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Reductant-Induced Free Radical Fluoroalkylation of Nitrogen Heterocycles and Innate Aromatic Amino Acid Residues in Peptides and Proteins

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Abstract: A series of fluoroalkylated cyclic λ^3-iodanes and their hydrochloride salts was prepared and used in a combination with sodium ascorbate in buffer or aqueous methanol mixtures for radical fluoroalkylation of a range of substituted indoles, pyrroles, tryptophan or its derivatives, and Trp residues in peptides. As demonstrated on several peptides, aromatic amino acid residues Trp, Tyr, Phe and His are targeted with high selectivity to Trp. The functionalization method is biocompatible, mild, rapid and transition metal-free. Proteins myoglobin, ubiquitin and human carbonic anhydrase I were also successfully functionalized.

Introduction

Selective methods for late-stage functionalization of organic molecules and biomolecules are highly sought after. Of special interest for basic biological research and drug development are methods that allow fast and selective protein functionalization targeting specific amino acid residues.[1-4] Typical standard methods include lysine functionalization with N-hydroxysuccinimide ester of carboxylic acid, a reaction of highly nucleophilic cysteine in alkylation with haloaceamides or reaction with maleimides or related Michael acceptors. In comparison, methods that enable direct functionalization of aromatic amino acids are less common. Examples include ene-type functionalization of tyrosine residues with triazolodiones[5] or azo-coupling using aryldiazonium reagents.[6] Tryptophan residues were selectively modified using metallocarbenes in aqueous media. A carbene derived from a vinyl diazo compound and a rhodium catalyst reacted with nitrogen and C(2) of tryptophan indole ring.[7] A transition metal-free procedure based on stabilized aminoxyl radical for tryptophane-selective bioconjugation was reported recently.[8] Radical trifluoromethylation of Trp residues using sodium trifluoromethanesulfinate (CF_3SO_2Na) and *t*-butylhydroperoxide allowed the preparation of fluorinated protein constructs for ^{19}F NMR studies.[9] However, there are no biocompatible and rapid methods targeting His and Phe residues.

Trifluoromethylated cyclic λ^3-iodanes (Togni reagents) and their tetrafluoroethylene-containing analogues **1** have been previously used as electrophilic fluoroalkylating species in reactions with various sulfur-, oxygen-, phosphorus- and carbon-centered nucleophiles and also sulfur-centered biomolecules including bioconjugations of Cys residues (Figure 1A).[10-12] The concept of reductive trifluoromethylation of small molecules such as alkenes or aromatics using Togni reagents and transition-metal (Cu, Fe and photochemically using Ru or Ir) or non-metallic (NaI, TEMPONa) reductants has been realized successfully on number of examples (Figure 1B).[13-14] However, reported conditions did not allow to perform this functionalization with biomolecules in aqueous environment under ambient conditions. Here we describe the development of a radical functionalization method based on the reduction of λ^3-iodanes with water-soluble and biocompatible reductant targeting small molecules (indoles, pyrroles) and peptides or proteins on aromatic amino acid residues with high selectivity to tryptophan (Figure 1C). The reactions operate in water under ambient conditions, at micromolar concentration and with high reaction rates, thus expanding the protein functionalization toolbox. Related to this work is the report of ethynylation of Trp-containing peptides (in acetonitrile and trifluoroacetic acid) using an ethynyl-containing λ^3-iodane and a gold catalyst.[15]

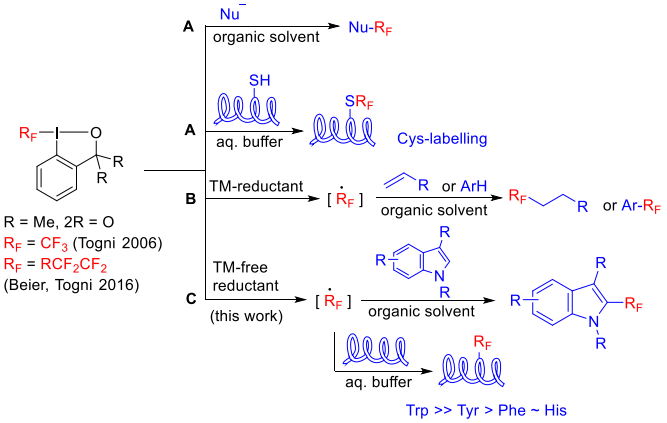

Figure 1. Previously reported (**A**, **B**) and hereby presented (**C**) fluoroalkylations of small organic substrates and biomolecules using λ^3-iodanes.

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Results and Discussion

Structures of λ^3-iodanes used in this study are shown in Figure 2. They are all stable compounds and include commercial Togni reagents **1a** and **1b**, previously reported tetrafluoroethyle-containing iodanes **1c**, **1d** and **1i** and reagents **1e**, **1f** and salts **1g**, **1h**, **1j**. The salt **1i** was shown to undergo functionalization on the amino group using acyl chlorides or sulfonyl chlorides for late-stage customization with a functional group (demonstrated by an attachment of fluorescent groups, biotin, short oligo(ethyleneglycol) chain, and various functional groups, including CHO, I, Bpin, N_3, ethynyl, and other).[12] Iodanes **1e-1h** and **1j** were prepared by umpolung reactions of appropriate fluoroalkylsilanes with fluoro- or acetoxyiodanes followed by protonation with HCl or reaction with acetyl chloride (Scheme 1).[16]

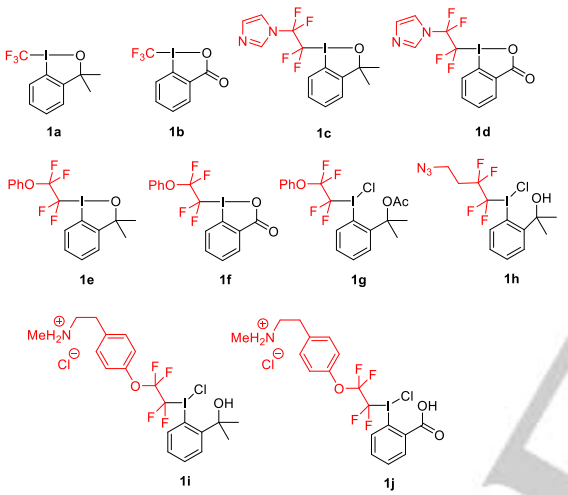
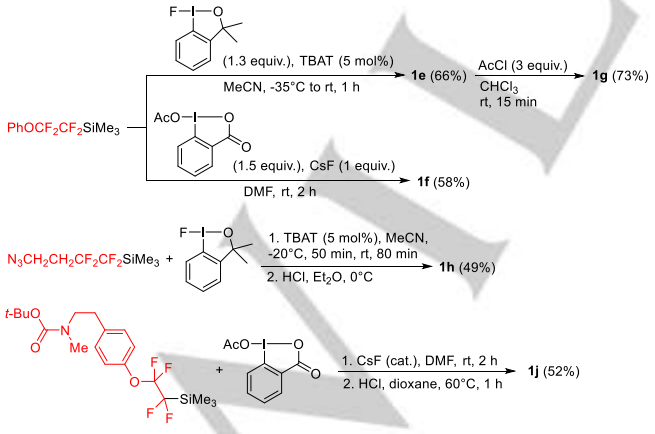


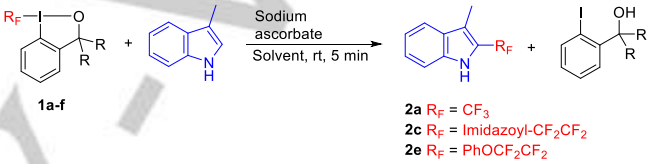
Figure 2. Structures of λ^3-iodanes **1** used in this study.



Scheme 1. Synthesis of new reagents **1**.

For optimization study, an electron-rich aromatic substrate 3-methylindole (skatole) was used. It has been previously fluoroalkylated using **1a-1c** activated by $Zn(NTf_2)_2$, $ZnBr_2$, TMSOTf, CuOAc or $MeReO_3$ (20–80 °C).[11,17-19] We evaluated reagent **1f** with skatole and a number of reductants in methanol or ethanol (with minimum water when necessary). While reductants such as Na_2SO_3, $FeCl_2$, CuOAc, NaH_2PO_3 and Et_3B afforded the product **2e** in maximum 5–10% ^{19}F NMR yields, the use of sodium ascorbate afforded **2e** in 42% ^{19}F NMR yield (Table 1, entry 1). Optimization of solvent, reaction time, temperature, amount of reagent, and ascorbate revealed that the best conditions were a slight excess of **1f** and 50 mol% of sodium ascorbate in aqueous methanol (Table 1). The reaction was finished in less than 5 min at ambient temperature and delivered

Table 1. Optimization of radical fluoroalkylation of 3-methylindole with hypervalent iodine reagents **1** and sodium ascorbate.



Entry	**1** (equiv.)	Na Asc. (equiv.)	Solvent	Yield (%)[a]
1[b]	**1f** (1.0)	0.5	EtOH	**2e**, 42
2	**1f** (1.0)	0.5	EtOH/H_2O (1:1)	**2e**, 44
3	**1f** (1.2)	0.5	Dioxane/H_2O (1:1)	**2e**, 30
4	**1f** (1.0)	0.5	MeOH/H_2O (6:1)	**2e**, 49
5	**1f** (1.2)	0.5	MeOH/H_2O (6:1)	**2e**, 53
6	**1f** (1.5)	0.5	MeOH/H_2O (6:1)	**2e**, 59
7[c]	**1f** (1.2)	0.5	MeOH/H_2O (6:1)	**2e**, 53
8[d]	**1f** (1.2)	0.5	MeOH/H_2O (6:1)	**2e**, 32
9	**1f** (1.2)	1.0	MeOH/H_2O (6:1)	**2e**, 46
10	**1f** (1.2)	0.05	MeOH/H_2O (6:1)	**2e**, 39
11[e]	**1f** (1.2)	0.5	MeOH/H_2O (6:1)	**2e**, 98
12[e]	**1e** (1.2)	0.5	MeOH/H_2O (6:1)	**2e**, 91
13[e]	**1a** (1.2)	0.5	MeOH/H_2O (6:1)	**2a**, 34
14[e]	**1b** (1.2)	0.5	MeOH/H_2O (6:1)	**2a**, 53
15[e]	**1c** (1.2)	0.5	MeOH/H_2O (6:1)	**2c**, 63
16[e]	**1d** (1.2)	0.5	MeOH/H_2O (6:1)	**2c**, 54

[a] ^{19}F NMR yield using (2,2,2-trifluoroethanol) as an internal standard. [b] Reaction time was 1 h. [c] Reaction temperature was –78 °C. [d] Reaction temperature was 40 °C. [e] Slow addition (over 5 min) of a solution of **1** in MeOH to the solution of skatole and sodium ascorbate in aq. MeOH.

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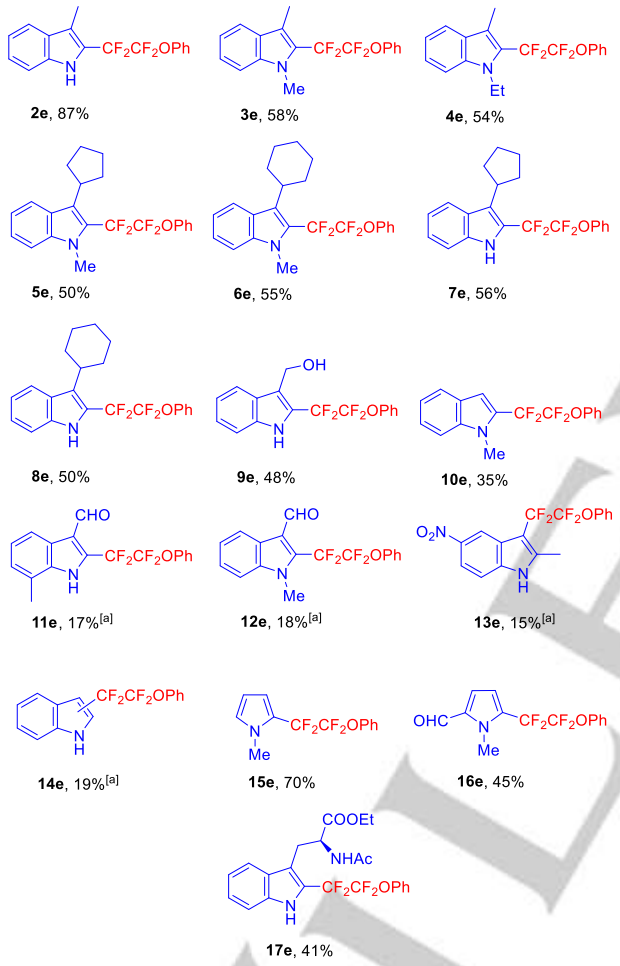
2e in high yields, while for **1a-d** the yields were somewhat reduced. The order of addition was very important for achieving high product yields. Hypervalent iodine reagents **1** needed to be added last to achieve the best results.

When exploring the scope of radical fluoroalkylation of indole derivatives under optimal reaction conditions (Table 1, Entry 11), it was found that indoles with alkyl, cycloalkyl or other electron-rich groups in position 1, 2 or 3 underwent smooth conversion to indoles fluoroalkylated in position 2 or 3 (Scheme 2). Indoles substituted with electron-acceptor groups (such as formyl or nitro) afforded fluoroalkylated products in low yields.

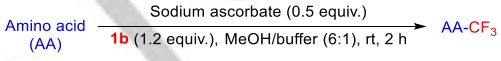


Scheme 2. Fluoroalkylation of indoles and pyrroles with **1f**. Isolated yields are shown. [a] ^{19}F NMR yield using (2,2,2-trifluoroethanol) as an internal standard.

A similar outcome was observed with unsubstituted indole and *N*-methyl pyrroles. *N*-Methyl indole afforded two isomers in a 65:35 ratio. The major isomer **10e** was isolated in 35% yield. Protected tryptophan was fluoroalkylated in position 2 in moderate yield. A number of substrates such as caffeine, adenosine monophosphate, but also electron-rich phenol or 2-methylthiophene were resistant to fluoroalkylation under the applied reaction conditions (not shown in Scheme 2).

Evaluation of aromatic amino acid derivatives in trifluoromethylation with a small excess of **1b** revealed that Trp was much more reactive than Tyr, Phe or His. Conducting the reaction in basic buffer increased the initially low reactivity of Tyr and His which is consistent with the formation of more electron-rich substrates by the deprotonation of aromatic OH group of Tyr or imidazolium ring of His (Table 2). A clear correlation between electron density of aromatic amino acid derivative and reactivity in trifluoromethylation with **1b** was found.

Table 2. Fluoroalkylation of amino acid derivatives with **1b**.



Entry	Amino acid deriv.	Product yield (%)[a]
1	N-Ac Trp	56 + 6[b][c]
2	N-Ac Tyr	11[b], 18 (pH 9)
3	Phe[d]	0 (pH 5), 4 (pH 9)
4	His·HCl	2 (pH 5), 11 (pH 9)

[a] ^{19}F NMR yield using (trifluoroethanol) as an internal standard. [b] Water instead of buffer was used. [c] Mono- and bis(trifluoromethylation), respectively. [d] Phenylalanine ethyl ester hydrochloride was used.

In a competitive experiment, an equimolar mixture of all natural amino acids (5 mM, pH 7.5) was used for trifluoromethylation with **1a** and of sodium ascorbate (10 equiv. to each amino acid) and half the amount of sodium ascorbate. Semiquantitative LCMS analysis showed reactivity order as follows: Trp >> Cystine > Tyr > Phe > His (Figure 3). Other amino acids were not reactive. Extracted ion chromatograms indicated that with a large excess of **1a**, Trp afforded mono(trifluoromethylated) product and two isomers of the bis(trifluoromethylated) product and Phe provided two isomers of mono(trifluoromethylated) product. Reactivity of cystine can be explained by the presence of trifluoromethyl radicals (mechanism of the process vide infra) which cause homolytic cleavage of the S-S bond followed by radical recombination. When only reactive aromatic amino acids were evaluated in a competitive experiment using moderate excess of **1b** and **1d** and a large preference for the reaction with Trp to mono- or bis(fluoroalkylated) product was observed (Table 3).

Next, several short peptides containing aromatic amino acids were evaluated. A small peptide with the amino acid sequence AFRIPLYWGRI was fluoroalkylated with excess of **1e**, **1f** or **1h** (10 equiv.) and sodium ascorbate in aqueous acetonitrile. MALDI MS analysis showed the formation of conjugates with one fluoroalkyl modification (major product) and two modification (minor product) (Figure 4 and Figures S1-S3). The subsequent

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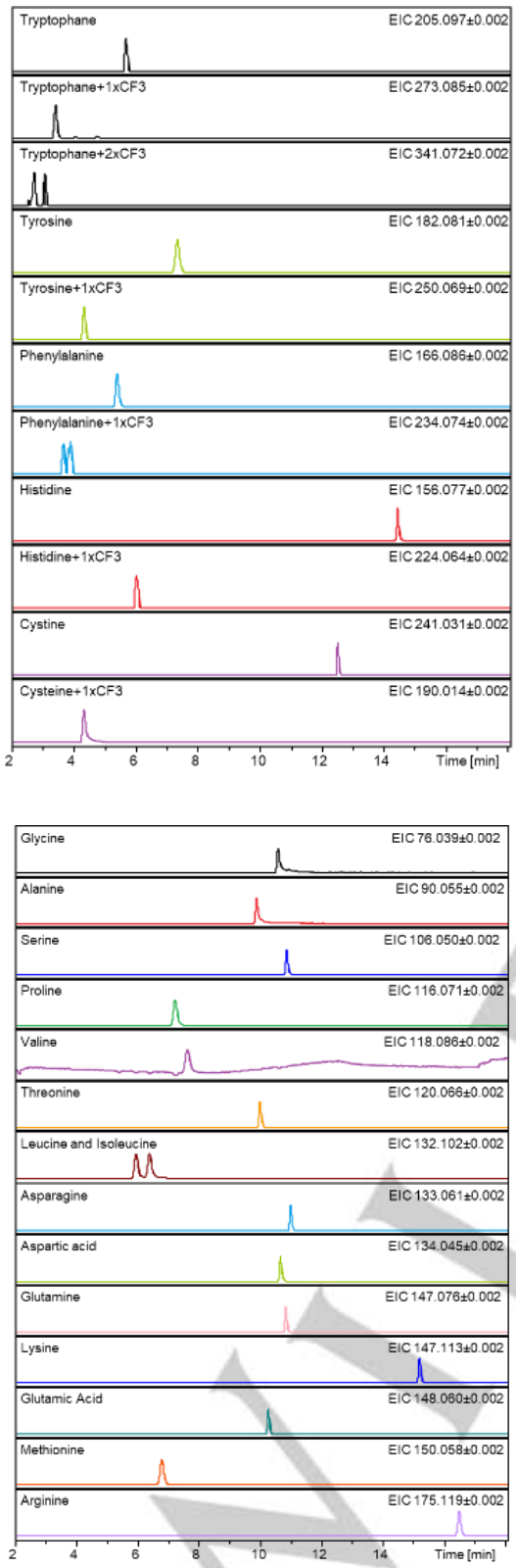

Figure 3. Extracted ion chromatograms of HPLC-MS analysis of a mixture of all natural amino acids in trifluoromethylation with **1a** (10 equiv. calculated to each amino acid) and sodium ascorbate (5 equiv. calculated to each amino acid) in buffer (pH 7.5), rt, 15 min. Reactive amino acids are shown on the left, unreactive amino acids are shown on the right.

Table 3. MS conversions of fluoroalkylated aromatic aminoacids in reactions with **1b** and **1d** and sodium ascorbate.[a]

Entry	Reagent	MS conversion (%)			
		Trp-R_F + Trp-$(R_F)_2$	Tyr-R_F	Phe-R_F	His-R_F
1	**1b** (10 equiv.)	75 + 8	12	2	<1
2	**1b** (50 equiv.)	38 + 62	40	10	7
3	**1d** (10 equiv.)	72 + 14	19	5	0
4	**1d** (50 equiv.)	0 + 100	26	9	0

[a] Sodium ascorbate (5 or 25 equiv.), buffer (pH 7.5), rt, 5 min.

MS/MS analysis confirmed that both modifications took place exclusively on the Trp residue (Figures S4 and S5). Formation of small amount of oxidized fluoroalkylated product (probably oxindole of the Trp residue) was often observed. The formation of this side-product was minimized by using an additive methionine or using one half of sodium ascorbate relative to hypervalent iodine reagent. Peptide TEVNAWLVHRDP in reaction with **1g** (10 or 100 equiv.) afforded mono(fluoroalkylation) and bis(fluoroalkylation) on the Trp residue (Figures S6-S9). The efficiency of fluoroalkylation was concentration dependent; at higher concentrations a complete fluoroalkylation was observed even with lower excess of **1g**.

Peptide bradykinin (RPPGFSPFR), an inflammatory mediator, causing dilatation of blood vessels required a large excess of **1j** for the fluoroalkylation to take place. This is consistent with the observed low reactivity of Phe residues and no reactivity of other amino acid residues present in bradykinin. MS analysis indicated partial formation of fluoroalkylated bradykinin on Phe residues (not selective) and only traces of bis(fluoroalkylated) bradykinin (Figure 4 and Figures S10 and S11). Cyclic peptide somatostatin is a hormone regulating human endocrine system, for example, it inhibits the secretion of insulin and glucagon. Somatostatin in reaction with **1j** afforded products of hydrogen substitution (not addition to the disulfide bridge): mono(fluoroalkylated) somatostatin as the main product, bis(fluoroalkylated) somatostatin as the minor product and traces of tris(fluoroalkylated) somatostatin (Figure 4 and Figure S12). Another investigated peptide was bombesin. It is a 14-amino acid peptide capable of stimulating gastrin release from G-cells. It is also a tumor marker for small cell carcinoma of lung, gastric cancer, pancreatic cancer, and neuroblastoma. Again, with a large excess of **1j** fluoroalkylation on Trp residue took place and the main products were mono- and bis(functionalized) with some traces of tris(fluoroalkylated) product and oxidized products (Figure 4 and Figures S13-S15).

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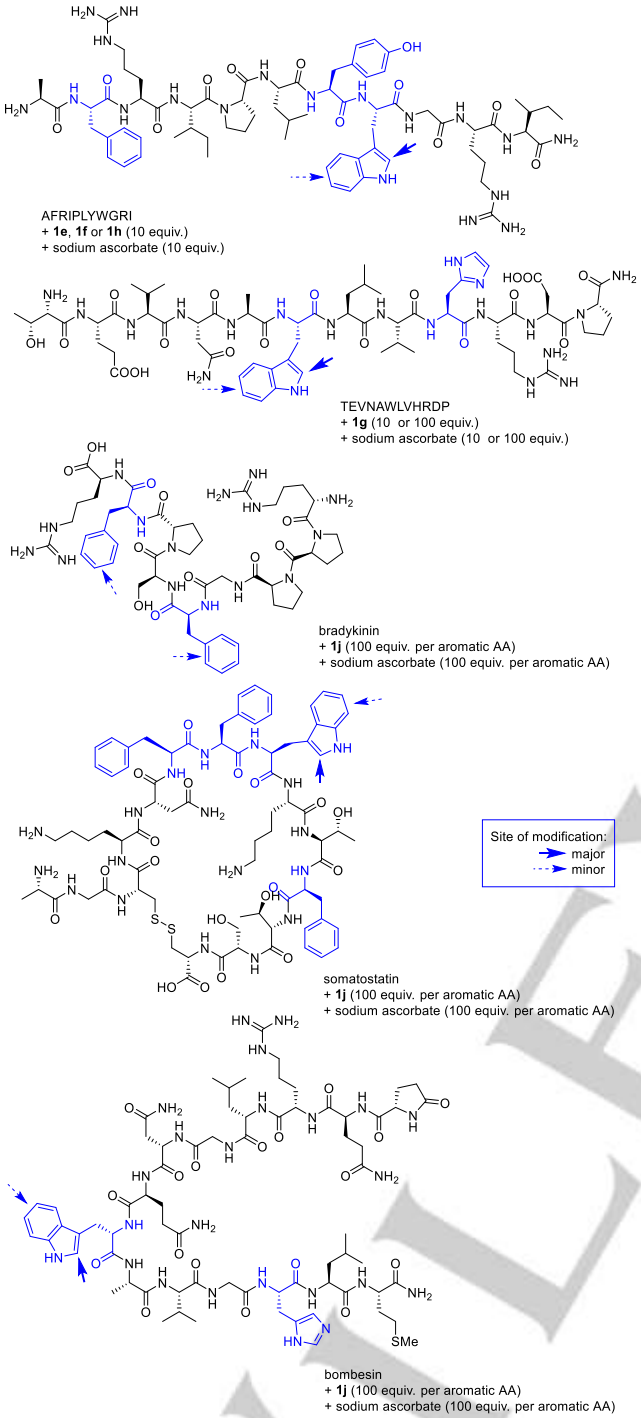

Figure 4. Structures of peptides AFRIPLYWGRI, TEVNAWLVHRDP, bradykinin, somatostatin and bombesin with aromatic amino acid residues highlighted in blue and their observed major and minor sites of functionalization using sodium ascorbate and **1** depicted by blue arrows. Sites of trace functionalization are not shown.

Human recombinant insulin in HEPES or TRIS buffer (pH 7-9) was subjected to fluoroalkylation with reagents **1c**, **1d**, **1i** and **1j** (20-100 equiv. to aromatic AA). This larger system consist of several aromatic amino acid residues (4 Tyr, 3 Phe, 2 His); however, no Trp residue. Comparison of **1c** and **1d** revealed that the 'acid type' reagent **1d** provided a deeper degree of fluoroalkylation (up to 8 modifications) than the 'alcohol type' reagent **1c** (Figures S16 and S17). Furthermore, at pH 9 the extent of fluoroalkylation was higher than at pH 7 or 8 for both reagents **1i** and **1j** (Figure S18), which is consistent with a higher degree of ionization of Tyr residues at higher pH and therefore creation of more electron rich reactive sites. Again, no signal corresponding to addition to the disulfide bridge was observed by MS.

To test the potential of fluoroalkylated hypervalent iodine compounds for the introduction of a specific tag on proteins, horse heart myoglobin and ubiquitin from bovine erythrocytes were selected as model proteins. Myoglobin (16.9 kDa) contains a number of aromatic amino acids (2 Trp, 2 Tyr, 7 Phe and 11 His). Fluoroalkylation of myoglobin with **1h** (100 equiv.) followed by product separation by gel filtration and click reaction with a dibenzocyclooctyne-amine (DBCO-amine) afforded myoglobin containing one and two fluoroalkyl-triazole modifications (Figure 5). Bottom-up approach revealed the site of modification being primarily on Trp14 residue and to a lesser degree also on Trp7 residue (Figure S19).

Since all above mentioned data show only modification of Trp residues, to shed light on the behavior of other aromatic amino acids, we carried out similar experiment with ubiquitin, a small (8.6 kDa) regulatory protein found in most eucaryotes. As far as aromatic amino acid residues are concerned, it contains one Tyr, one His, two Phe and no Trp residue. Ubiquitin was modified with **1h** (100 equiv.) and sodium ascorbate (50 equiv.) in 50 mM ammonium bicarbonate buffer (pH 7.5), followed by a click reaction with a DBCO-amine derivative. The extent of modification was similar to myoglobin. Prevalently, a mono(fluoroalkylated) protein was observed in MS data (Figure S20). Subsequent, top-down analysis determined the modification to take place on Tyr59 (Figure S21).

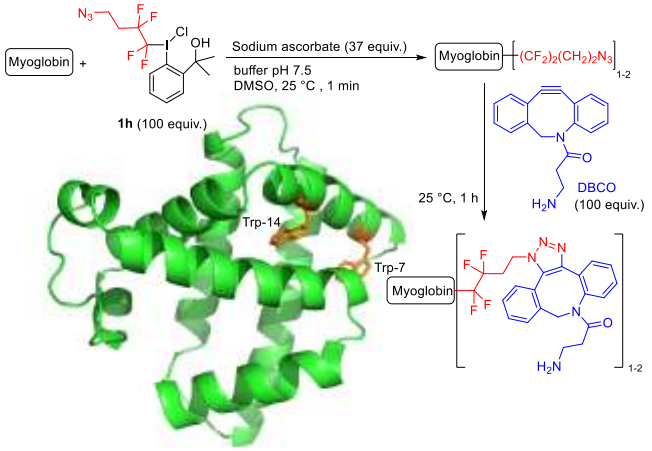

Figure 5. Structure of myoglobin with highlighted Trp7 and Trp14 residues, which underwent fluoroalkylation with **1h**, followed by the click reaction with DBCO and simplified fluoroalkylation reaction scheme of modification of myoglobin.

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Finally, carbonic anhydrase I from human erythrocyces (hCA I) (cca 29 kDa) at cca 17 μM concentration in ammonium bicarbonate buffer (pH 7.5) was fluoroalkylated with reagents **1a** (200 equiv.) and sodium ascorbate (150 equiv.). To determine the sites of fluoroalkylation, the reaction side products were removed using a polyacrylamide electrophoresis and the separated protein was analyzed using a bottom-up approach. Bands of interest were excised, digested by trypsin, and analyzed by LC-MS/MS. Fluoroalkylated residues were identified using Mascot search against hCA I sequence. For example, the analysis revealed that in the reaction with **1a** the residues Trp123, Tyr88, Phe84 and His67 were modified with one trifluoromethyl group (Figure S22). All modified residues are solvent accessible using 1hcb structural model of hCA I (Figure 6), which clearly demonstrated that after radical fluoroalkylation with **1a** the native structure of the protein was not perturbed.

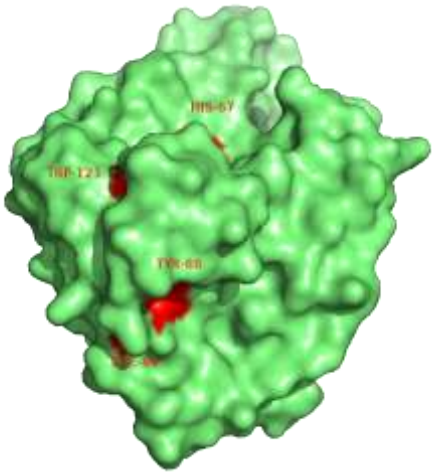
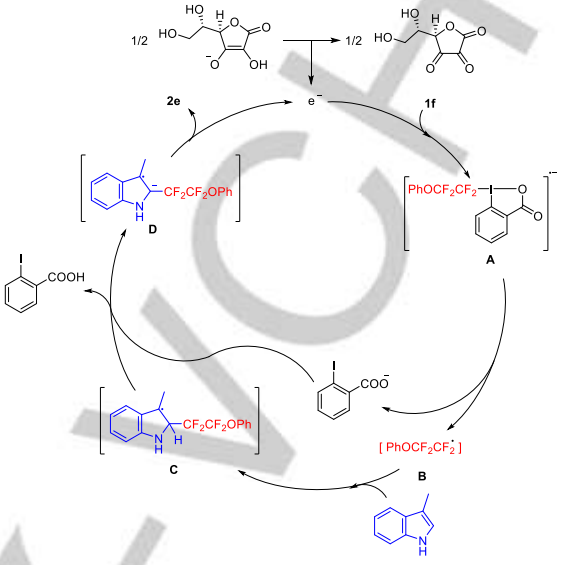


Figure 6. Structural model (1hcb) of hCA I with Trp123, Tyr88, Phe84 and His67 residues, which underwent fluoroalkylation highlighted in red.

The proposed simplified mechanism of sodium ascorbate-mediated fluoroalkylation of indole derivatives is shown in Scheme 3 (in the reaction of **1f** with 3-methylindole). Ascorbate is an efficient two-electron donor; however, under the conditions it donates one electron to the iodine reagent **1f** forming the transient radical anion **A**, which decomposes to 2-iodobenzoate and fluoroalkyl radical **B**. Radical addition of **B** to 3-methylindole leads to the formation of a stabilized benzylic radical **C**, which transfers proton to 2-iodobenzoate or any other base present in the reaction mixture to give the radical anion **D**. Finally, the reactive intermediate **D** is oxidatively aromatized by another SET process to afford the product **2e**, closing the catalytic cycle involving electron as the catalyst as proposed by Curran and Studer.[20] The oxidant involved in oxidative aromatization step of **D** to **2e** is either oxygen from air, **1f** (in the case of low ascorbate loading) or semidehydroascorbate radical (in the case of high ascorbate loading). Fluoroalkyl radical **B** is a relatively electron-poor species,[21] which prefers to react with electron-rich substrates, such as indoles or pyrroles. Further evidence for fluoroalkylation to proceed via radical pathway was obtained by performing a control experiment in which *N*-acetyl tryptophan with **1b** in the presence of TEMPO provided TEMPO-CF_3 adduct in 43% ^{19}F NMR yield and no product of trifluoromethylation of the tryptophan derivative (Figure S23).



Scheme 3. Proposed simplified reaction mechanism for sodium ascorbate-mediated fluoroalkylation of 3-methylindole with hypervalent iodine reagent **1f**.

Conclusion

In conclusion, we have demonstrated that sodium ascorbate acts as an efficient, biocompatible and mild reductant towards fluoroalkyl-substituted cyclic λ^3-iodanes for rapid fluoroalkylation (functionalization with the trifluoromethyl or RCF_2CF_2 groups) of small molecules (indoles, pyrroles), aromatic amino acids or aromatic amino acid residues in peptides and proteins with a high selectivity to tryptophan. With a small excess of λ^3-iodanes, Trp residues were selectively fluoroalkylated in position two. With a large excess of λ^3-iodanes, the second fluoroalkylation of the Trp residue and fluoroalkylation of other aromatic amino acid residues (Tyr, Phe and His) in peptides and proteins was observed. The reaction is rapid (less than 15 min), operates in water or buffer of a wide pH range (at least pH 5–9) at ambient temperature. A mechanism involving free-radical intermediates was proposed. The presented method is expected to find applications in Trp bioconjugation and construction of protein-small molecule conjugates, antibody-drug conjugates and post-translational protein modification in general. Further investigations in application of the method for protein surface and epitope mapping and protein crosslinking are underway.

Acknowledgements

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Keywords: bioconjugation • radical • fluorine • iodine • tryptophan

[1] C. D. Spicer, B. G. Davis, *Nat. Commun.* **2014**, *5*, 4740.
[2] D. M. Patterson, L. A. Nazarova, J. A. Prescher, *ACS Chem. Biol.* **2014**, *9*, 592-605.
[3] E. M. Sletten, C. R. Bertozzi, *Angew. Chem., Int. Ed.* **2009**, *48*, 6974-6998.
[4] O. Koniev, A. Wagner, *Chem. Soc. Rev.* **2015**, *44*, 5495-5551.
[5] H. Ban, M. Nagano, J. Gavrilyuk, W. Hakamata, T. Inokuma, C. F. Barbas, 3rd, *Bioconjug. Chem.* **2013**, *24*, 520-532.
[6] J. Gavrilyuk, H. Ban, M. Nagano, W. Hakamata, C. F. Barbas, 3rd, *Bioconjug. Chem.* **2012**, *23*, 2321-2328.
[7] J. M. Antos, M. B. Francis, *J. Am. Chem. Soc.* **2004**, *126*, 10256-10257.
[8] Y. Seki, T. Ishiyama, D. Sasaki, J. Abe, Y. Sohma, K. Oisaki, M. Kanai, *J. Am. Chem. Soc.* **2016**, *138*, 10798-10801.
[9] M. Imiolek, G. Karunanithy, W. L. Ng, A. J. Baldwin, V. Gouverneur, B. G. Davis, *J. Am. Chem. Soc.* **2018**, *140*, 1568-1571.
[10] P. Eisenberger, S. Gischig, A. Togni, *Chem. Eur. J.* **2006**, *12*, 2579-2586.
[11] V. Matoušek, J. Václavík, P. Hájek, J. Charpentier, Z. E. Blastik, E. Pietrasiak, A. Budinská, A. Togni, P. Beier, *Chem. Eur. J.* **2016**, *22*, 417-424.
[12] J. Václavík, R. Zschoche, I. Klimánková, V. Matoušek, P. Beier, D. Hilvert, A. Togni, *Chem. Eur. J.* **2017**, *23*, 6490-6494.
[13] T. Koike, M. Akita, *J. Fluorine Chem.* **2014**, *167*, 30-36.
[14] S.-M. Wang, J.-B. Han, C.-P. Zhang, H.-L. Qin, J.-C. Xiao, *Tetrahedron* **2015**, *71*, 7949-7976.
[15] M. B. Hansen, F. Hubalek, T. Skrydstrup, T. Hoeg-Jensen, *Chem. Eur. J.* **2016**, *22*, 1572-1576.
[16] J. N. Brantley, A. V. Samant, F. D. Toste, *ACS Cent. Sci.* **2016**, *2*, 341-350.
[17] M. S. Wiehn, E. V. Vinogradova, A. Togni, *J. Fluorine Chem.* **2010**, *131*, 951-957.
[18] R. Shimizu, H. Egami, T. Nagi, J. Chae, Y. Hamashima, M. Sodeoka, *Tetrahedron Lett.* **2010**, *51*, 5947-5949.
[19] M. Sodeoka, A. Miyazaki, R. Shimizu, H. Egami, *Heterocycles* **2012**, *86*, 979-983.
[20] A. Studer, D. P. Curran, *Nat. Chem.* **2014**, *6*, 765-773.
[21] W. R. Dolbier, *Chem. Rev.* **1996**, *96*, 1557-1584.

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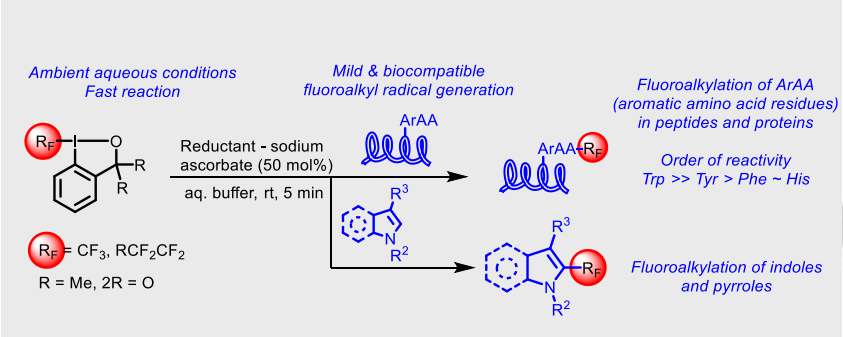

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Reductant-Induced Free Radical Fluoroalkylation of Nitrogen Heterocycles and Innate Aromatic Amino Acid Residues in Peptides and Proteins

Sodium ascorbate-induced radical fluoroalkylation of indoles, pyrroles and aromatic amino acids in peptides and proteins using cyclic hypervalent iodine reagents is reported.