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Triazole Appending Agent (TAAG): A New Synthon for Preparing Iodine-Based Molecular Imaging and Radiotherapy Agents

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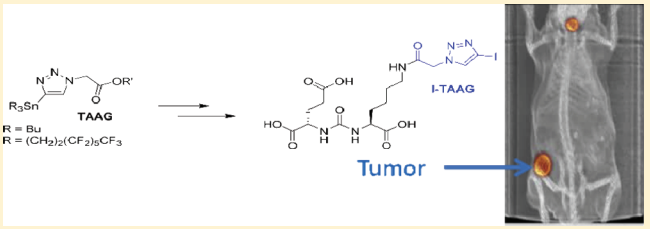
Supporting Information

ABSTRACT: A new prosthetic group referred to as the triazole appending agent (TAAG) was developed as a means to prepare targeted radioiodine-based molecular imaging and therapy agents. Tributyltin-TAAG and the fluorous analogue were synthesized in high yield using simple click chemistry and the products labeled in greater than 95% RCY with ^{123}I. A TAAG derivative of an inhibitor of prostate-specific membrane antigen was prepared and radiolabeled with ^{123}I in 85% yield where biodistribution studies in LNCap prostate cancer tumor models showed rapid clearance of the agent from nontarget tissues and tumor accumulation of 20% injected dose g^{-1} at 1 h. The results presented demonstrate that the TAAG group promotes minimal nonspecific binding and that labeled conjugates can achieve high tumor uptake and exquisite target-to-nontarget ratios.



KEYWORDS: *imaging, iodine, radiochemistry, radiopharmaceuticals, SPECT*

Molecular imaging of cancer using isotopes of iodine is becoming increasingly attractive because of the ability to develop isostructural theranostics: agents that can be used for both diagnosis and treatment.[1−4] By simply changing the isotope of iodine, it is possible to convert an effective positron emission tomography (PET) or single photon emission computed tomography (SPECT) imaging agent (based on ^{124}I or ^{131}I/^{123}I, respectively) into a targeted therapeutic compound (based on ^{125}I or ^{131}I) without altering the structure of the molecule. Development of these types of agents is hindered by the limited number of iodine-containing prosthetic groups that are synthetically accessible, resistant to catabolism, and can penetrate and be retained in tumors when appended to the appropriate targeting vector.[5−10] The focus to date has largely been on iodobenzene-derived prosthetic groups, which do not meet these criteria and are typically lipophilic, thereby promoting nonspecific binding, particularly when appended to small molecules.[7]

Radioiodinated heterocycles are an attractive alternative to the existing benzene-derived prosthetic groups. Årstad et al.[11,12] reported the preparation of trifunctional reagents for multiscale imaging using both optical and nuclear techniques. ^{125}I triazoles were formed in situ when a click reaction was performed between an alkyne-derived fluorophore and an azide-derived active ester in the presence of NaI. Trigg and Avory in a recent patent[13] described the preparation of ^{123}I-labeled acetylene, which was "clicked" to form labeled heterocycles. We explored the feasibility of preparing bifunctional tin-triazoles[14−18] that can be linked to targeting vectors such that the bioconjugates can be isolated and ultimately radiolabeled and purified in a single efficient step. The targets included both butyl and fluorous tin derivatives where the latter is designed to allow for single step labeling and chemoselective filtration to isolate the desired product in high effective specific activity.[19−21] We discovered a versatile and stable class of compounds referred to as the triazole appending agents (TAAGs) that can be used to develop highly effective probes that clear rapidly from nontarget tissues and that are able to penetrate tumor cells when appended to the appropriate vector.

Tributyltinacetylene is commercially available and readily undergoes a catalyst-free cycloaddition reaction with methyl 2-azidoacetate (Scheme 1). Heating **1a** and the azide in toluene at reflux afforded the ester **2a** in 81% yield where following chromatographic purification only a single isomer was isolated. The product is stable for several months when stored in the freezer and protected from light. The fluorous analogue of **1a** was prepared by treating commercially available tris-(1*H*,1*H*,2*H*,2*H*-perfluorooctyl)phenyltin with iodine followed by ethynyl magnesium bromide. The product **1b** can be isolated or immediately combined with the appropriate azide

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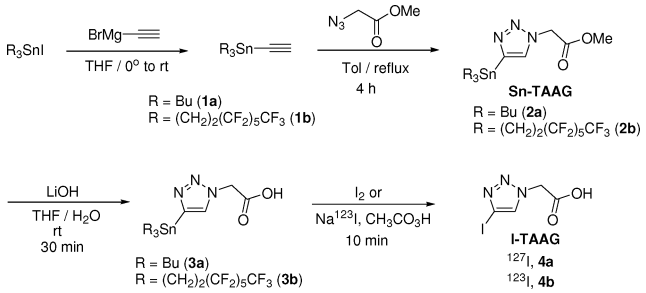
Scheme 1. Synthesis of I-TAAG



where in the case of methyl 2-azidoacetate, **2b** was obtained in 79% yield. There was no notable reactivity difference between the fluorous and the alkyl tin derivatives.

The methyl esters **2a** and **2b** were hydrolyzed using aqueous LiOH in greater than 90% yield. Iodination of **3a** produced the reference standard (**4a**) needed for the radiochemical reactions in 95% yield. X-ray quality crystals were obtained, and the structure (Figure 1), which was consistent with the ^{1}H and ^{13}C

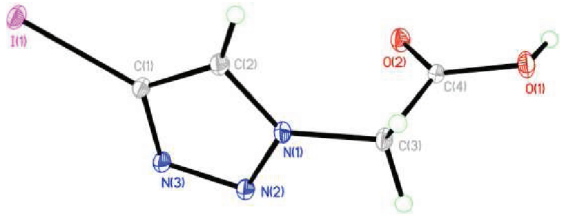


Figure 1. ORTEP representation (50% thermal probability ellipsoids) for I-TAAG (**4a**).

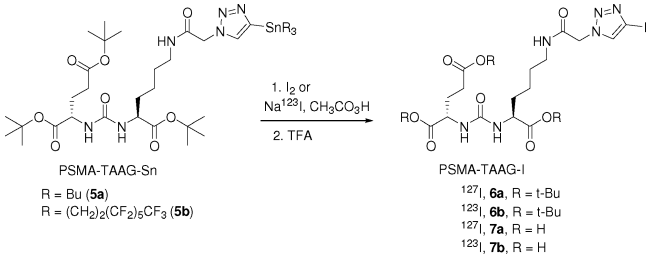
NMR data, showed that a single isomer was isolated. The length of the C−I bond in the triazole was 2.0617(18) Å as compared to 2.097(9) Å in *p*-iodobenzoic acid.[22] All bond lengths and angles were comparable to other reported triazoles.[23,24]

To test the reactivity and stability of the I-TAAG construct, **3a** was treated with $Na^{123}I$ in the presence of peracetic acid. Using 500 μg of precursor, the desired product **4b** was obtained in >95% radiochemical yield and >99% radiochemical purity. Reactions were complete within 10 min, and the product was isolated by semipreparative HPLC.

The log *D* of **4b** was determined to be −2.58 ± 0.01 (at pH 7.4), which is more hydrophilic than *p*-iodobenzoic acid (log *D* of 0.07 at pH 7.4). Compound **4b** was stable over 48 h in solution with no signs of deiodination. A biodistribution study was performed to assess the extent to which the agent deiodinates in vivo and binds nonspecifically to key tissues such as the kidneys and liver. Compound **4b** clears all major organs quickly and collects in the bladder within 30 min (see the Supporting Information for quantitative data). This, as noted earlier, is a desirable feature of a prosthetic group being used to develop targeted imaging and therapy agents.[25]

As a model vector, we chose a glutamate-urea-lysine analogue, which is an inhibitor of prostate-specific membrane antigen (PSMA): a protein that is overexpressed in prostate cancer.[26,27] The glu-urea-lys construct has been derivatized with different radioisotopes including iodoaryl compounds and was therefore an attractive agent for assessing the iodotriazole synthon.[28−32] Methyl ester **2a** was coupled to *t*Bu protected glu-urea-lys at 60 °C for 24 h, and the product **5a** was isolated in 73% yield. The fluorous analogue **5b** was prepared from the ester **2b** in 77% yield. The iodine standard **7a** (Scheme 2) was

Scheme 2. Labelling of a TAAG-Derived PSMA Inhibitor



prepared by treating **5a** with I_2 followed by deprotection using trifluoroacetic acid (TFA) where the product was isolated in 71% yield.

The PSMA-TAAG-tin ligand was radiolabeled using the same oxidant system as for the free ligand, and the product **7b** was obtained in 85% yield and >99% radiochemical purity. The log *D* of the product was determined to be −3.23 ± 0.05 (at pH 7.4). The compound was administered to male NCr nude mice containing LNCap tumors, which are known to express PSMA. At 2 h postinjection, the images showed uptake of the agent in the kidneys, bladder, and a small amount in the tumor. At 24 h, the activity was found only in the tumor and in the thyroid (Figure 2). The thyroid uptake, which was higher than

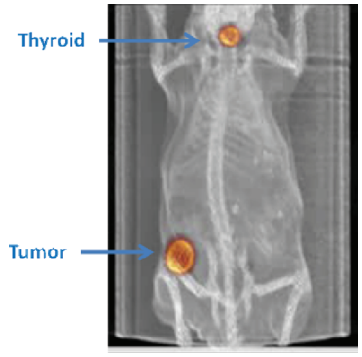


Figure 2. Fused scintigraphic-CT image (23 h postinjection; 37 MBq of **7b** administered via the tail vein) of NCr nude mice containing an LNCap tumor. Quantitative biodistribution data can be found in the Supporting Information.

for **4b**, is likely due to catabolism of the agent after prolonged retention in vivo.[33−37]

Quantitative biodistribution studies showed that tumor uptake was over 20% injected dose (ID) g^{-1} at 1 h, which could be blocked to less than 2% ID g^{-1} by administering a known PSMA blocking agent (PMPA). At 23 h, the agent still retained 15% ID g^{-1} in the tumor, and the remaining activity was in the thyroid (~10% ID g^{-1}).

The TAAG synthon is versatile in that it can be prepared by taking a simple tin-alkyne and combining it with virtually any azide, providing a scope of use that is analogous to ^{18}F-alkynes used to develop PET agents using click type chemistry.[38−40] TAAG conjugates can be readily isolated, fully characterized, and labeled using robust single step iodination methods, and the products purified by HPLC or SPE where the former was used here because of the high polarity of the compounds being labeled. The results presented here demonstrate that the TAAG

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group promotes minimal nonspecific binding and that labeled conjugates can achieve high tumor uptake and produce exquisite target-to-nontarget ratios. The use of TAAG and uniquely functionalized analogues provides an alternative to conventional benzene-derived prosthetic groups and should facilitate the development of a new generation of radioiodine-based theranostics.

■ ASSOCIATED CONTENT

Supporting Information

Synthesis and characterization of all new compounds, complete crystallographic data, and biodistribution study results. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

All authors have given approval to the final version of the manuscript. All animal experiments were performed in accordance with the guidelines laid out by the Canadian Council on Animal Care (CCAC) and McMaster University. The authors declare no competing financial interest.

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■ ABBREVIATIONS

TAAG, triazole appending agent; PET, positron emission tomography; SPECT, single photon emission computed tomography; PSMA, prostate-specific membrane antigen; ID, injected dose; TFA, trifluoroacetic acid

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