Multifunctional Deuterated and Tritiated 'Click' Molecular Probes via Palladium-Mediated Reductive Deiodination of 5-Iodo-1,2,3-Triazoles

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Abstract: Tritiated compounds are invaluable tools for in vitro and in vivo studies on bioactive molecules. Here we describe a 'click-chemistry'-based strategy for deuteration and tritiation of multifunctional compounds and multimodal molecular imaging probes. The method relies on a palladium-mediated reductive deiodination of 5-iodo-1,2,3-triazoles in the presence of $NaBD_4$, deuterium or tritium gas.

Key words: tritium, 'click' chemistry, 1,2,3-triazole, deuterium, bioconjugation

The copper-mediated alkyne–azide cycloaddition (CuAAC) is nowadays one of the most reliable 'click' reactions,[1] suitable for the synthesis of small molecules,[2] polymers,[3] pseudopeptides,[4] and nucleic acids[5] incorporating the 1,2,3-triazole function. An application of CuAAC which is rapidly growing in importance is the conjugation and tagging of biologically active molecules.[6] The CuAAC has been widely investigated for the labeling of bioactive molecules with radioisotopes,[7] especially for the synthesis of PET and SPECT tracers.[8] The click labeling reaction generally gives high yields in short reaction times, which makes it an invaluable tool for meeting the strict synthetic requirements of radiochemical processes. Importantly, not only terminal alkynes can react with azides to form 1,2,3-triazoles, but also constrained cycloalkynes[9] and iodoalkynes, which show enhanced reactivity, excellent regioselectivity, and broad substrate scope in CuAAC.[10] The resulting 5-iodo-1,2,3-triazoles[11] have found numerous synthetic applications in palladium-catalysed C–C bond-forming reactions leading to 1,4,5-trisubsituted 1,2,3-triazoles[10,12] and fused heterocyclic systems.[13] Recently, a practical protocol for the synthesis of 5-[^{125}I]iodo-1,2,3-triazoles has been developed for preparing multifunctional triazole molecules bearing fluorescent tags, radioactive iodine, and a reactive group as reagents for the 'double-labeling' of peptides.[14] Click chemistry has been successfully adapted to the radiosynthesis of PET tracers starting from easily accessible [^{18}F]azides or alkynes. However, introduction of the triazole prosthetic group can deeply modify both binding selectivity and affinity of the ligand for its receptor, especially in the case of small biomolecules. A tritium-labeled version of the fluorinated triazole ligand could be extremely useful to study in vitro the properties of 'click'-PET tracers prior to embarking into the laborious ^{18}F radiochemistry and imaging process. In fact, the tritiated compound can be safely handled and stored for long periods (tritium is a low energy, 18.6 keV, β emitter, half-life = 12.32 years) contrarily to the ^{18}F-labeled isotopic version (^{18}F is a higher energy, 634 keV positron emitter, half-life = 110 min). Thus tritiated triazoles can be used for preliminary in vitro/in vivo profiling of bioconjugated molecular tools prepared by click chemistry, such as SPECT or PET tracers, with the advantage that tritium-labeled compounds are easier to handle, store, and can still be detected in picomolar concentrations (Figure 1).

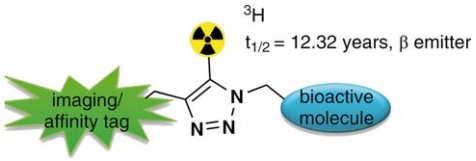

Figure 1 Schematic representation of dual modal probes with tritium as radioactive tag

To the best of our knowledge, however, no tritiation protocols of the 1,2,3-triazole ring have been published so far. Only deuterated triazoles, prepared via D_2O deuteration of organometallic intermediates (organolithium, magnesium, aluminium,[15] or cuprates),[16] have been reported (Scheme 1). The applicability of these protocols is limited to substrates that do not incorporate acidic or exchangeable protons, furthermore protecting groups are generally required. These limitations do not allow the labeling of triazole rings in complex functionalised molecules, and these methods generally require strictly anhydrous reaction conditions and complex experimental procedures.

In this work we report the development of a 'click-chemistry'-based strategy for the deuteration and tritiation of biologically active compounds and multimodal molecular imaging probes. The method relies on a palladium-medi-

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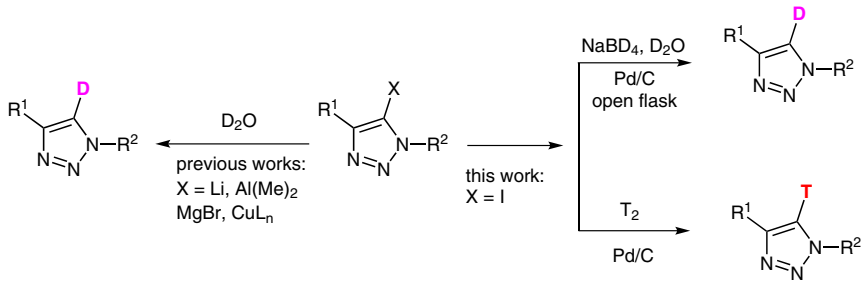


Scheme 1

ated reductive deiodination of 5-iodotriazoles in the presence of $NaBD_4$, deuterium or tritium gas (Scheme 1) and can be used for introducing deuterium or tritium on densely functionalised triazoles containing free COOH and OH groups, as well as basic moieties (such as amine or guanidine).

As a proof of concept application of the method, we labeled with tritium two 'click compounds' that could be used as probes for molecular imaging (Figure 2). The first is a fluorinated cholic acid derivative **1**, which we developed as a candidate PET tracer for evaluating the hepatic excretory function, cholestasis, and hepatic effects of drugs.[17] The second is a biotinylated c[-RGDfK-] peptide **2**, which is a high affinity ligand for the vitronectin receptor ($\alpha_v\beta_3$ integrin).[18] Different RGD peptides have been labeled with fluorescent tags,[19] biotin,[20] and radio nuclides (^{68}Ga, ^{64}Cu, and ^{18}F).[21] Biotinylation of c[-RGDfK-] was reported to produce a versatile biomarker that can be used for the development of enzyme-linked immunosorbent assay (ELISA) as well as immunohistochemical stains of cells and biological tissues.[22] Thus, tritium-labeled **2** was synthesised as a tool for the precise quantification of $\alpha_v\beta_3$ integrin expression in cells and tissues in competitive binding experiments.

Reductive deiodination of 5-iodotriazoles was initially explored using the triazole **3** (Table 1) as a model substrate and $NaBD_4$ or $NaBD_3CN$ as deuterium sources in the presence of Pd/C (Table 1, entries 1–4, 7, and 8), $Pd(PPh_3)_4$ (Table 1, entry 5), and $Pd(PPh_3)_2Cl_2$ (Table 1, entry 6) as catalysts, which performed similarly.[23] The reaction was ineffective using $NaBD_4$ in anhydrous THF (Table 1, entry 1), while in protic solvents such as NMR-grade CD_3OD (Table 1, entry 2) or D_2O (Table 1, entry 3) vigorous bubbling was observed, and the expected 5-deutero-1,2,3-triazole **4** was obtained as the only product. Not surprisingly, using EtOH as a cosolvent (Table 1, entry 4) the major product was the 5*H*-1,2,3-triazole. The low deuterium incorporation observed with nondeuterated protic solvents suggests that deiodination may occur through two concomitant processes: (1) reduction of a triazole–Pd–I intermediate by $NaBD_4$ and (2) a faster 5-iodo-triazole reduction by deuterium gas generated via palladium-catalysed solvolysis of $NaBD_4$ and adsorbed on the palladium surface. This would be consistent with the extremely short reaction times observed in protic solvents and with the previously reported mechanism of palladium-catalysed hydrolysis of $NaBH_4$ in water.[24] $NaBD_3CN$ (Table 1, entry 7) was also found to deiodinate the triazole ring in the presence of Pd/C, but the reaction times were much longer. Also in this case, the use of EtOH as a cosolvent (Table 1, entry 8) produced a mixture of 5*D*- and 5*H*-triazole **4**. Based on the considerations below, the conditions reported in entry 3 (Table 1) were selected to explore the scope of the reaction: (1) using Pd/C as a

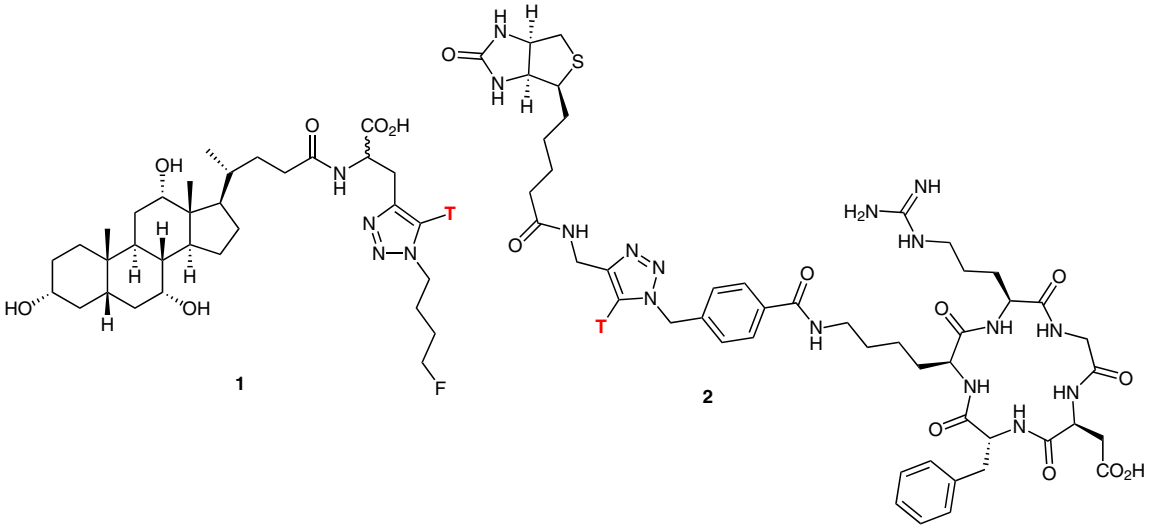
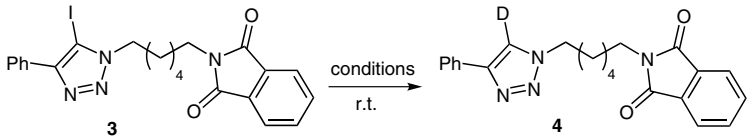


Figure 2

Table 1 Optimisation of Deuterium-Labeling Conditions



3 4

Entry	Pd cat.	Deuterium source	Additive[c]	Time	Yield of **4** (%)[d]	D (%)[e]
1	Pd/C[a]	$NaBD_4$	–	0.5 h	–	–
2	Pd/C[a]	$NaBD_4$	CD_3OD	5 min	82	100
3	Pd/C[a]	$NaBD_4$	D_2O	5 min	98	100
4	Pd/C[a]	$NaBD_4$	EtOH	5 min	98	29
5	$Pd(PPh_3)_4$[b]	$NaBD_4$	CD_3OD	2 h	98	100
6	$Pd(PPh_3)_2Cl_2$[b]	$NaBD_4$	CD_3OD	2 h	98	100
7	Pd/C[a]	$NaBD_3CN$	D_2O	24 h	55	100
8	Pd/C[a]	$NaBD_3CN$	EtOH	24 h	55	39

[a] Dry, 10% on charcoal.
[b] 0.1 equiv.
[c] 0.5 mL in 1.5 mL of THF.
[d] Isolated yield.
[e] Assessed by MS ion pattern analysis.

catalyst, no chromatographic purification was generally required; (2) the reaction was complete in less than ten minutes and in an open flask; (3) D_2O is the cheapest deuterated solvent.

Using the method reported by Hein,[10a] an array of 5-iodotriazoles (Table 2) was prepared from the iodoalkynes precursors and subjected to the iodine–deuterium exchange. All of the 5*D*-triazoles were obtained in high yields. The reaction tolerated several functional groups, such as acidic moieties (COOH), protic functions (OH and CONH), or hydrolysable groups (methyl ester and acetal) which affected neither the reaction course, nor deuterium incorporation. Furthermore, the readily reducible pyridine ring (**11**), an aromatic fluoride (**12**), cyclopropyl (**12**) and phthalimide (**4**) moieties were all found to be stable under the reaction conditions. A ^2H NMR experiment performed on compound **10** confirmed both the presence of deuterium and the labeling site (see Supporting Information).

Table 2 Substrate Scope

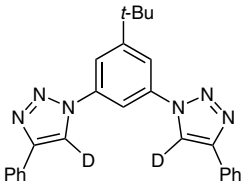
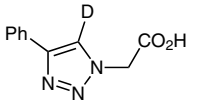
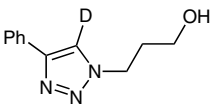
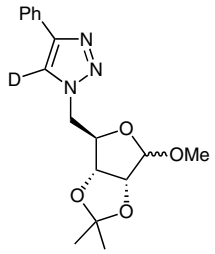
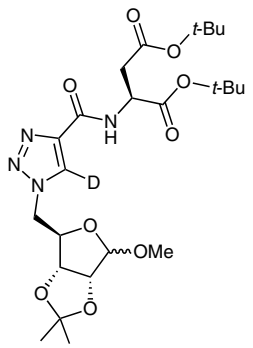
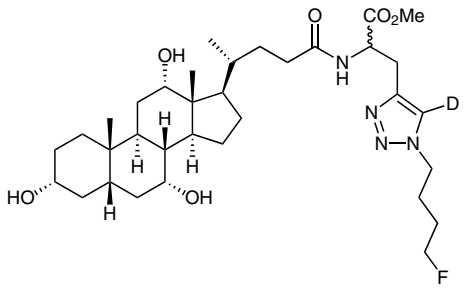
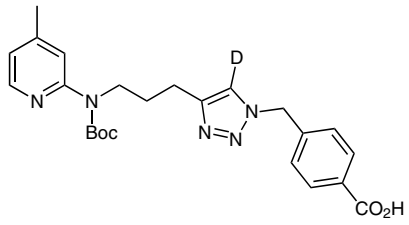
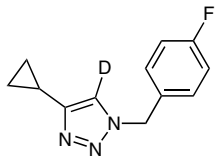
Entry	Triazole		Yield (%)[a]	D (%)[b]
1	**5**		92	100
2	**6**		98	100
3	**7**		90	100

Table 2 Substrate Scope (continued)

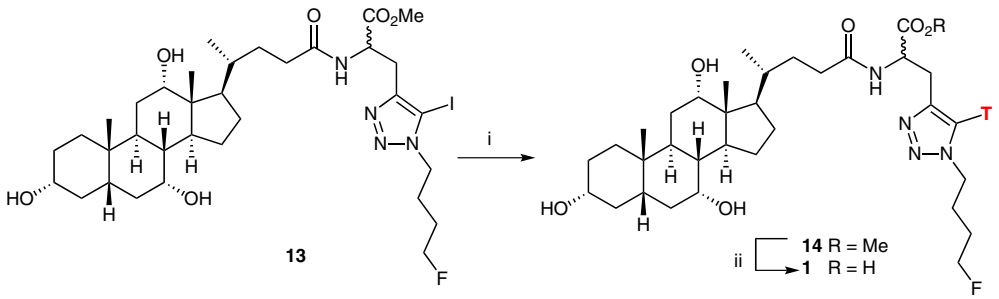
Entry	Triazole		Yield (%)[a]	D (%)[b]
4	**8**		95	100
5	**9**		92	100
6	**10**		90	98
7	**11**		98	100
8	**12**		80	100

[a] Isolated yield.
[b] Assessed by MS ion pattern analysis.

The deuteration reaction leading to compound **10** was also performed using deuterium gas (D_2) and Pd/C as a catalyst. The deiodination reaction required longer reaction times, however, complete conversion into **10** was achieved after two hours.

Introduction of tritium was investigated next: 2.9 μmol of iodinated precursor **13** (Scheme 2) were treated with 1640 mCi (28.4 μmol) of tritium gas (T_2) in methanol (200 μL) and Et_3N (35.9 μmol) in a tritium handling manifold. After 90 minutes, HPLC analysis showed complete conversion of **13** into the tritiated ester **14**. After routine workup, the crude ester was hydrolysed with 5 M NaOH, affording the crude product **1** (54 mCi, radiochemical purity 94%).

A portion of crude **1** (3.71 mCi) was purified by reverse-phase HPLC, affording the target ^{3}H-labeled cholic acid (**1**, 2.68 mCi) with a specific activity of 20 Ci/mmol (corresponding to 70% of tritium incorporation) and 99.8% radiochemical purity. ^{3}H NMR of **1** in DMSO-d_6 showed



Scheme 2 *Reagents and conditions*: i) T_2, Et_3N, Pd/C, MeOH, r.t., 1.5 h; ii) NaOH in EtOH, H_2O.

a singlet signal at 7.81 ppm, indicating a single labeled position on the triazole ring.

Synthesis of biotinylated c[-RGDfK-] peptide **2** started from the biotinylated propargylamide **15** (Scheme 3) which was reacted with the azide **16** affording either the 5-iodo-triazole **17** or the 5*H*-triazole **18**. After methyl ester hydrolysis, the biotinylated tags were obtained in good yields by precipitation.

Solid-phase synthesis of the cyclopeptide was accomplished as reported by McCusker et al.[25] After head-to-tail cyclisation, the lysine protecting group (4,4,-dimethyl-2,3-dioxocyclohexylidene)ethyl (Dde) was removed with a DMF (2%) hydrazine solution and the solid-supported peptide **19** (Scheme 4) was coupled with the biotinylated triazoles **17** and **18**. Resin cleavage and removal of the pentamethyl-dihydrobenzofuran-5-sulfonyl (Pbf) arginine protecting groups were accomplished using TFA in CH_2Cl_2 (50%). After HPLC purification, the 'click'-biotinylated c[-RGDfK-] peptides **20** and **21** were obtained as TFA salts in 18–19% overall yields, based on the initial resin loading. The 5-iodotriazole **20** (Scheme 4) was then subjected to deiodination with $NaBD_4$ and Pd/C in CD_3OD. Complete conversion into the deuterated compound **22** was observed by HPLC within five minutes. Deuterium incorporation was >95%, and **22** was the only product detected in the reaction mixture.

Reductive deiodination with D_2 gas was then performed on **20** in the presence of Et_3N and Pd/C. Deuterium incorporation in **22** was found to be 37% by HPLC–MS analysis after one hour, when the reaction was judged to be complete.

Tritiation of the precursor **20** to ^{3}H-c[-RGDfK-] **2** was performed under the same conditions used for deuteration. After two hours, HPLC–MS analysis showed 55% conversion. The specific activity was 4.3 Ci/mmol, which is lower than that measured for the bile acid derivative **1**. This may be due to the presence of several acidic protons in the TFA precursor **20**, and possibly also to the biotin sulfide function which may be responsible for partial poisoning of the palladium catalyst. The crude was then pu-

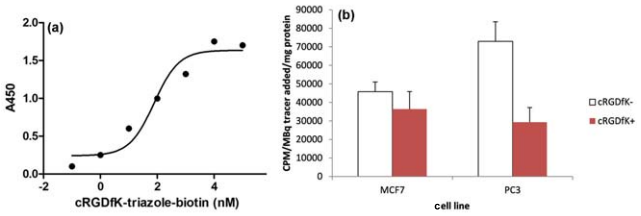
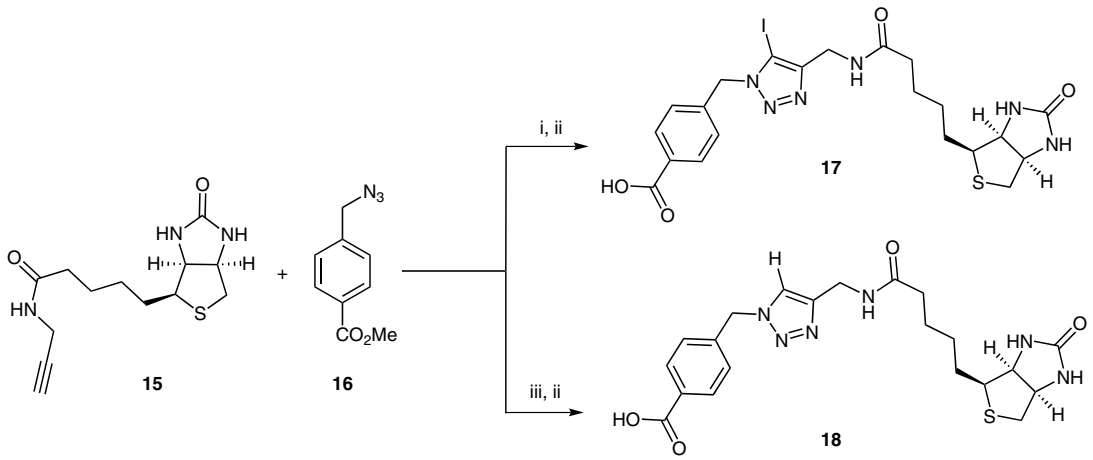
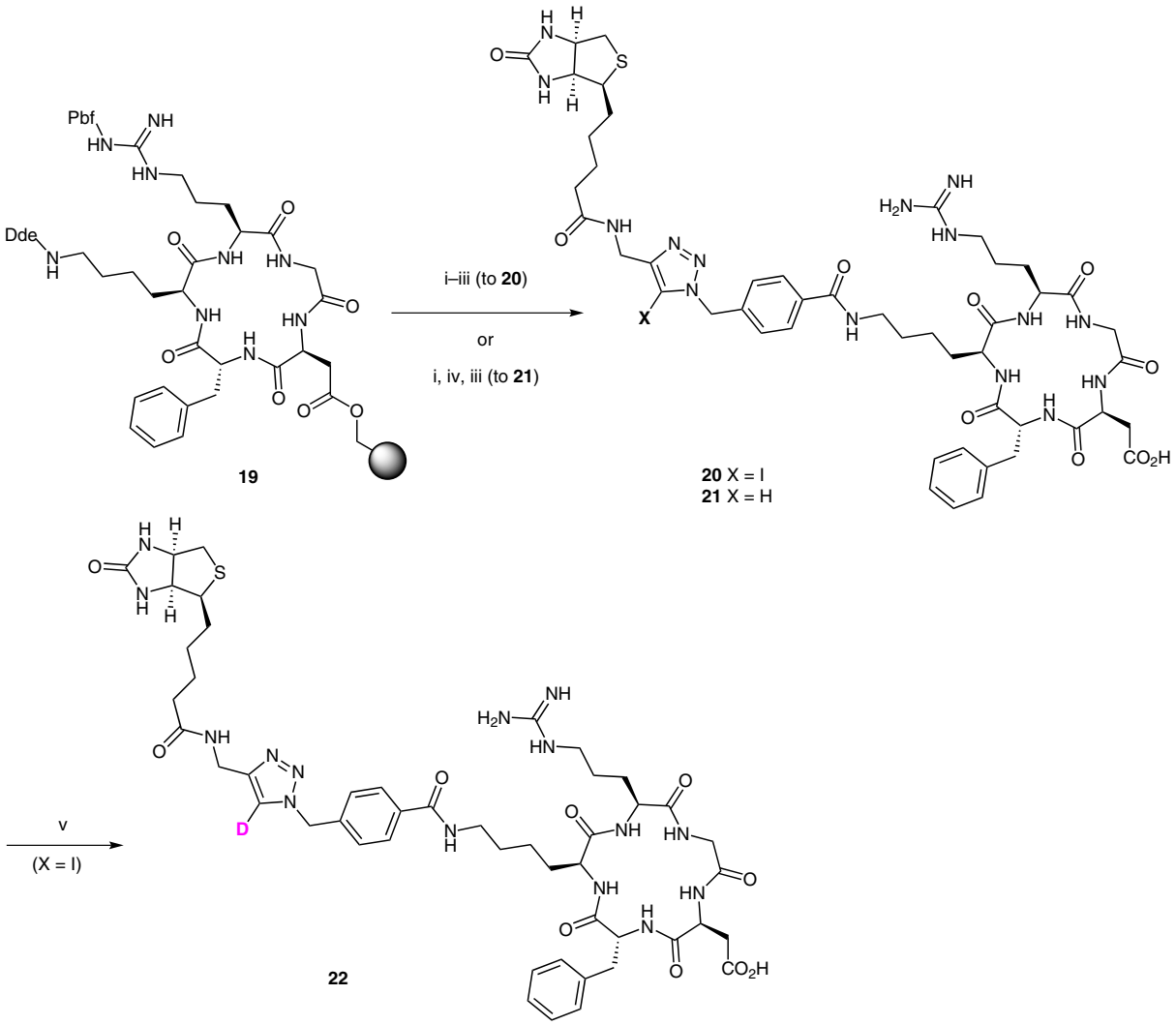


Figure 3 (a) ELISA experiment for measuring the $\alpha_v\beta_3$ affinity of **2**. Each data point is the average of two independent experiments, performed in triplicate; x-axis is a logarithmic scale. (b) Binding experiment of [^{3}H]-**2** to PC3 and MCF7 cells. Each data point is the average of four independent results.



Scheme 3 *Reagents and conditions*: i) CuI (1 equiv), NIS (1.1 equiv), Et_3N (1 equiv), DMF, r.t., 6 h, 56%; ii) LiOH, THF–H_2O (9:1), r.t., 36 h, quant.; iii) $CuSO_4$ (0.02 equiv), sodium ascorbate (0.1 equiv), *t*-BuOH–H_2O (2:1), r.t., 24 h.



Scheme 4 *Reagents and conditions*: i) hydrazine monohydrate in DMF (2%), r.t., 5 min; ii) **17** (2 equiv), HATU (2 equiv), DIPEA (4 equiv) in DMF, r.t., 2 h; iii) TFA in CH_2Cl_2 (50%), r.t., 2 h; iv) **18** (2 equiv), HATU (2 equiv), DIPEA (4 equiv) in DMF, r.t., 2 h; v) $NaBD_4$, Pd/C in CD_3OD, r.t., 5 min (>98% by HPLC–MS analysis).

rified by RP HPLC affording 21 μCi of labeled peptide in 95% radiochemical purity.

Binding of **2** to purified $\alpha_v\beta_3$ integrin protein was tested in an ELISA experiment. The results (Figure 3, a) confirmed that the biotinylated peptide **2** binds to $\alpha_v\beta_3$ integrin in a dose-dependent manner. An average binding affinity of 280 nM was calculated, slightly lower than the 140 nM value obtained with c[-RGDfK-] in an $\alpha_v\beta_3$ competition binding assay.[22] That may either be due to addition of the biotin moiety to the RGD peptide or the slightly different ELISA formats used. Nevertheless, the data clearly demonstrate that **2** is an effective substrate for an ELISA to assess binding to $\alpha_v\beta_3$ integrin. PC3 and MCF7 cells were chosen for the binding experiments as they are known to express high and no $\alpha_v\beta_3$ integrin, respectively.[21] As expected, binding of [^{3}H]-**2** was higher in PC3 than MCF7 cells (Figure 3, b), reflecting $\alpha_v\beta_3$ expression levels. Inclusion of 10μM of cold c[-RGDfK-] to competitively block specific binding to $\alpha_v\beta_3$ decreased [^{3}H]-**2** binding to PC3 cells by approximately 60%, but had little effect in MCF7 cells. A students t-test confirmed that the cold peptide caused a significant decrease in [^{3}H]-**2** binding to PC3 cells ($p < 0.001$), but not to MCF7 cells ($P = 0.15$). These findings indicate that the majority of bound radioactivity in PC3 cells can be attributed to specific binding to $\alpha_v\beta_3$, whereas the low level binding to MCF7 is nonspecific. These data are very similar to results obtained with c[-RGDfK-]-Aoa-[^{18}F]-FDR,[21d] confirming that addition of the biotin moiety does not appear to affect binding of c[-RGDfK-] to $\alpha_v\beta_3$ integrin.

In conclusion, the results above demonstrate that palladium-mediated reductive deiodination of 5-iodotriazoles synthesised by click chemistry is a versatile and straightforward method for producing isotopically labeled 1,2,3-triazole bioconjugates, in particular 5-[3*H*]-1,2,3-triazoles that can be used as multifunctional probes in biology and molecular imaging.

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Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

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