Dynamic Article Links

Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2011, **9**, 8346

www.rsc.org/obc

**PAPER**

# Facile aromatic radiofluorination of [$^{18}$F]flumazenil from diaryliodonium salts with evaluation of their stability and selectivity†

**Byung Seok Moon,[a,b] Hee Seup Kil,[c] Jun Hyung Park,[a] Ji Sun Kim,[a] Jimin Park,[d] Dae Yoon Chi,[c] Byung Chul Lee*[a,b] and Sang Eun Kim[a,b]**

***Received 28th July 2011, Accepted 22nd September 2011***
**DOI: 10.1039/c1ob06277h**



Aromatic radiofluorination of the diaryliodonium tosylate precursor with [$^{18}$F]fluoride ions has been applied successfully to access [$^{18}$F]flumazenil in high radiochemical yields of 67.2 ± 2.7% (decay corrected). The stability and reactivity of the diaryliodonium tosylate precursor plays a key role in increasing the production of $^{18}$F-labelled molecules under the fluorine-18 labelling condition. Various conditions were explored for the preparation of [$^{18}$F]flumazenil from different diaryliodonium tosylate precursors. Optimum incorporation of [$^{18}$F]fluoride ions in the 4-methylphenyl-mazenil iodonium tosylate precursor (**5f**) was achieved at 150 °C for 5 min by utilizing 4 mg of the precursor, $K_{2.2.2}/K_2CO_3$ complex, and the radical scavenger in *N*,*N*-dimethylformamide. This approach was extended to a viable method for use in automated synthesis with a radiochemical yield of 63.5 ± 3.2% (decay corrected, $n$ = 26) within 60.0 ± 1.1 min. [$^{18}$F]Flumazenil was isolated by preparative HPLC after the reaction was conducted under improved conditions and exhibited sufficient specific activity of 370–450 GBq μmol$^{-1}$, with a radiochemical purity of >99%, which will be suitable for human PET studies.

## Introduction

A fluorine-18 ion is one of the most attractive positron emitters produced by a cyclotron; it can yield up to multi-Curie levels, even with low-energy[1]. It also has a relatively moderate half-life ($t_{1/2}$ = 110 min) and is rapidly incorporated into aliphatic or aromatic sites of organic molecules through a nucleophilic substitution reaction, normally with a high radiochemical yield and specific activity.[2] However, the incorporation efficiency of fluorine-18 is significantly dependent upon the type and position of the substitution group. For instance, nucleophilic substitution at the aryl position commonly does not work well without electron-withdrawing groups such as an aldehyde, nitro, cyanide, ester, or ketone in the *ortho*- or *para*-position of the aryl ring, accompanied by leaving groups such as nitro, halogen, or trialkylammonium salt. Conversely, aromatic radiofluorination in electron-rich arenes has to follow the electrophilic fluorine-18 labelling method, *e.g.*, using the [$^{18}$F]$F_2$ gas. However, electrophilic radiofluorination of aromatic compounds has significant shortcomings, such as low radiochemical yields with various by-products and low specific activity of the $^{18}$F-labelled molecules (<37 GBq μmol$^{-1}$). This level of specific activity is not enough to account for a receptor with low density in the brain. Given the above reasons, fluorine-18 incorporation into electron-rich arenes remains a particular challenge due to the fact that classical aromatic nucleophilic substitution generally gives low or negligible yields.

Recently, diaryliodonium salts have been shown to be useful for labelling with a [$^{18}$F]fluoride ion at the aromatic position in small model compounds that have proven difficult to label using general aromatic fluorine-18 incorporation precursors.[3] This method is potentially more powerful for the introduction of fluorine-18 into aromatic organic compounds than former methods, such as the Balz–Schiemann[4] or Wallach reactions,[5] which are limited due to low radiochemical yields. Although diaryliodonium salts have potential advantages in radiofluorination, their application to give complicated chemical structures had little success. The main reason is that the diaryliodonium salt precursors that have functional substitution groups or more complex structures are difficult to synthesize and isolate from reaction mixtures. In addition, the diaryliodonium salt is chemically unstable under the basic conditions and high temperatures required for the fluorine-18 incorporation reaction. Nevertheless, diaryliodonium salts have excellent properties such as the possibility of efficient fluorine-18 incorporation in electron-rich arenes; we therefore investigated the application of diaryliodonium salt precursors for efficient fluorine-18 incorporation in [$^{18}$F]flumazenil ([$^{18}$F]FMZ, Fig. 1).

Radioisotope labelled flumazenil is known to be an important radiopharmaceutical product for the assessment of the central benzodiazepine receptor (cBZR) concentration in the brain.[6] This

[a]*Department of Nuclear Medicine, Seoul National University Bundang Hospital, Seoul National University College of Medicine, Seoul, 110-799, Korea. E-mail: leebc@snu.ac.kr; Fax: 82-31-787-4072; Tel: 82-31-787-2956*
[b]*Institute of Radiation Medicine, Medical Research Center, Seoul National University, Seoul, 110-744, Korea*
[c]*Department of Chemistry, Sogang University, Seoul, 121-742, Korea*
[d]*Department of Materials Science and Engineering, Seoul National University, Seoul, 151-744, Korea*

† Electronic supplementary information (ESI) available: See DOI: 10.1039/c1ob06277h

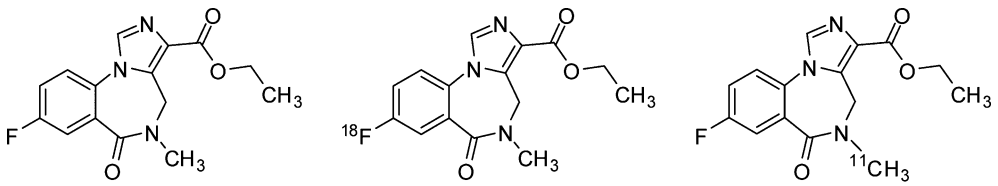



**Fig. 1** Structures of flumazenil and the radioisotope labelled flumazenil.



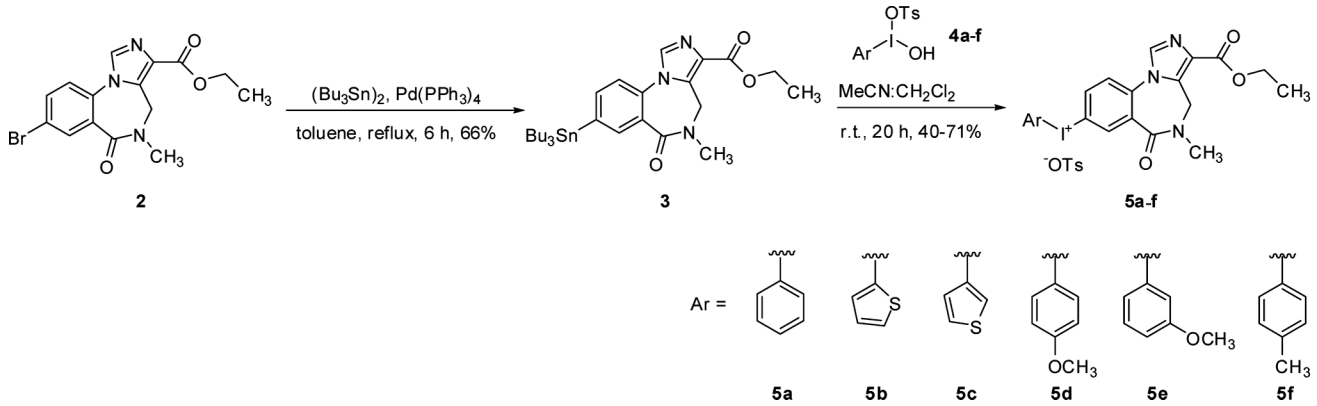
has been extensively studied for the quantitative evaluation of cBZR in epilepsy,[7] panic disorders,[8] the evaluation of cortical damage to the brain after an acute stroke,[9,10] and others[11–13] by positron emission tomography (PET). Carbon-11 labelled flumazenil, [$^{11}$C]flumazenil (ethyl-8-fluoro-5,6-dihydro-5-[$^{11}$C]methyl-6-oxo-4*H*-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate, [$^{11}$C]FMZ, [$^{11}$C]**1**, Fig. 1), shows itself to be more suitable than [$^{18}$F]FDG–PET (2-deoxy-2-[18F]fluoro-D-glucose) for providing valuable information in some fields. Its main limitation, however, is the physical half-life ($t_{1/2}$ = 20 min) of carbon-11, which means that only one or two patients can be treated from each production cycle in cyclotron-equipped centers. On the other hand, the fluorine atom in the native structure of flumazenil is an attractive feature which can introduce fluorine-18 by nucleophilic aromatic substitution without any structural modifications. The fluorine-18 labelled form, [$^{18}$F]flumazenil ([$^{18}$F]FMZ, [$^{18}$F]**1**), has the advantage of a longer half-life compared to the carbon-11-labelled form and a PET study on monkeys showed similar uptake patterns between [$^{18}$F]FMZ and [$^{11}$C]FMZ.[14] [$^{18}$F]FMZ also showed more stable estimates of binding potential values and a less noisy image than [$^{11}$C]FMZ in the human brain.[15] Many research groups have tried to synthesize [$^{18}$F]FMZ *via* a nucleophilic displacement from nitro-mazenil in order to overcome the short half-life, a limitation of [$^{11}$C]FMZ.[14,16,17] Direct nucleophilic fluorination showed a moderate radiochemical yield of about 15–30% (decay corrected, d.c.). However, other research groups reported that the overall decay corrected radiochemical yield after HPLC purification were very low, about 3–10% in contrast to the reported 15–30% yields.[15,17,18] Mandap *et al.* reported a more efficient method for [$^{18}$F]FMZ synthesis using a microwave-assisted system as an alternative method, but the effort produced a relatively moderate yield, about 26 ± 4% (d.c.) and microwave-assisted systems are not common at PET production sites.[16] Added to that, no attempts on high-scale production of flumazenil in commercial automatic devices have been addressed until now. In other trials, [$^{18}$F]flumazenil derivatives 2′-[$^{18}$F]fluoroflumazenil ([$^{18}$F]FFMZ)[19] and 5-(2′-[$^{18}$F]fluoroethyl)flumazenil ([$^{18}$F]FEFMZ)[20] were developed. However, these tracers did not achieve the same widespread use because their brain uptake and kinetics are different from those of [$^{11}$C]FMZ or [$^{18}$F]FMZ; this is because their structures are not exactly the same as that of native flumazenil.

We therefore adapted the aromatic [$^{18}$F]fluorination method, using a diaryliodonium salt that was recently studied in our group and by colleagues, to achieve efficient [$^{18}$F]FMZ synthesis.[21] These radiotracers showed good radiochemical yields by overcoming the limitations of general aromatic radiofluorination. In order to obtain high radiochemical yields and a reproducible synthesis of [$^{18}$F]FMZ, we have synthesized different diaryliodonium tosylate precursors, which introduces electron-rich heteroarenes, and attempted to optimize the reaction conditions based on their stability and the reactivity in the fluorine-18 incorporation environment. Here we describe the synthesis of [$^{18}$F]FMZ using the diaryliodonium tosylate precursors, which we believe to be highly efficient.

## Results and discussion

The limitations of the general aromatic [$^{18}$F]fluorination of flumazenil from nitro-mazenil allowed us to propose an alternative approach to aromatic fluorination. Diaryliodonium salts were already known as suitable precursors for the labelling of fluorine-18 into the aromatic ring by nucleophilic fluorination without additional activating groups (electron-withdrawing groups), such as the carbonyl or nitro groups. This approach also provides good yields, including electron-rich and electron-deficient arenes for which classical aromatic nucleophilic substitution is generally unfavorable and gives low or negligible yields. It is known that diaryliodonium salts can be used as the precursors for fluoroarenes,[22] and their values correspond well with those of the fluorine-18-labelled compounds produced using iodonium salts reported in our previous work.[21] In applying diaryliodonium salts to [$^{18}$F]FMZ synthesis, several heterodiaryl-introducing iodonium tosylate precursors were considered. In preparing the heterodiarene iodonium tosylate precursors, we synthesized the initial 8-bromoimidazobenzodiazepine (**2**) required for this study according to the procedures described in the literature with little modification,[23] as summarized in Scheme 1. Stannylation of ethyl-8-bromo-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate (**2**) with a tributyltin reagent and tetrakis(triphenylphosphine)palladium(0) [$(Ph_3P)_4Pd(0)$]afforded the corresponding stannylated compound **3**. This reaction was carried out according to a previously reported method with little modification.[24] Treatment of ethyl-5,6-dihydro-5-methyl-6-oxo-8-tributylstannyl-4*H*-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate (**3**) with a commercially available Koser's reagent (for **5a**) or various hydroxy(tosyloxy)-iodoarenes (for **5b–f**) afforded (8-benzodiazepine)iodonium tosylates **5a–f**, respectively. The electron-rich hydroxyl(tosyloxy)-iodoarenes are known to be unstable and to decompose violently at room temperature. Thus, we prepared **4a–f** according to the literature[22,25] and used them without purification under a nitrogen atmosphere to prepare the diaryliodonium tosylate precursors **5a–f**.

In aromatic [$^{18}$F]fluorination with the diaryliodonium salt precursors, the fluoride ion is expected to attack the more electron-deficient ring in the diaryliodonium tosylate precursor, so we




**Scheme 1** Sxynthesis of various diaryliodonium tosylate precursors.



prepared various electron-rich diaryliodonium salt precursors with a "dispensable ring" from **4a–f**. The electron density with respect to the dispensable ring was in the following order: phenyl- (**5a**) ≈ 3-methoxyphenyl- (**5e**) < 4-methylphenyl- (**5f**) < 4-methoxyphenyl- (**5d**) < 3-thiophenyl- (**5c**) < 2-thiophenyl-mazenil (**5b**) (from observed $^{13}$C NMR[26]). In addition to the effects of an electron-rich heteroaromatic ring, the effects of base and solvent also play a key role in aromatic radiofluorination with the diaryliodonium salt. Thus, various conditions were explored for the preparation of [$^{18}$F]FMZ, as summarized in Table 1. In the fluorine-18 incorporation experiments, [$^{18}$F]fluoride ion was dried with acetonitrile in the presence of a base (0.8 equiv. *versus* amounts of precursor) such as $TBAHCO_3$, TBAOH, or $K_{2.2.2}/K_2CO_3$. The generated [$^{18}$F]fluoride ion complex was then dissolved in different solvents (0.5 mL) in the presence of a diaryliodonium tosylate precursor (4 mg) and a radical scavenger (1 mg). This solution was heated at the desired temperature for 15 min. We performed a feasibility study for radiofluorination of the different diaryliodonium tosylate precursors (**5a–f**) and monitored their fluorination tendencies. The fluorine-18 incorporation yield was determined by radio-TLC in 10% $MeOH : CH_2Cl_2$ eluent.

Table 1 and Fig. 2 summarize the results of the [$^{18}$F]fluorine incorporation yields with different diaryliodonium tosylate

**Table 1** Aromatic [$^{18}$F]fluorination using various heterodiarene iodonium tosylates (**5a–f**)[a]

| | | | | Fluorine-18 incorporation yield of [$^{18}$F]**1** (%)[b] | | |
|---|---|---|---|---|---|---|
| | | | | Temperature (°C) | | |
| Entry | Precursor | Base | Solvent | 100 | 125 | 150 |
| 1 | **5a** | $n$Bu$_4$NHCO$_3$ | DMF | 6.3 ± 1.2 | 20.7 ± 3.1 | 24.6 ± 4.4 |
| 2[c] | **5a** | $n$Bu$_4$NHCO$_3$ | DMF | — | — | 2.8 ± 0.4 |
| 3 | **5b** | $n$Bu$_4$NHCO$_3$ | DMF | 1.2 ± 0.6 | 4.1 ± 2.0 | 6.3 ± 1.9 |
| 4 | **5c** | $n$Bu$_4$NHCO$_3$ | DMF | 1.8 ± 0.9 | 4.0 ± 2.5 | 3.4 ± 2.2 |
| 5 | **5d** | $n$Bu$_4$NHCO$_3$ | DMF | <1 | 1.3 ± 0.2 | 2.5 ± 1.2 |
| 6 | **5d** | $n$Bu$_4$NHCO$_3$ | DMSO | — | 2.1 ± 0.2 | — |
| 7 | **5e** | $n$Bu$_4$NHCO$_3$ | DMF | <1 | 1.2 ± 0.2 | 2.3 ± 1.0 |
| 8 | **5f** | $n$Bu$_4$NHCO$_3$ | DMF | 15.4 ± 1.6 | 23.1 ± 2.9 | 26.1 ± 4.1 |
| 9 | **5a** | $n$Bu$_4$NOH | DMF | 8.2 ± 2.7 | 11.0 ± 3.6 | 13.2 ± 3.6 |
| 10 | **5b** | $n$Bu$_4$NOH | DMF | 3.6 ± 1.4 | 5.7 ± 2.0 | 10.1 ± 4.1 |
| 11 | **5c** | $n$Bu$_4$NOH | DMF | 4.5 ± 2.9 | 7.1 ± 1.4 | 7.7 ± 2.7 |
| 12 | **5d** | $n$Bu$_4$NOH | DMF | <1 | <1 | 2.3 ± 0.3 |
| 13 | **5e** | $n$Bu$_4$NOH | DMF | <1 | 1.4 ± 0.6 | 2.7 ± 1.2 |
| 14 | **5f** | $n$Bu$_4$NOH | DMF | 11.7 ± 1.7 | 15.6 ± 2.7 | 17.7 ± 4.4 |
| 15 | **5a** | $K_{2.2.2}/K_2CO_3$ | DMF | 29.9 ± 6.2 | 32.5 ± 5.9 | 39.2 ± 9.1 |
| 16 | **5a** | $K_{2.2.2}/K_2CO_3$ | DMSO | — | — | 6.4 ± 3.2 |
| 17 | **5b** | $K_{2.2.2}/K_2CO_3$ | DMF | 1.7 ± 1.2 | 2.3 ± 1.0 | 2.8 ± 1.4 |
| 18 | **5c** | $K_{2.2.2}/K_2CO_3$ | DMF | 8.1 ± 2.1 | 15.8 ± 5.6 | 6.8 ± 2.1 |
| 19 | **5c** | $K_{2.2.2}/K_2CO_3$ | $CH_3CN$ | — | 11.7 ± 4.4 | — |
| 20 | **5c** | $K_{2.2.2}/K_2CO_3$ | DMSO | — | 1.8 ± 0.3 | — |
| 21 | **5d** | $K_{2.2.2}/K_2CO_3$ | DMF | <1 | <1 | 1.5 ± 0.3 |
| 22 | **5e** | $K_{2.2.2}/K_2CO_3$ | DMF | 1.4 ± 0.3 | 1.3 ± 0.5 | 1.4 ± 0.7 |
| 23 | **5f** | $K_{2.2.2}/K_2CO_3$ | DMF | 20.7 ± 4.2 | 55.1 ± 6.9 | 74.1 ± 5.8 |

[a] The reaction was carried out with 0.8 equiv. of base relative to the precursor (4 mg, **5a–f**) in 0.5 mL of solvent (DMF (*N*′,*N*′-dimethylformamide), DMSO (dimethyl sulfoxide) or $CH_3CN$ (acetonitrile)) and a radical scavenger (TEMPO, 1 mg) at the desired temperature for 15 min. [b] Radiochemical yield was determined by radio-TLC representing the percentage of [$^{18}$F]flumazenil in the reaction mixture (developing solvent: methanol : dichloromethane = 10 : 90, v/v; $n$ = 3 or 4). [c] No TEMPO used.

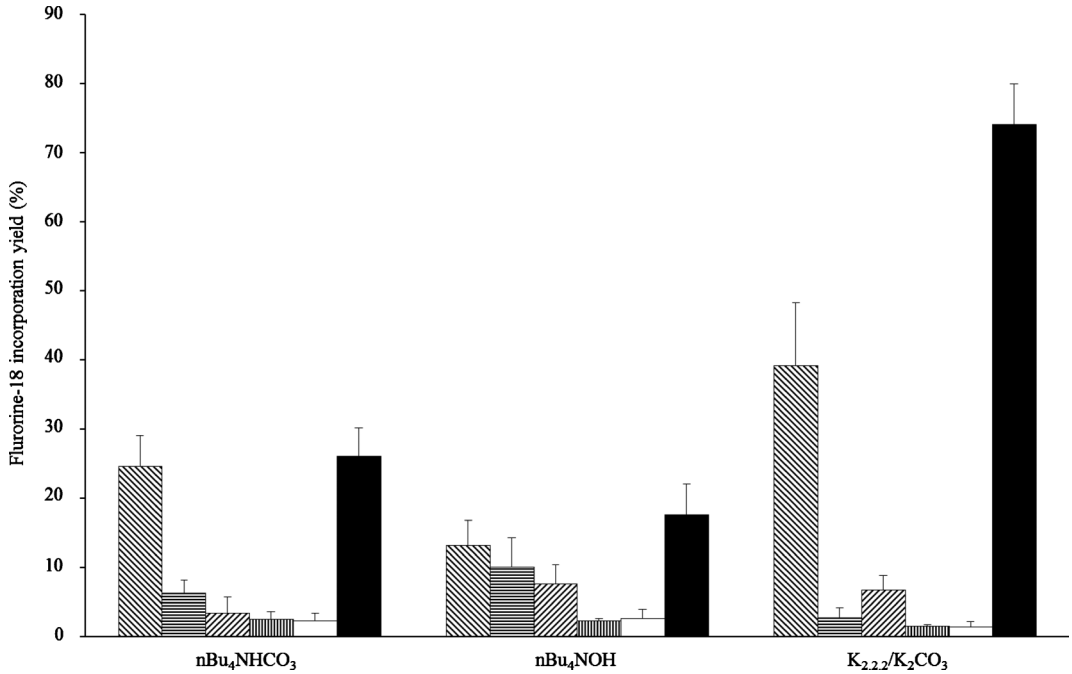



**Fig. 2** Comparison of the fluorine-18 incorporation yields of **5a–f** under different bases (left-handed striped bar: **5a**; horizontal striped bar: **5b**; right-handed striped bar: **5c**; vertical striped bar: **5d**; open bar: **5e**; closed bar: **5f**).

precursors. Among the various reaction conditions, promising results were achieved when a combination of cryptand (Kryptofix 2.2.2; $K_{2.2.2}$)/base ($K_2CO_3$) and 4-methylphenyl-mazenil iodonium tosylate (**5f**) were used in the presence of TEMPO in DMF as a reaction solvent (Table 1, entry 23). TEMPO was used as a radical scavenger and is also one of the important factors in the fluorine-18 incorporation reactions using a diaryliodonium salt precursor (Table 1, entry 2) because it is unstable under basic conditions and generates aromatic hydrocarbons by radical-induced decomposition.[27] Three commonly used solvent systems for radiolabelling reactions were tested, namely $CH_3CN$, DMF and DMSO. Among all these solvents under the equivalent conditions tested, DMF gave better radiolabelling results than those obtained with $CH_3CN$ and DMSO (Table 1 entries 15 *versus* 16 and 18 *versus* 19 or 20). The reactions in all of the solvents tested did not produce any remarkable fluorine-18 labelled by-products, as determined by the HPLC of the crude reaction mixture. The reaction temperature also had a considerable effect on the fluorine-18 incorporation yield. The optimal labelling temperature was determined to be 150 °C under most phase-transfer catalysts (also called the base) and with most types of precursor. For **5b–e** with bases at high temperature, the fluorine-18 incorporation yield was poor under most conditions. It could be considered that these low radiochemical yields might be the result of the stability of the diaryliodonium salts. Amongst the bases used, $K_{2.2.2}/K_2CO_3$ gave better fluorine-18-incorporation yields than TBAOH and $TBAHCO_3$, in the case of **5a** and **5f**, but the other precursors had similarly low incorporation yields. We evaluated the fluorine-18 incorporation effects according to the base concentration in the aromatic radiofluorination of [$^{18}$F]FMZ after the determining of the precursor, base, solvent, and temperature. The [$^{18}$F]fluorine incorporation yield was compared at molar ratios from the 0.4 to 1.0 equivalents of $K_2CO_3$ with that of the precursor, as summarized in Table 2. The results clearly show that the fluorine-18 incorporation yield is not quantitative (in the case of Table 2, entries 1–4) and that of aromatic radiofluorination from a diaryliodonium salt depends on the base : precursor ratio. The result is that diaryliodonium tosylate (**5f**)/$K_2CO_3$ in a ratio of 1 : 0.6 (80.4%) was sufficient to obtain the optimum yield (Table 2, entry 1), while increasing the amount of base resulted in a significant drop in radiochemical yield (Table 2, entries 3 and 4). The molar ratio of the base seemed to be an important factor in determining the efficiency of the fluorination because of the instability of the diaryliodonium salts in basic environments. Increasing the amount of precursor (**5f**) from 4 mg to 8 mg (Table 2, entry 6) was not significant in this experiment, while decreasing the amount resulted in a low fluorine-18 incorporation yield (Table 2, entry 5). Reduction of the heating time resulted in a similar outcome (Table 2, entries 2 *versus* 7). These results indicate that minimal amounts of precursor and short reaction times are readily available to apply the automated synthetic process for [$^{18}$F]FMZ production at low cost.

The results in Table 1 and Table 2 clearly showed the absence of the electron-rich heteroarene effect on the diaryliodonium salt. On comparison of the [$^{18}$F]fluoride incorporation yields for [$^{18}$F]flumazenil among the various precursors, the result obtained from 4-methylphenyl-mazenil iodonium tosylate (**8f**) was superior to those of the other precursors, in spite of the fact that 2-thiophenyl-(**8b**), 3-thiophenyl-(**8c**), and 4-methoxyphenyl-mazenil iodonium tosylate (**8d**) have relatively high electron densities. As mentioned above, in the fluoride substitution reactions of diaryliodonium salts that are functionalized on both aryl rings, the fluoride anion generally attacks the more activated arene (electron-deficient ring). This result therefore indicates that another factor, such as the stability of the diaryliodonium salt under basic conditions at a high temperature, is important for aromatic

**Table 2** Optimization of [$^{18}$F]flumazenil preparation under various conditions[a]

| Entry | Precursor (mg) | $K_2CO_3$ (equiv.) | Time (min) | Yield[b] (d.c.[c]) |
|---|---|---|---|---|
| 1 | 4 | 0.4 | 15 | 51.7 ± 7.8 |
| 2 | 4 | 0.6 | 15 | 80.4 ± 3.2 (66.5 ± 2.3) |
| 3 | 4 | 0.8 | 15 | 74.1 ± 5.9 (60.1 ± 3.6) |
| 4 | 4 | 1.0 | 15 | 42.2 ± 4.4 |
| 5 | 2 | 0.6 | 15 | 56.6 ± 5.3 |
| 6 | 8 | 0.6 | 15 | 76.4 ± 6.5 (56.8 ± 4.3) |
| 7 | 4 | 0.6 | 5 | 81.2 ± 4.1 (67.2 ± 2.7) |

[a] The reaction was carried out with 0.4–1.0 equiv. of $K_2CO_3$ ($K_{2.2.2}$ = 5.5 mg) relative to the precursor (**5f**) in 0.5 mL of DMF and a radical scavenger (TEMPO, 1 mg) at 150 °C for the desired reaction time. [b] The radiochemical yield is determined by radio-TLC and represents the percentage of [$^{18}$F]flumazenil in the reaction mixture ($n$ = 3 or 4). [c] The radiochemical yield is determined by isolating pure [$^{18}$F]flumazenil from a semi-preparative column using HPLC (20 : 80 acetonitrile : water, UV (254 nm), gamma-ray, flow rate: 3 mL min$^{-1}$, $n$ = 3).

**Table 3** Instabilities of diaryliodonium tosylates[a]

| Entry | Precursor | Decomposed compounds (μmol)[b] **6** | **7** | Instability (%)[c] |
|---|---|---|---|---|
| 1 | **5a** | 0.75 | 0.64 | 22.8 |
| 2 | **5b** | 0.94 | 0.90 | 30.4 |
| 3 | **5c** | 1.07 | 1.16 | 37.1 |
| 4 | **5d** | 0.51 | 1.25 | 30.3 |
| 5 | **5e** | 1.01 | 0.73 | 29.9 |
| 6 | **5f** | 0.45 | 0.78 | 20.8 |

[a] The reaction was performed using 4 mg of precursors (5.81–6.07 μmol), 0.5 mL of DMF, radical scavenger (TEMPO, 1 mg) and 0.8 equivalents of $K_2CO_3$ at 150 °C for 15 min. [b] Progress of the reaction was analyzed by HPLC (YMC-triart C18 column, 5 μm, 4.6 × 250 mm, acetonitrile : water, 254 nm, flow rate: 1.0 mL min$^{-1}$) and the values obtained by comparing the UV response of the sample with that of known concentrations of **6** and **7**. [c] Instability represents the percentage of decomposed products **6** and **7** compared with the concentration of starting materials.

fluorination, as well as the electron density of the counter arene. In fact, diaryliodonium salts are generally unstable at higher temperatures as previously reported,[28] and the fluorine-18 incorporation yields of [$^{18}$F]flumazenil are also dependent on the base concentration, as described above (Table 2, entries 1–4). To demonstrate convincingly the results displayed and as described above, we were interested in examining further the stabilities and reactivities of the prepared diaryliodonium salt precursors. If the diaryliodonium tosylate precursors (**5a–f**) decompose under basic conditions at high temperatures, the side products would be generated *via* two pathways; "*Pathway A*" would produce I-mazenil (**6**), and "*Pathway B*" would produce H-mazenil (**7**), as shown in Fig. 3. To monitor the stability of the diaryliodonium salts, each diaryliodonium salt precursor (**5a–f**) was reacted with $K_{2.2.2}/K_2CO_3$ in DMF at 150 °C for 15 min without the presence of the fluoride ion; the stability was evaluated by tracking the decomposed products (**6** and **7**) in the crude reaction mixture by HPLC.

As shown in Table 3, 4-methylphenyl-mazenil iodonium tosylate (**5f**) showed the best stability (20.8%) among the diaryliodonium tosylate precursors whilst in the presence of the base ($K_2CO_3$) at the optimized temperature (150 °C). Phenyl-mazenil iodonium tosylate (**5a**) had slightly less stability (Table 3, entry 1). Although the electron density in the dispensable ring is higher than in the phenyl ring in the case of the precursors **5b–d**, it was noted that the stability of the precursor was much decreased in comparison with those of the phenyl and the 4-methyl substituted phenyl rings. This observation indicates that the more electron-rich diaryliodonium tosylate precursors (**5b–d**) have lower stabilities; these results correspond with the tendency of the [$^{18}$F]fluoride incorporation yield (Table 1, entries 15, 17–18, and 21–23).

On the other hand, each diaryliodonium salt (**5a–f**) was treated with CsF (no base) in DMF at 90 °C for 2 h for selectivity measurements, thus checking the produced [$^{19}$F]flumazenil (**1**), I-mazenil (**6**) and H-mazenil (**7**) concentrations, as shown in Table 4. The crude product mixtures were analyzed by HPLC and then the molar absorptivity of each compound was examined with respect to their relative ratio. In all aspects of this experiment, [$^{19}$F]flumazenil was the main product, except in the case of precursor **5e**. The selectivities of the precursors were all quite different from each other, and it was verified that 4-methylphenyl-mazenil

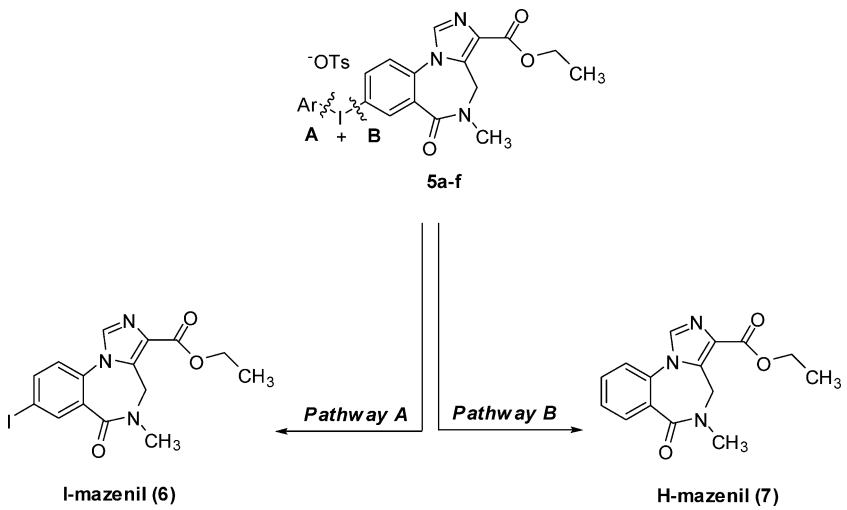



**Fig. 3** Proposed decomposition pathways.

**Table 4** The reaction of diaryliodonium tosylates with CsF[a]

| Entry | Precursor | Product ratios[b] | | | Selectivity for main product, **1** |
|---|---|---|---|---|---|
| | | **6** | **7** | **1** | |
| 1 | **5a** | 0.18 | 0.02 | 0.80 | 4.00 |
| 2 | **5b** | 0.08 | 0.06 | 0.86 | 6.14 |
| 3 | **5c** | 0.19 | 0.07 | 0.74 | 2.85 |
| 4 | **5d** | 0.07 | 0.10 | 0.83 | 4.88 |
| 5 | **5e** | 0.39 | 0.15 | 0.46 | 0.85 |
| 6 | **5f** | 0.07 | 0.02 | 0.91 | 10.11 |

[a] The reaction was performed using 4 mg of precursors (5.81–6.07 μmol), 0.5 mL of DMF, radical scavenger (TEMPO, 1 mg) and 5.0 equivalents of CsF at 90 °C for 2 h. [b] The relative ratios obtained from a mole of each compound, which was analyzed by comparing the UV response of the sample with that of known concentrations of **6**, **7**, and **1** (YMC-triart C18 column, 5 μm, 4.6 × 250 mm, acetonitrile : water, 254 nm, flow rate: 1.0 mL $min^{-1}$).

iodonium tosylate (**5f**) is the most attractive precursor for the efficient fluorine-18 incorporation of [$^{18}$F]FMZ. Phenyl-mazenil iodonium tosylate (**5a**) had a lower selectivity although its stability was similar to that of 4-methylphenyl-mazenil iodonium tosylate (**5f**) (Table 4, entry 1). Conversely, 2-thiophenyl-mazenil iodonium tosylate (**5b**) showed a reasonable selectivity value on the basis of its high electron density in the dispensable ring but it was unsuitable as a precursor because of its poor stability. It was well known that the fluorination of diaryliodonium salts with fluorine-18 occurred preferentially at a more electron-deficient arene, and showed higher efficiency with an electron-rich arene as the counter aryl ring. This tendency is in agreement with the result for the model compounds reported by other research groups. Our studies showed, however, that the stability and selectivity of precursors under basic conditions at a high temperature are important factors in addition to the electron density of the diaryliodonium salt for efficient aromatic [$^{18}$F]fluorination. Thus, our results regarding stability and selectivity will be useful in the design of diaryliodonium salt precursors that can be applied to the synthesis of $^{18}$F-labelled radiopharmaceuticals.

Overall, 4-methylphenyl-mazenil iodonium tosylate (**5f**) had greater stability and selectivity than the electron-rich diaryliodonium tosylates (**5b–d**) or phenyl-mazenil iodonium tosylate (**5a**), it also showed the best radiochemical yield among the diaryliodonium tosylate precursors. The best result was obtained for the reaction of 4 mg of the 4-methylphenyl-iodonium tosylate precursor with fluorine-18 with $K_{2.2.2}/K_2CO_3$ (0.6 equiv.) in DMF at 150 °C for 5 min. In separation steps, the crude product was diluted with water and pre-treated by a C18 plus Sep-pak cartridge to remove the DMF solvent and other polar decomposed mass prior to HPLC purification. The life-time of the HPLC column was shortened to about 4 months when the pretreatment step was not performed using the Sep-Pak cartridge. Finally [$^{18}$F]FMZ was isolated at around 28 min and did not show any remarkable fluorine-18-labelled by-products or UV detected mass surrounding this retention time, as determined by the HPLC of the crude reaction mixture (Fig. 4). Reformulation of the final product in an ethanol/saline solution can be achieved with the C18 plus Sep-pak cartridge. Under these conditions, the radiochemical yield (RCY) was 67.2 ± 2.7% (d.c.) with more than 99% radiochemical purity (RCP) after reverse HPLC purification. The total synthesis time for [$^{18}$F]FMZ ([$^{18}$F]**1**) was about 55 min, including HPLC purification, and the specific activity was in the range of 370–450 GBq $\mu mol^{-1}$. This high radiochemical yield showed the promising usefulness of diaryliodonium salt precursors which result in complex compounds as well as other radiopharmaceuticals which had 40–60% fluorine-18 incorporation yield.[21b,25c]

In order to explore additional applications, studies were extended to a high-scale production in the commercial automated synthetic module which is based on conductive heating. Employing the optimized conditions described above, we successfully obtained [$^{18}$F]**1** using the commercial automated device, with a high

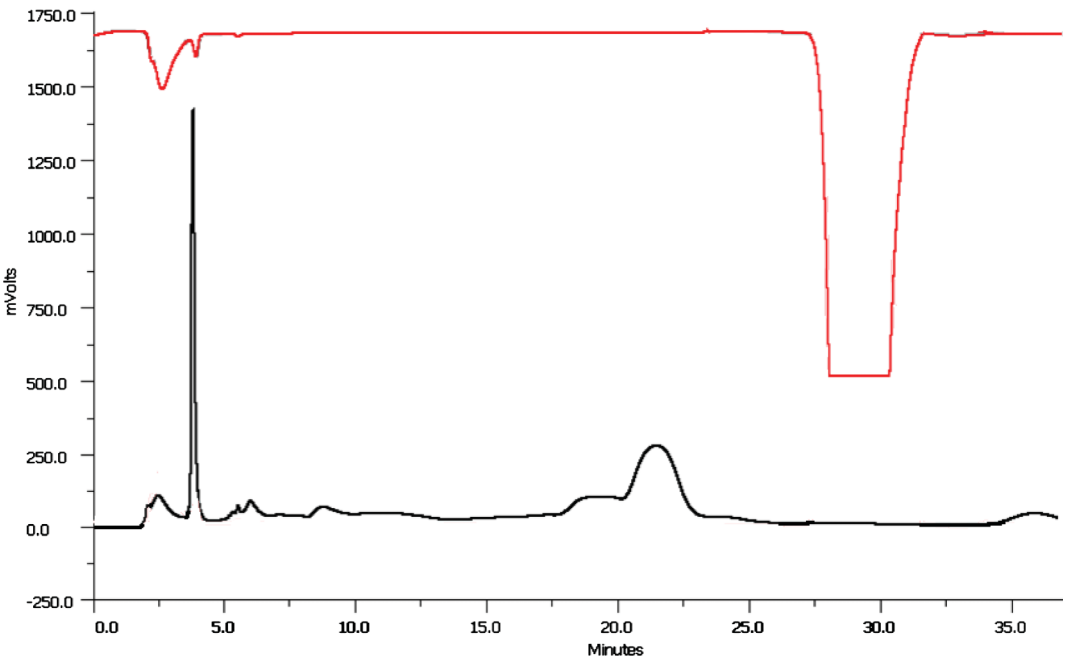



**Fig. 4** The radio-HPLC profile of [$^{18}$F]**1** from the reaction mixture (bottom: UV (254 nm); top: γ-ray).

radiochemical yield of about 63.5 ± 3.2 ($n$ = 26) within 60 ± 1.1 min which included azeotropic distillation, fluorine-18 incorporation, HPLC purification, and solid-phase purification without major modifications. We compared the results of previously reported syntheses based on conductive heating[14,17] or microwave dielectric heating[16] with this study. The elapsed time was shorter than that for the conductive heating system, which took 75–80 min using nitromazenil as the precursor and this method for [$^{18}$F]FMZ production has limitations in automated procedures because of the very low radiochemical yields, as described by other research groups.[15,17,18] Our experimental studies using the commercial automated device in accordance with literature routes also showed very low yields of about 0.4–1.1% ($n$ = 6). However, the automated production of [$^{18}$F]FMZ using a diaryliodonium salt as the precursor produced the high-efficiency yield (with no additional devices such as a microwave system) and the total synthetic time is comparable to microwave-assisted systems.[16] Our experiment also showed the high specific activity (over 370 GBq μmol$^{-1}$), reproducible yield ($n$ = 26 with no failure), simple operation, and without major modification of a commercial module. These results demonstrate that the diaryliodonium salt precursor can satisfy our desires for the high yield production of [$^{18}$F]FMZ and its applicability for clinical PET studies.

## Conclusions

For the efficient radiolabelling of [$^{18}$F]flumazenil, we developed an advanced [$^{18}$F]flumazenil radiosynthesis system using different diaryliodonium tosylate precursors. Among the various precursors, the 4-methylphenyl-mazenil iodonium tosylate precursor (**5f**) showed the highest radiochemical yield with high specific activity. Further studies showed that the optimized reaction conditions are well adapted to the high-scale automatic production of [$^{18}$F]flumazenil in a commercial module. In addition, stability and reactivity studies of different diaryliodonium salt precursors could be applied to the development of various radiopharmaceuticals having low radiochemical yield of fluorine substituted on the aryl positions.

## Experimental

All commercial reagents and solvents were used without further purification unless otherwise specified. Reagents and solvents were commercially purchased from Sigma–Aldrich (U. S.). Flash column chromatography was performed with silica gel (Merck, 230–400 mesh, ASTM). All reactions were monitored on precoated plates (Merck, silica gel 60F$_{254}$). $^1$H and $^{13}$C NMR spectra were recorded on a Varian 400-MR (400 MHz) spectrometer at ambient temperature. Chemical shifts were reported in parts per million (ppm, $\delta$ units). $H_2{}^{18}O$ was purchased from Taiyo Nippon Sanso Corporation (Japan). $^{18}$F-Fluoride was produced at Seoul National University Bundang Hospital by $^{18}$O(p,n)$^{18}$F reaction through proton irradiation using a KIRAMS-13 cyclotron (Samyoung Unitech Co., Ltd.). Chromafix® PS-HCO$_3$ (45 mg) cartridges were purchased from Macherey–Nagel Ins. (Germany). The automated production studies were performed in the TracerLab FX$_{FN}$ (GE Healthcare). C18 plus Sep-Pak® cartridges were purchased from Waters Corp. (U. S.). HPLC purification was performed with a Gilson 322 (Waters, semi-Preparative Xterra RP-18, 5 μm, 7.9 × 250 mm) or Thermo Separation Products System (Fremont, U. S.) (YMC, analytical YMC-triart C18, 5 μm, 4.6 × 250 mm) equipped with a NaI radiodetector (Raytest) and a UV-detector. HPLC-grade solvents (J. T. Baker, U. S.) were used for HPLC purification after membrane filtering (Whatman, 0.22 μm). Radio-TLC was analyzed on a Bioscan radio-TLC scanner (Washington DC, USA). All radioactivities were measured using a VDC-505 activity calibrator from Veenstra Instruments (Netherlands).

### Ethyl-5,6-dihydro-5-methyl-6-oxo-8-tributylstannyl-4*H*-imidazo-[1,5-a][1,4]benzodiazepine-3-carboxylate (3)

To a solution of ethyl 8-bromo-5,6-dihydro-5-methyl-6-oxo-4*H* - imidazo[1,5 - a][1 , 4]benzodiazepine - 3 - carboxylate (3.52 g, 9.67 mmol) in toluene (100 mL) was added tetrakis-(triphenylphosphine)palladium(0) (0.34 g, 0.290 mmol) and bistributyltin (16.8 g, 29.0 mmol). The reaction mixture was heated to reflux under an argon atmosphere for 6 h. The mixture was diluted with ethyl acetate (300 mL) and the extracted organic layer was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness *in vacuo*. Purification by flash column chromatography (5% MeOH : $CH_2Cl_2$) afforded ethyl-5,6-dihydro-5-methyl-6-oxo-8-tributylstannyl-4*H*-imidazo[1,5-a][1,4] benzodiazepine-3-carboxylate (**3**, 3.72 g, 66%) as a white solid: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 0.90 (t, $J$ = 7.2 Hz, 9H), 1.09–1.16 (m, 6H), 1.28–1.40 (m, 6H), 1.46 (t, $J$ = 7.2 Hz, 3H), 1.49–1.59 (m, 6H), 3.26 (s, 3H), 4.22–4.58 (m, 2H and 1H), 5.19 (d, $J$ = 14.8 Hz, 1H), 7.35 (d, $J$ = 8.0 Hz, 1H), 7.71 (dd, $J$ = 8.0, 1.2 Hz, 1H), 7.89 (s, 1H), 8.13 (d, $J$ = 0.8 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 10.1, 13.9, 14.6, 27.6, 29.2, 36.1, 42.6, 61.2, 121.0, 128.2, 128.8, 132.0, 135.2, 136.0, 140.2, 140.8, 144.3, 163.4, 167.3; CAS Registry No. provided by the author: 200408-05-5.

### General method for the diaryliodonium tosylate precursors (5a–f)

Hydroxyl(tosyloxy)iodo-aryl moieties (**4a–f**) were prepared according to the literature.[21,25] To a solution of hydroxyl-(tosyloxy)iodo-aryl moiety (**4a–f**, 1.5 equiv.) in $CH_3CN$ (2 mL) was added ethyl-5,6-dihydro-5-methyl-6-oxo-8-tributylstannyl-4*H*-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate (**3**, 1.0 equiv.) in $CH_2Cl_2$ (2 mL) at room temperature under argon atmosphere and stirred for 20 h. The solvent was evaporated by a stream of nitrogen. The crude mixture was dissolved in ethanol (1.0 mL) and transferred to the centrifuge tube. The solution was diluted with excess diethyl ether (20 mL). After centrifuging, the collected solid was dried *in vacuo* to give various heterodiarene iodonium tosylate precursors **5a–f**.

**Ethyl-8-phenyl(iodonium tosylate)-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate (5a).** M.p.: 215.5–217.9 °C; $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 1.41 (t, $J$ = 6.8 Hz, 3H), 2.34 (s, 3H), 3.21 (s, 3H), 4.30–4.51 (m, 2H and 1H), 5.13 (bs, 1H), 7.20 (d, $J$ = 8.0 Hz, 2H), 7.55 (td, $J$ = 7.8, 1.6 Hz, 2H), 7.66 (dd, $J$ = 6.8, 1.6 Hz, 2H), 7.71 (t, $J$ = 7.6 Hz, 1H), 7.81 (d, $J$ = 8.4 Hz, 1H), 8.27 (dd, $J$ = 8.4, 1.2 Hz, 2H), 8.30 (s, 1H), 8.48 (dd, $J$ = 8.8, 2.4 Hz, 1H), 8.72 (d, $J$ = 2.0 Hz, 1H); $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 14. 7, 21.3, 36.2, 43.2, 62.2, 114.69, 116.5, 127.0, 127.4, 129.7, 129.9, 132.8, 133.4, 134.0, 136.5, 136.7, 136.9, 137.9, 140.2, 140.3, 141.7, 143.6, 163.7, 166.2; MS (FAB) $m/z$ 488

($M^+$–OTs, 100%). HRMS calcd for $C_{21}H_{19}IN_3O_3$ 488.0466, found 488.0474.

**Ethyl-8-(2-thiophenyl)(iodonium tosylate)-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate (5b).** M.p.: 103.5–106.2 °C; $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 1.41 (t, $J$ = 7.0 Hz, 3H), 2.36 (s, 3H), 3.22 (s, 3H), 4.40–4.62 (m, 2H and 1H), 5.15 (bs, 1H), 7.20–7.23 (m, 3H), 7.69 (d, $J$ = 8.4 Hz, 2H), 7.84 (d, $J$ = 8.8 Hz, 1H), 7.94 (dd, $J$ = 5.2, 1.2 Hz, 1H), 8.10 (dd, $J$ = 3.6, 1.2 Hz, 1H), 8.31 (s, 1H), 8.47 (dd, $J$ = 8.8, 2.4 Hz, 1H), 8.73 (d, $J$ = 2.0 Hz, 1H); $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 13.2, 19.9, 34.8, 41.8, 60.7, 97.9, 115.9, 125.5, 125.9, 128.2, 128.4, 129.6, 131.3, 135.0, 135.2, 136.4, 137.7, 138.0, 138.1, 140.3, 141.4, 142.1, 162.2, 164.7.; MS (FAB) $m/z$ 494 ($M^+$–OTs). HRMS calcd for $C_{19}H_{17}IN_3O_3S$ 494.0035, found 494.0042.

**Ethyl-8-(3-thiophenyl)(iodonium tosylate)-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate (5c).** M.p.: 121.2–124.4 °C; $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 1.41 (t, $J$ = 7.2 Hz, 3H), 2.35 (s, 3H), 3.22 (s, 3H), 4.40–4.61 (m, 2H and 1H), 5.16 (bs, 1H), 7.20 (d, $J$ = 8.0 Hz, 2H), 7.67–7.71 (m, 3H), 7.73–7.75 (m, 1H), 7.82 (d, $J$ = 8.8 Hz, 1H), 8.30 (s, 1H), 8.44 (dd, $J$ = 8.4, 2.4 Hz, 1H), 8.60 (dd, $J$ = 2.8, 1.2 Hz, 1H), 8.70 (d, $J$ = 2.4 Hz, 1H); $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 14.6, 21.3, 36.2, 43.2, 62.2, 99.8, 115.4, 127.0, 127.3, 129.7, 129.8, 132.1, 132.69, 132.74, 136.5, 136.6, 137.8, 138.0, 139.8, 139.9, 141.7, 143.6, 163.7, 166.2; MS (FAB) $m/z$ 494 ($M^+$–OTs). HRMS calcd for $C_{19}H_{17}IN_3O_3S$ 494.0035, found 494.0033.

**Ethyl-8-(4-methoxyphenyl)(iodonium tosylate)-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate (5d).** M.p.: 101.3-104.0 °C; $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 1.41 (t, $J$ = 7.2 Hz, 3H), 2.35 (s, 3H), 2.35 (s, 3H), 3.22 (s, 3H), 3.84 (s, 3H), 4.39–4.61 (m, 2H and 1H), 5.16 (bs, 1H), 7.08 (d, $J$ = 9.2 Hz, 2H), 7.21 (d, $J$ = 8.0 Hz, 2H), 7.68 (d, $J$ = 8.0 Hz, 2H), 7.81 (d, $J$ = 8.4 Hz, 1H), 8.17 (d, $J$ = 8.8 Hz, 2H), 8.30 (s, 1H), 8.41 (dd, $J$ = 8.8, 2.4 Hz, 1H), 8.66 (d, $J$ = 2.0 Hz, 1H); $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 13.2, 19.9, 34.7, 41.8, 55.0, 60.7, 103.3, 113.6, 117.6, 125.5, 125.8, 128.2, 128.4, 131.2, 134.9, 135.2, 136.4, 137.5, 138.2, 138.4, 140.3, 142.0, 162.2, 163.3, 164.8; MS (FAB) $m/z$ 518 ($M^+$–OTs). HRMS calcd for $C_{22}H_{21}IN_3O_4$ 518.0576, found 518.0580.

**Ethyl- 8-(3-methoxypehnyl)(iodonium tosylate)-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate (5e).** M.p.: 93.7–96.5 °C; $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 1.40 (t, $J$ = 7.2 Hz, 3H), 2.35 (s, 3H), 3.21 (s, 3H), 3.85 (s, 3H), 4.39 –4.61 (m, 2H and 1H), 5.16 (bs, 1H), 7.23–7.26 (m, 3H), 7.46 (t, $J$ = 8.2 Hz, 1H), 7.68 (d, $J$ = 8.4 Hz, 2H), 7.78–7.88 (m, 3H), 8.29 (s, 1H), 8.46 (d, $J$ = 8.8, 2.0 Hz, 1H), 8.74 (d, $J$ = 2.0 Hz, 1H); $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 14.6, 21.3, 36.2, 43.2, 56.6, 62.2, 114.6, 116.1, 120.0, 122.0, 126.9, 127.3, 128.6, 129.4, 129.8, 132.7, 133.9, 136.4, 136.7, 137.9, 140.1, 140.2, 141.7, 143.5, 162.9, 163.5, 166.2; MS (FAB) $m/z$ 518 ($M^+$–OTs). HRMS calcd for $C_{22}H_{21}IN_3O_4$ 518.0577, found 518.0576.

**Ethyl-8-(4-methylphenyl)(iodonium tosylate)-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate (5f).** M.p.: 223.1–225.4 °C; $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ 1.41 (t, $J$ = 7.2 Hz, 3H), 2.35 (s, 3H), 2.41 (s, 3H), 3.22 (s, 3H), 4.39–4.61 (m, 2H and 1H), 5.16 (bs, 1H), 7.21 (d, $J$ = 8.4 Hz, 2H), 7.37 (d, $J$ = 8.4 Hz, 2H), 7.68 (d, $J$ = 8.0 Hz, 2H), 7.82 (d, $J$ = 8.4 Hz, 1H), 8.12 (d, $J$ = 8.4 Hz, 2H), 8.30 (s, 1H), 8.43 (dd, $J$ = 8.8, 2.4 Hz, 1H), 8.69 (d, $J$ = 2.4 Hz, 1H); $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ 13.2, 19.85, 19.94, 34.7, 41.8, 60.7, 111.2, 113.1, 125.5, 125.8, 128.2, 128.4, 131.3, 132.6, 135.0, 135.2, 135.4, 136.4, 138.5, 138.6, 140.2, 142.1, 144.1, 162.2, 164.8; MS (FAB) $m/z$ 494 ($M^+$–OTs). HRMS calcd for $C_{19}H_{17}IN_3O_3S$ 494.0035, found 488.0033.

**Ethyl-8-iodo-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-a]-[1,4]benzodiazepine-3-carboxylate (6).** Ethyl-5,6-dihydro-5-methyl-6-oxo-8-tributylstannyl-4*H*-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate (**3**, 0.282 g, 0.49 mmol) was dissolved in ethanol (30 mL). Aqueous sodium iodide (3.0 M, 1.60 mL, 4.90 mmol), peracetic acid (32%, 1.16 g, 4.90 mmol) and acetic acid (20 mL) were added. The reaction mixture was stirred at room temperature for 30 h. The solution was quenched with 10 mL of saturated sodium sulfite solution and basified with conc. ammonia water. The crude product was extracted with dichloromethane (3 × 20 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated to dryness *in vacuo*. Purification by flash column chromatography (5% $MeOH/CH_2Cl_2$) afforded iodinate **6** (0.184 g, 92%) as a white solid: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 1.45 (t, $J$ = 7.2 Hz, 3H), 3.25 (s, 3H), 4.20–4.62 (m, 2H and 1H), 5.06–5.40 (m, 1H), 7.17 (d, $J$ = 8.4 Hz, 1H), 7.88 (s, 1H), 7.95 (dd, $J$ = 8.4, 2.0 Hz, 1H), 8.39 (d, $J$ = 2.0 Hz, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 14.6, 36.2, 42.5, 61.3, 93.7, 123.6, 129.2, 130.8, 131.8, 134.9, 135.5, 141.7, 141.8, 163.2, 165.2; CAS Registry No. provided by the author: 268566-09-2.

**Ethyl-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate (7).** Ethyl-8-iodo-5,6-dihydro-5-methyl-6-oxo-4*H*-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate (**6**, 60 mg, 0.14 mmol) was dissolved in methanol (2 mL), followed by the addition of triethylamine (36 µL, 0.26 mmol), 10% Pd/C (30 mg) and hydrogen gas was bubbled using hydrogen balloon. After the reaction mixture stirred at room temperature for 1 h, the solution was filtered and evaporated. Purification by flash column chromatography (5% $MeOH/CH_2Cl_2$) afforded deiodinate **7** (38 mg, 92%) as a colorless oil:$^1$H NMR (400 MHz, $CDCl_3$) $\delta$ 3.03 (s, 3H), 3.91 (s, 2H), 7.02 (d, $J$ = 7.6 Hz, 1H), 7.29 (t, $J$ = 7.8 Hz, 1H), 7.47 (td, $J$ = 7.6, 1.6 Hz, 1H), 7.97 (dd, $J$ = 8.0, 1.6 Hz, 1H), 8.63 (s, 1H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 14.6, 36.1, 42.6, 61.2, 122.8, 128.8, 128.9, 129.3, 132.2, 132.9, 132.9, 135.2, 135.9, 163.3, 166.7; CAS Registry No. provided by the author: 3415-35-8.

**General method for aromatic fluorination of [$^{18}$F]flumazenil.** $^{18}$F was prepared by the $^{18}O(p,n)^{18}F$ reaction using $H_2{}^{18}O$ as the target material. [$^{18}$F]$F^-/H_2{}^{18}O$ was isolated from the enriched water by trapping on Chromafix-$HCO_3$ cartridge (pre-activated with 2 mL of ethanol and 5 mL of water), and then eluted with methanol : water (1 : 0.2 mL) dissolved the $TBAHCO_3$, TBAOH or $K_{2.2.2.}/K_2CO_3$.[29] This solution was dried by azeotropic distillation with acetonitrile under a nitrogen stream and then the iodonium tosylate precursor **5a–f** and TEMPO (1 mg) in various reaction solvents (0.5 mL) were added. The reaction mixture was heated at the desired temperature for 5–15 min. After cooling to room temperature, the reaction mixture was diluted with 10 mL of water. This solution was loaded into a C18 plus Sep-Pak, washed with 10 mL of water and eluted with 1 mL of $CH_3CN$. The combined

solution was separated by a semi-prep HPLC system (Waters, Xterra RP-18, 250 × 7.9 mm, 10 μ). The acetonitrile and water (20 : 80) were used as a mobile phase at a flow rate of 3 mL $min^{-1}$. The product fraction was collected at around 28 min.

**Instability of diaryliodonium tosylates.** The reaction was performed with 4 mg of heterodiarene iodonium salt precursors (5.81–6.07 μmol), TEMPO (1 mg), $K_{2.2.2.}/K_2CO_3$ (5.5 mg/0.8 equiv.) in DMF (0.5 mL) at 150 °C for 15 min. After cooling to room temperature, the reaction mixture was diluted with 10 mL of water and then loaded into a C18 plus Sep-Pak, followed by 10 mL of water. The decomposed compounds were collected with $CH_3CN$ (2 mL) and the progress of the reaction was analyzed by HPLC (YMC-triart C18 column, 5 μm, 4.6 × 250 mm; 25% acetonitrile–water (0 min), 25% acetonitrile–water (10 min), 80% acetonitrile–water (20 min), 80% acetonitrile–water (30 min); 254 nm, flow rate: 1.0 mL $min^{-1}$). The values (μmol) were obtained from comparing the UV responses of the samples with that of known concentrations of **6** and **7**. The standard curves were obtained in six points from $1.95 \times 10^{-6}$ to $6.23 \times 10^{-10}$ mol ($R^2$ = 1.00) for **6** and $2.81 \times 10^{-6}$ to $8.98 \times 10^{-10}$ mol ($R^2$ = 1.00) for **7**.

**Selectivity of diaryliodonium tosylates with CsF.** The reaction was performed with 4 mg of the heterodiarene iodonium salt precursors (5.81–6.07 μmol) and CsF (5.0 equiv.) in DMF (0.5 mL) at 90 °C for 120 min. After cooling to room temperature, the reaction mixture was diluted with 10 mL of water and then loaded into the C18 plus Sep-Pak, followed by 10 mL of water. The products were collected with $CH_3CN$ (2 mL) and the progress of the reaction was analyzed by HPLC (YMC-triart C18 column, 5 μm, 4.6 × 250 mm; 25% acetonitrile-water (0 min), 25% acetonitrile-water (10 min), 80% acetonitrile-water (20 min), 80% acetonitrile-water (30 min); 254 nm, flow rate: 1.0 mL $min^{-1}$). The relative ratios were obtained by comparison of the UV responses of the samples with that of known concentrations of **6**, **7** and **1**. The standard curves was obtained in six points from $1.95 \times 10^{-6}$ to $6.23 \times 10^{-10}$ mol ($I^2$ = 1.00) for **6**, $2.81 \times 10^{-6}$ to $8.98 \times 10^{-10}$ mol ($R^2$ = 1.00) for **7** and $2.64 \times 10^{-6}$ to $8.45 \times 10^{-10}$ mol ($R^2$ = 1.00) for **1**.

**Automated production of [$^{18}$F]flumazenil.** For the automatic production of [$^{18}$F]flumazenil, we used the TracerLab $FX_{FN}$ module (GE Healthcare) without any major modification. The [$^{18}$F]fluoride (37–55 GBq in $H_2{}^{18}O$) from the target was directly loaded to the Chromafix® PS-$HCO_3$ cartridge for trapping. The trapped fluorine-18 was eluted with $K_{2.2.2.}/K_2CO_3$ in MeOH : $H_2O$ (1.0 : 0.2 mL). After complete drying at 65–95 °C by vacuum and a He gas flow, the 4-methylphenyl-mazenil iodonium tosylate precursor (**5f**, 4 mg) and TEMPO (1 mg) in DMF (1.0 mL) was added to the reactor and incubated at 150 °C for 5 min. After cooling to 40 °C by air flow, the reaction mixture was diluted with 10 mL of water and then extracted with C18 plus Sep-Pak. The eluted solution with 1.2 mL of $CH_3CN$ was injected to HPLC system. The HPLC purification was performed in 20% $CH_3CN$–$H_2O$ at a flow rate of 4.5 mL $min^{-1}$, using a UV detector at 254 nm and a γ-ray detector in the module. The fraction of [$^{18}$F]flumazenil collected from HPLC at around 23 min was transferred to a diluted vial which was filled with 50 mL of $H_2O$. The diluted solution was exchanged to 5% EtOH–saline solution by C18 plus Sep-Pak to remove the clinically unavailable HPLC solvent.

## Acknowledgements

This work was supported in part by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (2011-0006320 and NRF-351-2009-2-E00063). The research was supported in part by the Converging Research Center Program through the Ministry of Education, Science and Technology (2010K001055). This study was also supported by a grant from the Korea Food Research Institute (Project No. E0112401) and Ministry of Health and Welfare (Project No. A062254).

## References

1 M. S. Berridge and T. J. Tewson, *Int. J. Radiat. Appl. Instrum., Part A*, 1986, **37**, 685.
2 L. S. Cai, S. Y. Lu and V. W. Pike, *Eur. J. Org. Chem.*, 2008, **17**, 2853.
3 (*a*) V. W. Pike and F. I. Aigbirhio, *J. Chem. Soc., Chem. Commun.*, 1995, (21), 2215; (*b*) A. Shah, V. W. Pike and D. A. Widdowson, *J. Chem. Soc., Perkin Trans. 1*, 1998, 2043; (*c*) R. Gail, C. Hocke and H. H. Coenen, *J. Label. Compd. Radiopharm.*, 1997, **40**, 50; (*d*) V. W. Pike and F. I. Aigbirhio, *J. Label. Compd. Radiopharm.*, 1995, **37**, 120; (*e*) S. Martin-Santamaria, M. A. Carroll, C. M. Carroll, C. D. Carter, V. W. Pike, H. S. Rzepa and D. A. Widdowson, *Chem. Commun.*, 2000, 649; (*f*) L. Ross, J. Ermert, C. Hocke and H. H. Coenen, *J. Am. Chem. Soc.*, 2007, **129**, 8018.
4 G. Balz and G. Schiemann, *Chem. Ber.*, 1927, **60**, 1186.
5 O. Wallach, *Justus Liebigs Ann. Chem.*, 1886, **235**, 242.
6 V. W. Pike, C. Halldin, C. Crouzel, L. Barré, D. J. Nutt, S. Osman, F. Shah, D. R. Turton and S. L. Waters, *Nucl. Med. Biol.*, 1993, **20**, 503.
7 P. Ryvlin, S. Bouvard, D. Le Bars, G. De Lamérie, M. C. Grégoire, P. Kahane, J. C. Froment and F. Mauguiére, *Brain*, 1998, **121**, 2067.
8 A. L. Malizia, V. J. Cunningham, C. J. Bell, P. F. Liddle, T. Jones and D. J. Nutt, *Arch. Gen. Psychiatry*, 1998, **55**, 715.
9 W-D. Heiss, M. Grond, A. Thiel, M. Ghaemi, J. Sobesky, J. Rudolf, B. Bernd and K. Wienhard, *Stroke*, 1998, **29**, 454.
10 W. D. Heiss, L. Kracht, M. Grond, J. Rudolf, B. Bauer, K. Wienhard and G. Pawlik, *Stroke*, 2000, **31**, 366.
11 V. A. Holthoff, R. A. Koeppe, K. A. Frey, J. B. Penney, D. S. Markel, D. E. Kuhl and A. B. Young, *Ann. Neurol.*, 1993, **34**, 76.
12 M. Meyer, R. A. Koeppe, K. A. Frey, N. L. Foster and D. E. Kuhl, *Arch. Neuro.*, 1995, **52**, 314.
13 J. E. Litton, J. Neiman, S. Pauli, L. Farde, T. Hindmarsh, C. Halldin and G. Sedvall, *Psychiatry Res., Neuroimaging*, 1993, **50**, 1.
14 N. N. Ryzhikov, N. Seneca, R. N. Krasikova, N. A. Gomzina, E. Shchukin, O. S. Fedorova, D. A. Vassiliev, B. Gulyas, H. Hall, I. Savic and C. Halldin, *Nucl. Med. Biol.*, 2005, **32**, 109.
15 I. Odano, C. Halldin, P. Karlsson, A. Varrone, A. J. Airaksinen, R. N. Krasikova and L. Farde, *NeuroImage*, 2009, **45**, 891.
16 K. S. Mandap, T. Ido, Y. Kiyono, M. Kobayashi, T. G. Lohith, T. Mori, S. Kasamatsu, T. Kudo, H. Okazawa and Y. Fujibayashi, *Nucl. Med. Biol.*, 2009, **36**, 403.
17 G. Massaweh, E. Schirrmacher, C. La Fougere, M. Kovacevic, C. Wängler, D. Jolly, P. Gravel, A. J. Reader, A. Thiel and R. Schirrmacher, *Nucl. Med. Biol.*, 2009, **36**, 721.
18 J. Woodcraft, C. Jones, A. Gaeta, W. Trigg, P. Jones, S. Plant, A. Jackson, *PCT Int Appl.* WO 2011042529, 2011.
19 (*a*) Y. S. Chang, J. M. Jeong, Y. H. Yoon, W. J. Kang, S. J. Lee, D. S. Lee, J. K. Chung and M. C. Lee, *Nucl. Med. Biol.*, 2005, **32**, 263; (*b*) M. Mitterhauser, W. Wadsak, L. Wabnegger, L-K. Mien, S. Tögel, O. Langer, W. Sieghart, H. Viernstein, K. Kletter and R. Dudczak, *Nucl. Med. Biol.*, 2004, **31**, 291; (*c*) W. Wadsak, M. Mitterhauser, L-K. Mien, S. Tögel, B. Keppler, R. Dudczak and K. Kletter, *J. Labelled Compd. Radiopharm.*, 2003, **46**, 1229; (*d*) Y. H. Yoon, J. M. Jeong, H. W. Kim, S. H. Hong, Y-S. Lee, H. S. Kil, D. Y. Chi, D. S. Lee, J-K Chung and M. C. Lee, *Nucl. Med. Biol.*, 2003, **30**, 521.
20 (*a*) S. M. Moerlein and J. S. Perlmutter, *Eur. J. Pharmacol.*, 1992, **218**, 109; (*b*) G. Grunder, T. Siessmeier, C. Lange-Asschenfeldt, I.

Vernaleken, H-G. Buchholz, P. Stoeter, A. Drzezga, H. Lüddens, F. Rösch and P. Bartenstein, *Eur. J. Nucl. Med. Mol. Imaging*, 2001, **28**, 1463; (*c*) P. Leveque, D. Labar and B. Gallez, *Nucl. Med. Biol.*, 2001, **28**, 809; (*d*) P. Leveque, S. Sanabria-Bohorquez, A. Bol, A. De Volder, D. Labar, K. Van Rijckevorsel and B. Gallez, *Eur. J. Nucl. Med. Mol. Imaging*, 2003, **30**, 1630.

21 (*a*) B. C. Lee, K. C. Lee, H. Lee, R. H. Mach and J. A. Katzenellenbogen, *Bioconjugate Chem.*, 2007, **18**, 514; (*b*) B. C. Lee, J. S. Kim, B. S. Kim, J. Y. Son, S. K. Hong, H. S. Park, B. S. Moon, J. H. Jung, J. M. Jeong and S. E. Kim, *Bioorg. Med. Chem.*, 2011, **19**, 2980; (*c*) B. C. Lee, C. S. Dence, H. Zhou, E. E. Parent, M. J. Welch and J. A. Katzenellenbogen, *Nucl. Med. Biol.*, 2009, **36**, 147.

22 (*a*) G. F. Koser, R. H. Wettach and C. S. Smith, *J. Org. Chem.*, 1980, **45**, 1542; (*b*) M. van der Puy, *J. Fluorine Chem.*, 1982, **21**, 385.

23 (*a*) Z-Q. Gu, G. Wong, C. Dominguez, B. R. de Costa, K. C. Rice and P. Skolnick, *J. Med. Chem.*, 1993, **36**, 1001; (*b*) R. I. Fryer, R. A. Schmidt and L. H. Sternbach, *J. Pharm. Sci.*, 1964, **53**, 264; (*c*) R. I. Fryer, P. Zhang, K-Y Lin, R. B. Upasani, G. Wong and P. Skolnick, *Med. Chem. Res.*, 1993, **3**, 183; (*d*) R. I. Fryer, Z-Q. Gu and C-G. Wang, *J. Heterocycl. Chem.*, 1991, **28**, 1661.

24 T. Gan, S. G. Van Ornum and J. M. Cook, *Tetrahedron Lett.*, 1997, **38**, 8453.

25 (*a*) E. A. Merritt, V. M. T. Carneiro, L. F. Silva Jr. and B. Olofsson, *J. Org. Chem.*, 2010, **75**, 7416; (*b*) T. Nabana and H. Togo, *J. Org. Chem.*, 2002, **67**, 4362; (*c*) M. R. Zhang, K. Kumata and K. Suzuki, *Tetrahedron Lett.*, 2007, **48**, 8632.

26 see Electronic Supplementary Information (ESI†).

27 (*a*) J. J. Lubinowski, J. W. Knapczyk, J. L. Calderon, L. R. Petit and W. E. McEwen, *J. Org. Chem.*, 1975, **40**, 3010; (*b*) M. A. Carroll, J. Nairne, G. Smith and D. A. Widdowson, *J. Fluorine Chem.*, 2007, **128**, 127.

28 (*a*) Y. Yamada and M. Okawara, *Bull. Chem. Soc. Jpn.*, 1972, **45**, 1860; (*b*) F. M. Beringer and M. Mausner, *J. Am. Chem. Soc.*, 1958, **80**, 4535.

29 B. S. Moon, J. H. Park, H. J. Lee, J. S. Kim, H. S. Kil, B. S. Lee, D. Y. Chi, B. C. Lee, Y. K. Kim and S. E. Kim, *Appl. Radiat. Isot.*, 2010, **68**, 2279.