ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Article

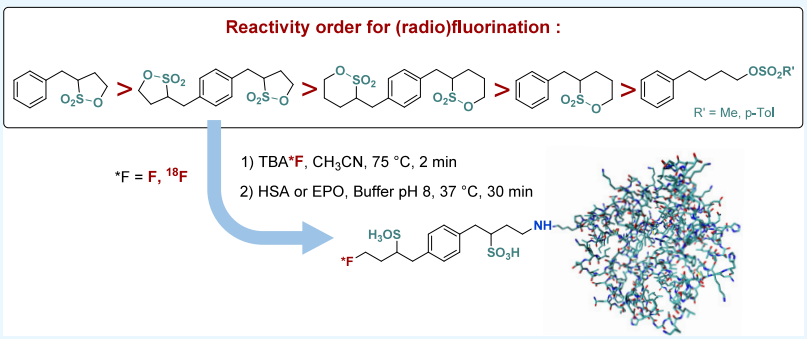
Sultones for Radiofluorination and Bioconjugation: Fluorine-18 Labeling of Native Proteins and Glycoproteins

Anne-Elodie Lafargue, Clémence Maingueneau, Benoit Bernay, Abdelaziz Ejjoummany, and Cécile Perrio*

Cite This: *ACS Omega* 2025, 10, 17983−17992

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information



ABSTRACT: Aliphatic nucleophilic substitution of a sulfonate ester group (such as triflate, mesylate, tosylate, or nosylate) represents a prominent reaction in fluorine-18 chemistry, as illustrated by the radiosynthesis of [^{18}F]FDG (fluorodeoxyglucose) routinely produced for clinical imaging by positron emission tomography (PET). In prior studies, radiofluorination of sultones (i.e., cyclic sulfonate esters) was shown to easily afford, by ring opening, [^{18}F]fluorosulfocompounds as a new class of promising hydrophilic radiopharmaceuticals. Herein, we first depict a further exploration of the ^{18}F-radiochemistry of sultones, including a comparative study with acyclic sulfonate esters. Propane sultones were found to be highly reactive toward [^{18}F]TBAF (tetra-*n*-butylammonium fluoride) under mild anhydrous conditions and far more reactive than acyclic analogues (mesylate and tosylate) and butane sultones. We then developed the ^{18}F-labeling of protein (human serum albumin) and glycoprotein (recombinant human erythropoietin) according to a double ring opening strategy from a bispropane sultone involving radiofluorination followed by subsequent bioconjugation in aqueous buffer solution to the ε-amino group in lysine residues. Overall, the results highlight the distinction of propane sultones vs acyclic analogues for radiofluorination, and they confirm the viability of the bispropane sultone as a novel key precursor for the ^{18}F-radiolabeling of biopolymers under biocompatible conditions. In addition, these findings open the way to the development of innovative radiopharmaceuticals that are especially appropriate for *in vivo* imaging by taking advantage of the anionic sulfo group.

INTRODUCTION

Thanks to their high target specificity and their low immunogenicity, biologics (i.e., peptides, proteins, and glycoproteins) constitute important candidates as diagnostic and theranostic agents, especially in nuclear oncology.[1] In particular, their use as radiopharmaceuticals for positron emission tomography (PET) imaging finds increasing preclinical and clinical applications.[2] Because of its favorable physical properties ($t_{1/2}$ = 109.7 min, 97% β+, E(β+) = 0.64 MeV) as well as its efficient production by bombardment of protons on the $H_2[^{18}O]O$ target according to the $^{18}O(p, n)^{18}F$ nuclear reaction, fluorine-18 represents the most attractive radioisotope for PET probe development and off-site distribution.[3] As a consequence, many efforts have been made to develop methods for the radiolabeling of biologics with fluorine-18. The main difficulties encountered in such a task are related to the high sensitivity of proteins toward temperature, pH, and organic medium leading to their denaturation and also to the need for "rapid" radiochemistry. Most of the recent methods involve the preconjugation of a proper prosthetic group to the native biologics for further direct radiolabeling according to $^{19}F/^{18}F$ exchange or $Al^{18}F$ strategy, or for ligation by inverse electronic demand Diels−Alder (IEDDA) reaction and copper-catalyzed azide−alkyne cycloaddition (CuAAC) to a radiofluorinated reagent (i.e., ^{18}F-

Received: February 12, 2025
Revised: March 19, 2025
Accepted: April 14, 2025
Published: April 24, 2025

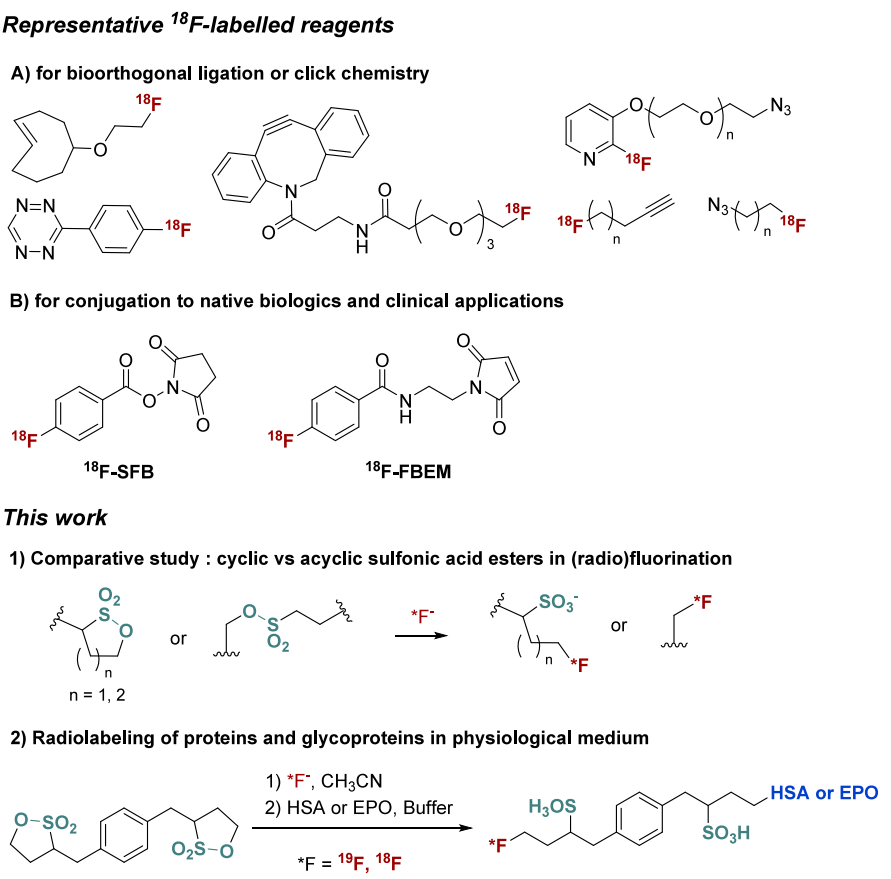


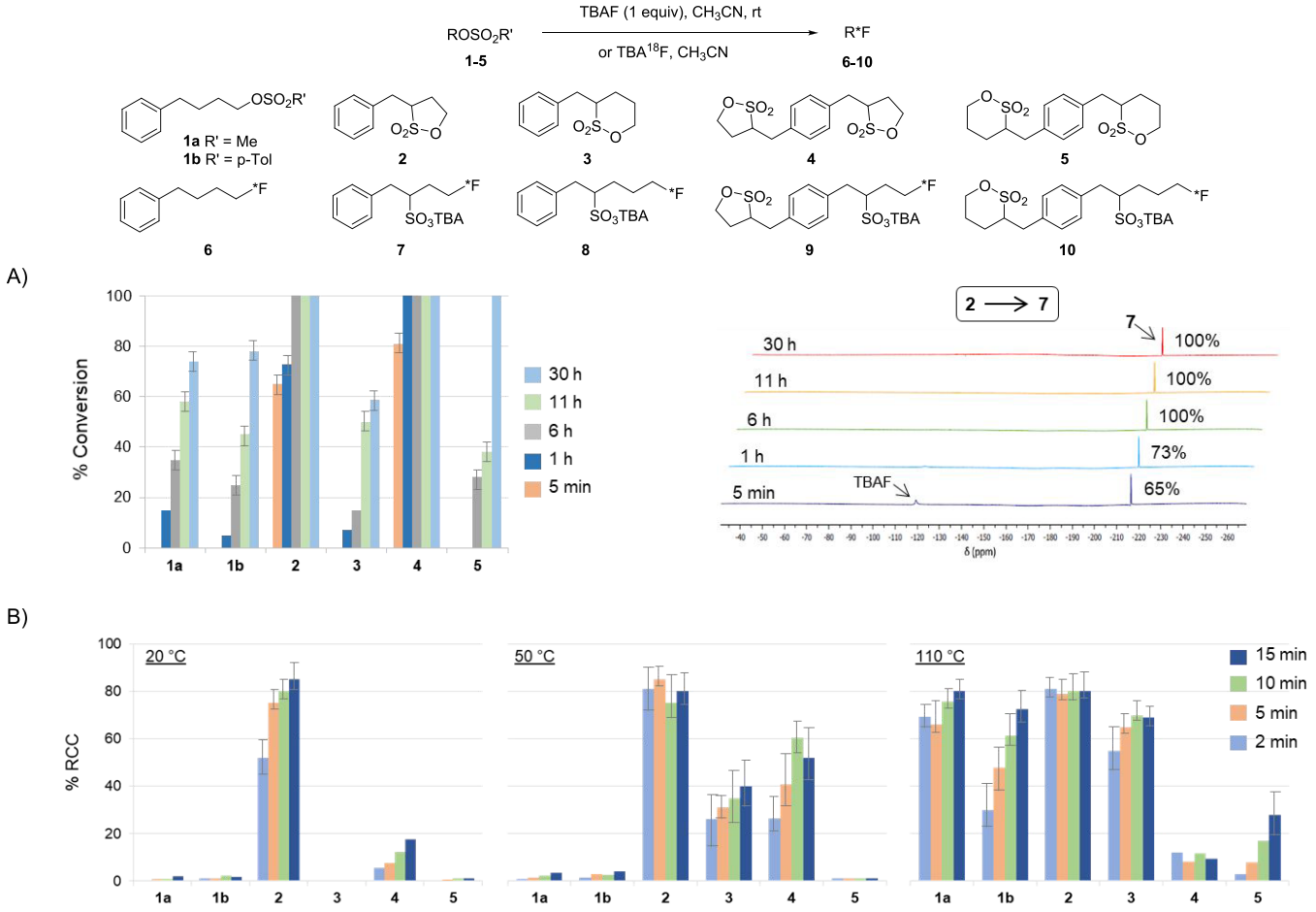
Figure 1. Radiofluorinated reagents for the ^{18}F-radiolabeling of biologics and proposed work.

tetrazine, ^{18}F-trans-cyclooctene, ^{18}F-cyclooctyne, ^{18}F-azide, ^{18}F-alkyne, Figure 1).[4] Those strategies require purification of the intermediate premodified biologics, making the overall processes rather long, tedious, and expensive. Thus, ^{18}F-labeling methods using unmodified biologics undeniably represent attractive alternatives. [^{18}F]SFB (*N*-succinimidyl-4-[^{18}F]fluorobenzoate) and [^{18}F]FBEM (*N*-[2-(4-[^{18}F]-fluorobenzamido)ethyl]maleimide) constitute the most popular radiofluorinated reagents able to label native biopolymers by reaction with lysine side chains or *N*-terminal primary amino groups and with thiol residues from cysteine, respectively.[5] Their radiosyntheses based on nucleophilic aromatic fluorination have to include HPLC purification to successfully perform conjugation to biologics afterward, rendering the overall radiosynthesis complex and time-consuming.

Aliphatic nucleophilic radiofluorination represents a prominent reaction in fluorine-18 chemistry, as illustrated by the radiosynthesis of [^{18}F]FDG (fluorodeoxyglucose) routinely produced for clinical PET imaging.[3] This reaction classically involves the displacement of a sulfonate leaving group (triflate, mesylate, tosylate, or nosylate) by purified cyclotron produced [^{18}F]fluoride anion (conventionally used as K[^{18}F]F/K_2CO_3/K_{222} complex) according to the S_N2 mechanism. The sulfonate/^{18}F exchange usually occurs under smooth heating anhydrous aprotic polar organic solvent conditions with acceptable radiochemical yields after relatively short reaction times (15−30 min). The main limitations are due to side elimination reactions, as well as hydrolysis of the sulfonate ester precursor. Both technical and chemical innovations (i.e., microwave activation,[6] microfluidic conditions,[7] addition of a tertiary alcohol[8] or ionic liquid as cosolvent,[9] in situ sulfonate ester formation,[10] titania catalysis using aqueous [^{18}F]-fluoride,[11] use of arylsulfonate nucleophile assisting leaving groups (NALGs)[12] bearing a potassium chelating unit for stabilization of charge in the transition state, and low basic reaction conditions)[13] have been proposed to optimize the substitution. We previously reported that sultones (i.e., cyclic sulfonate esters) were also prone to radiofluorination by ring opening with [^{18}F]fluoride to give the corresponding [^{18}F]-fluorosulfonic acid derivatives.[14] This reaction widened the range of aliphatic nucleophilic radiofluorinations with supplementary significant advantages. Due to their opposite polarities, sultone precursors and ^{18}F-fluorosulfonic acid products were easily separable by HPLC or even by solid phase extraction (SPE). Moreover, sulfoderivatives are known to possess hydrophilic properties that could be favorable for formulation and *in vivo* applications.[15,16] Based on such a concept, we developed [^{18}F]FLUSONIM as a [^{18}F]-fluorosulfonitroimidazole-based radiopharmaceutical that allowed high-performance PET imaging of hypoxia due to rapid clearance properties related to its high hydrophilicity.[16] We also introduced the double ring opening of bissultones for the ^{18}F-radiolabeling of lysine-based peptides according to radiofluorination-amination sequence (Figure 1).[14] Radiofluorination led to an intermediate ^{18}F-fluorosulfosultone that was then engaged after SPE isolation in the amination reaction with lysine residues. The overall process was found to be beneficial due to efficient radiochemistry and simple automation. To date, application was restricted to a dodecapeptide, with the

https://doi.org/10.1021/acsomega.5c01335
ACS Omega 2025, 10, 17983−17992

Scheme 1. Reaction of Sulfonic Esters 1−5 with TBA*F to Obtain Fluoroproducts 6−10[a]



[a](A) *F = F (left: % conversions calculated by ^{1}H and ^{19}F NMR, right: ^{19}F NMR follow-up for the conversion of **2** to **7**). (B) *F = ^{18}F (% radiochemical conversion (RCC) calculated by radio-TLC and HPLC, average of five assays at least, SEM < ± 12).

two-step sequence performed in anhydrous organic solvent.[17] Nevertheless, we considered that this two-step methodology could offer promising perspectives for the ^{18}F-radiolabeling of native biologics and merit further investigations. In addition, sultone radiofluorination remained under-studied. Our preliminary data just revealed a different chemical behavior between pentane and butane sultones toward [^{18}F]fluoride, with pentane sultones being found to be more reactive than butane sultones.[14] In this paper, we first report a careful examination of the reactivity of propane and butane sultones as well as of bis(sultones) in the (radio)fluorination reaction, including the comparison with traditional acyclic sulfonate esters (Figure 1). We then expanded the bissultone-based double-click process to the ^{18}F-radiolabeling of protein (HSA, human serum albumin) and glycoprotein (rhuEPO, recombinant human erythropoietin) while preserving the biologics in aqueous buffer solutions. Our results demonstrated that the sultone ring opening reaction may constitute a novel tool for bioconjugation strategies beyond ^{18}F-radiolabeling.

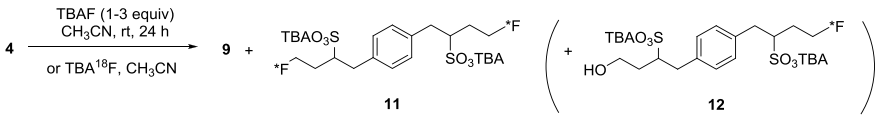
RESULTS AND DISCUSSION

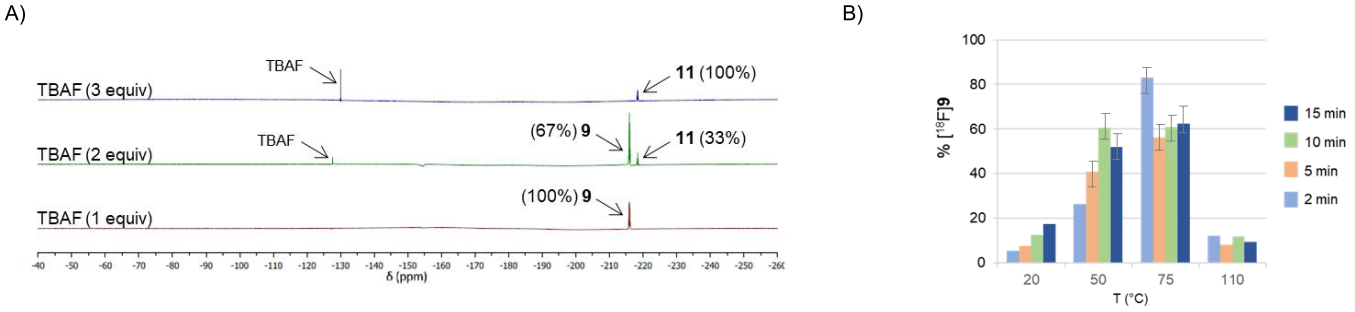
Cyclic versus Acyclic Sulfonic Acid Esters in (Radio)-fluororination. To compare the reactivity of sulfonate esters in cyclic and acyclic versions, mesylate **1a**, tosylate **1b**, sultones **2**,**3**, and bis-sultones **4**,**5** were chosen as model substrates (Scheme 1).[18] Fluorinations of **1**−**5** were first examined under nonradioactive reaction conditions using TBAF (tetra-*n*-butylammonium fluoride) in CH_3CN at room temperature, followed up by ^{19}F NMR analysis (Scheme 1). Both starting sulfonate and TBAF were taken at a 1/1 molar ratio. Recovered TBAF and the expected fluorinated products **6**−**10** were characterized by their typical signals at around −115 and −215 ppm, respectively. Fluorination of mesylate **1a** and tosylate **1b** progressed slowly, and conversions reached only 74−78% after 30 h. Substitutions of mesylate **1a** were significantly higher than those of tosylate **1b** at each intermediate time (i.e., 15 vs 5% at 1 h, 35 vs 25% at 6 h, and 58 vs 45% at 11 h), demonstrating that reactivity of mesylate **1a** was superior to that of tosylate **1b**. Propane sultone **2** displayed very high reactivity with 65% conversion at 5 min. At 6 h, the completion was observed. Butane sultone **3** was found to be less reactive than propane sultone **2** (in accordance with the lower ring strain of **3** versus **2**) and also less reactive than mesylate **1a** and tosylate **1b**, with a conversion rate of only 59% after 30 h. To summarize, propane sultone **2** displayed the highest reactivity, followed by mesylate **1a**, tosylate **1b**, and then butane sultone **3**.

Bissultones **4** and **5** were found to be more reactive than their monosultone analogues **2** and **3**, respectively, probably due to their two sulfonate ester functions. In particular, bispropane sultone **4** was remarkably reactive, with 81% conversion at 5 min and quantitative transformation after 1 h.

https://doi.org/10.1021/acsomega.5c01335
ACS Omega 2025, 10, 17983−17992

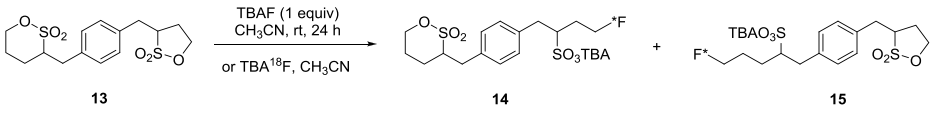
Scheme 2. Reaction of the Symmetric Bissultone 4 with TBA*F[a]

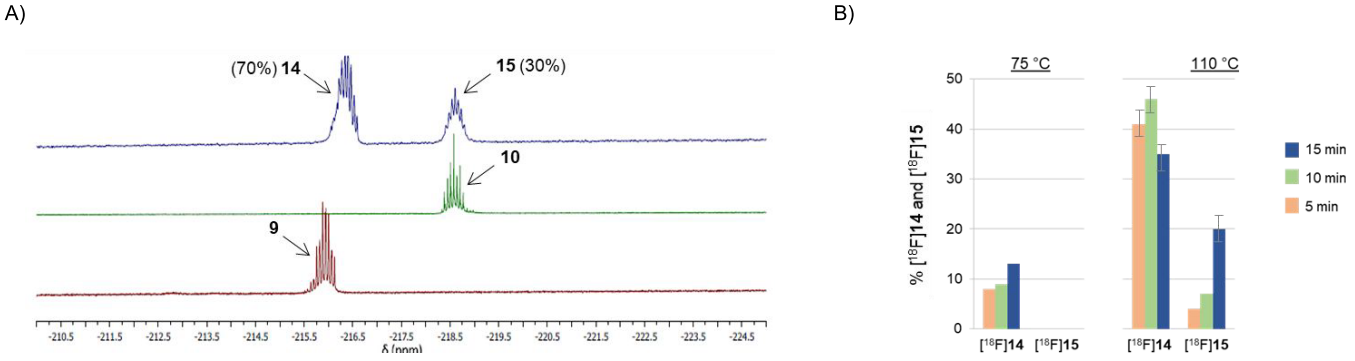




[a](A) *F = F ([19]F NMR spectra of the crude product using 1−3 equiv TBAF). (B) *F = [18]F (%[[18]F]**9** in the crude reaction mixture calculated by radio-TLC and HPLC, average of five assays at least, SEM < ± 9).

Scheme 3. Reaction of the Dissymmetric Bissultone 13 with TBA*F[a]





[a](A) *F = F ([19]F NMR spectra of the resulting crude 7/3 mixture **14** and **15**, and of fluorosulfonates **9** and **10**). (B) *F = [18]F (% [[18]F]**14** and [[18]F]**15** in the crude reaction mixture calculated by radio-TLC and HPLC, average of five assays at least, SEM < ± 8).

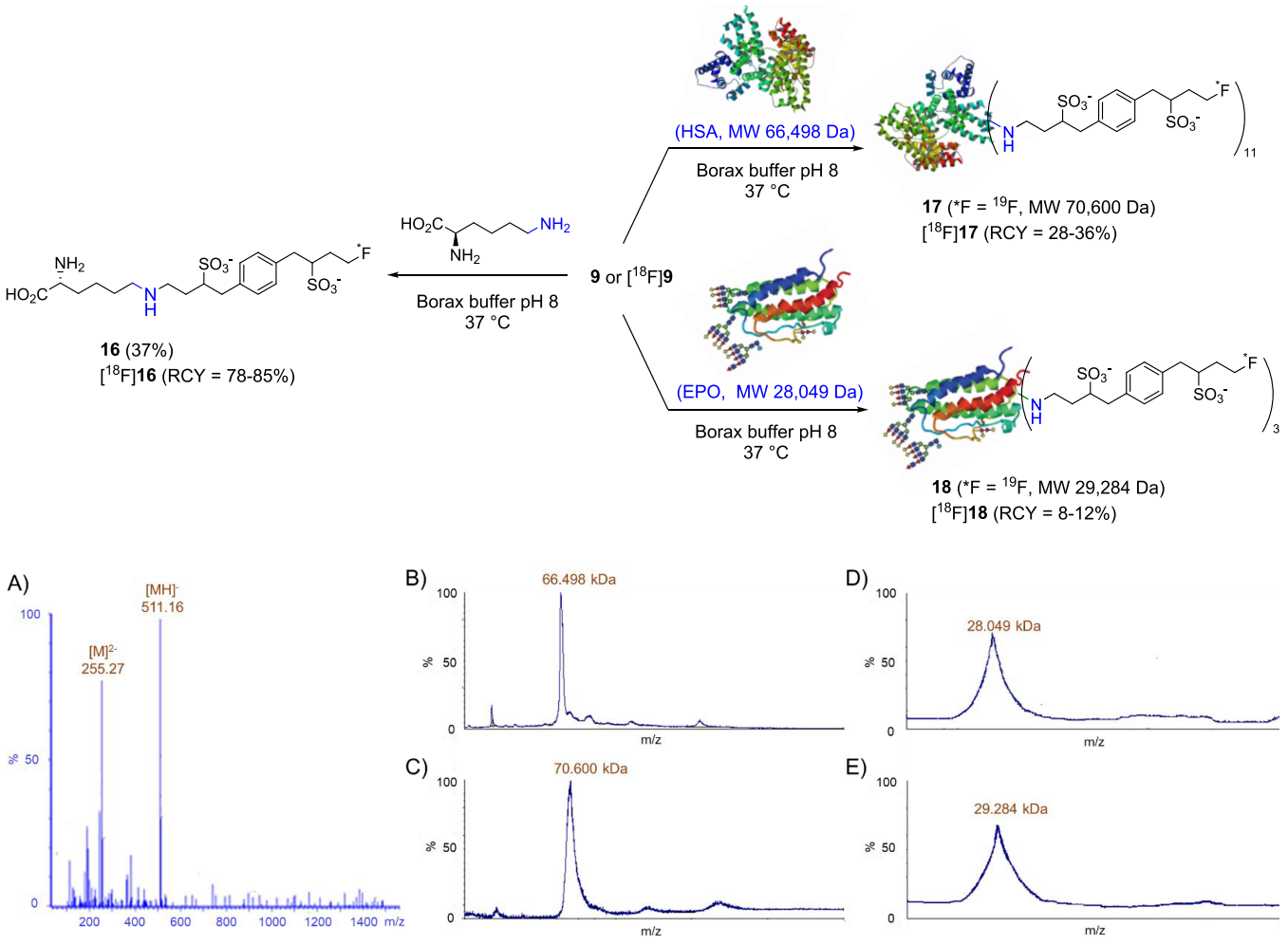
Fluorination of compound **5** was quite slow but complete after 30 h. The products formed from bissultones **4** and **5** were identified as monofluorinated products **9** and **10**. Nevertheless, because of the possibility of the double fluorination, we also characterized the difluorinated compounds. We carried out fluorination of bis-sultone **4** using 1, 2, and 3 equiv of TBAF and analyzed by [19]F NMR after 24 h (Scheme 2). With 1 equiv, only a single signal at −216 ppm was detected and was attributed to monofluoroproduct **9**. No trace of remaining TBAF was visible, in accordance with a quantitative conversion. With 2 equiv, a second signal at −218 ppm corresponding to the difluorocompound **11** appeared, and traces of TBAF were also detected. By integration of the two peaks at −216 and −218 ppm, a 2:1 ratio was evaluated for the **9** and **11** mixture. With 3 equiv of TBAF, the peak at −218 was still present but not the peak at −216, confirming the quantitative formation of the difluorinated product **11** and the total consumption of the monofluorinated compound **9**. The peak at −115 also visible was due to recovered unchanged TBAF used in excess. By analogy, as only one single peak was revealed for fluorination of bisbutane sultone **5** with 1 equiv of TBAF, the structure of **10** was assigned (identification of products **9**−**11** was also confirmed by [1]H, [13]C NMR and LC-MS analyses; see Supporting Information). These results suggested that the first fluorination of bissultones **4** and **5** was easier than the second one.

For radiofluorination, we used [[18]F]TBAF (in mixture with $TBAHCO_3$) rather than the conventional K[[18]F]F/K_{222}/K_2CO_3 complex to match the nonradioactive study. We also observed that propane sultone **2** rapidly reacted with Kryptofix K_{222}. All assays were performed manually, and they included independently, before radiofluorination, the preparation of dried [[18]F]TBAF from cyclotron produced [[18]F]fluoride (74−110 MBq). Radioactive reactions were carried out in CH_3CN at 20, 50, and 110 °C for 15 min using 5 mg of precursor (Scheme 1).

Unsurprisingly, the transformation of mesylate **1a** and tosylate **1b** to the corresponding radiofluorinated product [[18]F]**6** occurred only at 110 °C (70−78% RCC, radiochemical conversion). As for the nonradioactive reaction, mesylate **1a** was converted more rapidly than tosylate **1b**. Propanesultone **2** was remarkably reactive at 20 °C, with 85% RCC on average

https://doi.org/10.1021/acsomega.5c01335
ACS Omega 2025, 10, 17983−17992

Scheme 4. Bioconjugation Reactions from 9 or [^{18}F]9, with MS Spectrum of 16 (A) and MALDI-TOF MS Spectra of Native HSA (B), HSA-Conjugate 17 (C), Native rhuEPO (D), and rhuEPO Conjugate 18 (E)



after 15 min. Averaged RCC also reached 80% after only 2 min under heating at 50 or 110 °C. For butanesultone **3**, the RCC was the highest (about 65%) at 110 °C, moderate (close to 40%) at 50 °C, and zero at 20 °C. Butanesultone **3** was more reactive than mesylate **1a** and tosylate **1b** in radiofluorination, contrary to the results obtained for nonradioactive fluorination. Surprisingly, the RCC of bispropanesultone **4** to [^{18}F]**9** was poor (<18%) at 20 and 110 °C whatever the reaction time. At 50 °C, the formation of [^{18}F]**9** was moderate (around 60% RCC) after 10 min and then decreased. The radiofluorination of bisbutanesultone **5** did not occur at 20 and 50 °C and proceeded slowly at 110 °C to reach up to about 25% RCC after 15 min. The overall results for radiofluorination were globally consistent with those found under nonradioactive conditions, except for bispropanesultone **4**.

As the formation of [^{18}F]**9** dramatically fell down when the radiofluorination temperature passed from 50 to 110 °C, we studied the reaction at 75 °C (Scheme 2). RCCs reached 83% on average at 2 min and then decreased to around 60% at 15 min. From 5 min, we observed the formation of a novel radioactive product that was identified as [^{18}F]-fluorobissulfonate [^{18}F]**12** resulting from the ring opening of [^{18}F]**9**. The formation of [^{18}F]**12** was low (<5% RCC) at 50 °C and consistent (37% RCC) after 15 min at 110 °C. As a remark, bispropanesultone **4** in CH_3CN in the presence of $TBAHCO_3$ (used for the preparation of [^{18}F]TBAF) also underwent ring opening at 110 °C for 15 min and was totally consumed.

In further studies, we planned to exploit the difference of reactivity between the propane and butane sultone rings, and we synthesized the nonsymmetric bissultone **13** (Scheme 3). Indeed, we hypothesized that bissultone **13** would possess high reactivity for radiofluorination due to the propanesultone ring leading to preferentially the product [^{18}F]**14** and that [^{18}F]**14** would remain inert toward hydrolysis due to the stable butanesultone moiety. We also anticipated that the formation of regioisomer [^{18}F]**15** coming from radiofluorination of the butanesultone moiety would not be favored. First, bissultone **13** was treated with TBAF (1.1 equiv) in CH_3CN at room temperature for 24 h. The ^{19}F NMR spectrum of the resulting product displayed two signals at −216 and −219 ppm attributed to fluorosulfosultones **14** and **15**, respectively, by analogy with products **9** and **10**. The identification of monofluoro-compounds **14** and **15** was confirmed by LC-MS, and no difluoro-product was detected. The fluorosulfo-sultones **14** and **15** were in a 7:3 ratio, in accordance with the favored ring opening of the propanesultone versus the butanesultone. The radiofluorination of bissultone **13** was then realized with [^{18}F]TBAF at room temperature, 50, 75, 110, and 130 °C for 15 min. No radiofluorination was

https://doi.org/10.1021/acsomega.5c01335
ACS Omega 2025, 10, 17983−17992

observed at room temperature and 50 °C as well as at 130 °C probably due to degradation of precursor **13** (data not showed). At 75 °C, the RCC was low (about 13%) and only [^{18}F]**14** was formed (Scheme 3). At 110 °C, the two radiofluorinated products [^{18}F]**14** and [^{18}F]**15** were obtained in around 35 and 20% RCCs, respectively, after 15 min. We considered that the chemoselectivity of the (radio)fluorination was not high enough to pursue investigations with **13**. We then undertook to study conjugation and radiolabeling of proteins and glycoproteins with only sultone fluorosulfonates **9** and [^{18}F]**9**.

Bioconjugation and Radiolabeling of Biopolymers. Lysine residues are the most common targets in protein bioconjugation. This amino acid contains a nucleophilic ε-amine group often accessible for electrophilic reagents, and it is generally more abundant in proteins than other amino acids that also offer nucleophilic sites such as cysteine with its thiol function.[19] Prior to examination of conjugation and radiolabeling of biopolymers, we checked the reactivity of the sultone fluorosulfonates **9** and [^{18}F]**9** toward lysine in aqueous buffer (pH 8) solution at 37 °C (Scheme 4). Reaction of **9** (1 equiv) with lysine (1 equiv) lasted 14 h. LC-MS analysis revealed a 37/18/45 mixture of expected conjugate **16**, hydrolysis product **12**, and unchanged **9**. Although slow and incomplete, the ring opening of **9** was demonstrated to occur. We then performed the reaction of [^{18}F]**9** (15−45 MBq) with lysine for 30 min in buffer pH 8 at 37 °C. The RCC of [^{18}F]**9** to [^{18}F]**16** determined by HPLC analysis reached 85%. Unchanged [^{18}F]**9** and hydrolysis product [^{18}F]**12** were also detected at only about 3 and 12%, respectively. Although nonradioactive and radioactive conditions could not be compared in terms of stoichiometry and kinetics, the radioactive reaction performed much better than the nonradioactive one. This result was highly encouraging for biopolymers radiolabeling. We then used HSA and rhuEPO as protein and glycoprotein models (Scheme 4). Treatment of HSA (5 mg) or rhuEPO (25 μg) with excess sultone **9** in buffer (pH 8) for 14 h at 37 °C followed by PD-10 purification afforded conjugates **17** and **18**. Maldi-TOF MS analyses of conjugates **17** and **18** revealed an additional mass of 4102 and of 1235 Da, respectively (compared to native HSA and rhuEPO, respectively), corresponding to the grafting of 11 patterns **9** in HSA and only 3 patterns **9** in rhuEPO. The conjugation on 11 sites of HSA, and on 3 sites of rhuEPO, was consistent with the theoretical number of lysine residues available for bioconjugation, i.e., 15 for HSA[20] and 6 for rhuEPO.[21] Radiolabeling reactions were carried out by mixing HSA (0.5 mg) or rhuEPO (50 μg) with previously manually prepared [^{18}F]**9** (15−45 MBq) in buffer at pH 8 at 37 °C for 30 min. HPLC analyses of the crude mixtures revealed the formation of the expected conjugates [^{18}F]**17** (28−36% RCC) and [^{18}F]**18** (8−12% RCC) besides unchanged [^{18}F]**9** and [^{18}F]**12** (coming from hydrolysis of [^{18}F]**9**) detected as the sole byproducts. Given these promising results, and in order to obtain significant amounts of radioactivity of isolated [^{18}F]**17** and [^{18}F]**18**, the radiolabeling of HSA and rhuEPO was repeated using [^{18}F]**9** (110−185 MBq) prepared on a TRACERlab GE FX FN module (GE Healthcare) from cyclotron produced [^{18}F]fluoride (1−18 GBq). Crude [^{18}F]**17** and [^{18}F]**18** were recovered after the conjugation step, and they were purified manually on a PD-10 column. [^{18}F]**17** and [^{18}F]**18** were isolated in 15−21 and 4−9% activity yields (calculated from [^{18}F]fluoride, n = 5), respectively, after 45 min total radiosynthesis time. Extending the conjugation time to 60 min did not significantly increase the formation of [^{18}F]**17** and [^{18}F]**18**; hydrolysis of [^{18}F]**9** to [^{18}F]**12** was favored. It is noteworthy that [^{18}F]SFB was also subject to hydrolysis during conjugation to biopolymers, and in greater proportion than [^{18}F]**9**. Although the activity yield was low, the radiolabeling of rhuEPO was significant using rhuEPO on a scale of a few tens of μg. To our knowledge, our work represents the first ^{18}F-radiolabeling of native rhuEPO reported so far.[22]

CONCLUSIONS

In summary, propane sultones were demonstrated to possess a distinct reactivity compared to that of acyclic analogues and butane sultones. They were highly reactive toward $^{18/19}$F-fluoride, and the reactivity order for the sulfonate ester displacement by $^{18/19}$F-fluoride was propane sultones ≫ mesylate > tosylate ∼ butane sultones. Consequently, we finally valued bissultone **4** in a double ring opening sequence involving (radio)fluorination and then bioconjugation in aqueous buffer solution to HSA and rhuEPO presumably through the ε-amino group in lysine residues. Thus, HSA and rhuEPO were radiolabeled with fluorine-18 in 15−21 and 4−9% activity yields, respectively, within 45 min total radiosynthesis time. This two-step sequence only required the precursor bissultone **4** and the biologics as chemicals in addition to [^{18}F]fluoride, and it constitutes a new metal free methodology for the ^{18}F-radiolabeling of lysine containing native biopolymers. This method is advantageous compared to the [^{18}F]FSB strategy in terms of number of steps and efficiency. Moreover, it provides functionalization of biologics with a poorly bulky and charged bissulfogroup that made the radiolabeling totally original compared to the usual approaches. The impact of such a functionalization on *in vivo* properties and application to bioconjugation to native biopolymers beyond ^{18}F-radiolabeling will be reported in due course.

EXPERIMENTAL SECTION

General Information. All commercially available reagents, including HSA, and HPLC and LC-MS quality solvents, were purchased from Aldrich and used as received without further purification. rhuEPO was purchased from ProteoGenix. Sulfonate esters **1a** [RN 65512-08-5],[23] **1b** [RN 112775-09-4],[24] **1c** [RN 172147-79-4],[25] **2** [RN 75732-43-3],[14] and **3** [RN 1344707-66-9][14] were prepared as previously described. For preparation of borax buffer at pH 8, borax (0.025 M, 50 mL) was added to a mixture of HCl (0.1M, 20.5 mL) and H_2O (29.5 mL). Borax buffer was mixed for 30 min at room temperature before use. Flash chromatography was carried out on silica gel (Merck Kieselgel 60 F_{254}, 40−63 μm). Thin-layer chromatography (TLC) was performed on Merck plastic-backed plates precoated with silica gel 60F_{254}. Spots were revealed by a UV lamp at 254 nm and/or with $KMnO_4$. ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz), and ^{19}F NMR (376 MHz) spectra were recorded on a Bruker DPX 400 spectrometer. Chemical shifts δ are reported in parts per million (ppm) referenced to proton resonances resulting from incomplete deuteration of the NMR solvent. Coupling constants (J) are given in Hertz (Hz). Coupling patterns are abbreviated as follows: s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet), dd

https://doi.org/10.1021/acsomega.5c01335
ACS Omega 2025, 10, 17983−17992

(doublet of doublet), and dt (doublet of triplet). High-performance liquid chromatography (HPLC) was performed on a Waters Alliance e2695 separation module, a Waters 2998 photodiode array detector (190−380 nm), and a Berthold Herm LB 500 activity detector. Two chromatographic systems, A and B, were used for the analysis of radiosyntheses. [*System A:* analytical HPLC equipped with a Macherey-Nagel Nucleodur 100-3 Hilic column (150 × 4.6 mm, 3 μm) at 1 mL/min flow rate used in gradient method with acetonitrile and aqueous ammonium acetate 100 mM as eluents: isocratic 97% acetonitrile for 3 min, then linear gradient 97 to 50% acetonitrile over 2 min, and then isocratic 50% acetonitrile for 5 min. *System B:* analytical HPLC equipped with a Phenomenex BioSep SEC-s2000 column (7.8 × 300 mm) at a 1 mL/min flow rate with a buffer solution (pH 6.8) of sodium phosphate (50 mM) and NaCl (300 mM) as eluent used in isocratic mode]. LC-MS analyses were performed on a Waters Acquity UPLC LC apparatus equipped with a Waters reversed-phase Acquity UPLC BEH C18 column (2.1 mm × 75 mm, 1.7 μm) eluted with a gradient of MeOH/H_2O-0.1% formic acid [linear gradient from 10:90 to 90:10 (20 min), and then isocratic mode at 10:90 (10 min)] at a flow rate of 0.3 mL/min, linked to an electrospray MS Waters Q-TOF micro spectrometer. The source temperature of MS was 300 °C, and the analyses were performed in the appropriate electron ionization mode (ES^+ or ES^-). Maldi-TOF mass spectrum experiments were carried out on an AB Sciex 5800 proteomics analyzer equipped with TOF TOF ion optics and an OptiBeam on-axis laser irradiation with a 1000 Hz repetition rate. The system was calibrated immediately before analysis with a mixture of trypsinogen (bovine), enolase (yeast), and serum albumin (bovine), and mass precision was better than 500 ppm. For experiment, a 1 μL volume of protein sample was mixed with 10 μL volumes of solutions of sinapinic acid matrix prepared in a diluent solution of 40% ACN with 0.1% TFA. The mixture was spotted on a stainless steel Opti-TOF 384 target; the droplet was allowed to evaporate before introducing the target into the mass spectrometer. A laser intensity of 6500 nm was typically employed for ionizing. MS spectra were acquired in the positive high mass linear mode by summarizing 4000 single spectra (20 × 200) in the mass range from 20 000 to 120 000 Da.

Chemistry. *3,3′-[1,4-Phenyl-bis(methylene)]bis(1,2-oxathiolane-2,2-dioxide) **4**.* [RN 1344707-91-0] was obtained according to the modified literature procedure using LiHMDS as follows. In a two-neck round-bottom flask (A) under nitrogen were introduced anhydrous THF (50 mL) and 1,1,1,3,3,3-hexamethyldisilazane (HMDS, 3.4 mL, 16.4 mmol, 3.3 equiv). After cooling to 0 °C, *n*-butyllithium (1.6 M in hexane, 10.3 mL, 16.4 mmol, 3.3 equiv) was added dropwise. The mixture was stirred at 0 °C for 15 min and then at −78 °C for 10 min. In a second two-neck round-bottom flask (B) under nitrogen were introduced anhydrous THF (100 mL), propane-1,3-sultone (2.0 g, 16.4 mmol, 3 equiv), and 1,4-bis(bromomethyl)benzene (1.44 g, 5.5 mmol, 1 equiv). The mixture was cooled to −98 °C. The LiMDS solution in flask A was then transferred into flask B. The final mixture was stirred at −98 °C for 4 h, allowed to warm slowly to 0 °C, and then quenched with water. After addition of ethyl acetate, the organic phase was washed with water and then brine, dried over $MgSO_4$, and concentrated under reduced pressure. The residue was recrystallized with petroleum spirit at 0 °C to afford the title compound **4** as a white solid (1.74 g, 92%). Spectroscopic data were in accordance with those previously reported.[14]

*3,3′-[1,4-Phenyl-bis(methylene)]bis(1,2-oxathiane-2,2-dioxide) **5**.* In a two-necked round-bottom flask under nitrogen stream were introduced butane-1,4-sultone (0.75 mL, 7.3 mmol, 3 equiv) and anhydrous THF (5 mL). The mixture was cooled to −78 °C, and then *n*-butyllithium (1.6 M in hexane, 3.3 mL, 8.03 mmol, 3.3 equiv) was added dropwise. The mixture was stirred at −78 °C for 15 min, and then 1,4-bis-bromomethyl-benzene (0.64 g, 2.4 mmol, 1 equiv) in THF (2 mL) was added dropwise. The mixture was stirred at −78 °C for 4 h, allowed to warm slowly to 0 °C, and then quenched with H_2O. After extraction with AcOEt, the combined organic fractions were washed with H_2O, dried over $MgSO_4$, and then concentrated under reduced pressure. Recrystallization at room temperature with petroleum spirit afforded the title compound **5** as a white solid (700 mg, 76%). Mp: 240−241 °C. Rf: 0.46 (AcOEt). ^1H NMR ($CDCl_3$, 400 MHz): δ 7.14 (s, 4H), 4.58−4.41 (m, 4H), 3.44 (dd, J = 13.8 and 4.0 Hz, 2H), 3.26−3.20 (m, 2H), 2.73 (dd, J = 13.8 and 10.6 Hz, 2H), 1.98−1.75 (m, 8H). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz): δ 134.9, 129.7, 73.7, 60.6, 33.8, 27.6, 24.0. HRMS (ESI^+): calcd for $C_{16}H_{22}NaO_6S_2$: 397.0751 $[M + Na]^+$; found: 397.0756.

*3-(4-((2,2-Dioxido-1,2-oxathiolan-3-yl)methyl)benzyl)-1,2-oxathiane 2,2-Dioxide **13**.* The product **13** was prepared according to a two-step synthesis (see the Supporting Information). In a two-neck round-bottom flask (A) under nitrogen were introduced anhydrous THF (50 mL) and 1,1,1,3,3,3-hexamethyldisilazane (HMDS, 2.5 mL, 13.9 mmol, 1.7 equiv). After cooling to 0 °C, *n*-butyllithium (1.6 M in hexane, 8.6 mL, 13.9 mmol, 1.7 equiv) was added dropwise. The mixture was stirred at 0 °C for 15 min and then at −78 °C for 10 min. In a second two-neck round-bottom flask (B) under nitrogen were introduced anhydrous THF (100 mL), propane-1,3-sultone (1.5 g, 12.3 mmol, 1.5 equiv), and 1,4-bis(bromomethyl)benzene (2.2 g, 8.2 mmol, 1 equiv). The mixture was cooled to −98 °C. The LiMDS solution in flask A was then transferred into flask B. The final mixture was stirred at −98 °C for 4 h, allowed to warm slowly to 0 °C, and then quenched with water. After addition of ethyl acetate, the organic phase was washed with water and then brine, dried over $MgSO_4$, and concentrated under reduced pressure. Purification on silica gel using pentane/AcOEt 1:0 to 7:3 as eluent afforded 3-(4-(bromomethyl)benzyl)-1,2-oxathiolane 2,2-dioxide **13′** as a white solid (154 mg, 9%). Mp: 90−91 °C. Rf: 0.1 (pentane/AcOEt 9:1). ^1H NMR ($CDCl_3$, 400 MHz): δ 7.37 (d, J = 8.0 Hz, 2H), 7.21 (d, J = 8.0 Hz, 2H), 4.37 (s, 2H), 4.43 (dt, J = 8.8 and 3.8 Hz, 1H), 4.33 (dt, J = 8.8 and 7.2 Hz, 1H), 3.51−3.48 (m, 1H), 3.37 (dd, J = 14.1 and 5.7 Hz, 1H), 2.88 (dd, J = 14.1 and 9.5 Hz, 1H), 2.55−2.47 (m, 1H), 2.34 (dq, J = 13.3 and 8.5 Hz, 1H). $^{13}C\{^1H\}$ NMR ($CDCl_3$, 100 MHz): δ 137.1, 136.3, 129.7, 129.3, 66.9, 56.4, 34.3, 32.9, 29.2. HRMS (ESI^+): calcd for $C_{11}H_{13}BrNaO_3S$: 326.9666 $[M + Na]^+$; found: 326.9668.

In a two-necked round-bottom flask under nitrogen stream was introduced butane-1,4-sultone (0.4 mL, 3.93 mmol, 2 equiv) and anhydrous THF. The mixture was cooled to −78 °C and then *n*-butyllithium (1.6 M in hexane, 2.7 mL, 4.33 mmol, 2.2 equiv) was added dropwise. The mixture was stirred at −78 °C for 15 min, and then bromobenzylsultone **13′** (0.6 g, 1.97 mmol, 1 equiv) in THF (2 mL) was added dropwise. The mixture was stirred at −78 °C for 4 h, allowed to warm slowly to 0 °C, and then quenched with H_2O. After extraction

https://doi.org/10.1021/acsomega.5c01335
ACS Omega 2025, 10, 17983−17992

with AcOEt, the combined organic fractions were washed with H_2O, dried over $MgSO_4$, and then concentrated under reduced pressure. Purification on silica gel using 1:1 pentane/AcOEt as eluent afforded the title compound **13** as a white solid (87 mg, 12%). Mp: 223−224 °C. Rf: 0.40 (pentane/AcOEt 1:1). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.20−7.16 (m, 4H), 4.60−4.54 (m, 1H), 4.48−4.41 (m, 2H), 4.37−4.31 (m, 1H), 3.54−3.44 (m, 2H), 3.35 (dd, J = 14.2 and 6.0 Hz, 1H), 3.28−3.22 (m, 1H), 2.89 (dd, J = 14.2 and 9.1 Hz, 1H), 2.76 (dd, J = 13.7 and 10.7 Hz, 1H), 2.56−2.48 (m, 1H), 2.35 (dq, J = 13.2 and 8.5 Hz, 1H), 2.02−1.88 (m, 2H), 1.87−1.81 (m, 2H). ^{13}C{^{1}H} NMR ($CDCl_3$, 100 MHz): δ 136.1, 135.9, 130.2, 130.1, 129.8, 74.9, 68.3, 60.6, 57.0, 34.2, 34.1, 29.8, 28.2, 23.9. HRMS (ESI^+): calcd for $C_{15}H_{20}NaO_6S_2$: 383.0599 $[M + Na]^+$; found: 383.0602.

Fluorination Reactions. To a solution of acyclic or cyclic sulfonate esters **1**−**5** and **13** (1 equiv) in CD_3CN (1 mL) was added TBAF (1 M in THF, 1 or 2 or 3 equiv). The mixture was stirred at room temperature for 24 h and then concentrated under reduced pressure to afford the expected products **6**−**10**. Spectroscopic data for products **6** [RN 909912-96-5],[26] **7** [RN 1344707-75-0],[14] **8** [RN 1344707-77-2],[14] and **9** [RN 1344707-94-3][14] were in accordance with those previously reported.

1-[4-(2,2-Dioxo-[1,2]oxathian-3-ylmethyl)-phenyl]-5-fluoro-pentan-2-sulfonate Tetrabutyl Ammonium ***10***. Colorless oil. ^{1}H NMR (CD_3CN, 400 MHz): δ 7.23 (s, 4H), 4.34 (ddt, J = 47.6, 6.1, and 1.8 Hz, 2H), 4.26−4.22 (m, 2H), 3.47 (dd, J = 13.7 and 3.1 Hz, 1H), 3.28−3.22 (m, 8H), 2.79−2.71 (m, 2H), 2.67−2.65 (m, 2H), 2.60 (dd, J = 13.7 and 10.9 Hz, 1H), 1.97−1.81 (m, 4H), 1.77−1.66 (m, 8H), 1.64−1.54 (m, 4H), 1.50−1.39 (m, 8H), 1.06 (t, J = 7.4 Hz, 12H). ^{13}C{^{1}H} NMR (CD_3CN, 100 MHz): δ 138.9, 129.5, 84.9 (d, J = 160.8 Hz), 68.7, 58.8, 56.2, 37.4, 36.2, 28.9 (d, J = 19.0 Hz), 28.2, 27.6, 26.0 (d, J = 5.8 Hz), 23.9, 19.9, 13.5. ^{19}F NMR (CD_3CN, 376 MHz): δ (−219.0)-(−218.0) (m). LC-MS: t_R = 7.6 min, (ES^+): 242.35 $[TBA+H]^+$, (ES^-): 393.3 $[M-H]^-$.

3,3′-[1,4-Phenyl-bis(methylene)]bis(3-fluoropropane-sulfonate tetrabutyl ammonium) ***11***. Colorless oil. ^{1}H NMR (CD_3CN, 400 MHz): δ 7.16−7.14 (m, 4H), 4.48−4.46 (m, 4H), 3.45−3.39 (m, 2H), 3.19−3.14 (m, 16H), 2.80−2.74 (m, 2H), 2.55- 2.49 (m, 2H), 2.12−1.95 (m, 4H), 1.81−1.78 (m, 16H), 1.43−1.32 (m, 16H), 1.23−0.91 (t, J = 7.2 Hz, 24H). ^{13}C{^{1}H} NMR (CD_3CN, 100 MHz): δ 137.8, 128.9, 82.8 (d, J = 159.9 Hz), 58.1, 57.1 (d, J = 6.3 Hz), 36.7, 30.4 (d, J = 20.3 Hz), 23.2,19.2, 12.7. ^{19}F NMR (CD_3CN, 376 MHz): δ (−218.4)-(−217.9) (m). LC-MS: t_R = 3.2 min, (ES^+) 242.07 $[TBA+H]^+$, (ES^-) 192.2 $[M-H]^{2-}$.

4-Fluoro-1-(4-(4-hydroxy-2-sulfobutyl)phenyl)butane-2-sulfonate Tetrabutylammonium ***12***. To a solution of fluorosulfonate sultone **9** (20 mg, 0.033 mmol, 1 equiv) in H_2O (500 μL) was added NaOH (4 mg, 0.099 mmol, 3 equiv). The reaction mixture was stirred at room temperature for 48 h and concentrated under reduced pressure to give compound **9** as an oil (19 mg, 93%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.16−7.14 (m, 4H), 4.52−4.48 (m, 2H), 4.32−4.05 (m, 2H), 3.45−3.39 (m, 2H), 3.19−3.14 (m, 8H), 2.80−2.74 (m, 2H), 2.53−2.44 (m, 2H), 2.22−2.05 (m, 4H), 1.81−1.78 (m, 8H), 1.44−1.30 (m, 8H), 1.20−0.92 (t, J = 7.3 Hz, 12H). ^{19}F NMR ($CDCl_3$, 376 MHz): δ (−216.5)−(−216.2) (m, 1F). HRMS (ESI^-): calcd for $C_{14}H_{20}FO_7S_2^-$: 383.0634 $[M]^-$; found: 383.0637. LC-MS: t_R = 2.6 min, (ES^+) 242.35 $[TBA+H]^+$, (ES^-) 383.5 $[M]^-$.

Tetrabutylammonium 1-(4-((2,2-Dioxido-1,2-oxathiolan-3-yl)methyl)phenyl)-5-fluoropentan-2-sulfonate ***14*** *and Tetrabutylammonium 1-(4-((2,2-Dioxido-1,2-oxathian-3-yl)-methyl)phenyl)-4-fluorobutane-2-sulfonate* ***15***. Obtained in the mixture as a colorless oil. ^{19}F NMR (CD_3CN, 376 MHz): δ (−218.8)−(−218.4) (m), (−216.6)−(−216.1) (m). HRMS (ESI^-): calcd for $C_{15}H_{20}FO_6S_2^-$: 379.0685 $[M-H]^-$; found: 379.0688. LC-MS: t_R = 7.1 min, (ES^+) 242.07 $[TBA+H]^+$, (ES^-) 379.17 $[M-H]^-$.

Conjugation Reactions. *Sulfosultone* ***9*** *with Lysine*. To a solution of sulfosultone **9** (88 mg, 0.14 mmol, 1 equiv) in borax buffer, pH 8 (1 mL), was added lysine (21 mg, 0.14 mmol, 1 equiv). The reaction mixtures were then stirred at room temperature for 14 h and analyzed by LC-MS.

Sulfosultone ***9*** *with HSA*. To a solution of native HSA (5 mg, 0.076 μmol, 1 equiv) in borax buffer pH 8 (300 μL) was added freshly prepared sulfosultone **9** (2.6 mg, 4.41 μmol, 58 equiv). The reaction mixture was stirred at 37 °C for 14 h and then analyzed by MALDI-TOF MS.

Sulfosultone ***9*** *to rhuEPO*. To a solution of recombinant rhuEPO (25 μg, 0.83 nmol, 1 equiv) in borax buffer pH 8 (200 μL) was added freshly prepared sulfosultone **9** (0.010 mg, 16.6 nmol, 20 equiv). The reaction mixture was stirred at 37 °C for 14 h and then analyzed by MALDI-TOF MS.

Radiochemistry. *Radioisotope Production and Radiochemistry*. No-carrier added $[^{18}F]$fluoride was produced using the $^{18}O(p,n)^{18}F$ nuclear reaction. Irradiation occurred on the target filled with ^{18}O-enriched water (97%, Euriso-top) using Cyclone 18/9 (IBA) Cyclotron. Manual radiosyntheses were performed in a fume hood equipped with a 5 cm lead-shielded wall and lead-shielded glass screens starting from $[^{18}F]$fluoride radioactivity amounts below 185 MBq.[27] Automated radiosyntheses were performed using a TRACERlab FXFN module (GE Healthcare) in a lead-shielded cell starting from $[^{18}F]$fluoride radioactivity amounts below 2 GBq. Radioactivity measurements were carried out with a Capintec R15C.

Manual Radiosyntheses. *Preparation of [^{18}F]TBAF*. Cyclotron-produced $[^{18}F]$fluoride (74−111 MBq) was separated from ^{18}O-enriched water using ion-exchange resin (Waters Sep-Pak Light Cartridge, Accell Plus QMA Carbonate) eluted with a solution of tetra-*n*-butylammonium bicarbonate ($TBAHCO_3$) [200 μL of a 70 mM solution of $TBAHCO_3$ in CH_3CN/H_2O (20:80) and 800 μL of acetonitrile]. The water was removed azeotropically with acetonitrile (2 × 0.5 mL) at 110 °C for 10 min under a steam of nitrogen to afford dry $[^{18}F]$TBAF (>90% radiochemical yield in recovered $[^{18}F]$TBAF).

Radiofluorination Reactions. Sulfonate esters **1**−**5** and **13** (5 mg) in anhydrous acetonitrile (500 μL) were added to dried $[^{18}F]$TBAF (55−100 MBq), and the sealed reaction vials were heated at 20, 50, 75, or 110 °C for 2, 5, 10, or 15 min under stirring. An aliquot from each reaction mixture (20 μL) was dissolved in anhydrous acetonitrile (100 μL) and then analyzed by HPLC (system A).

Purification of Sulfosultone [^{18}F] ***9***. After radiofluorination of bis-sultone **4** (5 mg) in acetonitrile (500 μL) for 2 min at 75 °C, acetonitrile was removed at room temperature under a gentle steam of nitrogen. Borax buffer pH 8 (1 mL) was then added, and the reaction mixture was stirred at room temperature for 2 min until a white precipitate appeared. The supernatant was recovered by filtration on a 0.2 μm filter. Filtration allowed to remove >95% of starting bissultone **4**.

https://doi.org/10.1021/acsomega.5c01335
ACS Omega 2025, 10, 17983−17992

*Conjugation of [^{18}F]**9** to Lysine.* [^{18}F]**9** (15−45 MBq) in borax buffer pH 8 (100 μL) was added to lysine (1.3 mg), and the reaction mixture was stirred at 37 °C for 30 min. HPLC Analyses were performed using system A.

*Conjugation of [^{18}F]**9** to HSA and rhuEPO.* [^{18}F]**9** (110−185 MBq) in borax buffer pH 8 (50 μL) was added to native HSA (0.5 mg) or rhuEPO (50 μg). The reaction mixture was stirred at 37 °C for 30 min, and conjugates [^{18}F]**24** and [^{18}F]**25** were isolated after purification on a PD-10 column. HPLC analyses were performed using system B.

*Automated Radiosynthesis of [^{18}F]**9**.* [^{18}F]Fluoride (1−1.8 GBq) was trapped on an ion-exchange resin (Waters Sep-Pak Light Cartridge, Accell Plus QMA Carbonate) and eluted with a solution of tetra-*n*-butylammonium bicarbonate (TBAHCO$_3$) [200 μL of a 70 mM solution of TBAHCO$_3$ in CH$_3$CN/H$_2$O (20:80) and 800 μL of acetonitrile]. The resulting [^{18}F]TBAF was dried upon heating at 80 °C for 7 min, followed by heating at 100 °C for 3 min under a vacuum and a stream of helium. Upon cooling to below 30 °C, a solution of bissultone **4** (5 mg) in 600 μL of CH$_3$CN was added, and the mixture was heated at 75 °C for 2 min. The mixture was cooled down to below 30 °C, and acetonitrile was removed under a gentle steam of helium for 10 min. Borax buffer pH 8 (1 mL) was added, and the reaction mixture was stirred for 2 min. The supernatant containing [^{18}F]**9** was transferred into an external vial under a steam of helium and recovered for manual radiolabeling of HSA and rhuEPO.

■ ASSOCIATED CONTENT

Data Availability Statement

The data underlying this study are available in the published article and its Supporting Information.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.5c01335.

Additional experimental procedures; ^{1}H, ^{13}C, and ^{19}F NMR spectra for new compounds; and HPLC chromatograms for radiosyntheses (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Cécile Perrio − *CNRS, CEA, Caen Normandie University, Caen 14074, France;* orcid.org/0000-0001-6559-3861; Email: perrio@cyceron.fr

Authors

Anne-Elodie Lafargue − *CNRS, CEA, Caen Normandie University, Caen 14074, France*

Clémence Maingueneau − *CNRS, CEA, Caen Normandie University, Caen 14074, France;* orcid.org/0000-0002-8222-2226

Benoit Bernay − *UNICAEN, US EMerode, Proteogen Plateform, Normandie Université, Caen 14032, France*

Abdelaziz Ejjoummany − *CNRS, CEA, Caen Normandie University, Caen 14074, France*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.5c01335

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank MESRI, Région Normandie, FR3038 INC3M, French National Agency for Research called "Investissements d'Avenir" Labex IRON (ANR-11-LABX-0018-01), and Carnot I2C for the financial support.

■ REFERENCES

(1) (a) Sharma, R.; Suman, S. K.; Mukherjee, A. Antibody-based Radiopharmaceuticals as Theranostic Agents: An Overview. *Curr. Med. Chem.* **2022**, *29*, 5979−6005. (b) Kręcisz, P.; Czarnecka, K.; Królicki, L.; Mikiciuk-Olasik, E.; Szymański, P. Radiolabeled Peptides and Antibodies in Medicine. *Bioconjugate Chem.* **2021**, *32*, 25−42. (c) Opalinska, M.; Hubalewska-Dydejczyk, A.; Sowa-Staszczak, A. Radiolabeled Peptides: Current and New Perspectives. *Q. J. Nucl. Med. Mol. Imaging* **2017**, *61*, 153−167. (d) Aerts, A.; Impens, N. R.; Gijs, M.; D'Huyvetter, M.; Vanmarcke, H.; Ponsard, B.; Lahoutte, T.; Luxen, A.; Baatout, S. Biological Carrier Molecules of Radiopharmaceuticals for Molecular Cancer Imaging and Targeted Cancer Therapy. *Curr. Pharm. Des.* **2014**, *20*, 5218−5244.

(2) Wei, W.; Rosenkrans, Z. T.; Liu, J.; Huang, G.; Luo, Q. Y.; Cai, W. ImmunoPET: Concept, Design, and Applications. *Chem. Rev.* **2020**, *120*, 3787−3851.

(3) (a) Wang, Y.; Lin, Q.; Shi, H.; Cheng, D. Fluorine-18: Radiochemistry and Target-Specific PET Molecular Probes Design. *Front Chem.* **2022**, *10*, No. 884517. (b) Ajenjo, J.; Destro, G.; Cornelissen, B.; Gouverneur, V. Closing the Gap between ^{19}F and ^{18}F Chemistry. *EJNMMI Radiopharm. Chem.* **2021**, *6*, 33. (c) Goud, N. S.; Joshi, R. K.; Bharath, R. D.; Kumar, P. Fluorine-18: A Radionuclide with Diverse Range of Radiochemistry and Synthesis Strategies for Target Based PET Diagnosis. *Eur. J. Med. Chem.* **2020**, *187*, No. 111979. (d) Jacobson, O.; Kiesewetter, D. O.; Chen, X. Fluorine-18 Radiochemistry, Labeling Strategies and Synthetic Routes. *Bioconjugate Chem.* **2015**, *26*, 1−18.

(4) (a) Zhong, X.; Yan, J.; Ding, X.; Su, C.; Xu, Y.; Yang, M. Recent Advances in Bioorthogonal Click Chemistry for Enhanced PET and SPECT Radiochemistry. *Bioconjugate Chem.* **2023**, *34*, 457−476. (b) Morris, O.; Fairclough, M.; Grigg, J.; Prenant, C.; McMahon, A. A Review of Approaches to ^{18}F Radiolabelling Affinity Peptides and Proteins. *J. Label. Compd. Radiopharm.* **2019**, *62*, 4−23. (c) Kumar, K.; Ghosh, A. ^{18}F-AlF Labeled Peptide and Protein Conjugates as Positron Emission Tomography Imaging Pharmaceuticals. *Bioconjugate Chem.* **2018**, *29*, 953−975. (d) Yang, H.; Zhang, L.; Liu, H.; Zhang, Y.; Mou, Z.; Chen, X.; Li, J.; He, F.; Li, Z. Simplified One-pot ^{18}F-Labeling of Biomolecules with in situ Generated Fluorothiophosphate Synthons in High Molar Activity. *Theranostics* **2023**, *13*, 472−482. (e) Lin, D.; Wallace, M.; Allentoff, A. J.; Donnelly, D. J.; Gomes, E.; Voronin, K.; Gong, S.; Huang, R. Y.-C.; Kim, H.; Caceres-Cortes, J.; Bonacorsi, S., Jr. Chemoselective Methionine Bioconjugation: Site-Selective Fluorine-18 Labeling of Proteins and Peptides. *Bioconjugate Chem.* **2020**, *31*, 1908−1916.

(5) (a) Nagachinta, S.; Novelli, P.; Joyard, Y.; Maindron, N.; Riss, P.; Dammicco, S. Fully Automated ^{18}F-Fluorination of *N*-succinimidyl-4-[^{18}F]fluorobenzoate ([^{18}F]SFB) for Indirect Labelling of Nanobodies. *Sci. Rep.* **2022**, *12*, 18655. (b) Tang, G.; Zeng, W.; Yu, M.; Kabalka, G. Facile Synthesis of *N*-Succinimidyl 4-[^{18}F]-fluorobenzoate ([^{18}F]SFB) for Protein Labeling. *J. Label. Compd. Radiopharm.* **2008**, *51*, 68−71. (c) Vaidyanathan, G.; Zalutsky, M. R. Labeling Proteins with Fluorine-18 using *N*-Succinimidyl 4-[^{18}F]-fluorobenzoate. *Nucl. Med. Biol.* **1992**, *19*, 275−281. (d) Vaidyanathan, G.; Zalutsky, M. R. Improved Synthesis of *N*-Succinimidyl 4-[^{18}F]fluorobenzoate and its Application to the Labeling of a Monoclonal Antibody Fragment. *Bioconjugate Chem.* **1994**, *5*, 352−356.

(6) (a) Taylor, M. D.; Roberts, A. D.; Nickles, R. J. Improving the Yield of 2-[^{18}F]fluoro-2-deoxyglucose using a Microwave Cavity. *Nucl. Med. Biol.* **1996**, *23*, 605−609. (b) Kallmerten, A. E.; Alexander, A.; Wager, K. M.; Jones, G. B. Microwave accelerated labeling methods in

the synthesis of radioligands for positron emission tomography imaging. *Curr. Radiopharm.* **2011**, *4*, 343−354.
(7) Pascali, G.; Matesic, L.; Collier, T. L.; Wyatt, N.; Fraser, B. H.; Pham, T. Q.; Salvadori, P. A.; Greguric, I. Optimization of Nucleophilic ^{18}F Radiofluorinations using a Microfluidic Reaction Approach. *Nat. Protoc.* **2014**, *9*, 2017−2029.
(8) Kim, D. W.; Ahn, D.-S.; Oh, Y.-H.; Lee, S.; Kil, H. S.; Oh, S. J.; Lee, S. J.; Kim, J. S.; Ryu, J. S.; Moon, D. H.; Chi, D. Y. A New Class of SN2 Reactions Catalyzed by Protic Solvents: Facile Fluorination for Isotopic Labeling of Diagnostic Molecules. *J. Am. Chem. Soc.* **2006**, *128*, 16394−16397.
(9) (a) Kim, D. W.; Choe, Y. S.; Chi, D. Y. A New Nucleophilic Fluorine-18 Labeling Method for Aliphatic Mesylates: Reaction in Ionic Liquids Shows Tolerance for Water. *Nucl. Med. Biol.* **2003**, *30*, 345−350.
(10) Médoc, M.; Sobrio, F. Nucleophilic Fluorination and Radiofluorination via Aziridinium Intermediates: N-Substituent Influence, Unexpected Regioselectivity, and Differences between Fluorine-19 and Fluorine-18. *J. Org. Chem.* **2015**, *80*, 10086−10097.
(11) Sergeev, M. E.; Morgia, F.; Lazari, M.; Wang, C., Jr.; van Dam, R. M. Titania-Catalyzed Radiofluorination of Tosylated Precursors in Highly Aqueous Medium. *J. Am. Chem. Soc.* **2015**, *137*, 5686−5694.
(12) Lu, S.; Lepore, S. D.; Li, S. Y.; Mondal, D.; Cohn, P. C.; Bhunia, A. K.; Pike, V. W. Nucleophile assisting leaving groups: a strategy for aliphatic ^{18}F-fluorination. *J. Org. Chem.* **2009**, *74*, 5290−5296.
(13) (a) Bratteby, K.; Shalgunov, V.; Battisti, U. M.; Petersen, I. N.; van den Broek, S. L.; Ohlsson, T.; Gillings, N.; Erlandsson, M.; Herth, M. M. Insights into Elution of Anion Exchange Cartridges: Opening the Path toward Aliphatic ^{18}F-Radiolabeling of Base-Sensitive Tracers. *ACS Pharmacol. Transl. Sci.* **2021**, *4*, 1556−1566. (b) Bratteby, K.; Shalgunov, V.; Herth, M. M. Aliphatic ^{18}F-Radiofluorination: Recent Advances in the Labeling of Base-Sensitive Substrates. *ChemMedChem.* **2021**, *16*, 2612−2622. (c) Orlovskaya, V.; Antuganov, D.; Fedorova, O.; Timofeev, V.; Krasikova, R. Tetrabutylammonium tosylate as inert phase-transfer catalyst: The key to high efficiency SN_2 radiofluorinations. *Appl. Radiat. Isot.* **2020**, *163*, No. 109195.
(14) Schmitt, S.; Bouteiller, C.; Barré, L.; Perrio, C. Sultone Opening with [^{18}F]Fluoride: An Efficient ^{18}F-Labelling Strategy for PET Imaging. *Chem. Commun.* **2011**, *47*, 11465−11467.
(15) Beaufrez, J.; Guillouet, S.; Seimbille, Y.; Perrio, C. Synthesis, Fluorine-18 Radiolabeling, and In Vivo PET Imaging of a Hydrophilic Fluorosulfotetrazine. *Pharmaceuticals* **2023**, *16*, 636.
(16) Maingueneau, C.; Lafargue, A.-E.; Guillouet, S.; Fillesoye, F.; Cao Pham, T. T.; Jordan, B. F.; Perrio, C. ^{18}F-Fluorination of Nitroimidazolyl-Containing Sultone: A Direct Access to a Highly Hydrophilic Radiotracer for High-Performance Positron Emission Tomography Imaging of Hypoxia. *JACS Au* **2024**, *4*, 3248−3257.
(17) Priem, T.; Bouteiller, C.; Camporese, D.; Romieu, A.; Renard, P.-Y. Synthesis and Reactivity of a Bis-sultone Cross-linker for Peptide Conjugation and [^{18}F]-Radiolabelling via Unusual "Double click" Approach. *Org. Biomol. Chem.* **2012**, *10*, 1068−1078.
(18) The triflate **1c** was also prepared but all attempts to fluorination and radiofluorination failed due to its high instability (data not shown).
(19) Kang, M. S.; Kong, T. W. S.; Khoo, J. Y. X.; Loh, T. P. Recent Developments in Chemical Conjugation Strategies Targeting Native Amino Acids in Proteins and Their Applications in Antibody−drug Conjugates. *Chem. Sci.* **2021**, *12*, 13613−13647.
(20) (a) Barbosa, L. R. S.; Ortore, M. G.; Spinozzi, F.; Mariani, P.; Bernstorff, S.; Itri, R. The Importance of Protein-Protein Interactions on the pH-Induced Conformational Changes of Bovine Serum Albumin: A Small-Angle X-Ray Scattering Study. *Biophys. J.* **2010**, *98*, 147−157. (b) Niu, G.; Lang, L.; Kiesewetter, D. O.; Ma, Y.; Sun, Z.; Guo, N.; Guo, J.; Wu, C.; Chen, X. In Vivo Labeling of Serum Albumin for PET. *J. Nucl. Med.* **2014**, *55*, 1150−1156.
(21) (a) Llop, E.; Gallego, R. G.; Belalcazar, V.; Gerwig, G. J.; Kamerling, J. P.; Segura, J.; Pascual, J. A. Evaluation of Protein *N*-Glycosylation in 2-DE: Erythropoietin as a Study Case. *PROTEOMICS* **2007**, *7*, 4278−4291. (b) Singh, A. K.; Gupta, S. Analysis of Recombinant Human Erythropoietin and Darbepoietin in Spiked Plasma. *Proteomics: Clin. Appl.* **2007**, *1*, 626−639.
(22) Kostikov, A. P.; Chin, J.; Orchowski, K.; Schirrmacher, E.; Niedermoser, S.; Jurkschat, K.; Iovkova-Berends, L.; Wängler, C.; Wängler, B.; Schirrmacher, R. Synthesis of [^{18}F]SiFB: A Prosthetic Group for Direct Protein Radiolabeling for Application in Positron Emission Tomography. *Nat. Protoc.* **2012**, *7*, 1956−1963.
(23) Romeiro, L. A. S.; da Silva Ferreira, M.; da Silva, L. L.; Castro, H. C.; Miranda, A. L. P.; Silva, C. L. M.; Noël, F.; Nascimento, J. B.; Araújo, C. V.; Tibiriçá, E.; Barreiro, E. J.; Fraga, C. A. M. Discovery of LASSBio-772, a 1,3-Benzodioxole N-Phenylpiperazine Derivative with Potent Alpha 1A/D-Adrenergic Receptor Blocking Properties. *Eur. J. Med. Chem.* **2011**, *46*, 3000−3012.
(24) Zhao, X.; Poon, Z.; Engler, A. C.; Bonner, D. K.; Hammond, P. T. Enhanced Stability of Polymeric Micelles Based on Postfunctionalized: The Substituent Effect. *Biomacromolecules* **2012**, *13*, 1315−1322.
(25) Jolly, P. I.; Fleary-Roberts, N.; O'Sullivan, S.; Doni, E.; Zhou, S.; Murphy, J. A. Reactions of Triflate Esters and Triflamides with an Organic Neutral Super-Electron Donor. *Org. Biomol. Chem.* **2012**, *10*, 5807−5810.
(26) Bejot, R.; Fowler, T.; Carroll, L.; Boldon, S.; Moore, J. E.; Declerck, J.; Gouverneur, V. Fluorous Synthesis of ^{18}F Radiotracers with the [^{18}F]Fluoride Ion: Nucleophilic Fluorination as the Detagging Process. *Angew. Chem., Int. Ed.* **2009**, *48*, 586−589.
(27) Bixler, A.; Springer, G.; Lovas, R. Practical Aspects of Radiation Safety for using Fluorine-18. *J. Nucl. Med. Technol.* **1999**, *27*, 14−16.

https://doi.org/10.1021/acsomega.5c01335
ACS Omega 2025, 10, 17983−17992