Supplementary Materials

FOR THE ARTICLE

Triphenylphosphonium analogs of short peptide related to bactenecin 7 and oncocin 112 as antimicrobial agents

Andrey G. Tereshchenkov[1,2], Zimfira Z. Khairullina[1], Inna A. Volynkina[1], Dmitrii A. Lukianov[1], Pavel A. Nazarov[2#], Julia A. Pavlova[1,2], Vadim N. Tashlitsky[1], Elizaveta A. Razumova[1], Daria A. Ipatova[1], Yury V. Timchenko[1], Dmitry A. Senko[3], Olga V. Efremenkova[4], Alena Paleskava[5,6], Andrey L. Konevega[5,6,7], Ilya A. Osterman[1], Igor A. Rodin[1], Petr V. Sergiev[1,2,8], Olga A. Dontsova[1,2,3], Alexey A. Bogdanov[1,2], and Natalia V. Sumbatyan[1#]

1 Lomonosov Moscow State University, Department of Chemistry, 1/3 Leninskie Gory, 119991 Moscow, Russia
2 Lomonosov Moscow State University, A.N. Belozersky Institute of Physico-Chemical Biology, 1/40 Leninskie Gory, 119991 Moscow, Russia
3 Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, 117997 Moscow, Russia
4 Gause Institute of New Antibiotics, 11 B. Pirogovskaya Street, 119021 Moscow, Russia
5 Petersburg Nuclear Physics Institute, NRC "Kurchatov Institute", 188300 Gatchina, Russia
6 Peter the Great St.Petersburg Polytechnic University, 195251 Saint Petersburg, Russia
7 NRC "Kurchatov Institute", 123182 Moscow, Russia
8 Lomonosov Moscow State University, Institute of Functional Genomics, 119991 Moscow, Russia
Correspondence: sumbtyan@belozersky.msu.ru (N.V.S.); nazarovpa@gmail.com (P.A.N.)

This file includes:

I. DETAILED SYNTHESIS

Detailed synthesis of oncocin (Onc112)

Solid-phase synthesis of oncocin (VDKPPYLPRPRPPRrIYNr-NH_2) was carried out using custom-made automated parallel peptide synthesizer. Fmoc strategy with HATU/DIPEA activation was applied. TentaGel HL NH2 resin (loading 0.48 mmol/g) was used as solid support. Resin was functionalized by Fmoc-RAM linker. Standard protected amino acids used in this work had the following side protections: Fmoc-Arg(Pbf)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Asp(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Tyr(tBu)-OH. After the synthesis, linear peptide was totally deprotected and cleaved from the polymer with TFA/DTT/H_2O/TIS 89:5:5:1 cocktail. A crude peptide was isolated by ether precipitation and subsequent purification was performed by reverse-phase HPLC with YMC Triart C18 column (150 × 30 mm). Purity (>98%) of the oncocin was confirmed by UPLC/MS analysis: *m*/*z* calculated for $[C_{109}H_{177}N_{37}O_{24}+H]^+$: 2390.4; found 2390.8.

Detailed synthesis of peptides 1 and 2

Peptidyl-polymers *Fmoc-Arg(Pbf)Arg(Pbf)IleArg(Pbf)ProArg(Pbf)ProProTyr(tBu)Leu-**P*** and *Fmoc-Arg(Pbf)Arg(Pbf)IleArg(Pbf)ProArg(Pbf)ProProArg(Pbf)Leu-**P*** were synthesized according to the standard Fmoc solid phase peptide synthesis protocol using a 2-chlorotrityl resin (1.6 mmol Cl^-/g) [1] and HBTU as an activating agent. The volume of solvent was determined based on the calculation of 10 ml of solvent per 1 g of resin. The resin was prepared for synthesis by soaking it in DMF for 10–15 minutes, followed by washing with dioxane (1 × 4 min), DMF (1 × 4 min) and CH_2Cl_2 (1 × 4 min). The first amino acid addition was accomplished by adding Fmoc-Leu-OH (2 eq) and DIPEA (2 eq) in CH_2Cl_2 to the reactor and shaking for 10 minutes. An additional 3 equivalents of DIPEA were then added and mixed for an hour. At the end of the reaction, methanol was added and the mixture was shaken for 10 minutes. Then the solvent was separated by filtration, and the resin was washed successively with methylene chloride, DMF, and methanol three times each. The resin was dried in a vacuum desiccator, the loading was calculated. Further peptide chain elongation was done according to the standard protocol [1]: (1) washing resin with DMF (3 × 1 min); (2) removing of Fmoc group by 20% piperidine in DMF (2 × 15 min); (3) determination of loading using Fmoc-test [2]; (4) washing the peptidyl-resin with DMF (3 × 1 min); (5) amino acid activation: HBTU (3 eq) and DIPEA (3 eq) in DMF were mixed with amino- and side chain protected amino acid (3 eq) and shacked for 5 min; (6) condensation: the activated amino- and side chain protected amino acid was added to the peptidyl-resin and stirred for 1 h; (7) DIPEA (0.6 eq) was added after 10 min from the beginning of condensation; (8) the peptidyl-resin was filtered and washed alternately with DMF (2 × 1 min) and iPrOH (2 × 1 min); (9) completion of the condensation was monitored using Kaiser-test [3]. If the test was positive, then steps 5–9 were repeated, otherwise the next step was performed; (10) unreacted α-amino groups were blocked using a freshly prepared mixture of acetic anhydride – DIPEA – DMF (5:6:89, v/v) (1 × 5 min, 1 × 30 min); (11) the peptidyl-resin was washed with DMF (3 × 1 min). The protocol was repeated until required length of the peptide chain was reached. In the final stage, the resin was washed sequentially with DMF (1 × 5 min) and CH_2Cl_2 (1 × 5 min).

*H-ArgArgIleArgProArgProProArgLeu-OH*6TFA (**1**, Bac(1-10))*

Fmoc-Arg(Pbf)Arg(Pbf)IleArg(Pbf)ProArg(Pbf)ProProArg(Pbf)Leu-**P** was Fmoc-deprotected with 20% piperidine in DMF as described above, washed with DMF (3 × 1 min) and then treated with reagent K (TFA : phenol : water : thioanisole : mercaptoethanol = 82.5 : 5 : 5 : 5 : 2.5) (10 ml per 1 g of resin) for 4 h. Then solvent was separated by filtration, and the product was precipitated from the filtrate with diethyl ether. The target product was purified by preparative HPLC in a gradient of CH_3CN in 0.05% TFA from 5 to 30% for 15 min: τ = 12.0 min; MALDI-TOF MS: *m*/*z* calculated for $[C_{57}H_{105}N_{25}O_{11}+H]^+$: 1316.8; found 1316.8; HRMS: *m*/*z* calculated for $[C_{57}H_{105}N_{25}O_{11}+2H]^{2+}$: 658.9286; found 658.9285; calculated for $[C_{57}H_{105}N_{25}O_{11}+3H]^{3+}$: 439.6215; found 439.6215; calculated for $[C_{57}H_{105}N_{25}O_{11}+4H]^{4+}$: 329.9679; found 329.9681; calculated for $[C_{57}H_{105}N_{25}O_{11}+5H]^{5+}$: 264.1758; found 264.1758. See also Section III of Supplementary materials for detailed HRMS analysis (Figures S5.1–S5.5).

*H-ArgArgIleArgProArgProProTyrLeu-OH*5TFA (**2**, Bac(1-10, R/Y))* was prepared from Fmoc-Arg(Pbf)Arg(Pbf)IleArg(Pbf)ProArg(Pbf)ProProTyr(tBu)Leu-**P** according procedure described above for

compound **1**. The target product was purified by preparative HPLC in a gradient of CH_3CN in 0.05% TFA from 5 to 30% for 15 min: τ = 11.8 min; MALDI-TOF MS: m/z calculated for $[C_{60}H_{102}N_{22}O_{12}+H]^+$: 1323.8; found 1323.7; HRMS: m/z calculated for $[C_{60}H_{102}N_{22}O_{12}+2H]^{2+}$: 662.4097; found 662.4100; calculated for $[C_{60}H_{102}N_{22}O_{12}+3H]^{3+}$: 441.9422; found 441.9423; calculated for $[C_{60}H_{102}N_{22}O_{12}+4H]^{4+}$: 331.7085; found 331.7084. See also Section III of Supplementary materials for detailed HRMS analysis (Figures S6.1–S6.4).

Detailed Synthesis of Bac(1-10, R/Y)-C2-TPP (3)

(2-Bromoethyl)(triphenyl)phosphonium bromide ([TPP-C2-Br]Br)

1.54 g (0.0059 mol) of triphenylphosphine and 2.18 g (0.012 mol) of 1,2-dibromoethane were dissolved in methanol. The mixture was sealed in a screw-capped vessel and kept in an argon atmosphere at 85 °C for 72 h. The solvent was removed on a rotary evaporator, the resulting residue was dissolved in CH_2Cl_2 and precipitated with diethyl ether. The target product was isolated on a silica gel column eluting with a solvent system of $CHCl_3$:CH_3OH = 9:1. Yield: 2.54 g (96%); TLC: R_f ($CHCl_3$:CH_3OH, 9:1) 0.61; LC-MS *m/z* calculated for $[C_{20}H_{19}BrP]^+$: 369.04; found 369.30; τ (UPLC) = 1.53 min; ^{1}H NMR ($CDCl_3$): 7.75–7.87 (m, 12H, HC^2_{arom}, HC^3_{arom}, HC^5_{arom}, HC^6_{arom}), 7.65–7.71 (m, 3H, HC^4_{arom}), 4.57 (dt, 2H, HC^2, *J* = 11.7, 6.6), 3.74 (dt, 2H, HC^1, *J* = 19.6, 6.3); ^{13}C NMR ($CDCl_3$): 135.4 (d, HC^4_{arom}, *J* = 3.2), 133.9 (d, HC^2_{arom}, HC^6_{arom}, *J* = 10.3), 130.6 (d, HC^3_{arom}, HC^5_{arom}, *J* = 12.9), 117.3 (d, HC^1_{arom}, *J* = 86.6), 27.1 (d, HCP, *J* = 51.7), 22.9 (d, HCBr, *J* = 5.2); ^{31}P NMR ($CDCl_3$): 23.34 (s).

(2-Aminoethyl)(triphenyl)phosphonium bromide ([TPP-C2-NH_2]Br)

0.5 g (0.0011 mol) of [TPP-C2-Br]Br was dissolved in 10 ml of 7 N ammonia solution in methanol and the mixture was kept for 4 h at 85 °C. Then the mixture was cooled, and volatile components were removed on a rotary evaporator. The target product was isolated on a silica gel column eluting with a solvent system of $CHCl_3$:CH_3OH = 6:1. Yield: 0.246 g (58%); TLC: R_f ($CHCl_3$:CH_3OH, 6:1) 0.76; LC-MS *m/z* calculated for $[C_{20}H_{21}NP]^+$: 306.14; found 306.31; τ (UPLC) = 0.76 min; ^{1}H NMR (DMSO-d_6): 7.92 (m, 3H, HC^4_{arom}), 7.75–7.87 (m, 12H, HC^2_{arom}, HC^3_{arom}, HC^5_{arom}, HC^6_{arom}), 3.99 (m, 2H, HC^1), 3.07 (b.s., 2H, HC^2); ^{13}C NMR (DMSO-d_6): 135.8 (d, HC^4_{arom}, *J* = 3.2); 134.2 (d, HC^4_{arom}, HC^6_{arom}, *J* = 11.4), 130.9 (d, HC^3_{arom}, HC^5_{arom}, *J* = 12.7), 117.7 (d, HC^1_{arom}, *J* = 87.4), 33.8 (d, HCN, *J* = 2.3), 20.0 (d, HCP, *J* = 54.1); ^{31}P NMR (DMSO-d_6): 21.33 (s).

Fmoc-Arg(Pbf)Arg(Pbf)IleArg(Pbf)ProArg(Pbf)ProProTyr(tBu)Leu-C2-TPP

Peptidyl-polymer Fmoc-Arg(Pbf)Arg(Pbf)IleArg(Pbf)ProArg(Pbf)ProProTyr(tBu)Leu-**P** was treated with a 50% solution of HFIP in CH_2Cl_2 for 1h. Volatile components were removed using a rotary evaporator. Obtained peptide with protected groups Fmoc-Arg(Pbf)Arg(Pbf)IleArg(Pbf)ProArg(Pbf)ProProTyr(tBu)Leu-COOH (**6**) was isolated on a silica gel column eluting with a solvent system of $CHCl_3$:CH_3OH = 4:1 (R_f 0.33). Next, compound **6** (84 mg, 0.032 mmol), DIPEA (17 µl, 0.097 mmol), and HBTU (16 mg, 0.042 mmol) were dissolved in CH_2Cl_2 and kept for 5 min. Then [TPP-C2-NH_2]Br (19 mg, 0.048 mmol) was added to the solution. The obtained mixture was stirred for 1.5 h. Then the solvent was removed on a rotary evaporator, the resulting residue was dissolved

in ethyl acetate and washed sequentially with water, 0.1 N H_2SO_4, saturated $NaHCO_3$, and saturated NaCl, dried over anhydrous sodium sulfate, then the solvent was removed on a rotary evaporator. The target product was isolated on a silica gel column eluting with a solvent system of $CHCl_3$:CH_3OH = 9:1. Yield: 76 mg (81%); TLC: R_f ($CHCl_3$:CH_3OH, 9:1) 0.29; MALDI-TOF MS: *m*/*z* calculated for $[C_{151}H_{203}N_{23}O_{25}PS_4]^+$: 2897.4; found 2897.6.

*H-ArgArgIleArgProArgProProTyrLeu-C2-TPP*6TFA (**3**, Bac(1-10, R/Y)-C2-TPP)*

Fmoc-Arg(Pbf)Arg(Pbf)IleArg(Pbf)ProArg(Pbf)ProProTyr(tBu)Leu-C2-TPP (76 mg, 0.026 mmol) was treated with reagent K by the procedure described for compound **1**. After precipitation with diethyl ether, the residue was mixed with 1.5 mL of 20% piperidine in DMF and stirred for 1.5 h. The product was precipitated with diethyl ether, and the solid precipitate was washed three times with ether. The target product was isolated by preparative HPLC in a gradient of CH_3CN in 0.05% TFA from 20 to 40% for 18 min: τ = 14.5 min. Yield: 20 mg (47%); MALDI-TOF MS: *m*/*z* calculated for $[C_{80}H_{121}N_{23}O_{11}P]^+$: 1610.9; found 1610.9; HRMS: *m*/*z* calculated for $[C_{80}H_{121}N_{23}O_{11}P+H]^{2+}$: 805.9710; found 805.9708; calculated for $[C_{80}H_{121}N_{23}O_{11}P+2H]^{3+}$: 537.6498; found 537.6496; calculated for $[C_{80}H_{121}N_{23}O_{11}P+3H]^{4+}$: 403.4892; found 403.4892; calculated for $[C_{80}H_{121}N_{23}O_{11}P+4H]^{5+}$: 322.9928; found 322.9931. See also Section III of Supplementary materials for detailed HRMS analysis (Figures S7.1–S7.5).

Detailed Synthesis of Bac(1-10, R/Y)-C10-TPP (4)

(10-Bromodecyl)(triphenyl)phosphonium bromide ([TPP-C10-Br]Br)

3 g of triphenylphosphine (0.011 mol) and 4.5 g of 1,10-dibromodecane (0.015 mol) were dissolved in ethanol. The obtained mixture was sealed in a screw-capped vessel and kept in an argon atmosphere at 85 °C for 72 h. The solvent was removed on a rotary evaporator, the resulting residue was dissolved in CH_2Cl_2 and precipitated with diethyl ether. The target product was isolated on a silica gel column eluting with a solvent system of $CHCl_3$:CH_3OH = 9:1. Yield: 5.32 g (86%); TLC: R_f ($CHCl_3$:CH_3OH, 9:1) 0.33; LC-MS *m*/*z* calculated for $[C_{28}H_{35}BrP]^+$: 483.2; found 482.7; ^1H NMR ($CDCl_3$): 7.78–7.64 (15H, m, -$C_6\underline{H}_5$), 3.30 (2H, dt, *J* = 15.2, 5.9, 4.9 , Ph_3P-$C\underline{H}_2$-), 2.55 (2H, t, *J* = 7.3, -$C\underline{H}_2$-Br), 1.74–1.53 (6H, m, Ph_3P-CH_2-$C\underline{H}_2$-$C\underline{H}_2$-, -$C\underline{H}_2$-CH_2-Br), 1.36–1.23 (10H, m, -CH_2-$C\underline{H}_2$-CH_2-); ^{13}C NMR ($CDCl_3$): 135.10 (3C,d, J = 3.0, p-$\underline{C}_6H_5$), 133.60 (6C, d, J = 10.00, o-$\underline{C}_6H_5$), 130.58 (6C, d, J = 12.5, m-$\underline{C}_6H_5$), 117.56 (3C, d, J = 86.0, ipso-$\underline{C}_6H_5$), 34.19 (1C, -$\underline{C}H_2$-Br), 32.24-22.52 (9H, -$\underline{C}H_2$-).

(10-Aminodecyl)(triphenyl)phosphonium bromide ([TPP-C10-NH_2]Br) was prepared as described above for [TPP-C2-Br]Br from 1 g of [TPP-C10-Br]Br (0.0018 mol) and 20 ml of 7 N ammonia solution in methanol. The product was isolated on a silica gel column eluting with a solvent system of $CHCl_3$:CH_3OH = 4:1. Yield: 481 mg (53%); TLC: R_f ($CHCl_3$:CH_3OH, 4:1) 0.36; LC-MS *m*/*z* calculated for $[C_{28}H_{37}NP]^+$: 418.27; found 418.37; ^1H NMR ($CDCl_3$): 7.83–7.76 (15H, m, -C6H5), 3.00 (2H, dt, *J* = 15.2, 5.9, 4.9 , Ph_3P-$C\underline{H}_2$-), 2.26 (2H, t, *J* = 7.3, -$C\underline{H}_2$- NH_2), 1.89–1.63 (6H, m, Ph_3P-CH_2-$C\underline{H}_2$-$C\underline{H}_2$-, -$C\underline{H}_2$-CH_2-NH_2), 1.31–1.19 (10H, m, -CH_2-$C\underline{H}_2$-CH_2-), 3.73 (2H, t , -$N\underline{H}_2$); ^{13}C NMR ($CDCl_3$): 135.11 (3C, d, *J* = 3.0, p-$\underline{C}_6H_5$), 133.73 (6C, d, *J* = 10.00,

o-C$_6$H$_5$), 130.48 (6C, d, *J* = 12.5, m-C$_6$H$_5$), 117.58 (3C, d, *J* = 86.0, ipso-C$_6$H$_5$), 40.05 (1C, -CH$_2$-NH$_2$), 32.33–22.43 (9H, -CH$_2$-).

*H-ArgArgIleArgProArgProProTyrLeu-C10-TPP*6TFA (**4**, Bac(1-10, R/Y)-C10-TPP)*

Fmoc-Arg(Pbf)Arg(Pbf)IleArg(Pbf)ProArg(Pbf)ProProTyr(tBu)Leu-C10-TPP was obtained by the procedure described for Fmoc-Arg(Pbf)Arg(Pbf)IleArg(Pbf)ProArg(Pbf)ProProTyr(tBu)Leu-C2-TPP starting from compound **6** (100 mg, 0.038 mmol), DIPEA (14 μl, 0.076 mmol), HBTU (21.8 mg, 0.057 mmol), and [TPP-C10-NH$_2$]Br (18 mg, 0.042 mmol). The target product was isolated on a silica gel column eluting with a solvent system of CHCl$_3$:CH$_3$OH = 9:1. Yield: 45 mg (39%). TLC: R_f (CHCl$_3$:CH$_3$OH, 9:1) 0.42, R_f (CHCl$_3$:CH$_3$OH, 6:1) 0.56. Bac(1-10, R/Y)-C10-TPP (**4**) was obtained from Fmoc-Arg(Pbf)Arg(Pbf)IleArg(Pbf)ProArg(Pbf)ProProTyr(tBu)Leu-C10-TPP (20 mg, 0.0067 mmol) by the procedure described for compound **1**. The target product was isolated by preparative HPLC in a gradient of CH$_3$CN in 0.05% TFA from 20 to 40% for 18 min: τ = 16.7 min. Yield: 8.5 mg (52%); MALDI-TOF MS: *m*/*z* calculated for $[C_{88}H_{137}N_{23}O_{11}P]^+$: 1723.1; found 1723.0; HRMS: *m*/*z* calculated for $[C_{88}H_{137}N_{23}O_{11}P+2H]^{3+}$: 575.0249; found 575.0249; calculated for $[C_{88}H_{137}N_{23}O_{11}P+3H]^{4+}$: 431.5205; found 431.5209; calculated for $[C_{88}H_{137}N_{23}O_{11}P+4H]^{5+}$: 345.4178; found 345.4180. See also Section III of Supplementary materials for detailed HRMS analysis (Figures S8.1–S8.4).

Detailed synthesis of TPP-C10-Bac(1-10, R/Y) (5)

(10-Carboxydecyl)triphenylphosphionium bromide ([TPP-C10-COOH]Br)

1.57 g (6 mmol) of triphenylphosphine and 1.32 g (5 mmol) of 11-bromoundecanoic acid were dissolved in 4 ml of benzene. And the mixture was kept for 72 hours at 85°C. Then benzene was evaporated on a rotary evaporator. The obtained residue was dissolved in 4 ml of methylene chloride following to addition of fivefold excess of diethyl ether and left in the refrigerator to crystallize. The precipitate was separated by filtration, washed with ether and dried over CaCl$_2$. Yield: 2.13 g (81%); TLC: R_f (CHCl$_3$:MeOH, 9:1) 0.33; LC-MS *m*/*z* calculated for $C_{29}H_{36}O_2P$ $(M)^+$: 447.57, found 447.82; ^{1}H NMR (CDCl$_3$): 7.85–7.72 (15H, m, -C6H5), 3.40 (2H, dt, *J* = 15.2, 5.9, 4.9 , Ph$_3$P-CH$_2$-), 2.35 (2H, t, *J* = 7.3, -CH$_2$-COOH), 1.72–1.55 (6H, m, Ph$_3$P-CH$_2$-CH$_2$-CH$_2$-, -CH$_2$-CH$_2$-COOH), 1.36–1.23 (10H, m, -CH$_2$-CH$_2$-CH$_2$-); ^{13}C NMR (CDCl$_3$): 177.53 (1C,-COOH), 135.14 (3C,d, *J* = 3.0, p-C$_6$H$_5$), 133.61 (6C, d, *J* = 10.00, o-C$_6$H$_5$), 130.58 (6C, d, *J* = 12.5, m-C$_6$H$_5$), 118.22 (3C, d, *J* = 86.0, ipso-C$_6$H$_5$), 34.45 (1C, -CH$_2$COOH), 30.38-22.42 (9H, -CH$_2$-); ^{31}P NMR (CDCl$_3$) 23.80 (1P, s, Ph$_3$P-CH$_2$-).

*TPP-C10-C(O)NH-ArgArgIleArgProArgProProTyrLeu-OH*5TFA (**5**, TPP-C10-Bac(1-10, R/Y))*

150 mg of peptidyl-polymer Fmoc-Arg(Pbf)Arg(Pbf)IleArg(Pbf)ProArg(Pbf)ProProTyr(tBu)Leu-**P** was treated with 20% solution of piperidine in DMF for 15 min. Next, the solvent was removed by filtration and the resin was washed with DMF several times. [TPP-C$_{10}$-COOH]Br (211 mg, 0.40 mmol), HBTU (152 mg, 0.40 mmol), DIPEA (70 μL, 0.4 mmol) were added to peptidyl-polymer in DMF and stirred for 12 h. Then the peptidyl polymer was washed with DMF and CH$_2$Cl$_2$ and treated with 3 mL of reagent K for 4 h. The product was precipitated with diethyl ether, and the solid precipitate was washed three times with

diethyl ether. Yield: 82 mg (63%). The target product was isolated by preparative HPLC in a gradient of CH_3CN in 0.05% TFA from 20 to 40% for 18 min: τ = 11.5 min. MALDI-TOF MS: m/z calculated for $[C_{89}H_{136}N_{22}O_{13}P]^+$: 1752.0; found 1751.9; HRMS: *m*/*z* calculated for $[C_{89}H_{136}N_{22}O_{13}P+H]^{2+}$: 877.0248; found 877.0250; calculated for $[C_{89}H_{136}N_{22}O_{13}P+2H]^{3+}$: 585.0189; found 585.0185; calculated for $[C_{89}H_{136}N_{22}O_{13}P+3H]^{4+}$: 439.0160; found 439.0159; calculated for $[C_{89}H_{136}N_{22}O_{13}P+4H]^{5+}$: 351.4143; found 351.4144. See also Section III of Supplementary materials for detailed HRMS analysis (Figures S9.1–S9.2).

Detailed synthesis of BODIPY-ArgArgIleArgProArgProProArgLeu-OH*5TFA (BODIPY-Bac(1-10))

4 mg (0.0020 mmol) of Bac(1-10)*6TFA was dissolved in 200 μl of 0.2 M $NaHCO_3$ and 0.7 mg (0.0018 mmol) of succinimide ester BODIPY-FL-C3 in 40 μl of DMF was added. The resulting mixture was stirred for 2 hours at room temperature. The obtained mixture was diluted 10 times with water and the product was purified by preparative HPLC in a gradient of CH_3CN in 0.05% TFA from 5 to 40% for 20 min: τ = 17.3 min. Yield: 1.2 mg (31%); fluorescence (MeOH): λ_{ex} = 505 nm, λ_{em} = 510 nm; LC-MS *m*/*z* calculated for $[C_{71}H_{118}BF_2N_{27}O_{12}+2H]^{2+}$: 795.98; found 794.82; τ (UPLC) = 0.89 min; MALDI TOF MS *m*/*z* calculated for $[C_{71}H_{118}BF_2N_{27}O_{12}+H]^+$: 1591.0; found 1591.0.

II. SUPPLEMENTARY FIGURES

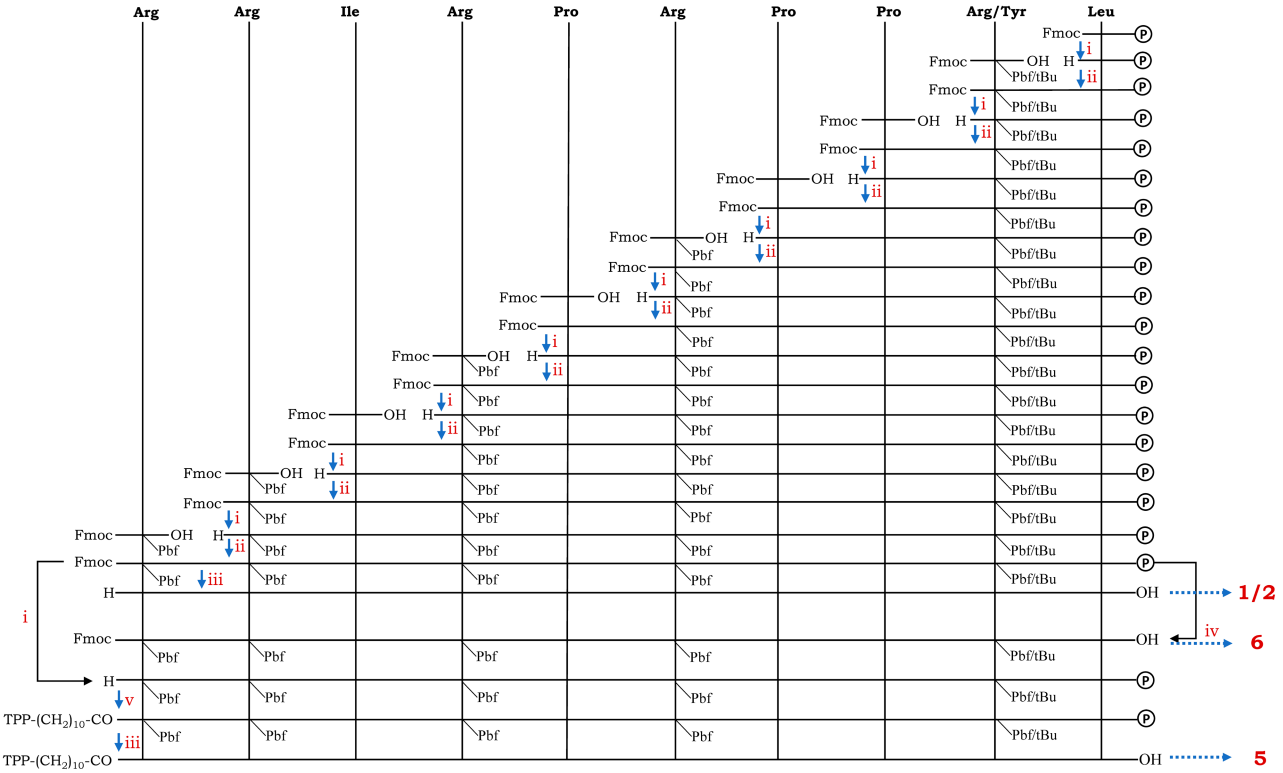


Figure S1. Scheme of the chemical synthesis of short peptide from Bac 7 (Bac(1-10), **1**), its R⁹/Y-analog (Bac(1-10, R/Y), **2**), their triphenylphosphonium analog TPP-C10-Bac(1-10, R/Y), **5**, and protected peptide **6**: i – 20% Pip/DMF, ii – HBTU/DIPEA/DMF, iii – reagent K, iv – HFIP/CH_2Cl_2, v – TPP-$(CH_2)_{10}$-COOH/HBTU/DIPEA/DMF.

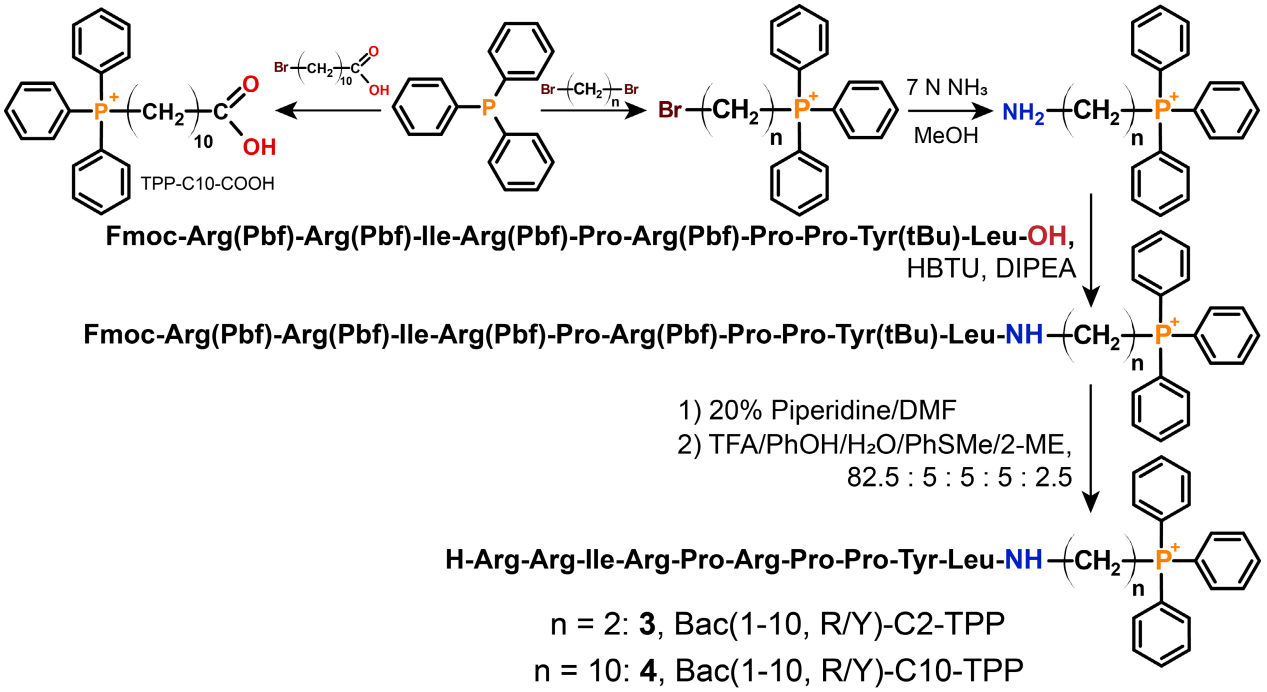


Figure S2. Scheme of the chemical synthesis of triphenylphosphonium analogs of short peptide from Bac7: (Bac(1-10, R/Y)-C2-TPP, **3**) and (Bac(1-10, R/Y)-C10-TPP, **4**).

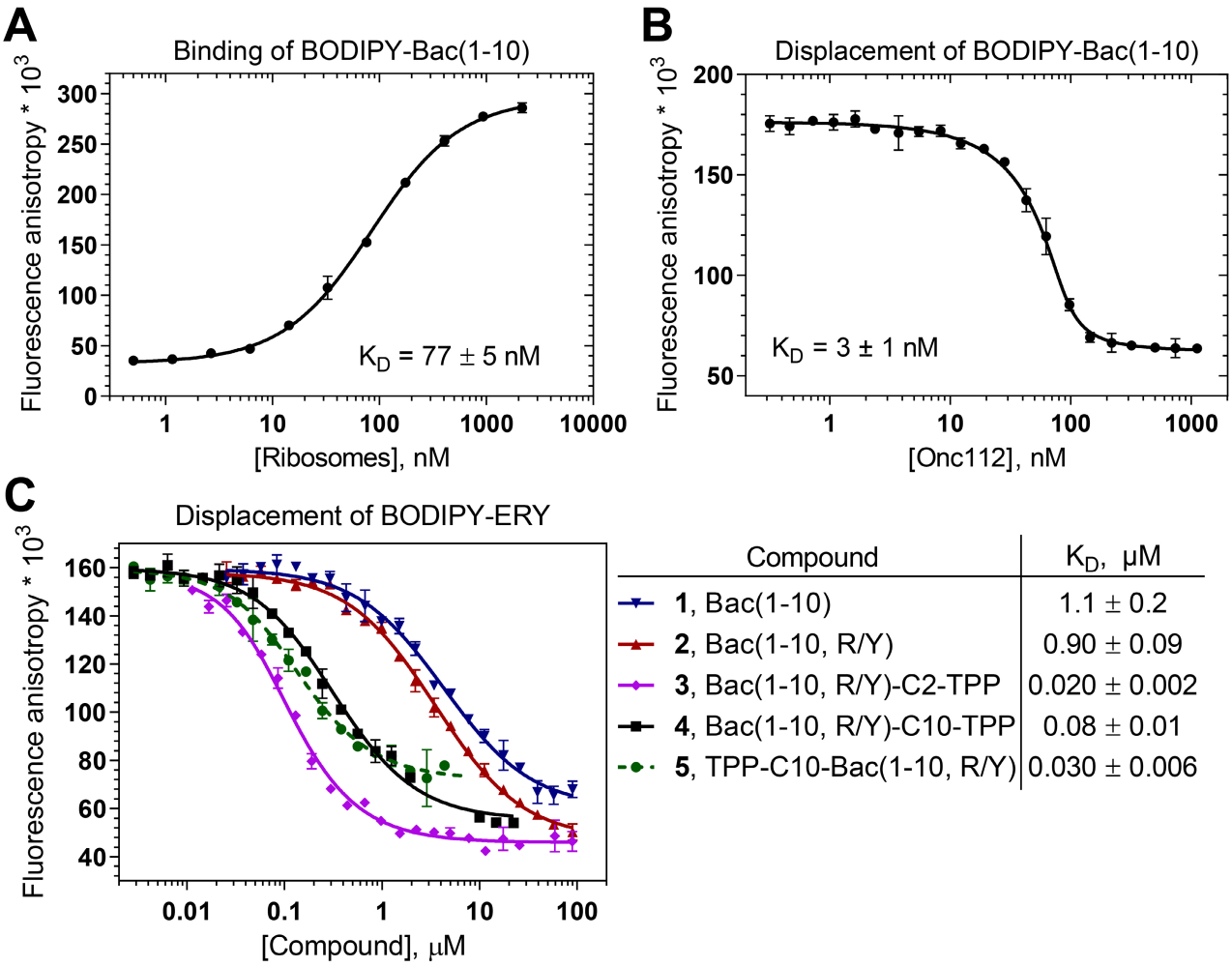
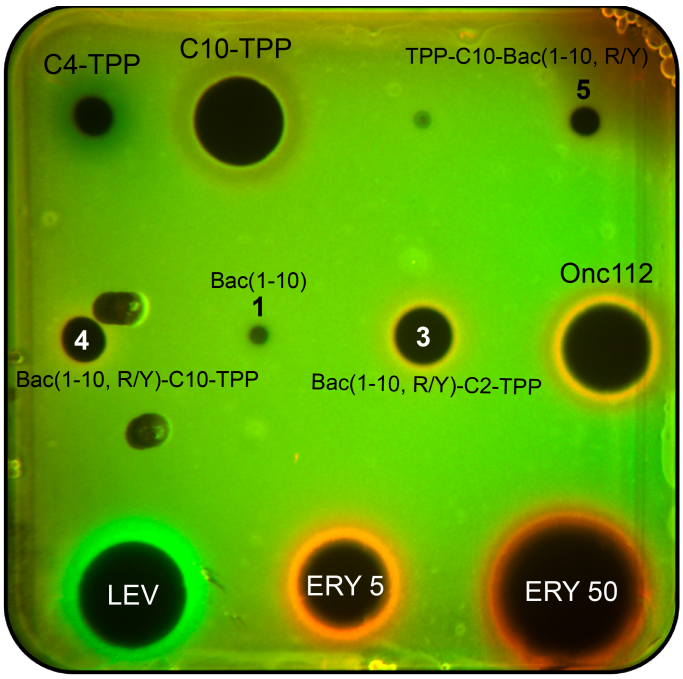


Figure S3. Binding affinity to bacterial ribosomes of triphenylphosphonium analogs of short peptide from Bac7. Average values of apparent dissociation constants (K_D) with CI (α = 0.05) are shown. All experiments were performed at least three times, error bars are SD. (**A**) Binding of BODIPY-Bac(1-10) to *E. coli* 70S ribosomes measured by fluorescence anisotropy. (**B**) A competitive binding assay to test the affinity of Onc112 to *E. coli* 70S ribosomes measured by displacement of BODIPY-Bac(1-10). (**C**) A competitive binding assay to test the affinity of peptides **1** and **2** and their TPP analogs **3–5** to *E. coli* 70S ribosomes measured by displacement of BODIPY-ERY.



E. coli (Δ*tolC*) pDualrep2

Figure S4. Testing of antibacterial activity of triphenylphosphonium derivatives of decapeptide related to Bac7 and Onc112 using *E. coli (ΔtolC)* pDualrep2 strain. Onc112, erythromycin at concentrations of 5 mg/mL (ERY 5) and 50 mg/mL (ERY 50), levofloxacin (LEV), C10-TPP, and C4-TPP are used as the controls. The induction of the red fluorescent protein expression (green halo around the inhibition zone, pseudocolor) is triggered by DNA damage, while the induction of Katushka2S protein (red halo, pseudocolor) occurs in response to ribosome stalling. Unlabeled spots correspond to substances not studied in this work.

III. HRMS DETAILED DATA

HRMS data for Bac(1-10) (1)

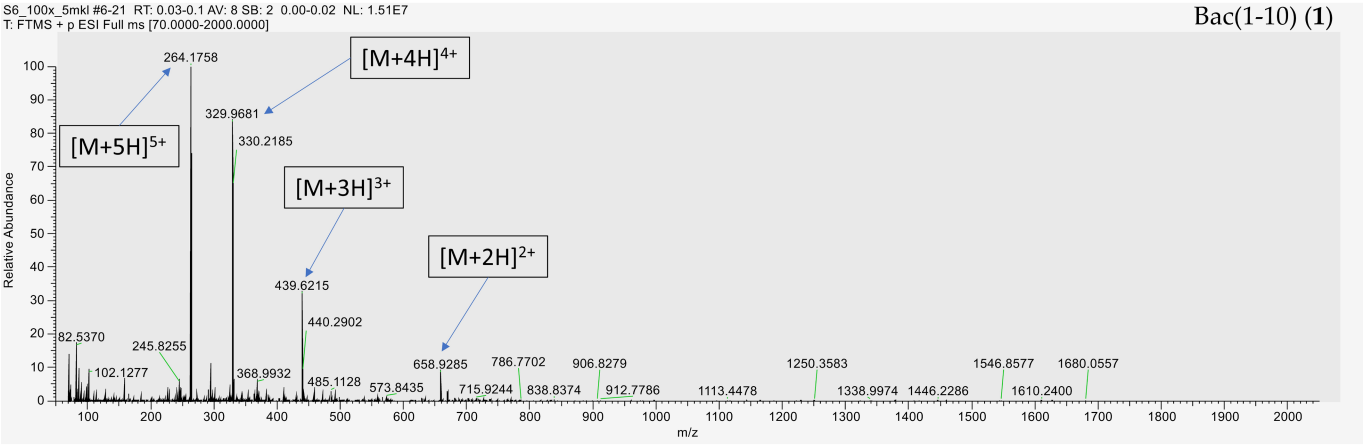


Figure S5.1. The HRMS spectrum of compound **1** in positive ion mode.

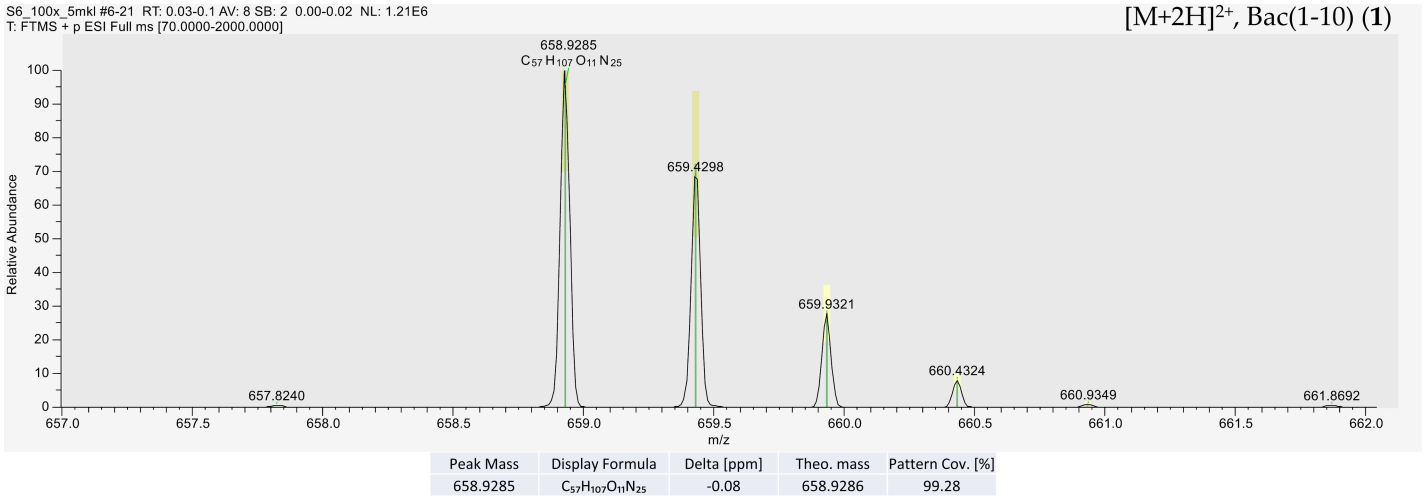


Figure S5.2. The HRMS data for $[M+2H]^{2+}$ ion of $[C_{57}H_{105}N_{25}O_{11}]$ (compound **1**).

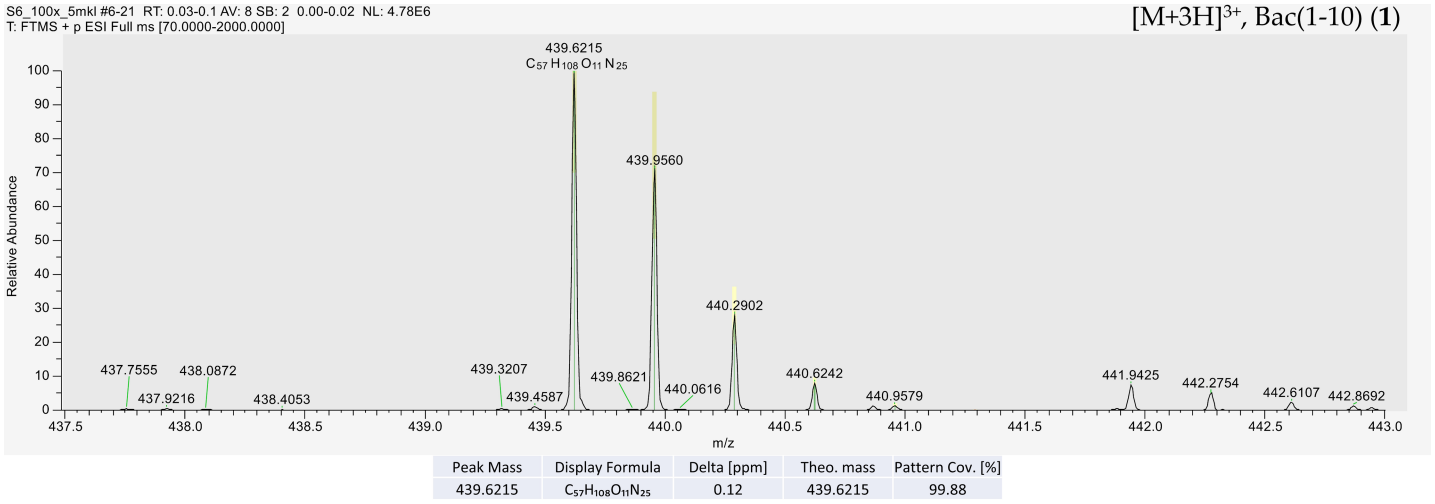


Figure S5.3. The HRMS data for $[M+3H]^{3+}$ ion of $[C_{57}H_{105}N_{25}O_{11}]$ (compound **1**).

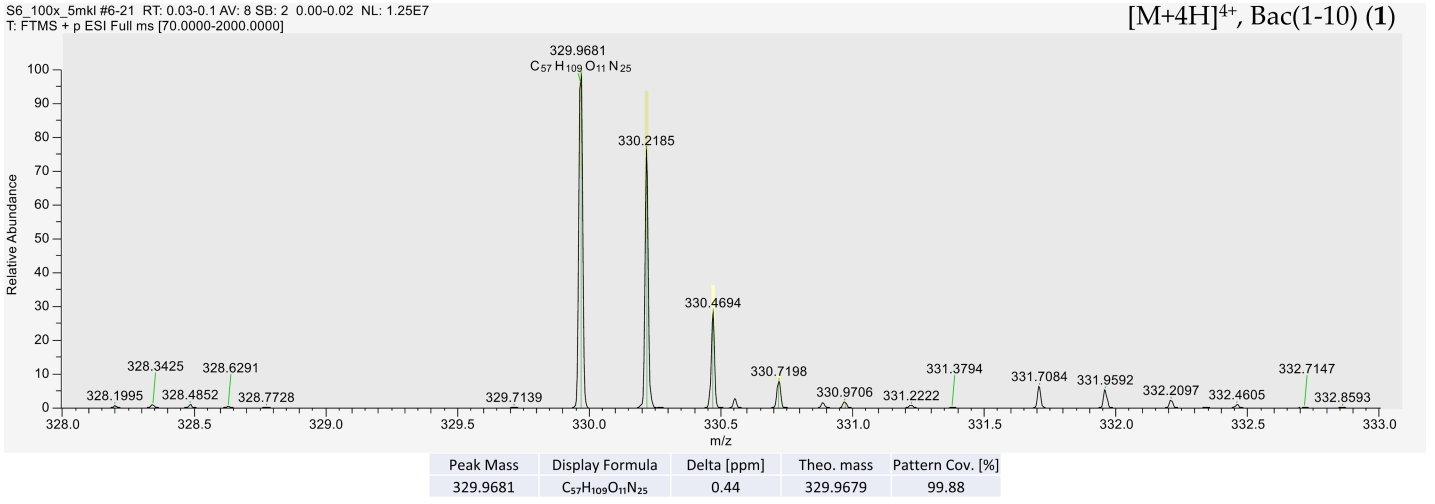


Figure S5.4. The HRMS data for $[M+4H]^{4+}$ ion of $[C_{57}H_{105}N_{25}O_{11}]$ (compound **1**).

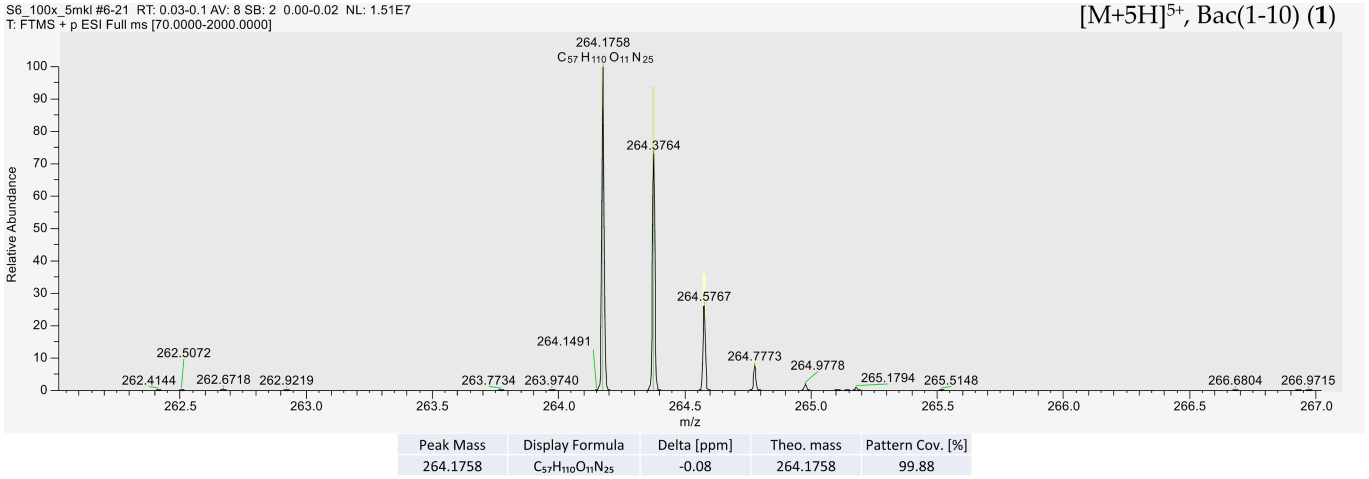


Figure S5.5. The HRMS data for $[M+5H]^{5+}$ ion of $[C_{57}H_{105}N_{25}O_{11}]$ (compound **1**).

HRMS data for Bac(1-10, R/Y) (2)

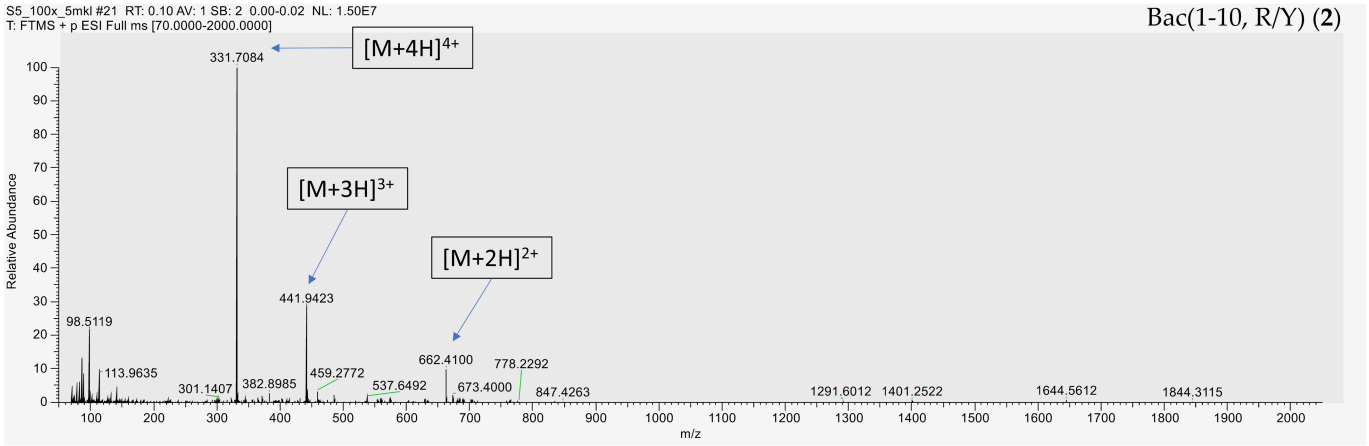


Figure S6.1. The HRMS spectrum of compound **2** in positive ion mode.

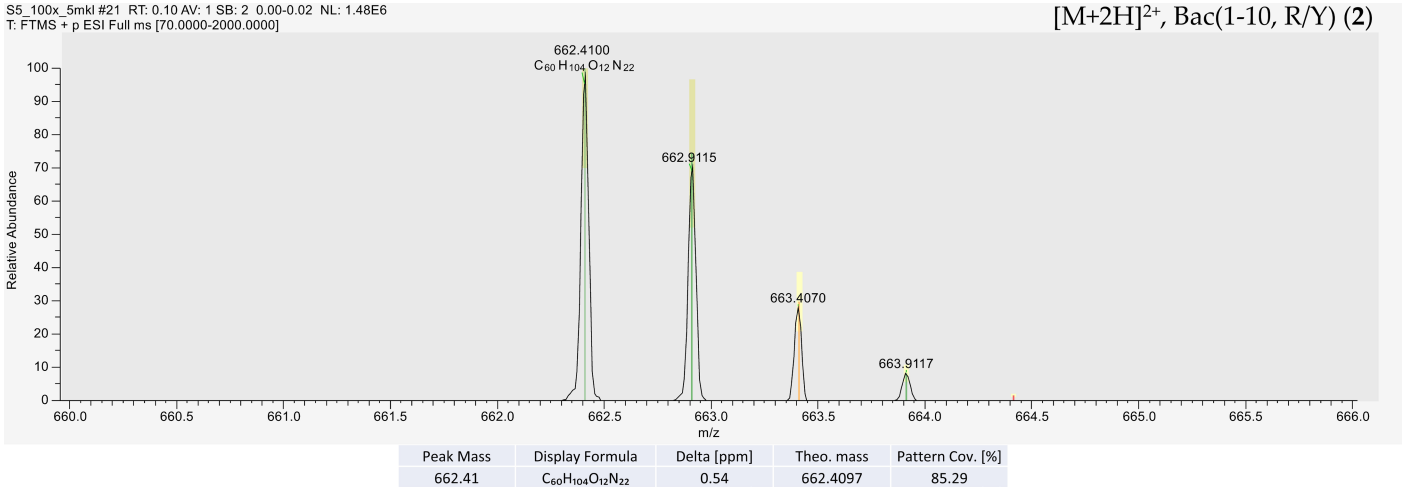


Figure S6.2. The HRMS data for $[M+2H]^{2+}$ ion of $[C_{60}H_{102}N_{22}O_{12}]$ (compound **2**).

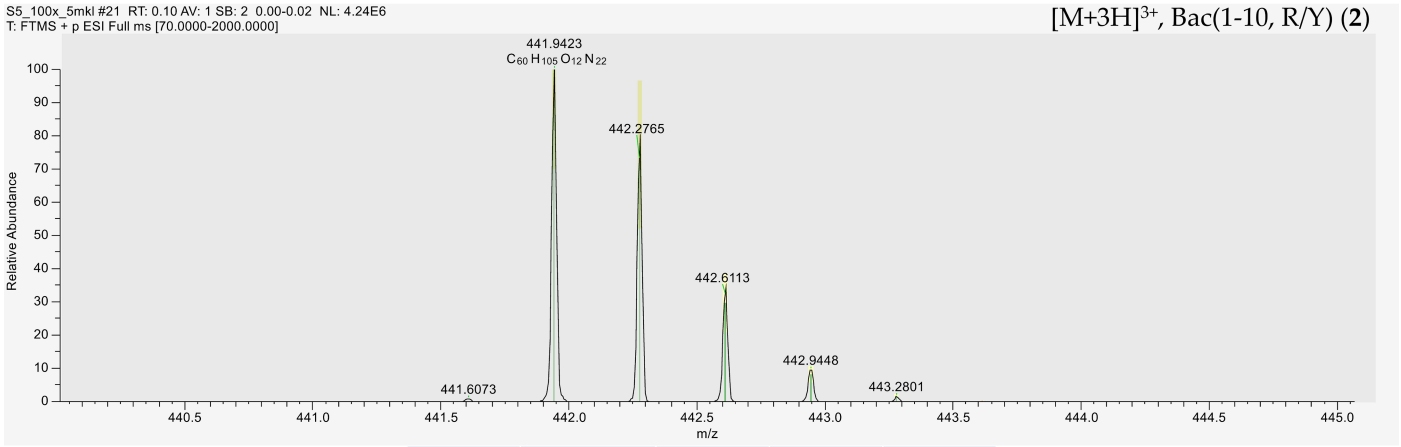


Figure S6.3. The HRMS data for $[M+3H]^{3+}$ ion of $[C_{60}H_{102}N_{22}O_{12}]$ (compound **2**).

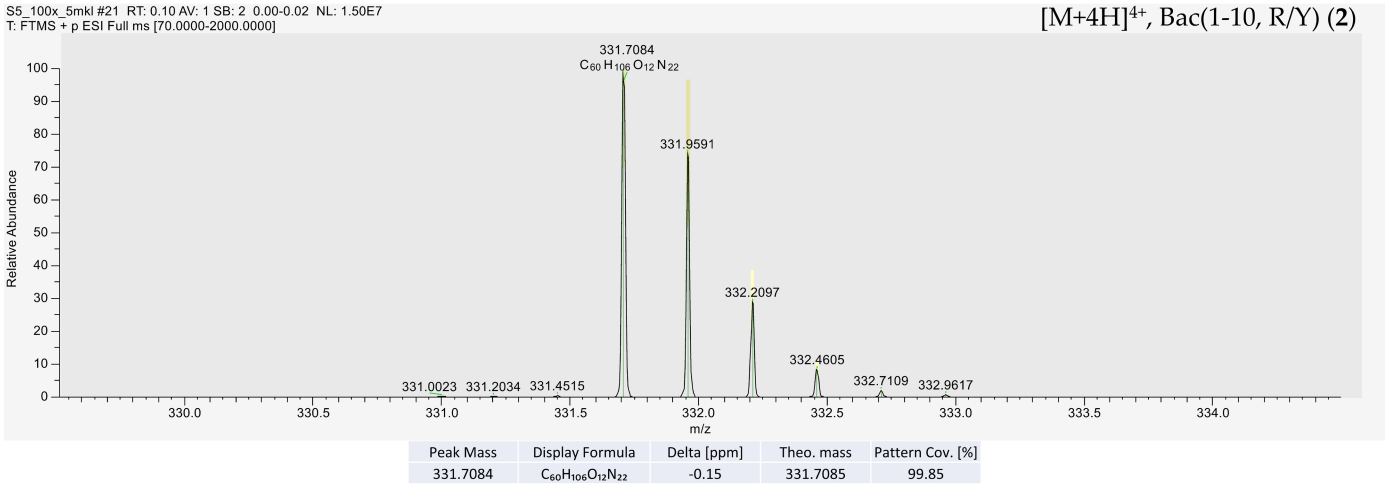


Figure S6.4. The HRMS data for $[M+4H]^{4+}$ ion of $[C_{60}H_{102}N_{22}O_{12}]$ (compound **2**).

HRMS data for Bac(1-10, R/Y)-C2-TPP (3)

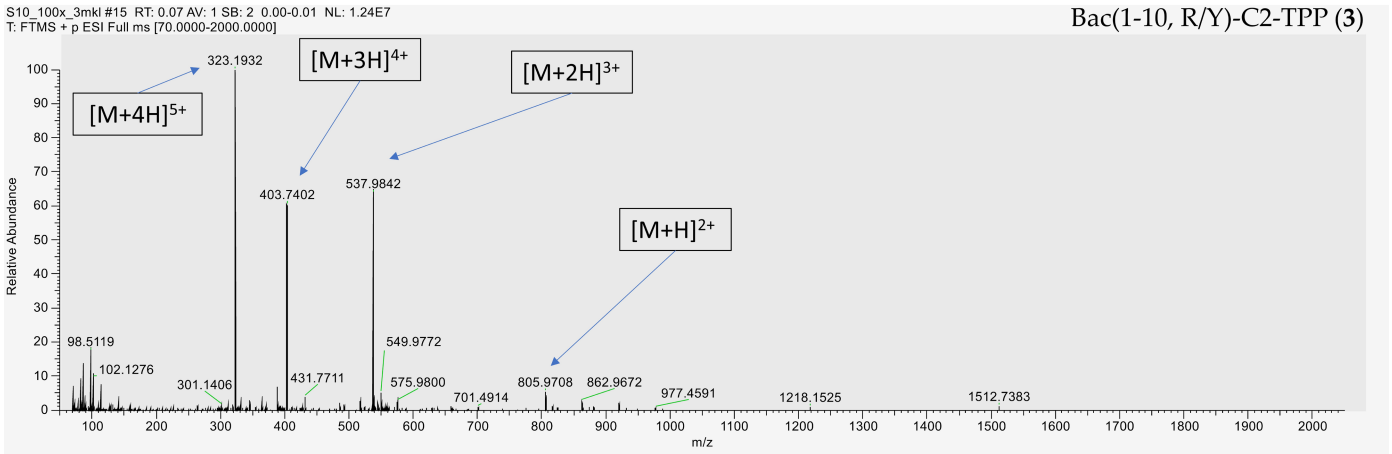
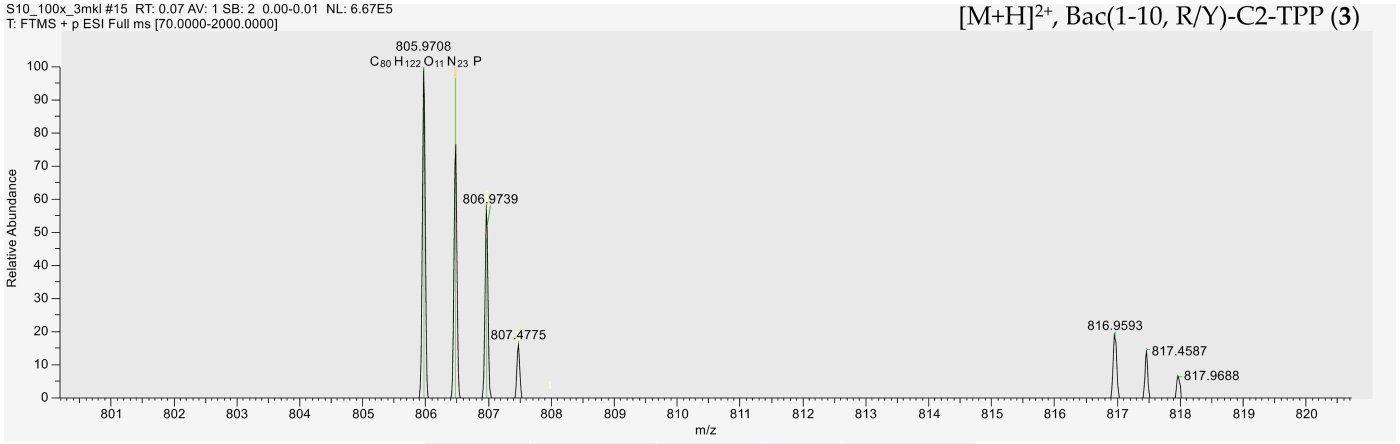
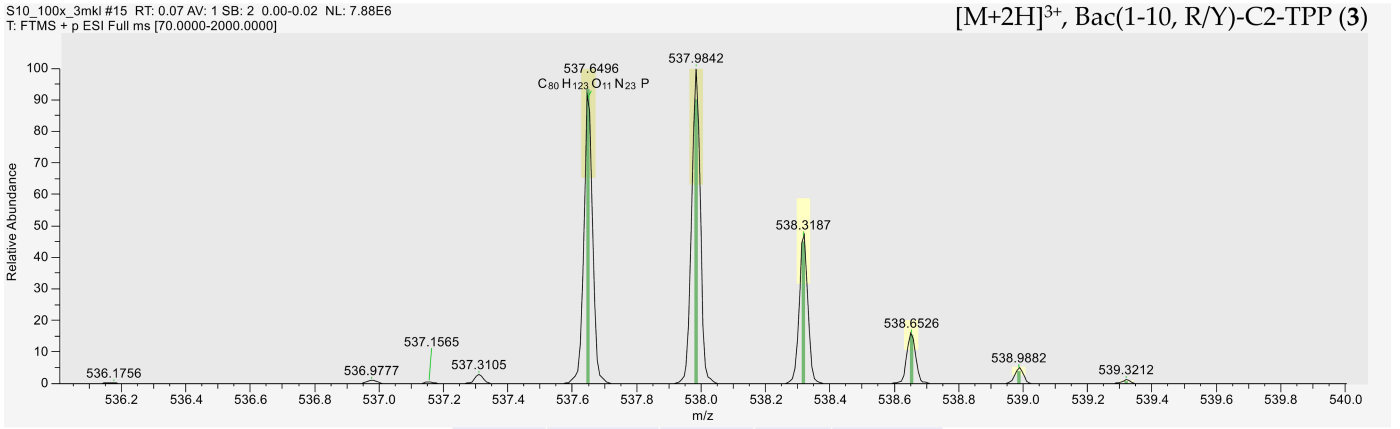


Figure S7.1. The HRMS spectrum of compound **3** in positive ion mode.



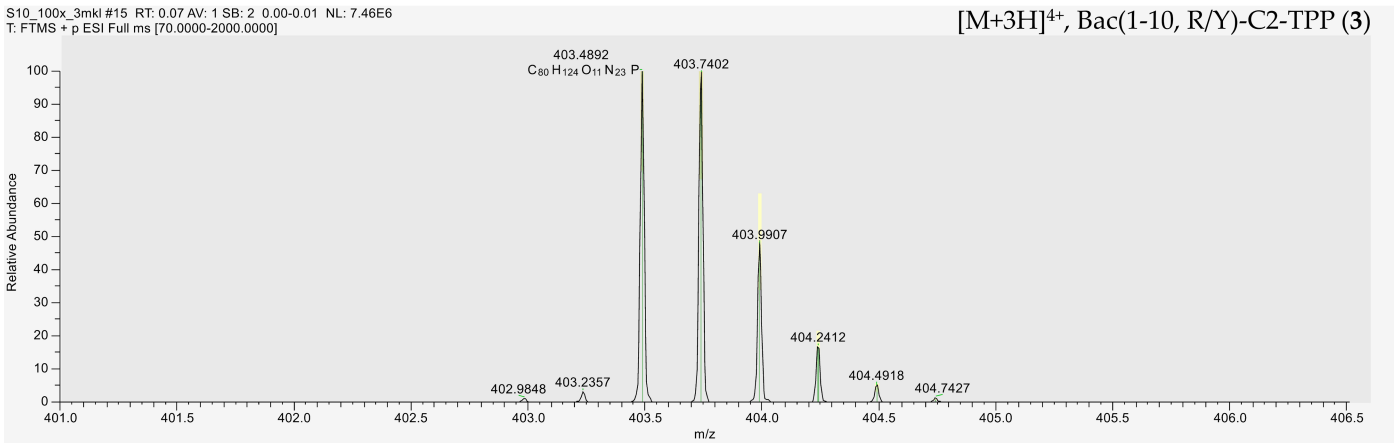
Peak Mass	Display Formula	Delta [ppm]	Theo. mass	Pattern Cov. [%]
805.9708	$C_{80}H_{122}O_{11}N_{23}P$	-0.3	805.971	98.48

Figure S7.2. The HRMS data for $[M+H]^{2+}$ ion of $[C_{88}H_{137}N_{23}O_{11}P]^{+}$ (compound **3**).



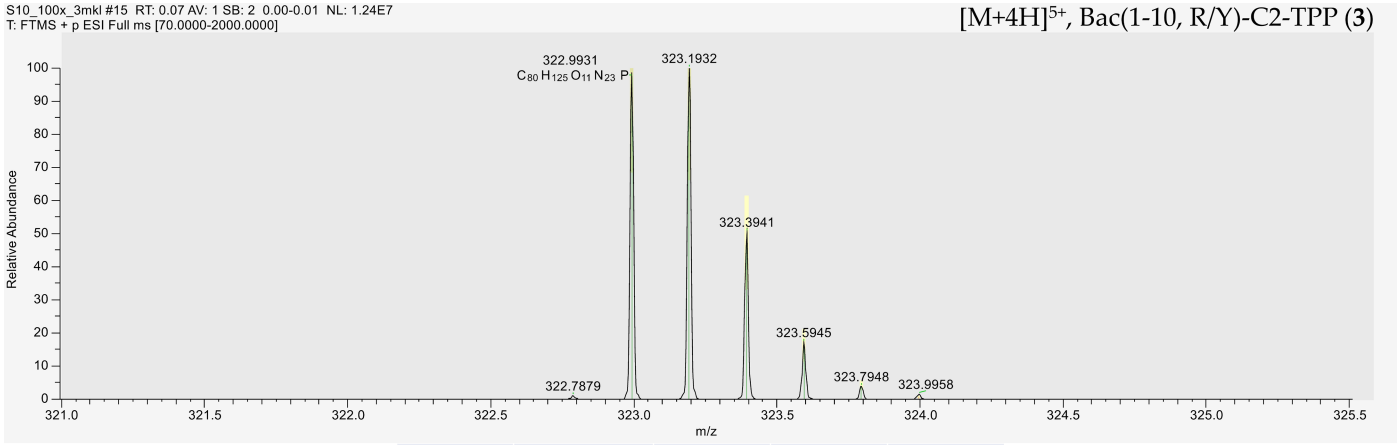
Peak Mass	Display Formula	Delta [ppm]	Theo. mass	Pattern Cov. [%]
537.6496	$C_{80}H_{123}O_{11}N_{23}P$	-0.32	537.6498	99.95

Figure S7.3. The HRMS data for $[M+2H]^{3+}$ ion of $[C_{88}H_{137}N_{23}O_{11}P]^{+}$ (compound **3**).



Peak Mass	Display Formula	Delta [ppm]	Theo. mass	Pattern Cov. [%]
403.4892	$C_{80}H_{124}O_{11}N_{23}P$	0.16	403.4892	99.95

Figure S7.4. The HRMS data for $[M+3H]^{4+}$ ion of $[C_{88}H_{137}N_{23}O_{11}P]^{+}$ (compound **3**).



Peak Mass	Display Formula	Delta [ppm]	Theo. mass	Pattern Cov. [%]
322.9931	$C_{80}H_{125}O_{11}N_{23}P$	0.89	322.9928	99.59

Figure S7.5. The HRMS data for $[M+4H]^{5+}$ ion of $[C_{88}H_{137}N_{23}O_{11}P]^{+}$ (compound **3**).

HRMS data for Bac(1-10, R/Y)-C10-TPP (4)

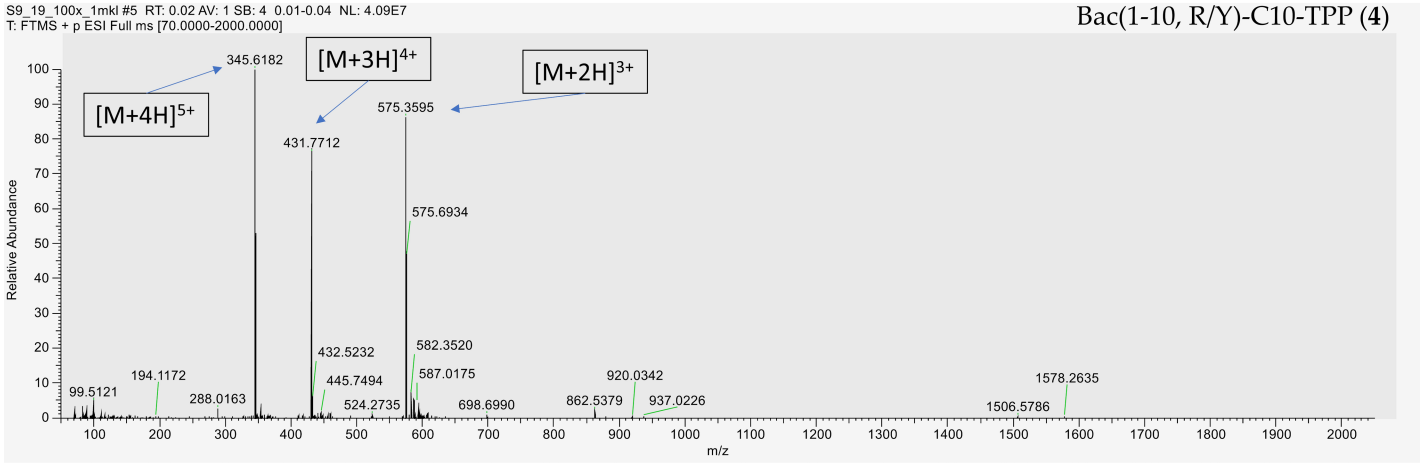
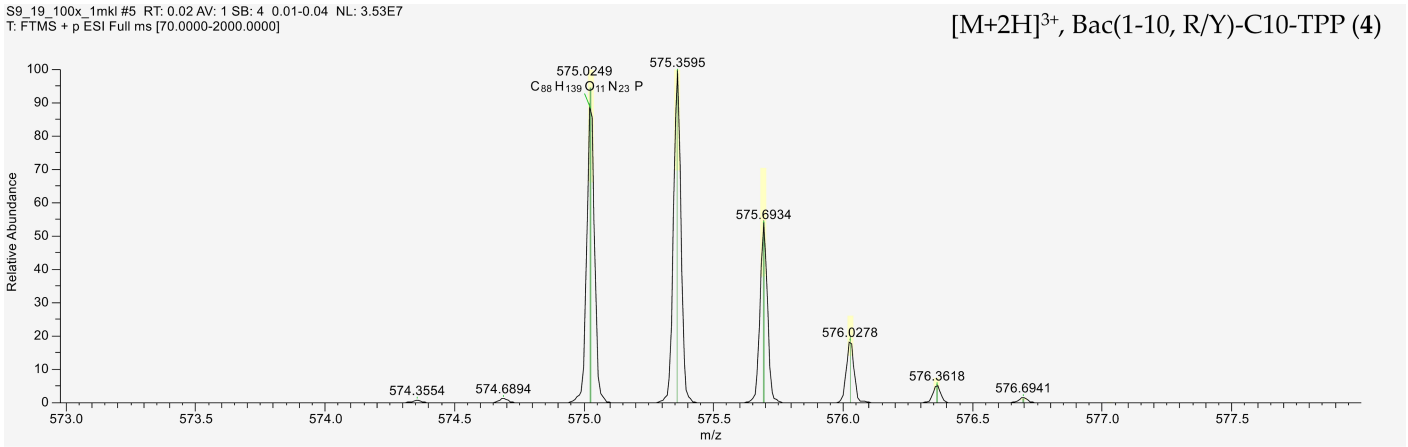


Figure S8.1. The HRMS spectrum of compound **4** in positive ion mode.



Peak Mass	Display Formula	Delta [ppm]	Theo. mass	Pattern Cov. [%]
575.0249	$C_{88}H_{139}O_{11}N_{23}P$	0.02	575.0249	99.92

Figure S8.2. The HRMS data for $[M+2H]^{3+}$ ion of $[C_{88}H_{137}N_{23}O_{11}P]^{+}$ (compound **4**).

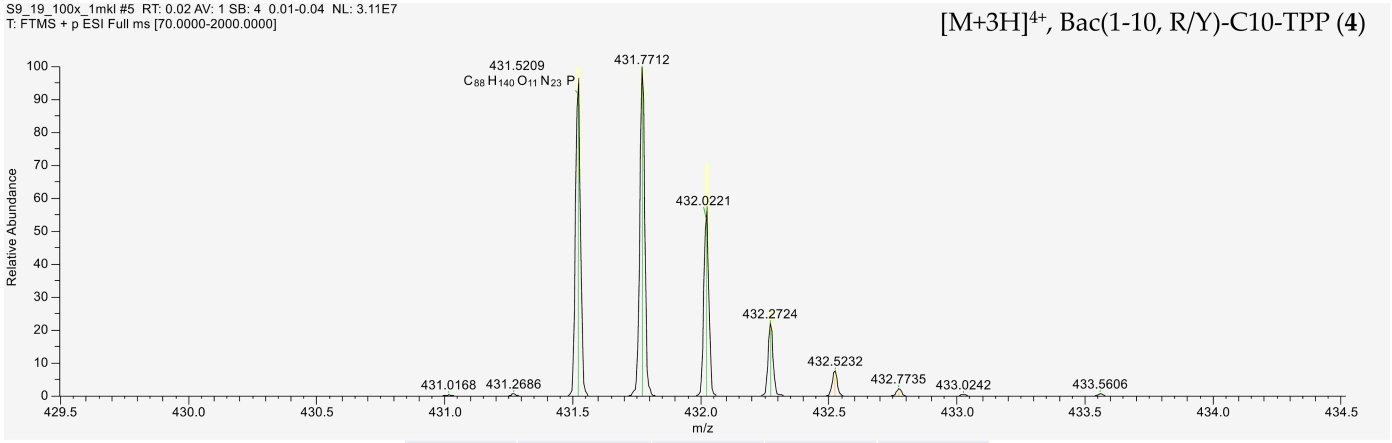


Figure S8.3. The HRMS data for $[M+3H]^{4+}$ ion of $[C_{88}H_{137}N_{23}O_{11}P]^{+}$ (compound **4**).

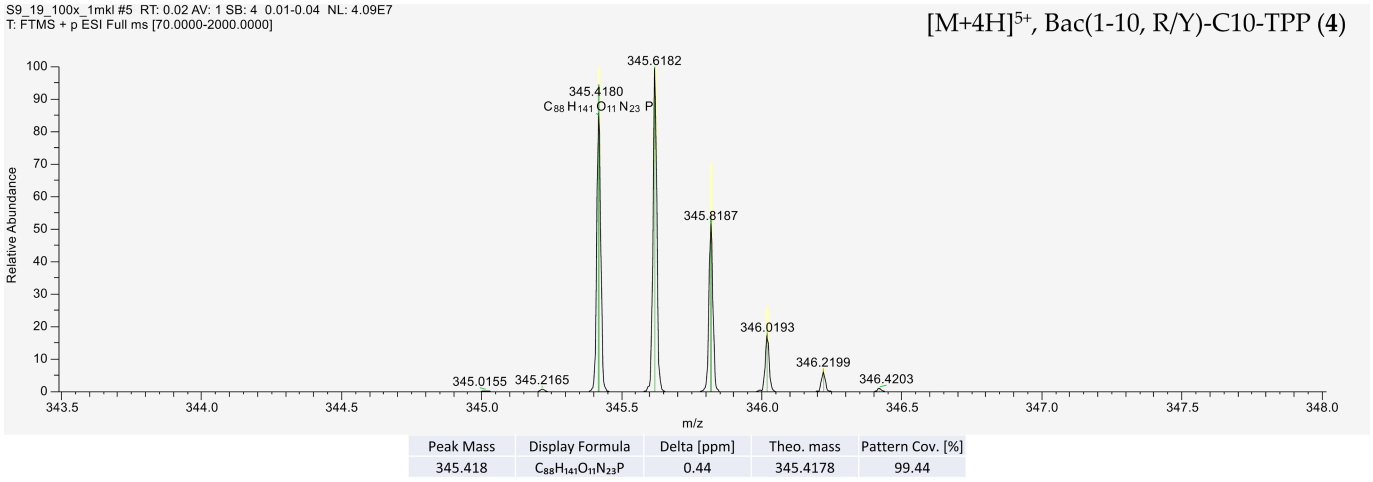


Figure S8.4. The HRMS data for $[M+4H]^{5+}$ ion of $[C_{88}H_{137}N_{23}O_{11}P]^{+}$ (compound **4**).

HRMS data for TPP-C10-Bac(1-10, R/Y) (5)

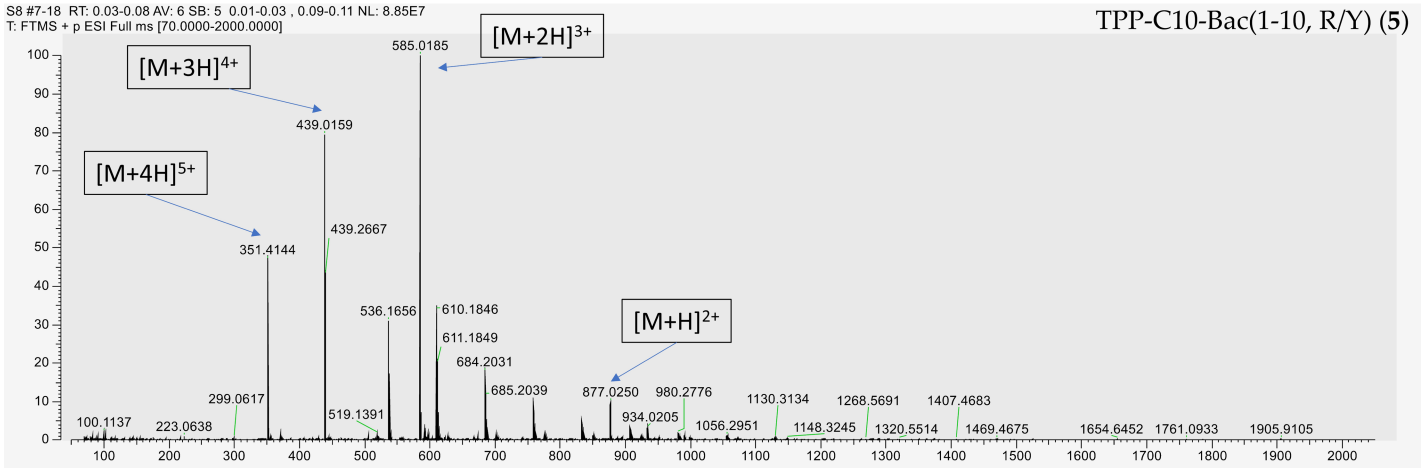


Figure S9.1. The HRMS spectrum of compound **5** in positive ion mode.

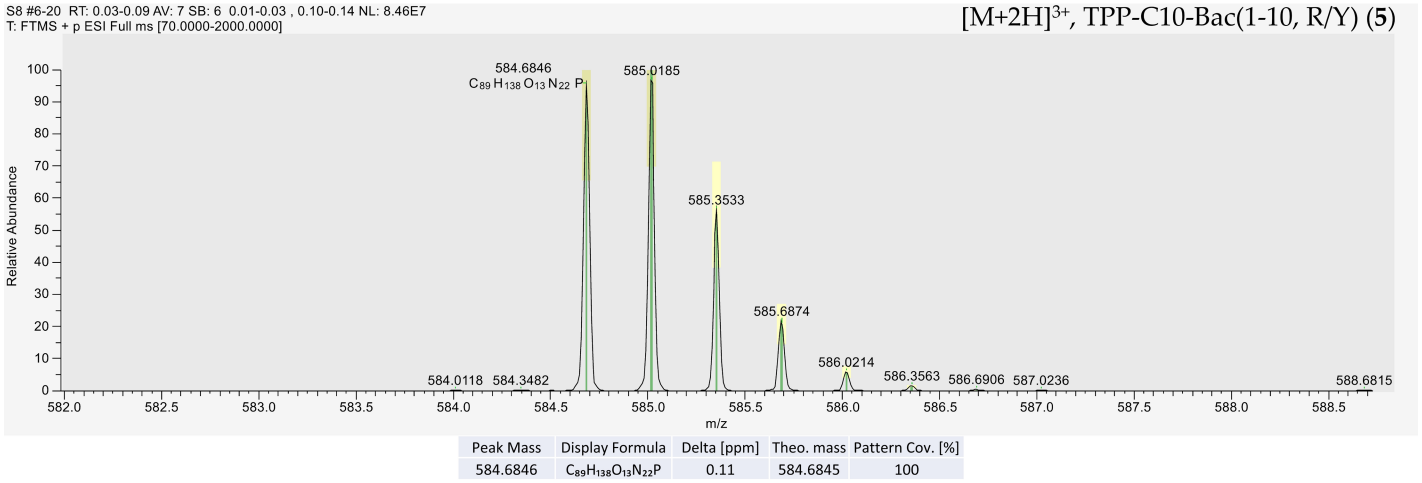


Figure S9.2. The HRMS data for $[M+2H]^{3+}$ ion of $[C_{89}H_{136}N_{22}O_{13}P]^{+}$ (compound **5**).

V. SUPPLEMENTARY REFERENCES

1. Hansen, P. R.; Oddo, A. Fmoc solid–phase peptide synthesis. *Methods Mol. Biol.* **2015**, *1348*, 33-50, doi: 10.1007/978-1-4939-2999-3_5.

2. Eissler, S.; Kley, M.; Bächle, D.; Loidl, G.; Meier, T.; Samson, D. Substitution determination of Fmoc-substituted resins at different wavelengths. *J. Pept. Sci*. **2017,** *23*, 757-762, doi: 10.1002/psc.3021.

3. Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. Color test for detection of free terminal amino groups in the solidphase synthesis of peptides. *Anal. Biochem*., **1970**, *34*, 595-598, doi: 10.1016/0003-2697(70)90146-6.