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The traceless Staudinger ligation for indirect ¹⁸F-radiolabelling[†]‡

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The Staudinger ligation of phosphine-substituted thioesters with ¹⁸F-fluoroethylazide has been successfully applied to access ¹⁸F-labelled molecules in radiochemical yields superior to 95%; the first fluorous variant of a Staudinger radio-ligation has been validated.

Introduction

Two transformations involving the azide group currently dominate bioorthogonal chemistry, the azide-alkyne 1,3-dipolar cycloaddition leading to triazoles, and the Staudinger ligation, a process forming an amide bond by reacting an azide with a phosphinesubstituted ester.¹ Both reactions have appealing features to study biomolecules in their native settings and have found endless applications for labelling and beyond.² The ability to radiolabel biomarkers with the nuclide ¹⁸F is essential to advance positron emission tomography (PET), an imaging modality used to conduct diagnostic clinical studies, understand biochemical processes and support drug development programmes.3 The short half-life of positron-emitting fluorine-18 ($t_1 = 109.7 \text{ min}$) demands rapid and efficient methodology to access²[18F]-labelled biomarkers in high radiochemical yield and purity.⁴ Fluorine-labelling of complex biomolecules such as peptides, antibodies or oligonucleotides remains challenging and is not always compatible with existing direct ¹⁸F-labelling strategies. Methods have therefore been developed that employ non carrier-added ¹⁸F-labelled prosthetic groups for chemoselective coupling under mild reaction conditions.⁴ For this multi-step indirect labelling approach, it is of the utmost importance for the coupling process to be highly efficient and for the new functionality installed upon labelling, not to perturb the physicochemical properties or in vivo behavior of the ¹⁸F-labelled biomarker. Copper-catalysed cycloaddition with either the alkyne or the azide carrying the ¹⁸F-tag has been successfully applied for labelling small molecules and peptides.⁵ The process generates a 1,2,3-triazole motif, a convenient bioisosteric replacement of the amide bond but the toxicity of copper(I) is limiting for some in vivo applications. The so-called traceless Staudinger ligation is a highly attractive process leading to a native amide bond without inclusion of the phosphine oxide in the final product.⁶ The synthetic value of this remarkable transformation has been demonstrated by Raines and coworkers with the ligation of complex peptide fragments, possibly best illustrated with the total synthesis of ribonuclease A.⁷

Herein, we report the first examples of Staudinger ligation with 2-[¹⁸F]fluoroethylazide **1** for the ¹⁸F-labelling of small molecules through formation of an amide bond. For this study, we selected the traceless Staudinger ligation of a phosphinothioester with an ¹⁸F-labelled azide fragment. Mechanistically, the attack of the phosphine onto the azide leads to an iminophosphorane, which rearranges to afford an amidophosphonium salt. After hydrolysis, the labelled amide is formed with concomitant release of the phosphine oxide (Scheme 1). This transformation benefits from the absence of metal catalyst but its value in the context of radiolabelling relies on the validation of a protocol with favorable reaction kinetics, and which allows for easy separation of the ¹⁸F-labelled amide from the phosphine oxide by-product.



Scheme 1 ¹⁸F-labelled azide 1 as a prosthetic group for indirect labelling.

Results and discussion

The borane-protected phosphinothiol **2** was chosen as a common building block to access masked (diphenylphosphino)methanethiol thioesters, which after deprotection, could

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undergo traceless Staudinger ligation with the azide **1**. Deprotonation of the commercially available (diphenylphosphonio)trihydroborate complex with sodium hydride in DMF at 0 °C followed by alkylation with (bromomethyl)ethanethioate **3** (easily prepared in two steps from thioacetic acid), gave the doubly protected trihydroborate complex **4** in a moderate yield of 60%.⁸ Treatment of **4** with sodium methoxide in methanol for ten minutes at room temperature revealed the borane-protected thiol moiety **2**. This thiol was engaged in the next step without purification (Scheme 2, Stage 1). Coupling of **2** with different carboxylic acids was undertaken using standard DCC conditions. Subsequent removal of the borane protecting group by treating the coupled

Stage 1: Synthesis of phosphino-borane 2



Stage 2: Synthesis of thioesters 5



Stage 3: Coupling of thioesters 5 with fluoroethylazide 1



Scheme 2 Staudinger ligation with fluorinated azide 1.

adduct with 1,4-diazabicyclo [2.2.2]octane (DABCO) in toluene at 60 °C for 1 h afforded adducts **5** in moderate yields over two steps (Scheme 2, Stage 2).⁹

Starting from 5-phenylpentanoic acid, 5a could be accessed in 40% overall yield. Compound 5b was synthesised in 60% yield over two steps and starting from N-acetylphenylalanine or Nacetyltryptophan, 5c and 5d were accessed in 55% and 63% overall vield respectively. The Staudinger ligation of 5a-d with 1 in THF- H_2O (3:1) under standard ligation conditions (RT, 16 h) gave the desired fluorinated targets 6 in moderate to excellent yields (Scheme 2, Stage 3, Method A). Reacting 5a and 5b with 1 accessed 6a and 6b in isolated yields of 79% and 82% respectively. Staudinger ligation of the (L)-phenylalanine-derived precursor 5c with 1 gave the fluorinated amide 6c in 82% yield. The ligation of 5d was slightly less efficient as the product was contaminated with the starting material both as free phosphine and phosphine oxide. Since the long reaction times employed in these initial studies are not compatible with fluorine-18 radiolabelling, further optimisation was undertaken (Scheme 2, Stage 3, Method B). Heating the ligation mixture at 80 °C enabled the reaction time to be reduced to 30 min with conversion up to 100%.

In addition to thioesters 5a-d, we also prepared the fluorous derivative 7 (Scheme 3).¹⁰ The fluorodetagging of fluorous precursors has recently been validated using direct ¹⁸F-fluorination,¹¹⁻¹³ however detagging upon displacement with a ¹⁸F-labelled prosthetic group has not been reported. The Staudinger ligation is an ideal platform for proof of concept as the fluorous tag can be attached onto the phosphino fragment of the starting thioester.¹⁴ The fluorous-tagged thioester 7 bearing two C₄F₉ tags on the phosphine was therefore synthesised following a reaction sequence similar to the one applied for the preparation of non fluorous thioesters. This compound was found to oxidise to the corresponding phosphine oxide faster than the non fluorous counterpart. The extent of oxidation was minimised during purification by performing the silica gel chromatography under an atmosphere of N₂. Staudinger ligation with 1 using standard conditions led to the formation of 6c in 27% yield with purification of the reaction mixture using FSPE. The decrease in isolated yield could be attributed to the propensity of the phosphane precursor 7 towards oxidation.



Scheme 3 Fluorous Staudinger ligation with 1.

With key studies of the Staudinger ligation with the fluorinated azide 1 in hand, focus turned to the validation of a radiochemical variant of this approach using 2-[¹⁸F]fluoroethylazide 1. This ¹⁸F-labelled azide was prepared by nucleophilic fluorination of the fluorous-tagged precursor 2-azido-ethyl-1H,1H,2H-perfluorodecane-1-sulfonate. The fluorination was performed in acetonitrile using [¹⁸F]-/K⁺-Kryptofix 222 and fluorous solid

Table 1	Staudinger	ligation	with	[¹⁸ F]1
Fable 1	Staudinger	ligation	with	[¹⁸ F]1

Entry	Starting material	Reaction conditions	RCY (%)	Product
1	5a	С	>95 (n = 2)	[¹⁸ F]6a
2	5b	С	>95 (n = 3)	
3	5c	C D	>95 (n = 6) >95 (n = 3)	[¹⁸ F]6c
4	7	D	>95 (n = 3)	⁰ → ^{Ph} H → ¹⁸ F [¹⁸ F]6c
				NH
5	5d	С	>95 (n = 6)	
				[¹⁸ F] 6d

^{*a*} Method C: THF–H₂O (4:1), 120 °C, 15 min; Method D: DMF–H₂O (6:1), 120 °C, 15 min.

phase extraction (FSPE) implemented to separate the radiolabelled product [¹⁸F]1 from the excess fluorous precursor (specific activity of ~10 GB q µmol⁻¹).11 This protocol is superior to previous radiosyntheses that rely on purification by distillation. The FSPE-purified azide [18F]1 was recovered in a mixture of H₂O and CH₃CN. The ligations were performed adding $[^{18}F]1$ to a solution of the thioester dissolved in THF- H_2O (4:1) followed by heating at 80 °C for 30 min. The reaction time could be reduced to 15 min by increasing the temperature to 120 °C (Method C). Under these conditions, the ligation of 5a-b with ¹⁸F]1 proceeded in excellent radiochemical yields superior to 95% (entries 1-2, Table 1). The reactions were equally successful when performed in a mixture of DMF-H₂O (6:1) (Method D).¹⁵ In contrast to couplings carried out in THF, the efficiency of the ligation process in DMF was not found to be dependent on concentration. Pleasingly, the labelling of both the non fluorous and fluorous alanine derivative 5c and 7 with [18F]1 in DMF- H_2O (6:1) at 120 °C for 15 min afforded [¹⁸F]6c in an excellent RCY in excess of 95% (entries 3-4, Table 1). For the ligation of 7, purification using FSPE led to an improvement in chemical purity although breakthrough of fluorous material into the fluorophobic eluted fraction was seen. HPLC was therefore necessary to obtain analytically pure sample of the ¹⁸F-labelled amide. The ligation of the tryptophan derivative 5d was also very high yielding with the totality of the azide converted into the ¹⁸F-labelled amide

(entry 5, Table 1). The overall synthesis duration was within 30 min. Pleasingly, all radio-HPLC traces typically showed only the presence of the ligated product in crude reaction mixture (Fig. 1).



Fig. 1 Radio-HPLC trace of crude material [¹⁸F]6a.

Conclusions

In conclusion, we have demonstrated that the Staudinger ligation is a suitable strategy for prosthetic group labelling using [¹⁸F]2fluoroethylazide. This catalyst-free ligation process benefits from short reaction times and very high radiochemical yields, a clear advantage for multi-step indirect ¹⁸F-labelling. Fluorous technology has been implemented for the fast and highly efficient purification of the prosthetic group [¹⁸F]2-fluoroethylazide and for the traceless Staudinger ligation itself. In the context of ¹⁸F-radiochemistry, this is the first example of fluorous detagging by indirect ¹⁸Flabelling.¹⁶ In recognition of the importance of the Staudinger ligation in the conjugation of biomolecules, we anticipate that this novel prosthetic group ¹⁸F-radiochemistry may find numerous applications in the context of peptide ¹⁸F-labelling as the new functionality installed upon ligation is the native amide bond.

Experimental

General methods

¹H NMRs were reported on Bruker DPX 200, DPX 400, AV 400 and AV 500 spectrometers, at a frequency of 200, 400 and 500 MHz respectively. 13C NMRs were recorded on Bruker AV 400 and AV 500 spectrometers at a frequency of 100 or 125 MHz respectively. Mass spectra (m/z) were obtained on a Bruker MicroTOF in Electrospray (ESI). Analytical thin layer chromatography (TLC) was performed on Merck Silica 60 F254 plates. Crude reaction mixtures were analysed by TLC and HPLC. HPLC analysis was performed with a Gilson 322 or Dionex Ultimate 3000 system, equipped with a NaI/PMT radiodetector (Raytest) and a UVdetector (Gilson). Radio-TLC was performed on Macherey-Nagel Polygram Silica Plates and eluted with EtOAc or 95% aq. MeCN. Analysis was performed with a plastic scintillator/PMT detector. FSPE separation was carried out using pre-assembled Waters Sep-Pak cartridges (Waters, Milford, MA) and FluoroFlash Silica gel (Fluorous Technologies Inc., Pittsburgh, PA). Pre-assembled Sep-Pak C₁₈SPE cartridges (Waters, Milford, MA) were used in the same way.

[¹⁸F]Fluoride was produced by the cyclotron of PETNET Solutions at Mont Vernon Hospital (UK) *via* the ¹⁸O(p,n)¹⁸F reaction and delivered as [¹⁸F]fluoride in [¹⁸O]water (1–2 GBq, 1–3 mL). This target solution was passed through a QMA anion exchange resin cartridge (20 mg, Waters). [¹⁸F]Fluoride adsorbed on the charged-resin was eluted into a reaction vial with a solution of Kryptofix 222 (15 mg) and K₂CO₃ (3 mg) in 1 mL acetonitrile– water (8 : 2). Excess water was removed under N₂ stream at 100– 110 °C, and the resulting complex was dried an additional 3 times by azeotropic distillation with 0.5 mL acetonitrile each under N₂ stream. The resulting dry complex of K¹⁸F/Kryptofix 222 was further dissolved by anhydrous acetonitrile (2–4 mL) and dispensed into reaction vials containing the precursor for nucleophilic fluorination.

Selected experimental data are given below—full experimental procedures and spectroscopic data for all compounds in the paper are given in the supporting information.

General procedure for compounds 5a-d

(Diphenylphosphino)methanethiol (2) (416 mg, 1.8 mmol) was dissolved in DCM (20 mL), to which DCC (408 mg, 1.98 mmol), DMAP (1 crystal) and then carboxylic acid (1.8 mmol) were added. The reaction was stirred for 16 h at room temperature, during which time, a white precipitate formed. The reaction mixture was passed through Celite® and concentrated to dryness. The residue was purified by flash chromatography (40% EtOAc in hexane) to afford the desired product which was subsequently dissolved in toluene (5 mL) and DABCO (92 mg, 0.82 mmol) was added. The reaction mixture was stirred at 60 °C for 1 h, at which point it was cooled to room temperature and concentrated to dryness. The residue was purified by flash chromatography (50% EtOAc in hexane) to afford the desired products **5**.

S-((Diphenylphosphino)methyl) 5-phenylpentanethioate (5a). Yellow oil. Yield: 40%; $\delta_{\rm H}$ (400 MHz, CDCl₃) 1.55–1.79 (4H, m, (*CH*₂)₂), 2.54 (2H, t, *J* = 6.6 Hz, CH₂*CH*₂CO), 2.59 (2H, t, *J* = 7.6 Hz, Ph*CH*₂CH₂), 3.53 (2H, dd, *J* = 3.8, 1.0 Hz, S*CH*₂P), 7.12–7.22 (3H, m, *Ph*), 7.25–7.29 (3H, m, Ph), 7.33–7.38 (5H, m, Ph), 7.40–7.48 (4H, m, Ph); $\delta_{\rm C}$ (100 MHz, CDCl₃) 25.2, 25.3, 25.5, 30.6, 35.5, 43.6, 125.8, 128.3, 128.4, 128.5, 128.6, 128.7, 129.1, 131.1 (d, *J* = 9.5 Hz), 132.7, 132.8, 136.7 (d, *J* = 13.4 Hz), 141.9, 198.1; $\delta_{\rm P}$ (162 MHz, CDCl₃) –14.8; *m/z* (ESI) C₂₄H₂₆OPS ([M + H]⁺) calc. 393.1436, found 393.1440.

S-((Diphenylphosphino)methyl) 4-formylbenzothioate (5b). Yellow oil. Yield: 60%; $\delta_{\rm H}$ (400 MHz, CDCl₃) 3.74 (2H, d, *J* = 3.8 Hz), 7.29–7.55 (10H, m), 7.80–8.10 (4H, m), 10.09 (1H, s, CHO); $\delta_{\rm C}$ (100 MHz, CDCl₃) 127.9, 128.9, 129.0, 129.8, 130.2, 131.9, 132.4, 132.5, 191.3; $\delta_{\rm P}$ (162 MHz, CDCl₃) –50.0; *m/z* (ESI) C₂₂H₁₇O₂PS ([M + H]⁺) calc. 365.0760, found 365.0755.

(*R*)-*S*-((Diphenylphosphino)methyl) 2-acetamido-3-phenylpropanethioate (5c)⁶. Yellow oil. Yield: 55%; $\delta_{\rm H}$ (200 MHz, CDCl₃) 1.94 (3H, s), 2.94 (1H, dd, *J* = 14.0, 7.5 Hz), 3.09 (1H, dd, *J* = 14.0, 5.2 Hz), 3.42–3.61 (2H, m), 4.91–5.03 (1H, m), 5.72 (1H, d, *J* = 8.2 Hz), 7.05–7.12 (2H, m), 7.22–7.29 (3H, m), 7.34–7.49 (10H, m); $\delta_{\rm C}$ (100 MHz, CDCl₃) 23.0, 25.6 (d, *J* = 24.5 Hz), 38.0, 59.6, 127.0, 128.47, 128.49, 128.8 (d, *J* = 36.0 Hz), 129.1 (d, *J* = 9.8 Hz), 132.6 (d, *J* = 19.4 Hz), 135.5, 169.9, 198.9; $\delta_{\rm P}$ (162 MHz, CDCl₃) –44.7; *m/z* (ESI) 422 ([M + H]⁺).

S-((Diphenylphosphino)methyl) 2-acetamido-3-(1*H*-indol-2yl)propanethioate (5d). Yellow oil. Yield: 55%; $\delta_{\rm H}$ (400 MHz, CDCl₃) 1.91 (3H, s, *OAc*), 3.26 (2H, t, *J* = 6.1 Hz, CH*CH*₂), 3.50 (2H, ddd, J = 28.6, 13.6, 3.8 Hz, SCH₂P), 5.05 (1H, dt, J = 8.6, 5.6 Hz, CHCH₂), 6.90 (1H, d, J = 2.3 Hz, NH), 7.14 (1H, td, J = 7.1, 1.0 Hz, Ar), 7.22 (1H, td, J = 8.1, 1.0 Hz, Ar), 7.35–7.46 (11H, m, Ar), 7.54 (1H, d, J = 7.5 Hz, Ar); $\delta_{\rm C}$ (100 MHz, CDCl₃) 23.2, 25.8 (d, J = 23.8 Hz), 28.0, 59.4, 109.4, 111.3, 118.4, 119.9, 122.3, 123.2, 127.8, 128.6 (d, J = 6.7 Hz), 129.2 (d, J = 5.7 Hz), 132.1 (d, J = 19.1), 132.7 (d, J = 19.1), 136.0, 136.7 (d, J = 14.3), 169.8, 199.5; $\delta_{\rm P}$ (162 MHz, CDCl₃) –15.6; m/z (ESI) C₂₆H₂₆N₂O₂PS ([M + H]⁺) calc. 461.1447, found 461.1450.

General procedure for compounds 6a-d

Phosphinothioate **5** (0.05 mmol) was dissolved in a mixture of THF and water (3:1, 1 mL) and 2-fluoroethylazide (150 μ L of a 0.33 M solution in THF, 0.05 mmol) was added. The reaction mixture was stirred at room temperature for 16 h then concentrated *in vacuo*. The residue was purified by flash chromatography (95:5 DCM–MeOH) to afford the desired products **6**.

N-(2-Fluoroethyl)-5-phenylpentanamide (6a). White solid. Yield: 79%; $\delta_{\rm H}$ (400 MHz, CDCl₃) 1.61–1.75 (4H, m, (*CH*₂)₂), 2.23 (2H, t, *J* = 6.6 Hz, CH₂*CH*₂CO), 2.64 (2H, t, *J* = 7.2 Hz, Ph*CH*₂CH₂), 3.57 (2H, dq, *J* = 28.1, 5.1 Hz, NH*CH*₂CH₂), 4.50 (2H, dt, *J* = 47.3, 4.8 Hz, CH₂*CH*₂F), 5.77 (1H, s, *NH*), 7.15–7.21 (3H, m, *Ph*), 7.26–7.31 (2H, m, *Ph*); $\delta_{\rm C}$ (100 MHz, CDCl₃) 25.2, 31.0, 35.5, 36.5, 39.7 (d, *J* = 22.5 Hz), 54.1, 82.9 (d, *J* = 166.2 Hz), 125.8, 128.3, 128.4, 173.0; $\delta_{\rm F}$ (377 MHz, CDCl₃) –224.3; *m/z* (ESI) C₁₃H₁₉FNO ([M + H]⁺) calc. 224.1445, found 224.1440.

N-(2-Fluoroethyl)-4-formylbenzamide (6b). White solid. Yield: 82%; $\delta_{\rm H}$ (400 MHz, CDCl₃) 3.79 (2H, d, J = 28.0 Hz, NH*CH*₂CH₂), 4.60 (2H, d, J = 47.1 Hz, CH₂*CH*₂F), 6.92 (1H, br s, *NH*), 7.94 (4H, app. s, *Ph*), 10.06 (1H, s, CHO); $\delta_{\rm C}$ (100 MHz, CDCl₃) 40.6 (d, J = 19.0 Hz), 82.5 (d, J = 168.2 Hz), 127.8, 129.8, 138.3, 139.2, 166.7, 191.6; $\delta_{\rm F}$ (377 MHz, CDCl₃) –223.9; *m/z* (ESI) C₁₀H₁₁FNO₂ ([M + H]⁺) calc. 196.0768, found 196.0776.

(*R*)-2-Acetamido-*N*-(2-fluoroethyl)-3-phenylpropanamide (6c). White solid. Yield: 92%; $\delta_{\rm H}$ (400 MHz, CDCl₃) 2.01 (3H, s, *OAc*), 2.99 (1H, dd, *J* = 13.4, 8.1 Hz, CHCHH), 3.13 (1H, dd, *J* = 13.6, 6.1 Hz, CHCHH), 3.40–3.55 (2H, m, NHCH₂CH₂), 4.21–4.48 (2H, m, CH₂CH₂F), 4.62 (1H, dt, *J* = 16.2, 7.8 Hz, *CH*CH₂), 5.90–5.98 (1H, m, *NH*), 6.15 (1H, d, *J* = 7.3 Hz, *NH*), 7.22 (2H, d, *J* = 8.1 Hz, *Ph*), 7.28–7.35 (3H, m, *Ph*); $\delta_{\rm C}$ (100 MHz, CDCl₃) 23.2, 38.6, 39.8 (d, *J* = 20.0 Hz), 53.4, 54.7, 81.9 (d, *J* = 167.9 Hz), 127.2, 128.8, 129.2, 136.4, 170.0, 171.0; $\delta_{\rm F}$ (377 MHz, CDCl₃) –224.0; *m*/*z* (ESI) C₁₃H₁₆FN₂O₂ ([M + H]⁺) calc. 251.1197, found 251.1201.

2-Acetamido-*N***-(2-fluoroethyl)-3-(1***H***-indol-3-yl)propan-amide (6d). White solid. Yield: 60%; \delta_{\rm H} (400 MHz, CDCl₃) 2.08 (3H, s,** *OAc***), 3.07 (1H, dd,** *J* **= 12.9, 7.6 Hz, CHC***H***H), 3.37 (\frac{1}{2}H, t,** *J* **= 3.8 Hz, NH***CH***₂CH₂F), 3.41 (1H, dt,** *J* **= 5.6, 3.7 Hz, NH***CH***₂CH₂F), 3.46 (\frac{1}{2}H, t,** *J* **= 3.8 Hz, NH***CH***₂CH₂F), 3.57 (1H, dd,** *J* **= 12.7, 7.9 Hz, CHCH***H***), 4.35 (2H, dt,** *J* **= 46.2, 3.9 Hz, CH₂***CH***₂F), 4.58 (1H, s,** *NH***), 4.93 (1H, t,** *J* **= 7.7 Hz, NH***CH***CO), 5.07 (1H, s,** *NH***), 7.04 (1H, s,** *Ar***), 7.18–7.24 (3H, m,** *Ar***), 7.32– 7.36 (2H, m,** *Ar***); \delta_{\rm C} (100 MHz, CDCl₃) 22.8, 30.5, 40.5 (d,** *J* **= 27.0 Hz), 54.7, 82.6 (d,** *J* **= 168.4 Hz), 108.5, 111.6, 119.5, 120.1, 121.7, 123.4, 128.0, 137.3, 172.6, 174.0.; \delta_{\rm F} (377 MHz, CDCl₃)** -222.0; m/z (ESI) $C_{15}H_{19}FN_3O_2$ ([M + H]⁺) calc. 292.1456, found 292.1460.

(*R*)-2-Acetamido-*N*-(2-fluoroethyl)-3-phenylpropanamide (6c) prepared from the reaction of 7 with 1. Phosphinothioate 7 (120 mg, 0.13 mmol) was dissolved in a mixture of THF and water (4:1, 1 mL) and 2-fluoroethylazide (390 μ L of a 0.33 M solution in THF, 0.13 mmol) was added. The reaction mixture was stirred at room temperature for 16 h then concentrated *in vacuo*. The residue was purified by FSPE to afford the desired product **6c** in 27% yield.

Radiochemical procedures

2-[¹⁸F]fluoroethylazide [¹⁸F]**1** was prepared as previously reported and purified by FSPE.¹¹

General procedure for ¹⁸F-labelled Staudinger ligation reactions

In a sealed reaction vial, the FSPE purified solution of 2-[¹⁸F]fluoroethylazide (20–100 MBq) in MeCN–H₂O (7:3, 0.5 mL) was mixed with a thiophosphane **5** (1 mg) in either 300 mL THF–H₂O (4:1) or 300 mL DMF–H₂O (6:1) and heated for 15 min at 120 °C. Analysis by reverse-phase HPLC gave retention times that correlated to the cold reference compounds, with >95% conversion from [¹⁸F]2-fluoroethylazide to the product [¹⁸F]**6** in each case.

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