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"Click to Chelate": Synthesis and Installation of Metal Chelates into Biomolecules in a Single Step

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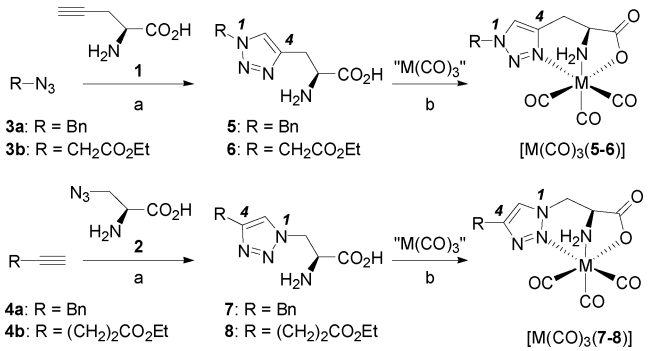
The radiolabeling of biologically active molecules has become an indispensable tool for the assessment of novel drug candidates. To keep pace with the growing number of new targets, innovative and efficient methodologies are needed for the firm attachment of molecules of biomedical interest to readily available radionuclides with suitable decay characteristics.

In recent years, new cores of technetium-99m ($T_{1/2}$ = 6 h, 140 keV γ-radiation) have been explored for the radiolabeling of biomolecules.[1] The organometallic precursor $[Tc(OH_2)_3(CO)_3]^+$ is a prominent example.[2] A variety of bifunctional tridentate ligand systems (e.g., based on amino acid scaffolds like cysteine, lysine, and histidine) have been designed for the tricarbonyl precursor.[3−6] However, the preparation of such chelators generally requires multiple-step syntheses, and their incorporation into biomolecules often lacks efficiency and is complicated by cross-reactivity with other functional groups present. To circumvent the problems associated with current strategies for functionalizing (bio)molecules with metal chelators, we set out to investigate the use of Sharpless' click chemistry in this context.[7,8]

Click chemistry (the Cu(I)-catalyzed [3 + 2] cycloaddition of alkynes and azides forming stable 1,2,3-triazole linkages) meets the requirements of an innovative functionalization strategy for biomolecules because it is efficient, selective, and devoid of side reactions. The mild reaction conditions are well suited for the modification of a wide variety of (bio)molecules, into which the required azide and alkyne functionalities can be incorporated by standard synthetic transformations or biochemical methods.[9] In addition, we recognized that 1,4-disubstituted 1,2,3-triazoles share structural and electronic features with 1,4-disubstituted imidazoles of N$^\epsilon$-derivatized histidines, which have been shown to be extraordinarily good chelators, particularly for organometallic cores of Mo, Tc, and Re.[10−12] Surprisingly few examples of 1,2,3-triazole chelators obtained via click chemistry are reported,[13,14] even though triazoles are known to be potent ligand systems for various transition metals.[15]

To probe the viability of a "click-to-chelate" approach, various commercial and noncommercial azide and alkyne derivatives were reacted with either L-propargyl glycine (**1**) or L-azido alanine (**2**)[16] to form 1,2,3-triazole-4-yl alanines **5**/**6** (referred to as a "regular" click ligand) or the isomeric products **7**/**8** ("inverse" click ligand, Scheme 1). In addition, representatives of four different classes of biomolecules (tumor affine peptide bombesin **9**, thymidine **10**, carbohydrate **11**,[17] and phospholipid **12**) were selected to demonstrate the versatility of the new approach (Figure 1).

Scheme 1 [a]



[a] Conditions: (a) $Cu(OAc)_2$, Na(ascorbate), water, 25 °C (15 h) or 100 °C (30 min); (b) M = ^{99m}Tc: $[^{99m}Tc(OH_2)_3(CO)_3]^+$, PBS, pH 7.4, 30 min, 100 °C; M = Re: $[ReBr_3(CO)_3]^{2-}$, water or alcohols, 50−65 °C, 1−4 h.

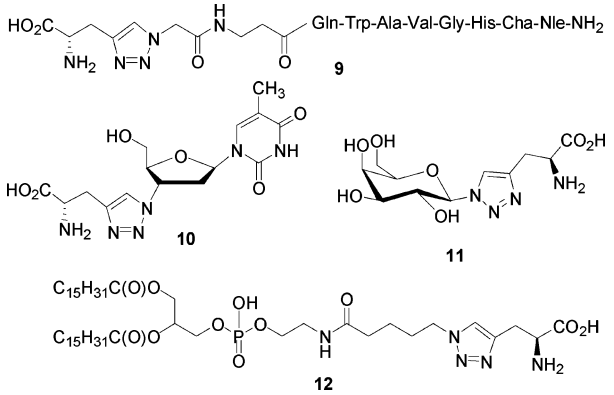


Figure 1. Derivatives of biomolecules functionalized via click chemistry with a tridentate 1,2,3-triazole containing metal chelator.

Unlike the multistep syntheses required for the preparation of N$^\epsilon$-derivatized histidines,[18] the click strategy avoids protective groups, which allowed the synthesis of optically pure histidine-like, 1,4-functionalized triazoles **5**−**8** and **10**−**12** in a single step and in quantitative yields.[19] These features are particularly appealing for the functionalization of biomolecules such as carbohydrates with polydentate metal chelates, which is notoriously inefficient using common synthetic strategies as we and others have experienced.[20,21] The azido bombesin derivative was prepared and clicked on solid support[22] to afford product **9**.

Reaction of ligands **5**−**12** with $[ReBr_3(CO)_3]^{2-}$ in aqueous or alcoholic media yielded the well-defined neutral complexes $[Re(CO)_3(\mathbf{5}-\mathbf{12})]$ (Scheme 1). ^{1}H and ^{13}C NMR analyses indicate that metal chelation occurs via N(3) (regular click) or N(2) (inverse click) of the 1,2,3-triazole heterocycle, the N$^\alpha$-amine, and

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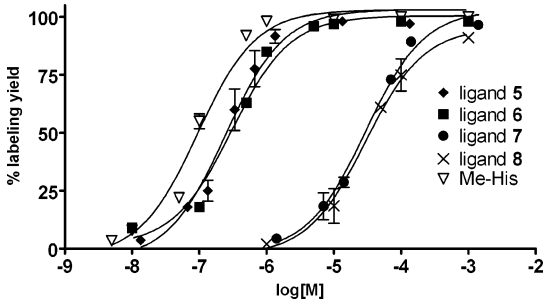
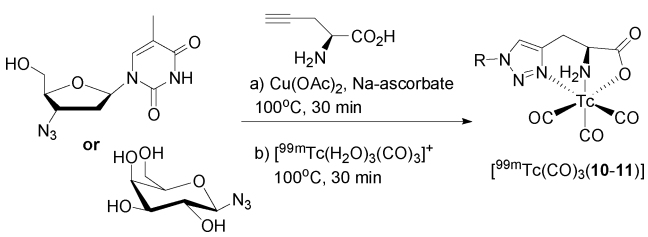


Figure 2. Labeling capacities of ligands **5**−**8** and N$^{\epsilon}$-methyl histidine (Me-His) with $[^{99m}Tc(OH_2)_3(CO)_3]^+$.

Scheme 2. One-Pot Procedure to Yield Radiolabeled Conjugates Directly



the carboxylate.[19] The characteristic features of the NMR spectra of isolated complexes are consistent with those reported for Tc/Re(CO)$_3$ complexes of N$^{\epsilon}$-functionalized histidine derivatives.[12]

Radioactive complexes $[^{99m}Tc(CO)_3(\mathbf{5}-\mathbf{12})]$ were also obtained as single products.[23,24] For ligands **5**−**8**, varying the ligand concentration gave rise to step-sigmoid curves (Figure 2). EC_{50} values of investigated regular click ligands ranged from 2 to 3 × 10^{-7} M. These values were comparable to that of N$^{\epsilon}$-methyl histidine ($EC_{50} = \sim 10^{-7}$ M), demonstrating the potency of the regular triazole ligands **5**/**6**. Values of inverse click ligands **7**/**8** were approximately 2 orders of magnitude higher ($EC_{50} = \sim 10^{-5}$ M), presumably a consequence of the lower electron density at N(2) compared to that at N(3) of the triazole heterocycle (DFT calculations).[19]

While the click products represent a class of extraordinarily good chelators, the individual substrates (e.g., alkyne **1** and azide **2**) do not form stable or defined complexes with $[M(OH_2)_3(CO)_3]^+$. This observation spurred the idea of a one-pot procedure to avoid isolation of the functionalized (bio)molecule prior to labeling with $[M(OH_2)_3(CO)_3]^+$. Thus, aqueous solutions of 3′-azido thymidine or 1-azido-1-deoxy-β-D-galactopyranose and alkyne **1**, $Cu(OAc)_2$, and Na(ascorbate) were heated to 100 °C for 30 min. $[^{99m}Tc(OH_2)_3(CO)_3]^+$ was added, and the mixtures were heated for further 30 min (Scheme 2). HPLC analysis confirmed the clean formation of complexes $[^{99m}Tc(CO)_3(\mathbf{10})]$ and $[^{99m}Tc(CO)_3(\mathbf{11})]$, identical to the products obtained with presynthesized and purified ligands **10** and **11**.[19]

The radiolabeled bombesin derivative $[^{99m}Tc(CO)_3(\mathbf{9})]$ was assessed in vitro and in vivo for its stability and receptor affinity.[19] These preliminary experiments revealed a high in vivo stability of the conjugate and an almost identical pharmacological profile to $[^{99m}Tc(CO)_3N^{\alpha}AcHis\text{-bombesin}]$,[25] a previously tested stable bombesin analogue with sequence homology but a histidine chelate (detailed results will be published elsewhere). These results suggest that the new triazole ligands represent a valuable alternative to histidine-derived chelators.

We have shown that click chemistry both simplifies the synthesis of efficient bifunctional ligands in which 1,4-disubstituted triazoles form an integral part of the metal chelating system and facilitates their incorporation into (bio)molecules. Labeling of the chelators and bioconjugates with *fac*-"Tc/Re(CO)$_3$" is efficient and results in complexes that are stable in vitro and in vivo. The one-pot procedure represents a remarkable improvement for the synthesis of metal-labeled conjugates, for example, for diagnostic purposes. Extension of the "click-to-chelate" approach to the preparation of structurally diverse ligand systems suitable for complexation of other metals is currently being investigated.

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Supporting Information Available: Experimental procedures and analytical data for all compounds, DFT calculations, and in vitro and in vivo data for ^{99m}Tc-labeled bombesin derivative **9**. This material is available free of charge via the Internet at http://pubs.acs.org.

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