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JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Communication
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Late Stage Benzylic C–H Fluorination with [^{18}F]Fluoride for PET Imaging

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Supporting Information

ABSTRACT: We describe the first late-stage ^{18}F labeling chemistry for aliphatic C–H bonds with no-carrier-added [^{18}F]fluoride. The method uses Mn(salen)OTs as an F-transfer catalyst and enables the facile labeling of a variety of bioactive molecules and building blocks with radiochemical yields (RCY) ranging from 20% to 72% within 10 min without the need for preactivation of the labeling precursor. Notably, the catalyst itself can directly elute [^{18}F]fluoride from an ion exchange cartridge with over 90% efficiency. Using this feature, the conventional and laborious dry-down step prior to reaction is circumvented, greatly simplifying the mechanics of this protocol and shortening the time for automated synthesis. Eight drug molecules, including COX, ACE, MAO, and PDE inhibitors, have been successfully [^{18}F]-labeled in this way.

Positron emission tomography (PET) is a molecular imaging modality that has wide-ranging applications in clinical oncology, cardiology, and neurology as well as basic biomedical research.[1] The characteristics that set PET apart from other imaging techniques are its ability for high sensitivity, noninvasive imaging and quantification of in vivo interactions at a molecular level.[1] Among all PET radioisotopes, ^{18}F is the most widely used and clinically relevant radionuclide.[1b,2] Furthermore, fluorinated derivatives of known drugs often show stronger binding to target sites, lower metabolic burden, and higher bioavailability.[3] By far the most prominent radiotracer to date is [^{18}F]fluorodeoxyglucose ([^{18}F]FDG), which has dominated the use of PET in oncology for over 20 years.[4]

Despite the great success of PET imaging in certain clinical and research domains, the development of new radiotracers remains a formidable challenge.[5] Currently, there are only seven approved PET tracers, three of which are simple radionuclides.[6] High throughput assessment of potential radiotracers would be highly advantageous to increase the rate of discovery as it is currently infeasible to predict whether a particular radiolabeled molecule will exhibit the required in vivo characteristics to serve as a target-specific radiotracer. One main challenge tempering PET throughput stems from constraints on applicable synthetic methods for radiolabeling and the synthesis of precursors.[7] Due to their short half-lives, PET radioisotopes must typically be incorporated into tracer molecules at a late stage of the overall synthesis process. Combined with other constraints, including solvent compatibility, low reaction concentrations, and the need for rapid process steps including product purification, PET radiotracer synthesis has a very limited toolbox of chemical reactions when compared to other synthetic organic disciplines. For ^{18}F labeling, the majority of the radiotracers and radiotracer candidates are synthesized through nucleophilic ^{18}F-substitution.[8] A recent upsurge in fluorination chemistry has revealed a number of novel ^{18}F labeling methods, including preparation of [^{18}F]fluoroaromatics through aryl iodonium salts,[9] Pd-catalyzed allylic fluorination with [^{18}F]fluoride,[10] the preparation of [^{18}F]4-fluorophenols via oxidative fluorination,[11] aromatic ^{18}F labeling through Pd^{IV} or Ni^{II} complexes,[12] copper-catalyzed ^{18}F labeling of aryltrifluoromethyl groups,[13] and enantioselective radiosynthesis of [^{18}F]fluorohydrins.[14] Some of these methods have been scaled up and optimized for high specific activity imaging applications,[15] while others have yet to be demonstrated in an imaging context due to practical limitations of the methodologies.

Methods currently available for ^{18}F labeling, including the newest advances, are dominated by a "prefunctionalization" approach in which a highly reactive chemical functional group (L) is preinstalled at the labeling site and substituted later by ^{18}F (Figure 1a). In most cases, multistep synthesis is required for the preparation of each "preactivated" precursor.[1c] Consequently, potential radiotracer candidates are often prioritized for radiolabeling based on synthetic accessibility of the precursor, which amplifies dramatically with increasing structural complexity. Frequently, harsh reaction conditions are required for the ^{18}F labeling step, which can further diminish the functional group compatibility of reaction.[2,16] The drawback of the "prefunctionalization" approach is most evident in the screening of PET tracers, where labeling of a diverse range of molecules is desired. Thus, iterative discovery through arduous trial and error is adopted.[5]

Received: March 6, 2014
Published: April 27, 2014

dx.doi.org/10.1021/ja5039819 | J. Am. Chem. Soc. 2014, 136, 6842–6845

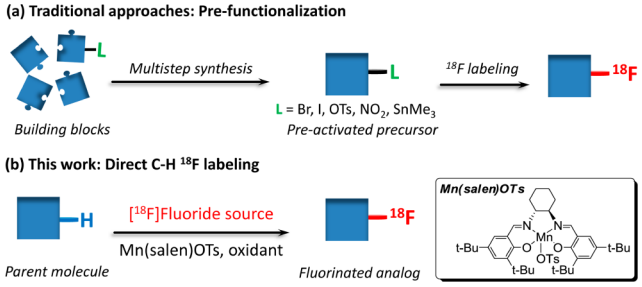


Figure 1. Approaches for labeling molecules with ^{18}F. (a) Multistep synthesis of preactivated labeling precursors, which is time and resource consuming. (b) Direct C−H ^{18}F-fluorination of parent molecules.

Here we describe an ^{18}F labeling strategy that implements a direct replacement of sp^3 hydrogen with fluorine (Figure 1b). The method avoids the need for target preactivation, enabling high throughput radiolabeling of parent compounds and building blocks. We demonstrate selective ^{18}F substitution at benzylic C−H bonds, which are common to drug and drug-like molecules. The method shows promise to significantly increase the efficiency of PET tracer synthesis and evaluation and to provide ready access to labeled molecules that are difficult to access or cannot be prepared by conventional methods.

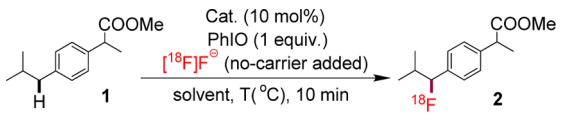
The concept of direct hydrogen substitution with ^{18}F was first demonstrated by Firnau et al. in the 1980s,[17] but there has been very limited development of ^{18}F labeling methods based on C−H fluorination since this pioneering work. Recently, several of us reported a series of manganese-catalyzed aliphatic C−H fluorination reactions that exhibit promising features for ^{18}F labeling applications.[18] These reactions utilize nucleophilic fluoride (F^-) as the fluorine source, in contrast to methods that require reactive, electrophilic fluorinating agents.[19] Although there are several C−H fluorination methods based on nucleophilic fluoride sources,[20] application to ^{18}F labeling has yet to be demonstrated.

The pivotal factor for adapting fluoromanganese(IV) fluorine transfer reactions to ^{18}F labeling is the formation of reactive ^{18}F-containing intermediates using substoichiometric, low-concentration [^{18}F]fluoride. Under catalytic ^{19}F reaction conditions, excess fluoride serves as the axial ligand for the oxomanganese(V) species in the hydrogen abstraction and subsequently as the fluorine transfer agent.[18a−c]

To address this challenge in the context of radiochemistry, we employed the exploratory conditions shown in Table 1 to evaluate the efficacy of various manganese salen catalysts for ^{18}F labeling. We found that while Mn(salen)Cl gave only trace amounts of ^{18}F-labeled product, manganese salen complexes with more labile triflate (OTf) or perchlorate counterions showed substantial higher radiochemical conversions (RCC) to ^{18}F-labeled product **2** (16% and 34%, respectively). Various more weakly associated ligands were evaluated, and *p*-toluenesulfonate (OTs) was found to afford the highest RCC (53%) in the test reaction. Further optimizations based on the Mn(salen)OTs catalyst achieved a maximum of 65% RCC for the initial substrate (ibuprofen) using iodosylbenzene (PhIO) as the oxidant (Table 1, entry 5). No labeling products were detected in control experiments in which the manganese salen catalyst or iodosylbenzene was omitted.

We found that this procedure allowed for the efficient ^{18}F labeling of benzylic C−H bonds in a wide range of substrates with RCC ranging from 20% to 68% (Figure 2). A range of

Table 1. Aliphatic C−H ^{18}F-Fluorination of Ibuprofen Ester



	catalyst	solvent	*T*	RCC
1	Mn(salen)Cl	CH_3CN	50	trace
2	Mn(salen)OTf	CH_3CN	50	16%
3	Mn(salen)ClO_4	CH_3CN	50	34%
4	Mn(salen)OTs	CH_3CN	50	53%
5	Mn(salen)OTs	acetone	50	65%
6	Mn(salen)OTs	acetone	25	45%
7	Mn(salen)OTs	acetone	90	50%

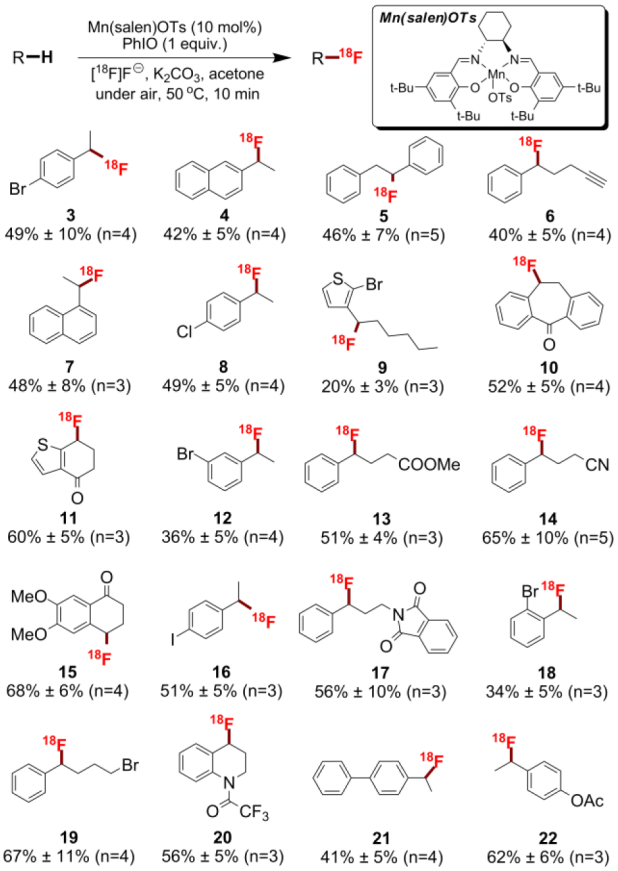


Figure 2. Direct ^{18}F labeling of aliphatic C−H bonds of substrates and decay-corrected radiochemical conversions (RCCs).

functional groups were well tolerated, including esters, amides, imides, ketones, alkynes, ethers, cyanides, heterocycles, carbamates, and aryl and aliphatic halides. The labeling was generally more efficient for substrates bearing electron-donating groups, presumably due to the electrophilic nature of the hydrogen-abstracting oxomanganese(V) intermediate. The method can be used to prepare ^{18}F-labeled synthons (in addition to direct labeling for radiotracer evaluation). For example, dibenzosuberone (**10**), the chemical precursor of a series of tricyclic antidepressant drugs (TCAs) including amitriptyline and nortriptyline, was readily labeled with ^{18}F in 50% RCC. The tolerance of reactive functional groups such as halogens and alkynes enables the rapid incorporation of ^{18}F-

dx.doi.org/10.1021/ja5039819 | J. Am. Chem. Soc. 2014, 136, 6842−6845

labeled motifs into complex structures through well-established methods such as nucleophilic substitutions or "click" reactions.[21] Notably, the ^{18}F labeling reaction can be performed under air and without rigorous exclusion of water, greatly simplifying the protocol and facilitating scale-up.

The major benefit of this mild C−H ^{18}F-fluorination reaction is its application to late-stage radiolabeling. To demonstrate this potential, we examined a variety of well-known biologically active molecules. The selected compounds encompass inhibitors of important biological and pharmacological targets including cyclooxygenase (COX), monoamine oxidase B (MAO-B), phosphodiesterase 10A (PDE_{10A}), and angiotensin-converting enzyme (ACE), as well as biomessenger molecules such as the neurotransmitter dopamine, and the immuno-modulating drug, fingolimod. Subjecting these molecules (or protected analogues) to Mn-catalyzed fluorination led to successful ^{18}F labeling specifically at benzylic positions (Figure 3). The RCC ranged from 22% to 72% at 50 °C within

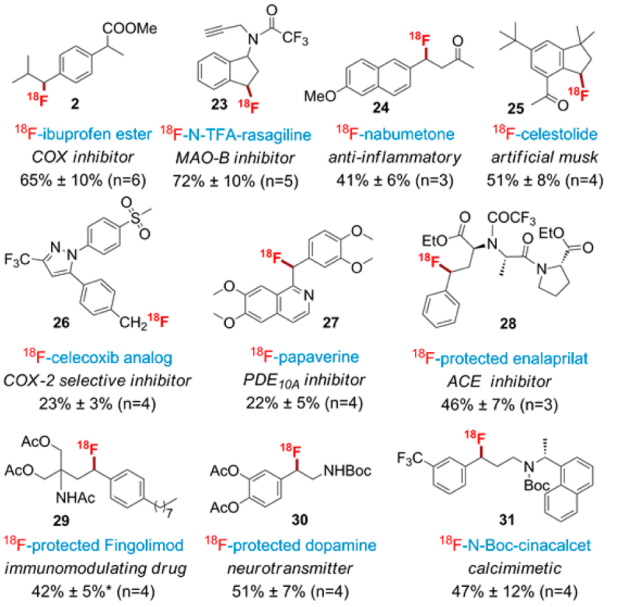


Figure 3. Direct C−H ^{18}F labeling of bioactive molecules. Reported radiochemical conversions (RCCs) are decay-corrected and averaged over (n) experiments. *0.35 equiv of oxidant, PhIO, was used.

10 min. In the case of fingolimod, **29**, high regioselectivity was observed for the protected amino diol side chain (see Supporting Information). Notably, the ^{18}F labeling reaction showed a much broader substrate scope than its ^{19}F counterpart. For example, fluorinating C−H bonds β to electron-withdrawing groups (e.g., a ketone or Boc-protected amine) was very challenging on a preparative scale in the ^{19}F reaction, but this position was readily labeled under ^{18}F reaction conditions with an RCC of 41% and 51%, respectively, for **24** and **30**. This seemingly counterintuitive phenomenon results from the very low concentration of [^{18}F]fluoride and the large excess of the manganese catalyst. Apparently, the low concentration of manganese [^{18}F]fluoride species present under ^{18}F labeling conditions is sufficient to capture the incipient substrate radicals with high efficiency.

Having demonstrated the enabling power of this new ^{18}F fluorination protocol, we performed initial process optimization to facilitate scale-up for PET imaging. While the method is compatible with typical "dry-down" procedures used in ^{18}F chemistry, we found that no drying procedure was required. [^{18}F]Fluoride deposited on an anion exchange cartridge (AEC) could be eluted using an organic solution of the catalyst, Mn(salen)OTs, with over 90% recovery of the radiolabel from the column and no erosion of the RCC in the subsequent fluorination reaction. For example, ^{18}F-labeled celestolide was obtained with 10% non-decay corrected RCY with a specific activity of 2.68 Ci/μmol (end of bombardment) (Figure 4). These results demonstrate the significant potential of the present method for PET imaging applications.

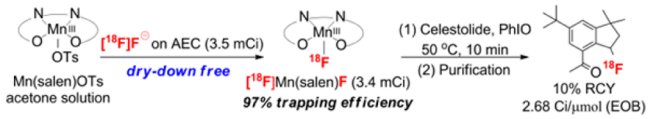


Figure 4. Dry-down free procedure for the synthesis of ^{18}F-celestolide.

It is of interest to compare and contrast the ^{18}F fluorinations described here using limiting fluoride ion to the ^{19}F reactions we have previously described that use a large excess of fluoride.[18] We suggest the mechanism shown in Figure 5a for

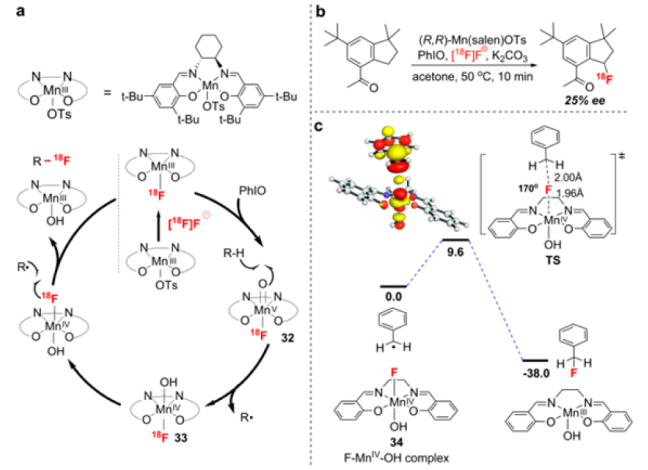


Figure 5. (a) Proposed mechanism for ^{18}F labeling of benzylic C−H bonds catalyzed by a manganese salen catalyst. (b) Detection of enantioselectivity of labeling products of celestolide by chiral radio-HPLC analysis. (c) Energy landscape of fluorine transfer from F−Mn^{IV}−OH intermediate (**34**) to a benzyl radical.

this ^{18}F labeling reaction. In ^{19}F chemistry, a *trans*-difluoromanganese(IV) complex was shown to be the reactive fluorine transfer intermediate. This compound was isolated and structurally characterized.[18c] However, due to the limiting amount of [^{18}F]fluoride in the labeling conditions, the formation of the [^{18}F]*trans*-difluoromanganese(IV) intermediate is not feasible. Therefore, the [^{18}F]fluorine transfer is more likely to proceed directly through a ^{18}F−Mn^{IV}−OH intermediate (**33**) even in the presence of a large excess of a manganese catalyst that has no fluoride ligand.

The involvement of a manganese salen-bound ^{18}F intermediate in the fluorine transfer step was demonstrated experimentally by analyzing the enantioselectivity of the resulting ^{18}F-labeled product. Using celestolide as the diagnostic substrate, we measured a 25% ee in the fluorinated product using chiral HPLC analysis and radio-detection (Figure 5b). Moreover, when the catalyst was changed from (*R,R*)-Mn(salen)OTs to (*S,S*)-Mn(salen)OTs, the same 25% ee was

dx.doi.org/10.1021/ja5039819 | J. Am. Chem. Soc. 2014, 136, 6842−6845

observed in the labeling product but with reversed enantioselectivity. The fluorine transfer reactivity of the $^{18}F-Mn^{IV}-OH$ complex was further supported by density functional theory (DFT) computations (Figure 5c). The activation barrier of fluorine transfer from the $F-Mn^{IV}-OH$ complex (**34**) to the benzyl radical was only 9.6 kcal/mol in an acetone solvent continuum. The molecular orbitals involved in the C−F bond formation are the $\sigma^*(d_{z^2})$ orbital of **34** and the benzyl radical SOMO. The overall fluorine transfer process is thermodynamically favored with a calculated free energy change of −38.0 kcal/mol.

In conclusion, we have developed a facile, no-carrier-added, ^{18}F labeling method that allows efficient late-stage labeling of a variety of organic molecules and known drugs. The reaction is operationally simple, requiring no dry-down operations, and is tolerant of both moisture and air. This protocol can be immediately adapted in any laboratory site with a basic PET chemistry infrastructure. We are working to expand the concept of ^{18}F labeling via direct C−H activation demonstrated in this study.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental procedures, spectroscopic data for all new compounds, and details for DFT calculation. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This research was supported by the Center for Catalytic Hydrocarbon Functionalization, an Energy Frontier Research Center, U.S. Department of Energy, Office of Science, Basic Energy Sciences, under Award No. DE SC0001298 (J.T.G.). Fluorination of biomolecules was supported by the US National Science Foundation award CHE-1148597 (J.T.G.). A portion of this research was carried out at Martinos Center for Biomedical Imaging using resources provided by the Center for Functional Neuroimaging Technologies, P41EB015896, and shared instrumentation grants S10RR017208 and S10RR023452. H.R. was supported by a US Dept. of Energy radiochemistry training grant DE-SC0008430 (J.M.H.). X.H. thanks the Howard Hughes Medical Institute for fellowship support. W.L. thanks Merck, Inc. for fellowship support. The authors also thank Prof. A. G. Doyle and T. Graham for helpful discussions.

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