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C-H radiocyanation of bioactive molecules via sequential iodination/copper-mediated crosscoupling[†]

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This report describes a net C-H radiocyanation reaction for the transformation of electron rich (hetero) aromatic substrates into ¹¹CN-labeled products. Electrophilic C(sp²)-H iodination of the (hetero)arene with N-iodosuccinimide is followed by Cu-mediated radiocyanation with K^{11} CN. This sequence is applied to a variety of substrates, including the nucleobases uracil and cytosine, the amino acids tyrosine and tryptophan, and the peptide LYRAGWRAFS, which undergoes selective C-H radiocyanation at the tryptophan (W) residue.

The incorporation of ¹¹C into organic molecules is a valuable approach for generating positron emission tomography (PET) radiotracers.¹ [¹¹C]Cyanide is a particularly valuable ¹¹C synthon, as it is readily available from small medical cyclotrons and is an excellent nucleophile for both organic and transition metal-mediated reactions.^{2,3} We recently reported a Cumediated method for the radiocyanation of aryl halides with ^{[11}C]cyanide.⁴ Using CuI in combination with 1,2-dimethylethylene diamine (dmeda) as a ligand⁵ enables the rapid reaction of aryl halides with $[^{11}C]CN^-$ to afford $[^{11}CN]aryl$ nitrile products. This transformation shows extremely high functional group tolerance and thus offers an attractive approach for the radiolabeling of biological molecules such as unprotected peptides. However, a key limitation of the method is that aryl halides are not native functional groups in biology. As such, de novo solid-phase peptide synthesis (SPPS) needs to be employed to access each of the aryl halide-containing precursors required for radiocvanation (Scheme 1A). For instance, the $[^{11}C]CN$ radiolabeling of opioid receptor agonist nociceptin necessitated the synthesis of this 17-amino acid peptide with an iodophenylalanine inserted in place of the native leucine at residue 14. This approach is particularly undesirable in cases where (1) the native peptide or protein is readily available (such that a more direct approach for [¹¹C]cyanation would greatly expedite radiolabeling), (2) the requisite iodo-amino acid building block for SPPS is not readily available (or is unstable), or (3) local SPPS

equipment and/or expertise is unavailable (which is the case for most PET radiochemistry facilities).

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A complementary approach would involve directly converting a native peptide (or other bioactive molecule) into a radiocyanated product in a single pot (net C-H radiocyanation; Scheme 1B). We reasoned that this could be accomplished by combining electrophilic C(sp²)-H iodination of a (hetero)arene with subsequent Cu-mediated radiocyanation of the in situgenerated aryl iodide. In this sequence, radiocyanation should occur selectively at the most electron rich $C(sp^2)$ -H site of the molecule. Notably, there is literature precedent for the



Scheme 1 (A) Our prior work: de novo solid phase peptide synthesis (SPPS) of iodine-containing peptides for ¹¹CN radiolabeling.⁵ (B) This work: net C-H radiocyanation of native peptides via electrophilic iodination/Cu-mediated radiocyanation sequence, which opens the door for ultimately labeling full proteins.

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electrophilic iodination of amino acids, peptides, and proteins, supporting the feasibility of the first step of this sequence on such scaffolds.⁶ In this *Edge Article*, we demonstrate the viability of this approach for the net C–H radiocyanation of diverse arene and heteroarene substrates, including unprotected peptides. Furthermore, we demonstrate the selective radiolabeling of tryptophan moieties in the presence of other aromatic amino acids.

Our initial studies focused on establishing a one-pot eleciodination/radiocyanation sequence trophilic for two substrates: 1,1'-biphenyl (1) and tyrosine (2). These were selected to represent the range of targets of interest, from hydrophobic molecules with modest nucleophilicity (exemplified by 1) to those that are hydrophilic and electron rich and contain other functional groups (exemplified by 2). Key considerations included (i) developing mild conditions for electrophilic iodination that minimize over-functionalization and other side reactions, (ii) identifying an electrophilic iodinating reagent whose by-products do not interfere with the radiocyanation step, and (iii) developing a sequence that is practical and reproducible for both substrates.

We surveyed four reagents that have been employed in the literature for the iodination of electron rich arenes and peptides: *N*-iodosuccinimide (NIS),⁷ I(Py)₂BF₄ (IPy₂),⁸ NaI/ chloramine (NaI/Chlor),⁹ and NaI/Selectfluor® (NaI/Select).¹⁰ Initial screens were carried out at 25 °C using dichloromethane/ acetonitrile/trifluoroacetic acid as the solvent system, since this mixture effectively dissolves both 1 and 2 and is also straightforward to remove *via* a nitrogen purge. The yield/selectivity of C-H iodination for each substrate was determined by ¹H NMR spectroscopic analysis of the crude reaction mixture (Table 1). For both 1 and 2, NIS and IPy₂ afforded similarly high (70–80%) yields of the mono-iodination product. In contrast, significantly lower yields (<1–35%) were obtained with NaI/Chlor and NaI/ Select.

We next moved forward to the C-H iodination/ radiocyanation sequence using NIS and IPy2. Both substrates 1 and 2 were subjected to the iodination conditions in Table 1, and then the volatiles were removed. Cu-mediated radiocyanation was performed on this crude material without further purification, using our previously reported conditions.⁴ The radiochemical yield (RCY) of each reaction was determined by radio-TLC and/or radio-HPLC. As summarized in Table 2, significant differences in RCY were observed as a function of the choice of I⁺ reagent. With NIS, 1-¹¹CN was formed in $68 \pm 14\%$ RCY (n = 6), while lower RCY $(40 \pm 4\%, n = 3)$ was obtained with IPy₂. Similarly, using 2 as a substrate, the NIS reaction afforded a higher RCY (68 \pm 4%, n = 4) than that with **IPy**₂ (54 \pm 7%, n =3). Based on these results, NIS was selected as the iodinating reagent moving forward.11

Under these optimal conditions, we evaluated the scope of C-H radiocyanation with a range of arene and heteroarene substrates (Scheme 2). In all cases, selectivity was confirmed by comparison to authentic standards of the ¹²CN products. Ether and amine-substituted aromatic substrates react under these conditions to form 2-9-¹¹CN in radiochemical yields ranging from 36–86% (Scheme 2A). As anticipated based on the

Table 1 Optimization of the $C(sp^2)$ -H iodination of 1 and 2^a



Entry	ArH	\mathbf{I}^+	Yield ArI
1	1	NIS	77%
2	1	IPy ₂	78%
3	1	NaI/chlor	$<1\%^{b}$
4	1	NaI/select	$9\%^b$
5	2	NIS	75%
6	2	IPy ₂	70%
7	2	NaI/chlor	35% ^c
8	2	NaI/select	$16\%^b$

^{*a*} Conditions: 1 equiv. (0.05 mmol) of ArH, 1 equiv. of $[I^+]$, 0.2 mL of trifluoroacetic acid (TFA), 2 mL of dichloromethane (DCM), 0.25 mL of acetonitrile (MeCN) at 25 °C for 2 h. ^{*b*} Mass balance was primarily unreacted starting material as determined by ¹H NMR spectroscopy. ^{*c*} Mass balance was primarily unreacted starting material and 4% diiodottyrosine as determined by ¹H NMR spectroscopy.

mechanism of electrophilic iodination, these transformations proceed with high selectivity for sites *ortho*- and/or *para*- to resonance electron donating substituents. This sequence is effective for simple aromatics (forming **3-5**-¹¹**CN**), as well as arene rings in more highly functionalized bioactive molecules, such as lidocaine (to form **6**-¹¹**CN**), a precursor to the radio-tracer PIMBA (to form **7**-¹¹**CN**), nimesulide (to form **8**-¹¹**CN**), and febuxostat (to form **9**-¹¹**CN**).



^{*a*} Iodination conditions: 1 equiv. (0.05 mmol) of ArH, 1 equiv. of [I⁺], 0.2 mL of trifluoroacetic acid (TFA), 2 mL of dichloromethane (DCM), 0.25 mL of acetonitrile (MeCN) at 25 °C. Radiocyanation conditions: $\rm H^{11}CN$, 0.5 equiv. of CuI, 1 equiv. of dmeda, 0.25 equiv. of K₃PO₄, 5.5 : 1 DMF/H₂O (163 µL). ^{*b*} 24 h. ^{*c*} 2 h. RCY = radiochemical yield.



Scheme 2 Substrate scope for net $C(sp^2)$ -H radiocyanation. RCY (radiochemical yield) calculated by radio-HPLC and radio-TLC as detailed in the ESI.† Unless otherwise noted a single regioisomer was detected in both the C-H iodination and radiocyanation steps. ¹2% of a regioisomeric product was observed.

Electron rich heterocycles are also viable substrates (Scheme 2B). For instance, benzothiophene, pyrazole, pyrazolylpyridine, and aminopyridine derivatives react to form **10-13**-¹¹CN in radiochemical yields ranging from 45–79%. The nucleobases uracil and cytosine afford the ¹¹CN-labeled analogues **14**-¹¹CN and **15**-¹¹CN in 69% and 84% radiochemical yield, respectively. Comparable radiochemical yield (75%) is obtained for the glycosylated base in cytidine (to form **16**-¹¹CN). Overall, the examples in Scheme 2 highlight the compatibility of this method with diverse functional groups, including 1° and 2° alcohols, carboxylic acids, amines, amides, and sulfonamides.

Finally, we evaluated three aromatic amino acids, phenylalanine, tyrosine (Tyr, Y), and tryptophan (Trp, W), along with peptides containing one or more of these residues. We sought to establish (1) which individual aromatic amino acids participate in this net C–H radiocyanation sequence and (2) the selectivity of the reaction when multiple aromatic amino acids are present. Consistent with literature reports,¹² phenylalanine proved inert towards electrophilic iodination/radiocyanation in all contexts examined (both as a free, unprotected amino acid and in various peptides). In contrast, tyrosine underwent high yielding iodination/ radiocyanation as the unprotected amino acid (yielding 2-¹¹CN in 68 ± 14% RCY, n = 4), as an acyl-methyl protected derivative (to form 17-¹¹CN in 44 ± 5 RCY, n = 4), and in the short peptide Ac-GYG-NH₂ (affording 18-¹¹CN in 67 ± 7% RCY, n = 3; Scheme 3A). These results are consistent with literature reports showing selective electrophilic iodination of tyrosine *ortho*-to the OH substituent as well as the compatibility of phenols with Cu-mediated radiocyanation of aryl halides.⁴ This radiosynthesis of 18-¹¹CN from tyrosine was automated in a commercial synthesis module, and the product was purified by a preparatory HPLC, affording a 23 ± 9% isolated RCY, 1.2 ± 0.1 Ci µmol⁻¹ molar activity, n = 2; see ESI Section 6.3[†]).

We anticipated challenges with both steps in the net C–H radiocyanation of tryptophan. First, the reaction of tryptophan derivatives with electrophilic iodinating reagents has been reported to form mixtures of products due to the low stability of 2-iodotryptophan as well as competing oxidation and cyclization pathways.¹⁴ Second, in our previous studies of Cu-mediated radiocyanation, 2-haloindoles were among the poorest performing substrates.⁴ These challenges are reflected in our initial results using unprotected tryptophan and the protected derivative Ac-Trp-NH₂ as substrates. Under the standard electrophilic iodination/radiocyanation conditions, both afforded very low yields of ¹¹CN-labeled products (none detected and 2%, respectively). We hypothesized that judicious selection of protecting groups (specifically eliminating nucleophilic NH₂



Scheme 3 (A) Amino acid substrates. (B) Competition study between tyrosine and tryptophan derivatives.

groups proximal to the 2-position) might enable the target transformation. Indeed, using Ac-Trp-OMe as a substrate, the radiolabeled product **20-¹¹CN** was formed selectively in $10 \pm 3\%$ RCY (n = 5).¹⁵ The selectivity of C–H radiocyanation for the 2-position was confirmed by comparison with a ¹²CN-containing authentic sample. While the yield of this transformation is modest, it is sufficient for most radiochemistry applications.

Our final objective was to achieve selective ¹¹CNradiolabeling of peptides containing multiple aromatic amino acids. To test the selectivity of this C–H iodination/ radiocyanation sequence, a competition experiment was conducted between Ac-Trp-OMe and Ac-Tyr-OMe. As shown in Scheme 3B, treatment of a 1:1 mixture of these protected amino acids with 1 equiv. of NIS followed by the standard radiocyanation conditions resulted in selective formation of **20-¹¹CN** (in 8 ± 1% RCY, n = 3). **17-¹¹CN** was not detected. The observed selectivity is consistent with the higher nucleophilicity of tryptophan *versus* tyrosine. The modest overall RCC of **20-¹¹CN** is likely due to a combination of side reactions during the iodination step, the modest stability of iodotryptophan intermediate, and low efficiency of the radiocyanation at the 2position of the indole.^{4,13}

Based on the high selectivity of this competition experiment, we pursued the net C–H radiocyanation of the peptide LYR-AGWRAFS, which contains three different aromatic amino acids (Scheme 4). Subjecting the peptide to our optimal electrophilic iodination/radiocyanation conditions in an automated radio-synthesis module afforded a single detectable radiocyanated product (as determined by radio-HPLC analysis) in 17 \pm 3% RCC. A series of experiments was conducted to establish the site selectivity of radiolabeling, including synthesis of an authentic sample of the ¹²CN-tyrosine peptide (which does not match the

observed product by HPLC) and trypsin digestion of the initial C–H iodination product (which shows that the iodine is incorporated exclusively on the tryptophan fragment). Overall, our data (see ESI,† p. S56–S61 (Section 7) for a complete summary) are fully consistent with selective radiocyanation of the tryptophan residue in this peptide as shown in Scheme 4. Notably, this ¹¹CN-labelled peptide would not be accessible *via* the original SPPS approach in Fig. 1A,† since the required 2-iodo-tryptophan building block is not sufficiently stable for isolation and use in peptide synthesis.

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Conclusions

In conclusion, this report demonstrates a simple two-step net $C(sp^2)$ -H radiocyanation reaction. This transformation is operationally simple, proceeds smoothly with peptides, and can be conducted in automated in a commercial radiosynthesis module (substrates **18-¹¹CN** and **21-¹¹CN**). It provides a straightforward approach to selectively introducing a ¹¹CN moiety on tryptophan residues in a peptide containing multiple other aromatic amino acids without the need to synthesize dedicated labelling precursors. Ongoing work is focused on applying this method to the ¹¹CN-radiolabeling of various bioactive molecules of interest to our PET imaging program, including proteins.

Data availability

Further details of experimental methods, additional analysis, and analytical data, as well as supplemental figures including NMR spectra, analytical TLC and HPLC traces, and additional screening and control experiments are included in the ESI.[†]

Author contributions

M. S. S., P. J. H. S. and A. K. M. conceived the idea, provided funding and directed the project. M. H., L. S. S. and S. T. J. synthesized reference standards and labeling precursors. M. H., L. S. S. and X. S. performed radiolabeling studies. M. H., M. S. S. and P. J. H. S. wrote the manuscript. All authors reviewed and approved the final version.

Conflicts of interest

The authors declare no competing financial interests.

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Scheme 4 Selective C–H radiocyanation of the tryptophan residue of LYRAGWRAFS.

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- 15 The reproducibility of reactions forming 20-¹¹CN and 21-¹¹CN was enhanced by the addition of sodium ascorbate (1 equiv.) to the mixture during the radiocyanation step. However, the sodium ascorbate additive did not have a similar favorable impact with the other substrates. With 20-¹¹CN and 21-¹¹CN there is often a significant amount of NIS remaining following the iodination step, and we hypothesize that the role of sodium ascorbate is to quench this so that it does not oxidize the Cu^I mediator to the unreactive Cu^{II} state.