Entwicklung von neuen Radiomarkierungsverfahren und deren Anwendung zur Synthese von Radiotracern für die Positronen-Emissions-Tomographie

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Kurzzusammenfassung

In den letzten 30 Jahren hat sich die Positronen-Emissions-Tomographie (PET) zu einem der wichtigsten Instrumente in der klinischen Diagnostik entwickelt. Neben den ständigen technischen Verbesserungen verdankt die PET ihren klinischen Stellenwert vor allem dem ihr inhärenten Potenzial, physiologische und biochemische Prozesse auf molekularer Ebene und in Echtzeit darzustellen. Aufgrund der wachsenden Zahl neuer und krankheitsspezifischer Radiotracer nimmt sie auch auf dem Gebiet der Wirkstoffentwicklung sowie beim Monitoring von pharmakologischen Interventionen eine immer wichtigere Rolle ein.

Grundlegend hierfür ist einerseits das intelligente Design innovativer und selektiver molekularer Sonden mit der Fähigkeit zur Visualisierung molekularer Targets, die in physiologischen und pathophysiologischen Prozessen involviert sind und andererseits die Entwicklung der dafür notwendigen fortschrittlichen Markierungsstrategien. Letzteres ist zentraler Bestandteil der radiochemischen Grundlagenforschung und Hauptgegenstand dieser kumulativen Promotionsarbeit, in dessen Rahmen ein neues „minimalistisches“ Protokoll zur Radiofluorierung ausgearbeitet wurde.

Die Entwicklung der sog. „minimalistische“ Methode ermöglicht eine vereinfachte und zeitsparende Herstellung von unterschiedlichsten ^{18}F-markierten Verbindungen, da sie weder der azeotropen Trocknung noch des Zusatzes einer Base oder anderer Additive bedarf. Das neue Radiomarkierungsverfahren umfasst eine direkte Elution von $^{18}F^-$ mittels alkoholischer Lösung der Ammonium-, Diaryliodonium- oder Triarylsulfoniumsalz Vorläufer. Nach Entfernung des Alkohols wird das resultierende [^{18}F]Fluoridsalz in einem geeigneten Lösungsmittel erhitzt. Die hohe Effizienz der auf der „minimalistischen“ Methode basierenden Synthese bietet somit auch einen schnellen Zugang zu ^{18}F-markierten Fluorbenzaldehyden ([^{18}F]FBAs) in großen Aktivitätsmengen, was einen entscheidenden Vorteil für die Entwicklung neuer Markierungsmethoden mit Hilfe dieses Radiomarkierungsbausteins darstellt.

Darauf aufbauend wurde, ausgehend von [^{18}F]FBA, auf der Basis der Seyferth-Gilbert Homologisierung, ein innovatives Verfahren zur Synthese von bisher unbekannten ^{18}F-markierten Fluorarylacetylenen entwickelt. Hierdurch konnten über Cycloadditions- und Kreuzkupplungsreaktionen unterschiedliche radiomarkierte Modellverbindungen sowie PET-Tracer hergestellt und somit die Vielseitigkeit der neuen radiomarkierten Synthone aufgezeigt werden. Die durch die „minimalistische“ Methode gesteigerte Effizienz der Synthese von [^{18}F]FBA ermöglicht es ebenfalls, den bereits in der Literatur bekannten Markierungsbaustein *C*-(4-[^{18}F]Fluorphenyl)-*N*-phenylnitron [^{18}F]FPPN in ausreichenden Mengen zu produzieren und für die zielgerichtete Synthese diverser radiofluorierter β-Lactame über die Kinugasa-

Reaktion einzusetzen. Mit dem Erhalt der radiofluorierten β-Lactam-Peptid und -Protein-Konjugate in hohen radiochemischen Ausbeuten unter sehr milden Bedingungen, konnte die Eignung der Kinugasa-Reaktion als neues und leistungsstarkes Radiofluorierungsverfahren eindrucksvoll demonstriert werden.

Abstract

Positron emission tomography (PET) plays not only an important role in clinical diagnostics but is also of great value in the area of drug development and therapy monitoring owing to its unique potential to visualize physiological and biochemical processes at the molecular level in real time. PET benefits from continuous technical improvements and, especially, the growing number of accessible novel disease-specific radiotracers. However, the great diagnostic potential of many PET-probes remains only partly exploited in current clinics since simple and efficient procedures for their preparation are missing. This hampers their widespread application. Consequently, the development of novel synthetic strategies is of central importance in radiochemistry and has been the main topic of this cumulative dissertation.

First, a novel "minimalist" approach to ^{18}F-labeling was developed. This concept eliminates the need of time-consuming azeotropic drying and avoids the application of a base or other additives which have been considered to be indispensable for nucleophilic radiofluorination recations. It was demonstrated for the first time that ^{18}F-labeled compounds could be efficiently prepared using only [^{18}F]fluoride and onium salt precursors. The radiolabeling method consists of a direct elution of [^{18}F]fluoride with alcoholic solutions of precursors bearing a quaternary ammonium, diaryliodonium or triarylsulfonium functionality followed by heating of the resulting [^{18}F]fluoride salt in a suitable solvent. The versatility and the exceptionally wide scope of the novel radiofluorination procedure were demonstrated by the preparation of several useful ^{18}F-labeled prosthetic groups and by the one-step preparation of a radiolabeled model peptide.

The radiolabeling procedure based on the "minimalist" protocol enables i.a. the convenient and fast preparation of [^{18}F]fluorobenzaldehydes ([^{18}F]FBA) in high radiochemical yields and excellent radiochemical purities on a large scale. As a result, [^{18}F]FBAs could be used as a radiolabeling building block.

Accordingly, [^{18}F]FBAs were applied for the synthesis of hitherto unknown [^{18}F]fluoro-phenylacetylenes using the Seyferth-Gilbert homologation. The versatility of these radiolabeled synthons was demonstrated by the preparation of different radiolabeled model compounds as well as potential PET-probes *via* various cycloaddition and cross-coupling reactions.

Moreover, the efficient production of [^{18}F]FBAs on a large scale allowed the preparation of *C*-(4-[^{18}F]fluorophenyl)-*N*-phenyl nitrone, a radiofluorinated 1,3-dipole for radiolabeling *via* (2+3) cycloadditions. This building block was utilized to transfer the Kinugasa reaction into radiochemistry. The Kinugasa reaction enabled the preparation of various radiofluorinated β-lactams, a radiofluorinated β-lactam-peptide and protein conjugates in high yields under exceptionally mild conditions within a short reaction time. This study demonstrated the

suitability of the Kinugasa reaction not only for radiolabeling of small molecules but also of sensitive biopolymers.

Inhaltsverzeichnis

1 Einleitung

Zur Früherkennung von Krankheiten sowie zur Entwicklung gezielt wirkender Medikamente, werden bildgebende Verfahren benötigt, die in der Lage sind, molekulare Ereignisse *in vivo* in Echtzeit abzubilden und zu überwachen. Unter den verfügbaren Bildgebungsverfahren spielt dabei vor allem die Positronen-Emissions-Tomographie (PET) eine wichtige und zentrale Rolle. Die PET erzeugt Schnittbilder von lebenden Organismen durch die Detektion des Verteilungsmusters eines zuvor applizierten radioaktiv markierten Pharmazeutikums und bildet somit durch spezifische Wechselwirkungsprozesse biochemische und physiologische Funktionen ab. Andere Bildgebungsverfahren, wie die Magnetresonanztomographie (MRT), Computer-Tomographie (CT) oder Ultraschallbestrahlung (US) zeigen strukturelle und physiologische Veränderungen im Gewebe, die die Folge von molekularen Prozessen, wie die Aktivierung oder Inhibition von Rezeptorsystemen, Enzymen oder die Änderung in der Signaltransduktion sind. Genau diese molekularen Prozesse können mittels PET einzeln und direkt visualisiert werden. Die PET ist daher von exklusiver Bedeutung für die Diagnostik und für das Monitoring von therapeutischen Interventionen. In der Kardiologie wird sie zum Beispiel für die Bildgebung von Herzmuskelperfusionen zur Charakterisierung von koronaren Herzkrankheiten verwendet.[1] Die Neurologie nutzt das Verfahren beispielweise zur Diagnostik und Beurteilung der biologischen Aggressivität von Gliomen.[2] Dyskinesien, wie Chorea Huntington, Dystonie, Tics und Myoklonien können ebenfalls mittels PET-Messungen diagnostiziert werden, die als Grundlage für das weitere therapeutische Vorgehen dienen.[3] Auch bei der Differentialdiagnose von primären Demenzen, wie der Alzheimer-Krankheit und bei hypokinetisch rigiden Syndromen (z.B. M. Parkinson) kommt der PET eine immer weiter wachsende Bedeutung zu.[4] Den mit Abstand größten Stellenwert hat die PET allerdings nach wie vor in der Onkologie. Mit Hilfe des PET-Tracers 2-[^{18}F]Fluor-2-desoxy-D-glucose ([^{18}F]FDG), der von den Zellen des menschlichen Körpers wie Glucose aufgenommen wird und sich insbesondere in Geweben mit einem erhöhten Glucosestoffwechsel (wie z.B. Tumorgewebe oder bei Inflammation) anreichert, ist eine genaue Beurteilung verschiedener Krebserkrankungen minimal-invasiv möglich.[5] Die PET bietet damit nicht nur eine maßgebliche Hilfestellung bei der Charakterisierung der Tumorerkrankung, sondern auch bei der Differenzierung zwischen malignen und benignen Läsionen, dem Staging eines bekannten Tumorleidens, der Suche nach einem unbekannten Primärtumor, der Ermittlung eines Tumorrezidivs, und nicht zuletzt auch bei der Strahlentherapieplanung.[5c]

Durch die Identifizierung immer neuer molekularer Targets, die mit einer Erkrankung assoziiert sind, wird das Anwendungsgebiet für die PET Diagnostik stetig erweitert und für die Untersuchung verschiedener Erkrankungen genutzt.

Der Prozess der Radiotracerentwicklung reicht von der Targetidentifikation über das Tracerdesign bis hin zur klinischen Anwendung und erfordert dadurch die interdisziplinäre Zusammenarbeit von Biologen, Chemikern und Medizinern. Neben der Identifizierung von Leitstrukturen ist die Einführung von Radionukliden in diese Strukturen ein wichtiger Schwerpunkt der Radiopharmakaentwicklung.

Daher kommt der Entwicklung von neuen Syntheseverfahren und -methoden, die es ermöglichen Moleküle einfach, effizient und schnell zu markieren, eine große Bedeutung zu. Dabei liegt das Forschungsgebiet an der Schnittstelle zwischen Labor und Klinik, sodass neben der Optimierung bereits bekannter und der Entwicklung neuer Markierungsbausteine auch der Verwendung dieser zur Herstellung innovativer, targetspezifischer und klinisch relevanter Radiotracer eine wichtige Rolle zukommt.

1.1 Grundlagen der Positronen-Emissions-Tomographie (PET)

Die PET ist ein hochauflösendes, quantitatives und nichtinvasives diagnostisches Verfahren zur Untersuchung und Visualisierung biochemischer und metabolischer bzw. funktioneller Zusammenhänge. Das Messprinzip basiert auf der räumlichen und zeitlichen Erfassung eines dreidimensionalen Radioaktivitätsverteilungsmusters eines mit einem β^+-Emitter markierten Radiopharmakons („Tracer“ oder „Sonde“). Im Gegensatz zu anderen morphologischen bildgebenden Verfahren, die in der Krankheitsdiagnostik meist auf die Darstellung von Fehlfunktionen, die mit einer Abnormität der Körperstruktur einhergehen, beschränkt sind, können mittels PET direkt biochemische Vorgänge im Körper untersucht werden.[6]

Die Basis solcher Untersuchungen ist die Anwendung von speziell für den jeweiligen Zweck entwickelten und mit Positronenstrahlern markierten Radiopharmaka. Diese werden dem Patienten entweder durch Inhalation aber vorwiegend, durch intravenöse Injektion appliziert. Anschließend verteilt sich der Radiotracer gemäß seiner inhärenten physiologischen Eigenschaften im Organismus und setzt bei der Umwandlung eines Protons (p) zu einem Neutron (n) im Kern des entsprechenden Radionuklids ein Positron (β^+) und ein Neutrino (ν_e) frei.[7]

$$ {}^{1}_{1}p \rightarrow {}^{1}_{0}n + e^{+} + v_e \qquad \text{Gl. 1.0} $$

Das durch den Zerfall entstandene und beschleunigte Positron legt bei abnehmender kinetischer Energie eine mittlere Weglänge von ca. 1–2 mm zurück, bis es dann nach vielen zufälligen und folgelosen Kollisionen auf ein Elektron trifft.[8]

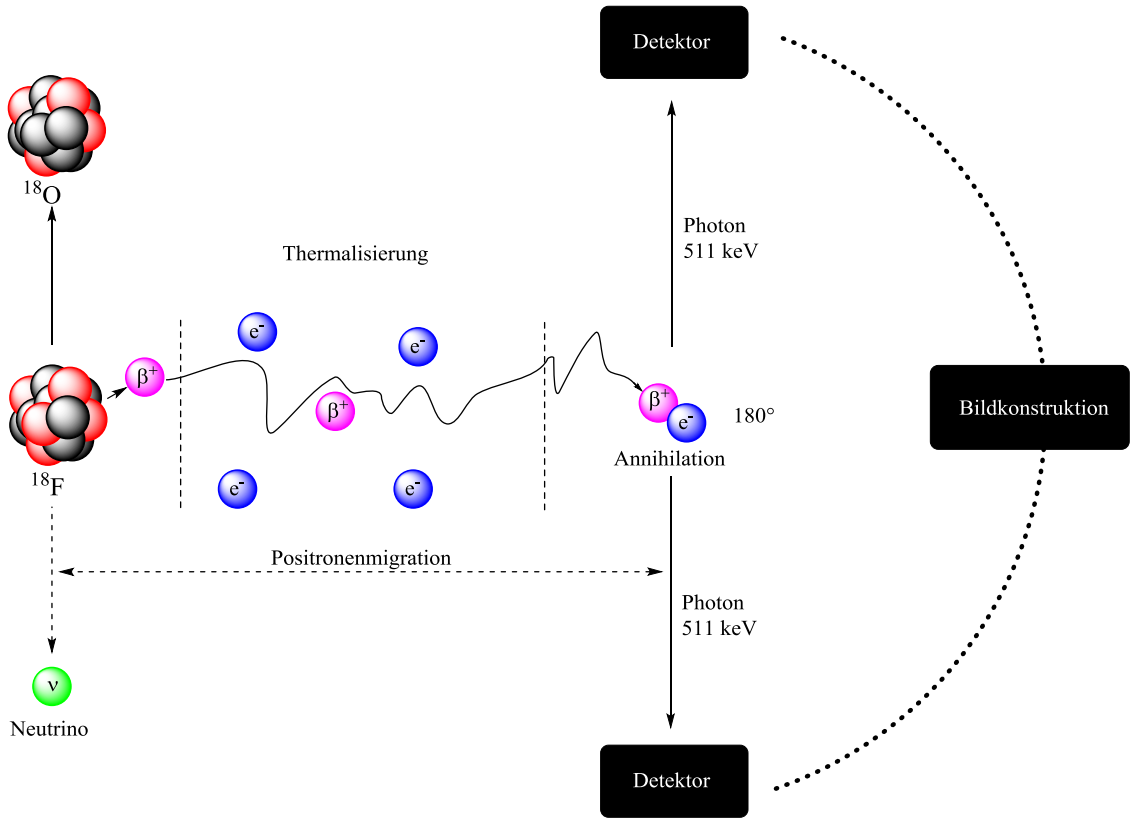

Abbildung 1.0: *Funktionsprinzip der Positronen-Emissions-Tomographie.*

Resultierend aus der darauf folgenden Annihilation des Elektrons und seines Antiteilchens, dem Positron, werden zwei hochenergetische Photonen (γ-Quanten) mit einer Energie von jeweils 511 keV diametral ausgesandt. Diese Energie entspricht nach der Einstein Gleichung den Ruhemassen von Positron und Elektron. Die mittlere freie Weglänge, die das Positron bis zur Annihilation zurücklegt, ist damit entscheidend für die Auflösung des resultierenden PET-Bildes. Je geringer die Energie des Positrons ist, desto geringer ist seine mittlere Reichweite und desto größer ist die Auflösung. (siehe Tabelle 1.0)

Ohnehin wird die Auflösung der PET durch zwei physikalische Effekte limitiert:

- Die Koinzidenzmessung bestimmt nicht den Ort der Positronenemission, sondern den Ort der Positronenannihilation und
- Restimpulse von Elektronen und Positronen bewirken, dass die beiden γ-Quanten nicht genau in entgegengesetzter Richtung zerstrahlen.

Infolgedessen beträgt die maximale PET-Auflösung ca. 1–3 mm.

Aufgrund ihrer geringen Wechselwirkungswahrscheinlichkeit mit Materie verlassen die beiden pro Zerfallsereignis entstehenden γ-Quanten nahezu ungehindert den Körper und werden registriert.

Tabelle 1.0: *Physikalische Größen der wichtigsten klinischen PET Isotope*.

PET Isotop	Halbwertszeit [min]	Eβ⁺max [MeV]	Reichweite max/mittel [mm]	Kernreaktion	Zerfallsprodukt
^{18}F	110	0,64	2,4/0,6	$^{18}O(p,n)^{18}F$	^{18}O
^{11}C	20	0,96	4,1/1,2	$^{14}N(p,\alpha)^{11}C$	^{11}B
^{13}N	10	1,2	5,4/1,6	$^{16}O(p,\alpha)^{13}N$	^{13}C
^{15}O	2	1,7	8,2/2,7	$^{13}N(d,n)^{15}O$	^{15}N

Hierfür enthält das PET-Gerät eine Vielzahl von Detektoren, die ringförmig um das zu untersuchende Objekt angeordnet sind. Zur Erfassung der Positronenstrahlung bzw. der Vernichtungsstrahlung ist eine sogenannte Koinzidenz-Schaltung gegenüberliegender Gammadetektoren notwendig, um die resultierenden γ-Quanten dem β⁺-Zerfall eindeutig zuzuordnen. Unter der Annahme einer im Rahmen der Geräteauflösung vernachlässigbaren Reichweite der Positronen liegt der Ort des Zerfalls direkt auf einer Linie zwischen den beiden Detektoren, in denen die zwei γ-Quanten registriert werden. Diese Linie wird auch als *line of response* (LOR) bezeichnet. Ein Zerfallsereignis wird nur dann als gültig gemessen, wenn die beiden Detektorpaare innerhalb der Koinzidenzauflösezeit je ein γ-Quant registrieren. Alle linear gemessen Koinzidenzereignisse werden mit Hilfe einer Ausleseelektronik und Bildbearbeitungssoftware in eine Bildaufnahme rekonstruiert, die die Lokalisation und Konzentration des Radiotracers wiedergibt.[9] Viele Millionen Zerfallsereignisse werden benötigt, um genügend Daten für die Erstellung eines PET-Bildes zu erhalten.

1.2 Auswahl geeigneter Radionuklide für die PET

Die Auswahl eines geeigneten Radionuklids für nuklearmedizinische Anwendungen ist an einige, zum Teil voneinander unabhängige Bedingungen bzw. Limitierungen geknüpft. So muss neben der Zerfallsart und der Zerfallsenergie auch die physikalische Halbwertszeit des Emitters präzise auf die Pharmakokinetik und –dynamik des jeweiligen Radiotracers abgestimmt werden. Entscheidend sind auch ökonomische und logistische Aspekte, wie

Produktionskosten und Verfügbarkeit des jeweiligen Radionuklids. Der Einsatz der für die PET gängigen, kurzlebigen Positronenemitter ^{11}C, ^{13}N, ^{15}O und ^{18}F (siehe Tabelle 1.0) erfordert beispielsweise ein hauseigenes Zyklotron für die Radionuklidproduktion oder zumindest, wie im Falle des ^{18}F möglich, die Einbindung in ein funktionierendes Versorgungsnetzwerk.[10]

Kohlenstoff, Stickstoff und Sauerstoff sind originäre Bestandteile vieler biologisch aktiver Verbindungen, wodurch die Möglichkeit besteht sie mittels ^{11}C, ^{13}N und ^{15}O-Markierungen entsprechender Vorläufer, als isotop radiomarkierte Moleküle zu erhalten. Mit Ausnahme von Isotopeneffekten kann eine potenzielle Beeinträchtigung ihrer biologischen Aktivität durch Fremdmarkierung damit nahezu ausgeschlossen werden, was zunächst den Anschein erweckt, die kurzlebigen Positronenstrahler (^{11}C, ^{13}N und ^{15}O) seien für diagnostische Zwecke besonders gut geeignet.[11] In Anbetracht ihrer kurzen Halbwertszeit können diese jedoch lediglich zur Untersuchung schneller Stoffwechselvorgänge eingesetzt werden. Deshalb wurden sogenannte Analogtracer entwickelt, die sterische und chemische Analogien, und infolge dessen Analogien im Bindungsverhalten aufweisen und gleichzeitig den Einbau langlebigerer Nuklide in Pharmaka erlauben, ohne dabei die pharmakokinetischen Eigenschaften des Moleküls zu verändern.[12] Auf Grund seines dem Wasserstoff (1,35 Å vs. 1,20 Å) sehr ähnlichen Van-der-Waals-Radius und der annähernden Übereinstimmung einer C-F- und einer C-OH-Bindungslänge,[13] hat sich ^{18}F als ein für diesen Zweck besonders gut geeignetes Radionuklid erwiesen. Deshalb und vor allem wegen seiner weiteren nachfolgend aufgelisteten Vorteile kommt dem ^{18}F eine außergewöhnliche Stellung zu und macht es damit zu dem am häufigsten eingesetzten PET-Nuklid:

- Die Halbwertszeit von 109,7 min ermöglicht komplizierte und zeitaufwendige Synthesen, inklusive Reinigung und Qualitätskontrolle, mit ausreichend Aktivität für nachfolgende PET-Untersuchungen, konkomittierend mit einer zeitlich begrenzten radioaktiven Belastung des Patienten.
- Seine geringe Positronenenergie von 0,64 MeV erlaubt eine sehr hohe Ortsauflösung in der Positronen Emissions Tomographie.
- ^{18}F ermöglicht es, eine hohe spezifische Aktivität zu erreichen, was unter anderem für die rezeptorbindenden Tracer maßgeblich von Bedeutung ist.

Zur Herstellung von ^{18}F sind über zwanzig Kernreaktionen bekannt, von denen die vier wichtigsten in Tabelle 1.1 aufgeführt sind.[14]

Tabelle 1.1: *Wichtige Kernreaktionen zur ^{18}F-Produktion.*

Kernreaktion	Target	Chem. Form des ^{18}F	Spez. Aktivität [Ci/mmol]
$^{18}O(p,n)^{18}F$	$H_2{}^{18}O$	$^{18}F^-_{(aq)}$	10^5
$^{16}O(^3He,p)^{18}F$	H_2O	$^{18}F^-_{(aq)}$	10^5
$^{20}Ne(d,\alpha)^{18}F$	Ne(0,1% F_2)	$[^{18}F]F_2$	1–10
$^{18}O(p,n)^{18}F$	$^{18}O_2$(0,1% F_2)	$[^{18}F]F_2$	1–50

Zum einen wird es als elementares $[^{18}F]F_2$ geträgert (carrier-added, c.a.) in elektrophilen Synthesen eingesetzt. Zum anderen wird es als $^{18}F^-$ ungeträgert (no-carrier-added, n.c.a.) für nucleophile Synthesen angewendet. Die Kernreaktion $^{18}O(p,n)^{18}F$ (erste Zeile Tabelle 1.1) ist in Folge ihres hohen Wirkungsquerschnitts und der damit einhergehenden hohen Targetausbeute sowie der hohen spezifischen Aktivität, die derzeit am meisten angewandte Produktionsmethode von n.c.a. ^{18}F. Die sich daraus ergebenden Möglichkeiten zur Markierung mit ^{18}F werden im folgenden Abschnitt 1.3 näher erläutert.

1.3 Strategien zur Markierung mit ^{18}F

Grundsätzlich lassen sich die Synthesestrategien zur Markierung von organischen Verbindungen in zwei Klassen unterteilen:[15]

- Die indirekten Fluorierungen ermöglichen unter Verwendung von kleinen ^{18}F-markierten Synthesebausteinen, die mit reaktiven funktionellen Gruppen (wie Amino-, Thiol-, Carboxylgruppe) umgesetzt werden, die Herstellung von komplexen und biologisch relevanten Molekülen.
- Die direkten Markierungsverfahren werden ihrerseits nochmals in nucleophile und elektrophile Fluorierungen unterteilt. ^{18}F wird hierbei in einem Schritt in das Zielmolekül eingeführt.

Obwohl den elektrophilen Fluorierungen eine bedeutende historische Rolle, bei der Entwicklung von ^{18}F-markierten Molekülen für die PET-Bildgebung zukommt, werden diese aufgrund geringerer spezifischer Aktivität (theoretisch maximal 50%, da $[^{18}F]F_2$ ein Trägermedium erfordert) und unerwünscht auftretender Mischungen der Markierungsprodukte ($[^{18}F]F_2$ reagiert i.d.R. unspezifisch) heute weniger bevorzugt.

Die Methoden der Wahl sind daher sowohl die nucleophile aromatische als auch die nucleophile aliphatische Substitution. Dazu wird [^{18}F]Fluorid gemäß der bereits oben beschriebenen Kernreaktion erzeugt und in einer wässrigen ([^{18}O]H_2O) Lösung erhalten. Als Anion wird $^{18}F^-$ dann auf einer Ionenaustauschersäule fixiert, wodurch [^{18}O]H_2O zurückgewonnen werden kann[16] und mit Kaliumcarbonat in Wasser/Acetonitril eluiert.[17] Das so erhaltene Fluorid-Ion ist in Folge seines hohen Solvatationsgrades, bedingt durch seine hohe Ladungsdichte, ein schwaches Nucleophil. Um die Reaktivität von $^{18}F^-$ soweit zu steigern, dass nucleophile Substitutionen möglich werden, müssen sogenannte Phasentransferkatalysatoren (z.B.: Kryptofix-2.2.2)[18] zugesetzt und Wasser entfernt werden.[19] Letzteres gelingt bisher nur durch mehrfache und zeitaufwendige azeotrope Trocknungen. Die Entwicklung des azeotropen trocknungsfreien ^{18}F-Fluorid-Vorbereitungs-Verfahren ist daher seit langem Gegenstand der Forschung.

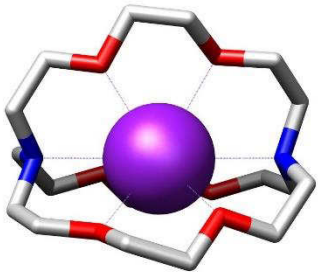

Abbildung 1.1: *Gittermodell der Komplexierung eines Kaliumkations (violett) durch Kryptofix-2.2.2.*[18]

Allerdings sind bisher alle vorgestellten Verfahren zur Herstellung von hoch reaktivem $^{18}F^-$ ohne die Verwendung azeotroper Trocknungen, stark limitiert und/oder unzureichend. Beispielsweise konnten Lemaire *et al*. auf die azeotrope Trocknung durch die Verwendung von Phosphazen Superbasen, wie P_2Et verzichten. Gelöst in einem Gemisch aus Acetonitril und Wasser ermöglichen sie eine nahezu quantitative Elution von $^{18}F^-$.[20] Das Eluat wird anschließend einem entsprechenden Markierungsvorläufer und 2-*tert*-Butyl-1,1,3,3-tetramethylguanidin (BTMG), gelöst in wasserfreiem MeCN, hinzugefügt und für eine kurze Reaktionszeit erhitzt. Eine Reihe von Verbindungen konnten auf diese Weise erfolgreich markiert werden. Allerdings werfen die extrem basischen Bedingungen die Frage auf, wie ein solches Reaktionsmilieu mit der Mehrheit der in der Radiochemie verwendeten Vorläufer-Moleküle zu vereinbaren ist. Außerdem erfordert die Methode hohe Mengen der sehr toxischen Verbindungen P_2Et und BTMG.

Aerts *et al*. hingegen verwendete mit Wasser benetzbare makroporöse Copolymere, deren Beladung mit langkettigen quartären Ammoniumcarbonaten eine direkte Wiedergewinnung von ^{18}F-Fluorid aus [^{18}O]H_2O ermöglicht.[21] Die Elution erfolgt mit MeCN als *n*-Tetradecyltrimethylammonium[^{18}F]fluorid zusammen mit *n*-Tetradecyltrimethylammoniumcarbonat. Das Eluat wird anschließend direkt zur Radiomarkierung eingesetzt. Nachteil dieser Methode ist die Notwendigkeit hoher Vorläufermengen und einer zusätzlichen Base (wie K_2CO_3/K2.2.2 oder Et_4NHCO_3).

Auch Wessmann *et al*. waren mit ähnlichen Problemen konfrontiert und daher ebenfalls gezwungen hohe Mengen an Markierungsvorläufer zu verwenden, um akzeptable radiochemische Umsätze zu erhalten.[22] Zur Elution bediente sich die Gruppe einem hoch konzentrierten Gemisch aus KOH/K2.2.2 in wasserfreiem MeCN und war somit zumindest in der Lage, unter Verwendung kleinerer Aliquote, effizient zu radiofluorieren.

Erst kürzlich veröffentlichte die Gruppe um Pike ihre Arbeit zur kryptandfreien Radiofluorierung von Diaryliodoniumtosylaten im organisch-wässrigen, mit einem Wasseranteil von bis zu 28%.[23] Diese Methode erlaubt die Durchführung der Radiomarkierung direkt mit bestrahlten [^{18}O]Wasser. Leider ist das Verfahren auf die Markierung von (4-Methoxyphenyl)aryliodonium-Salzen, die stark elektronenziehende Gruppen (wie CN, CO_2R und CO(4-EWGAr)) in 2- oder 4-Position zu Iod enthalten, begrenzt. Ferner ist die radioaktive Konzentration von n.c.a $^{18}F^-$ in bestrahltem [^{18}O]Wasser relativ gering, wodurch die verfügbare Menge der ^{18}F-markierten Produkte sehr stark begrenzt ist, was das Verfahren für praktische Anwendungen ungeeignet macht.

Wie aus der Literatur zu entnehmen ist, werden also überwiegend basische Salze, wie K_2CO_3, Et_4NHCO_3, $KHCO_3$, etc., zur Elution des [^{18}F]Fluorids verwendet. Gleichzeitig ist es in der Radiochemie gängige Praxis, Trimethylammonium- und Aryliodonium-Salze als Vorläufer-Moleküle in nucleophilen Substitutionsreaktionen zur Synthese radiomarkierter Verbindungen zu verwenden. Derartige Onium-Salze sind sehr gut in organischen Lösungsmitteln löslich und könnten sich daher potenziell zur direkten Elution von $^{18}F^-$ eignen. Dies und vor allem weiterführende Untersuchungen darüber, ob die resultierenden Onium-[^{18}F]Fluorid-Salze sich möglicherweise direkt zu den gewünschten radiofluorierten Produkten, ohne die Zugabe weiterer Zusätze und Basen, umsetzen lassen, galt es zu Beginn dieser kumulativen Promotionsarbeit zu überprüfen (vgl. Publikation 1).[24] Als erste Modellverbindungen wurden hierfür radiofluorierte Fluorbenzaldeyde (2-, 3- und 4-[^{18}F]FBA) ausgewählt. Bei den nachfolgenden Synthesen komplexer und/oder klinisch relevanter Verbindungen fungierten sie dann zunächst als kleine reaktive Synthesebausteine und anschließend in der Zielverbindung als radioaktiv markierter Anker.

Obgleich bereits eine Vielzahl derartiger Synthone zur Radiomarkierung von biologisch relevanten Verbindungen entwickelt wurden, sind bislang nur weinig geeignete Kopplungsreaktionen bekannt, die den hohen Ansprüchen der Radiochemie genügen.[17],[25] Vor allem in den letzten Jahren haben sich diesbezüglich die (2+3) Cycloadditionsreaktionen als äußerst zweckdienlich erwiesen. Sie sind daher auch ein wichtiger Bestandteil dieser Arbeit und werden als solcher entsprechend ausführlich in den folgenden Abschnitten behandelt.[26],[27]

1.4 Die (2+3) Cycloaddition und ihre Anwendung in der Radiochemie

In einer (2+3) Cycloaddition reagieren ungesättigte Verbindungen, sog. Dipolarophile (z.B. Alkine, Alkene, Schiffsche Basen, etc.), und 1,3 Dipole, die mindestens ein Heteroatom enthalten und mit mindestens einer mesomeren Grenzstruktur, die zwei getrennte Ladungen enthält (z.B. Nitriloxide, Azide, Nitrone, Ozon, N_2O, Sydnone, etc.), zu fünfgliedrigen Heterocyclen.[28] Die Reaktion erfolgt konzertiert unter Erhalt der Stereochemie.[29] Systematisch und mechanistisch wurde die (2+3) Cycloaddition bereits eingehend in den frühen 60er Jahren von R. Huisgen untersucht und daher in Anlehnung an seine Verdienste auch häufig als Huisgen 1,3-Dipolare Cycloaddition bezeichnet.

Die Regioselektivität der Reaktion ist stark abhängig von sterischen und elektronischen Effekten der Substituenten. Das Produkt kann daher entweder als 1,4- bzw. 1,5-Isomer regiospezifisch oder auch als Gemisch beider Regioisomere erhalten werden.[30]

Unter den (2+3) Cycloadditionen sind insbesondere die 1,3-dipolaren Cycloadditionen zwischen Aziden und Alkinen nach Huisgen beachtenswert. Sie dienen in der organischen Chemie als Verknüpfungsreaktionen zweier ungesättigter Verbindungen und ermöglichen den Zugang zu einer Vielfalt an fünf- und sechsgliedriger Heterocyclen.[28] Bereits 2001 erkannte die Gruppe um B. Sharpless ihr enormes Potential und modifizierte sie dahingehend, dass ihre Reaktionscharakteristika den von ihnen definierten, strengen Kriterien des Konzepts der sogenannten „Click"-Chemie genügen.[31] Derartige Reaktionen sind solche, die hohe Ausbeuten liefern, modulare und breite Anwendung finden, unter einfachen Reaktionsbedingungen durchzuführen sind, eine einfache Aufarbeitung und Isolierung der Produkte mittels Kristallisation oder Destillation ermöglichen, hohe thermodynamische Antriebskraft besitzen, eine hohe Atomeffizienz aufweisen, regio- und stereospezifisch (idealerweise regio- und stereoselektiv) sind und in umweltfreundlichen bzw. einfach zu entfernenden Lösungsmitteln ablaufen.[26b],[32]

Auch in der Radiochemie, wo fortwährend nach neuen, leistungsfähigen und –im Hinblick auf die Halbwertszeit der am häufigsten verwendeten Nuklide– schnellen Syntheseverfahren gesucht wird, fand und findet das Konzept der „Click-Chemie" breite Beachtung.

Im Jahr 2006 gelang erstmalig ihr Transfer aus der organischen- in die Radiochemie. Es waren Marik und Sutcliffe, die das große Potential der „Click-Reaktionen" erkannten und sie in der Radiochemie als neues Markierungskonzept etablierten.[33] Dabei machten sich die Autoren die schnelle Bildung von Triazolen über die Kupfer(I) katalysierte 1,3-dipolare Cycloaddition zwischen Alkinen und Aziden (CuAAC) zur Markierung zunutze. Das zunächst synthetisierte ^{18}F-markierte Alkin wurde dabei in einer nachfolgenden Cycloadditionsreaktion mit einem,

eine Azidfunktion tragenden Peptid verknüpft. Über die inverse Variante dieser Markierungsstrategie berichteten 2007 Glaser und Årstad.[34] Als ^{18}F-Markierungsbaustein diente ihnen der 1,3-Dipol 2-[^{18}F]Fluorethylazid ([^{18}F]FEA), welcher in Folgereaktionen über die CuAAC mit einer Reihe von Modellverbindungen mit terminaler Alkinfunktion umgesetzt wurde. Im Zuge ihrer Untersuchungen über die Reichweite der Methode berichteten sie über die erfolgreiche Markierung eines Modellpeptids und konnten mit den hohen Ausbeuten von 93% bei kurzen Reaktionszeiten demonstrieren, dass sich das Konzept der „Click-Chemie" prinzipiell für die Anwendung in der Radiochemie eignet.

Allerdings ist ihre Verwendbarkeit bei der Herstellung von Radiopharmazeutika durch den unverzichtbaren Einsatz von Kupfer, welches bei Anwesenheit *in vivo* zu einer Denaturierung von Peptiden und Proteinen führen und im Endprodukt zelltoxisch wirken kann, stark limitiert.[35] Eine attraktive Alternative dazu, bieten die sog. Cu-freien, spannungsgetriebenen Azid-Alkin-Cycloadditionsreaktionen (SPAAC). Die erfolgreiche Übertragung dieser Methode in die Radiochemie wurde erst jüngst von der Gruppe um Carpenter vorgestellt. Als Vorläufer verwendeten sie Azadibenzocyclooctinamin (ADIBOA). Dieses wurde zunächst mit Hilfe des ^{18}F-markierten Aktivesters *N*-Succinimidyl-4-[^{18}F]fluorbenzoat ([^{18}F]SFB) acyliert und anschließend in einer SPAAC mit einem Modellazid in das entsprechende ^{18}F-markierte Triazol überführt.[36]

Bis vor kurzem wurden in der Radiochemie lediglich die Azid-Alkin Kupplungen für Markierungsreaktionen eingesetzt, obwohl bereits zahlreiche andere (2+3) Cycloadditionen bekannt sind. Viele von ihnen sind bereits mit leicht verfügbaren Ausgangsverbindungen durchzuführen und liefern sowohl metabolisch, als auch chemisch stabile Cycloadditionsprodukte.[28] In diesem Zusammenhang wurde 2012 von Zlatopolskiy *et al.* die Entwicklung eines neuen 1,3 Dipols ([^{18}F]Fluorphenylnitron, [^{18}F]FPPN) ausgehend von [^{18}F]FBA zur Radiomarkierung in sog. Nitron-Alken-Cycloadditionen vorgestellt.[27b] Durch die nachfolgende, erfolgreiche Umsetzung des neuen Markierungsbausteins mit einem nicht gespannten, allerdings hinreichend stark aktivierten Maleimid-Derivat konnte die Gruppe die Eignung dieser 1,3-dipolaren Cycloaddition zur Radiomarkierung demonstrieren.

Unter Verwendung des neu entwickelten 1,3 Dipols wurde daraufhin eine weitere, ebenfalls den (2+3) Cycloadditionen zugehörige Reaktion, die sog. Kinugasa-Reaktion, im Rahmen dieser Promotionsarbeit auf ihre Verwendbarkeit in der Radiochemie geprüft und anschließend etabliert. Die Details zur Reaktion werden im Kapitel 1.6 näher erläutert.

1.5 Die Seyferth-Gilbert-Homologisierung

Die Seyferth-Gilbert-Homologisierung ist ein wertvolles Verfahren um Aldehyde und Ketone in Anwesenheit von Dimethyl-(diazomethyl)phosphonat schnell in „Ein-Kohlenstoff" homologe Alkine zu überführen. Die Reaktion wurde erstmalig 1970 von D. Seyferth veröffentlicht und 1979 von der Gruppe um J. C. Gilbert optimiert und weiter entwickelt.[37] Ein großer Nachteil dieses Verfahrens ist jedoch, dass das benötigte Dimethyl-(diazomethyl)phosphonat relativ instabil ist und daher vor jeder Reaktion frisch und in zeitaufwendigen mehrstufigen Reaktionen hergestellt werden muss. Außerdem ist die Reaktion aufgrund der stark basischen Reaktionsbedingungen (*n*-BuLi oder KO^{t}Bu) auf unempfindliche und basenstabile Reaktanden begrenzt.

Erst die 1989 von Ohira und 1996 von Bestmann veröffentlichte Modifikation der Seyferth-Gilbert-Homologisierung erhöhte die Kompatibilität dieses Verfahrens mit einer Vielzahl funktioneller Gruppen und eröffnete damit die Möglichkeit, diverse Alkine, entweder als Produkt selbst, oder als Zwischenprodukt in mehrstufigen (Total)-Synthesen zu erhalten.[38] Das für die Reaktion benötigte Dimethyl-(diazomethyl)phosphonat-Anion wird dabei *in situ* aus dem sog. Ohira-Bestmann Reagenz (Dimethyl-1-diazo-2-oxopropylphosphonat) in Anwesenheit von K_2CO_3 in MeOH gewonnen.

Die Möglichkeit eine ganze Bandbreite an unterschiedlichen Alkinen in guter bis sehr guter Ausbeute und Reinheit zu erhalten, macht die Seyferth-Gilbert Homologisierung zu einem beliebten Werkzeug in der organischen Chemie.[39] Auch in der Radiochemie besteht ein fortwährendes Interesse an neuen Synthesestrategien zur einfachen Herstellung von Alkinen, welche *via* „Click Chemie" den Zugang zu innovativen Radiotracern, die potenziell neue Targetstrukturen adressieren, ermöglicht. Ein wichtiger Teil dieser Arbeit bestand daher in der Entwicklung und Optimierung eines für die Radiochemie geeigneten Verfahrens zur Herstellung von ^{18}F-markierten Arylacetylen mittels Seyferth-Gilbert-Homologisierung und ausgehend von [^{18}F]FBA (Vgl. Publikation 2).[40]

1.6 Die Kinugasa-Reaktion

Benannt nach ihrem Entdecker M. Kinugasa, ist die Kinugasa-Reaktion eines der am häufigsten verwendeten Verfahren zur Konstruktion von β-Lactamen.[41] Sie beschreibt die Reaktion von terminalen Alkinen mit Nitronen in Anwesenheit von Cu(I)-Salzen. Streng genommen gehört sie zur Gruppe der (2+3) Cycloadditionen und liefert als solche bei Verwendung nicht terminaler Alkine, anstelle der β-Lactame, Isoxazoline. Herkömmlich wird

die Kinugasa-Reaktion in organischen Lösungsmitteln, wie MeCN, DMF oder wie von Kinugasa selbst, in wasserfreiem Pyridin unter Inertgasatmosphäre und bei Raumtemperatur durchgeführt. Sie gelingt aber auch im wässrigen Milieu unter „Click-Chemie" Bedingungen.[42]

Infolge des stetig wachsenden Bedarfs an neuen, hoch potenten β-Lactam-Antibiotika sowie an effektiveren β-Lactamase-Inhibitoren wurde im Laufe der Jahre konsequenterweise auch das Interesse an neuen und vor allem direkten Synthesewegen zur Konstruktion von β-Lactamen geweckt. Aufgrund ihrer diversen Vorteile rückte diesbezüglich die Kinugasa-Reaktion wieder stärker in den Fokus und zahlreiche Arbeiten zur ihrer Modifikation und Optimierung wurden in den vergangenen 50 Jahren nach ihrer Erstentdeckung publiziert.[43] Miura *et al.* beispielsweise berichteten über eine signifikante Erhöhung der Ausbeute durch die Verwendung von verschiedenen Katalysatoren (wie z.B. 1,10-Phenanthrolin) und stellte zusätzlich die erste intermolekulare asymmetrische Kinugasa-Reaktion mit chiralen Liganden vor.[44] Die Gruppe um Basak folgte diesem Ansatz und erweiterte ihn durch die Verwendung des Evans-Oxazolidinon als chiralem Hilfsreagenz. Ihnen gelang dadurch die Synthese enantiomerenreiner Mischungen von *cis*- und *trans*- β-Lactamen. Außerdem bestätigten die Autoren die Vermutung, dass die *cis*-β-Lactame in Gegenwart von basischen Reagenzien zu den *trans*-β-Lactamen isomerisieren.[45] Inspiriert durch die Arbeiten von Miura, stellten Lo und Fu die erste vollständig diastereo- und enatioselektive katalytische Kinugasa-Reaktion mit chiralen Liganden vor.[46] Verwendet wurde dazu *N*,*N*-Dimethylcyclohexylamin und der planar-chirale Ligand Bis(azaferrocen).

Eingehend bekannt ist, dass während der Kinugasa-Reaktion gelegentlich einige Nebenprodukte auftreten können. Den größten Anteil daran hat das sich in Folge einer oxidativen Dimerisierung bildende Kupplungs-Produkt der terminalen Acetylene (Glaser-Kupplung).[47] Der Grund hierfür liegt in der Anwesenheit von Cu(II), das sich potenziell im Laufe der Reaktion bildet oder auch in Spuren in den verwendeten Cu(I)-Salzen vorkommen kann. Basak *et al.* beschäftigte sich ausführlich mit dieser Problematik und berichtete 2007 über die Durchführung der Kinugasa-Reaktion unter „Click"-Chemie Bedingungen.[42] Sie erkannten die interessante Analogie der Mechanismen zwischen der Cycloaddition von terminalen Alkinen und Aziden bei Raumtemperatur in Anwesenheit von Cu(I) und der Kinugasa-Reaktion. Anstelle der bislang verwendeten Cu(I)-Salze, wurde $CuSO_4$ als Kupfer(I)-Quelle eingesetzt, das mittels Natriumascorbat *in situ* zu Cu(I) reduziert wurde. Im Gegensatz zum konventionellen Verfahren unter Inertgas, besteht der wesentliche Vorteil bei der Durchführung der Kinugasa-Reaktion unter „Click"-Chemie Bedingungen darin, dass der

unerwünschten Bildung von Cu(II) entgegen gewirkt und die Bildung von daraus resultierenden Nebenprodukten unterdrückt werden kann. Arbeiten unter Schutzgasatmosphäre sind damit überflüssig, was zusätzlich die Synthesen erheblich erleichtert.

Das Interesse der Radiochemie besteht nun einerseits darin, die Reaktion sowohl zur Markierung von klinisch relevanten Molekülen als auch zur gezielten Synthese von ^{18}F-markierten β-Lactamen zu verwenden, die sich prinzipiell zur Visualisierung bakterieller Infektionen mittels PET eignen sollten. Andererseits könnten ^{18}F-markierte β-Lactame aufgrund ihrer Fähigkeit nicht nur bakterielle Trans- und Carboxypeptidasen, sondern auch unterschiedliche Serinproteasen, wie das prostataspezifische Antigen (PSA), Thrombin und Elastasen irreversibel zu inhibieren, potenziell für die PET-Bildgebung von Prostatatumoren, Thrombosen und Emphysemen geeignet sein. Diesbezüglich werden in der hier vorliegenden Promotionsarbeit diverse Beispiele von ^{18}F-markierten β-Lactam-Derivaten vorgestellt, die erfolgreich über eine Kinugasa-Reaktion erhalten wurden. (vgl Publikation 3).[48]

2 Abdrucke der zur kumulativen Promotion eingereichten Publikationen

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Krapf, P., Richarz, R., Urusova, E. A., Neumaier, B., Zlatopolskiy, B. D., Seyferth–Gilbert Homologation as a Route to ^{18}F-Labeled Building Blocks: Preparation of Radiofluorinated Phenylacetylenes and Their Application in PET Chemistry. *European Journal of Organic Chemistry*, **2016**, *2016*, 430-433. Copyright Wiley VCH Verlag GmbH & Co. KGaA. Reproduced with permission.

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Neither azeotropic drying, nor base nor other additives: a minimalist approach to ^{18}F-labeling†

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A novel, efficient, time-saving and reliable radiolabeling procedure *via* nucleophilic substitution with [^{18}F]fluoride is described. Different radiolabeled aliphatic and aromatic compounds were prepared in high radiochemical yields simply by heating of quaternary anilinium, diaryliodonium and triarylsulfonium [^{18}F]fluorides in suitable solvents. The latter were obtained *via* direct elution of $^{18}F^-$ from an anion exchange resin with alcoholic solutions of onium precursors. Neither azeotropic evaporation of water, nor a base, nor any other additives like cryptands or crown ethers were necessary. Due to its simplicity this method should be highly suitable for automated radiosyntheses, especially in microfluidic devices.

Introduction

Positron emission tomography (PET) offers quantitative real time 3D-visualization of physiological and pathological processes *in vivo* by means of probes labeled with β^+-emitting nuclides (PET-nuclides). Among PET-nuclides, fluorine-18 (^{18}F) is the most widely used since it is easily accessible in >50 GBq quantities from $H_2{}^{18}O$ *via* the high-yielding $^{18}O(p,n)^{18}F$ nuclear reaction. Furthermore, ^{18}F exhibits a low β^+-energy [$E(\beta^+)$ = 630 keV, 97%] resulting in PET images with high spatial resolution.[1] In addition, the half-life of ^{18}F (109.8 min) enables multistep radiosynthesis and allows distribution of the radiolabeled tracers to regional medical centers.

Although numerous methods of ^{18}F-labeling have been developed, the vast majority of radiofluorinated compounds are prepared *via* aliphatic and aromatic nucleophilic substitution reactions with $^{18}F^-$.[2] In accordance with the production route, [^{18}F]fluoride is obtained in a $H_2{}^{18}O$ solution (1–3 mL). Owing to tenacious hydration, water significantly diminishes the nucleophilicity of $^{18}F^-$. To remove the bulk of water, $^{18}F^-$ is usually trapped on an anion-exchange resin[3] and then eluted with an aqueous solution of K_2CO_3. Thereafter, 2.2.2-cryptand (K2.2.2) in acetonitrile is added and the water is removed by time-consuming (7–15 min) repetitive azeotropic drying with acetonitrile. K2.2.2 captures K^+, and consequently increases the nucleophilicity of $^{18}F^-$ by means of charge separation. To prepare the radiofluorinated compound, the dried residue of [K2.2.2K$^+$]$^{18}F^-$ is usually taken up in a solution of the precursor in a polar aprotic solvent and heated for a short period of time. The precursor should contain a suitable leaving group for nucleophilic substitution with [^{18}F]fluoride. Instead of K_2CO_3, other basic salts such as $KHCO_3$ or $K_2C_2O_4$/K_2CO_3 in combination with a cryptand or Cs^+ or tetraalkylammonium carbonates or bicarbonates without a cryptand can be used.[4] For more demanding radiofluorinations, radiolabeling can be substantially hampered by adsorption of $^{18}F^-$ onto the vessel walls (up to >50%).

A plethora of methods describing the production of highly reactive [^{18}F]fluoride without the use of azeotropic drying were reported. However, all of them suffer from significant limitations.

Lemaire *et al.*[5] avoided azeotropic drying by applying solutions of phosphazene superbases like P_2Et in MeCN containing water for almost quantitative elution of $^{18}F^-$ from the anion exchange resin. The eluate was added to a solution of the appropriate labeling precursor (up to 40 mg) and BTMG (2-*tert*-butyl-1,1,3,3-tetramethylguanidine) as an additional base in anhydrous MeCN. Several radiofluorinated compounds were successfully prepared by heating of the resulting mixture for a short time. However, the extremely strong basic conditions raise the question whether this reaction milieu is compatible with the majority of precursors for radiofluorination. Additionally, this method requires high amounts of P_2Et and BTMG, which are known to be highly toxic.

Aerts *et al.*[6] avoided azeotropic drying using water-wettable macroporous copolymers loaded with a long alkyl chain quaternary ammonium carbonate to directly recover [^{18}F]fluoride

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from [^{18}O]H_2O. [^{18}F]Fluoride was eluted with MeCN in the form of *n*-tetradecyltrimethylammonium [^{18}F]fluoride together with *n*-tetradecyltrimethylammonium carbonate.

This eluate was directly used for ^{18}F-nucleophilic substitutions. This method was disadvantageous with respect to the need for high precursor amounts (15–40 mg) as well as in a majority of cases of high amounts of an additional base (K_2CO_3/K2.2.2 or Et_4NHCO_3) necessary to achieve high radiochemical yields.

Wessmann *et al.*[7] used highly concentrated KOH/K2.2.2 in anhydrous MeCN to efficiently elute [^{18}F]fluoride from an anion exchange resin. An efficient ^{18}F-incorporation *via* nucleophilic aliphatic substitution was demonstrated using small aliquots of $^{18}F^-$ eluate. In upscaling experiments high amounts of radiolabeling precursors were to be applied to achieve an acceptable level of radiochemical conversion (RCC).§

Quite recently, Chun *et al.*[8] published radiofluorination of diaryliodonium tosylates under organic-aqueous (up to 28% water) and cryptand-free conditions using K_2CO_3 as a base. Their method allowed to obtain moderate yields of ^{18}F-labeled compounds directly using the irradiated [^{18}O]water without the need for azeotropic drying and cryptand addition. Unfortunately, the narrow scope significantly confines the practical utility of this method. Only (4-methoxyphenyl)aryliodonium salts containing selected strong electron-withdrawing groups, CN, CO_2R and CO(4-EWGAr) in the 2- or 4-position to iodine, could be radiolabeled yielding the corresponding [^{18}F]-fluorobenzonitriles, [^{18}F]fluorobenzoates and [^{18}F]fluorobenzophenones in moderate RCYs. Furthermore, the relatively low concentration of $^{18}F^-$ in irradiated [^{18}O]H_2O limits the accessible amounts of ^{18}F-labeled compounds, especially using microfluidic devices (reaction volume <50 μL).

Usually, basic salts are used to elute [^{18}F]fluoride from an anion-exchange resin. At the same time, it can be quantitatively recovered using neutral salts (*e.g.* isotonic saline). On the other hand trimethylammonium and aryliodonium salts are widely used as precursors for the preparation of radiofluorinated aromatic compounds. These onium salts which are highly soluble in organic solvents could, we thought, be used directly to elute [^{18}F]fluoride from the anion-exchange resin. Furthermore, we examined if the resulting onium [^{18}F]fluoride salts could be directly converted into ^{18}F-labeled compounds without addition of a base or any other ingredients under "minimalist" conditions.

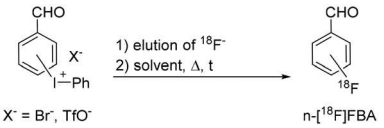
Results and discussion

Initial experiments

Radiofluorinated fluorobenzaldehydes (2-, 3- and 4-[^{18}F]FBA) were chosen as model compounds. They serve as versatile building blocks for different radiosyntheses.[9] First, the elution of [^{18}F]fluoride from an anion exchange resin with iodonium [^{18}F]FBA precursors in anhydrous DMF or DMSO was examined (Table 1). With (3- and 4-formylphenyl)phenyliodonium triflates, radioactivity recovery amounted up to 30% (in the case of bromides up to 20%). The precursor eluate was directly heated for 10–15 min.

Afterwards, an excess of water was added to quench the reaction and, most importantly, completely solubilize surface-adsorbed $^{18}F^-$.[10] Formation of 3-[^{18}F]FBA and 4-[^{18}F]FBA in RCCs up to 22 and 51%, respectively, was observed. Unreacted $^{18}F^-$ was the only impurity detected in the case of (*para*-formylphenyl)phenyl iodonium salt precursors. In the case of *meta*-substituted salts, formation of [^{18}F]fluorobenzene was also observed. In contrast to other nucleophilic ^{18}F-substitutions, the radiofluorination reactions of diaryliodonium salts tolerate the presence of water well.[8] Consequently, water was added to the corresponding solvent to improve the elution yield of $^{18}F^-$. When (*para*-formylphenyl)-phenyliodonium triflate in 90% DMF was used for elution, the radioactivity recovery amounted to 55% and 4-[^{18}F]FBA was obtained in radiochemical conversions (RCCs) of up to 51% within 15 min.[11]

Table 1 Preparation of [^{18}F]fluorobenzaldehydes using iodonium precursors without azeotropic drying and any additives[a]



Entry	LG^+X^-	n^b	Solvent	Temperature [°C], time [min]	Elution yield of $[^{18}F]^-$ [%]	RCC[c] [%]
1	TfO^-	2	95% DMF	130, 10	30	2
2[d]	TfO^-	3	DMSO	130, 10	30	22
3	Br^-	3	DMF	160, 10	6	34
4	Br^-	3	95% DMF	160, 15	30	5
5	Br^-	4	DMSO	90, 10	20	51
6	TfO^-	4	90% DMF	160, 15	55	51

[a] $^{18}F^-$ (200–500 MBq) was eluted from a QMA cartridge with a solution of the iodonium precursor in the corresponding solvent (5 mg in 500 μL). The resulting solution was heated at *T* °C for *t* min. [b] *n* – substitution position. [c] H_2O (4 mL) was added to quench the reaction and completely solubilize surface-adsorbed $^{18}F^-$ and RCC determined by radio-HPLC. [d] With 20 mg precursor. Each experiment was carried out at least in triplicates. The standard deviation of RCC did not exceed 20% of its mean value.

Elution of $^{18}F^-$ with onium salts

The elution of $^{18}F^-$ with onium salts in aprotic solvents like DMF or DMSO was rather ineffective (recovery <60%). However, after some pilot experiments we found that the elution of $^{18}F^-$ with onium salts in various alcohols was much more effective (Fig. 1). Especially, with solutions of these salts in MeOH or EtOH the total recovered radioactivity averaged 90–98%. Elution with precursors in higher alcohols was less efficient. In particular, low-boiling methanol not only enabled elution of more than 95% of the initially applied [^{18}F]fluoride but could also be completely removed at 70–80 °C within

§ Radiochemical conversion (RCC) refers to the amount of radiofluoride which is transformed to the desired ^{18}F-labeled compound. It was determined by radio-HPLC. Radiochemical yield (RCY) refers to the isolated yield of the radiochemically and chemically pure radiolabeled compound.

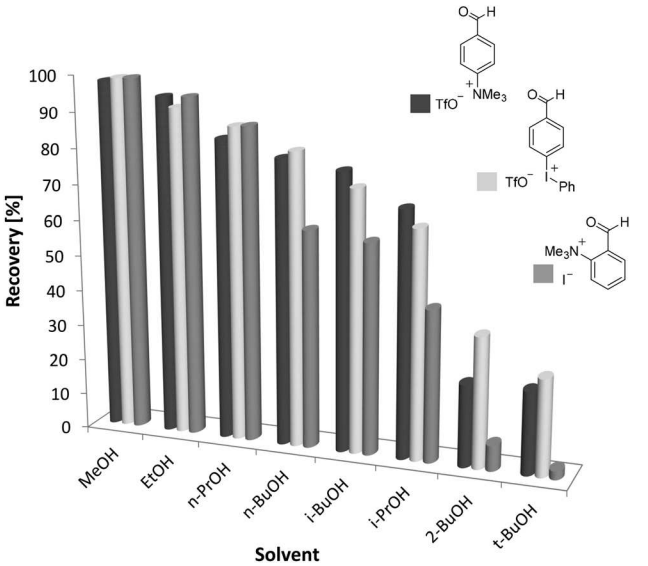


Fig. 1 Recovery yield for the elution of $^{18}F^-$ from a QMA cartridge with onium salts in anhydrous alcohols.

Table 2 Elution of $^{18}F^-$ from a QMA cartridge with different amounts of onium salts[a]

Entry	Amount [mg]	Recovery of $^{18}F^-$ [%]			
1	10	99	—	—	—
2	8	—	98	—	—
3	5	99	—	99	98
4	2.5	99	—	97	92
5	1	—	96	—	—
6	0.7	—	89	—	—
7	0.5	97	78	80	84
8	0.3	—	—	68	82
9	0.1	85	44	65	76

[a] Target [^{18}O]water was passed through an anion exchange resin. The cartridge was washed with anhydrous MeOH and $^{18}F^-$ was eluted by a solution of the corresponding precursor in MeOH (500 μL).

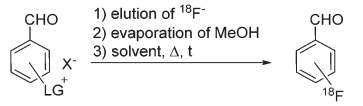
2–3 min without the need for azeotropic drying. Radioactivity loss during the evaporation step was negligible (<2%).

With regard to radiosynthesis in microfluidic devices, we studied the dependence of [^{18}F]fluoride elution yield on behalf of the precursor amount (Table 2). The elution yield of $^{18}F^-$ exceeded 85% with only 0.1 mg precursor in the case of the basic bicarbonate salt. Radioactivity recovery of more than 75% was reached using only 0.3–0.5 mg of non-basic iodide, triflate or perchlorate precursors.

[^{18}F]Fluorobenzaldehydes

Once a reliable method for the elution of [^{18}F]fluoride had been established, we focused on the preparation of [^{18}F]FBAs and an optimization of the radiosynthesis with respect to precursor, reaction solvent and temperature (Table 3).[11] In the case of 2- and 4-[^{18}F]FBA, the best RCCs were achieved with

Table 3 Preparation of [^{18}F]fluorobenzaldehydes[a]



$LG^+ = Me_3N^+$, PhI^+, $(4\text{-MeOPh})I^+$
$X^- = I^-$, Br^-, ClO_4^-, TfO^-, HCO_3^-
n-[^{18}F]FBA

Entry	n^b	LG^+X^-	Solvent	Temperature [°C], time [min]	RCC[c] [%]
1	4	$Me_3N^+I^-$	DMSO	80, 10	75(*65*)[d]
2	***4***	***$Me_3N^+HCO_3^-$***	***DMSO***	***80, 10***	***87***
3	***4***	***$Me_3N^+ClO_4^-$***	***DMSO***	***150, 10***	***90***
4	4	$Me_3N^+TfO^-$	DMF	115, 10	63
5[e]	4	$Me_3N^+TfO^-$	DMSO	80, 10	40–65
6	2	$Me_3N^+HCO_3^-$	MeCN	80, 10	68
7	2	$Me_3N^+I^-$	DMSO	80, 10	62(*62*)[d]
8	2	$Me_3N^+TfO^-$	DMSO	130, 15	64(*46*)[d]
9[e]	2	$Me_3N^+TfO^-$	DMSO	80, 1–2	30–46
10	***2***	***$Me_3N^+ClO_4^-$***	***DMSO***	***80–200, 10–15***	***63–80***
11	3	$Me_3N^+I^-$	Sulfolane	200, 10	6
12	***3***	***(4-MeOPh) $I^+ClO_4^-$***	***DMSO***	***150, 10***	***40***
13[f]	3	PhI^+Br^-	DMF	130, 10	45

[a] $^{18}F^-$ was eluted from a QMA cartridge with precursor (5–8 mg) in MeOH, methanol was evaporated under He flow (Ar/N_2 can be used instead) at 70–80 °C, the residue was dissolved in the appropriate solvent and the resulting solution was heated at T °C for t min. [b] n – substitution position. [c] H_2O (4 mL) was added to quench the reaction and solubilize surface-adsorbed $^{18}F^-$ and RCC determined by radio-HPLC. [d] RCC with 2 mg precursor in 100 μL solvent. [e] K2.2.2/K_2CO_3 protocol. [f] $CsHCO_3$/TEMPO protocol.[12b] Each experiment was carried out at least in triplicates. If the standard deviation of RCC exceeded 20% of its mean value the range of RCC is given. TEMPO – 2,2,4,4-tetramethylpiperidine-*N*-oxyl.

N,*N*,*N*-trimethylanilinium precursors. The counter-ion of the precursor salt significantly influenced radiolabeling. From the corresponding perchlorates in DMSO highest RCCs of 80% and 90% for 2- and 4-[^{18}F]FBA were achieved within 10 min, at 130 and 150 °C respectively. With bicarbonate or iodide salts, 2- and 4-[^{18}F]FBA were obtained in good to excellent RCCs (up to 68% and 87%, respectively) at 80 °C. In all cases, [^{18}F]fluoride was the only radioactive impurity detected after quenching the reaction mixture with an excess of H_2O. Reproducibility and scalability were excellent.

Thus, 4-[^{18}F]FBA was prepared in 65–75% RCY from 43–47 GBq [^{18}F]fluoride (n = 20, non-decay corrected) with >99% radiochemical purity (RP) within 23 min using the bicarbonate precursor. Unreacted [^{18}F]fluoride was removed by solid phase extraction (SPE). By comparison, using the conventional K_2CO_3/K2.2.2 protocol, 2- and 4-[^{18}F]FBA could be obtained best in RCYs of 30–55%. Furthermore, a concurrent formation of labeled side-products (5–25%) was observed. In addition very short reaction times and a narrow temperature range (60–120 s at 90 ± 5 °C) had to be applied to obtain reasonable RCCs and RCPs of 2-[^{18}F]FBA since 2-[^{18}F]FBA was unstable under basic reaction conditions. In contrast, the use of the perchlorate precursor under "minimalist" conditions yielded 2-[^{18}F]FBA in high RCCs of 63–80% within a broad reaction temperature and time range (8–15 min at 80–200 °C). The dependence of RCC on the

precursor amount was briefly examined. RCCs of 2- and 4-[^{18}F]FBA obtained from 2 mg of *N*,*N*,*N*-trimethylanilinium iodide salts were similar to those obtained from 5 mg precursor (Table 3, entries 1 and 7; given in parentheses). In the case of other onium salts RCCs were lower (Table 3, entry 8; given in parentheses; see also Table S4, entries 3, 10, 15, and Table S5, entries 3, 5, 8; given in parentheses in the ESI†).[11] Consequently, all further experiments were carried out with precursor amounts of 5–8 mg.

As with 2- and 4-isomers, 3-[^{18}F]FBA could be prepared from the corresponding *N*,*N*,*N*-trimethylanilinium salts. However, due to the weaker activating effect of the carbonyl group at the *meta*-position, RCCs were low (not more than 6%) even under harsh reaction conditions (Table 3). Using (4-methoxyphenyl)-iodonium salt precursors, 3-[^{18}F]FBA could be prepared in moderate RCCs of up to 40%, which were comparable to those obtained under conventional radiofluorination conditions.[11,12] Again $^{18}F^-$ was the only radioactive impurity which could be detected.

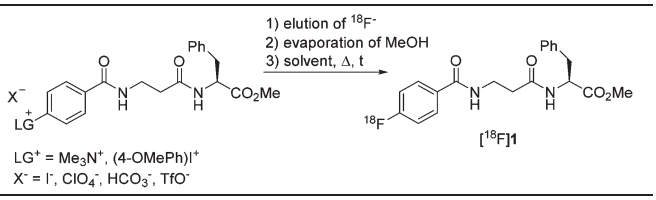
^{18}F-Labeled model peptide [^{18}F]1

To further explore the scope of the novel radiofluorination procedure, its applicability was tested for several practically relevant radiosyntheses. First, we studied the feasibility of the n.c.a. one-step preparation of ^{18}F-benzoylated peptides *via* direct aromatic nucleophilic radiofluorination.[13] In all radiosyntheses published so far additional electron withdrawing groups, such as CN, CF_3 and F, have to be used in order to increase the reactivity of the trimethylammonium or nitro leaving group and ensure acceptable labeling yields. These hydrophobic groups could noticeably increase the overall lipophilicity of the radiolabeled conjugates (especially in the case of short peptides) and, consequently, unfavourably affect their biodistribution. We studied whether direct ^{18}F-peptide labeling could also be carried out without an additional activating group. 4-[^{18}F]-Fluorobenzoyl-βAla-Phe-OMe [^{18}F]**1** was chosen as a model peptide. Initially, different *N*,*N*,*N*-trimethylammonium peptide precursors were investigated (Table 4). Using the conventional K_2CO_3/K2.2.2 radiofluorination procedure, no formation of [^{18}F]**1** was observed (entries 3, 4), whereas preparation of [^{18}F]**1** under "minimalist" conditions led to the desired radiolabeled conjugate as a single product in RCCs of up to 30%. Application of the corresponding (4-methoxyphenyl)iodonium iodide precursor allowed to reduce the reaction time from 15 to 10 min and to raise the RCC to 56%. These almost neutral reaction conditions are comparable to or even milder than those described in the literature for the preparation of radiolabeled bombesin analogs and RGD peptides using additional activating groups (DMSO, K2.2.2/K_2CO_3 or Cs_2CO_3, 70–130 °C, 4–15 min).[13] The scope and limitations of the application of radiofluorination under "minimalist" conditions for the one-step ^{18}F-labeling of more complicated peptides are left to be investigated in further studies.

^{18}F-Labeled active ester [^{18}F]2

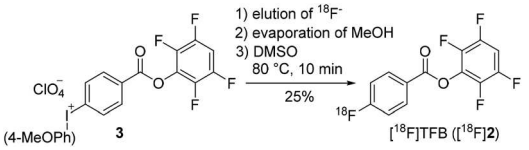
The novel radiofluorination method was applied to the preparation of an ^{18}F-labeled active ester, 2,3,5,6-tetrafluorophenyl

Table 4 Preparation of [^{18}F]**1** from onium precursors without azeotropic drying and addition of a base[a]



Entry	LG^+X^-	Solvent	Temperature [°C], time [min]	RCC[b] [%]
1	$Me_3N^+HCO_3^-$	Sulfolane	130, 15	30
2	$Me_3N^+HCO_3^-$	DMSO	200, 15	29
3[c]	$Me_3N^+TfO^-$	DMSO	130, 30	0
4[c]	$Me_3N^+I^-$	Sulfolane	200, 15	0
5	(4-MeOPh)I^+I^-	DMSO	130, 10	56
6[d]	(4-MeOPh)I^+I^-	DMSO	120, 10	56

[a] $^{18}F^-$ was eluted from a QMA cartridge with precursor in MeOH, MeOH was evaporated under He flow at 70–80 °C, the residue was dissolved in the appropriate solvent and the resulting solution was heated at *T* °C for *t* min. [b] H_2O (4 mL) was added to quench the reaction and solubilize surface-adsorbed $^{18}F^-$ and RCC determined by radio-HPLC. [c] K2.2.2/K_2CO_3 protocol. [d] $CsHCO_3$/TEMPO protocol. Each experiment was carried out at least in triplicates. The standard deviation of RCC did not exceed 10% of its mean value.



Scheme 1 Synthesis of the ^{18}F-labeled active ester [^{18}F]**2** under "minimalist" conditions.

4-[^{18}F]fluorobenzoate ([^{18}F]TFB, [^{18}F]**2**) (Scheme 1), which is the carbo-analog of 2,3,5,6-tetrafluorophenyl 6-[^{18}F]fluoronicotinate ([^{18}F]F-Py-Tfp).[14] [^{18}F]TFB is a novel amine-reactive prosthetic group which can be used for labeling of peptides and proteins similar to the well known *N*-succinimidyl 4-[^{18}F]fluorobenzoate ([^{18}F]SFB).[15] However, all published radiosyntheses of [^{18}F]SFB using the K2.2.2/K_2CO_3 protocol for radiofluorination consist of time-consuming and demanding procedures comprising 2–3 reactions and multiple operation steps.[16] In contrast, [^{18}F]F-Py-Tfp can be prepared in good yield *via* direct radiofluorination of the respective pyridine-2-*N*,*N*,*N*-trimethylaminium precursor using less basic $TBAHCO_3$ or $KHCO_3$/K2.2.2. Unfortunately, formation of [^{18}F]F-Py-Tfp is accompanied by the concurrent formation of 2,3,5,6-tetrafluorophenyl 6-(2,3,5,6-tetrafluorophenoxy)-nicotinate which should be completely separated from the radiolabeled active ester best by HPLC.

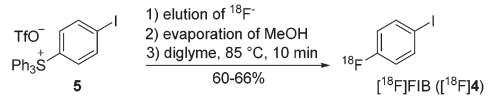
Under "minimalist" conditions the radiolabeled active ester [^{18}F]**2** was successfully prepared from the corresponding iodonium precursor **3** in one step in a fair 24% RCC. Under conventional K2.2.2/K_2CO_3 conditions an extensive decomposition of **3** was observed and only trace amounts of [^{18}F]**2** (<1%) could be detected in the reaction mixture.

Radiolabeling of sulfonium salt 5

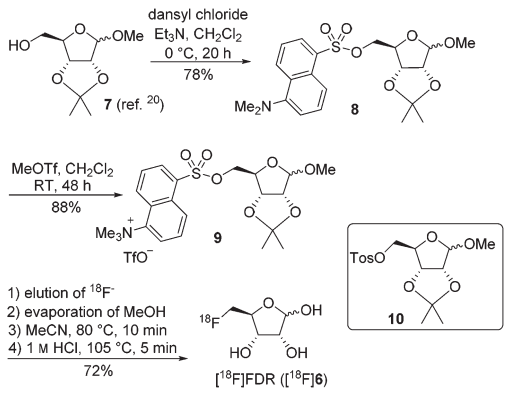
Recently, triarylsulfonium salts have been proposed as precursors for radiofluorination of aromatic compounds.[17] 1-[^{18}F]-fluoro-4-iodobenzene ([^{18}F]FIB, [^{18}F]**4**), a valuable building block for radiolabeling *via* transition metal-catalyzed cross-coupling reactions, was prepared from commercially available (4-iodophenyl)diphenylsulfonium triflate (**5**) (Scheme 2).[18] As with anilinium and iodonium salts, almost complete $^{18}F^-$ recovery from the anion exchange cartridge was achieved with sulfonium salt **5** in MeOH. [^{18}F]**4** was prepared under "minimalist" conditions in 60–66% RCC using diglyme as a solvent at 85 °C for 10 min. Concurrent formation of [^{18}F]fluorobenzene (20–25%) was observed. Using K_2CO_3/K2.2.2, [^{18}F]FIB was obtained in RCCs of up to 40–50%.

Aliphatic ^{18}F-labeling – preparation of [^{18}F]FDR

Finally, the novel radiolabeling procedure was used for the synthesis of 5-[^{18}F]fluoro-D-ribose ([^{18}F]FDR, [^{18}F]**6**) (Scheme 3). [^{18}F]FDR is a hydrophilic prosthetic group for radiolabeling of biopolymers *via* oxime ligation.[19,20] First, the onium group-containing precursor for radiolabeling has to be prepared. To this end, protected D-ribose **7**[20] was converted to the intermediate dansyl sulfonate ester **8** which in turn was treated with methyl triflate to give the desired precursor **9** with a 54% yield over two steps (Scheme 3). [^{18}F]Fluoride was eluted from the solid support with **9** in MeOH almost quantitatively. After MeOH removal, MeCN was added and the resulting solution was heated at 80 °C for 10 min. Deprotection of the labeled intermediate was accomplished by addition of 1 N HCl and heating at 105 °C for 5 min. [^{18}F]FDR was obtained in 72% RCC. By comparison using the conventional K_2CO_3/K2.2.2 protocol, [^{18}F]**6** was prepared from the corresponding tosylate precursor **10**[20] in 58% RCC.



Scheme 2 Preparation of [^{18}F]FIB from sulfonium precursor **5** under "minimalist" conditions.



Scheme 3 Preparation of [^{18}F]FDR.

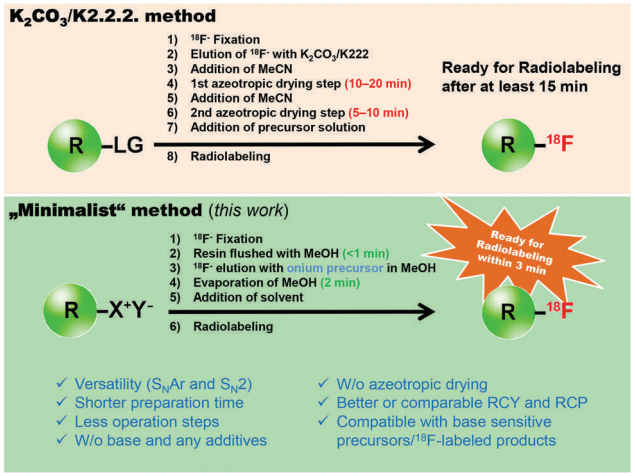


Fig. 2 Comparison between conventional K_2CO_3/K2.2.2 and the "minimalist" radiofluorination method.

Conclusion

In all nucleophilic radiosyntheses with $^{18}F^-$ published to date, a base often in combination with a cryptand or crown-ether has been used. We demonstrated for the first time that application of these ingredients is dispensable and in some cases even counterproductive. ^{18}F-Labeled compounds could be efficiently prepared using only [^{18}F]fluoride and onium salt precursors. The radiolabeling method based on this finding consists of direct elution of [^{18}F]fluoride with alcoholic solutions of precursors bearing a quaternary ammonium, diaryliodonium or triarylsulfonium functionality followed by heating of the resulting [^{18}F]fluoride salt in a suitable solvent. A comparison of conventional K_2CO_3/K2.2.2 and the novel radiofluorination method is shown in Fig. 2. The versatility and the exceptionally wide scope of the novel radiofluorination procedure were demonstrated by the preparation of several useful ^{18}F-labeled prosthetic groups and by the one-step preparation of a radiolabeled model peptide. Labeling yields were comparable to or in many cases even better than those achieved with the classical K2.2.2/K_2CO_3 method. Importantly, our novel method eliminates not only the need for a base or other additives but also the need for time-consuming azeotropic evaporation steps. Besides, it enables the synthesis of base-sensitive radiotracers and the use of base-sensitive precursors. Moreover, due to its simplicity and the fact that all starting materials and products are soluble in organic solvents, the novel procedure should be well suited for automated radiosyntheses, especially in microfluidic devices.

Acknowledgements

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10 If surface-adsorbed $^{18}F^-$ was not solubilized, false high radiochemical conversions (up to 40% higher) were observed; see Fig. S1 in ESI† for representative examples.

11 See ESI† for complete experimental details.

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17 L. Mu, C. R. Fischer, J. P. Holland, J. Becaud, P. A. Schubiger, R. Schibli, S. M. Ametamey, K. Graham, T. Stellfeld, L. M. Dinkelborg and L. Lehmann, *Eur. J. Org. Chem.*, 2012, 889–892.

18 J. D. Way and F. Wuest, *J. Labelled Compd. Radiopharm.*, 2013, **57**, 104–109.

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20 X.-G. Li, S. Dall'Angelo, L. F. Schweiger, M. Zanda and D. O'Hagan, *Chem. Commun.*, 2012, **48**, 5247–5249.

Supporting Information

Table of Contents

Materials and Methods

General: ^{1}H-NMR spectra: Bruker Avance II 300 (300 MHz) and Bruker Avance II+ 600 (600 MHz). ^{1}H chemical shifts are reported in ppm relative to residual peaks of deuterated solvents. Higher-order NMR spectra were approximately interpreted as first-order spectra, where possible. The observed signal multiplicities are characterized as follows: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, and br = broad. Coupling constants (*J*) were reported in Hertz (Hz). ^{13}C-NMR spectra [additional APT (Attached Proton Test)]: Bruker Avance II 300 (75.5 MHz) and Bruker Avance II+ 600 (125.9 MHz). ^{13}C chemical shifts are reported relative to residual peaks of deuterated solvents. Low resolution ESI-MS: Finnigan LCQ. High resolution ESI-MS: Bruker APEX IV 7T FTICR MS. TLC: Merck precoated sheets, 0.25 mm Sil G/UV$_{254}$. The chromatograms were viewed under UV light and/or by treatment with phosphomolybdic acid (10% in ethanol). Column chromatography: Merck silica gel, grade 60, 230–400 mesh. Solvent proportions are indicated in a volume:volume ratio. All reactions were carried out with magnetic

stirring unless otherwise stated and, in the case of air- or moisture-sensitive substrates and/or reagents, were handled in flame-dried glassware under argon or nitrogen. Organic extracts were dried with anhydrous $MgSO_4$. 2-Formyl-*N*,*N*,*N*-trimethylanilinium triflate,[1] 4-formyl-*N*,*N*,*N*-trimethylanilinium triflate,[1] 4-formyl-*N*,*N*,*N*-trimethylanilinium iodide,[2] 3-formyl-*N*,*N*,*N*-trimethylanilinium iodide,[2] HCl·H-βAla-Phe-OMe,[3] TFA·H-βAla-Phe-OMe,[3] (4-carboxyphenyl)(4-methoxyphenyl)iodonium tosylate,[4] 1-*O*-methyl-2,3-*O*-isopropylidene-D-ribofuranoside (**9**),[5] and 1-*O*-methyl-2,3-*O*-isopropylidene-5-*O*-toluenesulfonyl-D-ribofuranoside (**12**)[6] and $Ag(PPh_3)_2HCO_3$[7] were prepared according to the literature.

HPLC analyses and purifications were carried out on Dionex Ultimate 3000 System with Ultimate 3000 Diode Array Detector coupled in series with Berthold NaI detector. Unless otherwise stated, a Chromolith® SpeedROD RP-18e column (Merck, Darmstadt Germany), 50×4.6 mm, was used for analyses and purifications of radiofluorinated products. Aqueous MeCN solutions were used as a mobile phase. StrataX (RP polymeric phase) cartridges were obtained from Phenomenex (Aschaffenburg, Germany),* Sep-Pak Accell Plus QMA carbonate plus light cartridges,** 40 mg sorbent per cartridge from Waters GmbH (Eschborn, Germany) and Chromafix® 30-PS-HCO_3 cartridges from Macherey-Nagel (Düren, Germany).** ^{18}F-labeled compounds were identified by spiking of the reaction mixture with ^{19}F-reference substances.

[^{18}F]Fluoride was produced via the $^{18}O(p,n)^{18}F$ reaction by bombardment of enriched [^{18}O]water with 16.5 MeV protons using a MC16 cyclotron (Scanditronix, Uppsala, Sweden). All isolated radiochemical yields are not decay-corrected. Unless otherwise indicated, all radiochemical experiments were carried out at least in triplicates.

* Preconditioned with 1 mL EtOH followed by 10 mL H_2O.
** Preconditioned with 1 mL H_2O

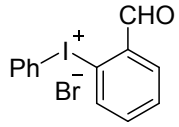
Chemistry

Preparation of (formylphenyl)(phenyl)iodonium bromides. General procedure 1 – (GP1): $BF_3 \cdot Et_2O$ (4.5 mmol) was added dropwise to an ice-cold solution of the appropriate formylphenylboronic acid (3.0 mmol) in anhydrous CH_2Cl_2 (15 mL) under Ar and the resulting mixture was stirred for 10 min. Thereafter, bis(acetoxy)iodobenzene (3.0 mmol) was added, the cooling bath was removed and the mixture was stirred for a further hour. The solvent was removed under reduced pressure. The rest was taken in CH_2Cl_2 (25 mL) and stirred vigorously with saturated NaBr (15 mL) for 15 min. Thereafter, CH_2Cl_2-insoluble (3- and 4-formylphenyl)(phenyl)iodonium bromides were filtered off, washed with H_2O (20 mL), MeCN (2×50 mL), Et_2O (2×50 mL) and dried. In the case of the more soluble (2-formylphenyl)(phenyl)iodonium bromide, the organic layer was repeatedly treated with saturated NaBr (4×15 mL), dried, filtered and concentrated under reduced pressure. The residue was recrystallized from CH_2Cl_2/Et_2O to give the desired iodonium salt.

Preparation of diaryliodonium and *N*,*N*,*N*-trimethylanilinium triflates, perchlorates and hydrogen carbonates using reaction of the corresponding bromides or iodides with the appropriate silver salts. General procedure 2 – (GP2): To prepare the corresponding triflate the appropriate bromide or iodide (0.5 mmol) was dissolved in MeOH (12 mL) and treated with AgOTf, $AgClO_4$ or $Ag(PPh_3)_2HCO_3$ (0.5 mmol). After vigorous shaking of the mixture for 2 min [or 1 h in the case of $Ag(PPh_3)_2HCO_3$] while shielded from light, the precipitate of silver halide was centrifuged off (4000 rpm, 10 min). The supernatant was concentrated under reduced pressure and the residue was recrystallized from CH_2Cl_2/Et_2O or acetone/Et_2O to give the desired salt. Diaryliodonium bicarbonate salts could not be prepared. In the case of *N*,*N*,*N*-trimethylanilinium bicarbonate salts incomplete anionic exchange was observed owing to the low solubility of

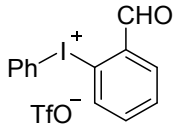
$Ag(PPh_3)_2HCO_3$ in MeOH. In this case the supernatant was used or radiochemical experiments. Solutions of 3- and 4-substituted *N*,*N*,*N*-trimethylanilinium bicarbonates could be stored at –80 °C for at least 4 weeks. 2-Formyl-*N*,*N*,*N*-trimethylanilinium bicarbonate was prepared directly before each experiment.

(2-Formylphenyl)(phenyl)iodonium bromide: The title compound (0.53 g, 54%) was obtained as



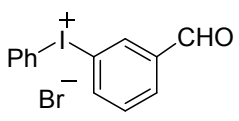
an off-white solid according to GP1 from 2-formylphenyl boronic acid (0.38 g, 2.53 mmol), $BF_3{\cdot}Et_2O$ (0.46 mL, 0.52 g, 3.66 mmol) and bis(acetoxy)iodobenzene (0.86 g, 2.53 mmol). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 7.58 (t, *J* = 7.6 Hz, 3 H), 7.55–7.75 (m, 2 H), 7.80–7.88 (m, 1 H), 8.20–8.32 (m, 3 H), 10.25 (s, 1 H); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ = 115.9, 118.8, 131.5, 131.8, 132.2, 132.6, 133.6, 136.2, 136.4, 137.5, 194.4. MS (ESI): positive mode *m/z* = 341.0 ($[M + MeOH]^+$), 309.0 ($[M + H]^+$); ESI HRMS: calcd for $C_{13}H_{10}OI^+$: 308.9771; found: 308.9779.

(2-Formylphenyl)(phenyl)iodonium triflate: The title compound (0.26 g, 74%) was obtained as a



colorless solid according to GP2 from (2-formylphenyl)(phenyl)iodonium bromide (0.3 g, 0.77 mmol) and AgOTf (198 mg, 0.77 mmol). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 7.39 (d, *J* = 7.9 Hz, 1 H), 7.55–7.75 (m, 2 H), 7.75–7.96 (m, 3 H), 8.31 (d, *J* = 7.5 Hz, 2 H), 8.38 (dd, *J* = 7.5, 1.6 Hz, 1 H), 10.29 (s, 1 H); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ = 111.1, 111.9, 113.0, 128.7 (q, *J* = 128.1 Hz), 131.9, 132.28, 132.30, 133.2, 137.1, 137.6, 138.1, 194.8. MS (ESI): positive mode *m/z* = 341.0 ($[M + MeOH]^+$), 309.0 ($[M + H]^+$); MS (ESI): negative mode *m/z* = 149.0 ($[CF_3SO_2]^-$); ESI HRMS: calcd for $C_{14}H_{14}O_2I^+$: 341.0033; found: 341.0024; calcd for $C_{13}H_{10}OI^+$: 308.9771; found: 308.9772

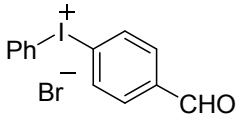
(3-Formylphenyl)(phenyl)iodonium bromide:[8] The title compound (1.23 g, 63%) was obtained as



a colorless solid according to GP1 from 3-formylphenyl boronic acid (0.75 g, 5 mmol), $BF_3{\cdot}Et_2O$ (1 mL, 1.07 g, 7.55 mmol) and bis(acetoxy)iodobenzene

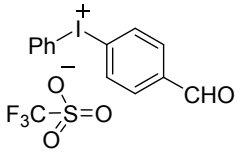
(1.61 g, 5 mmol). ^{1}H-NMR [$(CD_3)_2SO$, 300 MHz]: δ = 7.42–7.50 (m, 2 H), 7.55–7.62 (m, 1 H), 7.69 (t, *J* = 7.8 Hz, 1 H), 8.10 (dt, *J* = 7.7, 1.2 Hz, 2 H), 8.23 (dd, *J* = 8.3, 1.1 Hz, 1 H), 8.46–8.50 (m, 1 H), 8.67 (t, *J* = 1.4 Hz, 1 H), 9.98 (s, 1 H).

(4-Formylphenyl)(phenyl)iodonium bromide: The title compound (0.89 g, 71%) was obtained as



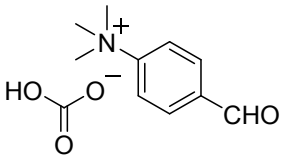
an off-white solid according to GP1 from 4-formylphenyl boronic acid (0.48 g, 3.2 mmol), $BF_3{\cdot}Et_2O$ (0.8 mL, 0.89 g, 6.32 mmol) and bis(acetoxy)iodobenzene (1.03 g, 3.2 mmol). ^{1}H-NMR [$(CD_3)_2SO$, 300 MHz]: δ = 7.35–7.52 (m, 2 H), 7.55–7.65 (m, 1 H), 7.93 (d, *J* = 8.3 Hz, 2 H), 8.21 (d, *J* = 7.4 Hz, 2 H), 8.39 (d, *J* = 8.3 Hz, 2 H), 10.00 (s, 1 H); ^{13}C-NMR [$(CD_3)_2SO$, 75.5 MHz]: δ = 120.77, 126.88, 131.99 (×2), 132.0, 135.5, 136.0, 137.9, 193.0. MS (ESI): positive mode *m/z* = 341.0 ([M + MeOH]$^+$), 309.0 ([M + H]$^+$); ESI HRMS: calcd for $C_{14}H_{14}O_2I^+$: 341.0033; found: 341.0035; calcd for $C_{13}H_{10}OI^+$: 308.9771; found: 308.9773.

(4-Formylphenyl)(phenyl)iodonium triflate: The title compound (0.58 g, 74%) was obtained as a



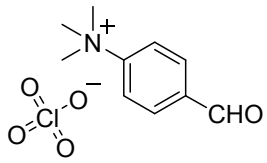
beige solid according to GP2 from (4-formylphenyl)(phenyl)iodonium bromide (0.814 g, 2.09 mmol) and AgOTf (0.538 g, 2.09 mmol). ^{1}H-NMR [$(CD_3)_2SO$, 300 MHz]: δ = 7.49–7.60 (m, 2 H), 7.63–7.75 (m, 1 H), 8.00 (d, *J* = 8.3 Hz, 2 H), 8.29 (d, *J* = 7.4 Hz, 2 H), 8.45 (d, *J* = 8.3 Hz, 2 H), 10.02 (s, 1 H); ^{13}C-NMR [$(CD_3)_2SO$, 75.5 MHz]: δ = 116.6, 122.2, 131.9 (×2), 132.5, 135.5, 135.8, 137.9, 192.5. MS (ESI): positive mode *m/z* = 341.0 ([M + MeOH]$^+$), 309.0 ([M + H]$^+$); MS (ESI): negative mode *m/z* = 149.0 ([CF_3SO_3]$^-$); ESI HRMS: calcd for $C_{14}H_{14}O_2I^+$: 341.0033; found: 341.0041; calcd for $C_{13}H_{10}OI^+$: 308.9771; found: 308.9777.

4-Formyl-*N*,*N*,*N*-anilinium bicarbonate: The title compound was prepared according to GP2 from



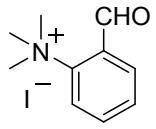
4-formyl-*N*,*N*,*N*-anilinium iodide (0.24 g, 0.82 mmol) and $Ag(PPh_3)_2HCO_3$ (0.572 g, 0.82 mmol) in MeOH (12 mL). The supernatant was used directly for radiolabeling experiments.

4-Formyl-*N*,*N*,*N*-anilinium perchlorate: The title compound was prepared according to GP2 from



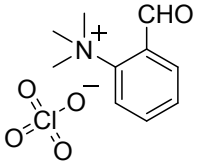
4-formyl-*N*,*N*,*N*-anilinium iodide (0.24 g, 0.82 mmol) and $AgClO_4$ (0.170 g, 0.82 mmol) in MeOH (12 mL). The supernatant was directly used for radiolabeling experiments.

2-Formyl-*N*,*N*,*N*-anilinium iodide:[2] NaI (0.23 g, 1.53 mol) was added to a stirred solution of 2-



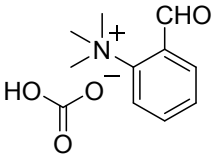
formyl-*N*,*N*,*N*-anilinium triflate[1] (0.48 g, 1.53 mmol) in acetone (10 mL). After 10 min stirring the precipitate was filtered off, washed with acetone and dried to give the title compound (0.35 g, 78%) as a colorless solid. The spectral data were in accordance with those reported in the literature.[2]

2-Formyl-*N*,*N*,*N*-anilinium perchlorate: $LiClO_4$ (0.13 g, 1.22 mmol) was added to a stirred



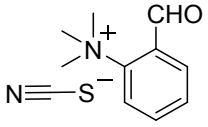
solution of 2-formyl-*N*,*N*,*N*-anilinium triflate[1] (0.38 g, 1.22 mmol) in acetone (20 mL). After 15 min stirring Et_2O (5 mL) was added and the precipitate was filtered off, washed with acetone/Et_2O and dried to give the title compound (0.23 g, 72%) as a colorless solid. ^{1}H-NMR [$(CD_3)_2SO$, 300 MHz]: δ = 3.76 (s, 9 H), 7.87–8.03 (m, 2 H), 8.05–8.12 (m, 1 H), 8.27–8.40 (m, 1 H), 10.2 (s, 1 H); ^{13}C-NMR [$(CD_3)_2SO$, 75.5 MHz]: δ = 56.7, 122.4, 129.5, 131.2, 135.8, 140.1, 144.2, 193.1. MS (ESI): positive mode *m/z* = 196.1 ([M + MeOH]$^+$), 164.1 ([M + H]$^+$); MS (ESI): negative mode *m/z* = 98.9 ([ClO_4]$^-$); ESI HRMS: calcd for $C_{11}H_{18}O_2N^+$: 196.1332.

2-Formyl-*N*,*N*,*N*-anilinium bicarbonate: The title compound was prepared according to GP2 from



2-formyl-*N*,*N*,*N*-anilinium iodide (0.12 g, 0.41 mmol) and $Ag(PPh_3)_2HCO_3$ (0.29 g, 0.42 mmol) in MeOH (6 mL). The supernatant was used directly for radiolabeling experiments.

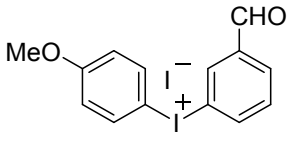
2-Formyl-*N*,*N*,*N*-anilinium thiocyanate: NaCNS (52 mg, 0.64 mmol) was added to a stirred



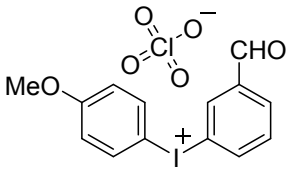
solution of 2-formyl-*N*,*N*,*N*-anilinium triflate[1] (0.2 g, 0.64 mmol) in acetone (10 mL). After 15 min stirring the reaction mixture was concentrated under

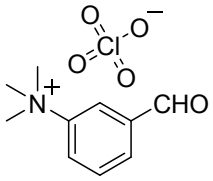
reduced pressure. The residue was recrystallized from acetone/Et_2O to give the title compound (0.23 g, 72%) as a colorless solid. ^{1}H-NMR [$(CD_3)_2SO$, 300 MHz]: δ = 3.77 (s, 9 H), 7.81–7.95 (m, 2 H), 8.05–8.17 (m, 1 H), 8.32 (dd, *J* = 6.8, 1.5 Hz, 1 H), 10.2 (s, 1 H); ^{13}C-NMR [$(CD_3)_2SO$, 75.5 MHz]: δ = 56.7, 122.5, 129.5, 131.2, 135.8, 140.2, 144.2, 193.1 (the signal of the thiocyanate carbon could not be observed). MS (ESI): positive mode *m/z* = 196.1 ([M + MeOH]$^+$), 164.1 ([M + H]$^+$); ESI HRMS: calcd for $C_{11}H_{18}O_2N^+$: 196.1332; found: 196.1335; calcd for $C_{13}H_{10}OI^+$: 164.1070; found: 164.1076.

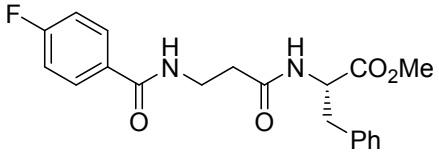
(3-Formylphenyl)(4-methoxyphenyl)iodonium iodide: Tos·H_2O (1.43 g, 7.50 mmol) was added



to a solution of 3-iodobenzaldehyde (1.16 g, 5.0 mmol), 3-chloroperoxybenzoic acid (1.29 g, 7.5 mmol, 89% purity) and anisol (0.85 g, 0.86 mL, 7.91 mmol) in CH_2Cl_2/2,2,2-trifluoroethanol 1:1 (25 mL) and the mixture was stirred for 3 days. The resulting suspension was concentrated under reduced pressure and the residue was triturated with Et_2O. The crude product was recrystallized from CH_2Cl_2/Et_2O to give known (3-formylphenyl)(4-methoxyphenyl)iodonium tosylate[9] (1.75 g, 69%) as a colorless solid. The latter was taken up in CH_2Cl_2 (40 mL) and the resulting solution was stirred vigorously with saturated NaI (4×20 mL). The precipitate was filtered off, washed with CH_2Cl_2 (30 mL), water (15 mL), acetone (15 mL), acetone/Et2O 1:1 (100 mL) and dried to give the title compound as a yellow solid. The CH_2Cl_2 washings were dried and concentrated under reduced pressure. The residue was recrystallized from acetone/Et_2O to give a second crop of product (total yield 1.07 g, 46% in two steps). ^{1}H-NMR [$(CD_3)_2SO$, 300 MHz]: δ = 3.79 (s, 3 H), 7.02–7.08 (m, 2 H), 7.71 (t, *J* = 7.9 Hz, 1 H), 8.10–8.21 (m, 3 H), 8.44 (dt, *J* = 7.9, 1.2 Hz, 1 H), 8.65 (t, *J* = 1.2 Hz, 1 H), 9.99 (s, 1 H); ^{13}C-NMR [$(CD_3)_2SO$, 75.5 MHz]: δ = 55.6, 106.6, 117.3, 118.8, 132.0, 132.5, 134.4, 137.1, 138.1, 139.7, 161.8, 191.4. MS (ESI): positive mode *m/z* = 371.0 ([M + MeOH]$^+$), 339.0 ([M + H]$^+$); MS (ESI): negative mode *m/z* = 126.9 ([I]$^-$); ESI HRMS: calcd for $C_{14}H_{12}O_2N^+$: 338.9876; found: 338.9878.

(3-Formylphenyl)(4-methoxyphenyl)iodonium perchlorate: The title compound was prepared  according to GP2 from (3-formylphenyl)(4-methoxyphenyl)iodonium iodide (0.12 g, 0.257 mmol) and $AgClO_4$ (50 mg, 0.245 mmol) in MeOH (12 mL). The supernatant was used directly for radiolabeling experiments.

3-Formyl-*N*,*N*,*N*-anilinium perchlorate: The title compound was prepared according to GP2 from  3-formyl-*N*,*N*,*N*-anilinium iodide[2] (0.11 g, 0.38 mmol) and $AgClO_4$ (74 mg, 0.36 mmol) in MeOH (9 mL). The supernatant was used directly for radiolabeling experiments.

4-Fluorobenzoyl-βAla-Phe-OMe (1): DIEA (0.27 mL, 0.198 g, 1.53 mmol) was added to a stirred  solution of HCl·H-βAla-Phe-OMe (0.22 g, 0.77 mmol) and 4-fluorobenzoyl chloride (90 µL, 0.12 g, 0.77 mmol) in CH_2Cl_2 (10 mL) and stirring was continued for 1 h. The mixture was concentrated under reduced pressure, the residue was taken up in EtOAc/H_2O (50 mL each), the organic layer was separated and washed with 1 N HCl (3×15 mL), H_2O (2×15 mL), 5% $NaHCO_3$ (3×15 mL), brine (2×15 mL), dried and concentrated under reduced pressure. The residue was recrystallized from EtOAc/hexane to give the title compound (0.26 g, 91%) as a colorless solid. R_f = 0.55, acetone:hexane = 1:1. ^{1}H-NMR ($CDCl_3$, 600 MHz): δ = 2.49 (t, *J* = 5.6 Hz, 2 H), 3.05 (dd, *J* = 13.9, 6.6 Hz, 1 H), 3.15 (dd, *J* = 13.9, 5.6 Hz, 1 H) 3.59–3.67 (m, 1 H), 3.69–3.76 (m, 1 H), 3.72 (s, 3 H), 4.83–4.88 (m, 1 H), 6.29 (d, *J* = 7.7 Hz, 1 H), 7.06–7.11 (m, 4 H), 7.16–7.25 (m, 4 H), 7.75–7.80 (m, 2 H); ^{13}C-NMR ($CDCl_3$, 150.9 MHz): δ = 35.3, 36.0, 37.7, 52.5, 53.3, 115.5 (d, *J* = 22.6 Hz), 127.3, 128.7, 129.1, 129.4 (d, *J* = 7.5 Hz), 130.5 (d, *J* = 3.0 Hz), 135.7, 165.1 (d, *J* = 365.2 Hz), 165.6, 171.7, 172.0. MS (ESI): positive mode *m/z* = 1057.4 ([2 M + Na]$^+$), 1035.4 ([2 M + H]$^+$), 540.2 ([M + Na]$^+$), 518.2 ([M + H]$^+$); MS (ESI): negative mode *m/z* = 630.2 ([M + 2 HCOOH – H]$^-$), 516.2 ([M – H]$^-$); ESI HRMS: calcd for

$C_{29}H_{28}FN_3O_5Na^+$: 540.1905; found: 540.1907; calcd for $C_{29}H_{29}FN_3O_5^+$: 518.2086; found: 518.2085; calcd for $C_{29}H_{27}FN_3O_5^-$: 516.1940; found: 516.1933.

4-*N*,*N*-Dimethylaminobenzoyl-βAla-Phe-OMe: 4-*N*,*N*-Dimethylaminobenzoyl chloride (0.44 g, 2.42 mmol) was added to a stirred solution of HCl·H-βAla-Phe-OMe (0.69 g, 2.42 mmol) and DIEA (0.843 mL, 0.63 g, 4.84 mmol) in CH_2Cl_2 (10 mL) and stirring was continued for a further hour. Thereafter, 4-*N*,*N*-dimethylaminobenzoyl chloride (0.15 g, 2.42 mmol) was added and the reaction mixture was stirred for a further 1 h. The mixture was concentrated under reduced pressure, the residue was taken up in EtOAc/5% $NaHCO_3$ (50 mL each), the organic layer was separated and washed with H_2O (2×15 mL), 5% $NaHCO_3$ (3×15 mL), brine (2×15 mL), dried and concentrated under reduced pressure. The residue was recrystallized from Et_2O/hexane to give the title compound (0.64 g, 67%) as a colorless solid. R_f = 0.33, acetone:hexane = 1:1. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 2.48 (t, *J* = 5.8 Hz, 2 H), 3.03 (s, 6 H), 3.05 (dd, *J* = 14.0, 6.7 Hz, 1 H), 3.15 (dd, *J* = 14.0, 5.8 Hz, 1 H), 3.62–3.73 (m, 2 H), 3.71 (s, 3 H), 4.86 (q, *J* = 7.3 Hz, 1 H), 6.30 (d, *J* = 7.7 Hz, 1 H), 6.67 (d, *J* = 8.9 Hz, 2 H), 6.86–6.91 (br, 1 H), 7.05–7.10 (m, 2 H), 7.16–7.23 (m, 3 H), 7.66–7.70 (m, 2 H); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ = 35.7, 36.8, 37.7 40.1, 52.3, 53.2, 111.1, 121.8, 127.1, 128.5, 128.6, 129.1; 135.7, 152.4, 167.4, 171.7, 171.9. MS (ESI): positive mode *m/z* = 817.4 ([2 M + Na]$^+$), 795.4 ([2 M + H]$^+$), 420.2 ([M + Na]$^+$), 398.2 ([M + H]$^+$); MS (ESI): negative mode *m/z* = 396.2 ([M – H]$^-$); ESI HRMS: calcd for $C_{22}H_{27}N_3O_4Na^+$: 420.1894; found: 420.1887; calcd for $C_{22}H_{28}N_3O_4^+$: 398.2074; found: 398.2072; calcd for $C_{22}H_{26}N_3O_4^-$: 396.1929; found: 396.1919.

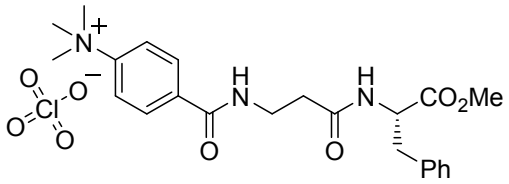
4-*N*,*N*,*N*-Trimethylammoniumbenzoyl-βAla-Phe-OMe iodide: Methyl iodide (0.28 mL, 0.64 g, 4.53 mmol) was added to a solution of 4-*N*,*N*-dimethylaminobenzoyl-βAla-Phe-OMe (0.6 g, 1.51 mmol) in acetone (10 mL) and the mixture was stirred under reflux for 16 h. Thereafter, the precipitate was

filtered off, washed with acetone (30 mL) and dried to give the title compound (0.63 g, 81%) as a colorless solid. ^{1}H-NMR [$(CD_3)_2SO$, 300 MHz]: δ = 2.35–2.47 (m, 2 H), 2.88 (dd, *J* = 13.6, 9.1 Hz, 1 H), 3.01 (dd, *J* = 8.3, 5.5 Hz, 1 H), 3.41 (q, *J* = 6.7 Hz, 2 H), 3.58 (s, 3 H), 3.64 (s, 9 H), 4.42–4.54 (m, 1 H), 7.12–7.27 (m, 5 H), 8.05 (q, *J* = 10.2 Hz, 4 H), 8.43 (d, *J* = 7.9 Hz, 1 H), 8.67 (d, *J* = 5.5 Hz, 1 H); ^{13}C-NMR [$(CD_3)_2SO$, 75.5 MHz]: δ = 34.7, 35.9, 36.6, 51.8, 53.5, 56.4, 120.7, 126.5, 128.2, 128.7, 128.9, 135.6, 137.2, 148.9, 164.4, 170.4, 172.0. MS (ESI): positive mode *m/z* = 412.2 ($[M]^+$); MS (ESI): negative mode *m/z* = 538.1 ($[M + I–H]^-$), 126.9 ($[I]^-$); ESI HRMS: calcd for $C_{23}H_{30}N_3O_4^+$: 412.2231; found: 412.2234; calcd for $C_{23}H_{30}N_3O_4I^-$: 538.1208; found: 538.1215.

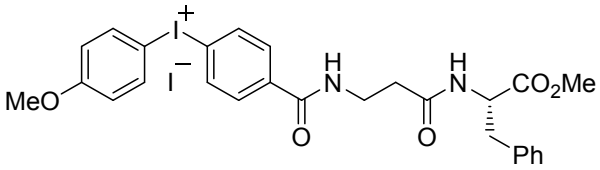
4-*N*,*N*,*N*-Trimethylammoniumbenzoyl-βAla-Phe-OMe triflate: The title compound (0.19 g, 95%) was obtained as a colorless solid according to GP2 from 4-*N*,*N*,*N*-trimethylammoniumbenzoyl-βAla-Phe-OMe iodide (0.2 g, 0.37 mmol) and AgOTf (95 mg, 0.37 mmol). ^{1}H-NMR [$(CD_3)_2SO$, 300 MHz]: δ= 2.31–2.47 (m, 2 H), 2.82–2.94 (m, 1 H), 3.01–3.08 (m, 1 H), 3.35–3.47 (m, 2 H), 3.58 (s, 3 H), 3.63 (s, 9 H), 4.47–4.53 (m, 1 H), 7.16–7.27 (m, 5 H), 7.99–8.10 (m, 4 H), 8.43 (d, *J* = 7.7 Hz, 1 H), 8.67 (t, *J* = 5.2 Hz, 1 H); ^{19}F-NMR [$(CD_3)_2SO$, 282.4 MHz]: δ= –77.74; ^{13}C-NMR [$(CD_3)_2SO$, 75.5 MHz]: δ = 34.7, 36.0, 36.7, 51.8, 53.5, 56.4, 120.6, 126.5, 128.2, 128.7, 129.0, 135.7, 137.2, 149.0, 164.4, 170.4, 172.0.

4-*N*,*N*,*N*-Trimethylammoniumbenzoyl-βAla-Phe-OMe bicarbonate: The title compound was prepared according to GP2 from 4-*N*,*N*,*N*-trimethylammoniumbenzoyl-βAla-Phe-OMe iodide (0.12 g, 0.22 mmol) and $Ag(PPh_3)_2HCO_3$ (0.154 g, 0.22 mmol) in MeOH (10 mL). The supernatant was used directly for radiolabeling experiments.

4-*N*,*N*,*N*-Trimethylammoniumbenzoyl-βAla-Phe-OMe perchlorate: The title compound was prepared according to GP2 from 4-*N*,*N*,*N*-trimethyl-ammoniumbenzoyl-βAla-Phe-OMe iodide (0.105 g, 0.19 mmol) and $AgClO_4$ (40 mg, 0.22 mmol) in MeOH (8.8 mL). The supernatant was used directly for radiolabeling experiments.

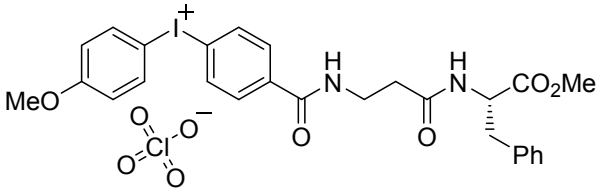


4-(4-Methoxyphenyliodonium)benzoyl-βAla-Phe-OMe iodide: TFA·H-βAla-Phe-OMe[3] prepared from Boc-βAla-Phe-OMe (0.3 g, 0.86 mmol) was taken up in toluene (25 mL) and the mixture was concentrated under reduced pressure to remove the traces of TFA (×3). The residue was dissolved in DMF (10 mL) under Ar, HOBt (12 mg, 0.089 mmol) and (4-carboxyphenyl)(4-methoxyphenyl)-iodonium tosylate (0.45 g, 0.85 mmol) were added and the heterogeneous mixture was cooled in an ice-water bath. DCC (0.176 g, 0.86 mmol) and thereafter 2,4,6-trimethylpyridine (0.23 mL, 0.21 g, 1.74 mmol) were added to the stirred reaction mixture. The cooling bath was removed and the mixture was stirred for a further 6 h. Afterwards, the reaction mixture was incubated at 4 °C for 16 h, DCU was filtered off and DMF was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (10 mL) and filtered. CH_2Cl_2 (20 mL) followed by saturated NaI (10 mL) were added and the mixture was stirred vigorously for 10 min. The resulting gel was centrifuged (4000 rpm, 15 min, 10 °C), the aqueous fraction was discarded, the pellet was resuspended in CH_2Cl_2 fraction, saturated NaI (10 mL) was added and the mixture was shaken vigorously for 10 min. The reaction mixture was centrifuged (4000 rpm, 15 min, 10 °C), the pellet was washed with CH_2Cl_2 (4×10 mL), H_2O (3×10 mL) and Et_2O (3×10 mL) (each time the pellet was suspended in the corresponding solvent, the suspension was centrifuged, the supernatant was discarded and the pellet was resuspended). Thereafter, the precipitate was triturated with acetone, filtered off and washed with acetone (30 mL) to give the title compound (0.31 g, 51%) as a colorless solid. ^{1}H-NMR [$(CD_3)_2SO$, 600 MHz]: δ=

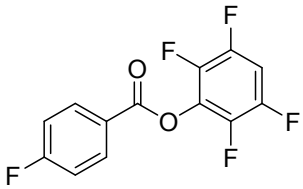


2.36 (dq, *J* = 18.0, 7.4 Hz, 2 H), 2.87 (dd, *J* = 13.8, 9.3 Hz, 1 H), 3.00 (dd, *J* = 13.8, 5.6 Hz, 1 H), 3.36–3.47 (m, 2 H), 3.55 (s, 3 H), 3.78 (s, 3 H), 4.47 (td, *J* = 8.4, 5.6 Hz, 1 H), 7.04 (d, *J* = 9.1 Hz, 2 H), 7.08–7.13 (m, 1 H), 7.15–7.20 (m, 4 H), 7.81 (d, *J* = 8.4 Hz, 2 H), 8.15 (d, *J* = 9.2 Hz, 2 H), 8.26 (d, *J* = 8.4 Hz, 2 H), 8.38 (d, *J* = 7.7 Hz, 1 H), 8.56 (t, *J* = 5.4 Hz, 1 H); ^{13}C-NMR [$(CD_3)_2SO$, 150.9 MHz]: δ = 34.6, 35.9, 36.6, 51.8, 53.4, 55.7, 117.3, 121.0, 126.4, 128.1, 128.9, 129.8, 134.6, 137.1, 137.2, 150.9, 161.8, 164.9, 169.7, 170.3, 172.0. MS (ESI): positive mode *m/z* = 587.1 ($[M]^+$); MS (ESI): negative mode *m/z* = 126.9 ($[I]^-$); ESI HRMS: calcd for $C_{27}H_{28}N_2O_5I^+$: 587.1037; found: 587.1038.

4-(4-Methoxyphenyliodonium)benzoyl-βAla-Phe-OMe perchlorate: The title compound was prepared according to GP2 from 4-(4-methoxyphenyliodonium)benzoyl-βAla-Phe-OMe iodide (0.126 g, 0.18 mmol) and $AgClO_4$ (36.6 mg, 0.18 mmol) in MeOH (10.5 mL). The supernatant was used directly for radiolabeling experiments.

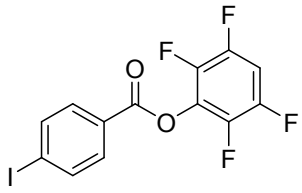


2,3,5,6-Tetraphenyl 4-fluorobenzoate (2): Et_3N (0.54 mL, 0.39 g, 3.87 mmol) was added dropwise to a vigorously stirred solution of 4-fluorobenzoyl chloride (0.46 mL, 0.61 g, 3.87 mmol) and 2,3,5,6-tetrafluorophenol (0.64 g, 3.87 mmol) in Et_2O (30 mL) and the stirring was continued for a further 10 min. The reaction mixture was filtered, washed with H_2O (15 mL) and brine (2×10 mL), dried and concentrated under reduced pressure. The residue was recrystallized from hexane to give **4** (0.41 g) as a colorless solid. The mother liquor was concentrated under reduced pressure and the residue was recrystallized from hexane to give a second crop of **4** (0.45 g, total 77%). R_f = 0.62, EtOAc:hexane = 1:10. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 7.07 (tt, J = 9.9, 7.1 Hz, 1 H), 7.21–7.30 (m, 2 H), 8.21–8.37 (m, 2 H); ^{19}F-NMR ($CDCl_3$, 282.4 MHz): δ = –152.75, –138.93, –102.24; ^{13}C-NMR($CDCl_3$, 75.5 MHz): δ = 103.4 (t, *J* = 21.9 Hz), 116.2 (d, *J* = 21.9 Hz), 123.5 (d, *J* = 1.5 Hz),



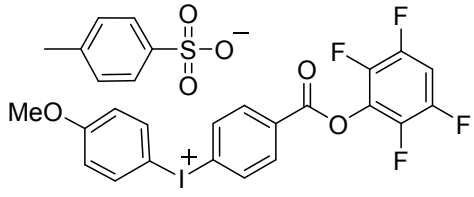
133.5 (d, J = 2.3 Hz), 139.0–142.7 (m), 144.5–145.1 (m), 147.3–148.2 (m), 163.3 (d, J = 259.7 Hz), 168.5. MS (ESI): positive mode *m/z* = 288.3 ($[M]^+$). MS (EI, 70 eV): m/z (%): 165.0 [$C_6HF_4O^+$] (10), 123.0 [$C_6H_4FO^+$] (100), 95.0 [$C_6H_4F^+$] (10).

2,3,5,6-Tetraphenyl 4-iodobenzoate: Et_3N (1.504 mL, 0.76 g, 7.51 mmol) was added dropwise to



a vigorously stirred solution of 4-iodobenzoyl chloride (2 g, 7.51 mmol) and 2,3,5,6-tetrafluorophenol (1.25 g, 7.51 mmol) in Et_2O (60 mL) and the stirring was continued for a further 10 min. The reaction mixture was filtered, the filter cake was washed with Et_2O (30 mL) and the filtrate was concentrated under reduced pressure. The residue was dissolved Et_2O (10 mL) and filtered. The filtrate was concentrated under reduced pressure. The residue was recrystallized from Et_2O/hexane to give the title compound (1.38 g, 48%) as a colorless solid. R_f = 0.46, EtOAc:hexane = 1:10. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 7.07 (tt, J = 9.9, 7.1 Hz, 1 H), 7.82–7.98 (m, 4 H); ^{19}F-NMR ($CDCl_3$, 282.4 MHz): δ= –152.70, –138.80; ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ = 103.0, 103.4 (t, J = 23.0 Hz), 126.6, 131.9, 138.3, 138.9–142.7 (m), 144.4 (dt, J = 3.8, 12.1 Hz), 147.7 (dt, J = 4.5, 12.1 Hz), 162.2. MS (ESI): positive mode *m/z* = 397.3 ($[M + H]^+$). MS (EI, 70 eV): m/z (%): 395.9 [M^+] (3), 230.9 [$C_7H_4OI^+$] (100), 202.9 [$C_6H_3I^+$] (100), 104.0 [$C_7H_4O^+$] (10).

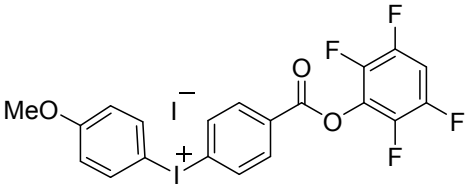
(4 - Methoxyphenyl)[4 - (2,3,5,6 - tetrafluorophenoxycarbonyl)phenyl]iodonium tosylate:



$Tos \cdot H_2O$ (0.72 g, 3.79 mmol) was added to a solution of 2,3,5,6-tetraphenyl 4-iodobenzoate (1 g, 2.52 mmol), mCPBA [1.44 g, 85% purity, 7.09 mmol; commercially available 77% mCPBA (Aldrich) was dried at 2 mbar and 40 °C for 3 h before use] and anisole (0.51 mL, 0.51 g, 4.72 mmol) in 50% CF_3CH_2OH in CH_2Cl_2 (20 mL) and the mixture was stirred for 3 days. The reaction mixture was added to vigorously stirred Et_2O (450 mL) and stirring was continued for a further 45 min. The precipitate was filtered off and washed with Et_2O (100 mL),

redissolved in CH_2Cl_2 (20 mL) and filtered through Celite®. The filtrate was concentrated under reduced pressure. The residue was recrystallized from CH_2Cl_2/Et_2O to give the title compound (1.53 g, 90%) as a colorless solid. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 2.28 (s, 3 H), 3.77 (s, 3 H), 6.80 (d, *J* = 9.0 Hz, 2 H), 6.97 (d, *J* = 6.0 Hz, 2 H), 7.01–7.14 (m, 1 H), 7.36 (d, *J* = 9.0 Hz, 2 H), 7.98–8.03 (m, 4 H), 8.18 (d, *J* = 9.0 Hz, 2 H); ^{19}F-NMR ($CDCl_3$, 282.4 MHz): δ= –152.70, –138.55; ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ = 21.2, 55.5, 103.7 (t, *J* = 23.0 Hz), 104.7, 117.4, 123.0, 125.9, 128.5, 129.3, 132.7, 135.3, 137.9, 138.8–142.3 (m), 139.6, 142.2, 144.4 (dt, *J* = 3.8, 15.9 Hz), 147.7 (dt, *J* = 4.5, 16.6 Hz), 161.3, 162.4. MS (ESI): positive mode *m/z* = 503.0 ($[M]^+$); MS (ESI): negative mode *m/z* = 171.0 ($[C_7H_7SO_3]^-$); ESI HRMS: calcd for $C_{20}H_{12}F_4O_3I^+$: 502.9762; found: 502.9769.

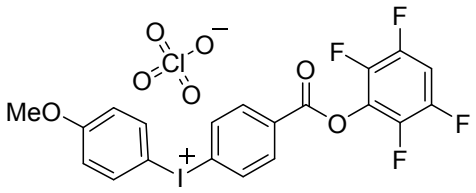
(4‐Methoxyphenyl)[4‐(2,3,5,6‐tetrafluorophenoxycarbonyl)phenyl]iodonium iodide:



(4‐Methoxyphenyl)[4‐(2,3,5,6‐tetrafluorophenoxy-carbonyl)phenyl]iodonium tosylate (1.19 g, 1.76 mmol) was dissolved in CH_2Cl_2 (20 mL). After addition of saturated NaI (10 mL), the mixture was vigorously stirred for 15 min and centrifuged (4000 rpm, 15 °C, 10 min). The aqueous solution and precipitate were separated off, saturated NaI (10 mL) was added and the mixture was vigorously stirred for 15 min and centrifuged (×3). The organic fraction was filtered, dried and concentrated under reduced pressure. The residue was recrystallized from CH_2Cl_2/Et_2O, the precipitate was filtered off, washed with acetone (10 mL) and Et_2O (80 mL) to give the title compound (0.29 g, 26%) as an off-white solid. The substance could be stored at 4 °C under Ar at least for 4 months. However, it was unstable in solution especially at elevated temperatures (dissolved in DMF or DMSO it was unstable already at ambient temperature). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 3.79 (s, 3 H), 6.65–6.73 (m, 2 H), 6.95–7.10 (m, 1 H), 7.56 (d, *J* = 8.9 Hz, 2 H), 7.81–8.05 (m, 3 H), 8.21–8.35 (m, 1 H); ^{19}F-NMR ($CDCl_3$, 282.4 MHz): δ= –152.63, –138.72; ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ = 55.5, 82.3, 103.0, 103.5 (t, *J* = 22.6 Hz), 116.4, 126.6, 128.8, 131.9,

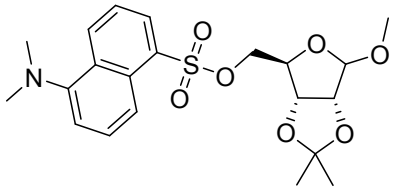
138.3, 138.6–142.5 (m), 144.2–144.7 (m), 144.5–144.9 (m), 159.5, 162.2. MS (ESI): positive mode *m/z* = 502.9 ([M]$^+$); MS (ESI): negative mode *m/z* = 126.9 ([I]$^-$); ESI HRMS: calcd for $C_{20}H_{12}F_4O_3I^+$: 502.9762; found: 502.9741.

(4‑Methoxyphenyl)[4‑(2,3,5,6‑tetrafluorophenoxycarbonyl)phenyl]iodonium perchlorate:



The title compound (168 mg, 88%) was obtained as a colorless solid according to GP2 from (4-methoxyphenyl)[4-(2,3,5,6-tetrafluorophenoxycarbonyl)phenyl]iodonium iodide (0.2 g, 0.32 mmol) and $AgClO_4$ (66 mg, 0.32 mmol) in acetone (10 mL). ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 3.80 (s, 3 H), 6.88–6.98 (m, 2 H), 7.06 (tt, *J* = 9.9, 7.1 Hz, 1 H), 8.04–8.13 (m, 2 H), 8.14–8.18 (m, 2 H), 8.21–8.26 (m, 2 H); ^{19}F-NMR ($CDCl_3$, 282.4 MHz): δ= –152.52, –138.48; ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ = 55.5, 101.1, 103.8 (t, *J* = 21.1 Hz), 118.4, 120.1, 130.6, 133.5, 135.1, 138.3, 138.7–139.3 (m), 142.1–144.4 (m), 147.5–147.7 (m), 161.0, 163.4. MS (ESI): positive mode *m/z* = 503.0 ([M]$^+$); ESI HRMS: calcd for $C_{20}H_{12}F_4O_3I^+$: 502.9762; found: 502.9769. MS (ESI): positive mode *m/z* = 503.0 ([M]$^+$); MS (ESI): negative mode *m/z* = 171.0 ([$C_7H_7SO_3$]$^-$); ESI HRMS: calcd for $C_{20}H_{12}F_4O_3I^+$: 502.9762; found: 502.9749.

1-*O*-Methyl-2,3-*O*-isopropylidene-5-*O*-[5-(*N*,*N*-dimethylamino)naphthalene-1-sulfonyl]-D-ribofuranoside (8):



Dansyl chloride (2.9 g, 10.75 mmol) was added to a stirred ice-cold solution of 1-*O*-methyl-2,3-*O*-isopropylidene-D-ribofuranoside (**9**) (1.7 g, 8.32 mmol) and Et_3N (1.6 g, 15.81 mmol) in CH_2Cl_2 (25 mL), the cooling bath was removed and the reaction mixture was stirred for further 16 h. The mixture was concentrated under reduced pressure. Et_2O and H_2O (100 mL each) were added to the residue, the organic fraction was separated and washed with H_2O (3×20 mL), 5% $NaHCO_3$ (3×20 mL) and brine (2×20 mL), then dried and concentrated under reduced pressure. The residue was purified by column chromatography [EtOAc/hexane = 1:4, silica gel (0.1% CaO)] and

recrystallization from Et_2O/pentane gave **10** (2.85 g, 78%) as a yellow solid. R_f = 0.11, EtOAc:hexane = 1:8. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 1.24 (s, 3 H), 1.42 (s, 3 H), 2.90 (s, 6 H), 3.14 (s, 3 H), 3.93–4.0 (m, 2 H), 4.32 (t, *J* = 7.2 Hz, 1 H), 4.43–4.56 (m, 2 H), 4.88 (s, 1 H), 7.18–7.27 (m, 1 H), 7.53–7.65 (m, 2 H), 8.25–8.33 (m, 2 H), 8.63 (d, *J* = 8.5 Hz, 1 H); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ = 24.8, 26.3, 45.4, 54.9, 69.5, 81.4, 83.5, 84.8, 109.4, 112.6, 115.7, 119.4, 123.0, 128.8, 129.8, 129.9, 130.6, 130.9, 131.7. MS (ESI): positive mode *m/z* = 897.3 ([2 M + Na]$^+$), 875.3 ([2 M + H]$^+$), 460.2 ([M + Na]$^+$), 438.2 ([M + H]$^+$); ESI HRMS: calcd for $C_{22}H_{27}N_3O_4Na^+$: 420.1894; found: 420.1887; calcd for $C_{21}H_{27}NO_7Na^+$: 460.1400; found: 460.1394; calcd for $C_{21}H_{26}NO_7^+$: 438.1581; found: 438.1579.

1-*O*-Methyl-2,3-*O*-isopropylidene-5-*O*-[5-(*N*,*N*,*N*-trimethylammonium)naphthalene-1- sulfon-yl]-D-ribofuranoside triflate (9): MeOTf (0.251 mL, 0.376 g, 2.29 mmol) was added to a solution **10** (1 g, 2.29 mmol) in CH_2Cl_2 (5 mL) in a dry box under Ar. The reaction flask was then removed from the dry box and the reaction mixture was incubated at ambient temperature for 3 days. Thereafter, Et_2O (50 mL) was added and the precipitated oil was separated, recrystallized twice from CH_2Cl_2/Et_2O, sonicated with Et_2O (3×20 mL) and thoroughly dried under reduced pressure to give **11** (1.05 g, 69%) as a colorless foam. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 1.26 (s, 3 H), 1.41 (s, 3 H), 3.17 (s, 3 H), 3.95–4.15 (m, 2 H), 4.08 (s, 9 H), 4.30 (dt, *J* = 0.6, 14.1 Hz, 1 H), 4.50–4.54 (m, 1 H), 4.58–4.62 (m, 1 H), 4.90 (s, 1 H), 7.83 (t, *J* = 8.3 Hz, 1 H), 8.0 (dd, *J* = 8.9, 7.6 Hz, 1 H), 8.21 (d, *J* = 8.3 Hz, 1 H), 8.47 (d, *J* = 8.9 Hz, 1 H), 8.89–8.97 (m, 2 H); ^{13}C-NMR ($CDCl_3$, 75.5 MHz): δ = 24.8, 26.3, 55.1, 58.7, 70.5, 81.2, 83.5, 84.7, 109.5, 112.8, 120.6 (q, *J* = 320.1 Hz), 122.7, 125.2, 127.2, 127.6, 129.4, 131.16, 131.22, 133.9, 142.1. MS (ESI): positive mode *m/z* = 452.3 ([M]$^+$); MS (ESI): negative mode *m/z* = 149.0 ([CF_3SO_3]$^-$); ESI HRMS: calcd for $C_{22}H_{30}NO_7S^+$: 452.1737; found: 452.1739.

Radiochemistry

Synthesis of [^{18}F]fluorobenzaldehydes from iodonium precursors alone without any evaporation steps. General procedure 3 – (GP3): [^{18}F]Fluoride (100–330 MBq) was fixed on a QMA cartridge. The cartridge was washed with the appropriate solvent (1 mL) and the radiofluoride was eluted with a solution of the corresponding iodonium salt (5 mg, unless not otherwise stated) in the appropriate solvent (0.5 mL). The elution yield was determined by comparing the radioactivity eluted into the reaction vial with the total radioactivity trapped on the anion exchange resin. The reaction mixture was stirred at the temperature and for the indicated time in Table S1. Subsequently the mixture was cooled down to room temperature, water (4 mL) was added and the reaction mixture was shaken vigorously for 30 s. Thereafter, RCC was determined by radio-HPLC.

Unless stated, HPLC conditions: Eluent: 30% MeCN, flow rate: 1.5 mL/min.

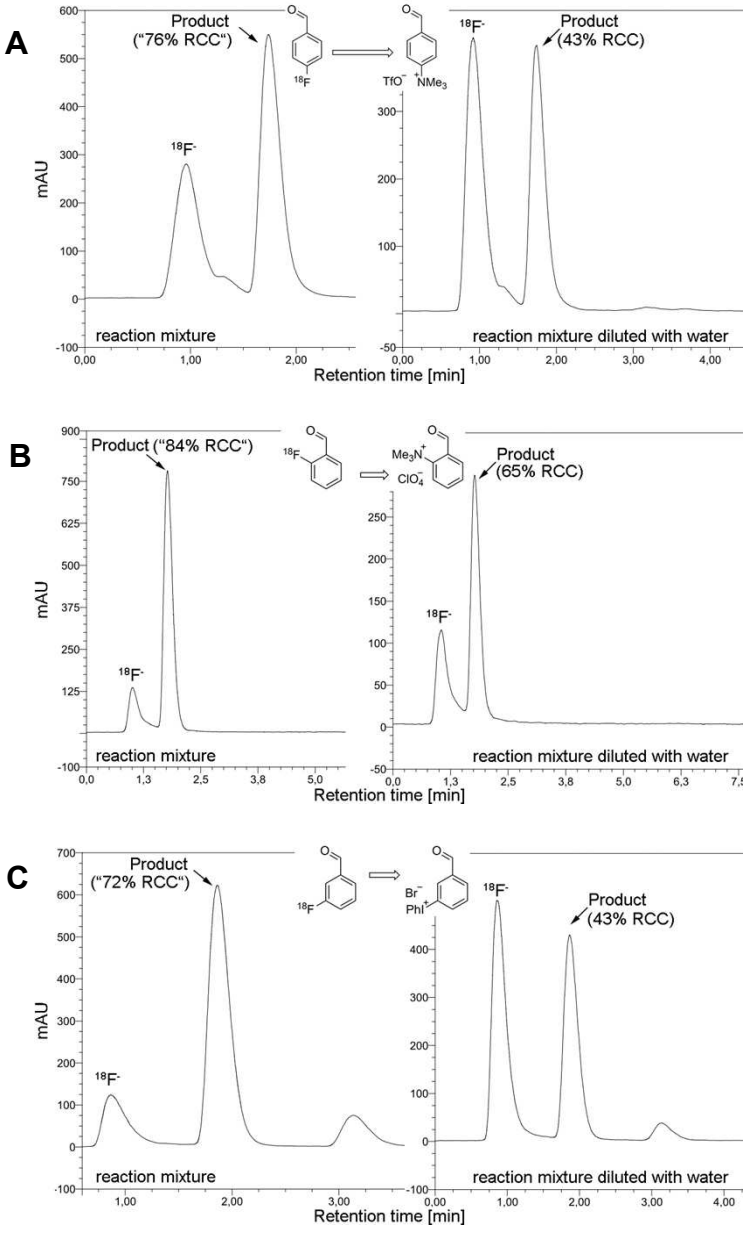
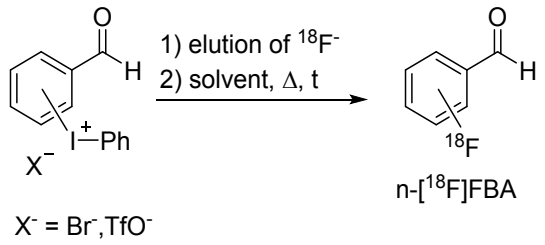


Figure S1. False high *vs.* correct radiochemical conversions obtained by the direct HPLC analysis of reaction mixtures and the HPLC analysis of reaction mixtures diluted with an excess of water: A – 4-[^{18}F]FBA (from 4-formyl-*N*,*N*,*N*-trimethylanilinium triflate, K2.2.2/K_2CO_3 protocol, GP5), B – 2-[^{18}F]FBA (from only 2-formyl-*N*,*N*,*N*-trimethylanilinium perchlorate, GP4), C – 3-[^{18}F]FBA (from (3-formylphenyl)(phenyl)iodonium bromide, $CsHCO_3$/TEMPO protocol, GP6).

Table S1. Synthesis of [^{18}F]fluorobenzaldehydes from iodonium precursors alone without any evaporation steps[a]

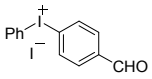
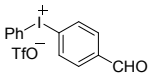
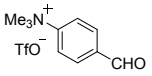


entry	LG$^+$X	n	solvent	temperature [°C], time [min]	elution yield [%]	RCC [%]
1	TfO$^-$	2	95% DMF	130, 10	30	2
2[b]	TfO$^-$	3	DMSO	130, 10	30	22
3	Br$^-$	3	DMF	160, 10	6	34
4	Br$^-$	3	95% DMF	160, 15	30	4
5	Br$^-$	4	DMSO	90, 10	20	51
6	TfO$^-$	4	90% DMSO	160, 15	30	35
7	TfO$^-$	4	90% DMF	160, 15	55	51

[a] All experiments were carried out as described in GP3; [b] with 20 mg precursor

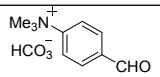
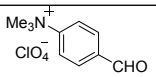
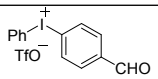
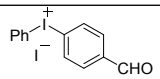
Elution of ^{18}F$^-$ from a QMA cartridge with onium salts in anhydrous alcohols (Table S2): [^{18}F]Fluoride (50–200 MBq) was fixed on a QMA cartridge. The cartridge was washed with the appropriate alcohol (1 mL), and the radiofluoride was eluted with a solution of the corresponding onium salt (5 mg) in the appropriate alcohol (0.5 mL). The elution yield was determined by comparing the radioactivity eluted into the reaction vial with the total radioactivity trapped on the anion exchange resin.

Table S2. Recovery yield for the elution of $^{18}F^-$ from a QMA cartridge with onium salts in anhydrous alcohols.

entry	solvent	 recovery [%]	 recovery [%]	 recovery [%]
1	MeOH	99	99	98
2	EtOH	95	92	95
3	1-PrOH	88	88	84
4	*i*-BuOH	61	82	80
5	*i*-PrOH	59	73	78
6	*n*-BuOH	43	64	69
7	2-BuOH	7	37	23
8	*t*-BuOH	2	27	23

Elution of $^{18}F^-$ from a QMA cartridge with different amounts of onium salts (Table S3): [^{18}F]Fluoride (50–500 MBq) was fixed on a QMA cartridge. The cartridge was washed with the appropriate alcohol (1 mL) and the radiofluoride was eluted with a solution of the corresponding onium salt (see Table S3) in MeOH (0.5 mL). The elution yield was determined by comparing the radioactivity eluted into the reaction vial with the total radioactivity trapped on the anion exchange resin.

Table S3. Elution of $^{18}F^-$ from a QMA cartridge with different amounts of onium salts.

entry	precursor amount [mg]	 recovery [%]	 recovery [%]	 recovery [%]	 recovery [%]
1	10	99	-	-	-
2	8	-	98	-	-
3	5	99	-	99	98
4	2.5	99	-	97	92
5	1	-	96	-	-
6	0.7	-	89	-	-
7	0.5	97	78	80	84
8	0.3	-	-	68	82
9	0.1	85	44	65	76

Synthesis of ^{18}F-labeled compounds from only onium precursors. General procedure 4 – (GP4): Aqueous [^{18}F]fluoride (0.05–50 GBq) was trapped on a anion-exchange resin (QMA or Chromafix® 30-PS-HCO_3 cartridge). It should be noted, that in the case of QMA cartridges, the aqueous [^{18}F]fluoride was loaded onto the cartridge from the male side, whereas MeOH flushing and $^{18}F^-$ elution were done from the female side of the cartridge. If the QMA has been loaded, flushed and eluted from the female side only, sometimes a significant amount of [^{18}F]fluoride remained on the resin (this is probably because QMA-light (46 mg) cartridges have a single frit on the male side but four on the female side).

Unless otherwise stated the cartridge was washed with MeOH (1 mL) and [^{18}F]fluoride was eluted into a reaction vial with a solution of the appropriate precursor (5 mg; unless otherwise stated) in MeOH (0.5 mL). Methanol was evaporated under reduced pressure at 70 °C within 2–3 min. After cooling to room temperature the appropriate solvent (500 µL) was added. The reaction mixture was

stirred under the conditions given in Tables S4–S10. Subsequently the mixture was cooled down to room temperature, water (4 mL) was added and the reaction mixture was shaken vigorously for 30 s. Thereafter, RCC was determined by radio-HPLC.

Unless stated, HPLC conditions: Eluent: 30% MeCN, flow rate: 1.5 mL/min.

Preparation of ^{18}F-labeled compounds using K2.2.2/K_2CO_3 protocol. General procedure 5 – (GP5): [^{18}F]Fluoride (0.05–50 GBq) was fixed on a QMA cartridge and eluted with 0.066 M K_2CO_3 (360 µL) into a solution of K2.2.2 (20 mg, 53.1 µmol) in MeCN (700 µL). After evaporation, the corresponding onium precursor (5 mg) dissolved in DMSO (500 µL) was added to the dry cryptate ([K⊂2.2.2]$^+$/^{18}F$^-$) and the reaction mixture was stirred at the temperature and for the indicated time (Tables S4–5, 8). The mixture was cooled down to room temperature, water (4 mL) was added and the reaction mixture was shaken vigorously for 30 s. Thereafter, RCC was determined by radio-HPLC.

Unless otherwise stated, HPLC conditions: Eluent: 30% MeCN, flow rate: 1.5 mL/min.

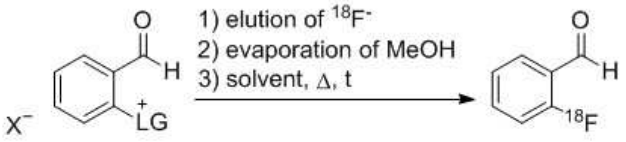
Synthesis of ^{18}F-labeled compounds using $CsHCO_3$/TEMPO protocol. General procedure 6 – (GP6): [^{18}F]Fluoride (0,05–50 GBq) was fixed on an anion-exchange resin and eluted with an aqueous solution of $CsHCO_3$ (400 µL; 2.5 mg/mL) into the reaction vial containing MeCN (1 mL). After azeotropic drying under helium at 110 °C (×3), a solution of the appropriate iodonium salt (1–2 mg) and 2,2,6,6-tetramethylpiperidine-*N*-oxyl (TEMPO) (0.5–1 mg) in DMF (1 mL) was added and the reaction mixture was stirred at the temperature and for the indicated time (Tables S6). The mixture was cooled down to room temperature, water (4 mL) was added and the reaction mixture was shaken vigorously for 30 s. Thereafter, RCC was determined by radio-HPLC.

Unless otherwise stated, HPLC conditions: Eluent: 30% MeCN, flow rate: 1.5 mL/min.

Preparative synthesis of 2-/4-[^{18}F]fluorobenzaldehydes (2-/4-[^{18}F]FBA): The ^{18}F-labeled compounds were prepared according to GP4. After cooling to ambient temperature, the reaction

mixture was diluted with water (9 mL) and loaded onto a Strata X cartridge. The cartridge was washed with 0.1 M HCl (10 mL) and H_2O (5 mL) and the corresponding [^{18}F]fluorobenzaldehyde (up to 35 GBq, 65–75% EOB; not decay corrected) was eluted with EtOH (0.3 mL). The radiochemical and chemical purities after solid phase extraction (SPE) purification were > 99%.

Table S4. Synthesis of 2-[^{18}F]FBA.[a]



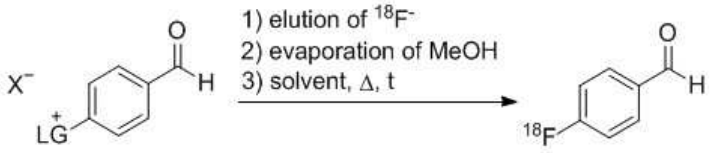
$LG^+ = Me_3N^+, PhI^+$

$X^- = I^-, Br^-, ClO_4^-, TfO^-, SCN^-, HCO_3^-$

entry	LG^+X^-	solvent	temperature [°C], time [min]	RCC [%]
1	$Me_3N^+HCO_3^-$	DMF	80, 10	58
2	$Me_3N^+HCO_3^-$	MeCN	80, 10	68
3	$Me_3N^+I^-$	DMSO	80, 10	62(*62*)[c]
4	$Me_3N^+I^-$	DMSO	130, 10	63
5	$Me_3N^+I^-$	MeCN	130, 10	24
6	$Me_3N^+SCN^-$	DMSO	80, 10	26
7	$Me_3N^+SCN^-$	DMSO	130, 10	56
8	$Me_3N^+SCN^-$	MeCN	130, 10	46
9	PhI^+TfO^-	DMF	160, 15	35
10	$Me_3N^+TfO^-$	DMSO	130, 15	64(*46*)[c]
11	PhI^+Br^-	DMSO	80, 2	10
12	PhI^+Br^-	DMSO	160, 15	61
13	PhI^+Br^-	DMF	160, 10	5
14	$Me_3N^+ClO_4^-$	DMSO	80, 15	63
15	$Me_3N^+ClO_4^-$	DMSO	150, 10	80(*30*)[c]
16	$Me_3N^+ClO_4^-$	DMSO	200, 10	65
17	$Me_3N^+ClO_4^-$	MeCN	80, 10	37
18[b]	$Me_3N^+TfO^-$	DMSO	80, 2	46

[a] Unless otherwise stated, all experiments were carried out as described in GP4; [b] carried out according to the K2.2.2/K_2CO_3 protocol (GP5); [c] RCC with 2 mg precursor in 100 µL solvent.

Table S5. Preparation of 4-[^{18}F]FBA.[a]

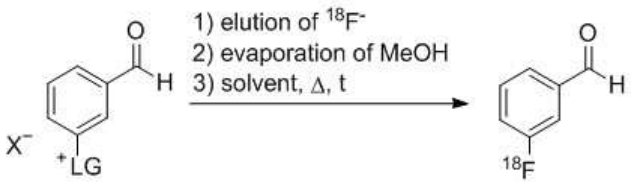


$LG^+ = Me_3N^+$, PhI^+
$X^- = I^-$, Br^-, ClO_4^-, TfO^-, HCO_3^-

entry	LG^+X^-	solvent	temperature [°C], time [min]	RCC [%]
1	$Me_3N^+HCO_3^-$	DMF	80, 10	28
2	$Me_3N^+HCO_3^-$	MeCN	130, 10	45
3	$Me_3N^+HCO_3^-$	DMSO	80, 10	87(*40*)[c]
4	$Me_3N^+HCO_3^-$	sulfolane	200, 10	80
5	$Me_3N^+I^-$	DMSO	80, 10	75(*65*)[c]
6	PhI^+TfO^-	DMF	160, 15	50
7	PhI^+Br^-	DMF	115, 5	61
8	$Me_3N^+ClO_4^-$	DMSO	150, 10	90(*33*)[c]
9	$Me_3N^+ClO_4^-$	sulfolane	200, 10	89
10	$Me_3N^+TfO^-$	DMF	115, 10	63
11[b]	$Me_3N^+TfO^-$	DMSO	80, 10	65

[a] Unless otherwise stated, all experiments were carried out as described in GP4; [b] carried out according to the K2.2.2/K_2CO_3 protocol (GP5); [c] RCC with 2 mg precursor in 100 µL solvent.

Table S6. Preparation of 3-[^{18}F]FBA.[a]

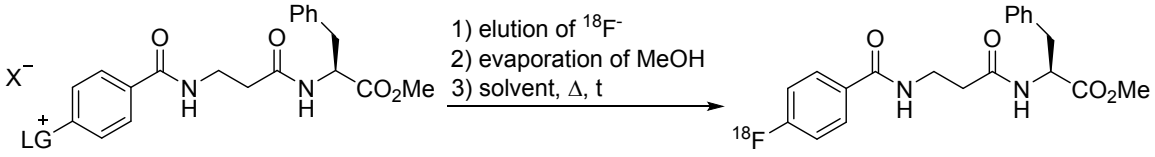


$LG^+ = Me_3N^+, PhI^+, (4\text{-}OMePh)I^+$
$X^- = I^-, Br^-, ClO_4^-, TfO^-, HCO_3^-$

entry	LG^+X^-	solvent	temperature [°C], time [min]	RCC [%]
1	PhI^+I^-	95% DMSO	130, 10	14
2	PhI^+I^-	95% DMF	130, 10	12
3	PhI^+I^-	95% MeCN	130, 10	5
4	$(4\text{-}OMePh)I^+I^-$	DMSO	80, 10	12
5	$(4\text{-}OMePh)I^+I^-$	DMSO	130, 10	31
6	PhI^+Br^-	DMSO	80, 10	0
7	$Me_3N^+HCO_3^-$	DMSO	80, 10	0
8	$Me_3N^+I^-$	DMSO	130, 10	0
9	$Me_3N^+I^-$	sulfolane	200, 10	6
10	PhI^+TfO^-	DMF	160, 15	10
11	$(4\text{-}OMePh)I^+ClO_4^-$	DMSO	80, 10	22
12	$(4\text{-}OMePh)I^+ClO_4^-$	DMSO	150, 10	40
13	$(4\text{-}OMePh)I^+ClO_4^-$	95% MeCN	80, 10	2
14	$(4\text{-}OMePh)I^+ClO_4^-$	95% DMSO	80, 10	6
15[b]	PhI^+Br^-	DMF	130, 10	45

[a] Unless otherwise stated, all experiments were carried out as described in GP4; [b] carried out according to the TEMPO/$CsHCO_3$ protocol (GP6).

Table S7. Synthesis of dipeptide [^{18}F]**1**.[a]

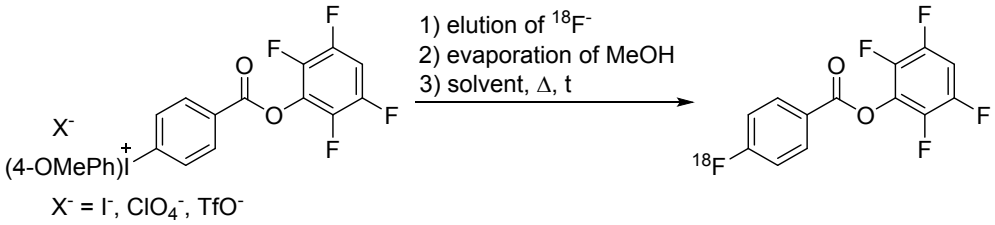


$LG^+ = Me_3N^+$, $(4\text{-OMePh})I^+$
$X^- = I^-$, ClO_4^-, HCO_3^-

entry	LG^+X^-	solvent	temperature [°C], time [min]	RCC [%]
1	$Me_3N^+HCO_3^-$	sulfolane	130, 15	30
2	$Me_3N^+HCO_3^-$	sulfolane	200, 20	31
3	$Me_3N^+HCO_3^-$	diglyme	200, 15	4
4	$Me_3N^+HCO_3^-$	DMSO	130, 15	29
5	$Me_3N^+ClO_4^-$	DMSO	140, 15	12
6	$(4\text{-OMePh})I^+I^-$	DMSO	130, 10	56
7	$(4\text{-OMePh})I^+ClO_4^-$	DMSO	140, 15	12
8[b]	$(4\text{-OMePh})I^+I^-$	DMF	120, 10	56
9[b]	$(4\text{-OMePh})I^+I^-$	diglyme	120, 10	16
10[c]	$Me_3N^+I^-$	DMSO	90, 10	0
11[c]	$Me_3N^+I^-$	sulfolane	150, 10	0
12[c]	$Me_3N^+TfO^-$	DMSO	150, 10	0
13[c]	$Me_3N^+TfO^-$	sulfolane	150, 10	0

[a] Unless otherwise stated, all experiments were carried out as described in GP4; [b] carried out according to the K2.2.2/K_2CO_3 protocol (GP5); [c] carried out according to the TEMPO/$CsHCO_3$ protocol (GP6).

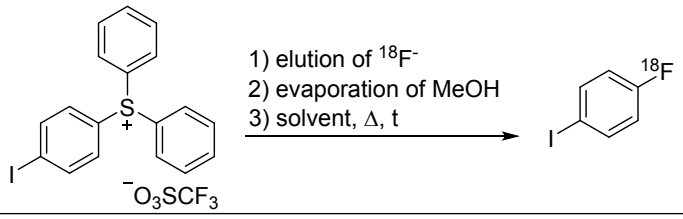
Table S8. Synthesis of 2,3,4,6-tetrafluoro 4-[^{18}F]fluorobenzoate (4-[^{18}F]TFP, [^{18}F]**2**).[a]



entry	X^-	solvent	temperature [°C], time [min]	RCC [%]
1[b]	I^-	DMSO	130, 10	10–25
2	ClO_4^-	DMSO	130, 10	23–25
3	$TosO^-$	DMSO	130, 10	11

[a] All experiments were carried out as described in GP4; [b] large fluctuations of RCCs were observed. HPLC conditions: Eluent: 50% MeCN, flow rate: 3 mL/min

Table S10. Synthesis of 1-[^{18}F]fluoro-4-iodobenzene ([^{18}F]FIB, [^{18}F]**4**).[a]

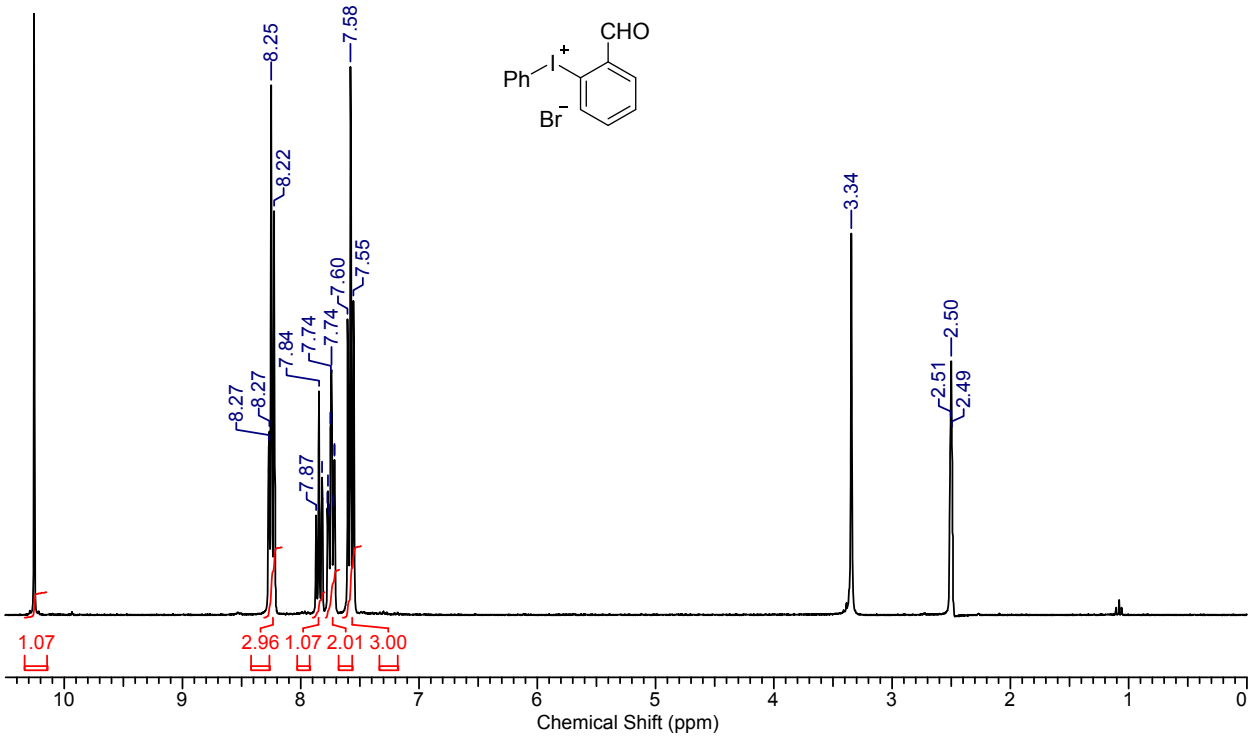


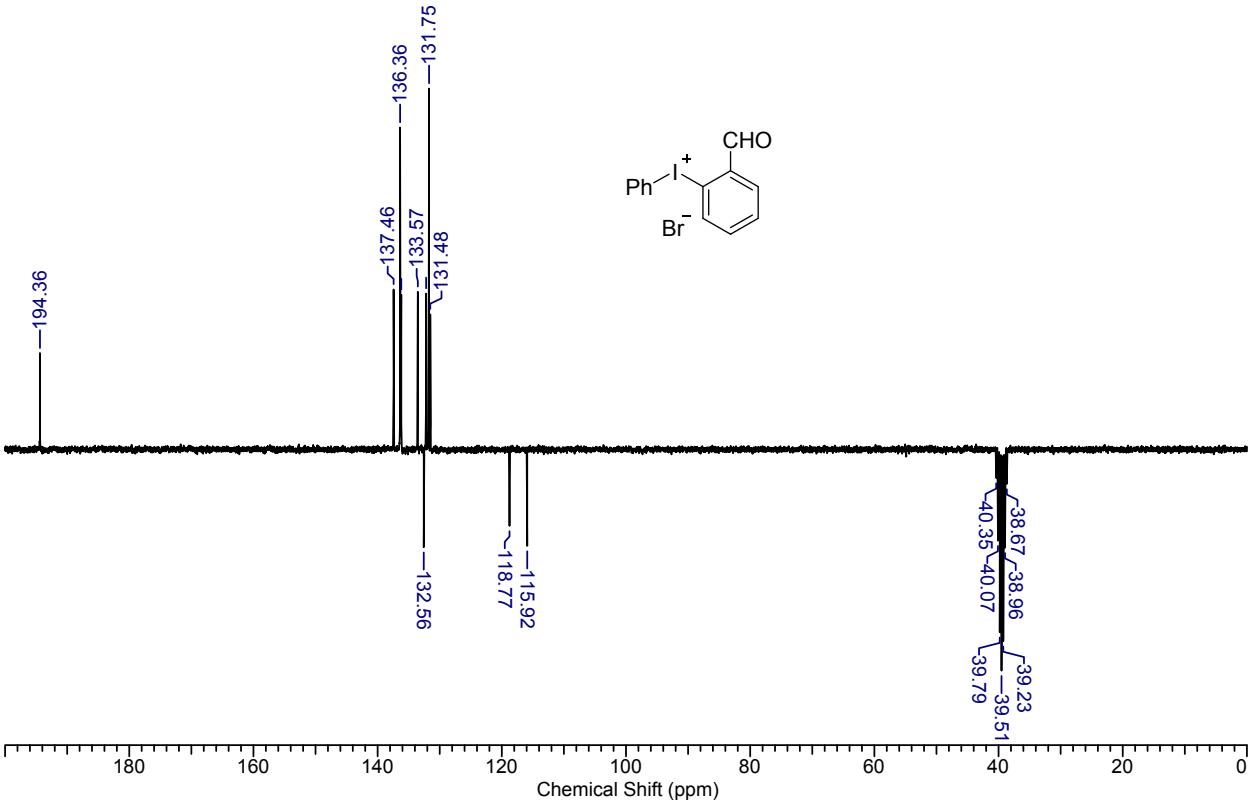
entry	solvent	temperature [°C], time [min]	RCC [%]
1	diglyme	85, 15	66
2	MeCN	85, 15	41
3	DMSO	85, 15	16
4	sulfolane	85, 15	41
5	DMF	85, 15	29
6[b]	MeCN	85, 15	50
7[b]	diglyme	85, 15	42

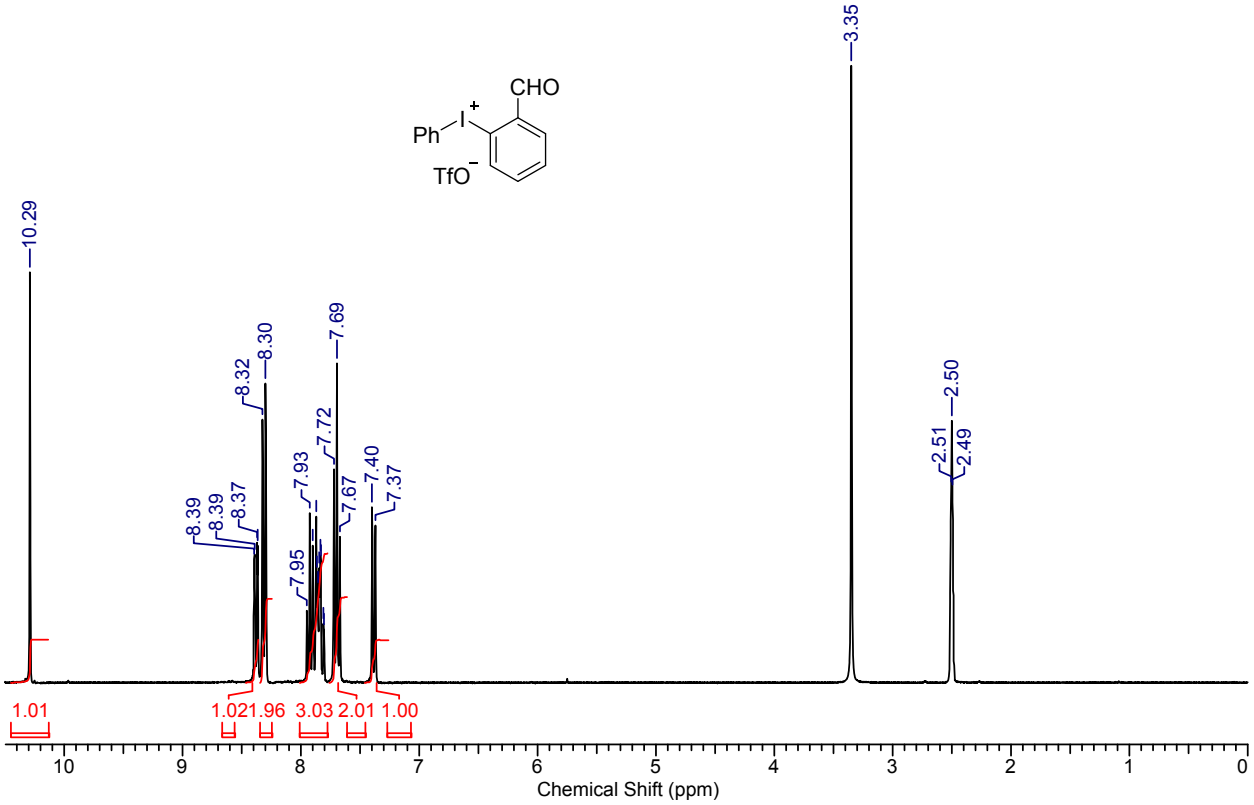
[a] All experiments were carried out as described in GP4. [b] carried out according to the K2.2.2/K_2CO_3 protocol (GP5). HPLC conditions: Eluent: 50% MeCN, flow rate: 1.5 mL/min.

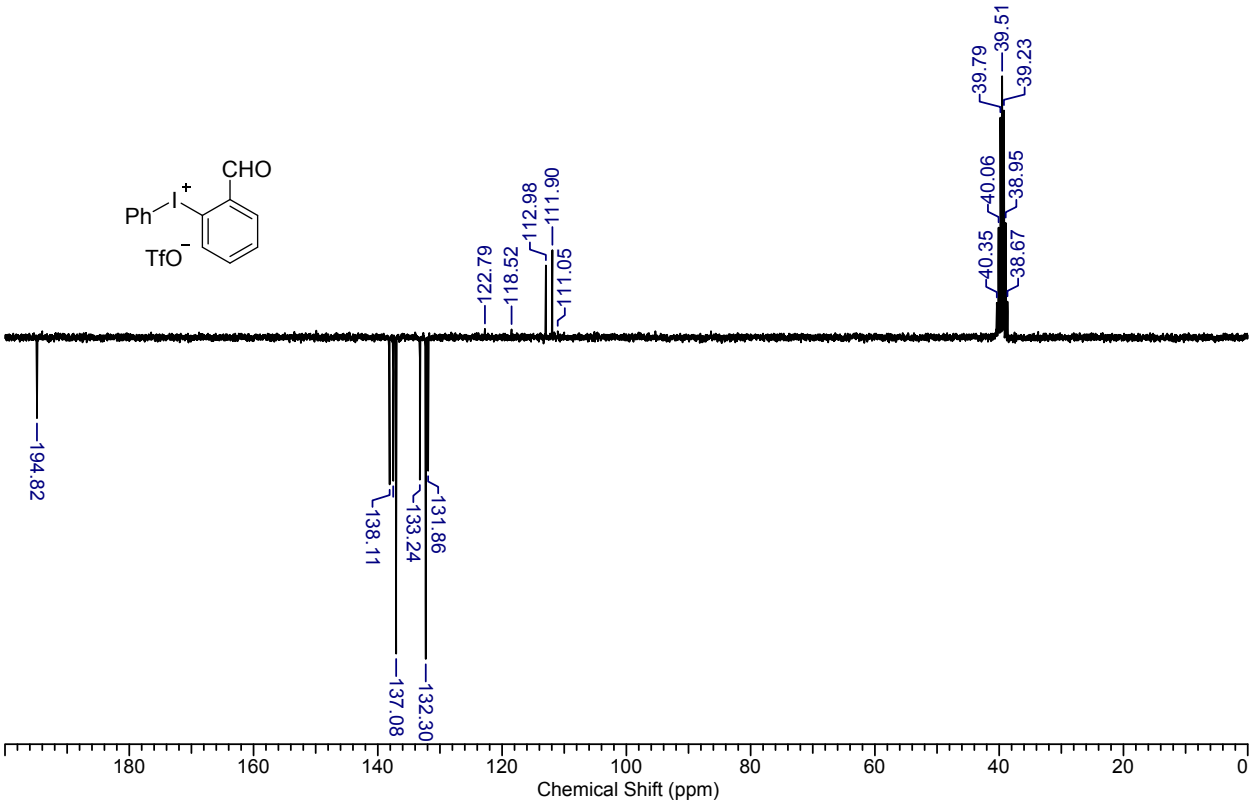
Preparation of 5-[^{18}F]fluoro-D-ribose ([^{18}F]FDR, [^{18}F]4): The protected intermediate of [^{18}F]FDR was obtained from precursor **11** according to GP4 using MeCN as solvent. The reaction was carried out at 80 °C for 10 min. Removal of the protective groups was performed by incubation of the crude reaction mixture with 1 M HCl (400 µL) for 5 min at 105 °C. Thereafter, the reaction mixture was neutralized with 1 M NaOH (400 µL) to give [^{18}F]**8** in 74% RCC (determined by radio-HPLC). [^{18}F]**8** was also prepared using the conventional K2.2.2/K_2CO_3 protocol according to GP5. The subsequent deprotection of the radiolabeled intermediate gave [^{18}F]**8** in 58% RCC (determined by radio-HPLC). HPLC conditions: Column: Luna C-18 (2) 5µm (250×4.6 mm, Phenomenex), eluent: 55% MeCN, flow rate: 1.5 mL/min.

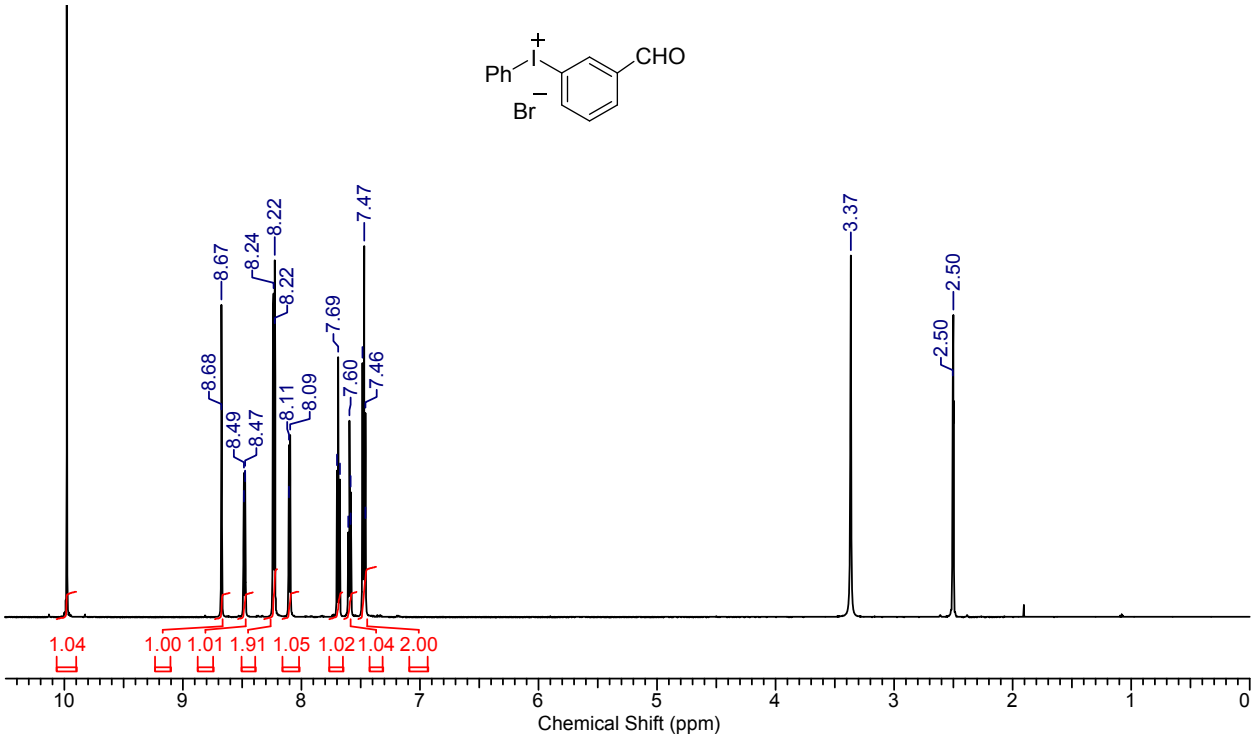
[1]H- and APT-NMR Spectra

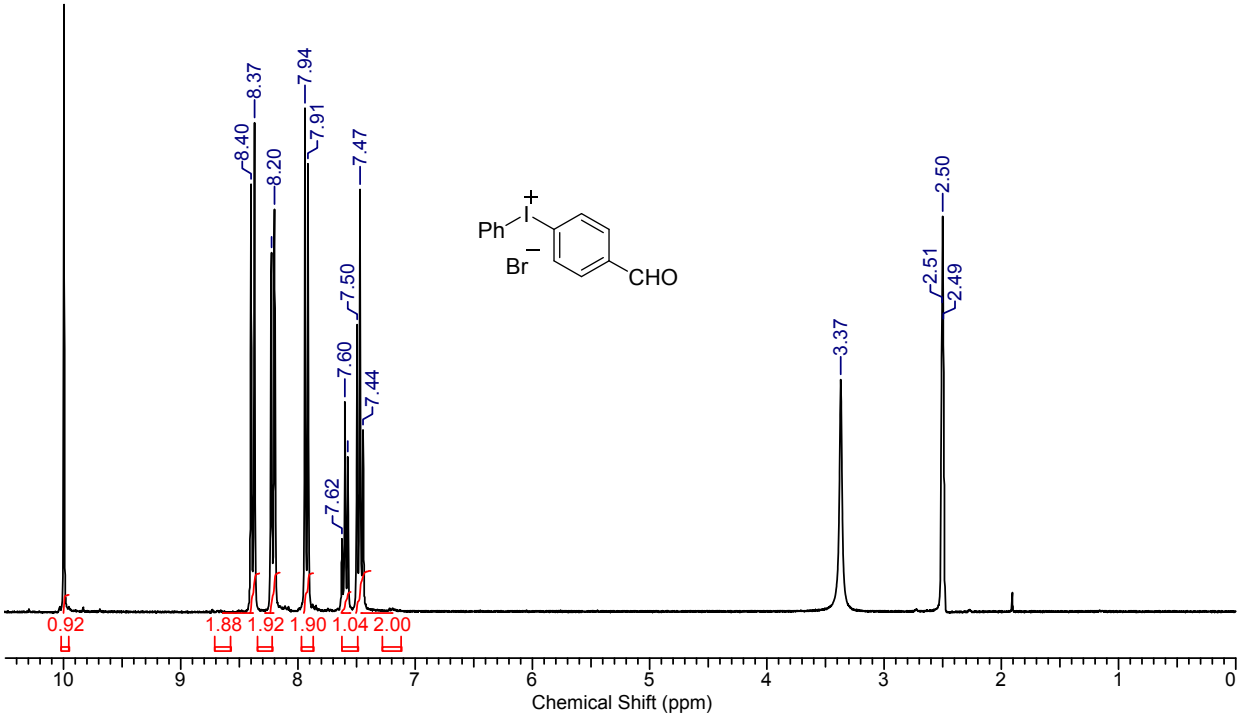


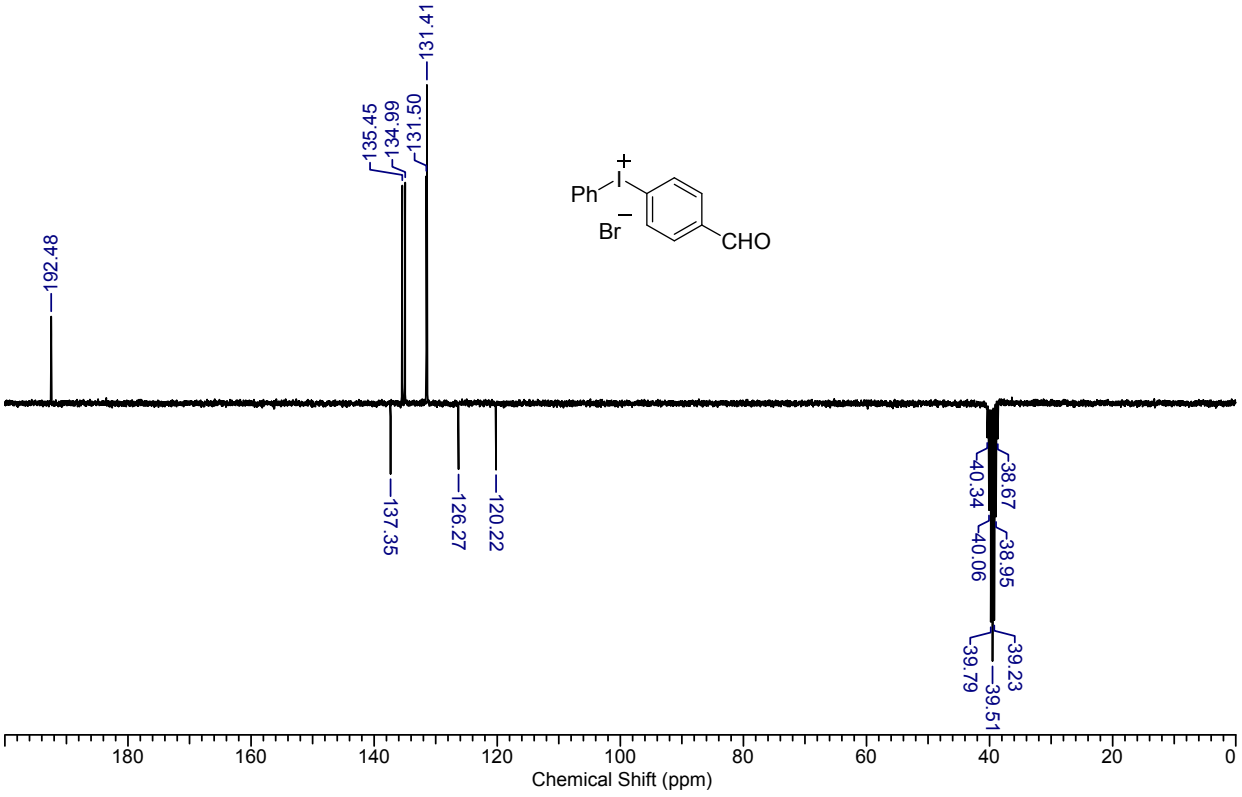


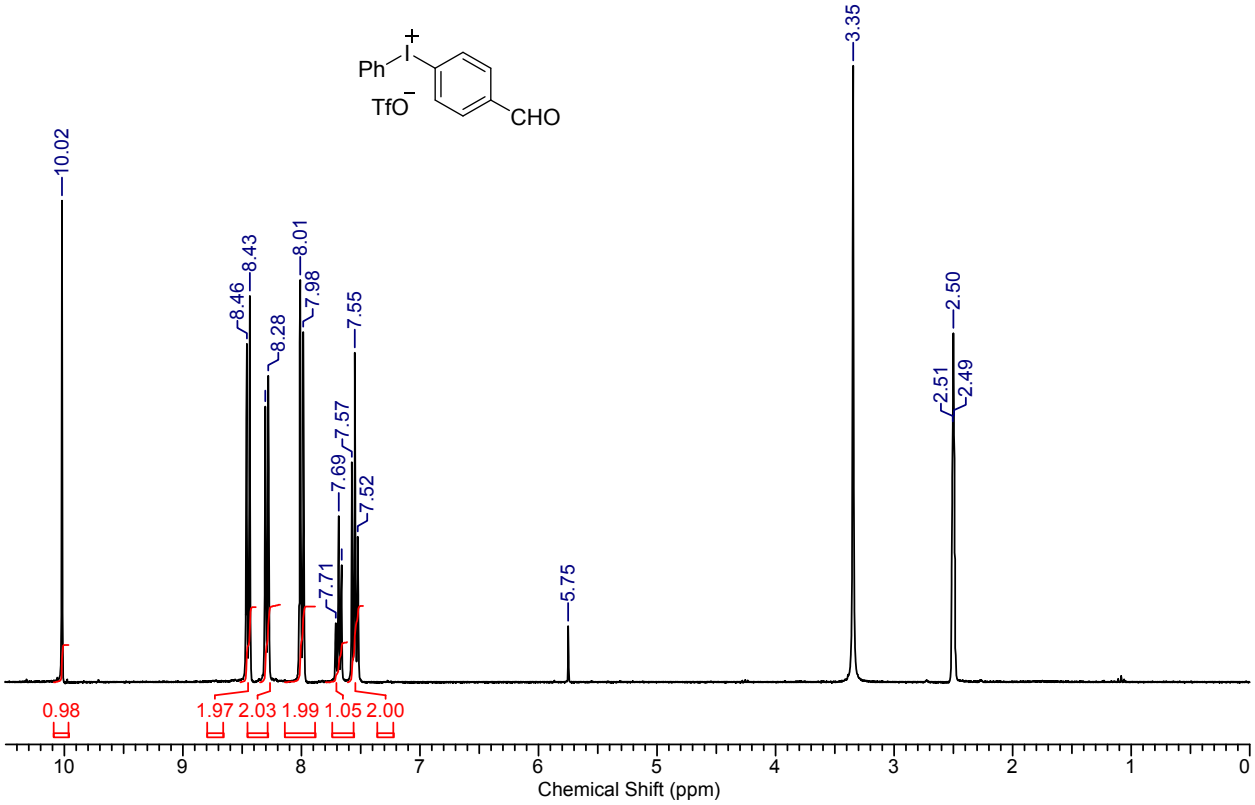

CHO
Ph
I
+
TfO
−
10.29
8.39
8.39
8.37
8.32
8.30
7.95
7.93
7.72
7.69
7.67
7.40
7.37
3.35
2.51
2.50
2.49
1.01
1.02
1.96
3.03
2.01
1.00
Chemical Shift (ppm)

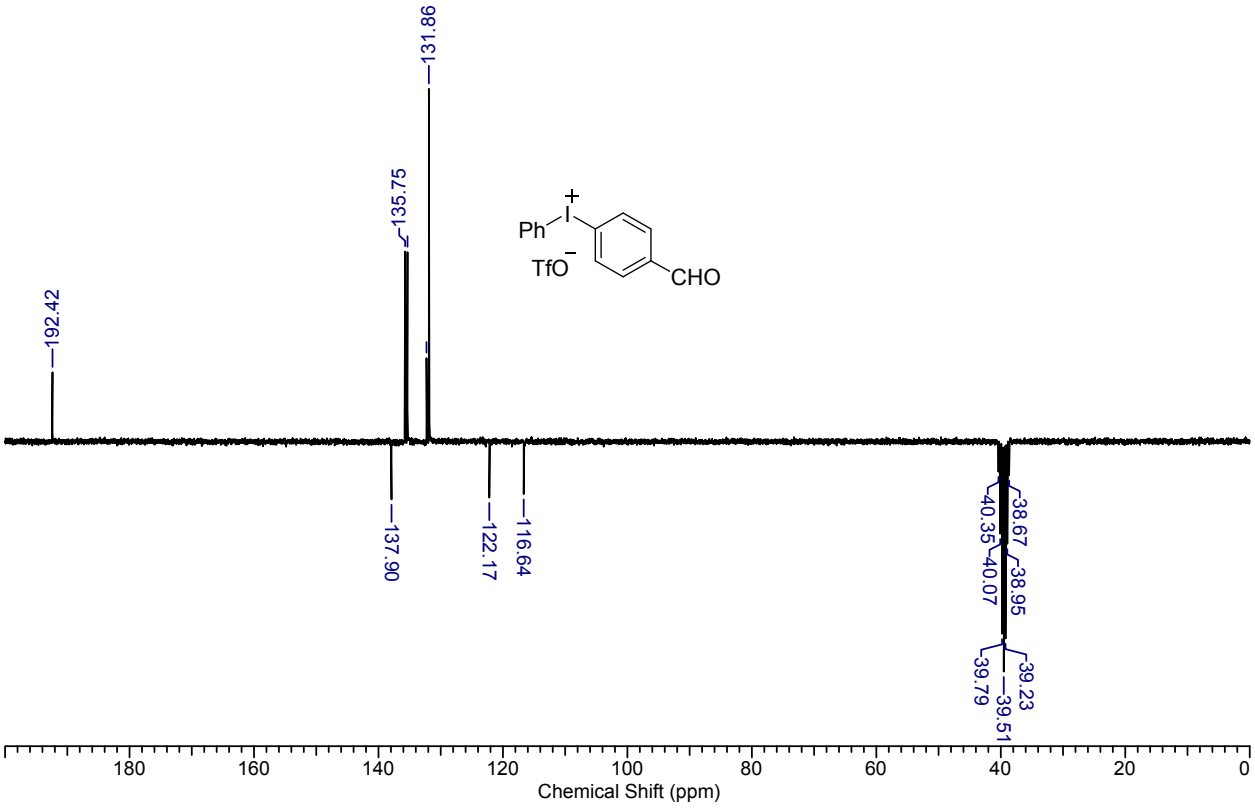

CHO
Ph
I
+
TfO
−
194.82
138.11
137.08
133.24
132.30
131.86
122.79
118.52
112.98
111.90
111.05
39.79
39.51
39.23
40.06
38.95
40.35
38.67
Chemical Shift (ppm)

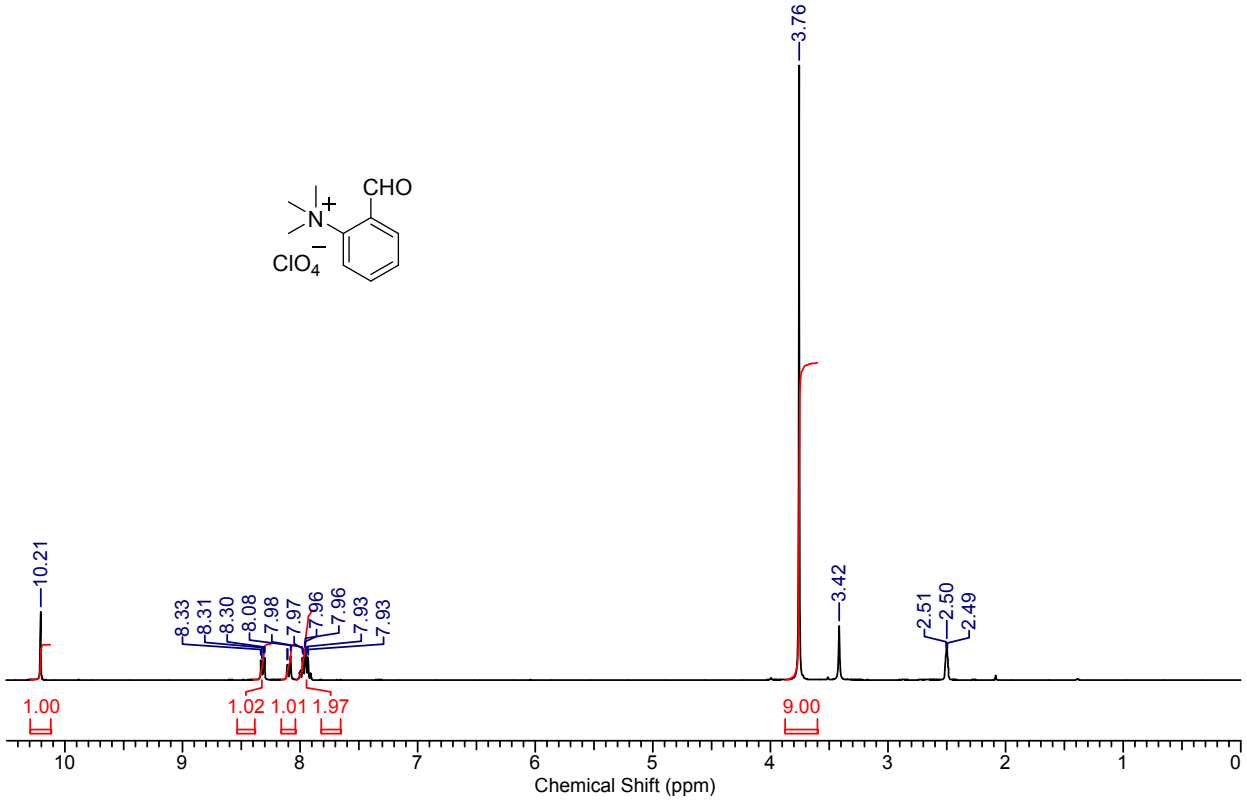

Ph
I
+
Br
−
CHO
8.67
8.68
8.49
8.47
8.24
8.22
8.22
8.11
8.09
7.69
7.60
7.47
7.46
3.37
2.50
2.50
1.04
1.00
1.01
1.91
1.05
1.02
1.04
2.00
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

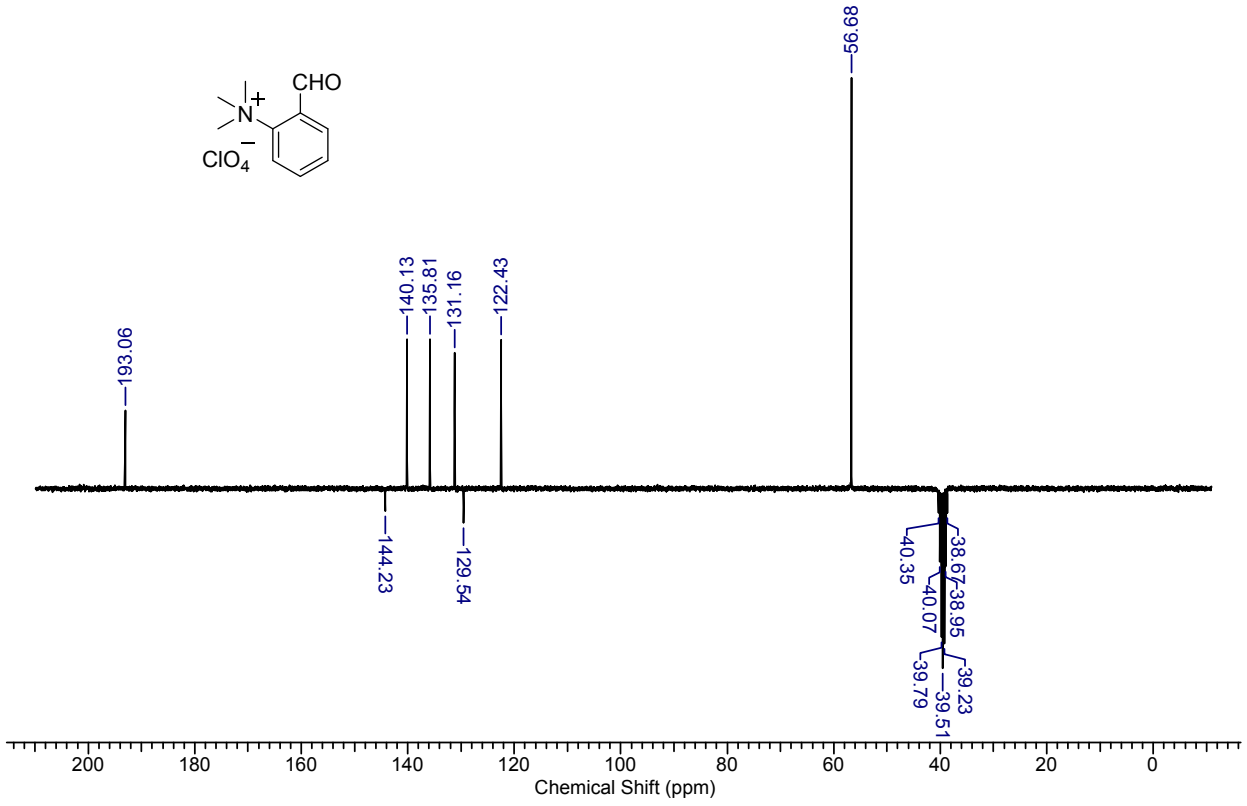

8.40
8.37
8.20
7.94
7.91
7.62
7.60
7.50
7.47
7.44
3.37
2.51
2.50
2.49
Ph
I
Br
CHO
0.92
1.88
1.92
1.90
1.04
2.00
Chemical Shift (ppm)

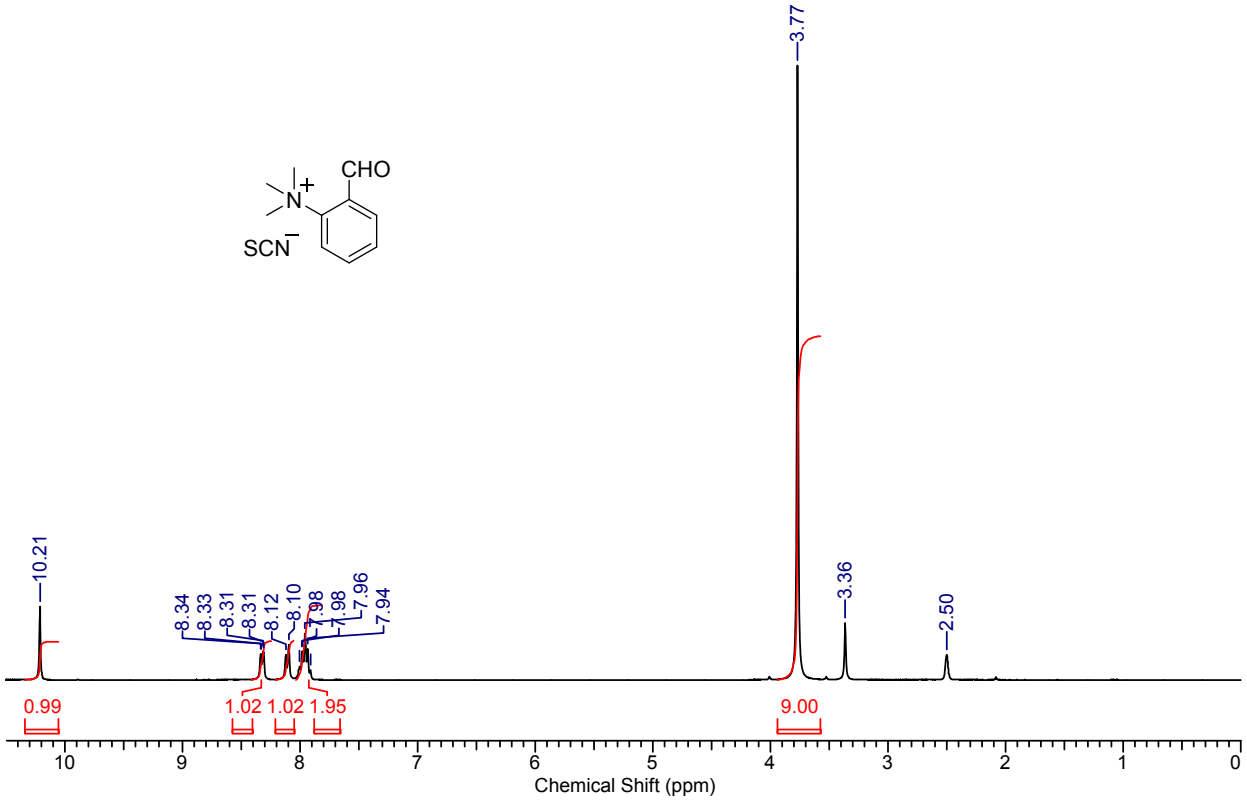

192.48
135.45
134.99
131.50
131.41
137.35
126.27
120.22
40.34
40.06
39.79
39.51
39.23
38.95
38.67
Ph
I
Br
CHO
Chemical Shift (ppm)

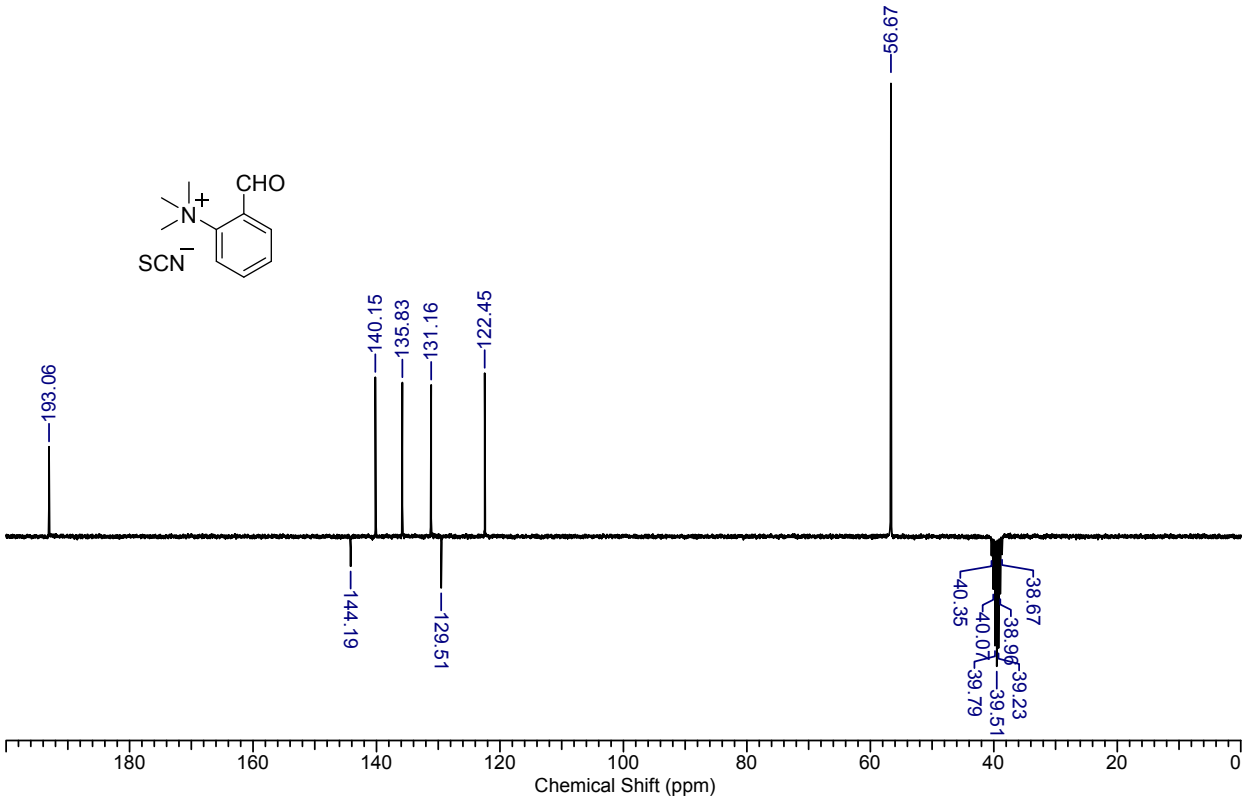

Ph
I
TfO
CHO
10.02
8.46
8.43
8.28
8.01
7.98
7.71
7.69
7.57
7.55
7.52
5.75
3.35
2.51
2.50
2.49
0.98
1.97
2.03
1.99
1.05
2.00
Chemical Shift (ppm)

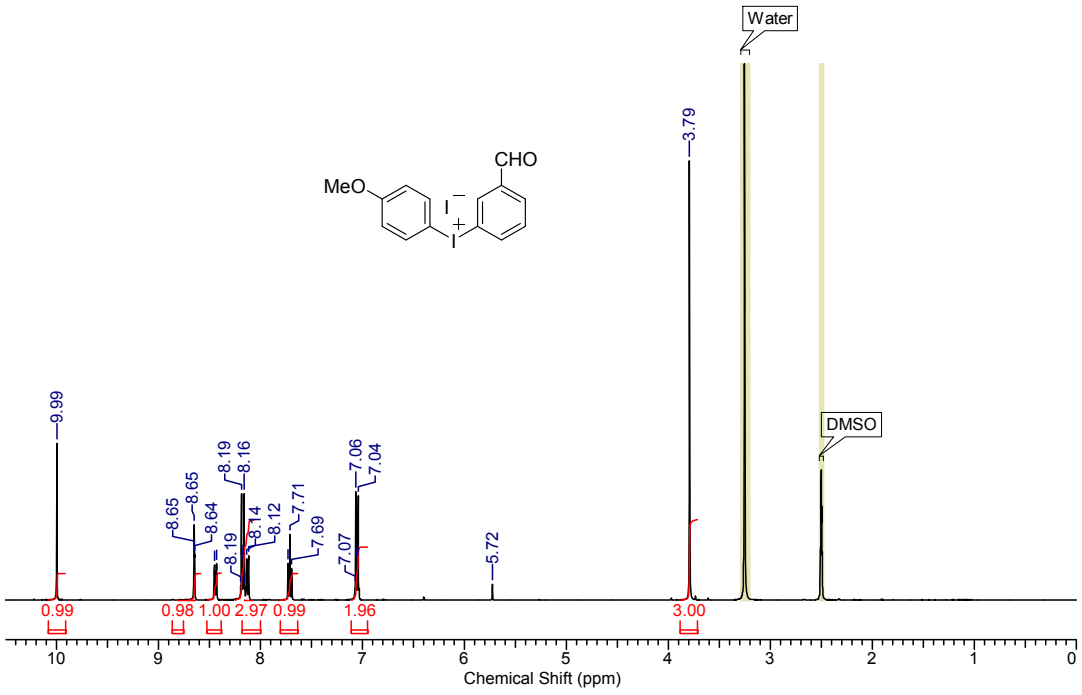

Ph
I
TfO
CHO
192.42
135.75
131.86
137.90
122.17
116.64
40.35
40.07
39.79
38.67
38.95
39.23
39.51
Chemical Shift (ppm)

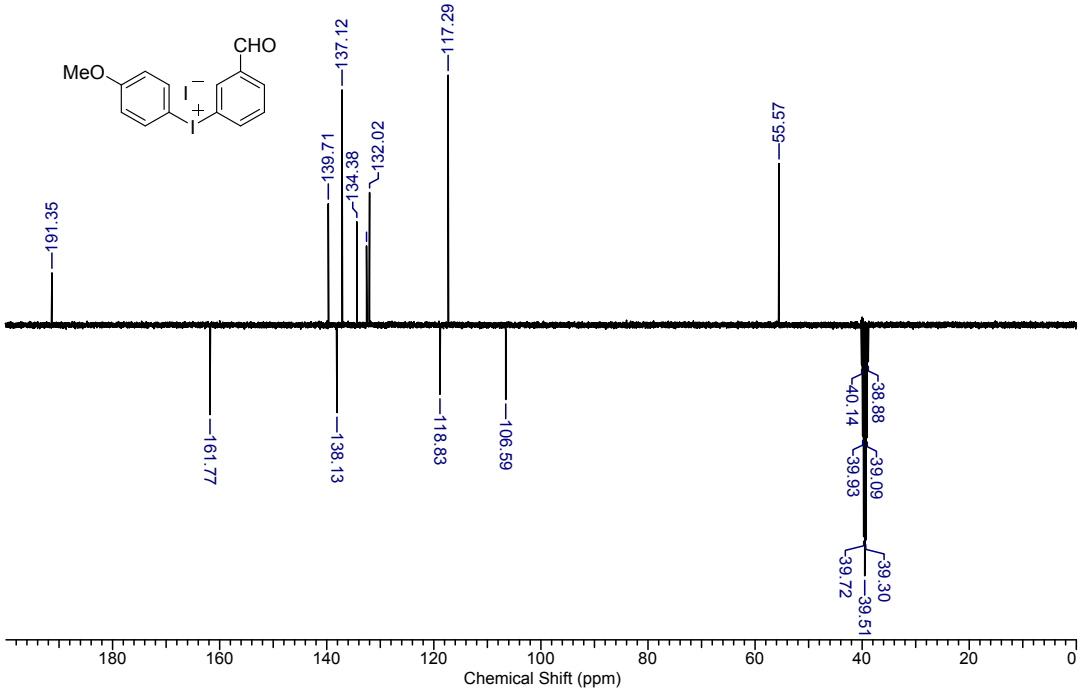

CHO
N+
ClO4−
3.76
10.21
8.33
8.31
8.30
8.08
7.98
7.97
7.96
7.96
7.93
7.93
3.42
2.51
2.50
2.49
1.00
1.02
1.01
1.97
9.00
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

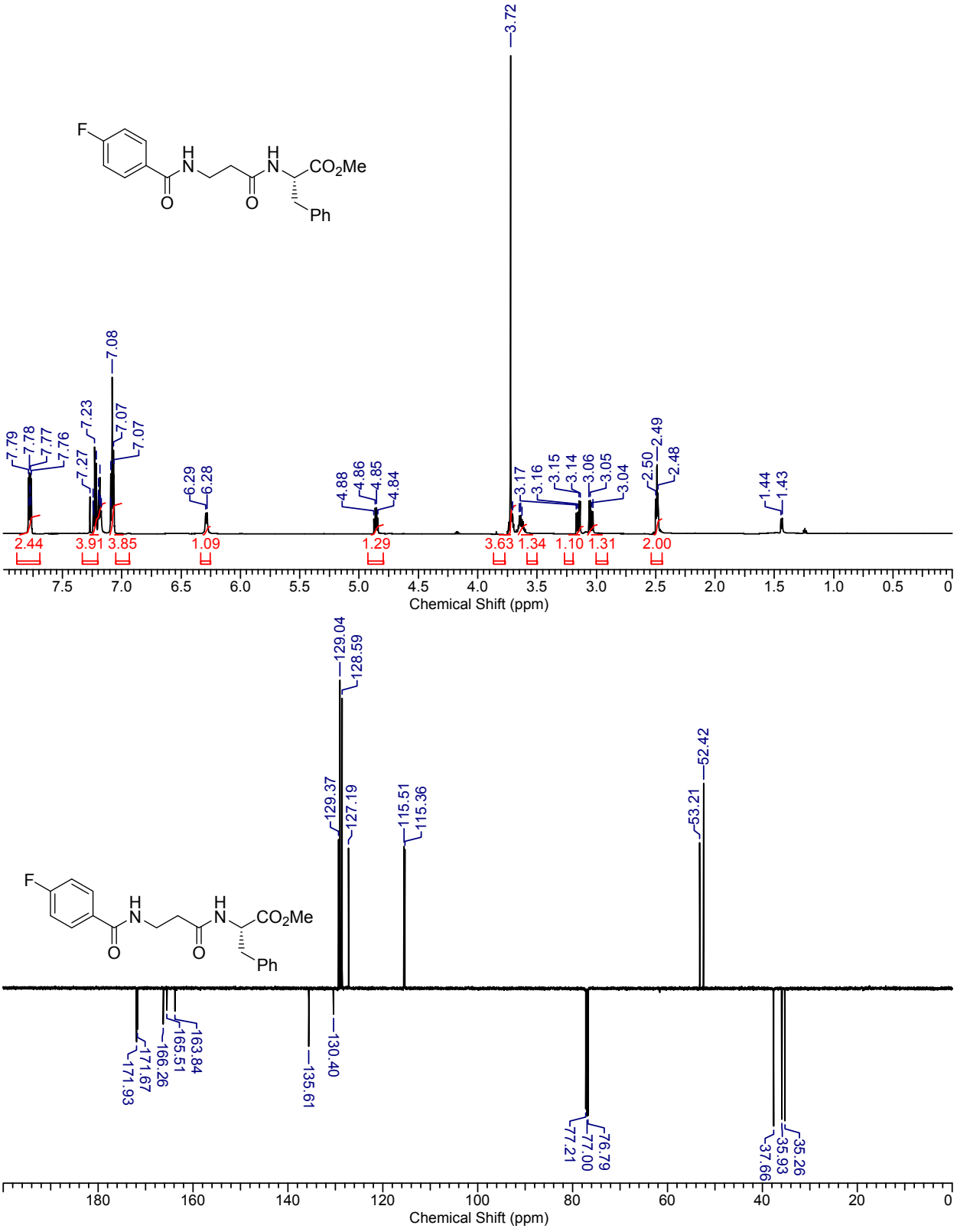

CHO
N+
ClO4−
56.68
193.06
140.13
135.81
131.16
122.43
144.23
129.54
40.35
40.07
39.79
39.51
39.23
38.95
38.67
200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

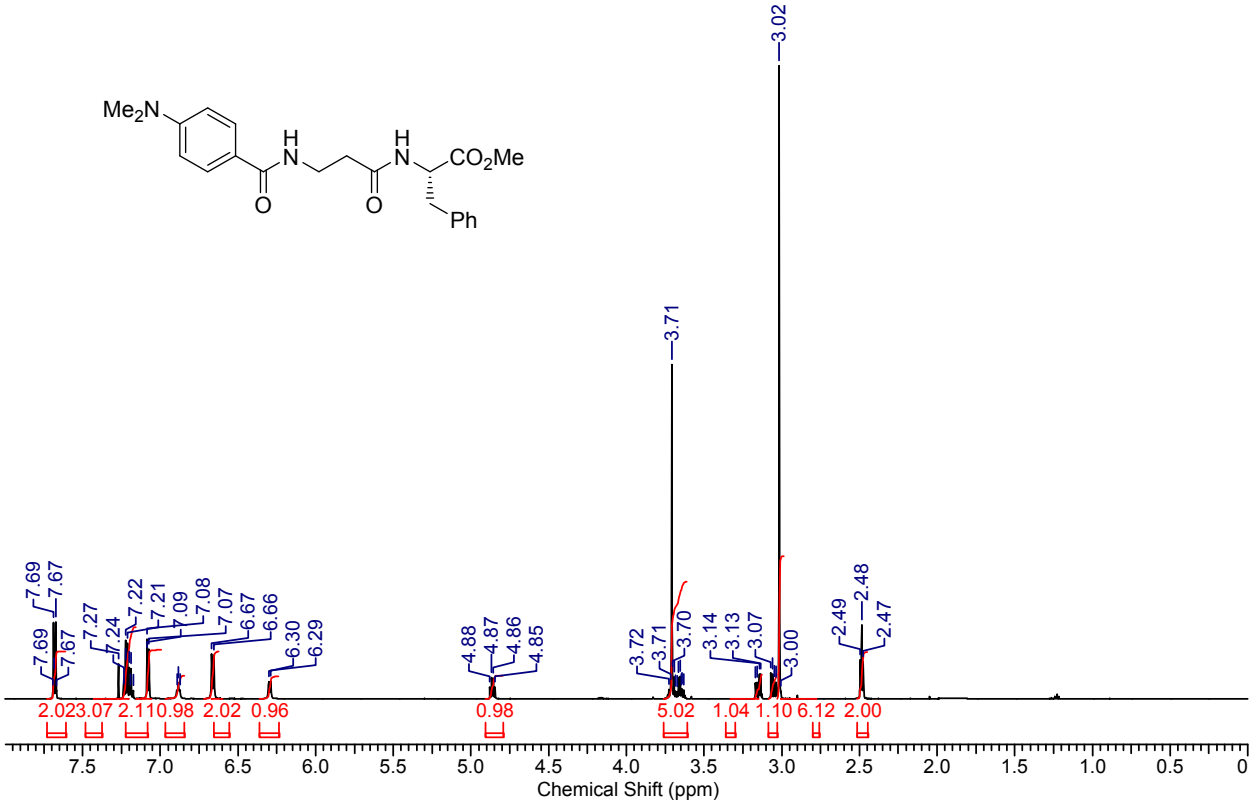

CHO
N+
SCN−
10.21
8.34
8.33
8.31
8.31
8.12
8.10
7.98
7.98
7.96
7.94
3.77
3.36
2.50
0.99
1.02
1.02
1.95
9.00
Chemical Shift (ppm)

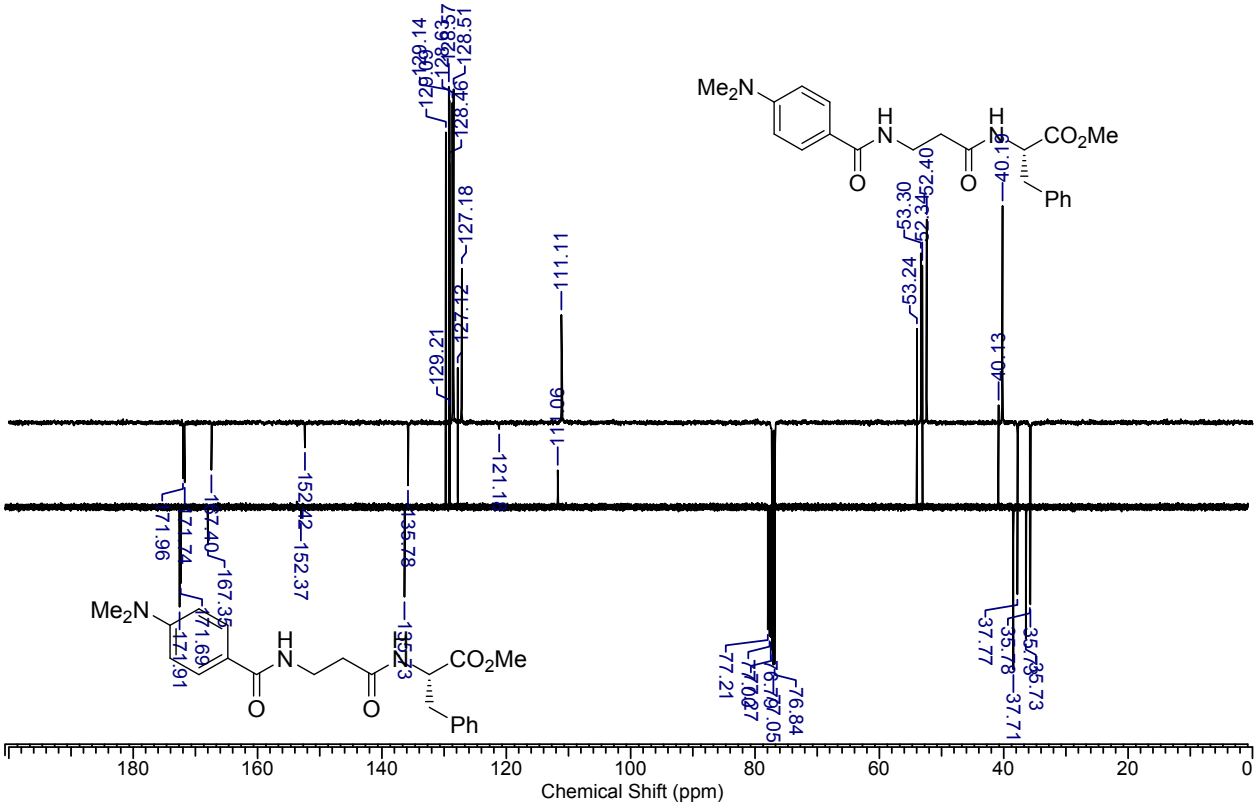

CHO
N+
SCN−
193.06
140.15
135.83
131.16
122.45
56.67
144.19
129.51
40.35
40.07
39.79
39.51
39.23
38.98
38.67
Chemical Shift (ppm)

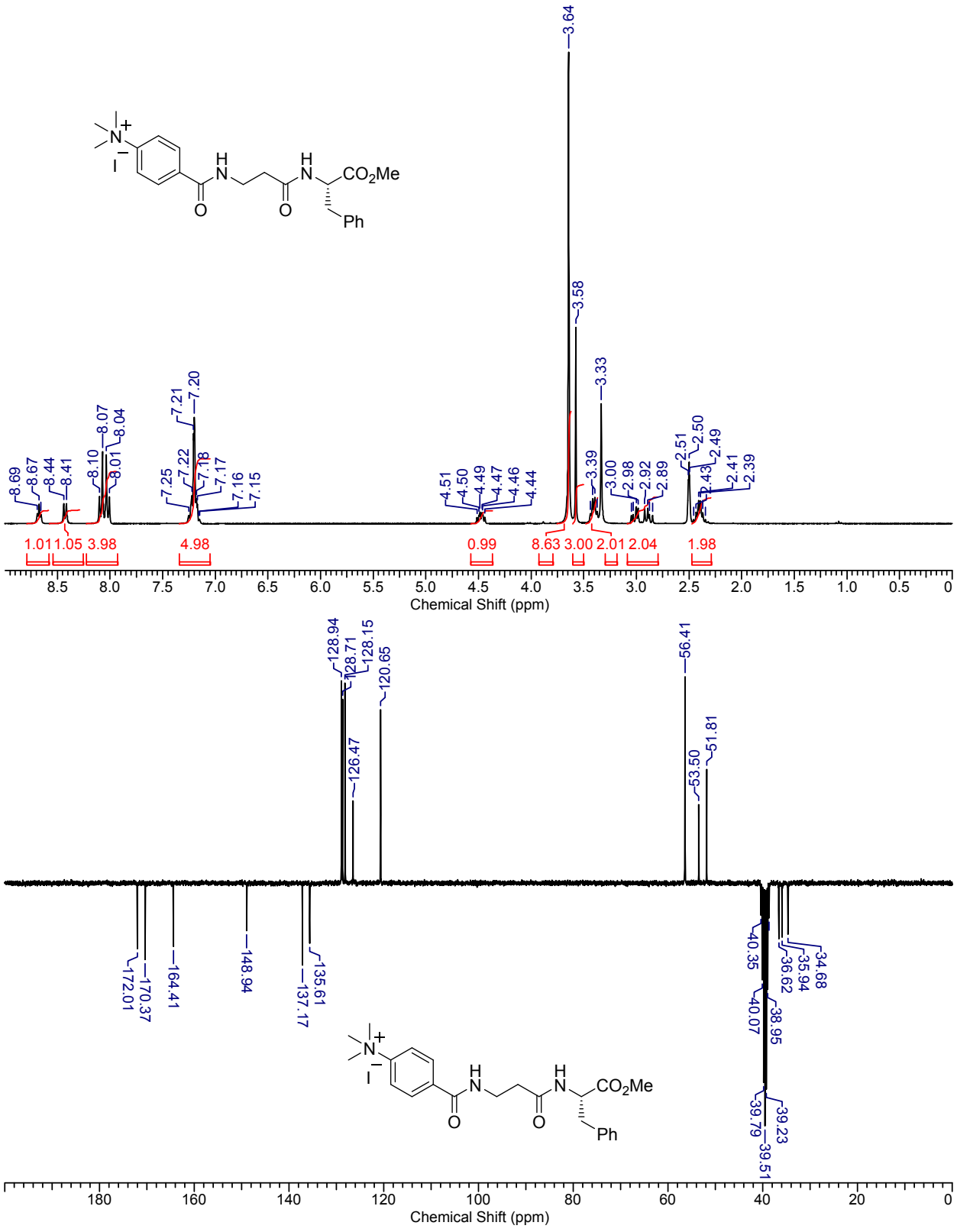

Water
DMSO
CHO
MeO
9.99
8.65
8.65
8.64
8.19
8.19
8.16
8.14
8.12
7.71
7.69
7.07
7.06
7.04
5.72
3.79
0.99
0.98
1.00
2.97
0.99
1.96
3.00
Chemical Shift (ppm)

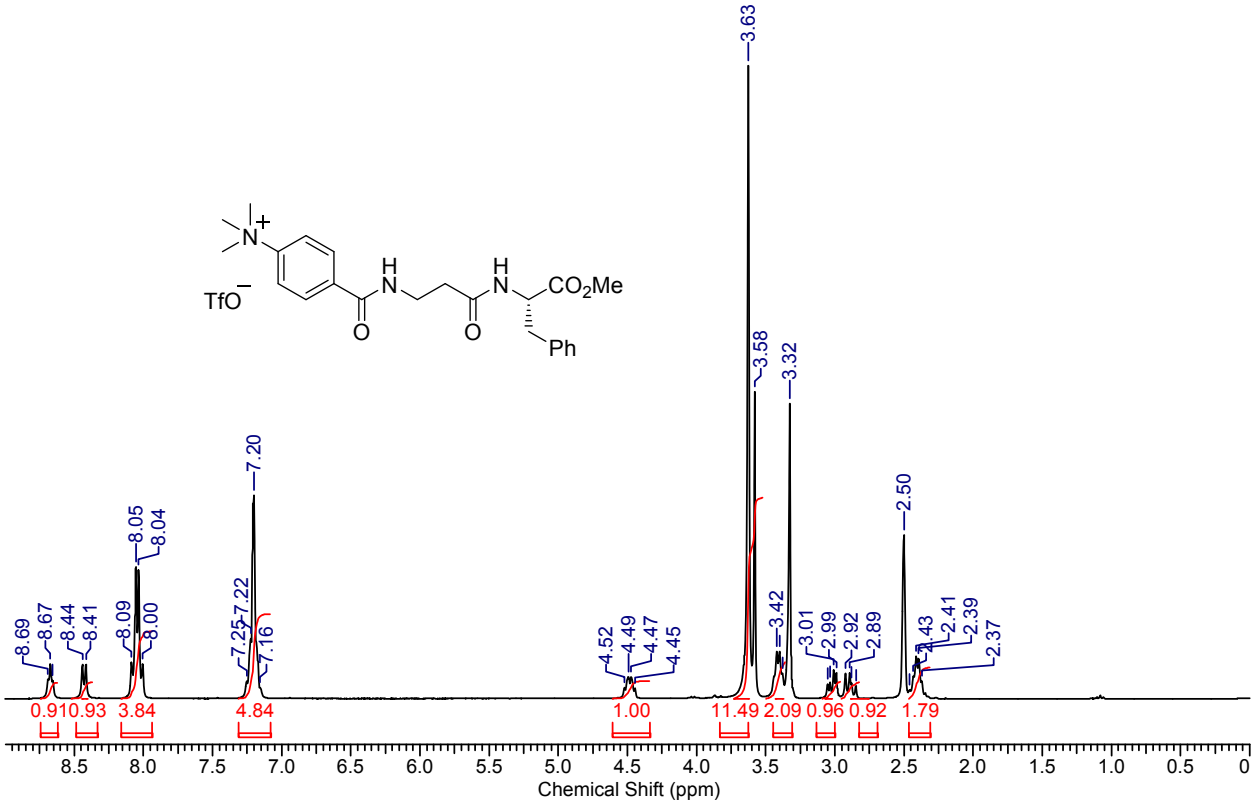

CHO
MeO
191.35
161.77
139.71
138.13
137.12
134.38
132.02
118.83
117.29
106.59
55.57
40.14
39.93
39.72
38.88
39.09
39.30
39.51
Chemical Shift (ppm)

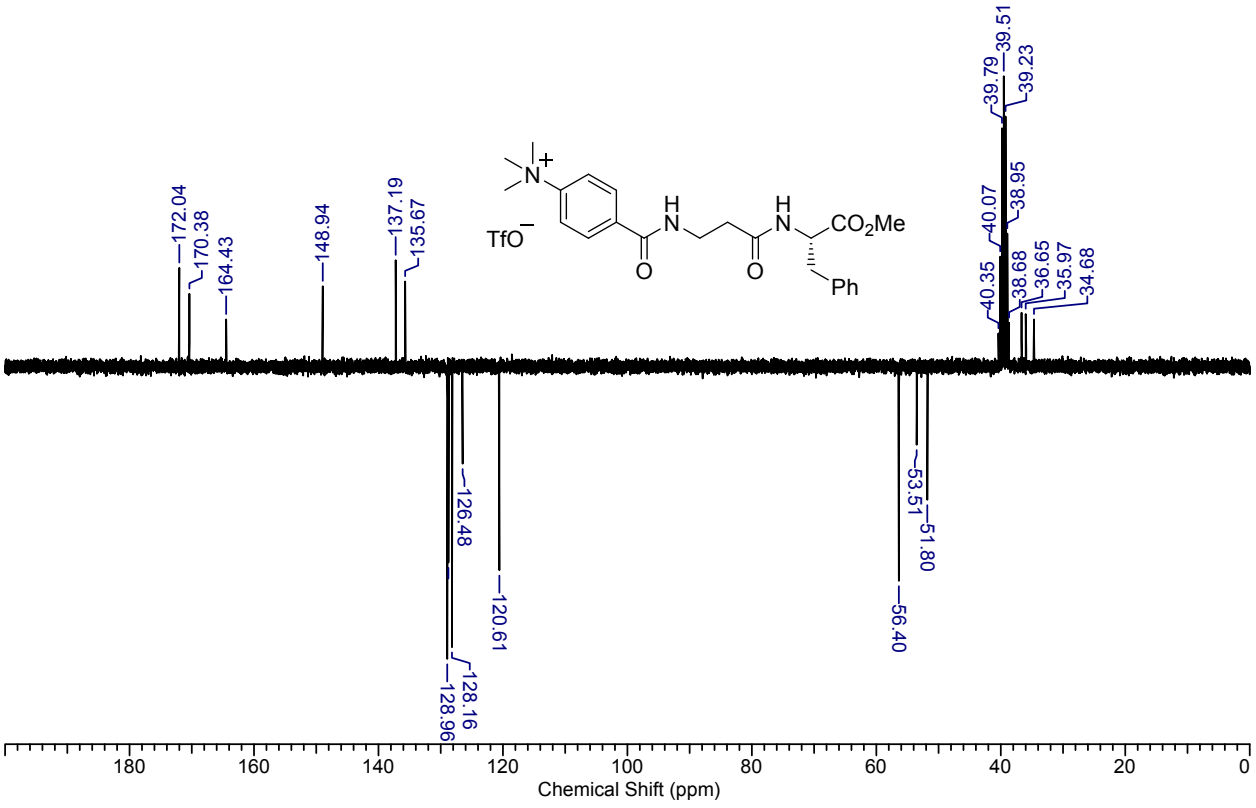

F
H
N
O
H
N
O
CO2Me
Ph
3.72
7.79
7.78
7.77
7.76
7.27
7.23
7.08
7.07
7.07
6.29
6.28
4.88
4.86
4.85
4.84
3.17
3.16
3.15
3.14
3.06
3.05
3.04
2.50
2.49
2.48
1.44
1.43
2.44
3.91
3.85
1.09
1.29
3.63
1.34
1.10
1.31
2.00
Chemical Shift (ppm)
129.04
128.59
129.37
127.19
115.51
115.36
52.42
53.21
171.93
171.67
166.26
165.51
163.84
135.61
130.40
77.21
77.00
76.79
37.66
35.93
35.26
Chemical Shift (ppm)

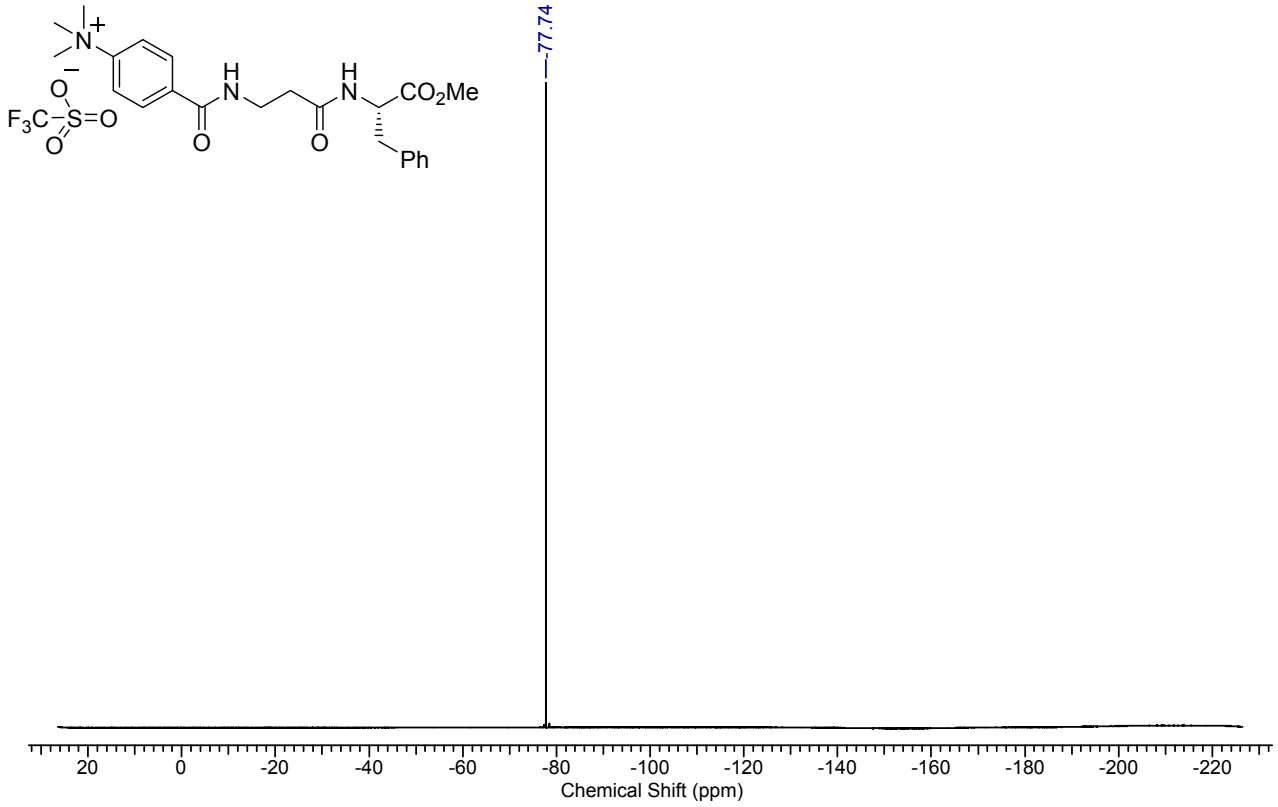

Me2N
H
N
O
H
N
O
CO2Me
Ph
3.02
3.71
7.69
7.67
7.27
7.24
7.22
7.21
7.09
7.08
7.07
6.67
6.66
6.30
6.29
4.88
4.87
4.86
4.85
3.72
3.71
3.70
3.14
3.13
3.07
3.00
2.49
2.48
2.47
2.02
3.07
2.11
0.98
2.02
0.96
0.98
5.02
1.04
1.10
6.12
2.00
Chemical Shift (ppm)

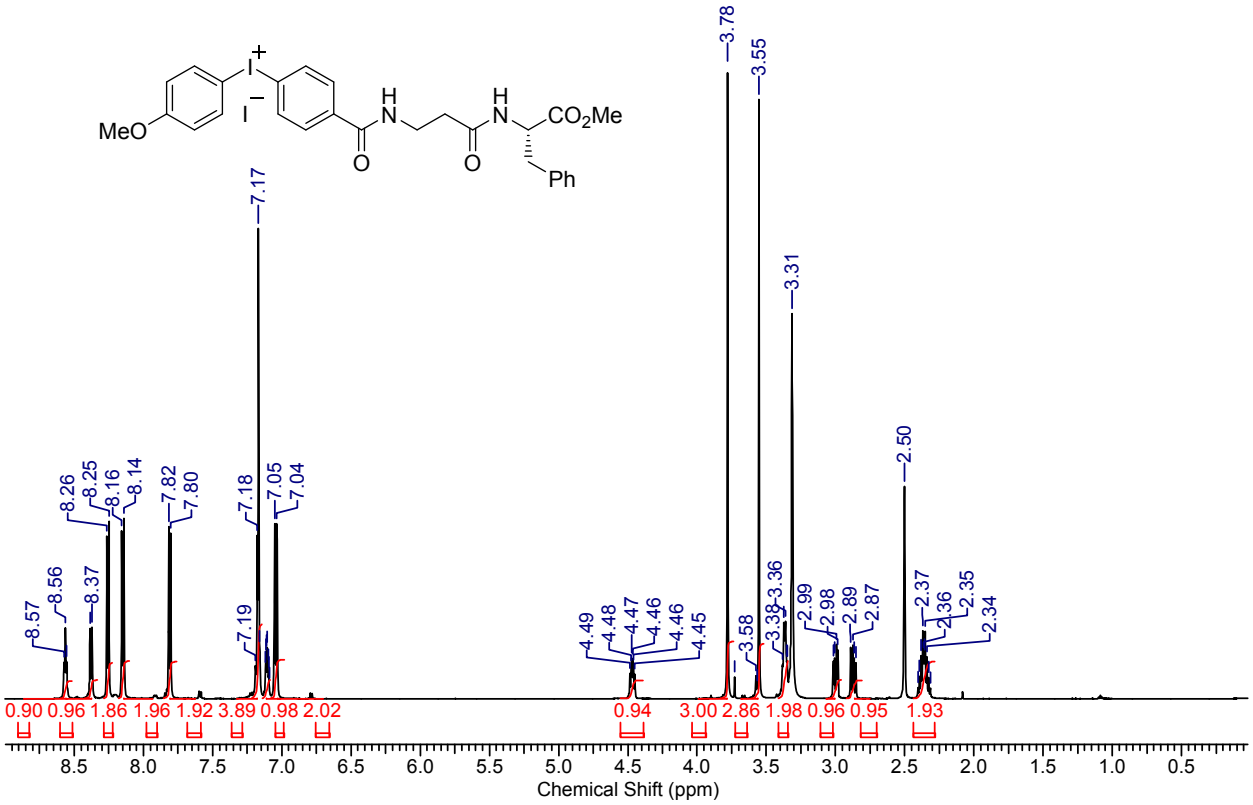

Me2N
H
N
O
H
N
O
CO2Me
Ph
129.21
127.18
127.12
111.11
53.30
52.40
53.24
40.13
152.42
152.37
135.78
77.21
76.84
37.77
35.78
37.71
Chemical Shift (ppm)

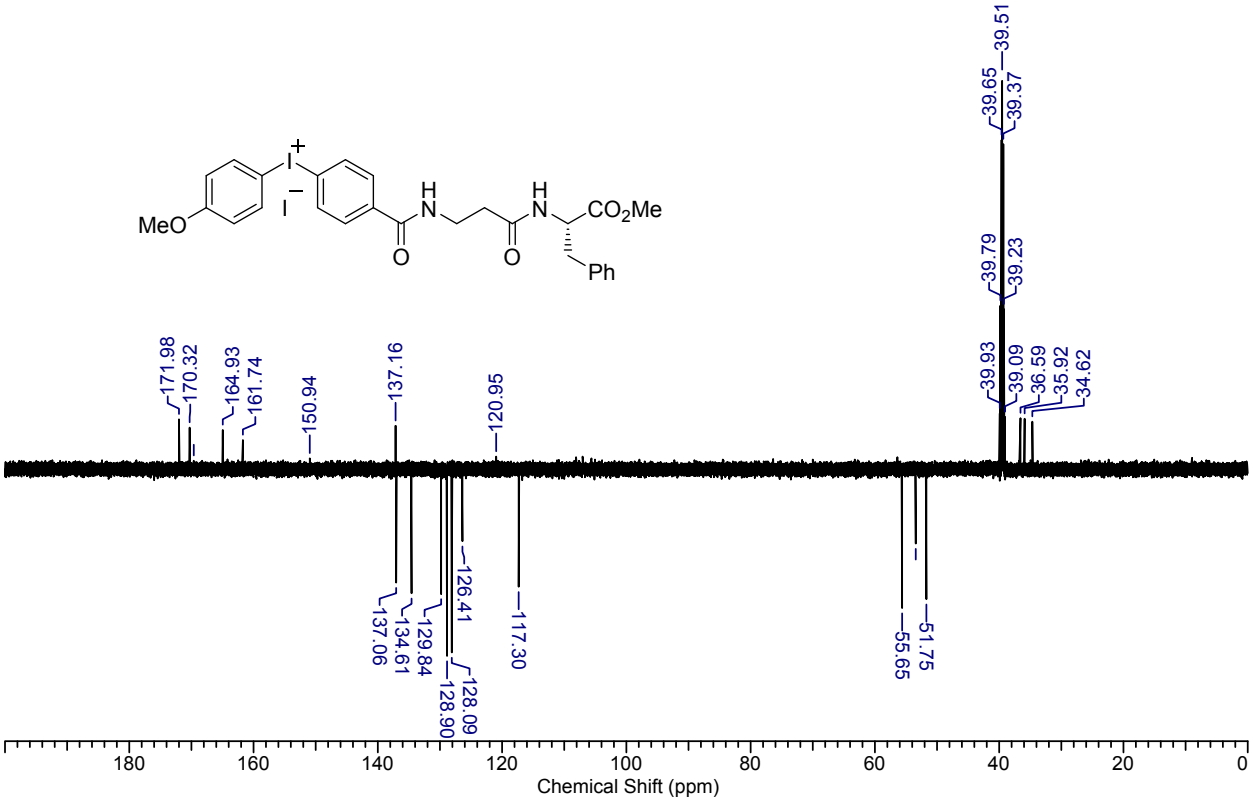

3.64
3.58
3.33
8.69
8.67
8.44
8.41
8.10
8.07
8.04
8.01
7.25
7.22
7.21
7.20
7.18
7.17
7.16
7.15
4.51
4.50
4.49
4.47
4.46
4.44
3.39
3.00
2.98
2.92
2.89
2.51
2.50
2.49
2.43
2.41
2.39
1.01 1.05 3.98
4.98
0.99
8.63 3.00 2.01 2.04
1.98
Chemical Shift (ppm)
128.94
128.71
128.15
120.65
126.47
56.41
53.50
51.81
172.01
170.37
164.41
148.94
137.17
135.61
40.35
40.07
39.79
39.51
39.23
38.95
36.62
35.94
34.68
Chemical Shift (ppm)

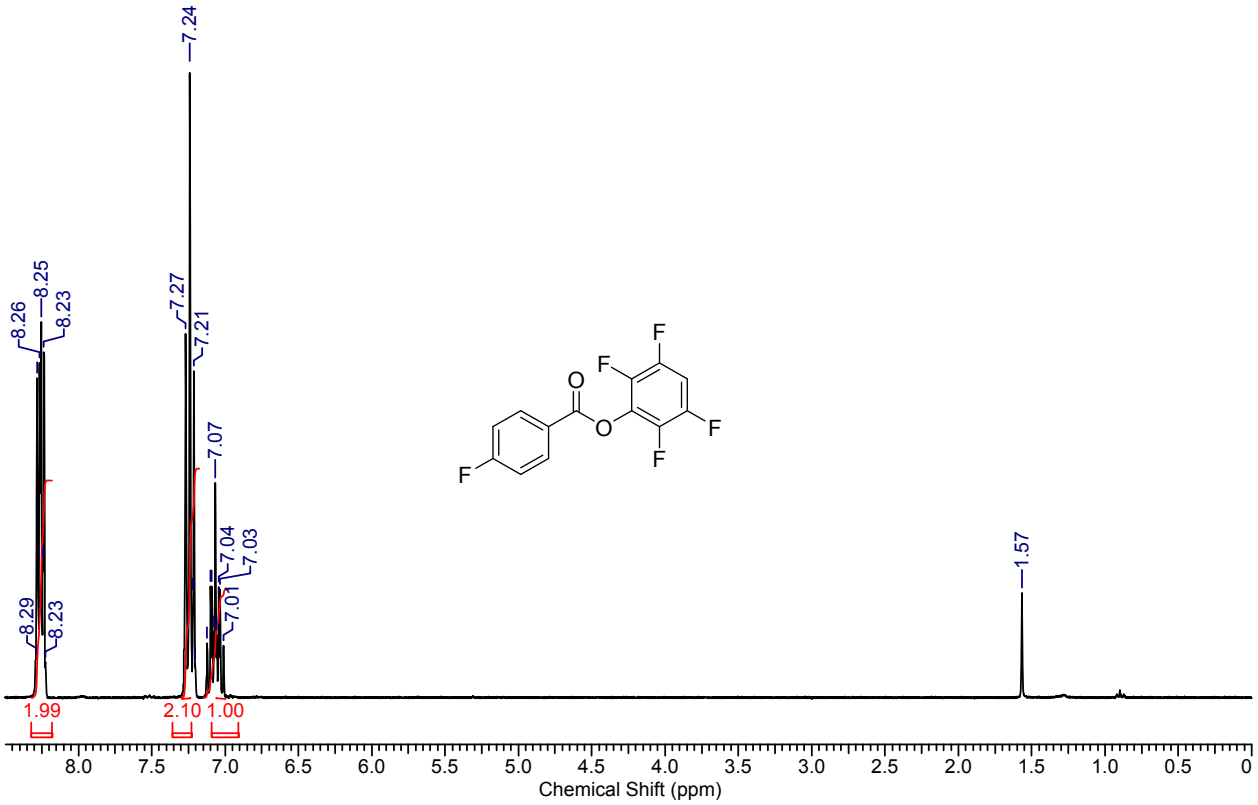

3.63
3.58
3.32
2.50
7.20
8.05
8.04
8.69
8.67
8.44
8.41
8.09
8.00
7.25
7.22
7.16
4.52
4.49
4.47
4.45
3.42
3.01
2.99
2.92
2.89
2.43
2.41
2.39
2.37
0.91 0.93 3.84 4.84 1.00 11.49 2.09 0.96 0.92 1.79
TfO⁻
CO2Me
Ph
Chemical Shift (ppm)

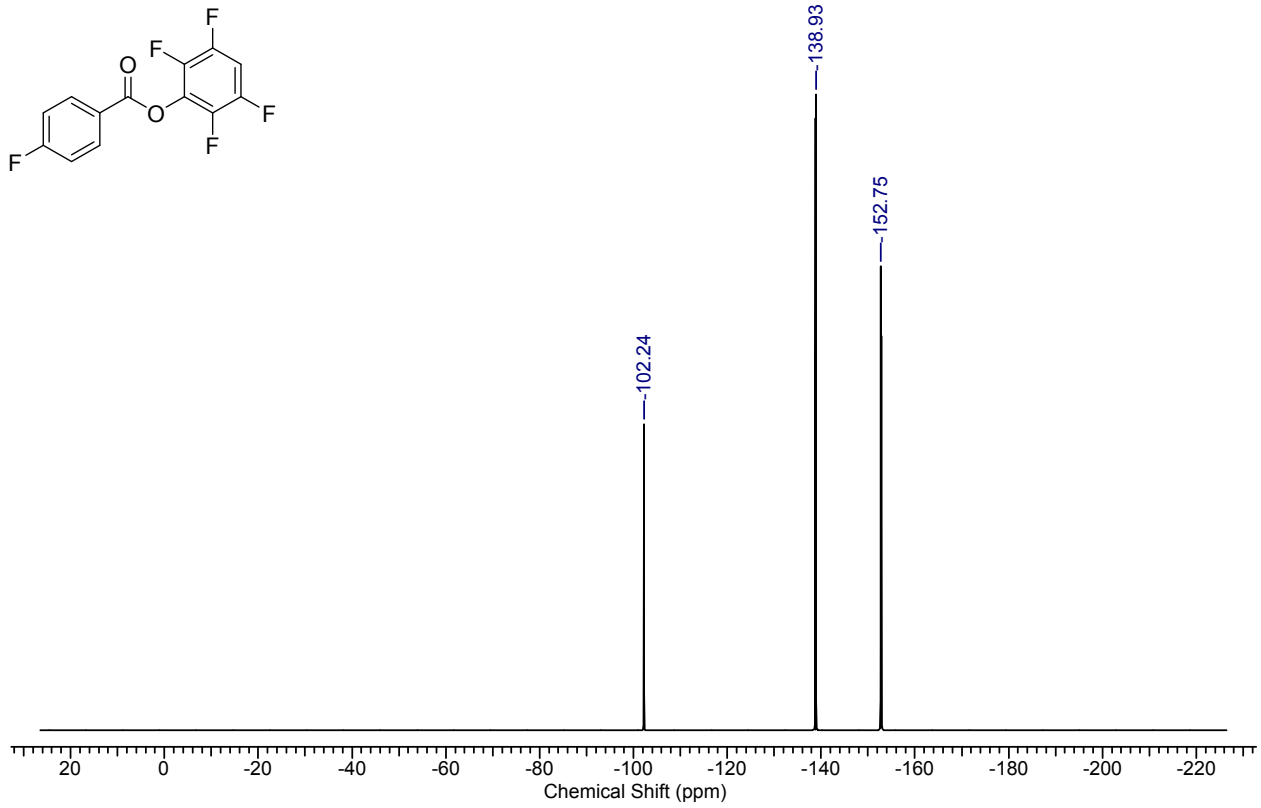

39.79
39.51
39.23
40.07
38.95
40.35
38.68
36.65
35.97
34.68
172.04
170.38
164.43
148.94
137.19
135.67
126.48
128.96
128.16
120.61
53.51
51.80
56.40
TfO⁻
CO2Me
Ph
Chemical Shift (ppm)

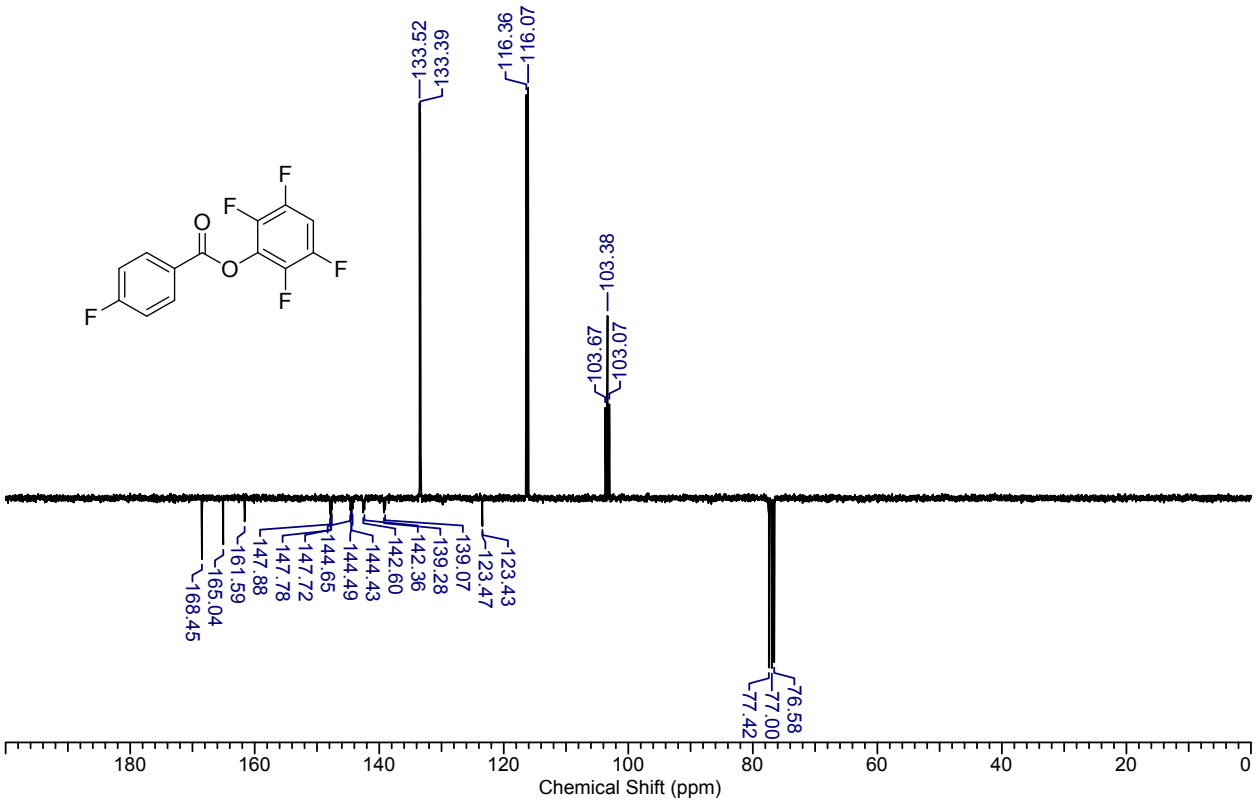

77.74
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
-220
Chemical Shift (ppm)

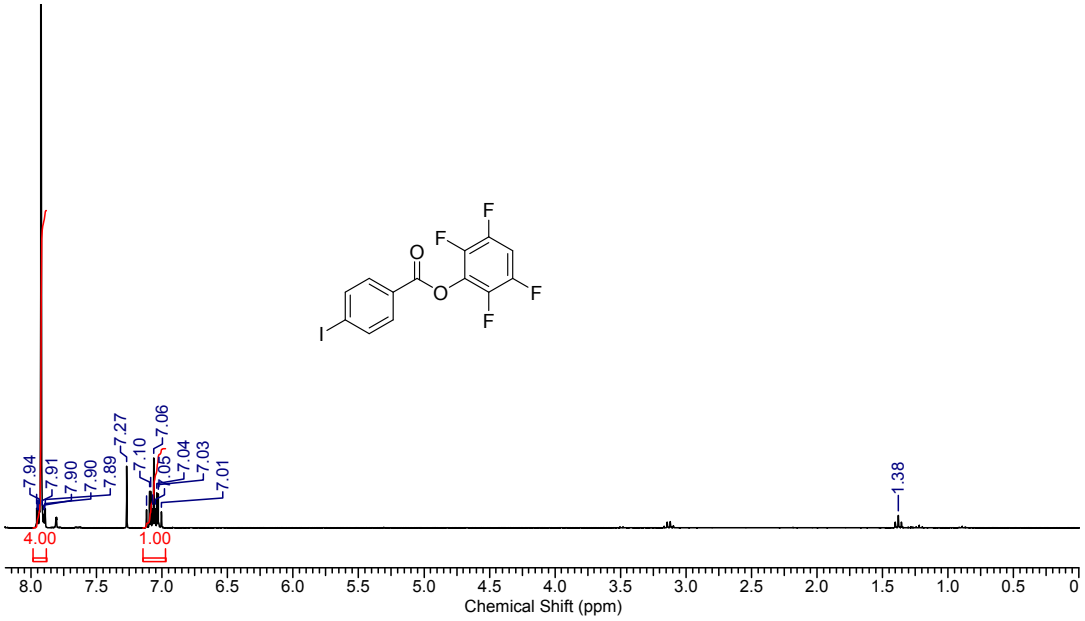

MeO
I
I
H
N
O
H
N
O
CO2Me
Ph
3.78
3.55
7.17
3.31
2.50
8.26
8.25
8.16
8.14
7.82
7.80
7.18
7.05
7.04
8.57
8.56
8.37
7.19
4.49
4.48
4.47
4.46
4.46
4.45
3.58
3.39
3.36
2.99
2.98
2.89
2.87
2.37
2.36
2.35
2.34
0.90 0.96 1.86 1.96 1.92 3.89 0.98 2.02
0.94 3.00 2.86 1.98 0.96 0.95 1.93
Chemical Shift (ppm)

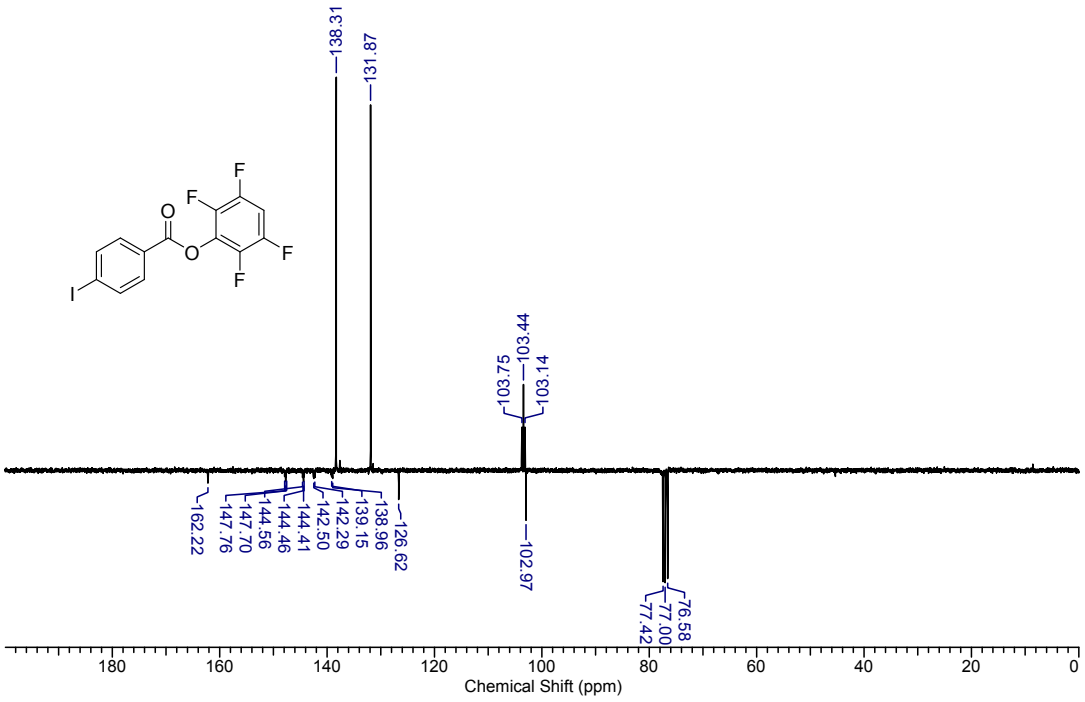

MeO
I
I
H
N
O
H
N
O
CO2Me
Ph
39.51
39.65
39.37
39.79
39.23
171.98
170.32
164.93
161.74
150.94
137.16
120.95
39.93
39.09
36.59
35.92
34.62
137.06
134.61
129.84
128.90
128.09
126.41
117.30
55.66
51.75
Chemical Shift (ppm)

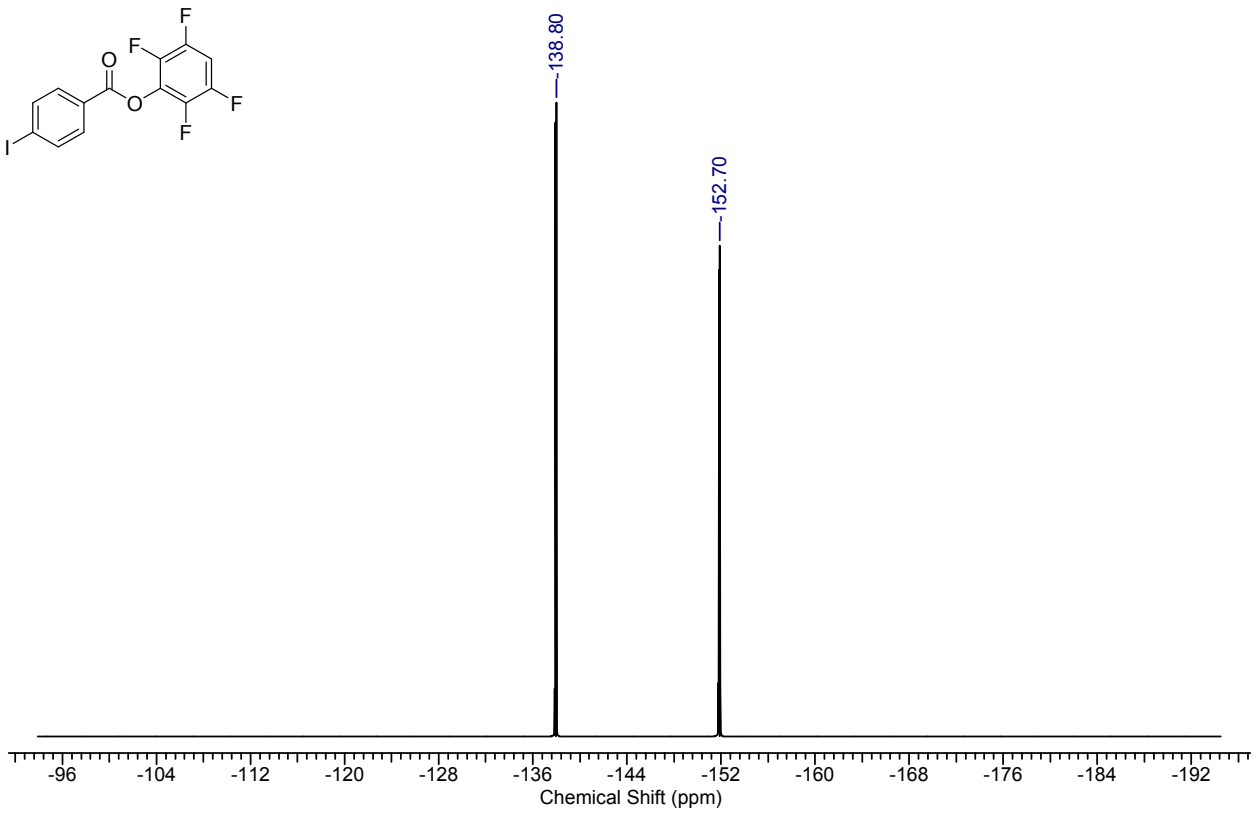

7.24
8.26
8.25
8.23
7.27
7.21
7.07
7.04
7.03
7.01
8.29
8.23
1.57
1.99
2.10
1.00
F
F
O
O
F
F
F
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Chemical Shift (ppm)

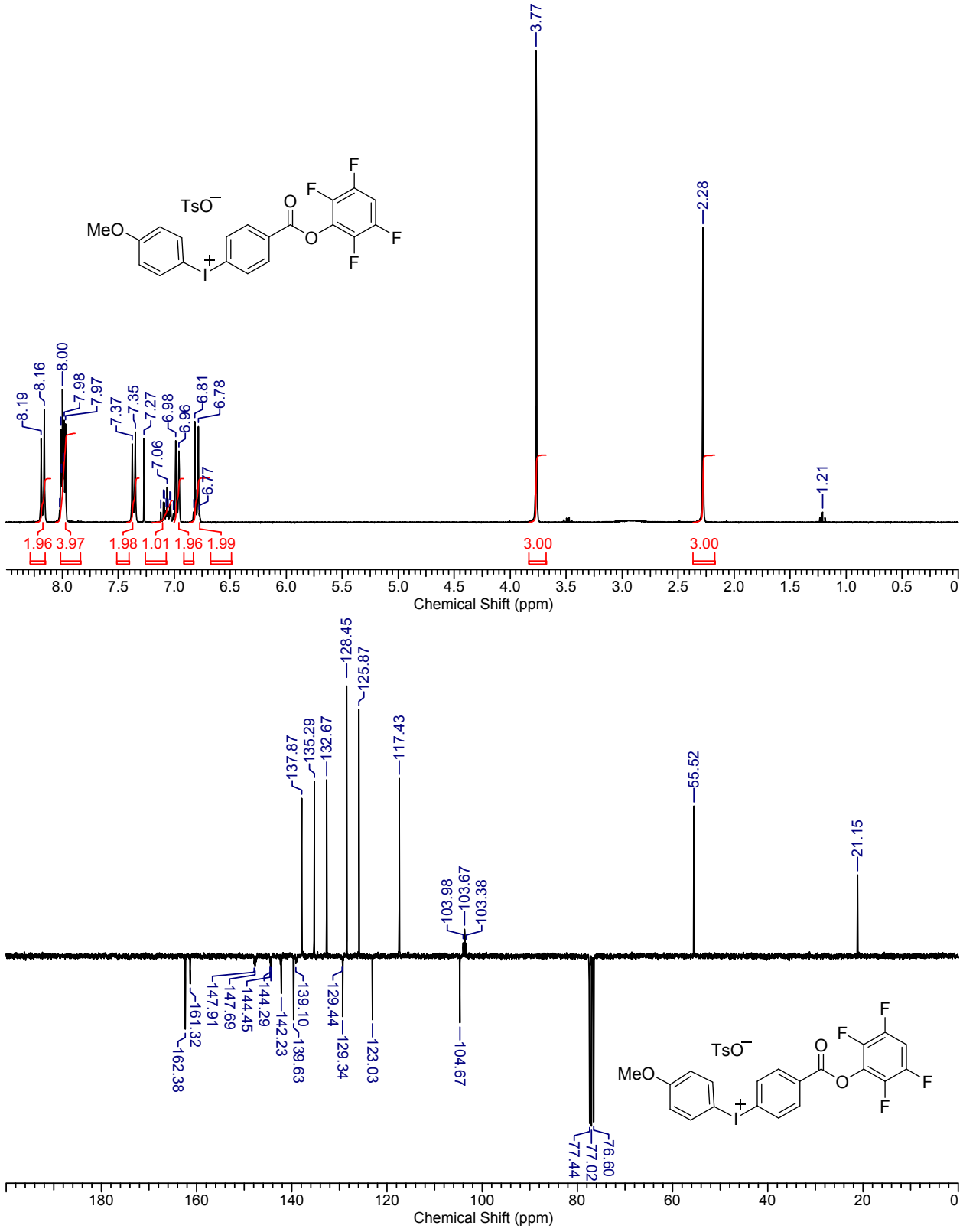

F
F
O
O
F
F
F
138.93
152.75
102.24
20
0
-20
-40
-60
-80
-100
-120
-140
-160
-180
-200
-220
Chemical Shift (ppm)

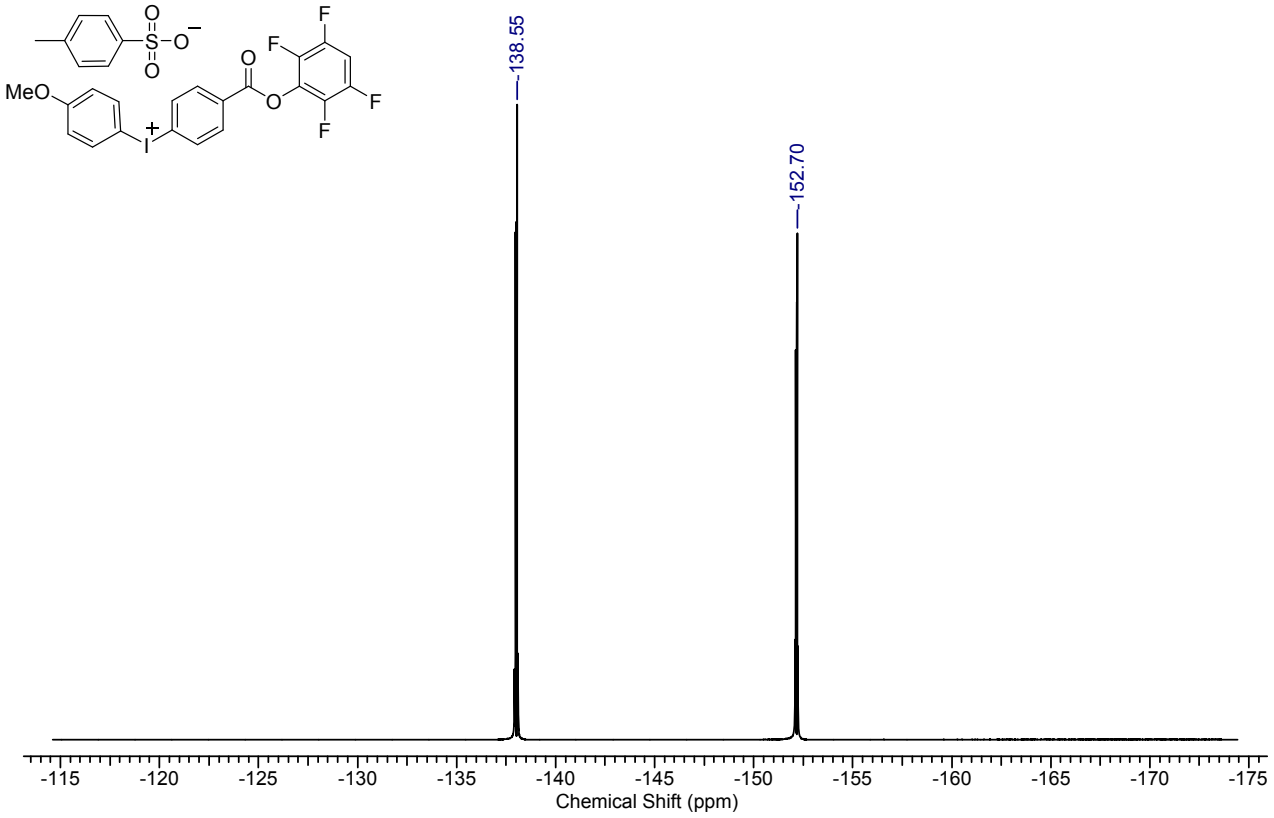

F
F
O
O
F
F
F
133.52
133.39
116.36
116.07
103.38
103.97
103.07
168.45
165.04
161.59
147.88
147.78
147.72
144.65
144.49
144.43
142.60
142.36
139.28
139.07
123.47
123.43
77.42
77.00
76.58
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

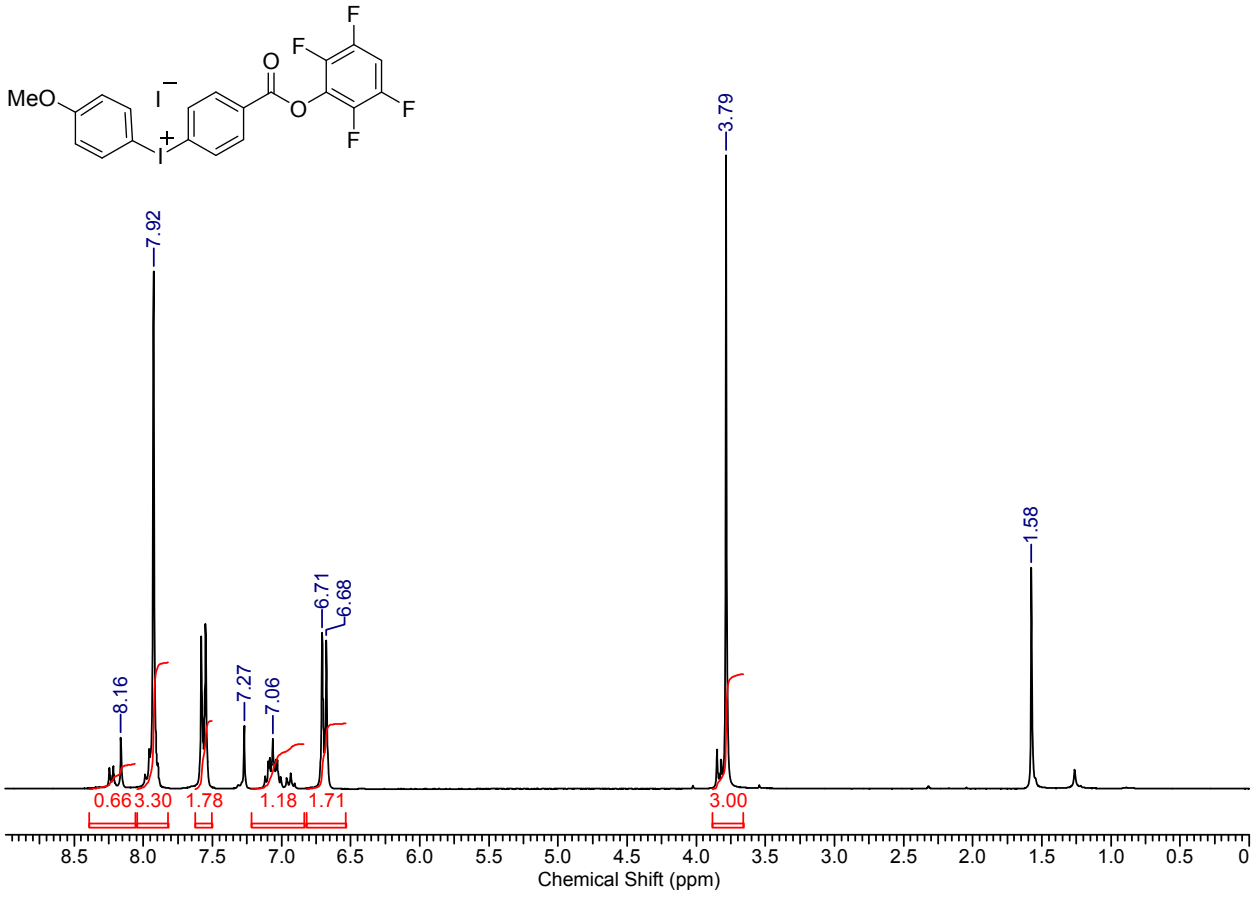

F
F
O
O
F
F
I
7.94
7.91
7.90
7.90
7.89
7.27
7.10
7.06
7.05
7.04
7.03
7.01
1.38
4.00
1.00
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Chemical Shift (ppm)

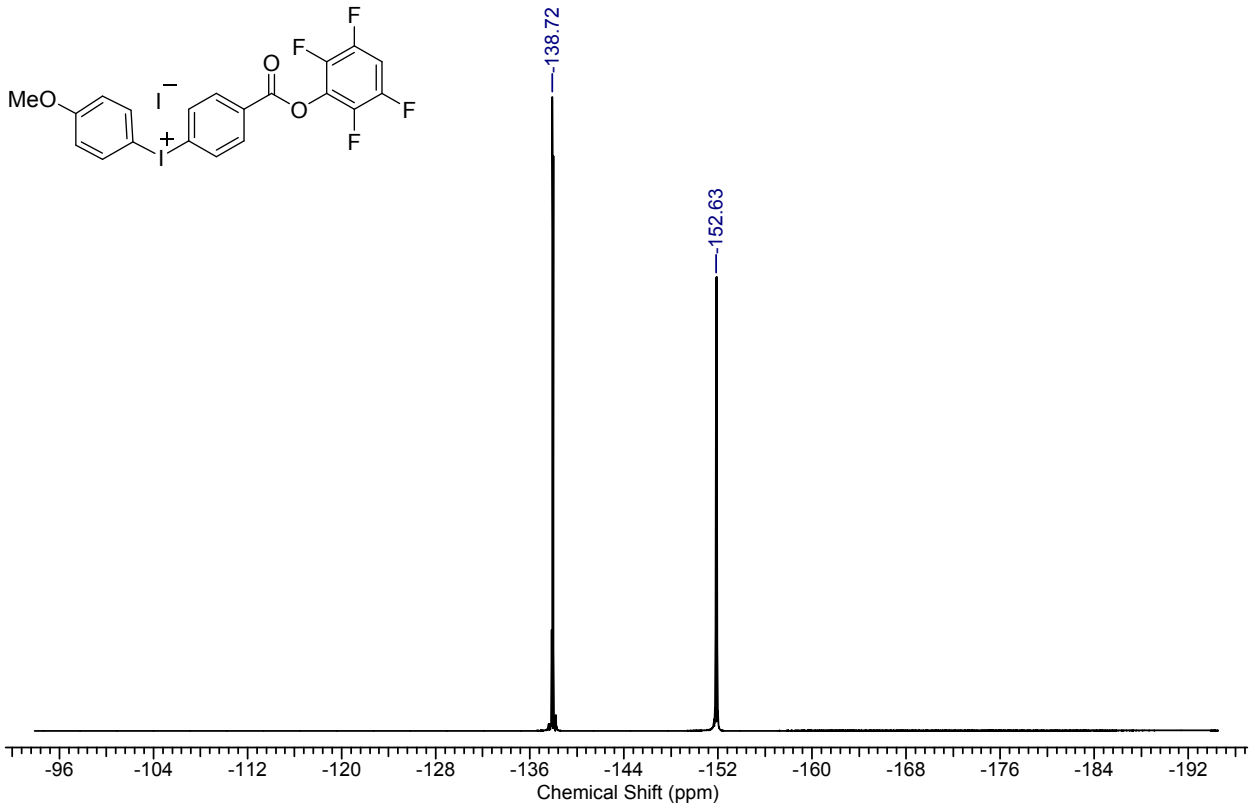

F
F
O
O
F
F
I
138.31
131.87
103.75
103.44
103.14
162.22
147.76
147.70
144.56
144.46
144.41
142.50
142.29
139.15
138.96
126.62
102.97
77.42
77.00
76.58
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

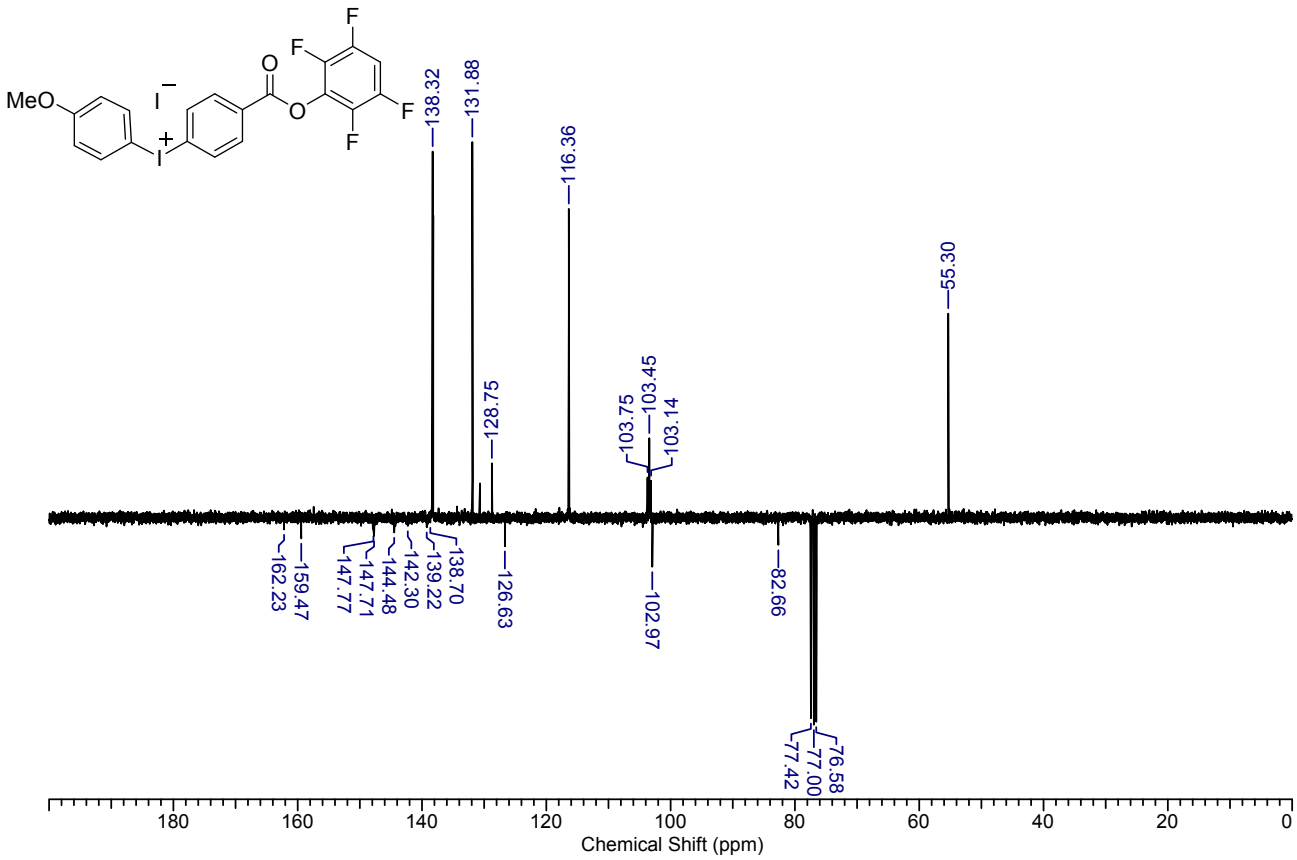

F
F
O
O
F
F
I
138.80
152.70
-96
-104
-112
-120
-128
-136
-144
-152
-160
-168
-176
-184
-192
Chemical Shift (ppm)

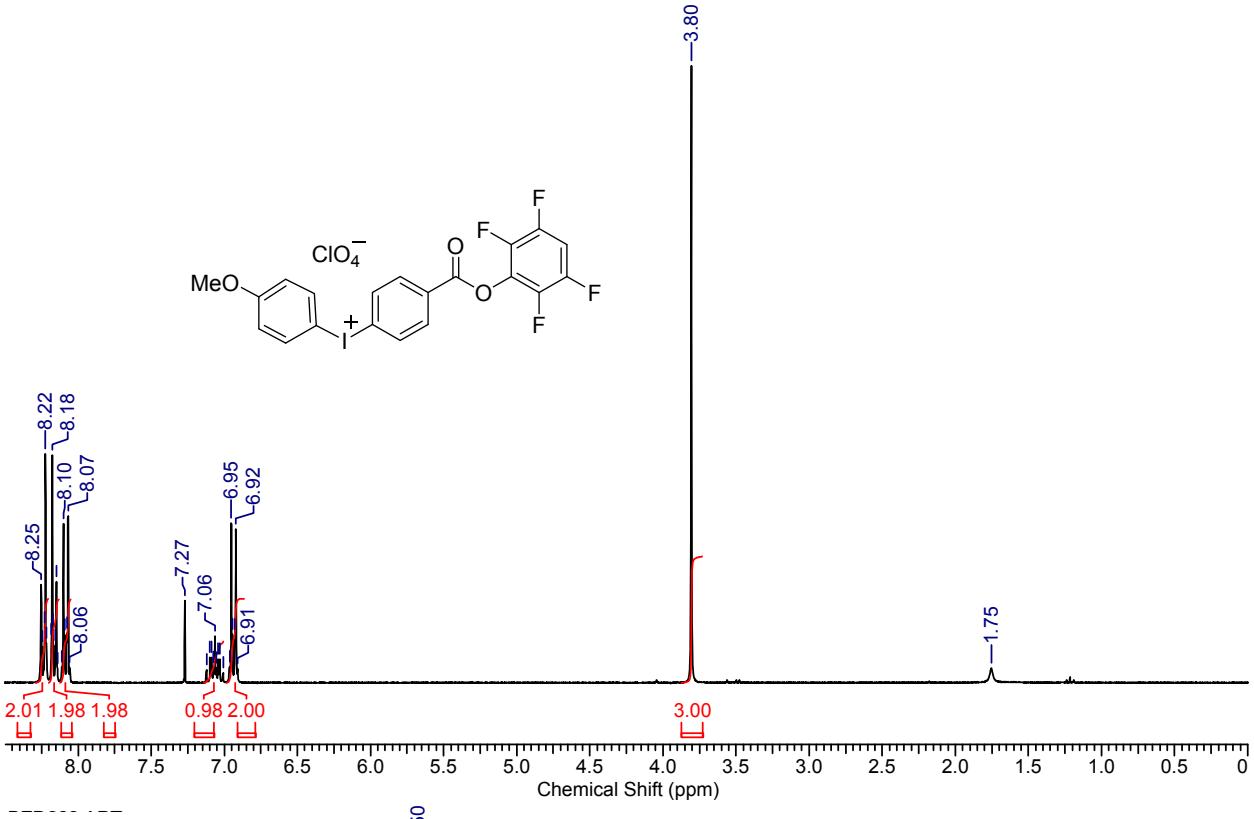

TsO⁻
MeO
I
O
O
F
F
F
F
8.19
8.16
8.00
7.98
7.97
7.37
7.35
7.27
7.06
6.98
6.96
6.81
6.78
6.77
3.77
2.28
1.21
1.96
3.97
1.98
1.01
1.96
1.99
3.00
3.00
Chemical Shift (ppm)
128.45
125.87
137.87
135.29
132.97
117.43
55.52
21.15
103.98
103.67
103.38
162.38
161.32
147.91
147.69
144.45
144.29
142.23
139.10
139.63
129.44
129.34
123.03
104.67
77.44
77.02
76.60
Chemical Shift (ppm)

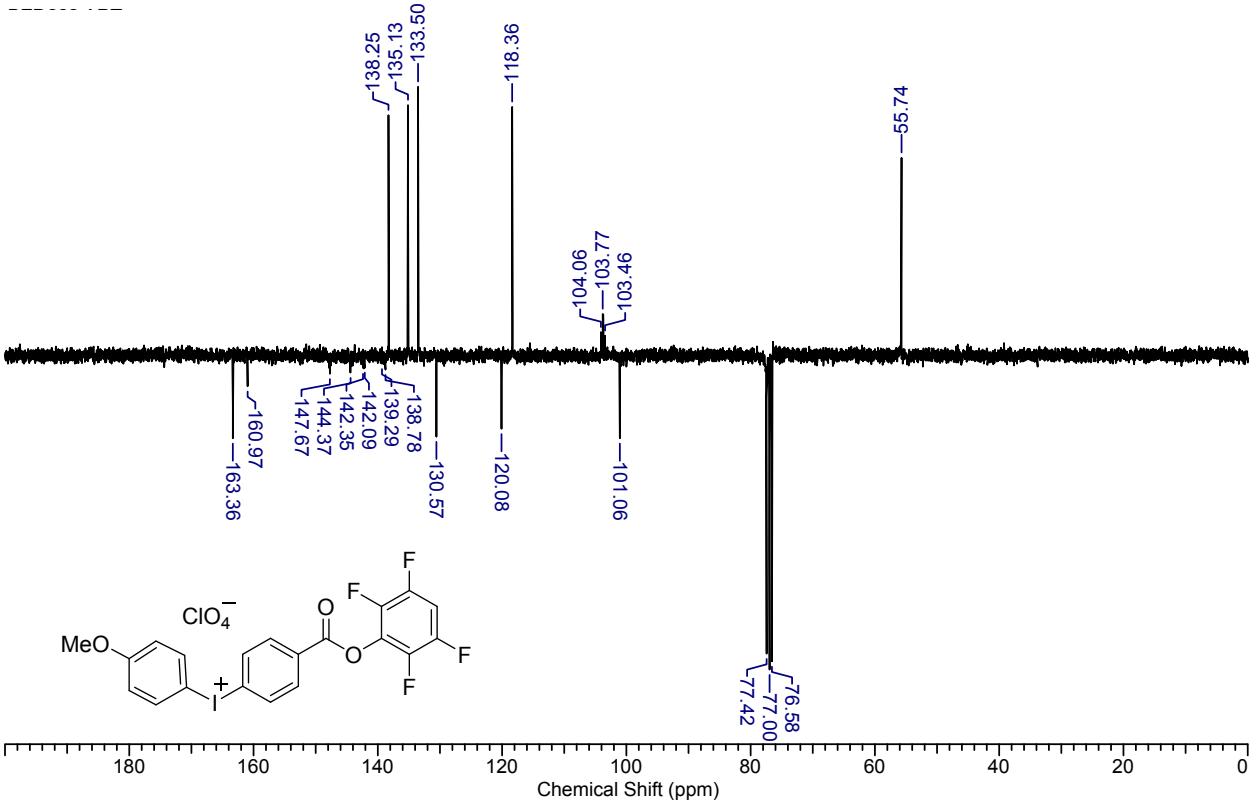

MeO
O
S
O
O
O
I
O
O
F
F
F
F
138.55
152.70
-115
-120
-125
-130
-135
-140
-145
-150
-155
-160
-165
-170
-175
Chemical Shift (ppm)

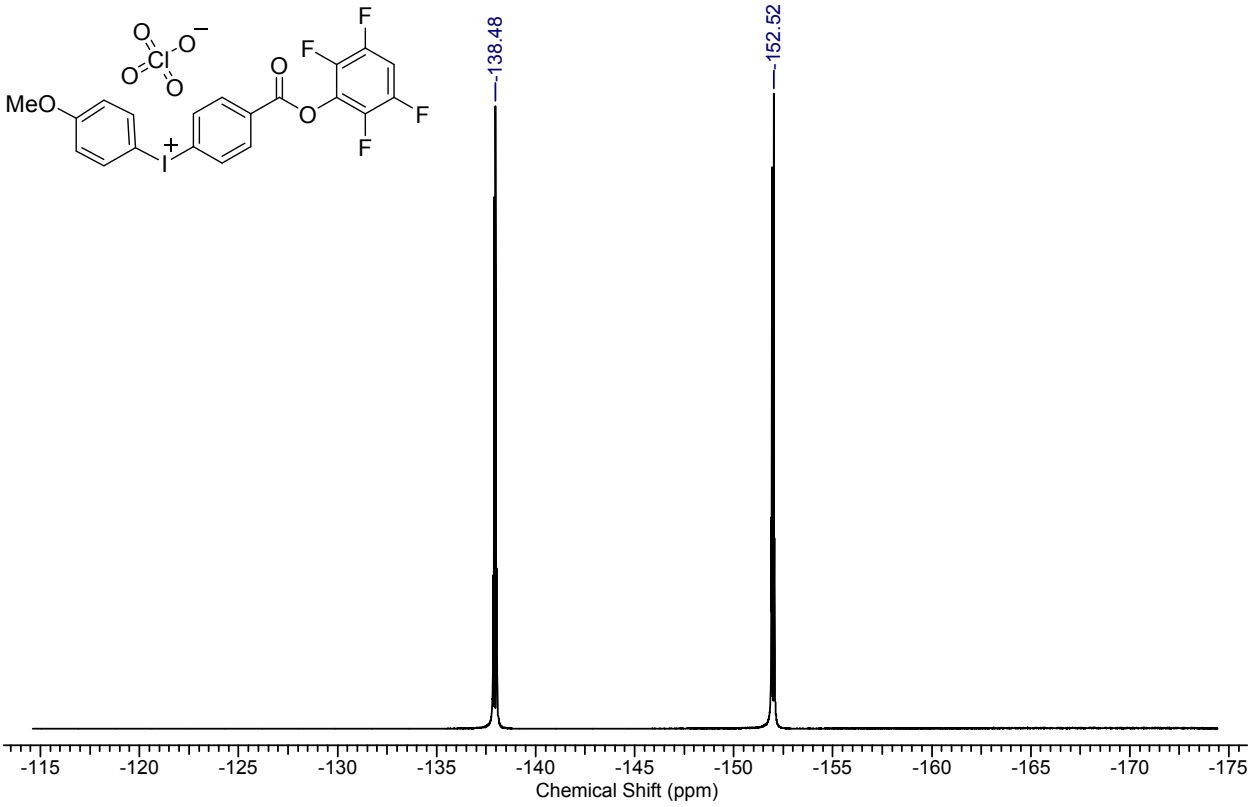

MeO
I
O
O
F
F
F
F
3.79
7.92
1.58
6.71
6.68
8.16
7.27
7.06
0.66
3.30
1.78
1.18
1.71
3.00
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Chemical Shift (ppm)

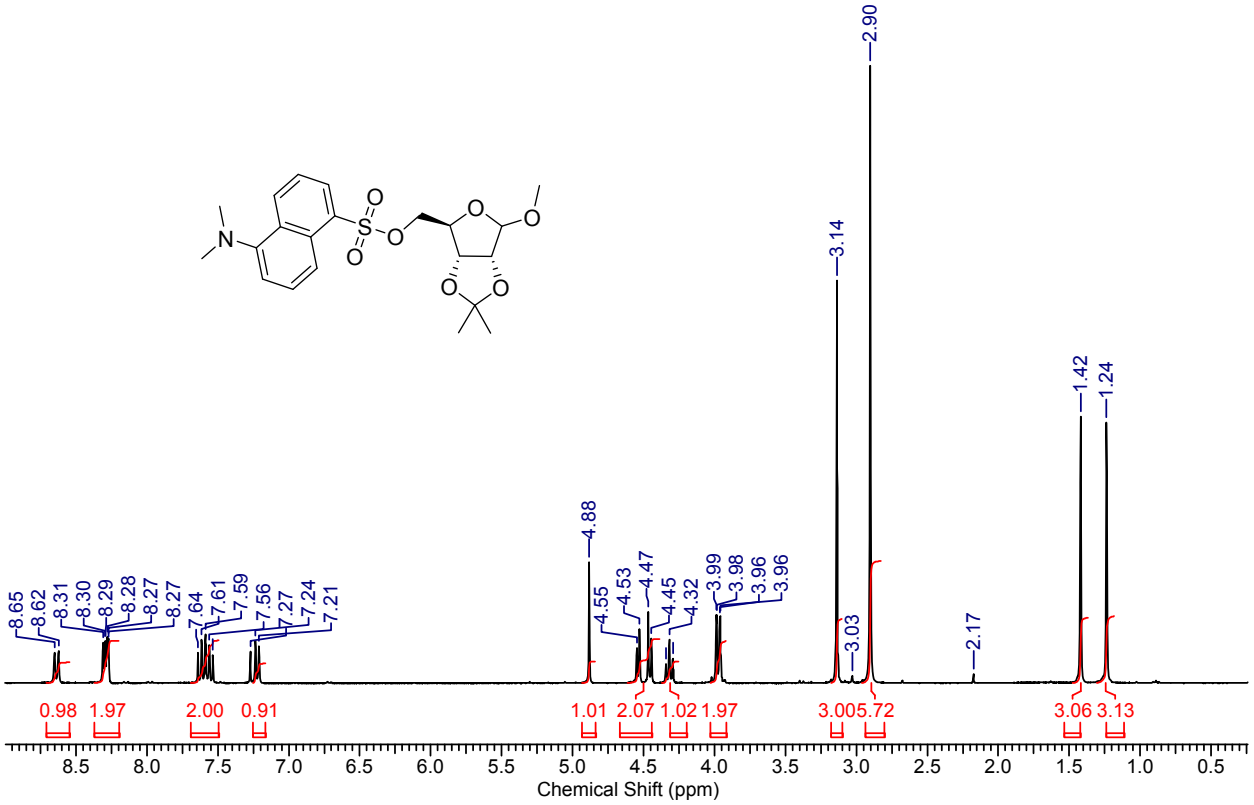

MeO
I
O
O
F
F
F
F
138.72
152.63
-96
-104
-112
-120
-128
-136
-144
-152
-160
-168
-176
-184
-192
Chemical Shift (ppm)

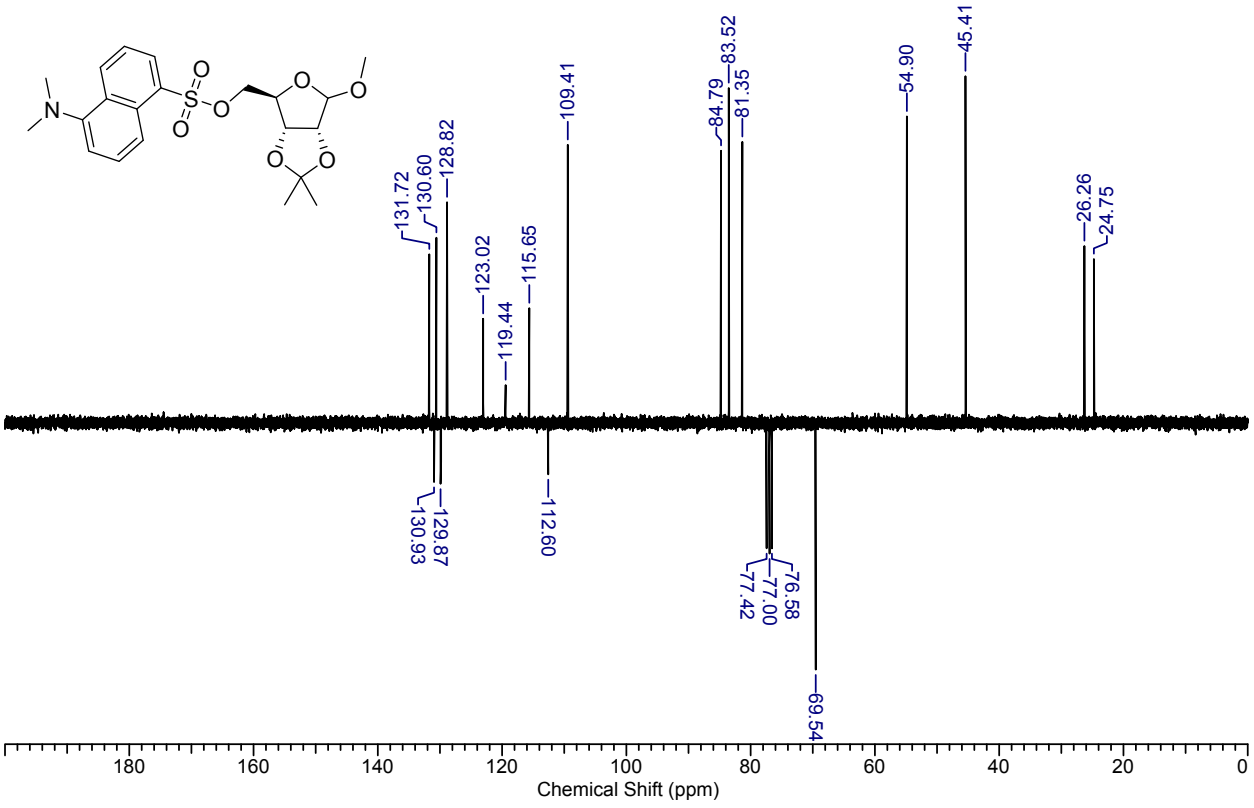

MeO
I
I
O
O
F
F
F
F
138.32
131.88
116.36
128.75
103.75
103.45
103.14
55.30
162.23
159.47
147.77
147.71
144.48
142.30
139.22
138.70
126.63
102.97
82.66
77.42
77.00
76.58
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

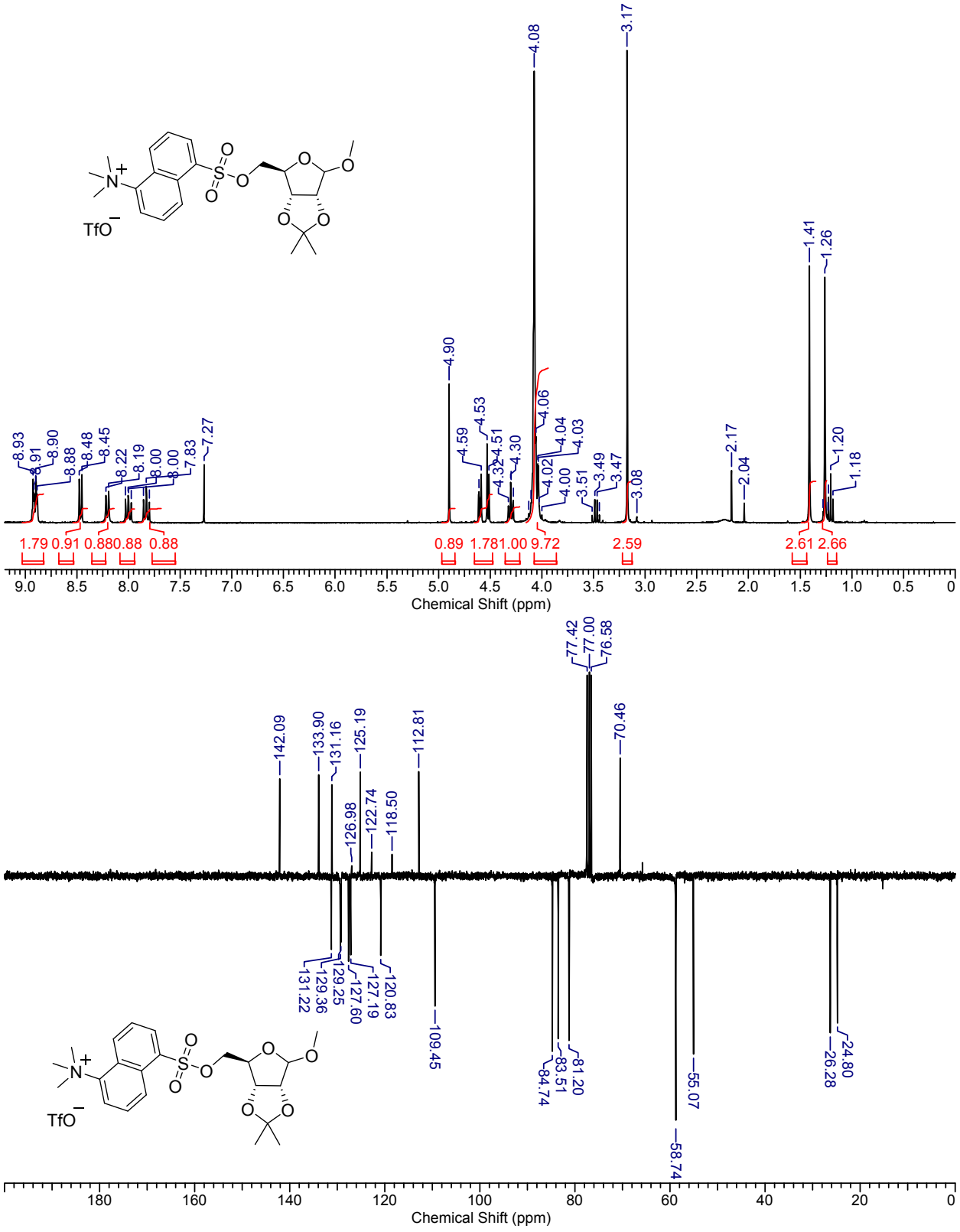

3.80
8.22
8.18
8.10
8.07
8.25
8.06
7.27
7.06
6.95
6.92
6.91
1.75
2.01 1.98 1.98 0.98 2.00 3.00
Chemical Shift (ppm)


138.25
135.13
133.50
118.36
55.74
104.06
103.77
103.46
160.97
163.36
147.67
144.37
142.35
142.09
139.29
138.78
130.57
120.08
101.06
77.42
77.00
76.58
Chemical Shift (ppm)

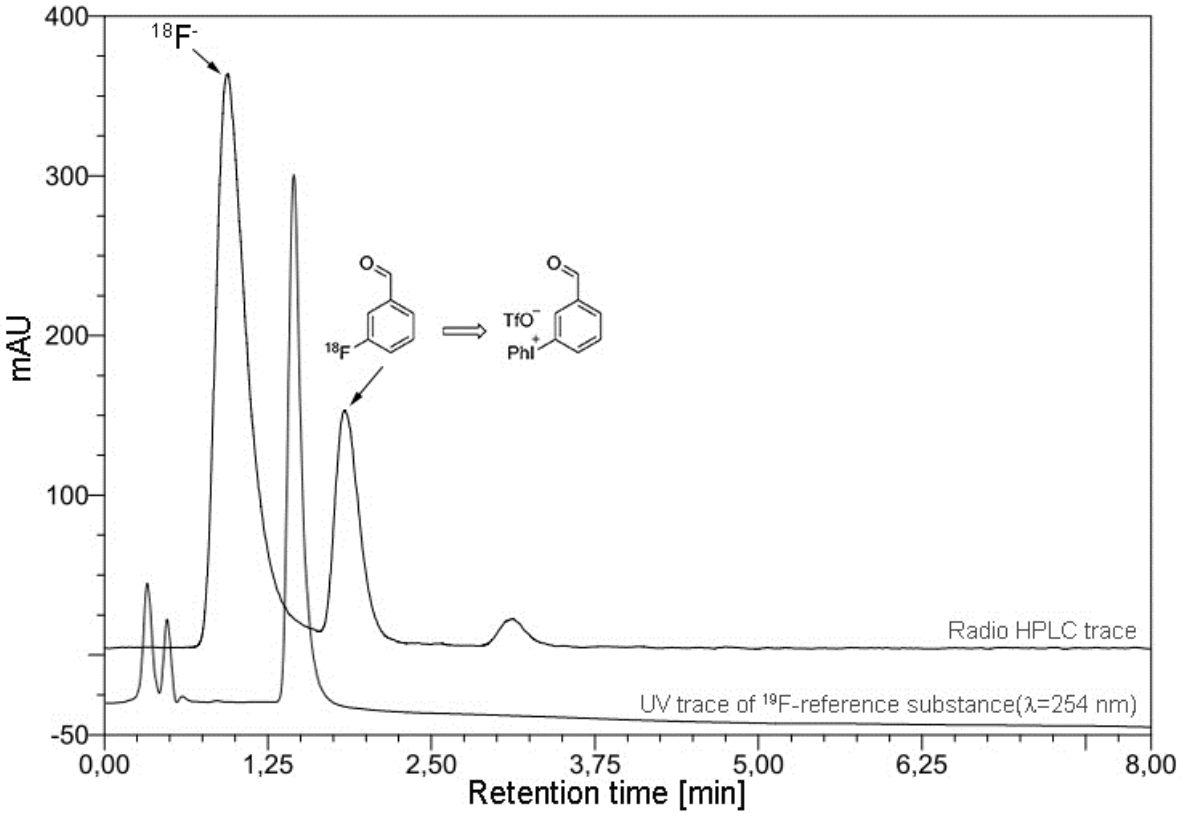

MeO
O
Cl
O
O
O
I
+
O
O
F
F
F
F
-138.48
-152.52
-115
-120
-125
-130
-135
-140
-145
-150
-155
-160
-165
-170
-175
Chemical Shift (ppm)

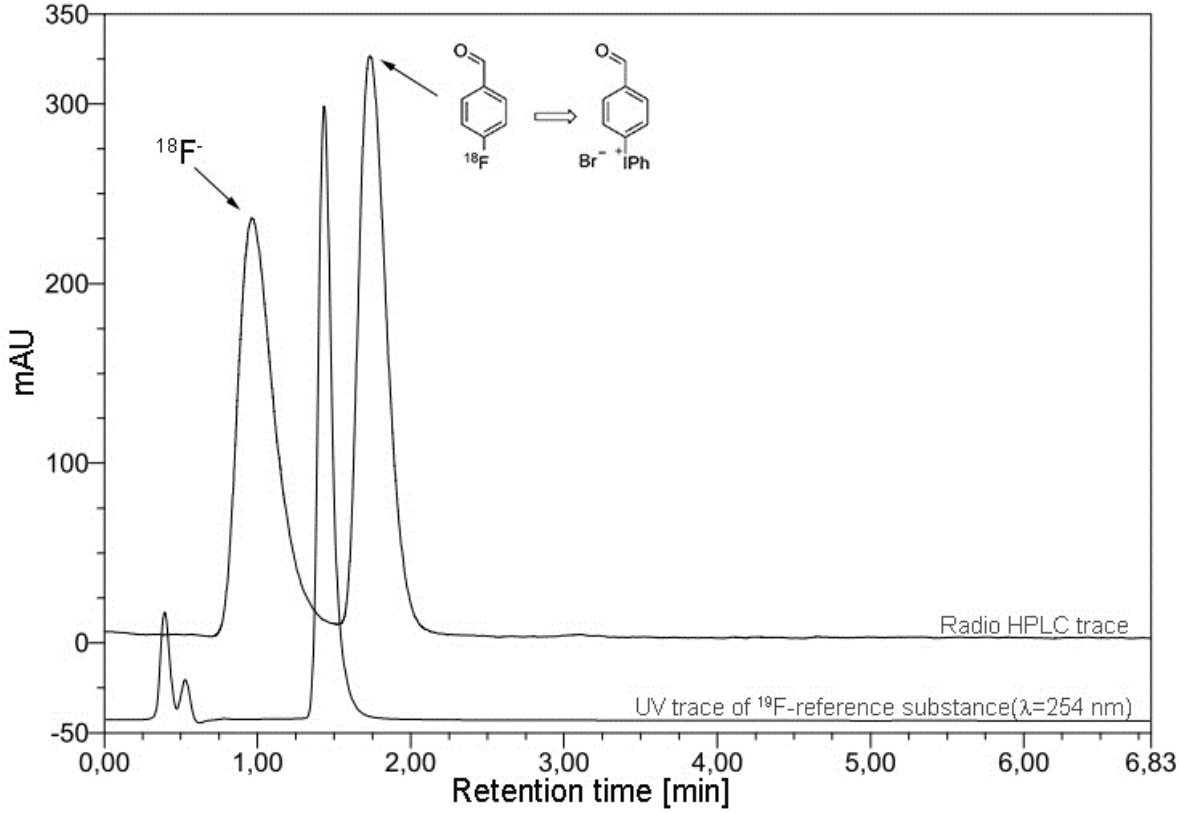

8.65
8.62
8.31
8.30
8.29
8.28
8.27
8.27
7.64
7.61
7.59
7.56
7.27
7.24
7.21
4.88
4.55
4.53
4.47
4.45
4.32
3.99
3.98
3.96
3.96
3.14
3.03
2.90
2.17
1.42
1.24
0.98 1.97 2.00 0.91 1.01 2.07 1.02 1.97 3.00 5.72 3.06 3.13
Chemical Shift (ppm)

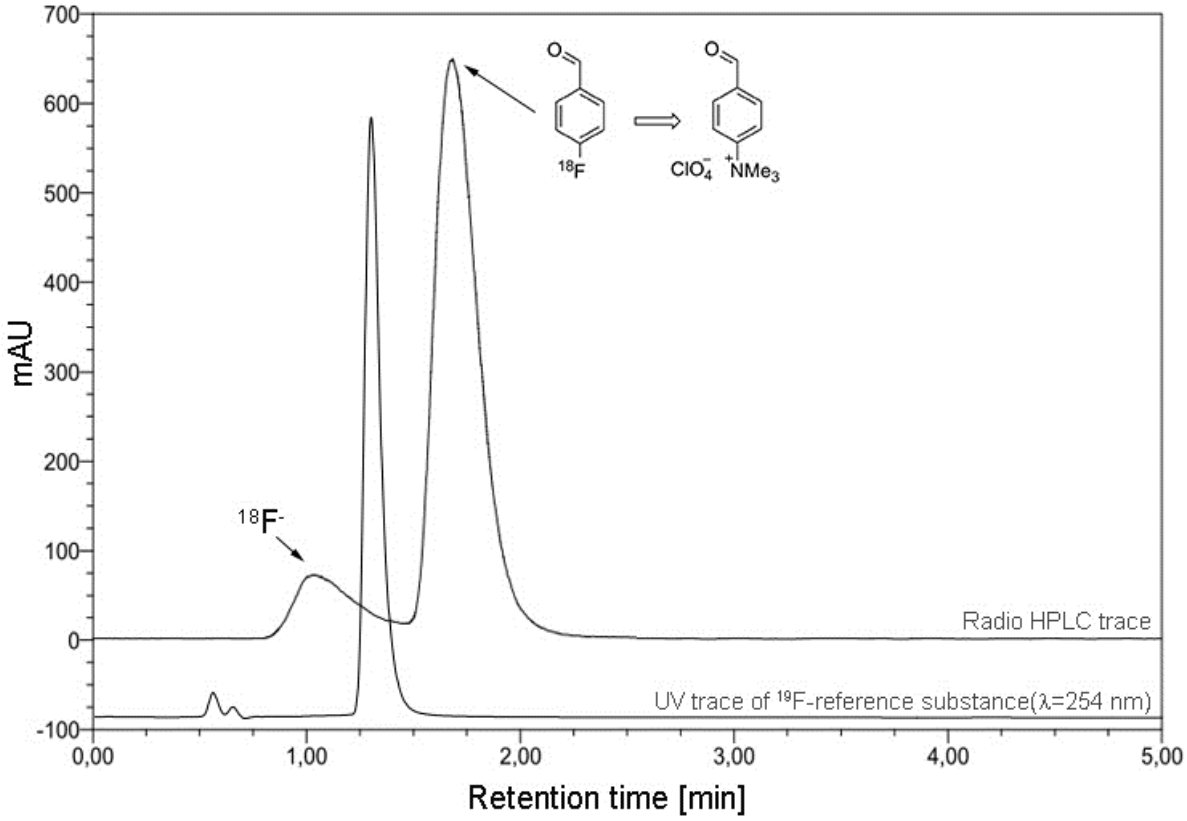

131.72
130.60
128.82
123.02
119.44
115.65
109.41
84.79
83.52
81.35
54.90
45.41
26.26
24.75
130.93
129.87
112.60
77.42
77.00
76.58
69.54
Chemical Shift (ppm)

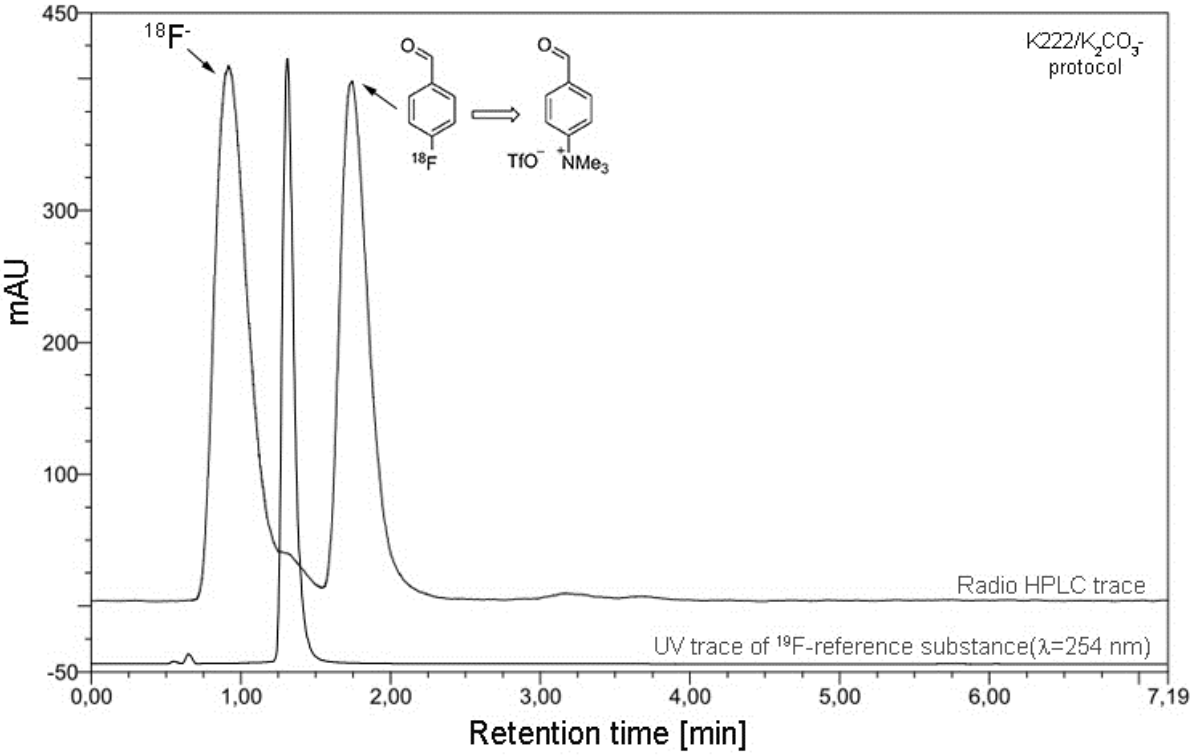

TfO−
8.93
8.91
8.90
8.88
8.48
8.45
8.22
8.19
8.00
8.00
7.83
7.27
4.90
4.59
4.53
4.51
4.32
4.30
4.08
4.06
4.04
4.03
4.02
4.00
3.51
3.49
3.47
3.17
3.08
2.17
2.04
1.41
1.26
1.20
1.18
1.79 0.91 0.88 0.88 0.88
0.89 1.78 1.00 9.72
2.59
2.61 2.66
Chemical Shift (ppm)
142.09
133.90
131.16
125.19
126.98
122.74
118.50
112.81
77.42
77.00
76.58
70.46
131.22
129.36
129.25
127.60
127.19
120.83
109.45
84.74
83.51
81.20
58.74
55.07
26.28
24.80
TfO−
Chemical Shift (ppm)

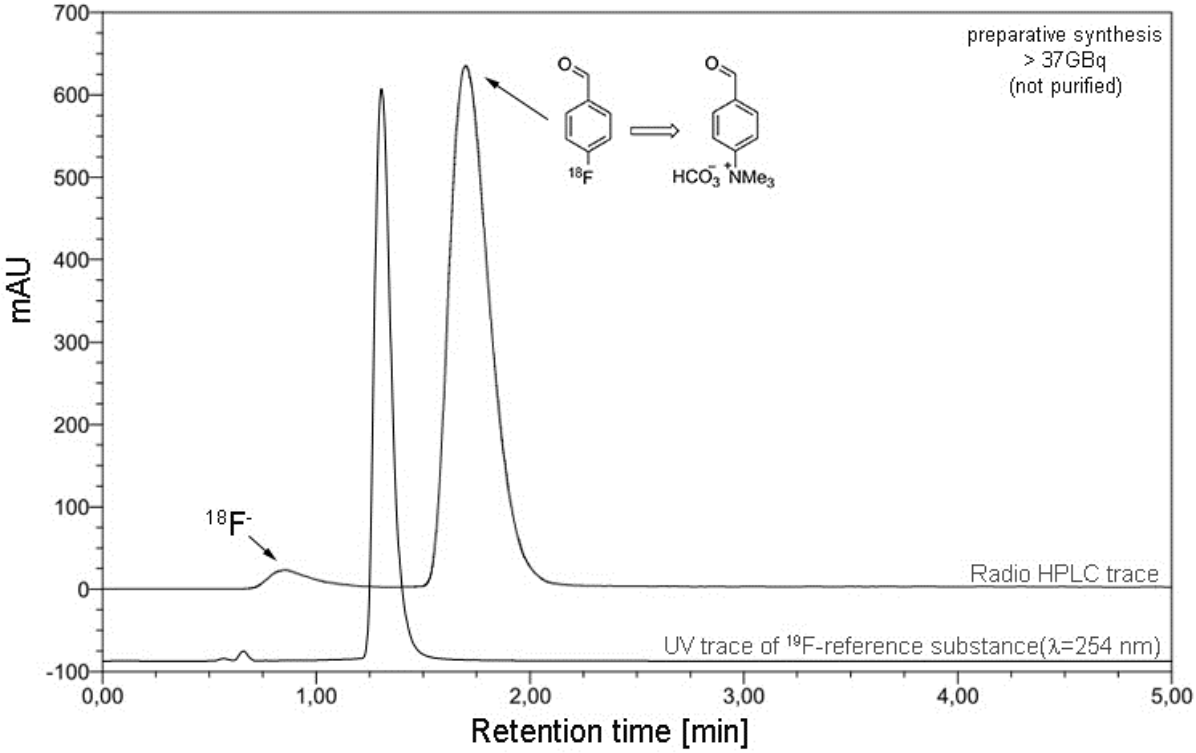
HPLC Traces

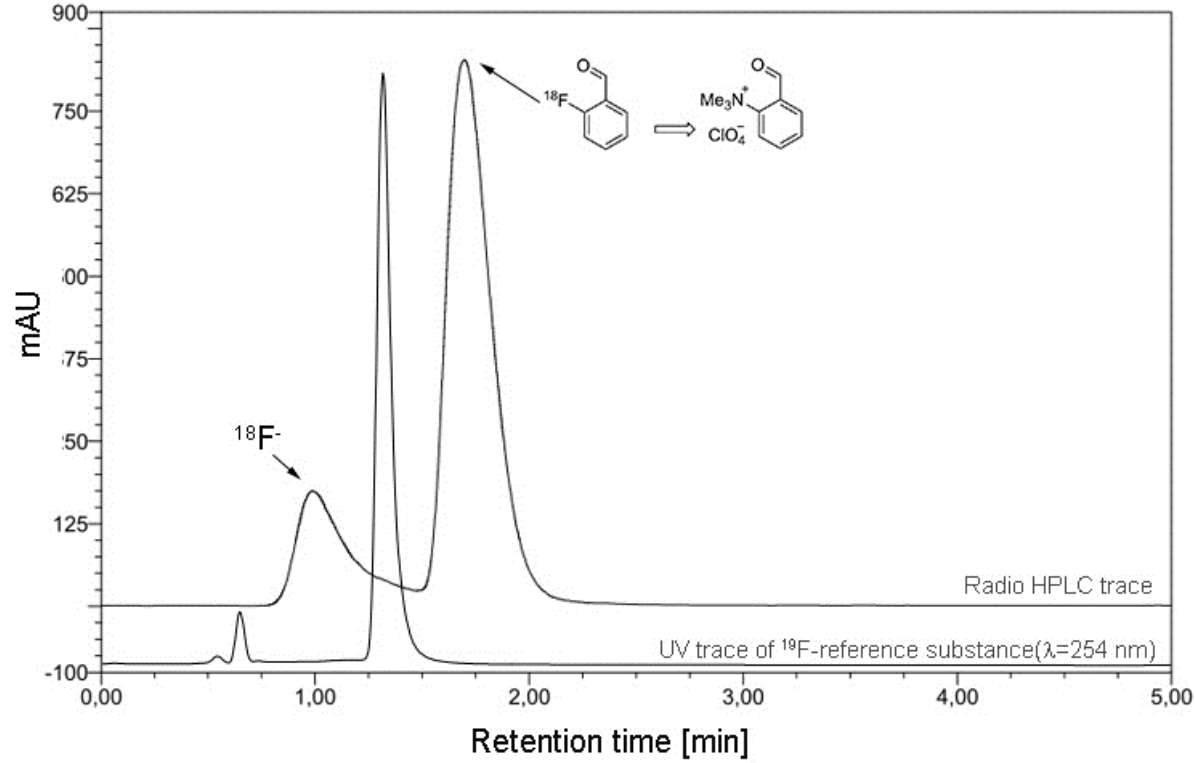

400
300
200
100
-50
mAU
$^{18}F^-$
TfO⁻
PhI⁺
^{18}F
Radio HPLC trace
UV trace of ^{19}F-reference substance(λ=254 nm)
0,00
1,25
2,50
3,75
5,00
6,25
8,00
Retention time [min]

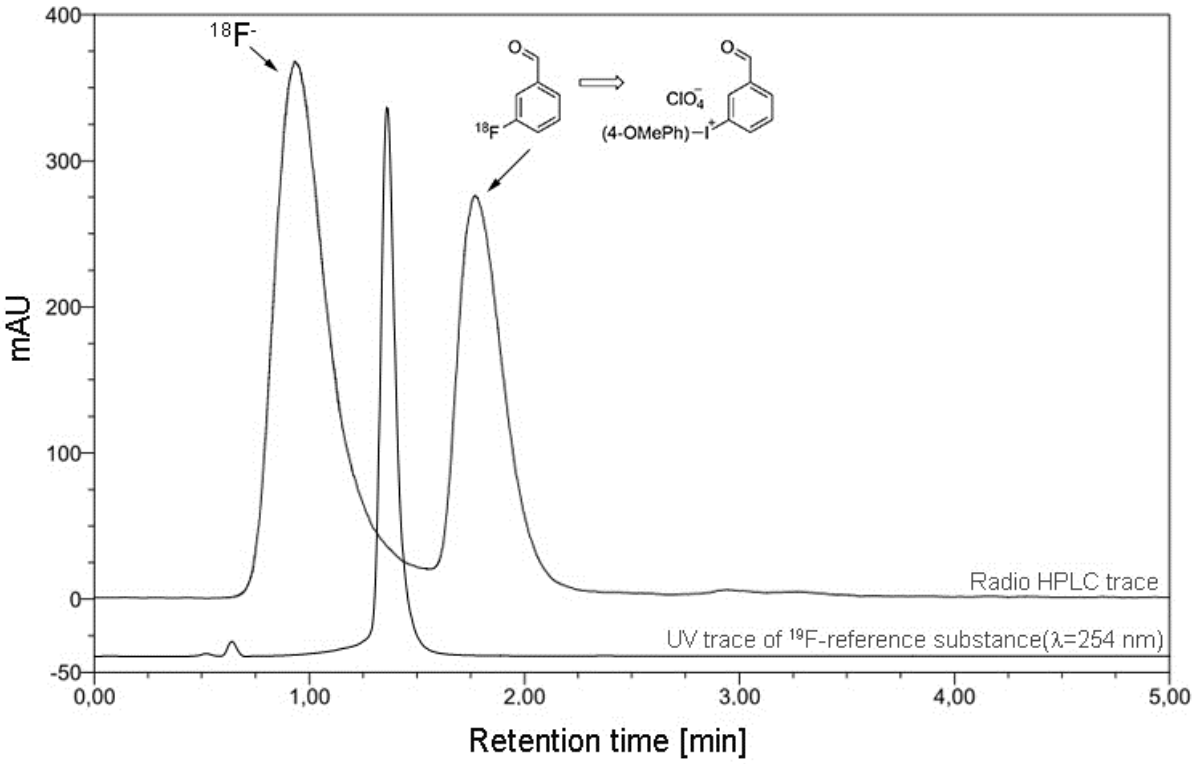

350
300
200
100
0
-50
mAU
$^{18}F^-$
^{18}F
Br⁻
IPh
Radio HPLC trace
UV trace of ^{19}F-reference substance(λ=254 nm)
0,00
1,00
2,00
3,00
4,00
5,00
6,00
6,83
Retention time [min]

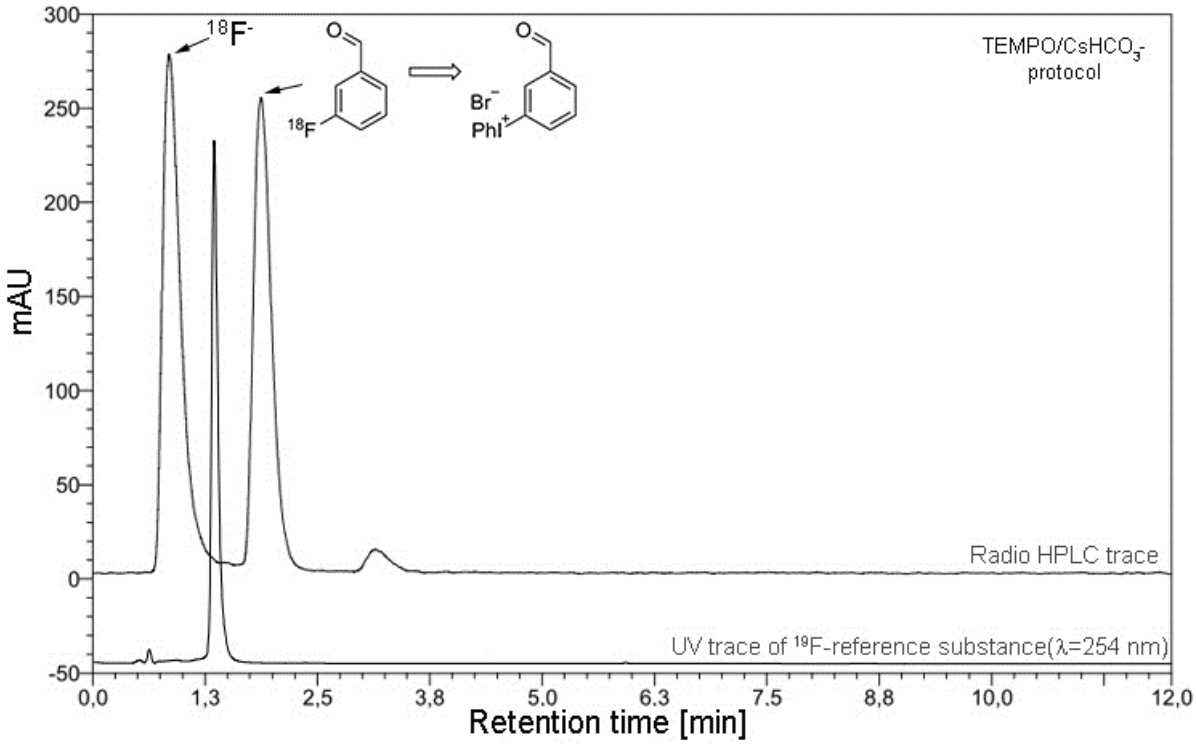

mAU
Retention time [min]
$^{18}F^-$
Radio HPLC trace
UV trace of ^{19}F-reference substance(λ=254 nm)
^{18}F
ClO_4^-
$^+NMe_3$

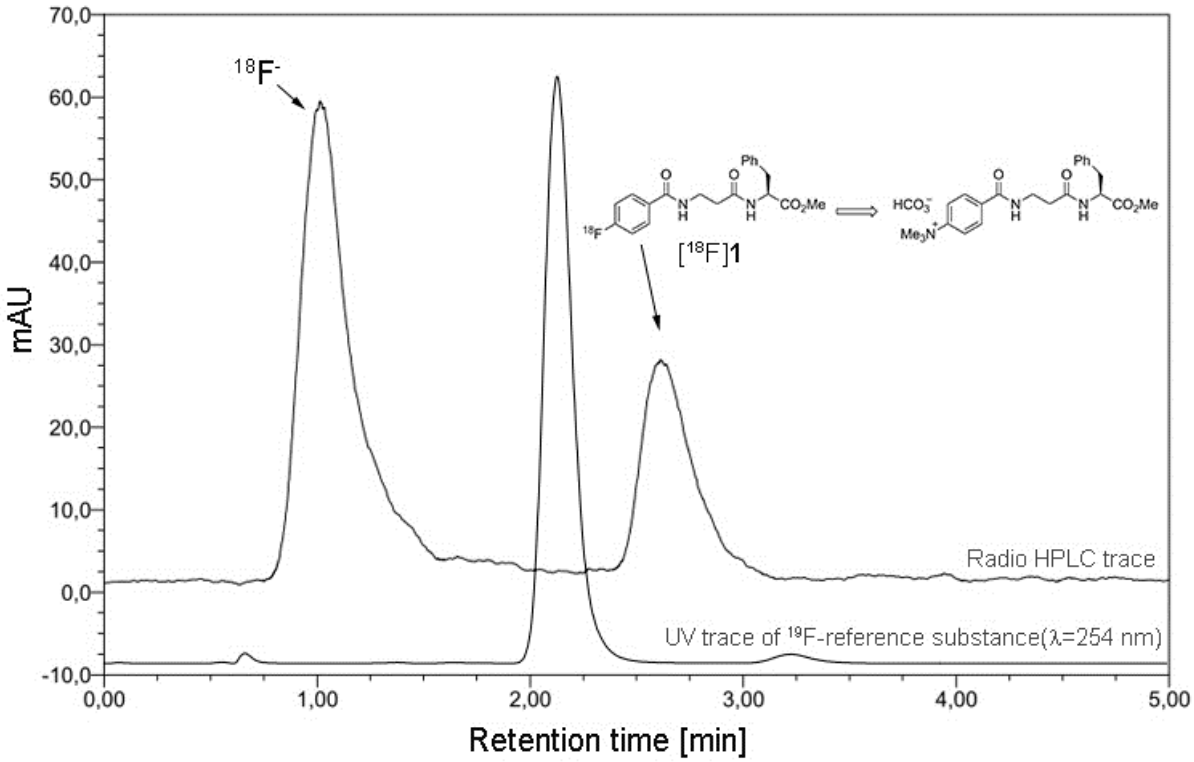

mAU
Retention time [min]
$^{18}F^-$
K222/K_2CO_3-protocol
Radio HPLC trace
UV trace of ^{19}F-reference substance(λ=254 nm)
^{18}F
TfO^-
$^+NMe_3$

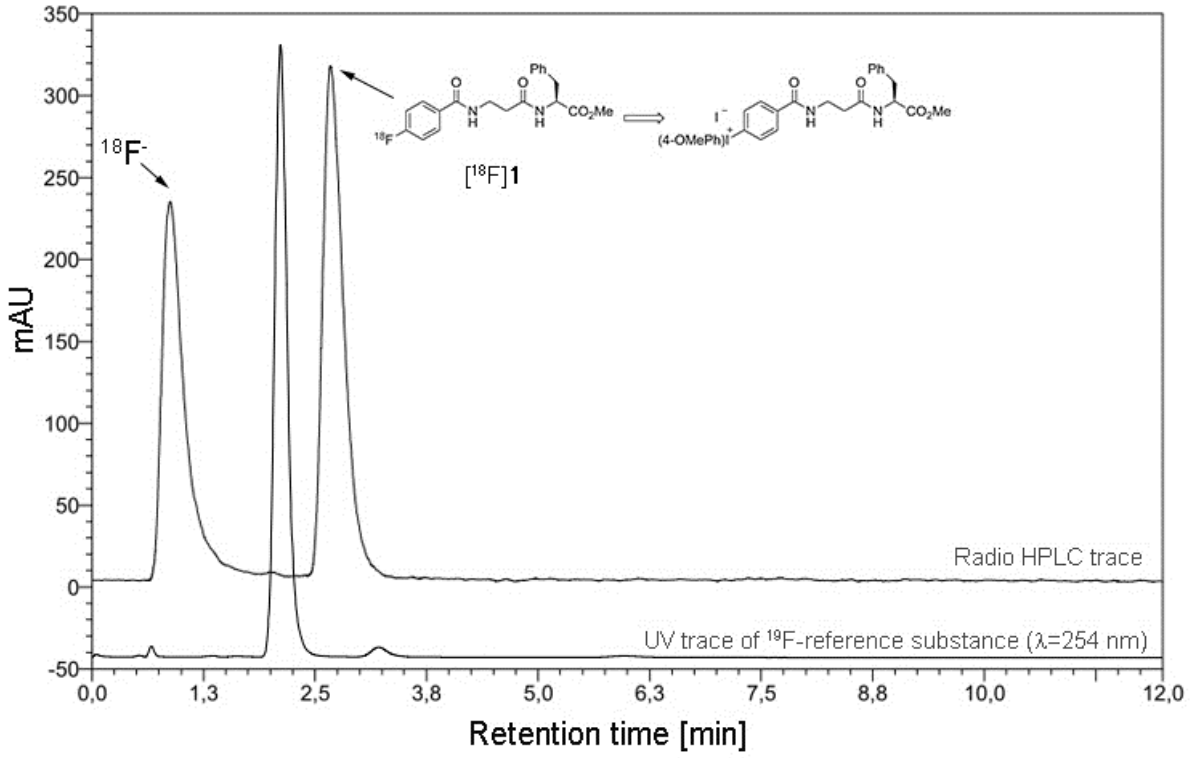

preparative synthesis
> 37GBq
(not purified)
mAU
700
600
500
400
300
200
100
0
-100
$^{18}F^-$
Radio HPLC trace
UV trace of ^{19}F-reference substance(λ=254 nm)
0,00
1,00
2,00
3,00
4,00
5,00
Retention time [min]

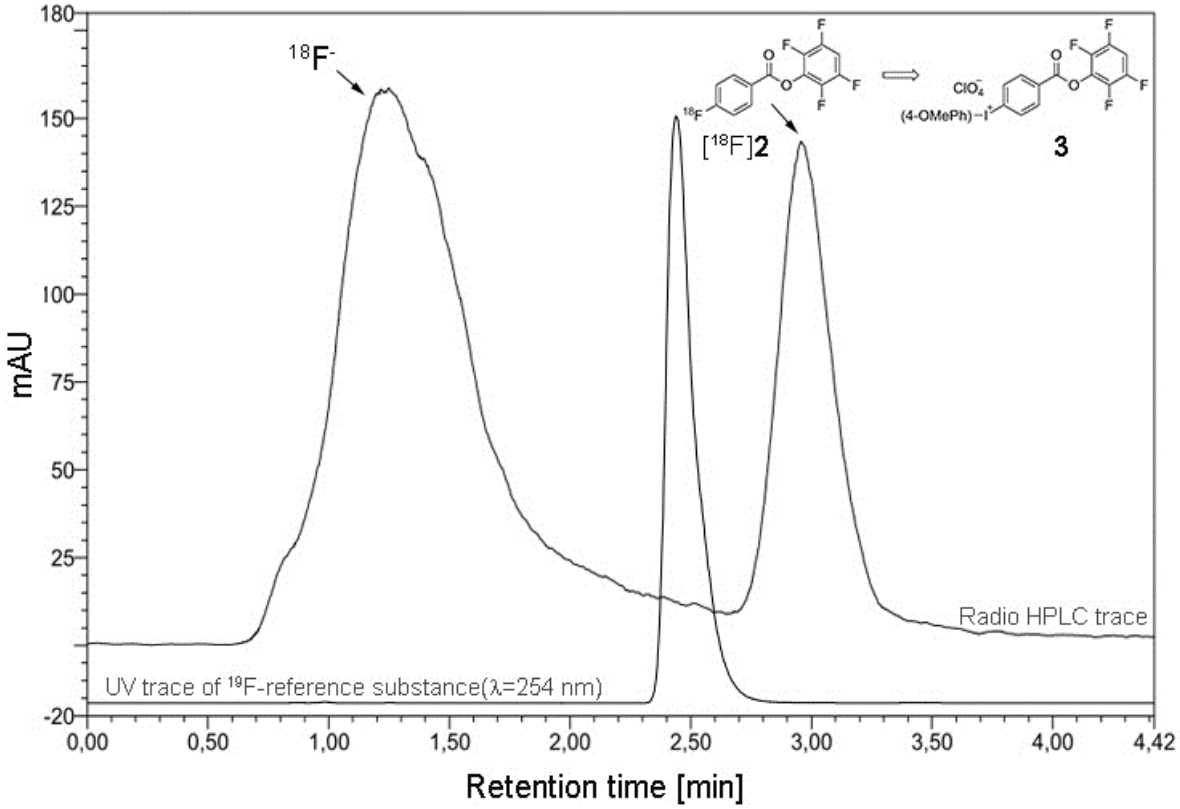

mAU
900
750
625
125
$^{18}F^-$
Radio HPLC trace
UV trace of ^{19}F-reference substance(λ=254 nm)
-100
0,00
1,00
2,00
3,00
4,00
5,00
Retention time [min]

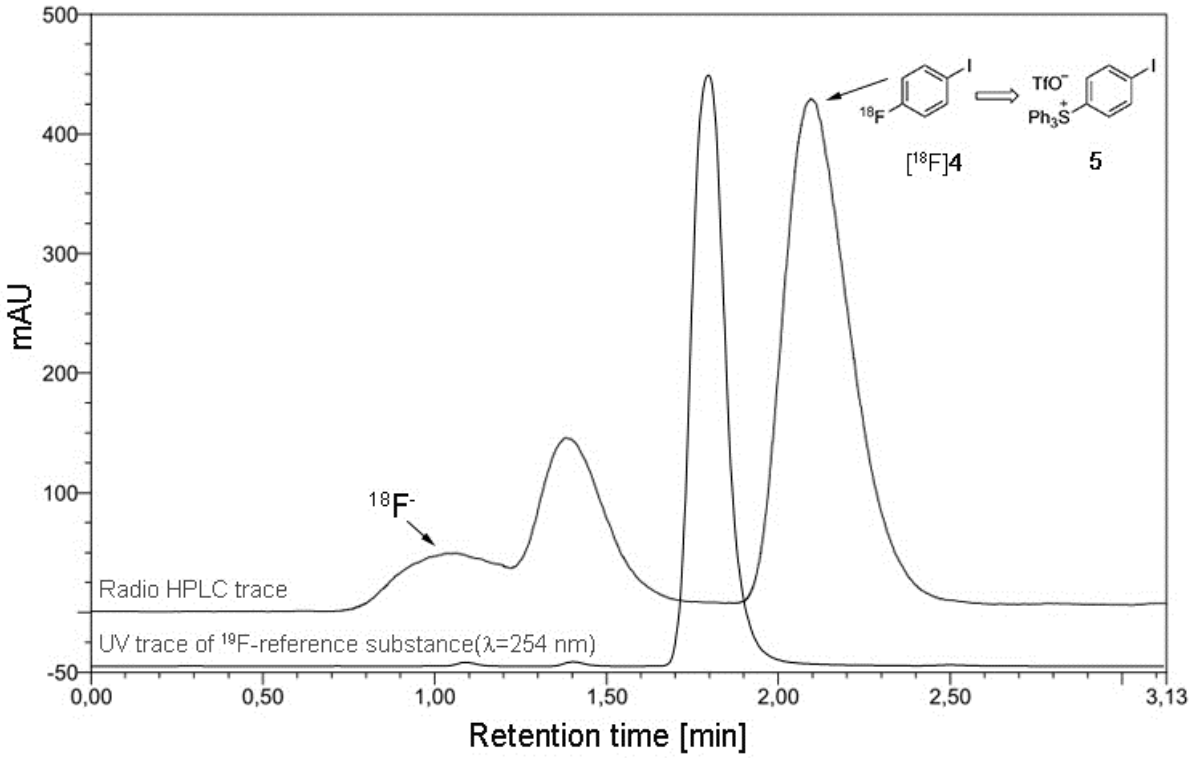

400
300
200
100
0
-50
mAU
$^{18}F^-$
ClO_4^-
(4-OMePh)–I$^+$
^{18}F
Radio HPLC trace
UV trace of ^{19}F-reference substance(λ=254 nm)
0,00
1,00
2,00
3,00
4,00
5,00
Retention time [min]

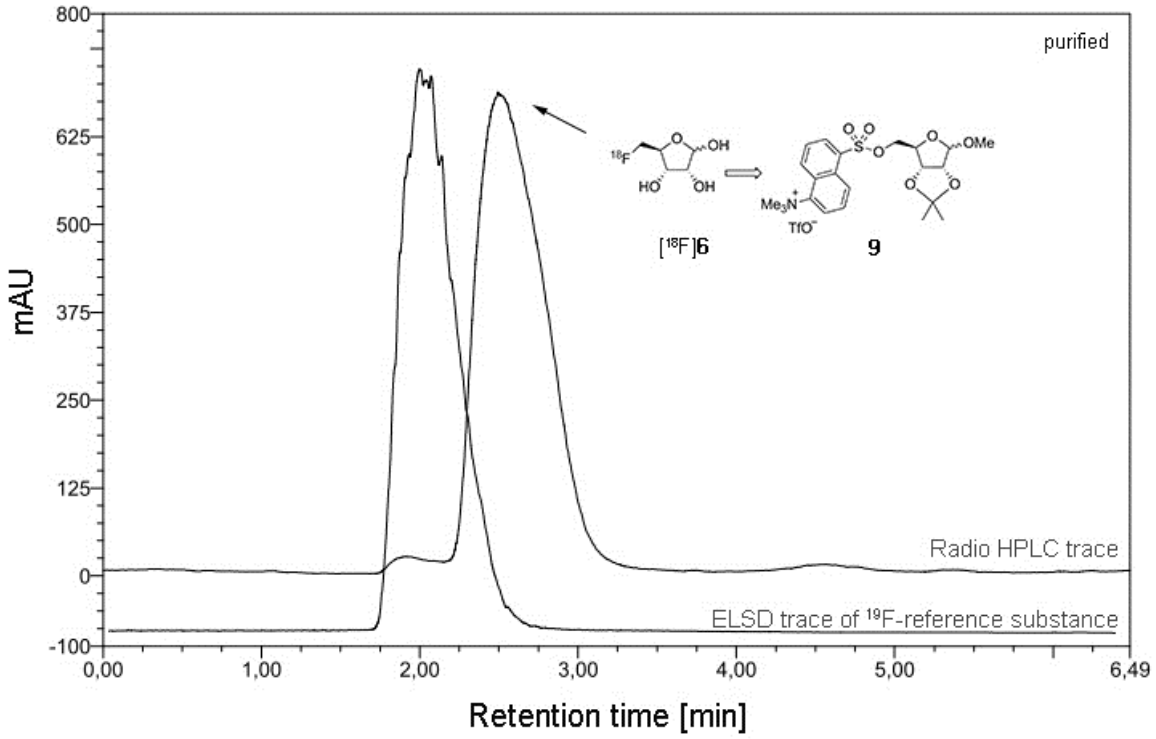

300
250
200
150
100
50
0
-50
mAU
$^{18}F^-$
^{18}F
Br^-
PhI$^+$
TEMPO/CsHCO$_3$-protocol
Radio HPLC trace
UV trace of ^{19}F-reference substance(λ=254 nm)
0,0
1,3
2,5
3,8
5,0
6,3
7,5
8,8
10,0
12,0
Retention time [min]


70,0
60,0
50,0
40,0
30,0
20,0
10,0
0,0
-10,0
mAU
0,00
1,00
2,00
3,00
4,00
5,00
Retention time [min]
$^{18}F^-$
[^{18}F]**1**
Radio HPLC trace
UV trace of ^{19}F-reference substance(λ=254 nm)


350
300
250
200
150
100
50
0
-50
mAU
0,0
1,3
2,5
3,8
5,0
6,3
7,5
8,8
10,0
12,0
Retention time [min]
$^{18}F^-$
[^{18}F]**1**
Radio HPLC trace
UV trace of ^{19}F-reference substance (λ=254 nm)


180
150
125
100
75
50
25
-20
mAU
$^{18}F^-$
[^{18}F]2
3
Radio HPLC trace
UV trace of ^{19}F-reference substance(λ=254 nm)
0,00
0,50
1,00
1,50
2,00
2,50
3,00
3,50
4,00
4,42
Retention time [min]


500
400
300
200
100
-50
mAU
$^{18}F^-$
[^{18}F]4
5
Radio HPLC trace
UV trace of ^{19}F-reference substance(λ=254 nm)
0,00
0,50
1,00
1,50
2,00
2,50
3,13
Retention time [min]



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Communication

Radiolabeled Synthons

Seyferth–Gilbert Homologation as a Route to ^{18}F-Labeled Building Blocks: Preparation of Radiofluorinated Phenylacetylenes and Their Application in PET Chemistry

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Abstract: A convenient method for the preparation of hitherto unknown ([^{18}F]fluorophenyl)acetylenes ([^{18}F]FPAs) using the Seyferth–Gilbert homologation is reported. The novel building blocks were efficiently prepared from easily accessible [^{18}F]fluorobenzaldehydes by using the Bestmann–Ohira reagent. High radiochemical yields and excellent radiochemical purities were achieved within only 20 min of reaction time; 2- and 4-[^{18}F]FPAs were applied to prepare radiofluorinated heterocycles by using different cycloaddition and cross-coupling reactions. Additionally, these building blocks were used to prepare three novel PET tracers. Thus, an artificial radiofluorinated protected amino acid [^{18}F]**10**, a COX-2-specific ligand [^{18}F]**14**, and a PSMA-selective inhibitor [^{18}F]**16** were obtained in high radiochemical yields.

Introduction

Positron emission tomography (PET) is an important non-invasive imaging modality widely used in clinical diagnostics. PET allows the real-time visualization of physiological and pathological processes on the molecular level by using in vivo tracing of biologically active molecules labeled with β^+-emitting nuclides. Among them, fluorine-18 represents the most popular PET radionuclide since no carrier added (n.c.a.) ^{18}F in the form of [^{18}F]fluoride is readily accessible in multi-Curie amounts by the high-yielding ^{18}O(p,n)^{18}F nuclear reaction at low-energy cyclotrons. Furthermore, it exhibits favorable decay properties. Thus, the energy of the emitted β^+-particles is low [$E(\beta^+)$ = 630 keV] resulting in PET scans with high intrinsic resolution due to the short range of the emitted particles in tissue. Furthermore, the half-life of ^{18}F (109.8 min) is well-suited for the majority of PET applications. Moreover, this relatively long half-life enables sophisticated multistep radiochemical syntheses and monitoring of biological processes in the range of hours. Additionally, the half-life of ^{18}F facilitates "satellite" distribution of ^{18}F-labeled radiopharmaceuticals to distant clinical centers.

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A broad spectrum of direct and indirect methods for ^{18}F-labeling has been developed.[1] Among them, the (3+2) azide-alkyne Huisgen cycloaddition (azide-alkyne click reaction),[2] originally transferred to radiochemistry by Marik et al.[2d] became a widely used method for the preparation of PET tracers. In particular, sensitive biomolecules including biopolymers could be efficiently radiofluorinated by using this method. The main advantages of this approach are: chemical and enzymatic stability of the resulting 1,2,3-triazoles, regioselectivity of radiolabeling, excellent compatibility with different functional groups, and fast reaction kinetics under mild reaction conditions. (3+2) Cycloadditions of radiofluorinated nitrile oxides and nitrones with unsaturated compounds have also been explored for radiolabeling.[2f] Furthermore, the Kinugasa reaction has been shown to provide easy access to radiofluorinated β-lactams starting from ^{18}F-labeled nitrones and alkynes.[2h]

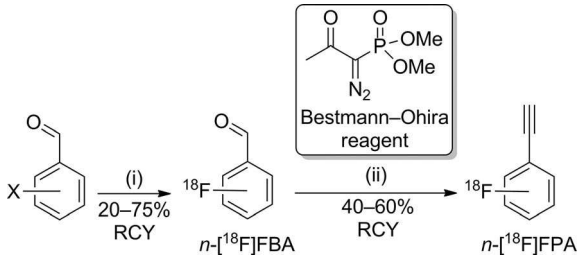
Several radiofluorinated alkynes have been synthesized and applied as radiolabeled synthons for click chemistry.[3] However, compared to the plethora of alkynes applied for nonradioactive applications only a few radiofluorinated alkynes have been developed for PET chemistry. Furthermore, their preparation often requires multiple reaction steps and time-consuming purification procedures, resulting in low radiochemical yields (RCY).

Radiofluorinated arylacetylenes represent potentially very useful building blocks, which could provide access to numerous novel radiolabeled compounds of high interest. Their radiosynthesis has not been reported yet, albeit a rapid development of radiofluorination methods has taken place in recent years.[4,5] Herein we describe the efficient preparation of hitherto unknown ([^{18}F]fluorophenyl)acetylenes ([^{18}F]FPAs). These synthons were synthesized from the corresponding [^{18}F]fluorobenzaldehydes ([^{18}F]FBAs) by using the Seyferth–Gilbert homologation with the Bestmann–Ohira reagent.[6] The application of 2- and

4-[^{18}F]FPAs as building blocks to produce various radiolabeled compounds was studied. The latter were prepared by (3+2) cycloaddition reactions, the radio-Kinugasa reaction, the copper-free Sonogashira coupling and the Rh-catalyzed (2+2+2) cycloaddition. Furthermore, these building blocks were used to produce PET tracers. Thereby, ^{18}F-labeled variants of an amino acid [^{18}F]**10**, a COX-2- ([^{18}F]**14**) and a PSMA-specific ([^{18}F]**16**) ligand were prepared.

Results and Discussion

[^{18}F]Fluorobenzaldehydes were produced according to the recently reported "minimalist" radiofluorination protocol.[7] Shortly, [^{18}F]fluoride was eluted from an anion exchange resin with the appropriate onium salt precursor in MeOH. MeOH was evaporated, the residual [^{18}F]fluoride salt was redissolved in DMSO and heated for 10 min to give *n*-[^{18}F]FBAs in 20–75 % radiochemical yields (RCYs; not corrected for decay) after purification by solid-phase extraction (SPE) within 20–25 min (Scheme 1). [Radiochemical yield (RCY) refers to the isolated yield of the radiochemically and chemically pure radiolabeled compound.]



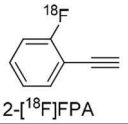
Scheme 1. Synthesis of *n*-[^{18}F]FPAs. (i) Preparation of *n*-[^{18}F]FBAs: 2-[^{18}F]FBA: X = $Me_3N^+ClO_4^-$, DMSO, 150 °C, 10 min (50–65 %); 3-[^{18}F]FBA: X = (4-$MeOC_6H_4$)I^+I^-, DMSO, 130 °C, 10 min (20–25 %); 4-[^{18}F]FBA: X = $Me_3N^+ClO_4^-$, DMSO, 150 °C, 10 min (65–75 %); (ii) Synthesis of *n*-[^{18}F]FPAs: dimethyl (1-diazo-2-oxopropyl)phosphonate (Bestmann–Ohira reagent) solution (10 % in MeCN), K_2CO_3, 120 °C for 15 min. Therafter, distillation at 80 °C under a gentle stream of helium was carried out {in the case of 4-[^{18}F]FPA, $NaBH_4$ (1–2 mg) was added before distillation}.

The radiolabeled benzaldehydes were allowed to react with the Bestmann–Ohira reagent [dimethyl (1-diazo-2-oxopropyl)-phosphonate] in the presence of K_2CO_3 in MeOH/MeCN at 120 °C for 15 min to afford the desired radiofluorinated alkynes in > 90 % radiochemical conversions (RCCs). [Radiochemical conversion yield (RCC) refers to the amount of [^{18}F]fluoride or another immediate radiolabeled precursor, which was transformed into the desired ^{18}F-labeled compound; it was determined by radio-HPLC.] The radiolabeled synthons were isolated by distillation in 40–60 % RCY (not corrected for decay; $n > 20$) and with more than 98 % radiochemical purity (RP) within 20 min. In the case of 4-[^{18}F]FPA, $NaBH_4$ was added to the reaction mixture before distillation to avoid contamination of the product with traces (< 5 %) of 4-[^{18}F]fluorobenzaldehyde. Notably, this preparation method was insensitive to electronic and steric effects of substituents and worked equally well for all three [^{18}F]FBAs.

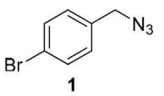
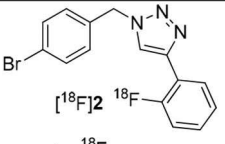
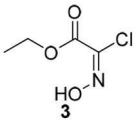
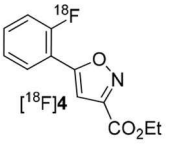
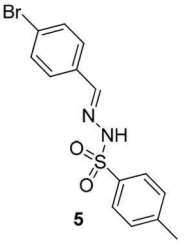
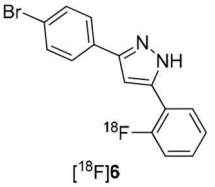
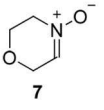
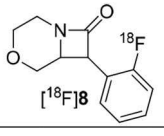
Once a reliable and simple method for the efficient preparation of [^{18}F]FPAs had been established, their versatility as synthons for radiolabeling was evaluated.

Initially, the utility of 2-[^{18}F]FPA for click labeling was studied. Accordingly, 2-[^{18}F]FPA was allowed to react with a model azide **1** (Entry 1, Table 1) in the presence of $CuSO_4$, sodium ascorbate, and histidine in aqueous MeOH at ambient temperature. The corresponding radiolabeled triazole [^{18}F]**2** was formed in an RCC of 53 % already after 10 min of reaction time. In following experiments the cycloaddition reactivity of 2-[^{18}F]FPA with 1,3-dipoles other than azides was examined (Entries 2–4, Table 1). Thus, 2-[^{18}F]FPA was allowed to react with nitrile oxide generated in situ from the corresponding *N*-hydroxyimidoyl chloride **3** by base-promoted elimination of HCl to afford the ^{18}F-labeled 3,5-substituted isoxazole [^{18}F]**4** in 52 % RCC.

Table 1. (3+2) Cycloadditions between 2-[^{18}F]FPA and different 1,3-dipoles.



2-[^{18}F]FPA

Entry[a]	Precursor	Product	RCC±SD [%]
1[b]	 **1**	 [^{18}F]**2**	53±5
2[c]	 **3**	 [^{18}F]**4**	52±6
3[d]	 **5**	 [^{18}F]**6**	20±8
4[e]	 **7**	 [^{18}F]**8**	52±5

[a] All syntheses were carried out manually. Before being analyzed, the reaction mixtures were cooled to ambient temperature and diluted with water (2 mL). Unless otherwise stated, each experiment was carried out at least in triplicate ($n \geq 3$). [b] 2-[^{18}F]FPA (50–500 MBq) in MeOH (50 µL), $CuSO_4$ (2.5 mg), histidine (3.8 mg), sodium ascorbate (9.8 mg) and **1** (5 mg) in H_2O (200 µL), 25 °C, 10 min. [c] 2-[^{18}F]FPA (50–500 MBq) in EtOH (200 µL), **3** (10 mg), Et_3N (10 µL), 120 °C, 30 min. [d] 2-[^{18}F]FPA (50–500 MBq) in dioxane (400 µL), **5** (10.5 mg), K_2CO_3 (8 mg), 150 °C, 30 min. [e] **7** (5 mg), CuI (9.8 mg), Et_3N (28 µL), MeCN (200 µL), 2 min, ambient temp. Then 2-[^{18}F]FPA (50–500 MBq) in MeOH (150 µL), 1,10-phenanthroline (18 mg) in MeCN (100 µL), 90 °C, 10 min.

Next the feasibility of the building block for the preparation of radiolabeled pyrazoles was studied. To this end, 2-[^{18}F]FPA was allowed to react with (4-bromophenyl)diazomethane generated in situ from the respective *N*-tosylhydrazone **5** (Entry 3, Table 1) at 150 °C for 30 min to give the radiolabeled 3,5-substi-

tuted pyrazole [^{18}F]**6** in 20 % RCC. Radiolabeled isoxazoles and pyrazoles could be of potential use as imaging agents targeting, i.e., HDAC 3 and 8,[8] αvβ3 integrins,[9] 20-HETE synthase[10] and COX1/COX2.[11]

Radiofluorinated β-lactams are potentially applicable in PET for the detection of bacterial and viral infections as well as thrombosis, emphysema, and tumors.[12] Consequently, the suitability of ([^{18}F]fluorophenyl)acetylenes for the preparation of ^{18}F-labeled bicyclic β-lactams by the radio-Kinugasa reaction[2h] using cyclic nitrone **7** and 2-[^{18}F]FPA was investigated (Entry 4, Table 1). In the presence of CuI as Cu^{I} source and 1,10-phenanthroline as Cu^{I}-stabilizing ligand this reaction provided the corresponding radiofluorinated fused β-lactam [^{18}F]**8** in an RCC of 52 % within 10 min.

Next, the ability of the radiofluorinated fluoroacetylenes to participate in late transition metal mediated transformations was studied.

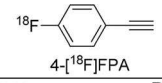
The Sonogashira reaction represents a powerful tool for the preparation of arylalkynes and conjugated enynes.[13] It has been extensively used for the synthesis of different materials, pharmaceuticals and natural products.[7] However, Sonogashira cross-coupling with radiofluorinated alkynes has not been reported yet. Only 4-[^{18}F]fluoroiodobenzene has been used as a building block for the radio-Sonogashira coupling.[14] We studied the Sonogashira reaction between 4-[^{18}F]FPA and protected (4-iodophenyl)alanine **9** (Table 2). Usually, radio-Sonogashira cross-couplings were carried out by using Pd complexes with triarylphosphine ligands and CuI as a metal co-catalyst. We applied the protocol of Yang et al.,[15] which eliminates the need of any co-catalyst and ligands. Accordingly, the radio-Sonogashira reaction was performed under air in water by using only $PdCl_2$ as a catalyst and pyrrolidine as a base. Under these conditions the protected radiofluorinated artificial amino acid [^{18}F]**10** was obtained in an RCC of 83 % after 10 min at 120 °C.

Alkyne trimerization represents a powerful tool for the de novo construction of polysubstituted heteroaromatic and aromatic systems. This reaction is widely used in the total synthesis of complex natural products.[16] Transfer of this method into radiochemistry could provide a fast access to otherwise hardly available ^{18}F-labeled polycyclic compounds.[17]

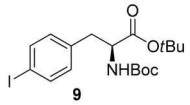
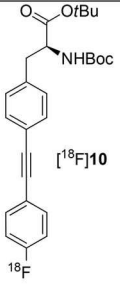
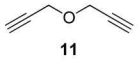
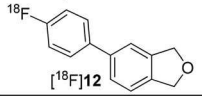
As a proof of principle, model 4-[^{18}F]fluorophenyl-substituted 1,2-dihydrobenzofuran [^{18}F]**12** was prepared by the reaction of propargyl ether (**11**) with 4-[^{18}F]FPA in the presence of Wilkinson's catalyst [$Rh(PPh_3)_3Cl$] in unoptimized 18 % RCC.

Finally, [^{18}F]**14** and [^{18}F]**16** were prepared to illustrate the potential of the novel labeling synthons for tracer production and development. [^{18}F]**14** represents a probe potentially suitable for the visualization of cyclooxygenase 2 (COX-2), an enzyme associated with inflammatory processes and tumor progression.[18] On the other hand [^{18}F]**16** containing the Lys-ureido-Glu fragment could be applied for imaging of the prostate-specific membrane antigen (PSMA),[19] e.g., in prostate carcinoma,[20] breast cancer[21] and tumor-associated neovasculature.[22] Both molecular probes were afforded in > 70 % RCCs by the azide-alkyne "click" cycloaddition between 2-[^{18}F]FPA and the corresponding azide precursors **13** and **15** under standard conditions (Table 3). [^{18}F]**14** and [^{18}F]**16** were isolated by semipreparative HPLC in RCYs of 25 % and 30 % (corrected for decay; starting from ^{18}F-fluoride), respectively, within an overall synthesis time of 60 min. The specific activity of [^{18}F]**14** and

Table 2. Late transition metal mediated reactions of 4-[^{18}F]FPA.

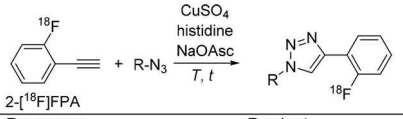


4-[^{18}F]FPA

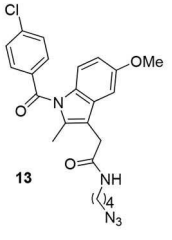
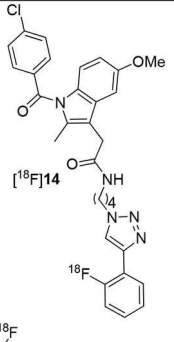
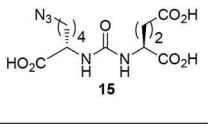
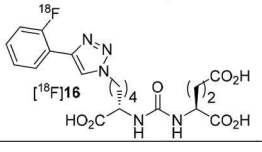
Entry[a]	Metal	Precursor	Product	RCC±SD [%]
1[b]	Pd	 **9**	 [^{18}F]**10**	83±2
2[c,d]	Rh	 **11**	 [^{18}F]**12**	18

[a] All syntheses were carried out manually. Before being analyzed, the reaction mixtures were cooled to ambient temperature and diluted with water (2 mL). Unless otherwise stated, each experiment was carried out at least in triplicate ($n \geq 3$). [b] **9** (35 mg), $PdCl_2$ (0.35 mg), pyrrolidine (100 μL) in H_2O (250 μL), 50 °C, 5 min. Then 4-[^{18}F]FPA (50–500 MBq) in MeCN (150 μL), 120 °C, 10 min. [c] 4-[^{18}F]FPA (50–500 MBq) in EtOH (150 μL), $Rh(PPh_3)_3Cl$ (1 mg), 40 °C, 5 min. Thereafter, **11** (20 μL), 120 °C, 15 min. [d] The synthesis of [^{18}F]**12** was carried out one time ($n = 1$).

Table 3. Preparation of [^{18}F]**14** and [^{18}F]**16**.



2-[^{18}F]FPA

Entry[a]	Precursor	Product	RCC±SD [%]
1[b]	 **13**	 [^{18}F]**14**	75±4
2[c]	 **15**	 [^{18}F]**16**	84±3

[a] All syntheses were carried out manually. Before being analyzed, the reaction mixtures were cooled to ambient temperature and diluted with water (2 mL). Unless otherwise stated, each experiment was carried out at least in triplicate ($n \geq 3$). [b] $CuSO_4$ (2.5 mg), 2-[^{18}F]FPA (50–500 MBq) in MeOH (50 μL), histidine (3.8 mg, 50 μL), sodium ascorbate (9.8 mg) and **13** (15 mg) in H_2O (150 μL), 25 °C, 10 min. [c] 2-[^{18}F]FPA (50–500 MBq) in MeOH (50 μL), $CuSO_4$ (2.5 mg), histidine (3.8 mg), sodium ascorbate (9.8 mg) and **15** (4 mg) in H_2O (150 μL), 50 °C, 20 min.

[^{18}F]**16** was 140 GBq/μmol and 232 GBq/μmol, respectively. The biological evaluation of [^{18}F]**14** and [^{18}F]**16** is in progress and will be reported in due course.

Conclusions

The first application of the Seyferth–Gilbert homologation in PET chemistry is reported. This approach enables the fast and simple access to radiofluorinated phenylacetylenes. A wide application scope of the novel synthons was demonstrated by the preparation of various radiolabeled model compounds and PET tracers using (3+2) cycloadditions and cross-coupling reactions.

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Keywords: Radiopharmaceuticals · Fluorine-18 · Click chemistry · Seyferth-Gilbert homologation · Alkynes

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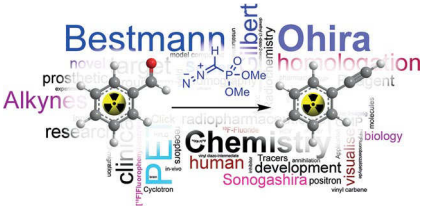
Radiolabeled Synthons



A convenient method for the preparation of hitherto unknown ([^{18}F]fluorophenyl)acetylenes ([^{18}F]FPAs) utilizing the Seyferth–Gilbert homologation is reported. The novel building blocks were applied to prepare radiofluorinated heterocycles and PET tracers utilizing different cycloaddition and cross-coupling reactions.

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SUPPORTING INFORMATION

DOI: 10.1002/ejoc.201501377
Title: Seyferth–Gilbert Homologation as a Route to ^{18}F-Labeled Building Blocks: Preparation of Radiofluorinated Phenylacetylenes and Their Application in PET Chemistry
Author(s): Philipp Krapf, Raphael Richarz, Elizaveta A. Urusova, Bernd Neumaier,* Boris D. Zlatopolskiy

Materials and Methods

General

^{1}H-NMR spectra: Bruker Avance II 300 (300 MHz) and Bruker Avance II+ 600 (600 MHz). ^{1}H chemical shifts are reported in ppm relative to residual peaks of deuterated solvents. Higher-order NMR spectra were approximately interpreted as first-order spectra, if possible. The observed signal multiplicities are characterized as follows: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, as well as br = broad. Coupling constants (*J*) were reported in hertz (Hz). ^{13}C-NMR spectra [additional APT (Attached ProtonTest)]: Bruker Avance II 300 (75.5 MHz) and Bruker Avance II+ 600 (125.9 MHz). ^{13}C chemical shifts are reported relative to residual peaks of deuterated solvents. Low resolution ESI-MS: Finnigan LCQ. High resolution ESI-MS: Bruker APEX IV 7T FTICR MS. EI-MS: Finnigan MAT 95, 70 eV, high resolution EI-MS spectra with perfluorokerosene as reference substance. TLC: Merck precoated sheets, 0.25 mm Sil G/UV$_{254}$. The chromatograms were viewed under UV light and/or by treatment with phosphomolybdic acid (10% in ethanol). Column chromatography: Merck silica gel, grade 60, 230–400 mesh. Solvent proportions are indicated in a volume:volume ratio. All reactions were carried out with magnetic stirring if not stated otherwise and, if air or moisture sensitive substrates and/or reagents, were handled in flame-dried glassware under argon or nitrogen. Organic extracts were dried with anhydrous $MgSO_4$.

2-Formyl-*N*,*N*,*N*-trimethylanilinium perchlorate,[1] (3-formylphenyl)(4-anisyl)iodonium iodide, 4-formyl-*N*,*N*,*N*-trimethylanilinium perchlorate,[1] 4-bromobenzyl azide (**1**),[2] 4-bromobenzaldehyde tosylhydrazone (**5**),[3] *N*-hydroxymorpholine,[4] *N*-Boc 4-iodophenylalanine *tert*-butyl ester (**9**),[5] indomethacyl pentafluorophenolate,[6] 4-azidobutylamine-1,[7] and LysO*t*Bu-ureido-Glu(O*t*Bu)$_2$[8] were prepared according to literature.

StrataX cartridges were obtained from Phenomenex (Aschaffenburg, Germany) and Sep-Pak Accell Plus QMA carbonate plus light cartridges, 46 mg sorbent per cartridge from Waters GmbH (Eschborn, Germany).

HPLC analyses were carried out on Dionex Ultimate® 3000 HPLC systems and a DAD UV detector coupled in series with a Berthold NaI detector. A Chromolith® SpeedROD RP-18e column (Merck, Darmstadt Germany), 50 × 4.6 mm was used for analysis if not otherwise stated. The mobile phase for 2-,3- and 4-[^{18}F]FBA as well as for 2- and 4-[^{18}F]FPA was 30% aq. MeCN at a flow rate of 1.5 mL/min. Retention times: 2-[^{18}F]FBA: 1.7−1.8 min, 3-[^{18}F]FBA: 1.8−1.9 min, 4-[^{18}F]FBA: 1.7 min; 2-[^{18}F]FPA: 4.3 min, 4-[^{18}F]FPA: 4.6 min. The mobile phase for 3-[^{18}F]FPA was 45% aq. MeCN at a flow rate of 1.5 mL/min. Retention time for 3-[^{18}F]FPA: 2.4 min.

UV and radioactivity detectors were connected in series, giving a time delay of 0.5–0.9 min depending on a flow rate. ^{18}F-labeled compounds were identified by spiking of the reaction mixture with unlabeled standards using HPLC.

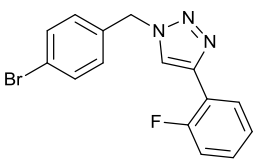
A Chromolith® SemiPrep RP-18 endcapped 100-10 column (Merck, Darmstadt Germany), was used for semi-preparative HPLC purifications. The mobile phase for [^{18}F]**14** was 50% aq. MeCN at a flow rate of 3.0 mL/min. The mobile phase for [^{18}F]**16** was 10% EtOH in 0.02 M NaH_2PO_4 (pH 2.0) at a flow rate of 3.0 mL/min.

[^{18}F]Fluoride was produced via the ^{18}O(p,n)^{18}F reaction by bombardment of enriched [^{18}O]water with 16.5 MeV protons using a MC16 cyclotron (Scanditronix, Uppsala, Sweden). All isolated radiochemical yields are not decay-corrected. If not otherwise indicated all radiochemical experiments were carried out at least in triplicates.

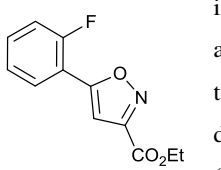
For all radiosyntheses with [^{18}F]FPAs conducted under elevated temperatures radioactivity in the reaction vial was measured before and after the heating step using a dose calibrator (CRC-55tr, Capintec, NJ). The loss of radioactivity never exceeded 5% of its decay-corrected value.

Chemistry

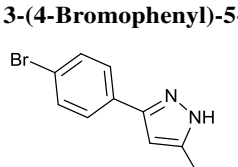
1-(4-Bromobenzyl)-4-(2-fluorophenyl)-1,2,3-triazole (2): To a solution of 4-bromobenzyl azide (**1**)[2] (0.3 g, 1.41 mmol) in 70% *t*BuOH (10 mL) 4-fluorophenylacetylene (0.17 g, 152 μL, 1.42 mmol), $CuSO_4 \cdot 5H_2O$ (70 mg, 0.28 mmol), L-histidine (43 mg, 0.28 mmol) and sodium ascorbate (0.22 g, 1.11 mmol) were added and the reaction mixture was stirred for 16 h. Thereafter, the mixture was concentrated under reduced pressure and the residue was taken up in Et_2O (50 mL). The ethereal solution was washed with H_2O (3×50 mL), brine (2×10 mL), dried and concentrated under reduced pressure to give **2** (0.3 g, 64%) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.47–5.57 (m, 2 H) 7.02–7.15 (m, 2 H) 7.14–7.23 (m, 2 H) 7.46–7.57 (m, 2 H) 7.64 (s, 1 H) 7.70–7.86 (m, 2 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 53.5, 115.8 (d, *J*=21.9 Hz) 119.2, 123.0, 126.6 (d, *J*=3.3 Hz), 127.4 (d, *J*=8.1 Hz), 129.6, 132.3, 133.6, 147.5, 162.7 (d, *J*=247.5 Hz). MS (ESI): positive mode m/z = 687.0 ([2M + Na]$^+$), 354.0 ([M + Na]$^+$), 332.0 ([M + H]$^+$); MS (ESI): negative mode m/z = 330.0 ([M – H]$^-$); ESI HRMS: calcd for $C_{15}H_{11}BrN_3Na^+$: 354.0013; found: 354.0005; calcd for $C_{15}H_{12}BrN_3^+$: 332.0193; found: 332.0186. Correct isotopic pattern.



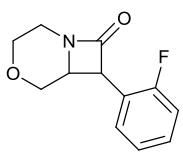
Ethyl 5-(2-fluorophenyl)-isooxazole-3-carboxylate (4): A solution of ethyl 2-chloro-2-(hydroxyimino)acetate (0.3 g, 1.98 mmol) in EtOH (10 mL) was added dropwise to a refluxing solution of 2-fluoroacetylene (1 g, 0.94 mL, 8.3 mmol) and the reaction mixture was heated under reflux for 24 h. After that, the mixture was cooled to ambient temperature, diluted with Et_2O (150 mL). The solution was washed with H_2O (3×50 mL), brine (2×10 mL), dried and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc:hexane = 1:10) affording the title compound (0.32 g, 68%) as a green oil which slowly solidified in a green solid. R_f = 0.68, EtOAc:hexane = 1:1.6, R_f = 0.46, EtOAc:hexane = 1:5. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (t, *J*=7.18 Hz, 3H), 4.48 (q, *J*=7.18 Hz, 2H), 7.10 (d, *J*=3.78 Hz, 1H), 7.15–7.36 (m, 2H), 7.40–7.57 (m, 1H), 7.99 (td, *J*=7.60, 1.70 Hz, 1H) ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.5, 160.3 (d, *J*=72.5 Hz), 157.4, 157.2, 132.3 (d, *J*=8.3 Hz), 127.6, 124.8 (d, *J*=3.8 Hz), 116.4 (d, *J*=20.4 Hz), 115.1 (d, *J*=12.8 Hz), 103.9 (d, *J*=11.3 Hz), 62.2, 14.1. MS (ESI): positive mode *m/z* = 493.1 ([2M + Na]$^+$), 258.1 ([M + Na]$^+$), 236.1 ([M + H]$^+$); ESI HRMS: calcd for $C_{12}H_{10}NFONa^+$: 258.0537; found: 258.0536; calcd for $C_{12}H_{11}NFO^+$: 236.0717; found: 236.0717.



3-(4-Bromophenyl)-5-(2-fluorophenyl)pyrazole (6): A suspension of K_2CO_3 (0.7 g, 5.06 mmol) in a solution of 4-bromobenzaldehyde tosylhydrazone (**5**)[3] (0.88 g, 2.49 mmol), 2-fluorophenylacetylene (0.6 g, 0.56 mL, 5 mmol) in dioxane (20 mL) in a closed thick-walled reaction vial was stirred at 110 °C for 24 h. Afterwards, the resulting slurry was diluted with Et_2O (100 mL) and washed with H_2O (3×50 mL) and brine (2×20 mL). The ethereal fraction was cooled in a fridge for 1 h, the resulting precipitate was filtered off, washed with ether and dried under reduced pressure to give **6** (0.45 g, 57%) as a colorless solid. R_f = 0.45, EtOAc:hexane = 1:1.6. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.12 (m, 1H) 7.23–7.50 (m, 3H) 7.55–7.93 (m, 5H) 13.22–13.76 (m, 1H). ^{13}C NMR (125.8 MHz, DMSO-d_6) δ 150.6, 146.5, 142.6, 137.9, 133.2, 132.2 (d, *J*=44.0 Hz), 130.2 (d, *J*=110.7 Hz), 128.5, 127.7, 125.2 (d, *J*=110.7 Hz), 121.4 (d, *J*=86.7 Hz), 116.7, 103.1 (d, *J*=42.8 Hz). The signal of C_q—F was not observed. MS (ESI): positive mode m/z = 341.0 ([M + Na]$^+$), 317.0 ([M + H]$^+$); MS (ESI): negative mode m/z = 315.0 ([M – H]$^-$); ESI HRMS: calcd for $C_{15}H_{10}N_2FBrNa^+$: 338.9904; found: 338.9897; calcd for $C_{15}H_{11}N_2FBr^+$: 317.0084; found: 317.0086; calcd for $C_{15}H_9N_2FBr^-$: 314.9939; found: 314.9937. Correct isotopic pattern.

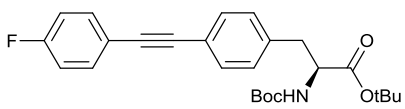


7-(2-Fluorophenyl)-4-oxa-1-azabicyclo[4.2.0]octan-8-one (8): To $CuSO_4 \cdot 5H_2O$ (1.94 g, 7.76 mmol) concentrated aqueous ammonia (6 mL) and H_2O (17 mL) were added. The solution was stirred with ice cooling for 15 min, while a stream of Ar was passed through the solution. Solid $NH_2OH \cdot HCl$ (1.5 g, 21.6 mmol) was added to the reaction mixture under Ar and

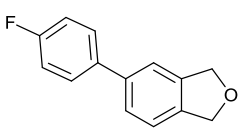


stirring was continued for a further 1 h. A solution of 1-ethynyl-2-fluorobenzene (0.94 mL, 1 g, 8.33 mmol) in EtOH (30 mL) was added rapidly to the resulting pale blue solution. An additional water (30 mL) was added, the reaction flask was shaken by hand, the yellow precipitate was separated by filtration, washed with H_2O (3 × 50 mL), MeOH (3 × 50 mL), Et_2O (3 × 50 mL) and dried at 40 °C and 4 mbar for 1 h to give copper(I) (2-fluorophenyl)acetylide (0.73 g, 52%) which was directly used for the next step. Yellow HgO (1.2 g, 5.54 mmol) was added to a solution of *N*-hydroxymorpholine (0.4 g, 3.88 mmol) in $CHCl_3$ (30 mL). The suspension was vigorously stirred for 45 min under Ar to yield 3,6-dihydro-2*H*-1,4-oxazine-4-oxide (TLC control). The mixture was filtered through Celite® directly into the flask with copper(I) (2-fluorophenyl)acetylide (0.65 g, 3.56 mmol). Pyridine (0.4 mL, 0.39 g, 4.95 mmol) was added and the reaction mixture was stirred for 4 h. The mixture was washed with H_2O (3 × 10 mL), brine (2 × 10 mL), dried, filtered and concentrated under reduced pressure. The residual dark brown tar was extracted with boiling hexane (5 × 20 mL). Hexane was removed under reduced pressure and the residue was purified by column chromatography (EtOAc:hexane = 1:2). Three fractions were isolated: the fraction containing mainly *cis*-**8** (0.11 g, 14%) as a brown solid, *trans*-**8** (70 mg, 9%) as a brown semisolid as well as the mixed fraction (30 mg; overall 34%). *cis*/*trans*-**8** were used to identify [^{18}F]**8**. *cis*-**8**: R_f = 0.19, EtOAc:hexane = 2:1. ^{1}H-NMR($CDCl_3$, 300 MHz): δ = 2.94 (dt, *J* = 12.8, 1.0 Hz, 1 H), 3.0–3.15 (m, 1 H), 3.29 (td, *J* = 11.4, 3.6 Hz, 1 H), 3.76 (dd, *J* = 12.8, 3.6 Hz, 1 H), 3.92–4.02 (m, 2 H), 4.71 (d, *J* = 4.8 Hz,1 H), 7.0–7.10 (m, 1 H), 7.11–7.19 (m, 1 H), 7.23–7.34 (m, 1 H), 7.65–7.74 (m, 1 H); ^{13}C-NMR($CDCl_3$, 75.5 MHz): δ = 38.9, 48.6, 51.3, 66.0, 69.5, 115.1 (d, *J* = 20.4 Hz), 120.1 (d, *J* = 16.6 Hz), 124.4 (d, *J* = 3.8 Hz), 129.3 (d, *J* = 7.6 Hz), 129.6 (d, *J* = 3.8 Hz), 158.6, 164.2 (d, *J* = 366.2 Hz). MS (ESI): positive mode *m/z* = 465.2 ([2 M + Na]$^+$), 244.1 ([M + Na]$^+$), 222.1 ([M + H]$^+$); ESI HRMS: calcd for $C_{12}H_{12}FNO_2Na^+$: 244.0744; found: 244.0744; calcd for $C_{12}H_{13}FNO_2^+$: 222.0925; found: 222.0927; calcd for $C_{12}H_{11}FNO_2^-$: 220.0779; found: 220.0773. *trans*-**8**: R_f = 0.15, EtOAc:hexane = 2:1. ^{1}H-NMR($CDCl_3$, 300 MHz): δ= 3.12 (ddd, *J* = 13.3, 11.7, 4.6 Hz, 1 H), 3.40–3.51 (m, 2 H), 3.61 (ddd, *J* = 10.3, 4.6, 2.2 Hz, 1 H), 3.77 (ddd, *J* = 13.3, 3.6, 0.4 Hz, 1 H), 3.91 (dd, *J* = 11.7, 4.6, 1 H), 4.24 (s, 1 H), 4.40 (dd, *J* = 11.2, 4.4 Hz, 1 H), 7.05 (ddd, *J* = 9.9, 8.4, 1.2 Hz, 1 H), 7.10–7.18 (m, 1 H), 7.23–7.33 (m, 1 H), 7.44 (td, *J* = 7.6, 1.8 Hz, 1 H); ^{13}C-NMR($CDCl_3$, 75.5 MHz): δ = 39.1, 53.0 (d, *J* = 2.3 Hz), 54.2 (d, *J* = 1.5 Hz), 66.1, 71.9, 115.4 (d, *J* = 21.1 Hz), 122.0 (d, *J* = 15.0 Hz), 124.5 (d, *J* = 3.0 Hz), 128.4 (d, *J* = 3.8 Hz), 129.4 (d, *J* = 8.3 Hz), 158.8, 163.8 (d, *J* = 250.7 Hz). MS (ESI): positive mode *m/z* = 465.2 ([2 M + Na]$^+$), 244.1 ([M + Na]$^+$), 222.1 ([M + H]$^+$); ESI HRMS: calcd for $C_{12}H_{12}FNO_2Na^+$: 244.0744; found: 244.0744; calcd for $C_{12}H_{13}FNO_2^+$: 222.0925; found: 222.0928; calcd for $C_{12}H_{11}FNO_2^-$: 220.0779; found: 220.0779.

***N*-Boc *tert*-Butyl (*S*)-2-Amino-3-{4-[2-(4-fluorophenyl)ethynyl]phenyl}propanoate (10):** A solution of Boc-4-IPhe-O*t*Bu (**9**)[5] (0.25 g, 0.56 mmol), $PdCl_2$ (4 mg, 22.6 µmol) and pyrrolidine (0.27 ml, 3.29 mmol) in H_2O (0.8 mL) was stirred at ambient temperature for 72 h. The reaction mixture was concentrated under reduced pressure, the residue was taken up in Et_2O (50 mL) and was washed with H_2O (3×20 mL), 1 N $NaHSO_4$ (3×20 mL), H_2O (3×20 mL), brine (2×20 mL), dried and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc:hexane = 1:8) affording the title compound (0.12 g, 47%) as a brown oil. R_f = 0.42, EtOAc:hexane = 1:8 (two time development). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (s, 9 H), 1.44 (s, 9 H), 3.08 (d, *J*=5.5 Hz, 2 H), 4.39–4.53 (m, 1 H), 5.02 (d, *J*=7.6 Hz, 1 H), 6.99–7.09 (m, 2 H), 7.17 (d, *J*=8.2 Hz, 2 H), 7.40–7.56 (m, 4 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 162.4 (d, *J*=249.2 Hz), 155.0, 136.9, 133.4 (d, *J*=249.2 Hz), 133.4, 131.5, 129.6, 121.5, 119.4 (d, *J*=3.6 Hz), 115.6 (d, *J*=21.9 Hz), 88.9, 88.3, 82.2, 79.8, 54.7, 38.5, 28.3, 28.0. MS (ESI): positive mode *m/z* = 901.4 ([2M + Na]$^+$), 879.5 ([2M + H]$^+$), 462.2 ([M + Na]$^+$), 440.2 ([M + H]$^+$); MS (ESI): negative mode *m/z* = 438.2 ([M – H]$^-$); ESI HRMS: calcd for $C_{26}H_{30}NO_4FNa^+$: 462.2051; found: 462.2044; calcd for $C_{26}H_{29}NO_4F^-$: 438.2086; found: 438.2067.

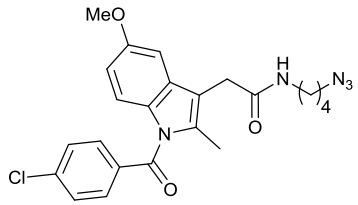


5-(4-Fluorophenyl)-1,3-dihydro-2-benzofuran (12): To an ice-cold solution propargyl ether (0.45 mL, 0.4 g, 4.25 mmol) in EtOH (20 mL) 4-fluorophenylacetylene (1.45 mL, 1.5 g, 12.49 mmol) was added followed by

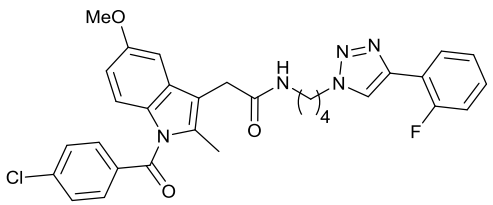


$RhCl(PPh_3)_3$ (19 mg, 0.21 mmol). The cooling bath was removed and the reaction mixture was stirred for 20 h. The mixture was concentrated under reduced pressure, the residue was dissolved in Et_2O (50 mL) and the ethereal solution was washed with H_2O (3×20 mL), 1 N $NaHSO_4$ (3×20 mL), 5% $NaHCO_3$ (3×20 mL), H_2O (3×20 mL), brine (2×20 mL), dried and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc:hexane = 1:8) affording the title compound (80 mg, 9%) as an off-white solid. R_f = 0.43, EtOAc:hexane = 1:8. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.17 (s, 4H) 7.08–7.19 (m, 2H) 7.28–7.35 (m, 1H) 7.39–7.48 (m, 2H) 7.50–7.61 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 162.5 (d, *J*=245.3 Hz), 139.9 (d, *J*=20.3 Hz), 138.2, 137.2, 137.1, 128.7 (d, *J*=8.3 Hz), 126.4, 121.3, 119. 6, 115.6 (d, *J*=21 Hz), 73.5, 73.4. MS (EI, 70 eV), *m/z* (%) = 214.1 (81) [M^+], 213.1 (42) [M^+ – H], 186.1 (69) [M^+ – CO], 185.1 (100) [M^+ – HCO], 183.1 (47) [M^+ – CH_3O], 170.1 (20) [M^+ – C_2H_4O], 165.1 (43) [M^+ – CH_2OF]; ES HRMS: calcd for $C_{14}H_{11}FO^+$: 214.0794; found: 214.0803; calcd for $C_{14}H_{10}FO^+$: 213.0716; found: 213.0724.

***N*-(4-Azidobut-1-yl)-indometacin amide (13):** 4-Azidobutylamine-1[7] (2.8 mL, 2.24 g, 19.6 mmol) was added to a solution of indomethacyl pentafluorophenolate[6] (6.91 g, 13.19 mmol) in CH_2Cl_2 (25 mL) and the reaction mixture was incubated at ambient temperature for 2 h. The reaction mixture was diluted with EtOAc (150 mL), washed with H_2O (3×20 mL), 1 N $NaHSO_4$ (3×20 mL), 5% $NaHCO_3$ (3×20 mL), H_2O (3×20 mL), brine (2×20 mL), dried and concentrated under reduced pressure. The residue was crystallized from CH_2Cl_2/Et_2O affording the title compound (5.35 g, 89%) as a colorless solid. The mother liquor was concentrated under reduced pressure and the residue was crystallized from CH_2Cl_2/Et_2O to give the second crop of **13** (0.29 g, total 94%). R_f = 0.19, EtOAc:hexane = 1:1. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.40–1.58 (m, 4H), 2.39 (s, 3H), 3.23 (t, *J*=2.83 Hz, 4H), 3.65 (s, 2H), 3.82 (s, 3H), 5.74 (t, *J*=5.57 Hz, 1H), 6.70 (dd, *J*=9.06, 2.46 Hz, 1H), 6.81–6.92 (m, 2H), 7.42–7.54 (m, 2H), 7.59–7.72 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.9, 168.3, 156.3, 139.6, 136.3, 133.5, 131.1, 130.9, 130.2, 129.2, 115.1, 112.7, 112.3, 100.8, 55.7, 50.9, 39.0, 32.2, 26.8, 26.1, 13.2. MS (ESI): positive mode *m/z* = 929.3 ([2M + Na]$^+$), 476.2 ([M + Na]$^+$), 454.2 ([M + H]$^+$); MS (ESI): negative mode *m/z* = 452.1 ([M – H]$^-$); ESI HRMS: calcd for $C_{23}H_{24}N_5O_3ClNa^+$: 476.1460; found: 476.1452; calcd for $C_{23}H_{23}N_5O_3Cl^-$: 452.1484; found: 452.1495.



***N*-4-[4-(2-Fluorophenyl)-1,2,3-triazol-1-yl]but-1-yl-indometacine amide (14):** $CuSO_4·5H_2O$ (82 mg, 0.33 mmol), histidine (0.102 g, 0.66 mmol) and sodium ascorbate (0.174 g, 0.88 mmol) were added to a solution of **15** (0.5 g, 1.1 mmol) and 2-fluorophenylacetylene (0.15 mL, 0.158 g, 1.32 mmol) in 85% *t*BuOH (50 mL) and the reaction mixture was stirred for 24 h. Thereafter, $CuSO_4·5\,H_2O$ (82 mg, 0.33 mmol), histidine (0.102 g, 0.66 mmol) and 2-fluorophenylacetylene (0.15 mL, 0.158 g, 1.32 mmol) in 60% *t*BuOH (15 mL) followed by sodium ascorbate (0.174 g, 0.88 mmol) were added to the reaction mixture and stirring continued for further 24 h (two times). Afterwards, the mixture was concentrated under reduced pressure, the residue was taken up in Et_2O (75 mL) and washed with H_2O (3×50 mL), brine (2×10 mL), dried and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc:hexane=1:2) to give **14** (0.2 g, 32%) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.48 (q, *J*=7.3 Hz, 2H), 1.88 (q, *J*=7.3 Hz, 2H), 2.37 (s, 3H), 3.26 (q, *J*=6.9 Hz, 2H), 3.64 (s, 2H), 3.78 (s, 3H), 4.37 (t, *J*=6.9 Hz, 2H), 5.81–5.90 (m, 1H), 6.67 (dd, *J*=9.0, 2.5 Hz, 1H), 6.83 (d, *J*=9.0 Hz, 1H), 6.88 (d, *J*=2.5 Hz, 1H), 7.09–7.16 (m, 1H), 7.21–7.26 (m, 1H), 7.28–7.35 (m, 1H), 7.42–7.52 (m, 2H), 7.59–7.67 (m, 2H), 7.87 (d, *J*=3.7 Hz, 1H), 8.14–8.33 (m, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 170.1, 168.3, 159.1 (d, *J*=247.7 Hz), 156.2, 141.1 (d, *J*=2.5 Hz), 139.5, 136.4, 133.5, 131.1, 130.6 (d, *J*=78.0 Hz), 129.3 (d, *J*=8.8 Hz), 129.2, 127.7 (d, *J*=2.5 Hz), 124.6 (d, *J*=2.5 Hz), 122.7 (d, *J*=12.6 Hz), 118.4 (d, *J*=12.6 Hz), 115.6 (d, *J*=21.4 Hz), 115.1, 112.7, 112.1, 100.8, 55.7, 49.7, 38.7, 32.2, 27.5, 26.5, 13.2. MS (ESI): positive mode m/z = 1169.4 ([2M + Na]$^+$), 1147.4 ([2M + H]$^+$), 633.2 ([2M + HCO_2Na + H]$^+$), 596.2 ([M +



Na]$^+$), 574.2 ([M + H]$^+$); MS (ESI): negative mode m/z = 572.2 ([M – H]$^–$); ESI HRMS: calcd for $C_{31}H_{29}ClFN_5O_3Na^+$: 596.1835; found: 596.1827; calcd for $C_{31}H_{30}ClFN_5O_3^+$: 574.2016; found: 574.2014; calcd for $C_{31}H_{28}ClFN_5O_3^–$: 572.1870; found: 572.1866.

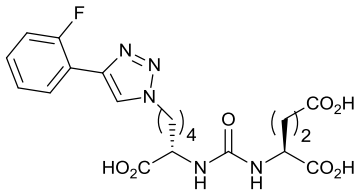
1,5-Di-*tert*-butyl (*S*)-2-({[(*S*)-6-azido-1-(*tert*-butoxy)-1-oxohexan-2-yl]carbamoyl}amino)pentanedioate (S1): To a suspension of K_2CO_3 (0.495 g, 3.59 mmol) and $CuSO_4 \cdot 5\,H_2O$ (50 mg, 0.20 mmol) in a solution of LysO*t*Bu-ureido-Glu(O*t*Bu)$_2$[8] (1 g, 2.05 mmol) in MeOH (10 mL) was added imidazole-1-sulfonyl azide hydrochloride (0.515 g, 2.46 mmol) and the reaction mixture was stirred for 24 h. Thereafter, the mixture was quenched with H2O (40 mL) and extracted with Et_2O (100 mL). The ethereal layer was washed with H_2O (3×20 mL), 1 N $NaHSO_4$ (3×20 mL), brine (2×20 mL), dried and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc:hexane=1:3) affording the title compound (0.67 g, 64%) as a colorless compound. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37–1.51 (m, 2H), 1.43 (s, 9H), 1.46 (s, 9H), 1.47 (s, 9H), 1.54–1.70 (m, 3H) 1.72–1.94 (m, 2H) 1.99–2.16 (m, 1H) 2.19–2.44 (m, 2H) 3.26 (t, *J*=6.81 Hz, 2H) 4.28–4.38 (m, 2H) 5.14 (br, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.4, 172.3, 172.1, 156.7, 82.1, 81.9, 80.5, 53.3, 53.1, 51.1, 32.8, 31.6, 28.5, 28.4, 28.1, 28.0 (×2), 22.3. MS (ESI): positive mode m/z = 1049.6 ([2M + Na]$^+$), 573.4 ([2M + HCO_2Na + H]$^+$), 536.3 ([M + Na]$^+$), 514.3 ([M + H]$^+$); ESI HRMS: calcd for $C_{24}H_{43}N_5O_7Na^+$: 536.3055; found: 536.3053; calcd for $C_{24}H_{44}N_5O_7^+$: 514.3235; found: 514.3234.

(2S)-2-({[(1S)-5-Azido-1-carboxypentyl]carbamoyl}amino)pentanedioic acid (15): A solution of **S1** (0.3 g, 0.58 mmol) in TFA/TIS/H_2O (97.5/2.5/2.5) (5 mL) was incubated at ambient temperature for 3 h. The mixture was concentrated under reduced pressure, the residue was dissolved in TFA (5 mL), the resulting solution was incubated at room temperature for 2 h and concentrated under reduced pressure. The residue was triturated with Et_2O (×3). The residual oil was taken up in MeOH (30 mL) and the resulting solution was concentrated under reduced pressure. The residue was triturated with Et_2O (×3) and dried under reduced pressure to give the crude desired product (0.185 g, crude 92%) as a colorless oil which was used without any further purification. ^{1}H NMR (300 MHz, CD_3OD) δ 1.27–2.02 (m, 8H), 2.07–2.27 (m, 2H), 2.34–2.53 (m, 1H), 2.99 (t, *J*=7.5 Hz, 1H), 4.31 (dd, *J*=8.8, 4.6 Hz, 2H). ^{13}C NMR (75 MHz, CD_3OD) δ 175.0, 174.6, 174.5, 158.8, 52.2, 52.0, 48.8, 31.7, 29.7, 27.3, 25.0, 22.1. MS (ESI): negative mode m/z = 344.1 ([M – H]$^–$); ESI HRMS: calcd for $C_{12}H_{18}N_5O_7^–$: 344.1212; found: 344.1210.

1,5-Di-*tert*-butyl (2S)-2-({[(2S)-1-(*tert*-butoxy)-6-[4-(2-fluorophenyl)-1,2,3-triazol-1-yl]-1-oxohexan-2-yl]carbamoyl}amino)-pentanedioate (S2): An emulsion of 2-fluorophenylacetylene (0.132 mL, 0.14 g, 1.12 mmol) in a solution $CuSO_4 \cdot 5\,H_2O$ (10 mg, 40.1 µmol) and histidine (6 mg, 38.7 µmol) in 60% MeCN (10 mL) followed by sodium ascorbate (0.174 g, 0.88 mmol) were added to a solution of **S1** (0.2 g, 0.39 mmol) in 70% MeCN (50 mL) and the reaction mixture was stirred for 24 h. Thereafter, An emulsion of 2-fluorophenylacetylene (0.132 mL, 0.14 g, 1.12 mmol) in a solution $CuSO_4 \cdot 5\,H_2O$ (10 mg, 40.1 µmol) and histidine (6 mg, 38.7 µmol) in 60% MeCN (10 mL) followed by sodium ascorbate (0.174 g, 0.88 mmol) were added to the reaction mixture and stirring continued for further 24 h (two times). Afterwards, the mixture was concentrated under reduced pressure, the residue was taken up in Et_2O (100 mL) and washed with H_2O (3×50 mL), brine (2×10 mL), dried and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc:hexane=1:1.5) to give **S2** (0.1 g, 41%) as a colorless solid. R_f = 0.29, EtOAc:hexane = 1:1.5. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.39–1.53 (m, 1H), 1.41 (d, *J*=0.8 Hz, 9H), 1.42 (d, *J*=0.6 Hz, 9H), 1.45 (d, *J*=0.8 Hz, 9H), 1.61–1.74 (m, 1H), 1.77–1.91 (m, 2H), 1.91–2.15 (m, 4H), 2.22–2.39 (m, 2H), 4.27–4.38 (m, 2H), 4.41 (td, *J*=7.3, 2.5 Hz, 2H), 5.15–5.38 (br, 2H), 7.08–7.16 (m, 1H), 7.23 (t, *J*=6.0 Hz, 1H), 7.27–7.33 (m, 1H), 7.94 (d, *J*=3.5 Hz, 1H), 8.29 (t, *J*=7.3 Hz, 1H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.4, 172.2, 172.1, 159.1 (d, *J*=247.7 Hz), 156.8, 141.1, 129.2 (d, *J*=7.5 Hz), 127.8 (d, *J*=2.5 Hz), 124.5 (d, *J*=3.8 Hz),

122.7 (d, *J*=12.6 Hz), 118.5 (d, *J*=1.3 Hz), 115.6 (d, *J*=21.4 Hz), 82.0, 81.9, 80.5, 53.1, 53.0, 50.0, 32.5, 31.5, 29.8, 28.3, 28.0, 28.0, 27.9, 22.0. MS (ESI): positive mode m/z = 1289.7 ([2M + Na]$^+$), 656.4 ([M + Na]$^+$), 634.4 ([M + H]$^+$); ESI HRMS: calcd for $C_{36}H_{48}FN_5O_7Na^+$:656.3430; found: 656.3432; calcd for $C_{36}H_{49}FN_5O_7^+$: 634.3611; found: 634.3608.

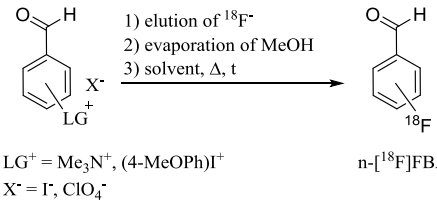
(2*S*)-2-({[(1*S*)-1-Carboxy-5-[4-(2-fluorophenyl)-1,2,3-triazol-1-yl]pentyl]carbamoyl}amino)pentanedioic acid (16): A solution of **S2**



(0.098 g, 0.15 mmol) in TFA/TIS/H_2O (97.5/2.5/2.5) (5 mL) was incubated at ambient temperature for 3 h. The mixture was concentrated under reduced pressure, the residue was dissolved in TFA (5 mL), the resulting solution was incubated at room temperature for 2 h and concentrated under reduced pressure. The residue was triturated with Et_2O (×3). The residual oil was taken up in MeOH (30 mL) and the resulting solution was concentrated under reduced pressure. The residue was triturated with Et_2O (×3) and dried under reduced pressure affording the title product [36 mg, according to ^{1}H-NMR spectrum the product contained 14 mol. % (1 mass %) of MeOH, 49%] as an off-white solid. ^{1}H NMR (300 MHz, CD_3OD) δ 1.31–1.54 (m, 2H), 1.57–2.24 (m, 6H), 2.27–2.47 (m, 2H), 4.26 (m, 2H), 4.44 (t, *J*=7.0 Hz, 2H), 7.11–7.40 (m, 3H), 7.96–8.13 (m, 1H), 8.25 (d, *J*=3.6 Hz, 1H). ^{13}C NMR (75 MHz, CD_3OD) δ 176.5, 176.4, 175.9, 160.6 (d, *J*=247.6 Hz), 160.10, 142.2, 130.9 (d, *J*=9.1 Hz), 128.7 (d, *J*=3.78 Hz), 125.8 (d, *J*=3.8 Hz), 124.8, 119.6 (d, *J*=12.8 Hz), 116.9 (d, *J*=21.9 Hz), 53.9, 53.8, 51.3, 32.9, 31.1 30.9, 28.9, 23.5. MS (ESI): positive mode m/z = 953.3 ([2M + Na]$^+$), 488.2 ([M + Na]$^+$), 466.2 ([M + H]$^+$); MS (ESI): negative mode m/z = 929.3 ([2M – H]$^-$), 464.2 ([M – H]$^-$); ESI HRMS: calcd for $C_{20}H_{24}FN_5O_7Na^+$: 488.1552; found: 488.1548; calcd for $C_{20}H_{25}FN_5O_7^+$: 466.1733; found: 466.1717; calcd for $C_{20}H_{23}FN_5O_7^-$: 464.1587; found: 464.1589.

Radiochemistry

Preparation of n-[^{18}F]FBAs. General procedure 1 – (GP1): Aqueous [^{18}F]fluoride (0.05–10 GBq) was trapped on a anion-exchange resin



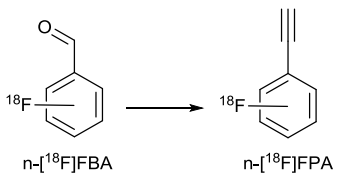
$LG^+ = Me_3N^+$, (4-MeOPh)I^+
$X^- = I^-$, ClO_4^-
n-[^{18}F]FBA

(QMA or Chromafix® 30-PS-HCO_3 cartridge). The cartridge was washed with MeOH (1 mL) and [^{18}F]fluoride was eluted into a reaction vial with a solution of the appropriate precursor (5 mg) in MeOH (0.5 mL). Methanol was evaporated under reduced pressure at 70 °C within 2–3 min. After cooling to room temperature the appropriate solvent (500 µL) was added. The reaction mixture was stirred under the conditions given in Table 1. After cooling to ambient temperature, the reaction mixture was diluted with water (9 mL) and loaded onto a polymer RP cartridge (the cartridge was preconditioned with 2 mL EtOH followed by 30 mL H_2O). The cartridge was washed with 0.1 M HCl (10 mL) and H_2O (10 mL) and the corresponding [^{18}F]fluorobenzaldehyde was eluted with MeOH (0.5 mL). The radiochemical and chemical purities after solid phase extraction (SPE) were > 99%.

Table 1. Preparation of n-[^{18}F]FBAs

Entry	n	LG^+X^-	Solvent	Temperature [°C], Time [min]	RCC ±SD [%]
1	2	$Me_3N^+ClO_4^-$	DMSO	150, 10	80±2
2	3	(4-OMePh)I^+I^-	DMSO	130, 10	40±2
3	4	$Me_3N^+ClO_4^-$	DMSO	150, 10	90±1

Synthesis of n-[^{18}F]FPAs. General procedure 2 – (GP2): n-[^{18}F]FPA was prepared by the reaction of the corresponding n-[^{18}F]FBA (6–12 GBq in 200 µL methanol) with dimethyl (1-diazo-2-oxopropyl)phosphonate solution (10% in MeCN, 200 µL) and potassium carbonate (5 mg, 36 µmol) at 120 °C for 15 min. Remaining [^{18}F]FBA was destroyed by subsequent addition of sodium borohydride (2 mg, 53 µmol). The product was purified by distillation at 80 °C under a flow of helium (20 mL/min) into a trapping vial (cooled below −50 °C).



Preparation of ^{18}F-labeled 1,2,3-triazoles. General procedure 3 – (GP3): To an aqueous solution of $CuSO_4$ (2.5 mg, 10 umol, 50 µL), the corresponding azide (5 mg) dissolved in MeCN (50 µL) was added. Thereafter, a solution of L-histidine (3.9 mg, 25 µmol) in H_2O (50 µL) was added followed by the addition of an aqueous solution of sodium ascorbate (9.8 mg, 50 µmol, 50 µL) and [^{18}F]fluorophenylacetylene (50–500 MBq) in MeCN/MeOH (1:1) (50 µL). The order of the addition of reagents is very important. If it was changed, no product formation could be detected. The reaction mixture was allowed to stir under the conditions given in Table 2 diluted with H_2O (2 mL) and subsequently analyzed by radio-HPLC.

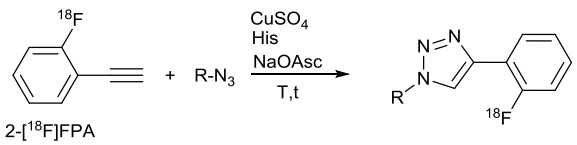
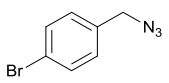
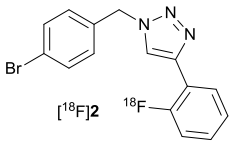
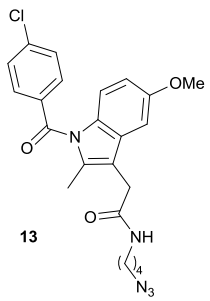
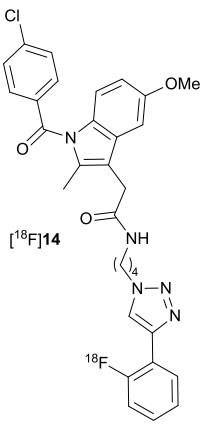
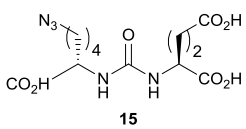
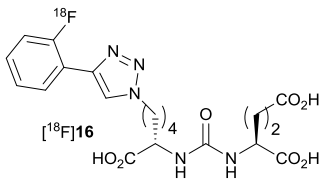
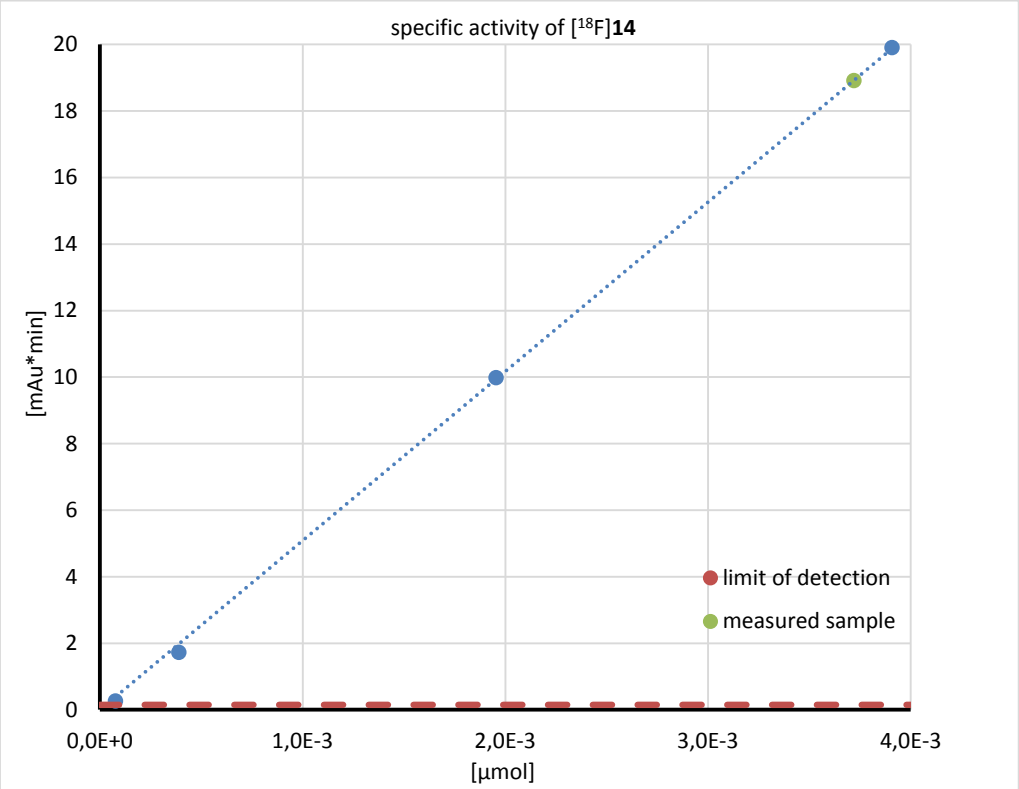


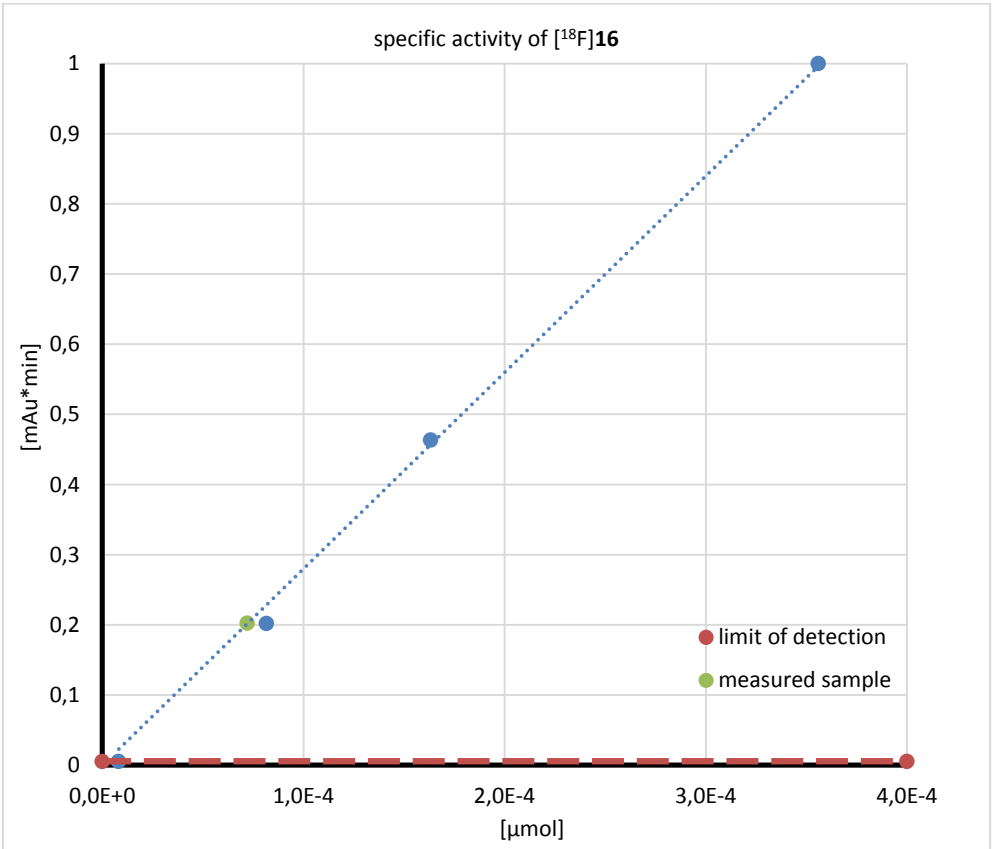
Table 2. Preparation of ^{18}F-labeled compounds using „click"-chemistry conditions.

Entry	Azide	Product	Temperature [°C], Time [min]	RCC±SD [%]
1	 **1**	 [^{18}F]**2**	25, 10	53±5
2	 **13**	 [^{18}F]**14**	50, 20	75±4
3	 **15**	 [^{18}F]**16**	25, 10	84±3

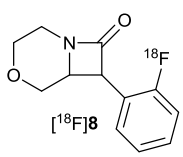
Specific activity calculation of [^{18}F]14 and [^{18}F]16:

The specific activities (GBq/µmol) were calculated by dividing the radioactivity of the radiolabeled product by the amount of the unlabeled tracer determined from the peak area in the UV-HPLC chromatograms (λ= 254 nm for [^{18}F]**14** and [^{18}F]**16**). The amounts of unlabeled compounds were determined from the UV-absorbance/concentration calibration curve. The solutions of [^{18}F]**14** and [^{18}F]**16** obtained after HPLC purification were concentrated under reduced pressure, the residues were redissolved in EtOH/H_2O 1:4 (500 µL) and the resulting solution was completely injected. The specific activity of [^{18}F]**14** and [^{18}F]**16** was 140 GBq/µmol and 232 GBq/µmol, respectively.

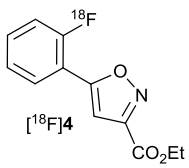




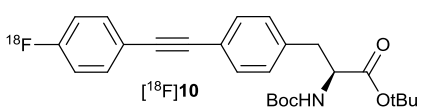
Preparation of 7-(2-[^{18}F]fluorophenyl)-4-oxa-1-azabicyclo[4.2.0]octan-8-one ([^{18}F]8): To a suspension of CuI (9.5 mg, 50 µmol) in MeCN (200 µL), triethylamine (28 µL, 20.4 mg, 0.202 mmol) was added under argon atmosphere and the mixture was allowed to stir for 2 min at ambient temperature. Subsequently, 1,10-phenanthroline (18.0 mg, 100 µmol) and a solution of 2-[^{18}F]fluorophenylacetylene (50–500 MBq) in MeCN/MeOH = 1:1 (150 µL) were added. The reaction mixture was allowed to stir for 2 min at room temperature. Thereafter, the freshly prepared 3,6-dihydro-2H-1,4-oxazine-4-oxide (3 mg, 29.7 µmol) in MeCN (200 µL) was added, the reaction mixture was stirred for 10 min at 90 °C, cooled to ambient temperature and analyzed by HPLC.



Preparation of ethyl 5-(2-[^{18}F]fluorophenyl)isoxazole-3-carboxylate ([^{18}F]4): To a solution of ethyl 2-chloro-2-(hydroxyimino)acetate (10 mg, 66 µmol) and 2-[^{18}F]fluorophenylacetylene (50–500 MBq) in EtOH (400 µL) and triethylamine (10 µL) were added. The reaction mixture was allowed to stir for 30 min at 120 °C, cooled to ambient temperature and analyzed by radio-HPLC.

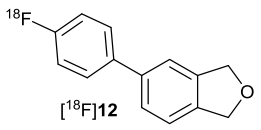


Preparation of (S)-*tert*-butyl 2-((*tert*-butoxycarbonyl)amino)-3-(4-(4-[^{18}F]fluorophenyl)ethynyl)phenylpropanoate ([^{18}F]10): To a suspension of (S)-*tert*-butyl 2-((*tert*-butoxycarbonyl)amino)-3-(4-iodophenyl)-propanoate (35 mg, 78 µmol) in H_2O (150 µL), an aqueous solution of $PdCl_2$ (0.35 mg, 2 µmol) (100 µL) and pyrrolidine (100 µL) were added and allowed to stir at 50 °C for



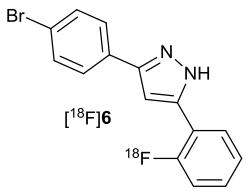
5 min. Afterwards 2-[^{18}F]fluorophenylacetylene (50–500 MBq) in MeCN (150 µL) was added, the reaction mixture was stirred at 120 °C for 10 min, cooled to ambient temperature and analyzed by Radio-HPLC.

Preparation of 5-(4-[^{18}F]fluorophenyl)-1,3-dihydroisobenzofuran ([^{18}F]12): To a solution of tris(triphenylphosphine)rhodium(I) chloride (1 mg, 1.1 µmol) and 4-[^{18}F]fluorophenylacetylene (50–500 MBq) in EtOH (400 µL) was added and the mixture was allowed to stir at 40 °C for 5 min. Thereafter, propargyl ether (20 mg, 212 µmol) was added and the reaction mixture was stirred at 120 °C for 10 min, cooled to ambient temperature and analyzed by radio-HPLC.



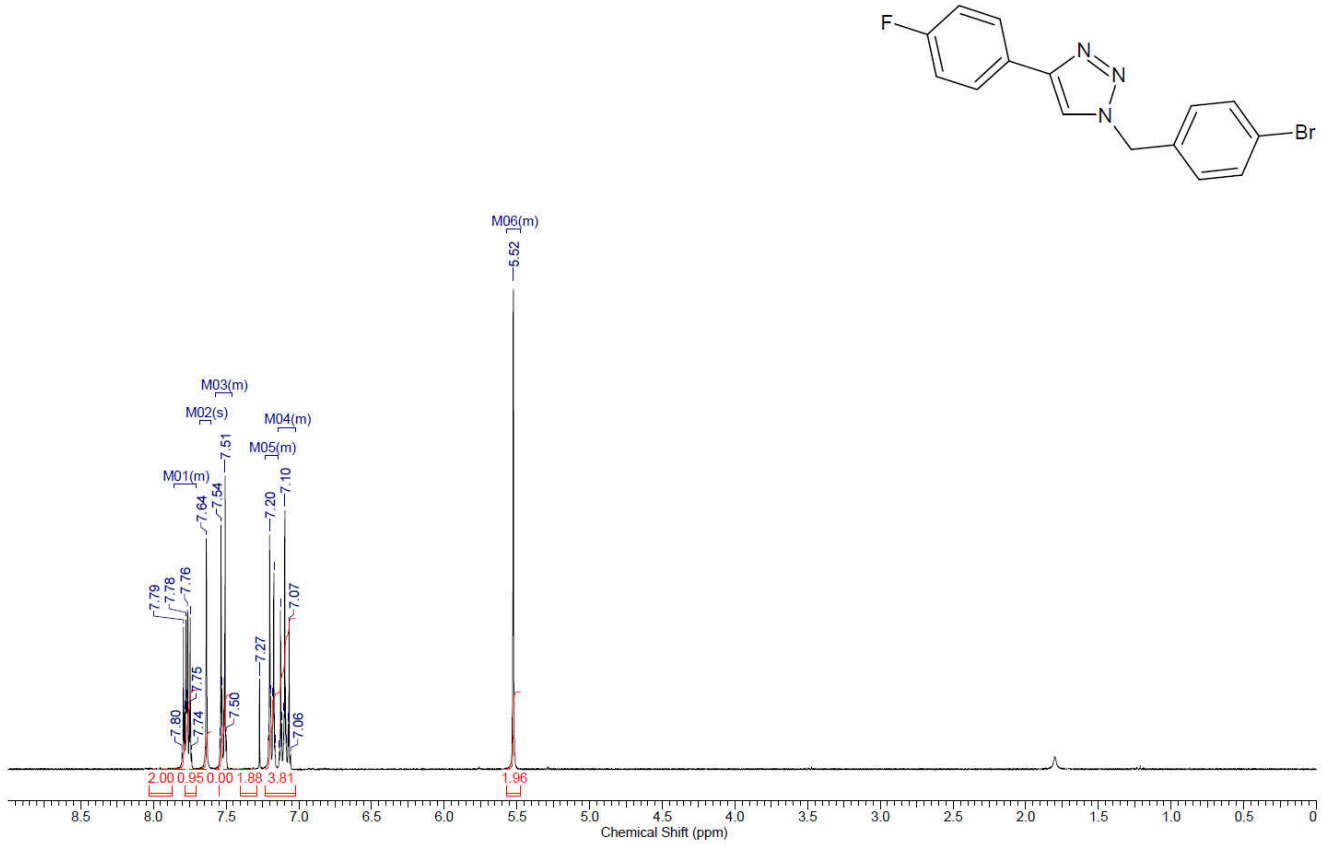
[^{18}F]**12**

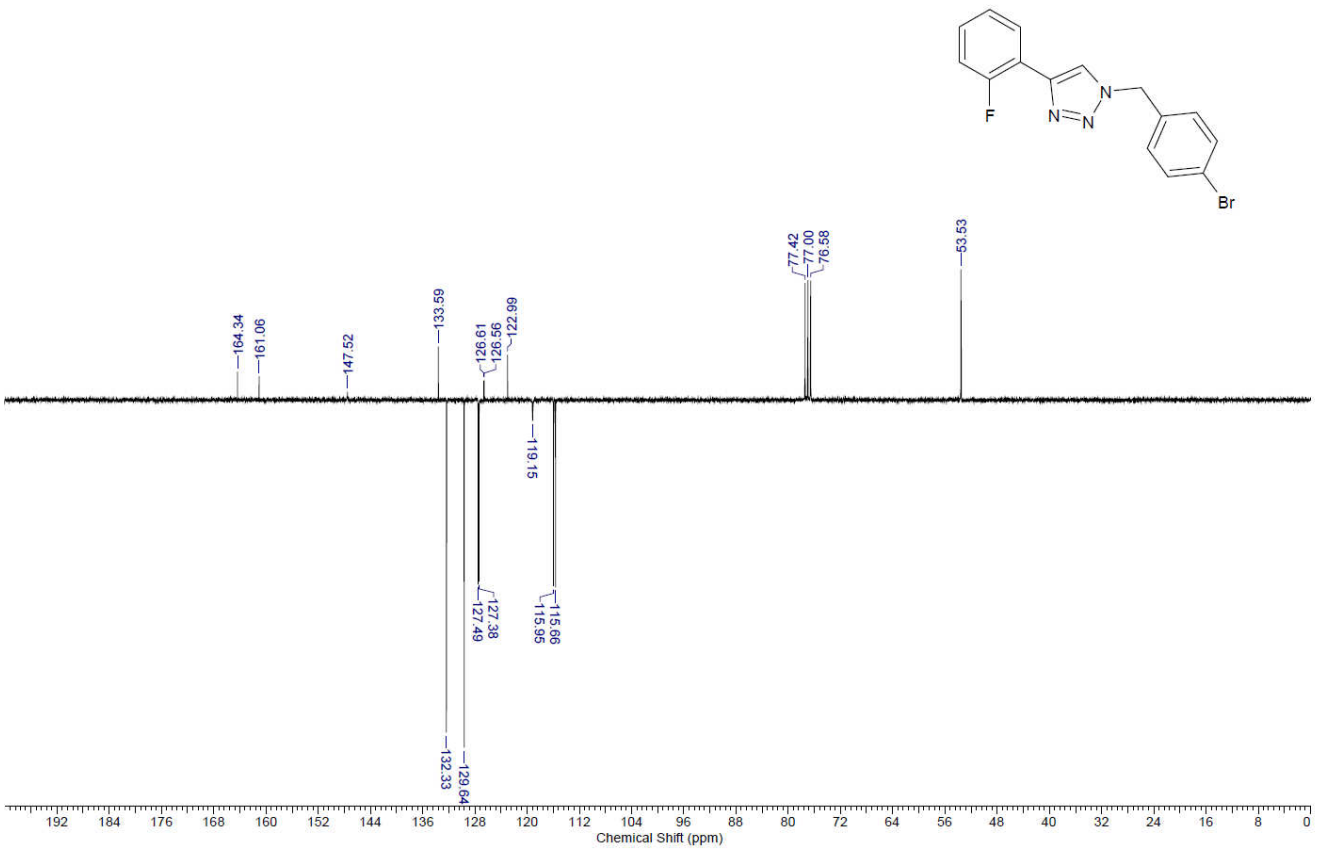
Preparation of 3-(2-bromobenzyl)-5-(2-[^{18}F]fluorophenyl)-3*H*-pyrazole ([^{18}F]6): To a stirred suspension of K_2CO_3 (8 mg, 58 µmol) in a solution of *N´*-(4-bromobenzylidene)-4-methylbenzensulfonohydrazide (10 mg, 28 µmol), 2-[^{18}F]fluorophenylacetylene (50–500 MBq) in dioxane (400 µL) was added and the reaction mixture was stirred at 150 °C for 30 min, cooled to ambient temperature and analyzed by radio-HPLC.

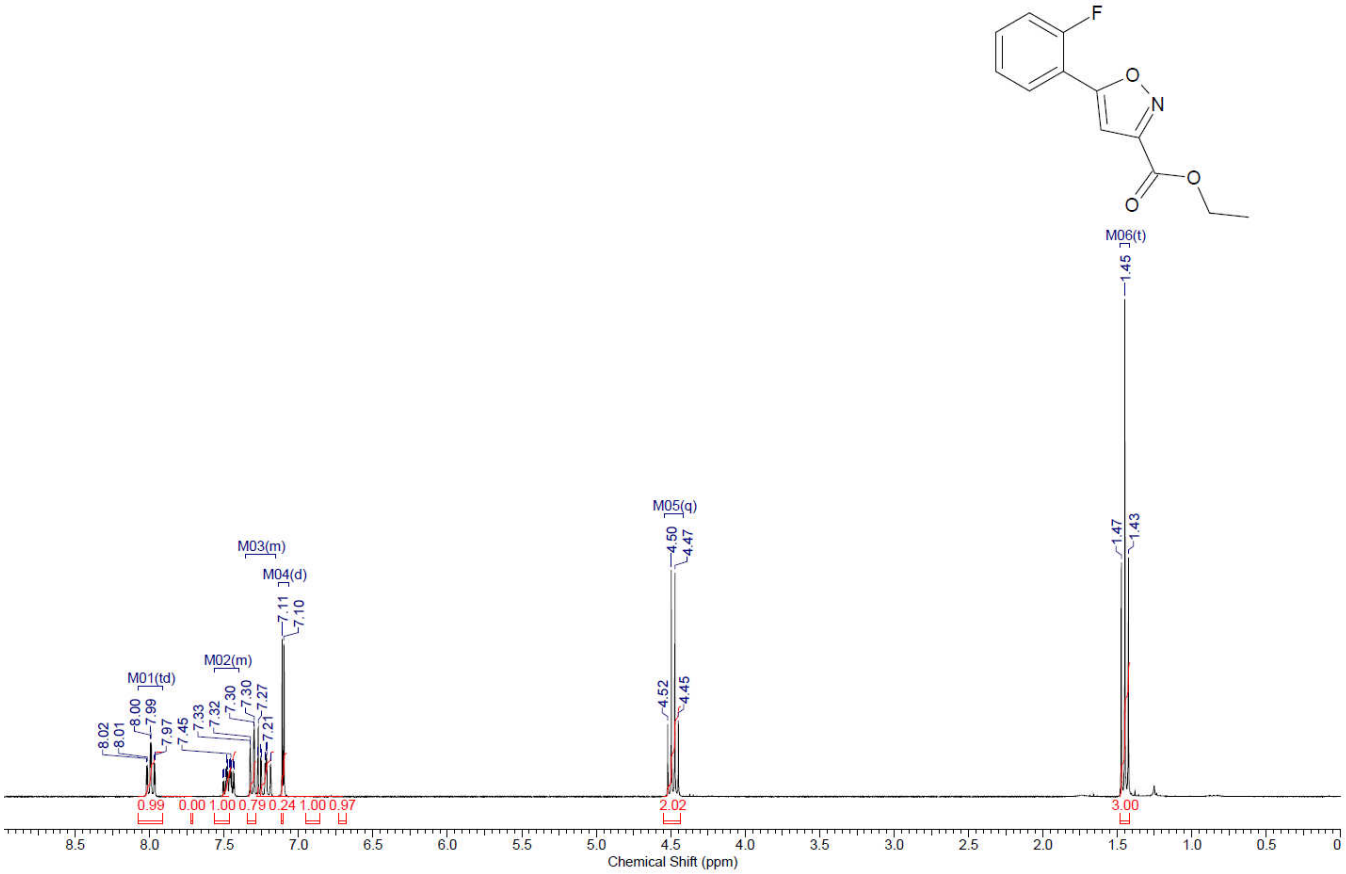


[^{18}F]**6**

^{1}H- and APT-NMR



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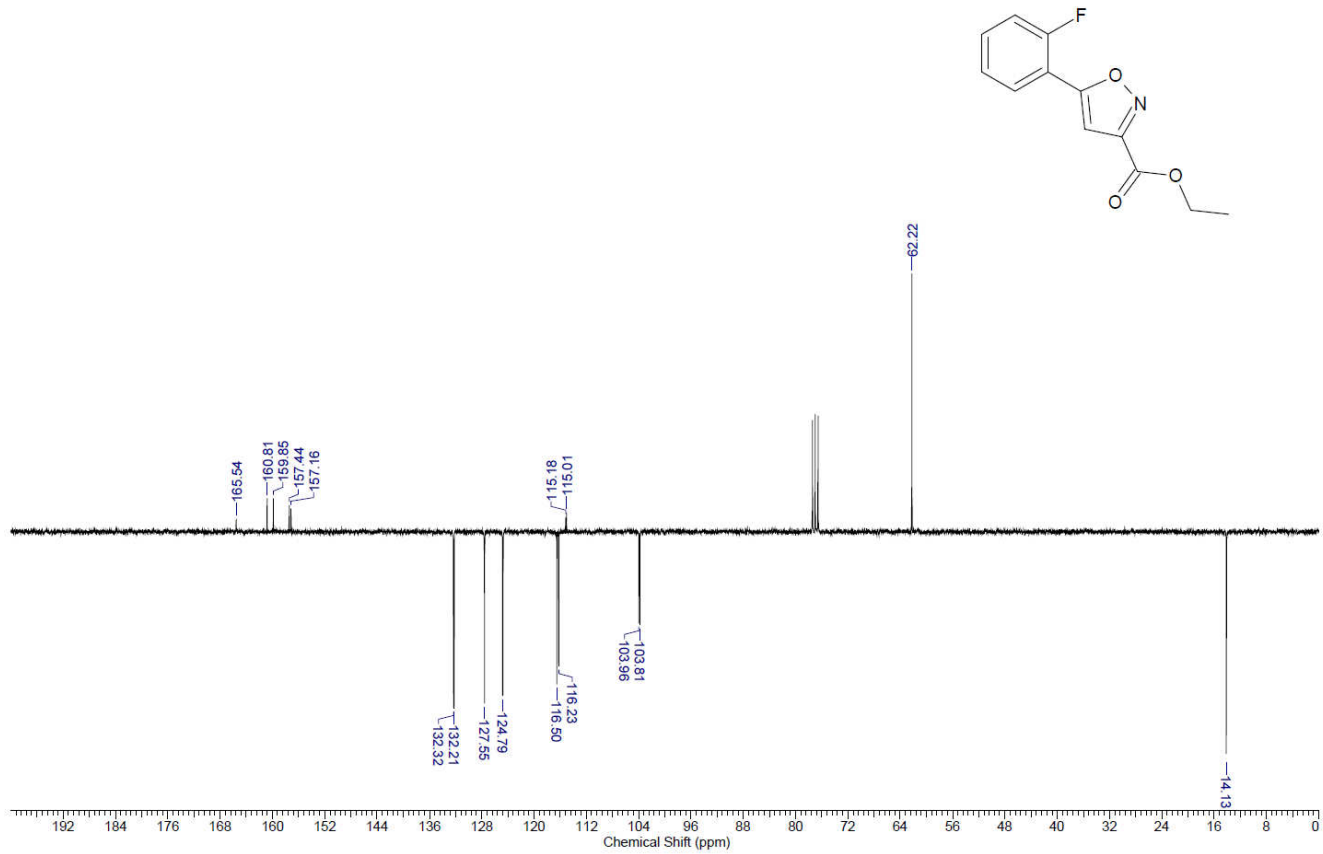
O
N
O
O
M06(t)
1.45
M05(q)
4.50
4.47
M03(m)
M04(d)
7.11
7.10
M02(m)
M01(td)
8.02
8.01
8.00
7.99
7.97
7.45
7.33
7.32
7.30
7.27
7.21
4.52
4.45
1.47
1.43
0.99 0.00 1.00 0.79 0.24 1.00 0.97
2.02
3.00
Chemical Shift (ppm)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N
O
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159.85
157.44
157.16
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115.01
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103.96
116.23
116.50
132.21
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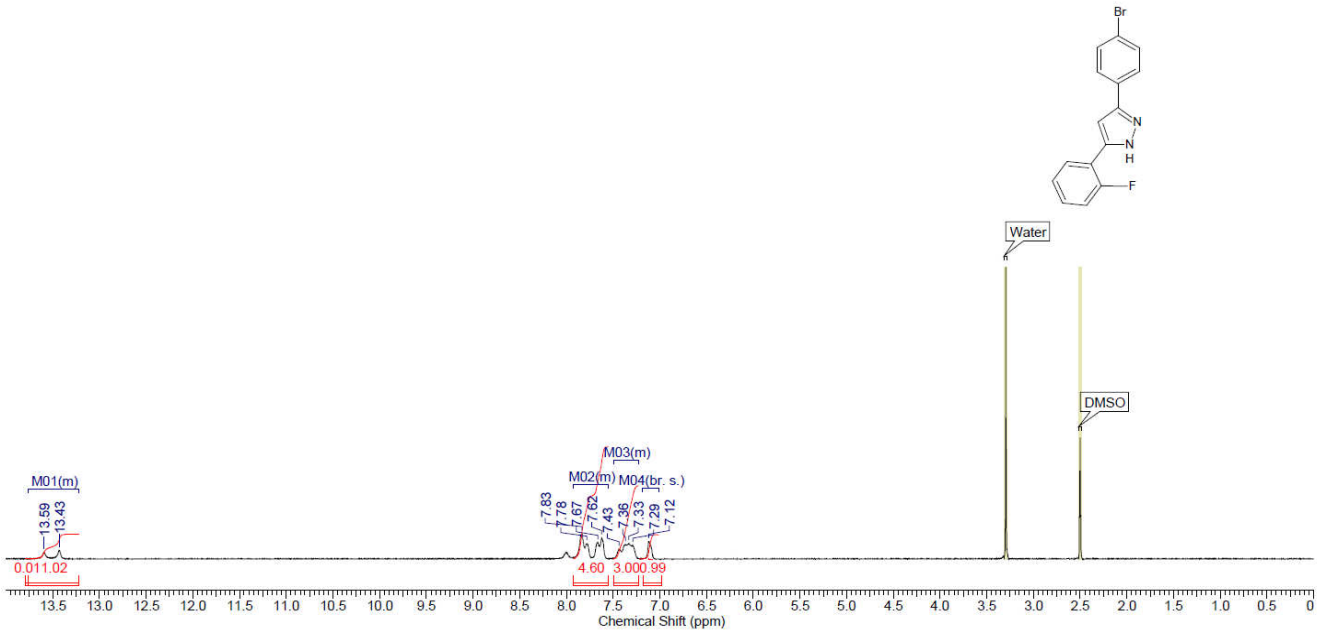
127.55
124.79
14.13
Chemical Shift (ppm)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N
N
H
F
Water
DMSO
M01(m)
M02(m)
M03(m)
M04(br. s.)
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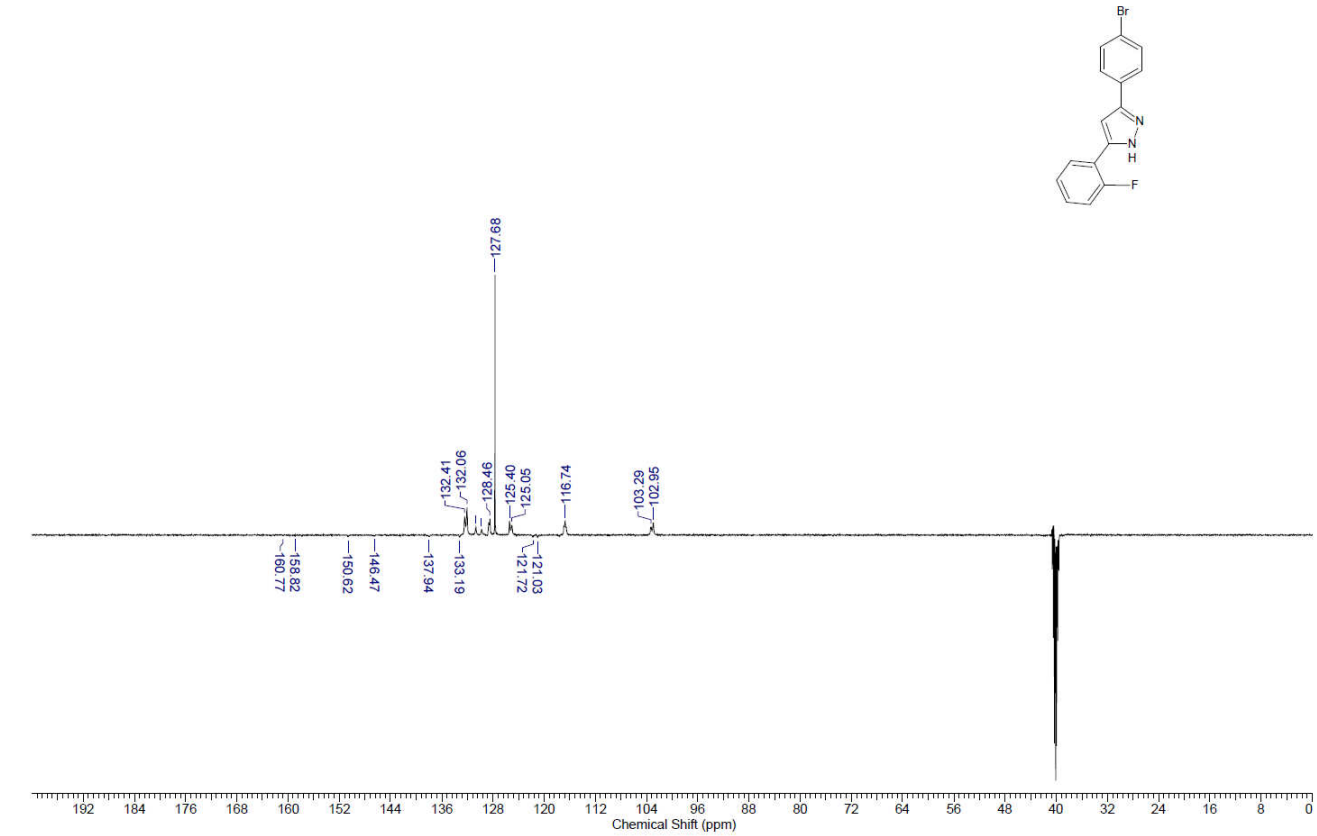
7.78
7.67
7.62
7.43
7.36
7.33
7.29
7.12
0.01
1.02
4.60
3.00
0.99
Chemical Shift (ppm)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N
N
H
F
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132.06
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125.05
116.74
103.29
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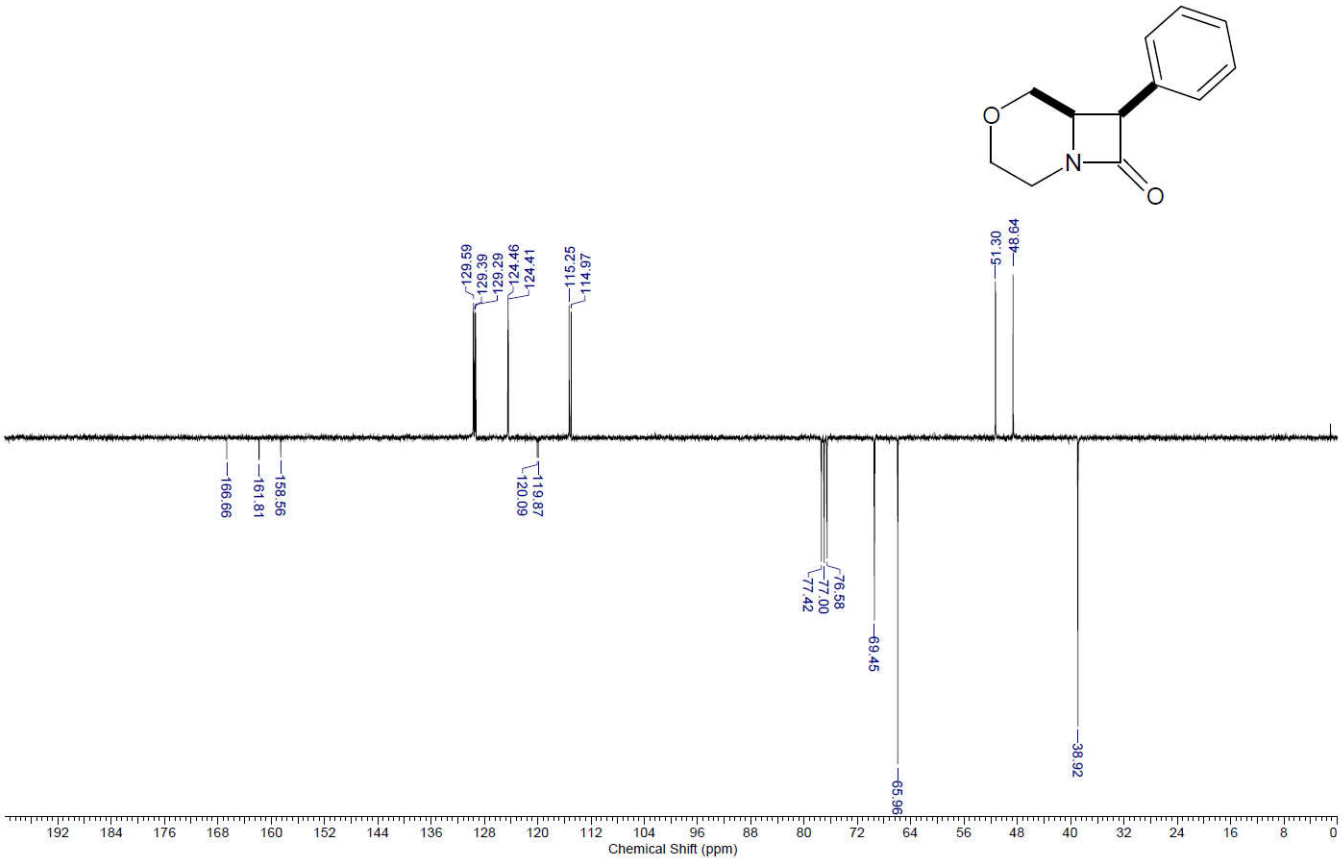
158.82
150.62
146.47
137.94
133.19
121.72
121.03
Chemical Shift (ppm)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N
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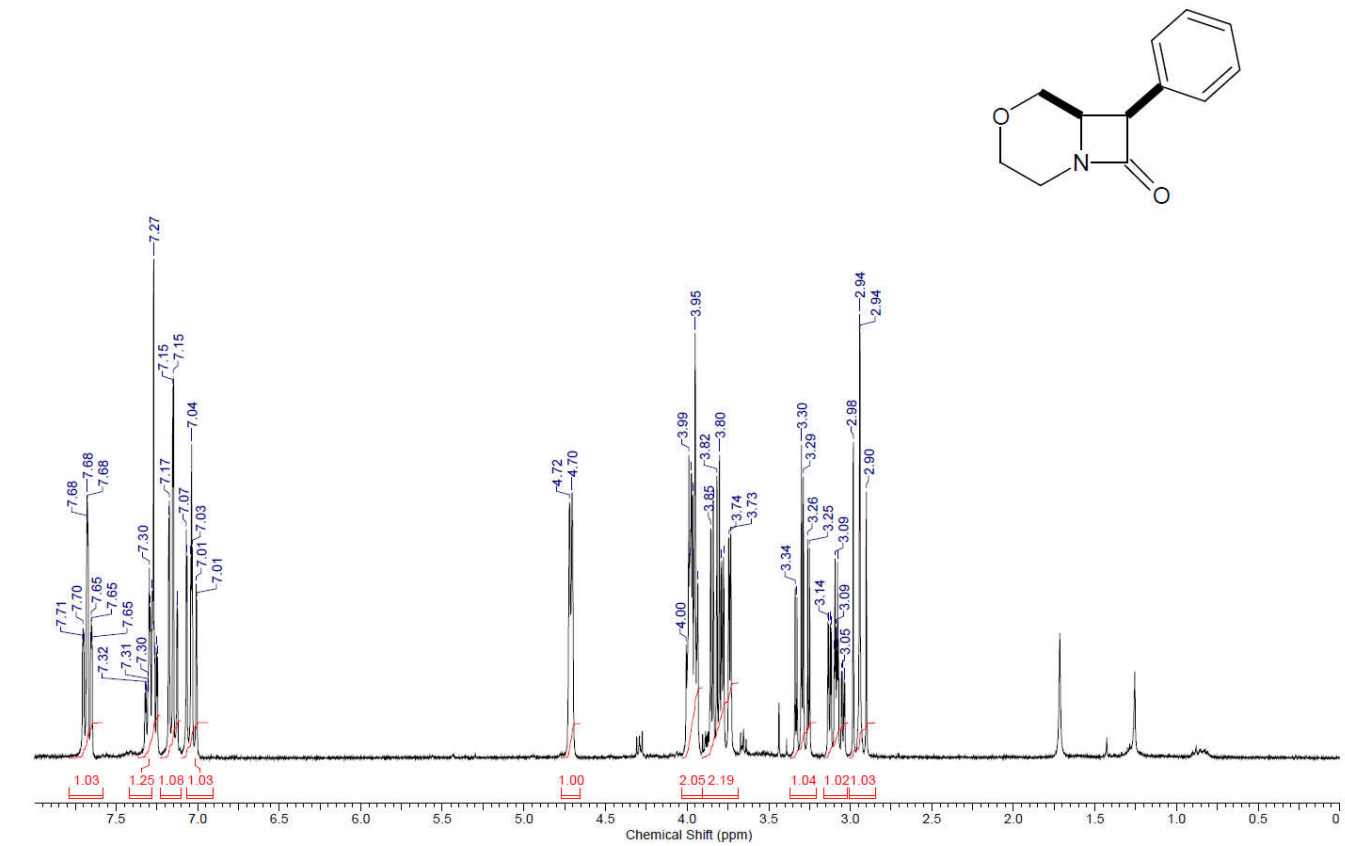
124.41
115.25
114.97
51.30
48.64
166.66
161.81
158.56
120.09
119.87
77.42
77.00
76.58
69.45
66.96
38.92
192 184 176 168 160 152 144 136 128 120 112 104 96 88 80 72 64 56 48 40 32 24 16 8 0
Chemical Shift (ppm)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N
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7.65
7.65
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7.32
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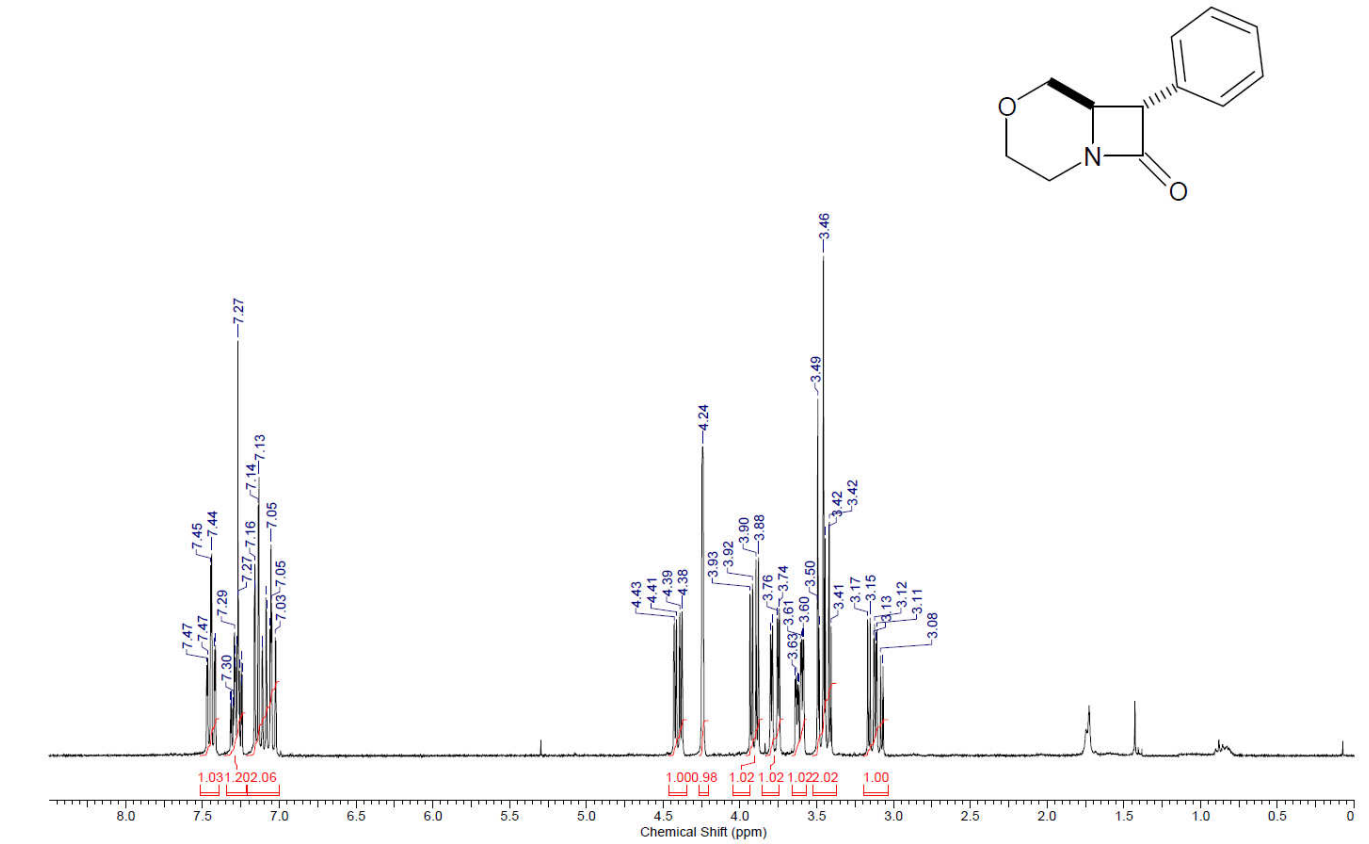
7.30
7.30
7.15
7.15
7.17
7.07
7.04
7.03
7.01
7.01
4.72
4.70
3.95
3.99
3.82
3.80
3.85
3.74
3.73
4.00
3.30
3.29
3.34
3.26
3.25
3.14
3.09
3.09
3.05
2.94
2.94
2.98
2.90
1.03 1.25 1.08 1.03 1.00 2.05 2.19 1.04 1.02 1.03
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0
Chemical Shift (ppm)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N
O
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7.44
7.30
7.29
7.27
7.27
7.16
7.14
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7.05
7.05
7.03
4.43
4.41
4.39
4.38
4.24
3.93
3.92
3.90
3.88
3.76
3.74
3.63
3.61
3.60
3.50
3.49
3.46
3.42
3.42
3.41
3.17
3.15
3.13
3.12
3.11
3.08
1.03
1.20
2.06
1.00
0.98
1.02
1.02
1.02
2.02
1.00
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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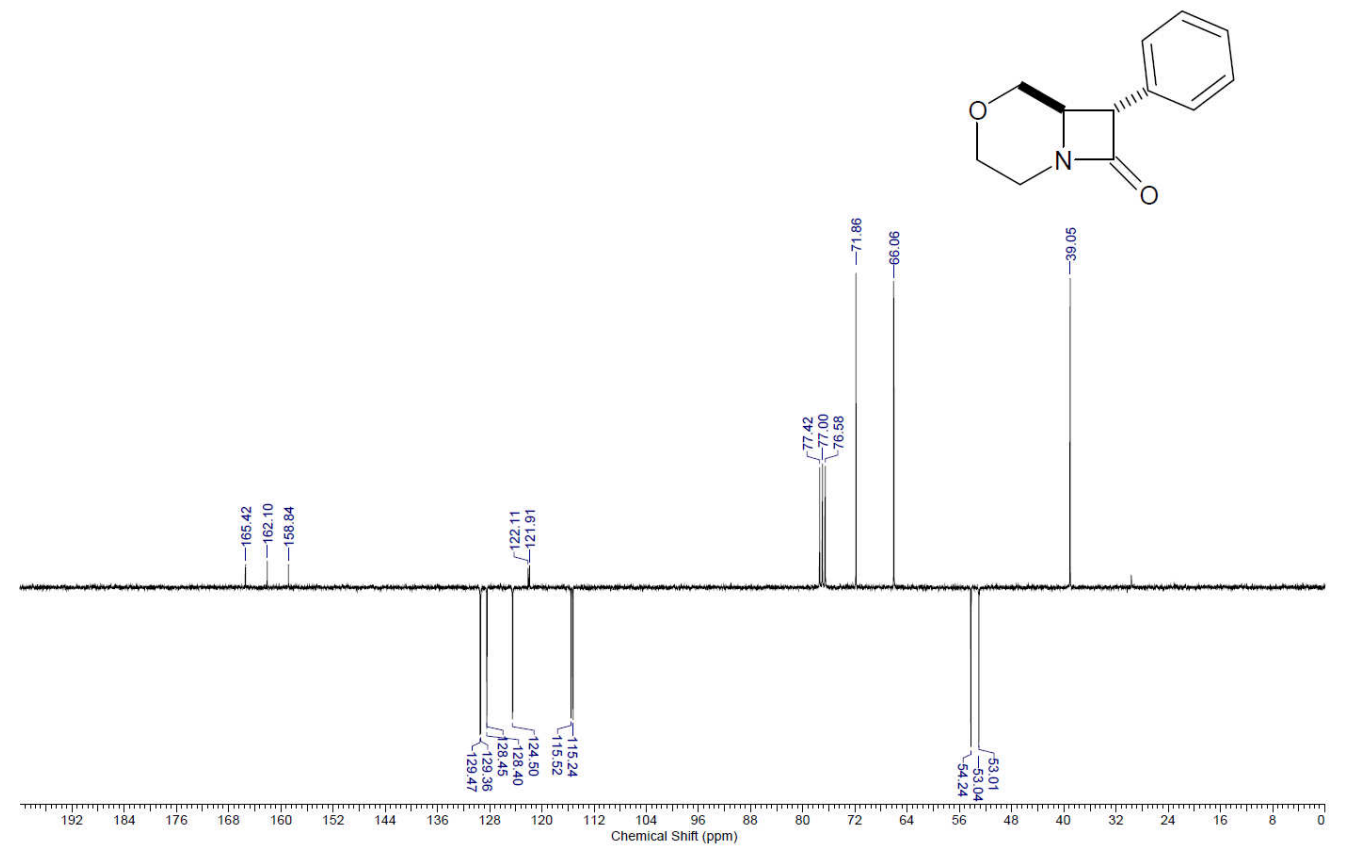
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Chemical Shift (ppm)


O
N
O
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158.84
122.11
121.91
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76.58
71.86
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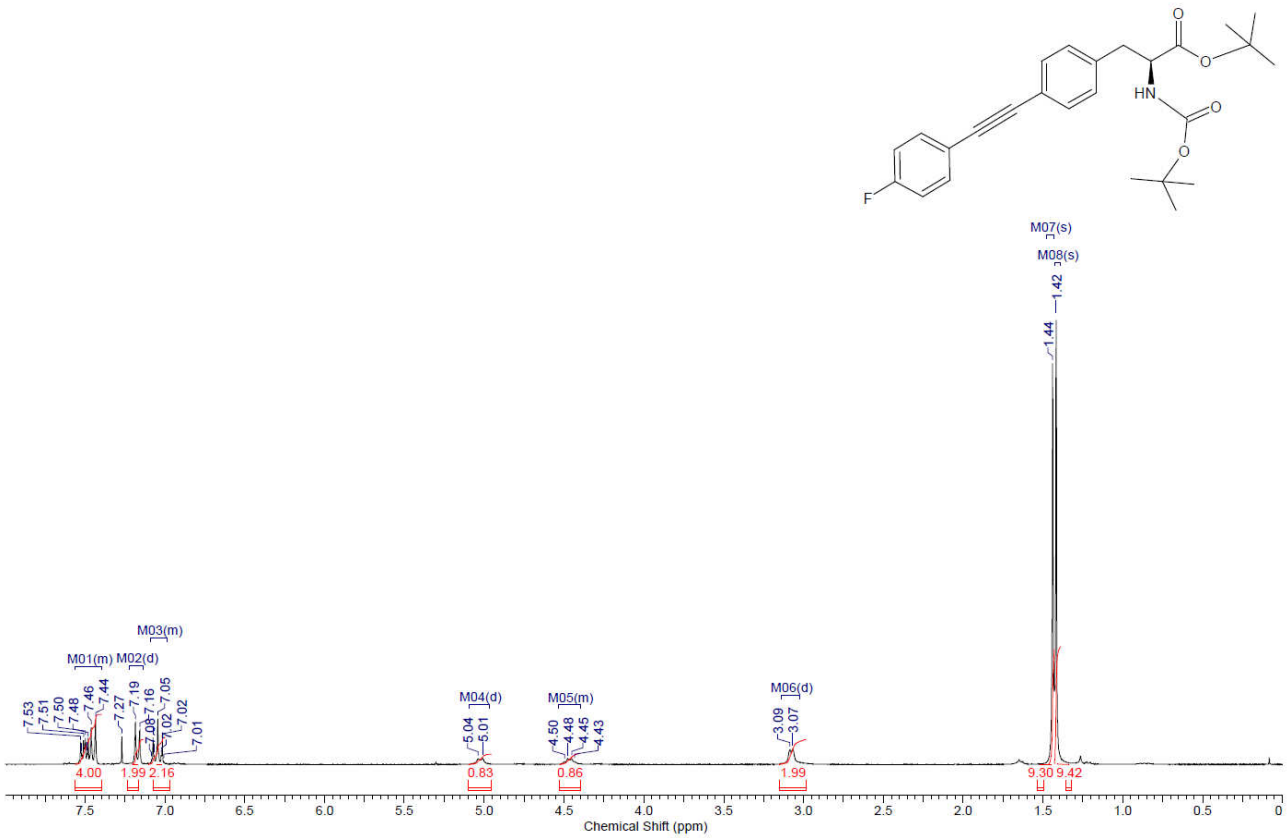
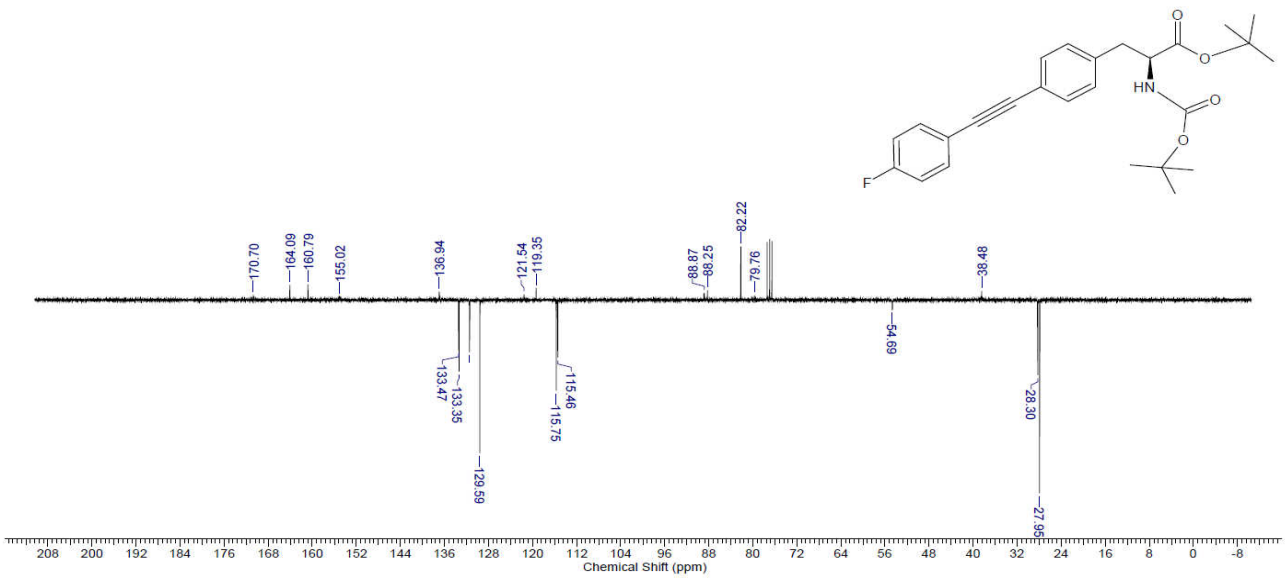
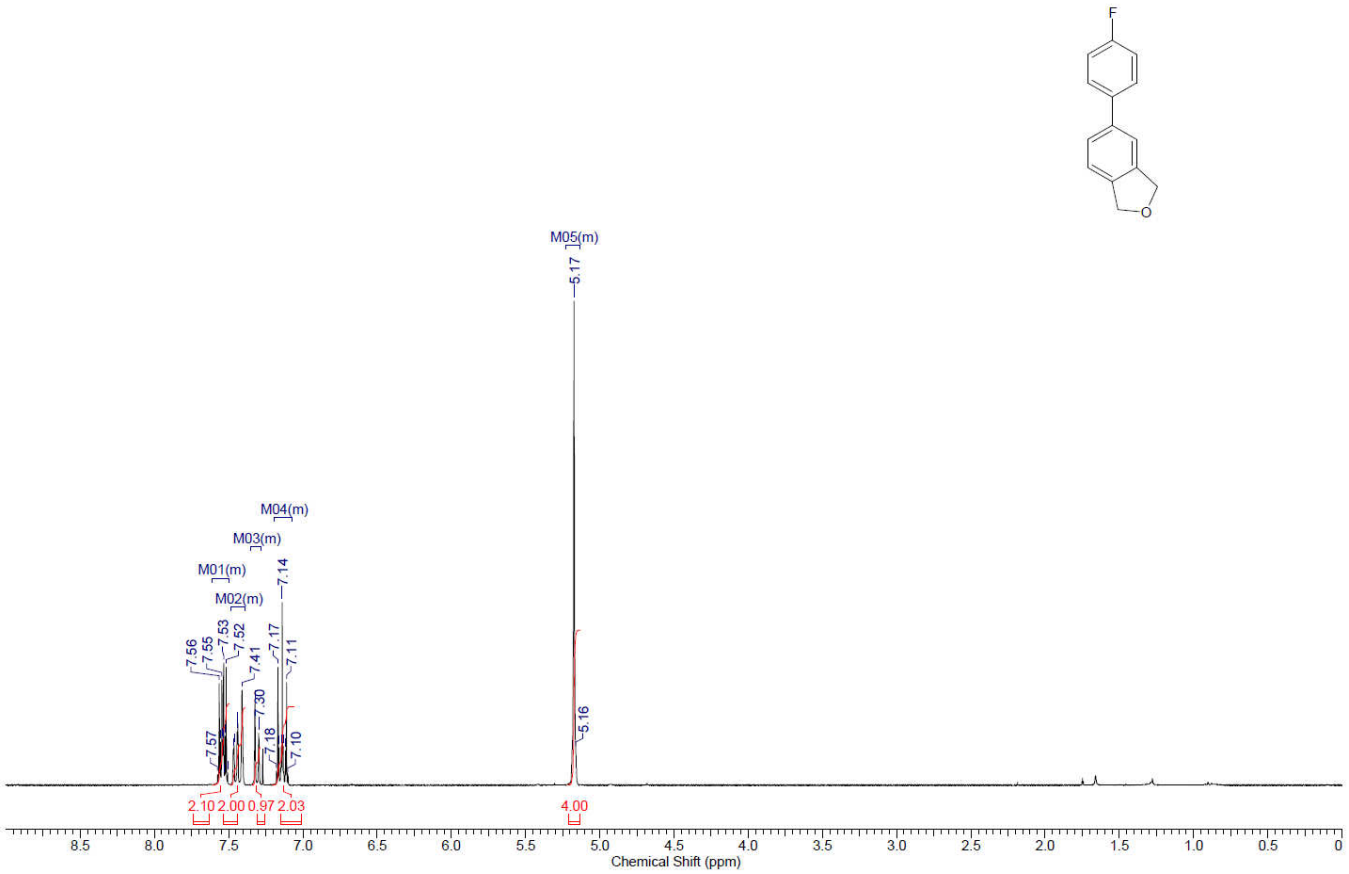
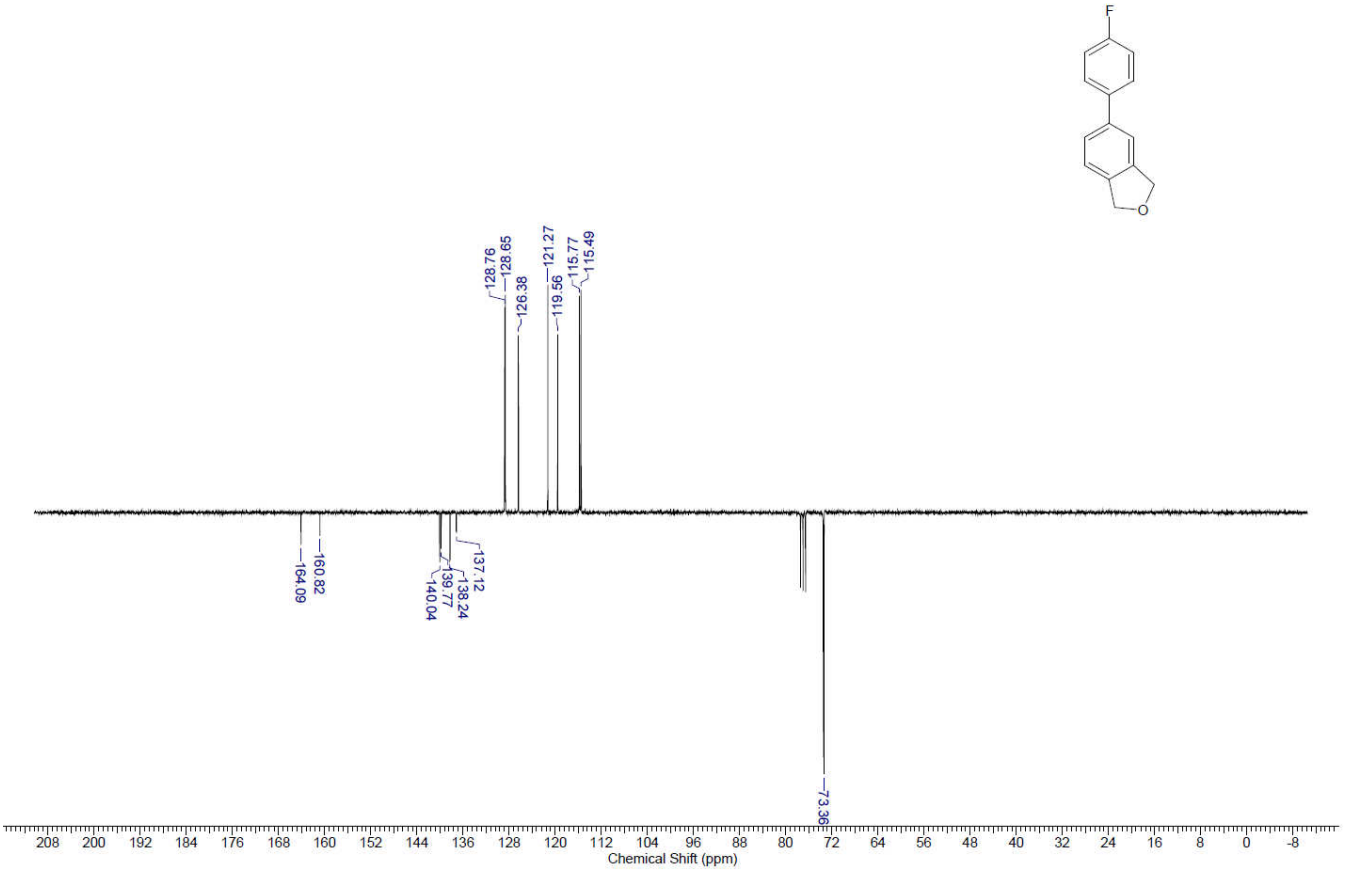
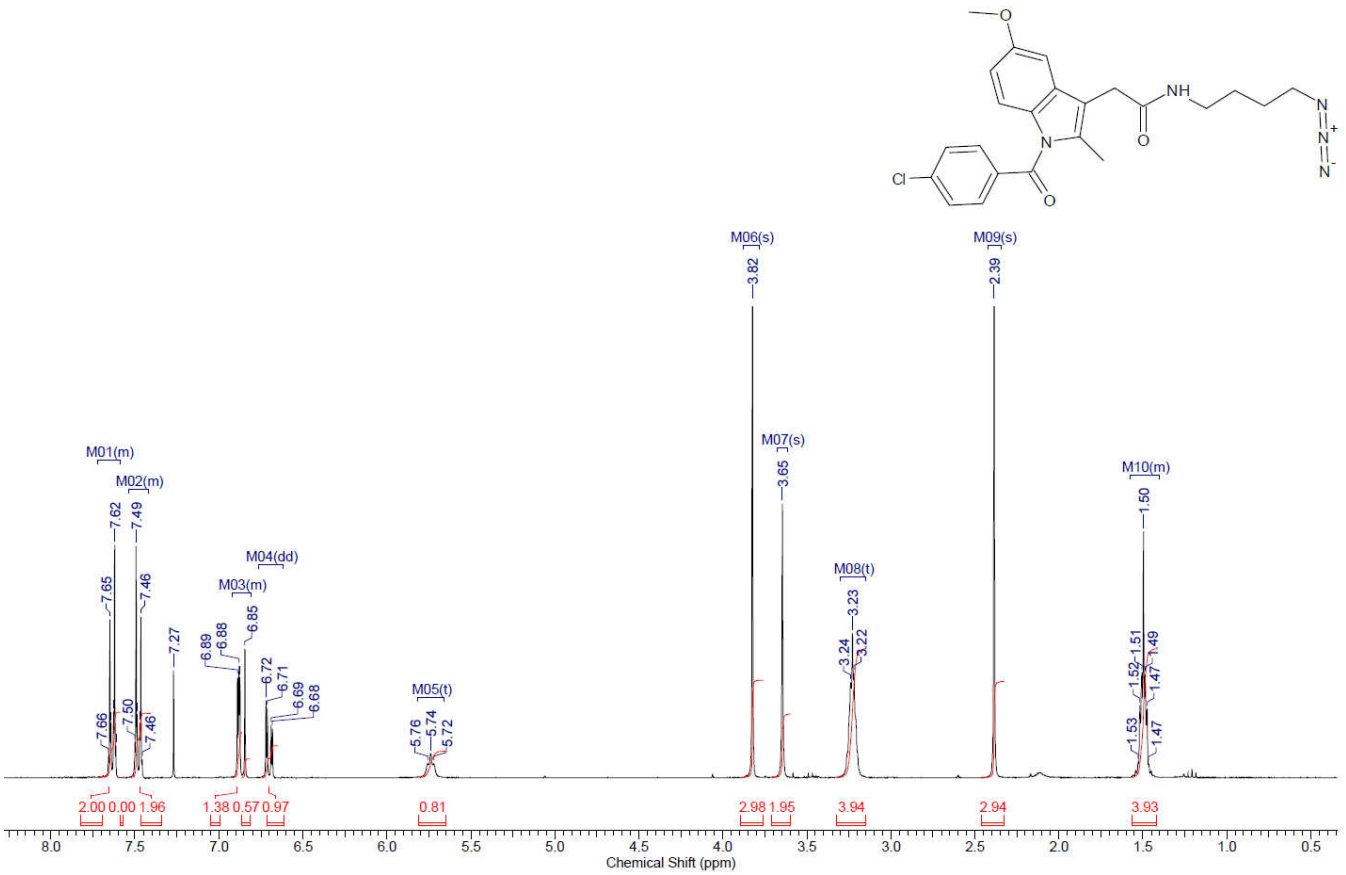
129.47
129.36
128.45
128.40
124.50
115.52
115.24
54.24
53.04
53.01
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0
Chemical Shift (ppm)

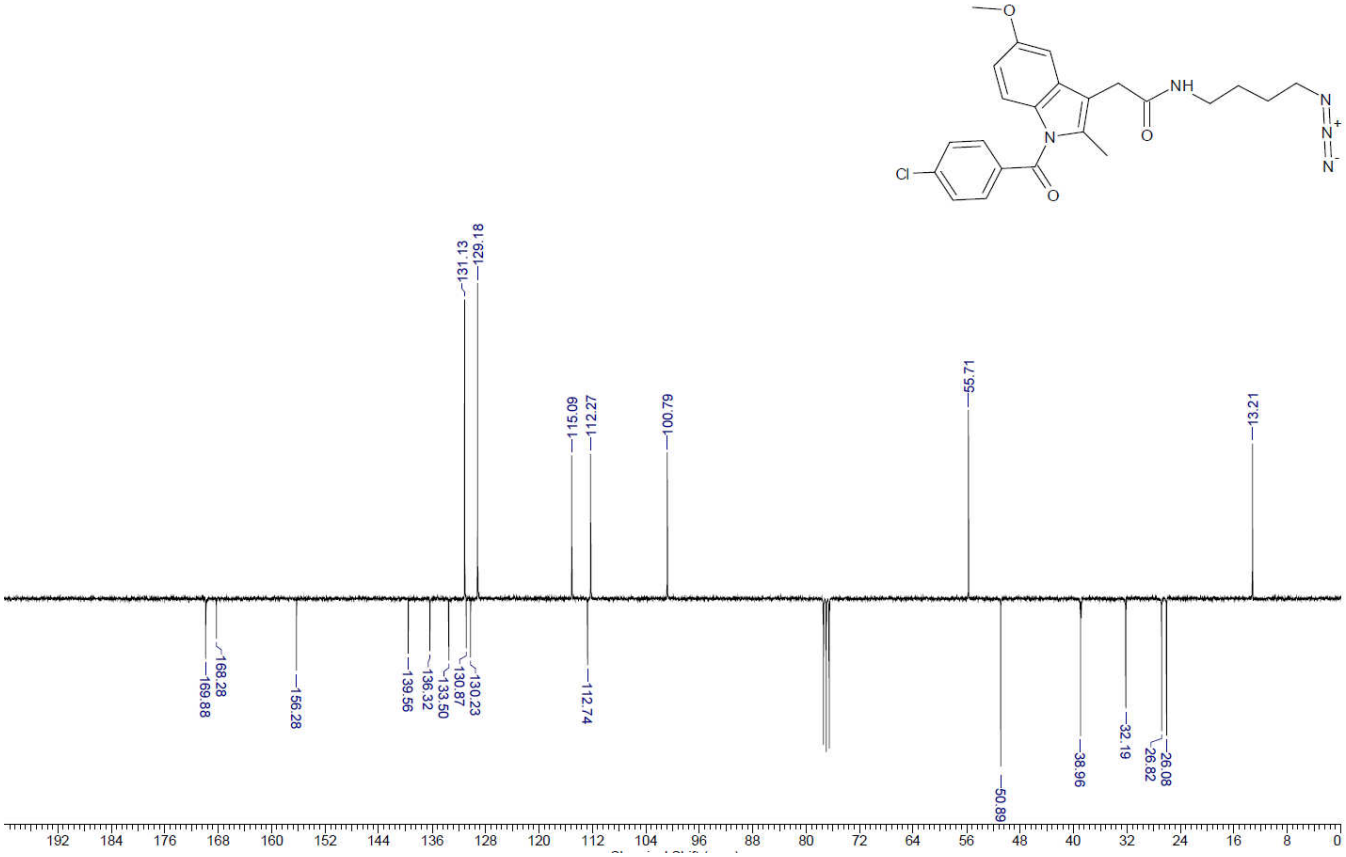

M07(s)
M08(s)
1.42
1.44
M03(m)
M01(m)
M02(d)
7.53
7.51
7.50
7.48
7.46
7.44
7.27
7.19
7.16
7.08
7.05
7.02
7.01
M04(d)
5.04
5.01
M05(m)
4.50
4.48
4.45
4.43
M06(d)
3.09
3.07
4.00
1.99
2.16
0.83
0.86
1.99
9.30
9.42
Chemical Shift (ppm)

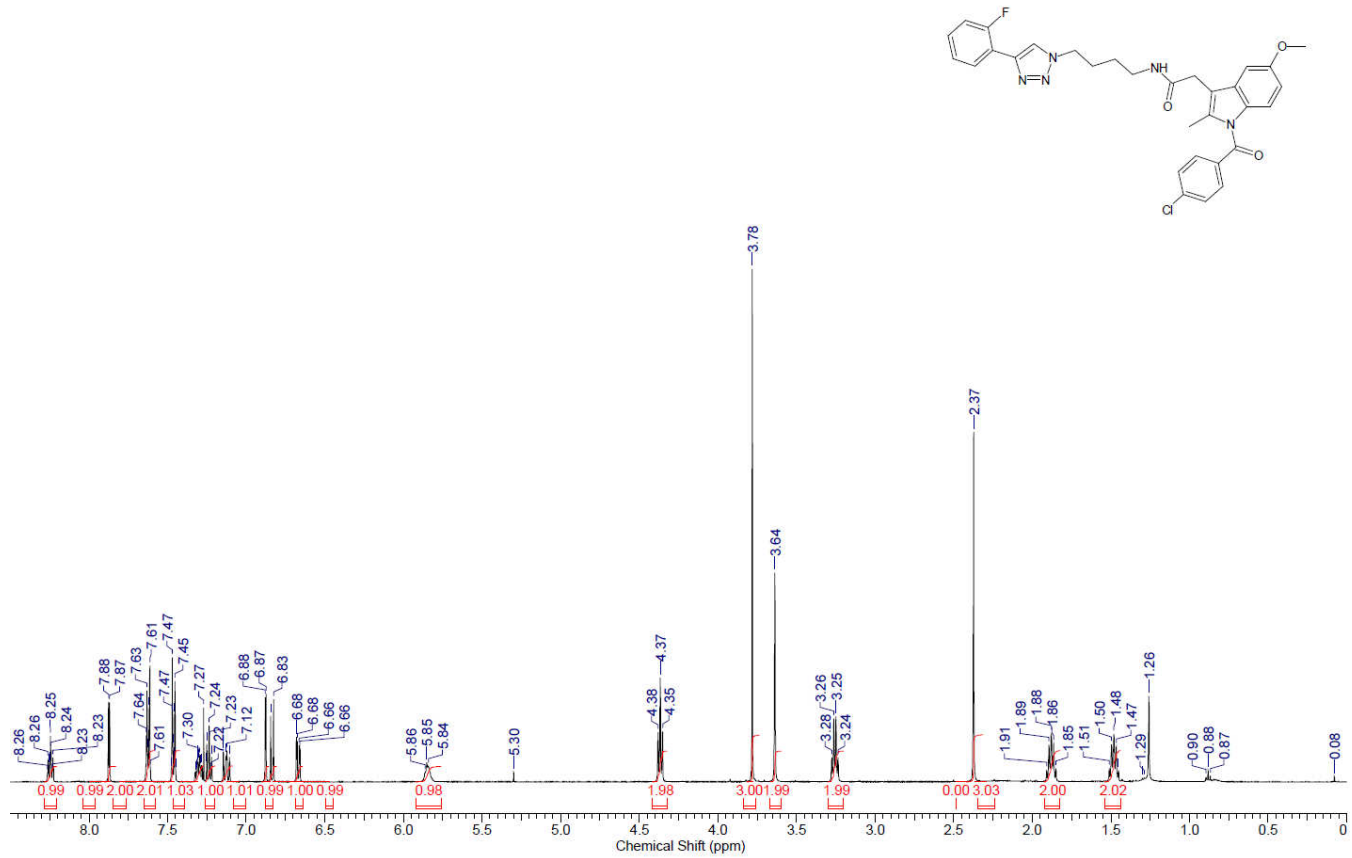

170.70
164.09
160.79
155.02
136.94
133.47
133.35
129.59
121.54
119.35
115.46
115.75
88.87
88.25
82.22
79.76
54.69
38.48
28.30
27.96
Chemical Shift (ppm)

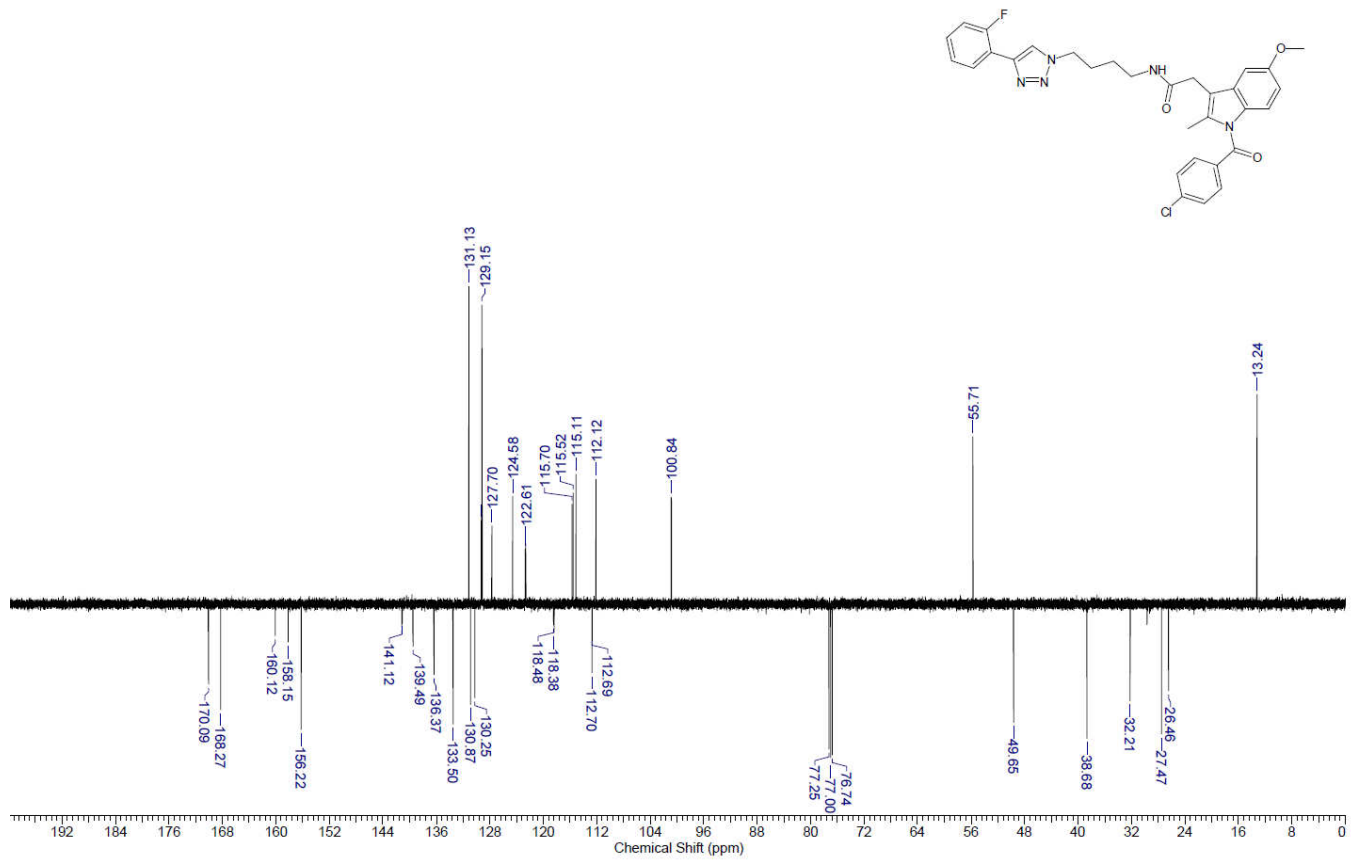

F
O
M05(m)
5.17
M04(m)
M03(m)
M01(m)
M02(m)
7.14
7.56
7.55
7.53
7.52
7.41
7.17
7.11
7.30
7.67
7.18
7.10
5.16
2.10 2.00 0.97 2.03
4.00
Chemical Shift (ppm)

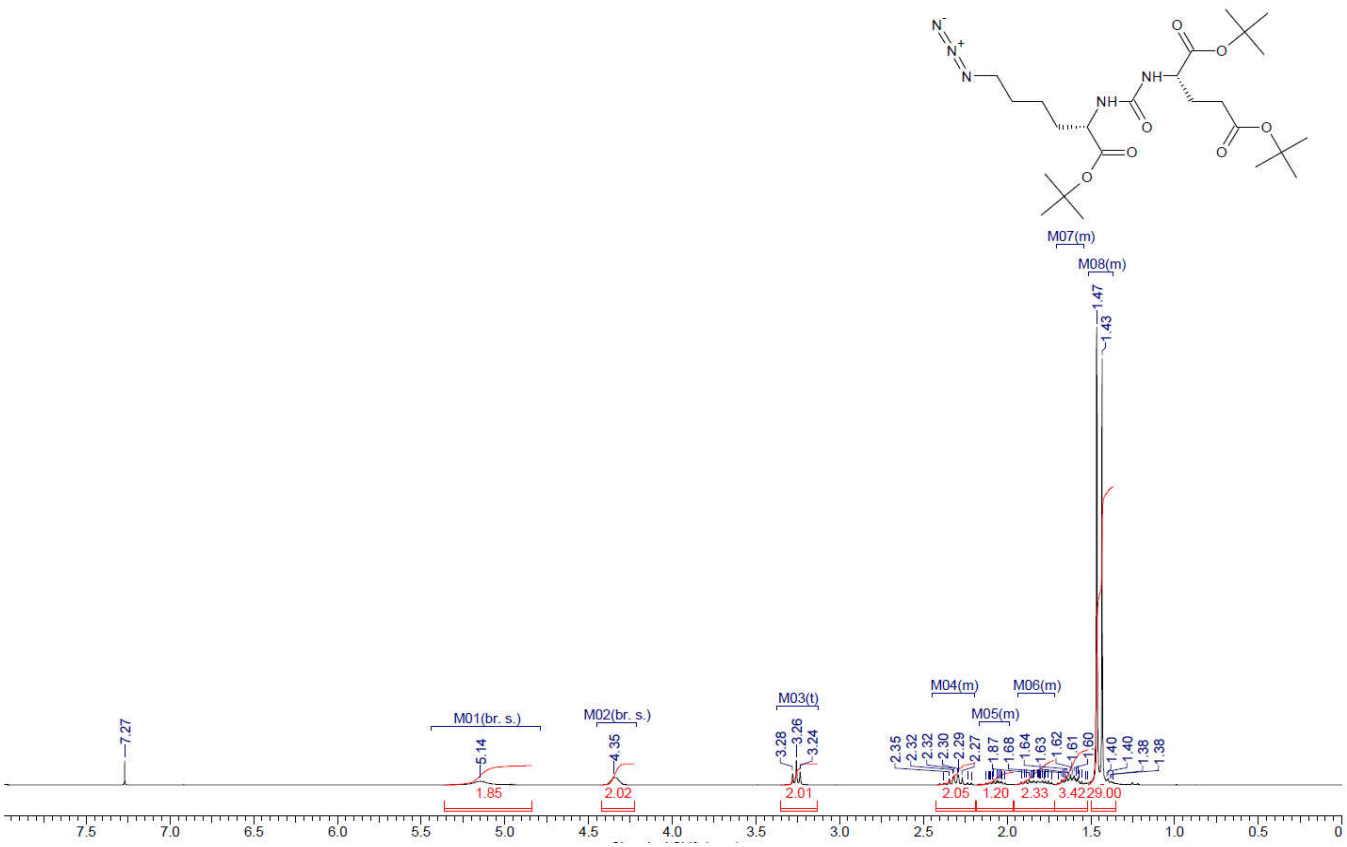

F
O
128.76
128.65
126.38
121.27
119.56
115.77
115.49
164.09
160.82
140.04
139.77
138.24
137.12
73.36
Chemical Shift (ppm)

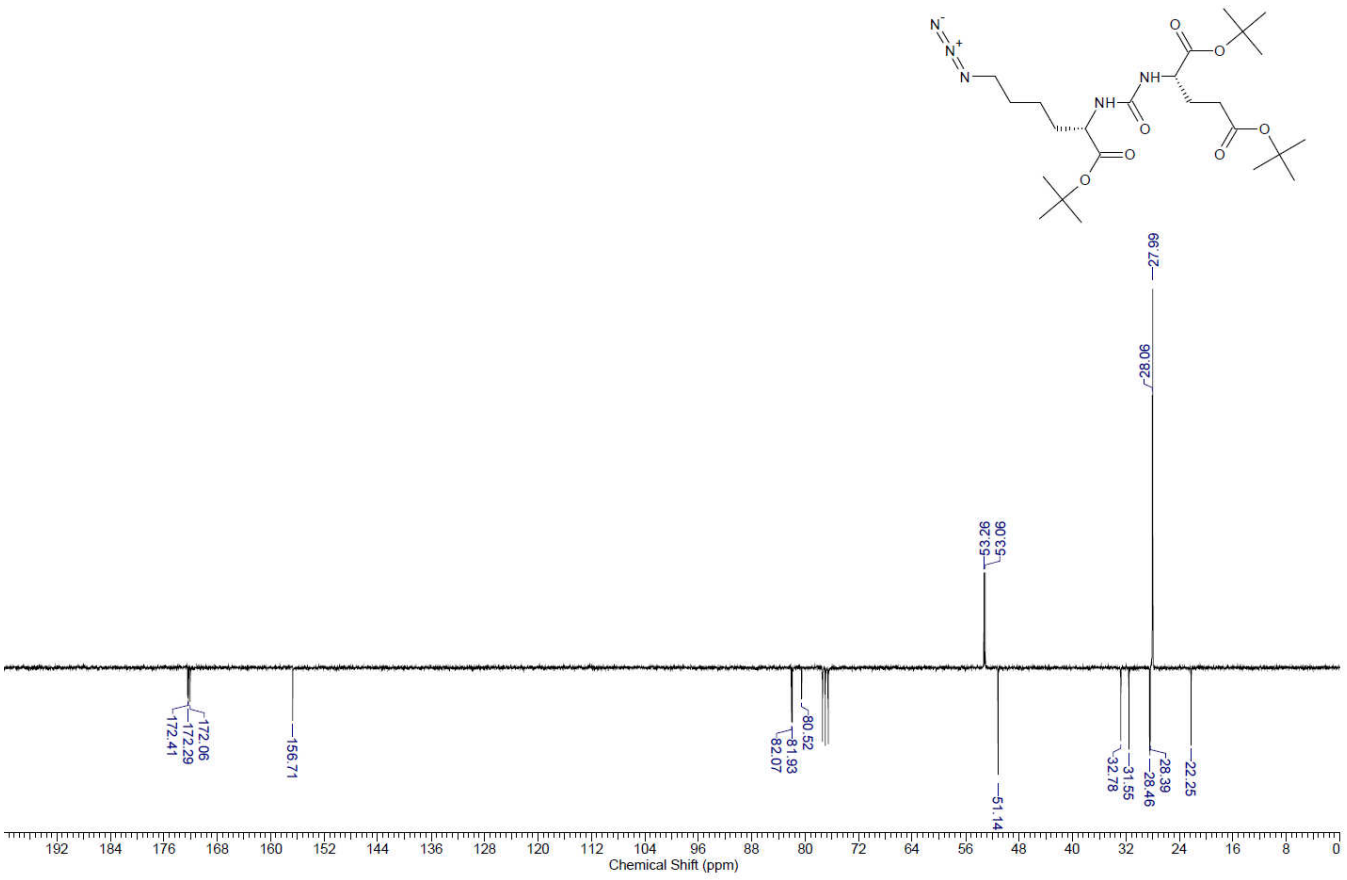

M01(m)
M02(m)
M03(m)
M04(dd)
M05(t)
M06(s)
M07(s)
M08(t)
M09(s)
M10(m)
7.66
7.65
7.62
7.50
7.49
7.46
7.46
7.27
6.89
6.88
6.85
6.72
6.71
6.69
6.68
5.76
5.74
5.72
3.82
3.65
3.24
3.23
3.22
2.39
1.53
1.52
1.51
1.50
1.49
1.47
1.47
2.00 0.00 1.96
1.38 0.57 0.97
0.81
2.98 1.95
3.94
2.94
3.93
Chemical Shift (ppm)

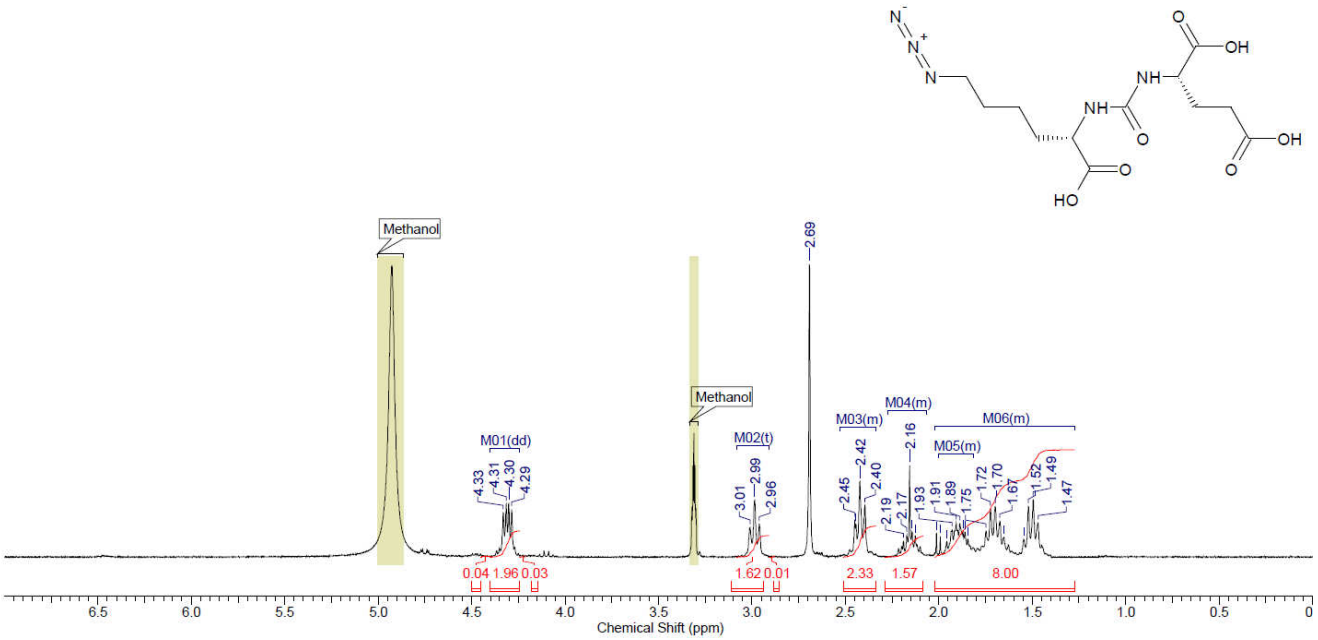

131.13
129.18
115.09
112.27
100.79
55.71
13.21
169.88
168.28
156.28
139.56
136.32
133.50
130.87
130.23
112.74
50.88
38.96
32.19
26.82
26.08

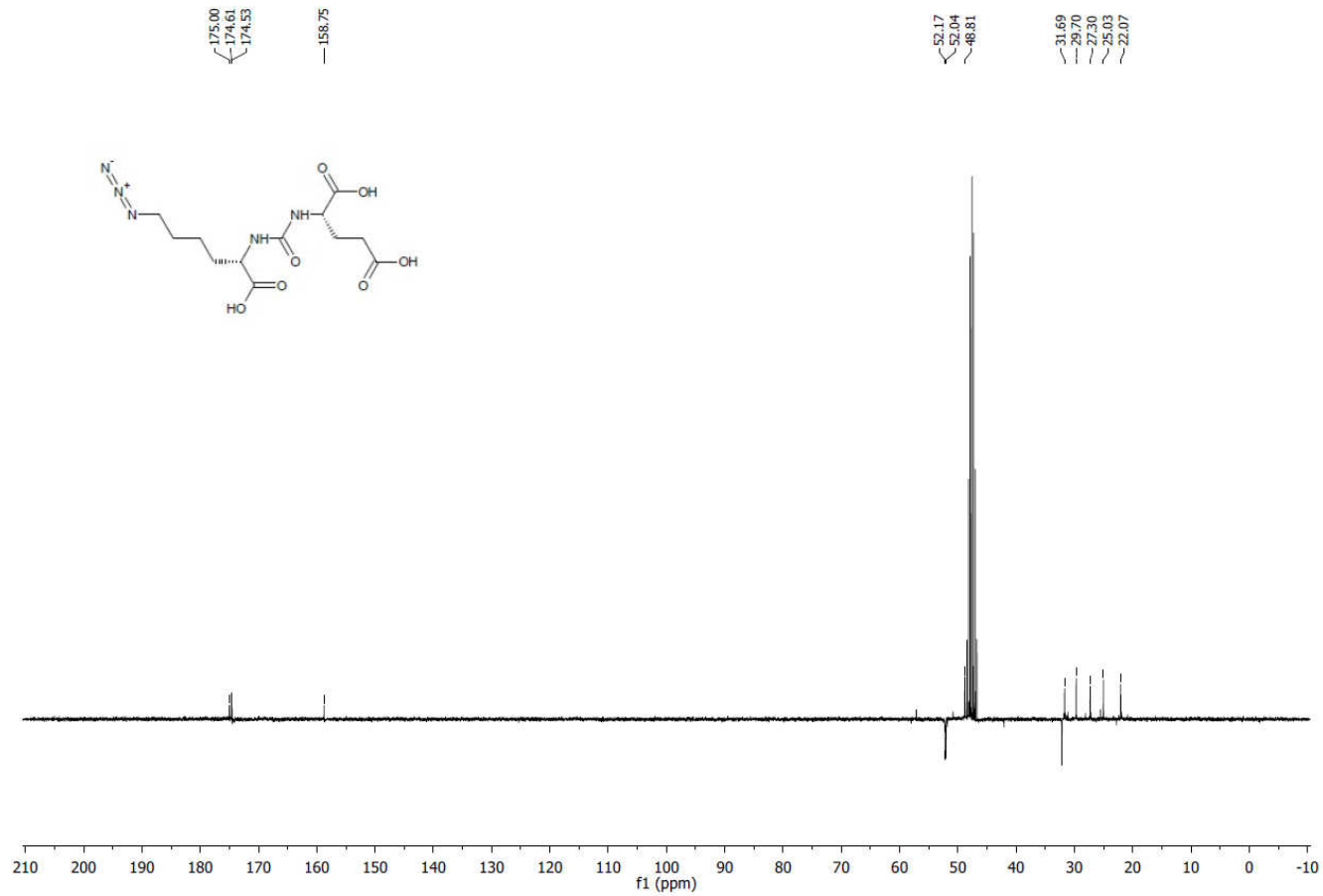

F
N
N=N
NH
O
O
N
O
Cl
3.78
3.64
2.37
4.38
4.37
4.35
3.28
3.26
3.25
3.24
1.91
1.89
1.88
1.86
1.85
1.51
1.50
1.48
1.47
1.29
1.26
0.90
0.88
0.87
0.08
5.86
5.85
5.84
5.30
7.88
7.87
7.64
7.63
7.61
7.61
7.47
7.47
7.45
7.30
7.27
7.24
7.23
7.22
7.12
6.88
6.87
6.83
6.68
6.68
6.66
6.66
0.99 0.99 2.00 2.01 1.03 1.00 1.01 0.99 1.00 0.99
0.98
1.98
3.00 1.99
1.99
0.00 3.03
2.00
2.02
Chemical Shift (ppm)

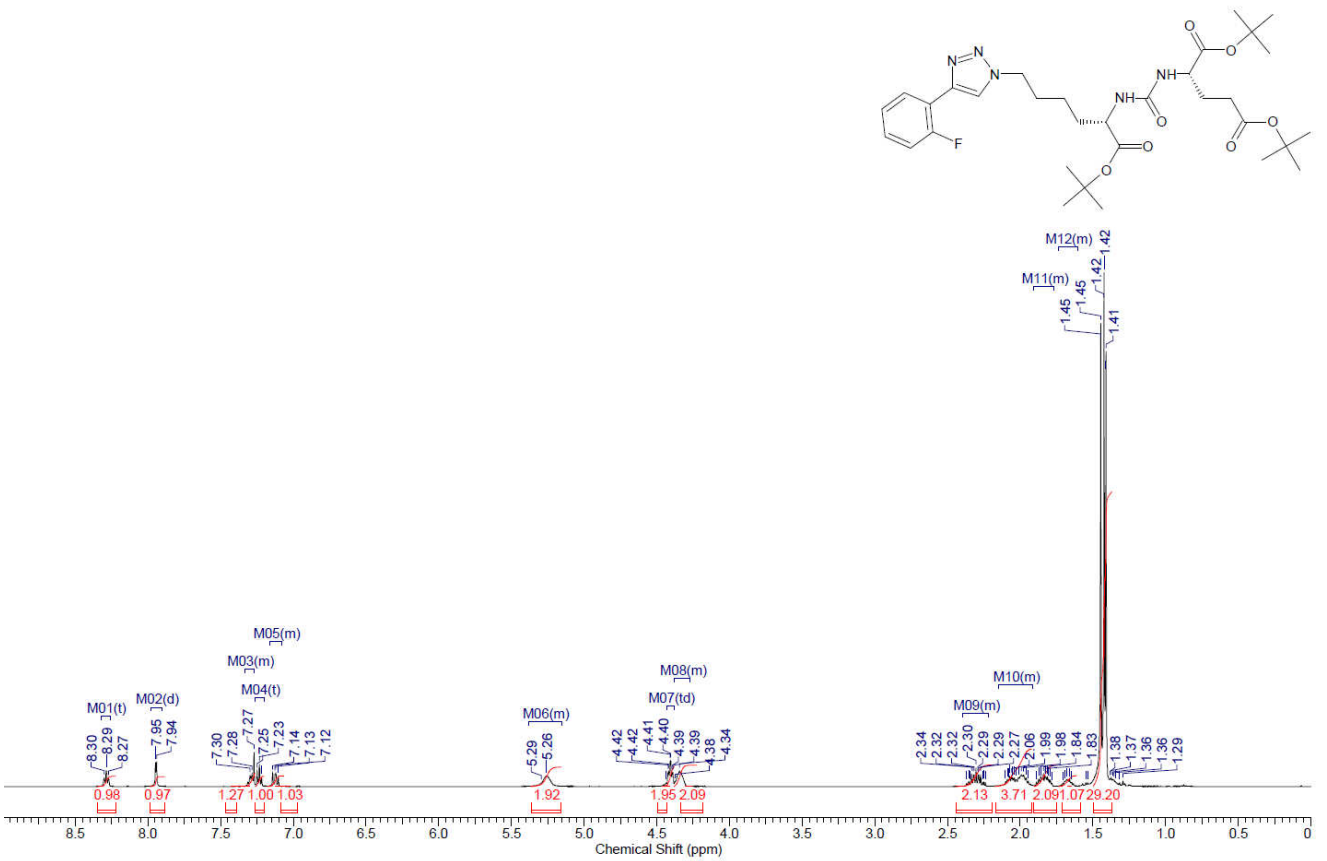

F
N
N=N
NH
O
O
N
O
Cl
131.13
129.15
127.70
124.58
122.61
115.70
115.52
115.11
112.12
100.84
55.71
13.24
170.09
168.27
160.12
158.15
156.22
141.12
139.49
136.37
133.50
130.87
130.25
118.48
118.38
112.70
112.69
77.25
77.00
76.74
49.65
38.68
32.21
26.46
27.47
Chemical Shift (ppm)

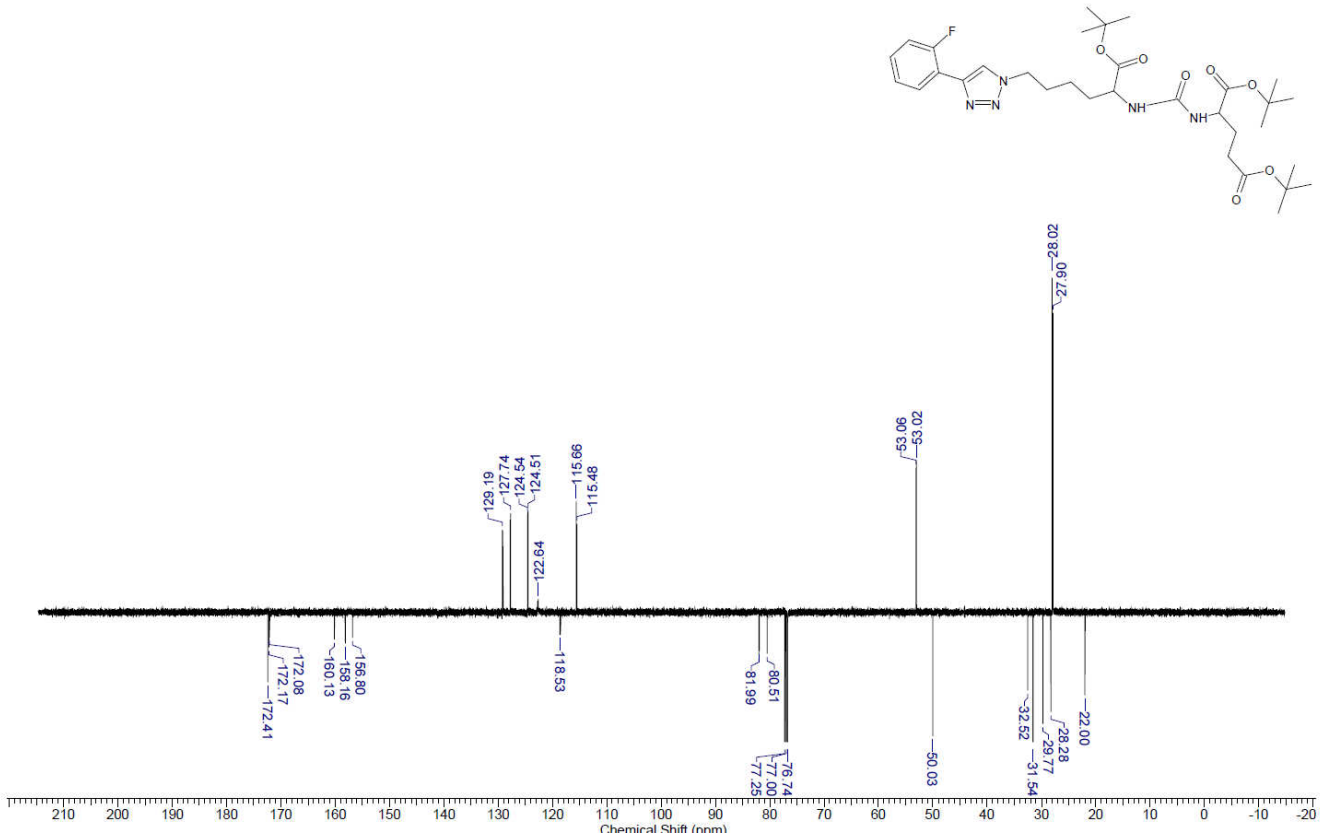

M07(m)
M08(m)
M01(br. s.)
M02(br. s.)
M03(t)
M04(m)
M06(m)
M05(m)
7.27
5.14
4.35
3.28
3.26
3.24
2.35
2.32
2.30
2.29
2.27
1.87
1.68
1.64
1.63
1.62
1.61
1.60
1.47
1.43
1.40
1.38
1.38
1.85
2.02
2.01
2.05
1.20
2.33
3.42
29.00

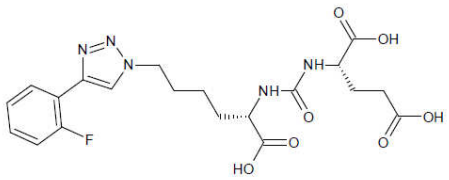

69.27
28.06
53.26
53.06
172.41
172.29
172.06
156.71
82.07
81.93
80.52
32.78
31.55
28.46
28.39
22.25
51.14
Chemical Shift (ppm)

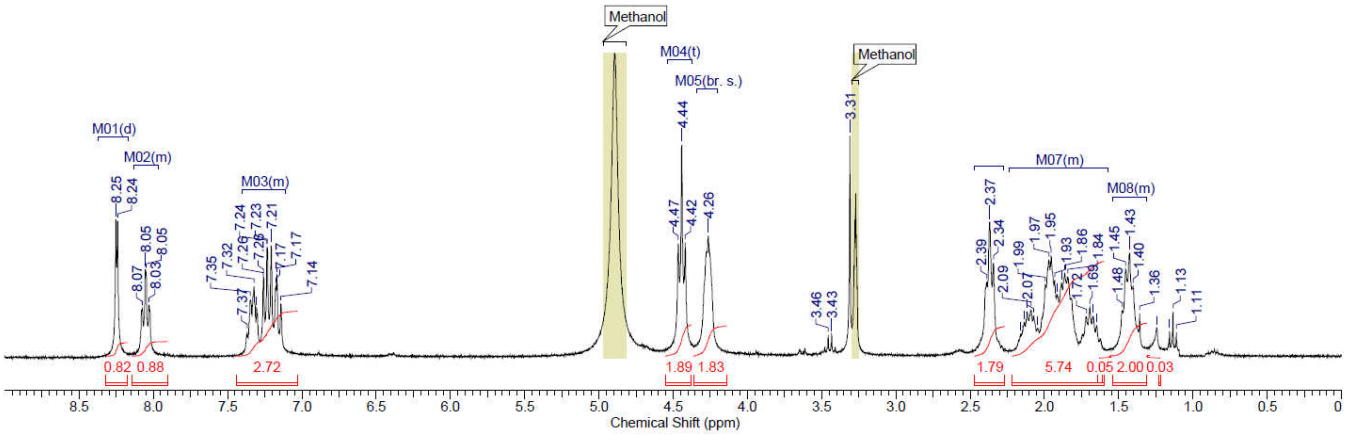

Methanol
M01(dd)
Methanol
M02(t)
M03(m)
M04(m)
M05(m)
M06(m)
Chemical Shift (ppm)

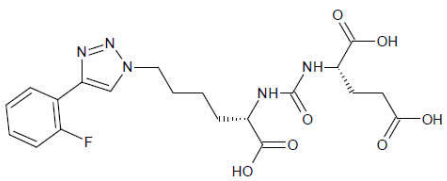

f1 (ppm)

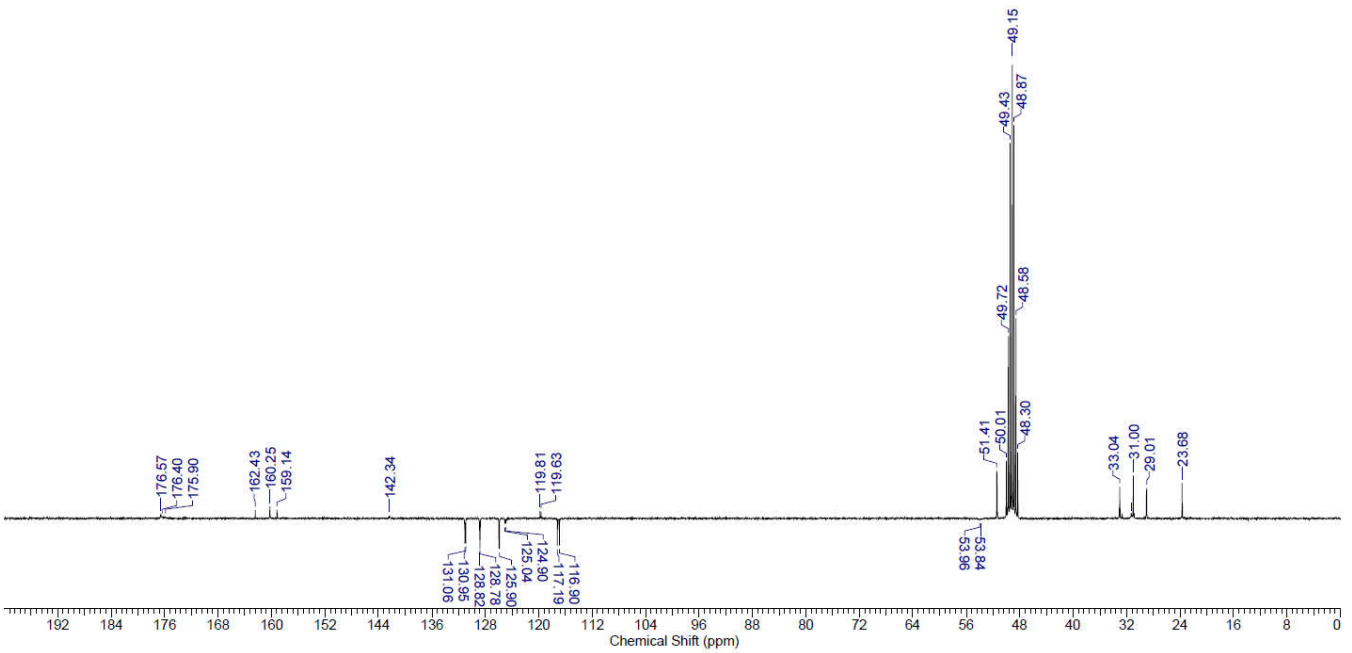

M01(t)
M02(d)
M03(m)
M04(t)
M05(m)
M06(m)
M07(td)
M08(m)
M09(m)
M10(m)
M11(m)
M12(m)
Chemical Shift (ppm)


Chemical Shift (ppm)

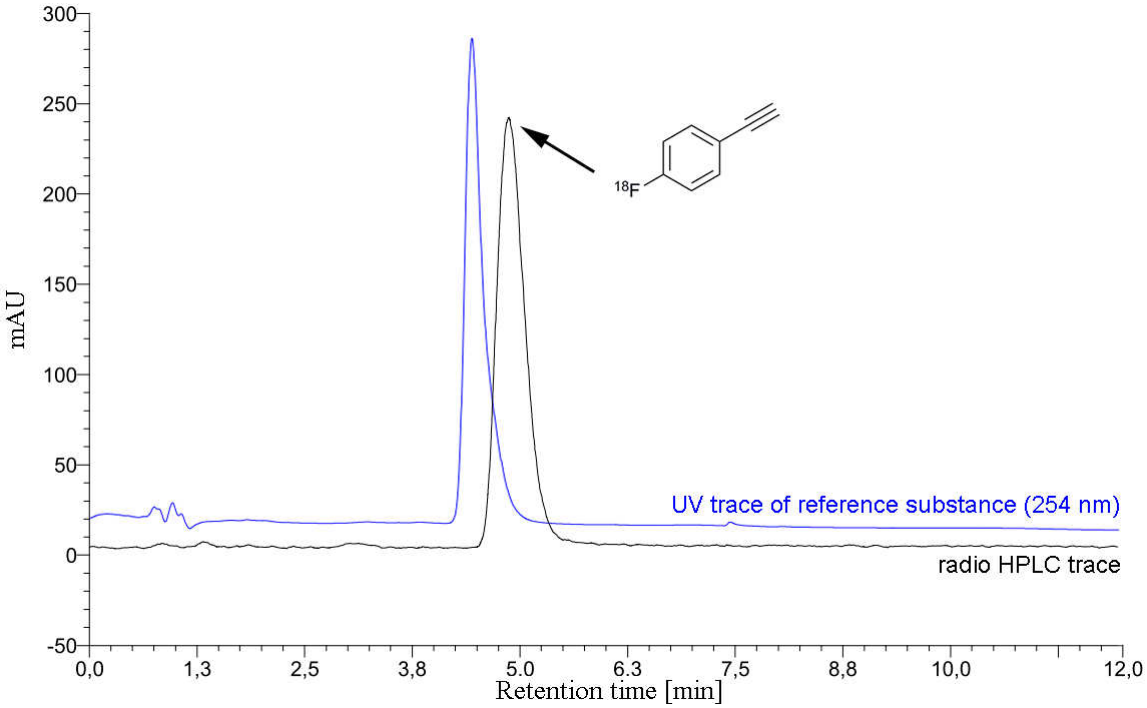


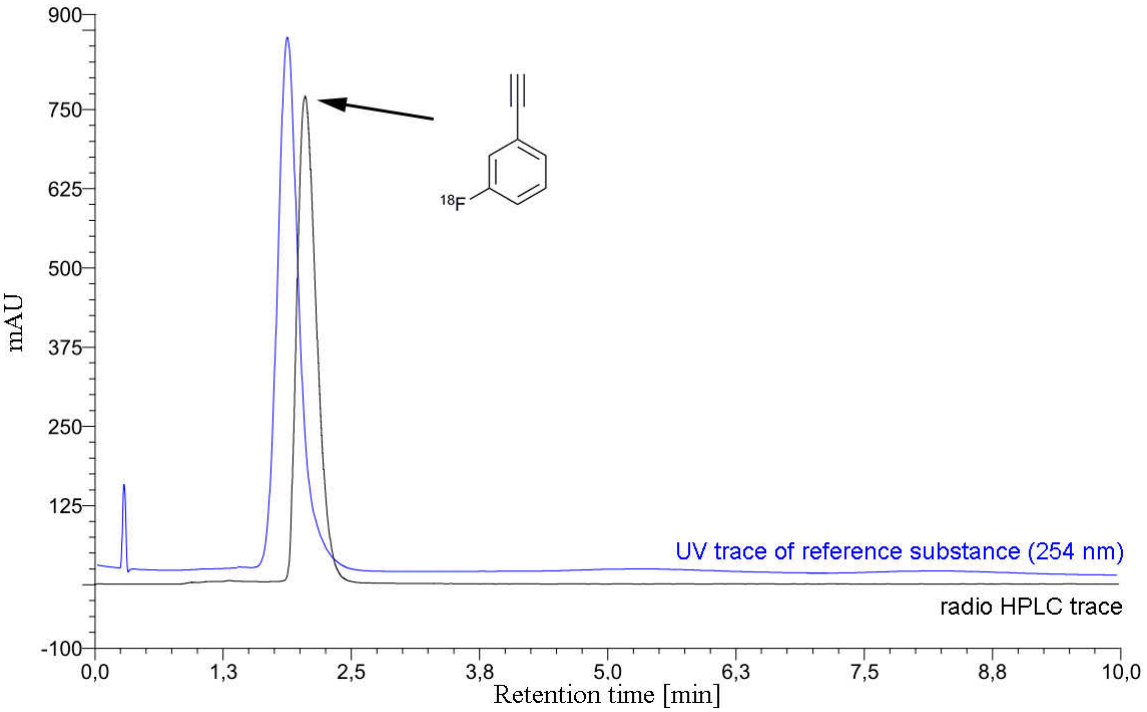

Methanol
M04(t)
M05(br. s.)
Methanol
M01(d)
M02(m)
M03(m)
M07(m)
M08(m)
8.25
8.24
8.07
8.05
8.03
8.05
7.35
7.32
7.31
7.26
7.24
7.23
7.21
7.17
7.17
7.14
4.47
4.44
4.42
4.26
3.46
3.43
3.31
2.39
2.37
2.34
2.09
1.99
2.07
1.97
1.95
1.93
1.86
1.84
1.72
1.69
1.48
1.45
1.43
1.40
1.36
1.13
1.11
0.82
0.88
2.72
1.89
1.83
1.79
5.74
0.05
2.00
0.03
Chemical Shift (ppm)

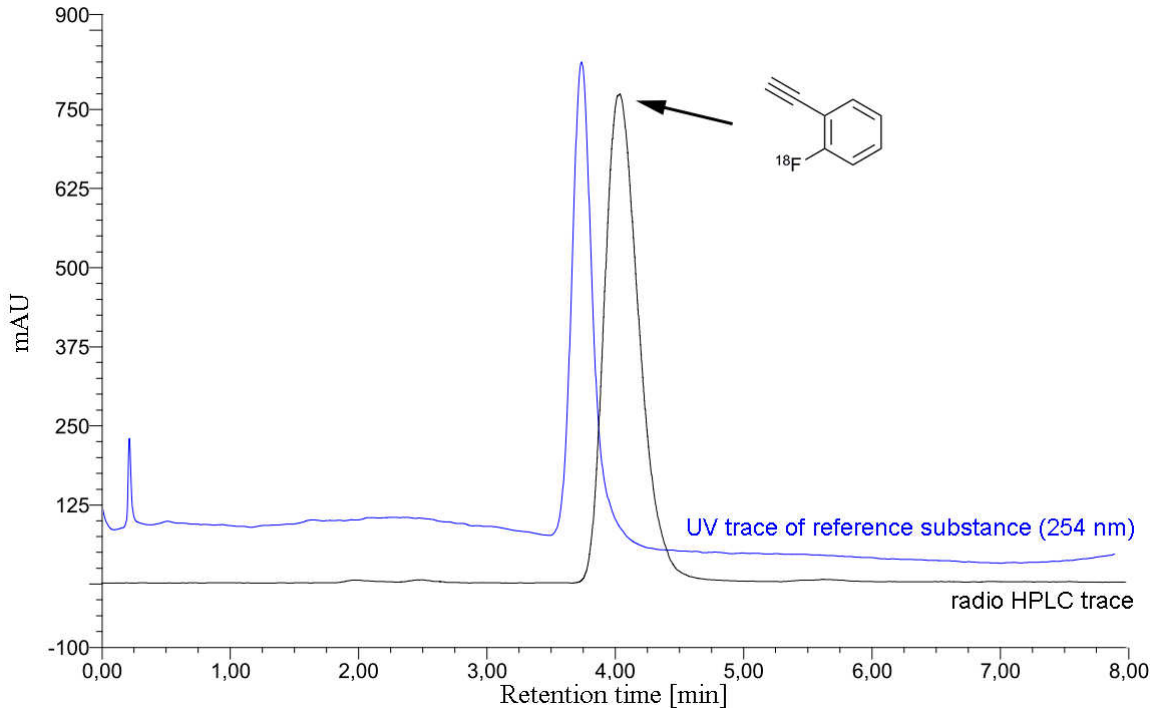


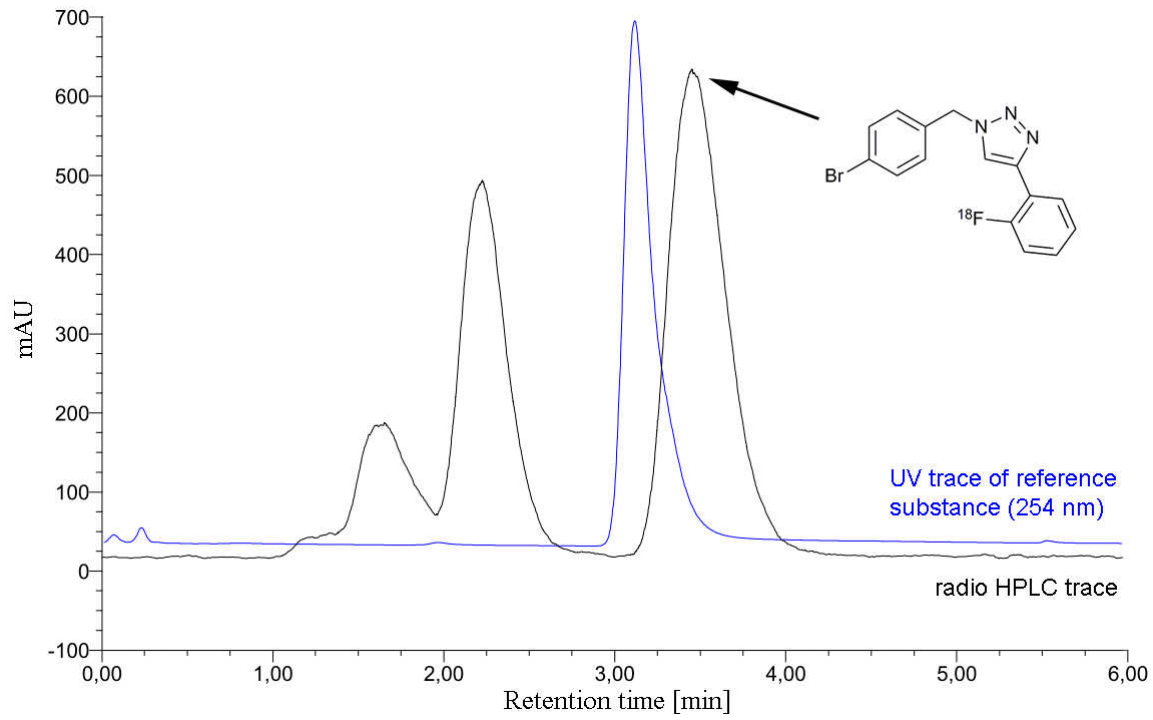

176.57
176.40
175.90
162.43
160.25
159.14
142.34
131.06
130.95
128.82
128.78
125.90
125.04
124.90
119.81
119.63
117.19
116.90
53.96
53.84
51.41
50.01
49.72
49.43
49.15
48.87
48.58
48.30
33.04
31.00
29.01
23.68
Chemical Shift (ppm)

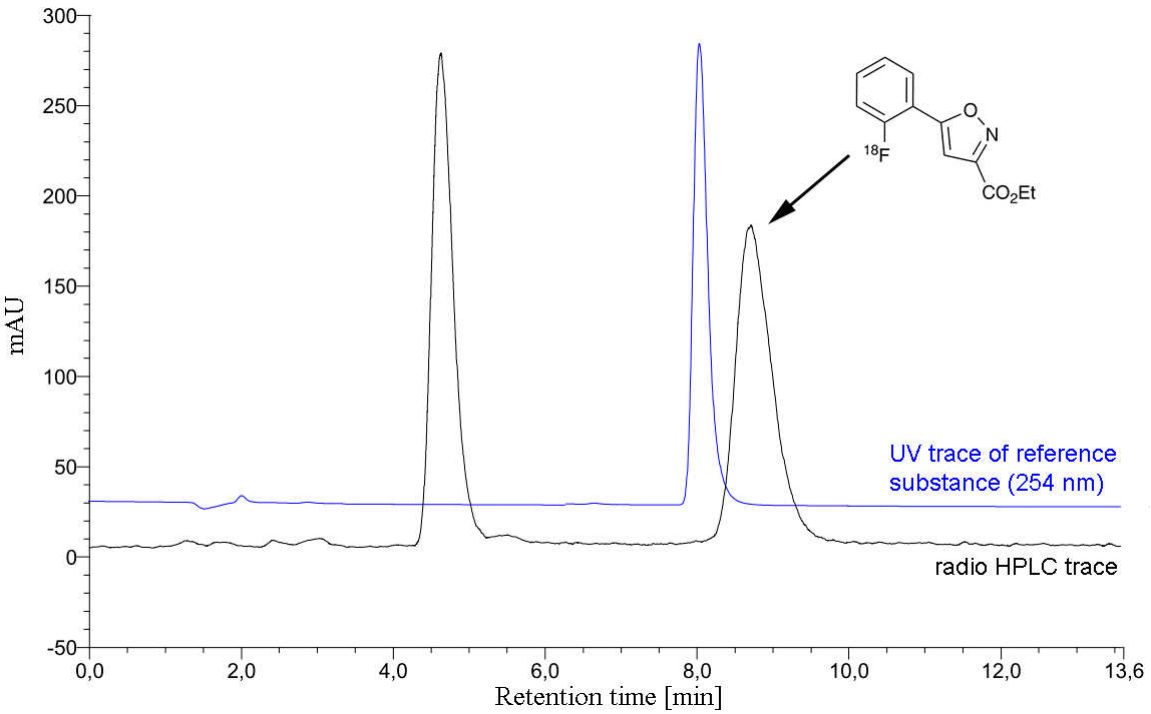
HPLC Traces

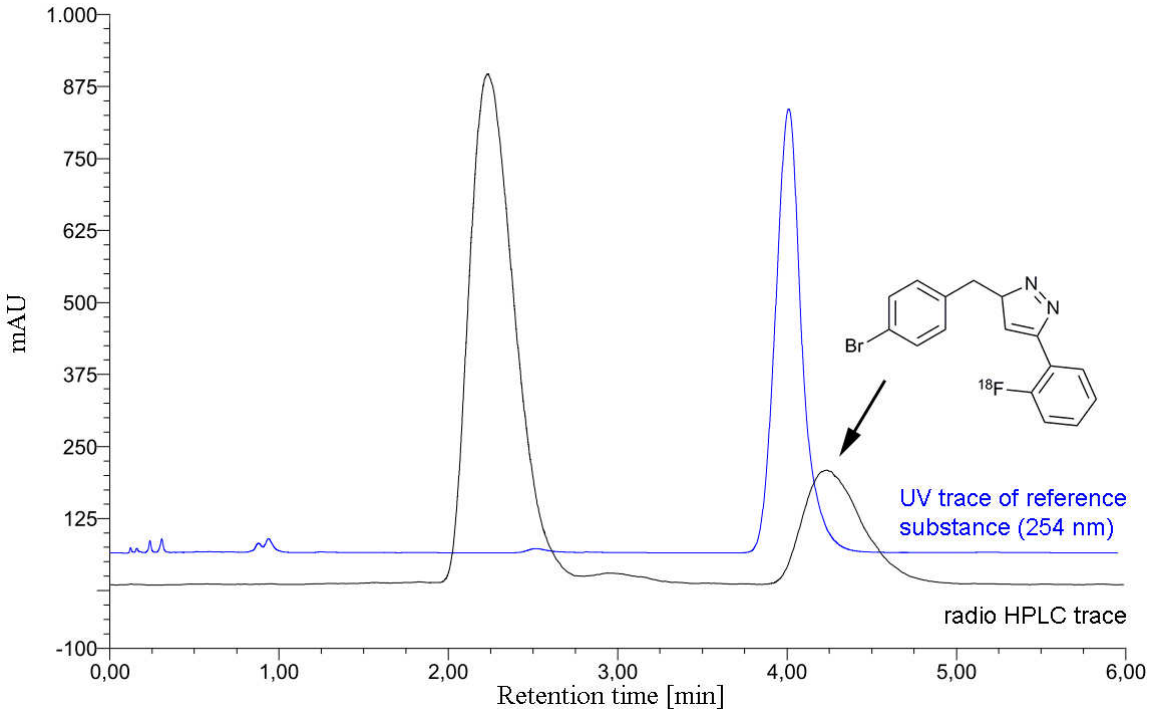

300
250
200
150
100
50
0
-50
mAU
^{18}F
UV trace of reference substance (254 nm)
radio HPLC trace
0,0
1,3
2,5
3,8
5.0
6.3
7,5
8,8
10,0
12,0
Retention time [min]

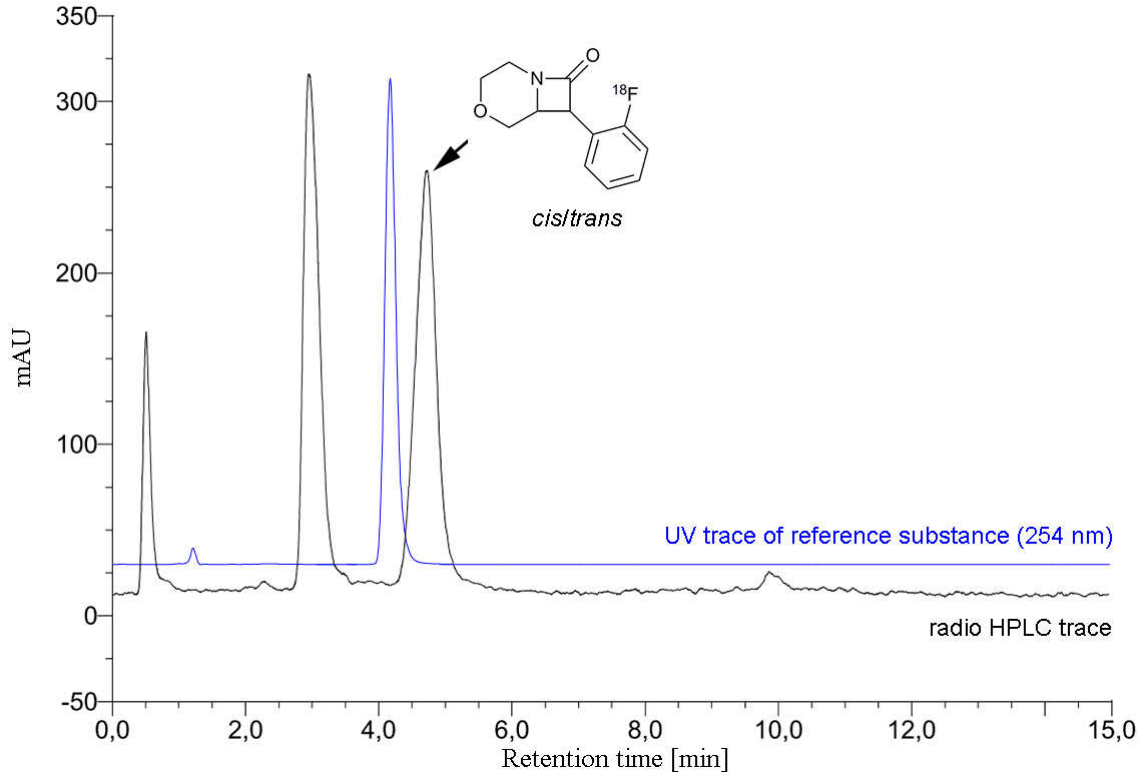

900
750
625
500
375
250
125
-100
mAU
^{18}F
UV trace of reference substance (254 nm)
radio HPLC trace
0,0
1,3
2,5
3,8
5.0
6,3
7,5
8,8
10,0
Retention time [min]

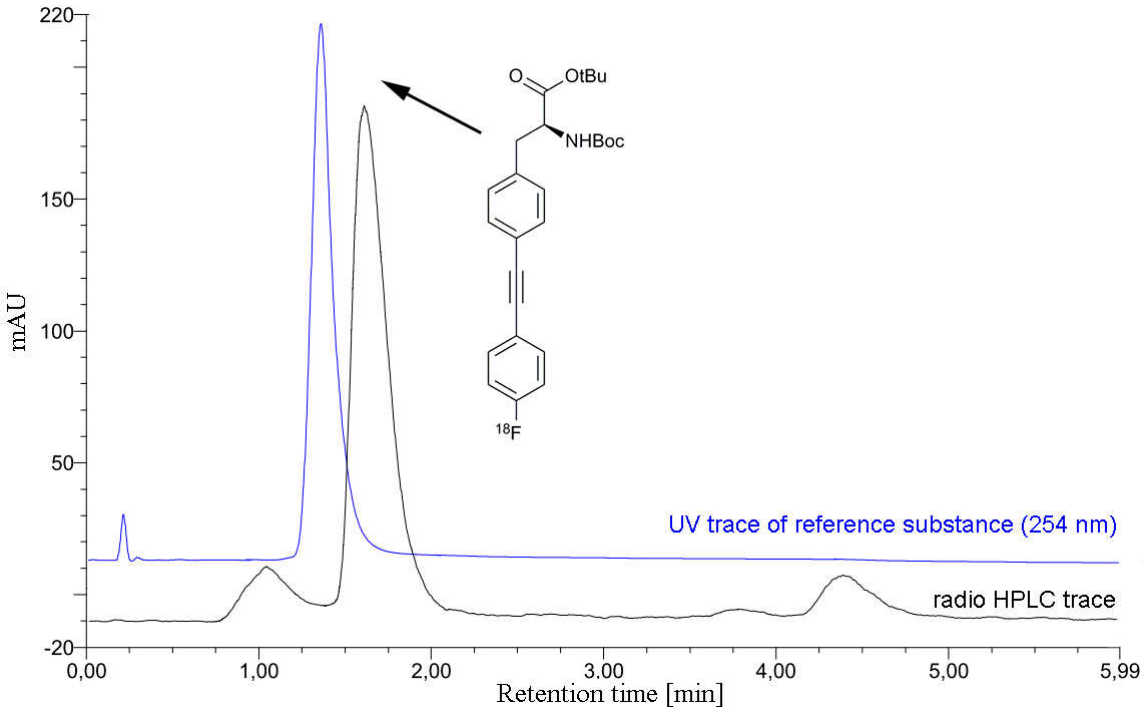

900
750
625
500
375
250
125
-100
mAU
0,00
1,00
2,00
3,00
4,00
5,00
6,00
7,00
8,00
Retention time [min]
18F
UV trace of reference substance (254 nm)
radio HPLC trace

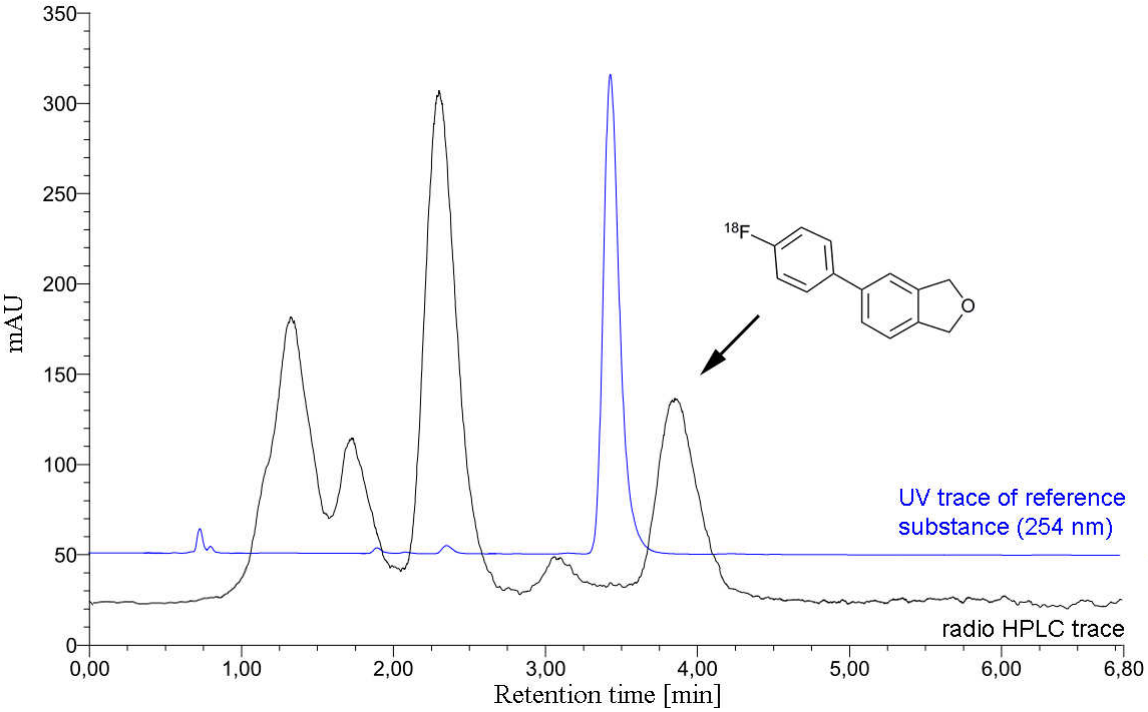

700
600
500
400
300
200
100
0
-100
mAU
0,00
1,00
2,00
3,00
4,00
5,00
6,00
Retention time [min]
Br
N
N
N
18F
UV trace of reference substance (254 nm)
radio HPLC trace

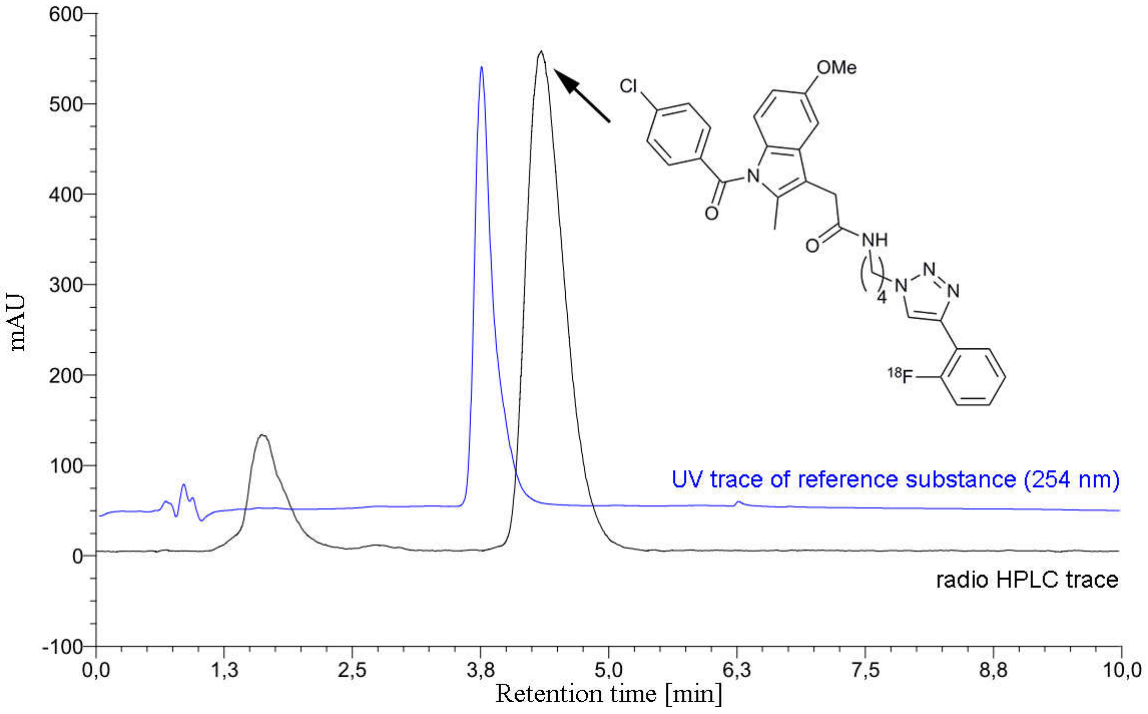

300
250
200
150
100
50
0
-50
mAU
0,0
2,0
4,0
6,0
8,0
10,0
12,0
13,6
Retention time [min]
UV trace of reference substance (254 nm)
radio HPLC trace
O
N
18F
CO2Et

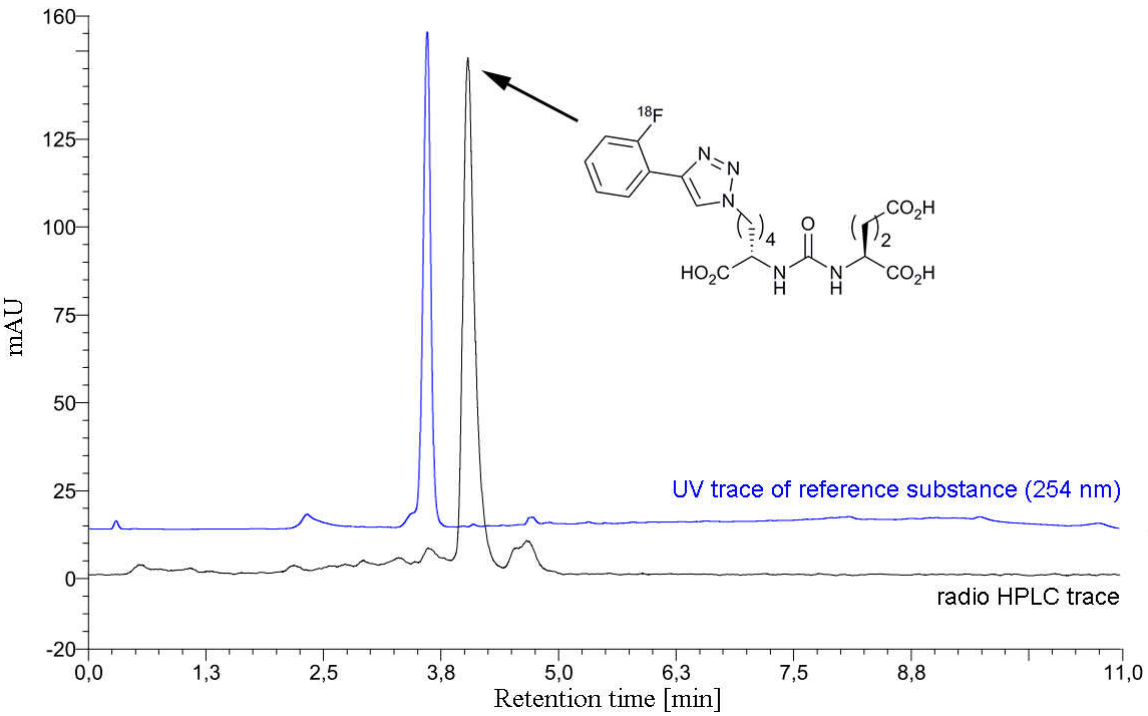

1.000
875
750
625
500
375
250
125
-100
mAU
0,00
1,00
2,00
3,00
4,00
5,00
6,00
Retention time [min]
UV trace of reference substance (254 nm)
radio HPLC trace
Br
N
N
18F


350
300
200
100
0
-50
mAU
0,0
2,0
4,0
6,0
8,0
10,0
12,0
15,0
Retention time [min]
N
O
O
18F
cis/trans
UV trace of reference substance (254 nm)
radio HPLC trace


220
150
100
50
-20
mAU
0,00
1,00
2,00
3.00
4,00
5,00
5,99
Retention time [min]
O
OtBu
NHBoc
18F
UV trace of reference substance (254 nm)
radio HPLC trace


mAU
350
300
250
200
150
100
50
0
0,00
1,00
2,00
3,00
4,00
5,00
6,00
6,80
Retention time [min]
18F
O
UV trace of reference substance (254 nm)
radio HPLC trace


mAU
600
500
400
300
200
100
0
-100
0,0
1,3
2,5
3,8
5,0
6,3
7,5
8,8
10,0
Retention time [min]
Cl
OMe
N
O
O
NH
4
N
N
N
18F
UV trace of reference substance (254 nm)
radio HPLC trace



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Isotopic Labeling

Synthesis of ^{18}F-Labelled β-Lactams by Using the Kinugasa Reaction

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Abstract: Owing to their broad spectrum of biological activities and low toxicity, β-lactams are attractive lead structures for the design of novel molecular probes. However, the synthesis of positron emission tomography (PET)-isotope-labelled β-lactams has not yet been reported. Herein, we describe the simple preparation of radiofluorinated β-lactams by using the fast Kinugasa reaction between ^{18}F-labelled nitrone [^{18}F]-**1** and alkynes of different reactivity. Additionally, ^{18}F-labelled fused β-lactams were obtained through the reaction of a cyclic nitrone **7** with radiofluorinated alkynes [^{18}F]-**6a**,**b**. Radiochemical yields of the Kinugasa reaction products could be significantly increased by the use of different Cu^{I} ligands, which additionally allowed a reduction in the amount of precursor and/or reaction time. Model radiofluorinated β-lactam-peptide and protein conjugates ([^{18}F]-**10** and ^{18}F-labelled BSA conjugate) were efficiently obtained in high yield under mild conditions (aq. MeCN, ambient temperature) within a short reaction time, demonstrating the suitability of the developed method for radiolabelling of sensitive molecules such as biopolymers.

Introduction

Amongst the available imaging technologies, positron emission tomography (PET) plays a very important role due to its outstanding potential to visualize physiological processes at the molecular level in real time. PET is therefore essential in clinical diagnostics and has gained major significance in drug development. Beside technical improvements, PET benefits from innovations in the field of tracer development, comprising both progress in labelling strategies and an intelligent design of selective molecular probes with the capability to visualize molecular targets involved in physiological and pathophysiological processes. A prerequisite for the latter is a detailed understanding of the biology underlying normal or diseased states at the molecular level. Molecular probes for PET-imaging must be labelled with suitable β^+-emitting nuclides. Among the spectrum of easily available radionuclides ^{18}F-fluorine is still the nuclide with the highest impact in PET research. This is mainly due to the excellent nuclear properties of ^{18}F in comparison to other cyclotron-produced nuclides. Decay characteristics of ^{18}F [$E(\beta^+) = 630$ keV, abundance: 97%; $t_{1/2} =$ 109.8 min] make it an ideal PET-isotope with respect to half-life and resolution. However, although much effort has been spent on the development of novel methods for incorporation of ^{18}F into molecules of interest, radiofluorination methods are still rather rare in comparison to fluorination methods used in conventional organic chemistry.[1] This is due mainly to the tiny amount of no-carrier-added (n.c.a.) $^{18}F^-$ (subnanomolar range) as well as to time restrictions and radiation safety measures. Hence, even promising modern fluorination techniques cannot easily be transferred from synthetic organic chemistry to radiochemistry.[2] Despite this, there are several successful examples of such translations, including the azide–alkyne "click" reactions[3] and metal-catalyzed fluorination methods, which enable easy access to otherwise inaccessible novel radiotracers.[4,5] Furthermore, a simple and efficient metal-free preparation of ^{18}F-labelled compounds through (3+2) cycloaddition reactions of radiofluorinated 1,3-dipoles other than azide to double or triple C–C bonds has recently been reported.[6]

β-Lactam antibiotics are amongst the most successful therapeutic agents developed to date. They exert their activity by inhibition of bacterial cell wall biosynthesis. The mechanism of action involves an irreversible inactivation of penicillin binding proteins (PBPs), which are serine proteases with transglycosy-

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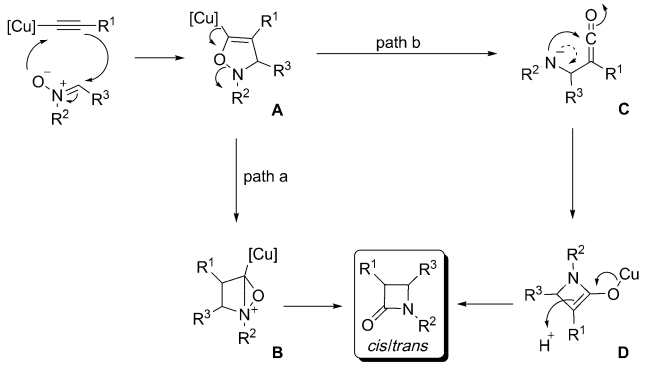
lase, DD-transpeptidase and DD-carboxypeptidase activities, that act through acylation of the nucleophilic serine residue at the enzyme active site.[7] Beside β-lactam antibiotics, numerous β-lactams with nonantibiotic activities have been discovered.[8] They exert their effects by inhibition of further serine proteinases such as thrombin, elastases, prostate specific antigen (PSA), human cytomegalovirus (HCV) protease as well as matrix metalloproteinases, cysteine proteases, and tubulin polymerization. Consequently, radiolabelled β-lactams can potentially be used for PET-imaging of bacterial and viral infections as well as thrombosis, emphysema, and tumors. However, no PET-tracers with a β-lactam structural motif have been published to date.

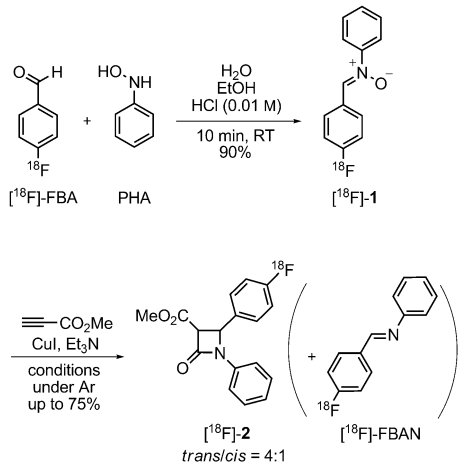
The Kinugasa reaction[9] (Scheme 1) is a valuable tool in preparative organic chemistry, offering easy access to β-lactams through copper(I)-catalyzed reaction between nitrones and terminal alkynes. Two plausible mechanisms of the Kinugasa reaction have been proposed. According to both, the transformation begins with the formation of a copper acetylide in situ (in the initial report[9a] preformed copper acetylides were used) (Scheme 1). Subsequent 1,3-cycloaddition of the latter to the nitrone gives 5-isoxazolinyl cuprate **A**. According to Ding and Irwing (path a),[9b,10a,b] the initially formed cuprate **A** rearranges into the highly strained, extremely unstable intermediate bicyclic oxaziridinium salt **B**, which undergoes spontaneous opening of the oxaziridinium ring to give (after protonation) the corresponding β-lactam as a mixture of *cis*/*trans* isomers. In an alternative mechanism, proposed by Ye et al.[10c] (path b), diastereomeric 2-azetidinones are obtained from cuprate **A** through ring-opening fragmentation followed by recyclization and protonation. Although the Kinugasa reaction usually gives β-lactams as mixtures of racemic diastereoisomers, numerous stereoselective variants of this transformation using the appropriate chiral Cu^{I} ligands or chiral auxiliaries have been reported.[10d–f] Application of the Kinugasa reaction to radiochemistry could allow simple access to radiolabelled 2-azetidinones. Herein, we describe for the first time a method for the preparation of radiofluorinated monocyclic β-lactams through the Kinugasa reaction between the easily accessible ^{18}F-labelled *C*-4-fluorophenyl-*N*-phenyl nitrone ([^{18}F]-**1**)[6b] and a range of terminal alkynes. Furthermore, a model cyclic nitrone **7** was reacted with radiolabelled fluorophenylacetylenes [^{18}F]-**6a**,**b** under Kinugasa conditions to give the corresponding labelled bicyclic β-lactams [^{18}F]-**8a**,**b**. Finally, a study was carried out on the applicability of the radio-Kinugasa reaction for labelling of peptides and biomolecules, using H-βAla-Phe-OMe and bovine serum albumin (BSA) as model peptide and protein, respectively.



Scheme 1. Proposed mechanisms of the Kinugasa reaction. Path a: via oxaziridinium intermediate **B**;[9b,10b] path b: via β-aminoketene intermediate **C**.[10c]

Results and Discussion

[^{18}F]-**1** was prepared by acid-catalyzed condensation of 4-[^{18}F]-fluorobenzaldehyde ([^{18}F]-FBA) with *N*-phenylhydroxylamine, as described earlier,[6b] in a radiochemical yield of 89–92% (Scheme 2). The ^{18}F-labelled synthon was allowed to react with methyl propiolate in the presence of CuI and Et_3N in MeCN at ambient temperature. The reaction was carried out under Ar to avoid the consumption of alkyne through Glaser coupling. The corresponding radiolabelled β-lactam [^{18}F]-**2** was formed after only 10 min reaction time in a radiochemical yield (RCY) of 65%, as a mixture of diastereomers. The thermodynamically favored *trans*-isomer was the predominant isomer obtained (*trans*/*cis* = 4:1). A prolonged reaction time of 20 min resulted in a slightly improved radiochemical yield of 67%. The main impurity (up to 25%) was identified as *N*-4-[^{18}F]-fluorobenzylidene aniline ([^{18}F]-FBAN).



Scheme 2. Preparation of ^{18}F-labelled β-lactam [^{18}F]-**2** under an inert atmosphere.

These promising initial results prompted us to optimize the Kinugasa reaction with respect to precursor amount, reaction time, solvent, Cu^{I}-stabilizing ligands and temperature.

In initial experiments, 10 μmol methyl propiolate precursor was used. Normally, this is sufficiently low for labelling of small molecules. However, this precursor amount is unacceptably high, for example in the case of peptides and proteins for

which precursors are generally difficult to separate from the labelled products. High precursor content can impair the quality of PET-images and/or even cause undesirable effects in patients, impeding the clinical application of the tracer.

To optimize the Kinugasa reaction with respect to precursor amount, nitrone [^{18}F]-**1** was allowed to react with different amounts of methyl propiolate [0.05–10 μmol at ambient temperature and 10 or 20 min reaction time (Figure 1)]. Reducing

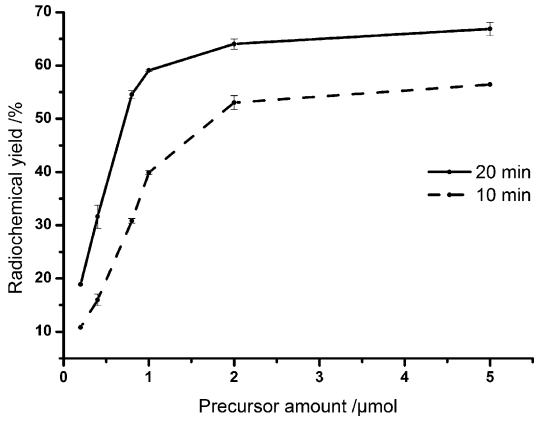


Figure 1. Radiochemical yield of [^{18}F]-**2** as a function of precursor amount.

the amount of the alkyne precursor from 10 to 0.8 μmol caused a gradual decrease of RCYs from 65 and 67% to 31 and 55% after 10 and 20 min, respectively. By further reducing the precursor amount to 0.2 μmol, the RCYs of [^{18}F]-**2** decreased to 11 and 19% after 10 and 20 min, respectively. All further optimization experiments were carried out with 0.8 μmol methyl propiolate, at a reaction time of 10 min at ambient temperature.

Formation of ^{18}F-labelled β-lactam [^{18}F]-**2** was dependent on temperature (Figure 2). Increasing the temperature from 25 to 60 °C improved the yield of the product from 31 to 68% within 10 min. Simultaneously, competitive formation of the side product [^{18}F]-FBAN (up to 30%) was observed. Further elevation of temperature did not increase RCY.

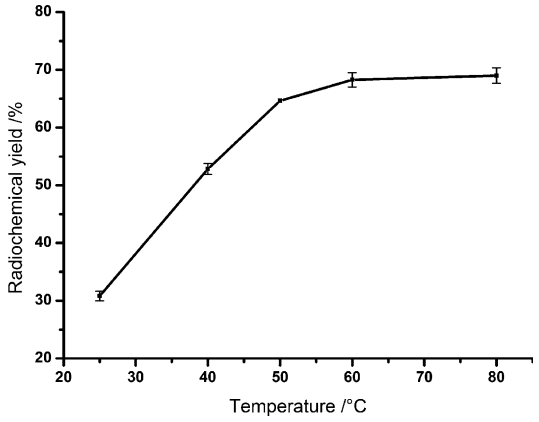


Figure 2. Dependence of radiochemical yield of [^{18}F]-**2** on temperature.

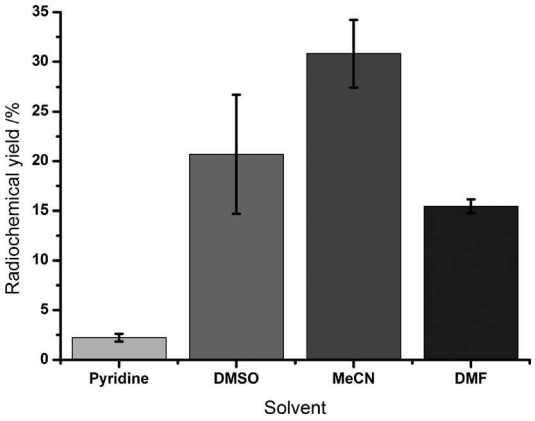


Figure 3. Radiochemical yield of [^{18}F]-**2** in different solvents.

The Kinugasa reaction between [^{18}F]-**1** and methyl propiolate was tested in different solvents (Figure 3). In dimethyl sulfoxide (DMSO) and *N*,*N*-dimethylformamide (DMF) formation of [^{18}F]-**2** was significantly lower than that observed in MeCN (21 and 16% vs. 31%, respectively). When the radiosynthesis was carried out in pyridine only traces of the product were formed (ca. 2%), presumably due to almost instantaneous consumption of methyl propiolate in a Michael reaction with pyridine.[11]

Miura et al.[10b] found that application of particular nitrogen ligands accelerated the Kinugasa reaction due to efficient stabilization of the reactive monomeric copper acetylide, preventing Cu^{I} oxidation and/or disproportionation to Cu^{0} and Cu^{II}.[12] Accordingly, in the presence of pyridine, a moderate increase of radiochemical yield (from 31 to 43%) was observed (Figure 4). A more efficient ligand was 1,10-phenanthroline

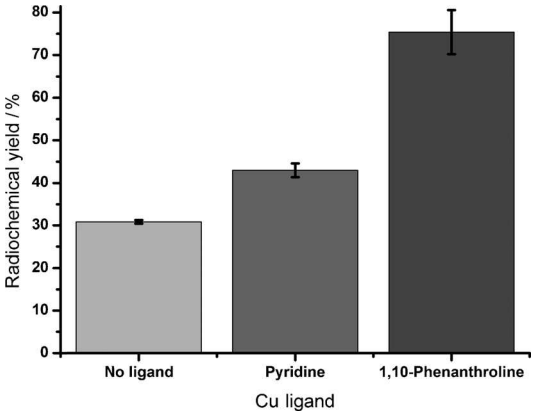
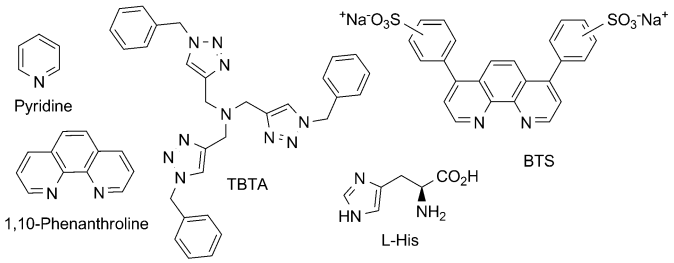


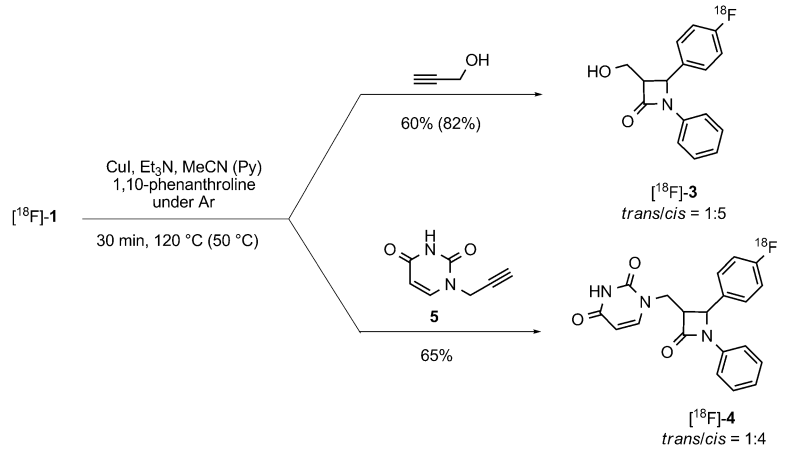
Figure 4. Influence of Cu^{I}-stabilizing ligands on the radiochemical yield of [^{18}F]-**2**.

(Scheme 3); in the presence of this ligand a maximum radiochemical yield of 75% was observed for [^{18}F]-**2**.

Having established the optimized reaction conditions for the radio-Kinugasa reaction with highly reactive alkynes, attention was focused on less reactive alkynes. Propargyl alcohol was chosen as a representative less reactive alkyne (Scheme 4). Preliminary experiments had shown that higher reaction tempera-



Scheme 3. Cu^{I}-stabilizing ligands used in this work.

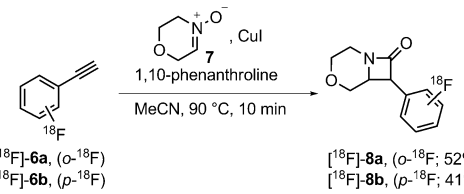


Scheme 4. Synthesis of labelled β-lactam alcohol [^{18}F]-**3** and β-lactam-nucleobase chimera [^{18}F]-**4**.

tures were mandatory to obtain [^{18}F]-**3** in reasonable radiochemical yields within a time-frame that was compatible with the half-life of ^{18}F. Reaction of [^{18}F]-**1** with 10 μmol propargyl alcohol in the presence of 1,10-phenanthroline at 120 °C for 30 min in MeCN produced [^{18}F]-**3** in a radiochemical yield of 60%. Similar results were obtained by using tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine (TBTA),[12] which is widely used for ligand-promoted acceleration of the copper-catalyzed azide-alkyne cycloaddition reactions. In contrast, a radiochemical yield of 82% of the desired product was obtained at 50 °C in pyridine without ligand. In this case, the preferential formation of the kinetically favored *cis*-isomer (*trans*/*cis* ratio = 1:5) was observed. The main side product detected in the reaction mixture was [^{18}F]-FBA, originating from decomposition of [^{18}F]-**1**. In MeCN solvent, the radiochemical yields decreased rapidly with decreasing alkyne amount. When 1 μmol precursor was used, only 10% [^{18}F]-**3** was formed in the reaction mixture. In contrast, using 1 μmol precursor in pyridine, radiofluorinated β-lactam was obtained in a satisfactory yield of 50%.

With optimized conditions for the Kinugasa reaction between radiofluorinated nitrone [^{18}F]-**1** and moderately activated alkynes to hand, we tried to prepare ^{18}F-labelled β-lactam-nucleobase chimera [^{18}F]-**4** (Scheme 4). Radiofluorinated compounds of this type are of significant potential for the imaging of bacterial infections. To this end, [^{18}F]-**1** was allowed to react with 1-propargyl uracyl (**5**)[13] in MeCN to give the uracyl conjugate [^{18}F]-**4** as a mixture of *cis*/*trans* isomers (4:1) in a radiochemical yield of 65%. In contrast, only traces of [^{18}F]-**4** could be detected when pyridine was used. Instead of the expected product, two hydrophobic ^{18}F-labelled byproducts were observed in the reaction mixture. This unexpected result can probably be attributed to elimination of the nucleobase from the nucleotide analogue [^{18}F]-**4** to give the corresponding 3-methylene-substituted β-lactam.[14] The latter could react further with pyridine or 1-propargyluracil under formation of the respective *aza*-Michael adducts.

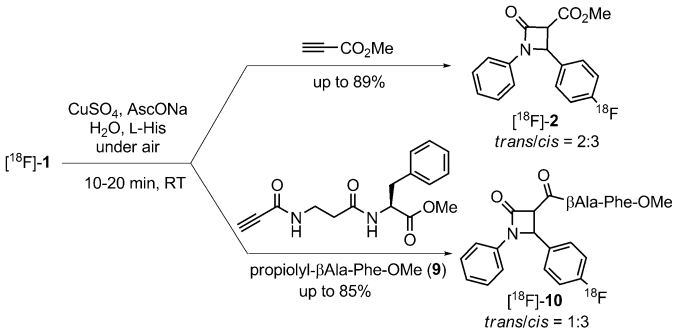
Having developed a method for the preparation of radiofluorinated monocyclic β-lactams through the radio-Kinugasa reaction, the application of the Kinugasa reaction in the synthesis of ^{18}F-labelled fused β-lactams was studied. In this case, easily accessible *o*- and *p*-[^{18}F]-fluorophenyl acetylenes ([^{18}F]-**6a** and [^{18}F]-**6b**, respectively) were used as radiolabelled building blocks (Scheme 5). In the presence of 1,10-phenanthroline these compounds reacted with the model nitrone 3,6-dihydro-2*H*-1,4-oxazine-4-oxide (**7**)[9b] to give the corresponding radiofluorinated bicyclic β-lactams [^{18}F]-**8a** and [^{18}F]-**8b** in radiochemical yields of 52 and 41%, respectively, within 10 min.



Scheme 5. Preparation of the labelled bicyclic β-lactams [^{18}F]-**8a** and [^{18}F]-**8b**.

Given the rapid kinetics of the Kinugasa reaction between C,N-diaryl nitrones and activated alkynes at ambient temperature, we investigated whether this approach might also be suitable for the radiofluorination of peptides and biopolymers. The original Kinugasa reaction should be carried out under an inert atmosphere in anhydrous organic solvents. This is a significant drawback with respect to labelling of the unprotected peptides and proteins because they are often not readily soluble in organic solvents such as acetonitrile. Basak et al.[15] exploited Cu^{I} generation in situ through reduction of $CuSO_4$ with sodium ascorbate as is usual for the azide-alkyne click reaction.[3] They were able to prepare several β-lactams in moderate yields in aqueous MeCN (or DMF) under an inert atmosphere by using Et_3N as a base.

In preliminary experiments we tested the "cold" Kinugasa reaction between **1** and methyl propiolate under "Basak" condi-



Scheme 6. Conjugation of nitrone [^{18}F]-**1** with methyl propiolate and the model dipeptide **9** under click conditions.

tions at ambient temperature and were able to detect only traces of β-lactam **2** in the reaction mixture. In contrast to the published results, we observed formation of the desired product in suitable yields within 10 min *without* base addition. Moreover, it should be pointed out that this modification of the Kinugasa reaction works equally well under air. Nevertheless, reasonable yields of [^{18}F]-**2** from [^{18}F]-**1** could only be achieved with alkyne amounts of more than 50 μmol (Scheme 6). To reduce the precursor amount, we tested the influence of the Cu^{I}-stabilizing ligands TBTA,[12] bathophenanthroline disodium disulfonate (BTS)[16] and L-histidine[17] (Scheme 3), which are known from click chemistry, on the yields of the radio-Kinugasa reaction. In the presence of each of the three ligands only 0.4 μmol methyl propiolate was necessary to obtain [^{18}F]-**2** in 80% radiochemical yields (Figure 5). Nontoxic and inexpensive

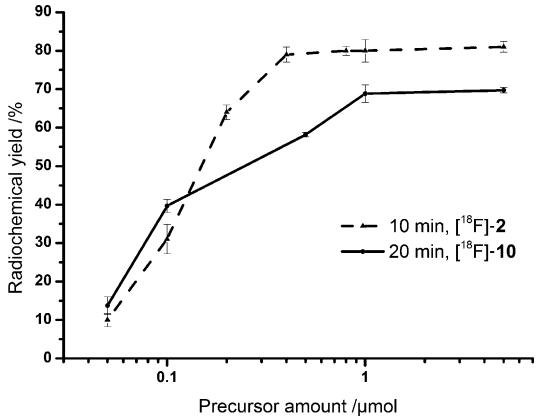


Figure 5. Dependence of radiochemical yields of [^{18}F]-**2** and [^{18}F]-**10** on the amount of alkyne precursors (methyl propiolate and dipeptide **9**, respectively) under click conditions.

L-histidine was chosen for further experiments. In contrast to the preferential formation of the thermodynamically more stable *trans*-isomer under basic "classical" Kinugasa reaction conditions (see above), the kinetically favored *cis*-isomer (*cis*/*trans* = 3:2) was preferentially formed under weak acidic click conditions. In this case, [^{18}F]-FBA (up to 20%), formed through hydrolysis of [^{18}F]-**1**, was identified as the main side product.

We next studied the labelling of propiolyl-substituted βAla-Phe-OMe **9** through conjugation with [^{18}F]-**1** (Scheme 6). Radiofluorinated depsipeptide [^{18}F]-**10** (*cis*/*trans* = 3:1) was obtained in a radiochemical yield of 58% within 10 min. A prolonged reaction time of 20 min was necessary to obtain the maximal radiochemical yields of 68–70%.

The dependency of radiochemical yields of [^{18}F]-**2** and [^{18}F]-**10** on the amount of alkyne precursor was studied under click conditions after 10 and 20 min reaction time, respectively (Figure 5). The maximum radiochemical yield of [^{18}F]-**2** (80%) was already achieved at 0.4 μmol, whereas for [^{18}F]-**10** (69%) the use of 1 μmol precursor was necessary. Minimization of the amount of alkyne to 100 nmol still produced the radiolabelled β-lactams [^{18}F]-**2** and [^{18}F]-**10** in moderate radiochemical yields of 31 and 40%, respectively.

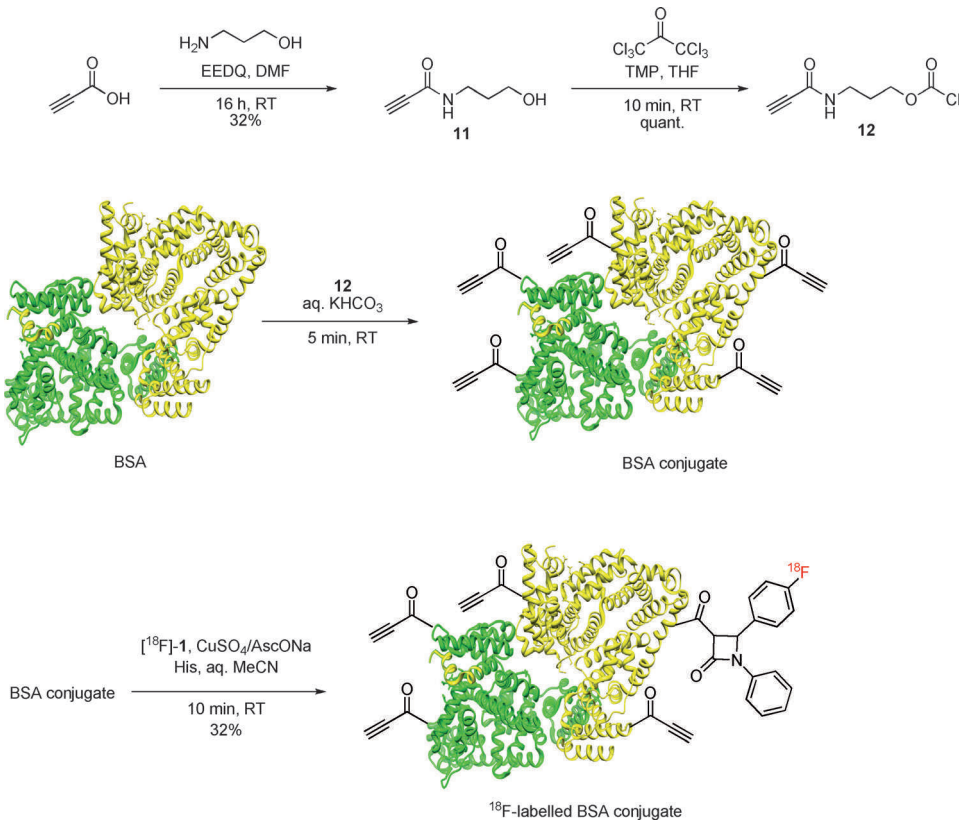
Once the protocol for the radio-Kinugasa reaction under click conditions had been established, we turned to the development of a simplified variant of this radiosynthesis. After reaction of [^{18}F]-FBA with *N*-phenylhydroxylamine at ambient temperature for 10 min in the presence of traces of HCl and subsequent addition of the reaction mixture to an aqueous solution containing the alkyne precursor (1 μmol) and the other reagents ($CuSO_4 \cdot 5H_2O$, AscONa, L-histidine), [^{18}F]-**2** and [^{18}F]-**10** were obtained in excellent radiochemical yields of 89 and 85%, respectively (Scheme 6).

The hydrolytic and serum stability of [^{18}F]-**10** was briefly studied. No decomposition of [^{18}F]-**10** was observed within a pH range from 1 to 9 or in human blood serum at 37 °C for at least 3 h.

The results detailed above raised the question of whether the radio-Kinugasa reaction might be suitable for the radiolabelling of propiolated proteins. To this end, bovine serum albumin (BSA; M_r = 66.5 kDa), chosen as a prototypical substrate, was acylated with chloroformate **12** in 0.15 M $KHCO_3$ for 5 min at ambient temperature (Scheme 7). Compound **12** was prepared as follows: propiolic acid was coupled with 3-aminopropanol-1 by using 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) to give 3-propionamidopropanol-1 (**11**) in moderate yield. The latter was treated with triphosgene in the presence of 2,4,6-trimethylpyridine (TMP) in tetrahydrofuran (THF) to give acylating agent **12**, which was immediately used for protein modification. The crude functionalized protein was purified by ultrafiltration. Propiolated BSA (19 nmol) was labelled with [^{18}F]-**1** (400–500 MBq) under click conditions in aqueous MeCN for 10 min at ambient temperature. Finally, the radiofluorinated BSA conjugate was isolated by simple filtration through a PD10 desalting cartridge to give the desired product in 32% radiochemical yield and in excellent radiochemical and chemical purity (Figure 6). In a control experiment, native BSA was treated with [^{18}F]-**1**. In this case radiolabelling of protein did not take place.

Conclusion

We have demonstrated that the Kinugasa reaction is an efficient tool that can be used for the simple and high-yielding preparation of ^{18}F-labelled β-lactams. The rapid kinetics of the



Scheme 7. Preparation of propiolated BSA and its ^{18}F-labelling through the radio-Kinugasa reaction. EEDQ: 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline, TMP: 2,4,6-trimethylpyridine.

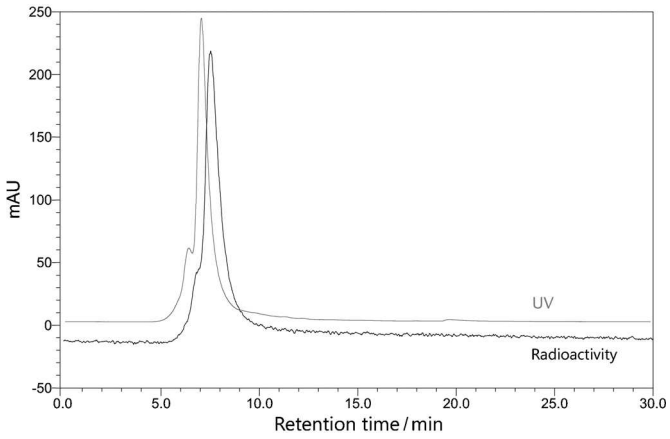


Figure 6. Analytical HPLC analysis of ^{18}F-labelled BSA conjugate: UV ($\lambda = 254$ nm; gray) and radioactivity (black) traces.

radio-Kinugasa reaction with activated alkynes under mild click conditions make it well-suited for the labelling of proteins, peptides and other biopolymers.

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Keywords: click chemistry · imaging agents · isotopic labelling · β-lactams · radiopharmaceuticals

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Supporting Information

Synthesis of ^{18}F-Labelled β-Lactams by Using the Kinugasa Reaction

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Materials and Methods

General: ^{1}H-NMR spectra: Bruker Avance II 300 (300 MHz) and Bruker Avance II+ 600 (600 MHz). ^{1}H chemical shifts are reported in ppm relative to residual peaks of deuterated solvents. Higher-order NMR spectra were approximately interpreted as first-order spectra, if possible. The observed signal multiplicities are characterized as follows: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, as well as br = broad. Coupling constants (*J*) were reported in hertz (Hz). ^{13}C-NMR spectra [additional APT (Attached Proton Test)]: Bruker Avance II 300 (75.5 MHz) and Bruker Avance II+ 600 (125.9 MHz). ^{13}C chemical shifts are reported relative to residual peaks of deuterated solvents. Low resolution ESI-MS: Finnigan LCQ. High resolution ESI-MS: Bruker APEX IV 7T FTICR MS. TLC: Merck precoated sheets, 0.25 mm Sil G/UV_{254}. The chromatograms were viewed under UV light and/or by treatment with phosphomolybdic acid (10% in ethanol). Column chromatography: Merck silica gel, grade 60, 230–400 mesh. Solvent proportions are indicated in a volume:volume ratio. All reactions were carried out with magnetic stirring if not stated otherwise and, if air or moisture sensitive substrates and/or reagents, were handled in flame-dried glassware under argon or nitrogen. Organic extracts were dried with anhydrous $MgSO_4$. *C*-4-Fluorophenyl-*N*-phenyl nitrone (**1**),[1] *N*-4-fluorobenzylidene aniline,[2] 1-propargyl uracyl (**5**),[3] *N*-hydroxymorpholine[4] and Boc-βAla-Phe-OMe[5] were prepared according to literature.

HPLC analyses and purifications were carried out on Dionex UltiMate 3000 System with Ultimate 3000 Diode Array Detector coupled in series with Berthold NaI detector. If not otherwise stated, a Chromolith® SpeedROD RP-18e column (Merck, Darmstadt Germany), 50×4.6 mm, was used for analyses and purifications of radiofluorinated products. Aqueous MeCN solutions were used as a mobile phase. StrataX cartridges were obtained from

Phenomenex (Aschaffenburg, Germany) and Chromafix® 30-PS-HCO_3 cartridges from Macherey-Nagel (Düren, Germany).

[^{18}F]Fluoride was produced via the ^{18}O(p,n)^{18}F reaction by bombardment of enriched [^{18}O]water with 16.5 MeV protons using a MC16 cyclotron (Scanditronix, Uppsala, Sweden). All radiochemical yields are not decay-corrected. If not otherwise indicated all radiochemical experiments were carried out in triplicates.

Chemistry

3-Carbomethoxy-4-(4-fluorophenyl)-1-phenylazetidin-2-one (2): Et_3N (0.19 mL, 0.14 g, 1.39 mmol) was added to a suspension CuI (0.27 g, 1.39 mmol) in anhydrous MeCN (15 mL) under Ar. The resulting colorless solution was cooled in a water-ice bath and methyl propiolate (0.28 mL, 0.28 g, 2.79 mmol) was added dropwise. To a resulting yellow suspension a solution of **1** (0.20 g, 0.93 mmol) in MeCN (5 mL) was added. Thereafter, the cooling bath was removed and the reaction mixture was stirred at ambient temperature for 45 min. 1 M H_2SO_4 (3 mL) was added and the mixture was extracted with Et_2O (2 × 25 mL). The organic phase was washed with 1 M $NaHSO_4$ (3 × 10 mL), brine (2 × 5 mL), dried, filtered and concentrated under reduced pressure. The residual oil was purified by column chromatography (EtOAc:hexane = 1:4) to give **2** (0.18 g, 65%) as a mixture of *cis*/*trans*-isomers (1:2.5, ^{1}H-NMR) as a faint yellow viscous oil which solidified slowly in a refrigerator into a colorless solid. R_f = 0.21, EtOAc:hexane = 1:4. ^{1}H-NMR($CDCl_3$, 300 MHz): δ = 3.80 (s, 3 H), 5.41 (s, 1 H), 5.47 (s, 0.7 H, *trans*), 5.51 (s, 0.3 H, *cis*), 7.03–7.16 (m, 3 H), 7.21–7.30 (m, 4 H), 7.32–7.37 (m, 0.5 H), 7.42–7.49 (m, 1.5 H); ^{13}C-NMR($CDCl_3$, 75.5 MHz): δ = 52.5 (*cis*), 53.4 (*trans*); 57.8 (*cis*), 58.3 (*trans*); 116.6 (d, *J* = 22.7 Hz, *cis*), 117.2 (d, *J* = 21.9 Hz, *trans*); 118.5; 125.79 (*cis*), 125.84 (*trans*); 129.9 (d, *J* = 8.3 Hz, *trans*), 130.4 (d, *J* = 9.1 Hz, *cis*); 130.3; 131.3 (d, *J* = 3.8 Hz, *cis*), 133.8 (d, *J* = 3.8 Hz, *trans*); 138.3 (*trans*), 138.5 (*cis*); 161.3; 164.4 (d, *J* = 246.1 Hz, *cis*), 164.5 (d, *J* = 246.8 Hz, *trans*); 168.3. MS (ESI): positive mode *m/z* = 322.1 ([M + Na]$^+$); MS (ESI): negative mode *m/z* = 298.1 ([M – H]$^-$); ESI HRMS: calcd for $C_{17}H_{14}FNO_3Na^+$: 322.0850; found: 322.0837; calcd for $C_{17}H_{13}FNO_3^-$: 298.0885; found: 298.0886.

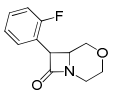
4-(4-Fluorophenyl)-3-hydroxymethyl-1-phenylazetidin-2-one (3): 1,10-Phenanthroline (0.34 g, 1.86 mmol) was added to a solution of Et_3N (0.26 mL, 0.19 g, 1.86 mmol), CuI (0.35 g, 1.86 mmol) and propargyl alcohol (0.11 mL, 0.10 g, 1.78

mmol) in anhydrous MeCN (10 mL) under Ar. The resulting red suspension was cooled in a water-ice bath and a solution of **1** (0.2 g, 0.93 mmol) in MeCN (5 mL) was added. Thereafter, the cooling bath was removed and the reaction mixture was stirred at ambient temperature for 48 h. The mixture was concentrated under reduced pressure, the residue was diluted with ether (70 mL) and the resulting suspension was filtered through Celite®. The filtrate was washed with 1 M $NaHSO_4$ (3 × 10 mL), water (3 × 10 mL), brine (2 × 5 mL), dried, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (EtOAc:hexane = 1:1) to give **2** (99 mg, 39%) as a mixture of *cis*/*trans*-isomers (10:1, ^{1}H-NMR) as a colorless solid. R_f = 0.4, EtOAc:hexane = 1:1. ^{1}H-NMR($CDCl_3$, 300 MHz): δ = 3.24–3.34 (m, 0.1 H, *trans*), 3.46–3.64 (m, 0.9 H, *cis*), 3.73–3.82 (m, 0.9 H, *cis*), 4.02–4.10 (m, 0.1 H, *trans*), 4.13–4.25 (m, 0.1 H, *trans*), 5.07 (d, *J* = 2.1 Hz, 0.1 H, *trans*), 5.29 (d, *J* = 5.7 Hz, 0.9 H, *cis*), 7.02–7.15 (m, 3 H), 7.22–7.43 (m, 6 H); ^{13}C-NMR($CDCl_3$, 75.5 MHz): δ = 56.7; 56.8 (*cis*), 62.5 (*trans*); 57.9 (*cis*), 58.7 (*trans*); 116.0 (d, *J* = 27.9 Hz, *cis*), 116.2 (d, *J* = 21.1 Hz, *trans*); 117.1; 124.2 (*cis*), 124.1 (*trans*); 127.7 (d, *J* = 9.1 Hz, *trans*), 128.6 (d, *J* = 8.3 Hz, *cis*); 129.07 (*cis*), 129.3 (*trans*); 129.9 (d, *J* = 3.0 Hz); 137.2; 162.6 (d, *J* = 248.4 Hz); 165.3. MS (ESI): positive mode *m/z* = 565.2 ($[2 M + Na]^+$), 294.1 ($[M + Na]^+$), 272.1 ($[M + H]^+$); MS (ESI): negative mode *m/z* = 270.1 ($[M – H]^–$); ESI HRMS: calcd for $C_{16}H_{14}FNO_2Na^+$: 294.0901; found: 294.0897; calcd for $C_{16}H_{15}FNO_2^+$: 272.1081; found: 272.1079; calcd for $C_{16}H_{13}FNO_2^–$: 270.0936; found: 270.0934.

1-{[2-(4-Fluorophenyl)-4-oxo-1-phenylazetidin-3-yl]methyl}-uracil (4): Pyridine (0.43 mL, 0.42 g, 5.33 mmol) was added to a solution of Et_3N (0.28 mL, 0.20 g, 2.0 mmol), CuI (0.38 g, 2.0 mmol) and 1-propargyluracil (**5**)[3] (0.2 g, 1.33 mmol) in anhydrous MeCN (15 mL) under Ar. The resulting suspension was cooled in a water-ice bath and **1** (0.4 g, 1.87 mmol) was added. Thereafter, the cooling bath was removed and the reaction mixture was stirred at ambient temperature for 72 h. The mixture was filtered through Celite®, the filtrate was concentrated under reduced pressure, and the residue was taken in EtOAc (70 mL). The organic solution was washed with 1 M HCl (3 × 10 mL), water (3 × 10 mL), brine (2 × 5 mL), dried, filtered and concentrated under reduced pressure. The residue was purified by column chromatography ($CHCl_3$:MeOH = 10:1) to give after recrystallization from MeOH/Et_2O **4** (0.17 mg, 35%) as a mixture of *cis*/*trans*-isomers (5:1, ^{1}H-NMR) as a colorless solid. R_f = 0.45, $CHCl_3$:MeOH = 10:1, two time development. ^{1}H-NMR[$(CD_3)_2SO_2$, 300 MHz]: δ = 3.47 (dd, *J* = 14.0, 7.6 Hz, 1 H), 3.68 (dd, *J* = 14.0, 9.2 Hz, 1 H), 4.04–4.15 (m, 0.85 H, *cis*), 4.18–4.27 (m, 0.15 H, *cis*), 5.19 (d, *J* = 2.3 Hz, 0.15 H,

trans), 5.46 (d, *J* = 7.6 Hz, 0.85 H, *cis*), 5.51 (d, *J* = 5.9 Hz, 0.85 H, *cis*), 5.60 (d, *J* = 8.1 Hz, 0.15 H, *trans*), 7.02–7.11 (m, 1 H), 7.14 (d, *J* = 7.9 Hz, 1 H), 7.18–7.35 (m, 7 H), 7.38–7.50 (m, 2 H), 11.26 (s, 0.85 H, *cis*), 11.33 (s, 0.15 H, *trans*); ^{13}C-NMR[$(CD_3)_2SO_2$, 75.5 MHz]: δ = 44.0; 51.3 (*cis*), 57.2 (*trans*); 55.5 (*cis*), 58.0 (*trans*); 100.6 (*cis*), 101.1 (*trans*); 115.8 (d, *J* = 21.9 Hz, *cis*), 115.9 (d, *J* = 21.1 Hz, *trans*); 116.69 (*trans*), 116.73 (*cis*); 123.8 (*cis*), 123.9 (*trans*); 128.3 (d, *J* = 1.5 Hz, *trans*), 129.3 (d, *J* = 7.6 Hz, *cis*); 129.2; 130.3 (d, *J* = 3.0 Hz); 136.96 (*cis*), 136.98 (*trans*); 145.4 (*cis*), 145.7 (*trans*); 150.7 (*cis*), 151.0 (*trans*); 160.4; 163.9 (d, *J* = 45.3 Hz, *trans*), 164.2 (d, *J* = 100.4 Hz, *cis*). MS (ESI): positive mode *m/z* = 1118.4 ([3 M + Na]$^+$), 753.2 ([2 M + Na]$^+$), 388.1 ([M + Na]$^+$), 366.1 ([M + H]$^+$); MS (ESI): negative mode *m/z* = 364.1 ([M – H]$^-$); ESI HRMS: calcd for $C_{20}H_{16}FN_3O_3Na^+$: 388.1068; found: 368.1065; calcd for $C_{20}H_{17}FN_3O_3^+$: 366.1248; found: 366.1245; calcd for $C_{20}H_{15}FN_3O_3^-$: 364.1103; found: 364.1096.

7-(2-Fluorophenyl)-4-oxa-1-azabicyclo[4.2.0]octan-8-one (8a): To $CuSO_4 \cdot 5H_2O$ (1.94 g, 7.76 mmol) concentrated aqueous ammonia (6 mL) and H_2O (17 mL) were added. The solution was stirred with ice cooling for 15 min, while a stream of Ar was passed through the solution. Solid $NH_2OH \cdot HCl$ (1.5 g, 21.6 mmol) was added to the reaction mixture under Ar and stirring was continued for a further 1 h. A solution of 1-ethynyl-2-fluorobenzene (**6a**) (0.94 mL, 1 g, 8.33 mmol) in EtOH (30 mL) was added rapidly to the resulting pale blue solution. An additional water (30 mL) was added, the reaction flask was shaken by hand, the yellow precipitate was separated by filtration, washed with H_2O (3 × 50 mL), MeOH (3 × 50 mL), Et_2O (3 × 50 mL) and dried at 40 °C and 4 mbar for 1 h to give copper(I) (2-fluorophenyl)acetylide (0.73 g, 52%) which was directly used for the next step.

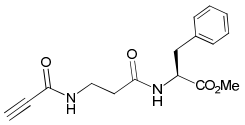


Yellow HgO (1.2 g, 5.54 mmol) was added to a solution of *N*-hydroxymorpholine[4] (0.4 g, 3.88 mmol) in $CHCl_3$ (30 mL). The suspension was vigorously stirred for 45 min under Ar to yield 3,6-dihydro-2*H*-1,4-oxazine-4-oxide (**7**) (TLC control). The mixture was filtered through Celite® directly into the flask with copper(I) (2-fluorophenyl)acetylide (0.65 g, 3.56 mmol). Pyridine (0.4 mL, 0.39 g, 4.95 mmol) was added and the reaction mixture was stirred for 4 h. The mixture was washed with H_2O (3 × 10 mL), brine (2 × 10 mL), dried, filtered and concentrated under reduced pressure. The residual dark brown tar was extracted with boiling hexane (5 × 20 mL). Hexane was removed under reduced pressure and the residue was purified by column chromatography (EtOAc:hexane = 1:2). Three fractions were isolated: the fraction containing mainly *cis*-**8a** (0.11 g, 14%) as a brown solid, *trans*-**8a** (70 mg, 9%) as a brown semisolid as well as the mixed fraction (30 mg; overall 34%). *cis*/*trans*-**8a** were used to

identify not only [^{18}F]**8a** but also [^{18}F]**8b**. *cis*-**8a**: R_f = 0.19, EtOAc:hexane = 2:1. ^{1}H-NMR($CDCl_3$, 300 MHz): δ = 2.94 (dt, *J* = 12.8, 1.0 Hz, 1 H), 3.0–3.15 (m, 1 H), 3.29 (td, *J* = 11.4, 3.6 Hz, 1 H), 3.76 (dd, *J* = 12.8, 3.6 Hz, 1 H), 3.92–4.02 (m, 2 H), 4.71 (d, *J* = 4.8 Hz,1 H), 7.0–7.10 (m, 1 H), 7.11–7.19 (m, 1 H), 7.23–7.34 (m, 1 H), 7.65–7.74 (m, 1 H); ^{13}C-NMR($CDCl_3$, 75.5 MHz): δ = 38.9, 48.6, 51.3, 66.0, 69.5, 115.1 (d, *J* = 20.4 Hz), 120.1 (d, *J* = 16.6 Hz), 124.4 (d, *J* = 3.8 Hz), 129.3 (d, *J* = 7.6 Hz), 129.6 (d, *J* = 3.8 Hz), 158.6, 164.2 (d, *J* = 366.2 Hz). MS (ESI): positive mode *m/z* = 465.2 ([2 M + Na]$^+$), 244.1 ([M + Na]$^+$), 222.1 ([M + H]$^+$); ESI HRMS: calcd for $C_{12}H_{12}FNO_2Na^+$: 244.0744; found: 244.0744; calcd for $C_{12}H_{13}FNO_2^+$: 222.0925; found: 222.0927; calcd for $C_{12}H_{11}FNO_2^-$: 220.0779; found: 220.0773.

trans-**8a**: R_f = 0.15, EtOAc:hexane = 2:1. ^{1}H-NMR($CDCl_3$, 300 MHz): δ = 3.12 (ddd, *J* = 13.3, 11.7, 4.6 Hz, 1 H), 3.40–3.51 (m, 2 H), 3.61 (ddd, *J* = 10.3, 4.6, 2.2 Hz, 1 H), 3.77 (ddd, *J* = 13.3, 3.6, 0.4 Hz, 1 H), 3.91 (dd, *J* = 11.7, 4.6, 1 H), 4.24 (s, 1 H), 4.40 (dd, *J* = 11.2, 4.4 Hz, 1 H), 7.05 (ddd, *J* = 9.9, 8.4, 1.2 Hz, 1 H), 7.10–7.18 (m, 1 H), 7.23–7.33 (m, 1 H), 7.44 (td, *J* = 7.6, 1.8 Hz, 1 H); ^{13}C-NMR($CDCl_3$, 75.5 MHz): δ = 39.1, 53.0 (d, *J* = 2.3 Hz), 54.2 (d, *J* = 1.5 Hz), 66.1, 71.9, 115.4 (d, *J* = 21.1 Hz), 122.0 (d, *J* = 15.0 Hz), 124.5 (d, *J* = 3.0 Hz), 128.4 (d, *J* = 3.8 Hz), 129.4 (d, *J* = 8.3 Hz), 158.8, 163.8 (d, *J* = 250.7 Hz). MS (ESI): positive mode *m/z* = 465.2 ([2 M + Na]$^+$), 244.1 ([M + Na]$^+$), 222.1 ([M + H]$^+$); ESI HRMS: calcd for $C_{12}H_{12}FNO_2Na^+$: 244.0744; found: 244.0744; calcd for $C_{12}H_{13}FNO_2^+$: 222.0925; found: 222.0928; calcd for $C_{12}H_{11}FNO_2^-$: 220.0779; found: 220.0779.

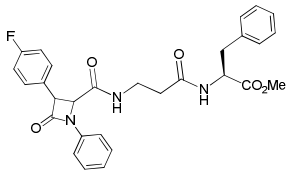
Propioloyl-βAla-Phe-OMe (9): To an ice-cold solution of TMS-propiolic acid (0.51 g, 3.59 mmol) and HOSu (0.42 g, 3.65 mmol) in THF (20 mL) DCC (0.75g, 3.63 mmol) was added and the reaction mixture was stirred for an additional 10 min at the same temperature. Thereafter, the cooling bath was removed and the mixture was stirred for a further 1 h. Afterwards, a solution of crude H-βAla-Phe-OMe trifluoroacetate in CH_2Cl_2 (20 mL) prepared from Boc-βAla-Phe-OMe (1.1 g, 3.14 mmol) as follows: deprotection with 50% TFA in CH_2Cl_2 (20 mL) for 10 min at ambient temperature, concentration under reduced pressure, addition of toluene to the residue and concentration of the resulting emulsion under reduced pressure (3 × 50 mL) and DIEA (0.77 mL, 0.57g, 4.41 mmol) were added and stirring continued for another 3 h. The reaction mixture was filtered, concentrated under reduced pressure, the residue was dissolved in Et_2O (80 mL) and washed with H_2O (4 × 20 mL), 1 M $NaHSO_4$ (3 × 20 mL), H_2O (20 mL), 10% $NaHCO_3$ (3 × 20 mL), brine (2 × 10 mL), dried, filtered and concentrated under reduced pressure. The residue was



purified by column chromatography (acetone:hexane = 1:1) to give TMS-propiolyl-βAla-Phe-OMe (0.82 g, 70%) as a colorless solid which was directly used for the next step.

To a solution of TMS-propiolyl-βAla-Phe-OMe (0.82 g, 2.19 mmol) in THF (5 mL) 1 M TBAF in THF (50 µL, 5 mmol) was added immediately followed by water (250 µL) and the reaction mixture was stirred for 10 min. EtOAc (50 mL) was added, the organic solution was washed with H_2O (3 × 20 mL), brine (2 × 10 mL), dried, filtered and concentrated under reduced pressure. The residue was purified by column chromatography (acetone:hexane = 1:1) to give **9** (0.6 g, 63% over 2 steps) as a colorless solid. R_f = 0.29, acetone:hexane = 1:1. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ = 2.44 (dt, *J* = 6.7, 4.9 Hz, 2 H), 2.81 (s, 1 H), 3.07 (ddd, *J* = 31.6, 13.2, 6.1 Hz, 2 H), 3.48–3.61 (m, 2 H), 3.76 (s, 3 H), 4.88 (dd, *J* = 14.1, 6.1 Hz, 1 H), 6.24 (d, *J* = 7.7 Hz, 1 H), 6.85 (br, 1 H), 7.10–7.15 (m, 2 H), 7.25–7.35 (m, 3 H); ^{13}C-NMR($CDCl_3$, 75.5 MHz): δ = 34.8, 35.5, 37.8, 52.4, 53.2, 73.3, 77.2, 127.2, 128.7, 129.1, 135.7, 152.1, 170.9, 171.9. MS (ESI): positive mode *m/z* = 627.3 ($[2 M + Na]^+$), 325.1 ($[M + Na]^+$), 303.1 ($[M + H]^+$); MS (ESI): negative mode *m/z* = 347.1 ($[M + HCOOH – H]^-$), 301.1 ($[M – H]^-$); ESI HRMS: calcd for $C_{16}H_{18}N_2O_4Na^+$: 325.1159; found: 325.1159; calcd for $C_{16}H_{19}N_2O_4^+$: 303.1139; found: 303.1340; calcd for $C_{16}H_{17}N_2O_4^-$: 301.1194; found: 301.1188.

Depsipeptide 10: Et_3N (70 µL, 50 mg, 0.49 mmol) was added to a suspension CuI (93 mg, 0.49 mmol) in anhydrous MeCN (10 mL) under Ar. The resulting colorless solution was cooled in a water-ice bath, propiolylated peptide **9** (0.1 g, 0.33 mmol) was added and the mixture was stirred for 5 min. Thereafter, **1** (86 mg, 0.40 mmol) was added. The cooling bath was removed and the reaction mixture was stirred at ambient temperature for 3 h. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in EtOAc (25 mL), washed with 1 M HCl (3 × 10 mL), brine (2 × 5 mL), dried, filtered and concentrated under reduced pressure. The residual yellow solid was purified by column chromatography (EtOAc:hexane = 1:1) followed by recrystallization from EtOAc/hexane to give *trans*-**10** (86 mg, 50%) as a mixture of diastereomers 1:1 as a colorless solid. The mother liquor was concentrated under reduced pressure to give a *cis*/*trans*-**10** (22 mg, overall 63%) (*cis*/*trans* = 1:2). The mixture of geometric isomers was separated by HPLC, the fraction corresponding to *cis*-**10** was collected and concentrated under reduced pressure to give *cis*-**10** (4 mg, 2%, mixture of diastereomers 1:1) as a colorless foam. A partial isomerization (~15%, HPLC) into the more thermodynamically stable *trans*-isomer was observed. HPLC: Chromolith® SemiPrep RP-18e column 100×10 mm (Merck, Darmstadt Germany), eluent:



40% MeCN, flow rate: 1.5 mL/min, t_R = 13.1 min (*cis*-**10**), 17.6 min (*trans*-**10**). *trans*-**10**: R_f = 0.4, EtOAc:hexane = 1:1. ^{1}H-NMR($CDCl_3$, 300 MHz; mixture of two diastereomers 1:1): δ = 2.35 (t, *J* = 5.5, 1.0 Hz, 1 H), 2.38–2.49 (m, 1 H), 3.06–3.27 (m, 2 H), 3.36 (dq, *J* = 12.7, 5.6 Hz, 0.5 H), 3.57 (q, *J* = 5.6 Hz, 1 H), 3.69 (s, 1.5 H), 3.69 (s, 1.5 H), 3.76 (d, *J* = 2.3 Hz, 0.5 H), 3.82 (d, *J* = 2.3 Hz, 0.5 H), 3.70–3.75 (m, 0.5 H), 3.78–3.87 (m, 0.5 H), 4.80–4.91 (m, 1 H), 5.38 (d, *J* = 2.2 Hz, 0.5 H), 5.48 (d, *J* = 2.2 Hz, 0.5 H), 6.14 (d, *J* = 7.8 Hz, 0.5 H), 6.56 (d, *J* = 8.1 Hz, 0.5 H), 6.74–6.81 (m, 0.5 H), 7.03–7.11 (m, 4 H), 7.12–7.18 (m, 0.5 H), 7.2–7.31 (m, 8 H), 7.35–7.44 (m, 2 H); ^{13}C-NMR($CDCl_3$, 75.5 MHz; mixture of two diastereomers 1:1): δ = 35.5, 35.8; 36.4, 36.5; 37.0, 37.6; 52.4, 52.6; 53.1, 53.9; 56.4, 56.8; 63.8, 64.0; 116.2 (d, *J* = 12.8 Hz), 116.3 (d, *J* = 12.8 Hz); 117.06, 117.13; 124.4, 124.5; 127.0, 127.1; 127.9, 128.0; 128.6 (d, *J* = 1.5 Hz), 129.09 (d, *J* = 3.0 Hz); 129.12; 132.3 (d, *J* = 7.6 Hz), 132.4 (d, *J* = 8.3 Hz); 135.8, 136.3; 136.97, 136.99; 161.8, 162.0; 163.2 (d, *J* = 235.6 Hz); 163,77, 163.80; 170.8, 171.7; 172.0, 173.1. MS (ESI): positive mode *m/z* = 1057.4 ([2 M + Na]$^+$), 1035.4 ([2 M + H]$^+$), 540.2 ([M + Na]$^+$), 518.2 ([M + H]$^+$); MS (ESI): negative mode *m/z* = 630.2 ([M + 2 HCOOH – H]$^-$), 516.2 ([M – H]$^-$); ESI HRMS: calcd for $C_{29}H_{28}FN_3O_5Na^+$: 540.1905; found: 540.1907; calcd for $C_{29}H_{29}FN_3O_5^+$: 518.2086; found: 518.2085; calcd for $C_{29}H_{27}FN_3O_5^-$: 516.1940; found: 516.1933.

cis-**10**: R_f = 0.31, EtOAc:hexane = 1:1. ^{1}H-NMR($CDCl_3$, 600 MHz; mixture of two diastereomers 1:1): δ = 2.07–2.18 (m, 2 H), 3.07–3.13 (m, 1 H), 3.15–3.20 (m, 1 H), 3.22–3.32 (m, 1 H), 3.35–3.42 (m, 1 H), 3.73 (s, 1.5 H), 3.74 (s, 1.5 H), 4.40 (d, *J* = 6.2 Hz, 1 H), 4.41 (d, *J* = 6.2 Hz, 1 H), 4.87–4.92 (m, 1 H), 5.31 (d, *J* = 6.2 Hz, 1 H), 5.91 (dd, *J* = 7.4, 4.7 Hz, 1 H), 6.95–7.04 (m, 2 H), 7.07–7.12 (m, 3 H), 7.19–7.23 (m, 1 H), 7.25–7.33 (m, 9 H); ^{13}C-NMR($CDCl_3$, 150.9 MHz; mixture of two diastereomers 1:1): δ = 35.14, 35.21; 35.16; 37.65, 37.74; 52.4, 52.5; 53.08, 53.09; 57.37, 57.38; 59.6, 59.70; 115.8 (d, *J* = 17.6 Hz), 116.3 (d, *J* = 12.8 Hz); 117.18, 117.21; 124.6; 127.22, 127.26; 126.68 (d, *J* = 8.8 Hz); 128.68, 128.70; 129.09, 129.14; 129.16, 129.17; 129.73 (d, *J* = 3.0 Hz), 129.74 (d, *J* = 3.0 Hz); 135.68, 135.70; 136.80, 136.83; 162.0, 162.1; 162.96 (d, *J* = 220.3 Hz), 163.00 (d, *J* = 176.3 Hz); 163,66, 163.71; 170.83, 170.86; 172.0, 172.02. MS (ESI): positive mode *m/z* = 1057.4 ([2 M + Na]$^+$), 1035.4 ([2 M + H]$^+$), 540.2 ([M + Na]$^+$), 518.2 ([M + H]$^+$); MS (ESI): negative mode *m/z* = 630.2 ([M + 2 HCOOH – H]$^-$), 516.2 ([M – H]$^-$); ESI HRMS: calcd for $C_{29}H_{28}FN_3O_5Na^+$: 540.1905; found: 540.1907; calcd for $C_{29}H_{29}FN_3O_5^+$: 518.2086; found: 518.2083; calcd for $C_{29}H_{27}FN_3O_5^-$: 516.1940; found: 516.1933.

3-Propionamidopropanol-1 (11): 3-Aminopropanol-1 (1.08 mL, 1.07 g, 14.3 mmol) was added to an ice-cold solution of propiolic acid (0.885 mL, 1 g, 14.3 mmol) and EEDQ (2.57 g, 14.3 g) in DMF (10 mL). The reaction mixture was allowed to warm to ambient temperature and stirred for further 16 h. Thereafter, the reaction mixture was concentrated under reduced pressure. The oily residue was dissolved in CH_2Cl_2 (20 mL) and extracted with H_2O (3 × 20 mL). The combined aqueous washings were saturated with solid $NaHSO_4$ and extracted with EtOAc (3 × 20 mL). The combined EtOAc extracts were washed with brine (3 × 20 mL), dried and concentrated under reduced pressure. The oily residue was purified by column chromatography (acetone:hexane = 1:1) to give **11** (0.6 g, 33%) as a yellow oil. R_f = 0.12, acetone:hexane = 1:1. ^{1}H-NMR($CDCl_3$, 300 MHz; mixture of two rotamers): δ = 1.75 (dt, *J* = 11.9, 6.1 Hz, 1.8 H), 1.81 (dt, *J* = 11.9, 6.0 Hz, 0.2 H), 2.50–2.76 (br, 0.9 H), 2.83 (s, 0.9 H), 3.16 (s, 0.1 H), 3.46 (q, *J* = 6.1 Hz, 1.8 H), 3.60 (q, *J* = 6.1 Hz, 0.2 H), 3.70 (t, *J* = 5.8 Hz, 1.8 H), 3.77 (t, *J* = 5.8 Hz, 0.2 H), 4.60–4.85 (br, 0.1 H), 6.55–6.85 (br, 0.9 H), 8.27–8.50 (br, 0.1 H); ^{13}C-NMR($CDCl_3$, 75.5 MHz; mixture of two rotamers): δ = 31.5, 32.3; 37.3, 41.2; 60.0, 60.2; 73.6, 74.1; 77.1, 79.6; 56.4, 153.0. MS (ESI): positive mode *m/z* = 173.1 ([M + 2 Na]$^+$), 150.1 ([M + Na]$^+$), 128.1 ([M + H]$^+$); ESI HRMS: calcd for $C_6H_9NO_2Na^+$: 150.0525; found: 150.0528; calcd for $C_6H_{10}NO_2^+$: 128.0706; found: 128.0704.

3-Propionamidopropyl-1-oxycarbonyl chloride (12): TMP (65.7 mg, 71.6 μL 0.542 mmol) was added dropwise to an ice-cold solution of **11** (68.9 mg, 0.542 mmol) and triphosgene (54 mg, 0.181 mmol) in THF (4 mL) under Ar. The reaction mixture was stirred for 5 min, filtered and concentrated under reduced pressure. Crude chloroformate **12** obtained as a colorless semisolid was immediately used for derivatization of BSA.

Propiolated BSA: A solution of BSA (0.3 g, 4.51 μmol; fraction V, heat shock isolation) in 0.15 M $KHCO_3$ (15 mL) was added to crude **12** and the mixture was stirred until **12** was completely dissolved. The crude derivatizated BSA was purified by ultrafiltration (to separate low- and high-molecular impurities ultrafiltration units with cut-off of 30 and 100 kDa, respectively, were used). Propiolated BSA was obtained as a colorless aqueous solution. Determination of the protein concentration (0.63 nmol/μL) was accomplished by the measurement of the absorbance at 280 nm by comparison of the peak area with that of the native BSA (concentration range: 0.01–0.75 nmol/μL). The average acylation degree was estimated by comparison of molecular weights of the native and modified BSA (determined

using MALDI-TOF) giving a value of 48–49 3-propionamidopropyl-1-oxycarbonyl groups/protein molecule.

Radiochemistry

4-[^{18}F]Fluorobenzaldehyde ([^{18}F]FBA): [^{18}F]FBA was produced as described by Haka et al..[6] The synthesis was carried out in a remotely controlled synthesis module (Scintomics, Fürstenfeldbruck, Germany). [^{18}F]Fluoride (12–15 GBq) was fixed on a Chromafix® PS-HCO_3 cartridge and eluted with 0.066 M K_2CO_3 solution (360 µL) into a solution of K2.2.2 (20 mg, 53.1 µmol) in MeCN (700 µL). After evaporation and azeotropic drying with MeCN, 4-formyl-*N,N,N*-trimethylanilinium triflate (5 mg, 16.0 µmol) dissolved in DMSO (500 µL) was added to the dry cryptate ($[K\subset2.2.2]^+/^{18}F^-$) and heated at 85 °C for 10 min. After cooling, the reaction mixture was diluted with water (5 mL) and loaded onto a C_{18} cartridge (the C_{18} cartridge was preconditioned with 2 mL EtOH followed by 30 mL H_2O). The cartridge was washed 0.1 M HCl (10 mL) and H_2O (5 mL) and 4-[^{18}F]fluorobenzaldehyde (4–10 GBq, 30–50% EOB) was eluted with EtOH (0.8–1 mL). Radiochemical yields were 30–50% (end of synthesis yield: 4–10 GBq) and the synthesis was completed within 50 min. The radiochemical purity after solid phase extraction (SPE) purification was 85–95%. Further purification steps were not necessary since there was no interference of the ^{18}F-labelled by-products with the subsequent reaction steps. The main concern was the removal of the precursor, which would affect [^{18}F]**1** formation. The applied SPE purification was effective for removing the precursor nearly quantitatively. Radiochemical yields of [^{18}F]**1** were calculated based on [^{18}F]FBA purity. Quality control: eluent: 30% MeCN, flow rate: 1.5 mL/min, t_R = 1.8 min.

***C*-4-[^{18}F]Fluorophenyl-*N*-phenyl nitrone ([^{18}F]1):** [^{18}F]**1** was produced as described by Zlatopolskiy et al..[5] To an ethanolic solution of [^{18}F]FBA (100 µL, 100–200 MBq), *N*-phenylhydroxylamine (5 mg, 46 mmol) in EtOH (100 µL) and 0.01 M HCl (100 µL) were added. The reaction volume was adjusted with H_2O to 400 µL and the mixture was stirred at 25 °C for 10 min. After that, the reaction mixture was diluted with H_2O (9 mL) and loaded onto a preconditioned StrataX C18 cartridge. The cartridge was washed with 0.5 M NaH_2PO_4 (5 mL), H_2O (5 mL), 0.1 M $NaHCO_3$ (5 mL) and H_2O (5 mL) and dried with a slight flow of argon for 1 min. Finally, [^{18}F]**1** was eluted with MeCN (300 µL). Radiochemical yields amounted to 90%. Radiochemical purities were determined by HPLC and were ≥ 93%

referred to [^{18}F]FBA. Radiochemical yields of the radio-Kinugasa reaction were calculated based on [^{18}F]**1** purity.
Quality control: eluent: 30% MeCN, flow rate: 1.5 mL/min, t_R = 2.2 min.

Preparation of [^{18}F]2 under inert atmosphere – General procedure (GP1): To a suspension of CuI (9.5 mg, 50 µmol) in MeCN (972 µL), triethylamine (28 µL, 20.4 mg, 0.202 mmol) was added under argon atmosphere and the resulting colorless solution was allowed to stir for 2 min at ambient temperature. Subsequently, if not otherwise indicated 16 µL (0.8 µmol) of the CuI stock solution was transferred into an argon-flushed conical vial. Thereafter, if not otherwise indicated 16 µL (0.8 µmol) of a freshly prepared solution of methyl propiolate (4.2 µL, 4.0 mg, 50 µmol) in MeCN (1 mL) was added followed by the addition of [^{18}F]**1** (50–100 MBq) in MeCN (50 µL). Afterwards, the reaction volume was adjusted to 400 µL with MeCN or if indicated with another solvent. Then, the mixture was stirred for 10 min (if not otherwise indicated) at the given temperature. RCYs of [^{18}F]**2** were determined by HPLC.
HPLC conditions: gradient: 30% MeCN – 0–3 min, 30→43% MeCN – 3–4 min, 43% MeCN – 4–10 min, flow rate: 1.5 mL/min, t_R = 5.8 min (*cis*-isomer), t_R = 6.6 min (*trans*-isomer).

Dependence of [^{18}F]2 yield on the amount of methyl propiolate: The study was carried out according to GP1 using 100 µL, 40 µL, 20 µL, 16 µL, 10 µL and 4 µL of the stock solution of CuI (5 µmol, 2 µmol, 1 µmol, 0.8 µmol, 0.5 µmol and 0.2 µmol, respectively) and 100 µL, 40 µL, 20 µL, 16 µL, 10 µL and 4 µL of the stock solution of methyl propiolate (5 µmol, 2 µmol, 1 µmol, 0.8 µmol, 0.5 µmol and 0.2 µmol, respectively) for 10 min and 20 min at room temperature.

Dependence of [^{18}F]2 yield on the reaction temperature: The study was carried out according to GP1 at 40 °C, 50 °C, 60 °C and 80 °C.

Effect of the reaction solvent on [^{18}F]2 yield: The study was carried out according to GP1 at ambient temperature using DMF, DMSO and pyridine to adjust the reaction volume.

Effect of the Cu-ligand on [^{18}F]2 yield: The study was carried out according to GP1 at ambient temperature with the following modification. To the stock solution of methyl

propiolate 1,10-phenanthroline (18.0 mg, 100 mmol, 2 eq relative to CuI) or pyridine (15.5 µL, 15.8 mg, 200 mmol, 4 eq relative to CuI) was added.

Preparation of [^{18}F]3 and [^{18}F]4 – General procedure (GP2): In MeCN: To a suspension of CuI (9.5 mg, 50 µmol) in MeCN (200 µL) triethylamine (28 µL, 20.4 mg, 0.202 mmol) was added under argon atmosphere and the mixture was allowed to stir for 2 min at ambient temperature. Subsequently, 1,10-phenanthroline (18.0 mg, 100 µmol) and alkyne were added followed by the addition of [^{18}F]**1** (50–100 MBq) in acetonitrile (50 µL). The reaction volume was adjusted to 400 µL with acetonitrile, the mixture was stirred for 30 min at 120 °C and analyzed by radio-HPLC. In pyridine: CuI (9.5 mg) was dissolved in pyridine (200 µL). Subsequently 1,10-phenanthroline (18.0 mg, 100 µmol) and alkyne were added followed by the addition of [^{18}F]**1** (50–100 MBq) in MeCN (50 µL). The reaction volume was adjusted to 400 µL with pyridine; the mixture was stirred for 30 min at 50 °C and analyzed by HPLC.
HPLC conditions: [^{18}F]**3**: eluent: 30% MeCN, flow rate: 1.5 mL/min, t_R = 3.1 min (*cis*-isomer), t_R = 4.3 min (*trans*-isomer). [^{18}F]**4**: eluent: 30% MeCN, flow rate: 1.5 mL/min, t_R = 2.8 min (*cis*-isomer), t_R = 3.4 min (*trans*-isomer).
[^{18}F]**3:** It was prepared according to GP2 using propargyl alcohol (1 or 10 µmol). RCYs: in MeCN: 10 and 60% (with 1 and 10 µmol precursor, respectively); in pyridine: 50 and 82% (with 1 or 10 µmol precursor, respectively).
[^{18}F]**4:** It was prepared according to GP2 using 1-propargyluracil (1.5 mg, 10 µmol). RCYs: in MeCN: 65%; in pyridine: < 2%.

Preparation of [^{18}F]8a,b – General procedure (GP3): In MeCN: To a suspension of CuI (9.5 mg, 50 µmol) in MeCN (200 µL) triethylamine (28 µL, 20.4 mg, 0.202 mmol) was added under argon atmosphere and the mixture was allowed to stir for 2 min at ambient temperature. Subsequently 1,10-phenanthroline (18.0 mg, 100 µmol) and a solution of the corresponding [^{18}F]fluorophenylacetylene (50–100 MBq) in MeCN/MeOH = 1:1 (150 µL) were added and the mixture was allowed to stir for 2 min at room temperature. Thereafter, the freshly prepared 3,6-dihydro-2*H*-1,4-oxazine-4-oxide (**7**) [3 mg, 29.7 µmol; prepared as described in the preparation of **8** (see above) but in MeCN instead of $CHCl_3$] in MeCN (200 µL) was added and the reaction mixture was stirred for 10 min at 90 °C and analyzed by HPLC.

HPLC conditions: [^{18}F]**8a**: gradient: 10% MeCN – 0–8 min, 10→40% MeCN – 8–10 min, 40% MeCN – 10–14 min, flow rate: 1.5 mL/min, t_R = 4.7 min. [^{18}F]**8b**: gradient: 10% MeCN

– 0–8 min, 10→40% MeCN – 8–10 min, 40% MeCN – 10–14 min, flow rate: 1.5 mL/min, t_R = 4.6 min (*cis*-isomer), t_R = 4.9 min (*trans*-isomer).

[^{18}F]8a: It was obtained according to GP3 using 1-ethynyl-2-[^{18}F]fluorobenzene ([^{18}F]**6a**) in RCY of 52%.

[^{18}F]8b: It was obtained according to GP3 using 1-ethynyl-4-[^{18}F]fluorobenzene ([^{18}F]**6b**) in RCY of 41%.

Synthesis of [^{18}F]2 and [^{18}F]10 under "click" conditions – General procedure (GP4): To the indicated volume of 0.2 M $CuSO_4$ an appropriate amount of a freshly prepared 50 mM solution of methyl propiolate or dipeptide **9** in MeCN was added. Thereafter, a solution of L-histidine (3.9 mg, 25 µmol) in H_2O (50 µL) was added followed by the addition of an aqueous solution of sodium ascorbate (9.8 mg, 50 µmol in 50 µL H_2O) and, afterwards, of [^{18}F]**1** (50–100 MBq) in MeCN (50 µL). The order of the addition of reagents is very important. If it was changed, no product formation could be detected. If necessary, the reaction volume was adjusted to 400 µL with H_2O and the mixture was stirred for 10 min ([^{18}F]**2**) or 20 min ([^{18}F]**10**) at ambient temperature and analyzed by HPLC.
HPLC conditions: [^{18}F]**2**: see GP1. [^{18}F]**10**: gradient: 30% MeCN – 0–3 min, 30→43% MeCN – 3–4 min, 43% MeCN – 4–10 min, flow rate: 1.5 mL/min, t_R = 5.7 min (*cis*-isomer), t_R = 6.4 min (*trans*-isomer).

Dependence of yields of the radio-Kinugasa reaction under "click" conditions on amount of alkyne precursor: The study was carried out according to GP4 using 50 µL, 20 µL, 16 µL, 10 µL, 4 µL, 2 µL, 1 µL and 0.5 µL of 0.2 M $CuSO_4$ and 200 µL, 100 µL, 20 µL, 16 µL, 10 µL, 4 µL, 2 µL and 1 µL of 50 mM acetonitrile solution of the corresponding precursor (methyl propiolate or **9**), respectively.

Simplified synthesis of [^{18}F]2 and [^{18}F]10 under "click" conditions: To 0.2 M $CuSO_4$ (50 µL) a freshly prepared 50 mM solution of methyl propiolate or dipeptide **9** in MeCN (20 µL) was added followed by the addition of 50 mM L-histidine (50 µL). Thereafter, 0.1 M sodium ascorbate (50 µL) and, finally, crude [^{18}F]**1** (complete reaction mixture) prepared as described above were added. The mixture was allowed to stir for 10 min at ambient temperature and analyzed by HPLC.

Synthesis of ^{18}F-labelled BSA conjugate: To 0.2 M $CuSO_4$ (50 µL) 1.3 mg (19 nmol, 30 µL) of BSA conjugate in H_2O was added. Thereafter, a solution of L-histidine (3.9 mg, 25 µmol) in H_2O (50 µL) was added followed by the addition of an aqueous solution of sodium ascorbate (9.8 mg, 50 µmol in 50 µL H_2O) and [^{18}F]**1** (50–100 MBq) in MeCN (50 µL). The mixture was stirred for 10 min at ambient temperature. The radiofluorinated BSA conjugate was isolated by filtration via a PD10 desalting cartridge and analyzed by HPLC on a Bio-Sil® R SEC 250 gel filtratrion column (BIO-RAD, USA), 300×7.8 mm. HPLC conditions: Phosphate buffer (0.05 M Na_2HPO_4, 0.05 M NaH_2PO_4, 0.15 M NaCl) (pH = 6.8) flow rate: 1.0 mL/min, t_R = 8.0 min.

Serum stability: In human blood serum (1.5 mL) the purified depsipeptide [^{18}F]**10** was shaken at 37 °C for 3h. At different time points (between 0 and 180 min) aliquots (100 µL) were taken and proteins were separated by precipitation with ice-cooled methanol (200 µL) followed by centrifugation for 5 min (12900 rpm). Finally, the supernatant was analyzed by radio-HPLC (Figure 1).

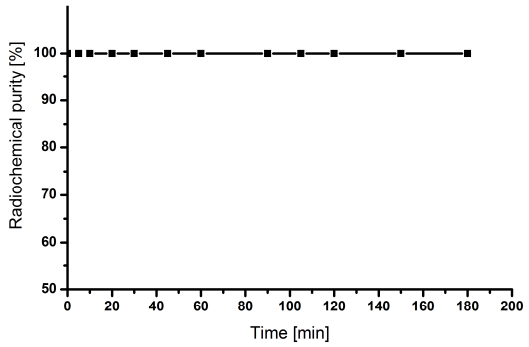


Figure 1. Blood serum stability of depsipeptide [^{18}F]**10**.

^{1}H- and APT-NMR Spectra

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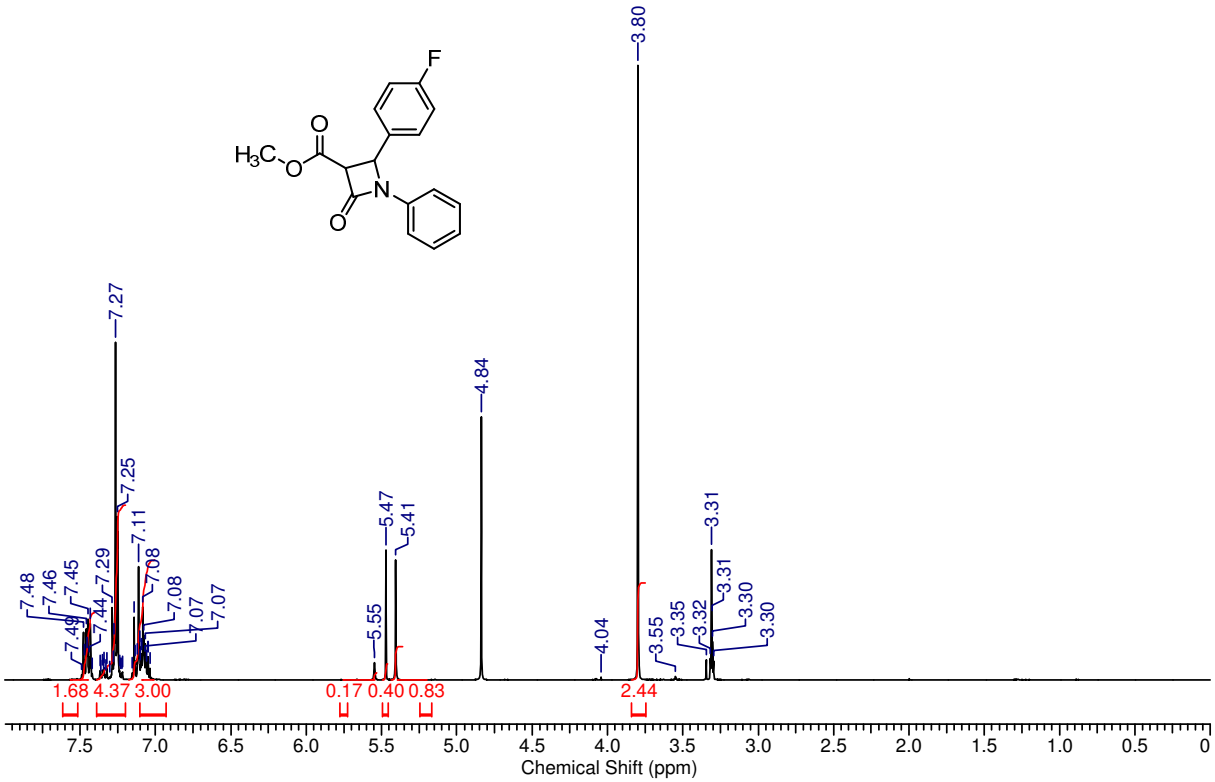

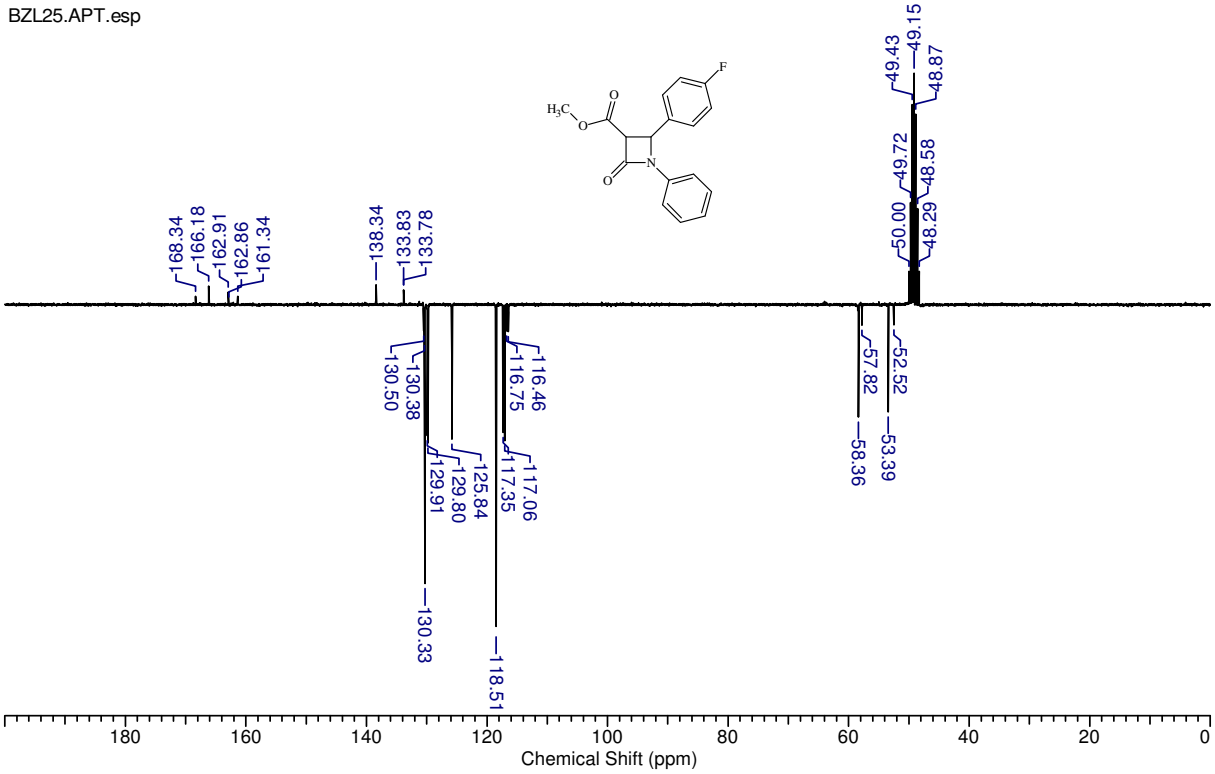

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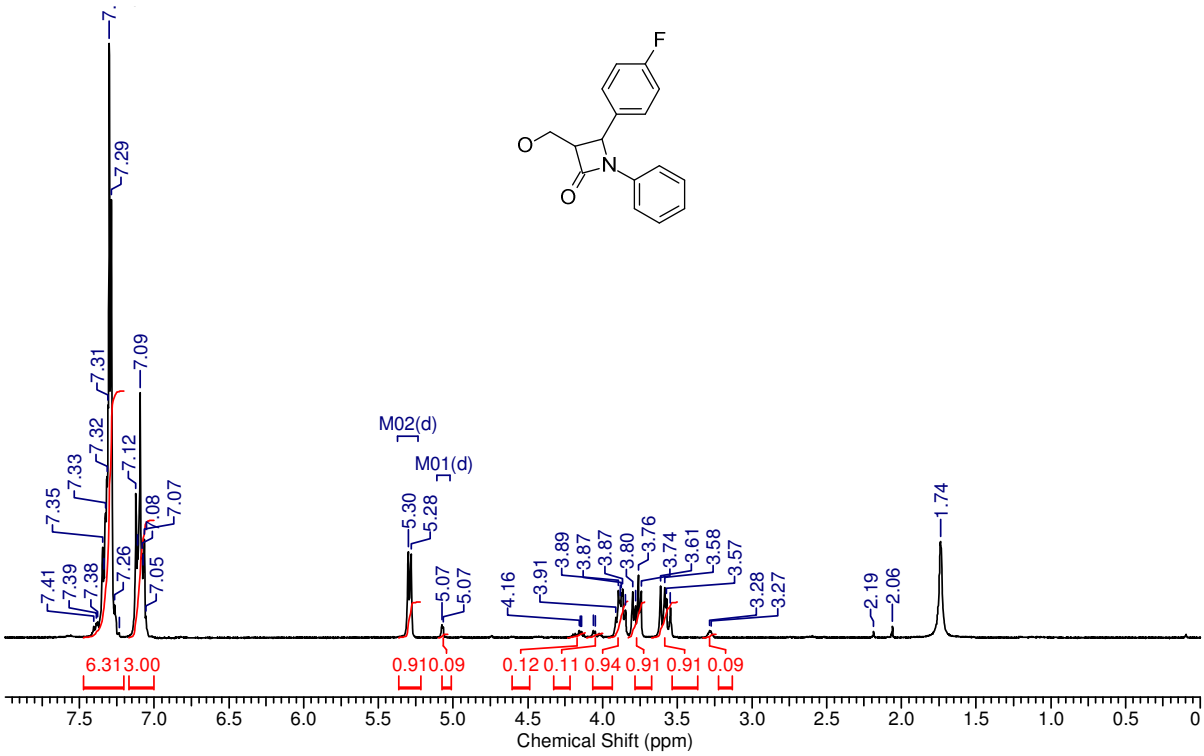
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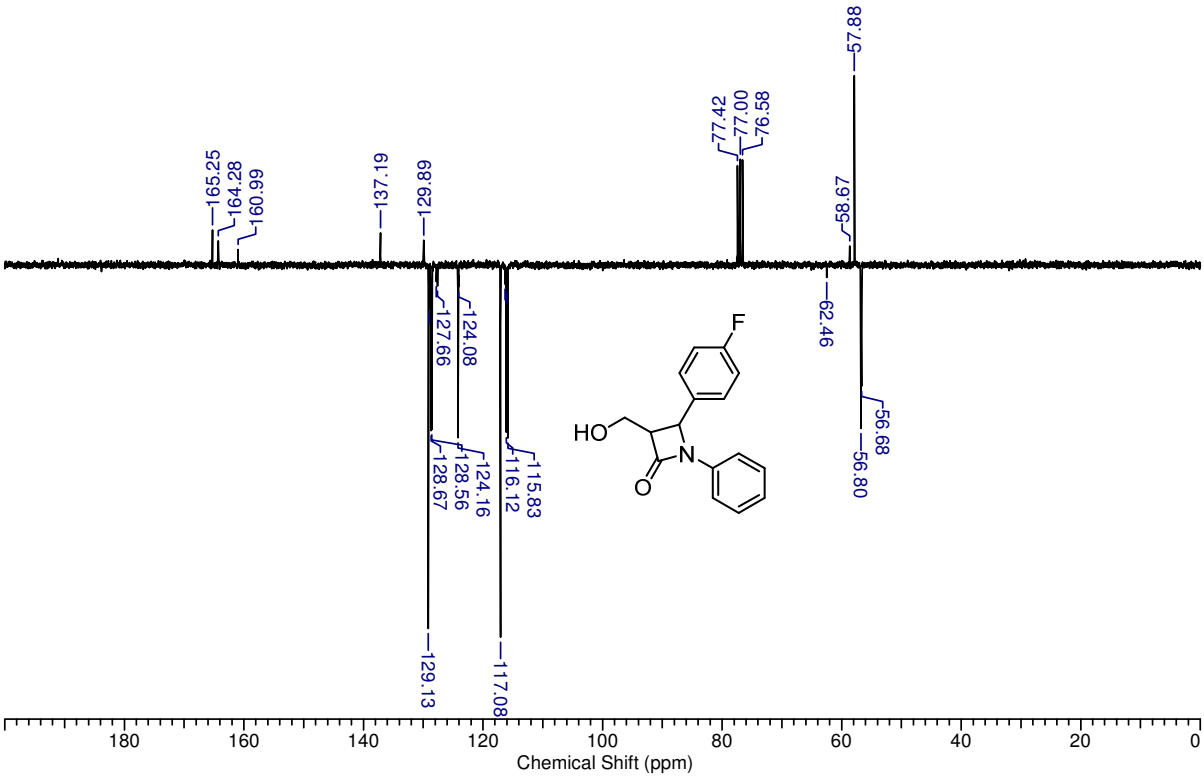
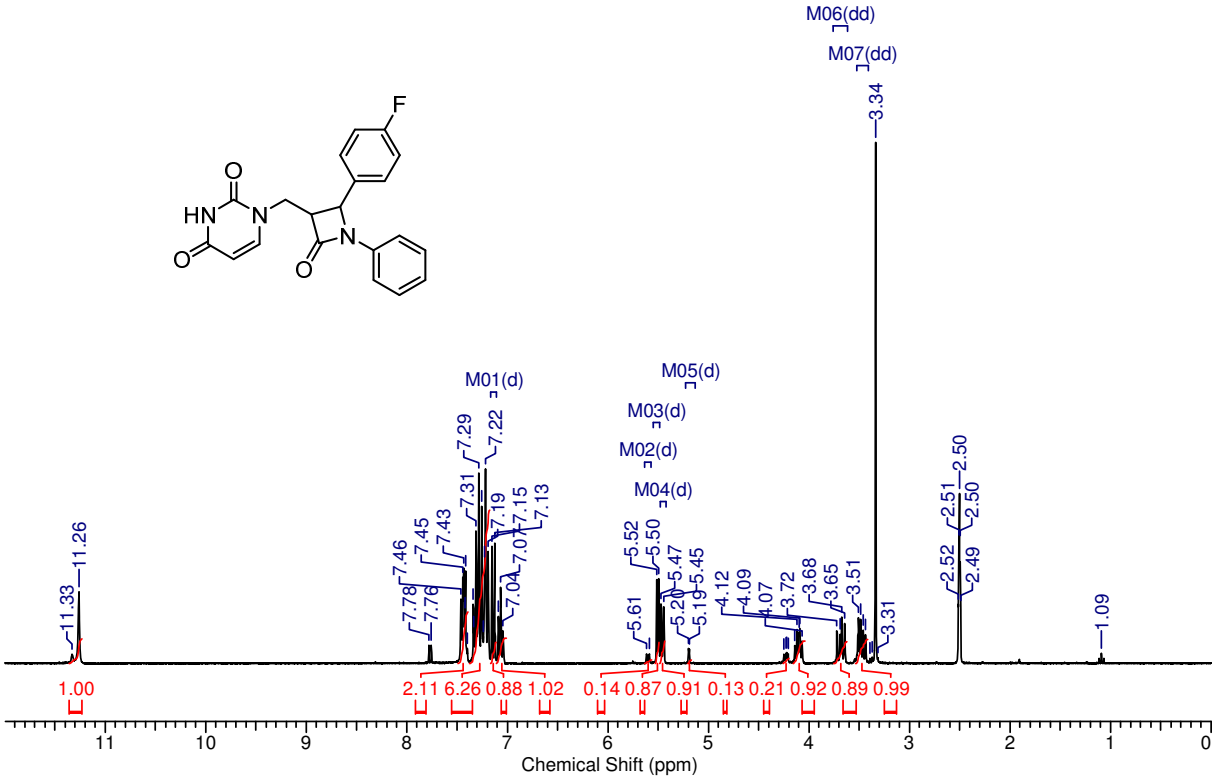
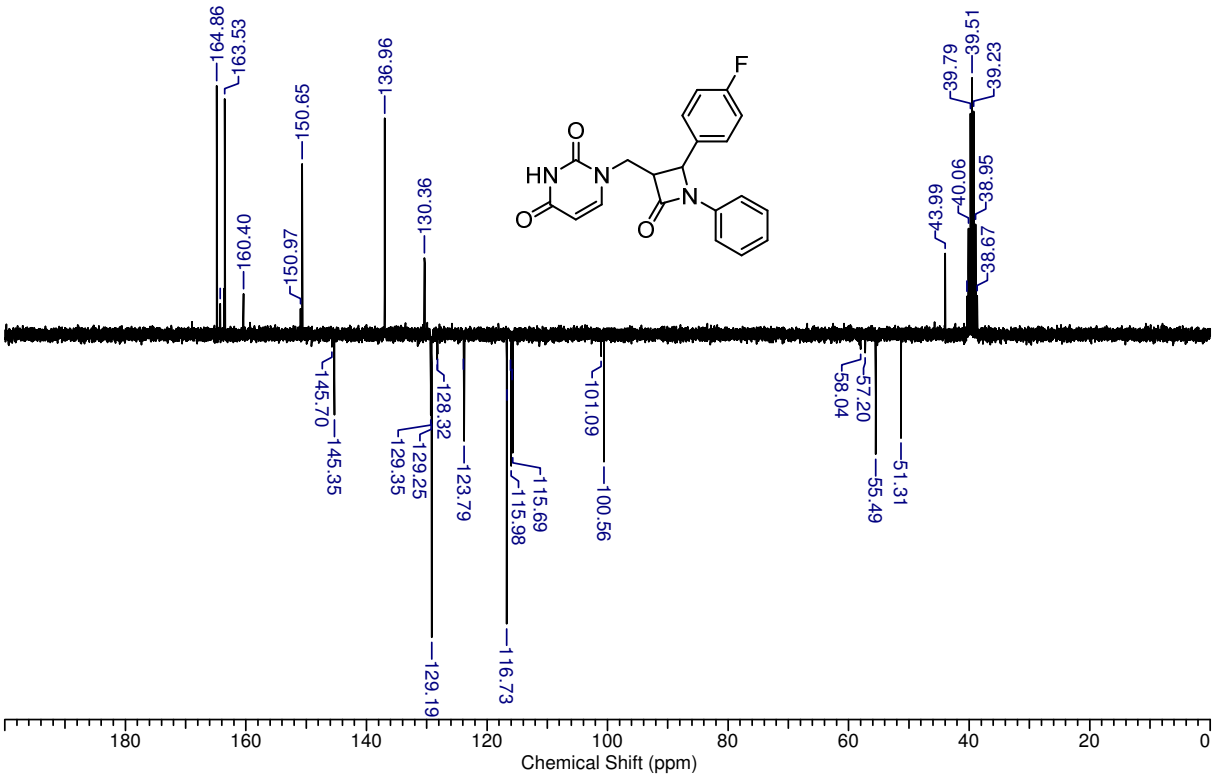
7.31
7.09
7.32
7.12
7.33
7.35
7.08
7.07
7.41
7.39
7.38
7.26
7.05
M02(d)
M01(d)
5.30
5.28
5.07
5.07
4.16
3.91
3.89
3.87
3.87
3.80
3.76
3.74
3.61
3.58
3.57
3.28
3.27
2.19
2.06
1.74
6.31
3.00
0.91
0.09
0.12
0.11
0.94
0.91
0.91
0.09
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Chemical Shift (ppm)

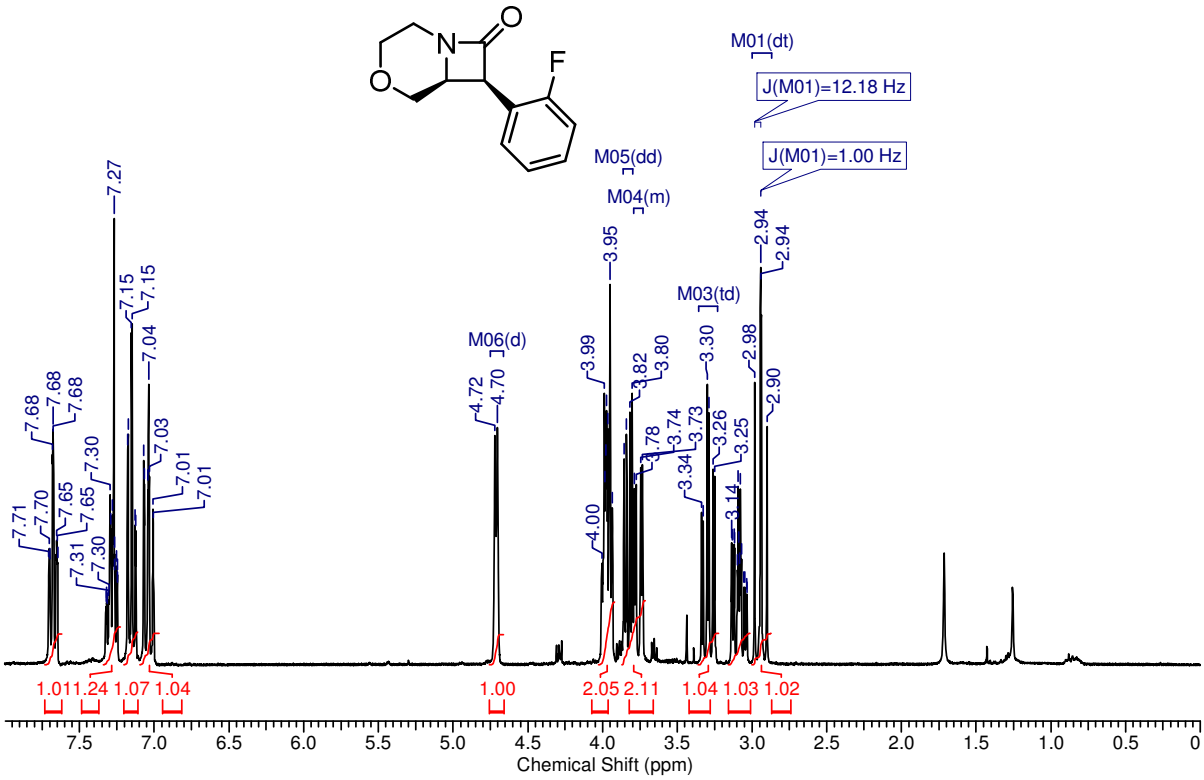

165.25
164.28
160.99
137.19
129.89
77.42
77.00
76.58
57.88
58.67
62.46
56.68
56.80
127.66
124.08
128.67
128.56
124.16
116.12
115.83
129.13
117.08
F
HO
N
O
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

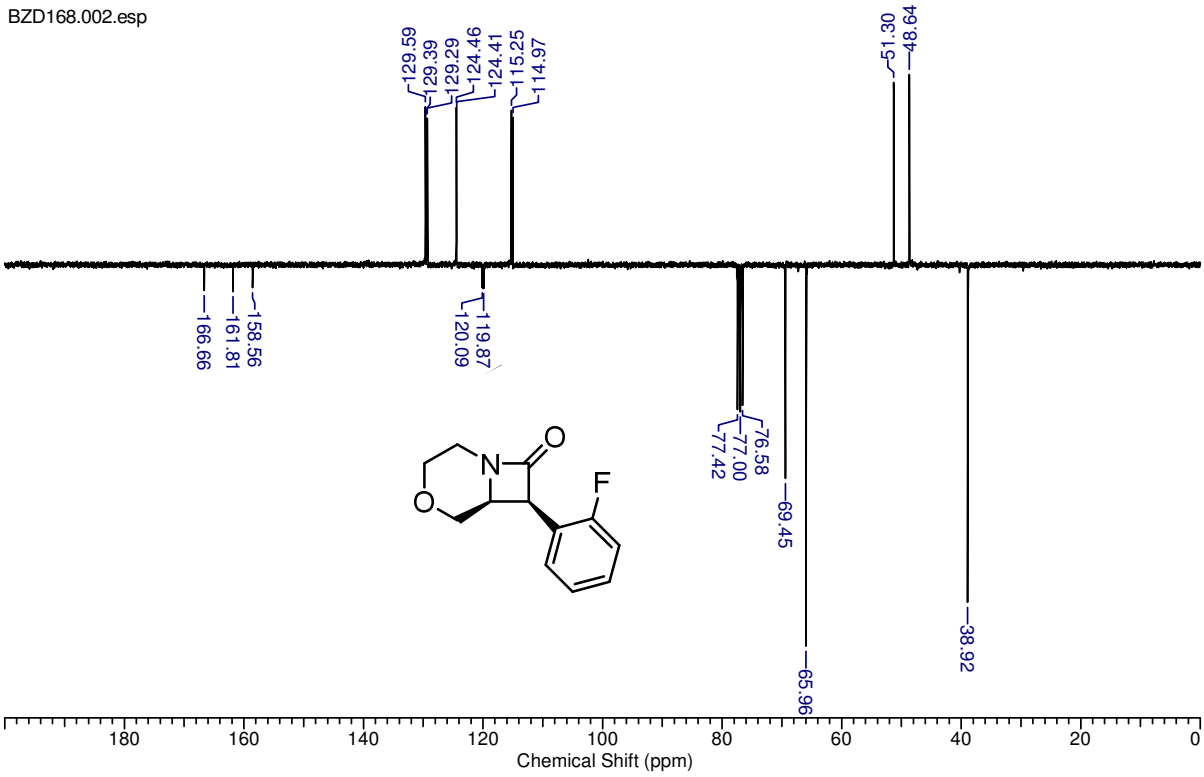
4:


F
O
HN
N
O
N
O
M06(dd)
M07(dd)
3.34
M01(d)
M05(d)
M03(d)
M02(d)
M04(d)
11.33
11.26
7.78
7.76
7.46
7.45
7.43
7.29
7.31
7.22
7.19
7.15
7.13
7.04
7.07
5.61
5.52
5.50
5.47
5.45
5.20
5.19
4.12
4.09
4.07
3.72
3.68
3.65
3.51
3.31
2.52
2.51
2.50
2.50
2.49
1.09
1.00
2.11
6.26
0.88
1.02
0.14
0.87
0.91
0.13
0.21
0.92
0.89
0.99
11
10
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

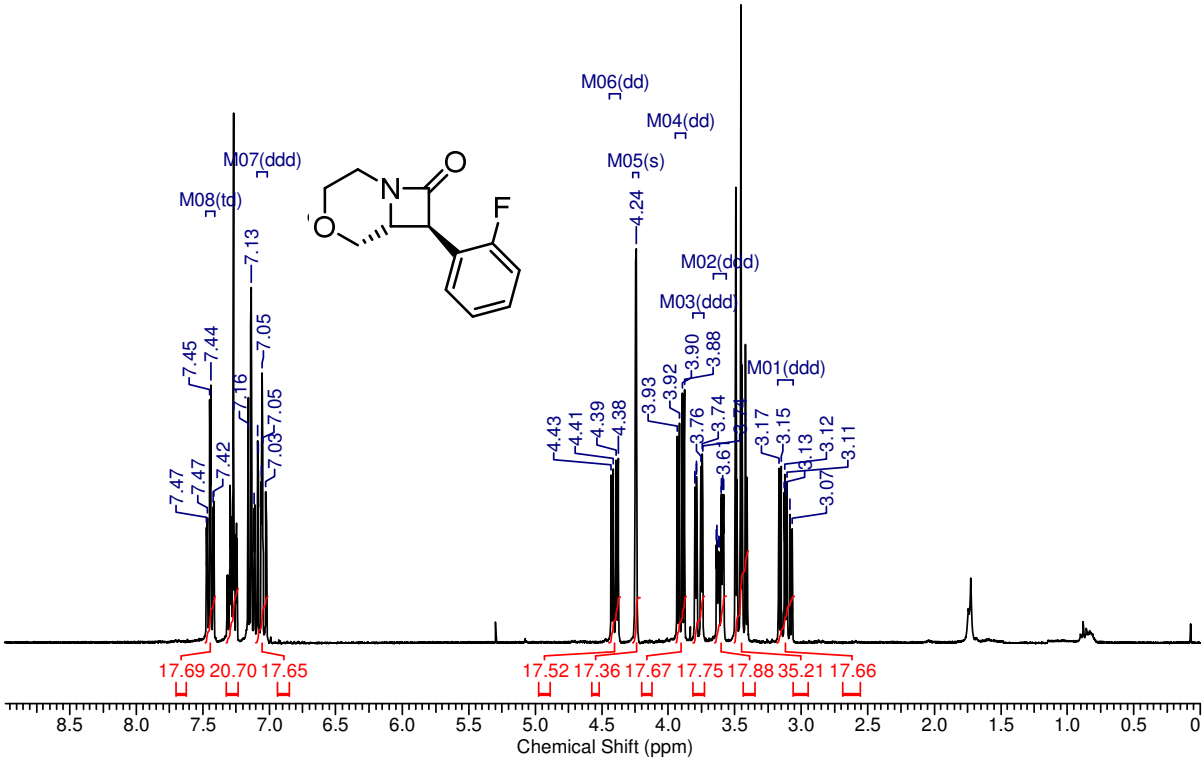

164.86
163.53
160.40
150.97
150.65
136.96
130.36
145.70
145.35
129.35
129.25
128.32
123.79
129.19
116.73
115.98
115.69
101.09
100.56
58.04
57.20
55.49
51.31
43.99
40.09
39.79
39.51
39.23
38.95
38.67
F
HN
O
N
O
N
O
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

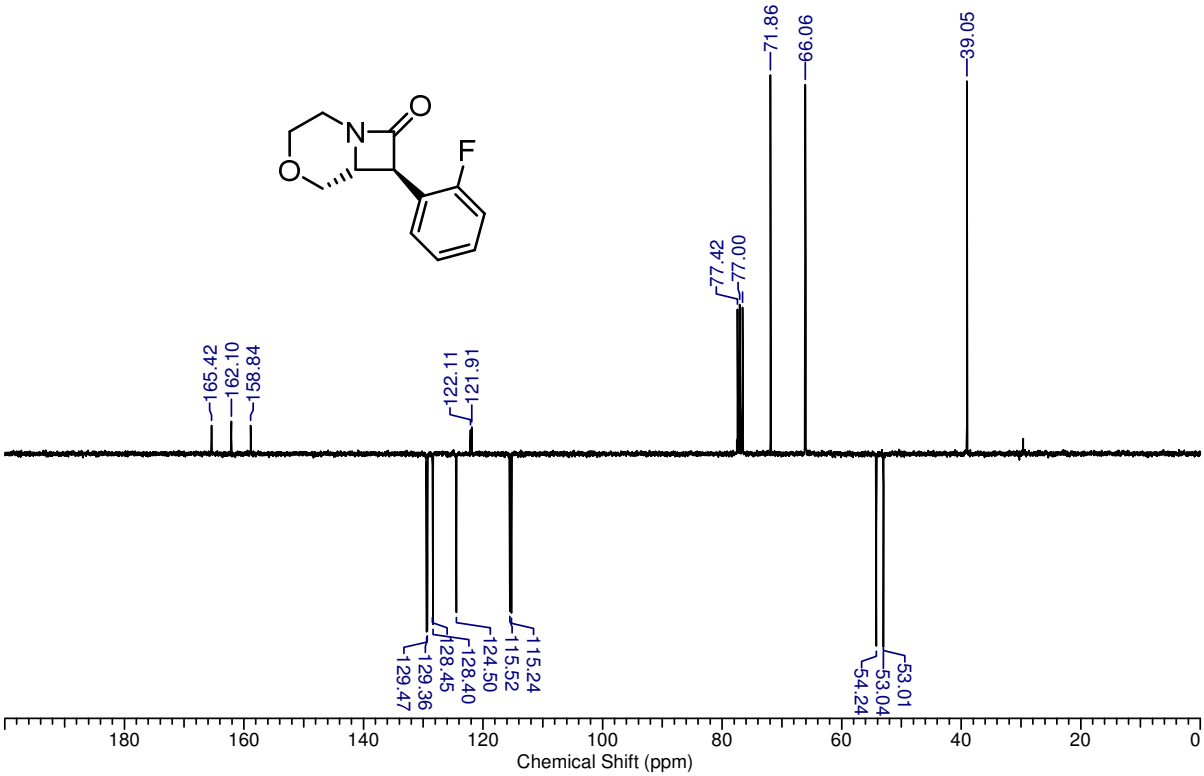
cis-**8a**:


M01(dt)
J(M01)=12.18 Hz
J(M01)=1.00 Hz
M05(dd)
M04(m)
M03(td)
M06(d)
Chemical Shift (ppm)

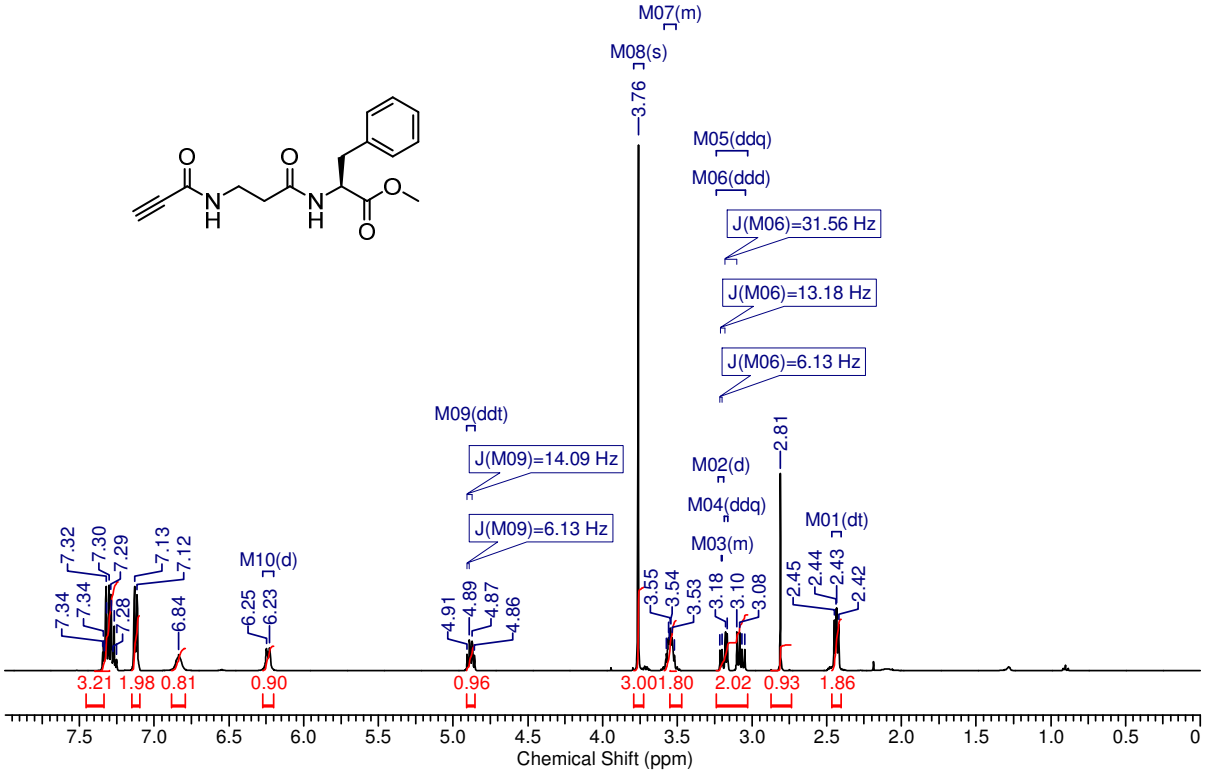

BZD168.002.esp
Chemical Shift (ppm)

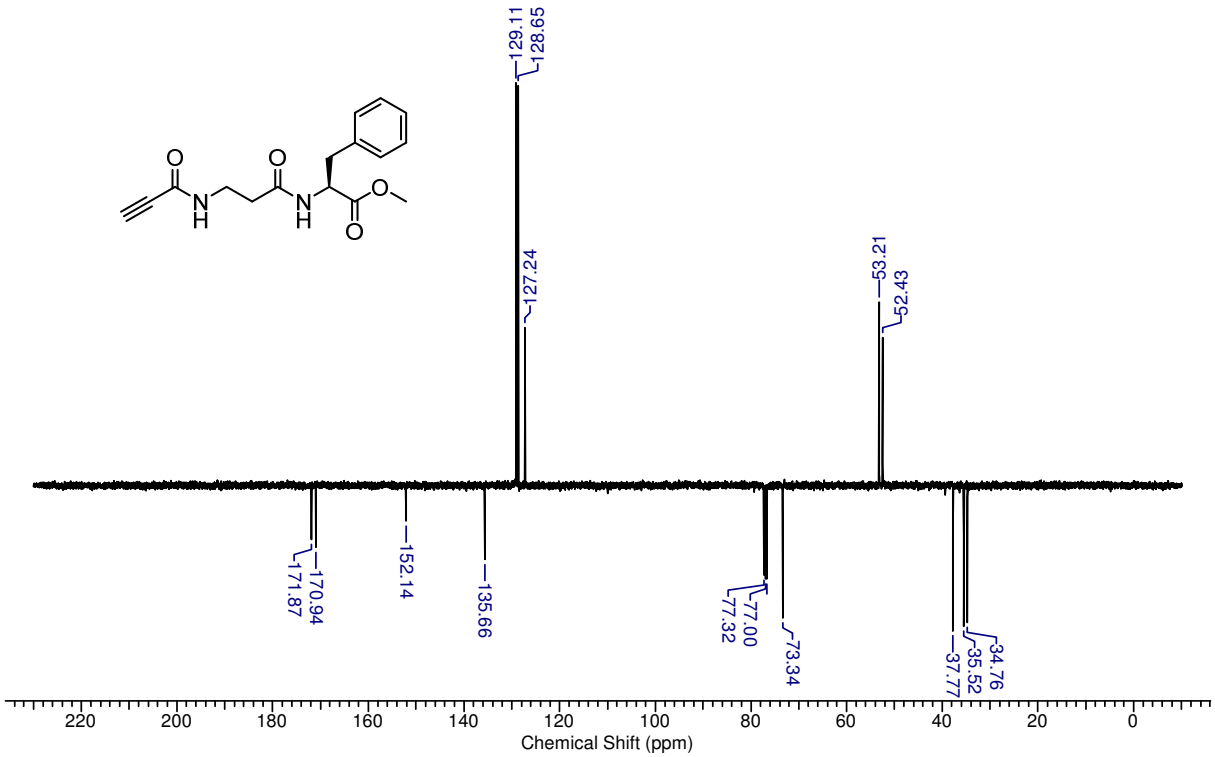
trans-**8a**:


M06(dd)
M04(dd)
M07(ddd)
M05(s)
M08(td)
M02(ddd)
M03(ddd)
M01(ddd)
7.45
7.44
7.16
7.13
7.05
7.05
7.03
7.47
7.47
7.42
4.43
4.41
4.39
4.38
4.24
3.93
3.92
3.90
3.88
3.76
3.74
3.61
3.17
3.15
3.13
3.12
3.11
3.07
17.69 20.70 17.65
17.52 17.36 17.67 17.75 17.88 35.21 17.66
Chemical Shift (ppm)

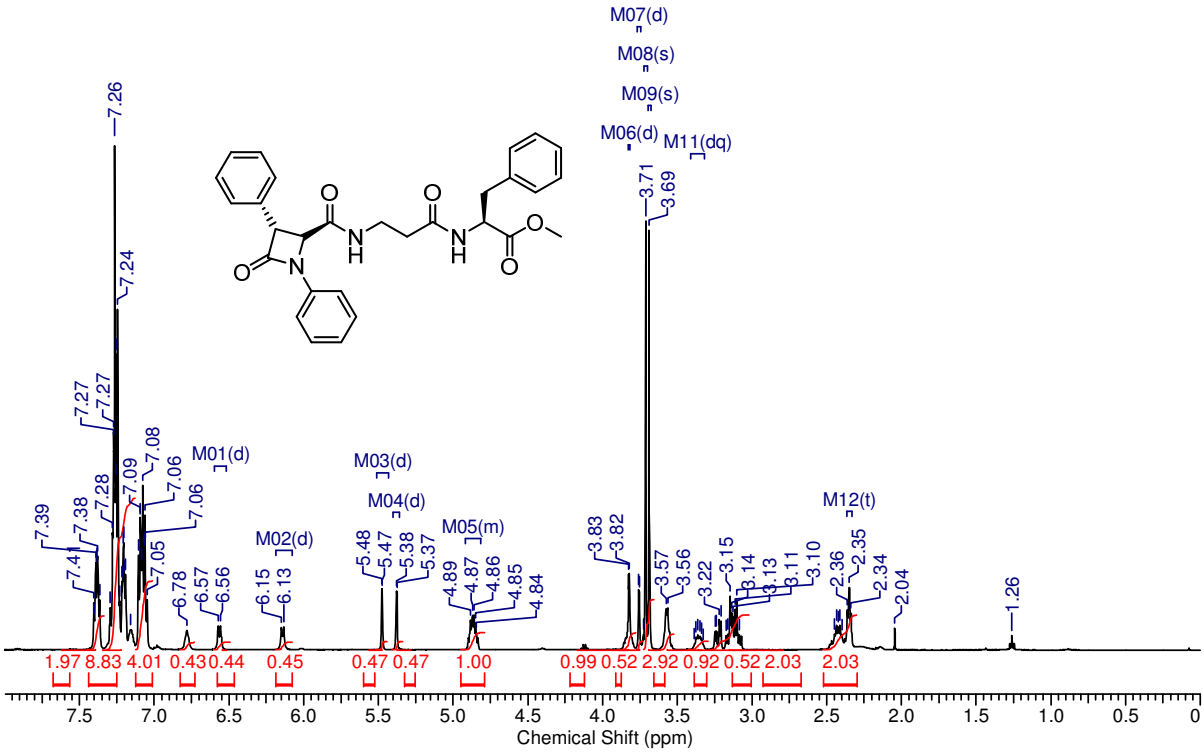

71.86
66.06
39.05
77.42
77.00
165.42
162.10
158.84
122.11
121.91
129.47
129.36
128.45
128.40
124.50
115.52
115.24
54.24
53.04
53.01
Chemical Shift (ppm)

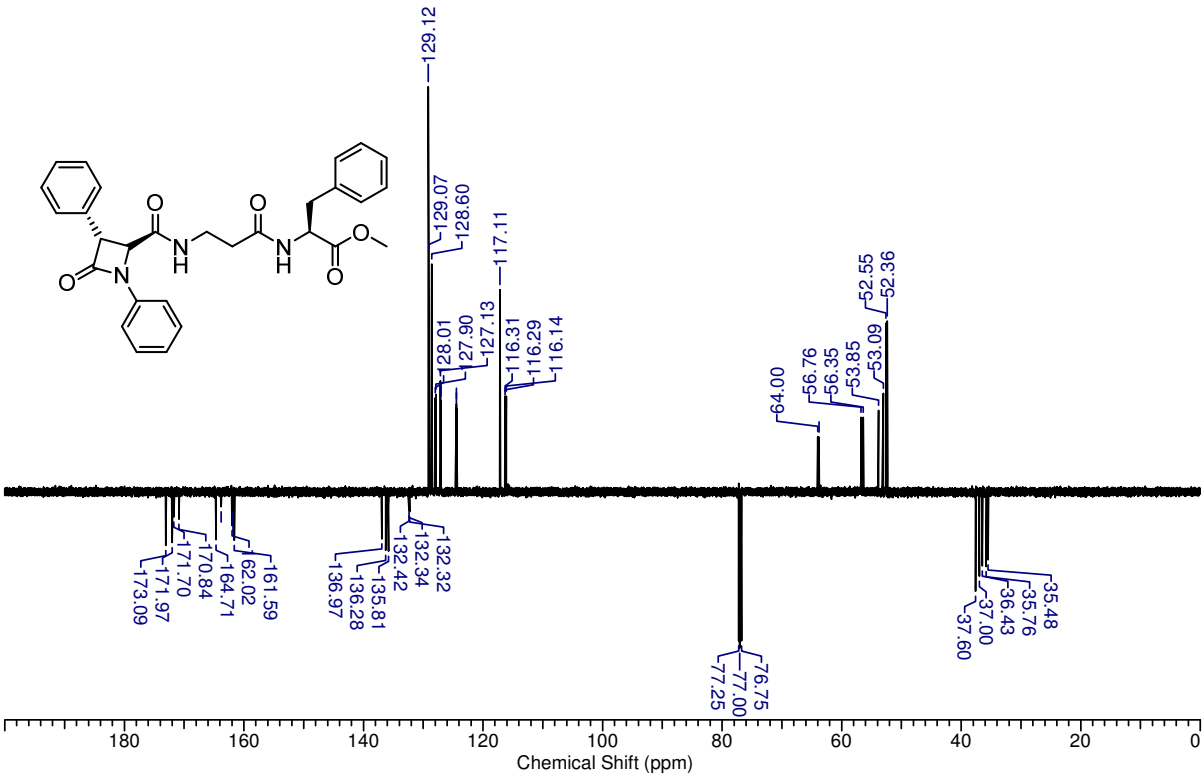
9:


M07(m)
M08(s)
3.76
M05(ddq)
M06(ddd)
J(M06)=31.56 Hz
J(M06)=13.18 Hz
J(M06)=6.13 Hz
M09(ddt)
J(M09)=14.09 Hz
J(M09)=6.13 Hz
M02(d)
M04(ddq)
M03(m)
M01(dt)
M10(d)
7.32
7.30
7.29
7.13
7.12
7.34
7.34
7.28
6.84
6.25
6.23
4.91
4.89
4.87
4.86
3.55
3.54
3.53
3.18
3.10
3.08
2.81
2.45
2.44
2.43
2.42
3.21 1.98 0.81 0.90 0.96 3.00 1.80 2.02 0.93 1.86
Chemical Shift (ppm)

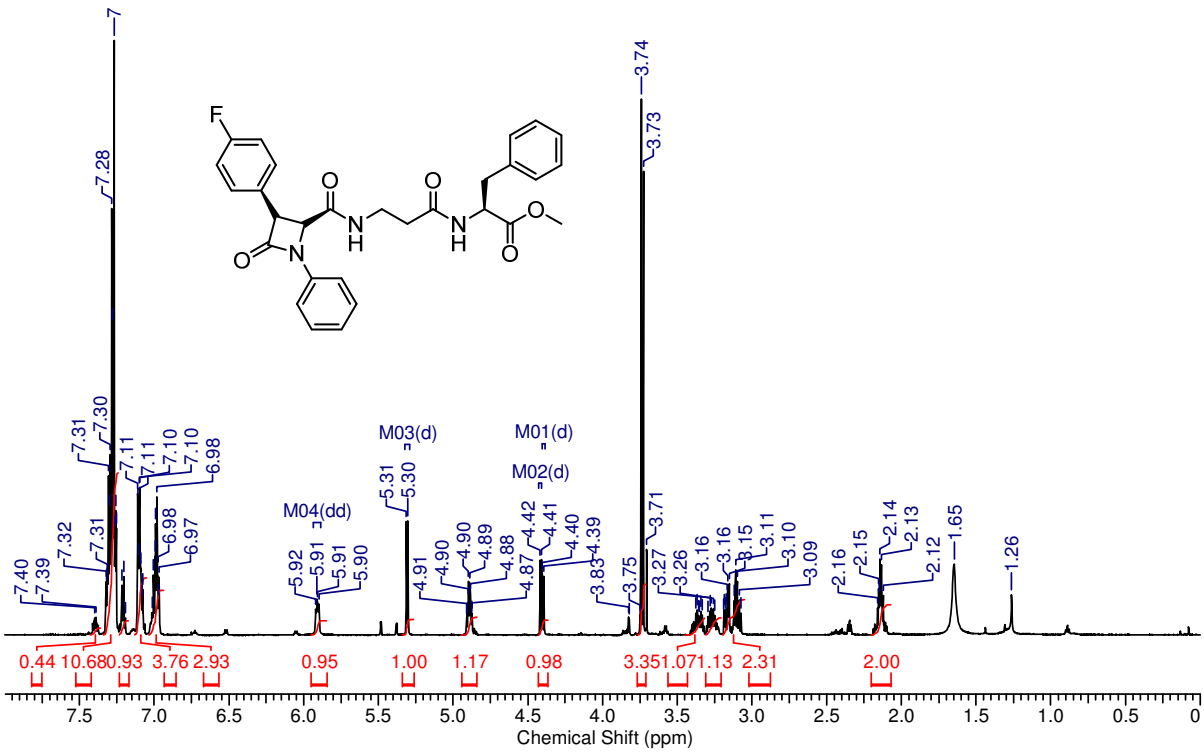

129.11
128.65
127.24
53.21
52.43
171.87
170.94
152.14
135.66
77.32
77.00
73.34
37.77
35.52
34.76
Chemical Shift (ppm)

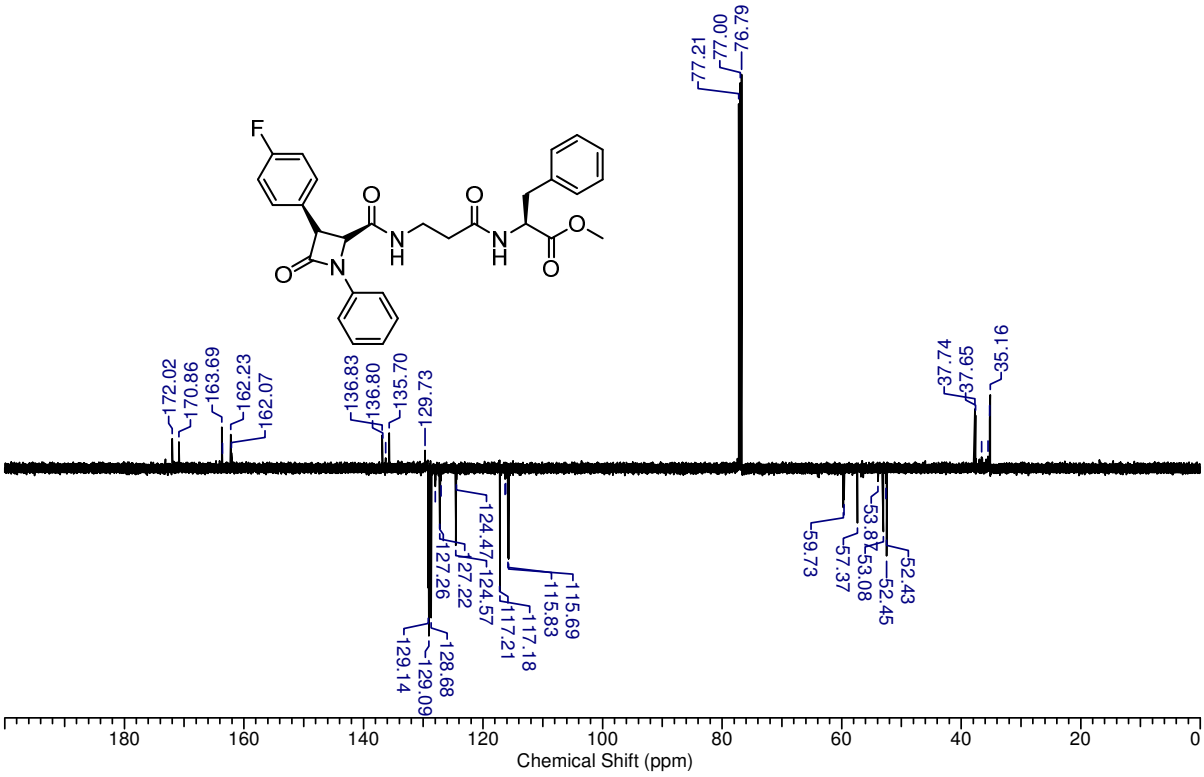
trans-**10**:


M07(d)
M08(s)
M09(s)
M06(d)
M11(dq)
7.26
7.24
7.27
7.27
7.08
7.09
7.06
7.06
M01(d)
7.39
7.38
7.28
7.05
6.78
6.57
6.56
M02(d)
6.15
6.13
M03(d)
M04(d)
5.48
5.47
5.38
5.37
M05(m)
4.89
4.87
4.86
4.85
4.84
3.83
3.82
3.71
3.69
3.57
3.56
3.22
3.15
3.14
3.13
3.11
3.10
M12(t)
2.36
2.35
2.34
2.04
1.26
1.97 8.83 4.01 0.43 0.44 0.45 0.47 0.47 1.00 0.99 0.52 2.92 0.92 0.52 2.03 2.03
Chemical Shift (ppm)

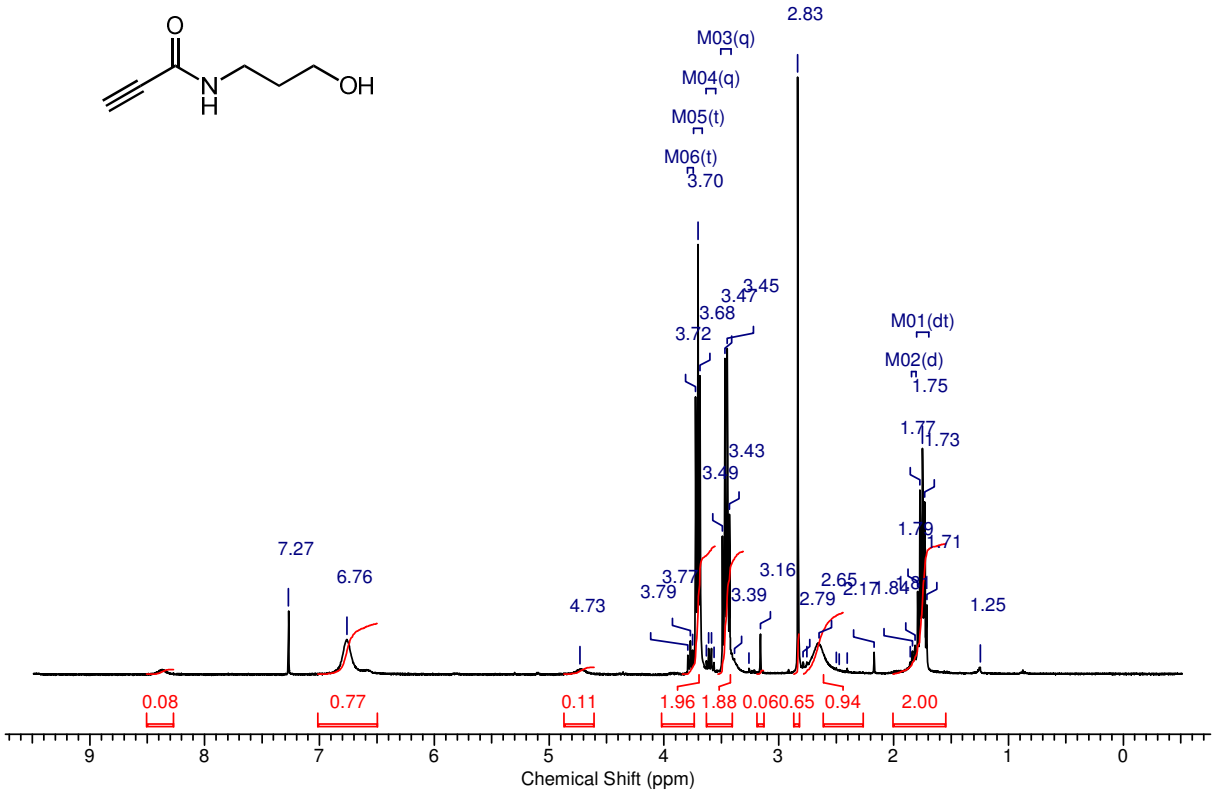

129.12
129.07
128.60
128.01
127.90
127.13
117.11
116.31
116.29
116.14
64.00
56.76
56.35
53.85
53.09
52.55
52.36
173.09
171.97
171.70
170.84
164.71
162.02
161.59
136.97
136.28
135.81
132.42
132.34
132.32
77.25
77.00
76.75
37.60
37.00
36.43
35.76
35.48
Chemical Shift (ppm)

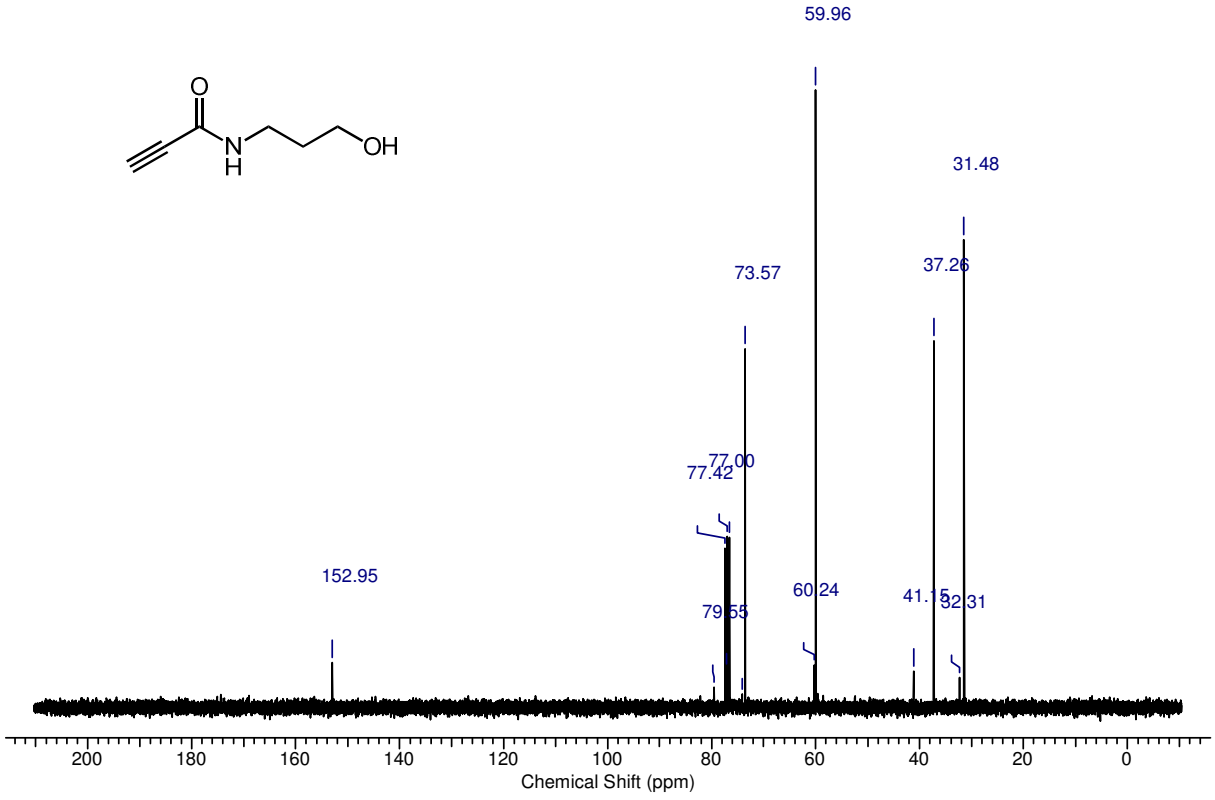
cis-**10**:


F
O
N
H
O
N
H
O
O
N
O
7.28
7.31
7.30
7.11
7.11
7.10
7.10
6.98
7.40
7.39
7.32
7.31
6.98
6.97
M04(dd)
5.92
5.91
5.91
5.90
M03(d)
5.31
5.30
4.91
4.90
4.90
4.89
4.88
M01(d)
M02(d)
4.87
4.42
4.41
4.40
4.39
3.83
3.75
3.74
3.73
3.71
3.27
3.26
3.16
3.16
3.15
3.11
3.10
3.09
2.16
2.15
2.14
2.13
2.12
1.65
1.26
0.44
10.68
0.93
3.76
2.93
0.95
1.00
1.17
0.98
3.35
1.07
1.13
2.31
2.00
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
Chemical Shift (ppm)


F
O
N
H
O
N
H
O
O
N
O
77.21
77.00
76.79
172.02
170.86
163.69
162.23
162.07
136.83
136.80
135.70
129.73
37.74
37.65
35.16
124.47
124.57
127.26
127.22
129.14
128.68
129.09
115.69
115.83
117.21
117.18
59.73
57.37
53.08
52.45
52.43
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

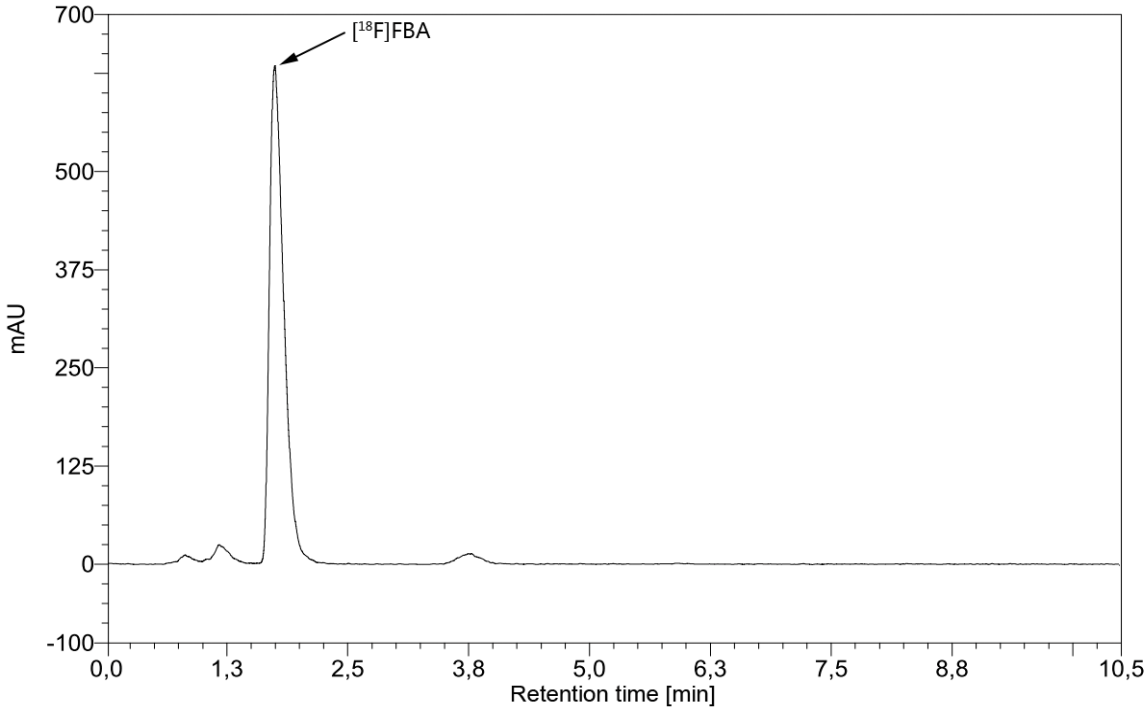
11:


O
N
H
OH
2.83
M03(q)
M04(q)
M05(t)
M06(t)
3.70
3.45
3.47
3.68
3.72
M01(dt)
M02(d)
1.75
1.77
1.73
3.43
3.49
7.27
6.76
4.73
3.77
3.79
3.39
3.16
2.79
2.65
2.17
1.84
1.71
1.25
0.08
0.77
0.11
1.96
1.88
0.06
0.65
0.94
2.00
9
8
7
6
5
4
3
2
1
0
Chemical Shift (ppm)

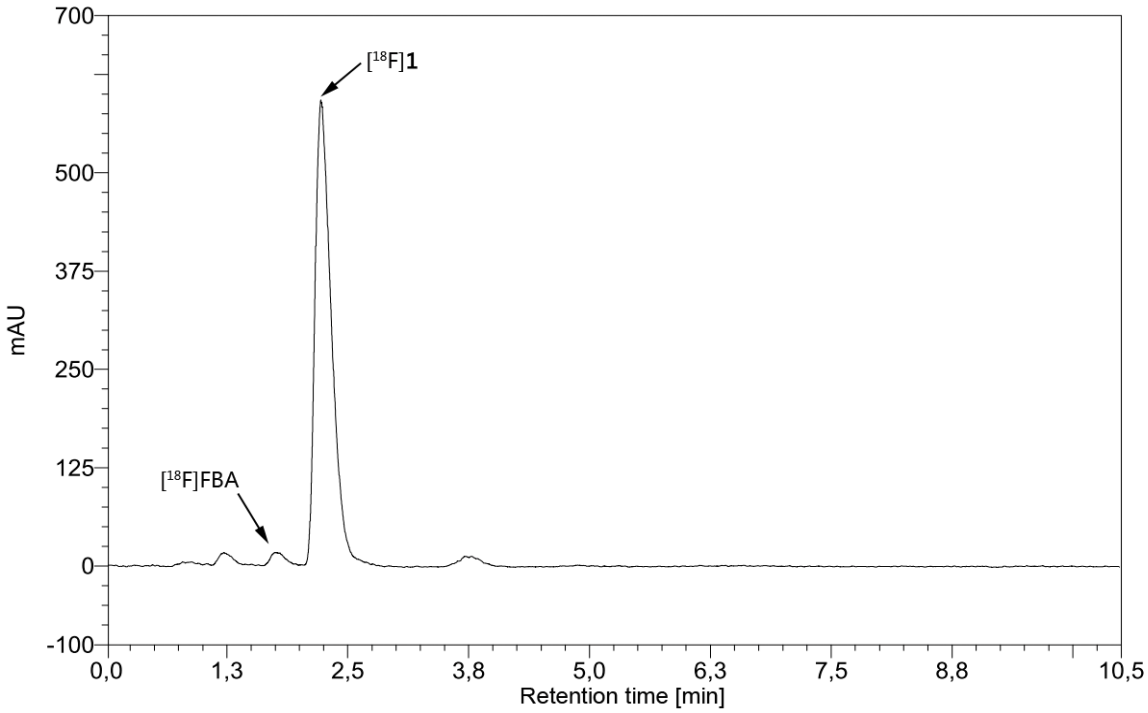

O
N
H
OH
59.96
31.48
73.57
37.26
77.00
77.42
152.95
60.24
41.15
32.31
79.55
200
180
160
140
120
100
80
60
40
20
0
Chemical Shift (ppm)

HPLC Traces

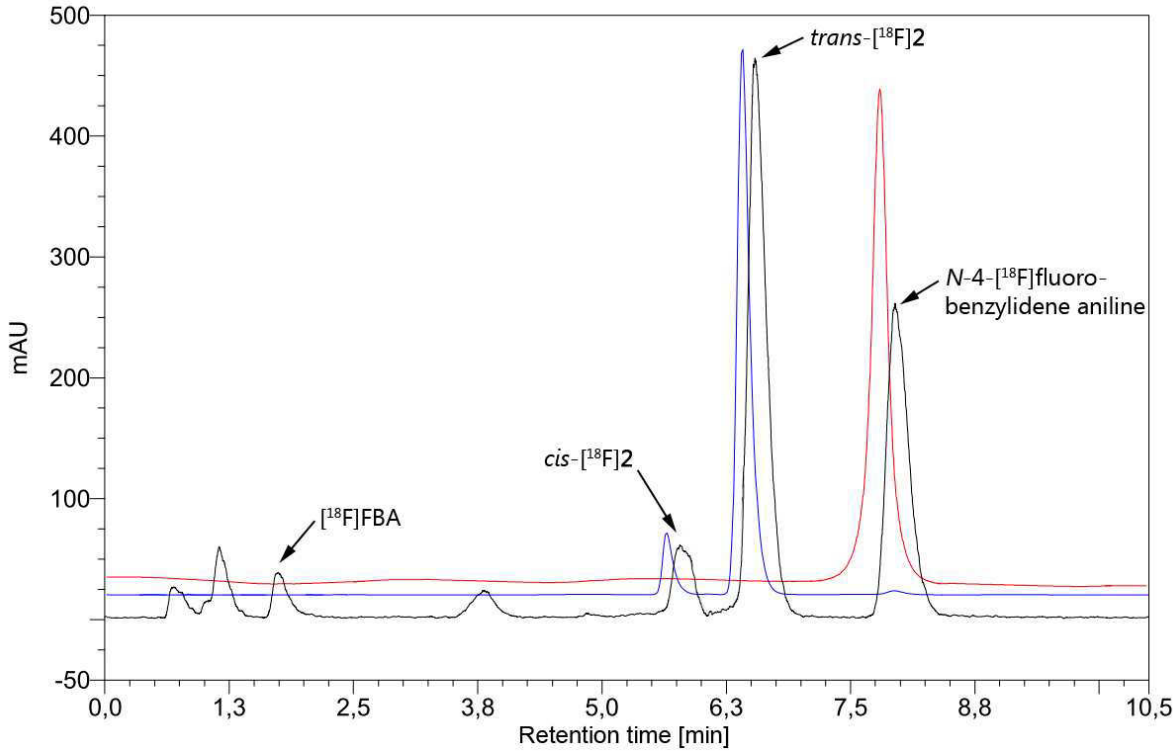
[^{18}F]FBA:



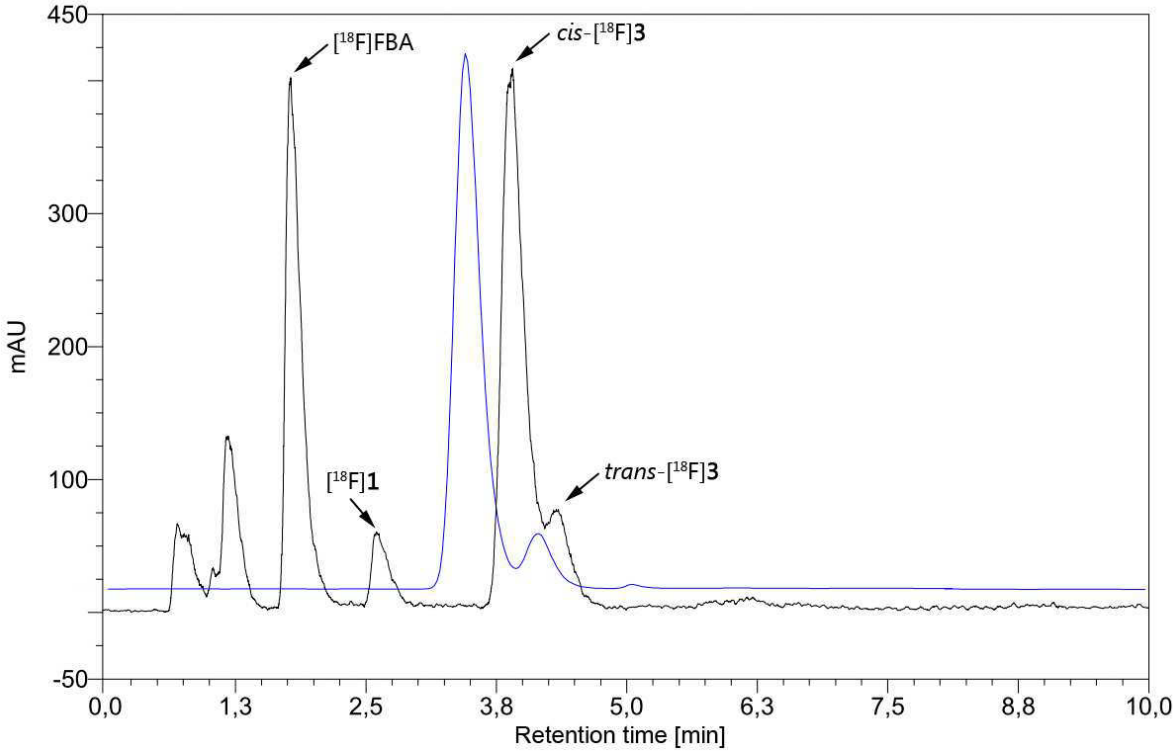
[^{18}F]**1**:



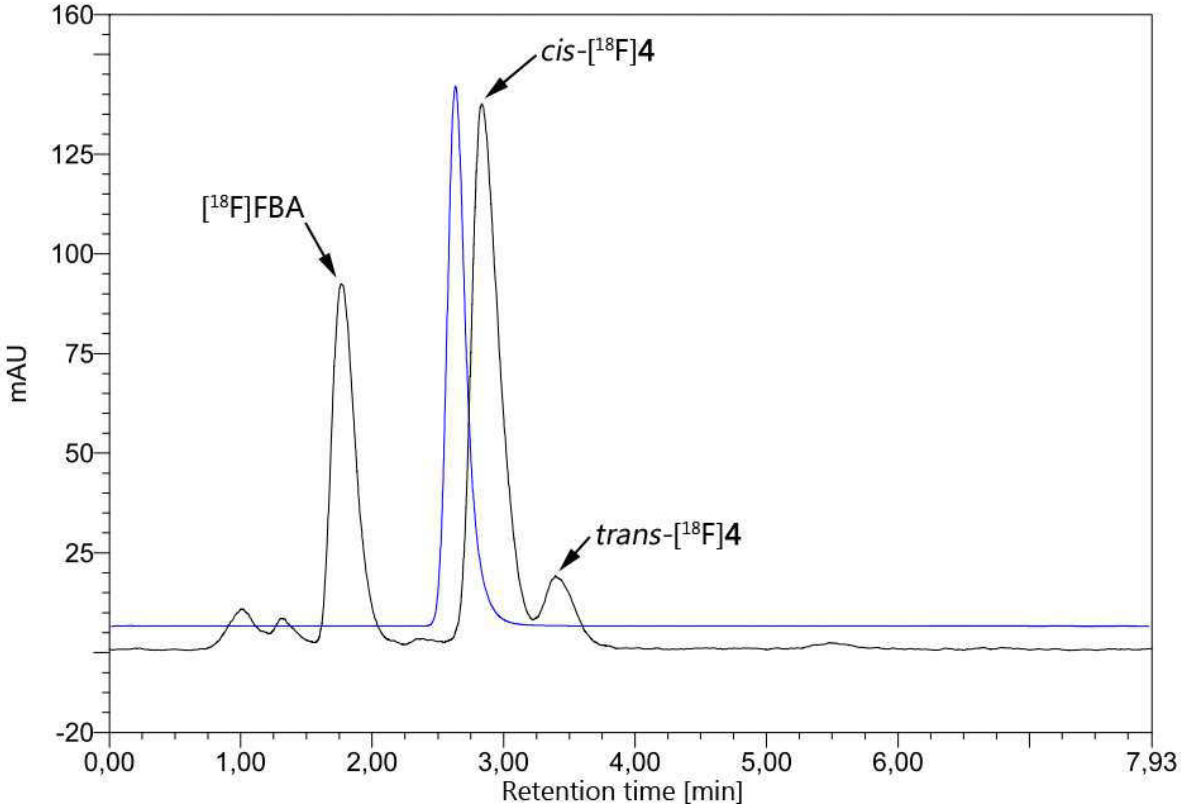
[^{18}F]**2** [under inert conditions; blue trace – **2** (reference compound), red trace –FBAN (reference compound)]:



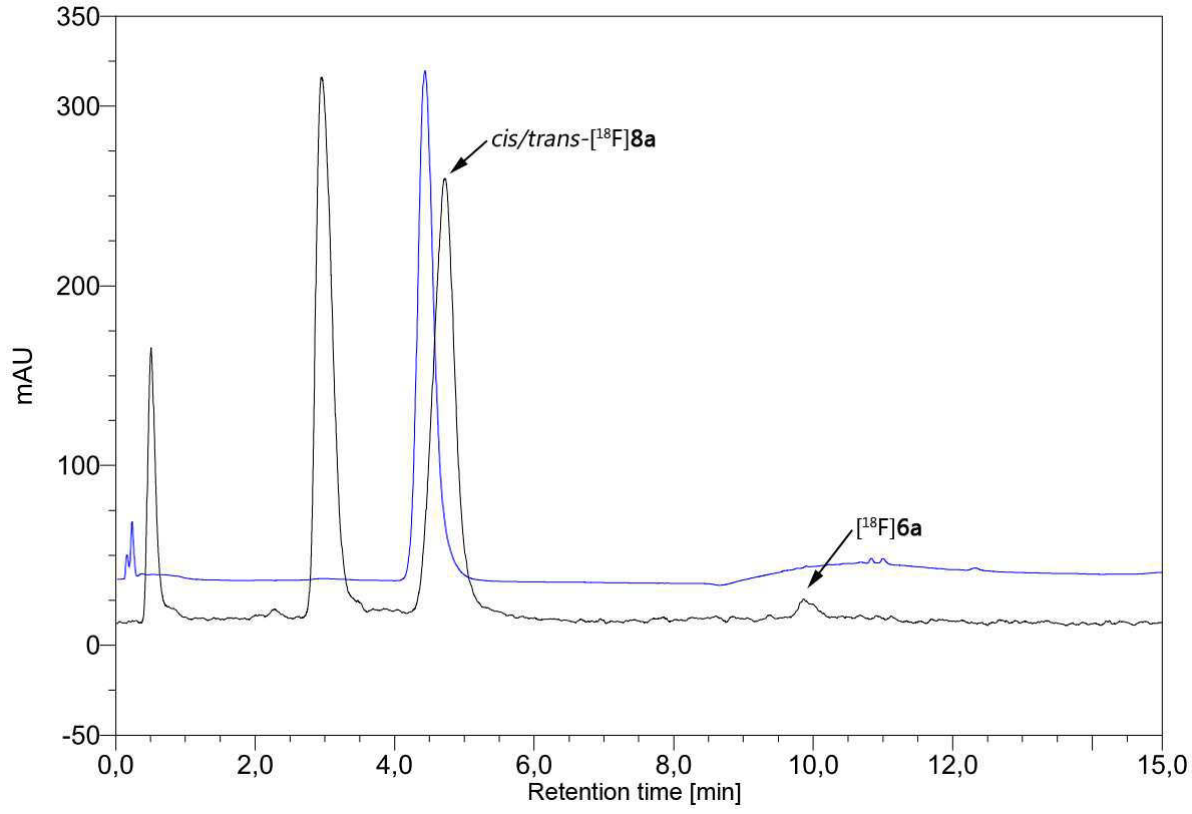
[^{18}F]**3** [blue trace – **3** (reference compound)]:



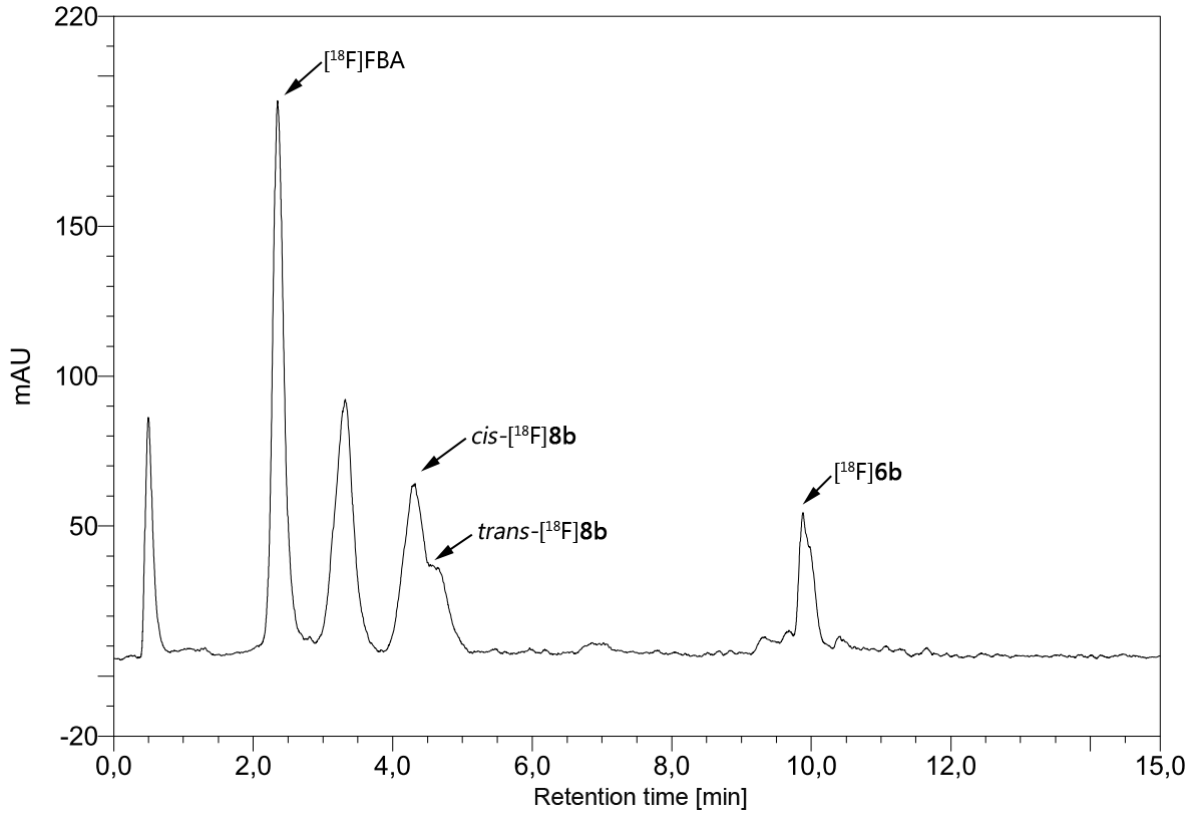
[^{18}F]**4** [blue trace – **4** (reference compound)]:



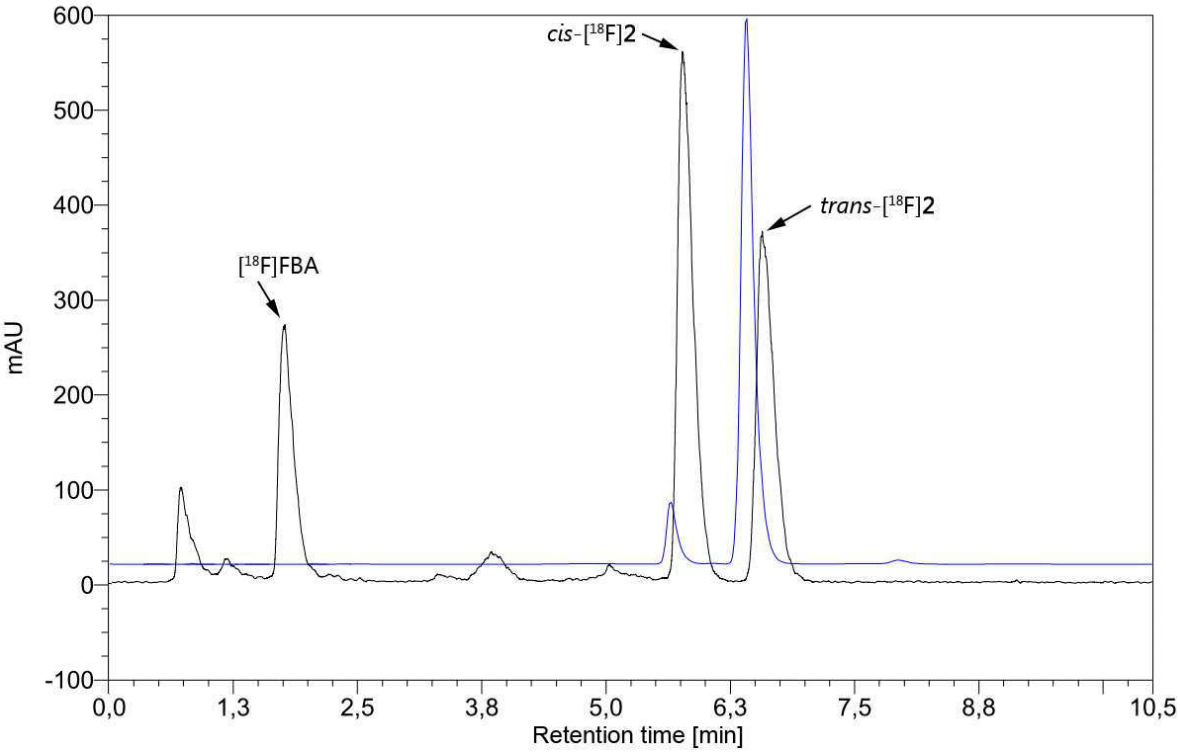
[^{18}F]**8a** [blue trace – **8a** (reference compound)]:



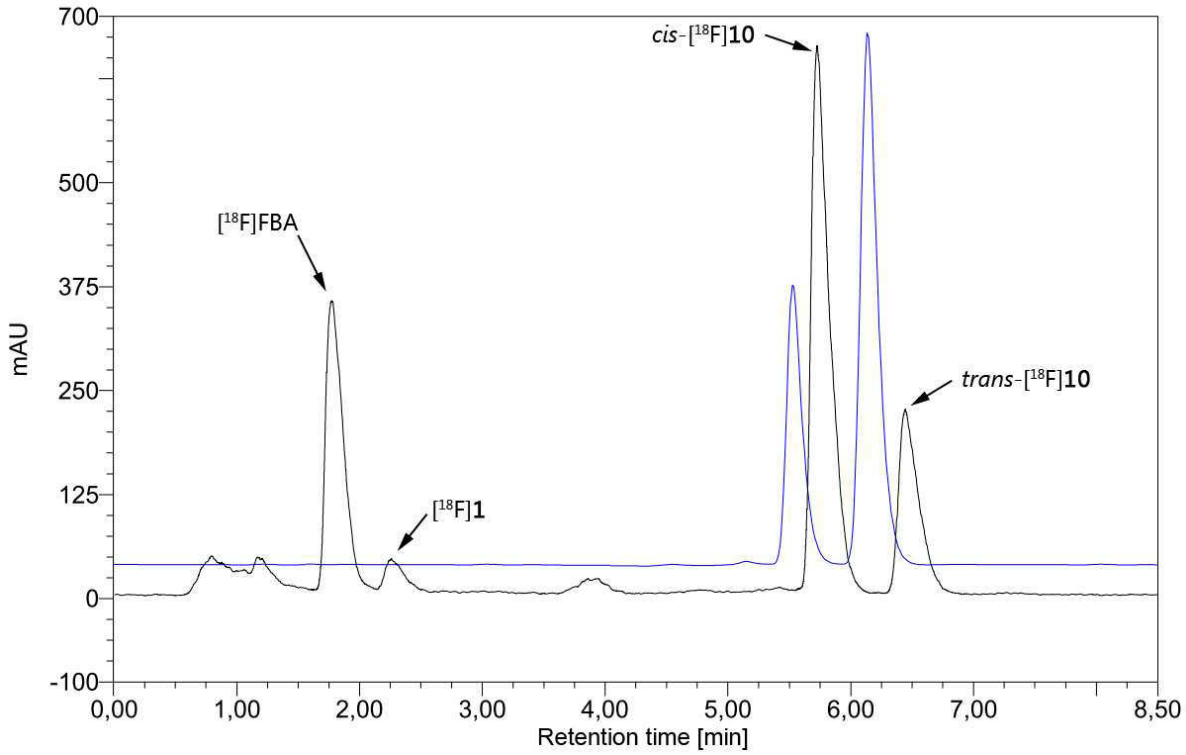
[^{18}F]**8b**:



[^{18}F]**2** [under "click" conditions; blue trace – **2** (reference compound)]:



[^{18}F]**10** [under "click" conditions; blue trace – **10** (reference compound)]:



References

[1] L. Tian, G. Xu, Y. L. L. Ye, *Synthesis* **2003**, 1329–1334.
[2] V. G. Koshechko,; V. E. Titov,; V. N. Bondarenko,; V. D. Pokhodenko, *J. Fluorine Chem.* **2008**, *129*, 701–706.
[3] J. Lu, J. Hu, Y. Song, Y. Ju, *Org. Lett.* **2011**, *13*, 3372–3375.
[4] G. Zinner, W. Kliegel, *Arch. Pharm. Ber. Dtsch. Pharm. Ges.* **1966**, *299*, 166–173.
[5] B. D. Zlatopolskiy, R. Kandler, D. Kobus, F. M. Mottaghy, B. Neumaier, *Chem. Commun*, **2012**,*48*, 7134–7136.
[6] M. S. Haka, M. R. Kilbourn, G. L. Watkins and S. A. Toorongian, *J. Labelled Comp. Radiopharm.* **1989**, *27*, 823.
[7] B. D. Zlatopolskiy, R. Kandler, F. M. Mottaghy, B. Neumaier, *Appl. Radiat. Isot.* **2012**, *70*, 184–192.

3 Zusammenfassende Diskussion

3.1 Entwicklung eines neuen Radiofluorierungsverfahren und dessen Übertragung auf verschiedene Modellreaktionen

Aus der Literatur sind bereits die unterschiedlichsten Methoden zur Einführung von ^{18}F in zahlreiche Zielmoleküle bekannt. Die Radiofluorierung mit $^{18}F^-$ erfolgt überwiegend *via* aliphatischer oder aromatischer nucleophiler Substitution. Produktionsbedingt wird [^{18}F]Fluorid jedoch als wässrige ($H_2{}^{18}O$) Lösung in stark hydratisierter und daher unreaktiver Form erhalten. Um $^{18}F^-$ in die erforderliche hochnukleophile Form zu überführen, sind eine zeitaufwendige, mehrstufige Aufbereitung sowie der Zusatz einer Base und weiterer Additive zwingend notwendig. Eine Verkürzung der Aufbereitung bzw. der Verzicht auf diesen Produktionsschritt sowie die Optimierung der eigentlichen Radiomarkierung würde die Herstellungszeit deutlich verringern und daher die Syntheseausbeute erheblich verbessern. Die höheren Ausbeuten führen dazu, dass u.a. Radiomarkierungsbausteine in ausreichend hohen Aktivitätsmengen zur Verfügung stehen und somit für Folgereaktionen zur Herstellung von bisher nur schwer zugänglichen klinisch relevanten molekularen Sonden genutzt werden können. Des Weiteren sind Produktionsprozesse mit weniger Operationsschritten wesentlich einfacher zu automatisieren und folglich bedeutend leichter in die klinische Routine zu implementieren.

Ein wichtiges Beispiel für ein solchen Radiomarkierungsbaustein ist [^{18}F]Fluorbenzaldehyd ([^{18}F]FBA). Aufgrund der verschiedenen durchführbaren Reaktionen mit [^{18}F]FBA, wird der Zugang zu einer ganzen Reihe von ^{18}F-markierten Verbindungen ermöglicht. Daher ist [^{18}F]FBA eines der am häufigsten verwendeten Markierunssynthone in der Radiochemie. Die bisher bekannten Syntheseverfahren für [^{18}F]FBA sind aufgrund langer Synthesezeiten und geringer radiochemischer Ausbeuten zur Herstellung klinisch relevanter Radiotracer jedoch nur bedingt bis gar nicht geeignet.

Das vorrangige Ziel dieser kumulativen Promotionsarbeit bestand daher in der Entwicklung eines neuen möglichst einfachen Radiofluorierungsverfahrens, welches sich nur noch auf die absolut nötigen Operationsschritte und Reagenzien beschränkt („minimalistsische“ Methode).

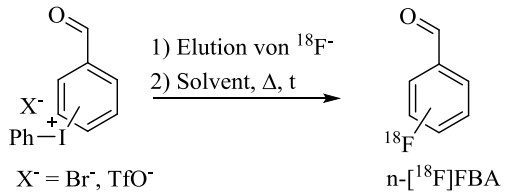
Die Herstellung diverser aromatischer und auch aliphatischer Verbindungen erfolgte dabei ausschließlich durch das Erhitzen ihrer entsprechenden quartären Onium-[^{18}F]Fluorid-Salze in den jeweils geeigneten Lösungsmitteln. Der Zusatz weiterer Additive, wie Kryptanden Kronenether oder Basen war nicht länger erforderlich.

3.2 Elution von $^{18}F^-$ mit Onium-Salzen

Bei ersten Experimenten wurde zunächst das $^{18}F^-$-Elutionsvermögen verschiedener in wasserfreiem DMF oder DMSO gelöster Onium-Salz-Vorläufer von [^{18}F]FBA von einer Ionenaustauscher-Kartusche untersucht. Dabei zeigte sich, dass die Rückgewinnung von $^{18}F^-$, unter Verwendung aprotischer Lösungsmitteln sehr gering war. Deshalb wurde in weiteren Experimenten der Fokus auf protische Lösungsmittel gelegt. Es stellte sich dabei heraus, dass sich die $^{18}F^-$ Wiederfindungsraten durch die Verwendung von verschiedenen Alkoholen als Elutionsmittel mit Onium-Salz-Vorläufer deutlich steigern ließen.

So konnte insbesondere bei Verwendung von MeOH oder EtOH, eine Elution von über 98% des adsorbierten $^{18}F^-$ erzielt werden.

Tabelle 3.0: *Erste Experimente zur Herstellung von [^{18}F]Fluorbenzaldehyden mittels Iodoniumsalz-Vorläufer ohne azeotrope Trocknung (Auszug).*



Eintrag	X^{-}	n	Lösungsmittel	Temp. [°C], Zeit [min]	Elutionsausbeute von $^{18}F^-$ [%]	RCC [%]
1	TfO^-	2	95% DMF	130, 10	30	2
2	TfO^-	3	DMSO	130, 10	30	22
3	TfO^-	4	DMSO	90, 10	20	51
4	Br^-	4	90% DMF	160, 15	55	51

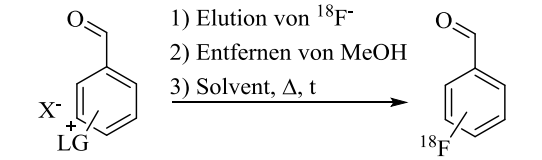
Darüber hinaus kann das niedrig siedende MeOH bereits bei 70–80 °C innerhalb von 2–3 min wieder entfernt werden. Die Verluste an Radioaktivität können dabei mit < 2% vernachlässigt werden.

Im Vergleich zum klassischen Elutionsverfahren mittels K_2CO_3/K2.2.2, welches eine mindestens 20–30 minütige azeotrope Trocknungsprozedur erfordert, wird über dieses neue Verfahren eine erhebliche Zeitersparnis erreicht. Da letzteres weder einer azeotropen Trocknung noch den Zusatz weiterer Additive, wie Basen, Kryptanden und Kronenethern bedarf, werden im Folgenden alle auf diesem Prinzip der ^{18}F-Vorverarbeitung basierenden Radiosynthesen als „minimalistische“ Methoden bezeichnet.

3.3 Herstellung von ^{18}F-markierten Fluorbenzaldeyden über die „minimalistische“ Methode

Nach der erfolgreichen Entwicklung einer geeigneten Methode zur Elution von $^{18}F^-$ wurde [^{18}F]FBA zunächst in einem Vorversuch über die „minimalistische Methode“ synthetisiert und anschließend im Hinblick auf Vorläufermenge, Temperatur und Lösungsmittel optimiert. Die besten radiochemischen Umsätze wurden dabei im Fall von 2- und 4-[^{18}F]FBA mit den *N*,*N*,*N*-Trimethylanilinium Vorläufern erzielt. Zudem zeigte sich, dass die Gegenionen einen signifikanten Einfluss auf die Radiomarkierung haben. Ausgehend von den entsprechenden Perchloratvorläufern wurden so die höchsten radiochemischen Ausbeuten von 90% für 4-[^{18}F]FBA innerhalb von 10 min bei 150 °C in DMSO erzielt.

Tabelle 3.1: *Herstellung von [^{18}F]Fluorbenzaldehyden über die „minimalistische“ Methode (Auszug).*



Eintrag	n	LG^+X^{--}	Lösungsmittel	Temp. [°C], Zeit [min]	RCC [%]
1	4	$Me_3N^+HCO_3^-$	DMSO	80, 10	87
2	2	$Me_3N^+I^-$	DMSO	80, 10	62
3	4	$Me_3N^+ClO_4^-$	DMSO	150, 10	80–90
4	3	$(4\text{-MeOPh})I^+ClO_4^-$	DMSO	150, 10	40

Mit den jeweiligen Bicarbonat- und Iodidsalzen konnte bereits bei 80 °C ein radiochemischer Umsatz von 87% bzw. 62% für 2- bzw. 4-[^{18}F]FBA erreicht werden. Die Reproduzierbarkeit war in allen durchgeführten Experimenten -auch bei der Verwendung sehr großer Startaktivitäten- exzellent.

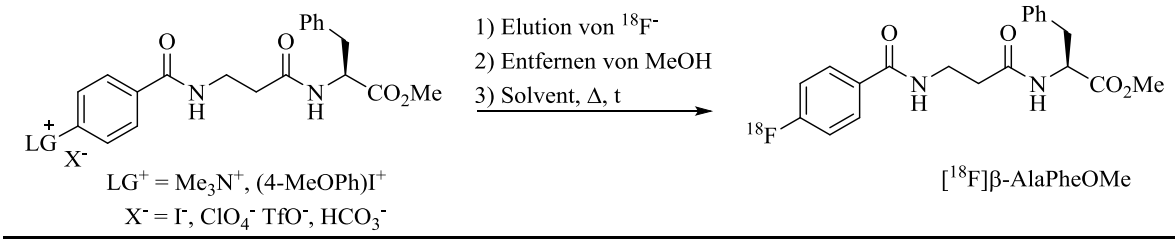
So ist es mit Hilfe des neu entwickelten Verfahrens gelungen, ausgehend von 47 GBq [^{18}F]Fluorid Startaktivität, 4-[^{18}F]FBA mit einer radiochemischen Ausbeute von 75% (nicht zerfallskorrigiert) und einer radiochemischen Reinheit von > 99% (nach SPE) innerhalb von 23 min herzustellen. Bei dem konventionellen Verfahren (K2.2.2/K_2CO_3) wurde hingegen eine signifikant schlechtere Ausbeute von 55% und eine radiochemische Reinheit von nur 90% erhalten. Darüber hinaus mangelt es dieser Methode an Robustheit und Reproduzierbarkeit.

Im Falle des 3-Isomers von [^{18}F]FBA wurden die besten radiochemischen Umsätze unter Verwendung des (4-Methoxyphenyl)-iodonium-Salz Vorläufers bei 150 °C innerhalb von 10 min erreicht. Aufgrund des schwächeren aktivierenden Effekts der Carbonyl-Gruppe in der *meta*-Position fiel der radiochemische Umsatz mit bis zu 40% nur moderat aus. Nichts desto trotz ist sie mit dem im konventionellen Verfahren ($CsHCO_3$/2,2,6,6-Tetramethylpiperidinyl-oxyl (TEMPO)-Protokoll) erhaltenen Umsatz vergleichbar.

3.4 ^{18}F-Markierung des Modellpeptids β-AlaPheOMe

Um das Anwendungspektrum des neuen Radiofluorierungsverfahrens zu untersuchen, wurde seine Verwendbarkeit anschließend bei verschiedenen praktisch relevanten Radiosynthesen untersucht. Als erste Modellsynthese diente hierfür die direkte aromatische nucleophile Radiofluorierung von ^{18}F-benzoylierten Peptiden.

Tabelle 3.2: *Herstellung eines ^{18}F-benzoylierten Peptids ([^{18}F]β-AlaPheOMe) über die „minimalistische“ Methode (Auszug).*



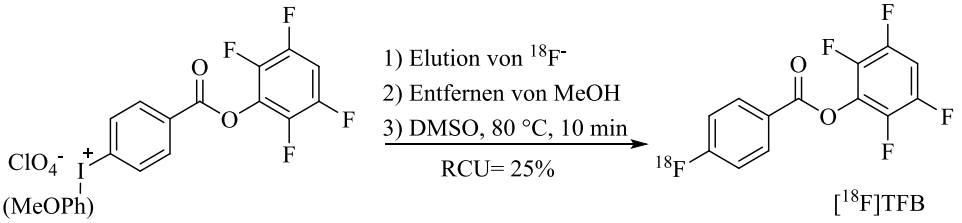
Eintrag	LG^+X^-	Lösungsmittel	Temp. [°C], Zeit [min]	RCC [%]
1	$Me_3N^+HCO_3^-$	Sulfolan	130, 15	30
2	$Me_3N^+HCO_3^-$	DMSO	200, 15	29
3	$(4\text{-MeOPh})I^+I^-$	DMSO	130, 10	56
4[a]	$Me_3N^+TfO^-$	DMSO	130, 30	0

[a] *Anwendung des konventionellen Verfahrens über K2.2.2/K_2CO_3. Keine Produktbildung.*

Bei den bisher beschriebenen Radiosynthesen von Peptiden ist die Einführung von elektronenziehenden funktionellen Gruppen, wie CN, CF_3, und F, zur Erhöhung der Reaktivität der Trimethylammonium- oder Nitro-Abgangsgruppe unabdingbar, um akzeptable Markierungsausbeuten sicherzustellen.[49] Allerdings führen solche aktivierenden hydrophoben Gruppen insbesondere bei kleinen Peptiden zu einer signifikanten Erhöhung ihrer gesamten

Lipophilie, was sich folglich negativ auf die Biodistribution der biogenen Verbindungen auswirken kann. Um dieses Problem zu umgehen, wurde versucht mit Hilfe des neu entwickelten Verfahrens die direkte Markierung eines Modellpeptids, ohne den Zusatz weiterer funktioneller Gruppen, zu realisieren. Dazu wurden verschiedene *N*,*N*,*N*-Trimethylammonium Vorläufer des Peptids [^{18}F]Fluorbenzoyl-β-AlaPheOMe auf ihre Eignung untersucht. Ausbeuten von bis zu 30% ergaben sich dabei durch den Einsatz des *N*,*N*,*N*-Trimethylammonium-Hydrogencarbonat Vorläufers. Im Gegensatz dazu konnte durch die Anwendung des konventionellen Verfahrens keine Produktbildung beobachtet werden. Die besten radiochemischen Umsätze von 56% konnten schließlich bei 120 °C durch Verwendung des (4-Methoxyphenyl)-iodonium-iodid Vorläufers in DMSO innerhalb von 10 min erzielt werden.

3.5 Radiosynthese des Aktivesters 4-[^{18}F]Tetrafluorbenzoat ([^{18}F]TFB)

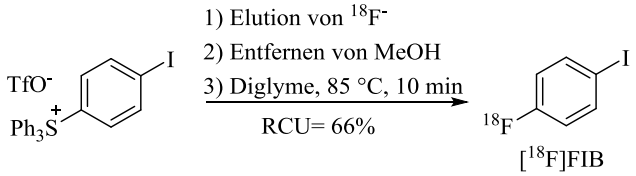


Schema 3.0: *Synthese des Aktivesters[^{18}F]TFB über die „minimalistische" Methode.*

Anschließend wurde über die „minimalistischen" Methode ein neues aminreaktives Markierungssynthon (4-[^{18}F]Tetrafluorbenzoat, [^{18}F]TFB), welches sich potentiell zur Radiomarkierung von Peptiden und Proteinen eignet, hergestellt.

Für die Markierung von Peptiden und Proteinen wird häufig *N*-Succinimidyl-4-[^{18}F]fluorbenzoat ([^{18}F]SFB) als aminreaktives Markierungssynthon eingesetzt.[50] Jedoch erfordert die Synthese von [^{18}F]SFB nicht nur mindestens 2–3 Reaktionsschritte, sondern vor allem eine zeitaufwendige azeotrope Trocknung (K2.2.2/K_2CO_3-Protokoll). Das Verfahren ist daher i.d.R. ungeeignet bzw. stark limitiert im Hinblick auf die Herstellung und insbesondere auf die automatisierte Herstellung von klinisch relevanten Tracern. Als Alternative zu [^{18}F]SFB ist es mit dem neu entwickelten Verfahren gelungen, 4-[^{18}F]Tetrafluorbenzoat ([^{18}F]TFB) ausgehend von dem entsprechenden Iodoniumsalz Vorläufer, in einem Schritt und in radiochemischen Umsätzen von bis zu 25% herzustellen. Hingegen war die konventionelle Radiosynthese von [^{18}F]TFB über K2.2.2/K_2CO_3 erfolglos.

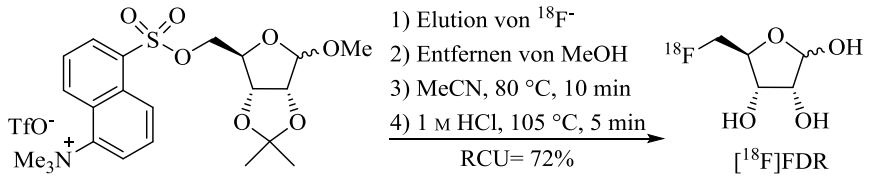
3.6 Radiofluorierung von Sulfoniumsalzen



Schema 3.1: *Herstellung von [^{18}F]FIB unter „minimalistischen" Bedingungen.*

Erst kürzlich wurde über den Einsatz von Triarylsulfoniumsalzen als Vorläufer zur Radiofluorierung von aromatischen Verbindungen berichtet.[51] Ihre Eignung als Vorläufer in Radiosynthesen unter „minimalistischen" Bedingungen wurde daraufhin überprüft und konnte bestätigt werden. Dazu wurde als erste Modellverbindung 1-[^{18}F]Fluor-4-iodbenzol ([^{18}F]FIB), welches als wertvoller Baustein in Metall-katalysierten Kreuzkupplungsreaktionen genutzt wird, ausgewählt. Unter Verwendung des neuen Radiofluorierungsverfahrens gelang die Herstellung von [^{18}F]FIB in Diglyme innerhalb von 10 min mit einem max. radiochemischen Umsatz (RCU) von 66%. Eine vergleichende Radiosynthese über das konventionelle Radiofluorierungsprotokoll (K2.2.2/K_2CO_3) lieferte das Produkt in einem radiochemischen Umsatz von ca. 50%.

3.7 ^{18}F-Markierung aliphatischer Verbindungen: Herstellung von 5-[^{18}F]Fluor-5-deoxy-D-ribose ([^{18}F]FDR)



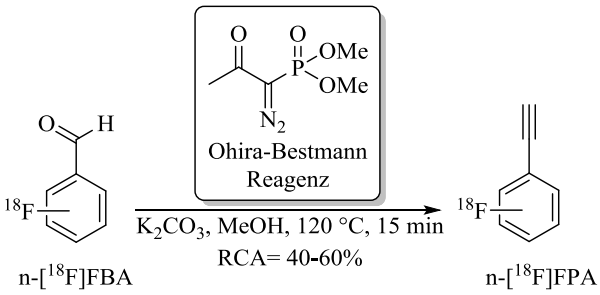
Schema 3.2: *Herstellung von [^{18}F]FDR unter „minimalistischen" Bedingungen.*

Zur Erweiterung des Spektrums an ^{18}F-Markierungsbausteinen, die über die „minimalistische" Methode leicht zugänglich sind, wurde schließlich versucht neben den aromatischen, auch aliphatische Verbindungen zu radiofluorieren. In diesem Zusammenhang sind vor allem die Aldose-Zucker (z.B.: FDG oder FDR) von großem Interesse. Da sie sowohl in cyclischen anomeren Formen, auch als offenkettiger Aldeyhd vorzuliegen können, sind sie die idealen Kandidaten für eine zielgerichtete und schnelle Radiomarkierung *via* Oximligation.[52] Zu diesem Zweck wurde 5-[^{18}F]Fluor-5-deoxy-D-ribose ([^{18}F]FDR) ausgehend vom geschützten Trimethylammoniumtriflat-Salz Vorläufer über die

„minimalistische" Methode hergestellt. Der gewünschte radiomarkierte Zucker konnte so nach einer Gesamtsynthesezeit von 15 min inklusive Entschützung mit einem radiochemischen Umsatz von 72% erfolgreich erhalten werden. Mit K2.2.2/K_2CO_3 wurde unter gleichen Reaktionsbedingungen ein im Vergleich deutlich geringerer Umsatz von 58% erreicht.

3.8 Seyferth-Gilbert Homologisierung zur Radiosynthese von [^{18}F]Fluorphenylacetylenen als neue Markierungsbausteine

Auf der Suche nach neuen Syntheseverfahren, haben sich insbesondere die (3+2) Cycloadditionen von Aziden mit Alkinen („Click"-Chemie) als überaus dienlich erwiesen. Grundvoraussetzung für eine erfolgreiche Radiosynthese klinisch relevanter Tracer über die „Click"-Chemie ist immer die Entwicklung einer effizienten *ab initio* Radiofluorierung entsprechender Azide und/oder Alkine als Markierungsbausteine. Einige wenige Alkine werden bereits erfolgreich in der Radiochemie eingesetzt.[53] Im Vergleich jedoch mit der Fülle an verschiedensten Alkinen, die der organisch präparativen Chemie zu Verfügung stehen, sind sie in der Radiochemie, aufgrund der Notwendigkeit mehrstufiger Syntheserouten und zeitaufwendiger Reinigungsprozeduren für ihre Herstellung, deutlich unterrepräsentiert. Allerdings könnten vor allem radiofluorierte Arylacetylene potentiell den Zugang zu einer ganzen Reihe an neuen und höchst interessanten radiomarkierten Verbindungen mit klinischer Relevanz eröffnen. Ihre Radiosynthese ist in der Literatur erst seit kurzem bekannt.[54] Ausgehend von dem entsprechenden Trimethylammoniumtriflat-Vorläufer konnten Roberts *et al.* über das klassische K2.2.2/K_2CO_3-Protokoll das gewünschte Arylacetylen in einer RCA von 14% erhalten. Bei derartig geringen Ausbeuten stellt sich jedoch die Frage, inwieweit dieses Radiofluorierungsverfahren zur Herstellung der benötigten Markierungssynthone geeignet ist wenn anschließend die Synthese der klinisch relevanten Tracer in einer oder mehreren Stufen, erst noch erfolgen muss.



Schema 3.3: *Synthese der radiofluorierten [^{18}F]Fluorphenylacetylene via Seyferth-Gilbert-Homologisierung.*

Basierend auf der „minimalistischen" Methode wurde daher ausgehend von [^{18}F]FBA ein neues Verfahren zur effizienten Herstellung von [^{18}F]Fluorphenylacetylenen über die Seyferth-Gilbert Homologisierung entwickelt.[40] Dazu wurden die radiomarkierten [^{18}F]Fluorbenzaldeyde (*ortho*-, *meta*- und *para*-), in Anwesenheit von K_2CO_3, gelöst in MeCN/MeOH,

mit dem sog. Ohira-Bestmann Reagenz (Dimethyl (1-diazo-2-oxopropyl)phosphonat) bei 120 °C und in 15 min Reaktionszeit zu den entsprechenden [^{18}F]Fluorphenylacetylenen (*ortho*-, *meta*- und *para*-) umgesetzt. Dabei konnte ein radiochemischer Umsatz von > 90% erzielt werden.

Die Isolierung der Produkte über Destillation ergab schließlich eine nicht zerfallskorrigierte radiochemische Ausbeute von 40–60% mit einer radiochemischen Reinheit von > 98% innerhalb von 20 min. Bemerkenswert ist außerdem, dass die verwendete Herstellungsmethode unempfindlich gegenüber elektronischen und sterischen Effekten ist und sich daher zur Synthese aller drei Regioisomere von [^{18}F]Fluorphenylacetylen gleich gut eignet.

3.9 [^{18}F]Fluorphenylacetylene in verschiedenen Beispielreaktionen

Nach erfolgreicher Entwicklung der neuen Markierungsbausteine, wurde anschließend ihre Eignung in „Click"-Chemie Reaktionen untersucht. Dazu wurde 2-[^{18}F]Fluorphenylacetylen mit einem Modellazid (**1**) (siehe Seite 176, Abb. 3.4) in Anwesenheit von $CuSO_4$, Natriumascorbat und L-Histidin in wässrigem Methanol bei Raumtemperatur umgesetzt. Das entsprechende Triazol [^{18}F]**2** (siehe Seite 176, Abb. 3.4) konnte mit einem radiochemischen Umsatz von 53% bereits nach 10 min Reaktionszeit erfolgreich erhalten werden. Nachfolgend wurden weitere 1,3 Dipole ausgewählt, um die Reaktivität von 2-[^{18}F]FPA in 1,3 dipolaren Cycloadditionen eingehender zu untersuchen. Zunächst wurde mittels baseninduzierter Eliminierung von HCl aus *N*-Hydroxyimidoylchlorid (**3**) (siehe Seite 176, Abb. 3.4) das entsprechende Nitriloxid *in situ* generiert und in Anwesenheit von 2-[^{18}F]FPA zu einem 3,5-substituierten Isoxazol [^{18}F]**4** (siehe Seite 176, Abb. 3.4) umgesetzt. Das Produkt wurde mit einem RCU von 52% erhalten.

Auch ein 3,5-substituiertes Pyrazol [^{18}F]**6** (siehe Seite 176, Abb. 3.4) konnte durch Reaktion von 2-[^{18}F]FPA mit (4-Bromphenyl)diazomethan, welches *in situ* aus dem korrespondierenden *N*-Tosylhydrazon (**5**) (siehe Seite 176, Abb. 3. 4) hergestellt wurde, mit einem radiochemischen Umsatz von 20% bei 150 °C in 30 min erhalten werden. Nach 10 min Reaktionszeit wurde auf diese Weise ein radiochemischer Umsatz von 52% erzielt.

Radiofluorierte β-Lactame könnten sich potenziell für die PET-Bildgebung von verschiedenen physiologischen und pathophysiologischen Prozessen eignen. Zu diesem Zweck wurde das Vermögen der radiofluorierten Phenylacetylene untersucht, in einer Radio-Kinugasa-Reaktion als geeignetes terminales Alkin zur Herstellung von ^{18}F-markierten β-Lactamen zu fungieren. Als zu markierende Modellverbindung diente das cyclische Nitron **7** (siehe Seite 176, Abb. 3.4), das in Anwesenheit des Cu(I) stabilisierenden Liganden 1,10-

Phenanthrolin und 2-[^{18}F]FPA in das entsprechende bicyclische radiofluorierte β-Lactam [^{18}F]**8** (siehe Seite 176, Abb. 3.4) überführt werden konnte.

Alkine als Edukte sind nicht nur Gegenstand aktueller Forschung im Bereich der Cycloadditionsreaktionen, sondern spielen auch eine wichtige Rolle bei übergangsmetall vermittelten Kreuzkupplungen. Als solche repräsentiert die Sonogashira-Reaktion ein leistungsfähiges Verfahren zur Herstellung von Aryl- und Alkenylalkinen sowie von Eninen.[55] Insbesondere die Aryl- und Alkenylalkine dienen häufig als Zwischenprodukte bei Totalsynthesen verschiedener Naturstoffe und Pharmazeutika.[56] Der Transfer der Sonogashira-Reaktion in die Radiochemie ist bisher nur unter Verwendung des Markierungsbausteins 4-[^{18}F]Fluoriodbenzol beschrieben.[57]

Im Rahmen dieser Promotionsarbeit wurde daher die Reaktion zwischen dem neu entwickelten Markierungsbaustein 4-[^{18}F]FPA und der geschützten Aminosäure (4-Iodphenyl)alanin **9** (siehe Seite 176, Abb. 3.4) eingehend untersucht. Jedoch wurde die Reaktion nicht wie gewöhnlich in Anwesenheit eines Pd-Komplexes in Verbindung mit Triarylphosphinliganden und CuI als Co-Katalysator, sondern unter den von Yang *et al*. beschriebenen Reaktionsbedingungen durchgeführt.[58] Der Vorteil besteht darin, dass sich die Kreuzkupplung, aufgrund der Verwendung von $PdCl_2$ als einzigem Katalysator und mit Pyrrolidin als Base, unter Luft und im wässrigen Milieu realisieren lässt. Auf diese Weise konnte die geschützte radiofluorierte artifizielle Aminosäure [^{18}F]**10** (siehe Seite 176, Abb. 3.4) bereits nach 10 min bei 120 °C mit einem Umsatz von 83% erhalten werden.

Eine weitere, ebenfalls häufig in *de novo* Synthesen zur Herstellung von Naturstoffen verwendete Reaktion ist die Alkin Trimerisierung.[59]

Ihr Transfer in die Radiochemie könnte möglicherweise den Zugang zu einer ganzen Reihe, auf anderen Synthesewegen, eher schwer zugänglichen ^{18}F-markierten polycyclischen Verbindungen eröffnen. In einer ersten Machbarkeitsstudie, konnte durch die Reaktion des Markierungsvorläufers 4-[^{18}F]FPA und dem Propargylether **11** (siehe Seite 176, Abb. 3.4) als Modelldialkin ein ^{18}F-markiertes, 4-[^{18}F]Fluorphenyl-substituiertes 1,2-Dihydrobenzofuran ([^{18}F]**12)** (siehe Seite 176, Abb. 3.4) in Anwesenheit des Wilkinson Katalysators [($RhCl(PPH_3)_3$] erhalten werden. Ohne weitere Optimierung konnte das Produkt mit einem radiochemischen Umsatz von 18% erhalten werden.

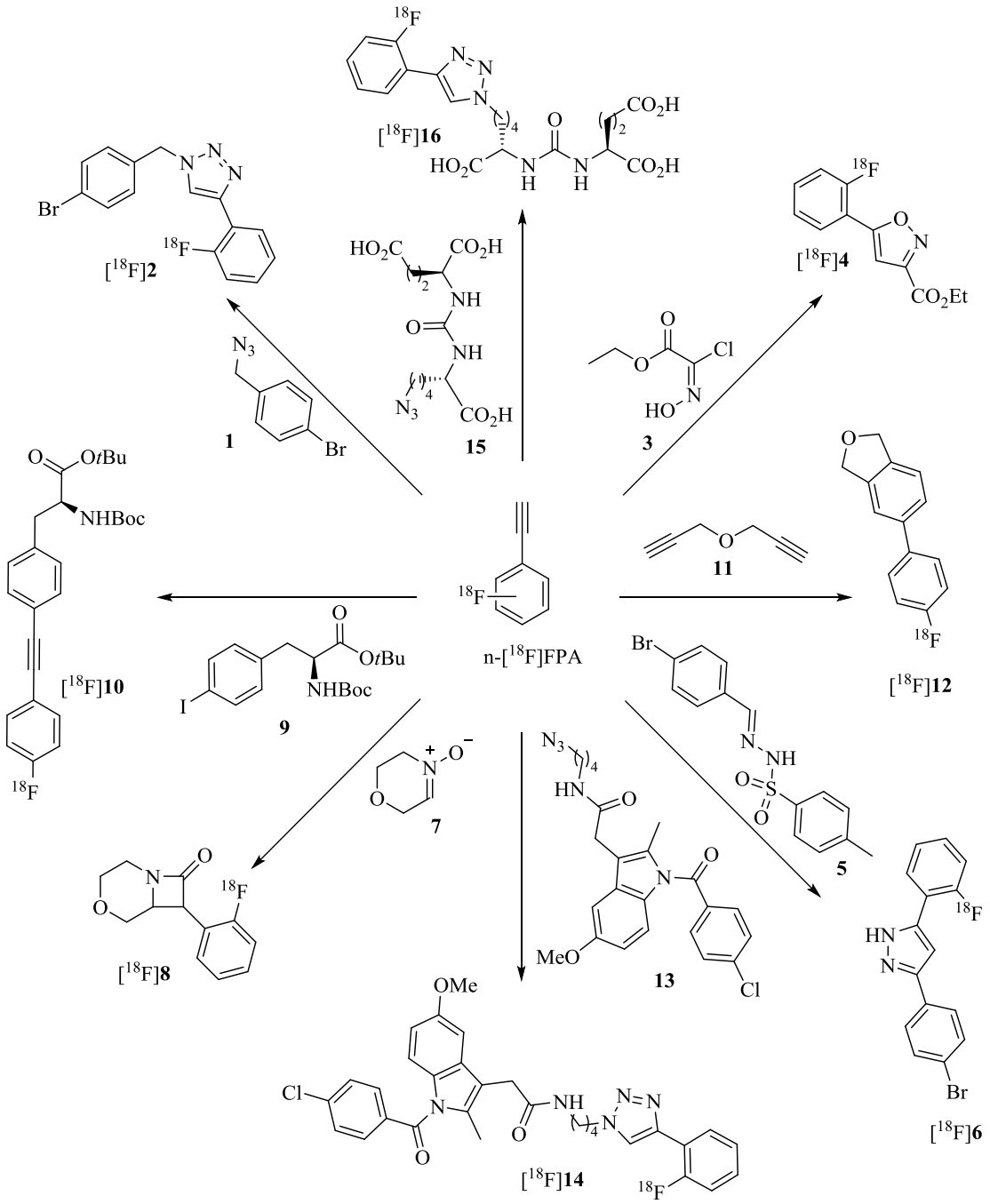


Abbildung 3.4: *Übersicht aller, im Rahmen dieser Publikation, hergestellten ^{18}F-markierten Verbindungen und deren jeweiligen Vorläufer.*

Nachdem die Vielseitigkeit der neuen Radiomarkierungsbausteine in eindrucksvoller Weise an verschiedenen Modellreaktionen demonstriert werden konnte, galt es schließlich ihr Potential zur Herstellung von klinisch relevanten Tracern zu ermitteln.

Dazu wurde ein ^{18}F-markiertes Indomethacinderivat [^{18}F]**14** (siehe Seite 176, Abb. 3.4) synthetisiert, das aufgrund seiner Eigenschaft als selektiver Cyclooxygenase 2 (COX-2)

Inhibitor möglicherweise zur Darstellung inflammatorischer Prozesse und Tumorprogression eingesetzt werden kann.[60]

Die das Lys-Urea-Glu Fragment enthaltene Verbindung [^{18}F]**16** (siehe Seite 176, Abb. 3.4) sollte hingegen potenziell in der Lage sein das prostataspezifische Membranantigen (PSMA) zu adressieren und sich damit zur Visualisierung von Prostata-, Brustkrebs und tumorassoziierte Neovaskularisation eignen.[61]

Beide molekularen Sonden konnten mit über 70% radiochemischem Umsatz erfolgreich über die Azid-Alkin „Click“ Cycloaddition zwischen 2-[^{18}F]FPA und dem jeweiligen Azid-Vorläufer hergestellt werden. Die RCA war in beiden Fällen > 25% (zerfallskorrigiert) bei einer gesamt Reaktionszeit von 60 min. inklusive semipräparativer Reinigung,

3.10 Radio-Kinugasa-Reaktion

Mit Hilfe der ^{18}F-markierten Phenylacetylene konnte in einem ersten Beispiel bereits erfolgreich gezeigt werden, dass die Kinugasa-Reaktion als neues, indirektes Radiomarkierungsverfahren einen wertvollen Beitrag im Bereich der Entwicklung innovativer und selektiver Tracer leisten kann. Außerdem kann sie prinzipiell als Instrument zur direkten und gezielten Synthese von ^{18}F-markierten β-Lactamen eingesetzt werden, um molekulare Prozesse, pathologischen Ursprungs mittels PET zu visualisieren.

Zur Herstellung von radiofluorierten β-Lactamen besteht neben der Verwendung von ^{18}F-markierten Alkinen und Nitronen ebenso die Möglichkeit, zunächst einen Vertreter der Substanzklasse der Nitrone zu radiofluorieren und diesen anschließend *via* Kinugasa-Reaktion mit einem geeigneten, terminalen Alkin in das entsprechende ^{18}F-markierte β-Lactam zu überführen. Grundvoraussetzung dafür ist die vorherige Entwicklung eines geeigneten Syntheseverfahrens zur schnellen und vor allem leistungsfähigen Herstellung radiofluorierter Nitrone. Den Grundstein dafür hat die Arbeitsgruppe Neumaier bereits 2012 gelegt, als sie im Rahmen ihrer Arbeiten auf dem Gebiet der metallfreien (3+2) Cycloadditionen, den 1,3 Dipol *C*-(4-[^{18}F]Fluorphenyl)-*N*-phenylnitron ([^{18}F]FPPN) als neuen Markierungsbaustein vorstellten.[27b] Mit einem Umsatz von über 90% war der eigentliche Syntheseschritt zum gewünschten Nitron zwar sehr effizient, dennoch gelang es diesen Markierungsbaustein nur durch den Einsatz großer Startaktivitäten, in ausreichender Aktivität für Folgereaktion zu Verfügung zu stellen. Grund dafür war das Fehlen eines geeigneten Syntheseverfahrens zur Herstellung des *ab initio* benötigten [^{18}F]Fluorbenzaldehyds.

Im Rahmen dieser Promotionsarbeit ist es über die „minimalistische“ Methode gelungen [^{18}F]FBA in ausreichenden Mengen für den Einsatz in mehrstufigen Herstellungsprozessen zur Synthese klinisch relevanter Verbindungen, zu erhalten.

4-[^{18}F]FBA wurde daher gemäß der „minimalistischen“ Methode und, je nach Bedarf, in einer absoluten Menge von bis zu 37 GBq synthetisiert. Nach Literaturvorschrift erfolgte anschließend, über eine säure-katalysierte Kondensationsreaktion zwischen [^{18}F]FBA und *N*-Phenylhydroxylamin, der Umsatz zu dem benötigten 1,3 Dipol [^{18}F]FPPN. Nach Reinigung mittels Festphasenextraktion lag die radiochemische Ausbeute bei über 90%.

3.11 Verwendung der Kinugasa-Reaktion zur Radiosynthese verschiedener Verbindungen und Markierung von Peptiden und Proteinen

Nachfolgend wurde in einem ersten orientierenden Experiment das ^{18}F-markierte Nitron in Anwesenheit von CuI und Et_3N in MeCN (Variante **a**) mit dem sehr reaktiven terminalen Alkin Methylpropiolat **17** (siehe Seite 176, Abb. 3.4), in das entsprechende radiomarkierte β-Lactam [^{18}F]**18** (siehe Seite 176, Abb. 3.4) überführt. Um den Verbrauch des Alkins (**17**), durch die in Konkurrenz stattfindende Glaser-Kupplung zu unterdrücken, wurde die Reaktion zunächst unter Argon durchgeführt. Das gewünschte Produkt konnte so bereits nach 10 min Reaktionszeit, als Diastereomerengemisch im Verhältnis 1:3 (*cis*:*trans*), in einer RCA von 65% erhalten werden. (diese und alle folgenden RCA sind, sofern nicht anders angegeben, als Summe der Ausbeuten beider Diastereomeren zu verstehen) Aufgrund dieser ersten, sehr vielversprechenden Ergebnisse wurde die Radio-Kinugasa-Reaktion anschließend im Hinblick auf Vorläufermenge, Reaktionszeit, Lösungsmittel, Cu(I)-stabilisierende Liganden und Temperatur optimiert.

Vorläufermengen im Bereich von 10 µmol, wie sie im ersten Markierungsversuch verwendet, wurden sind i.d.R. zur Markierung kleiner Moleküle ausreichend niedrig. Zur Radiomarkierung von biologisch relevanten Molekülen ist es hingegen zwingend erforderlich, die Menge der Vorläuferverbindung auf ein minimal nötiges Maß zu reduzieren. Bei der Radiomarkierung von Peptiden oder Proteinen gilt es beispielsweise allgemein als sehr schwierig den Vorläufer von dem eigentlichen radiomarkierten Produkt zu trennen. Hohe Vorläuferkonzentrationen im Produkt können sich aber negativ auf die Qualität der PET-Bilder auswirken und gegebenenfalls sogar zu unerwünschten Nebenwirkungen bzw. -effekten im Patienten führen. Die Reduktion der Vorläufermenge stand daher zunächst im Fokus der Untersuchungen.

Das radiomarkierte Nitron ([^{18}F]FPPN) wurde dazu mit verschiedenen Mengen an Methylpropiolat (0,05-10 µmol) bei Raumtemperatur für 10 min bzw. 20 min umgesetzt. Die

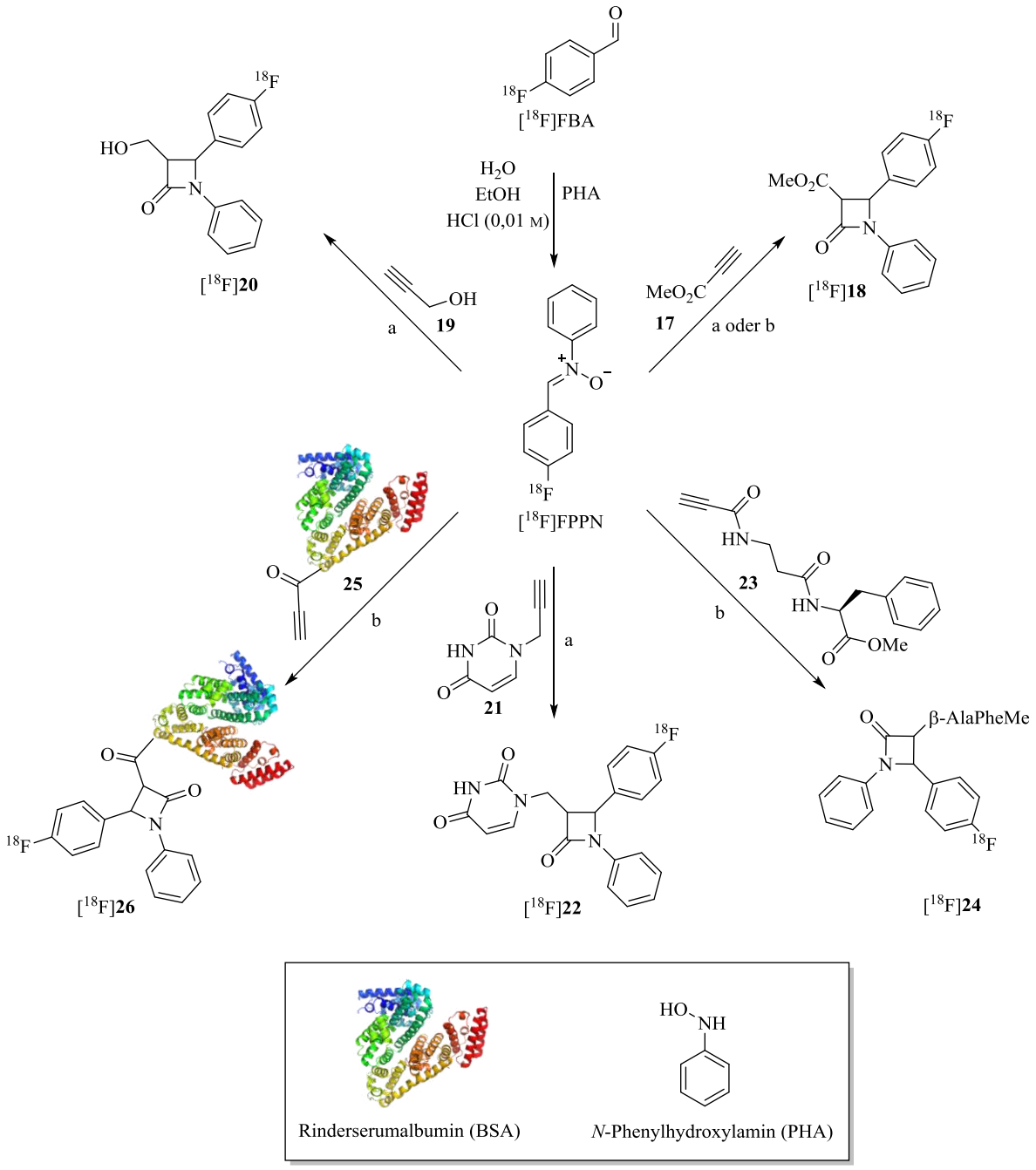
Reduktion der Vorläufermenge von 10 auf 0,8 µmol bewirkte eine allmähliche Abnahme der RCA von 65% und 67% auf 31% und 55% nach 10 min bzw. 20 min Reaktionszeit. Die weitere Reduzierung der Menge auf 0,2 µmol erniedrigt auch die RCA auf 11% und 19% nach 10 min bzw. 20 min. Um Verbesserungen bzw. Verschlechterungen der RCA genauer abbilden zu können, wurden alle weiteren Optimierungen bei einer Vorläufermenge von 0,8 µmol durchgeführt.

Zunächst wurde die Abhängigkeit der Temperatur auf die Produktbildung näher untersucht. Maximale Umsätze von 68% wurden bei 60 °C beobachtet. Eine weitere Temperaturerhöhung hatte keinen signifikanten Einfluss. Ebenso war die Ausbeute der Reaktion stark abhängig von dem verwendeten Lösungsmittel. Die beste RCA wurde dabei in MeCN (31%) erzielt. Deutlich niedrigere RCA wurden in DMSO bzw. DMF mit 21% bzw. 16% gefunden. In Pyridin hingegen waren, vermutlich aufgrund des sofortigen Verbrauchs von Methylpropiolat in einer konkurrierend auftretenden Michael Addition mit Pyridin, nur Spuren (< 2%) des Produktes zu detektieren.[62] Des Weiteren wurde der Einfluss von Cu(I) stabilisierenden Liganden auf die Kinugasa-Reaktion untersucht. Bereits 1995 stellten Miura *et al*. fest, dass insbesondere die Anwendung von Stickstoff-Liganden zu einer erheblichen Beschleunigung der Kinugasa-Reaktion führt.[44a]

Demgemäß wurde bei Anwesenheit von Pyridin als stabilisierender Ligand ein moderater Anstieg (von 31% auf 43%) beobachtet. Wesentlich effizienter hingegen war der Einsatz von 1,10 Phenanthrolin, womit eine maximale RCA von 75% erzielt werden konnte. Nach Ermittlung der optimalen Reaktionsparameter für die Radio-Kinugasa-Reaktion mit hoch reaktiven terminalen Alkinen, lag der Fokus anschließend auf weniger stark aktivierten. Als Modellverbindung für letztere diente Propargylalkohol. Die Hydroxymethyl-Gruppe des Modellalkins **19** (siehe Seite 176, Abb. 3.4) reduziert die Reaktivität erheblich und das Alkin konnte daher nur erschwert zur Reaktion gebracht werden, weshalb harsche Reaktionsbedingungen notwendig waren, um das Konjugationsprodukt zu erhalten. Durch den Wechsel von MeCN zu Pyridin wurde die RCA auf 82% beträchtlich gesteigert. Außerdem konnte die Reaktion bereits bei 50 °C und ohne zusätzlichen Cu-Liganden durchgeführt werden.

Nach Optimierung der Radio-Kinugasa-Reaktionen wurde das Spektrum auf klinisch relevante Anwendungsmöglichkeiten ausgeweitet. Als erstes Modell diente dazu die chimäre ^{18}F-markierte Nucleobase [^{18}F]**22** (siehe Seite 176, Abb. 3.5). Derartige radiofluorierte Verbindungen sollten in der Lage sein, bakterielle Infektionen mittels PET darzustellen. Zu diesem Zweck wurde 1-Propargyluracil mit Hilfe des Markierungsbausteins [^{18}F]FPPN und

nach optimierten Reaktionsbedingungen in das gewünschte Uracil-Konjugat [^{18}F]**22** überführt. Dabei konnte eine RCA von 65% erzielt werden.



Schema 3.5: *Übersicht aller, via Radio-Kinugasa-Reaktion hergestellten ^{18}F-markierten Verbindungen. a: CuI, Et_3N, Ar, wasserfrei, Cu-Ligand. b: $CuSO_4$, AscONa, H_2O, Cu-Ligand.*

Zudem wurde untersucht, ob sich FPPN als Markierungsbaustein zur Radiofluorierung von Peptiden und Proteinen eignet. Wie bereits erläutert wird die Kinugasa-Reaktion konventionell unter Inertgasatmosphäre und in wasserfreien organischen Lösungsmitteln durchgeführt. Ungeschützte Peptide und Proteine sind nicht in oder nur sehr schwer in organischen Lösungsmitteln wie MeCN löslich, was ihre Markierung unter den genannten

Reaktionsbedingungen stark limitiert bzw. erschwert. In Anlehnung an die von Basak *et al*. veröffentlichten Arbeiten wurde daher ein Verfahren entwickelt, das Arbeiten unter Schutzgas und insbesondere die Verwendung wasserfreier Lösungsmittel überflüssig machte.[63] In Vorversuchen wurde dieses Verfahren zunächst in einer "kalten" Kinugasa-Reaktion zwischen [^{19}F]FPPN und Methylpropiolat unter den von Basak *et al.* beschriebenen Bedingungen getestet. Allerdings konnten lediglich Spuren des gewünschten Produktes detektiert werden. Nach einigen weiteren Versuchen zeigte sich jedoch, dass ohne den Zusatz einer Base, zumindest moderate Ausbeuten erhalten wurden. Außerdem konnte auf den Einsatz von Schutzgas verzichtet werden. Nichtsdestotrotz lieferte die Reaktion nur unter Verwendung von Vorläufermengen größer 50 µmol akzeptable radiochemische Ausbeuten.

Zur Minimierung der Vorläufermenge wurde daher anschließend der Einfluss, der in „Click"-Reaktionen häufig verwendeten, Cu(I)-stabilisierenden Liganden, L-Histidin, BTS und TBTA,[64] auf die RCA in der Radio-Kinugasa-Reaktionen zwischen [^{18}F]FPPN und Methylpropiolat, unter „Click"-Chemie-Bedingungen (Variante **b**), untersucht. Unabhängig davon, welcher der Liganden verwendet wurde, konnte die RCA bereits bei einer Vorläufermenge von 0,4 µmol auf bis zu 80% gesteigert werden.

Daraufhin wurde Propiolyl substituiertes β-AlaPheOMe **23** (siehe Seite 176, Abb. 3.4) als Modellpeptid ausgewählt und mit [^{18}F]FPPN in einer Kinugasa-Reaktion unter „Click"-Chemie Bedingungen in das entsprechende ^{18}F-markierte Depsipeptid [^{18}F]**24** (siehe Seite 176, Abb. 3.4) überführt. Bereits nach 10 min Reaktionszeit wurde eine RCA von 58% erreicht.

Die Ergebnisse warfen anschließend die Frage auf, ob die Kinugasa-Reaktion sich möglicherweise auch zur Radiomarkierung von propiolierten Proteinen eignet. Zu diesem Zweck wurde zunächst natives Rinderserumalbumin (BSA; M = 66,5 kDa), als ein prototypisches Substrat, mit einem speziellen, eine C-C-Dreifachbindung enthaltenden, Chlorameisensäureester in Anwesenheit von 0,15 M $KHCO_3$ bei Raumtemperatur acyliert. Die anschließende Radio-Kinugasa-Reaktion zwischen dem propiolierten BSA **25** (siehe Seite 176, Abb. 3.4) und dem Markierungsbaustein [^{18}F]FPPN unter „Click"-Chemie Bedingungen lieferte das gewünschte ^{18}F-markierte Protein [^{18}F]**26** (siehe Seite 176, Abb. 3.4). Nach Reinigung mittels Gelausschluss-Chromatographie lag es in einer radiochemischen Ausbeute von 32% und einer radiochemischen Reinheit von > 98% vor. In dem zugehörigen Kontrollexperiment mit nativem BSA konnte keine Produktbildung beobachtet werden.

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Abkürzungsverzeichnis

Å

Å ..... Ångström

A

ADIBOA ..... Azadibenzocyclooctinamin

B

Bq ..... Bequerel
BSA ..... Rinderserumalbumin
BTMG 2-tert-Butyl-1,1,3,3-tetramethylguanidin
BTS ..... Bathophenanthrolin-disulfonat-dinatriumsalz

C

c.a. ..... Carrier-added
COX-2 ..... Cyclooxygenase 2
CT ..... Computer-Tomographie
CuAAC ..... Copper-Catalyzed Azide-Alkyne Cycloaddition

D

Da ..... Dalton
DMF ..... Dimethylformamid
DMSO ..... Dimethylsulfoxid

E

EtOH ..... Ethanol
eV ..... Elektronenvolt
EWG ..... electron withdrawing group

F

FBA ..... Fluorbenzaldehyd
FDG 2-Fluor-2-desoxy-D-glucose
FDR 5-Fluor-5-deoxy-D-Ribose
FEA ..... Fluorethylazid
FIB 1-Fluor-4-iodbenzol
FPA ..... Fluorphenylacetylen
FPPN ..... Fluorphenylnitron

G

Glu ..... Glutaminsäure

K

K2.2.2 ..... 1,10-diaza-4,7,13,16,21,24-hexaoxabicyclo[8.8.8]hexacosan

L

LOR ..... line of response
Lys ..... Lysin

M

MeOH ..... Methanol
min ..... Minute
mm ..... Millimeter
MRT ..... Magnetresonanztomographie

N

n.c.a ..... no-carrier-added

P

PET ..... Positronen-Emissions-Tomographie
PSA ..... Prostataspezifische Antigen

R

RCA ..... Radiochemische Ausbeute
RCU ..... Radiochemischer Umsatz

S

SFB *N*-Succinimidyl-4-fluorbenzoat
SPAAC ..... Strain-promoted alkyne-azide cycloaddition

T

TBTA ..... Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amin)
TEMPO 2,2,6,6-Tetramethylpiperidinyloxyl
TFB ..... Fluorbenzoat

U

US ..... Ultraschall

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Anhang I

A. Eigener Beitrag an den dieser Arbeit zugrundeliegenden Publikationen

R. Richarz,† **P. Krapf**,† F. Zarrad, E. A. Urusova, B. Neumaier*, B. D. Zlatopolskiy, *Org. Biomol. Chem*. **2014**, *12*, 8094–8099.

Das Konzept dieser Publikation wurde maßgeblich im Kollektiv aller Autoren erarbeitet und erstellt. Die experimentelle Umsetzung erfolgte durch P. Krapf und R. Richarz. Alle erforderlichen Optimierungen, welche u.a. Temperatur, Vorläufermenge und Zeit einschließt, wurden eigenverantwortlich von P. Krapf und R. Richarz zu gleichen Teilen durchgeführt. Die Erstellung des Manuskripts erfolgte gemeinsam durch Prof. B. Neumaier, Dr. B. D. Zlatopolskiy, P. Krapf und R. Richarz.

P. Krapf,† R. Richarz,† E. A. Urusova, B. Neumaier*, B. D. Zlatopolskiy, *Eur. J. Org. Chem.* **2016**, *2016* 430–434.

Die Konzeption dieser Publikation erfolgte, gemeinschaftlich, durch P. Krapf und R. Richarz, unterstützt durch Prof. B. Neumaier und Dr. B. D. Zlatopolskiy. Die radiochemische Umsetzung, einschließlich der Experimente und Datenerhebung, wurde von P. Krapf und R. Richarz zu gleichen Teilen durchgeführt. Die Entwicklung aller HPLC-analytischen Verfahren, sowie der semi-präparativen Herstellungsprozesse etwaiger Radiotracer erfolgte durch P. Krapf. Das Manuskript wurde gemeinschaftlich durch P. Krapf, R. Richarz, Prof. B. Neumaier und Dr. B. D. Zlatopolskiy erstellt.

B. D. Zlatopolskiy,† **P. Krapf**,† R. Richarz, H. Frauendorf, F. M. Mottaghy, B. Neumaier*, *Chem. Eur. J.* **2014**, *20*, 4697–4703.

Das Konzept des Forschungsansatzes wurde gemeinsam von Prof. B. Neumaier, Dr. B. D. Zlatopolskiy und P. Krapf entwickelt. Die Planung der Experimente, die Datenerhebung und –interpretation, sowie alle dazugehörigen radiochemischen Versuche wurden von P. Krapf durchgeführt. Die HPLC-analytischen Methoden für den Nachweis der neuen Verbindungen und die, teilweise erforderlichen, Reinigungsverfahren wurden von P. Krapf erarbeitet. Die Erstellung des Manuskripts erfolgte gemeinsam durch Dr. B. D. Zlatopolskiy und P. Krapf, sowie Prof. B. Neumaier.

† *Geteilte Erstautorenschaft*
* *Korrespondierender Autor*

B. Erklärung gemäß § 4 Abs. 1 Punkt 9

"Ich versichere, dass ich die von mir vorgelegte Dissertation selbständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und die Stellen der Arbeit − einschließlich Tabellen, Karten und Abbildungen −, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind, in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie – abgesehen von unten angegebenen Teilpublikationen − noch nicht veröffentlicht worden ist sowie, dass ich eine solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde.

Die Bestimmungen der Promotionsordnung sind mir bekannt. Die von mir vorgelegte Dissertation ist von Univ. Prof. Dr. Bernd Neumaier betreut worden."

Köln, 02.05.2016……

(Philipp Krapf)