Synthesis and use of 2-[^{18}F]fluoromalondialdehyde, an accessible synthon for bioconjugation

Project Description: We proposed methods for the synthesis and purification of 2-[^{18}F]fluoromalondialdehyde, which will be a readily accessible synthon for bioconjugation. Our achievements in these areas will specifically address a stated goal of the DOE providing a transformational technology for macromolecule radiolabeling. Accomplishment of our aims will serve both DOE mission-related research as well as nuclear medicine research supported by the NIH and industry. At the heart of our proposal is the aim to "improve synthetic methodology for rapidly and efficiently incorporating radionuclides into a wide range of organic compounds."

Final report: Shortly after the submission of the first-year progress report, we determined FDG defluorinated under most oxidation conditions that we explored for 2-deoxyglucose. Thus, we pivoted the project to leverage new reaction methods that employ palladium, with the overall goal being the same – to develop rapid and predicable labeling of peptides and other macromolecules. The updated strategy relied on our previous success using Pd-complexes to rapidly label aromatic compounds with carbon-11 cyanide and is depicted in Figure 1. The work was accomplished in collaboration with Steve Buchwald's group at MIT. A manuscript on the work is being prepared, which is excerpted below for this final report.

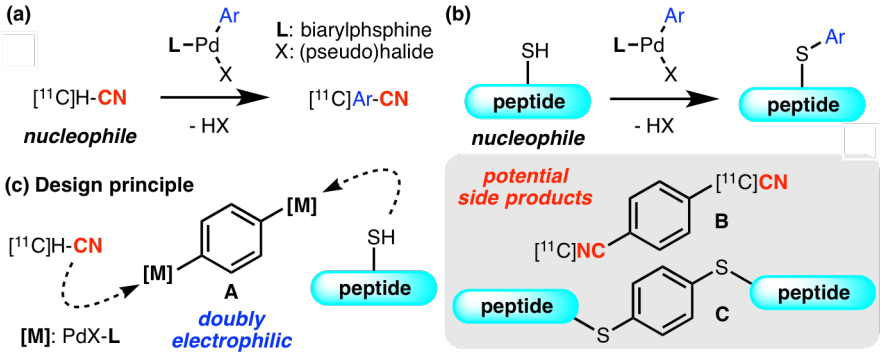
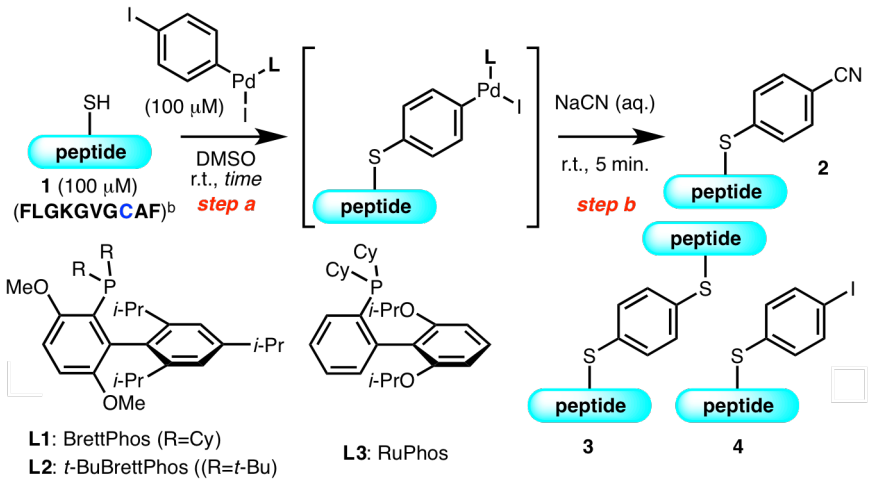


Figure 1. Design principle. (a) Recently, we have demonstrated that Pd-based oxidative addition complexes supported by biarylphosphine ligands are able to mediate challenging cross-coupling reactions in highly specialized settings. Using this strategy, extremely mild and efficient [^{11}C]-cyanation of aryl (pseudo)halides and (b) and arylation of a cysteine residue in native peptides. (c) We envisioned that a combination of these two approaches might find utility as a direct radiolabeling method for unmodified peptides. We hypothesized that the use of a doubly electrophilic organometal-lic entity (A) would be able to adjoin the precious radionuclide and equally important peptide substrate.

To evaluate our revised peptide labeling strategy, we studied the introduction of a non-radioactive cyanide source to model peptide 1 bearing an intrachain cysteine residue (Table 1). The peptide substrate was first treated with an oxidative addition complex derived from 1,4-diiodobenzene in DMSO, followed by an aqueous solution of NaCN. In the presence of the Pd complex supported by BrettPhos (L1), an efficient ligand for the [11C]-cyanation,12 the desired double cross-coupling product (2) was consistently observed in 34% average yield (Table 1, entry 1). Importantly, throughout the investigation double coupling product 3 and the intermediate from the first cross-coupling 4 were observed as the only peptidic side products, which were easily separable from the desired product. With the cyanide source as the limiting reagent, a condition that is usually employed in radiosyn-thesis, the product was obtained in consistently high yield, suggest-ing application to radiosynthesis was possible (Table 1, entry 2-3). Other variations in the reaction conditions, such as the use of an excess amount of Pd complex (Table 1, entry 4), elongated reaction time for step a (Table 1, entry 5), or buffered reaction media, a con-dition that should allow for wider application of the method, (Table 1, entry 6), did not significantly affect the outcome. Another effective ligand for [11C]-cyanation, t-BuBrettPhos (L2), also exhibited a high level reactivity for the desired transformation (Table 1, entry 7).12 On the other hand, the use of RuPhos, the ligand utilized in cysteine arylation,13 was much less effective and provided 4 as the major product (Table 1, entry 8). The optimized conditions were tested in a radiosynthesis using aqueous [11C]HCN as a [11C]-cyanide source (Table 2). While the results with the non-radioactive cyanide source were successfully extended to the radioactive system using L1 (Table 2, entry 1),17 the Pd complex supported by L2 or L3 showed significantly reduced reactivity (Table 2, entries 2 and 3). The contrast in reactivity com-pared to

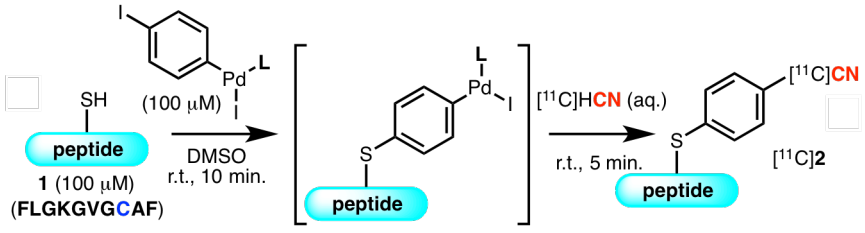
Table 1. Non-radioactive Cyanation of a Peptide.[a]



entry	L	reaction time of ***step a*** (min.)	stoichimotetry of NaCN (equiv.)	yield (%)[c]
1	**L1**	10	1	34[d]
2	**L1**	10	0.2	46
3	**L1**	10	0.1	61
4[e]	**L1**	10	1	31
5	**L1**	30	1	33
6[f]	**L1**	10	1	31
7	**L2**	10	1	64
8	**L3**	10	1	13

[a]Reaction conditions: **1** (20 nmol), Pd complex (20 nmol), DMSO (200 μL), r.t., 10-30 min.; NaCN (aq.) in 10 μL H_2O, 5 min. [b]AcNH-**FLGKGVGCAF**-CO_2H. [c]Determined by integration of HPLC peak area with calibration. [d]Average of three runs. [e]Results with 1.5 equivalent of Pd complex. [f]Result with 0.1 M Tris buffer in DMSO/H_2O (9:1) in place of DMSO.

Table 2. [^{11}C]-Cyanation of a Peptide.[a]



entry	L	RCY (%)
1	**L1**	50
2	**L2**	7
3	**L3**	< 5

[a]Reaction conditions: **1** (20 nmol), Pd complex (20 nmol), DMSO (200 μL), r.t., 10 min.; [^{11}C]HCN in H_2O (20 μL, 1-10 mCi), r.t., 5 min.

the non-radioactive system exemplifies the challenges as-sociated with the extension of a synthetic method to a radiochemical setting. Nonetheless, L1 exhibited desirable reactivity under both non-radioactive and radiosynthetic conditions, and thus was chosen as the ligand for subsequent studies. We further optimized reaction conditions and scaled the process to demonstrate that the reaction can make high specific activity radiolabeled peptides. This strategy exploits the nucleophilicity of both the peptide and labeling reagent, [11C]-cyanide, which enables “nucleophile-nucleophile coupling” in a direct manner. The method tolerates a wide range of reactive functional groups due to its mild reaction conditions and high chemoselectivity. A key feature of this method is that it can be successfully used on an extremely small amount of the peptide precursor (20 nmol), which not only simplifies purification, but is also economi-cally advantageous. The ease of operation of this method should allow for the development of 11C-peptide PET probes and also the evaluation of the therapeutic efficacy of peptides through PET imaging.