Insights into Elution of Anion Exchange Cartridges: Opening the Path toward Aliphatic $^{18}$F-Radiolabeling of Base-Sensitive Tracers

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ABSTRACT: Aliphatic nucleophilic substitution (SN2) with $[^{18}$F]fluoride is the most widely applied method to prepare $^{18}$F-labeled positron emission tomography (PET) tracers. Strong basic conditions commonly used during $^{18}$F-labeling procedures inherently limit or prohibit labeling of base-sensitive scaffolds. The high basicity stems from the tradition to trap $[^{18}$F]fluoride on anion exchange cartridges and elute it afterward with basic anions. This sequence is used to facilitate the transfer of $[^{18}$F]fluoride from an aqueous to an aprotic organic, polar reaction medium, which is beneficial for SN2 reactions. Furthermore, this sequence also removes cationic radioactive contaminations from cyclotron-irradiated $[^{18}$O]water from which $[^{18}$F]fluoride is produced. In this study, we developed an efficient elution procedure resulting in low basicity that permits SN2 $^{18}$F-labeling of base-sensitive scaffolds. Extensive screening of trapping and elution conditions (>1000 experiments) and studying their influence on the radiochemical yield (RCY) allowed us to identify a suitable procedure for this. Using this procedure, four PET tracers and three synthons could be radiolabeled in substantially higher RCYs (up to 2.5-fold) compared to those of previously published procedures, even from lower precursor amounts. Encouraged by these results, we applied our low-basicity method to the radiolabeling of highly base-sensitive tetrazines, which cannot be labeled using state-of-art direct aliphatic $^{18}$F-labeling procedures. Labeling succeeded in RCYs of up to 20%. We believe that our findings facilitate PET tracer development by opening the path toward simple and direct SN2 $^{18}$F fluorination of base-sensitive substrates.

KEYWORDS: fluorine-18, aliphatic radiolabeling, anion-exchange, QMA, base sensitivity, elution conditions

Positron emission tomography (PET) is a powerful and versatile molecular imaging tool to diagnose disease or monitor treatment progress.1-3 The most widely used PET radionuclide is fluorine-18 ($^{18}$F), as it can be produced in large amounts (>300 GBq) and possesses almost ideal nuclear decay characteristics for molecular imaging.4 Its low positron energy ensures high image resolution, while the half-life of approximately 110 min allows for production of $^{18}$F radiopharmaceuticals for a large number of patients and their distribution to remote sites several hundred kilometers away.5,6 Nucleophilic aliphatic $^{18}$F fluorination (SN2) is one of the most widely applied $^{18}$F-radiolabeling methods.6,7 However, the standard approach (Figure 1) to purify and concentrate $[^{18}$F]fluoride requires strong bases.8-10 The resulting basic environment hinders (or even prevents) $^{18}$F fluorination of base-sensitive substrates while triggering side reactions such as hydrolysis, elimination and/or decomposition of precursors/products.8,11-13 To address this challenge, a wide variety of methods to carry out SN2 $^{18}$F fluorinations under less basic conditions have been developed over the last decades.8,14-20 However, none of these methods appear to be ideal, as they only utilize a fraction of the available...
radioactivity, need special and nonstandard precursors/equipment, or are difficult to implement.21–23 Recently, Mossine et al. have shown that replacement of strong basic anions with weak organic bases significantly increased the radiochemical yields (RCY) for Cu-mediated aromatic 18F fluorinations.24 In light of this, we decided to explore the relationships between the [18F]fluoride elution efficiency for a given preconditioning/eluting anion combination and its potential to activate [18F]fluoride for Sn2 reactions (Figure 1). We hypothesized that by carefully mapping elution conditions and analyzing the corresponding Sn2 18F fluorinations we would be able to tailor the anion combination in such way as to efficiently radiolabel base-sensitive substrates, which cannot currently be radiolabeled using standard reaction conditions (Figure 1).

 RESULTS AND DISCUSSION

Nomenclature and Rationale. The RCY of a labeling procedure is a measure of the proportion of decay corrected and isolated product with respect to the starting radioactivity. Consequently, all steps of a labeling procedure contribute to the RCY.25 We reasoned that the RCY of an Sn2 18F fluorination is primarily determined by two factors: (1) the elution efficiency (EE) of the trapped [18F]fluoride and (2) the reaction efficiency (radiochemical conversion, RCC) (Figure 2A).25,26 The latter can be optimized with respect to temperature, solvent, and the basicity of the reaction medium and the solubility and activation of [18F]fluoride. Whereas the first four parameters are frequently optimized,27 the last three are often neglected, even though they are crucial for the labeling of base-sensitive substrates, as they determine the base concentration of the reaction. The aforementioned parameters are strongly dependent on the anion exchanged.28 In order to study the influence of preconditioning and elution conditions on the RCY, we decided to investigate the EE and the RCC independently. To approximate the expected efficiency of the whole labeling procedure (RCY), we defined a theoretical measure, which we named the pseudo radiochemical yield (pRCY) — pRCY = EE × RCC. This measure was used to evaluate the applied labeling conditions.

Screening of Elution Conditions. Initially, we screened a broad set of different elution conditions (>500 experiments) with the aim to identify a sufficient EE that simultaneously resulted in a low basicity eluate. For simplicity, only the commonly used Sep-Pak Light QMA (130 mg of resin loading) AEC was investigated.29 Furthermore, we explored how different preconditioning anions influence the EE, as this could be a major contributing factor in subsequent fluorinations (Figure 2A). We decided to precondition the cartridges with different preconditioning anions. As elution solvents, we studied water and two different MeCN/H2O mixtures. These elution solvents were chosen to find the best compromise between the better EEs of a higher water content and the considerably shorter azeotropic distillation process associated with a lower water content.

In all experiments, cyclotron-produced aqueous [18F]fluoride was quantitatively trapped. The concentrations resulting in an EE of 90% were calculated by fitting the Hill equation to the data (Table 1). We decided to use this value as we believe that the initial activity loss during the trapping and elution steps should be minimized to ≤10%. Various types and concentrations of eluting anions were screened to identify minimal concentrations. In addition to commonly applied eluting anions such as carbonates, bicarbonates, or oxalates, we investigated organic bases such as DBU, Et3N, DIPEA, and DMAP. These bases deprotonate water molecules, forming OH− anions in situ, which displace [18F]fluoride from AECs. During the subsequent drying
Table 1. Results from EE Screening Using Different Preconditioning and Eluting Anions over a Range of Concentrations

| High conc. | Low conc. | "Standard reagents" | "Organic bases" | "Neutral salts" |
|-----------|-----------|---------------------|-----------------|-----------------|
|           |           | K₂CO₃ /K₂22,18C₆ | DBU             | SO₄²⁻ (Bu₄N)₃⁺ |
|           |           | K₃CO₃ /K₂12,18C₆ | Et₃N            | OMe             |
|           |           | K₃CO₃ /K₂22,18C₆ | PDIPEA          | KOTf            |
| QMA - CF  | H₂O       | 15                  | 18 >200         | 76              |
|           | MeCN/H₂O  | 48                  | >200            | 30              |
|           | (50:50)   | 12                  | 18 >200         | 138             |
|           | MeCN/H₂O  | 15                  | >200            | 76              |
|           | (90:10)   | 15                  | >200            | 30              |

"The table displays concentrations of eluting anions in mM required to elute 90% of [¹⁸F]fluoride from the QMA cartridge. These values were determined by fitting the Hill equation to a set of 7 elutions (5–100 mM of the eluting anion in 1 mL of eluting solvent (5–100 μmol)). Further details can be found in Table S1. Colors indicate concentrations required to obtain EE 90%, with white representing the lowest concentration and gradually darker blue for higher concentrations. K₂22 = Kryptofix 222, 18C₆ = 18-crown-6.

Figure 3. Model radiolabeling reaction using precursor 2 to form [¹⁸F]3. (A) Reaction scheme. (B) Results from initial radiolabeling experiments.

This starting amount is accessible even at radiopharmaceutical centers that lack direct access to a cyclotron and therefore are dependent on [¹⁸F] deliveries. The radioactivity amount used for a single human PET scan is approximately 300 MBq, and such, 375 MBq of labeled tracer is sufficient as a lower limit for this purpose. To reduce the number of experiments, we decided to determine the pRCY on elution conditions that result in an EE of 20, 50, 90 and ~100%. From our initial elution experiments (Table 1), we further decided to test only elutions based on a 50:50 MeCN/H₂O mixture. This decision is a compromise between the diminishing EE observed with a 90:10 mixture and the prolonged drying procedure (~30 min compared to 10–15 min) when pure water was used.

Procedure, bases are removed through distillation, resulting in the low basicity of the reaction mixture. We also investigated a range of neutral salts as eluting anions. In all cases, the EE showed a sigmoidal curve progression with a sharp decrease at a specific concentration depending on the preconditioning of the AECs and the eluting anions (Figure 2B). Bicarbonate preconditioned AECs generally required lower concentrations of eluting anion compared to those for chloride preconditioned AECs. This effect is driven by the weaker interaction of chloride with the quaternary methyl groups of the resin compared to bicarbonates. As expected, the EE was higher for solvent mixtures containing more water. This improvement in EE was especially pronounced for organic bases, as higher water concentrations promoted in situ formation of OH⁻ ions. The most efficient eluting anions were bivalent "standard reagent" anions (K₂CO₃/K₂22, 18C₆; K₃CO₃/K₂22, K₃C₆O₄/18C₆) and "in situ formed OH⁻-anions" with organic bases (DBU and DIPEA) in higher water concentrations, whereas the neutral salts generally required higher concentrations of anions. For this reason, we decided to study the influence of eluting conditions on the RCC.

Trade-Off between the EE and the RCC. In a previous study of elution conditions for aromatic [¹⁸F] fluordeboronations, the highest RCCs were achieved using the lowest concentrations of eluting anions. The best RCYs could be reached using a trade-off between the concentration of eluting anions that yielded high RCCs and acceptable, but incomplete EE. The authors explained the observed trade-off with the base sensitivity of [¹⁸F] fluordeboronations. This observation inspired us to explore if such a trade-off between the EE and the RCC also exists for aliphatic [¹⁸F]-radiolabeling for base-sensitive reactions. A pRCY of 10% was defined as the lowest acceptable limit. This limit was set since it would theoretically allow isolation of 375 MBq of final product from 5 GBq of starting activity with a 45 min synthesis time taken into account.
Initial Radiolabeling Screen Using a Model Reaction.

In order to determine the trade-off between the EE and the RCC, 23 reactions were carried out to determine the minimal anion (base) concentration needed to obtain a pRCY of >10% for our model compound (Figure 3A, Table S2). Details of the workflow for the reactions and analyses can be found in Figure S2. The anion concentration was varied, with one eluting reagent from each category chosen, namely, K₂CO₃/K₂22 for standard reagents, Et₃N for organic bases, and Bu₄NOMs for neutral salts, and two preconditioning anions (HCO₃⁻ or Cl⁻) were tested for each combination. The results are summarized in Figure 3B. No trade-off between the EE (as an indicator of the anion elution concentration) and the RCC could be identified for any of the reactions. For QMAs preconditioned with HCO₃⁻ and eluted with K₂CO₃/K₂22, a linear dependency between the EE and the RCC was observed. A lower eluting anion concentration, and subsequently lower EE, was accompanied by lower RCC. This can be explained by the increased capability of the vessel’s glass wall to adsorb [¹⁸F]fluoride when the (bi)carbonate concentration in the eluate decreases. Absorbed [¹⁸F]fluoride is not accessible for labeling reactions, and consequently, the RCC drops. A pRCY of >10% could be achieved using >3 mM K₂CO₃/K₂22 (interpolated from the elution curves, Figure 3B). As such, we suggest that this should be the minimal elution concentration as a starting point to explore if base-sensitive structures can be labeled using HCO₃⁻ preconditioned QMAs and the according K₂CO₃/K₂22 elution mixture. Lower K₂CO₃/K₂22 concentrations would not be expected to result in acceptable RCYs for base-sensitive substrates. Consequently, [¹⁸F]labeling attempts would be futile if they cannot withstand 3 mM HCO₃⁻. QMA cartridges preconditioned with Cl⁻ and eluted with K₂CO₃/K₂22 showed a similar trend. However, the RCC was further reduced in an exponential fashion with lower EE. This decrease stems from the capacity of the cationic QMA resin to adsorb (bi)carbonate ions. At lower concentrations, no or very little amounts of (bi)carbonate ions can pass through the QMA and are as such not available in the eluate to promote [¹⁸F]fluorination. In contrast, Cl⁻ ions from the Cl⁻-preconditioned QMA are released from the resin during the elution process leading to competing chlorination and thus reducing the RCC further (Figures 3B and S3). Elutions using Et₃N or other organic bases such as DBU or DIPEA resulted in the loss of [¹⁸F] activity (5–50%) during azeotropic distillation of the eluate, and no [¹⁸F] incorporation of the remaining activity into the precursor was observed (Figure 3B, Table S2). This indicates that the organic bases do not generate conditions that are basic enough to promote S_n2 fluorinations. Surprisingly, elution of the HCO₃⁻-preconditioned QMA using Bu₄NOMs resulted in stable RCCs of around 50% independent of the elution anion concentration. pRCY values of >10% could be reached for all tested conditions. Since [¹⁸F]fluorination requires a base and the OMs eluting anion is nonbasic, the then basicity must stem from the HCO₃⁻-preconditioning anion that coelutes with the [¹⁸F]fluoride when eluting the QMA with Bu₄NOMs. No product was formed using the same conditions but preconditioning with the nonbasic anions: OMs⁻ and SO₄²⁻ (Table S3). A previous study reported an [¹⁸F]labeling strategy using neutral elution and preconditioning conditions and then subsequently basifying the eluted mixture with KHCO₃, KOH, or K₂CO₃ before [¹⁸F]fluorination. Unfortunately, in our hands, this strategy resulted in diminishing pRCYs for [¹⁸F]3 with lower concentrations of base, in line with our previous results using potassium (bi)carbonates. This was due to the fact that up to 50% of [¹⁸F]fluoride was adsorbed to the glass wall, despite using a protic solvent and high concentrations of K₂22. Rigorous stirring during the reaction to promote higher resolubilization of the adsorbed [¹⁸F]fluoride did not improve the RCC (Table S4). This prompted us to investigate further how preconditioning of the QMA cartridge combined with neutral elution could promote high pRCYs for low-base conditions.

Investigating the Role of the Preconditioning Anion.

Our data suggest that it is possible to utilize the basicity of the QMA cartridge preconditioning anion to promote [¹⁸F] fluorinations when using nonbasic salts for an efficient elution process. This combination could be used to minimize the base concentration in the reaction and protect base-sensitive precursors/tracers against degradation or to reduce base-promoted side-reactions. Therefore, we decided to test a number of preconditioning anions in combination with Bu₄NOMs elution to determine their influence on the EE, RCC, and ultimately, the pRCY (Table 2). Interestingly, the EE was mainly dependent on the valency of the preconditioning anion rather than the pKₐ with a higher valency increasing the EE (Tables 2 and S5). Nucleophilic preconditioning anions such as C₂O₄²⁻, AcO⁻, or Cl⁻ should be avoided as they lower the RCC by outcompeting the [¹⁸F]fluoride nucleophile, as confirmed by LC-MS analysis (Figures S3 and S5). For any [¹⁸F] fluorination, a certain basicity of the preconditioning anion is needed to promote the reaction. In our setup, the reaction

| preconditioning anion | pKₐ | EE (%) | RCC (%) | pRCY (%) |
|-----------------------|-----|-------|--------|---------|
| Cl⁻                   | −7.0| 24    | 0      | 0       |
| OMs⁻                  | −1.9| 28    | 0      | 0       |
| SO₄²⁻                 | 2.0 (−9.0) | 96   | 0      | 0       |
| H₂PO₄⁻                | 2.1 | 86b   | 0      | 0       |
| C₂O₄²⁻                | 4.2 (1.3) | 99   | traces | traces |
| AcO⁻                  | 4.7 | 45    | traces | traces |
| HCO₃⁻                 | 6.4 | 91.0 ± 5.4c | 56.7 ± 8.9c | 52.1 ± 6.9c |
| H₂PO₄⁺                | 7.2 | 95.6 ± 0.9c | 53.4 ± 4.3c | 51.0 ± 5.4c |
| CO₃²⁻                 | 10.3| 92.0 ± 6.3c | 55.1 ± 1.1c | 50.7 ± 4.3c |
| PO₄³⁻                 | 12.7| 97.0 ± 0c | 74.3 ± 14.2c | 72.0 ± 11.3c |

*pKₐ for second protonation if only one of the divalent anion was investigated. ¹Higher elution could be due to a mixture of mono- and divalent anions formed in aqueous solution. ²Reactions carried out in triplicates.
could proceed if preconditioning anions with a pKₐ of around 4 were used. For univalent preconditioning anions, a higher pKₐ resulted in a higher EE, in line with what has previously been reported. This observation follows the electrosensitivity theory which is based on the Donnan potential. It allows us to determine the electrosensitivity of anions in heterogeneous systems, i.e., the selectivity coefficient between ions in solution and bound to the resin. For anions of the same valency at low concentrations, the dominating factor for the affinity to the resin is the Debye–Hückel activity coefficient which in turn is proportional to the pKₐ, i.e., compounds with higher pKₐ values bind stronger to the resin. As such, preconditioning anions with a higher pKₐ than the fluoride ion facilitate elution of [¹⁸F]fluoride from the QMA cartridge, since eluting anions can more easily displace fluoride from the resin compared to the more strongly bound preconditioning anions.

**Quantifying the Breakthrough of Precondition Anions.** Given that the amount of base in the reaction mixture is determined by the EE of the preconditioning anion when the selected amount of each is used in nonbasic elution approaches are used, a precise quantification of the amount of preconditioning anion that is eluted into the reaction vessel would allow us to understand more thoroughly how these anions affect [¹⁸F] fluorinations, especially for base-sensitive structures. In order to quantify the breakthrough of the preconditioning anions from the QMA cartridge, we estimated their concentration in the eluate by (i) pH measurements (Table S6) and (ii) quantitative NMR (qNMR) (Table S7). In general, qNMR measurements provided a higher precision than pH measurements, but this approach could only be applied to the monovalent anions, HCO₃⁻, H₂PO₄⁻, and OMs⁻. Respective quantifications showed that the monovalent HCO₃⁻ preconditioning anion was proportionally displaced by OMs⁻, whereas the di- and trivalent CO₃²⁻ and PO₄³⁻ showed only minor displacement, even with high concentrations of OMs⁻. This observation can be explained by the Donnan potential. Due to their multiple charges, multivalent anions interact with the cationic groups on the anion-exchange resin more strongly than with monovalent anions. This effect is stronger than the one promoted by the pKₐ-dependent Debye–Hückel activity effect. Therefore, perhaps counterintuitively, when the QMA cartridge is preconditioned with more basic multiple-charge anions, e.g., PO₄³⁻, the basicity of the final elution mixture is lower than when a less basic anion with a lower charge, e.g., HCO₃⁻, is used for QMA preconditioning. This is because the breakthrough of the multiple-charge anion is considerably lower. Finally, qNMR results also showed that the more acidic H₂PO₄⁻ anion (pKₐ: 2.14 in H₂O) remained in its diprotonated form after it was eluted from the QMA. As such, it is able to reduce the basicity of the reaction mixture. However, the mixture remains basic enough to promote the [¹⁸F]-labeling step.

**Improved Resolubilization of [¹⁸F]Fluoride.** Adsorption of [¹⁸F]fluoride on the wall of the glass reaction vessels is a commonly observed phenomenon reducing RCCs under low basicity conditions. In comparison to standard systems using cryptands such as [¹⁸F]KF/K₂₁₂₂₂, tetraalkylammonium[¹⁸F]-fluoride is more lipophilic (cLog D₂₄ calculated with Chemic lapse software for Bu₄NF is 1.32 and for KF/K₂₁₂₂₂ is −0.41). Consequently, the solubility of such salts is higher in organic, polar aprotic solvents which are commonly used for fluorinations. For example, the use of Bu₄NOMs resulted in 10% less glass adsorption compared to that when using the corresponding K⁺/K₂₁₂₂₂ mixture (Tables S8 and S9). As a result, [¹⁸F]fluoride adsorption to glass walls is minimized, and the amount available in the reaction solution increased. To further explore the potential of tetraalkylammonium salts with respect to reaction basicity and to increase the resolubilization process of [¹⁸F]fluoride, three additional salts with different physicochemical properties were studied. Table 3A displays the rationale behind the selection of the respective salts. We decided to study the influence of these tetraalkylammonium salts in combination with the most promising preconditioning anions (carbonate, bicarbonate, phosphate, and hydrogen phosphate) that we identified in the preconditioning screening and three solvents (DMSO, MeCN, and BuOH) which are commonly used solvents for aliphatic [¹⁸F] fluorinations. The solvents were based on their different ability to act as hydrogen bond donors (HBD) and/or hydrogen bond acceptors (HBA) (Table 3B). These factors can affect the solubility of the anions, thus influence the basicity and thus RCYs when labeling base-sensitive structures.

**Multiparametric Screening Using Selected Preconditioning Anions and Elution Reagents for the Model Reaction.** All possible combinations of preconditioning anions, eluting reagents, and reaction solvent were tested (Figure 3A, Table 4). The nonbasic eluting anions OMs⁻ and OTF⁻ resulted in the highest pRCY in combination with multicharged preconditioning anions, especially phosphates. These conditions led to a very low preconditioning anion breakthrough and consequently to a lower base concentration in the eluate. This resulted in surprisingly high pRCYs while retaining high elution of the intact precursor (2) (Figures S14 and S15). H₂PO₄⁻, as an acidic eluting anion, only resulted in good pRCYs when applied with carbonate or bicarbonate preconditioning anions, especially phosphates. These conditions resulted in the inconsistent pRCYs of the same elution of the phosphate preconditioning QMAs con coinciding with the possible variations in elution of the basic preconditioning anion (PO₄³⁻) at this

| tetraalkylammonium salts | rationale |
|--------------------------|-----------|
| Bu₄NOTf                  | The lower pKₐ of OTF⁻ compared to that of the OMs⁻ of Bu₄NOMs should displace lower amounts of preconditioning anions during the elution process, resulting in a less basic eluate and therefore enabling labeling of traces under milder reaction conditions. |
| Bu₄NH₂PO₄                  | Due to the buffering capabilities of Bu₄NH₂PO₄, we decided to test this compound. This salt should neutralize more basic preconditioning anions. |
| Et₄NHCO₃                  | This salt is commonly used for elution in nucleophilic [¹⁸F]-radiolabeling and is used as a comparison. |

Table 3. Rationales Behind the Choice of Tetraalkylated Eluting Anions and Physiochemical Properties of the Different Solvent Used for the Multiparametric Screen of Elution Conditions

solvent    | proton affinity | H-bonding properties |
-----------|-----------------|-----------------------|
DMSO       | aprotic         | no HBD and HBA exist  |
MeCN       | aprotic         | no HBD and very weak HBA |
BuOH       | amphiprotic     | both HBD and HBA properties |

*Mixed with ~17% v/v MeCN added to make it liquid at room temperature.*
Tetraalkylammonium Salts in Combination with Various Preconditioning Anions in Either MeCN, DMSO, or t-BuOH/MeCN (5:1)  

Lower reaction e degradation of the precursor/tracer, but at the expense of less resolubilization. (iv) For anions should be used. (iii) Tetrabutylammonium counterions be followed: (i) Nonbasic anions should be used for the elution process. (ii) Multicharged, non-nucleophilic preconditioning anions should be used. (iii) Tetrabutylammonium counterions should be used for elution to increase resolubilization. (iv) For very base-sensitive compounds, t-BuOH could be used in the reaction solvent to reduce degradation (with the expense of lowered reaction efficiency).

Concentration range. The balance of acidic elution and basic preconditioning is regulated for the carbonates by H2CO3 formation escaping as CO2. However, for phosphate preconditioning all acidity from the elution remains in the eluate. This could probably be optimized with lower concentrations of Bu4NH2PO4 but then at the expense of a lower EE. Finally, the more frequently used elution reagent Et4NHCO3 resulted, as expected for base-insensitive preconditioners/tracers, in good pRCY for all preconditioning anions, comparable with those of the aforementioned high-yielding elution conditions.

Previous studies have reported efficient 18F fluorinations using t-BuOH.12 Surprisingly, the use of t-BuOH/MeCN in this case only resulted in relatively low RCC for all tested elution conditions. However, the amount of intact precursor at the end of the reaction was significantly higher compared to reactions using MeCN or DMSO and otherwise identical conditions (Figure S15). This indicates that the use of t-BuOH in the solvent could be beneficial for very base-sensitive substrates, since the resulting mild labeling conditions lead to less degradation of the precursor/tracer, but at the expense of less efficient 18F incorporation.40

**Reaction Time, Temperature, Precursor Concentration, and Leaving Groups.** From the literature it is known that the reaction time, temperature, precursor concentration, and the chosen leaving group have a strong, but structure-dependent, influence on RCYs. Therefore, we decided not to optimize these parameters for our model reaction and recommend that this should be investigated for individual syntheses.

**Intermediate Findings.** In order to minimize the base content during SN2 18F fluorinations while simultaneously maintaining good RCYs, the following key parameters should be followed: (i) Nonbasic anions should be used for the elution process. (ii) Multicharged, non-nucleophilic preconditioning anions should be used. (iii) Tetrabutylammonium counterions should be used for elution to increase resolubilization. (iv) For very base-sensitive compounds, t-BuOH could be used in the reaction solvent to reduce degradation (with the expense of lowered reaction efficiency).

**Values given as mean values with standard deviation, n = 3. Italic numbers and letters are used to indicate combinations of elution and preconditioning, for example, 1A representing HCO3− preconditioning with Bu4NOMs elution.**

### Table 4. Pseudo Radiochemical Yields (pRCY) of the Model Compound ([18F]3, Figure 3A) Using Different Tetraalkylammonium Salts in Combination with Various Preconditioning Anions in Either MeCN, DMSO, or t-BuOH/MeCN (5:1)

| Preconditioning | Solvent   | Bu4NOMs (A) | Bu4NOTY (B) | Bu4NH:PO4 (C) | Et4-NHCO3 (D) |
|-----------------|----------|-------------|-------------|---------------|---------------|
| HCO3− (f)      | MeCN     | 52.1±15.4   | 1.9%        | 73.6±9.9      | 74.6%         |
|                 | DMSO     | 51.6±17.5   | 2.6%        | 75.9±6.6      | 58.4±13.3     |
|                 | t-BuOH/MeCN | 20.1%    | 0.3%        | 18.3%         | 13.4%         |
| HPO4− (p)      | MeCN     | 50.5±5.1%   | 1.6±2.4%    | 10.5±6.6%     | 73.1±0.7%     |
|                 | DMSO     | 49.4±6.4%   | 56.8±0.1%   | 24.5±11.3%    | 81.7±1.4%     |
|                 | t-BuOH/MeCN | 13.7%    | 8.9%        | 14.7%         | 8.1%          |
| CO3− (q)       | MeCN     | 53.8±12.6%  | 33.5±11.6%  | 85.3±13.9%    | 75.2±2.2%     |
|                 | DMSO     | 75.4±2.3%   | 55.9±16.3%  | 85.2±2.6%     | 58.1±2.1%     |
|                 | t-BuOH/MeCN | 18.5%    | 1.9%        | 13.4%         | 7.7%          |
| PO4− (r)       | MeCN     | 79.9±3.6%   | 70.4±9.6%   | 48.2±21%      | 78.4±5.9%     |
|                 | DMSO     | 74.1±16.1%  | 79.3±7%     | 24.7±29.8%    | 78.5±2.4%     |
|                 | t-BuOH/MeCN | 18%       | 17.7%       | 4.7%          | 13.3%         |

**Improving the Labeling Procedures of Known Radio-pharmaceuticals/Synthons.** Next, we aimed to apply our findings (from Table 4) to the synthesis of a set of well-described PET tracers and radiolabeled building blocks and increase the RCYs of those structures thereby. We set out two criteria for compounds to be studied: (I) Selected structures should possess a reported RCY < 50%, and more importantly, (II) base-insensitive and -sensitive structures should be included to study the beneficial effect of the identified conditions. We were also interested to cover a broad set of structural motifs which could be affected by a basic environment (Table 5). Preconditioning and elution conditions were selected on a rational analysis or by reported data of the base-sensitivity of compounds to be labeled and selected from Table 4.

First, four relatively base-insensitive tracers were tested. We hypothesized that even these structures could benefit from elution with tetralkylammonium salts in respect to increasing the 18F-resolubilization from the glass wall into the reaction solvent. [18F]FETO, [18F]FETC-146, [18F]F-PEG1-N9, and [18F]FE-TCO have been reported to be stable under “standard” basic labeling conditions.41−43 No degradation adducts were observed using those conditions. We assumed that eluting a QMA which was preconditioned with HCO3− or the slightly more basic CO3− (conditions 1C or 3C, Table 4) with tetralkylammonium salts would result in higher 18F-resolubilization, while simultaneously the preconditioning anion would provide enough basicity to promote the labeling step. For all four compounds, an increased isolated RCY was achieved spanning from approximately 40 to 170% increase using the optimized conditions (Table 5). Retrospective analysis of the 18F-resolubilization data showed that this parameter was indeed in all reaction increased and significant contributed to the improvement RCY (10−30% of the observed increase). One additional factor that might have improved the yields is the lower base content used. This condition rather favors SN2 labeling over additional factor that might have improved the yields is the lower base content used. This condition rather favors SN2 labeling over...(Table 4...PET tracers and radiolabeled building blocks and increase the RCYs of those structures thereby. We set out two criteria for compounds to be studied: (I) Selected structures should possess a reported RCY < 50%, and more importantly, (II) base-insensitive and -sensitive structures should be included to study the beneficial effect of the identified conditions. We were also interested to cover a broad set of structural motifs which could be affected by a basic environment (Table 5). Preconditioning and elution conditions were selected on a rational analysis or by reported data of the base-sensitivity of compounds to be labeled and selected from Table 4.

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The relatively base-sensitive structure that was investigated in this study was [18F]FTHA. This compound is labeled at a secondary carbon atom and thus, is more prone to...
undergo E2 elimination, especially under strong basic conditions.\textsuperscript{44} We hypothesized that less basic conditions should consequently lead to a high RCY. Preconditioning with PO$_4^{3-}$ and using Bu$_4$OMs for elution resulted in the lowest basicity of the eluent (conditions \textsuperscript{4A}, Table 4). Applying these conditions doubled the isolated RCY compared to the reference procedure using \textquotedblleft standard\textquotedblright conditions (Table 5).\textsuperscript{45} The next compound tested was a building block which can be used to label a broad set of radiopharmaceuticals.\textsuperscript{39} $[^{18}$F]$2^R$,3$^R$,4$^S$,5$^R$,6$^R$]-2-Azido-6-(fluoromethyl)tetrahydro-$2^H$-pyran-3,4,5-triyl triacetate ($[^{18}$F]sugarazide) is labeled via a two-step labeling procedure. First, a hydroxy-group protected precursor is $^{18}$F-labeled and then deprotected. The acetyl protection groups are base-labile. In the reported labeling procedure, partial hydrolysis of those protecting groups occurred using \textquotedblleft standard\textquotedblright basic labeling conditions. Free hydroxy groups typically form H-bonds with $^{18}$F$^-$ and reduce its nucleophilicity, thus decreasing RCYs. We applied our low-basicity conditions using a PO$_4^{3-}$ preconditioned QMA and Bu$_4$NOTf (\textsuperscript{4B}) for elution in order to reduce the basicity and consequently reduce premature deprotection. No deprotection was observed using these conditions, and the

Table 5. Tracers Tested with the Derived Conditions from the Model Reaction$^a$

| Tracer name | Structure | Reference pRCY | pRCY (This work) | Reference RCY | RCY (This work) | Conditions$^d$ | RCY Increase |
|-------------|-----------|----------------|------------------|---------------|----------------|---------------|--------------|
| $[^{18}$F]FETO$^b_k$ | ![Image](https://example.com/image1) | 43.8±1.2% | 71.5±2.8% | 20±3% | 54.5±7.0% | 1C (DMSO) | ↑166% |
| $[^{18}$F]FETO-146$^l$ | ![Image](https://example.com/image2) | 31.7±1.8% | 42.4±3.2% | 12.3±1.8% | 24.6±2.7% | 3C (DMSO) | ↑100% |
| $[^{18}$F]PEG$_2$Ni$^n$ | ![Image](https://example.com/image3) | 63.6±6% | 88.2±2.7% | 37±8% | 66.4±9.3% | 3C (DMSO) | ↑76% |
| $[^{18}$F]EtCO$^o$ | ![Image](https://example.com/image4) | 46.5±9.8% | 81.5±1.4% | 44±9% | 61.8±4.7% | 3C (MeCN) | ↑40% |
| $[^{18}$F]THA$^p$ | ![Image](https://example.com/image5) | 28.3±11.7% | 38.7±17.8% | 13±6.3% | 26.5±4.8% | 4A (MeCN) | ↑104% |
| $[^{18}$F]sugarazide$^q$ | ![Image](https://example.com/image6) | 28±2.9% | 64.7±3.3% | 19% | 41.8±7.8% | 4B (MeCN) | ↑120% |
| $[^{18}$F]FPE$^r$ | ![Image](https://example.com/image7) | 37.6±17.9% | 72.5±13.1% | 18±2.2% | 47.8±7.9% | 4A (DMSO) | ↑165% |
| $[^{18}$F]23 | ![Image](https://example.com/image8) | - | 25.8±3.8% | - | 22.8±3.9% | 4A (tBuOH/DMSO) | - |
| $[^{18}$F]29 | ![Image](https://example.com/image9) | - | 11.5±3.5% | - | 5.2±2.8% | 4A (tBuOH/DMSO) | - |

$^a$Reference procedures were reproduced manually and compared to derived conditions. Automated synthesis was carried out and isolated RCY was compared to references. All results created within this work are based on $n = 3$. Synthetic schemes for precursors can be found in the Schemes S3—S5. Further details on the syntheses can be found in the Figures S20—S49 and Tables S11—S20.\textsuperscript{39} Earlier reported syntheses do not use quantitative analysis methods (only HPLC) and do not report isolated RCY and was therefore not suitable for comparison.\textsuperscript{39} MeCN/H$_2$O, (50:50) was used for elution instead of methanol.\textsuperscript{4} tBuOH/MeCN used instead.\textsuperscript{4} In-house data ($n = 7$). Tracers that were not accessible via standard $^{18}$F-labeling approaches are colored beige.
Tetrazines (Tz) are a class of compounds which can be applied in pretargeted imaging.\textsuperscript{47–53} Currently, only low-reactivity Tzs can be radiolabeled via direct aliphatic SN2.54 Typically, these structures display too low a reactivity for in vivo bioorthogonal chemistry approaches.\textsuperscript{55,56} Highly reactive structures such as monounsubstituted tetrazines (H-Tzs) have been reported to be highly sensitive to base.\textsuperscript{57} Extensive degradation is observed which prevents isolation of meaningful amounts for imaging studies. Using “standard” conditions, no or only trace amounts of the radiolabeled product could be isolated in the reaction mixture.\textsuperscript{58} We hypothesized that our mildest labeling conditions (4A) in combination with t-BuOH/DMSO could provide sufficiently low basicity labeling conditions to label a H-Tz. Initial attempts using a mesylate precursor resulted in an increase in traces of labeled product to a pRCY of approximately 2% of [18F]23. In a next step, we investigated the influence of different leaving groups. In addition to the mesylate (OMs) group, we tested tosylate (OTs)- and nosylate (ONs)-based precursors.

As mentioned previously, different leaving groups can influence the labeling yield substantially, but no trend with respect to increased RCY has been observed. The yields varied on a case-by-case basis depending on the individual molecular structure of the precursor.\textsuperscript{58} To facilitate the reaction, the solvent was further changed to t-BuOH mixed with DMSO which decreased the evaporation of solvent during automated synthesis while maintaining the RCC (Table S20). The nosylate precursor with t-BuOH/DMSO and the low basic elution condition (4A) resulted in a RCY of approximately 22% (Figure 4). Control experiments were also carried out to investigate if low-basicity conditions (4A in combination with a t-BuOH solvent) are needed to promote the reaction. These experiments yielded no or only trace amount of the [18F]-labeled Tz.\textsuperscript{[1563]} In order to test the applicability of the identified conditions to label H-Tzs, we decided to radiolabel an even more reactive Tz. The chosen structure displays a 4-fold increased reactivity toward TCO (2676 vs 682 M$^{-1}$ s$^{-1}$ Table S21)\textsuperscript{58} and should as such be even more difficult to label, since reactivity is proportional to the Tz’s base instability (Tables S5 and S20). In line with the aforementioned observations, the more reactive Tz \textsuperscript{(18F)29} could only be radiolabeled using the mildest labeling conditions. As expected, the compound could be isolated from the ONs precursor in a lower RCY (ca. 5% RCY) than the less reactive Tz. This reflects the higher base sensitivity of the structure.

**Recommendations for Aliphatic 18F-Radiolabeling Attempts.** Our results indicate that the following labeling conditions should be used as a starting point to label aliphatic structures (Figure 5): (i) Base-sensitive tracers/precursors: t-BuOH-mixtures or similar hindered protic solvents should be used in combination with condition 4A or 4B. (ii) Moderately base-sensitive compounds: Conditions 4A or 4B in combination with MeCN or DMSO should be used. (iii) Base-insensitive structures: 1C or 3C in DMSO are robust high-yield conditions; alternatively conventional methods using tetraalkylated carbonates should be used.
CONCLUSIONS

By carefully studying the key parameters involved in the trapping of \([^{18}\text{F}]\)fluoride on an anion exchange cartridge and its subsequent elution, we were able to identify conditions that result in low basic elutions. These conditions enable us to radio-label base-sensitive structures with significantly improved RCYs. Even structures that were previously not accessible by applying 'standard' aliphatic \(^{18}\text{F}\)-labeling strategies could be radio-labeled. The developed methodology can easily be implemented on all synthesis modules and is only dependent on the preconditioning of the anion exchange cartridge, its nonbasic elution, and on the selection of the right reaction solvent. This places new classes of \(^{18}\text{F}\)-fluorinated compounds within the reach of classical labeling approaches (S\(_N\)2 labeling).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsptsci.1c00133.

Detailed experimental procedures, characterization of novel structures and labeling protocols (PDF)

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Author Contributions

The elution screening experiments were conducted by K.B., V.S., and I.N.P. Precursor synthesis was done by K.B., U.B., and S.L.B., and subsequent radiolabeling experiments were carried out by K.B. The study concept was designed and the manuscript written by K.B., V.S., and M.H.H. with contribution from all authors. All authors have given approval to the final version of the manuscript.

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Notes

The authors declare no competing financial interest.

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