Electronic Supplementary Information

Short Peptide Based Hydrogels: Incorporation of Graphene into the Hydrogel

Bimalendu Adhikari and Arindam Banerjee*

Department of Biological Chemistry, Indian Association for the Cultivation of Science, Jadavpur, Kolkata- 700 032, India

Fax: +91-332473-2805; E-mail: bcab@iacs.res.in

Table of Contents

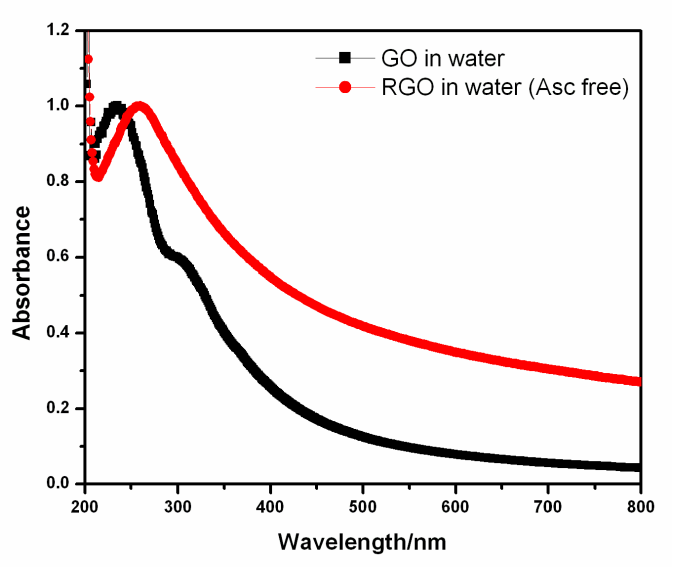

Fig. S1 UV-Vis spectra during the reduction of graphene oxide (GO) to reduced graphene oxide (RGO).

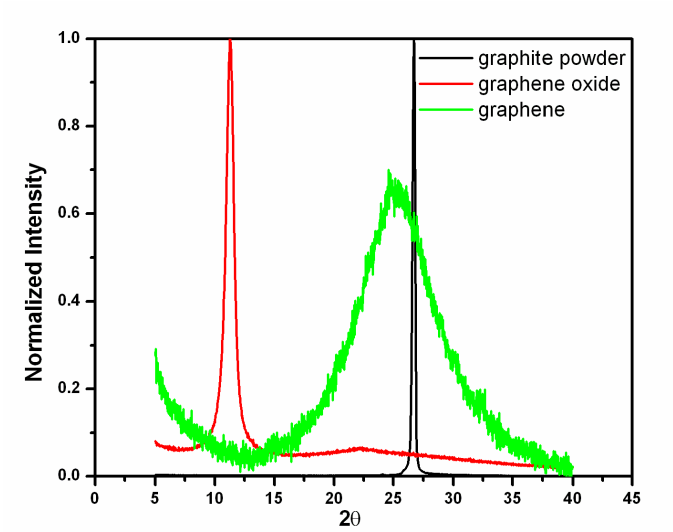

Fig. S2 X-ray diffraction patterns during the preparation of graphite powder to graphene oxide (GO) to graphene (RGO).

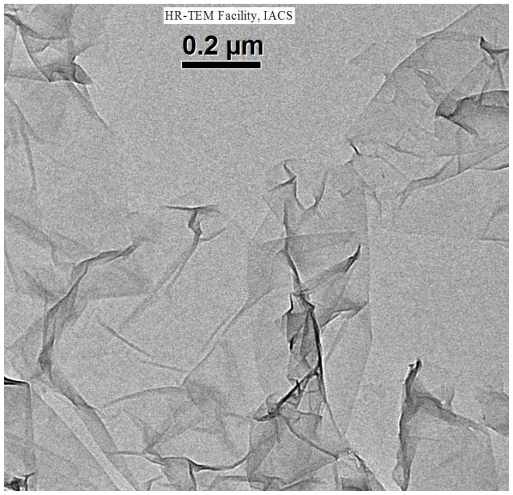

Fig. S3 Transmission electron microscopic image of reduced graphene oxide (RGO) by ascorbic acid.

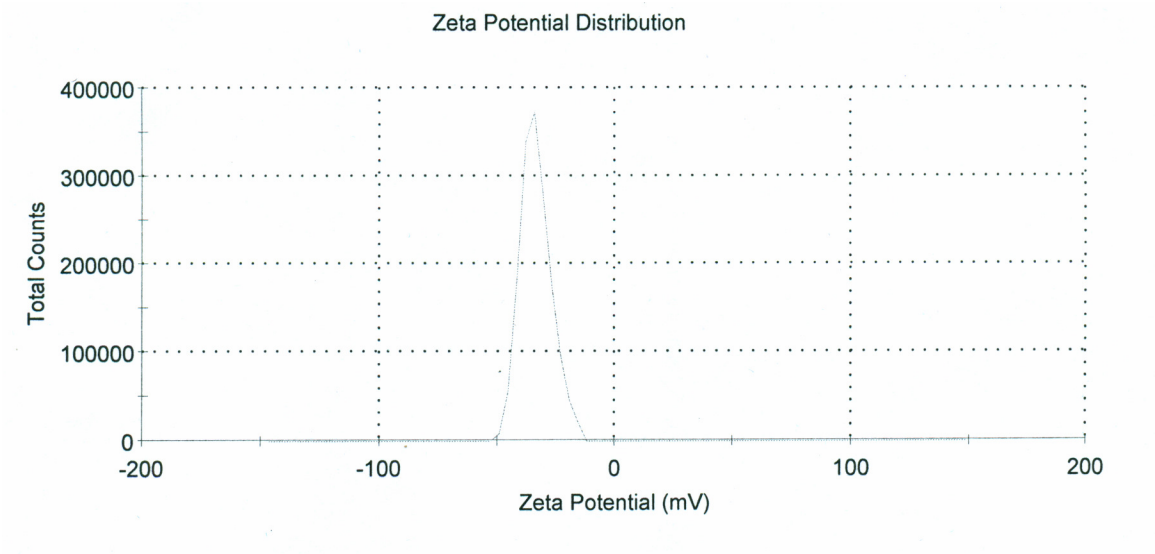

Fig. S4 Zeta potential distribution of RGO dispersed in dilute hybrid hydrogel obtained from peptide 1.

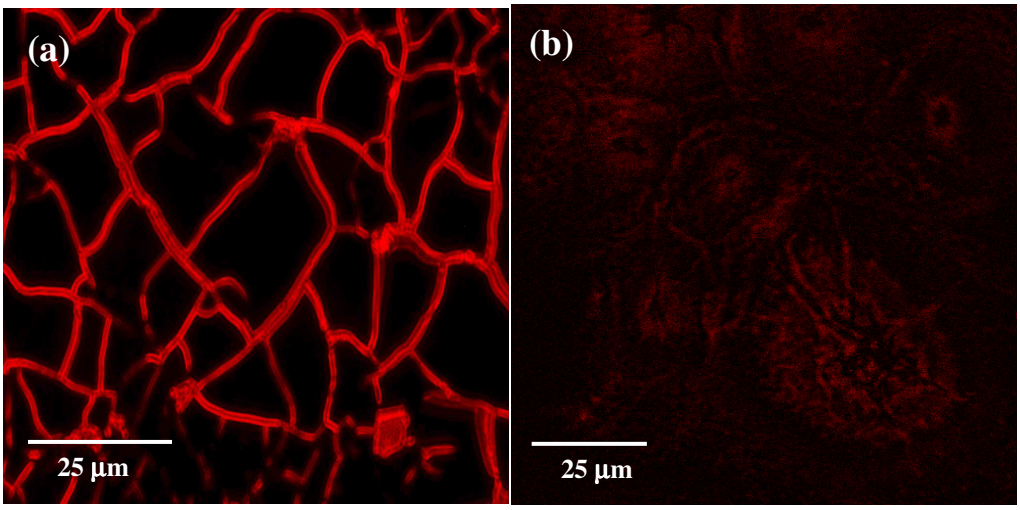

Fig. S5 Confocal microscope images of (a) a wet native hydrogel and (b) hybrid hydrogel stained with a fluorescent dye nile red dye. This hydrogel was obtained from peptide 1.

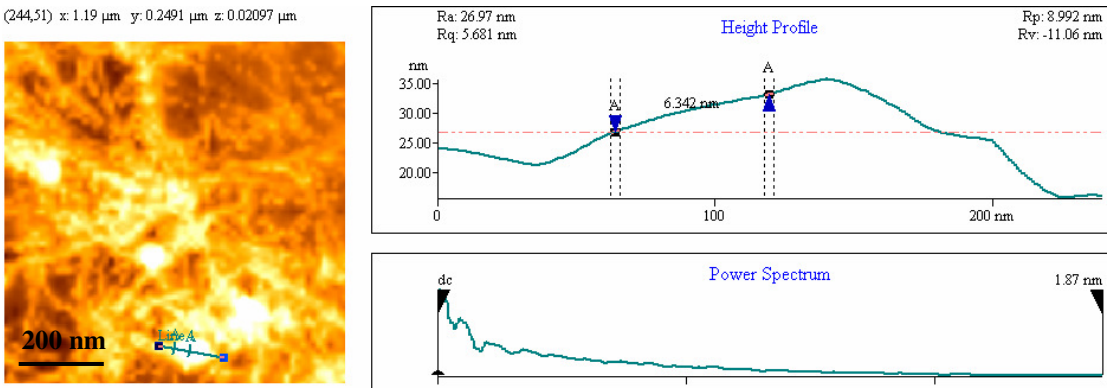

Fig. S6 Atomic force microscopic image and section analysis of RGO containing hydrogel obtained from peptide1. This image shows the presence of gel nanofibers and graphene sheet in a hybrid system. Height profile diagram indicates the presence of a graphene sheet with thickness almost 6 nm in the hybrid system.

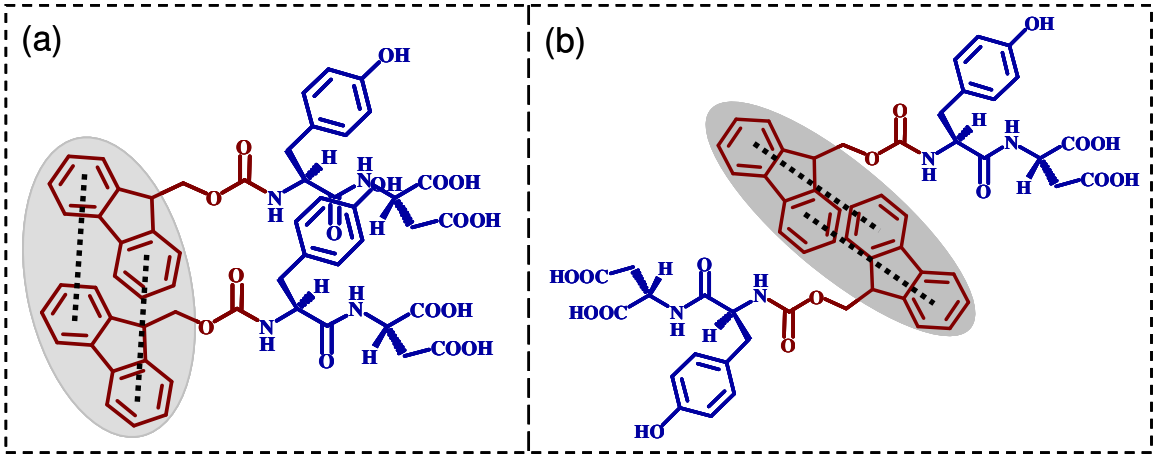

Fig. S7 Pictorial representation of both parallel (a) and antiparallel (b) π–π interactions among the gelator peptides (obtained from peptide 1) in the gel state.

Synthetic Procedure of Peptides:

Synthesis of Peptide 1:

(i) Boc-Tyr-OH: A solution of L-Tyr (3.621 g, 20 mmol) in a mixture of dioxane (40 mL), water (20mL) and 1M NaOH (20 mL) was stirred and cooled in an ice water bath. Di-tert-butyl pyrocarbonate (4.8 g, 22 mmol) was added and stirring was continued at room temperature for 6 h. Then the solution was concentrated in vacuum to about 20–25

mL, cooled in an ice water bath, covered with a layer of ethyl acetate (about 20 mL), and acidified with a dilute solution of $KHSO_4$ to pH 2-3 (congo red). The aqueous phase was extracted with ethyl acetate and this operation was done repeatedly. The ethyl acetate extract were pooled, washed with water, dried over anhydrous Na_2SO_4 and evaporated in vacuum. The pure material was obtained as a waxy solid.

Yield: 5.204 g (18.5 mmol, 92.5%).

(ii) Boc-Tyr-Asp-$(OMe)_2$: 4.782 g (17 mmol) of Boc-Tyr-OH was dissolved in 20 mL of dry DMF in an ice-water bath. H-Asp-$(OMe)_2$ was isolated from 5.76 g (34 mmol) of the corresponding methyl ester hydrochloride by neutralization; subsequent extraction with ethyl acetate and ethyl acetate extract was concentrated to 15 mL. It was then added to the reaction mixture, followed immediately by 3.5 g (17 mmol) of dicyclohexyl carbodiimide (DCC) and 2.30 g (17 mmol) of HOBt. The reaction mixture was allowed to come to room temperature and stirred for 2 days. The residue was taken up in ethyl acetate (40 mL) and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 1 M HCl (3 × 30 mL), brine (2 × 30 mL), 1M sodium carbonate (3 × 30 mL) and brine (2 × 30 mL) respectively. This washed organic solution was dried over anhydrous sodium sulfate and evaporated in vacuum. A white material was obtained.

Yield: 6.069 g (14.30 mmol, 84.12 %).

^{1}H NMR (300 MHz, $CDCl_3$, 25° C, TMS): δ 7.17 (d, ^3J(H, H) = 7.83 Hz, 1H; NH), δ 6.94–6.91 (d, 2H; aromatic H),), δ 6.69–6.66 (d, 2H; aromatic H), δ 5.24 (d, ^3J(H, H) = 7.68 Hz, 1H; NH), δ 4.74 (br, 1H; αCH), δ 4.28 (br, 1H; αCH), δ 3.63 (s, 3H; OCH_3), δ 3.58 (s, 3H; OCH_3), δ 2.92 – 2.73 (m, 4H; β CH_2), δ 1.33 (s, 9H; Boc–CH_3); HRMS: m/z 425.07 $[M+H]^+$, 447.04 $[M+Na]^+$; ^{13}C NMR (75 MHz, $CDCl_3$, 25° C): δ 171.83 (1C of

COOMe), δ 171.21 (1C of COOMe), δ 170.69 (C of CONH), δ 155.62 (C of CONH), δ 155.45 (1C, aromatic C of Tyr), δ 130.22 (1C, aromatic C of Tyr), δ 127.03 (2C, aromatic C of Tyr), δ 115.55 (2C, aromatic C of Tyr), δ 80.26 (1C, tertiary C of Boc), δ 77.58-76.74 (C of $CDCl_3$), δ 52.72 (1C, α C), δ 51.99 (1C, α C), δ 48.95 (1C, C of OCH_3), 48.62 (1C, C of OCH_3), δ 37.26 (1C, C of β CH_2), δ 35.85 (1C, C of β CH_2), δ 28.31 (3C, primary C of Boc).

(iii) Boc-Tyr-Asp-$(OH)_2$: To 5.093 g (12 mmol) of Boc-Tyr-Asp-$(OMe)_2$ were added 40 mL MeOH and 30 mL of 1(M) NaOH and the progress of saponification was monitored by thin-layer chromatography (TLC). The reaction mixture was stirred. After 10 h, methanol was removed under vacuum and the residue was taken in 50 mL of water and washed with diethyl ether (2 × 50 mL). The pH of the aqueous layer was then adjusted to 2-3 using 1(N) HCl, the aqueous layer was extracted with ethyl acetate (2 × 50 mL). The extract were pooled, dried over anhydrous sodium sulfate and evaporated in vacuum. Yield: 4.205 g (10.608 mmol, 88.40 %).

^{1}H NMR (300 MHz, [D_6]DMSO, 25° C): δ 9.15 (br, 1H; Tyr–OH), δ 8.13 (d, ^3J(H, H) = 7.8 Hz, 1H; NH), δ 7.10–6.95 (m, aromatic H, 2H; NH), δ 6.75 (d, ^3J(H, H) = 7.9 Hz, 1H; NH), δ 6.69–6.62 (m, aromatic H, 2H; NH), δ 4.53–4.51 (m, 1H; αCH), δ 4.06–3.99 (m, 1H; α CH), δ 3.45–3.43 (m, 2H; β CH), δ 2.85–2.61 (m, 2H; β CH), δ 1.31 (s, 9H; Boc–CH_3); HRMS: m/z 419.15 $[M+Na]^+$, 433.16 $[M+K]^+$; ^{13}C NMR (75 MHz, [D_6]DMSO, 25° C): δ 172.81 (C of COOH), δ 172.19 (C of COOH), δ 172.11 (C of CONH), δ 156.16 (C of CONH), δ 155.60 (1C, aromatic C of Tyr), δ 130.54 (1C, aromatic C of Tyr), δ 128.59 (2C, aromatic C of Tyr), δ 115.25 (2C, aromatic C of Tyr), δ 78.48 (1C, tertiary C of Boc), δ 60.20 (1C, αC), δ 56.34 (1C, αC), δ 40.75–39.08 (C of

[D_6]DMSO), δ 37.05 (1C, C of β CH_2), δ 36.70 (1C, C of β CH_2), δ 28.31 (3C, primary C of Boc).

(iv) H-Tyr-Asp-$(OH)_2$: To 3.5676 g (9 mmol) of Boc-Tyr-Asp-$(OH)_2$ was added 4 mL of 98% formic acid, and the removal of the Boc group was monitored by TLC. After 8 h, formic acid was removed under a vacuum. The residue was taken in water (20 mL) and washed with diethyl ether (2×30 mL). The pH of the aqueous solution was then adjusted to 7 with 30% aqueous NH_3. The aqueous solution was lyophilized to yield white solid product. Yield: 2.374 g (8.01 mmol, 89 %).

^{1}H NMR (300 MHz, [D_6]DMSO, 25° C): δ 8.36 (1H, NH), δ 8.13 (1H; NH), δ 7.01–6.61 (m, 5H; aromatic H), δ 4.06–3.98 (m, 1H; α CH), δ 3.66–3.52 (m, 1H; α CH), δ 2.95–2.88 (m, H; β CH_2), δ 2.74–2.62 (m, 1H; β CH), δ 2.27–2.15 (m, H; β CH_2), δ 1.96 (m, 1H; β CH); HRMS: m/z 297.05 $[M+H]^+$.

(v) Fmoc-Tyr-Asp-$(OH)_2$: 1.777 g of (6 mmol) of H-Tyr-Asp-$(OH)_2$ was dissolved in a basic sodium carbonate solution (15 mL). It was cooled in an ice-water bath. Cooled solution of Fmoc-Cl (2.31 g) in dioxane (15 mL) was added to it. The reaction mixture was allowed to come to room temperature and it was stirred for 24 hrs. Then the solution was concentrated in vacuum to about 15 mL, cooled in an ice water bath, covered with a layer of ethyl acetate (about 30 mL), and acidified with a dilute HCl to neutral pH. The aqueous phase was extracted with ethyl acetate and this operation was done twice. The ethyl acetate extract were pooled, dried over anhydrous Na_2SO_4 and evaporated in vacuum. A white material was obtained. Yield: 2.83 g (5.45 mmol, 91%); m.p. 135° C.

^{1}H NMR (300 MHz, [D_6]DMSO, 25° C): δ 12.59 (br, 2H; COOH), δ 9.13 (s, 1H; aromatic OH), δ 8.33 (d, ^{3}J(H, H) = 7.8 Hz, 1H; NH), δ 7.87– 7.85 (m, 2H; aromatic CH), δ 7.65– 7.60 (m, 2H; aromatic CH), δ 7.53 (d, ^{3}J(H, H) = 9 Hz, 1H; NH), δ 7.41–7.37 (m, 2H; aromatic CH), δ 7.33– 7.27 (m, 2H; aromatic CH), δ 7.10– 7.08 (m, 2H; aromatic CH of Tyr), δ 6.64– 6.61 (m, 2H; aromatic CH of Tyr), δ 4.57–4.54 (m, 1H; α CH), δ 4.18–4.09 (m, 4H; CH, CH_2 and αCH), δ 3.58–3.55 (m, 2H; β CH_2), δ 2.92–2.87 (m, 1H; β CH_2), δ 2.69–2.56 (m, 1H; β CH_2); HRMS: m/z 541.32 $[M+Na]^+$, 557.26 $[M+K]^+$; ^{13}C NMR (75 MHz, [D_6]DMSO, 25ºC): δ 172.87 (2C, C of COOH), δ 172.21 (C, C of CONH), δ 156.29 (C of CONH), δ), δ 144.37 (C, aromatic C), δ 141.19 (2C, aromatic C), δ 130.72 (2C, aromatic C), δ 128.73 (C, aromatic C), δ 128.17 (2C, aromatic C), δ 127.62 (2C, aromatic C), δ 125.91 (2C, aromatic C), δ 120.61 (2C, aromatic C), δ 120.41 (2C, aromatic C), δ 115.37 (2C, aromatic C), δ 66.20 (C of CH_2), δ 56.87 (C, α C), δ 49.13 (C, αC), δ 47.11 (C of CH), δ 40.88–39.49 (C of [D_6]DMSO), δ 37.29 (C, β C), δ 36.57 (C, β C).

Synthesis of Peptide 2:

(i) Boc-Phe-OH: A solution of L-Phe (3.303 g, 20 mmol) in a mixture of dioxane (40 mL), water (20 mL) and 1M NaOH (20 mL) was stirred and cooled in an ice water bath. Di-tert-butyl pyrocarbonate (4.8 g, 22 mmol) was added and stirring was continued at room temperature for 6 h. Then the solution was concentrated in vacuum to about 20-25 mL, cooled in an ice water bath, covered with a layer of ethyl acetate (about 20 mL), and acidified with a dilute solution of $KHSO_4$ to pH 2-3 (congo red). The aqueous phase was extracted with ethyl acetate and this operation was done repeatedly. The ethyl acetate

extract were pooled, washed with water, dried over anhydrous Na_2SO_4 and evaporated in vacuum. The pure material was obtained as a waxy solid.

Yield: 4.931 g (18.59 mmol, 93%).

(ii) Boc-Phe-Asp-$(OMe)_2$: 4.507 g (17 mmol) of Boc-Phe-OH was dissolved in 20 mL of dry DMF in an ice-water bath. H-Asp-$(OMe)_2$ was isolated from 5.76 g (34 mmol) of the corresponding methyl ester hydrochloride by neutralization; subsequent extraction with ethyl acetate and ethyl acetate extract was concentrated to 15 mL. It was then added to the reaction mixture, followed immediately by 3.5 g (17 mmol) of dicyclohexyl carbodiimide (DCC) and 2.30 g (17 mmol) of HOBt. The reaction mixture was allowed to come to room temperature and stirred for 2 days. The residue was taken up in ethyl acetate (40 mL) and dicyclohexylurea (DCU) was filtered off. The organic layer was washed with 1 M HCl (3 × 30 mL), brine (2 × 30 mL), 1M sodium carbonate (3 × 30 mL) and brine (2 × 30 mL) respectively. This washed organic solution was dried over anhydrous sodium sulfate and evaporated in vacuum. A white material was obtained. Yield: 6.939 g (14.501 mmol, 85.3 %).

^{1}H NMR (300 MHz, $CDCl_3$, 25° C, TMS): δ 7.16–7.00 (m, 5H; aromatic H and 1H; NH), δ 6.84 (d, ^3J(H, H) = 9.6 Hz, 1H; NH), δ 4.73–4.69 (m, 1H; α CH), δ 4.31 (br, 1H; α CH), δ 3.64 (s, 3H; OCH_3), δ 3.55 (s, 3H; OCH_3), δ 3.04 – 2.88 (m, 2H; β CH_2), δ 2.77 – 2.73 (m, 2H; β CH_2), δ 1.37 (s, 9H; Boc–CH_3); HRMS: m/z 430.96 $[M+Na]^+$, 431.97 $[M+Na+H]^+$, 446.93 $[M+K]^+$; ^{13}C NMR (75 MHz, $CDCl_3$, 25° C): δ 171.27 (1C of COOMe), δ 171.14 (1C of COOMe), δ 170.30 (1C of CONH), δ 155.32 (C of CONH), δ 136.46 (1C, aromatic C of Phe), δ 129.61 (1C, aromatic C of Phe), δ 129.42 (1C,

aromatic C of Phe), δ 128.71(1C, aromatic C of Phe), δ 128.40 (1C, aromatic C of Phe), δ 127.11(1C, aromatic C of Phe), δ 80.26 (1C, tertiary C of Boc), δ 77.41–76.90 (C of $CDCl_3$), δ 55.61 (1C, α C), δ 52.84 (1C, C of OCH_3), 52.07 (1C, C of OCH_3), δ 48.83 (1C, α C), δ 38.31(1C, C of β C), δ 36.01 (1C, C of β C), δ 28.02 (3C, primary C of Boc).

(iii) Boc-Phe-Asp-$(OH)_2$: To 4.9014 g (12 mmol) of Boc-Phe-Asp-$(OMe)_2$ were added 40 mL MeOH and 30 mL of 1(M) NaOH and the progress of saponification was monitored by thin-layer chromatography (TLC). The reaction mixture was stirred. After 10 h, methanol was removed under vacuum and the residue was taken in 50 mL of water and washed with diethyl ether (2 × 50 mL). The pH of the aqueous layer was then adjusted to 2-3 using 1(N) HCl, the aqueous layer was extracted with ethyl acetate (2 × 50 mL). The extract were pooled, dried over anhydrous sodium sulfate and evaporated in vacuum. Yield: 3.9425 g (10.364 mmol, 86.37 %).

^{1}H NMR (300 MHz, [D_6]DMSO, 25° C): δ 8.87 (d, 1H; NH), δ 7.30–7.15 (m, aromatic H, 5H), δ 7.01 (d, 1H; NH), δ 4.21–4.19 (m, 1H; α CH), δ 4.01 (m, 1H; α CH), δ 3.11–3.06 (m, 2H; β CH), δ 2.98–2.88 (m, 2H; β CH), δ 1.30 (s, 9H; Boc–CH_3); HRMS: m/z 403.14 $[M+Na]^+$.

(iv) H-Phe-Asp-$(OH)_2$: To 3.4225 g (9 mmol) of Boc-Phe-Asp-$(OH)_2$ was added 4 mL of 98% formic acid, and the removal of the Boc group was monitored by TLC. After 8 h, formic acid was removed under a vacuum. The residue was taken in water (20 mL) and washed with diethyl ether (2×30 mL). The pH of the aqueous solution was then adjusted to 7 with 30% aqueous NH_3. The aqueous solution was lyophilized to yield white solid product. Yield: 2.205 g (7.867 mmol, 87.40 %).

^{1}H NMR (300 MHz, [D_6]DMSO, 25° C): δ 8.25 (d, 1H, ^3J(H, H) = 6.3 Hz, NH), δ 8.13 (d, ^3J(H, H) = 6.3 Hz, 1H; NH), δ 7.22–6.93 (m, 5H; aromatic H), δ 4.10–4.07 (m, 1H; α CH), δ 3.62–3.58 (m, 1H; αCH), δ 3.05–2.92 (m, H; β CH_2), δ 2.85–2.80 (m, 1H; β CH), δ 2.70–2.63 (m, H; β CH_2), δ 2.41–2.30 (m, 1H; β CH); HRMS: m/z 281.26 $[M+H]^+$, 303.25 $[M+Na]^+$; ^{13}C NMR (75 MHz, [D_6]DMSO, 25° C): δ 173.89 (C of COOH), δ 173.33 (C of COOH), δ 171.47 (C of CONH), δ 137.69 (1C, aromatic C of Phe), δ 130.73 (1C, aromatic C of Phe), δ 130.00 (1C, aromatic C of Phe), δ 128.83(1C, aromatic C of Phe), δ 128.63 (1C, aromatic C of Phe), δ 127.24 (1C, aromatic C of Phe), δ 55.38 (C, α C), δ 49.37 (C, αC), δ 40.79–38.77 (C of [D_6]DMSO and 2 β C).

(v) Fmoc-Phe-Asp-$(OH)_2$: 1.682 g of (6 mmol) of H-Phe-Asp-$(OH)_2$ was dissolved in a basic sodium carbonate solution (15 mL). It was cooled in an ice-water bath. Then cooled solution of Fmoc-Cl (2.31 g) in dioxane (15 mL) was added to it. The reaction mixture was allowed to come to room temperature and stirred for 24 hrs. Then the solution was concentrated in vacuum to about 15 mL, cooled in an ice water bath, covered with a layer of ethyl acetate (about 30 mL), and acidified with a dilute HCl to neutral pH. The aqueous phase was extracted with ethyl acetate and this operation was done twice. The ethyl acetate extract were pooled, dried over anhydrous Na_2SO_4 and evaporated in vacuum. A white material was obtained. Yield: 2.755 g (5.487 mmol, 91.45 %); m.p. 149° C.

^{1}H NMR (300 MHz, [D_6]DMSO, 25° C): δ 12.62 (br, 2H; COOH), δ 8.39 (d, ^3J(H, H) = 7.5 Hz, 1H; NH), δ δ 7.87– 7.85 (m, 2H; aromatic CH), δ 7.69– 7.61 (m, 3H; aromatic CH), δ 7.42– 7.16 (m, 9H; aromatic CH and NH), δ 4.58–4.56 (m, 1H; α CH), δ 4.29–4.24 (m, 1H, αCH), δ 4.18–4.08 (m, 3H; CH, CH_2), δ 3.05–3.01 (m, 2H; β CH_2), δ

2.79–2.63 (m, 2H; β CH_2); HRMS: m/z 525.45$[M+Na]^+$, 541.44 $[M+K]^+$; ^{13}C NMR (75 MHz, $[D_6]$DMSO, 25ºC): δ 172.94 (C, C of COOH), δ 172.29 (C, C of COOH), δ 172.03 (C, C of CONH), δ 156.32 (C of CONH), δ 144.32 (C, aromatic C), δ 141.20 (2C, aromatic C), δ 138.73 (2C, aromatic C), δ 129.82 (C, aromatic C), δ 128.57 (C, aromatic C), δ 128.18 (C, aromatic C), δ 127.62 (C, aromatic C),), δ 127.34 (C, aromatic C), δ 126.78 (C, aromatic C), δ 125.85 (C, aromatic C), δ 120.62 (2C, aromatic C), δ 66.24 (C of CH_2), δ 56.55 (C, α C), δ 49.24 (C, α C), δ 47.11 (C of CH), δ 40.89–39.22 (C of $[D_6]$DMSO), δ 38.05 (C, β C), δ 36. 86 (C, β C).

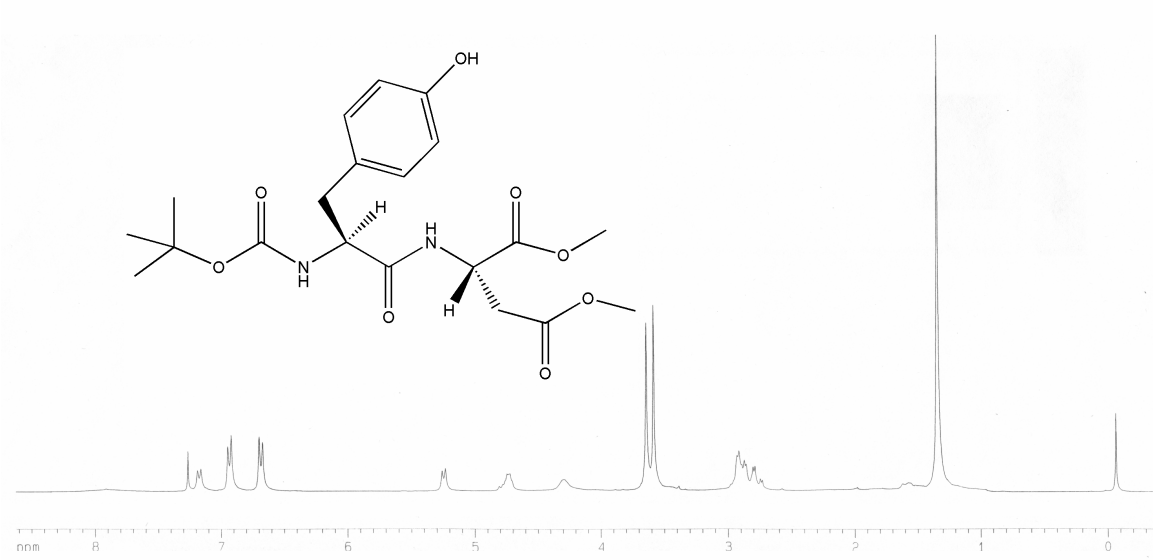

Fig. S8 ^1H NMR Spectrum of the Peptide Boc-YD-$(OMe)_2$.

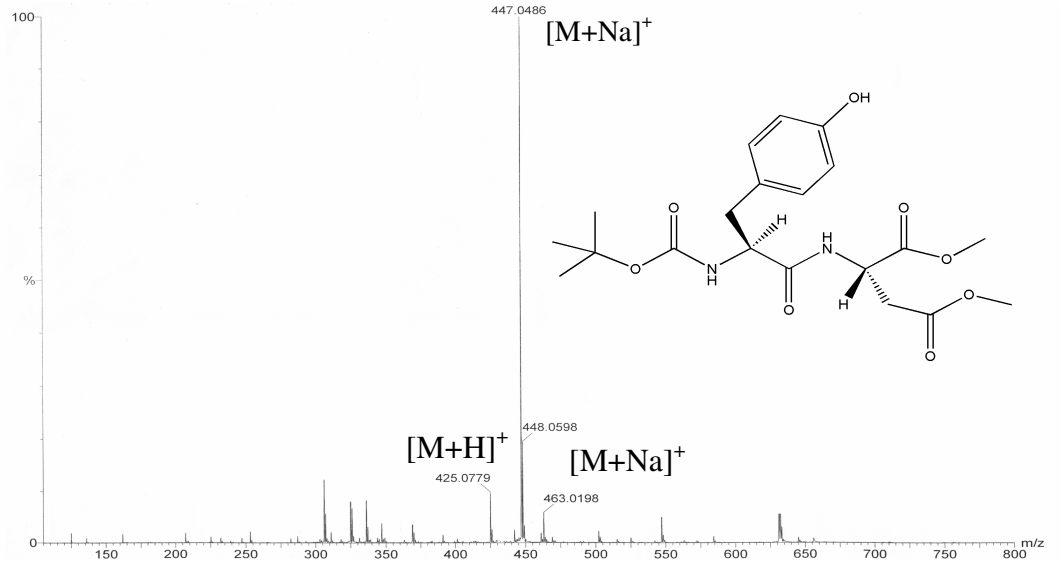

Fig.S9 HRMS Spectrum of the Peptide Boc-YD-$(OMe)_2$.

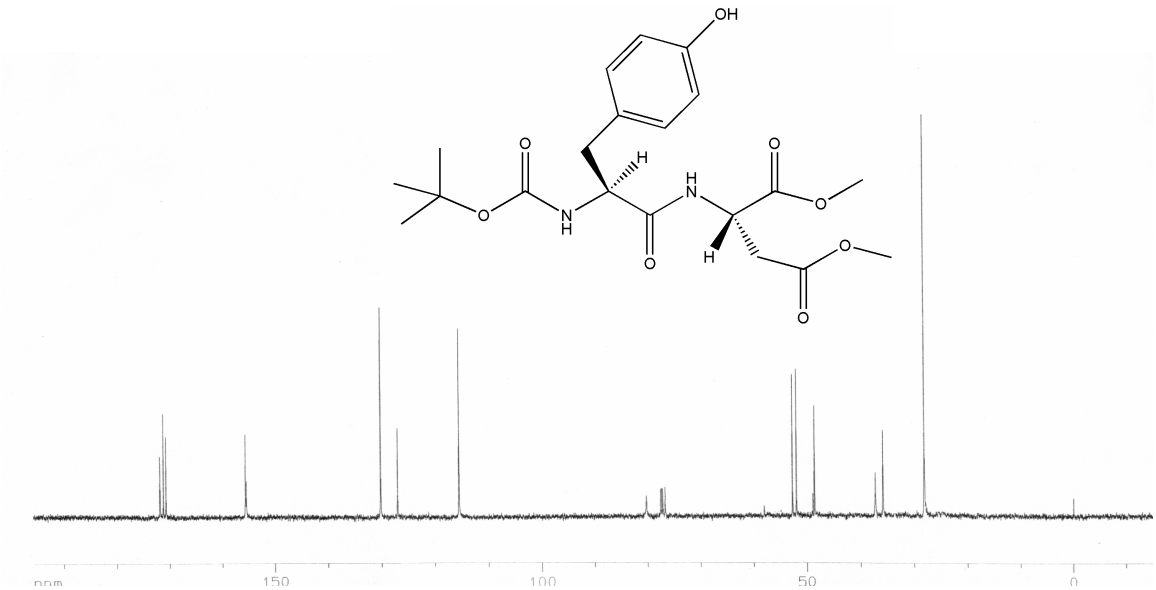

Fig.S10 ^{13}C NMR Spectrum of the Peptide Boc-YD-$(OMe)_2$.

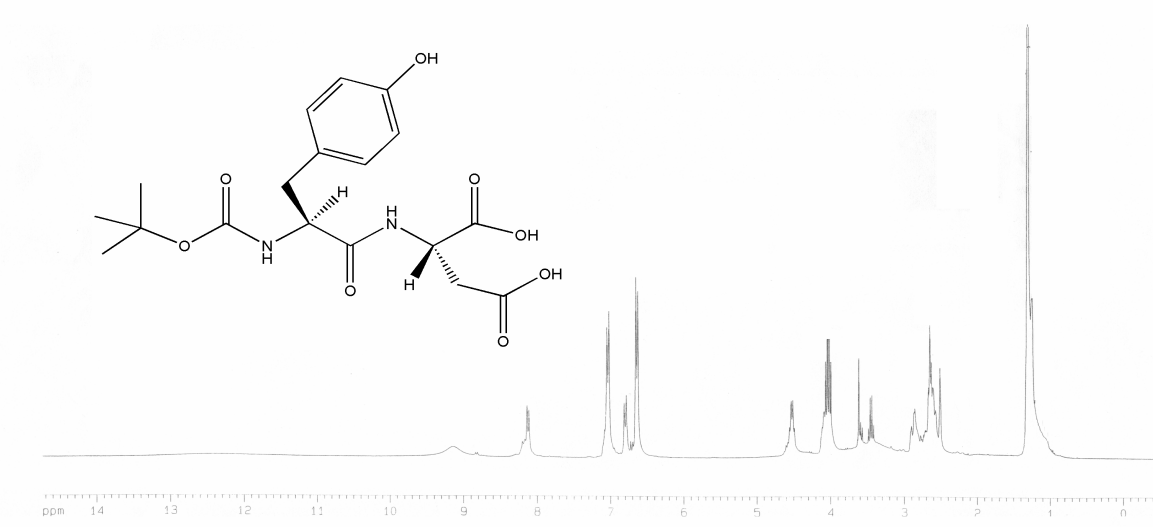

Fig. S11 ^{1}H NMR Spectrum of the Peptide Boc-YD-$(OH)_2$.

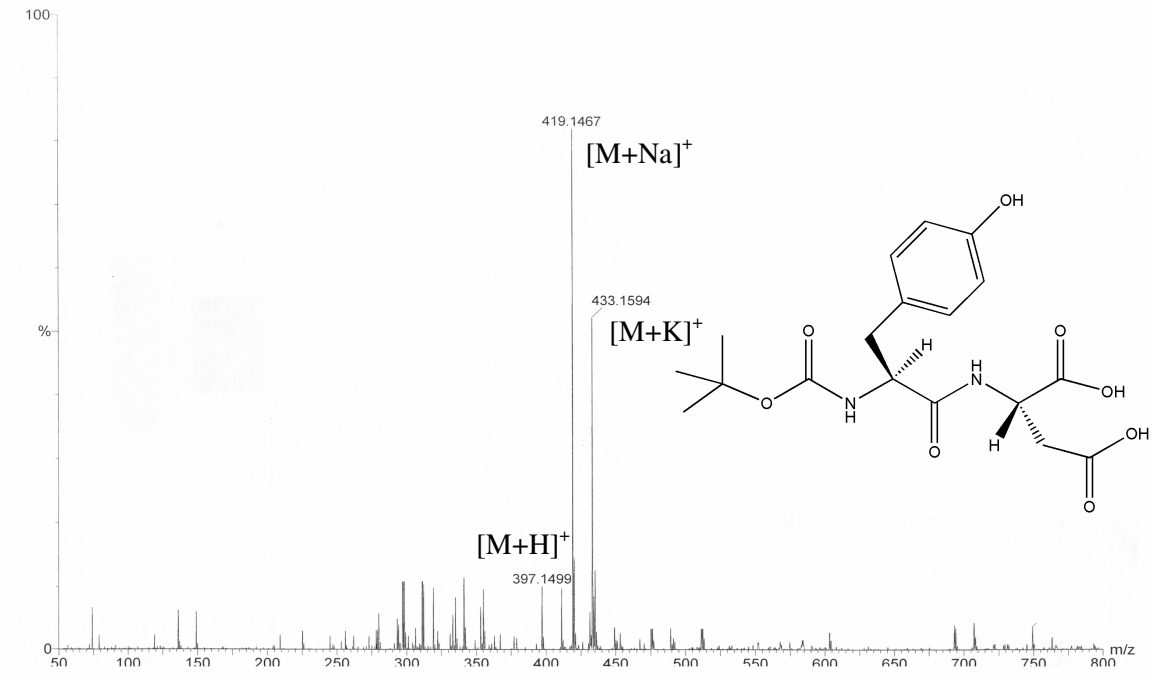

Fig. S12 HRMS Spectrum of the Peptide Boc-YD-$(OH)_2$.

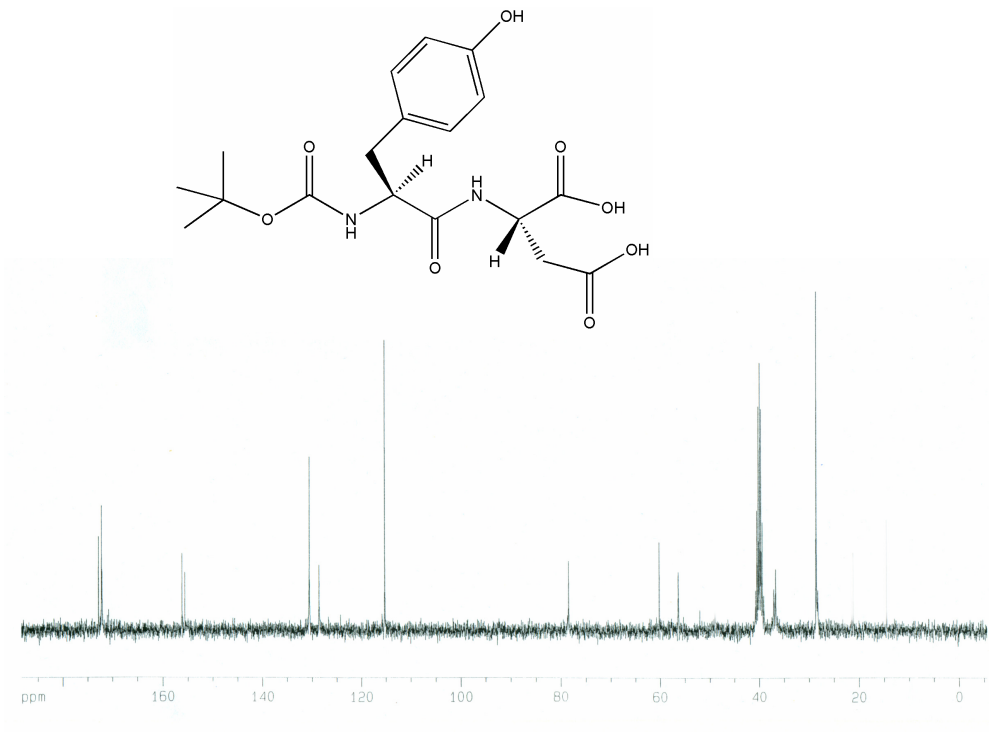

Fig. S13 ^{13}C NMR Spectrum of the Peptide Boc-YD-$(OH)_2$.

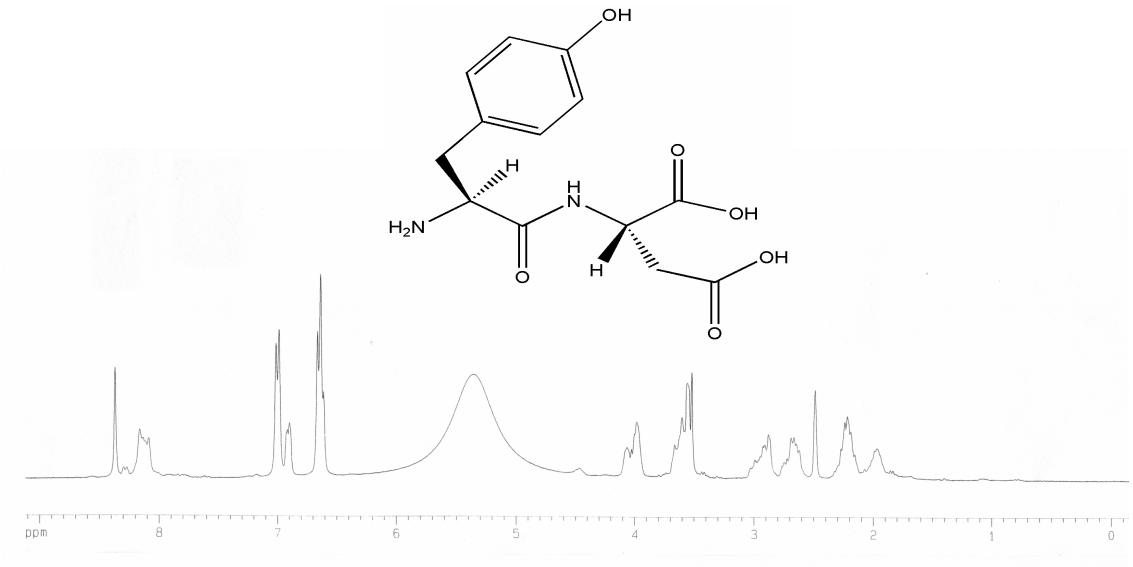

Fig.S14 ^{1}H NMR Spectrum of the Peptide H-YD-$(OH)_2$.

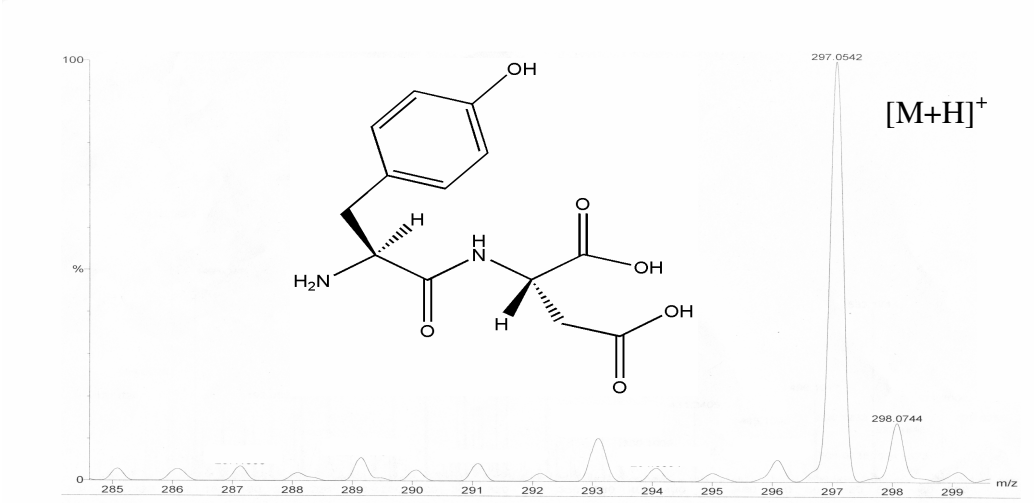

Fig.S15 HRMS Spectrum of the Peptide H-YD-$(OH)_2$.

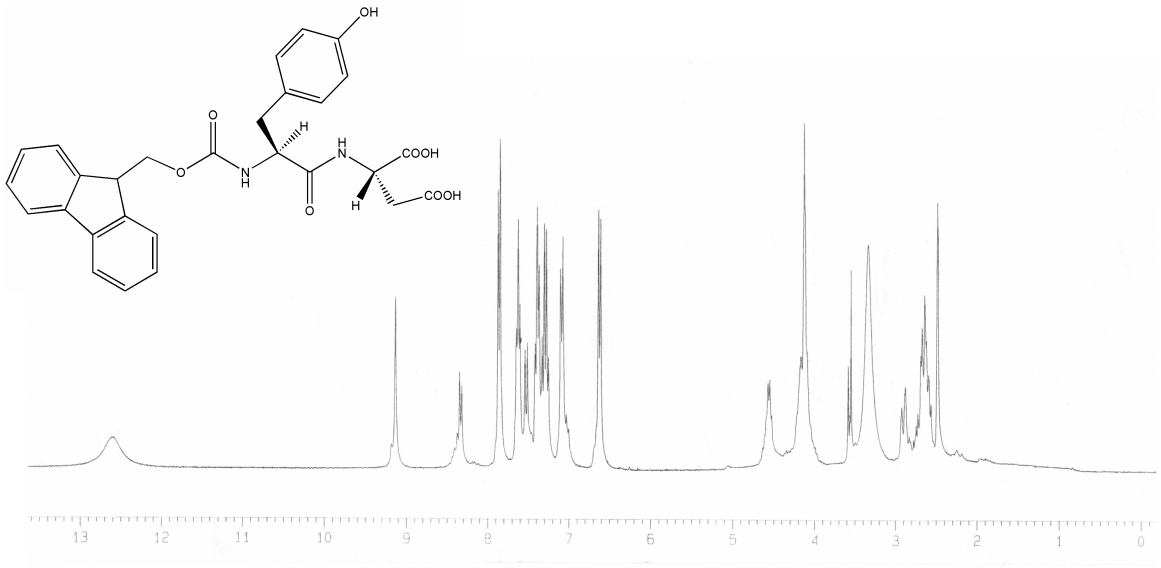

Fig. S16 ^{1}H NMR Spectrum of the Peptide Fmoc-YD-$(OH)_2$.

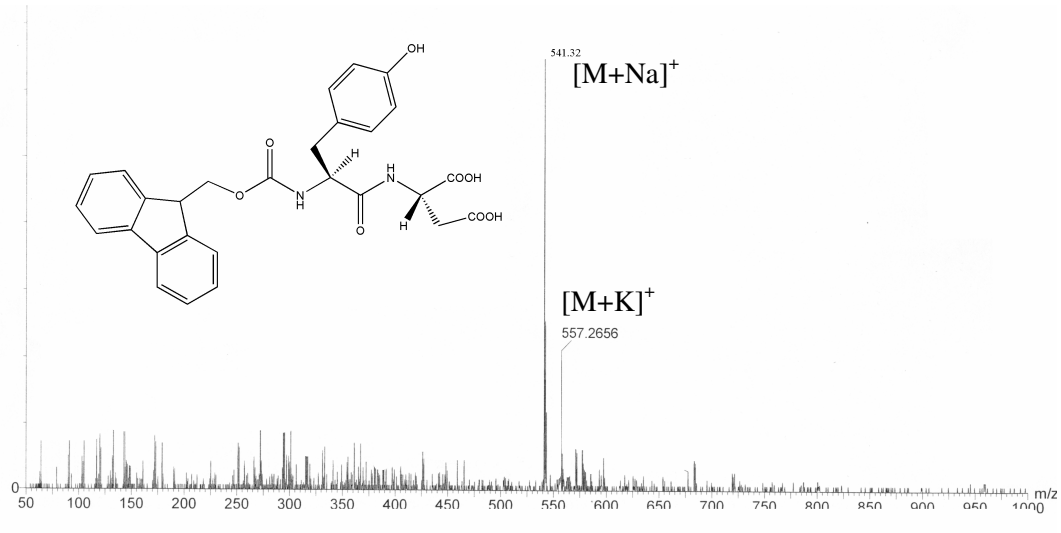

Fig. S17 HRMS Spectrum of the Peptide Fmoc-YD-$(OH)_2$.

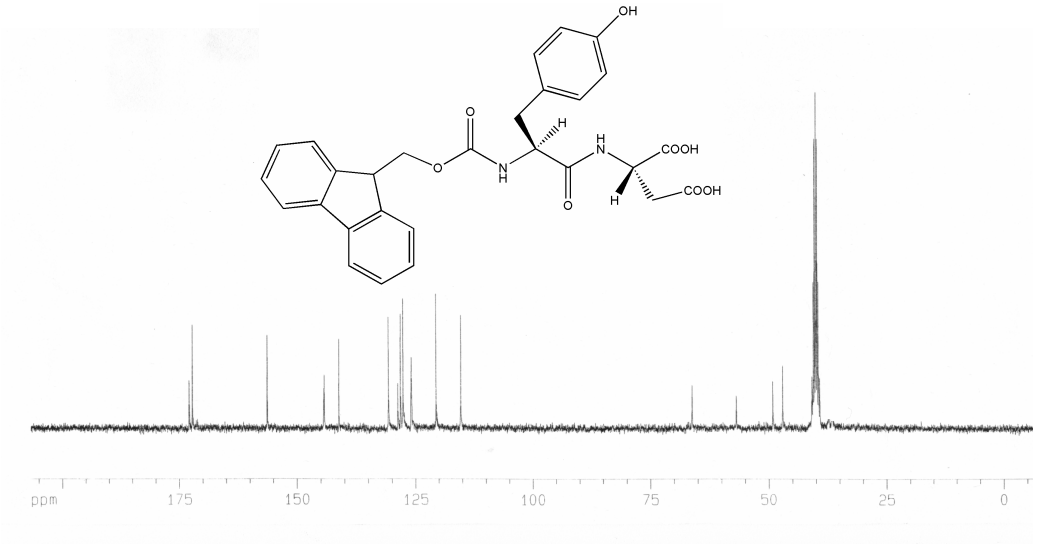

Fig.S18 ^{13}C NMR Spectrum of the Peptide Fmoc-YD-$(OH)_2$.

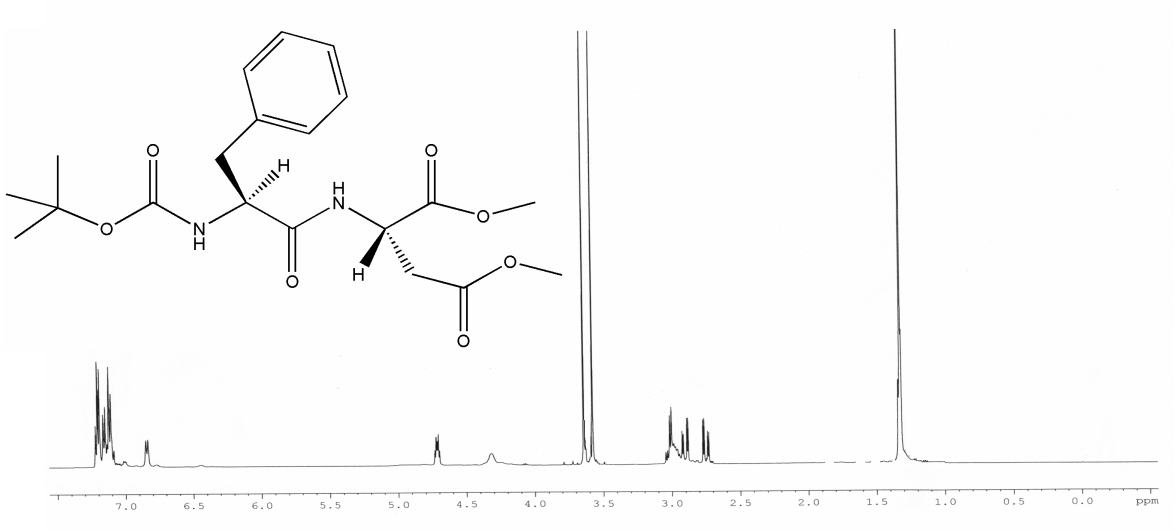

Fig. S19 ^{1}H NMR Spectrum of the Peptide Boc-FD-$(OMe)_2$.

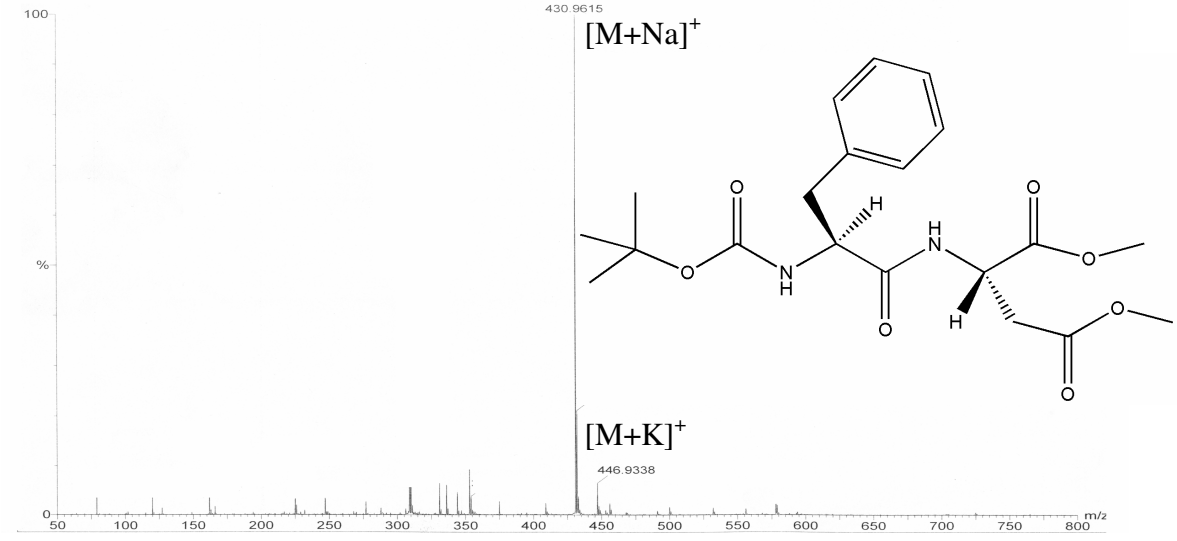

Fig. S20 HRMS Spectrum of the Peptide Boc-FD-$(OMe)_2$.

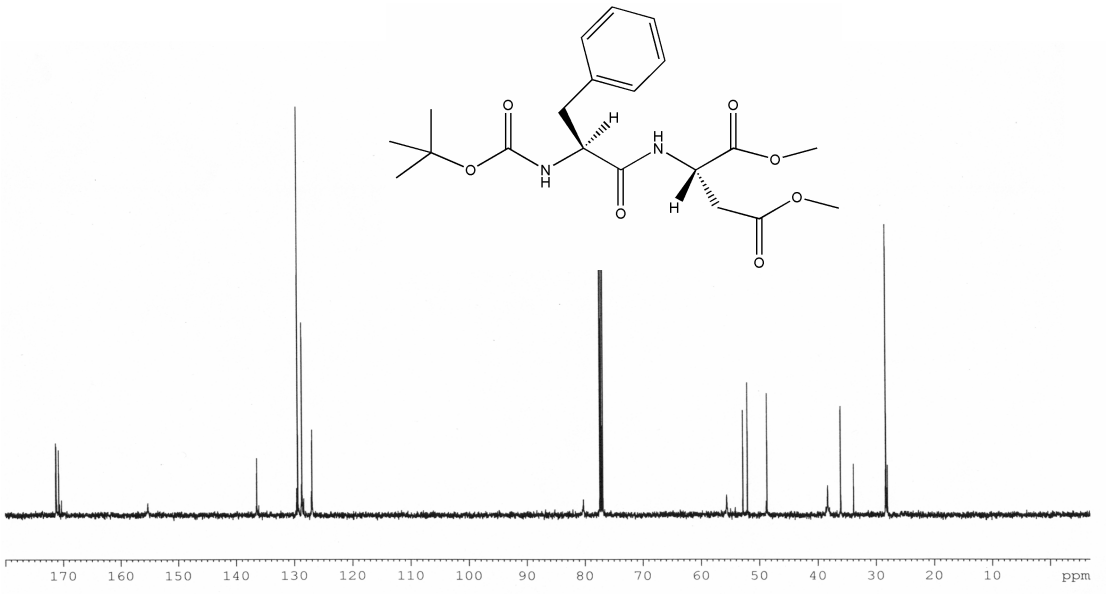

Fig. S21 ^{13}C NMR Spectrum of the Peptide Boc-FD-$(OMe)_2$.

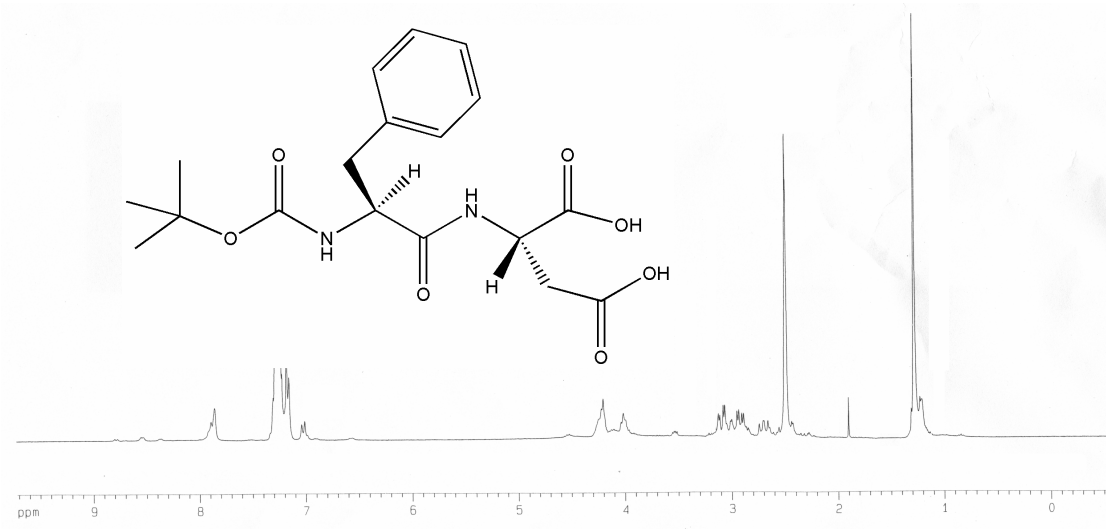

Fig. S22 ^{1}H NMR Spectrum of the Peptide Boc-FD-$(OH)_2$.

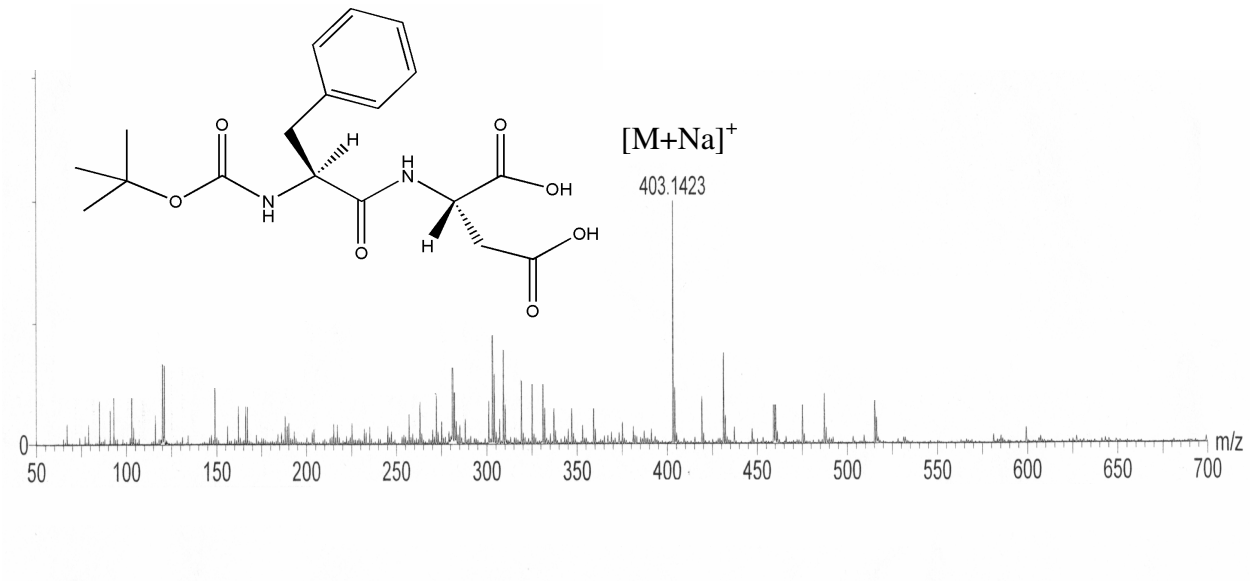

Fig. S23 HRMS Spectrum of the Peptide Boc-FD-$(OH)_2$.

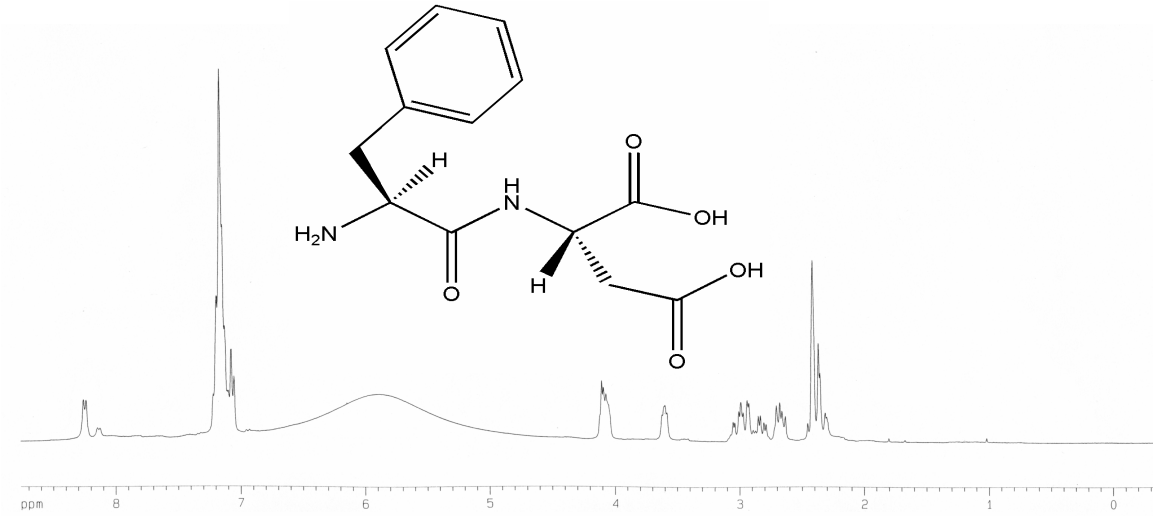

Fig. S24 ^{1}H NMR Spectrum of the Peptide H-FD-$(OH)_2$.

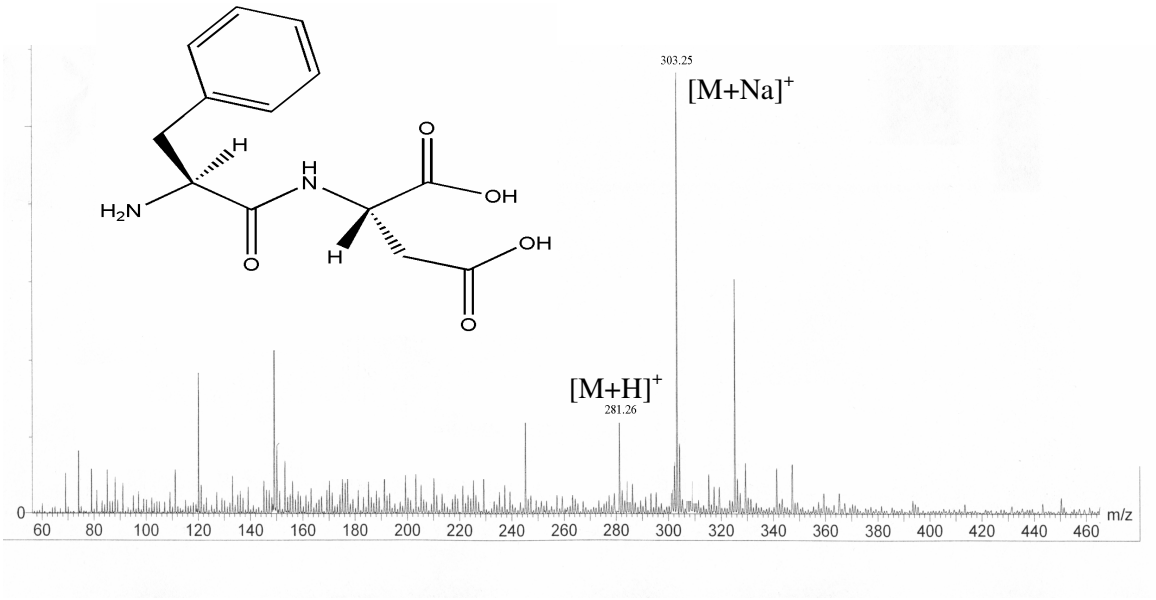

Fig. S25 HRMS Spectrum of the Peptide H-FD-$(OH)_2$.

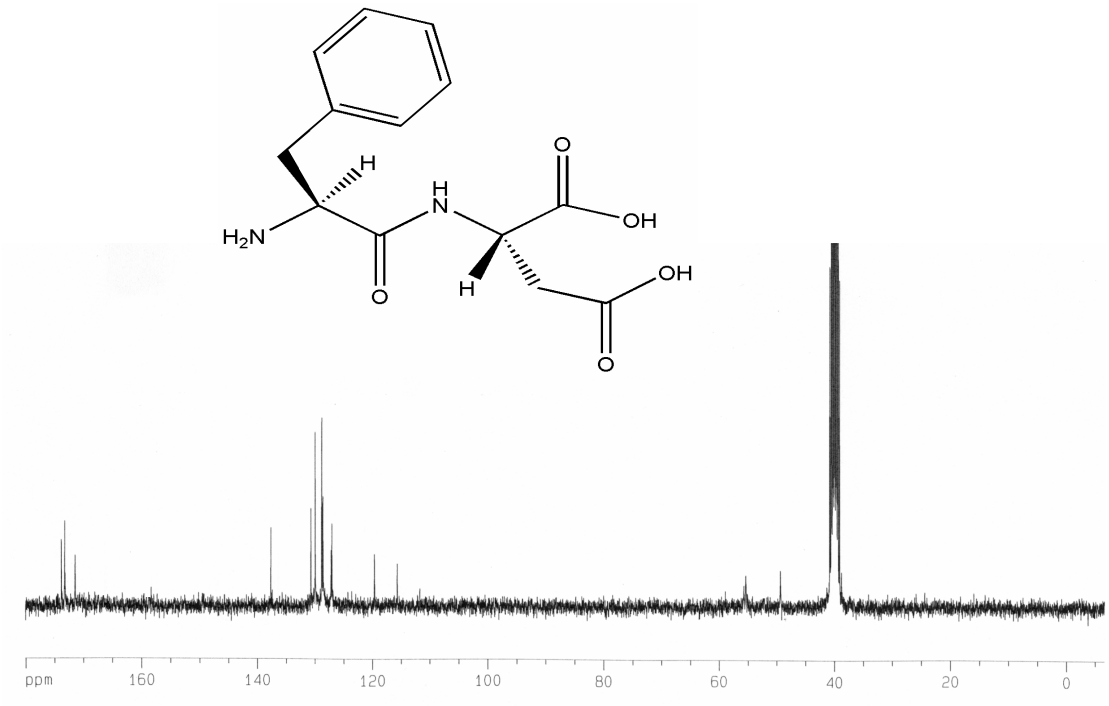

Fig. S26 ^{13}C NMR Spectrum of the Peptide H-FD-$(OH)_2$.

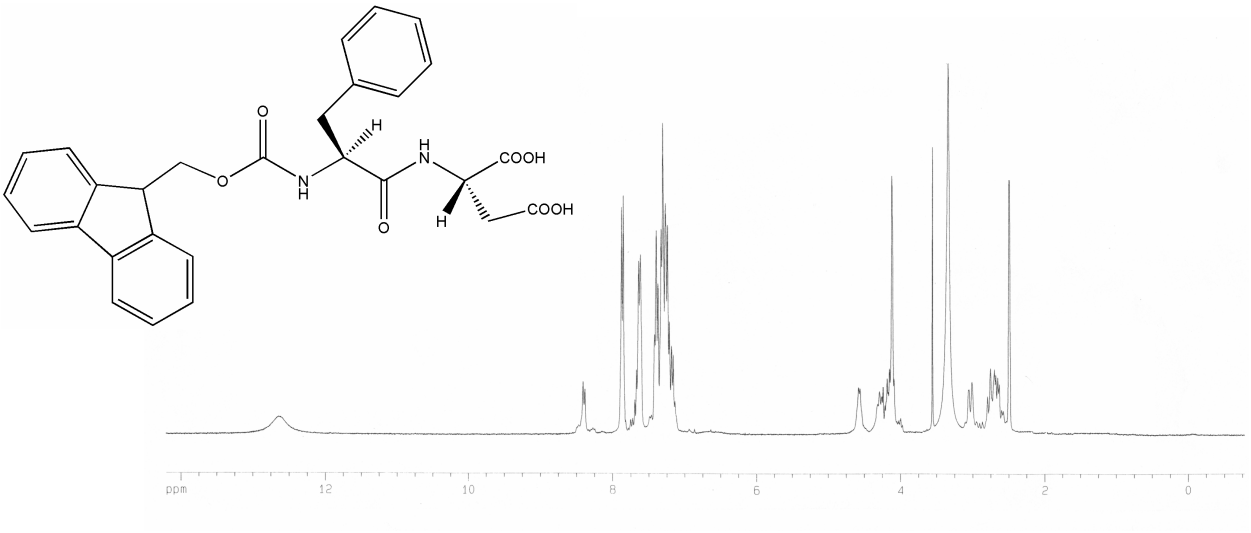

Fig. S27 ^{1}H NMR Spectrum of the Peptide Fmoc-FD-$(OH)_2$.

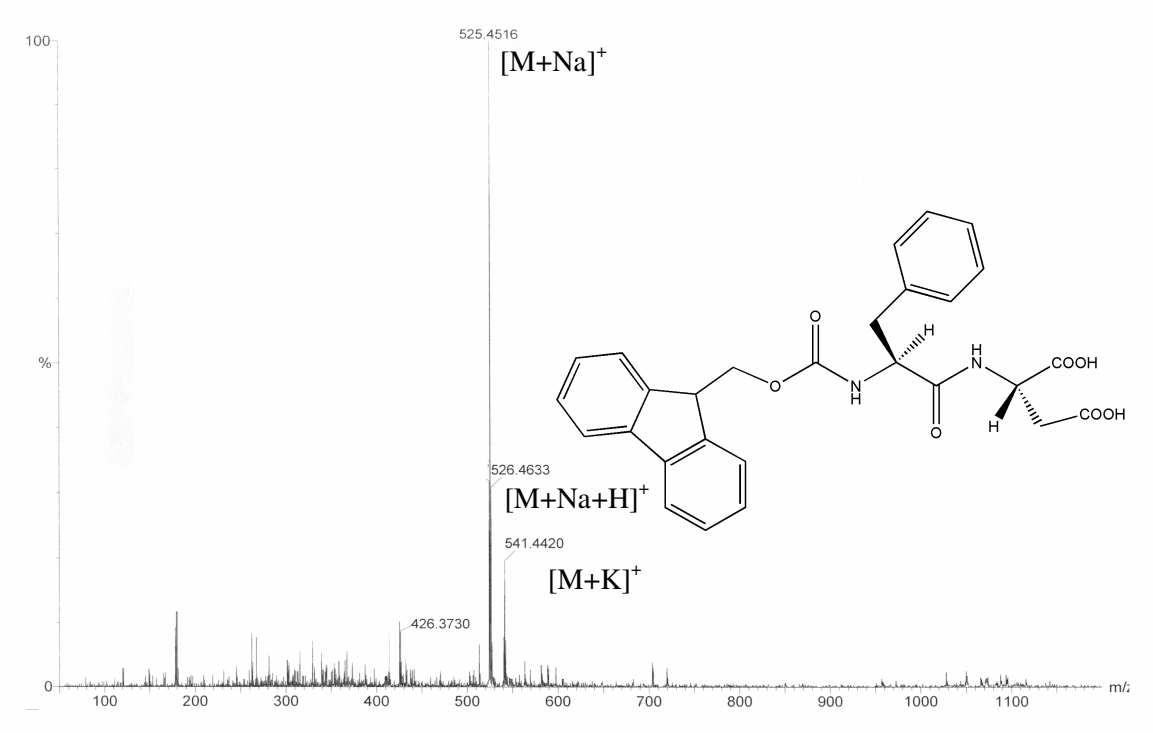

Fig. S28 HRMS Spectrum of the Peptide Fmoc-FD-$(OH)_2$.

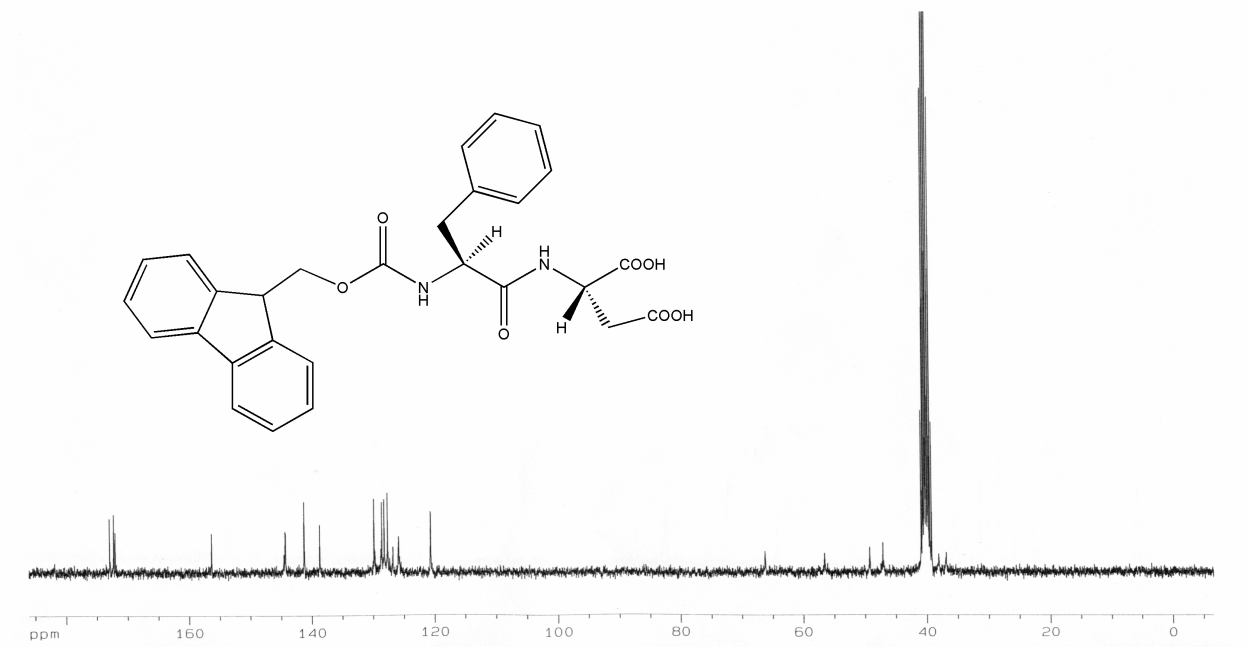

Fig. S29 ^{13}C NMR Spectrum of the Peptide Fmoc-FD-$(OH)_2$.