

# Automated synthesis of $^{18}\text{F}$ radiolabelled indole containing Oncrasin-like molecules; a comparison of iodonium salts and boronic ester chemistry.

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## Research article

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# Abstract

**Background:** Oncrasin-1 is a small molecule which was identified from a screen of KRAS mutant cancer cells and has shown specificity for KRAS mutant cell killing. We aimed to develop a radiolabelled form of Oncrasin-1 to enable in-vivo imaging of mutant KRAS expression in malignant tumours. This work outlines the synthesis of 3 fluorinated derivatives and development of iodonium salt and boronic ester precursors for radiolabelling with the  $^{18}\text{F}$  isotope.

**Results:** In our hands, synthesis of iodonium salts were not easily accessible due to the 3-carbaldehyde indole structure being preferentially oxidized by conditions required for iodonium salt formation, rather than benzyl iodide. Synthesis and radiolabelling of boronic acid pinacol ester precursors were successful, with the products being obtained in yields of  $10.76\% \pm 0.96\%$  (n=5),  $14.7\% \pm 8.58\%$  (n=3) and  $14.92\% \pm 3.9\%$  (n=3) for  $^{18}\text{F}$  KAM001,  $^{18}\text{F}$  KAM002 and  $^{18}\text{F}$  KAM003 respectively, with radiochemical purity of greater than 99%.

**Conclusions:** The successful synthesis of these tracers has been undertaken utilizing boronic ester radio-fluorination methods and will allow for investigation of Oncrasin based molecules as potential diagnostics for cancers expressing mutant KRAS protein.

## Background

Human RAS genes have been identified as one of the most frequently mutated oncogene family in all cancers, with estimations of up to a third of all cancers harbouring a mutation(1). Normal function of this family includes regulation of transcription, cell cycle progression, growth, survival, proliferation and cell migration signalling(2). For the KRAS protein, single point mutations at positions 12, 13, and 62 have been shown to result in constitutively active protein which in turn causes over expression or increased activity in a wide range of downstream signalling pathways(3).

The high frequency of mutation in cancer has made this gene family a target of significant interest within the field of oncology, however, despite extensive efforts to exploit these proteins as potential therapeutic targets, molecules designed for RAS mutant therapies have not made progress into the clinic.

Since identification of this oncogenic driver, efforts to develop new molecules to target KRAS have been ongoing; a molecule, denoted Oncrasin-1 shown in Figure 1, was discovered in 2008 through a synthetic lethal screen with KRAS mutation in breast cancer cell lines(4). In the initial screen the compound showed selective toxicity in both a KRAS mutant cell line and a cell line with wild type KRAS which was previously resistant but became sensitive after transfection with mutant KRAS.

Further optimization of the structure has been reported, with some analogues and derivatives showing improved potency against the KRAS mutant cell lines, including analogues with fluorine at the *meta* and *ortho* positions on the benzyl ring(5), shown in Figure 1.

As these compounds can potentially identify mutant KRAS in different cancer types, they may have potential for development as a diagnostic imaging approach for stratification of patients to different treatment groups. This would be a valuable tool for treatment planning as the presence of KRAS mutant protein is a predictor of poor response to anti-EGFR antibody treatments such as Cetuximab and Gefetinib, due to EGFR-independent downstream signalling through this pathway(6-8).

Positron emission tomography (PET) is an imaging technique which enables non-invasive imaging of metabolic processes and biochemical function in living tissues. PET imaging currently plays a central role in the detection, staging and response assessment of cancer patients, with clinical management based on based on PET imaging results being an accepted part of cancer patient care.

Radiosynthesis of  $^{18}\text{F}$  substituted aryl systems is currently a significant challenge in the field of radiofluorination, with limited means to effectively incorporate a radiolabel into an aryl system(9, 10). Many methods which are used for synthesis of  $^{19}\text{F}$  fluorination of aryl systems have long reaction times, which are unacceptable in the context of a 109.7-minute half-life, or rely on electrophilic sources of fluorine, which are not easily accessible when working with  $^{18}\text{F}$ . Preliminary evaluation of radiosynthetic methods indicated a limited number of potential precursors for fluorination reactions which could be used to label not only the *para* and *ortho* positions but also the *meta* position, which cannot be accessed through traditional electrophilic aromatic substitution radiochemistry. All three substitution patterns present a potential radiotracer target, therefore, establishing a method which could be used for radiofluorination reactions at all three positions is essential. To this end, two classes of precursors were selected for investigation.

Iodonium salt chemistry has become an area of great interest for radiofluorination of aryl systems, with many publications providing synthetic routes which have allowed access to radiotracers through this chemistry(11-13). While these are an exciting class of precursor, not all structures are compatible with the chemistry required to produce them, reaction conditions for labelling are very harsh, and the precursors can have a limited shelf life.

In addition to the iodonium salt precursor class, boronic acid pinacol ester precursors have become of significant interest for aryl radio fluorination, with the functional group being known for good long-term stability and chemical resilience(9, 10, 14, 15). The synthetic routes for these compounds are also well characterized due to their prevalence in synthetic chemistry, and the conditions for radiolabelling are not as harsh as those for iodonium salts.

Utilizing these two types of precursor, this paper presents the synthesis and automated radiolabelling of Oncrasin-like molecules for the purpose of development as PET imaging agents.

## Methods

### *General*

Solvents and reagents other than boron containing compounds were purchased from Sigma Aldrich and used without further purification. Boron compounds were purchased from Advanced Molecular Technologies and used without further purification.

#### *Sodium hydride coupling reaction with benzyl halides*

Sodium hydride (60%, 97mg, 2.4 mmol) was washed with petroleum spirits (20 mL) twice before addition of a chilled solution of indole 3 carboxaldehyde (200 mg, 1.37 mmol) in THF (20 mL) to afford a pink coloured solution. The desired benzyl bromide (1.57 mmol) was added to the solution, with the ice bath being removed 5 minutes after this addition. This reaction mixture was stirred at room temperature overnight before termination of the reaction by removal of solvent under vacuum. The resultant residue was then redissolved in 30 ml of diethyl ether and washed with saturated sodium bicarbonate (2x 50 mL) and distilled water (2x 50 mL). The organic layer was then dried onto silica for column chromatography. Fractions were combined and concentrated under reduced pressure to afford the product. Crystals were grown either through evaporation from dichloromethane or vapour diffusion methods from either ethyl acetate, ethanol, or methanol with petroleum spirits

#### *Miyarua borylation*

A flask was charged with N-(4-iodobenzyl)-indole-3-carbaldehyde (500 mg, 1.38 mmol), potassium acetate (407 mg, 4.14 mmol), bispinacolato diborane (1.75 g, 6.9 mmol) and palladium dichloride diphenyl phosphine ferrocene catalyst (30 mg, 0.04 mmol). The mixture was dissolved in 20 mL of DMF and heated to 70°C for 3 hours before being diluted into 300 mL of water and extracted 3 times with dichloromethane. The organics were combined and then dried onto silica for column chromatography. Fractions were combined and concentrated under reduced pressure to yield a mass of 360 mg (72%)

### **Radiochemistry**

#### *Production of Fluoride*

No-carrier-added [ $^{18}\text{F}$ ] fluoride was produced by the  $^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$  nuclear reaction with an 10 MeV proton beam generated by the IBA Cyclone 10/5 cyclotron in a titanium target using [ $^{18}\text{O}$ ]  $\text{H}_2\text{O}$  at Austin Health, Department of Molecular Imaging and Therapy. Typical irradiation parameters were 16 $\mu\text{A}$  for 30 min, which resulted in 10.4–11.8 GBq (281–318 mCi) of [ $^{18}\text{F}$ ] fluoride being transferred into the synthesis module. These yields are lower than the theoretical yield and are explained by the use of recycled [ $^{18}\text{O}$ ]  $\text{H}_2\text{O}$  of unknown isotopic enrichment.

#### *Preparation of copper catalyst*

15mg of Copper(II) triflate was dissolved in 400 $\mu\text{L}$  of DMF and combined with 100 $\mu\text{L}$  of a 50:50 mixture of DMF and pyridine. This mixture was sonicated to ensure proper solvation of the copper salt and added to the catalyst vial in the FlexLab as described in supplemental Table 1.

### *Preparation of potassium triflate eluent*

2.3mg of potassium triflate was dissolved in 500µL of distilled water and 500µL of acetonitrile to which 9mg of [2.2.2] Cryptand was added. The mixture was sonicated to ensure proper solvation and mixing of components and added to the eluent vial of the Flexlab module as described in supplemental Table 1.

### *Radiochemistry*

Radiolabelling reactions were undertaken using an iPhase Flexlab automated synthesis module. This module is equipped with multiple reaction vessels and two HPLCs, allowing the user to undertake complex, multistep radiosynthetic reactions. Depictions of the module and interactive interface used to control it can be found in Figure 3.

4mg of the appropriate radiotracer precursor was dissolved in 0.5ml of DMF and combined with the previously described catalyst system with a kryptofix chelated  $^{18}\text{F}$  fluoride source. The reaction was heated to 120°C for 20 minutes, with purging of the reactor every 5 minutes with atmospheric air for 3 seconds. After this time, the reaction was cooled to room temperature before dilution with 1ml of water and transfer to a loop vial in preparation for HPLC. The reaction vessel was then washed with a further 2ml of 50:50  $\text{H}_2\text{O}$ :ACN to ensure full recovery of the reaction mixture before subsequent HPLC purification of the combined fractions. After initial injection of the reaction mixture, the HPLC was run for 2 minutes with a 0.1% ammonium formate in  $\text{H}_2\text{O}$  solution before switching to a gradient starting at 80:20 0.1% ammonium formate in  $\text{H}_2\text{O}$ :ACN progressing to 10:90 over 18 minutes. Products collected between 20 and 23 minutes depending on the tracer. Products were trapped using a preconditioned C18 SPE cartridge. This cartridge was conditioned with 1ml of ethanol followed by 10ml of water and then drying before use. For elution of the product, 1ml of ethanol was used, with an additional 1ml of distilled water being used to rinse the cartridge and tubing into the product vial.

## **Results**

### *Synthesis of cold standards*

The synthesis of cold standards was carried out for spectroscopic characterization and identification, as well as a comparison with radiolabel products for confirmation of product by radio HPLC. Synthetic procedures were undertaken as outlined in Scheme 1 to yield cold standards in a good yield.

### *Synthesis of precursors*

#### *Coupling of indole-3-carbaldehyde and benzyl halides*

A general sodium hydride deprotonation of the indole followed by addition of a benzyl halide of the appropriate substituent and substitution pattern was used for generation of cold standards as well as precursors and iodinated intermediates, as shown in Scheme 1. Yields for these compounds were typically above 70%, with the *ortho* substituted boronic acid pinacol ester derivative yielding lower at 58%.

### *Miyaura borylation*

A standard Miyaura borylation was utilized for generation of the parasubstituted boronic acid pinacol ester precursor from an iodine substituted intermediate to afford the product in a 72% yield.

### *Iodonium salt precursors*

The initial synthetic route to an iodonium salt precursor was envisioned as shown in Scheme 1, with a sodium hydride coupling between commercially available indole-3-carbaldehyde and the appropriately substituted iodo benzyl bromide. This intermediate would then undergo a one-pot reaction to form an iodonium salt suitable for radiolabelling. To determine optimal conditions, initial reactions were carried out with the *para* substituted material, as shown in Scheme 2.

Synthesis of 1-(4-iodo-benzyl)-1H-indole-3-carbaldehyde (Compound 1) was undertaken as described in Scheme 1 and the product was obtained in quantitative yield. With Compound 1 in hand, a one-pot synthesis of the iodonium salt was trialled using reaction conditions described by Zhu, Jalalian and Olofsson(16), shown in Scheme 3. The resultant reaction mixture showed no indication of product formation. The characteristic proton signal correlating to the aldehyde proton at approximately 10ppm was absent and the expected mass for the desired product was not detected with high resolution mass spectrometry, suggesting an incompatibility between these reaction conditions and the aldehyde functional group. Literature indicated that sodium periodate(17) and peroxide(18) could also be employed as oxidants and were trialled with similar results. A summary of reactants attempted with Compound 1 shown in Table 1, with none of the combinations resulting in any identifiable products other than starting material.

Attempts to carry out iodonium salt forming reactions in a two-step process shown in Scheme 4 also yielded no identifiable compounds. Being unable to isolate a satisfactory salt from these conditions, a two-step reaction(20) produced a major product from the initial oxidation step and was isolated via column chromatography and crystallized using vapour diffusion methods for x-ray crystallography, with the structure shown in Figure 2.

### *Boronic acid pinacol ester precursors*

With an iodine containing intermediate already in hand, a Miyaura borylation reaction was undertaken to provide the *para* substituted precursor in a 72% yield, shown in Scheme 5. As previous coupling reactions had been undertaken successfully, commercially available sources of *para*, *meta* or *ortho* substituted boronic acid pinacol ester containing benzyl bromides were used to investigate a one-step coupling synthetic route. This method utilizing the standard benzyl halide coupling reaction conditions produced the desired product in yields 78%, 76% and 58% yields of the *para*, *meta*, and *ortho* boronic acid precursors respectively. Product identity was confirmed through spectroscopy as well as x-ray crystal structures.

With three precursors in hand, radiolabelling experiments were undertaken.

## Radiochemistry

Reaction conditions adapted from Tredwell et al.(15) were utilized for initial radiolabelling experiments with the *para* precursor, as shown in Scheme 6. Under these conditions, no radiolabelled products were isolated, with the majority of the starting material being unreacted.

As initial radiolabelling attempts yielded no discernible radiolabel products and previous literature suggested that these reactions may not be suitable for automated synthesis(15), possibly due to the inert gas systems they often operate under; the mechanism of catalysis for these reactions is unclear but may operate through a Cham-Lam coupling-like oxidation cycle(21), which would require atmospheric oxygen that is not present in standard, inert gas flushed automated systems. The reaction was attempted again with air being purged into the reaction vessel throughout the labelling, with no improvement in radiolabel incorporation.

A 4-Methoxycarbonylphenylboronic acid, pinacol ester was utilized as a model for trouble shooting as it is chemically similar to reagents used in both the Tredwell paper and another paper authored by Mossine et al. (10), which had resulted in excellent yields, however under the previously stated conditions, no radiolabelling was observed.

As the system described by Tredwell was not able to produce radiolabelled products in our hands, another similar system, described by Mossine et al. and shown in Scheme 7, was investigated.

This system also produced no discernible radiolabelled products at the expected retention time. Mossine and co-workers had noted poor radiochemical yields prior to their own optimization for boronic acid labelling with regards to eluents. Development of a new eluent was required for successful synthesis, which utilized a minimized quantity of potassium carbonate and potassium triflate in combination with [2.2.2] kryptofix.

Attempts to carry out the radiosynthesis with the model system utilizing other standard eluents such as bicarbonate and tertbutyl amine were unsuccessful.

To determine if the eluent was the limiting factor in the radiosynthesis, a synthesis was carried out without QMA cartridge facilitated fluoride isolation, with evaporation of the  $^{18}\text{O}$  water being performed in the absence of additives such as kryptofix prior to the labelling reaction. This system yielded small amounts of previously unobserved radiolabelling products.

Adoption of a potassium triflate eluent system afforded radiolabelling of the model system as the major radiolabel product. Further optimization of the eluent showed that the preconditioned QMA cartridge used for an  $^{18}\text{F}$ -FDG synthesis contained enough bicarbonate for labelling and so this was removed from the eluent. When using QMA cartridges which had been reconditioned after initial use, significant variability was observed, so this was avoided for future synthesis. A summary of conditions trialled with the model compound is shown in Table 3

Having successfully produced a radiolabelled molecule in the model system, the BpinKAM001 system was revisited, utilizing the revised catalyst system and new eluent, with successful product formation being achieved, with conditions shown in Scheme 8. HPLC purification of the radio peak from the reaction mixture was undertaken in the Flexlab module, with a representative trace shown in the supplementary information, and confirmed to be the desired radiotracer by registration with the cold standard peak retention time, as shown in Figure 4. Using these conditions radiolabelling of the remaining BpinKAM002 and BpinKAM003 compounds was undertaken successfully, with HPLC traces shown in Figure 5 and Figure 6.

Decay corrected yields for the purified tracers were  $10.76\% \pm 0.96\%$  (n=5),  $14.7\% \pm 8.58\%$  (n=3) and  $14.92\% \pm 3.9\%$  (n=3) for  $^{18}\text{F}$  KAM001,  $^{18}\text{F}$  KAM002 and  $^{18}\text{F}$  KAM003 respectively. All tracers were shown to have a radiochemical purity greater than 99%, with a representative run of the  $^{18}\text{F}$  KAM002 producing a molar activity of  $1.09\text{GBq}/\mu\text{mol}$ .

## Discussion

### *Synthesis of iodonium salt precursors*

Initial reactions to form iodonium salts were planned as described in scheme 2; A standard sodium hydride coupling would be the first step to form the benzyl iodide. This compound could then be reacted to form an iodonium salt under various conditions using electron rich aryl systems denoted 'Ar'. The iodonium salt would then undergo radiolabelling.

Reactions to attempt to form an iodonium salt precursor using the specified reagents were incompatible with the indole scaffold contained in the Oncrasin molecule as the indole structure is preferentially being oxidized rather than the iodine in the benzyl ring. This process likely occurs with the oxidants utilized in the reactions undertaken, with a wide range of intermediates being produced, resulting in complex mixtures of products. The structure obtained from the peracetic acid reaction suggests a multi-step Baeyer–Villiger oxidation, resulting in a ring opening reaction which is consistent with oxidation processes proposed by Marchand et. Al.(22). While the trialled conditions were unable to produce the desired iodonium salt, Rotstein and coworkers have been able to access an indole iodonium salt through use of a sodium perborate oxidant (23), and oxidation at the '3 position to form iodonium salts has also been shown in literature(24). The difference between these works and labelling of these Oncrasin-like molecules is that we are attempting to form an iodonium salt on the benzyl group rather than the indole.

This improved understanding of indole sensitivity to the oxidation conditions required for many iodonium salt forming reactions is significant for  $^{18}\text{F}$  radiochemistry, as indoles are an important biological scaffold and are present in many structures which may be investigated as PET imaging agents including amino acids, peptides and small molecule drugs.

As the Oncrasin indole structure was not amenable to the trialled oxidation conditions for a direct iodonium salt synthesis, attention was directed towards the boronic acid pinacol ester precursors.



Pinacol boronic esters precursors were readily produced through two synthetic routes, with good yields for both routes, proving to be a superior precursor class for this indole containing compound.

The lower yield of the ortho product can be explained by a steric clash observed in the crystal structure of this product, which would make nucleophilic attack of the halogen less favoured, hindering product formation.

### *Radiochemistry*

Initial experiments trialling both Treadwell and Mossine catalyst systems were unsuccessful due to use of an eluent system which was too basic for the copper catalyst system. The bicarbonate in the eluent was likely able to displace the triflate and pyridine molecules co-ordinated with the copper, inactivating the catalyst.

Trialling the reaction of the boronic esters without the eluent system helped to identify this as the confounding factor for this chemistry, and this finding is consistent with other literature around these methods (25).

Yields for these Oncrasin-like molecules are somewhat low compared to other literature report for benzyl labelling but in a similar range to labelling of indoles. Recent work by Zarganes-Tzitzikas et. al (14) suggest that increasing the quantities of precursor approximately 4 fold could improve radiolabelling yields, however protodeborylation side products which were observed were difficult to separate and would likely be increased with the increase in precursor and potentially impact on the purity of the tracer. Protodeborylation products may also be contributing to the UV profile of the product, lowering the measured molar activity.

Both Mossine and Treadwell papers noted that precursors containing amines produced lower radiochemical yields when utilizing the copper catalysed labelling as well as lower yields for labelling indoles. It could be that the indole precursors could also competing with pyridine in forming complexes with the copper required for the catalyst system, both deactivating the catalyst and sequestering precursor so that it is not available for radio fluorination. Despite this, the quantity of tracer produced was in excess of what is required for preliminary biological evaluation and will allow for further investigation of the molecules.

## **Conclusions**

Synthesis of Oncrasin-like molecules was attempted through both an iodonium salt precursor and a boronic acid pinacol ester precursor. Reaction conditions for direct synthesis of an iodonium salt precursor were not compatible with the indole scaffold in the structure. Boronic acid pinacol ester precursors were successfully synthesized and radiolabelled utilizing modified reaction conditions reported by Mossine and co-workers. Identifying the incompatibility of the indole scaffold and common iodonium salt-forming reaction conditions is an important contribution to field of radiotracer

development. Synthesis of these radiotracers will allow for further work to determine suitability of Oncrasin-based molecules as potential diagnostics for cancer detection and tumour type differentiation.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Availability of data and materials

Crystal structure generated and/or analysed during this work are available in the Cambridge Crystal Structure Database repository. <https://www.ccdc.cam.ac.uk/>

All other data generated or analyzed during this study are included in this published article and its supplementary information files.

### Additional file

Supplementary Data.docx

### Competing interests

The authors declare that they have no competing interests.

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### Authors' contribution

AM undertook all synthetic procedures for compounds with advice from JW and drafted the publication. YG and UA assisted with radiochemistry development. AS provided advice on clinical relevance for tracer development. UA, JW and AS reviewed the paper for publication.

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## Abbreviations

RCY – Radio chemical yield

RAS – Rat Sarcoma

KRAS – Kirsten Rat Sarcoma

EGFR – Epidermal Growth Factor Receptor

PET – Positron Emission Tomography

DMF – Dimethylformamide

ACN – Acetonitrile

HPLC – High Performance Liquid Chromatography

GBq – Giga-becquerel

SPE – Solid Phase Extraction

MCPBA – *Meta*-chloro perbenzoic acid

OTf – Triflate anion

OTs – Tosylate anion

QMA – Quaternary Methyl Ammonium

FDG – Fluorodeoxy glucose

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## Tables

*Table 1: Reaction conditions trialled with for iodonium salt synthesis.*

Oxidant	Counter ion	Aryl System
MCPBA	OTf/OTs	<i>p</i> -Methoxybenzene (19)
Peroxide	OTs	1,3, Dimethoxy Benzene
Oxone	OTs	1,3,5 Trimethoxy Benzene
Sodium Periodate	Acetic acid/H <sub>2</sub> SO <sub>4</sub>	1,3,5 Trimethoxy Benzene

Table 2: Flexlab set up for radiolabelling reactions

Container	Reagents
Vial 1	Eluent
Vial 2	4mg of precursor in 500µl of DMF
Vial 3	1ml anhydrous acetonitrile
Vial 4	450µl of DMF. 50 µl of Pyridine, 13mg of Cu(OTf) <sub>2</sub>
Vial 5	1ml of Acetonitrile and 1ml of distilled water
Vial 6	
Vial 7	
Vial 8	
Vial 9	
Vial 10	
Vial 11	10ml of distilled water
Vial 12	1ml of ethanol
Vial 13	1ml of saline
Vial 14	
Vial 15	
Vial 16	
Vial 17	
Vial 18	
Vial 19	
QMA seppak	Quaternary Methyl Ammonium Cartridge
Seppak A	
Seppak B	
Seppak C	
Seppak D	C18 seppak
HPLC Vial 1	
HPLC Vial 2	40ml of distilled water
Reactor 1	1ml anhydrous acetonitrile
Reactor 2	
Loop Vial 1	
Loop Vial 2	1ml distilled water
HPLC eluent A	0.1% Ammonium formate
HPLC eluent B	Acetonitrile
HPLC eluent C	Water
HPLC eluent D	Ethanol

Table 3: Trialled reaction conditions for model systems

Reaction	1	2	3	4	5
<b>Eluent</b>	Carbonate	Tetrabutyl amine	Bicarbonat e	None	Triflate
<b>Catalyst</b>	Tetrakis Complex	Tetrakis Complex	Tetrakis Complex	Cu(OTf) <sub>2</sub> + Pyridine	Cu(OTf) <sub>2</sub> + Pyridine
<b>Kryptofix</b>	10mg	-	4.5mg		4.5mg
<b>Outcome</b>	Nil	Nil	Nil	Minor Product	Product

# Figures

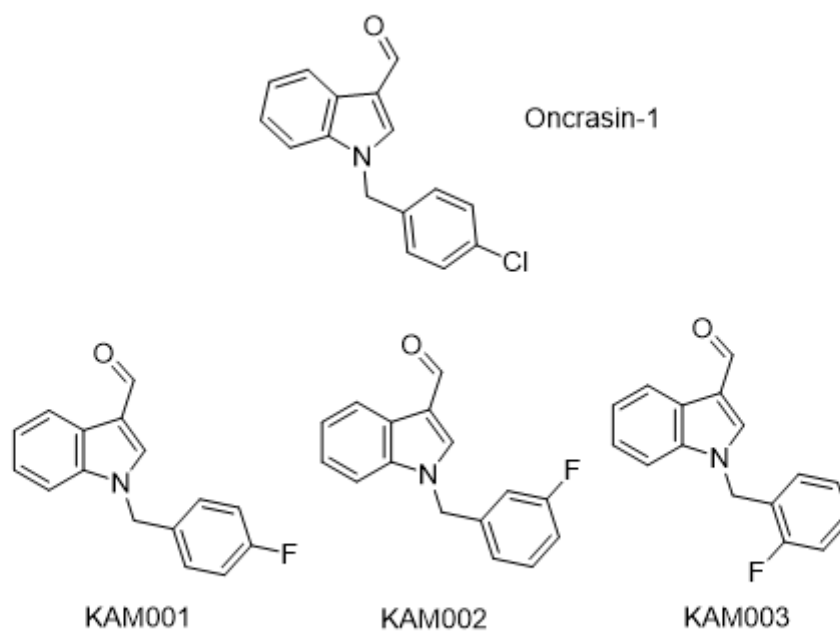


Figure 1: Parental compound, Oncrasin-1, and 3 fluorinated derivatives synthesized.

## Figure 1

Parental compound, Oncrasin-1, and 3 fluorinated derivatives synthesized.

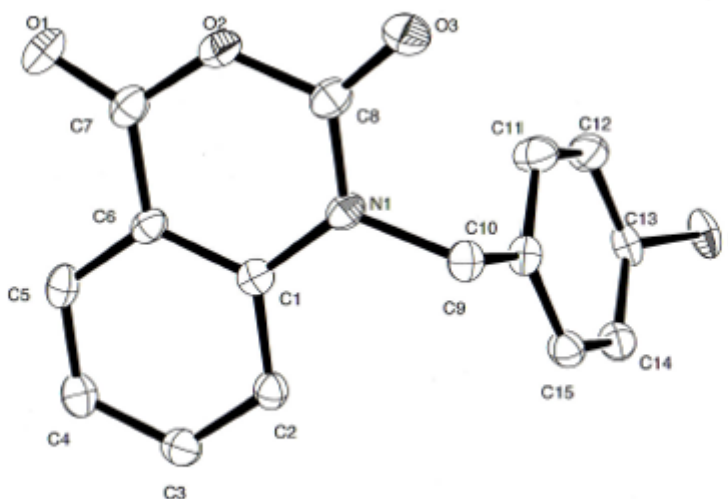


Figure 2: Crystal structure of a major product Step 1 in Scheme 4.

Figure 2

Crystal structure of a major product Step 1 in Scheme 4.

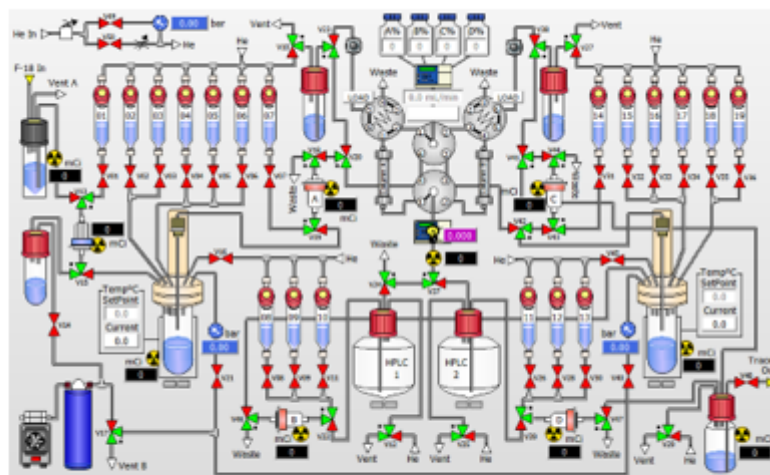


Figure 3: Flexlab module (left) and interactive control interface (right)

Figure 3

Flexlab module (left) and interactive control interface (right)



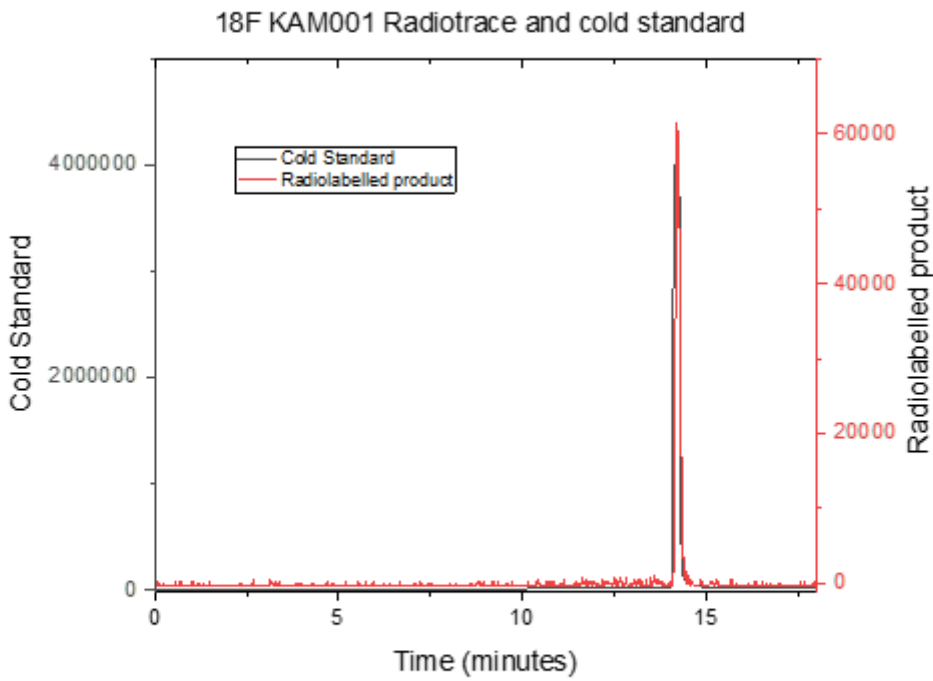


Figure 4:  $^{18}\text{F}$  KAM001 identity confirmation using cold standard retention.

**Figure 4**

$^{18}\text{F}$  KAM001 identity confirmation using cold standard retention.

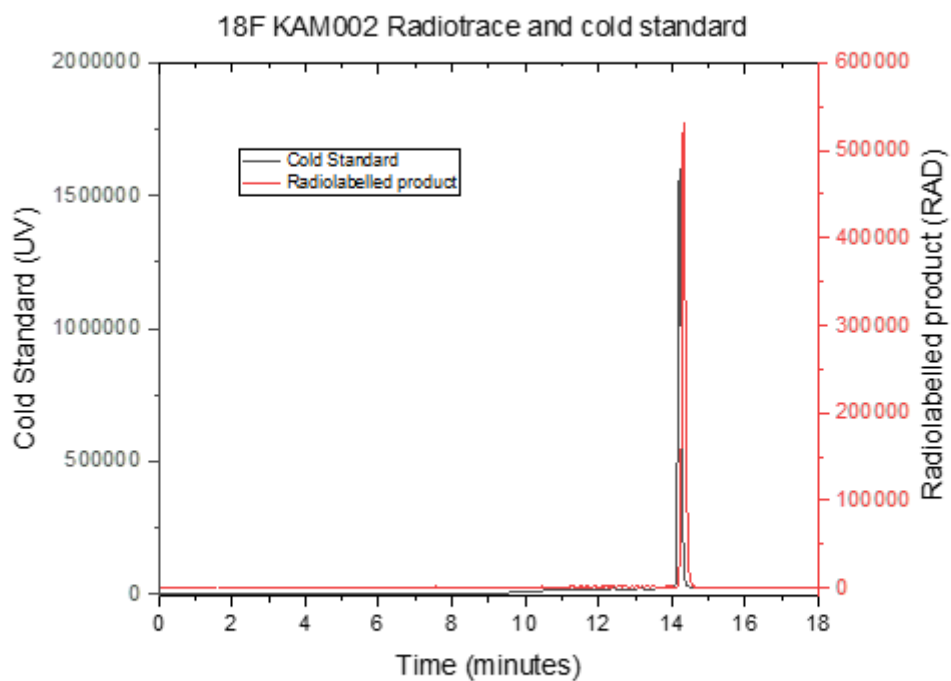


Figure 5:  $^{18}\text{F}$  KAM002 identity confirmation using cold standard retention.

### Figure 5

$^{18}\text{F}$  KAM002 identity confirmation using cold standard retention.

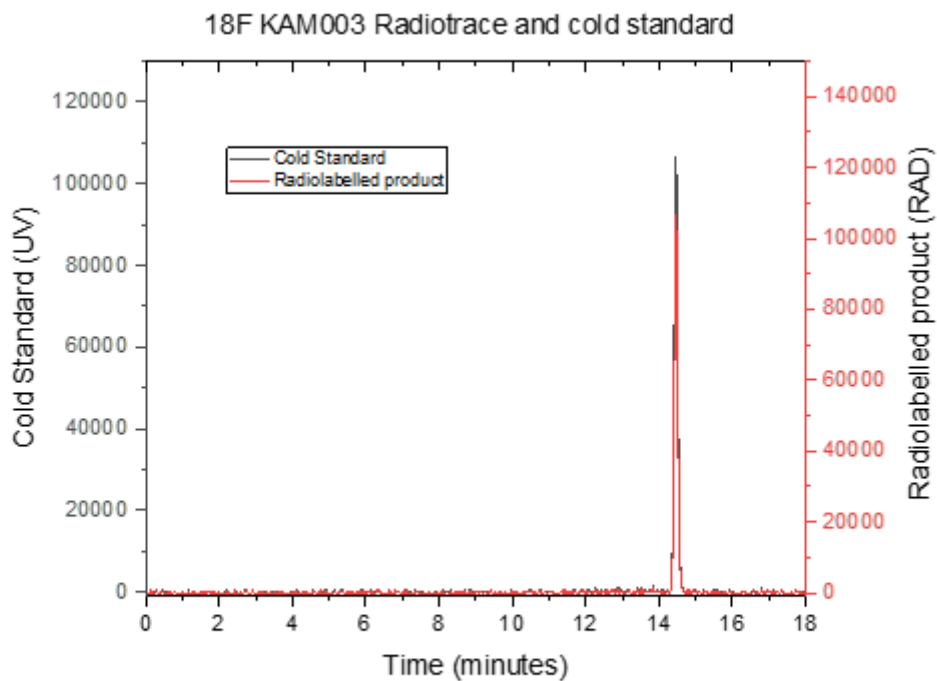


Figure 6:  $^{18}\text{F}$  KAM003 identity confirmation using cold standard retention.

## Figure 6

$^{18}\text{F}$  KAM003 identity confirmation using cold standard retention.

## Supplementary Files

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- [Supplementarydatareviewed20200625.docx](#)
- [schemes.docx](#)