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RESEARCH ARTICLE

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Evaluating *N*-difluoromethyltriazolium triflate as a precursor for the synthesis of high molar activity [^{18}F] fluoroform

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The trifluoromethyl group is a prominent motif in biologically active compounds and therefore of great interest for the labeling with the positron emitter fluorine-18 for positron emission tomography (PET) imaging. Multiple labeling strategies have been explored in the past; however, most of them suffer from low molar activity due to precursor degradation. In this study, the potential of 1-(difluoromethyl)-3-methyl-4-phenyl-1*H*-1,2,3-triazol-3-ium triflate as precursor for the synthesis of the [^{18}F]trifluoromethylation building block [^{18}F]fluoroform with high molar activity was investigated. The triazolium precursor was reacted under various conditions with [^{18}F]fluoride, providing [^{18}F]fluoroform with radiochemical yields (RCY) and molar activities (A_m) comparable and even superior with already existing methods. Highest molar activities ($A_m = 153 \pm 14$ GBq/μmol, dc, EOS) were observed for the automated procedure on the Neptis® perform module. Due to its easy handling and good RCY and A_m in the [^{18}F]fluoroform synthesis, the triazolium precursor is a valuable alternative to already known precursors.

KEYWORDS
[^{18}F]fluoroform, [^{18}F]trifluoromethylation, fluorine-18, high molar activity, triazolium precursor

1 | INTRODUCTION

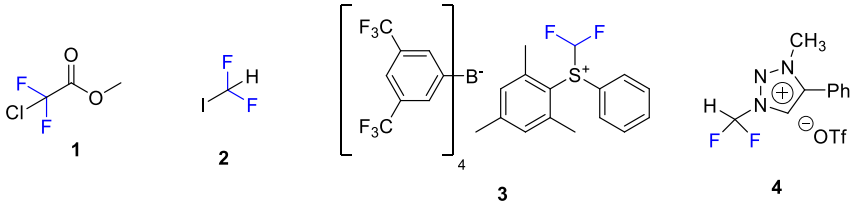
Despite the fact that naturally occurring fluorine-containing molecules are rare, fluorine is a very commonly used element in drug design.[1] This is due to its ability to positively influence the characteristics of a given molecule, for example, its pK_a, lipophilicity or pharmacokinetics.[1–3] Furthermore, fluorine and (poly) fluorinated groups can act as a bioisostere for hydrogen and many functional groups such as carbonyl, hydroxyl, and nitrile.[2,4] Besides the fluorine atom, the trifluoromethyl group is one of the most commonly used fluorine-containing structures.

The in vivo evaluation of a drug candidate by positron emission tomography (PET), a noninvasive imaging technique, is an emerging process in drug development.[1,5] It relies on biologically active molecules that are labeled with positron-emitting radionuclides, so-called PET tracers.[5] Of these radionuclides, fluorine-18 is one of the most popular ones. It has a convenient half-life (110 min)

and excellent beta decay characteristics.[4,6] Also, the role of this element in drug design contributes to the popularity of fluorine-18 as it allows the radiolabeling of many compounds without variation of the original structure.

Among the different radiofluorination strategies, the introduction of radiolabeled CF_3 groups into potential tracer molecules via [^{18}F]trifluoromethylation has gained increasing interest over the past years. Most of the reported methods are based on the use of the ^{18}F-labeled building block [^{18}F]fluoroform.[7] Different precursors have been explored, for example, methyl chlorodifluoroacetate **1**, difluoroiodomethane **2**, and the difluoromethylsulfonium salt **3** (Figure 1).[8–12] However, yielding [^{18}F]trifluoromethylated products with high molar activities has proven to be challenging. This is because the precursors decompose under the standard basic radiofluorination conditions releasing [^{19}F]fluoride that competes with [^{18}F]fluoride in the formation of [^{18}F]fluoroform resulting in high amounts of nonlabeled compound. Among the abovementioned precursors **1–3**, only difluoroiodomethane **2** provided [^{18}F]fluoroform with average molar activities suitable for PET tracer synthesis so far ($A_m = 97 \pm 20$ GBq/μmol, $n = 3$).[13] This precursor **2** is gaseous, and therefore, certain handling protocols need to be followed. Furthermore, molar activities still did not reach the levels of usual [^{18}F]fluorination reactions (0.1–100 GBq/μmol vs. >100 GBq/μmol in standard radiofluorination). As an alternative, a synthetic strategy via [^{18}F]fluoromethane and subsequent gas phase fluorination has been developed but on average only moderate molar activities were obtained ($A_m = 38 \pm 35$ GBq/μmol [$n = 20$] with max. $A_m = 163$ GBq/μmol).[14] The synthesis of triazolium salt **4** (1-(difluoromethyl)-3-methyl-4-phenyl-1*H*-1,2,3-triazol-3-ium triflate) has recently been reported in literature, and its reaction with [^{19}F]fluoride has been extensively studied, indicating that this precursor would be able to provide high molar activity [^{18}F]fluoroform (Figure 1).[15] Therefore, our aim was to investigate radioactive reactions of triazolium salt **4** with [^{18}F]fluoride to explore whether the triazolium salt **4** would be suitable as precursor for the synthesis of [^{18}F]fluoroform and would enable higher radiochemical yields (RCYs) and higher molar activities (A_m) than previously reported methods.

FIGURE 1 Different precursors for [^{18}F]trifluoromethylation: methyl chlorodifluoroacetate **1**, difluoroiodomethane **2**, difluoromethylsulfonium salt **3**, and triazolium salt **4**



2 | EXPERIMENTAL

2.1 | General methods and materials

All the chemicals were purchased from commercial sources and used without further purification. ^{1}H NMR spectra were recorded on a Varian 400-MHz NMR spectrometer. ^{1}H NMR chemical shifts were determined relative to CD_3CN at δ 1.94 ppm. All radioactive reactions and products were analyzed with high-performance liquid chromatography (HPLC) using a Shimadzu SPD-20A system and LabSolutions 5.85 software (Shimadzu Corporation, Japan) with a Grace Smart C18 column (5 μ 4.6 × 250 mm), MeCN/H_2O/TFA 30:70:0.1 or an Alltima C18 (5 μ 4.6 × 250 mm), MeCN/H_2O/TFA 20:80:0.1 as eluent and a flow of 1 ml/min. UV active compounds were detected at 254 nm. Radioactive products were identified by comparison with the unlabeled reference compounds. Radioactivity was quantified with a Veenstra VDC-304 dose calibrator.

2.2 | Synthesis of triazolium precursor 4

1-(Difluoromethyl)-3-methyl-4-phenyl-1*H*-1,2,3-triazol-3-ium triflate **4** was prepared in five steps from ethyl bromodifluoroacetate **5** following literature procedures.[15,16]

2.3 | Investigation of the stability of precursor 4 in presence of K_2CO_3 and K_{222} by ^{1}H NMR experiments

2.3.1 | K_2CO_3 stock solution

CD_3OD (2 ml) was added to ground K_2CO_3 (2.1 mg, 15 μmol) in a 4 ml vial containing a stirring bar. The solution was stirred until the salt was completely dissolved.

2.3.2 | K_{222} stock solution

$CDCl_3$ (0.8 ml) was added to K_{222} (5.3 mg, 14 μmol) in a 4 ml vial.

2.3.3 | Precursor **4** stock solution

$CDCl_3$ (0.8 ml) and CD_3OD (0.4 ml) were added to precursor **4** (7.2 mg, 20 μmol) in a 4 ml vial.

2.3.4 | General procedure for Table 1, entry 1

An aliquot of the K_2CO_3 stock solution (10 μl, 0.075 μmol) was added to an NMR tube. CD_3OD (0.2 ml) was added to the NMR tube. The solvent in the NMR tube was evaporated using a flow of Argon. An aliquot of the K_{222} stock solution (10 μl, 0.175 μmol) was added to a 4 ml vial (vial A), and the solvent was removed under vacuum. An aliquot of precursor **4** stock solution (30 μl, 0.5 μmol) was added to a second 4 ml vial (vial B), and the solvent was removed under vacuum. CD_3CN (0.15 ml) was added to K_{222} in vial A, and the solution in vial A was added to K_2CO_3 in the NMR tube. Vial A was rinsed with CD_3CN (0.1 ml), and the rinsing solution was added to the NMR tube. CD_3CN (0.15 ml) was added to precursor **4** in vial B, and the solution in vial B was added to the NMR tube. Vial B was rinsed with CD_3CN (0.1 ml), and the rinsing solution was added to the NMR tube. The NMR tube was sealed with a cap, and after 10 min at room temperature (rt), a ^1H NMR of the mixture was recorded. Note that when CD_3OD (0.2 ml) was added to the NMR tube, the solvent was added with the syringe tip touching the side of the NMR tube to move K_2CO_3 remaining on the side of the NMR tube to the bottom of the NMR tube.

Procedure for Table 1, entry 2: The same procedure as for entry 1 was used, except that the amounts of base and complexant were 10× higher: K_2CO_3 stock solution (100 μl, 0.75 μmol) and K_{222} stock solution (100 μl, 1.75 μmol) were used.

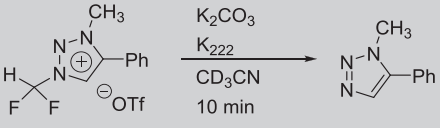
Procedure for Table 1, entry 3: The same procedure as for entry 1 was used, except that the reaction mixture was heated: the NMR tube was sealed with a cap, and after 10 min at 40°C, the NMR tube was cooled to 0°C. ^1H NMR of the mixture was recorded.

Procedure for Table 1, entry 4: The same procedure as for entry 1 was used, except that the reaction mixture was heated: the NMR tube was sealed with a cap, and after 10 min at 80°C, the NMR tube was cooled to 0°C. ^1H NMR of the mixture was recorded. NMRs can be found in the SI, Figure S1 to S8.

2.4 | Procedure for determining the reaction order by ^1H NMR experiments

CD_3CN (0.15 ml) was added to CsF (25.5 mg, 0.168 mmol) in an NMR tube. CD_3CN (0.6 ml) was added to precursor **4** (30.0 mg, 0.084 mmol) in a 20 ml vial, and then, the solution of **4** was transferred to the NMR tube. The NMR tube was sealed with a cap, and after the reaction time at 80°C, a ^1H NMR of the mixture was recorded (reaction times: 10 min, 20 min, 30 min, 2 h, 3 h, 5 h, and 6 h). NMRs can be found in the SI, Figure S9 and S10.

TABLE 1 Investigation of stability of precursor **4** in presence of K_2CO_3 and K_{222}[a]



4 **10**

				Yield (%)[b]	
Entry	**Temp.**	**K_2CO_3 (equiv)**	**K_{222} (equiv)**	**4**	**10**
1	rt	0.15	0.35	99.7	0.3
2	rt	1.5	3.5	40	60
3[c]	40°C	0.15	0.35	95	5
4[c]	80°C	0.15	0.35	91	9

[a]Reactions were carried out on a 0.5 μmol reaction scale of triazolium precursor **4** in 0.5 ml of CD_3CN in a sealed NMR tube. $n = 1$.
[b]^1H NMR yields.
[c]After reaction time, the mixture was cooled to 0°C.

2.5 | Radiochemistry

2.5.1 | Base-complexant stock solutions

150 µmol (20 mg) K_2CO_3 or 300 µmol (30 mg) $KHCO_3$, and 350 µmol complexant (93 mg 18-crown-6 or 130 mg kryptofix 222) were dissolved in 9 ml acetonitrile and 1 ml water.

2.5.2 | Dry [^{18}F]fluoride via [^{18}F]triflyl fluoride

[^{18}F]Fluoride was produced by irradiation of an ^{18}O-enriched water target and was trapped on a Chromafix® 30-PS-HCO_3 cartridge (Macherey-Nagel, Germany). It was eluted from the cartridge with 500 µl, 0.1 M aqueous potassium sulfate solution. The eluate was collected in a vessel containing 850 µl DMF, and 150 µl, 0.1 M *N*,*N*-bis (trifluoromethylsulfonyl)aniline in DMF was added. The vessel was heated to 40 °C, and the gaseous [^{18}F]triflyl fluoride was purged out of the reaction mixture with a flow of helium (10 ml/min) for about 5 min. The [^{18}F] triflyl fluoride was distilled over phosphorus pentoxide and trapped in a second reaction vessel containing a certain volume of base-complexant stock solution (**x**, volume depending on the amounts of base and complexant; base: 0.015 to 1.5 µmol K_2CO_3 or 0.3 µmol $KHCO_3$, complexant: 0.035 to 3.5 µmol K_{222} or 0.35 µmol 18-crown-6) and 900-**x** µl solvent (MeCN, THF, and DMF). [^{18}F]Fluoride was released in presence of the base and complexant.

The RCY of dry [^{18}F]fluoride was determined by dividing the decay-corrected radioactivity of the distillate by the radioactivity of the reaction solution before distillation. An overview of the RCYs can be found in the supporting information.

2.5.3 | [^{18}F]Fluoroform synthesis

Dry [^{18}F]fluoride was obtained from 25 GBq aqueous [^{18}F]fluoride as described under Section 2.5.2 by trapping gaseous [^{18}F]triflyl fluoride in a solution of 10 µl K_2CO_3/K_{222} stock solution in MeCN/H_2O (see Section 2.5.1) and 890 µl MeCN. After the trapping solution was heated to 80°C, 100 µl 10 mM triazolium precursor **4** (1 µmol) in MeCN was added, and the mixture reacted for 10 min at 80°C. [^{18}F]Fluoroform was purged out of the solution with a flow of helium (10 ml/min) for about 3 min, led over a Waters Sep-Pak® Plus Silica cartridge (long) and trapped in 1 ml DMF cooled to −60°C.

The RCY of [^{18}F]fluoroform was determined by dividing the decay-corrected radioactivity of the distillate by the radioactivity of the reaction mixture before distillation. For exemplary HPLC chromatograms of the analysis of the [^{18}F]fluoroform reaction see SI, Figure S11-14.

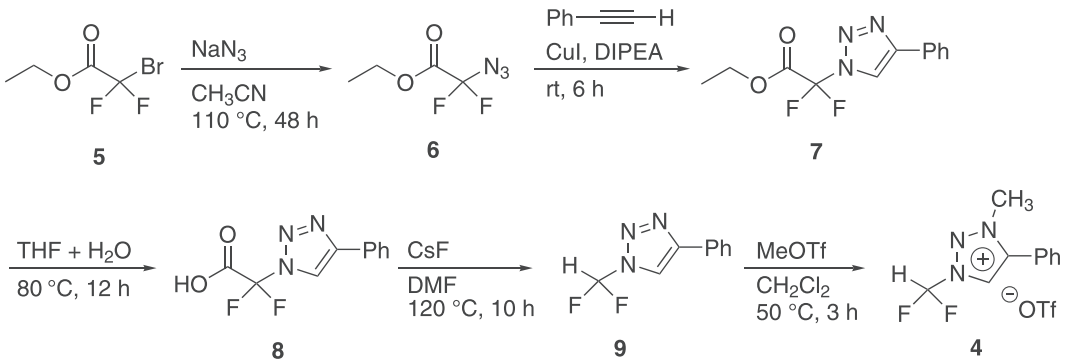
2.5.4 | Automated [^{18}F]fluoroform synthesis on the ORA Neptis® synthesizer

[^{18}F]Fluoroform was synthesized starting from 25 GBq [^{18}F]fluoride with the triazolium precursor **4** on the ORA Neptis® synthesizer according to the procedure described in literature.[13] Exactly the same setup and sequence as for $CHIF_2$ were used, except for two changes: 100 µl 10 mM triazolium precursor **4** (1 µmol) in MeCN was used instead of $CHIF_2$. The heating period of the oven of the synthesizer was prolonged to 2.5 min to ensure that a reaction temperature of 80°C was reached.

3 | RESULTS AND DISCUSSION

3.1 | Stability of precursor 4 in presence of K_2CO_3 and K_{222}

In contrast to difluoroiodomethane **2**, triazolium salt **4** is not commercially available. Precursor **4** was prepared according to literature procedures and was obtained as a white solid with a melting point of 104°C–109°C (Scheme 1).[15,16]



5 6 7 8 9 4

SCHEME 1 Synthesis of triazolium salt **4**

The stability of precursor **4** in pure solvent (MeCN and DMF) at elevated temperatures was studied thoroughly in the literature: when **4** was heated to 80°C for 60 h in acetonitrile or under reflux in DMF (bp = 153°C) for 1 h, no decomposition was observed.[15] However, because difluoroiodomethane proved to be particularly unstable under the basic radiofluorination conditions,[13] we investigated the stability of precursor **4** in presence of K_2CO_3/K_{222} (Table 1). When using 0.15 eq. K_2CO_3 at room temperature (Table 1, entry 1), only a trace amount (0.3%) of dedifluoromethylated triazole **10** was detected, and 99.7% of the precursor remained intact. Increasing the amount of base to 1.5 eq. led to significantly more degradation (60% methyl triazole **10**, entry 2). Also an increase of the temperature promoted degradation: the percentage of methyl triazole **10** in presence of 0.15 eq. K_2CO_3 increased to 5% at 40°C and 9% at 80°C (entries 3 and 4). We therefore concluded that as a consequence of this instability, low amounts of base and complexant are key for obtaining [^{18}F]fluoroform with high molar activity, as is the case with difluoroiodomethane.[13]

3.2 | Radiochemistry

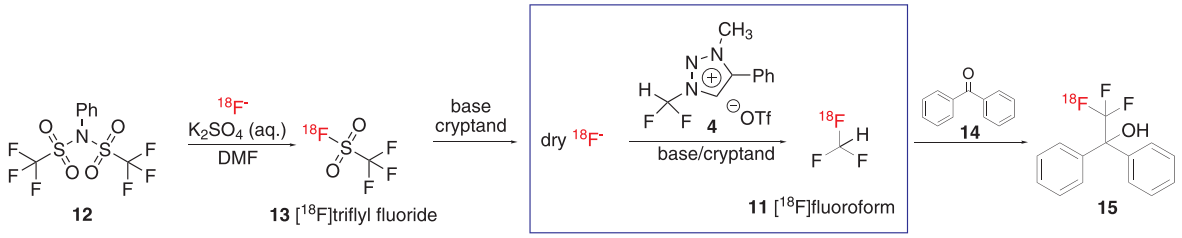
Under standard radiofluorination conditions (azeotropic drying; 15 μmol K_2CO_3, 35 μmol K_{222}) the reaction of triazolium precursor **4** to [^{18}F]fluoroform proceeded with a RCY of 61 ± 5% ($n = 3$) and a molar activity of 0.5 ± 0.1 GBq/μmol ($n = 3$), which is comparable with data obtained with difluoroiodomethane as precursor.[9,10] The molar activity of [^{18}F]fluoroform **11** was determined by formation of the UV active [^{18}F]trifluoromethylated product **15** (Scheme 2). Based on previous data with difluoroiodomethane as precursor[13] and the stability data obtained with triazolium precursor **4** (Table 1), the amounts of base and complexant were decreased 100-fold and the effect on the molar activity and RCY investigated. The molar activity obtained with triazolium precursor **4** could be improved to 102 ± 39 GBq/μmol while still having good RCY of 40± 3% ($n = 4$), which was higher than the results previously obtained with difluoroiodomethane (RCY = 35 ± 4%, A_m = 78 ± 38 GBq/μmol)[13] under same reaction conditions (25 GBq starting activity, 80°C, 10 min, 1 μmol precursor, 0.15 μmol K_2CO_3, 0.35 μmol K_{222}., and 1 ml MeCN).

Encouraged by these results, we studied the [^{18}F]fluoroform formation reaction with triazolium precursor **4** in more detail. To have full flexibility for the screening of the reaction conditions, the [^{18}F]triflyl fluoride method was used to produce dry [^{18}F]fluoride (see Scheme 2).[17] [^{18}F]Triflyl fluoride was generated by radiofluorination of bistriflate precursor **12** under aqueous conditions and was distilled into various solvents (MeCN, THF, and DMF; trapping temperatures according to melting point). [^{18}F]Fluoride was released in presence of various types and amounts of bases (K_2CO_3 and $KHCO_3$) and complexants (K_{222} and 18-crown-6). The RCY of [^{18}F]fluoride depending on solvents, bases, and complexants can be found in Table S1. For all conditions, good to excellent RCYs of dry [^{18}F]fluoride were obtained.

To optimize the RCY and molar activity of the subsequent [^{18}F]fluoroform formation with triazolium precursor **4**, the following reaction parameters were evaluated: (1) reaction temperature, (2) type and amounts of base and complexant, (3) precursor amount, (4) reaction time, and (5) solvent. In the following discussion, all reported RCYs are calculated from dry [^{18}F]fluoride.

The reaction temperature proved to be an important parameter for the optimal [^{18}F]fluoroform formation. The optimization reactions were carried out with about 500 MBq of dry [^{18}F]fluoride, 1 μmol of triazolium precursor **4**, 0.15 μmol of potassium carbonate, and 0.35 μmol of kryptofix 222. Under these conditions, 40°C was the optimal reaction temperature, resulting in RCYs of 52 ± 6% (dc, $n = 3$) (see Figure 2 and SI, Table S2).

In the subsequent reactions starting with higher amounts of [^{18}F]fluoride, however, the temperature optimum shifted towards higher temperatures: with 5 GBq [^{18}F]fluoride, the RCYs at 80°C were almost as high as at 40°C, and with 25 GBq [^{18}F]fluoride, 80°C was clearly preferred (see Table 2).



SCHEME 2 Generation of reactive [^{18}F]fluoride via gaseous [^{18}F]triflyl fluoride **13**, followed by [^{18}F]fluoroform **11** synthesis with the triazolium precursor **4** and subsequent model reaction to the UV active carbinol **15** for molar activity determination

Next, the effect of the amount of potassium carbonate and kryptofix 222 on the RCY and molar activity was investigated. An overview of the results using 5 GBq [^{18}F]fluoride is given in Table 3 and Table S4 of the SI. The results are in line with our previous results using difluoroiodomethane as precursor: the lower the amount of base, the higher the molar activity.[13] However, variation of the amount of potassium carbonate and kryptofix 222 had a much larger effect on the RCY observed for triazolium precursor **4** than observed for difluoroiodomethane at their optimal reaction temperatures (40°C and 80°C, respectively). Optimal results were obtained in the small range of 0.075 to 0.150 µmol potassium carbonate; outside this range, the yields significantly dropped, and at very low base amounts (0.015 µmol), no product was formed at all. With 0.038 µmol potassium carbonate, a very high molar activity of 314 GBq/µmol was obtained, but the A_m could only be determined once due to low RCYs (see Table 3, entry 2). Although this is $N = 1$, it is an interesting observation.

Changing from 5 GBq starting activity to 25 GBq resulted in a shift in the optimal base amount for obtaining good RCYs and molar activities. With 0.075 µmol potassium carbonate, 25 GBq of [^{18}F]fluoride and 40°C reaction temperature, no [^{18}F]fluoroform was formed. Only when the amount was increased to 0.30 to 0.45 µmol potassium carbonate (see Table 4) acceptable RCYs with low standard deviation could be obtained. Using this amount of base, molar activities were however lower.

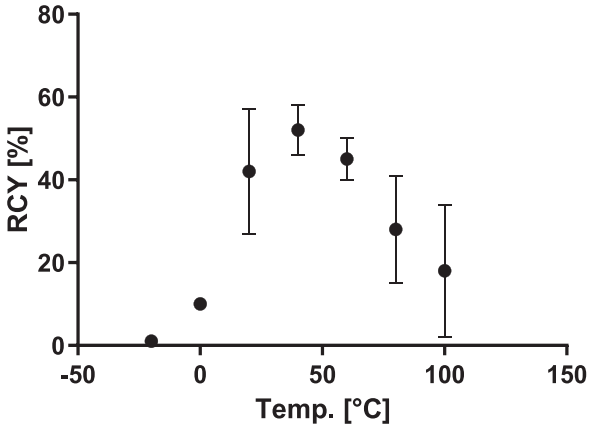


FIGURE 2 Temperature dependency of the [^{18}F]fluoroform synthesis with the triazolium precursor **4**; 500 MBq dry [^{18}F]fluoride, 1 µmol prec., 0.15 µmol K_2CO_3, 0.35 µmol kryptofix 222, 10 min, 1 ml MeCN; dc, $n = 3$. RCY, radiochemical yield

Good RCYs at low base amounts (0.15 µmol K_2CO_3 and 0.35 µmol kryptofix 222) and in consequence high molar activities could be restored by performing the reaction at 80°C: [^{18}F]fluoroform could be synthesized with a RCY of 40 ± 3% and a molar activity of 102 ± 39 GBq/µmol ($n = 4$) (Table 2, entry 6).

Based on the evaluation of the two parameters, reaction temperature and amounts of base and complexant, as described above, it can be concluded that low amounts of base lead to less precursor degradation and therefore high molar activity [^{18}F]fluoroform. However, a certain amount of base is needed to enable the [^{18}F]fluoroform formation at 40°C (see Table 3). When higher amounts of radioactivity are used (see Table 4) more base is consumed for the release of the [^{18}F]fluoride from [^{18}F]triflyl fluoride and as a consequence less base is available for the subsequent [^{18}F]fluoroform formation. Therefore, more base needs to be added to obtain comparable RCYs with high amounts of [^{18}F]fluoride.

The influence of the temperature is not yet fully understood. Our data show that high temperatures encourage precursor degradation, forming the dedifluoromethylated compound (see Table 1). This could explain the lower reaction temperature optimum that we found for low radioactivity levels (500 MBq [^{18}F]fluoride): at 40°C, the radiofluorination still proceeds well while precursor degradation is minimal. Assuming that at high radioactivity levels (25 GBq [^{18}F]fluoride) and low amounts of base (0.15 µmol), the base is almost completely consumed during the release of [^{18}F]fluoride from [^{18}F]triflyl fluoride (see calculations in the supporting information), it might be that under nearly base-free conditions, there are few side reactions

TABLE 2 Temperature dependency of the [^{18}F]fluoroform synthesis with the triazolium precursor **4** using different [^{18}F]fluoride amounts; 1 µmol prec., 0.15 µmol K_2CO_3, 0.35 µmol kryptofix 222, 10 min, 1 ml MeCN; dc

Entry	$^{18}F^-$ (GBq)	Temp. (°C)	RCY (%)	A_m (GBq/µmol)	n
1	0.5	40	52 ± 6	n.d.	3
2	0.5	80	28 ± 13	n.d.	3
3	5	40	55 ± 4	25 ± 7	3
4	5	80	46 ± 3	36 ± 13	3
5	25	40	25 ± 7	67 ± 20	3
6	25	80	40 ± 3	102 ± 39	4

Abbreviation: RCY, radiochemical yield.

TABLE 3 RCY and A_m of [^{18}F]fluoroform synthesized with the triazolium precursor **4** starting from 5 GBq [^{18}F]fluoride; 1 μmol prec., 10 min, 1 ml MeCN, 40°C, dc

			Triazolium precursor		$CHIF_2$ precursor (lit. data[13])[a]	
Entry	K_2CO_3 (μmol)	K_{222} (μmol)	RCY (%)	A_m (GBq/μmol)	RCY (%)	A_m (GBq/μmol)
1	0.015	0.035	0 ± 0	n.d.	18 ± 3	36 ± 30
2	0.038	0.088	11 ± 12	314 (n = 1)[b]	19 ± 6	38 ± 44
3	0.075	0.175	49 ± 10	75 ± 40	38 ± 2	25 ± 12
4	0.113	0.263	58 ± 3	39 ± 4	n.d.	n.d.
5	0.150	0.350	55 ± 4	25 ± 7	44 ± 1	18 ± 2
6	0.750	1.750	27 ± 2	7 ± 3	40 ± 1	5 ± 1
7	1.500	3.500	17 ± 2	5 ± 2	23 ± 2	4 ± 2

Note: n = 3.
Abbreviation: n.d., not determined; RCY, radiochemical yield.
[a]Reaction conditions: 80°C.
[b]Due to low yields, A_m could only be determined once.

TABLE 4 RCY and A_m of [^{18}F]fluoroform synthesized with the triazolium precursor **4** using 25 GBq [^{18}F]fluoride; 1 μmol prec., 10 min, 1 ml MeCN, 40°C; dc

Entry	K_2CO_3 (μmol)	K_{222} (μmol)	RCY (%)	A_m (GBq/μmol)	n
1	0.075	0.175	0 ± 0	n.d.	2
2	0.150	0.350	25 ± 7	67 ± 20	3
3	0.300	0.700	38 ± 1	57 ± 4	2
4	0.450	1.050	35 ± 1	30 ± 1	2

Abbreviation: n.d., not determined; RCY, radiochemical yield.

competing with the radiofluorination reaction, and the reaction temperature can be increased to improve the RCYs of the [^{18}F]fluoroform formation. However, more in-depth investigations are necessary to support this explanation, which is outside the scope of this manuscript.

Subsequently, the influence of the amount of precursor **4** on RCY and molar activity was investigated (see Figure 3 and SI, Table S3). The screening of amounts between 0.2 and 10 μmol with low starting activities (~500 MBq dry [^{18}F]fluoride) showed that the highest RCYs (~45%) could be obtained at precursor amounts of 0.75–2 μmol. Lower amounts of 0.2 μmol still resulted in an acceptable RCY of 34 ± 5%, whereas the RCY dropped dramatically at high precursor amounts (2 ± 0% at 10 μmol).

Based on these results, the effect of the precursor amount on molar activity was investigated with 25 GBq starting activity by comparing 0.75 μmol with 1 μmol precursor amount (see Table S6 in SI), assuming that reducing the precursor amount might reduce the amount of $^{19}F^-$ competing in the reaction and therefore lead to higher molar activities. However, this hypothesis could not be confirmed. Similar molar activities (92 ± 8 vs. 102 ± 39 GBq/μmol) were obtained using 0.75 and 1 μmol precursor, respectively, whereas the RCY slightly decreased using a lower amount of precursor (32 ± 8% vs. 40 ± 3%). Control experiments with a higher precursor amount (2 μmol) also did not result in higher RCY or molar activity (24 ± 5%, 99 ± 69 GBq/μmol).

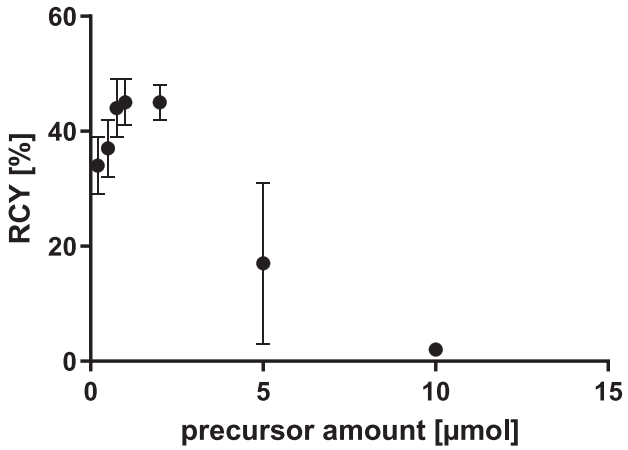


FIGURE 3 Dependency of the [^{18}F]fluoroform synthesis on the triazolium precursor amount; 500 MBq dry [^{18}F]fluoride, triazolium precursor **4**, 0.15 μmol K_2CO_3, 0.35 μmol kryptofix 222, 10 min, 40°C, 1 ml MeCN; dc, n = 3 (n = 6 for 1 and 5 μmol). RCY, radiochemical yield

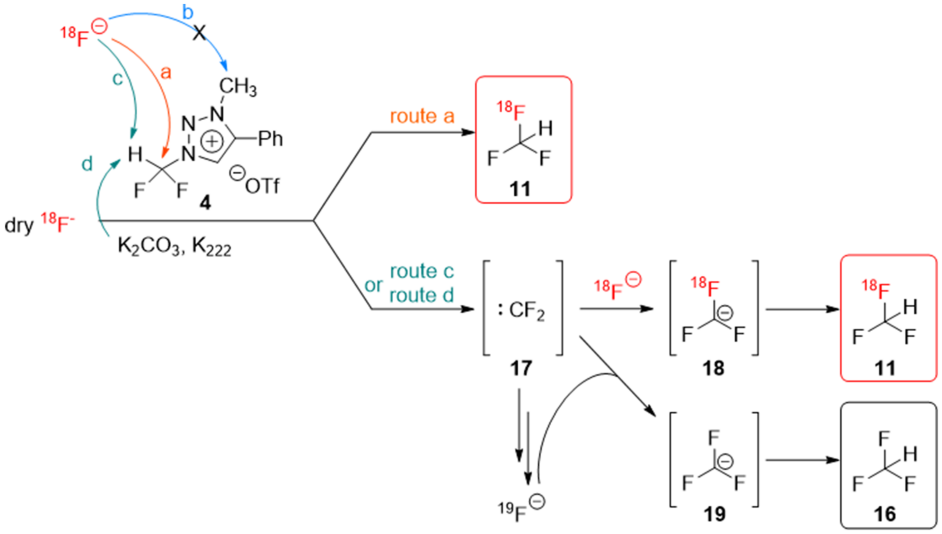
Furthermore, the influence of some other parameters was investigated: type of base and complexant, reaction time, and solvent (see Table S5 in SI). The parameters were chosen based on the optimization with difluoroiodomethane.[13] Variation of the type of base and complexant (at starting activities of 5 GBq) did not bring any improvement in RCY or molar activity, whereas $KHCO_3/K_{222}$ behaved similarly to K_2CO_3/K_{222} (RCY 57 ± 2% and A_m 27 ± 5 GBq/μmol vs. RCY 55 ± 4% and A_m 25 ± 7 GBq/μmol, respectively), K_2CO_3/18-cr-6 led to a drop in RCY (26 ± 12%) and a highly variable molar activity (42 ± 40GBq/μmol). The reaction time did not have an influence on the RCY or molar activity; similar results were obtained after 1 and 10 min reaction with 5 GBq starting activity (RCY 58 ± 2% and A_m 32 ± 21 GBq/μmol vs. RCY 55 ± 4% and A_m 25 ± 7 GBq/μmol, respectively). As alternative solvents, THF and DMF were tested in a reaction with 5 GBq starting activity. With THF, lower RCYs (38 ± 6%) but slightly higher molar activities (43 ± 8 GBq/μmol) were found compared with MeCN. In DMF, the reaction did not proceed very well, and RCYs were too low (10 ± 5%) for molar activity determination. Based on these results, it was decided to keep using K_2CO_3/K_{222} in MeCN and a reaction time of 10 min.

Finally, the performance of the triazolium precursor **4** in the [^{18}F]fluoroform synthesis was also evaluated using the Neptis® perform synthesis module. The same setup and synthesis sequence was used as we described earlier for difluoroiodomethane, and benzophenone was used as a model substrate for molar activity determination.[13] In initial experiments, we observed molar activities that surpassed our quantification limit (A_m > 300 GBq/μmol, n = 3). However, RCYs were highly variable and mostly very low (RCY = 10 ± 9% over three experiments), and the high molar activity results could not be repeated. After slightly adjusting the automated procedure to increase the yield and get more reliable results, we were able to obtain [^{18}F]fluoroform with an overall yield of 14 ± 2% and a molar activity of 153 ± 14 GBq/μmol (n = 3). The heating time of the reactor before precursor addition was crucial and should be at least 2 to 2.5 min to guarantee the reaction temperature to be 80°C. Compared with the results previously obtained with difluoroiodomethane (RCY = 9 ± 2%, A_m = 87 ± 13 GBq/μmol),[13] the triazolium precursor was superior in the automated synthesis because the molar activity was almost twice as high, whereas the RCY was comparable to slightly better as well.

3.3 | Considerations regarding the reaction mechanism

In the abovementioned investigation, triazolium precursor **4** provided good RCYs and molar activities in the manual (RCY = 40± 3%, A_m = 102 ± 39 GBq/μmol, Table 2) as well as in the automated synthesis (RCY = 14 ± 2%, A_m = 153 ± 14 GBq/μmol). We hypothesize that the high molar activities obtained with precursor **4** can be explained by the reaction mechanism of the precursor reacting with [^{18}F]fluoride and base.

In literature, it was proposed that the reaction of the triazolium precursor **4** with [^{19}F]fluoride proceeds via three competing routes (a), (b), and (c): route (a) as the fluoroform formation via S_N2 reaction, route (c) as the fluoroform formation via difluorocarbene, and route (b) as demethylation of the precursor (Scheme 3).[15] Additionally, under radiochemistry conditions, there could be a route (d) that is induced by the base present in



SCHEME 3 Proposed reaction mechanism for the reaction of triazolium precursor **4** with [^{18}F] fluoride

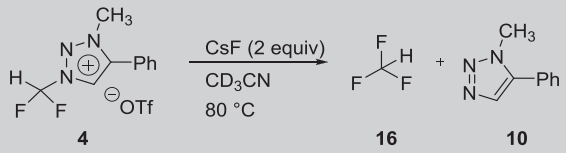


TABLE 5 Determining the reaction order

		Yield (%)[a]				
Entry	**Time (h)**	**4**	**10**	**[RX] (M)**	**ln[RX]**	**1/[RX] (1/M)**
1	0	100	0	0.11200	−2.189	8.929
2	0.167	82	18	0.09184	−2.388	10.889
3	0.333	74	26	0.08288	−2.490	12.066
4	0.5	67	33	0.07504	−2.590	13.326
5	2	34	66	0.03808	−3.268	26.261
6	3	24	76	0.02688	−3.616	37.202
7	5	15	85	0.01680	−4.086	59.524
8	6	13	87	0.01456	−4.229	68.681

Note: Reaction was carried out on a 0.084 mmol reaction scale of triazolium precursor **4** in 0.75 ml of CD_3CN in a sealed NMR tube.
[a]^{1}H NMR yields

the reaction and also leads to fluoroform formation via the difluorocarbene.

Our hypothesis was that the reaction mechanism of triazolium precursor **4** with [^{19}F]fluoride could be reflected in the radiochemistry results: if the reaction of triazolium precursor **4** with [^{18}F]fluoride under the basic radiofluorination conditions would proceed predominantly via routes (c) and (d), only [^{18}F]fluoroform **11** with low molar activity would be obtained due to formation of difluorocarbene **17** (Scheme 3). Difluorocarbene has previously been proposed to release [^{19}F]fluoride competing with [^{18}F]fluoride in the [^{18}F]fluoroform formation and therefore leading to isotopic dilution of the product.[12] Route (a) in contrast would be able to provide [^{18}F]fluoroform **11** with very high molar activity because no [^{19}F]fluoride is released via this route.

Our radiochemistry data support this hypothesis and indicate that there is an equilibrium between routes (a), (c), and (d) for the [^{18}F]fluoroform formation, which can be pushed towards one route or the other by controlling the amount of base present. High amounts of base result in [^{18}F]fluoroform with low molar activity, indicating that route (d) is predominant in this case. Low amounts of base result in less route (d) and result in high molar activity, suggesting that the predominant pathway here is route (a).

To support our radiochemistry findings regarding the reaction mechanism, we determined the reaction order of the reaction of triazolium precursor **4** with [^{19}F]fluoride to fluoroform **16** in a time-dependent ^{1}H NMR study (Table 5). Precursor **4** was reacted with two equivalents of CsF in CD_3CN at 80°C, and yields of **4** and **10** were determined by ^{1}H NMR. In the case of a first-order kinetic behavior, the plot of ln[RX] versus t will be linear ($\ln[RX] = -kt + \ln[RX]_0$ ([RX] = concentration of **4**)). In the case of a second-order behavior, the plot of 1/[RX]1/([RX]) versus t will be linear ($1/[RX] = 1/[RX]_0 + k't$). The two graphs from the experimental results showing ln [RX] versus t and 1/[RX] versus t are displayed in Figure 4A,B, respectively. When adding linear trendlines to both graphs, r^2 in graph 4B is higher than that in graph 4A (r = correlation coefficient). Thus, the plot of 1/[RX] versus t is closest to linearity. Next, theoretical half-lives ($t_{1/2}$) were calculated using the rate constants k and k' obtained from the trendlines of graphs 4A and 4B. In the case of the first-order behavior, $t_{1/2}$ will be 2.057 h ($t_{1/2} = \ln 2/k$). In the case of the second-order behavior, $t_{1/2}$ will be $1/(10.009[RX]_0)$ ($t_{1/2} = 1/k'[RX]_0$). As the number of $t_{1/2}$ elapsed increases to 1, 2, 3, the concentration of **4** decreases to 1/2, 1/4, and 1/8. The theoretical plots of [RX] versus t of first order and second order are shown in Figure 4C. When comparing the experimental values with theoretical values, experimental values are more similar to values of theoretical second order. Overall, we propose that the kinetics of the reaction is second order, which means that the reaction proceeds via a S_N2 process, route (a), under these reaction conditions.

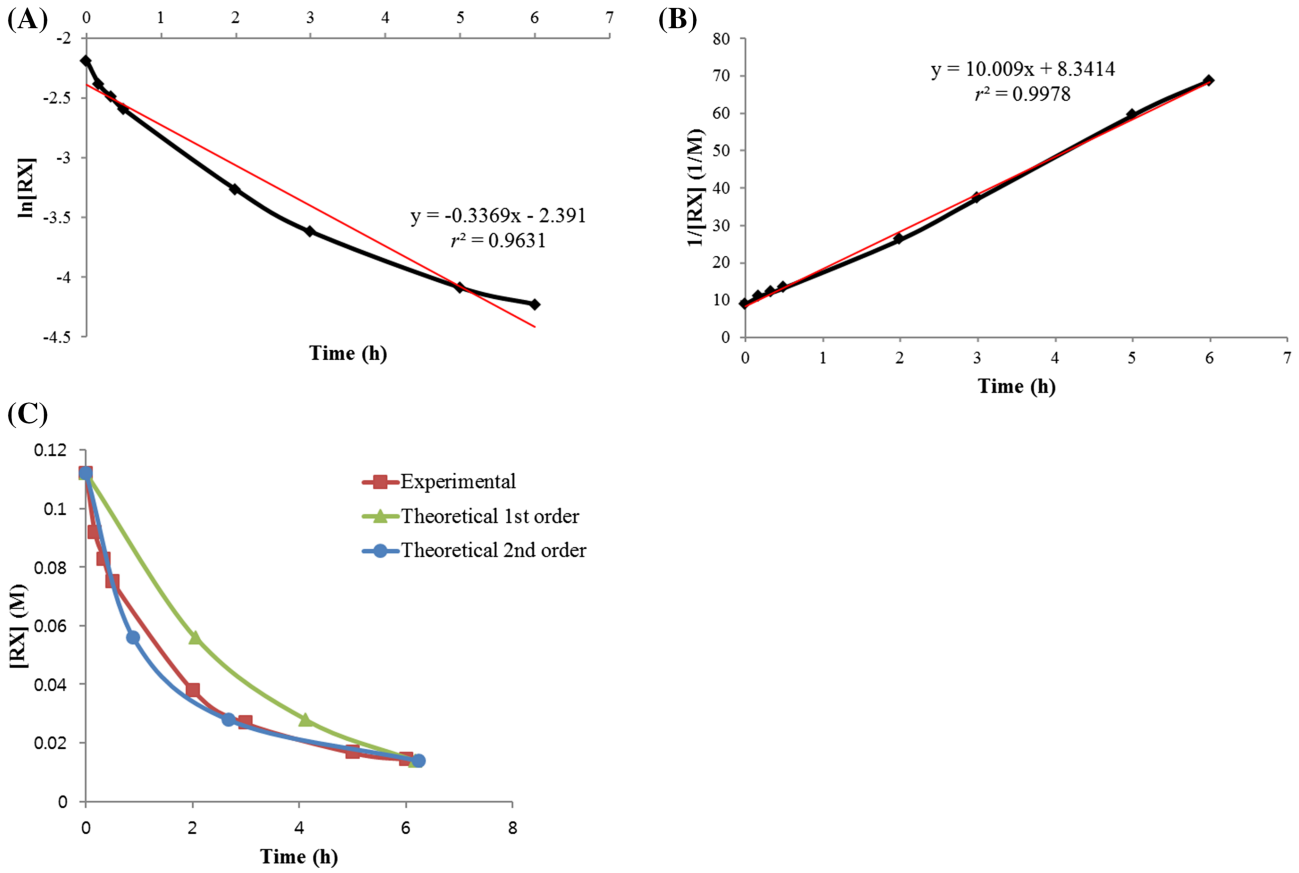


FIGURE 4 Plots of (A) ln[RX] versus *t*, (B) 1/[RX] versus *t*, and (C) [RX] versus *t*

4 | CONCLUSIONS

The triazolium precursor **4** is a valuable alternative to the already known precursors **1**–**3** for the [^{18}F]fluoroform synthesis due to several reasons: (1) the triazolium precursor is a solid and therefore easier to handle than the gaseous difluoroiodomethane, (2) most of the previous findings with difluoroiodomethane concerning the [^{18}F]fluoroform formation reaction also apply to the triazolium precursor and can therefore be adopted, (3) the triazolium precursor provides one of the highest molar activities of [^{18}F]fluoroform observed so far, especially in the automated synthesis on the Neptis® perform module ($A_m = 153 \pm 14$ GBq/μmol, dc, EOS). However, careful control of the reaction conditions is crucial, because the optimal range of base amount and temperature is very narrow. Furthermore, optimal conditions need to be adjusted for different amounts of [^{18}F]fluoride used in the reaction. This represents a major difference to difluoroiodomethane, which generally tolerates a broader range of reaction conditions. Nonetheless, triazolium precursor **4** is a valuable addition to the ^{18}F-trifluoromethylation chemistry toolbox and might be of particular value in the synthesis of PET tracers that require high molar activities.

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CONFLICT OF INTERESTS

Given her role as editor of this journal, Danielle J. Vugts had no involvement in the peer-review of this article. Full responsibility for the peer-review process for this article was delegated to Prof Michael Kassiou.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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