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Supporting Information

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New Regioselective Postsynthetic Modification of Phenylalanine Side Chains of Peptides Leading to Uncommon Ortho Iodinated Analogues.[**]

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General:

All products commercially available (including Aspartame **1**, β-Aspartame **2** and IPy_2BF_4) were purchased from Aldrich and used without further purification. Peptides **4**, **5**, and **6** were synthesized on solid support using standard Fmoc chemistry. Methylene chloride was distilled from calcium hydride and sodium metal. Liquid flash chromatography was carried out with Merck silica gel 60 (230-400 mesh) and analytical thin layer chromatography (TLC) was performed on 0.25 mm silica gel coated Kieselgel 60 F264 plates. The spots were visualized with UV light, and staining with nynhidrine followed by heat. NMR spectra were recorded on either a Bruker AMX-400 or AMX-300 spectrometer. Chemical shifts are reported in parts per million (ppm) on the scale, using residual solvent peaks as reference. Reactions were carried out under nitrogen atmosfere.

Analytical RP-HPLC was conducted using a C_{18} column (LiChrosorb, 250×4 mm). Solvents: A= 0.1% TFA in H_2O, B= 0.1% TFA in CH_3CN. Detection: λ= 214 nm

Compound	gradient	t_R
1a	80-20%A (25 min)	11.44
1b	80-20%A (25 min)	12.64
2a	80-20%A (25 min)	10.83
2b	80-20%A (25 min)	11.99
3a	80-20%A (25 min)	15.04
4a	80-20%A (25 min)	17.45
4b	80-20%A (25 min)	18.39
5a	80-20%A (25 min)	13.65
5b	80-20%A (25 min)	14.81
6a	70-20%A (20 min)	13.40
6b	0-20%A (20 min)	14.25

Semipreparative RP-HPLC was conducted using a 100 RP-8 column (LiChrospher, 250×10 mm, 10 μm). Solvents: A= 0.1% TFA in H_2O, B= 0.1% TFA in CH_3CN. Detection: λ= 214 nm.

MALDI-TOF-MS was conducted on a Voyager-DE STR Spectrometer, using α-cianohydroxycinnamic acid as matrix.

Synthesis of I-Aspartame derivatives:

Method A: 100 mg (0.34 mmol) of the peptide were dissolved on a mixture of 10 ml of CH_2Cl_2 and 1 ml of TFA. 0.51 mmol of IPy_2BF_4 were added (solution turns dark pink). Reaction was stirred for 0.5 hours at room temperature. Solvent was removed in vacuo. The piridinium salt formed in the reaction was eliminated by filtration through a short column of silica gel using MeOH: TFA (10:1) as eluent: R_f of peptides= 0.85 (stained with nynhidrine and visible with UV light). R_f of pyridinium salt= 0.43 (visible with UV light). The resulting solid was washed with ether and further purified by reversed phase HPLC.

Method B: 100 mg of peptide (0.34 mmol) were dissolved on a mixture of 100 ml of CH_2Cl_2 and 10 ml of TFA. 0.75 mmol of HBF_4 were added followed by addition of 0.37 mmol of IPy_2BF_4 (solution turns dark pink). The mixture was stirred at room temperature for o.5 hours. Work up of the reaction was the same as above.

L-Asp-L-(2-I)-Phe-OMe 1a:

^{1}H-RMN (300 MHz, CD_3OD): δ= 2.82-3.18 (m, 3H), 3.30-3.40 (m, 1H), 3.71 (s, 3H), 4.13 (m, 1H), 4.79-4.84 (dd, ^3J(H,H)= 5.9 Hz, ^3J(H,H)= 9.6 Hz, 1H), 6.97 (dt, ^4J(H,H)= 1.7 Hz, ^3J(H,H)= 7.9 Hz, 1H), 7.24-7.35 (m, 2H), 7.85 (dt, ^4J(H,H)= 1.1 Hz, ^3J(H,H)= 7.9 Hz, 1H).

^{13}C-RMN (300 MHz, CD_3OD): δ= 35.8 (CH_2), 42.6 (CH_2), 50.8 (CH), 53.0 (OCH_3), 54.1 (CH), 101.9 (C-I), 129.6 (CH), 130.0 (CH), 131.9 (CH), 140.5 (C), 141.0 (CH), 169.4 (CO), 172.7 (CO), 173.0 (CO).

L-Asp-L-(4-I)-Phe-OMe 1b:

^{1}H-RMN (300 MHz, CD_3OD): δ= 2.78-3.24 (m, 4H), 3.79 (s, 3H), 4.16 (dd, ^3J(H,H)= 3.3 Hz, ^2J(H,H)= 8.8 Hz, 1H), 4.73 (dd, ^3J(H,H)= 5.3 Hz, ^2J(H,H)= 8.8 Hz, 1H), 7.04 (d, ^3J(H,H)= 7.3 Hz, 2H), 7.66 (d, ^3J(H,H)= 7.3 Hz, 2H).

^{13}C-RMN (300 MHz, CD_3OD): 36.3 (CH_2), 37.7 (CH_2), 51.1(CH), 53.3 (CH_2), 55.6 (OCH_3), 93.3 (C-I), 132.6 (2 CH), 138.1 (C), 139.1 (2 CH), 169.7 (CO), 173.0 (2CO).

L-β-Asp-L-(2-I)-Phe-OMe 2a:

^{1}H-RMN (400 MHz, CD_3OD): δ= 2.80 (dd, ^3J(H,H)= 9.3 Hz, ^2J(H,H)= 8.0 Hz, 1H), 2.99 (dd, ^2J(H,H)= 8.0 Hz, ^3J(H,H)= 3.6 Hz, 1H), 3.11 (dd, ^3J(H,H)= 13.9 Hz, ^2J(H,H)= 9.5 Hz, 1H), 3.35 (dd, ^3J(H,H)= 6.1 Hz, ^3J(H,H)= 5.9 Hz, 1H), 3.70 (s, 3H), 4.13 (dd, ^3J(H,H)= 9.3 Hz, ^3J(H,H)= 3.6 Hz, 1H), 4.82 (dd, ^2J(H,H)= 9.5 Hz, ^3J(H,H)= 5.9 Hz, 1H), 6.97 (t, ^3J(H,H)= 7.7 Hz, 1H), 7.24 (d, ^3J(H,H)= 7.5 Hz, 1H), 7.32 (t, ^3J(H,H)= 7.5 Hz, 1H), 7.86 (d, ^3J(H,H)= 7.7 Hz, 1H).

^{13}C-RMN (400 MHz, CD_3OD): δ= 36.1 (CH_2), 42.7 (CH_2), 50.9 (CH), 53.0 (OCH_3), 54.0 (CH), 101.0 (C-I), 129.6 (CH), 130.1 (CH), 131.9 (CH), 140.6 (C), 141.0 (CH), 169.4 (CO), 172.6 (CO), 172.9 (CO).

L-β-Asp-L-(4-I)-Phe-OMe 2b:

^{1}H-RMN (400 MHz, CD_3OD): δ= 2.69 (dd, ^{3}J(H,H)= 9.2 Hz, ^{2}J(H,H)= 7.9 Hz, 1H), 2.92 (m, 2H), 3.21 (dd, ^{3}J(H,H)= 4.0 Hz, ^{3}J(H,H)= 5.0 Hz, 1H), 3.72 (s, 3H), 4.08 (dd, ^{2}J(H,H)= 9.5 Hz, ^{3}J(H,H)= 4.0 Hz, 1H), 4.71 (dd, ^{2}J(H,H)= 9.5 Hz, ^{3}J(H,H) = 5.0 Hz, 1H), 7.03 (d, ^{3}J(H,H)= 8.4 Hz, 2H), 7.65 (d, ^{3}J(H,H)= 8.4 Hz, 2H).

^{13}C-RMN (400 MHz, CD_3OD): 36.8 (CH_2), 37.5 (CH_2), 51.3(CH), 53.0 (OCH_3), 55.2 (CH), 93.0 (C-I), 132.3 (2 CH), 137.9 (C), 138.9 (2 CH), 169.7 (CO), 172.7 (CO),174.0 (CO).

Ac-L-β-Asp(OMe)-L-(2-I)-Phe-OMe 3a:

^{1}H-RMN (300 MHz, $CDCl_3$): δ= 2.01 (s, 3H), 2.70-2.77 (dd, ^{3}J(H,H)= 4.6 Hz, ^{2}J(H,H)= 15.9 Hz, 1H), 2.88-2.95 (dd, ^{3}J(H,H)= 4.6 Hz, ^{2}J(H,H)= 15.9 Hz, 1H), 3.08-3.15 (dd, ^{3}J(H,H)= 8.3 Hz, ^{2}J(H,H)= 14.0 Hz, 1H), 3.26-3.332 (dd, ^{3}J(H,H)= 8.3 Hz, ^{2}J(H,H)= 14.0 Hz, 1H), 3.68 (s, 3H), 3.72 (s, 3H), 4.75-4.89(m, 2H), 6.31 (d, ^{3}J(H,H)= 7.9 Hz, 1H), 6.71 (d, ^{3}J(H,H)= 7.7 Hz, 1H), 6.93 (t, ^{3}J(H,H)= 6.0 Hz, 1H), 7.17 (d, ^{3}J(H,H)= 6.0 Hz, 1H), 7.28-7.31 (m, 1H), 7.82 (d, ^{3}J(H,H)= 6.8 Hz, 1H).

^{13}C-RMN (300 MHz, $CDCl_3$): δ= 23.5 (CH_3), 37.7 (CH_2), 42.6 (CH_2), 49.1 (CH), 53.0 (2 OCH_3), 53.1 (CH) 101.3 (C-I), 128.8 (CH), 129.2(CH), 130.6 (CH), 139.4 (C), 140.1 (CH), 170.1 (CO), 170.5 (CO), 171.6 (CO), 172.0 (CO).

For-NLeu-Leu-(2-I)-Phe-OMe 5a:

^{1}H-RMN (400 MHz, CD_3OD+D_2O): δ= 0.95-1.02 (m, 10H), 1.39 (m, 4H), 1.59-1.79 (m, 5H), 3.16 (dd, ^{3}J(H,H)= 9.0 Hz, ^{3}J(H,H)= 13.9 Hz, 1H), 3.31 (m, 1H), 3.39 (m, 1H), 3.76 (s, 2H), 4.12 (dd, ^{2}J(H,H)= 6.0 Hz, ^{3}J(H,H)= 7.5 Hz, 1H), 4.49 (dd, ^{2}J(H,H)= 6.0 , ^{3}J(H,H)= 9.3, 1H), 7.04 (t, ^{3}J(H,H)= 7.9 Hz, 1H), 7.21 (d, ^{3}J(H,H)= 7.5 Hz, 1H), 7.39 (t, ^{3}J(H,H)= 7.5 Hz, 1H), 7.90 (d, ^{3}J(H,H)= 7.9 Hz ,1H), 8.17 (s, 1H).

^{13}C-RMN (400 MHz, CD_3OD+D_2O): δ= 14.8 (CH_3), 22.6 (CH_3), 23.7 (CH_3+CH_2), 26.1 (CH_3), 29.2 (CH_2), 33.3 (CH_2), 42.3 (CH_2), 43.4 (CH_2), 53.4 (CH), 53.6 (OCH_3), 53.9 (CH), 54.2 (CH), 101.6 (C-I), 130.1 (CH), 130.4

(CH), 132.4 (CH), 141.0 (C), 141.2 (CH), 164.5 (CHO), 173.8 (CO), 174.2 (CO), 174.9 (CO).

H_2N-Gly-Gly-(2-I)-Phe-Leu-OMe 4a:

^{1}H-RMN (300 MHz, D_2O): δ= 0.92 (d, ^3J(H,H)= 5.9 Hz, 3H), 0.96 (d, ^3J(H,H)= 6.2 Hz, 3H), 1.65 (m, 3H), 3.20 (dd, ^3J(H,H)= 7.4 Hz, ^2J(H,H)= 13.6 Hz, 1H), 3.34 (dd, ^3J(H,H)= 7.9 Hz , ^2J(H,H)= 13.6 Hz , 1H), 3.75 (s, 3H), 3.94 (s, 2H), 4.04 (s, 2H), 4.50 (t, ^3J(H,H)= 8.5 Hz, 1H), 4.95 (m, 1H), 7.09 (t, ^3J(H,H)= 7.7 Hz, 1H), 7.32 (d, ^3J(H,H)= 7.7 Hz, 1H), 7.44 (t, ^3J(H,H)= 7.5 Hz, 1H), 7.99 (d, ^3J(H,H)= 7.9 Hz, 1H).

^{13}C-RMN (300 MHz, D_2O): δ= 20.8 (CH_3), 22.1 (CH_3), 24.3 (CH), 39.6 (CH_2), 40.5 (CH_2), 41.8 (CH_2), 42.1 (CH_2), 51.5 (OCH_3), 53.0 (CH), 53.8 (CH_3), 100.3 (C-I), 128.7 (CH), 129.2 (CH), 130.8 (CH), 138.8 (C), 139.8 (CH), 167.7 (CO), 170.6 (CO), 172.3 (CO), 174.4 (CO).

Ac-Ala-Asp-Ala-Thr-(2-I)-Phe-NH_2 6a:

^{1}H-RMN (400 MHz, CD^3CN): δ= 0.84 (d, ^3J(H,H)= 6.1 Hz, 3H), 1.74 (d, ^3J(H,H)= 7.0 Hz, 8H), 2.46 (m, 3H), 3.49 (dd, ^3J(H,H)= 9.8 Hz, ^3J(H,H)= 14.0 Hz, 3H), 3.70-3.75 (dd, ^3J(H,H)= 5.5 Hz, ^3J(H,H)= 14.0 Hz, 1H), 4.39-4.45 (m, 1H), 4.54 (d, ^3J(H,H)= 6.7 Hz, 1H), 4.99 (t, ^3J(H,H)= 6.7 Hz, 1H), 5.09 (m, 1H), 7.40 (t, ^3J(H,H)= 6.4 Hz, 1H), 7.68-7.77 (m, 2H), 8.28 (d, ^3J(H,H)= 7.7 Hz, 1H).

Pd coupling of L-Asp-L-(2-I)-Phe-OMe **1a** with phenylboronic acid:

100 mg of L-Asp-L-(2-I)-Phe-OMe **1a** (0.24 mmol) were dissolved in 3ml of toluene and 2 ml of MeOH. 58 mg of $PhB(OH)_2$ (0.48 mmol) and 10 mg of $Pd(PPh_3)_4$ (5% mmol) were added. Reaction was stirred at 80ºC for 5 hours. After cooling at room temperature, mixture was filter through celite and solvents were removed in vacuo. Residue was purified by semipreparative reversed phase HPLC (C-18 column (250×16 mm), flow= 4ml/min, gradient 80-20%A in 30 min, t_R= 14.33 min)

L-Asp-L-(2-Phenyl)-Phe-OMe 7:

^{1}H-RMN (300 MHz, CD_3OD): δ= 2.78-2.88 (m, 1H), 2.89-3.09 (m, 2H), 3.40 (s, 3H), 4.38 (m, 1H), 4.64 (m, 1H), 4.99-5.13 (m, 1H), 7.10-7.33 (m, 7H), 7.97-8.09 (m, 2H).

^{13}C RMN (300 MHz, CD_3OD): (Some signals are double due to the presence of rotamers). δ= 31.5 (CH_2), 32.6 (CH_2), 34.6 (CH_2), 38.2 (CH_2), 53.7 (CH), 55.8 (CH), 60.7 (OCH_3), 61.3 (OCH_3), 116.7 (CH), 117.2 (CH), 126.0 (CH), 126.1 (CH), 126.4 (CH), 131.3 (C), 131.4 (C), 141.1 (C), 142.2 (C), 142.6 (C), 166.1 (CO), 166.4 (CO), 170.5 (CO), 171.4 (CO), 171.6 (CO), 173.6 (CO).

HPLC-ES: 370 (M+1).