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Supporting Information

Utilizing Reversible Copper(II) Peptide Coordination in a Sequence Selective Luminescent Receptor

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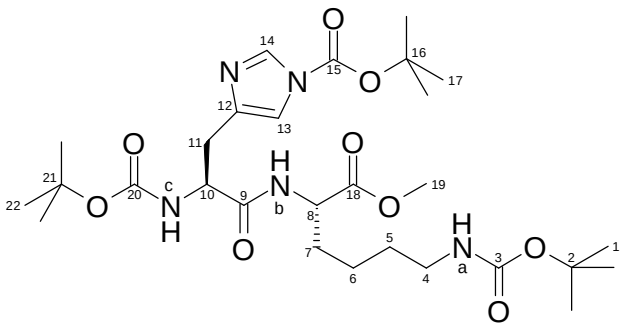
Supporting Information

1. Syntheses

1.1 Synthesis of small peptides in solution

H-His-OMe[1] and H-Lys(Boc)-OMe[2] were synthesised in solution according to literature known procedures.



Boc-His(Boc)-Lys(Boc)-OMe

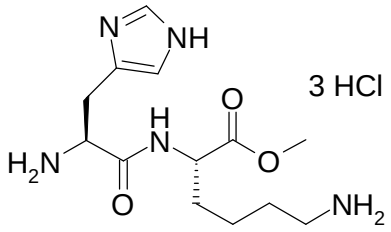
4-[(S)-2-tert-Butoxycarbonylamino-2-((S)-5-tert-butoxycarbonylamino-1-methoxy-carbonyl-pentylcarbamoyl)-ethyl]-imidazole-1-carboxylic acid tert-butyl ester: Under N_2-atmosphere Boc-His(Boc)-OH DCH salt (567 mg, 1.06 mmol), DIPEA (0.58 mL, 3.36 mmol), EDC (0.20 mL, 1.16 mmol), and HOBt (157 mg, 1.16 mmol) were dissolved in DMF (6 mL) under ice cooling. H-Lys(Boc)-OMe (250 mg, 0.96 mmol) dissolved in a little DMF was added slowly. The reaction was allowed to warm to room temp. and was stirred over night (20 h) at 40°C. The reaction progress was monitored by TLC (ethyl acetate). After completion of the reaction, water (25 mL) was added and the mixture was extracted with ethyl acetate. The organic layer was dried over $MgSO_4$, the solvent was evaporated and the crude product was purified by flash column chromatography on silica gel (ethyl acetate / petrol ether 3:1, R_f (ethyl acetate) = 0.75) yielding Boc-His(Boc)-Lys(Boc)-OMe (59 mg, 10 %) as a colourless solid.

^{1}H-NMR (600 MHz; $CDCl_3$): δ = 1.17 (bs, 2 H, HSQC, COSY: C^6H_2), 1.42 (s, 11 H, HSQC: C^5H_2, Boc-CH_3), 1.43 (s, 9 H, HSQC: Boc-CH_3), 1.58 (s, 10 H, HSQC: Boc-CH_3, C^7H_2), 1.68-1.80 (m, 1 H, HSQC: C^7H_2), 2.94 (dd, ^3J = 5.7 Hz, ^1J = 14.8 Hz, 1 H, HSQC, COSY: $C^{11}H_2$), 3.00-3.13 (m, 3 H, HSQC, COSY: $C^{11}H_2$ diastereotope, C^4H_2), 3.68 (s, 3 H, HSQC, COSY: $C^{19}H_3$), 4.43 (bs, 1 H, COSY: $C^{10}H$), 4.49-4.56 (m, 1 H, COSY: C^8H), 4.72 (bs, 1 H,

[1] Z. Hongjun; H. Bangyou; S. Guangliang, Patent No. CN 1557834, 29 Dec **2004**

[2] C. Mandl, *Dissertation*, University of Regensburg **2004**

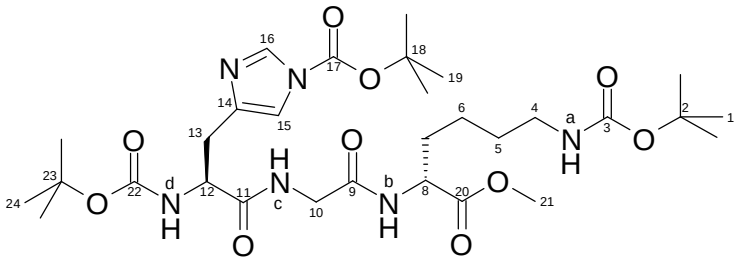
COSY: NH^a), 6.13 (bs, 1 H, COSY: NH^c), 7.16 (s, 1 H, COSY: $C^{13}H$), 7.23 (bs, 1 H, COSY: NH^b), 8.01 (s, 1 H, COSY: $C^{14}H$). – **^{13}C-NMR** (150 MHz; $CDCl_3$): δ = 22.2 (-, 1 C, HSQC: C^6), 27.8 (+, 3 C, HSQC: Boc-CH_3), 28.3 (+, 3 C, HSQC: Boc-CH_3), 28.4 (+, 3 C, HSQC: Boc-CH_3), 29.6 (–, 1 C, HSQC: C^5), 30.1 (–, 1 C, COSY: C^{11}), 32.1 (–, 1 C, COSY: C^7), 40.1 (–, 1 C, HSQC: C^4), 51.8 (+, 1 C, COSY: C^8),52.2 (+, 1 C, COSY: C^{19}), 54.2 (+, 1 C, COSY: C^{10}), 79.0 (C_{quat}, 1 C, COO^t*Bu*), 80.0 (C_{quat}, 1 C, COO^t*Bu*), 85.7 (C_{quat}, 1 C, COO^t*Bu*), 114.8 (+, 1 C, COSY: C^{13}), 136.8 (+, 1 C, COSY: C^{14}), 139.2 (C_{quat}, 1 C, HMBC: C^{12}), 146.9 (C_{quat}, 1 C, HMBC: C^9), 155.6 (C_{quat}, 1 C, HMBC: C^{15}), 155.9 (C_{quat}, 1 C, HMBC: C^3), 171.3 (C_{quat}, 1 C, HMBC: C^{20}), 172.3 (C_{quat}, 1 C, HMBC: C^{18}). – **MS** (ESI(+), DCM/MeOH + 10 mmol NH_4Ac): m/z (%) = 598.5 (100) [MH^+], 498.5 (19) [MH^+ - Boc].



H-His-Lys-OMe

***(S)-6-Amino-2-[(S)-2-amino-3-(1H-imidazol-4-yl)-propionylamino]-hexanoic acid methyl ester*:** The protected dipeptide Boc-His(Boc)-Lys(Boc)-OMe (55 mg, 0.09 mmol) was dissolved in DCM under ice cooling and mixed with HCl/ether. The reaction was allowed to warm to room temp. and was stirred for additional 3 hours. The reaction progress was monitored by TLC (ethyl acetate), and after completion the reaction mixture was evaporated to dryness and the residue was redissolved in water and lyophilised yielding H-His-OMe·3 HCl (36 mg, quantitative), as a colourless hygroscopic solid.

^{1}H-NMR (300 MHz; D_2O): δ = 1.39-1.53 (m, 2 H, Lys-CH_2), 1.61-2.05 (m, 4 H, Lys-CH_2), 2.93 (t, ^3J = 7.7 Hz, 2 H, Lys-CH_2), 3.34-3.50 (m, 2 H, His-CH_2), 3.79 (s, 3 H, OMe), 4.30 (dd, ^3J = 6.6 Hz, 7.7 Hz, 1 H, Lys-α-CH), 4.43 (dd, ^3J = 5.4 Hz, 8.5 Hz,1 H, His-α-CH), 7.50 (s, 1 H, imidazole-CH), 8.78 (s, 1 H, imidazole-CH). – **^{13}C-NMR** (75 MHz; D_2O): δ = 22.0 (–, 1 C, CH_2), 26.1 (–, 1 C, CH_2), 26.3 (–, 1 C, CH_2), 30.1 (–, 1 C, CH_2), 39.2 (–, 1 C, CH_2), 52.1 (–, 1 C, CH_2), 52.9 (+, 1 C, CH), 53.1 (+, 1 C, CH), 118.8 (+, 1 C, CH), 125.7 (C_{quat}, 1 C), 134.4 (+, 1 C, CH), 168.1 (C_{quat}, 1 C, His C=O), 173.6 (C_{quat}, 1 C, COOMe). – **MS** (ESI(+), H_2O/MeCN/TFA): m/z (%) = 298.2 (70) [MH^+], 190.6 (59) [M + 2 H^+ + 2 MeCN]$^{2+}$, 170.0 (100) [M + 2 H^+ + MeCN]$^{2+}$, 149.7 (13) [M + 2 H^+]$^{2+}$.

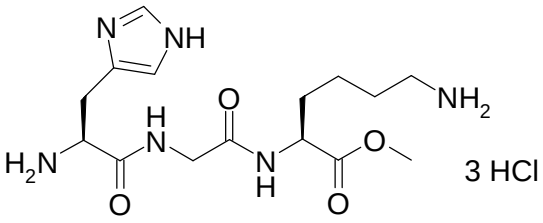


Boc-His(Boc)-Gly-Lys(Boc)-OMe

***4-((S)-2-tert-Butoxycarbonylamino-2-{[((R)-5-tert-butoxycarbonylamino-1-methoxycarbonyl-pentylcarbamoyl)-methyl]-carbamoyl}-ethyl)-imidazole-1-carboxylic acid tert-butyl ester*:** Under N_2-atmosphere Boc-His(Boc)-OH DCH salt (276 mg, 0.51 mmol), DIPEA (0.70 mL, 4.07 mmol), HBTU (215 mg, 0.57 mmol), and HOBt (76 mg, 0.57 mmol) were dissolved in DCM (10 mL) under ice cooling. H-Gly-Lys(Boc)-OMe (136 mg, 0.43 mmol) dissolved in a little DCM was added slowly. The reaction was allowed to warm to room temp. and was stirred for additional 20 h at room temperature. The reaction progress was monitored by TLC (ethyl acetate). After completion water (20 mL) was added, the organic layer was extracted with sat. $NaHCO_3$ (3x) and dried over $MgSO_4$. The solvent was evaporated and the crude product was purified by flash column chromatography on silica gel (dichloromethane/MeOH 97:3, R_f = 0.1) yielding Boc-His(Boc)-Gly-Lys(Boc)-OMe (55 mg, 19 %) as a colourless solid.

^{1}H-NMR (400 MHz; $CDCl_3$): δ = 1.33 (dd, ^2J = 7.3 Hz, ^3J = 14.7 Hz, 2 H, HMBC: C^7H_2), 1.41 (s, 20 H, Lys-Boc-CH_3, His-Boc-CH_3, HMBC: C^5H_2), 1.59 (s, 10 H, His-Boc-CH_3, HMBC: C^6H_2), 1.76-1.86 (m, 1 H, HMBC: C^6H_2), 2.92-3.09 (m, 3 H, HMBC: C^4H_2, $C^{13}H_2$), 3.19 (dd, ^2J = 4.2 Hz, ^3J = 14.8 Hz, 1 H, HMBC: $C^{13}H_2$), 3.70 (s, 3 H, HMBC: $C^{21}H_3$), 3.95 (bs, 2 H, COSY: $C^{10}H_2$), 4.43 (bs, 1 H, HMBC: $C^{12}H$), 4.60 (bs, 1 H, HMBC: C^8H), 4.97 (bs, 1 H, COSY: NH^a), 5.76 (bs, 1 H, COSY: NH^d), 7.18 (s, 1 H, HMBC: $C^{15}H$), 7.29 (bs, 1 H, COSY: NH^c), 7.75 (bs, 1 H, COSY: NH^b), 8.04 (s, 1 H, HMBC: $C^{16}H$). – **^{13}C-NMR** (100 MHz; $CDCl_3$): δ = 22.5 (–, 1 C, HSQC: C^7), 27.8 (+, 3 C, C^{Boc}), 28.3 (+, 3 C, C^{Boc}), 28.4 (+, 3 C, C^{Boc}), 29.2 (–, 1 C, HSQC: C^5), 29.8 (–, 1 C, HMBC: C^{13}), 31.2 (–, 1 C, HSQC: C^6), 39.9 (–, 1 C, HMBC: C^4), 43.4 (–, 1 C, HMBC: C^{10}), 51.8 (+, 1 C, HMBC: C^8), 52.2 (+, 1 C, HSQC: C^{21}), 52.9 (+, 1 C, HMBC: C^{12}), 78.9 (C_{quat}, 1 C, HMBC: C^{23}), 80.3 (C_{quat}, 1 C, HMBC: C^2), 85.8 (C_{quat}, 1 C, HMBC: C^{18}), 115.2 (+, 1 C, HMBC: C^{15}), 137.4 (+, 1 C, HMBC: C^{16}), 138.3 (C_{quat}, 1 C, HMBC: C^{14}), 146.7 (C_{quat}, 1 C, HMBC: C^3), 155.5 (C_{quat}, 1 C, HMBC: C^{17}), 156.1 (C_{quat}, 1 C, HMBC: C^{11}), 156.8 (C_{quat}, 1 C, HMBC: C^9), 171.9 (C_{quat}, 1 C,

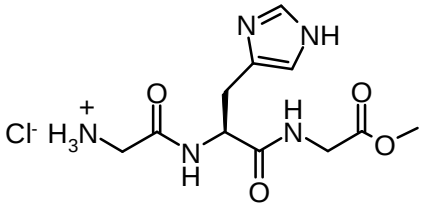
HMBC: C^{22}), 172.8 (C_{quat}, 1 C, HMBC: C^{20}). – **MS** (ESI(+), DCM/MeOH + 10 mmol NH_4Ac): m/z (%) = 655.5 (100) [MH^+].



H-His-Gly-Lys-OMe

***(S)-6-Amino-2-{2-[(S)-2-amino-3-(1H-imidazol-4-yl)-propionylamino]-acetylamino}-hexanoic acid methyl ester hydrochloride*:** The protected tripeptide Boc-His(Boc)-Gly-Lys(Boc)-OMe (55 mg, 0.08 mmol) was dissolved in DCM under ice cooling and mixed with HCl/ether. The reaction was allowed to warm to room temp. and was stirred for additional 3 hours. The reaction progress was monitored by TLC (EE). The reaction mixture was evaporated to dryness and the residue was redissolved in water and lyophilised. H-His-Gly-Lys-OMe·3 HCl (37 mg, quantitative) was obtained as a colourless hygroscopic solid.

^{1}H-NMR (300 MHz; D_2O): δ = 1.31-1.53 (m, 2 H, Lys-CH_2), 1.57-2.00 (m, 4 H, Lys-CH_2), 2.95 (t, ^3J = 7.7 Hz, 2 H, Lys-CH_2), 3.40 (d, ^3J = 6.6 Hz, 2 H, His-CH_2), 3.71 (s, 3 H, OMe), 4.00 (dd, ^2J = 29.9 Hz, ^3J = 17.0 Hz, 2 H, Gly-CH_2), 4.35 (t, ^3J = 6.4 Hz, 1 H, Lys-α-CH), 4.40 (dd, ^2J = 8.8 Hz, ^3J = 5.2 Hz, 1 H, His-α-CH), 7.43 (s, 1 H, imidazole-CH), 8.67 (s, 1 H, imidazole-CH). – **^{13}C-NMR** (75 MHz; D_2O): δ = 22.1 (–, 1 C, CH_2), 26.1 (–, 1 C, CH_2), 26.3 (–, 1 C, CH_2), 30.0 (–, 1 C, CH_2), 39.3 (–, 1 C, CH_2), 42.1 (–, 1 C, Gly-CH_2), 52.1 (+, 1 C, CH, Gly α-CH), 52.9 (+, 1 C, CH), 53.1 (+, 1 C, CH), 118.8 (+, 1 C, CH), 125.6 (C_{quat}, 1 C), 134.6 (+, 1 C), 168.7 (C_{quat}, 1 C, Gly C=O), 171.0 (C_{quat}, 1 C, His C=O), 174.2 (C_{quat}, 1 C, Lys C=O). – **MS** (ESI(+), H_2O/MeCN/TFA): m/z (%) = 177.9 (100) [M + 2 H^+]$^{2+}$, 198.4 (75) [M + 2 H^+ + MeCN]$^{2+}$, 355.0 (55) [MH^+].

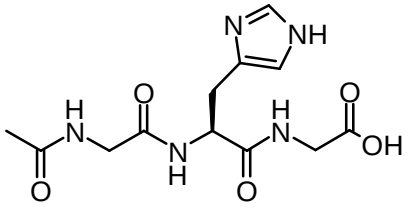


H-Gly-His-Gly-OMe

***[(S)-2-(2-Amino-acetylamino)-3-(1H-imidazol-4-yl)-propionylamino]-acetic acid methyl ester hydrochloride*:** H-Gly-His-Gly-OH (10.2 mg, 0.06 mmol) was suspended in 2 mL methanol and $SOCl_2$ (20 µL, 0.28 mmol) was added to the stirred solution. The reaction mixture was stirred over night, methanol was removed in vacuo and the reaction process was monitored by NMR. The crude product was dried in high vacuum obtaining H-Gly-His-Gly-OMe hydrochloride as white crystalline solid (19 mg, quantitative).

^{1}H-NMR (300 MHz; CD_3OD): δ = 3.17 (dd, ^3J = 7.0 Hz, ^2J =15.5 Hz, 1 H, CH), 3.30 (dd, ^3J = 7.0 Hz, ^2J = 15.5 Hz, 1 H, CH and solvent peak), 3.73 (s, 3 H, OCH_3), 3.76 (s, 2 H, CH_2), 3.97 (dd, ^{3}J = 17.6 Hz, 2 H, CH_2), 7.42 (s, 1 H, CH), 8.80 (s, 1 H, CH).

^{13}C-NMR (75 MHz; CD_3OD): δ = 28.3 (–, 1 C, CH_2), 41.7 (–, 1 C, CH_2), 41.9 (–, 1 C, CH_2), 52.8 (+, 1 C, OMe), 53.6 (+, 1 C, CH), 118.9 (C_{quat}, 1 C, CH), 130.6 (C_{quat}, 1 C), 135.1 (+, 1 C, CH), 167.5 (C_{quat}, 1 C, CONH), 171.8 (C_{quat}, 1 C, CONH), 172.2 (C_{quat}, 1 C, CONH). – **MS** (CI - MS (NH_3)): m/z (%) = 284.2 [MH^+].



Ac-Gly-His-Gly-OH

***[(S)-2-(2-Acetylamino-acetylamino)-3-(1H-imidazol-4-yl)-propionylamino]-acetic acid*:** H-Gly-His-Gly-OH (10.8 mg, 0.04 mmol) was suspended in 2 mL water and Ac_2O (40 µL, 0.40 mmol) was added to the stirred solution. The reaction mixture was stirred for 4 h at room temp., water and excess of acetic anhydride were removed in vacuo and the reaction process was monitored by NMR. The crude product was dried in high vacuum giving Ac-Gly-His-Gly-OH as a white crystalline solid (12.5 mg, quantitative).

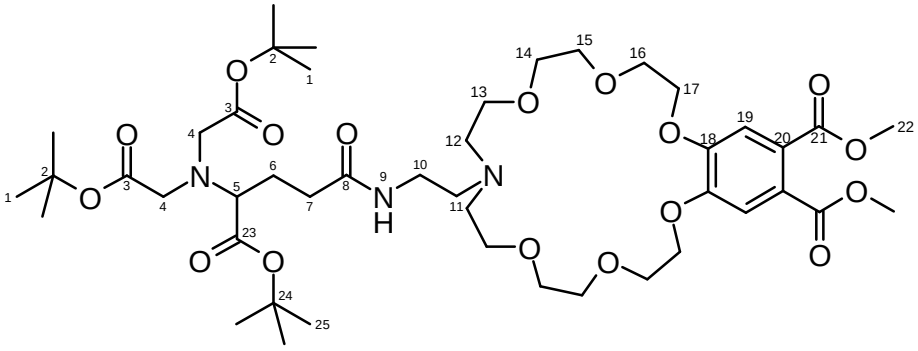
^{1}H-NMR (300 MHz; CD_3OD): δ = 2.00 (s, 3 H, CH_3), 3.19 (dd, ^3J = 5.3 Hz, ^2J = 15.2 Hz, 1 H, CH_2), 3.30 (dd, ^3J = 5.3 Hz, ^2J = 15.2 Hz, 1 H, CH_2 and solvent peak), 3.68 (d, ^{2}J = 17.3 Hz, 1 H, CH_2), 3.83 (d, 2 H, ^{2}J = 3.6 Hz, CH_2), 3.99 (d, ^2J = 17.1 Hz, 2 H, CH_2), 4.72 (t, ^3J = 5.3 Hz, 1 H, CH), 7.26 (s, 1 H, CH), 8.42 (s, 1 H, CH). – **^{13}C-NMR** (75 MHz; CD_3OD): δ 22.4 (+, 1 C, CH_3), 28.9 (-, 1 C, CH_2), 43.8 (-, 2 C, CH_2), 53.3 (+, 1 C, CH), 119.6 (+, 1 C, CH), 130.5 (C_{quat}, 1 C), 135.6 (+, 1 C, CH), 171.5 (C_{quat}, 1 C, CONH), 171.8 (C_{quat}, 1 C, CONH), 174.2 (C_{quat}, 1 C, CONH), 176.5 (C_{quat}, 1 C, CONH). – **MS** (ESI(+), H_2O/MeOH + 10 mmol NH_4Ac): m/z (%) = 312.0 (100) [MH^+], 334.0 (21) [MNa^+].

1.2 Solid phase synthesis of the peptide library for screening

All peptides were synthesized on an Advanced Chemtech 496 MOS synthesizer. Rink Amide MBHA resin and Fmoc protecting group strategy were used for the entire library. Coupling was done by TBTU / HOBt / DIPEA. HOBt was used as a 0.45 M solution, TBTU as a 0.44 M solution and DIEA as a 1.2 M solution, all in DMF. The Fmoc protected amino acids were dissolved in NMP as 0.4 M solutions. The syntheses were carried out in a 96 well reactor block. Every peptide was synthesized on 50 mg of resin. The lot of the resin used had a loading of 0.72 mmol/g (manufacturer's claims). Before each synthesis the resin was allowed to preswell in DMF for 30 min. Each coupling was done twice using a 5 fold excess of HOBt and slightly less than 5 fold excess of TBTU. DIEA was used in 10 fold excess. Fmoc deprotection was done by shaking the resin with 40 % piperidine in DMF for 3 minutes, subsequent washing and addition of 20 % piperidine in DMF followed by shaking for 10 minutes. When the syntheses were complete, the resin was washed with MeOH and DCM (5 x 2 mL each). Where acylation was necessary, it was conducted using 5 eq of Ac_2O, 5 eq of DIPEA in DMF for 30 minutes. Cleavage from the resin was afforded by shaking the resin for 3 h after addition of 1.5 mL of TFA / TIS / H_2O (90:5:5) (v/v). Where the peptides contained cysteine, 2 % EDT were added to the cleavage mixture. After filtering off the resin, the TFA solution was reduced in volume to about 0.5 mL. It was then transferred to a Falcon tube and precipitated with cold Et_2O. The precipitate was centrifuged at –4 °C for 10 minutes. The solution was then carefully decanted off and the precipitate resuspended in cold Et_2O before being centrifuged again. This resuspending/centrifuging step was repeated five times. Finally, the Et_2O was decanted off again and the peptide dried under vacuum. The peptides were analysed by ES-MS, LC-MS and analytical HPLC

Abbreviations:
DCM: dichlormethane; DMF: N,N-dimethylformamide; DIPEA: N,N'-diisopropylethylamine; EDC: 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; HOBt: hydroxylbenzo-triaole; TBTU: O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate; NMP: N-methyl-2-pyrrolidone; TIS: triisopropylsilane; TFA: trifluoro acetic acid; EDT: 1,2-ethane-dithiol.

1.3 Spectroscopic assignment for the *tert*-butyl ester of compound 4



14-{2-[4-(Bis-tert-butoxycarbonylmethyl-amino)-4-tert-butoxycarbonyl-butyryl-amino]-ethyl}-6,7,9,10,13,14,15,16,18,19,21,22-dodecahydro-12H-5,8,11,17,20,23-hexaoxa-14-aza-benzocyclohenicosene-2,3-dicarboxylicacid dimethylester: ^{1}H-NMR (600 MHz; $CDCl_3$): δ = 1.40 (s, 18 H, HSQC: $C^{1}H_3$), 1.41 (s, 9 H, HSQC: $C^{25}H_3$), 1.79-1.88 (m, 1 H, COSY: $C^{6}H_2$), 1.98-2.07 (m, 1 H, COSY: $C^{6}H_2$), 2.33-2.45 (m, 2 H, COSY: $C^{7}H_2$), 2.63 (bs, 2 H, COSY: $C^{11}H_2$), 2.77 (bs, 4 H, $C^{12}H_2$), 3.22-3.32 (m, 3 H, COSY: $C^{5}H$, $C^{10}H_2$), 3.36 (d, ^{2}J = 17.2 Hz, 1 H, $C^{4}H_2$), 3.44 (d, ^{2}J = 17.2 Hz, 1 H, $C^{4}H_2$), 3.57 (bs, 4 H, HMBC: $C^{13}H_2$), 3.61-3.65 (m, 4 H, HMBC: $C^{14}H_2$), 3.72-3.76 (m, 4 H, HMBC: $C^{15}H_2$), 3.85 (s, 6 H, COSY: $C^{22}H_3$), 3.88-3.91 (m, 4 H, HMBC: $C^{16}H_2$), 4.15-4.23 (m, 4 H, HMBC: $C^{17}H_2$), 6.88 (bs, 1 H, HSQC: $N^{9}H$), 7.18 (s, 2 H, HSQC: $C^{19}H$). – ^{13}C-NMR (150 MHz; $CDCl_3$): δ = 28.1 (+, 6 C, HSQC: C^{1}), 28.2 (+, 3 C, HSQC: C^{25}), 26.3 (-, 1 C, HSQC: C^{6}), 32.5 (-, 1 C, HSQC: C^{7}), 37.4 (-, 1 C, HSQC: C^{10}), 52.5 (+, 2 C, HSQC: C^{22}), 54.1 (-, 2 C, HMBC: C^{4}), 54.2 (-, 3 C, COSY: C^{11}, HSQC: C^{12}), 64.8 (+, 1 C, HSQC: C^{5}), 68.8 (-, 2 C, COSY: C^{13}), 69.5 (-, 2 C, HMBC: C^{16}), 69.7 (-, 2 C, HMBC: C^{17}), 70.6 (-, 2 C, HMBC: C^{14}), 71.1 (-, 2 C, HMBC: C^{15}), 80.7 (C_{quat}, 2 C, HMBC: C^{2}), 81.2 (C_{quat}, 1 C, HMBC: C^{24}), 113.7 (+, 2 C, HSQC: C^{19}), 125.4 (C_{quat}, 2 C, HMBC: C^{20}), 150.5 (C_{quat}, 2 C, HMBC: C^{18}), 167.7 (C_{quat}, 2 C, HMBC: C^{21}), 170.7 (C_{quat}, 2 C, HMBC: C^{3}), 171.8 (C_{quat}, 1 C, HMBC: C^{8}), 172.9 (C_{quat}, 1 C, HMBC: C^{23}). – IR (KBr) [cm^{-1}]: $\tilde{n}$ = 3056, 2976, 2931, 2823, 1724, 1658, 1600, 1520, 1363, 1138, 736. – UV (MeOH): λ_{max} (log ε) = 202 (3.381), 224 (3.933), 267 (3.423). – MS (ESI(+), DCM/MeOH + 10 mmol NH_4Ac): m/z (%) = 928.5 (100) [MH^{+}]. – HRMS Calcd for $C_{45}H_{74}N_3O_{17}$: 928.5018; Found: 928.5034 ± 0.0012. – Elemental analysis calcd. (%) for $C_{45}H_{73}N_3O_{17}$: C 58.24, H 7.93, N 4.53; found C 58.42, H 8.16, N 4.97.

2. Fluorescence data

All fluorescence experiments were performed on a Varian Cary Eclipse Fluorimeter. To determine the binding constants and the $K_{0.5}$ values, fluorescence titration experiments were carried out.

2.1 Screening of binding affinity using the peptide library

The screening of the synthesized peptide library was conducted in a microtiter plate (96 wells). The fluorescence intensity was found to be constant after 15 min.

Instrument Parameters

Excitation wavelength: λ_{ex} = 305 nm

Detection wavelength: λ = 320 – 550 nm

Temperature: T = 298 K

PMT voltage 800 volts

Screening conditions

Solvent: 50 mM HEPES buffer, pH 7.5

Well volume: 400 µL

Concentration (**5**): 3.75 x 10^{-5} M

Concentration [peptide]: 1.875 x 10^{-4} M ? 5 eq of peptide with regard to **5**

Procedure:

To each column in the microtiter plate samples of **5** with 5 eq of the corresponding peptide and **5** without added peptide as well as **5** with 5 eq of GHG were given as reference. Emission spectra for all wells were recorded.

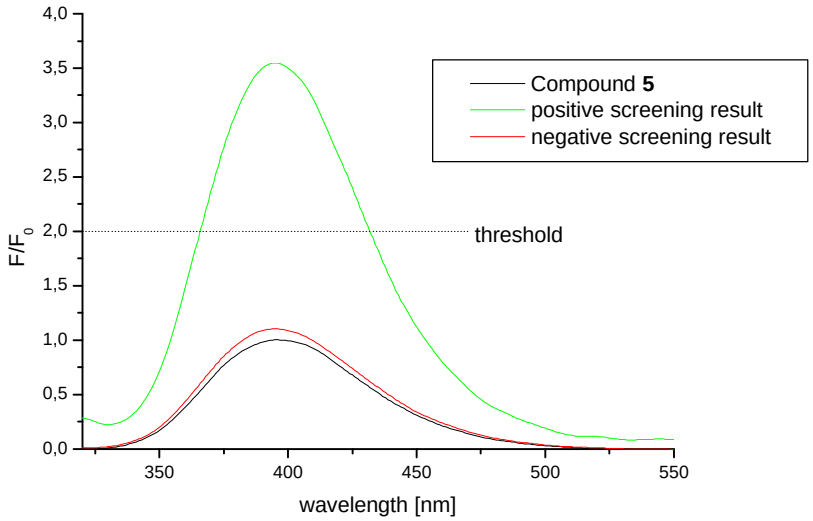


Figure S-1. For peptides inducing a significant increase in emission intensity over the parent compound **5** (F/F_0 = 2.0), the binding constant and stoichiometry of the respective peptide to **5** were determined by emission titration in a cuvette.

2.2 Emission titrations

Instrument Parameters

Excitation wavelength: λ_{ex} = 305 nm

Detection wavelength: λ = 320 – 550 nm or 320 – 600 nm

Temperature: T = 298 K

PMT voltage 600 volts

Titration conditions:

Solvent: 50 mM HEPES buffer, pH 7.5

Starting volume: 1.2 mL or 2.5 mL

Concentration (**5**): 5.0 10^{-5} M

Concentration [peptide]: $7.5 \cdot 10^{-4}$ M or $4.2 – 4.3 \cdot 10^{-3}$ M

Procedure:

To a cuvette with 1.2 mL or 2.5 mL of **5** in HEPES buffer were added 40 µL (? 0.5 eq) aliquots of the peptide solution, while the cuvette filled with 2.5 mL of Cu-NTA CE **5** in HEPES buffer was titrated stepwise with small amounts 5 – 60 µL, depending on the initial emission response, of the substrate solution. After each addition the solution was allowed to

equilibrate for 15 min before the fluorescence intensity and the UV spectrum (see Figure S-16 for a representative example) were recorded. The stoichiometry was determined by Job´s plot analysis extracted from titration data.[3] To determine the binding constant the obtained fluorescence intensities were volume corrected, plotted against the concentration of peptide and evaluated by non linear fitting methods.

H-Gly-His-Gly-Gly-NH_2

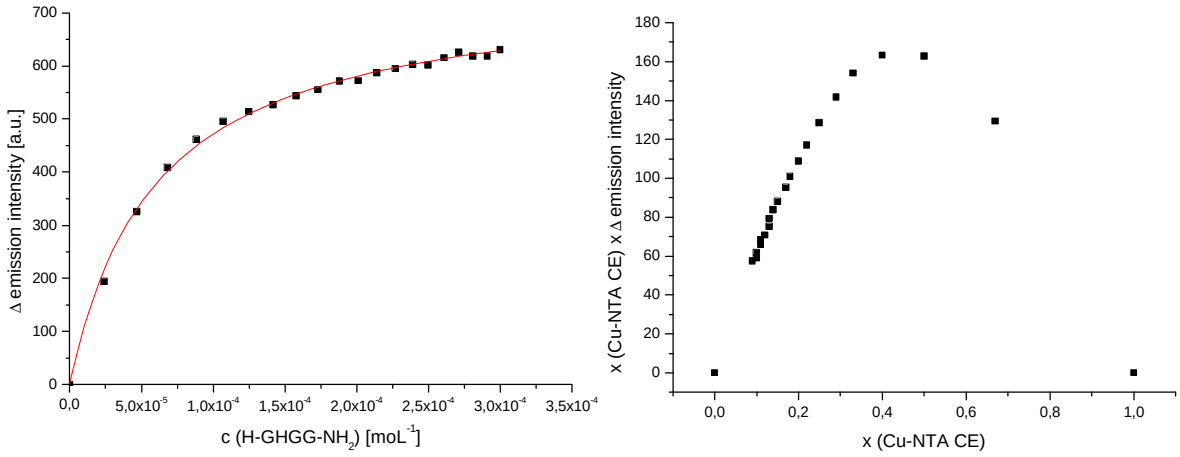


Figure S-2. Emission titration of **5** with peptide H-Gly-His-Gly-Gly-NH_2

H-Gly-His-Gly-OH

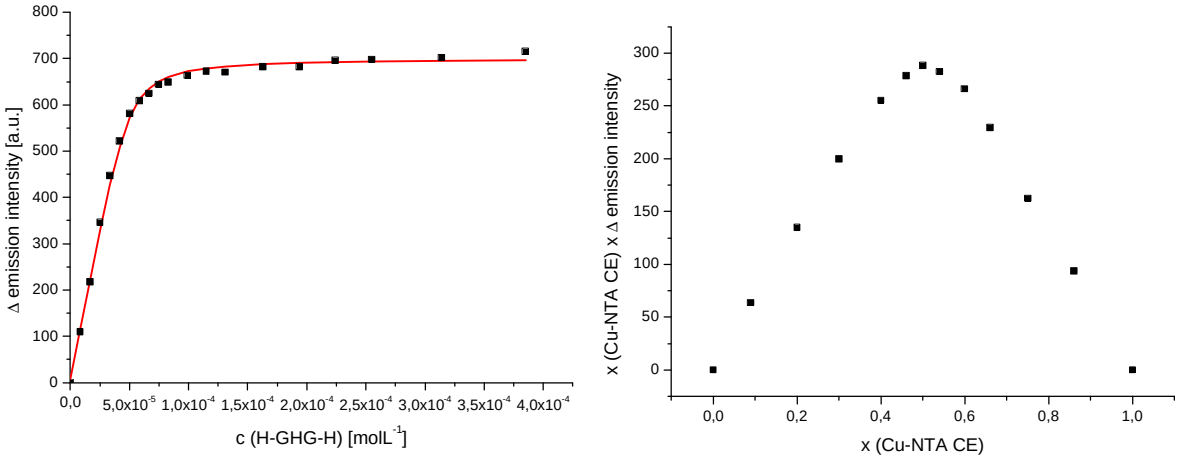


Figure S-3. Emission titration of **5** with peptide H-Gly-His-Gly-OH

[3] P. MacCarthy *Anal. Chem.* **1978**, 50, 2165, *Angew. Chem. Int. Ed.* **2006**, 45,4277-4281.

H-Gly-His-Gly-OMe

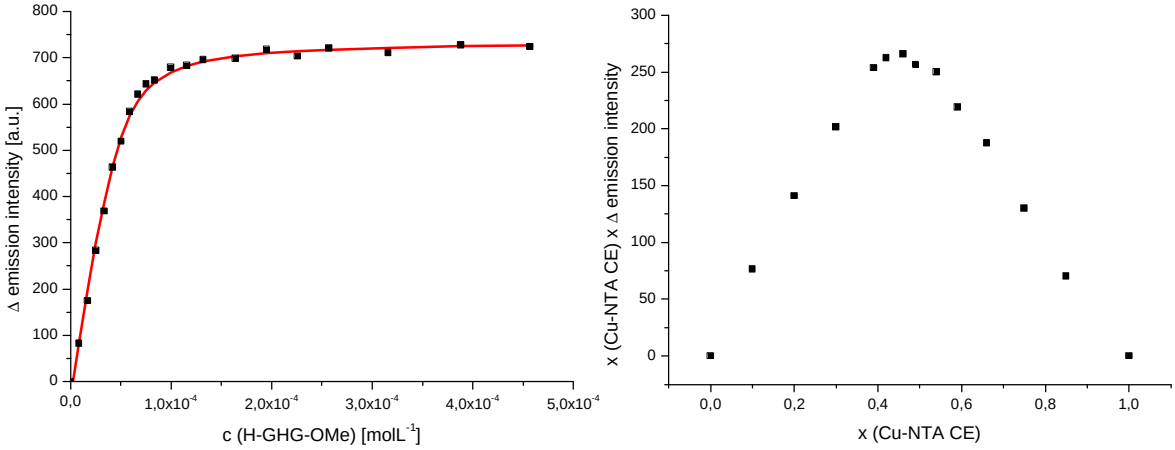


Figure S-4. Emission titration of **5** with peptide H-Gly-His-Gly-OMe

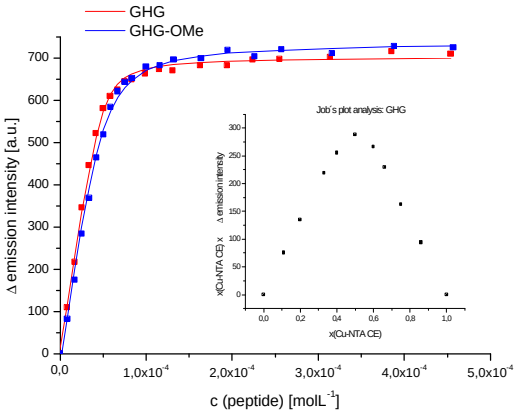


Figure S-5. Comparison of emission titrations of **5** with peptides H-Gly-His-Gly-OH and H-Gly-His-Gly-OMe

Ac-Gly-His-Gly-OH

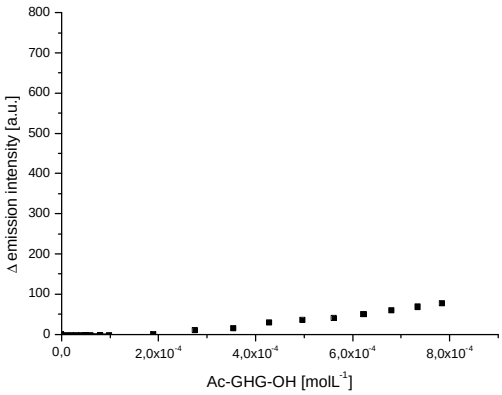


Figure S-6. Emission titration of **5** with peptide Ac-Gly-His-Gly-OH

H-Gly-Gly-His-OH

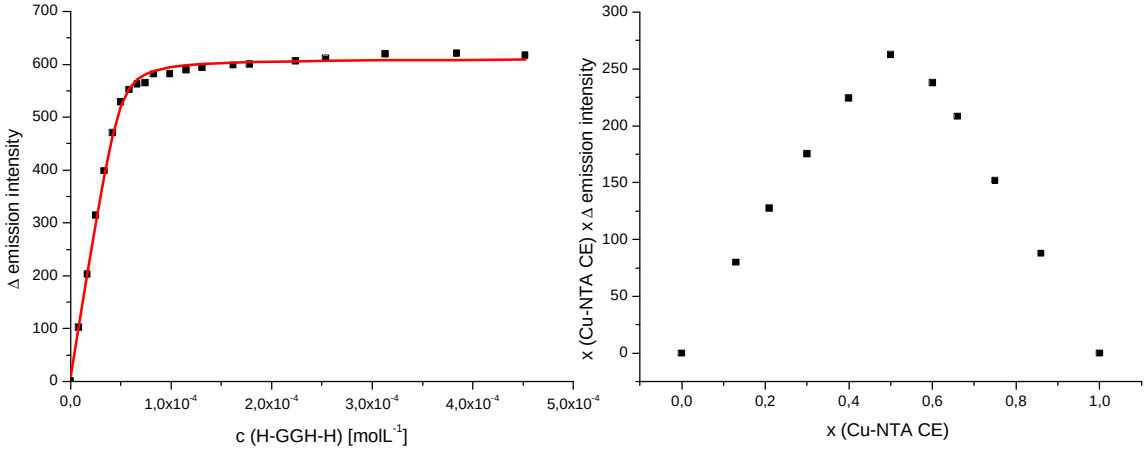


Figure S-7. Emission titration of **5** with peptide H-Gly-Gly-His-OH

H-Gly-His-OH

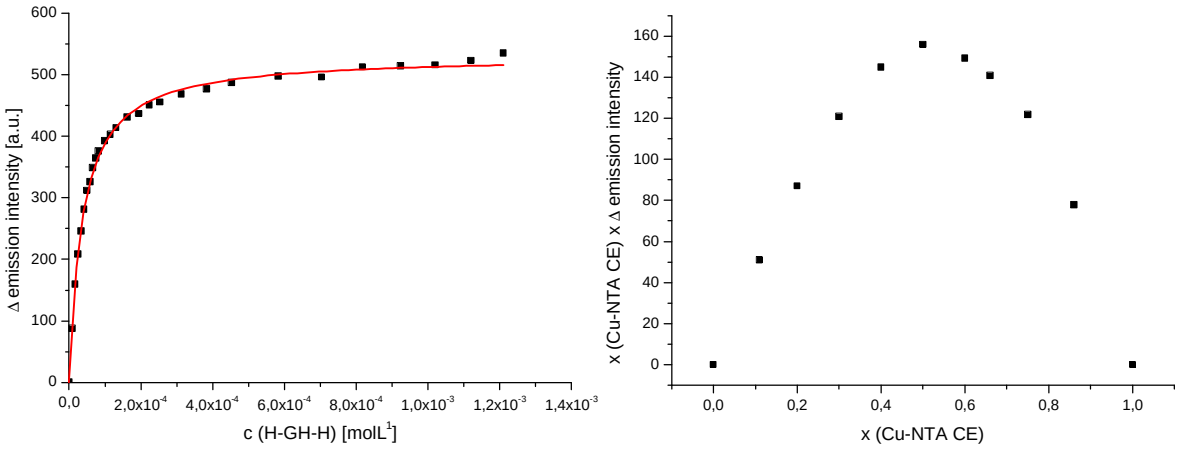


Figure S-8. Emission titration of **5** with peptide H-Gly-His-OH

H-His-OMe

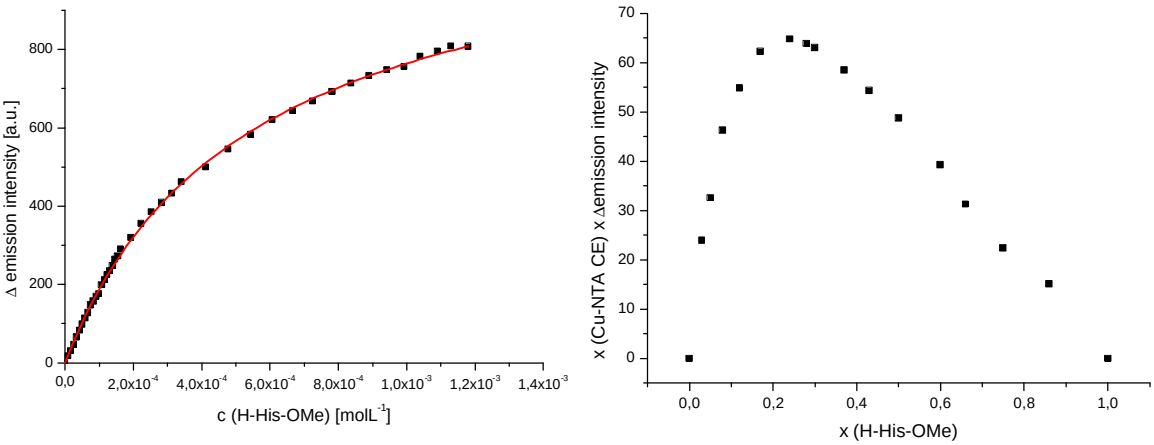


Figure S-9. Emission titration of **5** with peptide H-His-OMe

H-His-Lys-OMe

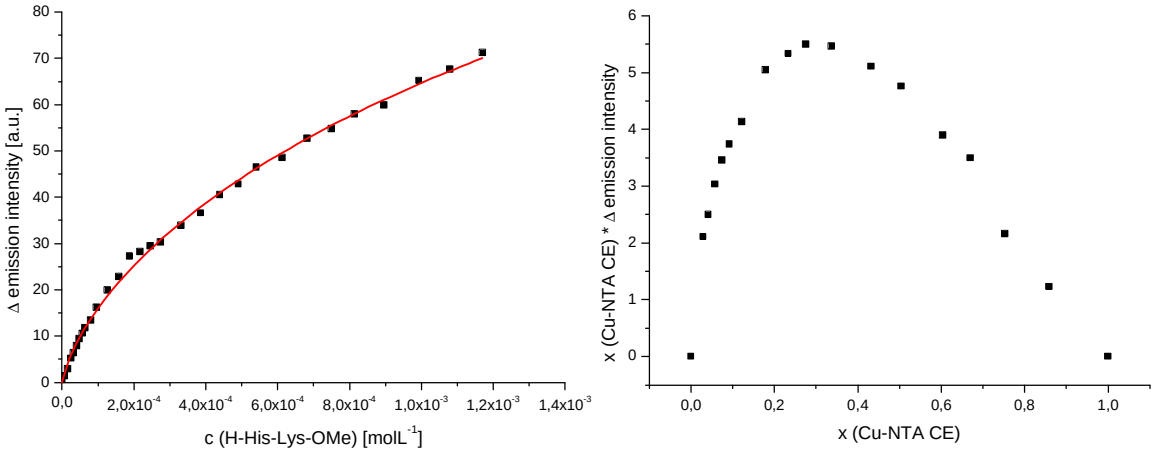


Figure S-10. Emission titration of **5** with peptide H-His-Lys-OMe

H-His-Gly-Lys-OMe

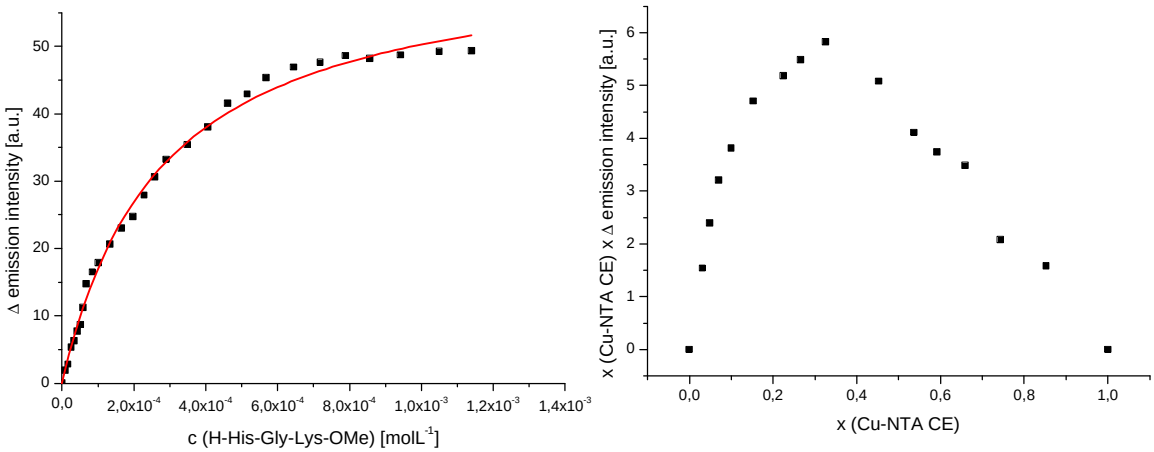


Figure S-11. Emission titration of **5** with peptide H-His-Gly-Lys-OMe

H-His-Gly-Gly-OH

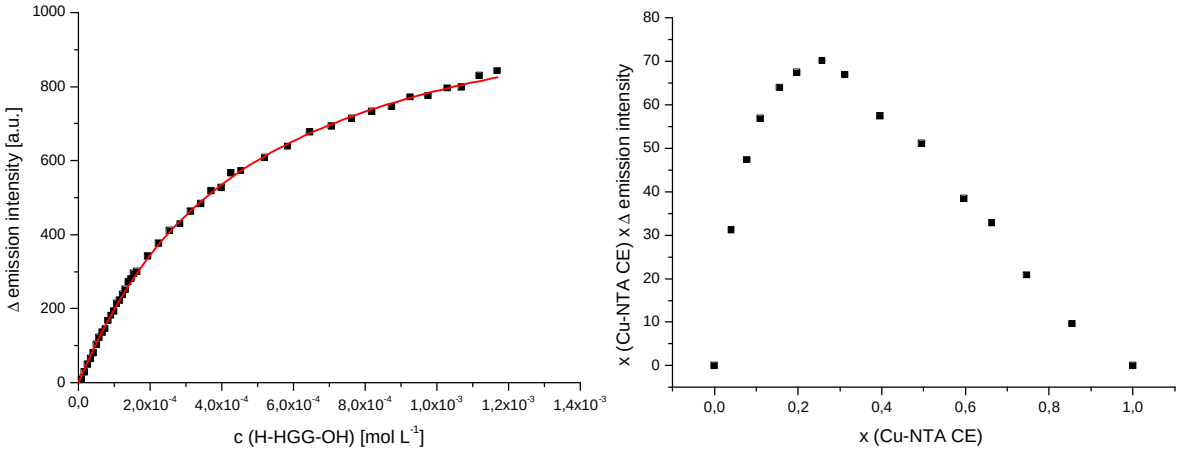


Figure S-12. Emission titration of **5** with peptide H-His-Gly-Gly-OH

H-Ala-His-Gly-Gly-NH_2

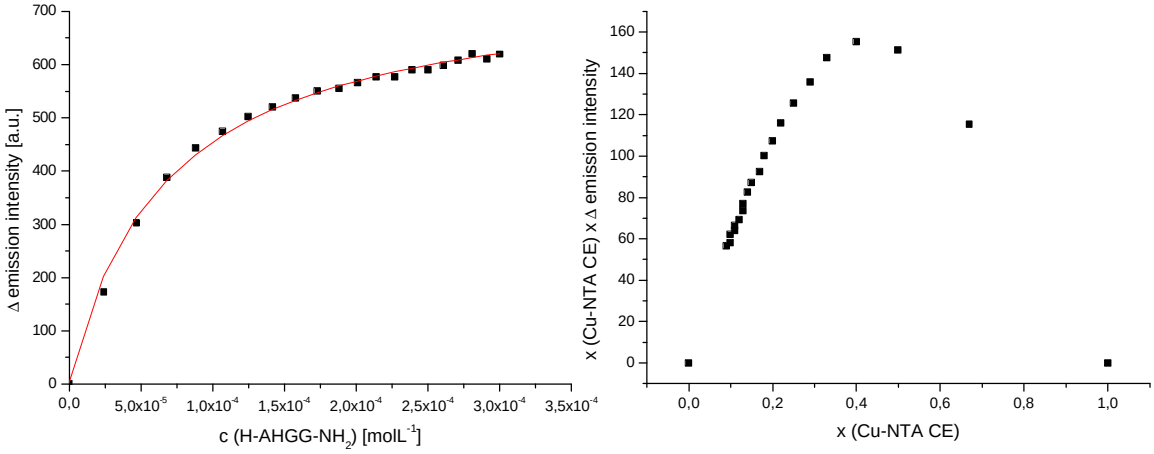


Figure S-13. Emission titration of **5** with peptide H-Ala-His-Gly-Gly-NH_2

H-Leu-His-Gly-Gly-NH_2

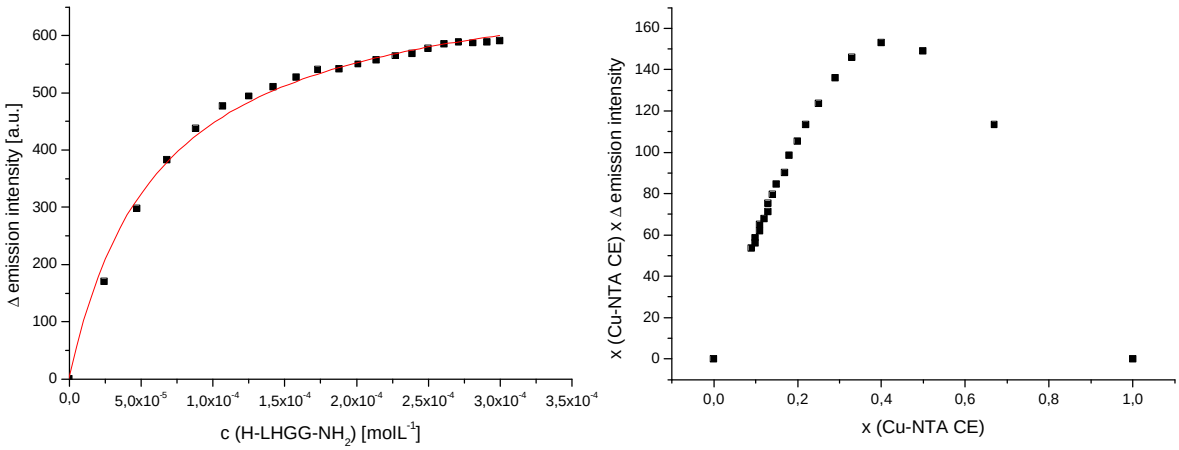


Figure S-14. Emission titration of **5** with peptide H-Leu-His-Gly-Gly-NH_2

H-Gln-His-Gly-Gly-NH_2

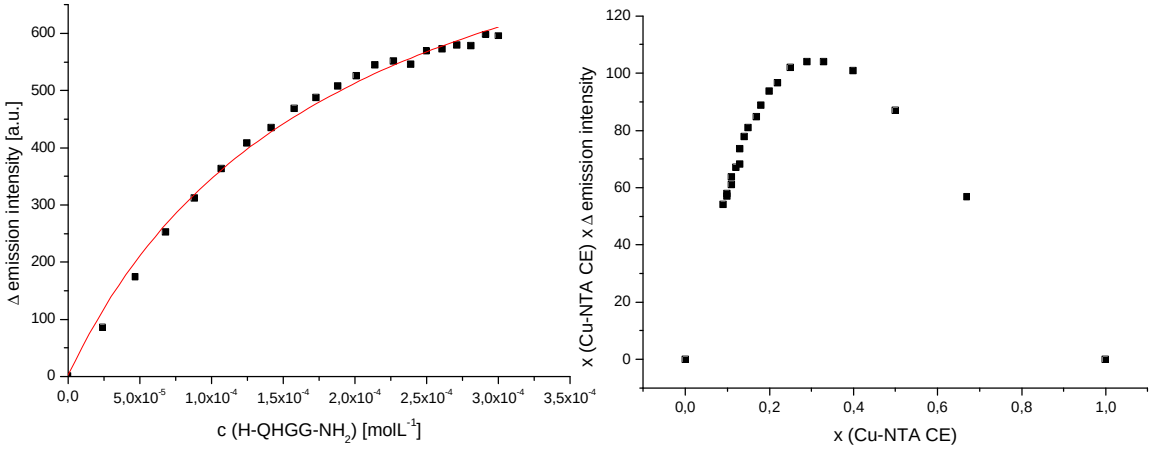


Figure S-15. Emission titration of **5** with peptide H-Gln-His-Gly-Gly-NH_2.

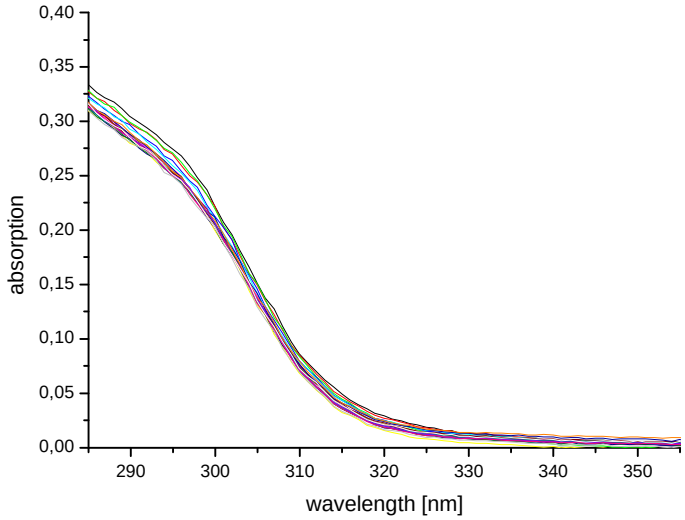


Figure S-16. UV monitoring of the titration of **5** with peptide Gly-His-Gly.

3. Determination of NTA-Cu complex stability in compound 5 in the presence of peptides

a) Fluorescence study

The tripeptide GGH and the dipeptide GH themselves show affinity to copper ions as reported in literature.[4] Therefore the stability of the copper-NTA complex of **5** in the presence of these peptides was investigated. To a HEPES (50 mM, pH 7.5) buffered solution of **5** (2.5 mL, $5.0 \cdot 10^{-5}$ M) 1.2 eq of GGH were added. After addition of GGH the fluorescence spectrum of the mixture was recorded in certain intervals (3 min, 12 min, 23, min, 53 min, 2 h 53 min). The fluorescence intensity increased if compared to the receptor due to the binding process and gave a constant output after approx. 15 min after adding. The over time recorded spectra showed no significant difference from each other thus only one is shown representatively. The sample was further kept in the fridge for 18 days and subsequently a fluorescence spectrum was collected. The emission intensity of the sample after 18 days (*blue*) was still the same as at the beginning of the experiment (*green*). This provides evidence that the fluorescence intensity enhancement during binding experiments has its origin in the binding event of the peptides to the receptor. If decomplexation would occur, the quenching of the benzocrown ether unit by the copper ion would disappear and the fluorescence intensity would be expected to reach the same level as in the ligand **4**. Figure S-17 summarizes the result. Fluorescence spectra of ligand **4** (blue) and receptor **5** (pink) are given for comparison.

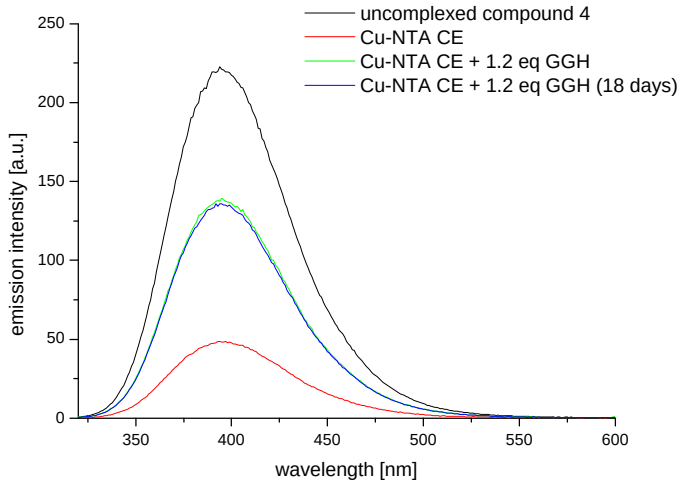


Figure S-17. Determination of complex stability of Cu-NTA CE **5** in the presence of GGH (1.2 eq) in buffered aqueous solution (HEPES, pH 7.5).

[4] S. J. Lau, B. Sarkar, *Biochem. J*. **1981**, *199*, 649-656.

b) UV absorption study

To a HEPES (50 mM, pH 7.5) buffered solution of Cu-NTA CE **5** ($5.0 \cdot 10^{-5}$ M) 5.0 eq GGH were added. Subsequently the UV absorption was measured every four minutes after addition and after 21 hours. Figure S-18 shows the recorded spectra and the absorption spectra of compound **4** and **5** without peptide present for comparison.

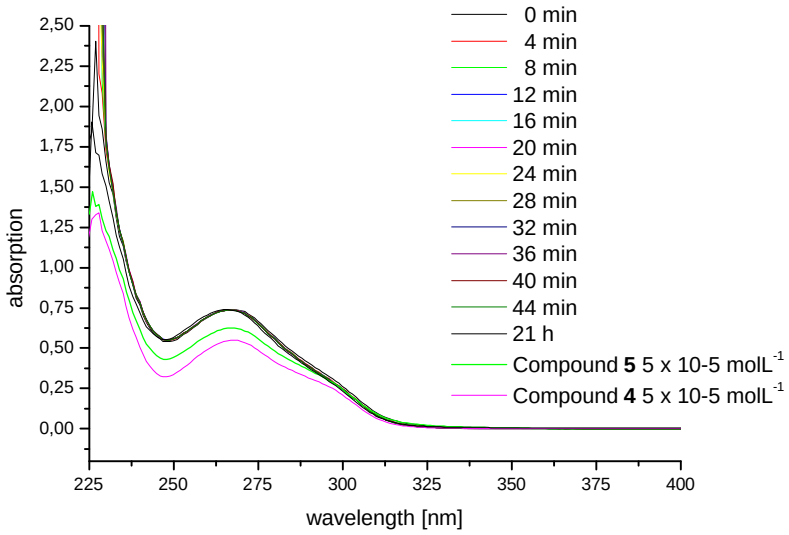


Figure S-18. UV absorption spectra of Cu-NTA CE **5** with GGH (5 eq) over time. The UV absorption of the ligand **4** (pink) and Cu-NTA CE **5** (*green*) are given for comparison.

4. Change of UV- and emission spectra of NTA-Cu and 5 in the presence of mercapto-ethanol

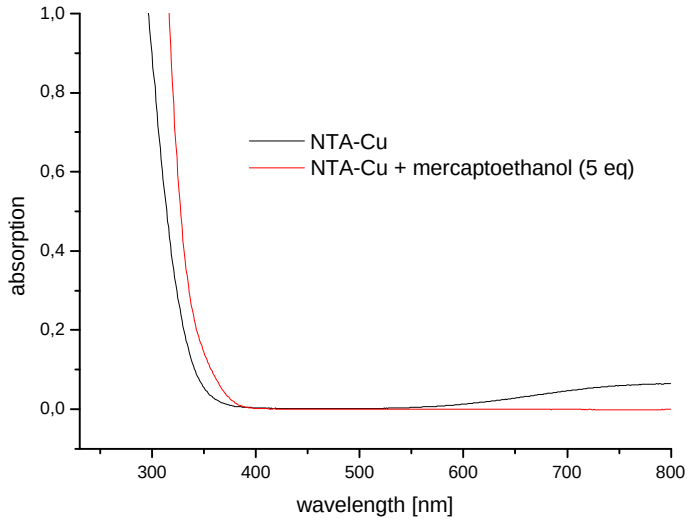


Figure S-19. UV absorption spectra of NTA-Cu (black) and NTA-Cu + 5 equivalents of mercaptoethanol (red)

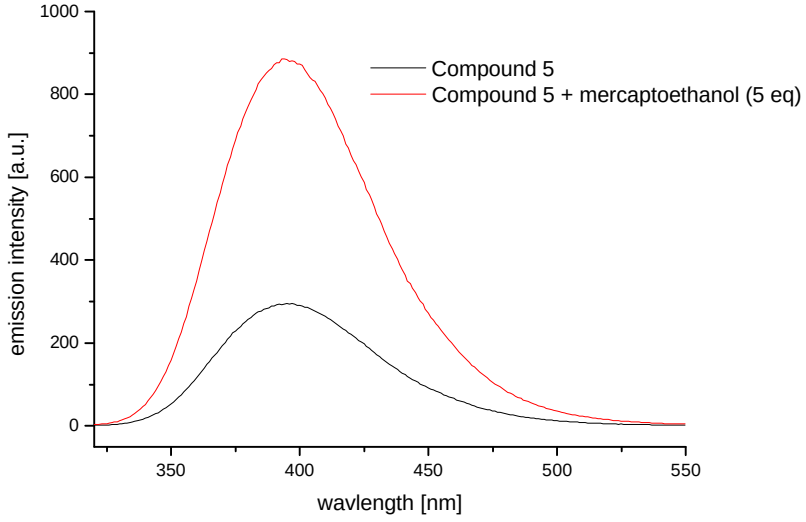


Figure S-20. Fluorescence spectra of compound **5** (*black*) and compound **5** + 5 equivalents of mercaptothiol (*red*); concentration of compound **5**: c = 5.0 x 10^{-5} molL^{-1}.

5. Binding Studies by Isothermal Titration Calorimetry (ITC)

In order to verify the results of the emission titrations, the binding processes of the peptides GHG and Ac-GHG with compound **5** were determined by isothermal titration calorimetry.
All ITC experiments were performed in buffered aqueous solution (HEPES, 50 mM, pH 7.5) at 25 °C using an ultrasensitive VP-ITC calorimeter from MicroCal (Northampton, MA, U.S.A.). The sample solutions were made from stock buffer solution (HEPES, 50 mM, pH 7.5). Concentration of Cu-NTA CE **5** (titrant) in the 300 µL syringe was set to 1.2 mM, whereas the concentration of the peptide GHG or Ac-GHG in the 1.436 mL calorimetric cell was 0.05 mM. Before each titration both experimental solutions (titrant, cell) were thoroughly degassed under vigorous stirring. During the ITC experiment the cell solution was stirred at 300 rpm by syringe to ensure rapid mixing and 60 x 5 µL of titrant were injected over 10 s with a spacing time between each injection of two minutes in order to allow complete equilibration. Before data analysis the total observed heat of binding was corrected for the heat of dilution yielding the effective heat of binding. Therefore an analogue ITC experiment with the calorimeter cell filled with HEPES and Cu-NTA CE as titrant was carried out. The data were analyzed by non linear fitting methods using the MicroCal Origin software (Windows based). The values for the binding stoichiometry (*n*) and the thermodynamic parameters of interaction: enthalpy of binding (Δ*H*) and binding constant (lg *K*), and, consequently, free energy change (Δ*G*) and entropy change (Δ*S*) were obtained using a "one-set of site" model for the fitting routine. Table 1 summarizes the results of the ITC experiments. Figure S-21 and figure S-22 show the raw ITC titration data and the processed curve.

	n	**lg *K*** [M^{-1}]	D***H*** [cal mol^{-1}]	D***S*** [cal mol^{-1} K^{-1}]	D***G*** [cal mol^{-1}]
H-GHG-OH	1.04 ± 0.01	5.10 ± 0.02	- 4168 ± 22.25	9.37	-6962
Ac-GHG-OH	1.18 ± 0.08	3.99 ± 0.04	- 4364 ± 47.40	3.61	- 5440

Table S-1. Results of the ITC experiments of Cu-NTA CE vs GHG and Ac-GHG, respectively.

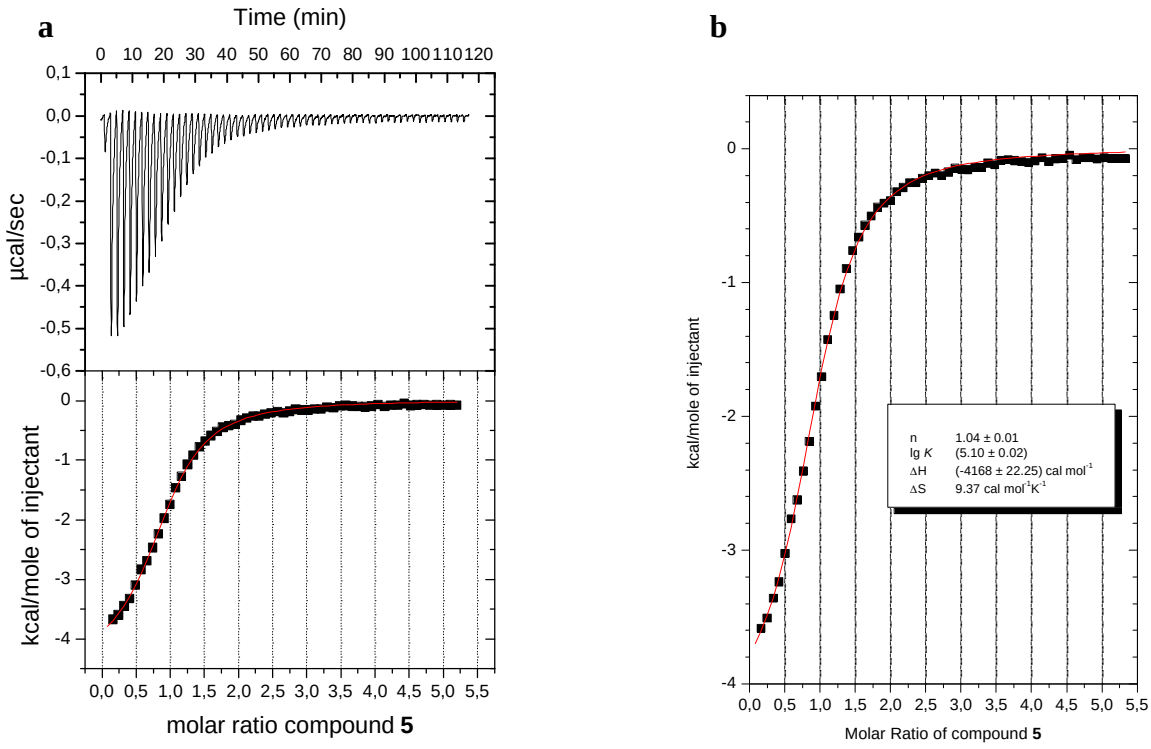


Figure S-21. ITC of GHG (0.05 mM) and Cu-NTA CE (**5**) (1.20 mM) in HEPES (50 mM, pH 7.5) at 25 °C; (**a**) raw ITC data, (**b**) heat of dilution corrected and fitted ΔH diagram.

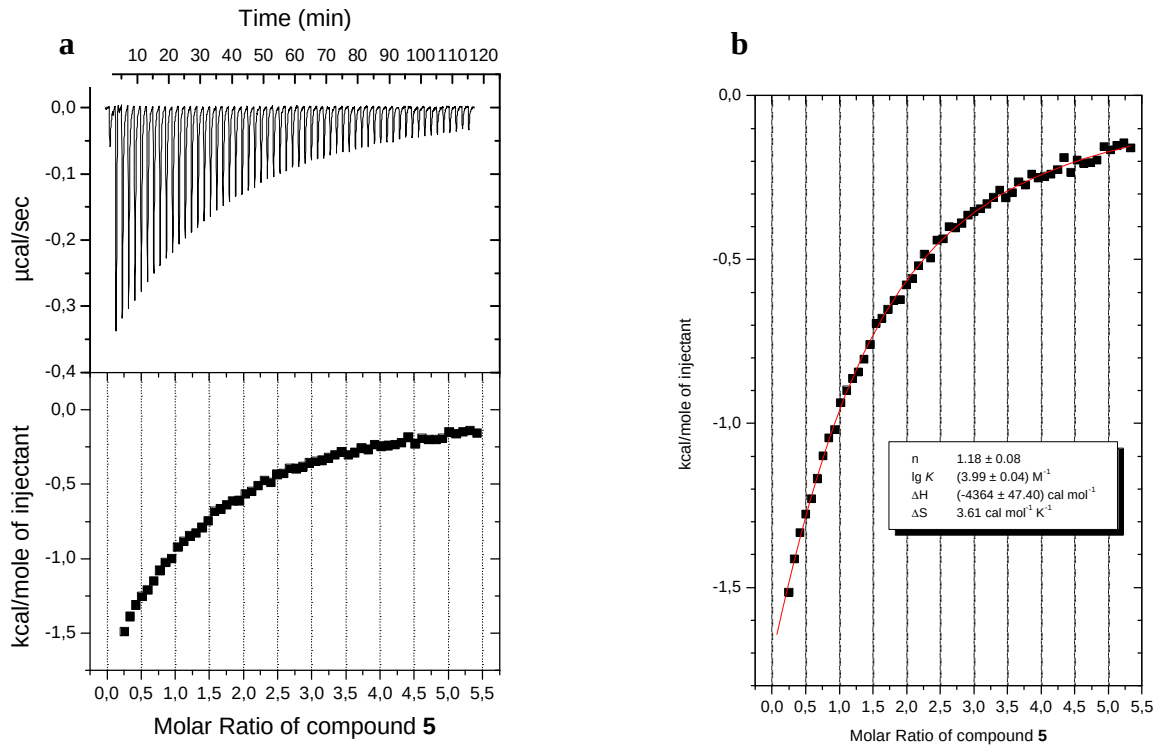


Figure S-22. ITC of Ac-GHG (0.05 mM) and Cu-NTA CE (**5**) (1.20 mM) in HEPES (50 mM, pH 7.5) at 25 °C; (**a**) raw ITC data, (**b**) heat of dilution corrected and fitted ΔH diagram.

6. Change of emission spectra of ligand **4** in the presence of GGH

Compound **4** was titrated under the conditions described above with GGH to confirm no change of its emission spectrum in the presence of the target peptides.