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Progress Towards the Synthesis of a Difluorinated Combretastatin Analogue

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Abstract

Combretastatin A-4, an anti-tumor agent isolated from the *Combretum caffrum* bush, and related synthetic derivatives, have been found to be effective in tumor reduction via vasculature collapse. While investigations into increasing the natural product's activity have focused mainly on including a central ring to gain conformational locking, these additions can have the undesired effect of creating a molecule too large or planar to attach to the corresponding binding site. This research focuses on utilizing fluorine substituted CA-4 derivatives to obtain the same outcomes desired with CA-4, with the avoidance of steric hindrance. A total synthesis of fluorine substituted CA-4 analogs is being performed with the use of diazonium salts as intermediates towards the final fluorinated analogues. The versatility of the diazonium salts are also being investigated for creating interesting heterocyclic molecules as well.

Introduction

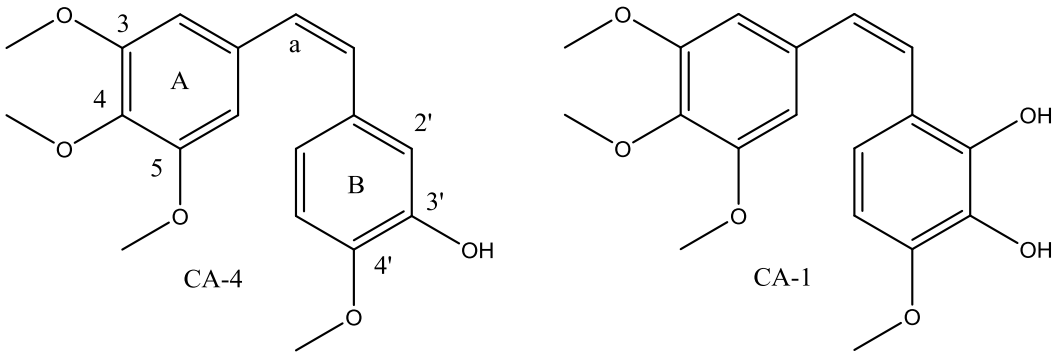

Figure 1. Combretastatins A-4 and A-1

In the past three decades, a major area of research in cancer drug development has been the disruption of the polymerization of α and ß tubulin, whether through stabilization or destabilization of microtubules.[1] Two of the more potent compounds known are combretastatin A-4 and combretastatin A-1, CA-4 and CA-1 respectively (Figure 1). The entire series of combretastatin drugs (A, B, C, D) were extracted from the *Combretum caffrum* bush, from which they derive their namesake, by George R. Pettit.[2] Over the past twenty years, there has been a steady increase of research into the potential activity of combretastatin analogs.

There are three main parts of the combretastatin skeleton where modifications have been investigated: The A-ring, containing the trimethoxy moiety at positions 3, 4, and 5, the *cis*-double bonded two carbon-bridge, and the B-ring, containing a *para*-methoxy, and a *meta*-hydroxyl.[3,4,5] The trimethoxy moiety has been found to be necessary to retain the high cytotoxic activity of CA-4 and CA-1, and the olefin bridge required to keep the molecule in the correct shape (*cis* conformation) for anti-tubulin polymerization (however the trans conformation of some resveratrol analogs

similar to combretastatin show anti-proliferative effects.)[3,6,1,7] Although the *para*-methoxy on the B-ring seems to be required for hydrogen bonding activity, the *meta*- and *ortho*- positions seem to have the most room for improvement.[3,8]

The majority of research into this area has been with modifications to the B-ring and the replacement of the olefinic group with heterocyclic ring structures. As stated earlier, the 4'-methoxy group is necessary to retain cytotoxic effects, however by substituting different groups onto the 3' position more knowledge is obtained about which functional groups would better serve the cytotoxic and anti-proliferative effects of the compound. A general trend is noticed between electron withdrawing groups and a general decrease and potency, and electron donating groups and a general increase in potency (amines). However, although chlorine, bromine, and iodine substitution on the 3' position showed a decrease in potency, fluorine showed an increase in cytotoxic activity and no increase or decrease in anti-tubulin effects.[3]

Work by Lawrence and coworkers found that retention of the 3'-hydroxy group is not necessary, and the resulting analogue shows very little difference from CA-4.[4] However, the lack of the hydroxyl substituent prevents the creation of the phosphorylated pro-drug, which increases the solubility of combretastatin in biological media.[5] Although the replacement of hydrogen with fluorine in the 2' position diminishes anti-proliferative effects,[4] the replacement with a hydroxyl group does not greatly diminish activity.[1] This would allow for the creation of a phosphorylated pro-drug while retaining the 3'-fluoro substitution.

In the olefin bridge, activity is dependent on the retention of *cis* stereochemistry and a non-planar orientation.[6] Analogs where the double-bonded bridge was replaced with heterocyclic compounds to maintain a *cis* conformation have been synthesized. Although replacement of the olefin bridge with most heterocyclic compounds decreased anti-proliferative activity, some pyrazoline compounds demonstrated only a minor decrease in this activity with an increase in solubility, and tetrazole compounds lacking the hydroxyl group on the 3' position showed an increase in activity.[6] Compounds in which a sulfonate group replaced the double bond also showed an increase in anti-proliferative activity, although its effectiveness *in vivo* would need to be determined.[3] The substitution of fluorine into the α-position of the double bond has been shown to increase cytotoxic activity because of three reasons: The fluorine in the α-position prevents interconversion between *cis* and *trans* isomers in vivo, increases the biological half-life, and interacts with a fatty residue in the colchicine binding site.[5]

Prior published research within this group involving *cis*-stilbenoids has included modifications to the olefinic bridge, mainly, by replacing the double bond with heterocyclic rings, such as pyrazoline and cyclohexenone derivatives, in hopes of retaining the shape of the molecule.[9] Cyclohexenone bridges have the ability to undergo undesired reactions *in vivo*, and thus pyrazoline bridges were investigated as an alternative. Although having less cytotoxic activity than CA-4, its pyrazoline derivative showed only a minor decrease in anti-proliferative effects, and combined with its solubility in water, shows promise as a basis for further experimentation. MacSpartan 2004 was used to model the conformation of the molecules in the colchicine binding site, which could be used to extrapolate information about its activity.[9]

Typically, analogues are tested for anti-cancer activity by testing the cytotoxicity, anti-tubulin, and colchicine displacement activity.[3] Cytotoxicity is tested by IC_{50} assays, anti-proliferative effects (polymerization interference) by GI_{50}, and the ability to bind to the colchicine site by measuring colchicine displacement. Common cell lines that are tested are HeLa, Bel-7403, PC-3, A549, and K562.[3,4,2,1] The prevention of vascular formation of tumor cells corresponds to the anti-proliferative effects of vascular disrupting agent. Generally, it is more favorable for these compounds to have a greater anti-vascular effect, rather than being cytotoxic. By attacking vasculature, only a small percentage of the tumor needs to be directly affected, however a compound which exerts its effects through cytotoxicity must attack the tumor as a whole. High cytotoxicity can also increase the risk of side effects.[10] While the binding of the therapeutic agents in question can be modelled by biological methods, it can also be modeled using molecular modeling programs such as MacSpartan and Sybyl to simulate the size, shape, and location of residues of binding sites and the drugs affinity for binding.[8,9]

In the present paper, fluorinated combretastatin analogs are of interest. In many modern pharmaceuticals, fluorine atoms as substituents have provided a number of utilities. Due to its deactivating nature when attached to an arene ring, it can delay or prevent metabolism of the compound, which in turn can potentially increase its half-life.[4,5] With fluorine's unique electronic properties lipophilicity can occur, which can increase its binding affinity for oily residues in proteins.[4,5] However, this may be a problem in the context of cis-stilbenoids because of their already poor solubility in *in vivo*.[5] Fluorine has the ability to form hydrogen bonds, which are an important mechanism in the locking of combretastatins into the B binding site for colchicine in ß-tubulin,[5] although some studies have shown that increased h-bonding in the A site of the αß-tubulin dimer can cause a decrease in activity.[1]

Fluorinated analogs that have shown an increase of anti-proliferative activity are: 3'-fluoro-combretastatin A-4 and α-fluoro-CA-4, with 3'-fluoro-CA-4 showing similar activity to its parent compound.[3,4,5] However, compounds such as 3',5'-difluoro-CA-4, ß-fluoro-CA-4, and 2',3'-difluoro-CA-4 showed a decrease in activity compared to CA-4 and

CA-1.[4] Unfortunately, there has not been investigations into the substitutions of fluorine in the α and 3' positions on the same molecule, or the potency of a 3'-fluoro-2'-hydroxy analog, which theoretically should not reduce activity while increasing solubility, along with increasing the ease of separating the cis and trans isomers. Due to the promise of fluorinated analogs, and fluorinated pharmaceuticals in general, and the lack of research on these combinations, further research into the field seems necessary and prudent. The ultimate goal is a more complete investigation into the effects of fluorine substitution on drugs that interact with the colchicine binding site of α and ß tubulin.

2. Results and Discussion

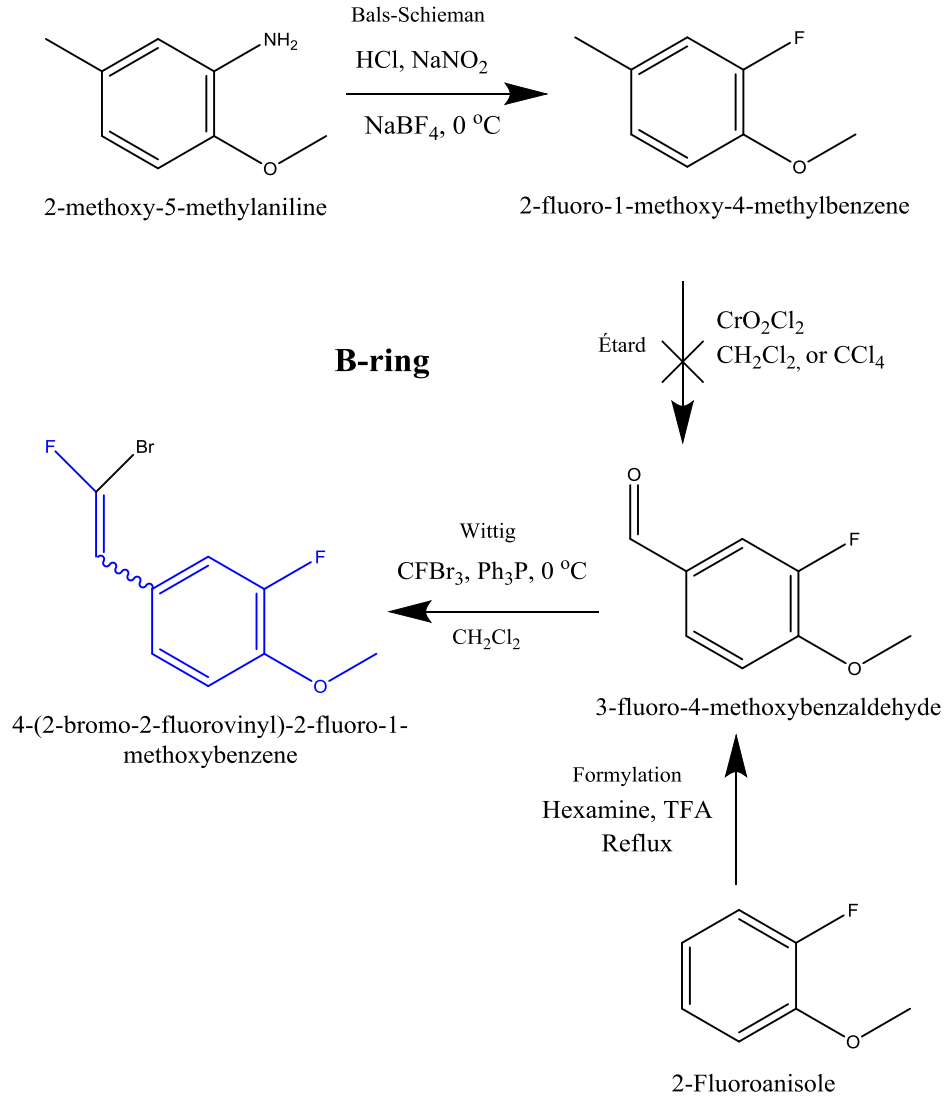

Figure 2. Synthesis scheme of the B-ring and olefin bridge.

In the course of the synthesis of α,3'-difluorocombretastatin, the majority of the reagents were synthesized prior to the final synthesis, with the main starting material originally 2-methoxy-5-methylaniline and later 2-fluoroanisole and 5-bromo-1,2,3-methoxybenzene, supplied by Sigma-Aldrich. The original B-ring synthesis starting with the aniline, underwent a modification of the Sandmeyer reaction, the Balz-Schiemann reaction, in which the aniline was diazotized and salted with alkali tetrafluoroborate. The diazonium tetrafluoroborate salt was subsequently destructively distilled under anhydrous conditions giving the aryl fluoride, 2-fluoro-4-methylanisole.[11] This aryl fluoride was then to be transformed into the aldehyde via an Étard reaction of the 2-fluoro-4-methylanisole with chromyl chloride.[12] However,

this part of the pathway was discarded for multiple reasons, including the difficulty of obtaining intermediates, the toxic nature of the byproducts, and also the economic viability of an alternative starting material: 2-fluoroanisole. In the process of tailoring the diazotization reaction to obtain a usable amount of product, other anilines were used to determine good conditions for the reaction: Aniline, 4-methoxyaniline, and 3,4,5-trimethoxyaniline, all providing usable diazonium tetrafluoroborate salts. In an effort to remain efficient in the use of materials, it was decided that these will play a part in a later part of this research. The diazonium salts will be used along with sulfonyl hydrazones to synthesize 2,5-disubstituted tetrazole analogues to combretastatin. 2,5 disubstituted tetrazoles allow conformational locking of the pharmacophores of combretastatin, but do not create the undesirable steric bulk that other heterocycle bridges do.[3]

The same aldehyde (3-fluoro-4-methoxybenzaldehyde) that would have been achieved from the Balz-Schiemann and subsequent Étard reaction was synthesized from 2-fluoroanisole via a formylation reaction with hexamethylene tetramine and trifluoroacetic acid, in underwhelming but usable yield. This aldehyde underwent the Wittig reaction with an ylide generated from tribromofluoromethane and Ph_3P in DCM,[8] utilizing methyl iodide to remove the residual Ph_3P. This halogenated alkene has been isolated with a minimal aldehyde contamination, as a mixture of *cis/trans* 2-bromo-2-fluoro-1-(3-fluoro-4-methoxybenzene)ethene.

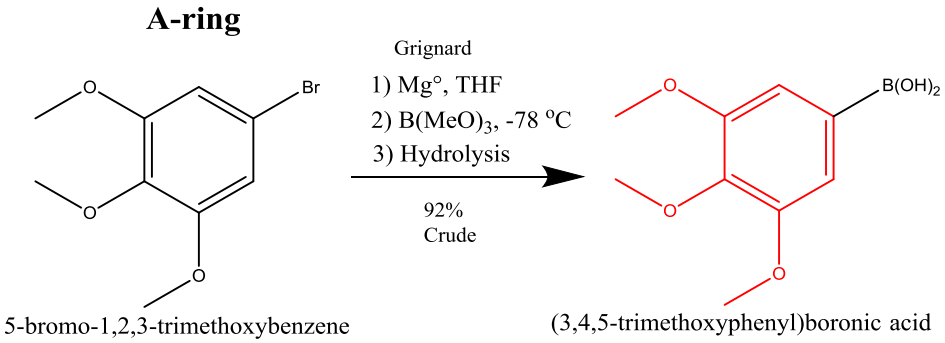

Figure 3. The A-ring synthesis.

The A-ring path was initiated with a Grignard reagent generated from the bromobenzene, to which boronic ester electrophile trimethylborate was added, and upon hydrolysis would yield 3,4,5-trimethoxybenzeneboronic acid.[5,11] Unfortunately, the reaction products have been difficult to isolate thus far. An alternative route via borylation of the diazonium salt synthesized from 3,4,5-trimethoxyaniline was investigated, and gave low yields (13%) of the target boronic acid. The boronic acid, along with the halogenated alkene, will undergo Suzuki coupling using tetrakis(triphenylphosphine)palladium(0) as a catalyst with a sodium carbonate regenerator[5] to form a cis/trans mixture of the final product, α,3'-difluorocombretastatin.

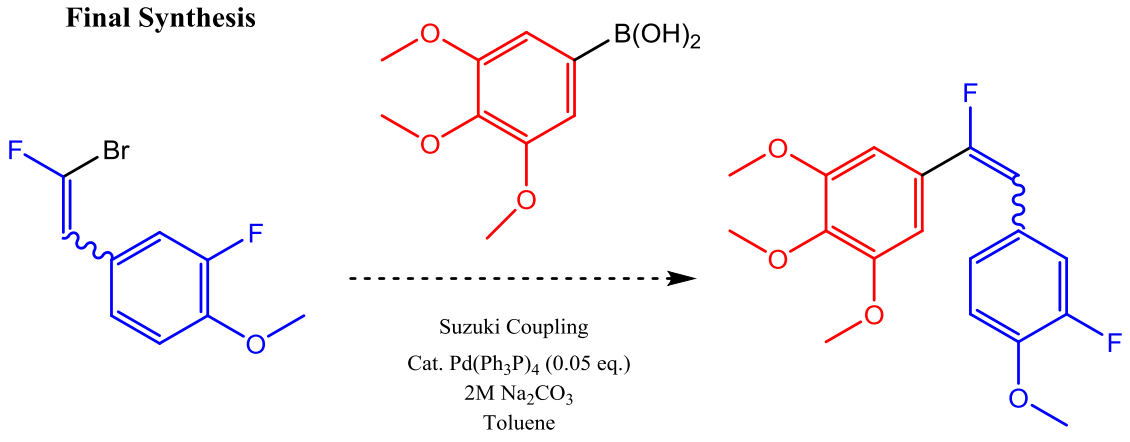

Figure 4. Final bridging synthesis.

Although the Suzuki coupling synthesis mirrors the synthesis utilized by Alloatti's group, it was necessary to synthesize the required intermediate boronic acid.[5] However, although this plan uses a Suzuki coupling similar to Alloatti's, it differs in that there is a fluorinated substituent on the B-ring of the final product, rather than a hydroxyl or amine group. In essence, this is where this research differs from the prior work of others: To create a previously unsynthesized CA analog containing two substituent modifications which individually have shown improved cytotoxicity and anti-proliferative effects in two separate analogs. The synthesis scheme was also planned so that relatively cheap, common, and later environmentally sound reagents would be utilized, excluding fluorobromoform. This would allow for the optimization of the beginning stage reactions, specifically the historically under-producing Balz-Schiemann reaction.[11]

3. Problems in Synthesis

Trimethylborate, one of the necessary reagents was synthesized, with an approximate yield of 20 ml of the azeotropic (70/30 MeOH) corresponding to a 34% yield, but a pure form sufficient to use in a Grignard reaction was unable to be isolated. Two qualitative tests confirming its identity were performed. Firstly, a flame test performed on a few drops of the solution produced a brilliant green flame, the exhaust depositing a fine white powder on the glass in which it was contained. Secondly, a few drops were introduced to a flask of cold water, producing a white smoke. This is concurrent with the properties of trimethylborate.[13,14] Unfortunately, the mixture decomposed before being able to undergo further purification. There are three reasons that have been considered as to why the yield was so low. Firstly, not enough sulfuric acid catalyst was used, secondly, distillation was not slow enough, and thirdly, fractions that came off from 65-69°C were not kept, and these fractions also would contain diminishing amounts of trimethylborate.[13,14] The decision was made to use trimethylborate obtained from commercial suppliers, rather than synthesizing on site, due to economic and environmental efficacy.

As mentioned previously, a problem arose initially during the synthesis of the 2-methyl-5-methoxybenzenediazonium tetrafluoroborate salt. During the first several syntheses, no usable product was obtained, which was later discovered to be the result of too high of a solubility and the unstable nature of the salt in solution, coupled with solvent incompatibilities preventing further attempts to isolate the compound using normal extractive techniques. Using other substituted anilines that were abundant the reaction was fine tuned to parameters that where eventually able to afford the desired product in 32% yield in the first successful reaction, and later up to a 55% yield. The other substituted anilines yielded diazonium tetrafluoroborate salt products ranging in yield from 25 – 81%.

During the synthesis of the 2-fluoro-4-methylanisole, product was obtained, however the gaseous boron trifluoride byproduct of this reaction made this process unpleasant and environmentally questionable. Yields ranged from 62 – 85% yield, however being a beginning stage reaction that would potentially be performed frequently, and the subsequent oxidation via chromyl chloride being undesirable because of the reaction conditions and toxic hexavalent chromium byproduct, this route was abandoned. Prior to the decision not to pursue this pathway, dibromination of the benzylic methyl and hydrolysis of the resulting product was considered, however the formylation of 2-fluoroanisole seemed a more favorable option.

Although the yield of the Wittig reaction involving the benzaldehyde product was not yet known, another Wittig reaction using copper (I) iodide to complex the unreacted triphenylphosphine and separate it from the reaction mixture was performed. Although TLC showed a complete removal of the triphenylphosphine from the extracted product, upon drying *in vacuo* with heat, the product decomposed, and NMR showed the presence of alkyl hydrogens, suggesting a polymerization. The original Wittig product was isolated from the methylated triphenylphosphine after column chromatography.

In future iterations of the synthesis of boronic acid, the Grignard reaction will be added to the trimethylborate via a pressure equalized addition funnel under argon atmosphere, to reduce the side reaction of two Grignard reagents attacking one boronic ester, creating the undesired boronic acid. Of the two initial Grignard reactions, the boronic ester was added to the formed Grignard reagent, with the first reaction cooled with dry ice and acetone, and the second cooled in an ice water bath. Both reaction products were subjected to acid base extractions, to attempt an isolation of boronic and borinic acids. The final extract showed six spots and ten spots in thin layer chromatography for the first and second reactions respectively. Under all solvent systems, the spots were too close to isolate an appreciable amount of uncontaminated product. Steam distillation of the crude products was attempted, however the boronic acid was not present in the aqueous distillate

4. Conclusion

To date both the A and B pathways have been completed.. The final stage of the synthesis will be the Suzuki coupling of the two sides. Ideally, this difluorinated combretastatin analogue will be tested against cancer cell lines to determine its IC_{50} values, if resources permit. Due to the enhanced binding affinities for colchicine binding sites in tubulin provided by the fluorine substitutions, it can be estimated that potency and half-life of the combretastatin analogue will be elevated over those of combretastatin A-4. Unfortunately, the water-solubility of the compound will likely be decreased, reducing the possibility of its effectiveness as an *in vivo* drug. However, if this compound proves effective, an analogue containing a hydroxyl in the 2' position will be synthesized, which would allow for the creation of a phosphorylated prodrug without diminishing activity.[3]

Also as a continuation to the research already performed, substituted 2,5-diphenyl tetrazoles will be synthesized from the diazonium salts already created and additional ones, utilizing sulfonyl hydrazones made from sulfonyl hydrazides and various substituted aromatic aldehydes. The 2,5-tetrazole bridge not only solves the issue of conformational flexibility white retaining activity, but also can increase the solubility of the drug. The synthesis of these tetrazoles will use a one-pot technique developed by Cunha et al. using phenylsulfonyl chloride, hydrazine, and an aromatic or aliphatic aldehyde in EtOH under mild conditions.[15] The possibility of extending this reaction to a one-pot synthesis of tetrazoles is worthy of investigation, as the reaction can be tailored *in situ* to provide potentially favorable conditions for the final step of 1,3-cycloaddition between the hydrazone and the diazonium.

5. Experimental

5.1 Synthesis of trimethylborate:

A solution of 160 ml anhydrous methanol and 30.90 g of orthoboric acid (H_3BO_3) was prepared. To this was added 10 ml of concentrated sulfuric acid in small portions over 10 minutes through a reflux condenser. Addition of sulfuric acid caused boiling on contact. Reflux was maintained via oil bath for 5 hours. A flame test was performed on the solution, giving a green flame and demonstrating the presence of product. After storing for two days, fractional distillation was performed with a 200 mm vigreux column. A fraction of approximately 100 ml came over between 52-54.5° C over the course of 3 hours (54.5°C azeotropic 75.5% $B(MeO)_3$ in methanol, literature).[13,14] To this fraction, 15 ml of concentrated sulfuric acid was added, and the formed layers separated, keeping the upper boronic ester layer. The boronic ester layer was stored over 10 ml of sulfuric acid for 1 week. After each addition of sulfuric acid, the volume of the trimethylborate/methanol solution was greatly reduced. However, after storing for two weeks, the product decomposed and was unable to be further isolated.

5.2 Synthesis of 2-methoxy-5-methylbenzenediazonium tetrafluoroborate:

Finely pulverized *p*-cresidine (2-methoxy-5-methylaniline), 0.688 g, was dissolved into 6 ml water, 1 ml conc. HCl. Contents were cooled on ice to 3 °C and precipitation was formed, 3 ml H_2O was added. Once dissolved, 0.354 g $NaNO_2$/0.6 ml H_2O was added dropwise, with stirring, temperature not exceeding 6 °C, over 15 minutes. The vessel was insulated from light with Al foil and allowed to stir for 30 minutes. After, 0.555 g $NaBF_4$/0.8 ml H_2O was added, and the mixture allowed to stir for 20 minutes at -2 °C. The precipitate was then filtered through P8 paper, washed with 2 ml cold water, 2 ml cold EtOH, and 3 ml cold Et_2O. The precipitate was dried in a drierite desiccator for 2 days, providing a red brown powder, weight of 1.183 g (31% yield). IR spectra, 2284 cm^{-1} N_2^+, 1068 cm^{-1} B-F. See attached data.

5.3 Synthesis of 2-fluoro-4-methylanisole:

To a short path distillation apparatus 0.893 g of 2-methoxy-5-methylbenzenediazonium tetrafluoroborate was added, with a gas adapter ending in a base trap. A self-sustaining reaction was initiated with a propane torch, and a distillate started coming over, along with white smoke. The reaction vessel was placed on oil bath and more distillate came over. After distillation was stopped, the remaining distillant was extracted with 2x15 ml of Et_2O. Due to low yields of distillate, the extracted and distilled fractions were combined, and filtered through celite. The combined fractions were dried over Na_2SO_4, and solvent evaporated in vacuo. Weight of 0.329 g (62% yield).

5.4 Synthesis of 3-fluoro-4-methoxybenzaldehyde:

A refluxing setup was charged with 5.596 g hexamethylene tetramine and 35 ml of trifluoroacetic acid. The reflux vessel was put under argon atmosphere, and 4.2 ml of 2-fluoroanisole was added. Reflux was spontaneous and then maintained for 24 hours.50 ml chloroform was added along with 300 ml sat. sodium bicarbonate solution, to afford a pH of 8. The layers were separated and aqueous extracted 3x25 ml with chloroform. The combined organic layers were washed with 2x100 ml sat. $NaHCO_3$ sol., 1x50 ml brine, and dried over Na_2SO_4. Solvent was evaporated in vacuo affording 6.357 g (over theoretical of 5.769). No crystals formed upon refrigeration (m.p. of product 30-31 °C).[4] The crude product was extracted with 25 ml chloroform and 50 ml sat. $NaHCO_3$ sol. The layers separated and aqueous washed with 10 ml chloroform. The combined organic layers dried over Na_2SO_4 and solvent evaporated in vacuo. The crude product purified via Biotage column chromatography using 12/88 EA/Hex to 100 EA gradient with 3 column volume flush of 100 Hex. Fractions were collected when Biotage indicated eluting product. Fractions 2-47 of 20 ml were combined and evaporated in vacuo, affording a slightly yellow oil, crystallizing to an off white waxy solid upon cooling. Compound stored at -40 °C, away from light, under argon. Weight of 1.660, a 29% yield. IR: 2846 cm^{-1} aldehyde H, 1670 cm^{-1} C=O, 1020 cm^{-1} arom. C-F. H^1NMR: 1H 9.87 ppm d, 2H 7.64 ppm t, 1H 7.09 ppm t, 3H 3.99 ppm s. Spectral data attached.

5.5 Synthesis of 4-[(E/Z)-2-bromo-2-fluorovinyl]-2-fluoroanisole.

To a vessel on an ice bath, 50 ml DCM and 2.620 g triphenylphosphine (9.989 mmol) added. To this solution was added 1.355 g (5.005 mmol) tribromofluoromethane, and allowed to stir for 0.5 h. To the solution was added 0.617 g of 3-fluoro-4-methoxybenzaldehyde (4.003 mmol) was added in chunks. Upon dissolving, the mixture was allowed to stir on ice for 0.5 h. The reaction mixture was diluted with 60 ml DCM, and washed 3 x 30 ml water. This organic layer dried over Na_2SO_4 and evaporated in vacuo to afford a yellowish oil with white crystals present. To remove excess triphenylphosphine, the crude product was redissolved in 25 ml chloroform and 2 molar equivalents (compared to TPP) of methyl iodide was added. This was allowed to stir for 4 hours, until the TPP spot disappeared on TLC. Purified using column chromatography wet load using 90/10 hex/EtOAc eluent. Fractions 8-44 kept and dried *in vacuo*, all showing a product spot and a minor aldehyde contamination. Proton NMR showed the presence of a vinyl hydrogen and an aldehyde hydrogen. Product was a deep purple oil.

5.6 Synthesis of 3,4,5-Trimethoxybenzeneboronic acid:

Trimethylborate previously dried over 10-12 mesh 4Å molecular sieves. Reflux apparatus charged with 0.147 g of hexane rinsed Mg^0 and a stirbar, and flame dried with propane torch, and put under nitrogen gas. The system was briefly opened and 1.235 g of 1,2,3-trimethoxy-5-bromobenzene and 15 mg I_2 were added, and the system was put under argon. To this mixture 10 ml THF was added with stirring. Gentle reflux was maintained for 24 hrs. At this time ~1/2 of the volume of solvent had evaporated, so 10 ml of THF was added. Mg metal was almost entirely reacted. The vessel was put on a $CO_{2(s)}$/acetone bath and 2 ml of trimethylborate was added with vigorous stirring. This was allowed to warm to room temperature over 24 hours. The reaction was quenched with 30 ml 1 N HCl slowly on an ice bath. 20 ml 1 N HCl was then added rapidly. The mixture was extracted with 3x20 ml Et_2O, and the combined organic layers were dried over Na_2SO_4 and evaporated in vacuo. Crude weight of 0.974 g of an orange/brown oil with white cyrstals. Crude yield of 91.9%. Product re-dissolved in 20 ml diethyl ether, and washed 5x20 ml of 10% NaOH solution in water w/v. Combined basic layers were washed with 2x12 ml Et_2O, and the basic layer was then acidified to ~1-2 pH using concentrated sulfuric acid. This acidic phase was then extracted using 4x20 ml of Et_2O. TLC of combined acidic extraction diethyl ether layers showed removal of bromobenzene, however 6 spots were still present otherwise. The combined acidic extracted organic layers were washed with brine, and dried over anhydrous Na_2SO_4. Aliquots of the ethereal extract were tested for crashing in hexane, EtOAc, DCM, acetone, iPrOH, benzene, and CCl_4. The extract was evaporated to ~15-20 ml and crashed with 40 ml of hexane. A precipitate crashed out of solution and the flask was refrigerated. TLC of the solution and the precipitate showed no appreciable difference in presence of spots, so both were re-dissolved in a minimal amount of ether and the ether layer was steam distilled using ~225 ml of water. Aqueous distillate was saturated with sodium chloride and extracted 4x20 ml and 1x40 ml Et_2O. Combined organic layer dried over anhydrous sodium sulfate and solvent evaporated *in vacuo* to afford a yellow oil, 50 mg crude.

5.7 Alternative Synthesis of 3,4,5-Trimethoxybenzeneboronic acid:

To a 50 ml round bottom flask 913 mg of 3,4,5-trimethoxyaniline was added. The purplish powder was dissolved in 10 ml of 2 M HCl and 5 ml of MeOH. To this solution, 345 mg $NaNO_2$ in 2.5 ml water was added. Addition was slow as to not raise the temperature above 5 ºC. Solution stirred for 30 minutes. To the solution, 675 mg $B_2(OH)_4$ was added as a powder in one addition. Solution allowed to stir for 1 hour. To the reaction, 30 ml of water was added and allowed to stir for an additional 30 minutes. Reaction mixture extracted 3x20 ml and 3x10 ml $CHCl_3$. Organic layers dried over sodium sulfate and evaporated *in vacuo*. Crude product, a purple wax, recrystallized twice from toluene, and washed with ~3 ml ice cold toluene. Yield was 126 mg, 12 % of theoretical. IR: 3352 cm^{-1} BO-H, 1348 cm^{-1} B-O.

5.8 Synthesis of (E)-5-(1-fluoro-2-(3-fluoro-4-methoxyphenyl)vinyl)-1,2,3-trimethoxybenzene:

4-[(E/Z)-2-bromo-2-fluorovinyl]-2-fluoroanisole, 26 mg weighed into 25 ml round bottom flask with 3 ml toluene. To this vessel, 63 mg 3,4,5-trimethoxyphenyl boronic acid and 0.3 ml 2 M Na_2CO_3 added. Vessel flushed with argon and 10 mg tetrakis(triphenylphosphine)palladium added. Vessel put on reflux condenser and entire apparatus evacuated and refilled with argon. Reaction refluxed for 22 hours. Diluted with 10 ml toluene and passed through a micro column of celite, followed by 20 ml of toluene and 10 ml EtOAc. Solvent evaporated *in vacuo* to afford a crude yellow oil.

6. Acknowledgements

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