Supporting Information

Rapid and High-Yielding Cysteine Labelling of Peptides with *N*-Succinimidyl 4-[^{18}F]Fluorobenzoate

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Experimental Procedures

General methods

All chemicals, solvents, and materials were commercially available and used as received without further purification. Silica gel (230-400 mesh) from Scharlau was used for column chromatography. Analytical and semi-preparative HPLC runs were performed using an Agilent 1100 Series coupled to a MS (API-ES positive ionization mode), UV-Vis diode array, and a Raytest Gina isotopic detectors. As stationary phase, analytical and semi-preparative Teknokroma Mediterranea Sea_{18} columns (5 μm) were employed. The cyclotron used for ^{18}F production was an 18/9 model from IBA. ^{1}H, ^{13}C and other routine NMR experiments (COSY, TOCSY, HSQC and HMBC) were usually obtained on a Bruker Avance III 600 MHz spectrometer equipped with a 5 mm TBI probe with Z-gradients. For some samples a Bruker Avance 500 MHz spectrometer equipped with a 5 mm triple channel (^{1}H, ^{13}C, ^{15}N) cryoprobe with Z-gradients. ^{19}F NMR experiments were performed with a Bruker Avance III 400 spectrometer equipped with a 5 mm BBOF probe with Z-gradients.

Preparation of Fmoc-Cys-OH (1)

Compound **1** was synthesised according to a procedure previously described by Zhao et al.; H.-Y Zhao, G. Liu, *J. Comb. Chem.*, 2008, **9**, 756.

Preparation of Fmoc-Cys-Arg(Pmc)-OH (2)

Fmoc-Arg(Pmc)-OH (0.75 mmol) was dissolved in acetonitrile/piperidine (4/1 v/v, 5 mL) at room temperature. After one hour, the crude mixture was dried and purified by silica gel flash chromatography using ethyl acetate/methanol/trietylamine (7.9/2/0.1 v/v/v) as eluent. Then, the compound was solved (0.46 mmol) in dichloromethane (3

mL) and drop-wise added during 30 minutes over a solution of Fmoc-Cys(Trt)-OH (0.5 mmol), 1-hydroxybenzotriazole (0.5 mmol), and 1,3-diisopropylcarbodiimide (0.5 mmol) in dichloromethane (7 mL). The mixture was allowed to react for 2 hours at room temperature. After washing the reaction crude with water, the dipeptide was purified by silica gel chromatography using a solvent gradient from chloroform to chloroform/methanol (9/1 v/v). Finally, the dipeptide was reacted with TFA (1.0 mmol) and triethylsilane (1.0 mmol) in dichloromethane (0.6 mL) at room temperature for 2 hours. Then, the crude was dried under vacuum and purified by flash chromatography using a solvent gradient from chloroform to chloroform/methanol (9/1 v/v). **2** was obtained as a white solid. ^{1}H NMR (500 MHz, DMSO) δ 7.76 (d, J = 7.8 Hz, 2H), 7.60 (d, J = 7.0 Hz, 1H), 7.59 (d, J = 7.3, 1H), 7.55 (b, 0.8H), 7.41 (t, J = 7.2 Hz, 1H), 7.40 (t, J = 7.3 Hz, 1H), 7.31 (d, J = 7.2, 1H), 7.30 (d, J = 7.2, 1H), 4.67 (b, 0.8H), 4.62 (b, 1H), 4.51 (b, 1H), 4.44 (b, 1H), 4.37 (b, 1H), 4.20 (t, J = 7.0, 1H), 3.21 (b, 2H), 2.99 (m, 1H), 2.88 (m, 1H), 2.61 (t, J = 6.4, 2H), 2.54 (s, 3H), 2.52 (s, 3H), 2.10 (s, 3H), 1.31 (s, 3H), 1.28 (s, 3H). MS-ESI (positive mode) for **2**: $[M+H]^{+}$, m/z = 766.

Preparation of Fmoc-Cys-Arg-OH (3)

2 (0.06 mmol) was reacted in TFA (0.5 mL) for one hour at room temperature. The reaction mixture was diluted with acetonitrile and purified by semi-preparative HPLC (25×1 cm, water/acetonitrile/TFA from 9/1/0.01 v/v/v to 6/4/0.01 v/v/v in 25 min, 5 mL·min^{-1}). The collected fractions were dried under reduce pressure and compound **3** was obtained as a white solid. ^{1}H NMR (500 MHz, DMSO): δ 8.31 (t, J = 9.3, 1H), 7.91 (d, J = 7.1, 1H), 7.89 (d, J = 7.8, 1H), 7.74 (m, 2H), 7.65 (m, 0.8H), 7.63 (d, J = 7.2, 0.8H), 7.43 (t, J = 7.4, 1H), 7.39 (t, J = 7.2, 1H), 7.34 (t, J = 7.6, 1H), 4.32 (m, 1H), 4.24 (m, 1H), 4.23 (m, 1H), 4.21 (m, 1H), 4.20 (m, 1H), 3.09 (m, 2H), 2.82 (m, 1H), 2.67 (m, 1H), 1.78 (m, 1H), 1.62 (m, 1H), 1.51 (m, 2H). ^{13}C NMR (125 MHz, DMSO):

δ 174.0, 171.2, 157.6, 156.8, 144.6, 141.6, 128.5, 128.0, 126.2, 121.0, 66.6, 58.0, 52.5, 47.5, 41.1, 28.8, 27.2, 26.0. MS-ESI (positive mode) for **2**: $[M+H]^+$, *m/z* = 500.

Preparation of peptide Ac-GCRGYGRGDSPG-NH$_2$ (4)

The synthesis of peptide **4** was accomplished according to a published procedure; S, VandeVondele, J. Vörös, J. A. Hubbell, *Biotechnol. Bioeng.*, 2003, **82**, 784.

Reaction of Cys thiols 1-4 with N-succinimidyl 4-fluorobenzoate (SFB)

To a solution of the cysteine thiols (0.01 mmol) in a 2/1 (v/v) mixture (300 μL) of DMSO/phosphate buffer (pH= 8.5, 0.2 M), SFB (0.015 mmol) solved in DMSO (200 μL) was added. The progress of the reactions were monitored by HPLC/UV/MS (15×0.46 cm, water/acetonitrile/TFA from 8/2/0.01 v/v/v to 2/8/0.01 v/v/v in 30 min, 2 mL·min^{-1}). The labelled compounds **1a**, **2a**, **3a**, and **4a** were purified by semi-preparative HPLC and the collected fractions were concentrated under vacuum to obtain white solids. Chemical yields were determined based on HPLC measurements by using isolate conjugates as standards.

Purification of Fmoc-Cys(FB)-OH (1a) and Fmoc-Cys(FB)-Arg(Pmc)-OH (2a)

Compound **1a** and **2a** were purified by semi-preparative HPLC (25×1 cm, water/acetonitrile/TFA from 5/5/0.01 v/v/v to 3/7/0.01 v/v/v in 10 min, 5 mL·min^{-1}). ^{1}H NMR (500 MHz, $CDCl_3$) for **1a**: δ 8.00 (d, 2H), 7.76 (d, *J* = 7.3 Hz, 2H), 7.57 (d, *J* = 7.6 Hz, 1H), 7.55 (d, *J* = 7.9 Hz, 1H), 7.40 (t, *J* = 6.7 Hz, 2H), 7.26 (t, *J* = 7.1 Hz, 2H), 7.14 (m, 2H), 5.82 (b, 1H), 4.69 (b, 1H), 4.41 (m, 1H), 4.36 (m, 1H), 4.23 (t, *J* = 6.2 Hz, 1H), 3.70 (m, 1H), 3.57 (m, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) for **1a**: δ 167.7, 166.3, 144.0, 141.7, 132.9, 130.7, 130.6, 128.1, 127.5, 125.5, 120.4, 116.4, 116.2, 68.0, 54.6, 47.4, 31.0. MS-ESI (positive mode) for **1a**: $[M+Na]^+$, *m/z* = 488.

^{1}H NMR (500 MHz, DMSO) for **2a:** δ 8.34 (d, *J* = 7.2, 0.9H), 7.99 (d, *J* = 8.5, 1H), 7.98 (d, *J* = 8.5, 1H), 7.89 (d, *J* = 7.2, 2H), 7.79 (d, *J* = 8.5, 0.8H), 7.71 (d, *J* = 7.0, 1H),

7.70 (d, *J* = 7.1, 1H), 7.41 (t, *J* = 7.2, 2H), 7.39 (t, *J* = 8.5, 2H), 7.29 (t, *J* = 7.2, 2H), 6.7 (b, 0.9H), 6.39 (b, 0.9H), 4.32 (m, 1H), 4.30 (m, 1H), 4.24 (m, 1H), 4.21 (m, 1H), 4.17 (m, 1H), 3.51 (dd, J = 13.3, *J* = 4.1, 1H), 3.23 (dd, 13.3, *J* = 9.5, 1H), 3.04 (m, 2H), 2.56 (t, *J* = 6.5, 2H), 2.48 (s, 3H), 2.47 (s, 3H), 2.02 (s, 3H), 1.76 (t, *J* = 6.5, 2H), 1.74 (m, 1H), 1.59 (m, 1H), 1.46 (m , 2H), 1.25 (s, 6H). ^{13}C NMR (125 MHz, DMSO) for **2a**: δ 190.2, 174.0, 170.7, 166.2, 156.8, 156.7, 153.2, 144.6, 141.5, 135.4, 135.0, 133.7, 130.7, 128.5, 127.9, 126.1, 123.5, 121.0, 118.6, 117.0, 74.3, 66.7, 54.5, 52.7, 47.4, 40.3, 33.0, 31.9, 29.1, 27.3, 26.4, 21.6, 19.0, 18.0, 12.8. MS-ESI (positive mode) for **2a**: $[M+H]^+$, *m/z* = 888.

Purification of Fmoc-Cys(FB)-Arg-OH (3a)

Conjugate **3a** was isolated by injection of the reaction mixture into a semipreparative HPLC system (25×1 cm, water/acetonitrile/TFA from 6/4/0.01 v/v/v to 4.5/5.5/0.01 v/v/v in 15 min, 5 mL·min^{-1}). ^{1}H NMR (500 MHz, DMSO): δ 8.28 (b, 0.6H), 7.99 (m, 2H), 7.90 (d, *J* = 7.3, 2H), 7.83 (d, *J* = 8.7, 1H), 7.71 (d, *J* = 7.3, 2H), 7.58 (b, 0.7H), 7.42 (t, *J* = 7.4, 2H), 7.39 (t, *J* = 7.3, 2H), 7.30 (m, 2H), 4.33 (m, 1H), 4.32 (m, 1H), 4.23 (m, 1H), 4.22 (m, 1H), 4.20 (m, 1H), 3.53 (dd, *J* = 13.4, *J* = 4.2, 1H), 3.10 (q, *J* = 6.1, 2H), 1.77 (m, 1H), 1.63 (m, 1H), 1.52 (q, *J* = 7.1, 2H). ^{13}C NMR (125 MHz, DMSO): δ 190.2, 157.5, 156.7, 144.6, 144.5, 145.6, 133.7, 130.6, 130.7, 128.5, 127.9, 126.2, 121.0, 117.2, 117.0, 66.7, 54.6, 52.9, 47.4, 41.2, 32.0, 29.1, 26.0. MS-ESI (positive mode) for **3a**: $[M+H]^+$, *m/z* = 622.

Purification of the peptide-FB conjugate (4a)

The reaction crude was submitted to semi-preparative HPLC (25×1 cm, water/acetonitrile/TFA from 9/1/0.01 v/v/v to 6/4/0.01 v/v/v in 30 min, 5 mL·min^{-1}). MS-ESI (positive mode) for **4a**: $[M+2H]^{+2}$, *m/z* = 672.5. See below for NMR characterisation.

Radiosynthesis of N-succinimidyl 4-[^{18}F]fluorobenzoate ([^{18}F]SFB)

[^{18}F]SFB was synthesised according to the well established three-step one-pot procedure with some modifications by using assembled Eckert & Ziegler modules (Isotope Products, Inc.). Thus, ^{18}F[F$^-$] was produced in a cyclotron by bombardment of [^{18}O]H_2O with high energy protons (18 MeV). Then, radioactivity was delivered to the automatic synthesiser system where ^{18}F[F$^-$] aqueous solution was passed through a Sep-Pak light QMA cartridge (Waters, Inc.) to trap the fluoride. The ^{18}F[F$^-$] was eluted from the trapping cartridge to the reaction vessel with a solution of potassium carbonate (5.5 μmol) and 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane (K_{222}, 18.7 μmol) in acetonitrile/water (1/1 v/v, 0.8 mL). The solvent was evaporated by applying He flow and vacuum at 110ºC. To ensure that the drying process was done successfully, additional anhydrous acetonitrile (1 mL) was added and the mixture was dried as before. To the anhydrous K_{222}/[^{18}F]KF complex, a solution of ethyl 4-(trimethylammonium triflate) benzoate (20 mmol) in anhydrous acetonitrile (1 mL) was added and the mixture was heated at 90ºC for 10 min. Then, tetrabutylammonium hydroxide (1 M in methanol, 25 μL) in anhydrous acetonitrile (0.4 mL) was added and the reaction vessel was heated at 120ºC for 5 min. Subsequently, a solution of *N*,*N*,*N*',*N*'-tetramethyl-O-(*N*-succinimidyl)uranium tetrafluoroborate (0.05 mmol) in dry acetonitrile (0.6 mL) was added and the reaction was heated at 90ºC for 2 min. The crude mixture was cooled down to 40ºC and neutralized with aqueous acetic acid (5%, 3 mL). The final solution was purified by semipreparative HPLC (25×1 cm, water/acetonitrile/TFA 6/4/0.01, 7.5 mL·min^{-1}). The desired fraction (t_r ~ 9-11 min) was collected over 0.9 % saline solution (20 ml) and passed through a Sep-Pak C18 cartridge (Waters, Inc.). The cartridge was rinsed with water (10 mL) and [^{18}F]SFB was eluted with acetonitrile (2 mL). The solvent was removed by bubbling N_2 at room temperature to provide a dry residue.

Chemical and radiochemical purity was determined by analytical HPLC (15×0.46 cm, water/acetonitrile/TFA from 9/1/0.01 v/v/v to 6/4/0.01 v/v/v in 30 min, 2 mL·min^{-1}). [^{18}F]SFB was obtained in a radiochemical yield (RCY) of 37 ± 5 % (decay-corrected), with chemical and radiochemical purity exceeding 98 %. A specific activity of 102 ± 7 GBq/μmol was estimated. [^{18}F]SFB was prepared in 74 ± 5 min.

Radiolabeling of cysteine thiols 1-4 with [^{18}F]SFB

To a solution of the thiol (0.15 μmol) in a 2/1 (v/v) mixture (30 μL) of DMSO/phosphate buffer (pH= 8.5, 0.2 M), [^{18}F]SFB (*ca.* 20 MBq) in DMSO (20 μL) was added. The reaction was kept at room temperature and analysed at different reaction times. An analytical HPLC system (15×0.46 cm, water/acetonitrile/TFA from 8/2/0.01 v/v/v to 2/8/0.01 v/v/v in 30 min, 2 mL·min^{-1}) were used to identify the thioesters (by co-elution with the corresponding ^{19}F-standards) and to determine the RCY obtained for the conjugates.

Radiosynthesis of [^{18}F]Fmoc-Cys(FB)-OH (1b)

The amino acid derivative **1** (1.5 μmol) was solved in a 2/1 (v/v) mixture (150 μL) of DMSO/phosphate buffer (pH 8.5, 0.2 M) and added to a solution of [^{18}F]SFB (~2 GBq) in DMSO (100 μL). After reacting at room temperature for 30 min, the crude was diluted with aqueous TFA (0.1%, 2.6 mL) and injected into a semipreparative HPLC (25×1 cm, water/acetonitrile/TFA from 8/2/0.01 v/v/v to 2/8/0.01 v/v/v in 15 min, 5 mL·min^{-1}). The fraction with product (t_r ~ 16-18 min) was collected and passed through a Sep-Pak light C18 cartridge (Waters, Inc.) after dilution with saline (0.9 %, 20 ml). The cartridge was rinsed with water (10 mL) and compound **1b** was eluted with ethanol (1 mL) over phosphate buffer solution (PBS, pH = 7.4, 9 mL). Chemical and radiochemical purity were determined by analytical HPLC (25×0.46 cm, water/acetonitrile/TFA from 8/2/0.01 v/v/v to 2/8/0.01 v/v/v in 30 min, 2 mL·min^{-1}) to

be > 95 % for > 4 hours with a specific activity of 59 ± 9 GBq/μmol. **1b** was obtained in a RCY of 9 ± 3 % (decay-corrected, 56 ± 6 min).

Radiosynthesis of [^{18}F]Fmoc-Cys(FB)-Arg(Pmc)-OH (2b)

[^{18}F]SFB dry residue (~2 GBq) was solved in DMSO (100 μL) and added to solution of **2** (0.75 μmol) in a 2/1 (v/v) mixture (150 μL) of DMSO/phosphate buffer (pH 8.5, 0.2 M). After reacting at room temperature for 10 min, the reaction mixture was diluted with aqueous TFA (0.1%, 2.6 mL) and purified by semipreparative HPLC (25×1 cm, water/acetonitrile/TFA 8/2/0.01 v/v/v to 2/8/0.01 v/v/v in 15 min, 5 mL·min^{-1}). The fraction of interest (t_r ~ 17-20 min) was collected and diluted with saline solution (0.9 %, 30 ml) and passed through a Sep-Pak light C18 cartridge (Waters, Inc.). The cartridge was rinsed with water (10 mL) and **2b** was eluted with ethanol (1 mL) over phosphate buffer solution (PBS, pH = 7.4, 9 mL). Chemical and radiochemical purity was determined by analytical HPLC (25×0.46 cm, water/acetonitrile/TFA from 8/2/0.01 v/v/v to 2/8/0.01 v/v/v in 30 min, 2 mL·min^{-1}). Peptide **2b** was obtained in a RCY of 26 ± 3 % (decay-corrected, 37 ± 3 min). Chemical and radiochemical purity exceed 95 % for > 4 hours. A specific activity of 76 ± 8 GBq/μmol was obtained.

Radiosynthesis of [^{18}F]Fmoc-Cys(FB)-Arg-OH (3b)

Peptide **3** (0.75 μmol) was solved in a 2/1 (v/v) mixture (150 μL) of DMSO/phosphate buffer (pH 8.5, 0.2 M) and added to a solution of [^{18}F]SFB (~2 GBq) in DMSO (100 μL). After reacting at room temperature for 10 min, the crude was diluted with aqueous TFA (0.1%, 2.6 mL) and injected into a semipreparative HPLC (25×1 cm, water/acetonitrile/TFA from 8/2/0.01 v/v/v to 2/8/0.01 v/v/v in 15 min, 5 mL·min^{-1}). The fraction with product (t_r ~ 12-14 min) was collected and passed through a Sep-Pak light C18 cartridge (Waters, Inc.) after dilution with saline (0.9 %, 20 ml). The cartridge was rinsed with water (10 mL) and compound **3b** was eluted with ethanol

(1 mL) over phosphate buffer solution (PBS, pH = 7.4, 9 mL). Chemical and radiochemical purity were determined by analytical HPLC (25×0.46 cm, water/acetonitrile/TFA from 8/2/0.01 v/v/v to 2/8/0.01 v/v/v in 30 min, 2 $mL \cdot min^{-1}$) to be > 95 % for > 4 hours with a specific activity of 79 ± 9 GBq/μmol. **3b** was obtained in a RCY of 57 ± 4 % (decay-corrected, 31 ± 3 min).

Radiosynthesis of the [^{18}F]peptide-FB conjugate (4b)

[^{18}F]SFB dry residue (~2 GBq) was dissolved in DMSO (100 μL) and added to a solution of **4** (0.75 μmol) in a 2/1 (v/v) mixture (150 μL) of DMSO/phosphate buffer (pH 8.5, 0.2 M). After reacting at room temperature for 10 min, the reaction mixture was diluted with aqueous TFA (0.1%, 2.6 mL) and purified by semipreparative HPLC (25×1 cm, water/acetonitrile/TFA from 9/1/0.01 v/v/v to 6/4/0.01 v/v/v in 30 min, 5 $mL \cdot min^{-1}$). The desired fraction (t_r ~ 18-20 min) was diluted with saline solution (0.9 %, 30 ml) and passed through a Sep-Pak light C18 cartridge (Waters, Inc.). The cartridge was rinsed with water (10 mL) and **4b** was eluted with ethanol (1 mL) over PBS (pH = 7.4) solution (9 mL). Chemical and radiochemical purity was estimated by analytical HPLC (25×0.46 cm, water/acetonitrile/TFA from 8/2/0.01 v/v/v to 2/8/0.01 v/v/v in 30 min, 2 $mL \cdot min^{-1}$). **4b** was obtained in a RCY of 54 ± 4 % (decay-corrected, 38 ± 3 min), with chemical and radiochemical purity over 95 % for > 4 hours. A specific activity of 70 ± 5 GBq/μmol was estimated.

^{1}H and 2D TOCSY NMR spectra of **2**

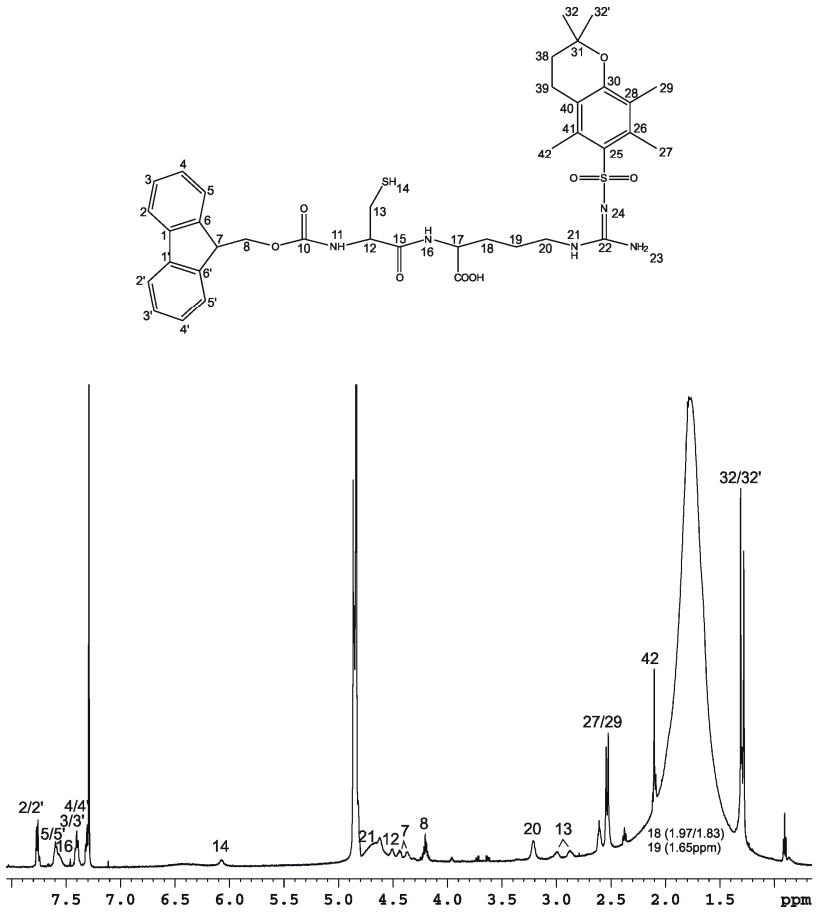


Figure S1. ^{1}H NMR spectrum of **2** acquired in a 600 MHz Bruker spectrometer ($CDCl_3$).

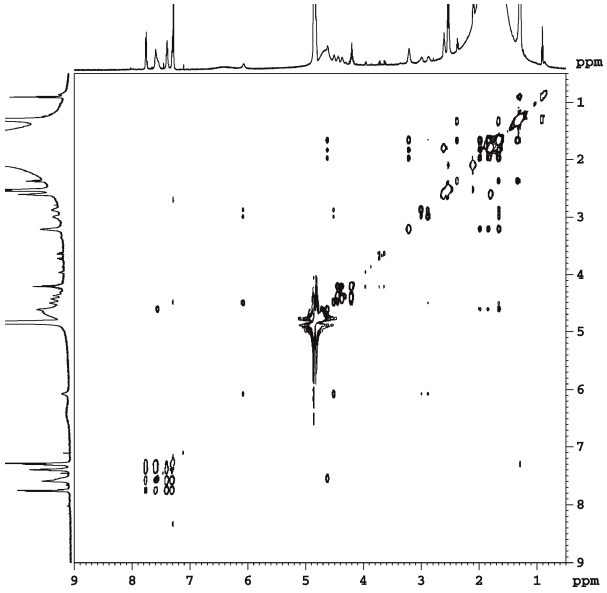


Figure S2. 2D TOCSY NMR spectrum of **2** acquired in a 600 MHz Bruker spectrometer ($CDCl_3$).

^{1}H and ^{13}C NMR spectra of **3**

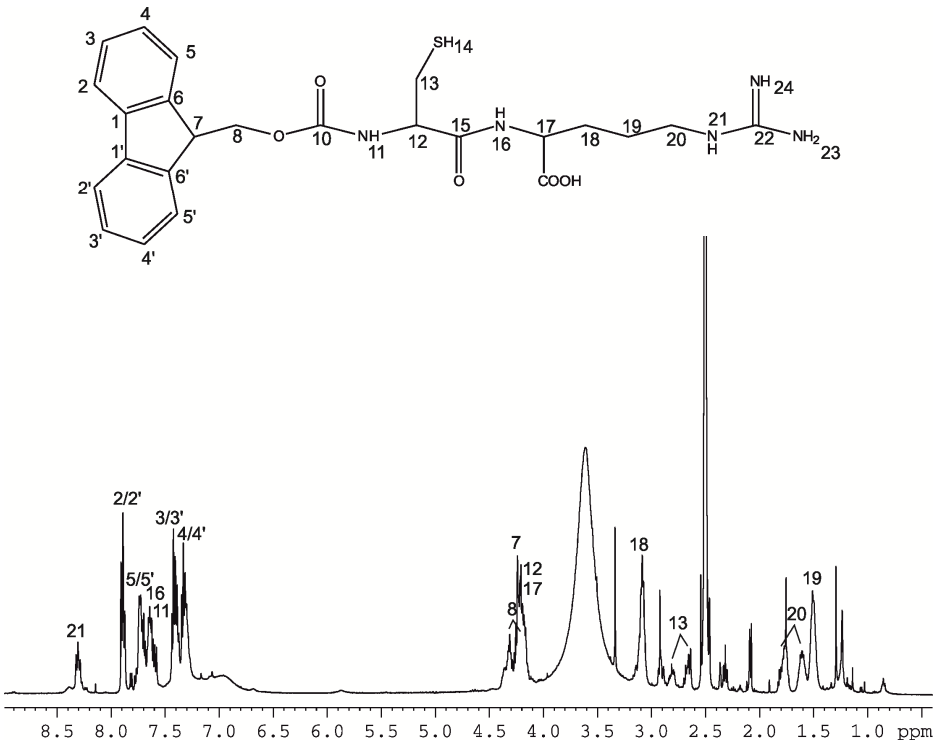


Figure S3. ^{1}H NMR spectrum of **3** acquired in a 500 MHz Bruker spectrometer (DMSO).

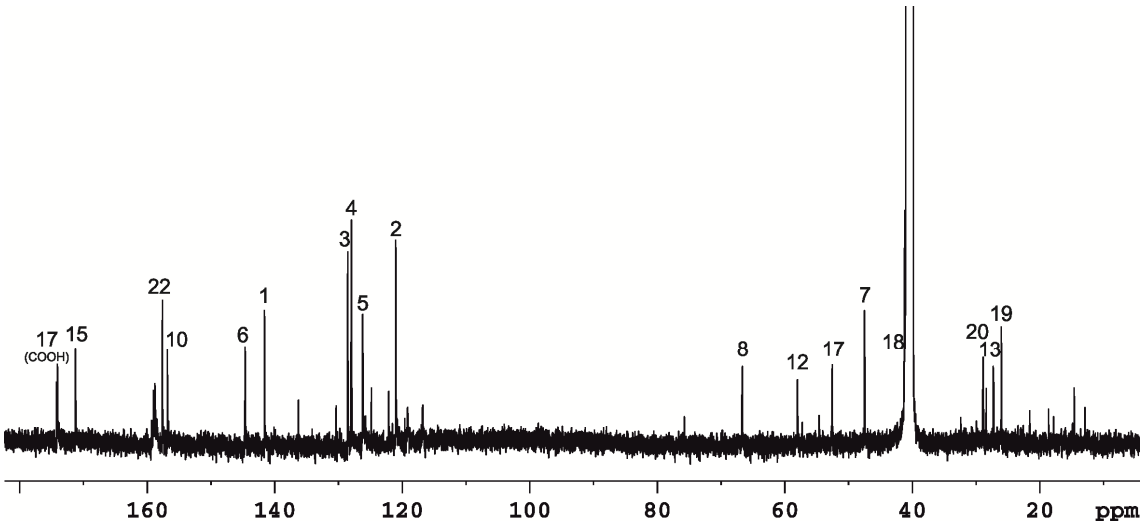


Figure S4. ^{13}C NMR spectrum of **3** acquired in a 500 MHz Bruker spectrometer (DMSO).

^{1}H, ^{13}C, 2D COSY, 2D HSQC, and 2D HMBC NMR spectra of **1a**

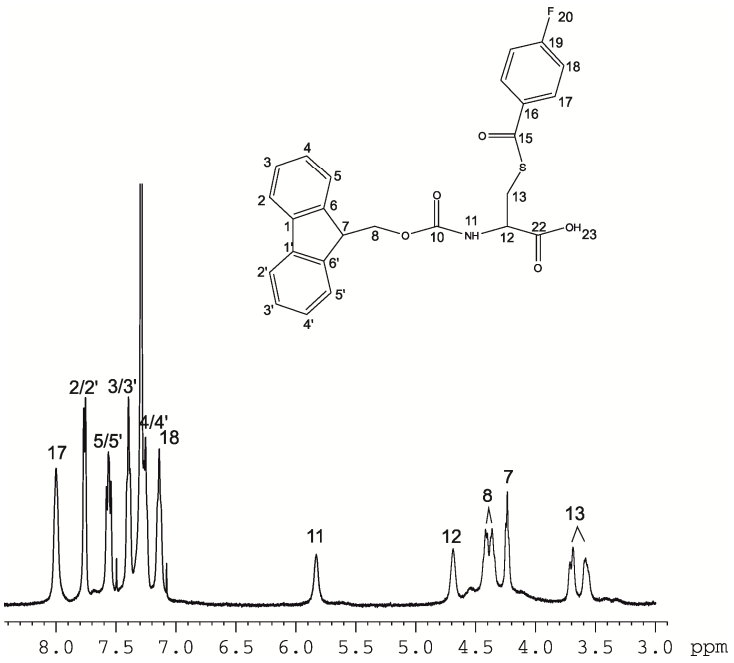


Figure S5. ^{1}H NMR spectrum of **1a** acquired in a 500 MHz Bruker spectrometer ($CDCl_3$).

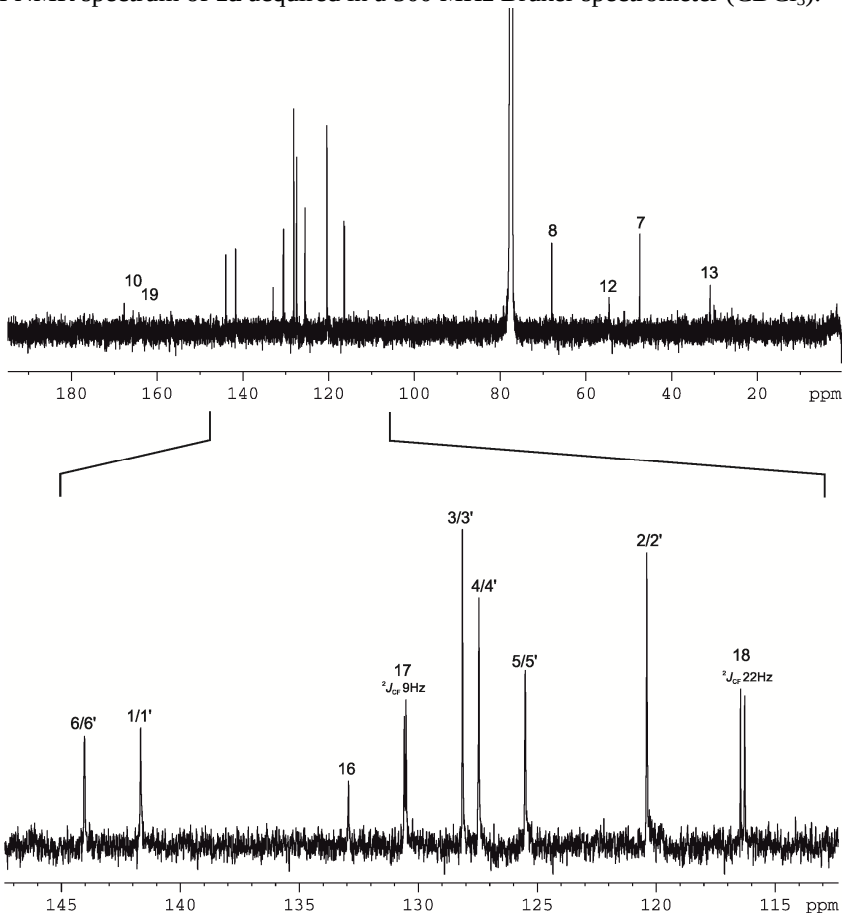


Figure S6. ^{13}C NMR spectrum of **1a** acquired in a 500 MHz Bruker spectrometer ($CDCl_3$).

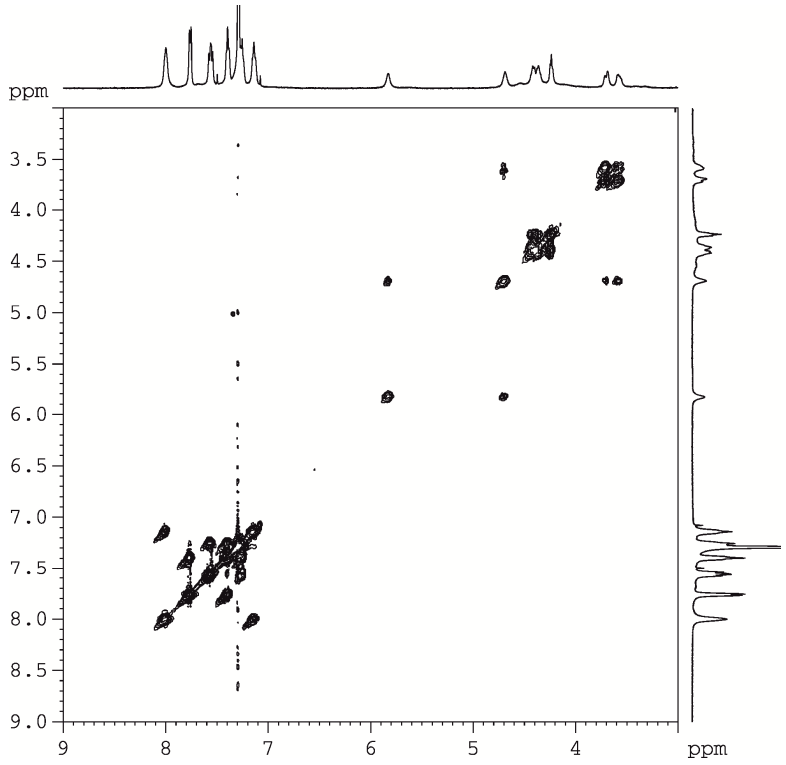


Figure S7. 2D COSY NMR spectrum of **1a** acquired in a 500 MHz Bruker spectrometer ($CDCl_3$).

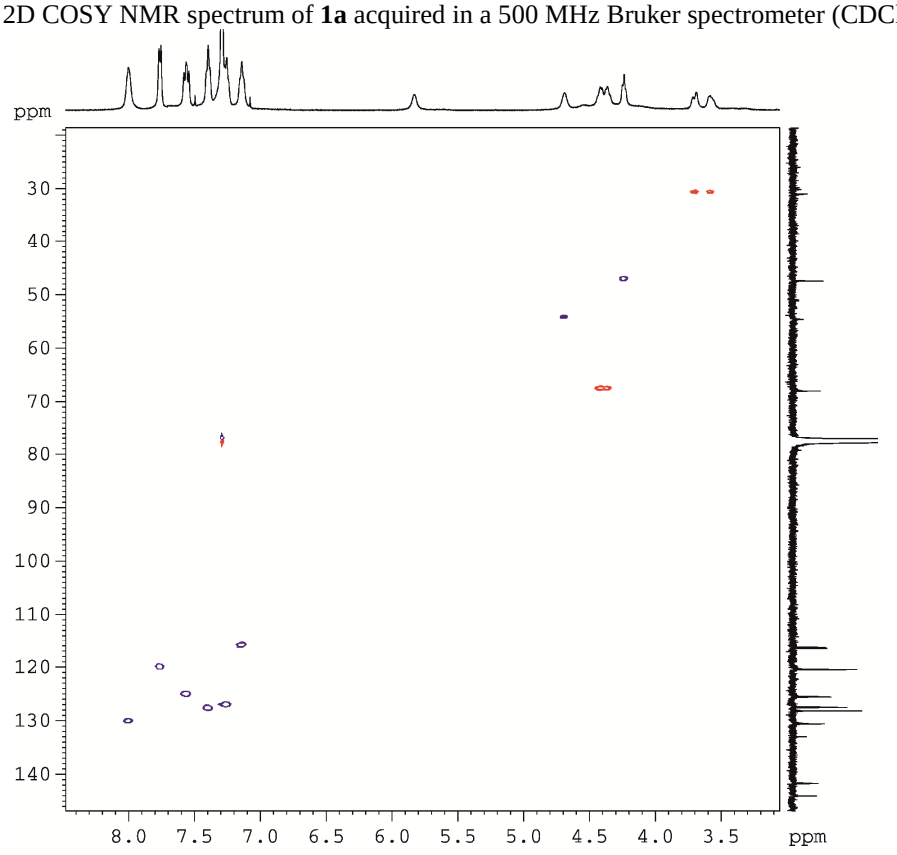


Figure S8. Multiplicity edited 2D HSQC NMR spectrum of **1a** acquired in a 500 MHz Bruker spectrometer ($CDCl_3$). CH are marked blue and CH_2 are marked red.

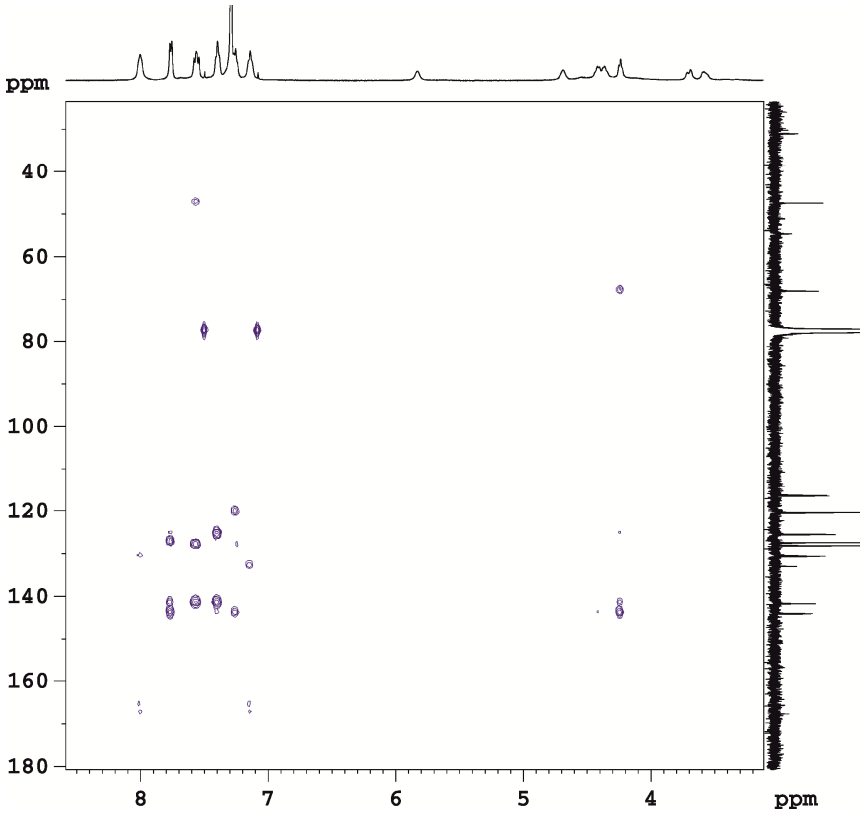


Figure S9. 2D HMBC NMR spectrum of **1a** acquired in a 500 MHz Bruker spectrometer ($CDCl_3$).

^{1}H, ^{13}C, 2D COSY, 2D HSQC, and 2D HMBC NMR spectra of **2a**

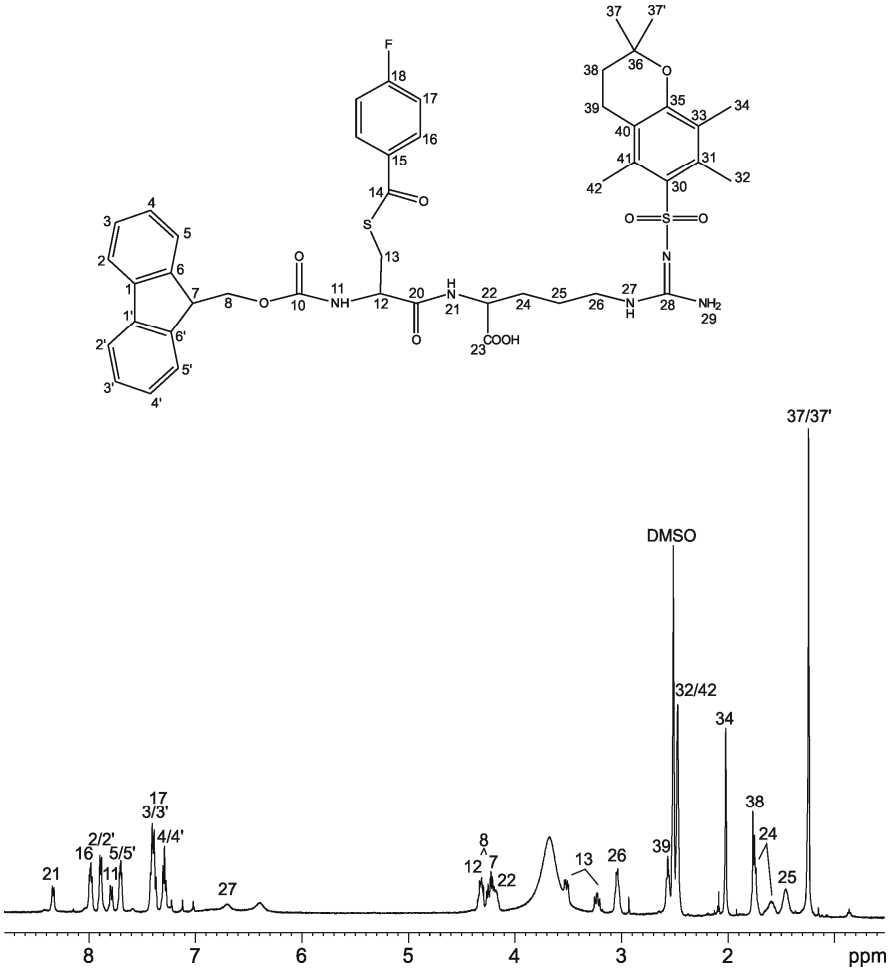


Figure S10. ^{1}H NMR spectrum of **2a** acquired in a 500 MHz Bruker spectrometer (DMSO).

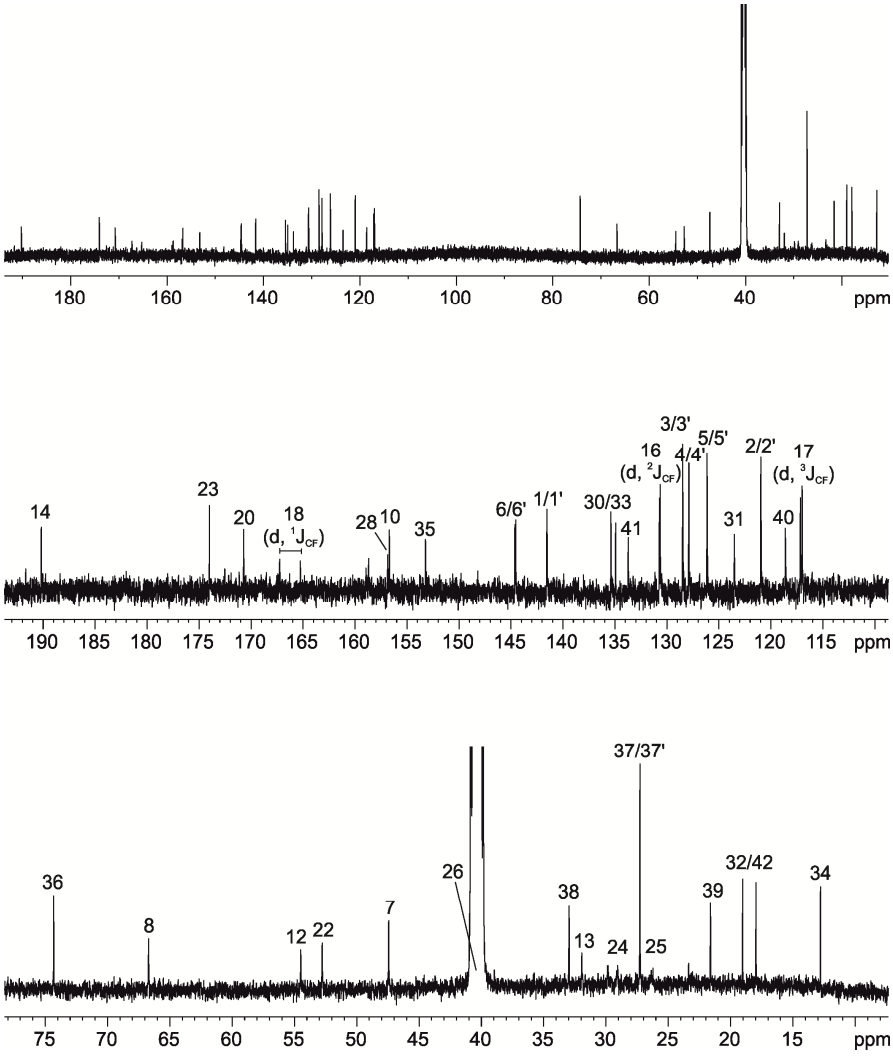


Figure S11. ^{13}C NMR spectrum of **2a** acquired in a 500 MHz Bruker spectrometer (DMSO).

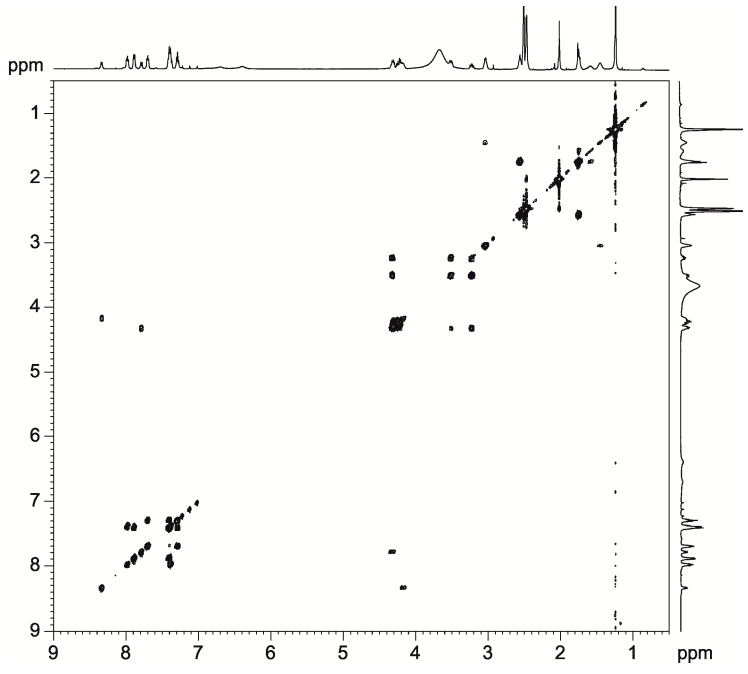


Figure S12. 2D COSY NMR spectrum of **2a** acquired in a 500 MHz Bruker spectrometer (DMSO).

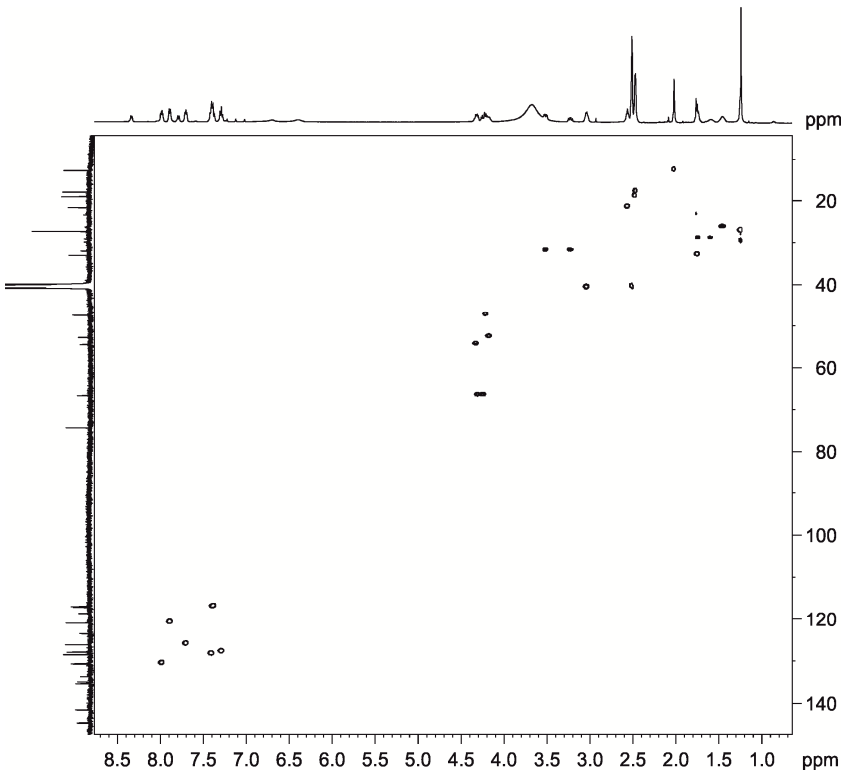


Figure S13. 2D HSQC NMR spectrum of **2a** acquired in a 600 MHz Bruker spectrometer (DMSO).

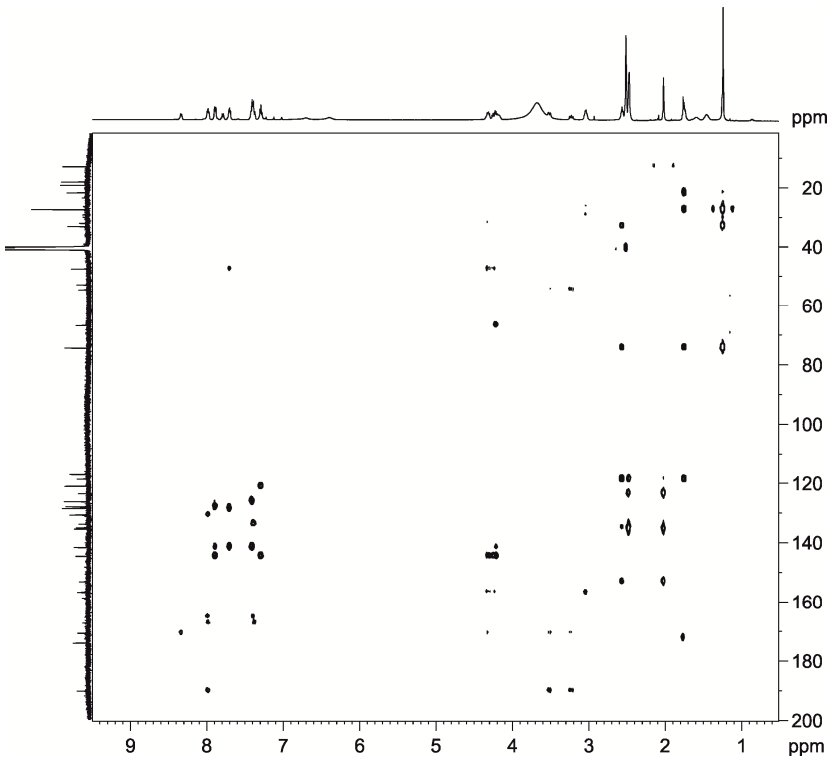


Figure S14. 2D HMBC NMR spectrum of **2a** acquired in a 500 MHz Bruker spectrometer (DMSO).

^{1}H, ^{13}C, 2D TOCSY, 2D HSQC, and 2D HMBC NMR spectra of **3a**

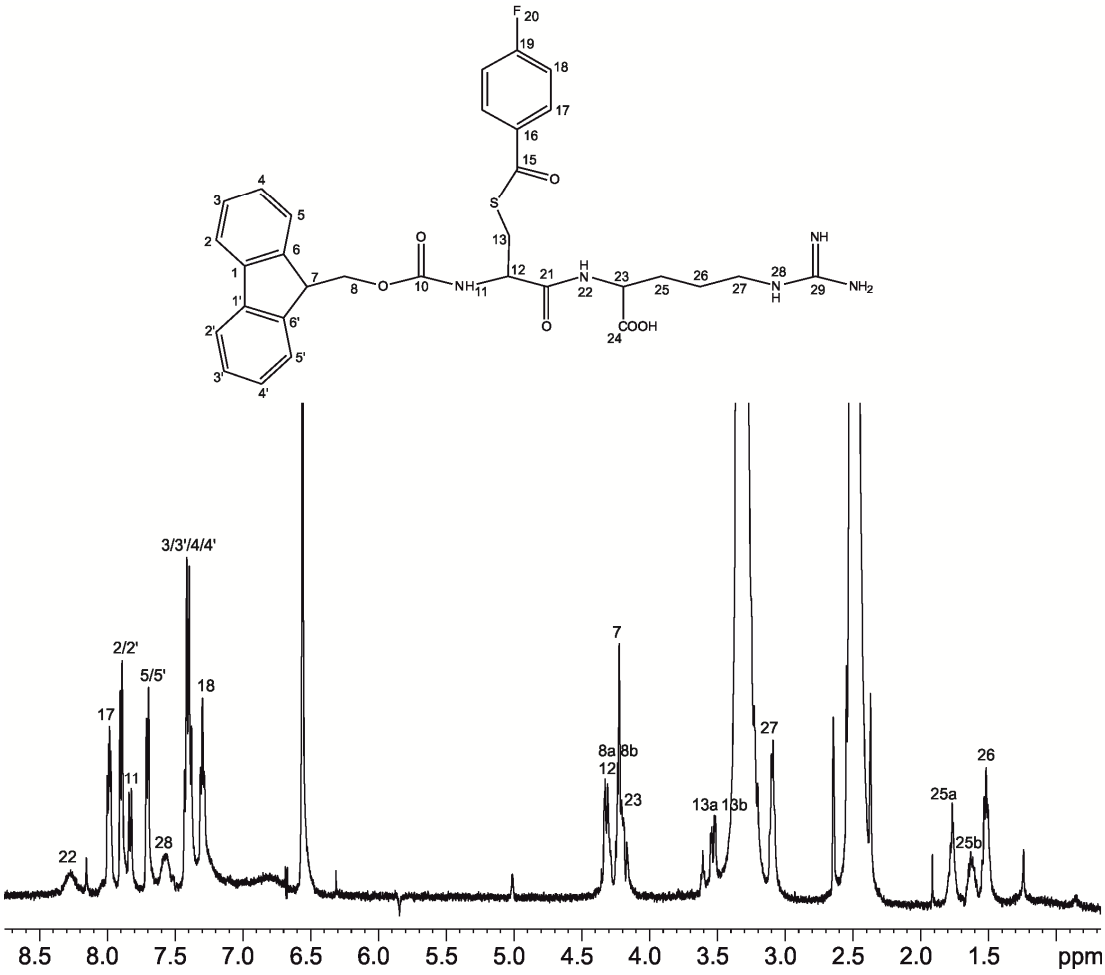


Figure S15. ^{1}H NMR spectrum of **3a** acquired in a 500 MHz Bruker spectrometer (DMSO).

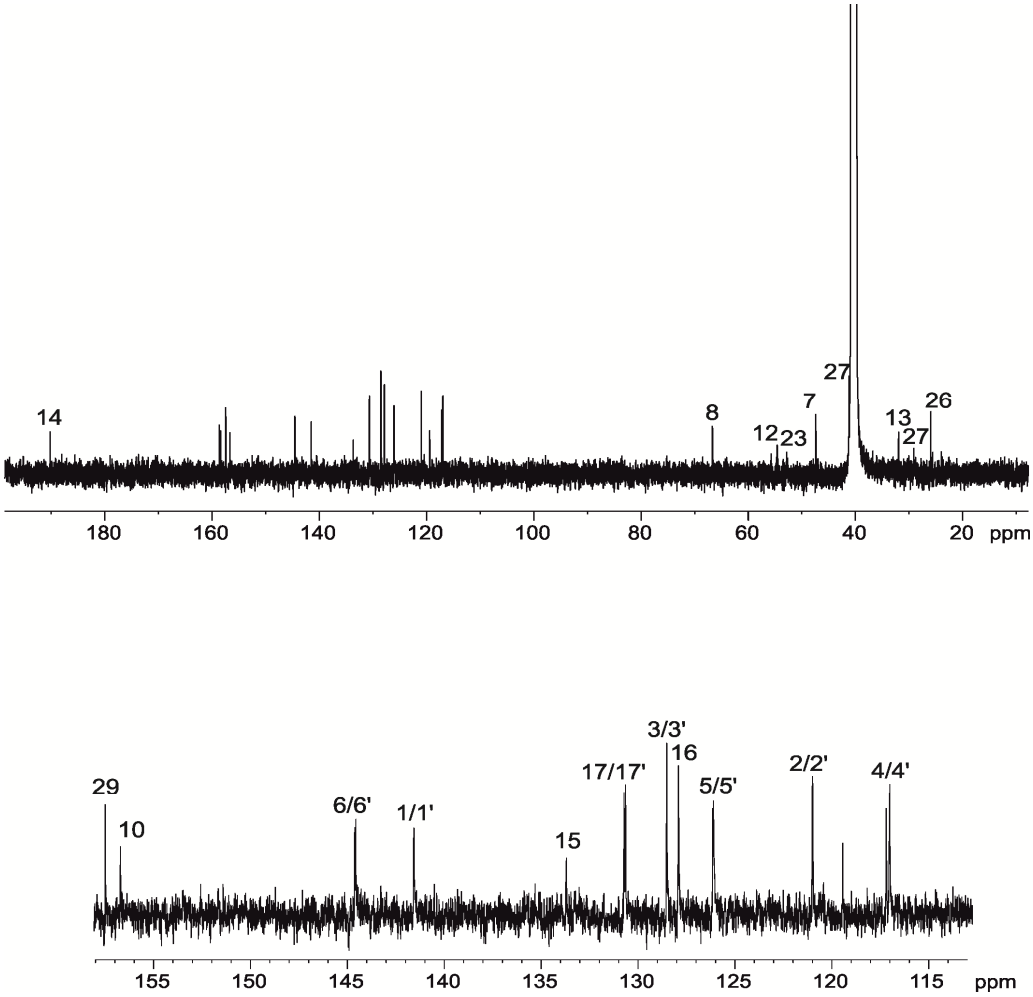


Figure S16. ^{13}C NMR spectrum of **3a** acquired in a 500 MHz Bruker spectrometer (DMSO).

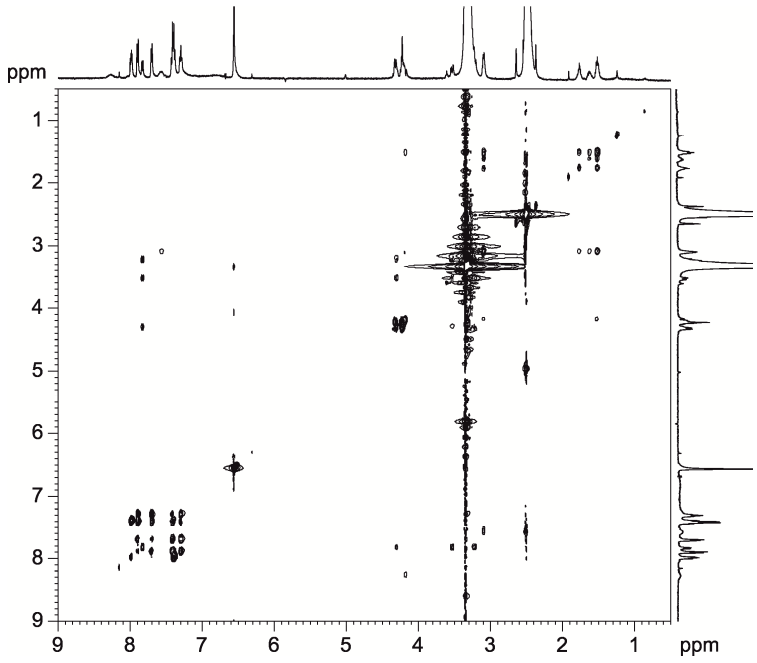


Figure S17. 2D TOCSY NMR spectrum of **3a** acquired in a 500 MHz Bruker spectrometer (DMSO).

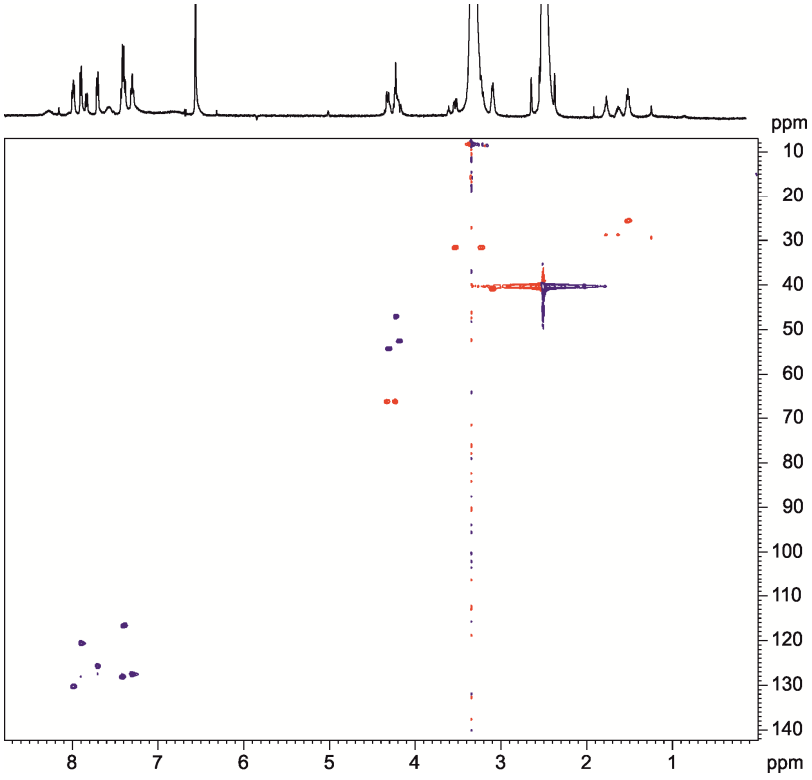


Figure S18. Multipliciy edited 2D HSQC NMR spectrum of **3a** acquired in a 500 MHz Bruker spectrometer (DMSO). CH are marked blue and CH_2 are marked red.

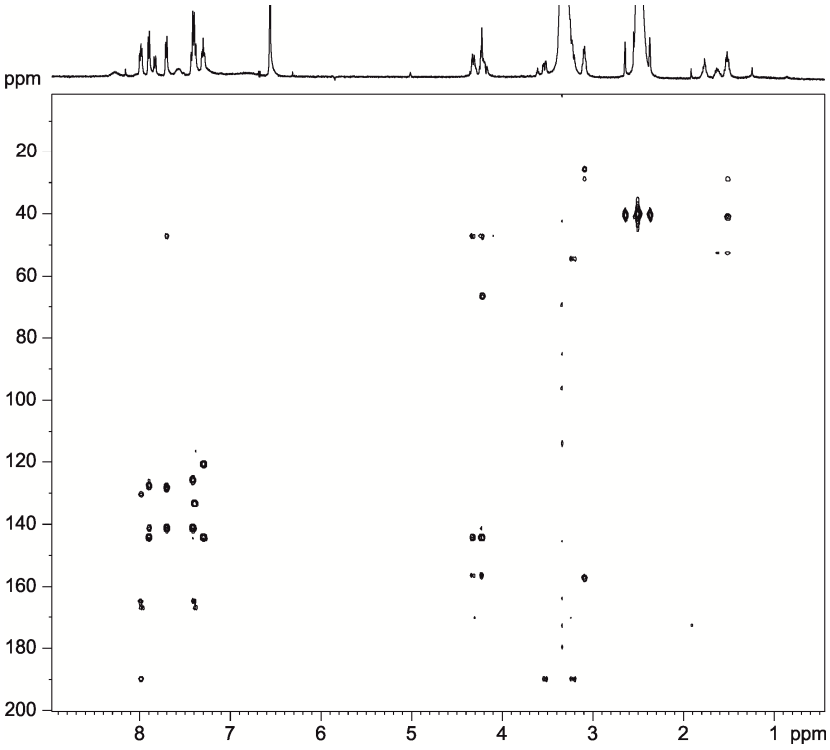
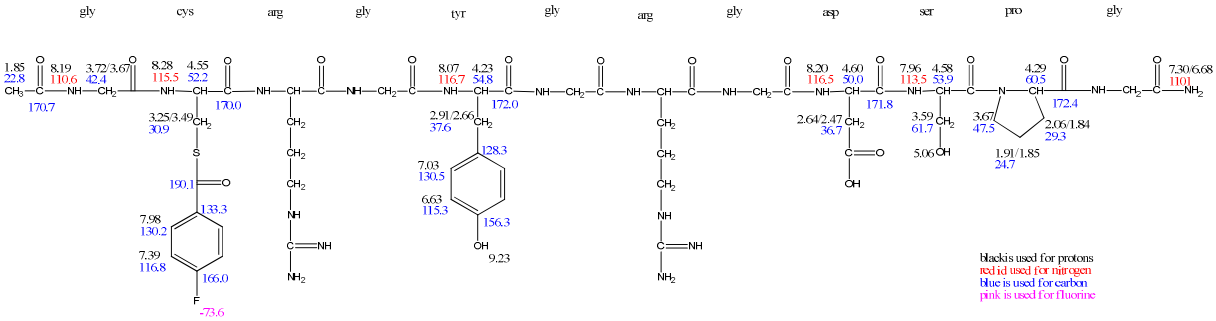


Figure S19. 2D HMBC NMR spectrum of **3a** acquired in a 500 MHz Bruker spectrometer (DMSO).

NMR characterisation of **4a**

The complete ^{1}H, ^{13}C and ^{15}N chemical shift assignment residue by residue has been possible by standard NMR experiments. Figure S10, shows the expanded CHα region of the $^{1}H/^{13}C$-HSQC experiment and Figure S11 shows an expanded region of the multiplicity edited $^{1}H/^{15}N$-HSQC. In both experiments all residues are clearly seen. A key experiment for the assignment of residue spin systems was HSQC-TOCSY (Figure S12). Scheme S1 and Table S1 summarize the ^{1}H, ^{13}C, ^{15}N and ^{19}F chemical shift assignments.



Scheme S1. Chemical shift assignment of ^{1}H, ^{13}C, ^{15}N and ^{19}F for peptide **4a**

Glycine 1	NH 106.4/8.26 CH_2 42.3/3.76
Glycine 2	NH 106.4/8.26 CH_2 42.3/3.73
Glycine 3	NH 105.8/8.08 CH_2 42.3/3.78
Glycine 4	NH 105.3/8.03 CH_2 42.2/3.59
Arginine 1	NH 118.9/8.16 CH 53.8/4.25 CH_2 29.4/(1.75-1.69)/(1.60-1.53) CH_2 25.4/(1.54-1.43) CH_2 41.2/(3.13-3.04) $NHCNHNH_2$ (exchangeable protons)
Arginine 2	NH 117.4/8.07 CH 52.5/4.30 CH_2 29.4/(1.75-1.69)/(1.60-1.53) CH_2 25.4/(1.54-1.43) CH_2 41.2/(3.12-3.04) $NHCNHNH_2$ (exchangeable protons)

Table S1: Assignment of glycine and arginine residues. Although each residue can be totally assigned independently, due to a lack of resolution in both carbonyl and alfa carbon regions we cannot accurately assign which signals belong to each glycine or arginine into the peptide chain.

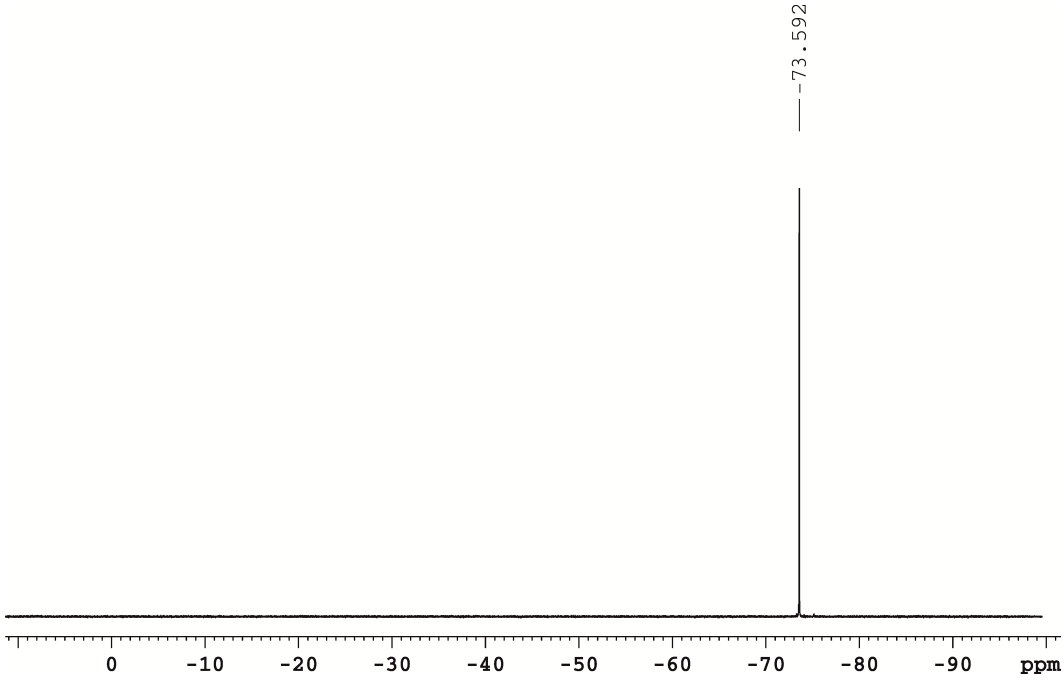


Figure S20. $^{19}F\{^{1}H\}$-NMR spectrum of **4a** acquired in a Bruker 400 MHz spectrometer (DMSO).

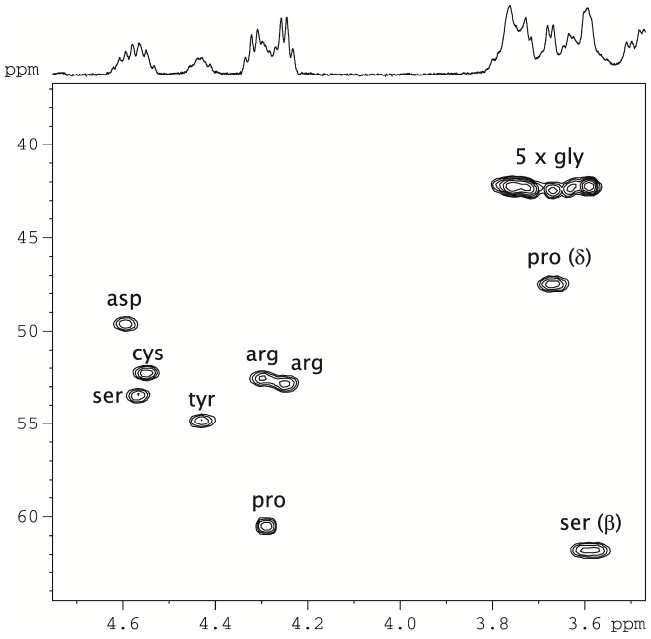


Figure S21. CHα region of $^{1}H/^{13}C$-HSQC NMR spectrum of **4a** acquired in a Bruker 500 MHz spectrometer (DMSO).

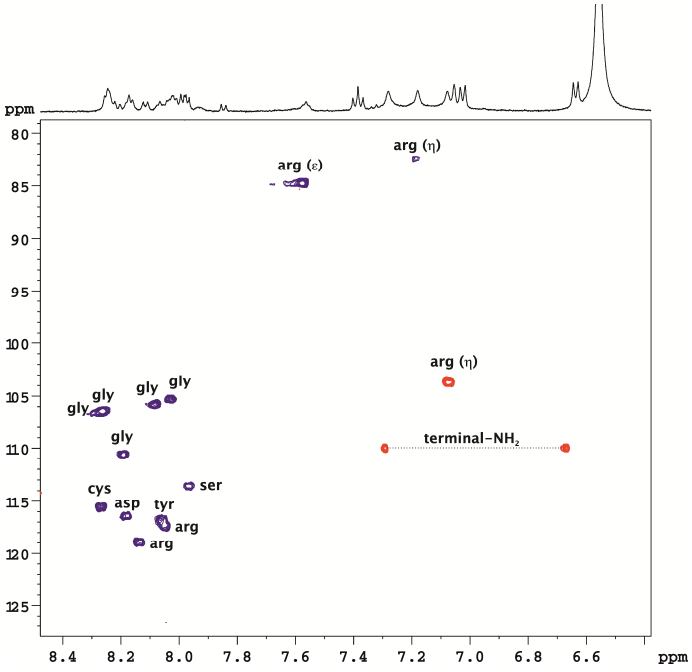


Figure S22. $^{1}H/^{15}N$-HSQC NMR spectrum of **4a** acquired in a Bruker 500 MHz spectrometer (DMSO).

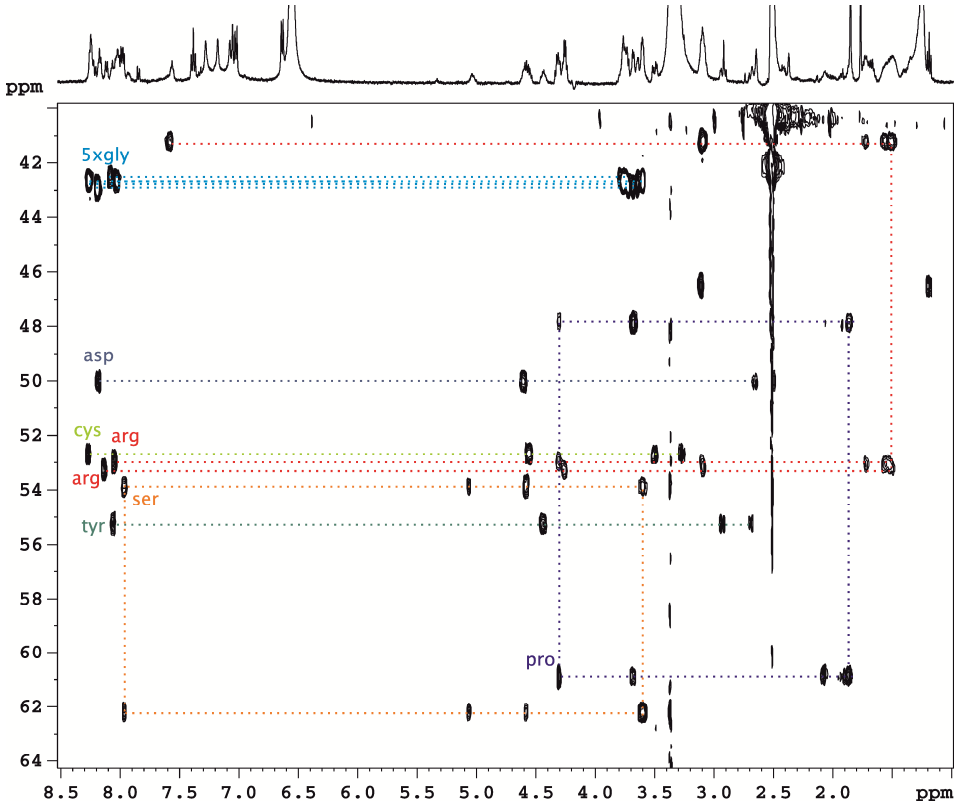


Figure S23. Assignment of residue spin system in $^{1}H/^{13}C$-HSQC-TOCSY NMR spectrum of **4a**. Experiment is acquired in a Bruker 500 MHz spectrometer (DMSO).

The key NMR experiment to confirm that undoubtedly 4-fluorobenzoyl was attached to the Cys residue was the $^{1}H/^{13}C$-HMBC experiment, which allowed to link $CH_2\beta$ protons of Cys with the carbonyl of 4-fluorobenzoyl group, as schematically indicated in Figure S11.

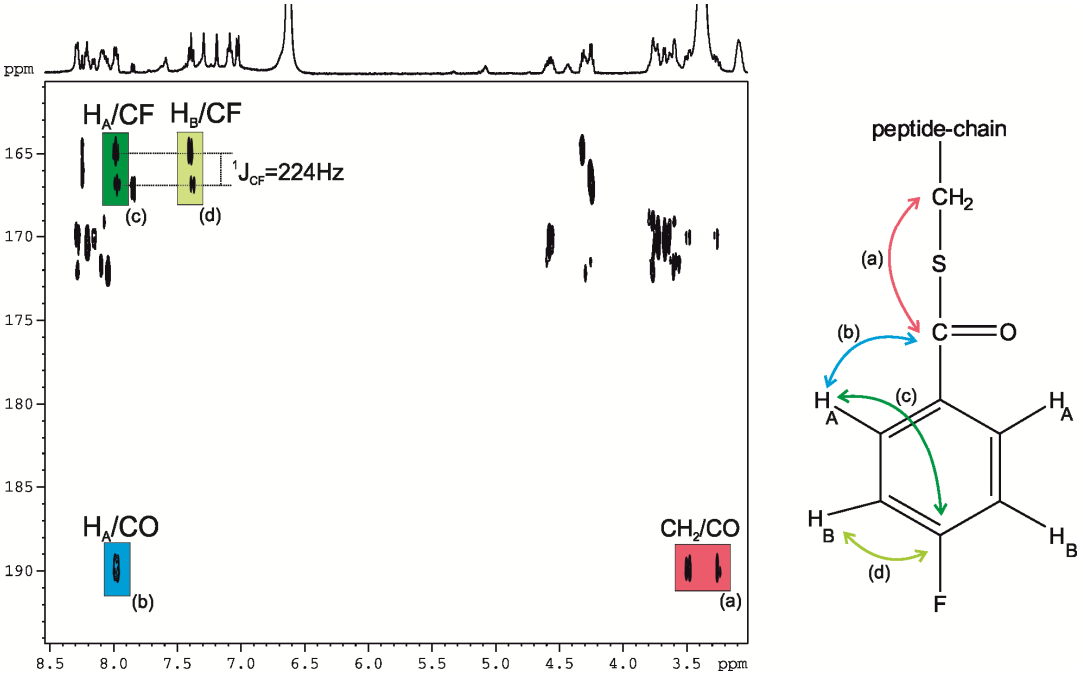


Figure S24. $^{1}H/^{13}C$ HMBC NMR spectrum (DMSO) demonstrates that 4-fluorobenzoyl group is linked to Cys residue.