Facile $^{18}F/^{19}F$ isotopic exchange at Ga^{III}

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Rapid Aqueous Late-Stage Radiolabelling of [GaF_3(BnMe$_2$-tacn)] by $^{18}F/^{19}F$ Isotopic Exchange – Towards New PET Imaging Probes

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Abstract. ***A simple and rapid method for ^{18}F radiolabelling of [GaF_3(BnMe$_2$-tacn)] by $^{18}F/^{19}F$ isotopic exchange is described. Using MeCN:H_2O or EtOH:H_2O (75:25) and $^{18}F^-$(aq) (up to 200 MBq) with heating (80 °C, 10 mins.), gives 66±4% ^{18}F incorporation at 268 nM concentration and 37±5% ^{18}F incorporation at even lower (27 nM) concentration, without the need for a Lewis acid promoter. An SPE purification method has been established, giving 99% radiochemical purity (RCP) of [$Ga^{18}F^{19}F$(BnMe$_2$-tacn)] in an EtOH/H_2O mixture.***

Fluorine-18 is the most widely utilised radioisotope in PET (Positron Emission Tomography) imaging due to its physical and nuclear characteristics: short but manageable half-life (~110 min), short positron linear range in tissue (2.3 mm), lack of side emission (97 % decay by positron emission), low energy of the positron ($E_{\beta max}$ = 635 keV) and wide availability of cyclotrons for its production. The ^{18}F half-life allows a certain degree of manipulation of the synthesis, provided that the radiolabelling occurs in the later stages of the synthesis (ideally in the final step). The most commonly used PET radiotracers are organic molecules where the radioactive fluorine is attached to a carbon atom, often requiring multistep syntheses and/or purification post-labelling, which can be time consuming and inefficient. This has driven recent work by several groups to investigate the ^{18}F radiolabelling properties of inorganic molecules, where the strong bond between (typically) a main group element and the fluorine can be exploited to allow fast, late-stage radiolabelling. Other important aspects to be considered are pH tolerance and the temperature required for radiolabelling, as these will influence significantly the compatibility with biomolecules. An ideal target for ^{18}F radiolabelling would be a method requiring a single step where the $[^{18}F]F^-$ target water is introduced directly without further purification, at very low precursor concentration (e.g. 10 nM), without the need for post-labelling purification, giving a high molar activity product that is stable in the formulation matrix.

A recent review from Gabbaï and co-workers describes some of the key advances in the development of Group 13 element based tracers towards PET applications.[1] Within the Group 13 elements, boron has the highest bond dissociation energy with fluorine (> 730 kJ mol^{-1})[2] and, after carbon, has been the most studied element for PET applications. Several different types of molecules have been successfully radiolabelled with ^{18}F, including, aryl-trifluoroborates, [3][4][5] zwitterionic onium-trifluoroborates,[6][7][8] and BODIPY-based dyes.[9][10] Typically, radiofluorination is achieved by either converting a boronic ester moiety into a fluoroborate species or by an isotopic exchange reaction. Very recently, the development of $[^{18}F]BF_4^-$ and $[^{18}F]CF_3SO_3^-$ as PET probes for imaging the sodium-iodide symporter have been reported.[11][12][13]

Work on the 'Al-F' system developed by McBride and co-workers demonstrated that radiofluorination can be achieved by heating $AlCl_3$, $[^{18}F]F^-$ and a pentadentate NOTA-derived ligand together in aqueous solution at pH 3.9-4.2 and 100 °C (NOTA = 1,4,7-triazacyclononane-1,4,7-triacetic acid).[14] This was an important breakthrough, providing the first example of a metal-chelate system for $[^{18}F]F^-$ capture in water, and exploiting coordination chemistry to determine fluoride incorporation. A related gallium species was reported subsequently; in this case the [$Ga^{18}F$(L)] moiety (L = 1-benzyl-1,4,7-triazacyclononane-4,7-dicarboxylate) being formed readily by radiofluorination from the pre-formed chloride complex, [GaCl(L)], in aqueous MeCN under mild conditions. This radio-complex is stable to at least pH 6, but unlike the 'Al-F' system, exhibits reduced stability in phosphate buffered saline (PBS) and human serum albumin (HSA) at pH 7.4.[15] This instability at higher pH was attributed, at least in part, to the lower stability of the carboxylate bonds to Ga^{III} *cf.* Al^{III}.

In 2014, we reported a series of trifluoride complexes of aluminium, gallium and indium with *neutral* tridentate tacn-based macrocycles, including [MF_3(BnMe$_2$-tacn)] (M = Al, Ga, In; BnMe$_2$-tacn = 1-benzyl-4,7-dimethyl-1,4,7-triazacyclononane), as potential $[^{18}F]F^-$ carrier molecules. Notably, these trifluoro-complexes are extremely stable in water.[16] Furthermore, we were able to demonstrate that the trichloro-analogue, [$GaCl_3$(BnMe$_2$-tacn)], can be radiofluorinated easily at room temperature and close to neutral pH, by treating an MeCN/H_2O solution of the complex with 2.99 mol. equiv. of aqueous KF doped with $[^{18}F]$KF (100–500 MBq). The resulting [$Ga^{18}F^{19}F_2$(BnMe$_2$-tacn)] shows very high radiochemical stability in PBS at pH 7.4 (RCP 98% after 120 mins.).

In the fluorination of the metal-*trichloro* species, the strength of the M-F bonds being formed undoubtedly provides a significant thermodynamic driving force for rapid introduction of F^-. It is also clear that the stability of the resultant radiofluorinated metal complexes in competitive media (PBS or serum) is also subtly dependent upon the choice of metal ion acceptor and any co-ligands present in the metal coordination sphere.[15]

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Supporting information for this article, including full experimental details, NMR spectroscopic and MS data for [GaF_3(BnMe$_2$-tacn)] and the radio-HPLC traces for the isotopic exchange experiments, is available on the WWW under http://www.angewandte.org.

Whilst radiofluorination was readily achieved for [MCl_3(BnMe$_2$-tacn)] (M = Ga, Al) at 2.6 μM concentration,[16],[17] to offer real prospects for the development of a viable imaging probe for PET based upon this system, it is desirable for the ^{18}F incorporation to be efficient at lower, i.e. nanomolar concentration. However, we found that the [MCl_3(BnMe$_2$-tacn)] did not undergo radiofluorination at 260 nM (0.1 mg/mL) concentration. We suggested that this could be due to the hydrolytic sensitivity of the M-Cl groups in the [MCl_3(BnMe$_2$-tacn)], resulting in competition between slow hydrolysis and Cl/F exchange under the labelling conditions.[16]

In view of the apparent resistance of [GaF_3(BnMe$_2$-tacn)] to hydrolysis, we first sought to probe the kinetic stability of this (inactive) trifluoro-complex in aqueous solution under a variety of conditions, before exploring the possibility of using ^{18}F/^{19}F isotopic exchange reactions in order to produce the radiofluorinated product more conveniently and preferably, using less material.

A more convenient synthesis of (inactive) [Ga^{19}F$_3$(BnMe$_2$-tacn)] was established as an alternative to the hydrothermal method we described previously,[16] *via* the direct reaction of the molecular [GaF_3(dmso)(OH_2)$_2$] complex with BnMe$_2$-tacn in CH_2Cl_2 at room temperature, giving the characteristic IR and ^{1}H, ^{19}F and ^{71}Ga NMR spectroscopic signatures (Figs. S1-S3); 87% isolated yield. ES$^+$ MS (MeCN/H_2O) shows *m/z* consistent with [GaF_3(BnMe$_2$-tacn)+Li]$^+$ and [GaF_2(BnMe$_2$-tacn)]$^+$ (Fig. S4); the strong affinity of [MF_3(R_3-tacn)] to alkali metal cations has been demonstrated previously.[18]

Solution ^{19}F NMR studies confirm that [GaF_3(Me$_3$-tacn)] is very stable in water (spectrum unchanged) at elevated temperature (80 °C), even after several hours. This is in contrast to the [$GaCl_3$(RMe$_2$-tacn)] (R = Me or Bn), which hydrolyses within minutes when small amounts of water are added to a solution of the complex in MeCN at room temperature[16]. The trifluoro-complex also shows a very good pH tolerance, with no detectable degradation between pH 4–11, and similarly, the spectra are unchanged by addition of a ten-fold excess of the (potentially) competitive chloride, carbonate, acetate or phosphate anions (as sodium salts in H_2O) to the [GaF_3(RMe$_2$-tacn)].

This stability of the [Ga^{19}F$_3$(RMe$_2$-tacn)] (R = Bn, Me) in water led us to consider the prospect of radiolabelling [Ga^{19}F$_3$(BnMe$_2$-tacn)] by ^{18}F/^{19}F isotopic exchange. The trifluoro-complex should be a more convenient precursor due to its ease of handling, together with its much longer shelf-life, compared to [$GaCl_3$(BnMe$_2$-tacn)]. Most importantly, we considered that the [Ga^{19}F$_3$(BnMe$_2$-tacn)] might facilitate radiofluorination at lower concentration, since the likelihood of hydrolysis is considerably less.

Previous work by Schirrmacher [19] on silicon-fluoride based systems and by Blower *et al*,[20] Gabbaï *et al* [6] and Perrin *et al* [21] using boron-fluoride systems, has demonstrated the value of ^{18}F/^{19}F isotopic exchange in a number of PET tracers. A Lewis acid promoter such as $SnCl_4$ is sometimes required to activate the fluorine-element bond in order to facilitate [^{18}F]F$^-$ incorporation in these systems.

Initial radiofluorination experiments were performed at 2.6 μM concentration of [Ga^{19}F$_3$(BnMe$_2$-tacn)] in MeCN/H_2O, using [^{18}F]F$^-$ target water directly (up to 200 MBq). The solvent conditions (MeCN/H_2O, EtOH/H_2O) and ratio, as well as the temperature (25 °C and 80 °C) were varied to establish the effect on the [^{18}F]F$^-$ incorporation. The results are summarised in Table 1.

The results show that incorporation of [^{18}F]F$^-$ into [GaF_3(BnMe$_2$-tacn)] occurs readily and reproducibly even at room temperature in 75:25 MeCN:H_2O, with [Ga^{18}F^{19}F$_2$(BnMe$_2$-tacn)] being the major radio-labelled species, the only other being unreacted [^{18}F]F$^-$. Moreover, heating the unbuffered mixture to 80 °C for 10 mins. leads to high [^{18}F]F$^-$ incorporation (typically between 65–73% using both 2.68 μM and 268 nM solutions of the complex), and without the need for a Lewis acid promoter. Radio-HPLC traces are shown in Figure 1 and in the Supporting Information (Figs. S5-S6). Confirmation of the identity of the radio-product comes from the corresponding UV traces (Figs. S11-S13). Using the relatively low activity [^{18}F]F$^-$ employed in this work (*ca*. 200 MBq), the molar activity determined for the 27 nanomol precursor concentration was *ca.* 675 MBq/μmol.

A simple purification protocol using a HLB solid-phase extraction (SPE) cartridge has also been established (see Experimental Section), giving radiochemical purity (RCP) = ~99%.

Table 1. Conditions used for ^{18}F/^{19}F radiofluorination experiments (RCY = radiochemical yield). All experiments were performed at least 3 times.

[GaF_3(BnMe$_2$-tacn)] (mass/mg)	Scale (nmol)	Organic solvent : H_2O ratio	Organic solvent	T/°C (time/ min.)	RCY* (%)
1	2680	8:92	MeCN	25 (45)	4±2
1	2680	8:92	MeCN	80 (30)	18±4
1	2680	50:50	MeCN	80 (60)	23±4
1	2680	75:25	MeCN	80 (10)	73±4
0.1	268	75:25	MeCN	25 (80)	8±4
0.1	268	75:25	MeCN	80 (10)	66±4
0.01	27	75:25	MeCN	80 (10)	37±5
1	2680	75:25	EtOH	80 (10)	81±1
0.1	268	75:25	EtOH	80 (10)	50±4

* RCY determined from HPLC.

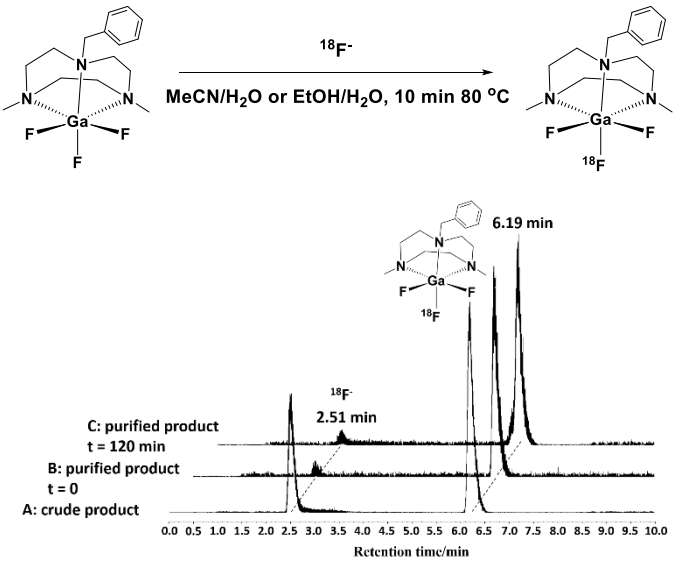

Figure 1**. A**: radio-HPLC chromatogram of the crude product. Peak 1: Rt = 2.51 min 35% (^{18}F$^-$). Peak 2: Rt = 6.19 min 65% ([Ga^{18}F^{19}F$_2$(BnMe$_2$tacn)]); **B**: radio-HPLC chromatogram of the purified product eluted from a HLB cartridge (formulated in 20% EtOH/H_2O). Peak 1: Rt = 2.49 min 1% (^{18}F$^-$). Peak 2: Rt = 6.18 min 99% ([Ga^{18}F^{19}F$_2$(BnMe$_2$tacn)]); **C**: radio-HPLC chromatogram of the purified product after 120 minutes (formulated in 20% EtOH/H_2O). Peak 1: Rt = 2.53 min 12% (^{18}F$^-$). Peak 2: Rt = 6.20 min 88% ([Ga^{18}F^{19}F$_2$(BnMe$_2$tacn)]).

This methodology has also been demonstrated successfully using an even lower (27 nM) precursor complex concentration, resulting in 37±5% ^{18}F incorporation using similar labelling conditions (Table 1), i.e. a decrease in concentration of two orders of magnitude compared to the radiofluorination of [MCl_3(BnMe$_2$-tacn)] reported previously by Cl/F exchange.

The radiochemical stability of the purified [Ga^{18}F^{19}F$_2$(BnMe$_2$-tacn)] was also monitored over time (typically 2-3 h) for a series of

samples. After SPE purification with an HLB cartridge, we found that the RCP decreased to between 88% and 77% after 120 mins. at room temperature, through loss of $[^{18}F]F^-$ from the radio-product. An alternative purification procedure via an HLB cartridge, followed by an alumina cartridge (at t = 0 min.) was also performed, leading to similar results, whereas cooling the purified $[Ga^{18}F^{19}F_2(BnMe_2\text{-tacn})]$ solution to –20 °C led to RCP = ~93% after 4 h (Fig. S7).

Since ΔG for the isotopic exchange is ~0, and some (~4%) $[^{18}F]F^-$ incorporation is seen at room temperature (with much higher incorporation at 80 °C – Table 1), the following equilibrium reaction must apply:

$$[Ga^{18}F^{19}F_2(BnMe_2\text{-tacn})] + {}^{19}F^- \leftrightarrows [Ga^{19}F_3(BnMe_2\text{-tacn})] + {}^{18}F^-$$

The effect of added fluoride or chloride was tested by the addition of either 10% $K^{19}F$ solution or a 0.9% saline solution to the purified $[Ga^{18}F^{19}F_2(BnMe_2\text{-tacn})]$ at 25 °C. These have no significant effect on the RCP over ca. 2 h. These results are consistent with the isotopic exchange proceeding via a dissociative mechanism, i.e. 1st order in $[GaF_3(BnMe_2\text{-tacn})]$, and independent of the concentration of the entering ligand. Performing the radiofluorination in dmso, a much more competitive (strongly coordinating) solvent, leads to a significant drop in the RCY, which is also consistent with a predominant dissociative mechanism at the distorted octahedral Ga(III) complex, via a 5-coordinate intermediate.[1]

The stability of the radio-product in 90% human serum albumin (HSA) / 10% EtOH showed RCP = 97% at t = 0 min. and 83% at t = 120 min. (Fig. S8).

The possibility of radiolysis leading to a decrease in RCP over time was also tested, both by formulating the purified radio-product in 10% EtOH/PBS, and by the addition of ascorbic acid to the purified radio-product; neither had any appreciable effect (Figs. S9-S10).

Performing the radiofluorination of $[Ga^{18}F^{19}F_2(BnMe_2\text{-tacn})]$ in 75%/25% EtOH/H_2O under the same conditions (80 °C/10 min.) also led to high $[^{18}F]F^-$ incorporation (Table 1) with an RCP of 82% after 2 h.

In summary, this study has demonstrated $^{18}F/^{19}F$ isotopic exchange on a metal-chelate based system for the first time. The new method leads to high ^{18}F incorporation using $[^{18}F]F^-$ target water directly from the cyclotron and without the need for a Lewis acid promoter. Furthermore, we have shown that the method also allows the concentration of the $[GaF_3(BnMe_2\text{-tacn})]$ used for the radiofluorination to be scaled down by at least two orders of magnitude (27 nM, 0.01 mg), representing a very significant decrease in the quantity of material needed compared to the $Cl^-/^{18}F^-$ exchange reaction that we reported previously.[16]

The results reported here suggest that $[Ga^{19}F_3(BnMe_2\text{-tacn})]$ offers a promising basis for the development of new PET probes. Future work will explore this system further (i) using computational and experimental work to determine the effects that parameters such as the choice of the Group 13 metal and altering the steric protection around the M-F groups have on the ^{18}F incorporation, and (ii) conjugation of peptides (via the benzyl pendant group) to the most promising candidates to evaluate them as radiotracers via biodistribution studies.

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[1] Radiofluorination of $[Ga^{19}F_3(BnMe_2\text{-tacn})]$ in 75%/25% dmso/H_2O (80 °C / 10 min.) leads to significantly lower $[^{18}F]F^-$ incorporation (14%) compared to either MeCN/H_2O or EtOH/H_2O (Fig. S14).

Keywords: gallium · radiofluorination · isotopic exchange · tacn · PET · fluorine-18

[1] K. Chansaenpak, B. Vabre, F. P. Gabbaï, *Chem. Soc. Rev*. **2016**, *45*, 954.
[2] G. E. Smith, H. L. Sladen, S. C. G. Biagini, P. J. Blower, *Dalton Trans.* **2011**, *40*, 6196.
[3] C. W. Harwig, R. Ting, M. J. Adam, T. J. Ruth, D. M. Perrin, *Tetrahedron Lett.* **2008**, *49*, 3152.
[4] R. Ting, C. W. Harwig, U. auf dem Keller, S. McCormick, P. Austin, C. M. Overall, M. J. Adam, T. J. Ruth, D. M. Perrin, *J. Am. Chem. Soc.* **2008**, *130*, 12045.
[5] Y. Li, R. Ting, C. W. Harwig, U. auf dem Keller, C. L. Bellac, P. F. Lange, J. A. H. Inkster, P. Schaffer, M. J. Adam, T. J. Ruth, C. M. Overall, D. M. Perrin, *Med. Chem. Commun.* **2011**, *2*, 942.
[6] K. Chansaenpak, M. Wang, Z. Wu, R. Zaman, Z. Li, F. P. Gabbaï, *Chem. Commun.* **2015**, *51*, 12439.
[7] Z. Li, K. Chansaenpak, S. Liu, C. R. Wade, P. S. Conti, F. P. Gabbaï, *Med. Chem. Commun.* **2012**, *3*, 1305.
[8] J. Bernard, R. Malacea-Kabbara, G. S. Clemente, B. P. Burke, M.-J. Eymin, S. J. Archibald, S. Jugé, *J. Org. Chem.* **2015**, *80*, 4289.
[9] J. A. Hendricks, E. J. Keliher, D. Wan, S. A. Hilderbrand, R. Weissleder, R. Mazitschek, *Angew. Chem. Int. Ed.* **2012**, *51*, 4603.
[10] S. Liu, T.-P. Lin, D. Li, L. Leamer, H. Shan, Z. Li, F. P. Gabbaï, P. S. Conti, *Theranostics*, **2013**, *3*, 181.
[11] A. Khoshnevisan, M. Jauregui-Osoro, K. Shaw, J. B. Torres, J. D. Young, N. K. Ramakrishnan, A. Jackson, G. E. Smith, A. D. Gee, P. J. Blower, *EJNMMI Res.* **2016**, *6*, 1.
[12] S. Diocou, A. Volpe, M. Juarequi-Osoro, M. Boudjemeline, K. Chuamsaamarkkee, F. Man, P. J. Blower, T. Ng, G. E. D. Mullen, G. O Fruhwirth, *Sci. Rep.* **2017**, *7*, 946.
[13] A. Khoshnevisan, K. Chuamsaamarkkee, M. Boudjemeline, A. Jackson, G. E. Smith, A. D. Gee, G. O. Fruhwirth, P. J. Blower, *J. Nucl. Med.* **2017**, *58*, 156.
[14] P. Leverman, W. McBride, R. Sharkey, A. Eek, L. Joosten, W. Oyen, D. Goldenberg, O. Boerman, *J. Nucl. Med.* **2010**, *50*, 454; W. McBride, C. D'Souza, R. Sharkey, H. Karacay, E. Rossi, C. Chang, D. Goldenberg, *Bioconjugate Chem.* **2016**, *21*, 1331; W. McBride, R. Sharkey, H. Karacay, C. D'Souza, E. Rossi, P. Laverman, C. Chang, O. Boerman, D. Goldenberg, *J. Nucl. Med.* **2009**, *50*, 991; W. McBride, C. D'Souza, H. Karacay, R. Sherkey, D. Goldenberg, *Bioconjugate Chem.* **2012**, *23*, 538.
[15] R. Bhalla, W. Levason, S. K. Luthra, G. McRobbie, G. Sanderson, G. Reid, *Chem. Eur. J.* **2015**, *21*, 4688.
[16] R. Bhalla, C. Derby, W. Levason, S. K. Luthra, G. McRobbie, G. Sanderson, W. Zhang, G. Reid, *Chem. Sci*. **2014**, *5*, 381.
[17] W. Levason, S. K. Luthra, G. McRobbie, F. M. Monzittu, G. Reid, *Dalton Trans.* **2017**, *46*, 14519.
[18] R. Bhalla, W. Levason, S. K. Luthra, G. McRobbie, G. Reid, G. Sanderson, W. Zhang, *Chem Commun.* **2014**, *50*, 12673.
[19] R. Schirrmacher, G. Bradtmöller, E. Schirrmacher, O. Thews, J. Tillmanns, T. Siessmeier, H. G. Buchholz, P. Bartenstein, B. Wängler, C. M. Niemeyer, K. Jurkschat, *Angew. Chem. Int. Ed*. **2006**, *45*, 6047.; A. P. Kostikov, J. Chin, K. Orchowski, E. Schirrmacher, S. Niedermoser, K. Jurkschat, L. Iovkova-Berends, C. Wängler, B. Wängler, R. Schirrmacher, *Nat. Protoc.* **2012**, *7*, 1956.
[20] M. Jauregui-Osoro, K. Sunassee, A. J. Weeks, D. J. Berry, R. L. Paul, M.. Cleij, J. P. Banga, M. J. O'Doherty, P. K. Marsden, S. E. M. Clarke, J, R. Ballinger, I. Szanda, S.-Y. Cheng, P. J. Blower, *Eur. J. Nucl. Med. Molec. Imaging*, **2010**, *37*, 2108.
[21] Z. Liu, K. Lin, F. Bénard, M. Pourghiasian, D. O. Kiesewetter, D. M. Perrin, X. Chen., *Nat. Protoc.* **2015**, *10*, 1423.
[22] The radiochemistry nomenclature used in this paper is in accordance with the new guidelines recommended by the Society of Radiopharmaceutical Sciences. See: http://www.srsweb.org/nomenclature-guidelines and: P. H. Elsinga, *EJNMMI Radiopharmacy and Chemistry*, **2017**, 2:2.

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Facile $^{18}F/^{19}F$ isotopic exchange at Ga^{III}

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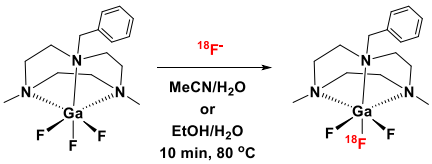

High ^{18}F incorporation is readily achieved by $^{18}F/^{19}F$ isotopic exchange into $[GaF_3(BnMe_2\text{-tacn})]$ using $[^{18}F]F^-(aq)$ in (unbuffered) aqueous MeCN or EtOH without the need for a Lewis acid promoter and allowing radiofluorination at low (sub-30 nM) concentration.