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Click Chemistry and Radiochemistry: The First 10 Years

Jan-Philip Meyer[†,#], **Pierre Adumeau**[§,#], **Jason S. Lewis**[†,‡,||,*], and **Brian M. Zeglis**[†,§,||,⊥,*]

[†]Department of Radiology, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, New York 10065, United States

[‡]Molecular Pharmacology and Chemistry Program, Memorial Sloan Kettering Cancer Center, 1275 York Avenue, New York, New York 10065, United States

[§]Department of Chemistry, Hunter College of the City University of New York, 413 East 69th Street, New York, New York 10028, United States

[||]Department of Radiology, Weill Cornell Medical College, 520 East 70th Street, New York, New York 10065, United States

[⊥]Ph.D. Program in Chemistry, The Graduate Center of the City University of New York, 365 5th Avenue, New York, New York 10016, United States

Abstract

The advent of click chemistry has had a profound influence on almost all branches of chemical science. This is particularly true of radiochemistry and the synthesis of agents for positron emission tomography (PET), single photon emission computed tomography (SPECT), and targeted radiotherapy. The selectivity, ease, rapidity, and modularity of click ligations make them nearly ideally suited for the construction of radiotracers, a process that often involves working with biomolecules in aqueous conditions with inexorably decaying radioisotopes. In the following pages, our goal is to provide a broad overview of the first 10 years of research at the intersection of click chemistry and radiochemistry. The discussion will focus on four areas that we believe underscore the critical advantages provided by click chemistry: (i) the use of prosthetic groups for radiolabeling reactions, (ii) the creation of coordination scaffolds for radiometals, (iii) the site-specific radiolabeling of proteins and peptides, and (iv) the development of strategies for in vivo pretargeting. Particular emphasis will be placed on the four most prevalent click reactions—the Cu-catalyzed azide-alkyne cycloaddition (CuAAC), the strainpromoted azide-alkyne cycloaddition (SPAAC), the inverse electron demand Diels-Alder reaction (IEDDA), and the Staudinger ligation —although less well-known click ligations will be discussed as well. Ultimately, it is our hope that this review will not only serve to educate readers but will also act as a springboard, inspiring synthetic chemists and radiochemists alike to harness click chemistry in even more innovative and ambitious ways as we embark upon the second decade of this fruitful collaboration.

Graphical Abstract

[*] lewisj2@mskcc.org. Phone: 646-888-3039. Fax: 646-888-3038. bz102@hunter.cuny.edu. Phone: 212-896-0443. Fax: 212-772-5332.

[#]**Author Contributions**

J.-P.M. and P.A. contributed equally to the preparation of this manuscript

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INTRODUCTION

A decade and a half have passed since Kolb, Finn, and Sharpless published the landmark review that introduced the concept of click chemistry.[1] In the intervening years, the influence of click chemistry has grown by leaps and bounds. To wit, the number of publications with "click chemistry" in the title has grown from 6 in 2003 to 252 in 2009 to 2014 in 2015![2]

In the words of the original authors, the criteria for a click chemistry ligation are as demanding as they are straightforward: [1]

> "The reaction must be modular, wide in scope, give very high yields, generate only inoffensive byproducts that can be removed by non-chromatographic methods, and be stereospecific (but not necessarily enantioselective). The required process characteristics include simple reaction conditions (ideally, the process should be insensitive to oxygen and water), readily availably starting materials and reagents, the use of no solvent or a solvent that is benign (such as water) or easily removed, and simple product isolation."

A handful of reactions that satisfy (or, at the very least, come *close* to satisfying) these criteria have been uncovered, including nucleophilic ring opening reactions with epoxides, aziridines, and aziridinium ions; the formation of ureas, oximes, and hydrazones via nonaldol carbonyl chemistry; and oxidative and Michael additions to carbon–carbon double bonds.[3] Yet one particularly powerful reaction has emerged as the canonical click ligation and has proven remarkably useful in myriad applications: the copper-catalyzed [3 + 2] cycloaddition between an azide and a terminal alkyne (Figure 1A).[4,5] More recently, Bertozzi and others have pioneered a subset of click reactions that boast an additional boundary condition: bioorthogonality.[6–9] Bioorthogonal click ligations satisfy all of the requirements of standard click reactions but are also inert within biological systems. Not surprisingly, these reactions are hard to come by, yet a handful (most notably the Staudinger ligation, the strain-promoted azide–alkyne cycloaddition reaction, and the inverse electron demand Diels–Alder cycloaddition) have been developed and proven powerful in the hands of chemical biologists, biochemists, and biomedical scientists (Figure 1B–D).[7,10–16]

Click chemistry has had a paradigm-shifting influence on a wide range of chemical fields, from drug development[17,18] and polymer chemistry[19,20] to chemical biology[21] and nanoscience.[22] However, it is hard to imagine a field that has more to gain from harnessing click chemistry than radiochemistry. The principal reason for this lies in what makes radiochemistry unique: the inexorable physical decay of radioisotopes during synthesis. As a

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result, radiolabeling reactions—and especially radiolabeling reactions using short-lived isotopes such as ^{11}C ($t_{1/2} \approx 20$ min) and ^{68}Ga ($t_{1/2} \approx 68$ min)—must be rapid and efficient tomaximize yield as well as selective and clean to eliminate time-sapping purification steps. Furthermore, the widespread use of biomolecules as targeting vectors has also placed a premium on bioconjugation reactions that are both selective and unencumbered by water. Finally, the proliferation of an ever-growing list of prosthetic groups and radiometal chelators has made modularity a critical feature of radiosynthetic protocols as well. Remarkably, all of these traits can be found in click chemistry ligations.

In light of these benefits, it is somewhat surprising that the first publications describing radiopharmaceuticals synthesized using click chemistry came rather late: a 2006 work from Mindt et al. describing the use of click chemistry to create coordination scaffolds for ^{99m}Tc and a 2007 report from Wuest and co-workers detailing the use of the CuAAC reaction to create an ^{18}F-labeled variant of neurotensin(8–13).[23] Yet in the years since this somewhat belated start, work at the nexus of these two fields has expanded dramatically.[24–27] This growth means that an *exhaustive* review covering every instance in which click chemistry has been applied to nuclear imaging would almost certainly be an *exhausting* read. Instead, in the pages that follow, it is our goal to highlight the most interesting, exciting, and useful points of intersection between click chemistry and nuclear medicine. More specifically, we will focus on the use of click chemistry for (*i*) radiolabeling reactions with prosthetic groups, (ii) the creation of novel chelation architectures, (iii) site-specific bioconjugation, and (iv) in vivo pretargeting. Taken together, we believe that these four areas underscore how the rapidity, efficiency, selectivity, modularity, and bioorthogonality of click chemistry have empowered radiochemists to create innovative agents for imaging and therapy. Ultimately, we sincerely hope that this review not only informs the reader about research at the intersection of chemistry and radiochemistry but also inspires new and seasoned researchers alike to apply this remarkably useful chemical technique to the development radiopharmaceuticals.

RADIOLABELING WITH PROSTHETIC GROUPS

One of the first reported, and still most extensively employed, applications of click chemistry to radiochemistry lies in the use of “clickable” prosthetic groups for radiolabeling. The everincreasing use of imaging agents based on biomolecular vectors has put a premium on radiosynthesis strategies that are both mild and selective. Put simply, peptides, proteins, and antibodies should be radiolabeled under aqueous conditions at room temperature to ensure that their structural integrity is preserved, yet critically, many radiolabeling reactions require elevated temperatures, nonaqueous solvents, or (at the very least) pH conditions outside of the physiological norm. This is especially true for ^{18}F-radiofluorination reactions, which often require organic solvents and high temperatures.

Radiolabeled prosthetic groups provide an efficient way to circumvent these issues. Prosthetic groups are radiolabeled reactive small molecules that can be appended to biomolecules under benign conditions. Until recently, the vast majority of prosthetic groups have relied upon reactions with natural amino acids (most notably, couplings between *N*-hydroxysuccinimidyl (NHS) esters and lysines and Michael additions between maleimides

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and cysteines).[28–30] Yet prosthetic groups of this ilk present a number of problems. Most concerning is the complete loss of regiochemical control during the labeling of a peptide or protein containing more than one lysine or cysteine. This, of course, can only be remedied by yield-sapping separations or the addition of time-consuming protection and deprotection steps.[31] On top of this, both NHS esters and their isothiocyanate cousins are unstable under aqueous conditions, and maleimide–thiol linkages are prone to reversible substitution reactions in vivo.[32]

In response to these limitations, radiochemists have increasingly turned to "clickable" prosthetic groups. Not surprisingly, the canonical CuAAC ligation leads the pack. In this regard, the relative age of the reaction certainly plays a role. Yet another critical advantage of the CuAAC ligation is that its "footprint" — a 1,2,3-triazole ring — is unlikely to perturb the structure or activity of the vector: the heterocycle is both relatively small and a rigid stereoisomer of an amide linkage. At this junction, we would be remiss if we did not mention the CuAAC reaction's lesser-known cousin: the ruthenium-catalyzed azide–alkyne cycloaddition (RuAAC).[33] The RuAAC reaction produces 1,5-disubstituted 1,2,3-triazoles as opposed to the 1,4-disubstituted 1,2,3-triazoles created by the Cu-catalyzed cycloaddition. Even though it is regarded as a "click reaction", the RuAAC ligation requires organic solvents, elevated temperatures, and inert gas atmosphere. Furthermore, the 1,5-disubstituted 1,2,3-triazoles produced by the reaction are—unlike 1,4-disubstituted 1,2,3-triazoles—metabolically active and can be degraded via enzymatic N^3 oxidation to produce highly reactive and potentially toxic metabolites.[34] Given both of these issues, it is not surprising that, to the best of our knowledge, the RuAAC reaction has not been applied to the synthesis of radiopharmaceuticals.

Moving back to the topic at hand, an extensive body of work has emerged on the design, synthesis, and optimization of radiolabeled CuAAC-ready building blocks. Much, although not all, of this work has focused on ^{18}F.[35–38] Indeed, a variety of radiosynthetic methods have been employed to create azide- and alkyne-bearing ^{18}F-labeled prosthetic groups (Figure 2A).[37,39,40] These tools and the CuAAC reaction have been harnessed with great success in the radiolabeling of a wide variety of vectors, including phosphonium ions,[41] peptides,[42–50] oligonucleotides,[39,47] and proteins.[27,47] This application of the CuAAC reaction is not without its flaws, however. These stem primarily from the two reagents needed to facilitate the cycloaddition: Cu(I/II) cations and a sacrificial reductant. The latter, most often ascorbic acid, can inadvertently reduce particularly fragile peptides and proteins.[27] The Cu cations can be even more of a problem. Peptides and proteins (specifically serine, histidine, and arginine residues) can coordinate Cu^{2+} ions, resulting in structural and functional alterations to the peptide.[51] For example, Pretze et al. observed the accidental formation of Cu–peptide complexes following the CuAAC-mediated ligation of an ^{18}F-labeled, alkyne-containing prosthetic group to an azide-bearing SNEW peptide.[45] The coordination of the oxidative Cu(I) species can also lead to dramatic alterations to the chelating amino acid residues, as demonstrated very recently.[52] These issues are compounded even further for radiometal-containing constructs. In these cases, not only can the chelator capture the copper catalyst and prevent the reaction from happening, but residual Cu^{2+} ions can also outcompete the far less abundant radiometal cations for coordination by the chelator.[53] On top of these coordination-related concerns, the presence

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of Cu^+ can also increase the likelihood of undesired side reactions such as Glaser couplings or the formation of copper-acetylides.[45,54,55] Some of these issues can be ameliorated through the use of Cu^+-stabilizing chelators such as THPTA or *N*-heterocyclic carbene complexes of Cu^+; however, these reagents can create their own set of complications.[56–58]

In light of the limitations of the CuAAC ligation, researchers have turned to a handful of "second generation" click reactions that are both bioorthogonal and catalyst-free. The most obvious place to start is the strain-promoted azide–alkyne cycloaddition (SPAAC). The SPAAC reaction is an azide–alkyne cycloaddition in which ring strain built into a cyclic alkyne—often a dibenzocyclooctyne (DBCO)—drives the reaction and eliminates the need for a catalyst.[59,60] Campbell-Verduyn et al. were among the first to use this approach for radiochemistry, creating a series of ^{18}F-labeled bombesin derivatives via the reaction of a DBCO-modified peptide with an array of ^{18}F-bearing, azide-containing prosthetic groups.[61] Following a similar strategy, another laboratory modified a series of $\alpha_v\beta_3$-targeting RGD peptides with DBCO and radiolabeled them using an [^{18}F]fluoro–PEG$_4$–azide prosthetic group.[50,62] In a creative twist, the authors scavenged excess unlabeled peptide using an azide-grafted resin, allowing them to achieve specific activities of up to 62.5GBq/μmol. Critically, all of the ^{18}F-labeled peptides bore biological affinity comparable to their unlabeled cousins and were shown to be effective for the visualization $\alpha_v\beta_3$-expressing U87MG xenografts (Figure 3). Of course, radiolabeling via the SPAAC reaction goes both ways: several laboratories have created ^{18}F-labeled cyclooctynes for the radiofluorination of azide-modified small molecules, sugars, and peptides (Figure 2B).[63–65]

The SPAAC reaction has also been used for radioiodinations and radiometalations. Choi et al., for example, used a DBCO-bearing cRGD peptide and a prosthetic group composed of a PEG$_4$–azide moiety grafted to an ^{125}I-labeled pyridine to create an ^{125}I-labeled cRGD.[66] Evans et al. labeled an azide-modified DOTA with ^{68}Ga for the radiometalation of several DBCO-modified peptides.[53] Likewise, the Anderson group has conjugated DIBO-bearing copper chelators to an azide-modified cetuximab antibody and an azide-bearing somatostatin analogue.[67,68]

Despite its utility, the SPAAC ligation has one critical limitation: its dibenzocyclooctatriazole "footprint". The work of Hausner and co-workers provides a particularly useful cautionary example.[69] Here, the authors radiolabeled an azide-modified A20FMDV2-peptide using an ^{18}F-labeled variant of DBCO. While in vitro experiments confirmed that the ^{18}F-labeled peptide retained its affinity and specificity for $\alpha_v\beta_6$-expressing cells, in vivo imaging suggested that the bulky and hydrophobic benzocyclooctatriazole footprint introduced by the SPAAC ligation led to dramatic changes in the pharmacokinetics of the tracer and significantly impaired its uptake in $\alpha_v\beta_6$-expressing xenografts.

The inverse electron demand Diels–Alder (IEDDA) cycloaddition between tetrazine (Tz) and a dienophile, most commonly *trans*-cyclooctene (TCO) but also norbornene (NB), has also provided fertile ground for the development of prosthetic groups. Like the SPAAC ligation, the IEDDA reaction is bioorthogonal and proceeds without a catalyst. The principal advantage of the IEDDA ligation is its extraordinary speed (vide infra), which makes it

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particularly well suited for applications with short-lived radioisotopes. In 2010, the laboratories of Fox and Conti reported the first ^{18}F-labeled TCO (Figure 2C).[70] This prosthetic group was used for the rapid ($t < 5$ min) radiolabeling of a range of tetrazine-bearing peptides, including RGD and the GLP agonist Exendin.[71–73] The ^{18}F-labeled Exendin proved particularly promising, enabling the PET imaging of GLP-1R-positive insulinoma xenografts in mice. The same ^{18}F–TCO was also used to great effect by Weissleder and co-workers for labeling a Tz-bearing analog of the PARP1 inhibitor AZD2281. In this work, however, the authors added a creative wrinkle: removing unlabeled AZD2281–Tz using a TCO-coated magnetic resin.[74,75] Finally, a number of ^{18}F-labeled tetrazines have also been synthesized, but the in vivo use of radiopharmaceuticals created using these moieties has thus far remained somewhat sparing.[76,77]

The utility of the IEDDA reaction extends beyond radiofluorination. [53] To wit, a handful of radioiodinated tetrazine constructs have been successfully developed (Figure 2C). Albu et al., for example, synthesized an ^{125}I-labeled tetrazine and conjugated this building block to a TCO-modified anti-VEGFR2 antibody.[78] Interestingly, in vivo studies using this tracer revealed an additional benefit of this approach: the ^{125}I-labeled antibody proved to be more than 10-fold more stable to deiodination over 48 h compared to traditionally radioiodinated analogs. More recently, Choi et al. used a similar strategy for the radiolabeleling of both a cRGD peptide and human serum albumin (HSA).[79] The ^{125}I-labeled HSA displayed impressive in vivo behavior, with a deiodination rate reduced by 50-fold compared to constructs created via traditional radioiodination. In 2011, Zeglis et al. employed the IEDDA reaction to create a modular strategy for the bioconjugation of a trastuzumab–TCO immunoconjugate with Tz–desferrioxamine (for $^{89}Zr^{4+}$) and Tz–DOTA (for $^{64}Cu^{2+}$).[80] More recently, Kumar and co-workers harnessed the IEDDA reaction to circumvent the incompatibility of antibodies with the high temperatures required to radiolabel the CB-TE2A-1C chelator with ^{64}Cu.[81] To this end, the authors modified the chelator with a norbornene moiety and grafted tetrazine onto an anti-PSMA antibody (YPSMA). After radiolabeling of the chelator-NB building block with ^{64}Cu at 85 °C, the ^{64}Cu–CBTE2A1C-NB synthon was attached to YPSMA–Tz under mild conditions, and the ^{64}Cu-labeled radioimmunoconjugate was successfully deployed for the PET imaging of PSMA-expressing tumors in a murine model of prostate cancer.

Although the rapidity of the IEDDA reaction provides a marked improvement over the sluggish SPAAC ligation, it fails to solve one of the latter's major issues: a bulky, hydrophobic footprint. As we have discussed, the SPAAC reaction leaves a benzocyclooctatriazole moiety in its wake. The IEDDA ligation creates an equally large footprint: a bicyclic [6.4.0] ring system. Both structures have the potential to interfere with the biological activity and pharmacokinetics of vectors, particularly small molecules and short peptides. The traceless version of the Staudinger ligation offers an exciting alternative (Figure 4A). This ligation relies on an initial reaction between a phosphine-based moiety and an azide followed by a rearrangement that produces a simple amide linkage. Along these lines, the radiolabeling of peptides with ^{18}F has been achieved via the reaction between (diphenylphosphanyl)methanethiol thioester-bearing peptides and an ^{18}F-labeled azide as well as that between a radiolabeled 2-(diphenylphosphanyl)phenol ester with an azide-bearing peptide (Figure 2D).[82–84] Unfortunately, however, the traceless Staudinger ligation

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requires high temperatures (90–130 °C) to achieve speeds that are compatible with short-lived isotopes. This undoubtedly limits its utility with fragile small molecules, peptides, and proteins; however, we are optimistic about the potential applications of this elegant transformation with longer-lived isotopes.

Finally, a handful of other, less-well-known click ligations have made sparing yet interesting appearances in the literature of prosthetic groups. In 2012, Zlatopolskiy et al. reported the formation of a reactive nitrone from ^{18}F-fluorobenzaldehyde and phenylhydroxylamine.[85] The authors showed that this ^{18}F-labeled nitrone could undergo a [3 + 2] cycloaddition with a maleimide, resulting in quantitative conversion in less than 15 min at 80 °C (Figure 4B). It must be said, however, these reaction conditions leave much to be desired when it comes to labeling biomolecules. Later the same year, the same group probed the potential of cycloaddition reactions between nitriloxides and dipolarophiles (Figure 4C).[86] An ^{18}F-labeled nitriloxide was synthesized from ^{18}F-*p*-fluorobenzaldehyde and reacted with a series of dipolarophiles, producing quantitative conversions in <10 min at 40 °C. However, these reactions were performed in alcohol, and no data was presented regarding the feasibility of this transformation under aqueous conditions. Recently, other groups have harnessed the reactivity of 2-cyanobenzothiazoles toward 1,2-aminothiols to radiolabel peptides and proteins containing *N*-terminal cysteines (Figure 4D).[87,88] To this end, ^{18}F-labeled 2-cyanobenzothiazoles were synthesized and appended to RGD and diRGD peptides bearing *N*-terminal cysteines as well as a genetically engineered variant of luciferase with a cysteine at the N-terminus. Lastly, just this year, Chiotellis et al. have explored phenyloxadiazole methylsulfones (PODS) as more stable alternatives to maleimides for conjugations with thiols (Figure 4E).[89] In this work, an ^{18}F-labeled PODS was used to radiolabel both a cysteine-bearing peptide and a cysteine-modified affibody, and the resulting constructs were used to HER2-positive tumors in a mouse model of breast cancer.

CREATING COORDINATION SCAFFOLDS

The use of click chemistry to create radiometal chelation architectures provides one of the best examples of the unique modularity conferred by this synthetic approach.[90,91] Easily the best known of these methods, dubbed "click-to-chelate" by its inventors, was introduced in 2006 by Mindt et al. (Figure 5).[92–94] This strategy employs the Cu^{I}-catalyzed azide–alkyne cycloaddition (CuAAC) reaction to attach small molecule "pro-chelators" to peptides and small molecules. However, the 1,2,3-triazole produced by the click ligation becomes far more than just a simple link between the subunits of the construct. Indeed, the heterocycle forms an integral part of a tripodal coordination scaffold capable of the rapid chelation of $[M(CO)_3]^+$ synthons, in which M can be the γ-emitting radiometal ^{99m}Tc ($t_{1/2}$ = 6.01 h) or the β-emitting radiometal ^{188}Re ($t_{1/2}$ = 16.98 h). In this way, "click-to-chelate" facilitates the creation of a chelator *and* its subsequent radiometalation in a rapid, robust, and reproducible one-pot reaction. This is particularly important given the mercurial coordination chemistry of ^{99m}Tc.

In their initial proof-of-concept report, the authors created seven different tripodal scaffolds —including N_3, N_2S, and N_2O ligand architectures—using a series of azide-modified small molecules. Subsequent labeling with $M(CO_3)$ [M = Re, ^{99m}Tc] synthons resulted in a series

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of highly stable, low-spin d^6-complexes despite differences in the size, molecular charge, and hydrophilicity of the prochelator.[92–95] The creation of a ^{99m}Tc-labeled variant of folate using "click-to-chelate" provides an excellent example of the approach (Figure 6). The 1,2,3-triazole ring formed in the first phase of the reaction between the azide-bearing folate construct (**1**) and the alkyne-modified amino acid (**2**) not only connects the pro-chelator to the folate vector but *also* serves as an essential part of the N_2O coordination scaffold for the $[^{99m}Tc(CO)_3]^+$ moiety. The incubation of the chelator-bearing construct with $[^{99m}Tc(CO)_3(H_2O)_3]^+$ reproducibly yields ^{99m}Tc-labeled folate (**3**) in high yield and specific activity.[92]

In subsequent work, this technique was applied to peptides as well as an array of other biologically active small molecules such as sugars, nucleosides, and steroids.[96–100] Fernandez et al., for example, developed a ^{99m}Tc-labeled glucose derivative as an imaging probe for glucose metabolism.[97] Similarly, Struthers et al. developed an elegant one-pot "click-to-chelate" synthesis of a ^{99m}Tc-labeled thymidine analogue as a SPECT surrogate for the clinically successful proliferation marker ^{18}F–FLT.[98] Taken together, this work clearly demonstrates that ^{99m}Tc-labeled tracers created using the "click-to-chelate" methodology demonstrate in vivo behavior that is comparable, and in some cases superior, to the current "gold standard" chelators for $[^{99m}Tc\text{-}(CO)_3]^+$: $N\tau$-derivatized histidine and $N\alpha$-acetylated histidine. Indeed, studies using ^{99m}Tc-labeled folate revealed that the click-to-chelate approach furnished compounds in purities and radiochemical yields equal to those achieved using traditional radiolabeling techniques. Furthermore, in this work, the click-to-chelate approach did not alter biodistribution patterns or pharmacodynamic parameters such as receptor affinities and selectivities. Finally, the superiority of the click-to-chelate methodology becomes most obvious in the context of synthetically challenging molecules. In the case of the azide-modified folate construct, for example, the differences in synthetic effort and yield are striking: "click-to-chelate" furnished an ^{99m}Tc-labeled tracer in 80% overall yield in 8 steps, whereas 10 steps were required to muster approximately 1% yield with a histidine-based chelator.[92]

From a chemical standpoint, it is important to note that the inherent asymmetry of the CuAAC reaction means that two different 1,2,3-triazoles can be formed when linking the vector and the chelator (Figure 7).[93,95] In the first, the "regular click ligand", the pro-chelator bears the alkyne moiety while the vector contains the azide group, and the N^3 atom of the triazole participates in the coordination of ^{99m}Tc. In the second, the "inverse click ligand", the pro-chelator boasts the azide moiety while the vector wields the alkyne group, and the N^2 atom of the triazole participates in the coordination of ^{99m}Tc. Somewhat surprisingly, the two different chelation environments display quite different behavior when radiolabeled with $[^{99m}Tc(CO)_3]^+$ and $[^{188}Re(CO)_3]^+$, with the "inverse click ligand" offering significantly lower labeling efficiency and decreased in vivo stability.[93] Although a concrete explanation for this phenomenon remains elusive, the most likely hypothesis points to the decreased electron density in the N^2 position compared to the N^3 site.

Before moving on, it is worth noting that a handful of other groups have also used click chemistry in the synthesis of radiometal chelators. Bailey et al., for example, used the CuAAC reaction in the synthesis H_4azapa: a carboxypyridine-based chelator for $^{111}In^{3+}$

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and $^{177}Lu^{3+}$ (Figure 8A).[91] In addition, Bottorff et al. have developed a synthetic strategy to generate isoxazole ligands via click chemistry (Figure 8B).[101] Yet in the end, it is undeniable that the "click-to-chelate" methodology represents the gold standard in this area. Indeed, this approach not only provides a cardinal example of the modularity and flexibility provided by click chemistry but also stands as one of the most useful and innovative developments in ^{99m}Tc chemistry of the past decade.[2–4]

SITE-SPECIFIC BIOCONJUGATION

The selectivity and bioorthogonality of click chemistry have also been leveraged for the site-specific modification of proteins and antibodies. This process has become ubiquitous in the synthesis of biomolecular therapeutics such as antibody-drug conjugates, and it is increasingly important in the creation of radiolabeled probes as well. Until recently, the overwhelming majority of bioconjugation methods were predicated on ligations between reactive bifunctional probes—e.g., *N*-hydroxysuccinimide-bearing chelators or maleimide-modified toxins—and amino acids within the biomolecule, most often lysines and cysteines. While these methods are undeniably simple, they are far from precise. Control over the location and frequency of these ligations is impossible because proteins have multiple copies of these amino acids distributed throughout their structures. As a result, these bioconjugation strategies produce constructs that are both heterogeneous and poorly defined. Furthermore, random conjugation strategies can decrease the reactivity of constructs if the cargo is inadvertently appended to the target-binding domains of the biomolecule.

In response to these issues, significant effort has been dedicated to the creation of strategies for the site-specific bioconjugation of proteins and antibodies. A wide variety of methods have been developed, including variants predicated on the selective reduction of disulfide bridges and the oxidative manipulation of the heavy chain glycans. Yet regardless of the exact strategy, a wealth of preclinical data makes the bottom line clear: site-specifically labeled proteins and antibodies are more homogeneous, better defined, and exhibit superior in vivo behavior compared to constructs synthesized using traditional, random bioconjugation techniques.[102–105] A handful of the most promising site-specific bioconjugation strategies combine the selectivity of enzymatic reactions with the modularity of chemical ligations. Generally speaking, these chemoenzymatic strategies have two steps. In the first, an enzyme is used to site-specifically incorporate a substrate bearing a reactive handle into the biomolecule. Then, in the second, a cargo bearing a complementary reactive handle is appended to its partner in the biomolecule. In this context, the selectivity and bioorthogonality of click chemistry are particularly valuable, as the two handles must *only* react with each other and not the enzyme or biomolecule.

A recently developed strategy for the site-specific modification of the heavy chain glycans (the biantennary sugar chains attached to the C_H2 domains of the F_C region of antibodies) provides an excellent example of a chemoenzymatic approach that employs the SPAAC ligation. Inspired by the work of Hsieh-Wilson and Qasba, this methodology employs two enzymes and has three steps: (i) the removal of the terminal sugars of the heavy chain glycans using β-(1,4)-galactosidase; (ii) the incorporation of azide-modified galactose residues (GalNAz) into the sugar chains with a promiscuous galactosyltransferase [GalT-

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(Y289L)]; and (iii) the attachment of dibenzocyclooctyne (DIBO)-bearing cargoes to the azide-presenting sugars (Figure 9).[106–108] Ultimately, this approach has the potential to yield highly homogeneous and well-defined immunoconjugates carrying up to four cargo molecules per antibody. In their initial report, Zeglis et al. used a DIBO-modified desferrioxamine (DFO) to create a site-specifically modified, ^{89}Zr-labeled radioimmunoconjugate based on the PSMA-targeting antibody J591. In subsequent work, the authors demonstrated the modularity and flexibility of this approach through the development of a series of immunoconjugates for the PET, NIRF, and multimodal PET/NIRF imaging of colorectal and pancreatic cancer. Importantly, in all three cases, the in vivo performance of the site-specifically modified imaging agents was equivalent, and in some respects superior, to that of analogous constructs synthesized using traditional techniques.[109,110] More recently, Geel et al. reported an interesting variation on this theme.[111] In this work, EndoS, an enzyme that trims each glycans chain down to its innermost residues, is used instead of β-(1,4)-galactosidase. This change ultimately produces an immunoconjugate with two azides per antibody after treatment with GalT(Y289L) and GalNAz. To date, this strategy has only been used in conjunction with DIBOmodified chemotherapeutics, but the modularity of the SPAAC ligation could easily facilitate the adaptation of this approach to the synthesis of radiopharmaceuticals.

Shifting gears to another family of enzymes, transglutaminases catalyze the formation of isopeptide bonds between the acyl functionality of glutamine residues and primary amines. While antibodies certainly contain multiple glutamine residues, transglutaminases have been found to react exclusively with the Q295 glutamines within the C_H2 domain of deglycosylated or aglycosylated IgGs. This unique reactivity has led a number of laboratories (most notably, that of Roger Schibli at ETH Zurich) to harness these enzymes for the site-specific modification of antibodies. One-step and two-step approaches have been developed (Figure 10). In the former, transglutaminase is used to directly append cadaverine-modified cargoes to the antibody. This method was used by Jeger et al. to site-specifically append two DFO chelators to a deglycosylated variant of the L1CAM-targeting antibody chCE7 for radiometalation with ^{64}Cu, ^{67}Ga and ^{89}Zr.[112] In an interesting twist, this group used transglutaminase to modify amutant version of the chCE7 antibody that contained two additional glutamines in place of the N297 residues, thereby creating an immunoconjugate with four DFO/mAb. More germane to the topic at hand, transglutaminase has also been used to modify proteins with azide- and cyclooctyne-modified cadeverines that can then be reacted via the SPAAC ligation with DIBO- or azide-bearing cargoes, respectively.[113,114] In a very recent proof-of-concept study, Puthenveetil et al. have used this strategy to label a model antibody with both Cy5.5 and BODIPY fluorophores. [115] While this approach has not yet been applied to radioimmunoconjugates, it could easily be adapted to create a modular route for the conjugation of radiometal chelators.

The last bioconjugation technique that we will discuss relies not on post-translational modifications but, rather, on harnessing the cell's translational machinery itself. The expansion of the genetic code to enable the incorporation of unnatural and noncanonical amino acids (uAAs and ncAAs, respectively) into proteins has quickly become a vital component of the molecular biologist's toolkit. The union of this technology and click chemistry has proven particularly powerful. *p*-Azido-L-phenylalanine (pAzF) is one of the

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most commonly used uAAs, and residues bearing trans-cyclooctene, tetrazine, cyclooctyne, and norbornene groups have been incorporated into proteins as well (Figure 11A).[116–119] pAzF and the SPAAC ligation have been used to create both antibody-drug conjugates and immunoglobulins modified with fluorophores. Yet until very recently, only one instance of the use of this technology to create a radiolabeled compound had been reported. In this work, Wållberg et al. developed affibodies containing selenocysteine, a natural ncAA, and exploited the unique reactivity of this residue with maleimides and iodomethane to site-specifically radiolabel the vectors with both ^{68}Ga and ^{11}C (Figure 11B).[120] Finally, just prior to the submission of this review, Wu et al. reported the first example of a radioimmunoconjugate created using an uAA. In this case, the authors incorporated an azide-bearing lysine residue (Az–K) into the heavy chain of the anti-CD20 antibody Rituximab and subsequently used the SPAAC ligation to attach a DIBO-bearing DOTA to the azide-containing immunoconjugate. [121] The site-specifically modified antibody showed in vitro and in vivo behavior comparable with an analogous construct synthesized using traditional methods. Ultimately, we are confident that more laboratories will use genetic engineering and click chemistry for the synthesis of radiopharmaceuticals as the technology underlying the former becomes more widely accessible and less technically demanding.

IN VIVO PRETARGETING

The next area of discussion, in vivo pretargeting, places the bioorthogonality and speed of click chemistry on center stage. To provide some background, in vivo pretargeting strategies have been developed in direct response to a core limitation of radiolabeled antibodies. Immunoglobulins are extraordinarily promising vectors for nuclear medicine due to their exquisite affinity and selectivity for their molecular targets. However, because antibodies have multiday biological half-lives, they must necessarily be labeled with isotopes with multiday physical half-lives such as ^{124}I ($t_{1/2} \approx 4.2$ days) or ^{89}Zr ($t_{1/2} \approx 3.2$ days) to create effective radioimmunoconjugates.[122] Unfortunately, however, this combination of lengthy circulation times and slow radioactive decay can create prohibitively high radiation dose rates to healthy organs.

Pretargeting methodologies seek to circumvent this problem by decoupling the antibody from the radioisotope and injecting the two components separately, in essence synthesizing the radioimmunoconjugate at the target tissue itself. A pair of components form the core of any pretargeting strategy: a small molecule radiolabeled hapten and an antibody capable of binding both an antigen and said hapten. The antibody is injected first and is given a number of days to accumulate at the target site and clear from the blood. After this interval, the radiolabeled hapten is administered. Because it is a small molecule, the hapten travels through the bloodstream quickly, either combining with its immunoconjugate partner or clearing from the body. This approach offers two distinct advantages over traditional immunoconjugates.[123,124] First, the rapid clearance of any unreacted radioligand limits the activity concentrations *in* and radiation dose *to* healthy organs. Second, and more importantly, this strategy facilitates the use of short-lived radioisotopes—e.g., ^{64}Cu ($t_{1/2}$ = 12.7 h), ^{18}F ($t_{1/2}$ = 109 min), and ^{68}Ga ($t_{1/2}$ = 68 min)—that would normally be incompatible with antibody-based vectors. The latter trait not only produces a dosimetric benefit but also has the potential to accelerate imaging workflows. A variety of approaches

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to in vivo pretargeting have been attempted, including the use of streptavidin-modified antibodies and biotin-based radioligands,[125–128] genetically engineered bispecific antibodies capable of binding radiometal chelate complexes,[124,129–131] and antibodies and radioligands conjugated to complementary oligonucleotide strands.[132–134] While all of these strategies have proven promising in the preclinical arena, their ultimate clinical implementation has been derailed somewhat by intrinsic issues such as the immunogenicity of streptavidin-modified bioconjugates.

In the end, it is not surprising that bioorthogonal click chemistry has attracted attention as a tool for in vivo pretargeting. Indeed, the selectivity, speed, and, above all, bioorthogonality of these reactions make them seem almost perfectly suited to the task. Attempts at in vivo pretargeting have been made using a variety of bioorthogonal click ligations. In 2011, for instance, Vugts et al. described the development of a pretargeting approach based on the Staudinger ligation between an azide-bearing antibody and phosphine-containing small molecule probes labeled with ^{68}Ga, ^{89}Zr, ^{177}Lu, and ^{123}I.[135] In this work, the authors reported that the Staudinger ligation product could not be observed in vivo, leading to the conclusion that in vivo pretargeting with the Staudinger ligation is not possible due to the reaction's sluggish kinetics, the inherent instability of the phosphine radioligands, or a combination thereof. The SPAAC reaction also has a history of in vivo use dating back to the groundbreaking work in zebrafish performed by Carolyn Bertozzi's laboratory.[136,137] Van den Bosch et al. investigated the feasibility of the SPAAC reaction for in vivo pretargeting using an ^{125}I- and azide-bearing Rituximab immunoconjugate (^{125}I–Rtx–N_3) and ^{177}Lu-labeled cyclooctyne radioligands.[138] Unfortunately, however, dual-isotope biodistribution experiments revealed disappointing activity concentrations of ^{177}Lu in the target tissue, suggesting that the somewhat slow reaction kinetics of the SPAAC ligation limit its application in vivo. Intriguingly, however, this position has been countered by recent work on the use of nanoparticles for SPAAC-mediated in vivo pretargeting (vide infra).[139]

Over the past 5 years, one of the newest additions of the click chemistry toolbox, the inverse electron demand Diels–Alder (IEDDA) reaction, has proven particularly well suited for pretargeting. [10,24,140,141] Like the Staudinger and SPAAC ligations, the IEDDA reaction is catalyst-free, clean, selective, and bioorthogonal. From a pretargeting perspective, the true advantage of the IEDDA cycloaddition is speed. The first-order rate constants for reactions between 1,2,4,5-tetrazines (Tz) and *trans*-cyclooctenes (TCO) hover in the range of 10^4–$10^5 M^{-1} s^{-1}$. In contrast, the rate constants for the Staudinger and SPAAC ligations are orders of magnitude slower: approximately 0.002 and $0.07 M^{-1} s^{-1}$, respectively.[142,143] Surely, this added speed could play a pivotal roll in the feasibility of click chemistry in the in vivo environment.

The vast majority of IEDDA-based pretargeting approaches employ a TCO-labeled antibody and a tetrazine-based radioligand (Figure 12A). Rossin et al. were the first to report a pretargeting strategy based on the ligation. The authors successfully employed a TCO-labeled immunoconjugate (CC49-TCO) and an ^{111}In-labeled dipyridyltetrazine radioligand to facilitate the pretargeted SPECT imaging of TAG72-expressing colorectal cancer xenografts (Figure 12B).[144] Since this initial report, this group has continued to be a pioneer in the field, producing investigations on alternative *trans*-cyclooctene moieties,[145] tetrazine-

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bearing clearing agents,[146] and pretargeting with antibody fragments and affibodies.[147,148] In parallel to the Dutch work, Zeglis et al. have developed and optimized a ^{64}Cu-based pretargeting approach for the PET imaging of colorectal carcinoma that produces images with excellent quality and contrast at only a fraction of the radiation dose to healthy tissue created by traditional radioimmunoconjugates (Figure 12C).[108,149,150] Even more recently, Houghton,[151] Meyer,[152] and colleagues used 5B1-TCO, a CA19.9-targeting immunoconjugate, as well as ^{64}Cu- and ^{18}F-labeled tetrazines to demonstrate the feasibility of the pretargeted imaging of an antigen that is both shed and internalized. Other laboratories have contributed to the advent of IEDDA-based pretargeting as well, developing ^{11}C-, ^{68}Ga-, and ^{99m}Tc-labeled tetrazine radioligands[153–156] as well as ^{18}F- and tetrazine-labeled nanoparticles (Figure 13).[157] Finally, Rossin et al. very recently expanded the scope of this methodology even further by harnessing the newly developed IEDDA pyridazine elimination reaction to trigger the selective in vivo cleavage of tumor-bound antibody-drug conjugates (ADCs).[158] This innovative "click-to-release" approach has the potential to add a powerful new tool to the arsenal of ADC therapies.

Of course, the extremely promising preclinical results for IEDDA-based pretargeting beg the question: *will it work in humans?* Not surprisingly, some legitimate concerns have been leveled on this front (most notably, the dramatic increase in blood volume upon moving from mice to humans). Although no trials have yet been reported, a number of laboratories are currently working toward bringing these exciting technologies to the clinic, and the field is collectively hopeful that the speed, selectivity, and bioorthogonality of the IEDDA reaction will be up to the task.

EMERGING APPLICATIONS

Of course, the four areas we have discussed so far are not the *only* points of intersection between click chemistry and radiopharmaceutical science. Indeed, the past few years have played witness to the increasingly innovative use of click chemistry in the synthesis of radiopharmaceuticals. For example, click chemistry has played an important role in the advent of nanoparticles as vectors for molecular imaging.[159] In this regard, the modularity, selectivity, and chemically mild nature of click chemistry have proven especially useful. Along these lines, Zeng et al. used the strain-promoted alkyne–azide cycloaddition (SPAAC) to modify the surface of azide-bearing shell-cross-linked nanoparticles with ~500 ^{64}Cu–DOTA moieties per particle, ultimately achieving specific activities of up to 975 Ci/μmol.[160] Similarly, Lee et al. reported the synthesis and in vivo evaluation of ^{64}Cu-labeled chitosan nanoparticles constructed via the SPAAC reaction between ^{64}Cu–DOTA–DBCO prosthetic groups and azide-modified chitosan NPs (Figure 14).[161] Click chemistry has also been used to enable pretargeted imaging using nanoparticulate vectors. For instance, Lee et al. have developed an SPAAC-based pretargeting strategy based on mesoporous silica nanoparticles (MSN).[139] In this work, DBCO-modified mesoporous silica nanoparticles were injected into mice bearing U87MG tumors. A total of 24 h later, the same mice were injected with an ^{18}F-labeled, azide-functionalized radioligand. This strategy successfully enabled the noninvasive visualization of tumor tissue (up to 1.4% ID/g at 2 h post-injection) with promising tumor-to-background contrast, a truly remarkable result given the somewhat sluggish kinetics of the SPAAC ligation.

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Shifting gears somewhat, a number of recent reports have emerged in which click chemistry (and the triazole-forming reactions, in particular) has been harnessed to enhance the in vivo stability of peptide-based imaging agents.[162,163] 1,2,3-Triazoles possess two critical physicochemical similarities to the amide bonds that normally link amino acids: planarity and the ability to act as hydrogen bond acceptors.[163,164] Yet unlike amide bonds, triazoles are resistant to protease or peptidase metabolism in vivo. As a result, radiolabeled peptides in which triazole linkages replace some of the traditional amide bonds offer enticing prospects for nuclear imaging. Just last year, Valverde et al.[165] demonstrated the potential of this approach by synthesizing a series of triazole-containing, ^{177}Lu-labeled peptidomimetic radiotracers that target the gastrin-releasing peptide receptor (GRPr) (Figure 15A). In vivo studies revealed a significant increase in the in vivo stability of the triazole-containing compounds, a result that is likely responsible for the observation that the amide-to-triazole substituted derivatives exhibited an approximately 2-fold increase in tumor uptake. Very recently, the same group applied this methodology to bombesin-derived peptides, aiming to create tracers with improved tumor-to-kidney activity concentration ratios.[166] This work demonstrated that the triazole-containing constructs boast improved (~5-fold) serum stability without sacrificing binding affinity. In vivo biodistribution experiments in mice bearing antigen-expressing PC3 and AR42J xenografts further revealed that the backbone-modified constructs possessed superior in vivo properties (Figure 15B).[166]

Finally, a very recent paper from Thurber and co-workers (although admittedly one that focuses on fluorescence rather than nuclear imaging agents) describes a complementary way to use click chemistry to enhance the in vivo stability of peptides.[167,168] In this work, the authors use an innovative "double-click" approach that simultaneously enables the conjugation of a fluorophore to the peptide and creates an internal cross-link that stabilizes the α-helical structure of the peptide.[168] The authors were able to demonstrate that the "double-clicked" peptides exhibited improved metabolic stability compared to analogous constructs. Furthermore, in vivo studies in C57BL/6 mice revealed that the click-stabilized peptides possessed increased protease resistance and significantly enhanced bioavailability.

CONCLUSIONS AND FUTURE DIRECTIONS

In the preceding pages, we have highlighted what we believe to be the most important and innovative advances from the first 10 years of research at the intersection of click chemistry and radiopharmaceutical chemistry. The bulk of this work has been concentrated in four areas: (i) the radiolabeling of molecules using prosthetic groups, (ii) the assembly of radiometal coordination architectures, (iii) the site-specific modification of immunoglobulins, and (iv) the creation of in vivo pretargeting strategies. Of course, in each, the details differ. Far more important, though, is that in every case, the refrain remains the same: the intrinsic selectivity and modularity of click chemistry can—and very often do—dramatically improve the construction of radiopharmaceuticals.

However, despite a wealth of preclinical data, click-based radiopharmaceuticals seem to have stalled just short of the clinic. This is somewhat surprising given the manifold advantages click chemistry offers for the construction of radiolabeled agents. For example, click chemistry could dramatically streamline the logistics of clinical probe production, as a

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single “clickable” prosthetic group could be used in the production of multiple radiotracers. Yet still, only a small handful of click-based radiopharmaceuticals have been the subject of clinical trials, most notably the $\alpha_v\beta_3$-targeting peptide ^{18}F–RGD–K5 and the somatostatin receptor targeting peptide ^{18}F–fluoroethyltriazole–Tyr^3–octreotate (Figure 16).[169–171] These two agents certainly represent a great start, but they must be considered just that: a start. We believe that as we move into the field’s second decade, clinical translation must be *the* top priority. To be sure, pushing back the frontiers of basic preclinical radiochemistry will remain vital. Yet ultimately, only the clinical translation of a variety of click-based probes will demonstrate once and for all the utility of this chemical technology in nuclear medicine.

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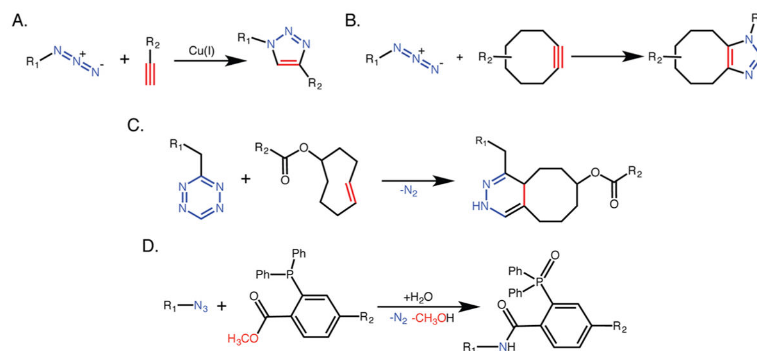

Figure 1.

Schematics of the (A) Cu-catalyzed azide–alkyne cycloaddition reaction, (B) the strain-promoted azide–alkyne cycloaddition, (C) the inverse electron demand Diels–Alder cycloaddition, and (D) the Staudinger ligation.

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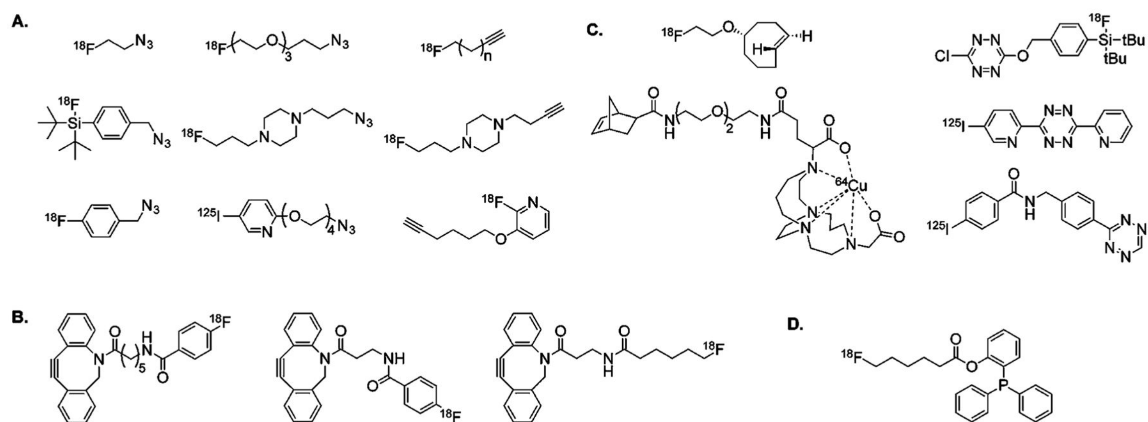

Figure 2.
An assortment of radiolabeled prosthetic groups used for the synthesis of radiopharmaceuticals via the (A) copper-catalyzed azide–alkyne cycloaddition, (B) strain-promoted azide–alkyne cycloaddition, (C) inverse electron-demand Diels–Alder cycloaddition, and (D) traceless Staudinger ligation.

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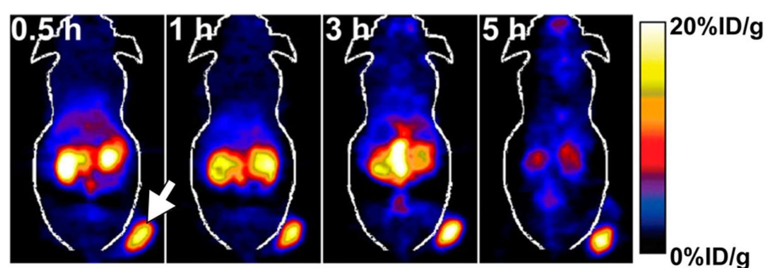

Figure 3.
Coronal PET images of a NOD/SCID mouse bearing a GLP-1R-positive insulinoma xenograft (white arrow) collected 0.5, 1, 3, and 5 h after the injection of an ^{18}F-labeled Exendin-4 radiotracer synthesized using a "clickable" prosthetic group. Adapted and reprinted with permission from Wu et al., copyright 2013 by the Society of Nuclear Medicine and Molecular Imaging, Inc.

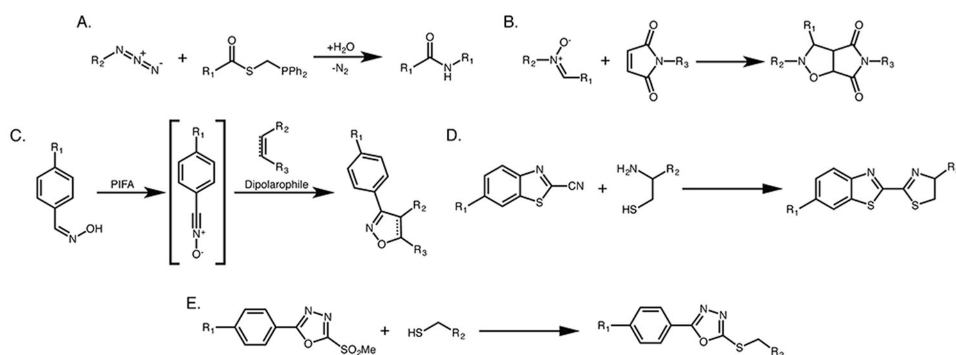

Figure 4.

Schematics of an assortment of click chemistry ligations (beyond those depicted in Figure 1) used for prosthetic group radiolabelings: (A) traceless Staudinger ligation, (B) nitrone–alkene cycloaddition, (C) nitrile–oxide cycloaddition, (D) 1,2-aminothiol–cyanobenzothiazole condensation, and (E) phenyloxadiazole methylsulfone–thiol conjugation.

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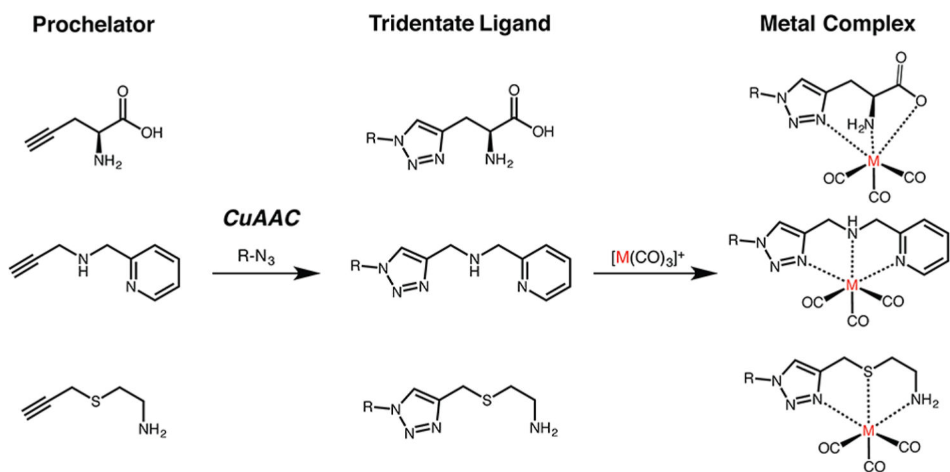

Figure 5.
"Click-to-chelate" approach: a variety of prochelators exhibiting electron-donating groups undergo the Cu^I-catalyzed azide–alkyne cycloaddition with an azide to from a tridentate ligand that can coordinate an organometallic $[M(CO)_3]^+$ synthon.

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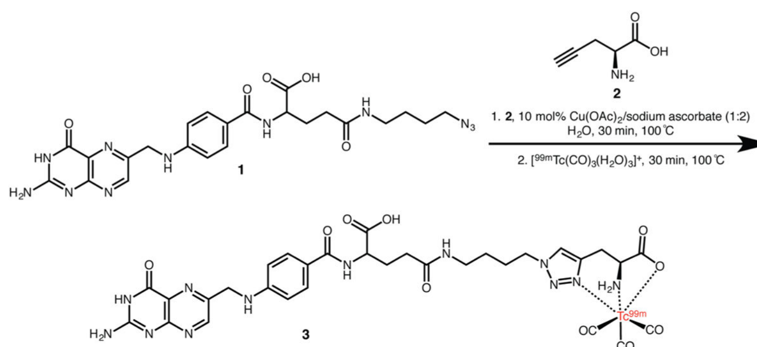

Figure 6.

Advantages of the one-pot “click-to-chelate” approach are particularly apparent in the context of synthetically challenging probes such as this ^{99m}Tc-labeled folate radiopharmaceutical (**3**).

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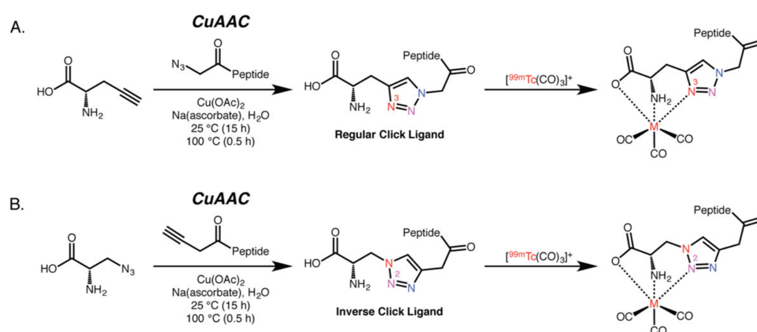

Figure 7.

The asymmetry of the CuAAC reaction creates two different coordination scaffolds depending on whether the prochelator contains the alkyne or azide functionality. The "regular click ligand" (A) is a more effective chelator for $[^{99m}Tc(CO)_3]^+$ and $[^{188}Re(CO)_3]^+$ than the "inverse click ligand" (B).

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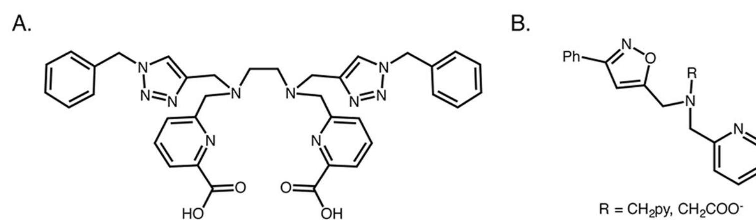

Figure 8.
Structures of the acyclic H_2azapa (A) and isoxazole (B) chelators for diagnostic and therapeutic radiometals such as ^{67}Ga, ^{64}Cu, ^{111}In (A), and ^{99m}Tc (B), respectively. The isoxazole ligand (B) was synthesized via click chemistry using the Cu-free 1,3-dipolar cycloaddition between an alkyne and an oxime.

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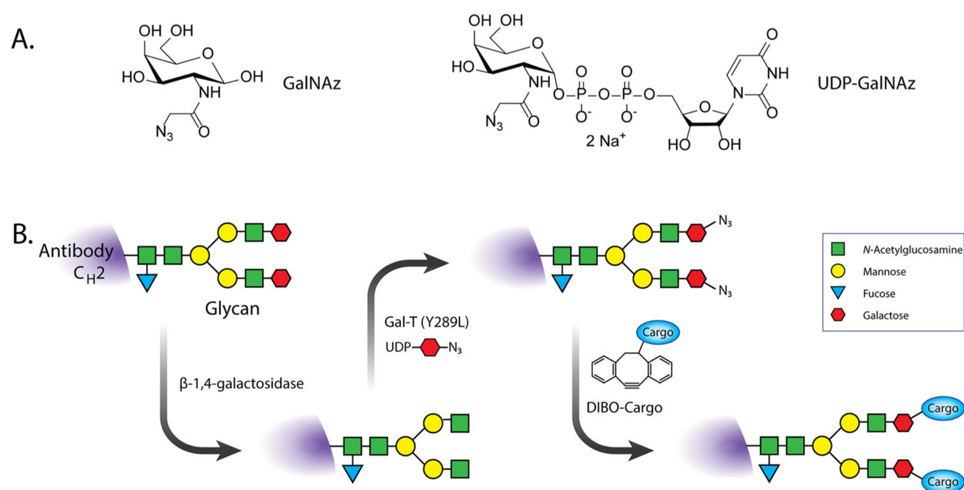

Figure 9.

(A) The structures of *N*-azidoacetylgalactosamine (GalNAz; left) and the enzyme substrate UDP–GalNAz (right); (B) schematic of the methodology for the chemoenzymatic incorporation of azide moieties in the heavy-chain glycans as well as the subsequent SPAAC-mediated grafting of cargo molecules.

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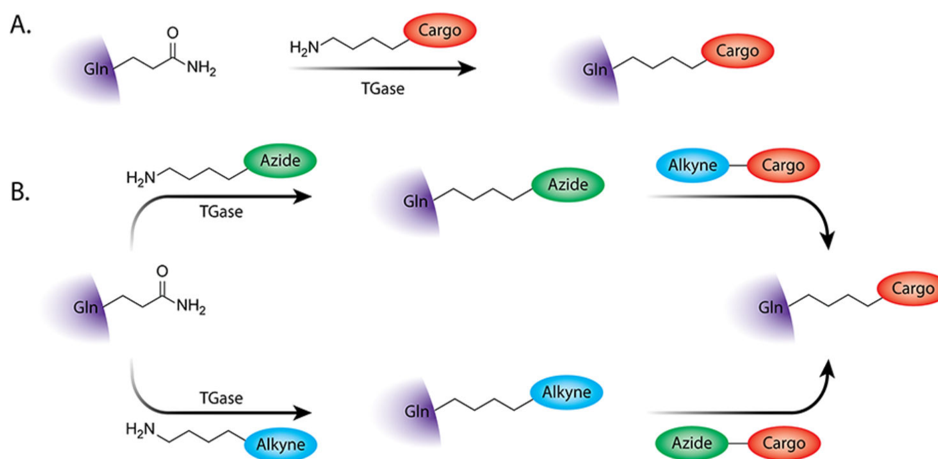

Figure 10.

Schematic of the one-step (A) and SPAAC-mediated two-step (B) approaches to the bioconjugation of antibodies using transglutaminase (TGase).

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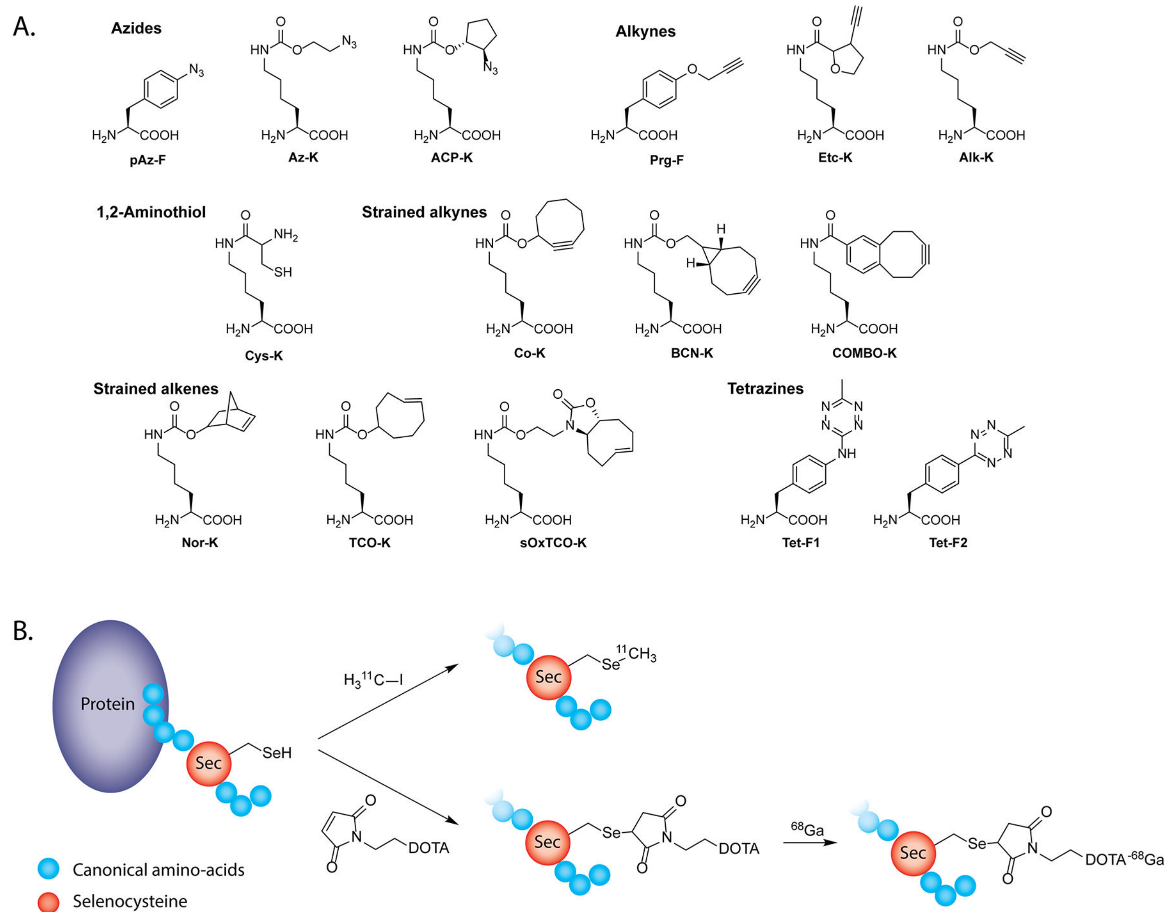

Figure 11.

(A) Examples of unnatural amino acids bearing bioorthogonally reactive functional groups; (B) schematic of the site-specific incorporation of the noncanonical amino acid selenocysteine into proteins and its subsequent radiolabeling with ^{11}C and ^{68}Ga.

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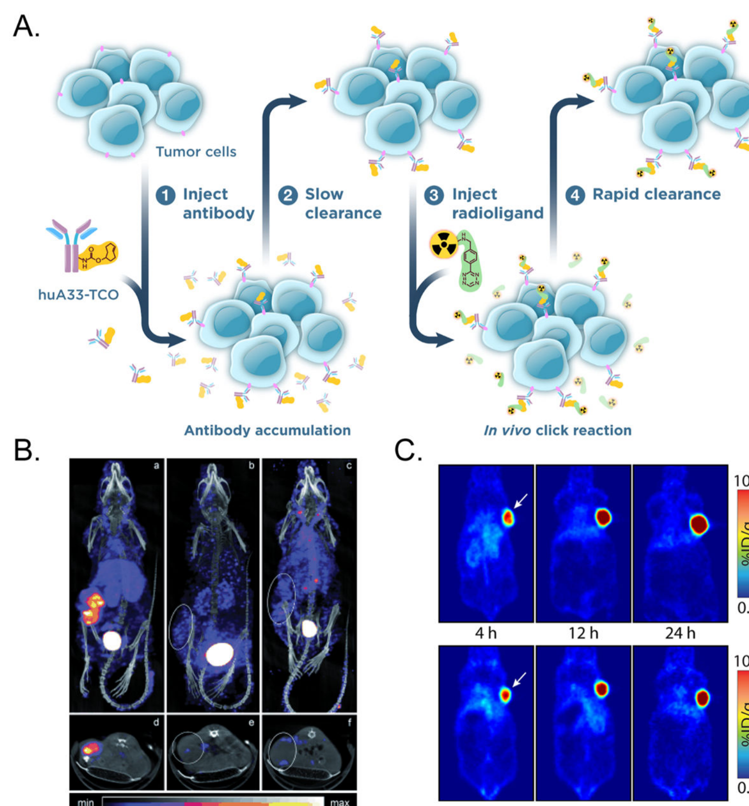

Figure 12.

(A) Schematic of the in vivo pretargeting approach using the IEDDA reaction. (B) Rossin et al. developed the first pretargeting approach using the in vivo IEDDA reaction to visualize TAG72-expressing LS174T xenografts (left) via SPECT imaging. To this end, a Tz-modified ^{111}In-DOTA radioligand was administered 24 h after the injection of a TCO-bearing immunoconjugate of the CC49 antibody. The use of either unmodified CC49 antibody (middle) or rituximab (no affinity for TAG72, right) instead of CC49-TCO confirmed that the accumulation of ^{111}In in the tumor was a product of in vivo click chemistry. (C) In a study conducted by Zeglis et al., pretargeted PET imaging was performed in mice bearing A33 antigen expressing SW1222 human colorectal carcinoma xenografts. To this end, the mice were first administered a TCO-bearing immunoconjugate of the A33 antigen-targeting antibody huA33 (huA33-TCO) followed, after a 24 h interval, by a ^{64}Cu- and Tz-modified radioligand (^{64}Cu–Tz–SarAr). Panels A and C were reprinted with permission from Zeglis et al., copyright 2015 American Chemical Society. Panel B was reprinted with permission from Rossin et al., copyright 2010 John Wiley and Sons.

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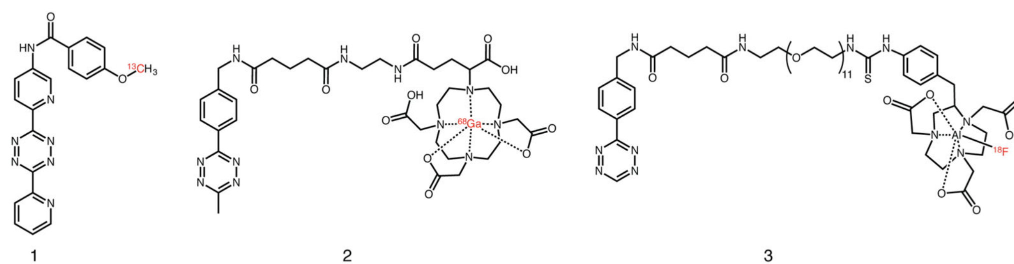

Figure 13.

Structures of selected tetrazines radiolabeled with the short-lives radionuclides ^{11}C ($t_{1/2} \approx 20$ min; **1**), ^{68}Ga ($t_{1/2} \approx 68$ min; **2**), and ^{18}F ($t_{1/2} \approx 110$ min; **3**).

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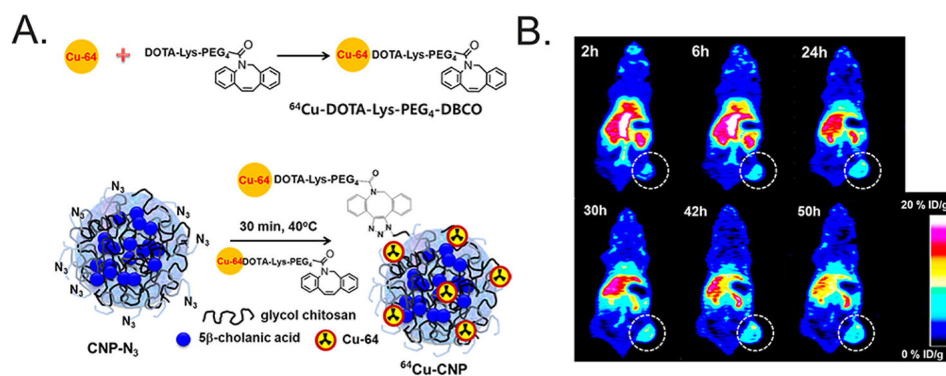

Figure 14.

(A) The SPAAC ligation was employed to combine ^{64}Cu–DOTA–Lys–PEG$_4$–DBCO prosthetic groups and azide-modified chitosan nanoparticles (CNP) to create ^{64}Cu-labeled CNPs. (B) MicroPET images of mice bearing SCC-7 xenografts acquired 2–50 h after the injection of the ^{64}Cu–CNPs (250 μCi, 200 μg per mouse) reveal tumoral activity concentrations of up to 11.3 ± 1.3% ID/g at 50 h post-injection. Reprinted with permission from Lee et al., copyright 2013 American Chemical Society.

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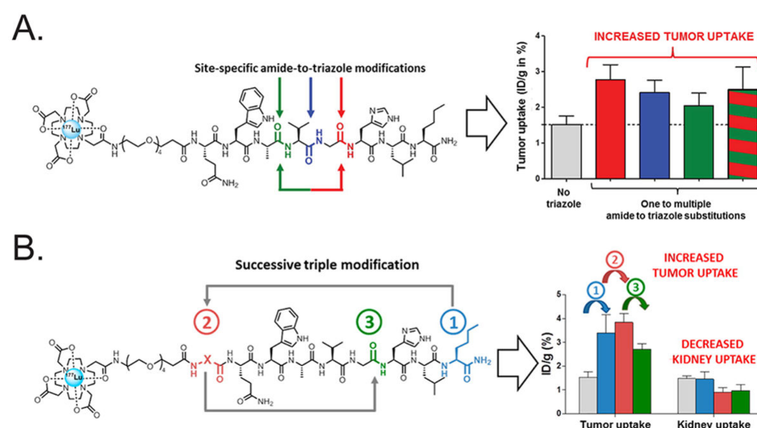

Figure 15.

(A) A series of ^{177}Lu-labeled peptidomimetics containing 1,4-disubstituted 1,2,3-triazole moieties were shown to exhibit significantly increased proteolytic stability while retaining nanomolar affinity for GRPr. These alterations are likely responsible for ~2-fold increases in the uptake of the tracers in target-expressing PC3 xenografts compared to their triazole-lacking cousins. (B) The same methodology was successfully applied to synthesize a series of GRPr-targeting bombesin derivatives that boast high tumor-to-kidney activity concentration ratios, a critical feature in the design of therapeutic radiopharmaceuticals. Panel A was reprinted with permission from Valverde et al., copyright 2015 American Chemical Society. Panel B was reprinted with permission from reference Valverde et al., copyright 2016 American Chemical Society.

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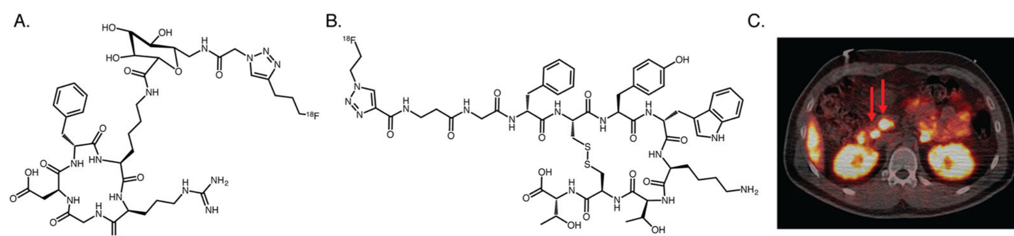

Figure 16.

(A) Structure of ^{18}F–RGD–K5; (B) structure of ^{18}F–fluoroethyltriazole–Tyr^{3}–octreotate; (C) ^{18}F–fluoroethyltriazole–Tyr^{3}–octreotate PET–CT image of a patient with multiple endocrine neoplasia type 1 syndrome and multiple pancreatic neuroendocrine tumors (red arrows). Adapted and reprinted with permission from Dubash et al., copyright 2016 by the Society of Nuclear Medicine and Molecular Imaging, Inc.