Electronic Supplementary Material (ESI) for ChemComm.

Electronic Supporting Information

Self-assembly of a tripeptide into a functional coating that resist fouling

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Experimental Details

Materials and methods

All chemicals, solvents, proteins and bacteria were purchased from commercially available companies and used as supplied unless otherwise stated. Fmoc-DOPA(ac)-COOH was obtained from Novabiochem/EMD chemicals (San-Diego, USA). L-4-fluoro phenylalanine, Boc-penta-Fluoro phe-COOH were purchased from chem-impex Inc. (Wood Dale, USA). Solvents and TFA were purchased from Bio-lab(Jerusalem, Israel). NMR solvents ($CDCl_3$ and DMSO-d_6) were supplied by Sigma-Aldrich (Jerusalem, Israel). Piperidine used for deprotection of Fmoc group was obtained from Alfa-Aesar (UK). The proteins BSA and lysozyme were obtained from Sigma-Aldrich (Jerusalem, Israel), Chem impex INC. (Wood Dale, USA) and Merck (Darmstadt, Germany) respectively. *Pseudomonas aeruginosa* (ATCC 27853) and *Eschrichia coli* (ATCC 1655) were purchased from ATCC (Virginia,USA). Crystal violet was obtained from Merck (Darmstadt, Germany).

Substrates

Silicon wafers (100) with a diameter of 2 inches were coated with 50 nm titanium (as measured by quartz crystal) by electron beam evaporation (TFDS-141E, VST) at a rate of 1

Å/sec. In the same manner silicon wafers were coated with 15 nm titanium and then 150 nm of gold. The coated wafers were diced (7100 2'' Pro-Vectus, ADT) into 1cm X 1cm pieces. Mica disks (V1 12mm, Ted Pella USA) with a diameter of 9.9 mm, were freshly cleaved before each use. Stainless steel L304 was obtained from Mashaf (Jerusalem, Israel), and supplied as 1cm X 1cm squares.

Surface modification

1cm X 1cm titanium surfaces were sonicated 5 minutes in ethanol, washed with TDW and dried under nitrogen. The clean surfaces were dipped in a peptide solution (0.5 mg/mL in methanol) and left for overnight at RT. Then, they were rinsed extensively with methanol and dried under nitrogen.

Contact angle measurements

Contact angle measurements were carried out using a Theta Lite optical tensiometer (Attension, Finland). Each experimental measurement consisted of three repeats, and the reported angles were averaged.

AFM analysis

Freshly cleaved mica surfaces were dipped overnight in different peptide solution at a concentration of 0.5 mg/mL in methanol. Then, the surfaces were washed with fresh methanol and dried under nitrogen. AFM images were taken in AC mode with Si_3N_2 tip with spring constant 3N/m in JPK instrument (NanoWizard 3).

ATR-FTIR analysis

ATR spectra were recorded using FT-IR (Thermo scientific, Model Nicolet 6700) with Ge-ATR arrangement (Harrick Scientific's VariGATR). For all the surfaces spectra were

collected with applied force of 350 N, at 4 cm^{-1} resolution with 3000 scans averaged signal and an incident angle of 65°.

QCM-D analysis

QCM-D(Q-sense, Biolin Scientific) was used for the study of peptide adhesion onto Ti surface. Measurements were performed in a flow module E1 system. Ti sensors with a fundamental resonant frequency of 5MHz were also purchased from Q-sense and used as supplied. Prior to each experiment Ti sensors were cleaned with Oxygen/Plasma (Atto, Diener Electronic), followed by rinsing with 2% SDS and TDW and finally dried under nitrogen. All QCM-D experiments were performed under flow-through conditions using a digital peristaltic pump (IsmaTec Peristaltic Pump, IDEX). The studied solutions were injected to the sensor crystal chamber at a rate of 0.1 mL/min. A tube and an O-ring compatible with organic solvent were used for the flow system. Peptides were dissolved in MeOH to a concentration of 0.5 mg/mL.

All data was analyzed using QTools software. Frequency data was fitted to the Sauerbrey equation and adsorbed mass was calculated according to the linear relation:

$$\Delta m = -\frac{C \cdot \Delta f}{n}$$

Where Δm is the adsorbed mass, Δf is the frequency change during adsorption, C=17.7 $ng \cdot cm^{-2} \cdot Hz^{-1}$,a sensitivity constant characteristic of the quartz crystal and n is the overtone number. Viscoelastic properties of the mass adsorbed were calculated by measuring the energy dissipation defined as:

$$D = \frac{E_{lost}}{2\pi E_{stored}}$$

Where E_{lost} is the energy lost in one oscillation cycle of the sensor, and E_{stored} is the total energy in the crystal. The dissipation is measured by recording the response of the oscillating sensor at its resonance frequency.

X-ray photoelectron spectroscopy (XPS) analysis

The X-ray Photoelectron Spectroscopy (*XPS*) measurements were performed using a Kratos AXIS Ultra X-ray photoelectron spectrometer (*Kratos Analytical Ltd., Manchester, UK*). Spectra were acquired using the Al-Kα monochromatic X-ray source (*1,486.7 eV*). Sample take-off angle was 90◦ (*i.e. normal to the analyzer*). The vacuum pressure in the analyzing chamber maintained to $2 \cdot 10^{-9}$ Torr. High-resolution XPS spectra were collected for F 1s, O 1s, C 1s and Ti 2 peaks with pass energy 20 eV and 0.1 eV step size. Data analyses were done using the Kratos Vision data reducing processing software (*Kratos Analytical Ltd.*) and Casa XPS (*Casa Software Ltd.*).

Evaluation of the layer thickness by XPS

Using the XPS measurements, it is possible to calculate the thickness of the assembled layers. We have done so using the standard attenuation relations of the photoelectrons emerging from different sample depths. The thickness calculation is based on the Briggs et al. method and others.[1] For the Au substrate, the overlay thickness *d* (*nm*) expressed as:

$$d = \lambda_o sin\theta ln\left(\frac{N_s\lambda_s I_o}{N_o\lambda_o I_s} + 1\right)$$

Where I_s and I_o are the intensities of the peaks from the substrate and the overlayer respectively, the substrate is the Ti 2p signal, and layer is the sum of the intensities of C 1s, O 1s, N 1s and F 1s peaks, θ is the takeoff angle (*in our case sin θ = 1*) and N_s and N_o are the volume densities. The inelastic mean free paths (IMFPs) parameters for substrate (λ_s) and for the overlayer (λ_o) assumed as 2.18 nm and 3.3 nm respectively. Calculated, using S. Tougard *QUASES-IMFP-TPP2M* software (*http://www.quases.com*). Inelastic electron mean free path calculated from the Tanuma, Powell and Penn algorithm.[2]

References

1. (a)D. Briggs and M. P. Seah, eds., *Practical surface analyses*, 2 edn., Wiley, New York, NY, USA, 1990;(b)B. R. Strohmeier, *Surf Interface Anal*, 1990, **15**, 51-56.

2. S. Tanuma, C. J. Powell and D. R. Penn, *Surf Interface Anal*, 1994, **21**, 165-176.

Ellipsometry

The thickness of the peptide-based coating was measured using α-SE spectroscopic ellipsometer (J.A. Woollam, Lincoln, Nebraska, USA). Measurements were performed at wavelengths from 380 to 900 nm, at a 70° angle of incidence. The optical properties of the substrate were fitted using Si with 50 nm Ti. The thickness of the layers and refractive indices were fitted according to the Cauchy model. The coefficients of the Cauchy equation were initially fixed for organic layers (A_n=1.45, B_n=0.01 and C_n=0), and an angle offset was permitted. Then, the parameters were allowed to be fitted to determine more accurate values.

Protein adsorption

50 μL of single protein solution of BSA, lysozyme (150 μM in PBS) applied onto the substrate in a Petri dish. The plate was placed in a humidified incubator at 37°C for 2 hours. The substrates were then rinsed 3 times with PBS

(pH=7.43, 10mM ,150 mM NaCl), and transferred into test tubes with 1 mL of 2% (w/w) SDS. The samples were shaken for 60 minutes and sonicated for 20 minutes at room temperature to detach the adsorbed proteins. Protein concentrations in the SDS solution were determined using the Non-interfering protein assay (Calbiochem, USA) according to the instructions of the manufacturer, using a microplate reader (Synergy 2, BioTek) at 480 nm. All measurements were performed in triplicates and averaged.

Biofilm formation

Pseudomonas aeruginosa and *Eschrichia coli* were grown in TSB medium (Fluka) and LB medium (BD Difco) respectively overnight at 37°C in loosely capped tubes with agitation (120 rpm) to the stationary phase. Then, cultures were diluted to 10^8 CFU/mL with TSB, and

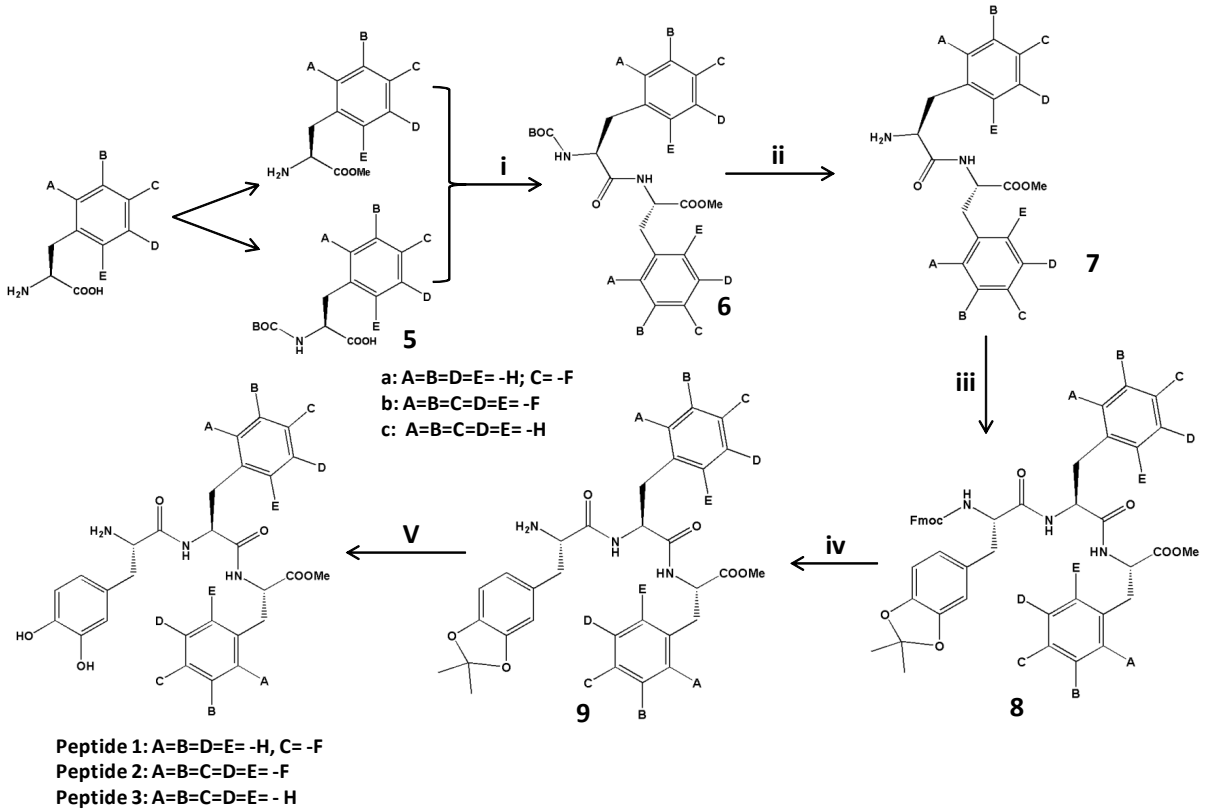
3 mL of each culture were transferred to a Petri dish. Substrates were placed horizontally in the plate and incubated at 37°C for 9 hours for the formation of biofilm by *P.aeruginosa* and 96 hours for the formation of biofilm by *E.coli*. Every 4.5 hours the medium was replaced with a fresh one to ensure sufficient supply of nutrients.

Crystal violet assay

After incubation, the substrates were gently rinsed 3 times with di-ionized water, and stained with 0.2% crystal violet for 15 minutes. The stained samples were washed with running water and left to dry in air. Eventually the bound dye was eluted with 30% acetic acid. Absorbance values were recorded at 590 nm in a microplate reader (Synergy 2, BioTek). All measurements were performed in triplicates and averaged.

Peptide synthesis

NMR spectra were obtained using Bruker DRX 400 or Bruker Avance II-500 spectrometer. The mass of the peptides was measured using Applied Biosystem Voyager-DE pro MALDI TOF mass spectrometer. The chirality of the peptides was confirmed using Chiralpak® AD-H column (4.6 mm ID, 250 mm L) (Daicel Chiral Technologies (china) co., LTD) on a Surviyor LC Pump Plus HPLC instrument (Thermo Scientific, USA). All the amino acids used for peptide synthesis were in L configuration. The peptides were synthesized by a conventional solution-phase method using a racemization free strategy. The Boc group and Fmoc group were used for N-terminal protection and the C-terminus was protected as a methyl ester. Couplings were mediated by dicyclohexylcarbodiimide/1-hydroxybenzotriazole (DCC/HOBt). The intermediate compounds were characterized by ^{1}H NMR and MALDI-TOF mass spectroscopy and final peptides were fully characterized by ^{1}H NMR, ^{13}C NMR, ^{19}F NMR, MALDI-TOF. The peptides were synthesized as described in the scheme bellow.



Scheme 1: Reactions and Conditions: (i) DCC, HOBT, Dry DCM, 0°C (ii) 20% TFA in DCM, (iii) Fmoc-DOPA(ac)-COOH, DCC, HOBT, Dry DCM, 0°C (iv) 20% Piperidine in DMF (v) 95% TFA/H_2O .

A. Synthesis of Peptide 1

A1. Boc-(4F)Phe-COOH 5a: A solution of NH_2-(4F)Phe-COOH 1.97 g (10 mmol) in a mixture of dioxane (20 mL), water (20 mL) and 1 M NaOH (10 mL) was stirred and cooled in an ice-water bath. Di-tert-butylpyrocarbonate 2.4 g (11 mmol) was added and stirring was continued at room temperature for 6 h. Then the solution was concentrated in vacuum to about 15–20 mL, cooled in an ice water bath, covered with a layer of ethyl acetate (about 30 mL) and a dilute solution of $KHSO_4$ was added to acidify the solution (pH 2–3). The aqueous phase was extracted with ethyl acetate. This operation was repeated three times. Then, the ethyl acetate extracts were collected, dried over anhydrous Na_2SO_4 and evaporated in a vacuum. The pure material was obtained as a waxy solid.

Yield: 2.115 g (7.25 mmol, 72.5%)

^{1}H NMR (DMSO-d_6, 400 MHz, δ_{ppm}): 12.60 [s, 1H COOH], 7.29-7.25 & 7.11-7.07 [m, 4H, Aromatic protons], 4.10-3.00 [m, 1H, CαH 4F Phe], 3.03-2.77 [m, 2H, CβH 4F Phe], 1.33 [s, 9H, Boc].

MALDI-TOF [matrix:α-cyano-4-hydroxy cinnamic acid (CHCA)]: m/z = $[M+H]^+$ 284.12 (calculated), 284.29 (observed), $[M+Na]^+$ 306.11 (calculated), 306.25 (observed).

A2. Boc-(4F)Phe(1)-(4F)Phe(2)-COOMe 6a: 500 mg (1.766 mmol) of Boc-(4F)Phe-OH were dissolved in 25 mL dry DCM in an ice-water bath. NH_2-(4F)Phe-OMe 697.13 mg (3.532 mmol) was isolated from the corresponding methyl ester hydrochloride by neutralization, subsequent extraction with ethyl acetate and solvent evaporation. Then, it was added to the reaction mixture, followed immediately by the addition of 365 mg (1.766 mmol) dicyclohexylcarbodiimide (DCC) and 239 mg (1.766 mmol) of HOBt. The reaction mixture was allowed cool to room temperature and stirred for 48 h. Then, DCM was evaporated, the residue was dissolved in ethyl acetate (60 mL) and dicyclohexyl urea (DCU) was filtered off. The organic layer was washed with 2 M HCl (3 × 30 mL), brine (2 × 30 mL), 1 M sodium carbonate (3 × 30 mL) and brine (2 × 30 mL), dried over anhydrous sodium sulfate and evaporated in a vacuum to yield compound **6a**, as a white solid. The product was purified by silica gel (100–200 mesh) using n-hexane–ethyl acetate (4:1) as eluent.

Yield: 616.6 mg (1.334 mmol, 75.5%)

^{1}H NMR ($CDCl_3$, 400 MHz, δ_{ppm}): 7.16-7.12 & 6.99-6.90 [m, 8H, Aromatic protons], 6.27-6.25 [d, 1H, NH 4F Phe(2)], 4.93 [b, 1H, NH 4F Phe(1)], 4.77-4.72 [m, 1H, CαH 4F Phe(2)], 4.28-4.27 [m, 1H, CαH 4F Phe(1)], 3.67 [s, 3H, OMe], 3.08-2.98 [m, 4H, CβH 4F Phe(1) and 4F Phe(2)], 1.41 [s, 9H, Boc].

MALDI-TOF [matrix:α-cyano-4-hydroxy cinnamic acid (CHCA)]: m/z = $[M+Na]^+$ 485.18 (calculated), 485.45 (observed), $[M+K]^+$ 501.16 (calculated), 501.32 (observed).

A3. NH_2-(4F)Phe(1)-(4F)Phe(2)-COOMe 7a: 600 mg (1.298 mmol) of compound **6a** were dissolved in 16 mL of DCM in an ice bath. Then, 4 mL of TFA were added and stirred for 2h. The progress of reaction was monitored by TLC (Thin Layer Chromatography). After the completion of reaction, all solvents were evaporated in a rotary evaporator. The product was then dissolved in water, neutralized with $NaHCO_3$ solution and extracted with ethyl acetate, dried over anhydrous sodium sulphate, and evaporated by rotary evaporator to obtain an oily product **7a.**

Yield: 435.3 mg (1.202 mmol, 92.6%)

^{1}H NMR (DMSO-d_6, 400 MHz, δ_{ppm}): 9.06-9.05 [d, 1H, NH 4F Phe(2)], 7.32-7.26 & 7.17-7.04 [m, 8H, Aromatic protons], 4.57-4.51 [m, 1H, CαH 4F Phe(2)], 4.04-3.96 [m, 1H, CαH 4F Phe(1)], 3.61 [s, 3H, OMe], 3.18-2.91 [m, 4H, CβH 4F Phe(1) and 4F Phe(2)].

MALDI-TOF [matrix:α-cyano-4-hydroxy cinnamic acid (CHCA)]: m/z = $[M+2H]^+$ 364.14 (calculated), 364.34 (observed), $[M+H_2O]^+$ 480.15 (calculated), 480.35 (observed).

A4. Fmoc-DOPA(ac)-(4F)Phe(1)-(4F)Phe(2)-COOMe 8a: 430 mg (1.187 mmol) of compound **7a** were dissolved in 25 mL dry DCM in an ice-water bath and 652.37 mg (1.42 mmol) of Fmoc-DOPA(ac)-COOH were added. Then 245 mg (1.187 mmol) dicyclohexylcarbodiimide (DCC) and 161 mg (1.187 mmol) of HOBt were added to the reaction mixture. Then, the reaction mixture was allowed to cool to room temperature and stirred for 48 h. DCM was evaporated and the residue was dissolved in ethyl acetate (60 mL). Dicyclohexylurea (DCU) was filtered off. The organic layer was washed with water, extracted, dried over anhydrous sodium sulfate and evaporated under vacuum to yield compound **8a**, as a white solid. The product was purified by silica gel (100–200 mesh) using n-hexane–ethyl acetate (4 : 1) as an eluent.

Yield: 594.8 mg (0.74 mmol, 62.4%).

^{1}H NMR ($CDCl_3$, 400 MHz, δ_{ppm}): 7.77-7.75, 7.54-7.50, 7.42-7.38, 7.33-7.29 [d & m, 8H, Fmoc aromatic protons], 7.05-6.86 [m, 8H, 4F Phe(1) and 4F Phe(2) aromatic protons], 6.62-6.55 [s & m, 3H, DOPA aromatic protons], 6.50 [b, 1H, NH 4F Phe(1)], 6.19 [b, 1H, NH 4F Phe(2)], 5.17 [b, 1H, NH DOPA], 4.68-4.66 [m, 1H, CαH DOPA], 4.54-4.52 [m, 1H, CαH 4F Phe(1)], 4.47-4.42 [m, 1H, CαH 4F Phe(2)], 4.31 (b, 2H, CβH Fmoc], 4.20-4.17 [m, 1H, CαH Fmoc], 3.65 [s, 3H, OMe], 2.98-2.92 [m, 6H, CβH 4F Phe(1) 4F Phe(2) & DOPA], 1.62 [s, 6H, 2×$COCH_3$].

MALDI-TOF [matrix:α-cyano-4-hydroxy cinnamic acid (CHCA)]: m/z = $[M+H]^+$ 804.31 (calculated), 804.70 (observed), $[M+Na+2H]^+$ 828.30 (calculated), 828.07 (observed), $[M+K+H]^+$ 843.27 (calculated), 843.60 (observed).

A5. NH_2-DOPA(ac)-(4F)Phe(1)-(4F)Phe(2)-COOMe 9a: 580 mg (0.721 mmol) of compound **8a** were treated with 15 mL of 20% Piperidine solution and stirred for 3h at room temperature. The completion of the reaction was monitored by TLC. Then, the solution was lyophilized and purified with column chromatography to obtain a pure compound **9a.**

Yield: 275.6 mg (0.474 mmol, 65.8%)

^{1}H NMR (DMSO-d_6, 400 MHz, δ_{ppm}): 8.53 [b, 1H, NH 4F Phe(1)], 7.96 [b, 1H, NH 4F Phe(2)], 7.24-7.23, 7.10-7.04 [m, 8H, 4F Phe(1) and 4F Phe(2) aromatic protons], 6.69-6.65, 6.55-6.53 [m, 3H, DOPA aromatic protons], 5.56 [m, 1H, CαH DOPA], 4.56 [m, 1H, CαH

4F Phe(1)], 4.47 [m, 1H, 4F Phe(2)], 3.61 [s, 3H, OMe], 3.12-2.73 [m, 6H, CβH 4F Phe(1) 4F Phe(2) & DOPA], 1.61-1.58 [d, 6H, 2×$COCH_3$].

MALDI-TOF [matrix:α-cyano-4-hydroxy cinnamic acid (CHCA)]: m/z = $[M+H]^+$ 582.23 (calculated), 582.25 (observed), $[M+Na]^+$ 604.22 (calculated), 604.37 (observed), $[M+K]^+$ 620.20 (calculated), 620.19 (observed).

A6. NH_2-DOPA-(4F)Phe(1)-(4F)Phe(2)-COOMe 1: 260 mg (0.447 mmol) of compound **9a,** were stirred in 10 mL of 95% TFA in water for 6h. The progress of the reaction was monitored by TLC. After completion of reaction the solvent was evaporated by a rotary evaporator. The product was then washed with hexane, cold ether and water (three times by each solvent) to obtain peptide **1**.

Yield: 139.1 mg (0.257 mmol, 57.5%)

^{1}H NMR (DMSO-d_6, 500 MHz, δ_{ppm}): 8.72-8.70 [d, 1H, NH 4F Phe(1)], 8.66-8.64 [d, 1H, NH 4F Phe(2)], 7.88 [b, 2H, OH DOPA], 7.29-7.23, 7.12-7.05 [m, 8H, 4F Phe(1) and 4F Phe(2) aromatic protons], 6.7-6.64, 6.5-6.47 [m, 3H, DOPA aromatic protons], 4.60-4.58 [m, 1H, CαH 4F Phe(1)], 4.53-4.52 [m, 1H, CαH 4F Phe(2)], 3.83 [m, 1H, CαH DOPA], 3.58 [s, 3H, OMe], 3.08-2.75 [m, 6H, CβH 4F Phe(1) 4F Phe(2) & DOPA]. ^{13}C NMR (DMSO-d_6, 125 MHz, δ_{ppm}): 171.9, 170.1, 168.5, 158.9, 158.54, 145.2, 144.5, 131.5, 125.2, 117.4, 115.5, 115.4, 115.3, 11.2, 114.5, 53.9, 52.3, 47.5, 36.2, 33.8, 25.8, 24.9. ^{19}F NMR (DMSO-d6, 470 MHz, δ_{ppm}): -116.42, -116.71.

MALDI-TOF [matrix:α-cyano-4-hydroxy cinnamic acid (CHCA)]: m/z = $[M+H]^+$ 542.20 (calculated), 542.57 (observed), $[M+Na]^+$ 564.19 (calculated), 564.46 (observed), $[M+K]^+$ 580.16 (calculated), 580.32 (observed).

B. Synthesis of peptide 2

B1. Boc-(F_5)Phe(1)-(F_5)Phe(2)-COOMe 6b.

We have purchased Boc-(F_5)Phe-COOH. We first deprotected the Boc group by treatment of TFA/DCM, then evaporate all the solvents and esterification of NH_2-Phe(F5)-COOH was done by treating with thionyl chloride and methanol. Then the compound **6b** was synthesized by coupling of Boc-(F5)Phe-COOH with NH_2-(F5)Phe -COOMe as described for compound **6a.**

^{1}H NMR ($CDCl_3$, 400 MHz, δ_{ppm}): 6.52 [b, 1H, NH Phe(2)], 4.93 [b, 1H, NH 4F Phe(1)], 4.92-4.85 [m, 1H, CαH Phe(2)], 4.42-4.29 [m, 1H, CαH Phe(1)], 3.81 [s, 3H, OMe], 3.42-2.95 [m, 4H, CβH Phe(1) and Phe(2)], 1.44 [s, 9H, Boc].

MALDI-TOF [matrix:α-cyano-4-hydroxy cinnamic acid (CHCA)]: m/z = $[M+Na+H]^+$ 630.11 (calculated), 630.08(observed), $[M+K+H]^+$ 646.08 (calculated), 646.13 (observed).

B2. NH_2-(F_5)Phe(1)-(F_5)Phe(2)-COOMe 7b.

The compound **7b** was prepared as described for compound **7a.**

^{1}H NMR (DMSO-d_6, 400 MHz, δ_{ppm}): 8.93-8.90 [d, 1H, NH Phe(2)], 8.40 [b, 1H, free NH_2], 4.72-4.70 [m, 1H, CαH Phe(2)], 3.90 [m, 1H, CαH Phe(1)], 3.61 [s, 3H, OMe], 3.17-2.99 [m, 4H, CβH Phe(1) and Phe(2)].

MALDI-TOF [matrix:α-cyano-4-hydroxy cinnamic acid (CHCA)]: m/z = $[M+Na+H]^+$ 530.05 (calculated), 530.16(observed), $[M+K+H]^+$ 546.03 (calculated), 646.53 (observed).

B3. Fmoc-DOPA(ac)-(F_5)Phe(1)-(F_5)Phe(2)-COOMe 8b.

The compound **8b** was prepared as described for compound **8a.**

^{1}H NMR (DMSO-d_6, 400 MHz, δ_{ppm}): 8.75-8.72 [d, 1H, NH Phe(1)], 8.36-8.34 [b, 1H, NH Phe(3)], 7.88-7.26 [m, 8H, Fmoc aromatic protons], 6.79-6.67 [m, 3H, DOPA aromatic protons], 5.57-5.55 [b, 1H, NH DOPA], 4.66-4.63 [m, 2H, CβH Fmoc], 4.14-4.09 [m, 3H, CαH DOPA, CαH Phe(1), CαH Phe(2)], 3.62 [s, 3H, OMe], 3.05-2.90 [m, 6H, CβH Phe(1), Phe(2) & DOPA], 1.56 [s, 6H, 2×$COCH_3$].

MALDI-TOF [matrix:α-cyano-4-hydroxy cinnamic acid (CHCA)]: m/z = $[M+Na]^+$ 970.21 (calculated), 970.22(observed), $[M+K]^+$ 986.19 (calculated), 986.04 (observed).

B4. NH_2-DOPA(ac)-(F_5)Phe(1)-(F_5)Phe(2)-COOMe 9b

The compound **9b** was prepared as described for compound **9a.**

^{1}H NMR (DMSO-d_6, 400 MHz, δ_{ppm}): 8.73-8.71 [d, 1H, NH Phe(1)], 6.69-6.55 [m, 3H, DOPA aromatic protons], 5.57-5.55 [d, 1H, NH Phe(2)], 4.64-6.63 [m, 1H, CαH DOPA], 4.54 [m, 1H, CαH Phe(1)], 4.13-4.08 [m, 1H, CαH Phe(2)], 3.61 [s, 3H, OMe], 3.15-2.67 [m, 6H, CβH Phe(1), Phe(2) & DOPA], 1.60 [s, 6H, 2×$COCH_3$].

MALDI-TOF [matrix:α-cyano-4-hydroxy cinnamic acid (CHCA)]: m/z = $[M+Na]^+$ 748.15 (calculated), 748.23(observed), $[M+K]^+$ 764.12 (calculated), 764.06 (observed).

B5. NH_2-DOPA-(F_5)Phe(1)-(F_5)Phe(2)-COOMe 2

The compound **2** was prepared as described for compound **1.**

^{1}H NMR (DMSO-d_6, 400 MHz, δ_{ppm}): 9.46 [b, 1H, NH Phe(1)], 9.25 [b, 1H, NH Phe(2)], 8.39 [b, 2H, free NH_2), 6.68-6.54 [m, 3H, DOPA aromatic protons], 4.69-4.65 [m, 2H, CαH Phe

(1) & Phe(2)], 4.55 [m, 1H, CαH DOPA], 3.61 [s, 3H, OMe], 3.01-2.95 67 [m, 6H, CβH Phe(1) Phe(2) & DOPA].^{13}C NMR (DMSO-d_6, 100 MHz, δ_{ppm}): 193.6, 158.5, 158.2, 144.3, 140.8, 139.5, 133.7, 129.9, 128.5, 127.8, 124.4, 53.8, 44.2, 33.8, 30.5, 29.4, 22.6, 17.6. ^{19}F (DMSO-d_6, 470 MHz, δ_{ppm}): -141.7, -142.4, -157.6, -163.1, -163.4.

MALDI-TOF [matrix:α-cyano-4-hydroxy cinnamic acid (CHCA)]: m/z = $[M+Na]^+$ 748.15 (calculated), 748.23(observed), $[M+K]^+$ 764.12 (calculated), 764.06 (observed).

C. <u>Synthesis of Peptide 3</u>

C1. Boc-Phe-COOH 5c:

The compound was synthesized as compound **5a**.

^{1}H NMR (DMSO-d_6, 400 MHz, δ_{ppm}): 12.654 [b, 1H, COOH], 7.29-7.18 [m, 5H, aromatic protons], 7.11-7.09 [d, 1H, NH], 4.11-4.02 [m, 1H, CαH], 3.03-2.81 [dd, 2H, CβH], 1.25 [s, 9H, Boc].

MALDI-TOF (matrix:α-cyano-4-hydroxy cinnamic acid (CHCA)):m/z = $[M+Na]^+$ 288.12 (calculated), 290.23 (observed).

C2. Boc-Phe(1)-Phe(2)-COOMe 6c:

The compound **6c** was synthesized as compound **6a**.

^{1}H NMR ($CDCl_3$, 500 MHz, δ_{ppm}): 7.31-7.21 & 7.03-7.01 [m, 10H, aromatic protons], 6.38-6.37 [d, 1H, NH Phe(2)] 5.03 [b, 1H, NH Phe(1)], 4.82-4.81 [m, 1H, CαH Phe (1)], 4.38 [m, 1H, CαH Phe (2)], 3.69 [s, 3H, OMe], 3.09-3.06 [m, 4H, CβH Phe(1) & Phe(2)], 1.43 [s, 9H, Boc].

MALDI-TOF [matrix:α-cyano-4-hydroxy cinnamic acid (CHCA)]: m/z = $[M+Na]^+$ 433.24 (calculated), 435.15 (observed).

C3. NH_2-Phe(1)-Phe(2)-COOMe 7c:

The compound **7c** was synthesized as compound **7a**.

^{1}H NMR (DMSO-d_6, 400 MHz, δ_{ppm}): 8.28-8.26 [d, 1H, NH Phe(2)], 7.28-7.13 [m, 10H, aromatic protons], 4.60-4.55 [m, 1H, CαH Phe (1)], 3.61 [s, 3H, OMe], 3.61-3.44-3.41 [m, 1H, CαH Phe (2)], 3.02-2.87 & 2.59-2.53 [m, 4H, CβH Phe(1) & Phe(2)], 1.79 [b, 2H, NH_2].

MALDI-TOF [matrix:α-cyano-4-hydroxy cinnamic acid (CHCA)]: m/z = $[M+Na]^+$ 265.18 (calculated), 265.68 (observed).

C4. Fmoc-DOPA(ac)-Phe(1)-Phe(2)-COOMe 8c.

The compound **8c** was synthesized as compound **8a**.

^{1}H NMR ($CDCl_3$, 500 MHz, δ_{ppm}): 7.77-7.75, 7.52-7.50, 7.40-7.39, 7.32-6.99 [d & m, 18H, Fmoc and Phe aromatic protons], 6.69 [b, 1H, NH Phe(1)], 6.60-6.49 [s & m, 3H, DOPA

aromatic protons], 6.43 [b, 1H, NH Phe(2)], 5.40 [b, 1H, NH DOPA], 4.74-4.72 [m, 1H, CαH DOPA], 4.64 [b, 1H, CαH Phe(1)], 4.42-4.39 [m, 1H, CαH Phe(2)], 4.22 (b, 2H, CβH Fmoc], 4.18-4.17 [m, 1H, CαH Fmoc], 3.62 [s, 3H, OMe], 3.04-2.89 [m, 6H, CβH Phe(1), Phe(2) & DOPA], 1.61[s, 6H, 2×$COCH_3$].

MALDI-TOF [matrix:α-cyano-4-hydroxy cinnamic acid (CHCA)]: m/z = $[M+H]^+$ 767.32 (calculated), 767.65 (observed), $[M+Na+2H]^+$ 792.31 (calculated), 792.23 (observed).

C5. NH_2-DOPA(ac)-Phe(1)-Phe(2)-COOMe 9c.

The compound **9c** was synthesized as compound **9a**.

^{1}H NMR (DMSO-d_6, 400 MHz, δ_{ppm}): 8.56-8.54 [d, 1H, NH Phe(1)], 7.96-9.94 [d, 1H, NH Phe(2)], 7.29-7.09 [m, 8H, Phe(1) and Phe(2) aromatic protons], 6.67-6.64, 6.54-6.52 [m, 3H, DOPA aromatic protons], 5.60-5.58 [m, 1H, CαH DOPA], 4.52-4.47 [m, 1H, CαH Phe(1)], 3.59 [s, 3H, OMe], 3.32 [m, 1H, Phe(2)], 3.29-2.68 [m, 6H, CβH Phe(1), Phe(2) & DOPA], 1.60-1.57 [d, 6H, 2×$COCH_3$].

MALDI-TOF [matrix:α-cyano-4-hydroxy cinnamic acid (CHCA)]: m/z = $[M+H]^+$ 546.25 (calculated), 548.17 (observed).

C6. **NH_2-DOPA-Phe(1)-Phe(2)-COOMe 3.**

The compound **3** was synthesized as compound **1**.

^{1}H NMR (DMSO-d_6, 500 MHz, δ_{ppm}): 8.75-8.74 [d, 1H, NH Phe(1)], 8.68-8.66 [d, 1H, NH Phe(2)], 7.91 [b, 2H, OH DOPA], 7.25-7.19 [m, 8H, Phe(1) and Phe(2) aromatic protons], 6.68-6.65, 6.50-6.48 [m, 3H, DOPA aromatic protons], 4.63-4.59 [m, 1H, CαH Phe(1)], 4.54-4.51 [m, 1H, CαH Phe(2)], 3.84-3.82 [m, 1H, CαH DOPA], 3.57 [s, 3H, OMe], 3.03-2.56 [m, 6H, CβH 4F Phe(1) 4F Phe(2) & DOPA]. ^{13}C NMR (DMSO-d_6, 125 MHz, δ_{ppm}): 171.9, 171.0, 168.6, 158.6, 158.3, 145.6, 144.9, 137.7, 137.4, 129.6, 129.4, 128.5, 126.9, 125.7, 120.7, 117.2, 116.0, 54.3, 53.9, 52.2, 38.0, 37.0.

MALDI-TOF [matrix:α-cyano-4-hydroxy cinnamic acid (CHCA)]: m/z = $[M+H]^+$ 506.22 (calculated), 507.72 (observed), $[M+Na+H]^+$ 529.21 (calculated), 529.79 (observed), $[M+K+H]^+$ 545.18 (calculated), 545.76 (observed).

D. Synthesis of peptide 4

D1. Fmoc-DOPA(ac)-(4F) Phe-COOMe

919 mg (2.0 mmol) of Fmoc-DOPA(ac)-COOH was dissolved in dry DCM and cooled in ice bath. NH_2-(4F)Phe-COOMe was isolated from 932mg (4.0 mmol) of its corrosponding methyl ester hydrochloride. This was done by neutralizing it with sodium bicarbonate, followed by extraction with ethyl acetate and evaporation. Then, NH_2-(4F)Phe-COOMe was

added to the reaction mixture. Immidiately after that, 915 mg (2.5 mmol) DCC and 338 mg (2.5 mmol) HOBt were added. After 48h of continuous stirring the DCM was evaporated and ethyl acetate was added while dicyclohexyl urea (DCU) was filtered off. The organic layer was washed with water, extracted, dried over anhydrous sodium sulfate and evaporated under vacuum. The product was purified by silica gel (100–200 mesh) using n-hexane–ethyl acetate (3 : 1) as an eluent.

Yield: 825 mg (1.29 mmol, 64.5%)

^{1}H NMR ($CDCl_3$, 500 MHz, δ_{ppm}): 7.77-6.88 [m, 13H, aromatic proton Fmoc and 4F Phe], 6.62-6.55 [m, 3H, aromatic protons DOPA], 6.22 [b, 1H, NH 4F Phe], 5.27 [b, 1H, NH DOPA], 4.79-4.75 [m, 1H, CαH DOPA], 4.53-4.46[m, 1H, CαH 4F Phe], 4.37-4.33 [m, 1H, CαH Fmoc], 4.16-4.06 [m, 2H, CβH Fmoc], 3.66 [s, 3H, OMe], 3.09-2.90 [m, 4H, CβH DOPA & 4F Phe], 1.63 [s, 6H, 2x COMe].

MALDI-TOF [matrix:α-cyano-4-hydroxy cinnamic acid (CHCA)]: m/z = $[M+Na+2H]^+$ 663.23 (calculated), 663.22(observed), $[M+K+2H]^+$ 679.20 (calculated), 679.53 (observed).

D2. NH_2-DOPA-(4F) Phe-COOMe 4:

The Fmoc group of Fmoc-DOPA(ac)-(4F)Phe-COOMe was deprotected with 20% piperidine in DMF, Lyophilized, and purified. Finally the product was treated with 95% TFA in water for 5h, evaporated all the solvents, washed with cooled diethyl ether, finally the product was lyophilized to get white powder of compound **4**.

^{1}H NMR (DMSO-d_6, 400 MHz, δ_{ppm}): 8.92 & 8.81 [s, 2H, 2× OH], 8.02 [b, 2H, free NH_2], 7.27-7.09 [m, 4H, aromatic proton Phe], 6.67-6.48 [m, 3H, aromatic protons DOPA], 4.58-4.52 [m, 1H, CαH DOPA], 3.90-3.86 [b, 1H, CαH Phe], 3.61 [s, 3H, OMe], 3.08-2.67 [m, 4H, CβH DOPA & Phe]. ^{13}C NMR(DMSO-d_6, 100 MHz, δ_{ppm}): 171.4, 168.7, 162.7, 160.4, 158.5, 145.6, 145.0, 133.3, 133.2, 131.4, 125.6, 120.6, 117.2, 115.9, 115.5, 115.3, 54.1, 53.9, 52.4, 41.0, 36.8, 36.2, 23.6.]. ^{19}F NMR(DMSO-d_6, 470 MHz, δ_{ppm}): -(116.25-116.29).

MALDI-TOF [matrix:α-cyano-4-hydroxy cinnamic acid (CHCA)] :m/z = [M+H] 377.15 (calculated), 377.25 (observed), $[M+Na]^+$ 399.13 (calculated), 399.24(observed).

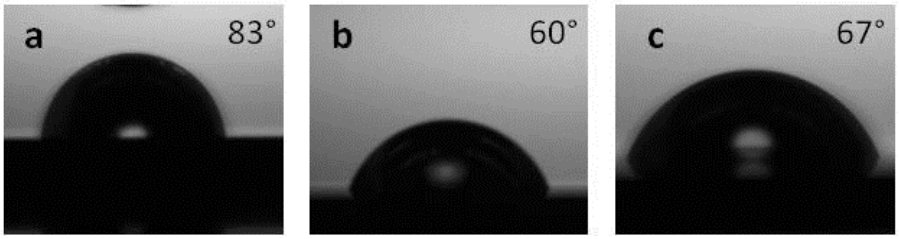


Figure S1. Contact angle measurements of titanium surfaces after dip coating in a solution containing peptide (**a**) **2**, (**b**) **3**, and (**c**) **4**. Peptide concentration was 0.5 mg/mL in methanol. The incubation time was 10h.

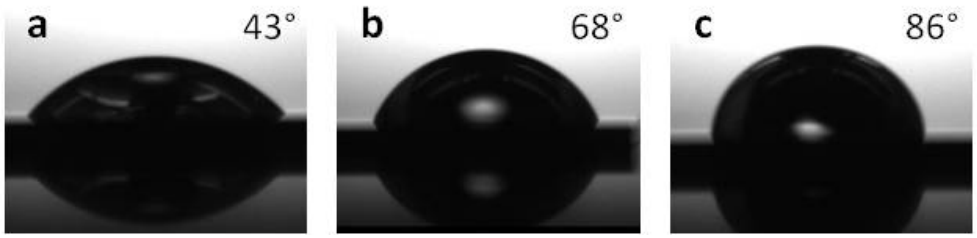


Figure S2. An increase in peptide concentration leads to an increase in the hydrophobicity of the surface. Contact angle measurements of (a) a bare titanium surface (b) a titanium substrate after dip coating in peptide **1** for 10h at a concentration of 0.5 mg/mL in methanol (c) a titanium substrate after dip coating for 10h in peptide **1** at a concentration of 1.0 mg/mL in methanol.

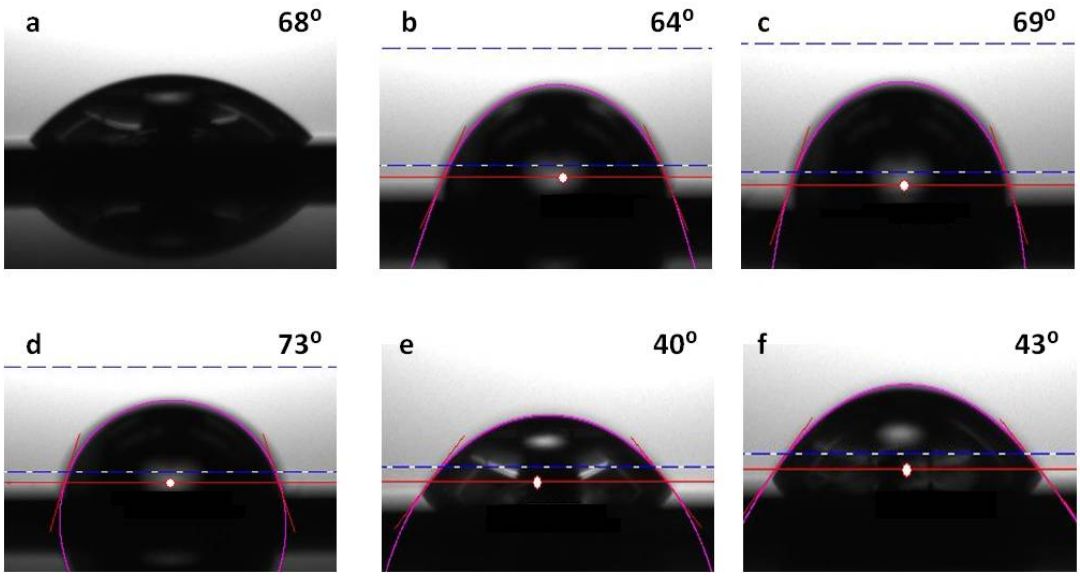


Figure S3. Contact angle measurements of a titanium substrate after dip coating in peptide **1** solution for 10h. The peptide was dissolved in (**a**) methanol (**b**) ethanol (**c**) isopropanol (**d**) acetone (**e**) dimethyl sulphoxide (DMSO) and (**f**) 1,1,1,3,3,3-hexafluoro-2-propanol (HFP).

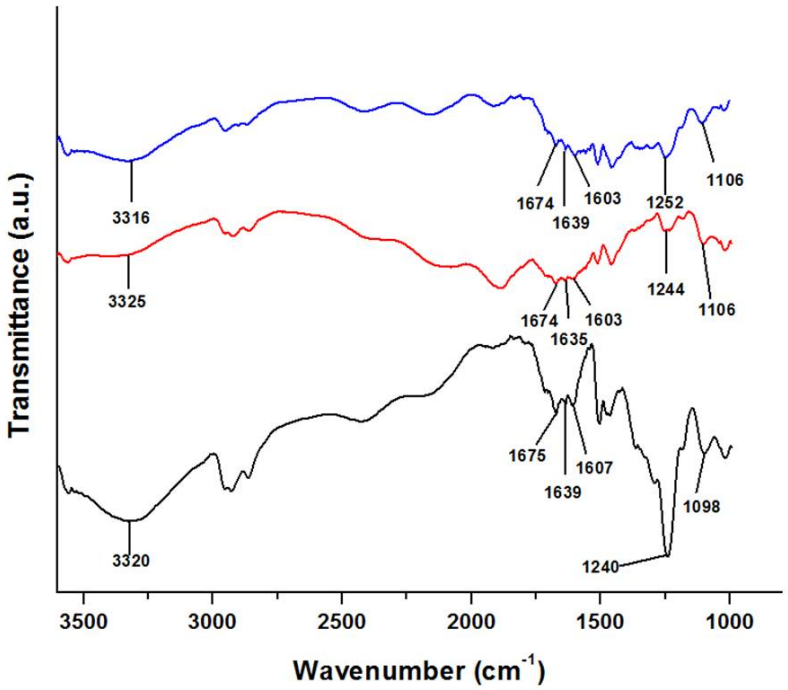


Figure S4. ATR-FTIR spectra of titanium substrates after dip coating in peptide **1** for 10h. The peptide was dissolved in acetone (black), ethanol (red) and isopropanol (blue).

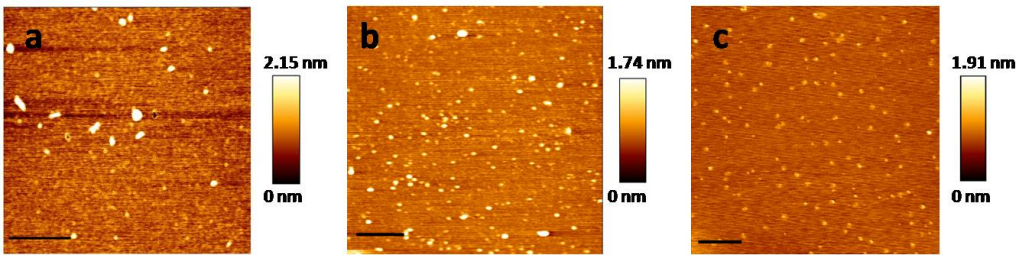


Figure S5. AFM topography images of a mica substrate modified with (a) peptide **2** (b) peptide **3** and (c) peptide **4**. The scale bars represent 500 nm.

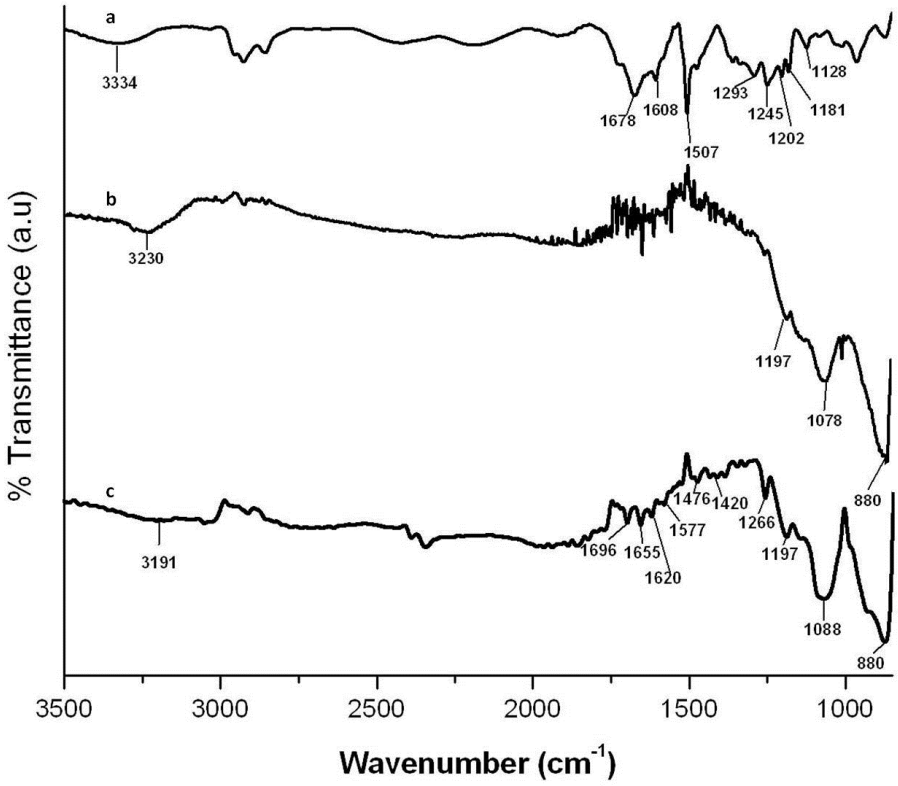


Figure S6. ATR-FTIR spectra of titanium substrates after dip coating with (**a**) peptide **2** (**b**) peptide **3** (**c**) peptide **4**.

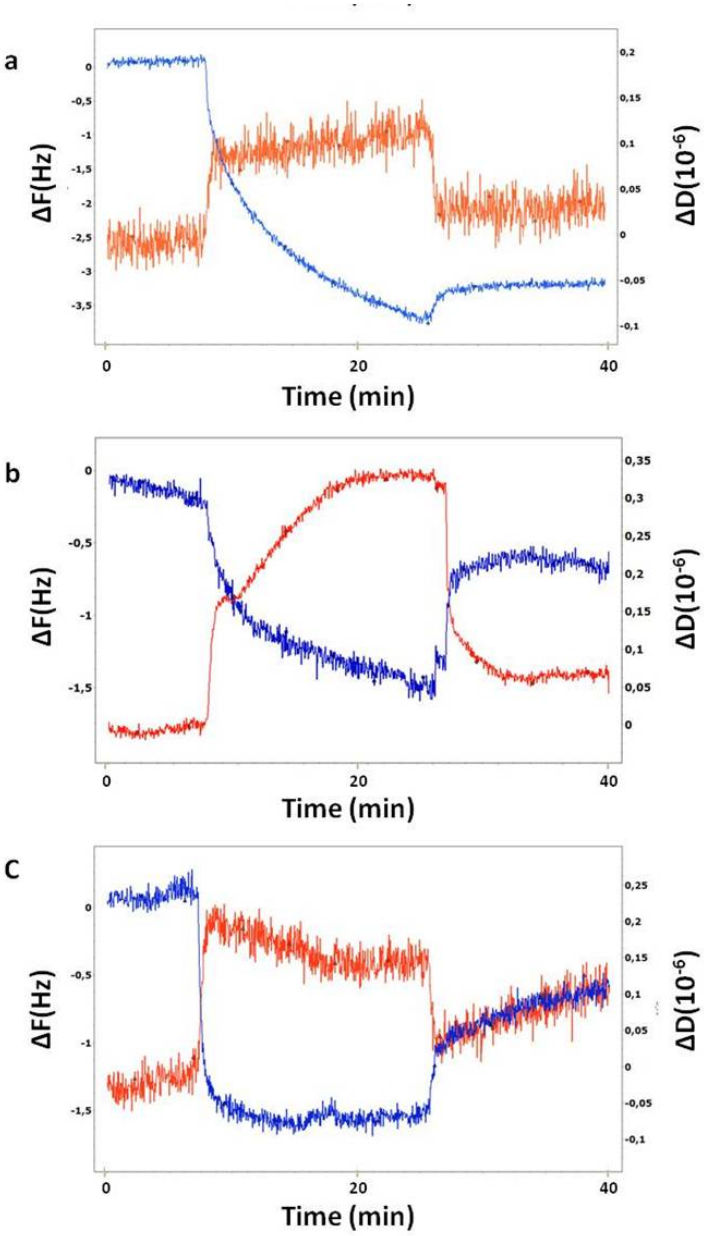


Figure S7. Real-time QCM-D measurements of peptide (**a**) **2**, (**b**) **3** and (**c**) **4** adsorption to a titanum sensor. Frequency overtone 5 presented in blue. Dissipation overtone 5 is presented in red.

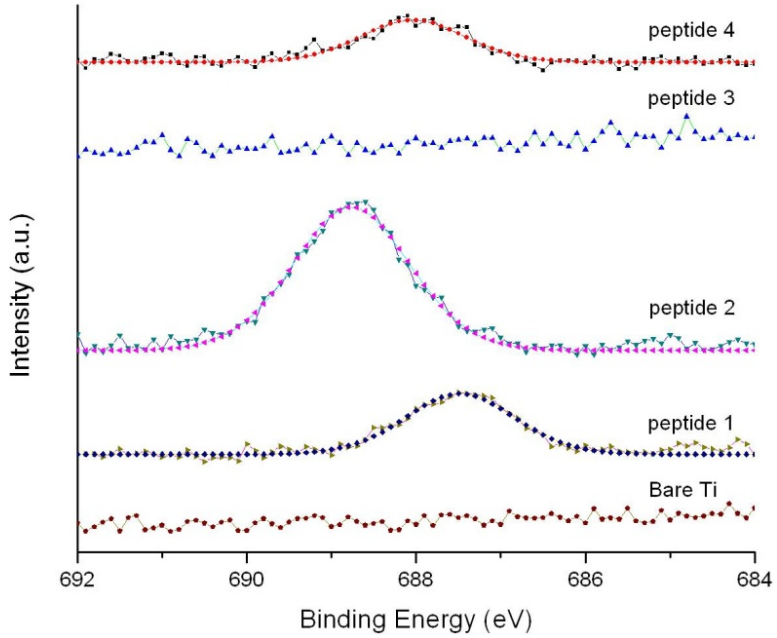


Figure S8. The fluorine signal obtained from XPS analysis of a bare titanium substrate, and substrates after dip coating with peptides **1-4**.

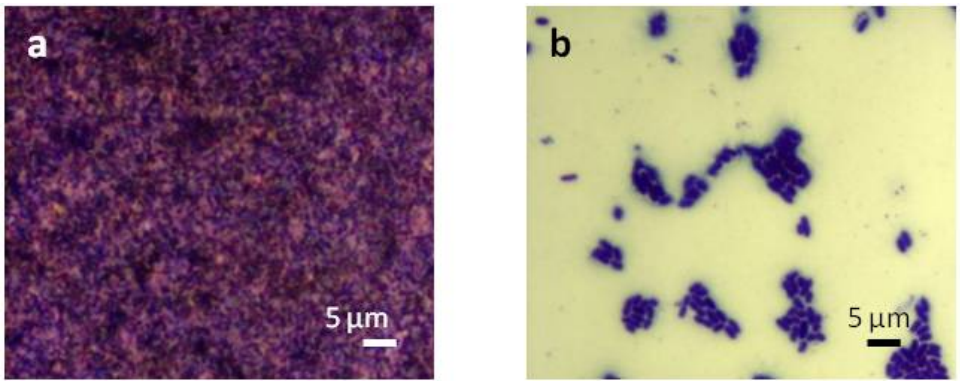


Figure S9. Optical microscopy micrographs of (a) a bare titanium substrate and (b) a titanium substrate coated with peptide 1 after incubation with *P.aeruginosa* for 9 hours and staining with crystal violet.

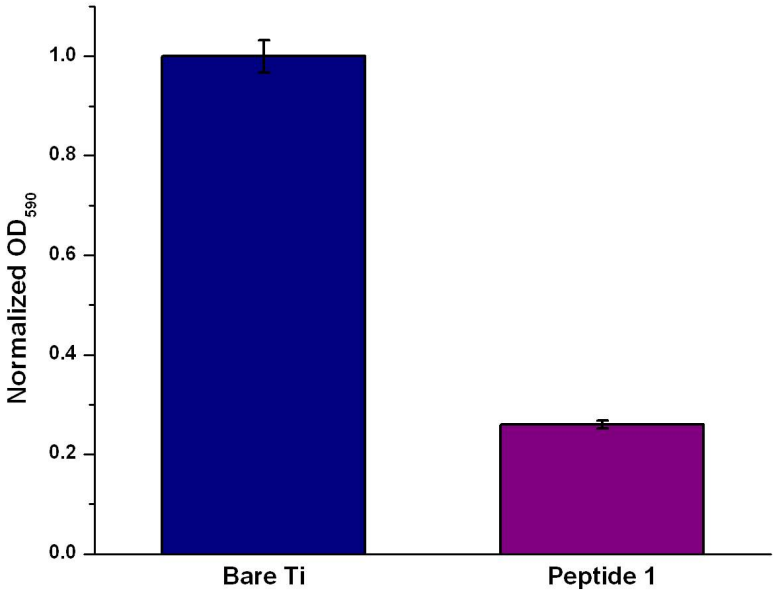


Figure S10. Optical density quantification of the accumulated bacteria, *E.coli*, on a bare titanium substrate and a substrate coated with peptide **1**. Error bars represent standard deviations (n=9)

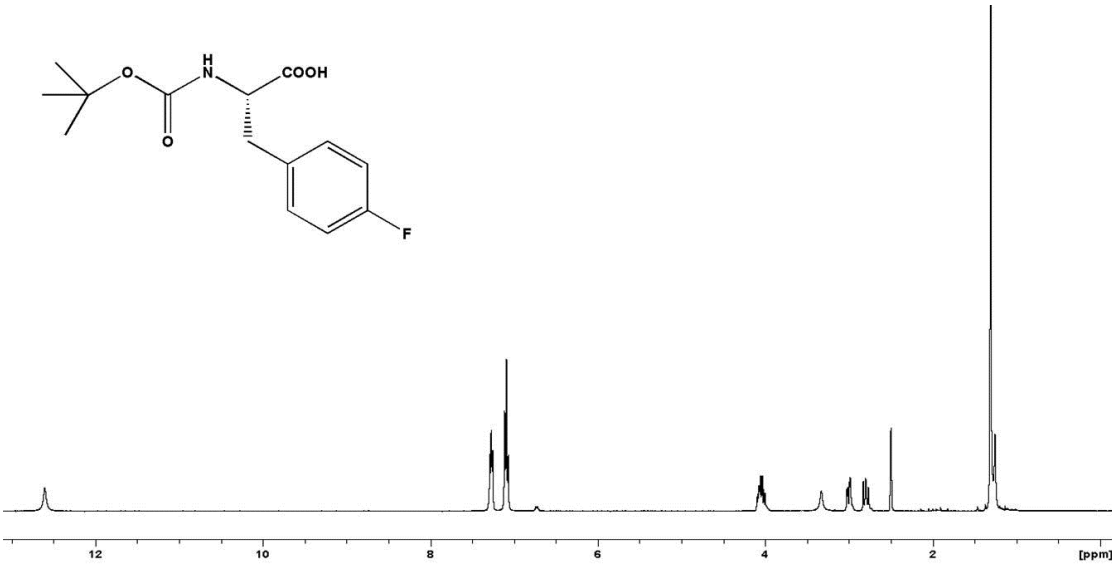


Figure S11. ^{1}H NMR (DMSO-d_6, 400 MHz, δ_{ppm}) Boc-(4F)Phe-COOH **5a**.

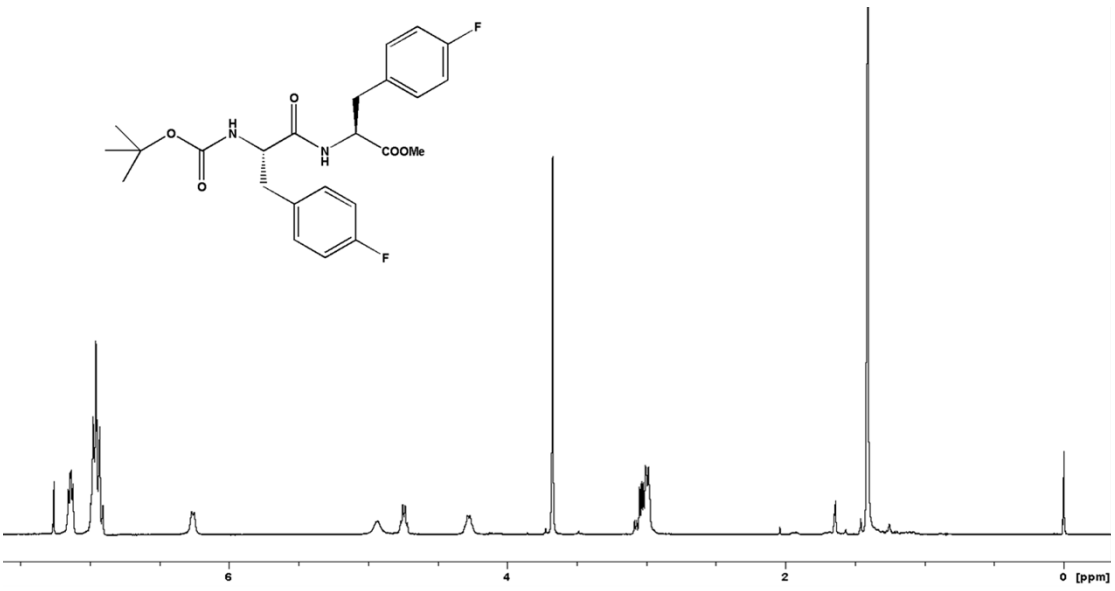


Figure S12. ^{1}H NMR ($CDCl_3$, 400 MHz, δ_{ppm}) of Boc-(4F)Phe(1)- (4F)Phe(2)-COOMe **6a**.

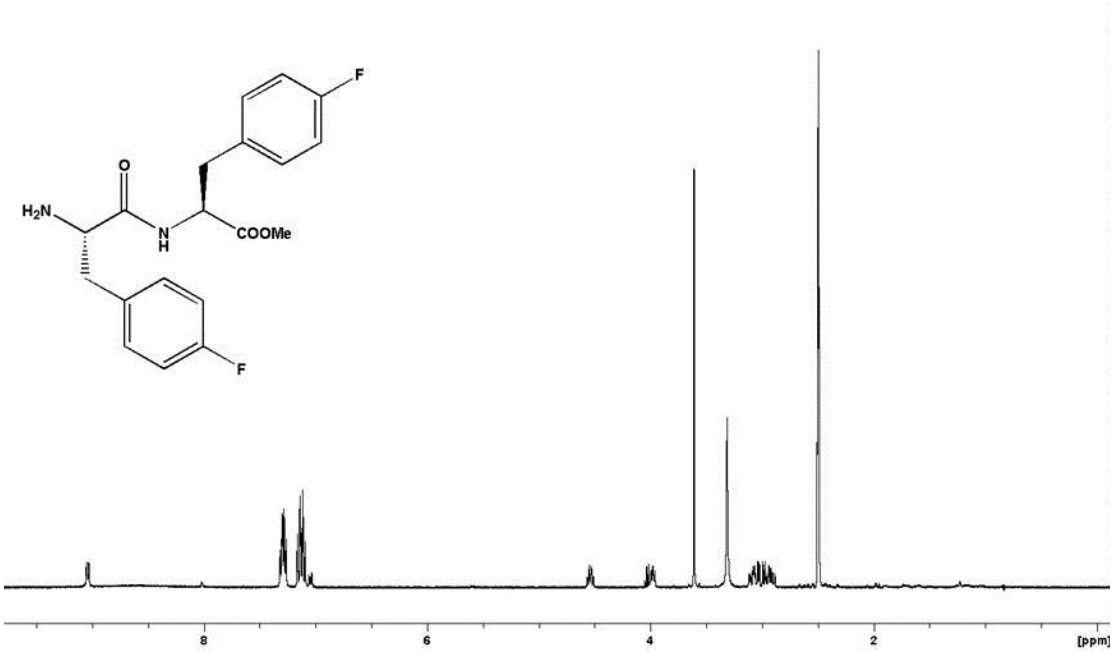


Figure 13. ^{1}H NMR (DMSO-d_6, 400 MHz, δ_{ppm}) of NH_2-(4F)Phe(1)- (4F)Phe(2)-COOMe **7a**.

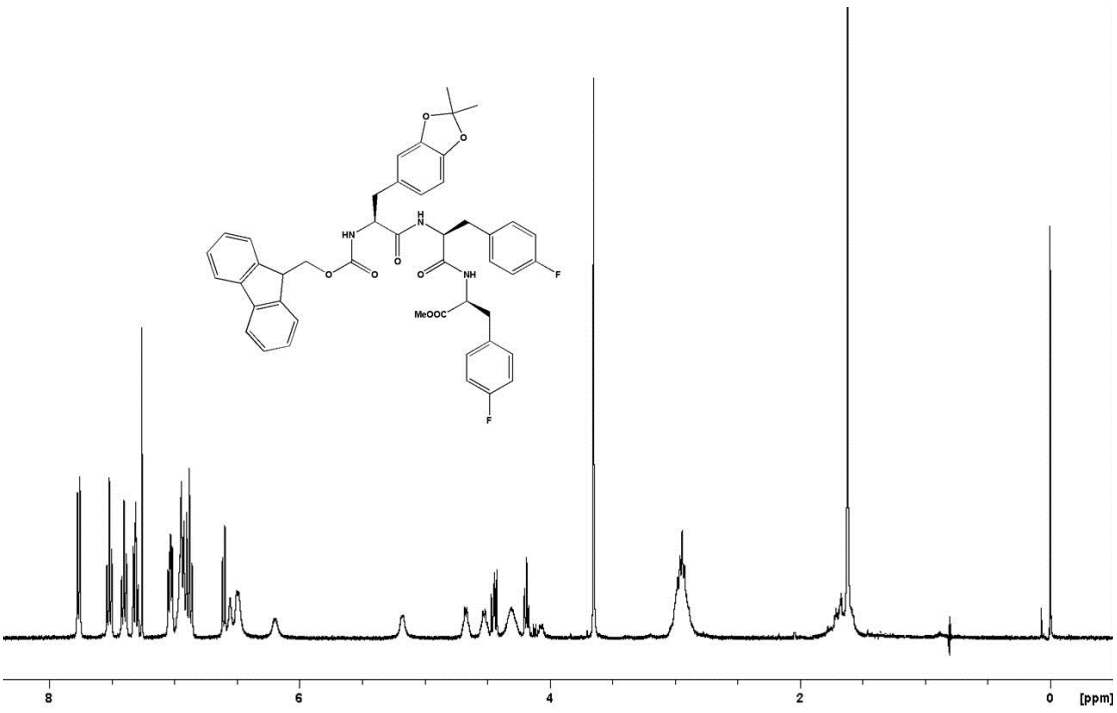


Figure S14. ^{1}H NMR ($CDCl_3$, 400 MHz, δ_{ppm}) of Fmoc-DOPA(ac)-(4F)Phe(1)- (4F)Phe(2)-COOMe **8a**.

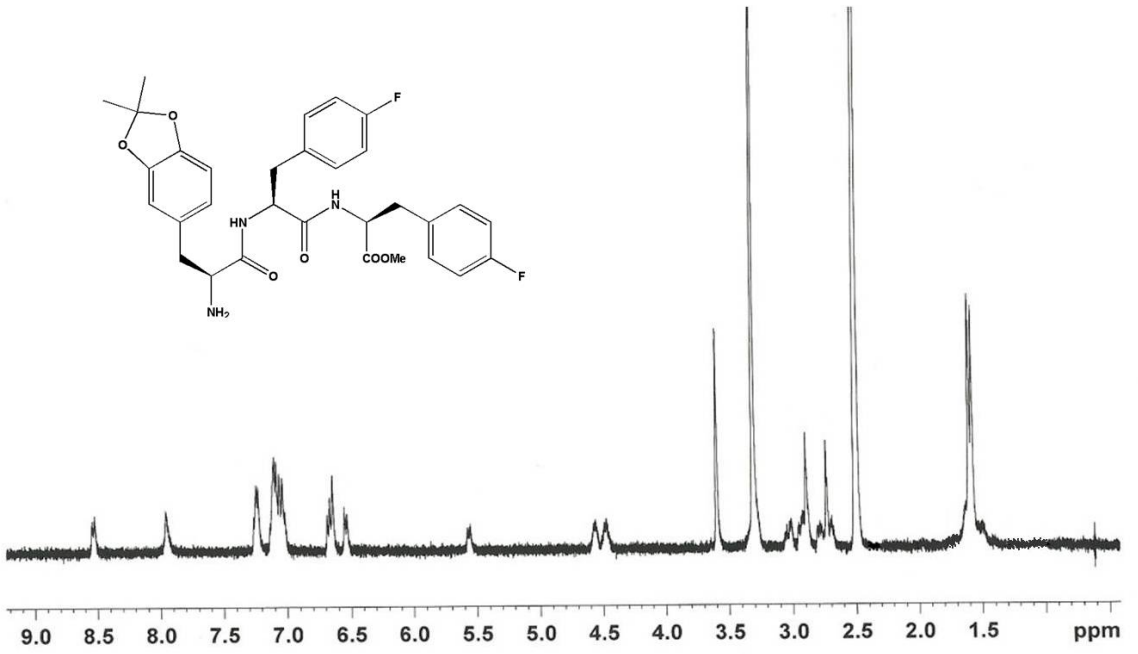


Figure S15. ^{1}H NMR (DMSO-d_6, 400 MHz, δ_{ppm}) of NH_2-DOPA(ac)-(4F)Phe(1)-(4F)Phe(2)-COOMe **9a**.

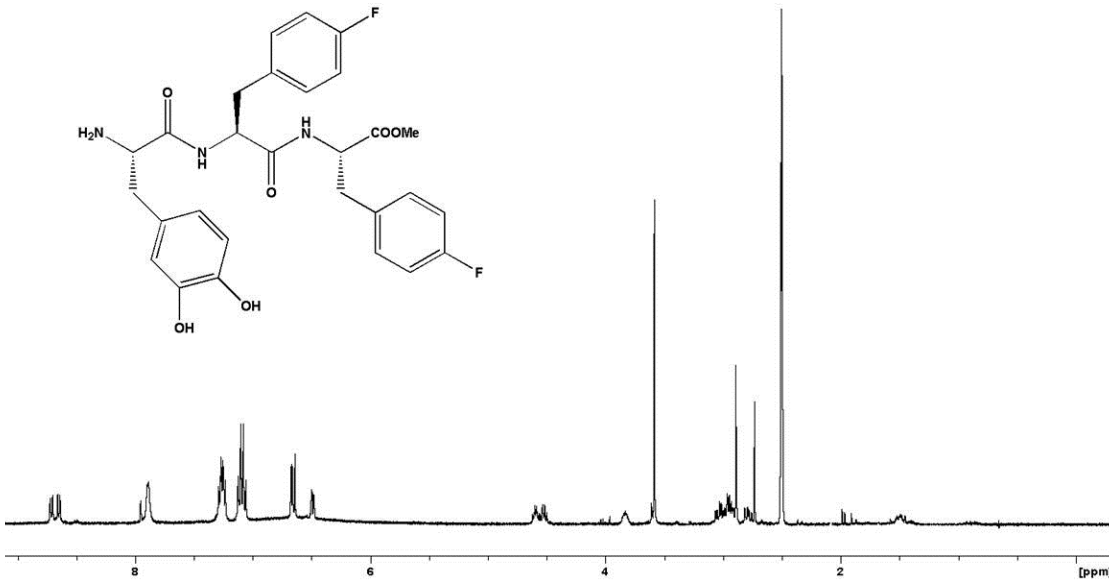


Figure S16. ^{1}H NMR (DMSO-d_6, 400 MHz, δ_{ppm}) of NH_2-DOPA-(4F)Phe(1)- (4F)Phe(2)-COOMe **1**.

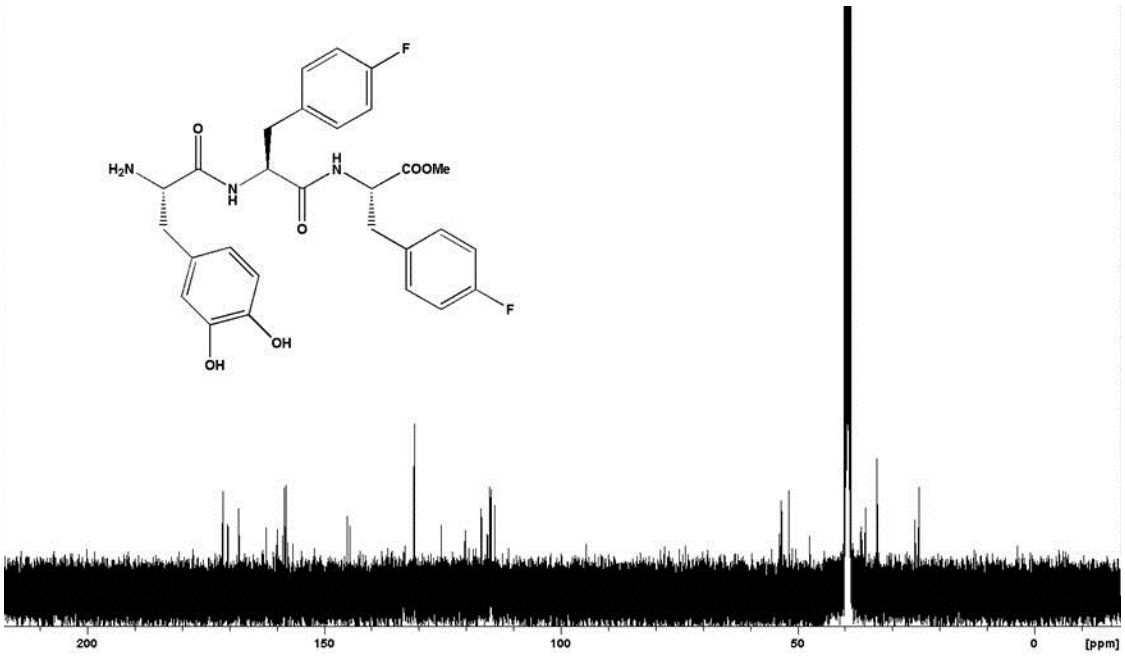


Figure S17. ^{13}C NMR (DMSO-d_6, 100 MHz, δ_{ppm}) of NH_2-DOPA-(4F)Phe(1)- (4F)Phe(2)-COOMe **1**.

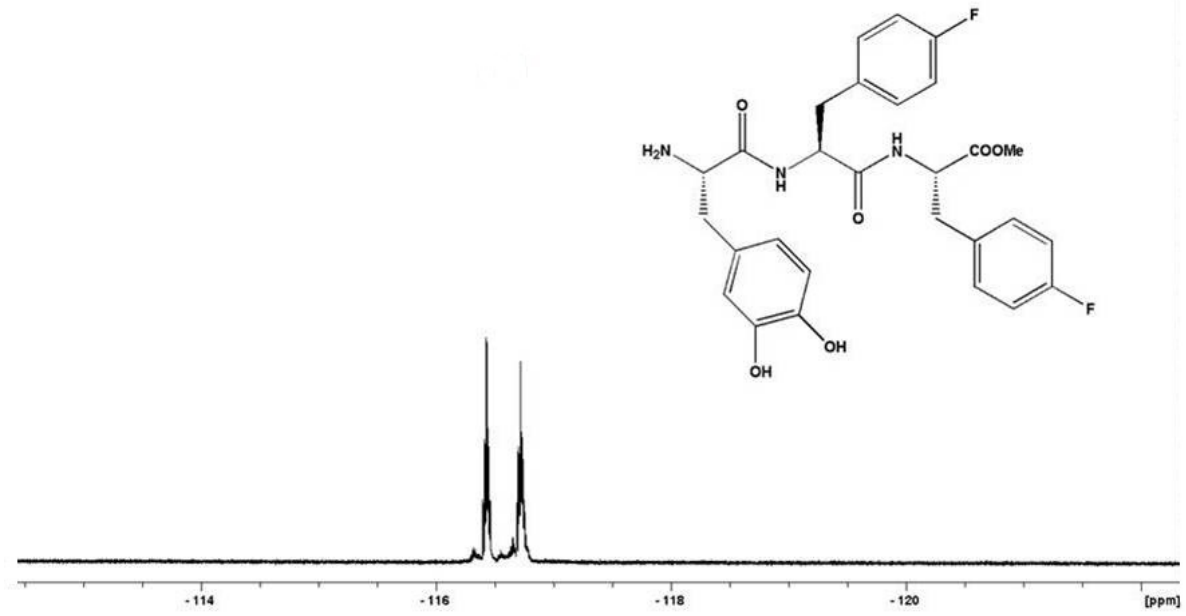


Figure S18. ^{19}F NMR (DMSO-d_6, 470 MHz, δ_{ppm}) of NH_2-DOPA-(4F)Phe(1)- (4F)Phe(2)-COOMe **1**.

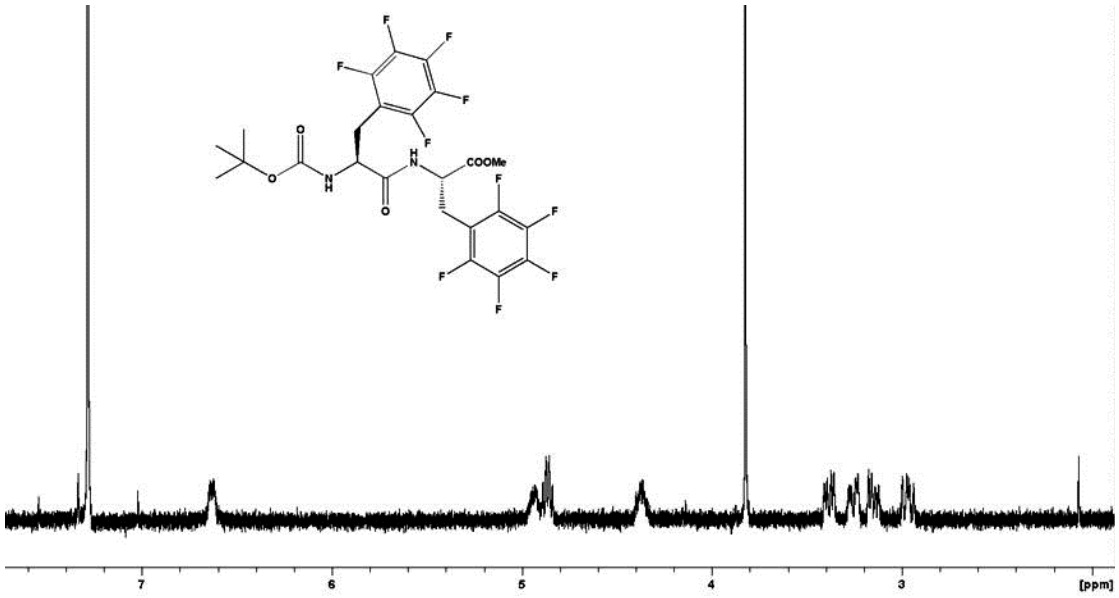


Figure S19. ^{1}H NMR ($CDCl_3$, 400MHz, δ_{ppm}) of Boc-(F_5)Phe(1)- (F_5)Phe(2)-COOMe **6b**.

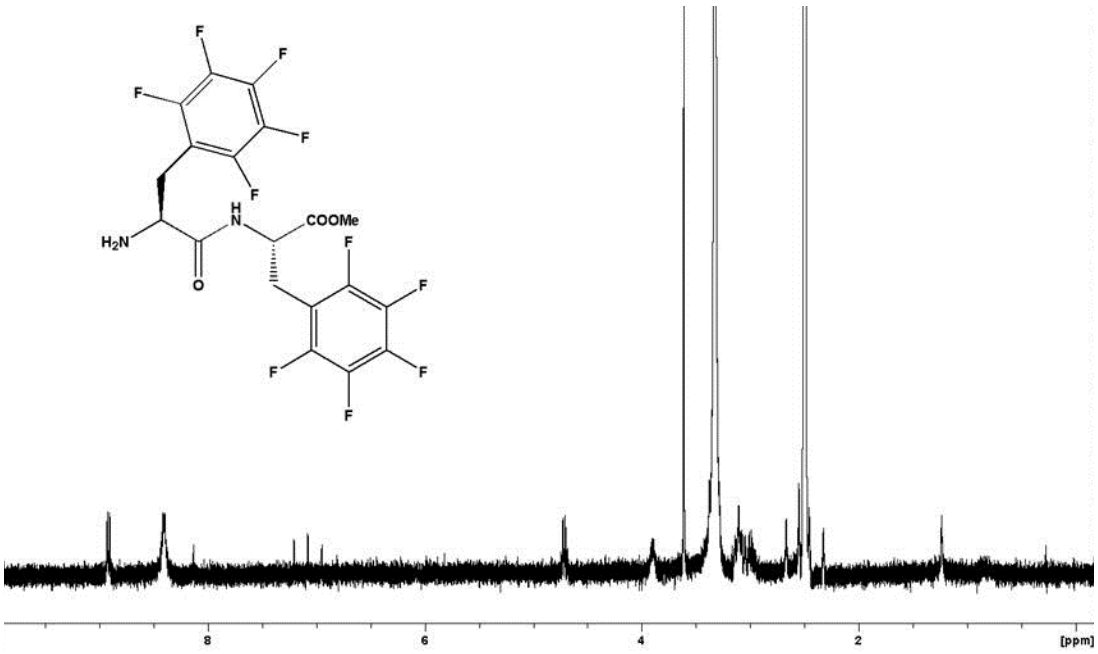


Figure S20. ^{1}H NMR (DMSO -d_6, 400MHz, δ_{ppm}) of NH_2-(F_5)Phe(1)- (F_5)Phe(2)-COOMe **7b.**

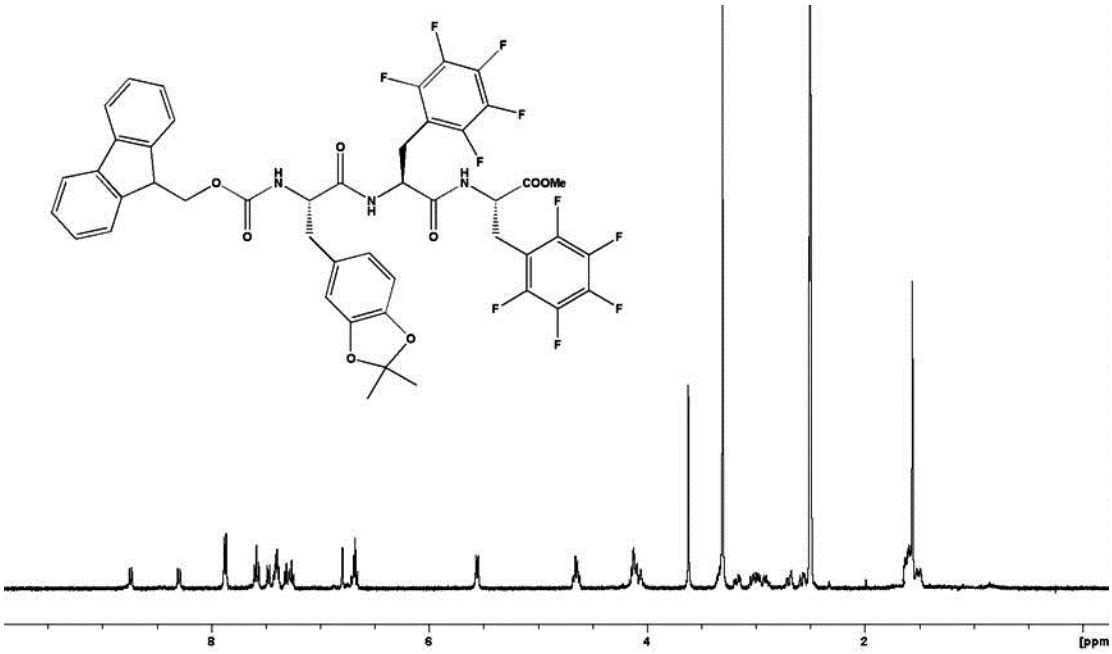


Figure S21. ^{1}H NMR (DMSO- d_6, 400MHz, δ_{ppm}) of Fmoc-DOPA(ac)-(F_5)Phe(1)-(F_5)Phe(2)-COOMe **8b.**

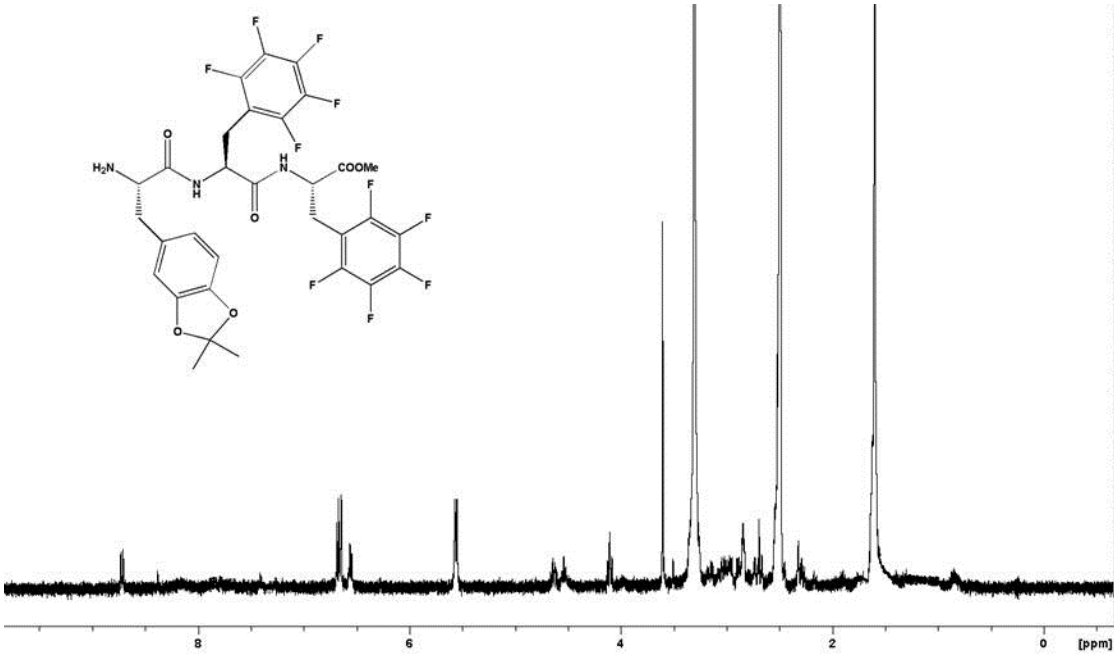


Figure S22. ^{1}H NMR (DMSO -d_6, 400MHz, δ_{ppm}) of NH_2-DOPA(ac)-(F_5)Phe(1)-(F_5)Phe(2)-COOMe **9b.**

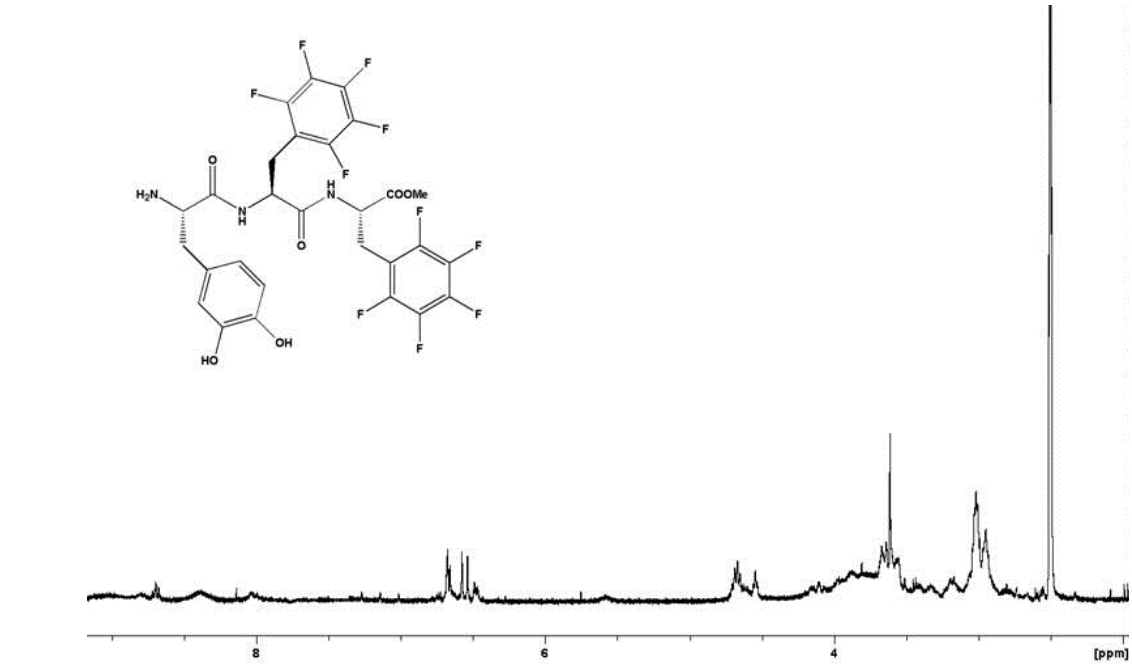


Figure S23. ^{1}H NMR (DMSO -d_6, 400MHz, δ_{ppm}) of NH_2-DOPA-(F_5)Phe(1)- (F_5)Phe(2)-COOMe **2.**

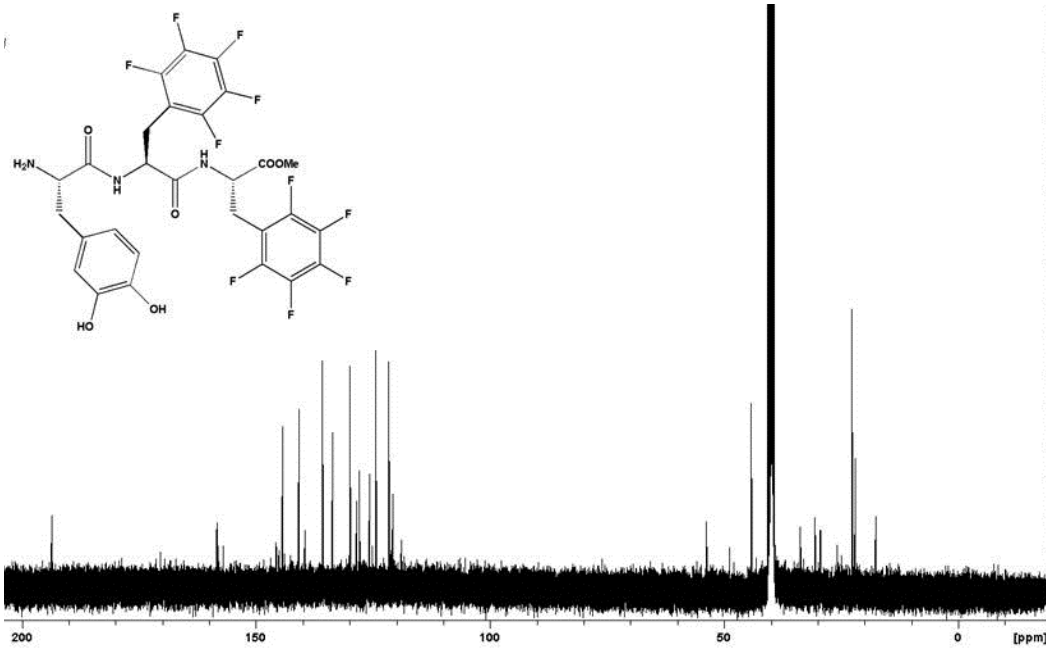


Figure S24. ^{13}C NMR (DMSO -d_6, 400MHz, δ_{ppm}) of NH_2-DOPA-(F_5)Phe(1)- (F_5)Phe(2)-COOMe **2.**

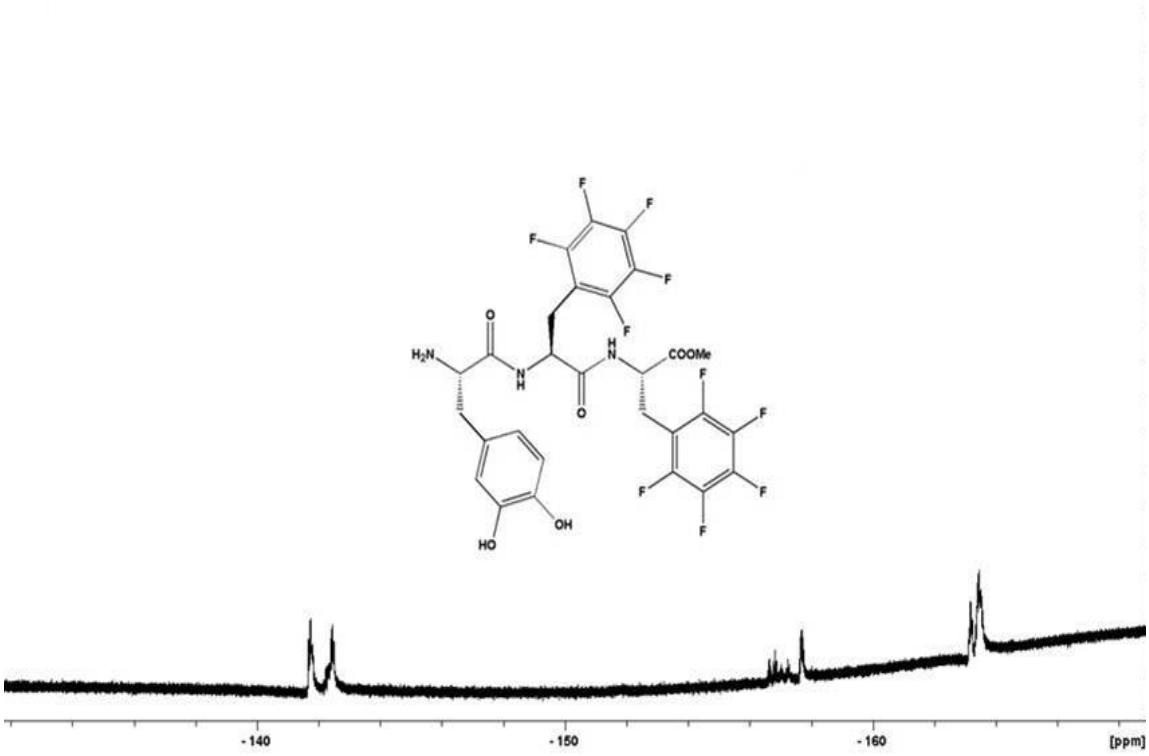


Figure S25. ^{19}F NMR (DMSO -d_6, 470MHz, δ_{ppm}) of NH_2-DOPA-(F_5)Phe(1)- (F_5)Phe(2)-COOMe **2.**

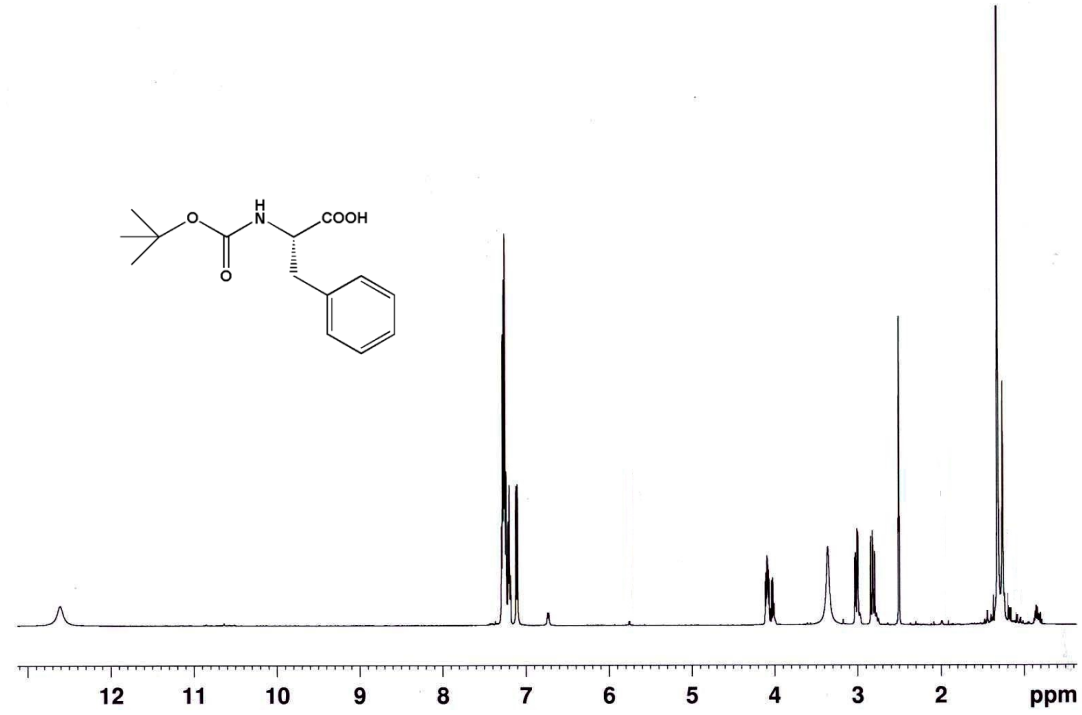


Figure S26: ^1H NMR (DMSO -d_6, 400MHz, δ_{ppm}) of Boc-Phe-OH **5c**.

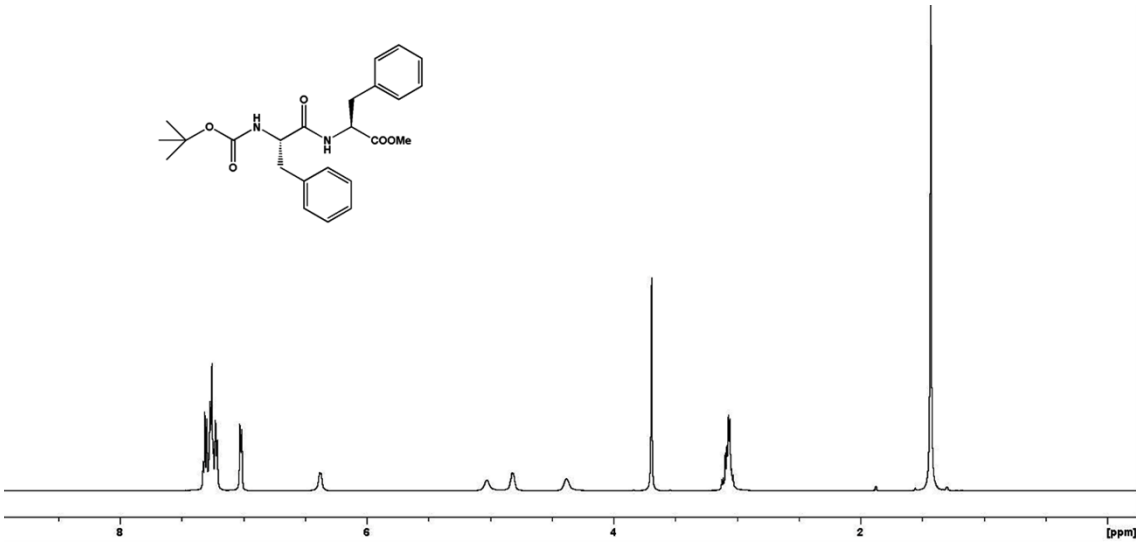


Figure S27: ^{1}H NMR ($CDCl_3$, 400MHz, δ_{ppm}) of Boc-Phe(1)-Phe(2)-COOMe **6c**.

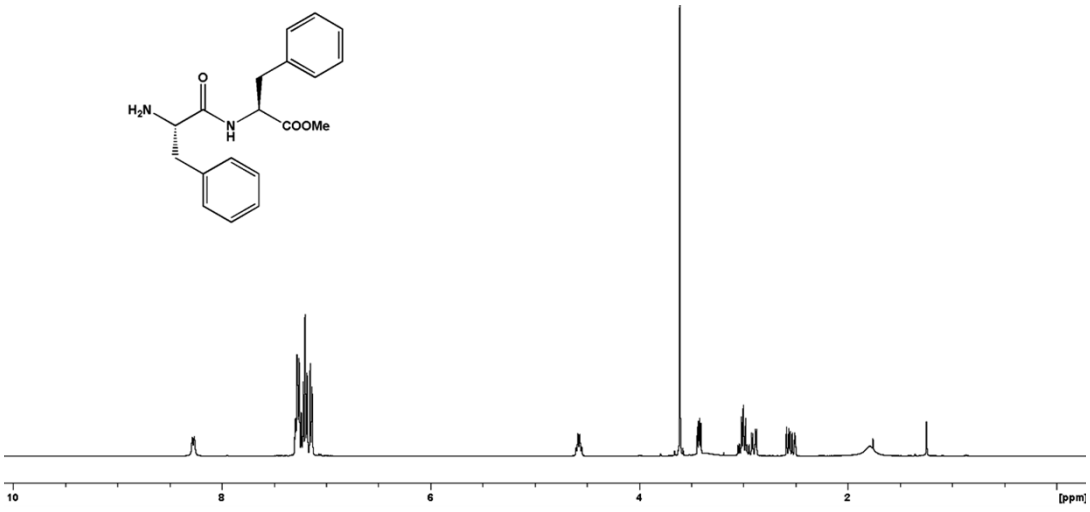


Figure S28: ^{1}H NMR (DMSO-d_6, 400MHz, δ_{ppm}) of NH_2-Phe(1)-Phe(2)-COOMe **7c**.

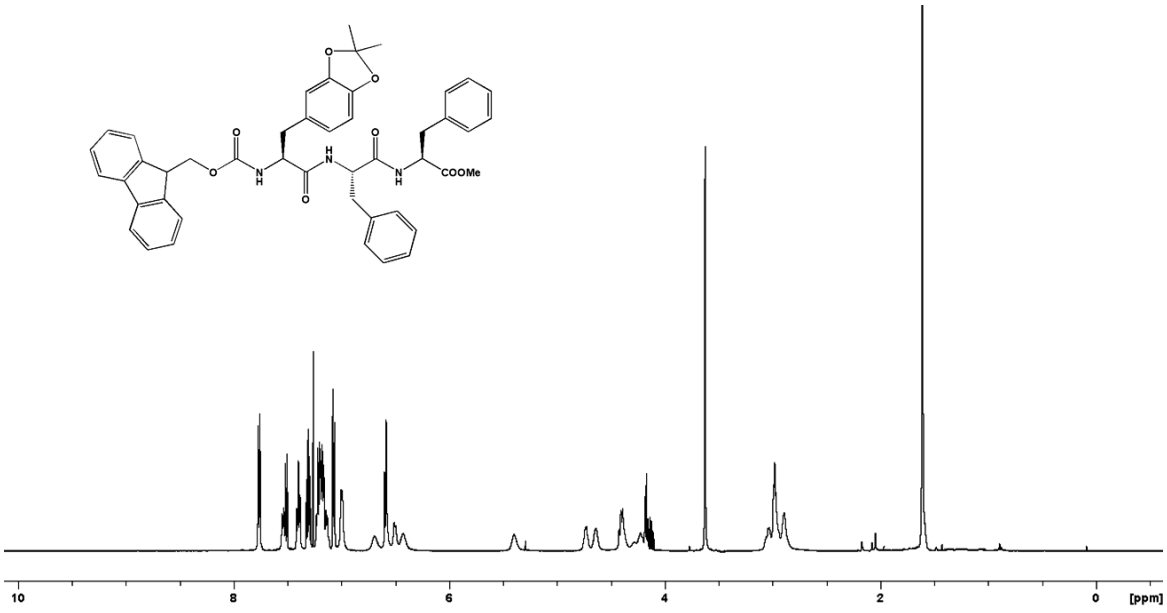


Figure S29: ^{1}H NMR ($CDCl_3$, 400MHz, δ_{ppm}) of Fmoc-DOPA(ac)-Phe(1)-Phe(2)-COOMe **8c**.

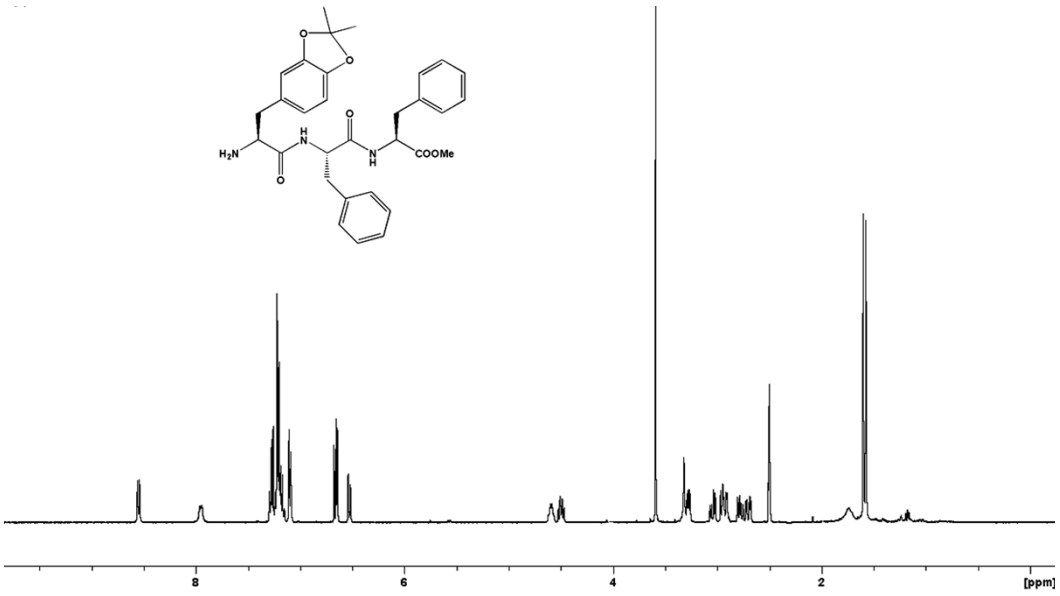


Figure S30: ^{1}H NMR (DMSO-d_6, 400MHz, δ_{ppm}) of NH_2 -DOPA(ac)-Phe(1)-Phe(2)-COOMe **9c**.

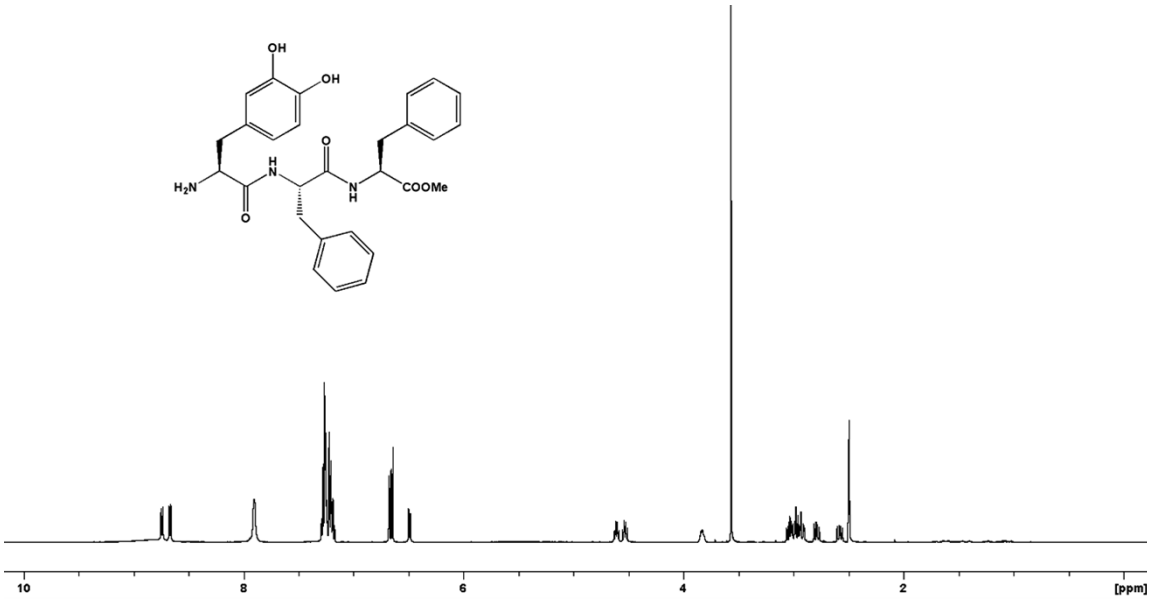


Figure S31: ^{1}H NMR (DMSO-d_6, 500MHz, δ_{ppm}) of NH_2 -DOPA-Phe(1)-Phe(2)-COOMe **3**.

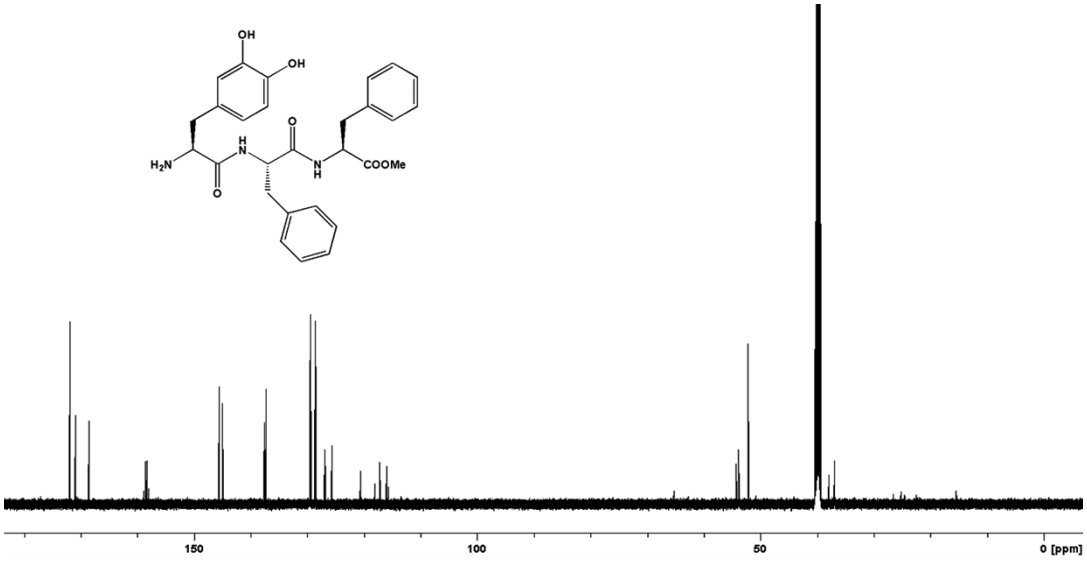


Figure S32: ^{1}H NMR (DMSO-d_6, 125MHz, δ_{ppm}) of NH_2 -DOPA-Phe(1)-Phe(2)-COOMe **3**.

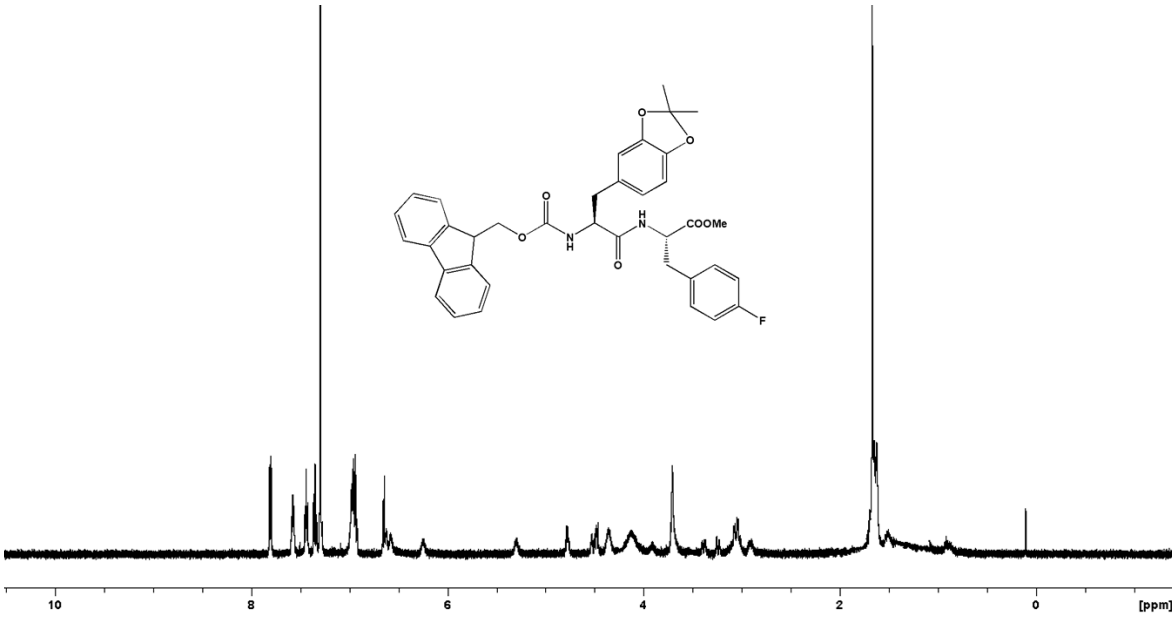


Figure S33: ^{1}H NMR ($CDCl_3$, 500MHz, δ_{ppm}) of Fmoc-DOPA(ac)-(4F)Phe-COOMe.

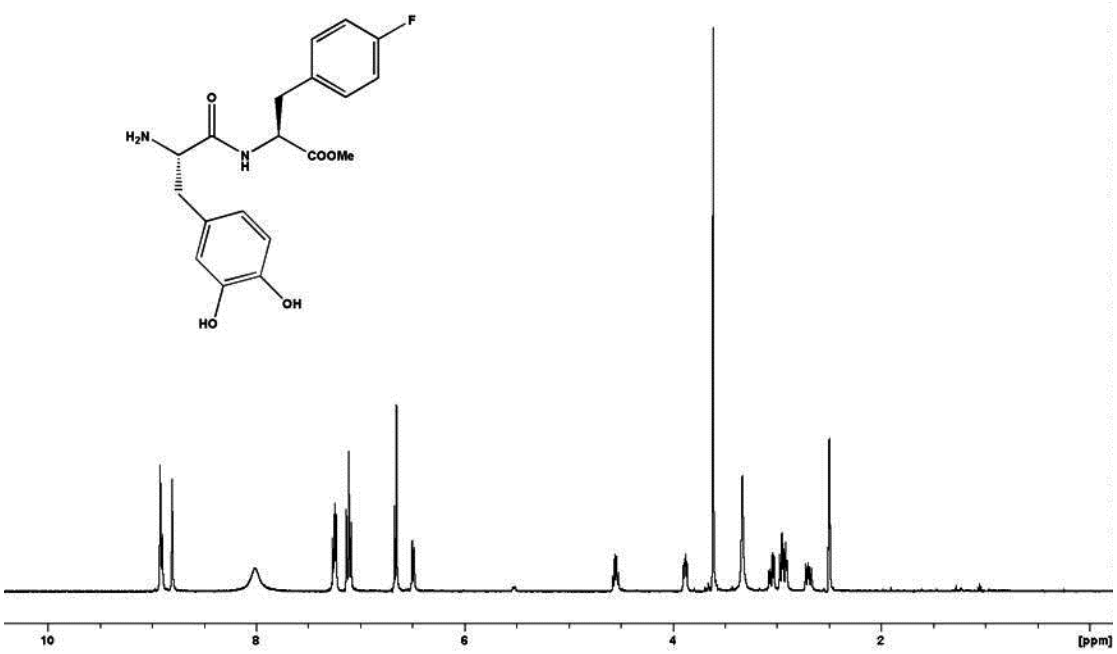


Figure S34. ^{1}H NMR (DMSO-d_6, 400MHz, δ_{ppm}) of NH_2-DOPA-(4F)Phe-COOMe **4**.

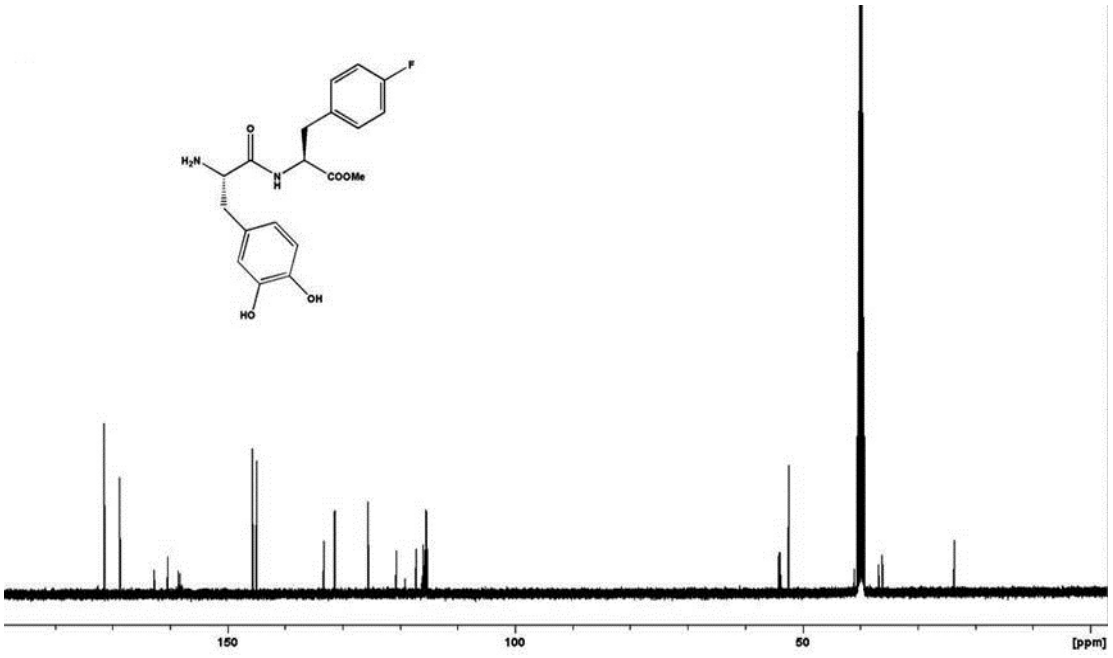


Figure S35. ^{13}C NMR (DMSO-d_6, 400MHz, δ_{ppm}) of NH_2-DOPA-(4F)Phe-COOMe **4**.

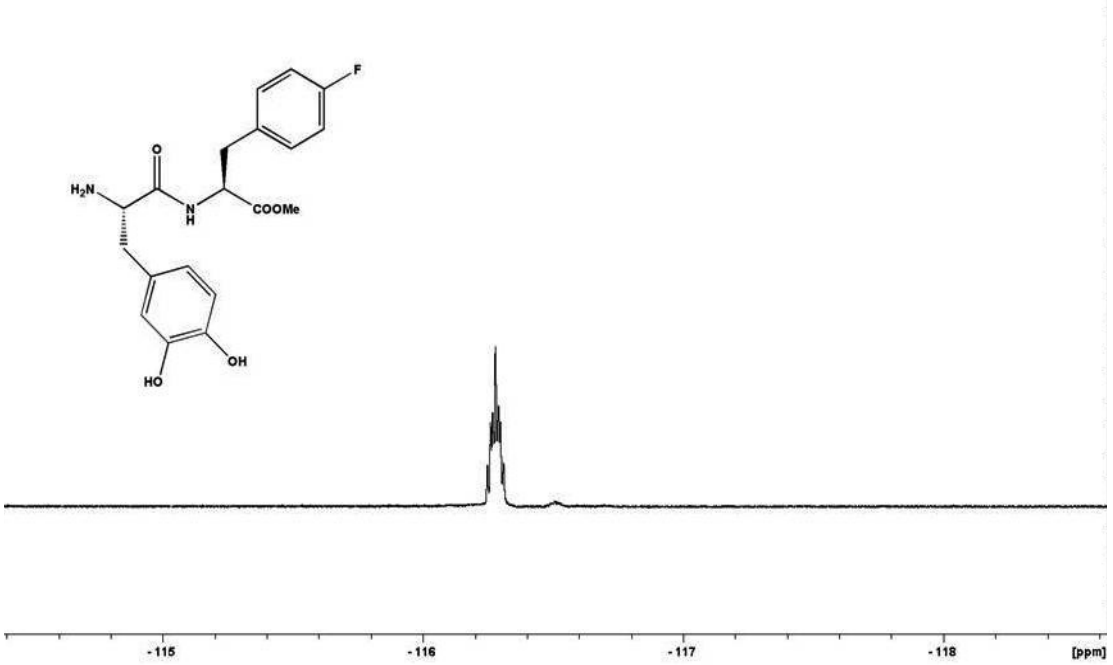


Figure S36. ^{19}F NMR ($CDCl_3$, 470MHz, δ_{ppm}) of NH_2-DOPA-(4F)Phe-COOMe **6**.

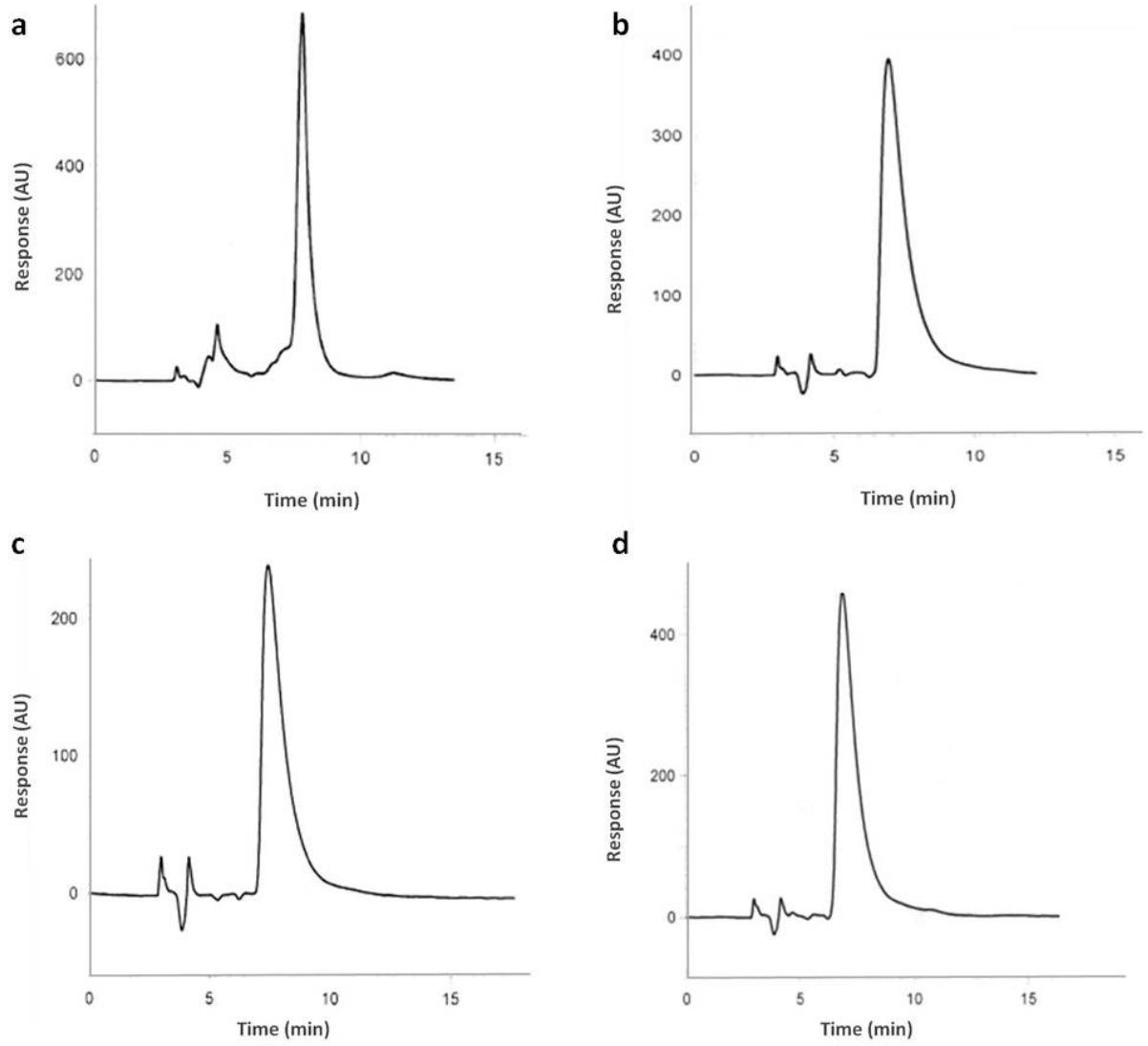


Figure S37. Chiral HPLC chromatograms of peptide (a) **1**, (b) **2**, (c) **3** and (d) **4**. Measurements were performed using 15% ethanol in n-heptane and 0.1% TFA as eluent. The flow rate was 1 ml/min. Prior to each measurement, the instrument was calibrated with Acetyl DL-tryptophan.