Supporting Information to

Selective binders of the tandem SH2 domains in Syk and ZAP-70 kinases by DNA-programmed spatial screening

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1 Synthesized Conjugates

1.1 Oligonucleotides

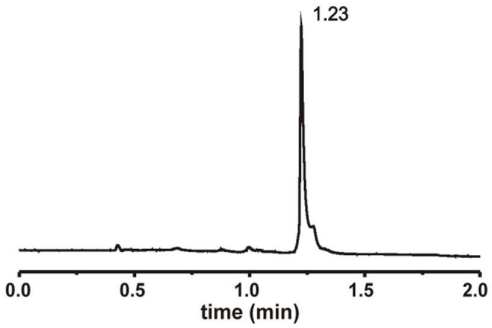
5'-GGC TGC XCA CTA-3' **ON1** X = 5-(Fmoc-Cys(StBu)-NH-C_3H_2)-U

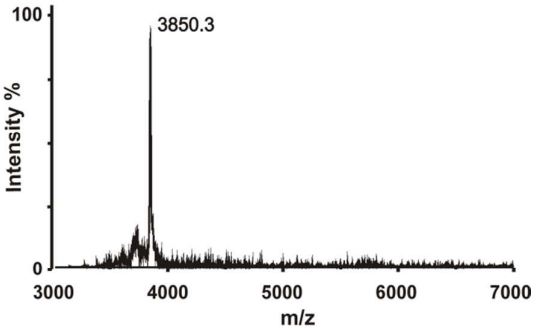
$C_{125}H_{162}N_{45}O_{72}P_{11}S_2$

OD_{260} = 34.2 (313 nmol, 31%); ε_{260} = 109300 L ·M^{-1}·cm^{-1}

HPLC: t_R = 1.23 min (5-50% ACN in 0.1 M TEAA in 2 min, 260 nm)

MALDI-TOF (m/z): calc.: 3850.70 $[M+H]^+$; found: 3850.3





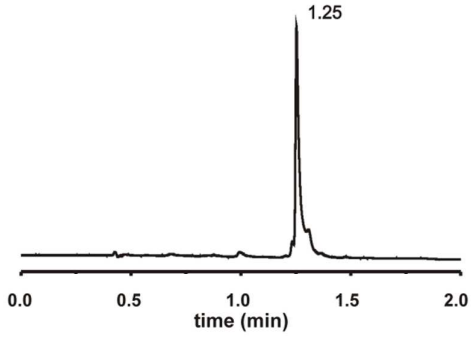
5'-CCA AGX TCG TGT-3' **ON2** X = 5-(Fmoc-Cys(StBu)-NH-C_3H_2)-U

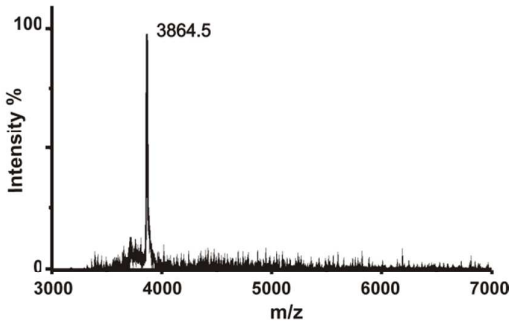
$C_{126}H_{163}N_{44}O_{753}P_{11}S_2$

OD_{260} = 31.4 (280 nmol, 28%); ε_{260} = 112000 L ·M^{-1}·cm^{-1}

HPLC: t_R = 1.25 min (5-50% ACN in 0.1 M TEAA in 2 min, 260 nm)

MALDI-TOF (m/z): calc.: 3865.70 $[M+H]^+$; found: 3864.5





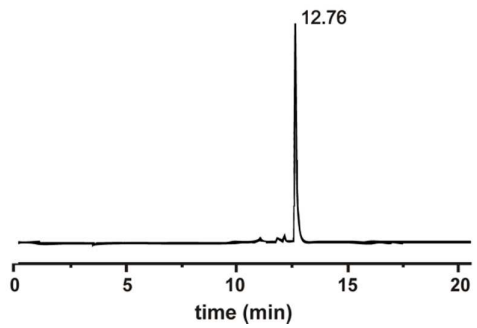
5'-GGC TGC TCA CTA Y-3' **ON3** Y = -PO_2-OC_3H_6-S-S-C_3H_6-OH

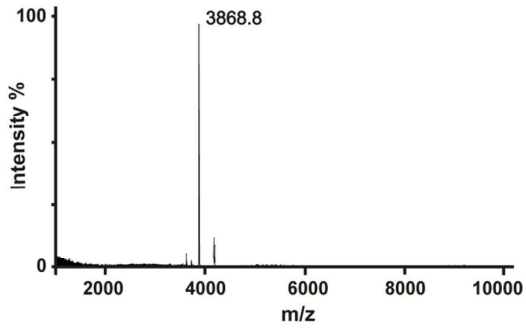
$C_{122}H_{161}N_{43}O_{75}P_{12}S_2$

OD_{260} = 43.5 (398 nmol, 40%); ε_{260} = 109300 L ·M^{-1}·cm^{-1}

HPLC: t_R = 12.76 min (5-50% ACN in 0.1 M TEAA in 20 min, 260 nm)

MALDI-TOF (m/z): calc.: 3864.64 $[M+H]^+$; found: 3868.8





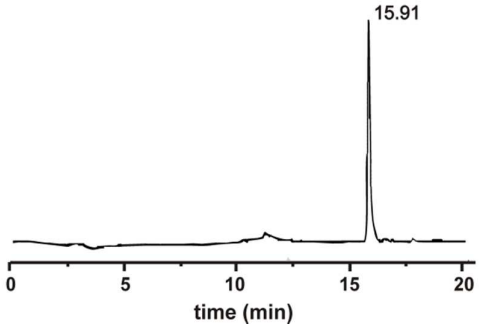
5'-ZCC AAG TTC GTG T-3' **ON4** Z = HO-C_6H_{12}-S-S-C_6H_{12}-O-PO_2-

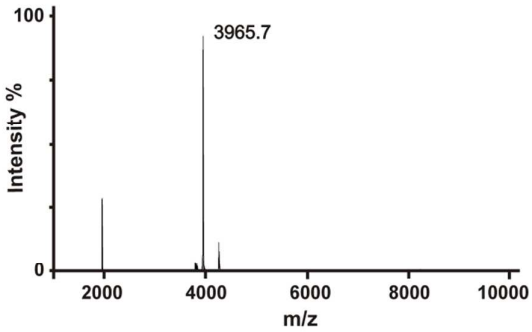
$C_{129}H_{174}N_{42}O_{76}P_{12}S_2$

OD_{260} = 48.0 (429 nmol, 43%); ε_{260} = 112000 L ·M^{-1}·cm^{-1}

HPLC: t_R = 15.91 min (5-50% ACN in 0.1 M TEAA in 20 min, 260 nm)

MALDI-TOF (m/z): calc.: 3963.74 $[M+H]^+$; found: 3965.7





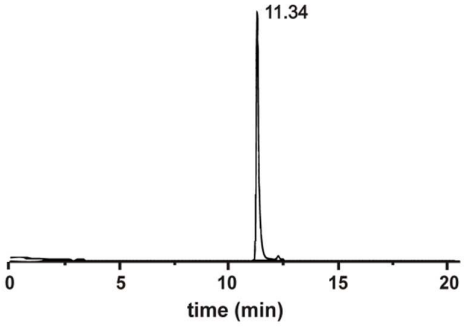
5'-ZGG CTG CTC ACT A-3' **ON5** Z = HO-C_6H_{12}-S-S-C_6H_{12}-O-PO_2-

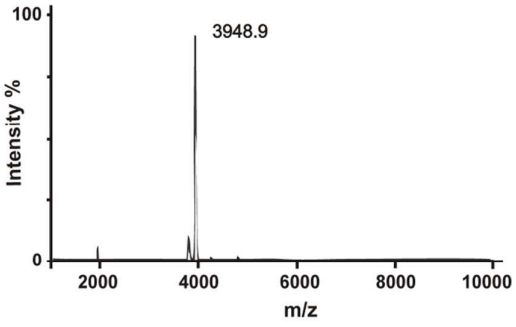
$C_{128}H_{173}N_{43}O_{75}P_{12}S_2$

OD_{260} = 49.4 (452 nmol, 58%); ε_{260} = 109300 L ·M^{-1}·cm^{-1}

HPLC: t_R = 11.34 min (5-50% ACN in 0.1 M TEAA in 20 min, 260 nm)

MALDI-TOF (m/z): calc.: 3948.74 $[M+H]^+$; found: 3948.9





1.2 Peptides

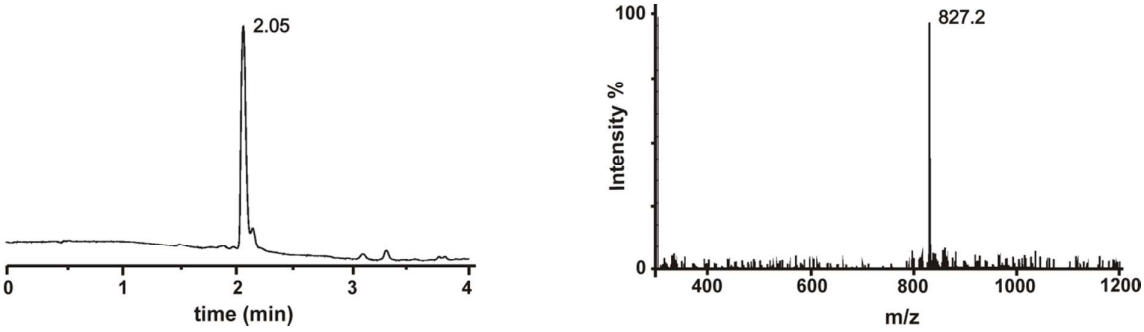
FAM-Lys-pTyr-Thr-Gly-Leu-Asn-Thr-Arg-Ser-Gln-Glu-Thr-pTy-Glu-Thr-Leu-Gly-OH **1**

$C_{105}H_{145}N_{23}O_{43}P_2$

OD_{267} = 259.0 (3.32 µmol, 31%); ε_{267} = 78000 L ·M^{-1}·cm^{-1}

HPLC: t_R = 2.05 min (5-50% ACN in H_2O, 0.01% TFA, 4 min, 210 nm)

ESI-MS (m/z): calc.: 2480.36 $[M+H]^+$; found.: 827.2 $[M+3H]^{3+}$



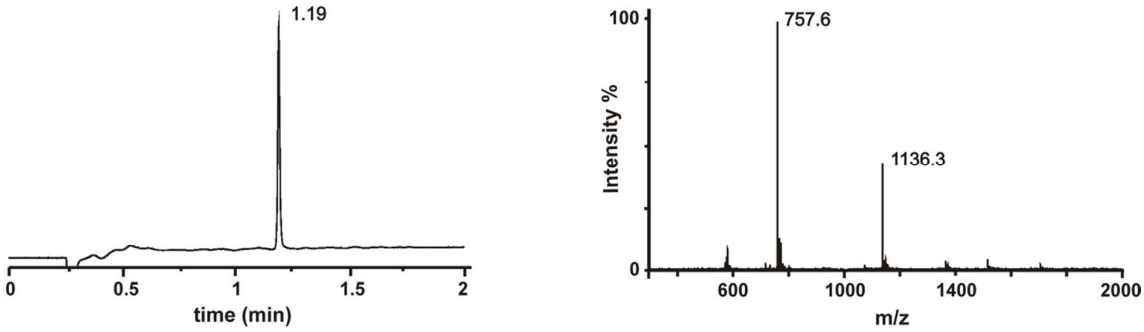
Ac-Lys-pTyr-Asn-Glu-Leu-Asn-Leu-Gly-Arg-Arg-Glu-Glu-pTyr-Asp-Val-Leu-Gly-NH_2 **2**

$C_{92}H_{147}N_{27}O_{36}P_2$

OD_{267} = 5.7 (4.39 µmol, 44%); ε_{267} = 1304 L ·M^{-1}·cm^{-1}

HPLC: t_R = 1.19 min (5-50% ACN in H_2O, 0.01% TFA, 2 min, 210 nm)

ESI-MS (m/z): calc.: 2270.06 $[M+H]^+$; found: 1136.3 $[M+2H]^{2+}$, 757.6 $[M+3H]^{3+}$, 568.7 $[M+4H]^{4+}$



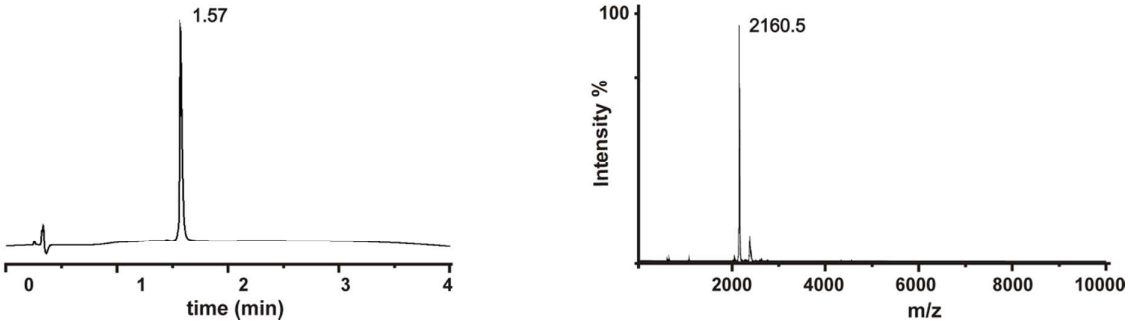
Ac-Lys-pTyr-Thr-Gly-Leu-Asn-Thr-Arg-Ser-Gln-Glu-Thr-pTyr-Glu-Thr-Leu-Gly **4**

$C_{86}H_{138}N_{24}O_{37}P_2$

OD_{267} = 3.9 (300 nmol, 30%); ε_{267} = 1304 L ·M^{-1}·cm^{-1}

HPLC: t_R = 1.57 min (5-50% ACN in H_2O, 0.01% TFA, 4 min, 210 nm)

MALDI-TOF (m/z): calc.: 2162.10 $[M+H]^+$; found: 2160.5

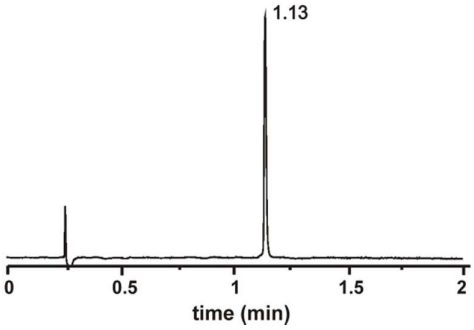


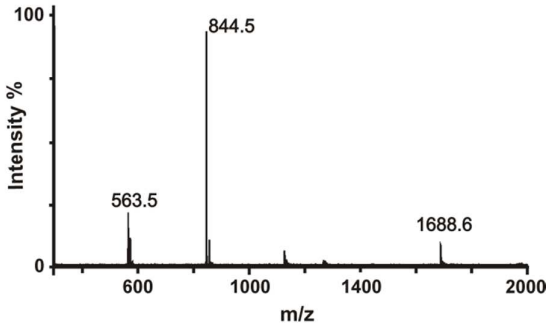
Ac-Lys-pTyr-Asn-Glu-Leu-*aeea*-pTyr-Asp-Val-Leu-Gly-Lys-NH_2 **7_1a**

OD_{267} = 0.81 (624 nmol, 31%); ε_{267} = 1304 L ·M^{-1}·cm^{-1}

HPLC: t_R = 1.13 min (5-50% ACN in H_2O, 0.01% TFA, 2 min, 210 nm)

ESI-MS (m/z): calc.: 1687.73 $[M+H]^+$; found: 1688.6$[M+1H]^{1+}$, 844.5 $[M+2H]^{2+}$, 563.5 $[M+3H]^{3+}$





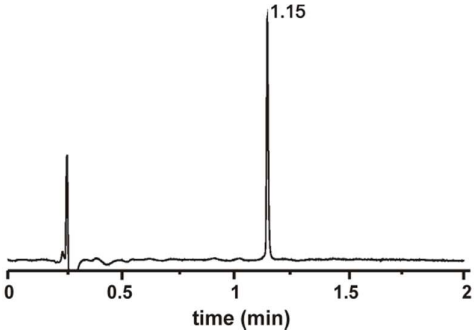
Ac-Lys-pTyr-Asn-Glu-Leu-(*aeea*)$_2$-pTyr-Asp-Val-Leu-Gly-Lys-NH_2 **7_2a**

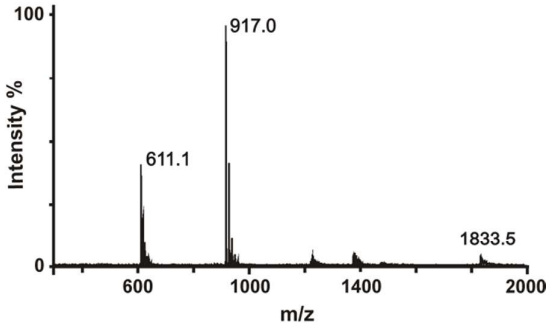
$C_{76}H_{123}N1_{27}O_{31}P_2$

OD_{267} = 1.36 (1046 nmol, 52%); ε_{267} = 1304 L ·M^{-1}·cm^{-1}

HPLC: t_R = 1.14 min (5-50% ACN in H_2O, 0.01% TFA, 2 min, 210 nm)

ESI-MS (m/z): calc.: 1832.80 $[M+H]^+$; found: 1833.5$[M+1H]^{1+}$, 917.0 $[M+2H]^{2+}$, 611.1 $[M+3H]^{3+}$





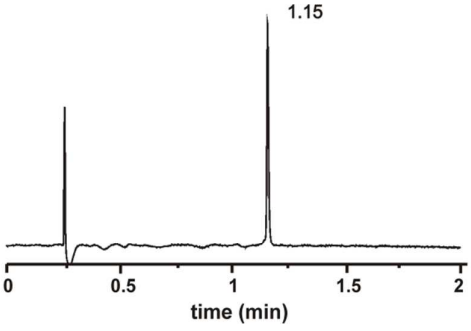
Ac-Lys-pTyr-Asn-Glu-Leu-(*aeea*)$_3$-pTyr-Asp-Val-Leu-Gly-Lys-NH_2 **7_3a**

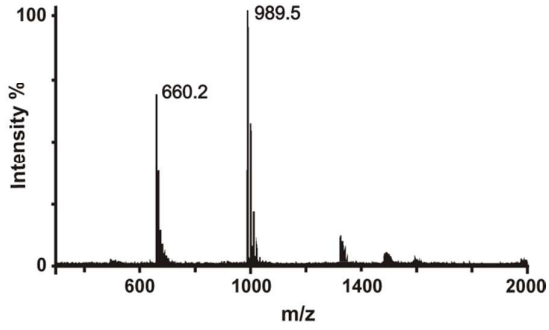
$C_{82}H_{134}N_{18}O_{34}P_2$

OD_{267} = 0.65 (496 nmol, 25%); ε_{267} = 1304 L ·M^{-1}·cm^{-1}

HPLC: t_R = 1.15 min (5-50% ACN in H_2O, 0.01% TFA, 2 min, 210 nm)

ESI-MS (m/z): calc.: 1977.6788 $[M+H]^+$; found: 989.5 $[M+2H]^{2+}$, 660.2 $[M+3H]^{3+}$





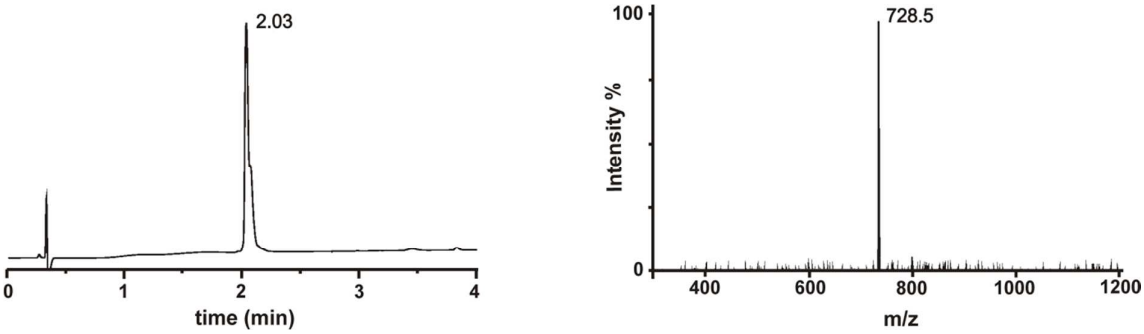
Ac-Lys-pTyr-Glu-Thr-Leu-*aeea*-pTy-Glu-Thr-Leu-Gly-OH **7_1b**

$C_{64}H_{101}N_{13}O_{28}P_2$

OD_{267} = 0.71 (546 nmol, 55%); ε_{267} = 1304 L ·M^{-1}·cm^{-1}

HPLC: t_R = 2.03 min (5-50% ACN in H_2O, 0.01% TFA, 4 min, 210 nm)

ESI-MS (m/z): calc.: 1562.64 $[M+H]^+$; found.: 782.5 $[M+2H]^{2+}$



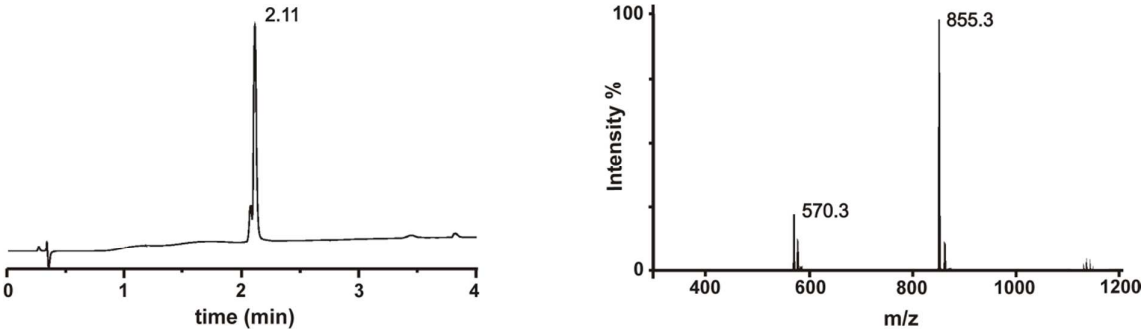
Ac-Lys-pTyr-Glu-Thr-Leu-(*aeea*)$_2$-pTy-Glu-Thr-Leu-Gly-OH **7_2b**

$C_{70}H_{112}N_{14}O_{31}P_2$

OD_{267} = 0.56 (430 nmol, 43%); ε_{267} = 1304 L ·M^{-1}·cm^{-1}

HPLC: t_R = 2.11 min (5-50% ACN in H_2O, 0.01% TFA, 4 min, 210 nm)

ESI-MS (m/z): calcr.: 1706.71 $[M+H]^+$; found: 855.3 $[M+2H]^{2+}$, 570.4 $[M+3H]^{3+}$



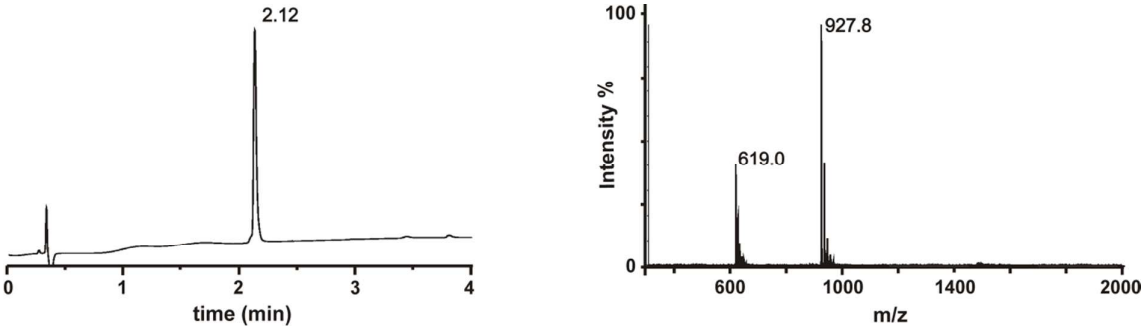
Ac-Lys-pTyr-Glu-Thr-Leu-(*aeea*)$_3$-pTy-Glu-Thr-Leu-Gly-OH **7_3b**

$C_{76}H_{123}N_{15}O_{34}P_2$

OD_{267} = 0.37 (283 nmol, 28%); ε_{267} = 1304 L ·M^{-1}·cm^{-1}

HPLC: t_R = 2.12 min (5-50% ACN in H_2O, 0.01% TFA, 4 min, 210 nm)

ESI-MS (m/z): calc.: 1852.56 $[M+H]^+$; found: 927.8 $[M+2H]^{2+}$, 619.0 $[M+3H]^{3+}$



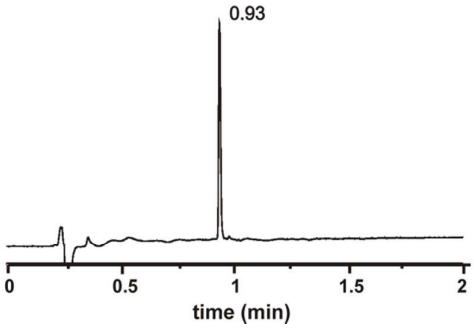
Ac-Lys-pTyr-Asp-Val-Leu-Gly-Lys-NH_2 **8**

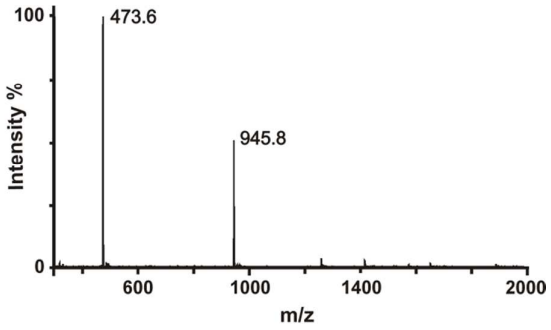
$C_{40}H_{66}N_{10}O_{14}P$

OD_{267} = 0.60 (918 nmol, 46%); ε_{267} = 652 L ·M^{-1}·cm^{-1}

HPLC: t_R = 0.93 min (5-50% ACN in H_2O, 0.01% TFA, 4 min, 210 nm)

MALDI-TOF (m/z): calc.: 944.47 $[M+H]^+$; found: 945.8 $[M+H]^+$, 473.6 $[M+2H]^{2+}$





1.3 PNA-peptide-conjugates

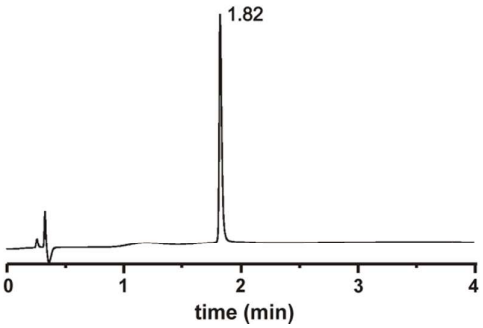
Ac-Lys-pTyr-Glu-Thr-Leu-attctacgca-pTyr-Glu-Thr-Leu-Gly-NH_2 **5b**

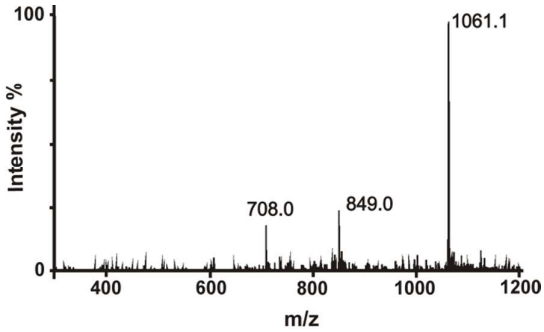
$C_{171}H_{234}N_{72}O_{55}P_2$

OD_{267} = 62.7 (640 nmol, 6%); ε_{267} = 98000 L $\cdot M^{-1} \cdot cm^{-1}$

HPLC: t_R = 1.82 min (5-50% ACN in H_2O, 0.01% TFA, 4 min, 210 nm)

ESI-MS (m/z): calc.: 4240.08 $[M+H]^+$; found: 1061.1 $[M+4H]^{4+}$, 849.0 $[M+5H]^{5+}$, 708.0 $[M+6H]^{6+}$





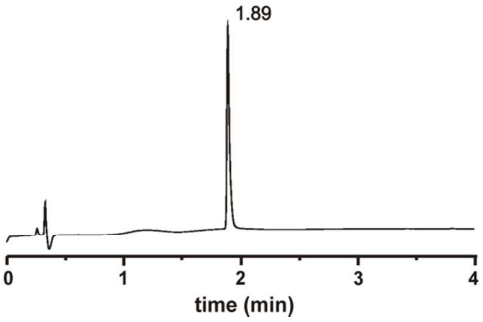
Ac-Lys-pTyr-Asn-Glu-Leu-attctacgca-pTyr-Asp-Val-Leu-Gly-NH_2 **5a**

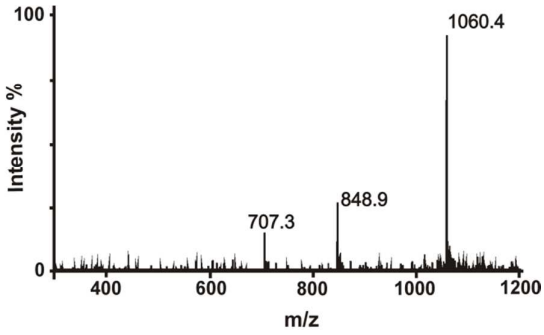
$C_{171}H_{233}N_{73}O_{54}P_2$

OD_{267} = 43.6 (439 nmol, 4%); ε_{267} = 99304 L $\cdot M^{-1} \cdot cm^{-1}$

HPLC: t_R = 1.89min (5-50% ACN in H_2O, 0.01% TFA, 4 min, 210 nm)

ESI-MS (m/z): calc.: 4237.08 $[M+H]^+$; found.: 1060.4 $[M+4H]^{4+}$, 848.9 $[M+5H]^{5+}$, 707.3 $[M+6H]^{6+}$





1.4 Maleimido Peptides

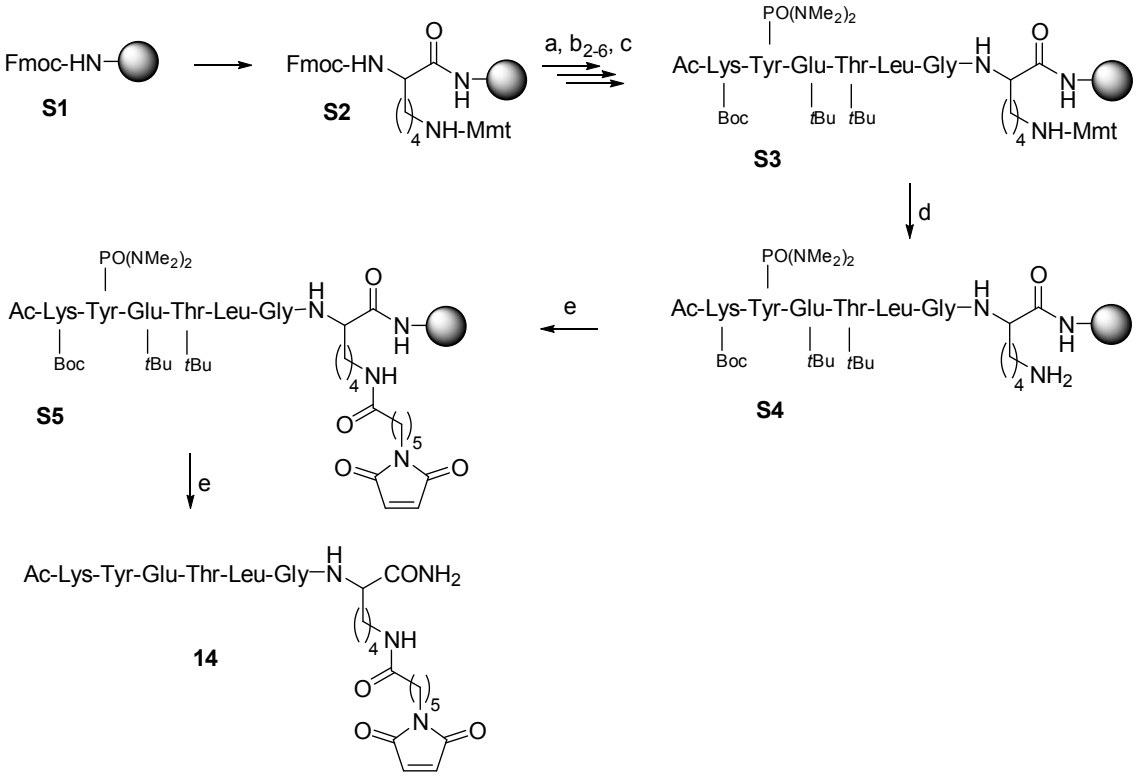


Figure S1: Examplary solid phase synthesis of a maleimido peptide. (a) Fmoc-Deprotection: Piperidine/DMF (1:4 [v/v]), 2 x 5 min; (b) amino acid coupling: 4 eq. Fmoc-amino acid [(b_1) Fmoc-Lys(Mmt)-OH, (b_2) Fmoc-Gly-OH, (b_3) Fmoc-Leu-OH, (b_4) Fmoc-Thr(*tBu*)-OH, (b_5) Fmoc-Glu(*tBu*)-OH, (b_6) Fmoc-pTyr((*NMe*$_2$)$_2$)-OH, (b_7) Fmoc-Lys(*Boc*)-OH], 3.6 eq HCTU, 3.6 eq. HOBT und 8 eq. NMM in DMF, 30 min; (c) Capping: Ac_2O/2,6-Lutidine/DMF (5:6:89 [v/v/v]), 2 x 3 min; (d) Mmt-Deprotection: 5% TFA und 2% TIS in DCM, 5 x 1 min; (e) Maleimide coupling: 4 eq. 6-Maleimidocaproic acid, 3.6 eq HCTU und 8 eq. NMM in DMF, 2 x 2h min; (f) final cleavage: 1. TFA/TIS/H_20 (95:2.5:2.5 [v/v/v]), 90 min; 2. addition of 10% H_20, 18 h at RT.

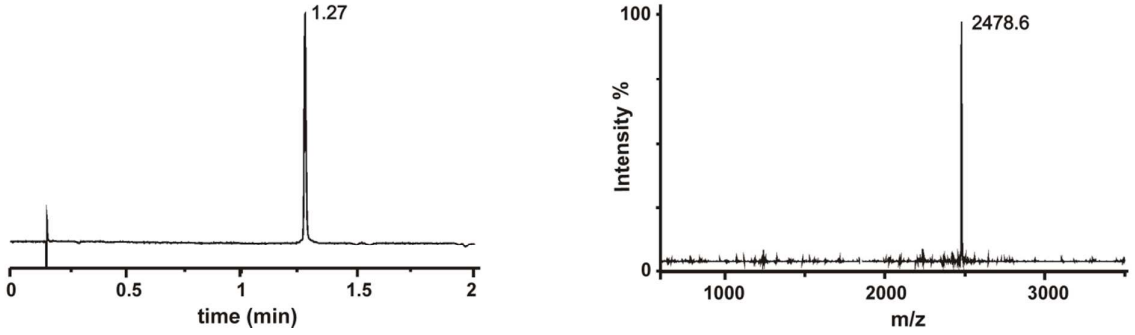
Ac- Lys(MIC)-pTyr-Glu-Thr-Leu-Asn-Leu-Gly-Arg-Glu-Glu-Gly-pTyr-Asp-Val-Leu-Lys-Gly-NH_2 **9**

$C_{104}H_{162}N_{26}O_{40}P_2$

ε_{267} = 1304 L ·M^{-1}·cm^{-1}

HPLC: t_R = 1.27 min (5-50% ACN in H_2O, 0.01% TFA, 2 min, 210 nm)

MALDI-TOF (m/z): calc: 2478.10 $[M+H]^+$; found: 2478.6 $[M+H]^+$



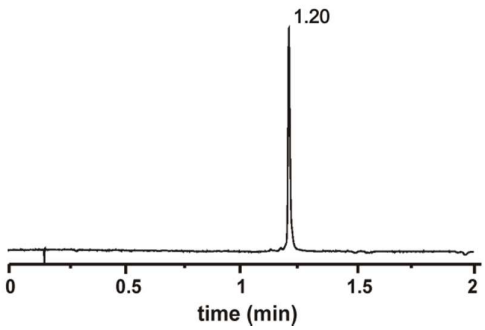
Ac- Lys-pTyr-Glu-Thr-Leu-Asn-Leu-Gly-Arg-Glu-Glu-Gly-pTyr-Asp-Val-Leu-Lys(MIC)-Gly-NH_2 **10**

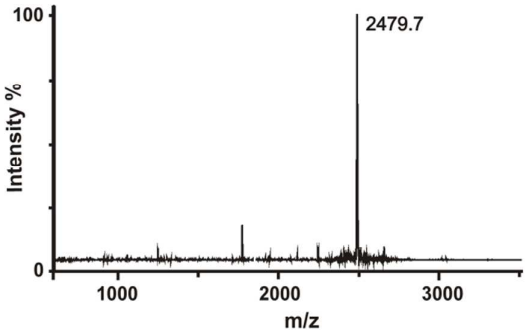
$C_{104}H_{162}N_{26}O_{40}P_2$

ε_{267} = 1304 L ·M^{-1}·cm^{-1}

HPLC: t_R = 1.20 min (5-50% ACN in H_2O, 0.01% TFA, 2 min, 210 nm)

MALDI-TOF (m/z): calc: 2478.10 $[M+H]^+$; found: 2479.7 $[M+H]^+$





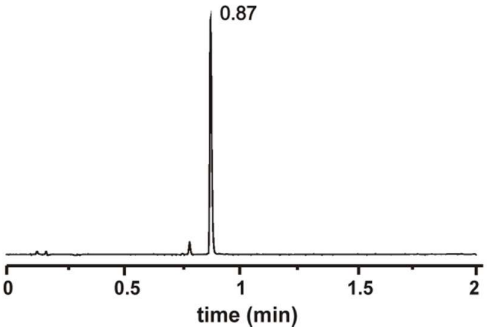
Ac-Lys(MIC)-pTyr-Glu-Thr-Leu-Gly-NH_2 **11**

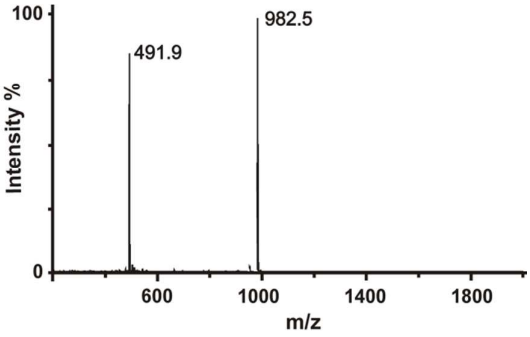
$C_{42}H_{63}N_8O_{17}P$

OD_{267} = 1.7 (2.61 µmol, 26%); ε_{267} = 652 L ·M^{-1}·cm^{-1}

HPLC: t_R = 0.87 min (5-50% ACN in H_2O, 0.01% TFA, 2 min, 210 nm)

ESI-MS (m/z): calc.: 982.4 $[M+1H]^{1+}$; found: 982.5 $[M+1H]^{1+}$





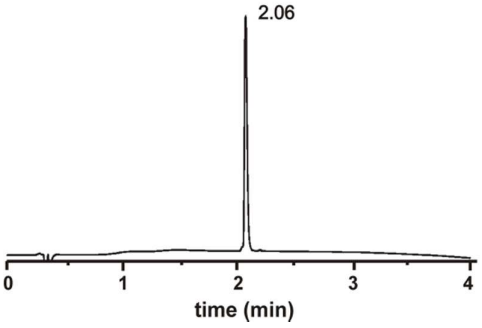
Ac-Lys(MIC)- pTyr-Asp-Val-Leu-Lys-NH_2 **12**

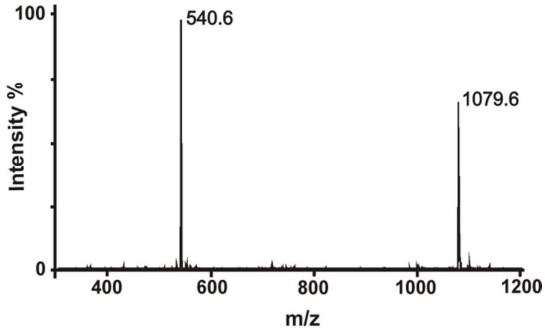
$C_{48}H_{75}N_{10}O_{16}P$

OD_{267} = 4.9 (7.56 µmol, 76%); ε_{267} = 652 L ·M^{-1}·cm^{-1}

HPLC: t_R = 2.06 min (5-50% ACN in H_2O, 0.01% TFA, 4 min, 210 nm)

ESI-MS (m/z): calc.: 1079.51 $[M+1H]^{1+}$; found: 1079.6 $[M+1H]^{1+}$, 540.6 $[M+2H]^{2+}$,





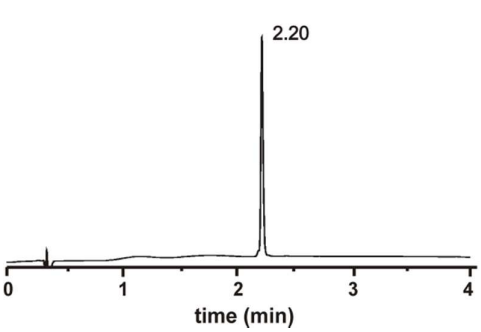
Ac-Lys(MIC)-pTyr-Asn-Gln-Leu-Lys-NH_2 **13**

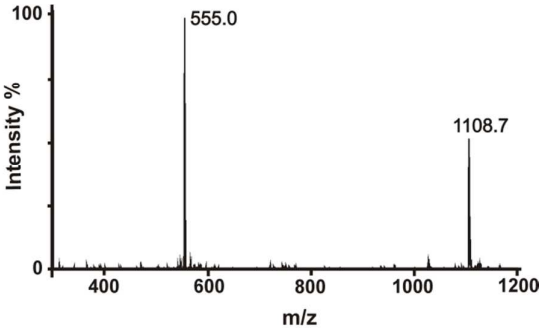
$C_{48}H_{74}N_{11}O_{17}P$

OD_{267} = 2.9 (4.51 µmol, 45%); ε_{267} = 652 L ·M^{-1}·cm^{-1}

HPLC: t_R = 2.20 min (5-50% ACN in H_2O, 0.01% TFA, 4 min, 210 nm)

ESI-MS (m/z): calc.: 1107.50 $[M+1H]^{1+}$; found: 1108.7 $[M+1H]^{1+}$, 555.0 $[M+2H]^{2+}$





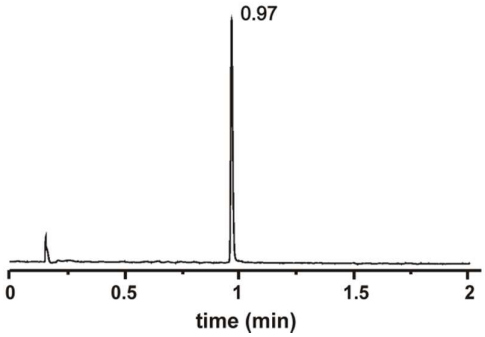
Ac-Lys-pTyr-Glu-Thr-Leu-Gly-Lys(MIC)-NH_2 **14**

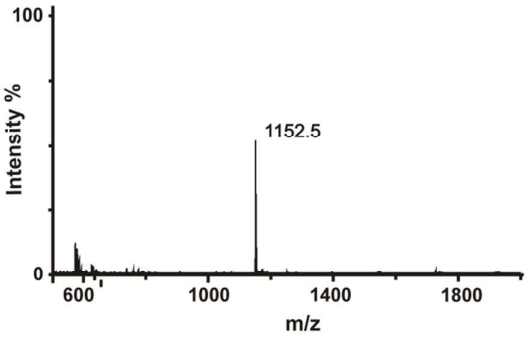
$C_{50}H_{78}N_{11}O_{18}P$

OD_{267} = 2.7 (4.13 µmol, 41%); ε_{267} = 652 L ·M^{-1}·cm^{-1}

HPLC: t_R = 0.97 min (5-50% ACN in H_2O, 0.01% TFA, 2 min, 210 nm)

ESI-MS (m/z): calc: 1152.53 $[M+1H]^{1+}$; found: 1152.5 $[M+1H]^{1+}$





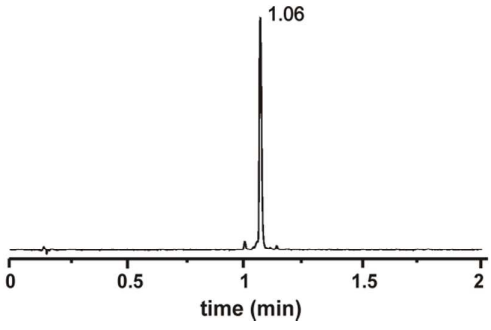
Ac- Lys-pTyr-Glu-Thr-Leu-Asn-Leu-Gly-Arg-Glu-Glu-Gly-Lys(MIC)-NH_2 **15**

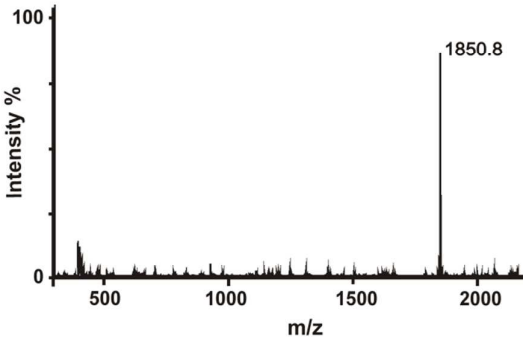
$C_{78}H_{124}N_{21}O_{29}P$

OD_{267} = 2.2 (3.44µmol, 34%); ε_{267} = 652 L ·M^{-1}·cm^{-1}

HPLC: t_R = 1.06 min (mobile Phase 3, 5-50% ACN in 2 min, 210 nm)

ESI-MS (m/z): ber.: 1850.86 $[M+H]^{+}$; gef.: 1850.8 $[M+H]^{+}$





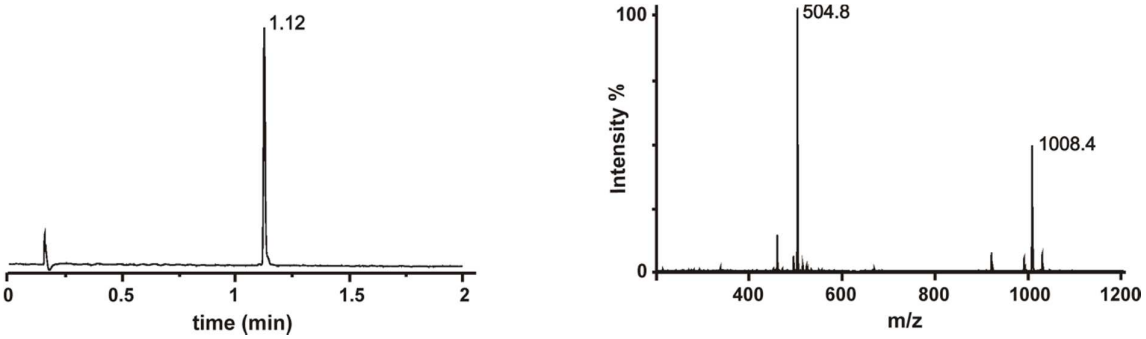
Ac-Gly-pTyr-Asp-Val-Leu-Lys(MIC)-NH_2 **16**

$C_{44}H_{66}N_9O_{16}P$

OD_{267} = 3.2 (4.88µmol, 49%); ε_{267} = 652 L ·M^{-1}·cm^{-1}

HPLC: t_R = 1.12 min (5-50% ACN in H_2O, 0.01% TFA, 4 min, 210 nm)

ESI-MS (m/z): calc: 1108.44 $[M+H]^+$; found: 1008.4 $[M+H]^+$, 504.8 $[M+H]^+$



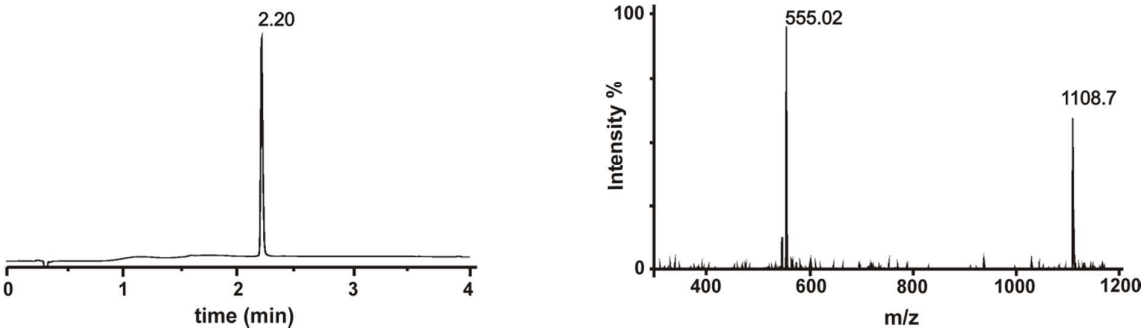
Ac-Lys-pTyr-Asn-Glu-Leu-Lys(MIC)-NH_2 **17**

$C_{48}H_{74}N_{11}O_{17}P$

OD_{267} = 3.2 (4.92 µmol, 49%); ε_{267} = 652 L ·M^{-1}·cm^{-1}

HPLC: t_R = 2.20 min (5-50% ACN in H_2O, 0.01% TFA, 4 min, 210 nm)

ESI-MS (m/z): calc.: 1107.50 $[M+1H]^{1+}$; found: 1108.7 $[M+1H]^{1+}$, 555.0 $[M+2H]^{2+}$



1.5 DNA-peptide-conjugates

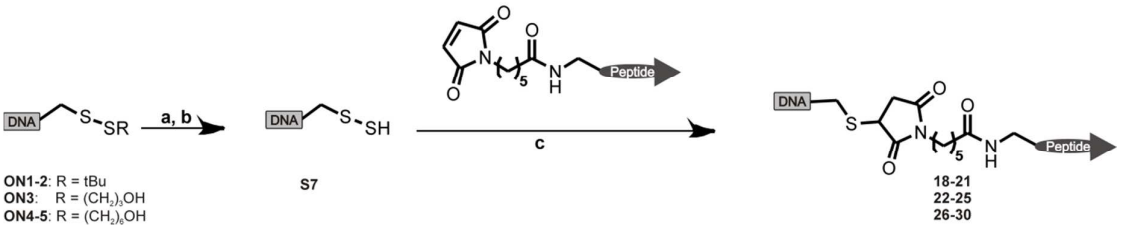


Figure S2: Synthesis of DNA-peptide conjugates **18**-**30** via maleimide-thiol coupling of thiol-modified oligonucleotides **ON1**-**ON5** and maleimide-containing peptides **9**-**17**. (a) 0.5 mM **ON1**-**5** in TCEP-buffer (5 mM TCEP, 10 mM NaCl, 10 mM NaH_2PO_4, pH 6.5), rt, 1-4h for **ON3**-**5** and up to 48 h for **ON1**-**2**, (b) Precipitation: 20% volume of a 3M NH_3OAc solution (pH 5.2) and isopropanol (c) 1 mM **ON1**-**5**, 2 eq. **9**-**17** in ligation buffer (10 mM NaCl, 10 mM NaH_2PO_4, pH 6.5), rt, 1-2h.

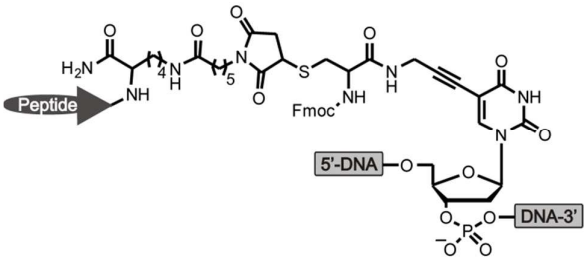


Figure S3: Chemical structure of the DNA-peptide linkage in the DNA-peptide conjugates **18**-**21**, which have been used for the construction of the monofunctionalized ternary DNA-peptide complexes **TC9** and **TC10**.

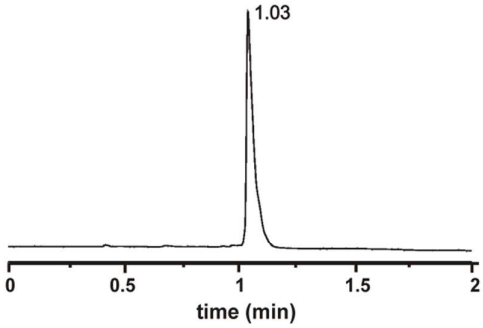
Ac-Lys(**ON1**)-pTyr-Glu-Thr-Leu-Gly-NH_2 **18**

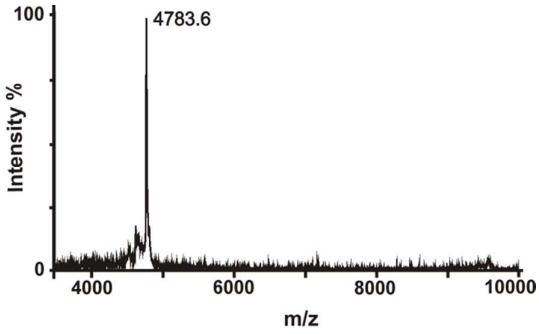
$C_{165}H_{220}N_{54}O_{89}P_{12}S$

OD_{260} = 43.5 (398 nmol, 40%); ε_{260} = 109300

HPLC: t_R = 1.03 min (5-50% ACN in 0.1 M TEAA in 20 min, 260 nm)

MALDI-TOF (m/z): calc.: 4786.1 $[M+H]^+$; found.: 4783.6 $[M+H]^+$





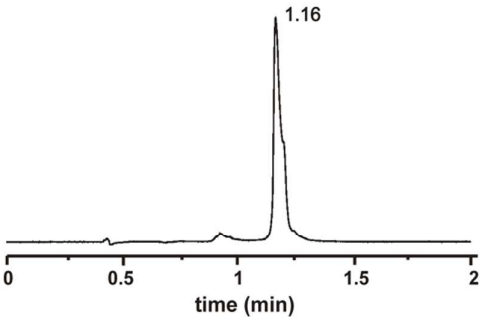
Ac-Lys(**ON1**)pTyr-Glu-Thr-Leu-Asn-Leu-Gly-Arg-Glu-Glu-Gly-pTyr-Asp-Val-Leu-Lys-Gly-NH_2 **19**

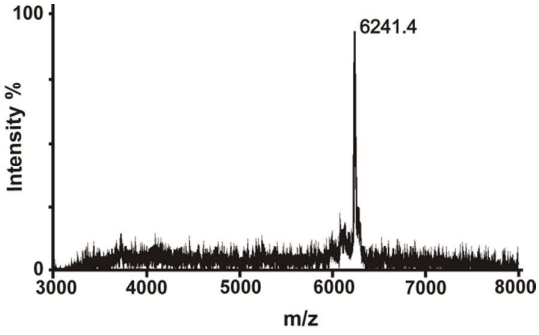
$C_{225}H_{316}N_{71}O_{112}P_{13}S$

OD_{260} = 36.9 (338 nmol, 34%); ε_{260} = 109300

HPLC: t_R = 1.16 min (5-50% ACN in 0.1 M TEAA in 20 min, 260 nm)

MALDI-TOF (m/z): calc: 6241.76 $[M+H]^+$; found: 6241.4 $[M+H]^+$





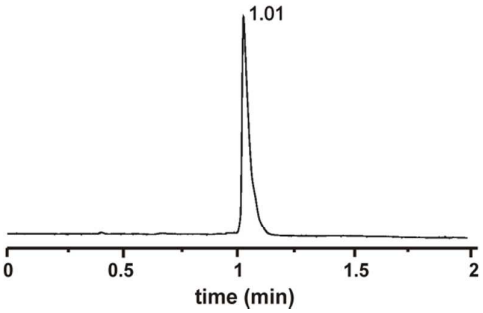
Ac- Lys-Gly-Arg-Glu-Glu-Gly-pTyr-Glu-Thr-Leu-Gly-Lys(**ON2**)-NH_2 **20**

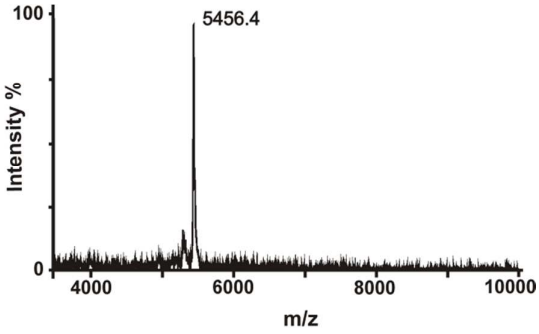
$C_{192}H_{265}N_{63}O_{100}P_{12}S$

OD_{260} = 41.0 (366 nmol, 37%); ε_{260} = 112000

HPLC: t_R = 1.01 min (mobile Phase 2, 5-50% ACN in 2 min, 260 nm)

MALDI-TOF (m/z): calc.: 5458.42 $[M+H]^+$; found 5456.4 $[M+H]^+$





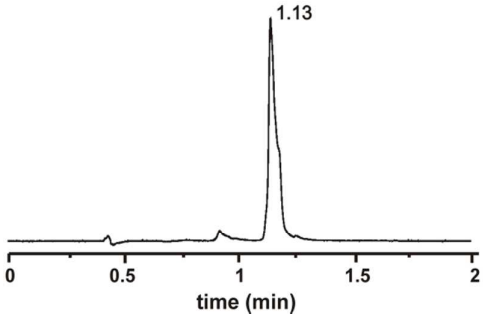
Ac-Lys-pTyr-Glu-Thr-Leu-Asn-Leu-Gly-Arg-Glu-Glu-Gly-pTyr-Asp-Val-Leu-Lys(**ON2**)-Gly-NH_2 **21**

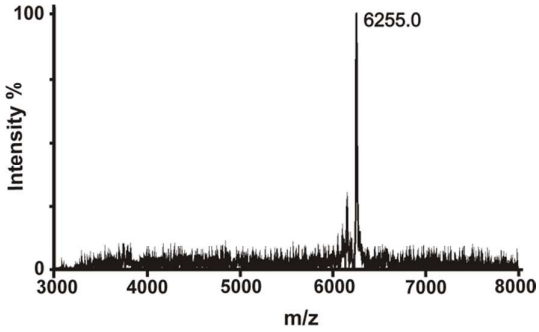
$C_{226}H_{317}N_{70}O_{113}P_{13}S$

OD_{260} = 10.5 (94 nmol, 9%); ε_{260} = 112000

HPLC: t_R = 1.13 min (5-50% ACN in 0.1 M TEAA in 20 min, 260 nm)

MALDI-TOF (m/z): calc.: 6256.76 $[M+H]^+$; found: 6255.0 $[M+H]^+$





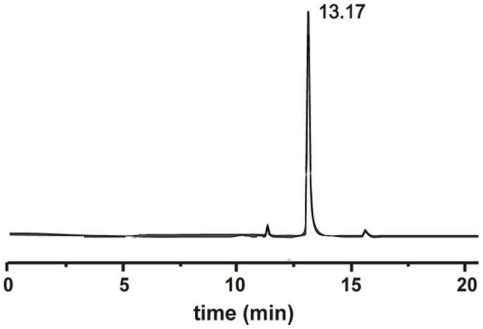
Ac-Lys(**ON3**)-pTyr-Asp-Val-Leu-Lys-NH_2 **22**

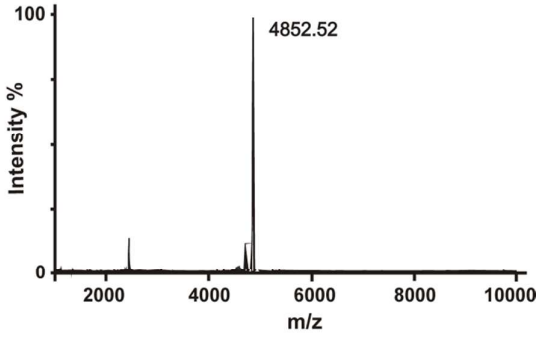
$C_{167}H_{230}N_{53}O_{90}P_{13}S$

OD_{260} = 63.7 (583 nmol, 58%); ε_{260} = 109300

HPLC: t_R = 13.17 min (5-50% ACN in 0.1 M TEAA in 20 min, 260 nm)

MALDI-TOF (m/z): calc.: 4852.14 $[M+H]^+$; found: 4852.5





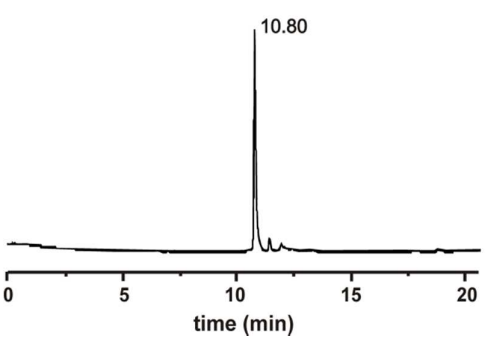
Ac-Lys(**ON3**)-pTyr-Glu-Thr-Leu-Gly-NH_2 **23**

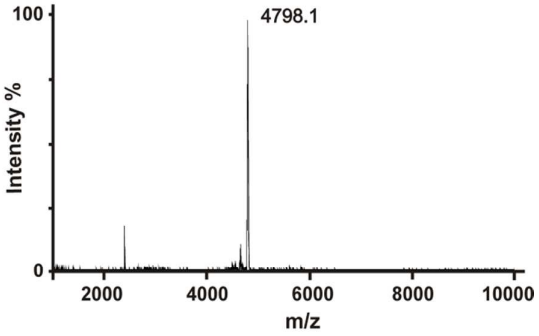
$C_{163}H_{220}N_{51}O_{92}P_{13}S$

OD_{260} = 63.6 (582 nmol, 58%); ε_{260} = 109300

HPLC: t_R = 10.80 min (5-50% ACN in 0.1 M TEAA in 20 min, 260 nm)

MALDI-TOF (m/z): calc.: 4799.04 $[M+H]^+$; found: 4798.1





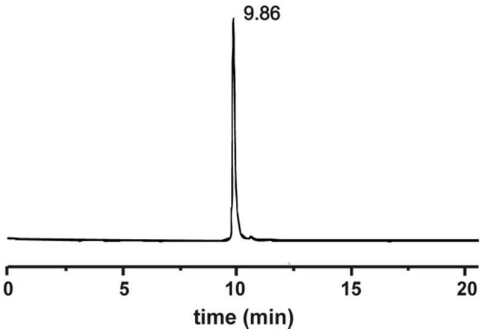
Ac-pTyr-Glu-Thr-Leu-Gly-Lys(**ON3**)-NH_2 **24**

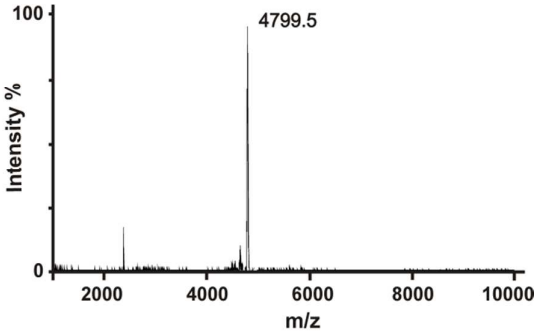
$C_{163}H_{220}N_{51}O_{92}P_{13}S$

OD_{260} = 80.1 (733 nmol, 73%); ε_{260} = 109300

HPLC: t_R = 9.86 min (5-50% ACN in 0.1 M TEAA in 20 min, 260 nm)

MALDI-TOF (m/z): calc.: 4799.04 $[M+H]^+$; found: 4799.5





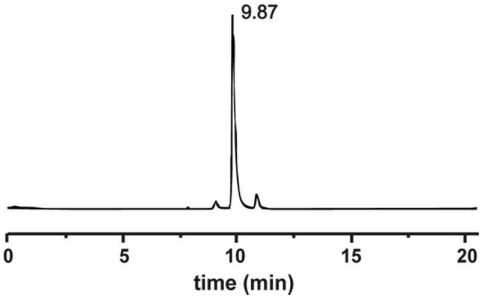
Ac-Gly-pTyr-Asp-Val-Leu-Lys(**ON3**)-NH_2 **25**

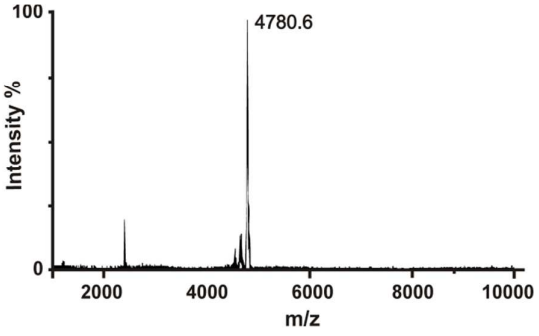
$C_{163}H_{221}N_{52}O_{90}P_{13}S$

OD_{260} = 59.1 (541 nmol, 54%); ε_{260} = 109300

HPLC: t_R = 9.87 min (5-50% ACN in 0.1 M TEAA in 20 min, 260 nm)

MALDI-TOF (m/z): calc.: 4781.07 $[M+H]^+$; found: 4780.6





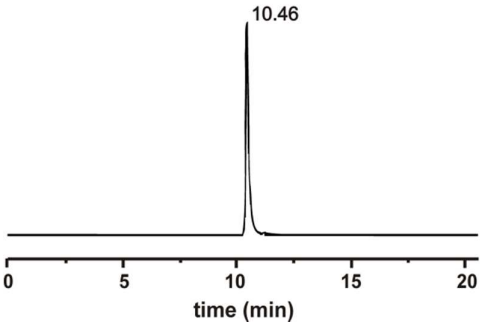
Ac-Lys(**ON4**)-pTyr-Asn-Glu-Leu-Lys-NH_2 **26**

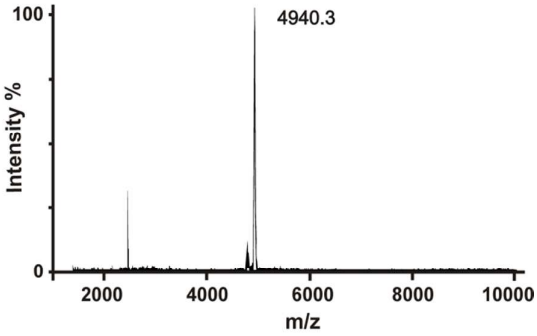
$C_{171}H_{236}N_{53}O_{92}P_{13}S$

OD_{260} = 83.2 (743 nmol, 74%); ε_{260} = 112000

HPLC: t_R = 10.46 min (5-50% ACN in 0.1 M TEAA in 20 min, 260 nm)

MALDI-TOF (m/z): calc.: 4940.18 $[M+H]^+$; found: 4940.3 $[M+H]^+$





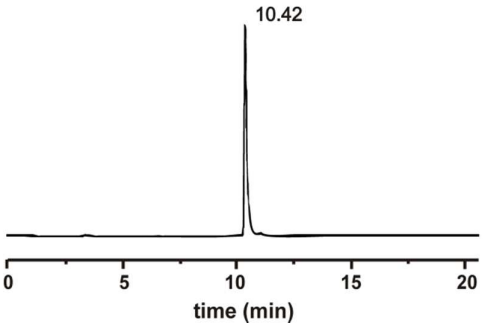
Ac-Lys(**ON4**)-pTyr-Glu-Thr-Leu-Gly-NH_2 **27**

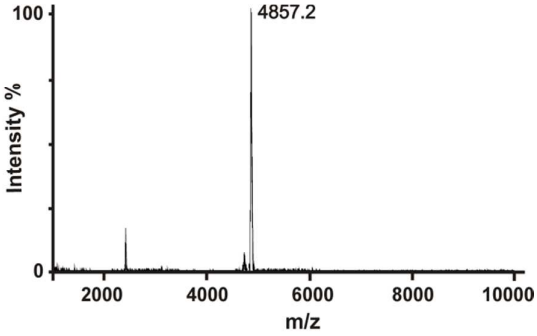
$C_{168}H_{230}N_{51}O_{91}P_{13}S$

OD_{260} = 81.9 (740 nmol, 72%); ε_{260} = 112000

HPLC: t_R = 10.42 min (5-50% ACN in 0.1 M TEAA in 20 min, 260 nm)

MALDI-TOF (m/z): calc.: 4856.13 $[M+H]^+$; found: 4857.2 $[M+H]^+$





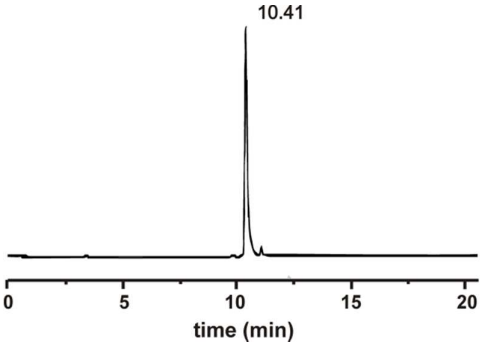
Ac-pTyr-Glu-Thr-Leu-Gly-Lys(**ON4**)-NH_2 **28**

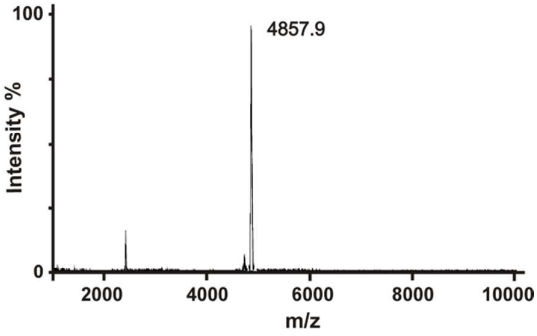
$C_{168}H_{230}N_{51}O_{91}P_{13}S$

OD_{260} = 77.7 (694 nmol, 69%); ε_{260} = 112000

HPLC: t_R = 10.41 min (5-50% ACN in 0.1 M TEAA in 20 min, 260 nm)

MALDI-TOF (m/z): calc.: 4856.13 $[M+H]^+$; found: 4857.9 $[M+H]^+$





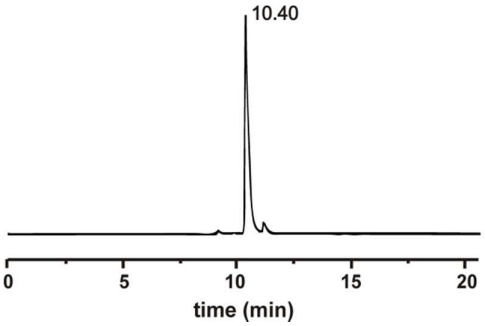
Ac-Lys-pTyr-Asn-Glu-Leu-Lys(**ON4**)-NH_2 **29**

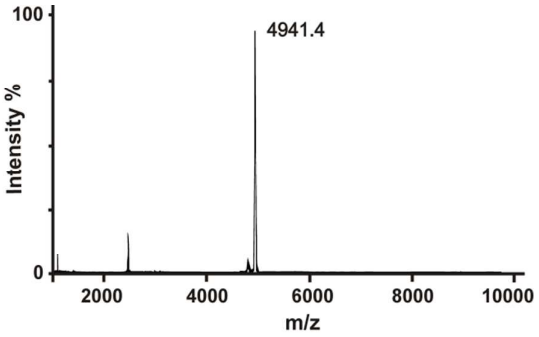
$C_{171}H_{236}N_{53}O_{92}P_{13}S$

OD_{260} = 64.2 (573 nmol, 57%); ε_{260} = 112000

HPLC: t_R = 10.40 min (5-50% ACN in 0.1 M TEAA in 20 min, 260 nm)

MALDI-TOF (m/z): calc.: 4940.07 $[M+H]^+$; found: 4041.3 $[M+H]^+$





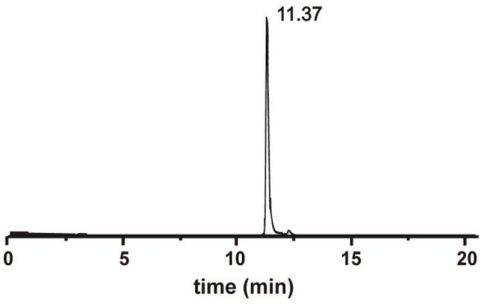
Ac-Lys(**ON5**)-pTyr-Asp-Val-Leu-Lys-NH_2 **30**

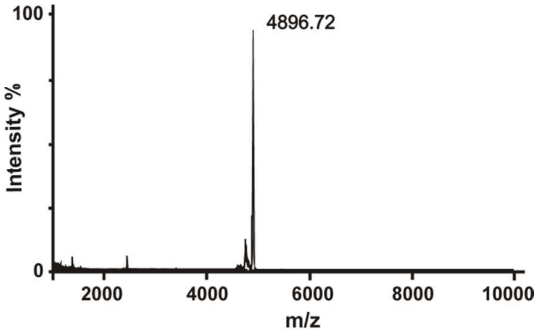
$C_{170}H_{235}N_{52}O_{91}P_{13}S$

OD_{260} = 81.3 (744 nmol, 74%); ε_{260} = 109300

HPLC: t_R = 11.37 min (5-50% ACN in 0.1 M TEAA in 20 min, 260 nm)

MALDI-TOF (m/z): calc.: 4897.17 $[M+H]^+$; found: 4896.72 $[M+H]^+$





2 Binding studies

Table S1. List of oligonucleotide complexes used in binding studies with tSH2 domains from Syk and ZAP-70 kinases.

Code		Oligonucleotide sequence	Peptide sequence
$TC1_n$ b	**27·23** **$T1_{0-9}$**	3' – TGT GCT TGA ACC **Z**(L1) · (L2)**Y** ATC ACT CGT CGG – 5' 5' – ACA CGA ACT TGG $(A)_{0-3}$ TAG TGA GCA GCC – 3'	L1: Ac-Lys(MIC-ON4)-pTyr-Glu-Thr-Leu-Gly-NH_2 L2: Ac-Lys(MIC-ON3)-pTyr-Glu-Thr-Leu-Gly-NH_2
$TC1_n$ a	**26·22** **$T1_{0-9}$**	3' – TGT GCT TGA ACC **Z**(L1) · (L2)**Y** ATC ACT CGT CGG – 5' 5' – ACA CGA ACT TGG $(A)_{0-10}$ TAG TGA GCA GCC – 3'	L1: Ac-Lys(MIC-ON4)-pTyr-Asn-Glu-Leu-Lys-NH_2 L2: Ac-Lys(MIC-ON3)-pTyr-Asp-Val-Leu-Lys-NH2
$TC2_n$ a	**26·22** **$T1_{0-9}$**	3' – TGT GCT TGA ACC **Z**(L1) · ATC ACT CGT CGG **Z**(L2) – 5' 5' – ACA CGA ACT TGG $(A)_{1-10}$ TAG TGA GCA GCC – 3'	L1: Ac-Lys(MIC-ON4)-pTyr-Asn-Glu-Leu-Lys-NH_2 L2: Ac-Lys(MIC-ON3)-pTyr-Asp-Val-Leu-Lys-NH_2
$TC3_n$ b	**28·24** **$T1_{0-9}$**	3' – TGT GCT TGA ACC **Z**(L1) · (L2)**Y** ATC ACT CGT CGG – 5' 5' – ACA CGA ACT TGG $(A)_{0-3}$ TAG TGA GCA GCC – 3'	L1: Ac-Lys-pTyr-Glu-Thr-Leu-Gly-Lys(MIC-ON4)-NH_2 L2: Ac-Lys-pTyr-Glu-Thr-Leu-Gly-Lys(MIC-ON3)-NH_2
$TC3_n$ a	**29·25** **$T1_{0-9}$**	3' – TGT GCT TGA ACC **Z**(L1) · (L2)**Y** ATC ACT CGT CGG – 5' 5' – ACA CGA ACT TGG $(A)_{0-10}$ TAG TGA GCA GCC – 3'	L1: Ac-Lys-pTyr-Asn-Glu-Leu-Lys(MIC-ON4)-NH_2 L2: Ac-Gly-pTyr-Asp-Val-Leu-Lys(MIC-ON3)-NH_2
$TC4_n$ b	**28·23** **$T1_{0-9}$**	3' – TGT GCT TGA ACC **Z**(L1) · (L2)**Y** ATC ACT CGT CGG – 5' 5' – ACA CGA ACT TGG $(A)_{0-3}$ TAG TGA GCA GCC – 3'	L1: Ac-Lys-pTyr-Glu-Thr-Leu-Gly-Lys(MIC-ON4)-NH_2 L2: Ac-Lys(MIC-ON3)-pTyr-Glu-Thr-Leu-Gly-NH_2
$TC4_n$ a	**29·24** **$T1_{0-9}$**	3' – TGT GCT TGA ACC **Z**(L1) · (L2)**Y** ATC ACT CGT CGG – 5' 5' – ACA CGA ACT TGG $(A)_{0-10}$ TAG TGA GCA GCC – 3'	L1: Ac-Lys-pTyr-Asn-Glu-Leu-Lys(MIC-ON4)-NH_2 L2: Ac-Lys(MIC-ON3)-pTyr-Asp-Val-Leu-Lys-NH_2
$TC5_n$ a	**29·30** **$T1_{0-9}$**	3' – TGT GCT TGA ACC **Z**(L1) · ATC ACT CGT CGG **Z**(L2) – 5' 5' – ACA CGA ACT TGG $(A)_{1-10}$ TAG TGA GCA GCC – 3'	L1: Ac-Lys-pTyr-Asn-Glu-Leu-Lys(MIC-ON4)-NH_2 L2: Ac-Lys(MIC-ON5)-pTyr-Asp-Val-Leu-Lys-NH_2
$TC6_n$ b	**27·24** **$T1_{0-9}$**	3' – TGT GCT TGA ACC **Z**(L1) · (L2)**Y** ATC ACT CGT CGG – 5' 5' – ACA CGA ACT TGG $(A)_{0-10}$ TAG TGA GCA GCC – 3'	L1: Ac-Lys(MIC-ON4)-pTyr-Glu-Thr-Leu-Gly -NH_2 L2: Ac-Lys-pTyr-Glu-Thr-Leu-Gly-Lys(MIC-ON3)-NH_2
$TC6_n$ a	**26·25** **$T1_{0-9}$**	3' – TGT GCT TGA ACC **Z**(L1) · (L2)**Y** ATC ACT CGT CGG – 5' 5' – ACA CGA ACT TGG $(A)_{0-10}$ TAG TGA GCA GCC – 3'	L1: Ac-Lys(MIC-ON4)-pTyr-Asn-Gln-Leu-Lys-NH_2 L2: Ac-Gly-pTyr-Asp-Val-Leu-Lys(MIC-ON3)-NH_2
TC 9 c	**20·ON6** **$T1_{0-9}$**	3' – TGT GCT **X**(L1)GA ACC · ATC ACT CGT CGG – 5' 5' – ACA CGA ACT TGG $(A)_{0-9}$ TAG TGA GCA GCC – 3'	L1: Ac-Lys(MIC-ON1)pTyr-Glu-Thr-Leu-Asn-Leu-Gly-Arg-Arg-Glu-Glu-Gly-pTyr-Asp-Val-Leu-Lys-Gly-NH_2
TC 10 d	**ON7·21** **$T1_{0-9}$**	3' – TGT GCT TGA ACC · ATC AC**X**(L1) CGT CGG – 5' 5' – ACA CGA ACT TGG $(A)_{0-9}$ TAG TGA GCA GCC – 3'	L1: Ac-Lys-pTyr-Glu-Thr-Leu-Asn-Leu-Gly-Arg-Arg-Glu-Glu-Gly-pTyr-Asp-Val-Leu-Lys(MIC-ON2)-Gly-NH_2
TC 9 b	**18·ON6** **$T1_{0-9}$**	3' – TGT GCT **X**(L1)GA ACC · ATC ACT CGT CGG – 5' 5' – ACA CGA ACT TGG $(A)_{0-9}$ TAG TGA GCA GCC – 3'	L1: Ac-Lys(MIC-ON1)-pTyr-Glu-Thr-Leu-Gly-NH_2
TC 10 c	**ON7·19** **$T1_{0-9}$**	3' – TGT GCT TGA ACC · ATC AC**X**(L1) CGT CGG – 5' 5' – ACA CGA ACT TGG $(A)_{0-9}$ TAG TGA GCA GCC – 3' '	L1: Ac-Lys-pTyr-Glu-Thr-Leu-Asn-Leu-Gly-Arg-Glu-Glu-Gly-Lys(MIC-ON2)-NH_2

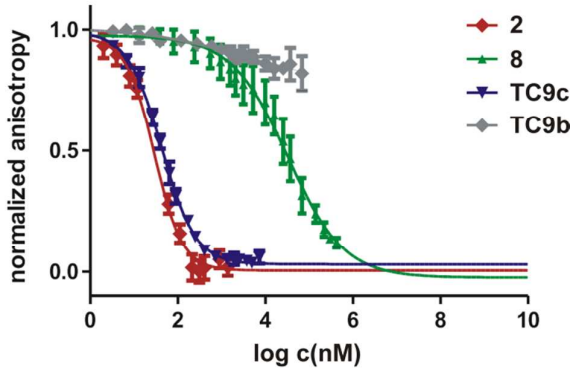


Figure S4: Competitive binding curves of the mono- and bivalent peptides **2** and **8** and the control measurements with the monovalent DNA-Peptide conjugate **TC9b** and the ITAM-DNA duplex conjugate **TC9c**.

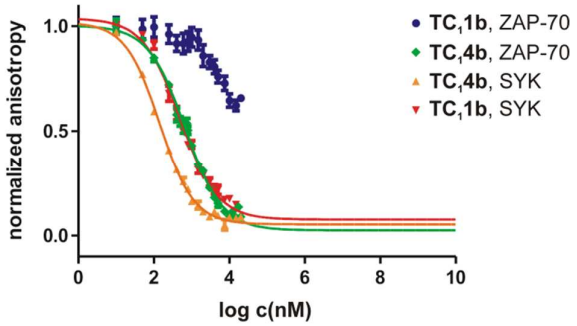


Figure S5: Competitive binding curves of the DNA-peptide conjugates **$TC_1$1b** and **$TC_1$4b** with ZAP-70 (blue, green) and Syk (red, orange).

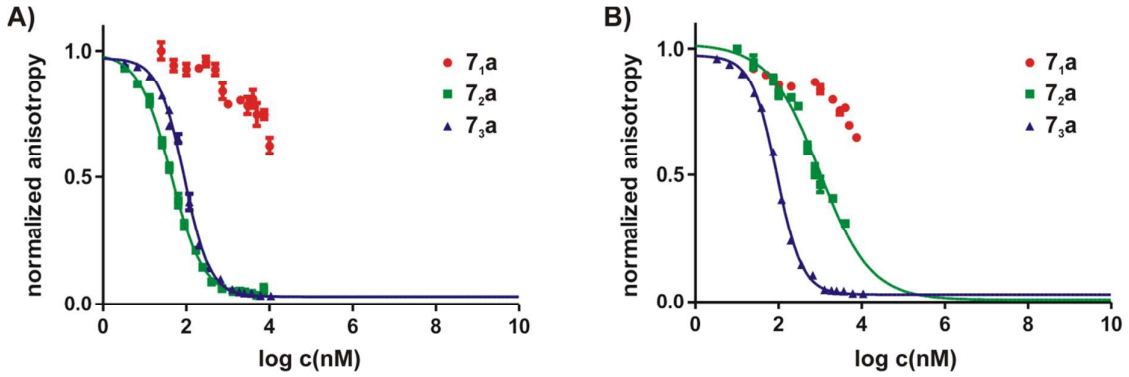


Figure S6: Competitive binding curves of peptides containing 1-3 aminoethylethoxyethyloxy acetic acid (*aeea*) spacer units to (A) ZAP-70 and (B) Syk.

Table S2. Distance-dependent IC_{50}-values for the interaction of the ternary peptide-DNA complexes **TC_n4a** and **TC_n5** with Zap-70 tSH2.

Distance n	IC_{50} [µM] – ZAP-70
1 nt	2.15
2 nt	3.42
4 nt	5.85
6 nt	16.60
9 nt	18.43
11 nt	> 20.0
13 nt	> 20.0
14 nt	> 20.0
16 nt	> 20.0
21 nt	> 20.0

Table S3. Orientation-dependent IC_{50}-values between bivalent pYETL displays in ternary complexes **$TC_1$1b**, **$TC_1$3b**, **$TC_1$4b** and **$TC_1$6b** and tSH2 domains of Syk tSH2 and Zap-70.

	IC_{50}-values in [µM]			
	$TC_1$1b	**$TC_1$3b**	**$TC_1$6b**	**$TC_1$4b**
ZAP-70	> 20	7.15	0.99	0.71
Syk	0.48	1.18	0.12	0.14

Table S4. IC_{50}-values for the interaction of peptide-oligoethylene glycol and peptide-PNA conjugates with the tSH2 domains of Syk and ZAP-70 kinases

conj	Sequence	IC_{50} [µM] ZAP-70	IC_{50} [µM] Syk
7_1a	KpYNEL*(aeea)*pYDVLGK	>10.000	>10.000
7_2a	KpYNEL*(aeea)*$_2$pYDVLGK	0.057	0.890
7_3a	KpYNEL*(aeea)*$_3$pYDVLGK	0.11	0.11
7_1b	KpYETL*(aeea)*pYETLG	4.63	2.96
7_2b	KpYETL*(aeea)*$_2$pYETLG	0.11	0.27
7_3b	KpYETL*(aeea)*$_3$pYETLG	0.142	0.115
5b	KpYETL*(attctacgca)*pYETLG	0.31	3.00
6b	KpYETL*(attctacgca)*pYETLG	0.91	8.12
5a	KpYNEL*(attctacgca)* pYDVLG	3.10	4.15
6a	KpYNEL*(attctacgca)*pYDVLG	6.60	7.67

Table S5. Interaction of the TCRζ TAM1-derived peptide Ac-NQLpYNELNLGREEpYDVLD with the tSH2 domains of Syk and Zap-70 kinases. Comparing K_d values obtained by means of a surface plasmon resonance assay (Ottinger, E. A., Botfield, M. C., and Shoelson, S. E. (1998) Tandem SH2 domains confer high specificity in tyrosine kinase signaling. *J. Biol. Chem. 273*, 729-735.) with IC_{50} values determined by means of the fluorescence polarization assay in solution.

K_d [nM] ZAP-70 (SPR assay)	**K_d [nM] Syk (SPR assay)**	**IC_{50} [nM] ZAP-70 (FP assay)**	**IC_{50} [nM] Syk (FP assay)**
8.3	120	1.6	6.7