Supporting Information for

Thiocarbamate-linked polysulfonate-peptide conjugates as selective hepatocyte growth factor receptor binders

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Abbreviations

The following abbreviations are used throughout the text of the Supporting Information: CDI, N,N-carbonyldimidazole; CHCA, α-cyano-4-hydroxycinnamic acid; CZE, capillary zone electrophoresis; DIEA, N,N-diisopropylethylamine; DMF, N,N-dimethylformamide; TEA, triethylamine; TFA, trifluoroacetic acid; TNBS, 2,4,6-trinitrobenzenesulfonic acid.

General

The following eluents were used for RP-HPLC analyses and purifications
Eluent A : water containing 0.5% TFA (v/v)

Eluent B : water/acetonitrile : 20/80 by vol containing 0.5% TFA (v/v)

The linear gradients used for RP-HPLC analyses and purifications are specified for each compound within the Supporting Information.

Preparation of the N-imidazolylcarbonyl derivative of taurine 13a. Solution A
Taurine (2.0 g, 16.0 mmol) was dissolved in deionized water (25 mL) and added to aq. nBu₄OH (16 mL, 1.0 M in water). The solution was stirred for a few min and then frozen and lyophilized. The tetra(n-butyl)ammonium salt of taurine (732.6 mg, 2 mmol) and CDI (324.3 mg, 2 mmol, 1 equiv.) were dissolved separately in dry DMF (2 mL for each reagent). The solutions were mixed and stirred at rt for 2 h. This solution was used directly for the coupling to the peptidyl resins (solution A, 0.5 M final concentration in 13a).

Preparation of the p-nitrophenyl carbonyl derivative of taurine 13b. Solution B
Taurine (2.0 g, 16 mmol) was dissolved in aq. nBu₄OH (16 mL, 1 M in water). This solution was diluted with dry DMF (128 mL). Bis(p-nitrophenyl)carbonate (4.86 g, 16 mmol, 1 equiv.) was dissolved in this solution which was stirred at rt for 30 min and then used directly in the coupling step (solution B, final concentration 100 mM in 13b).

Preparation of 19 for coupling to the peptidyl resins. Solution C
Amine 2 (82 mg, 0.4 mmol) was dissolved in a mixture of aqueous nBu₄OH (400 µL, 1.0 M in water) and DMF (4 mL). In this solution was dissolved bis(p-nitrophenyl)carbonate (121.7 mg, 0.4 mmol, 1 equiv.). The mixture was stirred for 30 min at rt and used directly for the coupling step (solution C, 100 mM final concentration in 19).
Synthesis and isolation of 13b

Taurine (21.5 mg, 172 µmol) was dissolved in an aqueous solution of nBu₄NOH (172 µL, 172 µmol, 1.0 M in water). This solution was diluted with CH₂Cl₂ (17.2 mL) after which bis(p-nitrophenyl)carbonate (52.3 mg, 172 µmol, 1 equiv.) was added in one portion. The resulting reaction mixture was stirred at rt for 1 h. Thereafter, the solvent was evaporated and the crude product was purified by RP-HPLC (0-10% B in 10 min, then 10-20% B in 30 min, C₁₈ Nucleosil, 215 nm, 6.0 mL/min) to give 30 mg of 13b (yield 60%).

LC-MS analysis (C₁₈ Nucleosil, source temperature 120 °C, desolvatation temperature 350 °C, cone voltage 30 V): purity > 99%; C₉H₉N₂O₇S calcd for [M - H] = 289.02, obs. 289.00.

Rf (CH₂Cl₂/MeOH, 9/1, v/v) = 0.2

Fig. S1. RP-HPLC analysis. C₁₈ Nucleosil column, 1.0 mL/min, λ = 215 nm, 0-100% B in 30 min, 30 °C.
Fig. S2. $^1$H NMR analysis for 13b (DMF-d7). $^1$H NMR (300 MHz, DMF-d7) $\delta$ ppm: 0.97 (24H, t, 7.2 Hz), 1.41 (16H, m), 1.77 (16H, m), 2.82 (2H, t, 6.6 Hz), 3.39 (16H, m), 3.56 (2H, m), 7.51 (2H, d, 9.3 Hz), 7.83 (1H, broad t), 7.51 (2H, d, 9.3 Hz).

The integration of the peak at 0.97 ppm (methyl protons of $n$Bu$_4$N$^+$ ions) suggests that 13b coelutes by RP-HPLC with 1 mol of CF$_3$CO$_2$N($n$Bu)$_4$. 
Fig. S3. $^1$H NMR analysis for 13b (DMF-$d_7$)

Fig. S4. $^1$H NMR analysis for 13b (300 MHz, DMF-$d_7$)
Fig. S5. COSY spectrum for 13b (300 MHz, DMF-$d_7$).
Fig. S6. COSY spectrum for 13b (300 MHz, DMF-d7).
Fig. S7. HSQC spectrum for 13b (300 MHz, DMF-d7).
Fig. S8. HSQC spectrum for 13b (300 MHz, DMF-d7).
Fig. S9. HSQC spectrum for 13b (300 MHz, DMF-$d_7$).
Fig. S10. \textsuperscript{13}C NMR JMOD spectrum for 13b (75.4 MHz, DMF \textit{d}7). \(\delta\) ppm: 15.6, 22.0, 26.0, 40.7, 52.7, 60.7, 125.0, 127.6, 147.0, 155.5, 159.3.

Fig. S11. \textsuperscript{13}C NMR JMOD spectrum for 13b (75.4 MHz, DMF \textit{d}7).
Fig. S12. $^{13}$C NMR spectrum for 13b (75.4 MHz, DMF $d_7$).

**Synthesis and isolation of 19**

Amine 2 (15 mg, 73 µmol) was dissolved in aq. $n$Bu$_4$NOH (73 µL, 1.0 M in water, 73 µmol, 1 equiv.) and diluted with CH$_2$Cl$_2$ (30 mM final concentration). Bis(p-nitrophenyl)carbonate (22 mg, 73 µmol, 1 equiv.) was added in one portion to this solution and the resulting reaction mixture was stirred at rt for 1 h. Thereafter, the solvent was evaporated and the residue was purified by RP-HPLC (0-25% B in 30 min, C$_{18}$ Nucleosil, 215 nm, 6.0 mL/min) to give 13 mg (yield 49%) of derivative 19.

RP-HPLC purity (0-100% B in 30 min, 30 ºC, C$_{18}$ Nucleosil): 91%.

MALDI-TOF (DHB matrix) C$_9$H$_8$N$_2$O$_{10}$S$_2$ calcd. for [M - H$^+$] m/z (monoisotopic) = 368.9, obs. 368.6.
Fig. S13. RP-HPLC of compound 19. C18 Nucleosil column, 1.0 mL/min, λ = 215 nm, 0-100% B in 30 min, 30 °C.
Fig. S14. $^1$H NMR spectrum for 19 (300 MHz, DMF-$d_7$). δ ppm: 0.97 (3H, t, 7.2 Hz), 1.42 (8H, m), 1.77 (8H, m), 3.39 (8H, m), 3.78 (1H, t, 6.0 Hz), 3.96 (2H, td), 7.52 (2H, d, 9.3 Hz), 7.61 (1H, m), 8.32 (2H, d, 9.3 Hz).

The NMR spectrum shows an excess of nBu$_4$N ions suggesting that as for compounds 13b some CF$_3$CO$_2$N(nBu)$_4$ (~ 1.5 mol) coelutes with compound 19 during RP-HPLC.
Fig. S15. $^1$H NMR spectrum for 19 (300 MHz, DMF-d$_7$).
Fig. S16. $^1$H NMR spectrum for 19 (300 MHz, DMF-$d_7$).
Fig. S17. COSY spectrum for 19 (300 MHz, DMF-d7).
Fig. S18. COSY spectrum for 19 (300 MHz, DMF-d7).
Fig. S19. COSY spectrum for 19 (300 MHz, DMF-$d_7$).
Fig. S20. HMBC spectrum for 19 (DMF-d7)
Fig. S21. HSQC spectrum for 19 (DMF-$d_7$)
Fig. S22. HSQC spectrum for 19 (DMF-$d_7$)
Fig. S23. $^{13}$C NMR spectrum for 19 (75.4 MHz, DMF d7). $\delta$ ppm: 15.6, 22.0, 26.0, 43.9, 60.7, 125.0, 127.5, 147.0, 155.5, 159.3.
Peptide 3

Peptide Fmoc-K(Fmoc)GY(OtBu)K(Mtt) was assembled on a NovaSyn TGR® resin (0.1 mmol, loading 0.25 mmol/g) using standard Fmoc-SPPS. The peptidyl resin was deprotected with a solution of piperidine 20% in DMF (v/v), washed with DMF and drained. Then, the amino groups were acetylated using TBTU (1.0 mmol)/AcOH (1.0 mmol)/DIEA (1.0 mmol) in DMF pre-activated during 1 min. The resin beads were agitated 1 h at rt, and then washed with DMF (5 × 2 min). The absence of free primary amino groups was confirmed using TNBS assay.2

Mtt protecting group was removed selectively by washing the peptidyl resin with TFA/CH2Cl2 : 1/99 ; v/v ; 19 × 6 mL) as described elsewhere.3 The resin was then swelled in THF (8 × 2 min). THF (4 mL), TEA (56 µL, 0.4 mmol) and PhSCOCl (57 µL, 0.4 mmol) were then added to the beads which were agitated during 1 h. The resin was then washed with THF (3 × 2 min) and DMF (3 × 2 min). The absence of free primary amino groups was confirmed using TNBS assay.2

The peptide was cleaved and deprotected with TFA/H2O/thioanisole (4 mL, 96.5/2.5/1.0, v/v/v) for 1 h 45 min at rt. The peptide was precipitated in cold Et2O/n-heptane (100 mL, 1/1 ; v/v) and centrifuged (10 min, 3500 rpm, 4 °C). The crude peptide was purified by RP-HPLC (0-18% B in 36 min, C18 Nucleosil, detection at 215 nm, 6.0 mL/min) to give 38 mg (53%) of peptide 3.

RP-HPLC purity (0-100% de B in 30 min, 30°C, C18 Nucleosil column): 97%. 

![Peptide 3](image-url)
Fig. S24. RP-HPLC analysis of peptide 3. C_{18} Nucleosil column, 1.0 mL/min, $\lambda = 215$ nm, 0-100% B in 30 min, 30 °C.

| RT  | Area | % Area | Height |
|-----|------|--------|--------|
| 1   | 9.454| 0.91   | 6105   |
| 2   | 13.178| 0.59  | 3932   |
| 3   | 15.110| 0.87  | 6405   |
| 4   | 10.381| 0.97  | 447369 |
| 5   | 20.438| 0.53  | 4018   |

Fig. S25. MALDI-TOF analysis of peptide 3. $\alpha$-cyano-4-hydroxy-cinnaminic acid (CHCA) matrix [M + Na$^+$] monoisotopic calcd. 736.3, obs. 736.0

**Peptide 4**

Synthesis using $N$-imidazolycarbonyl taurine derivative 13a
Peptide Fmoc-K(Fmoc)GY(OtBu)K(Mtt) was assembled on a NovaSyn TGR® resin (0.1 mmol, loading 0.25 mmol/g) using standard Fmoc-SPPS. The peptidyl resin was deprotected with a solution of piperidine 20% in DMF (v/v), washed with DMF and drained. Solution A (2.0 mmol, 0.5 M) and TEA (280 µL, 2.0 mmol) were sequentially added to the peptidyl resin. The resin beads were agitated at rt for 16 h, and then washed with DMF (5 × 2 min). The absence of free primary amino groups was confirmed using TNBS assay.

Mtt protecting group was removed selectively by washing the peptidyl resin with TFA/CH₂Cl₂ : 1/99 ; v/v ; 13 × 6 mL) as described elsewhere. The resin was then swelled in THF (8 × 2 min). THF (4 mL), TEA (56 µL, 0.4 mmol) and PhSCOCl (57 µL, 0.4 mmol) were then added to the beads which were agitated for 1 h. The resin was then washed with THF (3 × 2 min) and DMF (3 × 2 min). The absence of free primary amino groups was confirmed using TNBS assay.

The peptide was cleaved and deprotected with TFA/H₂O/thioanisole (4 mL, 96.5/2.5/1.0, v/v/v) at rt for 90 min. The peptide was precipitated in cold Et₂O/n-heptane (100 mL, 1/1 ; v/v) and centrifuged (10 min, 3500 rpm, 4 °C). The crude peptide was purified by RP-HPLC (0-18% B in 36 min, C₁₈ Nucleosil, detection at 215 nm, 6.0 mL/min) to give 23 mg (25%) of di-sulfonated-peptide 4.

RP-HPLC purity (0-100% in 30 min, 30 °C, C₁₈ Nucleosil): 99%.
CZE purity, citrate buffer (15 min, -15 kV): > 99%.
LC-MS (C₁₈ Nucleosil, T°C source 120 °C, T°C desolvatation 350 °C, cone voltage 30V):
[M + H]⁺ calcd. monoisotopic 932.3, obs. 932.5, [M - H]- calcd. 930.3, obs. 930.5.
Fig. S26. LC-MS analysis of di-sulfonated-peptide 4. LC traces. C18 column (buffer A H2O + 0.1%TFA, buffer B CH3CN/H2O (80/20 ; v/v) + 0.1% TFA, source temperature 120 °C, desolvatation temperature 350 °C, cone voltage 10-30V.
Fig. S27. LC-MS analysis of di-sulfonated-peptide 4. MS traces. C18 column (buffer A H2O + 0.1%TFA, buffer B CH3CN/H2O (80/20 ; v/v) + 0.1% TFA, source temperature 120 °C, desolvation temperature 350 °C, cone voltage 10-30V.

Synthesis using the p-nitrophenylcarbonyl derivative of taurine 13b

![Chemical structure of 13b](Image)
**Fig. S28.** RP-HPLC analysis of di-sulfonated-peptide 4. C₁₈ Nucleosil column, 1.0 mL/min, λ = 215 nm, 0-100% B in 30 min, 30 °C.

**Fig. S29.** MALDI-TOF analysis of dendrimer 4. CHCA matrix (negative mode). [M - H]⁻ monoisotopic calcd. 930.3, obs. 929.4.

**Peptide 5**

Peptide 5 was synthesized as previously described for peptide 4 by replacing Fmoc-L-Tyr(OtBu)-OH by Fmoc-L-Ala-OH during the SPPS.

0.1 mmol scale, 32 mg of peptide 5 after RP-HPLC purification (yield 39%).
RP-HPLC purity (0-100% de B in 30 min, 30°C, C18 Nucleosil column): 99%

Fig. S30. RP-HPLC analysis of di-sulfonated-peptide 5. C18 Nucleosil column, 1.0 mL/min, λ = 215 nm, 0-100% B in 30 min, 30 °C.
Fig. S3. MALDI-TOF analysis of di-sulfonated-peptide 5. CHCA matrix (negative mode). [M-H]\(^{-}\) monoisotopic calcd. 838.2, obs. 838.7

Peptide 6

Peptide Fmoc-K(Fmoc)GY(OtBu)K(Boc) was assembled on a NovaSyn TGR\(^{\circledR}\) resin (0.14 mmol, loading 0.25 mmol/g) using standard Fmoc-SPPS.\(^{1}\) The peptidyl resin was deprotected with a solution of piperidine 20% in DMF (v/v), washed with DMF and drained. Solution A (2.8 mmol, see general section) and TEA (390 \(\mu\)L, 2.8 mmol) were sequentially added to the beads. The resin beads were agitated at rt for 19 h and then washed with DMF (5 \(\times\) 2 min). The absence of free primary amino groups was confirmed using TNBS assay.\(^{2}\) The peptide was cleaved from the resin and deprotected using TFA/H\(_2\)O/TIS (5 mL, 95/2.5/2.5, v/v/v) at rt for 90 min. The peptide was precipitated in cold Et\(_2\)O/n-heptane (100 mL, 1/1; v/v) and centrifuged (10 min, 3500 rpm, 4 °C). The white pellet was dissolved in deionized water and lyophilized to give 87 mg (69%) of di-sulfonated-peptide 3 which was used without further purification.

RP-HPLC purity (0-100% B in 30 min, 70 °C, C\(_{18}\) Nucleosil): 95%.
CZE purity acetate buffer (10 min 10 kV): 94%.
Fig. S32. RP-HPLC analysis of peptide 6. C_{18} Nucleosil column, 1.0 mL/min, \( \lambda = 215 \) nm, 0-100% B in 30 min, 30 °C.

Fig. S33. MALDI-TOF spectrum for 6. CHCA matrix (negative mode). \([M - H]^–\) monoisotopic calcd. 794.3, obs. 795.0 and 817.1 \([M - 2H + Na]^+\).
Peptide 7

Peptide elongation
Peptide Fmoc-K(Fmoc)GY(OtBu)K(Boc) was assembled on a NovaSyn TGR® resin (0.1 mmol, loading 0.25 mmol/g). The Fmoc groups were removed using 20% piperidine in DMF (v/v, 5 min then 15 min). The peptidyl resin was then washed with DMF (5 × 2 min). Fmoc-K(Fmoc)-OH (472.6 mg, 0.8 mmol) was then coupled manually using standard TBTU (250.5 mg, 0.78 mmol)/DIEA (140 µL, 0.8 mmol) activation in DMF (1 h). The beads were then washed with DMF (5 × 2 min).

Coupling of taurine residues using 13a
Solution A (2 mmol) and TEA (280 µL, 2 mmol) were sequentially added to the beads. The suspension was agitated overnight and then washed with DMF (5 × 2 min). The coupling and washing steps was repeated four times, after which the TNBS assay proved the lack of free amino groups.

Cleavage and deprotection step
The peptidyl resin was deprotected and cleaved in TFA/H₂O/TIS (3 mL, 95/2.5/2.5, v/v/v) at rt for 1 h 30 min. The crude peptide was precipitated in cold Et₂O/n-heptane (100 mL, 1/1, v/v) and centrifuged (3500 rpm, 10 min, 4 °C). The peptide-pellet was washed twice with Et₂O/n-heptane (100 mL, 1/1 ; v/v), dissolved in deionized water and lyophilized. The crude peptide was purified by RP-HPLC (0-20% B in 30 min, C₁₈ Nucleosil, 215 nm, 6.0 mL/min) to give 12 mg of peptide 7 (yield 11%).

  RP-HPLC purity (0-100% B in 30 min, 30°C, C₁₈ Nucléosil): > 99%.
  CZE purity, sodium phosphate buffer (10 min ; 10 kV): > 99%.
Fig. S34. RP-HPLC analysis of tetra-sulfonated-peptide 7. C18 Nucleosil column, 1.0 mL/min, λ = 215 nm, 0-100% B in 30 min, 30 °C.

| RT  | Area  | % Area | Height |
|-----|-------|--------|--------|
| 1.6582 | 6216629 | 100.00 | 1215540 |

Fig. S35. MALDI-TOF spectrum for 7. CHCA matrix (negative mode). [M - H]⁻ monoisotopic calcd. 1352.46, obs. 1352.9 and 1374.7 [M - 2H + Na]⁻.
Fig. S36. CZE analysis of tetra-sulfonated-peptide 7 using pH 7.0 phosphate buffer (10 kV).

**Peptide 8**

Peptide elongation step
Peptide Fmoc-K(Fmoc)GY(OtBu)K(Mtt) was assembled on a NovaSyn TGR® resin (0.1 mmol, loading 0.25 mmol/g). The Fmoc groups were removed using 20% piperidine in DMF (v/v, 5 min then 15 min). The peptidyl resin was then washed with DMF (5 × 2 min). Fmoc-K(Fmoc)-OH (472.6 mg, 0.8 mmol) was then coupled manually using standard TBTU (250.5 mg, 0.78 mmol)/DIEA (140 µL, 0.8 mmol) activation in DMF (1 h). The beads were then washed with DMF (5 × 2 min).

**Coupling of taurine residues using 13a**
Solution A (4 mL, 2 mmol, 20 equiv.) and TEA (280 µL, 2 mmol, 20 equiv.) were sequentially added to the beads. The suspension was agitated overnight and then washed with DMF (5 × 2 min). The coupling and washing steps was repeated four times, after which the TNBS assay proved the lack of free amino groups.

**Coupling of taurine residues using 13b**

Alternatively, solution B (8 mL, 0.8 mmol, 8 equiv.) and DIEA (66 µL, 0.4 mmol, 4 equiv.) were sequentially added to the beads which were agitated at rt for 19 h. The coupling step was repeated once after which the TNBS assay proved the lack of free amino groups.

**Incorporation of PTC group**

Mtt protecting group was removed selectively by washing the peptidyl resin with TFA/CH₂Cl₂ : 1/99 ; v/v, 25 × 6 mL) as described elsewhere.³ The resin was then swelled in THF (8 × 2 min). THF (4 mL), TEA (56 µL, 0.4 mmol) and PhSCOCl (57 µL, 0.4 mmol) were then added to the beads which were agitated for 1 h. The resin was then washed with THF (3 × 2 min) and DMF (3 × 2 min). The absence of free primary amino groups was confirmed using TNBS assay.²

**Cleavage and deprotection step**

The peptide was cleaved and deprotected with TFA/H₂O/thioanisole (4.6 mL, 96.5/2.5/1, v/v/v) at rt for 90 min. The peptide was precipitated in cold Et₂O/n-heptane (100 mL, 1/1 ; v/v) and centrifuged (10 min, 3500 rpm, 4 °C). The crude peptide pellet was purified by RP-HPLC (0-20% B in 40 min, C₁₈ Nucleosil, detection at 215 nm, 6.0 mL/min) to give 24 mg (yield 16%) of tetra-sulfonated-peptide 8.

RP-HPLC purity (0-100% B in 30 min, 30 °C, C₁₈ Nucleosil): 99%.

![RP-HPLC analysis of tetra-sulfonated-peptide 8](image)

| RT  | Area | % Area | Height |
|-----|------|--------|--------|
| 0.553 | 7808357 | 100.00 | 111507 |

Fig. S37. RP-HPLC analysis of tetra-sulfonated-peptide 8. C₁₈ Nucleosil column, 1.0 mL/min, λ = 215 nm, 0-100% B in 30 min, 30 °C.
Peptide 9

Peptide elongation step
Peptide Fmoc-K(Fmoc)GY(OtBu)K(Boc) was assembled on a NovaSyn TGR® resin (0.1 mmol, loading 0.25 mmol/g). The Fmoc groups were removed using 20% piperidine in DMF (v/v, 5 min then 15 min). The peptidyl resin was then washed with DMF (5 × 2 min).

Coupling of sulfonate groups
Then, solution C (4 mL of DMF solution, 0.4 mmol, 4 equiv.) and TEA (28 µL, 0.2 mmol, 2 equiv.) were sequentially added to the beads. The resin was agitated for 19 h and then washed with DMF (5 × 2 min). The coupling step was repeated after which TNBS assay proved the lack of free amino groups.

Deprotection and cleavage step
The peptidyl resin was treated with a mixture of TFA/H₂O/TIS (3 mL, 95/2.5/2.5, v/v/v) at rt for 90 min. The peptide was then precipitated in cold Et₂O/n-heptane (100 mL, 1/1, v/v), centrifuged (3500 rpm, 10 min, 4 °C) and washed twice with cold Et₂O. The residue was dissolved in deionized water and lyophilized. The crude peptide was purified by RP-HPLC (0-20% B in 30 min, C₁₈ Nucleosil, detection at 215 nm, 6.0 mL/min) to give 11 mg of tetra-sulfonated-peptide 9 (yield 10%).

RP-HPLC purity (0-100% B in 30 min, 30°C, C₁₈ Nucleosil): > 99%.
CZE soldium phosphate buffer pH 7.0 (10 min, 10 kV): 95%; sodium citrate buffer pH 3.0 (15 min, 15 kV): > 99%.
Fig. S39. RP-HPLC analysis of tetra-sulfonated-peptide 9. C\textsubscript{18} Nucleosil column, 1.0 mL/min, $\lambda = 215$ nm, 0-100\% B in 30 min, 30 °C.

| RT  | Area | % Area | Height |
|-----|------|--------|--------|
| 5.281 | 2790555 | 100.00 | 830389 |

Fig. S40. MALDI-TOF spectrum for 9. CHCA matrix (negative mode). [M - H]\textsuperscript{−} monoisotopic calcd. 954.19, obs. 954.7.
Fig. S41. CZE analysis of tetra-sulfonated peptide 9 using sodium citrate buffer (20 mM, pH 3.0), 15 kV, capillary length 50 cm, Ø 75 µm.
Peptide 10

Peptide 9 (73 mg, 68 μmol) was dissolved in DMF (2.7 mL). TEA (38 μL, 272 μmol, 4 equiv.) and PhSCOC1 (39 μL, 272 μmol, 4 equiv.) were added in one portion. The reaction mixture was agitated at rt for 30 min, after which the peptide was precipitated by adding the reaction mixture to cold Et2O (40 mL). The precipitate was separated by centrifugation (3500 rpm, 10 min, 4°C). The residue was resuspended in cold Et2O and centrifuged again (twice). The crude product was purified by RP-HPLC (0-20% B in 40 min, C18 Nucleosil, detection at 215 nm, 6.0 mL/min) to give 32 mg of peptide 10 (yield 43%).

RP-HPLC purity (0-100% de B in 30 min, 30 °C, C18 Nucleosil): 99%.

Fig. S42. CZE analysis of tetra-sulfonated peptide 9 using sodium phosphate buffer (10 mM, pH 7.0), 10 kV, capillary length 50 cm, ∅ 75 μm.
Fig. S43. RP-HPLC analysis of tetra-sulfonated-peptide 10. C18 Nucleosil column, 1.0 mL/min, $\lambda = 215$ nm, 0-100% B in 30 min, 30 °C.

Fig. S44. MALDI-TOF spectrum for 10. CHCA matrix (negative mode). [M - H]$^-$ monoisotopic calcd. 1090.19, obs. 1089.3.
Synthesis of the conjugates using thiocarbamate ligation

General protocol
The general protocol is illustrated with the synthesis of conjugate 32.
Peptide 24 (8.9 mg, 4.3 µmol) and di-sulfonated-peptide thioester 4 (4.0 mg, 4.3 µmol) were dissolved in sodium phosphate buffer (0.2 M, pH 7.5, 432 µL, final peptide concentration: 10 mM). The solution was deaerated and stirred under a N2 atmosphere for 47 h. The reaction mixture was then diluted with deionized water and purified by RP-HPLC (0-30% B in 10 min, then 30-60% B in 60 min, C18 Nucleosil column, detection at 215 nm, 6.0 mL/min) to give 5 mg of conjugate 32 (yield 44%).

Conjugate 30
Reaction of peptide 22 (17.6 mg) with di-sulfonated-peptide thioester 4 (7.4 mg) yielded 15 mg (yield 61%) of conjugate 30 (RP-HPLC 0-10% B in 5 min, then 10-40% B in 60 min, C18 Nucleosil, detection at 215 nm, 6.0 mL/min).

Fig. S45. RP-HPLC analysis of peptide 30. C18 Nucleosil column, 1.0 mL/min, λ = 215 nm, 0-100% B in 30 min, 30 °C.
Fig. S46. MALDI-TOF spectrum for 30. CHCA matrix (negative mode). Monoisotopic mass [M - H]⁻ 2562.09, obs 2562.1.

Fig. S47. CZE analysis of conjugate 30 using sodium phosphate buffer (10 mM, pH 7.0), 10 kV, capillary length 50 cm, Ø 75 μm.
Fig. S48. CZE analysis of conjugate 30 using sodium borate buffer (20 mM, pH 9.5), 10 kV, capillary length 50 cm, Ø 75 µm.

Conjugate 31

Reaction of peptide 23 (11.5 mg) with di-sulfonated-peptide thioester 4 (4.9 mg) yielded 12 mg (yield 76%) of conjugate 31 (RP-HPLC 0-10% B in 5 min, then 10-30% B in 60 min, C18 Nucleosil, detection at 215 nm, 6.0 mL/min).

RP-HPLC purity (0-100% de B en 30 min, 30 °C, C18 Nucléosil): 99%.

CZE purity, phosphate buffer (10 min, 10 kV): 99%; borate buffer (10 min, 15 kV): 99%.
Fig. S49. RP-HPLC analysis of peptide 31. C₁₈ Nucleosil column, 1.0 mL/min, λ = 215 nm, 0-100% B in 30 min, 30 °C.

Fig. S50. MALDI-TOF spectrum for 31. CHCA matrix, monoisotopic mass [M - H]⁺ 2562.09, obs 2562.1.
Fig. S51. CZE analysis of conjugate 31 using sodium phosphate buffer (10 mM, pH 7.0), 10 kV, capillary length 50 cm, Ø 75 µm.
Fig. S52. CZE analysis of conjugate 31 using sodium borate buffer (20 mM, pH 9.5), 10 kV, capillary length 50 cm, Ø 75 µm.

Conjugate 32
Reaction of peptide 24 (8.9 mg) with di-sulfonated-peptide thioester 4 (4.0 mg) yielded 5 mg (yield 44%) of conjugate 32 (RP-HPLC 0-30% B in 10 min, then 30-60% B in 60 min, C18 Nucleosil, detection at 215 nm, 6.0 mL/min).
Fig. S53. RP-HPLC analysis of peptide 32. C$_{18}$ Nucleosil column, 1.0 mL/min, $\lambda = 215$ nm, 0-100% B in 30 min, 30 $^\circ$C.

| RT   | Area  | % Area | Height |
|------|-------|--------|--------|
| 1    | 17.862| 12356036 | 100.00 | 1067596 |

Fig. S54. MALDI-TOF spectrum for 32. CHCA matrix. Monoisotopic mass [M - H]$^-$ 2535.12, obs. 2535.3 and 2557.3 [M - 2H + Na]$^-$. 
Fig. S55. CZE analysis of conjugate 32 using sodium phosphate buffer (10 mM, pH 7.0), 10 kV, capillary length 50 cm, ∅ 75 µm.

| Time | Area  | Area % | Corrected Area | Corrected Area Percent |
|------|-------|--------|----------------|------------------------|
| 6.658| 21773 | 98.83  |               |                        |
| 7.346| 2378  | 1.17   |               |                        |
| Totals| 220351| 100.00 |               |                        |
Fig. S56. CZE analysis of conjugate 32 using sodium borate buffer (20 mM, pH 9.5), 10 kV, capillary length 50 cm, \( \varnothing \) 75 \( \mu \)m.

### Conjugate 33

Reaction of peptide 25 (9.8 mg) with di-sulfonated-peptide thioester 4 (4.5 mg) yielded 6.0 mg (yield 47\%) of conjugate 33 (RP-HPLC 0-20\% B in 10 min, then 20-60\% B in 60 min, C\(_{18}\) Nucleosil, detection at 215 nm, 6.0 mL/min).

![Chemical structure of conjugate 33](image)
Fig. S57. RP-HPLC analysis of peptide 33. C18 Nucleosil column, 1.0 mL/min, λ = 215 nm, 0-100% B in 30 min, 30 °C.

Fig. S58. MALDI-TOF spectrum for 33. CHCA matrix. Monoisotopic mass [M - H]+ 2535.12, obs 2535.2.
Fig. S59. CZE analysis of conjugate **33** using sodium phosphate buffer (10 mM, pH 7.0), 10 kV, capillary length 50 cm, Ø 75 µm.

| Time  | Area  | Area % | Corrected Area | Corrected Area Percent |
|-------|-------|--------|----------------|------------------------|
| 6.088 | 16158 | 100.00 |               |                        |

**Totals** | 16158 | 100.00 |
Fig. S60. CZE analysis of conjugate 33 using sodium borate buffer (20 mM, pH 9.5), 10 kV, capillary length 50 cm, Ø 75 µm.

| Time | Area | Area % | Corrected Area | Corrected Area Percent |
|------|------|--------|----------------|------------------------|
| 1.133 | 640735 | 100.00 |                |                        |

Totals | 640735 | 100.00 |

Conjugate 34
Reaction of peptide 26 (8.2 mg) with di-sulfonated-peptide thioester 4 (4.7 mg) yielded 7.0 mg (yield 59%) of conjugate 34 (RP-HPLC 0-30% B in 10 min, then 30-60% B in 60 min, C18 Nucleosil, detection at 215 nm, 6.0 mL/min).
Fig. S61. RP-HPLC analysis of peptide 34. C18 Nucleosil column, 1.0 mL/min, λ = 215 nm, 0-100% B in 30 min, 30 °C.

Fig. S62. MALDI-TOF spectrum for 34. CHCA matrix. Monoisotopic mass [M - H]⁺ 2339.08, obs. 2338.8 and 2360.8 [M - 2H + Na]⁺
Fig. S63. CZE analysis of conjugate 34 using sodium phosphate buffer (10 mM, pH 7.0), 10 kV, capillary length 50 cm, Ø 75 µm.

| Time (min) | Area  | Area % | Corrected Area | Corrected Area Percent |
|-----------|-------|--------|----------------|-----------------------|
| 5.513     | 2561041 | 97.52  |                |                       |
| 5.679     | 65127  | 2.48   |                |                       |
| Total     | 2626168 | 100.00 |                |                       |
Fig. S64. CZE analysis of conjugate 34 using sodium borate buffer (20 mM, pH 9.5), 10 kV, capillary length 50 cm, \( \Phi \) 75 \( \mu \)m.

Conjugate 35
Reaction of peptide 27 (7.4 mg) with di-sulfonated-peptide thioester 4 (4.3 mg) yielded 5.0 mg (yield 44\%) of conjugate 35 (RP-HPLC 0-20\% B in 10 min, then 20-60\% B in 60 min, C\textsubscript{18} Nucleosil, detection at 215 nm, 6.0 mL/min).
Fig. S65. RP-HPLC analysis of peptide 35. C18 Nucleosil column, 1.0 mL/min, $\lambda = 215$ nm, 0-100% B in 30 min, 30 °C.

Fig. S66. MALDI-TOF spectrum for 35. CHCA matrix. Monoisotopic mass $[M - H]^{-}$ 2339.08, obs 2338.8.
Fig. S67. CZE analysis of conjugate 35 using sodium phosphate buffer (10 mM, pH 7.0), 10 kV, capillary length 50 cm, Ø 75 µm.

| Time | Area  | Areas % | Corrected Area | Corrected Area Percent |
|------|-------|---------|----------------|------------------------|
| 6.790| 36207 | 100.00  |                |                        |

| Totals | 36207 | 100.00 |                |

**UV - 214nm**

**Results**
Fig. S68. CZE analysis of conjugate 35 using sodium borate buffer (20 mM, pH 9.5), 10 kV, capillary length 50 cm, Ø 75 µm.

**Conjugate 36**

Reaction of peptide 28 (11.0 mg) with di-sulfonated-peptide thioester 4 (4.8 mg) yielded 6.0 mg (yield 40%) of conjugate 36 (RP-HPLC 0-20% B in 10 min, then 20-40% B in 40 min, C₁₈ Nucleosil, detection at 215 nm, 6.0 mL/min).
Fig. S69. RP-HPLC analysis of peptide 36. C18 Nucleosil column, 1.0 mL/min, \( \lambda = 215 \) nm, 0-100% B in 30 min, 30 °C.

| RT  | Area   | % Area | Height |
|-----|--------|--------|--------|
| 1   | 15.120 | 45193  | 1.74   | 5534          |
| 2   | 16.325 | 2486943| 95.99  | 350752        |
| 3   | 17.083 | 58809  | 2.27   | 7839          |

Fig. S70. MALDI-TOF spectrum for 36. CHCA matrix. Average mass [M - H] calcd. 2587.1, obs. 2586.5.
Fig. S71. CZE analysis of conjugate 36 using sodium phosphate buffer (10 mM, pH 7.0), 10 kV, capillary length 50 cm, ∅ 75 µm.
Fig. S72. CZE analysis of conjugate 36 using sodium borate buffer (20 mM, pH 9.5), 10 kV, capillary length 50 cm, Ø 75 µm.

Conjugate 37

Reaction of peptide 29 (12.4 mg) with di-sulfonated-peptide thioester 4 (4.9 mg) yielded 4.0 mg (yield 25%) of conjugate 37 (RP-HPLC 0-20% B in 10 min, then 20-40% B in 40 min, C18 Nucleosil, detection at 215 nm, 6.0 mL/min).
Fig. S73. RP-HPLC analysis of peptide 37. C18 Nucleosil column, 1.0 mL/min, $\lambda = 215$ nm, 0-100% B in 30 min, 30 °C.

| Fit  | Area   | % Area | Height |
|------|--------|--------|--------|
| 1    | 15.518 | 5654259| 100.00 | 790241 |

Fig. S74. MALDI-TOF spectrum for 37. CHCA matrix. Average mass [M - H] calcd. 2743.2, obs. 2743.4, 2765.5 [M - 2H + Na] and 2787.4 [M - 3H + 2Na].
Fig. S75. CZE analysis of conjugate 37 using sodium citrate buffer (20 mM, pH 3.0), 15 kV, capillary length 50 cm, \( \varnothing \) 75 \( \mu \)m.

### Table

| Time | Area  | Area % | Corrected Area | Corrected Area Percent |
|------|-------|--------|----------------|------------------------|
| 3.046| 5849  | 0.25   |                |                        |
| 4.787| 234983| 99.75  |                |                        |
| Totals| 2355702| 100.00 |                |                        |

Conjugate 38

Reaction of peptide 22 (11.1 mg) with peptide thioester 3 (3.7 mg) yielded 3.4 mg (yield 25%) of conjugate 38 (RP-HPLC 0-20% B in 10 min, then 20-40% B in 40 min, C\(_{18}\) Nucleosil, detection at 215 nm, 6.0 mL/min).

RP-HPLC purity (0-100\% de B in 30 min, 30 °C, C\(_{18}\) Nucleosil): 99%

CZE purity, sodium acetate buffer (50 mM, pH 4.5) (10 min, 10 kV): 99%; sodium citrate buffer (20 mM, pH 3.0) (15 min, 15 kV): 99%.
Fig. S76. RP-HPLC analysis of peptide 38. C_{18} Nucleosil column, 1.0 mL/min, $\lambda = 215$ nm, 0-100% B in 30 min, 30 °C.

Fig. S77. MALDI-TOF spectrum for 38. CHCA matrix. Monoisotopic mass [M + H]$^+$ calcd. 2346.1, obs. 2346.2.
Fig. S78. CZE analysis of conjugate 38 using sodium citrate buffer (20 mM, pH 3.0), 15 kV, capillary length 50 cm, ∅ 75 µm.
Fig. S79. CZE analysis of conjugate 38 using sodium acetate buffer (50 mM, pH 4.5), 10 kV, capillary length 50 cm, Ø 75 µm.

**Conjugate 39**

Reaction of peptide 22 (11.2 mg) with di-sulfonated-peptide thioester 5 (4.3 mg) yielded 9.0 mg (yield 60%) of conjugate 39 (RP-HPLC 0-10% B in 5 min, then 10-40% B in 100 min, C\textsubscript{18} Nucleosil, detection at 215 nm, 6.0 mL/min).

RP-HPLC purity (0-100% de B in 30 min, 30 °C, C\textsubscript{18} Nucleosil): 99%.

CZE purity, sodium borate buffer (20 mM, pH 9.5) (10 min, 15 kV): 99%; sodium phosphate buffer (10 mM, pH 7.0) (15 min, 10 kV): 99%.
Fig. S80. RP-HPLC analysis of peptide 39. C_{18} Nucleosil column, 1.0 mL/min, λ = 215 nm, 0-100% B in 30 min, 30 °C.
Fig. S81. MALDI-TOF spectrum for 39. CHCA matrix. Monoisotopic mass calcd [M - H]$^-$ = 2470.06, obs. 2470.2.

Fig. S82. CZE analysis of conjugate 39, pH 7, 10 mM sodium phosphate buffer, 10 kV, capillary length 50 cm, Ø 75 µm.
Fig. S83. CZE analysis of conjugate 39 using sodium borate buffer (20 mM, pH 9.5), 10 kV, capillary length 50 cm, ∅ 75 µm.

Conjugate 40
Reaction of peptide 22 (9.9 mg) with tetra-sulfonated-peptide thioester 8 (6.7 mg) yielded 9.0 mg (yield 56%) of conjugate 40 (RP-HPLC 0-10% B in 5 min, then 10-40% B in 60 min, C18 Nucleosil, detection at 215 nm, 6.0 mL/min).

| Time | Area  | Area % | Corrected Area |
|------|-------|--------|----------------|
| 1.454| 475983| 99.22% |                |
| 1.825| 3758  | 0.78%  |                |
| Totals| 479741| 100.00%|                |

UV - 214nm
Results

**Ac-OHKTSTGTHLEPPG-NH₂**

**S71**
Fig. S84. RP-HPLC analysis of peptide 40. C18 Nucleosil column, 1.0 mL/min, λ = 215 nm, 0-100% B in 30 min, 30 °C.

Fig. S85. MALDI-TOF spectrum for 40. CHCA matrix. Average mass calcd [M - H]- 3122.4, obs. 3121.1, 3143.1 [M - 2H + Na]-, 3165.1 [M - 3H + 2Na]- and 3186.9 [M - 4H + 3Na]-.
Fig. S86. CZE analysis of conjugate 40 using sodium citrate buffer (20 mM, pH 3.0), 15 kV, capillary length 50 cm, Ø 75 µm.
Fig. S87. CZE analysis of conjugate 40 using sodium borate buffer (20 mM, pH 9.5), 10 kV, capillary length 50 cm, Ø 75 µm.

Conjugate 41
Reaction of peptide 22 (9.7 mg) with tetra-sulfonated-peptide thioester 10 (4.8 mg) yielded 9.0 mg (yield 64%) of conjugate 41 (RP-HPLC 0-10% B in 5 min, then 10-40% B in 60 min, C18 Nucleosil, detection at 215 nm, 6.0 mL/min).
Fig. S88. RP-HPLC analysis of peptide 41. C_{18} Nucleosil column, 1.0 mL/min, \( \lambda = 215 \) nm, 0-100% B in 30 min, 30 °C.

Fig. S89. MALDI-TOF spectrum for 41. CHCA matrix. Average mass calcd [M - H] calcd. 2723.9, obs. 2723.6.
Fig. S90. CZE analysis of conjugate 41 using sodium borate buffer (20 mM, pH 9.5), 10 kV, capillary length 50 cm, Ø 75 µm.

**Microarray experiments**

Phosphate buffered saline (PBS): prepare 0.01M solution (0.138 M NaCl, 0.0027 M KCl, pH 7.4) by dissolving 1 foil pouch (ref. P3813, Sigma-Aldrich) in 1 L of deionized water.

Semicarbazide microscope glass slides and peptide microarrays were prepared as described in detail elsewhere. Peptide microarrays were produced by printing the different probes (0.1 mM in PBS, triplicate) with a pin microarrayer (the diameter of the spots was ~ 200 µm). 24 microarrays were printed on each microscope glass slide.

All the incubations were performed in triplicate. For this, the microscope glass slides were inserted in a 96-well device (microplate microarray hardware 96 wells ref. MMH4 × 24, Arrayit Corp. CA, USA) equipped with a silicone gasket (ref. GMMH4X24, Arrayit Corp. CA, USA). The microarrays were blocked with 0.1 % bovine serum albumin (BSA) in PBS (5 min at rt) and then washed three times with Tween20® 0.05% in PBS.
The different proteins (human HGF receptor, C-Met/Fc chimera, Sigma ref H 0536; human VEGF R2 receptor, KDR/Fc chimera, R&D Systems ref 357-KD/CF; human EGF receptor, HER-1/Fc chimera, R&D Systems ref 344-ER) were dissolved in BSA 0.1% Tween20® 0.05% in PBS at the appropriate concentration (see figures). 50 µl were added to the well which were incubated 2 h at rt. The wells were then washed three times with Tween20® 0.05% in PBS.

Detection of the captured receptors was performed using an anti-human Fc tetramethylrhodamine-labelled antibody (diluted 1/500 in BSA 0.1% Tween20® 0.05% in PBS, 50 µL/well, 2 h at rt). Finally the wells were washed 6 times with Tween20® 0.05% in PBS, twice with deionized water and dried.

The microarrays were analyzed using a fluorescence microarray laser scanner (LS Reloaded, Tecan Group Ltd., Switzerland). 16 bits images were quantified using dedicated image analysis software (Array-Pro® Analyzer, Switzerland). The data correspond to the median (interquartile range) of the spot fluorescence intensities after background subtraction.

To verify that conjugation does not modify the adsorption properties of the compounds to a significant extent we have prepared a series of short peptides and conjugates derived from peptide 22 with a biotin modification (Fig. S91). The peptides were microarrayed as described above and detected using cyanine 3-labelled streptavidin (1 mg/mL diluted 1/500 in PBS with 0.1% BSA, 2 h at rt, 50 µL per well). The signal detected at 532 nm was similar for all biotinylated peptides and conjugates present on the microarray, whereas the different non biotinylated controls displayed background intensities.
Fig. S91. Influence of the conjugation on the adsorption properties of the compounds.

**Alphascreen® HGF/SF-MET binding assay**

Competition assays for binding of HGF to recombinant MET-Fc protein were performed in 384-well microtiter plates (OptiPlate™-384, PerkinElmer, CA, USA, 50 µL of final reaction volume). Final concentrations were: 0-100 µM for peptides 6, 22 or 30, 2.5 nM for hMET-Fc (recombinant extracellular MET domain fused with human IgG1-Fc, R&D System, MN, USA), 2.5 nM for pro-HGF (Peprotech, NJ, USA), 10 µg/mL for streptavidin coated Donor beads and protein A-conjugated Acceptor beads, 10 nM for biotinylated anti-HGF/SF antibodies (R&D Systems).

The buffer used for preparing all the peptide or protein solutions and the bead suspensions was: PBS, 5 mM HEPES pH 7.4, 0.1% BSA.
The peptide solution (5 µL, 0-1000 µM) was mixed with solutions of hMET-Fc (5 µL, 25 nM) and pro-HGF (5 µL, 25 nM). The mixture was incubated for 10 min (final volume 15 µL). Then, protein A-conjugated acceptor beads (10 µL, 50 µg/mL) were added to the vials. The plate was incubated at 23°C for 30 min in a dark box. Next, biotinylated anti-HGF/SF antibody was added to the vials (15 µL, 8.3 nM). Finally, streptavidin coated donor beads (10 µL, 50 µg/mL) were added and the plate was further incubated at 23°C for 30 min in a dark box. The emitted signal intensity was measured using an EnSpire® Multimode Plate Reader (PerkinElmer).

Because the peptides may scavenge singlet oxygen at high concentrations, typically > 10 µM, and thus artificially decrease the emitted signal intensity, the peptides were also incubated with biotinylated rabbit IgG (10 µM final concentration), streptavidin donor and protein A acceptor beads (10 µg/mL final concentration) for 30 min to determine the signal loss due to singlet oxygen quenching. The resulting data were used to correct the signal intensities collected in the first Alphascreen® experiment.

The results are presented as percentage of the signal obtained in the absence of peptide.

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