforts to develop NR coactivator binding inhibitors (CBIs) (Figure 1.18).
Figure 1.18 (a) Coactivator (SRC) binding can be inhibited by (b) conformational differences in antagonist bound receptor or (c) by direct inhibition by a CBI.65

Geistlinger and Guy have demonstrated the ability of macrolactam-constrained NR box-containing peptides (derived from the second NR box of SRC-2) as NR-CBIs with IC50 values approximating 0.1μM. Additionally, by systematically altering the identity of individual leucine residues through the incorporation of non-natural side-chains, they were able to induce NR specificity, evaluating the effectiveness of the resultant compounds in thyroid receptor-beta (TRβ) in addition to ERα and ERβ (Figure 1.19).20

Figure 1.19 Macrolactam-constrained LxxLL-containing peptides as NR box CBIs. NR-selectivity has been induced from mutations in single LxxLL residues.20
Thioether and disulfide bridges have been similarly employed by Spatola as constraints to NR box-containing peptides in order to encourage helicity and selectivity. Such constrained peptides have achieved Ki values in the low nanomolar range for ERα and ERβ. The resultant SAR revealed a delicate interplay between flexibility and helicity underlying the optimal constriction of peptide fragments, as increased helicity results in heightened selectivity while increased flexibility results in heightened affinity. The study was presented as an SAR of the binding constraints of the receptor docking sites, and underscores the relevance of such findings for the development of peptidyl as well as non-peptidyl approaches to alpha helix mimicry.\textsuperscript{19,66,67}

Katzenellenbogen has effectively pioneered the development of small-molecule ERα-CBIs, demonstrating low micromolar affinity for the ERα-CBD via the projection of flexible hydrophobic side chains generally from rigid aromatic cores (Figure 1.20).\textsuperscript{65,68,69,70,71}

![Figure 1.20](image)

**Figure 1.20** Small-molecule, rigid aromatic scaffolds mimic LxxLL peptides by projecting flexible hydrophobic substituents.\textsuperscript{68}

Additionally, as the slightly larger AR-CBD has frequently demonstrated a preference for NR-box variants that contain bulkier residues at the $i$, and $i+4$ positions (e.g. FxxLF, WxxVW),\textsuperscript{65,72}
derivatizing similar CBI scaffolds with larger substituents has resulted in complete selectivity for AR compared with ERα.

Recently, Hamilton’s group has introduced their poly-aryl proteomimetic approach to the investigation of NR-CBIs (*vide supra*). As the NR box composes only a single turn of an alpha helix, biaryl systems were employed. The use of a heteroaromatic scaffold in place of a biphenyl was intended to increase hydrophilicity while simultaneously facilitating the substitution of various functionalities from a pre-assembled core. The scaffold was substituted at the 2-pyridyl and the 1,5-pyridone, in an analogous fashion to a 3,2',6' substituted biphenyl (Figure 1.21).

![Figure 1.21 Trisubstituted-biaryl scaffold as NR box CBI.](image)

Superimposing the resultant x-ray structure of various derivatives on the alpha helical LxxLL motif of a coactivator-derived peptide demonstrated the energetic accessibility of complementary configurations (Figure 1.22). A small series of compounds were evaluated for their efficacy as ERα-CBIs with the most active compounds achieving Ki values in the micromolar range, comparable to the most active small-molecule ERα-CBIs reported to date.
These and other strategies have been incorporated into the design of more general NR-CBIs. This represents a nascent field of study, with non-peptidyl approaches to PPI inhibition not yet approaching the low nanomolar binding affinities frequently observed with NR-LBD modulators. However, side-effects and resistance mechanisms of these otherwise effective therapeutics, NR-CBIs constitute a novel and promising means of modulating NR-promoted transcription for the treatment of numerous human pathologies.

1.5 Design of Bipolar Biphenyl Proteomimetics as NR-CBIs

Encouraged by recent success in designing small molecules capable of disrupting ERα-CoA interactions, and intrigued by the potential of substituted poly-aromatics as flexible mimics of alpha helices, our group has designed a biphenyl scaffold functionalized to mimic the key interactions that mediate NR-CoA binding.

The design of our novel approach recognizes a number of highly desirable elements for incorporation in the design of an NR-CBI. An ideal NR-box mimetic should contain hydrophobic substituents oriented in such a way as to mimic the key hydrophobic contacts of the

Figure 1.22 Overlay of trisubstituted biaryl scaffold with LxxLL alpha helix.40
i, i+3, and i+4 leucine residues, considered necessary and sufficient for binding to the NR-CBD. As a therapeutic agent, it ideally would retain the ‘drug-like’ properties traditionally regarded as essential for effective pharmaceuticals while still incorporating some of the inherent flexibility of peptide fragments in order to effectively be oriented in the binding domain. Finally, it would be functionalized to complement the bipolar ‘charge clamp,’ providing added stability to the binding interaction while additionally serving as an internal compass that can orient the scaffold within the binding domain.

Three main elements are therefore incorporated in the design (Figure 1.23):

1) A biaryl core, intended to mimic the configuration of an alpha-helical peptide backbone.

2) Hydrophobic substituents, intended to mimic the projection and character (both steric and electronic) of leucine side-chains.

Figure 1.23  di-substituted 4,4’-bisoxybiphenyl as NR box mimetic.

3) Asymmetric polar termini, intended to interact with charged residues of the NR-CBD, mimicking the ‘charge clamp’ that stabilizes NR-CoA binding.

As an alpha helix mimetic, the biphenyl provides a core structure with a unique energetic profile (vide supra), the energetics of which can be further controlled through conscientious substitution
proximal to the biaryl linkage. The hydrophobic substituents therefore have a two-fold purpose, both in mimicking the hydrophobic interactions considered essential to binding in the NR-CBD, and in controlling the orientation of the biphenyl, thereby favoring optimal conformations for their own projection. Asymmetric amino and acidic termini substituted from the 4 and 4’ positions, provide an internal compass which can orient the scaffold within the binding domain by mimicking key ‘charge clamp’ interactions. Appending these termini via ethoxy linkers further solubilizes the compound while providing flexibility.

Ultimately, this approach provides a biaryl scaffold which projects hydrophobic substituents and is flanked by asymmetric bipolar substituents. A variety of derivations on this strategy may ultimately prove to be optimal for enhancing inhibition of NR-CoA interactions and inducing NR-specificity, including variations in the aromatic core, the identity and arrangement of the hydrophobic substituents, the identity of the polar termini as well the mode by which they are affixed, and the inclusion of further elements for interaction (e.g. mimicry of further residues within or flanking the NR box).

1.6 Suzuki-Miyaura Coupling of Biaryl Systems

Synthesis of the biphenyl scaffold was envisioned from the convergent coupling of the previously prepared, pseudo-symmetric aryl subunits, each of which might originate from simple substituted phenols (Figure 1.24).
The emergence of palladium-catalyzed coupling procedures has played an inestimable role in expanding the scope of organic synthesis and medicinal chemistry, as it provides, among other things, an efficient and functionality-tolerant means for carbon-carbon bond formation. Prior to the inception of palladium chemistry, carbon-carbon bonds were typically generated through activation of organyl-halides using organolithium or Grignard methods. Although these procedures were widely employed at the time and remain very valuable to this day, the harsh reaction conditions are intolerant of common functionalities (e.g. carbonyls), sharply restricting the scope of their use. The advent of more tolerant palladium-catalyzed approaches allowed for C-C ligation in the presence of such functional groups and consequently changed the face of synthesis. Palladium coupling methods have been well-examined over the last few decades and have been extended to include various metalations (B, Sn, Si, etc) as well as C-N and C-O coupling procedures, in addition to the coupling of activated C-C systems. A number of methods of activating various aryl, vinyl, and alkyl groups for these palladium mediated reactions exist.
but generally one component contains an electronegative functionality (most commonly a halide) while the other component contains an electropositive species (e.g. metalloids).  

The overwhelming emergence of palladium catalysis stems from the ability of Pd(0) complexes to activate electrophilic organic compounds (halogenated and pseudo-halogenated carbon bonds) through an oxidative addition of the C-X bond to the metal center. Palladium is a transition metal which most commonly exists in its 0 (d\(^{10}\)) and +2 (d\(^{8}\)) oxidation states and has a tendency to form stable 16e- square planar complexes. The active catalyst for palladium-mediated C-X activation is typically a 14e- phosphine-bound Pd(0) species and is often generated in situ from the exposure of Pd(II) or Pd(0) precursors to various phosphines. The catalytic cycle (Figure 1.25) is considered to be generally conserved throughout the various coupling approaches, although each step likely involves additional, reaction-specific detail. Generally speaking, an activated carbon species first oxidatively adds to the Pd(0) complex, forming a Pd(II) intermediate that is prone to reaction with nucleophiles, most frequently in the form of a transmetalation or insertion event. This is the stage where the reactions diverge creating a variety of possible products, but always resulting in the existence of the Pd(II) complex bound simultaneously to both of the desired subunits and the concurrent release of the activating groups. Finally, the resultant Pd(II) complex undergoes reductive elimination to yield the coupled product while regenerating the active Pd(0) species.
The Suzuki coupling reaction is among the most widely used variants of palladium reactions and generally refers to the Pd(0)-catalyzed coupling of an aryl or vinyl halide with an aryl or vinyl boronic acid (or derivatives thereof, including boronic esters and trifluoroborate salts). A seemingly infinite assortment of reaction conditions have been described in the literature, optimizing a variety of parameters (e.g. solvent, base, ligand) to suit the particular requirements of varyingly substituted and functionalized aryl and vinyl systems. Recognizing the versatility of this reaction and its potential use in assembling the target biphenyl scaffold, we envisioned the synthesis of our compounds to culminate with the Suzuki coupling of the fully substituted and functionalized phenyl subunits.

**Figure 1.25** General catalytic cycle of palladium-mediated couplings.75
Chapter 1: Background

Part B: Applications of Palladium Chemistry to the Development of Radiotracers
1.7 Radiotracers

The term radiotracer describes the incorporation of radioactive nuclides into compounds known to be functionally active in a given physical process in order to observe the progression of the substance within a system of interest.\(^{76,77}\) Among other applications, radiotracers are frequently employed for the evaluation of biological systems, allowing such events as cellular uptake, enzymatic catalysis, and ligand competition to be assessed by tracing the characteristic emission properties of the radionuclide.\(^ {78}\)

When designing radiotracers, it is essential to employ compounds with high affinity for the target system of interest, dosed at quantities that are significantly below therapeutic levels, and with detectable decay emissions and high specific activity. The effectiveness of radiotracers is therefore generally dependent on the following assumptions, outlined by Ruth: \(^ {79}\)

1) "the tracer behaves or interacts with the system to be probed in a known reproducible fashion,
2) "the tracer does not alter or perturb the system in any measurable fashion,
3) "the tracer concentration can be measured."

Radioactivity results from nuclear instability. A nucleus which is unstable in either its composition of subatomic particles or the arrangement thereof, will eventually undergo spontaneous decay. Particular radionuclides possess distinct decay mechanisms, having nuclide-specific kinetic properties and emission products.
While a vast assortment of radionuclides have been identified (>3000), only select nuclides possess favorable emissions properties for use as radiotracers. For example, the emission of nucleons (e.g. alpha particles, protons, neutrons) is highly undesirable for radiotracer distribution in mammalian systems because these large particles will generally only travel short distances upon emissions before coinciding with intracellular structures. These interactions can have profound biological effects, particularly carcinogenic, and additionally prevent the efficient detection of particle emissions by external spectrometers. Instead, nuclides which emit gamma radiation are preferable for mammalian imaging. Gamma radiation ($\gamma$) is a form of high energy electromagnetic radiation ($\nu > 10^{19}$ Hz). While high levels of gamma radiation do pose medical risks, the threat of intracellular damage from gamma emmiters is significantly reduced compared with particle-emitting nuclides. Additionally, gamma rays can effectively penetrate cellular membrane and the skin allowing their detection by external spectrometers. Positron emissions, a form of beta decay, also generate coincident gamma radiation upon annihilation with an electron (an event that generally occurs in close proximity to the initial point of emission), similarly allowing external detection of positron emissions. As a result, nuclides that emit either positrons or gamma rays have been favored in the development of radiotracers for \textit{in vivo} imaging.

In addition to considering the emission products of a radionuclide, the kinetics of the radioactive decay must be considered when determining an appropriate nuclide to incorporate in the design of a radiotracer. In order to effectively detect the radiotracer, a minimal activity, as measured in disintegrations per second, must be present. Activity will be simultaneously dependent on the quantity of tracer being generated and the half-life of the radionuclide. Nuclides with long half-lives will exhibit low activity unless large quantities of material are administered, potentiating
the likelihood of promoting a biological response. Additionally, administering radiotracers with longer than necessary half-lives to living organisms increases medical risks by prolonging radioactive exposure, while nuclides with very short half-lives are not appropriate if they do not allow sufficient time to effectively label (synthesize, purify, etc) and administer the radiotracer before the tracer’s activity is reduced below useful values.

Careful attention therefore must be paid to nuclear decay properties in choosing an appropriate radionuclide for a particular experiment. Available nuclides may additionally be limited by the accessibility of the radionuclide of interest, the ease of incorporation of that nuclide to the tracer compound, the availability of appropriate detection equipment, and the limitations of that equipment (e.g. detectable energy ranges, minimal detectable specific activity). Among the most effective nuclides to have emerged for the development of biological radiotracers are select radioisotopes of halogens, particularly fluorine-18 (\(^{18}\text{F}\)) and various isotopes of iodine.

\(^{18}\text{F}\) is a positron emitter with a half-life of 110 minutes which can readily be generated in cyclotrons by the proton bombardment of \(\text{H}_2^{18}\text{O}\). The emergence of positron emission tomography (PET), which generates 3-D images through the detection of gamma rays emitted from positron/electron collisions, made positron emitters particularly enticing for medical imaging. Subsequently, the development of \(^{18}\text{F}\)-fluorodeoxyglucose (\(^{18}\text{F}\)-FDG) as a common cancer imaging agent and the resultant proliferation of PET scanners and on-site cyclotrons to many hospitals, has resulted in numerous efforts to develop medically useful \(^{18}\text{F}\)-tracers.
The radioisotopes of iodine offer a distinct set of advantages compared with fluorine. A number of isotopes of iodine exist, all but one of which (\(^{127}\text{I}\)) are radioactive. Of these, three have emerged as particularly useful radiotracers of biological systems: \(^{123}\text{I} \) (\(\gamma\) emitter, \(t_{1/2} = 13\) hrs), \(^{125}\text{I} \) (\(\gamma\)-emitter, \(t_{1/2} = 59\) days), and \(^{131}\text{I} \) (\(\beta\) and \(\gamma\)-emitter, \(t_{1/2} = 8\) days). The thirteen hour half-life of \(^{123}\text{I} \) is well-suited for dealing with the time constraints of synthesis, purification, and biological distribution, while simultaneously short enough to limit the quantities of tracer that need to be administered for \textit{in vivo} imaging. The energy of the gamma rays emitted by \(^{123}\text{I} \) are well within the detection limits of single-photon emission computed tomography (SPECT). \(^{125}\text{I} \) has a much longer half-life of 59 days, making its use in humans inefficient. However, \(^{125}\text{I} \) is useful for studies run outside of the body, such as radioimmunoassays and cellular studies which typically can tolerate the higher concentrations of tracer which may be necessary to detect activity from the longer-lived nuclide. Additionally, the longer half-life of \(^{125}\text{I} \) provides the benefit of not requiring constant regeneration of radiolabeled materials. \(^{131}\text{I} \) has numerous decay products (positrons, electrons, antineutrinos, gamma rays) and therefore would generally be considered medically undesirable. However, due to the high uptake of iodine to the thyroid, \(^{131}\text{I} \) has been effectively employed for radiation therapy in thyroid cancer. Unfortunately, such use is inapplicable for the treatment of tumors in other tissues unless a targeting agent with extremely high selectivity is identified.

Numerous additional nuclides have found utility as radiotracers. Methods of incorporating radioactive isotopes of C, H, N, and O have been investigated, due to their ubiquity throughout biological and materials sciences. With the exception of \(^{3}\text{H} \), which is employed in competitive binding studies, the undesirable kinetics of these radionuclides tend to make them inefficient as
biological radiotracers. Approximately 80% of radiopharmaceuticals currently in use incorporate the radionuclide $^{99m}$Tc. However, this speaks to the efficient decay properties of $^{99m}$Tc, as well as its effective conversion from available precursor $^{99}$Mo, rather than the biological efficacy of technetium. In comparison, the halogens offer much more pharmacologically relevant radionuclides for incorporation into radiotracers of biological systems.

1.8 Radiolabeling of AM630

The cannabinoid receptors, of which two are known (cannabinoid receptor 1 and cannabinoid receptor 2, CB$_1$ and CB$_2$ respectively), are 7-transmembrane G-protein coupled receptors (GPCRs) which were identified by their ability to mediate the signaling of certain psychoactive compounds of the cannabis plant (e.g. $\Delta^9$-tetrahydrocannabinoid). In addition to its association with cannabis-promoted psychotropic effects, the endocannabinoid system has been implicated in such diverse pathologies as cancer, Alzheimer’s disease, liver disease, diabetes, pain, and inflammation.$^{80}$

CB$_1$ and CB$_2$ possess 44% sequence homology (68% in their transmembrane domains) and share affinities for numerous ligands, including various classes of endogenous, plant, and synthetic cannabinoids. Their divergent biological functions are mainly determined by their varying tissue distribution. Of note is the extensive expression of CB$_1$ in the brain while CB$_2$ has most frequently been identified in the peripheral nervous system and additionally plays significant rules in immune responses and inflammation.
Growing interest in the therapeutic potential of CB2 ligands for both pain modulation and inflammation has motivated efforts to develop CB2-selective ligands. CB2 selective radiotracers are highly desirable for research purposes in order to facilitate observations of uptake and pharmacokinetics of potential CB2-selective therapeutic candidates as well as comprehensive imaging of CB2 tissue distribution. AM630 (6-iodopravadoline), a synthetic aminoalkylindole, has been demonstrated to be an inverse agonist of CB2, with a Ki of 32nM for CB2 as well as >10² selectivity for CB2 compared with CB1. The pharmacological properties of AM630 (particularly its CB2 specificity) combined with the inclusion of an iodo substituent at the C6 position of the indole moiety, makes AM630 a highly promising candidate for development as a CB2 radiotracer.

1.9 Metalated Precursors for Radionuclide Incorporation

A radiolabeled derivative of AM630 was specifically desired due to its known pharmacological properties (particularly its CB2 specificity). It was therefore considered essential to conserve the precise chemical identity of AM630, and a method was required that would ensure site-specificity upon radioiodination. Due to the immediate progression of radioactive decay upon formation of a radioactive nuclide, radiolabeling should be the final step in synthesizing a radiotracer and the reaction should proceed quickly with limited need for purification. Both nucleophilic and electrophilic methods of site-specific radiohalogenation have been investigated, including methods which halogenate via direct addition as well as substitution.
This particular conversion presented as an ideal candidate for radioiodination via electrophilic halo-demetalation. Metalation can frequently be achieved from an aryl halide precursor, such as that presented by the 6-idoindole moiety of AM630. This would, in a single step, generate an intermediate from the bioactive molecule, which could immediately be subjected to radioiodination. The use of organometallics rather than other leaving groups allows high yielding site-specific halogenation under mild conditions. Various metals and metalloids have been employed for such conversions, including boron, silicon, germanium, and tin.  

Halo-demetalation methodologies originate from the recognition by Herbert Brown that organoboranes could be halogenated from diatomic halogens under basic conditions (Equation 1.1).

\[ \text{R}_3\text{B} + \text{X}_2 + \text{NaOC}_2\text{H}_5 \rightarrow \text{R}-\text{X} \]  

(eq. 1.1)

Initially the use of the harsh methoxide base precluded the radiohalogenation of compounds containing sensitive functional groups by radiodemetalation, however the recognition that the halogenations proceeded via reaction of an electropositive halogen with an electron-rich C-B bond led to the development of alternative strategies to affect this conversion. The rapid in situ oxidation of a halide species with an oxidant such as chloramine-T, N-chlorosuccinimide, or hydrogen peroxide, yields electropositive halides which efficiently undergo electrophilic substitution of C-M bonds (Equation 1.2).
Rapid, consistent methods of converting stable precursors to the radiolabeled products were desired, and numerous metal(loid)s were found to undergo halo-demetalation reactions with various efficiencies. A study of the effectiveness of radioiodination and radiobromination on aryltrimethyl Group IVB organometallics (ArSiMe₃, ArGeMe₃, ArSnMe₃) consistently identified organotins to be superior leaving groups compared with analogous silicon and germanium compounds. While Sn, Si, and Ge share approximately equivalent electronegativities, it is believed that the larger atomic radius of Sn is responsible for a reduction in the carbon-metal bond strength, facilitating the attack of the electropositive halide on the carbon.⁸⁸ Although stannanes are similarly more reactive to radiohalogenation in comparison to boronated materials, arguments still remain regarding the desirability of the heightened reactivity offered by organyltins versus the stability and lack of toxicity offered by organylboronates.⁸⁹,⁹⁰

While early work proved the efficiency of the halo-demetalation, methods for synthesizing the relevant organometallic compounds were very harsh, generally employing strong bases (e.g. hydrides) and organolithium or Grignard reagents. This greatly reduced the range of substrates that could undergo metalation or, for complex molecules, required metalation to be accomplished at a very early stage. However, the development of radiolabeling methods via halo-demetalation closely paralleled the onset of palladium catalysis and the 1976 demonstration that aryl stannanes
could be synthesized by the palladium-catalyzed reaction of aryl halides with hexaalkylditin reagents facilitated the assembly of complex organotins (Equation 1.3).  

\[
\begin{array}{c}
\text{aryl halide} \quad \text{Sn}_2\text{Me}_6 \quad [\text{Pd}] \\
\rightarrow
\text{aryl organotin}
\end{array}
\]  

(eq 1.3)

This led to further work in the development of such methods, eventually allowing the incorporation oftrialkylstannanes into complex molecules, providing useful precursors for radiohalogenation (as well as the expansion of Stille coupling methodology).

Development of other palladium-catalyzed metalations closely paralleled the use of organotins; for example, the palladium-catalyzed conversion of aryl halides to aryl silanes was first reported in 1977. However it wasn’t until 1995 that the palladium-catalyzed boronation of aryl halides from diborons was accomplished by Miyaura, finally providing functionality tolerant means of synthesizing organoborons (Equation 1.4).

\[
\begin{array}{c}
\text{diboron} \quad \text{X-Ar} \quad \text{PdCl}_2(\text{dppF}) \quad \text{KOA}c/\text{DMSO} \\
\rightarrow
\text{aryl organoboron}
\end{array}
\]  

(eq. 1.4)

When considering the challenge of radiolabeling AM630, I was attracted to the efficiency of the use of metalated intermediate species to tolerantly and site-specifically convert aryl halides to their radiohalogenated analogues. I thus planned to accomplish site-specific radioiodination at the indolic C6 position of AM630 by converting the 6-idoindole moiety to a 6-stannylindole,
followed by subjection of this compound to electrophilic aromatic iodination with an

electropositive radioactive iodine source. Due to my prior experience with palladium chemistry,
the potential need to optimize stannylation conditions was anticipated, as was the possibility of
employing another metal(loid)s should complications arise, whether in the synthesis of the
stannylated material or in the subsequent demetalation.
References

Chapter 1, Part A:


58. Adapted from: http://medlibrary.org/medwiki/Nuclear_hormone_receptor.


Chapter 1, Part B:


Chapter 2: Initial Synthesis and Evaluation of Bipolar Biphenyls as NR-CBIs
2.1 Introduction

The design of this proteomimetic scaffold incorporates three main components (Figure 2.1):

1) A biaryl core, intended to mimic the configuration of an alpha-helical peptide backbone.

2) Hydrophobic substituents, intended to mimic the projection and character (both steric and electronic) of leucine side-chains.

3) Asymmetric polar termini, intended to interact with charged residues of the NR-CBD, mimicking the ‘charge clamp’ that stabilizes NR-CoA binding.

Figure 2.1 Bipolar Biphenyl Scaffold

In addition to the ultimate goal of designing compounds capable of inhibiting the NR-CoA binding interaction, I was particularly interested in the potential of the biphenyl as a core scaffold which could more generally be exploited in the development of small-molecule alpha helix mimetics. Therefore, the nature of this core was never considered variable toward optimizing the scaffold design. However, optimization of the arrangement and identity of the functionalities projecting from the biphenyl was considered essential to selectively mimicking the key binding interactions.
Prior to synthesizing a complex library of biphenyl derivatives, it was deemed imperative to first synthesize and evaluate a small preliminary series to support the hypothesized potential of these compounds as NR-CBIs. The initial compound design employed a 3,3’ pattern for hydrophobic substitution, as the assembly of derivatives containing heightened steric hindrance about the biphenyl linkage may require significant synthetic optimization and the optimal substituent arrangement had not yet been assessed. Included in this initial series were symmetrically substituted di- isopropyl, sec-butyl, tert-butyl, and benzyl derivatives as well as a derivative lacking hydrophobic substituents in order to evaluate whether the core bipolar biphenyl structure possessed any intrinsic binding affinity. The inclusion of asymmetric ionizable ‘amino-acid’ termini was intended to orient the compounds in the NR-CBD through mimicry of the ‘charge clamp’ interactions. To assess the effectiveness of these ionized termini at enhancing CBI activity, the non-ionized ‘amino-ester’ precursors of each derivative were intended to additionally be subjected to biological evaluation (Figure 2.2).

**Figure 2.2** 3,3’-disubstituted biphenyl ‘amino-acids’ and ‘amino-esters’
2.2 Synthesis

Synthesis of this scaffold was therefore envisioned from the convergent coupling of the previously prepared fully functionalized aryl subunits, each of which initially derive from identical ortho-substituted phenols (Scheme 2.1). [*Synthesis of this initial compound system was performed as a joint effort between myself and Patrick Weiser. For completeness all compounds are here reported, regardless of which of us were directly responsible for the synthesis of each particular compound.]

![Scheme 2.1](image)

**Scheme 2.1** Retrosynthesis of 3,3′-disubstituted biphenyl amino-acid originally envisioned as emerging from the coupling of varyingly functionalized derivatives of ortho-substituted phenols.

A variety of palladium-mediated methods for biaryl coupling exist. We particularly favored the use of Suzuki Miyaura coupling due to its high functionality tolerance and the extensive literature precedent that exists for this methodology. Due to complications that arose from the incorporation of the amine terminus prior to the coupling reaction (detailed in Chapter 3), the
Synthetic route was ultimately modified, coupling the otherwise functionalized aromatic components prior to appending the amine terminus (Scheme 2.2).

Scheme 2.2 Altered retrosynthesis couples the biphenyl prior to appending the amine terminus.

Synthesis was then accomplished from the relevant commercially available alkyl- and aryl-substituted phenols (Scheme 2.3). Para-bromination of the ortho-substituted phenols (1) was accomplished using tetrabutyl ammonium tribromide in chloroform. Syntheses here diverged. The 4-bromophenols (2) were converted under Miyaura conditions to the hydroxyphenylboronic ester (3) as well as separately functionalized to the ethyl bromophenoxyacetate (4) when reacted with ethyl bromoacetate under Williamson ether conditions. These phenyl subunits were ligated via Suzuki coupling methodology to yield the phenolic-esters (5) in 27-61% yields. The desired amino esters (6) were obtained by addition of the dimethylaminoethyl terminus to the coupled phenolic esters (5) by means of Williamson ether substitution with 2-(dimethylamino)ethyl
chloride. Finally, the amino-esters underwent ester hydrolysis and were isolated as ammonium chloride salts (7).¹

*5a was analogously prepared from 4-iodophenol and 4-hydroxyphenylboronic acid.

[Please note that synthetic efforts toward this series as well as further compound libraries, and particularly the Suzuki coupling of the biphenyl core, proved to be far from trivial, requiring significant optimization which is discussed in detail in Chapter 3.]

As a result of the decision to append the amine terminus following coupling, the altered synthetic route provided an additional class of 4,4'-asymmetrically functionalized biphenyls, namely the phenolic-esters (5). These compounds varied from the amino-esters (6) and amino-acids (7) in the truncation of the amine terminus which is intended to assist in properly orienting the scaffold via interactions with charged residues in the CBD.

**Scheme 2.3** Synthesis of 3,3’-disubstituted bipolar biphenyls.¹
2.3 Biological Evaluation

Biological assays on the resultant 3,3’-disubstituted compounds (5, 6, 7) were performed in collaboration with John Katzenellenbogen’s group (University of Illinois, Urbana-Champaign) and Donald McDonnell’s group (Duke University Medical Center). Initial screens were intended to establish the capability of our scaffold to facilitate ERα-CoA binding inhibition and therefore evaluated only the final biphenyl amino-acid compounds (7). Subsequently a number of assays were extended to include the amino-ester (6) and phenolic-ester (5) precursors in order to assess the effect of varying the identity of the polar termini (Figure 2.3).

![Chemical structures of compounds 5, 6, and 7](image)

**Figure 2.3** Biological evaluation was performed on the 3,3’disubstituted biphenyl phenolic-esters (5), amino-esters (6) and amino-acids (7).
Coactivator binding inhibition was first assayed by means of time-resolved fluorescence resonance energy transfer (TR-FRET), employing terbium (FRET-donor, $\lambda_{em}=495\text{nm}$) and fluorescein (FRET-acceptor, $\lambda_{em}=520\text{nm}$). Terbium is a lanthanide known to possess a long-emission half-life in comparison with organic fluorophores. Because terbium is difficult to excite through external sources, the label is appended via complexation with streptavidin, a protein with particularly high-affinity ($K_d = 10^{-14}$) for biotin, and excitation is instead accomplished through energy transfer from organic complexes. Terbium will then emit its fluorescence, and, if near-in-space to a FRET-acceptor, will excite a measurable fluorescence signal.

In this assay, an ER$\alpha$ fragment, containing both the LBD and the CBD and mutated to contain only one cysteine residue (Cys-417, within the LBD), was labeled at its lone cysteine residue with maleimide-biotin. The labeled receptor was then placed in solution with the known ER$\alpha$ endogenous agonist estradiol (E$_2$) and a streptavidin-terbium complex and incubated with varying concentrations of the potential biphenyl CBIs. Fluorescein-SRC-3-NRD, an NR box-containing, coactivator-derived peptide, labeled nonspecifically at its four cysteine residues with iodoacetamide-fluorescein, was then added to the solution. Following an incubation period, the resultant TR-FRET was measured. As increasing CBI activity would correspond with a reduced level of fluorescein-SRC-3-NRD binding to the ER$\alpha$-CBD thereby distancing the fluorophores, a reduction in fluorescein fluorescence emission is indicative of enhanced CBI efficacy (Figure 2.4).
Figure 2.4 Schematic of ER-CoA TR-FRET assay. ER-CoA binding in the absence of inhibitor places terbium and fluorescein in proximity to one another, allowing fluorescence transfer. In presence of CBI, FRET signal will diminish.³

Decrease in the FRET signal was observed as a function of increasing concentrations of the biphenyl CBIs. An unlabeled, coactivator-derived, NR Box containing (SRC1-Box II) peptide was used as a control. Assays were performed in duplicate and reported inhibitory constants of approximately $K_i = 10^{-5}$ M (10-100μM) were reported, with the isopropyl 7b and sec-butyl 7c derivatives exhibiting highest inhibition (Figure 2.5).

Figure 2.5 Dose-dependent reduction in FRET signal observed when CBIs inhibit the binding of ERα and a coactivator-derived (SRC3) peptide fragment.¹
2.3.2 Cell-Based Co-transfection Reporter-Gene CBI Assay (J.A.K.)

The amino-acids (7) were then subjected to a cell-based co-transfection reporter-gene assay to further evaluate their efficacy as CBIs. Studies were performed in human endometrial cancer (HEC-1) cells, which express NR-coactivator proteins but do not express ERα. The cells were transfected with an ERα-expression vector, the estrogen-responsive reporter gene 2ERE-Luc, and pCMV β-galactosidase (ER-independent vector, used as an internal control). Once transfected, the cells were subjected to a hormone-reducing serum, to reduce the levels of endogenous hormone, and exposed to varying concentrations of the potential biphenyl CBIs in the presence of E₂ (1nM). After 24 hours, the cells were assayed for estrogen-promoted luciferase activity as well as for transcription of the control.4

Luciferases exhibit bioluminescence, allowing assaying of inhibition of ERα-promoted gene transcription to be performed via reduction in observable luminescence. The only biphenyl amino-acid to exhibit repeatable inhibition of this luminescence was the sec-buty derivative 7c, and, although an IC₅₀ of 2μM was measured, the reduction in luminescence was significantly less substantial than that observed from a guanylhydrazone CBI5 used as a control (Figure 2.6). It has been proposed, but not confirmed, that the inefficiency of these compounds in cell-based assays may be due to inherent limitations in cell-permeability.1
Figure 2.6 Reporter gene assay confirm inhibition of ERα-promoted transcriptional activity, particularly for the di-sec-butyl derivative 7c.

2.3.3 Radiometric Binding Assay (J.A.K.)

To ensure that these compounds were not acting as tradition ER-antagonists through interactions with the ER-LBD, a radiometric competitive binding assay was performed against tritiated estradiol. Briefly, ER (ERα or ERβ) was diluted in a Tris-containing binding buffer at pH 7.5 and incubated simultaneously with the ligand of interest and [3H]-estradiol ([2,4,6,7-3H] estradiol-1,3,510-triene-3,17β-diol, 84-85 Ci/mmol). After 24 hours, the receptor was adsorbed onto a hydroxylapatite pellet, free ligand was washed away, and the pellet was re-suspended in ethanol and scintillation fluid. Reduction in the scintillation count was used to detect competition for the ER-LBD. Ligand binding affinity is therefore reported as a relative binding affinity compared to that of estradiol.

Of the 3,3’ biphenyl amino-acids, only the di-tert-butyl derivative 7d was found to have any measurable affinity for the either the ERα- or ERβ-LBD (Table 2.1). Although this analysis
cannot confirm that antagonism is occurring at the ER-CBD, it does ensure that any observed
inhibitor effects are occurring via an alternative mode of inhibition to traditional antagonists.¹

<table>
<thead>
<tr>
<th>Compound</th>
<th>ERα</th>
<th>ERβ</th>
</tr>
</thead>
<tbody>
<tr>
<td>7a</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>7b</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>7c</td>
<td>~0.001</td>
<td>~0.001</td>
</tr>
<tr>
<td>7d</td>
<td>0.055</td>
<td>0.018</td>
</tr>
<tr>
<td></td>
<td>0.038</td>
<td>0.017</td>
</tr>
<tr>
<td></td>
<td>0.0049±0.016</td>
<td>0.018±0.001</td>
</tr>
<tr>
<td>7e</td>
<td>&lt;0.001</td>
<td>~0.001</td>
</tr>
</tbody>
</table>

**Table 2.1** Of the 3,3’-disubstituted bipolar biphenyls, only the di-tert-butyl derivative 7d demonstrated any measurable competition for the ER-LBD.

2.3.4 Cell-based ERα Reporter Gene Assay (D.P.McDonnell)

The ability of these compounds to inhibit ERα-promoted gene transcription was evaluated in MCF-7 cells, an ERα-positive breast cancer cell line. MCF-7 cells were pre-treated with 20μM of each biphenyl amino-acid for two hours followed by exposure to E₂ or vehicle for fourteen hours. RNA was then harvested and quantitative-PCR (polymerase chain reaction) was employed to evaluate the effects of these compounds at mediating ERα-promoted transcription. Transcription of three known ERα-target genes, SDF-1, PR, and PS2, was evaluated, as was transcription of IDH3A, an ERα-independent gene used as a control. Results were reported in terms of the fold induction of gene transcription (ratio of experimental versus control transcription) that was observed.
At 20μM, the benzyl 7e, isopropyl 7b, and sec-butyl 7c derivatives showed slight inhibition of transcription of all three ERα-target genes. The tert-butyl 7d and un-substituted 7a derivatives were ineffective, instead exhibiting agonist behavior both in the presence and absence of E2 (Figure 2.7).7

![Figure 2.7](image)

**Figure 2.7** Reporter gene assays in MCF-7 cells demonstrated the di-isopropyl 7b, di-sec-butyl 7c, and di-benzyl 7e derivatives as inhibitors of ERα- promoted transcription of three ERα-target genes (SDF1, PR, PS2). IDH3A, which is an ERα-independent gene, was evaluated as a control.

2.3.5 Mammalian Two-Hybrid Assay (D.P.M.)

The biphenyl amino-acids were additionally subjected to a two-hybrid assay in mammalian cells to observe their effectiveness at inhibiting the interaction between an activated ERα and an NR-coactivator derived NR box-containing peptide (GRIP1 NR box II; LKEKHKILHQLQDSSSPV).8 Cells were transfected with the activated fusion receptor
VP16-ERα, the LxxLL peptide fused with the yeast Gal4-DBD, and the 5xGal4Luc3 reporter gene. Binding of ERα to the LxxLL peptide activated transcription of the luciferase gene, the extent of which was assessed by its resultant bioluminescence. In the presence of E₂, effective CBIs should reduce luciferase transcription, while no transcriptional effect should be exhibited in the absence of LBD-bound agonist.

The benzylation compound (7e) demonstrated the most significant CBI activity in this assay. The sec-butyl derivative (7e) and, to a lesser extent, the isopropyl derivative (7b), additionally exhibited some capacity to interrupt ERα-CoA binding. While the tert-butyl derivative (7d) actually inhibited E₂-induced ERα-CoA binding, it enhanced this interaction in the absence of E₂, potentially to functioning as an LBD-bound agonist. The un-substituted scaffold (7a) was similarly capable of agonizing ERα in the absence of E₂, and had no significant effect in its presence (Figure 2.8).⁷

**Figure 2.8** Two-hybrid assay demonstrated the di-isopropyl 7b, di-sec-butyl 7c, and di-benzyl 7e amino-acid derivatives as inhibitors of ERα-CoA induced binding from the dose-dependent reduction in CoA linked transcription.

---

⁷ Figure 2.8: Two-hybrid assay demonstrated the di-isopropyl 7b, di-sec-butyl 7c, and di-benzyl 7e amino-acid derivatives as inhibitors of ERα-CoA induced binding from the dose-dependent reduction in CoA linked transcription.
In the interest of expanding these studies to evaluate the effectiveness and necessity of our bipolar termini, the substituted biphenyl phenolic-esters (5) and amino-esters (6), generated as precursors in the synthesis of the desired amino-acids (7), were additionally subjected to this mammalian two-hybrid assay. These assays were run analogously to that previously performed on the amino-acid derivatives, and employed 5-40μM concentrations in the presence and absence of E2. Our previous observations regarding the identity of the 3,3’-substituents re-emerged as trends, with the protonated and di-tert-butyl derivatives continuing to function as ERα-agonists in the absence of E2 while the di-benzyl, isopropyl, and sec-butyl derivatives demonstrated modest but effective inhibition of ERα-CoA interactions. Because the results of these assays are reported as normalized responses, no specific deductions could be made at this time regarding the effect of the variant phenolic-ester and amino-ester termini relative to the previously analyzed amino-acid termini. It is clear that the variously functionalized compounds are all capable of producing an inhibitory effect, although the amino-esters did exhibit an undesirable increased affinity for the LBD as deduced from the agonist activity of the amino-esters in the absence of E2 (Figure 2.9).7

![Figure 2.9](image)

Figure 2.9 Two-hybrid assay was repeated on the phenolic-esters (5) and amino-esters (6). Members of each series demonstrated CBI activity, encouraging further evaluation of the optimal terminal functionalities.
The substituted bipolar biphenyl scaffold was not designed specifically as an ERα-CBI, but as a broader NR-CBI which could ultimately be modified to induce receptor-selectivity. Therefore, the derivatives should be similarly capable of inhibiting other NR-CoA interactions. Thus, an AR-reporter gene assay was performed to assess the efficacy of these compounds as AR-CBIs. CV-1 cells, a cell-line derived from the kidneys of adult male African green monkeys which lacks any endogenous known steroid, thyroid hormone, or glucocorticoid receptors, were transfected with an AR-expression vector to convert them to AR-positive cells. These cells were additionally transfected with an AR-responsive luciferase gene (MMTV-luc). Following incubation with the compounds of interest in the absence and presence of R1881, a potent steroidal AR agonist, luciferase expression was assessed by its characteristic luminescence.

The biphenyl amino-acids were largely ineffective, with only the sec-butyl 7c demonstrating any hint of CBI activity and the un-substituted 7a, tert-butyl 7d, and isopropyl 7b derivatives enhancing luciferase transcription. Similarly the phenolic esters showed little efficacy in this assay, with only the sec-butyl 5c and possibly the isopropyl 5b derivatives interrupting the AR-CoA interaction. The sec-butyl (5c) and tert-butyl (5d) phenolic esters also exhibited modest AR-agonist activity in the absence of R1881 (data not shown). The substituted amino-esters (6b, 6c, 6d, 6e) were by far the most effective modulators of AR-CoA activity. However it should be noted that the amino-esters frequently did exhibit toxicity at high concentrations, as evaluated based on >50% decrease in the transcription of a co-expressed β-galactosidase gene which was employed for normalization of the results (Figure 2.10).
Figure 2.10 The biphenyls should inhibit NR-CoA binding over a broader range of NRs. When evaluated as AR-CBIs, the biphenyl amino-esters (6) showed increased CBI activity compared to the phenolic-esters (5) and amino-acids (7).

Additionally proliferation assays were performed in LNCaP cells (AR-sensitive prostate cancer cells). The cells were stripped of endogenous hormones using a charcoal-serum and exposed to varied concentrations of the biphenyls in the presence or absence of R1881. Unfortunately, no significant inhibition of cell proliferation was observed in these assays, which may be explainable by the measurable but weak inhibition of AR-CoA interactions observed with these compounds. Although an unfavorable result, I would expect further optimization of binding affinity to be necessary before obtaining significant macroscale physiological results.

2.4 Conclusions from 1st Generation Compound Series

The analysis of this initial series accomplished three major objectives.
1) **Proof-of-concept**, confirmed that 3,3’-substituted-4,4’-bipolar biphenyls are capable of inhibiting NR-CoA interactions through non-LBD interactions.

2) Established that the identity of the hydrophobic substituents from the biphenyl core is essential both to the capability and to subsequently optimizing efficacy of this approach to developing NR-CBIs. The benzyl and sec-butyl, and to a lesser extent the isopropyl derivatives were demonstrated as effective inhibitors of ERα-CoA interactions, regardless of the identity of the polar termini. The tert-butyl and unsubstituted derivatives were observed as LBD-bound agonists, although the tert-butyl derivatives also indicated a simultaneous inhibition of CoA recruitment in the presence of external agonist. AR affinity was lower and was increasingly dependent upon termini identity, yet similar trends in substituent preferences were observed.

3) Provided evidence that varying the identity of the terminal functionalities can exert a measurable effect on binding affinity but that mimicking the specific ‘charge clamp’ interactions may not be essential to binding. The results suggest that the inclusion of the charged amino-acid termini may hinder membrane permeability and the AR-reporter gene assay strongly indicated that the phenolic-esters and amino-esters were more effective than the amino-acid derivatives as inhibitors of AR-CoA interactions. This same study suggested that the amino-ester variation may be most effective of the three as an NR-CBI, however the amino-esters also demonstrated likely LBD-promiscuity in agonizing of ERα in the absence of E2.
Encouraged by the confirmation that this scaffold can effectively inhibit NR-CoA interactions, I considered the design of future analogues. However, because these compounds did not nearly approach the binding potency of effective therapeutics or molecular probes, I considered immediate derivatization of the 3,3’-disubstituted bipolar biphenyl scaffold without prior scaffold optimization to be inappropriate.

My interest in biphenyls as mimics of alpha-helical conformation stemmed from the conformational constraints that energetic barriers pose to biphenyl. I thus became interested in the potential effects of varying the disposition of substituents about the core in order to affect the energetics required for inducing alpha-helical behavior within the CBD. The bulk of my subsequent work is derived from this interest, first in developing efficient synthetic methodology towards the expansion of the biphenyls to incorporate varying substitution, and subsequently in evaluating the effectiveness of the synthesized derivatives, both as NR-CBIs and as conformational alpha helix mimetics.
References

Chapter 2:


Chapter 3: Synthesis of 2nd generation bipolar biphenyl NR-CBIs, and Development of efficient synthetic conditions for library expansion.
3.1 Introduction:

Having successfully demonstrated the capacity of substituted bipolar biphenyls to inhibit NR-CoA interactions, attention was turned towards further optimizing the design of the scaffold. Following the evaluation of the 1\textsuperscript{st} generation compound series, I became interested in evaluating the effects of varying the positioning of the leucine-mimicking substituents about the biphenyl.

A 3,3’-substitution pattern had been incorporated in the synthesis of the 1\textsuperscript{st} generation compound series. This arrangement was biased by uncertainty in the optimal positioning of substituents and the anticipated synthetic complications that would likely arise upon increasing the proximity of substituents about the biaryl linkage. In synthesizing the initial series, my focus had primarily been directed towards successfully assembling a small series of compounds so that the potential of the scaffold could be evaluated. At this juncture, it became critical to develop robust synthetic methods that could tolerate the extensive variations in substitution which may ultimately prove desirable.

The purpose of the subsequent synthetic efforts was therefore two-fold:

(1) Evaluate a series of mono- and di- substituted biphenyls which held constant the identity of the hydrophobic substituents while varying their arrangement.

(2) Optimize a robust synthetic route which would allow for the inclusion of varied hydrophobic substitution.
3.2 Design of Second Generation

I anticipated that the arrangement of substituents along the biphenyl would be vital to ultimately achieve success with the biphenyl NR box mimetics. This arrangement is not only essential for mimicking the distance separation of the side-chain mimics, but also is itself responsible for the rotational constraints of the biphenyl core from which the substituents protrude. Biphenyls are not generally planar two-dimensional molecules and, while free rotation is generally assumed about the biaryl bond, this rotation in fact is controlled by complex energetic constraints. Substituents proximal to the biaryl linkage will disfavor coplanarity, however conjugation across the biaryl system is favored and would be broken in an orthogonal conformation. As a result, energetic wells emerge, representing conformations that offer an energetic compromise between steric and electronic concerns (Figure 3.1).¹

![Graph showing energy levels with rotational angle of biphenyl](image)

**Figure 3.1** Preferred conformations are favored that offer a compromise between energetic constraints at co-planarity (steric) and orthogonality ( electronic).

Therefore, manipulating the steric constraints proximal to the biaryl linkage will affect the accessible conformations of the scaffold and ultimately influence the energetic costs of orienting
the molecule within the NR-CBD. In order to investigate the optimal substitution pattern, mono- and di-substituted derivatives were synthesized which contained at least one substituent positioned ortho to the biaryl linkage (Figure 3.2).

![Figure 3.2](image)

**Figure 3.2** Internally mono- and di-substituted biphenyls which contain one or more substituent ortho- to the biaryl linkage. (Please note the non-equivalence of the termini projecting from the 4 and 4’ positions.)

Biological evaluation of the initial 3,3’-disubstituted compound series had suggested a preference of the NR-CBD for the inclusion of larger hydrophobic substituents, with the di-sec-butyl and di-benzyl compounds emerging as leads for further investigation. From these, the benzyl substituents were incorporated in the design of the second-generation, as the inclusion of sec-butyl groups would introduce additional chiral centers, adding further complexity to the evaluation of the scaffold. Anticipating that synthetic challenges would arise when coupling the increasingly hindered biphenyls, I chose to first optimize the synthetic methodology around an analogous methylated series. Additionally, I anticipated subjecting these compounds to the same biological evaluation as the benzylated series as they might assess the importance of substituent arrangement in affecting resultant substituent projection, essentially independently from evaluating the efficacy of substituent identity.
The biphenyl amino-acid salts of the initial series were generally less effective as NR-CBIs compared with the corresponding amino-esters and phenolic-esters. I therefore chose to exclude the amino-acid derivatives from my 2nd generation library and aimed to synthesize the 2-mono, 2'-mono, 2,3'-di, 3,2'-di, and 2,2'-di methylated and benzylated bis-oxy biphenyl- amino-esters and phenolic-esters (Table 3.1).

![Chemical Structures](image)

**Table 3.1** Anticipated 2nd-generation bipolar biphenyls.

3.3 Synthesis of Second Generation

The design of this NR box mimetic is founded upon the use of a biphenyl scaffold which contains hydrophobic substitution from one or both phenyl rings and is flanked from the 4,4'-positions by asymmetric ether-linked polar termini. Beyond that, there are many derivatives might ultimately prove desirable for future SAR. Consequently, an important goal of this work was to design methodology that could accommodate a wide assortment of variations on the scaffold. The pseudo-symmetric scaffold can be envisioned from the ligation of two
appropriately substituted and functionalized 4-bromophenols, thereby providing three major retrosynthetic objectives: the preparation of 2- and 3- substituted 4-bromophenols, the further functionalization of these subunits, and the coupling of the biphenyl (Figure 3.4).

![Figure 3.4 Retrosynthesis of biphenyl amino-esters](image)

3.3.1 Synthesis of 2- and 3- Substituted 4-Bromophenol Building Blocks

In order to vary the arrangement and identity of hydrophobic substituents on the biphenyl, means of preparing non-commercially available substituted-bromophenols was required. In preparing the 3,3’-disubstituted series, the requisite 4-bromophenols were obtained in nearly quantitative yields through para-bromination of the commercially available 2-substituted-phenols using tetrabutylammonium tribromide (Equation 3.1).

![Equation 3.1](image)
However a method was needed for para-bromination of the 3-substituted phenols as well as for the prior incorporation of their substituents.

Preparation of 3-benzyl-4-bromophenol was achieved in four steps and 65% yield from 3-bromoanisole. 3-bromoanisole was converted to the corresponding arylmagnesium bromide to which benzaldehyde was added. The resultant secondary alcohol was reduced under H₂ in the presence of Pd/C. Bromination was performed under ambient conditions using N-bromosuccinimide in acetonitrile, and confirmation that bromination occurred selectively at the 4-position was provided using a 1D-NOESY NMR experiment. Finally the anisole was deprotected to yield the desired 3-benzyl-4-bromophenol (Scheme 3.1).²

![Scheme 3.1 Synthesis of 3-substituted-4-bromophenols.](image)

This analogous sequence was additionally performed using methyl ethyl ketone in place of benzaldehyde to yield the 4-bromo-3-(sec-butyl)phenol. Although a sec-butyl compound series was not ultimately prepared, this confirmed that secondary, as well as primary, substituents could be incorporated using this methodology.

Fortuitously, the 2-methyl and 2-benzyl phenols were available commercially from which bromination was easily accomplished using TBATB in chloroform. However, our group has since shown that an analogous sequence can be used to generate the non-commercially available
2-substituted 4-bromophenols from the Grignard activation of 2-bromoanisole in the presence of the carbonyl derivative of the desired substituent (Scheme 3.2) \(^3\).

![Scheme 3.2 Synthesis of 2-substituted-4-bromophenols](image)

3.3.2 Functionalization of the Termini

The inclusion of asymmetric termini necessitated the differentiation of the phenolic building blocks prior to coupling. Coupling the biphenyl prior to termini differentiation is unproductive as selective mono-etherification of a symmetric or pseudo-symmetric bi-phenol would be inefficient. However, it became clear very early on that the inclusion of the amine terminus adds complexity to the already problematic biaryl coupling reaction (\textit{vide infra}) while additionally altering the physical properties of the compounds, complicating chromatography associated with any subsequent reactions. Therefore, while the carboxy terminus was incorporated prior to the biaryl coupling, the amine terminus was appended subsequent to the coupling, as the final step in the generation of the scaffold (Figure 3.5).
Figure 3.5 Biphenyl coupling is performed prior to appending the amine terminus.

Etherification of both termini was accomplished under Williamson ether conditions, through which the presence of a strong base deprotonates the phenol, generating the phenoxide which is capable of nucleophilic substitution to a relevant alkyl halide. Specifically, the carboxy terminus was appended through the addition of ethyl bromoacetate to the substituted-4-bromophenoxide which was previously deprotonated by sodium hydride. The aromatic subunits then underwent coupling (vide infra), following which the amine terminus was added through etherification with 2-(dimethylamino)ethyl chloride hydrochloride in the presence of carbonate.

It should be noted that various Mitsunobu conditions were attempted for the addition of the amine terminus but proved relatively ineffective. Should derivatization of the amine terminus become desirable for scaffold optimization, Mitsunobu conditions should be further explored. This is particularly important due to the relative stability of ethanolamines compared with haloalkylamines (and, as a result, the much more extensive library of ethanolamines available commercially). However, numerous variations of the termini were not being evaluated at this juncture and etherification proved efficient for the given purpose.
3.3.3 Suzuki Coupling

The Suzuki reaction was selected for the coupling of the biphenyl scaffold. This palladium-mediated approach to coupling activated aromatic or vinylic systems offers high functionality tolerance compared with other methods of C-C bond ligation, as well as adaptability for the coupling of electronically-diverse precursors. The Suzuki reaction generally describes the palladium-catalyzed coupling of aryl or vinyl halides with aryl or vinyl boronic acids (or derivatives thereof). It proceeds via an analogous catalytic cycle to other palladium-mediated coupling reactions where oxidative addition of the halogenated species to a Pd(0) complex is followed by a transmetalation event by which both subunits become ligated to the metal center (see chapter 1). Reductive elimination allows for complexation of the desired product while effectively regenerating active catalyst (Figure 3.6).4,5

![Figure 3.6 General catalytic cycle of palladium-mediated couplings.](image)

In my early attempts to synthesize the initial 3,3’-disubstituted series, I had observed that the amine terminus contributed both to the generation of side-products and to challenging chromatographic separations. These issues, as well as literature precedent indicating that the
presence of tertiary amines increases the propensity for the homocoupling of the aryl halide (*vide infra*), led to the decision to couple the biphenyl prior to the addition of the amine terminus. This did alter the convergent synthetic approach that was originally envisioned, but this adjustment proved acceptable because the new route yielded a full series of biphenyl phenolic-esters in addition to the biphenyl amino-esters, allowing for analysis of the efficacy of the amine terminus in affecting binding affinity.

Suzuki coupling procedures have been extensively reviewed and are widely employed in the chemical literature. However, the wide range of reactant-specific reaction conditions contribute greatly to this versatility. A number of side reactions are commonly observed to compete with the desired reaction and significant optimization often is necessary to obtaining acceptable product yields. In synthesizing the 1st generation series, the coupling of the 3,3 ’-disubstituted biphenyls, while not sterically complex, did result in low and sometimes inconsistent yields which were attributed to the electronically-rich nature of both aromatic precursors. Increasing steric constraints, through the inclusion of substituents at the 2- and/or 2’- positions, would likely further reduce the efficiency of this reaction. Optimization of the coupling conditions required for these electronically-rich, sterically-complex biphenyls was therefore considered to be essential.

Identification of the competing side reactions and comprehension of the mechanisms by which they occur was essential to suppressing competition in order to optimize the desired biphenyl coupling. Let us first consider the catalytic cycle relevant for the desired cross-coupling reaction (*Figure 3.7*).
**Figure 3.7** General catalytic cycle for the Suzuki coupling reaction of the desired biphenyl from aryl bromide and aryl boronic ester precursors.

Homocoupling of both the halide species and the boronate are frequently observed to compete with the desired cross-coupling. Simplistically, one might expect these to result from the double addition of the aryl halide or of the aryl boronate to the palladium center, followed by reductive elimination of the resultant homocoupled product (**Figure 3.8**) However, consideration of the electronic character of the precursors and the palladium intermediates suggests more complex variables must be at play.
Figure 3.8 Homocoupling side reactions of aryl halides and boronates are sometimes observed.

Palladium catalysis stems in part from the redox cycling of Pd(0) and Pd(II) complexes. The homocoupling of the aryl halide would require the subsequent oxidative additions of two equivalents of the aryl halide to the metal center, thereby producing a Pd(IV) species. Although this occasional occurrence has not been ruled out, the presence of a reducing agent certainly encourages competition from this side reaction, reducing the Pd(II) complex formed upon oxidative addition of the first equivalent of aryl halide back to a Pd(0) species, allowing a second equivalent of aryl halide to add to the palladium center and thereby facilitating homocoupling of the aryl halide (Figure 3.9).6

Figure 3.9 The presence of a reducing agent allows a second equivalent of aryl halide to add to the palladium center, facilitating homocoupling of the aryl halide.6
In fact, the incorporation of both inorganic and organic reducing agents has been demonstrated to promote the formation of both symmetric and asymmetric biaryls from the palladium-catalyzed reductive coupling of aryl halides. Upon recognizing that tertiary amines, particularly DIEA (N,N-diisopropylethylamine), are effective at promoting this homocoupling in competition with the desired cross-coupled product, the synthetic approach was adjusted to append the tertiary amine terminus of our biphenyl scaffold following the coupling reaction.

An additional reductive side-reaction that is sometimes observed results in the protonation of the aryl halide. This particular side reaction was not observed to compete with the coupling of the biphenyl scaffold.

Similar disruption of the desired catalytic cycle can result from the presence of oxidants. Most commonly this occurs due to the presence of O₂, hence the importance of performing these reactions under inert conditions. Under oxidative conditions, the homocoupling of the organic nucleophile, specifically the organyl boronic acid (or derivatives thereof, e.g. boronic esters, trifluoroborates) is frequently observed. This requires the activation of two equivalents of the nucleophilic species, an event generally understood to be mediated by a Pd(II) complex. A recent study of the mechanism of palladium-catalyzed homocoupling of boronic acids points to the oxidation of Pd(0) to Pd(II) through formation of a peroxo-palladium species in the presence of dioxygen as the key mediator for this reaction (Figure 3.10). Additionally, this side-reaction can yield a phenol as a second by-product of the same mechanism. In my early attempts to cross-couple the biphenyl scaffold, homocoupling of the boronic ester was sometimes observed.
However, the phenol was never isolated as a side-product and ultimately, potentially through changes in reaction methodology adopted for alternative reasons, the homocoupling of the boronic ester was not identified as a major side reaction that was necessary to rationally suppress.

![Chemical Reaction Diagram](image)

**Figure 3.10** Homocoupling of the aryl boronic ester can be facilitated by the presence of O₂ through a peroxo-palladium intermediate.¹⁰

Upon completion of the 1ˢᵗ generation series of 3,3’-derivatives (*Chapter 2*), the low product yields repeatedly obtained when coupling the scaffold still lacked an explanation. With renewed resolve to optimize this coupling in order to tolerate increasing substitution, it became essential to identify the main source(s) of competition so that such might be suppressed.

The Suzuki coupling of the 3,3’ di-sec-butyl phenolic ester was therefore repeated. In addition to the recovery of 27% product, a pure sample of a new material was recovered and ultimately
identified as 4-phenyl-2-(sec-butyl)phenol (Figure 3.11). I could not immediately explain the origin of this compound, considering that the potential stripping of the substituents from either aryl subunit seemed extremely unlikely, particularly in the quantities recovered (40%).

![Chemical reaction diagram]

**Figure 3.11** 4-phenyl-2-(sec-butyl)phenol was recovered as a major side product of the Suzuki coupling of the 3,3’di-sec-butyl biphenyl

A comprehensive literature search revealed that, although seldom discussed in comparison to other side-products of palladium catalysis, this side-product can emerge fairly frequently from the transfer of an aromatic substituent from the phosphine ligand.\(^{11,12}\) Mechanistic studies have demonstrated that reductive elimination of palladium-bound aryl and phosphine ligands can form a tetra-substituted phosphonium which can then oxidatively add back to the metal center via any of its four aromatic substituents (one that originated from the aryl halide). This effectively transfers a phenyl ring to the palladium which then continues through the normal catalytic cycle (Figure 3.12).\(^{13}\)
Figure 3.12 Aryl transfer side reaction proceeds via the intermediate formation of tetra-aryl phosphonium.$^{13}$

I was able to confirm that the triphenylphosphine ligand provided the source of the 4-phenyl substituent by incorporating deuterated triphenylphosphine in place of the normal protonated triphenylphosphine ligand while otherwise repeating the reaction conditions. (Note that approximately half of the phosphine present in this reaction was deuterated while the other half was protonated, as we still used the commercially available Pd(PPh$_3$)$_2$Cl$_2$ catalyst. The resultant 4-phenyl-2-(sec-butyl)phenol illustrated an approximate 50% decrease in integration of the phenyl resonances when characterized by $^1$H NMR, and confirmation was established by the existence of an M+5 peak by GCMS, indicating that the deuterated phenyl had indeed been incorporated into this side-product (Data included in Appendix 3).

Finally understanding the major side-reaction that required suppression, I proceeded with attempts to optimize this coupling.

[Please note, optimization was not performed on the 3,3’ di-sec-butyl system discussed above, but on mono- and di- methylated derivatives. Initial optimization attempts were performed on the 2-methyl derivative. As acceptable yields were obtained, the same conditions were applied to the 2’-methyl. I then moved to the 2,3’-dimethyl derivative which, due to increased steric
hindrance, required further optimization to obtain ‘acceptable’ yields. These adjusted conditions were then applied to the 3,2’-dimethyl successfully, but the further increase of steric constraints when synthesizing the 2,2’-dimethyl derivative required that the coupling conditions again be further optimized. Finally, the optimized conditions for synthesizing the 2,2’-dimethyl were applied without further modification to an analogous mono- and di-benzylated series.]

In order to suppress the aryl-transfer side-reaction, it was necessary to restrict the reductive elimination of the tetra-aryl phosphonium. Two key strategies were implemented in order to do so.

The first change to be made was in the nature of the solvent. The 3:1 solution of THF/H₂O was replaced with dichloromethane. The use of a less polar solvent is expected to reduce the tolerance of phosphonium formation. Unfortunately, in 100% dichloromethane, no reaction occurred. The reaction was repeated with 10:1 and 4:1 solutions of DCM/H₂O. In 10:1 DCM/H₂O the reaction barely began. In 4:1 DCM/H₂O, the reaction did proceed, yielding 32% product. However substantial (although unquantified) amounts of side-product were also observed under these conditions, as the addition of H₂O used to encourage the desired reaction simultaneously allowed the reemergence of competition from the aryl-transfer reaction via formation of the tetra-aryl phosphonium.

The second, and perhaps the most substantial, change was to replace the triphenylphosphine ligand with the bulkier tri(ortho-tolyl)phosphine variant (Figure 3.13). It is expected that the bulkier substituents suppress this reaction by affecting the kinetics of reductively eliminating the
tetra-aryl phosphonium. Repeating the above reaction conditions, with 4:1 DCM/H2O while replacing the Pd(PPh3)2Cl2 with Pd(P(o-tol)3)2Cl2 and the added PPh3 ligand with P(o-tol)3, succeeded in a greater than two-fold increase in reaction yield to 68%. These reaction conditions were applied to the 2’-methyl derivative, yielding 69% product.

Figure 3.13 Tri(ortho-toly)phosphine is a bulkier alternative to the traditional triphenylphosphine ligand.

The above conditions generated 50% product when applied to the 2,3’-dimethyl derivative. Increasing the water content encouraged further re-emergence of the aryl-transfer side-reaction, and replacing DCM with dioxane significantly reduced product formation. Upon subjecting the phenyl bromide and phenylboronic ester subunits separately to the otherwise unaltered reaction conditions, the phenyl bromide showed no reaction while the phenylboronic ester again underwent the aryl-transfer side-reaction, even in the absence of phenyl bromide. Up to this point, the phenyl bromide had been used in slight excess compared with the phenylboronic ester, as the boronic ester was deemed more expensive in terms of ease of preparation. As it became clear that the side reaction of the phenylboronic ester would not be fully suppressed, the stoichiometry was switched (1 equiv phenyl bromide: 1.2 equiv phenylboronic ester). Upon
applying the new stoichiometry to the conditions used to synthesize the mono-methylated compounds, the 2,3’-dimethyl was obtained in 83% yield and the 3,2’-dimethyl in 90.% yield.

The 2,2’-dimethyl derivative demonstrated higher energetic barriers when subjected to these reaction conditions, an unsurprising observation when one considers the increase in hindrance. After two days, the reaction had not gone to completion. Increasing the quantity of phenylboronic ester to two equivalents caused significant formation of the aryl-transfer side-product but yielded only 30% isolated product. Neither increasing the catalyst loading to 7.5%, nor further increasing the quantity of aryl boronic ester to 3 equivalents pushed the reaction to completion. When the reaction was attempted over a 48 hour period, an accidental spike in the temperature on the second night occurred and the reaction reached completion, indicating a high thermodynamic barrier to reaction progression. Further adjustments to the temperature and the ratios of DCM/H₂O ultimately generated the 2,2’-dimethyl product in 73% yield, using a 3:2 ratio of DCM/H₂O at 110°C. (Note that reactions were performed in a sealed tube so, although the temperature is greater than the reflux temperature of water, it may be presumed approximately accurate. No attempts were made to quantify the resultant generation of pressure that may have existed in the tube at these elevated temperatures.) Lower temperatures retarded the reaction, although prolonged reaction times may ultimately have achieved similar success.

Accepting the yields of these reaction conditions (Scheme 3.3), the same conditions were then invoked in synthesizing the benzylated series. Table 3.2 presents the resultant yields, with 82%-96% generation of the desired products for the 2- and 2’- monobenzylated and 2,3’- and 3,2’- dibenzylated compounds. The highly hindered 2,2’-dibenzylated derivative was produced under
these conditions in 47% yield. This was acceptable as the main goal in developing this methodology was synthetic access to any desired mono- and di-substituted compound. Should a library of 2,2’-disubstituted variants become desirable in the future, further optimization of this coupling should then be performed.

Scheme 3.3 Efficient method for coupling variants of the biphenyl scaffold.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Bn</td>
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</tr>
<tr>
<td>2’-Bn</td>
<td>96%</td>
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<tr>
<td>2,3’-diBn</td>
<td>93%</td>
</tr>
<tr>
<td>3,2’-diBn</td>
<td>86%</td>
</tr>
<tr>
<td>2,2’-diBn</td>
<td>47%</td>
</tr>
</tbody>
</table>

Table 3.2 Product yields when optimized Suzuki method was applied to mono- and di-benzylated biphenyls.
3.4 Identity of 2\textsuperscript{nd} Generation Compounds

Ultimately I was interested in the biological analysis of the resultant phenolic-esters as well as the amino-ester derivatives. The biphenyl coupling represented the final step in generating the phenolic-esters. Samples of these compounds were additionally subjected to etherification with 2-(dimethyl)aminoethyl chloride under Williamson ether conditions (\textit{vide supra}) to yield the desired amino-esters. Interestingly, it was observed that the presence of the tertiary amine auto-catalyzed the partial trans-esterification of the ethyl ester to the corresponding methyl ester when exposed to even minimal amounts of methanol. As the specific identity of the ester was not essential to the scaffold design, this modification was accepted in the interest of purity, and the full library of methylated and benzylated amino ethyl esters was transesterified to amino methyl esters. For the sake of completeness, I additionally prepared the 2,3’-dimethylated and 2,3’-dibenzylated phenolic methyl esters on the chance that the ester identity resulted in significant biological effects. This synthesis therefore ultimately produced a 22-compound library (Table 3.3) of mono- and di- methylated and benzylated phenolic and amino esters as proteomimetic inhibitors of NR-CoA binding interactions.
Table 3.3 2nd-generation library of bipolar biphenyls.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Name</th>
<th>Description</th>
<th>Structure</th>
<th>Name</th>
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Chapter 3


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3. Weiser, P.T.; Hanson, R.N. *Manuscript in preparation*.


Chapter 4: Evaluation of 2nd-generation as alpha helix mimetics
4.1 Introduction

Ultimately, the goal of this project is to evaluate the potential of substituted bipolar biphenyl scaffolds as small-molecule inhibitors of NR-CoA interactions through mimicry of key features of the NR box. Two divergent methods were utilized to analyze the effectiveness of the 2\textsuperscript{nd} generation biphenyls.

1) \textit{Biological Evaluation}: Inhibitory assays were performed in order to assess the ability of various derivatives to competitively inhibit NR-promoted transcription of target genes via non-LBD derived inhibition of NR-CoA interactions.

2) \textit{Spectroscopic Evaluation}: Certain derivatives of this library contained diastereotopic protons on secondary methylenes located proximal to the biaryl linkage, the geminal protons of which were detectable as distinct resonances by $^1$H NMR. The potential of these resonances as internal probes of biphenyl configuration and rotational dynamics has been investigated.

4.2 Biological Evaluation

Like the 1\textsuperscript{st} generation series (Chapter 2), the 2\textsuperscript{nd} generation compounds were not designed to include receptor-specific elements that would impart specificity towards specific nuclear receptors. At this time, the scaffold itself remains in developmental stages, following which receptor-specific elements can (and should) be incorporated. Because estrogen receptors, and
particularly ERα, have been extensively studied, and because the development of non-traditional ERα-selective antagonists is attractive for the treatment of breast cancer, the 2nd generation biphenyl proteomimetics are again being primarily evaluated as ERα-CBIs. Additionally, in an effort to generalize these results as applicable to the broader range of NRs, these compounds are undergoing further evaluation as AR-CBIs. Biological studies are performed in the labs of two key collaborators: Donald P. McDonnell’s group at Duke University Medical Center performed the initial screening of the compound series and John A. Katzenellenbogen’s group at the University of Illinois, Urbana-Champaign has since subjected some of the lead compounds revealed by McDonnell’s studies to further biological evaluation.

The NR-CoA interaction represents a model system for the broader development of biphenyls as alpha helix mimetics. Ability to inhibit this interaction therefore has two-fold importance, both in the optimization of such compounds as a therapeutic approach for the inhibition of NR-CoA binding interactions, and as a more generalized approach towards the use of substituted biaryls as alpha helix mimetics.

The following results represent what is known to date about the capacity of these compounds to affect NR activity (08/04/2011).
4.2.1 Initial Compound Screening (D.P. McDonnell)

4.2.1.1 Two-Hybrid Assay

The compounds were first evaluated for their efficacy at inhibiting the interaction between ERα and an NR box-containing peptide derived from the coactivator protein GRIP1.\textsuperscript{1} The conditions of this assay were very similar to that discussed in Chapter 2 for the evaluation of the 1\textsuperscript{st}-generation compounds. SK-BR-3 cells, an ER-negative breast cancer cell line, were transfected with the 5xGal4-Luc3 reporter gene, the LxxLL-containing peptide fusion possessing the Gal4-DBD (pM-GRIP1 LxxLL2), VP16-activated ERα (pVP16-ERα), and a β-galactosidase vector (pCMV-βgal) which was used as an internal control. After an incubation period, the cells were exposed to varying concentrations of the CBIs, both in the presence and absence of E\textsubscript{2}. The fusion of the CoA-derived GRIP1 peptide to the Gal4-DBD necessitates ERα-CoA binding for the promotion of 5xGal4-Luc3 transcription (Figure 4.1). Efficacy at inhibiting the interactions between ERα and the NR box peptide is thus measurable by transcription of the luciferase gene, which is assayed by the resultant luminescence produced by luciferase activity.

![Diagram of ER-CoA Two-Hybrid Assay](image)

**Figure 4.1** Schematic of ER-CoA Two-Hybrid Assay
CBI activity was therefore assessed by the reduction in E₂-promoted luminescence as a result of increasing concentrations of biphenyl. Notably, of those compounds that emerged as potential ERα-CBIs, only one was mono-substituted (AW-Bn-02) and its inclusion in the list of successful compounds is debatable based on the collected data. From this assay, a total of nine compounds demonstrated potential as ERα-CBIs, including both methylated and benzylated derivatives, functionalized as both amino-esters and phenolic-esters (Table 4.1).

![Table 4.1 Lead compounds of Two-Hybrid Assay](image)

4.2.1.2 Reporter Gene Assay

Compounds were next evaluated for their ability to directly disrupt ERα-promoted transcription using an ER-promoted luciferase reporter gene. Effective inhibition of ERα-CoA (ERα-GRIP1)

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interactions would prevent transcription of the ER-reporter gene and was measured by assaying luciferase activity. This assay was again conducted in SK-BR-7 cells, ER-negative breast cancer cells, which were transfected to contain ERα (RST7-ERα), the 3xERE tata-Luc reporter gene, and pCMV-βgal which was used as an internal control for data normalization. Following transfection, varied concentrations of the biphenyls were added in the presence or absence of E2, and, after forty hours, the cells were assayed by luminescence to establish the extent of luciferase transcription (Figure 4.2).

![Figure 4.2 Schematic of ER Reporter Gene Assay.](image)

From this assay, six compounds emerged as potential CBIs (Table 4.2), all of which had previously exhibited CBI potential in the two-hybrid assay (vide supra). Preliminary IC\textsubscript{50} values of these compounds were calculated to be between 11-27\textmu M. Notably, all successful CBIs were di-substituted, and five of the six contained either a 3,2’ or 2,3’ substitution pattern, similar to the substituent arrangement favored by Hamilton for mimicking i, i+4 side chain projection.\textsuperscript{2} Particular trends in preference for methyl versus benzyl substituents were not noted, as the
effective compounds were exactly divided between three di-methylated and three di-benzylated derivatives, nor was a preference noted for N-terminus inclusion or truncation.

![Chemical Structures]

Table 4.2 Lead compounds of ER Reporter Gene Assay

4.2.1.3 Competitive Binding Assay against Estradiol

The six compounds identified as potential ERα-CBIs by prior two-hybrid and reporter gene assays were subjected to a competitive binding assay against E2 in order to establish their mechanism of inhibition. The previous assays demonstrated the capability of these compounds to disrupt ERα-CoA binding interactions, but were incapable of demonstrating whether inhibition resulted from direct competition at the CBD as desired, from traditional antagonism at the LBD, or from an alternate mode of inhibition that rendered the receptor incapable of CoA binding. This assay cannot confirm that inhibition occurs through direct NR-CoA competition, but can exclude the possibility that NR-CoA interactions are being inhibited through LBD-mediated antagonism.
To eliminate the possibility that the compounds were competing for the LBD, reporter gene assays were repeated using varying concentrations of estradiol to generate dose-response curves. If the compound is competing for the LBD, a negative shift in the overall EC$_{50}$ of E$_2$ will be observed as concentrations of biphenyl are increased. For example, see the effect of titrating E$_2$ in the presence of 4-hydroxytamoxifen, a known ER$\alpha$ LBD antagonist (Figure 4.3).

**Figure 4.3** Direct competition with E$_2$ will demonstrate dose-dependent alterations in E$_2$ EC$_{50}$, such as in the case of 4-hydroxytamoxifen (4OH-Tam).

A CBI acting through an alternative mode of inhibition, will generate dose-response curves which depict retention of E$_2$ EC$_{50}$ with a simultaneous drop in normalized luciferase response, indicating that inhibition of ER$\alpha$-promoted transcription is occurring through a LBD-independent mechanism. None of the six compounds that were evaluated demonstrated competition for the ER$\alpha$-LBD (Figure 4.4). From this assay, two lead compounds emerged as particularly effective inhibitors of ER$\alpha$ activity: AW-Me-08 (2,3’-dimethyl biphenyl amino-methyl ester) and AW-Bn-10 (2,2’-dibenzyl biphenyl amino-methyl ester).
Figure 4.4 Competitive binding assays of CBIs. None exhibited competition with E$_2$. AW-Me-08 and AW-Bn-10 demonstrated notable inhibition of luciferase response.

4.2.1.4 Androgen Receptor - Reporter Gene Assay

Characteristic ER$\alpha$-specific elements have not been incorporated in designing these compounds, therefore these results should be indicative of their potential to inhibit a broader class of NR-CoA interactions. Consequently an AR reporter gene assay used to assess the efficacy of the substituted biphenyls at inhibiting AR-promoted gene transcription. While the AR binds CoA’s through a similar mechanism as other NRs, including ER$\alpha$, it should be stated that AR-CBI activity cannot be entirely generalized to other NRs because the AR-CBD is known to have some notable differences compared with other NR-CBDs, particularly in its preference for bulkier NR box peptides.
This assay is largely analogous to the previously described ER reporter gene assay (vide supra). HepG2 cells, an AR-negative cell line derived from liver cancer, were transfected to express AR (SG5-AR) as well as an AR-promoted luciferase reporter gene (MMTV-Luc) and the control vector pCMV-βgal, used for normalization. The transfected cells were exposed to the biphenyls in the presence and absence of R1881, a potent AR agonist. AR-promoted transcription was assayed via the resultant luminescence associated with luciferase activity.

Of the 2\textsuperscript{nd}-generation substituted biphenyls, only AW-Me-08 demonstrated notable inhibition of AR-promoted transcription (Figure 4.5).

![Figure 4.5](image.png)

**Figure 4.5** Inhibition of AR-promoted transcription by AW-Me-08.

This is somewhat surprising as the AR-CBD is known to prefer bulkier NR box variants (e.g. FxxLF) compared with other NRs. Not only was preference for the bulkier benzylated biphenyls not observed, but it is difficult to conceive of the methyl substituents as capable of forming effective hydrophobic contacts within the AR-CBD to disrupt AR-CoA binding.
However, this observation may in fact be particularly noteworthy as it potentially reflects tangible evidence of the dual role of the substituents, not merely in penetrating the binding domain but in affecting the conformational constraints of the biphenyl. These energetic biases are important for effectively orienting the substituents to achieve analogous configurations to those of the $i, i+4$ side chains of the NR box, in order to allow appropriate contacts within the binding site. Although the analogous benzyl derivative, AW-Bn-08, might have been expected to have more pronounced activity as an AR-CBI than AW-Me-08, the increased steric bulk of the benzyl substituents may be resulting in insurmountable intramolecular conformational barriers, preventing their appropriate penetration into the CBD.

4.2.1.5 Summary of McDonnell’s Biological Evaluation

The data generated thus far supports the importance of di-substituted biphenyls. This is likely indicative of more efficient binding when mimicking two, rather than one, leucine side chains. It may additionally suggest the efficacy of di-substituted compounds at affecting the rotational energy barriers of the biphenyl core, reducing conformational mobility in such a way as to energetically favor binding interactions with the receptor. Of the six lead compounds that emerged, five contain either 3,2’ or 2,3’ substitution, analogously staggering substituents in the same arrangement which Hamilton previously described as favorable to mimicking $i, i+4$ side chain projection. Of these, the 2,3’-dimethyl biphenyl amino-methyl ester, AW-Me-08, is considered the best lead compound for further development of biphenyl NR box proteomimetics (in terms of inhibitory potential, solubility, and toxicity). Additionally, the one compound that emerged as an anomaly in terms of substituent arrangement, the 2,2’-dibenzyl biphenyl amino-
methyl ester AW-Bn-10, is of particular interest as it has exhibited distinctive structural properties by $^1$H NMR that I became interested in further investigating (vide infra).

No preference was observed by McDonnell’s studies for methyl compared with benzyl substitution, although this series was not intended as a comprehensive SAR of substituent identity. Additionally, no preference has been observed for the inclusion or truncation of the amine terminus. This likely indicates that mimicking the ‘charge clamp’ is not essential for binding, however, it also suggests that the dimethylamino-ethoxy is not efficiently interacting with the complementary ‘charge clamp’ residues of the CBD. Further optimization of the 4,4’-terminal functionalities may ultimately result in a significant enhancement of binding affinity if this interaction can be effectively exploited.

4.2.2 Further Evaluation of Lead Compounds (J.A.Katzenellenbogen)

Subsequently, Katzenellenbogen’s group has subjected the six compounds revealed by McDonnell’s initial screens as most successful in inhibiting NR-CoA interactions to further competition assays. TR-FRET (time-resolved fluorescence resonance energy transfer) assays were employed to evaluate competition of the purported CBI compounds against a coactivator-derived peptide. Additionally, a radiometric binding assay (RBA) was performed against $[^3]$H-estradiol to assess whether inhibition may be attributable to undesired competition for the ligand binding domain.
4.2.2.1 *In Vitro* TR-FRET CBI Assays

Efficacy of the biphenyls at inhibiting ERα-CoA binding was evaluated using a TR-FRET assay, performed analogously to that presented in chapter 2. Inhibition was assessed by fluorescence transfer between a terbium-labeled ERα fragment (residues 304-554) and a fluorescein-labeled SRC3 fragment (residues 627-829, includes three distinct NR box regions). These protein fragments were expressed in E.Coli. Subsequently, ERα was selectively labeled at Cys-417 with thio-reactive biotin which ultimately conjugated the fluorescing streptavidin-terbium complex, and SRC-3 was labeled non-specifically with 5-iodoacetamide-fluorescein. For the TR-FRET assessment, an excitation filter of 340/10nm was employed as well as emission filters of 495/20nm (terbium) and 520/25nm (fluorescein). Varying concentrations of compounds (10⁻⁶ to 10⁻³M) were incubated with the ERα complex in the presence of E₂ for 15 minutes prior to the addition of the SRC-3 complex (5nM). After incubation for one hour, fluorescence was observed.

The benzylated compounds (AW-Bn-04, AW-Bn-10, AW-Bn-11) demonstrated modest efficacy in inhibiting the fluorescence transfer between the ERα and SRC-3 complexes, with Kᵢ values ranging from 30.2-54.4μM. The methylated compounds were far less effective. Only AW-Me-04 was at all able to affect the fluorescence transfer (Kᵢ=142.8), while AW-Me-08 and AW-Me-09 were essentially ineffective even at 10⁻³M concentrations (*Table 4.3*).
Table 4.3 TR-FRET data indicates that ERα-CoA binding is still occurring.

In an effort to determine whether these compounds were acting through direct competition with SRC-3, two compounds (AW-Bn-04 and AW-Me-04) TR-FRET evaluation was repeated using varied concentrations of SRC-3. As the concentration of SRC-3 is increased, a larger concentration of CBI should be required to effectively inhibit protein-protein binding, as is demonstrated by an NR-box containing peptide used as a control (Figure 4.6).

Figure 4.6 Competitive Binding Assay using varied concentrations of SRC-3. Above is the competition of an NR-box containing peptide used as a control to demonstrate the expected appearance of dose-dependent alterations in ERα-CoA binding.
The observation that the reduction in fluorescence transfer observed by AW-Bn-04 and AW-Me-04 was independent of SRC-3 competition (Figure 4.7) suggested that these compounds may actually be modulating ERα activity through an alternative mechanism than the direct obstruction of the ERα-CBD.

![Graphs showing competitive binding assay results for AW-Bn-04 and AW-Me-04](image)

**Figure 4.7** Competitive Binding Assay using varied concentrations of SRC-3. Neither AW-Bn-04 or AW-Me-04 demonstrate the expected dose-dependent modifications in ERα-CoA binding.

4.2.2.2 Radiometric Binding Assay

The six lead compounds which had emerged from McDonnell’s studies were additionally subjected to a radiometric binding assay to assess whether they compete with estradiol for ERα-CBD.\(^5\)\(^6\) While McDonnell’s group had previously examined this possibility by reporter gene assays with varying concentration of E\(_2\), Katzenellenbogen instead uses a radiotracer approach to analyze this competition.
This assay (previously described in chapter 2) was accomplished by incubating the full-length human ERα protein with 2nM [³H]-estradiol ([2.4.6.7-3H] estra-1.3.5(10)-triene-3, 17β-diol), 82 Ci/mmol) in the presence of known concentrations of the inhibitor for 18-24 hours. The ligand-bound proteins were then adsorbed on hydroxyapatite pellets, allowing unbound ligand to be washed away. Binding affinities of the compound for the ERα-LBD are then observed by reduction in radioactivity and reported as relative binding affinities compared to E₂ (setting E₂ affinity as equal to 100%) (Table 4.4).

<table>
<thead>
<tr>
<th>Compound</th>
<th>RBA</th>
</tr>
</thead>
<tbody>
<tr>
<td>AW-Bn-04</td>
<td>0.496</td>
</tr>
<tr>
<td>AW-Bn-10</td>
<td>0.002</td>
</tr>
<tr>
<td>AW-Bn-11</td>
<td>0.001</td>
</tr>
<tr>
<td>AW-Me-04</td>
<td>0.010</td>
</tr>
<tr>
<td>AW-Me-08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AW-Me-09</td>
<td>0.002</td>
</tr>
</tbody>
</table>

**Table 4.4** Radiometric Binding Assay. AW-Bn-04 reduced the affinity of E₂ for the ERα-LBD, suggesting direct LBD competition.

While most of the ligands demonstrated no competition for the ligand-binding domain, a relative binding affinity of 0.496% was reported for AW-Bn-04. This compound may be interfering with E₂ binding in CBI assays, potentially affecting ERα-CoA binding through LBD-mediated antagonism.

4.2.2.3 Summary of Katzenellenbogen’s Biological Evaluation

The biological assays performed by Katzenellenbogen’s group do complicate our interpretation of the biological efficacy of these compounds, as they indicate that the biphenyl CBIs may not be
acting through the desired mechanism, inhibiting NR-CoA interactions through direct competition for the NR-CBD. When evaluating the results of McDonnell’s evaluations, it now is important to consider alternative, yet unspecified mechanisms which may be responsible for the observed inhibition of NR-promoted gene transcription. This is very important in terms of rational design efforts of further generations. As such efforts move forward, caution must be taken to develop assays that specifically probe whether the NR-CoA interactions of interest are being directly inhibited.

4.3 Spectroscopic Evaluation

4.3.1 Unexpected Spectral Complexity Revealed by $^1$H NMR

Upon synthesizing the 2,2’-dibenzyl phenolic ethyl ester (AW-Bn-05), routine characterization by $^1$H NMR revealed the possible occurrence of structural abnormalities. While much of the spectrum had a relatively predictable appearance, a complex resonance pattern emerged between 3.4-3.7ppm, a region where the bridging methylene protons of the benzyl substituents had typically been observed (Figure 4.8). My initial assumption was sample impurity, however this was negated by a combination of factors. No other extraneous peaks were present in the spectrum and the peaks that did appear in the region of interest did correctly integrate with four protons. Furthermore, $^{13}$C NMR revealed that the sample contained the correct number of carbon resonances and LCMS observed the presence of a single product with the expected molecular weight.
Although an explanation for this splitting remained unknown, confident that I had isolated the correct phenolic ester, I carried on with the final synthetic conversions yielding the desired 2,2’-dibenzyl amino methyl ester (AW-Bn-10).

Once again, full characterization (e.g. $^{13}$C NMR, HRMS) of the resultant product confirmed the desired product identity. However, the otherwise normal $^1$H NMR spectrum again revealed unpredicted multiplicity with regard to the methylene resonances. Further complicating matters, the observed methylene resonances in AW-Bn-10 were not only unpredictable, but differed from that observed in the precursor AW-Bn-05 (Figure 4.9).
I felt confident enough in the characterization of these products to submit them biological evaluation (*vide supra*). However, as the concept of using biphenyls as alpha helix proteomimetics is based upon the complex conformational constraints known to be exhibited by hindered biphenyls, I felt certain that the spectroscopic abnormalities warranted further investigation.

4.3.2 Predictions of Methylene Coupling

Recall that biphenyl is known to exhibit atropisomerism, an emergent chirality which results from energetic restrictions to the rotation of the biaryl linker (**Figure 4.10**). (Please see chapter 1 for a more complete discussion).\textsuperscript{7,8}

**Figure 4.10** Biaryl rotation is restricted by energetic barriers about the planar and orthogonal configurations.
While I was unsure of the exact source of the $^1$H NMR anomalies, I postulated that they were a concrete visualization of biphenyl atropisomerization. The use of biphenyls as alpha helix mimetics indeed hinges on the favoritism of certain conformers that emerge from inherent energetic restrictions to rotation. I therefore was interested in whether the methylene protons might serve as intrinsic probes by which conformational preferences and rotational dynamics of substituted biphenyls could be analyzed.

Perplexed by the spectral complexity, I first hypothesized that I may be observing multiple rotamers, each exhibiting unique methylene singlets. This explanation would be consistent with the isolation of a single product from achiral chromatography having the expected molecular weight and a normal $^{13}$C NMR spectrum. Presuming that this was the case, I further hypothesized that the integration of the methylene resonances would then be directly proportional to the concentration of the given conformer.

In fact, these conjectures reveal my initial unfamiliarity with the effects exerted by atropisomerism on nearby nuclei. Upon restricting the rotation of the biphenyl, the proximity of the methylenes to the biphenyl linker would cause its protons to exhibit diastereotopicity, as molecular asymmetry would restrict each proton to nonequivalent chemical environments. The $^1$H NMR resonances of these protons should then exhibit slightly different chemical shifts which would additionally allow observation of previously concealed geminal coupling.

Considering that the abnormal splitting of the methylene protons may result from diastereotopic protons, let us predict the splitting that should be observed by $^1$H NMR: $^9$
If the compound did not experience any significant rotational constraints on the NMR time-scale, then each methylene would experience coalescence, and consequently its protons would resonate as a singlet with an integration of two. The two methylene singlets (as the compound is di-benzylated) would likely experience slightly different chemical environments and therefore subtle differences in their chemical shifts (Figure 4.11).

Figure 4.11 Predicted appearance of methylenes, as two coalescing singlets with similar chemical shifts, and each having an integration of 2.

If instead, the protons within each methylene experience different chemical environments due to inherent molecular asymmetry, subtle differences in their local magnetic fields should be reflected in observable chemical shift differences. Moreover, the distinct chemical shifts of each proton would allow observation of geminal coupling within each methylene, therefore each proton would resonate as an individual doublet (Figure 4.12).
**Figure 4.12** Predicted appearance of the methylenes if each proton was in a unique chemical environment (due to proximal chirality). Geminal coupling should occur, therefore each proton would resonate as a doublet with an integration of 1.

However, this model still failed to represent the splitting that was observed (**Figure 4.13**).

**Figure 4.13** The observed splitting pattern differs from that predicted for diastereotopic methylenes.

Ultimately, this unique pattern was explained by second-order splitting that emerged between each pair of geminally coupled protons. In the simplest case of a coupled two-spin system with widely variant chemical shifts (A-X spin system), each proton would resonate as a doublet with
equal integration and with each component peak of the doublet representing half its intensity. If the two peaks instead experienced identical chemical environments, and therefore identical local magnetic fields (A₂ spin system), then the spins would be expected to coalesce into one singlet. Although physically speaking, coupling of the nuclei would occur, it would have zero net effect on the chemical shift and would therefore be unobservable by standard NMR techniques.

In between these two extremes lies the emergence of second-order spectra which result as the chemical shifts of coupled resonances approach one another (A-B spin systems), particularly once the ratio of the chemical shift difference (Hz, not ppm) to the coupling constant (Hz) \((\delta H_a - \delta H_b)/J_{ab}\) is less than 10. (As measurement of the chemical shift in Hz is dependent on the field strength of the magnet, these spectra can sometimes be simplified with the use of higher field magnets.) Second-order spectra cease to be interpretable by the general rules regarding peak multiplicity, intensity, and integration which are applied to first-order spectra.

The NMR resonances of second-order A-B spin systems frequently exhibit the ‘roof effect,’ a spectral phenomenon resulting, for example, in the increased peak intensity of the inner lines of a multiplet (Figure 4.14).^9

![Figure 4.14](image-url) Roof effect exhibited by second-order A-B spin systems.
In analyzing such roofed systems, the coupling constants still correspond with the peak separation, but the chemical shift is found at the center of gravity of the multiplet, rather than the midpoint. Chemical shifts of second-order A-B systems are determined by calculating the difference in frequency between the resonating protons ($\Delta\nu_{AB}$) and adding and subtracting this value from the midpoint (M) of the doublets (Equations 4.1, 4.2, 4.3).

\[
\Delta\nu_{AB} = \sqrt{[(a_2-b_1)(a_1-b_2)]} \quad \text{(eq. 4.1)}
\]

\[
\nu_A = M + \frac{1}{2} \Delta\nu \quad \text{(eq. 4.2)}
\]

\[
\nu_B = M - \frac{1}{2} \Delta\nu \quad \text{(eq. 4.3)}
\]

In this case, the geminal protons of each methylene are still experiencing very similar chemical environments and therefore have very similar chemical shifts. Additionally the pseudo-symmetry of the 2,2’-dibenzyl-4,4’-bisphenoxy core places both methylenes in very similar chemical environments which would be reflected in the very similar resonance frequencies of the nuclei.

I therefore examined the $^1$H NMR spectra of AW-Bn-05 and AW-Bn-10 for evidence of overlapping A-B spin systems.

Upon re-evaluating the spectrum of AW-Bn-05, the previously unexplainable splitting pattern immediately revealed itself as two overlapping roofed spin-systems, presumably arising from the geminal coupling of diastereotopic protons in each bridging methylene (Figure 4.15). This was
further supported by complimentary spectra taken in a different solvent (d\textsuperscript{6}-acetone, rather than CDCl\textsubscript{3}) which again revealed an A-B splitting pattern having slightly altered chemical shifts.

**Figure 4.15** Recognition of AW-Bn-05 as two overlapping A-B spin systems.

Supported by the spectral analysis of AW-Bn-05, I again turned to AW-Bn-10, the amino-ester derivative which had appeared to exhibit even more unlikely splitting, as it contained only six distinguishable peaks, unlike the eight that should emerge from four distinct doublets. \(^1\text{H} \) NMR experiments of AW-Bn-10 were performed using various solvents (d-chloroform, d\textsuperscript{6}-acetone, d\textsuperscript{4}-methanol, d\textsuperscript{6}-benzene, d\textsuperscript{6}-dimethyl sulfoxide) and provided enough chemical shift deviation to reveal the existence of eight total peaks, some of which had been previously shrouded by significant chemical shift overlap (**Figure 4.16**).
The only source of chirality within these compounds is that which may be induced by rotationally constraining the biphenyl core. The observation of diastereotopic protons at room temperature on an NMR time scale therefore provides an intrinsic intramolecular probe which can potentially allow further studies to measure the rotational dynamics of the biphenyl core and observe the structural preferences of the favored conformers. Excited by the potential of such knowledge to support future efforts at rationally optimizing the use of substituted biphenyls as alpha-helical proteomimetics, I pursued the further NMR analysis of these compounds.

4.3.3 Methylene Resonances as a Probe for Evaluating Rotational Dynamics

The original use of biphenyls as alpha helix mimetics rested around its possession of a pseudo-flexible core, generally with the ability for axial rotation, but with energetically favored conformers which can potentially project substituents in a similar disposition to that of a monofacially-functionalized alpha helix. Successful derivatives should therefore retain modest flexibility, demonstrated by considerable yet surmountable rotational energy barriers.
The spectral observation of diastereotopic protons on this otherwise achiral biphenyl provides us with a tool to directly observe biphenyl atropisomerism. Using variable temperature NMR energetic barriers to overcoming rotational restriction can be construed.

By increasing the temperature while continually monitoring the appearance of the methylenes, direct measurements can be taken of the coalescence temperature, at which chirality can no longer be observed. Coalescence is indicative that the rotational exchange rate between the protons exceeds the NMR time scale, preventing the observation of separate resonances by standard NMR techniques. At this point, the geminal protons should cease to exhibit separate chemical shifts, instead collapsing into a singlet with an integration of two, as they would experience identical chemical environments.\textsuperscript{10}

The free energy barrier ($\Delta G$) toward overcoming co-planarity (presumed to be rate-limiting) can be calculated from the Eyring equation (Equation 4.4):\textsuperscript{10,11}

$$k_{Tc} = \frac{R T_C}{N h} e^{-\Delta G / R T_c} \quad \text{(eq. 4.4)}$$

where $k$ is the rate constant of the interconversion between rotamers, $R$ is the ideal gas constant, $T_C$ is the coalescence temperature, $N$ is the Loschmidt number (the particle density of an ideal gas), and $h$ is Planck’s constant. In order to calculate the free energy, the rate constant must first be inferred from the NMR spectra as follows (Equation 4.5):

$$k_{Tc} = \frac{\pi}{2}(\nu_A - \nu_B) \quad \text{(eq. 4.5)}$$
where the relevant frequencies ($\nu_A$ and $\nu_B$) are measured in the absence of exchange (at
temperature substantially below coalescence). We then can solve the Eyring equation for $\Delta G$
(Equations 4.6, 4.7):

$$\Delta G = RT_C \ln \left[ R T_C \sqrt{2} \right] / \left[ \pi Nh(\nu_A - \nu_B) \right] \quad \text{(eq. 4.6)}$$

$$\Delta G = 19 \times 10^{-3} T_C \left[ 9.97 + \log T_C - \log(\nu_A - \nu_B) \right] \quad \text{(eq. 4.7)}$$

In recent years, the emergence of computer-aided dynamic line shape analysis tools have further
improved the accuracy of these techniques, aiding in the potential of dynamic NMR to provide
energetic analysis.$^{12,13}$

Initial experiments were performed toward the goal of extrapolating rotational energy barriers
from the presence of the diastereotopic methylene protons. A sample of AW-Bn-10, dissolved in
d$^6$-benzene, was taken to temperatures of +70°C. However no coalescence was observed at this
temperature, and due to temperature constraints on the gradient probes, higher temperatures
could not be attempted. Using a broadband probe, the materials of which were not as
temperature-sensitive, temperatures of 130°C were achieved. However, because this probe was
not suitably designed for $^1$H NMR, attempts to obtain spectra and observe whether coalescence
had yet been achieved proved futile and energetic data was not ultimately attained.

Similarly, low temperature $^1$H NMR was performed on samples of 2,3’-dibenzyl amino methyl
ester (AW-Bn-08) and 3,2’-dibenzyl amino methyl ester (AW-Bn-09), both of which exhibit
coalessence of their methylenes at room temperature. The methylene protons of these samples
were observed by $^1$H NMR at temperatures as low as -34.9°C however diastereotopic resonances
did not emerge at these temperatures.

Although thwarted by instrumentation availability in my efforts to extrapolate the rotational
energy barriers of these compounds, the discovery of secondary methylenes as intrinsic probes of
biaryl energetics bears significant relevance to the development of biaryl alpha helix
proteomimetics.

4.3.4 Methylene Resonances as a Probe for Evaluating Conformation

4.3.4.1 Distinction of NMR Resonances

The methylene resonances may additionally provide probes for assessing the preferred
conformation(s) being frozen out by restricted biaryl rotation using the Nuclear Overhauser
Effect (NOE), a nuclear phenomenon which allows observation of through-space interactions
between non-bonded but closely oriented nuclei (vide infra).\textsuperscript{14}

If NOE experiments were to be performed it was essential that individual protons first be
associated with their respective NMR resonances. While some peaks could be easily identified
by chemical shift and splitting patterns, others contained significant overlap (e.g. aromatics and
methylenes) and required comparative spectra taken while varying solvents and temperatures
and, in the most challenging cases, 2D NMR experiments.
Prediction of multiplicity patterns and chemical shifts allowed a number of resonances in the $^1$H NMR to be unambiguously distinguished. Others, for example the 3 and 3’ pseudo-symmetric singlets of the biphenyl core, exhibited very similar splitting patterns and chemical shifts but were distinguishable through readily observed NOE interactions with clearly identifiable alkyl resonances of the asymmetric termini. However, the pseudo-symmetry of the 2,2’-dibenzyl-4,4’-bisoxy biphenyl system led to a number of undifferentiable resonances, some of which exhibited significant overlap. Specifically three categories of extensively overlapping pseudo-symmetric resonances were identified: the aromatic protons of the benzyl substituents, the 6 and 6’ protons of the biphenyl core, and the methylene protons of the 2 and 2’ benzyl substituents.

The ortho, meta, and para protons of the benzyl substituents could clearly be discerned, displaying significant chemical shift differences. [$^1$H NMR (500MHz, d-acetone, room temperature, measured downfield from TMS): ortho: 6.92ppm, meta: 7.20ppm, para: 7.14ppm.] (Figure 4.17) However multiplicity was complicated by the direct overlap of protons at identical positions of the pseudo-symmetric substituents. The resonances of the 2-benzyl, compared with the 2’-benzyl, substituent proved unresolvable, even when variances in solvent, temperature, and magnetic field strength were employed. While this was not regarded as a major concern for attempts to discern the methylene resonances, this did limit the NOE studies that could be performed on this derivative. For example, interactions between the substituents would not be discernable, nor could any observed NOE enhancements between the substituents and the core scaffold be unambiguously associated with a particular substituent. If such ultimately proved desirable, synthetic manipulations of the molecular structure would be necessary (e.g. deuterium labeling, inclusion of alternative functionalities).
Figure 4.17 The ortho, meta, and para positions of the benzylic substituents. These substituents are indistinguishable by $^1$H NMR.

Presuming some measure of rigidity of the constrained biphenyl, as was indicated by the non-coalescence of the geminal methylene protons, I became very interested in the potential of NOE to observe the internal dihedral angle of the biphenyl. Lack of 6- and 6’- substitution (i.e. availability of protons at the 6 and 6’ positions) encouraged the possibility that such NOE studies might be performed by observing through-space interactions between the 2 and 2’ methylene protons and the 6 and 6’ protons (Figure 4.18).

Figure 4.18 Through-space interactions between the 2 and 2’ methylenes and the 6 and 6’ protons could potentially be valuable conformational probes.
However, the extensive overlap of the corresponding $^1$H NMR resonances severely hampered this objective. A 500MHz $^1$H NMR spectrum, taken at room temperature in d$_6$-acetone revealed the chemical shifts of the associated resonances (doublets, $J = 8.5$Hz) to vary by only 0.007ppm. Attempts were made to find a more suitable environment (solvent and temperature) to observe NOE enhancements of these resonances. As the temperature was reduced they were demonstrated to entirely overlap, appearing as a single doublet with an integration of two.

By far the most critical resonance associations to be made was to distinguish the methylene resonances of the pseudo-symmetric 2 and 2’ benzyl substituents (Figure 4.19). These resonances alone revealed the presence of molecular asymmetry and observation of their individual through-space interactions may hopefully reveal the extent of biaryl rotation and the resultant substituent disposition preferred in this and related biphenyl systems.

![Figure 4.19](image)

**Figure 4.19** The $^1$H NMR resonances of 2 and 2’ methylene protons experience significant overlap but, if distinguished, could be valuable as intramolecular probes.

The resonances of the 2 and 2’ methylenes exhibit very subtle differences in chemical shift, distinction of which ultimately required a combination of 2D-NMR techniques: HMBC
(Heteronuclear Multiple Bond Correlation) to trace long-range through-bond C-H coupling interactions, and HSQC (Heteronuclear Single-Quantum Correlation) to correlate single bond C-H couplings.

HMBC spectroscopy employs a heteronuclear pulse sequence and filters out larger single bond couplings allowing the detection of subtle couplings across longer bond ranges (2,3,4 bonds). By tracing resonances via long-range $^{13}\text{C} \rightarrow ^1\text{H}$ bond, coupling connectivity of hydrocarbon skeletons can be revealed.$^{15}$

Prior to employing HMBC to differentiate the 2 and 2’ methylenes, sufficient preliminary assignments of both $^1\text{H}$ and $^{13}\text{C}$ resonances be accomplished. Just as the $^1\text{H}$ resonances had exhibited significant overlap, molecular pseudo-symmetry similarly influenced the chemical shifts of the $^{13}\text{C}$ resonances and their differentiation was not straight-forward. HSQC spectroscopy, an alternative 2D NMR technique which correlates heteronuclei ($^{13}\text{C}$ and $^1\text{H}$) connected by one bond, was employed to assign $^{13}\text{C}$ resonances.$^{16}$ High resonance overlap as well as the quantity of quaternary carbons in the molecule (9, including 8 aromatic) prevented assignment of a number of $^{13}\text{C}$ resonance, however sufficient information was gained from this experiment to proceed.

Using HMBC, long-range through-bond coupling interactions were traced, originating from unmistakable resonances, I was ultimately able to assign the methylene resonances to their specific benzyl substituent (2 or 2’). Absolute confidence in the assignment of the alkyl resonances of the asymmetric termini made these appropriate foundations from which to trace.
The $^1$H resonance of the C terminal methylene (singlet, 4.70ppm) was determined to be connected to a quaternary aromatic carbon (157.66ppm). This was therefore presumed to be position 4 (C4) of the biphenyl, an assignment which was confirmed by HMBC long-range interactions with protons substituted from C3 (H-3, 6.75ppm) and C-6 (6.99ppm). C4 was then traced to the proton at position 3 (H3), and subsequently H3 was found to exhibit long-range coupling with three other carbon resonances: C5 (111.8ppm), as well as a quaternary aromatic carbon (134.1ppm) and an alkyl carbon known by HSQC known to belong to one of the two methylenes of interest (39.3ppm and 39.4ppm). Initially it was unknown whether the H3 coupled $^{13}$C resonance at 134.1ppm corresponded with C1 or C2, however, it was identified as the C1 resonance as tracing the HMBC of this resonance revealed it was coupled to H4 as well as either H6 or H6’ (overlapping resonances) (**Figure 4.20**). Additionally, the C2 resonance was ultimately assigned as one of four quaternary aromatic resonances with highly confirmed chemical shifts(140.9, 141.0, 141.3, 141.5), further confirming the correlation of the 134.1ppm with C1. An analogous trace was performed from the amine terminus, and identified the $^{13}$C resonance at 133.1ppm as C1’ (**Figure 4.21**)

**Figure 4.20** HMBC trace from the C-terminus to C1 of the biphenyl.

**Figure 4.21** HMBC trace from the N-terminus to C1’ of the biphenyl.
While a number of carbon resonances displayed long-range coupling with one or both methylene substituents, discerning the $^{13}$C resonances of C1 and C1’ was critical to ultimately distinguishing the two methylene resonances. Whether attributable to poor resolution or simultaneous coupling to both $^1$H resonances by either a single or multiple overlapping $^{13}$C resonances, all other $^{13}$C resonances which demonstrated coupling with the methylene protons of interest were ineffective at distinguishing the methylenes. However HMBC unequivocally demonstrated the C1 resonance (134.1ppm) to couple with the geminal protons of the more shielded methylene (3.530ppm, 3.617ppm) (Figure 4.22) and the C1’ resonance (133.1ppm) to couple with the protons of the slightly more deshielded methylene (3.563ppm, 3.617ppm) (Figure 4.23).

![Figure 4.22](image1.png) **Figure 4.22** HMBC trace from C1 of the biphenyl to the 2-substituted methylene.

![Figure 4.23](image2.png) **Figure 4.23** HMBC trace from C1’ of the biphenyl to the 2’-substituted methylene.
This finally allowed the unambiguous distinction of the methylene resonances (Figure 4.24).

[Similar treatment of AW-Bn-05 likewise associated its diastereotopic methylene protons with their corresponding 2<sup>nd</sup>-order <sup>1</sup>H resonances.]

![Diagram](image)

**Figure 4.24** Distinction of the methylene resonances of AW-Bn-10. The more upfield resonance corresponds to the 2-substituent; the more deshielded resonance to the 2’-substituent.

4.3.4.2 NOE Experiments

Having finally succeeded in differentiating the 2 and 2’ methylene resonances, I employed NOE experiments to examine the conformation of the biphenyl.\(^\text{17}\)
NOE (Nuclear Overhauser Effect) results from dipolar coupling interactions between nuclei that are near-in-space. Spectroscopically, it is observable from positive and negative enhancements in the NMR resonance intensities of proximal nuclei when the spin-states of a nucleus is perturbed, either by saturation or inversion.\textsuperscript{14,18}

Two categories of NOE experiments exist. Steady-state NOE experiments employ low-power radiofrequency over a prolonged time to saturate a specific resonance and, upon sampling, enhancement of nearby nuclei are observed which are proportional to their distance from the saturated nucleus. Difference spectra are generally used to observe enhancements, subtracting a normal unperturbed spectrum from the NOE spectrum. Transient NOE experiments instead invert nuclear spin populations through the use of selective pulse and gradient sequences. Rather than allowing equilibrium spin populations to be established prior to sampling, short mixing times are employed, allowing observations of spin transitions that occur immediately upon inversion of the primary nucleus. Although transient NOE is less capable of providing specific distance information, efficient experiment time and reduction in subtraction artifacts make transient NOE experiments appealing.

A variety of transient NOE experiments exist including both 1D and 2D variants. Generally, both 1D and 2D NOESY experiments first apply a 90° pulse and allow spins to precess for a given time under the influence of both dipolar and scalar coupling (scalar coupling effects are ultimately filtered out in both 1D and 2D experiments). This is followed by a second 90° pulse which inverts the spin populations. A short mixing time is allowed for the effects of this inversion to begin to influence nearby nuclei, following which the NMR spectrum is obtained.
In a 1D-NOESY experiment, a single inversion pulse is directed at a specified frequency window, generating a one-dimensional spectrum which depicts only the inverted and enhanced resonances and excludes any spins that do not experience dipolar coupling with the inverted spin. While only one inversion is performed at a time, the experiment does not require significant time and, by setting up an array of experiments, information about numerous frequency windows can conveniently be generated. 2D-NOESY experiments instead use a broad frequency range to simultaneously pulse all spins. The cross-peaks of the resultant two-dimensional spectrum then coincide with dipolar coupled nuclei.14,18

In investigating the conformation of AW-Bn-10, 1D-NOESY (Double Pulsed Field Gradient Spin Echo (DPFGSE) NOESY) experiments were most frequently used. 1D-NOESY experiments were performed in d6-acetone on a 400MHz NMR and employed various mixing times between 0.5-2.0s. Initial experiments at room temperature were successful in identifying NOE interactions between alkyl protons from each of the termini (C and N) and aromatic protons of each ring of the biphenyl (3,5 and 3’,5’ respectively). However, attempts to selectively invert specific spins were complicated by heightened resonance overlap between pseudo-symmetric nuclei (primarily nuclei of the biphenyl core and of the benzyl substituents). This issue was significantly alleviated by repeating the experiments at -45°C. The success of lower temperatures may have been two-fold. Certainly the more distinct chemical shifts of the various resonances aided pulse selectivity, but additionally enhanced rigidity at lower temperatures should allow more prolonged interactions between nuclei and a better indication of through-space interactions that occur in the lowest energy conformer(s).
Numerous NOE effects were observed in the spectrum, many of which could be easily depicted from knowledge of the rigid skeletal structure (e.g. NOE was observed between the C-terminal methylene and the protons of the 3 and 5 positions of the biphenyl core). My particular interest was in using the methylene protons to visualize the disposition of the benzyl substituents.

For simplicity, let us designate the methylene at position 2 to be methylene a, and it’s protons $H_a$. Then similarly, the methylene at position 2’ will be referred to as methylene b, and it’s protons $H_b$ (Figure 4.25).

![Figure 4.25 Reference nomenclature for the discussion of NOE correlations](image)

Protons $H_a$ were observed to experience NOE enhancements with the proton at the 3 position of biphenyl as well as one or more protons at the ortho positions of the benzyl substituents. (Unfortunately, the overlap of the benzyl substituents could not be reconciled so we cannot know whether any unpredictable interactions may be occurring between $H_a$ and the 2’ benzylic substituents. NOE enhancements of the 2 benzyl substituents by $H_a$ would be expected due to
their skeletal proximity.) Protons $H_b$ experienced analogous NOE enhancements with the proton at the 3’ biphenyl position and the ortho positions of the benzyl substituents (Figure 4.26).

![Diagram](image)

**Figure 4.26** NOE interactions between $H_a$ and proton at the 3-position of the biphenyl, and between $H_b$ and the proton at the 3’-position.

Observation that both methylenes $a$ and $b$ are closer in space to the meta protons (3 and 3’) on the portions of the biphenyl to which they are directly ligated implies that the methylenes themselves, which may otherwise be expected to undergo free rotation, may be in a fairly rigid environment. A more accurate depiction of the substituents might be to represent $H_a$ as oriented towards the proton at the 3 position of biphenyl, and $H_b$ towards the 3’ proton (Figure 4.27).
**Figure 4.27** Potential implication of the observed interactions between $H_a$ and $H_b$ and the 3 and 3’ protons.

Additionally, this observation provoked the question of whether the benzyl substituents themselves, whether due to their aromaticity or their steric bulk, might indeed be promoting biphenyl rigidity. For example, pi-stacking interactions of the benzylic substituents, or pi-stacking interactions between one (or both) of the benzylic substituents and one (or both) of the phenyl rings of the biphenyl core, may be rigidifying the compound (**Figure 4.28**).

**Figure 4.28** Pi-stacking interactions may be constraining the substituents.
If pi-stacking interactions were indeed occurring, NOE enhancements should be observable between the participating aromatic groups. Due to the resonance overlaps of the benzyl substituents I could not observe whether pi-stacking of the benzyl substituents actually was indicated, and no NOE experiment has yet indicated through-space interactions between core aromatic protons and the remote protons (meta and para) of the benzyl substituents.

Interestingly, additional NOE enhancements were observed between H_b and the 6 and/or 6’ proton(s) of biphenyl (Figure 4.29).

![Figure 4.29](image)

**Figure 4.29** Subtle NOE enhancements observed between H_b and the 6 and/or 6’ proton.

The resonances of the 6 and 6’ protons completely overlap at -45°C, therefore it could not be ascertained whether one or both nuclei were influenced by the inversion of H_b. However, consideration of the skeletal structure suggests that significant molecular strain would be
necessary for $H_b$ to experience dipolar coupling with the 6’ proton, and that more likely rotation of the biaryl situated methylene $b$ near-in-space to position 6.

This was not a merely an anomaly appearing in a sole NOE experiment. Multiple 1D NOESY experiments at room temperature as well as a 2D-ROESY experiment demonstrated consistent observations. However, in each of these experiments, attempts to separately distinguish the resonances of $H_a$ and $H_b$ proved futile, and all that can be said is that interactions between the 6 and/or 6’ protons and $H_a$ and/or $H_b$ were observed, but between which of these resonances specifically was unclear. Additionally, while inversion of $H_a$ did not sufficiently enhance the 6 and/or 6’ proton enough that their proximity can unambiguously be known, the opposite inversion of the overlapping 6/6’ resonance did enhance $H_a$ in addition to $H_b$, implying that $H_a$ may be similarly disposed.

Observation of $H_b$, as well as potentially $H_a$, does imply a likely trans- regiochemistry of substituents. Recall that I initially was interested in using steady-state NOE experiments to perform distance measurements in hopes of further discerning the favorable rotational angles of the biphenyl. However, as is apparent by now from the numerous hesitancies in this NOE analysis, the extreme pseudo-symmetry of this molecule makes it an inappropriate choice for further study of the biphenyl rotation. Derivatization, perhaps by way of alternate substitution, functionalization of one or both substituent, or isotopic-labeling, would be enormously helpful for further investigating the conformational constraints of this and related molecular structures. What has here been demonstrated is preliminary information regarding the favorable conformers
of the 2,2’-biphenyls and the potential of/methodology towards exploiting internal biphenyl substituents as molecular probes for investigations of their conformers.

4.3.5 Implications of NMR Investigations

Biaryl atropisomerism is the foundation from which the application of biphenyls as potential alpha helix mimics has emerged. While retention of molecular flexibility is likely important in eliciting binding interactions within dynamic protein systems, energetic inclinations for preferred binding conformation should aid in inducing and stabilizing binding interactions. The dual purpose of substituents upon the biphenyl has previously been explained, as substituents can mimic the identity of side-chains in projecting from a helical backbone while simultaneously influence the rotational constraints of that backbone itself. Described above is a potential third purpose for substituents, as they can in fact serve as intrinsic probes for investigation of their own energetic and conformational constraints. The potential of methylene linked substituents on biphenyl has here been described and, although precise data regarding the energetics and conformation of AW-Bn-10 was elusive due to molecular pseudo-symmetry, future libraries of poly-aryl proteomimetics should continue to incorporate these or related probes to permit the joint investigation of intramolecular conformational preferences and biological activity.
References

Chapter 4:


Chapter 5: Future Directions for the Development of Bipolar Biphenyl Proteomimetics
5.1 Introduction

The work presented in this dissertation represents early stage development of biphenyl inhibitors of alpha helix mediated PPIs, therefore extensive work remains for optimizing the use of biphenyls towards the eventual development of proteomimetic-based therapeutics. The full range of future directions of this project is beyond the scope of this discussion. My personal interests in this project are less motivated by the application of biphenyls to the exact constraints of particular protein binding interactions (e.g. NR-CoA), but instead by the potential to mimic alpha helical conformations through the deliberate manipulation of biphenyl disposition. Therefore, the particular aims that I enumerate here focus on the continued optimization of internal constraints so as to favor alpha helix-like conformers of biphenyl.

Briefly, I will mention a couple of points that should be explored by further SAR. Optimization of the ideal substituents for most effectively mimicking the $i$ and $i+4$ NR box side chains will likely be necessary to improve affinity and invoke receptor selectivity. Additionally mimicry of the second NR box leucine residue ($i+3$) may eventually prove desirable and would likely be most efficiently incorporated synthetically through di-substitution of the biphenyl. Another option that might be more effective biologically is to replace one of the core benzenes of biphenyl with an indane, in accordance with the prior demonstration that di-substituted indanes mimic $i, i+1$ alpha helix side chains.\textsuperscript{1,2} Also, although no preference has been observed thus far for the inclusion or truncation of the amine terminus, I am inclined to believe that the charge clamp has not been effectively exploited by the dimethylaminoethoxy terminus currently being employed, but that the model of stabilizing CBIs through mimicry of charge clamp interactions
remains sound. Therefore further SAR of the terminal functionalities may be beneficial for exploiting this potentially stabilizing interaction.

However, prior to performing significant SAR, additional optimization of the core biphenyl should be performed in order to further understand ways of manipulating this core for helical mimicry. It is not essential, nor even likely preferable, to rigidify the scaffold in an alpha helix-like conformation, but I anticipate that reducing the energetic burden exerted by the binding domain to promote optimal binding interactions would greatly enhance NR-CoA binding inhibition.

In the specific aims that follow, two complementary approaches are taken towards optimization of biphenyl alpha helical mimetics.

**Fundamental Objectives:** An enhanced understanding of the ability of substituents to affect the energetics of biphenyl, and specifically to favor or preclude particular rotational configurations, can guide future endeavors to consciously manipulate this system.

**Applied Objectives:** Various strategies of constraining the scaffold can be attempted in order to reduce conformational mobility and favor conformers which most effectively mimic alpha helix-like disposition.
5.2 Fundamental Objectives

5.2.1 Dynamic NMR for the Evaluation of Rotational Energy Barriers

The observation of distinct resonances associated with diastereotopic geminal protons adjacent to the biaryl linker of certain dibenzylated biphenyls was previously discussed. This was particularly exciting due to the potential of such resonances to function as internal probes by which the energetic barriers to biaryl rotation could be evaluated. Due to limitations in instrumentation, this potential is yet to be fully investigated. Additionally, similar studies may be possible on other less hindered derivatives, should a low enough temperature be attained to eliminate biphenyl coalescence. As the specific nature of this work has already been outlined in chapter 4, I will not further describe it here, however I do feel that knowledge of the rotational energy barriers of varyingly substituted derivatives has the potential to be very beneficial to the rational development of substituted biphenyls as alpha helix mimetics.

5.2.2 Chiral Chromatography for the Potential Distinction of Atropisomers

Chiral chromatography has been demonstrated to successfully distinguish biphenyl enantiomers containing stringent enough energetic barriers to prevent instantaneous coalescence. This has primarily been accomplished using Chiracel® OD columns, which are manufactured by Daicel for use in chiral HPLC and which employ a chiral polysaccharide stationary phase. To illustrate, consider biphenyl as viewed down its biaryl axis. High energetic barriers about
coplanarity (0°, 180°) can allow distinction, and even purification, of the right and left handed conformers (Figure 5.1).

![Diagram of coplanarity](image)

**Figure 5.1** Biphenyl viewed down the biaryl axis. High energetic barriers at coplanarity can lead to chiral distinction of certain biphenyls.

Chiral HPLC methods may serve a number of purposes for the development of biphenyl proteomimetics. Even on the analytical scale, resolution of atropisomers can demonstrate the co-existence of right and left handed conformers, potentially guiding further efforts toward the rational design of effective biphenyl alpha helix mimetics. Additionally, dynamic chromatography can be employed, whether at high temperatures for hindered biphenyls or at low temperatures for biphenyls which exhibit free rotation at room temperature, in order to further evaluate the energetic barriers of the various derivatives.\(^5\,6\) Assuming high energetic barriers about coplanarity, it may be possible to separately isolate the right- from the left- handed conformers and, if ultimately stable, the relative binding potencies of the enantiomers as NR-CBIs could then be evaluated. Short of the extreme energetic barriers that would be necessary to eliminate coalescence to this extent, preparative-scale chiral HPLC may still be able to isolate
the atropisomers, allowing further spectroscopic study in the interim, and allowing observations
to be made regarding the kinetics of the rotational coalescence.

5.3 Applied Objectives

5.3.1 Covalent Linkers as Regiospecific Constraints

Significant research efforts have been directed at the incorporation of covalent and non-covalent
linkers to stabilize alpha helical conformations in peptide fragments. Peptides are naturally
extremely flexible molecules and their higher-order conformations rely on very delicate balances
of enthalpic and entropic contributions. The inclusion of non-natural intra-peptide linkers can
induce the formation of desired secondary structure by controlling the proximity of essential
functionalities for the requisite intramolecular interactions.\textsuperscript{7,8,9}

I propose the investigation of similar constraints between the aromatic components of biphenyl
as well as related bi- and poly- aryls in order to induce the desired regiochemistry of substituents.
It is essential in mimicking the binding of amphipathic alpha helices that substituents project
mono-facially into the receptor’s binding domain. However, disubstitution of biphenyl with
bulky hydrophobic substituents will likely favor a trans arrangement of substituents (e.g. $60^\circ$
biphenyl rotation preferred over $120^\circ$) as depicted in Figure 5.2.
**Figure 5.2** Regiospecific arrangement of biphenyl substituents.

Left: The biphenyl is experiencing a 60° rotation (substituents are in cis-arrangement).

Right: The biphenyl is experiencing a 120° rotation (substituents are in trans-arrangement).

The introduction of covalent linkers (e.g. amides, alkenes) should reduce the extent of biaryl rotation and project substituents monofacially. This selection could then be illustrated by recognizing a restricted regiochemistry of substituents to +/-90° (**Figure 5.3**).

**Figure 5.3** Introducing a covalent linker between the phenyl rings can force a cis arrangement of substituents (+/- 90°).
Moreover, combining the use of chiral HPLC (vide supra) with this method of restricting regiochemistry, right-handed, monofacially-substituted biphenyl derivatives could potentially be isolated and evaluated as alpha helix mimetics (Figure 5.4).

![Figure 5.4](image)

**Figure 5.4** A covalently linked biphenyl could be further subjected to chiral chromatography, potentially allowing conformers to be isolated that are both regiospecific and enantiospecific.

Enantioselective synthesis may allow similar conformations to be attained through the incorporation of chiral elements on the linkers. For example, employing D vs L amino acid linkers may effectively induce chiral selection, potentially yielding right-handed, monofacially-substituted biphenyls without the simultaneous synthesis of the undesired left-handed diastereomer (Figure 5.5).

![Figure 5.5](image)

**Figure 5.5** Introduction of chiral elements on the covalent linker may favor the isolation of conformers that are both regiospecific and enantiospecific.
The above discussion by no means encompasses all possibilities for constraining biphenyls. For example, non-covalent linkers may instead be employed, and incorporation of heteroatoms to create a hydrophilic wet-edge may ultimately be desirable. I believe that the use of internally constrained biphenyls possesses considerable promise for the further optimization of small-molecule alpha helix mimetics and warrants significant further investigation.

5.3.2 Chimeric Derivations with Peptides of High Alpha Helical Propensity

In order to increase the propensity for the biphenyl core to adopt an alpha helix-like conformation, I propose the development of peptide-biphenyl chimeras, flanking the biphenyl proteomimetic with peptide chains predisposed to adopt alpha helical conformations. For example, all alanine chains may be appended to the biphenyl termini. An alternative approach would be to modify that taken by Shepartz, who grafted peptidomimetics within aPP (avian pancreatic polypeptide), a mini-protein with a remarkably high alpha helical propensity. The alpha helical propensity of aPP could potentially induce helical formations within the peptidomimetic or, in this case, proteomimetic implanted within it. This would theoretically encourage a similar right-handed monofacial display of substituents to the chirally resolved constrained biphenyls proposed above (Figure 5.6).
Figure 5.6 Inserting biphenyl proteomimetic within a larger peptide with high alpha helical propensity may encourage adoption of an alpha helix-like conformation.

Aside from the possibility of inducing helical propensity, chimeras may also be desirable due to interest in mimicking functionalities from beyond the biphenyl core. For example, the AR-CBD has demonstrated some preference to bind NR box motifs that contain a lysine residue at the $i-1$ position, and work is currently being performed in our group to append this and other related residues from various substituted biphenyls to evaluate the effect of mimicking this interaction. Mimicking flanking functionalities may ultimately be an effectual way to improve binding affinity as well as to enhance target selectivity. The peptide-like functionalities of the bipolar termini, as well as the ether linkages connecting the termini to the core biphenyl, provide convenient synthetic access for chimeric derivatization.

5.3.3 Enantiospecific Biphenyl Synthesis

Discussion of conformationally constrained biphenyls could not be complete without considering the possibility that the chiral, non-derivatized products may be directly attained through
enantioselective synthesis. However, such a goal relies on two fundamental challenges: the rotational kinetics of the resultant biphenyls to preserve chirality rather than coalescing into racemic mixtures, and the development of effective enantioselective synthetic methods.

The first goal of controlling the energetic barriers is attainable through manipulation of the identity and arrangement of substituents on the biphenyl. This objective is the foundation for the 2nd-generation substituted bipolar biphenyl compound series presented in this thesis (chapters 3 and 4) and additionally forms the basis for aims 1-4 presented as future directions of this project. Should an effective understanding of the energetic requirements for controlling chirality be met, as determined by isolation of stable atropisomers or successful evaluation of rotational energy barriers, then further exploration of asymmetric means of directly synthesizing the desired enantiomer should be sought.

Efforts have been made to develop enantiospecific synthetic methods of generating atropisomeric biphenyls, due particularly to the utility of such compounds in organometallic catalysis and materials science. Some success has been demonstrated with enantioselective biaryl coupling, but results have been variable and generally rely on the inclusion of very bulky substituents (e.g. bi-naphthyls)12 or directing groups (e.g. sulfinyl groups).13 The incorporation of chiral auxiliaries have allowed for asymmetric dynamic resolution, for example, the resolution of enantiomers of biaryl aldehydes has been accomplished via condensation with (-)-ephedrine.14
While direct asymmetric biaryl coupling methods would ultimately be preferable, recent advances by Scott Miller may greatly enhance the further development of chirally resolved biphenyl proteomimetics. Miller has previously demonstrated the chiral resolution of biphenyl atropisomers through peptide-catalyzed, asymmetric, electrophilic aromatic bromination.\textsuperscript{15} For initial optimization of reaction conditions, substrates were chosen which exhibited some measurable atropisomerism, with low enough rotational energy barriers that they could rapidly interconvert in solution, but that would encounter significantly increased rotational barriers when further substituted ortho to the biaryl linkage, preventing racemization of the products. Asymmetric electrophilic bromination was accomplished using an electropositive bromide source (e.g. NBS, NBP) in the presence of various chiral peptides, with eventual optimization of this approach yielding tribrominated product in 80\% yield and 97:3 enantiomer ratio, far exceeding that which has been generated by most other synthetic approaches to chiral biaryl resolution (\textbf{Scheme 5.1}).

![Scheme 5.1 Chiral resolution of biphenyls via asymmetric electrophilic bromination.\textsuperscript{16}](image)

The enantiopure biphenyls then could potentially be subjected to any number of reactions for their further functionalization. Of particular interest was the possibility of employing regioselective palladium coupling reactions to yield asymmetrically substituted, enantiopure biphenyls (\textbf{Scheme 5.2}).
Scheme 5.2  Chirally resolved tri-brominated biphenyls can regiospecifically undergo palladium-catalyzed coupling with retention of stereochemistry.\textsuperscript{16}

The high temperatures often required for palladium coupling reaction raised concerns regarding racemization of the biphenyl. Similarly, there was some apprehension that changes in the biphenyl rotational barrier may result upon complexation to the palladium center. However, the homo-substitution of the tri-brominated biphenyl proceeded in excellent yields with full preservation of enantiopurity in both reflexing THF/H\textsubscript{2}O and under microwave conditions at 100°C for 1 hour. Therefore the group proceeded, regiospecifically differentiating substituent identities from positions A, B, and C respectively, under Suzuki coupling conditions facilitated by MIDA (N-methyl-iminodiacetic acid) boronates.\textsuperscript{16,17} This then allowed for the tri-hetero-substitution of the biaryl without compromising the stereochemical integrity of the chiral biphenyl.

Enantioselective biphenyl synthesis relies upon sufficiently high barriers about coplanarity as to eliminate coalescence. The relatively few examples presented above of chiral coupling and chiral resolution of biphenyls represent the current state of knowledge. However, these techniques have been developed only recently and should not be regarded as exhaustive, but as a
foundation for further exploring chiral synthesis. Guided by theoretical indications of the energetic barriers of desirable biphenyls, enantiospecific synthetic methods should be further explored.

5.4 Summary

A range of possible methods of investigating and generating structurally biased biphenyls have here been presented. Administering regio- and/or enantio specific atropisomeric biphenyls should significantly reduce the amount of work that must be exerted by the receptor’s binding domain in affecting the appropriate conformation of the biphenyl core necessary for mimicking key binding interactions, thereby promoting the initial binding and stabilizing the resultant interactions between the alpha helix mimic and the protein binding domain.

The capacity of biphenyls as alpha helix mimetics remains to be fully explored. Although ultimately this particular scaffold may not prove to be the most effective alpha helix mimetic or NR-box mimetic, investigations of such flexible small-molecule scaffolds for PPI inhibition represent an extremely valuable approach for the development of novel therapeutics.
References

Chapter 5:


Chapter 6: Applications of Palladium Chemistry Towards the Development of Radiotracers
6.1 Introduction

Interest in further investigating the extent and behavior of the cannabinoid system, as well as growing recognition of the therapeutic potential of cannabinoid receptor 2 (CB₂) for the treatment of pain and inflammation, has contributed to the development of CB₂-selective compounds.¹,² A CB₂-selective radiotracer is particularly desirable for evaluation of the pharmacodynamics of potential therapeutic agents and for imaging the tissue distribution of CB₂. Due to its $10^2$ binding selectivity for cannabinoid receptor 2 (CB₂) compared with the related CB₁ receptor and possession of an aryl iodide, a radiotracer of AM630 presents as a useful and feasible target compound. In fact, radioiodinated-AM630 has reportedly been achieved by iodo-destannylation of a relevant stannylated derivative, however synthetic methods and characterization data for the stannylated compound have not been reported.³,⁴ AM630 contains sensitive functionalities including a ketone moiety which make its stannylation via the use of harsh alkali bases unfavorable, however palladium-catalyzed stannylation of AM630 should be sufficiently effective and functionality-tolerant. Halo-demetalation should then afford the desired radiotracer via exposure of the stannylated material to an electropositive halogen source.

6.2 Initial Attempts to Radiolabel AM630

6.2.1 Synthesis of Metalated-AM630

AM630 is an aminoalkylindole which is iodinated at the C6 position of the indole moiety. Stannylation of this 6-iodo-indole position, followed by site-selective electrophilic aromatic
iodination, should effectively generate a radioiodinated AM630 differing only in the identity of the iodine isotope (Equation 6.1).

As only a small quantity of AM630 was available, palladium-catalyzed stannylation was first attempted on relevant commercially available materials.

An initial attempt to stannylate 6-bromoindole-3-carbaldehyde, a compound which closely resembles the indolic core of AM630 was unsuccessful, as no reaction was observed to proceed. Exchanging the solvent for triethylamine again was unsuccessful at facilitating the reaction (Equation 6.2).

The stannylation of 4-nitroiodobenzene was performed to ensure that these reaction conditions could accomplish the stannylation of aryl halides, and was observed to proceed easily by reaction with hexabutylditin in toluene in the presence of Pd(PPh₃)₄ (Equation 6.3).
Stannylation of the 6-bromoindole-3-carbaldehyde was then attempted under basic reaction conditions (KH, tBuLi, SnBu3Cl). Although new aromatic peaks which may correspond with stannylated materials were observed by 1H NMR of the crude reaction mixture, it was apparent from the observation of numerous aromatic products and the altered integrations of the amine and aldehyde resonances that the diversely functionalized AM630 was unlikely to withstand such harsh reaction conditions (Equation 6.4).

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\begin{align*}
\text{eq. 6.4} \\
\end{align*}
\]

Recognizing that the inclusion of a protonated indole on the 6-bromoindole-3-carbaldehyde may be presenting a complication unparalleled by AM630, the compound was converted to its N-methylated analogue. However, essentially identical results were observed when reactions were repeated on the N-methylated 6-bromoindole-3-carbaldehyde.

While halo-destannylation has arguably been demonstrated as the most effective metal-halogen exchange reaction for radiohalogenation, a wealth of literature exists demonstrating the analogous use of alternatively metalated complexes.\(^5\) Boron is a particularly notable alternative to tin. While halo-deboronation does not generally proceed as efficiently as halodestannylation, boronates are non-toxic, stable alternatives to stannanes and can be employed for electrophilic exchange reactions to aryl, vinyl, and even alkyl systems.\(^6\) Initial attempts to prepare the boronic ester derivative of the N-methylated indole via Miyaura conditions demonstrated immediate success. Following minor modifications of the methodology (e.g. solvent, stoichiometry), the boronic ester derivative of AM630 was successfully synthesized in 73% yield (Equation 6.5).
Although radioiodination of AM630 had initially been envisioned from the conversion of a stannylated intermediate, the boronated derivative should undergo a very similar halodemetalation reaction and afford desired radioiodinated-AM630.

6.2.2 Radioiodination of Boronated-AM630

Radioiodination of boronated-AM630 was performed in collaboration with George Kabalka’s group at University of Tennessee, Knoxville. Kabalka is an organoboron chemist who additionally specializes in nuclear medicine and has extensively investigated the use of organylboron complexes for the generation of radiotracers containing a variety of radioactive nuclides.

Prior to performing iodinations, cold materials were used to determine appropriate HPLC conditions were examined to determine an effective method of separating residual boronated-AM630 from AM630. As the two compounds showed very similar elution profiles, displaying retention times within 1 minute on multiple HPLC columns and with various solvent conditions, the decision was made to convert residual boronic ester to a trifluoroborate salt following
iodination (Equation 6.6). This was confirmed by HPLC to be an effective method of isolating AM630.

The cold iodination was next attempted on a semi-preparative scale. The results indicated that a byproduct may be forming, however its retention overlapped with the trifluoroborate salt and, while the reaction yield was low, the desired AM630 product was isolated (as determined by its characteristic retention time).

Radioiodination was then executed. Radio-TLC was performed immediately following the reaction and suggested a 70% radiochemical yield. Subsequently, KHF$_2$ was added to convert residual boronic ester to the trifluoroborate salt and the reaction mixture was separated by semi-prep HPLC. However, HPLC revealed that no radiolabeled AM630 had formed. Instead, an unforeseen side product with a unique retention time (12.30min) was revealed which was both UV-active (indicating a radiolabeled organic structure) and radioactive.

The identity of this compound could not be ascertained as it was not observed in significant quantities under cold reaction conditions and could not be characterized by traditional means on the radiochemical scale. It has been postulated that this byproduct was non-selectively
radioiodinated, retaining its boronic ester (now trifluoroborate) while additionally undergoing iodination at an undesired position, potentially at the C2 (ortho) position of the anisole moiety (Scheme 6.1).

**Scheme 6.1** Radioiodination of boronated AM630 produced an unidentified radioiodinated byproduct.

6.2.3 Discussion of Initial Studies

Radiohalogenation by boron-halogen exchange should proceed according to established methodology wherein an organyl boronate complex undergoes electrophilic substitution with an electrophilic halide source.\(^7\) In this case, an electrophilic \(^{123}\)I species was generated under oxidative conditions by reaction of Na\(^{123}\)I with chloramine-T. Radiohalogenation was then
expected to occur at the most nucleophilic carbon of AM630, presumably the boronated position, via electrophilic aromatic substitution.

Aromatic metalation, whether using boron, tin, or any of various other metal(loid)s should generally be sufficient to render the site of substitution as the most reactive position for electrophilic halogenation. The observation of non-specific iodination of boronated AM630 at an alternative site, perhaps at the C2 position of the anisole moiety, indicates that boronation at the C6-indole position is insufficient to render that position most reactive to electrophilic halogenation.

While it was tempting to attribute the failed stannylation of AM630 and radioiodination of boronated-AM630 to ‘bad luck,’ a more rational approach was to acknowledge that the problems associated with these reactions may stem from a single source; that this seemingly simple aromatic system may be exhibiting unforeseen complexity with regards to the reactivity of the C6 position. It became clear that an increased understanding of indole reactivity would be important for successful manipulation of this system.

6.3 Challenges to Manipulating the C6 Position of Indole

6.3.1 Indole Structure and Reactivity

Indole (benzo(b) pyrrole, Figure 6.1) is a bicyclic system which represents a fusion of benzene and pyrrole. Due to its ubiquity in both naturally-occurring and synthetically relevant bioactive
molecules (e.g. tryptophan, plant hormones, GPCR modulators), the indole scaffold has been designated as a ‘privileged structure’ for the design of bioactive ligands.\(^8\)

![Indole](image)

**Figure 6.1** Indole.

A number of methods of directly synthesizing functionalized indoles have been explored in the literature,\(^9,10,11\) and these are complemented by an assortment of options for further functionalizing the pyrrole ring of indole (N1, C2, C3 positions).\(^12,13,14\) However, a detailed literature search reveals a conspicuous paucity of methods for derivatizing the benzo positions of indoles (C4, C5, C6, C7). The established utility of the indole scaffold for drug discovery efforts corroborates suspicion that this gap is not coincidental and is more likely indicative that the benzo ring of indole is enigmatically unreactive.

The 10\(\pi\) conjugated electron system of the cyclic, planar indole core is generally considered to be aromatic, however theoretical studies insinuate that indoles may not consistently act as fully aromatic systems, and some have asserted that \(\pi\)-electron density of unfunctionalized indoles may be more accurately viewed as a three-component system, consisting of the benzene ring, the N1-lone pair, and the C2-C3 alkene.\(^15\) Modest interactions likely remain between these components, but destabilization may be necessary to encourage the level of delocalized electron-flow that is generally characteristic of aromatic compounds.
Theoretical evidence supports this notion of indole. While significant $\pi$-bonding of the N1 lone pair is calculated in pyrrole, this diminishes upon fusion with other aromatics (to some extent with indole, and to an even greater extent with tricyclic carbazole). This is in contrast to benzo(c)indole (isoindole) which exhibits the predictable behaviors of a 10$\pi$ aromatic system. The ionic character of the N1-H bond further supports this, as carbazole exhibits the greatest ionic character implying the localization of significant electron density around the nitrogen.

Microwave and x-ray spectroscopy both have been employed to evaluate the bond lengths and bond angles of indole (and related pyrrole systems). The results further support such assertions of reduced aromaticity, demonstrating that the bond angles about N1, C2, C3, and C3a all approach that of an sp$^3$-hybridized system (109°) rather than the projected sp$^2$ bond angles (120°) of the fully aromatized system.

The localization of electron density about a nucleus can be inferred from the observed chemical shift of its NMR resonance. Correlating the changes in chemical shifts upon substitution can allow inferences to be drawn regarding the flow of electrons through a conjugated system. A series of $^{13}$C NMR spectra of simple substituted indoles have been catalogued for this purpose. While simple alkyl/arylations on the pyrrole ring (N1, C2, C3) don’t generally have significant effects on the benzo carbons, conjugated substituents on the pyrrole generally have a deshielding effect, noted with particular consistency at C5, C6, and C7. It should be noted that these chemical shifts are not drastic, frequently being observed on the order of 3ppm, but it does imply that conjugation of the N-ring can draw electron density from the benzo carbons. An analysis of C5-substituted indoles demonstrated no significant effects on C3 and C3a, however, whether
substituted with an electron-donating or an electron-withdrawing substituent, the C5 substituent caused a ~1-3ppm downfield shift on C2, perhaps indicating that the \( \pi \)-orbital containing the N lone pair may be enticed to partake in enhanced electron delocalization with the fused benzene.\(^{15}\)

Considered as a whole, these various studies of indole structure support the hypothesis that the energetics of unsubstituted indole \( \pi \)-system may not support full bicyclic aromaticity in the ground state. Aromatic compounds are most frequently categorized by their obedience of Huckel’s rules regarding electron count (4n+2 \( \pi \)-electrons), conjugation, cyclicity, and planarity. However, aromaticity is ultimately defined by resonance stabilization imparted from electron delocalization. Electron delocalization relies upon the overlap of symmetrical and energetically similar \( \pi \)-orbitals, and, if considering a fused system containing multiple \( \pi \)-molecular orbitals of largely variant energies, it may at times be disfavored.\(^{16}\) That conjugated substituents on the N-ring can deshield the benzo carbons may be an indication that the withdrawal of electron-density from the N-ring sufficiently affects the \( \pi \)-orbital energetics to allow orbital overlap and therefore resonance between the two ring systems.

Granted, these assertions are postulation rather than fact but, if such orbital energetics are indeed occurring, one would assume that electron-withdrawing substituents on the N-ring should correlate well with increased reactivity at the otherwise stable benzo positions. In fact the relatively sparse literature precedent for reactions at positions C4-C7 of indole frequently do employ conjugative substituents from N1, C2, and/or C3. Other strategies that have effectively been employed to activate the fused benzene moiety include metal conjugation of the \( \pi \)-system
(e.g. Ru\(^+\), Cr\(^{3+}\)) and intramolecular reaction with a substituent at a proximal position (e.g. intramolecular Heck reaction, Claisen condensation).\(^{12,13}\)

While substituents at C4, C5, C6, and C7 of indoles are reported infrequently in the literature compared to what might be expected, there certainly are numerous examples of their inclusion. Most frequently, appropriate functionalization is accomplished by incorporating substituents in the precursors prior to indole synthesis, a strategy that is inefficient for radiolabeling. Radioactive decay makes the incorporation of radionuclides inherently time-sensitive, therefore labeling procedures require rapid reactions with high radiochemical yield, limited need for complex purification, and requiring little to no subsequent synthetic modifications prior to administration. While halo-deboronation of AM630 was clearly demonstrated to be inefficient, the reputed stability of boronates made me optimistic of the potential of more reactive metals as precursors to radioiodinated AM630. I therefore proceeded with efforts to alternatively metalate the 6-iodoindole of AM630.

6.3.2 Radiolabeling Indole at C6 via Stannyl-Halogen Exchange

Initially, when challenged to efficiently convert the 6-iodoindole moiety of AM630 to its radioiodinated analogue, I sought to stannylate the material so that it might undergo iodo-destannylation to generate the desired product. When traditional palladium-catalyzed stannylation methods proved ineffective, I hypothesized that product formation was energetically disfavored, and posited inherent instability of the product. For decades, halo-deboronation methods have competed with halo-destannylation methods as precursors for radiohalogenation
and particularly radioiodination, and the boronic acid derivatives (acids, esters, trifluoroborates) are generally considered as more stable (although less reactive) alternatives to the stannanes. My suspicion that the stannylation of AM630 was energetically disfavored led to my interest in attempting boronation procedures and subsequent radioiodo-deboronation. However, the stability of the carbon-boron bond proved to be the downfall of the relatively accessible boronated derivative of AM630.

The need for an activating group capable of generating stronger carbanion character in order to promote site-specific electrophilic aromatic radioiodination at the C6-position of AM630 rejuvenated my interest in stannylating AM630 and related C6-indoles.

6.3.3 Precedent for Stannylation of 5- and 6- Haloindoles

The emergence of halo-destannylation and halo-deboronation procedures for radiohalogenation closely paralleled the advent of palladium-catalyzed coupling chemistry, and particularly the development of the Stille and Suzuki reactions. Therefore, significant emphasis was simultaneously being placed on the development of functionality-tolerant means of generating relevant metalated compounds. Previously the use of strong alkali bases (e.g. KH, BuLi) had been required to generate metalated (e.g. stannylated, boronated) compounds. While the success of the halo-demetalation approach to radiohalogenation ultimately is based on the efficacy of the radiolabeling reaction, the discovery that palladium catalysis could facilitate aryl/vinyl metalation from the reaction of functionally-complex, halogenated precursors with di-metal
complexes (e.g. Sn<sub>2</sub>But, bis(pinacolato)diboron) greatly broadened the scope of substrates amenable to further palladium-catalyzed coupling.

The dual use of aryl-tin precursors in facilitating both radiohalogenation<sup>5,17</sup> and carbon-carbon coupling,<sup>18</sup> as well as the known versatility of the indole scaffold as a privileged structure for drug development, make the lack of 6- stannylated indoles reported in the literature particularly perplexing. Upon broadening the literature search to include 5-stannylated indoles, a total of only four sources were found which reported the palladium-catalyzed stannylation of 5- or 6-indoles.

The lone peer-reviewed reference to report the palladium-catalyzed C6 stannylation of an indole was Yamada’s 2005 report of a stannylated tryptophan derivative, which was reportedly synthesized from a 6-iodoindole precursor in 45% yield (Equation 6.7)<sup>19</sup>

![Chemical Structure](image)

(eq. 6.7)

Additionally, a 2009 patent application reported the synthesis of a tricyclic fused indole derivative, stannylated at the 6-indole position from the palladium-catalyzed conversion of the 6-bromoindole precursor. The product was purportedly synthesized under standard stannylation conditions (Sn<sub>2</sub>Me<sub>6</sub>, Pd(PPh<sub>3</sub>)<sub>4</sub>), however no yield was reported as this compound was reported to be used without further purification (Equation 6.8).<sup>20</sup>
Considering that the C5 position may be similar in reactivity to C6, palladium-catalyzed synthesis of 5-stannylindoles were additionally investigated.

In 2010, Watanabe reported the stannylation of a series of 5-stannylated indoles from brominated precursors. All stannylation reactions were catalyzed using palladium tetrakis(triphenylphosphine) and employed either the hexamethylditin reagent in dioxane or hexabutylditin in a mixed solution of dioxane and triethylamine. Reported yields were entirely inconsistent, ranging from 2.1% to 89.8%, with four of the six compounds resulting in 12.4% yield or less. However, chromatography was performed on standard silica gel which is known to promote proteo-destannylation of unstable organostannanes, establishing the possibility that the reaction efficiency is not appropriately reflected by the reported product yield (Equations 6.9, 6.10, 6.11).\textsuperscript{21}
Additionally, Furuya synthesized a number of aryl-stannanes as precursors for electrophilic aromatic substitution, including in his library two activated indole derivatives. While stannylation of these compounds proceeded efficiently, it is questionable whether the electron-withdrawing groups on the N-ring facilitate the reaction, and notable that other indoles were not reported (Equations 6.12, 6.13).
The ubiquity of indole structures and palladium-catalyzed metalations makes the scarcity of reported palladium-catalyzed stannylations of 5- and 6- indoles in the literature particularly notable. A recent update on a perennial review, titled ‘Synthesis and Functionalization of Indoles Through Palladium-Catalyzed Reactions,’ further substantiates the presumption that this literature gap represents ineffective reaction methodology rather than a lack of product utility.14

With a heightened awareness of the challenges of stannylating 6-haloindoles, I proceeded in my attempts to stannylate AM630. Familiar with some of the hurdles frequently presented by palladium catalysis, and aware that radioiodinated AM630 has purportedly been achieved in the past through halo-destannylation, I did not regard this goal to be unattainable; however, I did recognize that non-trivial modifications in the reaction methodology would likely prove essential to accomplish this goal.

6.4 Synthesis of 5- and 6- Stannyl Indoles

6.4.1 Stannylation of 6-Bromoindole-3-carbaldehyde

While the finite reported examples of palladium-catalyzed stannylations of 5- and 6- indoles generally resulted in low and inconsistent yields, the occasional occurrence of higher-yields generally did correlate with the presence of electron-withdrawing functionalities on the N-ring. Fortuitously, AM630 does contain such a substituent, having a carbonyl at the 3-position that ultimately may be exploited to facilitate the desired reactivity of the C6 position. In previous attempts to synthesize metalated AM630, I had employed 6-bromoindole-3-carbaldehyde as well
as the related N-methylated derivative (*vide supra*). As the complexity of C6 indole reactivity seems to be independent of the protonation state of the pyrrolic nitrogen, the commercially-available protonated indole rather than the methylated analogue was selected as a suitable substrate in further developing synthetic methods for stannylation of AM630.

Palladium-catalyzed reactions are generally considered to occur via a highly conserved mechanism, wherein oxidative addition of an activated C-X bond to a Pd(0) center is followed by an apparently redox-independent transmetallation or insertion event, altering the substitution of the Pd(II) center. Depending on the proximity of the palladium-bound reactive species, isomerization events additionally are frequently observed. Finally reductive elimination occurs from a cis-substituted palladium complex, ligating the reactive components upon dissociation and regenerating an active Pd(0) catalyst (Figure 6.2).

![Figure 6.2](image)

*Figure 6.2* “Textbook mechanism of cross-coupling reactions.”

While the redox cycling of the palladium catalyst does generally follow this depiction, the vague nature of this description reflects the highly variant reactants that may be employed and the significant mechanistic differences which exist between palladium-catalyzed reactions. In order to promote the production of desired products, particularly at the expense of other competing
reactions which may concurrently occur, variable reaction conditions can be employed, for example by modifying the identity of spectator ligands, solvents, and other additives such as base.

Attempts to use standard methods to stannylate 6-bromo-3-carbaldehyde had previously demonstrated no reaction. (From here on, the term ‘standard conditions’ will be used to refer to the reaction of an aryl halide with hexabutylditin in the presence of palladium tetrakis(triphenylphosphine), in toluene, at 100-110°C, overnight.) Observation that the lack of product did not result from an overwhelming competing side-reaction, but instead from non-reactivity, implied an excessive energetic hurdle preventing the occurrence of one or more steps of the catalytic cycle.

The efficient stannylation of 4-nitroiodobenzene under standard conditions confirmed that these reaction conditions are suitable for stannylating aryl halides. Any of a number of explanations may be responsible for the lack of reaction progress when these same conditions are applying to 6-bromoindole-3-carbaldehyde. For example, this substrate may not undergo oxidative addition to the palladium catalyst. Alternatively, assuming that oxidative addition does occur, it is possible that a stable palladium complex forms with insuperable energetic barriers to transmetalation; or, that upon transmetalation, the formed complex is stable against reductive elimination. Beyond the generally discussed steps in the catalytic cycle, a host of other less understood events additionally occur. For example, cis-trans isomerization events have been observed to facilitate reductive elimination, and complex patterns of ligand association and
dissociation support both stability and reactivity of palladium complexes by altering the electron density about the metal center.  

Because the lack of formation of byproducts as well as products suggested the possibility that the standard methods were not facilitating the oxidative addition of the C-Br bond to the palladium center, rather than optimizing the reaction conditions based on the standard stannylation methods, I instead chose a nontraditional route. Having previously been successful in employing Miyaura boronation conditions to AM630 and the N-methylated 6-bromoindole-3-carbaldehyde, I attempted to modify stannylation procedures to mimic the Miyaura reaction conditions (1,1’-bis(pinacolato)diboron, KOAc, PdCl2dppf, DMF, 110°C).

6.4.2 Effects of Modifying Reaction Environment

I initially performed four reactions (Table 6.1). In the first, three singular components of the standard stannylation methods were altered to reflect those of the Miyaura reaction. To the first, potassium acetate was added (a); to the second Pd(PPh3)_4 was replaced with PdCl2dppf (b); and in the third reaction Pd(PPh3)_4 was co-administered with an additional catalytic equivalent of dppf (c). Unfortunately these modified reaction conditions remained unsuccessful in promoting any observable reaction. However, a fourth reaction (d) was also concurrently performed, employing standard Miyaura conditions with the lone modification being the replacement of 1,1’-bis(pinacolato)diboron with hexabutylditin. While reaction progress was fairly insubstantial, and no attempts were made to isolate the small quantity of product, this result was very exciting because it was the first indication that palladium catalysis could ultimately be
employed for the stannylation of 6-bromoindole-3-carbaldehyde and potentially other related haloindoles.

At this stage, in an effort to continue rational reaction optimization, the purpose of each reaction variable to have been altered in reaction d was considered.

Miyaura reactions specifically use potassium acetate is to facilitate transmetalation, as an exchange between acetate and halide on the palladium catalyst allows formation of a transmetalation byproduct containing an enthalpically favorable B-O bond (Figure 6.3).27

![Figure 6.3 Palladium-catalyzed Miyaura boronation reaction.27](image)

It was uncertain whether the inclusion of KOAc in stannylation methodology could analogously favor reaction progress through formation of an Sn-O bond.
Dppf (1,1”-bis(diphenylphosphino)ferrocene) and related bidentate phosphine ligands are capable of altering conformational biases of palladium intermediates, encouraging cis-positioning of the reactive species, which can favor certain steps of the catalytic cycle, particularly reductive elimination.\textsuperscript{18} Additionally bidentate ligands exhibit higher affinity for the palladium center than their monodentate counterparts, and the particular nature of the diphenylferrocene substituents on the phosphine may play a role in affecting the electron density, and hence the reactivity, of palladium bound complexes (\textit{vide infra}).

It may be that the combination of such conditions as dppf and KOAc were necessary for promoting the stannylation reaction, but an alternative variable that had not yet been assessed was also introduced at this stage. While toluene had originally been used as a solvent, DMF (dimethylformamide) was instead employed in reaction d. DMF, like toluene, is an aprotic solvent but is significantly more polar than toluene and can sometimes be better suited for reactions that proceed via charged or otherwise polarized transition states or intermediates.

Another series of reactions was then performed (\textit{Table 6.1}), employing reaction d as a foundation for further optimization. Uncertain whether it was the change in solvent that had facilitated the reaction or the effects of other variables such as the bidentate dppf ligand and/or the inclusion of potassium acetate, I repeated the standard stannylation reaction in DMF (e) as well as the Miyaura-like stannylation in toluene (f). In fact, DMF was observed to facilitate the reaction, as the Pd(PPh\textsubscript{3})\textsubscript{4} catalyst did promote some reactivity in DMF while the Miyaura-like conditions were ineffective in toluene. Additional variables of the Miyaura conditions were also examined, performing the reaction at 153°C (g) to evaluate whether reactivity was being
hindered by an elevated but surmountable energy barrier, and additionally assessing the result of eliminating KOAc (h). Significant retention of starting material continued to be observed in these reactions and products were not isolated. Observation of reaction progression determined h to have proceeded to the farthest extent in comparison with other reactions of this series.

While the desired stannylations were successfully progressing, they consistently showed significant retention of starting material. I therefore attempted to overcome energetic barriers by elevating the temperature to 153°C (i) and prolonging the reaction time to three days (j), but neither increased temperature or time significantly promoted the reaction. I additionally evaluated the effect of using dioxane in place of DMF in the presence (k) and absence (l) of KOAc. Dioxane is another aprotic solvent commonly employed in palladium-catalyzed reactions which has an intermediate polarity relative to toluene and DMF. Dioxane was effective in further promoting reaction progress, particularly in the absence of KOAc (l), and chromatography over triethylamine-stabilized silica gel provided 6-stannylinodole-3-carbaldehyde in 24% yield.
The recovery of 6-stannylindole-3-carbaldehyde established that palladium-catalyzed stannylation of 6-bromoindoles is possible and that 6-stannylindole-3-carbaldehyde is stable and can be isolated, providing a stannylated indole which can potentially be used for diverse applications including radiiodination and Stille coupling reactions. However, the overall goal of stannylation of AM630 was yet to be achieved and, while these reaction conditions might potentially be effective, more robust methods were desired.

Further method development was therefore attempted. As variations in solvents had shown significant effects on reactivity, the effect of water was next evaluated (Table 6.2). In choosing to do so, I was additionally influenced by methodology developed by Buchwald which employs...
stoichiometric quantities of water in the palladium-catalyzed silylation of aryl chloride, including the conversion of 5-chloroindole to a 5-silylindole.\textsuperscript{28} Water content was assessed on the stoichiometric scale, using 2 equivalents (m) and 10 equivalents (n), as well as the solvent scale, as 5\% (o), 25\% (p), and 50\% (q) aqueous solutions in dioxane. While the reaction appeared to be tolerant of small quantities of water, reaction progress was impeded as larger quantities were employed, and the reaction progress was completely halted in 1:1 H\textsubscript{2}O/dioxane (q). Ultimately no benefit was determined from the addition of water.

![Chemical Structure](image)

**Table 6.2** Attempts to optimize reaction 1 (Table 6.1) via addition of water.

<table>
<thead>
<tr>
<th>entry</th>
<th>H\textsubscript{2}O</th>
<th>dioxane</th>
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<tbody>
<tr>
<td>m</td>
<td>3.2 (\mu)L (2 equiv)</td>
<td>2.0 mL</td>
</tr>
<tr>
<td>n</td>
<td>16 (\mu)L (10 equiv)</td>
<td>2.0 mL</td>
</tr>
<tr>
<td>o</td>
<td>0.1 mL</td>
<td>1.9 mL</td>
</tr>
<tr>
<td>p</td>
<td>0.5 mL</td>
<td>1.5 mL</td>
</tr>
<tr>
<td>q</td>
<td>1.0 mL</td>
<td>1.0 mL</td>
</tr>
</tbody>
</table>

6.4.3 Effects of Modifying Catalyst

If all aspects of the reactant are presumed to be invariable (e.g. hybridization, halide identity, proximal substituents), then the only mode of manipulating an unreactive compound is by altering external variables. Lack of reactivity implies energetic stability of the compound in
comparison to the transition state by which the reaction proceeds. Reaction optimization is frequently accomplished by modifying the reaction environment, for example through changes in solvent or temperature to try to destabilize the compound and/or provide increased stability to unfavorable transition states. When environmental changes are ineffective, the reaction can only be accomplished by creating a more favorable energetic situation than the compound is currently experiencing. (Such is the nature of catalysis, to cause an energetically disfavored reaction to proceed through an alternative mechanism which has lower energy barriers.)

While perhaps a bit more difficult to predict qualitatively, I had a growing conviction that success in optimizing methods of palladium catalysis to effectively manipulate the reactivity of the halo-indole must ultimately derive from the nature of the spectator ligands. Various ligands can be used in transition metal catalysis and ligandless methodologies are sometimes explored. Tertiary phosphines have been extensively employed as spectator ligands due to their proficiency at influencing the energetics of the catalytic system by affecting the electron density about the metal center. The relative electron density of the metal in turn affects the energetics of its frontier orbitals, dictating their ability to effectively promote reactivity of substrate molecules.

As an example, let us consider the oxidative addition step of palladium catalysis, arguably the most conserved step of the assorted palladium-catalyzed transformations. [Please recognize that it is unknown whether the oxidative addition event is in fact limiting this reaction from occurring. I present increased detail of the oxidative addition step solely as a model of how the nature of the active catalyst can facilitate a given transformation.]
Oxidative addition of an aryl or vinyl halide (or pseudohalide) to a palladium center can proceed via nucleophilic substitution or a concerted mechanism, but in both cases involves the attack of a 4d electron pair of Pd upon the activated carbon of the C-X bond. This implies that the more electropositive the carbon, the more prone the C-X bond should be to oxidative addition, and questions have been raised regarding why less-polarized sp² C-X bonds (e.g. aryl and vinyl halides) are more prone to oxidative addition with palladium than their more polarized sp³ homologues (e.g. alkyl halides). DFT calculations indicate that orbital mixing of the σ* and π* orbitals of sp² hybridized carbons generates a hybrid orbital that can effectively overlap with the Pd 4d orbital, allowing the effective transfer of electron density and thereby favoring oxidative addition, while analogous oxidative addition at an sp³ carbon requires transfer of electron density from the 4d orbital of palladium to the rigidly oriented σ* orbital of the C-X bond, a far less energetically favorable event.²⁹

While the nature of the acceptor orbitals is influenced by the identity of the organyl halide complex, if we consider the identity of the organyl halide to be constant, then we must instead consider modifying the orbital energetics of the electron donor in order to promote an otherwise unfavorable oxidative addition. Tertiary phosphines (PR₃) are both electronically and sterically adept at affecting the electronics of transition metals. The phosphine initially forms σ-bonds to the metal center by donating its lone pair, contained with high-energy sp³ orbitals, to low-energy empty d-orbitals of the metal. Contrary to related amine complexes (hard base), phosphines additionally can exert π-acidity (soft base), which allows metallophosphine bonds to be additionally stabilized by back-bonding interactions.³⁰ The strength of the Pd-P bonding interactions can then be controlled through conscientious selection of phosphine substituents.
While triphenylphosphine is most commonly employed, numerous tertiary phosphines have been used to manipulate palladium-catalyzed reactions and assorted substrate classes.

Although substituent effects are widely acknowledged for their role in effecting Pd-P bond strength, questions persist regarding the specific energetic contributions and ultimate electronic effects which occur. Electron-rich phosphines (i.e. phosphines containing electron-donating substituents) are considered to be more effective σ-donors, imparting greater electron-density to the palladium species. This then should hypothetically heighten palladium nucleophilicity resulting in stronger bond formation between palladium and electrophilic substituents. Conversely, electron-withdrawing substituents on the phosphine may result in weaker Pd-P σ-bonds and increased electrophilicity of the palladium center. Empty d-orbitals on the phosphine were at one time posited to partake in back-bonding interactions with palladium, but this has since been discounted as orbital overlap is energetically implausible. Instead it is believed that P-R σ*-orbitals are responsible for the observed π-acidity of phosphines, although others have suggested that it is orbital mixing between the P-R σ*-orbitals and empty 3d-orbitals of phosphine that facilitate this π-acidity. The aryl and alkyl phosphines most commonly employed as ligands for palladium catalysis do not form nearly as strong back-bonding interactions as those observed with heteroatom substituents (e.g. PF₃, P(OR)₃), but their capacity to serve as weak π-acids is likely exploited in the formation of excited transition states.

The vast array of theoretical studies aimed at comprehending the electronic effects exerted by phosphine ligands is representative of the practical interest in promoting palladium-catalyzed transformations. Such efforts continue, and perhaps eventually it will be possible to rationally
select specific phosphine ligands to promote efficient catalysis. In the meantime, phosphine selection can in part be guided by a qualitative understanding of the effect of increasing phosphine nucleophilicity on the palladium center. The relative pKa values of various protonated phosphines might be used as a guide for predicting the effect they will impart on palladium charge density and, ultimately, reactivity.

Observation of the initial non-reactivity of 6-bromoindole-3-carbaldehyde to stannylation attempts indicated truncation of the catalytic cycle, and the significant retention of starting material once reaction progress was achieved suggested that significant energetic hurdle(s) still existed under these improved conditions. As related catalytic systems did promote the boronation reaction, I was fairly confident that oxidative addition of the aryl halide did occur and instead suspected that a stable intermediate palladium-complex was being generated which resisted further progression through the catalytic cycle. Although complex mechanistic studies were not performed, I hypothesized that alterations in the charge density of the palladium catalyst may affect necessary mechanistic events (e.g. ligand association/dissociation, cis-trans isomerization) in order to favor further reaction progression.

A series of five reactions was next performed, to assess the results of varying the electronic nature of the phosphine ligand in hopes of altering the energetics of the active catalyst and the intermediate palladium complexes (Table 6.3).
**Table 6.3** Attempts to optimize palladium reactivity via variations in the phosphine ligands.

Reaction methods were consistent with those used in reaction I (from which 24% product had been isolated) however, rather than employing PdCl$_2$dppe, the catalyst was generated *in situ* from bis(benzonitrile)palladium chloride (PdCl$_2$(PhCN)$_2$) and free phosphine ligand. PdCl$_2$(PhCN)$_2$ is a Pd(II) complex from which reduction as well as ligand dissociation/association events occur rapidly to generate a phosphine-ligated Pd(0) species. The phosphines evaluated included tris(4-fluorophenyl)phosphine (r), tris(4-methoxyphenyl)phosphine (s), tricyclohexyl phosphine (t), 1,2-bis(diphenylphosphino)ethane (dppe, u), and 1,1'-bis(diphenylphosphino)ferrocene (dpff, v). The aryl phosphines used in reactions r and s varied the electron-density of the phenyl substituent significantly but retained the phenyl character, while the alkyl phosphines, such as tricyclohexyl phosphine (t), should have significantly greater electron-donating affects than aryl phosphines and may significantly alter the strength of the P-Pd bond. As the reaction was previously performed using the bidentate ligand dppe, this was included as a control (v) to ensure
that the modified administration of the palladium catalyst did not significantly alter reactivity. Additionally the related bidentate ligand dppe, which substituted an alkyl linker (ethane) for the ferrocene linker, was also evaluated (u).

After 24 hours, product formation and the absence of significant byproduct formation was observed in every case. However, the lone reaction to employ a trialkylphosphine (tricyclohexylphosphine, PCy₃) rather than a triaryl phosphine (t), was solely capable of driving the reaction to completion. ¹H NMR reinforced TLC observations that the dominant aromatic component of the crude reaction mixture was 6-stannylindole-3-carbaldehyde, and following chromatography, the product was recovered in 82% yield (Equation 6.14).

![Chemical Reaction Image]

Clearly the character of the phosphine ligand played a major role in promoting the stannylation of 6-bromoindole-3-carbaldehyde, but it should be noted that comprehensive studies have not been performed to analyze what particular aspects of tricyclohexyl phosphine contributed to this. While I am admittedly biased to believe that the electronic nature of the alkyl phosphine was the key contributor, PCy₃ is also an extremely bulky ligand and its large cone angle may have played a substantial role, for example by controlling the geometry of key transition states or by reducing the number of accessible coordination sites on the palladium complex. The inability of bulky bidentate ligands to generate high yields disputes this but certainly does not eliminate the
possibility (comparison with bulky triarylphosphines such as tri(o-tolyl)phosphine would be more effective this potential steric influence). Prior to drawing any conclusions regarding the nature of the active catalyst required to efficiently promote this conversion, detailed mechanistic studies would be required.

6.4.4 Stannylation of 5-Bromoindole-3-carbaldehyde

My increased awareness of the hurdles to functionalizing alternative benzo positions of indole encouraged me to investigate whether the C5-brominated analogue could be stannylated under the same reaction conditions used for C6-stannylation. Therefore, stannylation of 5-bromoindole-3-carbaldehyde was attempted using the identical conditions employed in reaction t. Due to the observation of remaining starting material after 24 hours, the reaction was continued for a second 24 hour spell, following which the reaction was removed and the desired 5-stannylindole-3-carbaldehyde was successfully isolated. However, only 22% yield was achieved under these conditions. Furthermore, in addition to a substantial quantity of unreacted starting material, generation of the protonated byproduct (indole-3-carbaldehyde) was observed.

While a robust method for effectively stannylation various benzo-substituted indoles would be ideal, the ability of this methodology to at all affect the 5-bromo analogue was very encouraging. Recognizing the possibility that heightened enthalpic barriers might exist at C5, the reaction was repeated at 153°C in DMF. Although this reaction was observed to proceed by TLC, the reaction efficiency did not appear to be significantly heightened.
I then considered two aspects of the phosphine ligands that may have been responsible for the efficiency of PCy$_3$ at promoting the stannylation of C6 analogue: (1) Lewis basicity and (2) steric bulk. I selected to evaluate the efficiency of three phosphine ligands that varied in these properties. Tri-tert-butylphosphine (P(tBu)$_3$) is another bulky trialkyl phosphine and has an even greater basicity than PCy$_3$ (as implied by their relative pKa values) which may further increase the reactivity of the palladium center towards halo-indoles. However, both stannylated product and the protonated byproduct were suppressed when P(tBu)$_3$ was used, suggesting that the catalytic cycle may have been impeded at a very early stage, perhaps even preventing the oxidative addition of the halo-indole.

Although dppf and dppe demonstrated only modest ability to promote the related C6 stannylation, I considered the possibility that perhaps bulky phosphanes without such extreme basicity may in fact be desirable at C5. Interestingly, substitution of PCy$_3$ with dppf and dppe almost completely suppressed the formation of the protonated byproduct. While both reactions proceeded cleanly, unreacted starting material was retained in both cases. Analyzing the crude reaction mixtures by $^1$H NMR revealed that, although the major indolic component of the dppf-containing reaction was starting material, the use of dppe further enhanced product formation, and upon chromatography, a 60.% yield of 5-stannyldole-3-carbaldehyde was successfully isolated (Equation 6.15).

(eq. 6.15)
As in the case of the synthesis of 6-stannylinole-3-carbaldehyde, detailed mechanistic studies of 5-stannylinole-3-carbaldehyde have not been accomplished, but it is interesting to note the success of another sterically-constrained, alkylated phosphine in promoting palladium-catalyzed stannylation at a benzo position of indole-3-carbaldehyde.

6.4.5 Stannylation of AM630

Let us recall that methods of converting 6-bromoindole-3-carbaldehyde to 6-stannylinole-3-carbaldehyde were developed with the ultimate goal of stannylation the 6-iodo position of AM630.

AM630 was subjected to the conditions of reaction \( r \), which had previously yielded 6-stannylinole-3-carbaldehyde in 82% yield. After 24 hours, TLC and \(^1\)H NMR of the crude reaction mixture indicated formation of the desired stannylated derivative of AM630 without any competition from alternative byproducts, however significant concentrations of starting material remained. Encouraged by the lack of side reactions taking place, I decided to assess the results of prolonging the reaction. When the reaction was repeated over 40 hours, it did proceed to completion with minimal formation of byproducts, as revealed by TLC and \(^1\)H NMR of the crude reaction mixture. The reaction mixture was purified by manual flash chromatography using triethylamine-protected silica gel, and the desired 6-stannylated derivative of AM630 was isolated in 58% yield (Equation 6.16)
6.5 Radioiodination of Stannylated AM630

Radioiodination of stannylated AM630 was performed via electrophilic aromatic substitution at the 6-stannylated position. AM630 was reacted with a radioactive electropositive iodine, generated \textit{in situ} from the oxidation of $^{123}$I$^-$ with hydrogen peroxide at room temperature for thirty minutes (Equation 6.17). Sodium thiosulfate was then added to quench residual iodine and the product was isolated in high radiochemical yield via semi-preparative HPLC. Prior to performing radioiodination, elution profiles of cold samples of AM630 and stannylated AM630 had been evaluated by both analytical and semi-preparative HPLC, allowing conformation of the identity and purity of the isolated $^{123}$I-labelled AM630.
6.6 Radioiodination of 5-/6- Boronated/Stannylated Indole-3-Carbaldehydes

My interest in the unusual stability of substituents on the benzo ring of indoles, particularly at C5 and C6 had initially been sparked by observations of the inability of the 6-boronated AM630 to effectively undergo C6 specific radiolabeling via iodo-deboronation. Subsequently, my interest in the reactivity (or, perhaps more accurately, non-reactivity) of these C5 and C6 positions of indoles had been further stimulated by my efforts to develop robust methodologies to stannylate such systems and my growing recognition of the scarcity of literature reporting their effective functionalization.

The production of $^{123}$I-AM630 via iodo-destannylation of a 6-stannylindole derivative of AM630 can be directly contrasted with the previous attempt to iodo-deboronate a related boronated precursor. (Recall, that while the reaction appeared to yield a radiolabeled AM630 analogue, the label is believed to have been incorporated on an alternative position.) In order to more generally evaluate the relative competence of boronated versus stannylated indoles at effectively undergoing electrophilic radiohalogenation, it was determined that the 5- and 6- stannylated indole-3-carbaldehydes as well as 5- and 6- boronated indole-3-carbaldehydes would be subjected to radioiodination.

Synthesis of the requisite stannylated materials had already been achieved during the development of methods to stannylate AM630. Boronation of the 5- and 6- indoles, a process somewhat more commonly reported, was performed under standard Miyaura conditions and is discussed below. Methods for radioiodinating the stannylated and boronated materials should
mimic those employed for the radioiodination of AM630 and would again be accomplished in collaboration with George Kabalka’s group at UT-Knoxville.

6.6.1 Boronation of 5- and 6- Bromoindole-3-Carbaldehyde

The conversion of the 5- and 6- bromoindole-3-carbaldehydes to their complementary 5- and 6-boronic esters were both accomplished using standard Miyaura reaction conditions. Briefly, PdCl$_2$dpf was used to catalyze the boronation of the indolic bromides via reaction with 1,1'-bis(pinacolato)diboron in the presence of potassium acetate. The reaction was performed at 110°C in dioxane. The C5 boronic ester was recovered in 43% yield and the analogous C6 boronic ester in 52% yield.

No attempts were made to optimize these yields, as synthesis of the boronic esters were being generated purely to evaluate their capacity to undergo radioiodo-demetalation. However, I was curious whether the catalytic systems previously used for the stannylations of the 5- and 6-bromoindole-3-carbaldehydes could be effectively employed for the boronations.

Therefore, boronations were repeated under identical reaction conditions to those outlined above except substituting PdCl$_2$dpf with a combination of PdCl$_2$(PhCN)$_2$ and the relevant phosphine ligand. The dppe ligand which had successfully promoted the stannylation of 5-bromoindole-3-carbaldehyde proved entirely unsuccessful for the analogous boronation, resulting in complete retention of the starting material. However, the PCy$_3$ ligand, which had successfully stannylated the 6-bromoindole-3-carbaldehyde (82%) proved to additionally be useful in the boronation of 6-
bromoindole-3-carbaldehyde. While PCy3-promoted boronation of 6-bromoindole-3-carbaldehyde was not as efficient as the stannylation, the 48% recovery of the boronic ester rivals the 52% yield observed with the standard boronation catalyst PdCl2dppf.

6.6.2 Radio-iododemetalation of 5- and 6-Metalated Indole-3-Carbaldehydes

Radioiodination experiments will again be performed in collaboration with George Kabalka group, and will follow very similar protocols as detailed above for the radiolabeling of stannylated AM630. To date (08/04/2011) these results are not yet available.

6.7 Radiofluorination of AM630 and related 5- and 6- Stannylated Indoles

Organostannanes have effectively been used for radio-fluorination (18F labeling). The stannylated AM630 as well as 5- and 6- stannylindole-3-carbaldehydes will be subjected to selective electrophilic fluorination. Although the specific binding properties of a 6-fluorinated analogue of AM630 are yet unknown, this study may result in a CB2-selective PET imaging agent while additionally assessing the effectiveness of radiofluorination, in comparison to radiiodination, of stannylated indoles.

Radiofluorination experiments will be performed in collaboration with Henry F. Van Brocklin’s group at University of California at San Francisco. To date (08/04/2011) these experiments have not yet been attempted.
6.8 Summary and Future Directions

Radioiodination of AM630 originally presented as a relatively simple challenge. All that should hypothetically have been required was a simple stannylation of an aryl iodide followed by iodo-destannylation with an electropositive radioactive iodine source. Instead what emerged was a far more detailed study of a surprisingly complex system. Eventually the challenges of stannylating and subsequently radioiodinating AM630 were accomplished. Additionally, synthesis of structurally related 5- and 6-stannylated and boronated indoles has been accomplished and further studies are currently in progress to compare the effectiveness of radiofluoro- and radioiodo-demetalation of these compounds.

This work represents only a small foray into an under-explored research area. The indole scaffold represents one of nature’s most privileged structures, and as such is highly incorporated into natural products and exhibits significant therapeutic potential. Current approaches to functionalize the benzo positions of indoles (C4, C5, C6, C7) are currently very limited, frequently requiring substituents to be incorporated prior to indole synthesis.

For example, palladium-catalyzed reactions have found prolific use in synthetic chemistry over the past 30+ years, and the inability of indoles to undergo palladium-catalyzed substitution severely limits their versatility. This study attempts to use palladium catalysis only for metalation, but similar obstacles may restrict attempts to use palladium-catalysis to otherwise substitute the 5- and 6-indoles. Such an assumption is substantiated by the high degree of
stability demonstrated by both the 5- and 6-haloindoles and the 6-boronated derivative of AM630.

Numerous examples exist to demonstrate the utility of both stannanes and boronates as precursors for radiohalogenation. The inability of boronated-AM630 to undergo site-specific radiiodination can be directly contrasted with the efficient conversion of stannylated-AM630 to $^{123}$I-AM630. Whether various other aromatic systems exhibit similar preferences remains to be explored, as does the question of whether alternative organometallic compounds (e.g. germanes, silanes) may be more suitable for metal-halogen exchange reactions to radiolabel certain classes of substrates.

Finally, I would be remiss to disregard the relevance of a CB$_2$ selective radiotracer. Cannabinoid receptors are widely distributed, particularly in the central (CB$_1$) and peripheral (CB$_2$) nervous systems as well as other tissues, and have been demonstrated to be coupled with a wide-range of medically-relevant biological processes. Radioiodinated AM630, and potentially the radiofluorinated derivative of AM630 (should fluoro-destannylation prove effective), provide great potential for further understanding the endocannabinoid system and developing various receptor-selective therapeutics.
References

Chapter 6


20. Ehrhardt, Claus; Mcquire, Leslie Wighton; Rigollier, Pascal; Rogel, Olivier; Shultz, Michael; Tommasi, Ruben Alberto. PCT Int. Appl. (2009), WO 2009118292 A1 20091001. Language: English, Database: CAPLUS.


Appendix 1: Supplemental Synthetic Methods and Characterization for Chapter 2
General

All commercially available reagents were purchased from Sigma-Aldrich and used without further purification. Solvents were distilled and reactions requiring inert conditions were performed under N₂ or argon. Column chromatography was performed using silica gel unless otherwise indicated. Flash chromatography was performed using the Argonaut FlashMaster Solo with an FC204 fraction collector. Thin layer chromatography was used to monitor reactions using Selecto Scientific 200 micron silica gel flexible TLC plates. ¹H was recorded on a Varian Unity-INOVA 500MHz spectrometer. ¹³C NMR were recorded on a Varian 300MHz spectrometer. High resolution mass spectral data were obtained at the University of Massachusetts Mass Spectrometry Facility which is supported, in part, by the National Science Foundation.

Experimental Methods

Representative procedure for the synthesis of para-brominated 2-alkylphenols.

Preparation of 2-Benzyl-4-bromophenol (3e)

2-benzylphenol (4.61 g, 25 mmol) was dissolved in chloroform (200 mL). Tetra-n-butyl ammonium tribromide (14.47 g, 30 mmol) was added and the solution was stirred for 3 hours at room temperature. The solvent was removed by rotary evaporation and the crude product was partitioned between ether and water. The ether layer was washed sequentially with 1 N HCl (x2) and brine (x2). The organic layer was separated and dried over magnesium sulfate. Solvent was evaporated and the crude product was purified by chromatography on silica gel (Hexane/EtOAc, 80:20). The desired product (6.2 g, 95%) was isolated as a clear yellow oil. ¹H NMR (500 MHz, CDCl₃): δ = 7.30 (t, J = 7.5 Hz, 2H), 7.25-7.20 (m, 5H), 6.66 (d, J = 7.0 Hz, 1H), 3.94 (s, 2H) ppm.

2-Isopropyl-4-bromophenol (3b)

Same procedure as 3e. Product isolated in 88% yield as a yellow oil.
¹H NMR (500 MHz, CDCl₃): δ = 7.35 (d, J = 2.5 Hz, 1H), 7.19 (dd, J = 8.5, 2.5 Hz, 1H), 6.67 (d, J = 9 Hz, 1H), 3.32 (m, J = 7.0 Hz, 1H), 1.27 (d, J = 8.0 Hz, 6H) ppm
2-sec-Butyl-4-bromophenol (3c)

Same procedure as 3e. Product isolated in essentially quantitative yield as a yellow oil.
$^1$H NMR (500 MHz, CDCl$_3$-d): $\delta$ 7.24 (d, $J = 2.5$ Hz, 1H), 7.16 (dd, $J = 8$ Hz, 2.5 Hz, 1H), 6.65 (d, $J = 8$ Hz, 1H), 4.82 (br s, 1H), 2.93 (m, 1H), 1.62 (m, 2H), 1.22 (d, $J = 6.5$ Hz, 3H), 0.877 (t, $J = 7$ Hz, 3H) ppm.

2-tert-Butyl-4-bromophenol (3d)

Same procedure as 3e. Product isolated in essentially quantitative yield as a yellow oil.
$^1$H NMR (500 MHz, CDCl$_3$-d): $\delta$ 7.36 (d, $J = 2.5$ Hz, 1H), 7.17 (dd, $J = 8.5$ Hz, 2.5 Hz, 1H), 6.57 (d, $J = 8$ Hz, 1H), 5.01 (br s, 1H), 1.40 (s, 9H) ppm.

Representative procedure for the synthesis of para-hydroxyphenylboronate esters

Preparation of 2-Benzyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (4e)

![Borylation Reaction](image)

3e (0.79 g, 3.0 mmol), PdCl$_2$(dppt) (0.15 g, 6 mol %), and potassium acetate (0.59 g, 6.0 mmol) were added to the reaction vessel which was predried under vacuum and flushed with argon. Anhydrous dioxane (20 ml) was added via syringe. The reaction mixture was heated to 80 °C and stirred for 30 minutes. Bis(pinacolato) diboron (0.84 g, 3.3 mmol), dissolved in 5 mL dioxane, was added and the reaction was kept under inert atmosphere, stirred, and heated at 80 °C overnight. The crude mixture was filtered through activated carbon and Celite, and solvent was removed by rotary evaporation. The crude mixture was dissolved in ethyl acetate, washed with water (x2), brine (x2) and dried over magnesium sulfate. The product was purified by flash chromatography using silica gel (Hexane/EtOAc, 80:20) to afford 0.60 g (65%) of the desired product as a white solid (m.p. 119-121°C). $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ = 7.70 (s, 1H), 7.65 (dd, $J = 7.0$, 1.5 Hz, 1H), 7.30 (t, $J = 7.0$ Hz, 2H), 7.25 (d, $J = 8.0$ Hz, 2H), 7.22 (t, $J = 7.5$ Hz, 1H), 6.81 (d, $J = 7.0$ Hz, 1H), 4.04 (s, 2H), 1.37 (s, 12H) ppm.

2-Isopropyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (4b)

Same procedure as 4e. Product isolated in 95% yield as a white solid (m.p. 148-150 °C).
$^1$H NMR (500 MHz, CDCl$_3$): $\delta$ = 7.68 (s, 1H), 7.57 (dd, $J = 8.0$, 1.5 Hz, 1H), 6.76 (d, $J = 8.0$ Hz, 1H), 3.23 (m, $J = 7.0$ Hz, 1H), 1.37 (s, 12H), 1.30 (d, $J = 6.5$ Hz, 6H) ppm.
2-sec-Butyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (4c)

Same procedure as 4e. Product isolated in 58% yield as a white solid (m.p. 114-117 °C). 
$^1$H NMR (500 MHz, CDCl$_3$-d): δ 7.61 (d, $J = 1.5$ Hz, 1H), 7.55 (dd, $J = 7.5$ Hz, 1.5 Hz, 1H), 6.74 (d, $J = 7.5$ Hz, 1H), 4.93 (br s, 1H), 2.95 (m, 1H), 1.67 (m, 2H), 1.34 (s, 12H), 1.27 (d, $J = 6.5$ Hz, 3H), 0.88 (t, $J = 7$ Hz, 3H) ppm.

2-tert-Butyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (4d)

Same procedure as 4e. Product isolated in 61% yield as a white solid (m.p. 170-173 °C). 
$^1$H NMR (500 MHz, CDCl$_3$-d): δ 7.72 (d, $J = 1.5$ Hz, 1H), 7.55 (dd, $J = 7.5$ Hz, 1.5 Hz, 1H), 6.66 (d, $J = 8$ Hz, 1H), 4.96 (br s, 1H), 1.43 (s, 9H), 1.33 (s, 12H) ppm.

Representative procedure for the synthesis of ethyl 4-bromophenoxyacetates

**Preparation of Ethyl 2-(2-benzyl-4-bromophenoxy)acetate (5e)**

![Reaction scheme](image)

3e (2.10 g, 8 mmol) was dissolved in dry THF (75 mL). Sodium hydride (60 % in oil, 0.45 g, 11.2 mmol) was added and the solution was stirred at room temperature for 30 minutes. Ethyl bromoacetate (1.87 g, 11.2 mmol) was then added slowly and the solution was stirred for 2 hours at room temperature. The reaction mixture (milky-white suspension) was quenched with ethanol and concentrated. The concentrate was partitioned between ethyl acetate and water (x2). The combined organic layers were washed with brine (x2), dried, and concentrated. The mixture was purified by flash chromatography on silica gel (Hexane/EtOAc, 90:10) to afford 2.37 g (92%) of the desired product as a crystalline solid (m.p. 67-70 °C). 
$^1$H NMR (500 MHz, CDCl$_3$): δ = 7.34-7.22 (m, 7H), 6.65 (d, $J = 8.5$ Hz, 1H), 4.62 (s, 2H), 4.30 (q, $J = 6.5$ Hz, 2H), 4.05 (s, 2H), 1.32 (t, $J = 7.5$ Hz, 3H) ppm.

**Ethyl 2-(2-isopropyl-4-bromophenoxy)acetate (5b)**

Same procedure as 5e. Product was isolated in 78% yield as a yellow oil. 
$^1$H NMR (500 MHz, CDCl$_3$): δ = 7.35 (d, $J = 2.5$ Hz, 1H), 7.24 (dd, $J = 8.5$, 2.5 Hz, 1H), 6.61 (d, $J = 8.5$ Hz, 1H), 4.64 (s, 2H), 4.28 (q, $J = 7.5$ Hz, 2H), 3.41 (m, $J = 7.0$ Hz, 1H), 1.31 (t, $J = 7.5$ Hz, 3H), 1.26 (d, $J = 6.5$ Hz, 6H) ppm.
Ethyl 2-(2-sec-butyl-4-bromophenoxy)acetate (5e)

Same procedure as 5e. Product was isolated in 92% yield as a pale yellow oil. $^1$H NMR (500 MHz, CDCl$_3$-d): $\delta$ 7.28 (d, $J = 2.5$ Hz, 1H), 7.22 (dd, $J = 9$ Hz, 2.5 Hz, 1H), 6.60 (d, $J = 9$ Hz, 1H), 4.60 (s, 2H), 4.26 (q, $J = 7$ Hz, 2H), 3.16 (m, 1H), 1.60 (m, 2H), 1.29 (t, $J = 7.5$ Hz, 3H), 1.21 (d, $J = 7$ Hz, 3H), 0.86 (t, $J = 7.5$ Hz, 3H) ppm.

Ethyl 2-(2-tert-butyl-4-bromophenoxy)acetate (5d)

Same procedure as 5e. Product was isolated in 82% yield as a pale yellow oil. $^1$H NMR (500 MHz, CDCl$_3$-d): $\delta$ 7.41 (d, $J = 2.5$ Hz, 1H), 7.27 (dd, $J = 9$ Hz, 2.5 Hz, 1H), 6.62 (d, $J = 8$ Hz, 1H), 4.63 (s, 2H), 4.29 (q, $J = 7$ Hz, 2H), 1.42 (s, 9H), 1.32 (t, $J = 7$ Hz, 3H) ppm.

Representative procedure for the synthesis of biphenyl phenols.

Preparation of Ethyl 2-(1,1'-biphenyl-3,3'-dibenzyl-4'-ol-4-oxy) acetate (6e)

![Chemical structure](image)

5e (0.210 g, 0.60 mmol), PdCl$_2$(PPh$_3$)$_2$ (0.026 g, 6 mol %), PPh$_3$ (0.010 g, 6 mol %), and 2M aqueous sodium carbonate (0.8 mL, 1.2 mmol) were added to the reaction vessel which was vacuumed and flushed with argon. Tetrahydrofuran and water (7 mL : 2 mL) were degassed with argon and added via syringe. The reaction was heated to reflux and stirred for 30 minutes. 4e (0.224 g, 0.72 mmol), dissolved in 3 mL tetrahydrofuran, was then added and the reaction was stirred at reflux, overnight, under an inert atmosphere. Activated carbon was added to the crude solution and the resultant mixture was filtered through Celite and solvent was evaporated to dryness. The concentrate was dissolved in ethyl acetate, washed with water (x2) and brine (x2), dried over magnesium sulfate, and concentrated. The mixture was purified by flash chromatography on silica gel (Hexane/EtOAc, 80:20) to afford 0.165 g (61%) of the desired product as a white solid (m.p. 142-144 ºC). $^1$H NMR (500 MHz, CDCl$_3$): $\delta =$
7.34-7.27 (m, 12H), 7.24 (t, J = 7.5 Hz, 1H) 7.20 (t, J = 6.5 Hz, 1H), 6.83 (d, J = 7.0 Hz, 1H), 6.81 (d, J = 8.5 Hz, 1H), 4.64 (s, 2H), 4.30 (q, J = 7.0 Hz, 2H), 4.11 (s, 2H), 4.05 (s, 2H), 1.33 (t, J = 7.5 Hz, 3H) ppm.

**Ethyl 2-(1,1'-biphenyl-3,3'-diisopropyl-4'-ol-4-oxy) acetate (6b)**

Same procedure as 6e. Product was isolated in 49% yield as a white solid (m.p. 111-113 °C). $^1$H NMR (500 MHz, CDCl$_3$): δ = 7.40 (d, J = 2 Hz, 1H), 7.36 (d, J = 2.5 Hz, 1H), 7.29 (dd, J = 8.0, 2.5 Hz, 1H), 7.25 (dd, J = 7.8, 2.3 Hz, 1H), 6.80 (d, J = 6.5 Hz, 1H), 6.77 (d, J = 7.0 Hz, 1H), 4.68 (s, 2H), 4.29 (q, J = 7.0 Hz, 2H), 3.46 (7H, 1H), 1.32 (d, J = 6.5 Hz, 6H), 1.32 (t, J = 7.0 Hz, 3H), 1.30 (d, J = 8 Hz, 6H) ppm.

**Ethyl 2-(1,1'-biphenyl-3,3'-disec-butyl-4'-ol-4-oxy) acetate (6c)**

Same procedure as 6e. Product was isolated in 27% yield as a white solid (m.p. 119-121 °C). $^1$H NMR (500 MHz, CDCl$_3$-d): δ 7.36 (d, J = 2 Hz, 1H), 7.32 (d, J = 2 Hz, 1H), 7.30 (dd, J = 8.5 Hz, 2.5 Hz, 1H), 6.82 (d, J = 7.5 Hz, 1H), 6.78 (d, J = 8 Hz, 1H), 4.73 (s, 1H), 4.67 (s, 2H), 4.29 (q, J = 7 Hz, 2H), 3.23 (m, 1H), 3.01 (m, 1H), 1.73 (m, 2H), 1.65 (m, 2H), 1.31 (m, 9H), 0.93 (t, J = 7.5 Hz, 3H), 0.91 (t, J = 7.5 Hz, 3H) ppm.

**Ethyl 2-(1,1'-biphenyl-3,3'-ditert-butyl-4'-ol-4-oxy) acetate (6d)**

Same procedure as 6e. Product was isolated in 32% yield as a white solid (m.p. 174-177 °C). $^1$H NMR (500 MHz, CDCl$_3$-d): δ 7.48 (d, J = 2.5 Hz, 1H), 7.44 (d, J = 2.5 Hz, 1H), 7.32 (dd, J = 2 Hz, 8.5 Hz, 1H), 7.25 (dd, J = 2 Hz, 8.5 Hz, 1H), 6.78 (d, J = 8 Hz, 1H), 6.73 (d, J = 7.5, 1H), 4.68 (s, 2H), 4.30 (q, J = 7 Hz, 2H), 1.48 (s, 9H), 1.46 (s, 9H), 1.33 (t, J = 7.5 Hz, 3H) ppm.

**Representative procedure for the synthesis of the dimethylaminoethoxy ethyl ester**

**Preparation of Ethyl 2-(1,1'-biphenyl-3,3'-dibenzyl-4'-[2-(dimethylamino) ethoxy]-4- oxy) acetate (7e)**
A mixture containing 6e (81 mg, 0.18 mmol), 2-(dimethylamino)ethyl chloride hydrochloride (83 mg, 0.57 mmol), and potassium carbonate (132 mg, 0.95 mmol) in acetone (15 mL) was heated at reflux overnight. The reaction was monitored by thin layer chromatography using C18 plates. The reaction mixture was concentrated under reduced pressure. The resultant solid was then partitioned between ethyl acetate and water. The organic layer was washed with brine, dried over magnesium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by flash column chromatography on C18 gel (Hexane/EtOAc) to yield 55 mg (60%) of the desired product as a colorless oil. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ = 7.31-7.16 (m, 14H), 6.87 (d, $J$ = 9 Hz, 1H), 6.76 (d, $J$ = 8.5 Hz, 1H), 4.61 (s, 2H), 4.26 (q, $J$ = 7.5 Hz, 2H), 4.10 (t, $J$ = 5.8 Hz, 2H), 4.08 (s, 2H), 4.00 (s, 2H), 2.76 (t, $J$ = 5.5 Hz, 2H), 2.34 (s, 6H), 1.30 (t, $J$ = 7.3 Hz, 3H) ppm; $^{13}$C NMR (75.5 MHz, CDCl$_3$): $\delta$ = 169.2, 155.9, 155.1, 141.2, 141.0, 134.6, 133.6, 130.8, 130.1, 129.6, 129.4, 129.3, 129.2, 129.0, 128.8, 128.7, 128.3, 126.3, 126.0, 125.8, 125.6, 112.2, 112.1, 111.9, 111.7, 66.5, 66.1, 61.5, 58.3, 46.0, 45.7, 36.9, 36.4, 14.1 ppm.

**Ethyl 2-(1,1'-biphenyl-3,3'-diisopropyl-4'-[2-(dimethylamino) ethoxy]-4-oxy) acetate (7b)**

Same procedure as 7e. Product was isolated in 51% yield as a colorless oil. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ = 7.40 (d, $J$ = 2.5 Hz, 1H), 7.37 (d, $J$ = 3.0 Hz, 1H), 7.30 (d, $J$ = 8.5, 2.5 Hz, 1H), 7.29 (dd, $J$ = 8.5, 2.5 Hz, 1H), 6.89 (d, $J$ = 8.5 Hz, 1H), 6.76 (d, $J$ = 8.5 Hz, 1H), 4.66 (s, 2H), 4.27 (q, $J$ = 8.5 Hz, 2H), 4.13 (t, $J$ = 5.8 Hz, 2H), 3.43 (7let, $J$ = 7 Hz, 1H), 3.37 (7let, $J$ = 7 Hz, 1H), 2.81 (t, $J$ = 5.8 Hz, 2H), 2.38 (s, 6H), 1.30 (t, $J$ = 7.5 Hz, 3H), 1.29 (d, $J$ = 7 Hz, 6H), 1.26 (d, $J$ = 7 Hz, 6H) ppm; $^{13}$C NMR (75.5 MHz, CDCl$_3$): $\delta$ = 169.4, 155.4, 154.5, 137.9, 137.5, 135.3, 134.1, 125.5, 125.1, 125.0, 111.8, 111.8, 67.1, 66.1, 61.5, 58.7, 46.3, 27.4, 27.2, 23.0, 22.9, 14.4 ppm.

**Ethyl 2-(1,1'-biphenyl-3,3'-diseo-butyl-4'-[2-(dimethylamino) ethoxy]-4-oxy) acetate (7c)**
Same procedure as 7e. Product was isolated in 50% yield as a colorless oil. $^1$H NMR (500 MHz, CDCl$_3$-d): $\delta$ 7.35 (d, $J = 2$ Hz, 1H), 7.30 (m, 3H), 6.89 (d, $J = 8.5$ Hz, 1H), 6.76 (d, $J = 8$ Hz, 1H), 4.65 (s, 2H), 4.27 (q, $J = 7.5$ Hz, 2H), 4.11 (t, $J = 6$ Hz, 2H), 3.22 (m, 1H), 3.14 (m, 1H), 2.78 (t, $J = 6$ Hz, 2H), 2.73 (s, 6H), 1.71 (m, 2H), 1.59 (m, 2H), 1.27 (m, 9H), 0.89 (t, $J = 7.5$ Hz, 3H), 0.88 (t, $J = 7.5$ Hz, 3H) ppm; $^{13}$C NMR (75.5 MHz, CDCl$_3$-d): $\delta$ 169.42, 155.76, 154.80, 136.86, 136.53, 135.16, 133.96, 126.14, 125.74, 125.03, 124.95, 111.92, 111.85, 67.25, 66.13, 61.44, 58.68, 46.35, 34.28, 34.00, 30.18, 30.08, 20.67, 14.40, 12.54, 12.51 ppm.

**Ethyl 2-(1,1'-biphenyl-3,3'-ditert-butyl -4'-[2-(dimethylamino) ethoxy]-4-oxy) acetate (7d)**

Same procedure as 7e. Product was isolated in 54% yield as a colorless oil. $^1$H NMR (500 MHz, CDCl$_3$-d): $\delta$ 7.48 (s, 1H), 7.45 (s, 1H), 7.33 (d, 1H), 7.31 (d, 1H), 6.93 (d, $J = 8.5$ Hz, 1H), 6.77 (d, $J = 8$ Hz, 1H), 4.67 (s, 2H), 4.29 (q, $J = 7.5$ Hz, 2H), 4.15 (t, $J = 6.5$, 2H), 2.84 (t, $J = 6$ Hz, 2H), 2.37 (s, 6H), 1.47 (s, 9H), 1.43 (s, 9H), 1.32 (t, $J = 7$ Hz, 3H) ppm; $^{13}$C NMR (75.5 MHz, CDCl$_3$-d): $\delta$ 13.15, 28.75, 28.82, 33.92, 33.98, 45.11, 57.47, 60.24, 64.40, 65.62, 110.98, 111.45, 124.16, 124.27, 124.64, 124.95, 132.53, 133.59, 137.30, 137.64, 154.59, 155.78, 167.97 ppm.

*Representative procedure for the synthesis of the carboxylic acid and the hydrochloride salt.*

**Preparation of 2-(1,1'-Biphenyl-3,3'-dibenzyl-4'-[2-(dimethylamino) ethoxy]-4-oxy) acetic acid (1e)**

![Chemical structure](image)

1 N aqueous sodium hydroxide (0.10 mL, 0.10 mmol) was added to a solution of 7e (25 mg, 0.048 mmol) in methanol or ethanol (3 mL). The solution was heated to 40
ºC for 2-4 hours. The reaction was monitored by thin layer chromatography using C18 plates. Upon completion, the solution was concentrated under reduced pressure. The residue was acidified with 1 N hydrochloric acid (25 mL) and extracted with ethyl acetate. The organic layer was then washed with water and brine, dried with magnesium sulfate, filtered and concentrated under reduced pressure yielding the carboxylic acid (21 mg, 89%)

The carboxylic acid of 7e (21 mg, 0.042 mmol) was dissolved in ethyl acetate or dioxane (2 mL). To this solution was added 4 N hydrochloric acid in dioxane or ethyl acetate (3 mL). The reaction was allowed to stir at ambient temperature for 12-24 hours. The solvent was removed under reduced pressure. The resulting solid was rinsed with a minimal volume of ethyl acetate and hexanes, and the hydrochloride salt was collected by filtration (9 mg, 40%). ¹H NMR (500 MHz, CD₃OD): δ = 7.27 (s, 1H), 7.25 (s, 1H), 7.22-7.11 (m, 10H), 7.06 (s, 1H), 7.05 (s, 1H), 6.78 (d, J = 8.5 Hz, 1H), 6.74 (d, J = 9.0 Hz, 1H), 4.50 (s, 2H), 4.10 (t, J = 5.3 Hz, 2H), 4.04 (s, 2H), 3.94 (s, 2H), 3.34 (t, 2H), 2.72 (s, 6H) ppm

2-(1,1'-Biphenyl-3,3'-diisopropyl-4'-[2-(dimethylamino) ethoxy]-4-oxy) acetic acid (1b)

Same procedure as 1e. Hydrolysis resulted in 100% conversion. ¹H NMR (500 MHz, CD₃OD): δ = 7.37 (d, J = 1.5 Hz, 1H), 7.36 (d, J = 2 Hz, 1H), 7.22 (dd, J = 8.5, 2.5 Hz, 1H), 7.17 (dd, J = 8.5, 2.5 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 6.82 (d, J = 8.0 Hz, 1H), 4.57 (s, 2H), 4.33 (t, J = 4.5 Hz, 2H), 3.61 (t, J = 4.5 Hz, 2H), 3.49 (t, J = 6.8 Hz, 1H), 3.39 (t, J = 6.8 Hz, 1H), 3.01 (s, 6H), 1.27 (d, J = 7.0 Hz, 6H), 1.24 (d, J = 7.5 Hz, 6H) ppm. Further conversion to the hydrochloride salt proceeded in 59% yield.

2-(1,1'-Biphenyl-3,3'-disec-butyl-4'-[2-(dimethylamino) ethoxy]-4-oxy) acetic acid (1c)

Same procedure as 1e. Hydrolysis resulted in 72% isolated yield. ¹H NMR (500 MHz, CDCl₃-d): δ 7.34 (m, 4H), 7.07 (d, J = 8.5 Hz, 1H), 6.90 (d, J = 8 Hz, 1H), 4.70 (s, 2H), 4.41 (br s, 2H), 3.68 (br s, 2H), 3.24 (m, 2H), 3.06 (s, 6H), 1.70 (m, 2H), 1.28 (d, J = 4.5 Hz, 2H), 1.27 (d, J = 4 Hz, 2H), 0.90 (t, J = 7.5 Hz, 3H), 0.89 (t, J = 7.5 Hz, 3H) ppm. Further conversion to the hydrochloride salt proceeded in 40% yield.

2-(1,1'-biphenyl-3,3'-ditert-butyl-4'-[2-(dimethylamino) ethoxy]-4-oxy) acetic acid (1d)

Same procedure as 1e. Hydrolysis resulted in 89% isolated yield. ¹H NMR (500 MHz, CDCl₃-d): δ 7.37 (d, J = 1.5 Hz, 1H), 7.34 (d, J = 2.5 Hz, 1H), 7.26 (dd, J = 8.5 Hz, 2.5 Hz, 1H), 7.20 (dd, J = 8.5 Hz, 2 Hz, 1H), 6.98 (d, J = 8 Hz, 1H), 6.79 (d, J = 8.5 Hz, 1H), 4.57 (s, 2H), 4.33 (br t, J = 4.5 Hz, 2H), 3.58 (br t, J = 5 Hz, 2H), 2.95 (s, 6H), 1.36 (s, 9H), 1.35 (s, 9H) ppm. Further conversion to the hydrochloride salt proceeded in 79% yield.
Synthesis of compound 1a.

Preparation of Ethyl 2-(4-iodophenoxy)acetate (5a)

4-Iodophenol (1.1 g, 5 mmol) was dissolved in dry THF (45 mL). Sodium hydride, (60 % in oil, 0.28 g, 7 mmol) was added and the solution was stirred at room temperature for 30 minutes. Ethyl bromoacetate (1.2 g, 7 mmol) was then added slowly and the solution was stirred for 1.5 hours at room temperature. The reaction mixture was diluted with ethyl acetate and washed with water (x2). After drying over magnesium sulfate, the mixture was purified by column chromatography on silica gel (Hexane/EtOAc, 80:20) to afford 1.02 g (67%) of the desired product as a solid (m.p. 57-61 °C). $^1$H NMR (500 MHz, CDCl$_3$): δ = 7.57 (d, $J$ = 7 Hz, 2H), 6.69 (d, $J$ = 6.5 Hz, 2H), 4.59 (s, 2H), 4.27 (q, $J$ = 7 Hz, 2H), 1.30 (t, $J$ = 6.5 Hz, 3H) ppm.

Preparation of Ethyl 2-(1,1’-biphenyl-4’-ol-4-oxo) acetate (6a)

2-(4-Iodo-phenyl)-4-oxo) acetic acid (0.100g, 1equiv) and 4-hydroxyphenylboronic acid (0.0541g, 1.2 equiv) were combined with PdCl$_2$(PPh$_3$)$_2$ (0.0230g, 10 mol %), triphenyl phosphine (0.00858g, 10 mol %) and 2M sodium carbonate (0.0858g) in a sealed vial and dissolved in 4mL THF and 1mL H$_2$O. The reaction underwent microwave irradiation at 110°C for 20 minutes using a Biotage Initiator (TM) 2.0. The crude reaction mixture was concentrated and purified by flash chromatography on silica gel(Hexane/EtOAc 90:10) to afford 0.030g (34%) of the desire product as a white solid (m.p. 131-133 °C). $^1$H NMR (500 MHz, CDCl$_3$): δ = 7.45 (d, $J$ = 7 Hz, 2H), 7.41 (d, $J$ = 6.5 Hz, 2H), 6.95 (d, $J$ = 6.5 Hz, 2H), 6.87 (d, $J$ = 6.5 Hz, 2H), 4.97 (s, 1H), 4.66 (s, 2H), 4.29 (q, $J$ = 6.5 Hz, 2H), 1.31 (t, $J$ = 7.5 Hz, 3H) ppm.

Preparation of Ethyl 2-(1,1’-biphenyl-4’-[2-(dimethylamino) ethoxy]-4-oxo) acetate (7a)
Same procedure as 7e. Product was isolated in 74 % yield as a white solid. $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.47 (d, $J = 7.0$ Hz, 2H), 7.46 (d, $J = 6.5$ Hz, 2H), 6.97 (d, $J = 6.0$ Hz, 2H), 6.95 (d, $J = 6.0$ Hz, 2H), 4.65 (s, 2H), 4.29 (q, $J = 7.5$ Hz, 2H), 4.12 (t, $J = 6.0$ Hz, 2H), 2.79 (t, $J = 5.5$ Hz, 2H), 2.39 (s, 6H), 1.31 (t, $J = 7.5$ Hz, 3H) ppm. $^{13}$C NMR (75.5 MHz, CDCl$_3$): $\delta$ 207.2, 158.2, 157, 134.8, 134, 128.1, 128.0, 115.2, 115.1, 65.8, 61.7, 58.5, 46.0, 31.2, 14.3 ppm

Preparation of 2-(1,1'-Biphenyl-4'-[2-(dimethylamino) ethoxy]-4-oxy) acetic acid (1a)

1a was isolated as a sodium salt by stopping the procedure of 1e after the initial hydrolysis step and recovering the salt from ethyl acetate.
Appendix 2: Biological Methods and Data for Assays Presented in Chapter 2
Biological Data for 1st Generation Series of 3,3'-disubstituted Biphenyls:

Key to Compound Identity in Descriptions that Follow:

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
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<tr>
<td>7a</td>
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</tr>
<tr>
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<td>AW-III-82-56</td>
<td>tBu</td>
</tr>
<tr>
<td>7e</td>
<td>81</td>
<td>PW-I-81</td>
<td>Bn</td>
</tr>
</tbody>
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In Vitro TR-FRET CBI Assay (J.A.K.)

This assay was run in duplicate and repeated to confirm results. Compound 87 shows the most complete displacement of the SRC3 with the lowest IC50 of around 90 uM. The peptide control has an IC50 of ca 1 uM

This assay was performed as previously described. Briefly, an N-terminally Histagged ligand binding domain of ERα (residues 304-554; C381, 530S) was expressed, purified and labeled with biotin-maleimide (Quanta BioDesign) through the one remaining reactive cysteine (Cys417) while bound to a nickel column. The SRC3 nuclear receptor domain (NRD) (residues 627-829) includes all three NR-boxes and was nonspecifically labeled through four available cysteines using 5-iodoacetamido-fluorescein (Invitrogen).

The ER was subsequently tagged with a streptavidin-terbium complex (FRET donor, λem = 495 nm) and activated with a high concentration of agonist ligand estradiol to promote recruitment of fluorescein SRC3 (FRET acceptor, λem = 520 nm) and production of a high FRET signal. The biphenyl compounds were then serially diluted and added to each well of the assay mixture to test the ability of these compounds to disrupt the receptor/coactivator interaction and produce a subsequent decrease in FRET. The final concentrations of the reagents were as follows: ERα-417 (2 nM),
streptavidin–terbium (0.5 nM), estradiol (1 μM), test compound (0–1 mM), SRC3-NRD (50 nM).

A 15-mer SRC1-Box II peptide and a published guanylhydrazone coactivator binding inhibitor compound3 were used as positive controls in the TR-FRET and reporter gene assays, respectively. The compounds showed modest activity in this assay; compound 1c produced the most complete displacement of coactivator with a $K_i$ of 33μM in the TR-FRET assay.
This assay was also repeated to confirm results. Compound 87 gives a partial binding curve in each assay, but the results are not encouraging – potency is low, and inhibition is limited. See positive control, which is a guanyl hydrazone.

Summary of CBI activity

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<th>compound</th>
<th>TR-FRET IC$_{50}$ (µM)</th>
<th>TR-FRET Repeat (µM)</th>
<th>Reporter Gene IC$_{50}$ (µM)</th>
<th>Reporter Gene IC$_{50}$ (µM) -repeat</th>
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<td>87</td>
<td>227</td>
<td>1.8*</td>
<td>6*</td>
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*very small dynamic range compared to positive control peptide or guanylhydrazone CBI
**Radiometric Binding Assay (J.A.K.)**

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<th>ERβ RBA</th>
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<td>&lt;0.001</td>
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<tr>
<td>PW-I-81</td>
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<td>0.018</td>
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<td>0.049 ± 0.016</td>
<td>0.018 ± 0.001</td>
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<tr>
<td>AW-III-87-68</td>
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<td>~0.001</td>
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</tbody>
</table>

Performed as previously described. Relative binding affinities (RBA) were determined using 2 nM [3H]estradiol as tracer ([2,4,6,7-3H]estra-1,3,5,(10)-triene-3,17β-diol, 89 Ci/mmol, Amersham/GE Healthcare Bio-Sciences Corporation, Piscataway, NJ) and purified full-length human ERα and ERβ receptors (PanVera/Invitrogen, Carlsbad, CA). Incubations were for 18–24 h at 0 °C. Hydroxyapatite (Bio-Rad, Hercules, CA) was used to absorb the receptor–ligand complexes, and free ligand was washed away. The binding affinities are expressed as relative binding affinity (RBA) values with that of estradiol set to 100%. Estradiol binds to ERα with a $K_d$ of 0.2 nM and to ERβ with a $K_d$ of 0.5 nM. The most promising CBI, compound 1c, shows insignificant binding as a ligand and should not be considered a traditional antagonist.
MCF7 cells were pre-treated with 20 μM of each compound for 2 hours and then either vehicle or estradiol was added for 14h. RNA was harvested and quantitative-PCR was used to determine the effects of these compounds on the expression of ERα target genes.

SDF1, PR, and PS2 are known ERα target genes. IDH3A not an ERα target gene was used as a negative control to demonstrate the selectivity of these compounds.

At the concentration of 20 μM, #80, #81 and #87 showed some inhibition of the expression of SDF1 and PR, but the extent of inhibition is very subtle. We will try to repeat this again with higher concentrations of these compounds. #82 and #83-2 functioned as agonists with respect to ERα target genes, similar to what would be predicted from the mammalian two-hybrid assays described above. #82 further enhanced estradiol induction of PR and PS2.
Mammalian Two-Hybrid Assay (D.P.M.)

This assay assesses whether the test compounds interrupt the interaction between ERα and an LXXLL peptide derived from an ER coactivator (GRIP1).

**Compound #80:** didn’t have much effect, only a very slight inhibition at 100 μM.

**Compounds #81:** efficiently inhibited the estradiol (E2)-induced ERα/LXXLL interaction, although it is toxic at higher concentration (it’s OK up to ~50 μM)

**Compound #82:** also inhibited estradiol (E2)-induced ERα-LXXLL interaction in a dose-dependent manner. Interestingly, however, this compound by itself enhanced the interaction between ERα and LXXLL in the absence of estradiol, functioning almost as an ERα agonist in this setting. This is an interesting compound, we need to test it in other assays in order to determine if the effects seen are ERα-dependent and selective. Based on the inhibition curve, this compound has good affinity.

**Compound #83-2:** this compound by itself enhanced the interaction between ERα and LXXLL in the absence of estradiol, functioning almost as an ERα agonist. Unlike compound #82, this compound did not inhibit ERα/LXXLL interaction at the concentrations tested.

**Compound #87:** inhibited ERα/LXXLL interaction only at higher concentrations.

**OH-tam** is an ERα antagonists, was used as a positive control.

**TOXIC TO CELLS**
This is a very preliminary test of the 2nd batch of compounds just to determine the proper dose for use in cell culture: all compounds were used between 5-40 uM. This is again a mammalian two hybrid assay looking at the interaction between ERα and the LXXLL2 peptide from the coactivator GRIP1.

AW 77: this compound crashed out of solution, can’t determine the effect.
PW74: looks promising, inhibit at ~40 uM, no significant sign of toxicity at this dose.
AW78: also looks good from this assay.
AW84, AW73, PW82 and PW78 and maybe PW76 and AW79 all induced LXXLL-ER interaction. They maybe binding inside of the pocket. AW79 is toxic at high dose.
AW77: very toxic to cells at the concentrations tested, will test at much lower dose in the next round.
#82 and OH-tam were included as controls.
Androgen Receptor Assays (D.P.M.)

CV-1, MMtv-Luc
EtOH

CV-1 MMtv-Luc
+ 1 nM R1881

Assay:
Software:
Sample Map:
Detection Mode:
Plate Type:
WellRead Time (secs):
WellRepeats:
ExFilter1:
ExFilter2:
EmFilter1:
Ligh Source:
Gain:
G Filer Source:
Plate Size:
Plate Size:
Comment:
RLU Avg:

Lumi
Fusion 3.56
Default Map
Luminescence
Packard OptiPlate 96

Description:
Instrument Serial:
Description:
Shaking:
Temperature Control:
Plate Pre-Count Delay
2 (secs):
Plate Repeats:
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Gate/Delay2 (us/secs):
EmFilter2:
Number of Flashs:
1 PMT Voltage:
G Factor:
96 Lamp Intensity:
chk, pkg

Acquired:
May 02
2003 1:12 PM  Temperature Min/Max: 0.0/9.0°C

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Appendix 3: Supplemental Synthetic Methods and Characterization for Chapter 3
**Experimental**

Unless otherwise stated, all reactions were performed under argon atmosphere, in oven-dried glassware, using solvents which were either distilled or purchased as anhydrous and which were transferred by syringe. $^1$H (400 or 500 MHz) and $^{13}$C NMR (100 or 125 MHz) spectra were recorded in CDCl$_3$, d$_6$-acetone, or d$_4$-methanol. Chemical shifts are reported in ppm (δ units) downfield from TMS (δ 0.00 ppm). Analytical thin-layer chromatography (TLC) was performed by using polymer-backed silica gel plates coated with a 0.25 mm thickness of silica gel, and visualization was achieved by UV light, phosphomolybdic acid staining or exposure in an iodine chamber. Flash column chromatography was performed on silica gel of 40-63 μm particle size.

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 or negative] mode. Mass correction was done by an external reference using a Waters Lockspray accessory. Mobile phases were water and acetonitrile with 0.1% formic acid. The MS settings were: capillary voltage = 3kV, cone voltage = 35, source temperature = 120 °C and dissolvation temperature = 350°C [cone voltage = 10 and dissolvation temperature = 120 °C if experiment was carried out in low T and low V]. Also, we are grateful to the National Science Foundation for the purchase of the Waters high resolution mass spectrometer (CHE 0443618) used in this work. Elemental analysis was performed on a Model CE 440 CHN Analyzer.
Please refer to the following schemes for compound numbering for the methods and characterization that follows:
(3-methoxyphenyl)(phenyl)methanol (3). A two-neck flask equipped with a reflux condenser was loaded with magnesium turnings (78 mmol, 1.895g) and oven-dried overnight. The flask was subsequently flame-dried under Argon prior to the addition of tetrahydrofuran (50 mL) and iodine (1 chip). The temperature was cooled to 0°C and 3-bromoanisole (60mmol, 7.634mL) was slowly added via syringe. The solution was stirred for two hours while gradually warming to room temperature before heating at 60°C for four hours. The solution was then returned to room temperature and benaldehyde (12 mmol, 1.218 mL) was added by syringe and the reaction was stirred at room temperature overnight. A saturated solution of aqueous ammonium chloride (150 mL) was added to quench the magnesium and the solution was stirred at room temperature for two hours. The aqueous phase was extracted with ethyl acetate and the combined organic layers were washed with brine, dried with magnesium sulfate, and concentrated. Flash chromatography on silica gel (5% ethyl acetate/hexanes) yielded 1.02g of (3-methoxyphenyl)(phenyl)methanol (75%) as a yellow oil; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.36 (m, 4H), 7.26 (m, 2H), 6.96 (s, 1H), 6.95 (d, \(J = 6.8 \) Hz, 1H), 6.81 (dd, \(J = 7.2 \) Hz, 2.0 Hz, 1H), 5.82 (s, 1H), 3.79 (s, 3H), 2.24, (s, 1H) ppm.

1-benzyl-3-methoxybenzene (4). A round-bottom flask was loaded with compound 3 (3.65 mmol, 0.783g) and 10% palladium on carbon and flushed with argon. A 5:1 solution of ethanol (150 mL) and ethyl acetate (30 mL) was added. The reaction vessel was then flushed with hydrogen for ten minutes at room temperature. A hydrogen balloon was attached and the reaction progressed at room temperature for five hours. The crude reaction mixture was then filtered through celite (prepared with ethyl acetate) and concentrated. Flash chromatography (silica gel, 5% ethyl acetate/hexanes) yielded 0.669g of 1-benzyl-3-methoxybenzene (92%) as a colorless oil; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.27 (t, \(J = 7.2 \) Hz, 2H), 7.19 (t, \(J = 8.0 \) Hz, 4 H), 6.78 (d, \(J = 8.4 \) Hz, 1H), 6.74 (d, \(J = 6 \) Hz, 1H), 6.73 (s, 1H), 3.95 (s, 2H), 3.75 (s, 3H) ppm.

2-benzyl-1-bromo-4-methoxybenzene (5). Compound 4 (9.150 mmol, 1.814g) and N-Bromosuccinimide (3.33 mmol, 0.660g) were dissolved in acetonitrile (20 mL) and stirred at room temperature in daylight for one hour. The crude reaction was concentrated, re-dissolved in hexanes, and filtered to remove the succinimide bi-products (white solid). The filtrate was concentrated and further purified by flash chromatography (silica gel, 100% hexanes) to yield 0.867g 2-benzyl-1-bromo-4-methoxybenzene (94%) as a colorless oil; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.46 (d, \(J = 8.0 \) Hz, 1H, 7.301 (t, \(J = 7.6 \) Hz, 2H), 7.22 (t, \(J = 7.6 \) Hz, 1H), 7.20 (d, \(J = 7.2 \) Hz, 2H), 6.69 (d, \(J = 3.6 \) Hz, 1H), 6.66 (dd, \(J = 8.8 \) Hz, 3.6 Hz, 1H), 4.08 (s, 2H), 3.73 (s, 3H) ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 159.18, 141.58, 139.55, 133.55, 129.21, 128.73, 126.53, 117.21, 117.15, 115.56, 113.56, 55.64, 55.57, 42.12 ppm. Elemental analysis calculated (%) for C\(_{14}\)H\(_{13}\)BrO (276.0150): C 60.67, H 4.73; found: C 60.64, H 4.78.

3-benzyl-4-bromophenol (2e). To a solution of compound 5 (2.67mmol, 0.739g) in dichloromethane (30 mL) was added a 1M solution of boron tribromide in dichloromethane (8.00 mmol, 8.0 mL). The reaction was stirred at room temperature overnight. The reaction was quenched with water (40 mL) and extracted with ethyl acetate. The combined organic layers were washed with brine, dried with magnesium sulfate, and concentrated. Purification was accomplished on silica gel (5% ethyl acetate/hexanes) to yield 0.665g 3-benzyl-4-bromophenol (95%) as a yellow oil; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.39 (d, \(J = 8.4 \) Hz, 1H), 7.301 (t, \(J = 7.6 \) Hz, 2H), 7.23 (t, \(J =8.8 \) Hz, 1H), 7.19 (d, \(J = 8.8 \) Hz, 2H), 6.57 (dd, \(J\) =8.4 Hz, 2.8 Hz, 1H), 6.56
(d, J = 2.4 Hz, 1H), 4.03 (s, 2H), ppm; \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): δ 155.08, 141.98, 139.42, 133.80, 129.36, 128.81, 126.65, 118.18, 118.12, 115.55, 115.39, 41.95 ppm. Elemental analysis calculated (%) for C\(_{13}\)H\(_{11}\)BrO (261.9993): C 59.34, H 4.21; found: C 59.42, H 4.20.

**General Procedure for the Preparation of 2-substituted-4-bromophenol (2b, 2d).**
Tetrabutylammonium tribromide (9.39 mmol, 4.527g) was added to a solution of ortho-cresol (7.82 mmol, 0.820g) in chloroform (55 mL) and the solution was stirred at room temperature for 3 hours. The solvent was removed by rotary evaporation and the crude product was partitioned between ether (100 mL) and 1N hydrochloric acid (aq, 70 mL). The organic layer was washed sequentially with 1 N HCl and brine, dried over magnesium sulfate, and concentrated. The crude product was purified by chromatography on silica gel (10% ethyl acetate in hexanes) to yield product 2b (1.268 g, 87% yield) as a white solid (m.p. 58-61°C).

4-bromo-2-methylphenol (2b). White solid (m.p. 58-61°C); \(^1\)H NMR (500 MHz, CDCl\(_3\)): δ 7.24 (d, J = 2.0 Hz, 1H), 7.17 (dd, J = 8.5 Hz, 2.5 Hz, 1H), 6.65 (d, J = 8.0 Hz, 1H), 4.66 (s, 1H), 2.21 (s, 3H) ppm.

2-benzyl-4-bromophenol (2d). Pale yellow oil; \(^1\)H NMR (400 MHz, CDCl\(_3\)): δ 7.31 (t, J = 7.2 Hz, 2H), 7.23 (m, 5H), 6.67 (dd, J = 7.2 Hz, 1.2 Hz, 1H), 4.77 (s, 1H), 3.95 (s, 2H) ppm.

**General Procedure for the Preparation of the Aryl Boronic Esters (6a-e).**
Compound 2b (8.02 mmol, 1.5g), bis(pinacolato)diboron (8.82mmol, 2.240g), potassium acetate (24.06 mmol, 2.361g), and PdCl\(_2\)dpdpf (0.401 mmol, 0.328g) were dissolved in dioxane and heated to reflux (80°C) overnight. After cooling to room temperature, activated carbon was added and the resultant mixture was filtered through celite (prepared with ethyl acetate). The filtrate was concentrated and the concentrate was redissolved in ethyl acetate and washed with brine. The combined organic layers were dried with magnesium sulfate, concentrated and purified by flash chromatography (silica gel, 5% ethyl acetate/hexanes) to yield the desired product, 6b (1.2512g, 67% yield) as a white solid (m.p. 100-103°C).

4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (6a). Solid. mp = 114-116°C; \(^1\)H NMR (300 MHz, CDCl\(_3\)): δ 7.72 (d, J = 8.4 Hz, 2H), 6.82 (d, J = 8.7 Hz, 2H), 4.93 (br s, 1H), 1.33 (s, 12H) ppm.

2-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (6b). Solid. mp = 100-103°C; \(^1\)H NMR (300 MHz, CDCl\(_3\)): δ 7.59 (s, 1H), 7.55 (d, J = 7.5 Hz, 1H), 6.76, J = 8.1 Hz, 1H), 4.81 (s, 1H), 2.24 (s, 3H), 1.32 (s, 12H) ppm.

3-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (6c). Solid, mp = 90-93°C; \(^1\)H NMR (500 MHz, CDCl\(_3\)): δ 7.66 (d, J = 8.0 Hz, 1H), 6.62 (s, 1H), 6.62 (dd, J = 9 Hz, 2 Hz, 1H), 1.32 (s, 12H) ppm.

2-benzyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (6d). Solid, mp = 112-115°C; \(^1\)H NMR (400 MHz, CDCl\(_3\)): δ 7.67 (s, 1H), 7.61 (dd, J = 7.2 Hz, 1.6 Hz, 1H), 6.78 (d, J = 8.0 Hz, 1H), 5.43 (s, 1H), 1.34 (s, 12H) ppm.
3-benzyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (6e). Yellow oil; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.73 (d, \(J = 8.8\) Hz, 1H), 7.23 (d, \(J = 7.2\) Hz, 2H), 7.17 (t, \(J = 6.8\) Hz, 2H), 7.15 (t, \(J = 7.2\) Hz, 1H), 6.66 (dd, \(J = 8.4\) Hz, 2.4 Hz, 1H), 6.57 (d, 2.8 Hz, 1H), 4.83 (s, 1H), 4.28 (s, 2H), 1.27 (s, 12H) ppm. \(^1\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 158.56, 150.37, 142.38, 139.50, 129.31, 128.43, 125.89, 117.30, 117.25, 112.88, 83.68, 61.12, 41.08, 25.00, 21.36, 14.40 ppm. HRMS calcd for C\(_{19}\)H\(_{23}\)BO\(_3\), [M+Na] 332.1674, found 332.1685.

**General Procedure for the Preparation of the Ethyl (Phenoxy) Acetates (7a-e).**
To a solution of 4-bromo-3-methylphenol (5.349 mmol, 1.000 g) in anhydrous tetrahydrofuran (40 mL) was added sodium hydride (60% w/w) (8.024 mmol, 0.321 g). The reaction was stirred for 30 minutes prior to the slow addition of ethyl bromoacetate (8.024 mmol, 0.890 mL). The reaction (milky-white suspension) was stirred overnight at room temperature. Ethanol was added to quench the sodium hydride (color change to translucent yellow), and the solvent was evaporated. The crude product was partitioned in ethyl acetate and water and the organic layer was washed with brine, dried with magnesium sulfate, and concentrated. The product, 7c (1.424 g, 97% yield) was isolated as a solid (m.p. 36-38°C) and taken forward without further purification.

**ethyl 2-(4-bromophenoxy)acetate (7a).** Solid, mp = 55-57°C; \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 7.39 (d, \(J = 9.0\) Hz, 2H), 6.80 (d, \(J = 9.0\) Hz, 2H), 4.59 (s, 2H), 4.27 (q, \(J = 7.2\) Hz, 2H), 1.30 (t, \(J = 7.2\) Hz, 3H) ppm.

**ethyl 2-(4-bromo-2-methylphenoxy)acetate (7b).** Yellow oil; \(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 7.27 (s, 1H), 7.21 (dd, \(J = 8.7\) Hz, 2.4 Hz, 1H), 6.56 (d, \(J = 8.7\) Hz, 1H), 4.60 (s, 2H), 4.25 (q, \(J = 7.2\) Hz, 2H), 2.26 (s, 3H), 1.29 (t, \(J = 6.6\) Hz, 3H) ppm.

**ethyl 2-(4-bromo-3-methylphenoxy)acetate (7c).** Solid, mp = 36-38°C; \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 7.40 (d, \(J = 8.5\) Hz, 1H), 6.81 (d, \(J = 2.5\) Hz, 1H), 6.61 (dd, \(J = 9.7\) Hz, 3.5 Hz, 1H), 4.58 (s, 2H), 4.27 (q, \(J = 7.5\) Hz, 2H), 2.36 (s, 3H), 1.30 (t, \(J = 7.0\) Hz, 3H) ppm.

**ethyl 2-(2-benzyl-4-bromophenoxy)acetate (7d).** Solid, mp = 69-71°C; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.25 (m, 7H), 6.62 (d, \(J = 11.0\) Hz, 1H), 4.59 (s, 2H), 4.27 (q, \(J = 7.2\) Hz, 2H), 1.30 (t, \(J = 8.5\) Hz, 3H) ppm.

**ethyl 2-(3-benzyl-4-bromophenoxy)acetate (7e).** Solid, mp = 40-43°C; \(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 7.46 (d, \(J = 8.8\) Hz, 1H), 7.30 (t, \(J = 7.6\) Hz, 2H), 7.25 (m, 2H), 7.18 (d, \(J = 6.4\) Hz, 2H), 6.69 (d, \(J = 2.8\) Hz, 1H), 6.65 (dd, \(J = 8.8\) Hz, 2.8 Hz, 1H), 4.55 (s, 2H), 4.23 (q, \(J = 6.4\) Hz, 2H), 4.06 (s, 2H), 1.27 (t, \(J = 7.2\) Hz, 3H) ppm; \(^1\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 168.80, 157.40, 141.93, 139.30, 133.66, 129.29, 128.78, 126.64, 117.86, 116.71, 114.19, 65.66, 61.72, 42.10, 14.39 ppm. HRMS calcd for C\(_{17}\)H\(_{17}\)BrO\(_3\), [M+Na] 371.0259, found 371.0259.

**General Procedure for the Preparation of the Biphenyl Phenolic Esters (8a-j).**
To a screw-cap reaction vial was added compound 7g (0.193 mmol, 0.050 g), sodium carbonate (0.3860 mmol, 0.0409 g), PdCl\(_2\)(P(o-tol)\(_3\))\(_2\) (0.00965 mmol, 0.0076 g), and tri(o-tolyl)phosphine (0.00965 mmol, 0.0029 g). After flushing the vial with argon, a 3:2 mixture of dichloromethane (1.0 mL) and water (0.67 mL) was added and the vial was capped and heated to 115°C for 1
hour. The reaction mixture was cooled to room temperature and 3-benzyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenol (0.386 mmol, 0.1197g) was added as a solution in an additional 1.0 mL dichloromethane and 0.67 mL water. The vial was again capped and returned to 115°C overnight. The crude reaction mixture was taken up in ethyl acetate and washed with brine. The combined organic layers were dried with magnesium sulfate, concentrated, and purified by flash chromatography on silica gel (10% ethyl acetate/hexanes) to yield product 8g (0.067g, 95.8% yield) as a solid (m.p. 70-72°C).

**ethyl 2-((4'-hydroxy-2-methyl-[1,1'-biphenyl]-4-yl)oxy)acetate (8a).** Solid, mp = 100-102°C; ¹H NMR (500 MHz, CDCl₃): δ 7.12 (d, J = 8.0 Hz, 2H), 7.11 (d, J = 8.5 Hz, 1H), 6.84 (d, J = 9.0 Hz, 2H), 6.82 (d, J = 3.0 Hz, 1H), 6.75 (dd, J = 8.5 Hz, 3.0 Hz, 1H), 5.36 (s, 1H), 4.65 (s, 2H), 4.30 (q, J = 7.0 Hz, 2H), 2.22 (s, 3H), 1.32 (t, J = 7.0 Hz, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 169.67, 156.90, 154.74, 137.31, 135.55, 134.00, 131.15, 130.76, 116.77, 115.17, 111.81, 65.63, 61.79, 21.02, 14.42 ppm. HRMS calcd for C₁₇H₁₈O₄, [M+Na] 309.1103, found 309.1097.

**ethyl 2-((4'-hydroxy-2'-methyl-[1,1'-biphenyl]-4-yl)oxy)acetate (8b).** Solid, mp = 94-97°C; ¹H NMR (500 MHz, CDCl₃): δ 7.20 (d, J = 8.5 Hz, 2H), 7.04 (d, J = 8.5 Hz, 1H), 6.93 (d, J = 9.0 Hz, 2H), 6.74 (d, J = 3.0 Hz, 1H), 6.69 (dd, J = 8.0 Hz, 2.5 Hz, 1H), 5.40 (s, 1H), 4.66 (s, 2H), 4.30 (q, J = 7.5 Hz, 2H), 2.20 (s, 3H), 1.32 (t, J = 7.0 Hz, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 169.59, 156.75, 154.92, 137.28, 135.39, 134.18, 131.28, 130.75, 117.17, 114.47, 112.91, 65.75, 61.79, 20.85, 14.41 ppm. HRMS calcd for C₁₇H₁₈O₄, [M+Na] 309.1103, found 309.1114.

**ethyl 2-((4'-hydroxy-2,3'-dimethyl-[1,1'-biphenyl]-4-yl)oxy)acetate (8c).** Solid, mp = 89-91°C; ¹H NMR (400 MHz, CDCl₃): δ 7.11 (d, 8.8 Hz, 1H), 7.04 (s, 1H), 6.99 (d, J = 8.0 Hz, 1H), 6.82 (d, J = 2.0 Hz, 1H), 6.79 (d, J = 8.0 Hz, 1H), 6.75 (dd, J = 8.0 Hz, 2.0 Hz, 2H), 5.40 (s, 1H), 4.66 (s, 2H), 4.30 (q, J = 6.4 Hz, 2H), 2.28 (s, 3H), 2.24 (s, 3H), 1.32 (t, J = 7.2 Hz, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 169.16, 156.67, 152.61, 137.02, 135.39, 133.95, 131.97, 130.89, 127.99, 123.25, 116.50, 114.48, 111.55, 65.46, 61.39, 20.83, 15.82, 14.20 ppm. HRMS calcd for C₁₈H₂₀O₄, [M+Na] 323.1259, found 323.1260.

**ethyl 2-((4'-hydroxy-2,2'-dimethyl-[1,1'-biphenyl]-4-yl)oxy)acetate (8d).** Solid, mp = 107-109°C; ¹H NMR (500 MHz, CDCl₃): δ 6.99 (d, J = 8.0 Hz, 1H), 6.82 (d, J = 7.5 Hz, 2.0 Hz, 2H), 6.72 (d, J = 8.0 Hz, 1H), 6.72 (d, J = 8.0 Hz, 1H), 6.67 (dd, J = 8.0 Hz, 2.5 Hz, 1H), 5.06 (br s, 1H), 4.68 (s, 2H), 4.29 (q, J = 7.0 Hz, 2H), 2.32 (s, 3H), 2.21 (s, 3H), 1.31 (t, J = 7.0 Hz) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 169.66, 155.13, 154.74, 137.28, 134.99, 134.48, 132.38, 131.27, 127.76, 127.04, 117.07, 112.79, 110.99, 66.03, 61.61, 20.88, 16.54, 14.41 ppm. HRMS calcd for C₁₈H₂₀O₄, [M+H] 301.1440, found 301.1449.
ethyl 2-((2-benzyl-4'-hydroxy-[1,1'-biphenyl]-4-yl)oxy)acetate (8f). Solid, mp = 109-113°C; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.21 (t, $J = 6.4$ Hz, 2H), 7.14 (t, $J = 8.0$ Hz, 2H), 7.07 (d, $J = 8.0$ Hz, 2H), 6.98 (d, $J = 7.2$ Hz, 2H), 6.80 (d, $J = 8.8$ Hz, 2H), 6.78 (dd, $J = 6.4$ Hz, 3.0Hz, 1H), 6.73 (d, $J = 2.5$ Hz, 1H), 5.12 (s, 1H), 4.59 (d, 2H), 4.24 (q, $J = 6.4$ Hz, 2H), 3.90 (s, 2H), 1.28 (t, $J = 7.2$ Hz, 3H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 169.61, 156.93, 155.06, 141.36, 140.21, 135.06, 134.53, 131.68, 130.90, 129.14, 128.54, 126.11, 117.01, 114.48, 113.44, 65.75, 61.73, 39.34, 14.42 ppm. HRMS calcd for C$_{30}$H$_{28}$O$_4$, [M+Na] 475.1885, found 475.1884.

ethyl 2-((2'-benzyl-4'-hydroxy-[1,1'-biphenyl]-4-yl)oxy)acetate (8g). Solid, mp = 70-72°C; $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 7.22 (t, $J = 8.0$ Hz, 2H), 7.16 (t, $J = 4.4$ Hz, 2H), 7.14 (d, $J = 8.8$ Hz, 1H), 7.99 (d, $J = 9.0$Hz, 2H), 6.88 (d, $J = 6.4$ Hz, 2H), 6.72 (dd, $J = 8.0$ Hz, 2.8 Hz, 1H), 6.63 (d, $J = 2.4$ Hz, 1H), 4.98 (s, 1H), 4.67 (q, $J = 7.2$ Hz, 2H), 3.88 (s, 2H), 1.31 (t, $J = 6.4$ Hz, 3H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 169.42, 156.93, 155.06, 141.36, 135.06, 134.53, 131.06, 131.02, 116.65, 115.20, 65.69, 61.82, 39.47, 25.04, 14.38 ppm. HRMS calcd for C$_{23}$H$_{22}$O$_4$, [M+Na] 385.1416, found 385.1429.

ethyl 2-((2',3'-dibenzy1-4'-hydroxy-[1,1'-biphenyl]-4-yl)oxy)acetate (8h). Solid, mp = 104-105°C; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.26 (t, $J = 7.5$ Hz, 2H), 7.19 (t, $J = 7.5$ Hz, 4H), 7.15 (t, $J = 7.5$ Hz, 2H), 6.97 (dd, $J = 8.0$ Hz, 2.0 Hz, 1H), 6.95 (d, $J = 6.5$ Hz, 2H), 6.77 (dd, $J = 8.0$ Hz, 2.5 Hz, 1H), 6.75 (d, $J = 8.0$ Hz, 1H), 6.73 (d, $J = 2.5$ Hz, 1H), 4.86 (s, 1H), 4.58 (s, 2H), 4.24 (q, $J = 7.0$ Hz, 2H), 3.94 (s, 2H), 3.89 (s, 2H), 1.27 (t, $J = 7.0$ Hz, 3H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 169.41, 157.09, 152.97, 141.35, 140.16, 140.14, 135.88, 133.94, 132.31, 131.59, 129.06, 128.96, 128.53, 126.87, 126.55, 126.09, 116.76, 115.58, 112.34, 65.64, 61.72, 39.56, 36.57, 14.41 ppm. HRMS calcd for C$_{30}$H$_{28}$O$_4$, [M+Na] 475.1885, found 475.1892.

ethyl 2-((2',3'-dibenzyl-4'-hydroxy-[1,1'-biphenyl]-4-yl)oxy)acetate (8i). Solid, mp = 140-143°C; $^1$H NMR (500 MHz, d$_6$-acetone): $\delta$ 7.23 (m, 9H), 7.94 (d, $J = 8.5$ Hz, 1H), 7.05 (dd, $J = 8.0$ Hz, 2.0 Hz), 7.03 (d, $J = 2.0$ Hz, 1H), 6.99 (d, $J = 8.0$ Hz, 2H), 6.75 (d, $J = 8.5$ Hz, 1H), 6.71 (dd, $J = 8.5$ Hz, 3.0 Hz, 1H), 6.65 (d, $J = 2.5$ Hz, 1H), 5.22 (br s, 1H), 4.66 (s, 2H), 4.32 (q, $J = 7.0$ Hz, 2H), 4.05 (s, 2H), 3.88 (s, 2H), 1.33 (t, $J = 6.5$ Hz, 3H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 169.49, 154.99, 154.80, 141.40, 141.04, 140.14, 134.87, 134.65, 132.27, 131.66, 130.21, 129.28, 129.11, 128.52, 128.50, 126.08, 126.06, 117.01, 113.38, 111.38, 66.06, 61.63, 39.34, 36.20, 14.42 ppm. HRMS calcd for C$_{30}$H$_{28}$O$_4$, [M+Na] 475.1885, found 475.1884.

ethyl 2-((2',2'-dibenzyl-4'-hydroxy-[1,1'-biphenyl]-4-yl)oxy)acetate (8j). Oil; $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.20 (m, 4H), 7.15 (m, 2H), 6.99 (dd, $J = 7.0$ Hz, 1.5 Hz, 1H), 6.93 (d, $J = 8.5$ Hz), 6.88 (d, $J = 7.0$ Hz, 4H), 6.73 (dd, $J = 8.0$ Hz, 3.0 Hz, 1H), 6.72 (s, 1H), 6.67 (dd, $J = 7.5$ Hz, 2.0 Hz, 1H), 6.59 (d, $J = 2.5$ Hz, 1H), 4.73 (s, 1H), 4.58 (s, 2H), 4.25 (q, $J = 7.5$ Hz, 2H), 3.59 (d, $J = 15$ Hz, 1H), 3.56 (d, $J = 15$ Hz, 1H), 3.52 (d, $J = 15$ Hz, 1H), 3.46 (d, $J = 15.5$ Hz, 1H), 1.28 (t, $J = 7.0$ Hz, 3H) ppm; $^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 169.03, 156.98, 154.75, 141.22, 140.41, 140.36, 134.12, 132.95, 131.52, 131.48, 129.11, 129.04, 128.17, 125.87, 116.32, 116.16, 112.83, 111.68, 65.40, 61.39, 39.39, 39.25, 14.14 ppm. HRMS calcd for C$_{30}$H$_{28}$O$_4$, [M+Na] 475.1885, found 475.1884.
General Procedure for the Preparation of the Biphenyl Amino Methyl-Esters (1a-j).

Compound 8h, (0.0560 mmol, 0.0203g) was combined with cesium carbonate (0.280 mmol, 0.0913g), dissolved in acetone (2 mL), and heated to 80°C for 1-2 hours. After cooling to room temperature, 2-(dimethylamino)ethyl chloride hydrochloride (0.277 mmol, 0.0903g) was added and the reaction was returned to 80°C overnight. The reaction was again cooled to room temperature, concentrated, and taken up in ethyl acetate. The organic layer was then washed with brine, dried with magnesium sulfate, and concentrated. Flash chromatography on silica gel (75% ethyl acetate/hexanes) yielded the ethyl ester product (0.0209g, 73.9% yield) which, for stability reasons, was allowed to auto-transesterify at room temperature in methanol (10mL) over a period of a few days to yield the corresponding methyl ester, 1h as an oil.

methyl 2-((4’-(2-(dimethylamino)ethoxy)-2-methyl-[1,1’-biphenyl]-4-yl)oxy)acetate (1a). Oil; \(^{1}H\) NMR (400 MHz, d6-acetone): \(\delta\) 7.22 (d, \(J = 9.2\) Hz, 2H), 7.10 (d, \(J = 8.0\) Hz, 1H), 6.98 (d, \(J = 8.8\) Hz, 2H), 6.86 (d, \(J = 2.4\) Hz, 1H), 6.79 (dd, \(J = 8.8\) Hz, 2.8 Hz, 1H), 4.75 (s, 2H), 4.13 (t, 5.6 Hz, 2H), 3.75 (s, 3H), 2.72 (t, 5.6 Hz, 2H), 2.30 (s, 6H), 2.22 (s, 3H) ppm; \(^{13}C\) NMR (100 MHz, d6-acetone): \(\delta\) 169.33, 158.11, 157.35, 136.81, 135.22, 133.97, 130.90, 130.48, 116.57, 114.28, 111.89, 66.45, 64.92, 58.28, 51.48, 45.46, 20.25 ppm. HRMS calcd for C\(_{20}\)H\(_{25}\)NO\(_4\), [M+H] 344.1862, found 344.1863.

methyl 2-((4’-(2-(dimethylamino)ethoxy)-2’-methyl-[1,1’-biphenyl]-4-yl)oxy)acetate (1b). Oil; \(^{1}H\) NMR (400 MHz, d6-acetone): \(\delta\) 7.23 (d, \(J = 8.8\) Hz, 2H), 7.09 (d, \(J = 9.2\) Hz, 1H), 6.98 (d, \(J = 8.8\) Hz, 2.8 Hz, 1H), 4.78 (s, 2H), 4.10 (t, \(J = 5.6\) Hz, 2H), 3.76 (s, 3H), 2.68 (t, 5.6 Hz, 2H), 2.27 (s, 6H), 2.22 (s, 3H) ppm. \(^{13}C\) NMR (100 MHz, d6-acetone): \(\delta\) 170.06, 159.10, 157.95, 137.39, 135.80, 134.84, 131.66, 131.28, 117.24, 115.34, 112.72, 67.24, 65.77, 59.15, 52.27, 46.31, 21.00 ppm. HRMS calcd for C\(_{20}\)H\(_{25}\)NO\(_4\), [M+H] 344.1862, found 344.1864.

methyl 2-((4’-(2-(dimethylamino)ethoxy)-2,3’-dimethyl-[1,1’-biphenyl]-4-yl)oxy)acetate (1c). Oil; \(^{1}H\) NMR (400 MHz, d4-methanol): \(\delta\) 7.06 (d, \(J = 8.4\) Hz, 1H), 7.02 (m, 2H), 6.91 (d, \(J = 8.8\) Hz, 2H), 6.87 (d, \(J = 8.8\) Hz, 2H), 6.79 (dd, \(J = 8.8\) Hz, 2.8 Hz, 1H), 4.70 (s, 2H), 4.09 (t, \(J = 6.0\) Hz, 2H), 3.79 (s, 3H), 2.84 (t, \(J = 5.4\) Hz, 2H), 2.39 (s, 3H), 2.27 (s, 3H) ppm; \(^{13}C\) NMR (100 MHz, d4-methanol): \(\delta\) 170.41, 157.05, 155.90, 136.78, 135.58, 134.02, 131.47, 130.62, 127.59, 126.08, 116.13, 111.48, 110.44, 66.03, 64.82, 58.06, 44.92, 19.75, 15.36 ppm. HRMS calcd for C\(_{21}\)H\(_{27}\)NO\(_4\), [M+H] 358.2018, found 358.2017.

methyl 2-((4’-(2-(dimethylamino)ethoxy)-2’,3-dimethyl-[1,1’-biphenyl]-4-yl)oxy)acetate (1d). Oil; \(^{1}H\) NMR (400 MHz, d6-acetone): \(\delta\) 7.07 (m, 3H), 6.87 (d, \(J = 8.8\) Hz, 1H), 6.84 (d, \(J = 2.4\) Hz, 1H), 6.79 (dd, \(J = 8.0\) Hz, 2.8 Hz, 1H), 4.80 (s, 2H), 4.09 (t, \(J = 6.0\) Hz, 2H), 3.76 (s, 3H), 2.67 (t, \(J = 6.0\) Hz, 2H), 2.28 (s, 3H), 2.27 (s, 6H), 2.22 (s, 3H) ppm; \(^{13}C\) NMR (100 MHz, d6-acetone): \(\delta\) 169.42, 158.23, 155.30, 136.59, 134.77, 134.29, 131.91, 130.87, 127.73, 126.40, 116.40, 111.87, 111.08, 66.45, 65.23, 58.37, 45.55, 20.28, 15.76 ppm. HRMS calcd for C\(_{21}\)H\(_{27}\)NO\(_4\), [M+H] 358.2018, found 358.2024.

methyl 2-((4’-(2-(dimethylamino)ethoxy)-2,2’-dimethyl-[1,1’-biphenyl]-4-yl)oxy)acetate (1e). Oil; \(^{1}H\) NMR (400 MHz, d6-acetone): \(\delta\) 6.96 (m, 2H), 6.86 (m, 2H), 6.79 (m, 2H), 4.75 (s, 2H),
4.10 (t, J = 5.6 Hz, 2H), 3.76 (s, 3H), 2.68 (t, J = 6.8 Hz, 2H), 2.27 (s, 6H), 2.00 (s, 3H), 2.00 (s, 3H) ppm; $^{13}$C NMR (100 MHz, d$_6$-acetone): δ 169.36, 158.37, 157.42, 137.66, 137.43, 134.73, 133.64, 130.77, 130.68, 116.06, 115.93, 111.66, 111.62, 66.44, 64.93, 58.42, 45.56, 19.59 ppm. HRMS calcd for C$_{21}$H$_{27}$NO$_4$, [M+H] 358.2018, found 358.2018.

methyl 2-((2-benzyl-4'-(2-(dimethylamino)ethoxy)-[1,1'-biphenyl]-4-yl)oxy)acetate (1f). Oil; $^1$H NMR (400 MHz, d$_6$-acetone): δ 7.22 (t, J = 7.2 Hz, 2H), 7.16 (m, 4H), 7.01 (d, J = 8.8 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 6.84 (dd, J = 8.0 Hz, 2.8 Hz, 1H), 6.78 (d, J = 2.8 Hz, 1H), 4.71 (s, 2H), 4.12 (t, J = 5.6 Hz, 2H), 3.94 (s, 2H), 3.71 (s, 3H), 2.71 (t, J = 6.0 Hz, 2H), 2.27 (s, 6H) ppm; $^{13}$C NMR (100 MHz, d$_6$-acetone): δ 169.23, 158.25, 157.50, 141.53, 140.12, 135.37, 133.74, 131.37, 130.70, 128.96, 128.44, 126.02, 116.57, 114.31, 112.22, 66.47, 64.91, 58.27, 51.49, 45.47, 39.01 ppm. HRMS calcd for C$_{26}$H$_{29}$NO$_4$, [M+H] 420.2175, found 420.2162.

methyl 2-((2'-benzyl-4'-(2-(dimethylamino)ethoxy)-[1,1'-biphenyl]-4-yl)oxy)acetate (1g). Oil; $^1$H NMR (500 MHz, d$_6$-acetone): δ 7.16 (m, 6H), 7.00 (d, J = 6.8 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 6.85 (dd, J = 8.4 Hz, 2.0 Hz, 1H), 4.77 (s, 2H), 4.07 (t, J = 6.0 Hz, 2H), 3.94 (s, 2H), 3.75 (s, 3H), 2.67 (t, J = 6.0 Hz, 2H), 2.26 (s, 6H) ppm; $^{13}$C NMR (100 MHz, d$_6$-acetone): δ 169.27, 158.49, 157.34, 141.66, 139.98, 134.80, 134.39, 131.34, 130.74, 128.93, 128.43, 125.98, 116.62, 114.38, 112.19, 66.89, 65.01, 58.30, 41.42, 45.51, 39.04 ppm. HRMS calcd for C$_{26}$H$_{29}$NO$_4$, [M+H] 420.2175, found 420.2177.

methyl 2-((2,3'-dibenzyl-4'-(2-(dimethylamino)ethoxy)-[1,1'-biphenyl]-4-yl)oxy)acetate (1h). Oil; $^1$H NMR (400 MHz, d$_6$-acetone): δ 7.22 (m, 6H), 7.13 (m, 3H), 7.06 (dd, J = 8.0 Hz, 2.4 Hz, 1H), 7.03 (d, J = 2 Hz, 1H), 6.97 (dd, J = 8.0 Hz, 2 Hz, 2H), 6.81 (dd, J = 8.0 Hz, 2 Hz, 2H), 4.70 (s, 2H), 4.11 (t, J = 6 Hz, 2H), 3.94 (s, 2H), 3.91 (s, 2H), 3.70 (s, 3H), 2.70 (t, J = 6 Hz, 2H), 2.28 (s, 6H) ppm; $^{13}$C NMR (100 MHz, d$_6$-acetone): δ 169.23, 157.45, 155.87, 141.58, 141.49, 140.03, 135.50, 133.55, 131.69, 131.35, 129.72, 129.17, 128.91, 128.50, 128.43, 128.34, 125.98, 125.89, 116.62, 114.38, 112.19, 66.42, 65.01, 58.30, 41.42, 45.51, 39.04 ppm. HRMS calcd for C$_{33}$H$_{35}$NO$_4$, [M+H] 510.2644, found 510.2639.

methyl 2-((2',3-dibenzyl-4'-(2-(dimethylamino)ethoxy)-[1,1'-biphenyl]-4-yl)oxy)acetate (1i). Oil; $^1$H NMR (400 MHz, d$_6$-acetone): δ 7.28 (d, J = 7.2 Hz, 2H), 7.23 (t, J = 7.6 Hz, 2H), 7.18 (d, J = 8.4 Hz, 2H), 7.14 (dd, J = 7.2 Hz, 2.4 Hz, 1H), 7.10 (d, J = 8.0 Hz, 2H), 7.04 (m, 2H), 6.95 (d, J = 7.2 Hz, 2H), 6.91 (d, J = 8.8 Hz, 1H), 6.83 (dd, J = 8.0 Hz, 2.0 Hz, 1H), 6.79 (d, J = 2.8 Hz, 1H), 4.80 (s, 2H), 4.05 (t, J = 5.6 Hz, 2H), 4.01 (s, 2H), 3.90 (s, 2H), 3.77 (s, 3H), 2.64 (t, J = 6.0 Hz, 2H), 2.24 (s, 6H) ppm; $^{13}$C NMR (100 MHz, d$_6$-acetone): δ 169.31, 158.43, 154.88, 141.69, 141.32, 139.89, 134.92, 134.03, 133.55, 131.69, 131.35, 129.72, 129.17, 128.91, 128.50, 128.43, 128.34, 125.98, 125.89, 116.62, 112.22, 111.41, 66.89, 64.93, 58.46, 51.48, 45.59, 29.06, 36.00 ppm. HRMS calcd for C$_{33}$H$_{35}$NO$_4$, [M+H] 510.2644, found 510.2639.
General Procedure for Transesterification of Ethyl Esters to Methyl Esters

Compound 8h, (0.0403 mmol, 0.0121g) was dissolved in methanol. To this was added 2 equivalents of 1N H₂SO₄ (aq) (0.0806mmol, 0.0806mL). The reaction was stirred at room temperature overnight followed by heating to 70°C for 7 hours. The reaction was then quenched with 1N NaOH (aq) (0.0806mmol, 0.0806mL), concentrated, and taken up in ethyl acetate. The organic layer was washed with brine, dried with magnesium sulfate, and concentrated. Flash chromatography on silica gel (10% ethyl acetate/hexanes) yielded the methyl ester product (0.0096g, 81.9% yield) as a solid (mp = 124-127°C).

methyl 2-((4'-hydroxy-2,3'-dimethyl-[1,1'-biphenyl]-4-yl)oxy)acetate, AW-Me-11. Oil. ¹H NMR (400 MHz, CDCl₃): δ 7.12 (d, J = 8.8 Hz, 1H), 7.04 (s, 1H), 6.98 (dd, J = 8.0 Hz, 2.0 Hz, 1H), 6.82 (d, J = 2.8 Hz, 1H), 6.79 (d, J = 8.4 Hz, 1H), 6.75 (dd, J = 8.4 Hz, 2.8 Hz, 1H), 4.67 (s, 1H), 4.66 (s, 2H), 3.83 (s, 3H), 2.28 (s, 3H), 2.24 (s, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 169.85, 156.84, 152.86, 137.32, 135.71, 134.15, 132.20, 131.15, 128.22, 123.50, 116.70, 114.72, 111.71, 65.56, 52.56, 29.94, 21.07, 16.06 ppm. HRMS calcd for C₁₇H₁₈O₄, [M +Na] 309.1103, found 309.1107.

methyl 2-((2,3'-dibenzyl-4'-hydroxy-[1,1'-biphenyl]-4-yl)oxy)acetate, AW-Bn-11. Solid, mp = 124-127°C; ¹H NMR (400 MHz, CDCl₃): δ 7.28 (m, 2H), 7.18 (m, 7H), 6.97 (m, 4H), 6.77 (m, 2H), 6.73 (d, J = 2.0 Hz, 1H), 4.67 (s, 1H), 4.60 (s, 2H), 3.95 (s, 2H), 3.90 (s, 2H), 2.78 (s, 3H) ppm; ¹³C NMR (100 MHz, CDCl₃): δ 169.70, 157.04, 152.90, 141.29, 140.19, 139.99, 135.88, 134.03, 132.34, 131.59, 129.05, 129.00, 128.90, 128.52, 126.79, 126.60, 126.09, 116.76, 115.60, 112.22, 65.56, 52.54, 39.55, 36.62 ppm. HRMS calcd for C₃₃H₃₅NO₄, [M +Na] 461.1729, found 461.1721.
AW-III-88-71a

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Area Percent Report

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Appendix 4: Biological Methods and Data for Assays Presented in Chapter 4
## Two-Hybrid Assay (D.P.McDonnell)

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<td>Gate/Delay1 (usecs):</td>
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<tr>
<td>ExFilter2:</td>
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<td>Gate/Delay2 (usecs):</td>
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</tr>
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<td>Number of Flashes:</td>
<td>N/A</td>
</tr>
<tr>
<td>Gain:</td>
<td>1</td>
<td>G Factor:</td>
<td>N/A</td>
</tr>
<tr>
<td>Plate Size:</td>
<td>96</td>
<td>Lamp Intensity:</td>
<td>1</td>
</tr>
</tbody>
</table>

### Acquired:
- Plate Seq#: 4922
- Comment: Saturday
- Acquired: February 19 2011 12:14 PM
- Temperature Min/Max: 0.0/0.0°C
- File Report: C:/Fusion/Reports/CYC data2011/lumi\Lumi02-19-112180.CSV

### Luc

SKBR3 MM2H, split cells in 96 well plate (10,000 cells/well) in 0.10 ml white media for 2 days
tfn with lipofectin for 6h, add 50 ul of 3x ligand/media
assay ~40h after tmnt

<table>
<thead>
<tr>
<th>plasmid</th>
<th>conc. (ug/ul)</th>
<th>ng for trxn</th>
<th>total amount (ug)</th>
<th>ul stock</th>
</tr>
</thead>
<tbody>
<tr>
<td>5xGal4-Luc3</td>
<td>0.976</td>
<td>2300</td>
<td>14.904</td>
<td>13x</td>
</tr>
<tr>
<td>pCMV-bgal</td>
<td>1.080</td>
<td>100</td>
<td>0.648</td>
<td>7.8</td>
</tr>
<tr>
<td>pVP16-ERa new</td>
<td>0.535</td>
<td>400</td>
<td>2.592</td>
<td>63.0</td>
</tr>
<tr>
<td>pM-GRIP1 LXXLL2</td>
<td>1.300</td>
<td>200</td>
<td>2.696</td>
<td>13.0</td>
</tr>
<tr>
<td>pBSII</td>
<td>0.317</td>
<td>0</td>
<td>0.000</td>
<td>0.0</td>
</tr>
</tbody>
</table>

# of triplicates: 8.0 total ul 21.7123 282.3 +/- 1
make 12.5 x ul opti-MEM 628.2877 8167.7 nM E2

Lipofectin mix: Add ul lipofectin 32.5600
Note: AW-Bn-## compounds are frequently titled as AW-Bz-## compounds throughout the following assays. Please recognize that AW-Bn-## and AW-Bz-## compounds are in fact the same.
**Reporter Gene Assay (D.P.McDonnell)**

Assay: Lumi  
Description: chk bkg  
Software: Fusion 3.50  
Sample Map: cyc  
Description:  
Detection Mode: Luminescence  
Plate Type: 96 Well Plate  

**Well Read Time (secs):** 2  
**Well Repeats:** 2  
**Plate Repeats:** 1

**ExFilter1:** --Disabled--  
**ExFilter2:** --Disabled--  
**EmFilter1:** --Empty--  
**Light Source:** Not Defined  
**Gain:** 1  
**PMT Voltage:** 1100  
**Gate/Delay1 (usecs):** N/A  
**Gate/Delay2 (usecs):** N/A  
**EmFilter2:** --Disabled--  
**Number of Flashes:** N/A  
**Temperature Control:** Off  

**Plate Size:** 96  
**Lamp Intensity:** 1  

**Plate Seq#:** 4967  
**Comment:** Acquired: Sunday February 27 2011 12:45 PM  
**Temperature Min/Max:** 0.0/0.0 °C

**File Report:** C:\Fusion\Reports\CYC data\2011\lumi\Lumi02-27-112188.CSV

SKBR3 MM2H, split cells in 96 well plate (10,000 cells/well) in 0.10 ml white media for 2 days  
tfn with lipofectin for 6h, add 50 ul of 3x ligand/media  
assay ~40h after tmnt

<table>
<thead>
<tr>
<th>plasmid</th>
<th>conc. (ug/ul)</th>
<th>ng for trxn</th>
<th>total amount (ug)</th>
<th>ul stock</th>
</tr>
</thead>
</table>
| 3xERE tata-Luc | 0.686        | 2850        | 18.468            | 26.9213  | 363.4  
| pCMV-bgal      | 1.080        | 100         | 0.648             | 0.6000   | 8.1     
| RST7-ERa       | 0.100        | 50          | 0.324             | 3.2400   | 43.7    13x  
| pBSII          | 0.917        | 0           | 0.000             | 0.0000   | 0.0     |

**# of triplicates:** 8.0  
**total ul:** 30.7613  
**ul stock:** 415.3

**make 13.5 x ul opti-MEM:** 619.2387  
**E2**

**Lipofectin mix:**  
**Add ul lipofectin:** 32.5600  
617.4400  

1300.00
Preliminary IC$_{50}$ Values

Aw-Bz-04: 11.31 uM  
Aw-Bz-10: 18.44 uM  
Aw-Me-04: 23.55 uM  
Aw-Me-08: 26.82 uM  
Aw-Me-09: 21.10 uM
**Competitive Binding Assay against Estradiol**

(D.P. McDonnell)

**Assay:** Lumi  
**Description:** chk bkg

**Software:** Fusion 3.50  
**Instrument Serial:**

**Sample Map:** cyc  
**Description:**

**Detection Mode:** Luminescence  
**Shaking:** Disabled

**Plate Type:** 96 Well Plate  
**Temperature Control:** Off

Well Read Time (secs): 2  
Well Repeats: 1  
Plate Repeats: 1

ExFilter1: --Disabled--  
Gate/Delay1 (usecs): N/A

ExFilter2: --Disabled--  
Gate/Delay2 (usecs): N/A

EmFilter1: --Empty--  
EmFilter2: --Disabled--

Light Source: Not Defined  
Number of Flashes: N/A

Gain: 1  
PMT Voltage: 1100

G Factor Source: N/A  
G Factor: N/A

Plate Size: 96

**Plate Seq#: 5008**  
**Acquired:** March 04 2011 11:43 AM  
**Temperature Min/Max:** 0.0/0.0°C

**Comment:** SKBR3 MM2H, split cells in 96 well plate (10,000 cells/well) in 0.10 ml white media for 2 days  
tfn with lipofectin for 6h, add 50 ul of 3x ligand/media  
assay ~40h after tmnt

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<tr>
<th>plasmid</th>
<th>conc. (ug/ul)</th>
<th>ng for trxnl</th>
<th>total amount (ug)</th>
<th>ul stock</th>
<th>plasmid</th>
<th>conc. (ug/ul)</th>
<th>ng for trxnl</th>
<th>total amount (ug)</th>
<th>ul stock</th>
</tr>
</thead>
<tbody>
<tr>
<td>3xERE tata-Luc</td>
<td>0.509</td>
<td>2850</td>
<td>18.468</td>
<td>36.2829</td>
<td>3290.3</td>
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<td></td>
</tr>
<tr>
<td>pCMV-bgal</td>
<td>1.080</td>
<td>100</td>
<td>0.648</td>
<td>0.6000</td>
<td>4.8</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RST7-ERa</td>
<td>0.100</td>
<td>50</td>
<td>0.324</td>
<td>3.2400</td>
<td>25.9</td>
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<td></td>
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<tr>
<td>pBSII</td>
<td>0.917</td>
<td>0</td>
<td>0.000</td>
<td>0.0000</td>
<td>0.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

# of triplicates: 8.0  
**total ul** 40.1229

make 8 x ul opti-MEM 609.8771  
**4879.0** E2

Lipofectin mix: Add ul lipofectin 32.5600

617.4400
*3,2’-dimethyl phenol-methyl ester

*2,3’-dibenzyl phenol-methyl ester

*3,2’-dibenzyl phenol-ethyl ester

2,2’-dibenzyl amino-methyl ester
2,3’-dimethyl amino-methyl ester  

3,2’-dimethyl amino-methyl ester  

4-hydroxytamoxifen  

*toxicity concerns relative to necessary concentration for inhibition: AW-Bz-11, AW-Me-04, AW-Bz-04
Androgen Receptor - Reporter Gene Assay (D.P.McDonnell)

HepG2 (already grown in BME) seeded 30,000 cells/well in 96-well plate overnight in BME media (BME+8%CFS+NEAA+NaPyr+L-glutamine)
tfn directly (10 ul per well) add compounds a few hours later (10 ul 18x R1881 and 60ul of 3x inhibitors)
use only 60 wells/plate (fill outer wells with H2O)

<table>
<thead>
<tr>
<th>plasmid</th>
<th>conc. (ug/ul)</th>
<th>ng for trxn</th>
<th>total amount (ug)</th>
<th>ul stock</th>
<th>5x</th>
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<tbody>
<tr>
<td>MMTV-Luc</td>
<td>0.895</td>
<td>2800</td>
<td>18.144</td>
<td>20.2726</td>
<td>101.3</td>
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<tr>
<td>pCMVbgal</td>
<td>0.936</td>
<td>100</td>
<td>0.648</td>
<td>0.6923</td>
<td>3.6</td>
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<tr>
<td>SG5-AR</td>
<td>0.100</td>
<td>100</td>
<td>0.648</td>
<td>6.4800</td>
<td>32.4</td>
</tr>
<tr>
<td>pBSII</td>
<td>0.100</td>
<td>0</td>
<td>0.000</td>
<td>0.0000</td>
<td>0.0</td>
</tr>
</tbody>
</table>

# of triplicates: 8.0

uls

Assay: Lumi
Software: Fusion 3.50
Sample Map: cyc
Detection Mode: Luminescence
Plate Type: 96 Well Plate

Well Read Time (secs): 2
Well Repeats: 1
ExFilter1: --Disabled--
ExFilter2: --Disabled--
EmFilter1: --Empty--
Light Source: Not Defined
Gain: 1
G Factor Source: N/A
Plate Size: 96

Instrument Serial: chk bkg
Shaking: Disabled
Temperature Control: Off
Plate Pre-Count Delay (secs): 0
Gate/Delay1 (usecs): N/A
Gate/Delay2 (usecs): N/A
EmFilter2: --Disabled--
Number of Flashes: N/A
PMT Voltage: 1100
G Factor: N/A
Lamp Intensity: 1

use 10 ul per well
Data for Trial 1 of 2 is shown here.
In Vitro TR-FRET CBI Assays (J.A. Katzenellebogen)

The ERα and SRC proteins were expressed, purified and labeled as previously described.1-3 Briefly the ERα LBD (amino acids 304-554), with a single reactive cysteine at amino acid 417, and the SRC3 encompassing three NR boxes (amino acids 627-829), were expressed in E. coli from a pET-15b vector. The purified ERα 417 was labeled site-specifically with thio-reactive-biotin (MAL-dPEG4-biotin) (Invitrogen, Carlsbad, CA).4 The purified SRC3 was labeled non-specifically through cysteine residues with 5-iodoacetamido-fluorescein (Molecular Probes, Eugene, OR).

The time-resolved fluorescence resonance energy transfer (Tr-FRET) experiments were measured on a Wallac Victor II plate reader (Perkin Elmer, Shelton, CT) using an excitation filter at 340/10nm and emission filters for terbium and fluorescein at 495/20 and 520/25nm, respectively with a 100µs delay. Tr-FRET is expressed as (acceptor/donor) A/D x 1000.4

The CBI compounds were dissolved at 20mM in dimethylformamide (DMF) and then serially diluted in DMF to ensure solubility. The FRET buffer consisted of 20mM Tris, pH 7.5, 0.01% NP40, 50mM NaCl, 10% glycerol, 0.3mg/ml ovalbumin and 0.1mM butylated hydroxyl anisol (BHA). 9µl of buffer was placed in separate wells of a black 96-well Molecular Devices HE high efficiency microplate (Molecular Devices, Inc., Sunnyvale, CA). To this was added 1µl of the CBI dilutions, followed by 5µl of ER cocktail. This was incubated for 15 min, at room temperature, in the dark. Then 5µl of the SRC was added and incubated for 1 hr, at room temperature, in the dark. The plate was then measured in the fluorescence plate reader.

The ER cocktail consisted of the ERα-417-biotin, streptavidin-terbium (Invitrogen, Carlsbad, CA), and estradiol. The final concentrations in the simple co-activator competition assay were 1nM ERα-biotin, 0.25nM SATb, 1µM E2, 5nM SRC-fluorescein, 5% DMF and seven CBI concentrations ranging from 10⁻³M to 10⁻⁶M with a buffer control.

Included in the experiments were a background control that consisted of all of the components except the ER-biotin and a positive control that was competition with a 15 amino acid peptide encompassing the 2nd NR box of SRC1 (synthesized by the Roy J, Carver Biotechnology Center, UIUC).5

The experiments using various concentrations of the SRC were done in the same way except that the final concentrations of SRC were either 2nM, 5nM, or 10nM.

The data was analyzed using Prism 4 (Graph Pad Software, San Diego, CA). Ki was calculated from the Cheng-Prusoff equation

\[ IC_{50} = Kd(1 + To/Kd^*) \]

Where To is the total tracer added and Kd* is the Kd of the tracer. A Kd of 0.88nM was determined previously for the SRC3 fragment.5
<table>
<thead>
<tr>
<th>Compound</th>
<th>CBI Graph</th>
<th>IC50</th>
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<tbody>
<tr>
<td>AW-Bn-04</td>
<td><img src="image" alt="Graph" /></td>
<td>5nM-0.0001800</td>
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<tr>
<td>AW-Bn-10</td>
<td><img src="image" alt="Graph" /></td>
<td>5nM-0.0004855</td>
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<tr>
<td>AW-Bn-11</td>
<td><img src="image" alt="Graph" /></td>
<td>5nM-0.0001538</td>
</tr>
<tr>
<td>AW-Me-04</td>
<td>7-6-2011</td>
<td>5nM-0.0002491</td>
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<tr>
<td>----------</td>
<td>----------</td>
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</tr>
<tr>
<td><img src="image1.png" alt="Graph" /></td>
<td><img src="image2.png" alt="Graph" /></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>AW-Me-08</th>
<th>7-5-2011</th>
<th>5nM-2.474 (ambiguous)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image3.png" alt="Graph" /></td>
<td><img src="image4.png" alt="Graph" /></td>
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</tbody>
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<table>
<thead>
<tr>
<th>AW-Me-09</th>
<th>7-5-2011</th>
<th>Not Converged</th>
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<tbody>
<tr>
<td><img src="image5.png" alt="Graph" /></td>
<td><img src="image6.png" alt="Graph" /></td>
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</tr>
</tbody>
</table>
For comparison, a similar experiment done with the 15-mer peptide is shown. It is clear that the curves are shifted with the different concentrations of SRC. It has not been normalized and shows the full range of A/D*1000.

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC₅₀ (mM)</th>
<th>Ki (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AW-Bn-04</td>
<td>0.202 ± 0.031</td>
<td>30,240</td>
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<td>AW-Bn-10</td>
<td>0.309 ± 0.279</td>
<td>54,380</td>
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<tr>
<td>AW-Bn-11</td>
<td>0.308 ± 0.218</td>
<td>54,210</td>
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<tr>
<td>AW-Me-04</td>
<td>0.954 ± 0.997</td>
<td>142,814</td>
</tr>
<tr>
<td>AW-Me-08</td>
<td>too low to measure</td>
<td>--</td>
</tr>
<tr>
<td>AW-Me-09</td>
<td>too low to measure</td>
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</tr>
<tr>
<td>Control peptide</td>
<td>0.000219</td>
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</table>

- 10nM-0.0005237
- 5nM-0.0002239
- 2nM-0.0006162
- 10nM-11.97
- 5nM-1.976
- 2nM-8.813
(ambiguous for all values)


Radiometric Binding Assay (J.A.Katzenellenbogen)

Relative binding affinities were determined by competitive radiometric binding assays with 2nM [³H] estradiol ([2.4.6.7-3H] estra-1.3.5(10)-triene-3, 17β-diol), 82 Ci/mmol (Perkin Elmer, Waltham, MA) as tracer, as a modification of methods previously described.³,⁶ Unlabeled 17β-Estradiol (E₂) was obtained from Sigma (St. Louis, MO). Purified full-length human estrogen receptor (ERα) was purchased from Pan Vera/Invitrogen (Carlsbad, CA). Incubations were at 0°C for 18-24 h. Hydroxyapatite (BioRad, Hercules, CA) was used to absorb the receptor-ligand complexes and the unbound ligand was washed away. The binding affinities are expressed as a relative binding affinity (RBA) value, with that of E₂ set to 100%. Under these conditions, the K_d of E₂ for ERα is 0.2nM.

<table>
<thead>
<tr>
<th>Compound</th>
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</thead>
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<tr>
<td>AW-Bn-04</td>
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</tr>
<tr>
<td>AW-Bn-10</td>
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</tr>
<tr>
<td>AW-Bn-11</td>
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</tr>
<tr>
<td>AW-Me-04</td>
<td>0.010</td>
</tr>
<tr>
<td>AW-Me-08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AW-Me-09</td>
<td>0.002</td>
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</tbody>
</table>
Appendix 5: Supplemental NMR Data for Chapter 4
2,2'-dibenzyl amino-methylester

STANDARD PROTON PARAMETERS

Pulse Sequence: s2pul
Solvent: Acetone
Ambient temperature
File: AW-V-236_d-acetone
INOVA-500 "waters500"

Pulse 42.2 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
36 repetitions
OBSERVE H1, 499.7055537 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 32 sec
AW-V-236_d-acetone

d-acetone
500MHz NMR
1H-Spectrum
01/05/2011
2,2'-dibenzyl amino-methylester

STANDARD PROTON PARAMETERS

Pulse Sequence: s2pul
Solvent: Acetone
Ambient temperature
File: AW-V-236_d-acetone
INOVA-500 "waters500"

Pulse 42.2 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions

OBSERVE H1, 499.7055537 MHz
DATA PROCESSING
FT size 32768
Total time 3 min, 32 sec

3.68 3.66 3.64 3.62 3.60 3.58 3.56 3.54 3.52 3.50 3.48 3.46 3.44 3.42 ppm
d-chloroform
500MHz NMR
1H-Spectrum
01/05/2011
2,2'-dibenzyl amino-methylester
STANDARD PROTON PARAMETERS

Pulse Sequence: s2pul
Solvent: CDCl3
Ambient temperature
File: AW-V-236_d-chloroform
INOVA-500 "waters500"

Pulse 42.2 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 499.7029739 MHz
DATA PROCESSING
Fl size 32768
Total time 0 min, 32 sec
d-chloroform
500MHz NMR
1H-Spectrum
01/05/2011
2,2'-dibenzyl amino-methylester
STANDARD PROTON PARAMETERS
Pulse Sequence: s2pul
Solvent: CDC13
Ambient temperature
File: AW-V-236_d-chloroform
INOVA-500 "water500"

Pulse 42.2 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1,
99.7029739 MHz
DATA PROCESSING
FT size 32768
Total time 6 min, 32 sec
2,2'-dibenzyl amino-methylester

STANDARD PROTON PARAMETERS

Pulse Sequence: s2pul
Solvent: cd3od
Ambient temperature
File: AW-V-236_d-methanol
INOVA-500 "waters500"

Pulse 42.2 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions
OBSERVE H1, 499.7049412 MHz
DATA PROCESSING
FT size 32768
Total time 0 min, 32 sec
AW-V-236_d-methanol

d-methanol
500MHz NMR
1H-Spectrum
01/05/2011
2,2'-dibenzyl amino-methylester

STANDARD PROTON PARAMETERS

Pulse Sequence: 52pul
Solvent: cd30d
Ambient temperature
File: AW-V-236_d-methanol

INOVA-500 "waters500"

Pulse 42.2 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions

OBSERVE H1, 499.7049412 MHz

DATA PROCESSING

FT size 32768

Total time 0 min, 32 sec
2,2'-dibenzyl amino-methylester

STANDARD PROTON PARAMETERS

Pulse Sequence: s2pu1
Solvent: DMSO
Ambient temperature
File: AW-V-207_DMSO
INOVA-500 "waters500"

Pulse 42.2 degrees
Acq. time 2.048 sec
Width 8000.0 Hz
16 repetitions
OBSERVE Mt. 499.705338 MHz
DaTA PROCESSING
FT size 32768
Total time 8 min, 32 sec
2,2'-dibenzyl amino-methylester

**STANDARD PROTON PARAMETERS**

Pulse Sequence: s2pul

Solvent: DMSO

Ambient temperature

File: AV-V-207_DMSO

INOVA-900 "waters599"

Pulse 42.2 degrees

Acq. time 2.048 sec

Width 6000.9 Hz

16 repetitions

Observe 493.705338 MHz

Data Processing

FT size 32768

Total time 0 min, 32 sec

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**PROTON PARAMETERS Table**

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<th>3.64</th>
<th>3.62</th>
<th>3.60</th>
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<th>3.56</th>
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<th>3.50</th>
<th>3.48</th>
<th>3.46</th>
<th>3.44</th>
<th>3.42</th>
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AW-V-236

d-benzene
400MHz NMR
IH-Spectrum
1/24/2011

2,2'-dibenzyl amino-methylester

NU STANDARD IH OBSERVE

Pulse Sequence: s2pul
Solvent: Benzene
Ambient temperature
File: AW-V-236_d-benzene_01-24-2011
Mercury-400B "nmr400"

Relax. delay 1.000 sec
Pulse 34.6 degrees
Acq. time 1.365 sec
Width 6002.4 Hz
16 repetitions

OBSERVE H1, 399.1216661 MHz

DATA PROCESSING
Line broadening 0.2 Hz
FT size 10384
Total time 0 min, 47 sec

289
NU STANDARD 1H OBSERVE

Pulse Sequence: s2pul
Solvent: Benzene
Ambient temperature
File: AW-V-236 d-benzene_01-24-2011

Relax. delay 1.000 sec
Pulse 34.6 degrees
Acq. time 1.365 sec
Width 6002.4 Hz
16 repetitions
DATA PROCESSING
Line broadening 0.2 Hz
FT size 16384
Total time 0 min, 47 sec
AW-V-236_d-acetone_VT=7.7C
d-acetone
01/27/2011
Temperature = 7.7 degrees Celsius
2,2'-dibenzyl amino-methylester

NU STANDARD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: Acetone
Ambient temperature
File: AW-V-236_d-acetone_VT=7.7C
Mercury-400BB "nrm400"

Relax. delay 1.000 sec
Pulse 34.6 degrees
Acq. time 1.365 sec
Width 6002.4 Hz
4 repetitions
OBSERVE H1, 388.1237816 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 16384
Total time 0 min, 47 sec
AW-V-236 d-acetone VT=7.7C
d-acetone
01/27/2011
Temperature = 7.7 degrees Celsius
2,2'-dibenzyl amino-methylester

NU STANDARD IN OBSERVE
Pulse Sequence: s2pul
Solvent: Acetone
Ambient temperature
File: AW-V-236 d-acetone VT=7.7C
Mercur'y-400BB -nmr400"'

Relax. delay 1.000 sec
Pulse angle 34.6 degrees
Acq. time 1.365 sec
Width 6002.4 Hz
4 repetitions
OBSERVE H1, 399.1237016 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 1024
Total time 0 min, 47 sec

3.68 3.66 3.64 3.62 3.60 3.58 3.56 3.54 3.52 3.50 3.48 3.46 3.44 3.42 ppm
2,2'-dibenzyl amino-methylester

NU STANDARD 1H OBSERVE

Pulse Sequence: s2pul
Solvent: Acetone
Temp. = 35.0 °C / 238.2 K
File: AW-V-236_d-acetone_VTneg34.9
Mercury-400BB

Relax. delay 1.000 sec
Pulse 34.6 degrees
Acq. time 1.365 sec
Width 6002.4 Hz
10 repetitions
OBSERVE H1, 399.1236909 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 16384
Total time 0 min, 47 sec
2,2'-dibenzyl amino-methylester

NU STANDARD 1H OBSERVE

Pulse Sequence: s2pul
Solvent: Acetone
Temp. -35.0 C / 238.2 K
File: AW-V-236_d-acetone_VTneg34.9
Mercury-400BB "nmr400"

Relax. delay 1.000 sec
Pulse 34.6 degrees
Acq. time 1.365 sec
Width 6002.4 Hz
16 repetitions

DATA PROCESSING
Line broadening 0.2 Hz
FT size 16384
Total time 0 min, 47 sec

---

3.68 3.66 3.64 3.62 3.60 3.58 3.56 3.54 3.52 3.50 3.48 3.46 3.44 3.42 ppm

---

294
AW-V-236_d-acetone_VTneg40.9

d-acetone
VT = -40.9 degrees Celsius
400MHz NMR
1H Spectrum
02/05/2011
2,2'-dibenzyl amino-methylester
NU STANDARD 1H OBSERVE

Pulse Sequence: s2pul
Solvent: Acetone
Temp. -41.0 C / 232.2 K
File: AW-V-236_d-acetone_VTneg40.9
Mercury-490BB "nmr400"

Relax. delay 1.000 sec
Pulse 34.6 degrees
Acq. time 1.365 sec
Width 600.2 Hz
256 repetitions
OBSERVE H1, 399.1236894 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 10384
Total time 12 min, 46 sec

DATA PROCESSING
Line broadening 0.2 Hz
FT size 10384
Total time 12 min, 46 sec

9 8 7 6 5 4 3 2 1 0 ppm
AW-V-236\_d-acetone\_VTneg40.9
d-acetone
VT = -40.9 degrees Celsius
400MHz NMR
1H Spectrum
02/05/2011
2,2'-dibenzyl amino-methylester
NU STANDA RD 1H OBSERVE
Pulse Sequence: s2pul
Solvent: Acetone
Temp. = -41.0 C / 232.2 K
File: AW-V-236\_d-acetone\_VTneg40.9
Mercury=400BB "nmr400"

Relax. delay 1.000 sec
Pulse 34.6 degrees
Acq. time 1.355 sec
Width 6002.4 Hz
256 repetitions
OBSERVE 61, 399.12368894 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 16384
Total time 12 min, 46 sec
2,2'-dibenzyl amino-methyl ester

Pulse Sequence: gHSQC
Solvent: Acetone
Ambient temperature

File: AW-NMR-23_gHSQC

INOVA-500 "waters500"

Relax. delay 1.000 sec
Acq. time 0.201 sec
2D Width 21361.8 Hz
16 repetitions
2 x 256 increments

OBSERVE H1, 498.70559 MHz
DECouple C13, 125.66045 MHz
Power 39 dB
on during acquisition
off during delay
GARP-1 modulated

DATA PROCESSING
Gauss apodization 0.033 sec
F1 DATA PROCESSING
Gauss apodization 0.011 sec

FT size 2048 x 2048
Total time 2 hr, 54 min, 45 sec

File: AW-V-236
02/23/2011
d-acetone
RT
1H NMR

OBSERVE H1, 498.70559 MHz
DECouple C13, 125.66045 MHz
Power 39 dB
on during acquisition
off during delay
GARP-1 modulated

DATA PROCESSING
Gauss apodization 0.033 sec
F1 DATA PROCESSING
Gauss apodization 0.011 sec

FT size 2048 x 2048
Total time 2 hr, 54 min, 45 sec

AW-NMR-23_gHSQC
AW-NMR-23_gHSQC
02/23/2011
AW-V-236
d-acetone
300MHz NMR
RT
HSQC

2,2"-dibenzyl amino-methylester
2,2-dibenzyl amino-methylester
Pulse sequence: gHSQC
2,2-Dibenzyl amino-methylester

Pulse sequence: gHSQC

F1 (ppm)

6.8 6.9 7.0 7.1 7.2 7.3

116.5 116.0 115.5 115.0 114.5 114.0 113.5 113.0 112.5 112.0 111.5 111.0
2,2'-dibenzyl amino-methylester

Pulse Sequence: gHSQC
2,2'-dibenzyl amino-methylester

Pulse Sequence: gHMBC
Solvent: Acetone
Ambient temperature
File: AW-NMR-23_gHMBC
INOVA-500 "waters500"

Relax. delay 1.000 sec
Width 5106.3 Hz
20 Width 30154.5 Hz
500 increments
OBSERVE H1, 499.705654 MHz
DATA PROCESSING
Sine bell 0.100 sec
F1 DATA PROCESSING
Sine bell 0.013 sec
FT size 2048 x 2048
Total time 10 hr, 20 min, 57 sec
2,2'-dibenzyl amino-methyl ester

Pulse Sequence: gHMBC
Solvent: Acetone
Ambient temperature
File: AW-NMR-23_gHMBC
INOVA-500 "waters500"

Relax. delay 1.000 sec
Acq. time 0.201 sec
Width 5160.3 Hz
20 Width 30154.5 Hz
48 repetitions
660 increments
OBSERVE H1, 499.705694 MHz
DATA PROCESSING
Sine bell 0.100 sec
F1 DATA PROCESSING
Sine bell 0.013 sec
FT size 2048 x 2048
Total time 10 hr, 20 min, 57 sec
2,2'-dibenzyl amino-methylester

Pulse sequence: gHMBC

1H NMR

1H NMR-23_gHMBC 10 hr

02/23/2011
d-acetone
RT

1H NMR

400 MHz

F1 (ppm)

F2 (ppm)
AV-NMR-23_gHMBC 10 hr
02/23/2011
AV-V-236
d-acetone
500MHz NMR
RT
HMBC

2,2-di(tert-buty1) amino-methylester

Pulse sequence: gHMBC

Based on interpretation of AV-NMR-23, believe there are quaternary aromatic carbons of ether linkages. → tracing distinguishing these by C-term and then tracing through-bonds is good route to distinguishing C bond.
2,2- dibenzyl amino-methylester
Pulsed sequence: gHMBC
2,2'-dibenzyl amino-methylester

Pulsed field gradient: gHMBC
2,2'-dibenzyl amino-methylester

Pulse Sequence: gHMBC
Solvent: Acetone
Ambient temperature
File: AW-NMR-23_gHMBC
INOVA-500 "waters500"

Relax. delay 1.000 sec
Acq. time 0.201 sec
Width 516.3 Hz
2D Width 30154.5 Hz
48 repetitions
600 increments

OBSERVE H1. 488.7055694 MHz
DATA PROCESSING
Sine bell 0.100 sec
F1 DATA PROCESSING
Sine bell 0.013 sec
FT size 2048 x 2048
Total time 10 hr, 20 min, 57 sec
2,2-F2-benzyl amino-methylester
Pulse sequence: gHMBC

Based on AW-NMR-23, I think these are quaternary carbons of benzyl bond - which is present they are long-range through-bond coupled to is very important.
AW-NMR-23_gHMBC 10 hr
AW-v-236
02/23/2011
d-acetone
RT
H NMR

2,2'-diphenyl amino-methylester
Full Assignment: gHMBC
2,2-Fluorenyl amino-methylester
Pulse sequence: gHMBC
2,2-dimethylamino-methylester

Pulse sequence: gHMBC
2,2'-bipyridyl amino-methylester
Pulse sequence: gHMBC

F1 (ppm)

40.3 40.2 40.1 40.0 39.9 39.8 39.7 39.6


' h' through band connect with more shielded 12-methyl

'i' through band connect with more de-shielded 12-methyl
AW-NMR-18_IDNOESY_VT=deg45
AW-V-236
02/15/2011
d-acetone
VT= -45 degrees C
2,2'-dibenzyl amino-methylester
dS(3) -> 7.077-7.061ppm
STANDARD PROTON PARAMETERS
Pulse Sequence: NOESY1D
AW-NMR-18_1D_NOESY_VT= neg45
AW-V-236
02/15/2011
d-acetone
1D-NOESY
VT = -45 degrees C
2,2'-dibenzyl amino-methylester
dS(3) -> 7.077 - 7.061 ppm
STANDARD PROTON PARAMETERS
Pulse Sequence: NOESY1D
AW-NMR-18_1DNOESY_VT=neg45
AW-V-236
02/15/2011
d-acetone
1D-NOESY
VT= -45 degrees C
500MHz NMR
2,2'-dibenzyl amino-methylester
spectrum 4
Pulse Sequence: NOESY1D
AW-NMR-18_1DNOESY_VT=neg45
AW-V-236
02/15/2011
α-acetone
1D-NOESY
VT= -45 degrees C
500MHz NMR
2,2'-dibenzyl amino-methylester
spectrum 4
Pulse NOESY 1D
AW-NMR-18_1DNOESY_VT=neg45
AW-V-235
02/15/2011
d-acetone
1D-NOESY
VT= -45 degrees C
2,2'-diobenzyl amino-methylester
dS(9) -) 5.799-5.794ppm
STANDARD PROTON PARAMETERS
Pulse Sequence: NOESY1D

![NMR Spectrum](image)
AW-NMR-18_1DNOESY VT=neg45
AW-V-236
02/15/2011
d-acetone
1D-NOESY
VT=-45 degrees C
2,2'-dibenzyl amino-methylester
dS(9) -> 6.799-6.794ppm
STANDARD PROTON PARAMETERS
Pulse Sequence: NOESY10
AW-NMR-19_NOESY1D_mix=1.00s
02/16/2011
AW-V-236
2,2'-dibenzyl amino-methylester
d-acetone
RT (19.9 degrees C)
400 MHz NMR
NOESY1D, mix=1.00s, nt=256
spectrum 15
Pulse Sequence: NOESY1D

![NMR spectrum image]

ppm
2,2'-dibenzyl amino-methylester

Pulse Sequence: NOESY1D
AW-NMR-19_NOESYID_mix2.00seconds
02/18/2011
pH=7.35
2,2'-dibenzyl amino-methylester
d-acetone
RT (19.8 degrees C)
400 MHz NMR
NOESYID, mix = 2.00s, nt=256
spectrum 15, properly phased
Pulse Sequence: NOESYID
AW-NMR-19_NOESY1D_mix2.005seconds

07/16/2011
AW-V-236
2,2'-dibenzyl amino-methylester
c-acetone
RT (19.9 degrees C)
400 MHz NMR

NOESY1D, mix = 2.30s, nt=256
spectrum 15, properly phased
Pulse Sequence: NOESY1D
AW-NMR-23 ROESY
AW-V-236
02/23/2011
-d-acetone
RT
1H NMR
2,2'-dibenzyl amino-methyl ester
Pulse Sequence: ROESY
Solvent: Acetone
Ambient temperature
File: AW-NMR-23 ROESY
INOVA-500 "Waters500"
Relax. delay 1.000 sec
Acq. time 0.201 sec
Width 5106.3 Hz
2D Width 5106.3 Hz
16 repetitions
2 x 200 increments
OBSERVE H1, 499.7055694 MHz
DATA PROCESSING
Gauss apodization 0.083 sec
F1 DATA PROCESSING
Gauss apodization 0.083 sec
FT size 2048 x 2048
Total time 5 hr, 34 min, 34 sec

325
2,2'-dibenzyl amino-methylester

Pulse Sequence: ROESY
Solvent: Acetone
Ambient temperature

Relax. delay 1.000 sec
Mixing 0.200 sec
Acq. time 0.201 sec
Width 5106.3 Hz
2D Width 5106.3 Hz
16 repetitions
2 x 200 increments

OBSERVE H1, 499.7055694 MHz

DATA PROCESSING
Gauss apodization 0.093 sec

FT size 2048 x 2048
Total time 2 hr, 34 min, 34 sec
2,2'-dibenzyl amino-methylester

Pulse Sequence: ROESY
Solvent: Acetone
Ambient temperature
File: AW-NMR-23_ROESY
INOVA-500 "waters500"

Relax. delay 1.000 sec
Mixing 0.200 sec
Acq. time 0.201 sec
Width 5106.3 Hz
20 width 5106.3 Hz
16 repetitions
2 x 200 increments
OBSERVE H1, 499.705654 MHz
DATA PROCESSING

Gauss apodization 0.093 sec
F1 DATA PROCESSING
Gauss apodization 0.036 sec
FT size 2048 x 2048
Total time 2 hr, 34 min, 34 sec
2,2-diphenyl amino-methylester

Pulse sequence: ROESY

Fl (ppm)

3.46
3.48
3.50
3.52
3.54
3.56
3.58
3.60
3.62
3.64
3.66
3.68

7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4 6.2 6.0

I assume n w/c, but can't rule out l w/c unless see NOE enhancement at 3.511.

Overlapping region n/w c ⇄ c/d.
2,2-(benzyl amino)methylester
Pulse Sequence: ROESY
ROESY

2,2-diethyl amino-methyl ester

Pulse sequence: ROESY
Appendix 6: Supplemental Synthetic Methods and Characterization for Chapter 6
Tributylstannane analogue of AM630:

Molecular Weight = 667.5090
Exact Mass = 668.3000

Synthesis

To a screw-cap reaction vial was added AM630 (0.03965 mmol, 0.020g), PdCl$_2$(PhCN)$_2$ (0.001983 mmol, 0.0011g), and tricyclohexylphosphine (0.00397 mmol, 0.0011g). After flushing the vial with argon, hexabutylditin (0.1190mmol, 0.0590mL) and dioxane (2.0 mL) were added and the vial was capped and heated to 110°C for two nights (40 hours). The reaction mixture was cooled to room temperature and concentrated. The product was purified by chromatography (40% ethyl acetate/hexanes) on triethylamine protected silica gel (silica gel prepared in 1% TEA/Hexanes and washed with hexanes). Product was recovered as a solid in 58.2% yield (0.0154g).

$^1$H NMR (400 MHz, d-acetone): $\delta = 7.44$ (d, $J = 8.8$ Hz, 2H), 7.61 (s, 1H), 7.36 (d, $J = 8.0$ Hz, 1H), 7.15 (d, $J = 7.2$ Hz, 1H), 7.03 (d, $J = 8.8$ Hz, 2H), 4.41 (t, $J = 6.8$ Hz, 2H), 3.90 (s, 3H), 3.61 (t, $J = 5.2$ Hz, 4H), 2.75 (t, $J = 6.4$ Hz, 2H), 2.59 (s, 3H), 2.50 (br t, $J = 4.0$ Hz, 4H), 1.61 (m, $J = 8.0$ Hz, 6H), 1.37 (m, $J = 8.4$ Hz, 6H), 1.13 (m, $J = 8.4$ Hz, 6 H), 0.89 (t, $J = 7.6$ Hz, 9 H) ppm.

$^{13}$C NMR (100.36 MHz, d-acetone): $\delta = 191.45, 163.62, 144.09, 137.27, 135.14, 134.00, 132.16, 129.43, 128.52, 121.22, 118.40, 114.50, 114.36, 67.64, 58.55, 55.98, 55.19, 42.00, 28.20, 14.15, 12.78, 10.36$ ppm.


Appearance: Colorless oil; Prepared 05/2011; Store at or below 4°C.
Boronic ester analogue of AM630:

Molecular Weight = 504.42548
Exact Mass = 504.279552

Synthesis

To a screw-cap reaction vial was added AM630 (0.0492 mmol, 0.0248g), bis(pinacolato)diboron (0.1475mmol, 0.0375g), potassium acetate (0.1475mmol, 0.0145g), and PdCl2dpff (0.002459 mmol, 0.0020g). After flushing the vial with argon, dimethylformamide (2.0 mL) was added and the vial was capped and heated to 100°C overnight. The reaction mixture was cooled to room temperature and concentrated. The product was purified by chromatography on silica gel (2% methanol/dichloromethane). Product was recovered as an oil in 73.0% yield (0.0181g).

^1H NMR (500 MHz, d-acetone): δ = 7.91 (s, 1H), 7.753 (d, J = 9 Hz, 2H), 7.47 (d, J = 8.5 Hz, 1H), 7.38 (d, J = 7.5 Hz, 1H), 7.06, (d, J = 9 Hz, 2H), 4.47 (t, J = 6 Hz, 2H), 3.93 (s, 3H), 3.63 (t, J = 4.5 Hz, 4H), 2.79 (t, J = 6.5 Hz, 2H), 2.64 (s, 3H), 2.51 (t, J = 4 Hz, 4H), 1.38 (s, 12H) ppm.

^13C NMR (100.36 MHz, d-acetone): δ = 190.63, 162.95, 145.12, 135.96, 134.05, 131.50, 130.04, 127.04, 119.87, 116.75, 113.91, 113.62, 83.64, 66.80, 58.05, 55.19, 54.36, 41.42, 24.58, 12.11 ppm.

Properties: Polar. Known solubility in dichloromethane, ethyl acetate, acetone, methanol, dioxane, dimethylformamide, dimethyl sulfoxide. Solubility in water unknown, however when crude reaction mixture was partitioned between ethyl acetate and brine, product was recovered from the organic layer.

Appearance: Colorless oil; Prepared 03/2011, Repurified 06/2011; Store at or below 4°C
AW-M-55

6-(tributylstanny1)-1H-indole-3-carbaldehyde

Molecular Weight = 434.2028
Exact Mass = 435.1584

Synthesis

To a screw-cap reaction vial was added 6-bromoindole-3-carbaldehyde (0.0893 mmol, 0.020g), PdCl$_2$(PhCN)$_2$ (0.004465 mmol, 0.0026g), and tricyclohexylphosphine (0.00893 mmol, 0.0025g). After flushing the vial with argon, hexabutylditin (0.2679mmol, 0.133mL) and dioxane (2.0 mL) were added and the vial was capped and heated to 110°C overnight. The reaction mixture was cooled to room temperature and concentrated. The product was purified by chromatography (20% ethyl acetate/hexanes) on triethylamine protected silica gel (silica gel prepared in 1% TEA/Hexanes and washed with hexanes). Product was recovered as a solid in 82.0% yield (0.0318g).

$^1$H NMR (400 MHz, d-acetone): $\delta$ = 11.10 (br s, 1H), 10.03 (s, 1H), 8.22 (d, $J$ = 7.2 Hz, 1H), 8.16 (s, 1H), 7.69 (s, 1H), 7.36 (d, 8.0 Hz, 1H), 1.60 (m, $J$ = 7.6 Hz, 6H), 1.37 (m, $J$ = 7.2 Hz, 6H), 1.13 (m, $J$=8.0 Hz, 6H), 0.88 (t, $J$ = 7.2 Hz, 9H) ppm.

$^{13}$C NMR (100.36 MHz, d-acetone): $\delta$ = 184.60, 138.04, 136.96, 135.86, 129.90, 124.90, 121.24, 120.03, 119.43, 27.37, 13.31, 9.52 ppm.


Appearance: light pink, oily residue; Prepared 05/2011; Store at or below 4°C.
Molecular Weight = 271.1193
Exact Mass = 271.1380

Synthesis

To a screw-cap reaction vial was added 6-bromoindole-3-carbaldehyde (0.2232 mmol, 0.050g), bis(pinacolato)diboron (0.6696mmol, 0.1700g), potassium acetate (0.6696mmol, 0.06571g), and PdCl2dppf (0.0112 mmol, 0.0091g). After flushing the vial with argon, dioxane (4.0 mL) was added and the vial was capped and heated to 110°C overnight. The reaction mixture was cooled to room temperature and concentrated. The crude material was dissolved in ethyl acetate, washed with brine, dried with magnesium sulfate, and concentrated. The product was purified by chromatography on silica gel (10% ethyl acetate/hexanes). Product was recovered as a solid in 51.6% yield (0.0312g).

$^1$H NMR (400 MHz, d-acetone): $\delta =$ 11.22 (br s, 1H), 10.05 (s, 1H), 8.27 (d, $J =$ 2.8 Hz, 1H), 8.24 (d, $J =$ 8.0 Hz, 1H), 7.97 (s, 1H), 7.65 (d, $J =$ 8.0 Hz, 1H), 1.35 (s, 12H) ppm.

$^{13}$C NMR (100.36 MHz, d-acetone): $\delta =$ 184.70, 138.33, 137.29, 128.24, 137.26, 120.81, 119.42, 119.11, 119.10, 83.75, 24.59 ppm.

Properties: Polar. Known solubility in dichloromethane, ethyl acetate, acetone, dioxane. Solubility in water unknown, however when crude reaction mixture was partitioned between ethyl acetate and brine, product was recovered from the organic layer.

Appearance: white solid, m.p. = 250-253°C

Prepared 05/2011.

Store at or below 4°C
5-(tributylstannyl)-1H-indole-3-carbaldehyde

Molecular Weight = 434.2028
Exact Mass = 435.1584

Synthesis

To a screw-cap reaction vial was added 5-bromoindole-3-carbaldehyde (0.2232 mmol, 0.050g), PdCl₂(PhCN)₂ (0.01116 mmol, 0.0065g), and 1,2-Bis(diphenylphosphino)ethane (0.02232 mmol, 0.0089g). After flushing the vial with argon, hexabutylditin (0.6696mmol, 0.332mL) and dioxane (4.0 mL) were added and the vial was capped and heated to 110°C overnight. The reaction mixture was cooled to room temperature and concentrated. The product was purified by chromatography (20% ethyl acetate/hexanes) on triethylamine protected silica gel (silica gel prepared in 1% TEA/Hexanes and washed with hexanes). Product was recovered as a solid in 60.3% yield (0.584g).

¹H NMR (500 MHz, d-acetone): δ = 11.14 (br s, 1H), 10.07 (s, 1H), 8.45 (s, 1H), 8.18 (d, J = 2.5 Hz, 1H), 7.57 (d, J = 8.0 Hz, 1H), 7.41 (d, J = 6.4 Hz, 1H), 1.64 (m, J = 7.5 Hz, 6 H), 1.40 (m, J = 7.6 Hz, 6H), 1.16 (m, J = 8.0 Hz, 6H), 0.92 (t, J = 7.0 Hz, 9 H) ppm.

¹³C NMR (100.36 MHz, d-acetone): δ = 184.60, 137.88, 136.99, 133.55, 131.22, 129.74, 125.24, 119.11, 112.16, 27.37, 13.30, 9.54 ppm.


Appearance: light pink solid (m.p. = 84-87°C); Prepared 05/2011; Store at or below 4°C.
5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-indole-3-carbaldehyde

Molecular Weight = 271.1193
Exact Mass = 271.1380

Synthesis

To a screw-cap reaction vial was added 5-bromoindole-3-carbaldehyde (0.2232 mmol, 0.050g), bis(pinacolato)diboron (0.6696mmol, 0.1700g), potassium acetate (0.6696mmol, 0.06571g), and PdCl2dppf (0.0112 mmol, 0.0091g). After flushing the vial with argon, dioxane (4.0 mL) was added and the vial was capped and heated to 110°C overnight. The reaction mixture was cooled to room temperature and concentrated. The crude material was dissolved in ethyl acetate, washed with brine, dried with magnesium sulfate, and concentrated. The product was purified by chromatography on silica gel (10% ethyl acetate/hexanes). Product was recovered as a solid in 42.8% yield (0.0259g).

\[ \delta = 11.26 \text{ (br s, 1H)}, 10.09 \text{ (s, 1H)}, 8.80 \text{ (s, 1H)}, 8.25 \text{ (d, } J = 1.5 \text{ Hz, 1H)}, 7.70 \text{ (d, } J = 8.5 \text{ Hz, 1H)}, 7.57 \text{ (d, } J = 8.5 \text{ Hz, 1H)}, 1.38 \text{ (s, 12 H ppm).} \]

\[ \delta = 184.80, 139.64, 137.83, 129.97, 129.32, 124.43, 119.69, 111.69, 83.66, 24.62 \text{ ppm.} \]

Properties: Polar. Known solubility in dichloromethane, ethyl acetate, acetone, dioxane. Solubility in water unknown, however when crude reaction mixture was partitioned between ethyl acetate and brine, product was recovered from the organic layer.

Appearance: white solid, m.p. = 219-222°C; Prepared 05/2011; Store at or below 4°C
AM630

MW = 504.3607
Exact mass = 504.0910

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6-bromoindole-3-carbaldehyde

MW = 224.0540
Exact mass = 222.9633
CAS: 17826-04-9

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5-bromoindole-3-carbaldehyde

MW = 224.0540
Exact mass = 222.9633
CAS: 877-03-2