New synthetic methods for biologically active aromatic heterocycles

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New synthetic methods for biologically active aromatic heterocycles

by

Vinayak Gupta

A dissertation submitted to the graduate faculty
in partial fulfillment of the requirements for the degree of
DOCTOR OF PHILOSOPHY

Major: Organic Chemistry

Program of Study Committee:
George A. Kraus, Major Professor
Yan Zhao
Klaus Schmidt-Rohr
Arthur Winter
Thomas Bobik

Iowa State University
Ames, Iowa
2010
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GENERAL INTRODUCTION

Organic syntheses, which mainly constitute carbon-carbon and carbon-heteroatom bonds formation, is important because these bonds are found in practically all organic compounds that exhibit important biological, pharmaceutical and material properties. Due to the importance of these bonds, there has always been a need to develop and improve mild and general methods for their synthesis. In this context, we have investigated different strategies towards carbon-carbon and carbon-heteroatom bond formation which can then be utilized to develop various biologically active systems in concise ways. Further utilities of these routes have been shown by synthesizing various natural products.

Chapter one describes the use of novel phosphazene base P$_4$-$t$-Bu towards the synthesis of biologically important indolo[2,1-$a$]isoquinolines and 2,3-diarylbenzo[$b$furans and some other heterocyclic systems in a very concise way using commercially available or readily makeable organic intermediates.

Chapter two describes the development and structure-activity relationship (SAR) of pyrido[2,3-$d$]pyrimidines as effective inhibitors of the Ableson Kinase. This chapter also discusses synthesis and tagging of cyanin dyes with organic molecules for fluorescent studies.

Chapter three describes various attempts towards the synthesis of compounds belonging to flavonoid family like aurones, flavones and flavonols and outlines the development of a divergent approach to flavones via dihaloacrylic acid intermediates.
CHAPTER 1. Phosphazene base P₄-t-Bu: Application towards the synthesis of various heterocyclic compounds

Introduction

Neutral nitrogen bases such as sterically hindered tertiary amines or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) play an important role in organic synthesis.¹ The use of peralkylated sterically hindered amidine and guanidine bases has also been reported. In order to extend the collection of readily accessible, broadly applicable bases, Schwesinger and coworkers developed and arranged members of novel class of kinetically highly active uncharged phosphazene bases in order of strength and steric hindrance, to higher pKₐ values (Figure 1).¹ Among the strongest of these phosphazene bases, P₄-t-Bu is most readily available.¹

P₄-t-Bu (1) is synthesized starting from readily available phosphorous pentachloride (Scheme 1). Hexamethylphosphorotriamidate 3 is commercially available and can also be prepared by treating phosphorous pentachloride with dimethylamine, followed by the action of ammonia and potassium methoxide. Similarly, tert-butylphosphorimidic trichloride 4 can be prepared by condensing tert-butylamine hydrochloride with phosphorous pentachloride.
(Scheme 1). This sterically hindered base can then be prepared on a mole scale in a one-pot process from combining three equivalents of 3 and one equivalent of 4 and isolating as the HBF$_4$ or HClO$_4$ salts, which are only sparingly soluble in water. P$_4$-t-Bu (1) then can be liberated by using KNH$_2$ (Scheme 1).\textsuperscript{2} The strongly hygroscopic, colorless, crystalline base 1 is unusually stable and is very soluble in the conventional solvents. In 1.0 M D$_2$O solution, 1 remains unchanged even at about 160 °C over twenty hours; however, aqueous mineral acids readily hydrolyze it.\textsuperscript{3}

![Scheme 1: Synthesis of P$_4$-t-Bu](image)

P$_4$-t-Bu is probably the strongest neutral nitrogen base known at present; in THF solution, it is comparable to potassium(trimethylsilyl)aminde, but less nucleophilic. It deprotonates acetone to a large extent and establishes a clear equilibrium with triphenylmethane. Due to this extremely high basicity P$_4$-t-Bu has been used in a variety of different types of reaction systems.

In the presence of alkylating agents, in situ alkylation of low acidic substrates in concentrated (ca. 0.5 M) THF solution is generally extremely rapid on (gradual) addition of P$_4$-t-Bu at -100 °C to -78 °C. Due to the high solubilizing power of phosphazene bases, solubility problems are scarce. Separation of products from salts of the base is easily achieved, e.g. by direct precipitation of its halide salts with diethyl ether or benzene, by extraction (CH$_2$Cl$_2$) or precipitation (NaBF$_4$) of salts from aqueous solution, or by filtration over silica gel. The very low Lewis acidity of the huge cation contrasts sharply with the characteristics of lithium amide bases. Thus Lewis acid-catalyzed side reactions, e.g. aldol or
ester condensations in alkylations of enolates, are effectively suppressed; even \( \beta \)-lactones are easily mono- or peralkylated (Scheme 2, eq 1).\(^3\) In cases where the corresponding lithium derivatives decompose entirely via \( \beta \)-alkoxide elimination, ‘naked’ enolates of \( \beta \)-alkoxy esters undergo clean alkylation. Using bases like \( P_4-t\text{-}Bu \) enhances the formation of monoalkylation product considerably (Scheme 2, eq 2).\(^3\) This tendency also holds for selective monoalkylation of secondary carbon centers. Even sterically congested quaternary centers are formed with great ease (Scheme 2, eq 3).\(^4\) Alkylation of nitriles is not complicated by Thorpe condensation, as observed with LDA as base. Alkylation of 1,2-dinitriles with no elimination of hydrocyanic acid (Scheme 2, eq 4) is achieved in high yield.\(^5\)

Scheme 2: \( P_4-t\text{-}Bu \) reactions
Deprotonative functionalization of aromatics is one of the most useful transformations in organic synthesis, in which functionalized aromatic rings are directly generated via aromatic carbanions. P₄-t-Bu has also been extensively used for deprotonative functionalization of aromatic systems as shown by Kondo and coworkers.¹ᵇ ⁶ᵃ Traditionally, highly reactive metallic bases such as alkyllithiums or lithium dialkylamides are employed to generate arylmetals that function as aromatic carbanions. A lot of studies have focused on the chemo- and regioselectivity of this reaction. The use of highly reactive metallic bases often has undesirable side reactions such as nucleophilic attack of the intermediary aryl-metals on the electrophilic functional groups of the substrate. Therefore, the development of highly chemoselective reactions has been a challenge. In their research, Kondo and coworkers have examined the deprotonative functionalization of aromatics with P₄-t-Bu base because of its extreme basicity and low nucleophilicity, which allows for highly chemoselective reactions. Also, unlike Lewis acidic metal cation of cationic bases, the nonmetallic P₄-t-Bu base cannot function as a Lewis acid. Therefore, the reaction using P₄-t-Bu base proceeds without the “coordination mechanism”, and the reaction with unique regioselectivity is expected and observed.¹ᵇ ⁶ᵃ

In the first example to explore the deprotonative functionalization of P₄-t-Bu, the reactions of benzothiazole 15 were examined. When 15 was reacted with P₄-t-Bu in the presence of benzophenone in THF, adduct 16 was formed in 95% yield. Similar reactions of 15 with benzaldehyde and benzyl bromide gave addition products 17 and 18, respectively, in reasonable yields (Scheme 3).

![Scheme 3: Deprotonative 1,2-Addition of Benzothiazole with P₄-t-Bu](image)

Depronative functionalizations of π-deficient nitrogen heteroaromatics, which have relatively acidic ring-protons, were also reported. meta-Bromopyridine 19 was reacted with
P₄-t-Bu in the presence of benzophenone resulting in the deprotonative functionalization taking place at the expected 4-position to give 20a in only 3% yield, but was enhanced to 77% by using ZnI₂ as additive. Reactions with benzaldehyde and pivalaldehyde under similar conditions gave expected addition products 20b and 20c in excellent yields (Scheme 4).¹b, ⁶a

![Scheme 4: Deprotonative 1,2-Addition of 3-Bromopyridine with P₄-t-Bu](image)

Deprotonative functionalization of pyridazine 22 resulted in noteworthy regioselectivities. The reaction proceeded at the most remote position from the ring nitrogen and this system, when reacted with P₄-t-Bu base in the presence of benzophenone, benzaldehyde and pivalaldehyde displayed unique regioselectivity which is the opposite of the direct ortho metallation resulting in addition products 23a, 23b and 23c, respectively (Scheme 5).¹b, ⁶a

![Scheme 5: Deprotonative 1,2-Addition of Pyridazine with P₄-t-Bu](image)

Intrigued by regioselective results of azine 22, when pyrimidine 25 was subjected to similar conditions, it resulted in the formation of addition products 26a, 26b and 26c which followed the regioselectivity pattern of azines displaying regioselectivity which is the opposite of the direct ortho mettallation product (Scheme 6).¹b, ⁶a
These exciting results led Kondo and coworkers to investigate the deprotonative aromatization of substituted benzenes. para-Bromobenzonitrile (28) was treated with P₄-t-Bu base in the presence of benzophenone and ZnI₂ in THF, and the reaction proceeded chemoselectively at the 3-position (29a) in 88% yield without ZnI₂. Surprisingly, the orientation of the reaction is the opposite of the deprotonative metalation using TMP-zincate. Reactions with other electrophiles were also examined. Benzaldehyde and pivalaldehyde were used as electrophiles in the reaction of 4-bromobenzonitrile 28 in THF, and the appropriate 1,2-adducts 29b and 29c were obtained in 86% and 87% yield, respectively (Scheme 7).₁ᵇ, ₆ᵃ

The examples detailed above show the utility of P₄-t-Bu for many reactions which do not work with conventional reactive metallic bases such as LDA and Li-TMP etc. but shows remarkable success and specific regeioselectivities with P₄-t-Bu. The examples discussed below, will describe the utility of P₄-t-Bu for some more reaction systems including, but not limited to, nucleophilic aromatic substitution, functionalization of arylsilanes, Julia-Kocienski olefination and halogen-metal exchange.
Nucleophilic aromatic substitution (S_{N}Ar) reaction is one of the most fundamental and widely used transformations in synthetic organic chemistry. Various nucleophiles, such as alcohols, phenols, amines and 1,3-dicarbonyl compounds, have been employed for this transformation. Traditionally, highly reactive bases such as sodium hydride, potassium tert-butoxide etc. have been utilized for the deprotonation of nucleophiles in order to generate the reactive anions and the stoichiometric use of bases has been regarded as essential for the completion of the substitution reaction. However, catalytic use of strong bases is deemed desirable for selectivity, safety and environmental benignity and P_{4}-t-Bu has been shown to achieve these goals in the examples detailed next. The direct arylation of alcohols using P_{4}-t-Bu catalyzed coupling reactions has been a challenge, this was solved by using catalytic P_{4}-t-Bu for hydride generation from Et_{3}SiH with which sequential deprotonation and S_{N}Ar reaction can be carried out. Kondo and coworkers started their investigation with arylation various alcohols using ortho-fluoronitrobenzene 31. The reaction of ortho-fluoronitrobenzene 31 with n-hexanol was carried out in the presence of Et_{3}SiH and 10 mol% of P_{4}-t-Bu at 100 °C for two hours. The arylation reaction proceeded to give ether 32a in quantitative yield. The reaction with primary alcohols such as n-butanol and 2-phenylethyl alcohol, secondary alcohols like 2-butanol proceeded under the same reaction conditions to give ethers 32b-d in excellent yields (Scheme 8, Table). They next investigated the feasibility of Et_{3}SiH/catalytic P_{4}-t-Bu system to effect the S_{N}Ar reaction of C-nucleophiles. Diethyl methylmalonate was reacted with o-fluoronitrobenzene at 80 °C for 1 hour and the arylation was found to proceed in almost quantitative yield to give ether 35 (Scheme 8). Other alpha-substituted malonates also reacted with o-fluoronitrobenzene to give o-nitrophenylmalonates which are important precursors for the synthesis of oxindole derivatives. alpha-Substituted cyanoacetate and alpha-substituted malononitrile were also shown to be excellent C-nucleophiles (Scheme 8). Even the less reactive ortho-fluoro and para-fluorobenzonitriles were succesfully reacted with methylmalonate 34 to give the corresponding arylmalonates in reasonable yields (Scheme 8). Conventionally, aryl fluorides with weak electron withdrawing groups have not been used for S_{N}Ar reaction with these C-nucleophiles and this Et_{3}SiH/ catalytic P_{4}-t-Bu system provides a new and effective S_{N}Ar reaction protocol.
Another work from Kondo and coworkers describes the $P_4$-$t$-Bu promoted functionalization of aryltrimethylsilanes. They have demonstrated the utility of $P_4$-$t$-Bu for the activation of aryltrimethylsilanes. Initially, 1-phenyltrimethylsilane 36a was chosen as a substrate and its reaction with pivalaldehyde in the presence of 20 mol% $P_4$-$t$-Bu proceeded smoothly to give alcohol 37a in 91% yield (Scheme 9, Table: entry 1). Other conventional strong organic bases like DBU, BEMP and fluoride donors like CsF were found to be inactive. The reactions with benzaldehyde proceeded somewhat slowly at room temperature but at elevated temperature, the product 37b was obtained in 61% yield. Other aryl aldehydes with electron donating groups were also shown to successfully give products at room temperature. Other aryltrimethylsilanes such as 2-phenyltrimethylsilane 36c, 4-bromophenyltrimethylsilane 36d, 2-trifluoromethylphenyltrimethylsilane 36e and 4-methoxycarbonylphenyltrimethylsilane 36f were also successfully reacted with pivalaldehyde to give corresponding alcohols in moderate to excellent yields (Scheme 9, Table: entry c-f). The reactions of heteroaryltrimethylsilanes like 2-pyridyltrimethylsilane 36g and 3-pyridyltrimethylsilane 36h also proceeded smoothly to give corresponding alcohols 37g and 37h (Scheme 9, Table: entry g-h). In summary, they found that arylsilanes can be carbo-desilylated by the use of
catalytic $\text{P}_4$-$\text{t}$-$\text{Bu}$ as a promoter and the selective functionalizations of arylsilanes possessing no strong electron-withdrawing group can be accomplished.$^8$

Another work, reported by Nájera and coworkers use stoichiometric amount of $\text{P}_4$-$\text{t}$-$\text{Bu}$ in Julia-Kocienski olefination to synthesize various substituted olefins.$^{9a,b}$ They used various alkyl 3,5-bis(trifluoromethyl)phenylsulfones (BTFP sulfones) $\text{38}$ and generated stabilized anions by using stoichiometric amount of various bases like $\text{P}_4$-$\text{t}$-$\text{Bu}$, other inorganic bases like KOH and metallic bases like LDA and KHMDS and then condensed with different carbonyl compounds to give substituted olefins (Scheme 10).$^{9a,b}$

![Scheme 9: $\text{P}_4$-$\text{t}$-$\text{Bu}$ promoted functionalization of aryltrimethylsilanes.]

<table>
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<tr>
<th>#</th>
<th>Ar</th>
<th>R</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>1-Naphthyl</td>
<td>$\text{t}$-$\text{Bu}$</td>
<td>91</td>
</tr>
<tr>
<td>b</td>
<td>1-Naphthyl</td>
<td>Ph</td>
<td>61</td>
</tr>
<tr>
<td>c</td>
<td>2-Naphthyl</td>
<td>$\text{t}$-$\text{Bu}$</td>
<td>68</td>
</tr>
<tr>
<td>d</td>
<td>4-Br$\text{C}_6$H$_4$</td>
<td>$\text{t}$-$\text{Bu}$</td>
<td>73</td>
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<tr>
<td>e</td>
<td>2-CF$_3$C$_6$H$_4$</td>
<td>$\text{t}$-$\text{Bu}$</td>
<td>73</td>
</tr>
<tr>
<td>f</td>
<td>4-MeO$_2$CC$_6$H$_4$</td>
<td>$\text{t}$-$\text{Bu}$</td>
<td>46</td>
</tr>
<tr>
<td>g</td>
<td>2-Pyridyl</td>
<td>$\text{t}$-$\text{Bu}$</td>
<td>67</td>
</tr>
<tr>
<td>h</td>
<td>3-Pyridyl</td>
<td>$\text{t}$-$\text{Bu}$</td>
<td>80</td>
</tr>
</tbody>
</table>

Scheme 10: $\text{P}_4$-$\text{t}$-$\text{Bu}$ mediated Julia-Kocienski Olefination of BTFP sulfones.$^{9a,b}$

Though they showed successful examples with all the bases, from studies on the stability and reactivity of BTFP sulfones in different reactions, they concluded that $\text{P}_4$-$\text{t}$-$\text{Bu}$ is
most appropriate base for this type of coupling. Under $P_4$-t-Bu reaction conditions, BTFP sulfones were better substrates than other sulfones in terms of stability and reactivity.

Other work by Kondo and coworkers shows that the use of catalytic $P_4$-t-Bu dramatically improves the performance of halogen-zinc exchange of aryl iodides.\textsuperscript{10} In recent years, organozinc compounds have been widely used in organic synthesis and one of the most powerful methods for the preparation of functionalized organozinc derivatives is halogen-zinc exchange reaction.\textsuperscript{10} In their initial investigation, they chose ethyl 4-iodobenzoate $41$ as a substrate. They successfully reacted ethyl 4-iodobenzoate and diethylzinc in THF in the presence of 30 mol\% $P_4$-t-Bu at room temperature. The halogen-zinc exchange proceeded smoothly and the de-iodinated product was isolated quantitatively after hydrolysis. In the absence of $P_4$-t-Bu, the exchange was very slow and only a trace of de-iodinated product was detected at elevated temperatures. In order to expand the scope of this reaction, some functionalizations of aryl iodides were examined. As an example of 1,2-addition, aryl zinc prepared from ethyl 4-iodobenzoate $41$ and diethylzinc in the presence of $P_4$-t-Bu in THF was reacted with benzaldehyde to get benzydrol derivative $42$ in 78\% yield. As for the 1,4-addition reaction, aryl zinc prepared similarly in THF was reacted with chalcone and 1,4-adduct was obtained in 71\% yield. Using the same procedure, allylation was also carried out and various allyl arenes were prepared from corresponding aryl iodides in excellent yields (Scheme 11).\textsuperscript{10}

\begin{equation}
\begin{array}{c}
\text{R} \text{H} \\
\text{I} \\
\text{41} \\
(1) \text{ZnEt}_2, \\
P_4\text{-t-Bu (cat.)} \\
\text{THF, RT, 12h} \\
(2) E^+ \\
\text{42 (71\%-100\%)} \\
R = \text{o-CO}_2\text{Et, p-CO}_2\text{Et, o-Cl, o-OMe, o-Me} \\
E^+ = \text{PhCHO, Chalcone, Allyl bromide, H}_3\text{O}^+
\end{array}
\end{equation}

Scheme 11: $P_4$-t-Bu promoted halogen-Zn exchange reaction of aryl iodides.

As detailed above, many intriguing papers on $P_4$-t-Bu were published. Phosphazene base $P_4$-t-Bu has shown successful results where other bases have failed. Our research group became interested in this specific base when we explored the deprotonation of benzylic ethers as a way to synthesize benzofurans.\textsuperscript{11} Kraus and coworkers had originally planned to
prepare 44a from benzaldehyde 43a by the two-step sequence involving a photochemical hydrogen atom abstraction reaction followed by dehydration using POCl₃ and pyridine. Although this reaction sequence worked well on a millimole scale, the photochemical step proved difficult to scale-up. They next tried the base-mediated reaction. In view of the absence of an electron-withdrawing group on the benzyl moiety, the deprotonation of the benzylic ether 43a using strong bases in anhydrous media was examined. Treatment of 43a with LDA in THF from -78 to 25 °C returned recovered starting material. The reaction of 43a with LiTMP in THF from 0 to 25 °C afforded mostly recovered starting material with byproducts that were not derived from proton abstraction at the benzylic ether position. Benzaldehyde 43a did not react with sodium hydride or potassium hydride in THF or DMF. When benzaldehyde 43a was reacted with 1.1 equivalent of P₄-t-Bu in pivalonitrile at 90 °C, it successfully cyclized into benzofuran 44a in 49% yield. When benzene was used as the solvent, the yield was 47% (Scheme 12).

![Scheme 12: P₄-t-Bu promoted cyclization of o-substituted benzaldehyde.](image)

Using this model, the scope of this intramolecular cyclization with a series of aldehydes was explored and they were reacted with P₄-t-Bu. To explore the effect of electronics on the cyclization, o-alkylated aldehydes 43a-d were prepared and subjected to reflux conditions in benzene in the presence of P₄-t-Bu. In all cases, cyclization worked successfully to give corresponding benzofurans 44a-d in moderate yields. When electron deficient system 43g was subjected to general conditions, it worked best to give
corresponding benzofuran 44g in 78%. In conclusion, reaction of P₄-t-Bu with substituted o-benzyloxybenzaldehydes offered a new convenient and concise pathway to arylbenzofurans.

Scheme 13: P₄-t-Bu mediated cyclization of substituted benzaldehydes to benzofurans.¹¹

Results and Discussion

In connection with our studies of synthetic potential of hindered phosphazene base P₄-t-Bu, we turned our attention to indolo[2,1-a]isoquinolines 45 which represent a growing class of natural and synthetic compounds with useful biological activity. A sub-class whose members contain a quaternary ammonium salt is represented by mangochinine (46a),¹² cryptaustoline (46b),¹³ and ortho-methylcryptaustoline (46c)¹⁴ and is shown in Figure 2.

Figure 2

Certain indolo[2,1-a]isoquinolines have been reported to inhibit the growth of human mammary carcinoma cells,¹⁵ to treat multiple sclerosis,¹⁶ and to exhibit antiviral activity¹⁷. Compound 47 strongly inhibited tubulin polymerization.¹⁸
Four most versatile methods for the synthesis of indolo[2,1-\(a\)]isoquinolines have been reported. Orito reported the cyclization of 1-bromobenzyl-5,6-dihydroisoquinolines 48 by the nucleophilic addition of the dihydroisoquinoline nitrogen atom to the bromobenzyl moiety.\(^{19}\) This work is depicted in Scheme 14. He constructed several analogs with different patterns of oxygenation. Lautens and coworkers reported an innovative palladium-catalyzed tandem reaction sequence starting from a N-(2-bromoethyl)indole 49 and an aryl iodide.\(^{20}\) Importantly, this sequence can accommodate both electron-withdrawing and electron-donating groups on the aromatic ring. Saa and coworkers reported the synthesis of 45 from 3,4-dihydroisoquinolines 50 and benzyne.\(^{21}\) Although this pathway is a direct one, the yields were modest. Kametani reported the synthesis of an indolo[2,1-\(a\)]isoquinoline via an intramolecular benzyne reaction.\(^{22}\) Several groups reported intramolecular radical cyclizations onto indoles 51 to form the indolo[2,1-\(a\)]isoquinoline ring system.\(^{23, 24}\) The radicals were generated using either trialkyltin hydrides or trialkylgermanium hydrides. This pathway is flexible with regard to substitution on either the indole or the bromobenzene ring.

![Scheme 14](image)

Our approach to the synthesis of indolo[2,1-\(a\)]isoquinolines is depicted in Scheme 15. This approach involves the preparation of aldehyde 54 by the coupling of 52 and 53 followed by a base-induced cyclization to generate the indolo[2,1-\(a\)]isoquinoline system. Since tetrahydroisoquinolines are readily available,\(^{25}\) and several ortho-fluorobenzaldehydes are
commercially available, this approach has the potential to be a very flexible one. This synthetic strategy is distinctly different from the four general synthetic routes to indolo[2,1-
2]isoquinolines described above. Recently, De Koning reported the deprotonation and cyclization of N-benzyl pyrroles using potassium tert-butoxide to form related heterocyclic systems.\(^{26}\)

![Scheme 15: Retrosynthetic analysis](image)

In order to test the concept, we treated 2-fluorobenzaldehyde 53a with anhydrous potassium carbonate and tetrahydroisoquinoline 52a in DMF to generate 54a in 48% yield. Cyclization of aldehyde 54a was attempted using lithium diisopropylamide, lithium tetramethylpiperidine, sodium hydride, potassium hydride or potassium tert-butoxide and P\(_4\)-t-Bu (Scheme 16, table 1). Treatment of 543a with LDA in THF from -78 to 25 °C returned recovered starting material (Scheme 16, Table 1 – entry 1). The reaction of 54a with Li-TMP in THF from 0 to 25 °C afforded mostly recovered starting material with byproducts that were not derived from proton abstraction at the benzylic position (Scheme 16, Table 1 – entry 2). Benzaldehyde 54a did not react with sodium hydride, potassium hydride or potassium tert-butoxide in THF or DMF even at elevated temperatures (Scheme 16, Table 1 – entry 3, 4, 5). Only P\(_4\)-t-Bu (1) generated the desired tetracyclic product 45a (Scheme 16, Table 1 – entry 6). When benzaldehyde 54a was reacted with 1.1 equivalent of P\(_4\)-t-Bu in refluxing benzene for two hours, indolo[2,1-
2]isoquinoline 45a was produced in 35% isolated yield.
Scheme 16: Model system

With a successful two-step synthesis of dihydroindolo[2,1-a]isoquinolines, in order to explore the steric and electronic effects, we generated a number of related compounds from commercially available tetrahydroisoquinolines and ortho-fluorobenzaldehydes. Nucleophilic aromatic substitution reaction (S_NAr) of substituted tetrahydroisoquinolines (52) with various substituted ortho-fluorobenzaldehydes (53) worked in moderate to good yields to give substituted aldehydes (54a-j). The results of this effort are shown in Table 2. Since, with electron withdrawing groups on tetrahydroisoquinoline ring, generation of benzylic anion would not be hard, we decided to take the opposite route and thus used electron rich tetrahydroisoquinoline (52d) and explored the resultant effect on cyclization reaction. Similar approach was used with substituted ortho-fluorobenzaldehydes and mostly, electron rich systems were used.

### Table 1: Cyclization conditions

<table>
<thead>
<tr>
<th>#</th>
<th>Base</th>
<th>Solvent</th>
<th>Conditions</th>
<th>Result</th>
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<td>THF</td>
<td>-78 °C - RT</td>
<td>NR</td>
</tr>
<tr>
<td>2</td>
<td>LiTMP</td>
<td>THF</td>
<td>0 °C - RT</td>
<td>decomp.</td>
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<td>3</td>
<td>NaH</td>
<td>DMF</td>
<td>reflux</td>
<td>NR</td>
</tr>
<tr>
<td>4</td>
<td>KH</td>
<td>DMF</td>
<td>reflux</td>
<td>NR</td>
</tr>
<tr>
<td>5</td>
<td>t-BuOK</td>
<td>DMF</td>
<td>reflux</td>
<td>NR</td>
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<tr>
<td>6</td>
<td>P₄-t-Bu</td>
<td>Benzene</td>
<td>reflux</td>
<td>35%</td>
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</table>

Scheme 16: Model system
Table 2: Synthesis of 5,6-dihydroindolo[2,1-a]isoquinolines

<table>
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<tr>
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<th>R₁</th>
<th>R₂</th>
<th>R₃</th>
<th>R₄</th>
<th>R₅</th>
<th>% yield of 54a-j</th>
<th>% yield of 45a-1j</th>
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<td>a</td>
<td>H</td>
<td>H</td>
<td>H</td>
<td>H</td>
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<td>48</td>
<td>35</td>
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<td>b</td>
<td>H</td>
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<td>H</td>
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Except for one example (54k), which has a bromo substituent, all other compounds underwent successful cyclizations irrespective of the position of the electron donating methoxy groups (Table 2; Entry b-d). Even the presence of multiple methoxy groups was tolerated well by our intramolecular cyclization reaction and resulted in the formation of various di, tri or tetra methoxy substituted products (Table 2; Entry c-f). We also studied few examples with electronegative trifluoromethyl group as a substituent on benzaldehyde. Since trifluoromethyl group has significant electronegativity that is often described as being intermediate between the electronegativities of fluorine and chlorine, we thought it would be interesting to observe its effects on the cyclization. Also, trifluoromethyl group can be used to adjust the steric and electronic properties of a lead compound, or to protect a reactive methyl group from metabolic oxidation. Moreover, the trifluoromethyl group is often used as
a bioisostere to create derivatives by replacing a chloride or methyl group and is present in some notable drugs including efavirenz (Sustiva), an HIV reverse transcriptase inhibitor; fluoxetine (Prozac), an antidepressant; and celecoxib (Celebrex), a non-steroidal anti-inflammatory, it would be interesting to see the biological activities of the resultant cyclized compounds. All four systems (54g-j) underwent successful cyclization to give indolo[2,1-a]isoquinolines 45g-j in moderate yields. Though most of the aldehydes (54a-j) readily underwent intramolecular cyclization when reacted with 1.1 equivalent of P₄-t-Bu in refluxing benzene, there was no specific pattern or effect of electronics and stearics which could be discerned from the data.

Compound 45f could be used in a direct synthesis of ortho-methylcryptaustoline iodide 46c as shown in Scheme 17. The reduction of compound 45f using sodium cyanoborohydride in acetic acid at ambient temperature afforded the tetrahydro compound 55 which on treating with methanol containing excess methyl iodide over 48 hours afforded 46c in 73% overall yield from 45f. The NMR data and melting point of our synthetic compound were identical to that of the literature²⁷ compound.

Scheme 17: Synthesis of O-methylcryptaustoline iodide

The compounds 45a-j were evaluated for their ability to modulate immune response by our collaborators in immunobiology program at Iowa State University.²⁸ The cells obtained from influenza virus-infected mice were cultured in vitro with compounds 45a-j and influenza virus. Each compound (45a-j) suppressed the production of IL-10. IFNγ is secreted by cytotoxic T lymphocytes, and exhibits anti-viral activity, but may also result in immunopathology.¹⁹ Again, every compound (45a-j) inhibited production of IFNγ. Interleukin-2 (IL-2) is produced primarily by T-helper cells during influenza infection and plays an important role in activating T lymphocytes. Compounds 45a-j reduced production of IL-2.²⁸
In conclusion, this methodology constitutes a novel and direct route to dihydroindolo[2,1-a]isoquinolines. The route is flexible with respect to functionality and can be scaled up to prepare gram quantities of dihydroindolo[2,1-a]isoquinolines. The utility of this route is shown in the direct synthesis of ortho-methylcryptaustoline iodide 46c. Also, these compounds exhibited significant immunosuppressive activity against IL-2, IL-10 and IFN-γ and To the best of our knowledge, we have been the first to report that indolo[2,1-a]isoquinolines exhibit immunosuppressive activity.

In order to further explore the synthetic potential of the hindered phosphazene base P₄-t-Bu, we diverted our attention towards the synthesis of 2,3-diarylbenzo[b]furans (Figure 3).

Figure 3: General structure of diarylbenzo[b]furan.

2,3-Diarylbenzo[b]furans are a class of natural products that are broadly distributed and exhibit diverse biological activity. Amurensin H (48), isolated from Vitus amurensis, shows significant anti-inflammatory activity in mice models. Compound 48 may have therapeutic potential for the treatment of allergic airway inflammation. It has also been reported to treat chronic obstructive pulmonary disease. Gnetuhainin B (49), which shares the same basic skeleton with Amurensin H and differs only in the position and number of hydroxyl groups, was isolated from Gnetum hainanense which grows only in the southern part of the People’s Republic of China, especially in Hainan Province. Gnetuhainin G (50) is a novel furobenzofuran from Gnetum hainanense that is an antioxidant. alpha-Viniferin, structurally related to 48, has been reported to be a potent MRP1 transport inhibitor.
Several synthetic routes to benzofurans such as 47 are known.\textsuperscript{35} The majority of these syntheses take place via disconnection A (Figure 2). This includes the reaction of ortho-halophenols with acetylenes\textsuperscript{36}, and the oxidative cyclization\textsuperscript{37} of hydroxy stilbenes. A related pathway is the palladium-mediated arylation of benzofurans.\textsuperscript{38} In contrast, disconnection B (Figure 5) has only rarely been utilized to prepare diarylbenzofurans. A notable example is a photocyclization that takes place via an intramolecular hydrogen atom abstraction of the benzylic hydrogen atom followed by radical recombination and dehydration.\textsuperscript{39}

Our proposed retro-synthetic approach is depicted in Figure 6. We planned to use 2-hydroxybenzophenone (53) as our starting material, as many of these substituted 2-hydroxybenzophenones are commercially available or can be prepared easily by Friedel-Crafts acylation of substituted phenols (52) with various benzoyl chlorides (51). We envisioned that using P$_4$t-Bu, an anion can be generated at the benzylic position in O-arylated benzophenone (54) which could attack the carbonyl carbon of benzophenone (54) to cyclize the system and generate 2,3-diarylbenzo[\textit{b}]furan (47).
We first tested our hypothesis on a model system. Benzyl bromide was reacted with 2-hydroxybenzophenone (53a) under basic conditions to prepare 2-benzyloxybenzophenone (54a) in 83% yield. This substituted benzophenone 54a was then refluxed in benzene in the presence of 1.1 equivalents of P₄-t-Bu and 2,3-diphenylbenzo[ b]furan (47a) was isolate in quantitative yield (Scheme 18).

With this promising result in hand, we evaluated an array of benzophenones for the effect of electronic factors. Initially, we tested the effect of electron-donating groups on the cyclization reaction. Two different ortho-(aryloxy)benzophenones 54b and 54c were synthesized by reacting ortho-hydroxybenzophenone with appropriate benzyl bromides under basic conditions (Scheme 19). These substituted benzophenones had a methoxy on para position (54b) and methoxy on both meta and para positions (54c). Both of these systems cyclized in good yields when subjected to reflux conditions in the presence of P₄-t-Bu to give diarylbenzo[ b]furan 47b and 47c in 69% and 61% yield respectively (Scheme 19).
Scheme 19: Synthesis of 2,3-diarylbenzo[b]furans

Scheme 20 outlines another type of system where the cyclization conditions were attempted on benzophenones bearing electron donation methoxy and methylenedioxy groups. To synthesize these benzophenones, Friedel-Crafts acylation reaction conditions were utilized. Phenols 52a-b were condensed with benzoyl chloride in the presence of lewis acid aluminium trichloride in refluxing 1,2-dichloroethane to get ortho-hydroxybenzophenones 53b-c in decent yields. These substituted benzophenones were O-arylated with benzyl bromide and then subjected to cyclization conditions to get diarylbenzo[b]furans 47d-e in good yields (Scheme 20).
The success of diaryl systems motivated us to synthesize heteroaryl systems and try cyclization conditions on them. The synthesis began with Friedel-Crafts acylation of phenols 52a-b with 2-furoic acid chloride 51b in previously standardized conditions to give dimethoxy 53d and methylenedioxy 53e systems in 64% and 61% yield, respectively. Compounds 53d-e were O-arylated under usual conditions and then successfully cyclized in the presence of P4-t-Bu to give 2,3-diarylbenzo[b]furans 47f-g in decent yields (Scheme 21). These substituted diarylbenzofurans were novel because in addition to usual phenyl system, they have a furan as other aryl substitution.
With the success of all the above-mentioned systems, we designed a benzophenone system bearing five methoxy groups, three on one phenyl ring and two on the other. The idea was to maximize the electronic effects and to make the carbonyl carbon least reactive. The synthesis is shown in Scheme 22 and it started off with the Friedel-Crafts acylation of 3,4-dimethoxyphenol $52a$ with 3,4,5-trimethoxybenzoyl chloride $51c$ in 51% yield, followed by O-arylation using benzy bromide under basic conditions to give ortho-(benzyloxy)benzophenone $54h$ in 68% yield. This system, when refluxed in benzene in the presence of $P_4-t$-Bu, successfully cyclized to a pentamethoxy diarylbenzo[b]furan $47h$ in excellent yield (Scheme 22).
At this stage we had eight successful examples of P$_4$-t-Bu mediated cyclization to give 2,3-diarylbenzo[b]furans. Even the most electron rich systems like 54c and 54h underwent successful cyclization with high efficiency. But all those successful examples had only one site at which the anion can be generated. In order to show the flexibility of our method, we decided to design systems with more than one reactive site. In order to synthesize appropriate benzophenone system, we took the photochemical route which was developed and standardized in our laboratory.\textsuperscript{40} In the scheme described below, we reacted 1,4-benzoquinone 55 with 2-methoxybenzaldehyde 56a and 3,4,5-trimethoxybenzaldehyde 56b under ultraviolet conditions, in the presence of catalytic amount of benzophenone in benzene as solvent. Three days of continuous stirring under these conditions resulted in the successful formation of 2,4-dihydroxybenzophenone systems 53g-h in 68\% and 65\% yields respectively. These compounds were then di-O-arylated in the presence of sodium hydride and benzyl bromide to get compounds 54i-j in good yields. Subsequent cyclization with 1.1 equivalence of P$_4$-t-Bu resulted in the formation of only a very small amount of product and recovery of majority of the starting material. Compounds 54i-j underwent successful
cyclization reactions to give diarylbenzo[b]furans 47i-j when excess (2.5 equivalents) of P₄-t-Bu was used (Scheme 23). Moderate yields in both the cases can be attributed to the presence of another reactive site.

Scheme 23: Synthesis of 2,3-diarylfuran- effect of multiple reaction sites.

Success of the systems discussed above (Scheme 24) encouraged us to try another, yet more ambitious example with two sites to generate the anion and two sites to trap. This would result in the formation of benzo[1,2-b:5,4-b']difuran ring system (Scheme 24). The synthesis started with commercially available 1,3-dimethoxybenzene 57. Excess aluminium trichloride was used in Friedel-Crafts acylation to get the diacylation as well as demethylation of both the methoxy groups in one pot. Resorcinol derivative 53i was obtained in moderate yield but considering that dual purposes were served, we decided to move forward. O-arylation was done under standard basic conditions using sodium hydride and benzyl bromide to get the di O-arylated product 54k in 65% yield. This compound 54k was then subjected to cyclization reaction using 1.1 equivalents of P₄-t-Bu but reaction did not work even after prolonged heating. Successful cyclization was achieved with the use of
2.5 equivalents of $P_4$-$t$-Bu resulting in the formation of benzo[1,2-$b$:5,4-$b'$]difuran 47k in 61% yield (Scheme 24).

Scheme 24: Synthesis of benzo[1,2-$b$:5,4-$b'$]difuran ring system

Next, we tried few reactions to check the functional group tolerance as well as the effect of electron withdrawing substitution on the ring. Results are compiled below in Scheme 25.
As shown by the synthesis of substituted 2,3-diphenylnaphthofuran 47m, this method is compatible with an ester group. Also, the electron withdrawing effect of the ester did not influence the cyclization in a positive way. In fact, the yield of the cyclization reaction for compound having an ester in the para position (47m) was actually lower than the unsubstituted system (47l, Scheme 25). Chloro substitution is tolerated well during the cyclization step as indicated for compound 47n. When O-aryloxybenzophenone 54o containing a nitro substitution was subjected to cyclization conditions, it smoothly got converted into 2,3-diarylbenzo[b]phenone 47o in 65% yield. The same success could not be repeated with a cyano substituted benzophenone 54p as it failed to undergo cyclization under P₄-t-Bu conditions.

As the results detailed above illustrate, this cyclization is compatible with a variety of functional groups and represents a convenient way to synthesize aryl substituted benzofurans. As synthesis of 47k indicates, this chemistry is extendable to the benzo[1,2-\(b\):5,4-\(b\)]difuran ring system. Interestingly, this cyclization proceeds in good yield despite the presence of two different benzyl groups in the benzophenone (47i-j).
Scheme 26: Possible mechanistic pathway for the formation of 2,3-diarylbenzo[b]furans

The possible mechanism for these reactions is depicted in Scheme 26 which is based on the assumption that P₄-t-Bu successfully deprotonated the benzylic position which bears the most acidic hydrogen. The anion thus generated reacts with the carbonyl carbon giving rise to the intermediate which undergoes dehydration at elevated temperature to give 2,3-diarylbenzo[b]furan.

Scheme 27: Retrosynthetic analysis of amurensin H

This methodology can be used in a direct total synthesis of amurensin H (48). As shown in Scheme 27, our retrosynthetic consideration of 48 was focused on the efficient synthesis of substituted benzophenone 69 which subsequently can be cyclized by using our P₄-t-Bu mediated cyclization followed by exhaustive demethylation to give the natural
product 48. We envisioned that benzophenone 69 can be prepared from stilbene derivative 65. We based the synthesis of stilbene 65 on the work done by Snyder and coworkers.\textsuperscript{42}

The synthesis of substituted stilbene 65 is outlined in Scheme 28. Commercially available 3,5-dimethoxybenzaldehyde 59 was reduced using lithium aluminum hydride conditions to give dimethoxybenzyl alcohol 60 in 82% yield. Benzyl alcohol 60 was converted to dimethoxybenzyl bromide 61 using phosphorous tribromide and catalytic pyridine in dry ether in 84% yield. Ring halogenation of 61 was carried out by N-bromosuccinimide at 0 °C in 92% yield to give corresponding bromobenzyl bromide 62 in 92% yield. Benzyl bromide 62 was subjected to Michaelis-Arbuzov reaction conditions using triethyl phosphate to form phosphonate 63 in 92% yield. This phosphonate 63 underwent successful Horner-Wadsworth-Emmons reaction with \textit{para}-methoxybenzaldehyde 64 giving stilbene 65 in 74% yield.\textsuperscript{42}
Scheme 29: Total synthesis of amurensin H

Scheme 29 shows the completion of the total synthesis from stilbene 65. Starting from stilbene 65, metal-halogen exchange followed by reaction with 3,5-dimethoxybenzaldehyde 59 and oxidation with activated manganese dioxide affords benzophenone 67 in 77% yield from 65. This benzophenone 67 was selectively demethylated in 88% yield to give 68 using BBr₃ solution (1.0M in CH₂Cl₂) at -50 °C. The resulting phenol 68 is converted into benzyl ether 69 in 86% yield. Cyclization of 69 using P₄-t-Bu in dry benzene at 170 °C (sealed tube conditions) provided 70 in 42% yield. The higher temperature needed to effect the cyclization is likely a result of steric factors. Finally, the total synthesis of amurensin H 48 was achieved by exhaustive demethylation of benzofuran 70 using BBr₃ solution (1.0M in CH₂Cl₂) at room temperature in 67% yield. The analytical data for 48 matched with the previously reported data. In conclusion, we successfully completed the synthesis of amurensin H using the methodology developed for the synthesis of 2,3-diarylbenzo[b]furans. The synthesis was completed in eleven steps starting from commercially available materials in 7% overall yield.
As a final part of our studies regarding the use of P₄-t-Bu, we focused on the use of cyclization conditions for the synthesis of substituted pyrrolo[2,3-d] pyrimidine derivatives. For several decades, interest in pyrrole derivatives as antimicrobial agents has led to the preparation and antimicrobial evaluation of hundreds of such molecules. Pyrrole derivatives have antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* and an interesting antifungal activity against *Candida albicans*, as shown by Petruso et al. and Raimondi et al. Tubercidin, toyocamycin and sangivamycin are naturally occurring pyrrolo[2,3-d]pyrimidine antibiotics having significant activity against *Mycobacterium tuberculosis*, *Candida albicans* and *Streptococcus neoformans*, which was shown in many reports (Figure 7). 2,4-Diamino-5-methyl-6-substituted-pyrrolo[2,3-d]pyrimidines are potent and selective dihydrofolate reductase (DHFR) inhibitors against *Pneumocystis carinii*, *Toxoplasma gondii* and *Mycobacterium avium*, as reported by Gangjee et al. The aim of this study was to synthesize new pyrrolo[2,3-d]pyrimidine derivatives, hoping that they could be of promising chemical and biological interest.

Scheme 30: Retrosynthetic analysis
Our approach to the targeted pyrrolo[2,3-\(d\)]pyrimidine is shown in Scheme 30. We envisioned 3-carboxy-4,6-dichloropyrimidine \(76\) as a key component to our synthetic methodology. The reason for that is both chloro groups on pyrimidine \(76\) can be individually substituted with various amines \(78\) to prepare substituted carboxypyrimidine \(79\). We planned to try our \(P_4\)-t-Bu mediated cyclization reaction on this intermediate \(79\) to get substituted pyrrolo[2,3-\(d\)]pyrimidines \(74\).

Scheme 31: Synthesis

The synthesis started by subjecting commercially available 4,6-dihydroxypyrimidine \(75\) to Vilsmeier-Haack reaction conditions to get 3-carboxy-4,6-dichloropyrimidine \(76\) in 76% yield.\(^{51}\) This substituted pyrimidine \(76\) was then condensed with diethylamine under basic conditions to get \(N’N’\)-diethyl substituted pyridine \(77\) in 72% yield. This time, pyrimidine \(77\) was condensed with three different amines \(78a-c\) to get pyridine \(79a-c\) in excellent yields (Scheme 31).
Compound 79a-c were then subjected to previously developed P$_4$-t-Bu mediated cyclization reaction conditions to obtain substituted pyrrolo[2,3-d]pyrimidines 74a-c in good yields (Scheme 32). It was observed that compound 79b-c needed 2.2 equivalents of P$_4$-t-Bu to complete the cyclization because of the presence of two sites where anion can be generated.

In conclusion, we developed a very efficient and novel method to generate benzylic anions in electron rich systems and used this strategy to synthesize various heterocyclic aromatic compounds. We showed the utility of this method by synthesizing two natural products.

**Experimental**

**General procedure for the preparation of 54a-j:**
To a solution of 1,2,3,4-tetrahydroisoquinoline (0.56 g, 4.2 mmol) in dry DMF (6 mL), dry K$_2$CO$_3$ (0.58 g, 4.2 mmol) was added followed by solution of o-fluorobenzaldehyde (0.50 g, 4.0 mmol) in DMF at RT. Reaction mixture was heated to reflux for 20 h. After the completion of reaction, reaction mixture was cooled to RT, diluted with water and extracted.
with ethyl acetate (three times). Organic layer was then washed with water, brine and dried over MgSO₄. Excess solvent was evaporated in vacuo to obtain crude product. Crude product was subjected to column purification using 5% ethyl acetate: petroleum ether to obtain pure product (48% yield).

Spectroscopic Data for:

2-(3,4-Dihydroisoquinolin-2(1H)-yl)benzaldehyde (54a)

$^{1}$H-NMR (400MHz, CDCl₃) $\delta$ 3.07 (t, $J = 5.6$ Hz, 2H), 3.46 (t, $J = 5.6$ Hz, 2H), 4.34 (s, 2H), 7.10 – 7.13 (m, 2H), 7.19 – 7.22 (m, 4H), 7.54 (dt, $J = 7.8$ Hz, $J = 2$ Hz, 1H), 7.86 (dd, $J = 7.6$ Hz, $J = 1.6$ Hz, 1H), 10.34 (s, 1H); $^{13}$C-NMR (100MHz, CDCl₃) $\delta$: 29.1, 53.6, 54.8, 119.0, 122.3, 126.1, 126.4, 126.6, 128.6, 129.0, 130.0, 134.1, 134.2, 134.9, 155.2, 191.3; MS (m/z): 237, 149, 125, 123, 95, 83, 69, 55; HRMS: calcd for C$_{16}$H$_{15}$NO: 237.1154, found 237.1156.

2-(3,4-Dihydroisoquinolin-2(1H)-yl)-3-methoxybenzaldehyde (54b)

$^{1}$H-NMR (400MHz, CDCl₃) $\delta$ 2.98 (s, 2H), 3.49 (s, 2H), 3.86 (s, 3H), 4.35 (s, 2H), 7.00 – 7.02 (m, 1H), 7.14 – 7.19 (m, 4H), 7.26 (dt, $J = 7.6$ Hz, $J = 0.8$ Hz, 1H), 7.46 (dd, $J = 7.6$ Hz, $J = 1.6$ Hz, 1H), 10.58 (s, 1H); $^{13}$C-NMR (100MHz, CDCl₃) $\delta$: 30.3, 49.4, 54.0, 55.7, 117.5, 119.6, 125.8, 126.1, 126.3, 126.5, 129.3, 134.8, 134.9, 135.4, 143.8, 158.8, 193.7; MS (m/z): 267, 179, 93, 84, 77, 57, 49, 44, 40; HRMS: calcd for C$_{17}$H$_{17}$NO$_2$: 267.1259, found 267.1263.
2-(3,4-Dihydroisoquinolin-2(1H)-yl)-4,5-dimethoxybenzaldehyde (54c)

$^1$H-NMR (400MHz, CDCl$_3$) δ 3.05 (t, $J = 5.6$ Hz, 2H), 3.41 (t, $J = 6.0$ Hz, 2H), 3.90 (s, 3H), 3.94 (s, 3H), 4.27 (s, 2H), 6.70 (s, 1H), 7.07 – 7.09 (m, 1H), 7.17 – 7.20 (m, 3H), 7.37 (s, 1H), 10.29 (s, 1H); $^{13}$C-NMR (100MHz, CDCl$_3$) δ 29.4, 53.6, 54.1, 55.9, 56.2, 56.3, 102.8, 110.0, 122.4, 126.2, 126.4, 126.7, 129.2, 134.3, 145.5, 152.3, 155.0, 190.0; MS (m/z): 297, 282, 264; HRMS calcd for C$_{18}$H$_{19}$NO$_3$: 297.1365 found 297.1369.

2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)benzaldehyde (54d)

$^1$H-NMR (400MHz, CDCl$_3$) δ 2.96, (t, $J = 5.6$ Hz, 2H), 3.43 (t, $J = 6.0$ Hz, 2H), 3.86 (s, 3H), 3.87 (s, 3H), 4.26 (s, 2H), 6.59 (s, 1H), 6.67 (s, 1H), 7.11 (t, $J = 7.2$ Hz, 1H), 7.17 (d, $J = 7.6$ Hz, 1H), 7.52 (dt, $J = 7.6$ Hz, J = 1.6 Hz, 1H), 7.83 (dd, $J = 7.6$ Hz, $J = 1.6$ Hz, 1H), 10.32 (s, 1H); $^{13}$C-NMR (100MHz, CDCl$_3$) δ: 28.6, 53.7, 54.5, 56.0, 56.1, 109.1, 111.6, 119.1, 122.3, 125.9, 126.2, 128.5, 130.0, 134.9, 147.6, 147.8, 155.2, 191.4; MS (m/z): 297, 296, 282, 177, 149; HRMS calcd for C$_{18}$H$_{19}$NO$_3$: 297.1365, found 297.1369.

2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-3-methoxybenzaldehyde (54e)

$^1$H-NMR (400MHz, CDCl$_3$) δ 2.86 (s, 2H), 3.43 (s, 2H), 3.81 (s, 3H), 3.83 (s, 3H), 3.85 (s, 3H), 4.45 (s, 2H), 6.47 (s, 1H), 6.65 (s, 1H), 7.12 (d, $J = 8.4$ Hz, 1H), 7.22 (t, $J = 6.8$ Hz, 1H), 7.42 (d, $J = 7.2$ Hz, 1H), 10.54 (s, 1H); $^{13}$C-NMR (100MHz, CDCl$_3$) δ 29.9, 49.5, 53.7, 55.7, 56.1, 109.2, 112.0, 117.5, 120.0, 126.4, 126.7, 127.2, 134.9, 144.0, 147.4, 147.5,
158.8, 193.8; MS (m/z): 327, 326, 310, 270, 177, 164, 149, 77; HRMS calcd for C$_{19}$H$_{21}$NO$_4$: 327.1471, found 327.1474.

2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-4,5-dimethoxybenzaldehyde (54f)
$^1$H-NMR (400MHz, CDCl$_3$) $\delta$ 2.95 (t, $J = 5.6$ Hz, 2H), 3.38 (t, $J = 6.0$ Hz, 2H), 3.85 (s, 3H), 3.87 (s, 3H), 3.90 (s, 3H), 3.93 (s, 3H), 4.20 (s, 2H), 6.57 (s, 1H), 6.66 (s, 1H), 6.68 (s, 1H), 7.36 (s, 1H), 10.28 (s, 1H); $^{13}$C-NMR (100MHz, CDCl$_3$) $\delta$: 28.8, 54.1, 55.5, 56.1, 56.1, 56.2, 56.2, 102.7, 109.1, 109.9, 111.7, 122.2, 126.0, 126.2, 145.4, 147.6, 147.9, 152.3, 154.9, 190.0; MS (m/z): 357, 342, 296, 192, 164, 149; HRMS calcd for C$_{20}$H$_{23}$NO$_5$ 357.1576, found 357.1582.

2-(3,4-Dihydroisoquinolin-2(1H)-yl)-5-(trifluoromethyl)benzaldehyde (54g)
$^1$H-NMR (400MHz, CDCl$_3$) $\delta$ 3.09 (t, $J = 5.6$ Hz, 2H), 3.57 (t, $J = 6.0$ Hz, 2H), 4.43 (s, 2H), 7.12-7.14 (m, 1H), 7.22-7.25 (m, 4H), 7.71 (d, $J = 8.0$ Hz, 1H), 8.08 (s, 1H), 10.23 (s, 1H); $^{13}$C-NMR (100MHz, CDCl$_3$): $\delta$ 28.9, 53.2, 54.0, 118.6, 123.1 (q, $^2J = 33.2$ Hz), 124.1 (q, $^1J = 269.8$ Hz), 126.4, 126.9, 128.3, 128.4, 129.0, 131.1, 131.2, 133.3, 134.1, 156.5, 189.5; MS (m/z): 306, 305, 304, 286; HRMS calcd for C$_{17}$H$_{14}$F$_3$NO: 305.1027, found 305.1033.

2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-5-(trifluoromethyl)benzaldehyde (54h): $^1$H-NMR (400MHz, CDCl$_3$) $\delta$ 2.99 (t, $J = 5.6$ Hz, 2H), 3.55 (t, $J = 6.0$ Hz, 2H), 3.88
(s, 3H), 3.89 (s, 3H), 4.36 (s, 2H), 6.61 (s, 1H), 6.68 (s, 1H), 7.22 (d, J = 8.8 Hz, 1H), 7.71 (d, J = 8.8 Hz, 1H), 8.07 (s, 1H), 10.22 (s, 1H); $^{13}$C-NMR (100MHz, CDCl$_3$): $\delta$ 28.1, 53.1, 53.4, 55.6, 55.7, 108.9, 111.3, 118.4, 122.7 (q, $^2J = 33.4$ Hz), 123.9 (q, $^1J = 269.2$ Hz), 124.9, 125.8, 126.6, 127.9, 130.9, 147.5, 147.8, 156.4, 189.3; MS (m/z): 365, 364, 350, 332, 252, 140, 96, 85, 83, 48; HRMS calcd for C$_{19}$H$_{18}$F$_3$NO: 365.1239, found 365.1246.

2-(3,4-dihydroisoquinolin-2(1H)-yl)-4-(trifluoromethyl)benzaldehyde (54i)
$^1$H-NMR (400MHz, CDCl$_3$) $\delta$ 3.13 (t, J = 8.0 Hz, 2H), 3.51 (t, J = 8.0 Hz, 2H), 4.38 (s, 2H), 7.08-7.18 (m, 2H), 7.20 – 7.28 (m, 2H), 7.36 (d, J = 8.0 Hz, 1H), 7.45 (s, 1H), 7.95 (d, J = 8.0 Hz, 1H), 10.33 (s, 1H); $^{13}$C-NMR (100MHz, CDCl$_3$): $\delta$ 29.4, 53.7, 54.7, 115.9, 118.6, 123.8 (q, $^1J = 271$ Hz), 126.5, 126.7, 127.2, 129.2, 130.6, 131.0, 133.6, 134.1, 136.1 (q, $^2J = 32$ Hz), 155.1, 190.4; MS (m/z): 307, 306, 305, 304, 286, 218, 164; HRMS calcd for C$_{17}$H$_{14}$F$_3$NO: 305.1027, found 305.1036.

2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-4-(trifluoromethyl)benzaldehyde (54j): $^1$H-NMR (400MHz, CDCl$_3$) $\delta$ 3.01 (t, J = 8.0 Hz, 2H), 3.47 (t, J = 8.0 Hz, 2H), 3.87 (s, 3H), 3.88 (s, 3H), 4.30 (s, 2H), 6.61 (s, 1H), 6.68 (s, 1H), 7.33 (d, J = 8.8 Hz, 1H), 7.40 (s, 1H), 7.92 (d, J = 8.0 Hz, 1H), 10.29 (s, 1H); $^{13}$C-NMR (100MHz, CDCl$_3$): $\delta$ 28.9, 54.0, 54.3, 56.2, 56.3, 109.2, 111.7, 115.8, 118.5, 123.8 (q, $^1J = 272$ Hz), 125.3, 126.0, 130.5, 130.9, 136.0 (q, $^2J = 32$ Hz), 147.9, 148.2, 155.1, 190.4; MS (m/z): 365, 362, 333, 192, 177, 163, 145, 121, 91, 77, 49, 48; HRMS calcd for C$_{19}$H$_{18}$F$_3$NO: 365.1239, found 365.1246.

Procedure for the preparation of 45a-j
To a solution of aldehyde (0.10 g, 0.42 mmol) in freshly distilled, dry benzene (5 mL), $P_4-t$-Bu solution (0.46 mL, 0.46 mmol) was added at room temperature and reaction mixture was heated to reflux with monitoring (2 h). After the completion of reaction, benzene was partially evaporated and reaction mixture was purified by column purification using 3% ethyl acetate: petroleum ether to obtain pure product (35% yield).

**Spectroscopic Data for:**

![Chemical Structure](image)

**5,6-Dihydroindolo[2,1-a]isoquinoline (45a)**

$^1$H-NMR (400MHz, CDCl$_3$) $\delta$ 3.21 (t, $J = 6.8$ Hz, 2H), 4.27 (t, $J = 6.4$ Hz, 2H), 6.89 (s, 1H), 7.12 (t, $J = 7.2$ Hz, 1H), 7.20 – 7.36 (m, 5H), 7.65 (d, $J = 8.0$ Hz, 1H), 7.78 (d, $J = 8.0$ Hz, 1H); $^{13}$C-NMR (100MHz, CDCl$_3$) $\delta$: 29.4, 40.3, 96.6, 109.1, 115.5, 120.0, 120.9, 121.8, 124.5, 127.4, 128.6, 128.5, 128.9, 132.3, 135.8, 143.7; MS (m/z): 219, 109, 108; HRMS calcd for C$_{16}$H$_{13}$N: 219.1048, found 219.1052.

**8-Methoxy-5,6-dihydroindolo[2,1-a]isoquinoline (45b)**

$^1$H-NMR (400MHz, CDCl$_3$) $\delta$ 3.12 (t, $J = 6.4$ Hz, 2H), 3.93 (s, 3H), 4.70 (t, $J = 6.4$ Hz, 2H), 6.61 (d, $J = 8.0$ Hz, 1H), 6.83 (s, 1H), 6.98 (t, $J = 8.0$ Hz, 1H), 7.14 – 7.25 (m, 3H), 7.27 (t, $J = 7.8$ Hz, 1H), 7.72 (d, $J = 7.6$ Hz, 1H); $^{13}$C-NMR (100MHz, CDCl$_3$) $\delta$: 29.9, 43.3, 55.5, 97.3, 102.9, 113.8, 120.2, 124.4, 126.6, 127.2, 127.4, 128.2, 129.2, 130.7, 132.7, 136.1, 147.7; MS (m/z): 249, 248, 233, 232, 86, 84, 82, 50, 48; HRMS calcd for C$_{17}$H$_{15}$NO: 249.1154, found 249.1156.
9,10-Dimethoxy-5,6-dihydroindolo[2,1-\(\alpha\)]isoquinoline (45c)

\(^1\)H-NMR (400MHz, CDCl\(_3\)) \(\delta\) 3.19 (t, \(J = 6.8\) Hz, 2H), 3.94 (s, 3H), 3.97 (s, 3H), 4.20 (t, \(J = 6.4\) Hz, 2H), 6.76 (s, 1H), 6.82 (s, 1H), 7.09 (s, 1H), 7.18 (dt, \(J = 7.2\) Hz, \(J = 1.2\) Hz, 1H), 7.30 – 7.23 (m, 2H), 7.69 (d, \(J = 7.6\) Hz, 1H); \(^{13}\)C-NMR (100MHz, CDCl\(_3\)) \(\delta\) 29.4, 40.5, 56.4, 56.5, 92.6, 96.3, 102.5, 121.6, 123.9, 126.9, 127.4, 128.4, 129.5, 131.4, 131.5, 134.5, 145.4, 147.2; MS (m/z): 279, 264, 236; HRMS calcd for C\(_{18}\)H\(_{17}\)NO\(_2\): 279.1259, found 279.1264.

2,3-Dimethoxy-5,6-dihydroindolo[2,1-\(\alpha\)]isoquinoline (45d)

\(^1\)H-NMR (400MHz, CDCl\(_3\)) \(\delta\) 3.14 (t, \(J = 6.8\) Hz, 2H), 3.93 (s, 3H), 3.97 (s, 3H), 4.24 (t, \(J = 6.8\) Hz, 2H), 6.76 (s, 1H), 6.77 (s, 1H), 7.09 (t, \(J = 7.2\) Hz, 1H), 7.19 (t, \(J = 7.2\) Hz, 1H), 7.25 (d, \(J = 6.4\) Hz, 1H), 7.32 (d, \(J = 8.0\) Hz, 1H), 7.61 (d, \(J = 8.0\) Hz, 1H); \(^{13}\)C-NMR (100MHz, CDCl\(_3\)) \(\delta\) 29.0, 40.4, 56.2, 56.3, 95.3, 107.5, 109.0, 111.4, 120.0, 120.6, 121.5, 121.8, 125.1, 129.1, 136.0, 136.8, 148.5, 148.9; MS (m/z): 279, 264, 236; HRMS calcd for C\(_{18}\)H\(_{17}\)NO\(_2\): 279.1259, found 279.1262.

2,3,8-Trimethoxy-5,6-dihydroindolo[2,1-\(\alpha\)]isoquinoline (45e)

\(^1\)H-NMR (400MHz, CDCl\(_3\)) \(\delta\) 3.08 (t, \(J = 6.8\) Hz, 2H), 3.92 (s, 3H), 3.95 (s, 3H), 3.96 (s, 3H), 4.69 (t, \(J = 6.4\) Hz, 2H), 6.61 (d, \(J = 8.0\) Hz, 1H), 6.73 (s, 1H), 6.75 (s, 1H), 6.98 (t, \(J = 8.0\) Hz, 1H), 7.21 (d, \(J = 8.8\) Hz, 2H); \(^{13}\)C-NMR (100MHz, CDCl\(_3\)) \(\delta\) 29.5, 43.4, 55.5, 56.2, 56.3, 96.0, 102.7, 107.4, 111.2, 113.5, 120.1, 121.8, 125.5, 126.4, 130.9, 136.4, 147.5, 148.4,
148.8; MS (m/z): 310, 309, 294, 266; HRMS calcd for C_{19}H_{19}NO_3: 309.1365, found 309.1371.

2,3,9,10-Tetramethoxy-5,6-dihydroindolo[2,1-a]isoquinoline (45f)

\(^1\)H-NMR (400MHz, CDCl\(_3\)) \(\delta\) 3.12 (t, \(J = 6.4\) Hz, 2H), 3.92 (s, 3H), 3.94 (s, 3H), 3.96 (s, 6H), 4.17 (t, \(J = 6.4\) Hz, 2H), 6.64 (s, 1H), 6.75 (s, 1H), 6.81 (s, 1H), 7.07 (s, 1H), 7.17 (s, 1H); \(^{13}\)C-NMR (100MHz, CDCl\(_3\)) \(\delta\) 29.0, 40.7, 56.2, 56.3, 56.5, 56.6, 92.7, 95.0, 102.5, 107.0, 111.5, 121.8, 122.2, 124.2, 131.33, 134.8, 145.3, 146.9, 148.4, 148.5; MS (m/z): 357, 339, 324, 293, 280, 149; HRMS calcd for C\(_{20}\)H\(_{21}\)NO\(_4\): 339.1471, found 339.1476.

10-(Trifluoromethyl)-5,6-dihydroindolo[2,1-a]isoquinoline (45g)

\(^1\)H-NMR (400MHz, CDCl\(_3\)) \(\delta\) 3.16 (t, \(J = 6.4\) Hz, 2H), 4.22 (t, \(J = 6.4\) Hz, 2H), 6.89 (s, 1H), 7.24 – 7.40 (m, 5H), 7.73 (d, \(J = 7.6\) Hz, 1H), 7.88 (s, 1H); \(^{13}\)C-NMR (100MHz, CDCl\(_3\)) \(\delta\) 29.2, 40.6, 97.4, 97.4, 109.3, 118.5, 118.6, 122.4 (q, \(^2\)J = 32 Hz), 124.8, 125.6 (q, \(^1\)J = 270 Hz), 127.6, 128.3, 128.5, 128.6, 132.4, 137.5, 138.0; MS (m/z): 287, 217; HRMS calcd for C\(_{17}\)H\(_{12}\)F\(_3\)N: 287.0922 found 287.0929.

2,3-Dimethoxy-10-(trifluoromethyl)-5,6-dihydroindolo[2,1-a]isoquinoline (45h)

\(^1\)H-NMR (400MHz, CDCl\(_3\)) \(\delta\) 3.15 (t, \(J = 6.4\) Hz, 2H), 3.93 (s, 3H), 3.98 (s, 3H), 4.26 (t, \(J = 6.4\) Hz, 2H), 6.78 (s, 1H), 6.81 (s, 1H), 7.23 (s, 1H), 7.34 – 7.41 (m, 2H), 7.88 (s, 1H); \(^{13}\)C-
NMR (100MHz, CDCl₃) δ 28.8, 40.7, 56.3, 56.4, 96.1, 107.7, 109.1, 111.5, 118.1, 118.2, 121.1, 122.3 (q, ²J = 31 Hz), 125.3, 125.6 (q, ¹J = 269 Hz), 128.4, 137.8, 138.0, 148.7, 149.5; MS (m/z): 347, 287, 217, 84; HRMS calcd for C₁₉H₁₆F₃N₂: 347.1133, found 347.1141.

9-(Trifluoromethyl)-5,6-dihydroindolo[2,1-a]isoquinoline (45i)
¹H-NMR (400MHz, CDCl₃) δ 3.23 (t, J = 6.4 Hz, 2H), 4.31 (t, J = 6.4 Hz, 2H), 6.91 (s, 1H), 7.22 – 7.40 (m, 4H), 7.62 (s, 1H), 7.69 (d, J = 8.0 Hz, 1H), 7.78 (d, J = 8.0 Hz, 1H); ¹³C-NMR (100MHz, CDCl₃) δ 29.2, 40.5, 96.8, 106.7, 116.7, 121.1, 123.6 (q, ²J = 32 Hz), 124.9, 125.5 (q, ¹J = 269 Hz), 127.6, 128.4, 128.5, 128.6, 131.3, 132.6, 135.7, 138.4; MS (m/z): 287, 282, 217, 216; HRMS calcd for C₁₇H₁₂F₃N: 287.0922 found 287.0929.

2,3-Dimethoxy-9-(trifluoromethyl)-5,6-dihydroindolo[2,1-a]isoquinoline (45j)
¹H-NMR (400MHz, CDCl₃) δ 3.16 (t, J = 8.0 Hz, 2H), 3.94 (s, 3H), 3.97 (s, 3H), 4.27 (t, J = 8.0 Hz, 2H), 6.78 – 6.83 (m, 2H), 7.24 (s, 1H), 7.32 (d, J = 8.0 Hz, 1H), 7.59 (s, 1H), 7.66 (d, J = 8.0 Hz, 1H); ¹³C-NMR (100MHz, CDCl₃) δ 28.7, 40.6, 56.2, 56.3, 95.4, 106.4, 107.7, 111.4, 116.6, 120.6, 121.0, 123.2 (q, ²J = 32 Hz), 125.5, 125.6 (q, ¹J = 270 Hz), 131.4, 135.7, 138.7, 148.6, 149.5; MS (m/z): 347, 332, 304, 288, 273; HRMS calcd for C₁₉H₁₆F₃NO₂: 347.1133, found 347.1141.

**Representative procedure for the preparation of 2,3-diarylbenzofuran**

To a solution of 2-benzyloxybenzophenone (0.20 g, 0.69 mmol) in freshly distilled dry benzene (10 mL), P₄-t-Bu solution (0.77 mL, 0.76 mmol, 1.0 M solution in hexane) was added at room temperature and reaction mixture was heated to reflux with monitoring (3 h). After the completion of reaction, benzene was partially evaporated and reaction mixture was
purified by column purification using 10% ethyl acetate: petroleum ether to obtain pure product (100% yield).

2,3-Diphenylbenzo[b]furan (47a)
Mp 123-124.5 °C; \( ^1H \)-NMR (400 MHz) \( \delta \) 7.23 (t, \( J = 7.3 \) Hz, 1H), 7.28-7.35 (m, 4H), 7.38-7.52 (m, 6H), 7.55 (d, \( J = 8.3 \) Hz, 1H), 7.65-7.67 (m, 2H); \( ^{13}C \)-NMR (100 MHz) \( \delta \) 111.1, 117.5, 120.0, 122.9, 124.7, 127.0, 127.6, 128.3, 128.4, 129.0, 129.8, 130.2, 130.7, 132.8, 150.5, 154.0; MS (\( m/z \)): 271, 199, 183, 121; HRMS calcd for \( C_{20}H_{14}O \): 270.1044, found 270.11045.

2-(3,4-dimethoxyphenyl)-3-phenylbenzofuran (47c)
\(^1H\)-NMR (400 MHz) \( \delta \) 7.43 – 7.59 (m, 6H), 7.33 – 7.42 (m, 1H), 7.24 – 7.33 (m, 2H), 7.24 (t, \( J = 8.0 \) Hz, 1H), 7.16 (s, 1H), 6.83 (d, \( J = 8.0 \) Hz, 1H), 3.89 (s, 3H), 3.69 (s, 3H); \( ^{13}C \)-NMR (100 MHz) \( \delta \) 154.0, 150.8, 149.5, 148.9, 133.4, 130.7, 130.2, 129.2, 127.8, 124.6, 123.7, 123.1, 120.1, 120.0, 116.5, 111.3, 111.2, 110.3, 56.1, 55.8; MS (\( m/z \)): 331, 213; HRMS calcd for \( C_{22}H_{18}O_3 \): 330.1256, found 330.1257.

5,6-Dimethoxy-2,3-diphenylbenzofuran (47d)
$^1$H-NMR (300 MHz) δ 7.56 – 7.63 (m, 2H), 7.36 – 7.54 (m, 5H), 7.20 – 7.34 (m, 3H), 7.13 (s, 1H), 6.89 (s, 1H), 3.97 (s, 3H), 3.88 (s, 3H); MS (m/z): 331; HRMS calcd for C$_{22}$H$_{18}$O$_3$: 330.1256, found 330.1262.

![Chemical Structure](image1)

6,7-Diphenyl-[1,3]dioxolo[4,5-f]benzofuran (47e)

$^1$H-NMR (300 MHz) δ 7.56 – 7.63 (m, 2H), 7.36 – 7.51 (m, 5H), 7.23 – 7.34 (m, 3H), 7.05 (s, 1H), 6.85 (s, 1H), 5.99 (s, 2H); MS (m/z): 315, 247, 163; HRMS calcd for C$_{21}$H$_{14}$O$_3$: 314.0943, found 314.0934.

![Chemical Structure](image2)

3-(Furan-2-yl)-5,6-dimethoxy-2-phenylbenzofuran (47f)

$^1$H-NMR (400 MHz) δ 7.70 – 7.81 (m, 2H), 7.55 – 7.60 (m, 1H), 7.30 – 7.46 (m, 3H), 7.21 (s, 1H), 7.08 (s, 1H), 6.60 (dd, $^1J = 3.3$ Hz, $^2J = 0.6$ Hz, 1H), 6.55 (dd, $^1J = 3.3$ Hz, $^2J = 1.8$ Hz, 1H), 3.96 (s, 3H), 3.95 (s, 3H); $^{13}$C-NMR (100 MHz) δ 151.1, 149.0, 148.7, 147.4, 147.1, 142.2, 142.1, 131.1, 128.7, 127.3, 120.6, 111.5, 108.6, 108.4, 102.3, 102.2, 95.3, 95.2, 56.7, 56.5; MS (m/z): 321; HRMS calcd for C$_{20}$H$_{16}$O$_4$: 320.1049, found 320.1046.

![Chemical Structure](image3)

7-(Furan-2-yl)-6-phenyl-[1,3]dioxolo[4,5-f]benzofuran (47g)

$^1$H-NMR (400 MHz) δ 7.74 (d, $J = 8.0$ Hz, 2H), 7.51 – 7.56 (m, 1H), 7.31 – 7.44 (m, 3H), 7.17 (s, 1H), 7.02 (s, 1H), 6.57 (d, $J = 3.2$ Hz, 1H), 6.52 (dd, $^1J = 3.2$ Hz, $^2J = 2.0$ Hz, 1H),
6.01 (s, 2H); MS (m/z): 641, 391, 305, 239, 149; HRMS calcd for C₁₉H₁₂O₄: 304.0736, found 304.0735.

5,6-Dimethoxy-2-phenyl-3-(3,4,5-trimethoxyphenyl)benzofuran (47h)

¹H-NMR (300 MHz) δ 7.64 (dd, ¹J = 9.2 Hz, ²J = 2.0 Hz), 7.21 – 7.35 (m, 3H), 7.12 (s, 1H), 6.92 (s, 1H), 6.70 (s, 2H), 3.85 (s, 6H), 3.89 (s, 3H), 3.80 (s, 6H); MS (m/z): 421; HRMS calcd for C₂₅H₂₄O₆: 420.1573, found 420.1568.

6-Benzyl-3-(2-methoxyphenyl)-2-phenylbenzofuran (47i)

¹H-NMR (300 MHz) δ 7.56 – 7.63 (m, 2H), 7.20 – 7.45 (m, 10H), 7.18 (s, 1H), 6.94 – 7.07 (m, 3H), 6.86 (d, J = 2.4 Hz, 1H), 5.00 (s, 2H), 3.58 (s, 3H); ¹³C-NMR (100 MHz) δ 157.6, 155.3, 152.0, 149.2, 137.5, 132.0, 131.4, 131.3, 129.6, 128.7, 128.4, 128.2, 128.0, 127.7, 126.5, 121.8, 121.2, 114.2, 114.1, 111.7, 111.6, 104.5, 70.9, 55.5; MS (m/z): 407; HRMS calcd for C₂₈H₂₂O₃: 406.1515, found 406.1520.

6-Benzyl-2-phenyl-3-(3,4,5-trimethoxyphenyl)benzofuran (47j)
\( ^1 \text{H-NMR} \) (400 MHz) \( \delta \) 7.71 (d, \( J = 8.0 \) Hz, 2H), 7.43 – 7.50 (m, 3H), 7.29 – 7.43 (m, 6H), 7.06 (s, 2H), 6.69 (s, 2H), 5.09 (s, 2H), 3.98 (s, 3H), 3.79 (s, 6H); \( ^{13} \text{C-NMR} \) (100 MHz) \( \delta \) 155.5, 153.8, 151.4, 149.1, 137.6, 137.3, 130.8, 130.7, 128.7, 128.5, 128.4, 128.0, 127.5, 127.0, 117.8, 114.3, 111.8, 106.7, 103.9, 70.9, 61.2, 56.3; MS (\( m/z \)): 467, 359; HRMS calcd for \( \text{C}_{36}\text{H}_{26}\text{O}_5 \): 466.1740, found 466.1744.

![1,2-diphenynaphtho[2,1-b]furan (47l)](image)

1,2-diphenynaphtho[2,1-b]furan (47l)

\( ^1 \text{H-NMR} \) (400 MHz) \( \delta \) 7.94 (d, \( J = 8.4 \) Hz, 1H), 7.76 (d, \( J = 4.8 \) Hz, 2H), 7.52 – 7.63 (m, 7H), 7.41 (t, \( J = 7.2 \) Hz, 2H), 7.22 – 7.32 (m, 4H); \( ^{13} \text{C-NMR} \) (100 MHz) \( \delta \) 151.6, 150.3, 134.9, 131.2, 131.1, 130.8, 129.6, 129.2, 128.6, 128.5, 128.4, 128.0, 126.4, 126.2, 124.5, 123.9, 123.3, 119.8, 112.4; MS (\( m/z \)): 321, 249, 225, 209; HRMS calcd for \( \text{C}_{24}\text{H}_{16}\text{O} \): 320.1201, found 320.1196.

![Ethyl 4-(1-phenynaphtho[2,1-b]furan-2-yl)benzoate (47m)](image)

Ethyl 4-(1-phenynaphtho[2,1-b]furan-2-yl)benzoate (47m)

\( ^1 \text{H-NMR} \) (400 MHz) \( \delta \) 7.90 – 7.98 (m, 3H), 7.80 (d, \( J = 8.0 \) Hz, 1H), 7.74 (d, \( J = 8.0 \) Hz, 1H), 7.51 – 7.65 (m, 8H), 7.42 (t, \( J = 8.0 \) Hz, 1H), 7.29 (t, \( J = 8.0 \) Hz, 1H), 4.36 (q, \( J = 8.0 \) Hz, 2H), 1.38 (t, \( J = 8.0 \) Hz, 3H); \( ^{13} \text{C-NMR} \) (100 MHz) \( \delta \) 166.4, 152.0, 149.2, 135.1, 134.5, 131.2, 130.5, 130.4, 129.9, 129.8, 129.3, 129.2, 128.7, 128.5, 127.1, 126.5, 125.9, 124.7, 123.7, 123.2, 121.8, 112.4, 61.2, 14.6; MS (\( m/z \)): 393, 379, 359, 333; HRMS calcd for \( \text{C}_{27}\text{H}_{30}\text{O}_3 \): 392.1412, found 392.1412.
5-Chloro-2,3-diphenylbenzofuran (47n)

$^1$H-NMR (400 MHz) δ 7.63 – 7.71 (m, 2H), 7.41 – 7.54 (m, 7H), 7.27 – 7.37 (m, 4H); $^{13}$C-NMR (100 MHz) δ 152.6, 152.1, 132.4, 131.9, 130.4, 129.9, 129.3, 129.0, 128.9, 128.7, 128.2, 127.3, 125.1, 119.9, 117.3, 112.3; MS (m/z): 305, 286, 271; HRMS calcd for C$_{20}$H$_{13}$ClO: 304.0655, found 304.0657.

2-(3-Nitrophenyl)-3-phenylbenzofuran (47o)

$^1$H-NMR (400 MHz) δ 8.59 (s, 1H), 8.11 (d, $J = 8.0$ Hz, 1H), 7.89 (d, $J = 8.0$ Hz, 1H), 7.59 (d, $J = 8.0$ Hz, 1H), 7.46 – 7.56 (m, 6H), 7.37 – 7.47 (m, 2H), 7.28 (t, $J = 8.0$ Hz, 1H); $^{13}$C-NMR (100 MHz) δ 154.3, 148.7, 147.8, 136.5, 133.8, 132.6, 132.3, 132.1, 132.0, 130.1, 129.8, 129.6, 129.4, 128.6, 126.0, 123.6, 122.8, 121.7, 120.7, 111.6.

Preparation of (E)-2-bromo-1,5-dimethoxy-3-(4-methoxystyryl)benzene (65): In an oven dried 250 ml flask, equipped with a stirring bar, potassium tert-butoxide solution (20.0 ml, 20.0 mmol, 1.0 M solution in THF) was added dropwise to a solution of phosphonate ester (63) in freshly distilled dry THF (75 ml) at -78 °C under argon. Resulting reaction mixture
was stirred at -78 °C for 30 min followed by the addition of a solution of p-methoxybenzaldehyde (64) (2.47 g, 18.11 mmol) in dry THF (50 ml) at the same temperature. The resultant reaction mixture was stirred at -78 oC for 1 h and at room temperature for 12 h. Upon completion, the reaction mixture was quenched with saturated ammonium chloride solution and majority of the THF was evaporated in vacuo. Residue was partitioned between water and ethyl acetate and organic layer was separated. Aqueous layer was then extracted with ethyl acetate (3 x 75 ml); organic layers were combined, washed with brine and dried over anhydrous magnesium sulfate. The solvent was evaporated and residue was purified by column chromatography using 10% EtOAc in hexanes to obtain pure product (65) (4.92 g, 78% yield). $^1$H-NMR (400 MHz) δ 7.50 (d, J = 8.8 Hz, 2H), 7.41 (d, J = 16 Hz, 1H), 6.98 (d, J = 16.0 Hz, 1H), 6.91 (d, J = 8.4 Hz, 2H), 6.80 (d, J = 2.8 Hz, 1H), 6.41 (d, J = 2.4 Hz, 1H), 3.88 (s, 3H), 3.86 (s, 3H), 3.83 (s, 3H); $^{13}$C-NMR (100 MHz) δ 159.8, 159.7, 157.0, 139.1, 131.3, 129.9, 128.3, 125.9, 114.3, 105.1, 102.6, 98.9, 56.5, 55.7, 55.5.

Preparation of (E)-(2,4-dimethoxy-6-(4-methoxystyryl)phenyl)(3,5-dimethoxyphenyl) methanone (67): In an oven dried 250 ml round bottom flask, equipped with a stir bar, n-BuLi (3.91 ml, 9.77 mmol, 2.5 M solution in hexane) was added to a solution of stilbene (65) (3.25 g, 9.31 mmol) in dry THF (50 ml) at -78 °C under argon. Resulting yellow reaction mixture was stirred at -78 °C for 30 min followed by the addition of a solution of 3,5-dimethoxybenzaldehyde (59) (1.55 g, 9.31 mmol) in dry THF (25 ml). The resultant mixture was stirred at -78 °C for 1 h followed by stirring at room temperature for 2 h. Upon completion, the reaction mixture was quenched with saturated ammonium chloride solution and majority of the THF was evaporated in vacuo. Residue was partitioned between water and ethyl acetate and organic layer was separated. Aqueous layer was then extracted with
ethyl acetate (3 x 75 ml); organic layers were combined, washed with brine and dried over anhydrous magnesium sulfate. The solvent was evaporated and residue was purified by column chromatography using 30% EtOAc in DCM to obtain pure alcohol (3.0 g, 6.87 mmol) which was dissolved in benzene and activated manganese oxide (3.00 g, 34.36 mmol) was added to it. Resulting slurry was refluxed azeotropically for 6 h. Reaction mixture was filtered through celite and the filtrate was evaporated to dryness. The residue was purified by column chromatography using 40% EtOAc in hexanes to obtain the pure ketone (67) (1.77 g, 4.08 mmol) in 60% yield over two steps. $^1$H-NMR (400 MHz) $\delta$ 7.27 (d, $J = 8.0$ Hz, 2H), 6.95 – 7.05 (m, 3H), 6.70 – 6.87 (m, 4H), 6.63 (t, $J = 4.0$ Hz, 1H), 6.42 (d, $J = 2.0$ Hz, 1H), 3.90 (s, 3H), 3.79 (s, 3H), 3.77 (s, 3H), 3.68 (s, 3H); $^{13}$C-NMR (100 MHz) $\delta$ 197.5, 161.5, 161.0, 159.8, 158.6, 140.6, 137.9, 131.2, 129.8, 128.3, 123.3, 121.6, 114.2, 107.6, 107.5, 105.9, 105.8, 101.4, 98.0, 97.9, 56.0, 55.8, 55.7, 55.5.

### Preparation of (E)-(3,5-dimethoxyphenyl)(2-hydroxy-4-methoxy-6-(4-methoxystyryl) phenyl)methanone (68):

To a solution of ketone (67) (1.52 g, 3.50 mmol) in dry DCM was added boron tribromide (3.85 ml, 3.85 mmol, 1.0 M solution in DCM) at -78 °C under argon. Resulting reaction mixture was stirred for 3 h with steady increase in temperature to -50 °C. After the completion of reaction, the reaction mixture was neutralized by adding dilute HCl (1.0 M aqueous solution) and extracting the aqueous layer with DCM (4 x 25 ml). Organic layer was then subjected to brine wash and drying over anhydrous magnesium sulfate. Solvent was evaporated in vacuo to obtain crude product which was purified by column chromatography using 2% MeOH in DCM as elutent to obtain the pure alcohol (68) (1.30 g, 3.09 mmol) in 88% yield. $^1$H-NMR (400 MHz) $\delta$ 11.54 (s, 1H), 6.89 (d, $J = 8.0$ Hz, 2H), 6.71 – 6.78 (m, 4H), 6.67 (s, 1H), 6.63 (s, 1H), 6.43 – 6.52 (m, 3H), 3.88 (s, 3H), 3.78 (s, 3H),...
$^{13}$C-NMR (100 MHz) $\delta$ 200.4, 165.0, 164.9, 161.0, 159.8, 143.1, 130.5, 129.9, 128.0, 127.3, 114.2, 113.8, 107.3, 107.3, 106.6, 106.6, 104.8, 104.8, 100.4, 100.3, 55.9, 55.6; MS (m/z): 421, 420, 419, 418, 416, 313, 312, 311, 310, 300, 299, 298, 297, 295, 284, 283, 254; HRMS calcd for C$_{25}$H$_{24}$O$_6$: 420.15728, found 420.15831.

Preparation of (E)-(3,5-dimethoxyphenyl)(4-methoxy-2-((4-methoxybenzyl)oxy)-6-(4-methoxystyryl)phenyl)methanone (69): In an oven dried 100 ml flask equipped with a stir bar, benzophenone derivative (68) (0.50 g, 1.19 mmol) was taken in dry DMF (10 ml). To this, NaH (0.058 g, 1.43 mmol) was added at 0 $^{\circ}$C under argon and the reaction mixture was allowed to stir for 15 min. To this reaction mixture, a solution of $p$-methoxybenzyl bromide (0.26 g, 1.31 mmol) in dry DMF (5 ml) was added at 0 $^{\circ}$C and resulting reaction mixture was stirred at room temperature for 4 h with constant monitoring. After the completion of reaction, the reaction mixture was quenched with water and majority of the DMF was evaporated in vacuo. Residue was partitioned between water and ethyl acetate and organic layer was separated. Aqueous layer was then extracted with ethyl acetate (3 x 25 ml); organic layers were combined, washed with water and brine followed by drying over anhydrous magnesium sulfate. The solvent was evaporated and residue was purified by column chromatography using 20% EtOAc in hexane as eluent to obtain pure product (69) (0.56 g, 1.03 mmol) in 86% isolated yield. $^1$H-NMR (400 MHz) $\delta$ 7.31 (d, $J$ = 8.0 Hz, 2H), 6.90 – 7.05 (m, 5H), 6.77 – 6.89 (m, 4H), 6.74 (d, $J$ = 8.0 Hz, 2H), 6.66 (s, 1H), 6.45 (s, 1H), 4.89 (s, 2H), 3.88 (s, 3H), 3.78 (s, 3H), 3.76 (s, 3H); $^{13}$C-NMR (100 MHz) $\delta$ 197.6, 161.5, 161.1, 159.8, 159.4, 157.8, 141.4, 138.3, 131.3, 129.9, 128.9, 128.7, 128.4, 123.3, 122.2, 114.3,
Preparation of (E)-3-(3,5-dimethoxyphenyl)-6-methoxy-2-(4-methoxyphenyl)-4-(4-methoxystyryl)benzofuran (70): To a solution of 2-benzyloxybenzophenone derivative (69) (0.20 g, 0.37 mmol) in freshly distilled dry benzene (5 mL), P₄-t-Bu solution (0.44 mL, 0.44 mmol, 1.0 M solution in hexane) was added at room temperature and reaction mixture was heated to 170 °C in a sealed tube and stirred for 12 h. After the completion of reaction, benzene was partially evaporated and reaction mixture was purified by column purification using 40% ethyl acetate in hexanes to obtain pure product (70) (0.10 g, 0.19 mmol) in 52% yield. ¹H-NMR (400 MHz) δ 7.56 (d, J = 8.0 Hz, 2H), 7.14 (bs, 1H), 6.96 – 7.07 (m, 3H), 6.90 (bs, 2H), 6.77 – 6.88 (m, 4H), 6.64 – 6.72 (m, 3H), 3.93 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.76 (s, 3H); ¹³C-NMR (100 MHz) δ 161.9, 159.7, 159.6, 158.5, 155.3, 150.2, 137.4, 132.5, 130.7, 129.0, 127.9, 127.8, 124.0, 123.6, 122.4, 116.8, 114.3, 114.2, 109.1, 107.2, 101.0, 95.4, 56.2, 55.8, 55.7, 55.6; MS (m/z): 524, 523, 522, 521, 520, 508, 83, 81, 49, 48; HRMS calcd for C₃₃H₃₀O₆: 522.20423, found 522.20560.
Preparation of (E)-5-(6-hydroxy-2-(4-hydroxyphenyl)-4-(4-hydroxystyryl)benzofuran-3-yl)benzene-1,3-diol (48): To a solution of benzofuran derivative (70) (0.060 g, 0.12 mmol) in dry DCM was added boron tribromide (1.15 ml, 1.15 mmol, 1.0 M solution in DCM) at 0 °C under argon. Resulting reaction mixture was stirred for 6 h at room temperature. After the completion of reaction, the reaction mixture was neutralized by adding dilute HCl (1.0 M aqueous solution) and extracting the aqueous layer with DCM (4 x 25 ml). Organic layer was then subjected to brine wash and drying over anhydrous magnesium sulfate. Solvent was evaporated in vacuo to obtain crude product which was purified by column chromatography using 5% MeOH in DCM as eluent to obtain the pure Amurensin H (48) (0.035 g, 0.077 mmol) in 67% yield.

\[ \delta 7.47 (d, J = 8.0 \text{ Hz}, 1H), 7.43 (d, J = 8.0 \text{ Hz}, 1H), 6.95 - 7.05 (m, 2H), 6.86 - 6.95 (m, 2H), 6.77 - 6.85 (m, 2H), 6.63 - 6.73 (m, 2H), 6.54 (s, 1H), 6.52 (d, J = 2.5 \text{ Hz}, 1H), 6.38 - 6.43 (m, 2H), 6.30 (d, J = 2.5 \text{ Hz}, 1H); MS (m/z): 453, 452, 451, 448, 357, 327; HRMS calcd for C_{28}H_{20}O_6: 452.12598, found 452.12696.

Preparation of 4-Chloro-6-(diethylamino)pyrimidine-5-carbaldehyde (77): To a solution of 4,6-dichloropyrimidine-5-carbaldehyde (76) (1.00 g, 5.65 mmol) in anhydrous THF (50 ml) was added diethylamine (0.413 g, 5.65 mmol) followed by triethylamine (0.572 g, 5.65 mmol) at room temperature under argon. Resulting reaction mixture was refluxed for 3 h with monitoring. After the completion of reaction, the reaction mixture was filtered and filtrate was evaporated. Residue was purified by column chromatography using 15% EtOAc:Hexane as eluent to obtain the product (77) (0.871 g, 4.08 mmol) in 72% yield. \[ \delta 10.29 (s, 1H), 8.27 (s, 1H), 3.50 (q, J = 6.8 \text{ Hz}, 4H), 1.18 (t, J = 7.2 \text{ Hz}, 6H); 13C-NMR (100 MHz) \delta 187.8, 164.4, 160.5, 157.6, 110.4, 45.4, 12.5. \]

Representative procedure for the preparation of 79a-c: To a solution of aldehyde (77) (0.500 g, 2.34 mmol) in anhydrous THF (25 ml) was added N-methyl-1-phenylmethanamine
(78a) (0.340 g, 2.81 mmol) followed by triethylamine (0.284 g, 2.81 mmol) at room temperature under argon. Resulting reaction mixture was refluxed for 5 h with monitoring. After the completion of reaction, the reaction mixture was filtered and the filtrate evaporated. Residue was purified by column chromatography using 20% EtOAc:Hexane as eluent to obtain the pure product (79a).

![Chemical Structure](attachment:image.png)

4-(Benzyl(methyl)amino)-6-(diethylamino)pyrimidine-5-carbaldehyde (79a)
Yield: 85%; $^1$H-NMR (400 MHz) $\delta$ 9.37 (s, 1H), 8.06 (s, 1H), 7.26 – 7.33 (m, 2H), 7.20 – 7.26 (m, 3H), 4.91 (s, 2H), 3.66 (q, $J = 6.8$ Hz, 4H), 3.09 (s, 3H), 1.26 (t, $J = 6.8$ Hz, 6H); $^{13}$C-NMR (100 MHz) $\delta$ 181.9, 166.3, 165.6, 158.6, 137.1, 128.8, 128.0, 127.6, 96.4, 55.4, 45.0, 40.2, 13.7.

![Chemical Structure](attachment:image.png)

4-(Dibenzylation1amo)-6-(diethylamino)pyrimidine-5-carbaldehyde (79b)
Yield: 82%; $^1$H-NMR (400 MHz) $\delta$ 9.36 (s, 1H), 8.14 (s, 1H), 7.20 – 7.35 (m, 6H), 7.09 – 7.19 (m, 4H), 4.85 (s, 4H), 3.54 (q, $J = 6.4$ Hz, 4H), 1.21 (t, $J = 7.2$ Hz, 6H); $^{13}$C-NMR (100 MHz) $\delta$ 181.9, 166.2, 165.5, 158.6, 136.9, 128.7, 127.9, 127.5, 97.6, 54.0, 44.9, 13.6.
4-(Bis(4-methoxybenzyl)amino)-6-(diethylamino)pyrimidine-5-carbaldehyde (79c)
Yield: 80%; $^1$H-NMR (400 MHz) $\delta$ 9.33 (s, 1H), 8.13 (s, 1H), 7.04 (d, $J = 8.4$ Hz, 2H), 6.81 (d, $J = 8.0$ Hz, 2H), 4.75 (s, 4H), 3.77 (s, 6H), 3.55 (q, $J = 6.92$ Hz, 4H), 1.21 (t, $J = 8.0$ Hz, 6H); $^{13}$C-NMR (100 MHz) $\delta$ 182.1, 166.2, 165.6, 159.2, 158.8, 129.5, 129.0, 114.2, 97.7, 55.5, 53.4, 45.0, 13.8.

Representative procedure for the cyclization reaction to prepare 74a-c: To a solution of aldehyde (79a) (0.25 g, 0.84 mmol) in freshly distilled, dry benzene (10 mL), P$_4$-t-Bu solution (0.92 mL, 0.92 mmol) was added at room temperature and reaction mixture was heated to reflux with monitoring (5 h). After the completion of reaction, benzene was partially evaporated and reaction mixture was purified by column purification using 10% ethyl acetate: petroleum ether to obtain the pure product (74a).

N,N-Diethyl-7-methyl-6-phenyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (74a)
Yield: 83%; $^1$H-NMR (400 MHz) $\delta$ 8.37 (s, 1H), 7.45 – 7.55 (m, 4H), 7.38 – 7.44 (m, 1H), 6.47 (s, 1H), 3.79 (s, 3H), 3.78 (q, $J = 8.0$ Hz, 4H), 1.33 (t, $J = 8.0$ Hz, 6H); $^{13}$C-NMR (100 MHz) $\delta$ 156.1, 152.7, 151.8, 137.2, 132.6, 129.3, 128.9, 128.3, 102.6, 101.2, 43.7, 30.3, 14.0.
7-Benzyl-N,N-diethyl-6-phenyl-7H-pyrrolo[2,3-d]pyrimidin-4-amine (74b)
Yield: 58%; $^1$H-NMR (400 MHz) $\delta$ 8.37 (s, 1H), 7.30 – 7.43 (m, 5H), 7.16 – 7.23 (m, 3H), 6.93 – 6.99 (m, 2H), 6.51 (s, 1H), 5.46 (s, 2H), 3.79 (q, $J$ = 8.0 Hz, 4H), 1.34 (t, $J$ = 8.0 Hz, 6H); $^{13}$C-NMR (100 MHz) $\delta$ 156.0, 152.7, 152.0, 138.4, 137.0, 132.4, 129.4, 128.7, 128.6, 128.3, 127.2, 126.7, 102.5, 101.8, 46.2, 43.5, 13.8.

N,N-diethyl-7-(4-methoxybenzyl)-6-(4-methoxyphenyl)-7H-pyrrolo[2,3-d]pyrimidin-4-amine (74c)
Yield: 50%; $^1$H-NMR (400 MHz) $\delta$ 8.36 (s, 1H), 7.26 (d, $J$ = 8.0 Hz, 2H), 6.90 (d, $J$ = 6.7 Hz, 4H), 6.73 (d, $J$ = 8.0 Hz, 2H), 6.42 (s, 1H), 5.36 (s, 2H), 3.83 (s, 3H), 3.77 (q, $J$ = 8.0 Hz, 4H), 3.73 (s, 3H), 1.32 (t, $J$ = 8.0 Hz, 6H); $^{13}$C-NMR (100 MHz) $\delta$ 159.8, 158.8, 155.9, 152.4, 151.8, 136.9, 130.8, 128.2, 124.9, 114.1, 114.0, 102.5, 101.3, 101.2, 55.6, 55.4, 45.6, 43.6, 13.9.

References


CHAPTER 2. Development of pyrido[2,3-d]pyrimidines: New effective inhibitors of the Abelson Kinase

Introduction

Protein kinases are a group of enzymes that possess a catalytic subunit which transfers a gamma-phosphate group from nucleotide triphosphate (often ATP) to one or more amino acid residues in a protein substrate side chain. This transfer results in conformational change in protein structure, affecting its function. These enzymes fall into two broad classes, characterized with respect to their substrate specificity: serine/threonine specific and tyrosine specific.¹

The structural features that can be recognized in all protein tyrosine kinases are an ATP binding site, three residues that are thought to be associated with the function of the third phosphate group (often called the gamma-phosphate group) of an ATP molecule bound to the enzyme, and a possible catalytic site of the enzyme that is an amino acid (Figure 1).²

Phosphorylation of tyrosine residues control a wide range of properties in proteins, such as enzyme activity, subcellular localization and interaction between molecules.³ Furthermore, tyrosine kinases function in many signal transduction cascades, wherein extracellular signals are transmitted through the cell membrane to the cytoplasm and often to the nucleus where gene expression may be modified.³ Finally, mutations can cause some tyrosine kinases to become constitutively active, a nonstop functional state that may contribute to the initiation or progression of cancer.

Figure 1
Tyrosine kinases function in a variety of processes, pathways, and actions, and are responsible for key events in the body. The receptor tyrosine kinases function in transmembrane signaling, while tyrosine kinases within the cell function in signal transduction to the nucleus. Tyrosine kinase activity in the nucleus involves cell-cycle control and properties of transcription factors. In this way, in fact, tyrosine kinase activity is involved in the induction of mitosis (mitogenesis) in a cell. Proteins in the cytosol and proteins in the nucleus are phosphorylated at tyrosine residues during this process. Cellular growth and reproduction may rely in some part on tyrosine kinase. Tyrosine kinase function has been observed in the nuclear matrix, which is not comprised of chromatin, but of the nuclear envelope and a “fibrous web” that serves to physically stabilize DNA.

Fibroblasts cells that synthesizes the extracellular matrix and collagen and are involved in wound healing - that have been transformed by the polyomavirus possess higher tyrosine activity in the cellular matrix. Furthermore, tyrosine kinase activity has been determined to be correlated to cellular transformation. It has also been demonstrated that phosphorylation of a middle-T antigen on tyrosine is associated with cell transformation, a change that is similar to cellular growth or reproduction.

The transmission of mechanical force and regulatory signals is fundamental in the normal survival of a living organism. Protein tyrosine kinase plays a role in this task too. A protein tyrosine kinase called pp125 is likely at hand in the influence of cellular focal adhesions, as indicated by an immunofluorescent localization of the said kinase.

Cellular proliferation, explained in some detail above, may rely in some part on tyrosine kinase. Tyrosine kinase function has been observed in the nuclear matrix. Lyn, the first kinase discovered in the nuclear matrix, is part of the Src family of tyrosine kinases, which can be contained in the nucleus of differentiating, calcium-provoked kertinocytes. Lyn, in the nuclear matrix, among the nuclear envelope and the “fibrous web” that physically stabilizes DNA, was found functioning in association with the matrix.

Yet another function of protein tyrosine kinase is that in the event of circulatory failure and organ dysfunction caused by endotoxin in rats, where the effects of inhibitors tyrphostin and genistein are involved with protein tyrosine kinase. It has become clear that tyrosine kinase can be involved in some unfortunate things.
Tyrosine kinase is also involved in signaling. Signals in the surroundings received by receptors in the membranes of cells are transmitted into the cell cytoplasm. Transmembrane signaling due to receptor tyrosine kinases relies heavily on interactions. An example is the mediation of the SH2 protein domain; it has been determined via experimentation that the SH2 protein domain selectivity is functional in mediating cellular processes involving tyrosine kinase. Receptor tyrosine kinases may, by this method, influence growth factor receptor signaling. This is one of the more fundamental cellular communication functions of metazoans.\(^5\)

However, tyrosine kinase activity is also involved in some unfavorable events; for instance, enhanced activity of the enzyme has been implicated in the derangement of the function of certain systems, such as cell division. Also included are numerous diseases regarding local inflammation such as atherosclerosis and psoriasis, or systemic inflammation such as sepsis and septic shock.\(^4\) In fact, the polyoma virus affects tyrosine kinase activity in the nuclear matrix.\(^3\) The polyoma virus attacks fibroblasts, which are a cell type involved in wound healing and cell structure formation in mammalian animals. Fibroblasts that are transformed by the polyoma virus involve higher tyrosine activity in the cellular matrix. In this way, it has been determined that tyrosine kinase activity is correlated to cellular proliferation.\(^3\) Moreover, tyrosine kinase can sometimes function incorrectly in a way that leads to non-small cell lung cancer.\(^6\) A common, widespread cancer, non-small cell lung cancer is the cause of death in more people than the total number of breast, colorectal, and prostate cancers altogether.\(^6\) Tyrosine kinases are particularly important today because of their implications in the treatment of cancer. A mutation that causes certain tyrosine kinases to be constitutively active has been associated with several cancers. Imatinib (brand names Gleevec and Glivec) is a drug able to bind the catalytic cleft of these tyrosine kinases, inhibiting its activity.\(^7\)

It is known among certain members of the scientific community that protein phosphorylation occurs on residues of tyrosine by both transmembrane receptor- and membrane-associated protein tyrosine kinases in normal cells.\(^2\) This occurrence is likely quite significant to the activity of communications that are originally broadcasted a number and variety of growth factors.\(^2\) This is evidenced by the observation that cells affected by the
Rous sarcoma virus, a retrovirus that causes sarcoma in chickens, display obvious structure modifications and a total lack of normal cell growth regulation.\(^2\) Rous sarcoma virus-encoded oncoproteins are protein tyrosine kinases that are the cause of and are required for this unfortunate cellular transformation.\(^2\) This is the case, that is, when the Rous sarcoma virus-encoded oncoproteins are expressed. Also, tyrosine phosphorylation activity increases or decreases in conjunction with changes in cell composition and growth regulation.\(^2\) In this way, a certain transformation exhibited by cells is dependent on a role that tyrosine kinase demonstrates.\(^2\) Protein tyrosine kinases have a major role in the activation of lymphocytes.\(^2\) In addition, they are functional in mediating communication pathways in cells types such as adrenal chromaffin, platelets, and neural cells.\(^2\)

Tyrosine kinase can become a radically functioning enzyme within an organism due to influences discussed, such as mutations and more. This behavior causes havoc and essential processes become disorganized. Systems on which the organism relies malfunction, resulting often in cancers. Of course, the possibility of preventing this type of circumstance is a highly desirable notion to those that are able to conduct related research. Much research has already noted the significant effect that inhibitors of the radically functioning protein tyrosine kinase enzymes have on related ailments. Encouragingly, research has been quite prolific in a number of different cases and in our case, we directed our efforts towards the role of substituted pyrido[2,3-\(d\)]pyrimidines as tyrosine kinase inhibitors.

Tyrosine kinase inhibitors have the potential to reduce the spread of chronic myeloid leukemia.\(^8\) Chronic myelogenous leukemia (CML) is a hematopoietic stem cell disease that accounts for 15% of all adult leukemia and is characterized by the clonal expansion of cells carrying the Philadelphia (Ph) chromosome.\(^9\) The Ph chromosome, resulting from the translocation of genes from chromosomes 9 and 22 encodes the chimeric protein Bcr-Abl.\(^9,10\) It is now well recognized that the Bcr-Abl protein is both the initial cause and major driver of CML. The rearranged Bcr-Abl gene encodes a constitutively active Abl kinase, meaning a kinase whose activity is constantly “on”. In simpler terms, in chronic myelogenous leukemia, the Abelson tyrosine kinase is improperly activated by the accidental fusion of the \(bcr\) gene with the gene encoding the intracellular non-receptor tyrosine kinase, \(c\)-Abl. This constitutive nature of Bcr-Abl is widely recognized by the scientific community and others as an
unfavorable characteristic because it results in excessive and uncontrolled proliferation of the myelogenous cells in which it is expressed.\textsuperscript{11} 

![Chemical structure of STI571](image)

\textbf{Figure 2}

It is possible that a tyrosine kinase inhibitor could be a viable option for the treatment of Bcr-Abl-caused chronic myeloid leukemia.\textsuperscript{12} In fact, tyrosine kinase activity is crucial to the role of transformation of Bcr-Abl, so inhibiting it would be likely method to improve cancer symptoms. STI571 also known as Imatinib Mesylate and marketed under the trade name Gleevec, is an inhibitor specific to the Bcr-Abl tyrosine kinase (Figure 2). After clinical trials, it was concluded that the well-tolerated inhibitor has a significant favorable effect on chronic myeloid leukemia activity in patients after failure of interferon alpha treatment.\textsuperscript{12} One can also infer, using the evidence in the report of the potential of tyrosine kinase inhibitor STI571 to reduce the spread of chronic myeloid leukemia, that the role of Bcr-Abl tyrosine kinase activity in the cancer’s progression is vital. STI571 is, at present, the first-choice treatment for patients with chronic myeloid leukaemia in chronic phase. Despite the impressive rate of complete haematological response and complete cytogenetical remissions, some cases show primary resistance or relapse after an initial response - secondary or acquired resistance. Thus, new drugs that inhibit STI571-resistant kinases are needed.\textsuperscript{13}
In a detailed study by Panek and coworkers, PD089828 (Figure 3) was reported as a prototype of a novel structural class of tyrosine kinase inhibitors, the 6-aryl-pyrido-[2,3-d]pyrimidines, that is distinguished from previously reported protein tyrosine kinase inhibitors by possessing a pyrido[2,3-d]pyrimidine structure. This compound, which was identified by screening a compound library with assays that measured protein kinase activity, is ATP competitive for platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR) and fibroblast growth factor receptor (FGFR) tyrosine kinases; is uniquely noncompetitive for c-Src tyrosine kinase and demonstrates prolonged inhibition of a variety of growth factor-mediated cellular functions whose effects are reversible. In conclusion, for the most part, PD089828 is an ATP-competitive inhibitor with broad tyrosine kinase inhibitory activity. But two limitations prevented it from being applied in a clinical setting. These are its extreme lack of solubility in aqueous media and lack of the ability to form soluble addition salts with strong acids. For these reasons, PD 089828 was unsuitable for intravenous (iv) administration in animal models of proliferative diseases. Furthermore, in vivo studies found PD 089828 to be poorly available after oral or intraperitoneal (ip) administration in rats. Therefore, further SAR studies based on PD 089828 were conducted which focused not only on potency and selectivity but also on improving the bioavailability of this novel lead compound.
Three regions of the parent molecule were targeted for initial SAR studies. Modifications made to the 2-, 6-, and 7-positions of the initial lead compound 2b were explored (Figure 4).\textsuperscript{15}

Scheme 1: Synthesis of PD 089828 and analogs

Scheme 1 shows the general synthetic route used to prepare 2b and related analogs 2a, c-f. The condensation of aldehyde 3 with an arylacetonitrile (4a-c) under basic conditions afforded the corresponding 2,7-diamino-6-arylpyrido[2,3-d]pyrimidine intermediate (5a-c). Treatment of 5a-c with sodium hydride in DMF followed by the addition of the designated isocyanate to the reaction mixture afforded the ureas 2a-c. Under these conditions, acylation occurred predominately at the 7-amino position. The 2-amino moiety of the diamine
intermediates $5b$ and $5c$ was directly displaced at high temperatures (140-180 °C) using the appropriate reacting amine as solvent and two equivalents of sulfamic acid to afford compounds $6a-c$. Yields for this reaction were generally in the range 50-80%. Weak nucleophilic amines such as aniline did not react under these conditions. Elaboration of $6a-c$ to the targeted analogs $2d-f$ was accomplished as described above in Scheme 1 using NaH in DMF followed by the addition of the appropriate isocyanate.\(^{15}\)

The effects of phenyl substitution on tyrosine kinase inhibition were then investigated. It was found that disubstitution at the ortho positions of the phenyl ring by small groups such as 2',6'-dichloro ($2b$) and 2',6'-dimethyl ($2c$) resulted in a general increase in tyrosine kinase inhibitory activity relative to the unsubstituted compound $2a$. It was reported that larger groups in the ortho position such as ethyl or methoxy resulted in decreased tyrosine kinase inhibitory activity across the panel of kinases tested. It was also observed that ortho substitution restricts the phenyl group to an orthogonal conformation with respect to pyrido[2,3-$d$]pyrimidine ring. To improve the poor aqueous solubility of $2b$, several sites on the molecule were targeted for attaching aminoalkyl side chains. Unexpectedly, the 3-(diethylamino)propyl side chain of compound $2d$ was found to afford enhanced tyrosine kinase inhibitory activity activity for the PDGFr, FGFr, and c-src tyrosine kinases as well as improved aqueous solubility relative to the lead compound $2b$. Also, the incorporation of an 2-alkylamino side chain generally resulted in enhanced TKI potency, aqueous solubility, and bioavailability relative to the parent compound $2b$.\(^{15}\)

\[\text{Figure 5}\]

A related structure, 2-amino-6-(2,6-dichlorophenyl)-pyrido[2,3-$d$]pyrimidin-7(8$H$)-one ($7a$, Figure 5), which was also derived from mass screening, showed a similar profile toward the above kinases with micromolar inhibitory activity.\(^{16}\) Although both $2b$ and $7a$
possess the same core pyrido[2,3-d]pyrimidine ring system, Klutchko and coworkers expected that a systematic SAR development of both leads would generate unique profiles with respect to inhibitory activity, bioavailability, metabolism, and selectivity. They have reported detailed syntheses and structure activity relationships (SAR) toward several tyrosine kinases for a series of analogues of 7a. A wide range of alkyl and aryl substituents have been introduced at the C-2 nitrogen (Figure 5) while keeping the substituent at the N-8 nitrogen either as methyl or ethyl. They also reported cellular effects, in vivo anticancer activity, animal bioavailability, and metabolism for selected compounds drawn from this series.

![Scheme 2: Synthesis of new PD derivatives](image)

The synthesis of lead structure 7a and its C-2 acetamide derivative 7c is shown in Scheme 2. Hydrolysis of readily available 2,7-diaminopyridopyrimidine 5b in refluxing concentrated HCl provided the pyrimidin-7-ol in poor yield (19%) following recrystallization from N,N-dimethylformamide. N-8 methylation to give 7a was achieved in 49% yield with iodomethane in the presence of NaH in DMF at 60-80 °C for three hours. Heating 7a in refluxing acetic anhydride for twenty minutes produced the C-2 acetamide 7c in 61% yield. While suitable for making some simple C-2 amino analogues for initial SAR studies, the route outlined in Scheme 2 was too inefficient for generating a wide range of more elaborate analogs.
Scheme 3: Synthesis of PD precursors

A more expedient route for synthesizing C-2 amino analogues of 7a was developed wherein the amine could be introduced in the last step by displacement of either a C-2 methyl sulfide, sulfoxide, or sulfone leaving group (Schemes 3 and 4). Thus, as shown in Scheme 3, room temperature condensation of commercially available 5-pyrimidinecarboxylic acid ester 8 with either methyl- or ethylamine in THF provided 9a-b, which was reduced under standard reduction conditions to give benzylic alcohol 10a-b in high overall isolated yield. Oxidation with MnO₂ proceeded cleanly to the 5-pyrimidinecarboxaldehydes 11a-b, which were then condensed with 2,6-dichlorophenylacetonitrile in DMF at 105 °C in the presence of anhydrous K₂CO₃ as base to provide the 7-imino-pyridopyrimidine product 12a-b in 50% yield. The use of more strongly basic conditions was avoided to keep the somewhat base sensitive methylthio group intact. Imine 12a-b was first acetylated and then hydrolyzed to provide key intermediate 2-methylthio-7-oxo-pyridopyrimidine derivatives 13a-b.¹⁶
As detailed in Scheme 4, oxidation of 13a-b with 3-phenyl-2-(phenylsulfonyl)-oxaziridine in chloroform provided the sulfoxide 14 in 70% yield, whereas oxidation with two equivalents of m-chloroperbenzoic acid under similar conditions gave the corresponding sulfone 15a-b in 92% yield. The sulfone 15a was quite reactive and could readily be hydrolyzed to the 2-hydroxy compound 16 in refluxing aqueous acetic acid. The choice of intermediate 13, 14, or 15 for subsequent amine condensations was based on the relative reactivity of the leaving group and that of the amine to be introduced at the C-2 position. For example, condensations with more nucleophilic alkylamines were best carried out on sulfides 13a-b, whereas the less reactive arylamines were best condensed with the more reactive sulfoxide 14 or sulfone 15a-b. Generally, the reaction conditions for amine displacement involved heating the sulfide, sulfoxide, or sulfone with a 20-100% excess of amine either neat or diluted with a solvent at temperatures of 100 - 250 °C.\textsuperscript{16}

In summary, the initial SAR survey revealed that while C-2 amino analogues with chain extended aliphatic dibasic moieties improved aqueous solubility and potency relative to lead structure 7a, the introduction of simple arylamino moieties affected the potency much more dramatically. Since there appeared to be little, if any, increase in potency for N-8 ethyl
versus methyl substitution, further SAR studies within this series were continued with methyl at this position. Having established the essential requirement of C-2 arylamino substitution, a series of analogues with simple position substitution around the aniline-ring were explored to determine the effect on SAR. Relative to unsubstituted aniline-derivative 17 (R = H), mono-substitution of the aniline ring with simple electron-withdrawing or electron-donating group generally deceased the potency of these compounds towards most of the kinases tested. Disubstituted anilino compounds and mono-substituted compounds with larger substituents in the 3’- or 4’-positions showed the same general pattern of tyrosine kinase inhibition toward the PDGFr, FGFr, and c-Src kinases relative to unsubstituted aniline-derivative 17 (R = H). On the other hand, analogues possessing either phenolic (17, R = OH) or hydroxymethyl (17, R = -CH₂OH) functionality at these positions displayed potency generally equivalent to or better than 17 (R = H) toward the kinases profiled. The marked enhancement in potency for 17 (R = -CH₂OH), possessing improved aqueous solubility over the parent N-aryl compound 17 (R = H), argued for additional SAR development around the 3’- and 4’- positions with analogues that would impart even greater aqueous solubility. Compounds incorporating various cationic (amine) or anionic (acid) moieties to test this concept were developed and tested.¹⁶

Figure 6: Comparison PD derivatives and STI571 growth inhibition of Bcr-Abl cell line
Various comparative studies of substituted pyrido[2,3-d]pyrimidines with STI571 (1) resulted in the development of compounds that vary in their specificities for different tyrosine kinases and were more potent than STI571 (1) (Figure 6).\textsuperscript{17} Among these potential drug candidates, PD173955 (20), shown in Figure 6, was the most promising. PD173955 (20) inhibits the Abelson kinase (Abl) and the Src and Yes tyrosine kinases that are also often up-regulated in cancer.\textsuperscript{18} PD173955 inhibits the proliferation of many cancer cell types, but two limitations prevent it from being applied in a clinical setting. These are its ability to inhibit kinases other than the Abl kinase, which results in toxicity for proliferating normal cells\textsuperscript{19} and its low solubility in water. In its favor, its affinity is much lower for most kinases other than those of the Src family of which Abl is a member, and PD173955 inhibits both the active and inactive forms of Abl. By contrast, Imatinib (1) only inhibits the active form of the enzyme. In addition, the Ki for inhibition of Abl by PD173955 is very low, making it a more potent inhibitor of Abl and a more effective inhibitor of cancer cell proliferation than Imatinib.\textsuperscript{20, 22, 24} Thus, we speculated that, with further modification, PD173955 may be developed as an effective inhibitor of c-Abl to treat patients with CML and other cancers, including those that have developed Imatinib-resistance.

**Results and Discussion**

Clarkson and Duyster showed that 20 (Figure 7) and related compounds inhibit Bcr-Abl kinase activity with greater potency than Imatinib. Moreover, many of these compounds also inhibit kinase domain mutants of Bcr-Abl that are resistant to Imatinib.\textsuperscript{21} With the emerging need for kinase inhibitors that will kill cells that express Imatinib-resistant Bcr-Abl kinase domain mutants, PD173955 is a prime candidate for further development.

![Figure 7](image-url)
To investigate the structural elements of PD173955 that make it a good inhibitor of the Abl kinase, we planned to synthesize analogs of 20 (Figure 7). As reported earlier, the bicyclic ring and the halogen containing ring subunit of PD173955 interact with several amino acid residues in the crystal structure of the kinase complex and the orthogonal relationship between ortho-substituted phenyl ring and pyrido[2,3-d]pyrimidinone is important towards the activity of these compounds.\textsuperscript{22} We decided to conduct structure-activity relationship by varying the ortho-substitution on phenyl ring, thereby varying the orthogonal relationship. As discussed below, many analogs of 24 were also synthesized. These adducts were termed ‘PDC’ because of the extra carbon atom between the phenyl group and the secondary amino group (Figure 7). These were also expected to be good inhibitors of the Abl kinase because the crystal structure of Abl with PD173955 shows the methylthiophenyl ring extending into the solution space and not interacting extensively with the protein.\textsuperscript{22}

Scheme 5: Synthesis of PD precursor 11a

Scheme 5 outlines the synthesis of common intermediate 11a which started with the room temperature condensation of commercially available 5-pyrimidinecarboxylic acid ester 8 with aqueous methylamine in THF to provide 9a in 95% yield. Ester 9a was reduced under standard lithium aluminum hydride reduction conditions to give alcohol 10a in 94% yield.
Oxidation of alcohol 10a with activated manganese dioxide proceeded cleanly to 5-pyrimidinecarboxaldehyde 11a in 81% yield.\(^\text{16}\)

![Scheme 6: Synthesis of PD precursor 13a](image)

The condensation of aldehyde 11a with 2,6-dichlorophenylacetonitrile in DMF at 105 °C in the presence of anhydrous potassium bicarbonate as the base provided 7-imino-pyridopyrimidine product 12a. This reaction was hampered by problems related to solubility and low yields (Scheme 6). Subsequent acetylation and hydrolysis were also inefficient probably due to impure product formed at the previous step. At this stage, we looked for different and more efficient ways to assemble the basic skeleton of pyrido[2,3-d]pyrimidine 13a.

![Scheme 7: New method to prepare pyrido[2,3-d]pyrimidines](image)

Ensuing literature search resulted in a publication from Blass et al. reporting a facile, potassium fluoride/alumina mediated method for the preparation of functionalized pyrido[2,3-d]pyrimidines (Scheme 7).\(^\text{23}\) Their methodology used solid supported reagents and possessed the advantages of both solution and solid phase chemistry. Like solid phase synthesis, excess support bound reagent can be used and removed by filtration, avoiding cumbersome aqueous work-ups and decreasing solvent waste issues. Also, the products can
be isolated by filtration and removal of the solvents, eliminating the need for a cleavage step that is required in solid phase syntheses. Additional benefits included taking advantage of the strongly basic nature of potassium fluoride/alumina, which has allowed it to replace organic bases in a number of reactions including, but not limited to, selective N-alkylation of amides, epoxidation, diazetization, Sonogashira couplings, Suzuki couplings, Knoevenagel reactions, and Horner-Emmons chemistry.\textsuperscript{23} As explained earlier, one of the primary methods to prepare the requisite pyrimidinone scaffold core for this class of compounds is a tandem Knoevenagal and amide/ester exchange reaction between the functionalized pyrimidine aldehyde \textsuperscript{11} and a suitable phenyl acetic acid derivative. When these reactions were performed using standard bases, such as potassium carbonate, the yield and purity of the crude products was moderate at best and extended reaction times were required.\textsuperscript{16} Blass, however, reported that the application of potassium fluoride/alumina led to a substantial improvement in the overall yield and purity of the desired final products as well as significantly shorter reaction times.\textsuperscript{23} This pathway also reduced the number of steps required to synthesize pyrido[2,3-\textit{d}]pyrimidines (22) as acetylation and hydrolysis were not needed with this methodology unlike before (Scheme 7).

![Scheme 8: Synthesis of PD precursor 23]

<table>
<thead>
<tr>
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</table>

\textsuperscript{*}based on recovered starting material

Table 1: Summary of synthetic results
With the availability of this new methodology, we synthesized analogues of 22. Condensation of aldehyde 11a with ethyl-2,6-dichlorophenyl acetate was accomplished with KF/Al₂O₃ and dimethyl acetamide at room temperature to give functionalized pyrido[2,3-d]pyrimidinone 22a in poor yield. The main reason for the poor yield is lack of solubility of compound 22a in usual solvents. The yield of 22a was drastically increased when column chromatography was replaced by recrystallization from N,N-dimethylformamide as a way to purify the product. Other analogs (22b-e) were synthesized in excellent yields and were purified by usual column chromatography (Scheme 8, Table 1). Oxidation of 22a-e with two equivalents of meta-chloroperbenzoic acid in chloroform gave corresponding sulfones 23a-e in excellent yields (Scheme 8, Table 1).

![Scheme 9: Synthesis of PD and PDC analogs](image)

<table>
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<tr>
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<th>Y</th>
<th>R</th>
<th>Yield (%)</th>
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<td>Cl</td>
<td>NH₂</td>
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</tbody>
</table>

Table 2: Synthesis of PD and PDC analogues

Initially, the conversion of sulfone 23a into analogs of PD173955 using various anilines in boiling aprotic solvents failed. Interestingly, the reaction of sulfone 23a with 3-aminobenzylamine which is a more reactive nucleophile, in boiling DMF generated the PDC
adduct 24a in good yield (Scheme 9; Table 2 – entry 1). PD173955 (20) and its analog 25 were ultimately synthesized by reacting sulfone 23a with appropriate aniline derivative, using boiling diglyme as the solvent in a sealed tube, conditions much harsher than those for the synthesis of the PDC and its analogs (Scheme 9). The PDC adduct 24a was more soluble than PD173955 in aqueous solutions. Our interest in understanding the effect of changes in the PD structure on its ability to inhibit Abl kinase led us to synthesize various PD and PDC analogs 24a-f, 20 and 25 (Table 2). The reason to develop the analogs 26 and 27 was to circumvent the problem of low chemical reactivity of aromatic amine moieties of analogs 24a and 25. The synthetic route towards the synthesis of 26 and 27 consist of standard two step sequence where 24a and 25 were first reacted with bromoacetyl bromide under basic conditions followed by the treatment with ammonia to get 26 and 27 in good yields (Scheme 10).

These compounds were tested for Abl-kinase inhibition in Dr. Marit Nilsen-Hamilton’s laboratory, our collaborators in the biochemistry, biophysics and molecular biology department at Iowa State University. The results of inhibition of Abl kinase (Ki) are shown below in Figures 8 – 10. The number in parentheses after the Ki is the number of independent experiments performed to obtain the Ki. The asterisks represent the p values determined from the Pearson product moment correlation analysis (***, p<0.0005, **, p<0.005, *, p<0.03)
The 2,6-dichlorophenyl substituent 24a exhibited the strongest inhibition.

The activity of 20 and adducts 25 and 27 was tested using the standard protocol and the results are shown below.

Compounds 24a-e were synthesized to probe the steric and electronic influences on the aryl ring. Molecular models show that the 2,6-dichlorophenyl group in 20 is orthogonal to the pyrido[2,3-d]pyrimidine subunit as a result of nonbonded interactions of the chlorine atoms. The effectiveness with which c-Abl was inhibited by the synthesized analogs was determined from the Ki for each compound, which increases with decreasing inhibitory effectiveness. As shown above, compared with the 2-chlorophenyl substituent, the monochlorophenyl and phenyl substituents have increasingly higher Ki values. The 2,6-difluorophenyl substituent (24d), which is sterically the same size as the phenyl group, has about the same inhibitory efficiency as PDC with the 2-chloro (24b) and 2-bromo (24e)
substituents. Structural analysis of the interaction of PD173955 with the Abl kinase identified
the chloro substituents of the aryl ring as being embedded in the enzyme’s ATP-binding
pocket and held with multiple van der Waals interactions, which contribute to the affinity of
the molecule for the Abl kinase. The chlorine atoms limit the rotation of the aryl ring to a
rotational angle that is the same as for the central phenyl group of Imatinib. These phenyl
groups of Imatinib and PD173955 sit in the same position, similarly rotated, in the protein.
The removal of one or both chlorine atoms from 24a or their replacement with fluoro
substituents would allow rotational flexibility of the compound when not bound to the
enzyme and might increase the Ki compared with 24a by increasing k_{off} in the equilibrium
shown below due to increased entropy of the unbound 24a.

\[
\begin{align*}
\text{Abl} + \text{PD} & \xrightleftharpoons[k_{off}]{k_{on}} \text{Abl-PD} \\
\end{align*}
\]

Figure 11

The much higher Ki of 24c compared with 24b, 24d, or 24e suggests that electronic
effects also play an important role in the interaction of PDC with the enzyme. It is likely that
the relevant interacting amino residue might be lys271 that lies close enough to the inhibitor
in the enzyme pocket to interact with PD173955 (and presumably also PDC) through van der
Waals forces as described for the crystal structure, but which might also be close enough to
interact electrostatically with the aryl ring substituents through its epsilon amino group.

Although most van der Waals interactions reported for PD173955 with Abl are
located around the bicyclic substituent and the dichlorophenyl group, two of the ten
interactions are with amino acids that contact the methylthiophenyl segment of PD173955.
Alteration of the molecular structure in this region by insertion of an additional carbon
between the secondary amino group and the aryl groups resulted in an increase in Ki of about
200-fold, which is consistent with the loss of 2 of the 10 van der Waals interactions that hold
the inhibitor in place in the protein’s ATP-binding pocket. The 10-fold decrease in the Ki
with the replacement of the methylthio group by an amino group on either PD or PDC
probably reflects the gain in van der Waals or an electrostatic interaction with the enzyme by way of the amino group.

In summary, the effects of substituents on the aryl ring were studied by the preparation and testing of several PD173955 analogs. The results are consistent with the observation from the crystal structure that electronic and van der Waals forces are likely to be involved in the interaction of inhibitor and enzyme. They also show that inserting a single carbon atom into the C-N bond in the aniline subunit (PDC) reduced the kinase inhibition by a factor of 200, consistent with the loss of 20% of the van der Waals interactions between inhibitor and protein. Despite its decreased affinity for Abl compared with PD, PDC (24a) and PDC-Gly (26) exhibits a Ki very similar to that reported for Imatinib \(4 \times 10^{-8}\) and is significantly more water soluble compared with PD173955. Furthermore, replacing the thiomethyl group on PD173955 with either an amino (25) or a glycyl (27) group resulted in a decrease in the Ki of 10-fold, which is 1000-fold lower than the Ki reported for Imatinib.

![Figure 12: Structure of Cyanin dyes Cy3 and Cy5](image)

Since PDC-Gly 26 exhibit a Ki similar to that of Imatinib (1), we next decided to tag this compound with fluorescent dyes and use it for molecular imaging. The use of fluorescent dyes in biology is ever-increasing and is the basis for many advancements in science; for example, the sequencing of human genome.\(^25\) The fluorescent dyes short-listed for the imaging were cyanine dyes Cy3 (28a) and Cy5 (29a) (Figure 9). A generic cyanine dyes...
consists of a conjugated system based on a polymethine chain linking two nitrogen-containing heterocycles (e.g., indoles, benzothiazoles). They are generally named based on the number of carbon atoms in the polymethine chain. Trimethine and pentamethine dyes exhibit absorption maxima at 550 and 650 nm, respectively, and emission maxima around 570 and 670 nm, respectively, in the green or red part of the spectrum. Their spectroscopic and photophysical properties do not change significantly after covalent attachment to various molecules. These dyes are suitable for the imaging of single molecules in living cells and are useful reagents for the labeling of proteins and peptides. The application of cyanine dyes as donors/acceptors in fluorescence resonance energy transfer (FRET) based methods is especially popular. The dyes are generally converted into their N-hydroxysuccinimide esters for attachment to the proteins and peptides.

Scheme 11: Synthesis of common intermediates.

Our synthetic scheme for these dyes was based on the work done by Jung and coworkers. The synthesis of the Cy3 dye and Cy5 dye and their NHS esters respectively, began with the synthesis of common intermediates (Scheme 11). Commercially available trimethylindole was alkylated with methyl iodide in chloroform under sealed tube conditions to give the 1,2,3,3-tetramethylindolium iodide in 65% yield.
A similar reaction of trimethylindole 30 to give 5-carboxypentyl-2,3,3-trimethylindolium bromide 32 was done by heating the indole 30 with 6-bromohexanoic acid at 120 °C under neat conditions to get the addition product 32 in 78% yield (Scheme 11). Conversion of 1,2,3,3-tetramethylindolium iodide 31 to Fischer’s base 33 was accomplished in 61% yield by stirring 31 with 1.0 M potassium hydroxide solution at ambient temperature (Scheme 11).

Scheme 12: Synthesis of Cy3 dye and its NHS ester

Condensation of Fischer’s base 33 with commercially available N,N’-diphenylformamidine (37) in the presence of excess acetic anhydride as solvent was tried under various conditions, but acetanilidylvinyl indolium salt 38 couldn’t be prepared
Finally, we were able to prepare acetonilidylvinyl indolium salt 38 by condensing tetramethylindolium iodide 31 with N,N’-diphenylformamidine under refluxing conditions in the presence of acetic anhydride and few drops of acetic acid (Scheme 12). This compound was then reacted with the carboxypentylindolium salt 32 in ethanol in the presence of triethylamine to give the desired Cy3 dye 28a in 61% yield as a red powder. The dye was easily converted into the N-hydroxysuccinimide ester (NHS) by treatment with N,N-disuccinimidyl dicarbonate (DSC) in the presence of pyridine to give the activated dye 28b in 72% yield. All the pertinent spectroscopic data, especially NMR and mass spectrometry, were in agreement with the previously reported synthesis and structures assigned.\(^{25}\)

Scheme 13: Synthesis of Cy5 dye and its NHS ester

For the synthesis of the Cy5 dye 29a and its NHS ester 29b (Figure 12), the three-carbon spacer 35 was prepared (Scheme 11). Condensation of commercially available malondialdehyde bis(dimethyl acetal) with aniline under acidic conditions afforded the
anilino anilinium salt 35 in 85% yield (Scheme 11). The reaction of 35 with Fischer’s base 33 in refluxing acetic acid afforded the anilinobutadienyl salt 36 in 72% yield (Scheme 13). Finally, the reaction of the activated indolium salt 36 with the carboxypentylindolium salt 32 in ethanol in the presence of sodium acetate afforded the desired Cy5 dye 29a in 63% yield as a blue powder (Scheme 13). The dye was easily converted into the N-hydroxysuccinimide ester (NHS) by treatment with commercially available N,N’-disuccinimidyl dicarbonate (DSC) in the presence of pyridine to give the activated dye 29b in 78% yield (Scheme 13). Again, all spectroscopic data, especially NMR and mass spectrometry, were in agreement with the previously reported synthesis and structures assigned.

Activated Cy3-NHS ester (28b) was then reacted with previously developed abelson kinase inhibitor PDC-Gly (26) under basic conditions using N,N-diisopropylethylamine in N,N′-dimethylformamide at 60 °C to obtained fluorescent tagged compound 39 in 73% yield (Scheme 14). Similarly, compound 26 was tagged with activated Cy5-NHS ester (29b) under similar conditions to get the fluorescent tagged compound 40 in 75% yield (Scheme 14).
The synthetic route for the synthesis of Cy3 and Cy5 was fairly. However, the preparation of acetonilidylvinyl indolium salt 38 by condensing tetramethylindolium iodide 31 with N,N’-diphenylformamidine under refluxing conditions was the most difficult and limiting step as it was not reproducible every time (Scheme 12). In order to side-step this problem, we turned our attention to another Cy3 dye (41a, Scheme 15). A special structural characteristic of this Cy3 dye 41a was its symmetrical design due to the presence of two carboxypentyl side chains (Scheme 15). A major synthetic simplification was achieved due to this design as now, instead of condensing two different indoleium salts, we condensed carboxypentylindolium salt 32 with itself resulting in the reduction number of steps. The two common pathways used to synthesize this dye 41a are outlined in Scheme 15. In first pathway, carboxypentylindolium salt 32 was treated with N,N’-diphenylformamidine (37) in the presence of acetic anhydride, acetic acid and pyridine under reflux conditions to obtain
the Cy3 dye 41a in 68% yield. In other and still simpler pathway, carboxypentylindolium salt 32 was refluxed in the presence of triethylorthoformate in pyridine to give Cy3 dye 41a in 70% yield. Both pathways were easily reproducible with consistent yields and analytical data. This new Cy3 dye 41a was activated by converting it into the N-hydroxysuccinimide ester (NHS) by the usual method of treatment with N,N-disuccinimidyl dicarbonate (DSC) in the presence of pyridine to give the activated dye 41b in 72% yield (Scheme 15).

In order to explore and standardize the reactivity of this new Cy3-NHS ester 41b, we treated it with 1,6-hexanediame under usual basic conditions (Scheme 17). To our surprise, we recovered a twenty seven membered macrocycle 42 in excellent yield. To confirm this, we tried another experiment where instead of condensing Cy3-NHS ester 41b with equimolar amount of 1,6-hexanediame, we used excess diamine and even under these conditions, the only product obtained was twenty seven membered macrocycle 42. In order to check the scope of this novel method to form macrocycles, we treated Cy3-NHS ester 41b with commercially available meta-xylenediamine and para-xylenediamine. Both reactions successfully resulted in the formation of macrocycles 43 and 44 in 69% and 64% yield respectively (Scheme 17).
These results, although surprising, presented us with an opportunity to develop an entirely new Cy3 dye in the form of a macrocycle with a handle in order to attach it to various biomolecules (Figure 13).
Figure 13: Cy3 dye macrocycle with handle

We envisioned a bis-aminobenzoate type compound as it would serve the purpose by having an appropriate spacer as well as the handle attached on it. Scheme 18 outlines the synthesis of methyl-3,5-bisaminomethyl benzoate salt 50 starting from the commercially available 3,5-dimethylbenzoic acid 46. Acid 46 was converted to its methyl ester 47 by refluxing it in methanol under acidic conditions. Ester 47 was then converted to bis-bromo ester 48 using typical N-bromosuccinimide conditions, which in turn, was converted to azide 49 by the treatment with sodium azide. Azide 49 was reduced to amine and converted to ammonium salt 50 in one-pot reaction using triphenylphosphine.\(^{30}\)
With the appropriate ammonium salt 50 in hand, we reacted it with Cy3 dye 41b using excess base to successfully obtained the new Cy3 macrocycle based dye 45 in 67% yield (Scheme 19).
In another example, activated Cy3 41b was reacted with commercially available L-lysine methyl ester dihydrochloride to obtain second example of Cy3 macrocycle 51 with a handle in 65% yield (Scheme 20).

Scheme 20: Synthesis of the new Cy3 dye macrocycle

In conclusion, we successfully developed methods to tag biologically active molecules with various cyanin dyes for fluorescent imaging. We also improved the synthetic method for Cy3 dye by reducing the number of steps required as well as standardizing the reaction conditions. We were able to synthesize uncommonly large macrocycles 42 - 44 based on Cy3 dye and succeeded in utilizing this method to invent new Cy3 dyes 45 and 51 with a handle which can be used to tag various peptides and proteins.

Experimental

Unless otherwise noted, materials were obtained from commercial suppliers and used without purification. Tetrahydrofuran and diethyl ether were distilled from sodium and benzophenone. Dichloromethane, benzene and diisopropyl amine were distilled over calcium hydride. All experiments were performed under an argon atmosphere unless otherwise noted. Organic extracts were dried over anhydrous magnesium sulfate. Nuclear magnetic resonance experiments were performed with either a Varian 300 MHz or Bruker 400 MHz instrument. All chemical shifts are reported relative to CDCl3 (7.27 ppm for 1H and 77.23 ppm for 13C),
unless otherwise noted. Coupling constants \((J)\) are reported in Hz with abbreviations: \(s = \) singlet, \(d = \) doublet, \(t = \) triplet, \(q = \) quartet, \(m = \) multiplet. High resolution mass spectra were recorded on a Kratos model MS-50 spectrometer. Standard grade silica gel (60 Å, 32-63 μm) was used for flash column chromatography.

**Representative procedure for the preparation of 22a-e**

To a stirred solution of 0.400 g (2.19 mmol) of 4-amino-2-(methylthio) pyrimidine-5-carbaldehyde 11a and 0.638 g (2.74 mmol) of ethyl 2-(2,6-dichlorophenyl)acetate 21a in 10.0 mL of dry DMA, 2.536 g of KF/Al₂O₃ (40 wt %, KF, Sigma–Aldrich catalog #316385) was added in batches of 0.5 g every 30 min. The reaction mixture was then stirred at RT for 24 h under argon after which it was filtered through Celite, the residual solid was washed with methylene chloride and the combined filtrates were concentrated. Due to the poor solubility of dichloropyridopyrimidinones derivative 5a in most solvents, it was purified by recrystallization from DMF to obtain 0.480 g (62%). Other pyridopyrimidinones were purified by column chromatography over silica gel using 1:1 hexanes:EtOAc to give pure products.

6-(2,6-dichlorophenyl)-8-methyl-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (22a).

Yield 62%; \(^1\)H-NMR (400 MHz, CDCl₃): \(\delta\) 8.66 (s, 1H), 7.60 (s, 1H), 7.41 (d, \(J = 8.0\) Hz, 2H), 7.29 (d, \(J = 8.0\) Hz, 1H), 3.83 (s, 3H), 2.67 (s, 3H); \(^13\)C-NMR (100 MHz, CDCl₃): \(\delta\) 173.9, 161.1, 156.7, 154.6, 136.1, 135.6, 133.9, 130.4, 129.7, 128.3, 109.4, 28.7, 14.8; LRMS: 352.0, 200.0, 154.1; HRMS calculated \(M^+\) for \(C_{15}H_{11}Cl_2N_3OS\): 352.0073; found: 352.0074.
6-(2-chlorophenyl)-8-methyl-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (22b).
Yield 73%; $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 8.63 (s, 1H), 7.64 (s, 1H), 7.42 - 7.49 (m, 1H), 7.27 - 7.37 (m, 3H), 3.80 (s, 3H), 2.65 (s, 3H); $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ 173.5, 161.8, 156.5, 154.4, 135.0, 134.8, 133.8, 131.7, 131.5, 130.0, 129.9, 126.9, 109.5, 28.6, 14.7; LRMS: 318.0; HRMS calculated M$^+$ for C$_{15}$H$_{12}$ClN$_3$OS: 317.0390; found: 317.0396

8-methyl-2-(methylthio)-6-phenylpyrido[2,3-d]pyrimidin-7(8H)-one (22c).
Yield 89%; $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 8.61 (s, 1H), 7.67 (s, 1H), 7.61 - 7.66 (m, 2H), 7.34 - 7.45 (m, 3H), 3.79 (s, 3H), 2.63 (s, 3H); $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ 172.8, 162.4, 156.3, 153.9, 135.6, 132.8, 132.6, 128.9, 128.7, 128.4, 109.9, 28.5, 14.6; LRMS: 284.1, 176.1, 158.1; HRMS calculated M$^+$ for C$_{15}$H$_{13}$N$_3$OS: 283.0779; found: 283.0778

6-(2,6-difluorophenyl)-8-methyl-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (22d).
Yield 92%; $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 8.64 (s, 1H), 7.72 (s, 1H), 7.30 - 7.40 (m, 1H), 6.93 - 7.04 (m, 2H), 3.81 (s, 3H), 2.64 (s, 3H); $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ 173.9, 162.0 (d, $J = 28.0$ Hz), 161.2, 159.5 (d, $J = 28.0$ Hz), 156.7, 154.5, 136.9, 130.6 (t, $J = 40.0$ Hz), 122.4, 111.7 (d, $J = 100.0$ Hz), 109.3, 28.7, 14.7; LRMS: 320.1; HRMS calculated M$^+$ for C$_{15}$H$_{11}$F$_2$N$_3$OS: 319.0591; found: 319.0591.
6-(2-bromophenyl)-8-methyl-2-(methylthio)pyrido[2,3-d]pyrimidin-7(8H)-one (22e).
Yield 83%; $^1$H-NMR (400 MHz, CDCl₃): $\delta$ 8.63 (s, 1H), 7.65 (d, $J$ = 8.0 Hz, 1H), 7.61 (s, 1H), 7.29 – 7.40 (m, 2H), 7.21 – 7.28 (m, 1H), 3.80 (s, 3H), 2.64 (s, 3H); $^{13}$C-NMR (100 MHz, CDCl₃): $\delta$ 173.5, 161.8, 156.6, 154.5, 136.9, 135.0, 133.3, 133.1, 131.6, 130.2, 127.6, 123.9, 109.5, 28.7, 14.8; LRMS: 364.0, 362.0; HRMS calculated M⁺ for C$_{15}$H$_{12}$BrN$_3$O$_3$: 360.9884; found: 360.9876.

Representative procedure for the preparation of 23a-e

To a solution of 0.500 g (1.42 mmol) of 22a in 40 mL of chloroform, was added 0.800 g (3.12 mmol) of 75% m-chloroperoxybenzoic acid. The solution was stirred at RT for 6 h. After the completion of reaction, 2 mL of DMSO was added to the reaction mixture to neutralize unreacted m-chloroperoxybenzoic acid and it was further stirred for 15 minutes after which the reaction mixture was diluted with methylene chloride and washed with saturated NaHCO₃, water and brine. The organic phase was dried over anhydrous MgSO₄ and concentrated. The crude was used for the next step without further purification.

6-(2,6-dichlorophenyl)-8-methyl-2-(methylsulfonyl)pyrido[2,3-d]pyrimidin-7(8H)-one (23a). Yield 87%; $^1$H-NMR (300 MHz, CDCl₃): $\delta$ 9.03 (s, 1H), 7.78 (s, 1H), 7.42 – 7.48 (m, 2H), 7.31 – 7.38 (m, 1H), 3.91 (s, 3H), 3.44 (s, 3H); $^{13}$C-NMR (100 MHz, CDCl₃): $\delta$ 165.0, 160.5, 157.5, 155.5, 135.2, 135.0, 134.7, 132.9, 131.0, 128.4, 115.0, 39.5, 29.5; LRMS: 352.0, 200.0, 154.1; HRMS calculated M⁺ for C$_{15}$H$_{11}$Cl$_2$N$_3$O$_3$S: 383.9971; found: 383.9970
6-(2-chlorophenyl)-8-methyl-2-(methylsulfonyl)pyrido[2,3-d]pyrimidin-7(8H)-one (23b).
Yield 85%; $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 9.00 (s, 1H), 7.82 (s, 1H), 7.47 – 7.53 (m, 1H), 7.33 – 7.43 (m, 3H), 3.89 (s, 3H), 3.43 (s, 3H); $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ 164.6, 161.2, 157.3, 155.2, 136.6, 133.8, 133.7, 133.5, 131.3, 130.7, 130.1, 127.1, 115.1, 39.4, 29.4; LRMS: 452.3, 411.1, 391.3, 350.0, 331.1, 302.1, 268.1, 249.1, 215.1; HRMS calculated M$^+$ for C$_{15}$H$_{12}$ClN$_3$O$_3$S: 349.0310; found: 349.0299.

8-methyl-2-(methylsulfonyl)-6-phenylpyrido[2,3-d]pyrimidin-7(8H)-one (23c).
Yield 65%; $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 8.99 (s, 1H), 7.85 (s, 1H), 7.64 – 7.71 (m, 2H), 7.42 – 7.49 (m, 3H), 3.88 (s, 3H), 3.41 (s, 3H); $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ 164.2, 162.0, 157.0, 154.7, 137.7, 134.7, 131.2, 129.8, 129.1, 128.7, 115.7, 39.5, 29.4; LRMS: 318.0; HRMS calculated M$^+$ for C$_{15}$H$_{13}$N$_3$O$_3$S: 317.0390; found: 317.0396.

6-(2,6-difluorophenyl)-8-methyl-2-(methylsulfonyl)pyrido[2,3-d]pyrimidin-7(8H)-one (23d). Yield 82%; $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 9.02 (s, 1H), 7.90 (s, 1H), 7.37 – 7.47 (m, 1H), 7.03 (t, $J$ = 8.0 Hz, 2H), 3.89 (s, 3H), 3.42 (s, 3H); $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ 164.9, 161.9 (d, $J$ = 28.0 Hz), 160.6, 159.4 (d, $J$ = 28.0 Hz), 157.5, 155.4, 153.7, 131.4 (t, $J$ = 40.0 Hz), 127.8, 114.9, 111.9 (d, $J$ = 100.0 Hz), 39.5, 29.5; LRMS: 413.1, 352.0, 333.1,
304.1, 251.1, 209.2, 121.0; HRMS calculated M⁺ for C₁₅H₁₁F₂N₃O₃S: 351.0489; found: 351.0491.

![Chemical Structure](image_url)

6-(2-bromophenyl)-8-methyl-2-(methylsulfonyl)pyrido[2,3-d]pyrimidin-7(8H)-one (23e).
Yield 84%; ¹H-NMR (400 MHz, CDCl₃): δ 9.00 (s, 1H), 7.79 (s, 1H), 7.70 (d, J = 8.0 Hz, 1H), 7.42 (t, J = 8 Hz, 1H), 7.29 – 7.36 (m, 2H), 3.90 (s, 3H), 3.43 (s, 1H); ¹³C-NMR (100 MHz, CDCl₃): δ 164.7, 161.2, 157.3, 155.3, 138.3, 135.8, 133.7, 133.3, 131.2, 130.9, 127.8, 123.4, 115.2, 39.5, 29.5; LRMS: 457.0, 396.0, 348.0, 254.1, 215.1; HRMS calculated M⁺ for C₁₅H₁₂BrN₃O₃S: 392.9783; found: 392.9792.

Representative procedure for the preparation of 24a-f
A stirred mixture of 0.135 g (0.352 mmol) of sulfone 23a and 0.086 g (0.704 mmol) of 3-aminobenzylamine in DMF (5 mL) was refluxed overnight. The resultant reaction mixture was cooled to RT and diluted with water. This aqueous solution was extracted with ethyl acetate (3x 15 mL). The ethyl acetate layer was subjected to water wash (2x10 mL) and brine wash followed by drying over anhydrous MgSO₄ and concentrated in vacuo to give the crude product which was chromatographed over silica gel using 2% MeOH in CH₂Cl₂ to give 0.091 g (61%) of pure 24a.

2-(3-aminobenzylamino)-6-(2,6-dichlorophenyl)-8-methylpyrido[2,3-d]pyrimidin-7(8H)-one (24a). Yield 61%; ¹H-NMR (400 MHz, CDCl₃): δ 8.27 (bs, 1H), 7.44 (s, 1H), 7.40 (d, J = 8.0 Hz, 2H), 7.25 (t, J = 8.0 Hz, 1H), 7.14 (t, J = 8.0 Hz, 1H), 6.61 – 6.78 (m, 3H), 4.64 (d,
\[ J = 5.2 \text{ Hz, 2H}, \ 3.72 (s, \ 3H); \ \text{\^{13}}C-\text{NMR (100 MHz, CDCl}_3): \ \delta \ 161.67, \ 161.19, \ 158.67, \ 156.22, \ 147.00, \ 139.75, \ 136.71, \ 135.94, \ 134.55, \ 129.87, \ 129.79, \ 128.12, \ 120.27, \ 119.82, \ 118.58, \ 118.09, \ 114.42, \ 114.32, \ 113.83, \ 46.05, \ 28.33; \ \text{LRMS: 427.1, 425.1, 392.1, 390.1, 287.1, 285.1, 106.1; HRMS calculated M}^+ \text{ for C}_{21}H_{17}ClN_5O: 452.0810; \text{ found: 425.0813.} \]

\[ \text{2-(3-aminobenzylamino)-6-(2-chlorophenyl)-8-methylpyrido[2,3-d]pyrimidin-7(8H)-one (24b). Yield 77%; } \ \text{\^{1}H-\text{NMR (400 MHz, CDCl}_3): } \ \delta \ 8.32 (bs, \ 1H), \ 7.52 (s, \ 1H), \ 7.47 (t, \ J = 4.0 \ \text{Hz, 1H}), \ 7.30 - 7.36 (m, \ 3H), \ 7.14 (t, \ J = 8.0 \ \text{Hz, 1H}), \ 6.77 (d, \ J = 7.6 \ \text{Hz, 1H}), \ 6.70 (s, \ 1H), \ 6.62 (d, \ J = 7.6 \ \text{Hz, 1H}), \ 4.64 (d, \ J = 5.2 \ \text{Hz, 2H}), \ 3.71 (s, \ 3H); \ \text{\^{13}}C-\text{NMR (100 MHz, CDCl}_3): \ \delta \ 162.5, \ 161.6, \ 158.8, \ 156.1, \ 147.0, \ 140.0, \ 135.8, \ 135.5, \ 134.0, \ 131.9, \ 129.9, \ 129.6, \ 129.5, \ 126.8, \ 118.0, \ 114.4, \ 114.2, \ 46.0, \ 28.4; \ \text{LRMS: 391.1, 356.2, 251.1, 106.1, 69.0; HRMS calculated M}^+ \text{ for C}_{21}H_{18}ClN_5O: 391.1200; \text{ found: 391.1204.} \]

\[ \text{2-(3-aminobenzylamino)-8-methyl-6-phenylpyrido[2,3-d]pyrimidin-7(8H)-one (24c). Yield 53%; } \ \text{\^{1}H-\text{NMR (400 MHz, CDCl}_3): } \ \delta \ 8.20 (bs, \ 1H), \ 7.64 (d, \ J = 7.6 \ \text{Hz, 2H}), \ 7.55 (s, \ 1H), \ 7.43 - 7.32 (m, \ 3H), \ 7.13 (t, \ J = 7.6 \ \text{Hz, 1H}), \ 6.77 - 6.59 (m, \ 3H), \ 4.62 (d, \ J = 5.2 \ \text{Hz, 2H}), \ 3.71 (s, \ 3H); \ \text{\^{13}}C-\text{NMR (100 MHz, CDCl}_3): \ \delta \ 163.1, \ 161.3, \ 158.6, \ 155.6, \ 147.0, \ 139.9, \ 136.5, \ 133.7, \ 129.8, \ 129.6, \ 128.9, \ 128.4, \ 128.0, \ 120.4, \ 118.6, \ 118.0, \ 114.3, \ 46.0, \ 28.3; \ \text{LRMS: 358.2, 357.2, 356.2, 252.1, 121.1, 106.1, 77.0; HRMS calculated M}^+ \text{ for C}_{21}H_{19}N_5O: 357.1590; \text{ found: 357.1600.} \]
2-(3-aminobenzylamino)-6-(2,6-difluorophenyl)-8-methylpyrido[2,3-d]pyrimidin-7(8H)-one (24d). Yield 67%; \textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3}): \deltat 8.37 (bs, 1H), 7.59 (s, 1H), 7.32 (dt, J = 7.4 Hz, J = 2.0 Hz, 1H), 7.14 (t, J = 7.6 Hz, 1H), 6.97 (t, J = 8.0 Hz, 2H), 6.76 (d, J = 7.2 Hz, 1H), 6.69 (s, 1H), 6.61 (dd, J = 8.0 Hz, J = 2.0 Hz, 1H), 4.64 (d, J = 5.2 Hz, 2H), 3.71 (s, 3H); \textsuperscript{13}C-NMR (100 MHz, CDCl\textsubscript{3}): \deltat 162.3, 162.0, 161.7, 161.2, 159.8, 159.0, 156.3, 147.0, 139.8, 137.6, 129.9, 120.5, 119.9, 118.7, 118.0, 114.4, 111.8, 111.5, 105.6, 46.1, 28.5; LRMS: 394.1, 393.1, 392.1, 288.1, 269.1, 121.1, 106.1, 77.0, 69.0; HRMS calculated M\textsuperscript{+} for C\textsubscript{21}H\textsubscript{17}F\textsubscript{2}N\textsubscript{5}O: 393.1401; found: 393.1404.

2-(3-aminobenzylamino)-6-(2-bromophenyl)-8-methylpyrido[2,3-d]pyrimidin-7(8H)-one (24e). Yield 48%; \textsuperscript{1}H-NMR (400 MHz, CDCl\textsubscript{3}): \deltat 8.18 (bs, 1H), 7.65 (d, J = 7.6 Hz, 1H), 7.46 (bs, 1H), 7.37 – 7.32 (m, 2H), 7.26 – 7.20 (m, 2H), 7.13 (t, J = 7.6 Hz, 1H), 6.78 – 6.60 (m, 3H), 4.62 (d, J = 5.2 Hz, 2H), 3.71 (s, 3H); \textsuperscript{13}C-NMR (100 MHz, CDCl\textsubscript{3}): \deltat 162.5, 161.6, 161.3, 158.8, 156.1, 147.1, 139.9, 137.6, 135.8, 133.1, 131.9, 129.9, 129.7, 127.5, 124.3, 120.4, 118.7, 118.1, 114.4, 46.1, 28.4; LRMS: 435, 358, 356, 252, 237, 179, 106, 77; HRMS calculated M\textsuperscript{+} for C\textsubscript{21}H\textsubscript{18}BrN\textsubscript{5}O: 435.06947; found: 435.07033.
6-(2,6-dichlorophenyl)-8-methyl-2-(3-(methylthio)benzylamino)pyrido[2,3-d]pyrimidin-7(8H)-one (24f). Yield 64%; $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 8.19 (bs, 1H), 7.42 – 7.38 (m, 3H), 7.27 – 7.22 (m, 3H), 7.18 – 7.16 (m, 2H), 6.84 (bs, 1H), 4.68 (d, $J = 4.4$ Hz, 2H), 3.71 (s, 3H), 2.46 (s, 3H); $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ 161.6, 158.9, 156.3, 139.4, 139.3, 136.7, 136.6, 136.0, 134.6, 129.9, 129.4, 128.2, 126.0, 125.7, 124.6, 45.9, 28.4, 16.0; LRMS: 457, 422, 295, 188; HRMS calculated $M^+$ for C$_{22}$H$_{18}$Cl$_2$N$_4$S: 456.05783; found: 456.05881.

Representative procedure for the preparation of 20 and 25

To a solution of sulfone 23a (0.187 g, 0.5 mmol) in freshly distilled diglyme (5 ml), 1,3-phenylenediamine (0.120 g, 1.1 mmol) was added and resulting reaction mixture was refluxed for 12 h. After the completion of reaction, most of the solvent was evaporated invacuo and residue was purified by preparative TLC to get amine 9a (0.110 g, 55%).

6-(2,6-dichlorophenyl)-8-methyl-2-(3-(methylthio)phenylamino)pyrido[2,3-d]pyrimidin-7(8H)-one (20). Yield 57%; $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 8.60 (s, 1H), 7.75 (bs, 1H), 7.54 – 7.52 (m, 2H), 7.42 – 7.38 (m, 3H), 7.33 – 7.25 (m, 2H), 7.01 (d, $J = 8.0$ Hz, 1H), 3.82 (s, 3H), 2.53 (s, 3H); LRMS: 442, 407, 362, 313, 269, 203, 196, 180; HRMS calculated $M^+$ for C$_{21}$H$_{16}$Cl$_2$N$_4$OS: 442.04219; found: 442.04315.

2-(3-aminophenylamino)-6-(2,6-dichlorophenyl)-8-methylpyrido[2,3-d]pyrimidin-7(8H)-one (25). Yield 55%; $^1$H-NMR (400 MHz, CDCl$_3$): $\delta$ 8.58 (s, 1H), 7.53 (s, 1H), 7.46 – 7.40 (m, 3H), 7.28 – 7.25 (m, 1H), 7.23 – 7.11 (m, 2H), 7.07 – 6.98 (bs, 1H), 6.48 (d, $J = 8.0$ Hz,
1H), 3.81 (s, 3H); $^{13}$C-NMR (100 MHz, CDCl$_3$): $\delta$ 161.6, 159.3, 159.2, 158.6, 156.1, 147.4, 139.8, 136.5, 136.5, 136.4, 136.4, 135.9, 134.4, 130.1, 128.2, 126.3, 110.7, 110.3, 106.8, 106.5, 28.8; LRMS: 412, 377, 376, 284, 242; HRMS calculated M$^+$ for C$_{20}$H$_{15}$Cl$_2$N$_5$O: 411.06536; found: 411.06606.

**Representative procedure for tagging PDC-Gly (26) with Cy dyes**

![Chemical structure](image)

**Preparation of Cy3-PDC-Gly (39)** - In a 20 ml vial equipped with a stirring bar, Cy3-NHS ester (28b) (30.0 mg, 43.0 μmol), PDC-Gly (26) (25.0 mg, 52.0 μmol) and N,N-diisopropylethylamine (0.1 ml) were taken in DMF (5.0 ml) and stirred at room temperature under argon atmosphere in dark for 12 h. After the completion of reaction, the reaction mixture was purified by preparative TLC using 10% MeOH:DCM to give the pure product 39 in 72% yield. MS ($m/z$): 923.3910 (M$^+$ + 1), 471.2992; HRMS: calcd for C$_{53}$H$_{57}$C$_2$N$_8$O$_3$$^+$: 923.3931, found: 923.3915.
Preparation of Cy5-PDC-Gly (40) - In a 20 ml vial equipped with a stirring bar, Cy5-NHS ester (29b) (50.0 mg, 71.0 μmol), PDC-Gly (34.0 mg, 71.0 μmol) and N,N-diisopropylethylamine (0.5 ml) were taken in DMF (5.0 ml) and stirred at room temperature under argon atmosphere in dark for 12 h. After the completion of reaction, the reaction mixture was purified by preparative TLC using 10% MeOH:DCM to give the pure product 40 in 66% yield. MS (m/z): 947.5 (M+ - 1), 497.4, 383.3.

Representative procedure for the preparation of symmetrical Cy3 macrocycles

Symmetrical Cy3-NHS ester (41b) (0.2 g, 0.24 mmol) was taken in an oven dried 100 ml round bottom flask equipped with a stir bar. To this, 5 ml of dry DMF was added followed by the addition of 1,6-hexanediamine (0.03 g, 0.24 mmol) and diisopropylamine (0.06 g, 0.48 mmol) at room temperature under argon. Resulting reaction mixture was stirred for six hours with monitoring. After the completion of reaction, majority of DMF was removed under reduced pressure and residue was purified by column chromatography using 1% MeOH:DCM as elutent to obtain pure macrocycle 42 (0.114 g, 0.16 mmol) in 66% isolated yield.
(31aZ,33E)-31,31,35,35-tetramethyl-11,20-dioxo-6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,31,35-docosahydro-[1,7,14,21]tetraazacycloheptacosino[1,2-a:7,6-a']diindol-5-ium bromide (42). Yield 66%; 1H-NMR (400 MHz, CDCl₃): δ 8.42 (t, J = 13.4 Hz, 1H), 7.91 (t, J = 4.8 Hz, 1H), 7.33 – 7.44 (m, 4H), 7.22 – 7.30 (m, 2H), 7.13 – 7.20 (m, 2H), 7.10 (d, J = 8.0 Hz, 1H), 4.15 (t, J = 8.0 Hz, 4H), 3.28 (d, J = 4.0 Hz, 4H), 2.68 (s, 3H), 2.42 (t, J = 8.0 Hz, 3H), 1.75 – 1.97 (m, 7H), 1.72 (s, 12H), 1.55 – 1.65 (m, 7H), 1.40 – 1.50 (m, 3H), 1.20 – 1.37 (m, 3H); 13C-NMR (100 MHz, CDCl₃): δ 174.8, 174.0, 172.7, 151.3, 142.1, 140.9, 129.3, 125.7, 122.4, 111.3, 104.6, 49.3, 44.9, 38.8, 36.6, 28.5, 27.3, 26.6, 26.5, 25.8, 25.7, 25.6; LRMS: 637.45, 423.03, 339.07, 277.10; HRMS calculated M⁺ for C₄₁H₅₇N₄O₂: 637.4482; found: 637.4480.

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CHAPTER 3. New methodology towards the synthesis of flavones, flavonols and aurones

Introduction

Flavonoids are polyphenolic compounds that are ubiquitous in nature and categorized according to chemical structure into nine major subgroups: flavonols, flavones, flavanones, isoflavones, aurones, flavanediols, anthocyanidins, chalcones and tannins (Figure 1). They are synthesized exclusively in plants and many of them possess various biological functions such as acting as floral pigments, signal molecules and antimicrobial compounds. They occur in the plants as glycosides, meaning that they are bound to sugar molecules. Plants accumulate specific flavonoids and thus each species often exhibits a limited flower color range. For example, anthocyanin based delphinidin imparts violet to blue color and cyanidin gives red to magenta color to their corresponding flowers.

Figure 1: General structures of flavonoids

The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health. They have been reported to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, antitumor, antioxidant and estrogenic activities. Over
5,000 flavonoids have been identified but their applications have been hampered because they usually exist as a mixture of multiple compounds that are difficult to separate or their activities are not satisfactory.\textsuperscript{10}

Structurally, they consist of two aromatic rings linked by a three-carbon chain that forms an oxygenated heterocyclic ring (Figure 1). Flavonols are the most widespread flavonoids in plants. Some well known flavonols are quercetin and kaempferol. Flavonols can be found in many common foods such as onions, leeks, broccoli, red grapes, apples and blue berries. Flavones, such as luteolin and apigenin, are less common and can be found in green vegetables such as celery and parsley. Flavanones are mainly found in citrus fruits, in the juice but also the albedo. Isoflavones are also called plant estrogens because of their structural similarity to human estrogen. They are mainly found in soybeans. Anthocyanins, as mentioned above, are the water-soluble pigments which give the typical color to fruits and vegetables such as blueberries, strawberries, red wines and cabbages.

The bioavailability and metabolism of flavonoids are an important factor in determining their therapeutic effect. Some flavonoids such as anthocyanins are easily absorbed in the stomach, whereas most other flavonoids are only absorbed in the intestine. Enzymes released by the intestinal epithelium or by intestinal bacteria may change the chemical structure of flavonoids. Their metabolites are finally excreted in the bile or urine.\textsuperscript{11}

It is very difficult to estimate the total consumption of flavonoids because their content in foods has shown large variations. Most food consumption tables do not show data about flavonoids or other phytochemicals. However, the USDA website has comprehensive tables of flavonoids, proanthocyanidins and isoflavones of many foods. It is estimated that Americans consume daily about one gram of polyphenols, most of which is coming from tea, red wine, fruits, cacao, vegetables and legumes. The intake of individual flavonoids shows a large differences between different population groups. For example, Japanese adults consume about twenty five times more isoflavones that Western adults.\textsuperscript{11}

Because many foods are rich in flavonoids, they are generally recognized as safe and are well known for their health benefits. But they may also have adverse effects, such as antinutritional effects, thyroid toxicity, carcinogenic, development effects and drug interaction. Very high intakes of flavonoids have been associated with antinutritional effects,
such as a reduced intake of glucose or minerals. However, the slower absorption of glucose may have some use against diabetes mellitus. Some flavonoids have an effect on the thyroid function. They inhibit thyroid peroxidase and interfere with the production of the thyroid hormone. Soy isoflavones can, in theory, increase the risk of thyroid, cancer but some studies have shown that the intake of isoflavones actually decrease the risk of thyroid cancer. There were early reports that the intake of high quantities of isoflavones (from red clover) and coumestrol by cattle and sheep caused infertility, such effects have not been observed in primates. Epidemiological studies show no effect of isoflavones on fertility, miscarriage, abortion or ectopic pregnancy. Epidemiological studies and scientific experiments suggest a protective function of flavonoids against cardiovascular diseases. The effects of tea and red wine on cardiovascular disease have been studied intensively. It is estimated that the extra daily consumption of three cups of tea reduces the risk of cardiovascular risk by more than ten percent.\(^{11}\)

Epidemiological studies also show a relationship between red wine intake and a cardiovascular protective effect. Flavonoids act by inhibiting the initiation and progression of atherosclerosis. The high intake of flavonoids by the French explains the French paradox, which refers to the observation that the French have lower rates of heart attacks despite the fact that they consume a high amount of saturated fats.\(^{11}\)

The effects of flavonols, flavones and flavanones have been investigated in some large community health studies. Most studies show a protective effect for these phytochemicals. However, one English study showed an inverse relationship between intake of flavonols and flavones on cardiovascular. This could be attributed to the fact that the English consume tea with large quantities of milk, which might inhibit the absorption of flavonoids. More studies are required to prove the clinical effect of these flavonoids. Isoflavones form another group of well studied flavonoids. They can bind selectively with estrogen receptors (\textit{alpha-} and \textit{beta-}).\(^{11}\)

Animal experiments and epidemiological studies have suggested that isoflavones protect against cardiovascular diseases such as atherosclerosis. They seem to directly improve the health of blood vessels. Isoflavones help to reduce LDL oxidation through their antioxidant activity. Anthocyanidins, the phytochemicals which gives many fruits their red or
purple color, have many claimed health benefits such as antioxidant, anti-inflammatory, antimicrobial, anticarcinogenic activities, neuroprotective effects, induction of apoptosis and improvement of vision. Many *in vitro* studies have demonstrated the antioxidant effects of anthocyanins but the *in vivo* effects are less evident because of the low absorption.\textsuperscript{11}

![Scheme 1: General outline of common synthetic pathways of flavones.](image-url)

Though flavonoids consist of nine major subgroups, we focused our attention to the families of flavones (1) and flavonols (4) for the synthesis. Both flavones and flavonols are structurally very close. The only difference is the presence of a hydroxyl group at the 3-position in flavonols (Figure 1). For this reason, flavonols are also known as 3-hydroxyflavones. There have been many publications outlining different synthesis of flavones but a majority of these methods falls into the category of either oxidative cyclization of various substituted 2'-hydroxychalcones (2) or cyclodehydration of substituted 1-(2-hydroxyphenyl)-3-phenylpropane-1,3-dione (3, Scheme 1).
The preparation of $2''$-hydroxychalcone (2) for oxidative cyclization is usually carried out by condensing an appropriately substituted $2''$-hydroxyacetophenone with various substituted benzaldehydes under basic conditions. There have been several reports using various oxidative cyclization conditions and the most common of them are summarized in Scheme 2. In one of the earliest reported works on the synthesis of flavones, Doshi et al. reported the cyclization of $2''$-hydroxychromone (2) in presence of catalytic amount of iodine in boiling dimethylsulfoxide (Scheme 2, route 1). This catalytic iodine mediated method has been further explored and modified into a greener method by the use of iodine adsorbed on neutral alumina and microwave reactor assisted conditions.

Another often used method for the oxidative cyclization involves using selenium dioxide in isoamyl alcohol which requires prolonged heating. This, selenium dioxide mediated reaction, has also been improved over the years in order to make it more benign. The most common improvement of this method is the use of selenium dioxide and traces of...
Another very common pathway to flavones (1) from 2’-hydroxychromones (2) is the addition of bromine across the chromone double bond followed by base mediated cyclization (Scheme 2, route 3). Though, this method does not involve the use of high temperature or toxic reagents like dimethylsulfoxide. The one major disadvantage is the yields for the flavones (1) are moderate at best because of the formation of aurone (Figure 1) side products.

Cook and coworkers demonstrated an interesting and flexible synthesis of flavones (1) using a Wacker-Cook oxidation as the key step. They prepared the chalcone (2) starting material using a common base mediated condensation of 2’-hydroxyacetophenone and benzaldehyde. Their initial attempts using original Wacker conditions resulted in very poor conversions of chalcone to flavones (1). Moreover, Wacker conditions required the use of stoichiometric amounts of palladium. They improved this method by using excess tert-butyl hydroperoxide. This method needed only catalytic amounts of the palladium catalyst and moderately high temperatures (Scheme 2, route 4). Using this modified route, they made several substituted flavones (1) in good yields.

Another very common way to prepare flavones (2) and flavonols (4) is cyclodehydration of substituted 1-(2-hydroxyphenyl)-3-phenylpropane-1,3-dione (3, Scheme 1). The starting material 3 is usually prepared by a Baker-Venkataraman type rearrangement, which involves base induced transfer of the ester acyl group in an O-acylated phenol ester, which leads to a 1,3-diketone (Scheme 1). Like oxidative cyclization, the cyclodehydration pathway has also been very extensively used; studies have modified it as shown by the examples below in Scheme 3.

Scheme 3: Preparation of flavones via cyclodehydration pathway
Wilson Baker first showed the usefulness of 1,3-diketone 3 in the synthesis of flavones when an unsubstituted 3 was converted to a flavone using sodium acetate in acetic acid conditions (Scheme 3).\textsuperscript{19} Venkataraman and coworkers successfully attempted the same reaction under acidic conditions (Scheme 3).\textsuperscript{20} Many different acidic and basic conditions have been tried and reported since then. The use of hydrochloric acid in acetic acid\textsuperscript{21}, hydrobromic acid in acetic acid\textsuperscript{22}, para-toluenesulfonic acid in xylene\textsuperscript{23} and Lewis acids\textsuperscript{24} has been reported.

Some more cyclodehydration pathways are discussed in Scheme 4. Most of these routes use microwave conditions as a way to make the synthesis more environmentally friendly. Kabalka and coworkers reported a high yielding synthesis of flavones and chromones by using a catalytic amount of cupric chloride in a solution of the appropriate 1,3-diketone 3 in ethanol under microwave conditions (Scheme 4, route 1).\textsuperscript{25}

A solid-state synthesis, using high-speed ball milling (HSBM) was reported by Su and coworkers. Their route was an efficient, mechanically activated solid-state synthesis which used potassium bisulfate as a reagent to achieve the transformation. This route had the distinct advantage that it does not use strong acids like sulfuric acid, hydrochloric acid or hydrobromic acid for the synthesis. Moreover, this route was flexible, high yielding and environmentally benign, thus making it an attractive option to other routes (Scheme 4, route 2).\textsuperscript{26}
The first use of popular Vilsmeier-Haack reaction for the synthesis of flavones was reported by Su and coworkers. They used Vilsmeier-Haack conditions with bis-(trichloromethyl)carbonate/ N,N-dimethylformamide to cyclodehydrate the 1,3-diketone 3 to flavone 1 (Scheme 4, route 3). Many other research groups reported the use of various catalysts like gallium(III) triflate, indium(III) chloride and ionic liquids to achieve the cyclodehydration step in order to synthesize flavones efficiently (Scheme 4, route 4).

Another common method for the synthesis of flavones is detailed above in Scheme 5. This approach involves the coupling of substituted phenol 5 with acetylene 6 to
generate aryl propynoate 7 which, on irradiation, undergoes Photo-Fries rearrangement to give ortho-hydroxyaryl ethynyl ketone 8. This ketone 8 can then be cyclized to the corresponding flavone in 10-25% overall yield.\(^{31}\)

![Scheme 6: Conversion of flavones to flavonols](image)

Flavones 1 are usually converted to flavonols 4 by either of the methods shown in Scheme 6. In a method developed by Dean and coworkers, the 3-position of flavones are lithiated by LDA and then the 3-lithioflavone is reacted with methyl borate followed by hydrogen peroxide to give flavonols 4 in good yields.\(^{32}\) The second common method to carry out the conversion is to oxidize the flavone 1 with dimethyldioxirane, followed by the treatment with a catalytic of para-toluenesulfonic acid to give the flavonols in decent yields.\(^{33}\)

**Results and Discussion**

![Scheme 7: Retrosynthetic analysis of flavonols](image)

Our initial approach to develop a general synthesis of flavonols 4 is outlined above in Scheme 7. Our key step in this plan was the dehydrocyclization of arylketoester 11 to the flavonols 4 by using P$_4$-t-Bu chemistry developed previously. This arylketoester 11 could be prepared in a two step sequence starting from commercially available 3,4,5-trimethoxyphenol
9. Phenol 9 can be converted to ortho-hydroxy arylketoester 10 via a Friedel-Crafts acylation with ethyl oxalyl monochloride followed by a base mediated O-arylation with the appropriate benzyl bromide.

![Scheme 8: Synthesis of flavonols – model studies](image)

We first decided to try our key step on a model system. Trimethoxy phenol 9 was acylated by using ethyl oxalyl monochloride under aluminum trichloride mediated Friedel-Crafts conditions to give acylated phenol 10 in 80% yield. Compound 10 was then O-arylated with benzyl bromide using sodium hydride as the base to give compound 11 in 73% yield. This key intermediate 11 was then reacted with P₄-t-Bu in boiling benzene. The benzylic anion generated could potentially give rise to two products. The first possibility was an attack on the ester carbon to give flavonol 4. Potentially it could also attack on the carbonyl carbon to give a 2,3-disubstituted benzofuran. Unfortunately, even after prolonged heating, we just recovered the starting material (Scheme 8).
With the failure of our initial plan, we decided to modify compound 11 in such a way as to facilitate the anion formation at the benzylic position. For this purpose, we envisioned an electron-withdrawing group which could be removed easily at a later stage. A cyano group at the benzylic position seemed to fit the requirements perfectly thus giving rise to cyano compound 12a shown in Scheme 9.

The synthesis of compound 12a is shown in Scheme 10. Compound 13a was prepared by the benzylic bromination of phenylacetonitrile 14a in 66% yield. This alpha-bromo compound 13a was reacted with phenol 10 under basic conditions to give cyano compound 12a in 70% yield.

The synthesis of flavonols - model studies
With compound 12a in hand, we moved ahead and subjected it to a potassium tert-butoxide mediated intramolecular cyclization. At 0 °C or room temperature, the starting material remained unaffected, but at elevated temperatures it decomposed (Scheme 11). This led us to change the base to LDA. Compound 12a was reacted at 0 °C in the presence of LDA but instead of generation of the flavone, the intramolecular cyclization resulted into the formation of dihydro benzofuran type compound (15, Scheme 11). We then subjected this compound 15 to potassium hydride conditions in boiling tetrahydrofuran, hoping that it would rearrange into six-membered ring, but we ended up recovering compound 15.

![Scheme 12: Reduction of keto ester to alpha-hydroxy ester](image)

This experiment, though a failure, made us realize that in compounds like 12a, the carbonyl carbon is more reactive than the ester. With this crucial information in hand, we designed a new strategy where we reduced the keto group of compound 12a under sodium borohydride mediated conditions to give alpha-hydroxy ester 16a in 83% yield (Scheme 12).

![Scheme 13: Synthesis of flavonols – model studies](image)
This *alpha*-hydroxy ester 16a was then subjected to various basic conditions. Reaction with potassium *tert*-butoxide was unsuccessful even at elevated temperatures and resulted into the decomposition of the starting material (Scheme 13). Interesting results were observed when ester 16a underwent an LDA mediated cyclization reaction. At lower temperatures, 16a remained unaffected but when the temperature was increased to reflux, a new compound was formed. This compound was not the one which we were expecting to get. In fact, the intramolecular cyclization of compound 16a eliminated the cyano group and rearranged it into the flavonol 4a (Scheme 13). The analytical data of flavonols 4a was found to be in excellent agreement with literature data.\(^{34a-b}\)

Scheme 14: Synthesis of 3,5,6,7-tetramethoxyflavone

Tetramethoxy flavone 4b is a naturally occurring flavonol found in *Gomphrena martiana*\(^{35}\) and shows antimicobacterial\(^{36a-b}\), antitumoral\(^{36b}\) and antifungal activities\(^{37}\). This 3,5,6,7-tetramethoxyflavone was successfully synthesized by methylating trimethoxyflavonol 4a under basic conditions (Scheme 14). The analytical data for flavonol 4b matched with the reported data.\(^{38}\)

Figure 2: Flavonol based natural products
With the successful synthesis of one of the natural flavonols 4b, we decided to target some other naturally occurring flavonols, to further explore and develop our synthetic method. Some of the flavonols could directly be synthesized from our method are shown in Figure 2.

The synthesis started with benzylic bromination of commercially available (3,4-dimethoxy)phenylacetonitrile 14b and 3,4-(methylenedioxy)phenylacetonitrile 14c to give alpha bromo compounds 13b and 13c in 62% and 65% yields, respectively. Compounds 13b-c were then coupled with ortho-hydroxy compound 10 under basic conditions to give O-arylated compounds 12b-c in good yields. Reduction of ketoesters 12b-c using sodium borohydride gave key intermediates 16b-c in 75% and 79% yields, respectively. At that stage, we were set to try our crucial dehydrocyclization – rearrangement to give the corresponding flavonols. Unfortunately, even after repeated attempts, we were unable to get the required products. Changing the base to Li-TMP did not help, as we just recovered some of the starting material (Scheme 15).

Scheme 15: Synthesis of flavonols
These unfortunate results forced us to abandon a really elegant synthetic pathway and start afresh. Our next plan is outlined above in Scheme 16. We envisioned the final step to be an intramolecular Friedel-Crafts type acylation to close the ring to generate the flavonol 4 from key intermediate 17. Our plan was to make compound 17 via an anionic reaction of compound 18 with dimethyl oxalate.

Table 1

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<td>NO$_2$</td>
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Scheme 17: Attempted synthesis of 17

Synthetic efforts based on this new approach are shown in Scheme 17. We started with trimethoxyphenol 9 and reacted it with various substituted benzyl bromide compounds 19a-d to get O-arylated compounds 18a-d in 73 - 88% yields. Next, we tried a number of conditions to react 18a-d with dimethyl oxalate (Scheme 17, Table 1). Various bases were
tried but none of the conditions gave us the expected product. This unsuccessful endeavor led us to think further and design a completely new synthetic approach.

Our next plan is outlined above in Scheme 18. We postulated that in a benzofuran-
2,3-dione 19 type system, due to the presence of methoxy groups at the ortho and para position of the benzene, the carbonyl group at 3-position, the other carbon at 2-position would be more reactive and could be attacked by an appropriate nucleophile 20. We foresaw compounds like phenylacetonitrile, benzyl chloride and its triphenylphosphonium salt as potentially useful nucleophiles because the anion can be generated on the benzylic position which would attack the 2-position of benzofuran 19 followed by a furan ring opening. The phenoxide anion could attack the benzylic position bearing cyano, chloro or triphenylphosphonium group, which are good leaving groups, and result in the ring closing required to give required flavonols 4.
Benzofuran-2,3-diketone 19 was synthesized by following a literature procedure of condensing trimethoxyphenol 9 with oxalyl chloride at 100 °C under neat conditions in 78% yield. Next, we tried a number of conditions with either benzyl chloride or phenylacetonitrile. Various bases like sodium hydride, potassium tert-butoxide, LDA and Li-TMP were used, but all the reactions were unsuccessful resulting in either the recovery of starting material 19 or decomposition at elevated temperatures (Scheme 19, Table 2).

A breakthrough was achieved when we used benzyl triphenylphosphonium bromide 20a as a nucleophile under potassium tert-butoxide conditions. We recovered a couple of new products which were different from both the starting materials based on thin layer chromatography analysis as well as analytical data. It turned out that none of them were the expected flavonol, instead, they were found to be aurone 21a and isoaurone 22a. Even after
trying different bases like LDA and $n$-butyllithium, we just recovered aurone and isoaurone in various ratios and better yields (Scheme 20, Table 3). This was confirmed by comparing the analytical data with literature data.\textsuperscript{39}

Though, it was definitely a major setback to our synthetic plans, but we decided to turn this into an opportunity by designing two different pathways stemming from aurone 21a. In the first pathway, we decided to convert the double bond of aurone 21a into epoxide 24. Our plan was to subject this epoxide to various acidic or basic conditions to hopefully rearrange it into the flavonol 4a. Another idea based on Auwers synthesis\textsuperscript{40a,b} was planned, where bromine was added across the double bond to give dibromo compound 23. Dibromo 23 then can be subjected to basic conditions to rearrange into flavonol 4a. Scheme 21 outlines our approach to both of the pathways. Attempts to convert the aurone 21a into its epoxide were unsuccessful with both hydrogen peroxide as well as dimethyldioxirane conditions, thus bringing an end to this pathway. The second pathway based on Auwers synthesis successfully gave the product 23a in 88% yield. It turned out that compound 23a was actually a tribromide compound as the phenyl ring also underwent bromination to give the unexpected product. Unfortunately, even after repeated attempts, compound 23a produced a complex mixture when it was subjected to potassium hydroxide conditions (Scheme 21).

\begin{center}
Scheme 21: Synthesis of flavonols – model studies
\end{center}
After trying many unsuccessful strategies, we decided to completely overhaul the plan and approach the synthesis of flavonols 4 via flavones 1. As described above in Scheme 5, flavones can easily be converted into flavonols using various strategies. Since there are many different and efficient strategies available for the synthesis of flavones, we wanted to come up with a strategy which is different and yet more efficient than others. There was one common drawback in all the previously published strategies, that is, it is not possible to generate libraries of flavones efficiently as for every new substitution, one has to go back to the starting point and begin afresh. We decided to design a strategy which would overcome this point of contention and would be useful in making libraries of flavones more efficiently.

Our new synthetic plan is shown in Scheme 22. This strategy was based on an entirely new retro-synthetic approach. The key step in this new method is palladium mediated Suzuki coupling of chromone 30a with various substituted phenylboronic acids 31 to prepare flavones. The plan was to prepare chromone 30a from ortho-acylated phenol 29a. The acylated phenol 29a could be prepared from trimethoxyphenol 9a and dibromoacylate 27a, which in turn could be synthesized from commercially available tetrabromoacetone 25. The obvious advantage of this route is that few permutations and combinations of key
intermediates would result in large number of substituted flavones which could then be used for drug screening purposes.

![Scheme 23: Synthesis](image)

The synthesis started from commercially available tetrabromoacetone 25 which was subjected to Favorskii type rearrangement in the presence of aqueous sodium bicarbonate to give 3,3-dibromoacrylic acid 26a in 63% yield. This dibromoacrylate 26a was then converted into its corresponding acid chloride 27a by boiling it in thionyl chloride. This acrylate 27a was then used for Fridel-Crafts acylation of trimethoxyphenol 9a. Various different conditions were tried but the reactions were mostly unsuccessful. Refluxing with titanium tetrachloride in 1,2-dichloroethane returned mostly starting material (Scheme 23, Table 4 – entry 1). Neat conditions with titanium tetrachloride were unsuccessful but produced less than 5% of product with boron trifluoride etherate (Scheme 23, Table 4 – entry
2, 3). Even the use of aluminum trichloride was unsuccessful (Scheme 23, Table 4 – entry 4, 5). At this stage we changed the route and tried Steglich esterification conditions on 3,3-dibromoacrylic acid 26a and phenol 9a to give ester 28a in 71% yield. This ester 28a was then subjected to Fries rearrangement conditions using various Lewis acids (Scheme 23, Table 5). Initial attempts using titanium tetrachloride, boron trifluoride etherate and photochemical conditions did not result in the expected product (Scheme 23, Table 5 – entry 1-3). When aluminum trichloride was used in boiling chloroform, a small amount of product was obtained (Scheme 23, Table 5 – entry 4). This positive result was standardized by using higher boiling 1,2-dichloroethane as a solvent which gave 41% of rearranged product 29a (Scheme 23, Table 5 – entry 5). Even after repeated attempts and prolonged heating, we were unable to produce better yields. Usually, the demethylated ester would be recovered along with the expected product which accounts for the loss of yield.

After the successful Fries rearrangement to give compound 29a, it was subjected to various basic conditions resulting in the formation of compound 30a in good yields (Scheme 24, Table 6 – entry 1-3). This compound 30a, which we thought was a chromone derivative, was subjected to Suzuki coupling conditions with phenylboronic acid 31a in the presence of tetrakis(triphenylphosphine)palladium and anhydrous potassium bicarbonate in refluxing 1,4-dioxane. This Suzuki reaction gave us a new product whose analytical data did not match
with that of flavone 1a. After careful analysis and cross-checking, we realized that we have prepared aurone 21a (Scheme 24).

Scheme 25: Reassigning the structure

The above-mentioned formation of aurone 21a led us to realize that the cyclization of phenol 29a did not result in the formation of chromone 30a. Instead, an aurone precursor 32 was formed (Scheme 25).

Scheme 26: Suzuki coupling to form aurones

This compound 32 was then reacted with various phenylboronic acids 31a-c under previously used conditions and each resulted into successful formation of corresponding aurone 21a-c (Scheme 26). The analytical data of these compounds matched reasonably with their corresponding literature values.
Even though, this discovery was a setback to our plans of synthesizing flavones, it was an interesting way to prepare substituted aurones. We decided to use this setback in our favor and tried to explore the ways to make chromone. We decided to replace the two bromo groups with two chloro groups. We were expecting that this change would be sufficient to tilt the balance in favor of the formation of chromone instead of aurone precursor (Scheme 27).

Scheme 27: Aurone precursor versus flavone precursor

Scheme 28 outlines the new rterosynthetic analysis which is quite similar to the previous approach. The only difference is in the procedure for the preparation of 3,3-dichloroacrylic acid which is prepared by a literature procedure.
The synthesis started with the preparation of 3,3-dichloroacrylic acid 37 by a literature procedure.\textsuperscript{41} Vinyl acetate 33 and bromotrichloromethane 34 were refluxed together in the presence of azobisisobutyronitrile to give the addition product 35 in quantitative yield. This compound 35 was converted to 3,3-dichloroacrylaldehyde 36 by stirring it into a solution of 5% aqueous sulfuric acid for eight hours followed by azeotropic distillation and aqueous work-up to give aldehyde 36 in excellent yield. The aldehyde 36 was oxidized to 3,3-dichloroacrylic acid 37 by silver oxide oxidation in 60% yield. This acrylic acid derivative 37 was then subjected to Steglich esterification conditions in the presence of trimethoxyphenol 9a and dimethoxyphenol 9b to give ester 28b-c in good yields. Both esters 28b and 28c were subjected to Lewis acid catalyzed Fries rearrangement to get rearranged phenols 29b and 29c in 40% and 60% yields respectively. The yield of Fries rearranged product 29c was much higher than 29b because of the absence of para-methoxy group which usually gets demethylated thus reducing the overall yield of the reaction (Scheme 29).

Scheme 29: Synthesis

Scheme 30: Base mediated cyclization to form chromone
At that point, we were set to try the key cyclization experiment. When phenols \(29b\) and \(29c\) were subjected to basic conditions using 0.02 N sodium hydroxide solution (aqueous), we successfully accomplished corresponding chromones \(30b\) and \(30c\) in 65\% and 68\% yields respectively (Scheme 30).

Scheme 31 summarizes the efforts towards standardization of key coupling reaction. Initial attempts with using anhydrous potassium carbonate and cesium carbonate bases along with phase transfer catalysts like tetra-\(n\)-butylammonium bromide and tetra-\(n\)-butylammonium chloride in the presence of palladium (0) catalyst in refluxing diaoxane resulted in the successful product formation. But when the purifications on these products were attempted, we quickly discovered that it was a complicated mixture which was really difficult to resolve effectively (Scheme 31, Table 7 - entry 1-4).

![Scheme 31: Suzuki coupling – standardization of conditions](image)

Table 7

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</tbody>
</table>
We then attempted the coupling reaction with various bases in the absence of any additive. When anhydrous potassium fluoride was used as a base, even after eighteen hours of reflux, the reaction was not complete but it was observed that the pure product was getting formed without the formation of any impurities unlike previous cases (Scheme 31, Table 7 – entry 5). The coupling was successfully completed in excellent yields with both anhydrous cesium carbonate and potassium carbonate to give flavones in 76% and 81% yields respectively (Scheme 31, Table 7 – entry 6-7). Since, our best results were obtained by using powdered anhydrous potassium carbonate, we decided to use these conditions as the standard for all the future reactions. More flavones 1c and 1d were synthesized in 72% and 69% yields respectively using chromone 29c and phenylboronic acids 31b-c under standardized conditions (Scheme 32).

Scheme 32: Suzuki coupling – Synthesis of flavones

After the successes with chromone 29c, we successfully subjected chromone 29b to Suzuki coupling with phenylboronic acids 31a and 31c. Both reactions resulted in the formation of flavones 1a and 1e in 67% and 68% yields. Analytical data of all the flavones synthesized matched with literature values (Scheme 33).
In conclusion, we successfully developed a new methodology towards the synthesis of aurones and flavones. These flavones can be converted to flavonols by well known procedures. Though, there is no doubt that more work is needed to further generalize the invented routes, the flexibility offered by this route is unprecedented as by substituting the halogen from bromo to chloro in intermediate 29 resulted in different cyclized products, each of which can serve as a precursor to the library of corresponding aurones or flavones.

**Experimental**

Unless otherwise noted, materials were obtained from commercial suppliers and used without purification. Tetrahydrofuran and diethyl ether were distilled from sodium and benzophenone. Dichloromethane, benzene and diisopropyl amine were distilled over calcium hydride. All experiments were performed under an argon atmosphere unless otherwise noted. Organic extracts were dried over anhydrous magnesium sulfate. Nuclear magnetic resonance experiments were performed with either a Varian 300 MHz or Bruker 400 MHz instrument. All chemical shifts are reported relative to CDCl$_3$ (7.27 ppm for $^1$H and 77.23 ppm for $^{13}$C), unless otherwise noted. Coupling constants ($J$) are reported in Hz with abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. High resolution mass spectra were recorded on a Kratos model MS-50 spectrometer. Standard grade silica gel (60 Å, 32-63 μm) was used for flash column chromatography.
Preparation of ethyl 2-(6-hydroxy-2,3,4-trimethoxyphenyl)-2-oxoacetate (10)

To a solution of phenol 9 (2.0 g, 10.86 mmol) in CH₂Cl₂, was added titanium tetrachloride (2.276 g, 12.0 mmol) at -20 °C under argon. To this dark brown solution, ethyl chlorooxoacetate (1.64 g, 12.0 mmol) was added dropwise while maintaining temperature at below -15 °C. Resulting reaction mixture was stirred for 4 h with steady increase in temperature to 0 °C. After the completion of reaction, the reaction mixture was diluted with CH₂Cl₂ and poured over cold HCl (1.0 M) solution. Aqueous layer was separated and extracted with CH₂Cl₂ (3 x 25 ml). Combined organic extracts were washed with HCl (1.0 M) solution and brine followed by drying over anhydrous MgSO₄. Solvent was evaporated in vacuo to obtain the crude title compound. The crude compound was purified by silica gel column chromatography using 15% EtOAc + hexanes as solvent system to obtain pure title compound 10 in 80% yield as yellow solid.

¹H-NMR (400MHz, CDCl₃) δ 11.95 (s, 1H), 6.25 (s, 1H), 4.38 (q, J = 7.2 Hz, 2H), 3.91 (s, 3H), 3.91 (s, 3H), 3.77 (s, 3H), 1.40 (t, J = 7.2 Hz, 3H); ¹³C-NMR (100MHz, CDCl₃): δ 189.3, 164.2, 162.9, 154.2, 134.1, 104.7, 96.0, 62.0, 61.6, 61.1, 56.5, 14.1; MS (m/z): 307 (M+Na⁺), 239, 211; HRMS calcd for C₁₃H₁₆O₇: 284.0900, found: 284.0896.

Preparation of ethyl 2-(6-(cyano(phenyl)methoxy)-2,3,4-trimethoxyphenyl)-2-oxoacetate (12a)

To a slurry of NaH (0.041 g, 1.71 mmol) in dry DMF under argon, phenol 10 (0.44 g, 1.55 mmol) was added at 0 °C. Resulting reaction mixture was stirred at 0 °C for 15 minutes
followed by the addition of 2-bromo-2-phenylacetonitrile (0.33 g, 1.71 mmol) at same temperature. Reaction mixture was then stirred at 60 °C for 2 h. After the completion of the reaction, reaction mixture was quenched by adding saturated NH₄Cl solution. Reaction mixture was then extracted with EtOAc (3 x 100 ml). Combined organic extracts were then washed with water and brine, dried over anhydrous MgSO₄, filtered and evaporated in vacuo to obtain crude 12a. The crude compound was purified by column chromatography using 20% EtOAc/hexanes as eluent to get pure 12a in 70% yield; ¹H-NMR (400MHz, CDCl₃) δ 7.63 – 7.69 (m, 2H), 7.35 – 7.50 (m, 3H), 6.62 (s, 1H), 5.96 (s, 1H), 4.21 (m, 2H), 3.91 (s, 6H), 3.83 (s, 3H), 1.31 (t, J = 7.2 Hz, 3H); ¹³C-NMR (100MHz, CDCl₃): δ 184.7, 163.6, 158.5, 155.0, 152.9, 138.5, 132.5, 130.4, 129.3, 127.9, 116.9, 114.3, 100.0, 72.4, 62.4, 62.3, 61.2, 56.6, 14.2; MS (m/z): 399, 327, 325, 283, 282, 254, 211, 210, 209; HRMS calcd for C₂₁H₂₁NO₇: 399.1318, found: 399.1326

Preparation of ethyl 2-(6-(cyano(phenyl)methoxy)-2,3,4-trimethoxyphenyl)-2-hydroxyacetate (16a)

In a round bottom flask, starting material 12a (0.5 g, 1.25 mmol) was suspended in anhydrous ethanol under inert conditions in an ice-acetone bath. To this, NaBH₄ was added and the reaction mixture was stirred at room temperature for 3 h. After the completion of reaction, the reaction mixture was quenched with HCl (2.0 M) solution until the gas evolution stopped. Mixture was then diluted with water and extracted with EtOAc (2 x 100 ml). Combined organic extracts were then washed with water and brine, dried over anhydrous MgSO₄, filtered and evaporated in vacuo to obtain crude 16a. The crude compound was purified by column chromatography using 20% EtOAc/hexanes as eluent to get pure 16a in 83% yield; ¹H-NMR (400MHz, CDCl₃) δ 7.55 – 7.65 (m, 2H), 7.43 – 7.53 (m, 3H), 6.55 (d, J = 6.8 Hz, 1H), 5.81 (d, J = 13.2 Hz, 1H), 5.39 (dd, J = 24.4 Hz, J = 6.4
Hz, 1H), 4.18 (m, 2H), 3.89 (d, \( J = 9.2 \) Hz, 3H), 3.85 (d, \( J = 7.6 \) Hz, 3H), 3.82 (d, \( J = 5.2 \) Hz, 3H), 3.54 (m, 1H), 1.18 (dt, \( J = 7.2 \) Hz, \( dJ = 4.8 \) Hz, 3H).

**Preparation of 3-hydroxy-5,6,7-trimethoxy-2-phenyl-4H-chromen-4-one (4a)**

![Chemical structure of 3-hydroxy-5,6,7-trimethoxy-2-phenyl-4H-chromen-4-one (4a)](image)

To a solution of diisopropylamine (0.075 g, 0.75 mmol) in dry THF (10 mL) under argon, was added \( n \)-BuLi (2.5 M in hexanes; 0.30 mL, 0.71 mmol) at \(-78^\circ \)C. The mixture was warmed to \(-40^\circ \)C and stirred at this temperature for 45 minutes. The solution was returned to \(-78^\circ \)C and a solution of 16a (0.13 g, 0.32 mmol) in dry THF was added to it. Resulting reaction mixture was warmed to room temperature and then refluxed with monitoring. After the completion of the reaction, reaction mixture was quenched with HCl (1.0 M) solution and most of the solvent was evaporated *in vacuo*. Residue was then diluted with water and extracted with EtOAc (3 x 50 ml). Combined organic extracts were then washed with water and brine, dried over anhydrous MgSO\(_4\), filtered and evaporated *in vacuo* to obtain crude 4a. The crude compound was then purified by column chromatography using 40% EtOAc/hexanes as eluent to get pure flavonol 4a in 61% yield; \(^1\)H-NMR (400MHz, CDCl\(_3\)) \( \delta 8.21 \) (d, \( J = 8.6 \) Hz, 2H), 7.51 (t, \( J = 6.8 \) Hz, 2H), 7.44 (t, \( J = 7.2 \) Hz, 1H), 6.79 (s, 1H), 4.03 (s, 3H), 3.98 (s, 3H), 3.92 (s, 3H); \(^1^3\)C-NMR (100MHz, CDCl\(_3\)): \( \delta 172.0, 158.6, 154.0, 151.9, 142.7, 140.1, 138.3, 131.3, 130.0, 128.7, 127.5, 110.0, 96.3, 62.5, 61.8, 56.6; MS (\( m/z \)): 329, 328, 326, 314, 313, 267, 167, 105, 77, 69.

**Preparation of 3,5,6,7-tetramethoxy-2-phenyl-4H-chromen-4-one (4b)**

![Chemical structure of 3,5,6,7-tetramethoxy-2-phenyl-4H-chromen-4-one (4b)](image)
Flavonols 4a (0.04 g, 0.12 mmol) was taken in dry actone and anhydrous K$_2$CO$_3$ was added to it under argon. To this, methyl iodide (0.03 g, 0.18 mmol) was added and resulting reaction mixture was refluxed for 6 h. After the completion of reaction, the reaction mixture was filtered through celite and evaporated to dryness. Residue was then diluted with water and extracted with EtOAc (3 x 20 ml). Combined organic extracts were then washed with water and brine, dried over anhydrous MgSO$_4$, filtered and evaporated in vacuo to obtain crude 4b. The crude compound was then purified by column chromatography using 50% EtOAc/hexanes as eluent to get pure flavonol 4b in 61% yield; $^1$H-NMR (400MHz, CDCl$_3$) δ 8.06 (dd, $J = 8.0$ Hz, $J = 2.0$ Hz, 2H), 7.45 – 7.53 (m, 3H), 6.75 (s, 1H), 4.00 (s, 3H), 3.96 (s, 3H), 3.91 (s, 3H), 3.86 (s, 3H); $^{13}$C-NMR (100MHz, CDCl$_3$): δ 174.0, 157.9, 153.9, 153.5, 152.6, 141.6, 140.4, 131.0, 130.6, 128.7, 128.6, 128.4, 113.4, 96.3, 62.4, 61.8, 60.3, 56.5; MS (m/z): 342, 327, 323, 297, 284, 283, 241, 195, 167, 129, 105, 88, 76, 68; HRMS calcd for C$_{19}$H$_{18}$O$_6$: 342.1103, found: 342.1108.

Representative procedure for the preparation of 28a-c

A solution of 3,4,5-trimethoxyphenol 9 (2.30 g, 12.44 mmol), acrylic acid derivative 37 (1.93 g, 13.69 mmol) and DMAP (0.15 g, 1.24 mmol) in 10 ml of dry CH$_2$Cl$_2$ and 2 ml of dry DMF was treated at 0 °C under argon with DCC (2.60 g, 12.44 mmol). The mixture was stirred for 5 min at 0 °C and 30 min at room temperature. After the completion of reaction, reaction mixture was filtered through celite and diluted with CH$_2$Cl$_2$ followed by washing twice with HCl (1.0 M) and twice with saturated solution of NaHCO$_3$. The organic phase was washed with brine, dried over anhydrous MgSO$_4$ and evaporated in vacuo. Residue was purified by column chromatography using 12.5% EtOAc/hexanes as eluent to give pure product.

3,4,5-Trimethoxyphenyl 3,3-dibromoacrylate (28a)
Yield = 71%; $^1$H NMR (400MHz, CDCl$_3$) $\delta$ 7.20 (s, 1H), 6.36 (s, 2H), 3.82 (s, 6H), 3.81 (s, 3H); $^{13}$C-NMR (100MHz, CDCl$_3$): $\delta$ 161.1, 153.6, 146.2, 136.1, 127.1, 109.9, 99.0, 61.1, 56.4; MS (m/z): 396.9113; HRMS calcd for C$_{12}$H$_{12}$Br$_2$O$_5$: 393.9051, found: 393.9056.

3,4,5-Trimethoxyphenyl 3,3-dichloroacrylate (28b)

Representative procedure for Fries rearrangement to prepare 29a-c

Ester 28b (1.0 g, 3.26 mmol) was taken in dry 1,2-dichloroethane (75 ml) and added to a slurry of AlCl$_3$ (0.48 g, 3.60 mmol) in 1,2-dichloroethane (25 ml) at 0 °C under argon. Resulting dark brown solution was refluxed with monitoring. After the completion of reaction, the reaction mixture was poured over 1:1 mixture of ice and HCl (1.0 M) and stirred for 30 min. Organic phase was separated and aqueous layer was extracted with CH$_2$Cl$_2$ (3 x 100 ml). Combined organic phases were then washed with water, brine and dried over anhydrous MgSO$_4$. Solvent was evaporated under reduced pressure and crude product was purified by column chromatography using 10% EtOAc/hexanes as eluent.
3,3-Dibromo-1-(6-hydroxy-2,3,4-trimethoxyphenyl)prop-2-en-1-one (29a)

![Chemical Structure](image)

Yield: 41%; $^1$H-NMR (400MHz, CDCl$_3$) $\delta$ 12.78 (s, 1H), 7.86 (s, 1H), 6.24 (s, 1H), 3.94 (s, 3H), 3.88 (s, 3H), 3.77 (s, 3H); $^{13}$C-NMR (100MHz, CDCl$_3$): $\delta$ 191.1, 162.9, 161.6, 154.8, 137.6, 108.2, 97.6, 96.6, 96.5, 61.9, 61.4, 56.5; MS ($m/z$): 396.9113; HRMS calcd for C$_{12}$H$_{12}$Br$_2$O$_5$: 393.9051, found: 393.9056.

3,3-Dichloro-1-(6-hydroxy-2,3,4-trimethoxyphenyl)prop-2-en-1-one (29b)

![Chemical Structure](image)

Yield: 40%; $^1$H-NMR (400MHz, CDCl$_3$) $\delta$ 12.90 (s, 1H), 7.40 (s, 1H), 6.25 (s, 1H), 3.93 (s, 3H), 3.89 (s, 3H), 3.78 (s, 3H); $^{13}$C-NMR (100MHz, CDCl$_3$): $\delta$ 189.8, 162.8, 161.3, 154.7, 135.3, 131.4, 128.9, 108.4, 96.6, 61.9, 61.4, 56.4; MS ($m/z$): 307.0128; HRMS calcd for C$_{12}$H$_{12}$Cl$_2$O$_5$: 306.0062, found: 306.0055.

3,3-Dichloro-1-(2-hydroxy-4,6-dimethoxyphenyl)prop-2-en-1-one (29c)

![Chemical Structure](image)

Yield: 60%; $^1$H-NMR (400MHz, CDCl$_3$) $\delta$ 13.46 (s, 1H), 7.35 (s, 1H), 6.07 (d, J = 4.0 Hz, 1H), 5.90 (d, J = 2.2 Hz, 1H), 3.85 (s, 3H), 3.82 (s, 3H); $^{13}$C-NMR (100MHz, CDCl$_3$): $\delta$ 189.3, 168.4, 167.3, 162.3, 131.6, 129.3, 106.1, 94.0, 91.5, 56.3, 55.9; MS ($m/z$): 277.0026, 241.0263, 223.0596, 197.0805; HRMS calcd for C$_{11}$H$_{10}$Cl$_2$O$_4$: 275.9956, found: 275.9953.

Representative procedure for cyclization reaction to prepare 30a-c
Phenol 29b was taken in THF (5 ml) and to this, NaOH (33.0 ml, 0.02 N) solution was added at 0 °C. Resulting reaction mixture was stirred at room temperature for 3 h with monitoring. After the completion of reaction, reaction mixture was acidified by HCl (1.0 M) to a pH 5 and extracted with EtOAc (3 x 50 ml). Combined organic extracts were then washed with water and brine, dried over anhydrous MgSO₄, filtered and evaporated in vacuo to obtain crude chromone 30b. The crude compound was then purified by column chromatography using 50% EtOAc/hexanes as eluent.

2-(Bromomethylene)-4,5,6-trimethoxybenzofuran-3(2H)-one (30a)

![Chemical Structure](image)

Yield: 64%; ¹H-NMR (400MHz, CDCl₃) δ 6.74 (s, 1H), 6.48 (s, 1H), 4.19 (s, 3H), 3.94 (s, 3H), 3.78 (s, 3H); ¹³C-NMR (100MHz, CDCl₃): δ 177.4, 164.0, 162.4, 152.3, 152.0, 136.9, 107.2, 94.8, 90.8, 62.5, 61.8, 56.9; MS (m/z): 314.9858; HRMS calcd for C₁₂H₁₁BrO₅: 314.9863, found: 314.9858.

2-Chloro-5,6,7-trimethoxy-4H-chromen-4-one (30b)

![Chemical Structure](image)

Yield: 65%; ¹H-NMR (400MHz, CDCl₃) δ 6.70 (s, 1H), 6.24 (s, 1H), 3.94 (s, 6H), 3.89 (s, 3H); ¹³C-NMR (100MHz, CDCl₃): δ 175.8, 158.0, 154.7, 153.9, 152.8, 141.1, 112.1, 111.8, 96.3, 62.4, 61.7, 56.5; MS (m/z): 271.0361, 139.9717; HRMS calcd for C₁₂H₁₁ClO₅: 270.0295, found: 270.0288.

2-Chloro-5,7-dimethoxy-4H-chromen-4-one (30c)
Representative procedure for Suzuki coupling to prepare aurones 21a-c

A 100 ml oven dried round bottom flask, equipped with a stir bar is charged with anhydrous dioxane (10 ml), powdered anhydrous K$_2$CO$_3$ (0.26 g, 1.90 mmol), phenylboronic acid (31a) (0.1161 g, 0.95 mmol) and aurone precursor 30a (0.20 g, 0.635 mmol). Nitrogen was passed through the resulting reaction mixture for at least 20 min. To this was added the Pd(PPh$_3$)$_4$ (0.037g, 0.032 mmol) catalyst and resulting reaction mixture was heated to 90 °C and stirred with constant monitoring. After the completion of the reaction (6 h), reaction mixture was filtered through celite and evaporated under reduced pressure. The residue was partitioned between water and ethyl acetate and organic phase was separated. Aqueous layer was washed with ethyl acetate (2 x 30 ml) and all organic phases were mixed together, washed with brine and dried over anhydrous magnesium sulfate. Solvent was evaporated under reduced pressure to obtain crude aurone 21a which was purified by silica gel column chromatography using 50% EtOAc:hexanes as eluent to obtain pure aurone 21a in 85% isolated yield.

(Z)-2-benzylidene-4,5,6-trimethoxybenzofuran-3(2H)-one (21a)

Yield: 85%; $^1$H-NMR (400MHz, CDCl$_3$) $\delta$ 7.86 (d, $J = 8.0$ Hz, 2H), 7.43 (t, $J = 8.0$ Hz, 2H), 7.37 (d, $J = 4.0$ Hz, 1H), 6.76 (s, 1H), 6.54 (s, 1H), 4.25 (s, 3H), 3.97 (s, 3H), 3.82 (s, 3H);
$^{13}$C-NMR (100MHz, CDCl$_3$): $\delta$ 181.0, 164.3, 161.9, 151.9, 147.9, 136.8, 132.7, 131.4, 129.7, 129.0, 111.4, 107.2, 90.8, 62.6, 61.9, 56.9; MS (m/z): 313.11, 187.08, 121.05; HRMS calcd for C$_{18}$H$_{16}$O$_5$: 313.1071, found: 313.1074.

(Z)-4,5,6-trimethoxy-2-(4-methoxybenzylidene)benzofuran-3(2H)-one (21b)

Yield: 81%; $^1$H-NMR (400MHz, CDCl$_3$) $\delta$ 7.82 (d, $J = 12.0$ Hz, 2H), 6.95 (d, $J = 8.0$ Hz, 2H), 6.75 (s, 1H), 6.54 (s, 1H), 4.25 (s, 3H), 3.97 (s, 3H), 3.86 (s, 3H), 3.82 (s, 3H); $^{13}$C-NMR (100MHz, CDCl$_3$): $\delta$ 181.0, 164.0, 161.6, 160.9, 151.8, 146.8, 136.7, 133.2, 125.4, 114.6, 111.7, 107.5, 90.7, 62.7, 61.9, 56.8, 55.6; MS (m/z): 343.1169; HRMS calcd for C$_{19}$H$_{18}$O$_6$: 342.1103, found: 342.1096.

(Z)-2-(3,4-dimethoxybenzylidene)-4,5,6-trimethoxybenzofuran-3(2H)-one (21c)

Yield: 80%; $^1$H-NMR (400MHz, CDCl$_3$) $\delta$ 7.43 – 7.48 (m, 2H), 6.93 (d, $J = 8.0$ Hz, 1H), 6.74 (s, 1H), 6.51 (s, 1H), 4.26 (s, 3H), 3.99 (s, 3H), 3.97 (s, 3H), 3.94 (s, 3H), 3.82 (s, 3H); $^{13}$C-NMR (100MHz, CDCl$_3$): $\delta$ 180.9, 163.9, 161.6, 151.8, 150.7, 149.2, 146.8, 136.8, 125.7, 125.6, 113.7, 112.0, 111.4, 107.5, 90.7, 62.7, 61.9, 56.9, 56.2, 56.1; MS (m/z): 373.1287; HRMS calcd for C$_{20}$H$_{20}$O$_7$: 372.1209, found: 372.1214.

Representative procedure for Suzuki coupling to prepare flavones 1a-e

A 100 ml oven dried round bottom flask, equipped with a stir bar is charged with anhydrous dioxane (10 ml), powdered anhydrous K$_2$CO$_3$ (0.345 g, 2.49 mmol),
phenylboronic acid (31a) (0.2030 g, 1.66 mmol) and chromone 30c (0.20 g, 0.831 mmol). Nitrogen was passed through the resulting reaction mixture for at least 20 min. To this was added the Pd(PPh₃)₄ (0.048 g, 0.042 mmol) catalyst and resulting reaction mixture was refluxed with constant monitoring. After the completion of the reaction (18 h), reaction mixture was filtered through celite and evaporated under reduced pressure. The residue was partitioned between water and ethyl acetate and organic phase was separated. Aqueous layer was washed with ethyl acetate (2 x 30 ml) and all organic phases were mixed together, washed with brine and dried over anhydrous magnesium sulfate. Solvent was evaporated under reduced pressure to obtain crude flavone 1b which was purified by silica gel column chromatography using 2% MeOH:DCM as eluent to obtain pure flavone 1b in 74% isolated yield.

5,6,7-Trimethoxy-2-phenyl-4H-chromen-4-one (1a)

Yield: 67%; ¹H-NMR (400MHz, CDCl₃) δ 7.84 – 7.88 (m, 2H), 7.50 (d, J = 2.0 Hz, 2H), 7.48 (d, J = 2.0 Hz, 1H), 6.80 (s, 1H), 6.66 (s, 1H), 3.98 (s, 3H), 3.97 (s, 3H), 3.91 (s, 3H); ¹³C-NMR (100MHz, CDCl₃): δ 177.4, 161.3, 157.9, 154.7, 152.7, 140.5, 131.7, 131.5, 129.1, 126.1, 113.1, 108.6, 96.5, 62.4, 61.7, 56.5.

5,7-dimethoxy-2-phenyl-4H-chromen-4-one (1b)

Yield: 74%; ¹H-NMR (400 MHz, CDCl₃) δ 7.84 – 7.88 (m, 2H), 7.46 – 7.52 (m, 3H), 6.68 (s, 1H), 6.57 (d, J = 2.3 Hz, 1H), 6.37 (d, J = 2.3 Hz, 1H), 3.95 (s, 3H), 3.91 (s, 3H); ¹³C-NMR (100 MHz, CDCl₃): δ 177.8, 164.2, 161.0, 160.8, 160.1, 131.7, 131.4, 129.1, 126.1, 109.3,
109.2, 96.4, 93.0, 56.7, 56.0; MS (m/z): 283.0963, 269.0804; HRMS calcd for C_{17}H_{14}O_{4}: 282.0892, found: 282.0890.

5,7-dimethoxy-2-(4-methoxyphenyl)-4H-chromen-4-one (1c)

Yield: 72%; \(^1\)H-NMR (400MHz, CDCl\(_3\)) \(\delta\) 7.78 (d, \(J = 8.0\) Hz, 2H), 6.96 (d, \(J = 8.0\) Hz, 2H), 6.56 (s, 1H), 6.51 (d, \(J = 4.0\) Hz, 1H), 6.32 (d, \(J = 4.0\) Hz, 1H), 3.92 (s, 3H), 3.88 (s, 3H), 3.85 (s, 3H); \(^{13}\)C-NMR (100 MHz, CDCl\(_3\)): \(\delta\) 177.6, 163.8, 161.9, 160.7, 160.6, 159.7, 127.5, 123.7, 114.3, 109.1, 107.6, 96.0, 92.8, 56.4, 55.7, 55.4; MS (m/z): 313.1062; HRMS calcd for C_{18}H_{16}O_{5}: 313.0998, found: 313.0989.

2-(3,4-dimethoxyphenyl)-5,7-dimethoxy-4H-chromen-4-one (1d)

Yield: 69%; \(^1\)H-NMR (400MHz, CDCl\(_3\)) \(\delta\) 7.46 (d, \(J = 8.0\) Hz, 1H), 6.92 (d, \(J = 8.0\) Hz, 1H), 6.57 (s, 1H), 6.52 (s, 1H), 6.33 (s, 1H), 3.94 (s, 3H), 3.92 (s, 3H), 3.89 (s, 3H); \(^{13}\)C-NMR (100 MHz, CDCl\(_3\)): \(\delta\) 177.8, 164.1, 161.0, 160.8, 160.0, 151.8, 149.3, 124.1, 119.6, 111.2, 109.3, 108.6, 108.1, 108.0, 96.3, 93.0, 56.6, 56.3, 56.0; MS (m/z): 343.1177, 329.1014; HRMS calcd for C_{19}H_{18}O_{6}: 342.1103, found: 342.1104.

References


GENERAL CONCLUSION

In this dissertation, we have investigated the synthesis of various biologically important aromatic heterocycles.

In the first chapter, use of phosphazene base P₄-t-Bu towards generating benzylic anions is described. The synthetic route features the formation of three distinct aromatic heterocycles via a novel cyclodehydration step. Additionally, we synthesized indolo[2,1-a] based natural product ortho-methylcryptaustoline iodide and 2,3-diarylbenzo[b]furan based amurensin H. The key step in the total synthesis of both natural products was P₄-t-Bu mediated cyclization.

The second chapter describes our efforts towards the development of new abelson kinase inhibitors. Our investigation in this direction led us to successfully synthesize a variety of substituted pyrido[2,3-d]pyrimidines. Some of the compounds thus made turned out to be better inhibitors of Abelson kinase than currently available drugs.

The third chapter discusses our efforts towards the synthesis of various flavonoids. We initially targeted flavonols but eventually developed a novel methodology which can be used to synthesize either aurones or flavones by one small modification. We also successfully prepared many naturally occurring flavonols and flavones using this novel method.
Completing a Ph.D. is one of life’s greatest experiences and I have been privileged over these last five years to have had the opportunity to have met some truly inspiring people. Coming to contact with them during my time at ISU has given me a new perspective about the complexities of both life and science, and this has imprinted me with a new set of values to face life and its ever forthcoming intricacies. I immediately think of my research adviser, Dr. George A. Kraus, as I write down these lines. His pristine passion for chemistry and science has not skipped a beat— from the day I walked into his office as a first-year graduate student – until this day. I am deeply grateful to him to have simply transformed my thought process in science and showed me the true meaning of the words, ‘searching for excellence’. His unending energy and guidance has been the main driving force for me.

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I end with the inspiring words from Cynthia Heimel:

*When in doubt, make a fool of yourself. There is a microscopically thin line between being brilliantly creative and acting like the most gigantic idiot on earth. So, what the hell, leap.*