ChemComm

Cite this: *Chem. Commun.*, 2011, **47**, 11465–11467

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COMMUNICATION

Sultone opening with [^{18}F]fluoride: an efficient ^{18}F-labelling strategy for PET imaging†

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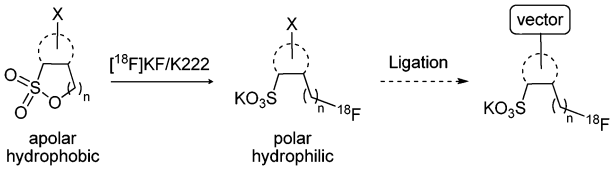
Received 21st July 2011, Accepted 13th September 2011
DOI: 10.1039/c1cc14435a

Sultones were subject to ring opening by nucleophilic attack with [^{18}F]fluoride to afford easily purified ^{18}F-labelled hydrophilic sulfonated products in high yields. A two-step sequence including radiofluorination and coupling to lysine was then developed from a bis-sultone precursor as a model approach for the labelling of biopolymers.

With its suitable decay properties [97% β^+, E_{max} (β^+) = 635 keV, $t_{1/2}$ = 109.7 min], fluorine-18 represents the ideal radionuclide for positron emission tomography (PET), a sensitive and powerful technique for molecular imaging in animal and human subjects *in vivo*.[1] However, incorporation of fluorine-18 into biopolymers (*i.e.* peptides, proteins, oligonucleotides or antibodies) that are becoming more widely used as *in vivo* imaging agents remains problematic.[2] Despite recent advances in a direct method by formation of ^{18}F–Si or ^{18}F–B bonds,[3] current labelling strategies involve multistep approaches that include introduction of readily prepared [^{18}F]F$^-$ into small organic prosthetic groups, their activation and subsequent coupling to specific functional groups within the macromolecule. Every method has limitations in terms of the number of reaction steps involved, overall reaction time, labelling yield of the prosthetic group used, efficiency of the subsequent conjugation to the biomolecule, chemoselectivity of the conjugation step, complexity for automation, easiness to purify the final radiolabelled product, specific activity and *in vivo* stability of the radiotracer and also the influence of the prosthetic group on ligand pharmacokinetics. Although a limited number of chemical reactions have been utilized to incorporate the prosthetic groups into biopolymers [mainly acylation, alkylation, oxime or hydrazine formation, and Huisgen 1,3-dipolar cycloaddition (click chemistry)], a variety of ^{18}F-labelled prosthetic groups have been reported. The latter usually are of lipophilic nature that has been proved to lead to increased hepatobiliary excretion.[4] To our knowledge, ^{18}F-PEGylation[5] and glycosylation[6] were the two main strategies developed so far to improve the biokinetics of labelled peptides. As an alternative, hydrophilic prosthetic groups were considered highly desirable.



Scheme 1 Labelling concept using the sultone opening with an [^{18}F]fluoride anion.

In recognition of the issues discussed above we set out to develop a platform technology based on the sultone opening with an [^{18}F]fluoride anion which would allow the production of water soluble sulfonated ^{18}F-labelled compounds (Scheme 1). Sultones are known to be prone to ring opening by nucleophilic attack.[7] Surprisingly, no example with fluoride has been reported so far. Access to ^{18}F-labelled biopolymers by our chosen method would be achieved starting from appropriately functionalized sultones according to a two-step procedure including radiofluorination then ligation to the target vector. We hypothesized that the apolar sultone/polar sulfonic derivatives conversion would be advantageous in terms of the purification of the radiofluorinated sulfonic acid derivatives, whilst facilitating automation of the process. Indeed, a simple reverse phase SPE would permit to recover the sulfonated product whereas any sultone precursor would be retained on the solid support, thus avoiding the presence of the excess reactive precursor in the conjugation step.

Initial efforts were focused on studying radiofluorination of simple model propane and butane sultones **1–6**. The non-radioactive (fluorine-19) fluorosulfonates **7–12** used as reference compounds for the corresponding labelled products were obtained quantitatively by reaction of the sultones **1–6** in acetonitrile at 110 °C for 3 h with either TBAF or KF in the presence of an equimolar amount of the Kryptofix2.2.2® (K222) cryptand. Under these conditions, no difference of reactivity between the sultones **1–6** was observed. Radiofluorination reactions were carried out in acetonitrile using azeotropically "dried" [^{18}F]fluoride in the form of its K$^+$/Kryptofix2.2.2®/^{18}F$^-$ complex in the presence of K_2CO_3. Conversion of sultones **1–6** to the corresponding [^{18}F]fluorosulfonates [^{18}F]**7**–[^{18}F]**12** was found to be strongly dependent on the reaction conditions and on the starting sultones.

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† Electronic supplementary information (ESI) available: Experimental procedures and characterization data. See DOI: 10.1039/c1cc14435a

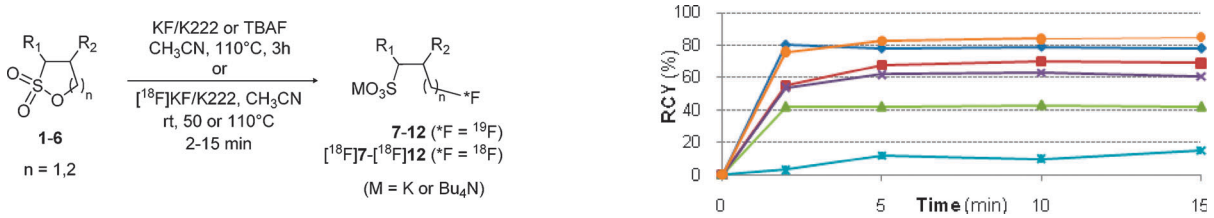


Fig. 1 Opening of sultones **1–6** with F^- or $^{18}F^-$ (scheme) and results for the synthesis of [^{18}F]fluorosulfonates [^{18}F]**7** (◆), [^{18}F]**8** (■), [^{18}F]**9** (▲), [^{18}F]**10** (×), [^{18}F]**11** (✱) and [^{18}F]**12** (●) at 110 °C (graph).

Results obtained for radiofluorinations at 110 °C showed that the highest radiochemical yields (RCYs > 80%) were obtained using the benzyl propane sultone **1** and the benzosultone **6** (Fig. 1). Benzyl and cyanobenzyl butane sultones **2** and **4** were slightly less reactive (60–65% RCYs). The RCY from cyanobenzyl propane sultone **3** was only moderate (45%), whereas it did not exceed 15% from the benzoyl propane sultone **5**. It is noteworthy that in all assays, the [^{18}F]fluorosulfonates [^{18}F]**7**–[^{18}F]**12** were the only radioactive products recovered besides the unreacted [^{18}F]fluoride. Moreover, the optimum RCYs were reached at 5 min, and did not move after a prolonged reaction time.

In order to find milder conditions, we examined the radiofluorination of sultones **1–6** at rt and 50 °C (Table 1). The optimum RCYs were obtained at rt for 5 min for the sultone **6** (entry 6), and at 50 °C for 2 min for both the sultones **1** (entry 1) and **3** (entry 3). The RCYs dramatically decreased for sultones **2**, **4** and **5** (entries 2, 4 and 5). In regard to the overall results, a significant or larger superiority in ring opening with [^{18}F]fluoride for benzyl sultones over benzoyl sultones, for benzyl sultones over cyanobenzyl sultones, and finally in general for propane sultones over butane sultones (as previously demonstrated with nucleophiles other than fluorides[7]) could be concluded. Although highly reactive, the benzosultone **6** may be of minor interest as the resulting sulfonate [^{18}F]**12** displays the fluorine atom at the poorly stable benzylic position.[8]

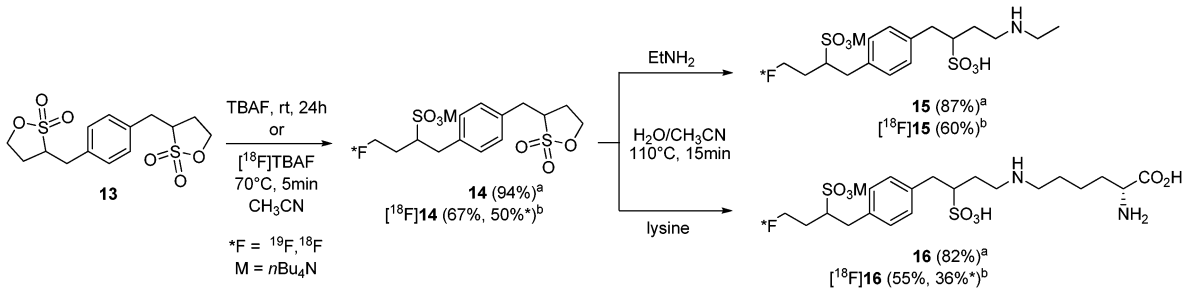
The facility for purification was checked with the conversion of the sultone **1** to the [^{18}F]fluorosulfonate [^{18}F]**7** taken as the representative example. After radiofluorination of the sultone **1** (5 mg) for 5 min at 50 °C in acetonitrile (500 µL), the reaction mixture was subject to purification by SPE using a Sep-pak C-18 cartridge. The overall radioactive fraction collected (1 mL) containing both [^{18}F]fluorosulfonate [^{18}F]**7** and unreacted [^{18}F]fluoride was injected into HPLC. The UV trace displayed the presence of sultone **1** in an amount less than 10 µg (< 50 nmol), lower than those of the final fluorosulfonate product **7** (92 µg; 0.4 µmol).

The next step was to validate the sultone opening with [^{18}F]fluoride for biopolymer labelling. The starting sultone was supposed to bear a functionality appropriate for ligation with a biopolymer and compatible with the radiofluorination. Having in hands the cyano[^{18}F]fluorosulfonate [^{18}F]**9**, we first thought to develop hydrolysis of the cyano group into carboxylic acid for further ligation to the biopolymer by acylation with a free amino group. However, attempts to perform hydrolysis of the non-radioactive cyanofluorosulfonate **9** under basic[9]

Table 1 Selected reaction conditions for the radiofluorination of sultones **1–6** to [^{18}F]fluorosulfonates [^{18}F]**7**–[^{18}F]**12**[a]

Entry	Sultone	[^{18}F]-Fluorosulfonate	T/°C	Time/min	RCY[b] (%)
1	**1**	[^{18}F]**7**	110	2	80
			50	2	80
			rt	10	83
2	**2**	[^{18}F]**8**	110	5	65
			50	15	32
3	**3**	[^{18}F]**9**	110	2	45
			50	2	42
4	**4**	[^{18}F]**10**	110	5	60
			50	15	20
5	**5**	[^{18}F]**11**	110	15	15
6	**6**	[^{18}F]**12**	110	5	82
			50	5	83
			rt	5	85

[a] ^{18}F-Labelling was carried out from 4–6 mg of sultones **1–6** in 500 µL of CH_3CN. [b] Radiochemical yield (RCY) of [^{18}F]fluorosulfonates [^{18}F]**7**–[^{18}F]**12**, calculated after HPLC isolation, decay corrected. Mean value from 4–7 runs (range: ±5%).



Scheme 2 Opening of the bis-sultone **13** with F^- or $^{18}F^-$ then with amine ([a] conversion rate determined by ^1H NMR; [b] RCY calculated from [^{18}F]TBAF, determined after HPLC separation, and decay corrected, mean value from 5 runs, range: ±2%, *—SPE included).

or acidic[10] conditions failed. Defluorination was observed in all cases (data not shown). Based on the results obtained with benzylsultone **1**, we then designed the bis-sultone **13** as an attractive labelling precursor (Scheme 2). The strategy for biopolymer labelling starting from the bis-sultone **13** would be the consecutive ring opening of the two sultone moieties first with [^{18}F]fluoride to lead to the intermediate [^{18}F]**14**, then with amine without the need for any additional reagents. In the non-radioactive synthesis, the fluorination of the bis-sultone **13** to the fluorosulfonate **14** was performed with TBAF in acetonitrile at rt for 24 h (94% conversion). Amination with ethylamine and lysine chosen as model amine compounds was achieved in an acetonitrile/water mixture at rt for 24 h to yield the disulfonates **15** and **16** (87 and 82% conversion from **13** respectively). Radiofluorination of the bis-sultone **13** was carried out in acetonitrile using azeotropically "dried" [^{18}F]TBAF in the presence of nBu$_4$NHCO$_3$. The highest RCY of [^{18}F]fluorosulfonate **14** (67% before SPE, 50% after SPE) was obtained at 70 °C for 5 min. Amination of the [^{18}F]fluorosulfonate **14** was performed in a water/acetonitrile mixture and was nearly quantitative when achieved at 110 °C for 15 min. According to a one-pot procedure (no SPE of [^{18}F]**14**), final disulfonates [^{18}F]**15** and [^{18}F]**16** were obtained in 60 and 55% RCYs, respectively, from [^{18}F]TBAF. The total synthesis of [^{18}F]**16** including SPE for both [^{18}F]**14** and [^{18}F]**16** was carried out in 36% RCY from [^{18}F]TBAF. No traces of the starting bis-sultone **13** were detected by HPLC analysis (UV trace) of [^{18}F]**16**.

In summary, we have developed a new efficient and clean radiofluorination method based on the sultone opening with [^{18}F]fluoride. This method has led for the first time to water soluble [^{18}F]fluoro sulfonates which can be easily purified by simple SPE. The methodology has been successfully extended to a two-step sequence including radiofluorination then coupling to lysine starting from a bis-sultone. This sequence was achieved with high radiochemical yields without any additional reagents. In this context, we believe that this procedure has a strong potential to label macromolecular substrates with fluorine-18. The overall work enlarged the scope of the nucleophilic radiofluorination approach and opened new perspectives in targeted PET imaging using biopolymers. Further developments in both radiochemistry and radiotracer development are underway in the laboratory.

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