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Supporting Information

Supporting Information

Synthesis and Evaluation of 3-Phenylpyrazolo[3,4-*d*]pyrimidine-Peptide Conjugates as Src Kinase Inhibitors

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1. Synthesis of peptides. All linear peptides were synthesized by the solid-phase synthesis strategy employing Fmoc-based chemistry as described in the general information section and described previously.[39] The synthesis of Ac-CIYKYY **(7)** is explained here as a representative example. Similarly, other peptide sequences were synthesized, purified, and their chemical structures were determined by a high-resolution electron spray time-of-flight mass spectrometer as described for **7**.

Ac-CIYKYY (7). The peptide sequence (**7**) was assembled on Fmoc-Tyr(*t*Bu)-Wang resin (115.0 mg; 0.88 mmol/g) by the Fmoc solid-phase peptide synthesis strategy on a PS3 automated peptide synthesizer at room temperature using Fmoc-protected amino acids [Fmoc-Tyr(*t*Bu)-OH (Y_5), Fmoc-Lys(Boc)-OH (K_4), Fmoc-Tyr(*t*Bu)-OH (Y_3), Fmoc-Ile-OH (I_2) and Fmoc-Cys(Trt)-OH (C_1)] as described in the general information section. The *N*-terminal was acetylated using acetic anhydride (2.0 mL). The side chain deprotection and cleavage was carried out by shaking of the resin with a mixture of TFA:anisole:water:EDT (95:2.0:2.0:1.0 v/v/v/v, 5.0 mL) for 1 h. The crude peptide was precipitated by the addition of cold diethyl ether (25 mL, Et_2O) and purified by preparative HPLC. The chemical structure of **7** was determined by a high-resolution electron spray time-of-flight mass spectrometer. HR-MS (ESI-TOF) (*m/z*): $C_{44}H_{59}N_7O_{11}S$ calcd, 893.3993; found, 893.4713 $[M]^+$.

Ac-YIYGSFK (**8**)**.** HR-MS (ESI-TOF) (*m/z*): $C_{46}H_{63}N_8O_{12}$ calcd, 919.4565; found, 919.0338 $[M]^+$; **Ac-EEIYGEFF.** HR-MS (ESI-TOF) (*m/z*): $C_{52}H_{67}N_8O_{17}$ calcd, 1075.4624; found, 1075.5815 $[M]^+$; **Ac-EIYGEFKK.** HR-MS (ESI-TOF) (*m/z*): $C_{50}H_{75}N_{10}O_{15}$ calcd, 1055.5413; found, 1055.6441 $[M]^+$, 528.3046 $[M + 2H]^{2+}$; **Ac-IYGEFKKK.** HR-MS (ESI-TOF) (*m/z*): $C_{51}H_{80}N_{11}O_{13}$ calcd, 1054.5937, found, 1054.6635 $[M]^+$, 527.8145 $[M +2H]^{2+}$; **Ac-AEEEIYGEFEAKKKK.** HR-MS (ESI-TOF) (*m/z*): $C_{83}H_{130}N_{19}O_{28}$ calcd, 1840.9333, found, 614.1799 $[M + 3H]^{3+}$.

2. Synthesis of PhPP-peptide conjugates.

PhPP-CH_2CONH-Cys-OH (22). The Fmoc-Cys(Trt)-Wang resin (172.0 mg, 0.58 mmol/g) was suspended in dry DMF (15 mL). Fmoc protecting group was removed with piperidine in DMF (twice, 20%, 25 mL) by shaking at room temperature for 10 min. The resin was collected by filtration and washed with DMF (2 × 20 mL), MeOH (2 × 20 mL), and DCM (2 × 20 mL), respectively, and dried under vacuum. The free amino group was coupled with PhPPCH_2COOH (**1**, 86 mg, 0.33 mmol) in the presence of a mixture of HBTU (258 mg, 0.68 mmol) and DIPEA (99%, 120 µl, 0.68 mmol) in DMF (20 mL) by shaking at room temperature for 6 h. The resin was collected by filtration and washed with DMF (2 × 20 mL) and DCM (2 × 20 mL), respectively, and dried under vacuum. The synthesized compound was cleaved and deprotected from the resin by shaking it with a mixture of TFA:anisole:water:EDT (95:2.0:2.0:1.0 v/v/v/v, 5.0 mL) for 1 h. The crude compound was precipitated by the addition of cold diethyl ether (25 mL, Et_2O), purified by preparative HPLC to afford **22**. HR-MS (ESI-TOF) (*m/z*): $C_{16}H_{16}N_6O_3S$ calcd, 372.1005; found, 373.0376 $[M + H]^+$.

PhPP-CH_2CONH-CIY-OH (23). The peptide CIY was assembled on Fmoc-Tyr(*t*Bu)-Wang resin (115.0 mg, 0.88 mmole/g) by Fmoc solid-phase peptide synthesis strategy using Fmoc protected amino acids [Fmoc-Ile-OH (I_2) and Fmoc-Cys(Trt)-OH (C_1)] on a PS3 automated peptide synthesizer at room temperature. Fmoc protecting group was removed with piperidine in DMF (twice, 20%, 25 mL) by shaking at room temperature for 10 min. The resin was collected by filtration, and washed with DMF (2 × 20 mL), MeOH (2 × 20 mL), and DCM (2 × 20 mL), respectively, and dried under vacuum. The free amino group was coupled with PhPPCH_2COOH (**1**, 58 mg, 0.22 mmol) in the presence of HBTU (174 mg, 0.46 mmol) and DIPEA (99%, 81 µL, 0.46 mmol) in DMF (20 mL) by shaking at room temperature for 6 h. The resin was collected by filtration and washed with DMF (2 × 20 mL) and DCM (2 × 20 mL), respectively, and dried under vacuum. The synthesized compound was cleaved and deprotected from the resin by shaking it with a mixture

of TFA:anisole:H_2O:EDT (95:2.0:2.0:1.0 v/v/v/v, 5.0 mL) for 1 h. The crude compound was precipitated by the addition of cold diethyl ether (25 mL, Et_2O), purified by preparative HPLC to afford **23**. HR-MS (ESI-TOF) (*m/z*): $C_{31}H_{36}N_8O_6S$ calcd, 648.2479; found, 649.1084 $[M + H]^+$.

PhPP-CH_2CONH-CIYKY-OH (24). Compound **24** was synthesized, purified, and characterized in a similar manner as described above for **23** by first synthesizing the peptide attached to Wang resin using Fmoc-Tyr(*t*Bu)-Wang resin (115.0 mg, 0.88 mmole/g) and Fmoc protected amino acids [Fmoc-Lys(Boc)-OH (K_4), Fmoc-Tyr(*t*Bu)-OH (Y_3), Fmoc-Ile-OH (I_2) and Fmoc-Cys(Trt)-OH (C_1)], and then *N*-terminal conjugation with PhPPCH_2COOH (**1**). HR-MS (ESI-TOF) (*m/z*): $C_{46}H_{57}N_{11}O_9S$ calcd, 939.4061; found, 940.0116 $[M + H]^+$, 470.4914 $[M + 2H]^{2+}$.

PhPP-CH_2CONH-CIYKF(4-NO_2)Y (26). Compound **26** was synthesized, purified, and characterized in a similar manner as described above for **23** by first synthesizing the peptide attached to Wang resin using Fmoc-Tyr(*t*Bu)-Wang resin (115.0 mg, 0.88 mmole/g) and Fmoc protected amino acids [Fmoc-Phe(4-NO_2)-OH (F_5), Fmoc-Lys(Boc)-OH (K_4), Fmoc-Tyr(*t*Bu)-OH (Y_3), Fmoc-Ile-OH (I_2) and Fmoc-Cys(Trt)-OH (C_1)] and then *N*-terminal conjugation with PhPPCH_2COOH (**1**). HR-MS (ESI-TOF) (*m/z*): $C_{55}H_{65}N_{13}O_{12}S$ calcd, 1131.4596; found, 1131.5396 $[M]^+$, 566.6261 $[M + 2H]^{2+}$.

PhPP-CH_2CONHCIYKF(4-I)Y (27). Compound **27** was synthesized, purified, and characterized in a similar manner as described above for **23** by first synthesizing the peptide attached to Wang resin using Fmoc-Tyr(*t*Bu)-Wang resin (115.0 mg, 0.88 mmol/g) and Fmoc protected amino acids [Fmoc-Phe(4-I)-OH (F_5), Fmoc-Lys(Boc)-OH (K_4), Fmoc-Tyr(*t*Bu)-OH (Y_3), Fmoc-Ile-OH (I_2) and Fmoc-Cys(Trt)-OH (C_1)] and then *N*-terminal conjugation with PhPPCH_2COOH (**1**). HR-MS (ESI-TOF) (*m/z*): $C_{55}H_{65}IN_{12}O_{10}S$ calcd, 1212.3712; found, 1212.5748 $[M]^+$, 606.6605 $[M + 2H]^{2+}$.

PhPP-CH(CH_3)CONH-CIYKYY (28). Compound **28** was synthesized, purified, and characterized in a similar manner as described for **25**. PhPP-CH(CH_3)COOH (**2**) was used instead of PhPP-CH_2COOH (**1**) for the conjugation with the peptide-attached resin. HR-MS (ESI-TOF) (*m/z*): $C_{56}H_{68}N_{12}O_{11}S$ calcd, 1116.4851; found, 1116.6211 $[M]^+$, 559.1588 $[M + 2H]^{2+}$.

PhPP-CH_2CH_2CONH-CIYKYY (29). Compound **29** was synthesized, purified, and characterized in a similar manner as described for **25**. PhPP-CH_2CH_2COOH (**3**) was used instead of PhPP-CH_2COOH (**1**) for the conjugation with the peptide-attached resin. HR-MS (ESI-TOF) (*m/z*): $C_{56}H_{68}N_{12}O_{11}S$ calcd, 1116.4851; found, 1116.7988 $[M]^+$.

PhPP-$CH_2CH_2CH_2$CONH-CIYKYY (30). Compound **30** was synthesized, purified, and characterized in a similar manner as described for **25**. PhPP-$(CH_2)_3$COOH (**4**) was used instead of PhPP-CH_2COOH (**1**) for the conjugation with the peptide-attached resin. HR-MS (ESI-TOF) (*m/z*): $C_{57}H_{70}N_{12}O_{11}S$ calcd, 1130.5008; found, 1131.0091 $[M]^+$, 565.9422 $[M + 2H]^{2+}$.

PhPP-CH(CH_3)CH_2CONH-CIYKYY (31). Compound **31** was synthesized, purified, and characterized in a similar manner as described for **25**. PhPP-CH(CH_3)CH_2COOH (**5**) was used instead of PhPP-CH_2COOH (**1**) for the conjugation with the peptide-attached resin. HR-MS (ESI-TOF) (*m/z*): $C_{57}H_{70}N_{12}O_{11}S$ calcd, 1130.5008; found, 1131.1193 $[M]^+$, 565.9531 $[M + 2H]^{2+}$.

Synthesis of PhPP-$(CH_2)_3$CONH-IYKYY (32). Compound **32** was synthesized, purified, and characterized in a similar manner as described for **25**. The peptide sequence IYKYY was assembled on Wang resin instead of CIYKYY. PhPP-$(CH_2)_3$COOH (**4**) was used instead of PhPP-CH_2COOH (**1**) for the conjugation with the peptide-attached resin. HR-MS (ESI-TOF) (*m/z*): $C_{54}H_{65}N_{11}O_{10}$ calcd, 1027.4916; found, 1027.5014 $[M]^+$, 514.0023 $[M + 2H]^{2+}$.

PhPP-$(CH_2)_3$CONH-CYKYY (33). Compound **33** was synthesized, purified, and characterized in a similar manner as described for **25**. The peptide sequence CYKYY was assembled on Wang resin instead of CIYKYY. PhPP-$(CH_2)_3$COOH (**4**) was used instead of PhPP-CH_2COOH (**1**) for the conjugation with the peptide-attached resin. HR-MS (ESI-TOF) (*m/z*): $C_{51}H_{59}N_{11}O_{10}S$ calcd, 1017.4167; found, 1017.5021 $[M]^+$, 508.9893 $[M + 2H]^{2+}$.

Synthesis of PhPP-CH_2CONH-GCIYKYY (34). Compound **34** was synthesized, purified, and characterized in a similar manner as described for **25**. The peptide sequence GCIYKYY was assembled on Wang resin instead of CIYKYY. HR-MS (ESI-TOF) (*m/z*): $C_{57}H_{69}N_{13}O_{12}S$ calcd, 1159.4909; found, 1159.8004 $[M]^+$, 580.7114 $[M + 2H]^{2+}$.

PhPP-CH_2CONH-?-Aba-CIYKYY (35). Compound **35** was synthesized, purified, and characterized in a similar manner as described for **25**. The peptide sequence ?-Aba-CIYKYY was assembled on Wang resin instead of CIYKYY. HR-MS (ESI-TOF) (*m/z*): $C_{59}H_{73}N_{13}O_{12}S$ calcd, 1187.5222; found, 1187.8407 $[M]^+$, 594.7434 $[M + 2H]^{2+}$.

PhPP-CH_2Ph-CONH-CIYKYY (36). Compound **36** was synthesized, purified, and characterized in a similar manner as described for **25**. PhPP-CH_2Ph-COOH (**6**) was used instead of PhPP-CH_2COOH (**1**) for the conjugation with the peptide-attached resin. HR-MS (ESI-TOF) (*m/z*): $C_{61}H_{70}N_{12}O_{11}S$ calcd, 1178.5008; found, 1179.2917 $[M + H]^+$, 589.9964 $[M + 2H]^{2+}$.

PP-CH(CH_3)CONH-CIYKYY (37). Compound **37** was synthesized, purified, and characterized in a similar manner as described for **25**. PP-CH(CH_3)COOH was synthesized as described for compound **2** and was used instead of PhPP-CH_2COOH (**1**) for the conjugation with the peptide-attached resin. HR-MS (ESI-TOF) (*m/z*): $C_{50}H_{64}N_{12}O_{11}S$ calcd, 1040.4538, found, 1040.5993 $[M]^+$, 521.1557 $[M + 2H]^{2+}$.

PhPP-CH_2CONH-YIYGSFK (38). The peptide sequence YIYGSFK was assembled on Fmoc-Lys(Boc)-Wang resin (213.0 mg, 0.47 mmol/g) by Fmoc solid-phase peptide synthesis strategy using Fmoc protected amino acids [Fmoc-Phe-OH (F_6), Fmoc-Ser(*t*Bu)-OH (S_5), Fmoc-Gly-OH (G_4), Fmoc-Tyr(*t*Bu)-OH (Y_3), Fmoc-Ile-OH (I_2), and Fmoc-Tyr(*t*Bu)-OH (Y_1)]. Using a similar method described above for **25**, *N*-terminal conjugation of the peptide-attached resin with PhPPCH_2COOH (**1**), cleavage, deprotection, and purification afforded **38**. HR-MS (ESI-TOF) (*m/z*): $C_{57}H_{69}N_{13}O_{12}$ calcd, 1127.5189; found, 1128.3235 $[M + H]^+$, 564.6595 $[M + 2H]^{2+}$.

PhPP-CH_2CONH-EEIYGEFF (39). The peptide sequence EEIYGEFF was assembled on Fmoc-Phe-Wang resin (100.0 mg, 1.0 mmol/g) by Fmoc solid-phase peptide synthesis strategy using Fmoc protected amino acids [Fmoc-Phe-OH (F_7), Fmoc-Glu(*t*Bu)-OH (E_6), Fmoc-Gly-OH (G_5), Fmoc-Tyr(*t*Bu)-OH (Y_4), Fmoc-Ile-OH (I_3), Fmoc-Glu(*t*Bu)-OH (E_2), and Fmoc-Glu(*t*Bu)-OH (E_1)]. Using a similar method described above for **25**, *N*-terminal conjugation of the peptide-attached resin with PhPPCH_2COOH (**1**), cleavage, deprotection, and purification afforded **39**. HR-MS (ESI-TOF) (*m/z*): $C_{63}H_{73}N_{13}O_{17}$ calcd, 1283.5247; found, 1284.7828 $[M + H]^+$, 642.8828 $[M + 2H]^{2+}$.

PhPP-CH_2CONH-EIYGEFKK (40). The peptide sequence EIYGEFKK was assembled on Fmoc-Lys(Boc)-Wang resin (213.0 mg, 0.47 mmol/g) by Fmoc solid-phase peptide synthesis strategy using Fmoc protected amino acids [Fmoc-Lys(Boc)-OH (K_7), Fmoc-Phe-OH (F_6), Fmoc-Glu(*t*Bu)-OH (E_5), Fmoc-Gly-OH (G_4), Fmoc-Tyr(*t*Bu)-OH (Y_3), Fmoc-Ile-OH (I_2), and Fmoc-Glu(*t*Bu)-OH (E_1)]. Using a similar method described above for **25**, *N*-terminal conjugation of the peptide-attached resin with PhPPCH_2COOH (**1**), cleavage, deprotection, and purification afforded **40**. HR-MS (ESI-TOF) (*m/z*): $C_{61}H_{81}N_{15}O_{15}$ calcd, 1263.6037; found, 1264.6379 $[M + H]^+$, 632.7722 $[M + 2H]^{2+}$, 422.2609 $[M + 3H]^{3+}$.

PhPP-CH_2CONH-IYGEFKKK (41). The peptide sequence IYGEFKKK was assembled on Fmoc-Lys(Boc)-Wang resin (213.0 mg, 0.47 mmol/g) by Fmoc solid-phase peptide synthesis strategy

using Fmoc protected amino acids [Fmoc-Lys(Boc)-OH (K_7), Fmoc-Lys(Boc)-OH (K_6), Fmoc-Phe-OH (F_5), Fmoc-Glu(*t*Bu)-OH (E_4), Fmoc-Gly-OH (G_3), Fmoc-Tyr(*t*Bu)-OH (Y_2), and Fmoc-Ile-OH (I_2)]. Using a similar method described above for **25**, *N*-terminal conjugation of the peptide-attached resin with $PhPPCH_2COOH$ (**1**), cleavage, deprotection, and purification afforded **41**. HR-MS (ESI-TOF) (*m/z*): $C_{62}H_{86}N_{16}O_{13}$ calcd, 1262.6560; found, 1263.7533 $[M + H]^+$, 632.3262 $[M + 2H]^{2+}$, 421.9538 $[M + 3H]^{3+}$.

PhPP-CH_2CONH-AEEEIYGEFEAKKKK (42). The peptide sequence AEEEIYGEFEAKKKK was assembled on Fmoc-Lys(Boc)-Wang resin (213.0 mg, 0.47 mmol/g) by Fmoc solid-phase peptide synthesis strategy using Fmoc protected amino acids [Fmoc-Lys(Boc)-OH (K_{14}), Fmoc-Lys(Boc)-OH (K_{13}), Fmoc-Lys(Boc)-OH (K_{12}), Fmoc-Ala-OH (A_{11}), Fmoc-Glu(*t*Bu)-OH (E_{10}), Fmoc-Phe-OH (F_9), Fmoc-Glu(*t*Bu)-OH (E_8), Fmoc-Gly-OH (G_7), Fmoc-Tyr(*t*Bu)-OH (Y_6) and Fmoc-Ile-OH (I_5), Fmoc-Glu(*t*Bu)-OH (E_4), Fmoc-Glu(*t*Bu)-OH (E_3), Fmoc-Glu(*t*Bu)-OH (E_2), and Fmoc-Ala-OH (A_1)]. Using a similar method described above for **25**, *N*-terminal conjugation of the peptide-attached resin with $PhPPCH_2COOH$ (**1**), cleavage, deprotection, and purification afforded **42**. HR-MS (ESI-TOF) (*m/z*): $C_{94}H_{136}N_{24}O_{28}$ calcd, 2048.9956; found, 1024.0379 $[M + 2H]^{2+}$, 683.6153 $[M + 3H]^{3+}$, 513.3387 $[M + 4H]^{4+}$.

Ac-Dpr(NHCOCH_2PhPP)IYKYY (52). The peptide sequence Ac-Dpr-IYKYY was assembled on Fmoc-Tyr(*t*Bu)-Wang resin (115.0 mg; 0.88 mmol/g) by Fmoc solid-phase peptide synthesis strategy using Fmoc protected amino acids [Fmoc-Tyr(*t*Bu)-OH (Y_5), Fmoc-Lys(Boc)-OH (K_4), Fmoc-Tyr(*t*Bu)-OH (Y_3), Fmoc-Ile-OH (I_2) and Fmoc-Dpr(ivDde)-OH $(Dpr)_1$]. The *N*-terminal was acetylated with acetic anhydride. The Dde protecting group of side chain of Dde (βAA) was selectively removed by removed by hydrazine monohydrate in DMF (twice, 2%, 5 mL) and shaking for 20 min at room temperature. The resin was collected by filtration and washed with DMF (2 × 20 mL), MeOH (2 × 20 mL), and DCM (2 × 20 mL), respectively, and dried under vacuum. The free amino group at side chain of $(Dpr)_1$ was coupled with PhPP-CH_2COOH (**1**) using HBTU/DIEA in

DMF for 6 h at room temperature as described above for **25**. The resin was collected by filtration and washed with DMF (2 × 20 mL), MeOH (2 × 20 mL), and DCM (2 × 20 mL), respectively. The side chain deprotection and cleavage was carried out by shaking the resin with a mixture of TFA:anisole:water:EDT (95:2.0:2.0:1.0 v/v/v/v, 5.0 mL) for 1 h. The crude compound was precipitated by the addition of cold diethyl ether (25 mL, Et_2O) and purified by preparative HPLC to afford **52**. HR-MS (ESI-TOF) (*m/z*): $C_{57}H_{69}N_{13}O_{12}$ calcd, 1127.5189; found, 1128.2123 $[M + H]^+$, 564.5722 $[M + 2H]^{2+}$.

Ac-CIF(4-NHCOCH$_2$PhPP)KYY (53). The peptide sequence Ac-CIF(4-NO_2)KYY was assembled on Fmoc-Tyr(*t*Bu)-Wang resin (115.0 mg; 0.88 mmol/g) by Fmoc solid-phase peptide synthesis strategy using Fmoc protected amino acids [Fmoc-Tyr(*t*Bu)-OH (Y_5), Fmoc-Lys(Boc)-OH (K_4), Fmoc-Phe(4-NO_2)-OH (F_3), Fmoc-Ile-OH (I_2), and Fmoc-Cys(Trt)-OH (C_1)]. The *N*-terminal was acetylated with acetic anhydride. The nitro group of 4-nitrophenylalanine in position 3 was reduced to amino group by shaking of the resin with $SnCl_2.H_2O$ in DMF (3M, 10 mL) at room temperature for 24 h. The resin was collected by filtration and washed with DMF (2 × 20 mL), MeOH (2 × 20 mL), and DCM (2 × 20 mL), respectively, and dried under vacuum. The amino group of 4-aminophenylalanine in position 4 was coupled with PhPP-CH_2COOH (**1**) using HBTU/DIEA in DMF for 6 h at room temperature as described above for **25**. The resin was collected by filtration and was washed with DMF (2 × 20 mL), MeOH (2 × 20 mL), and DCM (2 × 20 mL), respectively. The side chain deprotection and cleavage was carried out by shaking of the resin with a mixture of TFA:anisole:water:EDT (95:2.0:2.0:1.0 v/v/v/v, 5.0 mL) for 1 h. The crude compound was precipitated by the addition of cold diethyl ether (25 mL, Et_2O), purified by preparative HPLC to afford **53**. HR-MS (ESI-TOF) (*m/z*): $C_{57}H_{69}N_{13}O_{11}S$ calcd, 1143.4960; found, 1144.1511 $[M + H]^+$, 572.5094 $[M + 2H]^{2+}$.

Ac-CIYK(NHCOCH$_2$PhPP)YY (54). The peptide sequence Ac-CIYKYY was assembled on Fmoc-Tyr(*t*Bu)-Wang resin (115.0 mg; 0.88 mmol/g) by Fmoc solid-phase peptide synthesis strategy using

Fmoc protected amino acids [Fmoc-Tyr(*t*Bu)-OH (Y_5), Fmoc-Lys(Dde)-OH (K_4), Fmoc-Tyr(*t*Bu)-OH (Y_3), Fmoc-Ile-OH (I_2) and Fmoc-Cys(Trt)-OH (C_1)]. The *N*-terminal was acetylated with acetic anhydride. The Dde protecting group of the side chain of K4 was selectively removed by hydrazine monohydrate in DMF (twice, 2%, 5 mL) and shaking for 20 min at room temperature. The resin was collected by filtration and washed with DMF (2 × 20 mL), MeOH (2 × 20 mL), and DCM (2×20 mL), respectively, and dried under vacuum. The free amino group at side chain of K4 was coupled with PhPP-CH_2COOH (**1**) using HBTU/DIEA in DMF for 6 h at room temperature as described above for **25**. The resin was collected by filtration and washed with DMF (2 × 20 mL), MeOH (2 × 20 mL), and DCM (2 × 20 mL), respectively. The side chain deprotection and cleavage was carried out by shaking of the resin with a mixture of TFA:anisole:water:EDT (95:2.0:2.0:1.0 v/v/v/v, 5.0 mL) for 1 h. The crude compound was precipitated by the addition of cold diethyl ether (25 mL, Et_2O), purified by preparative HPLC to afford **54**. HR-MS (ESI-TOF) (*m/z*): $C_{57}H_{68}N_{12}O_{12}S$ calcd, 1144.4800; found, 1145.5019 $[M + H]^+$.

Ac-CIYKF(4-NHCOCH$_2$PhPP)Y (55). This compound was synthesized in a similar manner as described for **53**. The amino acid Fmoc-Phe(4-NO_2)-OH (F_5) was used at position 5 instead of position 3 in assembling the peptide. HR-MS (ESI-TOF) (*m/z*): $C_{57}H_{69}N_{13}O_{11}S$ calcd, 1143.4960; found, 1144.5926 $[M + H]^+$, 572.7971 $[M + 2H]^{2+}$.

Ac-CIYKYF(4-NHCOCH$_2$PhPP) (56). This compound was synthesized in a similar manner as for **53**. The peptide was assembled on Fmoc-Phe(4-NO_2)-OH-Wang resin (200 mg, 0.50 mmol/g) instead of Fmoc-Tyr(*t*Bu)-Wang resin. HR-MS (ESI-TOF) (*m/z*): $C_{57}H_{69}N_{13}O_{11}S$ calcd, 1143.4960; found, 1144.3780 $[M + H]^+$, 572.5859 $[M + 2H]^{2+}$.

Ac-YIYGSFK(NHCOCH$_2$PhPP) (57). The peptide sequence YIYGSFK was assembled on Fmoc-Lys(Dde)-Wang resin (200.0 mg; 0.5 mmol/g) by Fmoc solid-phase peptide synthesis strategy using Fmoc protected amino acids [Fmoc-Phe-OH (F_6), Fmoc-Ser(*t*Bu)-OH (S_5), Fmoc-Gly-OH (G_4),

Fmoc-Tyr(*t*Bu)-OH (Y_3), Fmoc-Ile-OH (I_2) and Fmoc-Tyr(*t*Bu)-OH (Y_1)]. The *N*-terminal was acetylated with acetic anhydride. The Dde protecting group of the side chain of K7 was selectively removed by hydrazine monohydrate in DMF (twice, 2%, 5 mL) and shaking for 20 min at room temperature. The resin was collected by filtration and washed with DMF (2 × 20 mL), MeOH (2 × 20 mL) and DCM (2 × 20 mL), respectively, and dried under vacuum. The free amino group at side chain of K7 was coupled with PhPP-CH_2COOH (**1**) using HBTU/DIEA in DMF for 6 h at room temperature as described above for **25**. The resin was collected by filtration and washed with DMF (2 × 20 mL), MeOH (2 × 20 mL), and DCM (2 × 20 mL), respectively. The side chain deprotection and cleavage was carried out by shaking the resin with a mixture of TFA:anisole:water:EDT (95:2.0:2.0:1.0 v/v/v/v, 5.0 mL) for 1 h. The crude compound was precipitated by the addition of cold diethyl ether (25 mL, Et_2O), purified by preparative HPLC to afford **57**. HR-MS (ESI-TOF) (*m/z*): $C_{59}H_{71}N_{13}O_{13}$ calcd, 1169.5294; found, 1170.0730 [M^+].

3. Analytical HPLC profile of compounds in two diverse systems. The purity of the final products was confirmed by analytical HPLC. The analytical HPLC analysis was performed on the Hitachi analytical HPLC system on a C18 Shimadzu Premier column (3 µm, 150 × 4.6 mm) using two gradient systems A and B (Tables 1 and 2) and a flow rate of 0.5 mL/min with UV detection at 250 nm. The HPLC system consists of a L-7100 low pressure gradient pump, 4-channel degasser, L-7200 sequential autosampler, high sensitivity diode array detector (190-800 nm), and a L-7485 fluorescence detector (emission: 200-850 nm; excitation 250-900 nm). This system is controlled by a D-700 HPLC System Manager software package. The chemical structures of compounds were confirmed by a high-resolution PE Biosystems Mariner API time-of-flight mass spectrometer. Table 3 shows the retention times of final products in two gradient systems A and B. Examples of analytical HPLC profiles for a number of compounds are shown.

Table 1. HPLC Gradient System A.

Time (min.)	**Water (% v/v) TFA (0.1%)**	**Acetonitrile (% v/v) TFA (0.1%)**
0	100	0
0-10	80	20
10-25	60	40
25-35	30	70
35-40	55	45
40-45	100	0

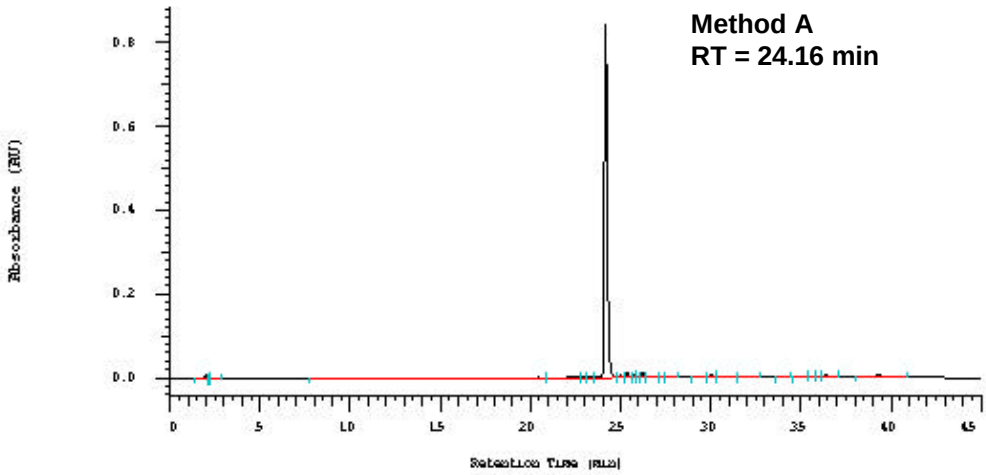
Table 2. HPLC Gradient System B.

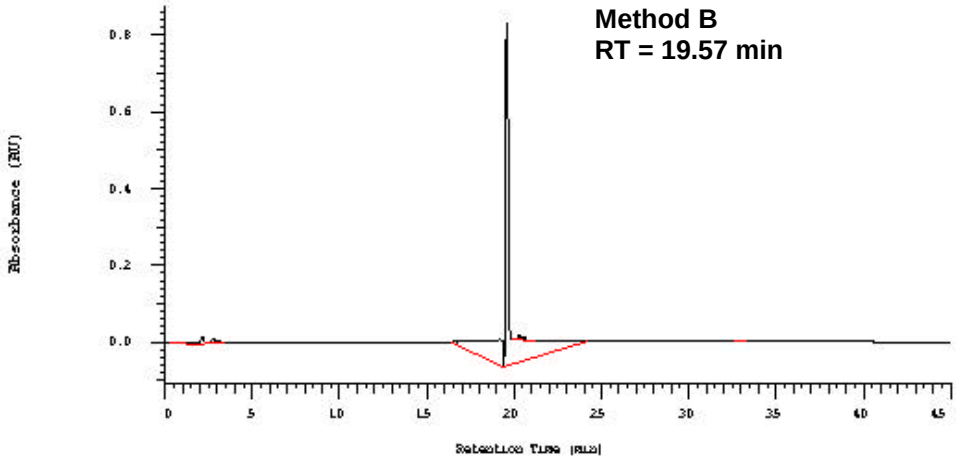
Time (min.)	**Water (% v/v) TFA (0.1%)**	**Acetonitrile (% v/v) TFA (0.1%)**
0	80	20
0-10	80	20
10-35	50	50
35-45	80	20

Table 3. The retention times (min.) of peptides and PhPP-peptide conjugates using two gradient systems A and B in analytical HPLC.

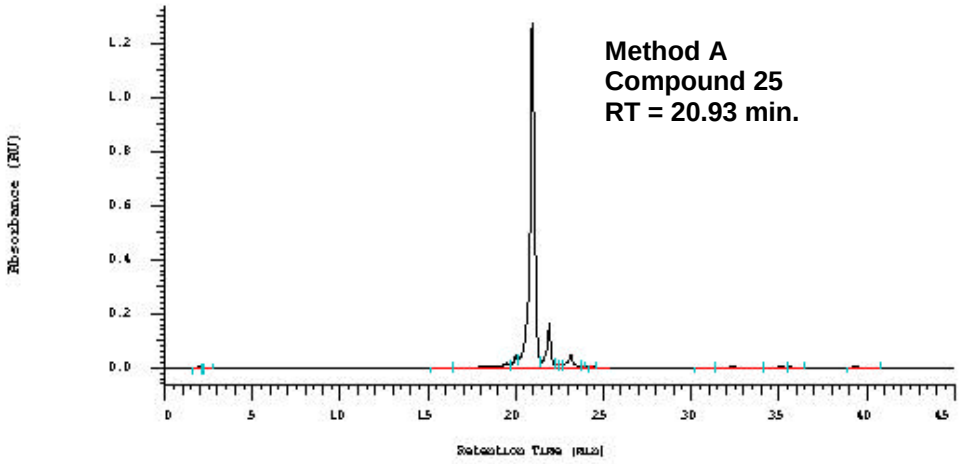
Compound No.	Retention Time (min.)		Compound No.	Retention Time (min.)	
	Method A	Method B		Method A	Method B
Ac-EEIYGEFF	24.16	19.57	**31**	22.05	18.45
Ac-EIYGEFKK	16.00	9.41	**34**	20.00	17.25
Ac-IYGEFKKK	15.44	9.12	**35**	20.48	17.63
Ac-AEEEIYGEFEAKKKK	13.20	6.27	**36**	23.73	19.33
7	18.64	15.81	**38**	20.93	17.89
8	15.63	7.60	**39**	24.75	19.79
22	14.19	6.59	**40**	18.35	15.81
23	20.56	17.41	**41**	17.73	15.23
24	19.33	16.67	**42**	19.76	16.43
25	20.93	17.76	**52**	19.04	16.51
26	23.63	19.44	**53**	20.48	17.60
27	29.01	21.87	**54**	30.48	21.89
28	22.19	18.51	**55**	20.48	17.55
29	20.77	18.21	**56**	20.91	17.89
30	21.31	18.21	**57**	22.08	18.51

Ac-EEIYGEFF

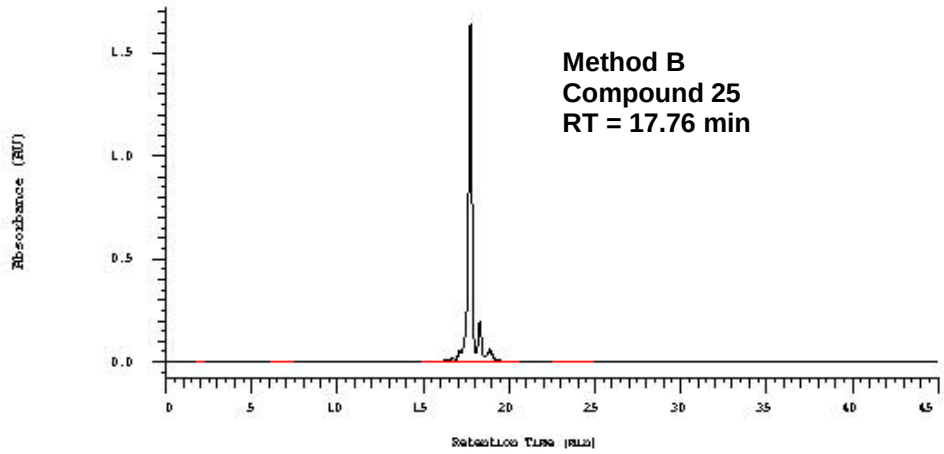

Method A
RT = 24.16 min
Absorbance (AU)
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25
30
35
40
45


Method B
RT = 19.57 min
Absorbance (AU)
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25
30
35
40
45

PhPP-CH_2CO-CIYKYY (25)

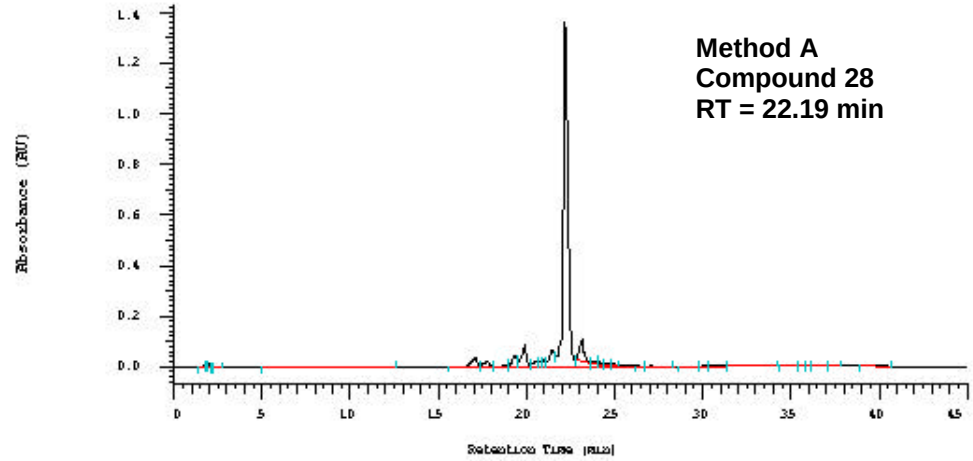


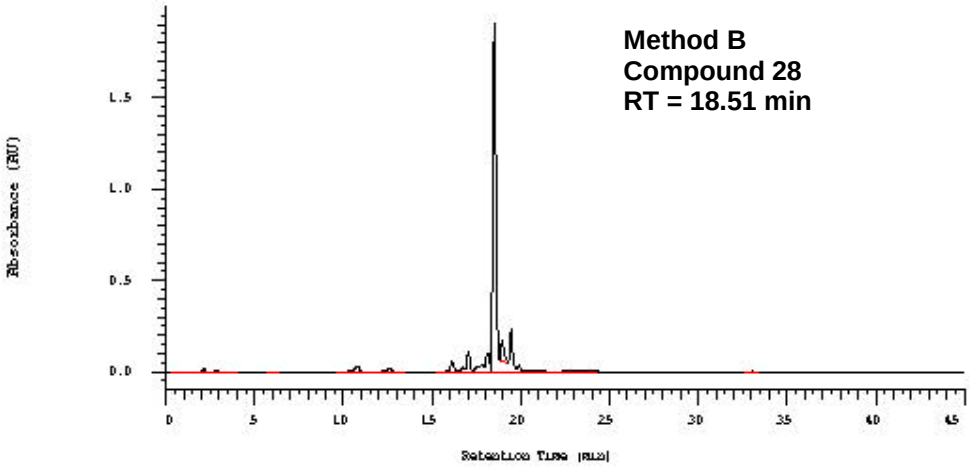
Method A
Compound 25
RT = 20.93 min.



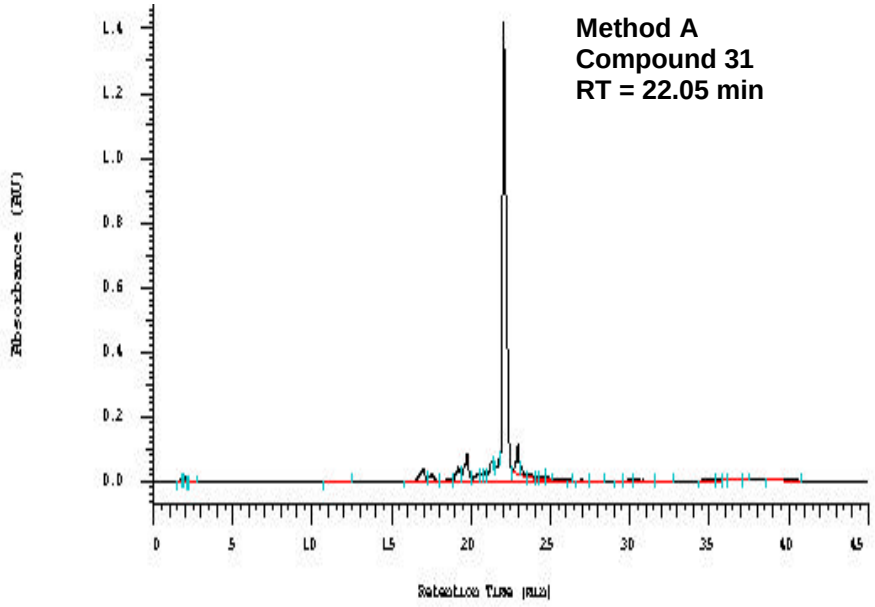
Method B
Compound 25
RT = 17.76 min

PhPP-CH(CH_3)-CO-CIYKYY (28)

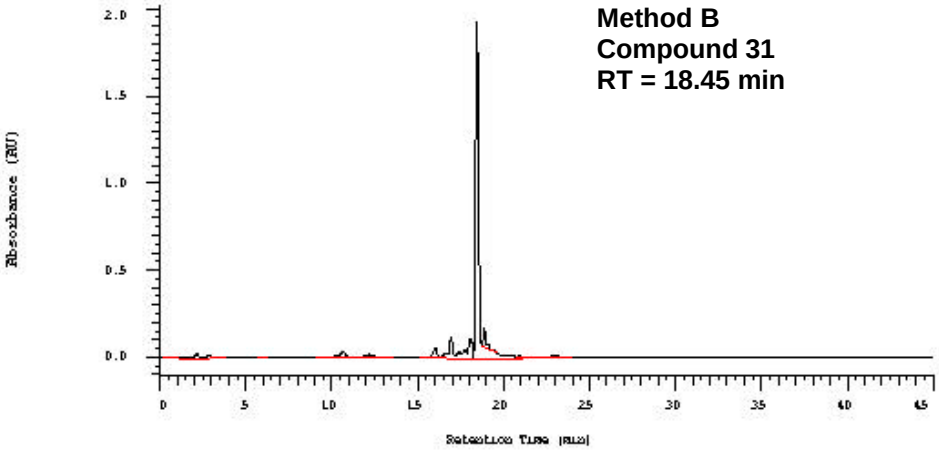




PhPP-CH(CH_3)CH_2-CO-CIYKYY (31)

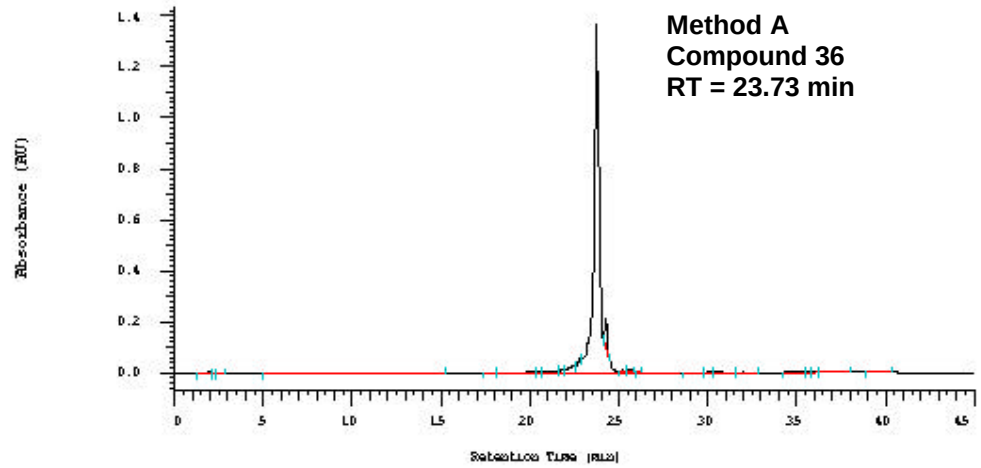


Method A
Compound 31
RT = 22.05 min

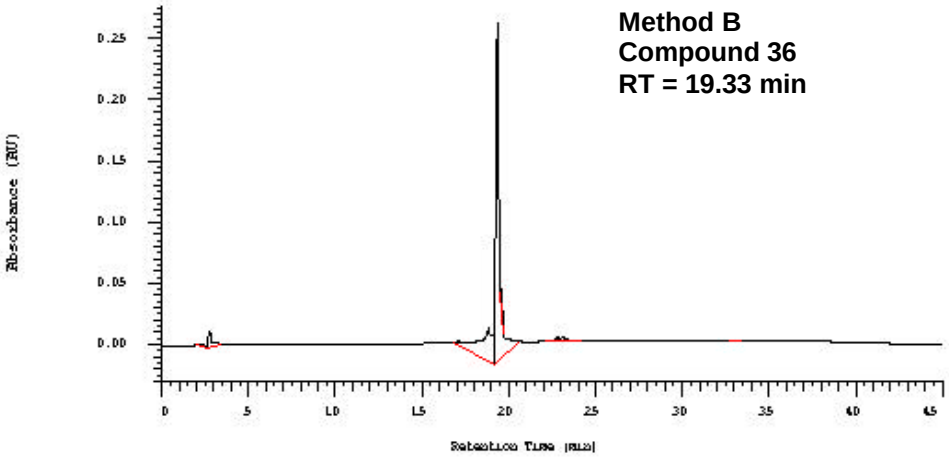


Method B
Compound 31
RT = 18.45 min

PhPP-CH_2PhCO-CIYKYY (36)

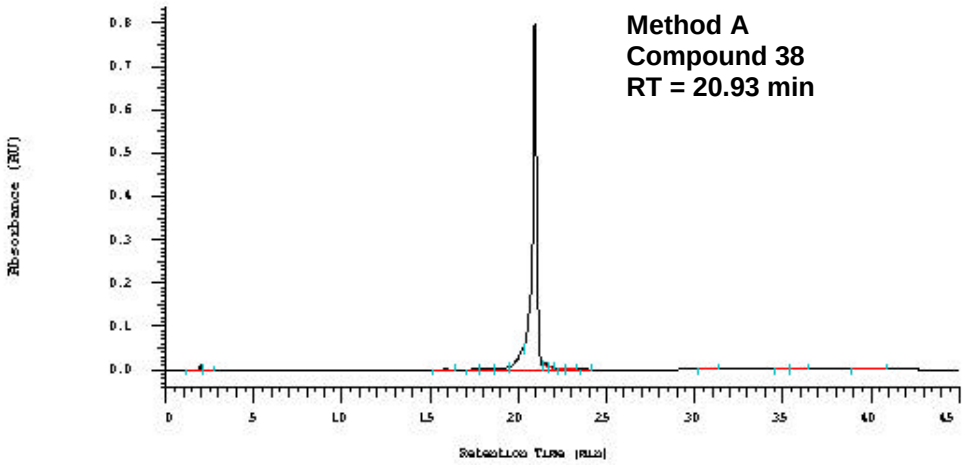


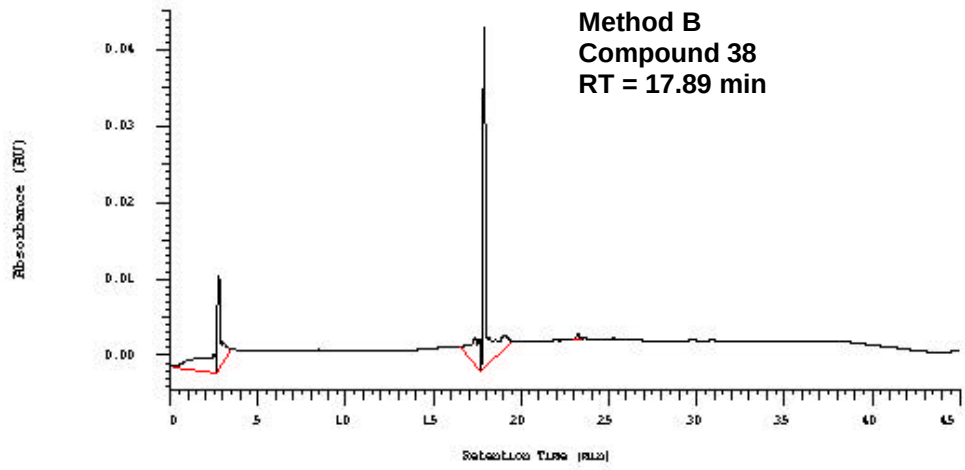
Method A
Compound 36
RT = 23.73 min



Method B
Compound 36
RT = 19.33 min

PhPP-YIYGSFK (38)





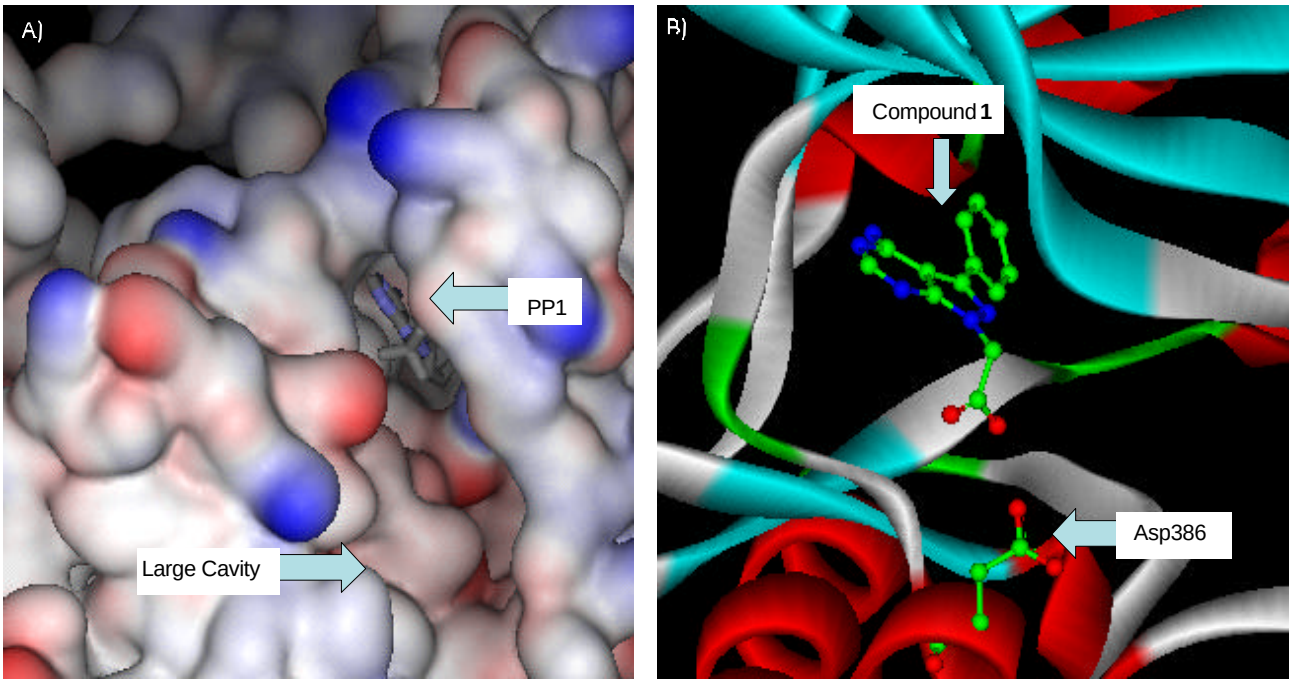


Figure 5 Simulated binding of PP1 (a) and compound **1** (b) to c-Src based on the crystal structures of PP1-Hck complex (1QCF)[19] and AMP-PNP complex with c-Src (2SRC)[54].

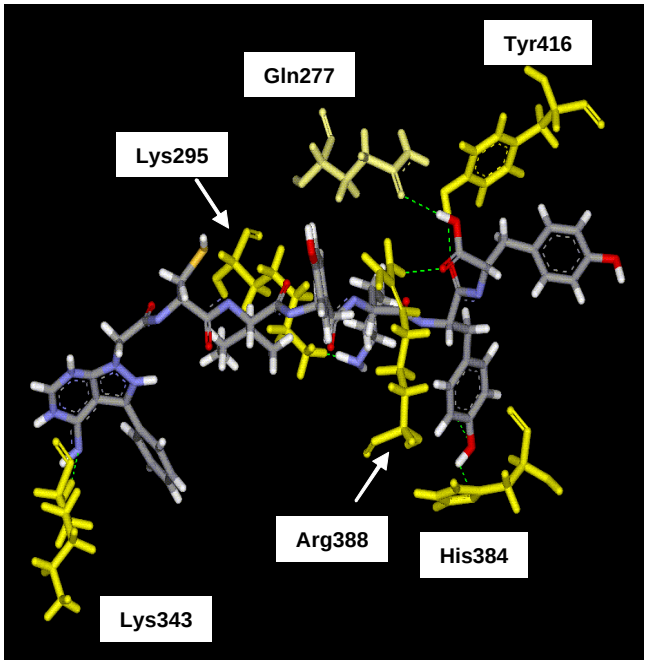


Figure 6 Predicted major interactions of compound **25** with amino acids of the Src kinase domain. Compound **25** and side chains of amino acids of the Src kinase domain are represented by sticks, respectively. The carbon skeleton of compound **25** is gray, oxygen atoms are red, and nitrogens are blue. Hydrogen atoms have been removed to improve clarity. The key amino acids residues (yellow) within the Src kinase domain are labeled. The binding pocket amino acids (yellow) form hydrogen bonds (green) with different functional groups of the PhPP-peptide conjugate. The Figure were drawn using the Accelrys visualization system.

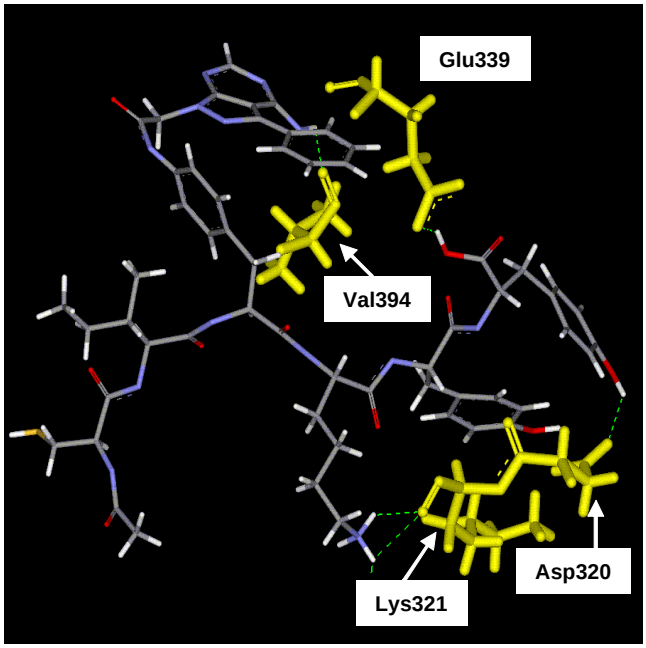


Figure 7 Predicted major interactions of compound 53 with amino acids of the Src kinase domain. Compound 53 and side chains of amino acids of the Src kinase domain are represented by sticks, respectively. The carbon skeleton of compound 53 is gray, oxygen atoms are red, and nitrogens are blue. Hydrogen atoms have been removed to improve clarity. The key amino acids residues (yellow) within the Src kinase domain are labeled. The Figure was drawn using the Accelrys visualization system.