Supporting Information

for

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69451 Weinheim, Germany
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 IPy₂BF₄) 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 atmosphere.
Analytical RP-HPLC was conducted using a C₁₈ column (LiChrosorb, 250x4 mm). Solvents: A= 0.1% TFA in H₂O, B= 0.1% TFA in CH₃CN.
Detection: λ= 214 nm
<table>
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<tr>
<th>Compound</th>
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<th>$t_R$</th>
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Semipreparative RP-HPLC was conducted using a 100 RP-8 column (LiChrospher, 250×10 mm, 10 µm). Solvents: A = 0.1% TFA in H$_2$O, B = 0.1% TFA in CH$_3$CN. Detection: $\lambda = 214$ 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$_2$Cl$_2$ and 1 ml of TFA. 0.51 mmol of IPy$_2$BF$_4$ 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 CH₂Cl₂ and 10 ml of TFA. 0.75 mmol of HBF₄ were added followed by addition of 0.37 mmol of IPy₂BF₄ (solution turns dark pink). The mixture was stirred at room temperature for 0.5 hours. Work up of the reaction was the same as above.

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

\(^1\)H-RMN (300 MHz, CD₃OD): \(\delta = 2.82-3.18 \text{ (m, 3H), } 3.30-3.40 \text{ (m, 1H), } 3.71 \text{ (s, 3H), } 4.13 \text{ (m, 1H), } 4.79-4.84 \text{ (dd, } ^3\text{J(H,H) = 5.9 Hz, } ^3\text{J(H,H) = 9.6 Hz, } 1\text{H), } 6.97 \text{ (dt, } ^4\text{J(H,H) = 1.7 Hz, } ^3\text{J(H,H) = 7.9 Hz, } 1\text{H), } 7.24-7.35 \text{ (m, 2H), } 7.85 \text{ (dt, } ^4\text{J(H,H) = 1.1 Hz, } ^3\text{J(H,H) = 7.9 Hz, } 1\text{H).}

\(^{13}\)C-RMN (300 MHz, CD₃OD): \(\delta = 35.8 \text{ (CH₂), } 42.6 \text{ (CH₂), } 50.8 \text{ (CH), } 53.0 \text{ (OCH₃), } 54.1 \text{ (CH), } 101.9 \text{ (C-I), } 129.6 \text{ (CH), } 130.0 \text{ (CH), } 131.9 \text{ (CH), } 140.5 \text{ (C), } 141.0 \text{ (CH), } 169.4 \text{ (CO), } 172.7 \text{ (CO), } 173.0 \text{ (CO).}

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

\(^1\)H-RMN (300 MHz, CD₃OD): \(\delta = 2.78-3.24 \text{ (m, 4H), } 3.79 \text{ (s, 3H), } 4.16 \text{ (dd, } ^3\text{J(H,H) = 3.3 Hz, } ^2\text{J(H,H) = 8.8 Hz, } 1\text{H), } 4.73 \text{ (dd, } ^3\text{J(H,H) = 5.3 Hz, } ^2\text{J(H,H) = 8.8 Hz, } 1\text{H), } 7.04 \text{ (d, } ^3\text{J(H,H) = 7.3 Hz, } 2\text{H), } 7.66 \text{ (d, } ^3\text{J(H,H) = 7.3 Hz, } 2\text{H).}

\(^{13}\)C-RMN (300 MHz, CD₃OD): 36.3 (CH₂), 37.7 (CH₂), 51.1(CH), 53.3 (CH₂), 55.6 (OCH₃), 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, CD₃OD): \(\delta = 2.80 \text{ (dd, } ^3\text{J(H,H) = 9.3 Hz, } ^2\text{J(H,H) = 8.0 Hz, } 1\text{H), } 2.99 \text{ (dd, } ^2\text{J(H,H) = 8.0 Hz, } ^3\text{J(H,H) = 3.6 Hz, } 1\text{H), } 3.11 \text{ (dd, } ^3\text{J(H,H) = 13.9 Hz, } ^2\text{J(H,H) = 9.5 Hz, } 1\text{H), } 3.35 \text{ (dd, } ^3\text{J(H,H) = 6.1 Hz, } ^3\text{J(H,H) = 5.9 Hz, } 1\text{H), } 3.70 \text{ (s, 3H), } 4.13 \text{ (dd, } ^3\text{J(H,H) = 9.3 Hz, } ^3\text{J(H,H) = 3.6 Hz, } 1\text{H), } 4.82 \text{ (dd, } ^2\text{J(H,H) = 9.5 Hz, } ^3\text{J(H,H) = 5.9 Hz, } 1\text{H), } 6.97 \text{ (t, } ^3\text{J(H,H) = 7.7 Hz, } 1\text{H), } 7.24 \text{ (d, } ^3\text{J(H,H) = 7.5 Hz, } 1\text{H), } 7.32 \text{ (t, } ^3\text{J(H,H) = 7.5 Hz, } 1\text{H), } 7.86 \text{ (d, } ^3\text{J(H,H) = 7.7 Hz, } 1\text{H).}

\(^{13}\)C-RMN (400 MHz, CD₃OD): \(\delta = 36.1 \text{ (CH₂), } 42.7 \text{ (CH₂), } 50.9 \text{ (CH), } 53.0 \text{ (OCH₃), } 54.0 \text{ (CH), } 101.0 \text{ (C-I), } 129.6 \text{ (CH), } 130.1 \text{ (CH), } 131.9 \text{ (CH), } 140.6 \text{ (C), } 141.0 \text{ (CH), } 169.4 \text{ (CO), } 172.6 \text{ (CO), } 172.9 \text{ (CO).}
L-β-Asp-L-(4-I)-Phe-OMe 2b:

$^1$H-RMN (400 MHz, CD$_3$OD): $\delta = 2.69$ (dd, $^3J(H,H) = 9.2$ Hz, $^2J(H,H) = 7.9$ Hz, 1H), 2.92 (m, 2H), 3.21 (dd, $^3J(H,H) = 4.0$ Hz, $^3J(H,H) = 5.0$ Hz, 1H), 3.72 (s, 3H), 4.08 (dd, $^2J(H,H) = 9.5$ Hz, $^3J(H,H) = 4.0$ Hz, 1H), 4.71 (dd, $^2J(H,H) = 9.5$ Hz, $^3J(H,H) = 5.0$ Hz, 1H), 7.03 (d, $^3J(H,H) = 8.4$ Hz, 2H), 7.65 (d, $^3J(H,H) = 8.4$ Hz, 2H).

$^{13}$C-RMN (400 MHz, CD$_3$OD): 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$_3$): $\delta = 2.01$ (s, 3H), 2.70-2.77 (dd, $^3J(H,H) = 4.6$ Hz, $^2J(H,H) = 15.9$ Hz, 1H), 2.88-2.95 (dd, $^3J(H,H) = 4.6$ Hz, $^2J(H,H) = 15.9$ Hz, 1H), 3.08-3.15 (dd, $^3J(H,H) = 8.3$ Hz, $^2J(H,H) = 14.0$ Hz, 1H), 3.26-3.332 (dd, $^3J(H,H) = 8.3$ Hz, $^2J(H,H) = 14.0$ Hz, 1H), 3.68 (s, 3H), 3.72 (s, 3H), 4.75-4.89 (m, 2H), 6.31 (d, $^3J(H,H) = 7.9$ Hz, 1H), 6.71 (d, $^3J(H,H) = 7.7$ Hz, 1H), 6.93 (t, $^3J(H,H) = 6.0$ Hz, 1H), 7.17 (d, $^3J(H,H) = 6.0$ Hz, 1H), 7.28-7.31 (m, 1H), 7.82 (d, $^3J(H,H) = 6.8$ Hz, 1H).

$^{13}$C-RMN (300 MHz, CDCl$_3$): $\delta = 23.5$ (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$_3$OD+D$_2$O): $\delta = 0.95$-1.02 (m, 10H), 1.39 (m, 4H), 1.59-1.79 (m, 5H), 3.16 (dd, $^3J(H,H) = 9.0$ Hz, $^3J(H,H) = 13.9$ Hz, 1H), 3.31 (m, 1H), 3.39 (m, 1H), 3.76 (s, 2H), 4.12 (dd, $^2J(H,H) = 6.0$ Hz, $^3J(H,H) = 7.5$ Hz, 1H), 4.49 (dd, $^2J(H,H) = 6.0$ Hz, $^3J(H,H) = 9.3$ Hz, 1H), 7.04 (t, $^3J(H,H) = 7.9$ Hz, 1H), 7.21 (d, $^3J(H,H) = 7.5$ Hz, 1H), 7.39 (t, $^3J(H,H) = 7.5$ Hz, 1H), 7.90 (d, $^3J(H,H) = 7.9$ Hz, 1H), 8.17 (s, 1H).

$^{13}$C-RMN (400 MHz, CD$_3$OD+D$_2$O): $\delta = 14.8$ (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).

H₂N-Gly-Gly-(2-I)-Phe-Leu-OMe 4a:

¹H-RMN (300 MHz, D₂O): 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, ³J(H,H) = 13.6 Hz, 1H), 3.34 (dd, ³J(H,H) = 7.9 Hz, ³J(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).

¹³C-RMN (300 MHz, D₂O): δ = 20.8 (CH₃), 22.1 (CH₃), 24.3 (CH), 39.6 (CH₂), 40.5 (CH₂), 41.8 (CH₂), 42.1 (CH₂), 51.5 (OCH₃), 53.0 (CH), 53.8 (CH₃), 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-NH₂ 6a:

¹H-RMN (400 MHz, CD₃CN): 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(PPh₃)₄ (5% mmol) were added. Reaction was stirred at 80°C 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, tₘₐₓ = 14.33 min)

L-Asp-L-(2-Phenyl)-Phe-OMe 7:
$^1$H-RMN (300 MHz, CD$_3$OD): $\delta$ = 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$_3$OD): (Some signals are double due to the presence of rotamers). $\delta$ = 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).