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Radiochemistry

[^{11}C]Fluoroform, a Breakthrough for Versatile Labeling of PET Radiotracer Trifluoromethyl Groups in High Molar Activity

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Abstract: Positron-emission tomography (PET) is an immensely important imaging modality in biomedical research and drug development but must use selective radiotracers to achieve biochemical specificity. Such radiotracers are usually labeled with carbon-11 ($t_{1/2}$ = 20 min) or fluorine-18 ($t_{1/2}$ = 110 min), but these are only available from cyclotrons in a few simple chemical forms. [^{18}F]Fluoroform has emerged for labeling tracers in trifluoromethyl groups but is severely limited in utility by low radioactivity per mass (low molar activity). Here, the synthesis of [^{11}C]fluoroform is described, based on CoF_3-mediated fluorination of cyclotron-produced [^{11}C]methane. This process is efficient and repetitively reliable. [^{11}C]Fluoroform shows versatility for labeling small molecules in very high molar activity (> 200 GBq μmol^{-1}), far exceeding that possible by using [^{18}F]fluoroform. Therefore, [^{11}C]fluoroform represents a major breakthrough for labeling prospective PET tracers in trifluoromethyl groups at high molar activity.

Positron-emission tomography (PET) is immensely important for biomedical research and for drug discovery and development. The value of PET for imaging molecular targets in a living human or animal subject depends on the availability of biochemically specific radiotracers, in which the radiolabel is usually one of the short-lived cyclotron-produced positron-emitters, carbon-11 ($t_{1/2}$ = 20.4 min) or fluorine-18 ($t_{1/2}$ = 109.8 min). The label position is often critical for avoiding troublesome radiometabolites that may confound attempts to quantify radiotracer interaction with the imaging target.[1] Therefore, in many cases it is preferable to label in one part of the structure rather than in another. A further very important consideration is the molar activity of the radiotracer, namely the radioactivity [Bq] per total mass of tracer [mol], in which the latter is predominantly the accompanying non-radioactive tracer known as carrier. For low-density imaging targets, such as enzymes, transporters, receptors, and plaques, the radiotracer molar activity needs to be very high. A low molar activity may result in far too high occupancy of the target by carrier with consequent violation of the tracer principle, or even an obliteration of any target-specific signal.[1] Minimal occupancy of the target by carrier may also be needed to avoid unwanted pharmacological effects.[2]

The most popular methods for labeling PET radiotracers at high molar activity use [^{11}C]methyl iodide or [^{18}F]fluoride ion as labeling agents.[1b] [^{11}C]Methyl iodide is produced from cyclotron-produced [^{11}C]methane or [^{11}C]carbon dioxide, whereas [^{18}F]fluoride ion is produced directly from a cyclotron.[1b] However, the use of these labeling agents or of others restricts the kind of groups that might be labeled in radiotracers, for example to methyl (Me) groups for [^{11}C]methyl iodide[1b,3] and to monofluoro (CF) groups for [^{18}F]fluoride ion.[1b,4]

Many drugs and potential PET radiotracers contain trifluoromethyl (CF_3) groups. A methyl, chloro, or other substituent can often be replaced with a CF_3 group with good retention of physicochemical and pharmacological properties.[5] Also, the CF_3 group is generally considered to be metabolically stable. Consequently, the pharmaceutical industry develops many drugs with CF_3 groups. In parallel, academic groups are developing methods for labeling such groups with fluorine-18,[6] with the most recent methods based on conversion of [^{18}F]fluoride ion into [^{18}F]fluoroform,[7] and then in situ generation of the reactive derivative [^{18}F]$CuCF_3$ (Figure 1).[8] These [^{18}F]fluoroform production methods at best deliver only moderate molar activity (< 32 GBq μmol^{-1}) owing to [^{18}F]fluoride ion dilution with carrier fluoride ion in the solution reaction systems. Generally, the radiotracer molar activities that are needed for PET imaging of low-density protein targets are several-fold higher.[1b,9] The range of useful PET radiotracers that may be produced from [^{18}F]fluoroform or [^{18}F]$CuCF_3$ that is produced by the best-performing methods is therefore limited to the low proportion not requiring such high molar activities.

We noted that ^{11}C-labeling of the trifluoromethyl group has never been achieved at any molar activity. [^{11}C]Methane is produced in high activity in numerous PET research facilities, either directly by the ^{14}N(p,α)^{11}C nuclear reaction on nitrogen-10% hydrogen or by reduction of cyclotron-produced [^{11}C]carbon dioxide. By either route, [^{11}C]methane typically has very high molar activity that well exceeds that of cyclotron sources of fluorine-18.[10,11] We reasoned that if [^{11}C]fluoroform could be produced from readily accessible [^{11}C]methane, very high molar activity might be retained and well exceed that currently achievable for [^{18}F]fluoroform or [^{18}F]$CuCF_3$. Moreover, [^{11}C]fluoroform would be expected to participate in labeling reactions without any further dilution with carrier, and therefore

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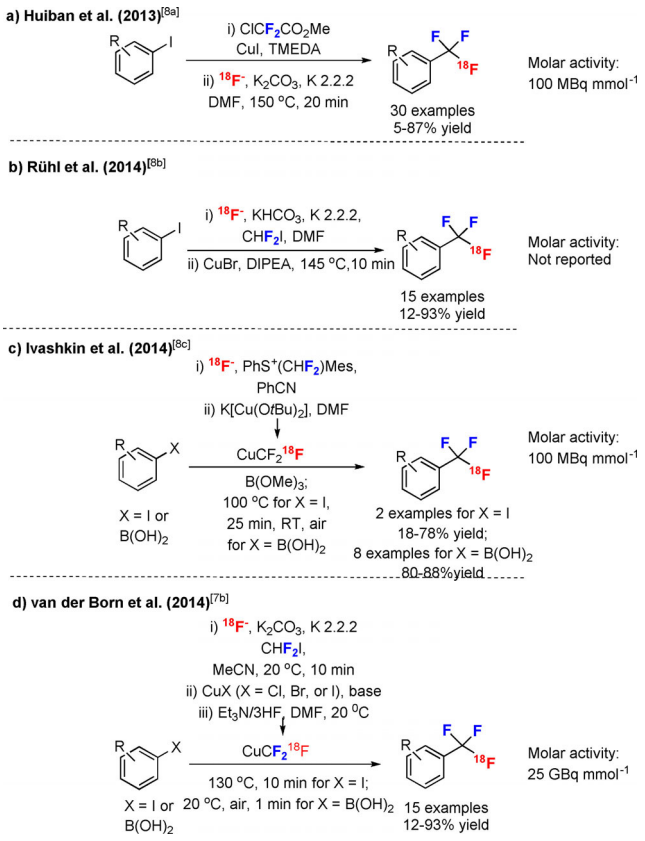


Figure 1. Methods for the synthesis of [^{18}F]fluoroform or the derivative [^{18}F]$CuCF_3$ for conversion into [^{18}F]trifluoromethylarenes.

the labeling of PET radiotracers in trifluoromethyl groups at high molar activity would then become possible. Therefore, we aimed to produce [^{11}C]fluoroform form [^{11}C]methane.

To meet our objective, a rapid and efficient fluorination method was required. To maintain high molar activity in the [^{11}C]fluoroform, the method would need to be applicable to [^{11}C]methane in the presence of only a low amount of carrier methane (typically $\ll 1$ μmol) and without introducing an appreciable amount of extra carrier. Moreover, the method would need to be amenable to easy remote-control within a lead-shielded "hot-cell" for radiation safety to personnel.

We noted that heated cobalt(III) fluoride (CoF_3) has been used to fluorinate hydrocarbons to yield mixtures of fluorinated products on a macro-scale.[12] An unappealing feature of a typical fluorination process was that the CoF_3 needed to be generated in situ by passing fluorine gas over heated CoF_2. Nonetheless, we noted that CoF_3 is now commercially available and might be used to avoid any need for noxious and highly reactive fluorine. Passage of [^{11}C]methane over heated CoF_3 therefore seemed an attractive possibility for producing [^{11}C]fluoroform, provided that neither over-fluorination of the sub-micromole amount of carrier methane nor the formation of other hydrocarbon byproducts would be a major issue. Therefore, we set out to test the feasibility of using commercially available CoF_3 for producing [^{11}C]fluoroform in useful yield.

Herein, we report that heated CoF_3 successfully mediates efficient conversion of [^{11}C]methane into [^{11}C]fluoroform. We further report a remotely controllable and reliable apparatus for [^{11}C]fluoroform production and illustrate the utility of the [^{11}C]fluoroform for labeling model compounds and examples of drug-like compounds by various chemical routes, some known and some new. These labeled compounds show very high molar activities that match those to be expected[6] from cyclotron-produced [^{11}C]methane in the absence of further dilution with carrier and far exceed those previously attained for [^{18}F]fluoroform or [^{18}F]$CuCF_3$. In particular, these molar activities rival those for PET radiotracers commonly used for imaging low-density protein targets, such as neurotransmitter receptors, transporters, enzymes, and plaques.

Our initial experiments showed that [^{11}C]methane readily passed through a heated column of commercial CoF_3 without any hold-up of radioactivity or any restriction of flow and with some production of [^{11}C]fluoroform. Figure 2 illustrates the apparatus that we finally developed for routinely preparing [^{11}C]fluoroform after further experimentation.

The temperature dependence of the conversion of [^{11}C]methane into [^{11}C]fluoroform was investigated in the apparatus with carrier He flow set at 20 mL min^{-1}. Radioactivity trapped in cold (≈ −94 °C) ethanol was used to calculate the yield (radioactivity breakthrough was relatively low). High yields of [^{11}C]fluoroform were obtained between 260 and

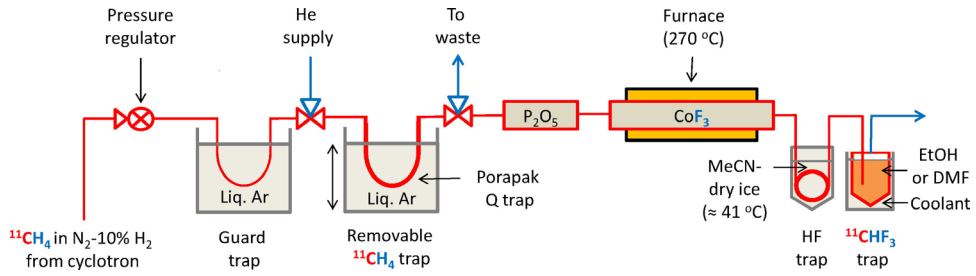


Figure 2. Apparatus for producing [^{11}C]fluoroform. Cyclotron-produced $^{11}CH_4$ in N_2-10% H_2 is passed first through a cold (−186 °C) stainless-steel U-tube and then another cold U-tube (−186 °C) containing Porapak Q. Waste gas goes to a collection bag. The U-tube is then raised and allowed to warm to RT over 4 min while the tube is also flushed with He gas (20 mL min^{-1}) to transfer the $^{11}CH_4$ over Sicapent and into a heated (270 °C) stainless-steel tube containing CoF_3 (17 g). The effluent passes through MeCN–dry ice (≈ −41 °C) to trap any trace HF (b.p. 19.5 °C) and then into a trap containing EtOH cooled with hexane–liquid N_2 (≈ −94 °C) or DMF cooled with MeCN–dry ice (≈ −41 °C) to trap the generated [^{11}C]CHF_3. See the Supporting Information for complete apparatus construction and operation details.

290 °C (Figure 3). The average yield of [^{11}C]fluoroform in this range was 53 ± 4 %, based on 11 experiments conducted with two different CoF_3 columns. The overall process for producing [^{11}C]fluoroform from [^{11}C]methane required only 7 min from the end of a cyclotron irradiation and was thus much less than one half-life of carbon-11.

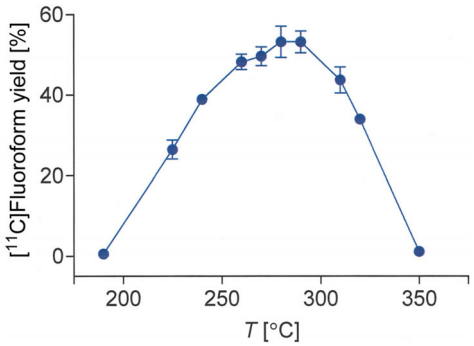


Figure 3. Dependence of the yield of [^{11}C]fluoroform from [^{11}C]methane on the temperature. Values are means ± standard deviation ($n > 3$).

Although we observed that heating a CoF_3 column to 350 °C many times resulted in severely impaired performance, initial conditioning of a newly installed CoF_3 column by heating it once to 350 °C while sealed under He subsequently resulted in optimal yields of [^{11}C]fluoroform at lower temperatures. Moreover, such a heat-conditioned CoF_3 column showed remarkably consistent performance over a very large number (> 80) of [^{11}C]fluoroform productions (Figure 4). Therefore, once set up

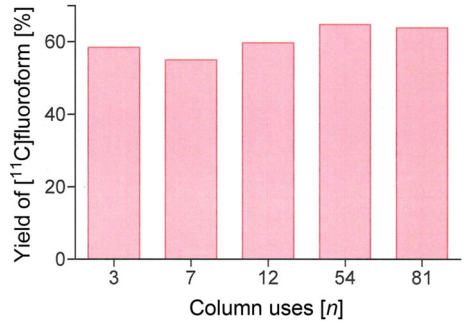


Figure 4. Dependence of [^{11}C]fluoroform yield on the number of CoF_3 column uses. Data are for the column operating at 270 °C with He flow set at 20 mL min^{-1}.

and conditioned, the apparatus required no significant maintenance other than to be kept filled with He. Additionally, the apparatus was easily adapted for automation and remote-control to ensure radiation protection to personnel (see the Supporting Information).

The robustly repeatable performance of a conditioned CoF_3 column suggests that the fluorination process does not depend on prior decomposition of CoF_3 to CoF_2 and fluorine. More likely is that the [^{11}C]methane interacts directly and stoichiometrically with CoF_3 to replace one hydrogen atom with fluorine to afford [^{11}C]fluoromethane, with this replacement process repeated until [^{11}C]fluoroform is formed.

The reactivity of [^{11}C]fluoroform was tested on model substrates (Figure 5). When [^{11}C]fluoroform in DMF was added to a solution of benzophenone (**1**) and *t*BuOK at RT, [^{11}C]α-(trifluoromethyl)-benzhydrol ([^{11}C]**2**) was obtained quantitatively in just 5 min. Treatment of 4-nitrophenylboronic acid (**3**) with [^{11}C]fluoroform under copper(I)-mediated conditions[7b,8c] for 1 min at RT afforded [^{11}C]1-nitro-4-(trifluoromethyl)benzene ([^{11}C]**4**) in 99 % yield with a molar activity of 551 GBq μmol^{-1}. This molar activity is over 20-fold higher than that reported for [^{18}F]**4** prepared from [^{18}F]fluoroform. Similarly, copper(I)-mediated treatment of 3-fluorophenylboronic acid (**5**) with [^{11}C]fluoroform yielded [^{11}C]1-fluoro-3-(trifluoromethyl)benzene ([^{11}C]**6**) in 98 % yield and with a molar activity of 242 GBq μmol^{-1}. Together, these results confirmed that [^{11}C]fluoroform was produced from [^{11}C]methane without appreciable dilution of molar activity.

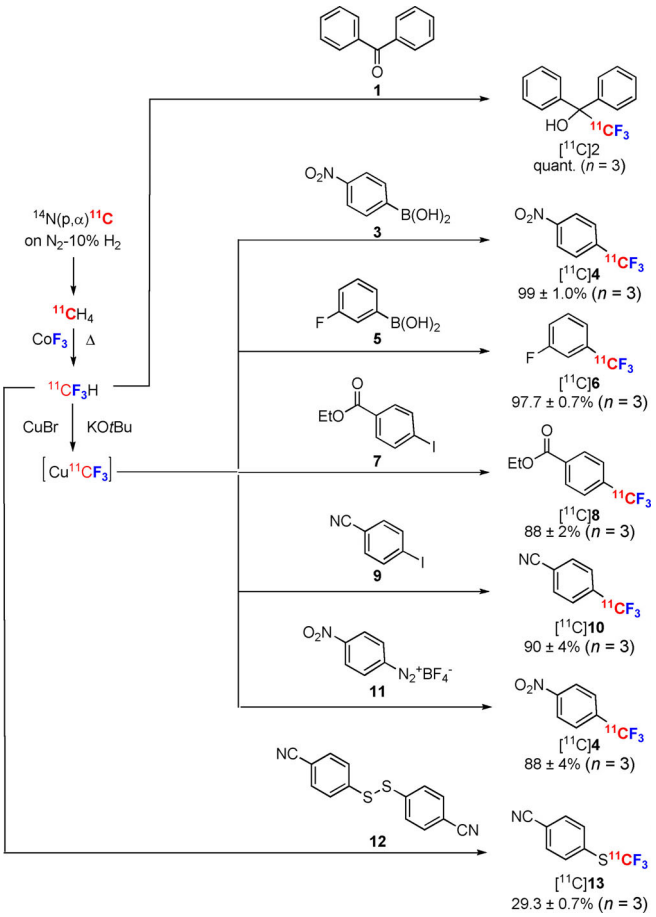


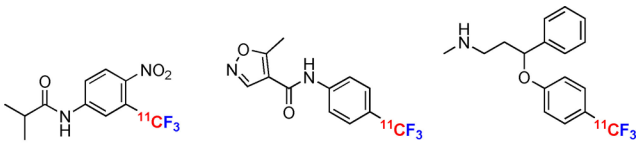
Figure 5. Preparation and use of [^{11}C]fluoroform for labeling model organic compounds with trifluoromethyl groups.

We also investigated the reactivity of [^{11}C]fluoroform towards other substrates. Thus, under copper(I)-mediated conditions,[5b] ethyl 4-iodobenzoate (**7**) and 4-iodobenzonitrile (**9**) were converted rapidly into ethyl [^{11}C]4-(trifluoromethyl)benzoate ([^{11}C]**8**) and [^{11}C]4-(trifluoromethyl)benzonitrile ([^{11}C]**10**) in 88

and 90% yield, respectively. These high yields attest to the absence of troublesome impurities in the collected [^{11}C]fluoroform; only low levels of minor unreactive fluorinated ^{11}C species were ever observed as contaminants.

The easy access to [^{11}C]fluoroform enabled us to explore the development of new labeling chemistries. Thus, we showed that treatment of a commercially available "wet" diazonium salt **11** with [^{11}C]$CuCF_3$ afforded [^{11}C]**4** in high yield approaching that from the boronic acid precursor **3**. Moreover, we showed the versatility of [^{11}C]fluoroform for labeling diverse functional groups through its reaction with the diaryldisulfane **12**, which afforded the labeled trifluoromethylsulfur arene [^{11}C]**13** rapidly (< 10 min) in 29% non-optimized yield.

Finally, we demonstrated the utility of [^{11}C]fluoroform for labeling trifluoromethyl groups with carbon-11 in drug-like or PET radiotracer-type molecules. For example, we used [^{11}C]fluoroform to label three known drugs, namely the antiandrogen flutamide (Eulexin) (**14**), the antirheumatic drug leflunomide (Arava) (**15**), and the antidepressant fluoxetine (Prozac) (**16**) (Figure 6). [^{11}C]**14** was obtained in 76% yield from the corresponding iodo-precursor (**17**) and [^{11}C]fluoroform under copper(I)-mediated conditions. This yield compares very favorably with that of [^{18}F]**14** prepared with the much lower molar activity method of Huiban et al.[8a] (55%). [^{11}C]**15** was labeled in 93% yield and with a high molar activity of 400 GBq μmol^{-1} (corrected to end of radionuclide production) from the copper(I)-mediated reaction on the corresponding boronic acid precursor (**19**). Finally, [^{11}C]**16** was obtained in 45% yield from a copper(I)-mediated reaction on a Boc-protected iodo precursor (**20**) followed by deprotection.



[^{11}C]**14**, [^{11}C]Flutamide ([^{11}C]Eulexin) 76 ± 8% (*n* = 3)

[^{11}C]**15**, [^{11}C]Leflunomide ([^{11}C]Arava) 93 ± 5% (*n* = 3) MA: 400 GBq μmol^{-1}

[^{11}C]**16**, [^{11}C]Fluoxetine ([^{11}C]Prozac) 45 ± 4% (*n* = 3)

Figure 6. ^{11}C-Labeled drugs prepared from no-carrier-added [^{11}C]fluoroform.

In conclusion, [^{11}C]fluoroform is readily and simply produced in usefully high yield and in high molar activity from cyclotron-produced [^{11}C]methane by passage over heated CoF_3. The process is rapid, repetitively robust, and low-maintenance. [^{11}C]Fluoroform represents a breakthrough as an agent for labeling PET radiotracers at trifluoromethyl groups in high molar activity sufficient for imaging low-density targets in human subjects. We now envisage access to an enhanced range of exciting radiotracers for PET applications based on adapting the known richly diverse chemistry of fluoroform[13] to [^{11}C]fluoroform for unprecedented ^{11}C-labeling at trifluoromethyl groups.

Experimental Section

Production of [^{11}C]fluoroform: The apparatus (Figure 2) was constructed, set-up, and operated as detailed in the Supporting Information. The collected [^{11}C]fluoroform was analyzed with HPLC (see the Supporting Information).

Conversion of [^{11}C]fluoroform into [^{11}C]$CuCF_3$: In a glovebox, *t*BuOK in DMF (0.3 M, 50 μL, 15 μmol) was added to CuBr (0.7 mg, 5 μmol) in a 1 mL vial. The vial was septum-sealed and removed from the glovebox. [^{11}C]Fluoroform (185–555 MBq) in DMF (50–300 μL) was added to the vial, mixed, and left at RT for 1 min. A solution of $Et_3N{\cdot}3HF$ in DMF (1.64% *v*/*v*; 5 μL) was then added. The mixture was mixed thoroughly and allowed to stay at RT for another minute.

Labeling reactions: For details of labeling reactions and product analyses see the Supporting Information.

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Conflict of interest

This work is the subject of a patent application.

Keywords: [^{11}C]fluoroform · carbon-11 · positron-emission tomography · radiochemistry · trifluoromethyl

[1] a) V. W. Pike, *Trends Pharmacol. Sci.* **2009**, *30*, 431; b) V. W. Pike, *Curr. Med. Chem.* **2016**, *23*, 1818.

[2] a) S. E. Lapi, M. J. Welch, *Nucl. Med. Biol.* **2012**, *39*, 601; b) S. P. Hume, R. N. Gunn, T. Jones, *Eur. J. Nucl. Med.* **1998**, *25*, 173; c) E. M. Jagoda, J. J. Vaquero, J. Seidel, M. V. Green, W. C. Eckelman, *Nucl. Med. Biol.* **2004**, *31*, 771.

[3] F. Wuest, M. Berndt, T. Kniess in *PET chemistry, The Driving Force in Molecular Imaging* (Eds.: P. A. Schubiger, L. Lehmann, M. Friebe), Springer-Verlag, Heidelberg, **2007**, pp. 183–213.

[4] L. Cai, S. Lu, V. W. Pike, *Eur. J. Org. Chem.* **2008**, 2853.

[5] a) W. Zhu, J. Wang, S. Wang, Z. Gu, J. L. Aceña, K. Izawa, H. Liu, V. A. Soloshonok, *J. Fluorine Chem.* **2014**, *167*, 37; b) M. Bassetto, S. Ferla, F. Pertusati, *Future Med. Chem.* **2015**, *7*, 527.

[6] V. T. Lien, P. J. Riss, *BioMed. Res. Int.* **2014**, *2014*, 380124.

[7] a) D. van der Born, J. D. M. Herscheid, R. V. A. Orru, D. J. Vugts, *Chem. Commun.* **2013**, *49*, 4018; b) D. van der Born, C. Sewing, J. D. M. Herscheid, A. D. Windhorst, R. V. A. Orru, D. J. Vugts, *Angew. Chem. Int. Ed.* **2014**, *53*, 11046; *Angew. Chem.* **2014**, *126*, 11226.

[8] a) M. Huiban, M. Tredwell, S. Mizuta, Z. Wan, X. Zhang, T. L. Collier, V. Gouverneur, J. Passchier, *Nat. Chem.* **2013**, *5*, 941; b) T. Rühl, W. Rafique, V. T. Lien, P. Riss, *Chem. Commun.* **2014**, *50*, 6056; c) P. Ivashkin, G. Lemonnier, J. Cousin, V. Grégoire, D. Labar, P. Jubault, X. Pannecoucke, *Chem. Eur. J.* **2014**, *20*, 9514.

[9] K. Madsen, L. Marner, M. Haahr, N. Gillings, G. M. Knudsen, *Nucl. Med. Biol.* **2011**, *38*, 1085.

[10] D. R. Christman, R. D. Finn, K. I. Karlstrom, A. P. Wolf, *Int. J. Appl. Radiat. Isot.* **1975**, *26*, 435.

[11] a) K. R. Buckley, J. M. Huser, S. Jivan, K. S. Chun, T. J. Ruth, *Radiochim. Acta* **2000**, *88*, 201; b) J. Andersson, P. Truong, C. Halldin, *Appl. Radiat. Isot.* **2009**, *67*, 106; c) H. Björk, K. Dahlström, J.-O. Bergström, P. Truong, C. Halldin in *10th Workshop on Targetry and Target Chemistry*, Madison,

WI, **2004**, Abstract A02, p. 12; d) V. Gómez-Vallejo, V. Gaja, J. Koziorowski, J. Llop in *Positron Emission Tomography—Current Clinical and Research Aspects* (Ed.: C.-H. Hsieh), InTech, Rijeka, **2012**, pp. 183 – 210.

[12] a) V. S. Asovich, V. V. Kornilov, R. Kostyaev, V. N. Maksimov, *Russ. J. Appl. Chem.* **1994**, *67*, 94; b) V. V. Kornilov, R. A. Kostyaev, B. N. Maksimov, B. A. Mel'nichenko, T. Fedorova, *Russ. J. Appl. Chem.* **1995**, *68*, 1227.

[13] a) A. Lishchynskyi, B. Guillaume, V. V. Grushin, *Chem. Commun.* **2014**, *50*, 10237; b) J. Russell, N. Roques, *Tetrahedron* **1998**, *54*, 13771; c) B. Folléas, I. Marek, J.-F. Normant, L. Saint-Jalmes, *Tetrahedron* **2000**, *56*, 275; d) G. K. S. Prakash, P. V. Jog, P. T. D. Batamack, G. A. Olah, *Science* **2012**, *338*, 1324; e) H. Kawai, Z. Yuan, E. Tokunaga, N. Shibata, *Org. Biomol. Chem.* **2013**, *11*, 1446.

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Radiochemistry

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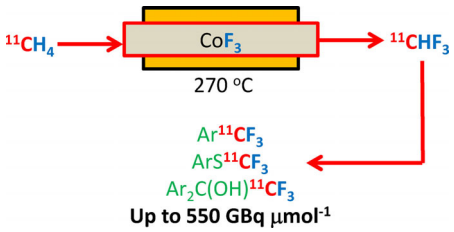

Putting a label on it: Cobalt(III) fluoride mediates efficient transformation of cyclotron-produced [^{11}C]methane into [^{11}C]fluoroform with high molar activity. [^{11}C]Fluoroform shows broad utility as an efficient labeling synthon for prospective positron emission tomography (PET) radiotracers.