I. ESTROGEN RECEPTOR-MEDIATED INHIBITION OF INFLAMMATORY SIGNALING: IMPLICATIONS FOR TREATMENT OF BREAST CANCER II. SMALL MOLECULE COACTIVATOR-BINDING INHIBITORS

BY

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DISSERATION

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ABSTRACT

Approximately two-thirds of breast cancers result from overexpression of the estrogen receptor, a ligand-dependent transcription factor. Traditional therapy employs drugs that bind directly to the receptor but do not mediate transcription. Unfortunately most breast cancers develop resistance to these antagonists. The mechanism of this resistance is not fully known. This thesis describes two different approaches to circumventing this resistance.

In Chapter 2, the unique anti-proliferative and anti-inflammatory activity of a lead compound, OBHS, is described. A crystal structure of OBHS bound to the estrogen receptor provided the basis for structural and functional analogs of OBHS. These compounds exhibited weaker binding to the estrogen receptor than OBHS; however some displayed comparable anti-inflammatory activity.

In Chapter 3, compounds were designed and synthesized in order to inhibit the binding of the estrogen receptor to coactivator proteins that facilitate transcription. We used a peptidomimetic approach to mimic conserved amino acids on the coactivator proteins. Compounds designed in this way did not inhibit the interaction between the estrogen receptor and a coactivator protein.
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Chapter 1: The Role of the Estrogen Receptor in Breast Cancer and Implications for Therapy

1.1 INTRODUCTION

The American Cancer Society estimated approximately 192,000 new cases of invasive breast cancer and 40,000 deaths caused by breast cancer in 2009. Only skin cancer has a higher incidence, and only lung cancer has a higher mortality rate. Approximately two-thirds of breast cancers are estrogen receptor (ER) positive, meaning they have elevated levels of this protein.

1.2 THE ESTROGEN RECEPTOR

The ER belongs to the nuclear hormone receptor superfamily of ligand-dependent transcription factors. Other nuclear hormone receptors, which are closely related to the estrogen receptor in structure and function, include the androgen, progesterone, and glucocorticoid receptors. The ER controls a wide variety of biological functions, partly due to its widespread and diverse tissue distribution. Both female and male reproductive systems express the ER; inappropriate activity of ER in this tissue leads to disease states such as breast, uterine and ovarian cancer. Additionally the ER can be found in the cardiovascular, skeletal and central nervous systems. Estrogens have important effects on maintaining bone health and various cognitive functions. Several endocrine-disrupting compounds (EDCs) exert some of their developmental toxicity through estrogenic pathways in the brain. Thus, the ER provides a target for understanding the causes of cancer and developing novel treatments.

1.3 MOLECULAR MECHANISM

ER and other nuclear hormone receptors contain five domains (Figure 1.1): (1) the N-terminal A/B domain containing an activation function-1 (AF-1) region; (2) the DNA binding domain (DBD), also called the C-domain; (3) the hinge region, also called the D-domain; (4) the E-domain, containing the ligand-binding domain (LBD) and an activation function-2 (AF-2)
region; and (5) the C-terminal F-domain. Both the AF-1 and the AF-2 mediate transcription; the
AF-1 acts in a ligand-independent fashion, but the response is not as strong as that of the ligand-
dependent AF-2. The AF-1 domain also contains sites for post-translational modification via
phosphorylation of certain amino acid residues.

The ER exists as one of two subtypes, ERα and ERβ, which have different distributions
among the various tissues and cell types.\(^1\) ERα is found in higher concentrations in the uterus,
prostate stromal cells, Leydig cells of the testes, epididymis, bone and breast. ERβ is found in
higher concentrations in the epithelium of the prostate, granulosa cells of the ovary, and bone
marrow. Various regions of the brain also contain different relative levels of ERα and ERβ. The
two ER subtypes have high sequence identity in the DBD (97%), some identity in the LBD (56%),
and little identity in the A/B, D, and F domains. Both ERα and ERβ bind the
endogeneous ligand for the ER, 17β-estradiol (E2), with high affinity; the affinity is slightly
higher for ERα than ERβ. Although overall the ligand binding domains of ERα and ERβ have
only modest sequence conservation, the ligand-binding pocket of ERβ is only different from ERα
in two significant ways. First, the volume of the ERα binding pocket is approximately 490 Å,
whereas the volume for the ERβ pocket is approximately 390 Å.\(^2\) The change in pocket volume
reflects the mutation of Leu 384 to a methionine and Met 421 to an isoleucine.

At the molecular level, the function of the estrogen receptor is dictated by its
conformation, which in turn depends on the nature of the bound ligand. E2 is a largely planar,
hydrophobic molecule with two hydrogen bond donors: a phenyl hydroxyl group on the A-ring,
which forms a hydrogen bond network with Glu353 and Arg394, and an aliphatic hydroxyl
group on the D-ring, which forms a hydrogen bond with His524. The A-ring phenol hydrogen
contributes more to the energy of binding than the D-ring hydroxyl hydrogen. The formation of
these hydrogen bonds favors a conformation of the receptor in which the C-terminal helix (helix 12) folds over the surface of the receptor and creates a hydrophobic groove on the surface, as shown in Figure 1.2. The coactivator proteins responsible for facilitating transcription contain conserved LXXLL motifs, which bind in this groove. At the ends of the grooves are two charged amino acids, K362 and E542, which form a charge clamp; this charge clamp forms hydrogen bonds with residues outside the LXXLL box. There are several classes of these coactivator proteins, including the p160 class and the p300 class. The p160 class contains the steroid receptor coactivator (SRC) proteins; the p300 class contains CBP and related proteins. These proteins were initially known for their ability to recruit transcription machinery. Later, it was discovered that some of these proteins possess histone acetyltransferase activity. Acetylation of lysines on the histone proteins, around which DNA is coiled, facilitates the uncoiling of DNA necessary for transcription. Figure 1.3 summarizes this process. Chapter 3 discusses attempts to inhibit the ER-coactivator interaction.

1.4 TYPES OF LIGANDS AND THERAPY

The ER binds an eclectic set of ligands, which can be steroidal or nonsteroidal, as well as synthetic or naturally occurring. (Figure 1.4) One of the earliest synthetic nonsteroidal estrogenic ligands was diethylstilbestrol (DES), which is a very potent agonist. DES was used in treatment for infertility and risk of premature delivery until it was observed that women who had been exposed to DES in utero developed a cancer rare form of vaginal cancer at an early age. Reproductive and developmental toxicity has also been observed in males. Faslodex, on the other hand, is a potent steroidal full antagonist. Faslodex contains a long sulfoxide-containing aliphatic side chain which extends from the binding pocket towards the coactivator binding groove. This prevents helix 12 from adopting the agonist conformation and subsequent
coactivator protein binding. Naturally occurring estrogenic compounds include genistein found in high levels in soy, and its analogs and metabolites. Various pesticides and herbicides have also been shown to have estrogenic activity, although the affinities of these compounds for the ER are often quite low.

Clinically, breast cancer and other diseases in which the ER is implicated are treated by a class of compounds called selective estrogen receptor modulators (SERMs). These compounds have an aminoethyl side chain that functions in a manner similar to the long side chain found on Faslodex (Figure 1.2c). These compounds may function as antagonists or agonists, depending on cellular context. Two of the most commonly used SERMS, tamoxifen and raloxifene, are shown in Figure 1.5. Tamoxifen acts as an agonist in uterus and bone tissue, but as an antagonist in breast tissue. Raloxifene, on the other hand, acts as an agonist in bone tissue, but an antagonist in both uterus and breast. Due to its antagonist action in breast tissue, tamoxifen has been effectively used for the treatment of breast cancer. However, prolonged treatment with tamoxifen leads to recurrence of cancer. It appears that, over time, tamoxifen begins to act as an agonist. Mechanisms that might explain this phenomenon will be discussed later in this introduction.

An alternative to SERM therapy is the class of compounds known as aromatase inhibitors (AIs). Two of these are shown in Figure 1.5. The biosynthesis of E2 concludes with oxidation of testosterone to E2, which generates the aromatic A ring of E2. The enzyme responsible for this process is a member of the cytochrome P450 family (CYP19A1), which is also known as aromatase. Inhibition of this enzyme starves ER-positive tumor cells of E2 needed for receptor function. Unfortunately, resistance is also seen with AIs; this may result from ligand-independent activation of ER, which will be described below.
1.5 THREE-DIMENSIONAL STRUCTURE OF POCKET

While the structure of estradiol is largely planar, it was predicted and later confirmed by x-ray crystallography that the binding pocket is significantly larger than estradiol. Unoccupied space exists above and below the plane of E2 (Figure 1.6); filling this space with hydrophobic groups might increase binding affinity. This partially explains the high binding affinity of DES; the ligand flips so that the ethyl chains extend vertically in the pocket (Figure 1.7). Many have tried to design ligands with inherent three-dimensional structures that would fill up the binding pocket and interact with the hydrophobic ceiling and floor of the binding pocket. Endo has designed bicyclooctanes and carboranes with phenol rings as three-dimensional estrogen ligands. The Katzenellenbogen group has synthesized cyclopentadienyl rhenium compounds with carbonyl ligands, giving the core a three dimensional structure as models for PET-imaging agents. The group has also synthesized oxabicyclic compounds with moderate binding affinities. One of these compounds displayed unusual and promising anti-inflammatory activity in addition to its antagonistic activity. Design of analogs based on this compound will be discussed in Chapter 2.

1.6 ER AND INFLAMMATION

The exact mechanism of tamoxifen resistance remains unclear, but it may be related to the ability of SERMS to act as agonists, when their structure indicates they should function as antagonists. This agonistic activity may proceed through extranuclear estrogenic pathways. In the cytoplasm, the ER interacts with complex signal transduction cascades. One example of these is the MAPK pathway, which is involved in cell growth and cell death. The MAPK pathway begins with various growth factors binding to receptors on the cell surface. These receptors phosphorylate kinases, which phosphorylate downstream kinases. Ultimately, transcription
factors are activated by phosphorylation, and gene expression is activated. Some of these kinases phosphorylate the ER, which leads to gene expression modulated by the ER in a ligand-dependent manner.\textsuperscript{10}

Another pathway involves cross-talk between the ER and NF-κB, a family of transcription factors activated in response to stress signals. Inflammatory triggers such as endotoxins (e.g., lipopolysaccharide), tumor necrosis factor α (TNFα), growth factors and reactive oxygen species bind to cell surface receptors in a manner analogous to the MAPK pathway. These transmembrane receptors initiate a similar phosphorylation cascade (Figure 1.8).

NF-κB is sequestered in the cytoplasm by IκB proteins, which block the nuclear localization sequence of NF-κB. An upstream kinase, IκK phosphorylates IκB, rendering it susceptible to post-translational modification by addition of a small protein called ubiquitin to lysine residues on the protein. After several more ubiquitin proteins are added to additional sites, or to previously added ubiquitins, IκB becomes susceptible to degradation by the proteasome. This process releases NF-κB, which is then free to enter the nucleus and exert its activation of transcription. The estrogen receptor is known to inhibit NF-κB, although the mechanism is not fully understood. As both the ER and NF-κB are transcription factors found in the nucleus and cytoplasm, there are many possible levels of interaction.

Several studies have investigated the interaction between NF-κB and the ER. In spinal cord astrocytes, addition of ER inhibited expression of the CCL2 cytokine, the expression of which is controlled by NF-κB.\textsuperscript{11} Interestingly, ER did not prevent the translocation of NF-κB to the nucleus, indicating that the cross-talk may involve interactions at the DNA-binding level. ChIP assays showed that ER inhibits binding of NF-κB to the CCL2 gene promoter. CCL2 expression levels decreased after treatment an ERα selective agonist.
Nettles et al. explored ability of the ER to mediate the expression of monocyte chemoattractant protein-1 (MCP-1), which is activated by NF-κB.\(^\text{12}\) MCP-1 expression was inhibited in MCF-7 cells using E2 and other ER agonists, but not by hydroxytamoxifen. Pull-down assays showed that removal of the LBD, but not any other domain, inhibited the ability of the ER to inhibit NF-κB activity. The coactivator protein CBP proved to be an essential component of the ER–NF-κB interaction. E2 caused the ER to displace CBP from NF-κB.

The crosstalk between the ER and NF-κB has provided a new target for breast cancer therapy, particularly those with underlying inflammation. Wyeth Pharmaceuticals developed several classes of ligands as anti-inflammatory compounds that would act in an ER-dependent manner (Figure 1.9). The first series of compounds, 3-arylindazoles, showed nanomolar inhibition in an NF-κB reporter gene assay in cells also transfected with ER.\(^\text{13}\) These compounds showed little or no uterine weight increase in vivo, and weak creatine kinase stimulation. One of these compounds, WAY-169916, has been investigated in further studies. In a high-throughput screen, an additional scaffold containing a hydroxyphenylsulfonamide was discovered.\(^\text{14}\) Derivatives of a lead compound showed comparable activity to WAY-169916. Finally, cyanopropionates were shown to have good NF-κB activity with low ER activity designed for the treatment of rheumatoid arthritis.\(^\text{15}\) In collaboration with Kendall Nettles at The Scripps Research Institute in Jupiter, Florida, we have identified a lead compound with low ER binding activity but strong inhibition of estrogen-dependent inflammation pathways; this compound, and efforts to design similar compounds, will be discussed in Chapter 2.
1.7 FIGURES

Figure 1.1 The functional domains of the ER mapped to its sequence.
Figure 1.2 (A) ER in the agonist conformation, with bound E2 (space-filling model). Shown in green is helix 12, which folds over E2 to create the coactivator binding groove (triangle). A fragment of an NR Box from a coactivator protein is shown in gold. (B) Electrostatic potential map of the coactivator binding groove. Areas with partial positive charge are colored red, areas with a partial negative charge are colored blue, and nonpolar areas are colored teal. The ILXXLL fragment of a coactivator peptide is shown as a red tube, with only the conserved leucine/isoleucine residues shown. (C) ER in the antagonist conformation, bound to TOT. The basic side chain protrudes towards the area occupied by helix 12 in the agonist conformation. Helix 12, which now blocks the coactivator binding groove, is shown in red.
Figure 1.3 Schematic of the mechanism of action by which ER activates expression of target genes.
Figure 1.4 Structures of endogeneous estrogens, synthetic agonists, phytoestrogens and xenoestrogens.
Figure 1.5 Structures of SERMs, antagonists and aromatase inhibitors.
**Figure 1.6** Representation of the binding pocket of ER. Key hydrogen bonds are made between ARG394 and GLU353 and the 3-hydroxyl group, as well as HIS524 and the 17-hydroxy group. The total volume of the binding pocket is represented by green dots. A representation of the molecular surface of E2 is shown in yellow.
Figure 1.7 The binding pocket of ER in the presence of DES. Green dots represent the receptor pocket volume. The electrostatic potential map of DES is colored yellow. The two ethyl groups extend into space not occupied by E2.
Figure 1.8 The NF-κB pathway. Signaling molecules are shown outside the cell, which initiate phosphorylation of proteins in the cytoplasm. Ultimately p50, p52, and members of the Rel family (which heterodimerize to form NF-κB units) are freed from IκBα and enter the nucleus to activate various genes.16
Figure 1.9 Inhibitors of NF-κB developed by Wyeth for treatment of inflammation.

1.8 REFERENCES


2.1 INTRODUCTION

Design behind initial oxabicyclic compounds

The search for synthetic estrogens and anti-estrogens has mostly focused on designing estrogens with planar structures, mimicking the near planarity of E2. Various heterocyclic cores have been synthesized with aryl and aliphatic substituents. Cores having similar arrangements of phenols and alkyl chains can have markedly different activities, despite overlaying very well with each other.\(^1\) As mentioned in Chapter 1, three dimensional ligands have been synthesized in order to fill the binding pocket more completely. With this in mind, the Katzenellenbogen group and others have designed ligands with a more three-dimensional structure. The diarylethylene motif found in tamoxifen was incorporated into cyclohexane\(^2\), bicyclononane\(^3\) and adamantane cores. The latter two ligands possess extraordinary binding affinities compared to E2. We developed a 7-oxabicyclo[2.2.1]heptene scaffold to create three-dimensional ligands with good affinity.\(^4\) Several compounds in this series were synthesized and tested for ER binding activity in our radiometric binding assay. In these assays the binding affinities are expressed as a percentage of the binding affinity of E2, or relative binding affinity (RBA). This value is calculated as \(K_a(E2) / K_a(\text{ligand})\). Most of these ligands exhibited weak affinities; the RBAs of the best compounds ranged from 1% to 10%.

These ligands were included in a library of compounds given to Kendall Nettles of The Scripps Research Institute at Jupiter, Florida, who was searching for new anti-inflammatory compounds; these compounds were also submitted to high-throughput crystal structure experiments. The Y537S ER mutant,\(^5\) which is constitutively active, was used in these crystal studies. This mutation stabilizes folding of helix 12 into the active conformation, creating a
binding pocket similar to that seen in agonist structures. Use of this mutant increases the likelihood that the ER will crystallize with a ligand bound even if the ligand is only a weak agonist. Nettles was able to obtain crystal structures of the ER with low-affinity ligands in a combinatorial fashion. Two of the crystal structures obtained by this method contained ligands from the oxabicyclic series with very low binding affinities. These crystal structures showed unusual distortions in the crystal structure, which will be described in more detail later. The Katzenellenbogen and Nettles groups are collaborating to determine whether these observations in the crystal structure lead to any novel biological activities.

The ER inhibits the NF-κB mediated inflammatory response, although the mechanism by which this inhibition occurs is unclear. Considering both ER and NF-κB are present in the cytoplasm and the nucleus, their interaction may occur in either location. They may bind directly, they may communicate via a bridging factor, they may compete for common coactivators, or they may influence shared signal transduction pathway.

Preliminary Biological Results from the Nettles Lab

Fortunately one of the oxabicycloheptene ligands showed promising results in anti-inflammatory assays. This compound also happened to be the oxabicyclic ligand with the highest ER activity. This ligand, termed OBHS (oxabicycloheptenesulfonate), was subjected to additional inflammatory assays in the Nettles group, which are described below. In these assays OBHS is compared to tamoxifen, which is not expected to inhibit the NF-κB pathway. In fact, the tamoxifen resistance mentioned in Chapter 1 may result from activation of this pathway by tamoxifen.

In preliminary studies from the Nettles lab OBHS showed promising activity in reporter gene assays using MCF-7 cells measuring either ER or NF-κB activity. ER transcription assays
showed that OBHS acted as an antagonist in a manner similar to tamoxifen. In NF-κB transcription assays, tamoxifen was unable to inhibit gene expression; both OBHS and E2 were able to inhibit NF-κB – mediated gene expression. Taken together, these results suggested that OBHS had the potential to address a dilemma in traditional endocrine cancer therapy. ER-positive cancer cells proliferate in the presence of agonists; the agonists allow the ER to inhibit the inflammatory response associated with a poorer prognosis in breast cancer. Traditional therapy that antagonizes the ER (such as SERMs) suppresses the anti-inflammatory activity of the ER. OBHS showed a biological profile reminiscent of the Wyeth compounds developed for the treatment of arthritis and other inflammation. OBHS has a better ER binding affinity than the Wyeth compounds; the significance of the ER binding affinity depends on the role of ER in inhibiting NF-κB.

Additional experiments were conducted in the Nettles lab to determine if ER is required for the unique activity of OBHS. In reporter gene assays conducted in ER-negative cells OBHS was unable to inhibit activation of pro-inflammatory genes. If ER was cotransfected into the same cells, OBHS regained its anti-inflammatory activity. Additionally anti-inflammatory assays were conducted in the presence of the pure ICI 182,780 antagonist in addition to OBHS or dexamethasone, a non-estrogenic inhibitor of NF-kB – mediated inflammation. ICI was able to block the activity of OBHS but not dexamethasone. These results taken together strongly suggest a role for ER in the activity of OBHS.

To further test the role of macrophages in stimulation of breast cancer cell growth, MCF-7 cells and macrophages were grown by the Nettles group in wells separated by a semi-permeable membrane. Thus, the cells did not contact each other directly, but communicated through cytokines and other signaling factors. The presence of macrophages caused increased
proliferation in the MCF-7 cells; this effect was increased by the addition of tumor necrosis factor α (TNF-α), a known initiator of the NF-κB pathway. In the absence of macrophages tamoxifen was able to inhibit proliferation. In the presence of macrophages and TNF-α, tamoxifen was unable to inhibit proliferation. Only OBHS was able to inhibit proliferation with or without the addition of macrophages, and was able to do so in a dose-dependent fashion. Tamoxifen was able to inhibit IL-6 mRNA production, as long as macrophages were not present. Even in the absence of macrophages, tamoxifen was unable to inhibit CCL5 mRNA production. OBHS, on the other hand, was able to inhibit IL-6 and CCL5 gene transcription, even in the presence of macrophages.

Analysis of proteins known to be regulated by NF-κB also showed that OBHS inhibits NF-κB mediated transcription. The mRNA levels for two proteins involved in inflammation and cell growth were measured. Both IL-6 and CCL5 were induced by TNFα in MCF-7 cells with or without the addition of macrophages. In addition to measuring cancer cell proliferation, the growth of macrophages was also examined. The same activity seen in MCF-7 cells was also seen in macrophages, indicating that OBHS inhibits both cancer and macrophage proliferation.

Inspired by these results we synthesized several grams of OBHS for use in animal studies. Professor Nettles and colleagues prepared a mouse model for breast cancer by injection of MCF-7 cells. The growth of these cells was inhibited by either tamoxifen or OBHS; the tamoxifen-induced inhibition of tumor growth is not surprising. We were pleasantly surprised to find that OBHS also decreased tumor progression (Figure 2.1). When macrophages were also injected into mice with MCF-7 cells, the tumors began to grow at a much faster rate, and under these conditions, tamoxifen was unable to prevent or decrease tumor growth. Only OBHS was able to cease tumor progression in some cases; in most cases OBHS treatment caused complete
tumor loss (Figure 2.2). Analysis of NF-κB activity and levels of IL-6 and CCL5 in treated animals confirmed the results seen from in vitro tamoxifen or OBHS treatment (Figure 2.3). We do not know of any other compounds which show such promising activity in a model of breast cancer exacerbated by inflammation. Therefore, we set out to design structural and functional analogs of OBHS as potential breast cancer therapeutic agents, and to further understand the unique and promising mechanism of action.

2.2 DESIGN AND SYNTHESIS

2.2.1 Crystal structure analysis

Professor Nettles obtained crystal structures of three oxabicyclic compounds: OBHS, and two diester derivatives with very low RBAs. The crystal structures of the low-binding esters showed some significant distortions from the E2 crystal structures. These effects were magnified in the OBHS structure. One of the main effects seen in the crystal structures is a deviation in helix 11, the start of which forms the dimer interface and the end of which can interact with helix 12 in certain circumstances. Helix 11 is the longest helix in the ER structure, although the length of this helix varies between structures of different ligands. In the crystal structure of OBHS, the helix begins to unwind at an earlier point than in other crystal structures. The loop between helix 11 and helix 12 is also greatly distorted in the crystal structure (Figure 2.4), although loops tend to be disordered in crystal structures. Additionally, His 524, which normally participates in hydrogen bonding with the 17-OH group of E2, is flipped out so that it is unable to participate in hydrogen bonding (Figure 2.5). The unusual distortions in the crystal structure of OBHS can be quantified by measuring the deviation at each residue between the E2 crystal structure and the OBHS crystal structure (Figure 2.6). The structures are almost identical through the middle of helix 11, except for some regions in the structure that correspond to unresolved residues in one
of the structures. The only significant difference in the crystal structure is seen in residues following His 524; the largest deviation is seen from residues 528 to 531. Computational studies indicate that His 524 also participates in a hydrogen bonding network which includes amino acids from several helices.\textsuperscript{7} Thus, it is possible that OBHS disrupts the dimer interface and creates a coactivator binding groove different from that formed by other ligands. Since the crystal structure was obtained using a mutant that favors the agonist conformation, it may not provide an accurate representation of the coactivator binding groove in the presence of OBHS.

We used the crystal structure as a basis for design of structural and functional analogs of OBHS. We first made additional compounds containing the oxabicyclic core as structural analogs. We were also interested in creating a general design for ligands which would exhibit the unusual NF-κB activity of OBHS. These compounds would not necessarily contain the oxabicyclic scaffold, but would contain functional groups that are necessary to induce a conformation of the ER induced by OBHS; necessary components would include the 1,2-diarylethylene moiety and a bulky pendant aryl group that would occupy the same space in the crystal structure as the phenyl sulfonate.

2.2.1 OBHS analogs containing a single phenol hydroxyl group

The first set of analogs synthesized were designed to investigate the role of the phenols, as one of the crystal structures of a low-affinity bicyclic analog indicated that only one of these phenols participated in a hydrogen bond. The original synthesis was used, with some modifications, to synthesize analogs of OBHS containing only one phenol (Scheme 2.1). A phenacyl bromide was alkylated with 4-methoxyphenylacetic acid with Et\textsubscript{3}N as the base in acetonitrile to generate ester 1. The phenacyl ester was treated with crushed KOH in acetonitrile to produce the diarylbutenolide 2. This butenolide could be reduced with DIBAL-H, but
deprotection of the resulting furan under acidic conditions caused decomposition due to the acid sensitivity of furan. Thus the butenolide was deprotected in neat pyridine hydrochloride at 220 °C, yielding 3 without need for further purification.

While these first three reactions could be carried out in high yields with minimal purification on a multi-gram scale, the selective DIBAL reduction proved to be more difficult, especially on a multi-gram scale. On smaller scales, adequate yields of the furan could be obtained after acidic workup. Unfortunately the yields could not be reproduced when using more than 10 mmol of 3. Several attempts were made to develop a synthesis that would be easily scalable, and allow for introduction of diversity on the two aryl groups. These attempts will be described later. Ultimately the problem was solved by switching to an inverse quench of the DIBAL into methanol. When methanol is added to large quantities of DIBAL and the intermediate hemiacetal, both will be quenched. If the aluminum adduct is hydrolyzed while DIBAL is still present, the hydroxyenal will be reduced to a butenediol, reducing the yield. By inverse quenching into a large excess of MeOH, the excess DIBAL and the aluminum adduct will be quenched quickly. Usage of this inverse quench procedure increased the yield of furan 4 to as high as 75%.

The final step in the synthesis of oxabicyclic ligands was the Diels-Alder reaction with diarylfurans and various dienophiles. In our group’s previous study, sulfonate and sulfone dienophiles were the most successful; accordingly we focused on these same dienophiles while exploring substitution patterns on the phenyl rings. The furan and diene were heated neat at 90 – 100 °C for 6 to 18 hours. The $^1$H NMR spectra were consistent with an exo adduct, using NMR analysis described by Zhou et al. Analogos 5 containing only one phenol hydroxyl group were obtained as a mixture of regiosomers, ranging from 1:1 to 4:1 after purification.
The oxabicyclics above were synthesized as racemates. Asymmetric Diels-Alder reactions are not known for the reaction between furans and vinyl sulfonates. Furan itself has been used in asymmetric auxiliary-based and catalyzed Diels-Alder reactions;\textsuperscript{8, 9} these reactions typically give \textit{endo} products under kinetic control, with a few exceptions. Before attempting to synthesize enantiopure OBHS, we wanted to know if there was a significant difference in binding affinities between the two enantiomers. Analytical chiral HPLC showed the two isomers could be readily separated using an (R,R)-Whelk OS-1 column. An (S,S)-Whelk OS-1 column was used for semi-preparative separation of the enantiomers. Approximately one milligram of each enantiomer was obtained. The HPLC traces for the racemate and each enantiomer are shown in Figure 2.7.

\textbf{2.2.2 Attempted improvements on OBHS synthesis}

Before improvements were made to the large scale synthesis of OBHS, an improved method was sought. An ideal synthesis would be more amenable to scale-up, and avoid low-yielding reactions at the end of the synthesis. Additionally, we were interested in modifying the two phenol-containing rings while avoiding the production of inseparable regioisomers. Towards this end, several routes to OBHS were investigated.

Zhou et al.\textsuperscript{4} prepared 3,4-diarylfuran by carbometalation of 3-phenyl-propynol with phenylmagnesium bromide, according to a general procedure developed by Fallis.\textsuperscript{10} This method generated highly substituted furans, albeit with low yields in many cases. However, this procedure did not work when changing the Grignard reagent to 4-methoxyphenylmagnesium bromide. The reaction appears to fail at the carbometalation step, as no consumption of propargyl alcohol was observed (Scheme 2.2)
3,4-Diarylfurans have also been synthesized by coupling of a 3,4-bisstannylfuran with aryl halides, or with 3,4-dihalofurans with aryl nucleophiles.\textsuperscript{11, 12} The 3,4-bisstannylfuran has been synthesized from a Diels-Alder / retro Diels-Alder sequence with bis(trialkylstannyl)acetylene and phenyloxazole.\textsuperscript{13} This reaction was carried out at very high temperature in the literature, but did not produce the product in high yield, and thus was not attempted (Scheme 2.3). Baldwin described a synthesis of 3,4-dibromofuran from \textit{trans}-2,3-dibromo-2-buten-1,4-diol using aqueous potassium dichromate. Unfortunately this procedure could not be reproduced (Scheme 2.4). The same product can be obtained by bis-stannylation of 2-butyn-1,4-diol catalyzed by Pd to yield the cis-distannyl alkene, followed by oxidation to the furan. Attempts at purification of the product of this reaction resulted in partial destannylation, giving a product that could not be separated from the distannane. \textit{cis}-2,3-Diaryl-2-buten-1,4-diols have been synthesized by a multi-component reaction using 1,4-dihydroxy-2-butyne, an aryl halide and an aryl boronic acid in the presence of palladium.\textsuperscript{14, 15} The arylpalladium intermediate produced from oxidative insertion into an aryl halide reacts with an alkyne to generate a vinylpalladium intermediate, which would then undergo a Suzuki coupling with the boronic acid. When carried out with 1,4-dihydroxy-2-butyne, 4-bromoanisole and 4-methoxyphenylboronic acid, only the unproductive side-product resulting from a Suzuki reaction between the aryl bromide and aryl boronic acid was isolated. Using the same alkyne with 4-methoxyphenylboronic acid under an atmosphere of oxygen (as a stoichiometric palladium oxidant), the same biphenyl byproduct was observed.

\textbf{2.2.3 Bicyclic OBHS analogs}

We turned to the synthesis of OBHS analogs in which the bridging oxygen would be replaced with a methylene or ethylene unit. We predicted that this change would lead to analogs
with higher ER binding affinity. The bridging oxygen in OBHS bound in the ER points either up or down towards a hydrophobic area of the binding pocket. Replacing the oxygen with carbon would provide a better match between ligand and receptor hydrophobicities. We attempted synthesis of both bicycloheptene and bicyclooctene; each has synthetic advantages and disadvantages.

*Bicyclo[2.2.1]heptene*

The bicyclo[2.2.1]heptene adduct could be synthesized from a Diels-Alder reaction between a vinyl sulfonate and a 3,4-diaircyclopentadiene. The desired cyclopentadiene has been synthesized by the Katzenellenbogen group as a precursor of a cyclopentadienyl ligand used in PET imaging.\(^{16}\) Cyclopentadiene itself is a very reactive diene in Diels-Alder reactions. However, substituted cyclopentadienes are less useful in Diels-Alder reactions, due to the presence of multiple regioisomers produced by rapid, reversible [1,5]-hydrogen shifts. In some cases mono- and di-substituted cyclopentadienes undergo regioselective Diels-Alder reactions catalyzed by Lewis acids.\(^{17-19}\) In order to obtain the *exo* product, we hoped that the same thermal conditions used to make OBHS might be used with cyclopentadienes. At elevated temperatures, the [1,5]-hydrogen shift occurs rapidly. The 2,3-diarylproduct was expected to react faster than either the 1,2-diaircyclopentadiene or the 1,5-diaircyclopentadiene, forming the desired adduct (Scheme 2.5).

The route described previously was briefly explored using an expensive cyclopentenone as the starting material. Two other more economical routes to the desired cyclopentadiene were explored. In the first route (Scheme 2.6) cyclopentenone was treated with iodine in pyridine / ether to generate alpha-iodoenone 6. This iodoenone underwent a Suzuki reaction with 4-methoxyphenylboronic acid using PdCl\(_2\)(PhCN)\(_2\) as the catalyst, triphenylarsine as the ligand,
and silver oxide as the base to yield 7. Addition of 4-methoxyphenyllithium (generated *in situ*), followed by dehydration produced the cyclopentadiene 8, albeit in low yield as a 2:1 mixture of isomers. A higher-yielding route was developed (Scheme 2.7), starting with a condensation between p-anisil and acetone to generate 9. The hydroxycyclopentenone was reduced to cyclopentenone 10 with either refluxing HI/AcOH, or TMSCl/NaI as a milder alternative. DIBAL reduction of the cyclopentenone, followed by dehydration, produced cyclopentadiene 8. Curiously the NMR of this compound indicated a ca. 20:1 ratio of 1,2-diarylcyclopentadiene to 2,3-diarylcyclopentadiene. Figure 2.8 shows the differences in the NMR spectra of 8 generated by the two routes described above. The product from DIBAL reduction was used in Diels-Alder reactions, as it was available in much larger quantities; 8 was subjected to Diels-Alder reaction conditions (with or without toluene as a solvent at temperatures ranging from 90 °C to 140 °C). Analysis of the crude product by HPLC, as well as attempts to purify the product by MPLC, indicated a complex mixture of at least 6 products (Figure 2.9). Deprotection of isolated peaks which showed NMR signals consistent with desired Diels-Alder adducts yielded <1 mg of product; as a result, satisfactory characterization of a pure compound could not be obtained.

*Bicyclo[2.2.2]octenes*

We then explored synthesis of diarylcyclohexadiene precursors to bicyclo[2.2.2]octenes. Cyclohexadienes do not undergo [1,5]-hydrogen shifts, leading to only one regioisomer when using a symmetrical diene. The drawback to using cyclohexadienes in the Diels-Alder reaction is that the distance between the termini of the diene is significantly larger than that of aliphatic dienes or cyclopentadienes and furans. With appropriate Lewis acid catalysts, or elevated temperatures cyclohexadienes will undergo Diels-Alder reactions. Unlike the cyclopentadiene system described above, the desired 2,3-diarylcyclohexadienes are not known in the literature.
Our attempts at cyclohexadienes began with alpha iodination of cyclohexenone to yield 11 in a manner analogous to cyclopentenone (Scheme 2.8). Enone 12 was produced by Suzuki coupling with 4-methoxyphenylboronic acid using the PdCl$_2$(PhCN)$_2$ / Ph$_3$As / Ag$_2$O system previously described for cyclopentenone 6. At this point there are no known ways to add the second aryl group with the appropriate double bond position. Addition of 4-methoxyphenylmagnesium bromide yielded a complex mixture of unknown products. Repeating the reaction with cerium chloride gave the cyclohexenol, but attempts to dehydrate the cyclohexenol regioselectively to the cyclohexadiene were unsuccessful. To install the second aryl group before the Diels-Alder reaction, we converted the cyclohexenone to the corresponding vinyl triflate 13 by generating the kinetic cross-conjugate enolate and quenching with Comins’ triflating reagent. This product could be obtained as a crude product in high yields. Attempts to further purify the product reduced the yield to 60%. The purified product only had limited stability when stored neat at -20 °C, so the crude product was usually used for attempted coupling reactions.

Cyclohexadienyl triflates have undergone coupling reactions with Grignard reagents in the presence of copper catalysts, or with preformed organocuprates. The substrates used in this methodology did not contain bulky substituents adjacent to the triflate. Attempts to repeat this methodology using triflate 13 were unsuccessful (Scheme 2.9). Application of Suzuki reaction conditions found to be effective for installation of an aromatic group ortho to an existing aromatic group were tried. The reaction did not yield the desired product; in fact, the new product still contained the triflate. Low-resolution mass spectrometry indicated a loss of H$_2$ resulting in 15; oxidation had seemingly occurred even though the reaction was conducted under argon.
Unless the oxidation occurred from adventitious oxygen, some other mechanism must account for the aromatization. One possibility invokes the ability of boronic acids to abstract a hydride, forming a borohydride intermediate and cyclohexadienyl cation. Under the basic conditions typically employed in Suzuki reactions, β-elimination could generate the benzene product. If this were true, then using a nucleophile without an empty p orbital might prove to be a better coupling partner. Kumada couplings with aryl Grignard reagents using palladium, iron, or nickel catalysts did not generate the desired product, producing only starting material. We also investigated the slow-release MIDA boronate developed by Burke, as this technology only generates low concentrations of boronic acid. Efficient transmetalation would prevent the boronic acid from acting as an oxidant. The slow-release conditions, however, did not generate product, nor did use of Molander’s potassium trifluoroborate. Unable to install the second aryl group prior to the Diels-Alder reaction, we considered carrying out the Diels-Alder reaction on a cyclohexadienyl ether. Generating of the kinetic enolate as described above, but quenching with TBSCI or TBSOTf yielded TBS ether 14. Heating this diene with phenylvinyl sulfonate did not yield a Diels-Alder adduct; only oxidized starting material 16 was obtained (Scheme 2.9).

We then moved from cyclohexadienes to acyclic butadienes to simplify the design. Acyclic dienes can exist in an s-trans conformation which is unreactive Diels-Alder reactions. 2,3-diphenylbutadiene, however, is known to react in a Diels-Alder reaction. The crystal structure of this compound shows a nearly s-cis conformation, which would be reactive in the Diels-Alder reaction. A dihedral drive calculation provides computational support for the stability of the s-cis conformation. We attempted a double Wittig reaction of p-anisil to prepare the diaryldiene in one step; only one ketone reacted, resulting in enone 17 (Scheme 2.10). 4,4’-Dimethoxybenzil reacted with methylmagnesium bromide to yield a mixture of dl- and meso 18.
Attempts to dehydrate the diol to form the diene resulted in 19 due to in a pinacol-type rearrangement.

2.2.4 Functional Analogs of OBHS

Unable to generate carbocyclic OBHS analogs, we sought to develop an “outside-in” approach to designing new analogs containing the structural motifs in OBHS found to give optimum binding: a 1,2-diarylethylene framework with a pendant aryl group at an appropriate distance from the diarylethylene unit.

Attempts chemical modifications of OBHS

The simplest approach was to replace the oxabicyclic core with a single ring. Dehydrating the core of OBHS proved to be more difficult than anticipated. Both acidic (HCl, TFA, POCl₃ and H₂SO₄) and basic conditions (KOtBu and LDA) were tried, but only starting material was observed. Treating OBHS with TMSI resulted in reduction of the oxabicyclic core, yielding a mixture of cyclohexenols. We then sought to synthesize dehydrated OBHS directly. 1,2-Bis(4-methoxyphenyl)benzene was readily prepared by a double Suzuki reaction between 1,2-dibromobenzene and 4-methoxyphenylboronic acid. Treatment of this compound with chlorosulfonic acid generated a sulfonic acid, which could not be converted to the sulfonyl chloride.

Thiophene carboxamides

We then sought to replace the trisubstituted benzene with a trisubstituted heterocyclic core. We chose thiophene as a bioisostere of benzene. The synthesis began with a Suzuki coupling between 3-bromothiophene and 4-methoxyphenylboronic acid to generate thiophene 21 (Scheme 2.11). This thiophene was regioselectively brominated at the 2 position with NBS. 2-Bromothiophene 21 underwent a second Suzuki reaction to generate 2,3-diaryltiophene 22.
Lithiation of this thiophene, followed by quenching with CO$_2$ provided the thiophene carboxylic acid 23. This carboxylic acid was coupled to primary aryl amines by either peptide coupling conditions or coupling to an *in situ* generated acid chloride. The resulting carboxamides 24 were deprotected to give the final products 25.

*Pyrazolo[1,5-a]pyrimidines*

We explored the use of pyrazolo[1,5-a]pyrimidines as scaffolds for OBHS functional analogs. This core has been used in ER ligands in the Katzenellenbogen group, with maximum RBA values around 1%. This is not necessarily a deterrent to making analogs of these compounds, as the relationship between ER affinity and NF-κB antagonism is not clear. In fact, modest NF-kB antagonism was shown with the highest binding pyrazolopyrimidine, containing trifluoromethyl groups at the C5 and C7 positions. We wondered whether replacing one of the trifluoromethyl groups with a pendant aromatic group would increase the NF-κB antagonism.

The synthesis began as previously reported (Scheme 2.12), with condensation between 4-methoxyphenylacetonitrile and methyl 4-methoxybenzoate to yield α-cyanoketone 26. This intermediate was converted to aminopyrazole 27 by condensation with hydrazine. Condensation with 1,3-dicarbonyl compounds would generate the pyrazolo[1,5-a]pyrimidine. In the previous report, only symmetrical 1,3-diketones and aldehydes were used, so regiochemistry was not an issue. However, the new desired target compounds needed substitution at either the 5 or the 7 position of the heterocyclic core. A follow-up report to our original study on pyrazolopyrimidines suggested a binding orientation, which we could use to determine where to position the pendant aromatic groups. Two sets of compounds were made in which one of the phenol hydroxyl groups was selectively alkylated with the basic dialkylaminoethyl side chain found in many SERMs. The phenol hydroxyl group on the aryl group at C2 was determined to be
more important than the one on the aryl group at C3, indicating that it acts as a mimic of the 3-
hydroxyl group of estradiol. Overlaying OBHS with the highest binding pyrazolopyrimidine
indicated that the 5-CF\textsubscript{3} group overlaps with the phenylsulfonate group.

With this knowledge, we sought to synthesize a pyrazolopyrimidine with a synthetic
handle for further functionalization at the 5- position. Regioselective syntheses of either 5- or 7-
pyrazolopyrimidinones have been accomplished by choice of base and dicarbonyl electrophile.
In one report, a condensation between aminopyrazoles and ethyl ethoxyacrylate in DMF, using
cesium carbonate as the base, yielded the pyrazolopyrimidin-5-one\textsuperscript{29}. The authors used NMR
data (chemical shift, coupling constant and NOE data) to determine the regioisomers.
Presumably, under these conditions the heterocyclic nitrogen adjacent to the exocyclic amino
group is the most reactive, and it undergoes a conjugate addition to the enoate, followed by
elimination and cyclization to form the fused heterocyclic product.

When these reaction conditions were repeated with the aminopyrazole to generate 28, the
NMR data were consistent with the data reported for the pyrazolopyrimidin-5-one. This product
was dehydrated to chloropyrazolopyrimidine 29 with POCl\textsubscript{3} in refluxing toluene. Pendant
aromatic groups were added by nucleophilic substitution with phenols in DMF, using potassium
carbonate as the base. Deprotection of 30 afforded the final pyrazolopyrimidines 31.

\textit{Benzo[b]thiophenes}

We also explored the possibility of converting traditional high affinity ligands into
antagonists possessing the unique \textit{in vitro} and \textit{in vivo} profile of OBHS. The antagonism would
come not from a basic side chain, as seen in SERMs, but from an appropriately positioned
pendant aryl group mimicking the phenylsulfonate of OBHS. Others in the Katzenellenbogen
group have converted tamoxifen, E2 and raloxifene into nontraditional antagonists. The binding
affinity of the tamoxifen derivatives remained high. The binding affinity of the E2 derivatives dropped roughly 10 fold, while the raloxifene derivatives bound very poorly. Further analysis of the raloxifene analogs docked into the ER binding pocket showed that flipping the location of the phenols and the pendant aryl group horizontally across the pocket might better position the sulfur near a very hydrophobic region of the pocket.

The initial synthetic strategy was modeled after the known syntheses of the benzo[b]thiophene core of raloxifene, which use an intramolecular Friedel-Crafts acylation. In this case the aryl thiol used needed to have a para substituent which could be transformed into the pendant aryl group. Desoxyanisoin was brominated with Br₂, generating 2-bromodesoxyanisoin. This compound did not readily couple to 4-bromothiophenol. While attempting to improve this reaction, attempts were also made to determine whether the cyclization would proceed efficiently. 4-Bromothiophenol was coupled to α-bromo-4-methoxyacetophenone. Cyclization with BF₃ etherate or Eaton’s reagent failed to produce a benzo[b]thiophene. This cyclization is not described in the literature, perhaps due to the lack of electron density on the thiophenol ring. Thus, alternate syntheses of benzo[b]thiophenes with better literature precedent were explored.

Larock has described the synthesis of fused heterocycles using an electrophilic iodo cyclization of ortho substituted ethers, thioethers and amines to generate iodinated benzofurans, benzothiophenes or indoles. A large library of benzo[b]thiophenes were made by this route, including some very similar to our target compounds.³⁰ This route was modified to allow for a handle at the 5-position of the benzo[b]thiophene core. A triflate was chosen, as it could be converted into a diarylamine, diarylether, or biaryl group via transition metal-catalyzed coupling reactions. To avoid the triflate reacting in coupling reactions in the synthesis, the
oxygen had to be masked with a protecting group that could be removed without removing methyl ethers protecting the required para substituted phenols. Initially the TBS group was investigated, but the isopropyl group was found to give better yields; TBS groups can occasionally be removed during silica gel chromatography, lowering yields.

The synthesis began with alkylation of 3-iodophenol with isopropyl bromide in DMF, yielding 3-iodo-1-isopropoxybenzene 32 (Scheme 2.13). Compound 33 was produced by bromination with NBS in DMF overnight. Selective Sonogashira coupling with 4-ethynylanisole in triethylamine yielded 34. The bromide was converted to a thiomethylether by lithium-bromine exchange followed by quenching with dimethyl disulfide to generate 35. Addition of I₂ in dichloromethane produced 3-iodobenzo[b]thiophene 36, which underwent a Suzuki coupling to yield diarylbenzo[b]thiophene 37. The isopropyl group was removed with AlCl₃ in dichloromethane to furnish 5-hydroxybenzo[b]thiophene 38.

Compound 38 could be directly coupled to aryl halides to generate biaryl ethers, which molecular modeling indicates would position the pendant aryl group in the same space that the phenyl group of the phenylsulfonate in OBHS occupies. Copper-catalyzed Buchwald-Hartwig etherification reactions were explored. Coupling to a few initial aryl iodides and halides using CuI and picolinic acid as the ligand did not generate the biaryl ether. We then decided to explore amination reactions. The triflate (39) and tosylate (40) of 38 were prepared and subjected to Buchwald-Hartwig amination conditions (Scheme 2.14). The tosylate was reacted with aryl amines using a highly active palladium catalyst generated by mixing Pd[(o-tol)₂P]₂ with a Josiphos ligand, CyPF. These conditions were reported to yield the product at room temperature; in our hands this was not the case. Even further heating to 80 °C did not effect product formation. Switching to the more reactive triflate, and using BINAP also did not produce the
desired diarylamine. Our group has had success using aryl bromides as the electrophile in these reactions. The LarocK synthesis was modified to place a bromine at the 5 position. This new synthesis while using a more expensive starting material required fewer steps.

4-Bromo-2-iodoaniline was converted to 4-bromo-2-iodothioanisole 41 by treatment with methyl disulfide and *in situ* diazotization with isoamyl nitrite (Scheme 2.15).31 The same chemoselective Sonogashira conditions used previously were applied to the thioanisole, yielding 42. Iodocyclization with I\(_2\) yielded 43. This benzo[b]thiophene underwent a Suzuki reaction selectively at the iodine to produce the 5-bromobenzo[b]thiophene 44, with varying amounts of 5-arylated material also formed as byproducts. Addition of bromide 43, anilines, and NaOtBu to a solution of Pd(OAc)\(_2\) and BINAP, previously heated to 60 °C to effect dissolution, and heating to reflux in a sealed tube resulted in formation of the Buchwald-Hartwig biaryl products 45. Deprotection of the methyl ethers revealed the desired bisphenol compounds 46.

### 2.3 ANALYSIS OF BIOLOGICAL ACTIVITY

The oxabicyclics containing only one phenol hydroxyl group bound to the ER with affinities approximately 20-fold lower than those of the analogous compound containing two phenol hydroxyl groups (Table 2.1). This is consistent with oxabicyclics containing one para phenol and either a para methyl ether or a meta phenol. These compounds had RBAs approximately 10-fold lower than the compounds containing two phenols. The crystal structures of several oxabicyclic compounds do not reveal a role for both phenols. However, the two phenols are oriented in the pocket in a similar fashion to the phenols in the high-affinity three-dimensional agonists cyclofenil and bicyclononane. Two phenols are also required for high affinity in these compounds. Although it does not appear as a strong hydrogen bond acceptor, Thr347 has been implicated in interacting with the phenol extending into the binding pocket in a
direction originating from the 11β position of a bound steroid. From this point forward all of the compounds we made contained two phenols.

The two separated enantiomers of OBHS were determined to have RBAs of 3.24% and 16.11% for ERα and 0.687% and 1.98% for ERβ. The average of these values agrees well with the observed value for the racemate. The absolute stereochemistry of the separated enantiomers has not been determined. In the crystal structure of OBHS shown in Figures 4 and 5 the oxygen bridge is pointing downward in the pocket. It is likely, but not necessarily true, that the OBHS enantiomer in this structure is the higher binding enantiomer. Each enantiomer was docked into the OBHS crystal structure; the key structural components (i.e. the two phenols and the phenyl sulfonate) overlay very well with each other (Figure 2.10). In the crystal structures of two oxabicyclic analogs the oxygen bridge is pointing up in the box. From these data we proposed that planar compounds lacking the oxygen bridge would bind with reasonable affinity.

The binding affinities of the planar functional OBHS analogs are summarized in Table 2.2. The pyrazolopyrimidine and thiophene ligands showed very low binding to the ER. This initially discouraged us from preparing additional analogs, as we had assumed ER affinity comparable to that of OBHS was necessary. OBHS had the highest binding affinity in its series, and was the only one to show the unusual NF-κB antagonism. The benzo[b]thiophenes exhibited the best RBAs of any of the planar functional analogs. These compounds are structurally analogous to the pyrazolopyrimidines; the only differences are the more nonpolar core, and the use of a nitrogen linkage instead of an oxygen linkage. The increase in RBA probably results from a more nonpolar core interacting more favorably with the nonpolar ER ligand binding pocket. Preliminary results from tamoxifen derivatives prepared by other Katzenellenbogen group members suggest that the NH- linkage affinity relative to the ether linker.
Recently, our collaborators performed a screen of a set of about 100 compounds in transcriptional assays using liver cells, including some ligands designed as OBHS structural or functional antagonists. The data for OBHS and tamoxifen are shown in Figure 10. The dose-response curves for E2 (solid line) and the indicated ligand (dashed lines) for ERα (red) and ERβ (blue). In this assay OBHS acts as an agonist with potency comparable to E2, but with diminished efficacy. When the assay was repeated in an antagonist mode, OBHS showed no antagonist activity on ERα, but good antagonist activity on ERβ. In our original studies using endometrial cancer cells, OBHS acted as an antagonist on both ERα and ERβ. In this screen pyrazolopyrimidine 31a showed poor ER activity, confirming what we had seen in our in vitro RBA assay, but also showed excellent inhibition of IL-6 expression.

2.4 CONCLUSIONS

OBHS, a novel oxabicyclic ER ligand, represents an exciting therapeutic candidate for breast cancer. The potential of OBHS arises from its unique ability to act as an ER antagonist while still maintaining antagonistic activity towards NF-κB. OBHS has proven to be effective in vitro and in vivo. Encouraged by these results, we designed and synthesized structural and functional analogs of OBHS. These compounds have low affinity for the ER; most were also ineffective in NF-κB assays. One compound so far, 31a has shown promising inhibition of NF-κB mediated expression of IL-6. Our ability to generate novel ER / NF-κB antagonists will continue to lead to promising lead compounds in the treatment of breast cancer.
2.5 FIGURES

**Figure 2.1** In vivo administration of OBHS or tamoxifen in a xenograft mouse model of breast cancer.

**Figure 2.2** In vivo administration of OBHS or tamoxifen in a xenograft mouse model of breast cancer with injection of macrophages. The blue line refers to animals in which the tumor volume stayed constant (N = 4); the red line refers to animals in which a decrease in tumor volume was seen (N = 14).
Figure 2.3 Analysis of levels of NF-kB activity, IL-6 and TNF-a levels in tissue samples.
Figure 2.4 The crystal structures of ERα bound to E2 (orange) or OBHS (teal).
Figure 2.5 The binding pocket of the ER bound to E2 (orange) and OBHS (teal).
Figure 2.6 RMSD between crystal structures of ER bound to three oxabicyclic ligands and E2. The spikes at residues 330 and 460 result from gaps in the crystal structure of unresolved residues. The important deviations in the crystal structure occur from residue 527 to 531.
Figure 2.7 Chiral HPLC chromatogram of racemic OBHS (red trace), or reinjection of purified enantiomers (green and blue traces) separated on a Whelk OS-1 column using isopropanol:hexanes.
Figure 2.8 Comparison of $^1$H NMR spectra of 8 produced in Scheme 5 (top) and Scheme 6 (bottom; crude NMR shown).
Figure 2.9 MPLC chromatogram of Diels-Alder adduct between 8 and phenylvinyl sulfonate.
Figure 2.10 Enantiomers of OBHS docked into the OBHS crystal structure.
Figure 2.11

(A) Intensity relative to 10 μM E2 versus log concentration (M)

(B) Intensity relative to 10 μM E2 for different proteins:
- ERα-LBD
- ERα
- ERα-ANT
- ERβ
- ERβ-ANT
- IL-6
- IL-6-repeat
Figure 2.11 (cont.)
Figure 2.11 (cont.)
Figure 2.11 (cont.) (A), (C), (E), (G) Transcription assays in HepG2 cells for OBHS (A), tamoxifen (C), 31a (E) and 31b (G). (B), (D), (F), (H) Single point transcription assays for OBHS (B), tamoxifen (C), 31a (F) and 31b. Assays were conducted with ERα containing only the LBD, full-length ERα or ERβ. ANT refers to assays conducted in the presence of E2. IL-6 data refer to mRNA levels of the IL-6 gene.
Scheme 2.1 Synthesis of OBHS and analogs.

\[
\text{MeO} - \text{O} \quad \text{Et}_3\text{N} \rightarrow \text{MeO} - \text{O} \quad \text{KOH, 18-crown-6} \\
\text{O} \quad \text{MeCN} \rightarrow \text{MeO} - \text{O} \quad \text{MeCN} \\
\text{Br} \quad 1\text{a: } R = \text{OMe: } 86\% \quad 2\text{a: } R = \text{OMe: } 97\% \\
\text{R} \quad 1\text{b: } R = \text{H: } 92\% \quad 2\text{b: } R = \text{H: } 100\% \\
\]

pyridine - HCl 220 °C 2 hr

\[
\text{HO} - \text{O} \rightarrow 90\degree \text{C} \\
\text{Z}_1 \quad \text{Z}_2 \rightarrow \text{HO} - \text{O} \\
\text{R} \quad 3\text{a: } R = \text{OH: } 99\% \quad 4\text{a: } R = \text{OH: } 75\% \\
\text{R} \quad 3\text{b: } R = \text{H: } 99\% \quad 4\text{b: } R = \text{H: } 47\% \\
\]

5a: \( R = \text{OH}, \ Z_1 = \text{SO}_2\text{Ph}, \ Z_2 = \text{H} \)
5b: \( R = \text{H}, \ Z_1 = \text{SO}_2\text{Ph}, \ Z_2 = \text{H} \)
5c: \( R = \text{H}, \ Z_1 = \text{SO}_2\text{Ph}, \ Z_2 = \text{H} \)
5d: \( R = \text{H}, \ Z_1 = \text{Z}_2 = \text{CO}_2\text{Et} \)
5e:
Scheme 2.2 Carbometalation strategy of Fallis for synthesis of diarylfurans.\textsuperscript{10}
Scheme 2.3 Diels-Alder approach to synthesis of diarylfurans.

\[ \text{R}_1\equiv\text{R}_2 + \text{N}^{\text{O}}\text{R}_3 \xrightarrow{\text{R}_1\equiv\text{R}_2} \text{R}_1\equiv\text{R}_2 + \text{R}_3\text{CN} \]

Scheme 2.4. Attempted syntheses of diarylfuran using double cross-coupling approaches.

\[ \text{Br}^{\equiv\equiv}\text{OH} \xrightarrow{\text{H}_2\text{SO}_4} \text{Br}^{\equiv\equiv}\text{Br} \xrightarrow{\text{K}_2\text{Cr}_2\text{O}_7} \]

\[ \text{R}^{\equiv\equiv}\text{R} \xrightarrow{\text{Pd}^0 \text{Bu}_6\text{Sn}_2} \text{Bu}_3\text{Sn}^{\equiv\equiv}\text{SnBu}_3 + \text{H}^{\equiv\equiv}\text{SnBu}_3 + \text{H}^{\equiv\equiv}\text{H} \]
Scheme 2.5 Expected distribution of products arising from Diels-Alder reaction of 8 with phenylvinyl sulfonate.
Scheme 2.6 Initial synthesis of diarylcyclopentadienes as precursors to OBHS analogs.
Scheme 2.7 Revised synthesis of diarylcylopentadienes.

1. DIBAL
2. HCl
87%

Diels-Alder adducts
Scheme 2.8 Synthesis of cyclohexadienes.

Scheme 2.9 Observed oxidation of cyclohexadienes under various reaction conditions.
Scheme 2.10 Attempted syntheses of 2,3-diarylbutadienes.

Scheme 2.10

![Scheme 2.10 Diagram](image-url)
Scheme 2.11 Synthesis of thiophene carboxamides.

\[
\text{Br-Thiophene} + \text{Ph-B(OH)\textsubscript{2}} \xrightarrow{\text{PdCl\textsubscript{2}(PP\textsubscript{3})\textsubscript{2}, base}} \text{Ph-S-Thiophene} \xrightarrow{\text{NBS, DMF}} \text{Ph-S-Thiophene-Br} 
\]

\[
\text{Ph-B(OH)\textsubscript{2}} \xrightarrow{\text{Pd(OAc)\textsubscript{2}, tBu\textsubscript{3}PHBF\textsubscript{4}, K\textsubscript{2}PO\textsubscript{4}, dioxane}} \text{Ph-S-Thiophene} \xrightarrow{\text{1. nBuLi, THF, 2. CO\textsubscript{2}}} \text{Ph-S-Thiophene-CO\textsubscript{2}H} 
\]

\[
\text{Ph-S-Thiophene-CO\textsubscript{2}H} \xrightarrow{\text{1. SOCl\textsubscript{2} or EDC/HOBT, 2. ArNH\textsubscript{2}}} \text{Ph-S-Thiophene-HN-aryl} \xrightarrow{\text{BBR\textsubscript{3}}} \text{Ph-S-Thiophene-HN-aryl} 
\]
Scheme 2.12 Synthesis of pyrazolopyrimidines.
Scheme 2.13 First Larock synthesis of benzothiophenes.

\[
\begin{align*}
\text{Scheme 2.13 First Larock synthesis of benzothiophenes.} \\
\text{32} & \xrightarrow{\text{NBS, DMF, r.t.}} \text{33a-c} + \text{34} \\
\text{34} & \xrightarrow{1. nBuLi, -78^\circ C} \xrightarrow{2. Me_2S_2, -78^\circ C \text{ to r.t.}} \text{35} \\
\text{36} & \xrightarrow{\text{PdCl}_2(\text{dpdf}), \text{Cs}_2\text{CO}_3, \text{dioxane, 110}^\circ \text{C}} \text{37} \\
\text{37} & \xrightarrow{\text{AlCl}_3, \text{CH}_2\text{Cl}_2} \text{38}
\end{align*}
\]
Scheme 2.14 Attempted coupling of benzothiophene sulfonates.

Scheme 2.15 Revised Larock synthesis of benzothiophenes.
Table 2.1 RBA data for oxabicyclic analogs.

<table>
<thead>
<tr>
<th>Compound</th>
<th>ERα</th>
<th>ERβ</th>
<th>4-OMe*</th>
<th>3-OH*</th>
</tr>
</thead>
<tbody>
<tr>
<td>5a (rac)</td>
<td>9.73</td>
<td>1.54</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OBHS-ent1</td>
<td>3.24</td>
<td>0.687</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OBHS-ent2</td>
<td>16.11</td>
<td>1.98</td>
<td></td>
<td></td>
</tr>
<tr>
<td>avg</td>
<td>9.67</td>
<td>1.33</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5b</td>
<td>0.592</td>
<td>0.196</td>
<td>0.98</td>
<td>0.90</td>
</tr>
<tr>
<td>5c</td>
<td>0.159</td>
<td>0.089</td>
<td>0.068</td>
<td>0.069</td>
</tr>
<tr>
<td>5d</td>
<td>0.03</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* RBA for ERα of analogs of 5b and 5c, except the phenyl group has been replaced by the indicated functional group. These data are taken from Zhou et al.\textsuperscript{4}
Table 2.2 RBA data for planar, functional OBHS analogs.

<table>
<thead>
<tr>
<th>Y =</th>
<th>ERα</th>
<th>ERβ</th>
</tr>
</thead>
<tbody>
<tr>
<td>26a</td>
<td>0.111%</td>
<td>0.005%</td>
</tr>
<tr>
<td>26b</td>
<td>0.062%</td>
<td>0.005%</td>
</tr>
<tr>
<td>26c</td>
<td>0.023%</td>
<td>0.005%</td>
</tr>
<tr>
<td>26d</td>
<td>0.004%</td>
<td>0.005%</td>
</tr>
<tr>
<td>31a</td>
<td>0.024%</td>
<td>0.004%</td>
</tr>
<tr>
<td>31b</td>
<td>0.015%</td>
<td>0.007%</td>
</tr>
<tr>
<td>46a</td>
<td>3.68%</td>
<td>1.04%</td>
</tr>
<tr>
<td>46b</td>
<td>1.51%</td>
<td>0.387%</td>
</tr>
<tr>
<td>46c</td>
<td>4.17%</td>
<td>2.75%</td>
</tr>
</tbody>
</table>

2.6 EXPERIMENTAL

All reagents used were purchased from Aldrich Chemical Company, Fisher Scientific, or TCI America and used without further purification unless otherwise specified. Anhydrous THF, ether, dichloromethane, toluene, dioxane, DMF, DMSO, and acetonitrile were obtained from a solvent delivery system. Anhydrous triethylamine, diisopropylethylamine, and diisopropylamine were obtained by distillation from calcium hydride. When necessary, organolithium and organomagnesium reagents were titrated with menthol / 1,10-phenanthroline. Reaction progress was monitored by thin-layer chromatography (TLC) using glass-backed silica gel plates with fluorescent indicator. Unless otherwise specified, reaction temperatures refer to external bath temperatures. Flash chromatography was performed using 40-63 μm particle size (230 – 400 mesh) silica gel from Silicycle. $^1$H and $^{13}$C NMR spectra were obtained on Varian 400 or 500 MHz FT-NMR instruments. Chemical shifts (δ) are reported in parts per million and are referenced to residual undeuterated solvent peaks. Mass spectra were obtained on a Micromass
Quattro or Micromass Q-Tof mass spectrometer. Melting points were obtained on a Thomas-Hoover apparatus and are uncorrected.

**General procedure A:** Bromoacetophenone (1.0 eq) and 4’-methoxyphenylacetic acid (1.1 eq) were dissolved in acetonitrile (0.5M in bromoacetophenone). Triethylamine (1.1 eq) was added at once. The reaction stirred for 2 hours at room temperature. The acetonitrile was removed by rotary evaporation, and redissolved in ethyl acetate. The organic layer was washed with 1N HCl and brine, dried over MgSO₄ and evaporated in vacuo, yielding a clear oil which solidified upon standing. This material was often of sufficient purity for the next reaction. If necessary, the product could be recrystallized from ethanol.

**2-(4-methoxyphenyl)-2-oxoethyl 2-(4-methoxyphenyl)acetate (1a)**

Following general procedure A, the product was obtained as white crystalline solid in 86% yield.

$^1$H NMR (499 MHz, CDCl₃) δ 7.81 - 7.93 (m, 2H), 7.20 - 7.35 (m, 2H), 6.92 - 7.00 (m, 2H), 6.83 - 6.92 (m, 2H), 5.32 (s, 2H), 3.88 (s, 3H), 3.81 (s, 3H), 3.77 (s, 2H)

$^{13}$C NMR (126 MHz, CDCl₃) δ 190.8, 171.7, 164.2, 159.0, 130.7, 130.3, 127.4, 125.9, 114.3, 114.2, 66.3, 55.8, 55.5, 40.3

HR-MS (ESI): Calculated for C$_{18}$H$_{19}$O$_5$ (M + H)$^+$: 315.1232; found 315.1227

**2-oxo-2-phenethyl 2-(4-methoxyphenyl)acetate (1b)**

Following general procedure A, the product was obtained as a cream-colored powder in 92% yield and used without further purification.

$^1$H NMR (400 MHz, CDCl₃) δ 7.83 - 7.94 (m, 2H), 7.55 - 7.65 (m, 1H), 7.41 - 7.52 (m, 2H), 7.20 - 7.32 (m, 2H), 6.82 - 6.95 (m, 2H), 5.35 (s, 2H), 3.80 (s, 3H), 3.77 (s, 2H)

$^{13}$C NMR (126 MHz, CDCl₃) δ 192.3, 171.6, 159.0, 134.4, 134.1, 130.7, 129.1, 128.0, 125.9, 114.3, 66.6, 55.5, 40.2
**General procedure B:**

Phenacyl ester and 18-crown-6 (0.04 eq) were dissolved in acetonitrile (0.2M). With stirring, crushed KOH pellets (1.1 eq) were added. The reaction stirred for one hour, during which time the reaction color changed from clear to yellow to orange to dark green. The reaction was quenched with 3N HCl (1 mL HCl / 10-15 mL acetonitrile). The reaction turned yellow-orange; after a minute of stirring a yellow-orange solid precipitated. This solid was filtered and recrystallized from ethanol.

**3,4-bis(4-methoxyphenyl)furan-2(5H)-one (2a)**

Following general procedure B, the product was obtained in 97% yield.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.76 - 7.95 (m, 2H), 7.24 - 7.29 (m, 2H), 6.91 - 6.98 (m, 2H), 6.84 - 6.90 (m, 2H), 5.31 (s, 2H), 3.87 (s, 3H), 3.80 (d, $J = 0.64$ Hz, 3H), 3.76 (s, 2H)

$^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 174.3, 161.5, 160.0, 154.7, 130.9, 129.2, 124.1, 123.6, 123.0, 114.6, 114.4, 70.6, 55.6, 55.5

**3-methoxyphenyl-4-phenylfuran-2(5H)-one (2b)**

Following general procedure B, the product was obtained as an orange solid in quantitative yield.

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.36 - 7.42 (m, 3H), 7.30 - 7.36 (m, 4H), 6.86 - 6.93 (m, 2H), 5.15 (s, 2H), 3.80 - 3.84 (m, 3H)

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 174.0, 160.2, 155.0, 131.4, 130.8, 130.7, 129.2, 127.7, 122.5, 114.4, 70.8, 55.5, 29.9

LR-MS: 266.1

**General procedure C:**
Butenolide and pyridine hydrochloride (10 eq) were heated with stirring at 220°C for two hours. After 2 hours, the reaction was cooled to room temperature and dissolved with ethyl acetate and 3N HCl. The aqueous layer was extracted with ethyl acetate. The combined organic layers were washed with brine, dried over MgSO₄, and concentrated by rotary evaporation to yield a dark brown solid in quantitative yield. This material was used without further purification.

**3,4-bis(4-hydroxyphenyl)furan-2(5H)-one (3a)**

\(^1\)H NMR (400 MHz, DMSO-\(d_6\)) \(\delta\) 10.05 (s, 1H), 9.67 (s, 1H), 7.19 - 7.27 (m, 2H), 7.09 - 7.15 (m, 2H), 6.69 - 6.80 (m, 4H), 5.25 (s, 2H)

**3-hydroxyphenyl-4-phenylfuran-2(5H)-one (3b)**

\(^1\)H NMR (500 MHz, acetone-\(d_6\)) \(\delta\) 7.34 - 7.49 (m, 5H), 7.18 - 7.31 (m, 2H), 6.78 - 6.89 (m, 2H), 5.27 (s, 2H)

HR-MS (ESI): calculated for C\(_{16}\)H\(_{12}\)O\(_3\)Na (M + Na): 275.0684; found: 275.0680

**General procedure D:**

Deprotected butenolide was dissolved in dry THF (0.2M) and cooled to -78°C with stirring. DIBAL-H (1.0M in CH\(_2\)Cl\(_2\), 5 eq) was added dropwise. The bright red solution was stirred at -78°C for 4 to 6 hours, until the reaction was complete, as judged by TLC. To monitor the reaction, an aliquot was taken with a syringe previously cooled to -78°C (using a flask filled with THF submerged in the dry ice / acetone bath), then quenched into a test tube at -78°C containing methanol. The reaction solution was transferred \(via\) cannula into rapidly stirring solution of methanol at -78°C. After this transfer was complete, the reaction mixture was warmed to 0°C in an ice bath. 10% H\(_2\)SO\(_4\) was added to the reaction mixture, which briefly caused the reaction mixture to form a gel at the bottom of the flask. The organic solvents were removed by rotary evaporation. The aqueous residue was thoroughly extracted with ethyl acetate. The combined
organic layers were washed with brine and dried over Na$_2$SO$_4$. The solvent was removed by rotary evaporation. The crude reaction product was purified by flash chromatography.

**3,4-bis(4-hydroxyphenyl)furan (4a)**

Following general procedure D, the product was obtained as an off-white solid in 75% yield.

$^1$H NMR (499 MHz, acetone-$d_6$) $\delta$ 7.64 (s, 2H), 7.05 - 7.14 (m, 4H), 6.76 - 6.83 (m, 4H)

$^{13}$C NMR (126 MHz, acetone-$d_6$) $\delta$ 156.9, 140.3, 129.8, 125.9, 123.6, 115.5

**3-hydroxyphenyl-4-phenylfuran (4b)**

Following general procedure D, the product was obtained as an off-white solid in 47% yield.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.54 (d, $J$ = 1.71 Hz, 1H), 7.51 (d, $J$ = 1.72 Hz, 1H), 7.26 - 7.32 (m, 3H), 7.21 - 7.25 (m, 2H), 7.07 - 7.13 (m, 2H), 6.74 - 6.80 (m, 2H), 4.84 (s, 1H)

$^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 154.9, 140.8, 140.5, 132.3, 130.1, 128.7, 128.6, 127.2, 126.1, 125.7, 124.8, 115.6

HR-MS (ESI): calculated for C$_{16}$H$_{13}$O$_2$ (M + H)$^+$: 237.0916; found: 237.0918

**General procedure E:**

Furan and dienophile (1.1 to 1.3 eq.) were combined in a 4mL vial. If both components were solids, drops of THF were added. The reaction was then heated to 95°C for 6 – 18 hr. The crude reaction mixture was purified by preparative TLC or flash chromatography. The product was then recrystallized from ethyl acetate/hexanes to yield pure product.

**5,6-Bis-(4-hydroxyphenyl)-7-oxabicyclo[2.2.1]hept-5-ene-2-sulfonic acid phenyl ester (OBHS) (5a)**

Following general procedure E, the product was obtained as a crystalline solid in 49% yield.

$^1$H NMR (500 MHz, acetone-$d_6$) $\delta$ 7.37 - 7.43 (m, 2H), 7.30 - 7.36 (m, 1H), 7.20 - 7.29 (m, 6H), 6.78 - 6.86 (m, 4H), 5.65 (d, $J$ = 1.29 Hz, 1H), 5.44 (dd, $J$ = 1.29, 4.50 Hz, 1H), 3.77 (dd, $J$ = 4.50, 8.36 Hz, 1H), 2.41 (ddd, $J$ = 4.50, 4.50, 12.10 Hz, 1H), 2.27 (dd, $J$ = 8.36, 12.01 Hz, 1H)
HR-MS (ESI) calculated for C_{24}H_{21}O_6S (M + H)^+: 437.1059; found: 437.1070

Elemental Analysis: Theoretical for C_{24}H_{20}O_6S: 66.04% C, 4.62% H; Found: 65.80% C, 4.59% H

(5/6)-(4-hydroxyphenyl)-(6/5)-phenyl-7-oxabicyclo[2.2.1]hept-5-ene-2-sulfonic acid phenyl ester (5b)

Following general procedure E, the product was obtained in 70% yield as a 4:1 ratio of regioisomers.

$^1$H NMR (500 MHz, CDCl$_3$) δ (major isomer only) 7.27 - 7.36 (m, 7H), 7.16 - 7.23 (m, 4H), 6.75 - 6.80 (m, 2H), 5.74 (d, J = 1.07 Hz, 1H), 5.43 (dd, J = 1.18, 4.39 Hz, 1H), 5.01 - 5.18 (m, 1H), 3.59 (dd, J = 4.50, 8.36 Hz, 1H), 2.54 - 2.64 (m, 1H), 2.21 (dd, J = 8.58, 12.22 Hz, 1H)

(5/6)-(4-hydroxyphenyl)-(6/5)-phenyl-7-oxabicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic acid diethyl ester (5c)

Following general procedure E, the product was obtained in 50% yield.

$^1$H NMR (400 MHz, CDCl$_3$) δ 7.20 - 7.36 (m, 5H), 7.09 - 7.19 (m, 2H), 6.73 - 6.81 (m, 2H), 6.06 (br. s., 1H), 5.46 - 5.53 (m, 2H), 4.12 - 4.25 (m, 4H), 3.14 (s, 2H), 1.23 - 1.36 (m, 6H)

$^{13}$C NMR (101 MHz, CDCl$_3$) δ 171.9, 171.7, 156.4, 141.7, 132.9, 129.3, 129.0, 128.3, 127.4, 124.3, 116.0, 85.6, 61.7, 61.6, 48.9, 14.4, 14.4

2-Benzencesulfonyl-(5/6)-(4-hydroxyphenyl)-(6/5)-phenyl-7-oxabicyclo[2.2.1]hept-5-ene (5d)

Following general procedure E, the product was obtained in 45% yield as a 1.4:1 ratio of regioisomers.

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.94 - 8.02 (m, 2H), 7.64 - 7.74 (m, 1H), 7.55 - 7.63 (m, 2H), 7.18 - 7.24 (m, 3H), 7.08 - 7.17 (m, 2H), 7.00 - 7.07 (m, 1H), 6.72 - 6.77 (m, 1H), 6.66 - 6.71 (m, 1H), 5.64 (d, J = 1.07 Hz, 1H), 5.61 (d, J = 1.07 Hz, 1H), 5.27 - 5.33 (m, 1H), 5.02 (s, 1H), 3.47 (dd, J
= 4.50, 8.36 Hz, 1H), 3.42 (dd, \( J = 4.29, 8.36 \) Hz, 1H), 2.41 (dd, \( J = 4.39, 12.11 \) Hz, 1H), 1.89 - 2.03 (m, 1H)

8,9-Bis-(4-hydroxyphenyl)-4-methyl-10-oxa-4-azatricyclo[5.2.1.0^{2,6}]dec-8-ene-3,5-dione (5e)

Following general procedure E, the product was isolated in 54% yield.

\(^1\)H NMR (500 MHz, acetone-\( d_6 \)) \( \delta \) 8.63 (br. s., 2H), 7.20 - 7.30 (m, 4H), 6.78 - 6.89 (m, 4H), 5.37 - 5.39 (s, 2H), 3.19 - 3.26 (m, 2H), 2.86 - 2.92 (m, 2H)

Elemental analysis: calculated 69.41% C, 4.72% H, 3.85% N; found: 68.28% C, 5.01% H, 3.67% N

**General procedure F**

Iodine was added in a three-neck flask with addition funnel to a solution of pyridine/ether (1:1 \( v/v \)) slowly with vigorous stirring. 2-Cyclohexenone was added to the addition funnel. After the iodine had been added and stirred for 10 minutes, a few drops of the enone were added. The flask was placed in an ice bath, and the remainder of the enone was added dropwise. The solution stirred for 2 hours. At this time, the reaction mixture was poured onto 3N HCl and extracted with ether. The ether was washed again with 3N HCl, three times with 20% w/w aqueous sodium thiosulfate, and brine. The ether layer was dried over MgSO\(_4\) and evaporated on the rotary evaporator.

**2-iodo-2-cyclohexenone (11)**

Following general procedure F, the product was obtained as a yellow solid in 76%.

\(^1\)H NMR (500 MHz, CDCl\(_3\)) \( \delta \) 7.75 (t, \( J = 4.40 \) Hz, 1H), 2.58 - 2.67 (m, 2H), 2.37 - 2.47 (m, 2H), 2.01 - 2.12 (m, 2H)

\(^{13}\)C NMR (126 MHz, CDCl\(_3\)) \( \delta \) 192.4, 159.8, 104.1, 37.5, 30.2, 23.1
HR-MS (EI): calculated for C₆H₇OI (M⁺): 221.9542; found: 221.9535

**2-iodo-2-cyclopentenone (6)**

Following general procedure F, the product was obtained as a yellow-orange solid in 63% yield.

**¹H NMR (499 MHz, CDCl₃)** δ 8.03 (t, J = 2.93 Hz, 1H), 2.76 - 2.82 (m, 2H), 2.49 - 2.55 (m, 2H)

HR-MS (EI): calculated for C₅H₃OI (M⁺): 207.93855; found: 207.93917

**General procedure G**

Iodoenone 11 (2.168g, 9.76 mmol), 4-methoxyphenylboronic acid (1.5 eq.), silver oxide (1.5 eq.), triphenylarsine (0.1eq.), and bis(benzonitrile)palladium(II) chloride (0.05 eq.) were combined in a round bottom flask. The flask was backfilled three times with argon before the addition of an 8:1 v:v THF:H₂O solution. The reaction was purged with a balloon of argon for 10 minutes, then stirred for an additional 45 minutes. Saturated NH₄Cl was added, and the reaction stirred for 1 hour. The reaction mixture was filtered over Celite, and extracted three times with ether. The combined ether extracts were washed with brine, dried over MgSO₄, and concentrated by rotary evaporation. The crude residue was purified by column chromatography (4:1 hexanes:ethyl acetate).

**2-(4-methoxyphenyl)-2-cyclopentenone (7)**

Following general procedure G, the product was obtained as a white solid in 67% yield.

**¹H NMR (499 MHz, CDCl₃)** δ 7.74 (t, J = 2.93 Hz, 1H), 7.64 - 7.70 (m, 2H), 6.87 - 6.96 (m, 2H), 3.82 (s, 3H), 2.67 - 2.72 (m, 2H), 2.56 - 2.60 (m, 2H)

**¹³C NMR (126 MHz, CDCl₃)** δ 208.3, 159.9, 157.5, 142.9, 128.5, 124.5, 114.0, 55.5, 36.0, 26.3

HR-MS (EI): calculated for C₁₂H₁₂O₂ (M⁺): 188.08373; found: 188.08424

**2-(4-methoxyphenyl)-2-cyclohexenone (12)**

Following general procedure G, the product was obtained as a white solid in 85% yield.
^1^H NMR (500 MHz, CDCl\textsubscript{3}) \( \delta \) 7.21 - 7.28 (m, 2H), 6.98 (t, \( J = 4.29 \text{ Hz} \), 1H), 6.83 - 6.91 (m, 2H), 2.55 - 2.62 (m, 2H), 2.48 - 2.55 (m, 2H), 2.10 (quin, \( J = 6.38 \text{ Hz} \), 2H)

^1^C NMR (126 MHz, CDCl\textsubscript{3}) \( \delta \) 198.5, 159.3, 147.2, 140.0, 130.0, 129.2, 113.7, 55.5, 39.4, 26.9, 23.2

HR-MS (EI): calculated for C\textsubscript{13}H\textsubscript{14}O\textsubscript{2} (M\textsuperscript{+}): 202.0994; found: 202.1003

6-(4-methoxyphenyl)-1,5-cyclohexadienyl trifluoromethanesulfonate (13)

0.5 mL dry THF and NaHMDS (1.1 mL, 1.0M in THF, 1.1 mmol) were added to a dry round bottom flask. The solution was cooled to -78°C for 30 minutes with stirring. Cyclohexenone 12 (200 mg, 0.99 mmol) was added as a solution in 1 mL THF. The solution turned light yellow. After 40 minutes of stirring at -78°C, N-(5-chloro-2-pyridy1)bis(trifluoromethanesulfonimide) (432 mg, 1.1 mmol) dissolved in 1 mL THF was added. The solution quickly turned red. The -78°C bath was removed after 30 minutes, and the reaction gradually allowed to warm to room temperature over 45 minutes. The THF was removed by rotary evaporation; the residue was dissolved in 30 mL ether, washed twice with 10 mL of saturated aqueous NaHCO\textsubscript{3}, once with 10 mL of brine, and dried over Na\textsubscript{2}SO\textsubscript{4}. Rotary evaporation yielded 292 mg (87%), which was used without further purification. Occasionally the material was purified by column chromatography (hexanes \( \rightarrow \) 10% EtOAc in hexanes), lowering the yield to ~60%.

^1^H NMR (499 MHz, CDCl\textsubscript{3}) \( \delta \) 7.16 - 7.23 (m, 2H), 6.85 - 6.92 (m, 2H), 6.04 (t, \( J = 4.52 \text{ Hz} \), 1H), 5.95 (t, \( J = 4.64 \text{ Hz} \), 1H), 3.83 (s, 3H), 2.29 - 2.48 (m, 4H)

6-(4-methoxyphenyl)-1,5-cyclohexadien-2-ol TBS ether (14)

A 1.0M solution of NaHMDS in THF (1.1 mL, 1.1 mmol, 1.0 eq.) and 0.5 mL THF were added to a dry round bottom flask. The solution was cooled to -78°C for 30 minutes. 12 was added as a solution in 1.5 mL dry THF. After 45 minutes of stirring at -78°C, TBSCl (166 mg, 1.1 mmol)
was added as a solution in 1 mL THF. The solution was stirred for 1 hour before the -78°C bath was removed and the reaction warmed to room temperature over 30 minutes. The THF was removed by rotary evaporation. The residue was dissolved in 25 mL ether and 7 mL saturated aqueous NaHCO₃. The aqueous layer was extracted three times with 10 mL ether. The combined ether layers were washed with 10 mL brine, dried over Na₂SO₄, and concentrated on a rotary evaporator. The crude product was purified by column chromatography (5% EtOAc in hexanes) to yield 202 mg (64%) of a clear oil.

¹H NMR (499 MHz, CDCl₃) δ 7.20 - 7.25 (m, 2H), 6.80 - 6.85 (m, 2H), 5.90 - 5.99 (m, 1H), 5.09 - 5.15 (m, 1H), 3.81 (s, 3H), 2.17 - 2.24 (m, 4H), 0.74 (s, 9H), -0.05 - -0.01 (m, 6H)

¹³C NMR (126 MHz, CDCl₃) δ 158.7, 148.8, 138.4, 131.8, 130.2, 126.6, 113.2, 104.9, 55.5, 25.8, 23.7, 22.4, 18.2, -4.6

2-(4-methoxyphenyl)phenyl triflate (15)

Diene triflate (39 mg, 0.12 mmol) 13, Pd(OAc)₂ (2 mg, 0.075 eq.), 4-methoxyphenylboronic acid (21 mg, 0.14 mmol, 1.2 eq.) and potassium carbonate (20 mg, 0.14 mmol, 1.2 eq.) were combined in a round-bottomed flask. The flask was evacuated and backfilled three times with argon. Dry dioxane (0.500 mL) was added, and the reaction heated to 90°C for 24 hours. The crude residue was purified by preparative TLC (6:1 hexanes:EtOAc) to yield 18 mg (46%) of 15.

¹H NMR (500 MHz, CDCl₃) δ 7.50 - 7.59 (m, 4H), 7.42 (t, J = 7.61 Hz, 2H), 6.96 - 7.01 (m, 2H), 3.86 (s, 3H)

2-(4-methoxyphenyl)phenyl TBS ether (16)

Cyclohexadiene 14 (22 mg, 0.069 mmol) and phenylvinyl sulfonate (15 mg, 0.083 mmol, 1.2 eq.) were heated to 100°C in a 4 mL vial for 20 hours. After cooling to room temperature, the
residue was purified by preparative TLC (10% EtOAc in hexane) to yield a thin film. $^1$H NMR analysis indicated the starting material had oxidized to form 16.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.42 - 7.47 (m, 2H), 7.29 - 7.33 (m, 1H), 7.17 - 7.23 (m, 1H), 7.03 (d, $J = 1.22$ Hz, 1H), 6.93 - 6.96 (m, 2H), 6.92 (dd, $J = 0.98, 8.06$ Hz, 1H), 3.86 (s, 3H), 0.84 - 0.87 (m, 9H), -0.04 - -0.02 (m, 6H)

2,3-bis(4-methoxyphenyl)-1,3-cyclopentadiene / 1,2-bis(4-methoxyphenyl)-1,3-cyclopentadiene (8)

4-bromoanisole (234mg, 1.25 mmol, 1.25 eq.) was dissolved in 1.5 mL THF and cooled to -78°C. nBuLi (1.6M in hexanes, 0.75 mL, 1.2 mmol, 1.2 eq.) was added dropwise. The resulting solution stirred at the same temperature for 1 hour before the addition of 6 in 2 mL THF. The solution stirred at -78°C for an additional 30 minutes; during this time the solution turned yellow. The dry ice / acetone bath was removed, and the reaction mixture warmed to room temperature. After stirring at room temperature overnight, the reaction mixture was quenched with 8 mL of 2N HCl and stirred for 1 hour. The resulting solution was then extracted three times with 20 mL ether. The combined organic layers were washed with 20 mL brine, dried over Na$_2$SO$_4$, and concentrated. The crude residue was purified by column chromatography (9:1 hexanes:EtOAc) to yield 40 mg (14%) of a white solid.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ (both isomers)7.26 - 7.30 (m, 2H), 7.18 - 7.24 (m, 2H), 7.07 - 7.14 (m, 2H), 6.82 - 6.86 (m, 2H), 6.75 - 6.82 (m, 4H), 6.68 (td, $J = 1.39, 5.36$ Hz, 1H), 6.42 - 6.48 (m, 2H), 3.82 (s, 3H), 3.80 (s, 3H), 3.79 (s, 3H), 3.49 (t, $J = 1.39$ Hz, 2H), 3.17 - 3.22 (m, 1H)

$^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ (both isomers) 158.7, 158.7, 158.3, 139.4, 139.2, 137.3, 131.5, 131.0, 130.3, 130.0, 129.8, 129.6, 129.2, 114.0, 113.9, 113.5, 55.4, 55.4, 45.9, 40.3
HR-MS (EI): calculated for C_{19}H_{18}O_{2} (M^+): 278.13068; found: 278.13131

4-hydroxy-3,4-bis(4-methoxyphenyl)-2-cyclopentenone (9)

In a round bottomed flask p-anisil (4.770 g, 17.6 mmol) and KOH (5.124 g, 91.3 mmol) were combined with 250 mL of absolute ethanol. The reaction mixture was heated to reflux; 10 mL acetone was added dropwise. The reaction stirred at reflux for an additional 2 hours. After cooling to room temperature the solvents were removed and replaced with 50 mL 3N HCl and 125 mL EtOAc. The aqueous layer was extracted twice with 50 mL ethyl acetate. The combined organic layers were washed twice with 20 mL brine, dried over Na$_2$SO$_4$, and concentrated to yield a dark red oil. This oil was purified by MPLC (hexanes/ethyl acetate gradient), yielding 4.346 g (79%) of a red oil, which turned into a foam upon drying under high vacuum.

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.47 - 7.57 (m, 2H), 7.31 - 7.38 (m, 2H), 6.83 - 6.89 (m, 2H), 6.75 - 6.82 (m, 2H), 6.58 (s, 1H), 3.78 (s, 3H), 3.78 (s, 3H), 2.77 - 3.02 (m, 3H)

$^{13}$C NMR (126 MHz, CDCl$_3$) δ 205.1, 173.8, 161.9, 159.0, 136.7, 131.5, 127.1, 126.1, 125.6, 114.4, 114.3, 81.6, 56.9, 55.6, 55.5

HR-MS (ESI): calculated for C$_{19}$H$_{19}$O$_{4}$ (M + H)$^+$: 311.1283; found: 311.1283

3,4-bis(4-methoxyphenyl)-2-cyclopentenone (10)

Hydroxycyclopentenone (2.04 g, 6.58 mmol) was dissolved in 40 mL MeCN. NaI (2.50 g, 16.67 mmol) was added, followed by TMSCl (2 mL, 16.28 g, 16.20 mmol). The reaction mixture stirred at room temperature for 2 hours. The acetonitrile was removed and replaced with 50 mL EtOAc. 50 mL 20% w/w aqueous Na$_2$S$_2$O$_3$ were added, and the layers separated. The aqueous layer was extracted twice with 50 mL EtOAc. The combined organic layers were dried over Na$_2$SO$_4$ and concentrated. The crude residue was purified by MPLC (hexanes/EtOAc gradient), yielding 1.378 g (71%) of a brown foam.
1H NMR (500 MHz, CDCl₃) δ: 7.48 (d, J = 8.8 Hz, 2H), 7.06 (d, J = 8.6 Hz), 6.81 (m, 4H), 6.64 (s, 1H), 4.54 (d, J = 7.3 Hz, 1H), 3.77 (s, 1H), 3.74 (s, 1H), 3.06 (dd, J₁ = 7.3 Hz, J₂ = 18.6 Hz, 1H), 2.38 (d, J = 18.7 Hz)

13C NMR (125 MHz, CDCl₃) δ: 208.49, 175.11, 161.85, 158.63, 135.11, 130.11, 128.26, 127.15, 126.02, 126.02, 114.66, 114.34, 55.553, 55.429, 47.084, 46.212

HR-MS (ESI): calculated for C₁₉H₁₉O₃ (M + H)⁺ : 295.1334; found: 295.1335

1,2-bis(4-methoxyphenyl)-1,3-cyclopentadiene (8)

Cyclopentenone (548 mg, 1.86 mmol) dissolved in 15 mL dry THF. The solution was cooled to -78°C for 25 minutes. DIBAL (4 mL of a 1.0M solution in hexanes, 4 mmol) was added dropwise. The dry ice bath was removed, and the reaction slowly warmed to room temperature over 1 hour. Drops of water were added to quench the excess DIBAL, followed by 10 mL 3N HCl. The solution was stirred for 2 hours; the THF was removed by rotary evaporation. The aqueous residue was extracted three times with 15 mL ether, washed with 15 mL brine, dried over Na₂SO₄, and concentrated to yield 453 mg (87%) of the diene product as an orange solid. Only the 1,2-diaryl isomer could be identified by NMR analysis.

1H NMR (500 MHz, CDCl₃) δ 7.26 - 7.30 (m, 2H), 7.20 - 7.23 (m, 2H), 6.81 - 6.87 (m, 2H), 6.74 - 6.80 (m, 2H), 6.68 (td, J = 1.42, 5.31 Hz, 1H), 6.46 (td, J = 1.50, 5.36 Hz, 1H), 3.82 (s, 3H), 3.79 (s, 3H), 3.49 (t, J = 1.50 Hz, 2H)

13C NMR (126 MHz, CDCl₃) δ 158.7, 158.4, 139.5, 139.2, 137.3, 131.5, 130.3, 130.1, 129.8, 129.6, 114.0, 113.9, 55.4, 45.9

1,2-bis(4-methoxyphenyl)-propenone (17)

In a flame-dried flask methyltriphenylphosphonium bromide (2.68 g, 7.5 mmol, 2.5 eq.) and potassium tert-butoxide (842 mg, 7.5 mmol, 2.5 eq.) were combined. The flask was cooled in an
ice bath for several minutes before the addition of 8 mL of dry THF. After stirring at this temperature for 45 minutes, p-anisil (811 mg, 3 mmol) was added in 10 mL THF. The solution was allowed to warm to room temperature overnight. After quenching with 5 mL 1N HCl, the THF was removed by rotary evaporation. 10 mL water was added, and the reaction mixture extracted twice with 25 mL EtOAc. The combined organic layers were washed with 15 mL brine, dried over MgSO₄ and concentrated by rotary evaporation. Purification of the residue by silica gel chromatography (3:1 hexanes : EtOAc) yielded 269 mg (33%) of the product as a yellow oil.

\[ ^1H \text{ NMR (500 MHz, CDCl}_3 \] δ 7.89 - 7.96 (m, 2H), 7.32 - 7.39 (m, 2H), 6.88 - 6.93 (m, 2H), 6.84 - 6.88 (m, 2H), 5.90 (s, 1H), 5.45 (s, 1H), 3.85 (s, 3H), 3.80 (s, 3H)

\[ ^13C \text{ NMR (126 MHz, CDCl}_3 \] δ 196.9, 163.9, 160.0, 148.0, 132.7, 130.1, 129.9, 128.3, 117.2, 114.2, 113.9, 55.7, 55.5

2,3-bis(4-methoxyphenyl)-2,3-butanediol (18)

In a dry round-bottom flask p-anisil (278 mg, 1.02 mmol) was dissolved in 10 mL dry THF, and cooled in an ice bath. To the resulting yellow solution was added 1.1 mL of a 3.0M solution of MeMgBr in ether (3.3 mmol, 3 eq.). The reaction warmed to room temperature for two hours, at which time the yellow color of the reaction disappeared. The reaction was quenched with 1N HCl, and concentrated by rotary evaporation. The residue was diluted with water and extracted with ethyl acetate. The combined organic extracts were dried over MgSO₄ and concentrated by rotary evaporation. The crude residue was purified by MPLC (hexanes : EtOAc) to yield 186 mg of the product as a white solid in 77% yield.

\[ ^1H \text{ NMR (500 MHz, CDCl}_3 \] (major isomer) δ 7.14 - 7.19 (m, 4H), 6.76 - 6.81 (m, 4H), 3.81 (s, 6H), 2.17 - 2.34 (m, 2H), 1.58 (s, 6H)
$^{13}$C NMR (126 MHz, CDCl$_3$) (major isomer) δ 158.6, 136.3, 128.3, 112.8, 78.7, 55.4, 25.4

3,3-bis(4-methoxyphenyl)-2-butanone (19)

Diol 19 (205 mg, 0.678 mmol) and Burgess’ reagent (355 mg, 1.492 mmol, 2.2 eq.) were dissolved in 5 mL toluene. The reaction mixture was heated to 50°C for 24 hours. The solvent was removed by rotary evaporation and the crude residue purified by MPLC (hexanes : EtOAc) to yield 83 mg (43%) of the product.

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.09 - 7.17 (m, 4H), 6.86 - 6.92 (m, 4H), 3.83 (d, $J = 0.73$ Hz, 6H), 2.12 (d, $J = 0.49$ Hz, 3H), 1.85 (s, 3H)

3-(4-methoxyphenyl)thiophene (20)

4-methoxyphenylboronic acid (966 mg, 6.4 mmol), 3.38 g K$_3$PO$_4$, and 224 mg PdCl$_2$(PPh$_3$)$_2$ combined in a round bottom flask with a stir bar. 864 mg 3-bromothiophene was transferred to the reaction flask with 25 mL dioxane. 5 mL water was added, and the reaction heated to 60°C overnight. After cooling to room temperature, the reaction was diluted with 60 mL ether and 60 mL 1N NaOH. The aqueous layer was extracted three times with 50 mL ether, washed with 20 mL brine, dried over Na$_2$SO$_4$, and concentrated on the rotary evaporator. The crude product was purified by column chromatography (3:1 → 2:1 hexanes:CH$_2$Cl$_2$), yielding 856 mg (85%) of a white solid.

$^1$H NMR (499 MHz, CDCl$_3$) δ 7.51 - 7.60 (m, 2H), 7.33 - 7.43 (m, 3H), 6.92 - 7.00 (m, 2H), 3.80 - 3.89 (s, 3H)

$^{13}$C NMR (126 MHz, CDCl$_3$) δ 159.1, 142.2, 129.0, 127.8, 126.5, 126.3, 119.2, 114.4, 55.6

HR-MS: calculated for C$_{11}$H$_{10}$OS (M$^+$): 190.0452; found 190.0454

2-bromo-3-(4-methoxyphenyl)thiophene (21)
Thiophene 20 (635 mg, 3.3 mmol) was dissolved in 20 mL CH$_2$Cl$_2$; the solution was cooled in an ice bath. N-bromosuccinimide (587 mg, 3.3 mmol) in 15 mL DMF added dropwise. The reaction mixture was allowed to warm to room temperature overnight. The reaction mixture was diluted with 100 mL brine, and the layers separated. The aqueous layer was extracted several times with CH$_2$Cl$_2$. The combined organic extracts were washed with 25 mL brine, dried over MgSO$_4$, concentrated on the rotary evaporator, and further dried on a high vacuum at 40°C yielding 810 mg (91%) of a clear oil, which was used without further purification.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.44 - 7.53 (m, 2H), 7.29 (d, $J = 5.79$ Hz, 1H), 7.01 (d, $J = 5.57$ Hz, 1H), 6.93 - 6.99 (m, 2H), 3.85 (s, 3H)

$^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 159.3, 141.1, 130.1, 129.3, 127.7, 126.0, 114.0, 108.1, 55.5

2,3-bis(4-methoxyphenyl)thiophene (22)

Bromothiophene 21 (746 mg, 2.77 mmol), 4-methoxyphenylboronic acid (631 mg, 4.15 mmol), K$_3$PO$_4$ (1.76g, 8.31 mmol), Pd(OAc)$_2$ (31 mg, 0.13 mmol) and tri(tert-butyl)phosphonium tetrafluoroborate (80 mg, 0.28 mmol) were combined in a round bottom flask fitted with a condenser. The apparatus was evacuated and backfilled with argon three times, before dry 1,4-dioxane (15 mL) were added. The reaction mixture was heated with stirring to 100°C for 16 hours. After cooling to room temperature, 30 mL water and 30 mL ether were added. The resulting mixture was filtered over celite. The layers were separated, and the aqueous layer extracted three times with 30 mL ether. The combined organic layers were dried over MgSO$_4$, and concentrated on the rotary evaporator. The crude residue was purified by column chromatography (40% $\rightarrow$ 80% dichloromethane/hexane) to yield 505 mg (62%) of a white solid.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.16 - 7.30 (m, 5H), 7.11 (d, $J = 5.15$ Hz, 1H), 6.82 (dd, $J = 7.18$, 8.68 Hz, 4H), 3.79 - 3.82 (m, 6H)
C NMR (126 MHz, CDCl₃) δ 159.1, 158.7, 137.9, 137.3, 130.7, 130.6, 130.4, 129.4, 127.2, 123.6, 114.1, 114.0, 55.5, 55.4

5-bromo-3,4-bis(4-methoxyphenyl)thiophene-2-carboxylic acid (23)

Thiophene 22 (440 mg, 1.49 mmol) dissolved in 7 mL dry THF. The reaction was then cooled to -78°C in a dry ice / acetone bath. nBuLi (1.0 mL, 1.6M in hexanes, 1.6 mmol) added dropwise. The reaction color changed from clear to green to blue over one hour. CO₂ from a lecture bottle was passed through a drying tube cooled with dry ice / acetone into the reaction mixture. After a few seconds, the reaction mixture turned orange. The solution was stirred at this temperature for 1.5 hours, before warming to room temperature. The THF was stripped, and 10 mL ethyl acetate added. 5 mL 1N HCl were added, which lowered the pH to <2. The aqueous layer was extracted three times with 10 mL ethyl acetate. The combined organic fractions were dried over Na₂SO₄ and concentrated on the rotary evaporator. The crude residue was recrystallized from chloroform to yield 248 mg (50%) of the thiophene carboxylic acid.

H NMR (500 MHz, CDCl₃) δ 7.90 (s, 1H), 7.25 - 7.33 (m, 2H), 7.17 - 7.24 (m, 2H), 6.86 (dd, J = 5.86, 8.55 Hz, 4H), 3.77 - 3.88 (m, 6H)

General procedure H

Thiophene-2-carboxylic acid was suspended in dichloromethane and cooled to 0°C. Thionyl chloride (1.21eq.) was added dropwise; the ice bath was removed and the mixture stirred for 3 hours. The solvent was removed, and the material dried on a high vacuum overnight. Dichloromethane was added, followed by aniline (1.1 eq.) and diisopropylethylamine (1.1 eq.). After stirring for 2 hours, the reaction mixture was poured into water and extracted exhaustively with dichloromethane. The combined organic layers were washed with water, brine, dried over
MgSO₄, and concentrated on the rotary evaporator. The crude product was purified by column chromatography or preparative TLC (3:1 hexanes:EtOAc).

**4,5-bis(4-methoxyphenyl)‑N‑phenylthiophene‑2‑carboxamide (24a)**

Following general procedure H, the crude product was isolated in 89% yield, and used without further purification.

\[ ^1H \text{NMR (400 MHz, CDCl}_3) \delta 7.98 (s, 1H), 7.56 - 7.68 (m, 3H), 7.32 (t, J = 7.93 Hz, 2H), 7.05 - 7.26 (m, 6H), 6.79 (dd, J = 1.10, 8.91 Hz, 4H), 3.77 (d, J = 2.20 Hz, 6H) \]

**4,5-bis(4-methoxyphenyl)‑N‑(4-fluorophenyl)thiophene‑2‑carboxamide (24b)**

Following general procedure H, the crude product was isolated in 46% yield, and used without further purification.

\[ ^1H \text{NMR (499 MHz, CDCl}_3) \delta 8.02 (br. s., 1H), 7.62 (s, 1H), 7.59 (dd, J = 4.76, 7.69 Hz, 2H), 7.22 (d, J = 7.81 Hz, 2H), 7.16 (d, J = 7.81 Hz, 2H), 7.03 (t, J = 8.18 Hz, 2H), 6.81 (d, J = 8.30 Hz, 4H), 3.80 (s, 6H) \]

**4,5-bis(4-methoxyphenyl)‑N‑(3-fluorophenyl)thiophene‑2‑carboxamide (24c)**

Following general procedure H, the product was isolated in 57% yield after column chromatography.

\[ ^1H \text{NMR (499 MHz, CDCl}_3) \delta 7.97 (s, 1H), 7.58 - 7.66 (m, 2H), 7.28 - 7.32 (m, 2H), 7.21 - 7.25 (m, 2H), 7.15 - 7.20 (m, 2H), 6.80 - 6.84 (m, 4H) \]

**4,5-bis(4-methoxyphenyl)‑N‑(3-chloro-2-methylphenyl)thiophene‑2‑carboxamide (24d)**

Following general procedure H, the product was isolated in 69% yield after column chromatography.

\[ ^1H \text{NMR (499 MHz, CDCl}_3) \delta 7.97 (s, 1H), 7.58 - 7.66 (m, 2H), 7.28 - 7.32 (m, 2H), 7.21 - 7.25 (m, 2H), 7.15 - 7.20 (m, 2H), 6.80 - 6.84 (m, 4H) \]
General procedure I

Thiophene carboxamide 25 was dissolved in dichloromethane and cooled to 0°C. Boron tribromide (1.0M in dichloromethane, 6 – 8 eq.) was added dropwise. The product was placed in a -20°C freezer overnight. After warming to room temperature, the reaction was quenched with methanol. The solvents were evaporated, and the residue purified by preparative TLC.

4,5-bis(4-hydroxyphenyl)-N-phenylthiophene-2-carboxamide (25a)

Following general procedure I, the product was isolated in 50% yield after preparative TLC (2:1 hexanes:EtOAc, 1:1 hexanes:EtOAc)

^1^H NMR (499 MHz, acetone-^d_6^) δ 8.44 - 8.79 (m, 1H), 7.93 (s, 1H), 7.77 - 7.87 (m, 2H), 7.36 (dd, J = 7.57, 8.55 Hz, 2H), 7.19 - 7.24 (m, 2H), 7.14 - 7.18 (m, 2H), 7.09 - 7.14 (m, 1H), 6.81 - 6.86 (m, 4H)

^1^3^C NMR (126 MHz, acetone-^d_6^) δ 160.1, 157.9, 157.0, 143.3, 139.4, 138.0, 137.3, 131.4, 130.6, 130.3, 128.9, 127.5, 123.9, 120.2, 115.8, 115.6

4,5-bis(4-hydroxyphenyl)-N-(4-fluorophenyl)thiophene-2-carboxamide (25b)

Following general procedure I, the product was isolated in 47% yield after preparative TLC (2:1 hexanes : EtOAc, 1:1 hexanes : EtOAc)

^1^H NMR (500 MHz, acetone-^d_6^) δ 9.57 (s, 1H), 8.67 (br. s., 1H), 8.50 (br. s., 1H), 7.89 (s, 1H), 7.79 - 7.86 (m, 2H), 7.20 (d, J = 8.58 Hz, 2H), 7.08 - 7.16 (m, 4H), 6.79 - 6.85 (m, 4H)

^1^3^C NMR (126 MHz, acetone-^d_6^) δ 160.0, 158.2, 160.0, 157.0, 143.4, 138.0, 137.0, 135.6, 131.5, 130.6, 130.2, 127.5, 125.2, 122.0, 115.7, 115.4, 115.3

4,5-bis(4-hydroxyphenyl)-N-(3-fluorophenyl)thiophene-2-carboxamide (25c)

Following general procedure I, the product was isolated in 26% yield after preparative TLC (1:1 hexanes:EtOAc)
$^1$H NMR (500 MHz, acetone-$d_6$) $\delta$ 9.31 - 9.49 (m, 1H), 8.59 - 9.03 (m, 1H), 7.93 (s, 1H), 7.48 (d, $J = 7.72$ Hz, 1H), 7.32 (d, $J = 7.72$ Hz, 1H), 7.12 - 7.26 (m, 5H), 6.81 (dd, $J = 6.75, 8.68$ Hz, 4H), 2.37 (s, 3H)

4,5-bis(4-hydroxyphenyl)-N-(3-chloro-2-methylphenyl)thiophene-2-carboxamide (25d)

Following general procedure I, the product was isolated in 44% yield after preparative TLC (1:1 hexanes:EtOAc)

$^1$H NMR (500 MHz, acetone-$d_6$) $\delta$ 9.29 - 9.50 (m, 1H), 8.50 - 9.05 (m, 1H), 7.93 (s, 1H), 7.48 (d, $J = 7.93$ Hz, 1H), 7.32 (d, $J = 7.29$ Hz, 1H), 7.22 - 7.27 (m, 1H), 7.12 - 7.22 (m, 4H), 6.77 - 6.85 (m, 4H), 2.37 (s, 3H)

2,3-bis(4-methoxyphenyl)-2-oxopropionitrile (26)

Methyl 4-methoxybenzoate (1.83g, 11 mmol) sodium hydride (880 of a 60% dispersion in mineral oil, 528 mg NaH, 22 mmol) were suspended in 50 mL dry THF in a 3-neck flask fitted with a condenser, septum and glass stopper. 4-methoxyphenylacetonitrile (1.47g, 10 mmol) was added via syringe to the stirring reaction mixture. The reaction mixture was refluxed overnight; after this time the reaction was cooled to room temperature. The reaction was quenched with 10 mL H$_2$O and the THF removed by rotary evaporation. The aqueous layer was extracted three times with 25 mL ether. The aqueous layer was acidified with 15 mL 1N HCl and extracted three times with 25 mL ethyl acetate. The combined organic layers were dried over MgSO$_4$ and concentrated by rotary evaporation to yield 1.88g (67%) of a yellow syrup.

$^1$H NMR (499 MHz, CDCl$_3$) $\delta$ 7.37 - 7.45 (m, 3H), 7.21 - 7.34 (m, 7H), 6.70 - 6.80 (m, 2H), 5.91 - 6.01 (m, 3H), 3.82 (s, 3H), 3.81 (s, 3H)

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 187.7, 164.6, 160.2, 131.9, 129.6, 126.6, 122.8, 117.3, 115.2, 114.4, 55.8, 55.6, 45.9
HR-MS (ESI): calculated for C_{17}H_{15}NO_3Na (M + Na)^+: 304.0950; found: 304.0949

5-amino-3,4-bis(4-methoxyphenyl)-1H-pyrazole (27)

Ketonitrile 26 (1.29 g, 4.57 mmol), 25 mL ethanol and 1.4 mL concentrated HCl were heated with stirring to 80°C. 711 μL (22.8 mmol) hydrazine hydrate was added dropwise. The reaction mixture was stirred for 16 hr. After cooling to room temperature, the solvent was removed on a rotary evaporator. The residue was partitioned in 5 mL water and the pH was adjusted to 7 with approximately 15 mL saturated NaHCO_3. The water was extracted three times with 15 mL ethyl acetate. The combined organic layers were washed with 10 mL brine, 10 mL water, dried over Na_2SO_4 and concentrated on a rotary evaporator. The residue was purified by flash chromatography (10% MeOH / CHCl_3) to yield 1.08 g (80%) of a white solid.

1H NMR (500 MHz, acetone-d_6) δ 7.27 - 7.34 (m, 2H), 7.13 - 7.21 (m, 2H), 6.89 - 6.94 (m, 2H), 6.83 - 6.88 (m, 2H), 4.13 (br. s., 2H), 3.80 (s, 3H), 3.79 (s, 3H)

2,3-bis(4-methoxyphenyl)pyrazolo[1,5-a]pyrimidin-5(4H)-one (28)

Aminopyrazole (880 mg, 2.98 mmol) and ethyl 3-ethoxyacrylate (650 mg, 4.5 mmol) were dissolved in 30 mL of DMF. Cesium carbonate (1.50g, 4.5 mmol) was added. The reaction mixture, which turned dark red, was heated to 110° C for 4 hours. After cooling to room temperature, the reaction was neutralized with acetic acid (10 mL). The quenched reaction mixture was diluted with 150 mL of brine and extracted twice with 50 mL of ethyl acetate. The combined organic layers were washed twice with 15 mL brine, dried over Na_2SO_4, and concentrated in vacuo to yield a brick red solid. The crude product was purified by passage through a plug of silica gel (5% MeOH in dichloromethane), yielding 518 mg (50%) of a dark red solid.
H NMR (499 MHz, DMSO-d$_6$) δ 8.38 (d, $J = 7.81$ Hz, 1H), 7.30 (d, $J = 8.55$ Hz, 2H), 7.12 (d, $J = 8.30$ Hz, 2H), 6.91 (d, $J = 8.55$ Hz, 2H), 6.82 (d, $J = 8.55$ Hz, 2H), 5.94 (d, $J = 7.81$ Hz, 1H), 3.75 (s, 3H), 3.70 (s, 3H)

**5-chloro-2,3-bis(4-methoxyphenyl)pyrazolo[1,5-a]pyrimidine (29)**

Pyrazolo[1,5-a]pyrimidinone (292 mg, 0.84 mmol) and 6 mL of toluene were combined in a round-bottomed flask. Phosphorous oxychloride (516 mg, 313 μL, 3.36 mmol) was added, and the reaction mixture was heated to 110°C. After stirring at this temperature for 3 hours, the mixture was cooled to room temperature. After the addition of 15 mL ethyl acetate, the reaction was quenched dropwise with 15 mL saturated NaHCO$_3$. The layers were separated and the aqueous layer extracted three times with 15 mL ethyl acetate. The combined organic layers were washed with 10 mL brine, dried with MgSO$_4$, and concentrated. The crude product was purified by column chromatography (2:1 hexanes:EtOAc), yielding 155 mg (50%).

H NMR (500 MHz, acetone-d$_6$) δ 8.95 (d, $J = 7.20$ Hz, 1H), 8.14 (s, 1H), 7.56 - 7.63 (m, 2H), 7.35 - 7.44 (m, 2H), 7.05 (d, $J = 7.20$ Hz, 1H), 6.93 - 7.02 (m, 4H), 3.86 (s, 3H), 3.85 (s, 3H)

C NMR (126 MHz, acetone-d$_6$) δ 160.5, 159.2, 154.5, 149.9, 137.4, 131.3, 130.2, 125.5, 123.8, 114.1, 114.0, 109.1, 54.9, 54.9

HR-MS (ESI): calculated for C$_{20}$H$_{17}$N$_3$O$_2$Cl (M+H)$^+$: 366.1.009; found 366.1004

**General procedure J**

Chloropyrazole, phenol (1.5 eq.), and potassium carbonate (1.5 eq.) were combined in DMF (0.2M with respect to chloropyrazole). The reaction mixture was heated with stirred at 80°C overnight. After cooling to room temperature, the reaction mixture was diluted with brine and extracted several times with ethyl acetate. The combined organic layers were washed with brine,
dried over Na$_2$SO$_4$, and concentrated. The crude residue was purified by column chromatography or preparative TLC (hexanes / ethyl acetate).

2,3-bis(4-methoxyphenyl)-5-phenoxyrazolo[1,5-a]pyrimidine (30a)

Following general procedure J, the product was isolated in 94% yield and used without further purification.

$^1$H NMR (499 MHz, CDCl$_3$) $\delta$ 8.54 (d, $J = 7.32$ Hz, 1H), 7.54 - 7.61 (m, 2H), 7.39 - 7.48 (m, 2H), 7.32 - 7.38 (m, 2H), 7.25 - 7.31 (m, 3H), 6.87 - 6.95 (m, 2H), 6.74 - 6.83 (m, 2H), 6.50 (d, $J = 7.32$ Hz, 1H), 3.85 (s, 3H), 3.80 (s, 3H)

$^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 160.9, 160.1, 158.2, 154.7, 144.6, 137.5, 130.5, 130.4, 129.6, 125.6, 121.8, 114.1, 113.8, 106.9, 99.7, 55.5, 55.4

HR-MS: calculated for C$_{26}$H$_{22}$N$_3$O$_3$ (M + H$^+$): 424.1661; found: 424.1669

2,3-bis(4-methoxyphenyl)-5-(4-fluorophenyl)pyrazolo[1,5-a]pyrimidine (30b)

Following general procedure J, the product was isolated in 89% yield and used without further purification.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.54 (d, $J = 7.29$ Hz, 1H), 7.48 - 7.60 (m, 2H), 7.28 - 7.36 (m, 2H), 7.20 - 7.25 (m, 2H), 7.03 - 7.13 (m, 2H), 6.87 - 6.94 (m, 2H), 6.75 - 6.82 (m, 2H), 6.49 (d, $J = 7.29$ Hz, 1H), 3.83 (s, 3H), 3.80 (s, 3H)

General procedure K

The crude pyrazolopyrimidines 30 were dissolved in CH$_2$Cl$_2$ and cooled on ice. Boron trifluoride complexed with methyl sulfide was added (50 eq.), and the reaction mixture warmed to room temperature overnight. The reaction was quenched with methanol, and the solvents evaporated. The residue was neutralized with saturated NaHCO$_3$, then extracted several times with ethyl acetate. The combined organic layers were washed with brine, dried over MgSO$_4$ and
concentrated. The crude product was purified by column chromatography (1:1 hexanes:EtOAc) to yield the product.

**2,3-bis(4-hydroxyphenyl)-5-phenoxyrazolo[1,5-a]pyrimidine (31a)**

Following general procedure K, the product was obtained in 49% yield as an off-white solid.

$^1$H NMR (500 MHz, acetone-$d_6$) δ 8.84 (d, $J = 7.32$ Hz, 1H), 8.65 (br. s, 1H), 8.30 (br. s, 1H), 7.48 (d, $J = 8.55$ Hz, 4H), 7.33 - 7.40 (m, 2H), 7.26 - 7.32 (m, 1H), 7.23 (d, $J = 8.79$ Hz, 2H), 6.87 (d, $J = 8.79$ Hz, 2H), 6.66 - 6.77 (m, 3H).

HR-MS (ESI): calculated for C$_{24}$H$_{18}$N$_3$O$_5$ (M+H)$^+$: 396.1348; found: 396.1347

**2,3-bis(4-hydroxyphenyl)-5-(4-fluorophenoxy)pyrazolo[1,5-a]pyrimidine (31b)**

Following general procedure K, the product was obtained in 57% yield as an off-white solid.

$^1$H NMR (499 MHz, acetone-$d_6$) δ 8.83 (d, $J = 7.32$ Hz, 1H), 8.59 (br. s., 1H), 8.25 (br. s., 1H), 7.47 (d, $J = 8.30$ Hz, 2H), 7.33 - 7.42 (m, 2H), 7.15 - 7.30 (m, 4H), 6.86 (d, $J = 8.30$ Hz, 2H), 6.67 - 6.78 (m, 3H).

$^{13}$C NMR (126 MHz, acetone-$d_6$) δ ppm 161.0, 158.0 (d, $J=5.4$ Hz), 156.0, 154.2, 144.3, 138.4, 130.7, 130.3, 125.2, 123.7 (d, $J=8.3$ Hz), 123.4, 115.9 (d, $J=22.9$ Hz), 115.3, 115.0, 106.7, 99.6

HR-MS (ESI): calculated for C$_{24}$H$_{17}$N$_3$O$_3$ (M+H)$^+$: 414.1254; found: 414.1257

**1-iodo-3-isopropoxybenzene (32a)**

3-iodophenol (4.856 g, 22.05 mmol) and K$_2$CO$_3$ (4.671 g, 33.80 mmol) were combined in a round bottom flask with 45 mL DMF. 2-bromopropane (4.0 mL, 5.244g, 42.6 mmol) was added and the reaction mixture heated to 75°C for 24 hours. After cooling to room temperature the reaction was poured into half-saturated brine and extracted with hexanes (3 x 100 mL). The combined organic layers were washed with 50 mL brine, dried over MgSO$_4$ and concentrated *in vacuo* to yield 5.132g (89%) of a clear oil.
$^1$H NMR (499 MHz, CDCl$_3$) δ 7.18 - 7.32 (m, 2H), 6.99 (t, $J = 8.18$ Hz, 1H), 6.81 - 6.90 (m, 1H), 4.52 (spt, $J = 6.02$ Hz, 1H), 1.34 (d, $J = 6.10$ Hz, 6H)

$^{13}$C NMR (126 MHz, CDCl$_3$) δ 158.8, 131.0, 129.8, 125.3, 115.6, 94.6, 70.5, 22.2

HR-MS (ESI): calc’d for C$_9$H$_{11}$OI (M+H)$^+$: 261.98455; found: 261.98550

**1-iodo-3-(tert-butyldimethylsiloxy)benzene (32b)**

3-iodophenol (4.40 g, 20 mmol), TBSCI (4.52 g, 30 mmol, 1.5 eq.), and imidazole (2.72 g, 40 mmol, 2.0 eq.) were combined with 40 mL of DMF. The reaction mixture was stirred at room temperature for 3 hours. The reaction mixture was diluted with 200 mL of saturated NaHCO$_3$, and extracted with hexanes (3 x 100 mL). The combined organic extracts were washed with brine (2 x 100 mL), dried over MgSO$_4$ and concentrated by rotary evaporation to yield 6.22 (93%) of a clear oil, which was used without further purification.

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.27 - 7.34 (m, 1H), 7.23 (t, $J = 1.82$ Hz, 1H), 6.95 (t, $J = 8.04$ Hz, 1H), 6.81 (td, $J = 1.18$, 8.15 Hz, 1H), 0.99 (s, 9H), 0.21 (s, 6H)

$^{13}$C NMR (126 MHz, CDCl$_3$) δ 156.5, 130.9, 130.7, 129.7, 119.7, 94.4, 25.9, 18.4, -4.2

**General procedure L**

Aryl iodide was dissolved in DMF. NBS (1.1 eq.) was added portionwise to the stirring solution, which turned yellow. The solution stirred for 18 hours, before being diluted with 50% brine. The aqueous layer was extracted with hexanes, dried over MgSO$_4$, and concentrated to yield product.

**1-bromo-2-iodo-4-isopropoxybenzene (33a)**

Following general procedure L, and purification by column chromatography (10% EtOAc in hexanes), the product was obtained as a yellow oil in 73% yield.

$^1$H NMR (499 MHz, CDCl$_3$) δ 7.46 (d, $J = 8.79$ Hz, 6H), 7.39 (d, $J = 2.69$ Hz, 6H), 6.75 (dd, $J = 2.93$, 8.79 Hz, 7H), 4.48 (spt, $J = 5.86$, 1H), 1.33 (d, $J = 5.86$ Hz, 6H)
$^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 157.3, 132.9, 127.7, 120.1, 117.8, 101.3, 70.9, 22.1

HRMS (EI): calc’d for C$_9$H$_{10}$OIBr (M$^+$): 339.89671; found: 339.89600

1-bromo-2-iodo-4-(tert-butyldimethylsilyloxy)benzene (33b)

Following general procedure L, the product was obtained as clear oil in 70% yield, following column chromatography (5% EtOAc/hexanes).

$^1$H NMR (499 MHz, CDCl$_3$) $\delta$ ppm 7.44 (d, $J$=8.8 Hz, 1 H), 7.37 (d, $J$=2.9 Hz, 1 H), 6.70 (dd, $J$=8.7, 2.8 Hz, 4 H), 0.98 (s, 9 H), 0.21 (s, 6 H)

$^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ ppm 155.2, 132.8, 132.1, 121.7, 121.3, 101.1, 25.8, 18.4, -4.3

4-bromo-3-iodoanisole (33c)

Following general procedure L, the product was obtained in 84% yield as a yellow oil, and used without further purification.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ ppm 7.46 (d, $J$=9.0 Hz, 1 H), 7.38 (d, $J$=3.0 Hz, 1 H), 6.76 (dd, $J$=8.8, 3.0 Hz, 1 H), 3.76 (s, 3 H)

$^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ ppm 158.9 (s), 132.9 (s), 125.6 (s), 120.5 (s), 116.3 (s), 101.3 (s), 55.9 (s)

HRMS (EI): calc’d for C$_7$H$_6$BrOI (M$^+$): 311.86358; found: 311.86470

1-bromo-4-isopropoxy-2-((4-methoxyphenyl)ethynyl)benzene (34)

Aryl iodide 33 (1.667 g, 4.74 mmol), 4-ethynylanisole (632 mg, 4.782 mmol), CuI (70 mg, 0.368 mmol) and bis(triphenylphosphine)palladium(II) chloride (60 mg, 0.085 mmol) were combined in a round bottom flask and fitted with a septum. The reaction mixture was backfilled three times with argon before the addition of dry Et$_3$N. Using liquid nitrogen the reaction mixture was further freeze-pump-thaw degassed. After the last cycle the flask was filled with argon and warmed to room temperature before being heated to 50°C for 5 hours. The reaction mixture was
diluted with 50 mL 50% saturated aqueous NH₄Cl and extracted twice with 100 mL ethyl acetate. The combined organic layers were washed with 25 mL brine, dried over MgSO₄, and concentrated in vacuo to yield a dark yellow oil. The crude product was purified by flash chromatography (hexanes → 10% EtOAc/hexanes) to yield 1.363 g (83%) of the bromoarylalkyne.

¹H NMR (500 MHz, CDCl₃) δ 7.52 (d, J = 8.58 Hz, 2H), 7.45 (d, J = 8.79 Hz, 1H), 7.06 (d, J = 2.79 Hz, 1H), 6.89 (d, J = 8.58 Hz, 2H), 6.72 (dd, J = 2.79, 8.79 Hz, 1H), 4.51 (d, J = 6.22 Hz, 1H), 3.83 (s, 3H), 1.33 (d, J = 6.00 Hz, 6H)

¹³C NMR (126 MHz, CDCl₃) δ 160.2, 157.0, 133.4, 133.3, 126.4, 119.9, 118.2, 116.1, 115.2, 114.3, 93.9, 87.2, 70.7, 55.6, 22.2

HRMS (ESI): calc’d for C₁₈H₁₈O₂: 345.0490 (M+H⁺); found: 345.0490

1-thiomethyl-4-isoproxy-2-((4-methoxyphenyl)ethynyl)benzene (35)

Aryl bromide 34 (3.31 g, 9.59 mmol) was dissolved in 75 mL dry THF and cooled to -78°C. nBuLi (1.6M in hexanes, 12 mL, 19.2 mmol, 2 eq.) was added dropwise over 5 minutes. The resulting orange solution was stirred at the same temperature for 1 hour. Methyl disulfide (1.984 g, 21 mmol, 2.2 eq.) was added. The solution turned black briefly before returning to an orange color. The solution was stirred at -78°C for 1.5 hours, before warming to room temperature. The reaction mixture was adsorbed onto Celite, dried under vacuum, and purified by MPLC using hexanes / ethyl acetate to yield 1.658 g of a thick yellow oil in 55% yield.

¹H NMR (499 MHz, CDCl₃) δ 7.49 - 7.58 (m, 2H), 7.17 (d, J = 8.55 Hz, 1H), 7.06 (d, J = 2.69 Hz, 1H), 6.88 - 6.93 (m, 2H), 6.86 (dd, J = 2.81, 8.67 Hz, 1H), 4.53 (d, J = 6.35 Hz, 1H), 3.84 (s, 3H), 2.50 (s, 3H), 1.34 (d, J = 6.10 Hz, 6H)
$^{13}$C NMR (126 MHz, CDCl$_3$) δ 160.0, 156.0, 133.3, 131.9, 128.5, 124.4, 119.6, 117.7, 115.6, 114.2, 95.3, 86.3, 70.7, 55.5, 22.2, 16.8

HR-MS (ESI): calc’d for C$_{19}$H$_{21}$O$_2$S (M+H)$^+$: 313.1262; found: 313.1262

3-ido-5-isopropoxy-2-(4-methoxyphenyl)benzo[b]thiophene (36)

Alkyne 35 (1.600 g, 5.12 mmol) was dissolved in dichloromethane (45 mL). Iodine (1.446 g, 5.67 mmol, 1.1 eq.) was added in portions to the stirring solution. The reaction was allowed to stir for an additional 45 minutes. 80 mL of a 20% w/w Na$_2$S$_2$O$_4$ solution was added, followed by extraction with dichloromethane (2 x 150 mL). The combined organic extracts were washed with 50 mL brine, dried over MgSO$_4$, and concentrated to yield 84% of a light brown solid. The product was pure enough for further reactions. If necessary the product could be purified by column chromatography (5% EtOAc/hexanes).

$^1$H NMR (499 MHz, CDCl$_3$) δ 7.58 - 7.67 (m, 3H), 7.31 (d, $J = 2.44$ Hz, 1H), 6.98 - 7.04 (m, 3H), 4.71 (spt, $J = 6.10$ Hz, 1H), 3.89 (s, 3H), 1.43 (d, $J = 6.10$ Hz, 6H)

$^{13}$C NMR (126 MHz, CDCl$_3$) δ 160.3, 156.9, 143.5, 143.4, 131.4, 131.2, 127.3, 123.1, 117.2, 114.1, 111.7, 78.8, 70.9, 55.6, 22.3

HRMS (ESI): calc’d for C$_{18}$H$_{18}$O$_2$Si (M+H)$^+$: 425.0072; found 425.0075

5-isopropoxy-2,3-bis(4-methoxyphenyl)benzo[b]thiophene (37)

Benzo[b]thiophene iodide 36 (1.338 g, 3.15 mmol), 4-methoxyphenylboronic acid (720 mg, 4.74 mmol, 1.5 eq), cesium carbonate (2.33 g, 7.15 mmol, 2.27 eq.) and PdCl$_2$(dppf) (208 mg, 0.25 mmol, 7.9 eq.) were combined in a dry round-bottom flask fitted with a condenser and septum. The reaction apparatus was backfilled with argon three times. 5 mL dry dioxane was added through the septum and placed in a preheated 110°C oil bath. The reaction mixture refluxed for 1.5 hours, before cooling to room temperature. 25 mL saturated NH$_4$Cl was added. The aqueous
layer was extracted with ethyl acetate (3 x 25 mL), dried over MgSO\textsubscript{4} and concentrated. The crude residue was purified by column chromatography (15% EtOAc in hexanes) to yield 1.020 g (80%) of a yellow solid.

\textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) \(\delta\) 7.70 (d, \(J = 8.58\) Hz, 1H), 7.22 - 7.26 (m, 4H), 7.05 (d, \(J = 2.36\) Hz, 1H), 6.97 (dd, \(J = 2.47, 8.68\) Hz, 1H), 6.92 - 6.96 (m, 2H), 6.75 - 6.81 (m, 2H), 4.51 (spt, \(J = 6.04\) Hz, 1H), 3.85 - 3.87 (m, 3H), 3.79 (s, 3H), 1.31 (d, \(J = 6.00\) Hz, 6H)

\textsuperscript{13}C NMR (126 MHz, CDCl\textsubscript{3}) \(\delta\) 159.3, 159.0, 156.0, 142.6, 140.5, 132.0, 131.7, 131.2, 130.9, 128.2, 127.2, 122.9, 115.9, 114.4, 114.0, 109.3, 70.7, 55.5, 55.4, 22.3

HR-MS (ESI): calc’d for C\textsubscript{25}H\textsubscript{25}O\textsubscript{3}S (M+H): 405.1524; found: 405.1525

\textbf{5-hydroxy-2,3-bis(4-methoxyphenyl)benzo[b]thiophene (38)}

Benzo[b]thiophene 37 (787 mg, 1.95 mmol) was dissolved in CH\textsubscript{2}Cl\textsubscript{2}. AlCl\textsubscript{3} (400 mg, 2.99 mmol, 1.5 eq) was added, and the reaction stirred for 48 hours. The reaction was quenched with methanol, loaded onto Celite, and purified by MPLC (hexanes/EtOAc gradient) to yield 481 mg (68%) of a yellow foam.

\textsuperscript{1}H NMR (499 MHz, CDCl\textsubscript{3}) \(\delta\) 7.69 (d, \(J = 8.55\) Hz, 1H), 7.17 - 7.32 (m, 4H), 7.00 (d, \(J = 2.20\) Hz, 1H), 6.89 - 6.97 (m, 3H), 6.80 (d, \(J = 8.55\) Hz, 2H), 5.04 (br. s., 1H), 3.86 (s, 3H), 3.81 (s, 3H)

\textsuperscript{13}C NMR (126 MHz, CDCl\textsubscript{3}) \(\delta\) 159.4, 159.0, 153.6, 142.8, 140.9, 131.7, 131.1, 130.9, 128.2, 127.1, 123.1, 114.4, 114.3, 114.1, 108.5, 55.5, 55.5

\textbf{2,3-bis(4-methoxyphenyl)benzo[b]thiophene-5-tosylate (39)}

38 (392 mg, 1.08 mmol) and tosyl chloride (224 mg, 1.17 mmol, 1.09 eq.) were dissolved in 3 mL dichloromethane. 1 mL pyridine was added, and the reaction stirred overnight. The reaction was diluted with 10 mL saturated NH\textsubscript{4}Cl and extracted three times with 10 mL of
dichloromethane. The combined extracts were dried over MgSO$_4$ and concentrated by rotary evaporation. The crude residue was purified by silica gel chromatography (40% EtOAc in hexanes) to yield 284 mg (51%) of 39 as an off-white powder.

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.58 - 7.77 (m, 3H), 7.27 (d, $J = 8.06$ Hz, 2H), 7.19 (d, $J = 8.55$ Hz, 2H), 6.93 - 7.06 (m, 4H), 6.82 - 6.89 (m, 2H), 6.76 (d, $J = 8.79$ Hz, 2H), 3.80 - 3.93 (m, 3H), 3.76 (s, 3H), 2.38 - 2.52 (m, 3H)

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 159.6, 159.1, 147.4, 145.4, 142.0, 141.6, 137.1, 132.5, 131.9, 131.4, 130.9, 129.9, 128.9, 127.1, 126.4, 123.1, 119.3, 116.8, 114.4, 114.1, 55.4, 22.0

2,3-bis(4-methoxyphenyl)benzo[b]thiophene-5-triflate (40)

38 (397 mg, 1.09 mmol) was dissolved in 1 mL dry pyridine. The reaction was cooled to 0°C for several minutes before triflic anhydride (325 mg, 1.18 mmol, 1.09 eq.) was added dropwise. The solution was stirred at this temperature for 40 minutes. The reaction mixture was diluted with 30 mL of ethyl acetate and washed sequentially with saturated NH$_4$Cl (10 mL) and brine (10 mL). The organic layer was dried over MgSO$_4$ and concentrated. The crude residue was purified by silica gel chromatography (15% EtOAc in hexane) to yield 374 mg (69%) of 40 as a waxy yellow solid.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.88 (d, $J = 8.79$ Hz, 1H), 7.44 (d, $J = 2.36$ Hz, 1H), 7.17 - 7.29 (m, 5H), 6.94 - 7.01 (m, 2H), 6.77 - 6.85 (m, 2H), 3.88 (s, 3H), 3.80 (s, 3H)

$^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 159.8, 159.4, 147.5, 142.8, 142.5, 138.2, 132.1, 131.5, 131.0, 126.9, 126.2, 123.7, 119.0, 117.5, 115.8, 114.7, 114.2, 55.5, 55.5

4-bromo-2-iodothioanisole (41)

4-bromo-2-iodoaniline (4.732g, 15.88 mmol) was dissolved in 40 mL acetonitrile in a three-neck round-bottomed flask with a stopper, condenser and septum. Methyl disulfide (1.464 g, 15.54
mmol, 0.979 eq.) was added dropwise to the dark purple solution. The reaction mixture was heated to 35°C, at which time isoamyl nitrite (2.0 mL, 1.961 g, 17.73 mmol, 1.05 eq.) was added dropwise. The reaction mixture was stirred at the same temperature for 10 minutes; the oil bath temperature was then increased to 80°C. During this time a yellow precipitate formed with simultaneous gas evolution. After 30 to 60 minutes, production of yellow precipitation and gas evolution ceased. The reaction mixture was transferred to a round bottom flask and concentrated by rotary evaporation. The crude residue was purified by silica gel chromatography (5% EtOAc in hexanes), yielding 2.204 g (42%) of a red oil.

\(^1^H\) NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.91 (d, \(J = 1.93\) Hz, 1H), 7.45 (dd, \(J = 2.04, 8.47\) Hz, 1H), 6.94 (d, \(J = 8.58\) Hz, 1H), 2.45 (s, 3H)

\(^1^3^C\) NMR (126 MHz, CDCl\(_3\)) \(\delta\) 142.8, 141.2, 131.9, 125.7, 118.3, 97.7, 17.4

HRMS (EI): calc’d for C\(_7\)H\(_6\)BrIS (M\(^+\)): 327.84185; found: 327.84125

**4-bromo-2-(4-methoxyphenylethynyl)thioanisole (42)**

4-bromo-2-iodothioanisole (2.196 g, 6.67 mmol), 4-ethynylanisole (950 mg, 7.168 mmol, 1.08 eq.), CuI (78 mg, 0.409 mmol, 0.06 eq.) and PdCl\(_2\)(PPh\(_3\))\(_2\) were combined in a round bottom flask. The flask was evacuated and backfilled three times with argon. Distilled Et\(_3\)N (13 mL) was added, and the reaction mixture heated to 50°C for 6 hours. The reaction was cooled to room temperature, diluted with 50 mL saturated aqueous NH\(_4\)Cl, then extracted 4 times with 50 mL ethyl acetate. The combined organic fractions were dried over MgSO\(_4\) and concentrated by rotary evaporation. The crude brown oil was purified by silica gel chromatography (9:1 hexanes : EtOAc) to yield 1.223 g (55%) of a yellow oil which solidified upon standing.

\(^1^H\) NMR (500 MHz, CDCl\(_3\)) \(\delta\) 7.59 (d, \(J = 2.14\) Hz, 1H), 7.48 - 7.53 (m, 2H), 7.38 (dd, \(J = 2.25, 8.47\) Hz, 1H), 7.00 (d, \(J = 8.58\) Hz, 1H), 6.85 - 6.91 (m, 2H), 3.83 (s, 3H), 2.48 (s, 3H)
\[ ^{13} \text{C NMR (126 MHz, CDCl}_3 \] \[ \delta \) 160.3, 140.9, 134.5, 133.4, 131.5, 125.7, 123.6, 117.6, 115.0, 114.3, 97.5, 84.6, 55.6, 15.4 \]

HRMS (ESI): calc’d for C\(_{16}\)H\(_{14}\)BrOS (M+H): 332.9949; found: 332.9956

**5-bromo-3-iodo-2-(4-methoxyphenyl)benzo[b]thiophene (43)**

42 (1.174 g, 3.52 mmol) was dissolved in 35 mL CH\(_2\)Cl\(_2\) at room temperature. Iodine (980 mg, 3.86 mmol, 1.1 eq.) was added portionwise. The reaction was allowed to stir for 30 minutes; at this time 100 mL saturated Na\(_2\)S\(_2\)O\(_3\) were added, and the aqueous layer extracted 3 times with 50 mL ethyl acetate. The combined organic extracts were dried over MgSO\(_4\) and concentrated by rotary evaporation to yield 1.450g (97%) of a pale yellow solid, which was used without further purification.

\[ ^{1} \text{H NMR (500 MHz, CDCl}_3 \] \[ \delta \) 7.96 (d, \( J = 1.72 \) Hz, 1H), 7.59 - 7.64 (m, 3H), 7.46 (dd, \( J = 1.93, 8.36 \) Hz, 1H), 6.98 - 7.05 (m, 2H), 3.88 (s, 3H) \]

\[ ^{13} \text{C NMR (126 MHz, CDCl}_3 \] \[ \delta \) 160.5, 144.2, 143.9, 137.7, 131.5, 129.1, 128.6, 126.6, 123.6, 119.7, 114.2, 55.6 \]

**5-bromo-2,3-bis(4-methoxyphenyl)benzo[b]thiophene (44)**

Iodobromothiophene (1.287 g, 2.89 mmol), 4-methoxyphenylboronic acid (465 mg, 3.06 mmol, 1.06 eq.), cesium carbonate (2.728 g, 8.37 mmol, 2.89 eq.) and PdCl\(_2\)(dppf)(CH\(_2\)Cl\(_2\)) were combined in a three neck flask fitted with a glass topper, condenser and septum. The flask was evacuated and backfilled three times with argon. Under argon, 5.8 mL dioxane was added, and the flask was placed in a 100°C bath for 1 hour. After cooling to room temperature the reaction mixture was diluted with 25 mL brine and extracted 4 times with 25 mL ethyl acetate. The combined organic extracts were dried over MgSO\(_4\), and concentrated by rotary evaporation. The
crude residue was purified by silica chromatography (9:1 hexanes:EtOAc) to yield 718 mg (58%) of a white solid.

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.69 (d, $J = 8.58$ Hz, 1H), 7.67 (d, $J = 1.93$ Hz, 1H), 7.39 - 7.44 (m, 1H), 7.19 - 7.27 (m, 4H), 6.93 - 6.99 (m, 2H), 6.77 - 6.82 (m, 2H), 3.87 (s, 3H), 3.79 (s, 3H)

$^{13}$C NMR (126 MHz, CHLOROFORM-d) δ 159.7, 159.3, 143.2, 141.2, 137.3, 131.7, 130.9, 127.4, 126.6, 126.0, 123.5, 118.8, 114.6, 114.2, 55.5, 55.5

HR-MS (ESI): calculated for C$_{22}$H$_{18}$O$_2$BrS (M+H)$^+$: 425.0211; found 425.0215

**General procedure M**

Pd(OAc)$_2$ and BINAP were combined in a pressure tube with 1 mL toluene. The tube was fitted with a septum, evacuated and backfilled with argon (this cycle was repeated two more times). The tube was heated to 60°C for 30 minutes. The resulting homogeneous orange solution was cooled to room temperature. Benzothiophene bromide (1.0 eq.), aniline (1.5 eq.) and NaOtBu (1.5 eq.) were added, and the tube was closed with a Teflon screw cap. The tube was heated to 150°C for 24 hours. After cooling to room temperature, the toluene solution was directly applied to a silica gel column and eluted with 5:1 hexanes : ethyl acetate, yielding the diarylamine product.

**N-(phenyl)-5-amino-2,3-bis(4-methoxyphenyl)benzo[b]thiophene (45a)**

Following general procedure M, the product was obtained as a white solid in 24% yield.

$^1$H NMR (500 MHz, CDCl$_3$) δ 7.73 (d, $J = 8.58$ Hz, 1H), 7.20 - 7.29 (m, 7H), 7.18 (dd, $J = 2.25$, 8.47 Hz, 1H), 7.00 (d, $J = 7.50$ Hz, 2H), 6.91 - 6.95 (m, 2H), 6.88 (t, $J = 7.40$ Hz, 1H), 6.77 - 6.83 (m, 2H), 5.72 (s, 1H), 3.84 (s, 3H), 3.78 - 3.81 (m, 3H)

$^{13}$C NMR (126 MHz, CDCl$_3$) δ 159.4, 159.0, 144.4, 142.7, 140.4, 140.1, 132.2, 131.8, 131.7, 130.9, 129.6, 128.1, 127.1, 122.9, 120.5, 118.0, 116.7, 114.4, 114.0, 113.2, 55.5
HR-MS (ESI): Calculated for C_{28}H_{24}NO_2S (M+H)^+: 438.1528; found: 438.1530

N-(4-fluorophenyl)-5-amino-2,3-bis(4-methoxyphenyl)benzo[b]thiophene (45b)

Following general procedure M, the product was obtained as an orange solid in 24% yield.

^1H NMR (500 MHz, CDCl_3) δ 7.71 (d, J = 8.58 Hz, 1H), 7.21 - 7.27 (m, 4H), 7.17 (d, J = 1.72 Hz, 1H), 7.09 (dd, J = 2.14, 8.58 Hz, 1H), 6.88 - 7.00 (m, 6H), 6.76 - 6.82 (m, 2H), 5.59 (s, 1H), 3.84 (s, 3H), 3.79 (s, 3H)

^13C NMR (126 MHz, CDCl_3) δ ppm 159.4, 159.0, 156.9, 142.7, 140.9, 140.5, 140.3 (d, J=2.8 Hz), 131.8, 131.7, 131.7, 130.9, 128.1, 127.1, 122.9, 119.1 (d, J=7.4 Hz), 117.1, 116.1 (d, J=23.0 Hz), 114.2, 112.0, 55.5

HR-MS (ESI): calculated for C_{28}H_{23}FNO_2S (M+H)^+: 456.1434; found: 456.1432

N-(3-fluorophenyl)-5-amino-2,3-bis(4-methoxyphenyl)benzo[b]thiophene (45c)

Following general procedure M, the product was obtained as an orange solid in 31% yield.

^1H NMR (500 MHz, CDCl_3) δ 7.76 (d, J = 8.58 Hz, 1H), 7.21 - 7.29 (m, 5H), 7.10 - 7.21 (m, 2H), 6.90 - 6.99 (m, 2H), 6.76 - 6.83 (m, 2H), 6.64 - 6.73 (m, 2H), 6.53 (m, 1H), 5.79 (s, 1H), 3.85 (s, 3H), 3.79 (s, 3H)

^13C NMR (126 MHz, CDCl_3) δ ppm 159.4, 159.0, 156.9, 142.7, 140.9, 140.5 (s), 140.3, 131.8, 131.7, 131.7, 130.9, 128.1, 127.1, 122.9, 119.1 (d, J=7.4 Hz), 117.1, 116.1 (d, J=22.1 Hz), 114.4, 114.0, 112.0, 55.5

HR-MS (ESI): calculated for C_{28}H_{23}FNO_2S (M+H)^+: 456.1434; found: 456.1436

General procedure I

Benzo[b]thiophene dimethyl ethers were dissolved in dichloromethane and cooled to 0°C. BBr_3 (1.0M in dichloromethane, 5.0 eq.) was added to the reaction. The reaction was stored at -20°C overnight, and then warmed to 0°C. Cold methanol was slowly added to quench the reaction.
The solvents were removed by rotary evaporation. The residue was dissolved in a small amount of ethyl acetate and purified by preparative TLC.

**N-(phenyl)-5-amino-2,3-bis(4-hydroxyphenyl)benzo[b]thiophene (46a)**

Following general procedure I, the product was isolated as a white solid in 80% yield.

$^1$H NMR (500 MHz, acetone-$d_6$) δ 8.64 (s, 1H), 8.54 (s, 1H), 7.78 (d, $J = 8.58$ Hz, 1H), 7.29 (d, $J = 2.14$ Hz, 1H), 7.08 - 7.25 (m, 9H), 6.87 - 6.94 (m, 2H), 6.75 - 6.84 (m, 3H)

$^{13}$C NMR (126 MHz, acetone-$d_6$) δ 163.6, 157.0, 142.8, 141.2, 140.0, 131.6, 130.8, 129.3, 126.9, 126.0, 122.7, 121.7, 119.9, 117.2, 116.7, 115.8, 115.7, 115.5, 115.4, 111.2

HR-MS (ESI): calculated for C$_{26}$H$_{20}$NO$_2$S (M+H)$^+$: 410.1215; found: 410.1216

**N-(4-fluorophenyl)-5-amino-2,3-bis(4-hydroxyphenyl)benzo[b]thiophene (46b)**

Following general procedure I, the product was isolated as a purple solid in 42% yield.

$^1$H NMR (500 MHz, acetone-$d_6$) δ 8.57 (s, 1H), 8.48 (s, 1H), 7.77 (d, $J = 8.58$ Hz, 1H), 7.41 (s, 1H), 7.07 - 7.27 (m, 8H), 6.96 - 7.05 (m, 2H), 6.85 - 6.94 (m, 2H), 6.71 - 6.81 (m, 2H)

$^{13}$C NMR (126 MHz, CD$_3$OD) δ ppm 157.2 - 157.3 (m), 156.7 - 156.7 (m), 142.6 (s), 140.2 (s), 140.0 (s), 131.7 (s), 131.4 (s), 130.5 (s), 130.2 (s), 130.1 (s), 126.9 (s), 125.9 (s), 122.3 (s), 117.5 (s), 115.4 (s), 115.0 (s), 112.2 (s), 111.3 (s), 105.1 (d, $J=21.2$ Hz)

HR-MS (ESI): calculated for C$_{26}$H$_{19}$NO$_2$FS (M+H)$^+$: 428.1121; found: 428.1117

**N-(3-fluorophenyl)-5-amino-2,3-bis(4-hydroxyphenyl)benzo[b]thiophene (46c)**

Following general procedure I, the product was isolated as a silver solid in 61% yield.

$^1$H NMR (500 MHz, CD$_3$OD) δ 7.71 (dd, $J = 0.43$, 8.58 Hz, 1H), 7.25 (dd, $J = 0.54$, 2.25 Hz, 1H), 7.06 - 7.18 (m, 6H), 6.80 - 6.86 (m, 2H), 6.78 (m, 1H), 6.71 (dd, $J = 2.25$, 11.68 Hz, 1H), 6.64 - 6.68 (m, 2H), 6.42 - 6.47 (m, 1H)

HR-MS (ESI): calculated for C$_{26}$H$_{19}$NO$_2$FS (M+H)$^+$: 428.1121; found: 428.1125
2.7 REFERENCES


Chapter 3: Inhibition of the Estrogen Receptor/Coactivator Protein Interaction

3.1 INTRODUCTION

One recent trend in breast cancer therapy involves inhibition of the ER/coactivator binding protein interaction. This approach may succeed in cases of SERM resistance. Tamoxifen resistance, in some cases, occurs when the ER undergoes post-translational modifications that render it active even in the absence of ligand. This form of ER still requires coactivator proteins for its transactivation function. Thus, inhibiting the ER/coactivator interaction would still inhibit ER function.

3.2 PEPTIDOMIMETICS

As mentioned in Chapter 1, the ER binds to many different types of steroid receptor coactivator (SRC) proteins containing LXXLL sequences which interact with a groove on the surface of the ER (Figure 3.1). Traditionally, inhibition of protein-protein interactions with a small molecule is considered to be difficult. First, the surface areas being targeted are typically larger than a typical binding pocket in a receptor protein. Second, the surfaces of proteins are flexible, resulting in a lack of a well-defined interaction surface. In the case of coactivator proteins, their widespread use by many different transcription factors makes selectivity an issue.

Despite these potential drawbacks, the development of effective inhibitors of protein-protein interactions represents a promising goal in medicinal chemistry. These inhibitors can be small molecules or modified peptides. Peptidomimetics refers to the use of small molecules which mimic key structural features of one of the proteins being involved in the interaction. Peptidomimetic compounds have been designed to mimic various secondary structures, including α-helices and β-sheets.
The α-helix motif plays a key role in many important protein-protein interactions, including p53/MDM2, Bcl-xL/Bak, and nuclear receptor / coactivator protein interactions. These helices typically contain hydrophobic residues along one face of the helix positions $i, i + 3$ or 4, and $i + 7$. Several different peptidomimetic cores have been designed for disrupting protein-protein interactions such as the ones mentioned above (Figure 3.2). Hamilton designed a terphenyl system with pendant alkyl chains to mimic these interactions. Terphenyl 1 inhibited the Bcl-xL / Bak interaction with a $K_i$ of 0.112 µM, mimicking the VXXLXXI motif found in Bak; any other pattern of alkyl substitution lowered the activity 10-fold. Compound 1 also inhibited the p53/DM2 interaction with a $K_i$ of 0.182 µM. The p53 protein contains an FXXWXXL α-helix similar to the one found in the Bcl-xL/Bak interaction. The ability of one compound to inhibit two different protein-protein interactions at different affinities could prove to be a problem. These initial compounds showed good binding affinity; however, they were unlikely to be good pharmaceutical candidates as they are very nonpolar. Hamilton developed a second generation of more polar scaffolds. Terephthalamide 2 inhibited the Bcl-xL/Bak interaction with a $K_i$ of 0.78 µM. These terephthalamides were not tested with other protein-protein interactions.

High throughput screening led to the identification of several imidazolines as potential inhibitors of p53/MDM2 interaction. Cis-imidazoline 3, also known as Nutlin-1, bound with an IC$_{50}$ of 0.26 µM. In 2010, inhibitors of the activity of importin α/β were identified in a screening process. The best of these, 4, exhibited an IC$_{50}$ of 106 µM.

All of the inhibitors described above mimic three turns of an α-helix, while the target sequence in the ER/SRC interaction is a shorter two-turn α-helix. Some inhibitors of the ER/SRC interaction are known in the literature (Figure 3.3). Lead guanylhydrazone 5 was identified by Wyeth in a high throughput screen; analogs of this compound were prepared by LaFrate et al.,
with some showing improved activity. The Katzenellenbogen group investigated an “outside-in” approach in which isobutyl chains mimicking leucine residues were placed around varying cyclic and heterocyclic cores. The trisubstituted pyrimidine core proved to be successful; further development of 6 led to several compounds with micromolar $K_i$ and $IC_{50}$ values. This approach has also been extended to the androgen receptor (AR) / coactivator interaction. The AR interacts with coactivators in which the leucines are replaced by phenylalanines or tryptophans; using substituents larger than isobutyl resulted in compounds selective for the AR over the ER. Hamilton created CBIs using biaryl compounds such as 7. These compounds had low micromolar $K_i$ values; one of these compounds was picked and shown to have very low affinity for the ER. Williams et al. attempted to make CBIs based on a biphenyl scaffold containing hydrogen bonding groups at the ends to interact with the charge clamp found in the coactivator binding groove. Even the best compound of these, compound 8, bound with poor potency and exhibited low efficacy. There is still a need for high-affinity ER CBIs.

### 3.3 DESIGN AND SYNTHESIS

Following Hamilton’s peptidomimetic scaffold, previous workers in the Katzenellenbogen group designed terphenyl CBIs to mimic a two-turn $\alpha$-helix (Figure 3.4), instead of the three-turn $\alpha$-helix mimicked by Hamilton’s design. These compounds proved difficult to make, due to the o,o’-dialkylbiphenyl moiety contained within the target scaffold.

#### 3.3.1 Diarylfurans

Following Hamilton’s strategy of using heterocycles to make the core more “drug-like”, we modified the original terphenyl design by replacing the middle ring with heterocycles that could be made by condensation reactions (Figure 3.5). The formation of these aromatic rings could provide a driving force to overcome the steric hindrance of the o,o’-dialky groups. We
proposed using an ether linkage for the alkyl group to allow for facile alkylation of the aromatic rings. The Katzenellenbogen group has synthesized several di-substituted and tri-substituted heterocycles from a common 1,4-diketone precursor. One successful method previously employed involved the reaction of aldehydes and enones using Stetter chemistry. While this reaction worked well for the para substituted chalcone, it did not produce the 1,4-diketone when the ortho substituted chalcone was used (Scheme 3.1).

Similar 1,4-diketones have been synthesized by oxidative coupling of enolates. We were able to couple 2’-methoxyacetophenone using CuCl2 to generate diketone 11 in low yields (Scheme 3.2). This compound could be effectively cyclized via a Paal-Knorr reaction to furan 12a using TsOH, or to pyrrole 12b using ammonium bicarbonate as an ammonia source.

Looking for a higher yielding diketone synthesis, we attempted homocoupling of 2’-methoxyacetophenone with 0.5 equivalents of I2 (Scheme 3.3). This approach generates 0.5 equivalents of α-iodo enone and 0.5 equivalents of the enolate of the starting ketone. This enolate typically undergoes an SN2 reaction with the α-halo ketone to generate the desired symmetrical 1,4-diketone. Repeated attempts to synthesize diketone 11 by this method were unsuccessful. 1H NMR analysis indicated that the product was not symmetrical, and the mass spectrum of the product indicated a loss of water. These two observations indicated that a coupling had taken place, followed by a dehydration that did not form 11. These data are consistent with 13 as the product. This type of furan synthesis is known as a Feist-Benary furan synthesis, in which a ketone or ester and α-halo ketone or α-halo ester couple to produce a furan. The likely mechanism (Scheme 3.4) generates the same intermediates as anticipated. Instead of the enolate alkylating at the α-carbon, however, it undergoes an aldol condensation followed by closure to
the epoxide in a manner analogous to the beginning of the Darzens condensation. Intramolecular opening of the epoxide followed by dehydration would produce the observed product.

Having demonstrated that diarylfurans could be prepared via a Feist-Benary furan synthesis, we moved forward towards the synthesis of an alkylated diarylfuran (Scheme 3.5). 2’-hydroxyacetophenone was alkylated with isobutyl bromide or isopentyl bromide in DMF to produce 14a-b. These products were treated with LDA, followed by quenching with iodine at -78 °C. The diarylfuran products 15a-b were isolated in low yield.

These furans only had substituents mimicking the i and i + 4 residues, so we attempted to incorporate an alkyl chain into the furan ring. We chose to replace the ketone with a β-keto ester; this component would be more reactive, and could later be transformed into an alkyl group. In a model system, 2’-methoxyacetophenone was acylated with diethyl carbonate in 66% yield (Scheme 3.6). β-Keto esters have been used in an interrupted Feist-Benary synthesis developed by Calter which uses the much milder triethylamine as a base and a preformed phenacyl bromide. Treatment of β-keto ester 16 with 2-bromo-2’-methoxyacetophenone did not yield the Feist-Benary product 17.

3.3.2 Diaryloxazoles

We then turned towards the synthesis of oxazoles as an alternative to the central furan ring. We briefly explored direct coupling and condensation between phenacyl bromides and benzamides, but these reactions did not reliably produce product. The Gabriel oxazole synthesis was then explored, in which an acyl α-amino ketone is cyclized under acidic conditions similar to the Paal-Knorr synthesis used to prepare furan 12a and pyrrole 12b.

2’-Methoxyacetophenone was brominated with Br₂ in ether to generate the α-bromo ketone (Scheme 2.7). The bromide was then displaced with sodium azide, yielding α-azido
ketone 18 in high yield. Initial attempts to hydrogenate the azide did not produce the α-amino ketone. Staudinger reduction with triphenylphosphine led to pyrazine 20; this reaction likely proceeded through an aza-Wittig mechanism (Scheme 2.7). We then retried the azide hydrogenation with the addition of drops of concentrated HCl. This allowed α-amino ketone 19 to be isolated as an HCl salt. This amine was acylated with acid chloride to generate the acyl α-amino ketone 21. Dehydration with TsOH did not induce cyclization. Nicolaou developed mild conditions (POCl₃/pyridine) to dehydrate acyl α-amino ketones to form oxazoles; these conditions were effectively used in the total synthesis of diazonamide A. These conditions were effectively used in our hands to convert 21 to oxazole 22.

Having generated a model oxazole, we then turned towards synthesizing the target oxazoles. We wanted to incorporate an alkyl chain on the internal oxazole, which would require generating branched α-amino ketones. Synthesizing the alkyl aryl ketones proved to be difficult. We then redesigned the diaryloxazole synthesis to use naturally occurring amino acids as the precursor to the amino ketones.

We designed a model system for a new route to diaryloxazoles beginning with amino acids. We proposed that the aminoketones could be synthesized by addition of an organometallic reagent to Weinreb amides derived from amino acids. This route has previously been employed to synthesize oxazoles.¹⁷ Screening of a variety of conditions for preparation of the Weinreb amide led to a high-yielding synthesis which did not require purification of the product (Scheme 3.8). Coupling of Weinreb amine with Boc-protected amino acids using EDCI/HOBt in DMF with ethyldiisopropylamine as the base produced Weinreb amide 23 in high yield without requiring further purification. In the next step we attempted adding Grignard and organolithium reagents to the Weinreb amide to generate the α-amino ketone.¹⁸ Compound 24 was synthesized
by first deprotonating with a sacrificial equivalent of isopropylmagnesium chloride, followed by addition of phenylmagnesium chloride. To test whether this approach would work for more functionalized Grignards, 27a was converted to the corresponding Grignard reagent, then added to Boc-Ala Weinreb amide to yield 25. This approach suffers from the requirement that excess Grignard reagent is used to deprotonate the carbamate before addition to the Weinreb amide. We utilized a one-pot methodology developed by Merck in which a mixture of an aryl iodide (27) and Weinreb amide was treated with isopropylmagnesium chloride.19 The first equivalent of Grignard reagent removed the carbamate proton. The second equivalent could either react with the Weinreb amide, resulting in an isopropyl alkyl ketone, or undergo an iodine-magnesium exchange. Fortunately for us, at low temperatures (< -50°C) the iodine-magnesium exchange proved to be faster than Grignard reaction with the Weinreb amide. The resulting Grignard reagent successfully reacted with the Weinreb amide to generate the desired α-amino ketone 26. This approach did not work using nBuLi.

The general methodology used to generate 26 was applied to the synthesis of substituted oxazoles (Scheme 3.9). The Boc group was readily removed using a solution of HCl in either dioxane or ether, allowing isolation of the hydrochloride salt 28. Compound 28b was carried on to test the final steps of the synthesis, as the oxazole derived from this product would likely be the best oxazole CBI. Coupling of this hydrochloride salt with 32 yielded acyl α-aminoketone 29. Cyclization using the POCl3 / pyridine system previously described successfully produced oxazole 30.
3.4 ANALYSIS

Coactivator Binding Inhibition

Furans 15 and diaryloxazole 31 were tested in a FRET-based assay measuring coactivator binding inhibition. Unfortunately, none of the synthesized compounds displayed any disruption of the ER/SRC interaction. The lack of activity shown by oxazole 31 suggests a flaw in the diaryloxazole design. Taking a closer look at the superposition of our model teraryl design with a coactivator peptide led us to realize our original design did not follow Hamilton’s design as closely as we had first thought.

Redesign of Ligands

Jacoby published a scaffold specifically designed to mimic the $i$, $i+1$, $i+3$, and $i+4$ residues of an α-helix; this design would mimic the SRC peptide better than Hamilton’s design which targets a larger α-helix. This scaffold contains a sterically hindered di-ortho substituted biaryl. Recent developments in transition metal-catalyzed cross-coupling reactions have allowed for synthesis of sterically hindered biaryls. Like the original Hamilton terphenyls, these compounds would likely be too nonpolar to be an effective drug. One or both of the phenyl rings could be replaced with heterocycles to create compounds like Hamilton’s biaryl CBIs.

3.5 CONCLUSIONS

We designed and synthesized potential inhibitors of the ER – SRC interaction. These compounds were proposed to effectively inhibit proliferation in ER-positive cancer cells, even in cases where these cancers have developed resistance to SERM treatment. Our peptidomimetic design did not produce effective CBIs. With a more carefully designed scaffold the ER – SRC interaction might be more effectively inhibited by peptidomimetic small molecules.
3.6 FIGURES

Figure 3.1 Representation of the coactivator binding groove. The electrostatic potential of the receptor is shown, with hydrophobic regions shown in brown and hydrophilic regions shown in blue. The key leucine side chains are shown, which bury themselves into the hydrophobic regions. At the top and the bottom of the groove are the hydrogen bonding charge clamp residues.
Figure 3.2 Previous inhibitors of protein-protein interactions based on α-helix mimicry.
Figure 3.3 Inhibitors of the ER/SRC interaction reported in the literature.
Figure 3.4 (A) The terphenyl scaffold designed by Hamilton to mimic a three-turn α-helix. The alkyl group on the central ring is reported to mimic either the $i + 3$ or the $i + 4$ residue. (B) Modification of the previous terphenyl scaffold to mimic a two-turn α-helix. (C) Second generation of the terphenyl scaffold in 4B.
Figure 3.5 The scaffolds from 4A and 4B (blue) are overlayed with a fragment from the coactivator peptide GRIP-1 (green). Only the hydrophobic sidechains are shown for clarity.
Scheme 3.1 Attempted Stetter synthesis of 1,4-diketones.

Scheme 3.2 Paal-Knorr synthesis of 2,5-diarylfuran and 2,5-diarylpyrrole.
Scheme 3.3 Unexpected synthesis of 2,4-diaryl furans.

Scheme 3.4 Mechanism of Feist-Benary reaction.
Scheme 3.5 Synthesis of 2,4-bis(2-alkoxyaryl)furans via Feist-Benary synthesis.

\[
\begin{align*}
\text{HO} & \quad \text{CH}_3 \quad + \quad \text{RBr} \quad \xrightarrow{\text{K}_2\text{CO}_3} \quad \text{OR} \quad \text{CH}_3 \\
10 & \quad 14\text{a} \quad \text{R} = \text{CH}_2\text{CH}-(\text{CH}_3)_2 & 70\% \\
14\text{b} \quad \text{R} = \text{CH}_2\text{CH}_2\text{CH}-(\text{CH}_3)_2 & 80\% \\
15\text{a} \quad \text{R} = \text{CH}_2\text{CH}-(\text{CH}_3)_2 & 20\% \\
15\text{b} \quad \text{R} = \text{CH}_2\text{CH}_2\text{CH}-(\text{CH}_3)_2 & 31\%
\end{align*}
\]

Scheme 3.6 Second attempt at Feist-Benary furan synthesis using a $\beta$-keto ester as nucleophile,
Scheme 3.7 Synthesis of α-amino ketones via reduction of azides.

- Reaction of 18 with H₂, Pd/C and HCl, MeOH to form 19 (99% yield).
- Reaction of 19 with PPh₃ in THF/H₂O to form 20.
- Reaction of 19 with chloroacetic acid in Et₃N, CH₂Cl₂ to form 21 (84% yield).
- Reaction of 21 with POCl₃ in pyridine to form 22a (81% yield).
- Reaction of 19 with Lawesson's reagent in toluene, reflux to form 22b (33% yield).
Scheme 3.8 Development of Weinreb amide route to diaryloxazoles.

Scheme diagram...
Scheme 3.9 Synthesis of diaryloxazole using Weinreb amide route.

3.7 EXPERIMENTAL

All reagents used were purchased from Aldrich Chemical Company, Fisher Scientific, or TCI America and used without further purification unless otherwise specified. Anhydrous THF, ether, dichloromethane, toluene, dioxane, DMF, DMSO, and acetonitrile were obtained from a solvent delivery system. Anhydrous triethylamine, diisopropylethylamine, and diisopropylamine were obtained by distillation from calcium hydride. When necessary, organolithium and organomagnesium reagents were titrated with menthol / 1,10-phenanthroline. Reaction progress was monitored by thin-layer chromatography (TLC) using glass-backed silica gel plates with fluorescent indicator. Unless otherwise specified, reaction temperatures refer to external bath temperatures. Flash chromatography was performed using 40-63 µm particle size (230 – 400
(mesh) silica gel from Silicycle. $^1$H and $^{13}$C NMR spectra were obtained on Varian 400 or 500 MHz FT-NMR instruments. Chemical shifts (δ) are reported in parts per million and are referenced to residual undeuterated solvent peaks. Mass spectra were obtained on a Micromass Quattro or Micromass Q-Tof mass spectrometer. Melting points were obtained on a Thomas-Hoover apparatus and are uncorrected.

**(E)-1,3-bis(2-methoxyphenyl)prop-2-en-1-one (9)**

2'-methoxyacetophenone (1.08g, 7.2 mmol) and o-anisaldehyde (1.08g, 7.92 mmol, 1.1 eq.) dissolved in 5 mL ethanol and 5 mL water. KOH (317g, 7.92 mmol, 1.1 eq.) was added, and the reaction stirred at room temperature for 1 hour. The ethanol was removed by rotary evaporation, and the crude residue exhaustively extracted with ethyl acetate. The combined organic extracts were washed with brine, dried over MgSO$_4$ and concentrated to afford to yield a yellow oil, which was used without further purification.

$^1$H NMR (499 MHz, CDCl$_3$) δ 7.95 (d, J = 16.11 Hz, 1H), 7.56 - 7.63 (m, 2H), 7.44 - 7.48 (m, 1H), 7.42 (d, J = 16.11 Hz, 1H), 7.32 - 7.38 (m, 2H), 6.94 - 7.06 (m, 5H), 3.89 (s, 3H), 3.87 (s, 3H)

**1,4-bis(2-methoxyphenyl)-1,4-butanedione (11)**

LDA (1.8M in heptanes/THF/ethylbenzene, 6.5 mL, 11.7 mmol) was added to 4 mL of dry THF and cooled to -78°. 2'-methoxyacetophenone (1.252g, 9 mmol) dissolved in 12 mL of THF was added dropwise. The solution stirred at this temperature for 30 minutes before a solution of CuCl$_2$ (1.344g, 10 mmol) in 15 mL of dry DMF was added. The reaction stirred at this temperature for 1 hour, before being allowed to warm to room temperature. The reaction mixture was diluted with brine and extracted several times with ethyl acetate. The combined organic extracts were washed with brine, dried over MgSO$_4$, and concentrated. The crude residue was
purified by column chromatography (4:1 hexanes:EtOAc) to yield 402 mg (30%) of the product as a tan powder.

\[ ^1H \text{ NMR (499 MHz, CDCl}_3\] \( \delta \) 7.75 (dd, \( J = 1.83, 7.69 \) Hz, 2H), 7.41 - 7.50 (m, 2H), 6.93 - 7.04 (m, 4H), 3.91 (s, 6H), 3.41 (s, 4H)

2,5-bis(2-methoxyphenyl)furan (12a)

Diketone 11 (600 mg, 2 mmol) and a few crystals of p-toluenesulfonic acid monohydrate were dissolved in 25 mL of toluene. The reaction flask was fitted with a Dean-Stark trap and heated at reflux for four hours. After cooling to room temperature a brown precipitate formed. This product was filtered and dried, yielding 492 mg (86%) of product.

\[ ^1H \text{ NMR (499 MHz, CDCl}_3\] \( \delta \) 8.00 (m, 2H), 7.22 - 7.25 (m, 2H), 7.02 - 7.09 (m, 4H), 6.97 (d, \( J = 8.30 \) Hz, 2H), 3.97 (s, 6H)

\[ ^13C \text{ NMR (126 MHz, CDCl}_3\] \( \delta \) 205.3, 155.7, 128.0, 126.1, 121.0, 112.6, 111.2, 55.6

HR-MS (ESI): calculated for C\(_{18}\)H\(_{17}\)O\(_3\) (M+H)\(^+\): 281.1178; found: 281.1167

2,5-bis(2-methoxyphenyl)pyrrole (12b)

Diketone 11 and ammonium formate were combined with acetic acid and heated to 100°C for 5 hours. After cooling to room temperature most of the acetic acid was removed by rotary evaporation. The remaining acid was quenched with saturated ammonium carbonate. The resulting solution was extracted exhaustively with ethyl acetate. The combined organic extracts were washed with brine and dried over Na\(_2\)SO\(_4\). The crude product was purified by column chromatography (3:1 hexanes:EtOAc) to yield 258 mg (58%) yield of a purple foam.

\[ ^1H \text{ NMR (400 MHz, CDCl}_3\] \( \delta \) 7.64 - 7.73 (m, 2H), 7.10 - 7.20 (m, 2H), 6.94 - 7.04 (m, 5H), 6.68 (d, \( J = 2.69 \) Hz, 2H), 4.02 (s, 6H)

HR-MS (ESI) calculated for C\(_{18}\)H\(_{18}\)NO\(_2\): 280.1338; found: 280.1322
**2,4-bis(2-methoxyphenyl)furan (13)**

LDA (1.8M in heptanes/THF/ethylbenzene, 6.7 mL, 12 mmol) was added to 20 mL dry THF and cooled to -78°C. 2’-methoxyacetophenone (1.09 g, 10 mmol) was added as a solution in THF. The resulting solution was stirred at this temperature for 30 minutes before a solution of I₂ (0.5 eq.) in THF was added. The reaction was allowed to warm to room temperature over 3 hours. The THF was removed by rotary evaporation. The residue was dissolved in 100 mL EtOAc and washed with 20 mL 10% Na₂S₂O₃, 20 mL of water, 0.5 M HCl, and 20 mL of brine. The organic layer was dried over MgSO₄ and concentrated. The crude residue was purified by flash chromatography (3:1 hexanes:EtOAc) to yield 624 mg (45%) of a white powder.

\(^1\)H NMR (499 MHz, CDCl₃) δ 8.05 (d, J = 0.98 Hz, 1H), 7.90 (dd, J = 1.83, 7.69 Hz, 1H), 7.63 (dd, J = 1.71, 7.57 Hz, 1H), 7.35 (d, J = 0.73 Hz, 1H), 7.23 - 7.29 (m, 2H), 7.01 - 7.07 (m, J = 1.00, 5.30, 7.50, 7.50 Hz, 2H), 6.96 - 7.01 (m, J = 1.00, 3.20, 8.20 Hz, 2H), 3.99 (s, 3H), 3.95 (s, 3H)

\(^13\)C NMR (126 MHz, CDCl₃) δ 156.8, 155.6, 149.9, 140.7, 128.3, 128.2, 127.8, 126.2, 123.6, 121.7, 121.0, 120.1, 111.2, 109.6, 55.7, 55.6

HR-MS (ESI): calculated for C\(_{18}\)H\(_{17}\)O\(_3\) (M+H): 281.1178; found: 281.1162

**General procedure A**

2’-hydroxyacetophenone, alkyl bromide, and potassium carbonate were combined in a round bottomed flask with DMF. The reaction mixture was stirred at room temperature for 6 hours. Water and brine were added, followed by extraction several times with ethyl acetate. The combined organic layers were washed with water, brine, dried over MgSO₄ and concentrated by rotary evaporation. The crude product contained some starting material, which was removed by dissolving in chloroform and washing with 1N NaOH, drying over MgSO₄ and concentrating.
2’-(2-isobutoxyphenyl)acetophenone (14a)

Following general procedure A, the product was obtained in 34% yield as a light yellow oil.

$^1$H NMR (400 MHz, CDCl$_3$) δ 7.72 (dd, $J = 1.71$, 7.81 Hz, 1H), 7.37 - 7.42 (m, 1H), 6.87 - 6.97 (m, 2H), 3.79 (d, $J = 6.35$ Hz, 2H), 2.63 (s, 3H), 2.13 (d, $J = 6.59$ Hz, 1H), 1.04 (d, $J = 6.59$ Hz, 6H)

$^{13}$C NMR (101 MHz, CDCl$_3$) δ 200.0, 158.8, 133.9, 130.6, 128.3, 120.5, 112.4, 75.2, 32.4, 28.5, 19.7

2’-(2-isopentoxyphenyl)acetophenone (14b)

Following general procedure A, the product was obtained in 82% as a light yellow oil.

$^1$H NMR (499 MHz, CDCl$_3$) δ 7.73 (dd, $J = 1.71$, 7.57 Hz, 1H), 7.38 - 7.47 (m, 1H), 6.91 - 7.01 (m, 2H), 2.62 (s, 3H), 1.85 (spt, $J = 6.70$ Hz, 1H), 0.98 (d, $J = 6.59$ Hz, 6H)

$^{13}$C NMR (101 MHz, CDCl$_3$) δ 200.1, 158.7, 133.6, 130.5, 128.4, 120.8, 112.4, 67.0, 38.2, 32.4, 25.4, 22.5

2,4-bis(2-isobutoxyphenyl)furan (15a)

Using the procedure as described for 13, 110 mg (20%) of a white powder was obtained.

$^1$H NMR (500 MHz, CDCl$_3$) δ: 8.12 (d, $J = 0.7$ Hz, 1H), 7.90 (dd, $J_1 = 1.7$ Hz, $J_2 = 7.8$ Hz, 1H), 7.56 (dd, $J_1 = 1.7$ Hz, $J_2 = 7.7$ Hz, 1H), 7.43 (d, $J = 0.8$ Hz), 7.26 – 7.21 (m, 2H), 7.04 – 7.00 (m, 2H), 6.97 (d, $J = 8.1$ Hz, 2H), 3.91 (d, $J = 6.3$ Hz), 3.86 ($J = 6.4$ Hz), 2.33 – 2.18 (m, 2H), 1.15 (d, $J = 6.5$ Hz, 6H), 1.10 (d, $J = 6.8$ Hz, 6H)

MS (Cl): 364.2

2,4-bis(2-isopentoxyphenyl)furan (15b)

Using the procedure as described for 13, 294 mg (31%) of a white powder was obtained.
$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ ppm 8.1 (d, $J$=0.9 Hz), 7.9 (dd, $J$=7.7, 1.7 Hz), 7.6 (dd, $J$=7.5, 1.7 Hz), 7.4 (d, $J$=0.6 Hz), 7.2 - 7.3 (m), 7.0 - 7.0 (m), 7.0 (d, $J$=8.1 Hz), 4.2 (t, $J$=6.4 Hz), 4.1 (t, $J$=6.6 Hz), 2.0 (spt, $J$=6.6 Hz), 1.8 - 2.0 (m), 1.0 (d, $J$=6.6 Hz), 1.0 (d, $J$=6.4 Hz)

$^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 155.1, 140.7, 129.1, 128.2, 127.8, 127.6, 126.0, 123.6, 121.7, 120.7, 120.7, 111.9, 111.9, 109.6, 66.9, 66.8, 65.7, 57.7, 38.4, 38.4, 25.5, 22.9

HR-MS (ESI): calculated for C$_{26}$H$_{33}$O$_3$ (M+H)$^+$: 393.2430; found: 393.2430

**Ethyl 3-(2-methoxyphenyl)-3-oxopropionate (16).** 2’-Methoxyacetophenone (6mL, 6.52g, 43.4 mmol) was dissolved in diethyl carbonate (50mL, 51.3g, 434 mmol) and 50 mL of dry THF in a 200 mL round-bottomed flask. NaH (2.08g, 60% dispersion in mineral oil) was washed with THF, then added slowly to the reaction mixture. The flask was then placed in an oil bath and heated at 85ºC for 2 hours. The reaction turned bright yellow, and then began to turn yellow-orange. After cooling to room temperature, the reaction mixture was slowly poured over an ice / acid mixture. Ethyl acetate was added to this solution, and the layers were separated. The ethyl acetate was washed with brine, dried over sodium sulfate, and concentrated to afford a yellow oil. The oil was purified by column chromatography (4:1 hexanes:ethyl acetate) to give 6.4g (66%) of β-keto ester. The NMR spectrum showed a 10:1 keto:enol ratio. NMR data is given for the keto form only.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.86 (dd, $J$ = 1.82, 7.83 Hz, 1H), 7.45 - 7.51 (m, 1H), 6.97 - 7.03 (m, 1H), 6.94 (dd, $J$ = 0.64, 8.36 Hz, 1H), 4.16 (q, $J$ = 7.07 Hz, 2H), 3.94 (s, 2H), 3.87 (s, 3H), 1.21 (t, $J$ = 7.07 Hz, 3H)

$^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 193.3, 168.4, 159.4, 134.9, 131.3, 126.4, 121.0, 111.8, 61.1, 55.5, 50.9, 14.3

**2-bromo-2’-methoxyacetophenone**
2’-methoxyacetophenone (10.9g, 72.3 mmol) was dissolved in 250 mL dry ether in a round bottom flask with stirring. A small scoop of AlCl₃ was added as a catalyst, which turned the color of the reaction pale yellow. Bromine (3.74 mL, 11.6g, 72.3, mmol) was added dropwise. During the course of the addition, the reaction transiently turned orange before fading. At the end of the addition the reaction maintained an orange color. After stirring for 30 minutes, the reaction was carefully poured into ice / NaHCO₃ / water and stirred until colorless. The organic layers were separated, washed with brine, dried over Na₂SO₄, and concentrated to yield 14.36g (87%) of a clear oil which solidified upon storage at -20°C. The product was used without further purification.

H NMR (400 MHz, CDCl₃) δ 7.82 (d, J = 2.20, 8.06 Hz, 1H), 7.48 - 7.55 (m, 1H), 6.96 - 7.06 (m, 2H), 4.61 (s, 2H), 3.94 (s, 3H)

C NMR (101 MHz, CDCl₃) δ 193.5, 159.2, 135.0, 131.8, 125.0, 121.3, 111.8, 55.8, 38.1

2-azido-2’-methoxyacetophenone (18)

2-bromo-2’-methoxyacetophenone (1.000g, 4.365 mmol) was dissolved in 30 mL acetone. Sodium azide (426 mg, 6.55 mmol) added in one portion. The reaction mixture was refluxed for 2 hours before cooling to room temperature. The solids were filtered off, and the acetone removed by rotary evaporation to yield 820 mg (100%) of product.

H NMR (400 MHz, CDCl₃) δ 7.93 (d, J = 1.71, 7.81 Hz, 1H), 7.49 - 7.58 (m, 1H), 7.03 - 7.09 (m, 1H), 6.96 - 7.02 (m, 1H), 4.52 (s, 2H), 3.94 (s, 3H)

C NMR (126 MHz, CDCl₃) δ 196.5, 139.7, 134.5, 132.8, 132.7, 128.6, 126.2, 56.7, 21.8

2-amino-2’-methoxyacetophenone hydrochloride (19)

150 mg 2-azido-2’-methoxyacetophenone was dissolved in MeOH in a glass bottle. 0.5 mL concentrated HCl and Pd/C (20 mg, 10% Pd/C) were added. The reaction vessel placed in a Parr
shaker and pressurized to 30 psi. After 4 hours, the reaction mixture was filtered through celite. The methanol was evaporated to yield 156 mg (99%) of a tan solid.

$^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 7.98 (dd, $J = 1.72, 7.93$ Hz, 1H), 7.61 - 7.70 (m, 1H), 7.23 (d, $J = 8.58$ Hz, 1H), 7.10 (t, $J = 7.50$ Hz, 1H), 4.91 (br. s., 3H), 4.43 (s, 2H), 4.01 (s, 3H)

$^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 191.7, 160.6, 136.3, 130.5, 123.1, 120.9, 112.3, 55.2, 49.2

HR-MS (ESI) calculated for C$_9$H$_{12}$NO$_2$ ($M^+$): 166.0868; found: 166.0873

2,5-bis(2-methoxyphenyl)pyrazine (20)

2-azido-2'-methoxyacetophenone (400 mg, 2.09 mmol) was dissolved in 25 mL dry THF. Triphenyphosphine (823 mg, 3.14 mmol) was added portionwise, and the reaction mixture heated to reflux for 18 hours. The reaction was cooled to room temperature and the THF removed by rotary evaporation. Ether was added to the crude residue. After filtration of undissolved solids, the ether was washed with saturated NaHCO$_3$, brine, dried over Na$_2$SO$_4$ and concentrated. The crude product was purified by column chromatography (3:1 → 1:2 hexanes:EtOAc) to yield 77 mg 25% of 20 as a yellow powder.

$^1$H NMR (499 MHz, CDCl$_3$) $\delta$ 9.26 (s, 2H), 7.94 (dd, $J = 1.59, 7.69$ Hz, 2H), 7.37 - 7.49 (m, 2H), 7.14 (dt, $J = 0.98, 7.45$ Hz, 2H), 7.06 (dd, $J = 0.98, 8.30$ Hz, 2H), 3.93 (s, 6H)

$^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 157.5, 149.0, 145.6, 131.3, 131.0, 126.1, 121.5, 111.6, 55.8

LR-MS (CI): 293.1 (M+H)$^+$

N-(2-methoxybenzoyl)-2-amino-2'-methoxyacetophenone (21)

Amino ketone hydrochloride 19 (350 mg, 1.73 mmol) was combined with 5 mL of CH$_2$Cl$_2$. 2-methoxybenzoyl chloride (281 mg, 1.65 mmol, 0.95 eq.) was added. The reaction was cooled in an ice-bath before the addition of triethylamine (337 mg, 3.3 mmol, 1.9 eq.). The reaction stirred

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at room temperature overnight. The solution was concentrated and the residue purified by column chromatography (2:1 CH$_2$Cl$_2$:EtOAc) to yield 415 mg (84%) of product as a white solid.

$^1$H NMR (499 MHz, CDCl$_3$) $\delta$ 9.11 (br. s., 1H), 8.25 (dd, $J$ = 1.71, 7.81 Hz, 1H), 8.00 (dd, $J$ = 1.83, 7.69 Hz, 1H), 7.56 (ddd, $J$ = 1.95, 7.08, 8.55 Hz, 1H), 7.48 (ddd, $J$ = 1.83, 7.26, 8.24 Hz, 1H), 7.00 - 7.13 (m, 4H), 4.98 (d, $J$ = 4.39 Hz, 2H), 4.10 (s, 3H), 4.00 (s, 3H)

$^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 195.5, 165.3, 160.0, 158.2, 135.1, 133.0, 132.4, 131.2, 125.0, 121.6, 121.3, 121.0, 112.0, 111.6, 56.3, 55.9, 52.2

2,5-bis(2-methoxyphenyl)oxazole (22)

Keto amide 22 (150 mg, 0.5 mmol) was dissolved in 2 mL pyridine. POCl$_3$ (0.25 mL) was slowly added to the reaction mixture, which turned bright yellow. The solution stirred at room temperature for 4 hours. The reaction mixture was diluted with 50 mL EtOAc and washed with 10 mL sat. NaHCO$_3$, 10 mL 0.5N HCl, and 10 mL brine. The organic layer was dried over Na$_2$SO$_4$ and concentrated. The crude residue was purified by column chromatography (1:1 EtOAc:hexanes) to yield 114 mg (81%) of the diaryloxazole.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.06 (dd, $J$ = 1.72, 7.72 Hz, 1H), 7.89 (dd, $J$ = 1.72, 7.72 Hz, 1H), 7.70 (s, 1H), 7.39 - 7.47 (m, 1H), 7.30 (ddd, $J$ = 1.72, 7.40, 8.25 Hz, 1H), 7.03 - 7.12 (m, 3H), 6.99 (d, $J$ = 8.36 Hz, 1H), 4.01 (s, 3H), 3.97 - 3.99 (m, 3H)

$^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 170.4, 158.6, 157.8, 155.9, 153.3, 131.7, 130.3, 129.1, 127.8, 126.2, 121.0, 120.8, 112.2, 111.1, 56.3, 55.7

2,5-bis(2-methoxyphenyl)thiazole (22b)

Ketoamide (100 mg, 0.33 mmol) and Lawesson’s reagent (202 mg, 0.5 mmol) were refluxed in 5 mL THF for 6 hours. After cooling to room temperature the THF was removed by rotary evaporation. The residue was dissolved in CH$_2$Cl$_2$, washed with brine, dried over MgSO$_4$ and
The crude product was purified by column chromatography (2:1 → 1:1 hexanes:EtOAc), yielding 32.7 mg (33%) of the product as a light yellow foam.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.42 (dd, $J = 1.72, 7.72$ Hz, 1H), 8.30 (s, 1H), 7.68 (dd, $J = 1.61, 7.61$ Hz, 1H), 7.35 - 7.44 (m, 1H), 7.27 - 7.33 (m, 1H), 7.07 - 7.14 (m, 1H), 6.96 - 7.06 (m, 3H), 4.04 (s, 3H), 3.95 (s, 3H)

$^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 162.0, 156.5, 155.9, 140.3, 135.2, 130.6, 129.2, 129.0, 128.3, 122.9, 121.3, 121.3, 111.7, 111.6, 55.8, 55.8

### General procedure B

N-Boc-protect amino acid (1.0 eq) and $N,O$-dimethylhydroxylamine hydrochloride (2.0 eq) were combined with isopropylidethylamine (3.0 eq) in DMF (0.33 M) and stirred for 5 minutes until solution is homogeneous. HOBt (1.2 eq) and EDC hydrochloride (1.2 eq) were added, and the solution stirred for two hours. The reaction mixture was diluted with cold water and extracted several times with ethyl acetate. The combined organic layers were washed with 0.5 M cold HCl, several times with brine, and dried over MgSO$_4$. The solvent was removed by rotary evaporation, leaving a thick oil, which was used without further purification.

### N-Boc-Ala Weinreb amide (23a)

Following general procedure B, the product was isolated as a tan solid in 99% yield

$^1$H NMR (499 MHz, CDCl$_3$) $\delta$ 5.17 - 5.32 (m, 1H), 4.56 - 4.77 (m, 1H), 3.77 (s, 3H), 3.21 (s, 3H), 1.44 (s, 9H), 1.31 (d, $J = 6.84$ Hz, 3H)

### N-Boc-Val Weinreb amide (23b)

Following general procedure B, the product was isolated as a thick syrup in 99% yield

$^1$H NMR (499 MHz, CDCl$_3$) $\delta$ 5.05 - 5.23 (m, 1H), 4.49 - 4.64 (m, 1H), 3.78 (s, 3H), 3.22 (s, 3H), 1.93 - 2.04 (m, 1H), 1.44 (s, 9H), 0.96 (d, $J = 6.84$ Hz, 3H), 0.91 (d, $J = 6.84$ Hz, 3H)
N-Boc-Leu Weinreb amide (23c)
Following general procedure B, the product was isolated as a thick syrup in 99% yield

$^1$H NMR (499 MHz, CDCl$_3$) $\delta$ 4.99 - 5.16 (m, 1H), 4.51 - 4.86 (m, 1H), 3.79 (s, 3H), 3.19 (s, 3H), 1.61 - 1.82 (m, 1H), 1.36 - 1.51 (m, 12H), 0.96 (d, $J = 6.59$ Hz, 3H), 0.92 (d, $J = 6.59$ Hz, 3H)

N-Boc-2-amino-1-phenylpropanone (24)
100 mg (0.43 mmol) of N-Boc-Ala Weinreb amide was dissolved in THF and cooled to -78°C. iPrMgCl (0.2 mL of a 2.0M solution in THF, 0.40 mmol, 0.95 eq.) was added dropwise. After stirring for 30 minutes, phenylmagnesium chloride (0.27 mL of a 2.0M solution in THF, 0.54 mmol, 1.25 eq) was added. The reaction was allowed to warm to room temperature and stirred overnight. The reaction was quenched with cold 0.5N HCl and extracted several times with ethyl acetate. The combined organic extracts were washed with brine, dried over Na$_2$SO$_4$ and concentrated by rotary evaporation. The crude residue was purified by 7:2 hexanes:EtOAc to yield 59.7 mg (56%) of a clear oil.

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.96 (d, $J = 7.57$ Hz, 2H), 7.54 - 7.61 (m, 1H), 7.44 - 7.51 (m, 2H), 5.48 - 5.67 (m, 1H), 5.29 (d, $J = 7.32$ Hz, 1H), 1.38 (d, $J = 7.08$ Hz, 3H)

$^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 199.7, 155.4, 134.4, 133.9, 129.0, 128.9, 79.9, 51.3, 28.6, 20.1

General procedure C
2-iodophenol, alkyl bromide, and potassium carbonate were heated to 95°C in DMF for 24 hours. After cooling to room temperature, the reaction mixture was neutralized slowly with 6M HCl and diluted with brine. The reaction mixture was extracted with ethyl acetate, dried over Na$_2$SO$_4$, and concentrated by rotary evaporation. The crude product was purified by column chromatography (6:1 hexanes : EtOAc) to yield the product.
1-iodo-2-isobutoxybenzene (27a)
Following general procedure C, the product was obtained as a clear oil in 64% yield.

$^1$H NMR (499 MHz, CDCl$_3$) $\delta$ 7.78 (dd, $J = 1.59$, 7.69 Hz, 1H), 7.20 - 7.38 (m, 1H), 6.79 (dd, $J = 1.34$, 8.18 Hz, 1H), 6.70 (dt, $J = 1.46$, 7.57 Hz, 1H), 3.79 (d, $J = 6.35$ Hz, 2H), 2.17 (spt, $J = 6.60$ Hz, 1H), 1.10 (d, $J = 6.84$ Hz, 6H)

$^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 157.8, 139.6, 129.6, 122.4, 112.1, 86.9, 75.9, 29.2, 20.5

1-iodo-2-isopropoxybenzene (27b)
Following general procedure C, the product was obtained as a clear oil in 62% yield.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.78 (dd, $J = 1.61$, 7.83 Hz, 1H), 7.24 - 7.30 (m, 1H), 6.84 (dd, $J = 1.18$, 8.04 Hz, 1H), 6.66 - 6.73 (m, 1H), 4.57 (spt, $J = 6.07$ Hz, 1H), 1.40 (d, $J = 6.00$ Hz, 6H)

N-Boc-2-amino-1-(2-isobutoxyphenyl)propanone (25)
In a flame-dried three-neck flask fitted with a condenser and septa magnesium turnings (106 mg, 4.4 mmol, 2.2 eq.) were combined with 2 mL dry THF. 1-iodo-2-isobutoxybenzene was added to the reaction mixture, followed by two crystals of iodine. The reaction mixture was heated to reflux until no more magnesium was being consumed. The reaction was cooled to room temperature. The procedure described for 24 was followed, with the Grignard reagent being added via cannulation. The crude residue was purified by silica gel chromatography (6:1 hexanes:EtOAc) to yield 164 mg (25%) of a clear oil.

$^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 7.73 (d, $J = 7.50$ Hz, 1H), 7.46 (t, $J = 7.93$ Hz, 1H), 6.99 (t, $J = 7.50$ Hz, 1H), 6.94 (d, $J = 8.36$ Hz, 1H), 5.51 - 5.64 (m, 1H), 5.28 - 5.40 (m, 1H), 3.84 - 3.92 (m, $J = 6.65$ Hz, 1H), 3.76 - 3.83 (m, 1H), 2.10 - 2.29 (m, 1H), 1.44 (s, 9H), 1.33 (d, $J = 7.07$ Hz, 3H), 1.08 (d, $J = 6.86$ Hz, 6H)
\[ ^{13} \text{C NMR (126 MHz, CDCl}_3\] \delta 201.5, 158.3, 155.4, 134.3, 131.3, 120.8, 112.5, 79.4, 75.6, 55.5, 28.6, 28.4, 19.7, 19.7, 19.2

**General procedure D**

Ortho-alkoxyiodide (1.25 eq.) and Weinreb amide were dissolved in dry THF. The reaction was cooled to -78°C and stirred for 15 minutes. Isopropylmagnesium chloride (2.0M in THF) was added dropwise. After completion of addition, the dry ice bath was removed, and the reaction mixture was allowed to warm to room temperature overnight. The reaction was diluted with ethyl acetate and carefully quenched with 0.5M ice-cold HCl. The organic layer was washed further with 0.5M HCl, brine, and dried over Na$_2$SO$_4$. The crude product was obtained by rotary evaporation. Column chromatography (hexanes/ethyl acetate) yielded the product as a clear oil.

**N-Boc-2-amino-1-(2-methoxyphenyl)propanone (26a)**

Following general procedure D, the product was isolated in 53% yield.

\[ ^1 \text{H NMR (500 MHz, CDCl}_3\] \delta 7.76 (d, \(J = 6.65\) Hz, 1H), 7.44 - 7.54 (m, 1H), 6.99 - 7.06 (m, 1H), 6.97 (d, \(J = 8.36\) Hz, 1H), 5.53 - 5.64 (m, 1H), 5.32 (d, \(J = 7.50\) Hz, 1H), 3.92 (s, 3H), 1.44 (s, 9H), 1.31 (d, \(J = 7.07\) Hz, 3H)

\[ ^{13} \text{C NMR (126 MHz, CDCl}_3\] \delta 201.1, 158.8, 155.5, 134.5, 131.5, 125.3, 121.1, 111.8, 79.5, 55.8, 55.5, 28.6, 19.0

**N-Boc-2-amino-1-(2-isobutoxy)-3-methylbutanone (26b)**

Following the general procedure D, the product was isolated in 27% yield.

\[ ^1 \text{H NMR (400 MHz, CDCl}_3\] \delta 7.65 (dd, \(J = 1.83\), 7.69 Hz, 1H), 7.38 - 7.50 (m, 1H), 6.88 - 7.03 (m, 2H), 5.43 (br. s., 2H), 3.91 (dd, \(J = 6.10\), 8.79 Hz, 1H), 3.73 (dd, \(J = 7.32\), 8.79 Hz, 1H), 2.05 - 2.31 (m, 2H), 1.45 (s, 9H), 1.03 - 1.10 (m, 6H), 1.00 (d, \(J = 6.84\) Hz, 3H), 0.65 (d, \(J = 6.84\) Hz, 3H)
N-Boc-2-amino-1-(2-isopropoxyphenyl)-4-methylpentanone (26c)

Following general procedure D, the product was isolated in 62% yield.

$^1$H NMR (499 MHz, CDCl$_3$) $\delta$ 7.62 (dd, $J = 1.59, 7.69$ Hz, 1H), 7.38 - 7.48 (m, 1H), 6.88 - 7.01 (m, 2H), 5.37 - 5.50 (m, 1H), 5.33 (br. s., 1H), 4.63 - 4.77 (m, 1H), 1.74 (br. s., 1H), 1.58 (br. s., 1H), 1.47 (d, $J = 6.10$ Hz, 3H), 1.43 (s, 9H), 1.39 (d, $J = 6.10$ Hz, 3H), 1.29 - 1.37 (m, 2H), 0.98 (d, $J = 6.35$ Hz, 3H), 0.85 (d, $J = 6.59$ Hz, 3H)

N-Boc-2-amino-1-(2-isobutoxyphenyl)-4-methylpentanone (26d)

Following general procedure D, the product was isolated in 64% yield.

$^1$H NMR (499 MHz, CDCl$_3$) $\delta$ 7.61 (dd, $J = 1.46, 7.57$ Hz, 1H), 7.41 - 7.47 (m, 1H), 6.99 (t, $J = 7.45$ Hz, 1H), 6.95 (d, $J = 8.06$ Hz, 1H), 5.25 - 5.43 (m, 2H), 3.91 (dd, $J = 6.10, 8.79$ Hz, 1H), 3.70 - 3.80 (m, 1H), 2.12 - 2.30 (m, 1H), 1.67 - 1.81 (m, 1H), 1.55 - 1.61 (m, 1H), 1.43 (s, 9H), 1.01 - 1.08 (m, 7H), 0.94 (d, $J = 6.35$ Hz, 3H), 0.84 (d, $J = 6.59$ Hz, 3H)

General procedure E

Crude amidoketone was dissolved in a solution of HCl (4M) in ether, and stirred at room temperature for up to 2 hours. The product was isolated as the hydrochloride salt by rotary evaporation of the solvent in nearly quantitative yield.

2-amino-1-(2-isobutoxyphenyl)-3-methylbutanone hydrochloride (28a)

Following general procedure E, the product was obtained in 100% yield.

$^1$H NMR (499 MHz, CD$_3$OD) $\delta$ 7.76 (dd, $J = 1.59, 7.93$ Hz, 1H), 7.56 - 7.66 (m, 1H), 7.20 (d, $J = 8.30$ Hz, 1H), 7.10 (dt, $J = 0.85, 7.51$ Hz, 1H), 5.10 (d, $J = 3.17$ Hz, 1H), 4.05 (dd, $J = 5.98, 9.16$ Hz, 1H), 3.90 (dd, $J = 7.08, 9.28$ Hz, 1H), 2.39 (d, $J = 3.42$ Hz, 1H), 2.11 - 2.25 (m, 1H), 1.06 - 1.20 (m, 9H), 0.83 (d, $J = 7.08$ Hz, 3H)

2-amino-1-(2-isobutoxyphenyl)-4-methylpentanone hydrochloride (28b)
Following general procedure E, the product was obtained in 93% yield.

\[ ^1H \text{ NMR (499 MHz, CD}_3\text{OD)} \delta 7.70 \text{ (dd, } J = 1.95, 7.81 \text{ Hz, 1H), 7.59 - 7.65 \text{ (m, 1H), 7.20 \text{ (d, } J = 7.81 \text{ Hz, 1H), 7.09 \text{ (dt, } J = 0.85, 7.51 \text{ Hz, 1H), 5.13 \text{ (dd, } J = 4.88, 8.79 \text{ Hz, 1H), 4.92 \text{ (s, 3H), 4.03 \text{ (dd, } J = 6.10, 9.52 \text{ Hz, 1H), 3.91 \text{ (dd, } J = 7.32, 9.28 \text{ Hz, 1H), 2.11 - 2.26 \text{ (m, 1H), 1.69 - 1.81 \text{ (m, 1H), 1.61 - 1.68 \text{ (m, 2H), 1.06 - 1.12 \text{ (m, 6H), 0.99 \text{ (d, } J = 6.59 \text{ Hz, 3H), 0.90 \text{ (d, } J = 6.59 \text{ Hz, 3H)}}\text{)}}

\text{Methyl isobutylsalicylate (31)}

Methyl salicylate (1.52 g, 10 mmol), isobutyl bromide (2.74 g, 20 mmol) and potassium carbonate (2.76 g, 20 mmol) were heated to 75°C in 20 mL for 20 hours. Upon cooling to room temperature the reaction mixture was slowly neutralized with 1N HCl. 100 mL brine was added, and the aqueous layer was extracted with ethyl acetate (3 x 50 mL). The combined organic extracts were washed with brine, dried over Na\textsubscript{2}SO\textsubscript{4}, and concentrated to yield the product as a light yellow oil in 99% yield.

\[ ^1H \text{ NMR (400 MHz, CDCl}_3\text{) \delta 7.74 - 7.81 \text{ (m, 1H), 7.43 \text{ (ddd, } J = 1.83, 7.45, 8.30 \text{ Hz, 1H), 6.91 - 6.98 \text{ (m, 2H), 3.89 \text{ (s, 3H), 3.79 \text{ (d, } J = 6.35 \text{ Hz, 2H), 2.14 \text{ (spt, } J = 6.30 \text{ Hz, 1H), 1.05 \text{ (d, } J = 6.84 \text{ Hz, 6H)}}\text{)}}

\text{2-isobutoxybenzoic acid (32)}

Methyl 2-isobutoxysalicylic acid (1.90 g, 9.1 mmol) was dissolved in 10 mL MeOH. 10 mL 2N NaOH was added, and the reaction mixture stirred at room temperature for 2 hours. The methanol was removed by rotary evaporation, and the residue carefully neutralized with 1N HCl. The aqueous layer was extracted several times with EtOAc; the combined organic extracts were washed with brine, dried over Na\textsubscript{2}SO\textsubscript{4}, and concentrated by rotary evaporation to yield 1.631 g (91%) of a clear oil which solidified upon standing.
\(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 8.16 (dd, \(J = 1.95, 7.81\) Hz, 1H), 7.51 - 7.57 (m, 1H), 7.07 - 7.14 (m, 1H), 7.01 - 7.05 (m, 1H), 4.01 (d, \(J = 6.59\) Hz, 2H), 2.22 (td, \(J = 6.62, 13.37\) Hz, 1H), 1.05 - 1.11 (m, 6H)

**N-(2-isobutoxybenzoyl)-2-amino-4-methyl-1-(2-isobutoxyphenyl)-1-pentanone (29)**

N-acyl aminoketone (66 mg, 0.22 mmol) and 2-isobutoxybenzoic acid (43 mg, 0.22 mmol, 1 eq.) dissolved in 0.66 mL DMF. Diisopropylethylamine (57 mg, 0.44 mmol, 2 eq) was added and the reaction mixture stirred at room temperature for 5 minutes. EDC hydrochloride (46 mg, 0.242 mmol, 1.1 eq.) and HOBt (33 mg, 0.242 mmol, 1.1 eq.) were added, and the reaction mixture stirred for 3 hours. The reaction mixture was diluted with brine and washed exhaustively with dichloromethane. The combined organic extracts were washed with 1N HCl, brine, dried over Na\(_2\)SO\(_4\), and concentrated by rotary evaporation to yield 90.1 mg (68%) of an off-white foam.

\(^1\)H NMR (499 MHz, CDCl\(_3\)) \(\delta\) 8.83 (d, \(J = 8.06\) Hz, 1H), 7.74 - 7.83 (m, 1H), 7.63 - 7.72 (m, 1H), 7.39 - 7.50 (m, 2H), 7.31 - 7.38 (m, 1H), 6.93 - 7.07 (m, 4H), 3.85 - 4.01 (m, 3H), 3.74 - 3.84 (m, 1H), 2.39 (td, \(J = 6.59, 13.18\) Hz, 1H), 2.24 (td, \(J = 6.62, 13.37\) Hz, 1H), 1.65 - 1.86 (m, 2H), 1.45 - 1.60 (m, 1H), 1.13 (dd, \(J = 6.84, 7.81\) Hz, 6H), 1.04 - 1.10 (m, 6H), 0.93 - 0.98 (m, 3H), 0.82 (d, \(J = 6.35\) Hz, 3H)

**4-isobutyl-2,5-bis(2-isobutoxyphenyl)oxazole (30)**

N-acylaminoketone (97 mg) was dissolved in 1.5 mL pyridine. 0.5 mL POCl\(_3\) was added dropwise; the reaction stirred at room temperature for 6 hours. The reaction was quenched slowly with ice-cold water. 25 mL EtOAc and 5 mL 1N HCl were added. The organic layer was washed with 15 mL brine, dried over Na\(_2\)SO\(_4\), and concentrated by rotary evaporation. The crude residue was purified by preparative TLC (3:1 hexanes/EtOAc) to yield 16.5 mg (20%) of a white solid.
$^1$H NMR (400 MHz, CDCl$_3$) δ 7.98 (d, $J = 6.84$ Hz, 1H), 7.43 (dd, $J = 1.71, 7.57$ Hz, 1H), 7.29 - 7.38 (m, 2H), 6.89 - 7.07 (m, 4H), 3.83 (d, $J = 6.59$ Hz, 2H), 3.74 (d, $J = 6.59$ Hz, 2H), 2.52 (d, $J = 7.32$ Hz, 2H), 1.90 - 2.25 (m, 3H), 0.99 - 1.06 (m, 6H), 0.92 (d, $J = 6.84$ Hz, 6H), 0.87 (d, $J = 6.59$ Hz, 6H)

### 3.8 REFERENCES


