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New synthetic methods for biologically active aromatic heterocycles

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by

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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 P_4-*t*-Bu: Application towards the synthesis of various heterocylic 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.[1a-b] 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 pK_a values (Figure 1).[1a-b] Among the strongest of these phosphazene bases, P_4-*t*-Bu is most readily available.[1a]

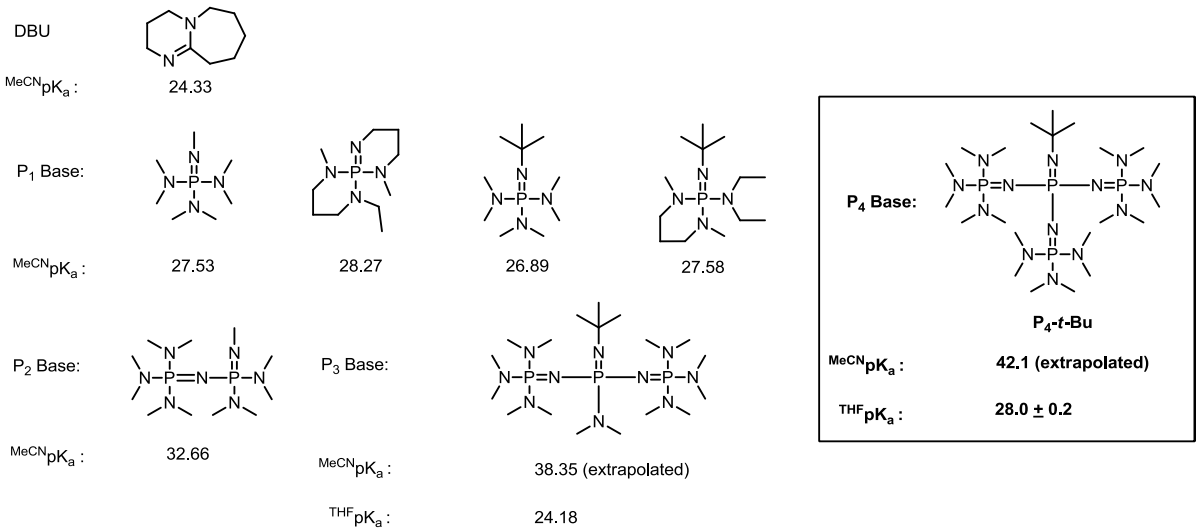
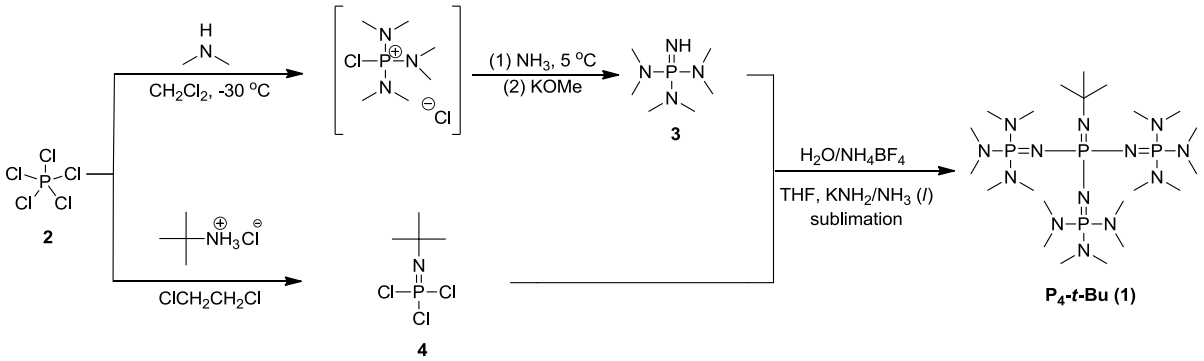


Figure 1: Structures and comparison of pK_a values of DBU and various phosphazene bases[1b]

P_4-*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).[2] The strongly hygroscopic, colorless, crystalline base **1** is unusually stable and is very soluble in the conventional solvents. In 1.0 M D_2O solution, **1** remains unchanged even at about 160 ^oC over twenty hours; however, aqueous mineral acids readily hydrolyze it.[3]

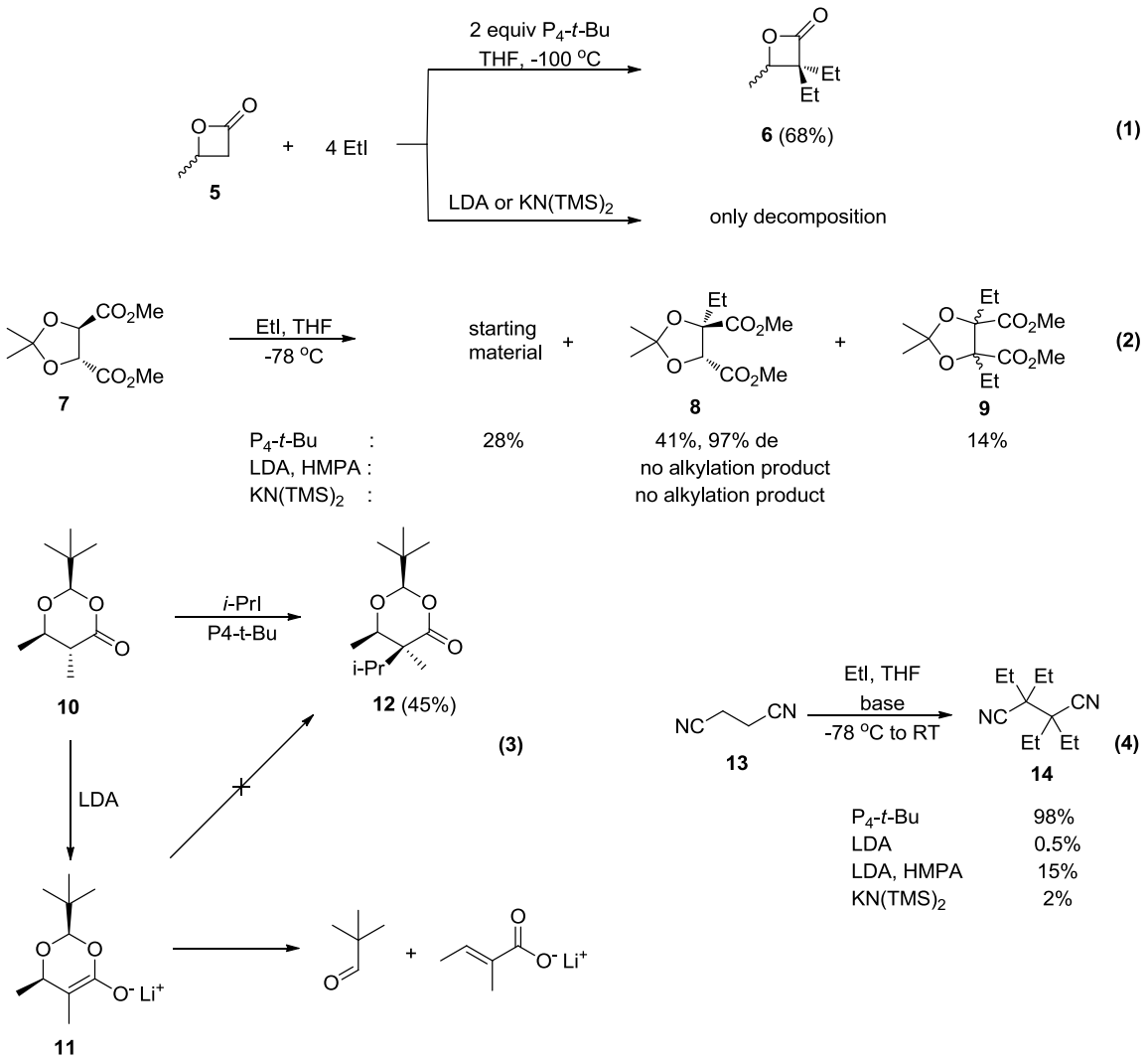


Scheme 1: Synthesis of P_4-*t*-Bu

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 ^oC to -78 ^oC. 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_2Cl_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

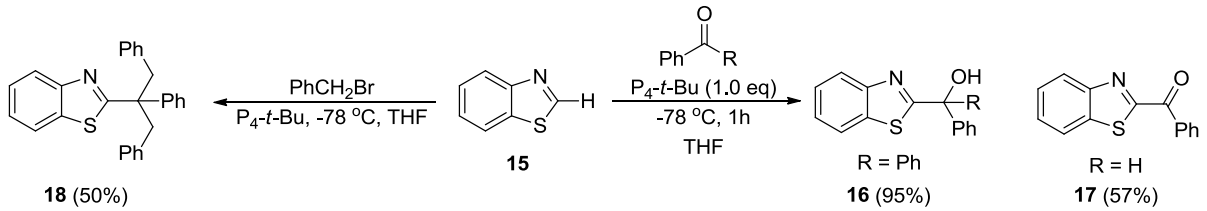
ester condensations in alkylations of enolates, are effectively suppressed; even β-lactones are easily mono- or peralkylated (Scheme 2, eq 1).[3] In cases where the corresponding lithium derivatives decompose entirely via β-alkoxide elimination, 'naked' enolates of β-alkoxy esters undergo clean alkylation. Using bases like P_4-*t*-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*-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. P_4-*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 P_4-*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 P_4-*t*-Bu base cannot function as a Lewis acid. Therefore, the reaction using P_4-*t*-Bu base proceeds without the "coordination mechanism", and the reaction with unique regioselectivity is expected and observed.[1b, 6a]

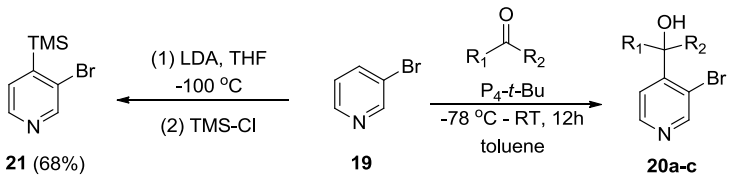
In the first example to explore the deprotonative functionalization of P_4-*t*-Bu, the reactions of benzothiazole **15** were examined. When **15** was reacted with P_4-*t*-Bu in the presence of benzophenone in THF, adduct **16** was formed in 95% yield. Similar reactions of **15** with benzaldehyde and benzyl bromide gave addition products **17** and **18**, respectively, in reasonable yields (Scheme 3).



Scheme 3: Deprotonative 1,2-Addition of Benzothiazole with P_4-*t*-Bu[1b, 6a]

Deprotonative functionalizations of π-deficient nitrogen heteroaromatics, which have relatively acidic ring-protons, were also reported. *meta*-Bromopyridine **19** was reacted with

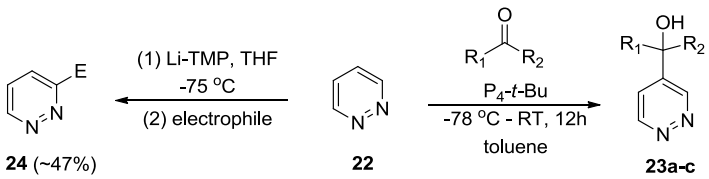
P_4-*t*-Bu in the presence of benzophenone resulting in the deprotonative functionalization taking place at the expected 4-position to give **20a** in only 3% yield, but was enhanced to 77% by using ZnI_2 as additive. Reactions with benzaldehyde and pivalaldehyde under similar conditions gave expected addition products **20b** and **20c** in excellent yields (Scheme 4).[1b, 6a]



R_1	R_2	Additive	P_4-*t*-Bu	Product (%)
Ph	Ph	none	1 eq	20a (3%)
Ph	Ph	ZnI_2	2 eq	20a (77%)
Ph	H	ZnI_2	2 eq	20b (85%)
t-Bu	H	ZnI_2	2 eq	20c (92%)

Scheme 4: Deprotonative 1,2-Addition of 3-Bromopyridine with P_4-*t*-Bu

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 P_4-*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]

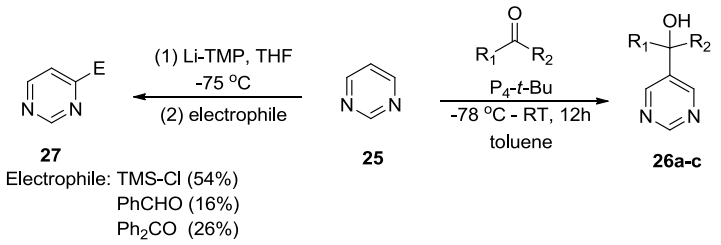


R_1	R_2	Additive	P_4-*t*-Bu	Product (%)
Ph	Ph	ZnI_2	1 eq	23a (57%)
Ph	Ph	ZnI_2	1.5 eq	23a (91%)
Ph	H	ZnI_2	1.5 eq	23b (0%)*
t-Bu	H	ZnI_2	1.5 eq	23c (73%)

* 36% of benzoyl pyridazine was obtained.

Scheme 5: Deprotonative 1,2-Addition of Pyridazine with P_4-*t*-Bu

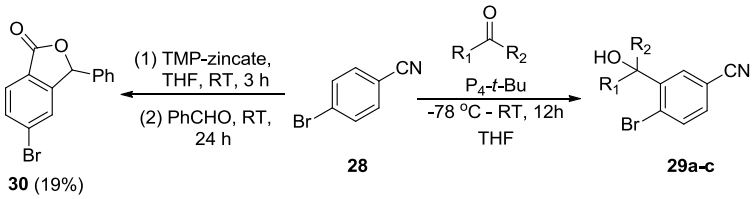
Intrigued by regioselective results of azine **22**, when pyrimidine **25** was subjected to similar conditions, it resulted in the formation of addition products **26a**, **26b** and **26c** which followed the regioselectivity pattern of azines displaying regioselectivity which is the opposite of the direct ortho metallation product (Scheme 6).[1b, 6a]



R_1	R_2	Additive	P_4-*t*-Bu	Product (%)
Ph	Ph	ZnI_2	1 eq	26a (19%)
Ph	Ph	ZnI_2	3 eq	26a (48%)
Ph	H	ZnI_2	3 eq	26b (80%)
t-Bu	H	ZnI_2	3 eq	26c (65%)

Scheme 6: Deprotonative 1,2-Addition of Pyrimidine with P_4-*t*-Bu

These exciting results led Kondo and coworkers to investigate the deprotonative aromatization of substituted benzenes. *para*-Bromobenzonitrile (**28**) was treated with P_4-*t*-Bu base in the presence of benzophenone and ZnI_2 in THF, and the reaction proceeded chemoselectively at the 3-position (**29a**) in 88% yield without ZnI_2. 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]

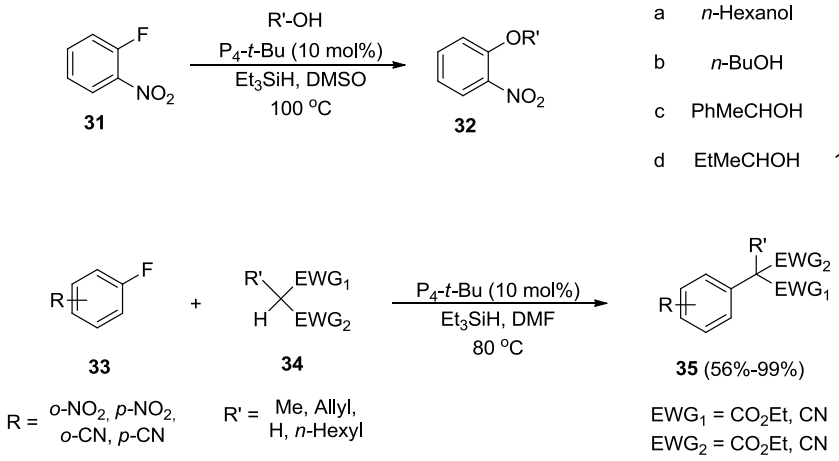


R_1	R_2	Additive	P_4-*t*-Bu	Product (%)
Ph	Ph	none	3 eq	29a (88%)
Ph	Ph	ZnI_2	3 eq	29a (60%)
Ph	H	ZnI_2	3 eq	29b (86%)
t-Bu	H	ZnI_2	3 eq	29c (87%)

Scheme 7: Deprotonative 1,2-Addition of 4-Bromobenzonitrile with P_4-*t*-Bu

The examples detailed above show the utility of P_4-*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 P_4-*t*-Bu. The examples discussed below, will describe the utility of P_4-*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_NAr) reaction is one of the most fundamental and widely used transformations in synthetic organic chemistry.[7] Various nuclephiles, 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_3SiH with which sequential deprotonation and S_NAr 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_3SiH and 10 mol% of P_4-*t*-Bu at 100 ^oC for two hours. The arylation reaction proceeded to give ether **32a** in quantitative yield.[7] 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_3SiH/catalytic P_4-*t*-Bu system to effect the S_NAr reaction of C-nucleophiles. Diethyl methylmalonate was reacted with *o*-fluoronitrobenzene at 80 ^oC 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.[7] 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 succefully 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_NAr reaction with these C-nuclephiles and this Et_3SiH/ catalytic P_4-*t*-Bu system provides a new and effective S_NAr reaction protocol.[7]

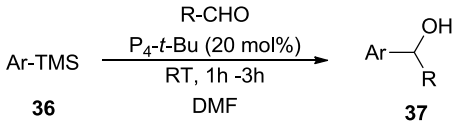


#	R'-OH	*t*(h)	%
a	*n*-Hexanol	2	100
b	*n*-BuOH	2	97
c	PhMeCHOH	2	92
d	EtMeCHOH	18	71

Scheme 8: S_NAr using Et_3SiH/P_4-*t*-Bu as a system for nucleophile activation[7]

Another work from Kondo and coworkers describes the P_4-*t*-Bu promoted functionalization of aryltrimethylsilanes.[8] Aryltrimethylsilanes have been used as important synthons and various desilylative functionalization have been investigated to date.[8] They have demonstrated the utility of P_4-*t*-Bu for the activation of aryltrimethylsilanes. Initially, 1-trimethylsilylnaphthalene **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-trimethylsilylnaphthalene **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 **37**g and **37h** (Scheme 9, Table: entry g-h). In summary, they found that arylsilanes can be carbo-desilylated by the use of

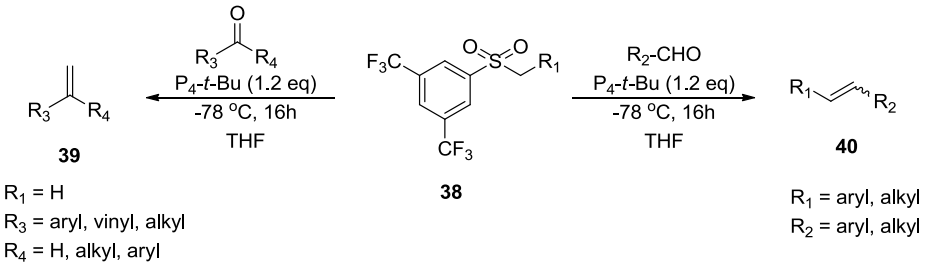
catalytic P_4-*t*-Bu as a promoter and the selective functionalizations of arylsilanes possessing no strong electron-withdrawing group can be acomplished.[8]



#	Ar	R	%
a	1-Naphthyl	*t*-Bu	91
b	1-Naphthyl	Ph	61
c	2-Naphthyl	*t*-Bu	68
d	4-BrC_6H_4	*t*-Bu	73
e	2-$CF_3C_6H_4$	*t*-Bu	73
f	4-$MeO_2CC_6H_4$	*t*-Bu	46
g	2-Pyridyl	*t*-Bu	67
h	3-Pyridyl	*t*-Bu	80

Scheme 9: P_4-*t*-Bu promoted functionalization of aryltrimethylsilanes.[8]

Another work, reported by Nájera and coworkers use stoichiometric amount of P_4-*t*-Bu in Julia-Kocienski olefination to synthesize various substituted olefins.[9a-b] They used various alkyl 3,5-bis(trifluoromethyl)phenylsulfones (BTFP sulfones) **38** and generated stabilized anions by using stoichiometric amount of various bases like P_4-*t*-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]

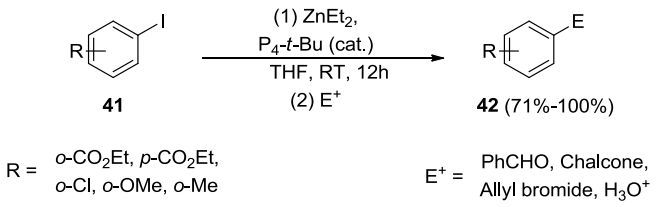


Scheme 10: P_4-*t*-Bu mediated Julia-Kocienski Olefination of BTFP sulfones.[9a,b]

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 P_4-*t*-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.

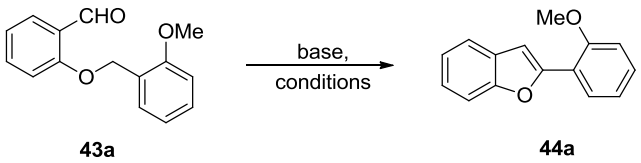
Other work by Kondo and coworkers shows that the use of catalytic P_4-*t*-Bu dramatically improves the performance of halogen-zinc exchange of aryl iodides.[10] In recent years, organiczinc compounds have been widely used in organic synthesis and one of the most powerful methods for the preparation of functionalized organozinc derivatives is halogen-zinc exchange reaction.[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 benzhydrol 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).[10]



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.[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 $POCl_3$ 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 P_4-*t*-Bu in pivalonitrile at 90 ^oC, it successfully cyclized into benzofuran **44a** in 49% yield. When benzene was used as the solvent, the yield was 47% (Scheme 12).

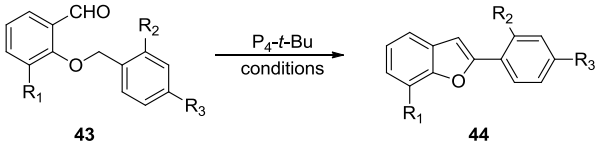


#	Base	Solvent	Conditions	Result
1	LDA	THF	-78 ^oC - RT	NR
2	LiTMP	THF	0 ^oC - RT	decomp.
3	NaH	DMF	reflux	NR
4	KH	DMF	reflux	NR
5	**P_4-*t*-Bu**	Benzene	reflux	**47%**
6	**P_4-*t*-Bu**	Pivalonitrile	90 ^oC	**49%**

Scheme 12: P_4-*t*-Bu promoted cyclization of *o*-substituted benzaldehyde.[11]

Using this model, the scope of this intramolecular cyclization with a series of aldehydes was explored and they were reacted with P_4-*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 P_4-*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 P_4-*t*-Bu with substituted *o*-benzyloxybenzaldehydes offered a new convenient and concise pathway to arylbenzofurans.

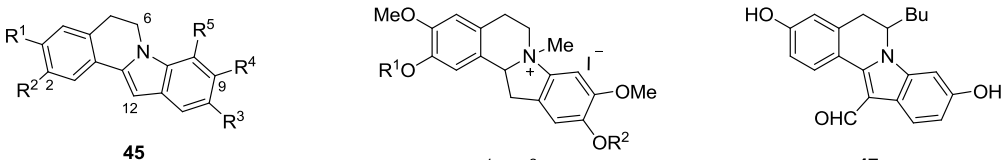


#	R_1	R_2	R_3	solvent	time (h)	*T* (°C)	% yield
a	H	OMe	H	benzene	12	reflux	47
b	H	OMe	H	pivalonitrile	8	90	49
c	OMe	OMe	H	benzene	14	reflux	48
d	OMe	OMe	H	pivalonitrile	12	90	51
e	H	H	H	benzene	14	reflux	69
f	H	MOM	H	benzene	12	reflux	50
g	H	H	NO_2	benzene	1	reflux	78

Scheme 13: P_4-*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 P_4-*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.

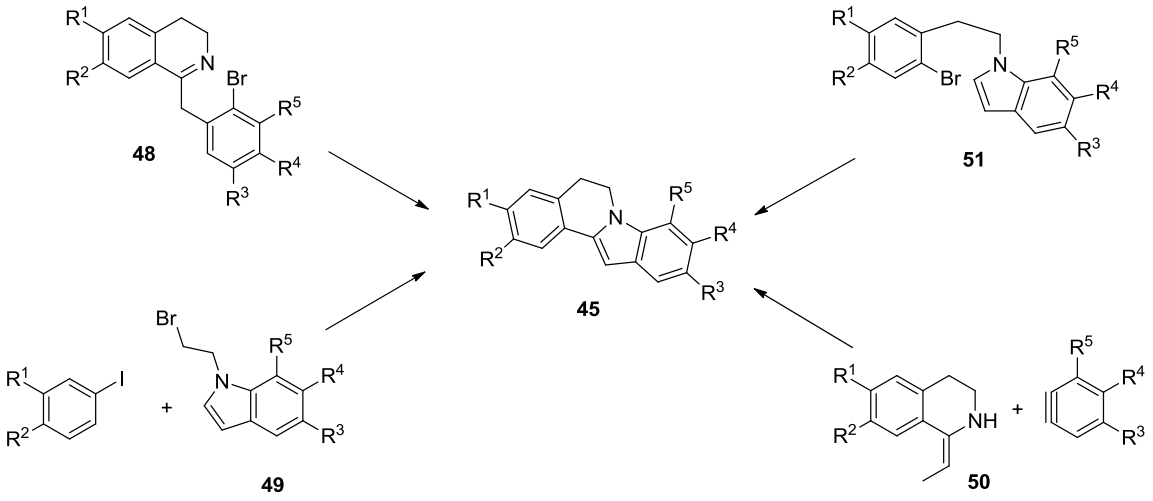


46a: $R^1 = R^2 = H$
46b: $R^1 = H$, $R^2 = Me$
46c: $R^1 = R^2 = Me$

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 activity[17]. Compound **47** strongly inhibited tubulin polymerization.[18]

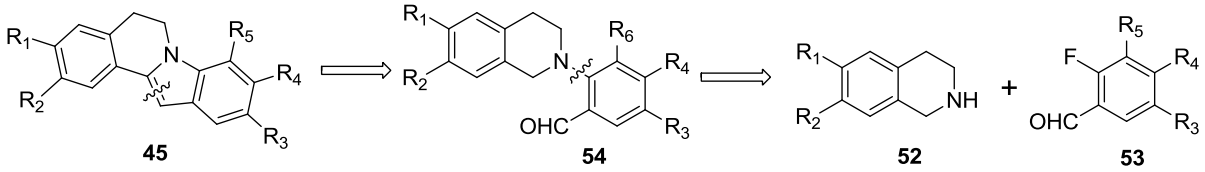
Four most versatile methods for the synthesis of indolo[*2,1-a*]isoquinolines have been reported. Orito reported the cyclization of 1-bromobenzyl-5,6-dihydroisoquinolines **48** by the nucleophilic addition of the dihydroisoquinoline nitrogen atom to the bromobenzyl moiety.[19] This work is depicted 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

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-a*]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: Reterosynthetic analysis

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-a*]isoquinoline **45a** was produced in 35% isolated yield.

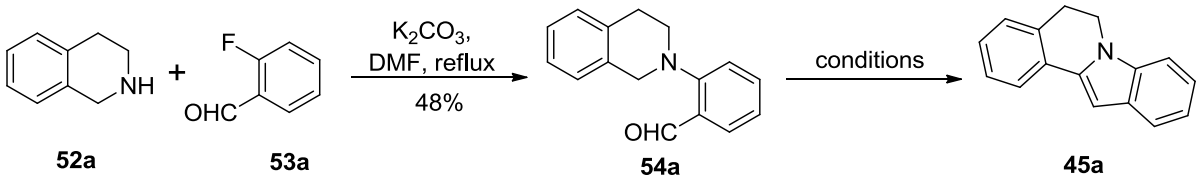
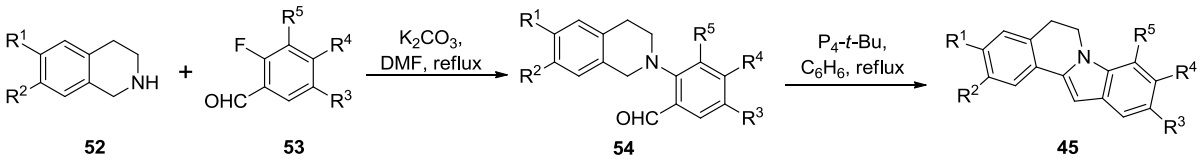


Table 1: Cyclization conditions

#	Base	Solvent	Conditions	Result
1	LDA	THF	-78 °C - RT	NR
2	LiTMP	THF	0 °C - RT	decomp.
3	NaH	DMF	reflux	NR
4	KH	DMF	reflux	NR
5	*t*-BuOK	DMF	reflux	NR
6	**P_4-*t*-Bu**	Benzene	reflux	**35%**

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.



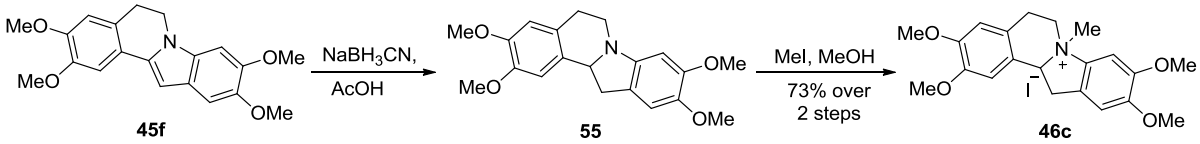
#	R_1	R_2	R_3	R_4	R_5	% yield of **54a - j**	% yield of **45a – 1j**
a	H	H	H	H	H	48	35
b	H	H	H	H	OMe	42	52
c	H	H	OMe	OMe	H	48	38
d	OMe	OMe	H	H	H	48	25
e	OMe	OMe	H	H	OMe	37	32
f	OMe	OMe	OMe	OMe	H	40	39
g	H	H	CF_3	H	H	60	44
h	OMe	OMe	CF_3	H	H	66	35
i	H	H	H	CF_3	H	52	42
j	OMe	OMe	H	CF_3	H	53	39
k	H	H	Br	H	H	56	0

Table 2: Synthesis of 5,6-dihydroindolo[*2,1-a*]isoquinolines

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, [27] 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 P_4-*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 literature[27] compound.

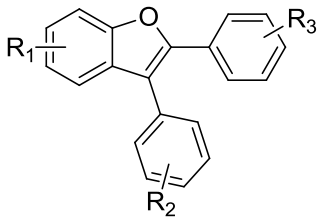


Scheme 17: Synthesis of *O*-methylcryptaustoline iodide

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 cyotoxic T lymphocytes, and exhibits anti-viral activity, but may also result in immunopathology.[19] Again, every compound (**45a-j**) inhibited production of IFNγ.19 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.

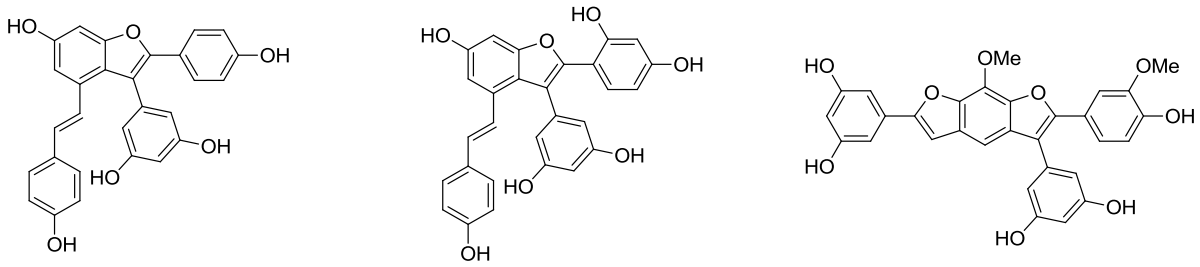
In order to further explore the synthetic potential of the hindered phosphazene base P_4-*t*-Bu,[12] we diverted our attention towards the synthesis of 2,3-diarylbenzo[*b*]furans (Figure 3).



Diarylbenzo[*b*]furan (**47**)

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.[29] 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.[2] It has also been reported to treat chronic obstructive pulmonary disease.[30] It has been synthesized by an oxidative coupling reaction according to its biogenetic pathway.[31] 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.[32] Gnetuhainin G (**50**) is a novel furobenzofuran from *Gnetum hainanense* that is an antioxidant.[33] alpha-Viniferin, structurally related to **48**, has been reported to be a potent MRP1 transport inhibitor.[34]



Amurensin H (**48**) Gnetuhainin B (**49**) Gnetuhainin G (**50**)

Figure 4

Several synthetic routes to benzofurans such as **47** are known.[35] The majority of these syntheses take place via disconnection A (Figure 2). This includes the reaction of *ortho*-halophenols with acetylenes[36], and the oxidative cyclization[37] of hydroxy stilbenes. A related pathway is the palladium-mediated arylation of benzofurans.[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.[39]

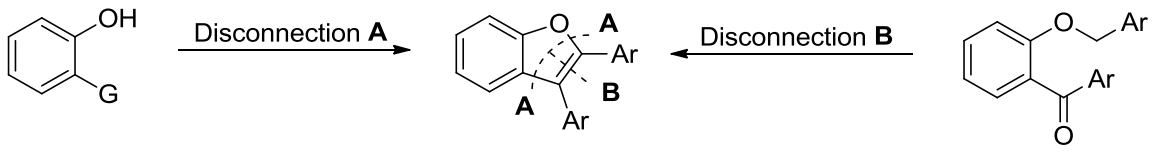


Figure 5

Our proposed reterosynthetic 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[*b*]furan (**47**).

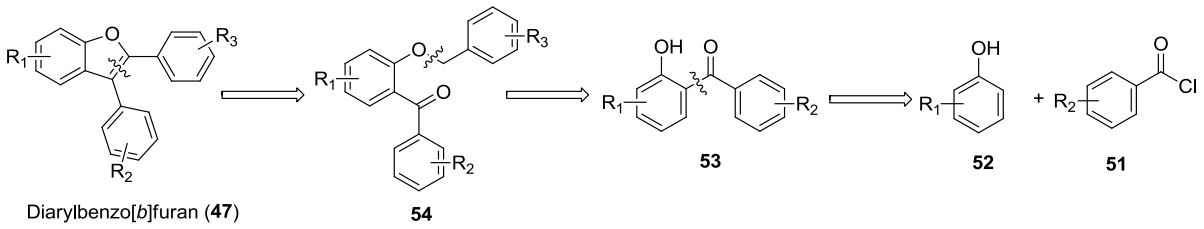
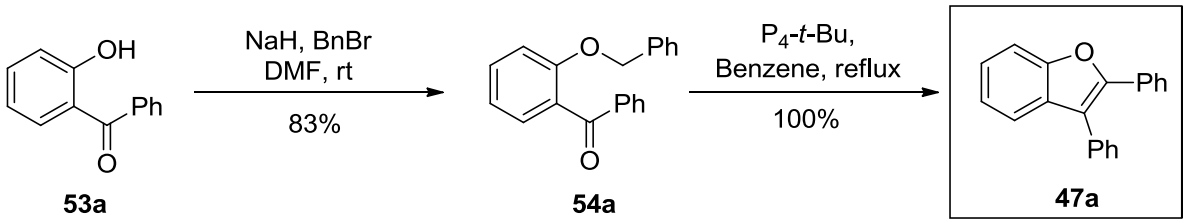


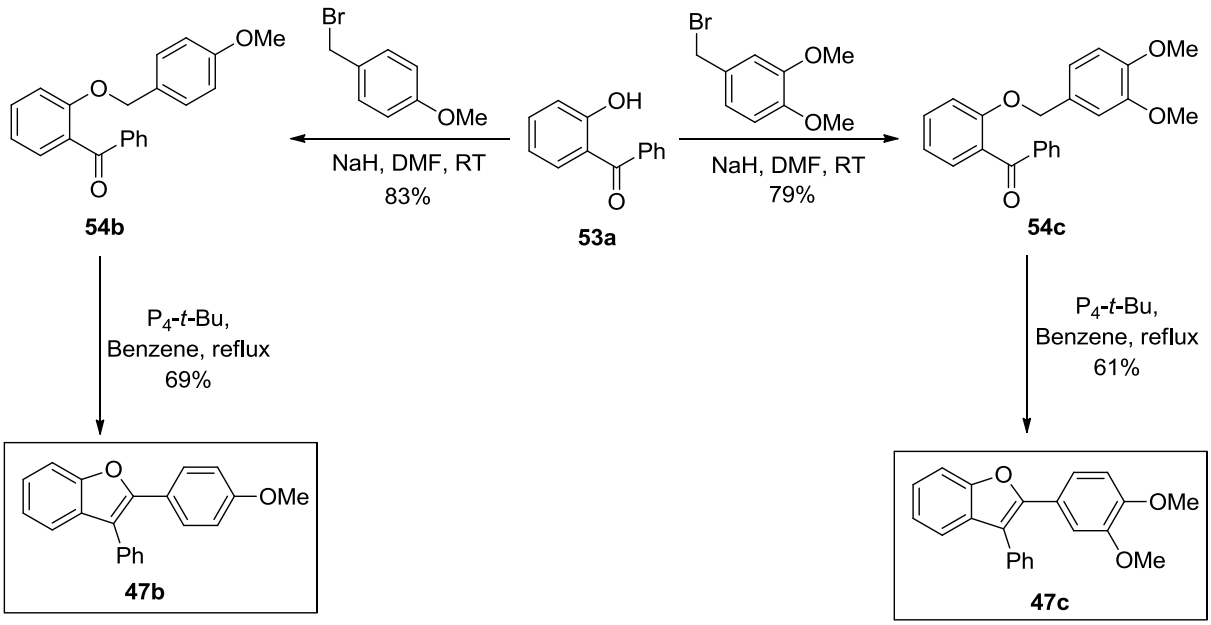
Figure 6: Reterosynthetic analysis

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 P_4-*t*-Bu and 2,3-diphenylbenzo[*b*]furan (**47a**) was isolate in quantitative yield (Scheme 18).



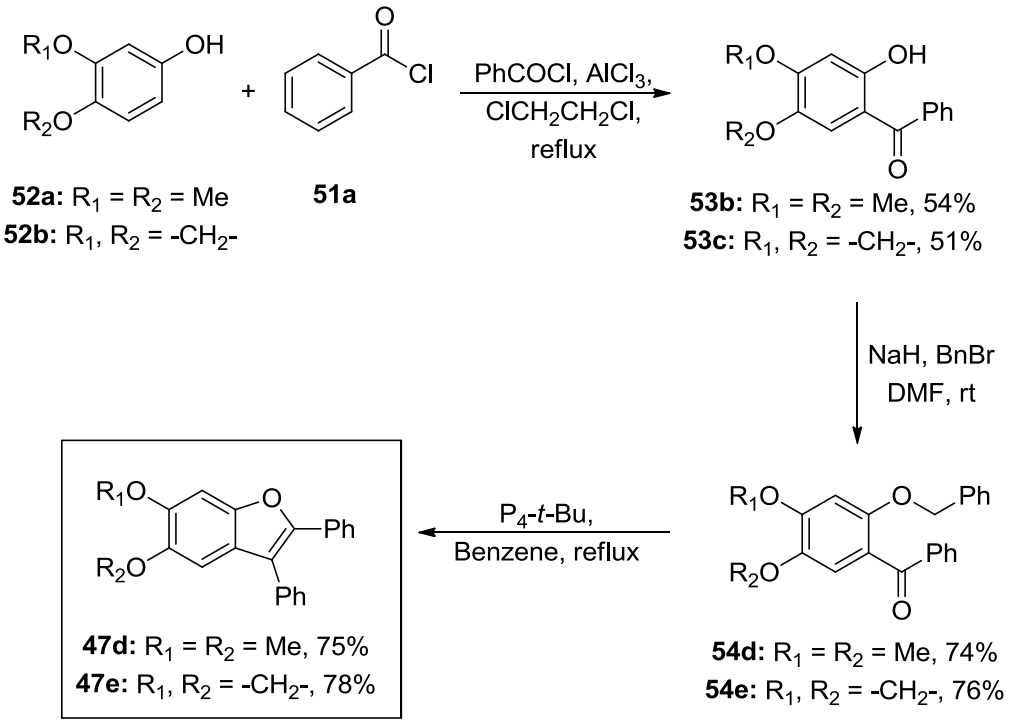
Scheme 18: Synthesis of model system

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 (Scheme19). 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 P_4-*t*-Bu to give diarylbenzo[*b*]furan **47b** and **47c** in 69% and 61% yield respectively (Scheme 19).



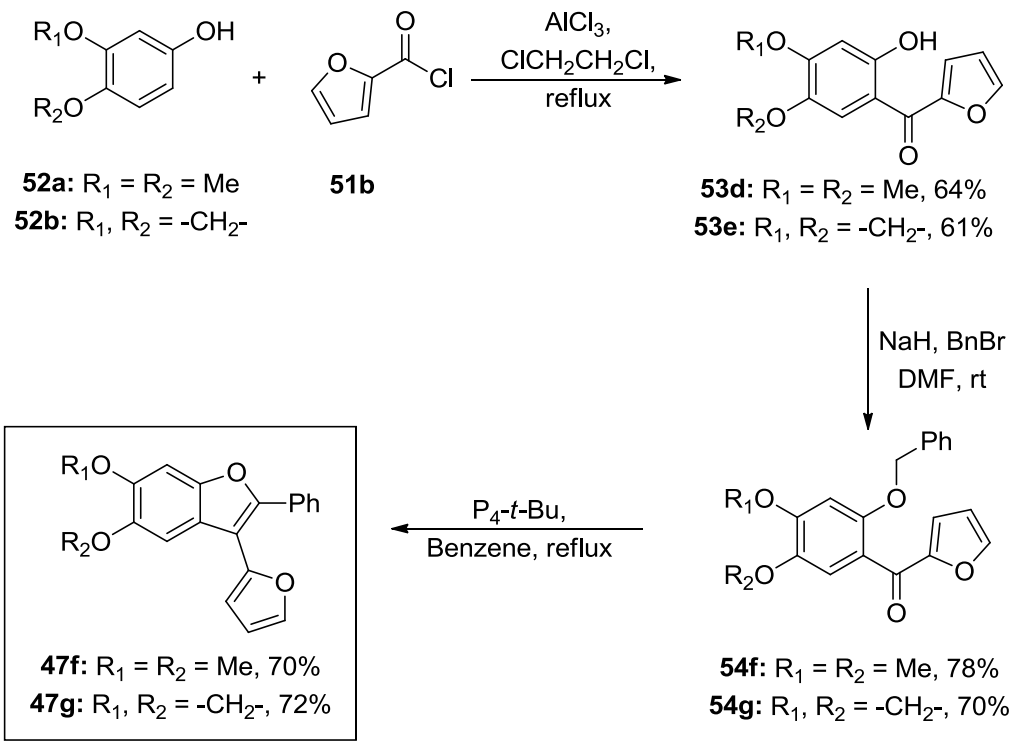
Scheme 19: Synthesis of 2,3-diarylbenzo[*b*]furans

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).



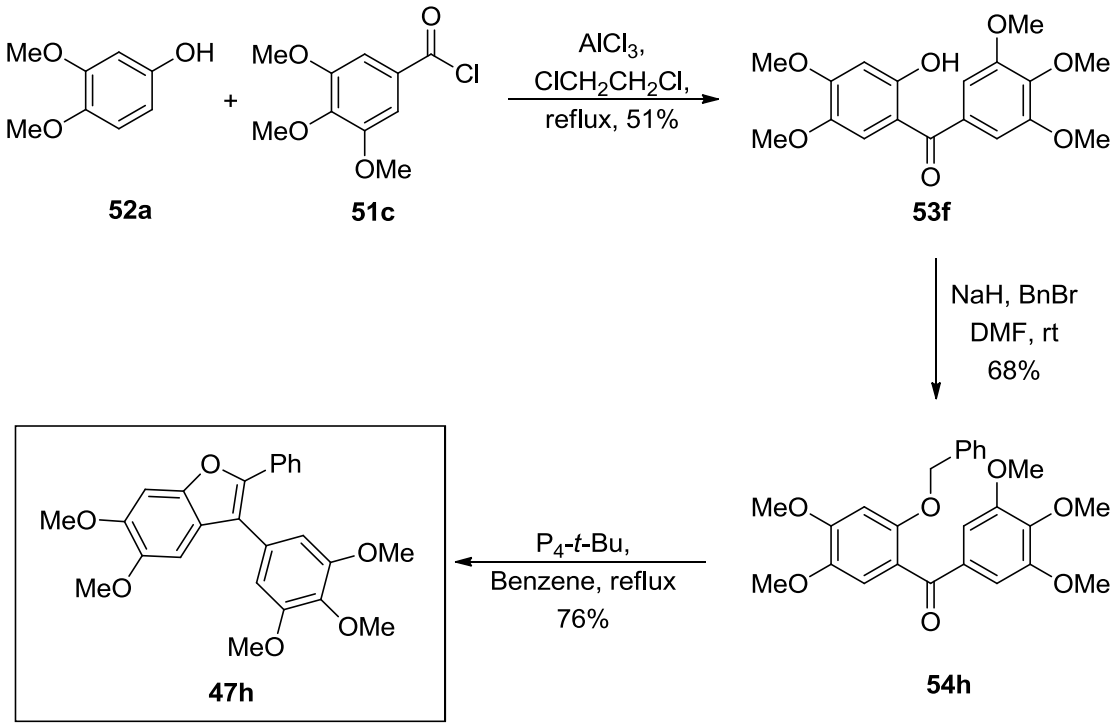
Scheme 20: Synthesis of 2,3-diarylbenzo[*b*]furans

The success of diaryl systems motivated us to synthesize hetroaryl 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 condtions and then successfully cyclized in the presence of P_4-*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.



Scheme 21: Synthesis of 2,3-diarylbenzo[*b*]furans

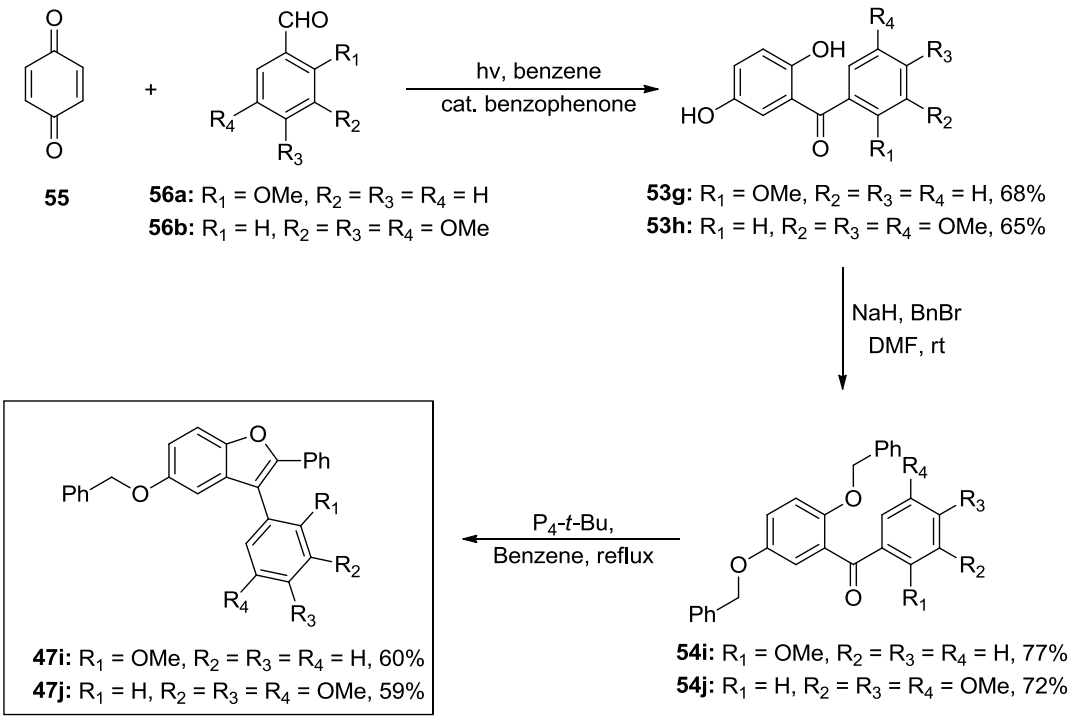
With the success of all the above-mentioned systems, we designed a benzophenone system bearing five methoxy groups, three on one phenyl ring and two on the other. The idea was to maximize the electronic effects and to make the carbonyl carbon least reactive. The synthesis is shown in Scheme 22 and it 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).



Scheme 22: Synthesis of 2,3-diarylbenzo[*b*]furans

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.[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. Componds **54i-j** underwent successful

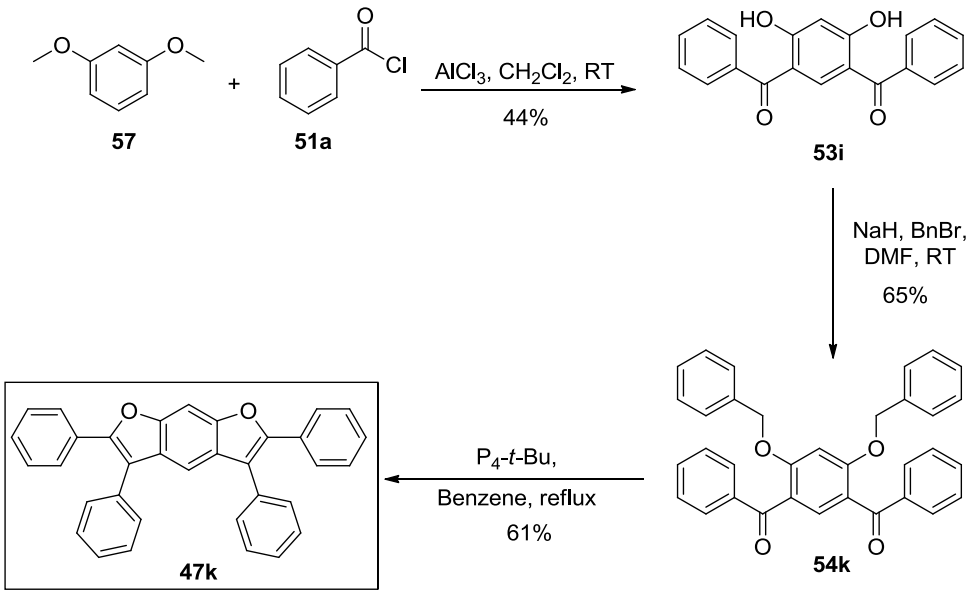
cyclization reactions to give diarylbenzo[*b*]furans **47i-j** when excess (2.5 equivalents) of P_4-*t*-Bu was used (Scheme 23). Moderate yields in both the cases can be attributed to the presence of another reactive site.



Scheme 23: Synthesis of 2,3-diarylbenzo[*b*]furans- effect of multiple reaction sites.

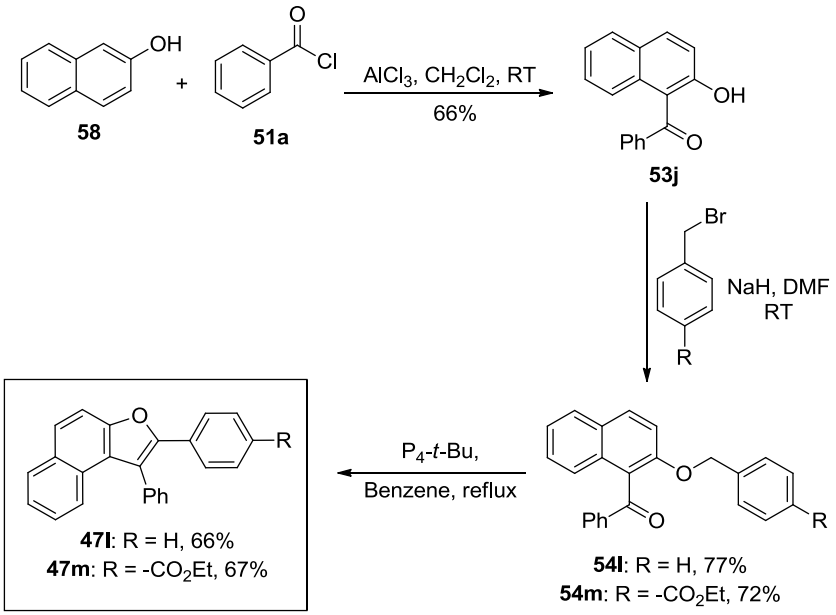
Success of the systems discussed above (Scheme 24) encouraged us to try another, yet more ambitious example with two sites to generate the anion and two sites to trap. This would result in the formation of benzo[1,2-*b*:5,4-*b'*]difuran ring system (Scheme 24). The synthesis started with commercially 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.[41] 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 P_4-*t*-Bu but reaction did not work even after prolonged heating. Successful cyclization was achieved with the use of

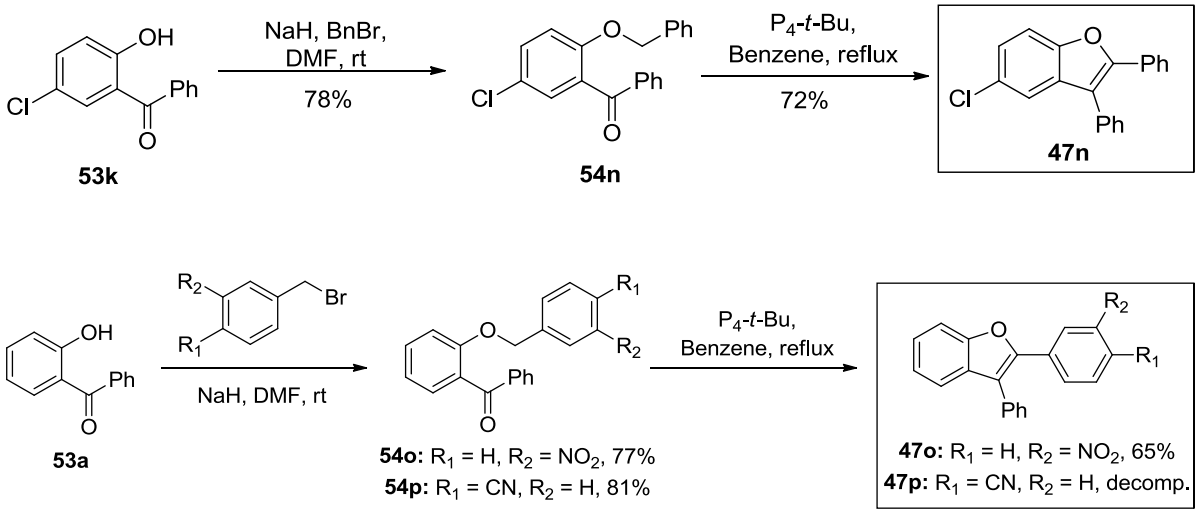
2.5 equivalents of P_4-*t*-Bu resulting in the formation of benzo[1,2-*b*:5,4-*b'*]difuran **47k** in 61% yield (Scheme 24).



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.

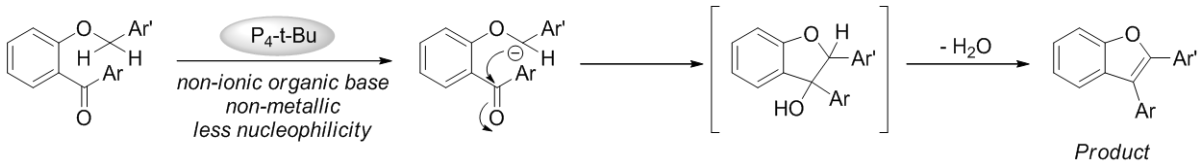




Scheme 25

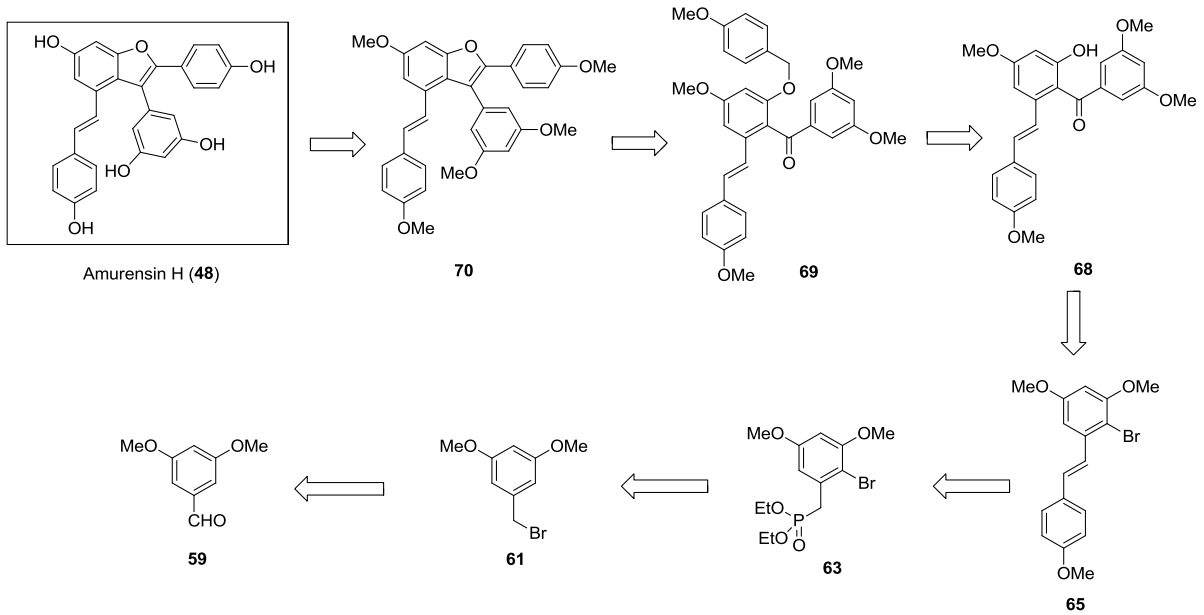
As shown by the synthesis of substituted 2,3-diphenylnaphthofuran **47m**, this method is compatible with an ester group. Also, the electron withdrawing effect of the ester did not influence the cyclization in a positive way. In fact, the yield of the cyclization reaction for compound having an ester 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 P_4-*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

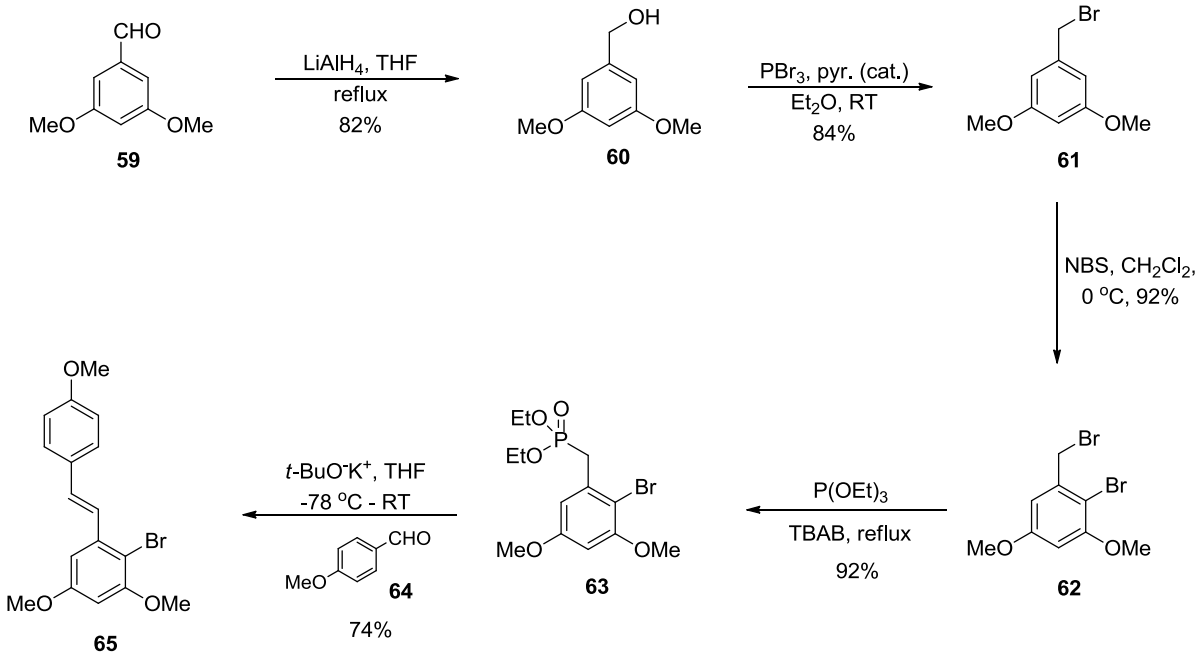
The possible mechanism for these reactions is depicted in Scheme 26 which is based on the assumption that P_4-*t*-Bu successfully deprotonated the benzylic position which bears the most acidic hydrogen. The anion thus generated reacts with the carbonyl carbon giving rise to the intermediate which undergoes dehydration at elevated temperature to give 2,3-diarylbenzo[*b*]furan.



Scheme 27: Reterosynthetic analysis of amurensin H

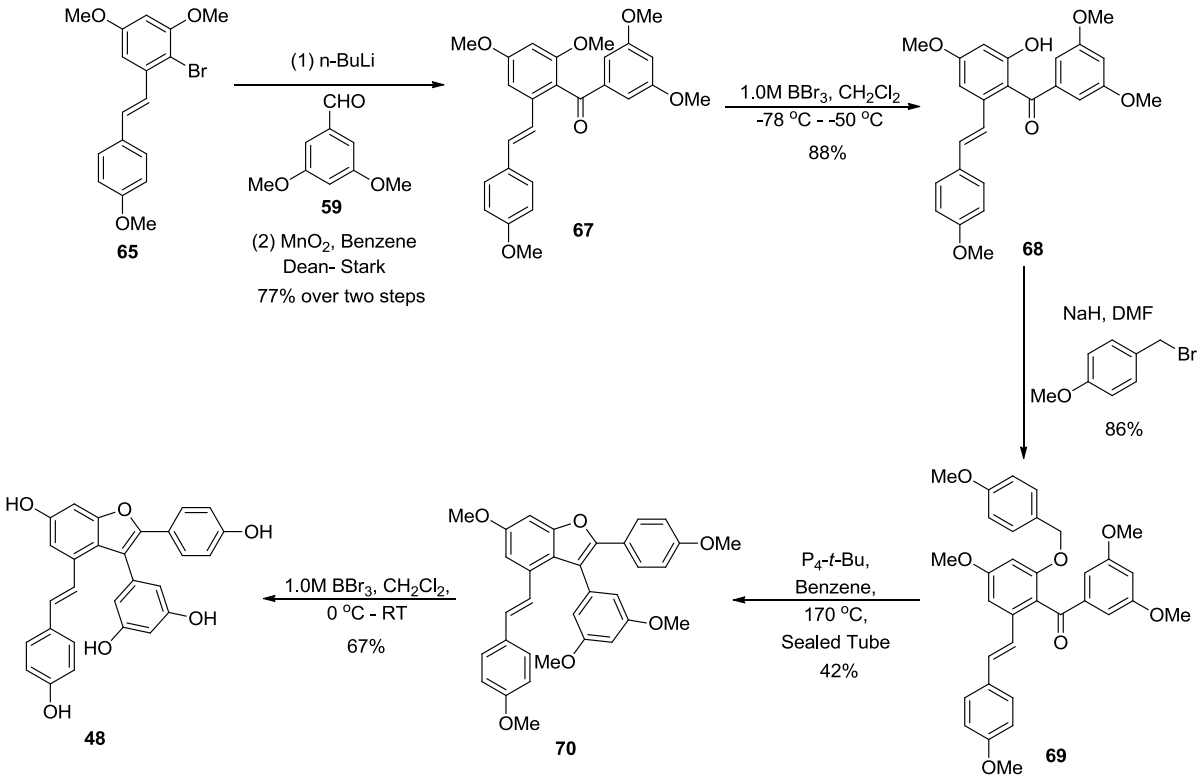
This methodology can be used in a direct total synthesis of amurensin H (**48**). As shown in Scheme 27, our reterosynthetic consideration of **48** was focused on the efficient synthesis of substituted benzophenone **69** which subsequently can be cyclized by using our P_4-*t*-Bu mediated cyclization 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.[42]



Scheme 28: Synthesis of the substituted stilbene **65**

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 ^{o}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 *para*-methoxybenzaldehyde **64** giving stilbene **65** in 74% yield.[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 BBr_3 solution (1.0M in CH_2Cl_2) at -50 ^oC. The resulting phenol **68** is converted into benzyl ether **69** in 86% yield. Cyclization of **69** using P_4-*t*-Bu in dry benzene at 170 ^oC (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 BBr_3 solution (1.0M in CH_2Cl_2) at room temperature in 67% yield. The analytical data for **48** matched with the previously reported data.[31, 43] 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.

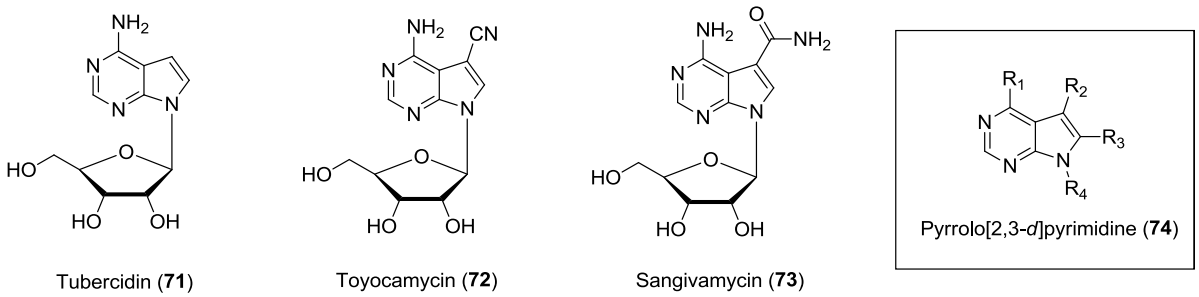
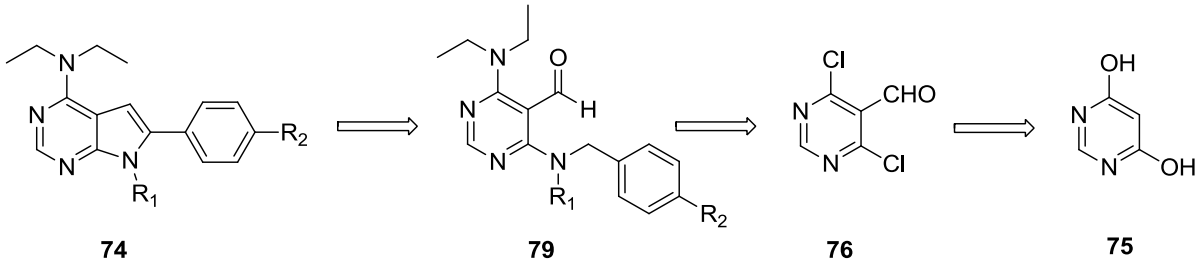


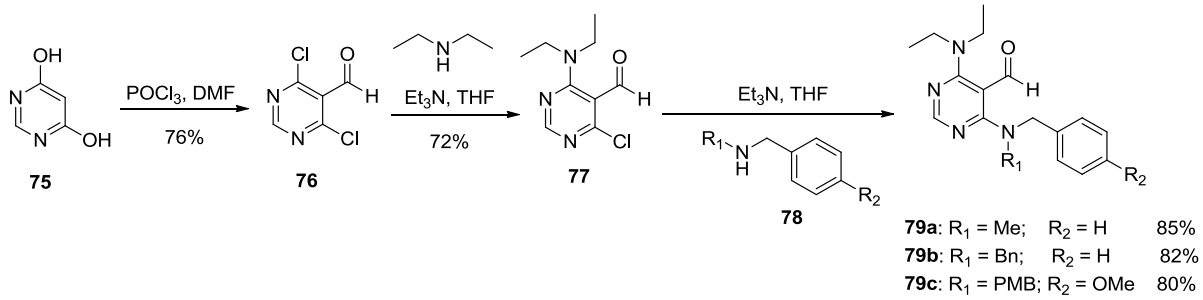
Figure 7: Naturally occurring pyrrolo[2,3-*d*]pyrimidine derivatives.

As a final part of our studies regarding the use of P_4-*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.[44] 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*.[45] and Raimondi *et al*.[46] 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).[47-49] 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*.[50] 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.



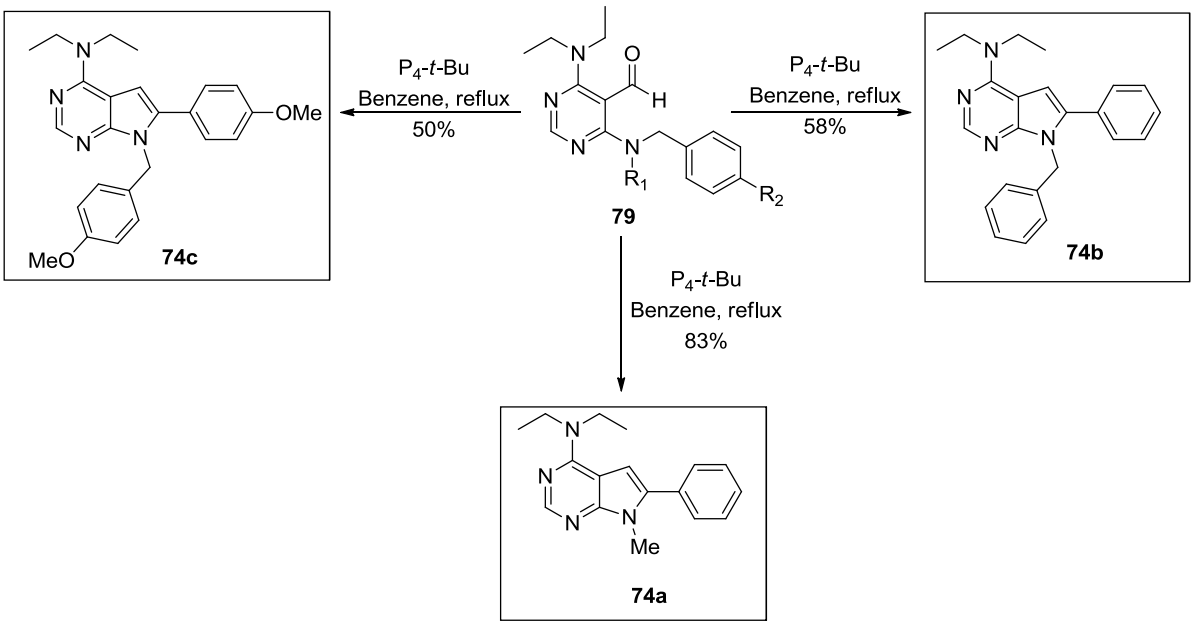
Scheme 30: Reterosynthetic analysis

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).



Scheme 32: Synthesis of pyrrolo[2,3-*d*]pyrimidines

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 heterocylic aromatic compounds. We showed the utility of this method by synthesizing two natural products.

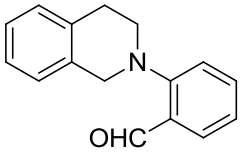
Experimental

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_2CO_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

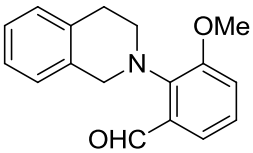
with ethyl acetate (three times). Organic layer was then washed with water, brine and dried over $MgSO_4$. Excess solvent was evaporated in vacuo to obtain crude product. Crude product was subjected to column purification using 5% ethyl acetate: petroleum ether to obtain pure product (48% yield).

Spectroscopic Data for:



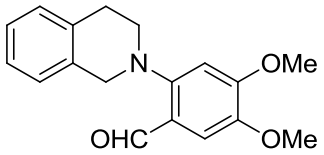
2-(3,4-Dihydroisoquinolin-2(1H)-yl)benzaldehyde (54a)

^{1}H-NMR (400MHz, $CDCl_3$) δ 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, $CDCl_3$) δ: 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.



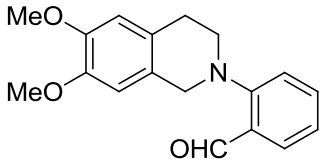
2-(3,4-Dihydroisoquinolin-2(1H)-yl)-3-methoxybenzaldehyde (54b)

^{1}H-NMR (400MHz, $CDCl_3$) δ 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, $CDCl_3$) δ: 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.



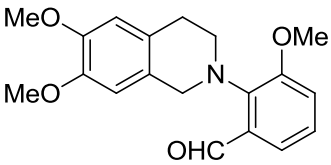
2-(3,4-Dihydroisoquinolin-2(1H)-yl)-4,5-dimethoxybenzaldehyde (54c)

^{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)

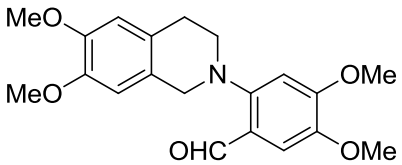
^{1}H-NMR (400MHz, $CDCl_3$) δ 2.96, (t, J = 5.6 Hz, 2H), 3.43 (t, J = 6.0 Hz, 2H), 3.86 (s, 3H), 3.87 (s, 3H), 4.26 (s, 2H), 6.59 (s, 1H), 6.67 (s, 1H), 7.11 (t, J = 7.2 Hz, 1H), 7.17 (d, J = 7.6 Hz, 1H), 7.52 (dt, J = 7.6 Hz, J = 1.6 Hz, 1H), 7.83 (dd, J = 7.6 Hz, J = 1.6 Hz, 1H), 10.32 (s, 1H); ^{13}C-NMR (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.



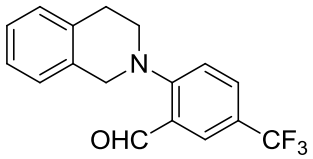
2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-3-methoxybenzaldehyde (54e)

^{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, 56.1, 109.2, 112.0, 117.5, 120.0, 126.4, 126.7, 127.2, 134.9, 144.0, 147.4, 147.5,

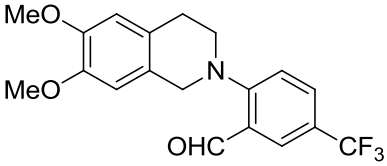
158.8, 193.8; MS (*m/z*): 327, 326, 310, 270, 177, 164, 149, 77; HRMS calcd for $C_{19}H_{21}NO_4$: 327.1471, found 327.1474.



2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-4,5-dimethoxybenzaldehyde (**54f**) ^{1}H-NMR (400MHz, $CDCl_3$) δ 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, $CDCl_3$) δ: 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.

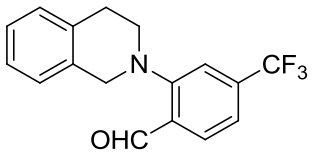


2-(3,4-Dihydroisoquinolin-2(1H)-yl)-5-(trifluoromethyl)benzaldehyde (**54g**) ^{1}H-NMR (400MHz, $CDCl_3$) δ 3.09 (t, J = 5.6 Hz, 2H), 3.57 (t, J = 6.0 Hz, 2H), 4.43 (s, 2H), 7.12-7.14 (m, 1H), 7.22 – 7.25 (m, 4H), 7.71 (d, J = 8.0 Hz, 1H), 8.08 (s, 1H), 10.23 (s, 1H); ^{13}C-NMR (100MHz, $CDCl_3$): δ 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 $C_{17}H_{14}F_3NO$: 305.1027, found 305.1033.



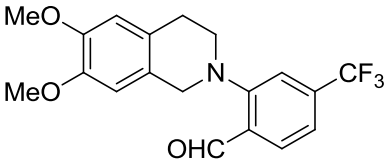
2-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-5-(trifluoromethyl)benzaldehyde (**54h):** ^{1}H-NMR (400MHz, $CDCl_3$) δ 2.99 (t, J = 5.6 Hz, 2H), 3.55 (t, J = 6.0 Hz, 2H), 3.88

(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, $CDCl_3$): δ 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 $C_{19}H_{18}F_3NO$: 365.1239, found 365.1246.



2-(3,4-dihydroisoquinolin-2(1H)-yl)-4-(trifluoromethyl)benzaldehyde (54i)

^{1}H-NMR (400MHz, $CDCl_3$) δ 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, $CDCl_3$): δ 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 $C_{17}H_{14}F_3NO$: 305.1027, found 305.1036.

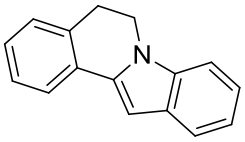


2-(6,7-dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)-4-(trifluoromethyl)benzaldehyde (54j): ^{1}H-NMR (400MHz, $CDCl_3$) δ 3.01 (t, J = 8.0 Hz, 2H), 3.47 (t, J = 8.0 Hz, 2H), 3.87 (s, 3H), 3.88 (s, 3H), 4.30 (s, 2H), 6.61 (s, 1H), 6.68 (s, 1H), 7.33 (d, J = 8.8 Hz, 1H), 7.40 (s, 1H), 7.92 (d, J = 8.0 Hz, 1H), 10.29 (s, 1H); ^{13}C-NMR (100MHz, $CDCl_3$): δ 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 $C_{19}H_{18}F_3NO$: 365.1239, found 365.1246.

Procedure for the preparation of 45a-j

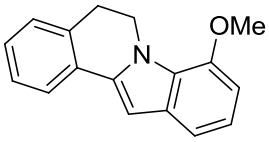
To a solution of aldehyde (0.10 g, 0.42 mmol) in freshly distilled, dry benzene (5 mL), P_4-*t*-Bu solution (0.46 mL, 0.46 mmol) was added at room temperature and reaction mixture was heated to reflux with monitoring (2 h). After the completion of reaction, benzene was partially evaporated and reaction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

Spectroscopic Data for:



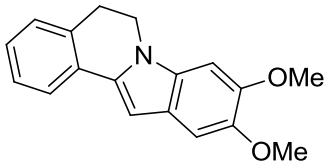
5,6-Dihydroindolo[2,1-*a*]isoquinoline (45a)

^{1}H-NMR (400MHz, $CDCl_3$) δ 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, $CDCl_3$) δ: 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.



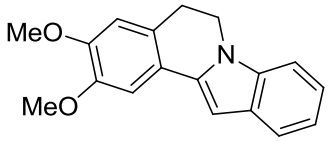
8-Methoxy-5,6-dihydroindolo[2,1-*a*]isoquinoline (45b)

^{1}H-NMR (400MHz, $CDCl_3$) δ 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, $CDCl_3$) δ: 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.



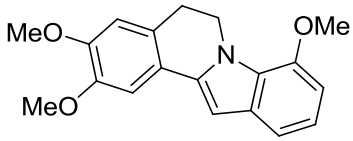
9,10-Dimethoxy-5,6-dihydroindolo[2,1-*a*]isoquinoline (45c)

^{1}H-NMR (400MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 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-*a*]isoquinoline (45d)

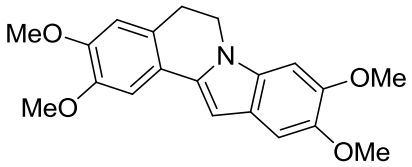
^{1}H-NMR (400MHz, $CDCl_3$) δ 3.14 (t, J = 6.8 Hz, 2H), 3.93 (s, 3H), 3.97 (s, 3H), 4.24 (t, J = 6.8 Hz, 2H), 6.76 (s, 1H), 6.77 (s, 1H), 7.09 (t, J = 7.2 Hz, 1H), 7.19 (t, J = 7.2 Hz, 1H), 7.25 (d, J = 6.4 Hz, 1H), 7.32 (d, J = 8.0 Hz, 1H), 7.61 (d, J = 8.0 Hz, 1H); ^{13}C-NMR (100MHz, $CDCl_3$) δ 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-*a*]isoquinoline (45e)

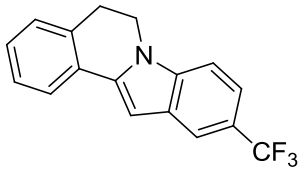
^{1}H-NMR (400MHz, $CDCl_3$) δ 3.08 (t, J = 6.8 Hz, 2H), 3.92 (s, 3H), 3.95 (s, 3H), 3.96 (s, 3H), 4.69 (t, J = 6.4 Hz, 2H), 6.61 (d, J = 8.0 Hz, 1H), 6.73 (s, 1H), 6.75 (s, 1H), 6.98 (t, J = 8.0 Hz, 1H), 7.21 (d, J = 8.8 Hz, 2H); ^{13}C-NMR (100MHz, $CDCl_3$) δ 29.5, 43.4, 55.5, 56.2, 56.3, 96.0, 102.7, 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.



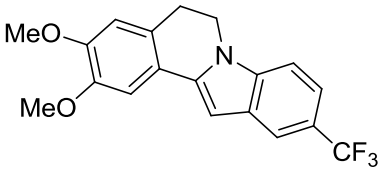
2,3,9,10-Tetramethoxy-5,6-dihydroindolo[2,1-*a*]isoquinoline (45f)

^{1}H-NMR (400MHz, $CDCl_3$) δ 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, $CDCl_3$) δ 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)

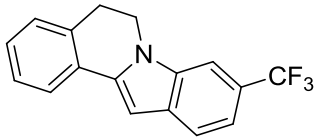
^{1}H-NMR (400MHz, $CDCl_3$) δ 3.16 (t, J = 6.4 Hz, 2H), 4.22 (t, J = 6.4 Hz, 2H), 6.89 (s, 1H), 7.24 – 7.40 (m, 5H), 7.73 (d, J = 7.6 Hz, 1H), 7.88 (s, 1H); ^{13}C-NMR (100MHz, $CDCl_3$) δ 29.2, 40.6, 97.4, 97.4, 109.3, 118.5, 118.6, 122.4 (q, ^2J = 32 Hz), 124.8, 125.6 (q, ^1J = 270 Hz), 127.6, 128.3, 128.5, 128.6, 132.4, 137.5, 138.0; MS (*m/z*): 287, 217; HRMS calcd for $C_{17}H_{12}F_3N$: 287.0922 found 287.0929.



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

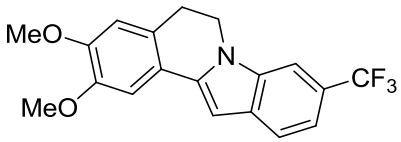
^{1}H-NMR (400MHz, $CDCl_3$) δ 3.15 (t, J = 6.4 Hz, 2H), 3.93 (s, 3H), 3.98 (s, 3H), 4.26 (t, J = 6.4 Hz, 2H), 6.78 (s, 1H), 6.81 (s, 1H), 7.23 (s, 1H), 7.34 – 7.41 (m, 2H), 7.88 (s, 1H); ^{13}C-

NMR (100MHz, $CDCl_3$) 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 $C_{19}H_{16}F_3NO_2$: 347.1133, found 347.1141.



9-(Trifluoromethyl)-5,6-dihydroindolo[2,1-*a*]isoquinoline (45i)

^{1}H-NMR (400MHz, $CDCl_3$) 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); ^{13}C-NMR (100MHz, $CDCl_3$) 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 $C_{17}H_{12}F_3N$: 287.0922 found 287.0929.



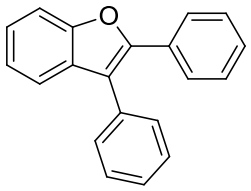
2,3-Dimethoxy-9-(trifluoromethyl)-5,6-dihydroindolo[2,1-*a*]isoquinoline (45j)

^{1}H-NMR (400MHz, $CDCl_3$) 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); ^{13}C-NMR (100MHz, $CDCl_3$) 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 $C_{19}H_{16}F_3NO_2$: 347.1133, found 347.1141.

Representative procedure for the preparation of 2,3-diarylbenzofuran

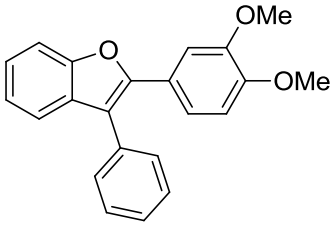
To a solution of 2-benzyloxybenzophenone (0.20 g, 0.69 mmol) in freshly distilled dry benzene (10 mL), P_4-*t*-Bu solution (0.77 mL, 0.76 mmol, 1.0 M solution in hexane) was added at room temperature and reaction mixture was heated to reflux with monitoring (3 h). After the completion of reaction, benzene was partially evaporated and reaction mixture was

purified by column purification using 10% ethyl acetate: petroleum ether to obtain pure product (100% yield).



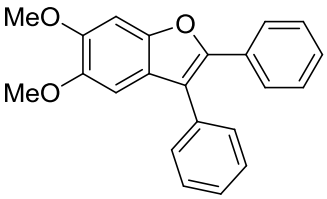
2,3-Diphenylbenzo[*b*]furan (47a)

Mp 123-124.5 ^{o}C; ^{1}H-NMR (400 MHz) δ 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) δ 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.



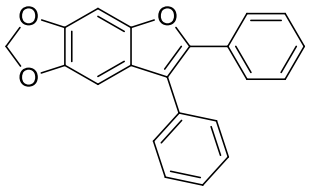
2-(3,4-dimethoxyphenyl)-3-phenylbenzofuran (47c)

^{1}H-NMR (400 MHz) δ 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) δ 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.



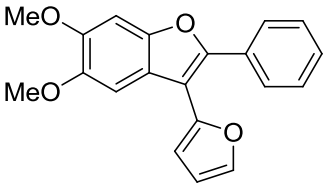
5,6-Dimethoxy-2,3-diphenylbenzofuran (47d)

^{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.



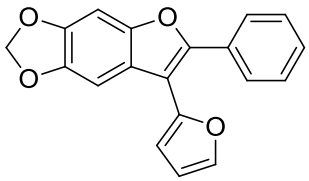
6,7-Diphenyl-[1,3]dioxolo[4,5-*f*]benzofuran (47e)

^{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.



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

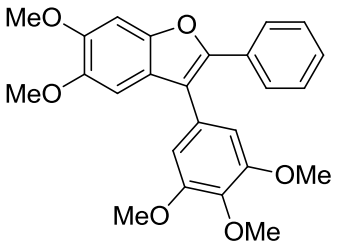
^{1}H-NMR (400 MHz) δ 7.70 – 7.81 (m, 2H), 7.55 – 7.60 (m, 1H), 7.30 – 7.46 (m, 3H), 7.21 (s, 1H), 7.08 (s, 1H), 6.60 (dd, ^{1}J = 3.3 Hz, ^{2}J = 0.6 Hz, 1H), 6.55 (dd, ^{1}J = 3.3 Hz, ^{2}J = 1.8 Hz, 1H), 3.96 (s, 3H), 3.95 (s, 3H); ^{13}C-NMR (100 MHz) δ 151.1, 149.0, 148.7, 147.4, 147.1, 142.2, 142.1, 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 caclcd for $C_{20}H_{16}O_4$: 320.1049, found 320.1046.



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

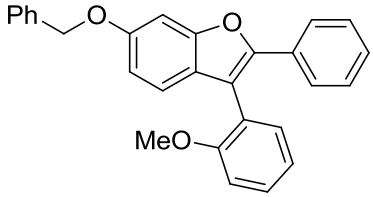
^{1}H-NMR (400 MHz) δ 7.74 (d, J = 8.0 Hz, 2H), 7.51 – 7.56 (m, 1H), 7.31 – 7.44 (m, 3H), 7.17 (s, 1H), 7.02 (s, 1H), 6.57 (d, J = 3.2 Hz, 1H), 6.52 (dd, ^{1}J = 3.2 Hz, ^{2}J = 2.0 Hz, 1H),

6.01 (s, 2H); MS (*m/z*): 641, 391, 305, 239, 149; HRMS calcd for $C_{19}H_{12}O_4$: 304.0736, found 304.0735.



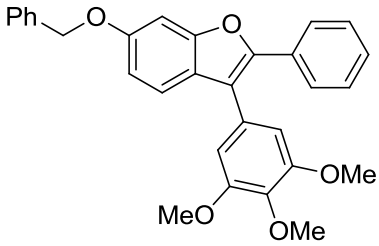
5,6-Dimethoxy-2-phenyl-3-(3,4,5-trimethoxyphenyl)benzofuran (47h)

^{1}H-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 $C_{25}H_{24}O_6$: 420.1573, found 420.1568.



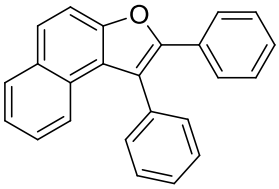
6-Benzyl-3-(2-methoxyphenyl)-2-phenylbenzofuran (47i)

^{1}H-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); ^{13}C-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 $C_{28}H_{22}O_3$: 406.1515, found 406.1520.



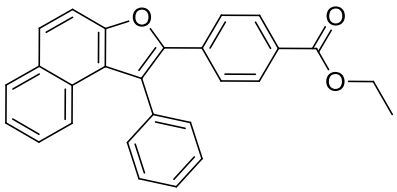
6-Benzyl-2-phenyl-3-(3,4,5-trimethoxyphenyl)benzofuran (47j)

^{1}H-NMR (400 MHz) δ 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}C-NMR (100 MHz) δ 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 $C_{30}H_{26}O_5$: 466.1740, found 466.1744.



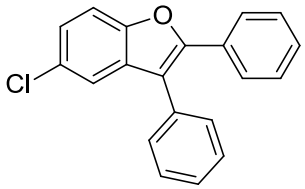
1,2-diphenylnaphtho[2,1-*b*]furan (47l)

^{1}H-NMR (400 MHz) δ 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}C-NMR (100 MHz) δ 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 $C_{24}H_{16}O$: 320.1201, found 320.1196.



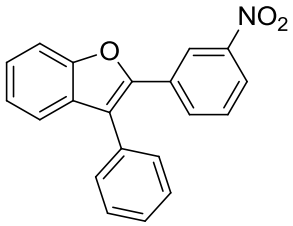
Ethyl 4-(1-phenylnaphtho[2,1-*b*]furan-2-yl)benzoate (47m)

^{1}H-NMR (400 MHz) δ 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}C-NMR (100 MHz) δ 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 $C_{27}H_{20}O_3$: 392.1412, found 392.1412.



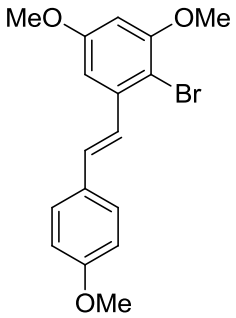
5-Chloro-2,3-diphenylbenzofuran (47n)

^{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.



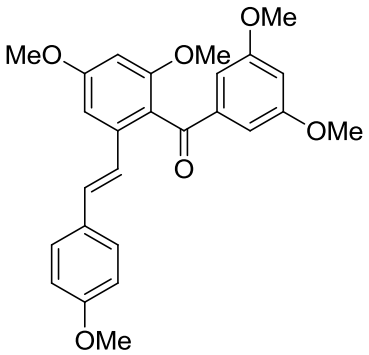
2-(3-Nitrophenyl)-3-phenylbenzofuran (47o)

^{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.



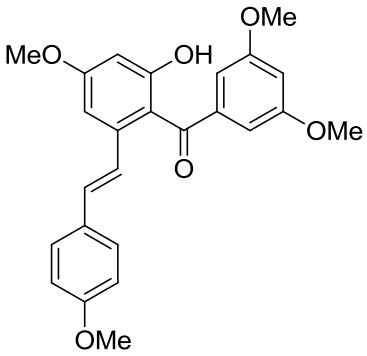
Preparation of (*E*)-2-bromo-1,5-dimethoxy-3-(4-methoxystyryl)benzene (65): In an oven dried 250 ml flask, equipped with a stirring bar, potassium *tert*-butoxide solution (20.0 ml, 20.0 mmol, 1.0 M solution in THF) was added dropwise to a solution of phosphonate ester (**63**) in freshly distilled dry THF (75 ml) at -78 ^{o}C under argon. Resulting reaction mixture

was stirred at -78 ^{o}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.



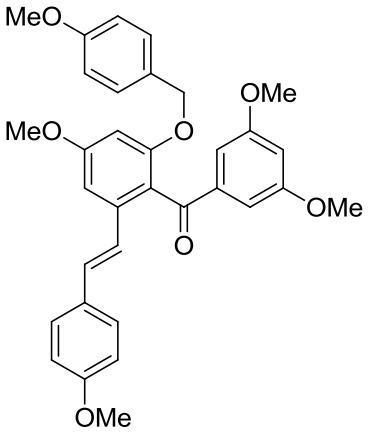
Preparation of (*E*)-(2,4-dimethoxy-6-(4-methoxystyryl)phenyl)(3,5-dimethoxyphenyl) methanone (67): In an oven dried 250 ml round bottom flask, equipped with a stir bar, *n*-BuLi (3.91 ml, 9.77 mmol, 2.5 M solution in hexane) was added to a solution of stilbene (**65**) (3.25 g, 9.31 mmol) in dry THF (50 ml) at -78 ^{o}C under argon. Resulting yellow reaction mixture was stirred at -78 ^{o}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 ^{o}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 filterate 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) δ 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) δ 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.



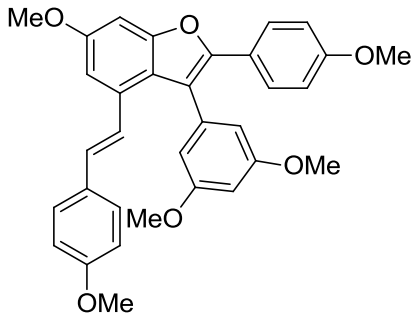
Preparation of (*E*)-(3,5-dimethoxyphenyl)(2-hydroxy-4-methoxy-6-(4-methoxystyryl) phenyl)methanone (68): To a solution of ketone (**67**) (1.52 g, 3.50 mmol) in dry DCM was added boron tribromide (3.85 ml, 3.85 mmol, 1.0 M solution in DCM) at -78 ^{o}C under argon. Resulting reaction mixture was stirred for 3 h with steady increase in temperature to -50 ^{o}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) δ 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),

3.68 (s, 3H); ^{13}C-NMR (100 MHz) δ 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, 301, 300, 299, 298, 297, 295, 284, 283, 254; HRMS calcd for $C_{25}H_{24}O_6$: 420.15728, found 420.15831.

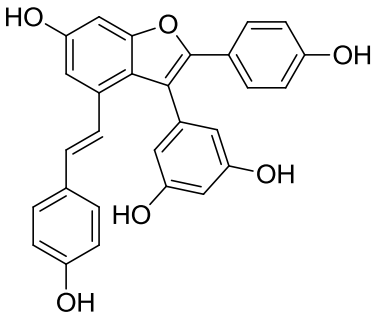


Preparation of (*E*)-(3,5-dimethoxyphenyl)(4-methoxy-2-((4-methoxybenzyl)oxy)-6-(4-methoxystyryl)phenyl)methanone (69): In an oven dried 100 ml flask equipped with a stir bar, benzophenone derivative (**68**) (0.50 g, 1.19 mmol) was taken in dry DMF (10 ml). To this, NaH (0.058 g, 1.43 mmol) was added at 0 ^{o}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 ^{o}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 elutent to obtain pure product (**69**) (0.56 g, 1.03 mmol) in 86% isolated yield. ^{1}H-NMR (400 MHz) δ 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) δ 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,

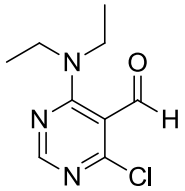
114.0, 107.4, 105.8, 101.8, 99.4, 70.4, 55.9, 55.8, 55.6, 55.5; MS (*m/z*): 540, 522, 418, 417, 121, 82, 40, 48; HRMS calcd for $C_{33}H_{32}O_7$: 540.21429, found 540.21563.



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), P_4-*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 ^{o}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. ^{1}H-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); ^{13}C-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 $C_{33}H_{30}O_6$: 522.20423, found 522.20560.



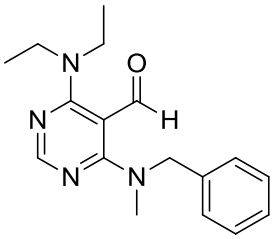
Preparation of (*E*)-5-(6-hydroxy-2-(4-hydroxyphenyl)-4-(4-hydroxystyryl)benzofuran-3-yl)benzene-1,3-diol (48): To a solution of benzofuran derivative (**70**) (0.060 g, 0.12 mmol) in dry DCM was added boron tribromide (1.15 ml, 1.15 mmol, 1.0 M solution in DCM) at 0 ^{o}C under argon. Resulting reaction 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 elutent to obtain the pure Amurensin H (**48**) (0.035 g, 0.077 mmol) in 67% yield. ^{1}H-NMR (400 MHz) δ 7.47 (d, J = 8.0 Hz, 1H), 7.43 (d, J = 8.0 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 Hz, 1H), 6.38 – 6.43 (m, 2H), 6.30 (d, J = 2.5 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 elutent to obtain the product (**77**) (0.871 g, 4.08 mmol) in 72% yield. ^{1}H-NMR (400 MHz) δ 10.29 (s, 1H), 8.27 (s, 1H), 3.50 (q, J = 6.8 Hz, 4H), 1.18 (t, J = 7.2 Hz, 6H); ^{13}C-NMR (100 MHz) δ 187.8, 164.4, 160.5, 157.6, 110.4, 45.4, 12.5.

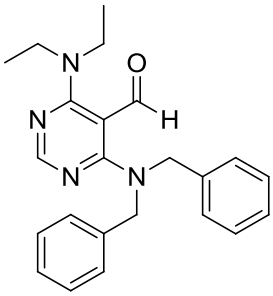
Representative procedure for the preparation of 79a-c: To a solution of aldehyde (**77**) (0.500 g, 2.34 mmol) in anhydrous THF (25 ml) was added *N*-methyl-1-phenylmethanamine

(**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 elutent to obtain the pure product (**79a**).



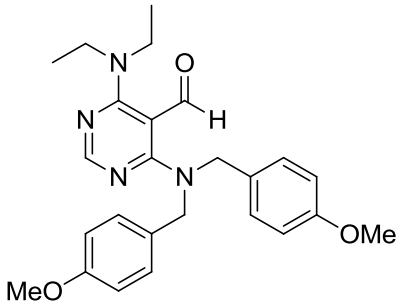
4-(Benzyl(methyl)amino)-6-(diethylamino)pyrimidine-5-carbaldehyde (79a)

Yield: 85%; ^{1}H-NMR (400 MHz) δ 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) δ 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.



4-(Dibenzylamino)-6-(diethylamino)pyrimidine-5-carbaldehyde (79b)

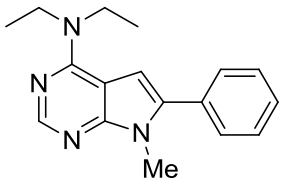
Yield: 82%; ^{1}H-NMR (400 MHz) δ 9.36 (s, 1H), 8.14 (s, 1H), 7.20 – 7.35 (m, 6H), 7.09 – 7.19 (m, 4H), 4.85 (s, 4H), 3.54 (q, J = 6.4 Hz, 4H), 1.21 (t, J = 7.2 Hz, 6H); ^{13}C-NMR (100 MHz) δ 181.9, 166.2, 165.5, 158.6, 136.9, 128.7, 127.9, 127.5, 97.6, 54.0, 44.9, 13.6.



4-(Bis(4-methoxybenzyl)amino)-6-(diethylamino)pyrimidine-5-carbaldehyde (79c)

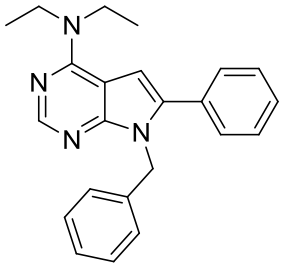
Yield: 80%; ^{1}H-NMR (400 MHz) δ 9.33 (s, 1H), 8.13 (s, 1H), 7.04 (d, J = 8.4 Hz, 2H), 6.81 (d, J = 8.0 Hz, 2H), 4.75 (s, 4H), 3.77 (s, 6H), 3.55 (q, J = 6.92 Hz, 4H), 1.21 (t, J = 8.0 Hz, 6H); ^{13}C-NMR (100 MHz) δ 182.1, 166.2, 165.6, 159.2, 158.8, 129.5, 129.0, 114.2, 97.7, 55.5, 53.4, 45.0, 13.8.

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**).



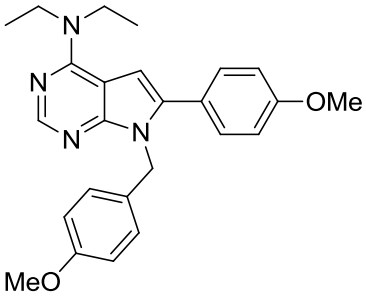
N,N-Diethyl-7-methyl-6-phenyl-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (74a)

Yield: 83%; ^{1}H-NMR (400 MHz) δ 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) δ 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-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (74b)

Yield: 58%; ^{1}H-NMR (400 MHz) δ 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) δ 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)-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine (74c)

Yield: 50%; ^{1}H-NMR (400 MHz) δ 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) δ 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

1. (a) Schwesinger, R.; Schlemper, H. *Angew. Chem. Intl. Ed. Engl.* **1987**, *26*(11), 1167-1169. (b) Imahori, T. *Yakugaku Zasshi* **2004**, *124*(8), 509-517.
2. Schwesinger, R.; Hasenfratz C.; Schlemper, H.; Walz, L.; Peters, Eva-Maria; Peters, K.; Schnering, H. G. von. *Angew. Chem. Intl. Ed. Engl.* **1993**, *32*(9), 1361-1363.

3. Schwesinger, R., *Nachr. Chem. Tech. Lab.* **1990**, *38*, 1214 (*Chem. Abstr.* **1991**, *114*, 23 099a).
4. Pietzonka, T.; Seebach, D., *Chem. Ber.* **1990**, *124*, 1837.
5. Schwesinger, R. Phosphazene Base P_4-t-Bu, *Encyclopedia of Reagents for Organic Synthesis* **2001**.
6. (a) Imahori, T.; Kondo, Y. *J. Am. Chem. Soc.* **2003**, 125, 8082-8083.
7. Ueno, M.; Yonemoto, M.; Hashimoto, M.; Wheatley, A. E. H.; Naka, H.; Kondo, Y. *Chem. Commun.* **2007**, 2264–2266.
8. Suzawa, K.; Ueno, M.; Wheatley, A. E. H.; Naka, H.; Kondo, Y. *Chem. Commun.* **2006**, 4850–4852.
9. (a) Alonso, D. A.; Nájera, C.; Varea, M. *Tet. Lett.* **2004**, *45*, 573–577 (b) Alonso, D. A.; Fuensanta, M.; Nájera, C.; Varea, M. *J. Org. Chem.* **2005**, *70*, 6404-6416.
10. Ueno, M.; Yonemoto, M.; Wheatley, A. E. H.; Kondo, Y. *Chem. Commun.* **2006**, 3549–3550.
11. Kraus, G. A.; Zhang, N.; Verkade, J. G.; Nagarajan, M.; Kisanga, P. B. *Org. Lett.* **2000**, *2*, 2409-2410
12. Vincze, Z.; Biro, A. B.; Csekei, M.; Timari, G.; Kotschy, A. *Synthesis* **2006**, 1375-1385.
13. Meyers, A. I.; Sielecki, T. M.; Crans, D. C.; Marshman, R. W.; Nguyen, T. H. *J. Am. Chem.Soc.* **1992**, *114* (22), 8483-8489.
14. Cruces, J.; Estevez, J. C.; Estevez, R. J.; Castedo, L. *Heterocycles* **2000**, *53* (5), 1041-1050.
15. Ambros, R.; Von Angerer, S.; Wiegrebe, W. *Archiv der Pharmazie* (Weinheim, Germany) **1988**, *321* (8), 481-486.
16. Merrill, J. E.; Funes, S.; Petko, W.; Wirtz-Brugger, F.; Chandross, K. (Aventis Pharmaceuticals Inc., USA). *PCT Int. Appl.* **2006**, 12 pp.
17. Hudyma, T. W.; Zheng, X.; He, F.; Ding, M.; Bergstrom, C. P.; Hewawasam, P.; Martin, S. W.; Gentles, R. G. (USA). *U.S. Pat. Appl. Publ.* **2006**, 318 pp., Cont.-in-part of U.S. Ser. No. 181,639.
18. Goldbrunner, M.; Loidl, G.; Polossek, T.; Mannschreck, A.; von Angere, E. *J. Med. Chem.* **1997**, *40*(22), 3524-3533.

19. Orito, K.; Harada, R.; Uchiito, S.; Tokuda, M. *Org Lett.* **2000**, *2*, 1799-1801.

20. Bressy, C.; Alberico, D.; Lautens, M. *J. Am. Chem. Soc.* **2005**, *127*, 13148-13149.

21. Atanes, N.; Guitian, E.; Saa, C.; Castedo, L.; Saa, J. M. *Tetrahedron Lett.* 1987, *28*,(7), 817-820.

22. Kametani, T.; Ogasawara, K. *J. Chem. Soc. C* **1967**, 2208-2212.

23. Allin, S. M.; Bowman, W. R.; Elsegood, M. R. J.; McKee, V.; Karim, R.; Rahman, S S. *Tetrahedron* **2005**, *61* (10), 2689-2696.

24. Yasuda, S.; Hirasawa, T.; Yoshida, S.; Hanaoka, M. *Chem. Pharm. Bull.* **1989**, *37* (6), 1682-1683.

25. Youn, S. W. *Org. Prep. Proced. Int.*, **2006**, *38* (6), 505-591.

26. Loetter, A. N. C.; Pathak, R.; Sello, T. S.; Fernandes, M. A.; van Otterlo, W. A. L.; de Koning, C. B. *Tetrahedron* **2007**, 63*(10)*, 2263-2274.

26. True, J.E.; Thomas, T.D.; Winter, R.W.; Gard, G.L. *Inorganic Chemistry*, **2003**, *42* (14), 4437–4441.

27. Elliott, Jr., I. W. *J. Org. Chem.* **1982**, *47*, (27), 5398-5400.

28. Kraus, G.A.; Gupta, V.; Kohut, M.; Singh, N. *Biorg. Med. Chem. Lett.* **2009**, *19*, 5539-5542.

29. Cheng, K.-W.; Wang, M.; Chen, F.; Ho, C.-T. *ACS Symposium Series* **2008**, 987, 36-58.

30. Li, Y.; Yao, C.; Bai, J.; Lin, M.; Cheng, G. *Acta Pharmacol. Sinica* **2006**, 27*(6)*, 735-740. Li, Y.; Cheng, G.; Lin, M.; Yao, C.; Huang, K.; Liu, B.; Bai, J.; Li, N. Faming Zhuanli Shenqing Gongkai Shuomingshu Application: CN 2006-10076299 20060421.

31. Huang, K.-S.; Lin, M.; Wang, Y.-H. *Chinese Chem. Lett.* **1999**, *10*, 817-820.

32. Huang, K.-S.; Wang, Y.-H.; Li, R.-L.; Lin, M. *J. Nat. Prod.* **2000,** *63*, 86-89.

33. Huang, K.-S.; Wang, Y.-H.; Li, R.-L.; Lin, M. *Phytochemistry* **2000**, *54(8)*, 875-881.

34. Bobrowska-Hagerstrand, M.; Lillas, M.; Mrowczynska, L.; Wrobel, A.; Shirataki, Y.; Motohashi, N.; Hagerstrand, H. *Anticancer Research* 2006, 26*(3A)*, 2081-2084.

35. Hou, X.-L.; Yang, Z.; Yeung, K.-S.; Wong, Henry N. C. *Progress in Heterocyc. Chem*. **2008**, 19, 176-207. Cacchi, S.; Fabrizi, G.; Goggiamani, A. *Current Org. Chem*. **2006**, 10*(12)*, 1423-1455.

36. Cho, C.-H.; Neuenswander, B.; Lushington, G. H.; Larock, R. C. *J. Combinatorial Chem*. **2008**, 10*(6)*, 941-947.

37. Bernini, R.; Cacchi, S.; De Salve, I.; Fabrizi, G. *Synthesis* **2007**, 873-882. Pan, C.; Yu, J.; Zhou, Y.; Wang, Z.; Zhou, M.-M. *Synlett* **2006**, 1657-1662. Colobert, F.; Castanet, A.-S.; Abillard, O. *European J. Org. Chem*. **2005**, 3334-3341.

38. Chiong, H. A.; Daugulis, O. *Org. Lett*. **2007**, *9(8)*, 1449-1451.

39. Abdul-Aziz, M.; Auping, J. V.; Meador, M. A. *J. Org. Chem*. **1995**, 60, 1303-8. Lappin, G. R.; Zannucci, J. S. *J. Chem. Soc. D: Chem. Commun*. **1969**, 1113.

40. Kraus, G. A.; Kirihara, M.; Wu, Y. *Benign by design* **1994**, 77-83.

41. Abdul-Aziz, M.; Auping, J. V.; Meador, M. A. *J. Org. Chem.***1995**, *60* (5), 1303-1308.

42. Snyder, Scott A.; Zografos, Alexandros L.; Lin, Y. *Angewandte Chemie, International Edition* **2007**, *46(43)*, 8186-8191.

43. Ito J.; Takaya Y.; Oshima Y.; Niwa M. *Tetrahedron* **1999**, *55*, 2529-2544.

44. Mohamed, M. S.; El-Domany, R. A.; El-Hameed, R. H. A. *Acta Pharm.* **2009**, *59*, 145–158.

45. Petruso, S.; Bonanno, S.; Caronna, S.; Ciofalo, M.; Maggio, B.; Schillaci, D. *J. Heterocyclic Chem*. **1994**, *31*, 941–945.

46. Raimondi, M. V.; Cascioferro, S.; Schillaci, D.; Petruso, S. *Eur. J. Med. Chem*. 2006, *41*, 1439–1445.

47. Misumo, Y.; Ikehara, M. J.; Watanabe, K. A.; Suzuki, S.; Itoh, T. *J. Org. Chem*. **1969**, *28*,3329–3331.

48. Dang, Q.; Gomez-Galeno, J. E. *J. Org.Chem.***2002**, *67*, 8703–8705

49. Rao, K. V. *J. Med. Chem.***1968,** *11*, 939–941.

50. Gangjee, A.; Lin, X.; Queener, S. F. *J.Med. Chem.***2004**, *47*, 3689–3692.

51. Gomtsyan, A.; Didomenico, S.; Lee, C-H.; Matulenko, M. A.; Kim, K.; Kowaluk, E. A.; Wismer, C. T.; Mikusa, J.; Yu, H.; Kohlhaas, K.; Jarvis, M. F.; Bhagwat, S. S. *J. Med. Chem.* **2002**, *45* (17), 3639-3648.

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]

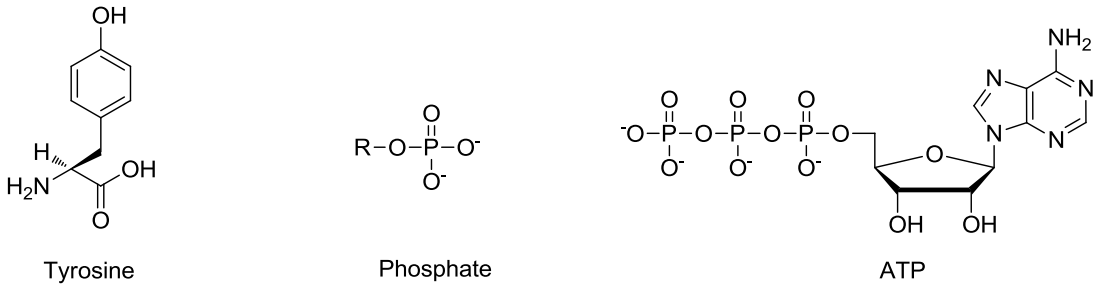


Figure 1

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.

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.[4] 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.[3]

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.[3] 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.[3]

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.[2]

Cellular proliferation, explained in some detail above, may rely in some part on tyrosine kinase.[3] 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.[3]

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.[4] 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]

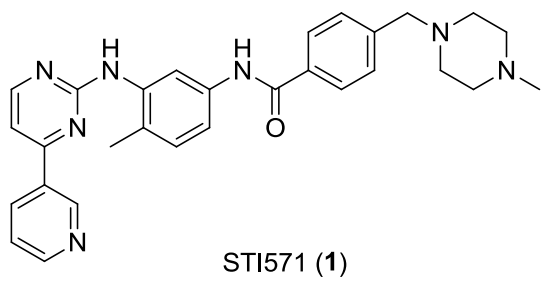
It is known among certain members of the scientific community that protein phosphorylation occurs on residues of tyrosine by both transmembrane receptor- and membrane-associated protein tyrosine kinases in normal cells.[2] This occurrence is likely quite significant to the activity of communications 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

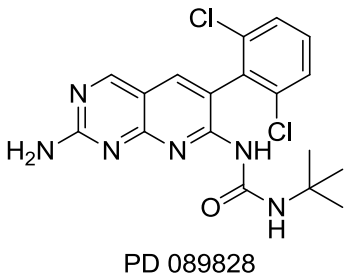
unfavorable characteristic because it results in excessive and uncontrolled proliferation of the myelogenous cells in which it is expressed.[11]



STI571 (**1**)

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.[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.[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.[13]



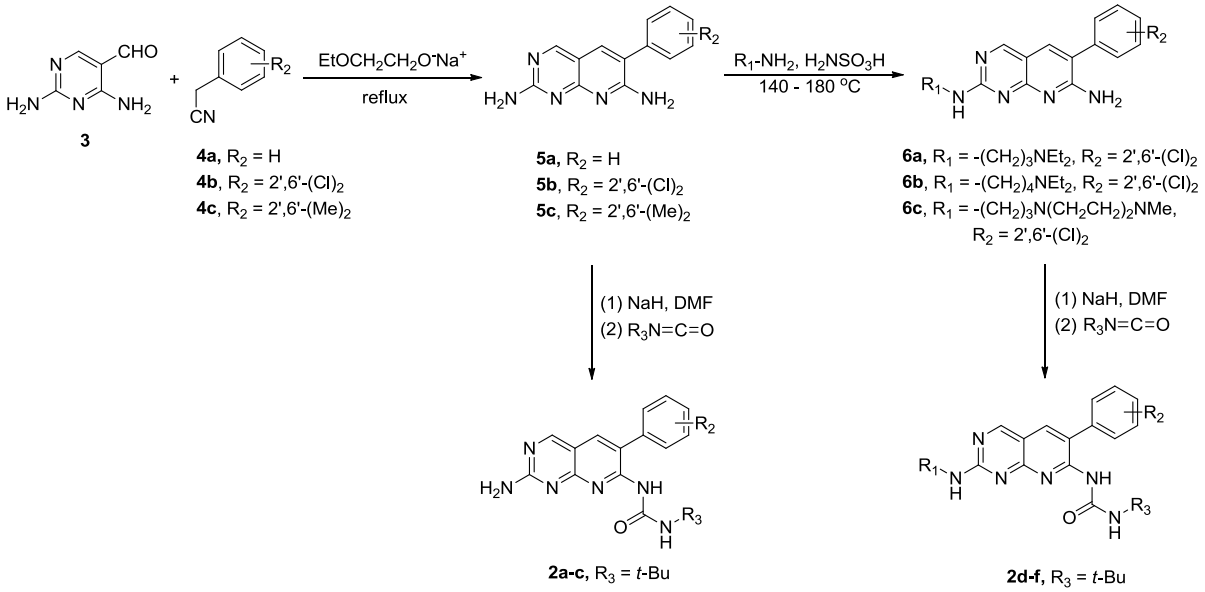
PD 089828

Figure 3

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 activitiy, 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.[14] 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.[15] 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.

Figure 4

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).[15]

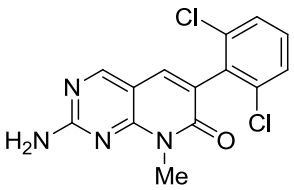


Scheme 1: Synthesis of PD 089828 and analogs

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]



7a

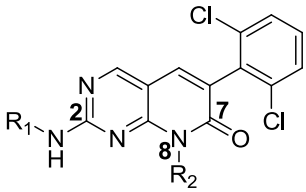


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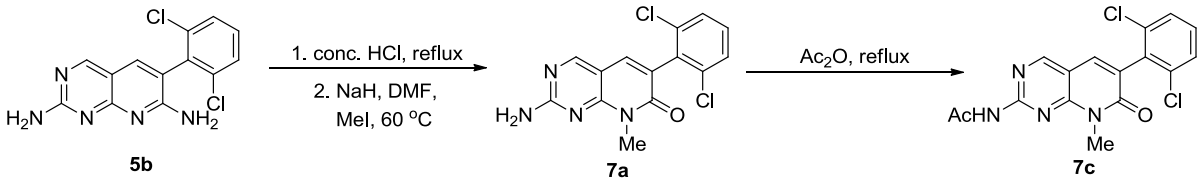
Figure 5

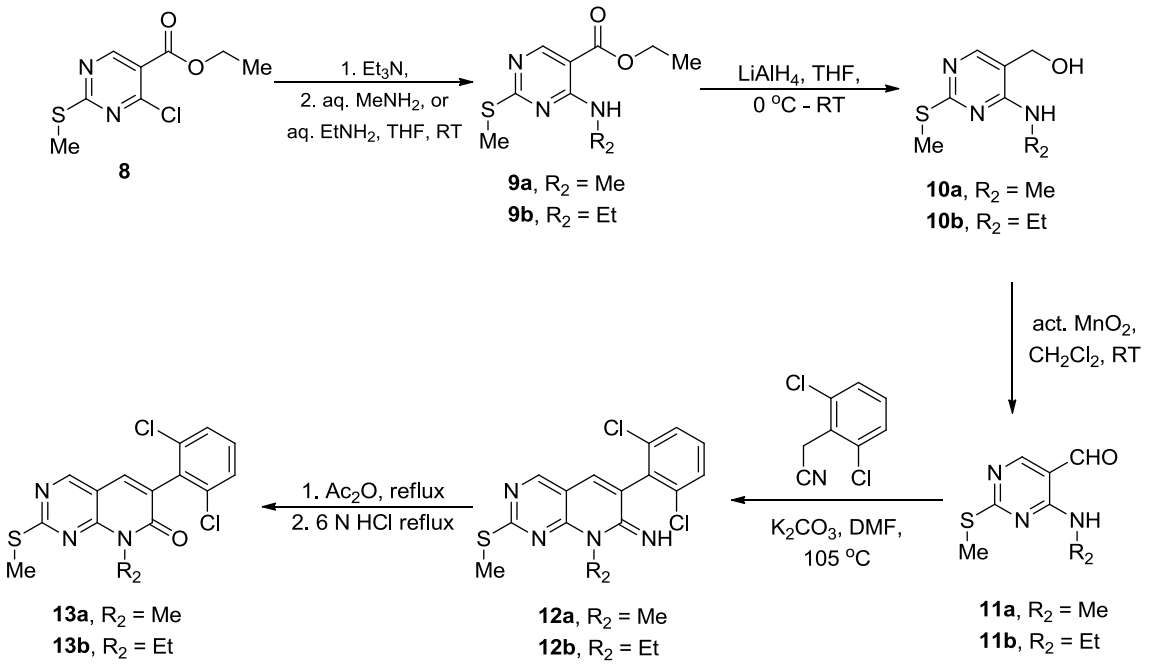
A related structure, 2-amino-6-(2,6-dichlorophenyl)-pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (**7a**, Figure 5), which was also derived from mass screening, showed a similar profile toward the above kinases with micromolar inhibitory activity.[16] Although both **2b** and **7a**

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.[16] 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.[16]



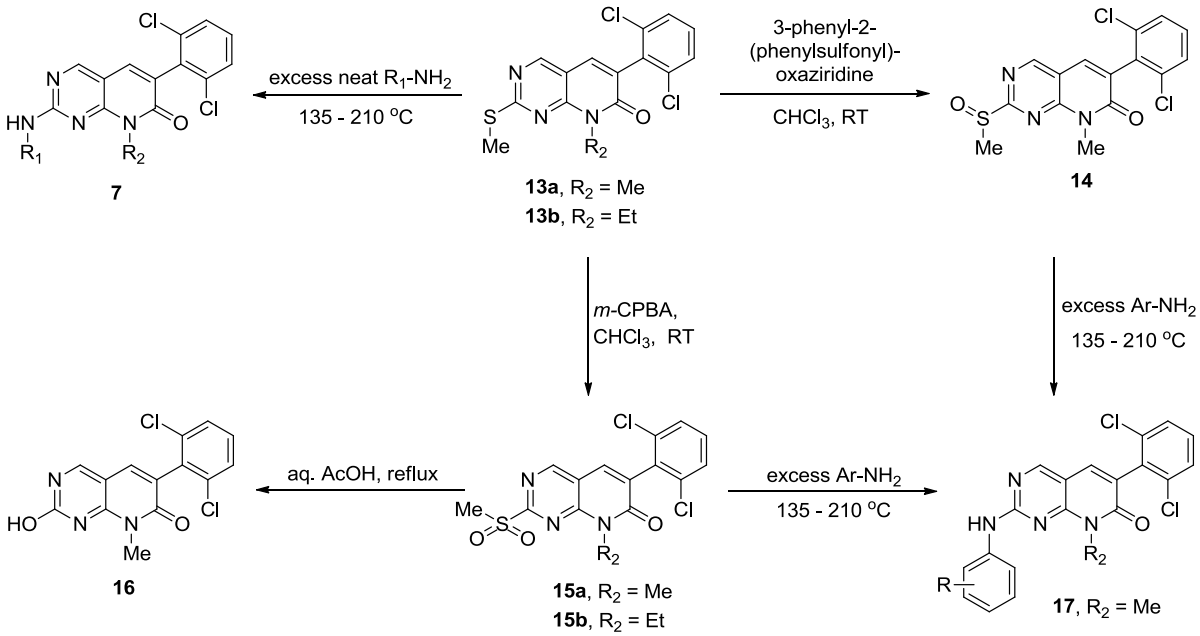
Scheme 2: Synthesis of new PD derivatives

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 MnO_2 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 K_2CO_3 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]

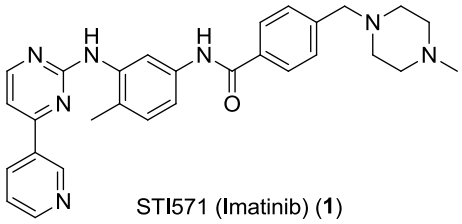


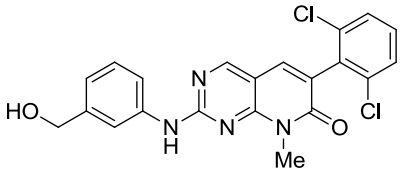
Scheme 4: Synthesis of PD derivatives

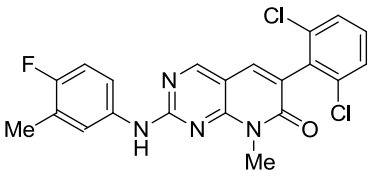
As detailed in Scheme 4, oxidation of **13a-b** with 3-phenyl-2-(phenylsulfonyl)-oxaziridine in chloroform provided the sulfoxide **14** in 70% yield, whereas oxidation with two equivalents of *m*-chloroperbenzoic acid under similar conditions gave the corresponding sulfone **15a-b** in 92% yield. The 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.[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. Di-substituted 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 = $-CH_2OH$) 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 = $-CH_2OH$), 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]







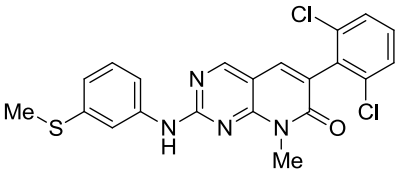
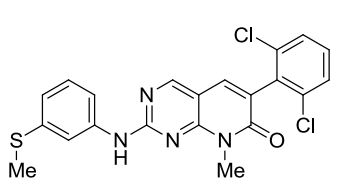


Figure 6: Comparison PD derivatives and STI571 growth inhibition of Bcr-Abl cell line

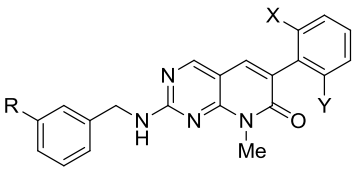
Various comparative studies of substituted pyrido[2,3-*d*]pyrimidines with STI571 (**1**) resulted in the development of compounds that vary in their specificities for different tyrosine kinases and were more potent than STI571 (**1**) (Figure 6).[17] Among these potential drug candidates, PD173955 (**20**), 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.[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 [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.[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.[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.



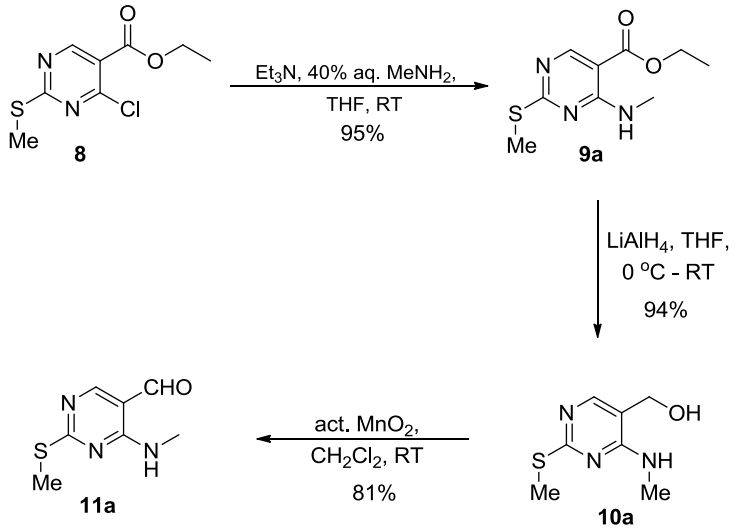
PD173955 (**20**)



PDC **(24)**

Figure 7

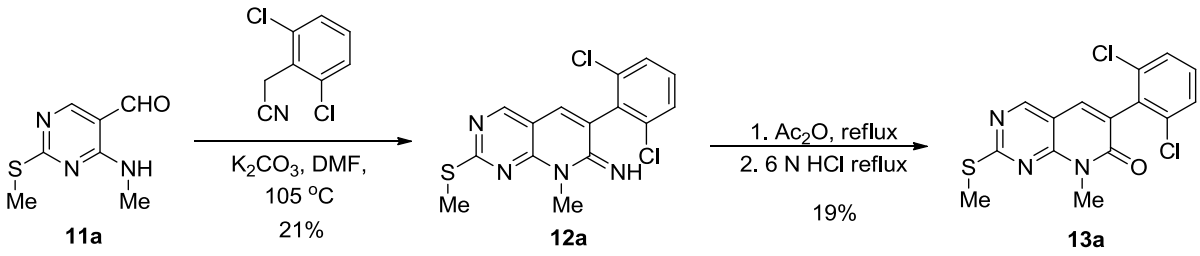
To investigate the structural elements of PD173955 that make it a good inhibitor of the Abl kinase, we planned to synthesize analogs of **20** (Figure 7). As reported earlier, the bicyclic ring and the halogen containing ring subunit of PD173955 interact with several amino acid residues in the crystal 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.[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.[22]



Scheme 5: Synthesis of PD precursor **11a**

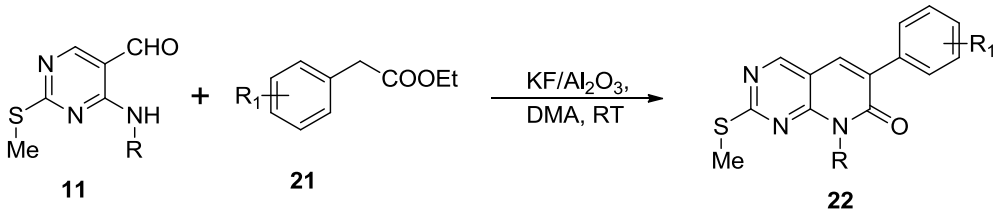
Scheme 5 outlines the synthesis of common intermediate **11a** which started with the room temperature condensation of commercially available 5-pyrimidinecarboxylic acid ester **8** with aqueous methylamine in THF to provide **9a** in 95% yield. Ester **9a** was reduced under standard lithium aluminum 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.[16]



Scheme 6: Synthesis of PD precursor **13a**

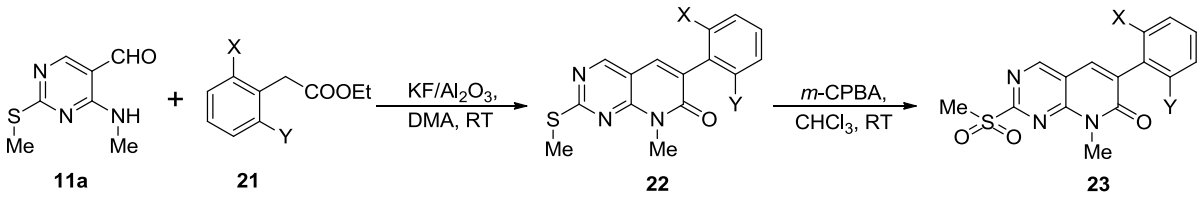
The condensation of aldehyde **11a** with 2,6-dichlorophenylacetonitrile in DMF at 105 °C in the presence of anhydrous potassium bicarbonate as the base provided 7-imino-pyridopyrimidine product **12a**. This reaction was hampered by problems related to solubility and low yields (Scheme 6). Subsequent 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.[23]

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).[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.[23] As explained earlier, one of the primary methods to prepare the requisite pyrimidinone scaffold core for this class of compounds is a tandem Knovenagal and amide/ester exchange reaction between the functionalized pyrimidine aldehyde **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.[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.[23] This pathway also reduced the number of steps required to synthesize pyrido[2,3-*d*]pyrimidines (**22**) as acetylation and hydrolysis were not needed with this methodology unlike before (Scheme 7).



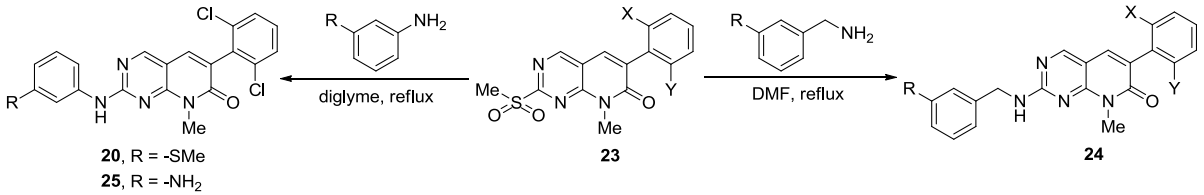
Scheme 8: Synthesis of PD precursor **23**

#	X	Y	**22** (% yield)	**23** (% yield)
a	Cl	Cl	62*	87
b	Cl	H	73	85
c	H	H	89	65
d	F	F	83	84
e	Br	H	92	82

*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/Al_2O_3 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).



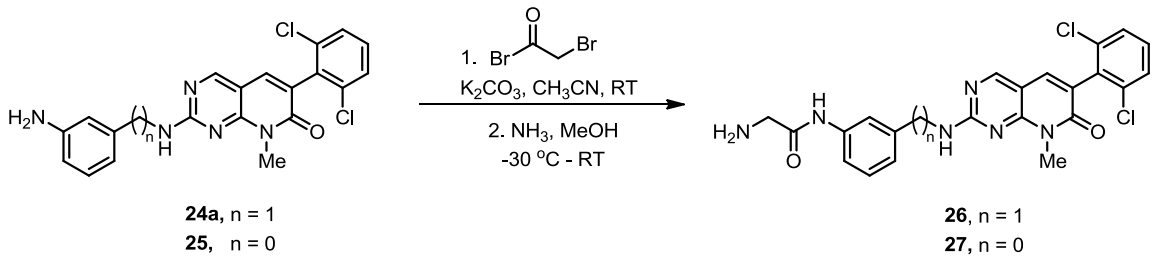
Scheme 9: Synthesis of PD and PDC analogs

#	X	Y	R	Yield (%)	Compound
1	Cl	Cl	NH_2	61	**24a**
2	Cl	H	NH_2	77	**24b**
3	H	H	NH_2	53	**24c**
4	F	F	NH_2	67	**24d**
5	Br	H	NH_2	48	**24e**
6	Cl	Cl	SMe	64	**24f**
7	Cl	Cl	SMe	57	**20**
8	Cl	Cl	NH_2	55	**25**

Table 2: Synthesis of PD and PDC analogues

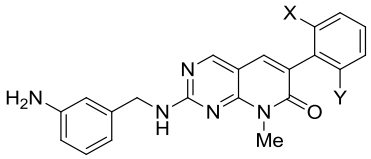
Initially, the conversion of sulfone **23a** into analogs of PD173955 using various anilines in boiling aprotic solvents failed. Interestingly, the reaction of sulfone **23a** with 3-aminobenzylamine which is a more reactive nucleophile, in boiling DMF generated the PDC

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).



Scheme 10: Synthesis of PD-Gly and PDC-Gly analogues

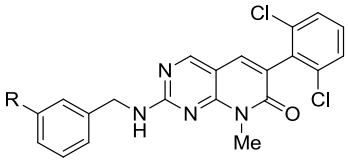
These compounds were tested for Abl-kinase inhibition in Dr. Marit Nilsen-Hamilton's laboratory, our collaborators in the biochemistry, biophysics and molecular biology department at Iowa State University. The results of inhibition of Abl kinase (Ki) are shown below in Figures 8 – 10. The number in parentheses 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 (***, $p<0.0005$, **, $p<0.005$, *, $p<0.03$)



24a: X = Cl, Y = Cl	Ki = 8 x 10^{-9} (16)***
24b: X = Cl, Y = H	6 x 10^{-8} (6)***
24c: X = H, Y = H	2 x 10^{-6} (5)**
24d: X = F, Y = F	4 x 10^{-8} (3)**
24e: X = Br, Y = H	4 x 10^{-8} (1)*

Figure 8

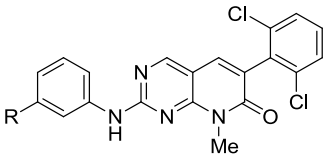
The 2,6-dichlorophenyl substituent **24a** exhibited the strongest inhibition.



24f: R = -SMe	Ki = 7 x 10^{-8} (3)***
26: R = $-NHCOCH_2NH_2$	2 x 10^{-8} (3)***

Figure 9

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



20 : R = -SMe	Ki = 2 x 10^{-10} (6)***
25 : R = $-NH_2$	2 x 10^{-11} (6)***
27 : R = $-NHCOCH_2NH_2$	2 x 10^{-11} (4)***

Figure 10

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.[22] 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.[22] 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**.

$$\text{Abl} + \text{PD} \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} \text{Abl-PD}$$

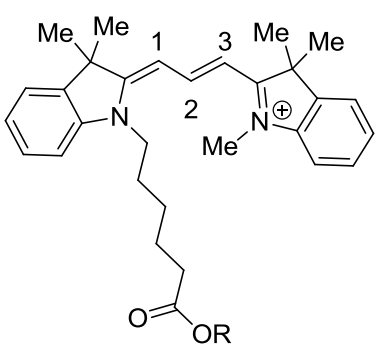
Figure 11

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.

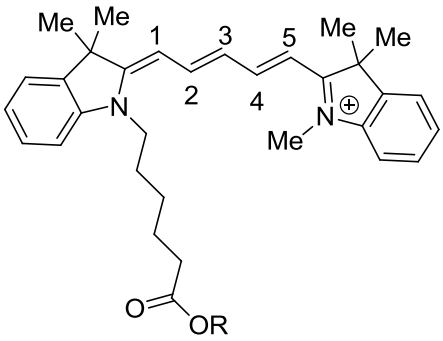
Although most van der Waals interactions reported for PD173955 with Abl are located around the bicyclic substituent and the dichlorophenyl group, two of the ten interactions are with amino acids that contact the methylthiophenyl segment of PD173955. Alteration of the molecular structure in this region 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 x 10^{-8}] [24] 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.



Cy3 dye
28a, R = H
28b, R = NHS

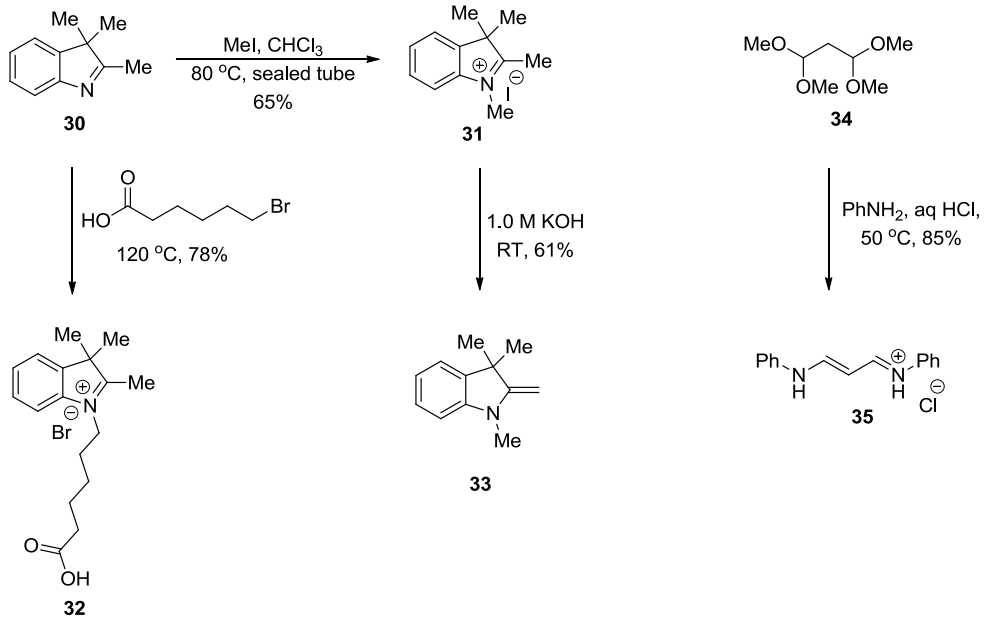


Cy5 dye
29a, R = H
29b, R = NHS

Figure 12: Structure of Cyanin dyes Cy3 and Cy5

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

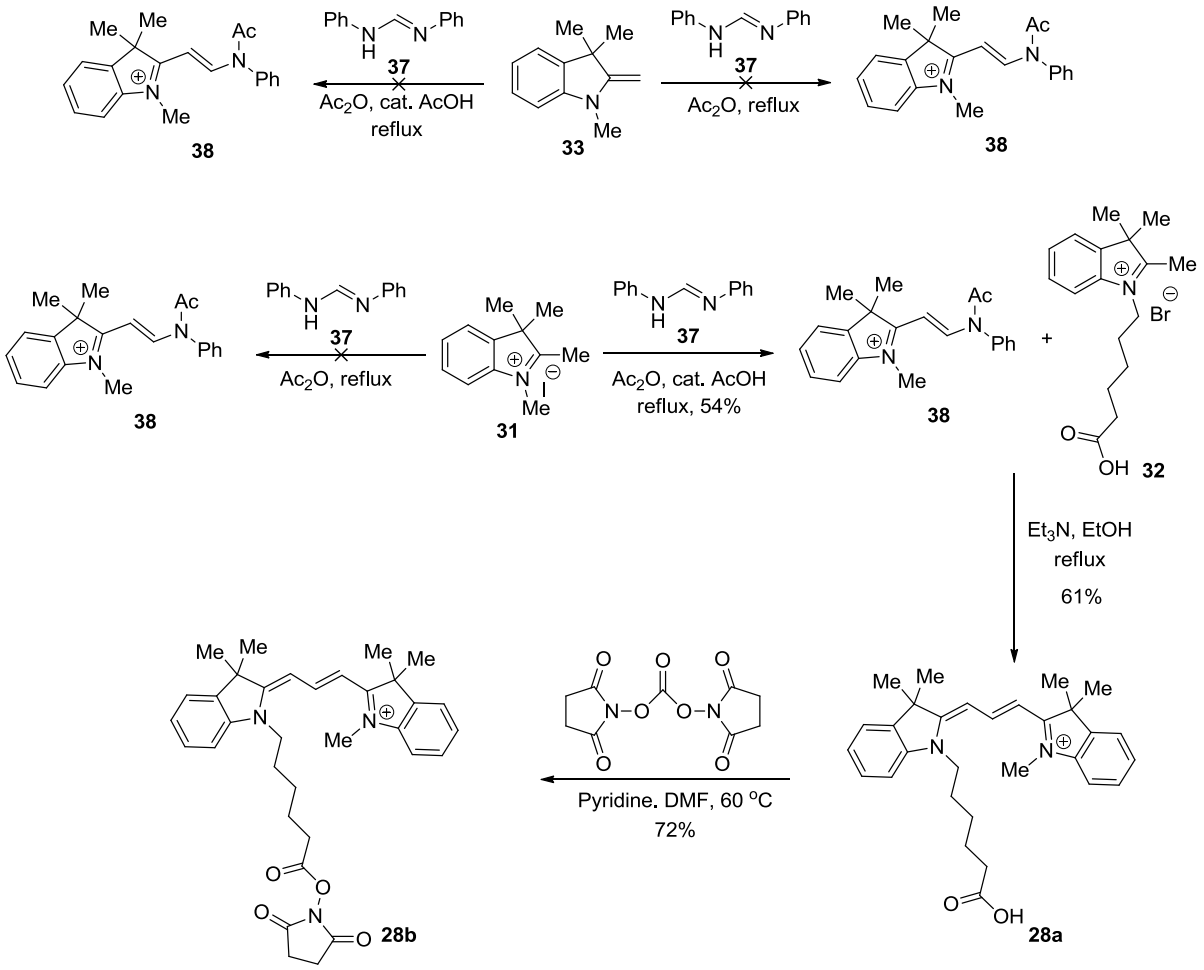
consists of a conjugated system based on a polymethine chain linking two nitrogen-containing heterocycles (e.g., indoles, benzothiazoles). They are generally named based on the number of carbon atoms in the polymethine chain. Trimethine and pentamethine dyes exhibit absorption maxima at 550 and 650 nm, respectively, and emission maxima around 570 and 670 nm, respectively, in the green or red part of the spectrum.[26] 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.[27] The dyes are generally converted into their N-hydroxysuccinimide esters **28b** and **29b** 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.[25] The synthesis of the Cy3 dye **28a** and Cy5 dye **29a** and their NHS esters **28b** and **29b** respectively, began with the synthesis of common intermediates (Scheme 11). Commercially available trimethylindole **30** was alkylated with methyl iodide in chloroform under sealed tube conditions to give the 1,2,3,3-tetramethylindolium iodide **31** in 65% yield

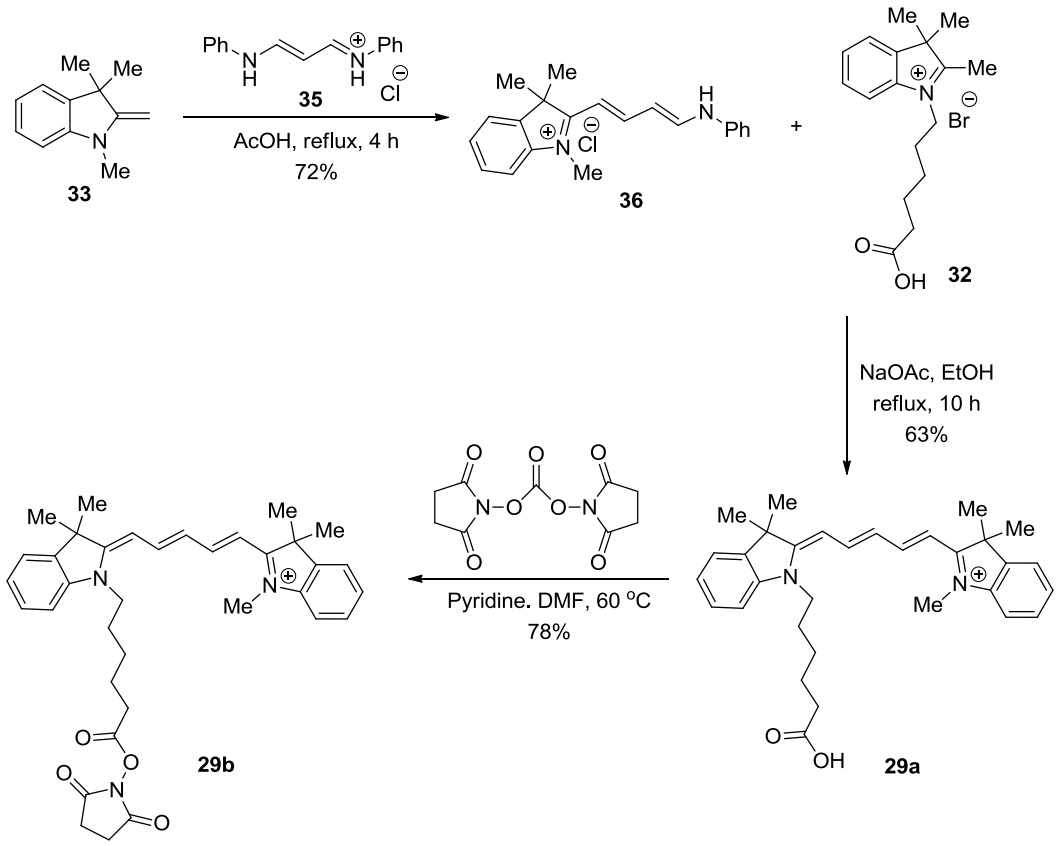
(Scheme 11). A similar reaction of trimethylindole **30** to give 5-carboxypentyl-2,3,3-trimethylindolium bromide **32** was done by heating the indole **30** with 6-bromohexanoic acid at 120 ^{o}C under neat conditions to get the addition product **32** in 78% yield (Scheme 11). Conversion of 1,2,3,3-tetramethylindolium 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

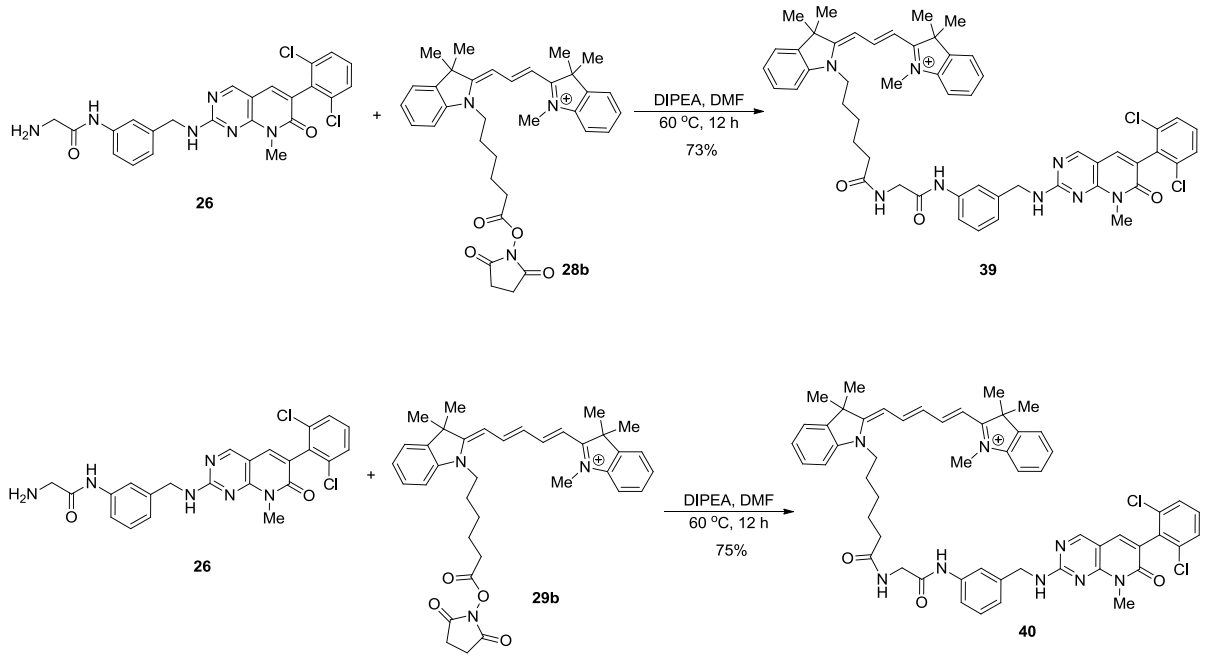
(Scheme 12). Finally, we were able to prepare acetanilidylvinyl indolium salt **38** by condensing tetramethylindolium iodide **31** with N,N'-diphenylformamidine under refluxing conditions in the presence of acetic anhydride and few drops of acetic acid (Scheme 12). This compound was then reacted with 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

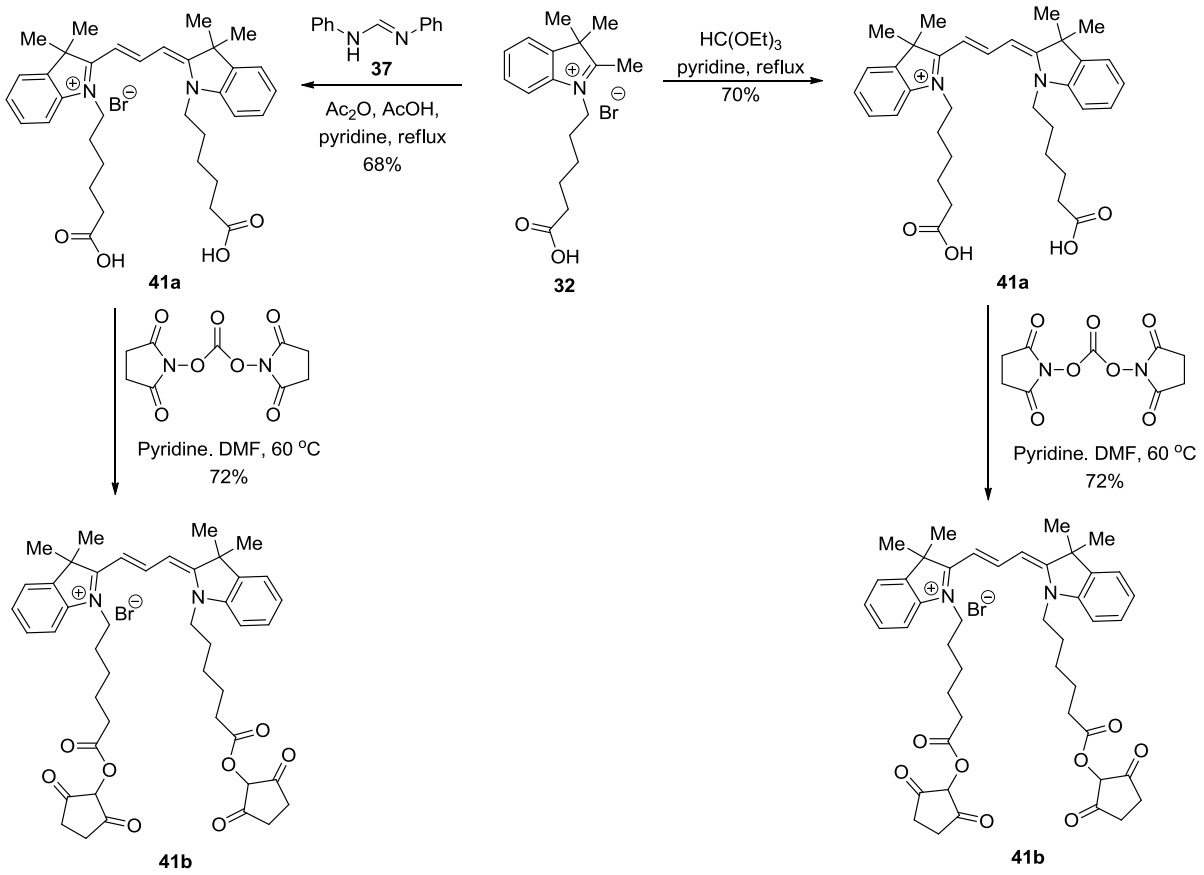
For the synthesis of the Cy5 dye **29a** and its NHS ester **29b** (Figure 12), the three-carbon spacer **35** was prepared (Scheme 11). Condensation of commercially available malondialdehyde bis(dimethyl acetal) with aniline under acidic conditions afforded the

anilino anilinium salt **35** in 85% yield (Scheme 11).[28] 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.[25]



Scheme 14: Conjugation of PDC-Gly with Cy3-NHS ester and Cy5-NHS ester

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 ^{o}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 (Scheme14).

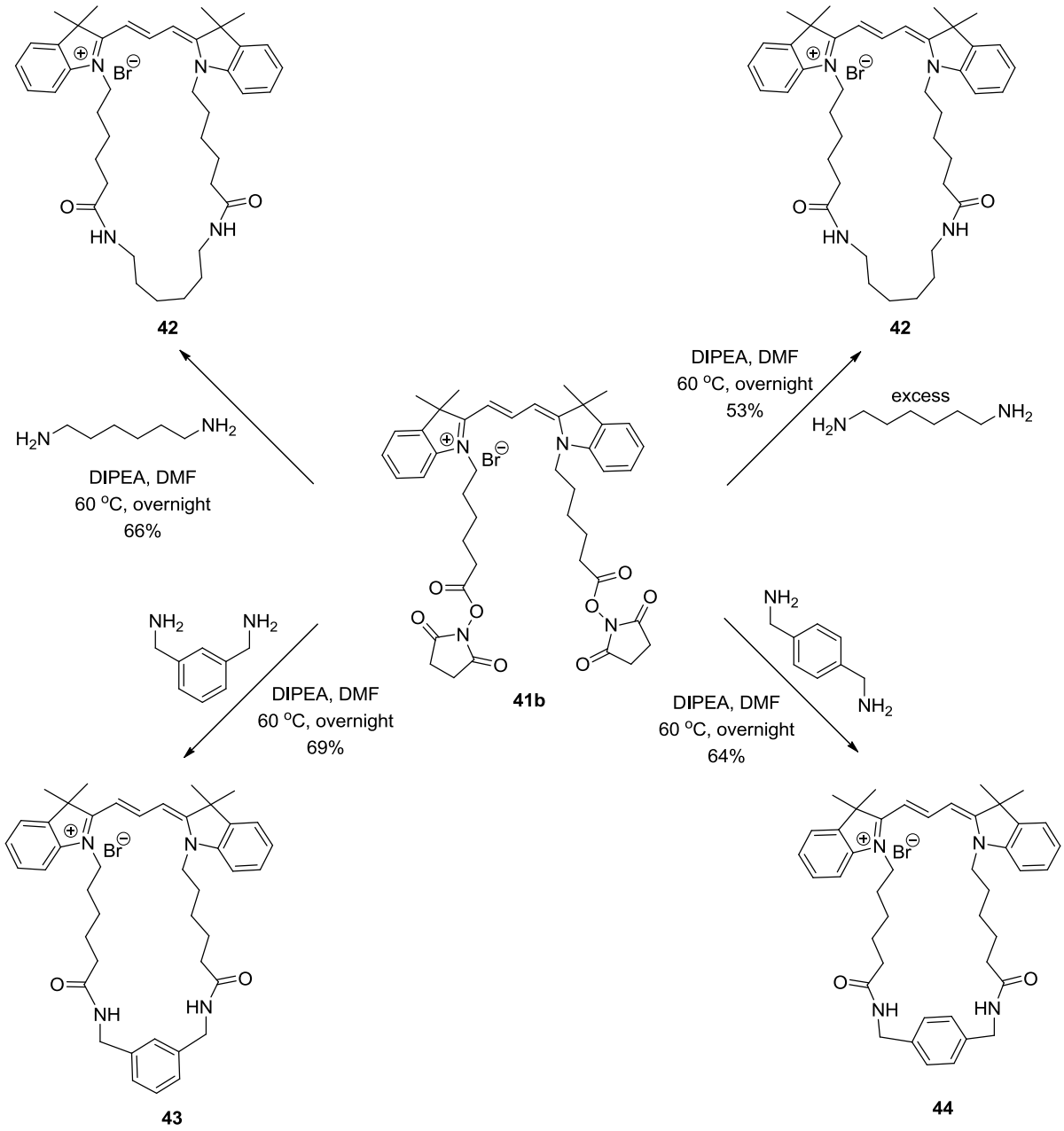


Scheme 15: Synthesis and activation of new Cy3 dye **41**

The synthetic route for the synthesis of Cy3 and Cy5 was fairly. However, the preparation of acetanilidylvinyl 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).[29] 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.[29] 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-hexanediamine 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-hexanediamine, 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).



Scheme 17: Synthesis of Cy3 macrocycles

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).

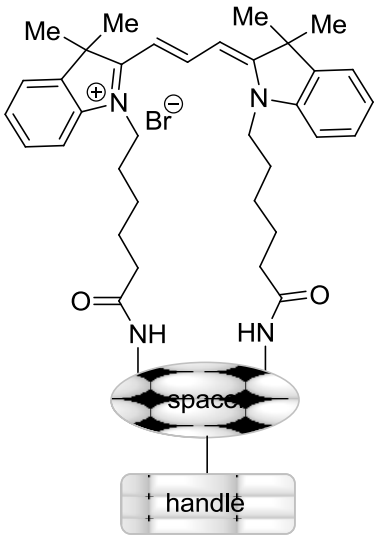
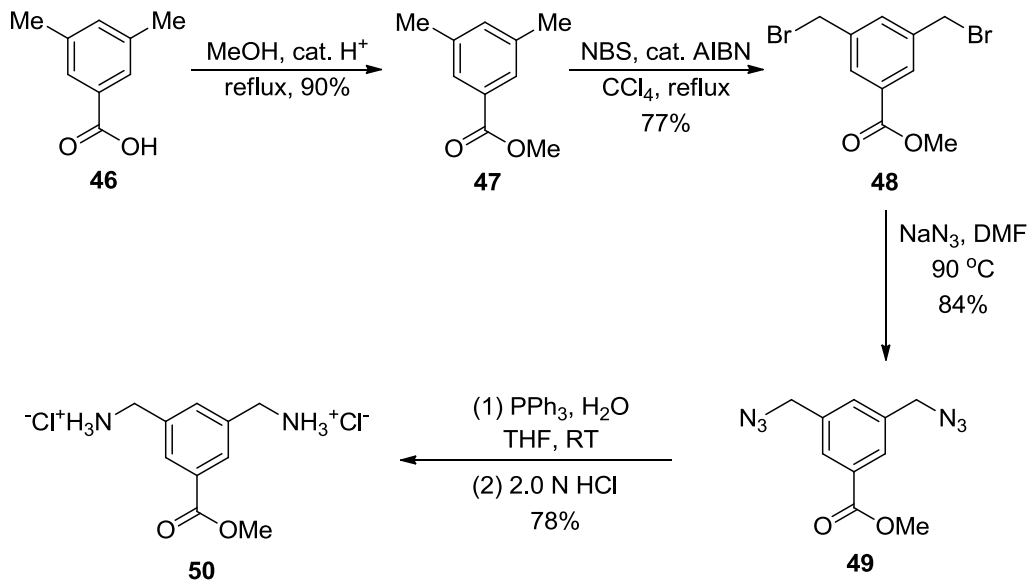


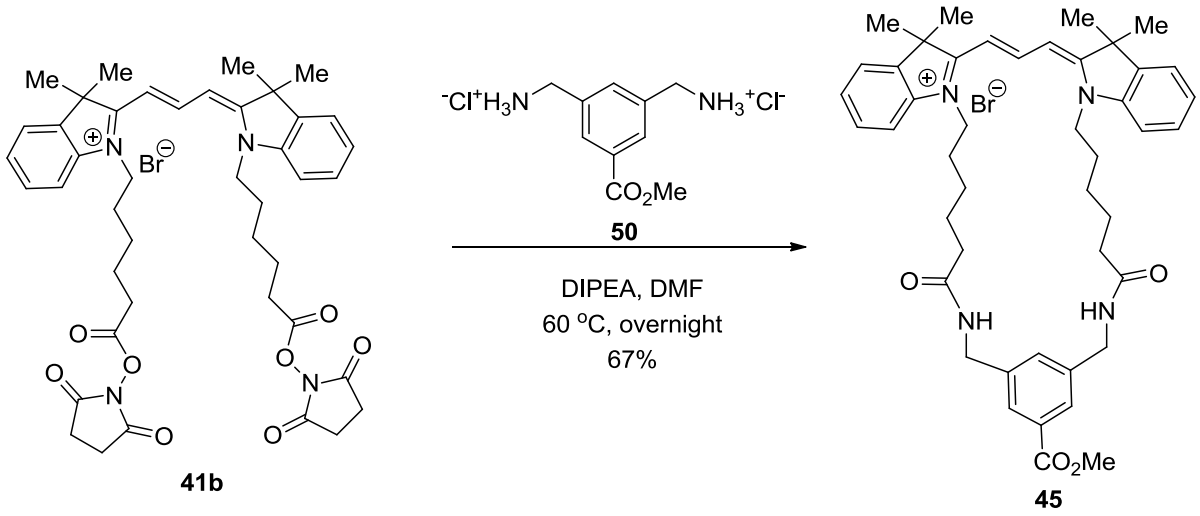
Figure 13: Cy3 dye macrocycle with handle

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]



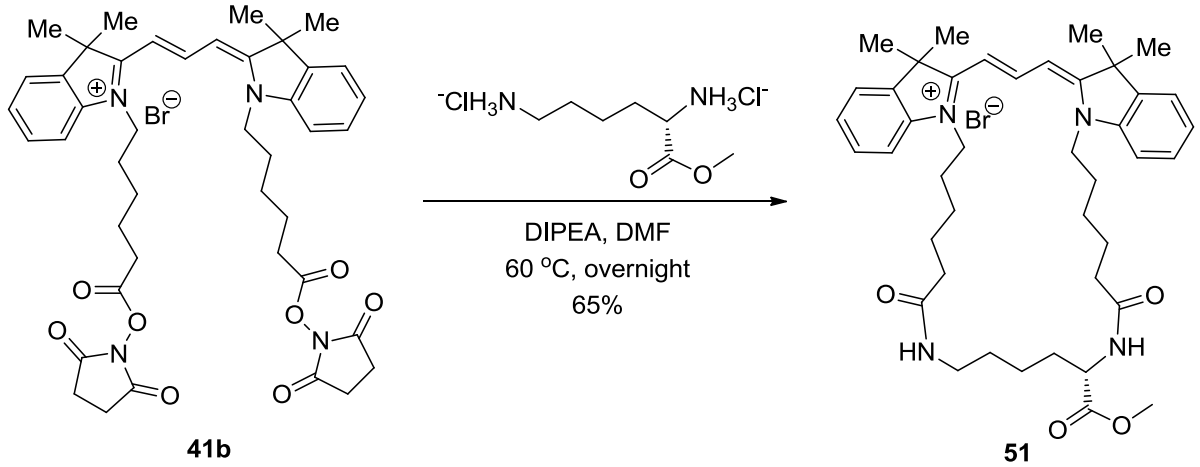
Scheme 18

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).



Scheme 19: Synthesis of the new Cy3 dye macrocyle

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 macrocyle

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 Cy 3 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 protiens.

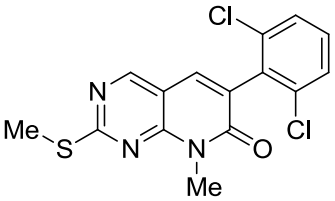
Experimental

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),

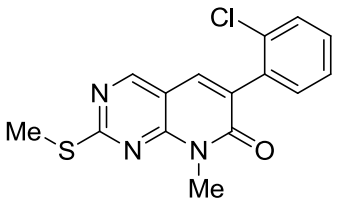
unless otherwise noted. Coupling constants (*J*) are reported in Hz with abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. High resolution mass spectra were recorded on a Kratos model MS-50 spectrometer. Standard grade silica gel (60 Å, 32-63 μm) was used for flash column chromatography.

Representative procedure for the preparation of 22a-e

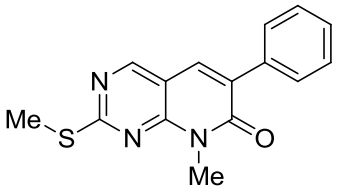
To a stirred solution of 0.400 g (2.19 mmol) of 4-amino-2-(methylthio) pyrimidine-5-carbaldehyde **11a** and 0.638 g (2.74 mmol) of ethyl 2-(2,6-dichlorophenyl)acetate **21a** in 10.0 mL of dry DMA, 2.536 g of KF/Al_2O_3 (40 wt %, KF, Sigma–Aldrich catalog #316385) was added in batches of 0.5 g every 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.



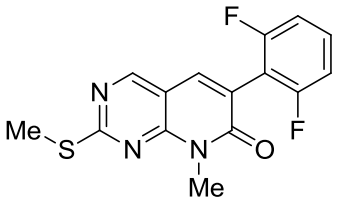
6-(2,6-dichlorophenyl)-8-methyl-2-(methylthio)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (22a). Yield 62%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.66 (s, 1H), 7.60 (s, 1H), 7.41 (d, J = 8.0 Hz, 2H), 7.29 (d, J = 8.0 Hz, 1H), 3.83 (s, 3H), 2.67 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 173.9, 161.1, 156.7, 154.6, 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.



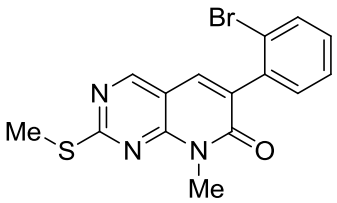
6-(2-chlorophenyl)-8-methyl-2-(methylthio)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (22b).
Yield 73%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.63 (s, 1H), 7.64 (s, 1H), 7.42 - 7.49 (m, 1H), 7.27 – 7.37 (m, 3H), 3.80 (s, 3H), 2.65 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 173.5, 161.8, 156.5, 154.4, 135.0, 134.8, 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 $C_{15}H_{12}ClN_3OS$: 317.0390; found: 317.0396



8-methyl-2-(methylthio)-6-phenylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (22c).
Yield 89%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 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, $CDCl_3$): δ 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 $C_{15}H_{13}N_3OS$: 283.0779; found: 283.0778



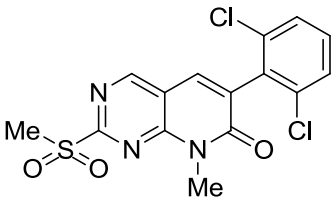
6-(2,6-difluorophenyl)-8-methyl-2-(methylthio)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (22d).
Yield 92%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.64 (s, 1H), 7.72 (s, 1H), 7.30 – 7.40 (m, 1H), 6.93 – 7.04 (m, 2H), 3.81 (s, 3H), 2.64 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 173.9, 162.0 (d, J = 28.0 Hz), 161.2, 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 $C_{15}H_{11}F_2N_3OS$: 319.0591; found: 319.0591.



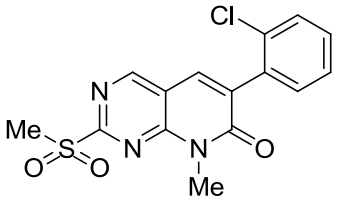
6-(2-bromophenyl)-8-methyl-2-(methylthio)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (22e). Yield 83%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.63 (s, 1H), 7.65 (d, J = 8.0 Hz, 1H), 7.61 (s, 1H), 7.29 – 7.40 (m, 2H), 7.21 – 7.28 (m, 1H), 3.80 (s, 3H), 2.64 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 173.5, 161.8, 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 $C_{15}H_{12}BrN_3OS$: 360.9884; found: 360.9876.

Representative procedure for the preparation of 23a-e

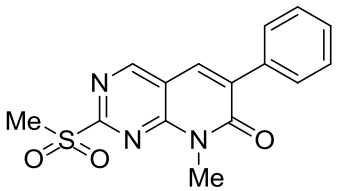
To a solution of 0.500 g (1.42 mmol) of **22a** in 40 mL of chloroform, was added 0.800 g (3.12 mmol) of 75% *m*-chloroperoxybenzoic acid. The solution was stirred at RT for 6 h. After the completion of reaction, 2 mL of DMSO was added to the reaction mixture to neutralize unreacted *m*-chloroperoxybenzoic acid and it was further stirred for 15 minutes after which the reaction mixture was diluted with methylene chloride and washed with saturated $NaHCO_3$, water and brine. The organic phase was dried over anhydrous $MgSO_4$ and concentrated. The crude was used for the next step without further purification.



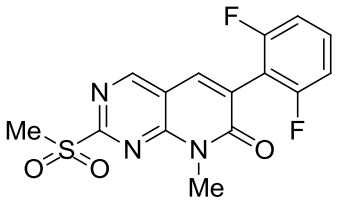
6-(2,6-dichlorophenyl)-8-methyl-2-(methylsulfonyl)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (23a). Yield 87%; ^{1}H-NMR (300 MHz, $CDCl_3$): δ 9.03 (s, 1H), 7.78 (s, 1H), 7.42 – 7.48 (m, 2H), 7.31 – 7.38 (m, 1H), 3.91 (s, 3H), 3.44 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 165.0, 160.5, 157.5, 155.5, 135.2, 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 $C_{15}H_{11}Cl_2N_3O_3S$: 383.9971; found: 383.9970



6-(2-chlorophenyl)-8-methyl-2-(methylsulfonyl)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (23b). Yield 85%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 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, $CDCl_3$): δ 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 $C_{15}H_{12}ClN_3O_3S$: 349.0310; found: 349.0299.

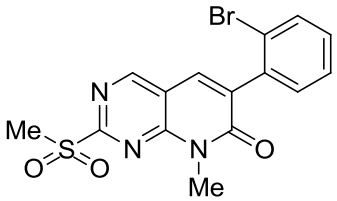


8-methyl-2-(methylsulfonyl)-6-phenylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (23c). Yield 65%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.99 (s, 1H), 7.85 (s, 1H), 7.64 – 7.71 (m, 2H), 7.42 – 7.49 (m, 3H), 3.88 (s, 3H), 3.41 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 164.2, 162.0, 157.0, 154.7, 137.7, 134.7, 131.2, 129.8, 129.1, 128.7, 115.7, 39.5, 29.4; LRMS: 318.0; HRMS calculated M^+ for $C_{15}H_{13}N_3O_3S$: 317.0390; found: 317.0396.



6-(2,6-difluorophenyl)-8-methyl-2-(methylsulfonyl)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (23d). Yield 82%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 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, $CDCl_3$): δ 164.9, 161.9 (d, J = 28.0 Hz), 160.6, 159.4 (d, J = 28.0 Hz), 157.5, 155.4, 135.7, 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,

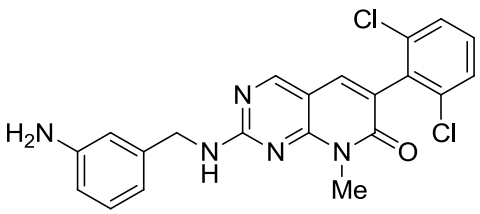
304.1, 251.1, 209.2, 121.0; HRMS calculated M^+ for $C_{15}H_{11}F_2N_3O_3S$: 351.0489; found: 351.0491.



6-(2-bromophenyl)-8-methyl-2-(methylsulfonyl)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (23e). Yield 84%; ^{1}H-NMR (400 MHz, $CDCl_3$): 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); ^{13}C-NMR (100 MHz, $CDCl_3$): 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 $C_{15}H_{12}BrN_3O_3S$: 392.9783; found: 392.9792.

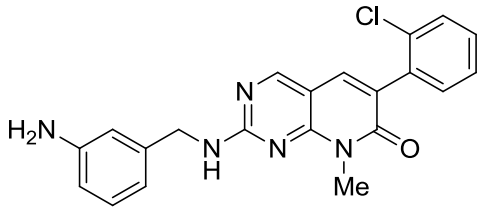
Representative procedure for the preparation of 24a-f

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 $MgSO_4$ and concentrated *in vacuo* to give the crude product which was chromatographed over silica gel using 2% MeOH in CH_2Cl_2 to give 0.091 g (61%) of pure **24a**.

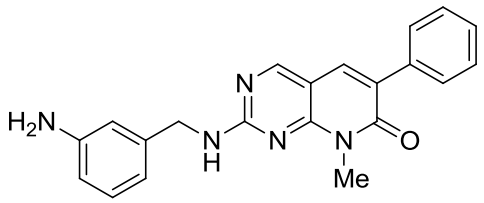


2-(3-aminobenzylamino)-6-(2,6-dichlorophenyl)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (24a). Yield 61%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.27 (bs, 1H), 7.44 (s, IH), 7.40 (d, J = 8.0 Hz, 2H), 7.25 (t, J = 8.0 Hz, 1H), 7.14 (t, J = 8.0 Hz, 1H), 6.61 – 6.78 (m, 3H), 4.64 (d,

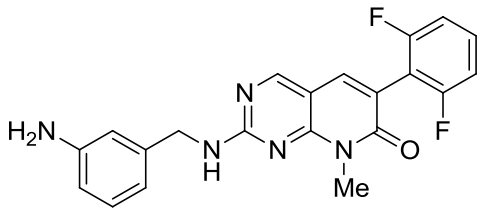
J = 5.2 Hz, 2H), 3.72 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 161.67, 161.19, 158.67, 156.22, 147.00, 139.75, 136.71, 135.94, 134.55, 129.87, 129.79, 128.12, 120.27, 119.82, 118.58, 118.09, 114.42, 114.32, 113.83, 46.05, 28.33; LRMS: 427.1, 425.1, 392.1, 390.1, 287.1, 285.1, 106.1; HRMS calculated M^+ for $C_{21}H_{17}Cl_2N_5O$: 452.0810; found: 425.0813.



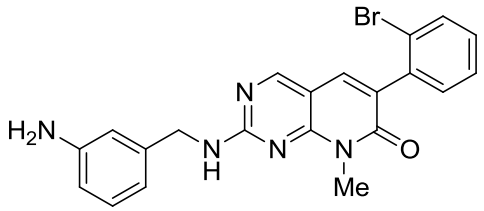
2-(3-aminobenzylamino)-6-(2-chlorophenyl)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (24b). Yield 77%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.32 (bs, 1H), 7.52 (s, IH), 7.47 (t, *J* = 4.0 Hz, 1H), 7.30 – 7.36 (m, 3H), 7.14 (t, *J* = 8.0 Hz, 1H), 6.77 (d, *J* = 7.6 Hz, 1H), 6.70 (s, 1H), 6.62 (d, *J* = 7.6 Hz, 1H), 4.64 (d, *J* = 5.2 Hz, 2H), 3.71 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 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; LRMS: 391.1, 356.2, 251.1, 106.1, 69.0; HRMS calculated M^+ for $C_{21}H_{18}ClN_5O$: 391.1200; found: 391.1204.



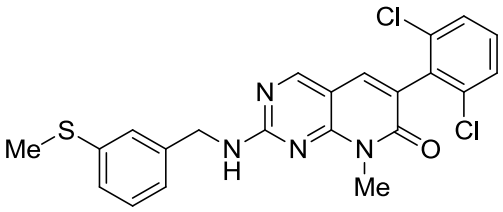
2-(3-aminobenzylamino)-8-methyl-6-phenylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (24c). Yield 53%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.20 (bs, 1H), 7.64 (d, *J* = 7.6 Hz, 2H), 7.55 (s, 1H), 7.43 – 7.32 (m, 3H), 7.13 (t, *J* = 7.6 Hz, 1H), 6.77 – 6.59 (m, 3H), 4.62 (d, *J* = 5.2 Hz, 2H), 3.71 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 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; LRMS: 358.2, 357.2, 356.2, 252.1, 121.1, 106.1, 77.0; HRMS calculated M^+ for $C_{21}H_{19}N_5O$: 357.1590; found: 357.1600.



2-(3-aminobenzylamino)-6-(2,6-difluorophenyl)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (24d). Yield 67%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.37 (bs, 1H), 7.59 (s, IH), 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); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 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, 111.7, 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^+ for $C_{21}H_{17}F_2N_5O$: 393.1401; found: 393.1404.



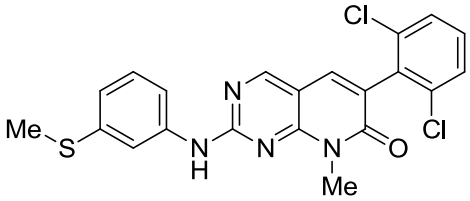
2-(3-aminobenzylamino)-6-(2-bromophenyl)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (24e). Yield 48%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.18 (bs, 1H), 7.65 (d, J = 7.6 Hz, 1H), 7.46 (bs, 1H), 7.37 – 7.32 (m, 2H), 7.26 – 7.20 (m, 2H), 7.13 (t, J = 7.6 Hz, 1H), 6.78 – 6.60 (m, 3H), 4.62 (d, J = 5.2 Hz, 2H), 3.71 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 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^+ for $C_{21}H_{18}BrN_5O$: 435.06947; found: 435.07033.



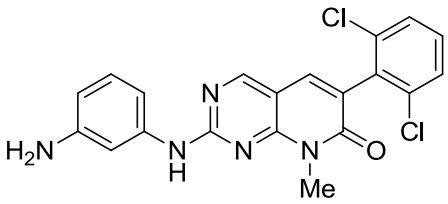
6-(2,6-dichlorophenyl)-8-methyl-2-(3-(methylthio)benzylamino)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (24f). Yield 64%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.19 (bs, 1H), 7.42 – 7.38 (m, 3H), 7.27 – 7.22 (m, 3H), 7.18 – 7.16 (m, 2H), 6.84 (bs, 1H), 4.68 (d, J = 4.4 Hz, 2H), 3.71 (s, 3H), 2.46 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 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 $C_{22}H_{18}Cl_2N_4OS$: 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%).



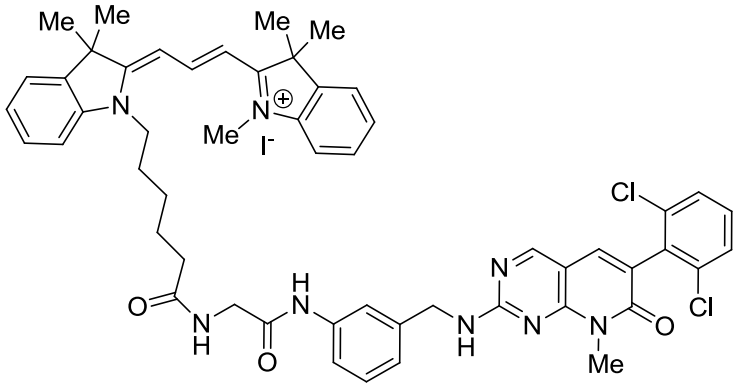
6-(2,6-dichlorophenyl)-8-methyl-2-(3-(methylthio)phenylamino)pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (20). Yield 57%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.60 (s, 1H), 7.75 (bs, 1H), 7.54 – 7.52 (m, 2H), 7.42 – 7.38 (m, 3H), 7.33 – 7.25 (m, 2H), 7.01 (d, J = 8.0 Hz, 1H), 3.82 (s, 3H), 2.53 (s, 3H); LRMS: 442, 407, 362, 313, 269, 203, 196, 180; HRMS calculated M^+ for $C_{21}H_{16}Cl_2N_4OS$: 442.04219; found: 442.04315.



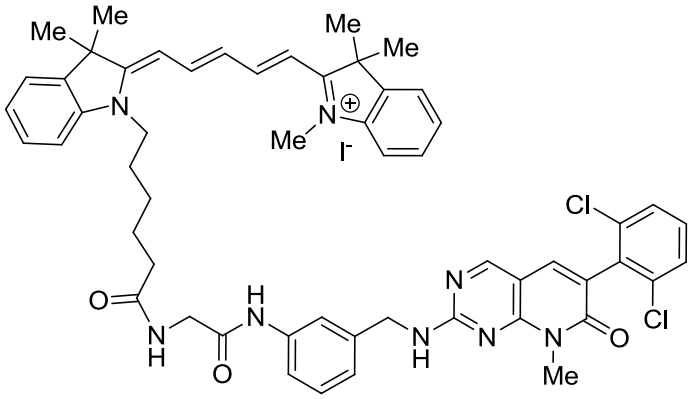
2-(3-aminophenylamino)-6-(2,6-dichlorophenyl)-8-methylpyrido[2,3-*d*]pyrimidin-7(8*H*)-one (25). Yield 55%; ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.58 (s, 1H), 7.53 (s, 1H), 7.46 – 7.40 (m, 3H), 7.28 – 7.25 (m, 1H), 7.23 – 7.11 (m, 2H), 7.07 – 6.98 (bs, 1H), 6.48 (d, J = 8.0 Hz,

1H), 3.81 (s, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 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 $C_{20}H_{15}Cl_2N_5O$: 411.06536; found: 411.06606.

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



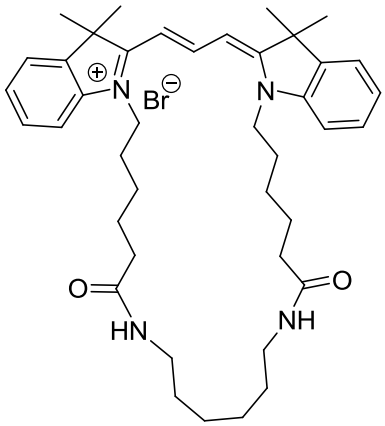
Preparation of Cy3-PDC-Gly (39) - In a 20 ml vial equipped with a stirring bar, Cy3-NHS ester (**28b**) (30.0 mg, 43.0 μmol), PDC-Gly (**26**) (25.0 mg, 52.0 μmol) and N,N-diisopropylethylamine (0.1 ml) were taken in DMF (5.0 ml) and stirred at room temperature under argon atmosphere in dark for 12 h. 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 $C_{53}H_{57}C_{l2}N_8O_3^+$: 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.



(31a*Z*,33*E*)-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%; ^{1}H-NMR (400 MHz, $CDCl_3$): 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); ^{13}C-NMR (100 MHz, $CDCl_3$): 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 $C_{41}H_{57}N_4O_2$: 637.4482; found: 637.4480.

References

1. Hanks, S. K.; Quinn, A. M.; Hunter T. *Science* **1988**, *241* (4861), 42–52.
2. Schaller M. D.; Borgman C. A.; Cobb B. S.; Vines R. R.; Reynolds A. B.; Parsons J. T. *Proc. Natl. Acad. Sci.* **1992**, *89* (11), 5192–5196.
3. Radha, V.; Nambirajan, S.; Swarup G. *Eur. J. Biochem.*, **1996**, *236* (2), 352–9.
4. Ruetten H.; Thiemermann C. *Br. J. Pharmacol*. **1997**, *122* (1), 59–70.
5. Dengjel J.; Kratchmarova I.; Blagoev B. *Mol. Biosyst.* **2009**, *5* (10), 1112–21.
6. Kris, M. G.; Natale, R. B.; Herbst, R. S.; Lynch, T. J.; Prager, D.; Belani, C. P.; Schiller, J. H.; Kelly, K.; Spiridonidis, H.; Sandler, A.; Albain, K. S.; Cella, D.; Wolf, M. K.; Averbuch, S. D.; Ochs, J. J.; Kay, A.C. *JAMA* **2003**, *290* (16), 2149–58.

7. Weinberg, R. A. *The Biology of Cancer. New York: Garland Science, Taylor & Francis Group, LLC.* **2007**, 757–759.
8. Klein, S.; Levitzki, A. *Advances in Cancer Research*, **2007**, *97*, 295-319.
9. *National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology: Chronic Myelogenous Leukemia* v.1.**2009**.
10. Schiffer, C. A. *N. Engl. J. Med.* **2007**, *357* (3), 258-265.
11. Wolf, D.; Tilg, H.; Rumpold, H.; Gastl, G.; Wolf, A. M. *Current Cancer Drug Targets* **2007**, *7*, 251-258.
12. Druker, B. J.; Talpaz, M.; Resta, D. J.; Peng, B.; Buchdunger, E.; Ford, J. M.; Lydon, N. B.; Kantarjian, H.; Capdeville, R.; Ohno-Jones, S.; Sawyers, C. L. *N. Engl. J. Med.* **2001**, *344* (14), 1031–7.
13. Ramirez, P.; DiPersio, J. F. *Oncologist*, **2008**, *13* (4), 424-434.
14. Dahring, T. K.; Lu, J. H.; Hamby, J. M.; Batley, B. L.; Kraker, A. J.; Panek, R. L. *J. Pharmacol. Exp. Ther.* **1997**, 281, 1446-1456.
15. Hamby, J. M.; Connolly, C. J.; Schroeder, M. C.; Winters, R. T.; Showalter, H. D.; Panek, R. L.; Major, T. C.; Olsewski, B.; Ryan, M. J.; Dahring, T.; Lu, G. H.; Keiser, J.; Amar, A.; Shen, C.; Kraker, A. J.; Slintak, V.; Nelson, J. M.; Fry, D. W.; Bradford, L.; Hallak, H.; Doherty, A. M. *J. Med. Chem.* **1997**, *40*, 2296-2303.
16. Klutchko, S. R.; Hamby, J. M.; Boschelli, D. H.; Wu, Z.; Kraker, A. J.; Amar, A. M.; Hartl, B. G.; Shen, C.; Klohs, W. D.; Steinkampf, R. W.; Driscoll, D. L.; Nelson, J. M.; Elliott, W. L.; Roberts, B. J.; Stoner, C. L.; Vincent, P. W.; Dykes, D. J.; Panek, R. L.; Lu, G. H.; Major, T. C.; Dahring, T. K.; Hallak, H.; Bradford, L. A.; Showalter, H. D. H.; Doherty, A. M. *J. Med. Chem.* **1998**, *41*, 3276-3292.
17. Wisniewski, D.; Lambek, C. L.; Liu, C.; Strife, A.; Veach, D. R.; Nagar, B.; Young, M. A.; Schindler, T.; Bornmann, W. G.; Bertino, J. R.; Kuriyan, J.; Clarkson, B. *Cancer Res.* **2002**, *62*, 4244–4255.
18. Moasser, M.M.; Srethapakdi, M.; Sachar, K.S.; Kraker, A.J; Rosen, N. *Cancer Res*. **1999**, *59*, 6145-6152.

19. Wissing, J.; Godl, K.; Brehmer, D.; Blencke, S.; Weber, M.; Habenberger, P.; Stein-Gerlach, M.; Missio, A.; Cotton, M.; Muller, S.; Daub, H. *Mol. Cell Proteomics* **2004**, *3*, 1181-1193.
20. Wisniewski, D.; Lambek, C. L.; Liu, C.; Strife, A.; Veach, D. R.; Nagar, B.; Young, M. A.; Schindler, T.; Bornmann, W. G.; Bertino, J. R.; Kuriyan, J. and Clarkson, B.. *Cancer Res.* **2002**, *62*, 4244–4255.
21. von Bubnoff, N.; Veach, D. R.; Miller, W. T.; Li, W.; Saenger, J.; Peschel, C.; Bornmann, W. G.; Clarkson, B.; Duyster, J. *Cancer Res.* **2003**, *63*, 6395-6404.
22. Nagar B.; Bornmann W. G.; Pellicena P.; Schindler T.; Veach D. R.; Miller W. T.; Clarkson B.; Kuriyan J. *Cancer Res.* **2002**, *62*, 4236-4243.
23. Blass, B. E.; Coburn, K.; Fairweather, N.; Sabat, M.; West, L. *Tet. Lett.*, **2006**, *47* (18), 3177-3180.
24. Schindler T.; Bornmann W.; Pellicena P.; Miller W.T.; Clarkson B.; Kuriyan J. *Science*, **2000**, *289*, 1938-1942.
25. Jung, M. E.; Kim, W-J. *Bioorganic and medicinal chemistry*, **2006**, *14*, 92-97.
26. Meguellati, K.; Spichty, M.; Ladame, S. *Org. Lett.* **2009**, *11* (5), 1123-1126.
27. Kvach, M. V.; Ustinov, A. V.; Stepanova, I. A.; Malakhov, A.D.; Skorobogatyi, M.V.; Shmanai, V.V.; Korshun, V.A. *Eur. J. Org. Chem.* **2008**, 2107–2117.
28. Reiko, T.; Machiko, O.; Shinzo, T. *Heterocycles*, **1986**, *24* (3), 755-769.
29. Xu, Y.; Liu, Y.; Qian, X. *Journal of Photochemistry and Photobiology A: Chemistry* **2007**, *190*, 1–8.
30. Ryu, E-H.; Yan, J.; Zhong, Z.; Zhao, Y. *J. Org. Chem.* **2006**, *71*, 7205-7213.

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).[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.[2] 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, anthocyanidin based delphinidin imparts violet to blue color and cyanidin gives red to magenta color to their corresponding flowers.[3]

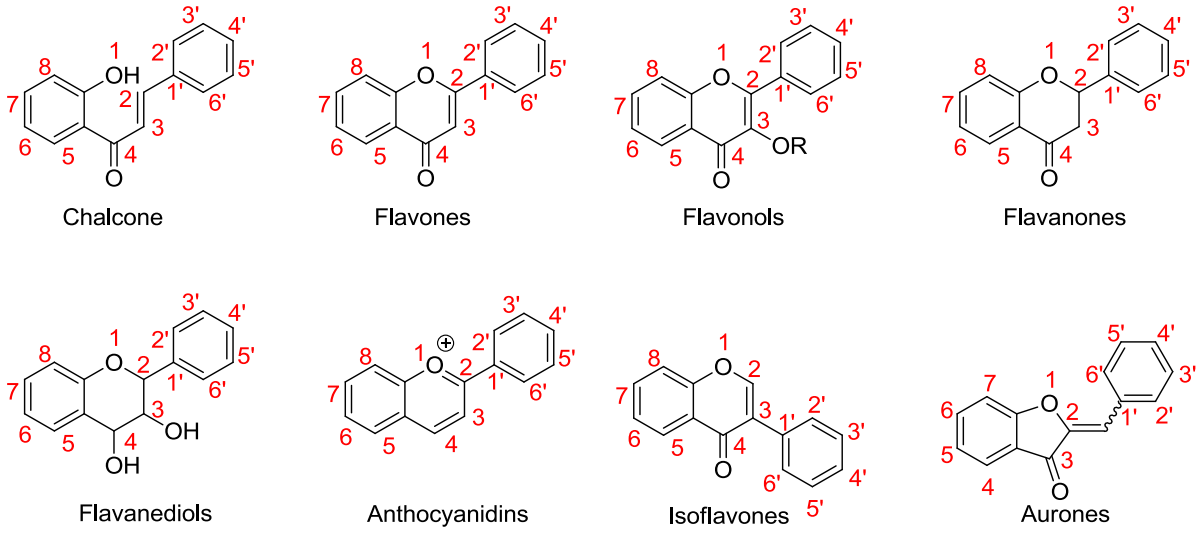


Figure 1: General structures of flavonoids

The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health. They have been reported to have antiviral[4], anti-allergic[5], antiplatelet[6], anti-inflammatory[8], antitumor[8], antioxidant[4,7] and estrogenic[9] 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.[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 us 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.[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.[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,

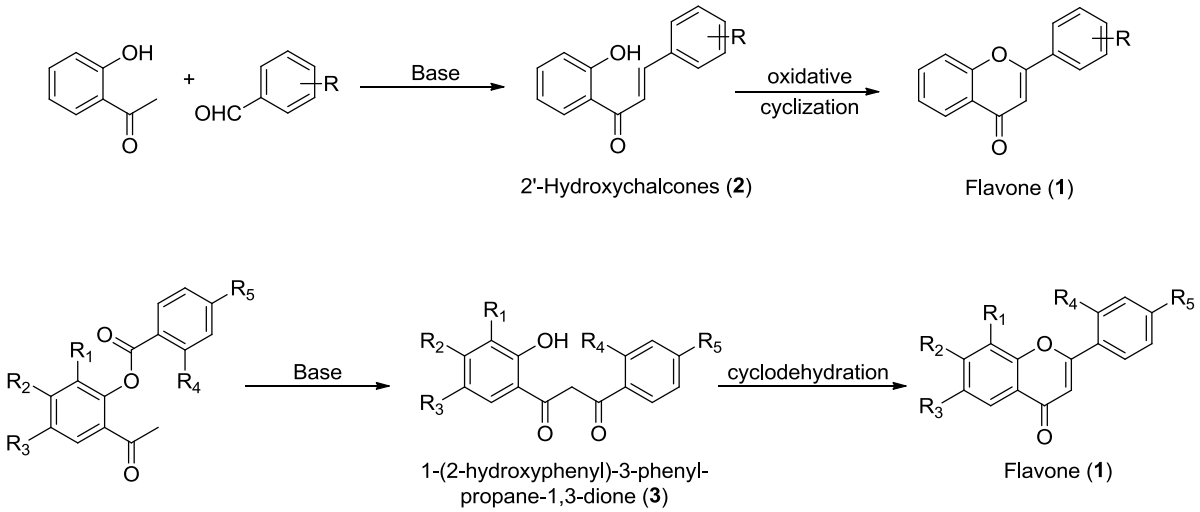
such as a reduced intake of glucose or minerals. However, the slower absorption of glucose may have some use against diabetes mellitus. Some flavonoids have an effect on the thyroid function. They inhibit thyroid peroxidase and interfere with the production of the thyroid hormone. Soy isoflavones can, 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 (*alpha*- and *beta*-).[11]

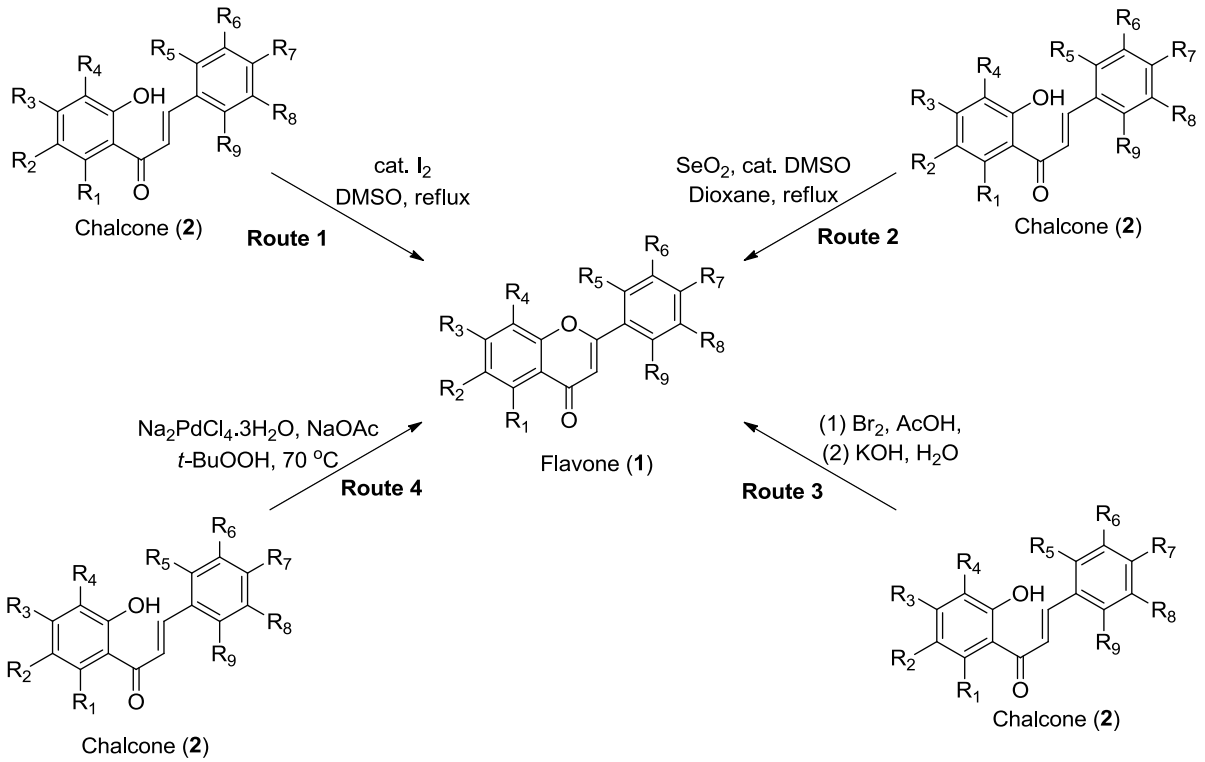
Animal experiments and epidemiological studies have suggested that isoflavones protect against cardiovascular diseases such as atherosclerosis. They seem to directly improve the health of blood vessels. Isoflavones help to reduce LDL oxidation through their antioxidant activity. Anthocyanidins, the phytochemicals 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.[11]



Scheme 1: General outline of common synthetic pathways of flavones.

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).



Scheme 2: Oxidative cyclization pathway to prepare flavones

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.[12] 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).[13] This catalytic iodine mediated method has been further explored and modified into a greener method by the use of iodine adsorbed on neutral alumina[14] and microwave reactor assisted conditions[15].

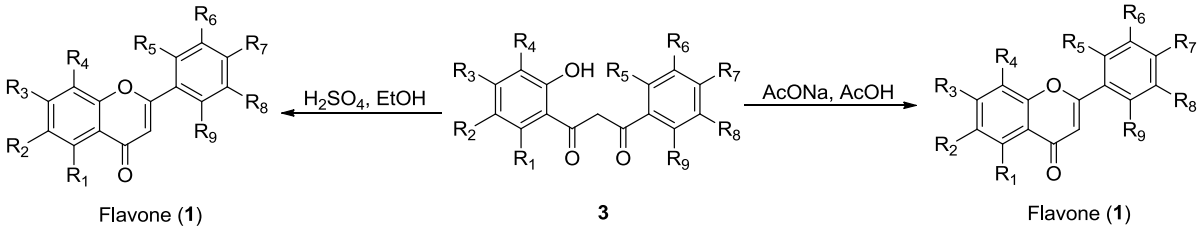
Another often used method for the oxidative cyclization involves using selenium dioxide in isoamyl alcohol which requires prolonged heating.[16a] This, selenium dioxide mediated reaction, has also been improved over the years in order to make it more benign. The most common improvement of this method is the use of selenium dioxide and traces of

dimethylsulfoxide over silica gel under microwave assisted conditions (Scheme 2, route 2).[16b]

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).[17a] 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.[17b-c]

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.[18]

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; studeies have modified it as shown by the examples below in Scheme 3.

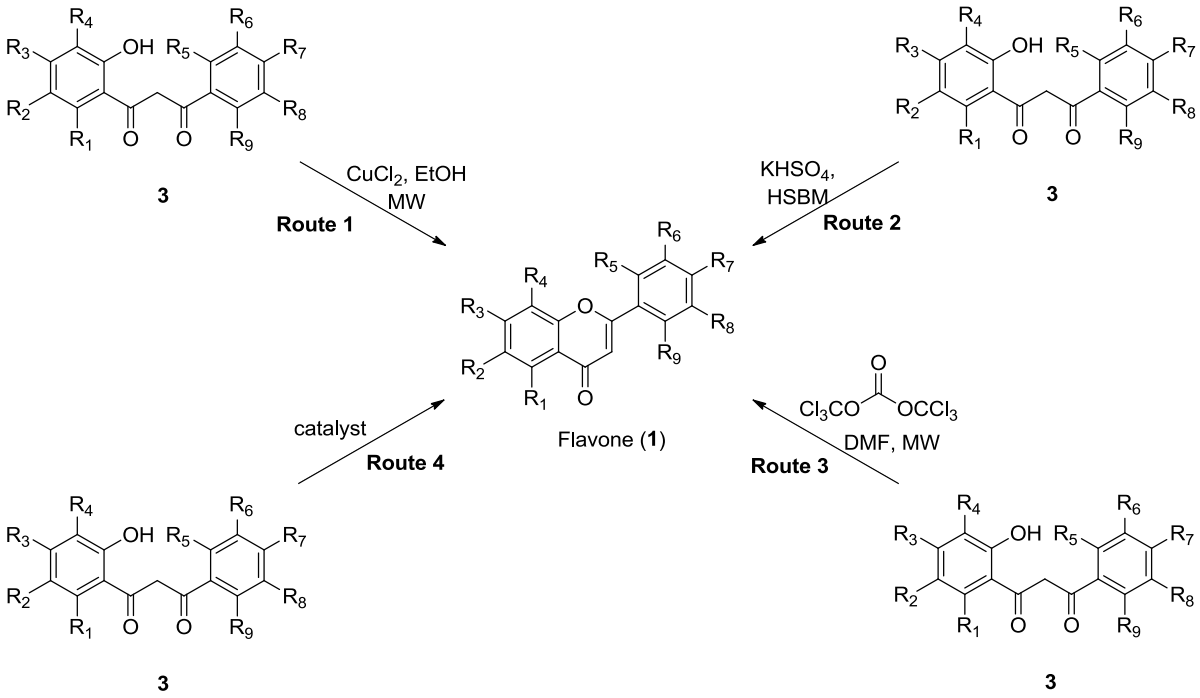


Scheme 3: Preparation of flavones via cyclodehydration pathway

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).[19] Venkataraman and coworkers successfully attempted the same reaction under acidic conditions (Scheme 3).[20] Many different acidic and basic conditions have been tried and reported since then. The use of hydrochloric acid in acetic acid[21], hydrobromic acid in acetic acid[22], *para*-toluenesulfonic acid in xylene[23] and Lewis acids[24] has been reported.

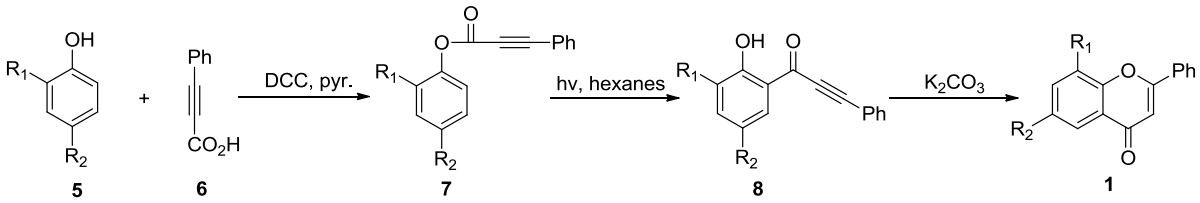
Some more cyclodehydration pathways are discussed in Scheme 4. Most of these routes use microwave conditions as a way to make the synthesis more environmentally friendly. Kabalka and coworkers reported a high yielding synthesis of flavones and chromones by using a catalytic amount of cupric chloride in a solution of the appropriate 1,3-diketone **3** in ethanol under microwave conditions (Scheme 4, route 1).[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).[26]



Scheme 4: Preparation of flavones via cyclodehydration pathway

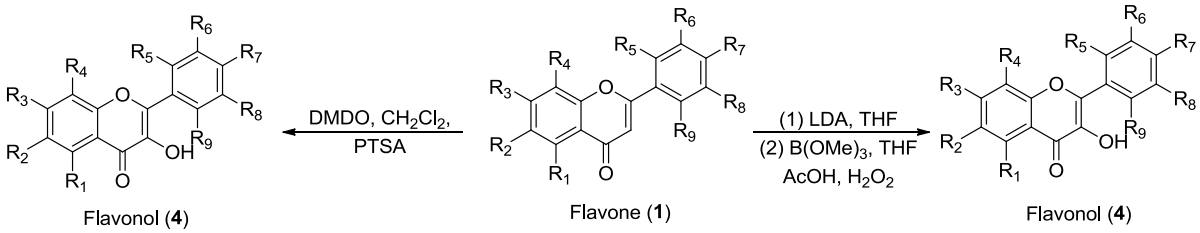
The first use of popular Vilsmeier-Haack reaction for the synthesis of flavones was reported by Su and coworkers. They used Vilsmeier-Haack conditions with bis-(trichloromethyl)carbonate/ N,N-dimethylformamide to cyclodehydrate the 1,3-diketone **3** to flavone **1** (Scheme 4, route 3).[27] Many other research groups reported the use of various catalysts like gallium(III) triflate[28], indium(III) chloride[29] and ionic liquids[30] to achieve the cyclodehydration step in order to synthesize flavones efficiently (Scheme 4, route 4).



Scheme 5: Synthesis of flavone (1)

Another common method for the synthesis of flavones is detailed above in Scheme 5. This approach flavones involves the coupling of substituted phenol **5** with acetylene **6** to

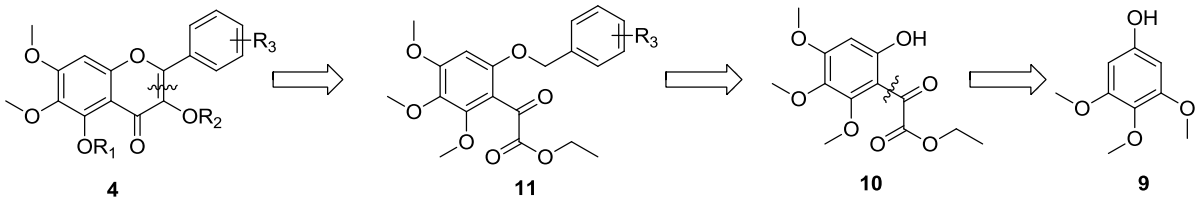
generate aryl propynoate **7** which, on irradiation, undergoes Photo-Fries rearrangement to give *ortho*-hydroxyaryl ethynyl ketone **8**. This ketone **8** can then be cyclized to the corresponding flavone in 10-25% overall yield.[31]



Scheme 6: Conversion of flavones to flavonols

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]

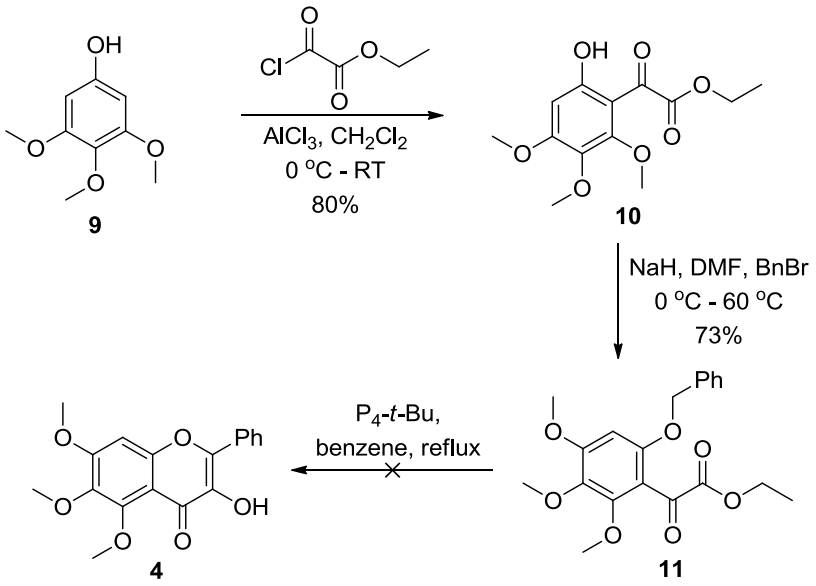
Results and Discussion



Scheme 7: Reterosynthetic analysis of flavonols

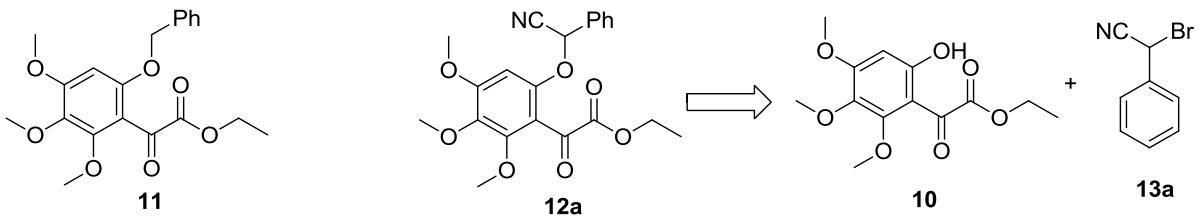
Our initial approach to develop a general synthesis of flavonols **4** is outlined above in Scheme 7. Our key step in this plan was the dehydrocyclization of arylketoester **11** to the flavonols **4** by using P_4-*t*-Bu chemistry developed previously. This arylketoester **11** could be prepared in 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.



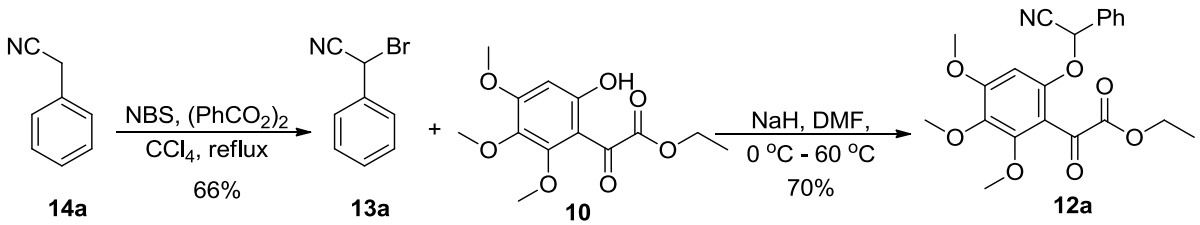
Scheme 8: Synthesis of flavonols – model studies

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 P_4-*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).



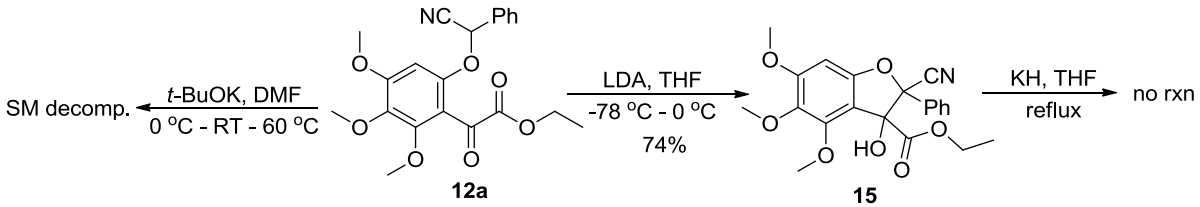
Scheme 9: New approach

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.



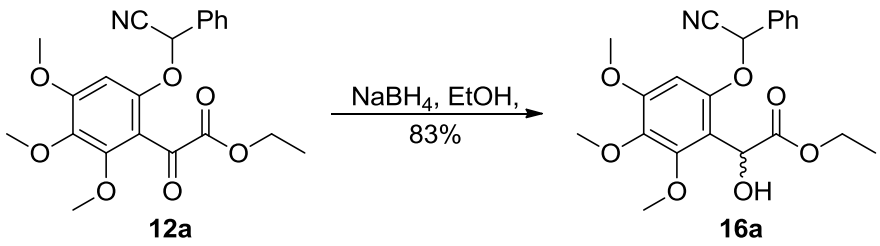
Scheme 10: Synthesis of compound **12a**

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.



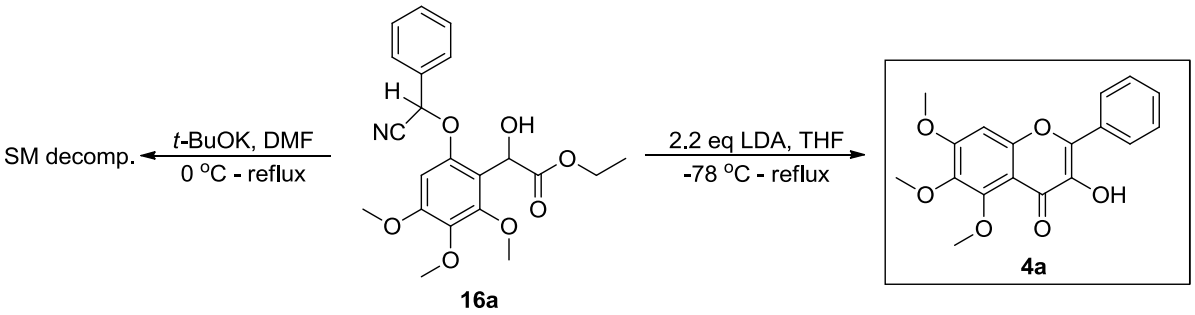
Scheme 11: Synthesis of flavonols - model studies

With compound **12a** in hand, we moved ahead and subjected it to a potassium *tert*-butoxide mediated intramolecular cyclization. At 0 ^{o}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 ^{o}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**.



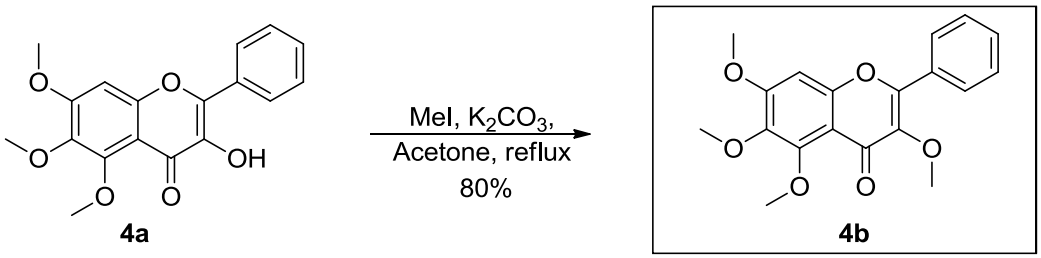
Scheme 12: Reduction of keto ester to alpha-hydroxy ester

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 ketone 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

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]

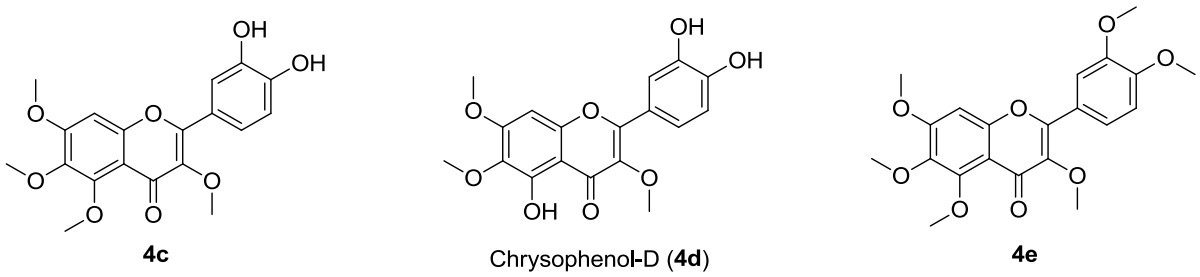
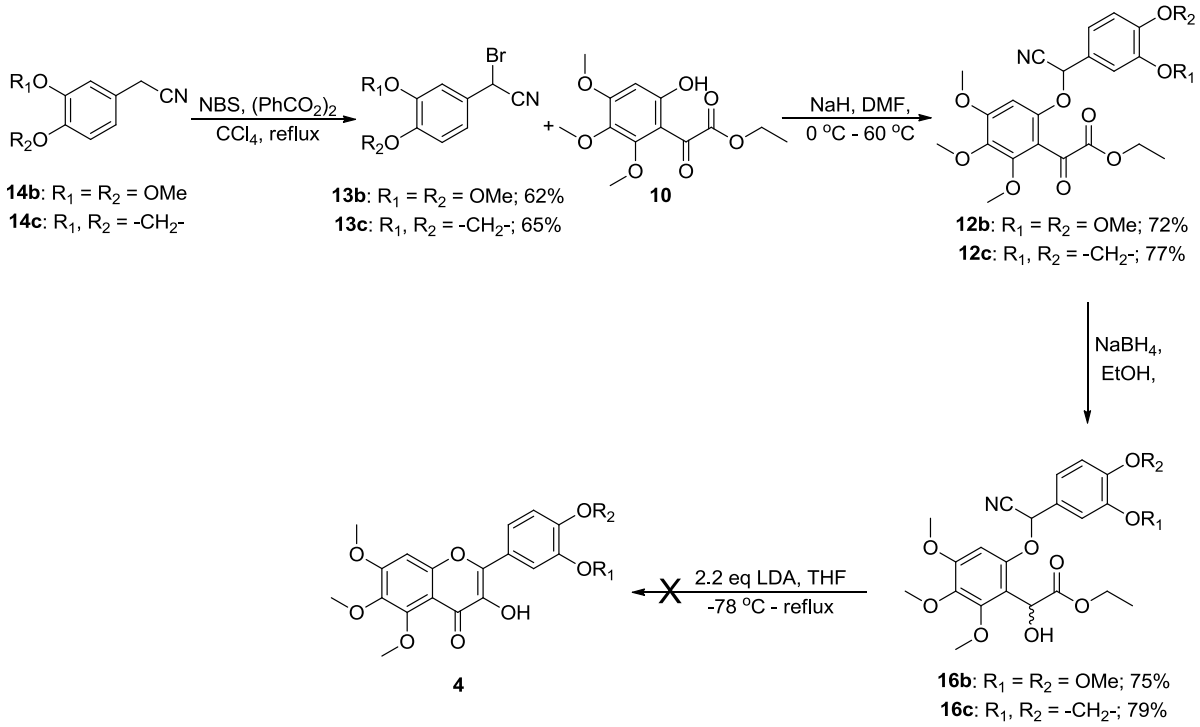


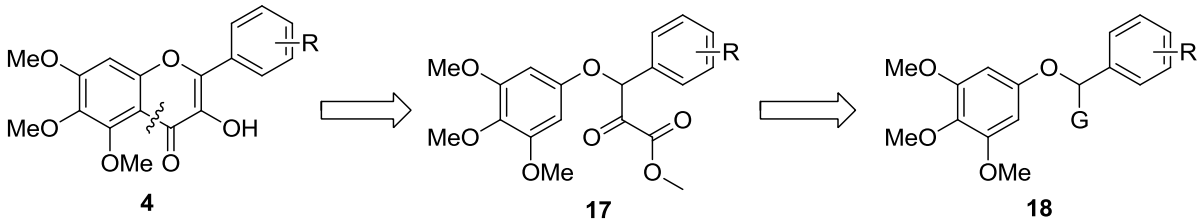
Figure 2: Flavonol based natural products

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.



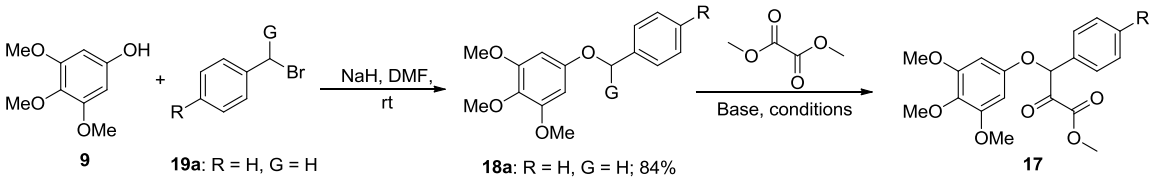
Scheme 15: Synthesis of flavonols

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 dehydocyclization – 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 16: New approach

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 intermediated **17**. Our plan was to make compound **17** via an anionic reaction of compound **18** with dimethyl oxalate.



19a: R = H, G = H
19b: R = H, G = CN
19c: R = NO_2, G = H
19d: R = OMe, G = CN

18a: R = H, G = H; 84%
18b: R = H, G = CN, 76%
18c: R = NO_2, G = H, 88%
18d: R = OMe, G = CN, 73%

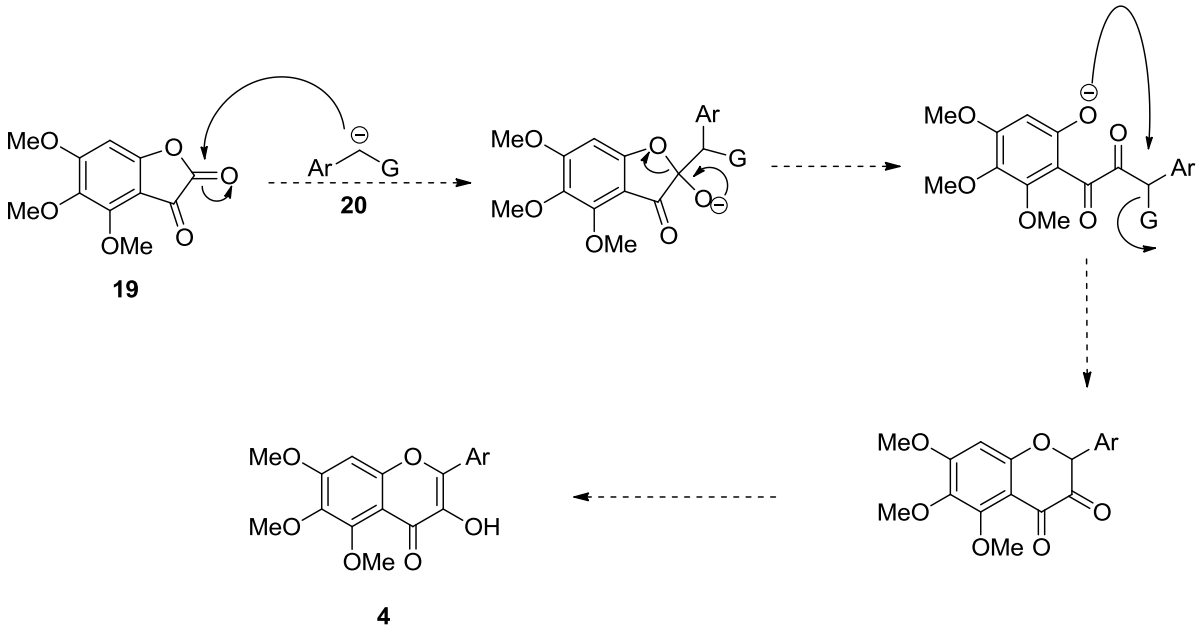
Table 1

#	R	G	Base	Product
1	H	H	LDA	nr
2	H	H	P_4-*t*-Bu	nr
3	H	CN	LDA	nr
4	OMe	CN	LDA	nr
5	NO_2	H	LDA	nr
6	NO_2	H	P_4-*t*-Bu	nr

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.



Scheme 18: New plan

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* postion 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 nuclephiles because theanion 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**.

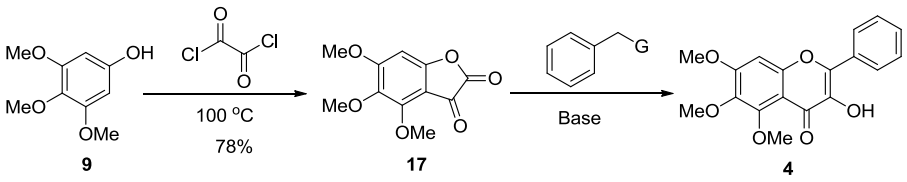


Table 2

#	G	Base	Product
1	Cl	LDA	nr
2	Cl	Li-TMP	nr
3	CN	*t*-BuOK	nr
4	CN	NaH	nr
5	CN	LDA	nr

Scheme 19: Synthesis of flavonols – model studies

Benzofuran-2,3-diketone **19** was synthesized by following a literature procedure of condensing trimethoxyphenol **9** with oxalyl chloride at 100 ^{o}C under neat conditions in 78% yield.[39] 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).

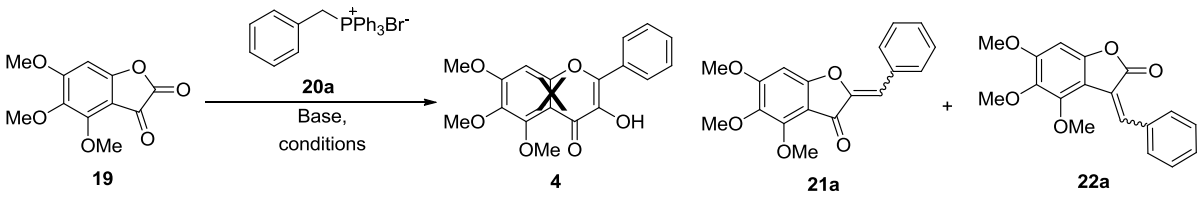


Table 3

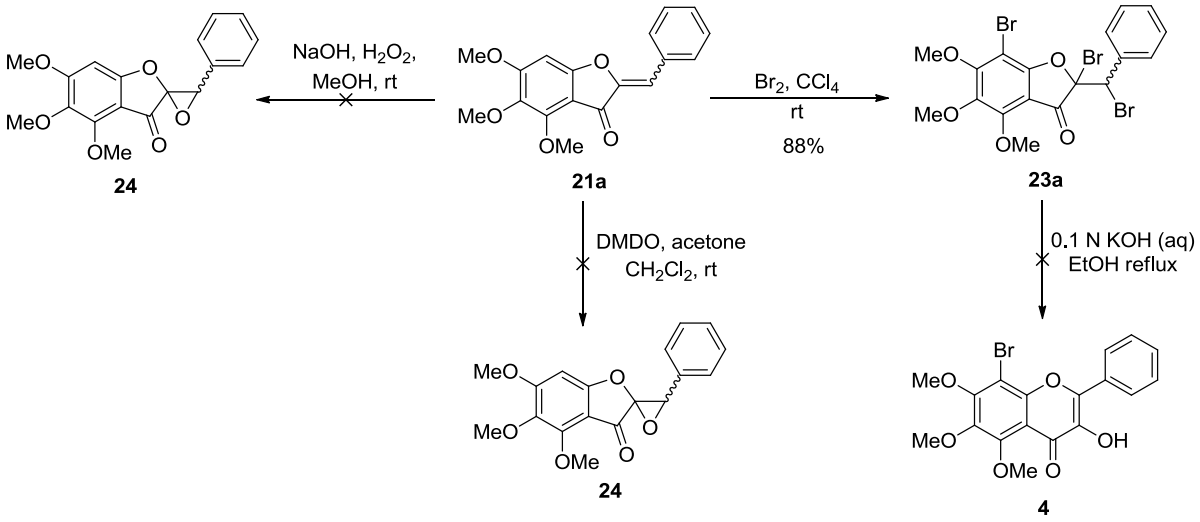
#	Base	Yield **21a**	Yield **22a**
1	*t*-BuOK	29%	21%
2	LDA	33%	25%
3	n-BuLi	61%	28%

Scheme 20: Synthesis of flavonols – model studies

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

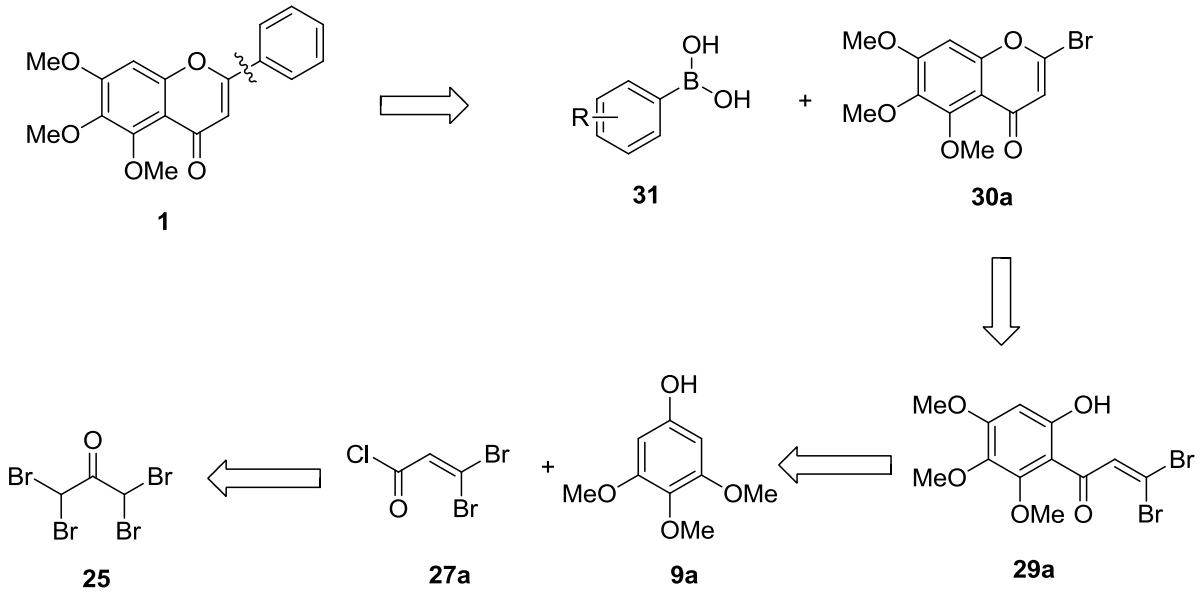
trying different bases like LDA and *n*-butyllithium, we just recovered aurone and isoaurone in various ratios and better yields (Scheme 20, Table 3). This was confirmed by comparing the analytical data with literature data.[39]

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 [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).



Scheme 21: Synthesis of flavonols – model studies

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.



Scheme 22: New plan – reterosynthetic analysis

Our new synthetic plan is shown in Scheme 22. This strategy was based on an entirely new reterosynthetic 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 dibromoacrylate **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.

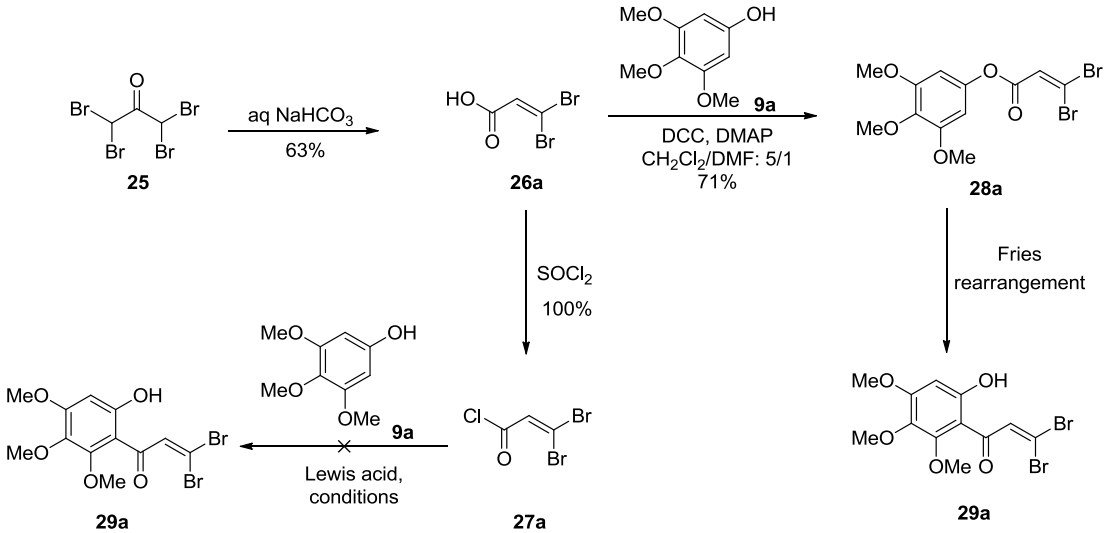


Table 4

#	Lewis acid	solvent	conditions	result
1	$TiCl_4$	$ClCH_2CH_2Cl$	reflux	nr
2	$TiCl_4$	neat	100 °C	decomp.
3	BF_3-Et_2O	neat	125 °C	< 5%
4	$AlCl_3$	$CHCl_3$	reflux	nr
5	$AlCl_3$	$ClCH_2CH_2Cl$	reflux	nr

Table 5

#	Lewis acid	solvent	conditions	result
1	$TiCl_4$	$ClCH_2CH_2Cl$	reflux	nr
2	none	C_6H_6	hv	nr
3	BF_3-Et_2O	neat	125 °C	< 5%
4	$AlCl_3$	$CHCl_3$	reflux	<10%
5	$AlCl_3$	$ClCH_2CH_2Cl$	reflux	41%

Scheme 23: Synthesis

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.[41] 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.

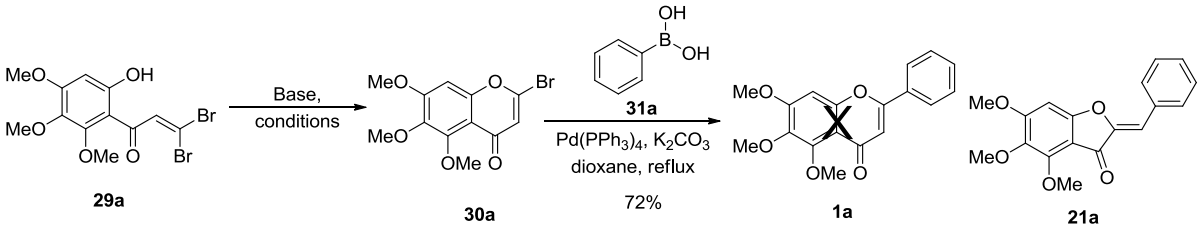


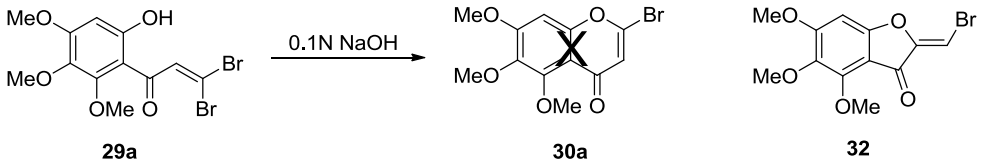
Table 6

#	Base	Solvent	Temperature	Yield (**30a**)
1	0.1N NaOH	EtOH	0 °C - RT	64%
2	0.2N NaOH	EtOH	0 °C - RT	61%
3	NaH	THF	0 °C - RT	63%

Scheme 24: Synthesis of flavones – model studies

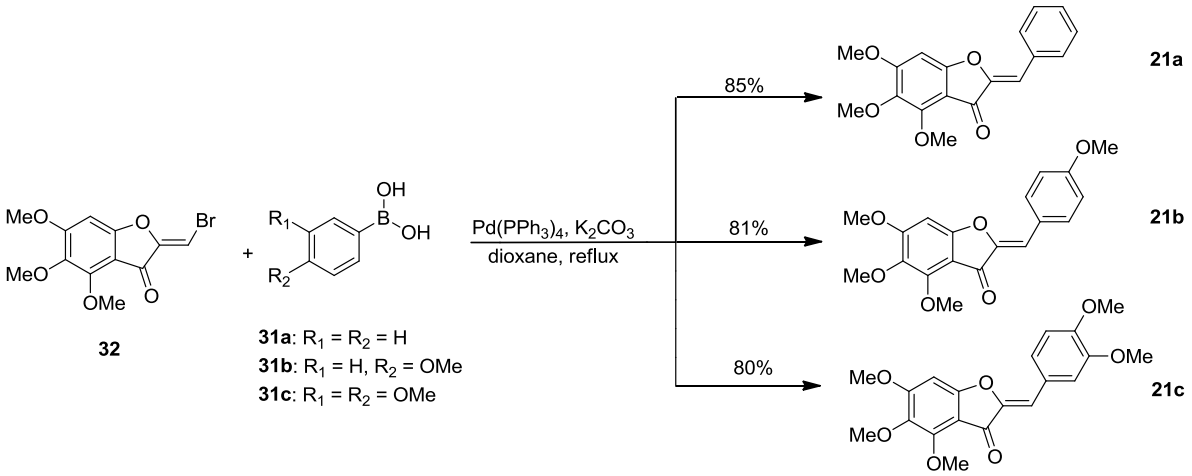
After the successful Fries rearrangement to give compound **29a**, it was subjected to various basic conditions resulting in the formation of compound **30a** in good yields (Scheme 24, Table 6 – entry 1-3). This compound **30a**, which we thought was a chromone derivative, was subjected to Suzuki coupling 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).



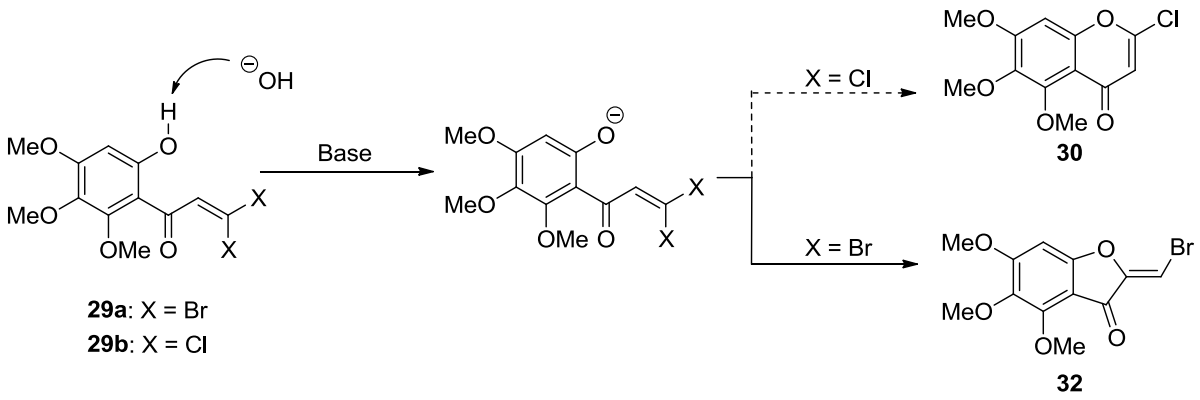
Scheme 25: Reassigning the structure

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).



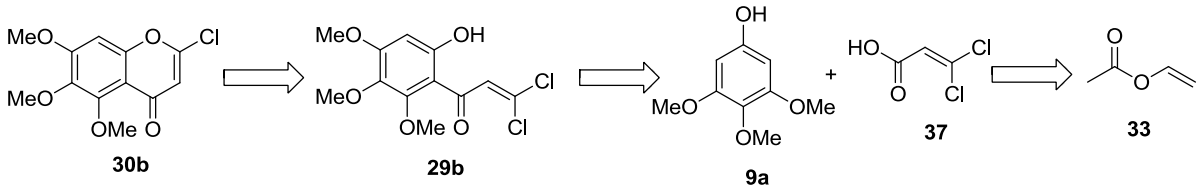
Scheme 26: Suzuki coupling to form aurones

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.



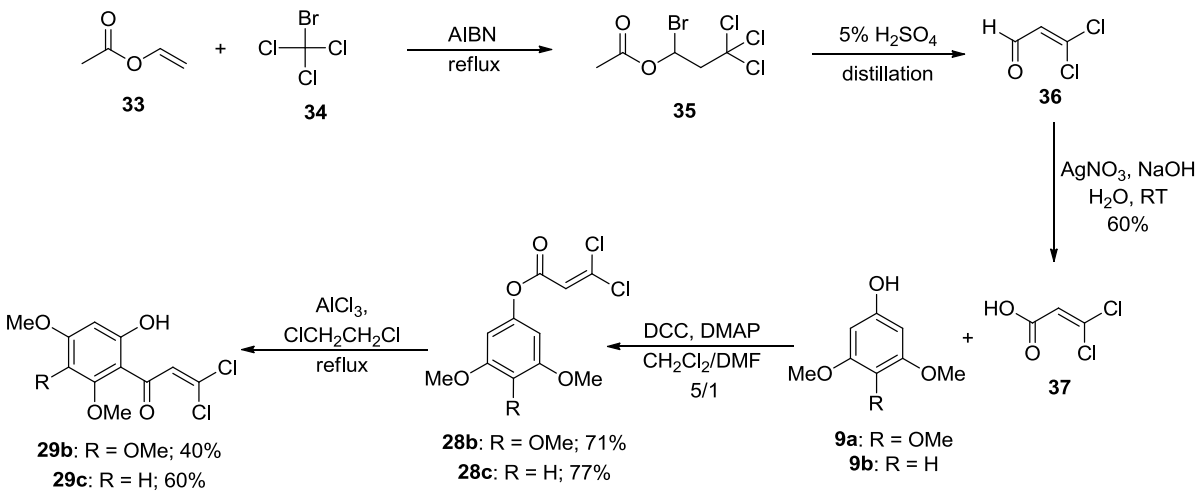
Scheme 27: Aurone precursor versus flavone precursor

Even though, this discovery was a setback to our plans of synthesizing flavones, it was an interesting way to prepare substituted aurones **21**. We decided to use this setback in our favor and tried to explore the ways to make chromone **30**. 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 **30** instead of aurone precursor **32** (Scheme 27).



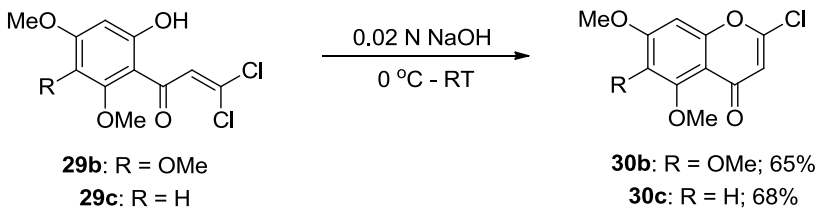
Scheme 28: Reterosynthetic analysis

Scheme 28 outlines the new reterosynthetic analysis which is quite similar to the previous approach. The only difference is in the procedure for the preparation of 3,3-dichloroacrylic acid **37** which is prepared by a literature procedure.



Scheme 29: Synthesis

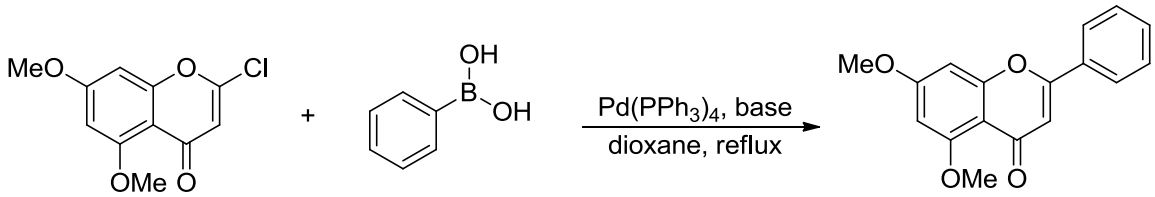
The synthesis started with the preparation of 3,3-dichloroacrylic acid **37** by a literature procedure.[41] Vinyl acetate **33** and bromotrichloromethane **34** were refluxed together in the presence of azobisisobutyronitrile to give the addition product **35** in quantitative yield. This compound **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 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, Table7 - entry 1-4).



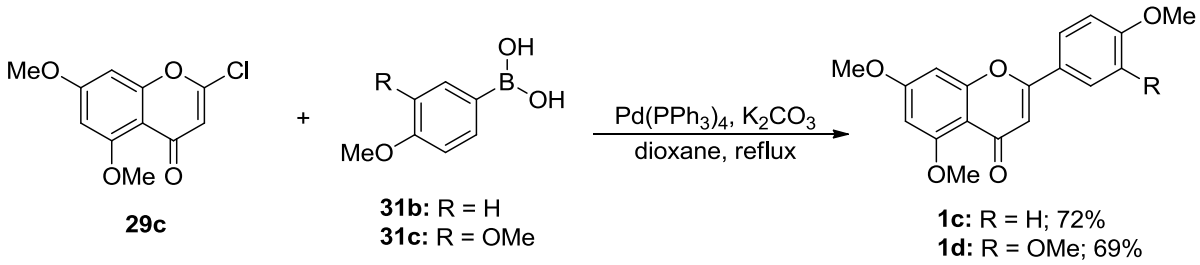
29c **31a** **1b**

Table 7

#	**29c** (equiv.)	**31a** (equiv.)	base 3.0 equiv.)	additive	time	Result
1	1.0	1.0	K_2CO_3	TBAB	6 h	mixture
2	1.0	1.0	Cs_2CO_3	TBAB	4 h	mixture
3	1.0	1.0	K_2CO_3	TBAC	8 h	mixture
4	1.0	1.0	Cs_2CO_3	TBAC	7 h	mixture
5	1.0	1.5	KF	-	18 h	< 60%
6	1.0	1.5	Cs_2CO_3	-	6 h	76%
7	1.0	1.5	K_2CO_3	-	18 h	81%

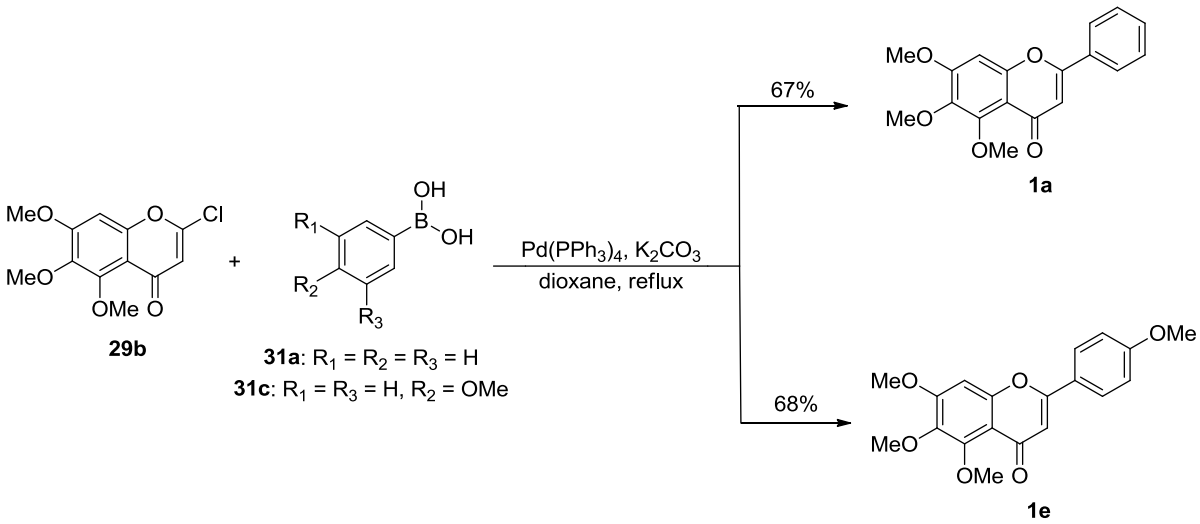
Scheme 31: Suzuki coupling – standardization of conditions

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).



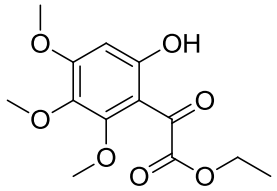
Scheme 35: Suzuki coupling – Synthesis of flavones

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 ^1H 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.

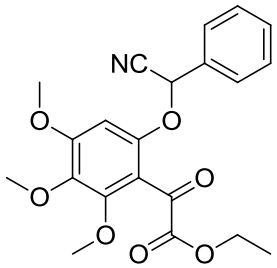
Preparation of ethyl 2-(6-hydroxy-2,3,4-trimethoxyphenyl)-2-oxoacetate (10)



To a solution of phenol **9** (2.0 g, 10.86 mmol) in CH_2Cl_2, was added titanium tetrachloride (2.276 g, 12.0 mmol) at -20 ^oC under argon. To this dark brown solution, ethyl chlorooxoacetate (1.64 g, 12.0 mmol) was added dropwise while maintaining temperature at below -15 ^oC. Resulting reaction mixture was stirred for 4 h with steady increase in temperature to 0 ^oC. After the completion of reaction, the reaction mixture was diluted with CH_2Cl^2 and poured over cold HCl (1.0 M) solution. Aqueous layer was separated and extracted with CH_2Cl_2 (3 x 25 ml). Combined organic extracts were washed with HCl (1.0 M) solution and brine followed by drying over anhydrous $MgSO_4$. Solvent was evaporated *in vacuo* to obtaine 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.

^{1}H-NMR (400MHz, $CDCl_3$) 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); ^{13}C-NMR (100MHz, $CDCl_3$): 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 $C_{13}H_{16}O_7$: 284.0900, found: 284.0896.

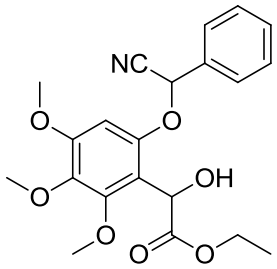
Preparation of ethyl 2-(6-(cyano(phenyl)methoxy)-2,3,4-trimethoxyphenyl)-2-oxo-acetate (12a)



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 ^oC. Resulting reaction mixture was stirred at 0 ^oC 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 ^{o}C for 2 h. After the completion of the reaction, reaction mixture was quenched by adding saturated NH_4Cl 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 $MgSO_4$, filtered and evaporated *in vacuo* to obtain crude **12a**. The crude compound was purified by column chromatography using 20% EtOAc/hexanes as elutent to get pure **12a** in 70% yield; ^{1}H-NMR (400MHz, $CDCl_3$) 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); ^{13}C-NMR (100MHz, $CDCl_3$): 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 $C_{21}H_{21}NO_7$: 399.1318, found: 399.1326

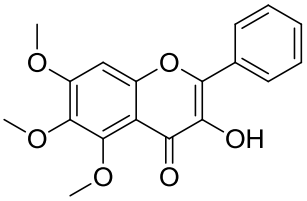
Preparation of ethyl 2-(6-(cyano(phenyl)methoxy)-2,3,4-trimethoxyphenyl)-2-hydroxy-acetate (16a)



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, $NaBH_4$ 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 $MgSO_4$, filtered and evaporated *in vacuo* to obtain crude **16a**. The crude compound was purified by column chromatography using 20% EtOAc/hexanes as elutent to get pure **16a** in 83% yield; ^{1}H-NMR (400MHz, $CDCl_3$) 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

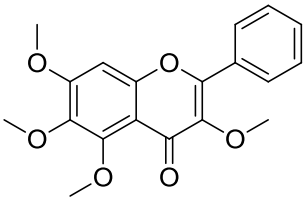
Hz, 1H), 4.18 (m, 2H), 3.89 (d, J = 9.2 Hz, 3H), 3.85 (d, J = 7.6 Hz, 3H), 3.82 (d, J = 5.2 Hz, 3H), 3.54 (m, 1H), 1.18 (dt, ^{t}J = 7.2 Hz, ^{d}J = 4.8 Hz, 3H).

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



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°C. The mixture was warmed to -40°C and stirred at this temperature for 45 minutes. The solution was returned to -78°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 elutent to get pure flavonol **4a** in 61% yield; ^{1}H-NMR (400MHz, $CDCl_3$) δ 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); ^{13}C-NMR (100MHz, $CDCl_3$): δ 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-4*H*-chromen-4-one (4b)

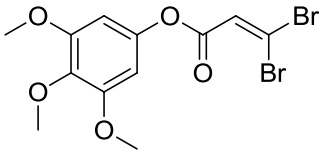


Flavonols **4a** (0.04 g, 0.12 mmol) was taken in dry actone and anhydrous K_2CO_3 was added to it under argon. To this, methyl iodide (0.03 g, 0.18 mmol) was added and resulting reaction mixture was refluxed for 6 h. After the completion of reaction, the reaction mixture was filtered through celite 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 elutent 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

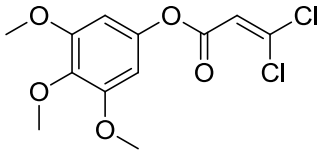
A solution of 3,4,5-trimethoxyphenol **9** (2.30 g, 12.44 mmol), acrylic acid derivative **37** (1.93 g, 13.69 mmol) and DMAP (0.15 g, 1.24 mmol) in 10 ml of dry CH_2Cl_2 and 2 ml of dry DMF was treated at 0 ^oC under argon with DCC (2.60 g, 12.44 mmol). The mixture was stirred for 5 min at 0 ^oC and 30 min at room temperature. After the completion of reaction, reaction mixture was filtered through celite and diluted with CH_2Cl_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 elutent to give pure product.

3,4,5-Trimethoxyphenyl 3,3-dibromoacrylate (28a)



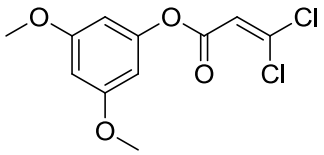
Yield = 71%; ^{1}H NMR (400MHz, $CDCl_3$) δ 7.20 (s, 1H), 6.36 (s, 2H), 3.82 (s, 6H), 3.81 (s, 3H); ^{13}C-NMR (100MHz, $CDCl_3$): δ 161.1, 153.6, 146.2, 136.1, 127.1, 109.9, 99.0, 61.1, 56.4; MS (*m/z*): 396.9113; HRMS calcd for $C_{12}H_{12}Br_2O_5$: 393.9051, found: 393.9056.

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



Yield = 71%; ^{1}H-NMR (400MHz, $CDCl_3$) δ 6.54 (s, 1H), 6.33 (s, 2H), 3.78 (s, 9H); ^{13}C-NMR (100MHz, $CDCl_3$): δ 160.7, 153.5, 146.1, 140.1, 135.9, 119.2, 119.1, 98.9, 98.9, 98.9, 60.9, 56.1; MS (*m/z*): 307.0128; HRMS calcd for $C_{12}H_{12}Cl_2O_5$: 306.0062, found: 306.0055.

3,5-Dimethoxyphenyl 3,3-dichloroacrylate (28c)

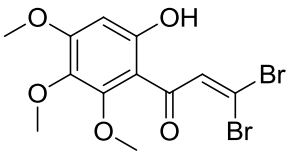


Yield = 77%; ^{1}H-NMR (400MHz, $CDCl_3$) δ 6.58 (d, J = 0.6 Hz, 1H), 6.36 (s, 1H), 6.28 – 6.32 (m, 2H), 3.77 (s, 6H); ^{13}C-NMR (100MHz, $CDCl_3$): δ 161.3, 160.7, 151.7, 140.2, 119.4, 119.3, 100.2, 100.1, 98.7, 55.7; MS (*m/z*): 277.0026, 225.1957; HRMS calcd for $C_{11}H_{10}Cl_2O_4$: 275.9956, found: 275.9953.

Representative procedure for Fries rearrangement to prepare 29a-c

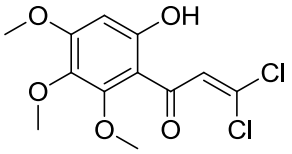
Ester **28b** (1.0 g, 3.26 mmol) was taken in dry 1,2-dichloroethane (75 ml) and added to a slurry of $AlCl_3$ (0.48 g, 3.60 mmol) in 1,2-dichloroethane (25 ml) at 0 ^{o}C under argon. Resulting dark brown solution was refluxed with monitoring. After the completion of reaction, the reaction mixture was 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_2Cl_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 elutent.

3,3-Dibromo-1-(6-hydroxy-2,3,4-trimethoxyphenyl)prop-2-en-1-one (29a)



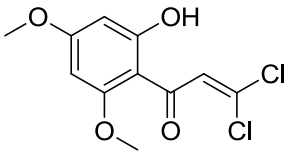
Yield: 41%; ^{1}H-NMR (400MHz, $CDCl_3$) δ 12.78 (s, 1H), 7.86 (s, 1H), 6.24 (s, 1H), 3.94 (s, 3H), 3.88 (s, 3H), 3.77 (s, 3H); ^{13}C-NMR (100MHz, $CDCl_3$): δ 191.1, 162.9, 161.6, 154.8, 137.6, 108.2, 97.6, 96.6, 96.5, 61.9, 61.4, 56.5; MS (*m/z*): 396.9113; HRMS calcd for $C_{12}H_{12}Br_2O_5$: 393.9051, found: 393.9056.

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



Yield: 40%; ^{1}H-NMR (400MHz, $CDCl_3$) δ 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, $CDCl_3$): δ 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 $C_{12}H_{12}Cl_2O_5$: 306.0062, found: 306.0055.

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

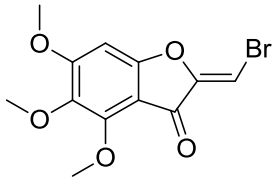


Yield: 60%; ^{1}H-NMR (400MHz, $CDCl_3$) δ 13.46 (s, 1H), 7.35 (s, 1H), 6.07 (d, J = 4.0 Hz, 1H), 5.90 (d, J = 2.2 Hz, 1H), 3.85 (s, 3H), 3.82 (s, 3H); ^{13}C-NMR (100MHz, $CDCl_3$): δ 189.3, 168.4, 167.3, 162.3, 131.6, 129.3, 106.1, 94.0, 91.5, 56.3, 55.9; MS (*m/z*): 277.0026, 241.0263, 223.0596, 197.0805; HRMS calcd for $C_{11}H_{10}Cl_2O_4$: 275.9956, found: 275.9953.

Representative procedure for cyclization reaction to prepare 30a-c

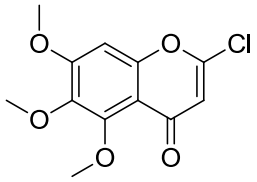
Phenol **29b** was taken in THF (5 ml) and to this, NaOH (33.0 ml, 0.02 N) solution was added at 0 ^{o}C. Resulting reaction mixture was stirred at room temperature for 3 h with monitoring. After the completion of reaction, reaction mixture was acidified by HCl (1.0 M) to a pH 5 and extracted with EtOAc (3 x 50 ml). Combined organic extracts were then washed with water and brine, dried over anhydrous $MgSO_4$, filtered and evaporated *in vacuo* to obtain crude chromone **30b**. The crude compound was then purified by column chromatography using 50% EtOAc/hexanes as elutent.

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



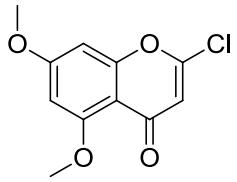
Yield: 64%; ^{1}H-NMR (400MHz, $CDCl_3$) δ 6.74 (s, 1H), 6.48 (s, 1H), 4.19 (s, 3H), 3.94 (s, 3H), 3.78 (s, 3H); ^{13}C-NMR (100MHz, $CDCl_3$): δ 177.4, 164.0, 162.4, 152.3, 152.0, 136.9, 107.2, 94.8, 90.8, 62.5, 61.8, 56.9; MS (*m/z*): 314.9858; HRMS calcd for $C_{12}H_{11}BrO_5$: 314.9863, found: 314.9858.

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



Yield: 65%; ^{1}H-NMR (400MHz, $CDCl_3$) δ 6.70 (s, 1H), 6.24 (s, 1H), 3.94 (s, 6H), 3.89 (s, 3H); ^{13}C-NMR (100MHz, $CDCl_3$): 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 $C_{12}H_{11}ClO_5$: 270.0295, found: 270.0288.

2-Chloro-5,7-dimethoxy-4H-chromen-4-one (30c)

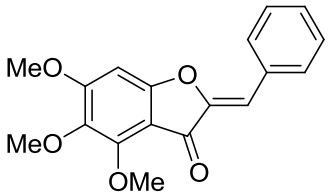


Yield: 68%; ^{1}H-NMR (400MHz, $CDCl_3$) δ 6.43 (d, J = 2.1 Hz, 1H), 6.36 (d, J = 2.0 Hz, 1H), 6.22 (s, 1H), 3.91 (s, 3H), 3.86 (s, 3H); ^{13}C-NMR (100MHz, $CDCl_3$): δ 176.2, 164.4, 161.1, 160.1, 153.5, 112.5, 108.4, 97.0, 92.9, 56.7, 56.1; MS (*m/z*): 241.0260, 223.0595, 197.0804; HRMS calcd for $C_{11}H_9ClO_4$: 240.0189, found: 240.0187.

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_2CO_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 ^{o}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 elutent to obtain pure aurone **21a** in 85% isolated yield.

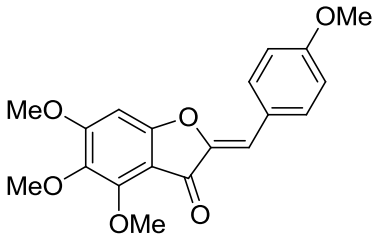
(*Z*)-2-benzylidene-4,5,6-trimethoxybenzofuran-3(2*H*)-one (21a)



Yield: 85%; ^{1}H-NMR (400MHz, $CDCl_3$) δ 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);

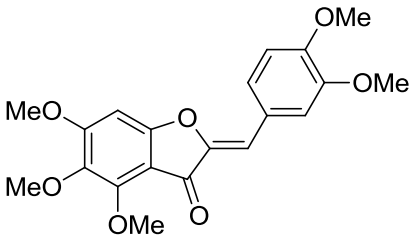
^{13}C-NMR (100MHz, $CDCl_3$): δ 181.0, 164.3, 161.9, 151.9, 147.9, 136.8, 132.7, 131.4, 129.7, 129.0, 111.4, 107.2, 90.8, 62.6, 61.9, 56.9; MS (*m/z*): 313.11, 187.08, 121.05; HRMS calcd for $C_{18}H_{16}O_5$: 313.1071, found: 313.1074.

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



Yield: 81%; ^{1}H-NMR (400MHz, $CDCl_3$) δ 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, $CDCl_3$): δ 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(2*H*)-one (21c)



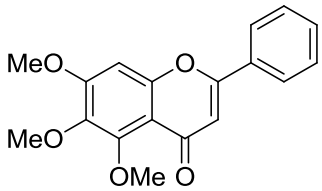
Yield: 80%; ^{1}H-NMR (400MHz, $CDCl_3$) δ 7.43 – 7.48 (m, 2H), 6.93 (d, J = 8.0 Hz, 1H), 6.74 (s, 1H), 6.51 (s, 1H), 4.26 (s, 3H), 3.99 (s, 3H), 3.97 (s, 3H), 3.94 (s, 3H), 3.82 (s, 3H); ^{13}C-NMR (100MHz, $CDCl_3$): δ 180.9, 163.9, 161.6, 151.8, 150.7, 149.2, 146.8, 136.8, 125.7, 125.6, 113.7, 112.0, 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_2CO_3 (0.345 g, 2.49 mmol),

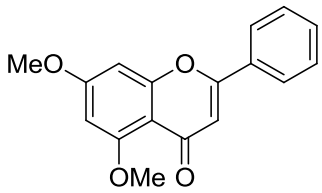
phenylboronic acid (**31a**) (0.2030 g, 1.66 mmol) and chromone **30c** (0.20 g, 0.831 mmol). Nitrogen was passed through the resulting reaction mixture for at least 20 min. To this was added the $Pd(PPh_3)_4$ (0.048 g, 0.042 mmol) catalyst and resulting reaction mixture was refluxed with constant monitoring. 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 elutent to obtain pure flavone **1b** in 74% isolated yield.

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



Yield: 67%; ^{1}H-NMR (400MHz, $CDCl_3$) 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); ^{13}C-NMR (100MHz, $CDCl_3$): 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.

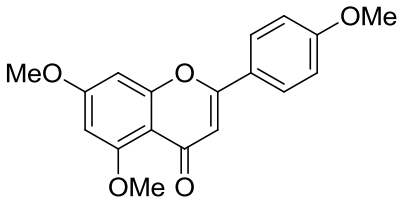
5,7-dimethoxy-2-phenyl-4*H*-chromen-4-one (1b)



Yield: 74%; ^{1}H-NMR (400 MHz, $CDCl_3$) 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); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 177.8, 164.2, 161.0, 160.8, 160.1, 131.7, 131.4, 129.1, 126.1, 109.3,

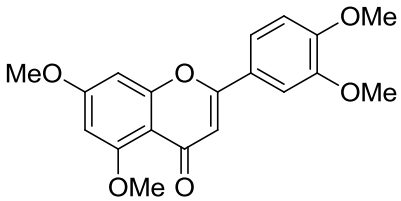
109.2, 96.4, 93.0, 56.7, 56.0; MS (*m/z*): 283.0963, 269.0804; HRMS calcd for $C_{17}H_{14}O_4$: 282.0892, found: 282.0890.

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



Yield: 72%; ^{1}H-NMR (400MHz, $CDCl_3$) δ 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, $CDCl_3$): δ 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-4*H*-chromen-4-one (1d)



Yield: 69%; ^{1}H-NMR (400MHz, $CDCl_3$) δ 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, $CDCl_3$): δ 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

1. Winkel-Shirley, B. *Plant Physiology* **2001**, *126*, 485-493.
2. Harborne, J.B.; Williams, C.A. *Phytochemistry* **2000**, *55*, 481–504.
3. Tanaka, Y. *Phytochem Rev.* **2006**, *5*, 283-291.

4. Nijveldt, R. J.; van Nood, E.; van Hoorn, D. E. C.; Boelens, P. G.; van Norren, K.; van Leeuwen, P. A. M. *Am. J. Clin. Nutr.* 2001, *74*, 418–25.
5. Kawai, M.; Hirano, T.; Higa, S.; Arimitsu, J.; Maruta, M.; Kuwahara, Y.; Ohkawara, T.; Hagihara, K.; Yamadori, T.; Shima, Y.; Ogata, A.; Kawase, I.; Tanaka, T. *Allergol Int.* **2007**, *56* (2), 113-23.
6. Kim, J. M.; Yun-Choi, H. S. *Arch. Pharm. Res.* **2008**, *31* (7), 886-890.
7. Yao, L. H.; Jiang, Y. M.; Shi, J.; Datta, N.; Singanusong, R.; Chen, S. S. *Plant Foods Hum. Nutr.* **2004**, *59*, 113–122.
8. Middleton, E. Jr.; Kandaswami, C.; Theoharides, T. C. *Pharmacol. Rev.* **2000**, *52*, 673–751.
9. Dixon, R.A.; Ferreira, D. *Phytochemistry* **2002**, *60*, 205–211.
10. Katsuyama, Y.; Funa, N.; Miyahisa, I.; Horinouchi, S. *Chemistry & Biology* **2007**, *14*, 613–621.
11. Erdman, J. W. Jr.; Balentine, D.; Arab, L.; Beecher, G.; Dwyer, J. T.; Folts, J.; Harnly, J.; Hollman, P.; Keen, C. L.; Mazza, G.; Messina, M.; Scalbert, A.; Vita, J.; Williamson, G.; Burrowes, J. *Journal of Nutrition* **2004**, *134* (6), 1529-35.
12. Song, L. L.; Kosmeder II, J. W.; Lee, S. K.; Gerhäuser, C.; Lantvit, D.; Moon, R. C.; Moriarty, R. M.; Pezzuto, J. M. *Canc. Res.* 1999, *59*, 578–585.
13. Doshi, A. G.; Soni, P. A.; Ghiya, B. *Ind. J. Chem., Sec. B* **1986**, *25B* (7), 759.
14. Sarda, S. R.; Jadhav, W. N.; Pawar, R. P. *Intern. J. ChemTech Res.* **2009,** *1* (3), 539-543.
15. Menezes, M. J.; Manjrekar, S.; Pai, V.; Patre, R. E.; Tilve, S. G. *Ind. J. Chem.* **2009**, *48B*, 1311-1314.
16. (a) Mahal, H. S.; Rai, H. S.; Venkataraman, K. *Journal of the Chemical Society* **1935**, 866-868. (b) Gupta, M.; Pault, S.; Gupta, R.; Loupy, A. *Organic Preparations and Procedures International* **2000**, *32* (3), 280-283.
17. (a) Dawane, B. S.; Vibhute, B.; Chawm, V. P.; Mane, A. S.; Shingare, M. S. *Korean J. of Med. Chem.*, **2000**, *10* (2) 78-83. (b) Donnelly, J. A.; Higginbotham, C. L. *Tetrahedron* **1990**, *46* (20), 7219-7226. (c) Reddy, B. J.; Reddy, K. S.; Parthasarathy, T. *Asian Journal of Chemistry* **2009**, *21*(5), 3855-3860.
18. Lorenz, M.; Kabir, M. S.; Cook, J. M.; *Tet. Lett.* 2010, *51*, 1095–1098.

19. Baker, W. *J. Chem. Soc.***1933**, 1381-1389.

20. Mahal, H. S.; Venkataraman, K. *J. Chem. Soc.***1934**, 1767-1769.

21. Nowlan, N. V.; Slavin, P. A.; Wheeler, T. S. *J. Chem. Soc.***1950**, 340-344.

22. Jucker, E.; Vogel, A. *Helvetica Chimica Acta* **1963**, *46*, 727-741.

23. Wilson, B.; Ollis, W. D.; Poole, V. D. *J. Chem. Soc.***1952**, 1505-1507.

24. Horie, T. *Nippon Kagaku Kaishi* **1978**, *5*, 748-52.

25. Kabalka, G. W.; Mereddy, A. R. *Tet. Lett.* **2005**, *46*, 6315–6317.

26. Zhu, X.; Li, Z.; Shu, Q.; Zhou, C.; Su, W. *Syn. Comm.* **2009** *39* (23), 4199-4211.

27. Su, W. K.; Zhu, X. Y.; Li, Z. H. *Organic Preparations and Procedures International* **2009**, *41*, 69–75.

28. Jina, C.; Heb, F.; Wub, H.; Chenb, J., Su, W. *J. Chem. Res.* **2009**, 27-29

29. Lee, Y. R.;Kang, K. Y. *Letters in Organic Chemistry*, **2007**, *4*, 440-444

30. Sarda, S. R.; Pathan, M. Y.; Paike, V. V.; Pachmase, P. R.; Jadhav, W. N.; Pawar, R. P. *Arkivoc* **2006** (xvi), 43-48.

31. McGarry, L. W.; Detty, M. R.; *J. Org. Chem.* **1990**, *55* (14), 4349-4356.

32. Costa, A. M. B. S. R. C. S.; Dean, F. M.; Jones, M. A.; Varma, R. S. *J. Chem. Soc., Perkin Trans.1: Org. and Bio-Org. Chem. (1972-1999)* **1985**, *4*, 799-808.

33. Horie, T.; Kawamura, Y.; Yamamoto, H.; Yamashita, K. *Chem. Pharm. Bull.* 1995, *43* (12), 2054-2063.

34. (a) McGarry, L. W.; Detty, M. R. *J. Org. Chem.* **1990**, *55* (14), 4349-4356. (b) Gao, H.; Kawabata, J. *Bioorg. Med. Chem.* **2005**, *13* (5), 1661-1671.

35. Buschi, C. A.; Pomilio, A. B.; Gros, E. G. *Phytochemistry* **1980**), *19* (5), 903-4.

36. (a) Pomilio, A. B.; Vacchino, M. N.; Vitale, A. A. *South American Medicinal Plants as a Potential Source of Bioactive Compounds* **2008**, 73-113. (b) Pomilio, A. B.; Buschi, C. A.; Tomes, C. N.; Viale, A. A. *Journal of Ethnopharmacology* **1992**, *36* (2), 155-161.

37. Tomas-Barberan, F. A.; Msonthi, J. D.; Hostettmann, K. *Phytochemistry* **1988**, *27* (3), 753-755.

38. Tomas-Lorente, F.; Iniesta-Sanmartin, E.; Tomas-Barberan, F. A.; Trowitzsch-Kienast, W.; Wray, V. *Phytochemistry* **1989**, *28* (6), 1613-15.

39. Takeuchi, Y.; Choshi, T.; Tomozane, H.; Yoshida, H.; Yamato, M. *Chem. Pharm. Bull.* 1990, *38* (8), 2265-7.

40. (a) von Auwers, K. *Berichte der Deutschen Chemischen Gesellschaft* **1916**, *49*, 809-818. (b) Minton, T. H.; Stephen, H. *Journal of the Chemical Society, Transactions* **1922**, *121*, 1598-1603.

41. Stack, D. P.; Coates, R. M. *Synthesis* **1984**, *5*, 434-6.

GENERAL CONCLUSION

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

In the first chapter, use of phosphazene base P_4-*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 P_4-*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.

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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.