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PAPER

The traceless Staudinger ligation for indirect ^{18}F-radiolabelling†‡

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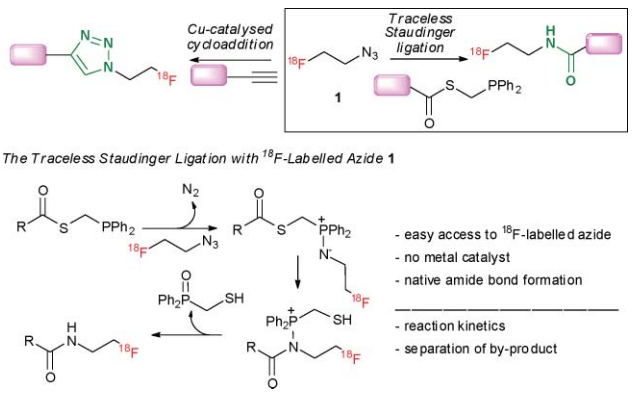
The Staudinger ligation of phosphine-substituted thioesters with ^{18}F-fluoroethylazide has been successfully applied to access ^{18}F-labelled molecules in radiochemical yields superior to 95%; the first fluorous variant of a Staudinger radio-ligation has been validated.

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 phosphine-substituted 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 ^{18}F 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 ($t_{\frac{1}{2}}$ = 109.7 min) demands rapid and efficient methodology to access [^{18}F]-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 ^{18}F-labelling strategies. Methods have therefore been developed that employ non carrier-added ^{18}F-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 ^{18}F-labelled biomarker. Copper-catalysed cycloaddition with either the alkyne or the azide carrying the ^{18}F-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-[^{18}F]fluoroethylazide **1** for the ^{18}F-labelling of small molecules through formation of an amide bond. For this study, we selected the traceless Staudinger ligation of a phosphinothioester with an ^{18}F-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 ^{18}F-labelled amide from the phosphine oxide by-product.



Scheme 1 ^{18}F-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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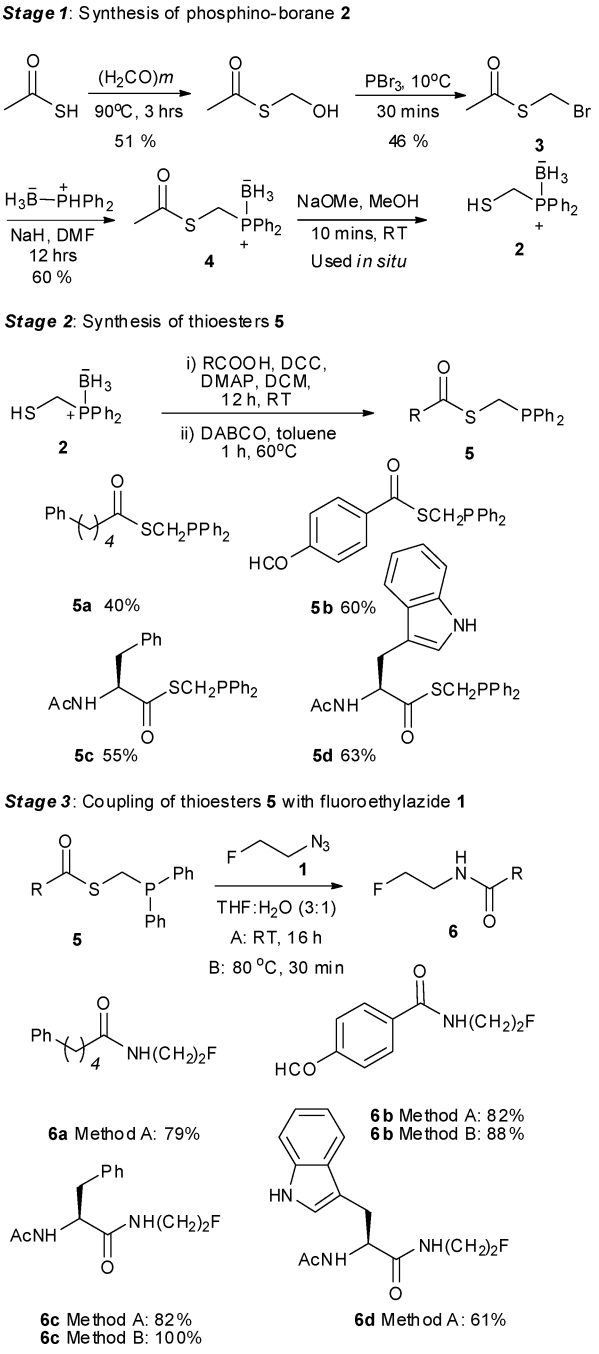
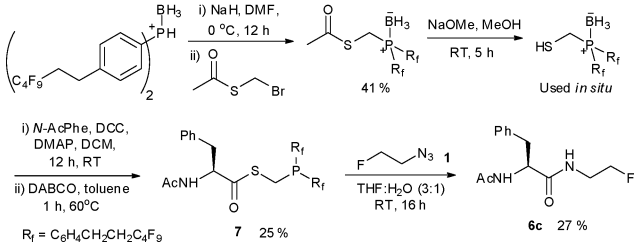
undergo traceless Staudinger ligation with the azide **1**. Deprotonation of the commercially available (diphenylphosphonio)trihydroborate complex with sodium hydride in DMF at 0 °C followed by alkylation with (bromomethyl)ethanethioate **3** (easily prepared in two steps from thioacetic acid), gave 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 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 *N*-acetyltryptophan, **5c** and **5d** were accessed in 55% and 63% overall yield 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 ^{18}F-fluorination,[11–13] however detagging upon displacement with a ^{18}F-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 C_4F_9 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 N_2. 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.



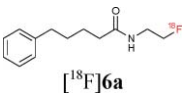
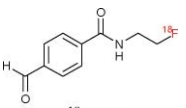
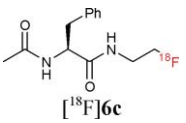
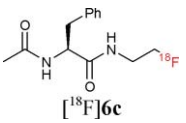
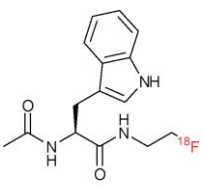
Scheme 2 Staudinger ligation with fluorinated azide **1**.



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-[^{18}F]fluoroethylazide **1**. This ^{18}F-labelled azide was prepared by nucleophilic fluorination of the fluorous-tagged precursor 2-azido-ethyl-1*H*,1*H*,2*H*,2*H*-perfluorodecane-1-sulfonate. The fluorination was performed in acetonitrile using [^{18}F]-/K^+-Kryptofix 222 and fluorous solid

Table 1 Staudinger ligation with [^{18}F]**1**

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

[a] Method C: THF–H_2O (4 : 1), 120 °C, 15 min; Method D: DMF–H_2O (6 : 1), 120 °C, 15 min.

phase extraction (FSPE) implemented to separate the radiolabelled product [^{18}F]**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 [^{18}F]**1** was recovered in a mixture of H_2O and CH_3CN. 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 [^{18}F]**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–H_2O (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 [^{18}F]**1** in DMF–H_2O (6 : 1) at 120 °C for 15 min afforded [^{18}F]**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 ^{18}F-labelled amide. The ligation of the tryptophan derivative **5d** was also very high yielding with the totality of the azide converted into the ^{18}F-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).

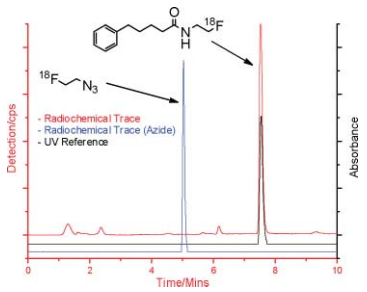


Fig. 1 Radio-HPLC trace of crude material [^{18}F]**6a**.

Conclusions

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

Experimental

General methods

^{1}H 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. ^{13}C 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 UV-detector (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 C_{18}SPE cartridges (Waters, Milford, MA) were used in the same way.

[^{18}F]Fluoride was produced by the cyclotron of PETNET Solutions at Mont Vernon Hospital (UK) *via* the ^{18}O(p,n)^{18}F reaction and delivered as [^{18}F]fluoride in [^{18}O]water (1–2 GBq, 1–3 mL). This target solution was passed through a QMA anion

exchange resin cartridge (20 mg, Waters). [^{18}F]Fluoride adsorbed on the charged-resin was eluted into a reaction vial with a solution of Kryptofix 222 (15 mg) and K_2CO_3 (3 mg) in 1 mL acetonitrile–water (8 : 2). Excess water was removed under N_2 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 N_2 stream. The resulting dry complex of K^{18}F/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%; δ_H (400 MHz, $CDCl_3$) 1.55–1.79 (4H, m, $(CH_2)_2$), 2.54 (2H, t, J = 6.6 Hz, CH_2CH_2CO), 2.59 (2H, t, J = 7.6 Hz, $PhCH_2CH_2$), 3.53 (2H, dd, J = 3.8, 1.0 Hz, SCH_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); δ_C (100 MHz, $CDCl_3$) 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; δ_P (162 MHz, $CDCl_3$) –14.8; *m/z* (ESI) $C_{24}H_{26}OPS$ ([M + H]$^+$) calc. 393.1436, found 393.1440.

***S*-((Diphenylphosphino)methyl) 4-formylbenzothioate (5b).** Yellow oil. Yield: 60%; δ_H (400 MHz, $CDCl_3$) 3.74 (2H, d, J = 3.8 Hz), 7.29–7.55 (10H, m), 7.80–8.10 (4H, m), 10.09 (1H, s, C*H*O); δ_C (100 MHz, $CDCl_3$) 127.9, 128.9, 129.0, 129.8, 130.2, 131.9, 132.4, 132.5, 191.3; δ_P (162 MHz, $CDCl_3$) –50.0; *m/z* (ESI) $C_{22}H_{17}O_2PS$ ([M + H]$^+$) calc. 365.0760, found 365.0755.

(*R*)-*S*-((Diphenylphosphino)methyl) 2-acetamido-3-phenylpropanethioate (5c)[6]. Yellow oil. Yield: 55%; δ_H (200 MHz, $CDCl_3$) 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); δ_C (100 MHz, $CDCl_3$) 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; δ_P (162 MHz, $CDCl_3$) –44.7; *m/z* (ESI) 422 ([M + H]$^+$).

***S*-((Diphenylphosphino)methyl) 2-acetamido-3-(1*H*-indol-2-yl)propanethioate (5d).** Yellow oil. Yield: 55%; δ_H (400 MHz, $CDCl_3$) 1.91 (3H, s, *OAc*), 3.26 (2H, t, J = 6.1 Hz, $CHCH_2$), 3.50 (2H, ddd, J = 28.6, 13.6, 3.8 Hz, SCH_2P), 5.05 (1H, dt, J = 8.6, 5.6 Hz, $CHCH_2$), 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*); δ_C (100 MHz, $CDCl_3$) 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; δ_P (162 MHz, $CDCl_3$) –15.6; *m/z* (ESI) $C_{26}H_{26}N_2O_2PS$ ([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 µL 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%; δ_H (400 MHz, $CDCl_3$) 1.61–1.75 (4H, m, $(CH_2)_2$), 2.23 (2H, t, J = 6.6 Hz, CH_2CH_2CO), 2.64 (2H, t, J = 7.2 Hz, $PhCH_2CH_2$), 3.57 (2H, dq, J = 28.1, 5.1 Hz, $NHCH_2CH_2$), 4.50 (2H, dt, J = 47.3, 4.8 Hz, CH_2CH_2F), 5.77 (1H, s, *NH*), 7.15–7.21 (3H, m, *Ph*), 7.26–7.31 (2H, m, *Ph*); δ_C (100 MHz, $CDCl_3$) 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; δ_F (377 MHz, $CDCl_3$) –224.3; *m/z* (ESI) $C_{13}H_{19}FNO$ ([M + H]$^+$) calc. 224.1445, found 224.1440.

***N*-(2-Fluoroethyl)-4-formylbenzamide (6b).** White solid. Yield: 82%; δ_H (400 MHz, $CDCl_3$) 3.79 (2H, d, J = 28.0 Hz, $NHCH_2CH_2$), 4.60 (2H, d, J = 47.1 Hz, CH_2CH_2F), 6.92 (1H, br s, *NH*), 7.94 (4H, app. s, *Ph*), 10.06 (1H, s, C*H*O); δ_C (100 MHz, $CDCl_3$) 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; δ_F (377 MHz, $CDCl_3$) –223.9; *m/z* (ESI) $C_{10}H_{11}FNO_2$ ([M + H]$^+$) calc. 196.0768, found 196.0776.

(*R*)-2-Acetamido-*N*-(2-fluoroethyl)-3-phenylpropanamide (6c). White solid. Yield: 92%; δ_H (400 MHz, $CDCl_3$) 2.01 (3H, s, *OAc*), 2.99 (1H, dd, J = 13.4, 8.1 Hz, CHC*H*H), 3.13 (1H, dd, J = 13.6, 6.1 Hz, CHCH*H*), 3.40–3.55 (2H, m, $NHCH_2CH_2$), 4.21–4.48 (2H, m, CH_2CH_2F), 4.62 (1H, dt, J = 16.2, 7.8 Hz, $CHCH_2$), 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*); δ_C (100 MHz, $CDCl_3$) 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; δ_F (377 MHz, $CDCl_3$) –224.0; *m/z* (ESI) $C_{13}H_{16}FN_2O_2$ ([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%; δ_H (400 MHz, $CDCl_3$) 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, $NHCH_2CH_2F$), 3.41 (1H, dt, J = 5.6, 3.7 Hz, $NHCH_2CH_2F$), 3.46 ($\frac{1}{2}$H, t, J = 3.8 Hz, $NHCH_2CH_2F$), 3.57 (1H, dd, J = 12.7, 7.9 Hz, CHCH*H*), 4.35 (2H, dt, J = 46.2, 3.9 Hz, CH_2CH_2F), 4.58 (1H, s, *NH*), 4.93 (1H, t, J = 7.7 Hz, NHC*H*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*); δ_C (100 MHz, $CDCl_3$) 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.; δ_F (377 MHz, $CDCl_3$)

–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 μL 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-[^{18}F]fluoroethylazide [^{18}F]**1** was prepared as previously reported and purified by FSPE.[11]

General procedure for ^{18}F-labelled Staudinger ligation reactions

In a sealed reaction vial, the FSPE purified solution of 2-[^{18}F]fluoroethylazide (20–100 MBq) in MeCN–H_2O (7 : 3, 0.5 mL) was mixed with a thiophosphane **5** (1 mg) in either 300 mL THF–H_2O (4 : 1) or 300 mL DMF–H_2O (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 [^{18}F]2-fluoroethylazide to the product [^{18}F]**6** in each case.

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References

1 M. Köhn and R. Breinbauer, *Angew. Chem., Int. Ed.*, 2004, **43**, 3106; J. M. Baskin and C. R. Bertozzi, *QSAR Comb. Sci.*, 2007, **26**, 1211; E. M. Sletten and C. R. Bertozzi, *Angew. Chem., Int. Ed.*, 2009, **48**, 6974.
2 For selected examples, see: E. Saxon and C. R. Bertozzi, *Science*, 2000, **287**, 2007; M. J. Hangauer and C. R. Bertozzi, *Angew. Chem., Int. Ed.*, 2008, **47**, 2394; E. M. Sletten and C. R. Bertozzi, *Angew. Chem., Int. Ed.*, 2009, **48**, 6974; G. Charron, M. M. Zhang, J. S. Yount, J. Wilson, A. S. Raghavan, E. Shamir and H. C. Hang, *J. Am. Chem. Soc.*, 2009, **131**, 4967; P. V. Chang, J. A. Prescher, E. M. Sletten, J. M. Baskin, I. A. Miller, N. J. Agard, A. Lo and C. R. Bertozzi, *Proc. Natl. Acad. Sci. U. S. A.*, 2010, **107**, 1821; J. C. Jewett, E. M. Sletten and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2010, **132**, 3688.
3 S. A. Ametamey, M. Honer and P. A. Schubiger, *Chem. Rev.*, 2008, **108**, 1501.
4 R. Schirrmacher, C. Wängler and E. Schirrmacher, *Mini-Rev. Org. Chem.*, 2007, **4**, 317; L. Cai, S. Lu and V. W. Pike, *Eur. J. Org. Chem.*, 2008, 2853; P. W. Miller, N. J. Long, R. Vilar and A. D. Gee, *Angew. Chem., Int. Ed.*, 2008, **47**, 8998.
5 J. Marik and J. L. Sutcliffe, *Tetrahedron Lett.*, 2006, **47**, 6681; M. Glaser and E. Årstad, *Bioconjugate Chem.*, 2007, **18**, 989; G. Smith, M. Glaser, M. Perumal, Q.-D. Nguyen, B. Shan, E. Årstad and E. O. Aboagye, *J. Med. Chem.*, 2008, **51**, 8057; M. Glaser, M. Solbakken, D. R. Turton, R. Pettitt, J. Barnett, J. Arukwe, H. Karlsen, A. Cuthbertson, S. J. Luthra and E. Arstad, *Amino Acids*, 2009, **37**, 717; M. Glaser and E. G. Robbins, *J. Labelled Compd. Radiopharm.*, 2009, **52**, 407.
6 B. L. Nilsson, L. K. Kiessling and R. T. Raines, *Org. Lett.*, 2000, **2**, 1939; E. Saxon, J. L. Armstrong and C. R. Bertozzi, *Org. Lett.*, 2000, **2**, 2141; B. L. Nilsson, L. K. Kiessling and R. T. Raines, *Org. Lett.*, 2001, **3**, 9.
7 B. L. Nilsson, R. J. Hondal, M. B. Soellner and R. T. Raines, *J. Am. Chem. Soc.*, 2003, **125**, 5269.
8 M. B. Soellner, B. L. Nilsson and R. T. Raines, *J. Org. Chem.*, 2002, **67**, 4993; Y. He, R. J. Hinklin, J. Chang and L. K. Kiessling, *Org. Lett.*, 2004, **6**, 4479.
9 Deprotection was performed immediately prior to ligation with **1** as adducts **6** were found to be susceptible to oxidation.
10 For selected reviews on fluorous synthesis see: W. Zhang, *Tetrahedron*, 2003, **59**, 4475; W. Zhang, *Chem. Rev.*, 2004, **104**, 2531; W. Zhang, *Chem. Rev.*, 2009, **109**, 749.
11 R. Bejot, T. Fowler, L. Carroll, S. Boldon, J. E. Moore, J. Declerck and V. Gouverneur, *Angew. Chem., Int. Ed.*, 2009, **48**, 586.
12 E. Blom, F. Karimi and B. Långström, *J. Label. Compd. Radiopharm.*, 2010, **53**, 24.
13 For an example of fluorous electrophilic fluorodetagging: S. Boldon, J. E. Moore and V. Gouverneur, *Chem. Commun.*, 2008, 3622.
14 For a fluorous Staudinger reaction: C. W. Lindsley, Z. Zhao, R. C. Newton, W. H. Leister and K. A. Strauss, *Tetrahedron Lett.*, 2002, **43**, 4467; D. P. Curran, X. Wang and Q. Zheng, *J. Org. Chem.*, 2005, **70**, 3716.
15 C. Mamat, A. Flemming, M. Köckerling, J. Steinbach and F. R. Wuest, *Synthesis*, 2009, 3311.
16 During the preparation of this manuscript, the Staudinger Ligation has been reported for the radiosynthesis of [^{18}F]$GABA_A$ receptor binding 4-quinolones: A Gaeta, J. Woodcraft, S. Plant, J. Goggi, P. Jones, M. Battle, W. Trigg, S. Luthra and M. Glaser, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 4649.

