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Efficient Regioselective Ring Opening of Activated Aziridine-2-Carboxylates with [^{18}F]Fluoride

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Aziridines can undergo a range of ring-opening reactions with nucleophiles. The regio- and stereochemistry of the products depend on the substituents on the aziridine. Aziridine ring-opening reactions have rarely been used in radiosynthesis. Herein we report the ring opening of activated aziridine-2-carboxylates with [^{18}F]fluoride. The aziridine was activated for nucleophilic attack by substitution of various groups on the aziridine nitrogen atom. Fluorine-18 radiolabelling was followed by ester hydrolysis and removal of the activation group. Totally regioselective ring opening and subsequent deprotection was achieved with *tert*-butyloxycarbonyl- and carboxybenzyl-activated aziridines to give α-[^{18}F]fluoro-β-alanine in good radiochemical yield.

Introduction

Nucleophilic ring opening of aziridines is an efficient way of synthesising a variety of organic compounds.[1,2] The strain in the three-membered ring of aziridine allows it to undergo a range of ring-opening reactions with nucleophiles.[3] The presence of specific substituents on the aziridine can control the regio- and stereochemistry of the ring-opening reaction.[4] For non-activated aziridines a strong nucleophile or acid catalyst is required to open the ring. The reactivity of the aziridine ring toward nucleophiles is increased by having an activation group on the aziridine ring nitrogen atom to stabilise the negative charge and to make the nitrogen a better leaving group.[5,6] The nucleophile can attack either of the two carbon atoms in the aziridine ring. For 2-substituted aziridines ring-opening can lead to two regioisomeric amine products. For cases in which an acyl substituent is used, attack is favoured on the more hindered aziridine ring carbon atom with azide, halide, or cyanide as the nucleophile, but is favoured on the less hindered carbon with alcohols as the nucleophile.[7]

Nucleophilic attack of aziridines by fluoride ions has been studied much less than other halides.[8] The synthesis of β-fluoroamines has been explored by ring opening of aziridines. Fluorination reagents such as HF, HF/pyridine, and diethylaminosulfur trifluoride (DAST) have been used.[9,10,11] Ring opening was recently carried out with the use of $KF{\cdot}2H_2O/Bu_4NHSO_4$, $KF{\cdot}2H_2O$, or KHF_2 in an ionic liquid and triethylamine trihydrofluoride.[12,13,14] However, these methods are not convenient when using the radionuclide fluorine-18 ($t_{1/2} = 109.7$ min).[15] Fluorine-18 is one of the most frequently used radionuclides for positron emission tomography (PET), which is an important technique for imaging and studying biochemical processes in vivo. PET uses organic compounds labelled with short-lived positron-emitting radionuclides and relies on rapid synthetic pathways that can incorporate radionuclides into these compounds.[16–18] Only a few publications can be found on the use of aziridines for radiosynthesis.[8,15,19–24]

The aim of this study was to investigate ring-opening reactions of aziridine-2-carboxylates with [^{18}F]fluoride in aziridines with various nitrogen-activating groups. Regioselectivity of ring opening was studied, and reaction conditions and deprotection protocols were optimised. Using aziridine-2-carboxylates, fluorine-18 ring opening followed by de-esterification and removal of the activating group will yield two possible isomers: α-[^{18}F]fluoro-β-alanine or β-[^{18}F]fluoroalanine (Scheme 1).

Results and Discussion

The ring-opening reaction with fluorine-18 was investigated by using methylaziridine-2-carboxylates activated with five different groups. The initial approach to synthesise these compounds was from commercially available triphenylmethyl (trityl)-protected methylaziridine-2-carboxylate. Removal of the trityl group proved to be problematic, and it was only possible to synthesise the *p*-nitrobenzenesulfonyl (nosyl)- and toluenesulfonyl (tosyl)-activated aziridine-2-carboxylates with this ap-

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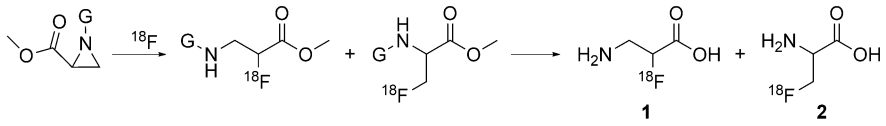
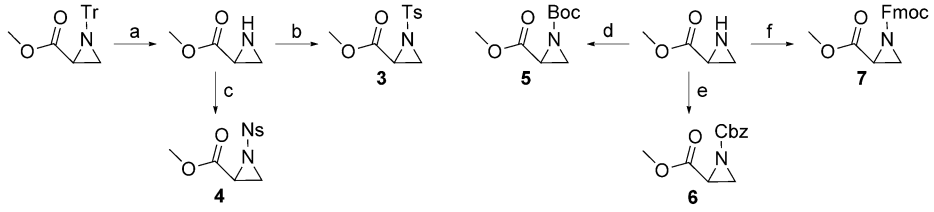


Scheme 1. Aziridine ring opening with [^{18}F]fluoride followed by removal of activating group G and methyl ester hydrolysis to afford α-[^{18}F]fluoro-β-alanine (**1**) or β-[^{18}F]fluoroalanine (**2**).

proach. The remaining aziridines with *tert*-butyloxycarbonyl (Boc), carboxybenzyl (Cbz), and fluorenylmethyloxycarbonyl (Fmoc) activating groups were synthesised directly from methylaziridine-2-carboxylate (Scheme 2). Because previous studies demonstrated the lack of reactivity of non-activated aziridines toward nucleophilic ring opening in the absence of a Lewis acid,[24] for example, the non-activated aziridine-2-carboxylate was not tested.



Scheme 2. Synthesis of activated methylaziridine-2-carboxylates. *Reagents and conditions*: a) TFA, $CHCl_3$, MeOH, 0 °C, 2.5 h; b) *p*-TsCl, EtOAc, H_2O, RT, 48 h; c) NsCl, EtOAc, H_2O, RT, 18 h; d) Boc_2O, Et_3N, MeCN, RT, 18 h; e) CbzCl, Et_3N, MeCN, RT, 18 h; f) Fmoc-OSu, Et_3N, THF, H_2O, RT, 2 h.

Labelling conditions for aziridine ring-opening with fluorine-18

The first step in aziridine ring opening was to find the optimal labelling conditions. [^{18}F]Fluoride was prepared by proton irradiation of enriched oxygen-18 water and thus initially available as an aqueous solution. To provide reactive [^{18}F]fluoride, it was firstly trapped on a small purification cartridge to remove (and recover) most of the water, and then eluted from the cartridge using a phase-transfer catalyst. Two different approaches were investigated for this procedure. After trapping of aqueous [^{18}F]fluoride on a quaternary methylammonium (QMA) column (anion exchange, silica based), it was eluted with either a Kryptofix solution containing potassium carbonate (K222/K_2CO_3) or a tetraethylammonium bicarbonate solution ($TEAHCO_3$), and subsequently dried by azeotropic distillation with acetonitrile. Recently, a faster and easier method for preparation of anhydrous [^{18}F]fluoride using Chromafix polymer cartridges (anion exchange) was published.[25] In this second approach [^{18}F]fluoride can be quantitatively trapped on the resin and, following rinsing with methanol to remove water, eluted with an anhydrous solution of tetrabutylammonium mesylate (TBAOMs) in methanol and dried without the need for numerous azeotropic drying cycles. The Chromafix columns are available in the bicarbonate (PS-HCO_3) form, but can be easily converted into less basic forms by flushing with a potassium phosphate solution (PS-K_2PO_4) prior to [^{18}F]fluoride trapping, for example. For all methods, after drying the [^{18}F]fluoride, it was dissolved in either *N*,*N*-dimethylformamide (DMF) or dimethyl sulfoxide (DMSO) and reacted with each of the five aziridine-2-carboxylates. The ring-opening reaction was investigated at various temperatures using conventional or microwave heating.

Initial radiolabelling experiments were performed using the Boc-activated aziridine, and the results are summarised in Table 1. Labelling with [^{18}F]fluoride using K222/K_2CO_3 as base gave lower yields than with $TEAHCO_3$. Microwave heating led to increased yields with decreased reaction times. Longer reaction times by microwave heating did not lead to higher yields owing to decomposition of the aziridine precursor during prolonged exposure to high temperature and base.

Seo et al. demonstrated that the milder base forms of Chromafix columns could be beneficial for radiolabelling yields by preserving the integrity of the radiolabelling precursor.[25] As can be seen in Table 1, the phosphate form gave much better results than the bicarbonate form (entries 10 and 11). Due to the simplicity of this approach, this may be advantageous, although ring-opening yields were not quite as high as with the QMA/$TEAHCO_3$ approach. Overall, the best yields for labelling of the Boc-activated aziridine were obtained with $TEAHCO_3$ as the base and microwave heating at 130 °C for 10 min.

Table 1. [^{18}F]Fluoride labelling of Boc-activated methylaziridine-2-carboxylate.

Entry	Base[a]	Solvent	*T* [°C][b]	*t* [min]	Yield [%][c]
1	K222/K_2CO_3	DMSO	100	20	5
2	K222/K_2CO_3	DMSO	130	20	17
3	K222/K_2CO_3[d]	DMSO	130 MW	10	15
4	$TEAHCO_3$	DMSO	100	20	15
5	$TEAHCO_3$	DMSO	130	20	25
6	$TEAHCO_3$	DMSO	130 MW	10	45
7	$TEAHCO_3$	DMF	130 MW	10	36
8	$TEAHCO_3$	DMSO	150	10	31
9	$TEAHCO_3$	DMSO	150 MW	10	43
10	TBAOMs/HCO_3^-	DMSO	130	20	3
11	TBAOMs/PO_4^{3-}	DMSO	130	20	40

[a] Base concentrations: K_2CO_3 8 mM, $TEAHCO_3$ 19 mM, TBAOMs 8 mM. [b] MW = microwave. [c] Ring-opening yields are based on percentage radiochemical conversion determined by radio-HPLC. [d] Concentration of K_2CO_3 solution: 19 mM.

The solvent was changed to DMF to see if this could improve the yield. The reaction with $TEAHCO_3$ and DMF was tested at various temperatures and also by microwave heating. All reactions in DMF (Boc, Cbz, tosyl) gave consistently lower yields than obtained for similar reactions in DMSO (for exam-

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ple, see entry 7 in Table 1). DMSO was thus chosen as solvent for all subsequent labelling reactions.

The ring-opening reaction of the Cbz-activated aziridine with [^{18}F]fluoride failed completely with K222/K_2CO_3. Ring opening was observed if the base was changed to $TEAHCO_3$, and microwave heating further increased the yields. Chromafix columns were tested with the microwave conditions, but the results were not promising. The best labelling conditions for the Cbz-activated aziridine were obtained using $TEAHCO_3$ with microwave heating at 130 °C for 10 min (Table 2).

Table 2. [^{18}F]Fluoride labelling of Cbz-activated methylaziridine-2-carboxylate.

Entry	Base[a]	Solvent	T [°C][b]	t [min]	Yield [%][c]
1	K222/K_2CO_3	DMSO	100	20	0
2	K222/K_2CO_3	DMSO	130	20	0
3	$TEAHCO_3$	DMSO	100	20	23
4	$TEAHCO_3$	DMSO	130	20	29
5	$TEAHCO_3$	DMSO	130 MW	10	48
6	$TEAHCO_3$	DMSO	150 MW	10	26
7	TBAOMs/HCO_3^-	DMSO	130 MW	10	4
8	TBAOMs/PO_4^{3-}	DMSO	130 MW	10	8

[a] Base concentrations: K_2CO_3 8 mM, $TEAHCO_3$ 19 mM, TBAOMs 8 mM. [b] MW = microwave. [c] Ring-opening yields are based on percentage radiochemical conversion determined by radio-HPLC.

Initial radiolabelling attempts using the Fmoc-activated aziridine failed. The labelling reactions were tested at 100 and 130 °C with K222/K_2CO_3 and $TEAHCO_3$. HPLC analysis showed decomposition of the aziridine precursor under these basic reaction conditions, presumably due to cleavage of the Fmoc group with the resultant decreased reactivity of the aziridine. The milder base TBAOMs in combination with the Chromafix columns (both PS-HCO_3 and PS-K_2PO_4) was tested, but again did not lead to any labelling. Fmoc was thus abandoned as an aziridine activating group.

[^{18}F]Fluoride labelling experiments with the nosyl-activated aziridine were first performed at 100 and 130 °C with K222/K_2CO_3 and $TEAHCO_3$. No labelling was observed after 20 min for both conditions. The UV trace on the HPLC indicated decomposition of the aziridine precursor. The stability of the nosyl-activated aziridine was thus investigated by heating in DMSO and showed that the optimal temperature was between 50 and 60 °C. Radiolabelling was not possible at such low temperatures, and the nosyl group was thus abandoned as a suitable aziridine activating group.

The best conditions for [^{18}F]fluoride labelling of the tosyl-activated aziridine involved the use of $TEAHCO_3$ and microwave heating at 100 °C (Table 3). These conditions led to a high radiochemical yield of 72%. The tosyl-activated aziridine was not stable at temperatures above 100 °C, and higher temperatures were detrimental to the radiochemical yield. As with the Boc- and Cbz-activated aziridines, labelling with K222/K_2CO_3 and the Chromafix method gave low yields.

Table 3. [^{18}F]Fluoride labelling of tosyl-activated methylaziridine-2-carboxylate.

Entry	Base[a]	Solvent	T [°C][b]	t [min]	Yield [%][c]
1	K222/K_2CO_3	DMSO	100	20	10
2	K222/K_2CO_3	DMSO	130	20	4
3	$TEAHCO_3$	DMSO	90	20	26
4	$TEAHCO_3$	DMSO	90 MW	20	34
5	$TEAHCO_3$	DMSO	100 MW	10	72
7	TBAOMs/HCO_3^-	DMSO	90	20	3
8	TBAOMs/PO_4^{3-}	DMSO	100 MW	10	1

[a] Base concentrations: K_2CO_3 8 mM, $TEAHCO_3$ 19 mM, TBAOMs 8 mM. [b] MW = microwave. [c] Ring-opening yields are based on percentage radiochemical conversion determined by radio-HPLC.

Concentration of activated aziridine for labelling

Different concentrations of the activated aziridine precursor for labelling were tested to determine the effect on ring-opening yield. For these experiments the Boc-activated aziridine-2-carboxylate was used, and labelling with [^{18}F]fluoride was performed with $TEAHCO_3$ and microwave conditions at 130 °C for 10 min. The concentration of $TEAHCO_3$ and the volume were kept constant. The correlation between aziridine precursor concentration and ring-opening yield determined by radio-HPLC is shown in Figure 1. At precursor concentrations of 10 and 20 mg mL^{-1} the radiochemical yield was ~60%. Increasing precursor concentrations only marginally increased the radiochemical yield, and thus a concentration of 10-20 mg mL^{-1} was considered to be adequate.

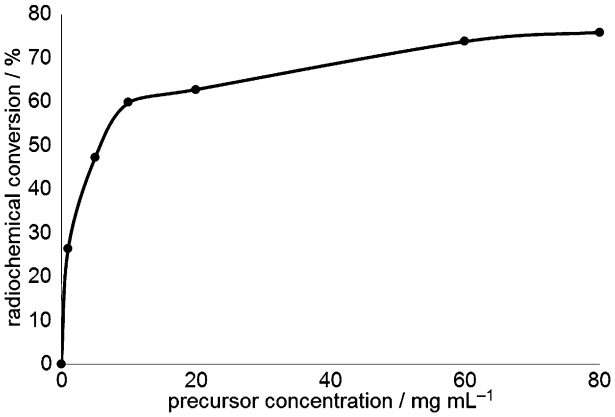

Figure 1. Correlation between Boc-activated methylaziridine-2-carboxylate concentration and radiochemical yield in ring opening with [^{18}F]fluoride (reaction conditions: $TEAHCO_3$, DMSO, 130 °C MW, 10 min).

Base concentration

Various concentrations of $TEAHCO_3$ were tested for labelling the Boc-activated aziridine-2-carboxylate using standard conditions. The volume and precursor concentration were kept constant. The correlation between base concentration in the reaction and ring-opening yield is illustrated in Figure 2. The highest radiochemical yield was achieved at a base concentration of 7.5 mM; higher base concentrations appeared to be slightly

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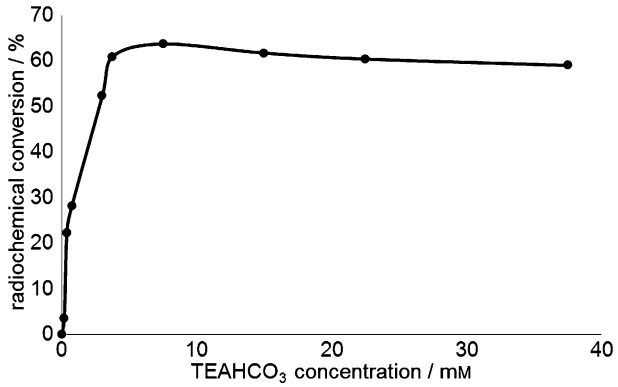

Figure 2. Correlation between $TEAHCO_3$ concentration and radiochemical yields in the ring opening of Boc-activated methylaziridine-2-carboxylate with [^{18}F]fluoride (reaction conditions: 5 mg precursor, DMSO, 130 °C MW, 10 min).

detrimental. The radiochemical yield was slightly higher than observed previously with an equivalent base concentration (Table 1, entry 6). This is explained by the use of a freshly prepared $TEAHCO_3$ solution.

Regioselectivity

Nucleophilic ring opening of aziridines can lead to two products due to reaction at both of the aziridine carbon atoms. For aziridine-2-carboxylates treated with [^{18}F]fluoride, both α-[^{18}F]fluoro-β-alanine and β-[^{18}F]fluoroalanine may be produced following ester hydrolysis and removal of N-activating groups. We succeeded in developing an HPLC analysis whereby these two compounds could be well separated (retention times of 3.3 and 5.8 min, Rs = 6), thus allowing for identification of the [^{18}F]-labelled products by co-injection of authentic reference compounds.

Ring opening with [^{18}F]fluoride was only successful for aziridine-2-carboxylates with Boc, Cbz, or tosyl as the activating group. The yields of the ring-opening reaction were similar for Boc and Cbz and highest for the more activating tosyl group. With all three aziridines, two initial radiolabelled products were observed on radio-HPLC analysis. The first eluting peak was identified as the N-activated ring-opening product after hydrolysis of the ester function. Surprisingly, the base in the labelling reaction and the high temperatures were able to hydrolyse the methyl ester. The later eluting peak was the intact N-activated ring-opening product. Unsurprisingly, the extent of ester hydrolysis during the labelling reaction was found to depend on the base concentration. No hydrolysis was observed at $TEAHCO_3$ concentrations below 0.2 mM, and only 4% ring-opening product was seen at this base concentration. To avoid ester hydrolysis, a more stable ester could be used. Previously we had prepared the N-Boc-activated aziridine with an isopropyl ester instead of the methyl, and reaction with [^{18}F]fluoride afforded intact ring-opening product (unpublished data). The yield of intact N-activated ring-opening product determined by HPLC was confirmed by TLC. Under the TLC conditions used, there was inadequate separation of the hydrolysed product from [^{18}F]fluoride to enable precise quantification of this product. Removal of the tosyl activating group was attempted by using hydrochloric acid and sulfuric acid.[26] Treatment with H_2SO_4 converted the two products into one radioactive product. The product was analysed by a second HPLC method to determine the regioselectivity, but it did not correspond to any of the two reference compounds. Due to the small masses involved in working with high specific radioactive fluorine-18, it was not possible to determine the identity of this product. Further work using the tosyl-activated aziridine was not pursued. The Boc group on the ^{18}F-ring-opened products could easily be removed by heating in aqueous HCl, and these conditions also hydrolysed the ester in the same step. The Cbz group was removed by adaptation of a published method using palladium acetate, triethylsilane, and triethylamine.[27] The small amount of triethylamine in the deprotection step was found to be sufficient to hydrolyse the methyl ester as well. This deprotection method was found to be far superior to hydrogenolysis, using hydrogen gas together with palladium over charcoal, and allowed Cbz cleavage and de-esterification in one step within 10 min, compared with 40 min for the reported hydrogenolysis approach.[8, 15]

The ring-opening product for labelling of the Boc- and Cbz-activated aziridines after ester hydrolysis and activation group removal was determined to be α-[^{18}F]fluoro-β-alanine (Figure 3). [^{18}F]Fluoride exclusively attacked the aziridine at the most substituted α-carbon, and none of the other regioisomer was detected. Interestingly, reports on ring opening of non-activated isopropylaziridine-2-carboxylate with HF/pyridine showed the complete opposite regioselectivity, with attack at the unsubstituted β-carbon atom.[28, 29] Ring opening of a 2-carboxylic ester substituted aziridinium salt by reaction with tetra-*n*-butylammonium fluoride demonstrated exclusive attack at the α-carbon atom, in agreement with our results, whilst the 2-methyl ether analogue gave only 25% attack at the α-carbon atom.[30] In contrast, a recent report using triethylamine

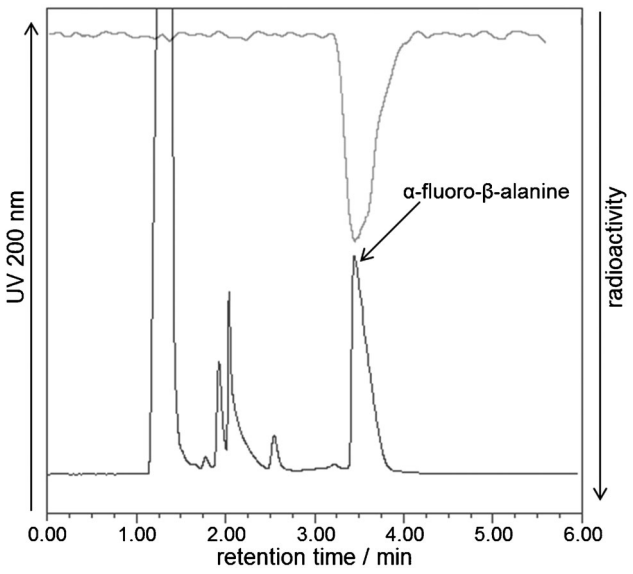

Figure 3. Analytical HPLC trace of radiolabelling product after deprotection spiked with reference α-fluoro-β-alanine. The radioactivity trace (top) is shifted to compensate for the time delay between the UV and radiodetectors.

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trihydrofluoride for ring opening of an N-deactivated ethylaziridine-2-carboxylate showed only 34% of the α-fluoro product, with the remainder due to attack at the β-carbon.[14] Van Oosten et al. reported a fluorine-18 attack on 2-methylaziridine with Cbz and benzoyl (Bz) as activating group on the nitrogen. Two regioisomers were formed for both aziridine ring opening reactions, but with opposite regioselectivity. The Cbz 2-methylaziridine was more reactive at the less hindered 2° carbon, whilst the Bz 2-methylaziridine showed opposite regioselectivity.[8,15] The authors also reported very different regioselectivity for both aziridines on fluorine-19 ring opening with HF. In this case, only attack at the methyl-substituted 3° carbon was observed. Roehn et al. tested aziridine ring opening with [^{18}F]fluoride on a model compound, a 2-carboxylic acid benzyl amide substituted aziridine. They tested Bz and three different sulfonyl activating groups. The Bz aziridine was unsuccessful, but the ring-opening yields were high with sulfonyl activating groups. The best activating group was used on three biomolecules: two peptides and a thymidine derivative. The ring opening with [^{18}F]fluoride for the peptide-based aziridines gave low yields, but the yield for the thymidine was 87%. The only product shown for the ring opening was with [^{18}F]fluoride attack at the less hindered carbon, but regioselectivity of ring opening was not discussed in the report. Furthermore, no attempts were made to remove the aziridine activating group subsequent to radiolabelling.[21] Recently an ApoSense compound, [^{18}F]2-(5-(dimethylamino)naphthalene-1-sulfonamido)-2-(fluoromethyl)butanoic acid ([^{18}F]NST732), was successfully prepared by nucleophilic ring opening of a sulfonyl-activated aziridine precursor.[22] In this case, there were two regioisomeric products formed at an approximate 60:40 ratio, with attack on the most substituted carbon atom slightly favoured. There are two earlier reports on fluorine-18 ring opening of aziridines, but in both cases radiochemical yields were low.[19,20]

Whilst there are numerous examples of ring opening of aziridines with fluoride, these predominantly use acidic conditions (e.g., HF/pyridine) or a Lewis acid (e.g., BF_3), often with non-activated aziridines, in which the reaction appears to be facilitated by initial protonation of the nitrogen to form an aziridinium ion.[7] Nucleophilic ring opening of activated aziridines is generally considered to occur via an S_N2 mechanism,[31] and there are numerous reports that support this.[32] Ring opening of a chiral aziridine-2-carboxylate with [^{11}C]cyanide was previously shown to lead to racemic products,[24] and preliminary results of ring opening of the same aziridine with [^{18}F]fluoride appeared to show the same racemisation (unpublished data). This lack of retention of stereochemistry can be rationalised by a base-catalysed racemisation of the initially formed ring-opened product under high temperature and basic reaction conditions.[24,33] For this reason, the current work was not focussed on stereoselectivity. The observed exclusive nucleophilic attack at the most substituted carbon atom in our experiments can be rationalised by the strong electron-withdrawing nature of the carboxylate group. For previously reported reactions of [^{18}F]fluoride with 2-methylaziridines,[8,15] there is no electron-withdrawing substituent on the aziridine ring; therefore a mixture of regioisomers is produced, and the regioselectivity depends more on the nitrogen-activating groups as previously noted.[34] The relatively small ionic radius of fluoride is assumed to minimise the influence of steric effects on regioselectivity. Thus, we can rationalise the observed regioselectivity of ring opening of activated aziridine-2-carboxylates with [^{18}F]fluoride and conclude that this is directed mainly by electronic effects due to the carboxylic ester substituent.

Conclusions

[^{18}F]Fluoride ring-opening reactions of five N-activated methylaziridine-2-carboxylates were studied. Fmoc activation was not suitable due to its instability under basic conditions. The nosyl group was only stable at temperatures up to 60 °C, and no ring opening occurred at this temperature. The tosyl-activated aziridine-2-carboxylate was somewhat unstable at high temperatures; nonetheless, good fluorine-18 incorporation was observed with microwave heating at 100 °C. Unfortunately, following deprotection no identifiable radioactive products were obtained. Both Boc- and Cbz-activated aziridine-2-carboxylates underwent successful regioselective ring opening with [^{18}F]fluoride in high radiochemical yield to produce α-[^{18}F]fluoro-β-alanine after ester hydrolysis and removal of the activating group. Nucleophilic attack by [^{18}F]fluoride occurred exclusively at the most substituted carbon atom.

α-Fluoro-β-alanine has long been known as a major catabolite of the chemotherapeutic drug 5-fluorouracil (5-FU), and is known to exhibit neurotoxicity in patients treated with 5-FU if its formation is not suppressed.[35] The radiosynthesis of α-[^{18}F]fluoro-β-alanine via a bromine-to-fluorine exchange reaction has been reported in two conference abstracts,[36,37] but no biological studies were reported. This radiopharmaceutical may be useful for non-invasively studying the fate of 5-FU in humans. Furthermore, fluorine-18 ring opening of aziridines may be extended to the preparation of [^{18}F]-labelled peptides.

Experimental Section

General

Methylaziridine-2-carboxylate was purchased from FluoroChem and H-β-fluoro-D,L-Ala-OH from Bachem. Other chemicals and solvents were purchased from Sigma–Aldrich. Silica gel 60 (40–63 μm) was purchased from Merck. QMA cartridges were purchased from Merck Millipore, and Chromafix PS-HCO_3 cartridges were purchased from ABX (Radeberg, Germany). ^{1}H and ^{13}C NMR spectra were acquired with a 400 MHz Bruker Avance II instrument equipped with TopSpin software. HRMS was performed using an Agilent 1200 HPLC system coupled via a T-splitter with a Bruker microTOF-Q II mass spectrometer with an electrospray ionisation interface. Mass spectra were acquired in positive mode. [^{18}F]Fluoride was produced by the ^{18}O(p,n)^{18}F reaction with proton irradiation of ^{18}O-enriched water (Taiyo Nippon Sanso Corp.) using an Eclipse™ HP Cyclotron (Siemens Healthcare). An automated Scansys module was used for drying the [^{18}F]fluoride and microwave reactions. HPLC analysis was performed on a Gilson HPLC with a Dionex UV lamp (UVD170U) and a Scansys radiodetector. Aziridine radiolabelling products were analysed on a Phenomenex Kinetex column (2.6 μm C_{18}, 50×4.6 mm) at a flow rate of 1.5 mL min^{-1}. The mobile phase

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was 0.1% TFA in MeCN/H_2O 15:85 for compounds **3** and **5**, 20:80 for compounds **4** and **6**, and 40:60 for compound **7** (HPLC method A). The reference compounds H-β-fluoro-D,L-Ala-OH and α-fluoro-β-alanine were analysed on a Phenomenex Luna column (5 μm C_{18} 100 Å, 150×4.6 mm) at a flow rate of 1 mL min^{-1}, and mobile phase consisting of 5 mM sodium decanesulfonate and 25 mM potassium dihydrogen phosphate, pH 2.6 (HPLC method B).

Synthesis

Methyl 1-tosylaziridine-2-carboxylate (3)[38]: Trifluoroacetic acid (TFA; 1.8 mL, 23.5 mmol) was added dropwise to a solution of methyl (*S*)-(−)-1-tritylaziridine-2-carboxylate (501 mg, 1.46 mmol) in $CHCl_3$ and MeOH (2.6 mL, 50:50) at 0 °C. The solution was stirred at 0 °C for 2.5 h. The solvents were removed, and the remaining solid was dried with Et_2O (5×4 mL). The residue was dissolved in Et_2O (30 mL) and H_2O (30 mL). The ether layer was extracted with H_2O (20 mL). The combined aqueous fractions were neutralised with solid $NaHCO_3$ and diluted with EtOAc (30 mL). The mixture was cooled to 0 °C and *p*-toluenesulfonyl chloride (278 mg, 1.46 mmol) was added. The solution was stirred at room temperature for 48 h. The organic and aqueous phases were separated, and the aqueous phase was extracted with EtOAc (40 mL). The organic phases were combined and concentrated. The compound was purified by flash chromatography in 25% EtOAc in heptane to give a colourless oil (253 mg, 68%). R_f = 0.61 (heptane/EtOAc 1:1); HPLC t_R: 7.4 min (method A); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.86 (d, 2H, J = 8.1 Hz), 7.37 (d, 2H, J = 8.1 Hz), 3.75 (s, 3H), 3.36 (dd, 1H, J = 4.1, 7.1 Hz), 2.78 (d, 1H, J = 7.1 Hz), 2.58 (d, 1H, J = 4.1 Hz), 2.47 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.2, 145.3, 134.0, 129.9, 128.2, 52.9, 35.7, 31.9, 21.7 ppm; HRMS (ESI): *m/z* calcd for $C_{11}H_{13}NO_4S + H^+$: 256.0644 [$M + H^+$]; found 256.0633.

Methyl 1-((4-nitrophenyl)sulfonyl)aziridine-2-carboxylate (4): TFA (1.8 mL, 23.5 mmol) was added dropwise to a solution of methyl (*S*)-(−)-1-tritylaziridine-2-carboxylate (502 mg, 1.46 mmol) in $CHCl_3$ and MeOH (2.6 mL, 50:50) at 0 °C. The solution was stirred at 0 °C for 2.5 h. The solvents were removed, and the remaining solid was dried with Et_2O (5×4 mL). The residue was dissolved in Et_2O (30 mL) and H_2O (30 mL). The ether layer was extracted with H_2O (20 mL). The combined aqueous fractions were neutralised with solid $NaHCO_3$ and diluted with EtOAc (30 mL). The mixture was cooled to 0 °C, and *p*-nitrobenzenesulfonyl chloride (324 mg, 1.46 mmol) was added. The solution was stirred at room temperature overnight. The organic and aqueous phases were separated, and the aqueous phase was extracted with EtOAc (40 mL). The organic phases were combined and concentrated. The compound was purified by flash chromatography in heptane/EtOAc (2:1) to give a yellow solid (332 mg, 79%). R_f = 0.32 (heptane/EtOAc 2:1); HPLC t_R: 8.4 min (method A); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.43 (d, 2H, J = 8.7 Hz), 8.21 (d, 2H, J = 8.7 Hz), 3.78 (s, 3H), 3.48 (dd, 1H, J = 4.2, 7.1 Hz), 2.91 (d, 1H, J = 7.1 Hz), 2.68 ppm (d, 1H, J = 4.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.6, 151.0, 143.0, 129.5, 124.5, 53.1, 36.2, 32.5 ppm; HRMS (ESI): *m/z* calcd for $C_{10}H_{10}N_2O_6S + H^+$: 287.0338 [$M + H^+$]; found 287.0330.

1-(*tert*-Butyl) 2-methylaziridine-1,2-dicarboxylate (5): Di-*tert*-butyl dicarbonate (475 mg, 2.18 mmol) was added to a solution of methylaziridine-2-carboxylate (200 mg, 1.98 mmol) in MeCN (5 mL) and Et_3N (0.28 mL). The solution was stirred at room temperature overnight. The mixture was concentrated, and the residue was dissolved in EtOAc (40 mL) and H_2O (20 mL). The layers were separated, and the aqueous layer was extracted with EtOAc (3×40 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated. The compound was purified by flash chromatography in 10 to 20% EtOAc in heptane to give a colourless oil (321 mg, 81%). R_f = 0.32 (heptane/EtOAc 3:1); HPLC t_R: 8.7 min (method A); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.79 (s, 3H), 3.05 (dd, 1H, J = 3.2, 5.4 Hz), 2.54 (dd, 1H, J = 1.4, 3.2 Hz), 2.43 (dd, 1H, J = 1.4, 5.4 Hz), 1.47 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.8, 159.5, 82.0, 52.5, 34.8, 31.2, 27.8 ppm; HRMS (ESI): *m/z* calcd for $C_9H_{15}NO_4 + Na^+$: 224.0899 [$M + Na^+$]; found 224.0893.

1-Benzyl 2-methylaziridine-1,2-dicarboxylate (6): Benzyl chloroformate (778 mg, 4.56 mmol) was added to a solution of methylaziridine-2-carboxylate (419 mg, 4.14 mmol) in MeCN (10 mL) and Et_3N (0.6 mL). The solution was stirred at room temperature overnight and concentrated. The residue was dissolved in CH_2Cl_2, washed with $NaHCO_3$, dried over $MgSO_4$ and concentrated. The compound was purified by flash chromatography in 10 to 20% EtOAc in heptane to give a colourless oil (639 mg, 66%). R_f = 0.19 (heptane/EtOAc 3:1); HPLC t_R: 12.7 min (method A); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.32–7.42 (m, 5H), 5.17 (s, 2H), 3.74 (s, 3H), 3.13 (dd, 1H, J = 3.2, 5.4 Hz), 2.63 (dd, 1H, J = 1.3, 3.2 Hz), 2.51 ppm (dd, 1H, J = 1.3, 5.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.6, 160.7, 141.1, 128.4, 68.6, 52.6, 34.8, 31.3 ppm; HRMS (ESI): *m/z* calcd for $C_{12}H_{13}NO_4 + H^+$: 236.0923 [$M + H^+$]; found 236.0912.

1-((9*H*-Fluoren-9-yl)methyl) 2-methylaziridine-1,2-dicarboxylate (7): 9-Fluorenylmethyl *N*-succinimidylcarbonate (1.51 g, 4.94 mmol) was added to a solution of methylaziridine-2-carboxylate (454 mg, 4.49 mmol) in THF (6 mL), H_2O (2 mL), and Et_3N. The solution was stirred at room temperature for 2 h at pH 8–9. The mixture was concentrated, and the residue was diluted with 10% citric acid and extracted with EtOAc. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated. The compound was purified by flash chromatography in 5 to 20% EtOAc in heptane to give a colourless oil (978 mg, 67%). R_f = 0.27 (heptane/EtOAc 3:1); HPLC t_R: 7.3 min (method A); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.79 (d, 2H, J = 7.5 Hz), 7.63 (d, 2H, J = 7.5 Hz), 7.43 (t, 2H, J = 7.5 Hz), 7.35 (t, 2H, J = 7.5 Hz), 4.51 (dd, 1H, J = 7.2, 10.5 Hz), 4.39 (dd, 1H, J = 7.2, 10.5 Hz), 4.27 (t, 1H, J = 7.2 Hz), 3.77 (s, 3H), 3.13 (dd, 1H, J = 3.2, 5.4 Hz), 2.64 (dd, 1H, J = 1.2, 3.2 Hz), 2.49 ppm (dd, 1H, J = 1.2, 5.4 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 168.7, 160.8, 143.5, 141.3, 127.8, 127.1, 125.1, 120.0, 68.7, 52.8, 46.9, 34.8, 31.2 ppm; HRMS (ESI): *m/z* calcd for $C_{19}H_{17}NO_4 + H^+$: 324.1236 [$M + H^+$]; found 324.1227.

Radiochemistry

[^{18}F] Fluorination with K222/K_2CO_3 and TEAHCO$_3$: Aqueous [^{18}F]fluoride was trapped on a Waters QMA column and eluted with either a K222/K_2CO_3 solution (1 mL; 35 mM K222 and 36 mM K_2CO_3, 6% H_2O in MeOH) or a $TEAHCO_3$ solution (1 mL; 75 mM, H_2O/MeOH 1:4). The solvent was removed by helium flow at 110 °C for 10 min, and excess H_2O was removed by azeotropic evaporation with MeCN (2×1 mL) at 110 °C. The dried [^{18}F]fluoride was dissolved in either DMF or DMSO (2 mL).

[^{18}F] Fluorination with Chromafix and HCO$_3^-$: Aqueous [^{18}F]fluoride was trapped on a Chromafix (PS-HCO_3) column. The column was washed with MeOH (2 mL) and the radioactivity eluted with TBAOMs in MeOH (0.05 M, 0.6 mL). The solvent was removed at 100 °C for 10 min with helium flow. The dried [^{18}F]fluoride was dissolved in DMSO (2 mL).

[^{18}F] Fluorination with Chromafix and $K_2PO_4^-$: A Chromafix (PS-HCO_3) column was washed with 0.2 M K_3PO_4 (5 mL) and dried with air. Aqueous [^{18}F]fluoride was trapped on the column, and the

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column was washed with MeOH (2 mL) and the radioactivity eluted with TBAOMs in MeOH (0.05 M, 0.6 mL). The solvent was removed at 100 °C for 10 min with helium flow. The dried [^{18}F]fluoride was dissolved in DMSO (2 mL).

General procedure for aziridine labelling with [^{18}F]fluoride: [^{18}F]Fluoride in DMSO (0.1–0.2 mL, 10–550 MBq) was added to the N-activated aziridine solution (5–8 mg) in DMSO. The solution was heated by either conventional heat or microwave heating. Microwave reactions were performed at 300 W in a closed vial. The yield of the reaction was determined by HPLC method A.

[^{18}F]Fluoride labelling with various precursor concentrations: [^{18}F]Fluoride in $TEAHCO_3$ and DMSO (0.1 mL, 170–460 MBq) was added to compound **5** (0.3–29.4 mg) in DMSO. The reaction was heated at 130 °C by microwave for 10 min. The yield of the reaction was determined by HPLC method A.

[^{18}F]Fluoride labelling with various base concentrations: [^{18}F]Fluoride in $TEAHCO_3$ and DMSO (2.5–500 μL, 40–1100 MBq) was added to compound **5** (5 mg) in DMSO. The volume of the reaction was kept constant, and the reaction was heated at 130 °C by microwave for 10 min. The yield of the reaction was determined by HPLC method A.

Synthesis of α-[^{18}F]fluoro-β-alanine (1)

From [^{18}F]-labelled compound 5: The two products formed in the [^{18}F]fluoride labelling of compound **5** were separated from the free [^{18}F]fluoride by collecting the peaks from HPLC. The Boc group and methyl ester were removed by addition of 1 M HCl and heating at 80 °C for 15 min to yield α-[^{18}F]fluoro-β-alanine with a radiochemical purity of >99 %. The product was analysed by HPLC method B and identified by co-injection of reference α-fluoro-β-alanine. Due to the lack of a good UV chromophore, the specific radioactivity of the product could not be determined, at it fell below the limit of detection of the UV detector.

From [^{18}F]-labelled compound 6: The two products formed in the [^{18}F]fluoride labelling of compound **6** were separated from free [^{18}F]fluoride by collecting the peaks from HPLC. The Cbz group and methyl ester were removed by addition of $Pd(OAc)_2$ (10 mg), Et_3SiH (0.1 mL), and Et_3N (10 μL) followed by heating at 80 °C for 10 min to yield α-[^{18}F]fluoro-β-alanine with a radiochemical purity of >99 %. The product was analysed by HPLC method B.

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