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PAPER

Synthesis and reactivity of a bis-sultone cross-linker for peptide conjugation and [^{18}F]-radiolabelling *via* unusual "double click" approach†

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A novel homobifunctional cross-linker based on a bis-sultone benzenic scaffold was synthesised. The potential utility of this bioconjugation reagent was demonstrated through the preparation of an original prosthetic group suitable for the [^{18}F]-labelling of peptides. The labelling strategy is based on the nucleophilic fluorination *via* the ring-opening of a first sultone moiety followed by the nucleophilic ring-opening of the second remanent sultone by a reactive amine of the biopolymer. Beyond the one-step radiolabelling of the peptide, the second main advantage of this strategy is the release of free sulfonic acid moieties making the separation of the targeted [^{18}F]-tagged sulfonated compound from its non-sulfonated precursor easier and thus faster. This first report of the successful use of a bis-sultone moiety as a versatile bioconjugatable group was demonstrated through a comprehensive reactivity study involving various nucleophiles, especially those commonly found in biopolymers. An illustrative example, highlighting the potential of this unusual and promising "double click" conjugation approach, was devoted to the radiolabelling of a biological relevant peptide.

Introduction

In the context of positron emission tomography (PET) imaging, it is generally accepted that fluorine-18 (^{18}F) is one of the widely used radioisotopes because it possesses unique physical and nuclear characteristics (*i.e.*, ease of formation, half-life of 109.7 min, high resolution, and relative minor radiation dose to patients) suitable for challenging biomedical diagnostic applications frequently involving peptide-based probes.[1,2,3] Two main conjugation strategies are theoretically possible for the radiolabelling of complex and fragile biomolecules/biopolymers (*e.g.*, peptides or proteins) with ^{18}F.

The first one is based on the direct no-carrier-added [^{18}F]-labelling. Indeed, despite the publication of a review claiming that the direct nucleophilic fluorination of biopolymers is impossible due to the presence of numerous H-acidic functions in such biomacromolecules,[4] there have been recently several attempts to implement this strategy to peptides.[5] Thus, some studies have shown that the direct [^{18}F]-labelling of peptides is feasible but requires the prior incorporation of a suitable precursor within the peptidyl scaffold, which must be highly reactive toward fluoride-18 anion through an S_N2 or S_NAr reaction, especially to avoid side-reactions leading to the decomposition of biopolymer. The second one, which is the most commonly used because of the limitations and drawbacks of the direct labelling approach (*vide supra*), is based on the use of pre-designed prosthetic groups. Thus, a large number of prosthetic groups allowing the mild and site-specific introduction of ^{18}F into bioactive molecules have been developed.[4] However, only a few of them have emerged as universal [^{18}F]-labelling reagents because specifications required for PET imaging applications are numerous and highly restrictive: 1) a limited number of reaction steps for its [^{18}F]-labelling and its subsequent introduction onto the biomolecule/biopolymer, 2) short overall reaction time, 3) high [^{18}F]-labelling yield, 4) high efficiency of the subsequent conjugation to the targeted biomolecule/biopolymer, 5) good chemoselectivity of the conjugation step, 6) ease of purification of the intermediates and final products from the precursors, and 7) no or limited influence of the introduced prosthetic group on ligand pharmacokinetics. Most of the prosthetic groups are designed to selectively react with a specific functional group of targeted peptide/protein. Thus, they are often classified according to the type of reaction leading to their covalent coupling to the biomolecular target. The most frequently used reactions are [^{18}F]-fluoroacylation, [^{18}F]-fluoroalkylation, [^{18}F]-fluoroamidation, thiol-alkylation (through Michael addition or S_N2 reaction)[6] or photochemical conjugation.[3,4] Theoretically, for an extensive and optimal use of the radioisotope, the key [^{18}F]-labelling step should occur at a late stage of the synthesis. Despite this general rule, most of the reported prosthetic groups involve

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one or more steps after the [^{18}F]-labelling for their subsequent covalent attachment to the targeted peptide/protein. For instance, this is particularly the case with the very popular amine-reactive [^{18}F]-labelling agent *N*-succinimidyl-4-[^{18}F]-fluorobenzoate ([^{18}F]-SFB).[7] Indeed, after the [^{18}F]-fluorination step, two steps are required to get the activated ester which is able to react with the selected peptide. Thus, current efforts of radiochemists are now devoted to the dramatic reduction in the number of steps between the [^{18}F]-labelling step and the final isolation of the radiolabelled probe. One-step [^{18}F]-labelling protocol and full-automation of the process remain two main challenges that would speed up the development of valuable PET-imaging probes.

Recently, "click chemistry" has emerged as a valuable and promising tool to reach such challenging goals.[8] All too frequently, the practical implementation of this approach is only focused on the use of the very popular azide–alkyne Huisgen cycloaddition[9] and more recently Diels–Alder type reactions (*e.g.*, tetrazine-*trans*-cyclooctene ligation)[10] and the Staudinger ligation.[11] However, "click chemistry" refers to a series of reactions that obey certain criteria, such as being modular, stereospecific, high-yielding and involving simple experimental procedures. They should also make use of readily available starting materials, environmentally friendly conditions (water as (co)solvent, or solvent-free conditions), and should avoid chromatographic isolations.[12] Usually, reactions included in such a group have a large thermodynamic driving force and involve the formation of a carbon–heteroatom bond. Thus, nucleophilic opening reactions of strained rings (*e.g.*, aziridines and epoxides) are often considered as belonging to the "click chemistry" repertoire.[13] Such reactions have been already used for [^{18}F]-labelling of biomolecules through fluoride-induced ring-opening of cyclic sulfamates and 2,3′-anhydronucleosides.[14] These pioneering works led us to consider the radiolabelling and bioconjugation ability of sultones (*i.e.*, internal esters of hydroxyl sulfonic acids), especially those with 5-membered rings (*e.g.*, 1,3-propanesultone).

In this article, we report the synthesis and reactivity studies of a bis-sultone cross-linking reagent which can be easily converted into an original [^{18}F]-prosthetic group meeting most of the above-cited criteria required for convenient and efficient radiolabelling of biopolymers.[15] Interestingly, the reactivity of the 1,3-propanesultone moiety toward various nucleophiles (*i.e.*, amines, alcohols, oxyamines, halides and thiols) under mild conditions constitutes the cornerstone of this novel strategy of labelling. Indeed, the mono-fluorinated labelling reagent can be directly obtained through the ^{18}F anion-mediated nucleophilic ring-opening of the first sultone moiety of this homobifunctional cross-linker, and does not require further activation before its final coupling with the targeted amine-containing bio-compound (amino acids and related peptides) through nucleophilic ring-opening of the remaining sultone (Fig. 1).

Results and discussion

Synthesis and bioconjugation ability of model [^{18}F]-reactive 1,3-propanesultones

Before starting the synthesis of the unusual bis-sultone cross-linker **3** theoretically suitable for radiolabelling of biologically active peptides and proteins by way of two sequential nucleophilic ring-opening reactions, we decided to first evaluate the reactivity of cognate compounds bearing a single 1,3-propanesultone moiety towards various nucleophiles (especially those found within biopolymers) and under mild conditions fully compatible with the stability of most (bio)molecules and biopolymers. Thus, the synthesis of 1-phenylacyl-1,3-propanesultone **1** was first considered (Scheme 1). This compound was readily obtained through

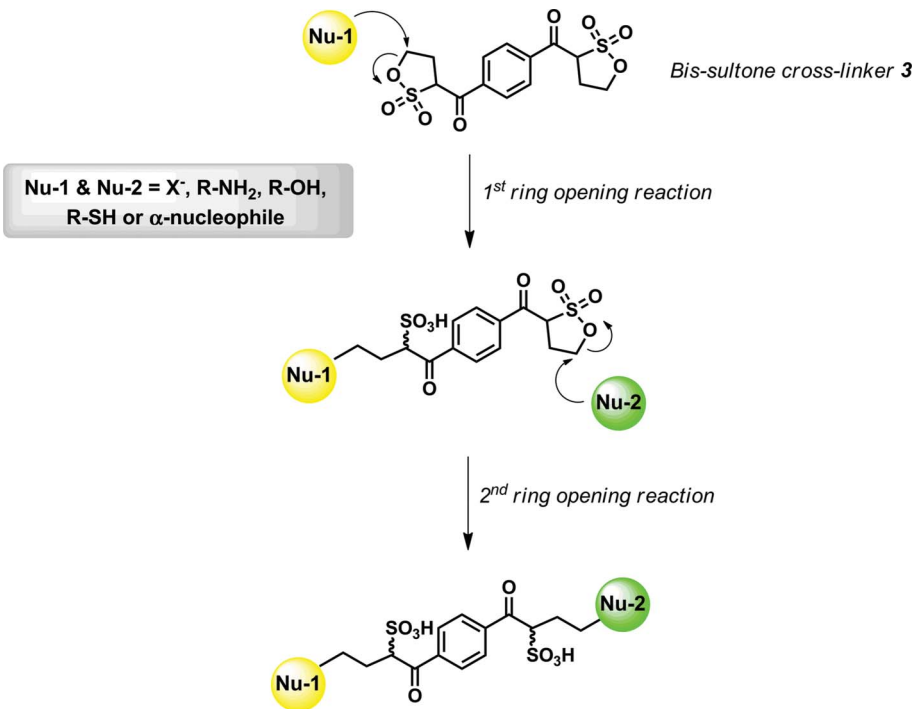
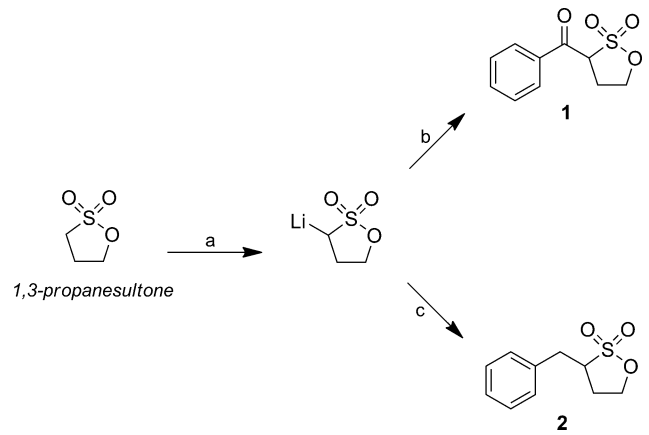


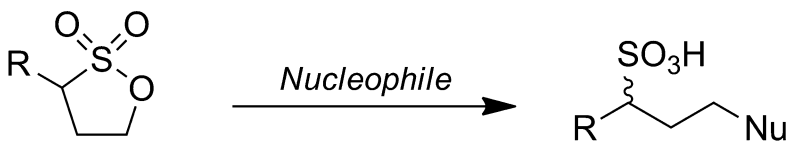
Fig. 1 Claimed reactivity of bis-sultone cross-linker **3** for bioconjugation applications.



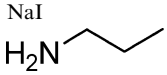
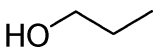
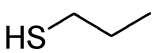
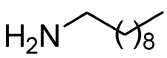
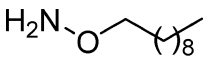
Scheme 1 *Reagents and conditions*: (a) *n*-BuLi, THF −78 °C, 1 h; (b) methyl benzoate, THF, −78 °C, 150 min then acetic acid, THF, −78 °C to rt, (60%); (c) benzyl bromide, THF, −78 °C, 150 min then acetic acid, THF, −78 °C to rt, (15%).

metalation of 1,3-propanesultone and its subsequent acylation with methyl benzoate. Thereafter, this compound was subjected to reaction with various nucleophiles: several potassium halides in the presence of Kryptofix[K222] at room temperature (Table 1). Among inorganic halides, KCl was found to react the fastest, followed by KBr, KI and finally KF (Table 1, entries 1–4). These results are in good agreement with those from previous studies related to reactions of γ and δ sultones with nucleophiles.[16a] Since the ability of tertiary amines to readily react with either 1,3-propanesultone or 1,4-butanesultone has been also reported by Ward *et al.*,[16b] it is important to check the lack of reactivity of **1** toward Kryptofix[K222] alone. Indeed, this latter cryptand possesses two tertiary amine moieties which may compete with the halide anions and thus lead to a wrong interpretation of the reactivity scale. Fortunately, we observed the formation of the non-desired quaternary alkylammonium sulfobetaine only after a prolonged reaction time (*ca.* 40% after 20 h), and the rate of its formation has dramatically dropped down to less than 5% in the presence of KI, KBr or KCl, and to 10% for the competitive reaction with KF. To suppress this side-reaction, the nucleophilic ring-opening of the sultone **1** with sodium halides without using cryptand was also investigated (Table 1, entries 5–8). Interestingly, the fastest ring-opening reaction of the sultone moiety was observed with NaI (entry 8). This latter reaction was found to be instantaneous and quantitative, and a gradual decrease in reactivity was observed from NaI to NaCl, with NaF no reaction was observed. To complete our comparative reactivity study in the halide series, we finally try to improve the fluorination rate of **1** by using alternative fluoride ion sources such as

Table 1 Reactivity of model [^{18}F]-reactive 1,3-propanesultones **1** and **2** toward various nucleophiles[a]



1, R = PhC(O)
2, R = Bn

Entry	Nucleophile	Conversion rate (HPLC) for **1** (%)	Conversion rate (HPLC) for **2** (%)
1[b]	KF	5	0
2[b]	KCl	65.5	27.5
3[b]	KBr	50.5	17
4[b]	KI	22.5	14.5
5[c]	NaF	0	—
6[c]	NaCl	1.5	—
7[c]	NaBr	25	—
8[d]	NaI	100	—
9[d]		18	5.5
10[d]		0	—
11[d]		0	—
12[d]		76	—
13[d]		6	—

[a] All reactions were conducted at rt with 1 equiv. of sultone and nucleophile; HPLC analysis was performed after 45 min of stirring except for entries 12 and 13 (6 h). [b] Ring-opening reaction in the presence of 1 equiv. of Kryptofix[K222] in CH_3CN. [c] Ring-opening reaction in CH_3CN–water (10 : 1, v/v). [d] Ring-opening reaction in CH_3CN.

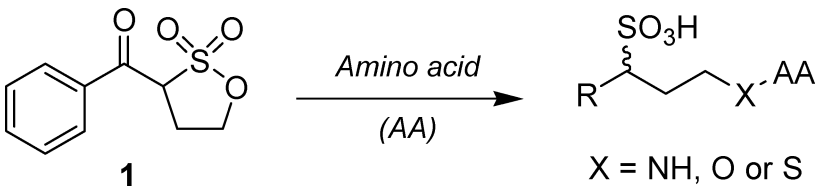
tetra-*n*-butylammonium fluoride (TBAF) and CsF. Reactions involving these two species were found to be slightly slower than the reaction using the standard "naked" fluoride (KF/Kryptofix[K222]) but no by-products derived from cryptand were observed. Finally, the optimal fluorination conditions selected for **1** were: 1 equiv. of KF, 1.2 equiv. of Kryptofix[K222] in a mixture of CH_3CN and water (20 : 1, v/v) and gave the monofluorosulfonic acid in a 38% isolated yield. Iodination of the sultone **1** using NaI in dry CH_3CN afforded the desired coupling product in a quantitative yield and was recovered by simple filtration. Bromination and chlorination of **1** were best performed using the potassium salt (KBr or KCl) and Kryptofix[K222] in dry CH_3CN (36% and 77% isolated yield after 6 h and 2 h 30 min reaction time respectively). The moderate yield for the bromo derivative is explained by the fact that a part of product was not recovered because it was not adequately resolved from Kryptofix[K222] through the RP-HPLC purification. The structure of the resulting halogeno-sulfonic acids were confirmed by detailed measurements including ESI mass spectrometry and NMR analyses, and their high purity was confirmed by HPLC analyses (see ESI†).

To get further insights on the reactivity of sultone **1**, especially toward representative nucleophiles found in biopolymers (*i.e.*, amino, hydroxyl and sulfhydryl groups), nucleophilic ring-opening reactions were also conducted with propylamine, propanol, propanethiol (Table 1, entries 9–11) and various L-amino acids (*i.e.*, cysteine ethyl ester (H-Cys-OEt), Boc-Lys-NH_2, tyrosine ethyl ester (H-Tyr-OEt) and valine methyl ester (H-Val-OMe), see Table 2). Such reaction was found to occur rapidly with propylamine (22% isolated yield) whereas no coupling products were observed with propanol and propanethiol under the mild experimental conditions we used (*i.e.*, rt and without any base). This point is of great interest, since it opens an avenue for selective labelling of peptides through their amine function at the *N*-terminal end or a lysine residue. This valuable chemical behavior was also confirmed by reactions involving amino acids. Indeed, free α- or ε-amino acids such as H-Val-OMe and Boc-Lys-NH_2 were readily reacted with **1** (Table 2, entries 2 and 4). The superior nucleophilicity of the ε-NH_2 group was illustrated by the fact that the reaction with Boc-Lys-NH_2 was not univocal with the significant formation of bis-alkylated lysine derivative resulting from the competitive reaction with a second equiv. of sultone **1**. For the ambident nucleophile H-Cys-OEt, two products were isolated, identified as the *N*- and the *S*-alkylated in 12% and 28% isolated yield, respectively (Table 2, entry 1). The superior reactivity of –SH group was explained by the use of K_2CO_3, an additive essential to solubilise and release the amino group of commercially available amino acid chlorhydrate, which generates the unprotonated thiolate. Finally, in agreement with the theory of nucleophilicity, H-Tyr-OEt was found to exclusively react with **1** *via* its α-amino group (Table 2, entry 3). Since the recent use of "super-nucleophiles" (*i.e.*, α-nucleophiles such as oxyamine, hydrazines and related compounds) constitutes a major breakthrough in the field of bioconjugation chemistry, especially to have access to sophisticated biomolecular assemblies through chemoselective oxime/hydrazone ligations,[17] it seemed particularly relevant for us to compare the reactivity oxyamine/amine toward sultone **1**. Thus, two independent reactions with 1 equiv. of decyloxyamine and decylamine, respectively, in dry CH_3CN at rt have clearly shown that the primary amine reacted faster than the oxyamine: a conversion rate of 76% (against only 6% for oxyamine) was obtained after 6 h (Table 1, entries 12 and 13). A more satisfying conversion rate was obtained with decyloxyamine only after adding 1 equiv. of K_2CO_3 (22% after 2 h).

Additionally, the influence of the adjacent carbonyl on the nucleophilic ring-opening efficacy was examined. In this aim, 1-benzyl-1,3-propanesultone **2** was synthesised from benzyl bromide and 1,3-propanesultone according to a literature protocol[18] (Scheme 1). Subsequent reactions with potassium halides and propylamine were performed under the same conditions than described for **1** (Table 1, entries 1–4 and 9) and conversion rates are always significantly lower than those observed with the keto derivative. However, the halogeno-sulfonic acids were isolated in moderate to good yields (39% for iodo, 45% for bromo and 83% for chloro derivative) after a prolonged reaction time (> 24 h).

As a result of this comprehensive reactivity study, the sultone **1** bearing a carbonyl group on the α-position of the sultone ring seemed to afford the best compromise between stability and reactivity, with a pronounced "hard" electrophilic character for its reactive centre. Furthermore, experimental conditions, reliability

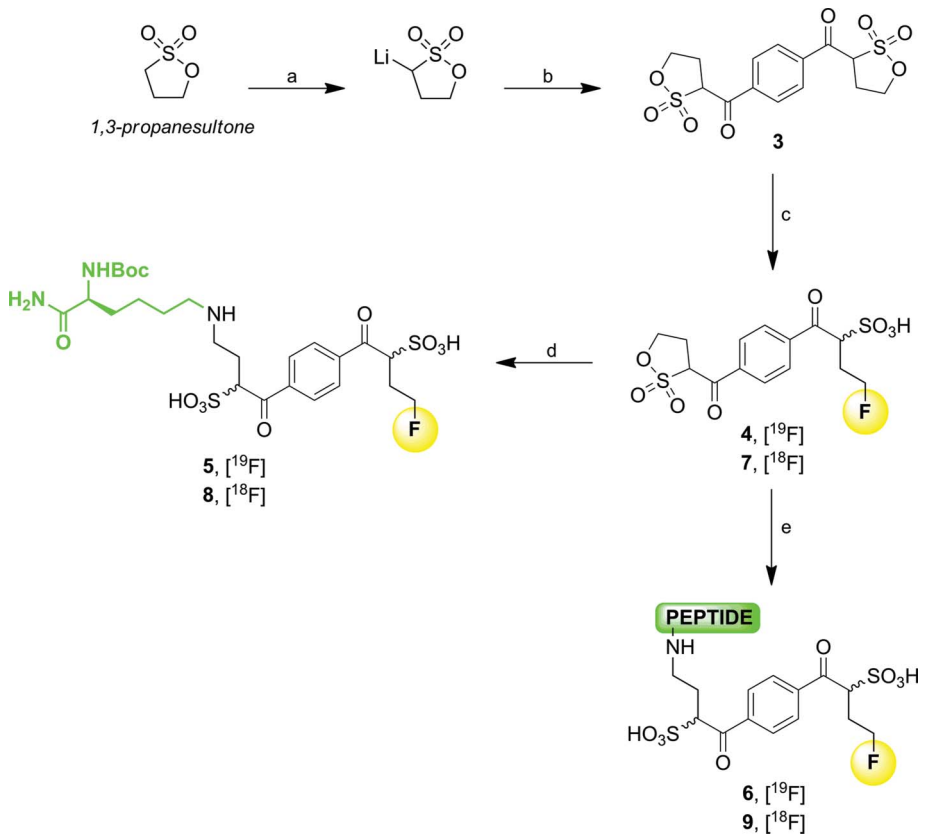
Table 2 Reactivity of model [^{18}F]-reactive 1,3-propanesultone **1** toward nucleophilic amino acids[a]



1

Entry	Amino acid	Isolated yield (*N*-alkylation) (%)	Isolated yield (*X*-alkylation) (%)
1	H-Cys-OEt	12%	28%
2	Boc-Lys-NH_2	22%	—[b]
3	H-Tyr-OEt	50%	0
4	H-Val-OMe	95%	—[b]

[a] All reactions were conducted at rt with 1 equiv. of sultone and amino acid, isolated yields after RP-HPLC purification and subsequent lyophilisation. [b] Non-ambident nucleophile.



Scheme 2 *Reagents and conditions*: (a) *n*-BuLi, THF −78 °C, 1 h; (b) methyl terephthalate, THF, −78 °C, 150 min then acetic acid, THF, −78 °C to rt, (40%); (c) KF, Kryptofix[K222], CH_3CN (containing 3% water), rt, 20 h, 50% (after RP-HPLC purification and subsequent lyophilisation); (d) Boc-Lys-NH_2, DMF, rt, 5 h, 7.5% (after RP-HPLC purifications, desalting over Dowex H^+ resin and subsequent lyophilisation); (e) dodecapeptide (TFA salt), DIEA, DMF, rt, 8 h, 5% (after RP-HPLC purification and subsequent lyophilisation). Some modifications have been made to these "cold" conditions for the [^{18}F]-radiosynthetic steps: time 20–30 min, temperature 80–100 °C and dry solvents (CH_3CN and DMF) were used.

and efficiency of the majority of investigated reactions support the fact that nucleophilic opening of sultones is a "click" reaction with a great potential in the field of bioconjugate chemistry.

Synthesis and chemical behavior of [^{18}F]-reactive bis-sultone cross-linker

First, bis-propanesultone **3**, the precursor of the targeted prosthetic group, was synthesised from dimethyl terephthalate and 1,3-propanesultone according to a similar protocol to the one designed for **1** (Scheme 2). All spectroscopic data, in particular NMR and mass spectrometry, are in agreement with the structure assigned (see ESI†). The stability of this homobifunctional cross-linker was assessed in different solvents currently used in radiolabelling protocols (*i.e.*, CH_3CN, deionised water and 0.1% aq. TFA). Compound **3** was found to be fully stable in CH_3CN even after several days of incubation. Conversely, storage in water led to a gradual degradation of **3** through the slow hydrolysis of its sultone moieties (Fig. 2). Such a phenomenon was more pronounced in acidic water. However, the moderate stability of **3** in neutral aq. buffers is not detrimental for its successful use in bioconjugation reactions involving nucleophiles more reactive than water. In the context of "cold" fluorine-labelling, the competitive water-mediated ring-opening reaction was prevented by performing both fluorination and subsequent conjugation to biomolecule/biopolymer under mild conditions (heating is avoided). Treatment of **3** with KF/Kryptofix[K222] in CH_3CN (containing 3% of water) at rt for 20 h gave the monofluoropropanesultone **4** which was isolated in pure form by RP-HPLC (50% isolated yield). Surprisingly, we have observed that this reaction is favoured by the presence of a small amount of water (30% isolated yield for the reaction conducted in dry CH_3CN). Instead of a one-pot, two-step reaction, which would certainly lead to a better yield, we have deliberately chosen to purify the intermediate **4** before its subsequent final coupling reaction with the targeted amine-containing compound. That allowed a reduction of the amount of excess peptide, which otherwise would have reacted with starting bis-sultone **3** or its mono-hydrolysed derivative to give a complex mixture difficult to rapidly resolve. Thus, the fluorinated propanesultone **4** recovered after lyophilisation was directly subjected to a second ring-opening reaction upon treatment with Boc-Lys-NH_2 or a biological active dodecapeptide (whose sequence is confidential and not disclosed within this article) bearing a primary amino group (*i.e.*, ε-NH_2 from a lysine residue).[19] These reactions were performed at rt in dry DMF (and in the presence of DIEA as a base for dodecapeptide). Purification was achieved by RP-HPLC to give the corresponding fluoro-conjugates **5** and **6** in modest isolated yields (7.5% and 5% respectively). Analysis of **5** and **6** using ESI mass spectrometry indicated the presence of fluorine and confirmed the integrity

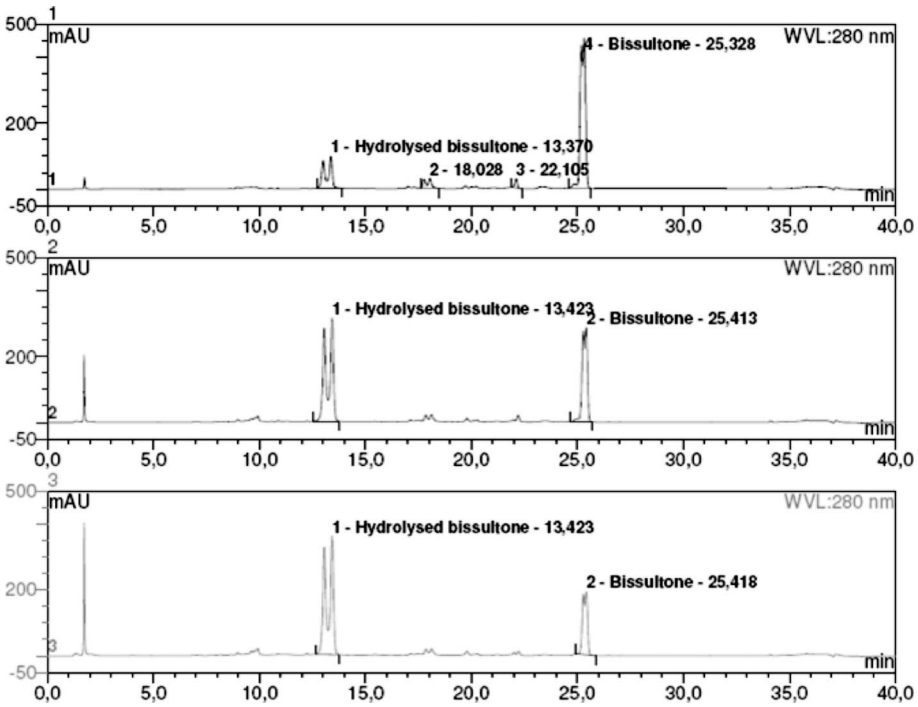


Fig. 2 RP-HPLC elution profiles (system A)[a] of bis-sultone **3** after incubation in deionised water: after 140 min (top, hydrolysis rate = 10%), 270 min (middle, hydrolysis rate = 50%) and 465 min (bottom, hydrolysis rate = 70%). [a] Doublet peaks were observed because compounds (**3** and its mono-hydrolysed derivative) are mixtures of two racemic diastereomers.

of the labelled biomolecule/biopolymer (see Fig. 3 for ESI mass spectrum of **6**). Although the use of RP-HPLC for purifications (followed by lyophilisation) and the small-scale chosen for these nucleophilic ring-opening reactions (2–5 µmol) induced significant losses of material, these reactions were found to give satisfying conversion rates around 30–40%. For further application in radiolabelling, these modest yields are not so detrimental, since the unreacted mono-sultone **4** can be easily removed taking into account the high difference in polarity compared with the desired conjugate bearing the two free sulfonate groups. However, to speed up the two-step bioconjugation methods involving the use of bis-sultone cross-linker **3**, further studies will be needed to find suitable conditions to perform the two derivatisation reactions through a one-pot procedure and so avoiding any intermediate RP-HPLC purification.[20]

Radiolabelling

[^{18}F]-fluorinated propanesultone **7** was prepared from the bis-sultone precursor **3** under the conditions described for the preparation of the "cold" standard (Scheme 2). The fully-automated synthesis and purification of **7** was performed using a TRACERlab FX device. An additional step of concentration of the resulting [^{18}F]-tagged product in CH_3CN was added to remove trace amounts of water which could lead to the premature hydrolysis of the second sultone moiety. Typically, 0.5–1.8 GBq of purified [^{18}F]-sultone **7** was obtained within 40 min, starting from 3–6 GBq of [^{18}F]-fluoride (25% average decay-corrected radiochemical yield for $n = 25$ and > 95% radiochemical purity, Fig. 4). It is essential to purify **7** before submitting it to the second ring-opening reaction, particularly to remove the large excess of starting bis-sultone **3**. Thanks to the difference of polarity between these two molecules, this was easily performed by solid-phase extraction using an HLB-SPE cartridge instead of the more conventional and time-consuming RP-HPLC. Finally, the conjugation of the peptide with the [^{18}F]-fluorinated propanesultone **7** was carried out in a mixture of dry CH_3CN-DMF (10 : 1, v/v) containing DIEA for 30 min at 80 °C. The reaction leading to [^{18}F]-labelled peptide **9** proceeded with a satisfying conversion (55%, for the radio-HPLC elution profile of the crude labelling mixture, see Fig. 4). Its identity was unambiguously confirmed by comparison of the RP-HPLC retention time with the corresponding "cold" reference previously synthesised. The [^{18}F]-prosthetic group **7** was also successfully coupled to Boc-Lys-NH_2 (conversion rate 58%, data not shown). Thus, these results clearly show the main advantage of the present labelling strategy compared with many published procedures involving the use of a prosthetic group, namely that the radiochemically feasible reagent is easily reachable through a one-step radiosynthesis which is actually the radiolabelling step of the targeted peptide. Furthermore, the high reactivity of the propanesultone moiety toward other halogens (especially iodine)[21] should enable rapidly expansion of the radioisotope–substrate scope of this radiosynthetic procedure, especially for multi-labelling purposes of selected bioactive peptides (*e.g.*, ^{76}Br, ^{77}Br, ^{123}I and ^{124}I for PET/SPECT imaging, ...).

Conclusions and future work

In this article, we have described for the first time the synthesis and a challenging bioconjugate application of an unusual homobifunctional cross-linking reagent whose the two propanesultone moieties are able to readily and sequentially react with two distinct

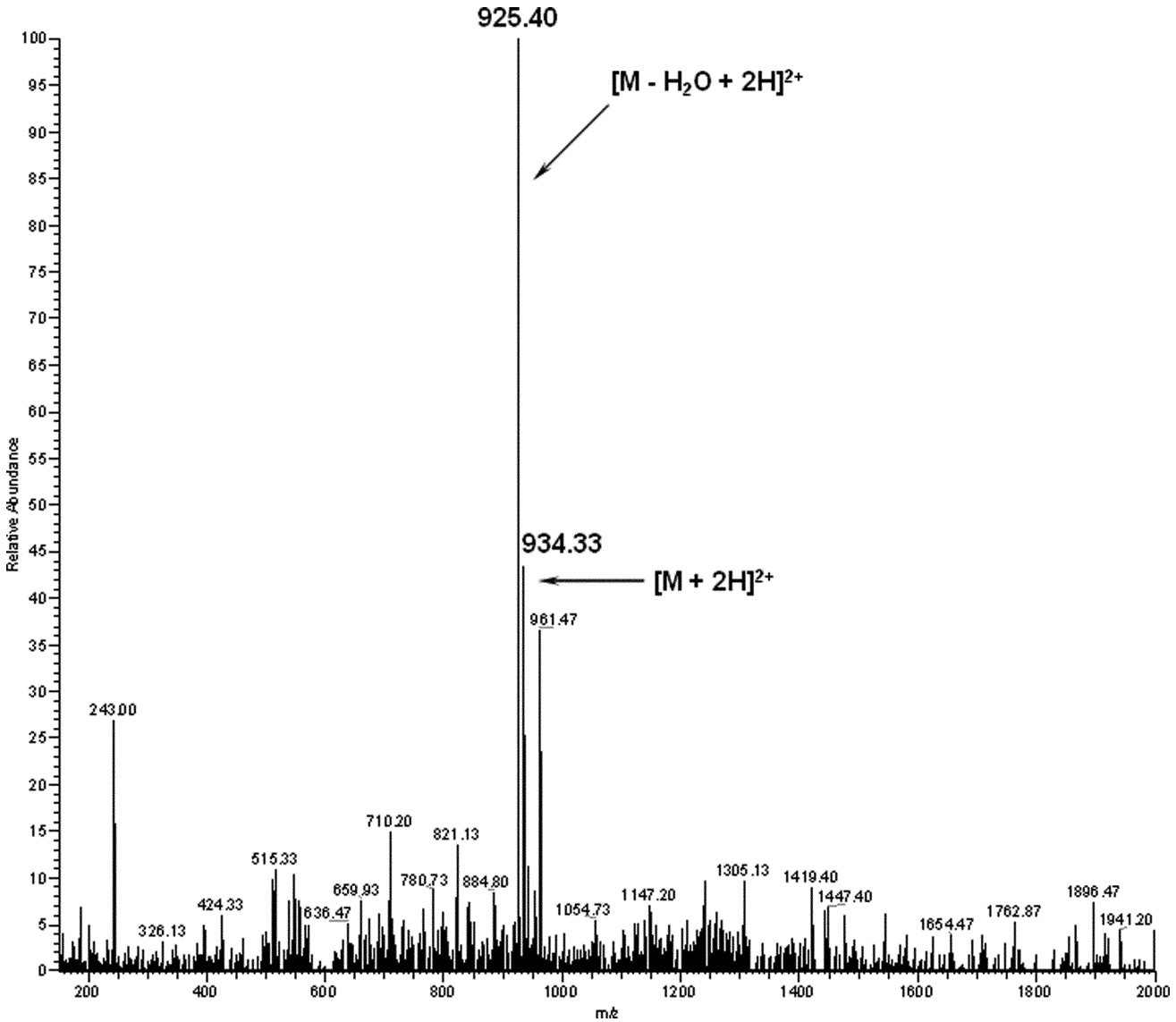


Fig. 3 ESI mass spectrum of the [^{19}F]-labelled dodecapeptide **6**. Both the doubly charged ions $[M + 2H]^{2+}$ and $[M - H_2O + 2H]^{2+}$ (calcd mass: 1865.89 and 1847.88) were observed under our ionisation conditions. The second molecular ion was assigned to the cyclic enamine derivative resulting from an intramolecular nucleophilic addition (of the secondary amine to the ketone moiety) and a subsequent dehydration reaction which were occurred into the ESI probe.

nucleophiles under mild conditions. Such valuable reactivity has been built on the development of an original [^{18}F]-propanesultone-base amine-selective reagent suitable for the prosthetic labelling with fluorine-18 of amino acids and peptides. Furthermore, we have demonstrated that this archetypal example of "click" nucleophilic ring-opening reaction is highly versatile but that its selectivity toward amines and oxyamines (compared to alcohols, phenols and thiols) can be easily tuned by using mild reaction conditions. Thus, this method could be potentially used to label a wide scope of peptides without requiring a previous chemical modification of the biopolymer with a specific and complementary functional group to the targeted prosthetic group. Interestingly, the two-step sequential derivatisation of bis-sultone cross-linker **3** leads to the post-synthetic introduction of two sulfonate groups within the (bio)molecular architecture which may be beneficial for the solubility of the resulting bioconjugate in water and related aq. buffers. Thus, further applications of this reagent devoted to the water-solubilisation of highly hydrophobic nanotools such as fluorophores[22] and organic supramolecular compounds (*e.g.*, fullerene and related compounds) are expected.

Experimental section

General

Unless otherwise noted, all other commercially available reagents and solvents were used without further purification. CH_3CN was dried through distillation over CaH_2. THF was dried through distillation over Na/benzophenone. Kryptofix[K222] (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane) and anhydrous DMF (99.8%) were purchased from Sigma–Aldrich. TLC was carried out on Merck DC Kieselgel 60

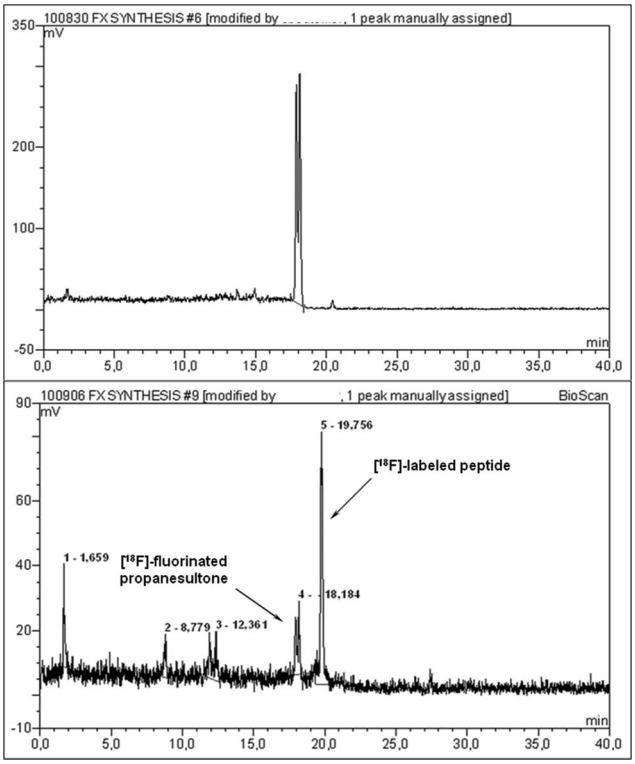


Fig. 4 Radio-HPLC elution profiles (system A) of purified [^{18}F]-fluorinated propanesultone **7** (top) and crude of [^{18}F]-labelled dodecapeptide **9**.

F-254 aluminium sheets. The spots were visualised by illumination with a UV lamp (λ = 254 nm) and/or staining with $KMnO_4$ solution. Flash column chromatography purifications were performed on Geduran® Si 60 silica gel (40–63 µm) from Merck. N^{α}-(*tert*-Butyloxycarbonyl)-L-lysine carboxamide (Boc-Lys-NH_2) was prepared from commercially available N^{α}-(*tert*-butyloxycarbonyl)-N^{ε}-(benzyloxycarbonyl)-L-lysine (Boc-Lys(Z)-OH, Bachem or Fluka), by using a conventional two-step synthetic procedure: aminolysis of mixed anhydride formed *in situ* by short treatment with isobutyl chloroformate and *N*-methylmorpholine (NMM), followed by hydrogenolysis to remove the Z protecting group.[23] The synthesis of dodecapeptides (N^{α}-Ac-lysine- or aminooxyacetic acid-terminated) was carried out on an Applied Biosystems 433A peptide synthesizer using standard Fmoc/*t*Bu chemistry[24] and Wang resin (Iris Biotech, loading 0.9 mmol g^{-1}) on a scale of 0.25 mmol. The HPLC-gradient grade acetonitrile (CH_3CN) and methanol (CH_3OH) were obtained from VWR. Aq. mobile phases for HPLC were prepared using water purified with a Milli-Q system (purified to 18.2 MΩ cm). Triethylammonium acetate (TEAA, 2.0 M) and triethylammonium bicarbonate (TEAB, 1.0 M) buffers were prepared from distilled triethylamine and glacial acetic acid or CO_2 gas. Ion-exchange chromatography (for desalting disulfonated derivatives **5**) was performed with an Econo-Pac® Disposable chromatography column (Bio-Rad, #732-1010) filled with an aq. solution of Dowex® 50WX8-400 (Alfa Aesar, *ca.* 5 g, 15 × 35 mm bed), regenerated using aq. 10% HCl solution and equilibrated with deionised water. NMR spectra (^{1}H, ^{13}C and ^{19}F) were recorded on a Bruker DPX 300 spectrometer (Bruker, Wissembourg, France). Chemical shifts are reported in parts per million (ppm) downfield from residual solvent peaks (acetone-d_6 (δ_H = 2.05, δ_C = 29.84 and 206.26), $CDCl_3$ (δ_H = 7.26, δ_C = 77.16), DMSO-d_6 (δ_H = 2.50, δ_C = 39.52) or CD_3OD (δ_H = 3.31, δ_C = 49.0)[25] and coupling constants are reported as Hertz (Hz). Splitting patterns are designated as singlet (s), doublet (d), double doublet (dd), double double doublet (ddd) and triplet (t). Splitting patterns that could not be interpreted or easily visualised are designated as multiplet (m). ^{13}C substitutions were determined with JMOD experiments, differentiating signals of methyl and methine carbons pointing "up" (+) from methylene and quaternary carbons pointing "down" (–). Melting points were determined on a Kofler bench device (Wagner and Munz). Infrared (IR) spectra were recorded with a universal ATR sampling accessory on a Perkin Elmer FT-IR Spectrum 100 spectrometer with frequencies given in reciprocal centimeters (cm^{-1}). The elemental analyses were carried out with a Flash 2000 Organic Elemental Analyzer (Thermo Scientific). Analytical HPLC was performed on a Thermo Scientific Surveyor Plus instrument equipped with a PDA detector. Semi-preparative HPLC was performed on a Thermo Scientific SPECTRASYSTEM liquid chromatography system (P4000) equipped with a UV-visible 2000 detector. Mass spectra were obtained with a Finnigan LCQ Advantage MAX (ion trap) apparatus equipped with an electrospray (ESI) source. Fluoride-18 was produced by the ^{18}O[p,n]^{18}F nuclear reaction using a GE Medical Systems PETtrace cyclotron [18 MeV proton beam] (Advanced Accelerator Applications, Saint-Genis-Pouilly, France) and ^{18}O-enriched water purchased from Marshall Isotopes Ltd. (98%, Tel Aviv, Israel). Solid-phase extraction (SPE) cartridges (SepPak QMA Light, Oasis HLB and CM) were obtained from ABX *advanced biochemical compounds* (Radeburg, Germany) and Waters (Guyancourt, France). The HLB cartridges were always pre-conditioned with ethanol (5 mL), water (5 mL) and dried with air. Radiosyntheses were performed on a TRACERlab FX (GE Medical Systems, Buc, France) automated synthesis unit in a shielded hot cell (8 cm lead, Comecer, Castel Bolognese, Italy). A flow-count radio-HPLC detector system from Bioscan was used only for HPLC analyses (performed on a Dionex UltiMate® 3000 LC system) of reactions involving ^{18}F.

HPLC separations

Several chromatographic systems were used for the analytical experiments and the purification steps: **System A**: RP-HPLC (Thermo Hypersil GOLD C_{18} column, 5 µm, 4.6 × 100 mm) with CH_3CN and 0.1% aq. trifluoroacetic acid (aq. TFA, 0.1%, v/v, pH 2.0) as eluents [100% TFA (5 min), linear gradient from 0% to 80% (40 min) of CH_3CN] at a flow rate of 1.0 mL min^{-1}. Dual UV detection was achieved at 254 and 265 nm. **System B**: RP-HPLC (Thermo Hypersil GOLD C_{18} column, 5 µm, 21.2 × 250 mm) with CH_3CN and 0.1% aq. TFA as eluents [100% TFA (10 min), linear gradient from 0% to 5% (2 min) and 5% to 70% (65 min) of CH_3CN] at a flow rate of 15.0 mL min^{-1}. Dual UV detection was achieved at 254 and 265 nm. **System C**: RP-HPLC (Thermo Hypersil GOLD C_{18} column, 5 µm, 4.6 × 100 mm) with CH_3CN and aq. triethylammonium acetate buffer (TEAA, 25 mM, pH 7.0) as eluents [100% TEAA (10 min), linear gradient from 0% to 80% (40 min) of CH_3CN] at a flow rate of 1.0 mL min^{-1}. UV-vis detection with the "Max Plot" (*i.e.*, chromatogram at absorbance

maximum for each compound) mode (220-450 nm). **System D**: RP-HPLC (Thermo Hypersil GOLD C_{18} column, 5 µm, 10 × 250 mm) with CH_3CN and aq. triethylammonium bicarbonate (TEAB, 50 mM, pH 7.5) as eluents [100% TEAB (10 min), linear gradient from 0% to 5% (2 min), 5% to 18% (13 min), 18% to 25% (14 min), 25% to 31% (6 min) and 31% to 61% (15 min) of CH_3CN] at a flow rate of 4.0 mL min^{-1}. Dual UV detection was achieved at 220 and 254 nm. **System E**: System D with Thermo Hypersil GOLD C_{18} column (5 µm, 10 × 100 mm). **System F**: RP-HPLC (Thermo Hypersil GOLD C18 column, 5 µm, 10 × 100 mm) with CH_3CN and 0.1% aq. TFA as eluents [100% TFA (10 min), linear gradient from 0% to 5% (2 min) and 5% to 70% (65 min) of CH_3CN] at a flow rate of 4.0 mL min^{-1}. Dual UV detection was achieved at 220 and 254 nm.

1-Phenylacyl-1,3-propanesultone (1)[26]

To a stirred solution of 1,3-propanesultone (502 mg, 4.1 mmol, 1 equiv.) in dry THF (7 mL), under an argon atmosphere, at −78 °C, was added dropwise *n*-BuLi (1.3 M in hexane, 3.15 mL, 4.1 mmol, 1 equiv.). After stirring at −78 °C for 1 h, a solution of methyl benzoate (0.51 mL, 4.1 mmol, 1 equiv.) in dry THF (5 mL) was added dropwise to the vigorously stirred mixture. The resulting reaction mixture was stirred at −78 °C for 2 h 30 min, then kept at −78 °C, and quenched by adding 1 mL of glacial acetic acid dissolved in dry THF (3 mL). Thereafter, the mixture was slowly warmed up to rt. The organic phase was diluted with CH_2Cl_2 (35 mL) and washed with deionised water (10 mL) and brine (10 mL). The organic phase was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The resulting residue was then purified by flash-chromatography on a silica gel column, using a mixture of cyclohexane–EtOAc (7 : 3, v/v) as the mobile phase, to give the desired product as a white powder (560 mg, yield 60%). R_f 0.46 (cyclohexane–EtOAc, 3 : 2, v/v); mp 67 ± 2 °C; ν_{max}/cm^{-1} 3063, 2963, 1690, 1449, 1342, 1291, 1235, 1181, 1149, 998, 974, 874, 836, 783; δ_H (300 MHz, $CDCl_3$) 8.02 (d, *J* 7.2, 2H), 7.58 (t, *J* 7.3, 1H), 7.48 (t, *J* 7.9, 2H), 5.08–5.03 (m, 1H), 4.62–4.55 (m, 1H), 4.52–4.44 (m, 1H), 3.27–3.16 (m, 1H), 2.72–2.60 (m, 1H); δ_C (75.5 MHz, $CDCl_3$) 187.5, 135.1, 134.8, 129.2, 129.1, 68.2, 59.7, 26.9; MS (ESI^-): *m/z* 225.02 $[M - H]^-$, calcd for $C_{10}H_{10}O_4S$ 226.03. HPLC (system A): t_R = 24.2 min (purity 99%); λ_{max} (recorded during the HPLC analysis)/nm 254; elemental analysis (%) calcd: C, 53.09; H, 4.45; S, 14.17. Found: C, 53.18; H, 4.18; S, 14.11.

1-Benzyl-1,3-propanesultone (2)[15]

Monosultone **2** was prepared from benzyl bromide and 1,3-propanesultone, according to a literature protocol.[18] The product was isolated as a colourless oil (yield 15%). R_f 0.41 (cyclohexane–EtOAc, 7 : 3, v/v); δ_H (300 MHz, $CDCl_3$) 7.39–7.24 (m, 5H), 4.49–4.31 (m, 2H), 3.59–3.48 (m, 1H), 3.42 (dd, *J* 5.5, 14.0, 1H), 2.89 (dd, *J* 9.8, 14.0, 1H), 2.56–2.31 (m, 2H); δ_C (75.5 MHz, $CDCl_3$) 136.1, 129.2, 128.9, 127.6, 67.0, 56.7, 34.6, 29.2; MS (ESI^-): *m/z* 229.27 $[M + H_2O - H]^-$ (hydration of sultone moiety occurred during the ionisation process), calcd for $C_{10}H_{12}O_3S$ 212.27; HPLC (system A): t_R = 23.6 min (purity 99%); λ_{max} (recorded during the HPLC analysis)/nm 254.

Bis-propanesultone (3)

To a stirred solution of 1,3-propanesultone (447 mg, 3.7 mmol, 2 equiv.) in dry THF (5 mL), under an argon atmosphere, at −78 °C, was added dropwise *n*-BuLi (1.3 M in hexane, 2.82 mL, 3.67 mmol, 2 equiv.). After 1 h at −78 °C, a solution of dimethyl terephthalate (358 mg, 1.84 mmol, 1 equiv.) in dry THF (10 mL) was added dropwise to the previous vigorously stirred mixture. The resulting reaction mixture was stirred at −78 °C for 2 h 30 min, then kept at −78 °C and quenched by adding 1 mL of glacial acetic acid dissolved in dry THF (3 mL). Thereafter, the mixture was slowly warmed up to rt then diluted with brine (20 mL) and EtOAc (50 mL). The formation of a solid residue was observed at the interface of the organic and aq. phases and was removed by filtration. The aq. phase was washed with EtOAc (30 mL). The combined organic phases were dried over anhydrous $MgSO_4$, filtered and then concentrated under reduced pressure. The resulting crude product was then purified by flash-chromatography on a silica gel column using a mixture of cyclohexane–acetone (3 : 2, v/v) as the mobile phase. The desired bis-propanesultone **3** was isolated as a beige solid (270 mg, yield 40%). R_f 0.42 (cyclohexane–acetone, 3 : 2, v/v); mp 210 ± 2 °C (dec.); ν_{max}/cm^{-1} 3350, 3013, 2978, 1682, 1508, 1442, 1407, 1343, 1300, 1227, 1191, 1137, 1091, 974, 948, 888, 861, 820, 777; δ_H (300 MHz, acetone-d_6) 8.20 (s, 4H), 5.63–5.57 (m, 2H), 4.59–4.49 (m, 4H), 3.17–3.08 (m, 2H), 2.80–2.71 (m, 2H); δ_C (75.5 MHz, acetone-d_6) 189.0, 140.4, 130.4 (2C), 69.6, 61.3, 27.7; MS (ESI^-): *m/z* 373.27 $[M - H]^-$, calcd for $C_{14}H_{14}O_8S_2$ 374.01; HPLC (system A): t_R = 26.7 min (purity >96%); λ_{max} (recorded during the HPLC analysis)/nm 265; elemental analysis (%) calcd: C, 44.91; H, 3.77; S, 17.13. Found: C, 43.10; H, 3.86; S, 17.50.

Monofluoropropanesultone (4)

200 µL of an aq. solution of KF (62.2 mg mL^{-1}) (12.44 mg, 0.21 mmol, 1 equiv.), Kryptofix[K222] (95 mg, 0.25 mmol, 1.2 equiv.) and 1 mL of CH_3CN were mixed in a 25 mL single-neck round-bottom flask. A solution of bis-propanesultone **3** (80 mg, 0.21 mmol, 1 equiv.) in dry CH_3CN (5.5 mL) was then added. The resulting reaction mixture was stirred at rt for 20 h and checked for completion by RP-HPLC (system A). Finally, the reaction mixture was quenched by dilution with aq. TFA and CH_3CN and purified by RP-HPLC (system B, 1 injection, t_R = 31.2–35.0 min). The product-containing fractions were lyophilised to give the monofluoropropanesultone **4** as a beige solid (27.4 mg, yield 32%, mixture of two racemic diastereomers). δ_H (300 MHz, CD_3OD) 8.16–8.09 (m, 4H), 5.53 (ddd, *J* 2.5, 6.6, 8.7, 1H), 5.04 (ddd, *J* 1.0, 4.0, 9.4, 1H), 4.61–4.46 (m, 2H), 4.45–4.37 (m, 1H), 4.29–4.21 (m, 1H), 3.15–3.04 (m, 1H), 2.71–2.58 (m, 1H), 2.55–2.33 (m, 2H). δ_F (282.5 MHz, CD_3OD) −217.6 (m, $-CH_2F$); MS (ESI^-): *m/z* 393.13 $[M - H]^-$, calcd for $C_{14}FH_{15}O_8S_2$ 394.02; HPLC (system A): t_R = 18.8 and 19.1 min (two racemic diastereomers, purity 92%); λ_{max} (recorded during the HPLC analysis)/nm 265.

Monofluoro-Boc-L-lysine-NH_2 conjugate (5)

Monofluoropropanesultone **4** (31.5 mg, 80 µmol, 1 equiv.) was dissolved in dry DMF (1.8 mL) and Boc-Lys-NH_2 (21.5 mg, 80 µmol, 1 equiv.) dissolved in dry DMF (0.8 mL) was added. The resulting reaction mixture was stirred at rt for 5 h and

checked for completion by RP-HPLC (system C). The crude product was then dissolved in aq. TEAB and twice purified by RP-HPLC (system D followed by system E). The product-containing fractions were lyophilised to give the desired lysine conjugate, which was rapidly desalted by ion-exchange chromatography (to remove triethylammonium salts) and again lyophilised to give the acid form of disulfonated monofluoro-Boc-L-lysine derivative **5**. Finally, a third RP-HPLC purification (system F) was performed to remove the free-amino lysine derivative formed during the ion-exchange chromatography through the premature cleavage of the Boc protecting group. Finally, the desired product was obtained as a white solid (2.8 mg, yield 7%). δ_H (300 MHz, DMSO-d_6) 8.18 (m, 2H), 7.79 (t, *J* 7.9, 2H), 4.93–4.91 (m, 1H), 4.51–4.42 (m, 1H), 4.24 (t, *J* 8.6, 2H), 3.77 (t, *J* 7.4, 2H), 2.67–2.60 (m, 2H), 1.77–1.74 (m, 2H), 1.65–1.55 (m, 2H), 1.37 (s, 9H), 1.31–1.24 (m, 2H); δ_F (282.5 MHz, DMSO-d_6) –216.7 (m, $-CH_2F$); MS (ESI$^-$): *m*/*z* 620.07 [M – H_2O – H]$^-$ (cyclic enamine formation occurred during the ionisation process), 638.07 [M – H]$^-$, calcd for $C_{25}FH_{38}N_3O_{11}S_2$ 639.19; HPLC (system C): t_R = 15.3 and 15.9 min (four diastereomers, purity 98%); λ_{max} (recorded during the HPLC analysis)/nm 265.

Monofluoro-peptide conjugate (6)

Monofluoropropanesultone **4** (1.6 mg, 4.06 µmol, 1.6 equiv.) was dissolved in dry DMF (0.6 mL) and a dodecapeptide (its sequence is confidential and not disclosed within this article but it contains a single reactive lysine residue, 5.0 mg, 2.6 µmol, 1 equiv.) previously dissolved in dry DMF (0.6 mL) and DIEA (10 µL, 57.4 µmol, 15 equiv.) was added. The resulting reaction mixture was stirred at rt for 8 h and checked for completion by RP-HPLC (system A). The crude product was then dissolved with aq. TFA and purified by RP-HPLC (system F). The product-containing fractions were lyophilised to give the desired monofluoro-peptide conjugate as a white solid (250 µg, yield 5%). MS (ESI$^+$): *m*/*z* 925.40 [M – H_2O + 2H]$^{2+}$ (cyclic enamine formation occurred during the ionisation process), 934.33 [M + 2H]$^{2+}$, calcd for $C_{79}FH_{128}N_{23}O_{24}S_2$ 1865.89; HPLC (system A): t_R = 20.8 min (four diastereomers, purity 94%); λ_{max} (recorded during the HPLC analysis)/nm 266.

Radiosyntheses

Mono-[^{18}F]-fluoropropanesultone (7). Following delivery of [^{18}F]-fluoride to the synthesizer module, the radioactivity was isolated on a QMA cartridge, allowing recovery of [^{18}O]-H_2O. The [^{18}F]-fluoride was eluted with a mixed solution of Kryptofix[K222] (19.8 mg) in CH_3CN (550 µL) and of K_2CO_3 (3.25 mg) in deionised water (50 µL), and transferred to the reaction vial. After azeotropic evaporation of water (3 × CH_3CN, 95 °C, with a stream of N_2 gas), bis-propanesultone **3** (3.5 mg) in dry CH_3CN (1 mL) was added. The radiolabelling was conducted in the reaction vial at 100 °C over 20 min. After dilution in a large volume of deionised water (40–50 mL), the reaction mixture was then loaded onto a HLB cartridge. After washing of the reactor vial with a 3% solution of CH_3CN in deionised water (10 mL), the product was finally eluted from the cartridge using a 17% solution of CH_3CN in deionised water (15 mL). Mono-[^{18}F]-fluoropropanesultone **7** was obtained within 40 min with a moderate 25% decay-corrected radiochemical yield (average value from *n* = 25 preparations) and with a 95% radiochemical purity. HPLC (system A): t_R = 18.2 min (two racemic diastereomers).

[^{18}F]-Labelled dodecapeptide (9). Dodecapeptide and a large excess of DIEA were put into a reaction vial and dry DMF (100 µL) was added. Then, 1 mL of the CH_3CN solution [^{18}F]-fluoropropanesultone **7** (*vide supra*) was added. The vial was put on a hotplate and heated at 80 °C for 30 min. Thereafter, the reaction was stopped and directly analysed by RP-HPLC (system A with radioactivity detection). HPLC (system A): t_R = 19.8 min (two racemic diastereomers). The retention time difference between the UV and radio traces (*ca.* 1 min) was caused by the serial arrangement of the detectors.

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