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A novel sulfonated prosthetic group for [^{18}F]-radiolabelling and imparting water solubility of biomolecules and cyanine fluorophores†‡

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Synthesis and some applications of a novel [^{18}F]-fluorinated prosthetic group based on the promising sultone radiochemistry and suitable for the labelling of amine-containing (bio)chemical compounds are described. The combined sequential use of two easy and efficient conjugation reactions namely the fluoride ring-opening of a 1,3-propanesultone moiety and the aminolysis of an *N*-hydroxysuccinimidyl ester is the key component of this original radiolabelling strategy. The mild reaction conditions and the release of a free sulfonic acid moiety as a result of the [^{18}F]-induced sultone ring-opening reaction, both make this [^{18}F]-conjugation method suitable for the radiofluorination of fragile and hydrophobic biomolecules and fluorophores, particularly by making the separation of the targeted [^{18}F]-tagged sulfonated compound from its starting precursor easier and thus faster. The ability of this unusual prosthetic group to readily introduce the radioisotope within complex (bio)molecular architectures has been demonstrated by (1) the preparation of the first [^{18}F]-labelled cyanine 5.5 (Cy 5.5) dye, a suitable precursor for the construction of hybrid positron emission tomography/near-infrared fluorescence (PET/NIRF) imaging probes and (2) the radiolabelling of a biologically relevant peptide bearing a single lysine residue.

Introduction

In the context of Positron Emission Tomography (PET) imaging, it is now widely established that the best and most convenient way to prepare the necessary radiotracers especially those derived from complex and fragile biomolecules (*e.g.*, peptides, proteins and antibodies) relies on the chemoselective conjugation of a functionalised prosthetic group carrying the chosen radionuclide.[1,2]

In the field of fluorine-18 (^{18}F) radiochemistry, the most popular radiofluorination agent for biomolecules is undoubtedly the amine-reactive [^{18}F]-labelling agent *N*-succinimidyl 4-[^{18}F]-fluorobenzoate ([^{18}F]-SFB). The synthesis and some biolabelling applications of this [^{18}F]-tagged acylation agent were first reported by the Vaidyanathan group in the early 90s.[3] Since this first report, numerous studies devoted to the improvement and/or automation of its radiosynthesis have been published.[4] [^{18}F]-SFB is generally prepared from the triflate salt of ethyl 4-(*N*,*N*,*N*-trimethylammonium)benzoate through a three-step synthetic approach using an [^{18}F]-FDG module (Fig. 1). The reported reaction times (70–100 min) are competitive for this fully automated radiosynthesis and lead to reasonable radiochemical yields (35–45%) suitable for some

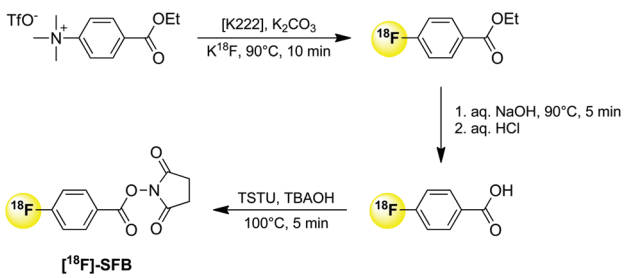

Fig. 1 General synthetic route currently used to prepare the amine-reactive [^{18}F]-labelling agent *N*-succinimidyl 4-[^{18}F]-fluorobenzoate ([^{18}F]-SFB). TfO^{-} = triflate, [K222] = Kryptofix, TSTU = *O*-(*N*-succinimidyl)-1,1,3,3-tetramethyluronium tetrafluoroborate, TBAOH = tetrabutylammonium hydroxide.

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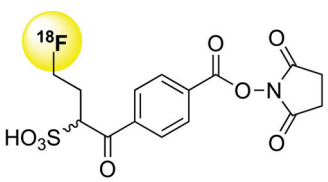
†A patent exploiting this original radiofluorination reagent is pending.
‡Electronic supplementary information (ESI) available: Detailed synthetic procedure for compound **6** and characterisation/spectral data for compounds **1**, **2–4** and **6–8**. See DOI: 10.1039/c2ob26659h

conjugation experiments. However, this tedious process is not easily automatisable and is not very well suited for routine production. A further not insignificant drawback is the lipophilic character of the [^{18}F]-SFB reagent which may complicate the purification step (by HPLC or solid-phase extraction) of the resulting [^{18}F]-labelled molecular bioprobes and negatively affect their biological properties. To circumvent these difficulties encountered with the use of [^{18}F]-SFB, we have recently explored the radiochemistry of an original homobifunctional bis-sultone cross-linker which can be easily converted into an [^{18}F]-prosthetic group through an unusual fluoride-mediated ring-opening reaction of its first sultone moiety.[5] The ability of this "alkylating" agent to radiolabel biologically relevant peptides by the nucleophilic ring-opening of its second remanent sultone by reactive lysine side-chains was also demonstrated. Satisfactory to very good radiochemical yields were obtained and the release of two "masked" sulfonic acid moieties is really beneficial to speed up the purification process of this [^{18}F]-prosthetic group and the subsequent [^{18}F]-peptides. Since the sultone moiety exhibits a higher reactivity towards a wide set of nucleophiles than the conventional *N*-hydroxysuccinimidyl (NHS) esters, our prosthetic group is more prone to hydrolysis especially during the conjugation of peptides bearing low reactive amino groups. In order to overcome this limitation, in cases where the radioactive biolabelling reaction yields have not been so high than expected, we have investigated the radiochemistry of a novel prosthetic group which can be regarded as a molecular hybrid between [^{18}F]-SFB and the [^{18}F]-fluorinated reagent derived from our bis-sultone cross-linker. Thus, our synthetic goal is to introduce both the sultone and the NHS ester moieties within the same benzenic scaffold and to reach the required chemical orthogonality between these two nucleophile-reactive functional groups through a simple/easy/time-unconsuming protecting group strategy fully compatible with the requirements of automation.

In this article, we report the synthesis of this novel heterobifunctional cross-linking reagent and its efficient conversion into the claimed mono-fluorinated prosthetic group **[^{18}F]-1** through an optimised multi-step protocol. Particular emphasis was placed on the discovery of unusual conditions for the clean and quantitative fluorination of 1,3-propanesultone moieties. The ability of **[^{18}F]-1** to radiolabel complex and fragile amine-containing (bio)molecules is illustrated by the preparation of the first [^{18}F]-tagged cyanine 5.5 (Cy 5.5) dye and a biologically active [^{18}F]-dodecapeptide.

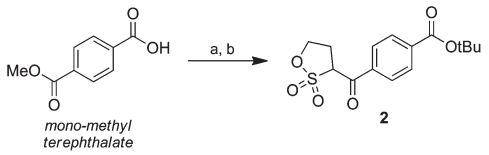


[^{18}F]-1

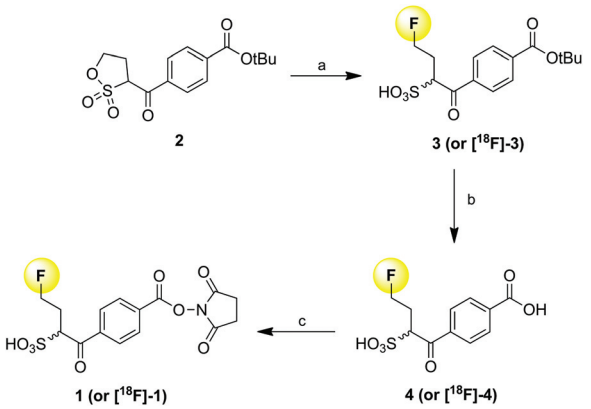
Results and discussion

Synthesis of the [^{18}F]-labelled prosthetic group and its non-radioactive fluorinated reference

By analogy with the synthesis of acylating agents currently used for labelling of biomolecules with fluorine-18, we have chosen a mono-sultone precursor derived from an aromatic carboxylic acid (*i.e.*, terephthalic acid) whose remnant –CO_2H moiety was protected through an acid-labile group namely *tert*-butyl ester. Thus, compound **2** was readily synthesised from mono-methyl terephthalate according to a two-step protocol (Scheme 1). First, esterification of the free carboxylic acid was achieved with *tert*-butyl trichloroacetamidate in dry CH_2Cl_2. This reagent has shown its superior efficacy as compared to the standard treatment with *tert*-butanol, water-soluble carbodiimide EDC and DMAP (isolated yield: 79% against 46%).[6] Thereafter, metalation of 1,3-propanesultone with *n*-BuLi and its subsequent acylation with the di-alkyl ester afforded the desired labelling precursor **2** in a good 51% overall yield. All spectroscopic data, in particular NMR and mass spectrometry, are in agreement with the structure assigned. The three-step synthetic scheme required to convert this mono-sultone derivative into the targeted fluorinated acylating agent **[^{18}F]-1** was firstly optimised through the preparation of the corresponding "cold" reference **1** (Scheme 2). Thus, fluorination of **2** was achieved using the standard "naked" fluoride (KF/

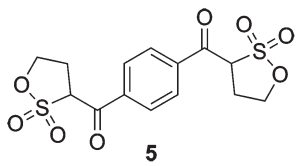


Scheme 1 *Reagents and conditions*: (a) *tert*-butyl 2,2,2-trichloroacetamidate, CH_2Cl_2, 35 °C, overnight; (b) 1,3-propanesultone, *n*-BuLi, THF, –78 °C, 3 h 30 then acetic acid, THF, –78 °C to rt, overall yield 51%.



Scheme 2 *Reagents and conditions*: (a) KF, Kryptofix[K222], CH_3CN–H_2O (98 : 2, v/v), rt, RP-HPLC purification, 63%; (b) TFA, CH_2Cl_2, rt, 1 h, quantitative yield; (c) DCC, NHS, CH_3CN, rt, 1 h or TSTU, DIEA, NMP, 30 min, quantitative yield. Some modifications have been brought to these "cold" conditions for the [^{18}F]-radiosynthetic steps: (a) see Table 1, entries 7–9; (b) 4.0 M aq. HCl, 80 °C, 5 min; (c) TSTU, DIEA, CH_3CN, 50 °C, 5 min.

Kryptofix[K222]). First attempt was conducted in dry CH_3CN at room temperature. Yet a significant amount of the expected fluoro-sulfonated derivative **3** was only obtained after a prolonged reaction time (4 days) and the formation of several side-products was also observed. In our previous work focused on the reactivity of the bis-sultone cross-linker **5** towards various nucleophiles, we have surprisingly observed that the addition of small amounts of water to the fluorination reaction medium (CH_3CN containing 3% of water) has significantly enhanced both its kinetic and univocal character without favouring the competitive and undesired hydrolysis of the sultone moieties of **5**. These unusual conditions were applied to the present fluorination reaction whose progress was carefully monitored by RP-HPLC. After 16 h of stirring at room temperature, the complete conversion of **2** into the desired fluoro-sulfonated derivative **3** was observed. The moderate 63% isolated yield was explained by material loss occurring during the RP-HPLC purification and lyophilisation steps. Thereafter, *tert*-butyl ester of **3** was quantitatively removed by treatment with a 25% TFA solution in CH_2Cl_2 to give the free benzoic acid **4**. Finally, the reaction with DCC and *N*-hydroxysuccinimide in dry CH_3CN led to the bioconjugatable NHS activated ester **1** in almost quantitative yields. Alternatively, this compound can be prepared by the reaction of **4** with the uronium coupling reagent TSTU and DIEA in dry NMP.[7] Due to its moderate stability, this NHS ester was not purified by RP chromatography and directly engaged in the amidification reactions leading to the required "cold" references (*vide infra*).



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Transposition of this high-yield synthetic plan to the preparation of the radioactive fluorinated derivative **[^{18}F]-1** was next explored. Such radiosynthesis and subsequent purification were performed using a TRACERlab MX device. The monosultone precursor **2** was reacted with [^{18}F]-fluoride at 90 °C for 10 min. Its conversion strongly depended on the choice of the reaction conditions (*i.e.*, solvent and composition of eluent solution used to transfer [^{18}F]-fluoride to the reaction vial). The results are summarised in Table 1. Standard conditions involving a mixture of K_2CO_3 and Kryptofix[K222] as the eluent and CH_3CN or DMSO as the solvent were firstly assayed. However, very low conversion rates were observed (entries 1 and 2). The use of Cs_2CO_3 instead of K_2CO_3 allowed us to obtain better results but the conversion rates were still lower than 15% (entry 3). As expected and already mentioned (*vide supra*), the presence of trace amounts of water in the reaction solvent has resulted in a slight increase of the reaction rate and yield which remain low (entries 4–6). Thus, we next investigated new experimental conditions to improve this radiofluorination step. It has been recently described that the combination of cesium [^{18}F]-fluoride in a bulky protic solvent such as *tert*-butanol (*t*BuOH) or *tert*-amyl alcohol (*t*AmylOH) could enhance the fluorination of some precursors compared to classical conditions involving [^{18}F]-TBAF as the "naked" fluoride source, in a dipolar aprotic solvent, namely acetonitrile. Kim *et al.* have studied the nucleophilic substitution of sulfonate-based leaving groups with fluoride ions, in *tert*-butanol and using a mixture of Cs_2CO_3–Kryptofix[K222] as the phase transfer agent.[8] They hypothesised that, among other factors, hydrogen bonds between the SO_2 part of leaving groups and the alcohol protons of the solvent contributed to enhance the leaving group ability of the sulfonate esters that resulted in an increase of the reaction efficiency. By analogy, the present sultone ring-opening process induced by the nucleophilic attack of the fluoride ion may be enhanced by the cooperative effect of the hydrogen bond formation between the alcohol solvent and the oxygen atoms of the propanesultone moiety. The radiofluorination of cyclic sulfonate ester **2** was therefore performed in bulky protic solvents such as *t*BuOH and *t*AmylOH (Table 1, entries 7 and 8). Interestingly, using *t*BuOH, the [^{18}F]-fluorinated *tert*-butyl ester **[^{18}F]-3** was obtained in significantly higher radiochemical yields and purity with respect to all previously performed reactions (*vide supra*). Typically, 4–5 GBq of purified [^{18}F]-fluorinated *tert*-butyl ester **[^{18}F]-3** were obtained within 30 min, starting from 8–10 GBq of [^{18}F]-fluoride (50–75% average decay-corrected radiochemical yield and 90–95% radiochemical purity). Furthermore, lower but still satisfying reaction yields were obtained when *t*AmylOH is used as a solvent. However, from an industrial point of view, the use of the above-mentioned alcohols is a serious problem. Indeed, the European Pharmacopoeia (Ph. Eur.) is very restrictive about the residual solvents present in a pharmaceutical preparation and the choice of the solvent often represents a challenging factor for the synthesis of new drugs and related radiopharmaceuticals. Thus, we have investigated the alternative use of isopropanol (iPrOH). This secondary alcohol is a class 3 solvent and its residual amount in final pharmaceutical preparations is less subjected to severe restrictions.[9] Once again, a high conversion (up to 90%) was

Table 1 Selected reaction conditions for the preparation of **[^{18}F]-3** from the mono-sultone precursor **2**

Entry	Solvent[a]	Base[b]	% Conversion rate (radio-HPLC)[c]
1	CH_3CN	K_2CO_3	3
2	DMSO	K_2CO_3	0
3	CH_3CN	Cs_2CO_3	12
4	CH_3CN + 3% H_2O	K_2CO_3	6.5
5	CH_3CN + 3% H_2O	Cs_2CO_3	32
6	CH_3CN + 6% H_2O	Cs_2CO_3	23
7	*t*BuOH	Cs_2CO_3	86
8	*t*AmylOH	Cs_2CO_3	70
9	iPrOH	Cs_2CO_3	90
10	EtOH	Cs_2CO_3	20

[a] [^{18}F]-labelling was carried out in 1.0 mL of the respective solvent (mixture) for 10 min at 90 °C except for entries 4–6 (1.6 mL). [b] With 1.8 equiv. of Kryptofix[K222]. [c] Ratio between product **[^{18}F]-3** and free fluorine-18 in the crude reaction mixture.

obtained using this unusual reaction solvent (Table 1, entry 9). Finally, the nucleophilic radiofluorination was also conducted in ethanol but a modest 20% conversion was observed (Table 1, entry 10), confirming that both the bulkiness and the hydrogen-bonding behavior of solvent molecules play a positive role in the reaction course. Thus, the present methodological study provides a novel example of nucleophilic radiofluorination reaction that did not work very well under standard conditions (K_2CO_3–Kryptofix[K222] with CH_3CN as a solvent) but was dramatically improved only by changing the reaction solvent mixture. Following the [^{18}F]-radiolabelling and purification steps, the *tert*-butyl ester of **[^{18}F]-3** was removed by treatment with 4.0 M aq. HCl instead of TFA (used for the synthesis of the "cold" reference). This change is mainly related to the better chemical compatibility of the hardware kit used during the automated synthesis, towards this strong acid. The reaction was performed at 80 °C for 5 min and the resulting free benzoic acid **[^{18}F]-4** was purified by solid-phase extraction (SPE) using an Oasis® HLB cartridge. Final elution with CH_3CN–DIEA (9 : 1, v/v) has allowed the recovery of the corresponding carboxylate anion which subsequently reacted with a solution of the TSTU uronium salt in CH_3CN at 50 °C for 5 min, to provide the corresponding NHS ester. Thus, 3–4 GBq of the targeted [^{18}F]-labelled prosthetic group **[^{18}F]-1** were obtained within 72 min, starting from 10–15 GBq of [^{18}F]-fluoride (35–45% average decay-corrected radiochemical yield for n = 15 and 80–95% radiochemical purity, see Fig. 2 for the RP-HPLC elution profile of **[^{18}F]-1**, **-3** and **-4**).

Synthesis of a cyanine-based PET/NIRF probe

To evaluate the utility of this novel amine-reactive prosthetic group both for radiolabelling and imparting water-solubility of fragile and hydrophobic molecular architectures, we first investigated its conjugation to a fluorescent amine derived from the pentamethine cyanine 5.5 (Cy 5.5) core. This far-red fluorescent marker often regarded as "the golden imaging standard"[10] is commercially available from GE Healthcare in a pre-activated form (NHS ester) and its core structure exhibits four sulfonic acid moieties to prevent its aggregation in aq. environments.[11,12] Since there is recent growing interest in the synthesis of [^{18}F]-labelled fluorophores for producing hybrid PET/NIRF imaging agents,[13] it seems relevant to develop a simple and efficient radiosynthetic methodology for incorporating fluorine-18 atoms into Cy 5.5-based dyes.[14] Interestingly, Li, Gabbai *et al.* have recently reported an elegant method to effectively convert BODIPY dyes into dual-labelled PET/fluorescence tracers through the ^{19}F/^{18}F formal exchange of one of the fluorine atoms within their canonical BF_2 dipyrromethene core.[15] However, this methodology cannot be applied to the vast majority of fluorophores which do not bear exchangeable fluorine atoms. In this context, a more versatile strategy is required, and the one involving an amidification reaction between the NHS ester of **[^{18}F]-1** and a primary amino group pre-introduced onto the cyanine scaffold appears to be a promising way to achieve this ambitious goal. In order to highlight the positive impact of our sulfonated prosthetic group to

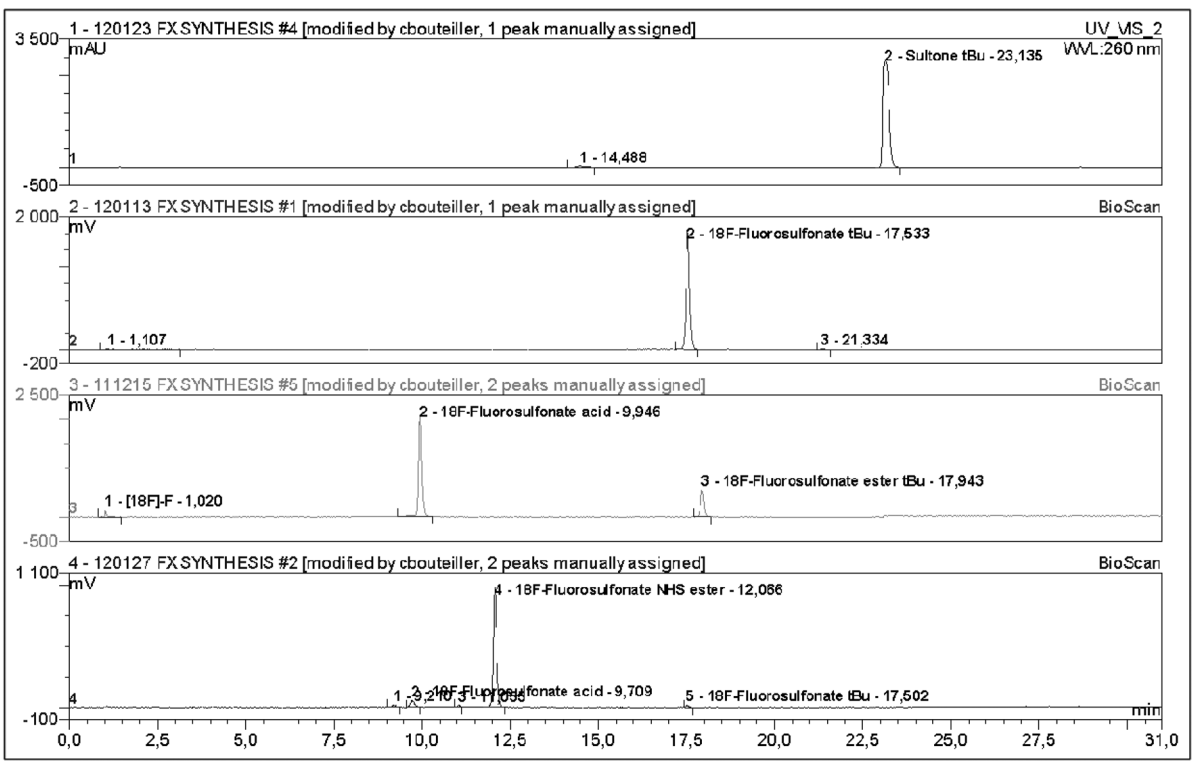
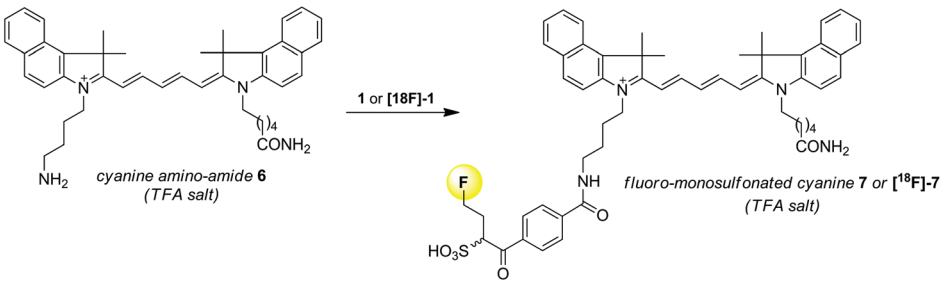


Fig. 2 HPLC elution profiles (system I) of purified mono-sultone precursor **2** "Sultone *t*Bu" (top, UV detection 260 nm) and crude of **[^{18}F]-3** "18F-fluorosulfonate *t*Bu", **[^{18}F]-4** "18F-fluorosulfonate acid" and **[^{18}F]-1** "18F-fluorosulfonate NHS ester" (radioactive detection).



Scheme 3 *Reagents and conditions*: DIEA, NMP, rt, overnight, RP-HPLC purification, 43%. The following modifications have been brought to these "cold" conditions for the [^{18}F]-radiosynthesis: DIEA, CH_3CN, rt, 1 min.

increase the hydrophilicity of the resulting [^{18}F]-labelled cyanine dye (particularly relevant for its rapid purification by SPE), we have chosen to work with a hydrophobic analogue of Cy 5.5 **6** that does not contain ionisable, negatively charged moieties (such as carboxylic, phosphonic or sulfonic acids)[16–18] but exhibits a single reactive amino group and a carboxamide function onto its linker arms. This amino-fluorophore was obtained through a two-step reaction sequence (*i.e.*, amidification followed by removal of the phthalimide protecting group, see ESI‡) from a cyanine phthalimide-acid derivative already reported by us.[19] The reactivity of this fluorescent primary amine towards the fluoro-sulfonated NHS ester **1** was firstly explored in order to obtain the "cold" reference (Scheme 3). Standard conditions currently used for the amidolysis of active esters (*i.e.*, a tertiary amine such as DIEA in a dry polar aprotic solvent such as NMP) led to the targeted fluorinated/sulfonated Cy 5.5 derivative **7**. Purification was achieved by RP-HPLC to give this fluorescent compound in a pure form and with a moderate 43% yield. Its structure was unambiguously confirmed by detailed measurements, including ESI mass spectrometry and NMR analyses (see ESI‡). Furthermore, this monosulfonated fluorescent dye was found to be soluble in water and related aq. buffers in the range 1–10 μM suitable for evaluating its optical properties under simulated physiological conditions (see Table 2 and Fig. 3). To prevent the formation of non-emissive aggregates, phosphate buffered saline (PBS, pH 7.5), containing BSA protein (5% w/v), was used as a model physiological fluid. Indeed, this protein is known to enhance the emission of many water-soluble fluorophores due to a combination of rigidisation, reduction in the polarity of the dye's microenvironment (binding in the hydrophobic pocket of BSA), and deaggregation.[20] **7** has absorption and emission bands that match closely those of the non-sulfonated Cy 5.5 derivatives such as its amino cyanine precursor **6** (see Table 2 and Fig. 3). High molar extinction coefficient (ε = 162 000 dm^3 mol^{-1} cm^{-1}) and good quantum yield (Φ_F = 38%) are comparable to those of original sulfobenzindocyanine dye Cy 5.5 (ε = 195 000 dm^3 mol^{-1} cm^{-1} and Φ_F = 23%). Thus, the corresponding [^{18}F]-labelled cyanine 5.5 derivative retains the valuable optical properties of this parent fluorescent core. This supports our labelling strategy to get "fluoro-dyes" (*i.e.*, fluorescent and fluorinated dyes) as suitable precursors of dual-function PET/NIRF imaging probes, in a fast and convenient way. Another interesting point concerns cyanine amino-amide **6** which was found to be also soluble and fluorescent in the same aq. buffer (range 1–10 μm) whereas its polyaromatic scaffold does not contain any ionisable, negatively charged water-solubilising moieties (see Table 2 and ESI‡). To our knowledge, few examples of water-soluble cyanine dyes bearing neutral and/or positively charged hydrophilic groups have been already reported in the literature; only the grafting

Table 2 Spectral properties of cyanine 5.5 derivatives

Cy 5.5 derivative	Solvent[a]	λ_{max}, abs/nm	ε/dm^3 mol^{-1} cm^{-1}	λ_{max}, em/nm	Φ_F [b]
Cy5.205 (GE Healthcare)	PBS	674[c]	195 000[c]	694[c]	0.23[c,d]
Amino-amide **6**	DMSO	688	195 000	712	0.45
Amino-amide **6**	PBS + 5% BSA	695	162 000	710	0.34
"Cold" reference **7**	DMSO	689	195 000	714	0.42
"Cold" reference **7**	PBS + 5% BSA	697	162 000	712	0.38

[a] All measurements were performed at 25 °C. [b] Cy5.205 (Cy 5.5 sym. From GE Healthcare) was used as standard (Φ_F = 0.23 in PBS),[12] excitation at 600 nm. [c] See Mujumdar *et al.*[12] [d] Quantum yield of Cy5.205 was also determined in PBS + 5% BSA by us, a value of 0.32 was found.

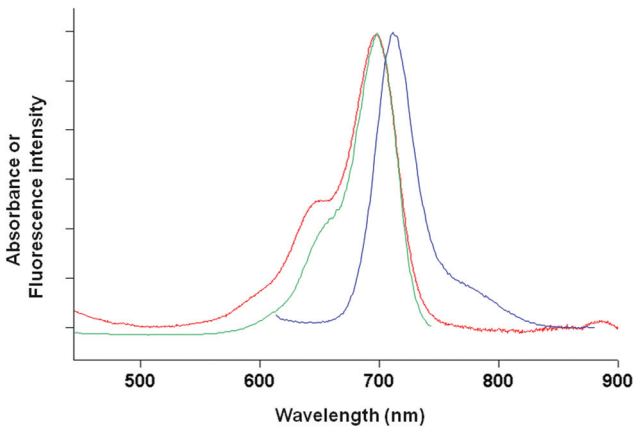


Fig. 3 Normalised absorption (—), emission (—) (Ex. 600 nm) and excitation (—) (Em. 760 nm) spectra of fluoro-monosulfonated cyanine **7** in PBS + 5% BSA at 25 °C.

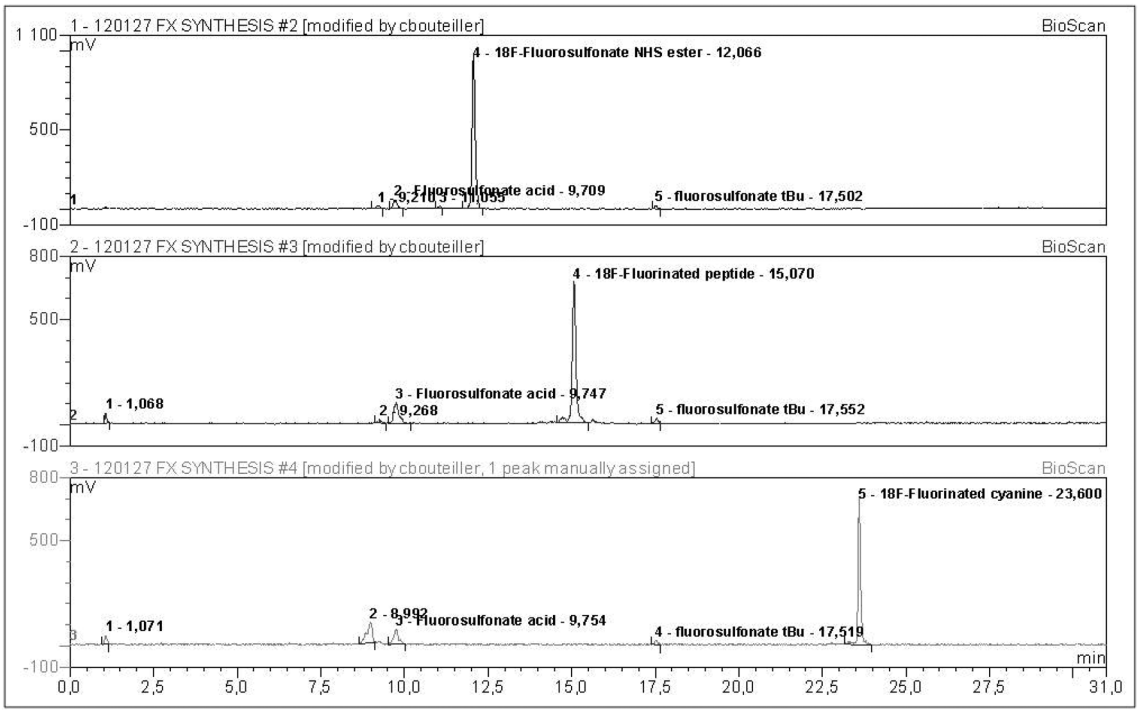


Fig. 4 Radio-HPLC elution profiles (system I) of purified **[^{18}F]-1** "18F-fluorosulfonate NHS ester" (top) and crude of [^{18}F]-labelled dodecapeptide **[^{18}F]-8** "18F-fluorinated peptide" (middle) and [^{18}F]-labelled cyanine **[^{18}F]-7** "18F-fluorinated cyanine" (bottom).

of carbohydrates, PEG linkers and tetralkylammonium moieties has been regarded and their synthesis often required non-trivial multi-step synthetic routes.[21] Thus, the present study enables us to expand the list of synthetic strategies relevant for increasing the hydrophilic character of cyanine dyes. Thereafter, the amino cyanine dye **6** was next subjected to the same amidification reaction involving the [^{18}F]-labelled prosthetic group **[^{18}F]-7**. The coupling reaction was found to occur instantaneously with a very good conversion rate (up to 70% within less than one minute) (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 **7** previously synthesised (see ESI‡ for the RP-HPLC elution profile of "cold" reference **7**).

[^{18}F]-Radiolabelling of a biologically active peptide

To demonstrate the scope of the present methodology for the conversion of various amine-containing (bio)molecular compounds into the corresponding [^{18}F]-based radiotracers, we have also explored the reactivity of the prosthetic group **[^{18}F]-1** towards a biologically active dodecapeptide (whose sequence is confidential and not disclosed within this article) bearing a single primary amino group (*i.e.*, ε-NH_2 from a lysine residue) and other nucleophilic amino acids (*i.e.*, arginine and tyrosine residues). The same experimental conditions previously optimised for the amino cyanine **6** were successfully applied to this biological macromolecule. As illustrated by the radio-RP-HPLC elution profile of the crude labelling mixture (Fig. 4, middle), the amidification reaction proceeded cleanly to produce the desired [^{18}F]-product **[^{18}F]-8** with a very good conversion rate (up to 80% within less than one minute). No undesired reactions with unprotonated guanidino groups of arginine residues and/or phenol moiety of tyrosines were observed. This provides tangible evidence of chemoselectivity of this peptide conjugation. The identity of the [^{18}F]-labelled dodecapeptide was unambiguously confirmed by comparison of the RP-HPLC retention time with the corresponding "cold" reference **8** synthesised according to the conditions described in Scheme 3 for **7** (see ESI‡ for the RP-HPLC elution profile of "cold" reference **8** and for the mass spectra of dodecapeptide before and after derivatisation with **1**).

Conclusions and future work

In this article, a novel prosthetic group **[^{18}F]-1** suitable for the [^{18}F]-radiolabelling of amine-containing (bio)molecular compounds was readily synthesised and its potential utility was clearly demonstrated through the efficient preparation of [^{18}F]-tagged fluorophores and peptides. This labelling reagent can be regarded as an analogue of [^{18}F]-SFB (probably the most often-used [^{18}F]-prosthetic group for PET labelling) whose fluorine-18 atom was pre-introduced through an unusual nucleophile-induced ring-opening reaction of the 1,3-propanesultone moiety of its precursor. For the first time, this nucleophilic substitution was found to be greatly favoured in bulky protic solvents (*e.g.*, iPrOH and *t*BuOH). This result promotes a better understanding of the sultones' reactivity towards nucleophiles

and should facilitate their future and broader use as bioconjugatable reactive handles. As claimed, the present radio-fluorination reaction led to the unveiling of a free sulfonic acid moiety which is greatly beneficial, since it accelerates and facilitates the purification of the resulting [^{18}F]-conjugates by drastically modifying their intrinsic hydrophilic character. Thus, these radiosynthetic procedures can be rapidly and easily implemented and automated, which are readily reproducible and give very satisfactory yields within very short reaction times (*ca.* 1 minute). These added-values make it clear that the novel prosthetic group **[^{18}F]-1** represents a viable complement to [^{18}F]-SFB, especially if hydrophilic radiotracers are targeted. Furthermore, the mild reaction conditions associated with the chemistry of the NHS active ester (found in **[^{18}F]-1**) will enable us to explore the [^{18}F]-radiolabelling of highly-functionalised and fragile fluorescent markers (bearing both water-solubilising moieties, bioconjugatable handles and recognition/targeting units) to convert them into [^{18}F]-PET/NIRF dual modality agents for biomedical imaging.

Experimental section‡

General

Unless otherwise noted, all other commercially available reagents and solvents were used without further purification. CH_2Cl_2 and CH_3CN were dried through distillation over P_2O_5 and CaH_2 respectively. Anhydrous THF was obtained through drying over Na/benzophenone. Anhydrous DMF was obtained from Carlo Erba-SdS or Fisher Scientific. Peptide synthesis-grade NMP was purchased from Carlo Erba-SdS. Bovine serum albumin (BSA) protein and Kryptofix[K222] (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8] hexacosane) were purchased from Sigma-Aldrich. TLC was carried out on Merck DC Kieselgel 60 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) or (63–200 μm for cyanine derivatives) from Merck. Cyanine amino carboxamide **6** was prepared according to a synthetic scheme described in the ESI‡ file. The synthesis of dodecapeptide (N^{α}-Ac-lysine-terminated) was carried out on an Applied Biosystems 433A peptide synthesizer using the standard Fmoc/*t*Bu chemistry[22] and the 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. Phosphate buffered saline (PBS, 100 mM phosphate + 150 mM NaCl, pH 7.5) and aq. mobile-phases for HPLC were prepared using water purified with a Milli-Q system (purified to 18.2 MΩ cm). Triethylammonium bicarbonate (TEAB, 1.0 M) buffer was prepared from distilled triethylamine and CO_2 gas.

Instruments and methods

NMR spectra (^{1}H, ^{13}C and ^{19}F) were recorded on a Bruker DPX 300 spectrometer (Bruker, Wissembourg, France) or with a Bruker AC 200. Chemical shifts are reported in parts per million (ppm) downfield from residual solvent peaks: $CDCl_3$ (δ_H = 7.26, δ_C = 77.16) or CD_3OD (δ_H = 3.31, δ_C = 49.0)[23] 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" (–). The elemental analyses were carried out with a Flash 2000 Organic Elemental Analyzer (Thermo Scientific). Analytical HPLC was performed using a Thermo Scientific Surveyor Plus instrument equipped with a PDA detector. Semi-preparative HPLC was performed using 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. High-resolution mass spectra (HRMS) were recorded on an LTQ Orbitrap Elite (Thermo Scientific). UV-visible absorption spectra were obtained on a Varian Cary 50 scan spectrophotometer by using a rectangular quartz cell (Varian, standard cell, Open Top, 10 × 10 mm, 3.5 mL). Fluorescence spectroscopic studies (emission/excitation spectra) were performed using a Varian Cary Eclipse spectrophotometer with a semi-micro-quartz fluorescence cell (Hellma, 104F-QS, 10 × 4 mm, 1400 μL). For details related to the determination of relative quantum yields, see ESI.‡ 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 using a TRACERlab MX (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 is used only for HPLC analyses (performed using 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: **S**ystem 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, 2.1 × 100 mm) with CH_3CN and 0.1% aq. trifluoroacetic acid (aq.

TFA, 0.1%, v/v, pH 2.0) as eluents [80% TFA (5 min), linear gradient from 20% to 40% (5 min) and 40% to 100% (50 min) of CH_3CN] at a flow rate of 0.25 mL min^{-1}. UV-vis detection with the "Max Plot" (*i.e.*, chromatogram at absorbance maximum for each compound) mode (220–798 nm). System C: RP-HPLC (Thermo Hypersil GOLD C_{18} column, 5 μm, 10 × 250 mm) with CH_3CN and 0.1% aq. TFA as eluent [100% TFA (5 min), linear gradient from 0% to 20% (10 min), 20% to 45% (25 min), 45% to 65% (10 min) and 65% to 100% (5 min) of CH_3CN] at a flow rate of 5.0 mL min^{-1}. Dual UV detection was achieved at 270 and 300 nm. System D: 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 (5 min), linear gradient from 0% to 10% (5 min), 10% to 30% (20 min), 30% to 50% (10 min) and 50% to 100% (15 min) of CH_3CN] at a flow rate of 15.0 mL min^{-1}. Dual UV detection was achieved at 270 and 300 nm. System E: RP-HPLC (Varian Kromasil C_{18} column, 10 μm, 21.2 × 250) with CH_3CN and aq. TEAB (50 mM, pH 7.5) as eluents [100% TEAB (5 min), linear gradient from 0% to 30% (10 min) and 30% to 100% (70 min) of CH_3CN] at a flow rate of 20 mL min^{-1}. Dual visible detection was achieved at 625 and 680 nm. System F: system C with the following gradient [90% TFA (5 min), linear gradient from 10% to 100% (36 min) of CH_3CN] at a flow rate of 4.0 mL min^{-1}. Dual visible detection was achieved at 625 and 680 nm. System G: system A with the following gradient [80% TFA (5 min), linear gradient from 20% to 100% (40 min) of CH_3CN] at a flow rate of 1.0 mL min^{-1}. Dual UV detection was achieved at 220 and 260 nm. System H: RP-HPLC (Thermo Hypersil GOLD C_{18} column, 5 μm, 10 × 100 mm) with CH_3CN and 0.1% aq. TFA as eluents [100% TFA (5 min), linear gradient from 0% to 80% (40 min) and 80% to 100% (5 min) of CH_3CN] at a flow rate of 4.0 mL min^{-1}. Dual UV detection was achieved at 227 and 261 nm. System I: system A with the following gradient [100% TFA (3.8 min), linear gradient from 0% to 44% (16.9 min) and 44% to 100% (3.8 min) of CH_3CN] at a flow rate of 1.3 mL min^{-1}. Dual UV detection was achieved at 254 and 265 nm.

Sultone-benzoic acid, *tert*-butyl ester (2)

(a) Esterification: To a stirred solution of mono-methyl terephthalate (500 mg, 2.78 mmol, 1 equiv.) in dry CH_2Cl_2 (15 mL), under an argon atmosphere, was added *tert*-butyl 2,2,2-trichloroacetimidate (1.25 g, 5.55 mmol, 2 equiv.). The resulting reaction mixture was stirred at 35 °C (with a warm water bath) overnight. Then, the crude mixture was filtrated to remove the remaining unreacted starting acid and was then purified by flash-chromatography on a silica gel column using a mixture of cyclohexane–ethyl acetate (9 : 1, v/v) as the mobile phase. The resulting pure solid was dried under vacuum and directly used in the next step. R_f 0.73 (cyclohexane–EtOAc, 3 : 7, v/v); δ_H (300 MHz, $CDCl_3$) 8.05 (m, 4H), 3.93 (s, 3H), 1.60 (s, 9H); δ_C (75.5 MHz, $CDCl_3$) 166.6, 165.0, 135.9, 133.5, 129.5 (2 × 2C), 81.8, 52.5, 28.2 (3C). All other spectroscopic data are identical to those reported by Nishimoto *et al.*[24]

(b) Acylation of 1,3-propanesultone: To a stirred solution of commercial 1,3-propanesultone (720 mg, 5.89 mmol, 2.1 equiv.) in dry THF (10 mL), under an argon atmosphere, at −78 °C, was added dropwise *n*-BuLi (2.0 M in hexane, 3 mL, 6 mmol, 2.2 equiv.). After 1 h of stirring at −78 °C, a solution of the previously isolated *tert*-butyl methyl diester (*vide supra*) in dry THF (10 mL) was added dropwise to the previously vigorously stirred mixture. The resulting reaction mixture was stirred at −78 °C for 2 h 30, then kept at −78 °C, and quenched by adding 1 mL of glacial acetic acid dissolved in dry THF (3 mL). Thereafter, the reaction mixture was slowly warmed up to rt then diluted with brine (20 mL) and CH_2Cl_2 (50 mL). The aq. phase was washed with CH_2Cl_2 (30 mL). The combined organic layers 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–EtOAc (gradient from 9 : 1 to 7 : 3, v/v) as the mobile phase. The desired product **2** was isolated as a white pasty solid (458 mg, overall yield for the two steps 51%). R_f 0.33 (cyclohexane–ethyl acetate, 3 : 7, v/v); δ_H (300 MHz, $CDCl_3$) 8.13 (m, 4H), 5.13 (dd, *J* 5.6, 8.7, 1H), 4.66–4.49 (m, 2H), 3.31–3.20 (m, 1H), 2.78–2.66 (m, 1H), 1.60 (s, 9H); δ_C (75.5 MHz, $CDCl_3$) 187.4, 164.5, 137.8 (2C), 137.3 (2C), 130.1, 129.0, 82.2, 68.3, 60.1, 28.2 (3C), 26.8; MS (ESI^-): *m*/*z* 325.27 $[M - H]^-$, 343.27 $[M + H_2O - H]^-$ (partial hydrolysis of the sultone moiety occurred during the ionisation process), calcd for $C_{15}H_{18}O_6S$ 326.26; HRMS (ESI^-): *m*/*z* 325.0751 $[M - H]^-$, calcd for $C_{15}H_{17}O_6S^-$ 325.0745; HPLC (system A): t_R = 31.4 min (purity 96%); λ_{max} (recorded during the HPLC analysis) 257 nm; elemental analysis (%) calcd: C, 55.20; H, 5.56; S, 9.82. Found: C, 54.40; H, 5.45; S, 9.21.

Mono-fluoro-sulfonated *tert*-butyl ester (3). To a stirred solution of Kryptofix[K222] (76.2 mg, 0.20 mmol, 3.3 equiv.) and KF (10.7 mg, 0.184 mmol, 3 equiv.) in a mixture of dry CH_3CN (1 mL) and deionised water (20 μL) was added sultone **2** (20 mg, 0.061 mmol, 1 equiv.). The resulting reaction mixture was stirred at rt and its completion was checked by analytical RP-HPLC (system A). Thereafter, the crude product was purified by semi-preparative RP-HPLC (system C). The product-containing fractions were immediately frozen in liq. nitrogen and lyophilised to give the desired sulfonic acid derivative **3** as a white amorphous powder (13.4 mg, yield 63%). This compound was partially obtained as the trialkylammonium (Kryptofix[K222]) salt (**3**-[K222], 1 : 0.4, determined by ^1H NMR) and could not be converted into the corresponding free sulfonic acid derivative by ion-exchange chromatography (Dowex H^+), due to the premature cleavage of its *tert*-butyl ester. δ_H (300 MHz, CD_3OD) 8.17 (m, 4H), 5.15 (dd, *J* 4.0, 9.5, –C*H*-(SO_3H), 1H), 4.70–4.32 (m, –CH_2F, 2H), 3.79–3.51 (m, –CH_2–Kryptofix[K222], 10H), 2.68–2.50 (m, –CH_2–CH_2F, 2H), 1.45 (s, *t*Bu, 9H); δ_C (75.5 MHz, CD_3OD) 27.9 (3 × *C*H_3–*t*Bu), 31.5 (d, *J* 19.3, –CH_2–CH_2–F), 55.0 (*C*H_2–Kryptofix[K222]), 63.7 (2C, –*C*H-(SO_3H) and Cq–*t*Bu), 64.6 (–*C*H_2–Kryptofix[K222]), 71.8 (–*C*H_2–Kryptofix[K222]), 83.0 (d, *J* 164.0, 1 × –*C*H_2–F), 130.1 (2 × *C*H–Ph), 130.7 (2 × *C*H–Ph), 135.7 (1 × Cq–Ph), 142.3 (1 × Cq–Ph), 168.7 (1 × Cq, *C*O_2*t*Bu), 196.1 (1 × Cq, *C*O–aryl ketone); δ_F (282.5 MHz, CD_3OD) −216.8 (m, –CH_2*F*, 1F); MS (ESI^-): *m*/*z* 670.80 $[2\ M - HF - H]^-$, 345.07 $[M - H]^-$, 289.07 [M – *t*Bu –

H]$^-$, calcd for $C_{15}H_{19}FO_6S$ 346.09. HPLC (system A): t_R = 23.2 min (purity 94%); λ_{max} (recorded during the HPLC analysis) 254 nm. Too hygroscopic compound for suitable elemental analysis.

Mono-fluoro-sulfonated benzoic acid (4). To a stirred solution of *tert*-butyl ester **3** (13.5 mg, 0.04 mmol) in CH_2Cl_2 (1 mL) was added a solution of TFA in CH_2Cl_2 (1 mL, 1 : 1, v/v). The resulting reaction mixture was vigorously stirred at rt for 1 h. Completion of the reaction was checked by analytical RP-HPLC (system A). Then, the reaction mixture was evaporated under reduced pressure and co-evaporated thrice with toluene (3 × 20 mL) to give the desired product **4** as a white solid (11.2 mg, quantitative yield). δ_H (300 MHz, CD_3OD) 8.14 (m, 4H), 5.12 (dd, *J* 4.2, 9.4, –C*H*(SO_3H), 1H), 4.70–4.29 (m, –C*H*$_2$F, 2H), 2.77–2.43 (m, –C*H*$_2$–CH_2F, 2H); δ_C (75.5 MHz, CD_3OD) 39.5 (d, *J* 30.2, –*C*H$_2$–CH_2–F), 72.0 (d, *J* 6.0, –*C*H(SO_3H)), 92.0 (d, *J* 242.1, 1 × –*C*H$_2$–F), 138.5 (2 × *C*H–Ph), 138.6 (2 × *C*H–Ph), 143.3 (1 × Cq–Ph), 150.6 (1 × Cq–Ph), 176.3 (1 × Cq, *C*O_2H), 204.4 (1 × Cq, *C*O-aryl ketone); δ_F (282.5 MHz, CD_3OD) −220.7 (tt, *J* 24.5, 48.0, –CH_2*F*, 1F); MS (ESI$^-$): *m*/*z* 289.00 [M − H]$^-$, calcd for $C_{11}H_{11}FO_6S$ 290.03; HRMS (ESI$^-$): *m*/*z* 289.0182 [M − H]$^-$, calcd for $C_{11}H_{10}FO_6S^-$ 289.0182; HPLC (system A): t_R = 13.5 min (purity 95%); λ_{max} (recorded during the HPLC analysis) 254 nm. Too hygroscopic compound for suitable elemental analysis.

[^{19}F]-Prosthetic group (1). Benzoic acid **4** (48 mg, 0.165 mmol) was dissolved in peptide synthesis-grade NMP (1.5 mL). TSTU (49.8 mg, 0.165 mmol, 1 equiv.) and DIEA (165 μL of a 2.0 M solution in NMP, 0.33 mmol, 2 equiv.) were sequentially added and the resulting reaction mixture was stirred at rt for 30 min. The reaction was checked for completion by ESI mass spectrometry. The crude NHS ester was used in the next amidification reactions without prior purification–isolation. MS (ESI$^-$): *m*/*z* 386.00 [M − H]$^-$, calcd for $C_{15}H_{14}FNO_8S$ 387.04.

Fluoro-monosulfonated cyanine "cold" reference (7). Cyanine amino-amide **6** (19.25 mg, 30.1 μmol) was dissolved in NMP (1 mL) and DIEA (180 μL of a 2.0 M solution in NMP, 360 μmol, 12 equiv.). 500 μL of a 90 mM solution of NHS ester **1** in NMP was added and the resulting reaction mixture was stirred at rt overnight. The reaction was checked for completion by RP-HPLC (system B). Thereafter, the reaction mixture was diluted with aq. TEAB and purified by semi-preparative RP-HPLC (system E, 1 injection, t_R = 42.0–46.0 min). The product-containing fractions were lyophilised and desalted by semi-preparative RP-HPLC (system F) to give the TFA salt of fluoro-monosulfonated cyanine **7** (12 mg, 13 μmol, yield 43%). δ_H (300 MHz, $CDCl_3$) 8.29 (bt, *J* 5.0, 1H, N*H*), 8.15–7.30 (m, N*H*, Ph-benzamide, Ph-benzoindole and 2 × CH=C*H*–CH=C, 17H), 7.01 (t, *J* 12.5, C*H*=CH–CH=C, 1H), 6.66 (d, *J* 13.8, C*H*=CH–CH=C, 1H), 6.33 (d, *J* 13.5, C*H*=CH–CH=C, 1H), 5.18 (m, C*H*(SO_3H), 1H), 4.64–4.07 (m, C*H*$_2$F and 2 × *N*(benzoindole)–C*H*$_2$, 6H), 3.52 (bm, C*H*$_2$–NH–C(O), 2H), 2.85–2.60 (m, –C*H*$_2$–CH_2F, 2H), 2.32 (bt, *J* 7.0, C*H*$_2$–$CONH_2$, 2H), 1.96 (s, 4 × C*H*$_3$(benzoindole), 12H) 1.90–1.44 (m, 5 × –C*H*$_2$–, 10H); δ_C (75.5 MHz, $CDCl_3$) 25.0, 26.1, 27.4, 27.8 (4 × *C*H_3), 30.5 (d, *J* 20.4, –*C*H_2–CH_2–F), 34.7, 39.5, 44.1, 44.5, 50.9 (1 × *C*$(CH_3)_2$), 51.1 (1 × *C*$(CH_3)_2$), 62.8 (1 × *C*H(SO_3H)), 77.4 (masked by $CHCl_3$), 82.4 (d, *J* 164.6, *C*H_2F), 103.6, 104.7, 110.7, 122.1, 125.0, 125.2, 126.8, 127.8, 128.3, 129.3, 130.3, 130.8, 130.9, 131.9, 132.0, 133.6, 134.0, 138.0, 139.4, 139.5, 139.6, 151.3, 152.2, 167.5, 173.6 (1 × *C*ONHR), 174.2 (2 × *C*H), 179.8 (1 × Cq, *C*ONH_2), 195.0 (1 × Cq, *C*O-aryl ketone); δ_F (282.5 MHz, $CDCl_3$) −75.5 (s, C*F*$_3$-TFA, 3F), −218.3 (m, –CH_2*F*, 1F); MS (ESI$^+$): *m*/*z* 911.20 [M + H]$^+$, 1011.93 [M + TEA + H]$^+$, MS (ESI$^-$): *m*/*z* 909.33 [M − H]$^-$, 1023.13 [M + TFA − H]$^-$, 1933.87 [2 M + TFA − H]$^-$, calcd for $C_{54}H_{59}FN_4O_6S$ 910.41; HRMS (ESI$^+$): *m*/*z* 911.4215 [M + H]$^+$, calcd for $C_{54}H_{60}FN_4O_6S^+$ 911.4217; HPLC (system B): t_R = 26.0 min, purity > 99%; HPLC (system I): t_R = 23.7 min; λ_{max} (DMSO) nm 689 (ε/dm^3 mol^{-1} cm^{-1} 195 000), λ_{max} em (DMSO) nm 714 (Φ_F 0.42); λ_{max} (PBS + 5% BSA) nm 697 (ε/dm^3 mol^{-1} cm^{-1} 162 000), λ_{max} em (PBS + 5% BSA) nm 712 (Φ_F 0.38).

Fluoro-monosulfonated dodecapeptide "cold" reference (8). Dodecapeptide (its sequence is confidential and not disclosed within this article but it contains a single reactive lysine residue, 2.5 mg, 1.3 μmol, 1 equiv.) was dissolved in H_2O–CH_3CN (1 : 1, v/v, 500 μL) and 2.6 μL of a 2.0 M solution of DIEA in NMP (5.2 μmol, 4 equiv.) was added. 15 μL of a 90 mM solution of NHS ester **1** in NMP was added and the resulting reaction mixture was stirred at rt overnight. The reaction was checked for completion by RP-HPLC (system G). Thereafter, the reaction mixture was diluted with aq. TFA 0.1% and purified by semi-preparative RP-HPLC (system H, 1 injection). The product-containing fractions were lyophilised to give the TFA salt of fluoro-monosulfonated dodecapeptide **8**. MS (ESI$^+$): *m*/*z* 873.13 [M + 2H]$^{2+}$, 1754.80 [M + H]$^+$, MS (ESI$^-$): *m*/*z* 1742.80 [M − H]$^-$, 1856.13 [M + TFA − H]$^-$, 1970.67 [M + 2TFA − H]$^-$, calcd for $C_{76}H_{122}FN_{23}O_{21}S$ 1743.89; HRMS (ESI$^+$): *m*/*z* 872.9517 [M + 2H]$^{2+}$, calcd for ($C_{76}H_{124}FN_{23}O_{21}S^{2+}$)/2 872.9523; HPLC (system A): t_R = 21.3 min, purity 96% (two diastereomers); HPLC (system I): t_R = 15.2 min; λ_{max} (recorded during the HPLC analysis) 261 nm.

Radiosyntheses

Prosthetic group ([^{18}F]-1). We performed the multistep synthesis of this novel prosthetic group on an automated synthesizer TRACERlab MX equipped with standard FDG cassettes which were modified according to our needs. A new Excel® sequence, defining every step of the synthetic procedure, was also developed to control the module *via* a computer. The FDG cassette is composed of three manifolds where solvents and reagents are charged: first manifold (positions 1 to 5), second manifold (positions 6 to 10) and third manifold (positions 11 to 15). The C_{18} and alumina cartridges were removed and the water bag (250 mL) was transferred from positions 7 to 13. A vial of CH_3CN (7 mL) and one containing a solution of TSTU in CH_3CN were respectively placed in positions 3 and 5. All the reactions took place in a single reactor which was cleaned with HCl and deionised water between purification and generation of the active ester. Appropriate detectors permit us to follow the radioactivity during the synthesis, on the QMA and HLB

cartridges, the reactor and the waste bottle. An activimeter was used to measure radioactivity into the final recovery vial. 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] (20.8 mg) in CH_3CN (400 μL) and of Cs_2CO_3 (9.8 mg) in deionised water (200 μL), and transferred to the reaction vial. After azeotropic evaporation of water with CH_3CN (3 × 1 mL, 95 °C, with a stream of N_2 gas), sultone–benzoic acid, *tert*-butyl ester **2** (3.7 mg) in iPrOH (1 mL) was added. The radiolabelling step was conducted in the reaction vial, at 90 °C during 10 min. After cooling, the reaction mixture was diluted with water and loaded onto an Oasis® HLB cartridge. The reaction vial and the cartridge were washed with water, then [^{18}F]-sulfonated *tert*-butyl ester **[^{18}F]-3** was eluted with an aq. solution of CH_3CN (H_2O–CH_3CN, 75 : 25, v/v, 3 mL) and transferred back to the reactor. The *tert*-butyl ester was then removed by treatment with 4.0 M aq. HCl (2 mL) at 80 °C for 5 min while the HLB cartridge was cleaned with CH_3CN (3 mL) and finally rinsed with water (30 mL). After cooling, the reaction mixture was diluted with water and the [^{18}F]-sulfonated benzoic acid **[^{18}F]-4** was trapped onto the Oasis® HLB cartridge. The reaction vial and the cartridge were washed with water, then [^{18}F]-sulfonated benzoic acid **[^{18}F]-4** was eluted with a 10% solution of DIEA in CH_3CN (2 mL) to the reactor. To the formed carboxylate anion was then added a solution of TSTU in CH_3CN (2 mg mL^{-1}, 2 mL, 1.2 equiv. compared with starting sultone–benzoic acid, *tert*-butyl ester **2**) from the second external line and activation was performed at 50 °C for 5 min. The reaction mixture was then transferred to the final vial. The reactor vial was washed with CH_3CN (2 mL) and the washing transferred to the final vial. Activity was measured with the activimeter. [^{18}F]-radiolabelling reagent **[^{18}F]-1** was obtained within 75 min with a moderate 20–30% decay-corrected radiochemical yield (average value from n = 10 preparations) and with a 95% radiochemical purity. HPLC (system I): t_R = 12.1 min.

[^{18}F]-MONOSULFONATED CYANINE ([^{18}F]-7). Cyanine amino-amide **6** was dissolved in CH_3CN containing 1% DIEA. Then, 1.0 mL of the CH_3CN solution of [^{18}F]-fluorosulfonated NHS ester **[^{18}F]-1** (*vide supra*) was added. The vial was vigorously stirred at rt for approximately one min. Thereafter, the reaction was stopped and directly analysed by RP-HPLC (system I with radioactivity detection). HPLC (system I): t_R = 23.6 min. The retention time difference between the UV and radio traces (*ca.* 0.1 min) is caused by the serial arrangement of the detectors. The crude mixture containing the PET/fluorescent tracer was diluted with a large amount of deionised water and purified by SPE using an Oasis® HLB cartridge. First, elution was performed with H_2O–CH_3CN (6 : 4, v/v) to remove unreacted **[^{18}F]-1** and its parent carboxylic acid **[^{18}F]-4**. Thereafter, elution with acetone followed by evaporation of acetone under a nitrogen gas flow provided the desired fluorinated cyanine **[^{18}F]-7** which was finally dissolved in physiological serum.

[^{18}F]-MONOSULFONATED DODECAPEPTIDE ([^{18}F]-8). Dodecapeptide was dissolved in water containing 1% DIEA. Then, 1.0 mL of the CH_3CN solution [^{18}F]-fluorosulfonated NHS ester **[^{18}F]-1** (*vide supra*) was added. The vial was vigorously stirred at rt for less than one minute. Thereafter, the reaction was stopped and directly analysed by RP-HPLC (system I with radioactivity detection). HPLC (system I): t_R = 15.1 min. The retention time difference between the UV and radio traces (*ca.* 0.1 min) was caused by the serial arrangement of the detectors. The crude mixture containing the peptide-based PET tracer was diluted with a large amount of deionised water and purified by SPE using an Oasis® HLB cartridge. First, elution was performed with H_2O–CH_3CN (82 : 18, v/v) to remove unreacted **[^{18}F]-1** and its parent carboxylic acid **[^{18}F]-4**. Thereafter, elution with acetone–citrate buffer (1 : 1, v/v) followed by evaporation of acetone under a nitrogen gas flow provided the desired aq. solution of **[^{18}F]-8** in citrate buffer (750 MBq, 1 mL).

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