ALPHA SELECTIVE EPOXIDE OPENING TO INTRODUCE 18F- INTO ORGANIC MOLECULES

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Objectives: Strained tricyclic ring-systems such as epoxides and aziridines are seldom used as precursors for the introduction of anionic fluorine-18 into organic compounds. Up until now only one example about the successful application of an epoxide precursor in the radiolabeling of [^{18}F]FMISO has been described in the literature by Welch and co-workers in 1985.[1] It was our objective to investigate the possibilities of using epoxides as precursors for ^{18}F-labeling reactions.

Methods: Various epoxides were reacted with "dried" [^{18}F]fluoride in different solvents such as dipolar-aprotic acetonitrile, DMF, DMSO and polar-protic tert-amyl-alcohol and tert-butanol at elevated temperatures. The crude reaction mixtures were investigated by means of radio-HPLC and the RCYs of the ^{18}F-fluorinated compounds determined. One epoxide in particular, bearing an aldehyde moiety was ^{18}F-fluorinated and used as a secondary labeling precursor for chemoselective conjugation to an amino-oxy derivatized peptide. In addition, ^{18}FMISO was synthesized in high RCYs using the appropriate epoxide precursor.

Results: We found that the reaction of various model epoxides with ^{18}F (Kryptofix2.2.2®/K_2CO_3 or $K_2[COOH]_2$] at elevated temperatures in tert-amyl-alcohol was 100% alpha selective and yielded the corresponding ^{18}F-fluoroalcohols in high radiochemical yields (RCYs) (80-85%). When dipolar aprotic solvents such as acetonitrile, DMF or DMSO were used, the yields dropped either significantly or no product could be detected at all. These findings were transferred to the synthesis of 4-(3-[^{18}F]fluoro-2-hydroxypropoxy)benzaldehyde which could be obtained in high RCYs of 80-87% and was subsequently used as a prosthetic group for the labeling of amino-oxy-derivatised peptides by chemoselective oxim formation (Fig.).

Conclusions: The finding that tert-alcohols facilitate the regioselective opening of epoxides with ^{18}F is perfectly in line with the previous reports on the facilitated ^{18}F-fluorination of tosylates and mesylates in these solvents.[2] The use of epoxides as labeling precusors has proven to be a valuable labeling reaction, showing a high level of regio-specificity and high RCYs of the corresponding ^{18}F-fluoro-alcohols. We are currently investigating the possibilities of removing the un-reacted epoxide from the ^{18}F-fluorinated products by means other than HPLC to render this method to be even more convenient.

References: [1] Jerabek, P. A. Appl. Radiat. Isot. 37 (1986) 599-605 [2] Kim et al. J. Am. Chem. Soc. 128 (2006) 16394

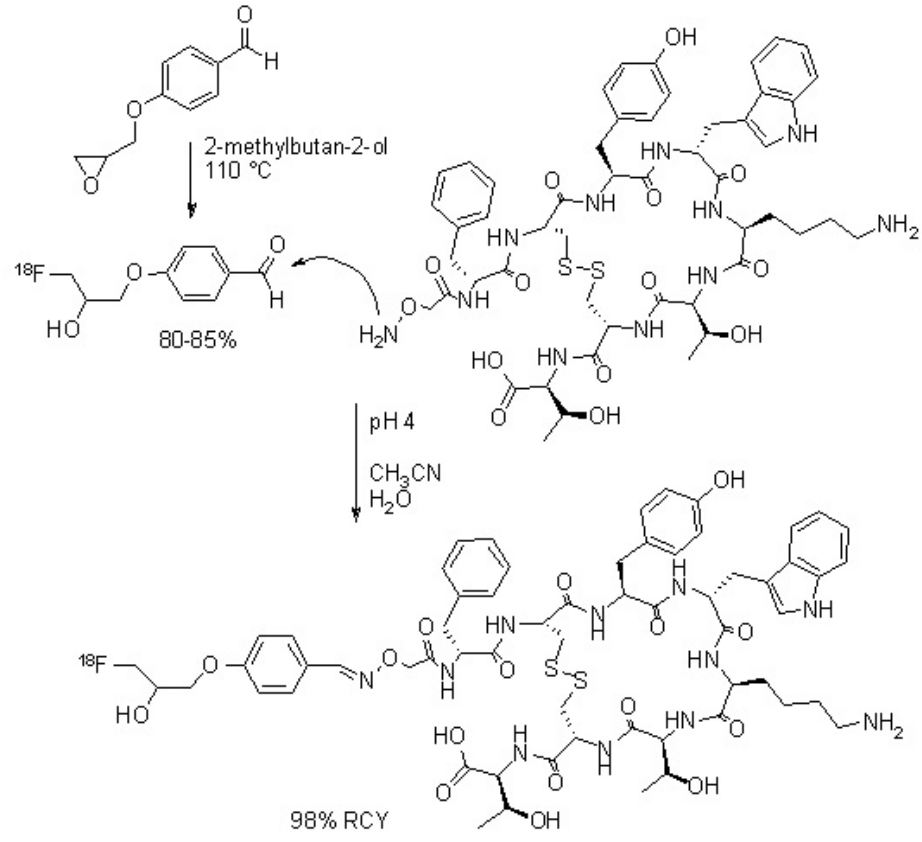

Fig. Synthesis of ^{18}F-labeled Tyr3-octreotate by chemoselective conjugation of 4-(3-[^{18}F]fluoro-2-hydroxypropoxy)benzaldehyde to an AO-derivatized Tyr3-octreotate

A CLICK CHEMISTRY APPROACH FOR THE DEVELOPMENT OF METALLIC RADIOTRACERS AND THERAPEUTIC AGENTS

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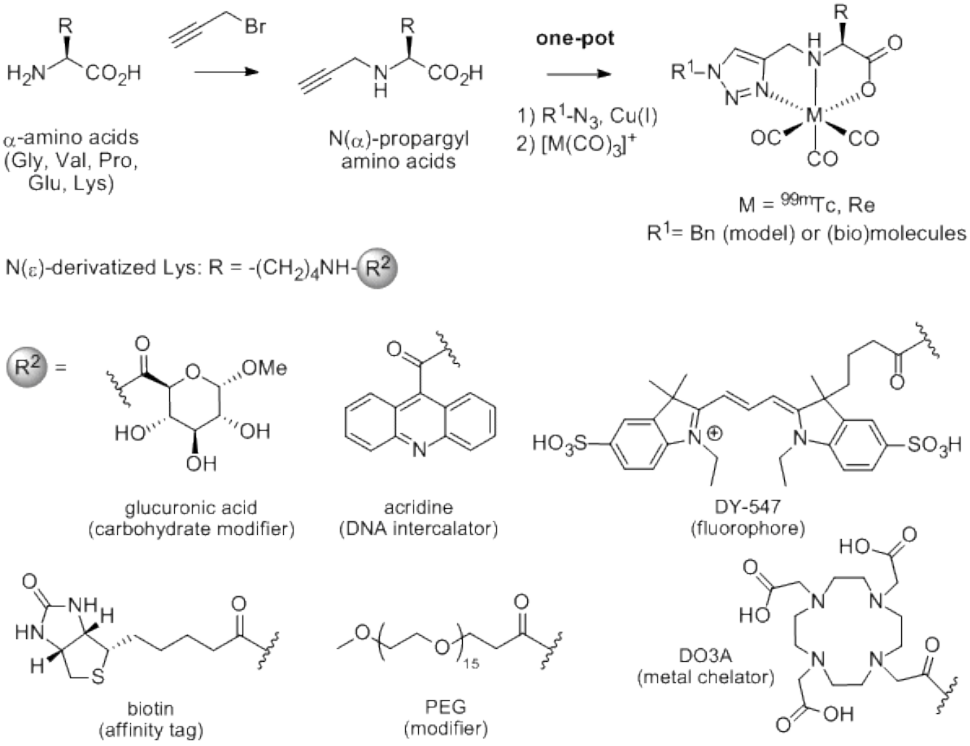
Objectives: We have recently reported that application of the Cu(I)-catalyzed [2+3] cycloaddition of terminal alkynes and azides (click chemistry) facilitates both the synthesis of structurally diverse chelating systems for $[M(CO)_3]^+$ (M= Re, ^{99m}Tc) and their attachment to (bio)molecules. The 1,2,3-triazole containing radiometal conjugates have been shown to be well suited for applications as SPECT tracers in vivo. We now wish to report an extension of the "click-to-chelate" strategy which not only allows the selective introduction of ^{99m}Tc-chelates into azide-functionalized (bio)molecules (R^1) of interest but also the simultaneous incorporation of other entities (R^2), e.g. pharmacological modifiers, affinity tags, therapeutic agents or, second imaging probes (Figure).



Methods: Click chemistry; one-pot Tc-99m labelling.

Results: N(α)-propargyl derivatives of commercial amino acids (AA) were identified as synthetically readily accessible precursors for the "click-to-chelate" approach providing structurally diverse building blocks. A set of "clickable" alkyne compounds based on AA scaffolds (Gly, Val, Pro, Glu, Lys) were synthesized, reacted with benzyl azide and the tridentate chelators obtained were subjected to complexation with fac$[M(CO)_3]^+$. In all cases, metal complex formation was quantitative at a ligand concentration in the low micromolar range. Conveniently, click reaction and metal labelling could also be achieved by efficient one-pot procedures, therefore simplifying further the synthetic procedures. The structure of model compounds was confirmed by spectroscopic methods (NMR, IR, MS) and x-ray crystallography of the corresponding rhenium analogues. Starting from a common N(α)-propargyl Lys precursor, a variety of N(ε)-amide derivatives were prepared including examples of the attachment of PEG, carbohydrates, DNA-intercalators, biotin, fluorophores and, metal chelators. Application of a selection of Lys derivatives for the labelling of a stabilized, tumour-targeting bombesin fragment with Tc-99m as well as the effect of the attached second entities in vivo will be presented.

Conclusions: "Click-to-chelate" now provides the possibility of the simultaneous introduction of second entities which will facilitate the modulation of pharmacologically relevant characteristics of the final metal conjugate as well as the development of potential multimodal imaging agents.

References: [1] Mindt et al. J. Am. Chem. Soc. 2006, 15096; [2] Mindt et al. ChemMedChem 2009, in print; [3] Struthers et al. Chem.-Eur. J. 2008, 6173; [4] Mindt et al. Bioconj. Chem. 2008, 1689.

CLICK FOR PET: DEVELOPMENT AND APPLICATION OF ACCELERATED 1,3-DIPOLAR CYCLOADDITIONS OF AZIDES AND ALKYNES TO [18F]-POSITRON EMISSION TOMOGRAPHY

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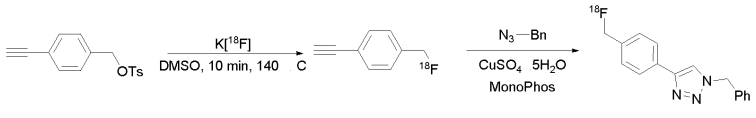
Objectives: The discovery by Sharpless in 2002 that copper (I) catalyzes the 1,3-dipolar cycloaddition of azides and alkynes to form 1,4- disubstituted triazoles (CuAAC) strongly contributed to the popularization of 'click' chemistry (1). Our interest in applying this reaction to time sensitive [^{18}F]-radiolabelling methodology for PET led us to consider recent advances in ligand accelerated CuAAC. The rate of the ligand free reactions can hinder the application of click chemistry to [^{18}F]-radiolabelling. Phosphoramidites are used as monodentate ligands for copper in a number of transformations and have demonstrated ligand accelerating effects (2). They are inexpensive, stable and easily accessible (3). We report the first example of dramatic rate acceleration of the [3+2] cycloaddition of azides and alkynes using phosporamidite ligands and application of our method to PET-imaging analogues.

Methods: A thorough screening of phosphoramidites and related ligands revealed that the addition of 1.1 mol % MonoPhos, the simplest dimethyl amino BINOL based phosphoramidite, decreases the reaction time of a standard click reaction from 24 h to 2 h (reaction with 1 mol % Cu in H_2O/DMSO). Similar accelerating effects were seen with other phosphoramidites (SI Table 1). Experiments were also conducted to determine the effect of catalyst loading upon the rate, demonstrating, as expected that an increase in the copper loading translates into a decreased reaction time (SI Table 2). As a copper source for the CuAAC, $CuSO_4 \cdot 5H_2O$ in combination with sodium ascorbate is overwhelmingly favoured. Although Cu(I) salts can also be used, they require an equivalent of nitrogen containing base to promote the reaction[ii]. We anticipated that the phosphoramidite might stabilize the catalytically active Cu(I) oxidation state. Our hypothesis was correct; we found similar rates and yields in our reaction using Cu (I) halide salts, and no evidence of the expected side products (see SI Table 3). Substrate screening showed that various functional groups, including fluorine, were tolerated in both substrates. Reactions remained fast and high yielding highlighting system versatility.

CuAAC Substrate Scope With Optimized Conditions

R-N_3	R-CCH	Time (h)	Yield (%)
-Bn	-Ph	1	91
-*o*-CH_2PhF	-Ph	1.5	84
-Ph	-Ph	4	88
-*o*-PhOMe	-Ph	2	80
-*o*-PhBr	-Ph	2	71
-*o*-PhCN	-Ph	2	81
-cinnamyl	-Ph	1.5	96
-C_8H_{17}	-Ph	2.5	99
-C_4H_8F	-Ph	1.5	90
-CH_2CO_2Et	-Ph	0.75	83
-Bn	-*o*-PhOMe	1.5	86
-Bn	-*p*-$PhNO_2$	5	62
-Bn	-CO_2Et	2	87
-Bn	-CO_2H	1	59
-Bn	-CH_2NH_2	1	65
-Bn	-*o*-$PhCH_2F$	2	93

Results: Testing our methodology for radiolabelling, we synthesized [^{18}F]-fluorinated 1-ethynyl-4-(fluoromethyl)benzene (Scheme 1). After fluorination, it was ligated to benzyl azide. Full conversion was detected after 10 min (HPLC and radio-TLC). Under identical conditions but in the absence of ligand, only minor conversion to the triazole was detected (<20 %).



Scheme 1 Synthesis of [18F]-labelled triazole

RCY=47 %

Conclusions: We have applied phosphoramidite copper complexes to the CuAAC and found that they dramatically enhance the reaction rate and stabilize the copper(I) oxidation state. The system is versatile and functional group independent. The methodology has been applied to the ligation of small [^{18}F]-labelled prosthetic groups to a model azide.

References: [1] R. Huisgen in 1,3-Dipolar Cycloaddition Chemistry (Ed.: A. Padwa),Wiley, New York, 1984, pp. 1-176; V.V. Rostovtsev, L.G. Green,V.V. Fokin and K. B. Sharpless, Angew. Chem. Int. Ed., 2002, 41, 2596. [2] D.J. Berrisford, C. Bolm, and K.B. Sharpless. Angew. Chem. Int. Ed., 1995, 34, 1059. [3] B.L. Feringa, Acc. Chem. Res., 2000, 33, 346.

LABELING OF PROTEINS WITH FLUORINE-18 VIA CLICK CHEMISTRY

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Objectives: The radiolabeling of peptides and proteins with the short-lived positron emitter ^{18}F requires rapid and mild reaction conditions compatible with the structural and functional integrity of these biomolecules. Over the last two years several approaches have been published focusing on the application of copper(I)-mediated 1,3-dipolar [3+2]cycloaddition of azides and alkynes for labeling peptides with ^{18}F. However, to date no ^{18}F labeling of proteins via click chemistry has been reported. In this work we describe for the first time the application of click chemistry for ^{18}F labeling of proteins as exemplified with azide-functionalized human serum albumin (HSA). Click chemistry was accomplished through 4-[^{18}F]fluoro-N-methyl-N-(prop-2-ynyl) benzenesulfonamide (p[^{18}F]F-SA) as novel alkine-containing ^{18}F-labeled click chemistry building block.

Methods: The novel click chemistry building block p[^{18}F]F-SA was prepared in a single step reaction in a remotely controlled synthesis module starting from readily available labeling precursor (Fig. 1).

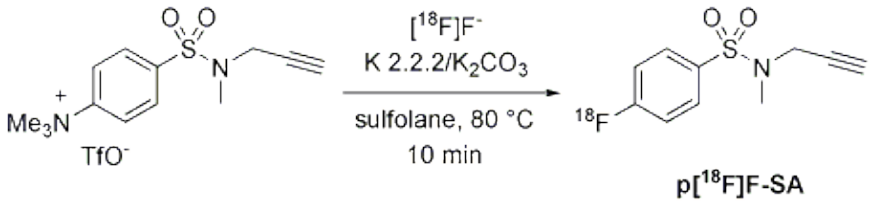

Fig. 1: Radiosynthesis of click chemistry building block **p[18F]F-SA**

HSA was modified with azide residues through conjugation of the lysine residues in HSA with an azide-functionalized active ester. Azide-modified HSA was subjected to digest with three different endoproteinases and subsequent MALDI-TOF MS analysis to assess the number of introduced azide residues. Radiolabeling of modified HSA was accomplished with p[^{18}F]F-SA in the presense of Cu(I)Br and the Cu(I) chelating ligand tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA). Radiolabeled HSA was purified with size-exclusion chromatography and analyzed with SDS-PAGE.

Results: Radiolabeled sulfonamide p[^{18}F]F-SA could be obtained in an automated synthesis unit in radiochemical yields of 21-35% (decay-corrected) within 75 min after HPLC purification. The radiochemical purity was >99%, and the specific activity was in the range of 71-128 GBq/μmol. Sulfonamide p[^{18}F]F-SA showed favorable lipophilicity (logP = 1.6) allowing application in aqueous reaction media. Tryptic digest and subsequent MALDI-TOF MS analysis of modified HSA revealed the introduction of an average of 28 azide residues into HSA. Click chemistry of azide-functionalized HSA (0.5 mg) with CuBr (0.2 mg) and TBTA in phosphate buffer (pH 7.4) gave 31% of ^{18}F-labeled HSA after size-exclusion chromatography.

Conclusions: The convenient radiosynthesis of p[^{18}F]F-SA as a novel ^{18}F-labeled sulfonamide-based click chemistry building block in an automated synthesis unit allows its wide application for a broad range of click chemistry reactions. For the first time, click chemistry could successfully be applied to the ^{18}F labeling of proteins, which further expands the scope of click chemistry as versatile tool for radiolabeling reactions.

PEPTIDE CLICK LABELING WITH 1-(AZIDOMETHYL)-4-[18F]-FLUOROBENZENE AND SOLID PHASE SYNTHESIS OF REFERENCE COMPOUNDS

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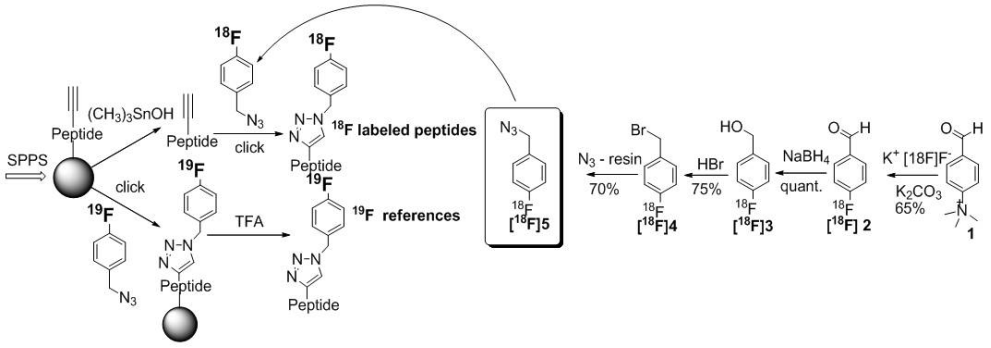
Objectives:



Methods: To test our methodology on the required time scale of radiolabelling, we designed a small azido prosthetic group, [18F]-fluorinated 1-azido-4-fluorobutane and [18F]-fluorinated 1-ethynyl-4-(fluoromethyl)benzene (Sheme 1,2)
After [18F]fluorination, the tag was attached to its complementary cold acetylene or azide in the presence of CuSO4•5H2O and MonoPhos. Further optimization reactions were performed by varying the amount of acetylene 0.01-0.5 mg (Fig.1) or azide 0.05-0.1 mg, to find the optimal yield of reaction in DMSO/H2O (1/3).

Results: Azide [^{18}F] 5 was synthetized within 75 min in 4 steps and with an overall decay-corrected radiochemical yield of 34%. These satisfying results for a four-step procedure have been obtained thanks to the exploitation of solid phase supported reactions and the absence of solvent evaporation process that allow to minimize the losses of time and radioactivity during reaction work-up and purification.This prosthetic group has been clicked very rapidly (10 minutes), at room temperature, with a diluted solution of a model alkyne-peptide (0.003M), in mild conditions and with an excellent yield (90%, decay corrected). Moreover, insulin, a fifty-one amino acid residues peptide hormone, has been labelled through this approach.Complementarily, as small peptides are most frequently prepared by solid phase synthesis, we have developed a fast and simple procedure to prepare the reference fluorine-19 peptides on solid support by click chemistry (figure 1). This technique has been demonstrated on linear and disulfide cyclic peptides with azide 5 and with two alkyne prostethic groups reported in the literature (2, 3).

Conclusions: In summary, an azide labeling agent (1-(azidomethyl)-4-[^{18}F]-fluorobenzene) has been produced in a four steps procedure in 75 minutes with a 34% radiochemical yield (decay corrected). Conjugation of [^{18}F]fluoroazide with a model alkyne-peptide produced the desired ^{18}F-radiolabeled peptide in less than 15 minutes with a yield of 90% and excellent radiochemical purity.Additionally, a click solid phase synthesis approach for the straightforward preparation of reference peptide compounds has been developed

Research Support: The authors wish to acknowledge the financial support from the Biowin Project of the Walloon Region (NeoFor and KeyMarker)

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LIGAND ACCELERATION AND EXPLORATION OF REACTION PARAMETERS OF F-18 CLICK CHEMISTRY

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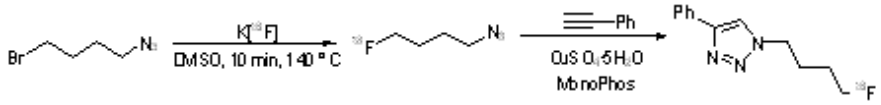
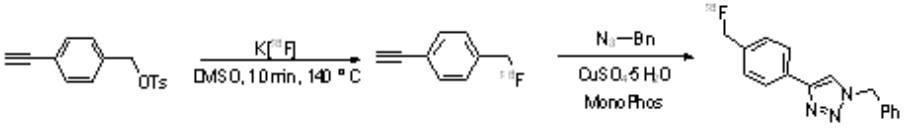
Objectives: The Huisgen 1,3-dipolar cycloaddition of azides and alkynes using copper(I) as catalyst, is recognized as the most commonly applied 'click reaction'. It finds application in bioorganic and medicinal research fields, as it proceeds under mild and tolerable conditions, in aqueous media, at neutral pH, and at room temperature, all within a reasonable reaction time. We found that phosporamidite ligands accelerate the reaction, and based on these findings, we optimized the radiolabelling conditions and concentrations. Phosphoramidites prove to be excellent, high yielding, easily recovered ligands. Preliminary studies to find optimal conditions for the two-step [F-18] labeling procedure were performed. 4-Methoxybenzyl azide and phenylacetylene were employed for the model reaction. With $CuSO_4$ and Na-ascorbate, aqueous DMSO was the best reaction media regardless of the water content. Monodentate phosphoramidites ligand are used for copper in a number of stereoselective transformations and have demonstrated strong ligand accelerating effects.

Methods: To test our methodology on the required time scale of radiolabelling, we designed a small azido prosthetic group, [^{18}F]-fluorinated 1-azido-4-fluorobutane and [^{18}F]-fluorinated 1-ethynyl-4-(fluoromethyl)benzene (Sheme 1,2)



Scheme 1 Synthesis of [18F]-labelled triazole using [18F]alkyl azide



Scheme 2 Synthesis of [18F]-labelled triazole using [18F]acetylene

. After [18F]fluorination, the tag was attached to its complementary cold acetylene or azide in the presence of $CuSO_4$□$5H_2O$ and MonoPhos. Further optimization reactions were performed by varying the amount of acetylene 0.01-0.5 mg (Fig.1) or azide 0.05-0.1 mg, to find the optimal yield of reaction in DMSO/H_2O (1/3).

Results: Full conversion to the labelled triazole was detected after 10 min (determined by HPLC and radio-TLC). [18F] fluoroalkynes and azides were prepared in yields ranging from 36% to 81%. Conjugation of [18F]fluoroalkynes and azides to various amount (> 0.01 mg) of acetylenes or azide with via Cu(I) mediated 1,3-dipolar cycloaddition yielded the desired [18F]-labeled products in 10 min with yields of 54–99% and excellent radiochemical purity (99%). The total synthesis time was 40 min from the end of bombardment. In the absence of MonoPhos under identical conditions, only minor conversion to the triazole product was detected (<20 %).With regard to [F-18], 1mol % of $CuSO_4$ showed sufficient activity within the constrains.(Figure2)

Conclusions: The Cu(I)-catalyzed, 1,3-dipolar cycloaddition 'click chemistry' reaction was applied successfully to the synthesis of small, F-18-labeled molecules, and optimal conditions were developed for one-pot, two-step reaction after purification by semipreparative HPLC.

Research Support: The authors wish to thank to the University Medical Center Groningen (UMCG) for financial support.

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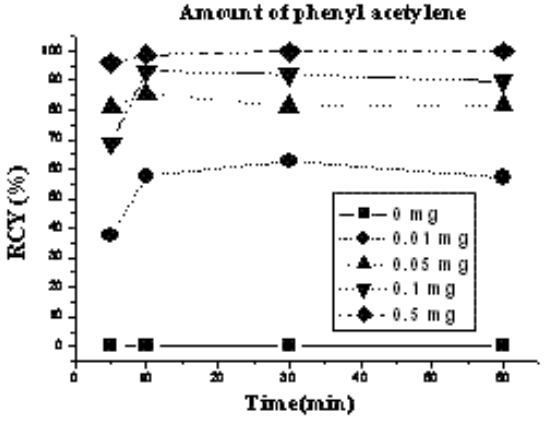

figure 1

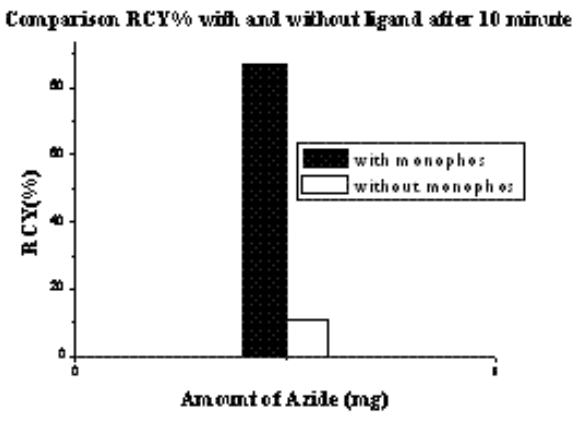

figure 2

SYNTHESIS OF AN F-18 LABELED RAPAMYCIN ANALOGUE FOR IMAGING THE EXPRESSION OF MAMMALIAN TARGET OF RAPAMYCIN (mTOR) IN BRAIN TUMORS

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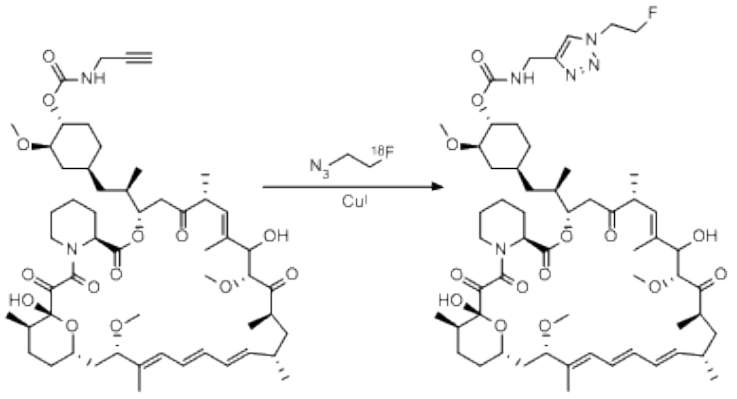
Objectives: The mammalian target of rapamycin (mTOR) is a key protein kinase that regulates signaling pathways involved in cell growth and proliferation. Rapamycin is a macrolytic lactone forma a complex with FKBP12 and inhibits mTOR activity. Rapamycin has been shown to exhibit activity against solid tumors, and rapamycin analogues are currently being evaluated in clinical trials as antitumor drugs. Therefore, the development of an F-18 labeled rapamycin analogue could provide a powerful tool for imaging the mTOR status of tumors in vivo.

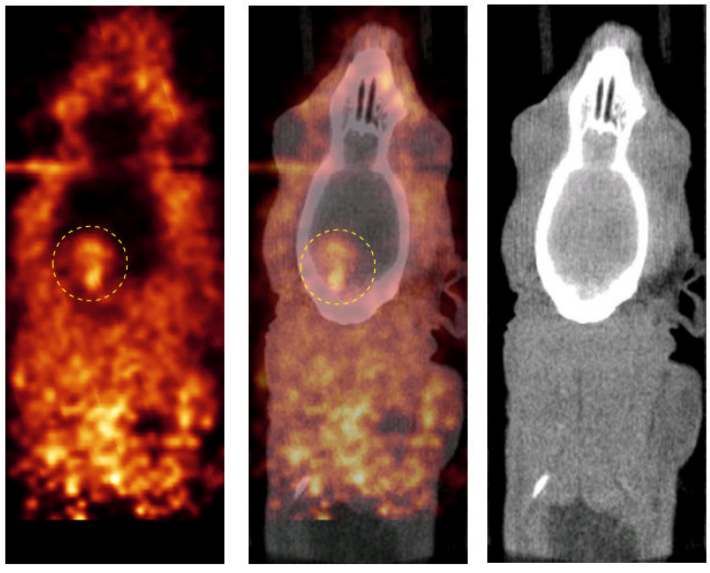
Methods: Two rapamycin analogues, that are suitable for F-18 labeling as PET tracers, have been synthesized by attaching a propargyl carbamate or but-3-ynyl carbonate group at the C40 hydroxy position, followed by formation of 1,2,3-triazole with 2-fluoroethyl azide in the presence of Cu(I) as catalyst. The F-18 labeled carbamate analogue was synthesized from the corresponding propargyl carbamate precursor with F-18 labeled 2-fluoroethyl azide in the presence of sodium ascorbate and copper sulfate. The F-18 labeled rapamycin analogue was purified by reversed phase HPLC and evaluated in an intracranial rat brain tumor model (9L glioma) by microPET.

Results: The rapamycin analogues were synthesized in good yields in three steps starting from rapamycin. The carbamate analogue shows higher stability than the carbonate analogue, and thus it was labeled with F-18 via using "click" chemistry. The one-pot labeling and click reaction failed to afford the desired product. However, the click reaction with distilled [F-18] 2-fluoroethyl azide proceeded smoothly to afford the desired product within 15 min. The total synthesis time is less than 120 min. The specific activity is ~ 300 mCi/μmol decay corrected to the end of synthesis. MicroPET imaging studies of the F-18 labeled rapamycin analogue in the intracranial 9L rat glioma model revealed a clear visualization of the tumor versus normal brain tissue whereas this tumor was not visible in the co-registered CT image.

Conclusions: A rapamycin analogue was labeled with F-18 at the C40 position via the "Click reaction" labeling method in good yield. Preliminary microPET studies in a rat glioma model indicate that the F-18 labeled rapamycin analogue is a potential radiotracer for imaging mTOR in vivo with PET.

Research Support: Funded by HL13851.





MicroPET Coregistration CT

MicroPET Imaging of Intracranial 9L Glioma in a Rat