



Supporting Information

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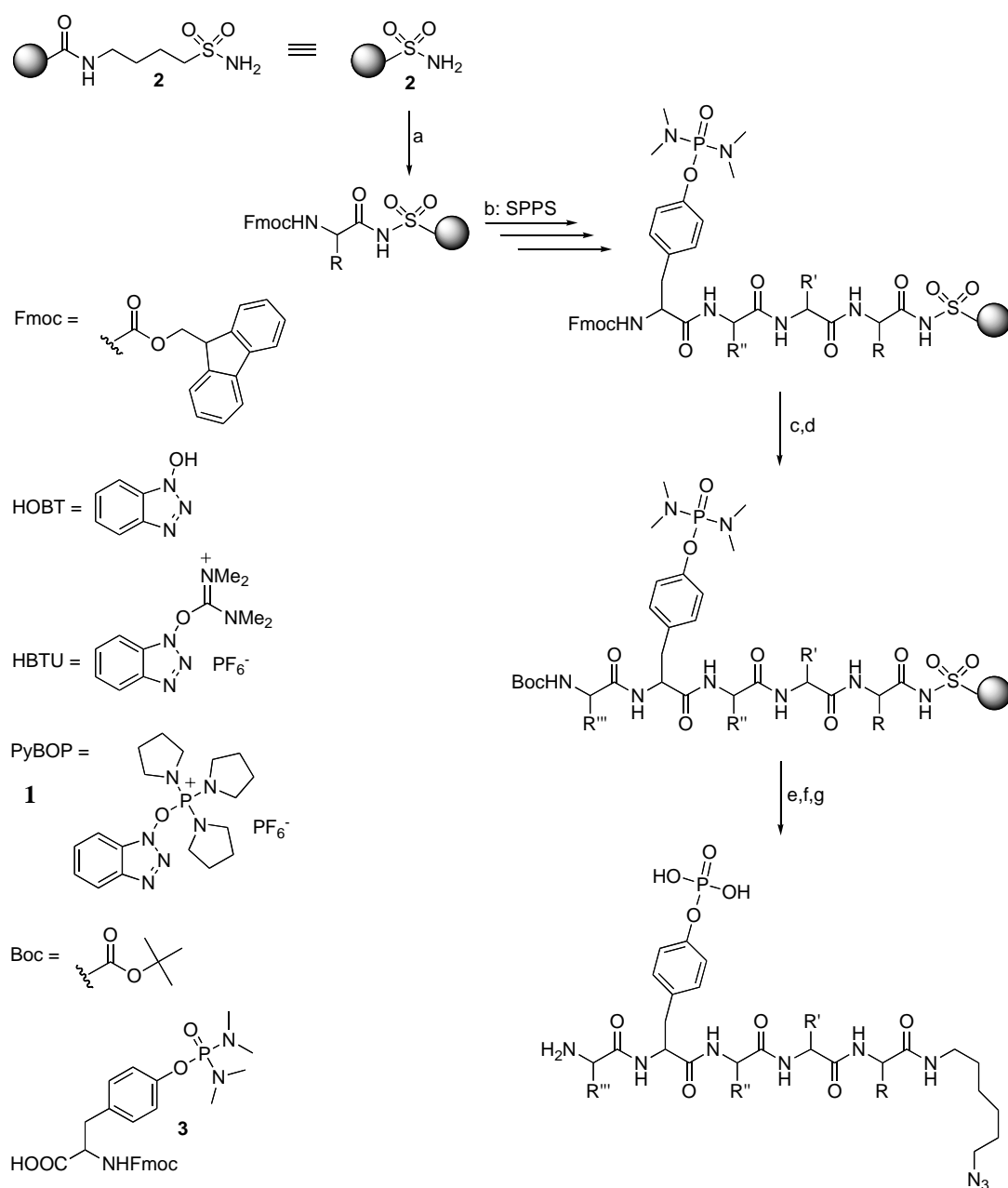
A Microarray Strategy for Mapping of Protein Tyrosine Phosphatase Substrate Specificity

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Synthesis of the phosphopeptides :

A library of pTyr-containing peptides composed of 1 tetra-, 1 hexa- and 46 pentapeptides was synthesized by means of solid phase peptide synthesis (SPPS) employing a resin equipped with the sulfamylbutyryl-linker¹ **2** which enables the introduction of the azide moiety within the safety-catch cleavage step. The pTyr-residue was introduced using PyBOP **1** and the pTyr building block **3**.² The pTyr was placed in second or third position of the peptide sequence counting from the *N*-terminus. To release the peptides from the solid support the *N*-acylsulfonamide was *N*-alkylated and treated with 6-azidoethylamine to yield the corresponding peptide-6-azidoethylamides (Supporting Scheme 1). After deprotection in solution and purification by HPLC, the pure peptides were directly used for spotting onto the glass slide.

The 48 peptides used for the microarray experiments were selected such that they would be sufficiently diverse in composition and position of amino acids, taking into account reported data about minimal requests concerning their substrates. Thus, the length of the peptides was chosen based on the finding that even tetrapeptides are substrates of PTPases.³



Supporting Scheme 1: Synthesis of a 48 member phosphopeptide library.

a: 3 equiv. Fmoc-amino acid, 5 equiv. (*i*Pr)₂NEt, 3 equiv. PyBOP, CHCl₃, -20°C, 8 h; b: Fmoc-SPPS: 4 equiv. Fmoc-amino acid, 4 equiv. HOBT, 3.6 equiv. HBTU, 8 equiv. (*i*Pr)₂NEt, *N*-dimethylformamide (DMF), 3 h or 3.5 equiv. Fmoc-pTyr **3**, 3.5 equiv. HOBT, 3.5 equiv. PyBOP, 7 equiv. (*i*Pr)₂NEt, DMF, 4 h; c: 20% piperidine in DMF, 2x20 min; d: 4 equiv. Boc-amino acid, 4 equiv. HOBT, 3.6 equiv. HBTU, 8 equiv. (*i*Pr)₂NEt, DMF, 3 h; e: 30 equiv. ICH₂CN, 5 equiv. (*i*Pr)₂NEt, *N*-methylpyrrolidinone, 24 h; f: 7.5 equiv. 6-azidoethylamine, tetrahydrofuran, 18 h; g: trifluoroacetic acid/CH₂Cl₂/triethylsilane (95:2.5:2.5), 3 h, then water (1:1, v/v), 21 h, 5-52% overall yield.

Analytical data of the pTyr-peptides:

aa = aminoacid; abbreviations of aminoacids in three-letter-code; DMSO = dimethylsulfoxide; FAB-HR = fast atom bombardment high resolution mass spectrometry; ESI-FTMS-HR = electron spray ionization Fourier transform high resolution mass spectrometry; HPLC = high pressure liquid chromatography. All peptides yielded single peaks on HPLC-ESI after HPLC-purification showing >95% purity. Standard Fmoc-protected amino acids, peptide reagents, and resins were purchased from Novabiochem. Pmp containing peptides were prepared by incorporating the corresponding non-natural amino acids during the coupling reactions. Fmoc-Pmp-OH was purchased from Advanced ChemTech and used without further modification.

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H₂N-Ala-Glu-pTyr-Gly-Asp-N-azidohexyl

$[\alpha]_D^{20} = -37.5^\circ$ (c = 1.09; H₂O).

¹H-NMR (400 MHz, D₂O): $\delta = 7.10$ (d, ³J = 8.4 Hz, 2H, 2 pTyr-ar-H), 7.00 (d, ³J = 8.2 Hz, 2H, 2 pTyr-ar-H), 4.56 (t, ³J = 6.6 Hz, 1H, pTyr- α -H), 4.53-4.46 (m, 1H, Asp- α -H), 4.26-4.22 (m, 1H, Glu- α -H), 3.92 (q, ³J = 7.1 Hz, 1H, Ala- α -H), 3.86-3.68 (m, 2H, Gly-CH₂), 3.16 (dd, ²J = 5.0 Hz, ³J = 6.7 Hz, 2H, N₃-CH₂), 3.19-3.01 (m, 3H, Tyr-CH_a, CH₂), 2.91-2.68 (m, 3H, pTyr-CH_b, CH₂), 2.27-2.22 (m, 2H, Glu-CH₂), 1.93-1.75 (m, 2H, Glu-CH₂), 1.49-1.31 (m, 7H, Ala-CH₃, 2 CH₂), 1.27-1.14 (m, 4H, CH₂).

¹³C-NMR (100 MHz, D₂O): $\delta = 177.0, 174.2, 172.8, 172.7, 172.0, 171.2, 170.7$ (7 C=O), 151.1 (pTyr-ar-C-O), 132.1 (pTyr-ar-C-CH₂), 130.5 (2 pTyr-ar-C *meta* O), 120.7 (2 pTyr-ar-C *ortho* zu O), 55.2, 55.1, 53.0, 51.3, 50.4, 49.1 (CH₂-N₃, pTyr- α -C, Asp- α -C, Gly- α -C, Glu- α -C, Ala- α -C), 42.8, 39.6, 35.7 (pTyr-CH₂, Asp-CH₂, CH₂-NH), 29.9, 28.3, 28.1, 26.4, 25.7, 25.6 (6 CH₂), 16.7 (Ala-CH₃).

³¹P-NMR (162 MHz, D₂O): $\delta = -3.3$ (PO₄).

FAB-HR: calc.: 758.2869 [M + H]⁺

found: 758.2870 [M + H]⁺

5

H₂N-Ala-Trp-pTyr-Gly-Thr-N-azidohexyl

$[\alpha]_D^{20} = -6.4^\circ$ (c = 0.36; DMSO).

¹H-NMR (400 MHz, DMSO-d₆): $\delta = 7.61$ (d, ³J = 7.8 Hz, 1H, Trp-ar-H), 7.29 (d, ³J = 8.0 Hz, 1H, Trp-ar-H), 7.09-6.93 (m, 7H, 3 Trp-ar-H, 4 pTyr-ar-H), 4.62-4.56 (m, 1H, Trp- α -H), 4.51-4.44 (m,

¹H, pTyr- α -H), 4.11 (dd, ²J = 4.1 Hz, ³J = 8.6 Hz, 1H, Thr- α -H), 4.01-3.95 (m, 1H, Ala- α -H), 3.80 (d, ³J = 5.5 Hz, 2H, Gly-CH₂), 3.65-3.20 (br, H₂O), 3.25 (t, ³J = 6.9 Hz, 2H, N₃-CH₂), 3.11-2.96 (m, 4H, pTyr-CH₂, NH-CH₂), 2.85 (dd, ²J = 9.5 Hz, ³J = 14.9 Hz, 1H, Trp-CHa), 2.74-2.67 (m, 1H, Trp-CHb), 1.51-1.43 (m, 2H, CH₂), 1.42-1.34 (m, 2H, CH₂), 1.30-1.21 (m, 4H, 2 CH₂), 1.16 (d, ³J = 6.9 Hz, 3H, Ala-CH₃), 1.02 (d, ³J = 6.4 Hz, 3H, Thr-CH₃).

¹³C-NMR (100 MHz, DMSO-d₆): δ = 172.2, 171.9, 170.4, 169.6, 169.5 (5 C=O), 136.8, 130.4, 127.9, 126.8, 124.5, 123.4, 121.5, 120.0, 119.1, 118.8 (10 ar-C), 112.0 (Trp-ar-C), 67.1 (Thr-CH), 59.1, 54.1 (2 aa- α -C), 51.2 (CH₂-N₃), 40.6, 39.2 (pTyr-CH₂, NH-CH₂), 29.5, 28.8, 26.5 (4 CH₂), 20.7 (Ala-CH₃), 17.6 (Thr-CH₃).

³¹P-NMR (162 MHz, DMSO-d₆): δ = -5.4 (PO₄).

FAB-HR: calc.: 801.3444 [M + H]⁺

found: 801.3447 [M + H]⁺

6

H₂N-Glu-Phe-pTyr-Met-Asp-N-azidohexyl

¹H-NMR (400 MHz, D₂O, NEt₃ added for solubility): δ = 7.23-7.12 (m, 3H, 3 ar-H), 7.08-6.98 (m, 6H, 6 ar-H), 4.47-4.35 (m, 4H, 4 aa- α -H), 4.19 (t, ³J = 7.0 Hz, 1H, aa- α -H), 3.18-3.06 (t, ³J = 6.9 Hz, 2H, N₃-CH₂), 2.82-2.69 (m, 4H, 2 CH₂), 2.56 (dd, ²J = 4.3 Hz, ³J = 15.9 Hz, 1H, CHb), 2.47-2.27 (m, 3H, CHb, CH₂), 1.95 (s, 3H, Met-CH₃), 1.94-1.80 (m, 6H, 3 CH₂), 1.59-1.31 (m, 6H, 3 CH₂), 1.21-1.09 (m, 4H, 2 CH₂).

³¹P-NMR (162 MHz, D₂O, NEt₃ added for solubility): δ = 1.1 (PO₄).

FAB-HR: calc.: 908.3372 [M + H]⁺

found: 908.3368 [M + H]⁺

7

H₂N-Leu-Cys-pTyr-Lys-Ala-N-azidohexyl

FAB-HR: calc.: 801.3847 [M + H]⁺

found: 801.3893 [M + H]⁺

8

H₂N-Ala-Asp-pTyr-Tyr-Ala-N-azidohexyl

¹H-NMR (400 MHz, DMSO-d₆): δ = 7.05-6.95 (m, 6H, 4 Tyr-ar-H, 2 pTyr-arH), 6.63 (d, ³J = 8.6 Hz, 2H, 2 pTyr-ar-H), 4.52-4.45 (m, 1H, pTyr- α -H), 4.43-4.35 (m, 2H, Tyr- α -H, Asp- α -H), 4.20 (q, ³J = 7.2 Hz, 1H, Ala- α -H), 3.63 (q, ³J = 7.0 Hz, 1H, Ala- α -H), 3.27 (t, ³J = 6.8 Hz, 2H, N₃-

CH₂), 3.08-2.86 (m, 4H, NH-CH₂, aa-CH₂), 2.71 (dd, ²J = 9.2 Hz, ³J = 13.9 Hz, 1H, aa-CHa), 2.61 (dd, ²J = 9.8 Hz, ³J = 14.0 Hz, 1H, aa-CHb), 2.35 (dd, ²J = 9.2 Hz, ³J = 17.2 Hz, 2H, aa-CH₂), 1.53-1.45 (m, 2H, CH₂), 1.40-1.13 (m, 12H, 3 CH₂, 2 Ala-CH₃).

¹³C-NMR (100 MHz, DMSO-d₆): δ = 172.4, 172.3, 171.5, 171.3, 170.6, 170.0 (6 C=O), 156.6 (2 Tyr-ar-C-O), 130.8, 130.5, 128.3, 120.4, 115.6 (10 ar-C), 55.1, 54.4 (2 Tyr-α-C), 51.3 (N₃-CH₂), 50.5, 49.0, 48.6 (Asp-α-C, 2 Ala-α-C), 39.6, 39.0, 37.3, 36.5 (CH₂-NH, 2 Tyr-CH₂, Asp-CH₂), 29.5, 28.9, 26.5, 26.4 (4 CH₂), 19.1, 17.6 (2 Ala-CH₃).

³¹P-NMR (162 MHz, DMSO-d₆): δ = -5.1 (PO₄).

FAB-HR: calc.: 806.3238 [M + H]⁺

found.: 806.3254 [M + H]⁺

9

H₂N-Leu-Tyr-pTyr-Lys-Gln-N-azidohexyl

[α]_D²⁰ = +18.4° (c = 0.43; DMSO).

¹H-NMR (400 MHz, DMSO-d₆): δ = 7.06-6.97 (m, 6H, 4 Tyr-ar-H, 2 pTyr-ar-H), 6.60 (d, ³J = 8.4 Hz, 2H, 2 pTyr-ar-H), 4.58-4.49 (m, 1H, pTyr-α-H), 4.46-4.37 (m, 1H, Tyr-α-H), 4.20-4.06 (m, 2H, Lys-α-H, Gln-α-H), 3.61-3.54 (m, 1H, Leu-α-H), 3.27 (t, ³J = 6.9 Hz, 2H, N₃-CH₂), 3.08-2.81 (m, 5H, NH-CH₂, Tyr-CHa, Tyr-CH₂), 2.73-2.55 (m, 3H, Tyr-CHb, Lys-CH₂), 2.09-2.02 (m, 2H, Gln-CH₂), 1.89-1.71 (m, 2H, Gln-CH₂), 1.64-1.07 (m, 17H, Leu-CH, Leu-CH₂, 3 Lys-CH₂, 4 CH₂), 0.83 (t, ³J = 5.5 Hz, 6H, 2 Leu-CH₃).

³¹P-NMR (162 MHz, DMSO-d₆): δ = -5.0 (PO₄).

FAB-HR: calc.: 918.4603 [M + H]⁺

found: 918.4623 [M + H]⁺

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H₂N-Gly-Met-pTyr-Met-Ala-N-azidohexyl

³¹P-NMR (162 MHz, DMSO-d₆): δ = -4.2 (PO₄).

FAB-HR: calc.: 776.2989 [M + H]⁺

found: 776.2953 [M + H]⁺

11

H₂N-Pro-Tyr-pTyr-Tyr-Val-N-azidohexyl

[α]_D²⁰ = -13.4° (c = 0.58; DMSO).

¹H-NMR (400 MHz, DMSO-d₆): δ = 7.03-6.90 (m, 8H, 4 Tyr-ar-H, 4 pTyr-ar-H), 6.63-6.57 (m, 4H, 4 Tyr-ar-H), 4.55-4.48 (m, 1H, pTyr-α-H), 4.45-4.39 (m, 1H, Tyr-α-H), 4.30-4.23 (m, 1H, Tyr-α-H), 4.08 (dd, ²J = 7.1 Hz, ³J = 8.6 Hz, 1H, Val-α-H), 3.72-3.67 (m, 1H, Pro-α-H), 3.27 (t, ³J = 6.8 Hz, 2H, N₃-CH₂), 3.07-2.83 (m, 8H, pTyr-CH₂, 2 Tyr-CH₂, NH-CH₂), 2.76-2.68 (m, 3H, Val-CH, Pro-CH₂), 2.04-1.87 (m, 2H, Pro-CH₂), 1.68-1.58 (m, 2H, Pro-CH₂), 1.51-1.44 (m, 2H, CH₂), 1.39-1.34 (m, 2H, CH₂), 1.31-1.24 (m, 4H, 2 CH₂), 0.82 (d, ³J = 6.7 Hz, 6H, 2 Val-CH₃).

¹³C-NMR (100 MHz, DMSO-d₆): δ = 171.6, 171.5, 171.1 (5 C=O), 156.6 (2 Tyr-ar-C-OH), 153.1 (pTyr-ar-C-O), 131.0, 130.8, 130.7, 131.4, 131.3, 128.2 (6 Tyr-ar-C, 3 pTyr-ar-C), 120.1 (2 pTyr-ar-C *ortho* zu O), 115.6 (4 Tyr-ar-C *ortho* zu O), 59.6, 58.6, 54.9, 51.3, 51.2, 46.2, 46.1 (5 aa-α-C, N₃-CH₂, Pro-CH₂), 39.6, 39.0, 37.3 (2 Tyr-CH₂, CH₂-NH, pTyr-CH₂), 31.3, 30.1, 29.5, 28.9, 26.6, 26.5, 24.4 (2 Pro-CH₂, Val-CH, 4 CH₂), 19.8, 18.9 (2 Val-CH₃).

³¹P-NMR (162 MHz, DMSO-d₆): δ = -4.2 (PO₄).

FAB-HR: calc.: 908.4072 [M + H]⁺

found: 908.4060 [M + H]⁺

12

H₂N-Phe-Ser-pTyr-Asp-Gly-N-azidohexyl

[α]_D²⁰ = -4.2° (c = 0.48; DMSO).

¹H-NMR (400 MHz, D₂O, NEt₃ added for solubility): δ = 7.21-7.15 (m, 3H, 3 ar-H), 7.10-6.99 (m, 6H, 6 ar-H), 4.41-4.36 (m, 2H, pTyr-α-H, Asp-α-H), 4.23 (t, ³J = 5.9 Hz, 1H, Ser-α-H), 3.73-3.66 (m, 3H, Phe-α-H, Gly-CH₂), 3.53 (d, ³J = 5.9 Hz, 2H, Ser-CH₂), 3.18-3.11 (m, 3H, CHa, N₃-CH₂), 3.07-2.92 (m, 1H, CHb), 2.88-2.75 (m, 4H, 2 CH₂), 2.50 (d, ³J = 6.3 Hz, 2H, Asp-CH₂), 1.47-1.32 (m, 4H, 2 CH₂), 1.25-1.11 (m, 4H, 2 CH₂).

³¹P-NMR (162 MHz, D₂O, NEt₃ added for solubility): δ = 0.9 (PO₄).

FAB-HR: calc.: 792.3082 [M + H]⁺

found: 792.3078 [M + H]⁺

13

H₂N-Pro-Ile-pTyr-Ala-Thr-N-azidohexyl

¹H-NMR (400 MHz, DMSO-d₆): δ = 7.09 (d, ³J = 7.8 Hz, 2H, 2 pTyr-ar-H), 6.97 (d, ³J = 8.1 Hz, 2H, 2 pTyr-ar-H), 4.52-4.47 (m, 1H, pTyr-α-H), 4.37-4.31 (m, 1H, Ala-α-H), 4.16-4.05 (m, 2H, Thr-α-H, Ile-α-H), 3.98-3.93 (m, 1H, Pro-α-H), 3.65-3.20 (br, H₂O), 3.28 (t, ³J = 6.9 Hz, 2H, N₃-CH₂), 3.11-2.95 (m, 6H, pTyr-CH₂, NH-CH₂, Pro-CH₂), 2.72-2.64 (m, 1H, Ile-CH), 1.89-1.81 (m, 2H, Pro-CH₂), 1.71-1.64 (m, 1H, Pro-CHa), 1.62-1.45 (m, 3H, Pro-CHb, CH₂), 1.43-1.20 (m,

11H, 3 CH₂, Ile-CH₂, Ala-CH₃), 1.16 (t, ³J = 7.3 Hz, 3H, Ile-CH₃), 1.00 (d, ³J = 6.4 Hz, 3H, Thr-CH₃), 0.81-0.77 (m, 3H, Ile-CH₃).

³¹P-NMR (162 MHz, DMSO-d₆): δ = -4.5 (PO₄).

FAB-HR: calc.: 768.3804 [M + H]⁺

found: 768.3829 [M + H]⁺

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H₂N-Ala-Arg-pTyr-Phe-Ala-N-azidohexyl

[α]_D²⁰ = +1.8° (c = 0.38; DMSO).

¹H-NMR (400 MHz, D₂O): δ = 7.25-7.07 (m, 5H, 5 Phe-ar-H), 7.00 (d, ³J = 8.3 Hz, 2H, 2 pTyr-ar-H), 6.94 (d, ³J = 8.3 Hz, 2H, 2 pTyr-ar-H), 4.48-4.39 (m, 2H, pTyr-α-H, Phe-α-H), 4.11-4.03 (m, 2H, Ala-α-H, Arg-α-H), 3.88 (q, ³J = 7.0 Hz, 1H, Ala-α-H), 3.17-3.12 (m, 3H, N₃-CH₂, pTyr-CHa), 3.01-2.72 (m, 7H, Phe-CH₂, pTyr-CHb, CH₂, NH-CH₂), 1.51-1.41 (m, 4H, 2 CH₂), 1.38-1.00 (m, 14H, 4 CH₂, 2 Ala-CH₃).

³¹P-NMR (162 MHz, D₂O): δ = -3.3 (PO₄).

FAB-HR: calc.: 831.4025 [M + H]⁺

found: 831.4047 [M + H]⁺

15

H₂N-Leu-Ser-pTyr-Arg-Gly-N-azidohexyl

[α]_D²⁰ = -6.5° (c = 1.00; H₂O).

¹H-NMR (400 MHz, D₂O): δ = 7.12 (d, ³J = 8.4 Hz, 2H, 2 pTyr-ar-H), 7.03 (d, ³J = 8.0 Hz, 2H, 2 pTyr-ar-H), 4.51 (t, ³J = 7.3 Hz, 1H, pTyr-α-H), 4.42 (t, ³J = 6.0 Hz, 1H, aa-α-H), 4.09 (dd, ²J = 5.7 Hz, ³J = 8.6 Hz, 1H, aa-α-H), 3.94 (t, ³J = 7.2 Hz, 1H, aa-α-H), 3.74-3.69 (m, 4H, 2 CH₂), 3.19 (t, ³J = 6.9 Hz, 2H, N₃-CH₂), 3.13-3.02 (m, 4H, 2 CH₂), 2.97 (d, ³J = 7.2 Hz, 2H, Tyr-CH₂), 1.75-1.35 (m, 11H, CH, 5 CH₂), 1.31-1.18 (m, 4H, 2 CH₂), 0.85 (t, ³J = 6.7 Hz, 6H, 2 Leu-CH₃).

³¹P-NMR (162 MHz, D₂O): δ = -3.1 (PO₄).

FAB-HR: calc.: 799.3974 [M + H]⁺

found: 799.4009 [M + H]⁺

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H₂N-Phe-Lys-pTyr-Gly-Asp-N-azidohexyl

[α]_D²⁰ = +3.5° (c = 1.00; H₂O).

$^1\text{H-NMR}$ (400 MHz, D_2O): $\delta = 7.26\text{-}7.22$ (m, 3H, 3 Phe-ar-H), 7.16-7.09 (m, 4H, 2 Phe-ar-H, 2 pTyr-ar-H), 7.05 (d, $^3\text{J} = 8.1$ Hz, 2H, 2 pTyr-ar-H), 4.58-4.52 (m, 1H, pTyr- α -H), 4.43 (t, $^3\text{J} = 7.5$ Hz, 1H, Asp- α -H), 4.17-4.10 (m, 2H, Phe- α -H, Lys- α -H), 3.88-3.67 (m, 2H, Gly- CH_2), 3.19-3.14 (m, 2H, $\text{N}_3\text{-CH}_2$), 3.10-3.00 (m, 5H, Tyr-CHa, 2 CH_2), 2.95-2.88 (m, 1H, pTyr-CHb), 2.83-2.75 (m, 4H, 2 CH_2), 1.52-1.33 (m, 8H, 4 CH_2), 1.20-1.06 (m, 6H, 3 CH_2).

$^{13}\text{C-NMR}$ (100 MHz, D_2O): $\delta = 174.2, 173.6, 172.7, 171.9, 171.2, 169.1$ (6 C=O), 151.3 (pTyr-ar-C-O), 133.8, 132.0, 130.6, 129.6, 129.4, 128.2, 120.7, 120.6 (11 ar-C), 55.5, 55.4, 54.4, 53.7, 51.3, 50.4 ($\text{CH}_2\text{-N}_3$, 5 aa- α -C), 42.7, 39.6, 39.3, 37.0, 36.1 (Phe- CH_2 , pTyr- CH_2 , Asp- CH_2 , Lys- $\text{CH}_2\text{-NH}_2$, NH- CH_2), 30.8, 28.3, 28.0, 26.5, 25.7, 25.6, 22.0 (7 CH_2).

$^{31}\text{P-NMR}$ (162 MHz, D_2O): $\delta = -3.1$ (PO_4).

FAB-HR: calc.: 833.3706 [M + H] $^+$

found: 833.3728 [M + H] $^+$

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$\text{H}_2\text{N-Ala-Lys-pTyr-Glu-Gly-N-azidohexyl}$

$[\alpha]_{\text{D}}^{20} = -15.3^\circ$ (c = 1.00; H_2O).

$^1\text{H-NMR}$ (400 MHz, D_2O): $\delta = 7.12$ (d, $^3\text{J} = 8.5$ Hz, 2H, 2 pTyr-ar-H), 7.02 (d, $^3\text{J} = 8.2$ Hz, 2H, 2 pTyr-ar-H), 4.54 (t, $^3\text{J} = 8.2$ Hz, 1H, pTyr- α -H), 4.23 (dd, $^2\text{J} = 5.5$ Hz, $^3\text{J} = 9.0$ Hz, 1H, Asp- α -H), 4.14 (t, $^3\text{J} = 7.2$ Hz, 1H, Lys- α -H), 3.95 (q, $^3\text{J} = 6.9$ Hz, 1H, Ala- α -H), 3.74 (s, 2H, Gly- CH_2), 3.18 (t, $^3\text{J} = 6.9$ Hz, 2H, $\text{N}_3\text{-CH}_2$), 3.13-3.08 (m, 2H, CH_2), 3.01 (dd, $^2\text{J} = 6.9$ Hz, $^3\text{J} = 13.9$ Hz, 1H, Tyr-CHa), 2.92 (dd, $^2\text{J} = 8.7$ Hz, $^3\text{J} = 13.8$ Hz, 1H, Tyr-CHb), 2.83 (t, $^3\text{J} = 7.6$ Hz, 2H, CH_2), 2.31 (t, $^3\text{J} = 7.3$ Hz, 2H, CH_2), 2.06-1.96 (m, 1H, Asp- β -CHa), 1.88-1.78 (m, 1H, Asp- β -CHb), 1.60-1.35 (m, 11H, Ala- CH_3 , 4 CH_2), 1.31-1.12 (m, 6H, 3 CH_2).

$^{31}\text{P-NMR}$ (162 MHz, D_2O): $\delta = -3.2$ (PO_4).

FAB-HR: calc.: 771.3555 [M + H] $^+$

found: 771.3562 [M + H] $^+$

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$\text{H}_2\text{N-Leu-Ala-pTyr-Glu-Asp-N-azidohexyl}$

FAB-HR: calc.: 814.3500 [M + H] $^+$

found: 814.3481 [M + H] $^+$

19

$\text{H}_2\text{N-Glu-Gly-pTyr-Gly-Gln-N-azidohexyl}$

$^1\text{H-NMR}$ (400 MHz, D_2O): $\delta = 7.11$ (d, $^3\text{J} = 8.4$ Hz, 2H, 2 pTyr-ar-H), 7.04 (d, $^3\text{J} = 8.3$ Hz, 2H, 2 pTyr-ar-H), 4.51 (dd, $^2\text{J} = 6.2$ Hz, $^3\text{J} = 8.5$ Hz, 1H, pTyr- α -H), 4.16 (dd, $^2\text{J} = 5.1$ Hz, $^3\text{J} = 9.2$ Hz, 1H, Gln- α -H) 3.91-3.71 (m, 5H, Glu- α -H, 2 Gly- CH_2), 3.19 (t, $^3\text{J} = 6.9$ Hz, 2H, N_3 - CH_2), 3.12-3.04 (m, 3H, pTyr-CHa, CH_2), 2.90 (dd, $^2\text{J} = 8.9$ Hz, $^3\text{J} = 14.1$ Hz, 1H, pTyr-CHb), 2.42-2.37 (m, 2H, CH_2), 2.23 (t, $^3\text{J} = 8.5$ Hz, 2H, CH_2), 2.10-1.98 (m, 3H, CHa, CH_2), 1.91-1.82 (m, 1H, CHb), 1.51-1.39 (m, 4H, 2 CH_2), 1.31-1.19 (m, 4H, 2 CH_2).

$^{31}\text{P-NMR}$ (162 MHz, D_2O): $\delta = -3.0$ (PO_4).

FAB-HR: calc.: 757.3034 [$\text{M} + \text{H}$] $^+$

found: 757.3014 [$\text{M} + \text{H}$] $^+$

20

$\text{H}_2\text{N-Glu-Lys-pTyr-His-Val-N-azidoethyl}$

$[\alpha]_{\text{D}}^{20} = -17.9^\circ$ ($c = 0.80$; H_2O).

$^1\text{H-NMR}$ (400 MHz, D_2O): $\delta = 8.51$ (s, 1H, His-ar-H), 7.12 (s, 1H, His-ar-H), 7.08 (d, $^3\text{J} = 8.4$ Hz, 2H, 2 pTyr-ar-H), 7.00 (d, $^3\text{J} = 8.1$ Hz, 2H, 2 pTyr-ar-H), 4.53 (t, $^3\text{J} = 7.2$ Hz, 1H, aa- α -H), 4.47 (t, $^3\text{J} = 8.4$ Hz, 1H, aa- α -H), 4.15 (t, $^3\text{J} = 6.9$ Hz, 1H, aa- α -H), 3.95 (t, $^3\text{J} = 6.4$ Hz, 1H, aa- α -H), 3.85 (d, $^3\text{J} = 8.0$ Hz, 1H, aa- α -H), 3.20 (t, $^3\text{J} = 6.8$ Hz, 2H, N_3 - CH_2), 3.16-2.87 (m, 6H, His- CH_2 , pTyr- CH_2 , CH_2), 2.83 (t, $^3\text{J} = 7.1$ Hz, 2H, NH- CH_2), 2.39-2.34 (m, 2H, CH_2), 2.06-1.98 (m, 2H, CH_2), 1.92-1.86 (m, 1H, CH), 1.58-1.38 (m, 8H, 4 CH_2), 1.26-1.12 (m, 6H, 3 CH_2), 0.85 (d, $^3\text{J} = 6.8$ Hz, 3H, Val- CH_3), 0.82 (d, $^3\text{J} = 6.8$ Hz, 3H, Val- CH_3).

$^{31}\text{P-NMR}$ (162 MHz, D_2O): $\delta = -3.1$ (PO_4).

FAB-HR: calc.: 879.4242 [$\text{M} + \text{H}$] $^+$

found: 879.4212 [$\text{M} + \text{H}$] $^+$

21

$\text{H}_2\text{N-Ala-Ala-pTyr-Lys-Gly-N-azidoethyl}$

$[\alpha]_{\text{D}}^{20} = -19.5^\circ$ ($c = 1.00$; H_2O).

$^1\text{H-NMR}$ (400 MHz, D_2O): $\delta = 7.13$ (d, $^3\text{J} = 8.4$ Hz, 2H, 2 pTyr-ar-H), 7.03 (d, $^3\text{J} = 8.3$ Hz, 2H, 2 pTyr-ar-H), 4.48 (t, $^3\text{J} = 7.6$ Hz, 1H, pTyr- α -H), 4.24 (q, $^3\text{J} = 7.1$ Hz, 1H, Ala- α -H), 4.08 (dd, $^2\text{J} = 6.1$ Hz, $^3\text{J} = 8.4$ Hz, 1H, Lys- α -H), 3.95 (q, $^3\text{J} = 7.1$ Hz, 1H, Ala- α -H), 3.92 (s, 2H, Gly- CH_2), 3.19 (t, $^3\text{J} = 6.8$ Hz, 2H, N_3 - CH_2), 3.15-3.06 (m, 2H, CH_2), 2.96 (d, $^3\text{J} = 7.6$ Hz, 2H, Tyr- CH_2), 2.86 (t, $^3\text{J} = 7.6$ Hz, 2H, CH_2), 1.73-1.38 (m, 11H, Ala- CH_3 , 4 CH_2), 1.33-1.16 (m, 9H, Ala- CH_3 , 3 CH_2).

$^{31}\text{P-NMR}$ (162 MHz, D_2O): $\delta = -3.1$ (PO_4).

FAB-HR: calc.: 713.3500 [M + H]⁺
found: 713.3477 [M + H]⁺

22

H₂N-Phe-Gln-pTyr-Gln-Asp-N-azidohexyl

[α]_D²⁰ = +10.8° (c = 0.36; DMSO).

¹H-NMR (400 MHz, DMSO-D₆): δ = 7.33-7.22 (m, 6H, 4 Phe-ar-H, 2 pTyr-ar-H), 7.13-6.99 (m, 3H, 1 Phe-ar-H, 2 pTyr-ar-H), 4.53-4.45 (m, 2H, pTyr-α-H, Asp-α-H), 4.35-4.09 (m, 3H, 2 Gln-α-H, Phe-α-H), 3.27 (t, ³J = 6.8 Hz, 2H, N₃-CH₂), 3.08-2.93 (m, 5H, pTyr-CHa, CH₂-NH, Phe-CH₂), 2.86-2.57 (m, 3H, pTyr-CHb, Asp-CH₂), 2.13-2.02 (m, 4H, 2 Gln-CH₂), 1.89-1.79 (m, 2H, Gln-CH₂), 1.78-1.67 (m, 2H, Gln-CH₂), 1.53-1.44 (m, 2H, CH₂), 1.41-1.33 (m, 2H, CH₂), 1.31-1.18 (m, 4H, 2 CH₂).

³¹P-NMR (162 MHz, DMSO-D₆): δ = -5.6 (PO₄).

FAB-HR: calc.: 904.3713 [M + H]⁺
found: 904.3759 [M + H]⁺

23

H₂N-Glu-pTyr-Ala-Pro-Gln-N-azidohexyl

[α]_D²⁰ = -57.2° (c = 0.65; H₂O).

¹H-NMR (400 MHz, D₂O): δ = 7.10 (d, ³J = 8.4 Hz, 2H, 2 pTyr-ar-H), 7.01 (d, ³J = 8.1 Hz, 2H, 2 pTyr-ar-H), 4.54-4.47 (m, 2H, pTyr-α-H, Ala-α-H), 4.29 (dd, ²J = 5.7 Hz, ³J = 8.3 Hz, 1H, Gln-α-H), 4.09 (dd, ²J = 5.3 Hz, ³J = 9.0 Hz, 1H, Pro-α-H), 3.82 (t, ³J = 6.6 Hz, 1H, Glu-α-H), 3.62-3.48 (m, 2H, Pro-CH₂-N), 3.18 (t, ³J = 6.9 Hz, 2H, N₃-CH₂), 3.12-3.04 (m, 2H, CH₂), 2.99 (dd, ²J = 6.7 Hz, ³J = 14.1 Hz, 1H, pTyr-CHa), 2.82 (dd, ²J = 9.1 Hz, ³J = 13.9 Hz, 1H, pTyr-CHb), 2.38-2.19 (m, 5H, CHa, 2 CH₂), 1.99-1.76 (m, 7H, CHb, 3 CH₂), 1.51-1.36 (m, 4H, 2 CH₂), 1.35-1.18 (m, 7H, 2 CH₂, Ala-CH₃).

³¹P-NMR (162 MHz, D₂O): δ = -3.1 (PO₄).

FAB-HR: calc.: 811.3504 [M + H]⁺
found: 811.3549 [M + H]⁺

24

H₂N-Gly-Ser-pTyr-Ala-Gly-N-azidohexyl

[α]_D²⁰ = -14.6° (c = 0.57; DMSO).

$^1\text{H-NMR}$ (400 MHz, DMSO-d_6): $\delta = 7.04$ (d, $^3\text{J} = 8.5$ Hz, 2H, 2 pTyr-ar-H), 6.94 (d, $^3\text{J} = 7.2$ Hz, 2H, 2 pTyr-ar-H), 4.48-4.40 (m, 2H, pTyr- α -H, Ala- α -H), 4.27-4.18 (m, 1H, Ser- α -H), 3.75-3.38 (m, 6H, 2 Gly- CH_2 , Ser- CH_2), 3.36-3.29 (m, 3H, pTyr-CHa, N_3 - CH_2), 3.09-2.96 (m, 3H, pTyr-CHb, NH- CH_2), 1.53-1.46 (m, 2H, CH_2), 1.43-1.35 (m, 2H, CH_2), 1.33-1.21 (m, 7H, 2 CH_2 , Ala- CH_3).

$^{13}\text{C-NMR}$ (100 MHz, DMSO-d_6): $\delta = 173.0, 171.6, 170.3, 169.1, 166.4$ (5 C=O), 152.9 (pTyr-ar-C-O), 131.6 (pTyr-ar-C- $\underline{\text{C}}$ - CH_2), 130.5 (2 pTyr-ar-C *meta* zu O), 119.9 (2 pTyr-ar-C *ortho* zu O), 62.1 (Ser- CH_2), 55.2, 54.6 (pTyr- α -C, Ser- α -C), 51.3 (CH_2 - N_3), 49.4, 46.3, 42.8 (2 Gly- α -C, Ala- α -C), 39.6, 39.1 (pTyr- CH_2 , CH_2 -NH), 29.6, 28.9, 26.5 (4 CH_2), 18.4 (Ala- CH_3).

$^{31}\text{P-NMR}$ (162 MHz, DMSO-d_6): $\delta = -4.2$ (PO_4).

FAB-HR: calc.: 658.2714 [M + H] $^+$

found: 658.2682 [M + H] $^+$

25

$\text{H}_2\text{N-Phe-pTyr-Arg-Ala-Asp-N-azidohexyl}$

FAB-HR: calc.: 875.3924 [M + H] $^+$

found: 875.3907 [M + H] $^+$

26

$\text{H}_2\text{N-Phe-pTyr-Tyr-Pro-Ala-N-azidohexyl}$

$[\alpha]_{\text{D}}^{20} = -12.3^\circ$ (c = 0.64; DMSO).

$^1\text{H-NMR}$ (400 MHz, DMSO-d_6): $\delta = 7.23$ -7.18 (m, 5H, 5 Phe-ar-H), 7.10-6.99 (m, 6H, 4 Tyr-ar-H, 2 pTyr-ar-H), 6.63 (d, $^3\text{J} = 8.2$ Hz, 2H, 2 pTyr-ar-H), 4.64-4.51 (m, 2H, pTyr- α -H, Tyr- α -H), 4.32 (dd, $^2\text{J} = 3.1$ Hz, $^3\text{J} = 8.0$ Hz, 1H, Pro- α -H), 4.18-4.12 (m, 1H, Ala- α -H), 3.96-3.91 (m, 1H, Phe- α -H), 3.60-3.54 (m, 1H, Pro-CHa), 3.44-3.35 (m, 1H, Pro-CHb), 3.26 (t, $^3\text{J} = 6.8$ Hz, 2H, N_3 - CH_2), 3.09-2.82 (m, 6H, NH- CH_2 , 2 aa- CH_2), 2.76-2.64 (m, 2H, aa- CH_2), 2.26-1.95 (m, 1H, Pro-CHa), 1.86-1.73 (m, 3H, Pro-CHb, Pro- CH_2), 1.52-1.44 (m, 2H, CH_2), 1.41-1.32 (m, 2H, CH_2), 1.30-1.15 (m, 7H, 2 CH_2 , Ala- CH_3).

$^{13}\text{C-NMR}$ (100 MHz, DMSO-d_6): $\delta = 172.7, 171.6, 171.0, 170.6, 168.6$ (5 C=O), 156.6, 153.0 (Tyr-ar-C-O, pTyr-ar-C-O), 135.5, 131.5, 131.2, 130.9, 130.8, 130.4, 130.3, 129.1, 128.3, 127.7, 120.3, 115.7 (16 ar-C), 60.3 (Pro- α -C), 54.7, 53.7, 53.1 (pTyr- α -C, Tyr- α -C, Phe- α -C), 51.2 (N_3 - CH_2), 49.2 (Ala- α -C), 47.6 (Pro- CH_2), 39.6, 39.0, 37.7 (3 CH_2), 29.5, 28.9, 26.5, 26.4, 25.1 (Pro- CH_2 , 4 CH_2), 18.9 (Ala- CH_3).

$^{31}\text{P-NMR}$ (162 MHz, DMSO-d_6): $\delta = -4.3$ (PO_4).

FAB-HR: calc.: 864.3810 [M + H]⁺
found: 864.3774 [M + H]⁺

27

H₂N-Phe-pTyr-Ser-Ser-Val-N-azidohexyl

$[\alpha]_D^{20} = +6.0^\circ$ (c = 0.48; DMSO).

¹H-NMR (400 MHz, DMSO-d₆): $\delta = 7.29-7.22$ (m, 5H, 5 Phe-ar-H), 7.12 (d, ³J = 8.4 Hz, 2H, 2 pTyr-ar-H), 7.01 (d, ³J = 8.4 Hz, 2H, 2 pTyr-ar-H), 4.66-4.59 (m, 1H, pTyr- α -H), 4.43-4.31 (m, 2H, 2 Ser- α -H), 4.08 (dd, ²J = 6.4 Hz, ³J = 8.8 Hz, 1H, Val- α -H), 3.94-3.90 (m, 1H, Phe- α -H), 3.66-3.62 (m, 2H, Ser-CH₂), 3.58-3.53 (m, 2H, Ser-CH₂), 3.27 (t, ³J = 6.8 Hz, 2H, N₃-CH₂), 3.18-2.88 (m, 6H, pTyr-CH₂, Phe-CH₂, NH-CH₂), 2.79-2.73 (m, 1H, Val-CH), 1.51-1.44 (m, 2H, CH₂), 1.40-1.32 (m, 2H, CH₂), 1.20-1.16 (m, 4H, 2 CH₂), 0.81 (d, ³J = 6.7 Hz, 3H, Val-CH₃), 0.79 (d, ³J = 6.8 Hz, 3H, Val-CH₃).

¹³C-NMR (100 MHz, DMSO-d₆): $\delta = 172.7, 172.2, 171.9, 171.6, 169.8$ (5 C=O), 154.1 (pTyr-ar-C-O), 137.5 (Phe-ar-C_q), 136.6, 132.5, 131.6, 131.4, 131.3 (4 Phe-arC, pTyr-ar-C-CH₂), 130.1 (2 pTyr-ar-C *meta* zu O), 128.7 (Phe-ar-C), 121.5 (2 pTyr-ar-C *ortho* zu O), 63.4, 63.2, 59.6, 56.9, 56.0, 54.8 (5 aa- α -C, 2 Ser-CH₂), 52.3 (N₃-CH₂), 40.6, 40.1, 38.6 (Phe-CH₂, CH₂-NH, pTyr-CH₂), 31.9, 30.4, 29.9, 27.5 (Val-CH, 4 CH₂), 20.9, 19.5 (2 Val-CH₃).

³¹P-NMR (162 MHz, DMSO-d₆): $\delta = -3.0$ (PO₄).

FAB-HR: calc.: 806.3597 [M + H]⁺
found: 806.3639 [M + H]⁺

28

H₂N-Gly-pTyr-Ser-Gly-Asp-N-azidohexyl

$[\alpha]_D^{20} = +0.2^\circ$ (c = 0.94; H₂O).

¹H-NMR (400 MHz, D₂O): $\delta = 7.14$ (d, ³J = 7.8 Hz, 2H, 2 pTyr-ar-H), 7.03 (d, ³J = 8.0 Hz, 2H, 2 pTyr-ar-H), 4.61-4.55 (m, 2H, pTyr- α -H, Asp- α -H), 4.34-4.31 (m, 1H, Ser- α -H), 3.85 (s, 2H, Gly-CH₂), 3.75-3.60 (m, 4H, Ser-CH₂, Gly-CH₂), 3.18 (t, ³J = 6.9 Hz, 2H, N₃-CH₂), 3.09-3.04 (m, 3H, pTyr-CH_a, CH₂), 2.93-2.70 (m, 3H, pTyr-CH_b, CH₂), 1.50-1.37 (m, 4H, 2 CH₂), 1.30-1.17 (m, 4H, 2 CH₂).

³¹P-NMR (162 MHz, D₂O): $\delta = -2.9$ (PO₄).

FAB-HR: calc.: 702.2607 [M + H]⁺
found: 702.2585 [M + H]⁺

29

H₂N-Glu-pTyr-Asp-Asp-Gln-N-azidohexyl

$[\alpha]_D^{20} = -7.4^\circ$ (c = 0.37; DMSO).

¹H-NMR (400 MHz, DMSO-d₆): $\delta = 7.08$ (d, ³J = 8.4 Hz, 2H, 2 pTyr-ar-H), 6.97 (d, ³J = 8.2 Hz, 2H, 2 pTyr-ar-H), 4.61-4.54 (m, 1H, pTyr- α -H), 4.53-4.42 (m, 2H, 2 Asp- α -H), 4.13-4.05 (m, 1H, Gln- α -H), 3.42-3.38 (m, 1H, Glu- α -H), 3.28 (t, ³J = 6.8 Hz, 2H, N₃-CH₂), 3.07-2.96 (m, 4H, NH-CH₂, pTyr-CH₂), 2.72 (t, ³J = 5.7 Hz, 1H, Asp-CH_a), 2.68 (t, ³J = 5.7 Hz, 1H, Asp-CH_b), 2.62-2.52 (m, 2H, Asp-CH₂), 2.23-2.00 (m, 4H, Gln-CH₂, Glu-CH₂), 1.95-1.85 (m, 1H, aa-CH_a), 1.81-1.61 (m, 3H, aa-CH_b, aa-CH₂), 1.53-1.45 (m, 2H, CH₂), 1.41-1.33 (m, 2H, CH₂), 1.31-1.19 (m, 4H, 2 CH₂).

¹³C-NMR (100 MHz, DMSO-d₆): $\delta = 174.8, 172.7, 172.6, 172.3, 172.0, 171.7, 171.6, 171.4, 170.9$ (9 C=O), 152.0 (pTyr-ar-C-O), 132.5 (pTyr-ar-C-CH₂), 130.7 (2 pTyr-ar-C *meta* zu O), 120.8 (2 pTyr-ar-C *ortho* zu O), 54.3, 53.3, 52.4 (pTyr- α -C, Gln- α -C, Glu- α -C), 51.3 (N₃-CH₂), 50.6 (2 Asp- α -C), 39.7, 39.1 (CH₂-NH, pTyr-CH₂), 36.4, 36.3 (2 Asp-CH₂), 32.3, 31.7 (Gln-CH₂, Glu-CH₂), 29.4, 28.9, 28.5, 26.7, 26.5, 26.4 (Gln-CH₂, Glu-CH₂, 4 CH₂).

³¹P-NMR (162 MHz, DMSO-d₆): $\delta = -5.2$ (PO₄).

FAB-HR: calc.: 873.3144 [M + H]⁺

found: 873.3159 [M + H]⁺

30

H₂N-Ala-Arg-pTyr-Gln-Asp-N-azidohexyl

FAB-HR: calc.: 856.3831 [M + H]⁺

found: 856.3814 [M + H]⁺

31

H₂N-Leu-pTyr-Glu-Asp-Ala-Asp-N-azidohexyl

¹H-NMR (400 MHz, CD₃OD): $\delta = 7.22$ (d, ³J = 8.2 Hz, 2H, 2 pTyr-ar-H), 7.13 (d, ³J = 8.6 Hz, 2H, 2 pTyr-ar-H), 4.68-4.63 (m, 3H, pTyr- α -H, 2 Asp- α -H), 4.34 (dd, ²J = 5.5 Hz, ³J = 8.0 Hz, 1H, Glu- α -H), 4.20 (q, ³J = 7.1 Hz, 1H, Ala- α -H), 3.81-3.77 (m, 1H, Leu- α -H), 3.27 (t, ³J = 6.8 Hz, 2H, N₃-CH₂), 3.21-3.12 (m, 2H, pTyr-CH₂), 3.02-2.86 (m, 4H, Asp-CH₂, NH-CH₂), 2.83-2.73 (m, 2H, Asp-CH₂), 2.40-2.34 (m, 2H, Glu-CH₂), 2.13-2.03 (m, 1H, Glu-CH_a), 1.97-1.87 (m, 1H, Glu-CH_b), 1.71-1.32 (m, 14H, Leu-CH, Leu-CH₂, 4 CH₂, Ala-CH₃), 1.00-0.96 (s, 6H, 2 Leu-CH₃).

³¹P-NMR (162 MHz, CD₃OD): $\delta = -3.1$ (PO₄).

FAB-HR: calc.: 929.3764 [M + H]⁺

found: 929.3744 [M + H]⁺

32

H₂N-Phe-Ile-pTyr-Ile-Val-N-azidohexyl

ESI-FTMS-HR: calc.: 858.46374 [M + H]⁺
found: 858.46381 [M + H]⁺

33

H₂N-Leu-pTyr-Arg-Ala-Gly-N-azidohexyl

[α]_D²⁰ = +6.7° (c = 1.00; H₂O).

¹H-NMR (400 MHz, D₂O): δ = 7.11 (d, ³J = 8.6 Hz, 2H, 2 pTyr-ar-H), 7.02 (d, ³J = 7.7 Hz, 2H, 2 pTyr-ar-H), 4.52 (t, ³J = 8.4 Hz, 1H, pTyr-α-H), 4.17-4.07 (m, 2H, 2 aa-α-H), 3.91-3.67 (m, 3H, Gly-CH₂, aa-α-H), 3.22-3.16 (dd, ²J = 6.8 Hz, ³J = 12.2 Hz, 2H, CH₂), 3.11-2.86 (m, 6H, 3 CH₂), 1.69-1.15 (m, 18H, Leu-CH, Ala-CH₃, 7 CH₂), 0.83 (t, ³J = 7.1 Hz, 6H, 2 Leu-CH₃).

³¹P-NMR (162 MHz, D₂O): δ = -3.1 (PO₄).

FAB-HR: calc.: 783.4031 [M + H]⁺
found: 783.4022 [M + H]⁺

34

H₂N-Ala-pTyr-Glu-Ala-Asp-N-azidohexyl

¹H-NMR (400 MHz, D₂O): δ = 7.11 (d, ³J = 8.6 Hz, 2H, 2 pTyr-ar-H), 7.02 (d, ³J = 8.6 Hz, 2H, 2 pTyr-ar-H), 4.53-4.49 (m, 2H, pTyr-α-H, Asp-α-H), 4.21 (dd, ²J = 5.6 Hz, ³J = 8.6 Hz, 1H, Glu-α-H), 4.12 (q, ³J = 7.2 Hz, 1H, Ala-α-H), 3.93 (q, ³J = 7.1 Hz, 1H, Ala-α-H), 3.22 (t, ³J = 6.7 Hz, 2H, N₃-CH₂), 3.09 (t, ³J = 6.8 Hz, 2H, pTyr-CH₂), 2.89 (t, ³J = 7.6 Hz, 2H, NH-CH₂), 2.82 (dd, ²J = 5.9 Hz, ³J = 16.4 Hz, 1H, Asp-CHa), 2.75 (dd, ²J = 7.3 Hz, ³J = 16.6 Hz, 1H, Asp-CHb), 2.32 (t, ³J = 7.2 Hz, 2H, Glu-CH₂), 1.99-1.90 (m, 1H, Glu-CHa), 1.85-1.75 (m, 1H, Glu-CHb), 1.61-1.45 (m, 4H, 2 CH₂), 1.39 (d, ³J = 6.6 Hz, 3H, Ala-CH₃), 1.33-1.19 (m, 7H, Ala-CH₃, 2 CH₂).

¹³C-NMR (100 MHz, D₂O): δ = 177.2, 177.1, 174.3, 172.8, 172.6, 171.9, 170.7 (7 C=O), 151.3 (pTyr-ar-C-O), 131.8, 130.5, 120.8, 118.3 (5 ar-C), 58.2, 52.7, 51.2, 50.5, 50.2, 49.0 (CH₂-N₃, 5 aa-α-C), 44.3, 39.6, 36.4, 35.6 (Asp-CH₂, pTyr-CH₂, NH-CH₂, CH₂), 29.9, 28.3, 27.9, 26.5, 25.6 (2 Glu-CH₂, 3 CH₂), 16.7, 16.5 (2 Ala-CH₃).

³¹P-NMR (162 MHz, D₂O): δ = -2.9 (PO₄).

FAB-HR: calc.: 772.3031 [M + H]⁺
found: 772.3062 [M + H]⁺

35

H₂N-Ala-pTyr-Gln-Ala-Gly-N-azidohexyl

$[\alpha]_D^{20} = +17.8^\circ$ (c = 0.55; DMSO).

¹H-NMR (400 MHz, D₂O): $\delta = 7.10$ (d, ³J = 8.4 Hz, 2H, 2 pTyr-ar-H), 7.02 (d, ³J = 8.2 Hz, 2H, 2 pTyr-ar-H), 4.50 (t, ³J = 7.4 Hz, 1H, pTyr- α -H), 4.19-4.09 (m, 2H, Gln- α -H, Ala- α -H), 3.93 (q, ³J = 7.0 Hz, 1H, Ala- α -H), 3.75 (dd, ²J = 17.0 Hz, ³J = 37.8 Hz, 2H, Gly-CH₂), 3.24-3.15 (m, 2H, N₃-CH₂), 3.13-3.04 (m, 2H, pTyr-CH₂), 2.95-2.85 (m, 2H, CH₂), 2.20-2.13 (m, 2H, Gln-CH₂), 1.93-1.77 (m, 2H, Gln-CH₂), 1.58-1.17 (m, 14H, 4 CH₂, 2 Ala-CH₃).

¹³C-NMR (100 MHz, D₂O): $\delta = 177.9, 175.6, 172.6, 172.3, 171.2, 170.7$ (6 C=O), 151.3 (pTyr-ar-C-O), 131.7, 130.5, 120.7 (5 ar-C), 55.4, 52.6, 51.3, 50.3, 49.2, 49.0 (CH₂-N₃, 5 aa- α -C), 42.8, 39.5, 36.5, 31.0 (Gln-CH₂, pTyr-CH₂, NH-CH₂, CH₂), 28.3, 27.9, 26.8, 25.6 (Gln-CH₂, 3 CH₂), 16.7, 16.4 (2 Ala-CH₃).

³¹P-NMR (162 MHz, D₂O): $\delta = -3.2$ (PO₄).

FAB-HR: calc.: 713.3136 [M + H]⁺

found: 713.3112 [M + H]⁺

36

H₂N-Ala-Val-pTyr-Trp-Thr-N-azidohexyl

¹H-NMR (400 MHz, D₂O): $\delta = 7.53$ (d, ³J = 7.9 Hz, 1H, Trp-ar-H), 7.41 (d, ³J = 8.0 Hz, 1H, Trp-ar-H), 7.19-7.09 (m, 5H, 3 Trp-ar-H, 2 pTyr-ar-H), 7.02 (d, ³J = 8.2 Hz, 2H, 2 pTyr-ar-H), 4.79-4.50 (br, H₂O), 3.98-3.90 (m, 3H, 3 aa- α -H), 3.25-2.80 (m, 9H, N₃-CH₂, Trp-CH₂, pTyr-CH₂, NH-CH₂, CH), 1.81-1.75 (m, 1H, CH), 1.53-1.20 (m, 11H, 4 CH₂, Ala-CH₃), 0.97 (d, ³J = 6.6 Hz, 3H, Thr-CH₃), 0.75 (d, ³J = 6.8 Hz, 3H, Val-CH₃), 0.65 (d, ³J = 6.7 Hz, 3H, Val-CH₃).

³¹P-NMR (162 MHz, D₂O): $\delta = -3.2$ (PO₄).

FAB-HR: calc.: 843.3919 [M + H]⁺

found: 843.3930 [M + H]⁺

37

H₂N-Leu-pTyr-Glu-Asp-N-azidohexyl

¹H-NMR (400 MHz, CD₃OD): $\delta = 7.07$ (d, ³J = 7.8 Hz, 2H, 2 pTyr-ar-H), 6.98 (d, ³J = 7.4 Hz, 2H, 2 pTyr-ar-H), 4.54-4.50 (m, 1H, pTyr- α -H), 4.49-4.45 (m, 1H, Asp- α -H), 4.18-4.14 (m, 1H, Glu- α -H), 3.80-3.75 (m, 1H, Leu- α -H), 3.13 (t, ³J = 6.8 Hz, 2H, N₃-CH₂), 3.06-3.02 (m, 2H, NH-CH₂), 2.97 (dd, ²J = 6.5 Hz, ³J = 14.5 Hz, 1H, pTyr-CHa), 2.82 (dd, ²J = 9.4 Hz, ³J = 14.5 Hz, 1H, pTyr-CHb), 2.75 (dd, ²J = 6.2 Hz, ³J = 17.0 Hz, 1H, Asp-CHa), 2.63 (dd, ²J = 7.0 Hz, ³J = 17.0 Hz, 1H, Asp-CHb), 2.24-2.19 (m, 2H, Glu-CH₂), 1.97-1.91 (m, 1H, Glu-CHa), 1.82-1.74 (m, 1H,

Glu-CHb), 1.55-1.32 (m, 7H, Leu-CH, Leu-CH₂, 2 CH₂), 1.25-1.15 (m, 4H, 2 CH₂), 0.80-0.76 (m, 6H, 2 Leu-CH₃).

¹³C-NMR (100 MHz, CD₃OD): δ = 176.6, 172.4, 172.2, 171.5, 170.5 (6 C=O), 147.3 (pTyr-ar-C-O), 133.8, 130.3, 120.6 (5 ar-C), 51.2, 50.4 (CH₂-N₃, aa- α -C), (48.5-47.7 CD₃OD), 40.1, 39.4, 35.6, 35.3 (Asp-CH₂, pTyr-CH₂, Leu-CH₂, NH-CH₂), 29.8, 28.4, 28.1, 26.0, 25.8, 25.7, 24.2 (Leu-CH, 2 Glu-CH₂, 4 CH₂), 21.8, 21.1 (2 Leu-CH₃).

³¹P-NMR (162 MHz, CD₃OD): δ = -3.2 (PO₄).

FAB-HR: calc.: 743.3129 [M + H]⁺

found: 743.3149 [M + H]⁺

38

H₂N-Met-pTyr-Gly-Asp-Ala-N-azidoheptyl

$[\alpha]_D^{20} = -3.2^\circ$ (c = 0.41; DMSO).

¹H-NMR (400 MHz, D₂O): δ = 7.12 (d, ³J = 8.3 Hz, 2H, 2 pTyr-ar-H), 7.02 (d, ³J = 8.3 Hz, 2H, 2 pTyr-ar-H), 4.54 (t, ³J = 7.5 Hz, 1H, aa- α -H), 4.13 (q, ³J = 7.1 Hz, 1H, Ala- α -H), 3.97 (t, ³J = 6.8 Hz, 1H, Met- α -H), 3.76 (dd, ²J = 17.0 Hz, ³J = 63.9 Hz, 2H, Gly-CH₂), 3.19 (t, ³J = 6.7 Hz, 2H, N₃-CH₂), 3.14-2.91 (m, 4H, pTyr-CH₂, NH-CH₂), 2.83 (dd, ²J = 6.0 Hz, ³J = 17.1 Hz, 1H, Asp-CHa), 2.74 (dd, ²J = 7.1 Hz, ³J = 17.2 Hz, 1H, Asp-CHb), 2.48-2.42 (m, 2H, Met-CH₂), 2.04-1.98 (m, 5H, Met-CH₂, Met-CH₃), 1.51-1.35 (m, 4H, 2 CH₂), 1.29-1.18 (m, 7H, 2 CH₂, Ala-CH₃).

³¹P-NMR (162 MHz, D₂O): δ = -3.0 (PO₄).

FAB-HR: calc.: 760.2848 [M + H]⁺

found: 760.2863 [M + H]⁺

39

H₂N-Leu-pTyr-Lys-Lys-Gln-N-azidoheptyl

$[\alpha]_D^{20} = -7.3^\circ$ (c = 1.09; H₂O).

¹H-NMR (400 MHz, D₂O): δ = 7.10 (d, ³J = 8.3 Hz, 2H, 2 pTyr-ar-H), 7.01 (d, ³J = 8.2 Hz, 2H, 2 pTyr-ar-H), 4.53 (t, ³J = 7.7 Hz, 1H, pTyr- α -H), 4.14-4.05 (m, 3H, Gln- α -H, 2 Lys- α -H), 3.90 (t, ³J = 7.2 Hz, 1H, Leu- α -H), 3.20 (t, ³J = 6.8 Hz, 2H, N₃-CH₂), 3.16-3.08 (m, 2H, pTyr-CH₂), 3.01-2.90 (m, 4H, 2 CH₂), 2.85 (t, ³J = 7.2 Hz, 2H, CH₂), 2.25 (t, ³J = 7.9 Hz, 2H, CH₂), 1.99-1.83 (m, 2H, CH₂), 1.70-1.32 (m, 19H, Leu-CH, 9 CH₂), 1.20-1.16 (m, 4H, 2 CH₂), 0.83 (t, ³J = 6.6 Hz, 6H, 2 Leu-CH₃).

¹³C-NMR (100 MHz, D₂O): δ = 178.0, 173.7, 172.9, 172.6, 172.0, 170.2 (6 C=O), 151.2 (pTyr-ar-C-O), 131.6 (pTyr-ar-C-CH₂), 130.5 (2 pTyr-ar-C *meta* zu O), 120.7 (2 pTyr-ar-C *ortho* zu O),

55.4, 53.9, 53.6, 53.0, 51.9, 51.3 (5 aa- α -C, CH₂-N₃), 40.1, 39.4, 39.3, 36.5 (CH₂-NH, pTyr-CH₂, Leu-CH₂, 2 Lys-CH₂-NH₂), 31.3, 31.1, 30.6, 28.8, 28.1, 27.2, 26.6, 26.5, 25.7, 25.6, 24.0, 22.3, 22.0 (12 CH₂, Leu-CH), 21.8, 21.4 (2 Leu-CH₃).

³¹P-NMR (162 MHz, D₂O): $\delta = -3.1$ (PO₄).

FAB-HR: calc.: 883.4919 [M + H]⁺

found: 883.4893 [M + H]⁺

40

H₂N-Leu-pTyr-Cys-Cys-Val-N-azidohexyl

³¹P-NMR (162 MHz, DMSO-d₆): $\delta = -4.1$ (PO₄).

C₃₂H₅₄N₉O₉PS₂ (803.32).

ESI-MS: calc.: 804.3 [M + H]⁺

found: 804.3 [M + H]⁺

41

H₂N-Phe-Phe-pTyr-Phe-Gln-N-azidohexyl

¹H-NMR (400 MHz, DMSO-d₆): $\delta = 7.35$ - 7.08 (m, 17H, 15 Phe-ar-H, 2 pTyr-ar-H), 7.07 - 6.96 (m, 2H, 2 pTyr-ar-H), 4.59 - 4.41 (m, 3H, pTyr- α -H, 2 Phe- α -H), 4.20 - 4.11 (m, 1H, Gln- α -H), 3.59 - 3.53 (m, 1H, Phe- α -H), 3.20 (t, ³J = 6.9 Hz, 2H, N₃-CH₂), 3.09 - 3.01 (m, 3H, aa-CHa, aa-CH₂), 2.99 - 2.81 (m, 4H, aa-CHb, NH-CH₂, aa-CHa), 2.71 - 2.56 (m, 3H, aa-CHb, aa-CH₂), 2.12 - 2.02 (m, 2H, Gln-CH₂), 1.89 - 1.71 (m, 2H, CH₂), 1.52 - 1.44 (m, 2H, CH₂), 1.42 - 1.20 (m, 6H, 3 CH₂).

ESI-FTMS-HR: calc.: 955.42260 [M + H]⁺

found: 955.42166 [M + H]⁺

42

H₂N-Gly-pTyr-Arg-Arg-Gln-N-azidohexyl

FAB-HR: calc.: 883.4416 [M + H]⁺

found: 883.4438 [M + H]⁺

43

H₂N-Gly-Trp-pTyr-Trp-Gln-N-azidohexyl

ESI-FTMS-HR: calc.: 943.39745 [M + H]⁺

found: 943.39783 [M + H]⁺

44

H₂N-Leu-pTyr-Ala-Ser-Gly-N-azidohexyl

$[\alpha]_D^{20} = +7.7^\circ$ (c = 0.52; DMSO).

¹H-NMR (400 MHz, CD₃OD): $\delta = 7.20$ (d, ³J = 8.0 Hz, 2H, 2 pTyr-ar-H), 7.12 (d, ³J = 8.2 Hz, 2H, 2 pTyr-ar-H), 4.68 (dd, ²J = 5.7 Hz, ³J = 9.6 Hz, 1H, pTyr- α -H), 4.35-4.29 (m, 2H, Ser- α -H, Ala- α -H), 3.92-3.75 (m, 5H, Ser-CH₂, Leu- α -H, Gly-CH₂), 3.26 (t, ³J = 6.8 Hz, 2H, N₃-CH₂), 3.21-3.12 (m, 3H, CH₂-CH₂-NH, pTyr-CHa), 2.93-2.86 (m, 1H, pTyr-CHb), 1.79-1.35 (m, 14H, 5 CH₂, Leu-CH, Ala-CH₃), 1.02-0.95 (m, 6H, 2 Leu-CH₃).

³¹P-NMR (162 MHz, CD₃OD): $\delta = -3.3$ (PO₄).

FAB-HR: calc.: 736.3159 [M + Na]⁺

found: 736.3179 [M + Na]⁺

45

H₂N-Pro-pTyr-Asp-Ile-Thr-N-azidohexyl

FAB-HR: calc.: 812.3702 [M + H]⁺

found: 812.3708 [M + H]⁺

46

H₂N-Gly-pTyr-Pro-Gly-Thr-N-azidohexyl

$[\alpha]_D^{20} = -34.0^\circ$ (c = 0.50; H₂O).

¹H-NMR (400 MHz, D₂O): $\delta = 7.19$ (d, ³J = 8.4 Hz, 2H, 2 pTyr-ar-H), 7.05 (d, ³J = 8.2 Hz, 2H, 2 pTyr-ar-H), 4.84 (dd, ²J = 5.2 Hz, ³J = 9.4 Hz, 1H, aa- α -H), 4.37 (dd, ²J = 5.6 Hz, ³J = 8.1 Hz, 1H, aa- α -H), 4.19-4.10 (m, 1H, Pro- α -H), 3.93-3.73 (m, 2H, Gly-CH₂), 3.70-3.60 (m, 2H, Gly-CH₂), 3.58-3.50 (m, 1H, Thr-CH), 3.22-3.02 (m, 6H, N₃-CH₂, Pro-CH₂, NH-CH₂), 2.91-2.75 (m, 2H, pTyr-CH₂), 2.24-2.16 (m, 1H, Pro-CHa), 1.97-1.85 (m, 3H, Pro-CHa, Pro-CH₂), 1.50-1.39 (m, 4H, 2 CH₂), 1.32-1.18 (m, 4H, 2 CH₂), 1.12 (d, ³J = 6.2 Hz, 3H, Thr-CH₃).

³¹P-NMR (162 MHz, D₂O): $\delta = -3.0$ (PO₄).

FAB-HR: calc.: 698.3022 [M + H]⁺

found: 698.3019 [M + H]⁺

47

H₂N-Leu-pTyr-Arg-Ile-Ala-N-azidohexyl

¹H-NMR (400 MHz, D₂O): $\delta = 7.12$ (d, ³J = 8.4 Hz, 2H, 2 pTyr-ar-H), 7.03 (d, ³J = 8.2 Hz, 2H, 2 pTyr-ar-H), 4.58 (t, ³J = 7.9 Hz, 1H, pTyr- α -H), 4.19 (t, ³J = 6.9 Hz, 1H, aa- α -H), 4.12 (q, ³J = 7.1 Hz, 1H, Ala- α -H), 4.01 (d, ³J = 7.7 Hz, 1H, aa- α -H), 3.87 (t, ³J = 7.0 Hz, 1H, aa- α -H), 3.20 (t,

$^3\text{J} = 6.9 \text{ Hz}$, 2H, $\text{N}_3\text{-CH}_2$), 3.10-3.03 (m, 5H, pTyr- CH_2 , CH_2 , Ile-CH), 2.97-2.92 (m, 2H, CH_2), 1.76-1.72 (m, 1H, Leu-CH), 1.60-1.35 (m, 12H, 6 CH_2), 1.28-1.20 (m, 7H, 2 CH_2 , Ala- CH_3), 0.86-0.79 (m, 12H, 2 Leu- CH_3 , 2 Ile- CH_3).

^{31}P -NMR (162 MHz, D_2O): $\delta = -3.0$ (PO_4).

FAB-HR: calc.: 839.4657 [$\text{M} + \text{H}$] $^+$

found: 839.4664 [$\text{M} + \text{H}$] $^+$

48

H_2N -Gly-pTyr-Cys-Ser-Ala-N-azidohexyl

$[\alpha]_{\text{D}}^{20} = +7.2^\circ$ ($c = 0.56$; DMSO).

^1H -NMR (400 MHz, DMSO-d_6): $\delta = 7.10$ (d, $^3\text{J} = 8.2 \text{ Hz}$, 2H, 2 pTyr-ar-H), 7.00 (d, $^3\text{J} = 8.2 \text{ Hz}$, 2H, 2 pTyr-ar-H), 4.65-4.59 (m, 1H, pTyr- α -H), 4.41 (dd, $^2\text{J} = 7.4 \text{ Hz}$, $^3\text{J} = 13.5 \text{ Hz}$, 1H, aa- α -H), 4.27 (q, $^3\text{J} = 6.3 \text{ Hz}$, 1H, Ala- α -H), 4.22-4.14 (m, 1H, aa- α -H), 3.64 (dd, $^2\text{J} = 5.9 \text{ Hz}$, $^3\text{J} = 10.8 \text{ Hz}$, 1H, aa-CHa), 3.54-3.47 (m, 2H, aa-CHb, aa-CHa), 3.37 (d, $^3\text{J} = 16.4 \text{ Hz}$, 1H, aa-CHb), 3.28 (t, $^3\text{J} = 6.8 \text{ Hz}$, 2H, $\text{N}_3\text{-CH}_2$), 3.05-2.93 (m, 3H, NH- CH_2 , aa-CHa), 2.83-2.65 (m, 3H, aa- CH_2 , aa-CHb), 1.53-1.45 (m, 2H, CH_2), 1.40-1.18 (m, 9H, 3 CH_2 , Ala- CH_3).

^{13}C -NMR (100 MHz, DMSO-d_6): $\delta = 172.4$, 171.8, 170.4, 170.3, 166.5 (5 C=O), 154.1 (pTyr-ar-C-O), 131.5 (pTyr-ar-C- CH_2), 130.5 (2 pTyr-ar-C *meta* zu O), 120.4 (2 pTyr-ar-C *ortho* zu O), 62.3 (Ser- CH_2), 56.0, 55.8, 55.0 (3 aa- α -C), 51.3 ($\text{N}_3\text{-CH}_2$), 49.2 (aa- α -C), 39.6, 39.1, 37.8 ($\text{CH}_2\text{-NH}$, pTyr- CH_2 , Gly- CH_2), 29.4, 28.9, 26.7, 26.5, 26.4 (Cys- CH_2 , 4 CH_2), 18.7 (Ala- CH_3).

^{31}P -NMR (162 MHz, DMSO-d_6): $\delta = -4.1$ (PO_4).

FAB-HR: calc.: 704.2591 [$\text{M} + \text{H}$] $^+$

found: 704.2614 [$\text{M} + \text{H}$] $^+$

49

H_2N -Phe-Lys-pTyr-Ser-Ala-N-azidohexyl

$[\alpha]_{\text{D}}^{20} = -14.7^\circ$ ($c = 1.10$; H_2O).

^1H -NMR (400 MHz, D_2O): $\delta = 7.27$ -7.24 (m, 3H, 3 Phe-ar-H), 7.16-7.08 (m, 4H, 2 Phe-ar-H, 2 pTyr-ar-H), 7.04 (d, $^3\text{J} = 7.8 \text{ Hz}$, 2H, 2 pTyr-ar-H), 4.48 (dd, $^2\text{J} = 6.3 \text{ Hz}$, $^3\text{J} = 8.8 \text{ Hz}$, 1H, pTyr- α -H), 4.27 (t, $^3\text{J} = 5.7 \text{ Hz}$, 1H, Ser- α -H), 4.19-4.10 (m, 3H, Lys- α -H, Ala- α -H, Phe- α -H), 3.74-3.63 (m, 2H, Ser- CH_2), 3.18 (t, $^3\text{J} = 6.9 \text{ Hz}$, 2H, $\text{N}_3\text{-CH}_2$), 3.10-2.88 (m, 6H, pTyr- CH_2 , Phe- CH_2 , CH_2), 2.82 (t, $^3\text{J} = 7.6 \text{ Hz}$, 2H, CH_2), 1.54-1.36 (m, 8H, 4 CH_2), 1.30-1.05 (m, 9H, 4 CH_2 , Ala- CH_3).

^{31}P -NMR (162 MHz, D_2O): $\delta = -3.2$ (PO_4).

FAB-HR: calc.: 819.3919 [M + H]⁺
found: 819.3885 [M + H]⁺

50

H₂N-Ala-pTyr-His-Ala-Gly-N-azidohexyl

¹H-NMR (400 MHz, D₂O): δ = 8.50 (s, 1H, His-ar-H), 7.10-7.05 (m, 3H, 2 pTyr-ar-H, His-ar-H), 6.99 (d, ³J = 8.8 Hz, 2H, 2 pTyr-ar-H), 4.47-4.40 (m, 2H, pTyr-α-H, His-α-H), 4.13 (q, ³J = 7.2 Hz, 1H, Ala-α-H), 3.94 (q, ³J = 7.3 Hz, 1H, Ala-α-H), 3.77 (dd, ²J = 16.9 Hz, ³J = 36.0 Hz, 2H, Gly-CH₂), 3.24-2.85 (m, 8H, His-CH₂, N₃-CH₂, pTyr-CH₂, CH₂), 1.60-1.18 (m, 14H, 4 CH₂, 2 Ala-CH₃).

¹³C-NMR (100 MHz, D₂O): δ = 175.6, 172.5, 171.2, 170.6, 170.5 (5 C=O), 151.3 (pTyr-ar-C-O), 135.3, 133.6, 131.5, 130.4, 128.4, 126.2, 120.7, 117.5 (8 ar-C), 55.5, 52.0, 51.2, 50.2, 49.2, 48.9 (CH₂-N₃, 5 aa-α-C), 42.8, 39.5, 36.6, 32.8 (His-CH₂, pTyr-CH₂, NH-CH₂, CH₂), 28.3, 27.9, 25.6 (3 CH₂), 16.8, 16.5 (2 Ala-CH₃).

³¹P-NMR (162 MHz, D₂O): δ = -3.0 (PO₄).

FAB-HR: calc.: 722.3139 [M + H]⁺
found: 722.3124 [M + H]⁺

51

H₂N-Gly-pTyr-Glu-Ser-Gly-N-azidohexyl

[α]_D²⁰ = -3.3° (c = 1.00; H₂O).

¹H-NMR (400 MHz, D₂O): δ = 7.10 (d, ³J = 7.5 Hz, 2H, 2 pTyr-ar-H), 7.01 (d, ³J = 8.1 Hz, 2H, 2 pTyr-ar-H), 4.53 (t, ³J = 7.7 Hz, 1H, pTyr-α-H), 4.31-4.23 (m, 2H, Ser-α-H, Glu-α-H), 3.93-3.62 (m, 6H, Ser-CH₂, 2 Gly-CH₂), 3.17 (dd, ²J = 6.8 Hz, ³J = 11.8 Hz, 2H, N₃-CH₂), 3.10-3.03 (m, 2H, CH₂), 3.00-2.85 (m, 2H, pTyr-CH₂), 2.30 (t, ³J = 7.2 Hz, 2H, Glu-CH₂), 2.00-1.93 (m, 1H, Glu-CHa), 1.87-1.77 (m, 1H, Glu-CHb), 1.49-1.33 (m, 4H, 2 CH₂), 1.29-1.13 (m, 4H, CH₂).

³¹P-NMR (162 MHz, D₂O): δ = -3.1 (PO₄).

FAB-HR: calc.: 716.2769 [M + H]⁺
found: 716.2742 [M + H]⁺

Peptide **52** and **53** were synthesized on Rink Amide MBHA resin on a Syro II synthesizer with Fmoc-deprot.: 2%DBU/2%pip/DMF, 3*3min and coupling: 3.9 eq. HATU, 4 eq. HOAt, 8 eq. DIPEA, 4 eq. Fmoc-aa. First 2 aa, single coupling 60 min; Pmp, last 2 aa and capping: double coupling 2 x 60 min.

52

Ac-NH-Glu-Phe-pTyr-Met-Asp-Amide

¹H-NMR (400 MHz, D₂O, NEt₃ added for solubility): δ = 7.23-6.98 (m, 9H, 5 ar-H), 4.47-4.35 (m, 3H, 3 aa- α -H), 4.19 (t, ³J = 7.0 Hz, 2H, 2 aa- α -H), 2.82-2.69 (m, 4H, 2 CH₂), 2.56 (dd, ²J = 4.3 Hz, ³J = 15.9 Hz, 1H, CHb), 2.47-2.27 (m, 3H, CHb, CH₂), 1.95 (s, 3H, Met-CH₃), 1.94-1.80 (m, 6H, 3 CH₂), 1.44 (s, 3H, CH₃).

FAB-HR: calc.: 824.2559 [M + H]⁺

found: 824.2538 [M + H]⁺

53

Ac-NH-Glu-Phe-Pmp-Met-Asp-Amide

¹H-NMR (400 MHz, D₂O, NEt₃ added for solubility): δ = 7.23-6.98 (m, 9H, 5 ar-H), 4.47-4.35 (m, 3H, 3 aa- α -H), 4.19 (t, ³J = 7.0 Hz, 2H, 2 aa- α -H), 2.82-2.69 (m, 4H, 2 CH₂), 2.56 (dd, ²J = 4.3 Hz, ³J = 15.9 Hz, 1H, CHb), 2.47-2.27 (m, 5H, CHb, CH₂, CH₂-Pmp), 1.95 (s, 3H, Met-CH₃), 1.94-1.80 (m, 6H, 3 CH₂), 1.44 (s, 3H, CH₃).

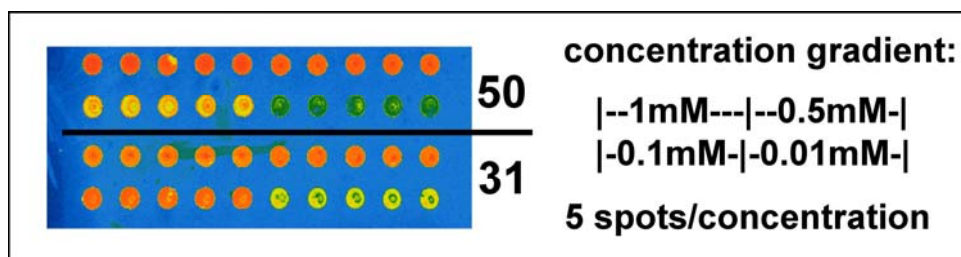
FAB-HR: calc.: 822.2769 [M + H]⁺

found: 822.2742 [M + H]⁺

Functionalization of the glass slides:

Dry *N,N*-dimethylformamide (60 mL) (DMF), *N,N*-diisopropylethyl amine (4.8 mM) (DIPEA), 1-hydroxybenzotriazole (4.8 mM) (HOBt) and *N,N'*-diisopropylcarbodiimide (4.8 mM) (DIC) were added to *N*-Fmoc-protected aminocaproic acid (4.8 mM) and stirred for 10 min under argon to activate the acid. Then, the solution was transferred into a Schlenk flask containing four NH₂-terminated polyamidoamine-dendrimer coated glass slides (Chimera Biotec, Dortmund) mounted on a teflon rack. The mixture was stirred overnight under argon, the supernatant solution was removed and the slides were rinsed twice with DMF, then stirred twice for 20 min in DMF for washing and rinsed again with DMF. Afterwards they were treated twice for 20 min with 60 mL of a 20% piperidine solution in DMF for deprotection. The washing procedure was repeated adding a 15 min stirring step in dichloromethane (DCM) as well as rinsing with DCM. During the whole procedure the slides were kept in the Schlenk flask under argon. The slides were then dried *in vacuo*. In the following step, the phosphane reagent carrying the free acid function (5.2 mM), dry DMF (65 mL), HOBt (5.2 mM), DIPEA (10.4 mM) and DIC (5.2 mM) were added subsequently under argon to the slides. The mixture was stirred overnight and the supernatant solution was again removed followed by a washing procedure consisting of rinsing twice with dry DMF, stirring in dry DMF/DCM (1:1) twice for 20 min, rinsing again twice with dry DMF. While stirring, the solution was degassed 3 times. Afterwards, the slides were treated twice for 10 min with a solution of acetic anhydride (4.8 mM) and pyridine (43.2 mM) in dry DMF (60 mL). After removing the supernatant, the slides were rinsed with dry DMF and dry DCM, stirred twice for 20 min in dry DCM, rinsed again with dry DCM, dried *in vacuo* and stored under argon until usage. Again, the solution was degassed 3 times while stirring as mentioned above.

Determination of optimal peptide spotting and antibody concentration



Supporting Figure 1: Optimization of detection of pTyr-peptides.

To determine the optimal spotting concentration for the pTyr-peptides, two peptides with very different amino acid properties, peptides **31** and **50**, were spotted in a concentration gradient from 0.01mM to 1mM. The slides were then incubated with anti-pTyr-antibody⁶-biotin-streptavidin-Cy5-conjugate (10nM, 50nM, 100 nM, only 50nM shown in Supporting Figure 1). The fluorescence read-out (intensity ranking colors: red (high), yellow, green, blue (low)) showed that for both peptides the optimal spotting concentration lies in the range of 0.5 to 1mM and that the optimal antibody⁶ concentration is 50nM. Therefore, these parameters were defined as assay conditions.

Spotting process and substrate specificity tests:

The spotting process was carried out using a GeSiM Nanoplotter (Gesim, Dresden, Germany) by spotting 0.25 nL droplets of peptide solutions (1 mM in dioxane/water⁷ = 2:1; spot size 400 μ m diameter) followed by incubation overnight. Peptides 5, 10, 11, 13, 22, 27, 32, 38, 40, 41, 43, 44, 45 and 48 were spotted in saturated solutions due to solubility problems, but the resulting fluorescence intensity from the buffer incubated assay was still within the observed intensity of the defined assay conditions (see above). To reduce nonspecific binding of reagents, all slides were incubated for 30 min with a blocking solution (Chimera Biotec GmbH).

Phosphopeptides were spotted onto the glass slide so that five assays containing the peptides and Cy5 in doublets were located on one slide. Before treatment with the phosphatase, hybridization chambers were tied on the slide (Gene Frame 125 μ L, Abgene, UK). In that way, incubation with four different phosphatase-concentrations (50 μ g/mL, 5 μ g/mL, 0.5 μ g/mL and 0.05 μ g/mL) and with the buffer for comparison could be carried out at the same time. Subsequent to incubation with phosphatase, the slide was washed with TETBS buffer (150 mM NaCl, 20 mM Tris, pH 7.5, 5 mM EDTA, 0.05% Tween20, 1mM DTE) and anti-pTyr-antibody⁶-biotin-streptavidin-Cy5-conjugate (50 nM; anti-pTyr-antibody⁶-biotin source: BIOTREND Chemicals; streptavidin-Cy5 source: Zyomed) in biotin-TETBS (TETBS containing 800 μ M biotin to block free binding sites of streptavidin) was added to the chambers. After incubation for 45 min, the slide was washed with TETBS and deionized H₂O. It was dried by centrifugation and fluorescence intensity was measured using a microarray laser scanning system (Axon) set to a pmt of 500 and 100% laser power. Signals were analyzed and quantified using GenePix pro 4.1 software (Axon).

Through the analysis of two microarrays, each phosphopeptide was tested four times resulting in an average value and standard deviation calculated with Microsoft Excel. The average deviation is given in the caption of Supporting Table 1 and 3. Each data point was normalized to the signal intensity of Cy5 and subsequently the signal intensities of the PTP-incubated array were compared to the ones that resulted from the buffer-incubated array. Through normalization using the Cy5-signal intensity, errors were eliminated that can occur due to varying signal intensities between arrays. All values given in Supporting Tables 1 and 3 are therefore Cy5-signal corrected

signal intensities after PTP incubation compared to buffer-only incubation. On the average of four Cy5-signal intensity measurements, the signal intensity of Cy5 on the buffer-incubated array was 2% lower than the signal intensity of phosphatase-incubated array, showing high reproducibility of the Cy5-signal intensity and thus the legitimacy of using it as standardization factor. Substrate specificity of PTP μ -D1 was determined at a concentration of 50 μ g/mL of the phosphatase and of PTP1B it was determined at a concentration of 2.5 μ g/mL.

In the phosphatase-incubated arrays, some spots exhibit higher fluorescence intensity on the outside than on the inner part (“doughnut-shaped” spots⁵). Here, this phenomenon appears to be concentration dependent as it only occurs with spots that have a low concentration of the detectable compound. Due to the nature of the assay, in which the decrease of the fluorescently detectable compound is monitored, this parameter is inherent to the assay. Since the spots of the buffer incubated assay are homogeneous, the value of all spot pixels was used for the interpretation of the results. The obtained intensity of a ring-spot is therefore an average of the signal of the whole spot. This was defined for the assay and applied throughout the analysis of the data.

To minimize interactions of the PTP and the peptides with the surface, the azide functionalized peptides and the phosphane functionalized surfaces carry an aminohexyl-linker (Figure 1) resulting in a spacer which consists of 19 atoms. Since linker of this length have been shown to be sufficient for functional assay microarray applications⁴, the spacer used here should be sufficient to allow the interaction between immobilized phosphopeptides and the active site of the interacting PTP.

Statistical methods

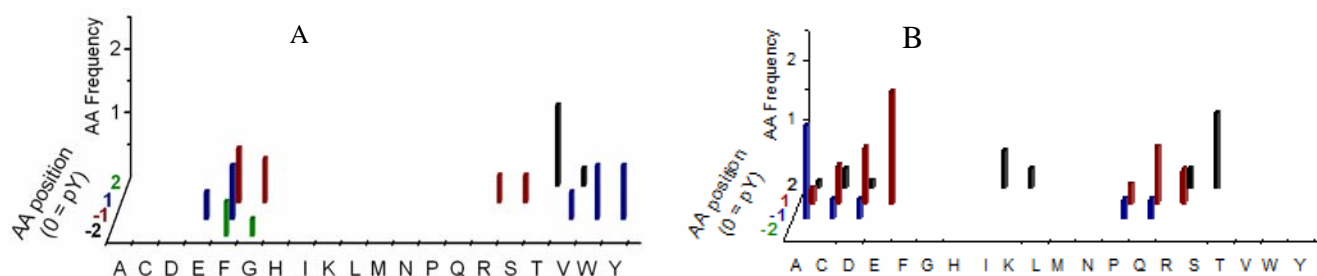
The specificity of the enzyme for a given amino acid at a given position within the phosphopeptides chosen for the microarray test was calculated according to the following reasoning: Assuming that n phosphopeptides were selected as the best substrates out of a total of N phosphopeptides tested, and given that r amino acids of a particular type were found at a given position, according to the scheme of the non-returned ball the probability to have exactly k amino acids of the given type are:

$$P(k) = [C(M,k) \times C(N-M, n-k)] / C(N,n)$$

Then the expected number of this type of amino acid at the given position is:

$$A_{\text{aminoacid}} = 1 \times P(1) + 2 \times P(2) + \dots + (n-1) \times P(n-1) + n \times P(n)$$

Consequently, the difference $A-r$ reflects the deviation from the number of amino acids expected in case of a random distribution: a positive value means a preference while a negative value means a non-preference for that amino acid at the given position.



Supporting Figure 2: A) Preference plot for amino acids at different positions in the pTyr-peptides from the microarray based on 26 peptides in which pTyr was the third amino acid. **B)** Plot for amino acids disfavoured by PTP μ (less than 40% signal decrease on the microarray).

Solution assay

Substrate specificity in solution was measured using a concentration of 10.8 $\mu\text{g/mL}$ of PTP μ -D1 as well as 0.5 $\mu\text{g/mL}$ of PTP1B, and 100 μM solutions of the substrates in TETBS buffer (150 mM NaCl, 20 mM Tris, pH 7.5, 5 mM EDTA, 0.05% Tween20, 1mM DTE) incubating for 10 min at 25°C with a reaction volume of 8 μL . The data for each peptide was determined four times resulting in an average value and standard deviation calculated with Microsoft Excel, the average deviation is given in the caption of Supporting Table 2 and 4. The reaction was stopped using hydrochloric acid containing malachit green solution. After incubation with this solution for 15 min the extinction of the malachit green-phosphate complex was measured at 620 nm. Correction was made for non enzymatic hydrolysis of the peptide substrates. Peptide **52** (70 μM) was incubated with PTP μ -D1 at the same conditions as stated before in the absence and presence of various concentrations of **53**. The assay was terminated using hydrochloric acid containing malachit green solution. After incubation with this solution for 15 min the extinction of the malachit green-phosphate complex was measured at 620 nm.

Docking experiments

Peptides **52** and **53** were constructed in silico. The protein structure of PTP μ was taken from the Protein Data Bank (PDB code 1RPM) and prepared using Sybyl (Tripos). Hydrogens were added to all atoms and then a short energy minimization was conducted to remove steric clashes. The docking experiments were conducted with GOLD version 1.2 (Cambridge Crystallographic Data Centre) and 3.0 for rescoring with the built-in GOLDScore function. All docking runs were conducted in parallel under IRIX Unix running on an SGI Origin 2000. We used the standard parameters except for early termination by RMSD. This function was disabled due to errors upon early termination. A distance constraint was set between the phosphorous atom of each peptide and the sulfur atom in the cysteine 1095 in chain A. The binding site was defined by a sphere centered also on the sulfur atom in cysteine 1095 with a radius of 15 Å. Results were analyzed by evaluation of the GOLDScore value (using also GOLD 3.0 for rescoring) and visual inspection of the binding modes using pymol version 0.98 and in house scripts

Hydrogens were added to all atoms and then an energy minimization was conducted to remove steric clashes. Subsequently, charges were corrected for neutral pH, i.e acidic side chains were deprotonated, lysines protonated and the like. Since it is known that the cysteine in the binding site of tyrosine phosphatases is rather acidic it was deprotonated as well.

The docking experiments were conducted with GOLD version 3.0 (Cambridge Crystallographic Data Centre, GB). All docking runs were conducted in parallel on an in-house linux cluster running on Suse Enterprise Server 9. Standard parameters were used if not stated otherwise. Several runs were conducted either with distance constraints or with simulated covalent linkage.

Distance constraints were set between the three oxygen atoms of the tyrosine phosphate group and the sulfur atom in cysteine 1095 in chain A of PTP μ . The distance interval was set between 3.1 and 3.5 Å with a force constant of 5.

The covalent binding was simulated between the phosphorous atom of the tyrosine phosphate and the sulphur atom of the cysteine 1095.

In both cases, the binding site was defined by a sphere centered on the sulfur atom in cysteine 1095 with a radius of 15 Å.

Results were analyzed by evaluation of the GOLDScore value and visual inspection of the binding modes using Maestro 7.5. A manual clustering of similar poses from the covalent linkage experiment was conducted. Two preferred conformations were identified which represent about 20 % of the top ranking 100 poses each.

Peptide number.	Amino acid							Microarray substrate ranking (signal intensities in %)
	-2	-1	pY	+1	+2	+3	+4	
13	P	I	pY	A	T			++ (12)
32	F	I	pY	I	V			++ (18)
11	P	Y	pY	Y	V			++ (20)
5	A	W	pY	G	T			++ (20)
4	A	E	pY	G	D			++ (23)
16	F	K	pY	G	D			++ (23)
6	E	F	pY	M	D			++ (25)
41	F	F	pY	F	Q			++ (30)
49	F	K	pY	S	A			++ (30)
9	L	Y	pY	K	Q			++ (33)
10	G	M	pY	M	A			++ (34)
14	A	R	pY	F	A			++ (34)
15	L	S	pY	R	G			++ (34)
36	A	V	pY	W	T			++ (35)
43	G	W	pY	W	Q			++ (35)
20	E	K	pY	H	V			+ (37)
17	A	K	pY	E	G			+ (40)
12	F	S	pY	D	G			+ (43)
19	E	G	pY	G	Q			+ (43)
23		E	pY	A	P	Q		+ (44)
45		P	pY	D	I	T		+ (44)
22	F	Q	pY	Q	D			+ (47)
30	A	R	pY	Q	D			+ (47)
40		L	pY	C	C	V		+ (47)
18	L	A	pY	E	D			+ (48)
26		F	pY	Y	P	A		+ (48)
7	L	C	pY	K	A			+ (50)
24	G	S	pY	A	G			+ (52)
25		F	pY	R	A	D		+ (52)
38		M	pY	G	D	A		+ (52)
21	A	A	pY	K	G			+ (51)
46		G	pY	P	G	T		+ (53)
8	A	D	pY	Y	A			+ (55)
27		F	pY	S	S	V		+ (55)
33		L	pY	R	A	G		0 (60)
47		L	pY	R	I	A		0 (61)
44		L	pY	A	S	G		0 (61)
31		L	pY	E	D	A	D	0 (68)
50		A	pY	H	A	G		0 (68)
34		A	pY	E	A	D		0 (70)
35		A	pY	Q	A	G		0 (71)
42		G	pY	R	R	Q		0 (72)
37		L	pY	E	D			0 (72)
29		E	pY	D	D	Q		0 (73)
28		G	pY	S	G	D		0 (77)
39		L	pY	K	K	Q		0 (77)
48		G	pY	C	S	A		0 (84)
51		G	pY	E	S	G		0 (99)

Supporting Table 1: Results of the microarray dephosphorylation experiments with PTP μ . Microarray substrate quality determination: signal intensities after PTP μ incubation compared to buffer-only incubation: 0-35% = ++ (preferred substrate); 35-60% = + (substrate); 60-100% = 0 (no substrate); average deviation 18%; phosphatase concentration: 50 μ g/mL; positions of the amino acids in regards to pY are indicated.

Peptide number	Solution phase assay (yield phosphate in nmol/min/mg enzyme)						
	-2	-1	pY	+1	+2	+3	+4
5	A	W	pY	G	T		
6	E	F	pY	M	D		
11	P	Y	pY	Y	V		
13	P	I	pY	A	T		
16	F	K	pY	G	D		
7	L	C	pY	K	A		
8	A	D	pY	Y	A		
9	L	Y	pY	K	Q		
10	G	M	pY	M	A		
12	F	S	pY	D	G		
14	A	R	pY	F	A		
15	L	S	pY	R	G		
17	A	K	pY	E	G		
18	L	A	pY	E	D		
19	E	G	pY	G	Q		
20	E	K	pY	H	V		
21	A	A	pY	K	G		
32	F	I	pY	I	V		
41	F	F	pY	F	Q		
49	F	K	pY	S	A		
22	F	Q	pY	Q	D		
23		E	pY	A	P	Q	
24	G	S	pY	A	G		
25		F	pY	R	A	D	
26		F	pY	Y	P	A	
27		F	pY	S	S	V	
30	A	R	pY	Q	D		
46		G	pY	P	G	T	
38		M	pY	G	D	A	
45		P	pY	D	I	T	
40		L	pY	C	C	V	
36	A	V	pY	W	T		
43	G	W	pY	W	Q		
48		G	pY	C	S	A	
28		G	pY	S	G	D	
29		E	pY	D	D	Q	
31		L	pY	E	D	A	D
33		L	pY	R	A	G	
34		A	pY	E	A	D	
35		A	pY	Q	A	G	
51		G	pY	E	S	G	
44		L	pY	A	S	G	
37		L	pY	E	D		
39		L	pY	K	K	Q	
47		L	pY	R	I	A	
42		G	pY	R	R	Q	
50		A	pY	H	A	G	

Supporting Table 2: Results of the dephosphorylation of the 48 peptides with PTP μ obtained with the Malachite Green assay. Phosphate yield in nmol/min/mg enzyme: >200 = ++ (preferred substrate); 50-200 = + (substrate); <50 = 0 (no substrate); average deviation 9%.

Peptide number.	Amino acid sequence							Microarray substrate ranking (signal intensities in %)
	-2	-1	pY	+1	+2	+3	+4	
14	A	R	pY	F	A			++ (19)
30	A	R	pY	Q	D			++ (22)
15	L	S	pY	R	G			++ (28)
18	L	A	pY	E	D			++ (29)
36	A	V	pY	W	T			++ (29)
4	A	E	pY	G	D			++ (30)
16	F	K	pY	G	D			++ (31)
17	A	K	pY	E	G			++ (32)
13	P	I	pY	A	T			++ (32)
44		L	pY	A	S	G		++ (33)
22	F	Q	pY	Q	D			++ (34)
25		F	pY	R	A	D		++ (35)
5	A	W	pY	G	T			++ (35)
6	E	F	pY	M	D			+ (39)
24	G	S	pY	A	G			+ (40)
12	F	S	pY	D	G			+ (41)
33		L	pY	R	A	G		+ (42)
21	A	A	pY	K	G			+ (43)
49	F	K	pY	S	A			+ (44)
20	E	K	pY	H	V			+ (46)
8	A	D	pY	Y	A			+ (48)
28		G	pY	S	G	D		+ (48)
37		L	pY	E	D			+ (51)
23		E	pY	A	P	Q		+ (54)
10	G	M	pY	M	A			+ (54)
31		L	pY	E	D	A	D	+ (55)
35		A	pY	Q	A	G		+ (57)
40		L	pY	C	C	V		+ (57)
51		G	pY	E	S	G		0 (61)
47		L	pY	R	I	A		0 (61)
45		P	pY	D	I	T		0 (62)
38		M	pY	G	D	A		0 (71)
26		F	pY	Y	P	A		0 (73)
7	L	C	pY	K	A			0 (74)
48		G	pY	C	S	A		0 (74)
46		G	pY	P	G	T		0 (77)
34		A	pY	E	A	D		0 (78)
50		A	pY	H	A	G		0 (100)

Supporting Table 3: Results of the microarray dephosphorylation experiments with PTP1B.

Microarray substrate quality determination: signal intensities after PTP1B incubation compared to buffer-only incubation: 0-35% = ++ (preferred substrate); 35-60% = + (substrate); 60-100% = 0 (no substrate); average deviation 16%; phosphatase concentration: 2.5 µg/mL; positions of the amino acids in regards to pY are indicated.

Peptide number	Amino acid sequence							Solution phase assay (yieldphosphate in nmol/min/mg enzyme)
	-2	-1	pY	+1	+2	+3	+4	
5	A	W	pY	G	T			++ (18740)
22	F	Q	pY	Q	D			++ (17126)
8	A	D	pY	Y	A			++ (13979)
48		G	pY	C	S	A		++ (13528)
4	A	E	pY	G	D			++ (13021)
13	P	I	pY	A	T			++ (12965)
14	A	R	pY	F	A			++ (12836)
16	F	K	pY	G	D			++ (12624)
15	L	S	pY	R	G			++ (12285)
25		F	pY	R	A	D		++ (11659)
7	L	C	pY	K	A			++ (11497)
18	L	A	pY	E	D			++ (11296)
44		L	pY	A	S	G		++ (11197)
6	E	F	pY	M	D			++ (11107)
49	F	K	pY	S	A			+ (10701)
38		M	pY	G	D	A		+ (10698)
24	G	S	pY	A	G			+ (10111)
30	A	R	pY	Q	D			+ (9750)
23		E	pY	A	P	Q		+ (9358)
21	A	A	pY	K	G			+ (8869)
12	F	S	pY	D	G			+ (8849)
20	E	K	pY	H	V			+ (8810)
10	G	M	pY	M	A			+ (8468)
37		L	pY	E	D			+ (8124)
40		L	pY	C	C	V		+ (8082)
35		A	pY	Q	A	G		+ (7087)
17	A	K	pY	E	G			+ (6635)
31		L	pY	E	D	A	D	+ (6582)
47		L	pY	R	I	A		0 (6329)
26		F	pY	Y	P	A		0 (5776)
33		L	pY	R	A	G		0 (5540)
45		P	pY	D	I	T		0 (5438)
51		G	pY	E	S	G		0 (4560)
28		G	pY	S	G	D		0 (3884)
36	A	V	pY	W	T			0 (3248)
50		A	pY	H	A	G		0 (2967)
34		A	pY	E	A	D		0 (2932)
46		G	pY	P	G	T		0 (267)

Supporting Table 4: Results of the dephosphorylation of the 48 peptides with PTP1B obtained with the Malachite green assay. Phosphate yield in nmol/min/mg enzyme: >11000 = ++ (preferred substrate); 11000-6500 = + (substrate); <6500 = 0 (no substrate); average deviation: 10%.

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