Supplementary Information

Simultaneous Identification and Quantification of Amino Acids in Biofluids by Reactive ^{19}F-Tag

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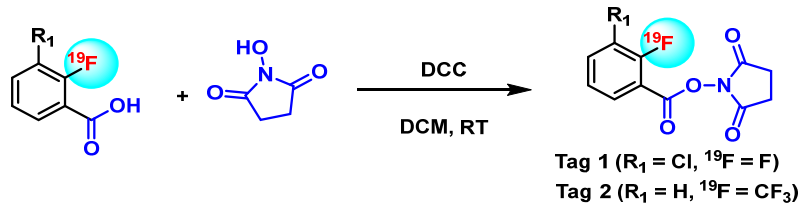
1. Synthesis and characterization of ^{19}F-tag



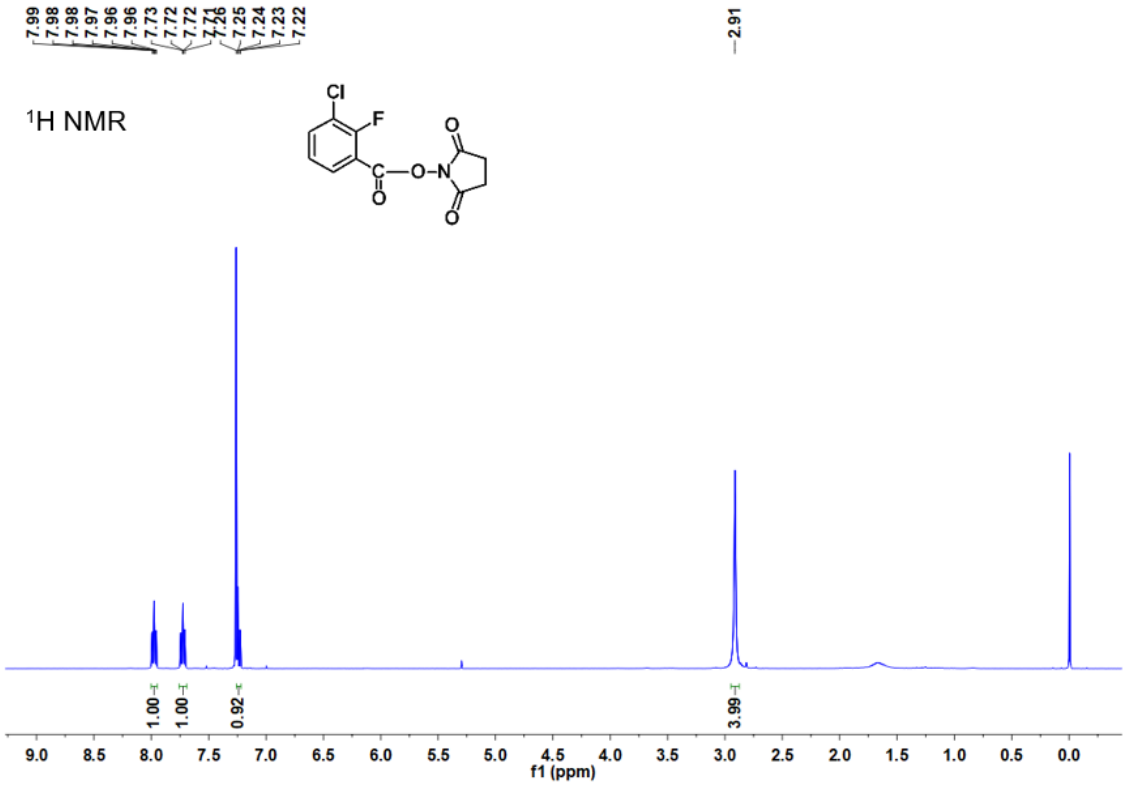
Tag 1 (R_1 = Cl, ^{19}F = F)
Tag 2 (R_1 = H, ^{19}F = CF_3)

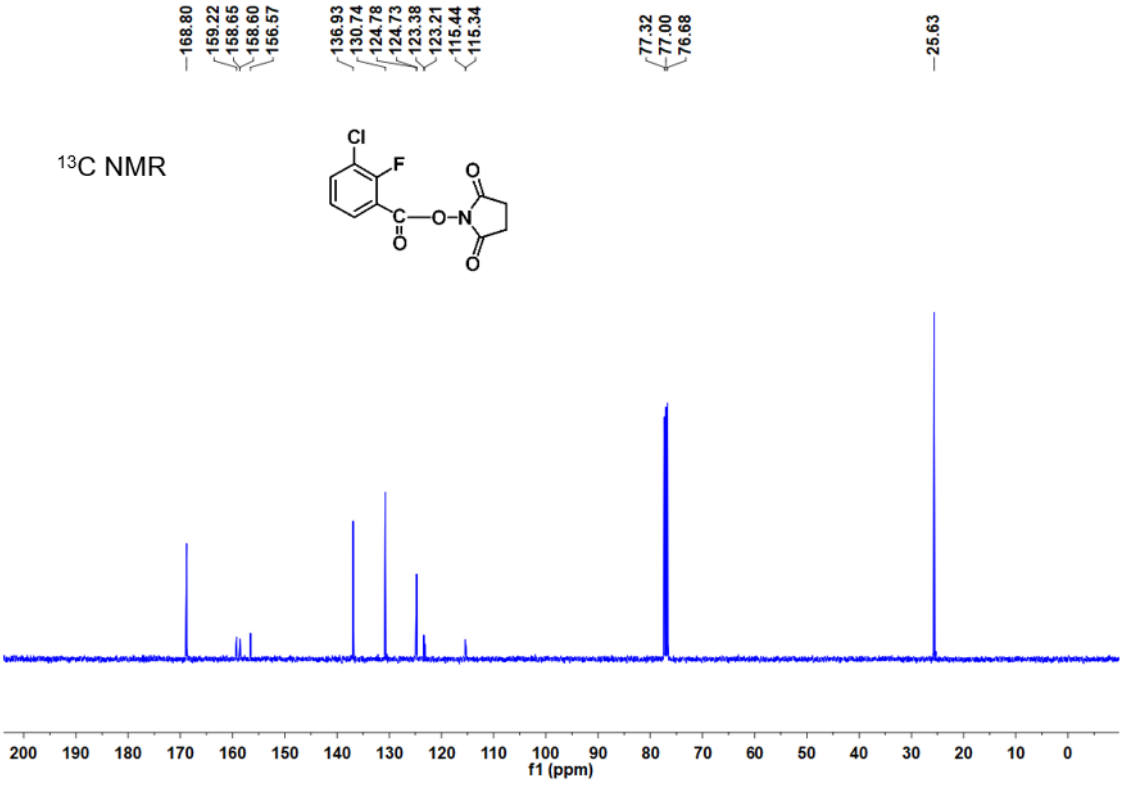
The general procedure is as follows: a mixture of 3-chloro-2-fluorobenzoic acid/2-(Trifluoromethyl)benzoic acid (10.0 mmol), *N*-Hydroxysuccinimide (10.0 mmol, 1.15 g) and *N,N*'-dicyclohexylcarbodiimide (DCC, 10.0 mmol, 2.06 g) in 40 mL dichloromethane was stirred for 3 h at room temperature. After filtration, the obtained organic solvent was concentrated under reduced pressure. The crude product obtained is recrystallized with ethyl ether to obtain the pure Tag 1 and Tag 2.

2,5-Dioxopyrrolidin-1-yl 3-chloro-2-fluorobenzoate (Tag 1), white solids (2.14 g), yield about 78%. ^{1}H NMR ($CDCl_3$, 400 MHz): 7.99-7.96 (m, 1H), 7.74-7.70 (m, 1H), 7.25-7.22 (m, 1H), 2.91 (s, 1H) ppm. ^{13}C NMR ($CDCl_3$, 101 MHz): 168.8, 158.6 (d, $J_{C\text{-}F}$ = 5.1 Hz), 157.9 (d, $J_{C\text{-}F}$ = 267.7 Hz), 136.9, 130.7, 124.8 (d, $J_{C\text{-}F}$ = 5.1 Hz), 123.3 (d, $J_{C\text{-}F}$ = 17.2 Hz), 115.4 (d, $J_{C\text{-}F}$ = 10.1 Hz), 25.6 ppm. ^{19}F NMR ($CDCl_3$, 376 MHz): -107.0 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{11}H_7ClFNO_4Na$, 293.9940; found, 293.9945.

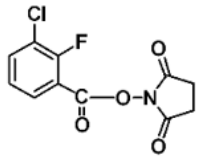
2,5-Dioxopyrrolidin-1-yl 2-(trifluoromethyl)benzoate (Tag 2), white solids (2.33 g), yield about 81%. ^{1}H NMR ($CDCl_3$, 400 MHz): 8.13 (d, *J* = 4.0 Hz, 1 H), 7.86 (d, *J* = 8.0 Hz, 1 H), 7.8-7.7 (m, 2H), 2.91 (s, 1H) ppm. ^{13}C NMR ($CDCl_3$, 101 MHz): 168.8, 161.0, 133.3, 132.0, 131.4, 130.4 (q, $J_{C\text{-}F}$ = 33.3 Hz), 127.3 (q, $J_{C\text{-}F}$ = 5.1 Hz), 125.4 (q, $J_{C\text{-}F}$ = 274.7 Hz), 125.1, 25.6 ppm. ^{19}F NMR ($CDCl_3$, 376 MHz): -59.7 ppm. HRMS (ESI) m/z: $[M+Na]^+$ calculated for $C_{12}H_8F_3NO_4Na$, 310.0298; found, 310.0310.

^{1}H NMR, ^{13}C NMR and ^{19}F NMR spectra of ^{19}F-tag

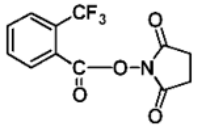


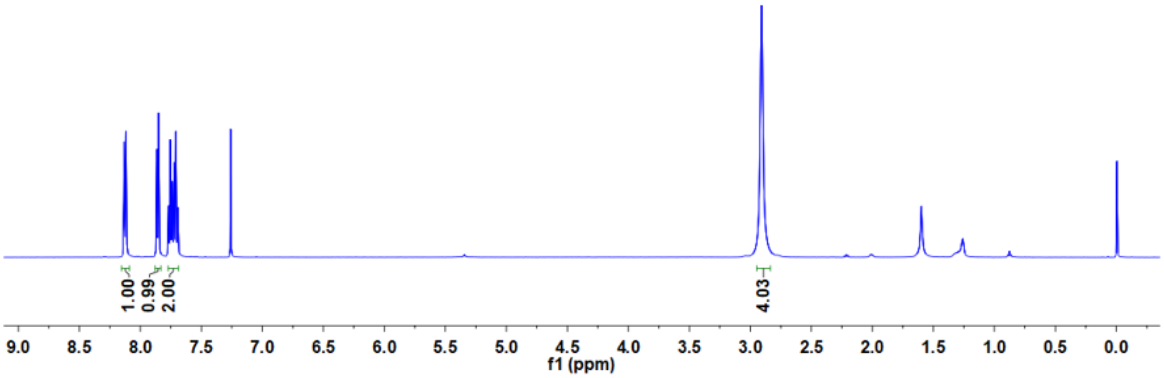


^{19}F NMR



^{1}H NMR





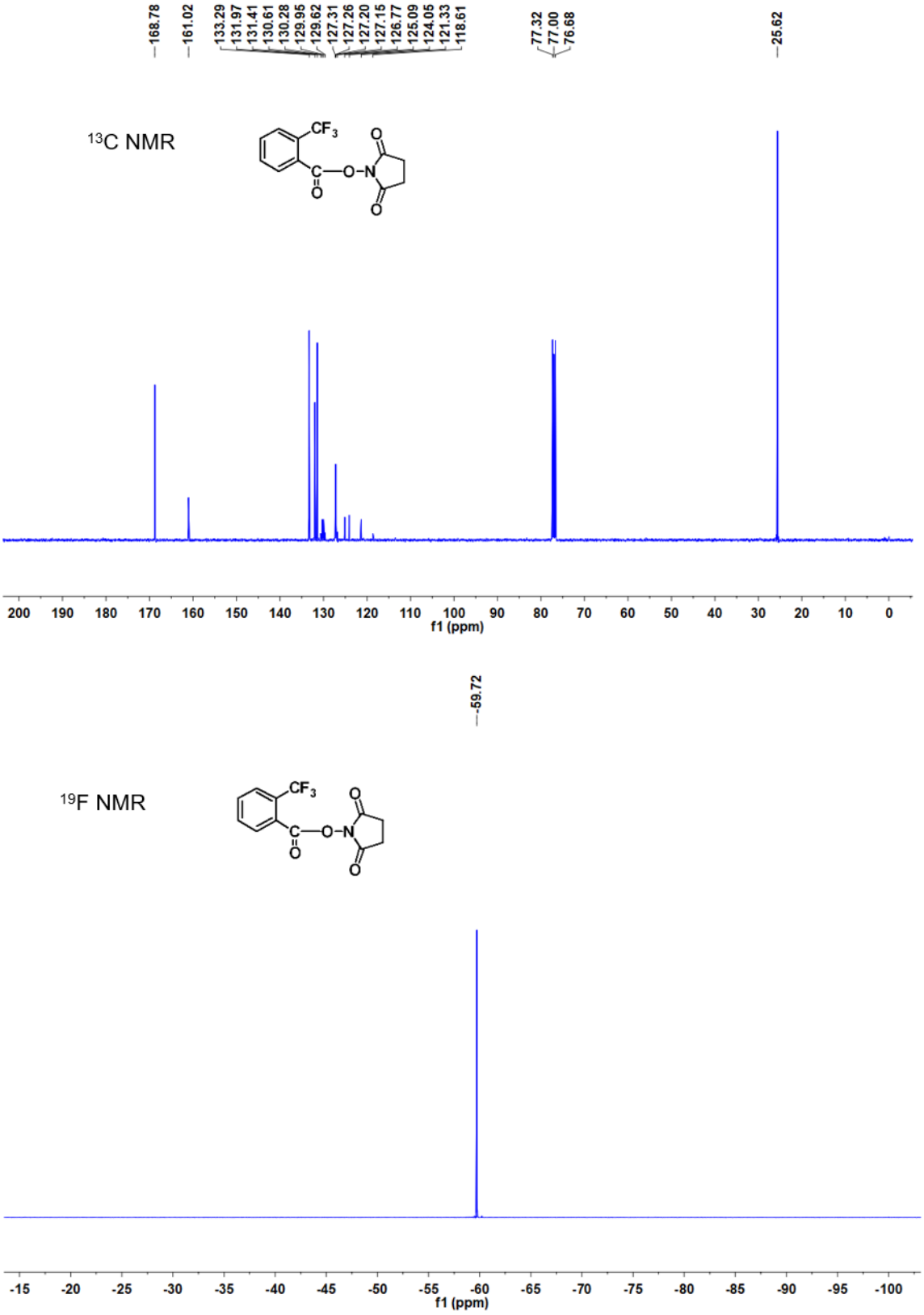


Figure S1. ^{1}H NMR, ^{13}C NMR and ^{19}F NMR spectra of ^{19}F-tag recorded in $CDCl_3$.

2. ^{19}F NMR sample preparation

Preparation of ^{19}F-tag solution. The stock solutions of 100 mM or 300 mM, 400 μL Tag 1 and Tag 2 in ultra-dry DMF were prepared and used for subsequent analysis.

Preparation of amino acid solution and their analogue. The stock solutions of 100 mM, 500 μL Gly, Ala, Val, Ser, Thr, Cys, Met, Asn, Gln, Lys, Arg, His, Pro and 4-methylimidazole were prepared in aqueous solution. The stock solutions of 50 mM, 500 μL Leu, Ile, Asp, Glu, Phe, Tyr, Trp, *N*-acetyl cysteine, *N*-acetyl tyrosine, *o*-methyl-tyrosine, *N*-acetyllysine were prepared in aqueous solution, and the pH was adjusted to 6-9 by using 5 M HCl or 5 M NaOH solution.

Preparation of phosphate solution. The 50 mM phosphate buffer of pH 7.5 was prepared by dissolving 0.0074 mol KH_2PO_4 and 0.0175 mol Na_2HPO_4 powder in 500 mL MilliQ water. The 50 mM phosphate buffer of pH 8.5 and 6.5 were prepared by adjusting the pH 8.5 or 6.5 with 5 M NaOH or 5 M HCl solution.

Chemical shifts of ^{19}F-tag labeled amino acids. 0.5 mM each amino acid was treated with 3.0 mM ^{19}F-tag in 50 mM phosphate buffer, pH 7.5 and the resulting mixture was incubated for 3 h and 4 h at 293 K for Tag 1 and Tag 2, respectively.

Simultaneous identification of 20 amino acids by reactive ^{19}F-tag. The solution of 0.1 mM 20 amino acids were mixed with 3 mM ^{19}F-tag in 50 mM phosphate buffer at pH 7.5, and the reaction mixture was incubated at 293 K for 2 h (Tag 1) and 8 h (Tag 2) followed by NMR spectra measurement.

Detection limit of amino acids by ^{19}F-tag. The detection limit of amino acids by Tag 2 was assessed by using methionine and arginine as an example. Different fractions of methionine and arginine mixture (sample 1: 200 μM Met, 200 μM Arg; sample 2: 1 μM Met, 1 μM Arg) were used for test. Sample 1 was incubated with 1.0 mM Tag 2 in 50 mM phosphate buffer at pH 7.5, 293 k for 6 h. Sample 2 was incubated with 1.0 mM Tag 2 in 50 mM phosphate buffer at pH 8.5, 293 k for 1 h and then heated at 70 ℃ for 10 minutes. The reaction mixture of sample 1 was diluted 200 times for NMR measurement. The samples were analyzed by NMR.

Work line of concentrations of amino acids with respect to peak heights of ^{19}F NMR signal. Nine different concentrations of amino acids (Val, Leu, Ile, Thr, Met, Phe, Trp, Lys and His) were selected. Each amino acid with concentrations of 0.05 mM, 0.1 mM, 0.2 mM, 0.3 mM, 0.4 mM, 0.5 mM, 0.6 mM, respectively was incubated with 1 mM ~ 6 mM ^{19}F-tag in 50 mM phosphate buffer, pH 7.5, for 2 h (Tag 1) and 10 h (Tag 2), and the respective ^{19}F NMR spectra were collected. A standard curve was therefore established between the ^{19}F peak heights of the amide product and the concentration of amino acid.

Quantitative determination of amino acids. A mixture of nine amino acids with concentrations of 0.05 mM and 0.1 mM, respectively, were treated with 3 mM and 6 mM Tag 2 in 50 mM phosphate buffer, pH 7.5. The reaction mixture was incubated 10 h at room temperature followed by ^{19}F NMR analysis. Each experiment was repeated three times and the ^{19}F NMR spectra were recorded at a magnetic

field with a ^{19}F frequency of 376 MHz. The concentrations of individual amino acid were calculated based on the standard curve.

Processing of fetal bovine serum (FBS) sample. The 2 mL commercial fetal bovine serum was first filtrated by a concentrator with molecular cutoff 3 kDa. The 1.2 mL filtrate was used for amino acids analysis. Commercialized the different batches (first batch and the second batch) of fetal bovine serum samples were processed in the same way to obtain FBS-1 and FBS-2, respectively.

Quantitative determination of amino acids in FBS. Sample 1: 100 μL FBS filtrate was mixed with 3.0 mM Tag 1 in 50 mM phosphate buffer at pH 7.5, and the resulting mixture was incubated for 1 h at room temperature followed by NMR measurement. Sample 2: 100 μL FBS filtrate was mixed with 9 mM Tag 1, 0.1 mM amino acids mixture of Thr, Gln, Ser, Val, Ile, Gly, Phe, Glu and Ala (each 0.1 mM), and the mixture was incubated for 1 h at room temperature followed by ^{19}F NMR measurements. The concentration of amino acid in FBS was determined by comparing the signal peak heights of the amino acid derivate in sample 1 and in sample 2.

Similarly, Tag 2 was used to identify the amino acid composition in FBS by preparing similar sample 1 as Tag 1. Sample 2 (sample 1 in addition of 0.1 mM Asp, 0.1 mM Thr, 0.1 mM Gln, 0.1 mM Ser, 0.1 mM Val, 0.1 mM Ile and 0.1 mM Leu), sample 3 (sample 1 in addition of 0.1 mM Thr, 0.1 mM Gln, 0.1 mM Leu and 0.1 mM Val), and sample 4 (sample 1 in addition of 0.1 mM Thr, 0.1 mM Ser and 0.1 mM Ile) were prepared to quantify the concentration of amino acids in FBS. All the samples treated with Tag 2 were incubated for 4 h at pH 8.5 before recording ^{19}F NMR spectra.

The quantification of amino acids in FBS-1 were repeated three times for Tag 1 and Tag 2, respectively. The quantification of amino acids in FBS-2 were repeated once for Tag 1 and Tag 2, respectively.

Preparation of *Escherichia coli* lysates. Bacterial cells were grown at 37 ℃ in 300 mL Luria broth (LB) rich medium shaken at 200 rpm. Once the optical cell density at 600 nm (OD600) reached about 0.9, the cell culture was harvested by ultracentrifuge at 6000 rpm for 8 min. The cell pellet was weighted about 0.9 g and lysed with 3.6 mL 20 mM PB buffer at pH 7.5. Then the lysates were centrifuged at 12000 rpm for 30 min, and the supernatant was centrifuged with a concentrated tube of 3 kDa cutoff.

Quantitative determination of amino acids in *Escherichia coli* lysates. Sample 1: 2 % (m/m) *Escherichia coli* lysates were mixed with 6.0 mM Tag 1 in 50 mM phosphate buffer at pH 7.5, and the resulting mixture was incubated for 1 h at room temperature followed by NMR measurement. Sample 2: 2 % (m/m) *Escherichia coli* lysates were mixed with 12.0 mM Tag 1, amino acids mixture of 0.1 mM Thr, 0.1 mM Gly, 0.1 mM Phe, 0.1 mM Glu, 0.1 mM Val and 0.1 mM Ile. Sample 3: 2 % (m/m) *Escherichia coli* lysates were mixed with 12.0 mM Tag 1, amino acids mixture of 0.1 mM Asp, 0.1 mM Ser, 0.1 mM Trp, 0.1 mM His, 0.1 mM Met and 0.1 mM Ala. All the samples treated with Tag 1 were incubated for 1 h at pH 7.5 before recording ^{19}F NMR spectra. The concentrations of amino acid in *Escherichia coli* lysates were determined by comparing the signal peak heights of the amino acid derivate in sample 1 and in sample 2, in sample 3.

3. NMR experiments

^{1}H NMR, ^{13}C NMR and ^{19}F NMR spectra of ^{19}F-tag were recorded with a Bruker 400 MHz NMR spectrometer. The amino acid analysis was performed in 50 mM phosphate buffer, pH 6.5, 7.5 and 8.5 and the NMR spectra were recorded in Bruker 400 MHz or 800 MHz NMR spectrometer. The chemical shift was calibrated by internal reference of trifluoroacetic acid (TFA, -75.55 ppm). Proton decoupling was performed for the mixture of Tag 1 and 20 amino acids, and FBS samples, and *Escherichia coli* lysates with a ^{19}F frequency of 376 MHz.

The NMR spectra of FBS samples and *Escherichia coli* lysates treated with Tag 2 was recorded with a proton frequency of 753 MHz equipped with a H(F)-TCI cryoprobe.

4. Formation rate constant (*k*) of amide complex by the reaction of ^{19}F-tag with amino acids

To further assess the apparent formation rate of the ^{19}F-tag and amino acids at different pH values, the reaction of single amino acid with ^{19}F-tag was monitored in 50 mM phosphate buffer, pH 6.5, 7.5 and 8.5, respectively. The hydrolysis of ^{19}F-tag was present in the reaction mixture, and the apparent formation rate of the backbone amide product formed by the ^{19}F-tag with amino acid was roughly determined by simulation of the product peak height with incubation time. Since the concentration of amino acids was excess (the concentration of amino acids used was 6 times the concentration of Tag 1 and 10 times the concentration of Tag 2), the formation of the backbone amide product can be regarded as a pseudo-first order reaction as shown in equation (1)

$$[P] = [P_0](1 - e^{-kt}) \quad (1)$$

where [P] and [P_0] represent the concentration of the backbone amide product at time t and the maximum concentration of backbone amide product, respectively. *k* is the apparent formation rate constant. The apparent formation rate constant was determined by fitting the increase of the backbone amide product with time.

5. Stability evaluation of ^{19}F-tag by ^{19}F NMR in aqueous solution

The overall half-lifetime of Tag 1 and Tag 2 at pH 7.5 is about 0.3 and 6 h, respectively. The chemical shifts of these intermediates are well resolved from the NMR signals of the amide products formed by the tags with the amino acids. These intermediates, though not known chemical structures, do not interfere the subsequent amino acid detections.

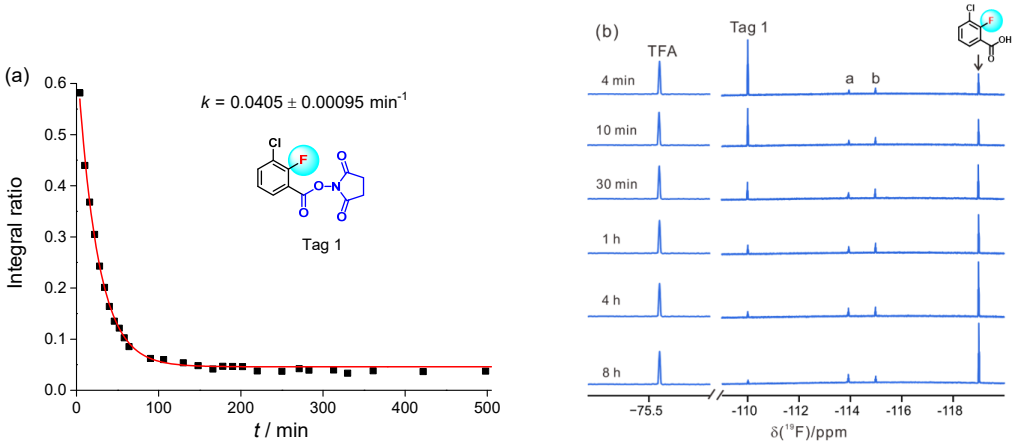


Figure S2. (a) Stability evaluation of 3.0 mM Tag 1 by ^{19}F NMR spectra in aqueous solution, and hydrolysis rate was determined by monitoring the peak area of Tag 1 in the ^{19}F NMR spectra as shown in (b). In b), the hydrolysis intermediates of Tag 1 are marked as a (-113.94 ppm) and b (-114.98 ppm), respectively. The ^{19}F NMR spectra were recorded at 293 K in 50 mM phosphate buffer, pH 7.5 with a ^{19}F frequency of 376 MHz.

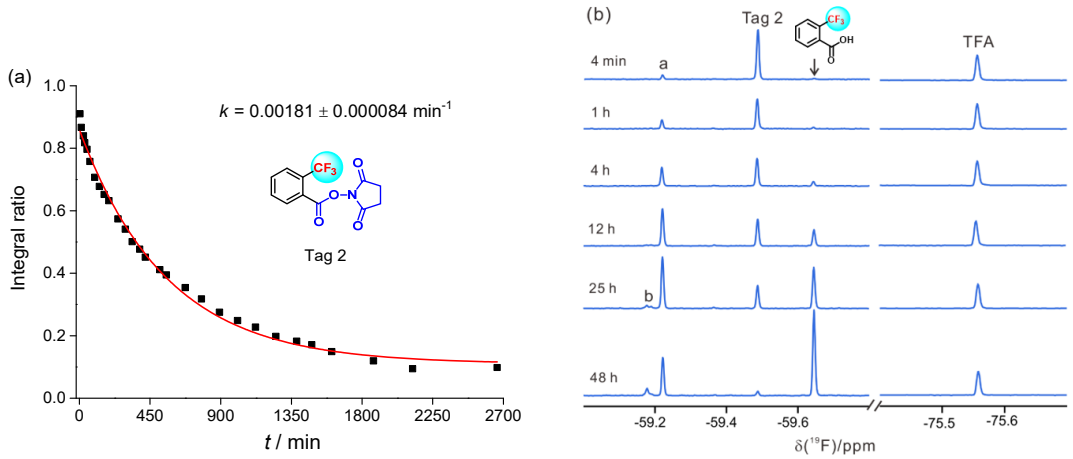


Figure S3. (a) Stability evaluation of 3.0 mM Tag 2 by ^{19}F NMR spectra in aqueous solution, and hydrolysis rate was determined by monitoring the peak area of Tag 2 in the ^{19}F NMR spectra as shown in (b). In b), the hydrolysis intermediates of Tag 2 are marked as a (-59.22 ppm) and b (-59.18 ppm), respectively. The ^{19}F NMR spectra were recorded at 293 K in 50 mM phosphate buffer, pH 7.5 with a ^{19}F frequency of 376 MHz.

6. Kinetic assay of ^{19}F-tag with amino acids

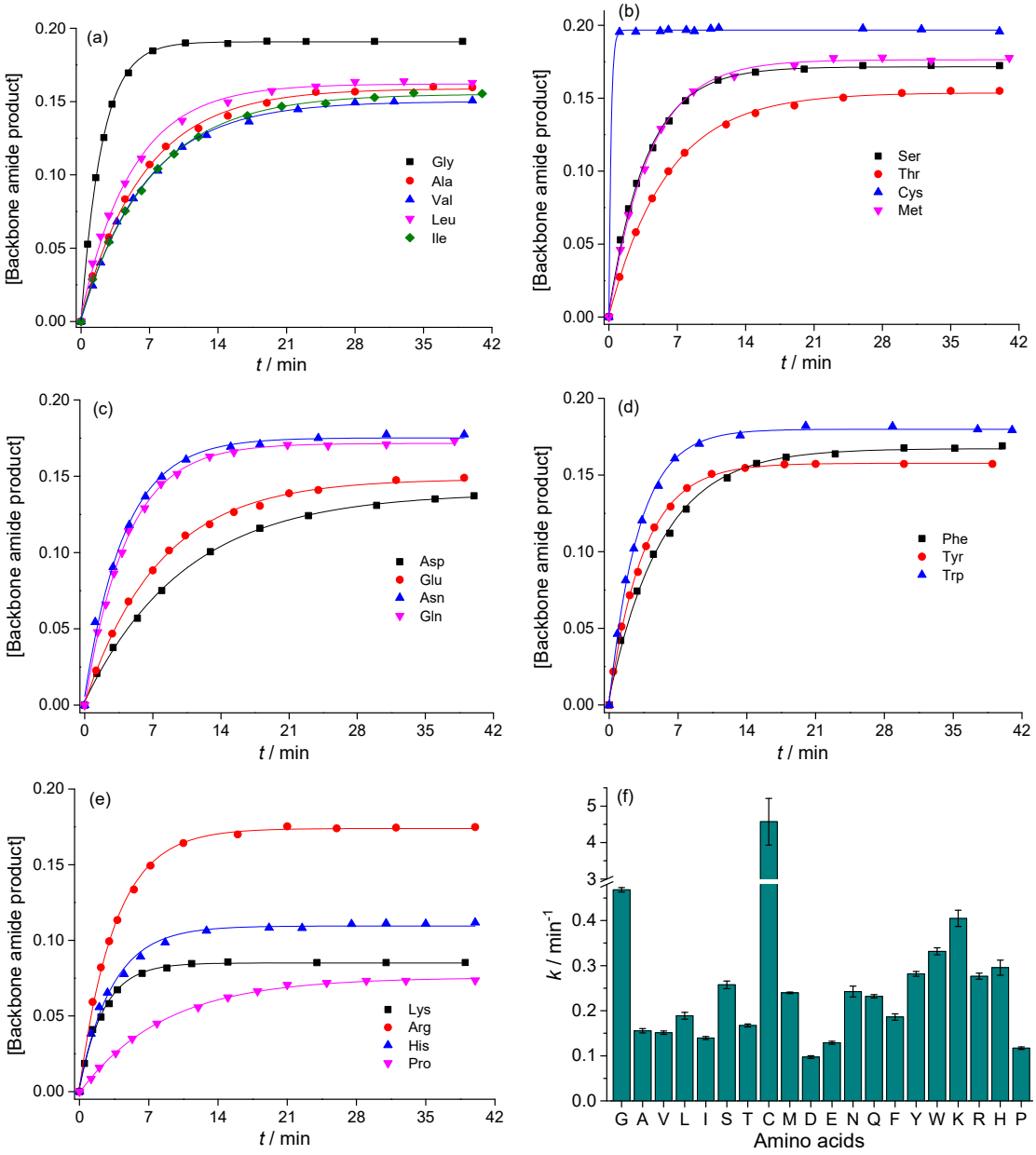


Figure S4. (a)-(e) Reaction profile of 0.2 mM Tag 1 with 1.2 mM each amino acid as monitored by ^{19}F NMR spectra. The concentration of the backbone amide product was monitored with incubation time at 293 K in 50 mM phosphate buffer, pH 7.5. (f) The overall formation rate of backbone amides produced by the reaction between the 0.2 mM Tag 1 with 1.2 mM amino acids at 293 K in 50 mM phosphate buffer, pH 7.5. The ^{19}F NMR spectra were recorded at a magnetic field with a ^{19}F frequency of 753 MHz.

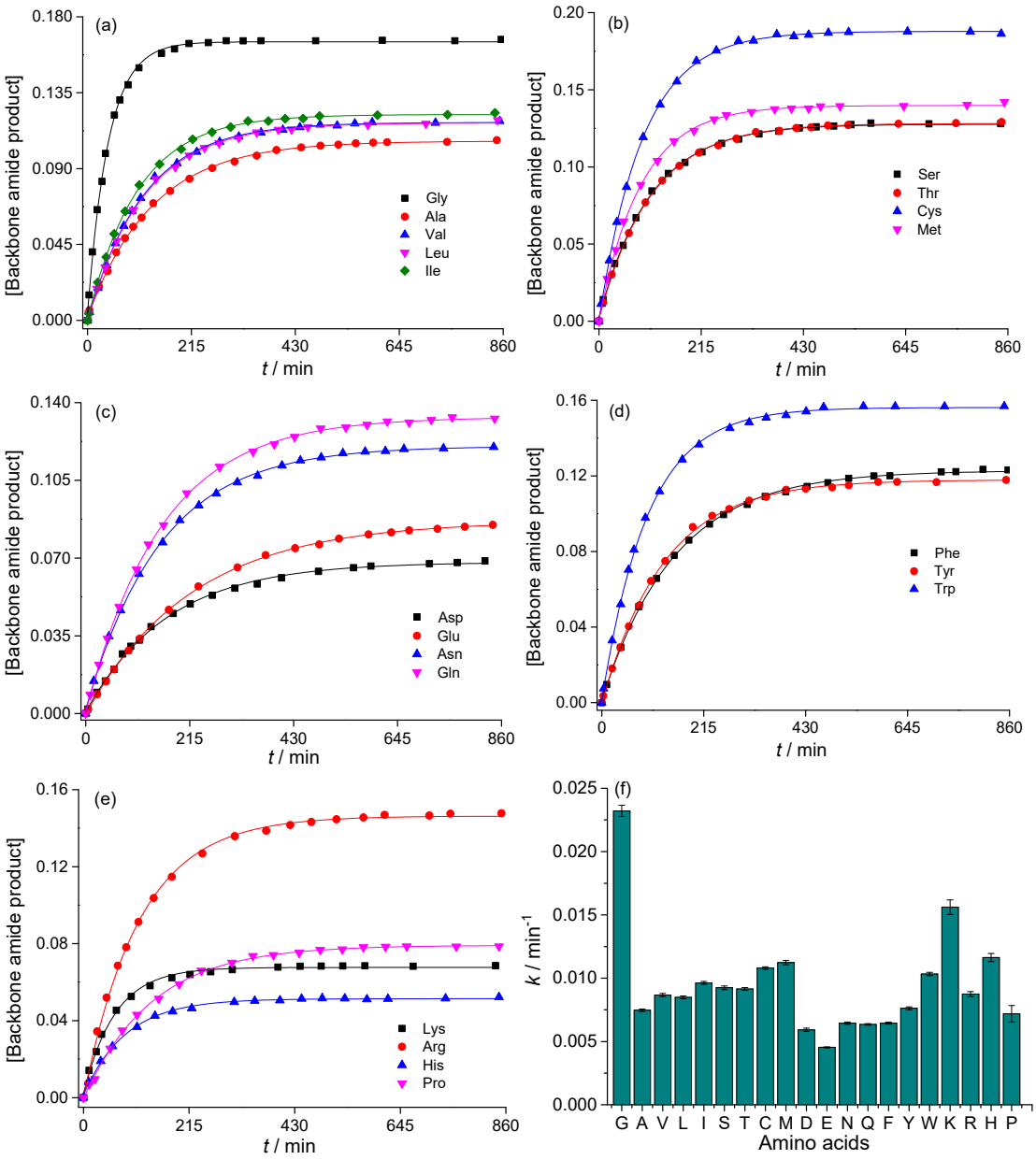


Figure S5. (a)-(e) Reaction profile of 0.2 mM Tag 2 with 2.0 mM each amino acid as monitored by ^{19}F NMR spectra. The concentration of the backbone amide product was monitored with incubation time at 293 K in 50 mM phosphate buffer, pH 7.5. (f) The overall formation rate of backbone amides produced by the reaction between the 0.2 mM Tag 2 with 2.0 mM amino acids at 293 K in 50 mM phosphate buffer, pH 7.5. The ^{19}F NMR spectra were recorded at a magnetic field with a ^{19}F frequency of 376 MHz.

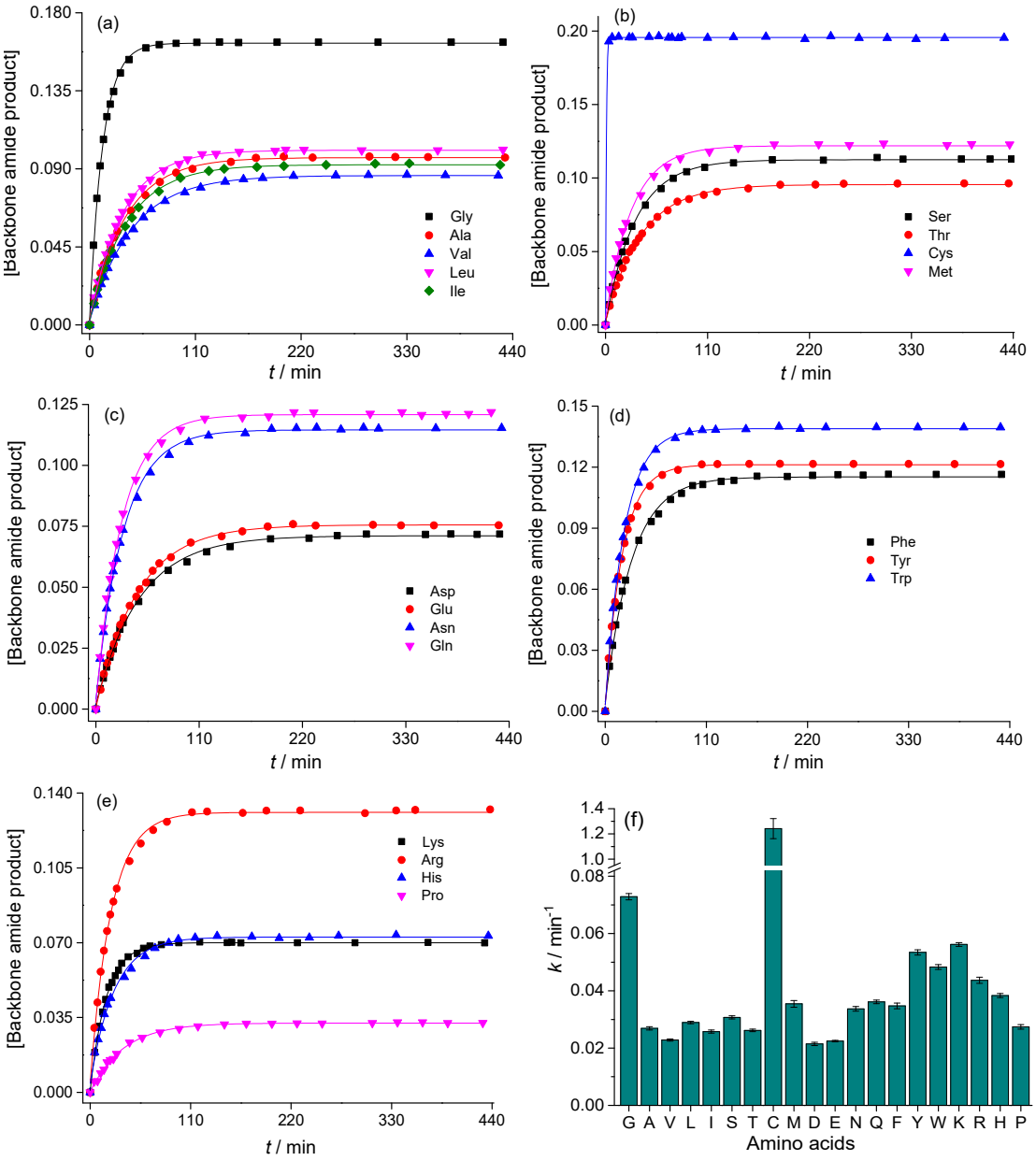


Figure S6. (a)-(e) Reaction profile of 0.2 mM Tag 1 with 1.2 mM each amino acid as monitored by ^{19}F NMR spectra. The concentration of the backbone amide product was monitored with incubation time at 293 K in 50 mM phosphate buffer, pH 6.5. (f) The overall formation rates of backbone amides produced by the reaction between the 0.2 mM Tag 1 with 1.2 mM amino acids at 293 K in 50 mM phosphate buffer, pH 6.5. The ^{19}F NMR spectra were recorded at a magnetic field with a ^{19}F frequency of 753 MHz.

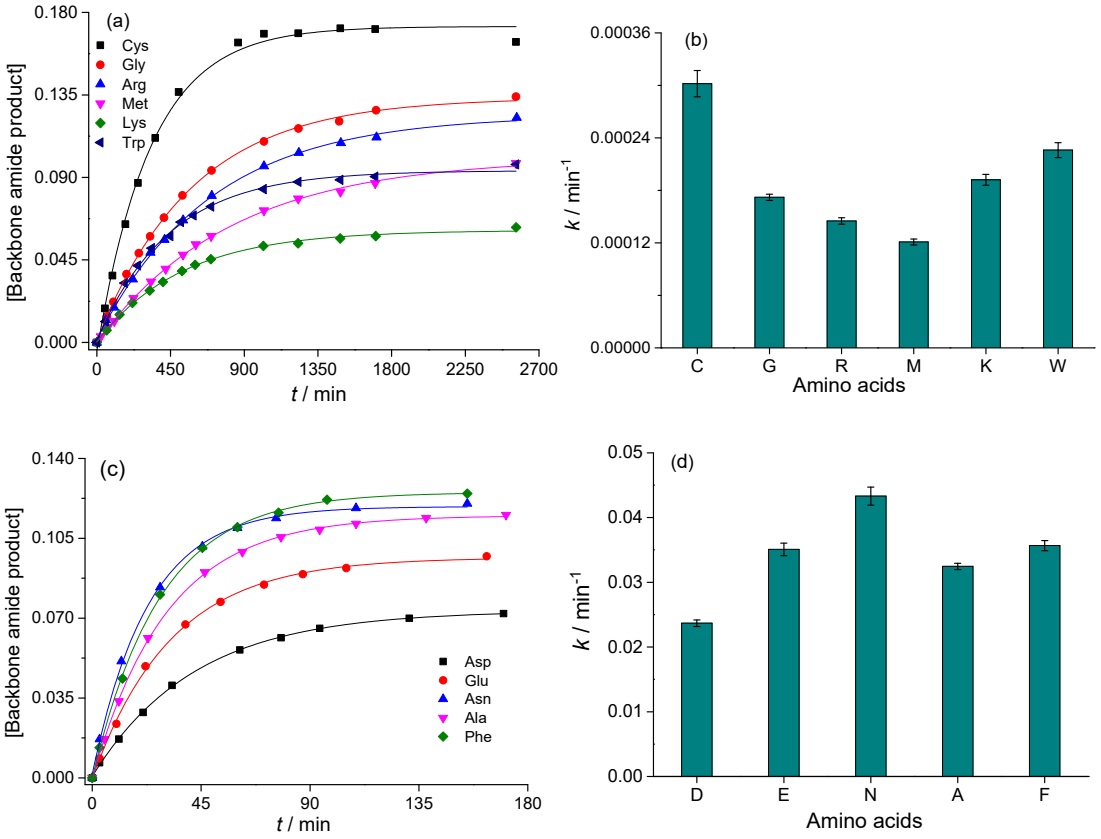


Figure S7. Reaction profile of 0.2 mM Tag 2 with 2.0 mM each amino acid as monitored by ^{19}F NMR spectra. The concentration of the backbone amide product was monitored with incubation time at 293 K in 50 mM phosphate buffer, pH 6.5 (a) and pH 8.5 (c). The overall formation rates of amides produced by the reaction between the 0.2 mM Tag 2 with 2.0 mM amino acids, in 50 mM phosphate buffer, pH 6.5 (b) and pH 8.5 (d) and at 293 K. The ^{19}F NMR spectra were recorded at a magnetic field with a ^{19}F frequency of 376 MHz (pH 6.5) and 753 MHz (pH 8.5).

7. Products produced by the ^{19}F-tag formed with Cys, Tyr, Lys and His

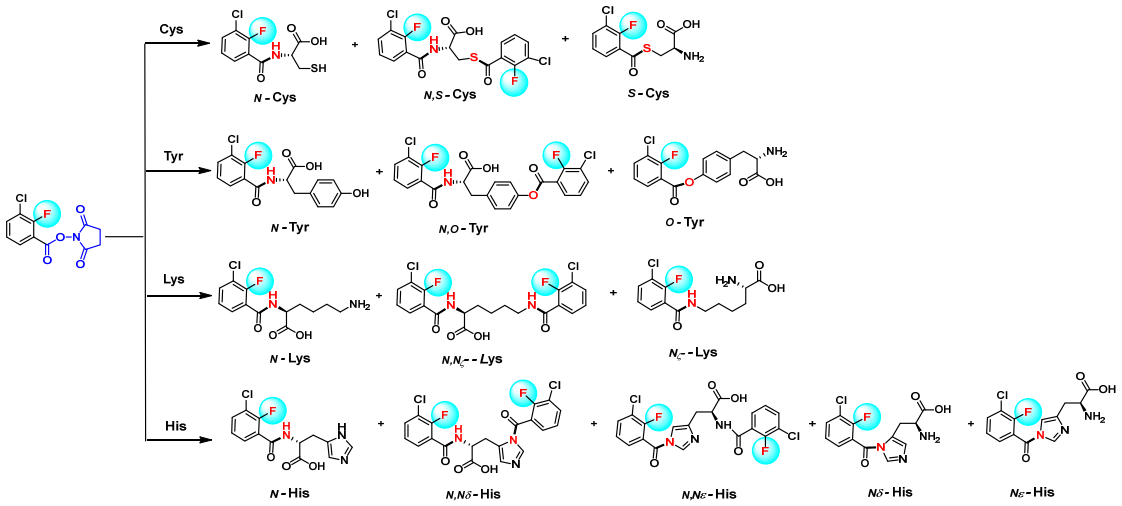
General description

Tag 1:

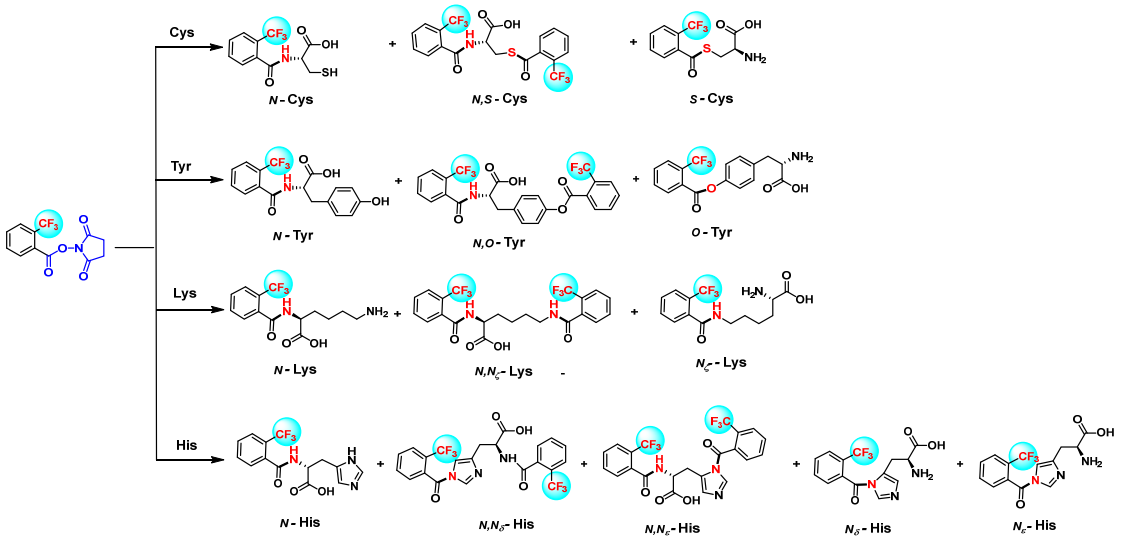
As to Cys, two F signals were determined, and thioester and amide were both formed (Figure S8). Four new ^{19}F signals were produced in that of Tyr with Tag 1, *N*-Tyr has chemical shift at -117.55 ppm, *O*-Tyr at -112.95 ppm and *N,O*-Tyr at -117.60 and -113.09 ppm (Figure S9), respectively. The products of Lys with Tag 1 have -117.88 ppm for *N*-Lys, -118.44 ppm for N_ζ-Lys, -117.80 ppm and -118.46 ppm for N,N_ζ-Lys (Figure S10). As to His, four F signals, *N*-His signals at -117.73 ppm, the signals of N_ε-His and N_δ-His were overlapped at -115.48 ppm, N,N_ε-His and N,N_δ-His at -118.16 and -115.81 ppm, respectively, were determined. The products of N_ε-His and N_δ-His were not stable and hydrolyze with time (Figure S11), resulting in the only stable amide complex in solution.

Tag2:

Tag 2 performs differently from Tag 1. Four main F signals were determined in the mixture of Cys with excess of Tag 2: -58.38 and -58.96 ppm were assigned to *N,S*-Cys, -58.97 ppm of *N*-Cys, and -58.36 ppm of *S*-Cys (Figure S12). It is noted that the thioester product, *S*-Cys, was firstly formed and then transformed to the amide, *N*-Cys. The consequent reaction of *N*-Cys with the ^{19}F-tag in formation of thioester was much slower than free Cys, resulting in the dual ^{19}F-labeled *N,S*-Cys product was less dominant, which is in great contrast to the Tag 1. When Tag 2 reacted with tyrosine, only two products, *N*-Tyr (-59.19 ppm) and *O*-Tyr (-59.03 ppm) were observed (Figure S13). Dual ^{19}F-labeled product, *N,O*-Tyr, was not produced in the first 8 h. Four F signals were determined for Lys, -59.08 ppm of *N*-Lys, -59.19 ppm of N_ζ-Lys, -59.14 ppm and -59.27 ppm of N,N_ζ-Lys were assigned (Figure S14). Three F signals, -58.93 ppm and -59.05 ppm of N_ε-His and N_δ-His, and -59.35 ppm of *N*-His, were produced in the mixture of His and Tag 2 (Figure S15).



Scheme S2. The chemical structures of possible products produced by the reaction of Tag 1 with Cys, Tyr, Lys and His.



Scheme S3. The chemical structures of possible products produced by the reaction of Tag 2 with Cys, Tyr, Lys and His.

Determination of resonance signals of the ^{19}F-tag labeled Cys, Tyr, Lys, and His by Tag 1 and Tag 2

1) Tag 1

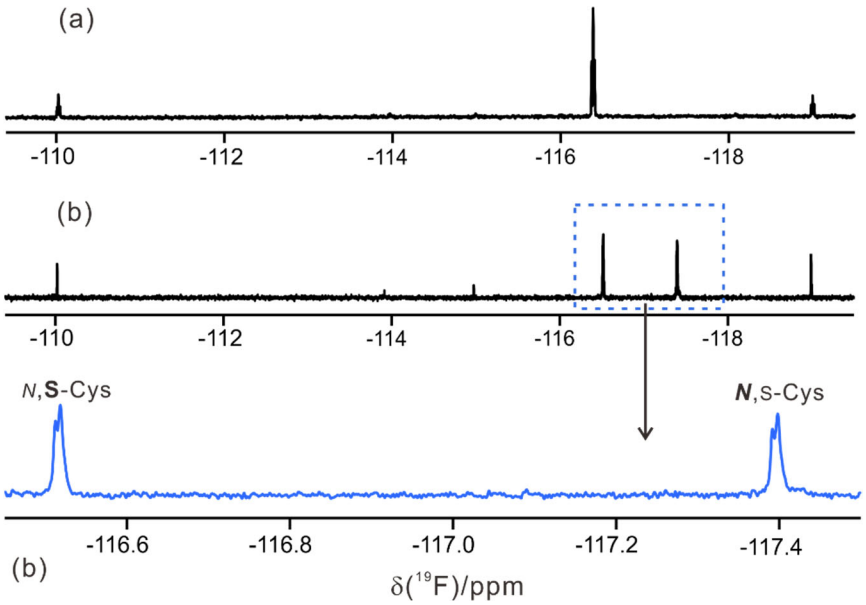


Figure S8. Comparison of the ^{19}F NMR spectra of the Tag 1 reaction with *N*-acetyl cysteine (NAC) (a) and Cys (b). The concentration of the Tag 1 and Cys was 3.0 and 0.5 mM, respectively. The ^{19}F NMR spectra were recorded after 0.5 h incubation at 293 K in 50 mM phosphate buffer, pH 7.5. The ^{19}F NMR spectra were recorded at a magnetic field with a ^{19}F frequency of 376 MHz. In b) proton decoupling was performed to resolve the overlapped signals.

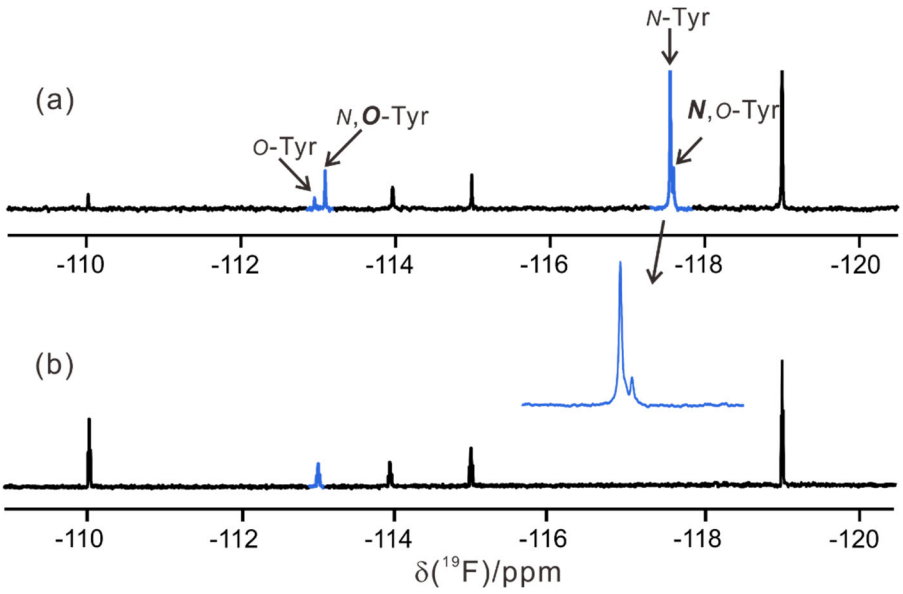


Figure S9. Comparison of the ^{19}F NMR spectra of the Tag 1 in reaction with Tyr (a) and *N*-acetyl tyrosine (b). The concentration of the Tag 1 and Tyr/*N*-acetyl tyrosine was 3.0 and 0.5 mM, respectively. The ^{19}F NMR spectra were recorded after 1 h incubation at 293 K in 50 mM phosphate buffer, pH 7.5. The ^{19}F NMR spectra were recorded at a magnetic field with a ^{19}F frequency of 376 MHz. In a) proton decoupling was performed to resolve the overlapped signals.

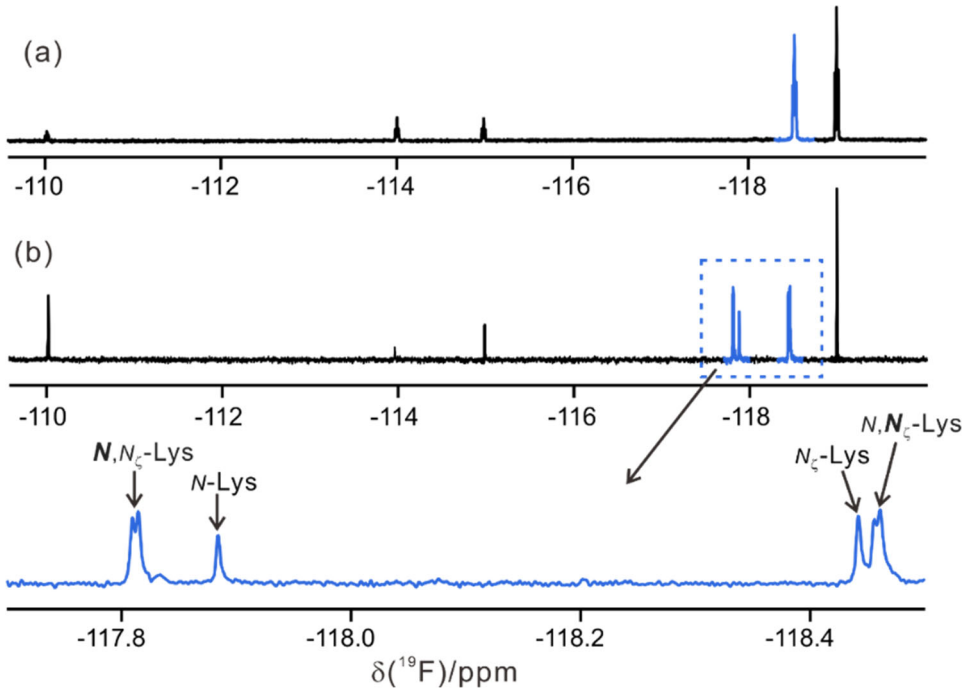


Figure S10. Comparison of the ^{19}F NMR spectra of the Tag 1 in reaction with N_6-acetyl lysine (a) and Lys (b). The concentration of the Tag 1 and N_6-acetyl lysine/Lys was 3.0 and 0.5 mM, respectively. The ^{19}F NMR spectra were recorded after 3 h and 0.5 h incubation for N_6-acetyl lysine and Lys at 293 K in 50 mM phosphate buffer, pH 7.5. The ^{19}F NMR spectra were recorded at a magnetic field with a ^{19}F frequency of 376 MHz. In b) proton decoupling was performed to resolve the overlapped signals.

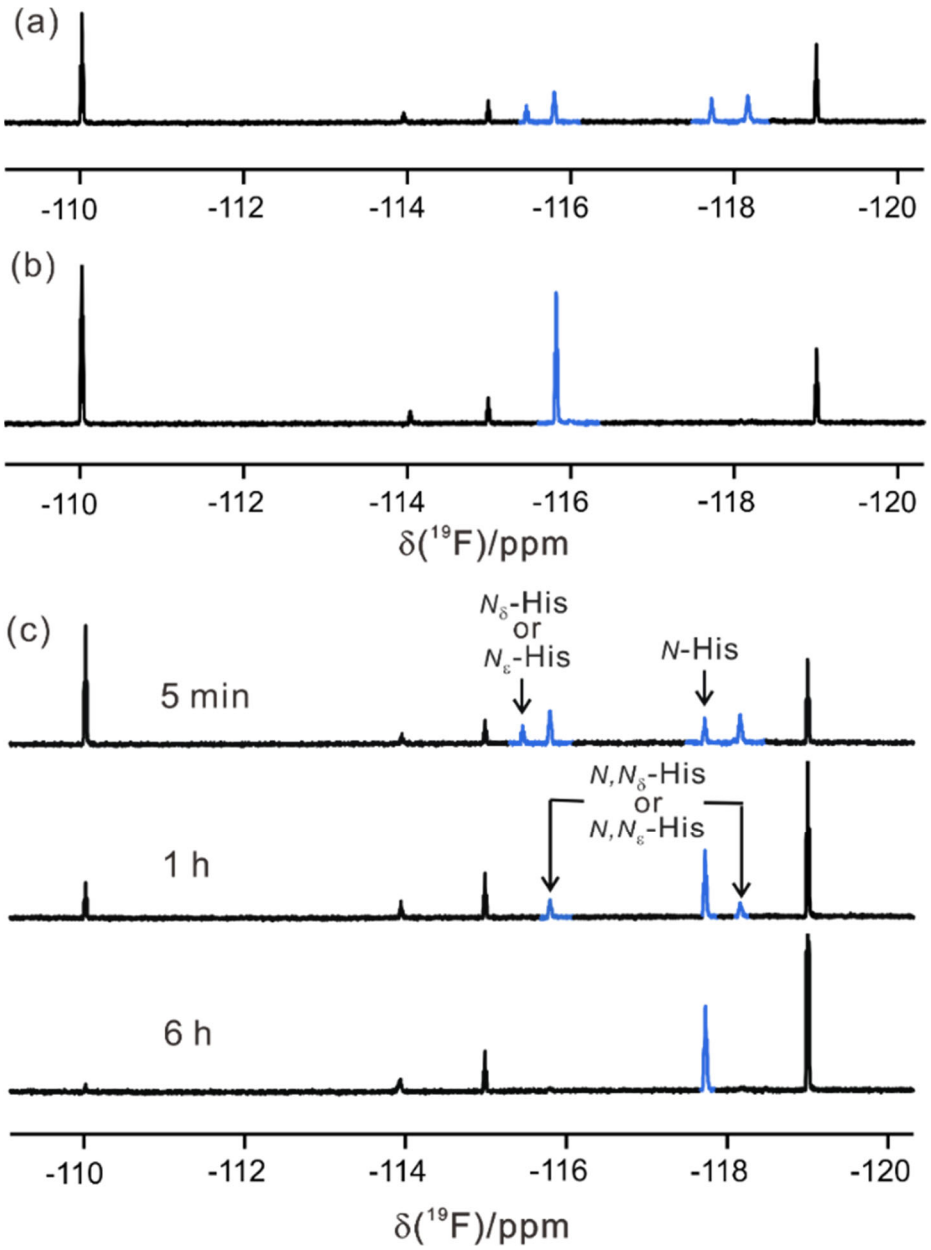


Figure S11. Comparison of the ^{19}F NMR spectra of the Tag 1 in reaction with His (a) and 4-methylimidazole (b). The ^{19}F NMR spectra were recorded after 0.5 h incubation at 293 K in 50 mM phosphate buffer, pH 7.5. (c) Time-dependence of ^{19}F NMR spectra recorded for the mixture of the Tag 1 and His. The concentration of the Tag 1 and His/4-methylimidazole was 3.0 and 0.5 mM, respectively. The ^{19}F NMR spectra were recorded at a magnetic field with a ^{19}F frequency of 376 MHz.

2) Tag 2

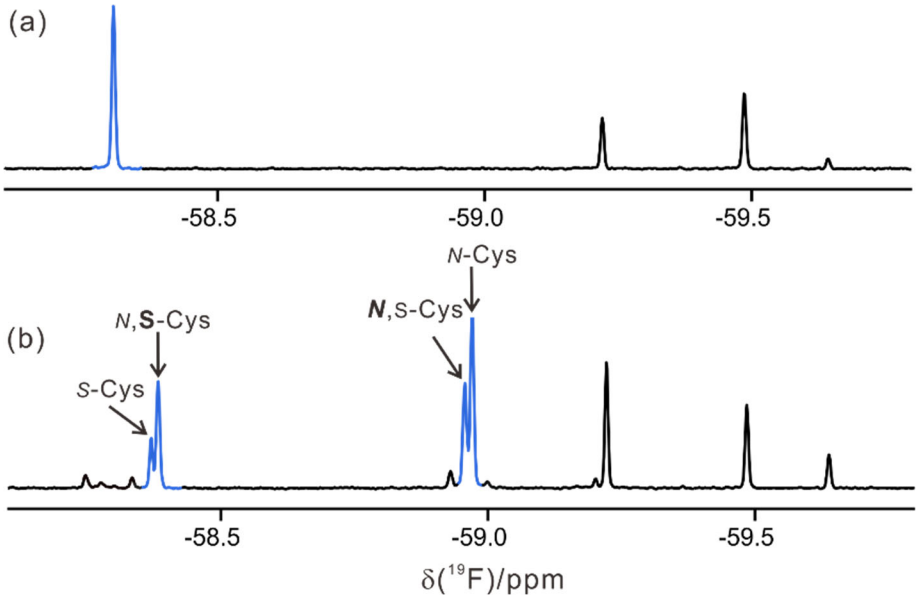


Figure S12. Comparison of the ^{19}F NMR spectra of the Tag 2 reaction with *N*-acetyl cysteine (NAC) (a) and Cys (b). The concentration of the Tag 2 and NAC/Cys was 3.0 and 0.5 mM, respectively. The ^{19}F NMR spectra were recorded after 3 h incubation at 293 K in 50 mM phosphate buffer, pH 7.5. The ^{19}F NMR spectra were recorded at a magnetic field with a ^{19}F frequency of 376 MHz.

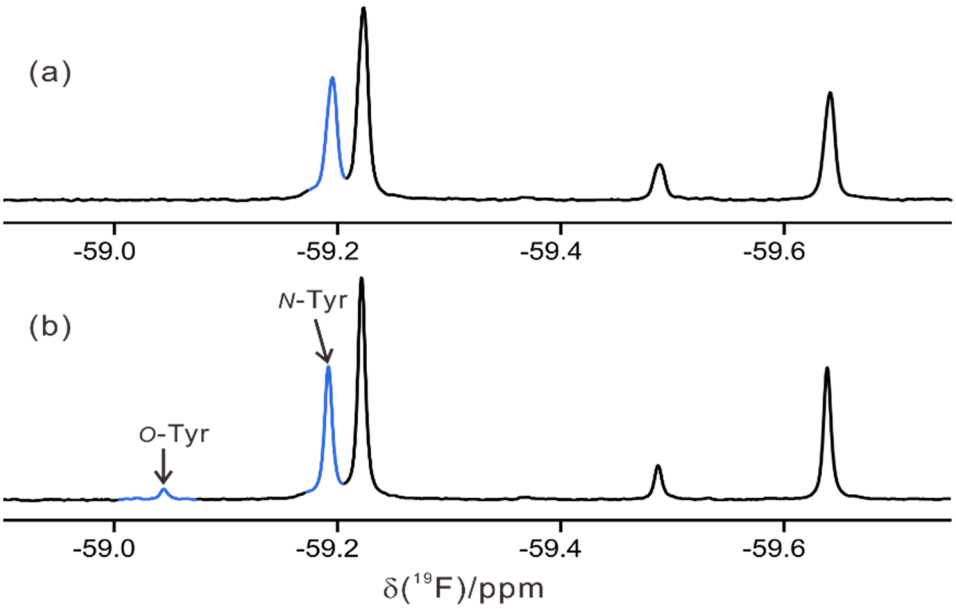


Figure S13. Comparison of the ^{19}F NMR spectra of the Tag 2 in reaction with *o*-methyl-tyrosine (a) and Tyr (b). The concentration of the Tag 2 and *o*-methyl-tyrosine/Tyr was 3.0 and 0.5 mM, respectively. The ^{19}F NMR spectra were recorded after 8 h incubation at 293 K in 50 mM phosphate buffer, pH 7.5. The ^{19}F NMR spectra were recorded at a magnetic field with a ^{19}F frequency of 376 MHz.

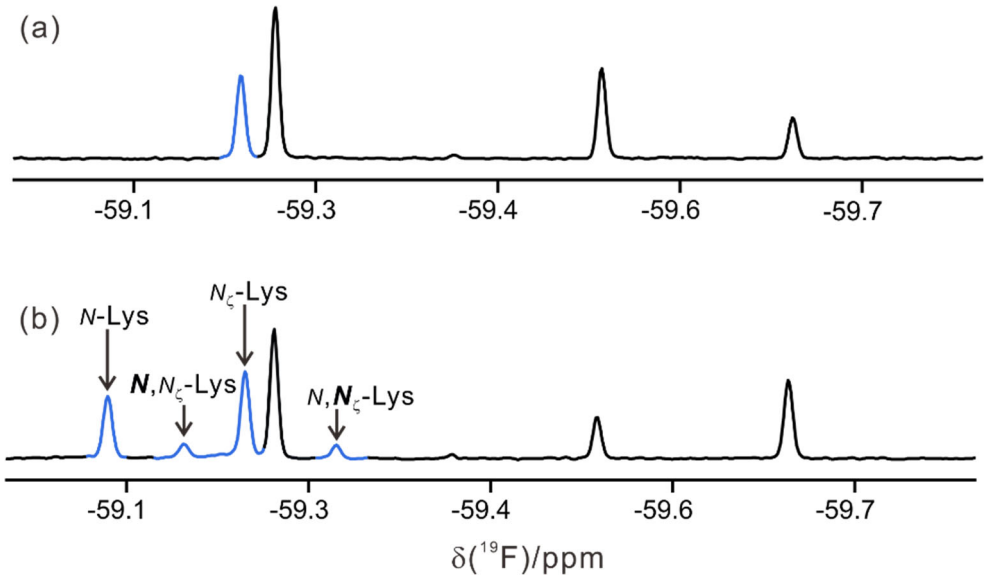


Figure S14. Comparison of the ^{19}F NMR spectra of the Tag 2 in reaction with N_6-acetyllysine (a) and Lys (b). The concentration of the Tag 2 and N_6-acetyllysine/Lys was 3.0 and 0.5 mM, respectively. The ^{19}F NMR spectra were recorded after 3 h incubation at 293 K in 50 mM phosphate buffer, pH 7.5. The ^{19}F NMR spectra were recorded at a magnetic field with a ^{19}F frequency of 376 MHz.

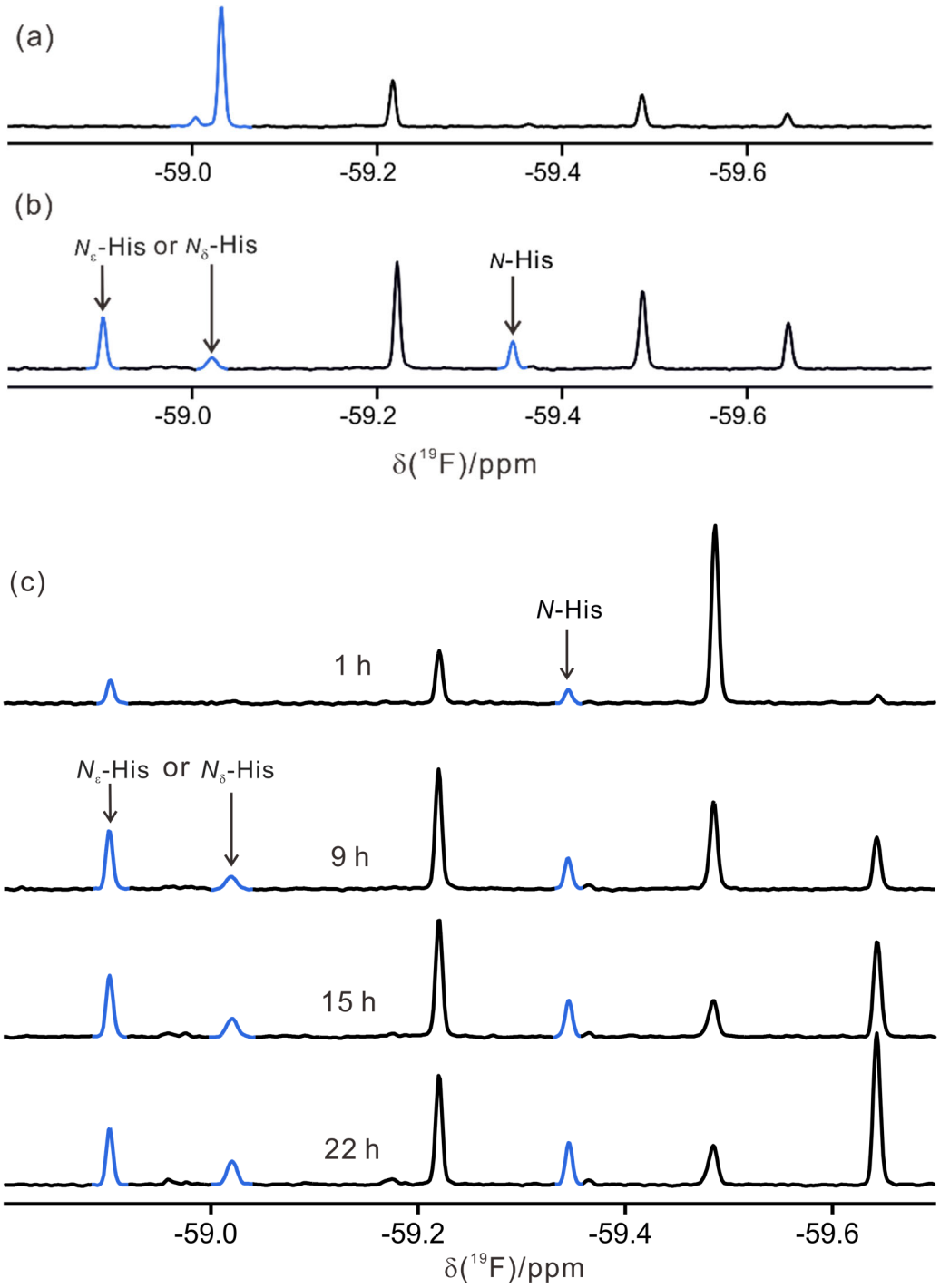


Figure S15. Comparison of the ^{19}F NMR spectra of the Tag 2 in reaction with 4-methylimidazole (a) and His (b), and the time-dependence of ^{19}F NMR spectra recorded for the mixture of the Tag 2 and His (c). The concentration of the Tag 2 and 4-methylimidazole/His was 3.0 and 0.5 mM, respectively. The ^{19}F NMR spectra were recorded after 3 h incubation at 293 K in 50 mM phosphate buffer, pH 7.5. The ^{19}F NMR spectra were recorded at a magnetic field with a ^{19}F frequency of 376 MHz.

8. The detection limit of amino acids by Tag 2

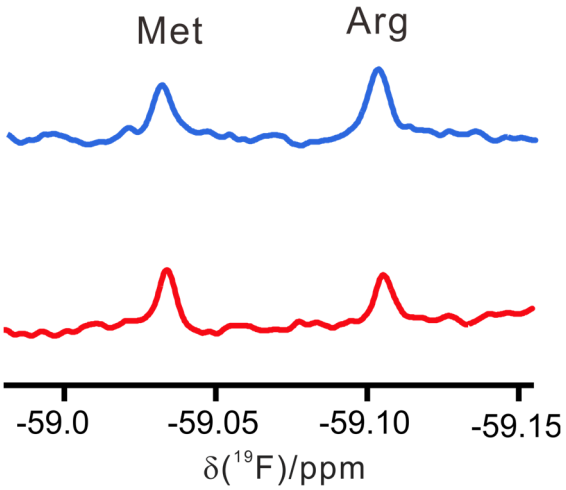


Figure S16. The detection limit of amino acids by Tag 2. 1.0 mM Tag 2 was mixed with 200 μM methionine and 200 μM arginine, and the resulting mixture was diluted 200 times followed by ^{19}F NMR measurement (blue). 1.0 mM Tag 2 was mixed with 1 μM methionine and 1 μM arginine followed by NMR measurement (red). The total measurement time was within 60 minutes. The ^{19}F NMR spectra was recorded after 6 h and 1 h incubation at 293 K in 50 mM phosphate buffer, pH 7.5 (blue) and 8.5 (red). The ^{19}F NMR spectra were recorded at a magnetic field with a ^{19}F frequency of 753 MHz.

9. Simultaneous identification of 20 amino acids by reactive ^{19}F-tag

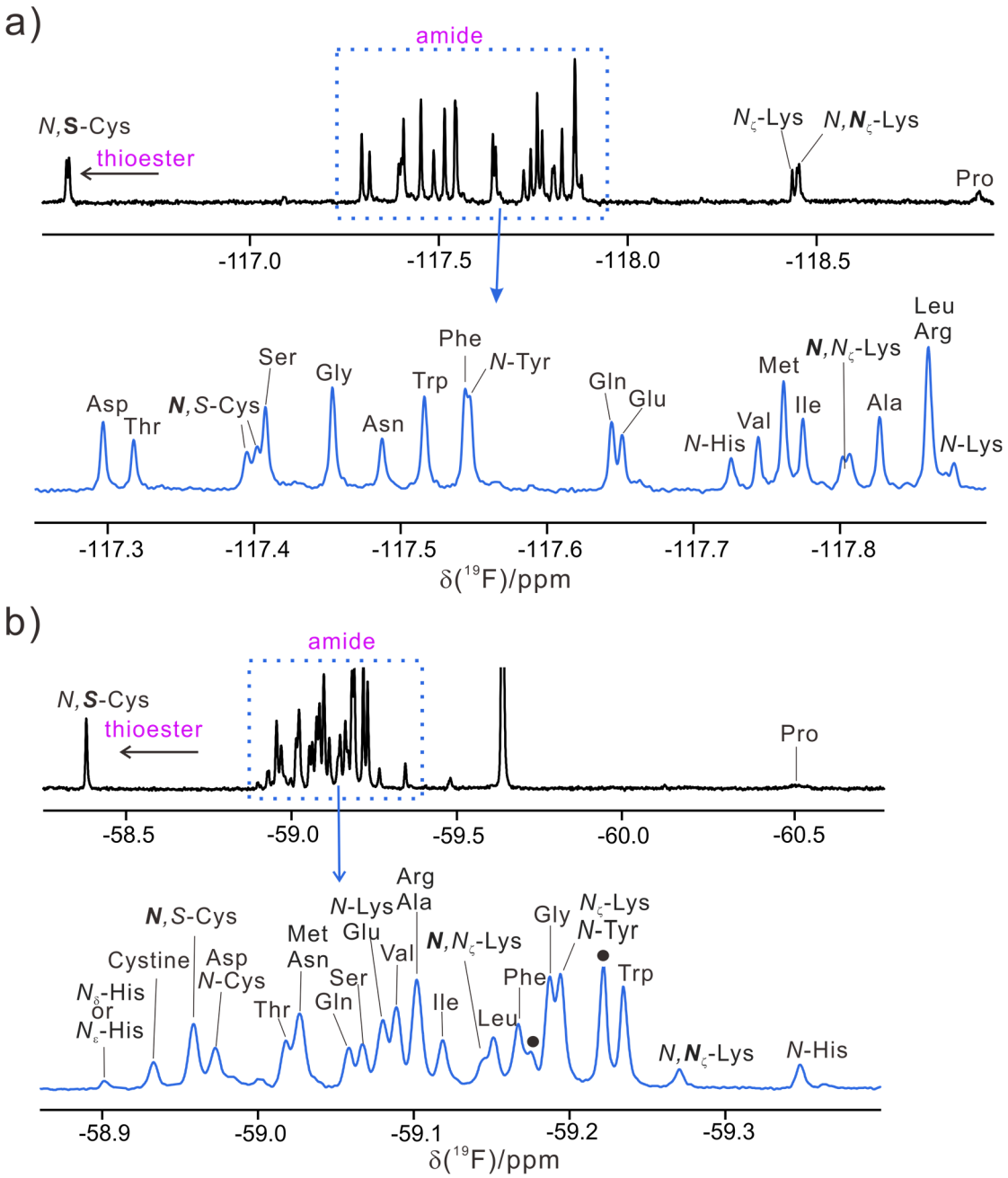
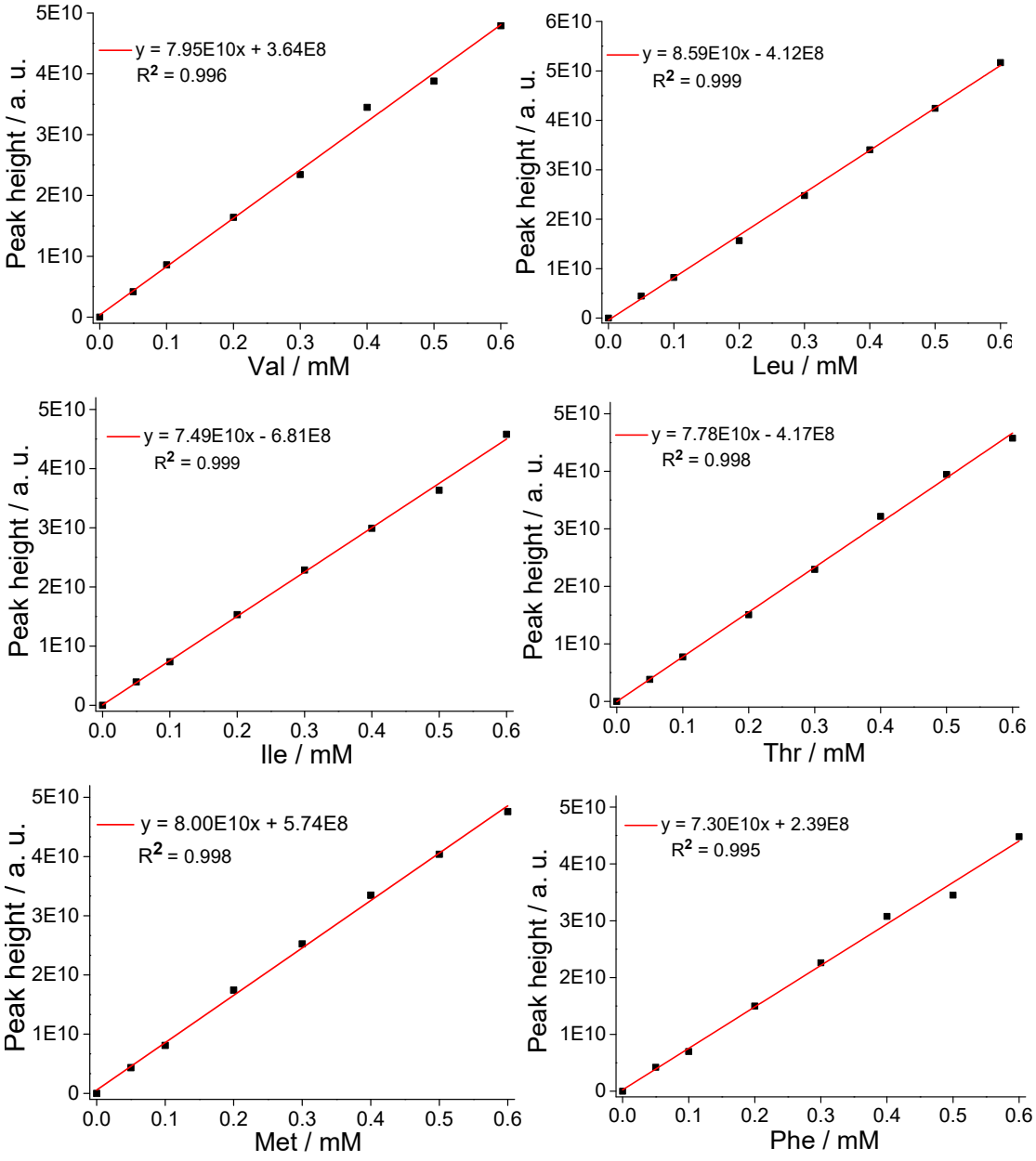
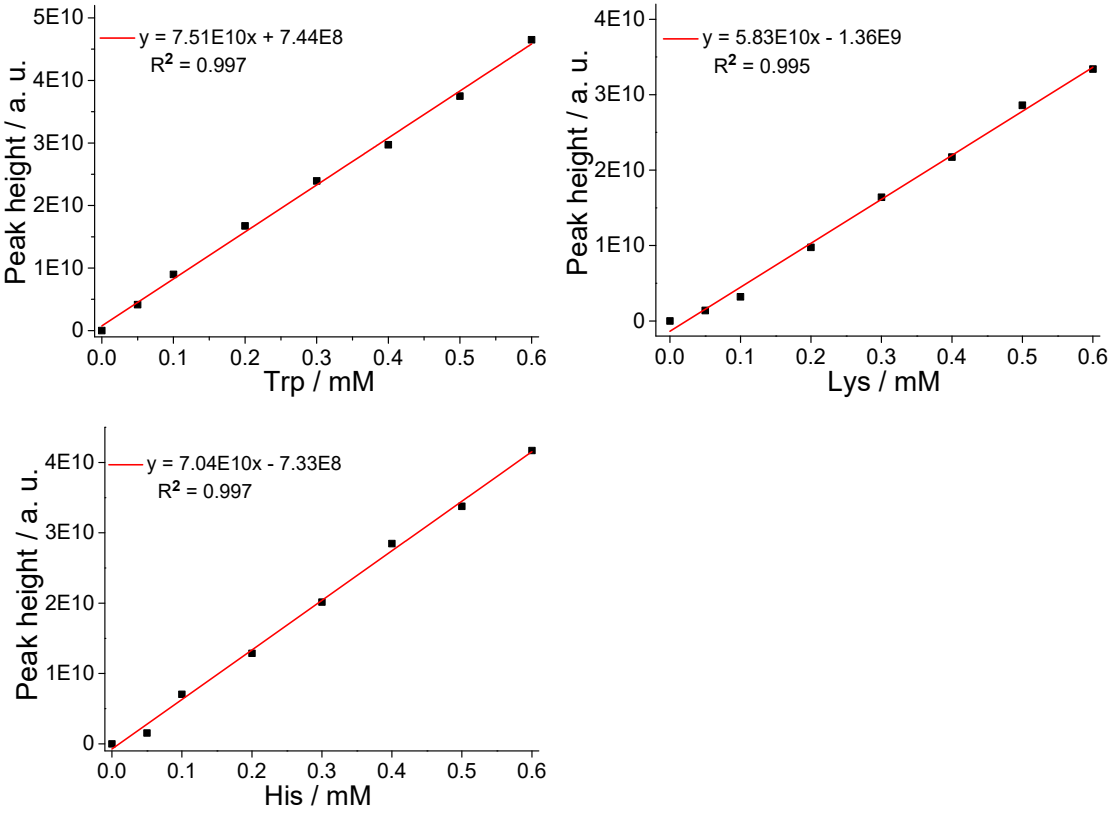


Fig. S16. 19F-NMR spectra recorded for the mixture of 0.1 mM 20 amino acids and 3 mM ^{19}F-tag in 50 mM phosphate buffer at pH 7.5, and the reaction mixture was incubated at 293 K for 2 h (Tag 1) (a) and 8 h (Tag 2) (b) followed by NMR spectra measurement, of which the spectra (a) were recorded with proton decoupling.

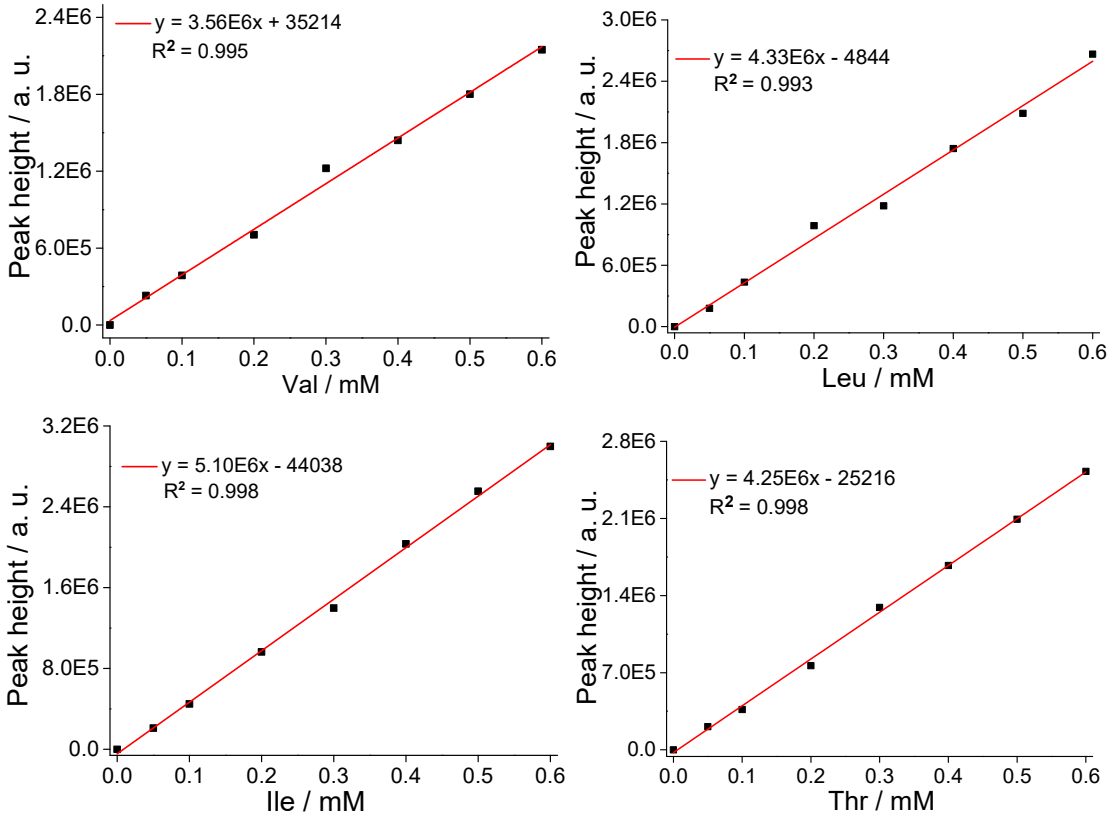
10. Correlation of peak heights of amide signals formed by ^{19}F-tag and concentrations of amino acids

Tag 1:




y = 7.51E10x + 7.44E8
R² = 0.997
Peak height / a. u.
Trp / mM
y = 5.83E10x - 1.36E9
R² = 0.995
Lys / mM
y = 7.04E10x - 7.33E8
R² = 0.997
His / mM

Tag 2:


y = 3.56E6x + 35214
R² = 0.995
Peak height / a. u.
Val / mM
y = 4.33E6x - 4844
R² = 0.993
Leu / mM
y = 5.10E6x - 44038
R² = 0.998
Ile / mM
y = 4.25E6x - 25216
R² = 0.998
Thr / mM

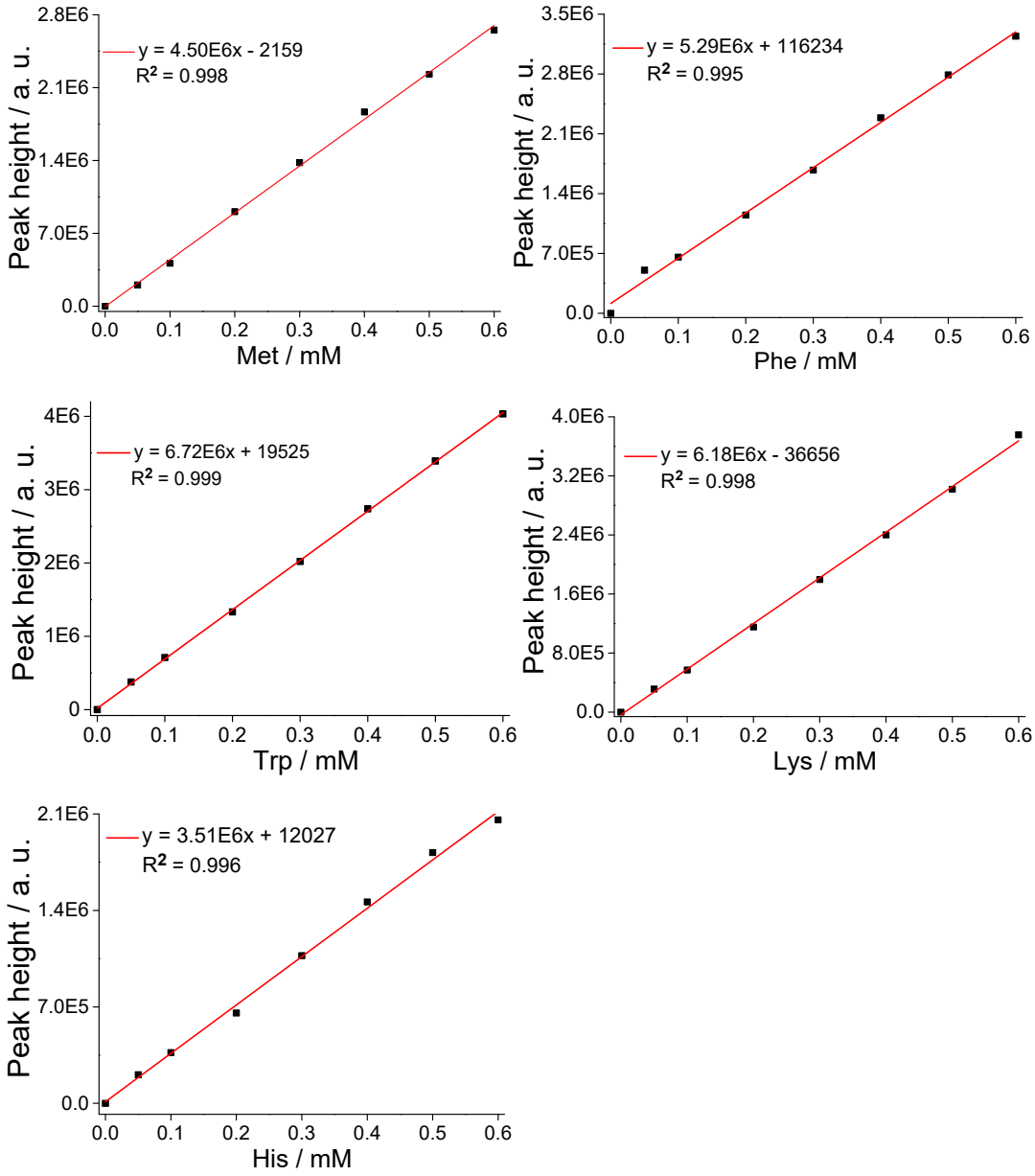


Figure S18. Plot of signal peak heights of amide product, formed by Tag 1 or Tag 2 with varied concentration of amino acids. The concentration of the Tag 1 and Tag 2 was 6 mM and 3 mM, respectively. The ^{19}F NMR spectra were recorded for the amino acid samples by mixing with Tag 1 (2 h) and Tag 2 (10 h) at 293 K in 50 mM phosphate buffer, pH 7.5. The ^{19}F NMR spectra were recorded at a magnetic field with a ^{19}F frequency of 753 MHz (Tag 1) and 376 MHz (Tag 2).

11. Simultaneous quantification of amino acids in a mixture by ^{19}F-tag

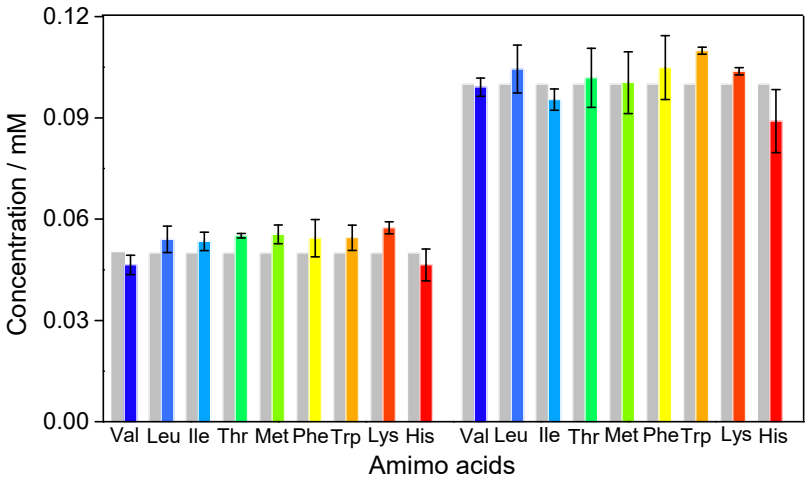


Figure S19. Simultaneous quantification of nine essential amino acids in the mixtures by using Tag 2. Two sets of amino acid mixture each containing 0.05 and 0.1 mM amino acids, respectively, were incubated with 4.0 mM Tag 2 at 293 K in 50 mM phosphate buffer, pH 7.5 for 10 h followed by NMR spectra detection. The determined concentration by ^{19}F-tag and the actual concentration (gray bar) was shown side by side for better comparison. Each experiment was repeated three times, and the ^{19}F NMR spectra were recorded at a magnetic field with a ^{19}F frequency of 376 MHz.

12. Amino acid analysis in fetal bovine serum by ^{19}F-tag

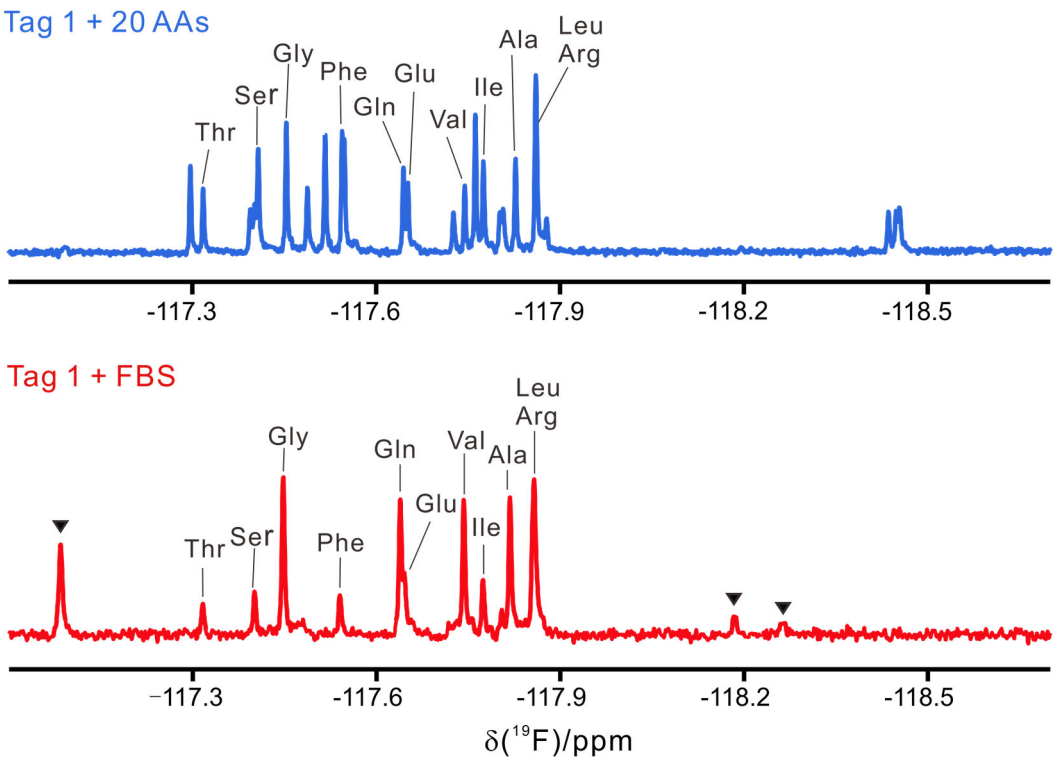


Figure S20. Comparison of the ^{19}F NMR spectra of the 3.0 mM Tag 1 mixed with 0.1 mM 20 amino acids (blue) and fetal bovine serum (red), respectively. The ^{19}F NMR spectra were recorded for the reaction mixture after 2 h incubation at 293 K in 50 mM phosphate buffer, pH 7.5. The triangles represent the unknown substances containing amino groups in FBS. The ^{19}F NMR spectra were recorded with proton decoupling at a magnetic field with a ^{19}F frequency of 376 MHz.

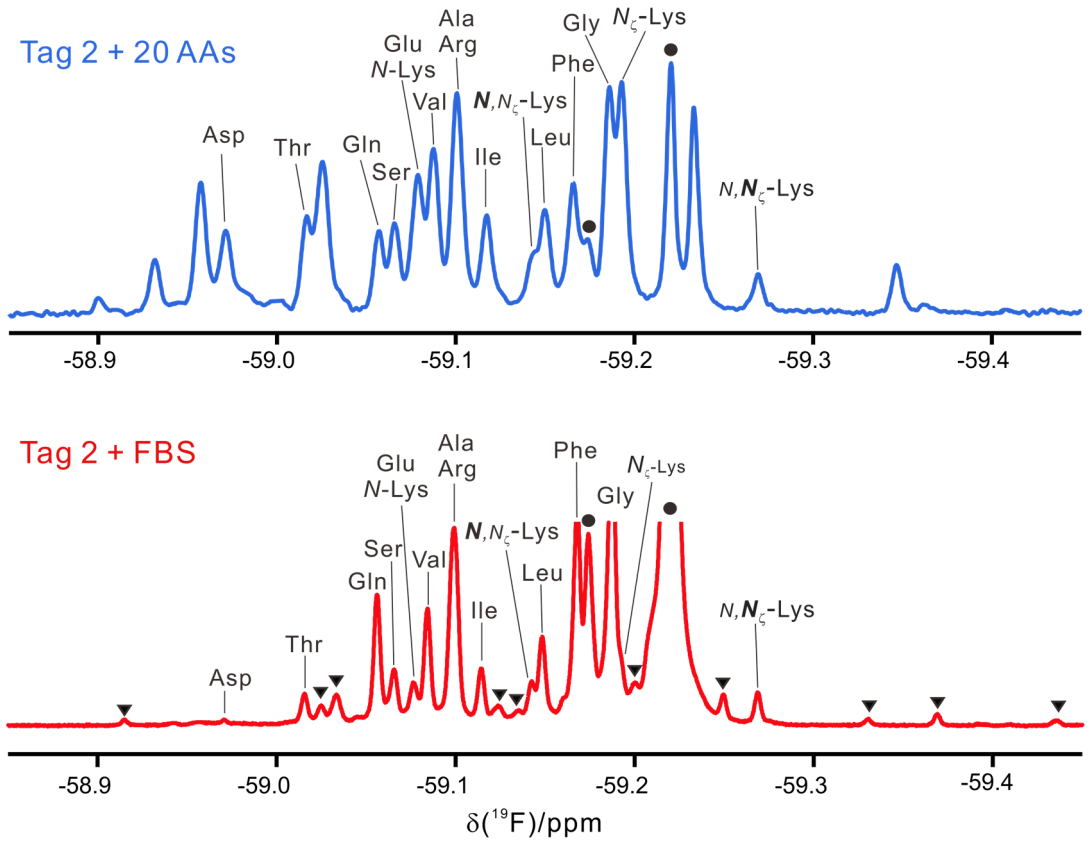


Figure S21. Comparison of the ^{19}F NMR spectra of the 3.0 mM Tag 2 mixed with 0.1 mM 20 amino acids (blue) and fetal bovine serum (red), respectively. The ^{19}F NMR spectra were recorded for the mixture after 8 h and 2 h incubation at 293 K in 50 mM phosphate buffer, pH 7.5 and 8.5. The triangles represent the unknown substances containing amino groups in FBS and the solid spheres represents the hydrolysis intermediate of free Tag 2. The ^{19}F NMR spectra were recorded at a magnetic field with a ^{19}F frequency of 376 MHz (Tag 2 with 20 amino acids) and 753 MHz (Tag 2 with FBS).

13. Simultaneous quantification of amino acids in fetal bovine serum by ^{19}F-tag

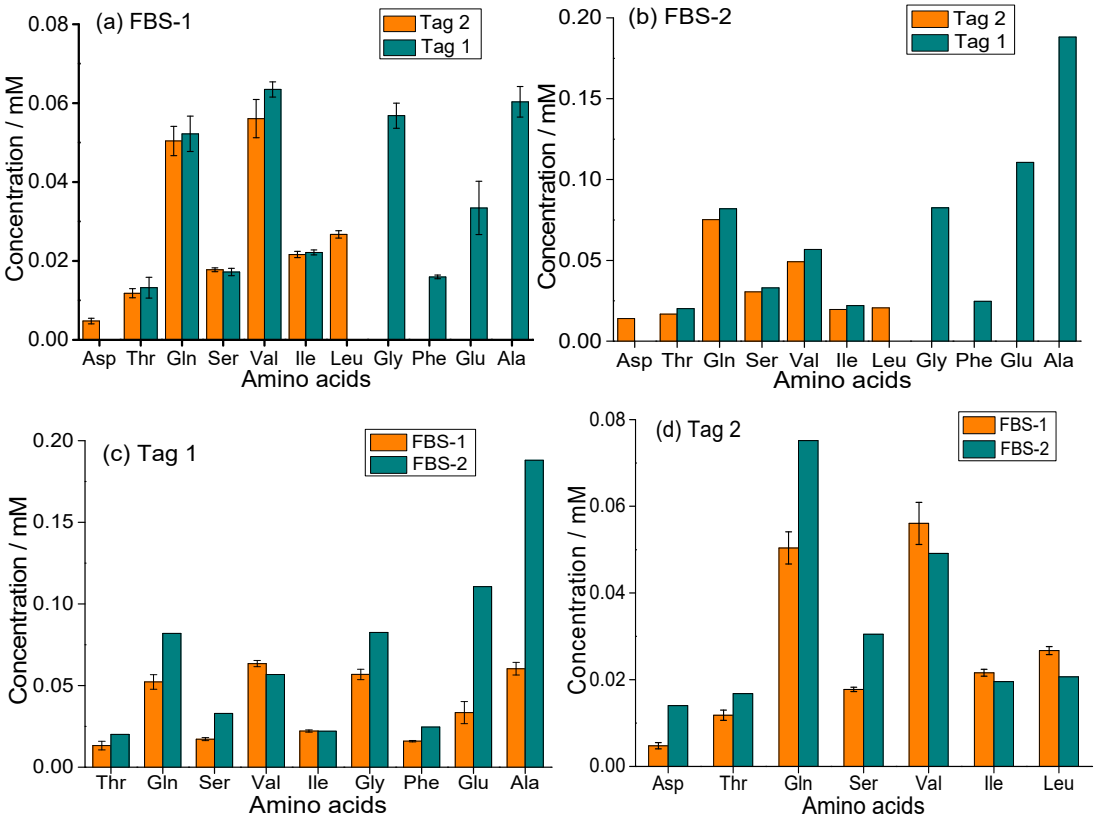


Figure S22. Concentration of amino acids in FBS from different batches determined by Tag 1 (cyan) or Tag 2 (orange). (a) and (b) are FBS samples from the first batch and the second batch respectively. (c) and (d) are for FBS samples of first batch (cyan), second batch (orange) treated by Tag 1 and Tag 2, respectively. The ^{19}F NMR spectra was recorded for the mixture after 1 h (Tag 1) and 4 h (Tag 2) incubation at 293 K in 50 mM phosphate buffer, pH 7.5 (Tag 1) and 8.5 (Tag 2). The ^{19}F NMR spectra were recorded at a magnetic field with a ^{19}F frequency of 376 MHz (Tag 1) and 753 MHz (Tag 2). Proton decoupling was performed for the Tag 1 mixture.

13. Variations of amino acids in fetal bovine serum from different batches detected by ^{19}F-tag

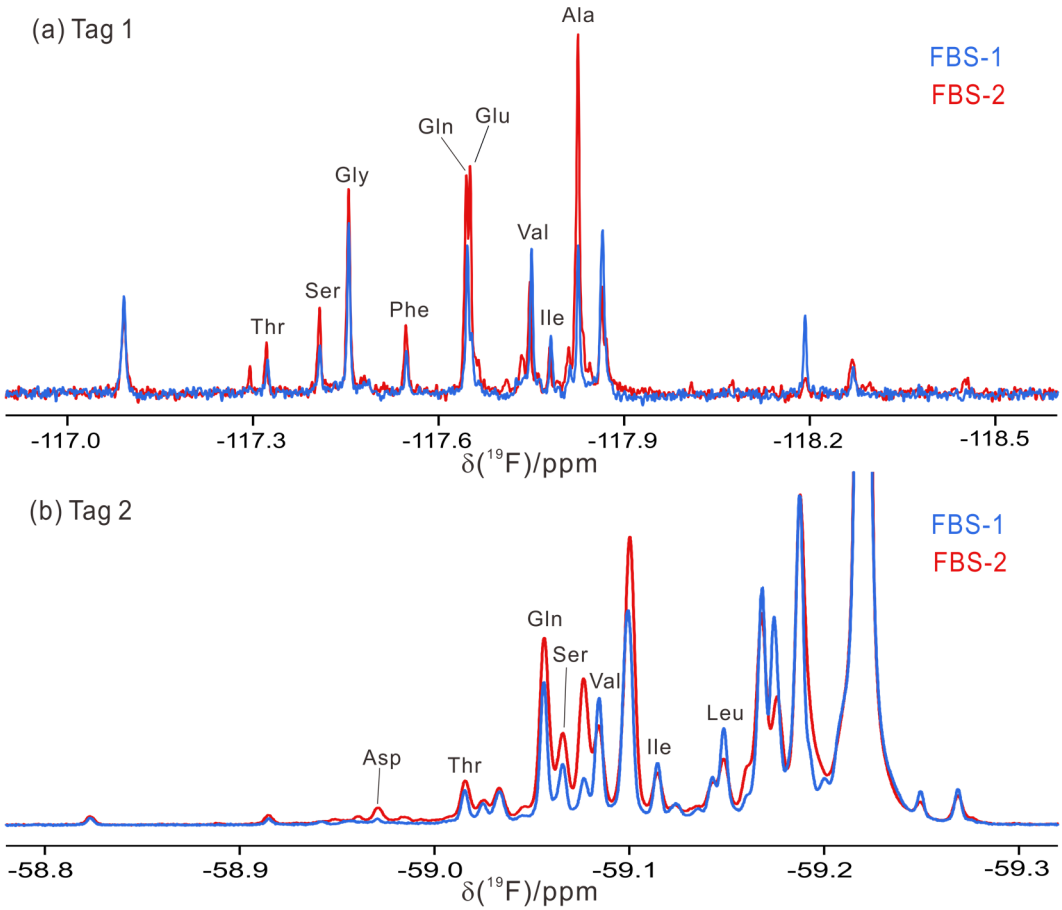


Figure S23. ^{19}F NMR spectra overlay of first batch FBS sample (blue) and second batch FBS sample (red) treated with Tag 1 (a) or Tag 2 (b), respectively. The ^{19}F NMR spectra were recorded for the mixture after 2 h (Tag 1) and 8 h (Tag 2) incubation at 293 K in 50 mM phosphate buffer, pH 7.5 (Tag 1) and 8.5 (Tag 2). The ^{19}F NMR spectra were recorded at a magnetic field with a ^{19}F frequency of 376 MHz (Tag 1) and 753 MHz (Tag 2). Proton decoupling was performed for the Tag 1 mixture.

14. Amino acid assay in *Escherichia coli* lysates by ^{19}F-tag

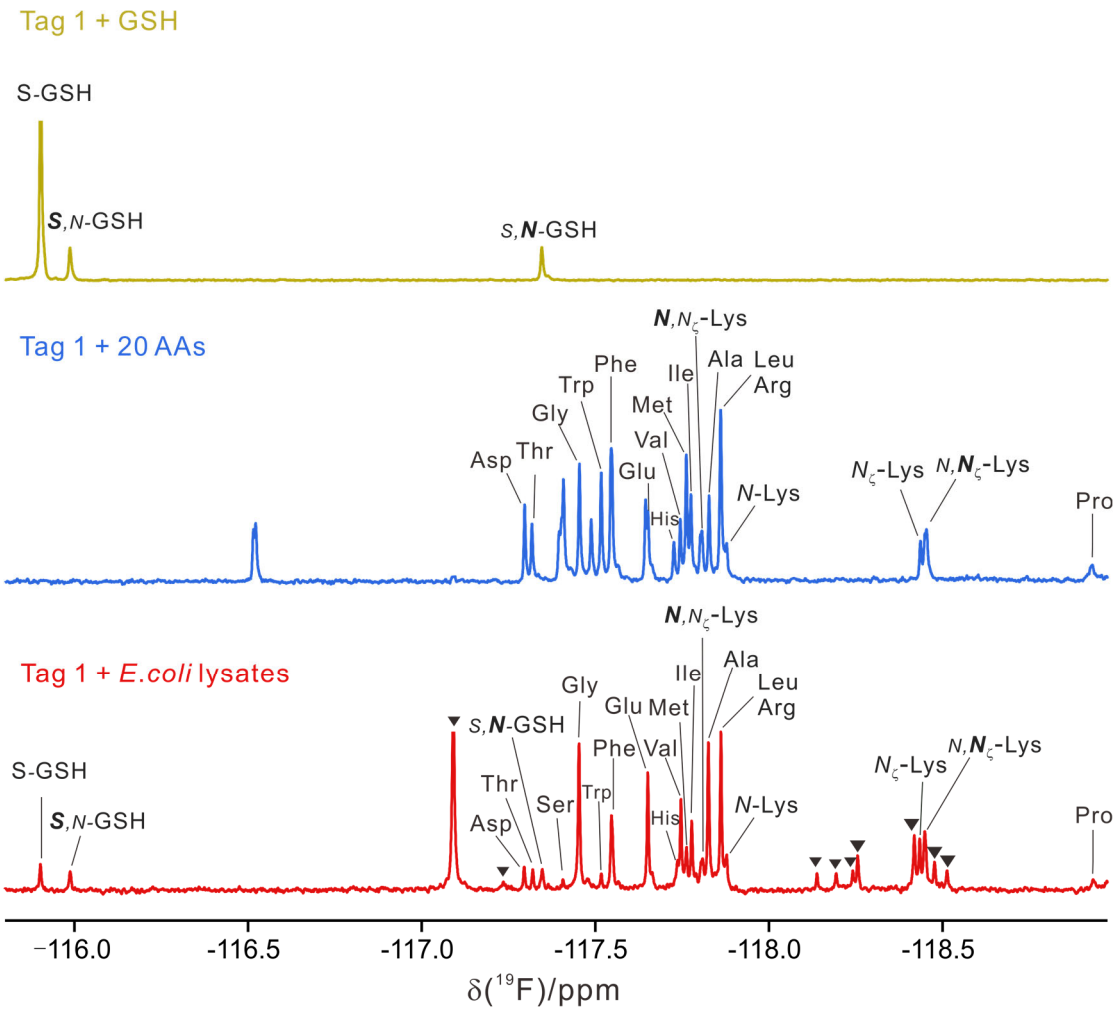


Figure S24. Comparison of the ^{19}F NMR spectra of the 3.0 mM Tag 1 mixed with 0.5 mM GSH (yellow), 0.1 mM 20 amino acids mixture (blue) and 2 % (m/m) *Escherichia coli* lysates (red), respectively. The ^{19}F NMR spectra were recorded for the reaction mixture after 2 h incubation at 293 K in 50 mM phosphate buffer, pH 7.5. The triangles represent the unknown substances containing amino groups in *Escherichia coli* lysates. The ^{19}F NMR spectra were recorded with proton decoupling at a magnetic field with a ^{19}F frequency of 376 MHz.

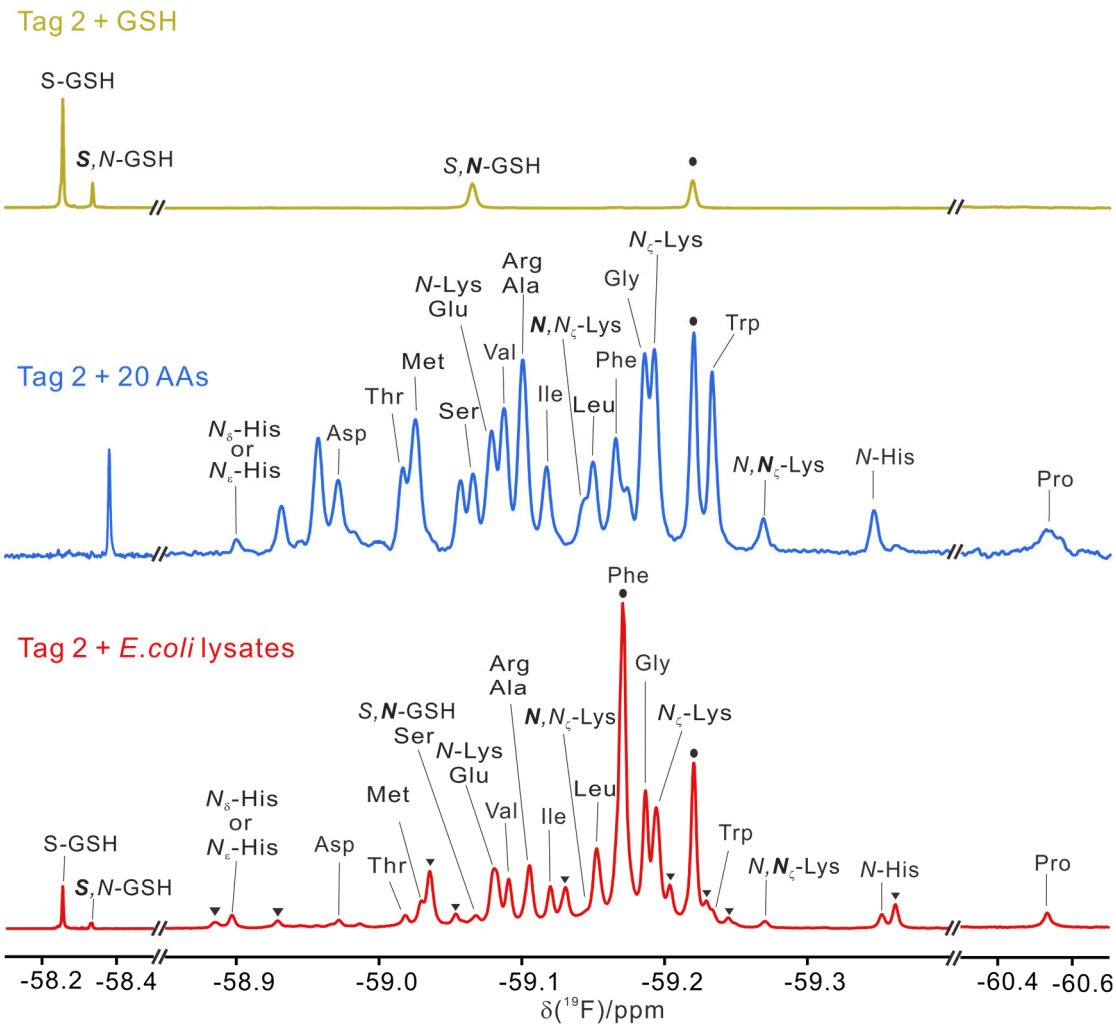


Figure S25. Comparison of the ^{19}F NMR spectra of the 3.0 mM Tag 2 mixed with 0.5 mM GSH (yellow), 0.1 mM 20 amino acids mixture (blue) and 2% (wet weight mass ratio) *Escherichia coli* lysates (red), respectively. The ^{19}F NMR spectra were recorded for the mixture after 2 h incubation at 293 K in 50 mM phosphate buffer, pH 8.5. The triangles represent the unknown substances containing amino groups in *Escherichia coli* lysates and the solid spheres represents the hydrolysis intermediate of free Tag 2. The ^{19}F NMR spectra were recorded at a magnetic field with a ^{19}F frequency of 376 MHz (Tag 2 with 20 amino acids) and 753 MHz (Tag 2 with GSH and *Escherichia coli* lysates).

Simultaneous quantification of amino acids in *Escherichia coli* lysates by ^{19}F-Tag

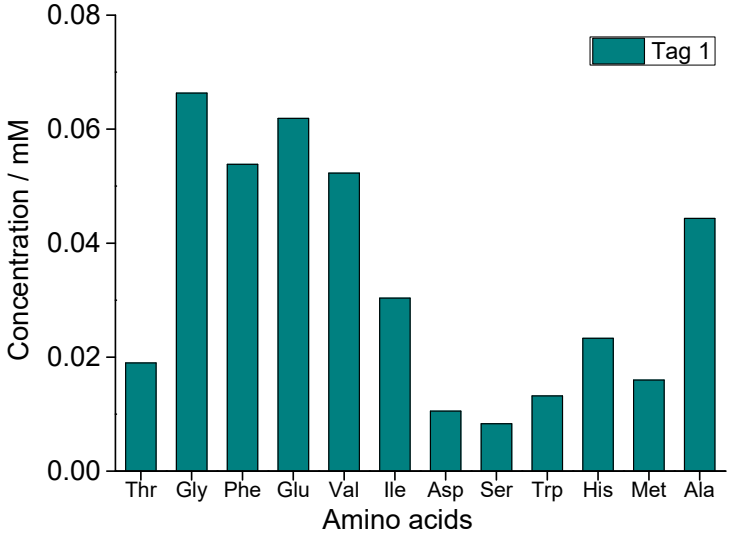


Figure S26. Quantification of amino acids in 2% (m/m) *Escherichia coli* lysates by Tag 1. The ^{19}F NMR spectra were recorded with proton decoupling at a magnetic field with a ^{19}F frequency of 376 MHz.