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Synthesis of ^{18}F-PET-sensitive Agents for Targeted Visualization of Brain Tumors

By

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Synthesis of ^{18}F-PET-sensitive Agents for Targeted Visualization of Brain Tumors

By

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B.S., Furman University, 2017

Advisor: Nathan T. Jui, Ph.D.

An abstract of a thesis submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
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Abstract

Synthesis of ^{18}F-PET-sensitive Agents for Targeted Visualization of Brain Tumors

High-grade gliomas (HGGs; grades III and IV by the World Health Organization) carry a dismal prognosis. In 2017 in the US alone, roughly 16,700 deaths resulted from a total of 23,800 cases of such gliomas. Current imaging techniques lack the selectivity necessary to properly visualize and detect the development of these malignant tumors. To address this gap in selective HGG visualization, we proposed a method of glioma detection using novel positron emission tomography (PET)-radiotracers with an alternative mechanism of action. By exploiting the phenomena of extensive binding of the fluorescent heme analog protoporphyrin IX (PpIX) to the peripheral benzodiazepine receptor (PBR) protein in several tumorous cell lines (including gliomas), we aimed to synthesize ^{18}F-radiolabeled 5-aminolevulinc acid (5-ALA) derivatives, where 5-ALA is a precursor to the biosynthesis of PpIX. These fluorinated species will potentially be detectable under PET scans, and aid in pre-operative assessment of gliomas. Precursors to the 2- and 3-^{18}F-5ALA were successfully made, but preliminary ^{18}F-labelling was unsuccessful for either isomer. Other methods of accessing 2- and 3-fluoro isomers were explored, but ultimately were unsuccessful due to competing E_1/E_2 reactions and inability to translate conditions to a radiochemical context. However, 5-ALA fluoro esters were successfully synthesized, with a ^{18}F-5-ALA-propyl ester being synthesized in 8% radiochemical yield. Unfortunately, initial tumor cell studies indicated no uptake of the ^{18}F-labeled ester. Currently, a catalytic fluorination method is being implemented for the 2-^{18}F-isomer along with further investigation into an amide-based fluorination strategy; for the 3-isomer, other leaving group activators (brosylate, nosylate, etc.) are being tested along with a Mn(salen) benzylic fluorination.

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List of abbreviations

ALA aminolevulinic acid

BBB blood-brain barrier

Boc_2O di-tert-butyl dicarbonate

DBU 1,8-diazabicycloundec-7-ene

DCC *N*,*N*-dicyclohexylcarbodiimide

DCM dichloromethane

DMAP *N*,*N*-4-(dimethylamino)pyridine

DMF dimethylformamide

EDCI 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide

Et ethyl

Et_3N triethylamine

EtOAc ethyl acetate

EtOH ethanol

equiv. equivalents

ESI electrospray ionization

FDG fluorodeoxyglucose

HGG high-grade gliomas

HOBt hydroxybenzotriazole

HPLC high performance liquid chromatography

LC/MS liquid chromatography mass spectrometry

LGG low-grade gliomas

LRMS low-resolution mass spectrometry

Me methyl

MeCN acetonitrile

mmol millimoles

MRI magnetic resonance imaging

Ms methanesulfonyl

NBS *N*-bromosuccinimide

NC isonitrile

NMR nuclear magnetic resonance

PBR peripheral benzodiazepine receptor

PET positron emission tomography

Ph phenyl

PIDA (diacetoxyiodo)benzene

PpIX protoporphyrin IX

RXN reaction

SKA silyl-ketene acetal

TBAT difluoro-triphenyl-tetrabutylammonium fluoride

TBS *tert*-butyldimethylsilyl

temp temperature

Tf_2O triflic anhydride

THF tetrahydrofuran

TLC thin layer chromatography

Ts toluenesulfonyl

Introduction

The standard protocol for assessment of high-grade gliomas (HGGs) typically involves magnetic resonance imaging (MRI), often with administration of contrast agents.[1] Tumor removal followed by radiation/chemotherapy is currently the general procedure for treating gliomas, however, dependence on MRI alone can lead to inaccurate grading of the tumor.[2] Current MRI contrast agents are not specific to molecular markers of brain tumors, and approximately 40% of HGGs demonstrate no enhancement/specificity.[3] Thus, total tumor resection as defined by MRI only gives a <5% five-year survival rate for HGGs.[4] This is due to incomplete tumor removal, leading to its recurrence. To circumvent the shortcomings of MRI tumor resection, molecular imaging with positron emission tomography (PET) is alluring. However, current PET radiotracers, the most common of which is fluorodeoxyglucose (FDG), cannot be used for brain tumor imaging due to the high FDG uptake of normal brain tissue. Therefore, we envisioned building a highly tumor-specific imaging probe that would aid neurosurgeons with brain tumor removal.

To design a tumor-specific probe, we considered biological processes that may be uniquely enhanced or upregulated in gliomas versus other cancers. One potential target involved the peripheral benzodiazepine receptor (PBR). This is an integral protein of the outer mitochondrial membrane involved with several cellular functions (cell proliferation, mitochondrial respiration, etc.). Interestingly, it has been shown that high PBR density is associated with enhanced glioma tumorigenicity and cellular proliferation.[5]

Protoporphyrin IX (PpIX) is the ferrous-ion free analog of heme and is a naturally occurring fluorescent porphyrin. PpIX has a high affinity for the PBR and has been shown to accumulate in the mitochondria of high-grade gliomas.[6] The synthesis of PpIX is typically tightly regulated, but in HGGs, the heme biosynthetic pathway enzymes are dramatically increased

resulting in high levels of PpIX. During the synthesis of PpIX, the production of the precursor, 5-aminolevulinic acid (5-ALA), is the rate-limiting intermediate step (eight equivalents of 5-ALA are required for synthesis of each PpIX molecule) (Figure 1).

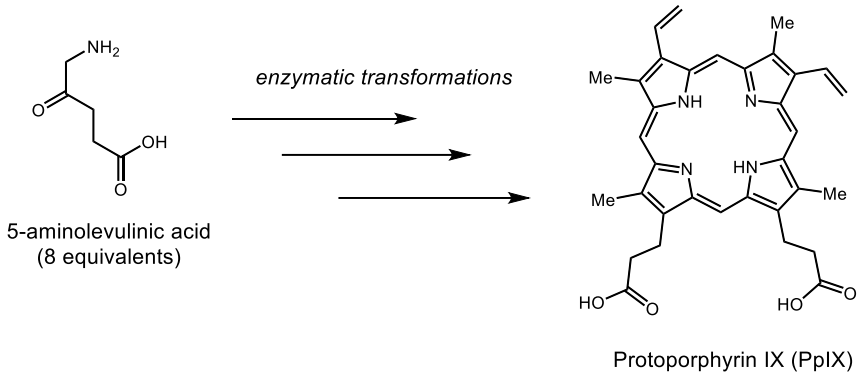


Figure 1. The heme biosynthetic pathway converts eight equivalents of 5-aminolevulinic acid (5-ALA) into one equivalent of PpIX. The biosynthesis of fluorescent PpIX can be exploited for potential tumor imaging applications.

Accordingly, oral administration of 5-ALA leads to selective uptake and increased PpIX synthesis in HGGs. Additionally, there is greater accumulation of PpIX in HGGs as compared to low-grade gliomas (LGGs) with areas of high proliferation demonstrating greater PpIX fluorescence.[7] The blood-brain barrier (BBB) in healthy individuals is impermeable to 5-ALA; however, in HGGs there is breakdown of the BBB resulting in selective passage of 5-ALA into HGGs.[8] This is crucial to our research plan, since we can exploit this leakage in our design of specific probes.

The structure of 5-ALA does not lend itself to practical installments of a PET radioisotope. Without altering the compound, the only usable PET isotopes are ^{11}C, ^{13}N, and ^{15}O. To date, the only synthesis of a ^{11}C derivative of 5-ALA has been 5-amino-4-oxo-[6-^{11}C] hexanoic acid ([^{11}C] MALA) (**1**, Figure 2).[9] However, more recently, Pippin et. al. reported a synthesis of [^{13}N]-5-aminolevulinic acid ([^{13}N]-5-ALA) in high radiochemical yield (RCY; 65%) (**2**, Figure 2).[8] Using microPET imaging, the radiolabeled 5-ALA was able to verify the location of the tumor within 10

minutes.[8] However, the half-lives of either ^{11}C or ^{13}N (20.33 min and 9.98 min, respectively) present an issue for distribution of these probes to smaller clinics without access to cyclotrons.

Encouraged by the finding that selective uptake of **2** in gliomas could be utilized as an imaging probe, we sought to develop and evaluate the fluorine-18 labeled PET tracers **3-6** (shown in Figure 2). In this application we chose to focus on ^{18}F because it is the ideal radionuclide for clinical PET imaging due to its longer half-life compared to ^{13}N and ^{11}C (109.77 min vs 9.98 min for ^{13}N and 20.3 min for ^{11}C), allowing for a more widespread clinical use. Another key advantage of ^{18}F is its short positron linear range, which results in the highest resolution of PET images thus far.[10] These structures were selected because they are structurally similar to 5-ALA, and they would retain fluorination upon bioconversion to PpIX.

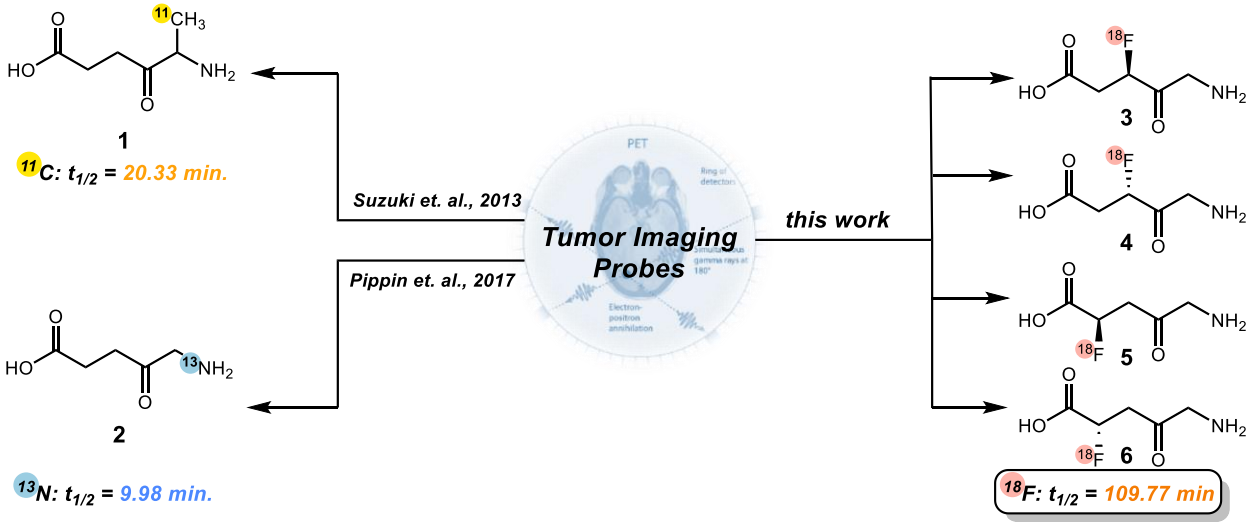


Figure 2. Both ^{11}C and ^{13}N-labelled 5-ALA derivatives have been made and tested for their utility as PET tracers. The longer half-life of the ^{18}F isotope and its ability to provide high resolution PET images make it an ideal candidate for 5-ALA modification.

Results and Discussion

We understood that the key synthetic challenge in the development of **3** – **6** would be appropriate protection of the α-amino ketone group, such that late-stage introduction of ^{18}F and reconstitution of the 5-ALA structure would be rapid. Both of our synthetic approaches utilize

displacement of an alkyl electrophile with fluoride and would quickly provide the chiral derivatives with regio- and stereo-control. Either route involves direct production of the amino ketone from an oxazole-4-carboxylic ester (based on a known procedure), where acidic treatment accomplishes oxazole hydrolysis and concurrent decarboxylation to give the desired α-amino ketone.[11]

The first synthetic strategy proposed to access 3-fluorinated 5-ALA derivatives **3** and **4** involves oxazole formation using the commercially available reagents cinnamoyl chloride (**7**) and ethyl isocyanoacetate (**8**) (Figure 3).[12,13] The desired product **9** was obtained in 55% yield from this method. The next step initially involved ozonolysis to aldehyde **10**, however, low yields (≤ 30%) along with substantial amounts of over-oxidized carboxylic acid side product compelled us to seek more efficient conditions (Figure 3).

After screening conditions for oxidative cleavage of the styryl alkene, the best results were seen using osmium tetroxide with sodium periodate, both from the perspective of a better yield (78%) and no formation of by-products (Table 1).[14] Moving forward, we proposed an aldol process to incorporate the last two carbons of the 5-ALA scaffold, along with the carboxy terminus. Our first attempts aimed for an enantioselective transformation using Evans chiral oxazolidinone auxiliaries.[15,16] Along with a less than desirable yield (35%), there was no apparent selectivity for either the (R) or (S) isomers. Despite this, we pursued a mesylation of the aldol intermediate **11**, which was obtained in quantitative yield (**12**) (Figure 3).[17] This mesylate was submitted to test potential radiofluorination, and, to our delight, the radiochemical yield was 38%.

The oxazole can be directly converted into the amino-ketone moiety present in 5-ALA through mild acid hydrolysis, which is essential considering the time-sensitive nature of radiolabeling.[12] However, the presence of both the oxazole and oxazolidinone protecting groups

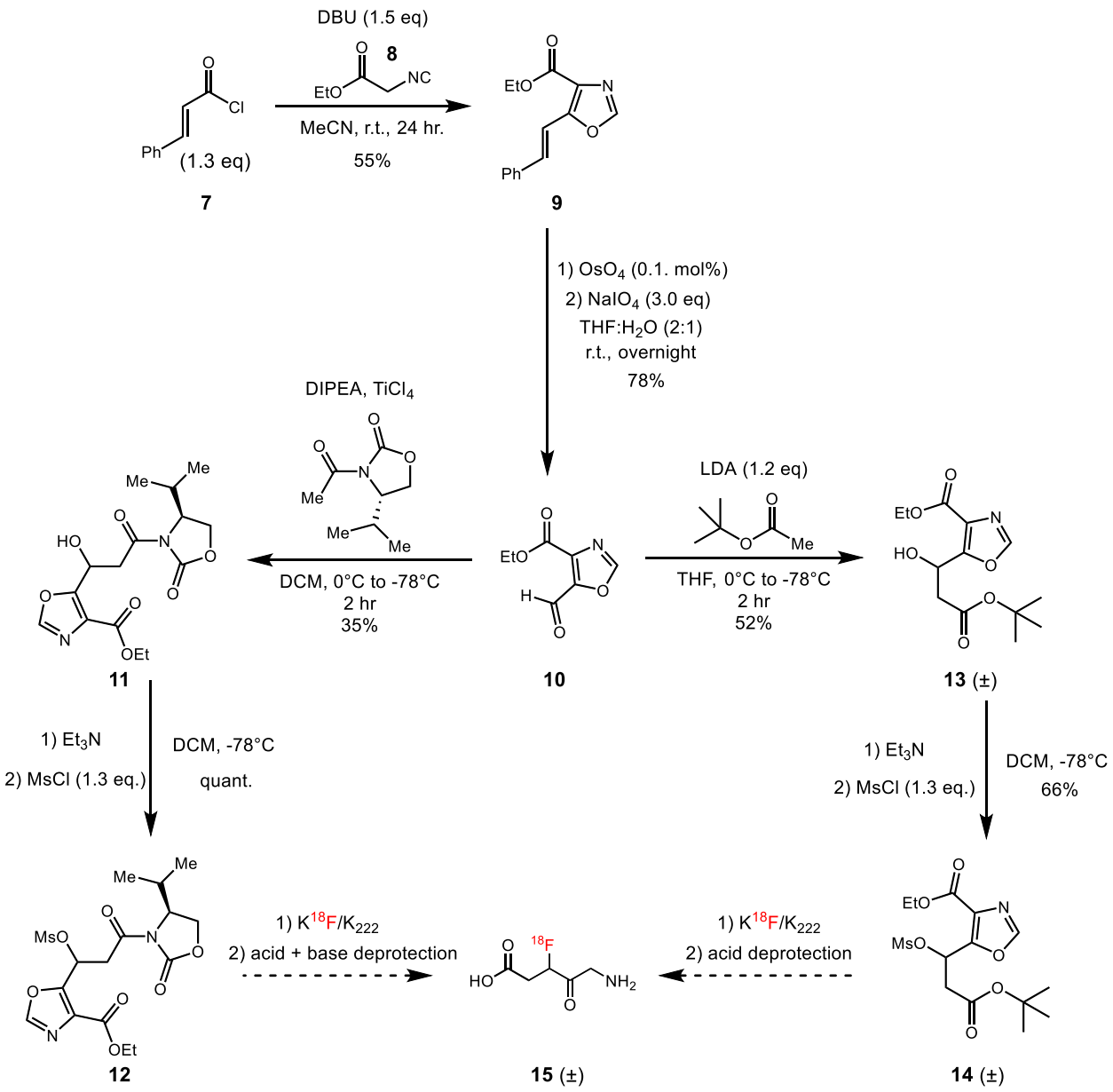
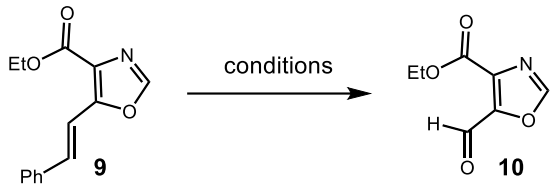


Figure 3. Synthetic routes to mesylates **12** and **14** from common intermediate **10**.

made deprotection more cumbersome, requiring sequential acid and base hydrolysis. As a solution, we subjected the same aldehyde intermediate through an aldol process with tert-butyl acetate (52%).[18] This would streamline the deprotection into a single mild acid hydrolysis. With aldol product **13** in hand, we were able to obtain mesylate **14** in 66% yield as a racemic mixture, with hopes of fully deprotecting after fluorination to yield **15** (Figure 3).

Table 1. Oxidative Cleavage Condition Screening for 9 to 10



entry	conditions	additive	result
1	$KMnO_4$ H_2O/DCM (1:3), 23 °C, 16 h	alumina solid support	0% yield; complex mixture
2	$KMnO_4$ H_2O/DCM (1:3), 23 °C, 16 h	$CuSO_4$•H_2O solid support	0% yield; complex mixture
3	$KMnO_4$ H_2O/THF (0.3:1), 40°C, 16 h	none	0% yield; complex mixture
4	$RuCl_3$•H_2O, PIDA, DCM/H_2O (4:1) 23 °C, 16 h	none	No conversion
5	$RuCl_3$•H_2O, oxone, H_2O/MeCN (1.2:2) 23 °C, 16 h	sodium bicarbonate	No conversion
6	$NaNO_2$, toluene, 80°C, O_2 balloon 16 h	benzoic acid	No conversion
7	**OsO_4 (cat.), $NaIO_4$, THF/H_2O (2:1) 16 h**	**none**	**78% yield; no side products**

Accessing the 2-fluoro-5-ALA derivatives proved to be challenging. The first synthetic strategy aimed to obtain an oxazole precursor again by addition of an acid chloride to ethyl isocyanoacetate (just as with the 3C-isomers), but with a terminal olefin-containing acid chloride **16**. The primary result was olefin isomerization to the more thermodynamically stable conjugated product **17**, something which we were unable to prevent across several reaction conditions. Another strategy employed the use of succinic anhydride, which underwent oxazole formation given our conditions (**18**, Figure 4). After transesterification of the carboxy terminus, alpha-halogenation was the last step before attempting fluorination. This was attempted through a silyl ketene acetal (SKA) intermediate.[19] We proposed that the greatest obstacle to halogenation was selectivity between two potential SKA intermediates; however, what we observed was no deprotonation to form either SKA. We surmised that the oxazole proton *p*k_a (~24) is less than that of the methylene protons (~29) and did not explore this route further (Figure 4).[20, 21]

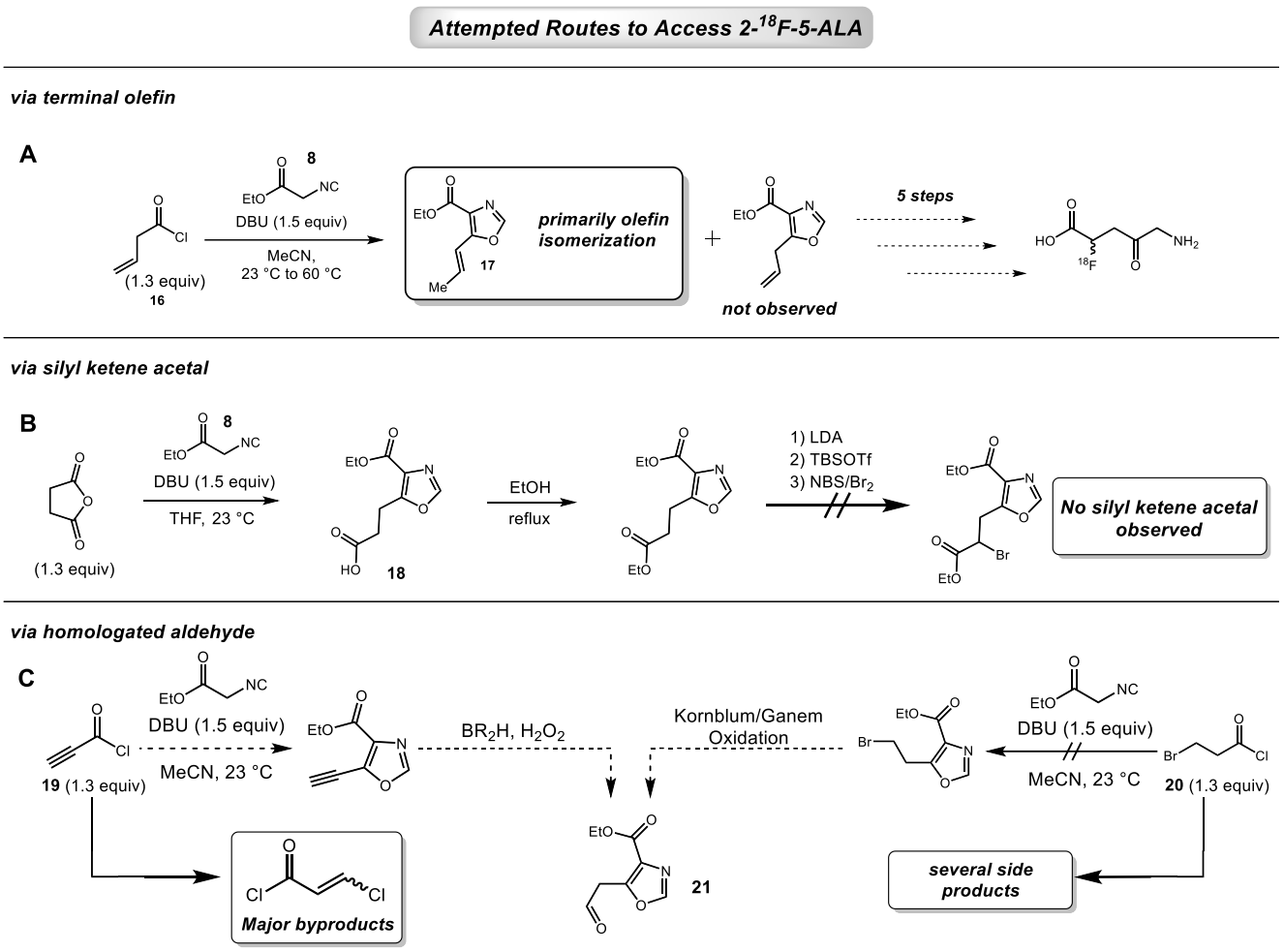


Figure 4. Attempts to access 2-^{18}F-5-ALA, along with primary roadblocks to each strategy attempted thus far.

We then considered synthesizing homologated aldehyde **21** from the oxazole — the aim being to activate the 2-position of 5-ALA for fluorination via addition to an aldehyde. Our first attempt explored the formation of an oxazole with a terminal alkyne, with hopes of converting the alkyne to **21** via hydroboration. The major obstacle here was accessing 2-proponoyl chloride (**19**), where the primary result was formation of Michael addition byproducts. Therefore, other routes were considered. Our second attempt involved the use of 3-bromopropionyl chloride (**20**) as the acid chloride for oxazole formation. Here, several other byproducts were observed, making this route unfavorable (Figure 4).

As a third attempt, we envisioned a diverted synthesis for both (2)- and (3)-fluorinated isomers with intermediate aldehyde **10** serving as the point of deviation. For 2-^{18}F-5-ALA, the new route involved a Wittig product as an intermediate (**22**), which was achieved in good yield (81%) with an *E/Z* of 1.7:1 (Figure 5).[22] Both *E* and *Z* isomers were subjected to mild acid hydrolysis of **22** to obtain **21**. After screening several conditions, the most success was seen with the use of oxalyl chloride and ethanol/water in chloroform, giving a crude mix with roughly 9:1 product to starting material.[23] This crude mixture was taken forward to the next step, involving formation of cyanohydrin **23**.[24] The yield was low (22%), primarily due to elimination occurring on silica during purification. Nonetheless, **22** was taken forward and successfully triflated in 96% yield, obtaining the product (**24**) (Figure 5).[25]

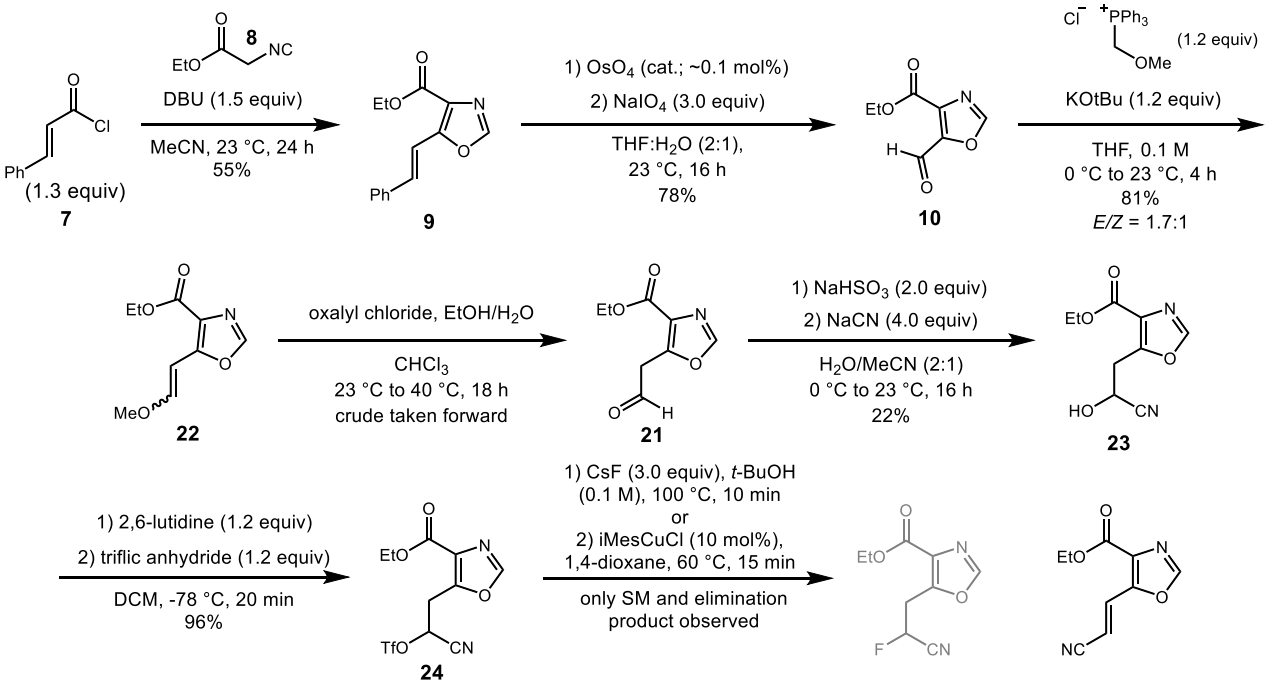


Figure 5. Revised synthesis to access 2-^{18}F-5-ALA via diverted route from common intermediate **10**.

Motivated by these results, we attempted fluorination of mesylate **14** and triflate **24** in collaboration with the Goodman lab (Winship Cancer Institute). Goodman's lab would oversee radiolabeling, deprotection, and biological testing of both precursors. Radiofluorination was

conducted via the [^{18}F]KF·K_{222} complex.[10] However, for both **14** and **24**, no incorporation of ^{18}F was detected via analytical HPLC or radio-TLC. When examining cold fluorination conditions with ^{19}F, only the conjugated elimination product and starting material were observed.

With this in mind, we changed our approach to fluorination from an acyclic to a cyclic substrate, based off previous success on fluorinating cyclic compounds.[26] Our substrate in this scenario was ribolactone **25**. In this route, we envisioned substituting the primary alcohol with a latent amine group and then activating the secondary alcohol as a leaving group. Then, under basic conditions, open the lactone ring and oxidation of the remaining alcohol up to the ketone. Based on the protecting group of the amine, a corresponding deprotection step would yield the fluorinated 5-ALA analog.

Activation of diol **25** through reaction with TsCl resulted in selective tosylation of the primary alcohol (**26**, 74%).[27] Then, to see if an amine could be used to substitute the tosylate, a few conditions were explored. Ultimately, the only successful formation of a C-N bond at the primary position was with sodium azide to obtain **27** (49%).[27] Taking this forward, the secondary alcohol was successfully mesylated (producing **28** in 58% yield).[17] But, when subjected to typical cold fluorination conditions (e.g. CsF in *tert*-butanol), only starting material and elimination product **30** were recovered (30% isolated) with no observation of fluorinated product **29** (Figure 6).

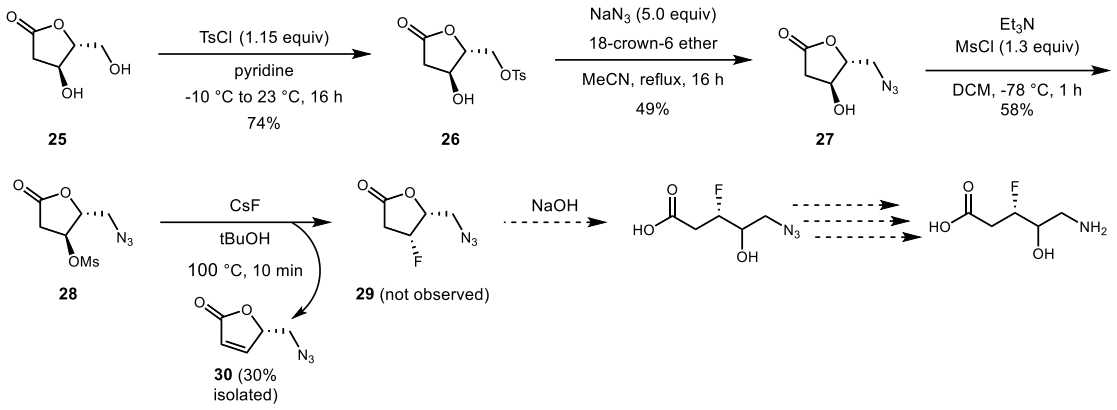
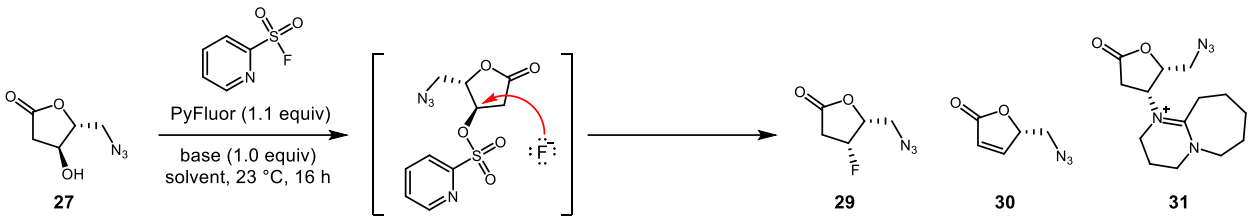


Figure 6. Synthetic route to 3-^{18}F-5-ALA via ribolactone **25**.

Considering this drawback of elimination occurring yet again, we planned out another approach involving the use of PyFluor, an electrophilic fluorinating agent developed by the Doyle group at Princeton University. This strategy takes advantage of the nucleophilicity of an alkoxy anion to generate both a fluorine nucleophile and activated leaving group in one pot, supposedly increasing the relative rate of substitution versus elimination. Furthermore, it has been shown that the ^{18}F-version of PyFluor can be synthesized in a straightforward manner from the starting 2-pyridinesulfonyl chloride, suggesting direct application to radiosynthetic methodology.[28]

Table 2. Base and Solvent Screening Conditions for Pyfluor Strategy

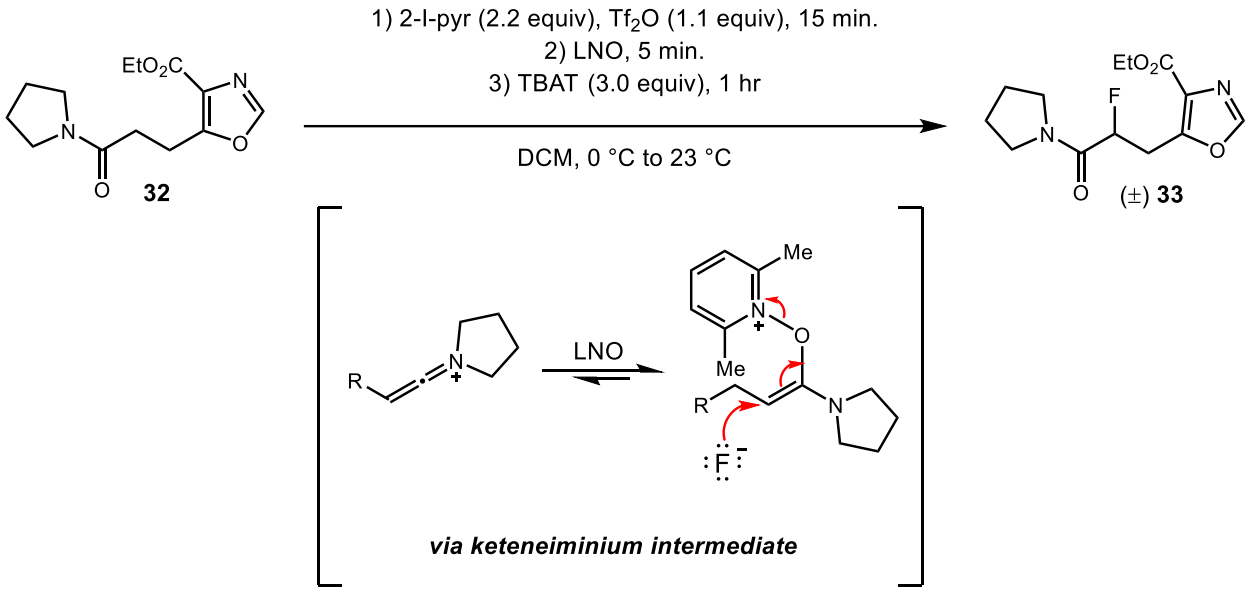


base	solvent	result
DBU	MeCN	Only observe **31** (not isolated)
DBU	DMF	Only observe **31** (not isolated)
DBU	THF	Only SM present
DBU	EtOAc	Only SM present
DIPEA	EtOAc	Only SM present
Et_3N	EtOAc	Only SM present
Pyridine	EtOAc	Only SM present

Despite changes in base and/or solvent, it was found that neither elimination product nor substitution product were present in the crude reaction mix. Interestingly, in the reactions utilizing Bronsted base DBU, a side product was observed like those reported by Doyle's group, but not isolated (**31**).[28] Other conditions were postulated, but ultimately this route was explored no further (Table 2).

Keeping the issue of elimination in mind, inspiration was found from a method of fluorination developed by the Maulide group from the University of Vienna, based on other previously established methods.[29a-d] This novel approach to fluorination takes advantage of both the high instability of a keteneiminium intermediate along with reverse polarity umpolung reactivity to achieve alkyl fluorination on otherwise inert substrates. This strategy follows the proposed mechanism: the amide carbonyl is activated with triflic anhydride to form an *O*-

trifyliminium species. This transient intermediate is then driven into an equilibrium with the corresponding keteneiminium with the aid of non-nucleophilic halopyridine base. The iminium species is then intercepted with a nitrogen oxide agent (such as 2,6-lutidine-N-oxide) to yield an enolate-type intermediate (aka "enolonium"; Scheme 1).[29]



Scheme 1. α-amide fluorination strategy via Nuno Maulide's group to access 2-^{18}F-5-ALA analog.

The now electrophilic character of the enolate carbon center enables the possibility of introducing a nucleophile (in this case, a fluoride) to obtain an α-fluorinated product. We envisioned using oxazole **18** from a previous route to make a tertiary amide, which could then be subjected to Maulide's fluorination conditions. The pyrrolidine oxazole amide was synthesized with relative ease (**32**, 60%; Figure 7).[29]

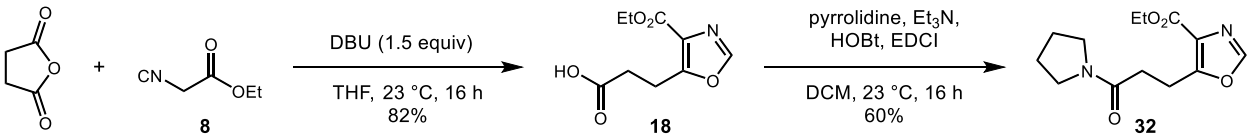


Figure 7. Synthetic route to amide oxazole **32** via previously accessed carboxy oxazole **18**.

Initially, when applying conditions identical to Maulide's fluorination technique, no fluorination product was observed. However, a simple switch in order of reagent addition (taking inspiration from Faigl et. al.'s mechanistic studies) resulted in fluorination product being observed and isolated (35% isolated of **33**).[30] Despite this initial success, the main concern here (as with every synthetic strategy attempted thus far) was successful translation of reaction conditions to those under a radiochemical context. First, to see if source of fluoride could be changed for something more amenable to radiolabeling conditions, it was possible to obtain fluorinated product with potassium fluoride by several subtle manipulations of reaction time and temperature (~32% ^{19}F-NMR yield; 21% isolated of **33**; Figure 8).

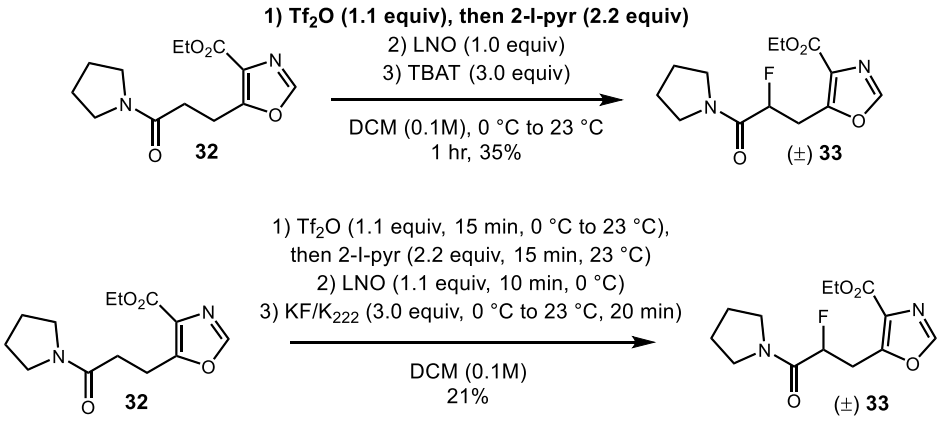
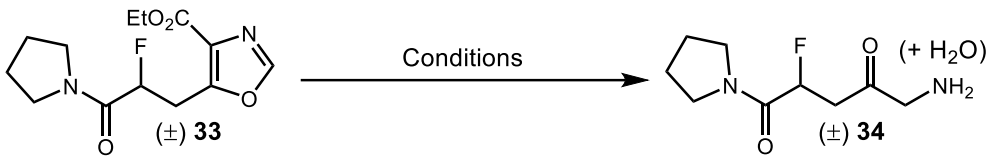


Figure 8. Maulide-esque conditions to access 2-^{18}F-5-ALA precursor. Note that addition of triflic anhydride and 2-Iodopyridine are switched from Maulide's original conditions.

An issue that presented itself in this scenario was product elimination. It was found that subjecting fluorinated product **33** to the same fluorination conditions in Step 3 of the reaction sequence resulted in complete elimination. However, it was possible to separate the desired product from the elimination side product via preparative HPLC. After addressing isolation of product, we decided to tackle other issues with this route.

A concern which arose during deprotection trials was the difficulty in hydrolyzing the amide to the desired carboxy terminus. This was observed during initial studies with the fluorinated product (Table 3).

Table 3. Initial Deprotection Attempts Resulting in Partial Deprotection



Conditions	Result*
3N HCl (aq), 100 °C, 20 min	100% conversion to **34**
3N HCl (aq), 100 °C, 20 min	100% conversion to **34**
12N HCl, 100 °C, 20 min	100% conversion to **34**

* Determined via ^{1}H-NMR and LC/MS

We decided to try different amides to favor complete hydrolysis. It was found that with the *N*-methylaniline amide (**35**; 52%), a two-step one-pot deprotection was required for global deprotection with sodium hydroxide and hydrochloric acid (Figure 9).[30,31]

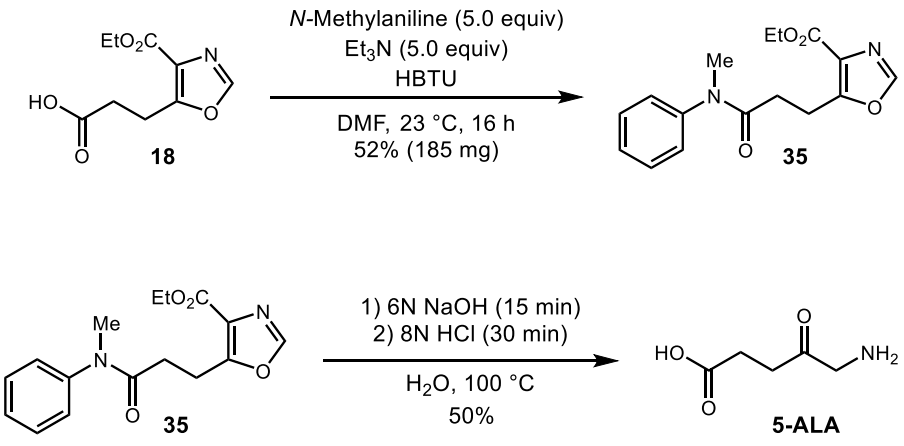


Figure 9. Synthesis of *N*-methylaniline oxazole amide **35** and global deprotection/isolation to yield 5-ALA.

However, the major drawback was now the stoichiometry of the reagents used. Under radiolabeling conditions, there would be an excess of substrate and the fluoride would be severely limited, due to the abundance (or lack thereof) of ^{18}F generated. So far, under cold fluorination conditions, we were able to achieve relative success with excess fluoride and limited substrate. But when mimicking the radiolabeling conditions, it was found that no product was formed. We regrettably decided to set aside this route as it stood.

With fluorination of the backbone presenting several hurdles, derivatization of the carboxy terminus of 5-ALA caught our attention. In this case, we can take inspiration from the 5-ALA derivative aminolevulinic acid hexyl ester, which has found use in fluorescence-guided surgery to help visualize tumorous tissue in bladder cancer diagnosis.[32,33] The aim here was to make new ^{18}F-5-ALA fluoroethyl and fluoropropyl esters to see potential uptake in brain tumor cells.

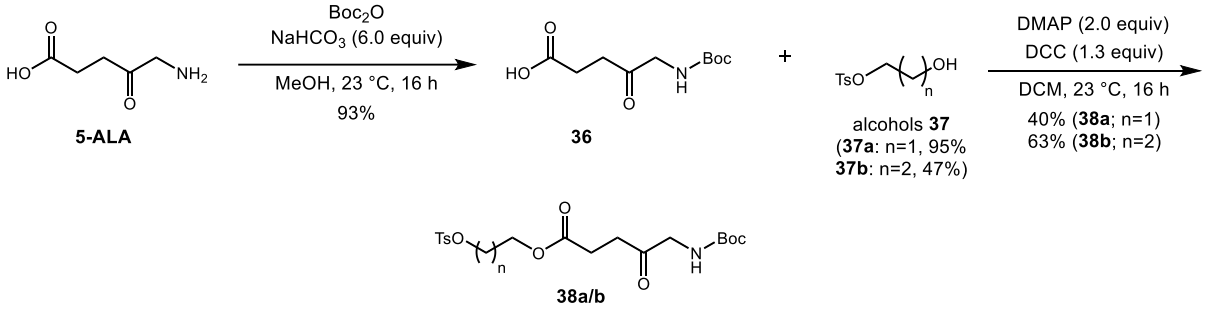


Figure 10. Synthetic route to ^{18}F-5-ALA ethyl and propyl ester precursors **38a** and **38b**, respectively.

The preparation here was straightforward: 5-ALA was boc-protected (**36**; 93%), while mono-tosylated ethylene glycol and 1,3-propanediol were separately prepared (**37a**, 95%; **37b**, 47%).[34,35] Through DCC-coupled transesterification, both the ethyl and propyl tosylate esters were successfully isolated and delivered to the Goodman lab (**38a**, 40%; **38b**, 63%, Figure 10).[36]

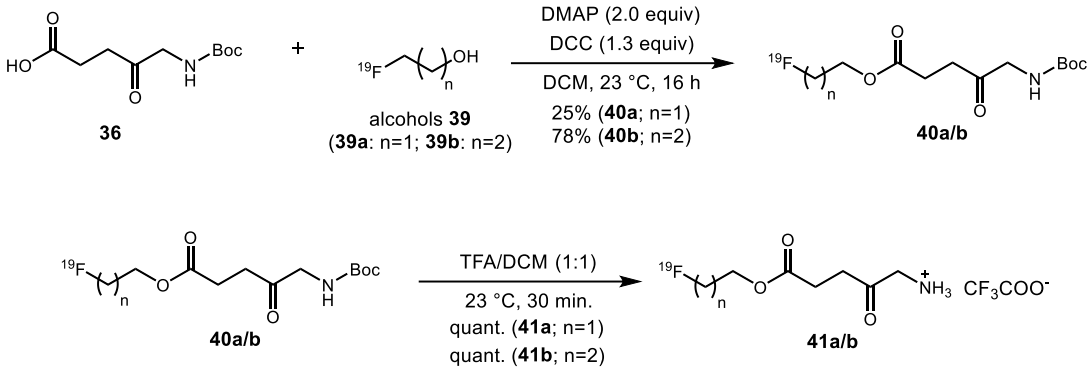


Figure 11. Synthetic route to ^{19}F-5-ALA ethyl and propyl ester HPLC standards **41a** and **41b**, respectively.

We also prepared cold ^{19}F standards of either analog, using either fluoropropanol (**39a**) or fluoroethanol (**39b**) as the esterification partner (**40a**, 25%; **40b**, 78%).[36] These fluorinated compounds were then successfully deprotected to have ^{19}F propyl and ethyl ester 5-ALA derivatives as HPLC standards for radiolabeling (**41a/b**, quant., Figure 11). To our delight, the propyl tosylate ester **42** was successfully radiolabeled and deprotected in a low RCY of 8% (Figure 12).

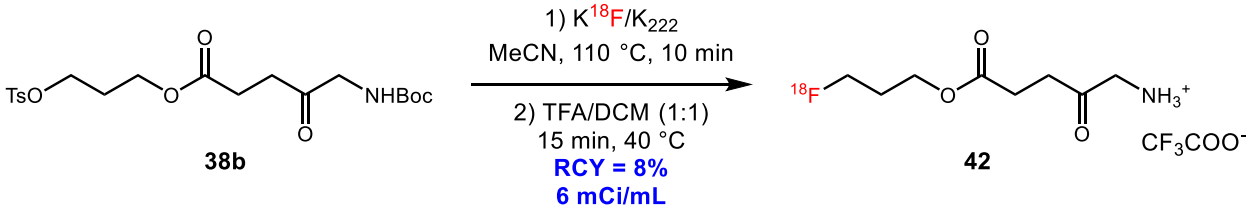


Figure 12. Radiosynthesis of ^{18}F-5-ALA propyl ester **42**, with 6 mCi/mL of remaining radioactivity.

This was enough to submit for cell studies in human and rat tumor cell lines. Aside from relatively greater uptake of **42** in human tumor cells as compared to rat cells, minimal uptake (<5%) of the fluoropropyl 5-ALA analog was observed across all cell lines tested (Table 4). One hypothesis is that the ester hydrolyzed upon cell incorporation.

Table 4. Decay-Corrected % Uptake Across Tumorous (9L, U87) and Non-Tumorous (DU145) Cell Lines.*

	9L (rat) Avg. % uptake	U87ΔEFGR (human) Avg. % uptake	DU145 (human control) Avg. % uptake
control	0.8	3.37	1.30
5-ALA	1.15	0.25	0.82
β-ALA	0.72	3.34	1.36
Ala+Gly	0.64	3.40	1.35
Phe+Tyr	0.8	3.56	1.81

*Radioactivity measured in μCi over 30 min. % uptake measured per 0.5 million cells. Values above are averaged over 5 trials.

Conclusion and Future Work

Precursors to the 2-position, 3-position, and esterified ^{18}F-5-ALA analogues were successfully synthesized and delivered to our collaborators for further biological testing. So far, only the ^{18}F-fluoropropyl ester analog was submitted for cell studies, and uptake into tumor cells was not observed. Further investigation into the PyFluor route is needed, based on the in-depth optimization studies done more recently by Doyle's group involving manipulation of both sulfonyl fluoride and base structure.[37] For the amide fluorination, there are several other amides to study to achieve a greater yield under radiolabeling reaction conditions. If results show significant uptake and fluorescence of ^{18}F-5-ALA in the model cell lines under PET scans, we will work to modify our current synthetic routes to achieve enantioselectivity, either using chiral auxiliaries/metal complexes to control aldol processes (for 3-fluoro-5-ALA precursors) or chiral Lewis acids to control cyanohydrin formation (for 2-fluoro-5-ALA precursors). For the 3-^{18}F-5ALA precursors (both cyclic and acyclic), if it is found that ^{18}F-S_N2-displacement is slow or results in primarily elimination product, the more reactive brosylate or nosylate may be employed in place of the mesylate leaving group. Another strategy with the 2-^{18}F-5-ALA precursor could be to employ catalytic conditions via Lalic's fluorination chemistry.[25]

It is possible that ^{18}F attached to a carbon adjacent to either the 1- or 4- carbonyl of 5-ALA could undergo *in vivo* defluorination. In the event of this occurrence, we will use the 2- or 3-deuterated analogues (^{18}FCD) to enhance the *in vivo* stability of these ^{18}F-5-ALA ligand candidates.

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Supporting Information

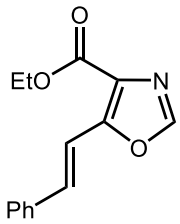
I. General Information

All reactions were carried out in oven-dried glassware, equipped with a stir bar and under a nitrogen atmosphere with dry solvents under anhydrous conditions, unless otherwise noted. All solvents were purified by passing over activated alumina and storing under argon. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogenous materials, unless otherwise stated. Reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. *n*-Butyllithium (*n*-BuLi) was used as a 2.5M solution in hexanes (Aldrich), was stored at 4°C and titrated prior to use. Organic solutions were concentrated under reduced pressure on a Buchi or Heidolf rotary evaporator using a water bath. Chromatographic purification of products was accomplished using forced-flow chromatography on 230-400 mesh silica gel according to the method of Still. Thin-layer chromatography (TLC) was performed on 250μm SiliCycle silica gel F-254 plates. Visualization of the developed chromatogram was performed by fluorescence quenching or by staining using $KMnO_4$, p-anisaldehyde, or ninhydrin stains. ^{1}H and ^{13}C NMR spectra were obtained from the Emory University NMR facility and recorded on an Inova (600 MHz), Bruker (600 MHz), Inova (500 MHz), VNMR (400 MHz), or Mercury (300 MHz), and are internally referenced to residual protio solvent signals. Data for ^{1}H NMR are reported as follows: chemical shift (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, b = broad), coupling constant (Hz), integration, and assignment. Data for decoupled ^{13}C NMR are reported in terms of chemical shift and multiplicity when applicable. IR spectra were recorded on a Thermo Fisher Diamond-ATR and reported in terms of frequency of absorption (cm^{-1}). Gas Chromatography Mass Spectrometry (GC-MS) was performed on an Agilent 5977A mass spectrometer with an Agilent 7890A gas chromatography inlet. Liquid Chromatography Mass Spectrometry (LC-MS) was performed on an Agilent 6120 mass 4 spectrometer with an Agilent 1220 Infinity liquid chromatography inlet.

II. Radiochemistry

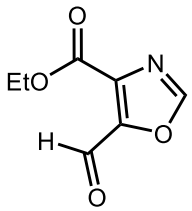
The [^{18}F]fluoride was produced at Emory University CSI with an 11MeV Siemens RDS 111 negative-ion cyclotron (Knoxville, TN) by the ^{18}O(p, n)^{18}F reaction using [^{18}O]H_2O (95%). Alumina N SepPaks and HLB Oasis cartridges were purchased from Waters, Inc. (Milford, MA).

II-A. Synthesis of 5-aminolevulinic acid (5-ALA) analogues



Ethyl (*E*)-5-styryloxazole-4-carboxylate (9): In an oven-dried flask open to air, ethyl isocyanoacetate (2.52 mL, 23 mmol) along with 30 mL dry ACN were added. To this stirred solution was added DBU (5.18 mL, 34.6 mmol), and the dark brown mixture was allowed to stir for 15 minutes. Then, cinnamoyl chloride (5.0 g, 30 mmol) was added in portions as HCl gas gradually developed. The flask was topped with a septum and needle to allow HCl gas to escape. The reaction was allowed to stir at room temperature for 16 h. The reaction was quenched with saturated sodium bicarbonate. The reaction mixture was then poured over water and extracted with ethyl acetate, dried with $MgSO_4$, and concentrated under reduced pressure to afford an oil. The crude mixture was purified by column chromatography in 15-50% EtOAc/Hexanes (2.0 g, 55% yield).[2]

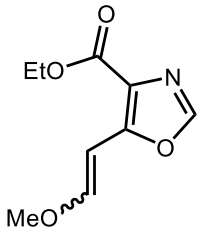
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.83 (s, 1H), 7.65 (d, J = 16.5 Hz, 1H), 7.62 – 7.57 (m, 2H), 7.45 – 7.34 (m, 4H), 4.47 (q, J = 7.1, 1.6 Hz, 2H), 1.47 (t, J = 7.2, 1.6 Hz, 3H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 161.9, 154.7, 149.0, 135.6, 135.3, 129.3, 128.9, 127.4, 126.7, 112.9, 61.2, 14.3.
LRMS (ESI, APCI) *m/z*: calc'd for $C_{14}H_{13}NO_3$ [M] 243.1, found $[M+H]^+$ 243.9



Ethyl 5-formyloxazole-4-carboxylate (10): To an oven-dried flask open to air and charged with a stir bar was added **ethyl (*E*)-5-styryloxazole-4-carboxylate** (500 mg, 2.1 mmol) along with THF/H_2O (14 mL:7 mL). To this stirred solution was added catalytic osmium tetroxide (~3 crystals). The solution immediately turned to black. Next, sodium periodate (1.32 g, 6.2 mmol) was added in portions to the reaction mixture. The flask was topped with a septum, and the mixture was left to stir for 16 h at room temperature. Over the course of the reaction, sodium iodate will precipitate out of solution as a pale-yellow solid. Reaction completion was determined by TLC. The reaction mixture was then diluted in EtOAc, extracted with H_2O (3x), and washed with $NaHCO_3$ and brine (1X each). The organic layers were dried with $MgSO_4$, filtered, and concentrated under reduced pressure to afford a dark-colored oil. The crude mixture was purified by column chromatography in 15-50% EtOAc/Hexanes (270 mg, 78% yield).[3]

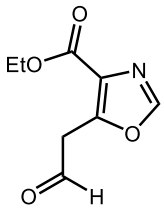
^{1}H NMR (600 MHz, $CDCl_3$) δ 10.34 (s, J = 1.0 Hz, 1H), 8.08 (s, J = 1.0 Hz, 1H), 4.50 (q, J = 7.2, 0.9 Hz, 2H), 1.45 (t, J = 7.2, 1.0 Hz, 3H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 178.1, 159.7, 152.8, 150.1, 138.1, 62.6, 14.1.
LRMS (ESI, APCI) *m/z*: calc'd for $C_7H_7NO_4$ [M] 169.0, found $[M\text{-}CHO]^+$ 140.9



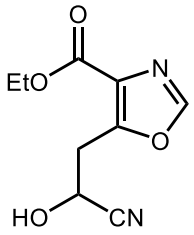
Ethyl (*E*/*Z*)-5-(2-methoxyvinyl)oxazole-4-carboxylate (22): To an oven-dried 3-neck flask under nitrogen equipped with a stir bar was added (methoxymethyl)triphenylphosphonium chloride (1.74 g, 5.08 mmol) along with THF (20 mL). The solution was cooled to 0 °C before the addition of potassium *tert*-butoxide (570 mg, 5.08 mmol) in one portion. The reaction mix was allowed to stir for 30 minutes or until solution turned a deep red. Next, while keeping the reaction mix at 0 °C, a solution of **ethyl 5-formyloxazole-4-carboxylate** (716 mg, 4.23 mmol) in 20 mL THF was added via addition funnel over 30 minutes. The dark yellow reaction mixture was allowed to stir at 0 °C and warm to room temperature over 4 h. The reaction mixture was quenched with saturated ammonium chloride, extracted with EtOAc, dried with $MgSO_4$, filtered, and concentrated under reduced pressure to afford a dark yellow oil. The crude mixture was purified by column chromatography in 30-75% EtOAc/Hexanes (672 mg, 81% yield, E/Z = 1.7:1).[4]

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.70 (s, 1H), 7.57 (s, 2H), 7.38 (d, J = 13.0 Hz, 2H), 6.32 – 6.26 (m, 3H), 5.99 (d, J = 7.1 Hz, 1H), 4.31 (qd, J = 7.1, 1.9 Hz, 7H), 3.82 (s, 4H), 3.70 (s, 6H), 1.33 (td, J = 7.1, 4.9 Hz, 10H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 162.3, 162.1, 154.9, 154.8, 153.3, 152.0, 148.3, 147.3, 123.5, 92.1, 91.6, 61.8, 60.9, 60.8, 56.9, 14.3, 14.3.
LRMS (ESI, APCI) *m/z*: calc'd for $C_9H_{11}NO_4$ [M] 197.0, found $[M+H]^+$ 197.9



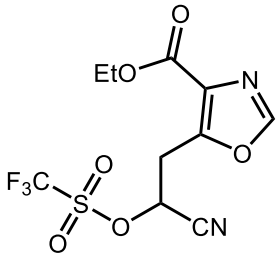
Ethyl 5-(2-oxoethyl)oxazole-4-carboxylate (21): In an oven-dried flask equipped with stir bar and open to air was added a solution of **ethyl (*E*/*Z*)-5-(2-methoxyvinyl)oxazole-4-carboxylate** (both isomers) (652 mg, 3.31 mmol) in chloroform (30 mL). Next, a solution of oxalyl chloride (0.28 mL, 3.31 mmol) in chloroform (3 mL) was added dropwise to the reaction mixture. Finally, ethanol (0.19 mL, 3.31 mmol) and water (0.06 mL, 3.31 mmol) were added, and the yellow reaction mixture was allowed to stir at room temperature for 6 h. Then, heat the reaction to 40 °C and allow to stir overnight. The reaction was quenched with saturated sodium bicarbonate, extracted with EtOAc, dried with $MgSO_4$, filtered, and concentrated under reduced pressure to afford a light yellow oil. The crude mix was taken forward without any further purification.[5]

^{1}H NMR (399 MHz, $CDCl_3$) δ 9.72 (s, 1H), 7.87 (s, J = 1.0 Hz, 1H), 4.46 – 4.28 (m, 2H), 4.20 (d, J = 1.4, 1.0 Hz, 2H), 1.48 – 1.30 (m, 3H).



Ethyl 5-(2-cyano-2-hydroxyethyl)oxazole-4-carboxylate (23): In an oven-dried flask equipped with stir bar and open to air was added sodium bisulfite (223 mg, 2.14 mmol) along with water (10 mL). The reaction mixture was cooled to 0 °C. Then, a solution of **ethyl 5-(2-oxoethyl)oxazole-4-carboxylate** (552 mg, 3.01 mmol) in ACN (10 mL) was added. Finally, a solution of sodium cyanide (590 mg, 12.04 mmol) in water (10 mL) was added to the flask. The reaction mixture turned a dark brown and was allowed to stir overnight. The mixture was diluted in EtOAc, extracted three times with water, washed with brine, dried with $MgSO_4$, filtered, and concentrated under reduced pressure to afford a light-yellow oil. The crude mixture was purified by column chromatography in 50-75% EtOAc/Hexanes (140 mg, 22% yield).[6]

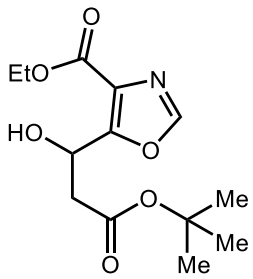
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.89 (s, 1H), 4.95 – 4.84 (m, 1H), 4.42 (q, J = 7.2, 1.0 Hz, 2H), 4.13 (d, J = 6.8, 1.0 Hz, 1H), 3.64 (qd, 2H), 1.42 (t, J = 7.1, 1.0 Hz, 3H).
^{13}C NMR (126 MHz, $CDCl_3$) δ 162.6, 152.9, 150.2, 130.1, 118.3, 62.1, 59.6, 32.2, 14.1.
LRMS (ESI, APCI) *m/z*: calc'd for $C_9H_{10}N_2O_4$ [M] 210.0, found $[M+H]^+$ 210.8



Ethyl 5-(2-cyano-2-(((trifluoromethyl)sulfonyl)oxy)ethyl)oxazole-4-carboxylate (24): In an oven-dried vial equipped with stir bar under N_2 was added **ethyl 5-(2-cyano-2-hydroxyethyl)oxazole-4-carboxylate** (130 mg, 0.62 mmol) along with CH_2Cl_2 (6 mL). The reaction mixture was cooled to -78 °C, and 2,6-lutidine (0.11 mL, 0.99 mmol) was added dropwise. While keeping the temperature at -78 °C, triflic anhydride (0.12 mL, 0.74 mmol) was added dropwise over 5 minutes. The reaction mixture was allowed to stir for 25 minutes. While still in the ice bath, the reaction was then quenched with 0.5M H_2SO_4 (6 mL). It is important that the work-up be conducted as quickly as possible, to prevent decomposition of the triflate. The vial was removed from the cold bath and the reaction mixture transferred to a separatory funnel. This was extracted 3X with CH_2Cl_2. The organic layers were washed with brine, dried with $MgSO_4$, filtered, and concentrated under reduced pressure to afford a light-yellow oil. By ^{1}H NMR, the crude mixture was determined to consist of desired triflate product in >95% purity (204 mg, 96% yield).

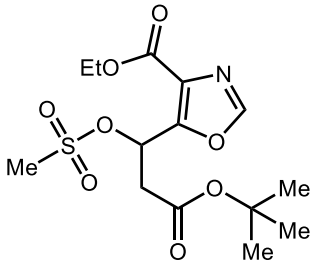
Thus, no further purification was conducted and the triflate was stored in benzene under N_2 at -20 °C prior to fluorination.[7]

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.95 (s, 1H), 5.78 (t, J = 7.7, 6.0 Hz, 1H), 4.44 (q, 2H), 3.90 (m, 2H), 1.43 (t, J = 7.2, 1.4 Hz, 3H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 161.2, 150.9, 149.1, 131.0, 118.2 (q, J = 320.1 Hz), 112.7, 68.2, 62.1, 30.8, 14.1.
LRMS (ESI, APCI) *m/z*: calc'd for $C_9H_{10}N_2O_4$ [M] 342.01, found $[M+H]^+$ 342.7



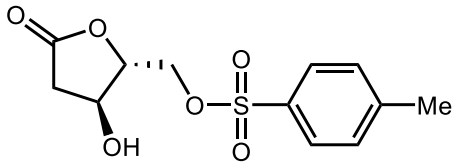
Ethyl 5-(3-(tert-butoxy)-1-hydroxy-3-oxopropyl)oxazole-4-carboxylate (13): To oven-dried three-neck round-bottom flask was added diisopropylamine (0.26 mL, 1.85 mmol) along with THF (1 mL). This reaction mixture was cooled to 0 °C, and then *n*-BuLi (0.65 mL, 2.5 M solution in hexanes) was added dropwise. This reaction mixture was left to stir for 20 minutes, and then was cooled to -78 °C. A solution of *tert*-butyl acetate (0.19 mL, 1.42 mmol) in THF (7 mL) was added dropwise to the flask, and this mixture was left to stir for 20 minutes. While maintaining the temperature at -78 °C, a solution of **ethyl 5-formyloxazole-4-carboxylate** (240 mg, 1.42 mmol) in THF (7 mL) was added very slowly dropwise to the reaction flask. The reaction mixture was allowed to stir for 1 h at -78 °C, and then removed from the ice bath to stir for 1 h while warming to room temperature. The mixture was quenched with saturated ammonium chloride, extracted three times with EtOAc, washed with brine, dried with $MgSO_4$, filtered, and concentrated under reduced pressure to afford a yellow oil. The crude mixture was purified by column chromatography in 20-50% EtOAc/Hexanes (209 mg, 52% yield).[8]

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.81 (s, 1H), 5.65 – 5.50 (m, 1H), 4.54 (d, 1H), 4.44 (q, J = 7.1, 1.5 Hz, 2H), 2.84 (qd, 2H), 1.50 – 1.39 (m, 12H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 169.78, 162.70, 159.36, 149.10, 127.89, 81.78, 63.03, 62.00, 40.75, 28.04, 14.22.
LRMS (ESI, APCI) *m/z*: calc'd for $C_{13}H_{19}NO_6$ [M] 285.12, found $[M\text{-}C(CH_3)_3]^+$ 229.9



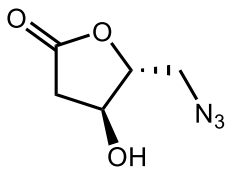
Ethyl 5-(3-(tert-butoxy)-1-((methylsulfonyl)oxy)-3-oxopropyl)oxazole-4-carboxylate (14): In oven-dried vial equipped with stir bar under N_2 was added a solution of **ethyl 5-(3-(tert-butoxy)-1-hydroxy-3-oxopropyl)oxazole-4-carboxylate** (199 mg, 0.70 mmol) in CH_2Cl_2 (5 mL). This reaction mixture was cooled to -78 °C, and triethylamine (0.1 mL, 0.70 mmol) was slowly added dropwise. While maintaining the temperature at -78 °C, methanesulfonyl chloride (0.07 mL, 0.91 mmol) was slowly added dropwise. The reaction mixture was allowed to stir at -78 °C for 45 minutes. While still in the ice bath, the reaction mixture was quenched with MeOH. The solution was then transferred to a separatory funnel, diluted in EtOAc, extracted with H_2O (3X), washed with saturated ammonium chloride (1X), and washed with brine (1X). The work-up was conducted as quickly as possible to prevent elimination/decomposition of the mesylate. The organic layers were dried with $MgSO_4$, filtered, and concentrated under reduced pressure to afford a yellow oil. The crude mixture was purified by column chromatography in 20-50% EtOAc/Hexanes (168 mg, 66% yield).[9]

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.92 (s, 1H), 6.63 (dd, J = 8.5, 5.9 Hz, 1H), 4.49 – 4.39 (m, 2H), 3.16 (dd, J = 16.5, 8.5 Hz, 1H), 3.09 (s, 3H), 2.93 (dd, J = 16.5, 5.8, 1.2 Hz, 1H), 1.47 – 1.40 (m, 4H), 1.42 (s, 9H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 167.19, 160.87, 152.51, 150.72, 129.58, 82.41, 69.25, 61.94, 39.34, 38.14, 27.92, 14.17.
LRMS (ESI, APCI) *m/z*: calc'd for $C_{14}H_{21}NO_8S$ [M] 363.1, found $[M\text{-}C(CH_3)_3]^+$ 307.8



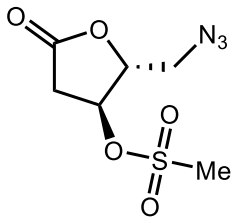
((2*R*,3*S*)-3-hydroxy-5-oxotetrahydrofuran-2-yl)methyl 4-methylbenzenesulfonate (26): In oven-dried round bottom flask equipped with stir bar was added (4*S*,5*R*)-4-hydroxy-5-(hydroxymethyl)dihydrofuran-2(3*H*)-one (2.5 g, 19 mmol). The atmosphere was exchanged with N_2 (3X), and dry pyridine (30 mL) was added to the flask. Then, the reaction mix was cooled to -10 °C. *p*-toluene sulfonyl chloride (4.15 g, 22 mmol) was added in one portion to the reaction flask. The reaction was warmed to room temperature and allowed to stir overnight. The reaction mixture was quenched with aqueous 0.5M HCl solution. The solution was then extracted with EtOAc (3X) and washed with brine (1X). The organic layers were dried with $MgSO_4$, filtered, and concentrated under reduced pressure to afford a yellow oil. The crude mixture was purified by column chromatography in 40-80% EtOAc/Hexanes (3.97 g, 74% yield). The physical properties and spectra were in accord with the previously reported values.[10]

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.75 (d, J = 7.7, 1.3 Hz, 2H), 7.39 – 7.33 (d, 2H), 4.67 – 4.55 (m, 1H), 4.52 – 4.44 (q, 1H), 4.27 (dd, J = 11.2, 3.1, 0.9 Hz, 1H), 4.14 (dd, J = 11.3, 3.5, 0.9 Hz, 1H), 2.89 (dd, J = 18.2, 7.1, 0.8 Hz, 1H), 2.58 (dd, J = 4.5, 0.7 Hz, 1H), 2.51 (d, J = 18.1, 3.7, 0.9 Hz, 1H), 2.45 (s, 3H).



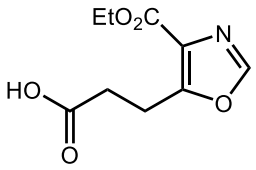
(4*S*,5*R*)-5-(azidomethyl)-4-hydroxydihydrofuran-2(3*H*)-one (27): In oven-dried round bottom flask equipped with stir bar was added solution of **((2*R*,3*S*)-3-hydroxy-5-oxotetrahydrofuran-2-yl)methyl 4-methylbenzenesulfonate** (500 mg, 1.75 mmol) and dry MeCN (17 mL). Next, sodium azide (568 mg, 8.74 mmol) and 18-crown-6 ether (463 mg, 1.75 mmol) were added rapidly to the reaction solution. Then, the reaction flask was equipped with a condenser and the reaction was set to reflux overnight. The reaction mixture was cooled to room temperature, and then filtered over a pad of celite. The filtrate was concentrated under reduced pressure to afford a light-yellow oil. The crude mixture was purified by column chromatography in 40-90% EtOAc/Hexanes (133 mg, 49% yield). The physical properties and spectra were in accord with the previously reported values.[11]

^{1}H NMR (600 MHz, $CDCl_3$) δ 4.51 – 4.43 (m, 2H), 3.67 (dd, J = 13.3, 4.1 Hz, 1H), 3.59 (dd, J = 13.3, 3.8 Hz, 1H), 3.00 – 2.89 (dd, 1H), 2.58 – 2.50 (dd, 1H), 2.39 (s, 1H).



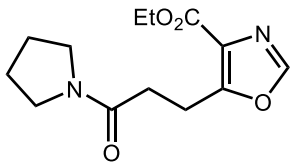
(2*R*,3*S*)-2-(azidomethyl)-5-oxotetrahydrofuran-3-yl methanesulfonate (28): In oven-dried vial equipped with stir bar under N_2 was added a solution of **(4*S*,5*R*)-5-(azidomethyl)-4-hydroxydihydrofuran-2(3*H*)-one** (15 mg, 0.096 mmol) in CH_2Cl_2 (0.5 mL). This reaction mixture was cooled to -78 °C, and triethylamine (13.4 µL, 0.096 mmol) was slowly added dropwise. While maintaining the temperature at -78 °C, methanesulfonyl chloride (10 µL, 0.124 mmol) was slowly added dropwise. The reaction mixture was allowed to stir at -78 °C for 1 h. While still in the ice bath, the reaction mixture was quenched with MeOH. The solution was then transferred to a separatory funnel, diluted in EtOAc, extracted with H_2O (3X), washed with saturated ammonium chloride (1X), and washed with brine (1X). The work-up was conducted as quickly as possible to prevent elimination/decomposition of the mesylate. The organic layers were dried with $MgSO_4$, filtered, and concentrated under reduced pressure to afford a yellow oil. The crude mixture was purified by column chromatography in 3:2 EtOAc/Hexanes (13.2 mg, 58% yield).[9]

^{1}H NMR (600 MHz, $CDCl_3$) δ 5.23 (m, J = 7.6, 2.3, 1.5 Hz, 1H), 4.78 (m, J = 3.2, 1.6 Hz, 1H), 3.78 – 3.70 (dd, 2H), 3.16 – 3.05 (m, 4H), 2.75 (dd, J = 18.9, 2.3, 1.4 Hz, 1H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 172.33, 82.34, 75.50, 52.57, 51.73, 38.67, 35.23, 30.93.
LRMS (ESI, APCI) *m/z*: calc'd for $C_6H_9N_3O_5S$ [M] 235.0, found $[M+H]^+$ 236.1



3-(4-(ethoxycarbonyl)oxazol-5-yl)propanoic acid (18): In an oven-dried flask under N_2 was added ethyl isocyanoacetate (0.84 mL, 7.69 mmol) along with 90 mL dry THF. To this stirred solution was added DBU (1.72 mL, 11.54 mmol), and the dark brown mixture was allowed to stir for 15 minutes. Then, a solution of succinic anhydride (1.0 g, 10 mmol) in dry THF (10 mL) was added to the reaction flask. The reaction was allowed to stir at room temperature overnight. The reaction was quenched with aqueous 1M potassium bisulfate solution. The reaction mixture was then extracted with ethyl acetate (3X), dried with $MgSO_4$, and concentrated under reduced pressure to afford a crude solid. The crude mixture was purified by column chromatography in 75% EtOAc/Hexanes (1.34 g, 82% yield).[2]

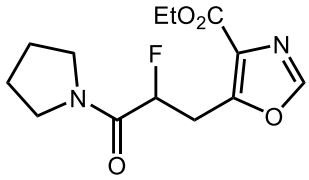
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (s, J = 1.3 Hz, 1H), 4.43 – 4.33 (q, 2H), 3.43 – 3.35 (t, 2H), 2.83 – 2.75 (t, 2H), 1.39 (t, J = 7.1, 0.8 Hz, 3H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 176.43, 161.79, 157.59, 149.25, 127.56, 61.24, 31.22, 21.25, 14.27.
LRMS (ESI, APCI) *m/z*: calc'd for $C_{13}H_{19}NO_6$ [M] 213.06, found $[M+H]^+$ 214.10



ethyl 5-(3-oxo-3-(pyrrolidin-1-yl)propyl)oxazole-4-carboxylate (32): In oven-dried vial equipped with stir bar was added HOBt (542 mg, 4.01 mmol) and EDCI (769 mg, 4.01 mmol). The atmosphere was exchanged with N_2 (3X). Next, triethylamine (0.56 mL, 4.01 mmol) and pyrrolidine (0.33 mL, 4.01 mmol) were added to the reaction mix along with CH_2Cl_2 (40 mL). Finally, a solution of **3-(4-(ethoxycarbonyl)oxazol-5-yl)propanoic acid** (856 mg, 4.01 mmol) in CH_2Cl_2 (10 mL) was added to the reaction flask, and the reaction allowed to stir at room temperature overnight. The reaction was quenched with 0.5M HCl solution. The solution was then transferred to a separatory funnel, diluted in EtOAc, extracted with H_2O (5X), washed with saturated sodium bicarbonate (5X), and washed with brine (1X). The organic layers were dried with $MgSO_4$, filtered, and concentrated under reduced pressure to afford a dark orange solid. The crude solid was taken forward without any further purification (640 mg, 60% yield).[11]

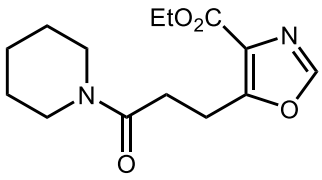
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.73 (s, J = 1.0 Hz, 1H), 4.36 (q, J = 7.1, 1.0 Hz, 2H), 3.50 – 3.30 (m, 6H), 2.72 – 2.59 (t, 2H), 1.89 (dt, J = 59.4, 6.8 Hz, 4H), 1.37 (t, J = 7.1, 1.0 Hz, 3H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 169.14, 161.98, 159.19, 148.93, 127.27, 61.07, 46.49, 45.79, 32.17, 26.10, 24.39, 21.55, 14.34.
LRMS (ESI, APCI) *m/z*: calc'd for $C_{13}H_{19}NO_6$ [M] 266.13, found $[M+H]^+$ 267.10



(±) ethyl 5-(2-fluoro-3-oxo-3-(pyrrolidin-1-yl)propyl)oxazole-4-carboxylate (33): In oven-dried vial equipped with stir bar under N_2 was added a solution of **ethyl 5-(3-oxo-3-(pyrrolidin-1-yl)propyl)oxazole-4-carboxylate** (53 mg, 0.2 mmol) in CH_2Cl_2 (2 mL). This reaction mixture was cooled to 0 °C, and triflic anhydride (37 µL, 0.22 mmol) was slowly added dropwise. The reaction vial was then removed from the ice bath and allowed to warm to room temperature over 15 minutes. Then, 2-iodopyridine (47 µL, 0.44 mmol) was added dropwise to the reaction vial, and the reaction allowed to stir for 15 minutes at room temperature. Next, the reaction vial was cooled down to 0 °C, and 2,6-lutidine-N-oxide (25 µL, 0.22 mmol) was added to the reaction vial. This was allowed to stir for 15 minutes at 0 °C. Finally, potassium fluoride (35 mg, 0.6 mmol) and Kryptofix-222 (226 mg, 0.6 mmol) were added as solids to the reaction vial in one portion. The reaction was then removed from the ice bath and allowed to stir for 30 minutes while warmed to room temperature. The reaction was quenched with saturated ammonium chloride. The solution was then transferred to a separatory funnel, diluted in CH_2Cl_2, extracted with H_2O (3X), and washed with brine (1X). The organic layers were dried with $MgSO_4$, filtered, and concentrated under reduced pressure to afford a brown oil. The crude mixture was purified by preparative HPLC in 20% MeOH/ H_2O (12 mg, 21% yield).[11]

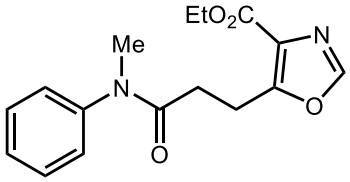
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.82 (s, J = 1.3 Hz, 1H), 5.44 – 5.29 (m, 1H), 4.42 – 4.33 (q, 2H), 3.74 – 3.45 (m, 6H), 2.00 – 1.91 (m, 2H), 1.89 – 1.82 (m, 2H), 1.40 – 1.35 (t, 3H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 161.86, 154.69, 149.77, 133.00, 132.11, 130.98, 129.97, 129.68, 129.64, 128.35, 88.44, 87.22, 69.23, 64.01, 61.33, 46.70, 46.34, 46.29, 30.93, 29.71, 29.37, 29.12, 28.97, 26.32, 23.67, 18.33, 14.30, 14.13.
LRMS (ESI, APCI) *m/z*: calc'd for $C_{13}H_{17}FN_2O_4$ [M] 284.1, found $[M+H]^+$ 285.1



ethyl 5-(3-oxo-3-(piperidin-1-yl)propyl)oxazole-4-carboxylate: In oven-dried vial equipped with stir bar was added HOBt (581 mg, 4.3 mmol) and EDCI (824 mg, 4.3 mmol). The atmosphere was exchanged with N_2 (3X). Next, triethylamine (0.6 mL, 4.3 mmol) and piperidine (0.42 mL, 4.3 mmol) were added to the reaction mix along with CH_2Cl_2 (40 mL). Finally, a solution of **3-(4-(ethoxycarbonyl)oxazol-5-yl)propanoic acid** (912 mg, 4.3 mmol) in CH_2Cl_2 (10 mL) was added to the reaction flask, and the reaction allowed to stir at room temperature overnight. The reaction

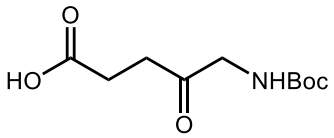
was quenched with 0.5M HCl solution. The solution was then transferred to a separatory funnel, diluted in EtOAc, extracted with H_2O (5X), washed with saturated sodium bicarbonate (5X), and washed with brine (1X). The organic layers were dried with $MgSO_4$, filtered, and concentrated under reduced pressure to afford a brown oil. The crude mixture was purified by column chromatography in 50-100% EtOAc/Hexanes (1.01 g, 84% yield).[11]

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.74 (s, J = 0.5 Hz, 1H), 4.42 – 4.32 (q, 2H), 3.54 (t, 2H), 3.42 – 3.33 (m, 4H), 2.72 (t, 2H), 1.62 – 1.50 (m, 6H), 1.38 (t, J = 7.2, 0.7 Hz, 3H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 168.83, 161.97, 159.18, 148.93, 127.28, 61.06, 46.45, 42.85, 30.81, 26.43, 25.52, 24.50, 21.97, 14.34.
LRMS (ESI, APCI) *m/z*: calc'd for $C_{14}H_{20}N_2O_4$ [M] 280.14, found $[M+H]^+$ 281.15



ethyl 5-(3-(methyl(phenyl)amino)-3-oxopropyl)oxazole-4-carboxylate (35): In oven-dried vial equipped with stir bar was added HBTU (444 mg, 1.17 mmol), **3-(4-(ethoxycarbonyl)oxazol-5-yl)propanoic acid** (250 mg, 1.17 mmol), and dry DMF (12 mL). Next, triethylamine (0.82 mL, 5.87 mmol) and *N*-methylaniline (0.64 mL, 5.87 mmol) were added to the reaction mix. The reaction was allowed to stir at room temperature overnight. The reaction was quenched with saturated ammonium chloride. The solution was then transferred to a separatory funnel, diluted in EtOAc, extracted with H_2O (3X), washed with 1M lithium chloride (5X), and washed with brine (1X). The organic layers were dried with $MgSO_4$, filtered, and concentrated under reduced pressure to afford a dark yellow oil. The crude mixture was purified by column chromatography in 40-90% EtOAc/Hexanes (185 mg, 52% yield).[11]

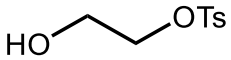
^{1}H NMR (600 MHz, $CDCl_3$) δ 7.68 (s, J = 1.3 Hz, 1H), 7.39 (t, J = 7.7, 1.7 Hz, 2H), 7.35 – 7.29 (t, 1H), 7.12 (d, J = 8.2, 1.3 Hz, 2H), 4.31 (q, J = 7.0, 1.7 Hz, 2H), 3.31 (t, 2H), 3.24 (s, J = 1.7 Hz, 3H), 2.43 (t, J = 7.5 Hz, 2H), 1.33 (t, J = 7.2, 1.8 Hz, 3H).
LRMS (ESI, APCI) *m/z*: calc'd for $C_{16}H_{18}N_2O_4$ [M] 302.13, found $[M+H]^+$ 302.9



5-((tert-butoxycarbonyl)amino)-4-oxopentanoic acid) (36): In oven-dried flask equipped with stir bar was added 5-aminolevulinic acid hydrochloride (5.0 g, 30 mmol), sodium bicarbonate (15.04 g, 179 mmol), and di-tert-butyl dicarbonate (6.51 g, 30 mmol). The atmosphere was exchanged with N_2 (3X). Next, dry MeOH (100 mL) was added to the reaction flask and the mixture was allowed to stir overnight at room temperature. The reaction mix was filtered over a pad of celite. The filtrate was concentrated under pressure, and the remaining crude residue was dissolved in H_2O. The aqueous solution was acidified with saturated potassium bisulfate until pH 2 was attained. This layer was extracted with EtOAc (3X). The organic layers were dried with

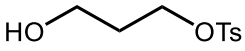
$MgSO_4$, filtered, and concentrated under reduced pressure to afford a light yellow solid. The crude mixture was taken forward without further purification (6.38 g, 93% yield). The physical properties and spectra were in accord with the previously reported values. [12]

^{1}H NMR (600 MHz, CD_3OD) δ 3.89 (s, J = 2.6 Hz, 2H), 2.69 (t, J = 7.5 Hz, 2H), 2.55 (t, J = 6.7, 2.5 Hz, 2H), 1.42 (m, J = 5.9, 2.8 Hz, 9H).



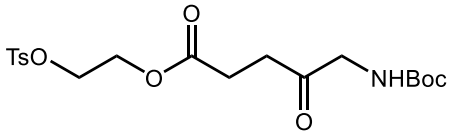
2-hydroxyethyl 4-methylbenzenesulfonate (37a): In an oven-dried flask equipped with stir bar under N_2 was added ethylene glycol (8.36 mL, 150 mmol). The reaction flask was cooled to 0 °C, and a solution of *p*-toluene sulfonyl chloride (1.90 g, 10 mmol) in dry pyridine (9.53 mL, 118 mmol) was added to the reaction mix over 18 minutes. The reaction was allowed to stir at 0 °C for 30 minutes. The reaction was quenched with saturated ammonium chloride (30 mL). The organic layers were extracted with $CHCl_3$ (3X), dried with $MgSO_4$, filtered, and concentrated under reduced pressure to afford a light-yellow oil. The crude mixture was purified by column chromatography in 50% EtOAc/Hexanes (2.1 g, 95% yield). The physical properties and spectra were in accord with the previously reported values.[13]

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.80 (d, J = 8.5, 2.2 Hz, 2H), 7.37 – 7.30 (d, 2H), 4.13 (m, J = 4.5, 2.5, 1.2 Hz, 2H), 3.81 (t, J = 4.4, 3.2 Hz, 2H), 2.44 (s, J = 1.9 Hz, 3H).



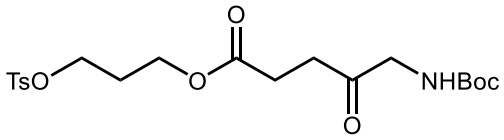
3-hydroxypropyl 4-methylbenzenesulfonate (37b): In oven-dried vial equipped with stir bar was added a solution of 1,3-propanediol (3.63 mL, 50 mmol) in CH_2Cl_2 (20 mL). Then, triethylamine (1.53 mL, 11 mmol) was added to the reaction flask. An addition funnel was attached to the reaction flask with a rubber septum. The atmosphere was exchanged for N_2 (3X) and the flask cooled to 0 °C. Next, a solution of *p*-toluene sulfonyl chloride (1.91 g, 10 mmol) in CH_2Cl_2 (15 mL) was added to the reaction flask dropwise over 30 minutes. Then, the reaction was allowed to warm to room temperature and stir for 18 h. The reaction mixture was concentrated under reduced pressure, yielding a viscous slurry with solid triethylamine·hydrochloride as precipitate. This slurry was taken up in minimal Et_2O (5 mL) and filtered. The filtrate was collected and concentrated under reduced pressure to yield a viscous oil. The crude mixture was purified by column chromatography in 60% EtOAc/Hexanes (1.08 g, 47% yield). The physical properties and spectra were in accord with the previously reported values.[14]

^{1}H NMR (600 MHz, $CDCl_3$) δ 7.79 (d, J = 8.3, 1.8 Hz, 2H), 7.37 – 7.31 (d, 2H), 4.17 (t, 2H), 3.71 (t, J = 6.1, 1.7 Hz, 2H), 2.44 (s, J = 1.7 Hz, 3H), 1.88 (tt, J = 6.0, 5.2 Hz, 2H).



2-(tosyloxy)ethyl 5-((tert-butoxycarbonyl)amino)-4-oxopentanoate (38a): In oven-dried flask equipped with stir bar was added **5-((tert-butoxycarbonyl)amino)-4-oxopentanoic acid** (2.8 g, 12.1 mmol), DCC (3.34 g, 16.2 mmol), and DMAP (2.97 g, 24.3 mmol). The atmosphere was exchanged for N_2 (3X). Then, dry DCM (70 mL) was added to the reaction flask. Next, a solution of **2-hydroxyethyl 4-methylbenzenesulfonate** (1.74 g, 8.1 mmol) in DCM (10 mL) was added to the reaction flask, and the reaction was allowed to stir at room temperature overnight. The reaction mixture was diluted in DCM, and the flask stored in freezer (-20 °C) for 30 minutes to precipitate out any DCU byproduct. The reaction mix was then filtered over a pad of celite. The filtrate was collected and diluted with 0.5M HCl. The solution was then transferred to a separatory funnel, extracted with $CHCl_3$ (3X) and washed with brine (1X). The organic layers were dried with $MgSO_4$, filtered, and concentrated under reduced pressure to afford a light-yellow oil. The crude mixture was purified by column chromatography in 20-70% EtOAc/Hexanes (1.4 g, 40% yield).[15]

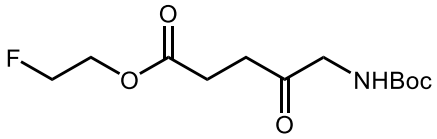
^{1}H NMR (600 MHz, CD_3OD) δ 7.79 (d, J = 8.3 Hz, 2H), 7.44 (d, J = 8.1 Hz, 2H), 4.20 (m, J = 3.6 Hz, 4H), 3.88 (s, J = 15.1 Hz, 2H), 2.69 (t, 2H), 2.48 (t, J = 6.6 Hz, 2H), 2.45 (s, 3H), 1.43 (s, 9H)
^{13}C NMR (151 MHz, $CDCl_3$) δ 202.27, 170.04, 153.79, 143.26, 130.89, 128.08, 126.08, 78.00, 65.58, 60.01, 48.39, 48.35, 48.24, 33.03, 32.27, 30.68, 28.89, 27.03, 26.43, 25.64, 24.28, 23.55, 23.45, 22.84, 19.78.
LRMS (ESI, APCI) *m/z*: calc'd for $C_{19}H_{27}NO_8S$ [M] 429.15, found $[M\text{-}CO_2C(CH_3)]^+$ 329.8



3-(tosyloxy)propyl 5-((tert-butoxycarbonyl)amino)-4-oxopentanoate (38b): In oven-dried flask equipped with stir bar was added **5-((tert-butoxycarbonyl)amino)-4-oxopentanoic acid** (680 mg, 3.0 mmol), DCC (825 mg, 4.0 mmol), and DMAP (733 mg, 6.0 mmol). The atmosphere was exchanged for N_2 (3X). Then, dry DCM (15 mL) was added to the reaction flask. Next, a solution of **3-hydroxypropyl 4-methylbenzenesulfonate** (451 mg, 2.0 mmol) in DCM (5 mL) was added to the reaction flask, and the reaction was allowed to stir at room temperature overnight. The reaction mixture was diluted in DCM, and the flask stored in freezer (-20 °C) for 30 minutes to precipitate out any DCU byproduct. The reaction mix was then filtered over a pad of celite. The filtrate was collected and diluted with 0.5M HCl. The solution was then transferred to a separatory funnel, extracted with $CHCl_3$ (3X) and washed with brine (1X). The organic layers were dried with $MgSO_4$, filtered, and concentrated under reduced pressure to afford a light-yellow oil. The crude mixture was purified by column chromatography in 20-70% EtOAc/Hexanes (558 mg, 63% yield).[15]

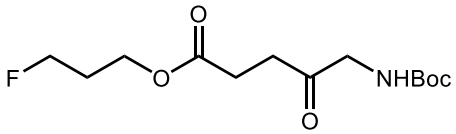
^{1}H NMR (600 MHz, CD_3OD) δ 7.77 (d, J = 7.9, 6.1, 1.8 Hz, 2H), 7.43 (d, 2H), 4.10 – 4.01 (m, J = 8.9, 6.3, 2.4 Hz, 4H), 3.86 (s, J = 11.9, 9.1, 4.0 Hz, 2H), 2.68 (t, J = 9.1, 6.4 Hz, 2H), 2.49 – 2.41 (m, 5H), 1.92 (dt, J = 9.3, 5.8 Hz, 2H), 1.42 (s, 9H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 202.39, 170.24, 153.76, 143.05, 130.99, 128.03, 126.04, 78.00, 64.91, 58.61, 48.37, 48.26, 32.37, 30.68, 28.88, 26.43, 26.28, 25.72, 24.28, 22.83, 19.76.
LRMS (ESI, APCI) *m/z*: calc'd for $C_{20}H_{29}NO_8S$ [M] 443.16, found $[M\text{-}CO_2C(CH_3)]^+$ 343.8



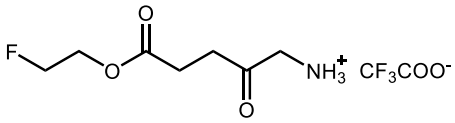
2-fluoroethyl 5-((tert-butoxycarbonyl)amino)-4-oxopentanoate (40a): In oven-dried flask equipped with stir bar was added **5-((tert-butoxycarbonyl)amino)-4-oxopentanoic acid** (100 mg, 0.433 mmol), DCC (119 mg, 0.577 mmol), and DMAP (106 mg, 0.867 mmol). The atmosphere was exchanged for N_2 (3X). Then, dry DCM (4 mL) was added to the reaction flask. Next, 2-fluoroethanol (17 µL, 0.289 mmol) was added to the reaction flask, and the reaction was allowed to stir at room temperature overnight. The reaction mixture was diluted in DCM, and the flask stored in freezer (-20 °C) for 30 minutes to precipitate out any DCU byproduct. The reaction mix was then filtered over a pad of celite. The filtrate was collected and diluted with 0.5M HCl. The solution was then transferred to a separatory funnel, extracted with $CHCl_3$ (3X) and washed with brine (1X). The organic layers were dried with $MgSO_4$, filtered, and concentrated under reduced pressure to afford a light-yellow oil. The crude mixture was purified by column chromatography in 15-60% EtOAc/Hexanes (20 mg, 25% yield).[15]

^{1}H NMR (500 MHz, CD_3OD) δ 4.65 – 4.60 (m, 1H), 4.54 – 4.50 (m, 1H), 4.34 – 4.29 (m, 1H), 4.29 – 4.23 (m, 1H), 3.90 (s, J = 2.4 Hz, 2H), 2.77 (m, J = 7.5, 5.7 Hz, 2H), 2.67 – 2.61 (m, 2H), 1.44 (s, J = 2.2 Hz, 9H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 209.90, 176.60, 160.95, 85.62, 84.51, 83.14, 67.55, 67.41, 53.33, 52.01, 51.87, 51.73, 51.59, 51.45, 51.31, 51.16, 37.46, 37.32, 35.76, 31.27, 31.08, 29.55, 28.57.
LRMS (ESI, APCI) *m/z*: calc'd for $C_{12}H_{20}FNO_5$ [M] 277.13, found $[M\text{-Boc}]^+$ 177.18



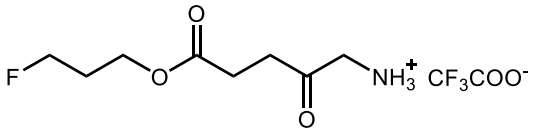
3-fluoropropyl 5-((tert-butoxycarbonyl)amino)-4-oxopentanoate (40b): In oven-dried flask equipped with stir bar was added **5-((tert-butoxycarbonyl)amino)-4-oxopentanoic acid** (100 mg, 0.433 mmol), DCC (119 mg, 0.577 mmol), and DMAP (106 mg, 0.867 mmol). The atmosphere was exchanged for N_2 (3X). Then, dry DCM (4 mL) was added to the reaction flask. Next, 3-fluoro-propan-1-ol (22 µL, 0.289 mmol) was added to the reaction flask, and the reaction was allowed to stir at room temperature overnight. The reaction mixture was diluted in DCM, and the flask stored in freezer (-20 °C) for 30 minutes to precipitate out any DCU byproduct. The reaction mix was then filtered over a pad of celite. The filtrate was collected and diluted with 0.5M HCl. The solution was then transferred to a separatory funnel, extracted with $CHCl_3$ (3X) and washed with brine (1X). The organic layers were dried with $MgSO_4$, filtered, and concentrated under reduced pressure to afford a light-yellow oil. The crude mixture was purified by column chromatography in 10-50% EtOAc/Hexanes (66 mg, 78% yield).[15]

^{1}H NMR (600 MHz, CD_3OD) δ 4.56 – 4.48 (m, 1H), 4.44 (m, J = 8.1, 3.4 Hz, 1H), 4.15 (dt, J = 10.6, 6.6 Hz, 2H), 3.87 (s, 2H), 2.79 – 2.68 (m, 2H), 2.62 – 2.53 (m, 2H), 1.98 (m, J = 25.4, 5.8 Hz, 2H), 1.43 (s, 9H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 209.96, 176.79, 160.96, 84.74, 83.65, 83.13, 64.37 (d, J = 5.7 Hz), 53.29, 37.40 (d, J = 30.1 Hz), 33.39 (d, J = 20.1 Hz), 31.21 (d, J = 10.9 Hz), 29.30, 28.60.
LRMS (ESI, APCI) *m/z*: calc'd for $C_{13}H_{22}FNO_5$ [M] 291.15, found $[M\text{-Boc}]^+$ 343.8



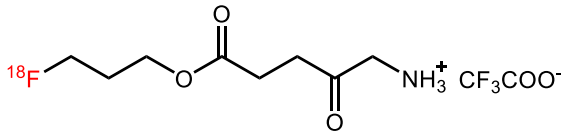
5-(2-fluoroethoxy)-2,5-dioxopentan-1-aminium trifluoroacetate (41a): In an oven-dried vial equipped with stir bar was added a solution of **2-fluoroethyl 5-((tert-butoxycarbonyl)amino)-4-oxopentanoate** (10 mg, 0.036 mmol) in CH_2Cl_2 (0.5 mL). Next, TFA (0.5 mL) was added to the reaction vial. The reaction was allowed to stir at room temperature for 30 minutes. Then, the reaction mix was concentrated under reduced pressure to afford desired salt in quantitative yield (10.4 mg).

^{1}H NMR (600 MHz, CD_3OD) δ 4.64 – 4.56 (m, 1H), 4.55 – 4.49 (m, 1H), 4.28 (dt, J = 27.7, 6.6, 2.3 Hz, 2H), 4.00 (s, J = 3.2 Hz, 2H), 2.82 (m, J = 7.5, 5.2 Hz, 2H), 2.74 – 2.68 (m, 2H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 205.53, 176.34, 85.56, 84.44, 67.61 (d, J = 19.9 Hz), 37.74, 30.94.
LRMS (ESI, APCI) *m/z*: calc'd for $C_7H_{13}FNO_3$ $[M]^+$ 178.09, found $[M+H]^+$ 179.12



5-(3-fluoropropoxy)-2,5-dioxopentan-1-aminium trifluoroacetate (41b): In an oven-dried vial equipped with stir bar was added a solution of **3-fluoropropyl 5-((tert-butoxycarbonyl)amino)-4-oxopentanoate** (10 mg, 0.034 mmol) in CH_2Cl_2 (0.5 mL). Next, TFA (0.5 mL) was added to the reaction vial. The reaction was allowed to stir at room temperature for 30 minutes. Then, the reaction mix was concentrated under reduced pressure to afford desired salt in quantitative yield (10.4 mg).

^{1}H NMR (600 MHz, CD_3OD) δ 4.53 (m, J = 7.7, 4.9 Hz, 1H), 4.45 (m, J = 5.8, 2.5 Hz, 1H), 4.22 – 4.11 (m, 2H), 4.00 (s, J = 3.4 Hz, 2H), 2.81 (m, 2H), 2.66 (m, 2H), 2.07 – 1.93 (m, 2H).
^{13}C NMR (151 MHz, $CDCl_3$) δ 205.59, 176.57, 84.71, 83.63, 64.58 (d, J = 5.5 Hz), 51.96, 51.82, 51.68, 51.53, 51.39, 51.25, 51.11, 50.64, 37.29, 33.36 (d, J = 20.2 Hz), 30.99.
LRMS (ESI, APCI) *m/z*: calc'd for $C_8H_{15}FNO_3$ $[M]^+$ 192.10, found $[M+H]^+$ 193.20



5-(3-[18]fluoropropoxy)-2,5-dioxopentan-1-aminium trifluoroacetate (42): Procedure for radiosynthesis is detailed in Voll et. al. (2005).[16] The crude mixture was purified by preparative HPLC in 30% EtOH/ H_2O (8% radiochemical yield; 6 mCi/mL activity).

III. Cell Study Procedure and Raw Data

U87ΔEFGR, DU145, and 9L cell lines were transfected using pEYFP-Cl (provided by the Emory Integrated Genomics Core (EIGC) in the laboratory of Professor Adam I. Marcus at the Emory University Winship Institute by the reported method of RJ Winn).[16] The 9L gliosarcoma cells were initially grown as monolayers in T-flasks containing Dulbecco's Modified Eagle's Medium (DMEM) under humidified incubator conditions (37 °C, 5% CO_2/95% air). The growth media was supplemented with 10% fetal calf serum and antibiotics (10,000 units/mL penicillin and 10 mg/ mL streptomycin). The growth media was replaced three times per week, and the cells were passaged so that the cells would reach confluency in a week's time. When the monolayers were confluent, cells were prepared for experimentation in the following manner. Growth media was removed from the T-flask, and the monolayer cells were exposed to 1X trypsin:EDTA for ~1 min to weaken the protein attachments between the cells and the flask. The flask was then slapped, causing the cells to release. Supplemented media was added to inhibit the proteolytic action of the trypsin, and the cells were aspirated through an 18 Ga needle until they were monodispersed. A sample of the cells was counted under a microscope using a hemocytometer, and the live/dead fraction was estimated through trypan blue staining (>98% viability). The remainder of the cells was placed into a centrifuge tube and centrifuged at 75G for 5 min, and the supernatant was removed. The cells were then resuspended in amino acid/ serum-free DMEM salts.[17]

In this study, approximately 0.5×10^6 cells were exposed to [18F]**42** (5 μCi) in 3 mL of amino acid free media ± transporter inhibitors 5-ALA, β-ALA, Ala+Gly, or Phe+Tyr (10 mM) for 30 min under incubator conditions in 12 mm × 75 mm glass vials. Each assay condition was performed in quintuplicate. After incubation, cells were twice centrifuged (75G for 5 min) and rinsed with ice-cold amino acid/serum-free DMEM salts to remove residual activity in the supernatant. The vials were placed in a Packard Cobra II Auto-Gamma counter, the raw counts were decay-corrected, and the activity per cell number was determined. The data from these studies (expressed as percent uptake relative to control) were catalogued using Excel.[17]

5/8/2019

F[18]-5-ALA-fluoropropyl ester

9L		Corrected counts/min (mCi)	% dose uptake per 0.5 million cells	Average % dose uptake per 0.5 million cells	Standard deviation
	Control			0.80	0.11
	1	120044.6	0.86		
	2	122574.5	0.87		
	3	94812.6	0.68		
	4	127618.6	0.91		
	5	94952.06	0.68		
	5-ALA			1.15	0.04
	1	174402.7	1.13		
	2	158589.8	1.15		
	3	161198.1	1.22		
	4	171775.9	1.12		
	5	157573.8	1.12		
	β-ALA			0.72	0.07
	1	116601.5	0.79		
	2	94526.16	0.75		
	3	110257.5	0.64		
	4	105230.1	0.76		
	5	90364.57	0.64		
	Ala+Gly			0.64	0.23
	1	106549.8	0.92		
	2	98197.38	0.40		
	3	119163.3	0.69		
	4	128995.8	0.80		
	5	56456.54	0.40		
	Phe+Tyr			0.80	0.11
	1	96786.37	0.69		
	2	112272.6	0.80		
	3	97743.72	0.70		
	4	131075.9	0.93		
	5	124178	0.89		
u87 delta EGFR					
	Control			3.37	0.56
	1	491719.7	3.51		
	2	454015.9	3.24		
	3	578486.9	4.13		

	4	476698.5	3.40		
	5	361654.4	2.58		
	5-ALA			0.25	0.11
	1	22063.89	0.16		
	2	28477.33	0.20		
	3	33199.28	0.24		
	4	27492.67	0.20		
	5	61272.8	0.44		
	β-ALA			3.34	0.29
	1	495257.9	3.53		
	2	435032	3.10		
	3	490877.1	3.50		
	4	504863.5	3.60		
	5	414163.2	2.95		
	Ala+Gly			3.40	0.42
	1	509030.8	3.63		
	2	510258.8	3.64		
	3	497170.3	3.55		
	4	494931.7	3.53		
	5	372047.1	2.65		
	Phe+Tyr			3.56	1.58
	1	652714.9	4.65		
	2	305051.6	2.18		
	3	374084	2.67		
	4	351402.9	2.51		
	5	810666.4	5.78		
DU145					
	Control			1.30	0.12
	1	133768.1	1.18		
	2	148094.2	1.31		
	3	170010.3	1.51		
	4	141370.8	1.25		
	5	141264.2	1.25		
	5-ALA			0.82	0.09
	1	91691.09	0.81		
	2	99746.73	0.88		
	3	77706.12	0.69		
	4	105625.5	0.94		
	5	89547.26	0.79		
	β-ALA			1.36	0.11
	1	161620.3	1.43		
	2	143048.5	1.27		
	3	151009	1.34		
	4	169231.6	1.50		
	5	140500.1	1.24		

Ala+Gly				1.35	0.20
	1	183273.4	1.62		
	2	165686.4	1.47		
	3	138362.7	1.22		
	4	151367.9	1.34		
	5	125557.9	1.11		
Phe+Tyr				1.81	0.83
	1	341995	3.03		
	2	167399.3	1.48		
	3	143162.7	1.27		
	4	112666.9	1.00		
	5	257241.5	2.28		

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