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Hydrogen-bond promoted nucleophilic fluorination: concept, mechanism and applications in positron emission tomography

Some protic solvents such as oligoethylene glycols and tertiary alcohols were found to be unprecedented powerful promoters for S_N2 -type nucleophilic fluorinations, which is in great contrast with conventional wisdom for S_N2 reactions.

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Hydrogen-bond promoted nucleophilic fluorination: concept, mechanism and applications in positron emission tomography

Ji-Woong Lee,^{†a} Maria Teresa Oliveira,^a Hyeong Bin Jang,^a Sungyul Lee,^{*b} Dae Yoon Chi,^{*c} Dong Wook Kim^{*d} and Choong Eui Song^{*a}

Due to the tremendous interest in carbon–fluorine bond-forming reactions, research efforts in this area have been dedicated to the development of facile processes to synthesize small fluorine-containing organic molecules. Among others, PET (Positron Emission Tomography) is one of the most important applications of fluorine chemistry. Recognizing the specific requirements of PET processes, some groups have focused on fluorination reactions using alkali metal fluorides, particularly through S_N2 -type reactions. However, a common “misconception” about the role of protic solvents and hydrogen bonding interactions in this class of reactions has hampered the employment of these excellent promoters. Herein, we would like to review recent discoveries in this context, showing straightforward nucleophilic fluorination reactions using alkali metal fluorides promoted by protic solvents. Simultaneous dual activation of reacting partners by intermolecular hydrogen bonding and the enhancement of the “effective fluoride nucleophilicity”, which is Nature’s biocatalytic approach with the fluorinase enzyme, are the key to this unprecedentedly successful nucleophilic fluorination.

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Key learning points

- (1) Importance of hydrogen bonding interactions in enzymatic S_N2 fluorination.
- (2) Alkali metal fluorides as efficient and safe nucleophilic sources in nucleophilic fluorination in protic solvents.
- (3) New mechanistic insights into the positive effects of protic solvents for S_N2 reactions; simultaneous “electrophile–nucleophile” dual activation by intermolecular hydrogen bonding.
- (4) Synthesis of ^{18}F -labeled PET radiotracers *via* protic solvent promoted nucleophilic fluorinations; high reaction rates and water tolerance.

1. Introduction

Nucleophilic substitution is a fundamental class of reactions in chemistry. In this type of reaction, a nucleophile replaces a leaving group by attacking the positive or partially positive charge of an atom referred to as the electrophile. In the case of aliphatic bimolecular nucleophilic substitutions (S_N2) at an sp^3 carbon,

a bond is broken and another bond is formed synchronously, *i.e.*, in one step. S_N2 reactions are usually severely affected by the solvent used.¹ In most organic chemistry textbooks, polar aprotic solvents (*e.g.* dimethylformamide, dimethylsulfoxide, *N*-methylpyrrolidone, *etc.*) have been described as optimal for this type of chemical reaction. On the other hand, protic solvents such as water and alcohols have been deemed to be unsuitable due to the formation of “trapped” anionic nucleophiles by the hydrogen bonding donors of protic solvents, thus generating inert nucleophiles. For example, halides (F^- , Cl^- , Br^- and I^-) can be trapped in aqueous solutions and become unreactive, so their use in organic synthesis has been quite limited to polar aprotic solvent systems. However, Nature has utilized aqueous fluorides for nucleophilic substitution reactions,² overcoming their low nucleophilicity in water, sometimes accelerating the reaction more than 10^8 fold over the non-catalyzed reaction. Unambiguous evidence of this operation has been obtained from the fluorinase

^a Department of Chemistry, Sungkyunkwan University, 2066, Seobu-ro, Jangan-gu, Suwon-si, Gyeonggi-do 440-746, Korea. E-mail: s1673@skku.edu

^b Department of Applied Chemistry, Kyung Hee University 1732, Duckyoung-daero 1732, Gihung-gu, Yongin-si, Gyeonggi-do 446-701, Korea. E-mail: sylee@khu.ac.kr

^c Department of Chemistry, Sogang University, 35 Baekbeomro Mapogu, Seoul 121-742, Korea. E-mail: dychi@sogang.ac.kr

^d Department of Chemistry, Inha University, 100 Inha-ro, Nam-gu, Incheon 402-751, Korea. E-mail: kimdw@inha.ac.kr

[†] Current address: Department of Chemistry, University of Copenhagen, Universitetsparken 5, 2100 Copenhagen Ø, Denmark

enzyme isolated from *Streptomyces cattleya*.² The active site for fluorination of SAM (*S*-adenosyl-L-methionine) is orchestrated to desolvate fluoride in a controlled manner by hydrogen bonding interactions, generating the fluoride nucleophile (Fig. 1).

Despite their low natural occurrence in Nature, fluorinated organic molecules have received considerable attention in life sciences, particularly during the last decade.^{3,4} Fluorine is often incorporated to enhance the potency, lipophilicity, bioavailability,

and metabolic stability of a molecule. Furthermore, radioactive fluorinated compounds (containing ¹⁸F) also serve as radiotracers for medical non-invasive imaging processes such as positron emission tomography (PET).^{5,6} In this regard, methods for introducing a single fluorine atom at a specific molecular site are considered to be of utmost importance.^{7,8}

One possible fluorination approach involves utilizing highly reactive electrophilic “F⁺” equivalents to achieve the fluorination



Ji-Woong Lee and Maria Teresa Oliveira

Ji-Woong Lee received his MSc in chemistry from Sungkyunkwan University under the guidance of Prof. Choong Eui Song (2009). In 2013 he obtained his PhD under the supervision of Prof. Dr Benjamin List at the Max-Planck-Institute. After postdoctoral research at the UC-Berkeley, he is currently an assistant professor at the University of Copenhagen.

Maria Teresa Oliveira received her MSc in chemistry from Instituto Superior Técnico, Lisbon (2008). In 2013 she obtained her PhD in organic chemistry under the supervision of Prof. Dr Nuno Maulide at the Max-Planck-Institute. Currently, she is a postdoctoral researcher at the University of Copenhagen.



Hyeong Bin Jang

Hyeong Bin Jang received her MSc in chemistry from Sungkyunkwan University under the guidance of Prof. Choong Eui Song (2011). She currently works as a research scientist at LG CHEM.



Sungyul Lee

Sungyul Lee received his PhD degree in physical chemistry from University of Chicago (1988). He has been appointed as an Assistant, Associate, and Professor at Kyung Hee University from 1989 to present. His research interests include the theoretical calculation of solvent catalysis and materials chemistry.



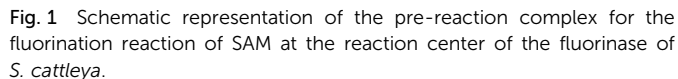
Dae Yoon Chi

Dae Yoon Chi received his PhD in organic chemistry from the University of Illinois at Urbana-Champaign (1986). Then he was appointed as a staff member of the Department of Nuclear Medicine, Samsung Medical Center in 1994. In 1995, he joined the Inha University and was promoted to a professor in 2000. In 2009, he moved to Sogang University. His main research spans medicinal chemistry and radiopharmaceutical chemistry.

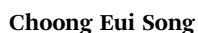


Dong Wook Kim

Dong Wook Kim received his PhD in chemistry from Inha University (2004). He was a post-doctoral fellow at University of Illinois at Urbana-Champaign. In 2006 he became a professor at Chonbuk National University. In 2013, he moved to Inha University as a professor. His research interests focus on radiolabeling, medicinal chemistry and molecular imaging.

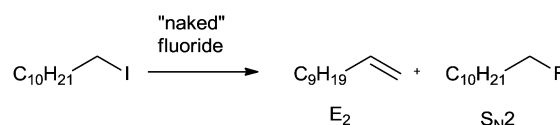


The typical method for introducing a single fluorine atom at a specific aliphatic site by nucleophilic fluorination involves the displacement of a sulfonate or halide by a fluoride anion. The use of anhydrous HF (b.p = 19.6 °C) suffers from safety and handling problems. Alkali MFs such as KF and CsF are perhaps



Choong Eui Song (b. 1955) received his PhD from RWTH Aachen (1988). Since 1989, he has been working as a Principal Research Scientist at the Korea Institute of Science and Technology. In 2004, he moved to Sungkyunkwan University as a professor. His research interests focus on the development of new methodologies and chiral catalysts for asymmetric reactions. He has received several scientific awards, including the Scientist of the Month Award of Korea (2001), the

Over the last decade, we have developed several new methodologies employing protic solvents, which have been known to be



Fluoride source (solvent)	E ₂ : S _N 2
KF/18-crown-6 (CH ₃ CN)	49:51
KF/[2.2.2]-cryptand (CH ₃ CN)	69:31
TBAF (THF)	89:11
TBAF (anhydrous THF)	92:8
TBAT (CH ₃ CN)	26:74
1 (THF)	99:1
2 (THF)	99.4:0.6

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inadequate for S_N2 reactions, for facile fluorination reactions. Inspired by hydrogen-bond assisted/catalyzed enzymatic fluorination reactions, our solvent engineering approach has allowed us to generate more “controllable” fluoride nucleophiles, thus enabling practical and chemoselective nucleophilic fluorination reactions.

Recently, the field of transition metal-catalyzed nucleophilic fluorinations^{9–12} to install fluorine substituents on a sp^3 -hybridized carbon¹⁵ has experienced rapid growth, offering a new and efficient synthetic strategy to prepare ^{18}F -labeled radiopharmaceuticals.¹⁶ Moreover, a number of examples of nucleophilic deoxyfluorination reagents have shown their potential in facile nucleophilic fluorination reactions, although their applications are quite limited due to the instability of the reagents under ambient conditions.^{7,8}

In this Tutorial Review, we focus on transition metal-free fluorination reaction protocols using alkali metal fluorides, mainly KF and CsF, as practical fluoride sources. Inspired by the operation of enzymatic fluorination reactions, we highlight the importance of weak forces such as hydrogen bonding interactions to promote selective fluorination reaction at sp^3 carbon centers. The selected recent examples demonstrating the action of polar protic solvents in nucleophilic fluorination reactions are exemplified with ionic liquids, bulky alcohols and oligoethylene glycols, in which the C–F bond formation reaction is promoted mainly by H-bondings. Summarized experimental and mechanistic details shed light on the origin of positive effects of H-bondings in protic media for facile nucleophilic fluorination reactions. Recent applications of protic solvents in PET techniques with significant improvement compared to traditional ^{18}F -fluorination protocols are also presented.

2. Fluorinase enzyme-catalyzed nucleophilic fluorination

In 2001, a seminal discovery regarding the unprecedented activity of two fluorinases for C–F bond formation was unveiled by the Withers group.^{17,18} They found that mutated enzymes such as glucosidase from *Agrobacterium sp.* can catalyse the β -glucosidic bond formation reaction of substrate **A** with R–OH in the presence of additional fluoride, KF (Scheme 2). In the absence of fluoride, the enzyme is totally inactive towards the β -glucosidic bond forming reaction. The intermediate, α -glucosyl fluoride **C**, was observed by 1H and ^{19}F NMR spectroscopy. Subsequently, the resulting electrophilic fluoride **C** can participate in the substitution reaction with an incoming nucleophile (R–OH) to generate the desired β -glucosidic bond (**D**). The importance of Ser-358 is presumed to be that the hydrogen bonding interaction may play a role in the desolvation and stabilization of the fluoride, which acts not only as a nucleophile but also as a leaving group.

Another example of an active fluorinase was reported by the O'Hagan group.¹⁹ The X-ray crystallographic structure of the fluorinase shows a snapshot of the fluorination reaction of SAM in the reaction pocket (Fig. 1).¹ The hydroxy group of the Ser-158 and Thr-80 residues replaces water molecules, extracting the fluoride anion from aqueous medium and placing it at the



Scheme 2 Enzymatic β -glucosidic bond formation reaction via nucleophilic fluorination and defluorination (DNP = 2,5-dinitrophenyl).

active center of the enzyme, consequently increasing the nucleophilicity of the fluoride in the reaction site. These weak hydrogen bonding interactions of Ser-158 and neighboring Thr-80 are enough to compensate the high energy penalty of dehydration (activation barrier = *ca.* 400 kJ mol^{−1}) to incorporate fluoride into the reaction center. Moreover, these hydrogen bonding interactions can reduce the basicity of fluoride, consequently enhancing “effective fluoride nucleophilicity”. These examples taken from Nature strongly suggest that the employment of hydrogen-bond interacting functional groups (*e.g.* serine and threonine) is critical for utilizing aqueous fluoride at extremely low concentrations. Therefore, it follows that fluorine chemistry in a flask may also benefit from hydrogen bonding interactions to employ inherently insoluble alkali fluoride in organic solvents.

3. S_N2 -type fluorination using alkali metal fluorides in aqueous ionic liquids

As mentioned before, the high lattice energy of alkali MFs such as KF and CsF often hinders their use in organic synthesis because of their low solubility in organic solvents. It is well known that ionic liquids (ILs), being highly polarized ionic compounds, can provide stabilizing effects during the course of a reaction with a polar transition state and high solubility for metal salts.²⁰

In 2002, we first reported a facile nucleophilic fluorination of alkyl mesylates using KF and an IL, [bmim][BF₄].²¹ For example, the fluorination of **3** with KF in a polar aprotic solvent such as CH₃CN at 100 °C had hardly formed any product even after 24 h. The use of a crown ether also afforded only 40% yield even after 24 h. However, surprisingly, using [bmim][BF₄] in the presence of water as the additive, the same reaction was completed within 1.5 h, affording the desired product **4** in up to 92% yield (Scheme 3). Our DFT calculations showed that the improved activity may be explained by the cooperative activity of the cation and the anion of ILs, *i.e.* the ionic liquid plays an ambiphilic “electrophile–nucleophile” dual activation role (Fig. 2).²²

Scheme 3 Ionic liquid promoted $\text{S}_{\text{N}}2$ -type fluorination.

The counteranion of the ionic liquid acts as a Lewis base toward M^+ , drastically reducing its electrostatic effects and thereby “freeing” the F^- nucleophile and the acidic C2-proton of the imidazolium cation interacts with the mesylate leaving group *via* hydrogen bonding, helping it to detach from the reactant. This nucleophilic substitution reaction in the presence of aqueous ILs can also successfully be extended to other nucleophiles such as other halogens, cyanide, acetate, azide and alkoxides to afford the corresponding substituted products with excellent yields.²³

Very interestingly, the presence of water also turned out to be critical for the high selectivity of the reaction. The protic environment of water may reduce the basicity of the fluoride anion. In the absence of water, the fluorination of **3** afforded the desired product **4** (85%) together with the alkene byproduct **5** (10%). Very interestingly, however, the addition of water (5 equiv.) completely eliminated the formation of the undesired alkene product, consequently increasing the yield (92%) of the product **4** (Scheme 3). Moreover, even in the fluorination of a highly base-sensitive substrate, **5**, which tends to undergo such an elimination reaction to produce styrene derivative **7**, the use of water as a protic cosolvent suppressed the elimination reaction. On the other hand, in the absence of water, the same reaction produced a significant amount of elimination product **7** (39%) (Scheme 4).²¹

From this finding, we became aware of the important role of protic media in chemoselective fluorination by reducing the basicity of the naked fluoride anion. Moreover, the possibility of performing the reaction in the presence of water is noteworthy and highly beneficial for the applications to PET processes, since ^{18}F fluoride is generated from ^{18}O -enriched water, and thus the

Fig. 2 Calculated transition state in $\text{S}_{\text{N}}2$ fluorination using KF in ILs: cooperative activation by ILs.Scheme 4 Nucleophilic fluorination of base-sensitive substrate **5** using KF.

obtained fluoride is in aqueous medium, which has to be completely dried before labeling of PET tracers. This is typically a time-consuming step required in conventional radiofluorination methods.

To enable facile purification of the final product, a polymer-supported IL **8** was reported (Scheme 5a).²⁴ By using a substoichiometric amount of Merrifield resin-anchored polymeric IL **8**, the fluorination of substrate **9** with CsF in acetonitrile/water for 2.5 h produced the desired product **10** in an excellent yield (98%). Surprisingly, the immobilized IL **8** showed superior activity to its non-immobilized version under identical reaction conditions. This could be ascribed to the synergistic effects between neighboring imidazolium salts, participating in efficient charge-charge interactions on the swellable polymer support. This bicipital effect of neighboring ion pairs has also been observed by Clark *et al.* using tetraarylphosphonium salt grafted silica gel as the catalyst for the iodination of alkyl bromides (Scheme 5b).²⁵

As described above, polar media such as ILs offer a convenient reaction environment by generating highly reactive fluoride anions from MFs, while diminishing by-product formation. However, the utility of ILs for fluorination reactions is limited by gradual thermal degradation of ILs in the presence of basic fluoride anions.²⁶ Therefore, we explored other more accessible, inexpensive, and robust reaction media for the activation of MFs towards fluorination reactions.

Scheme 5 (a) Application of polymer-supported IL **8** in a fluorination reaction. (b) Bicipital effect of neighboring ion pairs using a polymer-supported phosphonium salt.

4. *t*-Alcohol solvents for S_N2 fluorinations with alkali metal fluorides

In most organic chemistry textbooks, polar aprotic solvents are described as optimal for S_N2 -type reactions whereas protic solvents such as water and alcohols are unsuitable. However, as described previously, a fluorinase enzyme catalyses a nucleophilic fluorination with MF in aqueous medium. In the enzymatic reaction, the H-bonding between the enzyme, fluoride, and substrate plays a crucial role.^{1,19} Moreover, in 1994, Yonezawa and co-workers observed that the *t*-BuOH–TBAF complexes showed the best reactivity in the S_N2 fluorination reaction of benzyl bromide with a series of TBAF–alcohol complexes prepared from TBAF(H_2O)₃ and the corresponding alcohol solvents – such as *t*-BuOH, *i*-PrOH, *n*-BuOH, and *n*-PrOH.²⁷ This indicates that fine engineering of the H-bonding network could enable the use of protic solvents in nucleophilic fluorination reactions. Indeed, a remarkable and unprecedented effect was observed by using bulky aliphatic alcohols (e.g., *t*-BuOH and *t*-amyl alcohol) as the solvents in the fluorinations with CsF.²⁸ Scheme 6 highlights the superior efficiency of sterically bulky *t*-alcohols compared with the conventional polar aprotic solvent, acetonitrile, in fluorination. The relatively nonpolar and bulky *t*-alcohols such as *t*-BuOH and *t*-amyl alcohol dramatically increased the reactivity of CsF, allowing much faster reactions compared with acetonitrile (Scheme 6a). Although a relatively nonpolar protic solvent, *n*-BuOH, can also promote the same reactions, a significant amount (30%) of ether side-product was produced due to its smaller size and weaker steric effects. Bulkier *tert*-alcohols, on the other hand, suppressed the ether-formation to much larger degrees.

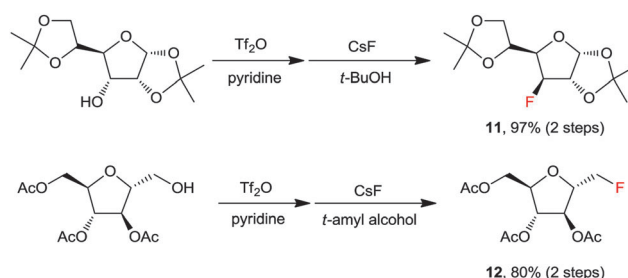


Scheme 6 (a) Reactivity of CsF and (b) chemoselectivity of fluorination in *t*-alcohols and acetonitrile.

More interestingly, in the fluorination of base-sensitive substrates such as **13** even with a “naked” fluoride source, the use of steric bulky *t*-alcohol solvents significantly suppressed the formation of elimination byproducts.²⁸ The protic environment of *t*-alcohols reduced the basicity of fluoride ions, thus effectively inhibiting the side reactions such as elimination or intramolecular cycloalkylation (Scheme 6b).²⁸ Recently, these *t*-alcohol media have been used for the preparation of many fluorinated bio-active molecules such as **11** and **12** (Scheme 7).^{29,30}

As shown in Fig. 3, *t*-alcohols are believed to affect the mechanism of this fluorination through the following H-bonding interactions: (1) the *t*-alcohol may form hydrogen bonds with the fluoride ion in the CsF lattice resulting in the selective solvation of the fluoride ion by *t*-alcohol molecules, thus reducing the strength of the CsF ionic bond. (2) The fluoride ion coordinated with bulky *t*-alcohols becomes a more “controllable” nucleophile, reducing the basicity of the fluoride ion and consequently suppressing side reactions such as eliminations. (3) The H-bonding of the *t*-alcohol solvents with the oxygen atoms of the sulfonate leaving group may improve its leaving group ability. Therefore, the use of bulky protic alcohols affords the possibility of generating a soluble, dissociated fluoride ion from CsF, while decreasing its Brønsted basicity.^{28,31}

More direct evidence that a “controllable” fluoride nucleophile can be generated during the *t*-alcohol media promoted fluorination process using an alkali metal fluoride was obtained by single crystal X-ray analysis of the TBAF(*t*-BuOH)₄ complex which was simply formed from the mixture of commercially available TBAF and *t*-BuOH.³² In the X-ray structure of Fig. 4c, four *t*-BuOH molecules coordinate around a single fluoride by hydrogen bonds, consequently reducing the basicity of fluoride.



Scheme 7 Selected examples for the preparation of fluorinated bio-active molecules by fluorination with CsF in a *t*-alcohol solvent.

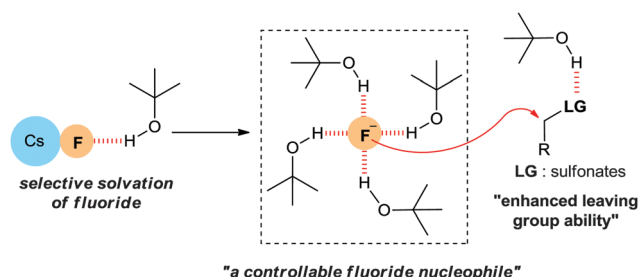


Fig. 3 Positive effects of a *t*-alcohol medium on nucleophilic aliphatic fluorination reactions.

The reduced basicity of TBAF(*t*-BuOH)₄, due to the H-bonding interaction of fluoride, significantly lowered its reactivity compared with anhydrous TBAF, which generates a perfectly “naked” fluoride source *in situ*. Therefore, TBAF(*t*-BuOH)₄ afforded higher yields of fluorinated product **14** while anhydrous TBAF led to the significant formation of elimination products (Fig. 4a). Additionally it possesses the inherent advantage of displaying lower hygroscopicity (Fig. 4b), as a consequence of its surrounding environment saturated with alcohol molecules, making it perfectly poised for strictly anhydrous reaction conditions.

Quite recently, Gouverneur and coworkers showed thorough examination of complexes of tetraalkyl ammonium fluoride with alcohol, 1,2-diols, 1,3-diols, triols and tetraols.³³ According to the extensive X-ray crystallographic analysis, the coordination number of hydrogen bonding donors to fluoride is critical, affecting nucleophilicity and basicity of the fluoride (Scheme 8). It is believed that the reactivity of fluoride is governed by the dissociation from the alcohol ligands, indicating the importance of equilibrium of complexation and dissociation. Depending on the steric bulk and the branching degree of alcohols, the coordination environment varies and generates diverse character of fluoride, which can be applied in various catalysis and selective substitution reactions.

The characteristics of nucleophilic substitution reactions with a “controllable” fluoride nucleophile are in stark contrast to the two aspects generally associated with conventional S_N2 reactions. First, as described previously, hindered protic solvents exhibited superior reactivity compared to aprotic dipolar solvents (CsF in *t*-BuOH *vs.* CsF in CH₃CN, Fig. 5a). More interestingly, the relative reactivity of halide nucleophiles reversed in a bulky alcohol solvent such as *t*-BuOH (F[−] ≫ I[−] and Br[−]) from that typical for halide ions in conventional protic solvents such as MeOH (F[−] < Cl[−] < Br[−] < I[−]); the latter sequence was predicted by simply considering the differential strength of H-bonding between the halide and protic solvent (Fig. 5a).^{28,31} Moreover, Fig. 5b shows that the fluorination rate of a mesylate is significantly faster than those of the corresponding halides such as iodide and bromide.

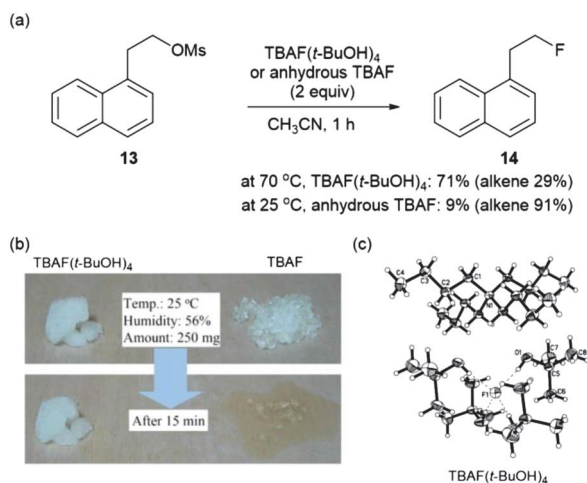


Fig. 4 (a) Comparison of the reactivity and selectivity of TBAF(*t*-BuOH)₄ and anhydrous TBAF in a nucleophilic fluorination reaction. (b) Reduced hygroscopicity of TBAF(*t*-BuOH)₄. (c) X-ray crystal structure of TBAF(*t*-BuOH)₄.



Scheme 8 Examples for the reactions of alcohol–fluoride complexes with **15**.

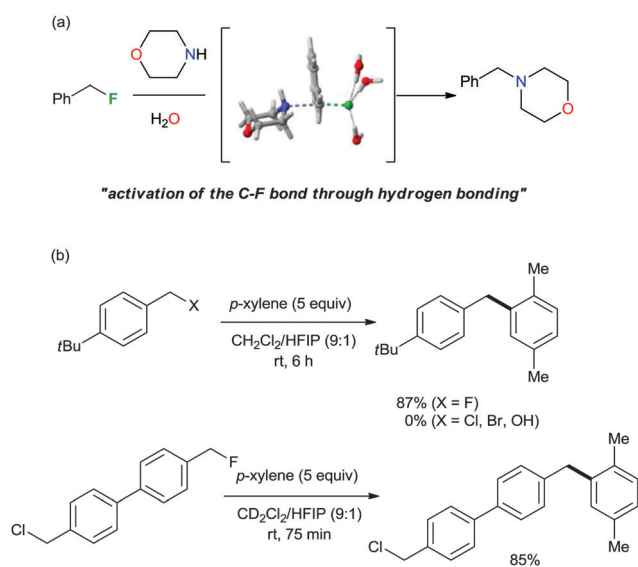
This result indicates that the reaction rate was determined not only by the nature of the leaving group, but also by other types of interactions such as those between the solvent (*t*-alcohol) and the leaving group. As proposed in Fig. 3, the H-bonding between the *t*-alcohol solvent and the oxygen atoms in the alkane-sulfonate leaving group may improve its nucleofugic (leaving group) character. Although it is believed that organofluorines are hardly hydrogen bonding acceptors, it was recently discovered that even the strong benzylic C–F bonds can be activated by H-bonding donor molecules such as water (Scheme 9a).³⁴ Similar to the mode of action of β-glycosidase (intermediate C in Scheme 2),¹⁸ hydrogen bonding interactions of water enabled the bimolecular nucleophilic substitution reactions of benzyl fluorides with diverse N-, O-, S-, and C-nucleophiles. The drastic change of leaving group ability of the reactant, benzyl fluoride, compared with benzyl bromide and chloride, was supported by performing an activation strain analysis in the gas phase. The most effective stabilization of the transition state was observed with benzyl fluoride in the presence of water. Soon after, the same group successfully applied this H-bonding strategy for the C–F bond activation of benzylic fluorides in the Friedel–Crafts benzylation reaction of arenes.³⁵ From the comparison and competition experiments, only benzylic C–F bonds showed significant reactivity in the presence of other substrates (X = Cl, Br, OH, Scheme 9b), highlighting the importance of the hydrogen bonding interaction of H-bond acceptor C_{sp3}–F with protic solvents.

Synergistic effects of two promoters – ionic liquid and *t*-alcohol in one molecule

Recognizing that ILs and *t*-alcohols are beneficial for the activation of MFs *via* electrostatic and H-bonding interactions, synergistic effects of ILs and *t*-alcohols in one molecule could



Fig. 5 (a) Reactivity of CsF, CsBr, and CsI in *t*-BuOH or CH₃CN. (b) Unusual leaving group ability in the *t*-BuOH reaction medium.



Scheme 9 (a) Activation of the C-F bond through H-bonding. (b) Selective activation of a C-F bond in the presence of a similar C-X bond. HFIP: hexafluoroisopropanol.

be expected to enhance selectivity and reactivity in fluorination reactions with MFs (Scheme 10a). Scheme 10b shows a comparison of different additives, including the combination of *t*-BuOH and nontethered [bmim][OMs]. In agreement with initial expectation,

t-alcohol-tethered IL 22 proved to be the most successful promoter, inducing the conversion of substrate 3 to fluorinated product 4 in excellent yields.³⁶ These intriguing cooperative effects of two promoters, *i.e.* ILs and *t*-BuOH, encouraged us to investigate a more efficient and easily accessible multifunctional promoter, which will be discussed in the next chapter.

5. Oligoethylene glycols for nucleophilic fluorinations with metal fluorides

Traditional crown ethers have been extensively utilized in organic synthesis by generating naked anions from inorganic salts. However, the application of crown ethers in practical fluorination reactions using a metal salt is rare because of their low promoting effects. Moreover, the generation of a "naked" fluoride can induce various by-product formation, due to the high basicity.

Oligoethylene glycols such as tetraethylene glycol (tetraEG) would be desirable candidates to overcome these drawbacks. By coordinating the potassium cation with polyethers, the solubility of potassium fluoride can be efficiently enhanced. On the other hand, one of the OH groups engages in controlled hydrogen bonding with the fluoride anion, thus decreasing the basicity, whereas the other OH group would interact with the leaving group, activating the electrophile towards nucleophilic attack (Fig. 6).³⁷

To verify this hypothesis, we evaluated the potential of oligoethylene glycols as the promoters for nucleophilic fluorination with KF, which is the most inexpensive and convenient source of nucleophilic fluoride.³⁷ Gratifyingly, in the presence of tetraEG or triethylene glycol (triEG), the reaction proceeded with unprecedented efficiency and completed within 1.5 h, giving the desired fluorinated product, 4, in almost quantitative yields (Scheme 11a). Notably, the thermal stability of oligoethylene glycols allowed the fluorination reaction to proceed under microwave conditions, affording the desired products in excellent yields in only 1 min (Scheme 11a). Considering the short half-life of the ¹⁸F radioisotope (*t*_{1/2}, 109.8 min), this rapid fluorination



Scheme 10 (a) Hybridization of ILs and *t*-alcohol in one molecule and (b) its application to fluorination with CsF.



To further verify the importance of the cooperative multifunctionality of the promoter, fluorination with KF was carried out in various glycol derivatives. When triethylene glycol dimethyl ether (triglyme) was used as the solvent, the reaction did not proceed. On the other hand, triethylene glycol monoethyl ether showed approximately half of the reactivity of triEG under the same reaction conditions (Scheme 11a).³⁷ Furthermore, the optimal reactivity with MFs can be easily achieved simply by varying the chain length of the oligoethylene glycols. As expected, in the case of CsF, the increase in chain length (*e.g.* penta- and hexaethylene glycol) resulted in an excellent yield of the product, while KF lost some reactivity in such solvents.³⁸ All these experimental results strongly indicate that a cooperative



More direct experimental evidence for the activation of KF with oligoethylene glycols was also obtained by the single crystal X-ray structure analysis of the complex of KF and a 1,1'-bi-2-naphthol(BINOL)-based chiral analog of oligoethylene glycol (Fig. 9).⁴⁰ As shown in the crystal structure (Fig. 9), the primary

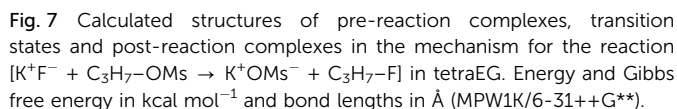




Fig. 8 Stabilization of the transition state of S_N2 reaction by using protic catalysts.

interaction (O–H–F) of terminal phenolic protons with fluoride is clear (O–F distance: 2.9–3.0 Å). Due to the dimer formation during the single crystal preparation, another hydrogen bonding interaction is observed with the other extremity phenolic protons of the other unit of the glycol-derivative (O–F distance: 3.2–3.3 Å), exhibiting weaker interactions. It is believed that the unique chelating environment using BINOL-based chiral oligoethylene glycol with KF can generate fluoride anions with a low coordination number, inducing higher reactivity for nucleophilic substitution reactions and other catalytic applications.^{40–42}

The simultaneous activation of both the nucleophile and the electrophile by oligoethylene glycols seems to be a more general phenomenon, promoting S_N2 reactions with different sources of nucleophiles (Scheme 12).^{37,40} Thus, the nucleophilic substitution reactions proceeded smoothly with a diverse set of carbon, nitrogen, oxygen, sulfur and halogen nucleophiles to give the corresponding products in almost quantitative yields. This reaction profile highlights the advantage of using oligoethylene glycols as the promoters for general nucleophilic substitution reactions. Furthermore, the concept for the ambiphilic activation using oligoEGs has been successfully extended to its asymmetric version.^{40–42} The employment of 1,1'-bi-2-naphthol-based chiral analogs of oligoethylene glycols showed unprecedented catalytic performance in desilylative kinetic resolution,⁴⁰ asymmetric Strecker synthesis⁴¹ and asymmetric silylation reactions.⁴² Owing to its robustness and simplicity, the fluorination protocol with oligoethylene glycol promoters can be applied in various fields. In fact, it



Scheme 12 Nucleophilic substitutions in oligoEGs using various salts.

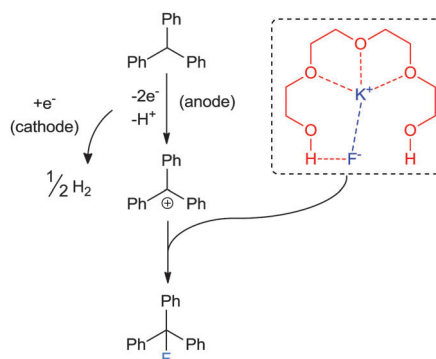
was recently applied to a selective electrochemical fluorination reaction with KF and tertiary carbon cation reactants by the Fuchigami research group (Scheme 13).⁴³

Bis-*tert*-alcohol-functionalized crown-6-calix[4]arene (BACCA)

Most recently, a bis-*tert*-alcohol-functionalized crown-6-calix[4]arene (BACCA) as another type of bis-terminal hydroxyl polyether was reported by Kim and his coworkers.⁴⁴ As shown in Scheme 14a, the binding of Cs^+ to the crown-6-calix[4]arene subunit of BACCA separates CsF to a large distance (> 8 Å) with the release of an essentially free fluoride, as established in a quantum chemical study. Moreover, H-bonding between the fluoride and the bis-terminal *t*-alcohol subunit of BACCA can reduce the basicity of fluoride, thus enhancing the effective nucleophilicity. As shown in Scheme 14b, BACCA showed a high performance in the nucleophilic fluorination reaction using CsF . On the other hand, the methylated BACCA, in which the two terminal *tert*-alcohol groups were methylated to prevent H-bonding with the fluoride, showed inferior activity in the same reaction. This result concludes that two H-bonds of OH groups towards fluoride and the leaving group seem to be important for promoting nucleophilic substitution reaction. Moreover, the use of BACCA allowed fluorination of base-sensitive substrates to proceed chemoselectively even in polar aprotic solvent such as CH_3CN (Scheme 14c).



Fig. 9 Experimental evidence for the activation of KF with a protic solvent such as tetraethylene glycol. (a) ORTEP structure as a dimeric form of the complex of KF and a binol-based chiral oligoethylene glycol. (b) BINOL backbone and bromine on 3,3'-positions are omitted for clarity; H-bond interactions between OH–F are highlighted in red and blue with distance (Å).



Scheme 13 Schematic representation of electrochemical fluorination using tetraEG.

6. ^{18}F -radiofluorination using $[\text{}^{18}\text{F}]\text{fluoride}$ for PET applications

Fluorine-18 is regarded as one of the best positron emitters because of its many desirable characteristics such as a small steric size, stable bonding to carbon, a suitable decay rate, low positron energy, and ease of production. As a result of our research program with protic solvents for nucleophilic fluorination reactions, clinically relevant $[\text{}^{18}\text{F}]\text{radiopharmaceuticals}$ now can be accessed for wide use in clinical fields and PET molecular imaging studies.

Synthesis of PET radiopharmaceuticals in ionic liquid media

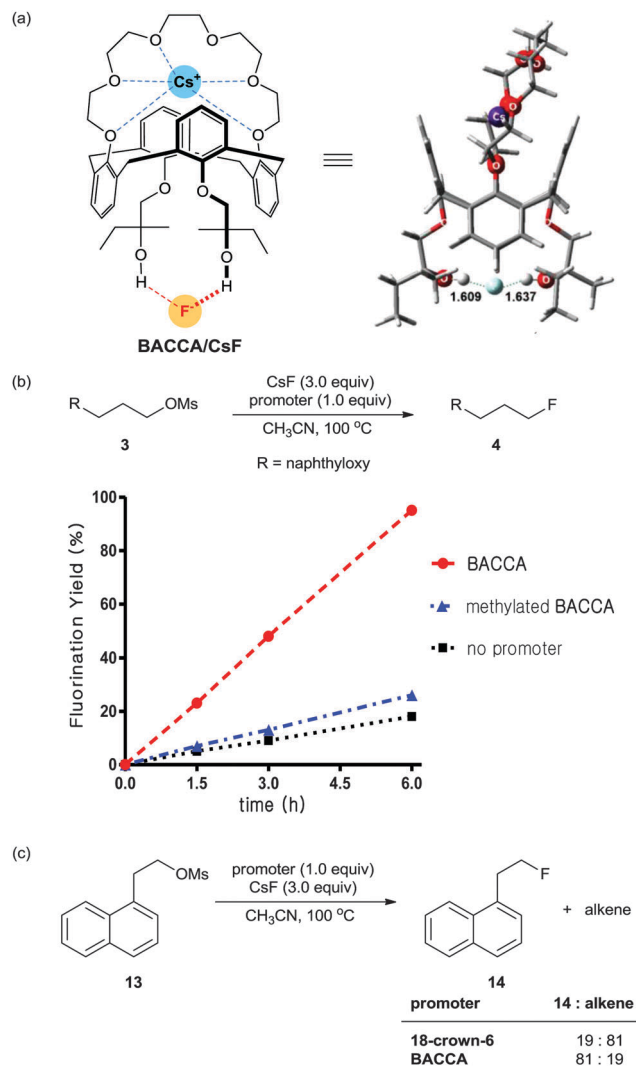
The practicability of the reaction implied by the fact that IL-based fluorinations do not require anhydrous conditions²¹ compelled us to apply them to the ^{18}F -labeling of various molecules, a process in which it is difficult to insure the complete absence

of water. In these experiments, we used no-carrier-added $[\text{}^{18}\text{F}]\text{fluoride}$ in water as obtained from the production target ($^{18}\text{O}(\text{p},\text{n})^{18}\text{F}$). The ^{18}F -radiofluorination of an alkyl mesylate precursor was attempted using $[\text{}^{18}\text{F}]\text{fluoride}$ in water with Cs_2CO_3 in IL, $[\text{bmim}][\text{OTf}]$. Gratifyingly, even in the presence of water, the reaction gave the corresponding ^{18}F -fluorinated product ($[\text{}^{18}\text{F}]\text{-4}$) in $93 \pm 1.2\%$ RCY (Scheme 15a).⁴⁵ This method not only provides the product rapidly in a good yield and with high effective specific activity, but is also particularly simple to operate. Practically, $[\text{}^{18}\text{F}]\text{fluoride}$ is obtained from the production target and it can be directly added to the reaction media without the dehydrating step (Scheme 15b).⁴⁵

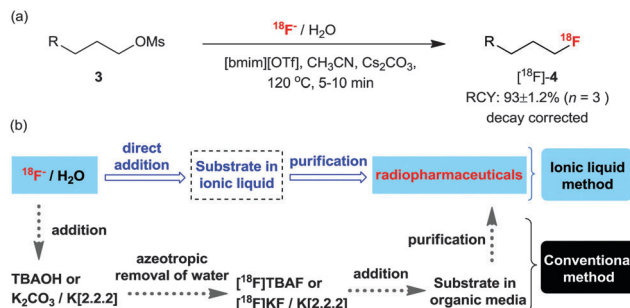
Taking advantage of the merits (high selectivity, high reaction rate, and convenient introduction of fluorine-18) of the ^{18}F -radiofluorination method in aqueous ILs, we successfully applied this protocol to an improved preparation of 3'-deoxy-3'- $[\text{}^{18}\text{F}]\text{fluoro}$ -thymidine ($[\text{}^{18}\text{F}]\text{FLT}$) (**23**)⁴⁶ and 2- $[\text{}^{18}\text{F}]\text{fluoro}$ -2-deoxyglucose ($[\text{}^{18}\text{F}]\text{FDG}$) (**24**)⁴⁷ which are the most popular radiopharmaceuticals for PET imaging (Scheme 16).

Synthesis of PET radiopharmaceuticals in *t*-alcohol media

The practical fluorination protocol in *t*-alcohol media renders it perfectly suited for application in the ^{18}F radiolabeling of important PET radiopharmaceuticals for automated mass production. Generally, when using no-carrier-added $[\text{}^{18}\text{F}]\text{fluoride}$, ^{18}F radiolabeling for the production of PET radiopharmaceuticals requires basic reaction conditions. Therefore, the protic atmosphere emerging from *t*-alcohol media could help to reduce the basicity of the labeling reaction condition and allow the radiofluorination to proceed chemo-selectively, consequently increasing the RCY. In addition, due to the lower boiling points of *t*-alcohols (e.g. bp of *t*-BuOH, 81 °C) than those of typical polar aprotic solvents such as DMF and DMSO, *t*-alcohols can easily be removed using nitrogen gas stream after the ^{18}F fluorination reaction. We aimed at radiopharmaceuticals that are challenging to label such as $[\text{}^{18}\text{F}]\text{FLT}$ (**23**), $[\text{}^{18}\text{F}]\text{FDG}$ (**24**), 1- $[\text{}^{18}\text{F}]\text{fluoro}$ -3-(2-nitroimidazol-1-yl)propan-2-ol ($[\text{}^{18}\text{F}]\text{FMISO}$) (**25**) and *N*- $[\text{}^{18}\text{F}]\text{fluoropropyl}$ -2 β -carbomethoxy-3 β -(4-iodophenyl)nortropane ($[\text{}^{18}\text{F}]\text{FP-CIT}$) (**26**).²⁸ As shown from the results in Scheme 16, all the examples showcase the utility of this *t*-alcohol medium radiofluorination compared to a conventional polar aprotic solvent, e.g. acetonitrile. Significantly, higher RCYs



Scheme 14 (a) Bis-*tert*-alcohol-functionalized crown-6-calix[4]arene (BACCA) and (b) $\text{S}_{\text{N}}2$ fluorination reaction using CsF in the presence of BACCA, methylated BACCA, or without any promoter. (c) Chemoselective fluorination of base-sensitive substrate **13** in the presence of BACCA.



Scheme 15 (a) ^{18}F -radiofluorination in IL $[\text{bmim}][\text{OTf}]$. (b) A schematic comparison of the conventional ^{18}F labeling process and the IL-based process.



Scheme 16 Synthesis of key ¹⁸F-labeled PET radiopharmaceuticals using *t*-alcohol solvent and their RCYs.

of ¹⁸F-fluorinated compounds **23–26** were obtained by automatic synthesis using the *t*-alcohol solvent system compared to other reported methods.^{28,47} In particular, [¹⁸F]FP-CIT (**26**) is an important radiopharmaceutical for the PET imaging of dopamine transporters. However, the extremely low RCY yield (1%) of **26** in CH₃CN hampered its application. A dramatic improvement was observed by using *t*-BuOH, affording 36% RCY. Furthermore, [¹⁸F]FP-CIT (**26**) can now be obtained in 42.5 ± 10.9% RCY from recent routine 192 productions for 1.5 years using an auto-synthesizer module in *t*-amyl alcohol.⁴⁸ As shown in Scheme 16, the protic atmosphere of *t*-alcohol as well as H-bonding between *t*-alcohol and the N atom of the FP-CIT precursor can suppress side reactions such as ring opening by two sequential steps of bimolecular elimination and



Scheme 17 Synthesis of *N*₄'-[¹⁸F]fluoropropyl-tanaproget and *N*₄'-[¹⁸F]fluoropropyl ciprofloxacin using *t*-alcohol solvent.

the intramolecular *N*-alkylation reaction, thereby increasing the RCY. Bayer Healthcare[®] has also tried to extend this methodology to automatic mass production of other PET radiopharmaceuticals.

It would also be instructive to note that the *t*-alcohol fluorination protocol was recently applied by us and other research groups to synthesize interesting PET radiotracers, such as [¹⁸F]fluorine-substituted tanaproget (**28**) as a progesterone receptor imaging agent⁴⁹ and *N*₄'-[¹⁸F]fluoropropyl ciprofloxacin (**29**) as a bacterial infection imaging agent (Scheme 17).⁵⁰ It is noteworthy here that mild radiofluorination reaction conditions allowed us to access biologically relevant molecules with diverse functional groups including nitriles, pyrroles, carbamates, esters, enone and cyclopropyl groups. Further applications of the ¹⁸F-radiofluorination reaction protocol using the protic engineering solvents will give facile access to different types of radiotracers of interest.

7. Conclusions

This Tutorial Review summarizes the recent breakthroughs in bimolecular nucleophilic fluorination reactions at an sp³-hybridized carbon, through the activation of alkali MFs in protic reaction media. Although conventional wisdom deems protic solvents as unsuitable for nucleophilic displacement reactions, we found that *t*-alcohols, oligoEGs, and aqueous ILs led to remarkable and unprecedented efficiency in nucleophilic fluorinations. Thus, by evolving from ILs to *t*-alcohols, and recently to oligoethylene glycols as the promoters, MFs have become superior fluoride reagents and thus now applicable to diverse base-sensitive substrates, affording excellent yields of the fluorinated products with high reaction rates. The simultaneous dual activation of reacting partners by intermolecular hydrogen bonding and the enhancement of the “effective fluoride nucleophilicity” by hydrogen bond interactions, mimicking Nature’s biocatalytic approach in the fluorinase enzyme, are the key to this unprecedentedly straightforward nucleophilic fluorination. The advantages of these protocols become particularly practical in the context of ¹⁸F labeling for PET imaging, where the short half-life of the radionuclide leads to an enormous dependency on reaction speed and efficiency.

Acknowledgements

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Notes and references

- 1 J. Xie and W. L. Hase, *Science*, 2016, **352**, 32–33.
- 2 D. O'Hagan and H. Deng, *Chem. Rev.*, 2015, **115**, 634–649.
- 3 K. Müller, C. Faeh and F. Diederich, *Science*, 2007, **317**, 1881–1886.

- 4 A. Harsanyi and G. Sandford, *Green Chem.*, 2015, **17**, 2081–2086.
- 5 L. Zhu, K. Ploessl and H. F. Kung, *Science*, 2013, **342**, 429–430.
- 6 S. Preshlock, M. Tredwell and V. Gouverneur, *Chem. Rev.*, 2016, **116**, 719–766.
- 7 F. H. Vaillancourt, E. Yeh, D. A. Vosburg, S. Garneau-Tsodikova and C. T. Walsh, *Chem. Rev.*, 2006, **106**, 3364–3378.
- 8 P. A. Champagne, J. Desroches, J.-D. Hamel, M. Vandamme and J.-F. Paquin, *Chem. Rev.*, 2015, **115**, 9073.
- 9 T. Liang, C. N. Neumann and T. Ritter, *Angew. Chem., Int. Ed.*, 2013, **52**, 8214–8264.
- 10 X. Yang, T. Wu, R. J. Phipps and F. D. Toste, *Chem. Rev.*, 2015, **115**, 826–870.
- 11 C. Hollingworth and V. Gouverneur, *Chem. Commun.*, 2012, **48**, 2929–2942.
- 12 E. Lee, A. S. Kamlet, D. C. Powers, C. N. Neumann, G. B. Boursalian, T. Furuya, D. C. Choi, J. M. Hooker and T. Ritter, *Science*, 2011, **334**, 639–642.
- 13 C. N. Neumann and T. Ritter, *Angew. Chem., Int. Ed.*, 2015, **54**, 3216–3221.
- 14 R. Schwesinger, R. Link, P. Wenzl and S. Kossek, *Chem. – Eur. J.*, 2006, **12**, 438–445.
- 15 J. Wu, *Tetrahedron Lett.*, 2014, **55**, 4289–4294.
- 16 S. H. Liang and N. Vasdev, *Angew. Chem., Int. Ed.*, 2014, **53**, 11416–11418.
- 17 O. Nashiru, D. L. Zechel, D. Stoll, T. Mohammadzadeh, R. A. J. Warren and S. G. Withers, *Angew. Chem., Int. Ed.*, 2001, **40**, 417–420.
- 18 D. L. Zechel, S. P. Reid, O. Nashiru, C. Mayer, D. Stoll, D. L. Jakeman, P. A. J. Warren and S. G. Withers, *J. Am. Chem. Soc.*, 2001, **123**, 4350–4351.
- 19 D. O'Hagan, C. Schaffrath, S. L. Cobb, J. T. G. Hamilton and C. D. Murphy, *Nature*, 2002, **416**, 279.
- 20 J.-W. Lee, J. Y. Shin, Y. S. Chun, H. B. Jang, C. E. Song and S. G. Lee, *Acc. Chem. Res.*, 2010, **43**, 985–994.
- 21 D. W. Kim, C. E. Song and D. Y. Chi, *J. Am. Chem. Soc.*, 2002, **124**, 10278–10279.
- 22 Y.-H. Oh, H. B. Jang, S. Im, M. J. Song, S.-Y. Kim, S.-W. Park, D. Y. Chi, C. E. Song and S. Lee, *Org. Biomol. Chem.*, 2011, **9**, 418–422.
- 23 D. W. Kim, C. E. Song and D. Y. Chi, *J. Org. Chem.*, 2003, **68**, 4281–4285.
- 24 D. W. Kim and D. Y. Chi, *Angew. Chem., Int. Ed.*, 2004, **43**, 483–485.
- 25 J. H. Clark, S. J. Tavener and S. J. Barlow, *Chem. Commun.*, 1996, 2429–2430.
- 26 C. B. Murray, G. Sandford and S. R. Korn, *J. Fluorine Chem.*, 2003, **123**, 81–84.
- 27 T. Yonezawa, Y. Sakamoto and K. Nogawa, *Jpn. Kokai Tokkyo Koho*, 1994, JP 06316551 A.
- 28 D. W. Kim, D.-S. Ahn, Y.-H. Oh, S. Lee, H. S. Kil, S. J. Oh, S. J. Lee, J. S. Kim, J. S. Ryu, D. H. Moon and D. Y. Chi, *J. Am. Chem. Soc.*, 2006, **128**, 16394–16397.
- 29 M. Egli, P. S. Pallan, C. R. Allerson, T. P. Prakash, A. Berdeja, J. Yu, S. Lee, A. Watt, H. Gaus, B. Bhat, E. E. Swayze and P. P. Seth, *J. Am. Chem. Soc.*, 2011, **133**, 16642–16649.
- 30 O.-M. Soueidan, B. J. Trayner, T. N. Grant, J. R. Henderson, F. Wuest, F. G. West and C. I. Cheeseman, *Org. Biomol. Chem.*, 2015, **13**, 6511–6521.
- 31 D. W. Kim, H.-J. Jeong, S. T. Lim, M.-H. Sohn, J. A. Katzenellenbogen and D. Y. Chi, *J. Org. Chem.*, 2008, **73**, 957–962.
- 32 D. W. Kim, H.-J. Jeong, S. T. Lim and M.-H. Sohn, *Angew. Chem., Int. Ed.*, 2008, **47**, 8404–8406.
- 33 K. M. Engle, L. Pfeifer, G. W. Pidgeon, G. T. Giuffredi, A. L. Thompson, R. S. Paton, J. M. Brown and V. Gouverneur, *Chem. Sci.*, 2015, **6**, 5293–5302.
- 34 P. A. Champagne, J. Pomarole, M. E. Therien, Y. Benhassine, S. Beaulieu, C. Y. Legault and J. F. Paquin, *Org. Lett.*, 2013, **15**, 2210–2213.
- 35 P. A. Champagne, Y. Benhassine, S. Beaulieu, J. Desroches and J. F. Paquin, *Angew. Chem., Int. Ed.*, 2014, **53**, 13835–13839.
- 36 S. S. Shinde, Y. S. Lee and D. Y. Chi, *Org. Lett.*, 2008, **10**, 733–735.
- 37 J.-W. Lee, H. Yan, H. B. Jang, H. K. Kim, S. W. Park, S. Lee, D. Y. Chi and C. E. Song, *Angew. Chem., Int. Ed.*, 2009, **48**, 7683–7686.
- 38 V. H. Jadhav, S. H. Jang, H.-J. Jeong, S. T. Lim, M.-H. Sohn, J. Y. Kim, S. Lee, J. W. Lee, C. E. Song and D. W. Kim, *Chem. – Eur. J.*, 2012, **18**, 3918–3924.
- 39 J. R. Pliego Jr., *Phys. Chem. Chem. Phys.*, 2011, **13**, 779–782.
- 40 H. Yan, H. B. Jang, J. W. Lee, H. K. Kim, S. W. Lee, J. W. Yang and C. E. Song, *Angew. Chem., Int. Ed.*, 2010, **49**, 8915–8917.
- 41 H. Yan, J. S. Oh, J. W. Lee and C. E. Song, *Nat. Commun.*, 2012, **3**, 1212.
- 42 S. Y. Park, J.-W. Lee and C. E. Song, *Nat. Commun.*, 2015, **6**, 7512.
- 43 T. Sawamura, K. Takahashi, S. Inagi and T. Fuchigami, *Angew. Chem., Int. Ed.*, 2012, **51**, 4413–4416.
- 44 V. H. Jadhav, W. Choi, S.-S. Lee, S. Lee and D. W. Kim, *Chem. – Eur. J.*, 2016, **22**, 4515–4520.
- 45 D. W. Kim, Y. S. Choe and D. Y. Chi, *Nucl. Med. Biol.*, 2003, **30**, 345–350.
- 46 B. S. Moon, K. C. Lee, G. I. An, D. Y. Chi, S. D. Yang, C. W. Choi, S. M. Lim and K. S. Chun, *J. Labelled Compd. Radiopharm.*, 2006, **49**, 287–293.
- 47 H. W. Kim, J. M. Jeong, Y.-S. Lee, D. Y. Chi, K.-H. Chung, D. S. Lee, J.-K. Chung and M. C. Lee, *Appl. Radiat. Isot.*, 2004, **61**, 1241–1246.
- 48 S. J. Lee, S. J. Oh, W. Y. Moon, M. S. Choi, J. S. Kim, D. Y. Chi, D. H. Moon and J. S. Ryu, *Nucl. Med. Biol.*, 2011, **38**, 593–597.
- 49 J. H. Lee, H.-B. Zhou, C. S. Dence, K. E. Carlson, M. J. Welch and J. A. Katzenellenbogen, *Bioconjugate Chem.*, 2010, **21**, 1096–1104.
- 50 K. Sachin, E.-M. Kim, S.-J. Cheong, H.-J. Jeong, S. T. Lim, M.-H. Sohn and D. W. Kim, *Bioconjugate Chem.*, 2010, **21**, 2282–2288.