A Transmetalation Reaction Enables the Synthesis of [18F]5-Fluorouracil From [18F]Fluoride for Human PET Imaging

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A Transmetalation Reaction Enables the Synthesis of $[^{18}\text{F}]{\text{5-Fluorouracil}}$ from $[^{18}\text{F}]{\text{Fluoride}}$ for Human PET Imaging

A dissertation presented

by

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to

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for the degree of

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A Transmetalation Reaction Enables the Synthesis of $^{18}$F5-Fluorouracil from $^{18}$FFluoride for Human PET Imaging

Abstract

5-Fluorouracil is a broad-spectrum chemotherapeutic for cancer treatment. $^{18}$F5-Fluorouracil, which contains the radioactive isotope fluorine-18, is a tracer for positron emission tomography (PET) imaging and has been applied for determining the location over time of 5-fluorouracil in the bodies of human cancer patients. Potential applications of $^{18}$F5-fluorouracil are for personalized chemotherapy and the development of new cancer treatments. All reported preparations of $^{18}$F5-fluorouracil for human PET imaging have used $^{18}$FF2 gas, which is challenging to produce and handle, and is less desired as a starting material in comparison to $^{18}$Ffluoride.

This dissertation describes the development and translation of new chemical reactions to produce human doses of $^{18}$F5-fluorouracil from $^{18}$Ffluoride. The first preparation of nickel(II) σ-aryl complexes by transmetalation from arylboronic acids was developed. This transmetalation reaction was applied to produce a nickel(II) σ-aryl complex that undergoes oxidative fluorination with $^{18}$Ffluoride for the synthesis of $^{18}$F5-fluorouracil. This oxidative fluorination reaction was translated for production of human doses of $^{18}$F5-fluorouracil. Although numerous transition metal-mediated fluorination reactions have been developed, none have previously been translated to enable human PET imaging.
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List of Abbreviations

18-crown-6: 1,4,7,10,13,16-hexaoxacyclooctadecane
4-OMe-pyridine: 4-methoxypyridine
5-FU: 5-fluorouracil
5-HT: 5-hydroxytryptamine
Ac: acetyl
Acac: acetylacetonate
Anal: analysis (elemental analysis)
aq.: aqueous
Bn: benzyl
Boc: tert-butoxycarbonyl
calcd: calculated
cGMP: current good manufacturing practice
Ci: curie
cod: cyclooctadiene
CT: computed tomography
Cy: cyclohexyl
DAM: bis(4-methoxyphenyl)methyl
dc: decay-corrected
DMF: dimethylformamide
DMSO: dimethylsulfoxide
DNA: deoxyribonucleic acid
F-DOPA: 6-fluoro-L-3,4-dihydroxyphenylalanine
dppf: 1,1'-bis(diphenylphosphino)ferrocene
dTMP: deoxythymidine monophosphate
dUMP: deoxyuridine monophosphate
EOS: end of synthesis
equiv.: equivalents
ESI: electrospray ionization
Et: ethyl
TOF: time of flight
EWG: electron-withdrawing group
FDA: Food and Drug Administration
F-dUMP: 5-fluorodeoxyuridine monophosphate
FIA: Flow Injection Analysis
g: grams
HPLC: high-performance liquid chromatography
HRMS: high resolution mass spectrometry
IEX: ion exchange
i-Pr: isopropyl
K\textsubscript{222}: 1,10-diaza-4,7,13,16,21,24-hexaoxabicyclo[8.8.8]hexacosane
L: neutral ligand, general
L\textsubscript{n}: neutral ligand, general, of n quantity
mCi: millicurie
Me: methyl
MHz: megahertz
min.: minutes
mL: milliliters
MOM: methoxymethyl
MR: magnetic resonance
Ndc: not decay-corrected
NFBS: N-fluorobenzenesulfonimide
NHP: non-human primate
NMR: nuclear magnetic resonance
Ox.: oxidative
PEEK: polyetheretherketone
Ts: para-toluenesulfonyl
USP: United States Pharmacopeia
UV: ultraviolet
W: watts
X: anionic ligand bound at heteroatom, general
Chapter 1. Introduction

1.1 \[^{18}\text{F}]5\text{-Fluorouracil}\n
5-Fluorouracil (5-FU) is a broad-spectrum chemotherapeutic whose applications include the treatment of cancers of the colorectal system,\(^1\) breast,\(^2\) pancreas,\(^3\) head and neck,\(^4\) and skin.\(^5\) The synthesis and properties of 5-FU were first reported in 1957 by Heidelberger and coworkers,\(^6\) and the first clinical studies of 5-FU for cancer treatment were reported shortly thereafter, in 1958.\(^7\) The cytotoxicity of 5-FU arises from inhibition of thymidylate synthase and by its incorporation into DNA and RNA.\(^8\) 5-FU is metabolized to fluorodeoxyuridine monophosphate (F-dUMP), which inhibits thymidylate synthase by formation of a ternary complex with the enzyme and its


cofactor, methylenetetrahydrofolate (Scheme 1.1). The structure of F-dUMP is identical to the natural thymidylate synthase substrate dUMP, except for the presence of fluorine in the position where deprotonation by the enzyme would normally occur. Therefore deprotonation and conversion to dTMP do not occur, and the amount of dTMP within the cell declines. The relative deficiency of dTMP in comparison to other nucleotides disrupts regulatory pathways for DNA synthesis and repair, and ultimately leads to cell death.

Scheme 1.1. Inhibition of Thymidylate Synthase by 5-Fluorouracil

Knowledge on the interaction of 5-FU with cancerous tumors could help to improve 5-FU chemotherapy. For this purpose, positron emission tomography (PET) imaging with $^{18}$F-5-FU has been applied to image 5-FU in human cancer patients. $^{18}$F-5-FU is a radioactive isotopologue of 5-FU that contains the $^{18}$F isotope, which decays to $^{18}$O by positron emission with a half-life of 109.771 minutes. When the emitted positron encounters an electron, annihilation occurs, and two gamma rays are emitted in opposite directions. These annihilation events can be measured

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quantitatively over time in three-dimensional space with PET instrumentation. After $^{18}$F-5-FU is administered to a human patient, the location of the molecule within the body is measured with PET. This data is acquired in a non-invasive fashion, and is usually acquired in combination with computed tomography (CT) or magnetic resonance (MR) images of anatomical features. The location of the $^{18}$F-5-FU can then be assigned to specific tumors, organs and other tissues. Other isotopes that emit a positron include $^{11}$C (half-life: 20 minutes), $^{13}$N (half-life: 10 minutes), and $^{15}$O (half-life: 2 minutes). In theory, 5-FU could be synthesized from any of these isotopes. However, $^{18}$F is the preferred positron-emitting isotope for PET imaging. The 110 minute half-life of $^{18}$F allows the most time for $^{18}$F-5-FU synthesis, purification, administration to human patient, metabolism, and imaging. The short half-life (at most 20.3 minutes) of the other positron-emitting isotopes $^{11}$C, $^{13}$N and $^{15}$O is a major drawback for their application. While the synthesis of $^{13}$C-5-FU has been reported, $^{18}$F-5-FU is the only isotopologue of 5-FU that has been applied for PET imaging.

PET imaging with $^{18}$F-5-FU has potential for prediction of 5-FU treatment efficacy in cancer patients. Because 5-FU can be severely toxic, cancer patients would benefit if efficacy (or lack thereof) could be predicted. PET imaging could potentially be performed with a trace, non-toxic dose of $^{18}$F-5-FU to predict efficacy before chemotherapy. The efficacy of 5-FU in human patients with liver metastases of colorectal cancer has been correlated with pharmacokinetic data.


obtained by PET imaging with $^{18}$F-5-FU.\textsuperscript{13} Progress has been reported toward application of such data to optimize 5-FU dosage for patients in a personalized fashion.\textsuperscript{14}

Another application of $^{18}$F-5-FU is to develop better cancer treatments through an understanding of mechanism of action of 5-FU, by imaging with PET. When new treatments are attempted, the change in 5-FU pharmacokinetics and tumor penetration can be measured by PET imaging with $^{18}$F-5-FU.\textsuperscript{15} For example, a $^{18}$F-5-FU PET study showed that administration of Interferon Alpha or N-phosphonoacetyl-L-aspartate altered 5-FU pharmacokinetics in tumors, plasma, and liver of human cancer patients.\textsuperscript{16} Another human PET study with $^{18}$F-5-FU showed that intra-arterial administration of 5-FU resulted in superior drug delivery to tumors, in comparison to intravenous administration.\textsuperscript{17}

A study in humans with $^{18}$F-5-FU validated a mechanism of action for how 5-FU metabolism is altered by Eniluracil (5-ethynyluracil). After Eniluracil treatment, PET imaging of $^{18}$F-5-FU demonstrated an improved 5-FU pharmacokinetic profile, and a decrease in delivery to the kidneys.\textsuperscript{18} This study validated, in live humans, the link between Eniluracil’s inhibition of


dihydropyrimidine dehydrogenase \(^{19}\) (an enzyme that promotes 5-FU excretion) and 5-FU pharmacokinetics. The improved bioavailability of 5-FU caused by Eniluracil allows for oral administration of 5-FU, instead of cumbersome intravenous administration. \(^{20}\) Results of a successful phase II clinical trial of oral Eniluracil plus 5-FU for treatment of metastatic breast cancer were reported in 2014. \(^{21}\)

Because of the 110 minute half-life of \(^{18}\)F, \([^{18}\text{F}]5\text{-FU}\) must be synthesized within several hours of PET imaging. In general, \(^{18}\)F-labeled PET tracers are made starting from one of two fluorine sources produced in a cyclotron: \([^{18}\text{F}]\text{fluoride}\) or \([^{18}\text{F}]\text{F}_2\) gas. \(^{22}\) The preferred starting material is \([^{18}\text{F}]\text{fluoride}\), because \([^{18}\text{F}]\text{fluoride}\) is much simpler to produce and handle than \([^{18}\text{F}]\text{F}_2\) gas, and therefore is more widely available. \(^{23}\) Despite the preference for \([^{18}\text{F}]\text{fluoride}\) as a starting material, production of a human dose of \([^{18}\text{F}]5\text{-FU}\) from \([^{18}\text{F}]\text{fluoride}\) has not been reported. All reported \([^{18}\text{F}]5\text{-FU}\) PET imaging studies have used \([^{18}\text{F}]5\text{-FU}\) that was made from \([^{18}\text{F}]\text{F}_2\) gas and uracil. \(^{24}\) This fluorination reaction was first reported in 1973 by Fowler and coworkers (Scheme 1.2, top), \(^{24a}\) and was translated to clinical application by 1980. \(^{25}\) Later modifications included

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conversion of $[^{18}\text{F}]\text{F}_2$ to $[^{18}\text{F}]\text{AcOF}$ prior to uracil fluorination (Scheme 1.2, bottom).\textsuperscript{26} A synthesis of $[^{18}\text{F}]5\text{-FU}$ for human PET imaging starting from $[^{18}\text{F}]\text{fluoride}$ is a desired alternative to the current reliance on impractical $[^{18}\text{F}]\text{F}_2$.

![Scheme 1.2](image_url)

**Scheme 1.2.** Previous Methods for Synthesis of Human Doses of $[^{18}\text{F}]5\text{-Fluorouracil}$

1.2 Fluorination of Arenes with $^{18}$FFluoride

1.2.1 Fluorination of Aryl Electrophiles with $^{18}$FFluoride

The synthesis of $^{18}$Faryl fluorides from $^{18}$Ffluoride$^{27}$ is of relevance to the synthesis of $^{18}$F5-FU, which contains a C(sp$^2$)–F bond. An early method for the synthesis of $^{18}$Faryl fluorides from $^{18}$Ffluoride was nucleophilic aromatic substitution on electron-deficient arenes. In 1983 Wolf reported substitution of nitro groups with $^{18}$Ffluoride on arenes with electron-withdrawing groups, in high radiochemical conversion (RCC: the conversion of $^{18}$Ffluoride to product that contains $^{18}$F) (Scheme 1.3).$^{28}$

![Scheme 1.3. Fluorination of Electron-Deficient Arenes by S_NAr Reaction with $^{18}$FFluoride](image)

Application of such reactivity to heterocycles is generally limited to activated positions, such as the 2- or 4-position of pyridine (Scheme 1.4).$^{29}$ Fluorination at the 3-position of pyridine

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only produced trace amounts of product. Early developments\textsuperscript{30} for the synthesis of electron-neutral \([^{18}\text{F}]\)aryl fluorides from \([^{18}\text{F}]\)fluoride were the fluorination of aryldiazonium salts (the Balz-Schiemann reaction\textsuperscript{31}) and triazines (the Wallach reaction\textsuperscript{32}), but broad substrate scope and functional group tolerance was not demonstrated.

\textbf{Scheme 1.4.} Synthesis of \([^{18}\text{F}]\)Fluoropyridines by S\textsubscript{N}Ar Reaction with \([^{18}\text{F}]\)Fluoride

The application of aryl iodine(III) electrophiles improved the substrate scope for the synthesis of electron-neutral and electron-rich \([^{18}\text{F}]\)aryl fluorides from \([^{18}\text{F}]\)fluoride. Early work by Pike and coworkers demonstrated that \([^{18}\text{F}]\)fluoride typically forms a C-F bond with the most electron-deficient aryl substituent in diaryliodonium(III) cation precursors (Scheme 1.5).\textsuperscript{33} In these experiments, the organic Kryptofix-222 ligand (K\textsubscript{222}) was employed to bind potassium and thereby solubilize K\textsuperscript{18}F. Subsequent work by Coenen et. al. employed diaryliodonium salts where one


substituent was the electron-rich 2-thiophenyl moiety, in order to direct fluorination onto the other substituent.\(^\text{34}\)

![Chemical structure and reaction scheme]

**Scheme 1.5.** \(^{18}\)F-Fluorination of Diaryliodonium(III) Salts with \([^{18}\text{F}]\text{fluoride}\)

Reports of structurally complex aryl iodine(III) precursors for late stage C(sp\(^2\))–\(^{18}\)F bond formation emerged in the patent literature in 2010. Dimagno reported the synthesis of \([^{18}\text{F}]\text{MTEB}\), a PET tracer for neuroimaging,\(^\text{35}\) from a diaryliodonium(III) salt and \([^{18}\text{F}]\text{fluoride}\).\(^\text{36}\) Subsequently, Dimagno and coworkers applied similar reagents for the syntheses of \([^{18}\text{F}]\text{fluorodopamine}\)\(^\text{37}\) and \([^{18}\text{F}]\text{F-DOPA}\) (Scheme 1.6).\(^\text{38}\)

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Scheme 1.6. $^{18}$F-Fluorination of Structurally Complex Diaryliodonium(III) Precursors

Scott, Sanford and coworkers developed a copper-mediated $^{18}$F-fluorination of diaryliodonium(III) salts that contain a mesityl group, which directs C–$^{18}$F bond formation to the non-mesityl group (Scheme 1.7).$^{39}$ Such discrimination between the two aryl groups is desired, because the $^{18}$F-fluorination of diaryliodonium(III) salts can involve undesired incorporation of [$^{18}$F]fluoride into one of the two aryl groups. For example, in the case of 2-thiophenyl(phenyl)iodonium tosylate, both aryl fragments were fluorinated with [$^{18}$F]fluoride, despite a significant difference in the electronic nature of the fragments.$^{40}$

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Scheme 1.7. Copper-Mediated $^{18}$F-Fluorination of Mesityl(aryl)iodonium(III) Precursors

An alternative approach to the use of diaryliodonium(III) salts is the use of iodine(III) precursors that contain only one aryl fragment for $^{18}$F-fluorination. A patent disclosed in 2010 described the $^{18}$F-fluorination of aryliodonium ylides derived from cyclic 1,3-dicarbonyl compounds such as Meldrum’s acid.\textsuperscript{41} Coenen applied such precursors for the synthesis of candidate ligands for serotonin and norepinephrine transporters (Scheme 1.8).\textsuperscript{42} Constitutional isomers were observed in the $^{18}$F-fluorination of one of two constitutional isomers.

Scheme 1.8. $^{18}$F-Fluorination of Iodonium(III) Ylides Derived from Meldrum’s Acid


In 2014, Vasdev, Liang and coworkers reported iodonium(III) ylides with a spirocycle in the ylide fragment (Scheme 1.9). The first synthesis of $[^{18}\text{F}]5$-FU from $[^{18}\text{F}]5$-fluoride was described. However, the $[^{18}\text{F}]5$-FU was isolated as an MeCN solution, which is toxic and not suitable for in vivo PET imaging. While typical human doses of $[^{18}\text{F}]5$-FU range from 5 – 10 mCi, the synthesis was described only on small scale (< 0.5 mCi of isolated $[^{18}\text{F}]5$-FU). The synthesis was performed manually, and required a technically cumbersome deprotection process involving multiple heating and cooling steps. To synthesize $[^{18}\text{F}]5$-FU for human use, all operations would need to be performed in a remote-controlled fashion, with automated equipment, in compliance with Current Good Manufacturing Practice (cGMP). While progress has been made for a different tracer, the application of iodonium(III) ylides for the synthesis of $[^{18}\text{F}]5$-FU has thus not overcome multiple challenges required for clinical translation and the synthesis of doses for human use.

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Scheme 1.9. $^{18}$F-Fluorination of Spirocyclic Iodonium(III) Ylides

In 2015, a titanium oxide-mediated fluorination of aryl tosylates with $[^{18}\text{F}]$fluoride has been reported.\textsuperscript{45} Although several $[^{18}\text{F}]$aryl fluorides were synthesized that were not prefunctionalized with electron-withdrawing groups, only simple products such as $[^{18}\text{F}]$fluorobenzene, $[^{18}\text{F}]$2-fluoronaphthalene, and $[^{18}\text{F}]$4-fluorotoluene were reported (Scheme 1.10).

Scheme 1.10. TiO$_2$-Mediated $^{18}$F-Fluorination of Aryl Tosylates

1.2.2 Oxidative Fluorination of Aryl Nucleophiles with $^{18}\text{F}$Fluoride

Scheme 1.11. Late-Stage Oxidative Fluorination with $^{18}\text{F}$Fluoride

Oxidative fluorination of aryl nucleophiles starting from $^{18}\text{F}$fluoride was not reported until 2011. The transformation of fluoride into a reagent for electrophilic fluorination can be thought of as a formal conversion of F$^{-}$ to F$^{+}$, which represents a conceptual challenge because fluorine is the most electronegative element. The Ritter group designed an electrophilic palladium(IV) fluorination reagent that was prepared from fluoride. The positron-emitting isotopologue, a Pd$^{IV}$–$^{18}$F reagent, was applied for the late-stage synthesis of complex $^{18}$Faryl.

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fluorides (Scheme 1.11). The aryl fragment originated from palladium(II) σ-aryl precursors with previously established electrophilic fluorination reactivity.\(^{48}\)

\[
\text{Scheme 1.12. Oxidative Fluorination of Nickel(II) σ-Aryl Complexes with } [^{18}\text{F}]\text{Fluoride}
\]

A subsequent report in 2012 from Lee, Hooker and Ritter described the oxidative fluorination of nickel(II) σ-aryl complexes with \([^{18}\text{F}]\text{fluoride (Scheme 1.12).}\(^{49,50}\) In contrast to the


fluorination of palladium(II) σ-aryl complexes, where fluorination was conducted over 10 minutes at 85 ºC, the fluorination of nickel(II) σ-aryl complexes occurred in less than 1 minute at 23 ºC. Short reaction times are desired in $^{18}$F chemistry, since $^{18}$F decays with a half-life of 110 minutes. Furthermore, water was tolerated in the fluorination reaction (0.5% by volume in MeCN), which permitted direct use of aqueous $[^{18}$F$]$fluoride solution produced by a cyclotron. Only one step was required for conversion of $[^{18}$F$]$fluoride to $[^{18}$F$]$aryl fluoride, whereas the previous method involving a Pd$^{IV}$-$^{18}$F intermediate required two steps.

These palladium and nickel-mediated late stage fluorination reactions were translated for the production of PET tracers for use in non-human primate (NHP) imaging.$^{51,52}$ Three tracers were made: $[^{18}$F$]$Paroxetine (a selective serotonin reuptake inhibitor antidepressant$^{53}$), an $^{18}$F-labeled agonist for the 5-HT$_{2C}$ receptor$^{54}$, and $[^{18}$F$]$MDL100907 (a ligand for the 5-HT$_{2A}$ receptor$^{55}$) (Scheme 1.13). These syntheses demonstrated the power of metal-mediated, late stage oxidative fluorination with $[^{18}$F$]$fluoride. The synthesis of tracers for NHP imaging served as an important milestone on the path toward human PET imaging.

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53 Katzman, M. CNS Drugs 2009, 23, 103.


Scheme 1.13. Translation of Late-Stage Pd and Ni-Mediated $^{18}$F-Fluorination for NHP Imaging

An alternative approach for oxidative fluorination with $[^{18}$F]$fluoride was reported in 2012 by Gouverneur and coworkers. Phenols with tert-butyl substituents were oxidized in the presence of $[^{18}$F]$fluoride to afford $^{18}$F-labelled fluorophenols without the use of protecting groups (Scheme 1.14).\textsuperscript{56} The substrate scope appears to be limited to para-fluorophenols.

Scheme 1.14. Oxidative Fluorination of tert-Butylphenols with $^{18}$FFluoride

In 2014, the Gouverneur laboratory reported the copper-mediated oxidative fluorination of arylboronic esters with $^{18}$Ffluoride (Scheme 1.15). Arylboronic esters are advantageous precursors for fluorination, because they typically are stable and may be prepared by a variety of methods. Although amines are reactive toward oxidation, the amine-containing K$_{222}$ ligand (employed for KF solubilization) is tolerated in this oxidation reaction, likely due to the mild oxidizing ability of copper(II). However, radiochemical conversions of $^{18}$Ffluoride for the formation of $^{18}$F4-fluorophenol and $^{18}$F4-fluoroaniline (arenes that are labile toward oxidation chemistry) were less than 10%, and improved about an order of magnitude when the O and N heteroatoms were masked with the tert-butoxycarbonyl protecting group. The fluorination of two heteroarenes (a quinoline and an N-protected indole) were described. However, in these cases, $^{18}$F

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was placed onto a separate ring than the heteroatom-containing ring. Incorporation of $^{18}$F onto the same ring in which a heteroatom is a constituent (with the exception of activated positions, e.g. the 2-position of pyridine) remains a challenge in the field of radiochemistry with $[^{18}$F]fluoride.

**Scheme 1.15.** Copper-Mediated Oxidative Fluorination of Arylboronic Esters

As discussed above, the field of oxidative arene fluorination with $[^{18}$F]fluoride has been actively expanding since the initial report by Ritter and coworkers in 2011.46 Numerous PET imaging studies have been enabled by these advances. However, all such studies were performed in animals. Clinical translation of a synthesis by oxidative fluorination to enable human PET imaging has not been reported.
1.3 Nickel(II) σ-Aryl Complexes

Nickel(II) σ-aryl complexes are precursors to [18F]aryl fluorides by oxidative fluorination with [18F]fluoride, as discussed in the previous section (Scheme 1.12 and Scheme 1.13). Nickel(II) σ-aryl complexes are also intermediates in catalysis. Nickel-catalyzed Suzuki-Miyaura coupling, first reported in 1995 by Percec and coworkers, likely proceeds through nickel(II) σ-aryl intermediates (Scheme 1.16).

Scheme 1.16. Plausible Mechanism for Nickel-Catalyzed Suzuki-Miyaura Coupling

In relation to their intermediacy in catalytic processes, nickel(II) σ-aryl complexes are important substrates for mechanistic investigation. Monfette and coworkers proposed that a dimeric NiII(Aryl)(OH) complex is the catalytic intermediate that undergoes transmetalation with boronic acid in Suzuki-Miyaura coupling under typically employed conditions (Scheme 1.17).

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Two nickel(II) σ-aryl complexes were synthesized: a NiII(Aryl)(OH) complex and its analogous NiII(Aryl)(Cl) complex. Both were shown to be kinetically competent for catalysis. Kinetic studies showed that NiII(Aryl)(OH) underwent reaction with boronic acid to form biaryl several orders of magnitude faster than NiII(Aryl)(Cl) did in the presence of base. Based on this and other data, the authors proposed that substitution of chloride with hydroxide is likely the rate-determining step in catalysis.

Scheme 1.17. Mechanistic Study of Ni(II) σ-Aryl Intermediates in Suzuki-Miyaura Coupling

Other mechanistic studies that have applied nickel(II) σ-aryl complexes\textsuperscript{61} include a study reported by Kochi in 1979 on the mechanism of biaryl formation from NiII(Aryl)(X) complexes

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and aryl halide, and a 2015 report from Sanford on the intermediacy of nickel(IV) in carbon-heteroatom bond formation.

Figure 1.1. Nickel(II) σ-Aryl Precatalysts

Nickel(II) σ-aryl complexes have been applied as precatalysts for Suzuki-Miyaura coupling, Buchwald-Hartwig coupling, ethylene dimerization and polymerization.

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63 Camasso, N. M.; Sanford, M. S. Science 2015, 347, 1218.
carbon dioxide reduction, Heck-type coupling, addition of alkene to aldehyde, cycloadditions, and oxidative esterification (Figure 1.1).

When nickel(II) σ-aryl complexes are prepared and isolated, they are most often synthesized by oxidative addition of Ni(0) to aryl (pseudo)halide, or transmetalation from an organometallic reagent to Ni(II). Preparation by oxidative addition of low-valent nickel to aryl halide was first reported by Fahey in 1970, by oxidative addition to a Ni(0) ethylene complex prepared in situ. Fahey’s report is representative of a general strategy where a Ni(II) precursor is reduced in situ to a low-valent nickel intermediate, which then reacts with aryl halide that is already present in the reaction pot (Scheme 1.18).

Scheme 1.18. In-Situ Reduction and Oxidative Addition for Ni(II) σ-Aryl Synthesis

Isolated nickel(0) precursors have also been used for preparation of nickel(II) σ-aryl complexes by oxidative addition. For example, Ritter and coworkers synthesized nickel(II) σ-aryl complexes...

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precursors for $^{18}$F-fluorination by oxidative addition of aryl bromides to nickel(0) biscyclooctadiene (Scheme 1.19), and Monfette et. al. prepared a nickel(II) bisphosphine $\sigma$-aryl complex for mechanistic studies in a similar manner from an aryl chloride (Scheme 1.17).

Scheme 1.19. Synthesis of Ni(II) $\sigma$-Aryl Complexes by Oxidative Addition of Aryl Bromides to Nickel(0) Bis(cyclooctadiene)

An alternative synthetic strategy for nickel(II) $\sigma$-aryl preparation involves transmetalation from an organometallic reagent to Ni(II). Early examples of transmetalation from aryllithium and aryl Grignard reagents to Ni(II) were reported by Chatt and Shaw in 1960. For example, an ortho-tolyl nickel complex was prepared from 2-methylphenylmagnesium bromide and trans-(PEt$_3$)$_2$NiBr$_2$ (Scheme 1.20).

Scheme 1.20. Ni(II) $\sigma$-Aryl Synthesis by Transmetalation from Grignard Reagent

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Nickel(II) σ-aryl complexes have also been prepared from arylzinc reagents.\textsuperscript{61,74} For example, Kurosawa and coworkers effected transmetalation from PhZnCl to Ni(η\textsuperscript{3}-allyl)(PPh\textsubscript{3})Cl to form Ni(η\textsuperscript{3}-allyl)(PPh\textsubscript{3})Ph, and also synthesized analogous complexes in this manner.\textsuperscript{75} When aryllithium reagents were used instead of arylzinc chlorides, more side products were observed. The application of arylzinc reagents for nickel(II) σ-aryl synthesis represents an advance compared to the application of aryllithium and aryl Grignard reagents, which have low functional group tolerance. However, to date there is no general method for nickel(II) σ-aryl synthesis from more robust p-block organometallics such as arylsilicon, aryltin, or arylboron reagents.


Chapter 2. Synthesis of Nickel(II) $\sigma$-Aryl Complexes by Transmetalation from Arylboron Reagents

2.1 Introduction

The ultimate goal of this thesis was the synthesis of $[^{18}\text{F}]5$-fluorouracil from $[^{18}\text{F}]$fluoride for human PET imaging. When this research was initiated, there was no reported synthesis of 5-fluorouracil from fluoride. The oxidative fluorination of nickel(II) $\sigma$-aryl complexes with $[^{18}\text{F}]$fluoride was considered as a promising method for the synthesis of $[^{18}\text{F}]5$-fluorouracil. As reported by Lee, Hooker and Ritter in 2012, nickel(II) $\sigma$-aryl complexes react to form $[^{18}\text{F}]$aryl fluorides at 23 ºC in less than 1 minute, by reaction with a 0.5% aqueous solution of $[^{18}\text{F}]$fluoride in acetonitrile, in the presence of a hypervalent iodine oxidant.\textsuperscript{49} In order to apply this method to synthesize $[^{18}\text{F}]5$-fluorouracil, an appropriate nickel(II) $\sigma$-aryl precursor A was necessary (Scheme 2.1). Complex A contains a 2,4-bisalkoxypyrimidine, which is a masked analog of uracil. Because uracil has poor solubility in organic solvents such as acetonitrile, and because uracil’s N–H bonds were perceived as a liability for chemistry with basic fluoride, protecting groups were desired in $[^{18}\text{F}]5$-fluorouracil precursor A.

![Scheme 2.1. Potential Ni(II) $\sigma$-Aryl Precursor A for $[^{18}\text{F}]5$-Fluorouracil Synthesis](image)

**Scheme 2.1.** Potential Ni(II) $\sigma$-Aryl Precursor A for $[^{18}\text{F}]5$-Fluorouracil Synthesis
There is one previously reported method for synthesis of nickel(II) σ-aryl complexes similar to A that contain the pyridylsulfonamide and pyridine ligands (which are required for oxidative fluorination). Oxidative addition of nickel(0) biscyclooctadiene to aryl bromides followed by ligand exchange afforded nickel(II) σ-aryl complexes similar to A, but with different aryl substituents. However, this method failed for the synthesis of A, even when the protecting groups were varied (Scheme 2.2). An alternative method to oxidative addition was desired, such as transmetalation to Ni(II). No broadly functional group-tolerant synthesis of nickel(II) σ-aryl complexes by transmetalation has previously been reported, but could be a powerful alternative to synthesis by oxidative addition with sensitive and strongly reducing Ni(0) reagents.

Scheme 2.2. Failed Synthesis of Ni(II) σ-Aryl Precursors for [18F]5-Fluorouracil Synthesis by Previously Reported Method Involving Oxidative Addition of Aryl Bromides to Ni(0)

In order to maximize the functional group tolerance, substrate scope, and practicality of a synthesis of nickel(II) σ-aryl complexes, the development of a transmetalation reaction starting from arylboronic acids and esters was desired. These reagents tolerate a wide variety of functional

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Acknowledgement is given to Martin Strebl for attempted syntheses of A by the synthetic method in Scheme 2.2.
groups, and several robust methods have been reported for their preparation, such as Miyaura borylation of aryl halides and iridium-catalyzed C-H borylation.

### 2.2 Development of a Nickel(II) Reagent for Transmetalation

The desired nickel(II) σ-aryl complex A has one aryl ligand and one sulfonamide ligand, and can be described by the formula Ni\(^{II}\)(Aryl)(X), where X is defined as an anionic ligand bound to the metal at a heteroatom. Transmetalation of the aryl fragment from arylboronic acids to inorganic Ni\(^{II}\)(X)\(_2\) complexes to form Ni\(^{II}\)(Aryl)(X) has precedent. Ni\(^{II}\)(X)\(_2\) complexes are used as precatalysts in Suzuki-Miyaura coupling, and are likely reduced by arylboronic acids through Ni\(^{II}\)(Aryl)(X) intermediates, to afford the biaryl product of homocoupling, along with low valent Ni that is active for cross-coupling catalysis (Scheme 2.3). However, there are no reports of direct observation of a Ni\(^{II}\)(Aryl)(X) intermediate (or any nickel(II) σ-aryl complex) that was formed by transmetalation from an arylboronic acid or ester. A faster transmetalation to Ni\(^{II}\)(Aryl)(X) than to Ni\(^{II}\)(X)\(_2\) (k\(_2\) > k\(_1\), Scheme 2.3) would preclude accumulation and observation of Ni\(^{II}\)(Aryl)(X).

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Scheme 2.3. Plausible Mechanism for Reduction of Ni(II) Precatalysts for Suzuki-Miyaura Coupling

A fundamental challenge of relative rates must be addressed in order to prepare Ni$^{II}$(Aryl)(X) from arylboronic acids. Transmetalation to form Ni$^{II}$(Aryl)(X) must be more facile than its destruction by homocoupling (i.e., $k_1 > k_2$, Scheme 2.3). To maximize transmetalation rate, a soluble Ni$^{II}$(X)$_2$ precursor with a sufficiently basic X-type ligand (e.g. hydroxide or alkoxide, but not chloride) to induce transmetalation was desired (Scheme 2.4). In support of this design, Monfette and coworkers have reported that a Ni$^{II}$(Aryl)(OH) complex forms biaryl by reaction with arylboronic acid (presumably via transmetalation) several orders of magnitude faster than Ni$^{II}$(Aryl)(Cl) does in the presence of base.$^{60}$ Furthermore, incorporation of the bidentate pyridylsulfonamide ligand (required for oxidative fluorination)$^{49}$ into the Ni$^{II}$(X)$_2$ precursor as the second X-type ligand was desired, in order to circumvent the need for additional ligand-exchange steps after transmetalation, and to render Ni$^{II}$(X)$_2$ soluble in organic solvents.

Scheme 2.4. Design of a Ni(II) Reagent for Transmetalation
Initial efforts to synthesize the desired Ni\textsuperscript{II}(pyridylsulfonamide)(X) complex were plagued by extremely facile formation of Ni\textsuperscript{II}(pyridylsulfonamide)\textsubscript{2} 1 (Scheme 2.5), the thermodynamic product of ligation. The formation of 1 occurs quickly upon mixing pyridylsulfonamide ligand with typical Ni\textsuperscript{II}(X)\textsubscript{2} precursors such as Ni(OAc)\textsubscript{2}(H\textsubscript{2}O)\textsubscript{4}, in several solvents that were evaluated, such as MeOH and DMSO. Transmetalation was not observed from complex 1 and arylboronic acid, likely because the sulfonamide ligand is not basic enough to activate boron. Complex 1 is an orange solid that is very insoluble in all organic solvents that were evaluated. The structure of 1 was determined by X-ray crystallography, and exhibits square-planar geometry.

Scheme 2.5. Thermodynamic Product of Pyridylsulfonamide Ligation to Ni(II)

In order to slow the formation of thermodynamic ligation product 1, pyridine was employed as solvent. Dissolution of Ni(OAc)\textsubscript{2}(H\textsubscript{2}O)\textsubscript{4} in pyridine affords a blue solution. When pyridylsulfonamide ligand is added, a green solution is obtained, and 1 does not precipitate after at least one hour at 23 °C, as long as the solution is sufficiently dilute (< 0.1 M of Ni\textsuperscript{II} in pyridine). Having established conditions where the pyridylsulfonamide ligand and Ni(II) coexist in solution without precipitation of 1, transmetalation from arylboronic acids and derivatives was attempted. Potassium tert-butoxide was selected as a base to induce transmetalation, because it is soluble in pyridine. When a solution of Ni(OAc)\textsubscript{2}(H\textsubscript{2}O)\textsubscript{4}, pyridylsulfonamide, and potassium tert-butoxide
in pyridine was treated with arylboronic acid and heated to 70 °C for one hour, transmetalation occurred. Complex 2a, a potential precursor for $[^{18}\text{F}]$5-fluorouracil, was isolated in 40% yield (Scheme 2.6). The structure of 2a was confirmed by X-Ray Crystallography (Figure 2.1).

**Scheme 2.6.** Assembly of 2a by Transmetalation Starting from Multiple Components

**Figure 2.1.** X-Ray Crystal Structure of 2a, a Potential Precursor to $[^{18}\text{F}]$5-Fluorouracil

This preliminary result of formation of 2a was a promising starting point for the development of a general transmetalation reaction from arylboron reagents. However, prior to proceeding to a thorough evaluation of substrate scope, simplification of the reaction conditions for transmetalation was desired. The composition of the nickel(II) species formed from
Ni(OAc)$_2$(H$_2$O)$_4$, pyridylsulfonamide, and potassium tert-butoxide in pyridine solution was unknown, and the formation of multiple species (some of which may be inactive for transmetalation) was possible. A single, pure nickel complex was desired that incorporated both pyridylsulfonamide and basic ligand for activation of arylboron reagent, in accordance with the original design for a Ni(II) transmetalation reagent presented in Scheme 2.4. Ultimately, complex 3 (Scheme 2.7) was prepared for this purpose.

Scheme 2.7. Synthesis of 3, a Reagent for Preparative Transmetalation

Complex 3 was prepared on multigram scale starting from nickel(II) acetate tetrahydrate, potassium tert-butoxide, and the bidentate pyridylsulfonamide ligand in a 1:2:1 molar ratio in pyridine solvent. This is the same mixture that gave rise to transmetalation product when treated with arylboronic acid. In order to isolate the reactive nickel(II) complex, the pyridine solvent was evaporated. A green solid of unknown composition was observed, and was contaminated with
orange solid that formed after most of the pyridine solvent had been evaporated. This orange solid was likely complex 1, the thermodynamic ligation product. After dissolving the green solid thus obtained in anhydrous THF and filtering off the orange solid, a dark green solution was obtained. When 3% water (by volume) was added, green solid 3 precipitated from the THF solution, likely by hydrolysis of a more soluble nickel(II) tert-butoxide intermediate.

The structure of 3 was determined by X-ray crystallography, and exhibits a pseudo-tetrameric structure of C\textsubscript{1} symmetry composed of four [Ni(pyridylsulfonamide)(OH)] units and potassium acetate. In the X-ray structure of 3 displayed in Scheme 2.7, the hydrogen atoms, solvent molecules, pyridylsulfonamide ligand atoms (except N bound to Ni), and potassium counterion are omitted for clarity. The nickel and hydroxide oxygen atoms in 3 are bound in a cubane-like structure. Two of the nickel atoms are bound to one acetate ligand, with Ni-O bonds that are about the same in length (2.05 and 2.07 Angstroms). The \textsuperscript{1}H NMR spectrum of 3 in pyridine-d\textsubscript{5} solvent exhibits a complex (but reproducible and characteristic) set of peaks from 40 ppm to –10 ppm. Complex 3 is stable when stored in a sealed glass vial under air at 23 ºC, and is manipulated under ambient atmosphere without apparent detriment.
Scheme 2.8. Preparation of Ni(II) σ-Aryl Complexes by Transmetalation from Arylboron Reagents

Complex 3 reacts by transmetalation with arylboronic acids and esters in pyridine to afford the desired nickel(II) σ-aryl complexes 2a – 2i after 1 hour at 70 °C (Scheme 2.8). Whereas the previously reported method for synthesis of complexes like 2 by oxidative addition to Ni(0) required two steps to be conducted in a glovebox,\textsuperscript{49} transmetalation with 3 can be conveniently set up at the bench. Complex 3 and arylboron reagent are mixed in a flask under air, which is replaced with N\textsubscript{2} prior to addition of dry pyridine and heating to effect transmetalation. The products 2a –
2i are conveniently purified through a column of SiO$_2$ / K$_2$CO$_3$ (9:1, w/w), without rigorous exclusion of air or water.

Complexes 2a – 2i all contain heteroaryl fragments bound to nickel, except for 2b, 2d and 2i (although the aryl fragments in 2d and 2i are linked to heterocycles). Such focus was given to heteroaryl complexes because the previously reported synthesis of structurally analogous complexes by oxidative addition$^{49}$ had only been applied to make one nickel(II) heteroaryl complex (N-Boc indole bound to Ni at carbon-5, which is not on the same ring as the nitrogen heteroatom). Complexes 2a, 2h and 2i were observed to be particularly labile toward self-condensation in solvents other than pyridine, likely due to the presence of unbound, basic heteroatoms in these complexes. For example, although 2i initially dissolves in CDCl$_3$, a yellow solid precipitates from solution within < 10 minutes, and pyridine is observed in solution. In order to maintain the presence of the pyridine ligand at nickel, complexes with Lewis-basic nitrogen-containing heterocycles were typically isolated by precipitation from pyridine.

2.3 Oxidative Fluorination of New Nickel(II) σ-Aryl Complexes

With the new complexes 2a – 2i in hand, their fluorination reactivity was surveyed. Complexes 2a – 2e afforded the desired $^{[18]}$Faryl fluorides $^{[18]}$F4a – $^{[18]}$F4e after treatment with $^{[18]}$Ffluoride and hypervalent iodine oxidant in MeCN (0.5% aq.) at 23 ºC (Scheme 2.9). However, under the same conditions, complexes 2g – 2i did not afford detectable $^{[18]}$Faryl fluoride product. A proposed explanation for the failed fluorination of 2g – 2i is that the Lewis basic nitrogen heteroatoms in these complexes inhibited C–F bond formation by coordination to putative high-valent Ni intermediates. The formation of a coordinately unsaturated, pentacoordinate metal center...
prior to C–F bond formation in the electrophilic fluorination of similar pyridylsulfonamido palladium(II) σ-aryl complexes has been supported by a mechanistic study. 48

\[ \text{Scheme 2.9. Oxidative Fluorination of Ni(II) σ-Aryl Complexes with } [18^F] \text{Fluoride} \]

The reaction of 2a with $[^{18}F]$fluoride to form $[^{18}F]4a$ represented a key milestone in the desired synthesis of $[^{18}F]5$-fluorouracil from $[^{18}F]$fluoride, because $[^{18}F]4a$ is a protected analog of $[^{18}F]5$-fluorouracil. Additionally, the tertiary amine in 2d was tolerated in the fluorination reaction that generated $[^{18}F]4d$, despite precedent for amine oxidation with iodine(III) oxidants. 80 Rapid oxidation of the Ni(II) center relative to amine oxidation is proposed to explain the tolerance

of the tertiary amine. The synthesis of thiophene derivative $[^{18}\text{F}]\text{4c}$ was unusual, as incorporation of $^{18}\text{F}$ from $[^{18}\text{F}]$fluoride into electron rich O/S heterocycles such as thiophene has little precedent.\textsuperscript{40,81}

### 2.4 Conclusions

The first preparation of nickel(II) σ-aryl complexes from arylboronic acids or esters was developed. Transmetalation is accomplished with a single reagent that contains Ni(II) prefunctionalized with hydroxide (for activation of arylboron reagent) and bidentate ligand (required for subsequent oxidative fluorination). This transmetalation reaction enabled the synthesis of several new nickel(II) σ-aryl and heteroaryl complexes. Because nickel(II) σ-aryl complexes are precursors to $[^{18}\text{F}]$aryl fluorides by oxidative fluorination, more $^{18}\text{F}$-labeled arenes can now be made. The transmetalation reaction was applied to make a nickel(II) pyrimidine complex that reacts oxidatively with $[^{18}\text{F}]$fluoride to afford protected $[^{18}\text{F}]$5-fluorouracil ($[^{18}\text{F}]\text{4a}$).

### 2.5 Experimental Section

#### 2.5.1 Materials and Methods

Thin layer chromatography (TLC) was performed using EMD TLC plates pre-coated with 250 μm thickness silica gel 60 F254 plates and visualized by fluorescence quenching under UV light. Flash chromatography was performed using silica gel (230 – 400 mesh) purchased from Silicycle Inc. NMR spectra were recorded on either a Varian Unity/Inova 600 spectrometer operating at 600 MHz for $^1\text{H}$ acquisitions, a Varian Unity/Inova 500 spectrometer operating at 500 MHz and 125


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MHz for $^1$H and $^{13}$C acquisitions, respectively, or a Varian Mercury 400 spectrometer operating at 400 MHz, 100 MHz, and 375 MHz for $^1$H, $^{13}$C, and $^{19}$F acquisitions, respectively. Chemical shifts for $^1$H and $^{13}$C acquisitions are reported in ppm with the solvent resonance as the internal standard ($^1$H: CDCl$_3$, $\delta$ 7.26; pyridine-d$_5$, $\delta$ 8.74), ($^{13}$C: CDCl$_3$, $\delta$ 77.16; pyridine-d$_5$, $\delta$ 135.91). Chemical shifts for $^{19}$F acquisitions are reported in ppm with PhF as the external standard ($^{19}$F: CDCl$_3$, $\delta$ – 113.15). Data are reported as follows: s = singlet, br = broad, d = doublet, t = triplet, q = quartet, m = multiplet; coupling constants in Hz; integration. All deuterated solvents were purchased from Cambridge Isotope Laboratories. High-resolution mass spectra were obtained using an Agilent ESI-TOF (6210) mass spectrometer or a Bruker q-TOF Maxis Impact mass spectrometer. FTIR spectra were obtained using a Bruker ALPHA Platinum ATR spectrometer. Concentration under reduced pressure was performed by rotary evaporation at 25–30 ºC at appropriate pressure. Purified compounds were further dried under high vacuum (0.2 Torr). Yields refer to purified and spectroscopically pure compounds unless otherwise indicated.

Dry pyridine used in nickel(II) $\sigma$-aryl synthesis was distilled from CaH$_2$ under N$_2$ (1 atm). Anhydrous pyridine (99.8%, <0.003% water, Aldrich Sure/Seal™) used in the synthesis of 1 was purchased from Sigma-Aldrich. Anhydrous THF (≤0.05% water) was purchased from BDH. Anhydrous diethyl ether (≤0.03% water) was purchased from EMD. Dry, degassed dioxane and DMSO were obtained by N$_2$ sparging of anhydrous dioxane and DMSO purchased from Sigma-Aldrich. Potassium acetate was dried in a glass flask under high-vacuum with a flame. All other commercial chemicals were used as received. Nickel(II) acetate tetrahydrate ($\geq$99.0%), benzothiophene-3-boronic acid ($\geq$95%), 5-bromobenzo[c] [1,2,5]thiadiazole (95%), 5-bromo-2-methoxyphenylboronic acid, 3-bromobenzo[b]thiophene (95%), 2.0M isopropylmagnesium bromide solution in THF, and 4-bromo-2-fluoroanisole (4a) (97%) were purchased from Sigma-
Aldrich. 2,4-Di-tert-butoxypyrimidine-5-boronic acid (98%) was purchased from Combi-Blocks. Palladium dichloride-bis(diphenylphosphino) ferrocene-dichloromethane complex, 5-fluorouracil, and 5-bromobenzo[c][1,2,5]thiadiazole (4d) were purchased from Oakwood. 6-Chloropyridine-3-boronic acid (96%) was purchased from Frontier Scientific. 5-Bromo-2-(2-methyl-2H-tetrazol-5-yl)pyridine (98%) was purchased from Astatech. 2-Nitro-N-(2-(pyridin-2-yl)phenyl)benzenesulfonamide was prepared from 2-bromopyridine in two steps.49 The oxidant (1,1’-(phenyl-λ^3^-iodanediyl)bis(4-methoxy)pyridinium)bis(trifluoromethanesulfonate) was prepared from iodobenzenediacetate.49 3-Bromo-5-(pyridin-2-ylethynyl)benzonitrile was a gift from Dr. Nickeisha Stephenson (Vasdev Laboratory, Harvard Medical School and Massachusetts General Hospital). 2,4-Dichloro-5-fluoropyrimidine was prepared from 5-fluorouracil.83

2.5.2 Synthetic Procedures

Synthesis of nickel(II) complex 1

To a 1 dram scintillation vial were added nickel(II) acetate tetrahydrate (35.0 mg, 0.141 mmol, 1.00 equiv.), methanol (2.5 mL), and a Teflon stirbar. The mixture was sonicated to afford a homogeneous light green solution. To this solution was added 2-nitro-N-(2-(pyridin-2-yl)phenyl)benzenesulfonamide.
yl)phenyl)benzenesulfonamide (100 mg, 0.281 mmol, 2.00 equiv.), and the vial was sealed with a Teflon-lined cap. An orange precipitate formed. The mixture was stirred at 23 °C for 3 days and 15 hours, and was then filtered on a glass frit. The collected solid was rinsed with methanol and dried in vacuo to afford 99.4 mg of the title compound (92% yield).

Complex 1 was not characterized by NMR, due to insolubility in all evaluated solvents.

Anal: calcd for C_{34}H_{24}N_{6}NiO_{8}S_{2}: C, 53.21; H, 3.15, N, 10.95; found: C, 53.25; H, 3.19; N, 10.83.

Crystals of 1 for X-ray analysis were obtained by slow formation from a soluble ligated nickel(II) complex, prepared as follows: To a 1-dram scintillation vial were added nickel(II) acetate tetrahydrate (14.0 mg, 56.3 μmol, 1.00 equiv.), a Teflon stirbar, and dry pyridine (0.8 mL) to afford, after mixing, a blue solution that was then treated with potassium tert-butoxide (0.200 mL of a solution prepared from 34.4 mg KOt-Bu and 1.05 mL of dry pyridine, 6.55 mg, 58.4 μmol, 1.04 equiv.). After stirring at 23 °C for 9 minutes, 2-nitro-N-(2-(pyridin-2-yl)phenyl)benzenesulfonamide (20.0 mg, 56.3 μmol, 1.00 equiv., as a solution in 0.60 mL dry pyridine) was added, followed by additional potassium tert-butoxide (0.200 mL of a solution prepared from 34.4 mg KOt-Bu and 1.05 mL of dry pyridine, 6.55 mg, 58.4 μmol, 1.04 equiv.).

The vial, sealed with a Teflon-lined cap, was heated at 90 °C for 5 minutes. After cooling to 23 °C, the solvent was removed in vacuo. The residue was treated with anhydrous THF to give a mixture that was filtered through celite, and the filtrate was concentrated in vacuo. A portion of the resulting solid was dissolved in CD_{2}Cl_{2} (in an NMR tube with plastic slip-on cap) to give a green solution that, over time, deposited orange crystals suitable for X-ray analysis. For crystallography data, see the X-ray Crystallography section.
Formation of nickel(II) complex 1 in MeOH and DMSO

To a 20 mL scintillation vial was added nickel(II) acetate tetrahydrate (31.1 mg, 0.125 mmol, 1.00 equiv.), a Teflon stirbar, and 5.00 mL of solvent (MeOH or DMSO), to afford a homogeneous green solution upon mixing. To the magnetically-stirred solution at 23 ºC was added 2-nitro-N-(2-(pyridin-2-yl)phenyl)benzenesulfonamide 1 (44.4 mg, 0.125 mmol, 1.00 equiv.). An orange precipitate formed (after 10 seconds in MeOH solvent, and after 5 minutes in DMSO solvent).

Synthesis of nickel(II) hydroxide cubane 3

To a 1 L round-bottomed flask were added nickel(II) acetate tetrahydrate (2.80 g, 11.3 mmol, 1.00 equiv.) and a Teflon-coated stirbar. The flask was fitted with a septum, and the headspace was
filled with nitrogen. Anhydrous pyridine (114 mL) was added, and a blue solution was observed after mixing. To this solution was added 2-nitro-N-(2-(pyridin-2-yl)phenyl)benzenesulfonamide\(^1\) (4.00 g, 11.3 mmol, 1.00 equiv., as a solution in 166 mL anhydrous pyridine) by cannula over 3 minutes, and a green-blue solution was observed. To this solution was added potassium tert-butoxide (2.53 g, 22.5 mmol, 2.00 equiv., as a solution in 80 mL anhydrous pyridine) by cannula over 5 minutes. A yellow-green solution with colorless precipitate was observed, which was stirred at 23 °C for 45 minutes, before being concentrated in vacuo (by rotary evaporation at 60 °C until all liquid pyridine was removed, and then under high vacuum at 23 °C) to give a mixture of green and orange solids. These solid residues were triturated with anhydrous THF (130 mL) in order to dissolve the green solid. The mixture was filtered through celite on a glass frit, which was then rinsed with anhydrous THF (2 × 20 mL). The THF filtrates were combined to give a dark green solution that was treated dropwise with \(\text{H}_2\text{O}\) (5.0 mL) over 30 minutes, with magnetic stirring, which caused a light green solid to precipitate. The solid was collected by filtration on a glass frit, rinsed with THF (2 × 20 mL), and dried in vacuo (0.2 Torr, 50 °C, 40 minutes; then 0.2 Torr, 150 °C, 2 hours) to afford 2.66 g of the title compound (as a solvate with 4 water molecules) as a green solid (50% yield).

NMR Spectroscopy: \(^1\)H NMR (600 MHz, pyridine-\(d_5\), 23 °C, \(\delta\)): 46.1, 45.2, 44.8, 44.5, 43.0, 41.8, 40.9, 40.7, 39.6, 39.4, 39.2, 37.4, 36.1, 33.6, 33.1, 32.2, 30.4, 29.4, 24.4, 22.0–19.0, 20.3, 19.9, 19.6, 18.3, 17.9, 17.7, 17.5, 16.7, 16.0, 14.8, 14.7, 14.4, 14.1, 13.8, 13.5, 13.1, 12.6, 11.9, 11.4, 10.9, 10.7, 10.6, 10.2, 9.9, 8.3, 8.1, 7.5, 6.9, 6.6, 6.3, 6.2, 6.1, 6.0, 5.8, 5.4, 1.9, 1.0, 0.5, 0.2, −0.2, −0.4, −0.7, −1.0, −1.9, −2.0, −2.6, −3.6, −4.0, −4.6, −5.0, −5.5, −6.5. Due to gradual decomposition in solution, \(^{13}\)C NMR analysis was not performed. Anal: calcd for \(\text{C}_{70}\text{H}_{55}\text{N}_{12}\text{S}_{4}\text{O}_{22}\text{KNi}_{4}(\text{H}_{2}\text{O})_{4}\): C, 44.47; H, 3.36, N, 8.89; found: C, 44.63; H, 3.07; N, 8.77. IR (neat, \(v, \text{ cm}^{-1}\)): 1594 (w), 1575 (w), 42
1535 (s), 1489 (m), 1476 (w), 1428 (m), 1367 (m), 1275 (m), 1234 (m), 1147 (s), 1129 (s), 1117 (s), 1061 (m), 972 (s), 852 (w), 824 (m), 753 (s), 730 (s), 651 (m), 630 (w), 593 (s), 562 (s), 528 (m), 434 (m).

Crystals for X-ray analysis were obtained as follows: The mixture of green and orange solids obtained after pyridine evaporation (by the above procedure) (20.0 mg) was treated with anhydrous THF (2.0 mL). To this mixture was then added anhydrous diethyl ether (1.8 mL) dropwise. The resulting mixture was filtered through celite into a scintillation vial that was then capped with a septum. A needle was inserted into the septum, so that water from wet air could slowly diffuse into the solution. Green crystals were obtained. For crystallography data, see the X-ray Crystallography section.
Synthesis of nickel(II) σ-aryl complex 2a

To a 250 mL 2-necked round-bottomed flask were added nickel cube 3 (185.4 mg, 98.1 μmol, 0.250 equiv.), 2,4-di-tert-butoxypyrimidine-5-boronic acid (105.1 mg, 0.392 mmol, 1.00 equiv.), and a Teflon-coated stirbar. The flask was fitted in one neck with a Teflon sleeve and glass joint to tube adapter (which was connected to a vacuum manifold), and in the other neck with a septum, and its atmosphere was evacuated and backfilled with nitrogen three times. Dry pyridine (15.5 mL) was added by syringe, and the septum was replaced with a glass stopper. The mixture was heated with stirring at 70 ºC for 1 hour. Once cooled to 23 ºC, hexanes (155 mL) was added with stirring. The mixture was filtered through celite on a glass frit, which was then rinsed with hexanes (20 mL). The hexanes filtrate was discarded, and the collected residue was dissolved in DCM/pyridine 95:5 (v/v), passed through the frit, and concentrated in vacuo to give a residue. The residue was quickly purified by chromatography on silica gel/K₂CO₃ 9:1 (w/w), eluting with DCM/pyridine 95:5 (v/v). The yellow/orange band was collected and concentrated in vacuo to remove DCM, to about 0.5 mL. Hexanes (0.8 mL) was added slowly, dropwise with mixing until turbid, and the sides of the vessel were scratched with a metal spatula to induce crystallization. More hexanes (9.2 mL) was added dropwise with mixing, and the resulting solid was triturated with a metal spatula, centrifuged, and the supernatant was decanted. The remaining solid was triturated with hexanes (10 mL), the mixture was centrifuged, and the supernatant was decanted.
The solid was dried in vacuo (0.2 Torr, 23 °C, 17 hours) to afford 139.4 mg of the title compound (as a solvate with 0.75 pyridine molecules) as a yellow solid (46% yield).

NMR Spectroscopy: $^1$H NMR (600 MHz, pyridine-d$_5$, 23 °C, $\delta$): 8.86 (br s, 1H), 8.64 (br s, 1H), 7.85 (d, $J = 8.0$ Hz, 1H), 7.81 (d, $J = 7.6$ Hz, 1H), 7.66–7.61 (m, 1H), 7.47 (dd, $J = 7.6$, 7.6 Hz, 1H), 7.43 (d, $J = 7.4$ Hz, 1H), 7.37–7.28 (m, 2H), 7.15–7.10 (m, 1H), 6.98–6.87 (br m, 1H), 6.59 (br s, 1H) 1.53 (s, 9H), 1.48 (br s, 9H). $^{13}$C NMR (125 MHz, pyridine-d$_5$, 23 °C, $\delta$): 175.0 (br), 164.1, 160.9 (br), 156.3, 152.4 (br), 148.1, 141.8, 138.4, 137.4, 136.9, 132.0, 131.3, 131.2, 130.9, 129.5, 129.2, 125.1, 123.62, 123.59, 123.0, 79.9 (br), 78.7, 29.1, 29.0. Anal: calcd for C$_{34}$H$_{36}$N$_6$NiO$_6$(C$_5$H$_5$N)$_{0.75}$: C, 58.52; H, 5.17, N, 12.20; found: C, 58.74; H, 5.22; N, 12.05. HRMS (ESI-TOF) (m/z): calcd for C$_{29}$H$_{32}$N$_5$NiO$_6$S [M – pyridine + H]$^+$, 636.1427; found, 636.1438.

Quantification of solvate pyridine stoichiometry was not trivial. In NMR solvents other than pyridine-d$_5$, complex 2a condenses with itself to form unidentified oligomeric species plus unligated pyridine. In pyridine-d$_5$ solvent, the residual pyridine solvent peaks are too intense compared to pyridine-H$_5$ from the analytical sample to permit unambiguous quantification. Therefore the following degradation experiment was performed to completely liberate the ligands bound to nickel, so that the relative amount of pyridine present could be directly observed and quantified. To a 4 mL 1-dram vial was added nickel complex 2a (2.2 mg), a Teflon-coated stirbar, and a large excess of KCN in CD$_3$OD (0.420 mL of a 0.292M solution of KCN in CD$_3$OD). The vial was sealed with a Teflon-lined cap, and heated at 60 °C for 1 hour to afford a light yellow homogeneous solution. Once cooled to 23 °C, the solution was analyzed by $^1$H NMR spectroscopy, and the ratio of pyridine to the potassium salt of the pyridylsulfonamide ligand was determined by integration to be 1.75 : 1.00. Because 2a already contains 1 pyridine covalently bound to nickel,
the balance of 0.75 pyridine molecules is attributed as solvate pyridine molecules contained within the lattice of the solid. This assignment is consistent with the results of CHN elemental analysis (vide supra).

Crystals of 2a for X-ray analysis were obtained as follows: To a 1-dram (4 mL) scintillation vial were added nickel(II) σ-aryl complex 2a (7.8 mg) and pyridine (0.410 mL), to give a yellow solution. This vial was placed in a 20 mL scintillation vial containing hexanes (5 mL), and the larger vial was sealed, so that crystallization by vapor diffusion would occur. After 2 days and 16 hours at 23 ºC, yellow crystals had formed. For crystallography data, see the X-ray Crystallography section.

**Synthesis of nickel(II) σ-aryl complex 2a (by a one-pot procedure from Ni(OAc)₂(H₂O)₄)**

![Chemical reaction](attachment:reaction.png)

Under air to a 50 mL 2 neck round-bottom flask was added nickel(II) acetate tetrahydrate (27.3 mg, 0.110 mmol, 1.00 equiv.) and a Teflon-coated stirbar. One neck was fitted with a Teflon sleeve and glass neck-to-tube (to manifold) adapter, and the other neck was fitted with a rubber septum. The flask’s atmosphere was evacuated and backfilled with nitrogen three times. Then 1.1 mL pyridine (all pyridine used in the reaction setup was distilled from calcium hydride under nitrogen) was added by syringe, to give a blue mixture upon stirring. A solution of 2-(2-pyridinyl)phenyl-2-nitrobenzenesulfonanilide (39.0 mg, 0.110 mmol, 1.00 equiv.) in 0.9 mL pyridine in a glass vial
was added by cannula under nitrogen to the flask, followed by 0.2 mL pyridine used to wash the flask and cannula. The flask’s contents turned to a dark blue-green solution. Then a solution of potassium tert-butoxide (24.7 mg, 0.220 mmol, 2.00 equiv.) in 0.9 mL pyridine in a glass vial (a homogeneous solution with no particulate solid, indicating good quality KOtBu that has not hydrolyzed to KOH) was added by cannula under nitrogen to the flask, followed by 0.2 mL pyridine used to wash the vial and cannula. The flask’s contents turned to a heterogeneous mixture consisting of a solution with a green-yellow color, and a suspended white solid. Then a solution of arylboronic acid (29.4 mg, 0.110 mmol, 1.00 equiv.) in 0.9 mL pyridine in a glass vial was added by cannula under nitrogen to the flask, followed by 0.2 mL pyridine used to wash the vial and cannula. The flask was immersed in a 70 °C oil bath for 1 hour with magnetic stirring under dynamic nitrogen atmosphere from a manifold, then removed and allowed to cool to room temperature. The flask was opened to air and 44 mL pentane was added slowly with magnetic stirring, and a yellow solid precipitated. The mixture was filtered over celite on a medium glass frit by vacuum suction, and the pad was rinsed with 5 mL pentane, and residual pentane removed by brief suction of air through the pad. Then in order to dissolve the collected solid, the celite pad was mixed into a slurry with dichloromethane/pyridine 95:5 (v/v), filtered, and the celite was treated with the same solvent mixture until the eluting filtrate was colorless. Then the collected dichloromethane/pyridine filtrate was concentrated in vacuo to afford a yellow residue. The residue was purified by chromatography on basic alumina (2.2 g, diameter = 1 cm, length = 2.5 cm), eluting with dichloromethane/pyridine 95:5 (v/v). The yellow band was collected and concentrated in vacuo to about 1 mL of an orange solution, and 15 mL pentane was added slowly with mixing to give a yellow solid that was triturated. After centrifugation, the supernatant was decanted and the solid was triturated in 15 mL pentane. After centrifugation, the supernatant was
decanted and residual solvent was removed in vacuo to give 33.9 mg of the title compound as a yellow solid (40% yield).

**Synthesis of nickel(II) σ-aryl complex 2b**

To a 250 mL 2-necked round-bottomed flask were added nickel cube 3 (141.8 mg, 75.0 µmol, 0.250 equiv.), 5-bromo-2-methoxyphenylboronic acid (69.0 mg, 0.300 mmol, 1.00 equiv.), and a Teflon-coated stirbar. The flask was fitted in one neck with a Teflon sleeve and glass joint to tube adapter (which was connected to a vacuum manifold), and in the other neck with a septum, and its atmosphere was evacuated and backfilled with nitrogen three times. Dry pyridine (12 mL) was added by syringe, and the septum was replaced with a glass stopper and Teflon sleeve. The mixture was heated with stirring at 70 °C for 1 hour. Once cooled to 23 °C, hexanes (120 mL) was added with stirring. The mixture was filtered through celite on a glass frit, which was then rinsed with hexanes (20 mL). The hexanes filtrate was discarded, and the collected residue was dissolved in DCM/pyridine 95:5 (v/v), passed through the frit, and concentrated in vacuo to a residue that was purified by chromatography on silica gel/K₂CO₃ 9:1 (w/w) (4.2 g, diameter = 1 cm, length = 12 cm), eluting with DCM/pyridine 95:5 (v/v). The yellow-orange band was collected and concentrated in vacuo to remove DCM, to about 1 mL. Hexanes (10 mL) was added dropwise with mixing. The resulting precipitate was triturated, the mixture was centrifuged, and the supernatant was decanted. The precipitate was dried in vacuo, and then dissolved in DCM (1.8 mL). To the
resulting solution was added pentane (9.0 mL), dropwise. The mixture was triturated, centrifuged, and the supernatant was decanted. The solid was dried in vacuo (0.2 Torr, 23 °C, 8 hours) to afford 119.2 mg of the title compound (as a solvate with 0.58 dichloromethane molecules) as an orange solid (55% yield).

NMR Spectroscopy [mixture of 2 rotamers]: $^1$H NMR (600 MHz, CDCl$_3$, 23 °C, $\delta$): 9.09 (br s, 2H), 8.29 (br s, 1H), 7.68 (br s, 1H), 7.58–7.53 (m, 2H), 7.53–7.46 (m, 2H), 7.37 (ddd, $J = 7.6$, 7.6, 1.2 Hz, 1H), 7.28 (dd, $J = 7.6$, 7.6 Hz, 1H), 7.20–7.04 (m, 5H), 6.99 (br s, 1H), 6.98 (d, $J = 7.6$ Hz, 1H), 6.78 (dd, $J = 8.4$, 2.4 Hz, 1H), 6.61 (br s, 1H), 6.15 (br s, minor rotamer, 1H), 5.94 (br s, major rotamer, 1H), 4.09 (br s, minor rotamer, 3H), 3.42 (br s, major rotamer, 3H). $^{13}$C NMR (125 MHz, CDCl$_3$, 23 °C, $\delta$): 162.3 (br), 156.1 (br), 151.4, 147.0 (br), 140.7 (br), 137.1, 136.7, 136.2 (br), 131.5, 130.3, 130.2, 129.8 (br), 128.8 (br), 127.9 (br), 126.4, 124.2, 124.1, 122.7, 122.3 (br), 121.8 (br), 112.3 (br), 109.1 (br), 55.6. Anal: calcd for C$_{29}$H$_{23}$BrN$_4$NiO$_5$S(CH$_2$Cl)$_{0.58}$: C, 48.84; H, 3.35; N, 7.70; found: C, 48.49; H, 3.25; N, 7.55. HRMS (ESI-TOF) (m/z): calcd for C$_{24}$H$_{19}^{79}$BrN$_3$NiO$_5$S [M – pyridine + H]$^+$, 597.9583; found, 597.9566.

2-(Benzo[b]thiophen-3-yl)-5,5-dimethyl-1,3,2-dioxaborinane (S1)

![Diagram showing the reaction between benzo[b]thiophene-3-boronic acid and 2,2-dimethyl-1,3-propanediol to form S1.](image)

To a 20 mL scintillation vial were added benzo[b]thiophene-3-boronic acid (534 mg, 3.00 mmol, 1.00 equiv.), 2,2-dimethyl-1,3-propanediol (328 mg, 3.15 mmol, 1.05 equiv.), a Teflon-coated stirbar, 10 mL of diethyl ether, and anhydrous magnesium sulfate (1.1 g). The vial was sealed with a Teflon-lined screw cap, shaken to mix, and stirred at 23 °C for 3 hours. Then the mixture was
filtered through filter paper, which was rinsed with ether, and the filtrate was concentrated in vacuo to afford a residue that was purified by chromatography on silica gel (diameter = 3 cm, length = 11 cm), eluting with hexanes/ethyl acetate 9:1 (v/v). A solid was obtained, which was tritutated with 3 mL of hexanes and dried in vacuo to afford 262.5 mg of the title compound as a light-pink solid (36% yield).

NMR Spectroscopy: ¹H NMR (600 MHz, CDCl₃, 23 °C, δ): 8.43 (d, J = 8.1 Hz, 1H), 8.04 (s, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.40 (dd, J = 8.2, 8.1 Hz, 1H), 7.34 (dd, J = 8.1, 8.1 Hz, 1H), 3.84 (s, 4H), 1.07 (s, 6H). ¹³C NMR (100 MHz, CDCl₃, 23 °C, δ): 142.9, 141.1, 137.9, 125.6, 124.2, 124.0, 122.3, 72.4, 32.1, 22.1. Anal: calcd for C₁₃H₁₅BO₂S: C, 63.44; H, 6.14; found: C, 63.11; H, 6.05. HRMS-FIA (ESI-TOF) (m/z): calcd for C₁₃H₁₆BO₂S [M + H]^+, 247.0964; found, 247.0953.

Synthesis of nickel(II) σ-aryl complex 2c

![Synthesis of nickel(II) σ-aryl complex 2c](image)

To a 250 mL 2-necked round-bottomed flask were added nickel cube 3 (141.8 mg, 75.0 μmol, 0.250 equiv.), arylboronic ester S₁ (73.8 mg, 0.300 mmol, 1.00 equiv.), and a Teflon-coated stirbar. The flask was fitted in one neck with a Teflon sleeve and glass joint to tube adapter (which was connected to a vacuum manifold), and in the other neck with a septum, and its atmosphere was evacuated and backfilled with nitrogen three times. Dry pyridine (12 mL) was added by syringe, and the septum was replaced with a glass stopper. The mixture was heated with stirring at 70 °C for 1 hour. Once cooled to 23 °C, hexanes (120 mL) was added with stirring. The mixture was
filtered through celite on a glass frit, which was then rinsed with hexanes (20 mL). The hexanes filtrate was discarded, and the collected residue was dissolved in DCM/pyridine 95:5 (v/v), passed through the frit, and concentrated in vacuo to give a residue. The residue was quickly purified by chromatography on silica gel/K$_2$CO$_3$ 9:1 (w/w) (5.1 g, diameter = 1 cm, length = 14 cm), eluting with DCM/pyridine 95:5 (v/v). The yellow-orange band was collected and concentrated in vacuo to remove DCM, to about 1.5 mL. Hexanes was added dropwise with mixing (2.6 mL), inducing gradual precipitation of yellow solid. A further 5.4 mL of hexanes was added dropwise with mixing. The solid was triturated and centrifuged, the supernatant was decanted, and the residual solid was dried in vacuo. The solid was dissolved in DCM (9.0 mL), and to this solution was added pentane (5 mL) dropwise with mixing, which induced gradual precipitation of a solid. A further 55 mL of pentane was added with mixing. The supernatant was decanted, leaving about 10 mL of mixture, which was triturated with a spatula, sonicated, centrifuged, and the remaining supernatant was decanted. The solid was dried in vacuo (0.2 Torr, 23 ºC, overnight) to afford 110.7 mg of the title compound as a yellow solid (59% yield).

NMR Spectroscopy: $^1$H NMR (500 MHz, CDCl$_3$, 23 ºC, δ): 9.13 (d, J = 5.1 Hz, 2H), 8.64 (br s, 1H), 8.32 (d, J = 5.6 Hz, 1H), 7.66–7.60 (m, 3H), 7.56 (d, J = 7.8 Hz, 1H), 7.51–7.42 (m, 2H), 7.22 (ddd, J = 7.8, 7.6, 1.5 Hz, 1H), 7.18 (d, J = 7.8 Hz, 1H), 7.16–7.04 (m, 7H), 7.01 (ddd, J = 7.8, 7.6, 1.2 Hz, 1H), 6.96 (d, J = 7.6 Hz, 1H), 6.52 (ddd, J = 5.8, 5.8, 1.2 Hz, 1H). $^{13}$C NMR (125 MHz, CDCl$_3$, 23 ºC, δ): 155.8, 152.1, 151.5, 147.1, 145.5, 142.5 (br), 140.6, 139.4, 137.3, 136.9, 136.5, 135.9, 132.0, 130.5, 130.3, 130.1, 128.8, 128.3, 126.3, 124.7, 124.1, 123.2, 122.92, 122.85, 122.42, 122.41, 122.0, 117.6. Anal: calcd for C$_{30}$H$_{22}$N$_4$NiO$_4$S$_2$: C, 57.62; H, 3.55; N, 8.96; found: C, 57.22; H, 3.45; N, 8.83. HRMS-FIA (ESI-TOF) (m/z): calcd for C$_{26}$H$_{18}$N$_3$NiO$_6$S$_2$ [M – pyridine + HCO$_2$\(^-\)]$, 589.9991; found, 589.9987.
Synthesis of nickel(II) σ-aryl complex 2d

To a 500 mL 2-necked round-bottomed flask were added nickel cube 3 (425.3 mg, 0.225 mmol, 0.25 equiv.), 4-(morpholinomethyl)phenylboronic acid (199.0 mg, 0.900 mmol, 1.00 equiv.), and a Teflon-coated stirbar. The flask was fitted in one neck with a Teflon sleeve and glass joint to tube adapter (which was connected to a vacuum manifold), and in the other neck with a septum, and its atmosphere was evacuated and backfilled with nitrogen three times. Dry pyridine (36 mL) was added by syringe, and the septum was replaced with a glass stopper and Teflon sleeve. The mixture was heated with stirring at 70 ºC for 1 hour. Once cooled to 23 ºC, hexanes (360 mL) was added with stirring. The mixture was filtered through celite on a glass frit, which was then rinsed with hexanes (20 mL). The hexanes filtrate was discarded, and the collected residue was dissolved in DCM/pyridine 95:5 (v/v), passed through the frit, and concentrated in vacuo to a residue that was purified by chromatography on silica gel/K$_2$CO$_3$ 9:1 (w/w) (4.2 g, diameter = 1 cm, length = 13 cm), eluting with DCM/pyridine 95:5 (v/v). The yellow band was collected and concentrated in vacuo to remove DCM, to about 2 mL. Hexanes (10 mL) was added dropwise with mixing, and a yellow solid precipitated. The precipitate was triturated, the mixture was centrifuged, and the supernatant was decanted. The solid was triturated in hexanes (10 mL), the mixture was centrifuged, and the supernatant was decanted. The solid was dried in vacuo (0.2 Torr, 23 ºC, 9.5 hours) to afford 34.2 mg of the title compound as a yellow solid (6% yield).
NMR Spectroscopy: $^1$H NMR (600 MHz, CDCl$_3$, 23 ºC, δ): 9.18 (d, J = 5.8 Hz, 2H), 8.21 (d, J = 5.8 Hz, 1H), 7.57–7.51 (m, 3H), 7.46 (d, J = 7.9 Hz, 1H), 7.37–7.32 (m, 3H), 7.32–7.27 (m, 1H), 7.18–7.10 (m, 4H), 7.07 (dd, J = 7.9, 1.0 Hz, 1H), 7.02–6.96 (m, 2H), 6.68 (d, J = 7.9 Hz, 2H), 6.59 (dd, J = 6.0, 5.9 Hz, 1H), 3.64–3.57 (m, 4H), 3.23 (s, 2H), 2.28 (br s, 4H). $^{13}$C NMR (125 MHz, CDCl$_3$, 23 ºC, δ): 156.0, 153.6, 152.6, 151.5, 147.1, 141.3, 137.1, 136.7, 136.6, 135.6, 135.2, 131.6, 131.5, 130.4, 130.1, 129.8, 128.8, 128.3, 127.1, 124.3, 124.1, 122.8, 122.6, 121.7, 67.1, 63.5, 53.7. Anal: calcd for C$_{33}$H$_{31}$N$_5$NiO$_5$S: C, 59.30; H, 4.67, N, 10.48; found: C, 59.18; H, 4.48, N, 10.84. HRMS (ESI-TOF) (m/z): calcd for C$_{29}$H$_{27}$N$_4$NiO$_7$S [M – pyridine + HCO$_2$]$^-$, 633.0954; found, 633.0948.

5-(5,5-Dimethyl-1,3,2-dioxaborinan-2-yl)benzo[c][1,2,5]thiadiazole (S2)

To a 100 mL 2-necked round-bottomed flask were added 5-bromobenzo[c][1,2,5]thiadiazole (430.1 mg, 2.00 mmol, 1.00 equiv.), 5,5,5',5'-tetramethyl-2,2'-bi(1,3,2-dioxaborinane (497 mg, 2.20 mmol, 1.10 equiv.), palladium dichloride-bis(diphenylphosphino)ferrocene-dichloromethane complex (163.3 mg, 0.200 mmol, 0.100 equiv.), potassium acetate (393 mg, 4.00 mmol, 2.00 equiv.), and a Teflon-coated stirbar. The flask was fitted in one neck with a Teflon sleeve and glass joint to tube adapter (which was connected to a vacuum manifold), and in the other neck with a septum, and its atmosphere was evacuated and backfilled with nitrogen three times. Dry, degassed dioxane (10 mL) was added by syringe, and the septum was replaced with a glass stopper. The mixture was heated with stirring at 90 ºC for 6 hours. Once cooled to 23 ºC, the mixture was filtered.
through celite on a glass frit, and the flask and celite were rinsed with dichloromethane. The combined filtrate was concentrated in vacuo to afford a residue, which was dissolved in dichloromethane and passed through sodium sulfate. The filtrate was concentrated in vacuo to afford a residue, which was purified by chromatography on silica gel (45 g, diameter = 3.5 cm, length = 14 cm), eluting with DCM/methanol 99:1 (v/v), to afford 137.6 mg of the title compound as a tan solid (28% yield).

NMR Spectroscopy: $^1$H NMR (500 MHz, CDCl$_3$, 23 ºC, δ): 8.47 (s, 1H), 7.95 (s, 2H), 3.83 (s, 4H), 1.05 (s, 6H). $^{13}$C NMR (125 MHz, CDCl$_3$, 23 ºC, δ): 156.3, 155.1, 133.5, 128.4, 120.4, 72.7, 32.1, 22.0. Anal: calcd for C$_{11}$H$_{13}$BN$_2$O$_2$S: C, 53.25; H, 5.28, N, 11.29; found: C, 53.45; H, 5.29; N, 11.25. HRMS (ESI-TOF) (m/z): calcd for C$_{11}$H$_{14}$BN$_2$O$_2$S [M + H]$^+$, 249.0869; found, 249.0861.

**Synthesis of nickel(II) $\sigma$-aryl complex 2e**

To a 250 mL 2-necked round-bottomed flask were added nickel cube 3 (141.8 mg, 75.0 μmol, 0.250 equiv.), arylboronic ester S2 (74.4 mg, 0.300 mmol, 1.00 equiv.), and a Teflon-coated stirbar. The flask was fitted in one neck with a Teflon sleeve and glass joint to tube adapter (which was connected to a vacuum manifold), and in the other neck with a septum, and its atmosphere was evacuated and backfilled with nitrogen three times. Dry pyridine (12 mL) was added by syringe, and the septum was replaced with a glass stopper. The mixture was heated with stirring at 70 ºC for 1 hour. Once cooled to 23 ºC, hexanes (120 mL) was added with stirring. The mixture was
filtered through celite on a glass frit, which was then rinsed with hexanes (20 mL). The hexanes filtrate was discarded, and the collected residue was dissolved in DCM/pyridine 95:5 (v/v), passed through the frit, and concentrated in vacuo to give a residue. The residue was quickly purified by chromatography on silica gel/K₂CO₃ 9:1 (w/w) (4.7 g, diameter = 1 cm, length = 13 cm), eluting with DCM/pyridine 95:5 (v/v). The yellow-orange band was collected and concentrated in vacuo to remove DCM, to about 0.5 mL. Hexanes was added dropwise with mixing (3 mL), inducing a biphasic mixture to form. The denser phase was ground with a metal spatula until completely converted to a yellow powder. A further 2 mL of hexanes was added. The solid was triturated, centrifuged, and the supernatant was decanted. Hexanes (10 mL) was added, and the mixture was triturated, sonicated for 2 minutes, centrifuged, and the supernatant was decanted. The solid was dried in vacuo (0.2 Torr, 23 ºC, 17 hours) to afford 106.5 mg of the title compound (as solvate with 0.8 pyridine and 0.08 hexane molecules) as a yellow solid (51% yield).

NMR Spectroscopy: ¹H NMR (600 MHz, pyridine-d₅, 23 ºC, δ): 8.54 (s, 1H), 8.46 (d, J = 5.9 Hz, 1H), 8.24 (d, J = 8.8 Hz, 1H), 7.86 (d, J = 7.6 Hz, 1H), 7.84 (dd, J = 7.6, 1.2 Hz, 1H), 7.67 (dd, J = 7.6, 7.6, 1.2 Hz, 1H), 7.51 (dd, J = 7.6, 7.6 Hz, 1H), 7.47 (d, J = 7.6 Hz, 1H), 7.38 (ddd, J = 7.6, 7.6, 1.2 Hz, 1H), 7.30 (dd, J = 8.2, 1.2 Hz, 1H), 7.25 (d, J = 7.6 Hz, 1H), 7.14 (ddd, J = 7.6, 7.6, 1.2 Hz, 1H), 6.93 (dd, J = 7.6, 7.6 Hz, 1H), 6.65 (ddd, J = 5.9, 5.9, 1.2 Hz, 1H). ¹³C NMR (125 MHz, pyridine-d₅, 23 ºC, δ): 164.1, 156.3, 154.6, 153.7, 152.8, 148.1, 142.2, 138.8, 137.7, 137.3, 136.6, 136.4, 132.2, 131.4, 131.3, 131.0, 129.5, 129.1, 126.9, 125.4, 123.1, 117.3. Anal: calcd for C₂₈H₂₀N₆NiO₄S₂(C₅H₅N)₀.₈(C₆H₁₄)₀.₀₈: C, 55.93; H, 3.63, N, 13.66; found: C, 55.98; H, 3.64; N, 13.61. HRMS (ESI-TOF) (m/z): calcd for C₂₈H₂₁N₆NiO₄S₂ [M + H]⁺, 627.0419; found, 627.0410.
Synthesis of nickel(II) σ-aryl complex 2f

To a 250 mL 2-necked round-bottomed flask were added nickel cube 3 (141.8 mg, 75.0 μmol, 0.250 equiv.), furan-3-boronic acid (33.6 mg, 0.300 mmol, 1.00 equiv.), and a Teflon-coated stirbar. The flask was fitted in one neck with a Teflon sleeve and glass joint to tube adapter (which was connected to a vacuum manifold), and in the other neck with a septum, and its atmosphere was evacuated and backfilled with nitrogen three times. Dry pyridine (12 mL) was added by syringe, and the septum was replaced with a glass stopper. The mixture was heated with stirring at 70 °C for 1 hour. Once cooled to 23 °C, hexanes (120 mL) was added with stirring. The mixture was filtered through celite on a glass frit, which was then rinsed with hexanes (20 mL). The hexanes filtrate was discarded, and the collected residue was dissolved in DCM, passed through the frit, and concentrated by rotary evaporation to a residue that was treated with hexanes (15 mL), sonicated. The mixture was then concentrated in vacuo. The resulting residue was quickly purified by chromatography on silica gel/K₂CO₃ 9:1 (w/w) (5.4 g, diameter = 1 cm, length = 13 cm), eluting with DCM/pyridine 95:5 (v/v). The yellow-orange band was collected and concentrated in vacuo to remove DCM, to about 0.5 mL. Hexanes (7 mL) was added dropwise with mixing, which caused a precipitate to form, which was triturated. The supernatant was decanted, the precipitate was dried in vacuo, and then dissolved in DCM (1 mL). To the resulting solution was added hexanes (10 mL) slowly, with mixing, to give a precipitate that was triturated. The mixture was centrifuged, the supernatant was decanted, and the solid was dried in vacuo (0.2 Torr, 23 °C, 15 hours) to afford
49.0 mg of the title compound (as a solvate with 0.32 dichloromethane molecules) as an orange solid (28% yield).

NMR Spectroscopy: \( ^1H \) NMR (600 MHz, CDCl\(_3\), 23 ºC, \( \delta \)): 9.14 (d, \( J = 5.3 \) Hz, 2H), 8.45 (dd, \( J = 5.9, 1.2 \) Hz, 1H), 7.62 (dd, \( J = 7.6, 7.6 \) Hz, 1H), 7.57–7.50 (m, 3H), 7.39–7.33 (m, 2H), 7.25–7.20 (m, 2H), 7.16–7.12 (m, 2H), 7.11–7.09 (m, 1H), 7.06 (d, \( J = 7.6 \) Hz, 1H), 7.03–6.97 (m, 2H), 6.72 (ddd, \( J = 5.9, 5.8, 1.2 \) Hz, 1H), 6.23 (s, 1H), 5.58 (d, \( J = 1.2 \) Hz, 1H). \( ^{13}C \) NMR (100 MHz, CDCl\(_3\), 23 ºC, \( \delta \)): 155.8, 154.1, 152.1, 147.0, 141.3, 140.8, 139.8, 137.5, 137.0, 136.2, 135.8, 131.8, 130.4, 130.3, 130.0, 128.8, 128.2, 124.5, 124.1, 122.8, 122.1, 121.6, 116.1, 113.3. Anal: calcd for C\(_{26}\)H\(_{20}\)N\(_4\)NiO\(_5\)S\((\text{CH}_2\text{Cl}_2)_{0.32}\): C, 53.91; H, 3.55, N, 9.55; found: C, 54.30; H, 3.15; N, 9.54. HRMS (ESI-TOF) (m/z): calcd for C\(_{22}\)H\(_{16}\)N\(_3\)NiO\(_7\)S [M – pyridine + HCO\(_2^-\)], 524.0063; found, 524.0065.

**Synthesis of nickel(II) \( \sigma \)-aryl complex 2g**

To a 250 mL 2-necked round-bottomed flask were added nickel cube 3 (141.8 mg, 75.0 \( \mu \)mol, 0.250 equiv.), 6-chloropyridine-3-boronic acid (47.2 mg, 0.300 mmol, 1.00 equiv.), and a Teflon-coated stirbar. The flask was fitted in one neck with a Teflon sleeve and glass joint to tube adapter (which was connected to a vacuum manifold), and in the other neck with a septum, and its atmosphere was evacuated and backfilled with nitrogen three times. Dry pyridine (12 mL) was added by syringe, and the septum was replaced with a glass stopper. The mixture was heated with stirring at 70 ºC for 1 hour. Once cooled to 23 ºC, hexanes (120 mL) was added with stirring. The
mixture was filtered through celite on a glass frit, which was then rinsed with hexanes (20 mL). The hexanes filtrate was discarded, and the collected residue was dissolved in DCM/pyridine 95:5 (v/v), passed through the frit, and concentrated in vacuo to give a residue. The residue was quickly purified by chromatography on silica gel/K$_2$CO$_3$ 9:1 (w/w) (4.75 g, diameter = 1 cm, length = 13 cm), eluting with DCM/pyridine 95:5 (v/v). The yellow-orange band was collected and concentrated in vacuo to remove DCM, to about 1 mL. Hexanes was added dropwise with mixing until turbid (0.3 mL), inducing precipitation of a yellow solid. A further 4.7 mL of hexanes was added dropwise with mixing. The solid was triturated, centrifuged, and the supernatant was decanted. Hexanes (5 mL) was added, and the mixture was triturated, centrifuged, and the supernatant was decanted. The solid was dried in vacuo (0.2 Torr, 23 ºC, 14 hours) to afford 82.9 mg of the title compound as a yellow solid (46% yield).

NMR Spectroscopy: $^1$H NMR (600 MHz, pyridine-d$_5$, 23 ºC, δ): 8.83 (d, J = 1.8 Hz, 1H), 8.27 (d, J = 5.3 Hz, 1H), 7.89 (dd, J = 8.2, 1.7 Hz, 1H), 7.81 (t, J = 9.1 Hz, 2H), 7.63 (t, J = 7.6 Hz, 1H), 7.49–7.44 (m, 2H), 7.41 (t, J = 7.6 Hz, 1H), 7.28–7.23 (m, 2H), 7.14 (t, J = 7.7 Hz, 1H), 6.97 (d, J = 8.2 Hz, 1H), 6.92 (t, J = 7.7 Hz, 1H), 6.69 (t, J = 6.5 Hz, 1H). $^{13}$C NMR (125 MHz, pyridine-d$_5$, 23 ºC, δ): 156.2, 155.2, 153.1, 149.2, 148.5, 148.0, 146.9, 141.8, 138.9, 137.1, 136.6, 132.3, 131.5, 131.4, 131.0, 129.4, 129.1, 125.4, 123.2, 123.0. Anal: calcd for C$_{27}$H$_{20}$Cl$_5$NiO$_{4}$S: C, 53.63; H, 3.33, N, 11.58; found: C, 53.75; H, 3.00; N, 11.49. HRMS-FIA(ESI-TOF) (m/z): calcd for C$_{22}$H$_{16}$Cl$_4$NiO$_4$S [M – pyridine + H]$^+$, 524.9935; found, 524.9916.
5-(5,5-Dimethyl-1,3,2-dioxaborinan-2-yl)-2-(2-methyl-2H-tetrazol-5-yl)pyridine (S3)

To a 2-necked round-bottomed flask were 5-bromo-2-(2-methyl-2H-tetrazol-5-yl)pyridine (717 mg, 3.00 mmol, 1.00 equiv.), 5,5,5',5'-tetramethyl-2,2'-bi(1,3,2-dioxaborinane) (1.017 g, 4.50 mmol, 1.50 equiv.), palladium dichloride-bis(diphenylphosphino)ferrocene-dichloromethane complex (243 mg, 0.298 mmol, 0.099 equiv.), potassium acetate (883 mg, 9.00 mmol, 3.00 equiv.), and a Teflon-coated stirbar. The flask was fitted in one neck with a Teflon sleeve and glass joint to tube adapter (which was connected to a vacuum manifold), and in the other neck with a septum, and its atmosphere was evacuated and backfilled with nitrogen three times. Dry, degassed DMSO (15 mL) was added by syringe, and the septum was replaced with a glass stopper. The mixture was heated with stirring at 80 ºC for 8 hours. Once cooled to 23 ºC, 15 mL of EtOAc and 15 mL of water were added, and the mixture was transferred to a separatory funnel and the phases were separated. The aqueous layer was extracted with EtOAc (2 × 10 mL), the combined organic layers were dried (Na₂SO₄), filtered, and concentrated in vacuo. The residue thus afforded was purified by chromatography on sodium sulfate (mesh size >50) (60 g, diameter = 4.5 cm, length = 13 cm), eluting with DCM/methanol 96:4 (v/v) to afford a solid that was triturated and sonicated in hexanes (4 mL), and filtered. The solid was triturated twice more with hexanes (2 × 4 mL), then washed with pentane (2 mL), and dried in vacuo to afford 508.2 mg of the title compound as a tan solid (62% yield).
NMR Spectroscopy: $^1$H NMR (500 MHz, CDCl$_3$, 23 ºC, δ): 9.10 (s, 1H), 8.20 (dd, J = 7.7, 1.3 Hz, 1H), 8.17 (d, J = 7.7 Hz, 1H), 4.43 (s, 3H), 3.78 (s, 4H), 1.03 (s, 6H). $^{13}$C NMR (125 MHz, CDCl$_3$, 23 ºC, δ): 165.3, 155.5, 148.1, 142.7, 128.6 (br), 121.5, 72.5, 39.8, 32.1, 22.0. HRMS-FIA(ESI-TOF) (m/z): calcd for C$_{12}$H$_{17}$BN$_5$O$_2$ [M + H]$^+$, 274.1476; found, 274.1472.

**Synthesis of nickel(II) σ-aryl complex 2h**

To a 250 mL 2-necked round-bottomed flask were added nickel cube 3 (181.8 mg, 96.2 µmol, 0.250 equiv. (1.00 equiv. of Ni)), arylboronic ester S3 (109.2 mg, 0.400 mmol, 1.04 equiv.), and a Teflon-coated stirbar. The flask was fitted in one neck with a Teflon sleeve and glass joint to tube adapter (which was connected to a vacuum manifold), and in the other neck with a septum, and its atmosphere was evacuated and backfilled with nitrogen three times. Dry pyridine (16 mL) was added by syringe, and the septum was replaced with a glass stopper. The mixture was heated with stirring at 70 ºC for 1 hour. Once cooled to 23 ºC, hexanes (160 mL) was added with stirring. The mixture was filtered through celite on a glass frit, which was then rinsed with hexanes (20 mL). The hexane filtrate was discarded, and the collected residue was dissolved in DCM/pyridine 95:5 (v/v), passed through the frit, and concentrated in vacuo to give a residue. The residue was quickly purified by chromatography on silica gel/K$_2$CO$_3$ 9:1 (w/w) (4.5 g), which was layered on top of basic alumina (2.1 g) within the same column (diameter = 1 cm), eluting with DCM/pyridine 95:5.
The yellow-orange band was collected and concentrated in vacuo to remove DCM, to about 3 mL. Hexanes was added dropwise with mixing (17 mL), the mixture was thoroughly ground with a metal spatula, and the supernatant was decanted. This process was repeated with 13 mL of hexanes. A further 10 mL of hexanes was added, and the solid was triturated, sonicated, centrifuged, and the supernatant was decanted. The solid was dried in vacuo (0.2 Torr, 23 ºC, 16 hours) to afford 110.4 mg of the title compound (as a solvate with 0.18 hexane molecules) as a yellow solid (43% yield).

NMR Spectroscopy: $^1$H NMR (500 MHz, pyridine-d$_5$, 23 ºC, δ): 9.35 (s, 1H), 8.36 (d, J = 5.9 Hz, 1H), 8.12 (d, J = 7.8 Hz, 1H), 7.89 (d, J = 7.8 Hz, 1H), 7.84 (d, J = 7.8 Hz, 1H), 7.81 (d, J = 7.8 Hz, 1H), 7.64 (dd, J = 7.9, 7.5 Hz, 1H), 7.50–7.43 (m, 2H), 7.40 (dd, J = 7.8, 7.7 Hz, 1H), 7.29 (d, J = 7.9 Hz, 1H), 7.25 (d, J = 8.2 Hz, 1H), 7.14 (dd, J = 7.8, 7.6 Hz, 1H), 6.93 (dd, J = 7.8, 7.6 Hz, 1H), 6.70–6.64 (m, 1H), 4.21 (s, 3H). $^{13}$C NMR (125 MHz, pyridine-d$_5$, 23 ºC, δ): 166.7, 156.4, 156.3, 155.4, 153.1, 148.1, 145.0, 143.3, 141.9, 138.8, 137.2, 136.6, 132.3, 131.44, 131.35, 131.0, 129.4, 129.1, 125.4, 123.2, 121.3, 39.7. Anal: calcd for C$_{29}$H$_{22}$N$_9$NiO$_4$S(C$_6$H$_{14}$)$_{0.18}$: C, 54.10; H, 3.85, N, 18.88; found: C, 54.26; H, 3.26; N, 18.34. HRMS-FIA(ESI-TOF) (m/z): calcd for C$_{24}$H$_{19}$N$_8$NiO$_4$S [M – pyridine + H]$^+$, 573.0604; found, 573.0587.
3-(5,5-Dimethyl-1,3,2-dioxaborinan-2-yl)-5-(pyridin-2-yethyl)benzonitrile (S4)

To a 50 mL 2-necked round-bottomed flask were added 3-bromo-5-(pyridin-2-yethyl)benzonitrile (400 mg, 1.41 mmol, 1.00 equiv.), 5,5,5',5'-tetramethyl-2,2'-bi(1,3,2-dioxaborinane) (479 mg, 2.12 mmol, 1.50 equiv.), palladium dichloride-bis(diphenylphosphino)ferrocene-dichloromethane complex (115 mg, 0.141 mmol, 0.100 equiv.), potassium acetate (416 mg, 4.24 mmol, 3.00 equiv.), and a Teflon-coated stirbar. The flask was fitted in one neck with a Teflon sleeve and glass joint to tube adapter (which was connected to a vacuum manifold), and in the other neck with a glass stopper, and its atmosphere was evacuated and backfilled with nitrogen three times. Dry, degassed DMSO (7.1 mL) was added. The mixture was heated with stirring at 80 °C for 6 hours. Once cooled to 23 °C, 7 mL of EtOAc and 7 mL of water were added, and the mixture was transferred to a separatory funnel and the phases were separated. The aqueous layer was extracted with 7 mL EtOAc, the combined organic layers were washed with 7 mL water, dried over anhydrous sodium sulfate, filtered, and concentrated in vacuo. The residue thus afforded was purified by chromatography on silica gel (diameter = 3 cm, length = 11 cm), eluting with DCM/methanol 97:3 (v/v). The residue thus obtained was dissolved in DCM (1 mL), and hexanes (14 mL) was added. The mixture was evaporated to about 5 mL, hexanes (5 mL) was added with mixing, and the supernatant was decanted. The remaining solid was dissolved in ether, and this solution was passed through sodium sulfate and concentrated in vacuo to afford 183.4 mg of the title compound as a beige solid (41% yield).
NMR Spectroscopy: $^{1}$H NMR (600 MHz, CDCl$_3$, 23 ºC, δ): 8.62 (d, J = 4.7 Hz, 1H), 8.21 (s, 1H), 8.03 (s, 1H), 7.88 (s, 1H), 7.69 (ddd, J = 7.6, 7.6, 1.8 Hz, 1H), 7.51 (d, J = 7.6 Hz, 1H), 7.26 (dd, J = 7.6, 4.7 Hz, 1H), 3.77 (s, 4H), 1.01 (s, 6H). $^{13}$C NMR (100 MHz, CDCl$_3$, 23 ºC, δ): 150.3, 142.9, 141.6, 137.5, 136.7, 136.4, 134.5 (br), 127.4, 123.3, 123.1, 118.4, 112.4, 90.3, 87.0, 72.5, 32.0, 21.9. HRMS-FIA(ESI-TOF) (m/z): calcd for C$_{19}$H$_{18}$BN$_2$O$_2$ [M + H]$^+$, 317.1462; found, 317.1469.

**Synthesis of nickel(II) σ-aryl complex 2i**

To a 250 mL 2-necked round-bottomed flask were added nickel cube 3 (118.2 mg, 62.5 µmol, 0.250 equiv.), arylboronic ester S4 (79.0 mg, 0.250 mmol, 1.00 equiv.), and a Teflon-coated stirbar. The flask was fitted in one neck with a Teflon sleeve and glass joint to tube adapter (which was connected to a vacuum manifold), and in the other neck with a septum, and its atmosphere was evacuated and backfilled with nitrogen three times. Dry pyridine (10 mL) was added by syringe, and the septum was replaced with a glass stopper. The mixture was heated with stirring at 70 ºC for 1 hour. Once cooled to 23 ºC, hexanes (100 mL) was added with stirring. The mixture was filtered through celite on a glass frit, which was then rinsed with hexanes (20 mL). The hexanes filtrate was discarded, and the collected residue was dissolved in DCM/pyridine 95:5 (v/v), passed through the frit, and concentrated in vacuo to give a residue. The residue was quickly purified by chromatography on silica gel/K$_2$CO$_3$ 9:1 (w/w) (4.9 g, diameter = 1 cm, length = 13 cm), eluting
with DCM/pyridine 95:5 (v/v). The yellow-orange band was collected and concentrated in vacuo to remove DCM, to about 0.5 mL. Hexanes (5 mL) was added with mixing, and a biphasic mixture formed, which was thoroughly ground with a metal spatula until the denser phase was converted to a yellow solid. The mixture was sonicated for 5 minutes, centrifuged, and the supernatant was decanted. The solid was triturated in hexanes (10 mL), the mixture was sonicated for 3 minutes, centrifuged, and the supernatant was decanted. The solid was dried in vacuo (0.2 Torr, 23 °C, 15 hours) to afford 95.4 mg of the title compound (as a solvate with 0.18 hexane molecules) as a yellow solid (54% yield).

NMR Spectroscopy: $^1$H NMR (500 MHz, pyridine-d$_5$, 23 °C, δ): $^{13}$C NMR (125 MHz, pyridine-d$_5$, 23 °C, δ): 160.4, 156.2, 152.7, 151.1, 148.1, 144.0, 143.2, 141.9, 139.4, 138.9, 137.1, 136.9, 136.5, 132.3, 131.5, 131.4, 131.0, 130.9, 129.5, 129.1, 128.1, 125.5, 124.3, 123.3, 120.6, 119.8, 110.2, 90.9, 88.7. Anal: calcd for C$_{36}$H$_{24}$N$_6$NiO$_4$S(C$_6$H$_{14}$)$_{0.18}$: C, 62.65; H, 3.76, N, 11.82; found: C, 62.26; H, 3.47; N, 11.59. HRMS (ESI-TOF) (m/z): calcd for C$_{31}$H$_{20}$N$_5$NiO$_4$S [M − pyridine + H]$^+$, 616.0590; found, 616.0581.

**2,4-Di-tert-butoxy-5-fluoropyrimidine (4a)**

![Reaction Scheme](image)

To a 50 mL round-bottomed flask were added 2,4-dichloro-5-fluoropyrimidine (334 mg, 2.00 mmol, 1.00 equiv.) and a Teflon-coated stirbar. The flask was fitted with a septum and connection to a vacuum manifold via a needle, and its atmosphere was evacuated and backfilled with nitrogen three times. Dry THF (10 mL) was added, resulting in a colorless solution. A solution of potassium
tert-butoxide (561 mg, 5.00 mmol, 2.50 equiv.) in 10 mL of THF was added dropwise over 8 minutes. The reaction mixture was stirred at 23 °C for 2 hours, and then 10 mL of EtOAc and 10 mL of water were added. The phases were separated, the aqueous layer was extracted with 10 mL of EtOAc, and the organic layers were combined and dried over MgSO₄, filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel (diameter = 2.5 cm, length = 24 cm), eluting with hexanes/EtOAc 96:4 (v/v) to afford 289 mg of the title compound as a colorless oil (60% yield).

NMR Spectroscopy: ¹H NMR (400 MHz, CDCl₃, 23 °C, δ): 7.98 (d, J = 2.6 Hz, 1H), 1.64 (s, 9H), 1.57 (s, 9H). ¹³C NMR (125 MHz, CDCl₃, 23 °C, δ): 159.1 (d, J = 3.7 Hz), 158.9 (d, J = 10.3 Hz), 143.6 (d, J = 252.5 Hz), 143.0 (d, J = 21.2 Hz), 83.3, 80.5, 28.51, 28.45. ¹⁹F NMR (376 MHz, CDCl₃, 23 °C, δ): −163.6. HRMS (ESI-TOF) (m/z): calcd for C₁₂H₂₀F₂N₂O₂ [M + H]⁺, 243.1509; found, 243.1497.

3-Fluorobenzo[b]thiophene (4c)⁸⁴

To a 100 mL 2-necked round-bottomed flask were added lithium chloride (890. mg, 21.0 mmol, 2.33 equiv.) and a Teflon-coated stirbar. The flask was fitted in one neck with a Teflon sleeve and glass joint to tube adapter (which was connected to a vacuum manifold), and in the other neck with a septum, and flame-dried under high vacuum to dry the LiCl. After cooling to room temperature and backfilling the atmosphere with N₂, isopropyl magnesium chloride (6.0 mL, 2.0M solution in THF, 12 mmol, 1.3 equiv.) was added, and the mixture was stirred at 23 °C for 1 hour, and then

cooled to –16 ºC (bath temperature) in a NaCl/ice bath. To the stirring mixture was added 3-fluorobenzo[b]thiophene (1.18 mL, 9.02 mmol, 1.00 equiv.) over 3 minutes. The mixture was then stirred in the -16 ºC bath for 10 minutes, then warmed to 0 ºC (ice/water bath) and stirred for 75 minutes, then the ice bath was removed, and the mixture was stirred for 45 minutes, and then cooled to 0 ºC. To the stirring mixture was added N-fluorobenzenesulfonimide (3.88 g, 12.3 mmol, 1.36 equiv., as a solution in 10 mL THF) over 7 minutes. After an additional 3 minutes at 0 ºC, the ice bath was removed, and the mixture was stirred for an additional 30 minutes. Water (20 mL) was then added, followed by 10 mL pentane, and after shaking, the phases were separated. The aqueous layer was extracted with pentane (3 × 15 mL), and the combined organic layers were dried (MgSO₄), filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel (diameter = 5.5 cm, length = 22 cm), eluting with pentane, to afford 336 mg of the title compound as a mixture with unreacted starting material (5.3:1 molar ratio). To a 100 mL round-bottomed flask was added 336 mg of this mixture, together with DMF (11.1 mL), palladium on activated carbon (235. mg, 10 wt% Pd, 23.5 mg Pd), and a Teflon stirbar. The flask was fitted with a septum and hydrogen balloon, and the headspace was purged with hydrogen. The mixture was stirred at 23 ºC for 65 minutes, and then water (20 mL) was added. The mixture was extracted with pentane (4 × 10 mL), and the combined organic layers were dried (MgSO₄), filtered, and concentrated in vacuo. The residue was purified by chromatography on silica gel (diameter = 5.5 cm, length = 18 cm), eluting with pentane, to afford 196 mg of the title compound as a colorless oil (14% yield).

NMR Spectroscopy: ¹H NMR (500 MHz, CDCl₃, 23 ºC, δ): 7.83–7.76 (m, 2H), 7.46–7.38 (m, 2H), 6.87 (d, J = 2.2 Hz, 1H). ¹³C NMR (125 MHz, CDCl₃, 23 ºC, δ): 152.1 (d, J = 262.5 Hz), 137.0 (d, J = 8.1 Hz), 129.2 (d, J = 24.6 Hz), 125.6, 124.6, 123.3, 120.2 (d, J = 2.5 Hz), 103.5 (d, J = 20.0
Hz). $^{19}$F NMR (376 MHz, CDCl$_3$, 23 ºC, δ): −135.7. These spectroscopic data correspond to previously reported data.$^{84}$

4-(4-Fluorobenzyl)morpholine (4d)$^{85}$

To a 25 mL round-bottomed flask were added potassium carbonate (415 mg, 3.00 mmol, 1.00 equiv.), a Teflon-coated stirbar, xylenes (4.5 mL), morpholine (0.263 mL, 3.00 mmol, 1.00 equiv.), and 4-fluorobenzyl chloride (0.360 mL, 3.01 mmol, 1.00 equiv.). The flask was fitted with reflux condenser, which was fitted with a glass joint to tube adapter (that connected to a vacuum manifold). The flask headspace was purged with nitrogen, and the reaction mixture was heated with magnetic stirring at 150 ºC for 2.5 hours. After cooling to 23 ºC, the reaction mixture was purified by chromatography on silica gel (diameter = 2.5 cm, length = 16 cm), eluting with DCM/methanol/triethylamine 97:3:0.2 (v/v/v) to afford 259 mg of the title compound as a yellow oil (44% yield).

NMR Spectroscopy: $^1$H NMR (500 MHz, CDCl$_3$, 23 ºC, δ): 7.31–7.26 (m, 2H), 7.03–6.97 (m, 2H), 3.73–3.68 (m, 4H), 3.46 (s, 2H), 2.42 (br s, 4H). $^{13}$C NMR (125 MHz, CDCl$_3$, 23 ºC, δ): 162.1 (d, J = 245.0 Hz), 133.6 (d, J = 3.1 Hz), 130.7 (d, J = 8.1 Hz), 115.1 (d, J = 21.2 Hz), 67.1, 62.7, 53.7. $^{19}$F NMR (376 MHz, CDCl$_3$, 23 ºC, δ): −115.8. (HRMS (ESI-TOF) (m/z): calcd for C$_{11}$H$_{15}$FNO

[M + H]+, 196.1138; found, 196.1132. The spectroscopic data correspond to previously reported data.\textsuperscript{85}
2.5.3 Radiochemistry

General methods

No-carrier-added $[^{18}\text{F}]$fluoride was produced from water 97% enriched in $^{18}\text{O}$ (ISOFLEX, USA) by the nuclear reaction $^{18}\text{O}(p,n)^{18}\text{F}$ using a Siemens Eclipse HP cyclotron and a silver-bodied target at Massachusetts General Hospital Athinoula A. Martinos Center for Biomedical Imaging. The produced $[^{18}\text{F}]$fluoride in water was transferred from the cyclotron target by helium push. Radioactivity was measured in a Capintec, Inc. CRC-25PET ion chamber.

Solvents and reagents for radiochemical experiments: Acetonitrile ($>99.9\%$, extra dry, $<0.005\%$ water) was purchased from Acros in bottles with a needle-penetrable barrier (AcroSeal®). Water was obtained from a Millipore Milli-Q Integral Water Purification System. 18-crown-6 (99%) was purchased from Alfa Aesar. Potassium phosphate tribasic (Reagent grade, $\geq 98\%$) was purchased from Sigma Aldrich.

Radiosynthesis of $^{18}\text{F}$-labeled molecules

In a nitrogen-filled glovebox, to an oven-dried 1-dram (4 mL) glass vial was added a nickel(II) $\sigma$-aryl complex 2 and hypervalent iodine oxidant (1,1′-(phenyl-$\lambda^3$-iodanediyl)bis(4-methoxypyridinium)bis(trifluoromethanesulfonate))$^{49}$ in a 1:1 mass ratio, and the two solids were mixed gently with a metal spatula to give a light yellow or light orange (depending on the color of the starting nickel complex) homogeneous admixture. To an oven-dried 1-dram glass vial was
added 2.0 mg of this admixture, and the vial was sealed with a screw cap with a Teflon-lined septum insert under nitrogen, and removed from the glovebox.

An $[^{18}\text{F}]$fluoride solution with 18-crown-6 and potassium phosphate tribasic was prepared as follows. To an oven-dried 1-dram (4 mL) glass vial was added dry 18-crown-6 (20.0 – 44.0 mg) under nitrogen, and this vial was sealed with a Teflon-lined cap. The vial was opened under air, dry MeCN (1.0 mL per 10.0 mg of 18-crown-6) was added quickly, and the vial was sealed and mixed until all 18-crown-6 had dissolved. The vial was opened, aqueous potassium phosphate (0.561M $\text{K}_3\text{PO}_4$ in water, 2.0 $\mu$L per 10.0 mg of 18-crown-6) was added quickly, and the vial was sealed, shaken, and then vortexed for 10 seconds. The vial was opened, aqueous $[^{18}\text{F}]$fluoride from the cyclotron (3.0 $\mu$L per 10.0 mg of 18-crown-6) was added quickly, and the vial was sealed, shaken, and then vortexed for 10 seconds.

The resulting solution (0.50 mL) was quickly added, with a 1-mL plastic syringe with 18-G disposable metal needle, to the vial containing nickel(II) $\sigma$-aryl complex and oxidant through the septum. After 1 minute at 23 °C, the radiochemical yield was then measured (see Measurement of Radiochemical yield, below), and HPLC analysis was also performed (see Characterization of $^{18}\text{F}$ Labeled Molecules, below).

**Measurement of radiochemical conversion by radio TLC**

Radiochemical conversion (RCC) was determined by multiplying the relative peak integrations of a radio TLC scan (radioTLC yield) and the fraction of radioactivity in solution (vide infra):

$$\text{RCC} = (\text{radioTLC yield}) \times (\text{fraction of radioactivity in solution})$$

The radioactivity of the reaction vial was measured. An aliquot of the solution was then taken with a capillary and spotted on a TLC plate. The remaining reaction solution was transferred to another
The reaction vial was rinsed with MeCN (0.5 – 1 mL) in order to remove residual reaction solution, and the radioactivity of the empty reaction vial was again measured to determine the radioactivity of $^{18}$F that was left on the walls of the reaction vial, and therefore was not in solution. The fraction of $^{18}$F not in solution was determined by dividing the radioactivity of the empty reaction vial by the initial radioactivity of the reaction vial + reaction solution (the second radioactivity measurement was decay corrected to the timepoint of the first, because there was a small delay time between measurements). This number was converted to % of $^{18}$F in solution by subtracting the number from 1, and multiplying the result by 100% to convert to percentage units.

The TLC plate was eluted with an appropriate solvent mixture, and then the TLC plate was scanned with a Bioscan AR-2000 RadioTLC Imaging Scanner. The Radiochemical TLC (RTLC) yield was calculated by dividing the area of the product peak by the total area of all peaks, and multiplying by 100% to convert to percentage units.

The radiochemical yield (RCC) was determined by multiplying the RTLC yield by the fraction of radioactivity in solution (typically 0.75–0.95).

**Table 2.5.1. Radiochemical Conversion Data**

<table>
<thead>
<tr>
<th>Entry</th>
<th>Molecule</th>
<th>RTLC yield (%)</th>
<th>$^{18}$F in solution (%)</th>
<th>RCC (%)</th>
<th>Average RCC (%)</th>
</tr>
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<tbody>
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<td>1</td>
<td></td>
<td>17</td>
<td>75</td>
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<td></td>
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<td></td>
<td>20</td>
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<td>$[^{18}$F$]_{4a}$</td>
<td>22</td>
<td>78</td>
<td>17</td>
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<td>4</td>
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Table 2.5.1 (Continued).

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<th>[\textsuperscript{18}F]4c</th>
<th>[\textsuperscript{18}F]4d</th>
<th>[\textsuperscript{18}F]4e</th>
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<tbody>
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<td>20</td>
<td>22</td>
</tr>
</tbody>
</table>
Example radioTLC scans

(Note: The startpoint and endpoint of the TLC elution path is approximately indicated by the vertical red lines).

![Radio TLC Scan Image](image)

**Figure 2.5.1.** Example radioTLC scan of crude reaction containing $[^{18}F]4a$ (Entry 1 of Table 2.5.1). TLC eluent: 9:1 hexanes/EtOAc (v/v). Percent of total integration is listed for $[^{18}F]4a$. 
Figure 2.5.2. Example radioTLC scan of crude reaction containing $[^{18}\text{F}]4\text{b}$ (Entry 7 of Table 2.5.1). TLC eluent: 9:1 hexanes/EtOAc (v/v). Percent of total integration is listed for $[^{18}\text{F}]4\text{b}$. 
**Figure 2.5.3.** Example radioTLC scan of crude reaction containing $[^{18}\text{F}]4\text{c}$ (Entry 13 of Table 2.5.1). TLC eluent: hexanes. Percent of total integration is listed for $[^{18}\text{F}]4\text{c}$. 
Figure 2.5.4. Example radioTLC scan of crude reaction containing $[^{18}\text{F}]4\text{d}$ (Entry 19 of Table 2.5.1). TLC eluent: 92:8:0.2 DCM/MeOH/Et$_3$N (v/v/v). Percent of total integration is listed for $[^{18}\text{F}]4\text{d}$. 
Figure 2.5.5. Example radioTLC scan of crude reaction containing $[^{18}\text{F}]4\text{e}$ (Entry 24 of Table 2.5.1). TLC eluent: 9:1 hexanes/EtOAc (v/v). Percent of total integration is listed for $[^{18}\text{F}]4\text{e}$. 

$[^{18}\text{F}]4\text{e}$

Entry 24: 22%
HPLC Characterization of $^{18}\text{F}$-labeled molecules

All $^{18}\text{F}$-labeled molecules were characterized by comparison of HPLC retention times to those of the corresponding authentic $^{19}\text{F}$-containing reference samples by coinjection analysis.

An Eclipse XDB-C18 HPLC column (5 µm, 4.6 x 150 mm) was used, with an Agilent / HP Series 1100 HPLC instrument for analytical HPLC analysis.

Note: Radioactivity chromatographs are delayed by +0.125 min (for 2 mL/minute flow rates) in comparison to the UV chromatographs, since the radioactivity detector is positioned after the UV diode array detector in the flow path of the analytical HPLC instrument.
Figure 2.5.6. Characterization of $[^{18}\text{F}]\text{4a}$. HPLC Method: 50% MeCN, 50% H$_2$O (10 mM ammonium formate), flow rate = 2 mL/min. Note: The radioactivity signal is delayed by +0.125 minutes relative to the UV signal.
Figure 2.5.7. Characterization of $[^{18}\text{F}]4\text{b}$. HPLC Method: 45% MeCN, 55% H$_2$O (10 mM ammonium formate), flow rate = 2 mL/min. Note: The radioactivity signal is delayed by +0.125 minutes relative to the UV signal.
Figure 2.5.8. Characterization of $[^{18}F]4c$. HPLC Method: 50% MeCN, 50% H$_2$O (10 mM ammonium formate), flow rate = 2 mL/min. Note: The radioactivity signal is delayed by +0.125 minutes relative to the UV signal.
Figure 2.5.9. Characterization of $[^{18}\text{F}]\text{4d}$. HPLC Method: Gradient: Started with 5% A (A = 99.9:0.1 MeCN/TFA, v/v) and 95% B (B = 99.9:0.1 water/TFA, v/v) at time = 0; from 0 – 10 minutes, increase linearly to 20% A and 80% B. Flow rate = 2 mL/min. Note: The radioactivity signal is delayed by +0.125 minutes relative to the UV signal.
Figure 2.5.10. Characterization of $[^{18}\text{F}]4\text{e}$. HPLC Method: 35% MeCN, 65% H$_2$O (10 mM ammonium formate), flow = 2 mL/min. Note: The radioactivity signal is delayed by +0.125 minutes relative to the UV signal.
2.5.4. X-ray Crystallographic Analysis

Experimental

A crystal was mounted on a diffractometer, and data was collected at 100 K. The intensities of the reflections were collected by means of a Bruker APEX II DUO CCD diffractometer (MoK$_\alpha$ radiation, $\lambda=0.71073$ Å), and equipped with an Oxford Cryosystems nitrogen flow apparatus. The collection method involved 0.5° scans in $\omega$ at 28° in 2$\theta$. Data integration down to 0.84 Å resolution was carried out using SAINT V7.46 A (Bruker diffractometer, 2009) with reflection spot size optimization. Absorption corrections were made with the program SADABS (Bruker diffractometer, 2009). The structure was solved by the direct methods procedure and refined by least-squares methods again $F^2$ using SHELXS-97 and SHELXL-97 (Sheldrick, 2008) with OLEX2 interface (Dolomanov, et al., 2009). Non-hydrogen atoms were refined anisotropically, and hydrogen atoms were allowed to ride on the respective atoms. Crystal data as well as details of data collection and refinement are summarized in Tables S3-S5.
Figure 2.5.11. The structure of 1. The atoms are depicted with 50% probability ellipsoids. Solvent and hydrogen atoms present in the crystal of 1 are omitted for clarity.

Table 2.5.2. Experimental details

<table>
<thead>
<tr>
<th></th>
<th>1</th>
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</thead>
<tbody>
<tr>
<td><strong>Crystal data</strong></td>
<td></td>
</tr>
<tr>
<td>Chemical formula</td>
<td>C\textsubscript{35}H\textsubscript{26}Cl\textsubscript{2}N\textsubscript{8}NiO\textsubscript{8}S\textsubscript{2}</td>
</tr>
<tr>
<td>(M_r)</td>
<td>880.37</td>
</tr>
<tr>
<td>Crystal system, space group</td>
<td>Triclinic, (P\bar{1})</td>
</tr>
<tr>
<td>Temperature (K)</td>
<td>100</td>
</tr>
<tr>
<td>(a, b, c) (Å)</td>
<td>9.134 (2), 9.293 (2), 12.052 (3)</td>
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<tr>
<td>(\alpha, \beta, \gamma) (°)</td>
<td>103.787 (5), 98.329 (5), 112.385 (5)</td>
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<td>(V) (Å\textsuperscript{3})</td>
<td>886.5 (4)</td>
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<td>(Z)</td>
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<tr>
<td>Radiation type</td>
<td>Mo K(\alpha)</td>
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<tr>
<td>(\mu) (mm\textsuperscript{-1})</td>
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### Table 2.5.2 (Continued).

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<th>Crystal size (mm)</th>
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<td><strong>Data collection</strong></td>
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</tr>
<tr>
<td>Diffractometer</td>
<td>CCD area detector</td>
</tr>
<tr>
<td>Absorption correction</td>
<td>Multi-scan SADABS</td>
</tr>
<tr>
<td></td>
<td>(Sheldrick, 2009)</td>
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<tr>
<td>T_{\text{min}}, T_{\text{max}}</td>
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<tr>
<td>No. of measured,</td>
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<tr>
<td>independent and observed I &gt; 2σ(I) reflections</td>
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</tr>
<tr>
<td>R_{\text{int}}</td>
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<tr>
<td>(sin θ/λ)_{\text{max}} (Å^{-1})</td>
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<tr>
<td><strong>Refinement</strong></td>
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<tr>
<td>R[F^2 &gt; 2σ(F^2)], wR(F^2), S</td>
<td>0.045, 0.139, 1.08</td>
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<td>No. of reflections</td>
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<tr>
<td>No. of parameters</td>
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<tr>
<td>No. of restraints</td>
<td>1</td>
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<tr>
<td>H-atom treatment</td>
<td>H-atom parameters constrained</td>
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<tr>
<td>Δρ_{\text{max}}, Δρ_{\text{min}} (e Å^{-3})</td>
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Computer programs: APEX2 v2009.3.0 (Bruker-AXS, 2009), SAINT 7.46A (Bruker-AXS, 2009), SHELXS97 (Sheldrick, 2008), SHELXL97 (Sheldrick, 2008), Bruker SHELXTL.
Figure 2.5.12. The structure of 2a. The atoms are depicted with 50% probability ellipsoids.

Solvent and hydrogen atoms present in the crystal of 2a are omitted for clarity.

Table 2.5.3. Experimental details

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<tr>
<th>Crystal data</th>
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<td><strong>Chemical formula</strong></td>
<td>C\textsubscript{39}H\textsubscript{41}N\textsubscript{7}NiO\textsubscript{6}S</td>
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<td><strong>M\textsubscript{r}</strong></td>
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<td><strong>Crystal system, space group</strong></td>
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<tr>
<td><strong>Temperature (K)</strong></td>
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<td><strong>a, b, c (Å)</strong></td>
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<td><strong>β (°)</strong></td>
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<td><strong>V (Å\textsuperscript{3})</strong></td>
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### Table 2.5.3 (Continued).

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**Data collection**

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<th>Diffractometer</th>
<th>Bruker D8 goniometer with CCD area detector diffractometer</th>
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<tr>
<td>Absorption correction</td>
<td>Multi-scan SADABS</td>
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<td>R\text{int}</td>
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<td>(sin θ/λ)\text{max} (Å⁻¹)</td>
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</table>

**Refinement**

| R[F² > 2σ(F²)], wR(F²), S       | 0.055, 0.119, 1.03                                         |
| No. of reflections              | 8271                                                       |
| No. of parameters               | 507                                                        |
| No. of restraints               | 73                                                         |
| H-atom treatment                | H-atom parameters constrained                              |
| Δρ\text{max}, Δρ\text{min} (e Å⁻³) | 1.00, -0.68                                                |

Computer programs: APEX2 v2014.3.0 (Bruker-AXS, 2014), SAINT 8.34C (Bruker-AXS, 2014), SHELXT-2014 (Sheldrick, 2015), SHELXL2014 (Sheldrick, 2015), Bruker SHELXTL (Sheldrick, 2015).
Figure 2.5.13. The structure of 3. The atoms are depicted with 50% probability ellipsoids. The solvent, potassium, hydrogen, and pyridylsulfonamide ligand atoms (except nitrogens bound to nickel) present in the crystal are omitted for clarity.

Table 2.5.4. Experimental details

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<td>Mr</td>
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<td>Temperature (K)</td>
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**Data collection**

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<table>
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<tr>
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<tr>
<td>Diffractometer</td>
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</tr>
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<td>Absorption correction</td>
<td>Multi-scan TWINABS</td>
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**Refinement**

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<td>1.24, -1.23</td>
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Computer programs: APEX2 v2014.3.0 (Bruker-AXS, 2014), SAINT 8.30C (Bruker-AXS, 2014), SHELXS97 (Sheldrick, 2008), SHELXL97 (Sheldrick, 2008), Bruker SHELXTL (Sheldrick, 2008).
2.6 Catalog of Spectra

$^1$H NMR of 2a, pyridine-$d_5$, 600 MHz, 23 °C
$^{13}$C NMR of 2a, pyridine-$d_5$, 125 MHz, 23 °C
$^1$H NMR of 3, pyridine-$d_5$, 600 MHz, 23 ºC
IR spectrum of 3, neat, 23 °C
$^1$H NMR of 2b, CDCl$_3$, 600 MHz, 23 °C

95
$^{13}$C NMR of 2b, CDCl$_3$, 125 MHz, 23 °C
$^1$H NMR of S1, CDCl$_3$, 600 MHz, 23 °C
$^{13}$C NMR of S1, CDCl$_3$, 100 MHz, 23 °C
$^1$H NMR of 2c, pyridine-$d_5$, 500 MHz, 23 ºC
$^{13}$C NMR of 2c, pyridine-$d_5$, 125 MHz, 23 °C
$^1$H NMR of 2d, CDCl$_3$, 600 MHz, 23 °C
$^{13}$C NMR of 2d, CDCl$_3$, 125 MHz, 23 °C
$^1$H NMR of S2, CDCl$_3$, 500 MHz, 23 °C
$^{13}$C NMR of S2, CDCl$_3$, 125 MHz, 23 °C
$^1$H NMR of 2e, pyridine-$d_5$, 600 MHz, 23 °C
$^{13}$C NMR of 2e, pyridine-$d_5$, 125 MHz, 23 °C
$^1$H NMR of 2f, CDCl$_3$, 600 MHz, 23 ºC
$^{13}$C NMR of 2f, CDCl$_3$, 100 MHz, 23 °C
$^1$H NMR of 2g, pyridine-$d_5$, 600 MHz, 23 °C
$^{13}$C NMR of 2g, pyridine-$d_5$, 125 MHz, 23 °C
$^1$H NMR of S3, CDCl₃, 500 MHz, 23 °C
$^{13}$C NMR of S3, CDCl$_3$, 125 MHz, 23 °C
$^{1}$H NMR of 2h, pyridine-$d_5$, 500 MHz, 23 °C
$^{13}$C NMR of 2h, pyridine-$d_5$, 125 MHz, 23 ºC
$^1$H NMR of $^{13}C_4$, CDCl$_3$, 600 MHz, 23 °C
$^{13}$C NMR of $\text{S}_4$, CDCl$_3$, 100 MHz, 23 °C
\textsuperscript{1}H NMR of 2i, pyridine-\textit{d}_5, 500 MHz, 23 \textdegree C
$^{13}$C NMR of 2i, pyridine-$d_5$, 125 MHz, 23 °C
$^1$H NMR of 4a, CDCl$_3$, 400 MHz, 23 °C
$^{13}$C NMR of $4a$, CDCl$_3$, 125 MHz, 23 °C
$^{19}$F NMR of 4a, CDCl$_3$, 125 MHz, 23 ºC
Chapter 3: Automated Synthesis of $[^{18}\text{F}]5$-Fluorouracil from $[^{18}\text{F}]$Fluoride

3.1 Introduction

$[^{18}\text{F}]5$-Fluorouracil ($[^{18}\text{F}]5$-FU) is a PET tracer for imaging of cancer in humans, with potential applications in personalized chemotherapy and the development of new cancer treatments (Chapter 1.1). All reported preparations of $[^{18}\text{F}]5$-FU for human use have employed $[^{18}\text{F}]F_2$ gas, even though $[^{18}\text{F}]$fluoride is the preferred $^{18}\text{F}$ starting material because it is easier to produce and handle. Chapter 2 of this dissertation described the development of a synthesis of $[^{18}\text{F}]5$-fluorouracil from $[^{18}\text{F}]$fluoride by oxidative fluorination of nickel(II) pyrimidine complex 2a (Scheme 3.1, top). Although this result was a promising milestone, production of $[^{18}\text{F}]5$-FU for human use was not yet possible. Several challenges remained for translation of the oxidative fluorination reaction for the production of human doses of $[^{18}\text{F}]5$-FU from $[^{18}\text{F}]$fluoride.

Scheme 3.1. Limited Scale of the Preliminary $[^{18}\text{F}]5$-Fluorouracil Synthesis
One challenge for translation relates to the scale of the reaction. A practical limit for the amount of $^{18}$F-fluoride starting material is 1.7 Ci, the production of which requires $^{18}$O-water to be bombarded with protons for 1 hour under typical cyclotron conditions. Linear production of more $^{18}$F-fluoride would require exponentially more bombardment time, and 1 hour of bombardment is a reasonable limit based on logistical constraints in a radiopharmaceutical production facility. The $^{18}$F-fluoride is typically produced in 2.4 mL of $^{18}$O-water, but only 2.5 μL of water is used in the oxidative fluorination reaction for $^{18}$F-4a synthesis. If the $^{18}$F-fluoride solution is not concentrated, then only 2.5 μL of the starting 2.4 mL solution could be used without reduction in RCC. Given a maximum of 1.7 Ci of $^{18}$F-fluoride in 2.4 mL of $^{18}$O-water, the maximum yield of $^{18}$F-5-FU would be 0.27 mCi (with oxidative fluorination conditions established for $^{18}$F-4a synthesis, and assuming quantitative yields for $^{18}$F-4a deprotection and purification) (Scheme 3.1, bottom). However, for PET imaging in humans, a typical dose of $^{18}$F-5-FU is 5 – 10 mCi. Therefore, production of at least 10 mCi of $^{18}$F-5-FU was desired. Concentration of the initial 2.4 mL $^{18}$F-fluoride solution was necessary to obtain sufficiently large amounts of $^{18}$F-fluoride starting material in 2.5 μL of water, given the constraint of producing at least 10 mCi of $^{18}$F-5-FU, with at most 15% radiochemical conversion (RCC). Extensive efforts to increase RCC of $^{18}$F-fluoride to $^{18}$F-4a above 15% were not successful.

Furthermore, remote-controlled automation of all operations with $^{18}$F was required because of radiation safety protocols. The production of human dose-grade $^{18}$F-5-FU would additionally require that all reagents, synthetic procedures, data collection and documentation were managed in compliance with current good manufacturing practice (cGMP) as required by the United States Food and Drug Administration (FDA). Purification and quality control to comply with United States Pharmacopeia (USP) regulations were also required. For example, the final $^{18}$F-5-FU
product must be formulated as a sterile solution, and have less than 5 ppm of nickel content. In addition to the above requirements, the automated synthesis of $[^{18}\text{F}]5$-FU must be highly reproducible. The synthetic process must be simple to set-up and execute, not only for the sake of facile automation, but also because of the potentially limited chemistry expertise of qualified technicians who execute the cGMP synthesis.

3.2 First Generation Automated Synthesis of $[^{18}\text{F}]5$-Fluorouracil

Automated synthesis was attempted with procedures that had previously been developed for the synthesis of $[^{18}\text{F}]\text{MDL100907}$ from a nickel(II) σ-aryl complex. A commercially available Siemens GN automated synthesizer was used to remove water from $[^{18}\text{F}]\text{fluoride}$, with fluoride concentration procedures that were optimized in order to provide a dry, buffered $[^{18}\text{F}]\text{fluoride}$ solution suitable for oxidative fluorination (Scheme 3.2, top). Oxidative fluorination was conducted by treating a mixture of nickel(II) pyrimidine complex 2a and hypervalent iodine oxidant with the dry $[^{18}\text{F}]\text{fluoride}$ solution, to produce $[^{18}\text{F}]4a$. After purification and quantitative deprotection of $[^{18}\text{F}]4a$ with HCl/EtOH over 2 minutes at 23 ºC, $[^{18}\text{F}]5$-FU was obtained (Scheme 3.2, bottom). However, the yield of 2.4 mCi (0.2% RCY, not decay corrected) was prohibitively low for human dose production. The low yield was due to low RCC in oxidative fluorination for the formation of $[^{18}\text{F}]4a$ (< 5%). In contrast, a previously reported nickel(II) σ-aryl complex used in the synthesis of $[^{18}\text{F}]\text{MDL100907}$ underwent oxidative fluorination with 35% RCC under the same conditions. The low RCC for formation of $[^{18}\text{F}]4a$ was due to the presence of tetrabutylammonium bicarbonate (TBAB) and PPTS buffer from the $[^{18}\text{F}]\text{fluoride}$ drying procedure. Without PPTS to buffer the TBAB, no $[^{18}\text{F}]4a$ was detected.

86 The first generation automated synthesis of $[^{18}\text{F}]5$-FU was developed in collaboration with Dr. Hong Ren.
Scheme 3.2. First Generation Automated Synthesis of $[^{18}\text{F}]5$-Fluorouracil

The yield of $[^{18}\text{F}]5$-FU was also impaired by low efficiency of $[^{18}\text{F}]$fluoride drying. Only 26% of the starting $[^{18}\text{F}]$fluoride remained after azeotropic drying procedures. Losses during drying were due to two factors: passage of time and imperfect mass transfer. Because $^{18}\text{F}$ decays with a half life of 110 minutes, and 45 minutes were required for the drying procedures, the maximum yield of dry $[^{18}\text{F}]$fluoride (with perfect mass transfer efficiency) starting from 0.98 Ci would be 0.74 Ci. Losses from incomplete mass transfer that further reduced the yield to 0.25 Ci are incurred during $[^{18}\text{F}]$fluoride trapping on an anion exchange cartridge, elution from the cartridge with TBAB, and incomplete resolubilization with PPTS/MeCN after azeotropic drying. An alternative approach to $[^{18}\text{F}]$fluoride concentration was desired that would not waste 74% of the starting material, and that would not require as much base to elute $[^{18}\text{F}]$fluoride from the anion exchange cartridge, because TBAB was observed to poison the oxidative fluorination reaction.
3.3 Second Generation Automated Synthesis of $[^{18}\text{F}]5$-Fluorouracil

A new process for automated $[^{18}\text{F}]$fluoride concentration was developed that affords 81% yield of $[^{18}\text{F}]$fluoride (Scheme 3.3). This $[^{18}\text{F}]$fluoride concentration process was accomplished with a new instrument$^\text{87}$ (Figure 3.1) that leverages a miniaturized anion-exchange cartridge and microfluidic lines to elute $[^{18}\text{F}]$fluoride with a total of only 4 $\mu$L of water and 1.5 $\mu$mol of K$_3$PO$_4$.

Scheme 3.3. A New Process for $[^{18}\text{F}]$Fluoride Concentration

Figure 3.1. Schematic of Instrument for Automated $[^{18}\text{F}]$Fluoride Concentration, and Reaction Setup for Oxidative Fluorination

$^\text{87}$Mark Lazari and Prof. Michael van Dam (Crump Institute and David Geffen School of Medicine, UCLA) are acknowledged for making the instrument.
The 4 µL of water used to elute $^{18}$F-fluoride from the anion-exchange cartridge is diluted with 1 mL of dry MeCN, affording a 0.4% aq. MeCN solution that is suitable for oxidative fluorination. Thus azeotropic drying was not necessary and time was saved. $^{18}$F-Fluoride mass transport was improved, relative to the mass transfer in the azeotropic drying procedure used in the first generation synthesis, because no $^{18}$F-fluoride resolubilization was necessary.

Scheme 3.4. Synthesis of Human Doses of $^{18}$F-5-Fluorouracil from $^{18}$F-Fluoride

This $^{18}$F-fluoride concentration process was integrated into a fully automated synthesis of $^{18}$F-5-FU (Scheme 3.4). $^{18}$F-Fluoride (range: 1.4 – 1.8 Ci) was produced in a cyclotron, transferred to the instrument, and processed as shown in Scheme 3.3. The resulting 0.4% aq. solution of $^{18}$F-fluoride in 1 mL MeCN was then rapidly added to a vial containing 2a and hypervalent iodine oxidant, and oxidative fluorination occurred in < 1 minute at 23 ºC to form $^{18}$F-4a. Purification of $^{18}$F-4a by reverse-phase semipreparative HPLC and solid phase extraction (SPE) techniques was facilitated by the hydrophobic tert-butyl protecting groups in $^{18}$F-4a. In all previous syntheses of $^{18}$F-5-FU for human use, a challenging separation of $^{18}$F-5-fluorouracil from uracil and multiple polar side products was required. After purification, deprotection of $^{18}$F-4a and formulation, $^{18}$F-5-FU was isolated (range: 13.5 – 18.8 mCi; 0.92% ± 0.18% yield, average of 3 runs), with a total synthesis time of about 1.5 hours (Scheme 3.4). The yield was
consistently greater than the target of 10 mCi for a human dose. High specific activity (> 10 Ci / \( \mu \)mol) was observed for the isolated \(^{18}\text{F}\)5-FU at the end of synthesis (EOS).

The above isolated yields refer to \(^{18}\text{F}\)5-FU that is formulated as a sterile solution in saline/ethanol, packaged in a vial in a cleanroom (Figure 3.2). The process was conducted in compliance with cGMP, and the product was suitable for injection to humans, based on standard quality control assays for radiopharmaceuticals.

![\(^{18}\text{F}\)5-Fluorouracil for Human Injection](image)

- > 10 mCi prepared
- SA > 10 Ci / \( \mu \)mol
- > 99% radiochemical purity
- < 0.1 ppm Ni
- < 10 \( \mu \)g non-USP impurities
- Sterile
- pH = 7.5
- 23 mL (9:1 saline/EtOH, v/v)

**Figure 3.2.** Product of cGMP \(^{18}\text{F}\)5-Fluorouracil Synthesis

The isolated yield of \(^{18}\text{F}\)5-FU, while low, is sufficient for human dose production. The drawback of the low yield is that a large amount of \(^{18}\text{F}\)fluoride must be produced for the synthesis. Under typical operating conditions, about 1 hour of cyclotron bombardment is required to produce approximately 1.7 Ci of \(^{18}\text{F}\)fluoride, from which production of >10 mCi of \(^{18}\text{F}\)5-FU for human injection can consistently be achieved (with the observed yield of 0.92% ± 0.18%). Improvement of yield would allow for the use of less \(^{18}\text{F}\)fluoride, and therefore shorter cyclotron bombardment time, which is desired for logistical reasons in the radiopharmacy. Although the new \(^{18}\text{F}\)fluoride concentration platform helped to improve yield (only about 20% of \(^{18}\text{F}\)fluoride is lost during the concentration process), the RCC for oxidative fluorination was low (2.89% ± 0.54%, average of 3
runs). Addition of PPTS buffer to the $[^{18}\text{F]}\text{fluoride} / \text{K}_3\text{PO}_4$ solution in MeCN prior to oxidative fluorination did not substantially improve RCC in the automated reaction (2.96% RCC was observed). The decay-corrected, average isolated yield of $[^{18}\text{F}]5$-FU is about 1.7%, which is similar in magnitude to the RCC. Therefore there is much potential for increasing the yield, by improving the RCC.

### 3.4 Conclusions

The first synthesis of $[^{18}\text{F}]5$-fluorouracil from $[^{18}\text{F]}\text{fluoride}$ that affords product suitable for in vivo use in humans was developed. A new instrument was applied for highly efficient $[^{18}\text{F}]\text{fluoride}$ concentration to enable the automated synthesis of human doses of $[^{18}\text{F}]5$-fluorouracil. This synthesis represents the first translation of transition metal-mediated fluorination to enable PET imaging in humans. In future work, this validated synthesis can routinely be applied to produce doses of $[^{18}\text{F}]5$-fluorouracil for clinical research imaging in oncology. Additionally, the production of other $^{18}\text{F}$-containing PET tracers may be possible by the combined application of the new automated synthesis platform (Figure 3.1) and the method for nickel(II) $\sigma$-aryl synthesis presented in Chapter 2.
3.5 Experimental Section

3.5.1. First Generation (not cGMP) Automated Synthesis and Isolation of $[^{18}\text{F}]4\text{a}$ and $[^{18}\text{F}]5$-Fluorouracil

Automated synthesis was accomplished using a Siemens Explora GN module in a lead-lined hot cell. The automated synthesis method was adapted from the previously reported automated synthesis of $[^{18}\text{F}]\text{MDL100907}$.\textsuperscript{52}

Preparation of Chemicals (Important: Unless otherwise specified, all chemicals should be prepared on the synthesis day within two hours).

- Prepare and condition Chromafix PS-HCO$_3$ IEX cartridge using aqueous 5 mg/mL TBAB as follows: Take out 30 mg of filling powder from Chromafix PS-HCO$_3$ IEX cartridge; condition the cartridge with 2 mL of 5 mg/mL aqueous TBAB solution followed by rinsing the cartridge with 10 mL of SWFI.

- Prepare a PTC solution utilizing the following masses and volumes: Transfer prepackaged 26 mg of TBAB (purchased from ABX) in 1.2 mL of water (in two plastic vials) to a 10 mL sterile vial; Add another 2.8 mL of SWFI and 6 mL of anhydrous MeCN to the solution.

- Prepare the PPTS anhydrous MeCN solution as follows: add 4.0 $\mu$L of a 0.25 M PPTS anhydrous MeCN stock solution to a conical GC vial; add 0.6 mL of anhydrous MeCN to the conical GC vial. The GC vial was then capped and sealed with parafilm. (0.25 M PPTS stock solution was prepared as follows: dissolve 63 mg of PPTS solid in 1.0 mL of anhydrous MeCN.)

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\textsuperscript{88} The first generation automated synthesis of $[^{18}\text{F}]5\text{-FU}$ was executed in collaboration with Dr. Hong Ren.
Explora GN Modules Cleaning and Preparation (IMPORTANT: Precursor and transfer lines need to be cleaned within two hours of synthesis with freshly opened anhydrous MeCN).

- Clean Explora modules and F18 line with SWFI (sterile water for injection) and acetonitrile.

- Clean precursor line 2 as follows: Load FRESH anhydrous MeCN to reagent 2 position; Use a new conical GC vial and clean the line with 10 mL of MeOH; Clean the line with 10 mL of acetone; Clean the line with 15 mL of anhydrous MeCN.

- Clean transfer line 2 as follows: Load FRESH anhydrous MeCN to reagent 2 position; Use a NEW test tube; Clean the line with anhydrous acetonitrile.

- Fill the Explora GN reagent vials with the following solvents: Reagent 1: SWFI; Reagent 2: MeCN; Reagent 3: Empty; Reagent 4: PTC; Reagent 5: Empty; Reagent 6: Empty.

- Prime the Explora GN with the “PTC” at position 4.

- Attach the IEX cartridge to F-18 line 2 on the Explora GN. Replace the old reaction vessel with a clean one.

Addition lines Cleaning and Preparation (IMPORTANT: Addition lines need to be cleaned within two hours of synthesis with freshly opened anhydrous MeCN).

- Clean main addition loop line as follows: Clean the line with 10 mL of anhydrous MeCN and then dry the line by passing high-flow N₂ through for 20 minutes to make sure the line is dry and there is NO residue MeCN left in the line. Purification Modules Cleaning (HPLC and columns may be cleaned one day prior to the synthesis.)
• Clean HPLC semiprep column and analytical column with a mixture of MeCN and H₂O (90:10 v/v).

• Rinse the semiprep column with 60% MeCN, 40% (10 mM ammonium formate in H₂O).

Main Addition Line Setup

Connect a 1 mL syringe to the main addition loop line, seal the 1 mL syringe with a new rubber bulb and place a G18 pink vent needle on the rubber bulb in accordance with Figure 3.5.1. The rubber bulb is then connected to “GN 2B line” from Explora GN in the hot cell. The main addition line is clamped on a stand as shown in Figure 3.5.1.

![Main Addition line](image)

**Figure 3.5.1.** Main addition line

NOTE: ALL needles are Gauge 18 pink needles.
Three-Way Addition Line Setup

Attach a three-way stopcock (Figure 3.5.2) with two female luers and 1 male luer to an HPLC Loop-in port, a line leading to a syringe with 3.0 mL of H₂O, and a line leading to a needle that will later be inserted to the reaction vial (containing Ni and oxidant).

![Three-Way Addition Line](image)

**Figure 3.5.2. Three-Way Addition Line**

Reaction Vial Setup

In a glovebox under an atmosphere of dry nitrogen, to an oven-dried 4 mL 1-dram glass vial, add 2.0 mg of 2 and 2.0 mg of 1,1'-(phenyl-λ³-iodanediy1)bis(4-methoxypyridinium)bis(trifluoromethanesulfonate)⁴⁹. Seal the vial with a screw cap containing an inserted Teflon-lined septum. Remove the vial from the glovebox.

Immediately prior to execution of the synthesis, perforate the reaction vial with needles leading to the Main Addition Loop Line, Three-Way Addition Line 1, and vacuum line (Figure 3.5.3). At this time, also attach precursor line 2 to the PPTS anhydrous MeCN solution.
Automated Synthesis Execution

The synthesis began when 0.98 Ci of $^{18}$F fluoride was loaded directly from the cyclotron to the IEX cartridge in the automated module. 0.6 Ci was eluted from the cartridge after 4 minutes. The automated azeotropic drying was then performed, and then 0.25 Ci of $^{18}$F fluoride as a solution in dry MeCN with TBAB and PPTS was passed through Transfer Line 2 into the Main Addition Loop Line. After this transfer was complete, vacuum was applied via the vacuum line leading to the reaction vial, which caused rapid (<1 second) addition of the $^{18}$F fluoride solution to the reaction vial (this rapid addition of the entire solution at once is essential) (addition occurred at 45 minutes after start of synthesis). Water (3.0 mL) was then added via Three-way Addition Line 1, resulting in a yellow mixture, which was then drawn to the HPLC Loop-in via the Three-way Addition Line 1, by slowly pulling on a syringe connected to the other side of HPLC Loop-in, so that the reaction mixture was loaded into HPLC Loop-in with minimal bubble formation.
Semiprep HPLC purification was conducted with a Phenomenex Luna C18(2) column (5 μm, 10.00 x 250 mm), eluting with 60:40 MeCN/(10 mM ammonium formate in water) v/v, flow rate = 5 mL/minute (Figure 3.5.4).

Compound $[^{18}F]4a$ was collected from the semiprep HPLC purification (at 23.6 minutes after injection, 0.6 minute collection window, Figure 3.5.4) in 2.9 mL of HPLC mobile phase. In order to remove MeCN and ammonium formate, the collected product was diluted with H$_2$O (20.0 mL) and passed through a Waters C18 Sep-Pak Plus (which had previously been preconditioned by washing first with ethanol and then water). The product was eluted from the Sep-Pak with ethanol (2.0 mL), to afford a solution containing 2.37 mCi of $[^{18}F]4a$ (at 95 minutes after the start of the synthesis).

![Figure 3.5.4.](image)

Figure 3.5.4. Semipreparative HPLC purification of $[^{18}F]4a$. HPLC method: 60:40 MeCN/(10 mM ammonium formate in water) v/v, flow rate = 5 mL/minute.
Deprotection was carried out by mixing the ethanol solution of $[^{18}F]4a$ with conc. HCl (0.23 mL, 37% by weight in water, 2.8 mmol HCl). The resulting solution was allowed to stand at 23 °C for 2 minutes, and was then neutralized with NaHCO$_3$ (2.9 mL, 1.0 M solution, 2.9 mmol NaHCO$_3$), and diluted with water (14.9 mL) to afford a solution of $[^{18}F]5$-fluorouracil (quantitative conversion).
Figure 3.5.5. HPLC characterization of isolated $[^{18}\text{F}]4\text{a}$. Method: 60:40 MeCN/(10 mM ammonium formate in water) v/v, flow rate = 2 mL/minute. Column: Eclipse XDB-C18 (5 μm, 4.6 x 150 mm).
Figure 3.5.6. TLC characterization of isolated $[^{18}\text{F}]4\text{a}$. Top: Eluent: 95:5 Hexanes/EtOAc (v/v). $[^{18}\text{F}]4\text{a}$: $R_f = 0.47$. $4\text{a}$ (Authentic Reference): $R_f = 0.48$. Radiochemical purity $> 98\%$. Bottom: Eluent: 96:4 Hexanes/THF (v/v). $[^{18}\text{F}]4\text{a}$: $R_f = 0.58$. $4\text{a}$ (Authentic Reference): $R_f = 0.59$. Radiochemical purity $> 98\%$. 
Figure 3.5.7. HPLC characterization of isolated $[^{18}\text{F}]$5-fluorouracil. HPLC Method: 65 mM ammonium formate in water, flow = 1 mL/min. Column: Luna Pentafluorophenyl (PFP) (2) (5 μm, 4.6 mm x 150 mm). Note: The radioactivity signal is delayed by +0.250 minutes relative to the UV signal.
Figure 3.5.8. TLC characterization of isolated $[^{18}\text{F}]}\text{5-FU}$. Top: RadioTLC of isolated $[^{18}\text{F}]}\text{5-FU}$. Eluent: 85:15:2 MeCN/H$_2$O/NH$_3$ (25% aq.) (v/v/v). $[^{18}\text{F}]}\text{5-FU}$: $R_f = 0.47$. 5-FU (Authentic Reference): $R_f = 0.46$. Radiochemical purity > 99%. Bottom: RadioTLC of isolated $[^{18}\text{F}]}\text{5-FU}$. Eluent: 75:25:2 DCM/MeOH/NH$_3$ (25% aq.) (v/v/v). $[^{18}\text{F}]}\text{5-FU}$: $R_f = 0.24$. 5-FU (Authentic Reference): $R_f = 0.23$. Radiochemical purity > 99%. 

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Determination of Specific Activity of $[^{18}\text{F}]4a$ from First Generation Synthesis

Specific activity of $[^{18}\text{F}]4a$ was determined by dividing the radioactivity of a sample of $[^{18}\text{F}]4a$ by the amount of ($[^{18}\text{F}]4a + 4a$) in the sample. The number of moles of ($[^{18}\text{F}]4a + 4a$) in an isolated sample was determined by measuring the UV signal at 272 nm, and converting the UV signal intensity to number of moles using a standard curve.

For 105 μCi of $[^{18}\text{F}]4a$ a UV absorbance (at 272 nm) of 8.2 was measured, corresponding to 0.335 nmol, for a specific activity of 0.31 Ci/μmol (12 GBq/μmol) at time of injection (TOI) to the analytical HPLC instrument.

The calibration curve was generated by integration of the UV absorbance signal intensity (at 272 nm) of 5 different known amounts of 4a, in triplicate for each amount (see Table 3.5.1 and Figure 3.5.9).
Table 3.5.1. Data for the standard curve of UV absorbance vs. amount of 4a

<table>
<thead>
<tr>
<th>Amount of 4a (nmol)</th>
<th>UV absorbance (272 nm)</th>
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<tbody>
<tr>
<td>5</td>
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</table>
Figure 3.5.9. Calibration curve for UV absorbance vs. amount of 4a
3.5.2. Second Generation (Automated, cGMP) Synthesis of $^{18}$F-5-Fluorouracil

General Information

The automated, large scale synthesis of $^{18}$F-5-FU was accomplished using a new concentrator instrument that is diagrammed in Figure 3.5.10. The instrument was assembled from Rheodyne 6-port switching valves, a 10-port Vici selector valve, and the following Upchurch Scientific tubings: 0.01”ID, 1/16”OD PEEK tubing (for eluent loop and anion exchange cartridge lines); 0.02”ID, 1/16”OD PEEK tubing (for line from common vial to valve, and eluent spout); 0.02”ID, 1/16”OD PFA HP plus tubing (for lines with liquid sensors); 0.03”ID, 1/16”OD ETFE tubing (for other liquid lines). 1/16”ID, 1/8”OD polyurethane tubing from McMaster-Carr was used for gas lines. The electronic valves were controlled via LabView® on a computer.

Wheaton V-Vials (3 mL) were used in combination with 20 mm butyl rubber septa and 20 mm aluminum crimp caps for the Common Vial and Addition Line Vial. For the Reaction Vial, a 10 mL 24-400 Wheaton V-Vial with a screw-on cap and insertable PTFE-lined septum was used.

The Opti-Lynx micro-anion exchange cartridge (and its holster with adapters to microfluidic lines) was purchased from Optimize Technologies, and filled with BioRad® AGMP-1 Resin by the manufacturer.

The radiopharmaceutical production facility, reagents, documentation, etc. were controlled in a cGMP environment. Ethanol, 37% aq. HCl, and 1M aq. sodium bicarbonate were USP grade. Sterile Water for Injection (SWFI) was used in purification and formulation. All liquids and air introduced to the concentrator instrument were filtered through 0.45 micron Phenomenex filters.

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89 Mark Lazari (Prof. Michael van Dam Lab, University of California, Los Angeles) is acknowledged for building the instrument.
(RC for water or 4:1 MeCN/water (v/v) solutions (including eluent for [¹⁸F]fluoride elution); PTFE for MeCN or 1 M NaOH). Dry MeCN was purchased from Acros in bottles sealed with a needle-permeable membrane. Aristar Ultra water was used for instrument cleaning, cartridge conditioning, and eluent solution preparation.

Figure 3.5.10. Schematic of instrument for [¹⁸F]fluoride concentration, and completed setup for automated production of [¹⁸F]5-fluourouracil
Full procedure for automated synthesis of $[^{18}\text{F}]$5-fluorouracil

1. The automation module was cleaned in preparation for $[^{18}\text{F}]$5-Fluorouracil production.

1.1 The flow pathways for elution were cleaned.

1.1.1 The green lines in Figure 3.5.11 panel A were cleaned with 4:1 MeCN / Aristar Ultra water (v/v).

1.1.2 The green lines in Figure 3.5.11 panel B were filled with 4:1 MeCN / Aristar Ultra water (v/v).

1.1.3 Nitrogen gas was applied as shown in the green lines in Figure 3.5.11 panel C, to clean the Elution Spout.

1.1.4 Steps 1.1.2 and 1.1.3 were repeated 9 times.
1.1.5 The green lines in Figure 3.5.11 panels A, B and C were emptied by passage of air or nitrogen.

1.2 The common vial and associated flow pathways were assembled and cleaned (Figure 3.5.12).

**Figure 3.5.12.** Common vial and associated flow pathways

1.2.1 The Common Vial was assembled from a 3 mL V-vial, septum, and crimped cap. The common vial was perforated with the PEEK tubing leading to the 6-port switching valve containing the trapping cartridge. The common vial septum was also perforated with needles on the termini of tubing leading to the block/vent line, and the selector valve.

**Important:** 18G needles must be used for attachment to the common vial, to prevent pressure build-up during F18 delivery from cyclotron.
1.2.2 Aristar Ultra Water (1 mL) was added through the selector valve to the common vial, through the pathway highlighted in green in Figure 3.5.12 panel A.

1.2.3 Nitrogen gas was applied (15 psi) to push the liquid from common vial to waste, as shown in Figure 3.5.12 panel B.

1.2.4 Steps 1.2.2 and 1.2.3 were repeated, but with MeCN (1 mL), and the lines were dried by passage of nitrogen.

1.3 The Addition Line Vial was assembled from a 3 mL V-vial, septum, and crimped cap. The cannula was perforated through the septum of the Addition Line Vial. Both the vial and cannula were cleaned with 4:1 MeCN / Aristar Ultra water (v/v), then with anhydrous MeCN, and dried with a stream of nitrogen until no trace of liquid remained.

1.4 The water dilution / HPLC loading line valve was assembled and cleaned as follows.

1.4.1 A Teflon three-way stopcock was attached to three lines as follows (Figure 3.5.13). **Position 1** was attached to tubing that leads to a 16G spinal needle. **Position 2** was connected via tubing to a female Luer so that wash solvents could be passed through the valve via syringe. **Position 3** was closed off, so that only **Position 1** and **Position 2** were connected.

**Figure 3.5.13.** Assembly of three-way water dilution / HPLC loading line valve
1.4.2 A solution of 4:1 MeCN / Aristar Ultra water (v/v) was passed through Position 2 to Position 1, to clean the valve and the 16 G needle. Then, anhydrous MeCN was passed through the same line. The 16 G spinal needle was thoroughly scrubbed with MeCN to remove residues from needle manufacture. The line was then dried with a stream of nitrogen until no trace of liquid remained.

2. Component Preparation

2.0 The flow rate of water, when passed through the Opti-Lynx cartridge as shown in Figure 3.5.19 (F18 trapping pathway), should be at least 2.4 mL per 10 minutes at nitrogen pressure of 15 psi. When flow rate was not adequate for a new cartridge, the cartridge was filled with water, placed in a 20 mL scintillation vial filled with water, and the vial was sonicated. Generally, water flow rate through the cartridge improved after sonication. Additionally, to improve flow rate, the lines can be disconnected from the ports on the switching valves, and MeCN can be passed through the valve ports (in the opposite direction to the usual direction of flow) to flush out accumulated particulates.

2.1 The Opti-Lynx miniature ion exchange cartridge was preconditioned as follows. To avoid introducing salt into the F18 trapping line, the Opti-Lynx cartridge inlet port was disconnected from the F18 trapping line. The eluent waste line (cleaned during step 1.1) was unscrewed, and then screwed into the inlet port to the Opti-Lynx cartridge. A 3 mL conical vial with septum and crimped cap was attached to the needle of the eluent waste line. The block/vent line, as well as the nitrogen line from the selector valve, were removed from the common vial septum, and affixed to the 3 mL vial via needles through septum. Sodium hydroxide solution (1M in water, 1 mL) was added via syringe, and was pushed with nitrogen (15 psi) through the Opti-Lynx cartridge. This process was then repeated, but with Aristar Ultra water (2 times 1 mL), then with MeCN (1 mL), followed by nitrogen to dry the lines and cartridge. The eluent waste line and F18 trapping line
were then reconfigured to their original connectivities prior to cartridge preconditioning. The needles on the block/vent line and selector valve line were replaced with clean 18G needles prior to reattachment to the common vial.

2.2 Three Waters® C18 Sep-Pak Plus SPE Cartridges were prepared, by rinsing each one, separately, with the following: 5 mL EtOH, then 10 mL of SWFI. Cartridges were left wet after conditioning. Two of the cartridges were attached so that they were in series.

2.3 One Waters® HLB Plus LP Extraction SPE Cartridge was prepared by rinsing with 5 mL EtOH, 10 mL of SWFI, and pass 3 mL of air through the cartridge.

2.4 One Grace Alltech® Maxi-Clean IC-Chelate SPE Cartridge was prepared as follows. The cartridge was filled with EtOH, shaken so that the contents were uniformly slurried in the EtOH, to ensure complete rinsing. EtOH was then passed through (total 1 mL). The cartridge was then rinsed with 20 mL of SWFI, and left wet.

2.5 K₃PO₄ / 18-crown-6 eluent solution (which will be used to elute [¹⁸F]fluoride) was prepared as follows.

2.5.1 K₃PO₄ (286 mg) was weighed to a 1-dram vial, and Aristar Ultra water (3.00 mL) was added. The vial was sealed with a PTFE-lined cap, and vortexed until contents were homogeneous with no solids.

2.5.2 To a 1-dram vial containing 400.0 mg of anhydrous 18-crown-6 was added 561 uL of the K₃PO₄ solution from step 2.5.1. Anhydrous MeCN (2.24 mL) was added, and the vial was sealed with a PTFE-lined cap, and vortex until the contents were homogeneous with no solids.
2.6 A solution of 18-crown-6 in anhydrous MeCN (which will be used as the oxidative fluorination reaction solvent) was prepared as follows. To a 1-dram vial containing 40.0 mg of anhydrous 18-crown-6 was added 4.0 mL of anhydrous MeCN. The vial was sealed quickly with a PTFE-lined cap to avoid absorption of water from the external atmosphere, and vortex until contents were homogeneous with no solids.

2.7 The Reaction Vial, containing 2a + I(III) oxidant, was prepared as follows. To ensure success, test that the RCC of oxidative fluorination of complex 2a is at least 10% (by the procedure described in Radiosynthesis of $^{18}$F-labeled Molecules, S23), within 2 weeks of production.

The following steps may be performed the day before production.

A clean 1-dram vial, and a clean 10 mL V-vial were dried in a 200 °C oven for at least 6 hours. While still hot, these materials were transferred into a nitrogen-filled glovebox, and cooled to room temperature in the glovebox under argon or nitrogen. To the 1-dram vial were added 13.0 mg of 2a, and 13.0 mg of I(III) Oxidant ([PhI(4-OMe-pyridine)$_2$][2OTf])$^{49}$. The two solids were gently mixed with a spatula, to make a homogeneous admixture. 20.0 mg of this admixture was added to the 10 mL V-vial, which was then sealed with a PTFE-lined septum and cap in the glovebox under nitrogen. The vial was then removed from the glovebox and transported under ambient atmosphere to the site of radiopharmaceutical production.

3. Hot cell set up

3.1 The hot cell was set up as follows. First, the Addition Line Vial was assembled as shown in Figure 3.5.14.
Figure 3.5.14. Assembly of addition line vial, cannula, eluent tube, and vent

The Addition Line Vial and cannula (which were assembled and cleaned as described in step 1.3) is attached to the eluent tube, with the eluent tube end near the bottom of the V vial. The end of the cannula was placed at the bottom of the V-vial. An 18G vent needle was inserted to the septum.

3.2 The water dilution / HPLC loading valve and lines were assembled as follows (Figure 3.5.15).

Figure 3.5.15. Assembly of water dilution and HPLC loading valve and lines.
As described in 1.4.1, **Position 1** leads to a clean 16G spinal needle (Transfer Line 1). **Position 1** was closed, so that only **Positions 2 and 3** were connected. To **Position 2**, the line leading to the semiprep HPLC loop-in was attached. To **Position 3**, a line leading to a syringe containing 4.0 mL of SWFI was attached.

3.3 Assemble the reaction vial as shown in Figure 3.5.16.

![Figure 3.5.16. Reaction vial assembly](image)

The **center** of the reaction vial septum was perforated with the cannula that is connected on its other side to the Addition Line Vial. The **sides** of the septum were perforated with the Transfer Line 1 16G spinal needle (connected to **Position 1** of the HPLC loading line valve, see Figure 3.5.15), and vacuum needle. Only the transfer line 1 needle touched the bottom of the V-vial. The Transfer Line 1 must be **closed off** from the three-way valve (**Position 1** must be closed off, see Figure 3.5.15). **The cannula and needles must fit tightly through the septum, with no open space around where they pass through the septum -- the setup must be airtight, to prevent premature addition of MeCN to the reaction vial during fluoride elution.**

3.4 The final complete hot cell setup for synthesis is displayed in Figure 3.5.17.
3.5 The purification and formulation module was prepared as shown in Figure 3.5.18.⁹⁰

---

⁹⁰ Judit Sore is acknowledged for assistance with building the purification and formulation module.
Figure 3.5.18. Purification and formulation module

As shown in Figure 3.5.18, a glass bottle connected to the HPLC collection line was filled with 200 mL of SWFI. Two C18 Sep Pak Plus cartridges connected in series (prepared in step 2.2) were connected to the valve. A 30 mL sterile vial was charged with 0.29 mL of 37% HCl. Additionally, 5 mL of SWFI, 2.0 mL of EtOH, and a solution of 18.1 mL of SWFI with 4.1 mL of 1M sodium bicarbonate were prepared for application as described in steps 6.3, 7.1 and 7.2. One Maxi Clean IC-Chelate, one conditioned C18 Sep pak and one conditioned HLB cartridge were connected in series to the product delivery line leading to the clean room.

4. Preparation for Synthesis

4.1 A syringe with 1 mL of anhydrous MeCN was attached to the MeCN input line.

4.2 The common vial (with attached PEEK tubing line, nitrogen, and vent lines), was placed in the dose calibrator, in order to measure the amount of F18 activity delivered from the cyclotron.
4.3 The F18 delivery line (from the cyclotron) was attached to the selector valve. The selector valve position was changed to match the position where the F18 line is connected, so that F18 could later be delivered from the cyclotron, to the common vial.

4.4 The anhydrous MeCN + 18-crown-6 solution (1.00 mL, prepared in step 2.6) was added to the Addition Line Vial (within 5 minutes before transfer of F18 from cyclotron, to prevent excessive wetting by exposure to air through the vent needle in the Addition Line Vial).

5. Synthesis Execution.

5.1 The $[^{18}\text{F}]$fluoride was delivered from the cyclotron to the common vial. The amount of activity delivered to the common vial was monitored by reading the dose calibrator.

---

**Figure 3.5.19.** Pathways for trapping, eluent loop filling, and elution

5.2 The $[^{18}\text{F}]$fluoride was trapped as follows. After the delivery of $[^{18}\text{F}]$fluoride in step 5.1 was complete, the selector valve was switched to the position for nitrogen gas. The $[^{18}\text{F}]$fluoride was pushed with 15 psi of nitrogen through the ion exchange cartridge (Figure 3.5.19, red line for F18...
Trapping Pathway). To the common vial was added MeCN (1.0 mL) through the selector valve, and this MeCN was pushed through the cartridge as above, followed with nitrogen to dry the lines and cartridge.

5.3 The $^{18}$F fluoride was eluted as follows. The eluent line was primed with K$_3$PO$_4$ / 18-crown-6 eluent (prepared in step 2.5.2). The eluent loop was then filled (Figure 3.5.19, purple line for Eluent Loading Pathway), the $^{18}$F fluoride was eluted by pushing (with nitrogen pressure) the eluent, from the eluent loop, through the cartridge, and out of the eluent spout into the Addition Line Vial (Figure 3.5.19, orange line for F18 Elution Pathway). This step was repeated two times, for a total of 3 elutions. At the end of the third elution, nitrogen was bubbled through the eluent spout at 15 psi, to thoroughly mix the eluted $^{18}$F fluoride with the MeCN in the Addition Line Vial.

5.4 When the elution finished, vacuum was applied to the needle connected to the Reaction Vial. Important: **When properly configured, the $^{18}$F fluoride solution is transferred from the Addition Line Vial, through the cannula, to the Reaction vial, instantaneously (< 1 second).** The vacuum was turned off once the addition was complete. A yellow homogeneous solution was observed in the Reaction Vial. After 1 minute, step 6.1 was initiated.

6. Purification

6.1 The reaction mixture was diluted with water as follows. The three-way water dilution / HPLC transfer line valve (Figure 3.5.15) was adjusted to connect the water addition line (Position 3) to the Transfer Line 1 (Position 1). SWFI (4.0 mL) was added to the reaction vial, followed by at least 20 mL of air, which was bubbled gently to mix the reaction mixture. A yellow mixture formed that should be mixed, with air bubbling, to homogeneity, to resemble freshly mixed orange juice.
6.2 Promptly, without letting the precipitate settle, the three-way valve was adjusted to connect the Transfer Line 1 (Position 1) with the HPLC loop-in line (Position 2), the valve was placed behind shielding, and the crude product was loaded into the loop-in line, and injected onto the semiprep HPLC (column: Phenomenex Luna C18(2) (5 µm, 10.00 x 250 mm)). Elution occurred with 60:40 MeCN/(10 mM ammonium formate in water) v/v, flow rate = 5 mL/minute, for 13 minutes, then 70:30 MeCN/(10 mM ammonium formate in water) v/v, flow rate = 5 mL/minute. The product $[^{18}\text{F}]4\text{a}$ eluted at approximately 18 minutes.

6.3 The $[^{18}\text{F}]4\text{a}$ was collected from the HPLC collection line into 200 mL of SWFI. After completion of collection, the diluted $[^{18}\text{F}]4\text{a}$ was passed (with nitrogen push) through two C18 Sep Pak cartridges (conditioned in step 2.2, and assembled in step 3.5, see Figure 3.5.18), in series, to trap the $[^{18}\text{F}]4\text{a}$ and remove MeCN and salt from the HPLC mobile phase. The cartridges were rinsed with SWFI (5 mL). EtOH (2.0 mL) was then passed through the cartridges, in order to elute $[^{18}\text{F}]4\text{a}$ into a 30 mL vial that contained 0.29 mL of 37% aq. HCl. The liquids were mixed as thoroughly as possible with a gentle nitrogen stream, and were allowed to sit for two minutes at 23 ºC. Deprotection of $[^{18}\text{F}]4\text{a}$ occurred during this time to form $[^{18}\text{F}]5\text{-FU}$.

7. Formulation

7.1 A solution prepared from 18.1 mL of SWFI and 4.1 mL of 1M sodium bicarbonate was added to the $[^{18}\text{F}]5\text{-FU}$ solution.

7.2 The resulting solution was passed (with nitrogen push, after removal of the delivery and vent lines) through the IC-Chelate, C18, and HLB cartridges (conditioned in steps 2.2, 2.3, 2.4), in series, into a line that lead to an intermediate sterile vial that was located in a clean room for sterile radiopharmaceutical packaging.
7.3 In the clean room, the collected $[^{18}\text{F}]$5-FU solution was passed from the intermediate vial, through a sterilizing Millex GP 0.22 micron filter, into a sterile 30 mL vial to afford pure, formulated, sterile $[^{18}\text{F}]$5-FU.

8. Quality control

8.1 Radiochemical Identity, Chemical & Radiochemical Purity, and Specific Activity of $[^{18}\text{F}]$5-FU were measured by HPLC. The column was a Phenomenex Luna® 5 µm PFP(2) 100 Å, 250 x 4.6 mm analytical column. The mobile phase was 65 mM ammonium formate in water, flow rate = 1 mL/minute.

8.2 The formulated $[^{18}\text{F}]$5-FU was visually inspected.

8.3 The pH was measured by coating pH indicator paper with several drops of the solution.

8.4 The formulated $[^{18}\text{F}]$5-FU was tested for bacterial endotoxins.

8.5 Radionuclidic Half-Life was measured.

8.6 The formulated $[^{18}\text{F}]$5-FU was analyzed for sterility.

8.7 The Sterilizing Filter Integrity Test was performed.

8.8 Radiochemical identity and purity were analyzed by Radio TLC.

8.9 Residual solvents were assayed by gas chromatography.

8.10 Nickel content was measured by ICP-MS.
Table 3.5.2. Results of $[^{18}F]$5-fluorouracil synthesis$^a$

<table>
<thead>
<tr>
<th></th>
<th>Synthesis 1</th>
<th>Synthesis 2</th>
<th>Synthesis 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isolated yield (mCi) of $[^{18}F]$5-FU at EOS $^b$</td>
<td>13.48</td>
<td>18.83</td>
<td>12.85</td>
</tr>
<tr>
<td>Starting $[^{18}F]$fluoride activity (mCi) $^c$</td>
<td>1785</td>
<td>1698</td>
<td>1433</td>
</tr>
<tr>
<td>Percent Yield</td>
<td>0.7552%</td>
<td>1.109%</td>
<td>0.8967%</td>
</tr>
<tr>
<td>Average % Yield ± Std. Dev.</td>
<td></td>
<td>0.92% ± 0.18%</td>
<td></td>
</tr>
<tr>
<td>Area of 5FU Peak (UV, 266 nm)</td>
<td>1.82437</td>
<td>0.927105</td>
<td>0.510297</td>
</tr>
<tr>
<td>Specific Activity$^d$ (SA) at EOS (Ci / µmol)</td>
<td>14.48</td>
<td>38.95</td>
<td>49.34</td>
</tr>
<tr>
<td>Average SA ± Std. Dev. at EOS (Ci / µmol)</td>
<td></td>
<td>34.3 ± 17.9</td>
<td></td>
</tr>
<tr>
<td>Synthesis Time (minutes)</td>
<td>120 (85$^e$)</td>
<td>84</td>
<td>90</td>
</tr>
<tr>
<td>Solution Volume (mL)</td>
<td>23.2</td>
<td>23.7</td>
<td>23.2</td>
</tr>
<tr>
<td>pH</td>
<td>7.5</td>
<td>7.5</td>
<td>7.5</td>
</tr>
<tr>
<td>Ni content (ppb)</td>
<td>45.7</td>
<td>94.2</td>
<td>40.3</td>
</tr>
<tr>
<td>Unknown Impurities (µg)$^f$</td>
<td>3.91</td>
<td>5.91</td>
<td>2.29</td>
</tr>
</tbody>
</table>

$^a$ Judit Sore, Kari Phan, and Garima Gautam are acknowledged for assistance with data collection.

$^b$ EOS refers to the end of synthesis, when the completely purified, formulated product in a sterile vial in a clean room, obtained after Step 7.3 of the synthesis, was measured for radioactivity.

$^c$ Radioactivity of $[^{18}F]$fluoride solution (in about 2.4 mL $[^{18}O]$water) delivered from cyclotron to the common vial (Step 5.1 of synthesis), immediately after the end of bombardment.

$^d$ Amount of 5FU, for the SA measurement, was calculated as follows: nmol 5FU = (5FU peak area) * (calibration curve slope) * (Solution Volume) / (Injection Volume). Injection volume = 0.100 mL; See Figure 3.5.20 for Calibration Curve.
### Table 3.5.2 (Continued).

[c] Corrected synthesis time for Synthesis 1, after subtracting extra time elapsed due to unexpected clogging, which did not occur in subsequent runs, after implementation of preventative measures.

[f] Estimated from UV areas (266 nm), as described above[c], and using the molecular weight of 5-FU.

### Table 3.5.3. Data for the standard curve of UV absorbance vs. amount of 5-FU[a]

<table>
<thead>
<tr>
<th>Amount of 5-FU (nmol)</th>
<th>UV absorbance (266 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.192189</td>
<td>72.7</td>
</tr>
<tr>
<td>0.192189</td>
<td>73.6</td>
</tr>
<tr>
<td>0.192189</td>
<td>75.3</td>
</tr>
<tr>
<td>0.384379</td>
<td>153.6</td>
</tr>
<tr>
<td>0.384379</td>
<td>156.1</td>
</tr>
<tr>
<td>0.384379</td>
<td>158.5</td>
</tr>
<tr>
<td>0.768758</td>
<td>321.8</td>
</tr>
<tr>
<td>0.768758</td>
<td>325.7</td>
</tr>
<tr>
<td>0.768758</td>
<td>328.8</td>
</tr>
<tr>
<td>1.153137</td>
<td>503.2</td>
</tr>
<tr>
<td>1.153137</td>
<td>499.8</td>
</tr>
<tr>
<td>1.153137</td>
<td>506.8</td>
</tr>
<tr>
<td>1.921894</td>
<td>855.9</td>
</tr>
<tr>
<td>1.921894</td>
<td>855.9</td>
</tr>
<tr>
<td>1.921894</td>
<td>862.5</td>
</tr>
</tbody>
</table>

[a] Hong Ren and Judit Sore are acknowledged for collecting data summarized in Table 3.5.3.
Figure 3.5.20. Calibration curve for nmol of 5-FU vs UV absorbance at 266 nm

\[
y = 0.002200x + 0.040661 \\
R^2 = 0.999741
\]
Table 3.5.4. Summary of quality control for produced \[^{18}\text{F}5\text{-Fluorouracil}\] (for doses produced from 3 validation syntheses)\(^a\)

<table>
<thead>
<tr>
<th>Analytical Test Method</th>
<th>Specifications</th>
<th>Result (PASS or FAIL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual Inspection</td>
<td>Clear and colorless solution, free from visible particles</td>
<td>PASS</td>
</tr>
<tr>
<td>Radionuclidic Purity of [^{18}\text{F}] by Gamma Spectroscopy</td>
<td>The final product centroid value is within 1% of the [^{68}\text{Ge}] calibration standard.</td>
<td>PASS</td>
</tr>
<tr>
<td>Radionuclidic Identity of [^{18}\text{F}] by Half-Life</td>
<td>Half-Life of 105 – 115 minutes</td>
<td>PASS</td>
</tr>
<tr>
<td>Radiochemical Purity [^{18}\text{F}5\text{-FU (TLC)}]</td>
<td>(\geq 90%) as [^{18}\text{F}] 5FU</td>
<td>PASS</td>
</tr>
<tr>
<td>Radiochemical Purity [^{18}\text{F}5\text{-FU (HPLC)}]</td>
<td>(\geq 90%) as [^{18}\text{F}] 5FU</td>
<td>PASS</td>
</tr>
<tr>
<td>Radiochemical Identity of [^{18}\text{F}5\text{-FU (HPLC)}]</td>
<td>Retention time of Standard and the Final product is ±5% of each other</td>
<td>PASS</td>
</tr>
<tr>
<td>pH</td>
<td>4.5-8.5</td>
<td>PASS</td>
</tr>
<tr>
<td>Filter Integrity</td>
<td>Meets Manufacture’s Bubble Point</td>
<td>PASS</td>
</tr>
<tr>
<td>Chemical Purity (HPLC)</td>
<td>(\leq 10\mu\text{g}) of Total Impurity</td>
<td>PASS</td>
</tr>
<tr>
<td>Residual Solvents (GC)</td>
<td>Ethanol (\leq 10%)</td>
<td>PASS</td>
</tr>
<tr>
<td></td>
<td>Acetonitrile (\leq 0.04%) (observed peak ≤ standard peak)</td>
<td>PASS</td>
</tr>
<tr>
<td></td>
<td>IPA (observed peak ≤ standard peak)</td>
<td>PASS</td>
</tr>
<tr>
<td>Bacterial Endotoxin</td>
<td>(&lt;6\text{ EU/mL (≤175EU/vial)})</td>
<td>PASS</td>
</tr>
</tbody>
</table>

**POSTRELEASE**

<table>
<thead>
<tr>
<th></th>
<th></th>
<th>PASS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sterility</td>
<td>No turbidity or growth (Sterile)</td>
<td>PASS</td>
</tr>
<tr>
<td>Nickel Content</td>
<td>(\leq 500\text{ppb})</td>
<td>PASS</td>
</tr>
<tr>
<td>Radionuclidic Purity of [^{18}\text{F}] by Gamma Spectroscopy</td>
<td>Main photopeak at 0.551± 0.02 MeV; (\geq 99.5%) [^{18}\text{F}]</td>
<td>PASS</td>
</tr>
</tbody>
</table>

\(^a\) Judit Sore, Kari Phan, and Garima Gautham are acknowledged for collecting data summarized in Table 3.5.4, and for preparing the table.
3.5.3. Measurement of Trapped and Eluted $[^{18}\text{F}]$fluoride, and RCC for $[^{18}\text{F}]4\text{a}$ formation, with the New Concentrator Instrument

The automated synthesis was performed (steps 1.1 – 5.4), with modifications A and E (below) and additional steps B – D to obtain the desired data:

A. In order to perform multiple experiments with one cyclotron bombardment, instead of using the entire 2.4 mL of $[^{18}\text{F}]$fluoride in $[^{18}\text{O}]$water, an aliquot (between 0.2 mL and 1.0 mL of the 2.4 mL $[^{18}\text{F}]$fluoride solution) was injected directly into the common vial by syringe, followed by a sufficient amount of Aristar Ultra water to bring the total volume in the common vial to 2.4 mL.

B. In order to measure trapped $[^{18}\text{F}]$fluoride, immediately after trapping $[^{18}\text{F}]$fluoride on the Opti-Lynx cartridge (step 5.2), the cartridge was removed from its holder, its radioactivity was measured, and then it was placed back into its holder.

C. In order to measure eluted $[^{18}\text{F}]$fluoride, after synthesis completion (step 5.4), the radioactivity of the Addition Line Vial, cannula, Reaction Vial, and Transfer Line 1 were measured and summed (since these are all of the components that came into contact with $[^{18}\text{F}]$fluoride after elution was completed, as defined by leaving the elution spout).

D. In order to measure RCC for conversion of $[^{18}\text{F}]$fluoride to $[^{18}\text{F}]4\text{a}$, after completion of step 5.4, the yellow solution in the Reaction Vial was assayed as described in Measurement of Radiochemical Yield by radio TLC (in this case, the Reaction Vial was rinsed with MeCN (2 times 1.0 mL, introduced via Transfer Line 1) to remove residual reaction solution from the walls of the vial). In all cases, radioactivity in solution was >90%.

E. In the case of Run #4 (Table 3.5.5), in order to test the effect of PPTS buffer on RCC, PPTS was added to the reaction solvent (prepared as follows instead of as described in step 2.6): 37.6
mg of 18-crown-6 was dissolved in 3.76 mL dry MeCN. A 1.00 M PPTS solution in dry MeCN was prepared, and 22.56 μL of this solution was added to the 18-crown-6 solution to give a homogeneous colorless solution.

**Table 3.5.5.** Data for trapping, elution, and RCC in automated synthesis with the concentrator instrument

<table>
<thead>
<tr>
<th>Run #</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclotron Target Water / Aristar Water (mL / mL)</td>
<td>0.2 / 2.2</td>
<td>0.4 / 2.0</td>
<td>0.8 / 1.6</td>
<td>1.0 / 1.4</td>
</tr>
<tr>
<td>Starting $[^{18}\text{F}]$fluoride activity (mCi) (t₀) (^a)</td>
<td>14.13</td>
<td>11.76</td>
<td>9.91</td>
<td>6.81</td>
</tr>
<tr>
<td>Trapped $[^{18}\text{F}]$fluoride activity (mCi), dc (^b)</td>
<td>13.53</td>
<td>11.59</td>
<td>9.69</td>
<td>6.44</td>
</tr>
<tr>
<td>Eluted $[^{18}\text{F}]$fluoride activity (mCi), dc (^b)</td>
<td>10.81</td>
<td>10.99</td>
<td>9.14</td>
<td>6.17</td>
</tr>
<tr>
<td>Elapsed Time for steps 5.2 – 5.4 (min)</td>
<td>15</td>
<td>11</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>Eluted $[^{18}\text{F}]$fluoride activity (mCi), ndc (^c)</td>
<td>9.84</td>
<td>10.25</td>
<td>8.42</td>
<td>5.72</td>
</tr>
<tr>
<td>Avg. ± Std. Dev.</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trapping Efficiency, %</td>
<td>95.75</td>
<td>98.55</td>
<td>97.78</td>
<td>94.57</td>
</tr>
<tr>
<td>Elution Efficiency, %</td>
<td>79.90</td>
<td>94.82</td>
<td>94.32</td>
<td>95.81</td>
</tr>
<tr>
<td>Overall Yield, % (ndc)(^c)</td>
<td>69.64</td>
<td>87.16</td>
<td>84.96</td>
<td>83.99</td>
</tr>
<tr>
<td>RCC, %</td>
<td>3.41</td>
<td>2.90</td>
<td>2.34</td>
<td>2.89 ± 0.54</td>
</tr>
<tr>
<td>RCC, % (with PPTS)</td>
<td>2.96</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) All decay-corrected (dc) measurements were corrected to t₀, which was the time at which the starting $[^{18}\text{F}]$fluoride activity was measured.

\(^b\) Decay-corrected to time = t₀. \(^c\) Not decay-corrected.
On large scale, the trapping and elution measurements were performed as follows. To the common vial was delivered $^{18}$F-fluoride (1708 mCi; the timepoint of this measurement was defined as $t_0$). Hereafter, $t_0$ denotes decay correction to time $= t_0$). After trapping on the cartridge, 32.06 mCi ($t_0$) remained in the common vial. The trapping waste ($^{18}$O-water recovery) contained 0.193 mCi ($t_0$). Therefore, $1708 \text{ mCi} - 32.06 \text{ mCi} - 0.193 \text{ mCi} = 1675.75 \text{ mCi} (t_0)$ of $^{18}$F-fluoride was trapped on the cartridge. After elution, 94.82 mCi ($t_0$) remained on the cartridge. Therefore, $1675.75 \text{ mCi} - 94.82 \text{ mCi} = 1580.93 \text{ mCi}$ was eluted ($t_0$). The total time for trapping and elution was 25 minutes. Applying 25 minutes of decay (half life = 109.771 minutes) to 1580.93 mCi, the non-decay-corrected amount of eluted $^{18}$F-fluoride was 1350.06 mCi.

Trapping efficiency = $1675.75 \text{ mCi} / 1708 \text{ mCi} \times 100\% = 98.1\%$

Elution efficiency = $1580.93 / 1675.75 \times 100\% = 94.3\%$

Overall not-decay-corrected yield = $1350.06 \text{ mCi} / 1708 \text{ mCi} \times 100\% = 79.0\%$

**Measurement of elution volume**

The eluent loop was filled with water, and an elution was performed by passing the contents of the eluent loop through the Opti-Lynx ion exchange cartridge, out of the elution spout, into a pre-weighed vial. This process was repeated (two elutions were performed in total). For two elutions, the measured volume was $12.4 \pm 0.4 \mu\text{L}$ (average of four measurements).

**Determination of K$_3$PO$_4$ and 18-crown-6 concentration in eluent**

As described in section 2.5 of the cGMP $^{18}$F-5-FU synthesis, an aqueous K$_3$PO$_4$ solution was prepared from 286 mg of K$_3$PO$_4$ and 3.00 mL of water. A 1.00 mL portion of the aqueous K$_3$PO$_4$ solution

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91 Mark Lazari is acknowledged for measuring the elution volume
solution weighed 1.077 g. To prepare the eluent solution, a portion of the aqueous $K_3PO_4$ solution (561 $\mu$L) was added to a vial containing 400.0 mg of 18-crown-6, dry MeCN (2.24 mL) was added, and the resulting mixture was sonicated until a homogeneous solution was obtained. A 1.00 mL portion of the eluent solution weighed 0.872 g. Based on these measured densities and component mass ratios, 1.00 mL of the eluent solution contains 78.1 $\mu$mol of $K_3PO_4$ and 477 $\mu$mol of 18-crown-6.