Supplementary information

The new multi-purpose fluorescent probe for simultaneous detection of more proteases

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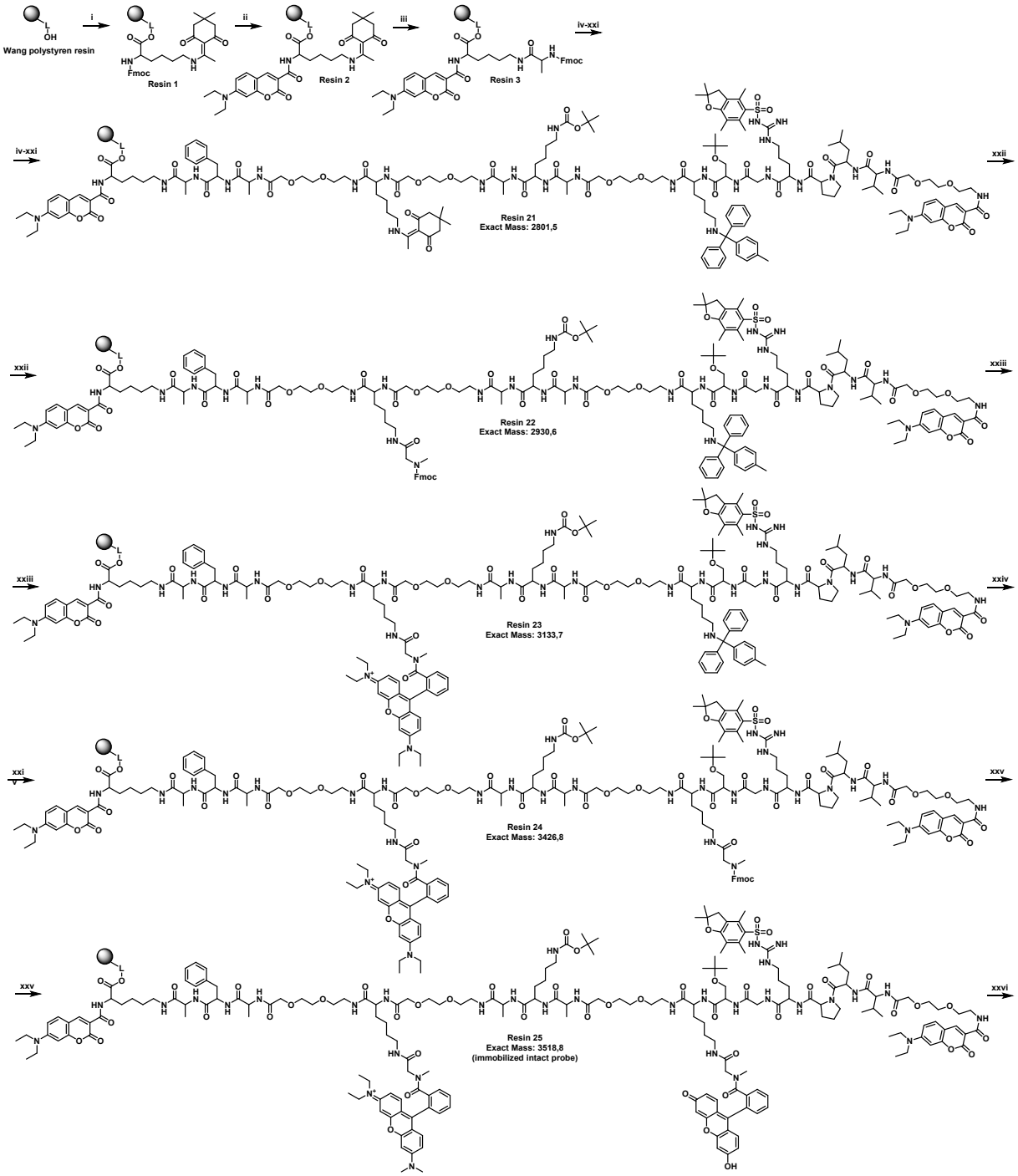
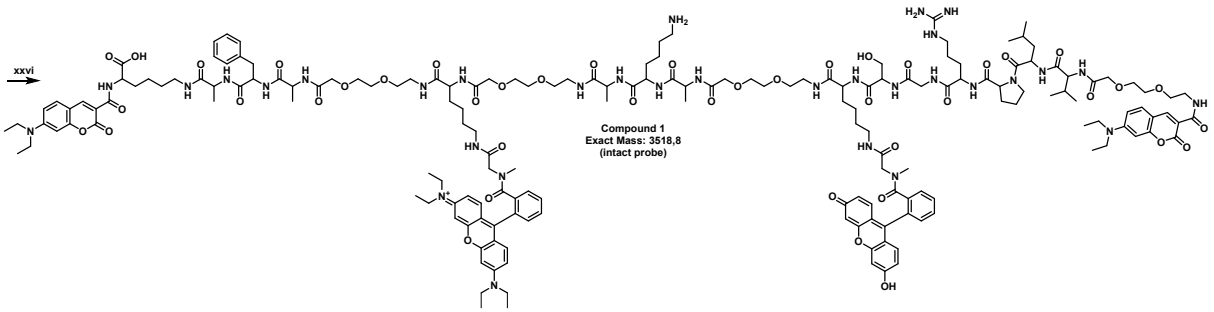
1. Synthesis

1.1. Synthesis of probe

Scheme S1: Synthesis of peptide probe **1**





i. Fmoc-Lys(Dde)-OH, HOBt, DMAP, DIC, DMF:DCM 1:1 (V/V), rt, 16h; ii. a.) 50% piperidine in DMF, rt, 30 min; b.) DEAC, HOBt, DMAP, DIC, DMF:DCM:DMSO 1:2:2 (V/V), rt, 20h; iii. a.) $HONH_2 \cdot HCl$, imidazole, NMP:DCM 3:2 (V/V), rt, 3h; b.) Fmoc-Ala-OH, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 22h – repeated twice; iv. a.) 50% piperidine in DMF, rt, 30 min; b.) Fmoc-Phe-OH, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 2h; v. a.) 50% piperidine in DMF, rt, 30 min; b.) Fmoc-Ala-OH, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 2h; vi. a.) 50% piperidine in DMF, rt, 30 min; b.) PEG, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 2h; vii. a.) 50% piperidine in DMF, rt, 30 min; b.) Fmoc-Lys(Dde)-OH, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 16h; viii. a.) 50% piperidine in DMF, rt, 30 min; b.) PEG, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 2h; ix. a.) 50% piperidine in DMF, rt, 30 min; b.) Fmoc-Ala-OH, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 2h; x a.) 50% piperidine in DMF, rt, 30 min; b.) Fmoc-Lys(Boc)-OH, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 2h; xi. a.) 50% piperidine in DMF, rt, 30 min; b.) Fmoc-Ala-OH, HOBt, DMF:DCM 1:1 (V/V), rt, 2h; xii. a.) 50% piperidine in DMF, rt, 30 min; b.) PEG, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 16h; xiii. a.) 50% piperidine in DMF, rt, 30 min; b.) Fmoc-Lys(Mtt)-OH, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 16h; xiv. a.) 50% piperidine in DMF, rt, 30 min; b.) Fmoc-Ser(tBu)-OH, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 3h; xv. a.) 50% piperidine in DMF, rt, 30 min; b.) Fmoc-Gly-OH, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 10h; xvi. a.) 50% piperidine in DMF, rt, 30 min; b.) Fmoc-Arg(Pbf)-OH, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 10h; xvii. a.) 50% piperidine in DMF, rt, 30 min; b.) Fmoc-Pro-OH, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 10h; xviii. a.) 50% piperidine in DMF, rt, 30 min; b.) Fmoc-Leu-OH, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 3h; xix. a.) 50% piperidine in DMF, rt, 30 min; b.) Fmoc-Val-OH, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 3h; xx. a.) 50% piperidine in DMF, rt, 30 min; b.) PEG, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 3h; xxi. a.) 50% piperidine in DMF, rt, 30 min; b.) DEAC, HOBt, DMAP, DIC, DMF:DCM:DMSO 1:2:2 (V/V), rt, 16h; xxii. a.) $HONH_2 \cdot HCl$, imidazole, NMP:DCM 3:2 (V/V), rt, 3h; b.) Fmoc-Sar-OH, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 3h; xxiii. a.) 50% piperidine in DMF, rt, 45 min; b.) RhB, HOBt, DMAP, DIC, DMF:DCM:DMSO 1:2:2 (V/V), rt, 16h; xxiv. a.) DCE, TES, HFIP, TFE, 60 °C, 6h; b.) Fmoc-Sar-OH, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 3h; xxv. a.) 50% piperidine in DMF, rt, 30 min; b.) FL, HOBt, DMAP, DIC, DMF:DCM:DMSO 1:2:2 (V/V), rt, 16h – repeated 3x; xxvi. 50% TFA in DCM, rt, 60 min.

Molecular weights of cleaved peptides are reported. Tert-butyl (tBu), tert-butyloxycarbonyl (Boc), 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) and 4-methyltrityl (Mtt) protecting groups are removed during the cleavage of the final compound from the resin with 50% TFA in DCM

Resin 1

Wang resin (500 mg) was washed with dichloromethane (5x), and subsequently reacted with Fmoc-Lys(Dde)-OH (1.0 mmol), HOBt (1.0 mmol), DMAP (0.25 mmol), and DIC (1.0 mmol) in DMF (2.5 mL) and DCM (2.5 mL). The reaction mixture was shaken for 16 hours at lab temperature. Afterwards, a solid support was washed with DMF (5x) and DCM (5x).

Resin 2

Resin 1 (500 mg) was subjected to 50% piperidine in DMF for 30 min, and subsequently washed with DMF (10x) and DCM (5x). Then, the resin was reacted with DEAC (1.0 mmol), HOBt (1.0 mmol), DMAP (1.0 mmol), and DIC (1.0 mmol) in DMF (1 mL), DCM (2 mL), and DMSO (2 mL). The reaction mixture

was shaken for 20 hours at lab temperature. Afterwards, a solid support was washed with DMSO (5X), DMF (5x) and DCM (5x).

Resin 3

To Resin 2 (500 mg), $HONH_2 \cdot HCl$ (4.5 mmol) and imidazole (3.4 mmol) in NMP (3 mL) and DCM (2 mL) were added, and obtained heterogeneous mixture was shaken for 3 hours at lab temperature. Afterwards, a solid support was washed with NMP (5X) and DCM (5x). Then, the resin was reacted with Fmoc-Ala-OH (1.0 mmol), HOBt (1.0 mmol), and DIC (1.0 mmol) in DMF (2.5 mL) and DCM (2.5 mL). The reaction mixture was shaken for 18 hours at lab temperature. Afterwards, a solid support was washed with DMF (5x) and DCM (5x). Repeated twice to achieve sufficient conversion.

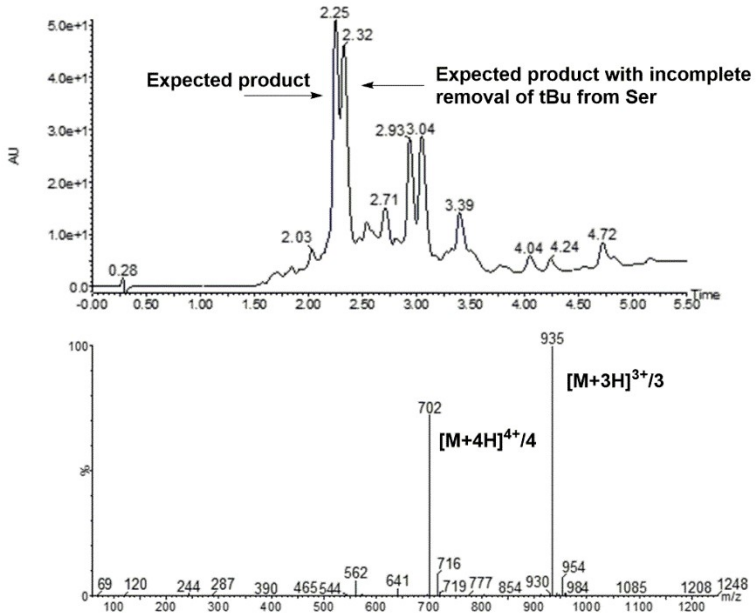
Resin 4–20

An appropriate resin (500 mg) was subjected to 50% piperidine in DMF for 30 min, and subsequently washed with DMF (10x) and DCM (5x). Then, a solid support was reacted with a suitable amino acid or PEG spacer (1.0 mmol), HOBt (1.0 mmol), and DIC (1.0 mmol) in DMF (2.5 mL) and DCM (2.5 mL). A reaction mixture was shaken at lab temperature. Afterwards, a solid support was washed with DMF (10x) and DCM (5x).

Resin 21

Resin 20 (250 mg) was subjected to 50% piperidine in DMF for 30 min, and subsequently washed with DMF (10x) and DCM (5x). Then, the resin was reacted with DEAC (0.6 mmol), HOBt (0.6 mmol), DMAP (0.6 mmol), and DIC (0.6 mmol) in DMF (0.75 mL), DCM (1.5 mL), and DMSO (1.5 mL). The reaction mixture was shaken for 16 hours at lab temperature. Afterwards, a solid support was washed with DMSO (5X), DMF (5x) and DCM (5x).

Figure S1: LC-MS analysis of chemically cleaved peptide from Resin 21 (Rt = 2.25 min)



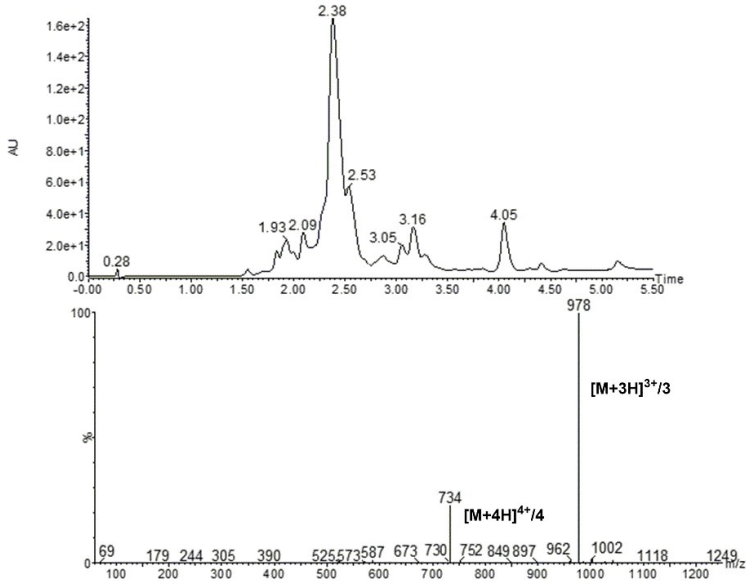
Method: Formic acid (0.1%) in ultrapure water (*V*/*V*) and acetonitrile (gradient 20–80% during the first 4.5 min). LC-MS column: (50 x 3.0 mm XSelect HSS T3 2.5 µm XP C18, Waters, Borehamwood, UK).

Resin 22

To Resin 21 (250 mg), $HONH_2 \cdot HCl$ (2.7 mmol) and imidazole (2.1 mmol) in NMP (1.8 mL) and DCM (1.2 mL) were added, and obtained heterogeneous mixture was shaken for 3 hours at lab temperature. Afterwards, a solid support was washed with NMP (5X), DMF (5x) and DCM (5x). Then, the resin was reacted with Fmoc-Sar-OH (0.6 mmol), HOBt (0.6 mmol), and DIC (0.6 mmol) in DMF (1.5 mL) and DCM

(1.5 mL). The reaction mixture was shaken for 16 hours at lab temperature. Afterwards, a solid support was washed with DMF (5x) and DCM (5x).

Figure S2: LC-MS analysis of chemically cleaved peptide from Resin 22 (Rt = 2.38 min)

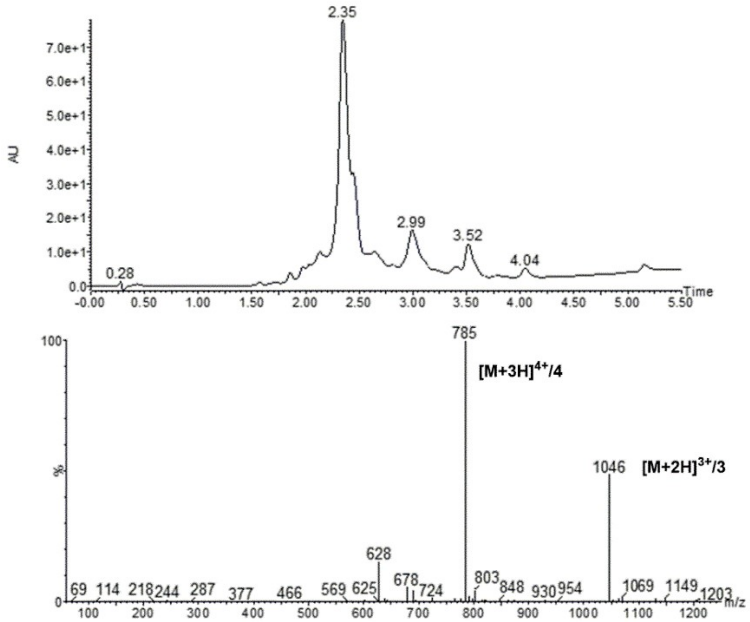


Method: Formic acid (0.1%) in ultrapure water (*V*/*V*) and acetonitrile (gradient 20–80% during the first 4.5 min). LC-MS column: (50 x 3.0 mm XSelect HSS T3 2.5 µm XP C18, Waters, Borehamwood, UK).

Resin 23

Resin 22 (250 mg) was subjected to 50% piperidine in DMF for 30 min, and subsequently washed with DMF (10x) and DCM (5x). Then, the resin was reacted with RhB (0.6 mmol), HOBt (0.6 mmol), DMAP (0.6 mmol), and DIC (0.6 mmol) in DMF (0.6 mL), DCM (1.2 mL), and DMSO (1.2 mL). The reaction mixture was shaken for 16 hours at lab temperature. Afterwards, a solid support was washed with DMSO (5X), DMF (5x) and DCM (5x).

Figure S3: LC-MS analysis of chemically cleaved peptide from Resin 23 (Rt = 2.35 min)

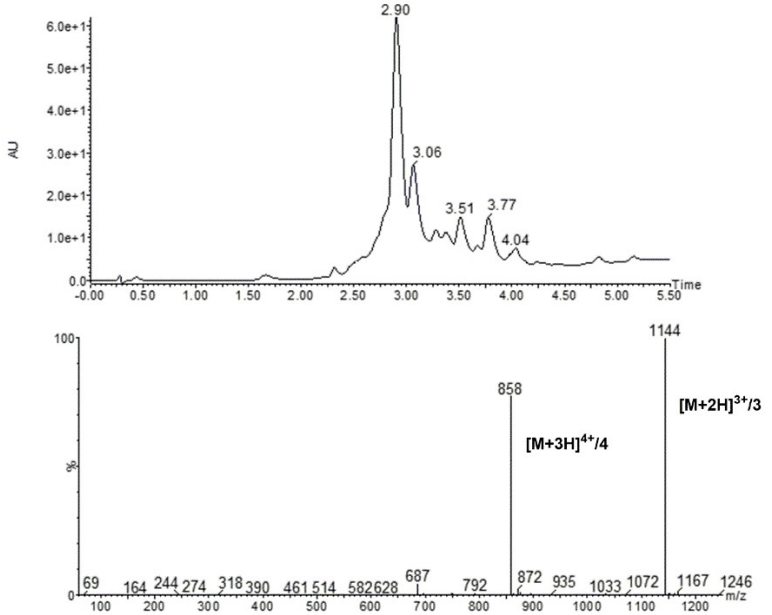


Method: Formic acid (0.1%) in ultrapure water (*V*/*V*) and acetonitrile (gradient 20–80% during the first 4.5 min). LC-MS column: (50 x 3.0 mm XSelect HSS T3 2.5 µm XP C18, Waters, Borehamwood, UK).

Resin 24

To Resin 23 (250 mg), 1,2-dichloroethane (9.75 mL), triethylsilane (3.0 mL), hexafluoroisopropanol (1.5 mL) and trifluoroethanol (0.75 mL) were added. This way obtained heterogeneous mixture was at 60 °C shaken for the time period of 6 hours. Afterwards, a solid support was washed with DCM (10x). Then, the resin was reacted with Fmoc-Sar-OH (0.9 mmol), HOBt (0.9 mmol), and DIC (0.9 mmol) in DMF (1.5 mL) and DCM (1.5 mL). The reaction mixture was shaken for 3 hours at lab temperature. Afterwards, a solid support was washed with DMF (10x) and DCM (5x).

Figure S4: LC-MS analysis of chemically cleaved peptide from Resin 24 (Rt = 2.90 min)

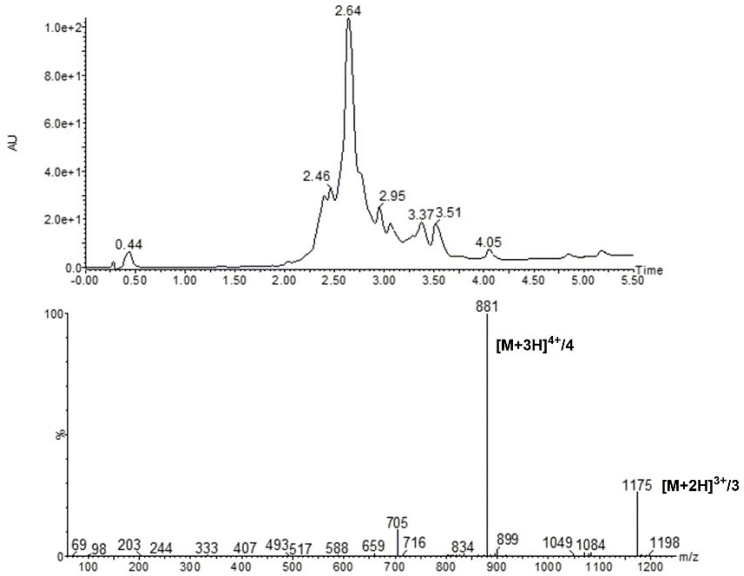


Method: Formic acid (0.1%) in ultrapure water (*V*/*V*) and acetonitrile (gradient 20–80% during the first 4.5 min). LC-MS column: (50 x 3.0 mm XSelect HSS T3 2.5 µm XP C18, Waters, Borehamwood, UK).

Resin 25

Resin 24 was subjected to 50% piperidine in DMF for 30min, and subsequently washed with DMF (10x) and DCM (5x). Then, the resin was reacted with FL (0.6 mmol), HOBt (0.6 mmol), DMAP (0.6 mmol), and DIC (0.6 mmol) in DMF (0.6 mL), DCM (1.2 mL), and DMSO (1.2 mL). The reaction mixture was shaken for 16 hours at lab temperature. Afterwards, a solid support was washed with DMSO (5X), DMF (10x) and DCM (10x). For reaching sufficient conversion, the reaction with fluorescein need to be performed in three repetition.

Figure S5: LC-MS analysis of chemically cleaved peptide from Resin 25 (Rt = 2.64 min)

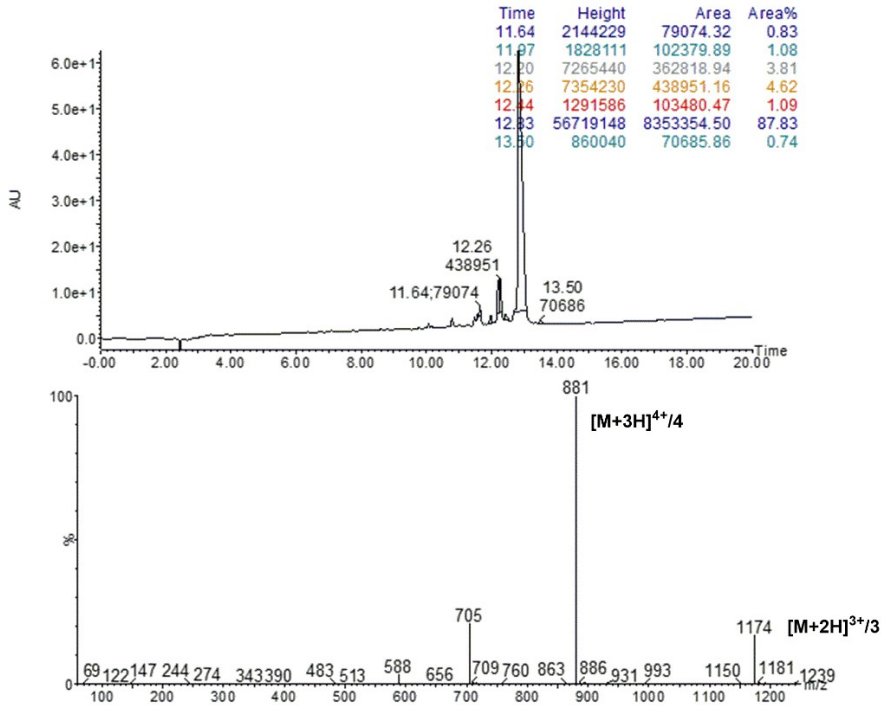


Method: Formic acid (0.1%) in ultrapure water (*V*/*V*) and acetonitrile (gradient 20–80% during the first 4.5 min). LC-MS column: (50 x 3.0 mm XSelect HSS T3 2.5 µm XP C18, Waters, Borehamwood, UK).

Compound 1

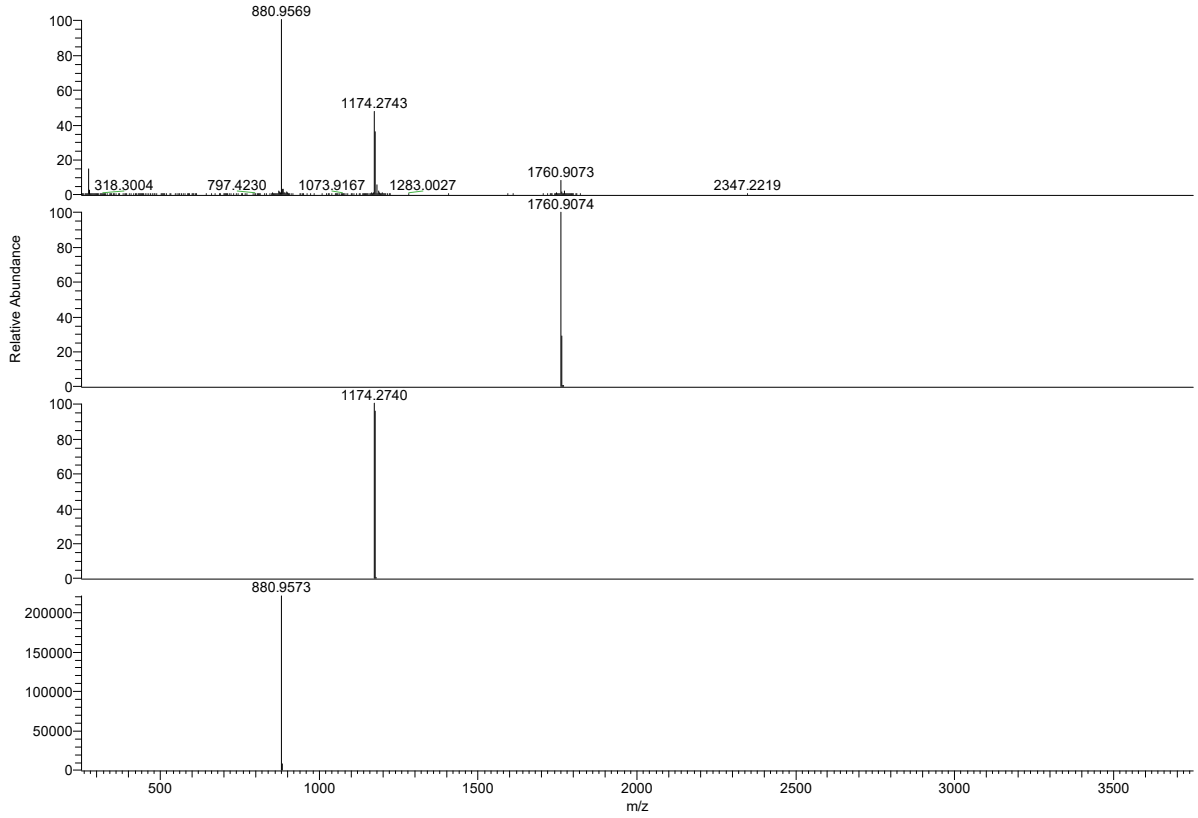
Resin 25 was subjected to 50% TFA in DCM (5 ml). The reaction mixture was shaken for 1 hour at lab temperature. Afterwards, TFA solution was collected and solvents were evaporated under stream of nitrogen. Oily residuum was dissolved in acetonitrile/water 1:1 and purification was performed on a semi-prep HPLC column (Aeris 5 µm 150 x 21.2 mm peptide XB-C18 100 Å, Phenomenex, California, USA), using a gradient of 50–80% acetonitrile in 0.1% TFA in ultrapure H_2O within 10 min. The combined fractions were concentrated in vacuo, and freeze-dried for 48 hours.

Figure S6: LC-MS analysis of isolated intact probe **1** (Rt = 12.83 min).



Method: Formic acid (0.1%) in ultrapure water (V/V) and acetonitrile (gradient 20–65% during the first 20 min). LC-MS column: (150 x 4.6 mm bioZen 2.6 µm peptide XB-C18, Phenomenex, USA)

Figure S7: HRMS analysis of isolated intact probe **1**.



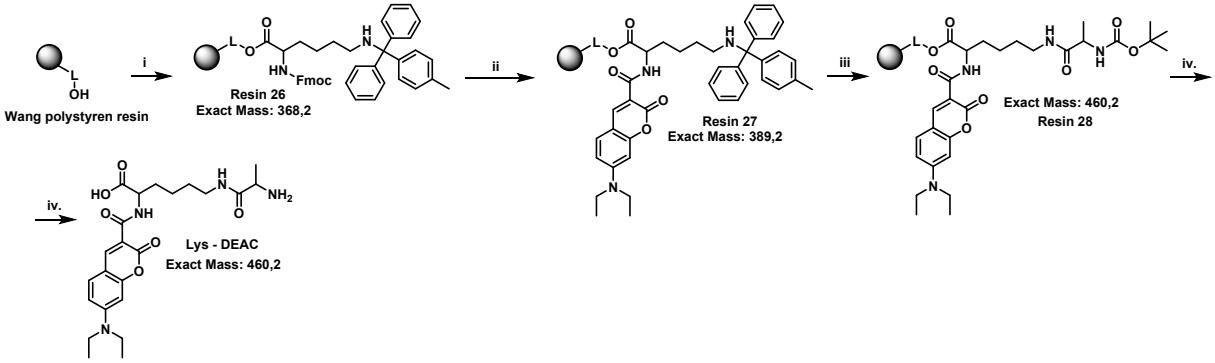
HRMS (ESI):

m/z calcd $C_{178}H_{245}N_{32}O_{43}^{+}$ for $[M+H]^{2+}/2$ = 1760.9074, found $[M+H]^{2+}/2$ = 1760.9073;
m/z calcd $C_{178}H_{245}N_{32}O_{43}^{+}$ for $[M+2H]^{3+}/3$ = 1174.2740, found $[M+2H]^{3+}/3$ = 1174.2743;
m/z calcd $C_{178}H_{245}N_{32}O_{43}^{+}$ for $[M+3H]^{4+}/4$ = 880.9573, found $[M+3H]^{4+}/4$ = 880.9569;

1.2. Synthesis of individual Fragments

1.2.1. Fragment Lys - DEAC

Scheme S2: Synthesis of fragment **Lys – DEAC**.



i. Fmoc-Lys(Mtt)-OH, HOBt, DMAP, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 16h; ii. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) DEAC, HOBt, DMAP, DIC, DMF:DCM:DMSO 1:2:2 (*V*/*V*/*V*), rt, 16h; iii. a.) DCE:TES:HFIP:TFE

13:4:2:1 (*V*/*V*/*V*/*V*), 60 °C, 9h; b.) Boc-Ala-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; iv. 50% TFA in DCM (*V*/*V*), rt, 30 min.

Molecular weights of cleaved peptides are reported. Tert-butyloxycarbonyl (Boc), and 4-methyltrityl (Mtt) protecting groups are removed during the cleavage of the final compound from the resin with 50% TFA in DCM

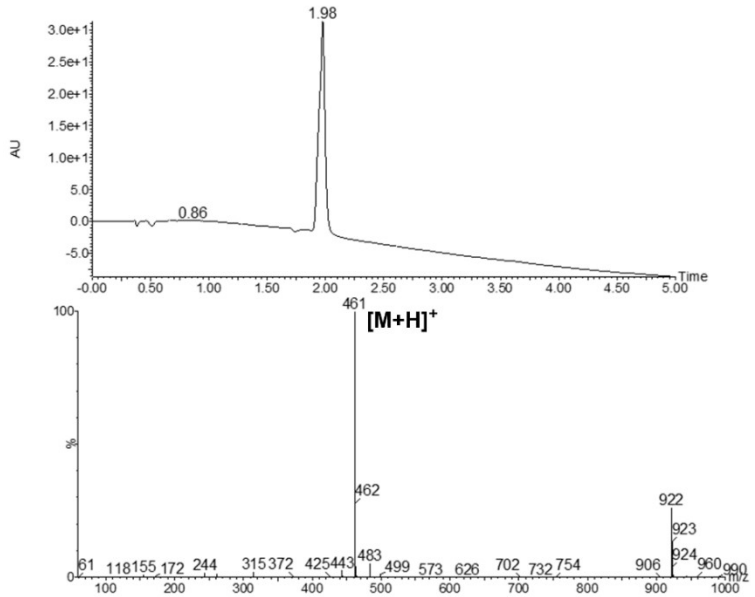
Resin 26
Wang resin (500 mg) was washed with dichloromethane (5x), and subsequently reacted with Fmoc-Lys(Mtt)-OH (1.0 mmol), HOBt (1.0 mmol), DMAP (0.25 mmol), and DIC (1.0 mmol) in DMF (2.5 mL) and DCM (2.5 mL). The reaction mixture was shaken for 16 hours at lab temperature. Afterwards, a solid support was washed with DMF (5x) and DCM (5x).

Resin 27
Resin 26 (500 mg) was subjected to 50% piperidine in DMF for 30 min, and subsequently washed with DMF (10x) and DCM (5x). Then, the resin was reacted with DEAC (1.0 mmol), HOBt (1.0 mmol), DMAP (1.0 mmol), and DIC (1.0 mmol) in DMF (1 mL), DCM (2 mL), and DMSO (2 mL). The reaction mixture was shaken for 16 hours at lab temperature. Afterwards, a solid support was washed with DMSO (5X), DMF (5x) and DCM (5x).

Resin 28
To Resin 27 (500 mg), 1,2-dichloroethane (16.25 mL), triethylsilane (5.0 mL), hexafluoroisopropanol (2.5 mL) and trifluoroethanol (1.25 mL) were added. This way obtained heterogeneous mixture was at 60 °C shaken for the time period of 9 hours. Afterwards, a solid support was washed with DCM (10x). Then, the resin was reacted with Boc-Ala-OH (1 mmol), HOBt (1 mmol), and DIC (1 mmol) in DMF (2.5 mL) and DCM (2.5 mL). The reaction mixture was shaken for 2 hour at lab temperature. Afterwards, a solid support was washed with DMF (10x) and DCM (5x).

Lys - DEAC
Resin 28 was subjected to 50% TFA in DCM (5 ml). The reaction mixture was shaken for 30 minutes at lab temperature. Afterwards, TFA solution was collected and solvents were evaporated under stream of nitrogen. Oily residuum was dissolved in acetonitrile/water 1:1 and purification was performed on a semi-prep HPLC column (Aeris 5 µm 150 x 21.2 mm peptide XB-C18 100 Å, Phenomenex, California, USA), using a gradient of 30–60% acetonitrile in ammonium acetate (10 mM) in ultrapure H_2O within 10 min. The combined fractions were concentrated in vacuo, and freeze-dried for 48 hours.

Figure S8: LC-MS analysis of isolated fragment **Lys - DEAC** (Rt = 1.98 min).



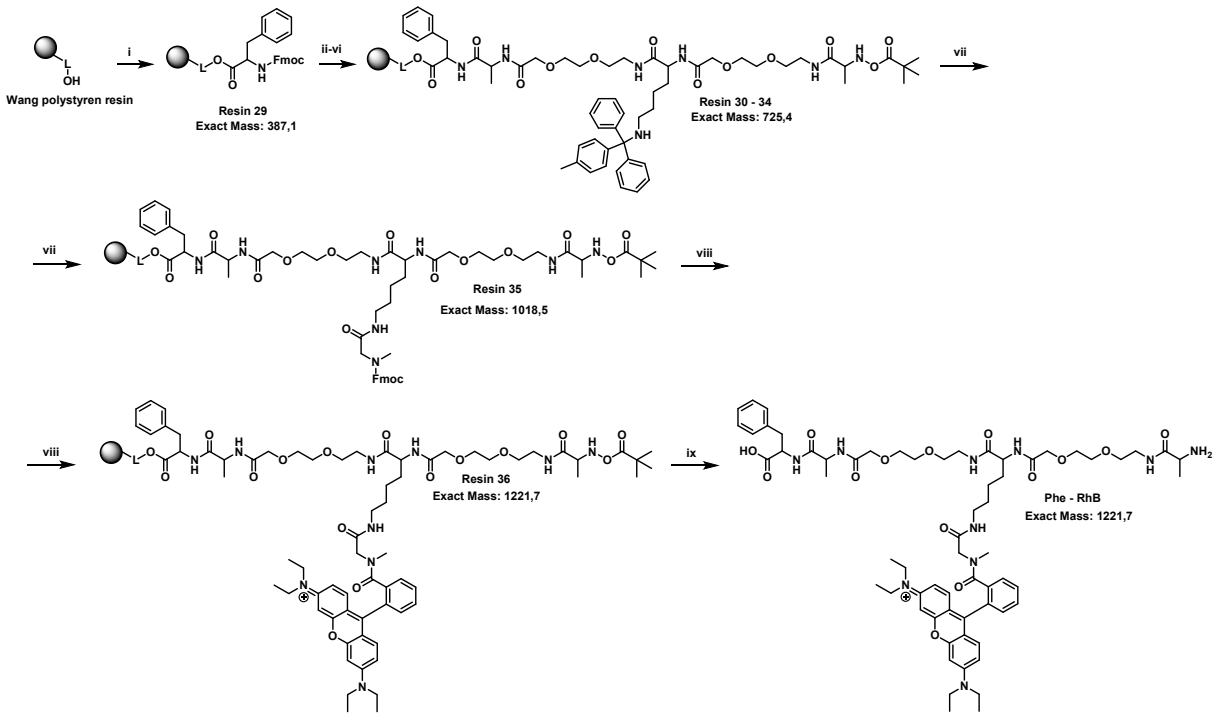
Method: Amonnium acetate (10 mM) in ultrapure water (*V*/*V*) and acetonitrile (gradient 20–80% during the first 4.5 min). LC-MS column: (50 x 3.0 mm XSelect HSS T3 2.5 µm XP C18, Waters, Borehamwood, UK).

HRMS (ESI):

m/z calcd $C_{23}H_{32}N_4O_6$ for $[M+H]^+$ = 461,2359, found $[M+H]^+$ = 461,2400;

1.2.2. Fragment Phe - RhB

Scheme S3: Synthesis of fragment **Phe - RhB**.



i. Fmoc-Phe-OH, HOBt, DMAP, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 16h; ii. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Fmoc-Ala-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; iii. a.) 50% piperidine in DMF (*V*/*V*), rt,

30 min; b.) Fmoc-PEG-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; iv. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Fmoc-Lys(Mtt)-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; v. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Fmoc-PEG-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; vi. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Boc-Ala-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; vii. a.) DCE:TES:HFIP:TFE 13:4:2:1 (*V*/*V*/*V*/*V*), 60 °C, 9h; b.) Fmoc-Sar-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; viii. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) RhB, HOBt, DMAP, DIC, DMF:DCM:DMSO 1:2:2 (*V*/*V*/*V*), rt, 16h; ix. 50% TFA in DCM (*V*/*V*), rt, 30 min.

Molecular weights of cleaved peptides are reported. Tert-butyloxycarbonyl (Boc), and 4-methyltrityl (Mtt) protecting groups are removed during the cleavage of the final compound from the resin with 50% TFA in DCM

Resin 29

Wang resin (500 mg) was washed with dichloromethane (5x), and subsequently reacted with Fmoc-Phe-OH (1.0 mmol), HOBt (1.0 mmol), DMAP (0.25 mmol), and DIC (1.0 mmol) in DMF (2.5 mL) and DCM (2.5 mL). The reaction mixture was shaken for 16 hours at lab temperature. Afterwards, a solid support was washed with DMF (5x) and DCM (5x).

Resin 30 - 34

An appropriate resin (500 mg) was subjected to 50% piperidine in DMF for 30 min, and subsequently washed with DMF (10x) and DCM (5x). Then, a solid support was reacted with a suitable amino acid or PEG spacer (1.0 mmol), HOBt (1.0 mmol), and DIC (1.0 mmol) in DMF (2.5 mL) and DCM (2.5 mL). A reaction mixture was shaken at lab temperature. Afterwards, a solid support was washed with DMF (10x) and DCM (5x).

Resin 35

To Resin 34 (500 mg), 1,2-dichloroethane (16.25 mL), triethylsilane (5.0 mL), hexafluoroisopropanol (2.5 mL) and trifluoroethanol (1.25 mL) were added. This way obtained heterogeneous mixture was at 60 °C shaken for the time period of 9 hours. Afterwards, a solid support was washed with DCM (10x). Then, the resin was reacted with Fmoc-Sar-OH (1 mmol), HOBt (1 mmol), and DIC (1 mmol) in DMF (2.5 mL) and DCM (2.5 mL). The reaction mixture was shaken for 2 hour at lab temperature. Afterwards, a solid support was washed with DMF (10x) and DCM (5x).

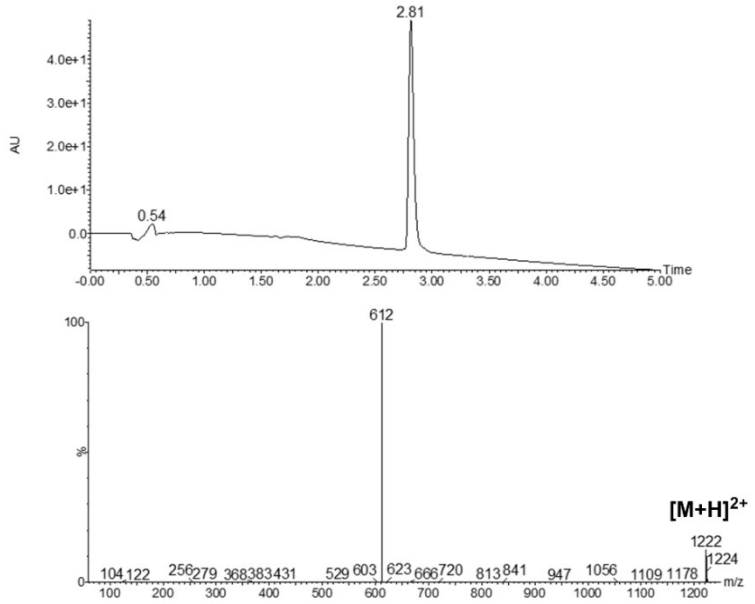
Resin 36

Resin 35 (500 mg) was subjected to 50% piperidine in DMF for 15 min, and subsequently washed with DMF (10x) and DCM (5x). Then, the resin was reacted with RhB (1 mmol), HOBt (1 mmol), DMAP (1 mmol), and DIC (1 mmol) in DMF (1 mL), DCM (2 mL), and DMSO (2 mL). The reaction mixture was shaken for 16 hours at lab temperature. Afterwards, a solid support was washed with DMSO (5X), DMF (5x) and DCM (5x).

Phe - RhB

Resin 36 was subjected to 50% TFA in DCM (5 ml). The reaction mixture was shaken for 30 minutes at lab temperature. Afterwards, TFA solution was collected and solvents were evaporated under stream of nitrogen. Oily residuum was dissolved in acetonitrile/water 1:1 and purification was performed on a semi-prep HPLC column (Aeris 5 µm 150 x 21.2 mm peptide XB-C18 100 Å, Phenomenex, California, USA), using a gradient of 30–60% acetonitrile in 0.1 % trifluoracetic acid in ultrapure H_2O within 10 min. The combined fractions were concentrated in vacuo, and freeze-dried for 48 hours.

Figure S9: LC-MS analysis of isolated fragment **Phe - RhB** (Rt = 2.81 min).



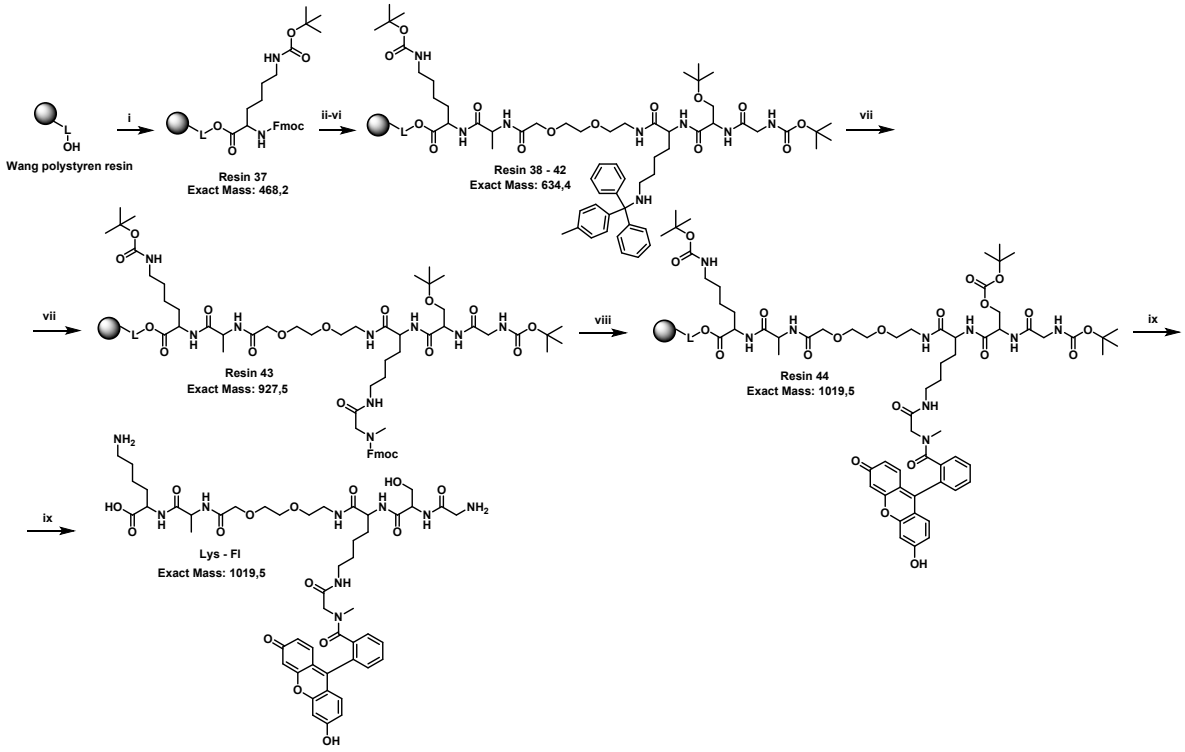
Method: Ammonium acetate (10 mM) in ultrapure water (*V*/*V*) and acetonitrile (gradient 20–80% during the first 4.5 min). LC-MS column: (50 x 3.0 mm XSelect HSS T3 2.5 µm XP C18, Waters, Borehamwood, UK).

HRMS (ESI):

m/z calcd for $C_{64}H_{89}N_{10}O_{14}^{+}$ = 1221.6554, found M = 1221.6548

1.2.3. Fragment Lys - Fl

Scheme S4: Synthesis of fragment **Lys - Fl**.



i. Fmoc-Lys(Boc)-OH, HOBt, DMAP, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 16h; ii. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Fmoc-Ala-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; iii. a.) 50% piperidine in DMF (*V*/*V*),

rt, 30 min; b.) Fmoc-PEG-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; iv. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Fmoc-Lys(Mtt)-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; v. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Fmoc-Ser(tBu)-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; vi. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Boc-Gly-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; VII. a.) DCE:TES:HFIP:TFE 13:4:2:1 (*V*/*V*/*V*/*V*), 60 °C, 9h; b.) Fmoc-Sar-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; viii. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Fl, HOBt, DMAP, DIC, DMF:DCM:DMSO 1:2:2 (*V*/*V*/*V*), rt, 16h; X. 50% TFA in DCM (*V*/*V*), rt, 45 min.

Molecular weights of cleaved peptides are reported. Tert-butyloxycarbonyl (Boc), tertbutyl (tBu) and 4-methyltrityl (Mtt) protecting groups are removed during the cleavage of the final compound from the resin with 50% TFA in DCM

Resin 37

Wang resin (500 mg) was washed with dichloromethane (5x), and subsequently reacted with Fmoc-Lys(Boc)-OH (1.0 mmol), HOBt (1.0 mmol), DMAP (0.25 mmol), and DIC (1.0 mmol) in DMF (2.5 mL) and DCM (2.5 mL). The reaction mixture was shaken for 16 hours at lab temperature. Afterwards, a solid support was washed with DMF (5x) and DCM (5x).

Resin 38 - 42

An appropriate resin (500 mg) was subjected to 50% piperidine in DMF for 30 min, and subsequently washed with DMF (10x) and DCM (5x). Then, a solid support was reacted with a suitable amino acid or PEG spacer (1.0 mmol), HOBt (1.0 mmol), and DIC (1.0 mmol) in DMF (2.5 mL) and DCM (2.5 mL). A reaction mixture was shaken at lab temperature. Afterwards, a solid support was washed with DMF (10x) and DCM (5x).

Resin 43

To Resin 42 (500 mg), 1,2-dichloroethane (16.25 mL), triethylsilane (5.0 mL), hexafluoroisopropanol (2.5 mL) and trifluoroethanol (1.25 mL) were added. This way obtained heterogeneous mixture was at 60 °C shaken for the time period of 9 hours. Afterwards, a solid support was washed with DCM (10x). Then, the resin was reacted with Fmoc-Sar-OH (1 mmol), HOBt (1 mmol), and DIC (1 mmol) in DMF (2.5 mL) and DCM (2.5 mL). The reaction mixture was shaken for 2 hour at lab temperature. Afterwards, a solid support was washed with DMF (10x) and DCM (5x).

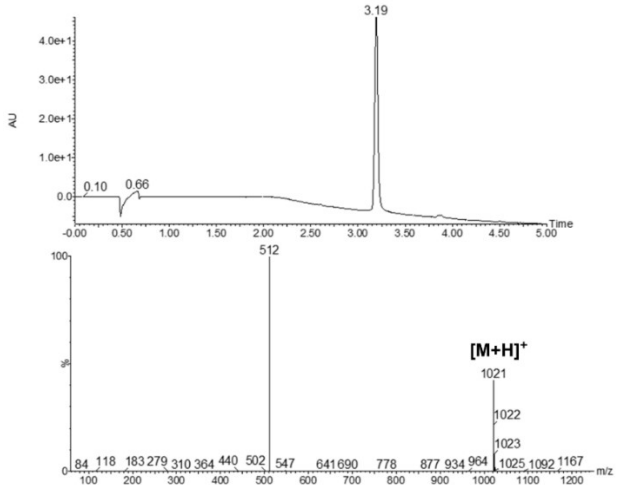
Resin 44

Resin 43 (500 mg) was subjected to 50% piperidine in DMF for 15 min, and subsequently washed with DMF (10x) and DCM (5x). Then, the resin was reacted with Fl (1 mmol), HOBt (1 mmol), DMAP (1 mmol), and DIC (1 mmol) in DMF (1 mL), DCM (2 mL), and DMSO (2 mL). The reaction mixture was shaken for 16 hours at lab temperature. Afterwards, a solid support was washed with DMSO (5X), DMF (5x) and DCM (5x).

Lys - Fl

Resin 44 was subjected to 50% TFA in DCM (5 ml). The reaction mixture was shaken for 30 minutes at lab temperature. Afterwards, TFA solution was collected and solvents were evaporated under stream of nitrogen. Oily residuum was dissolved in acetonitrile/water 1:1 and purification was performed on a semi-prep HPLC column (Aeris 5 µm 150 x 21.2 mm peptide XB-C18 100 Å, Phenomenex, California, USA), using a gradient of 30–60% acetonitrile in ammonium acetate (10 mM) in ultrapure H_2O within 10 min. The combined fractions were concentrated in vacuo, and freeze-dried for 48 hours.

Figure S10: LC-MS analysis of isolated fragment **Lys - Fl** (Rt = 3.19 min).



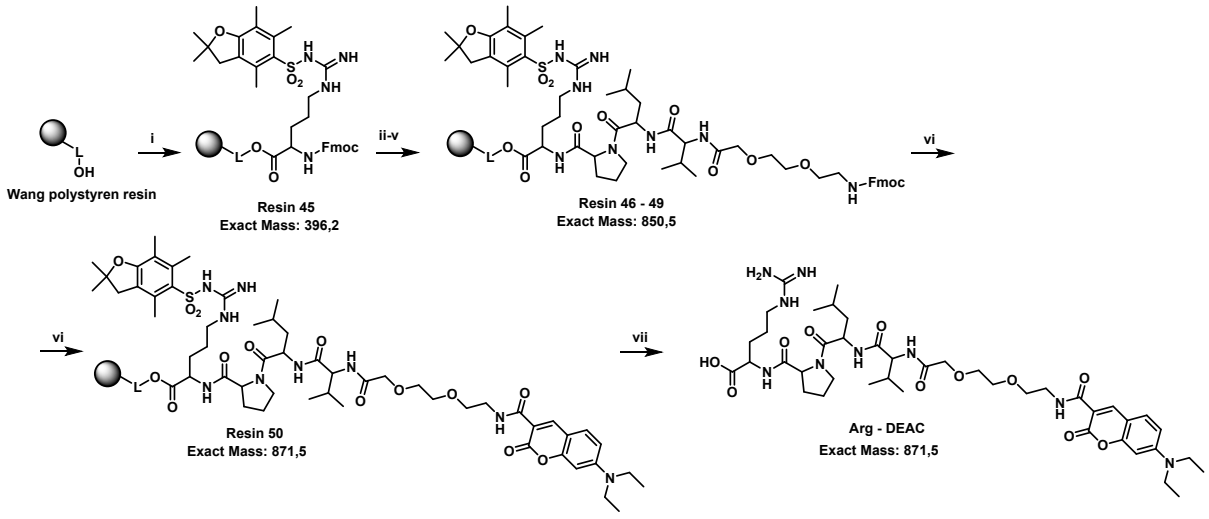
Method: Ammonium acetate (10 mM) in ultrapure water (*V*/*V*) and acetonitrile (gradient 5–70% during the first 4.5 min). LC-MS column: (50 x 3.0 mm XSelect HSS T3 2.5 µm XP C18, Waters, Borehamwood, UK).

HRMS (ESI):

m/z calcd $C_{49}H_{65}N_9O_{15}$ for $[M+H]^+$ = 1020.4673, found $[M+H]^+$ = 1020.4672

1.2.4. Fragment Arg - DEAC

Scheme S5: Synthesis of fragment **Arg - DEAC**.



i. Fmoc-Arg(Pbf)-OH, HOBt, DMAP, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 16h; ii. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Fmoc-Pro-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; iii. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Fmoc-Leu-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; iv. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Fmoc-Val-OH (0.2 M), HOBt (0.2 M), DIC (0.2 M), DMF:DCM 1:1 (*V*/*V*), rt, 2h; v. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Fmoc-PEG-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; vi. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) DEAC, HOBt, DMAP, DIC, DMF:DCM:DMSO 1:2:2 (*V*/*V*/*V*), rt, 16h; vii. 50% TFA in DCM (*V*/*V*), rt, 2h.

Molecular weights of cleaved peptides are reported. Tert-butyloxycarbonyl (Boc), and 4-methyltrityl (Mtt) protecting groups are removed during the cleavage of the final compound from the resin with 50% TFA in DCM

Resin 45

Wang resin (500 mg) was washed with dichloromethane (5x), and subsequently reacted with Fmoc-Arg(Pbf)-OH (1.0 mmol), HOBt (1.0 mmol), DMAP (0.25 mmol), and DIC (1.0 mmol) in DMF (2.5 mL) and DCM (2.5 mL). The reaction mixture was shaken for 16 hours at lab temperature. Afterwards, a solid support was washed with DMF (5x) and DCM (5x).

Resin 46 - 49

An appropriate resin (500 mg) was subjected to 50% piperidine in DMF for 30 min, and subsequently washed with DMF (10x) and DCM (5x). Then, a solid support was reacted with a suitable amino acid or PEG spacer (1.0 mmol), HOBt (1.0 mmol), and DIC (1.0 mmol) in DMF (2.5 mL) and DCM (2.5 mL). A reaction mixture was shaken at lab temperature. Afterwards, a solid support was washed with DMF (10x) and DCM (5x).

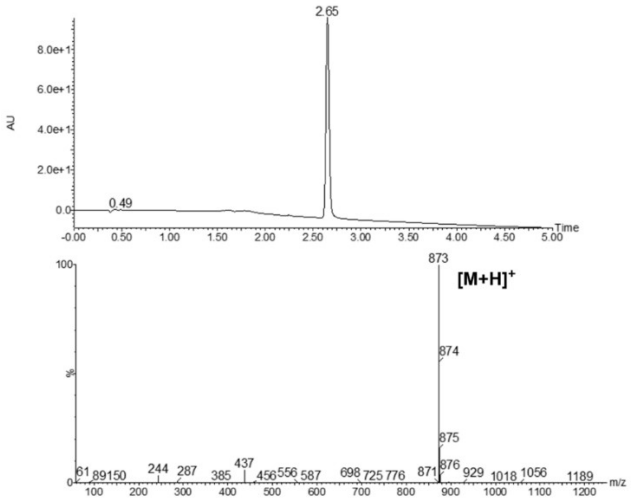
Resin 50

Resin 49 (500 mg) was subjected to 50% piperidine in DMF for 15 min, and subsequently washed with DMF (10x) and DCM (5x). Then, the resin was reacted with DEAC (1 mmol), HOBt (1 mmol), DMAP (1 mmol), and DIC (1 mmol) in DMF (1 mL), DCM (2 mL), and DMSO (2 mL). The reaction mixture was shaken for 16 hours at lab temperature. Afterwards, a solid support was washed with DMSO (5X), DMF (5x) and DCM (5x).

Arg - DEAC

Resin 50 was subjected to 50% TFA in DCM (5 ml). The reaction mixture was shaken for 30 minutes at lab temperature. Afterwards, TFA solution was collected and solvents were evaporated under stream of nitrogen. Oily residuum was dissolved in acetonitrile/water 1:1 and purification was performed on a semi-prep HPLC column (Aeris 5 µm 150 x 21.2 mm peptide XB-C18 100 Å, Phenomenex, California, USA), using a gradient of 40–70% acetonitrile in ammonium acetate (10 mM) in ultrapure H_2O within 10 min. The combined fractions were concentrated in vacuo, and freeze-dried for 48 hours.

Figure S11: LC-MS analysis of isolated fragment **Arg - DEAC** (Rt = 2.65 min).



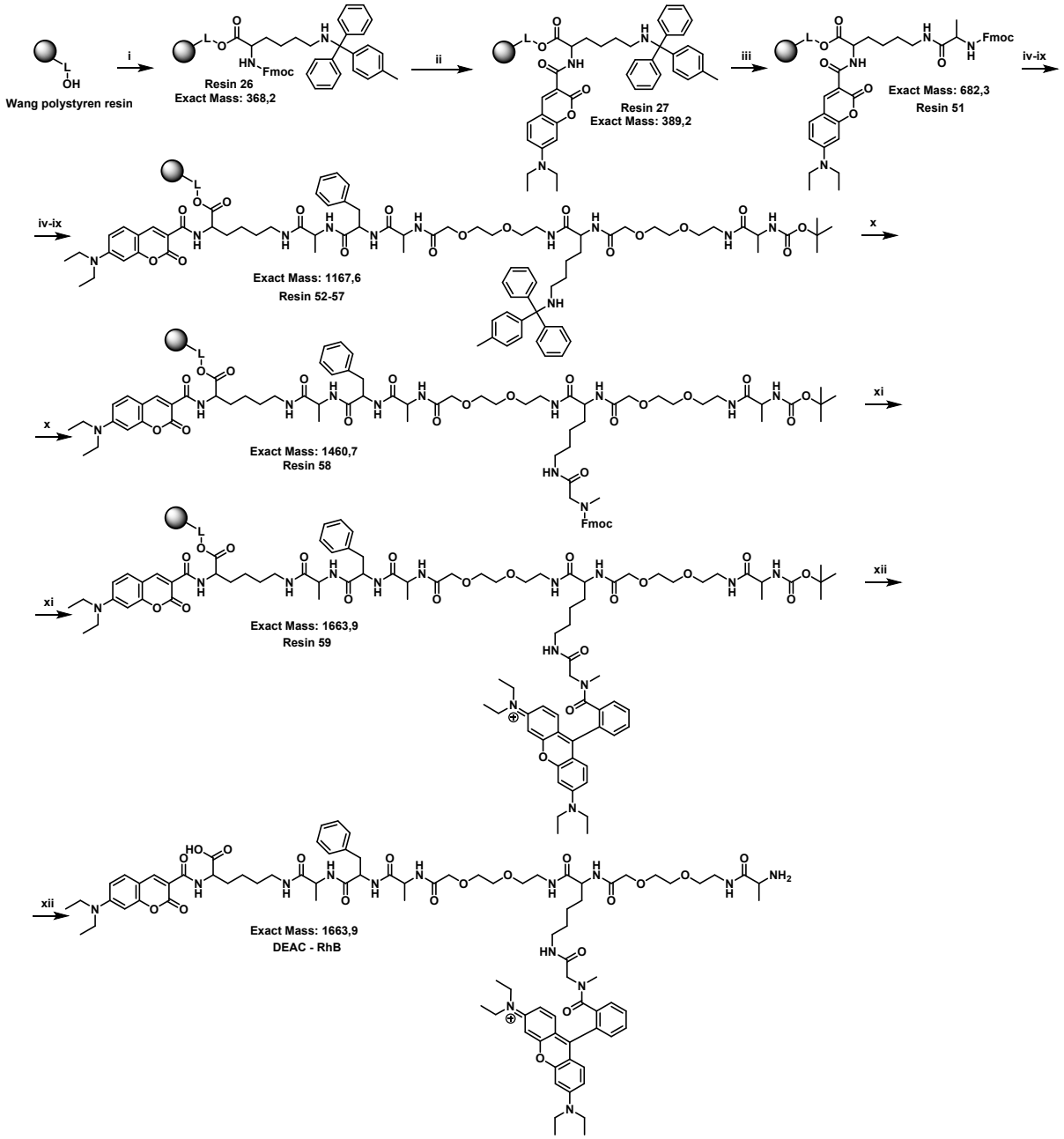
Method: Ammonium acetate (10 mM) in ultrapure water (*V*/*V*) and acetonitrile (gradient 20–80% during the first 4.5 min). LC-MS column: (50 x 3.0 mm XSelect HSS T3 2.5 µm XP C18, Waters, Borehamwood, UK).

HRMS (ESI):

m/z calcd $C_{42}H_{65}N_8O_{11}$ for $[M+H]^+$ = 872.4876, found $[M+H]^+$ = 872.4886

1.2.5. Fragment DEAC - RhB

Scheme S6: Synthesis of fragment **DEAC - RhB**.



i. Fmoc-Lys(Mtt)-OH, HOBt, DMAP, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 16h; ii. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) DEAC, HOBt, DMAP, DIC, DMF:DCM:DMSO 1:2:2 (*V*/*V*/*V*), rt, 16h; iii. a.) DCE:TES:HFIP:TFE 13:4:2:1 (*V*/*V*/*V*/*V*), 60 °C, 9h; b.) Fmoc-Ala-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; iv. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Fmoc-Phe-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; V. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Fmoc-Ala-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; VI. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Fmoc-PEG-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; VII. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Fmoc-Lys(Mtt)-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; viii. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Fmoc-PEG-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; ix. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Boc-Ala-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; x. a.) DCE:TES:HFIP:TFE 13:4:2:1 (*V*/*V*/*V*/*V*), 60 °C, 9h; b.) Fmoc-Sar-OH, HOBt, DIC, DMF:DCM 1:1

(*V*/*V*), rt, 2h; xi. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) RhB, HOBt, DMAP, DIC, DMF:DCM:DMSO 1:2:2 (*V*/*V*/*V*), rt, 16h; xii. 50% TFA in DCM (*V*/*V*), rt, 30 min.

Molecular weights of cleaved peptides are reported. Tert-butyloxycarbonyl (Boc), and 4-methyltrityl (Mtt) protecting groups are removed during the cleavage of the final compound from the resin with 50% TFA in DCM

Resin 51
To Resin 27 (500 mg), 1,2-dichloroethane (16.25 mL), triethylsilane (5.0 mL), hexafluoroisopropanol (2.5 mL) and trifluoroethanol (1.25 mL) were added. This way obtained heterogeneous mixture was at 60 °C shaken for the time period of 9 hours. Afterwards, a solid support was washed with DCM (10x). Then, the resin was reacted with Fmoc-Ala-OH (1 mmol), HOBt (1 mmol), and DIC (1 mmol) in DMF (2.5 mL) and DCM (2.5 mL). The reaction mixture was shaken for 2 hour at lab temperature. Afterwards, a solid support was washed with DMF (10x) and DCM (5x).

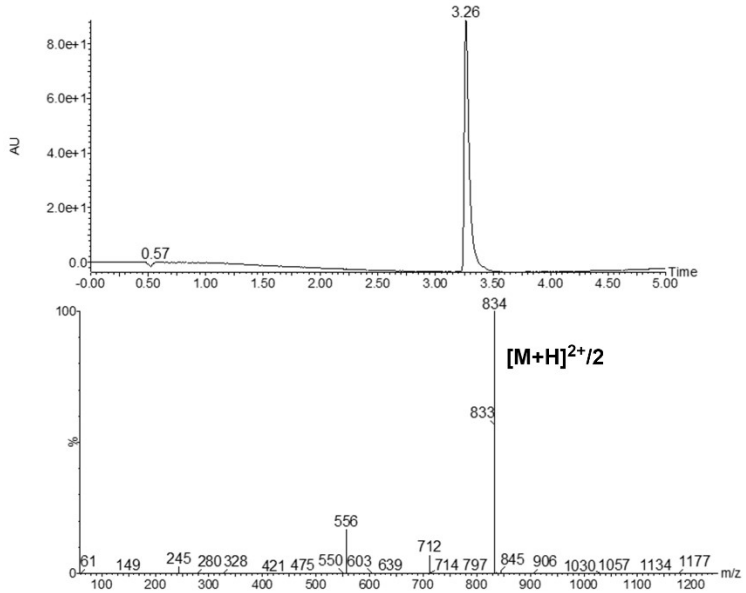
Resin 52 - 57
An appropriate resin (500 mg) was subjected to 50% piperidine in DMF for 15 min, and subsequently washed with DMF (10x) and DCM (5x). Then, a solid support was reacted with a suitable amino acid or PEG spacer (1.0 mmol), HOBt (1.0 mmol), and DIC (1.0 mmol) in DMF (2.5 mL) and DCM (2.5 mL). A reaction mixture was shaken at lab temperature for 30 min. Afterwards, a solid support was washed with DMF (10x) and DCM (5x).

Resin 58
To Resin 57 (500 mg), 1,2-dichloroethane (16.25 mL), triethylsilane (5.0 mL), hexafluoroisopropanol (2.5 mL) and trifluoroethanol (1.25 mL) were added. This way obtained heterogeneous mixture was at 60 °C shaken for the time period of 9 hours. Afterwards, a solid support was washed with DCM (10x). Then, the resin was reacted with Fmoc-Sar-OH (1 mmol), HOBt (1 mmol), and DIC (1 mmol) in DMF (2.5 mL) and DCM (2.5 mL). The reaction mixture was shaken for 1 hour at lab temperature. Afterwards, a solid support was washed with DMF (5x) and DCM (5x).

Resin 59
Resin 58 (500 mg) was subjected to 50% piperidine in DMF for 15 min, and subsequently washed with DMF (10x) and DCM (5x). Then, the resin was reacted with RhB (1 mmol), HOBt (1 mmol), DMAP (1 mmol), and DIC (1 mmol) in DMF (1 mL), DCM (2 mL), and DMSO (2 mL). The reaction mixture was shaken for 16 hours at lab temperature. Afterwards, a solid support was washed with DMSO (5X), DMF (5x) and DCM (5x).

DEAC - RhB
Resin 59 was subjected to 50% TFA in DCM (5 ml). The reaction mixture was shaken for 30 minutes at lab temperature. Afterwards, TFA solution was collected and solvents were evaporated under stream of nitrogen. Oily residuum was dissolved in acetonitrile/water 1:1 and purification was performed on a semi-prep HPLC column (Aeris 5 µm 150 x 21.2 mm peptide XB-C18 100 Å, Phenomenex, California, USA), using a gradient of 40–70% acetonitrile in 0.1% trifluoroacetic acid in ultrapure H_2O within 10 min. The combined fractions were concentrated in vacuo, and freeze-dried for 48 hours.

Figure S12: LC-MS analysis of isolated fragment **DEAC - RhB** (Rt = 3.26 min).



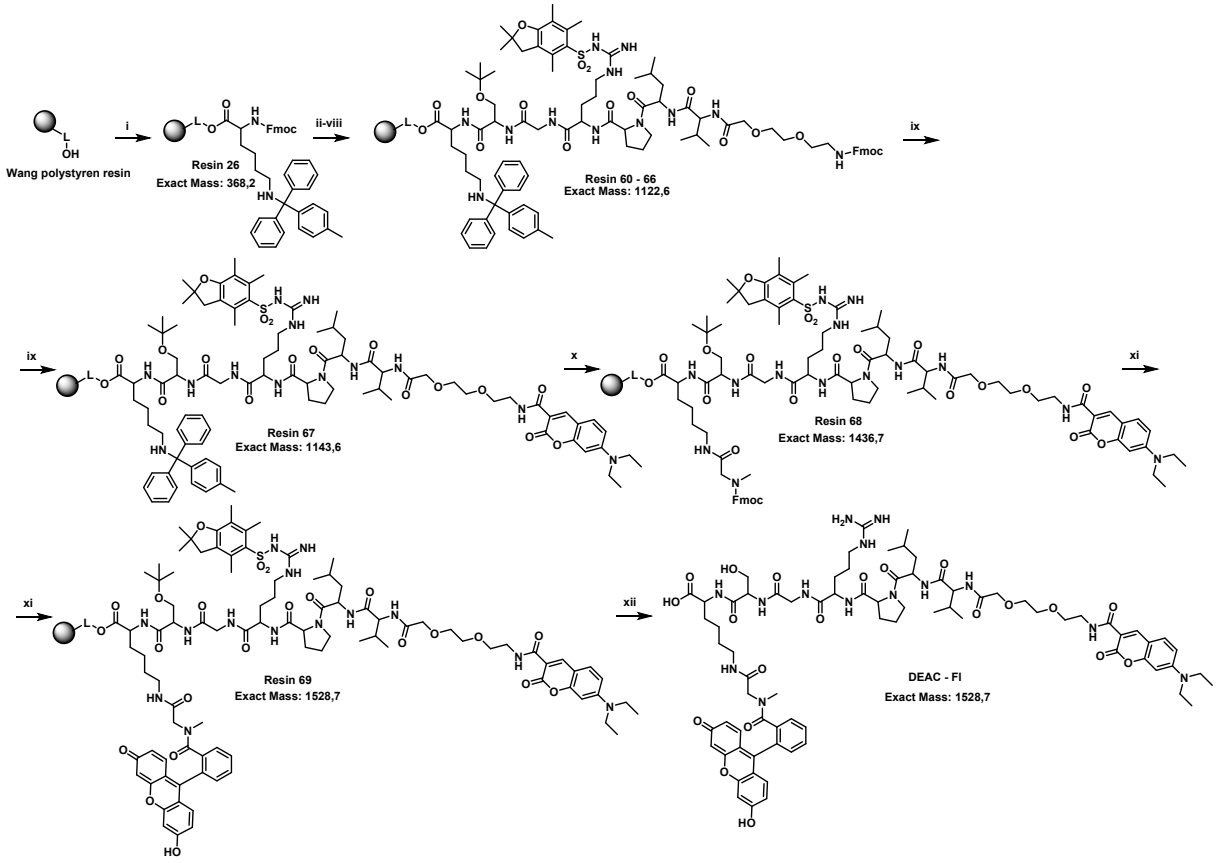
Method: Ammonium acetate (10 mM) in ultrapure water (*V*/*V*) and acetonitrile (gradient 20–80% during the first 4.5 min). LC-MS column: (50 x 3.0 mm XSelect HSS T3 2.5 µm XP C18, Waters, Borehamwood, UK).

HRMS (ESI):

m/z calcd $C_{87}H_{119}N_{14}O_{19}^{+}$ for $[M]^{+}$ = 1663.8770, found $[M]^{+}$ = 1663.8767

1.2.6. Fragment DEAC - Fl

Scheme S7: Synthesis of fragment **DEAC - Fl**.



i. Fmoc-Lys(Mtt)-OH, HOBt, DMAP, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 16h; ii. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Fmoc-Ser(tBu)-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; iii. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Fmoc-Gly-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; iv. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Fmoc-Arg(Pbf)-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; v. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Fmoc-Pro-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; vi. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Fmoc-Leu-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; vii. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Fmoc-Val-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; viii. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Fmoc-PEG-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; ix. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) DEAC, HOBt, DMAP, DIC, DMF:DCM:DMSO 1:2:2 (*V*/*V*/*V*), rt, 16h; x. a.) DCE:TES:HFIP:TFE 13:4:2:1 (*V*/*V*/*V*/*V*), 60 °C, 9h; b.) Fmoc-Sar-OH, HOBt, DIC, DMF:DCM 1:1 (*V*/*V*), rt, 2h; XI. a.) 50% piperidine in DMF (*V*/*V*), rt, 30 min; b.) Fl, HOBt, DMAP, DIC, DMF:DCM:DMSO 1:2:2 (*V*/*V*/*V*), rt, 16h; XIII. 50% TFA in DCM (*V*/*V*), rt, 2h.

Molecular weights of cleaved peptides are reported. Tert-butyloxycarbonyl (Boc), tert-butyl (tBu) and 4-methyltrityl (Mtt) protecting groups are removed during the cleavage of the final compound from the resin with 50% TFA in DCM

Resin 60 - 66

An appropriate resin (500 mg) was subjected to 50% piperidine in DMF for 30 min, and subsequently washed with DMF (10x) and DCM (5x). Then, a solid support was reacted with a suitable amino acid or PEG spacer (1.0 mmol), HOBt (1.0 mmol), and DIC (1.0 mmol) in DMF (2.5 mL) and DCM (2.5 mL). A

reaction mixture was shaken at lab temperature. Afterwards, a solid support was washed with DMF (10x) and DCM (5x).

Resin 67

Resin 66 (500 mg) was subjected to 50% piperidine in DMF for 15 min, and subsequently washed with DMF (10x) and DCM (5x). Then, the resin was reacted with DEAC (1 mmol), HOBt (1 mmol), DMAP (1 mmol), and DIC (1 mmol) in DMF (1 mL), DCM (2 mL), and DMSO (2 mL). The reaction mixture was shaken for 16 hours at lab temperature. Afterwards, a solid support was washed with DMSO (5X), DMF (5x) and DCM (5x).

Resin 68

To Resin 67 (500 mg), 1,2-dichloroethane (16.25 mL), triethylsilane (5.0 mL), hexafluoroisopropanol (2.5 mL) and trifluoroethanol (1.25 mL) were added. This way obtained heterogeneous mixture was at 60 °C shaken for the time period of 9 hours. Afterwards, a solid support was washed with DCM (10x). Then, the resin was reacted with Fmoc-Sar-OH (1 mmol), HOBt (1 mmol), and DIC (1 mmol) in DMF (2.5 mL) and DCM (2.5 mL). The reaction mixture was shaken for 1 hour at lab temperature. Afterwards, a solid support was washed with DMF (5x) and DCM (5x).

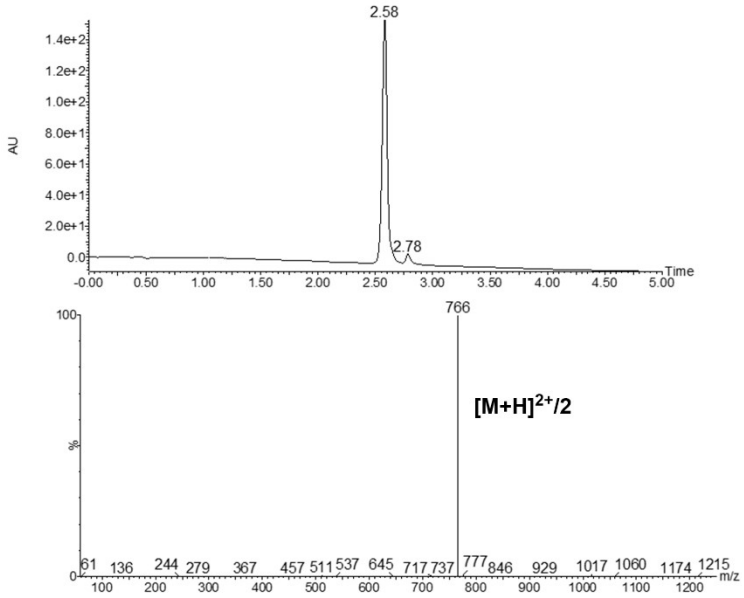
Resin 69

Resin 68 (500 mg) was subjected to 50% piperidine in DMF for 15 min, and subsequently washed with DMF (10x) and DCM (5x). Then, the resin was reacted with Fl (1 mmol), HOBt (1 mmol), DMAP (1 mmol), and DIC (1 mmol) in DMF (1 mL), DCM (2 mL), and DMSO (2 mL). The reaction mixture was shaken for 16 hours at lab temperature. Afterwards, a solid support was washed with DMSO (5X), DMF (5x) and DCM (5x).

DEAC - Fl

Resin 69 was subjected to 50% TFA in DCM (5 ml). The reaction mixture was shaken for 30 minutes at lab temperature. Afterwards, TFA solution was collected and solvents were evaporated under stream of nitrogen. Oily residuum was dissolved in acetonitrile/water 1:1 and purification was performed on a semi-prep HPLC column (Aeris 5 µm 150 x 21.2 mm peptide XB-C18 100 Å, Phenomenex, California, USA), using a gradient of 30–60% acetonitrile in 0.1% trifluoroacetic acid in ultrapure H_2O within 10 min. The combined fractions were concentrated in vacuo, and freeze-dried for 48 hours.

Figure S13: LC-MS analysis of isolated fragment **DEAC - Fl** (Rt = 2.58 min).



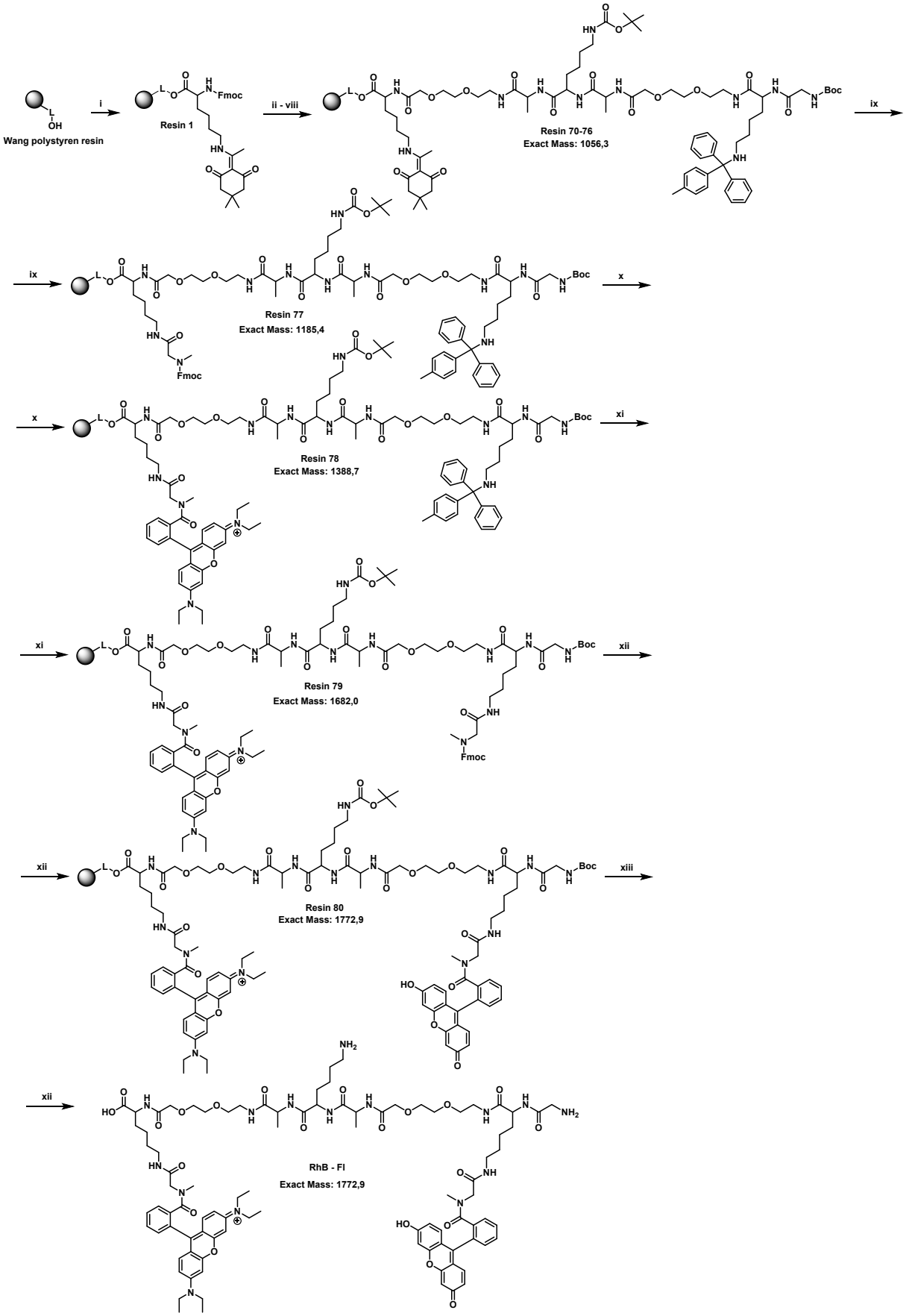
Method: Ammomnium acetate (10 mM) in ultrapure water (*V*/*V*) and acetonitrile (gradient 20–80% during the first 4.5 min). LC-MS column: (50 x 3.0 mm XSelect HSS T3 2.5 µm XP C18, Waters, Borehamwood, UK).

HRMS (ESI):

m/z calcd $C_{76}H_{100}N_{14}O_{20}$ for $[M+H]^+$ = 1529.7311, found $[M+H]^+$ = 1529.7312

1.2.7. Fragment RhB - Fl

Scheme S8: Synthesis of fragment **RhB - Fl**



i. Fmoc-Lys(Dde)-OH, HOBt, DMAP, DIC, DMF:DCM 1:1 (V/V), rt, 1h; ii. a.) 50% piperidine in DMF, rt, 15 min; b.) PEG, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 30 min; iii. a.) 50% piperidine in DMF, rt, 15 min; b.) Fmoc-Ala-OH, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 30 min; iv. a.) 50% piperidine in DMF, rt, 15 min; b.) Fmoc-Lys(Boc)-OH, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 30 min; v. a.) 50% piperidine in DMF, rt, 15 min; b.) Fmoc-Ala-OH, HOBt, DMF:DCM 1:1 (V/V), rt, 30 min; vi. a.) 50% piperidine in DMF, rt, 15 min; b.) PEG, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 30 min; vii. a.) 50% piperidine in DMF, rt, 15 min; b.) Fmoc-Lys(Mtt)-OH, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 30 min; viii. a.) 50% piperidine in DMF, rt, 15 min; b.) Fmoc-Gly(Boc)-OH, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 30 min; ix. a.) 2% $NH_2NH_2.H_2O$ in DMF (V/V), rt, 3x 3 min; b.) Fmoc-Sar-OH, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 1h; x. a.) 50% piperidine in DMF, rt, 15 min; b.) RhB, HOBt, DMAP, DIC, DMF:DCM:DMSO 1:2:2 (V/V), rt, 16h; xi. a.) DCE, TES, HFIP, TFE, 60 °C, 2,5h; b.) Fmoc-Sar-OH, HOBt, DIC, DMF:DCM 1:1 (V/V), rt, 30 min; xii. a.) 50% piperidine in DMF, rt, 15 min; b.) FL, HOBt, DMAP, DIC, DMF:DCM:DMSO 1:2:2 (V/V), rt, 16h; xiii. 50% TFA in DCM, rt, 60 min.

Molecular weights of cleaved peptides are reported. Tert-butyloxycarbonyl (Boc), and 4-methyltrityl (Mtt) protecting groups are removed during the cleavage of the final compound from the resin with 50% TFA in DCM

Resin 70 - 76

An appropriate resin (500 mg) was subjected to 50% piperidine in DMF for 15 min, and subsequently washed with DMF (10x) and DCM (5x). Then, a solid support was reacted with a suitable amino acid or PEG spacer (1.0 mmol), HOBt (1.0 mmol), and DIC (1.0 mmol) in DMF (2.5 mL) and DCM (2.5 mL). A reaction mixture was shaken at lab temperature for 30 min. Afterwards, a solid support was washed with DMF (10x) and DCM (5x).

Resin 77

To Resin 76 (500 mg), 2% $NH_2NH_2.H_2O$ in DMF (5 ml) was added, and obtained heterogeneous mixture was shaken at lab temperature. After 3 min the solution was removed and new portion was added and shaked for next 3 min. This step was repeated 3 times. Afterwards, a solid support was washed with DMF (5x) and DCM (5x). Then, the resin was reacted with Fmoc-Sar-OH (1 mmol), HOBt (1 mmol), and DIC (1 mmol) in DMF (2.5 mL) and DCM (2.5 mL). The reaction mixture was shaken for 1 hour at lab temperature. Afterwards, a solid support was washed with DMF (5x) and DCM (5x).

Resin 78

Resin 77 (500 mg) was subjected to 50% piperidine in DMF for 15 min, and subsequently washed with DMF (10x) and DCM (5x). Then, the resin was reacted with RhB (1 mmol), HOBt (1 mmol), DMAP (1 mmol), and DIC (1 mmol) in DMF (1 mL), DCM (2 mL), and DMSO (2 mL). The reaction mixture was shaken for 16 hours at lab temperature. Afterwards, a solid support was washed with DMSO (5X), DMF (5x) and DCM (5x).

Resin 79

To Resin 78 (500 mg), 1,2-dichloroethane (16.25 mL), triethylsilane (5.0 mL), hexafluoroisopropanol (2.5 mL) and trifluoroethanol (1.25 mL) were added. This way obtained heterogeneous mixture was at 60 °C shaken for the time period of 2.5 hours. Afterwards, a solid support was washed with DCM (10x). Then, the resin was reacted with Fmoc-Sar-OH (1 mmol), HOBt (1 mmol), and DIC (1 mmol) in DMF (2.5 mL) and DCM (2.5 mL). The reaction mixture was shaken for 1 hour at lab temperature. Afterwards, a solid support was washed with DMF (10x) and DCM (5x).

Resin 80

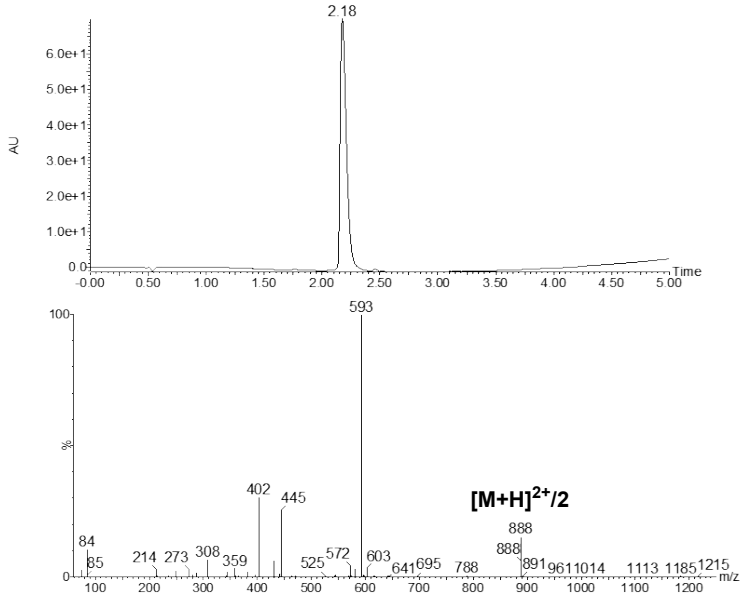
Resin 79 was subjected to 50% piperidine in DMF for 15 min, and subsequently washed with DMF (10x) and DCM (5x). Then, the resin was reacted with FL (1 mmol), HOBt (1 mmol), DMAP (1 mmol), and DIC (1 mmol) in DMF (1 mL), DCM (2 mL), and DMSO (2 mL). The reaction mixture was shaken for 16 hours

at lab temperature. Afterwards, a solid support was washed with DMSO (5X), DMF (10x) and DCM (10x). For reaching sufficient conversion, the reaction with fluorescein need to be performed in three repetition.

RhB - Fl

Resin 80 was subjected to 50% TFA in DCM (5 ml). The reaction mixture was shaken for 1 hour at lab temperature. Afterwards, TFA solution was collected and solvents were evaporated under stream of nitrogen. Oily residuum was dissolved in acetonitrile/water 1:1 and purification was performed on a semi-prep HPLC column (Aeris 5 µm 150 x 21.2 mm peptide XB-C18 100 Å, Phenomenex, California, USA), using a gradient of 20–40% acetonitrile in 0.1% HCOOH in ultrapure H_2O within 10 min. The combined fractions were concentrated in vacuo, and freeze-dried for 48 hours.

Figure S14: LC-MS analysis of isolated fragment RhB - Fl (Rt = 12.83 min).



Method: Formic acid (0.1%) in ultrapure water (V/V) and acetonitrile (gradient 20–65% during the first 20 min). LC-MS column: (150 x 4.6 mm bioZen 2.6 µm peptide XB-C18, Phenomenex, USA)

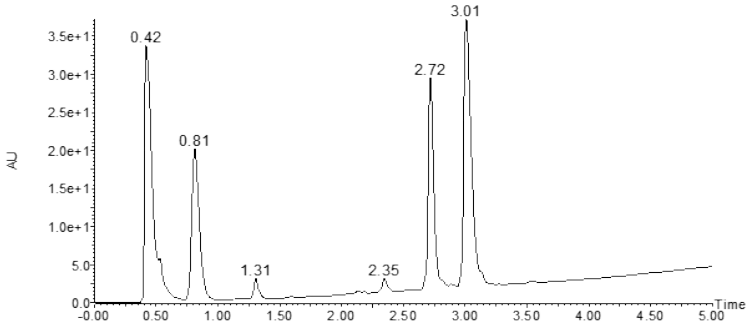
HRMS (ESI):

m/z calcd $C_{92}H_{123}N_{16}O_{20}^{+}$ for $[M+H]^{2+}$ = 1772.9167, found $[M+H]^{2+}$= 1772.9159

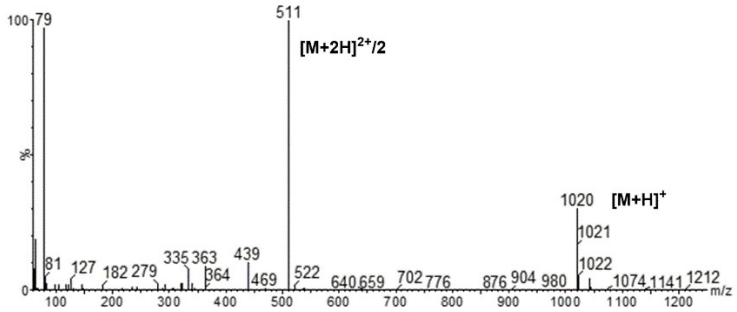
2. Biological assays

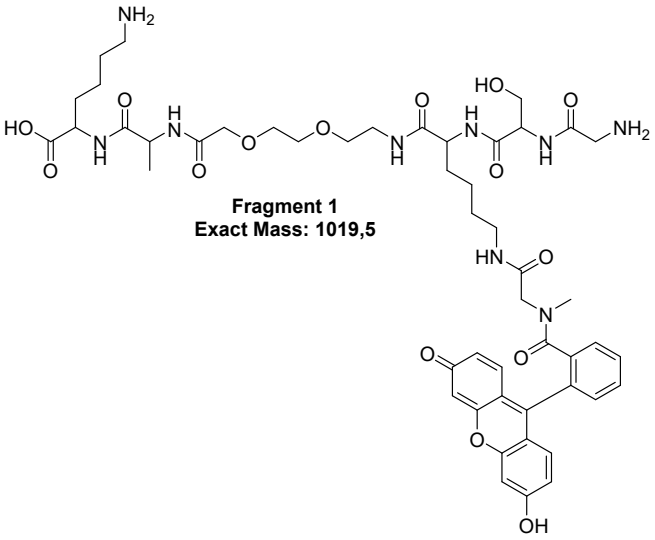
2.1. Trypsin cleavage

Figure S15: LC-MS analysis of the probe **1** cleaved by trypsin (5 ng/mL, 24 hours).



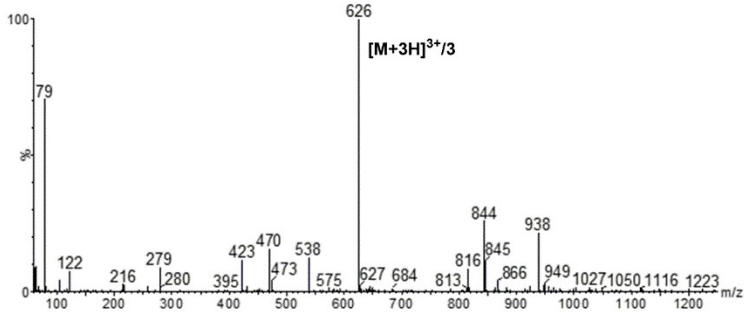
Fragment 1 (Rt = 0.81 min)

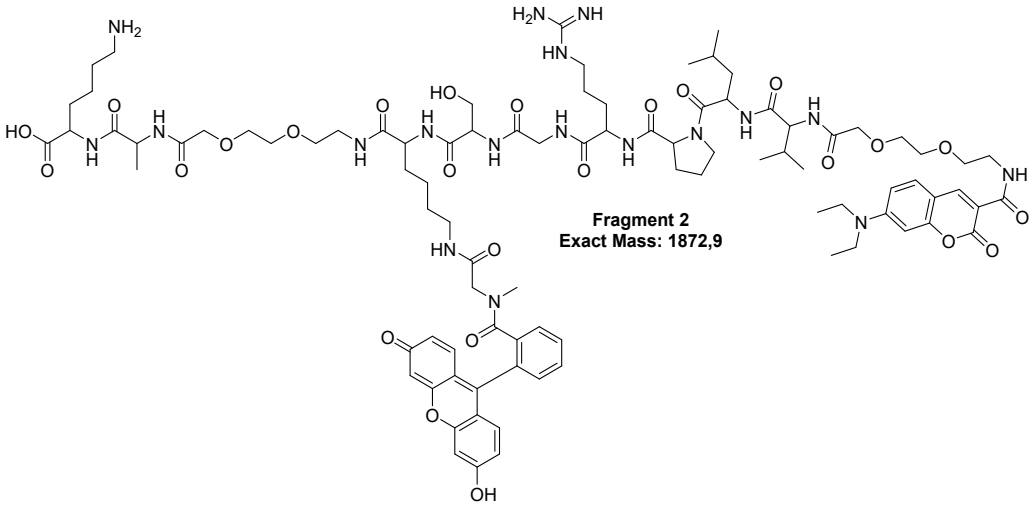




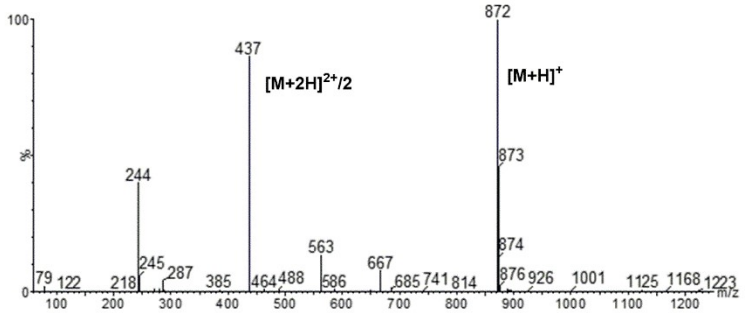
Fragment 1
Exact Mass: 1019,5

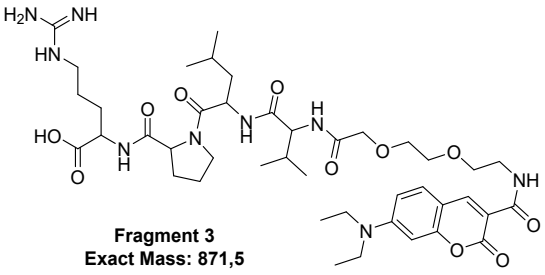
Fragment 2 (Rt = 2.35 min)



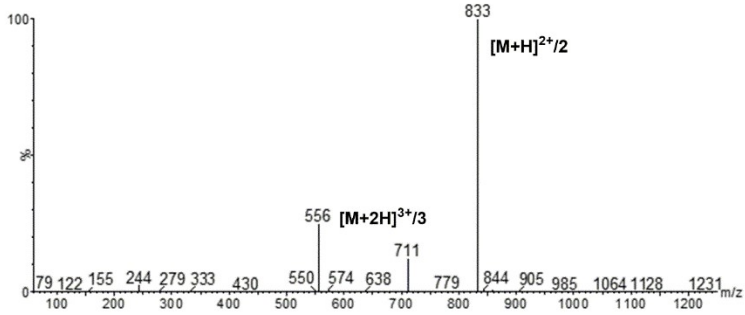


Fragment 3 (Rt = 2.72 min)





Fragment 4 (Rt = 3.01 min)



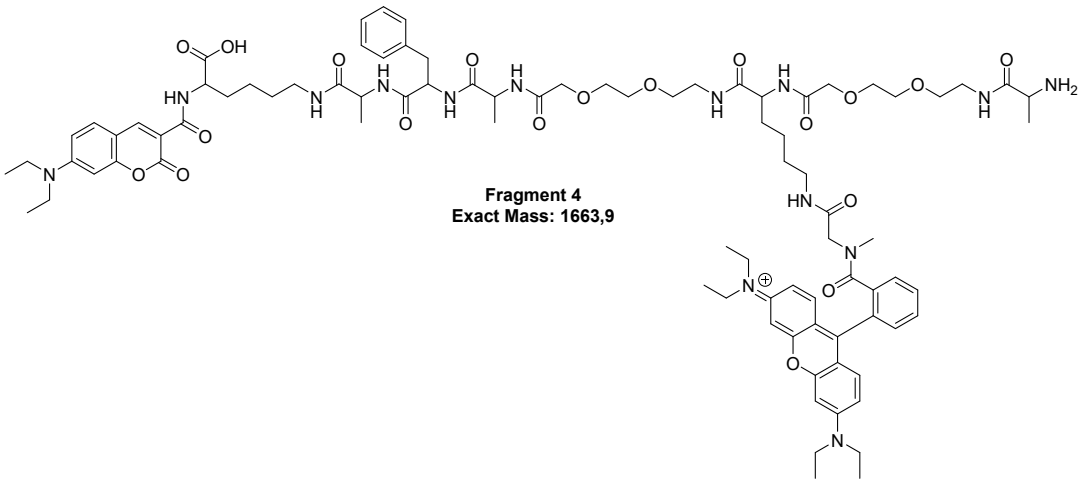
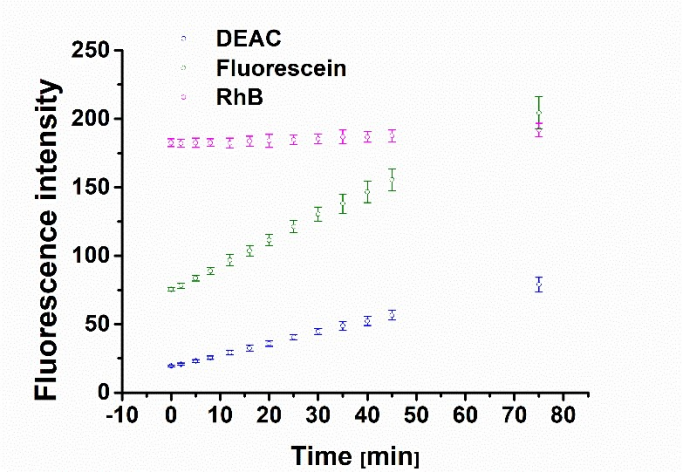
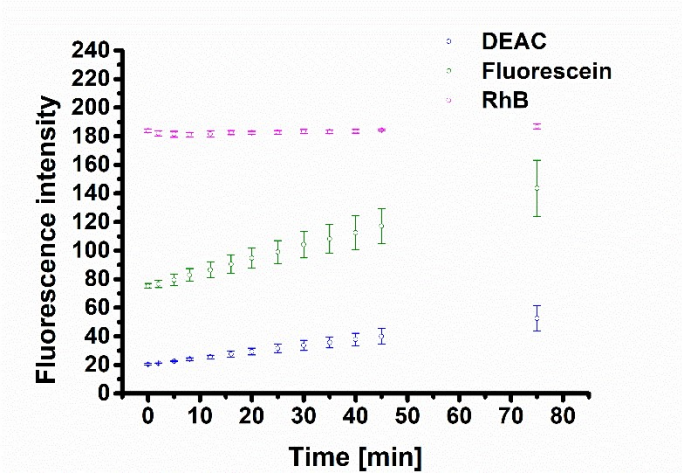


Figure S16: Fluorescence emission responses of the probe **1** (c=0.01 mM) within the time, in the presence of trypsin (c = 0.5 ng/mL).*



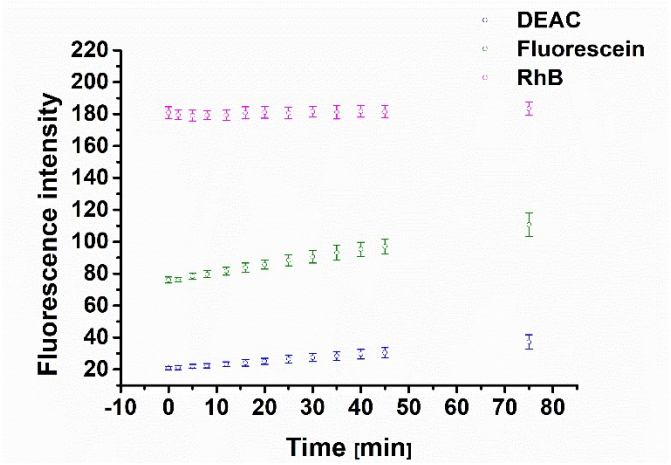
*λ_{EXC} = 425 nm, λ_{EM} = 475, 526 and 590 nm (DEAC, Fl, RhB). Each measurement was carried out in three independent parallels. The average values and standard deviations (n=3) were calculated and they are graphically presented.

Figure S17: Fluorescence emission responses of the probe **1** (c=0.01 mM) within the time, in the presence of trypsin (c = 0.25 ng/mL).*



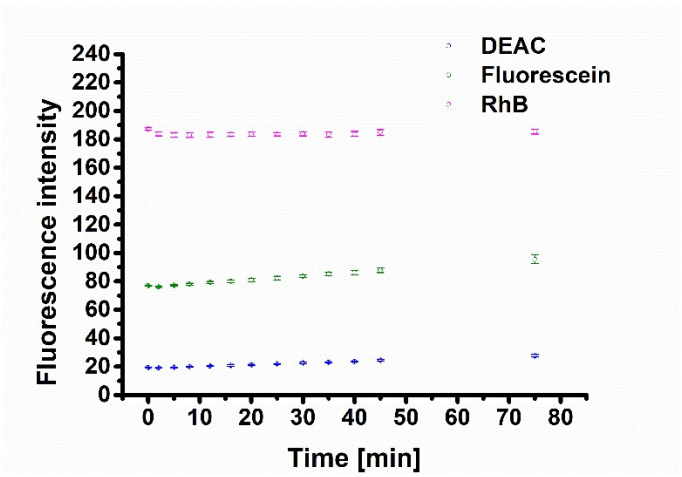
*λ_{EXC} = 425 nm, λ_{EM} = 475, 526 and 590 nm (DEAC, Fl, RhB). Each measurement was carried out in three independent parallels. The average values and standard deviations (n=3) were calculated and they are graphically presented.

Figure S18: Fluorescence emission responses of the probe **1** (c=0.01 mM) within the time, in the presence of trypsin (c = 0.125 ng/mL).*



*λ_{EXC} = 425 nm, λ_{EM} = 475, 526 and 590 nm (DEAC, Fl, RhB). Each measurement was carried out in three independent parallels. The average values and standard deviations (n=3) were calculated and they are graphically presented.

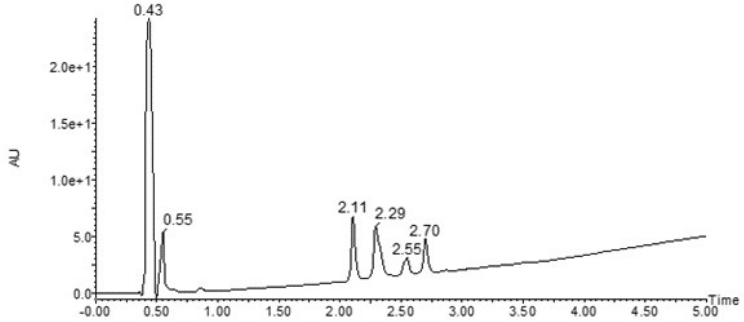
Figure S19: Fluorescence emission responses of the probe **1** (c=0.01 mM) within the time, in the presence of trypsin (c = 0.0625 ng/mL).*



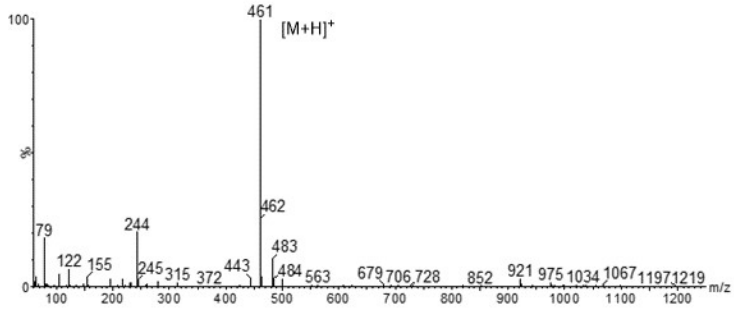
*λ_{EXC} = 425 nm, λ_{EM} = 475, 526 and 590 nm (DEAC, Fl, RhB). Each measurement was carried out in three independent parallels. The average values and standard deviations (n=3) were calculated and they are graphically presented.

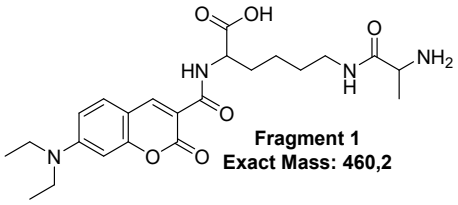
2.2. Chymotrypsin cleavage

Figure S20: LC-MS analysis of the probe **1** cleaved by trypsin (5 µg/mL, 2 hours).



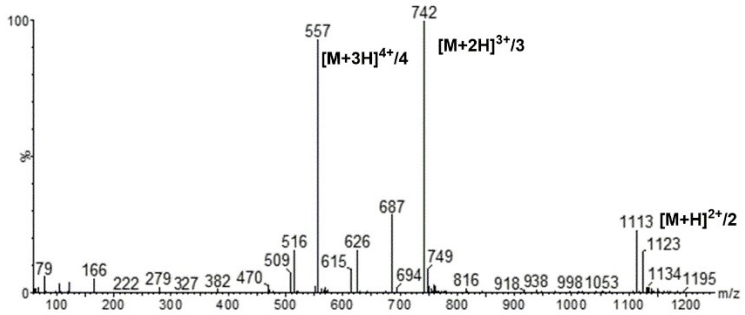
Fragment 1 (Rt = 2.11 min)

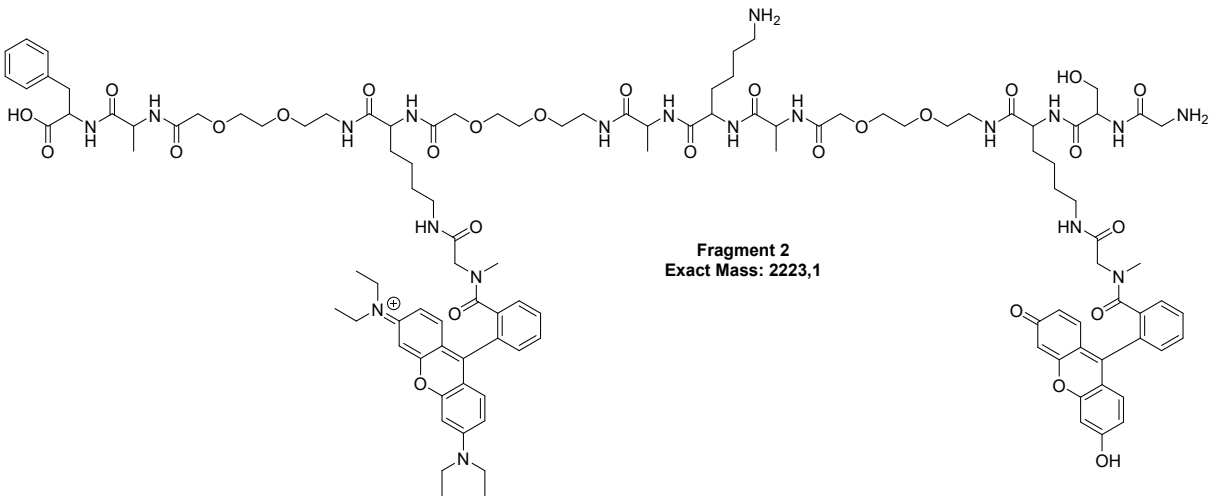




Fragment 1
Exact Mass: 460,2

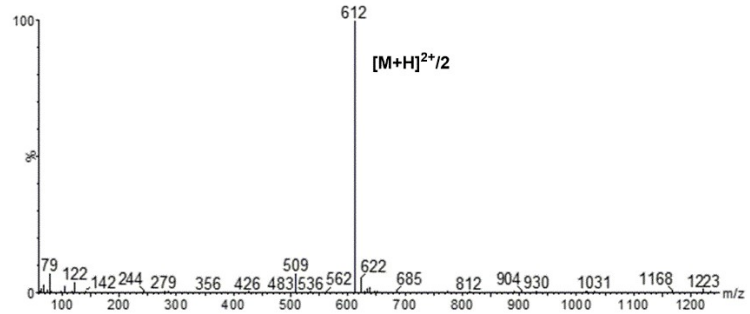
Fragment 2 (Rt = 2.29 min)

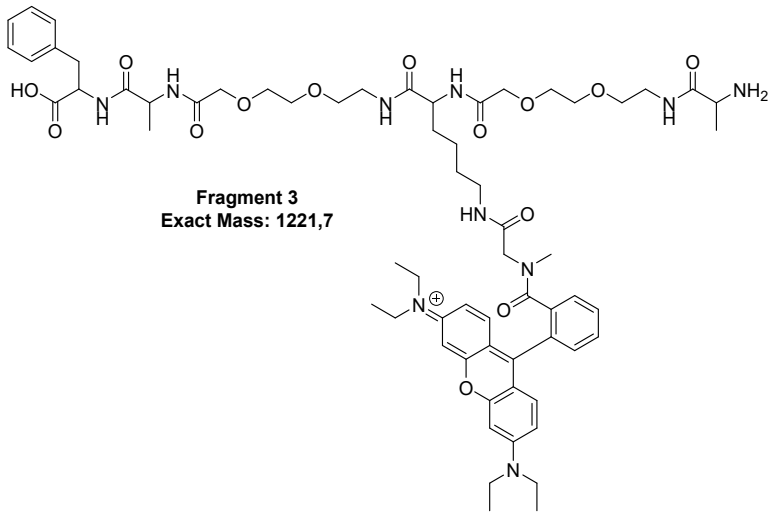




Fragment 2
Exact Mass: 2223,1

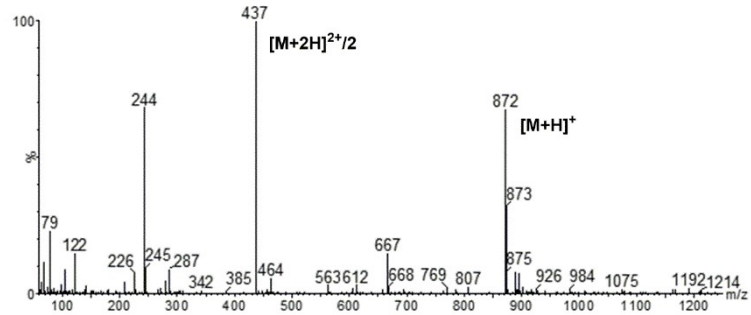
Fragment 3 (Rt = 2.55 min)

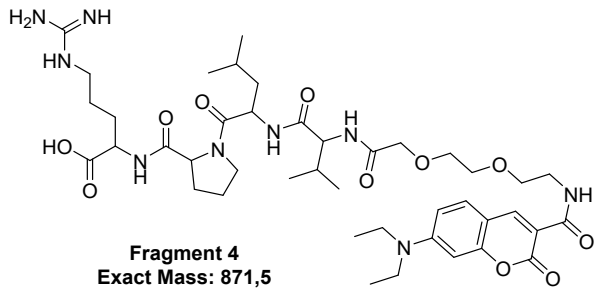




Fragment 3
Exact Mass: 1221,7

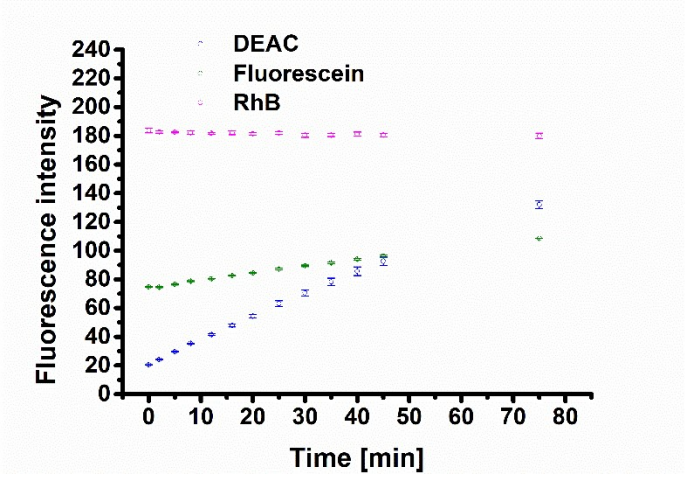
Fragment 4 (Rt = 2.70 min)





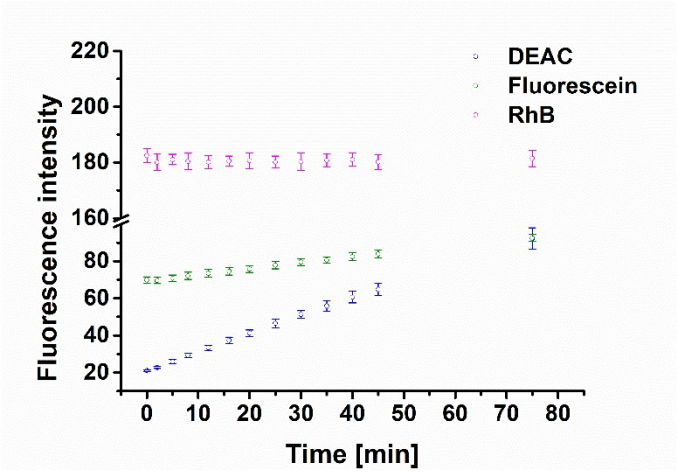
Fragment 4
Exact Mass: 871,5

Figure S21: Fluorescence emission responses of the probe **1** within the time, in the presence of chymotrypsin (c = *2* µg/mL).*



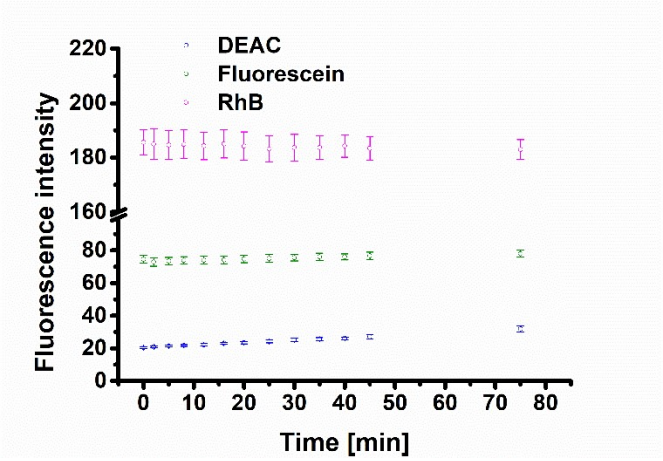
*λ_{EXC} = 425 nm, λ_{EM} = 475, 526 and 590 nm (DEAC, Fl, RhB). Each measurement was carried out in three independent parallels. The average values and standard deviations (n=3) were calculated and they are graphically presented.

Figure S22: Fluorescence emission responses of the probe **1** within the time, in the presence of chymotrypsin (c = 1 µg/mL).*



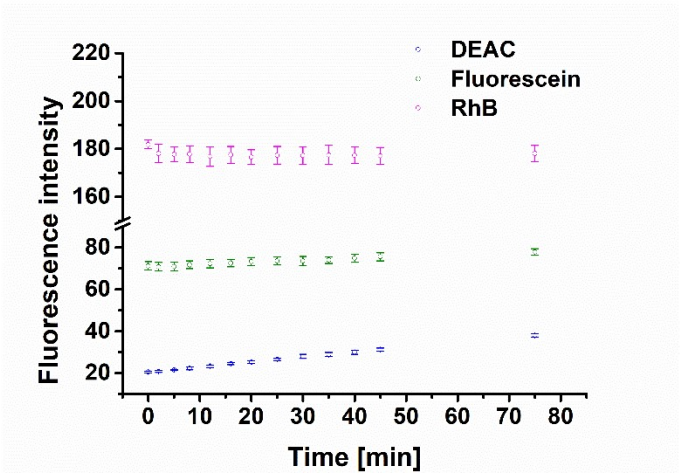
*λ_{EXC} = 425 nm, λ_{EM} = 475, 526 and 590 nm (DEAC, Fl, RhB). Each measurement was carried out in three independent parallels. The average values and standard deviations (n=3) were calculated and they are graphically presented.

Figure S23: Fluorescence emission responses of the probe **1** within the time, in the presence of chymotrypsin (c = 0.5 µg/mL).*



*λ_{EXC} = 425 nm, λ_{EM} = 475, 526 and 590 nm (DEAC, Fl, RhB). Each measurement was carried out in three independent parallels. The average values and standard deviations (n=3) were calculated and they are graphically presented.

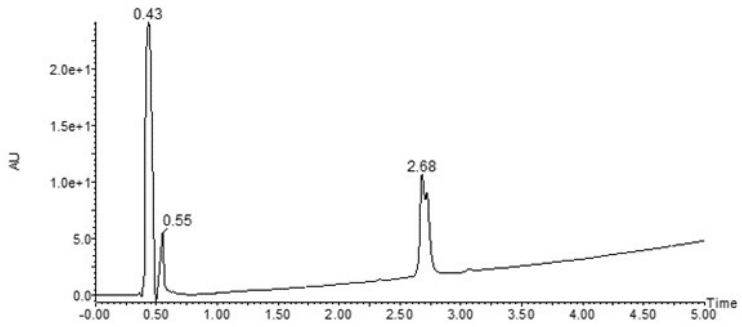
Figure S24: Fluorescence emission responses of the probe **1** within the time, in the presence of chymotrypsin (c = 0.25 μg/mL).*



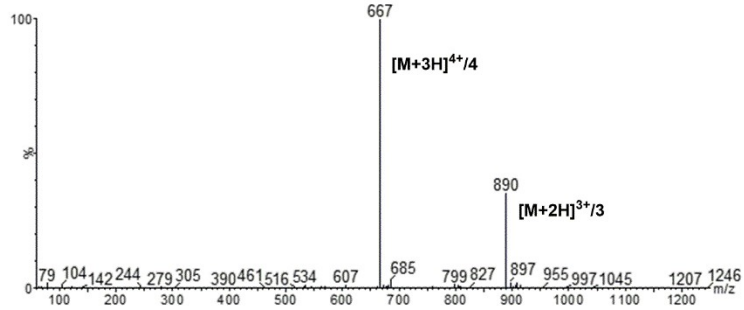
*λ_{EXC} = 425 nm, λ_{EM} = 475, 526 and 590 nm (DEAC, Fl, RhB). Each measurement was carried out in three independent parallels. The average values and standard deviations (n=3) were calculated and they are graphically presented.

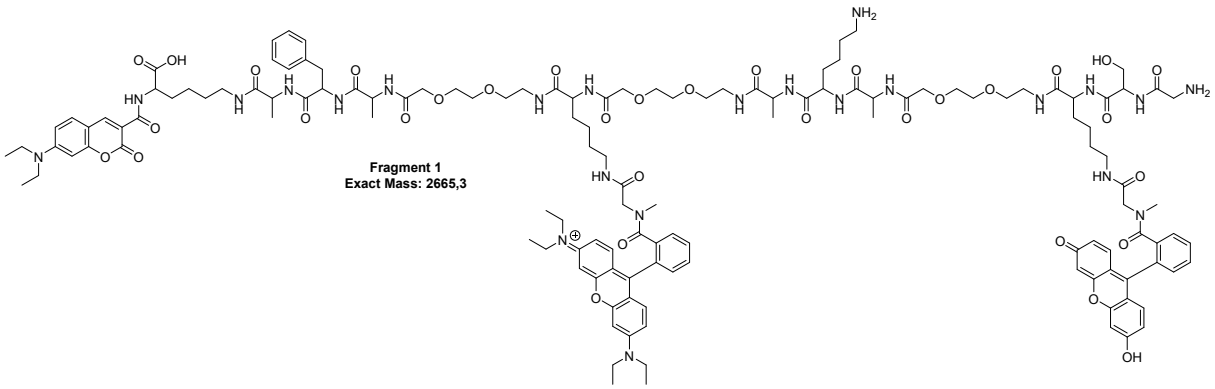
2.3. Thrombin cleavage

Figure S25: LC-MS analysis of the probe **1** cleaved by trypsin (0.05 U/mL, 2 hours).



Fragment 1 (Rt = 2.68 min)





Fragment 2 (Rt = 2.72 min)

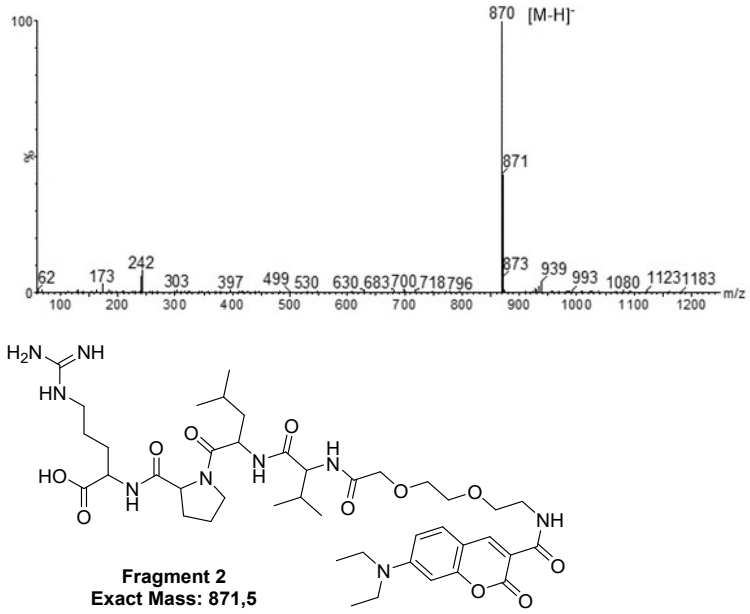
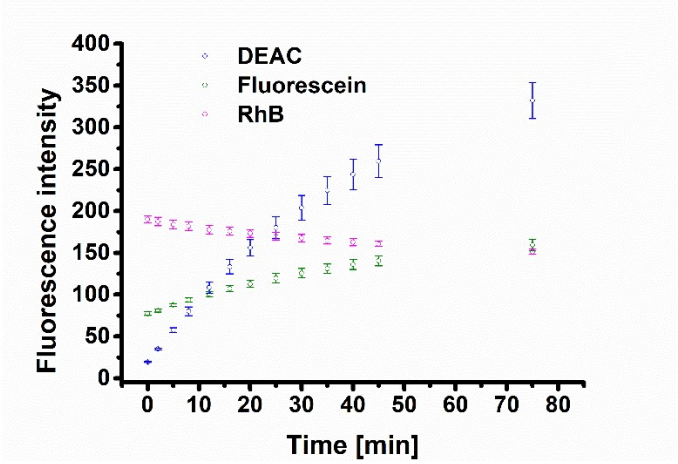
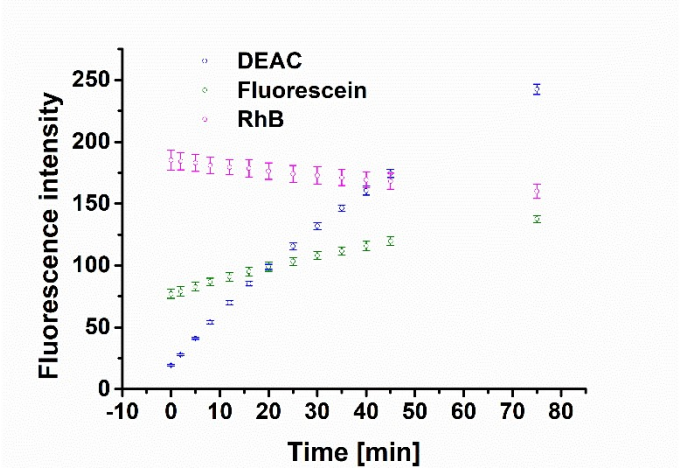


Figure S26: Fluorescence emission responses of the probe **1** within the time, in the presence of thrombin (c = 0.2 U/mL).*



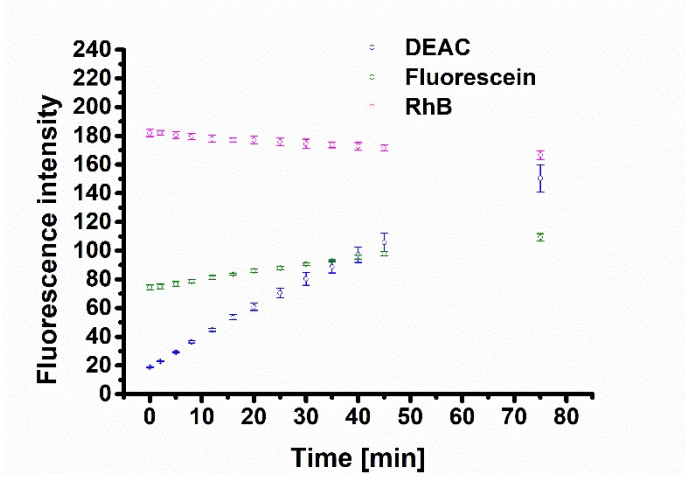
λ_{EXC} = 425 nm, λ_{EM} = 475, 526 and 590 nm (DEAC, Fl, RhB). Each measurement was carried out in three independent parallels. The average values and standard deviations (n=3) were calculated and they are graphically presented.

Figure S27: Fluorescence emission responses of the probe **1** within the time, in the presence of thrombin (c = 0.1 U/mL).*



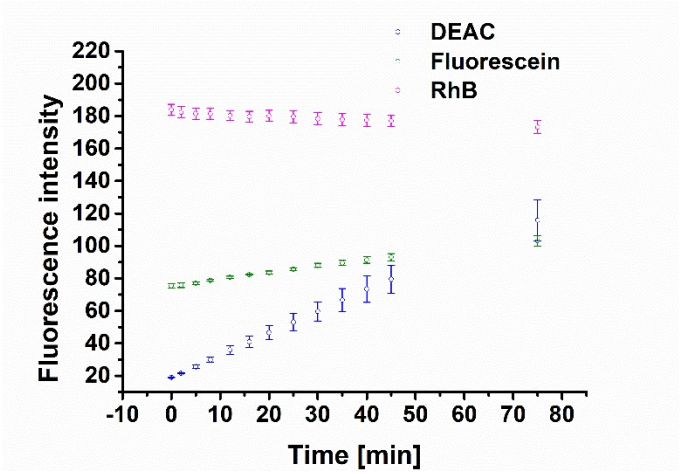
λ_{EXC} = 425 nm, λ_{EM} = 475, 526 and 590 nm (DEAC, Fl, RhB). Each measurement was carried out in three independent parallels. The average values and standard deviations (n=3) were calculated and they are graphically presented.

Figure S28: Fluorescence emission responses of the probe **1** within the time, in the presence of thrombin (c = 0.05 U/mL).*



λ_{EXC} = 425 nm, λ_{EM} = 475, 526 and 590 nm (DEAC, Fl, RhB). Each measurement was carried out in three independent parallels. The average values and standard deviations (n=3) were calculated and they are graphically presented.

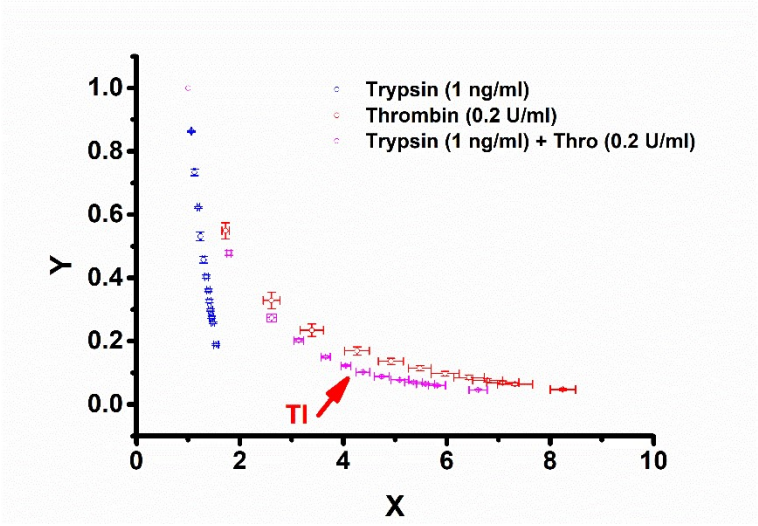
Figure S29: Fluorescence emission responses of the probe **1** within the time, in the presence of thrombin (c = 0.025 U/mL).*



λ_{EXC} = 425 nm, λ_{EM} = 475, 526 and 590 nm (DEAC, Fl, RhB). Each measurement was carried out in three independent parallels. The average values and standard deviations (n=3) were calculated and they are graphically presented.

2.4. Two-enzyme assay

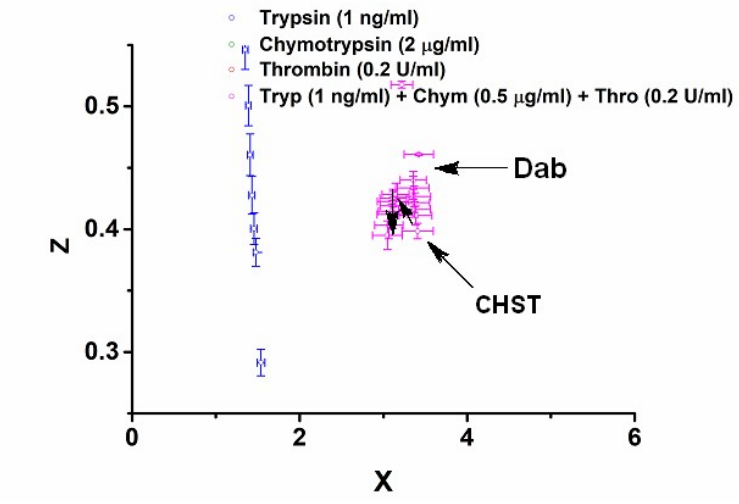
Figure S30: Fluorescence emission responses of probe **1** within the time, in the co-presence of trypsin (1 ng/mL) and thrombin (0.2 U/mL) and addition of trypsin inhibitor (TI).



Each measurement was carried out in three independent parallels. The average values and standard deviations (n=3) were calculated and they are graphically presented.

2.5. Three-enzyme assay

Figure S31: Detail of figure 12C - fluorescence emission response of the probe **1** within the time, in the co-presence of trypsin (1 ng mL^{-1}), chymotrypsin (0.5 µg mL^{-1}), thrombin (0.2 U mL^{-1}) and chymotrypsin inhibitor (CHST) and thrombin inhibitor (DAB)



Each measurement was carried out in three independent parallels. The average values and standard deviations (n=3) were calculated and they are graphically presented.

Figure S32: Fluorescence emission response of the probe **1** within the time, in the co-presence of trypsin (1 ng mL^{-1}), chymotrypsin (0.5 μg mL^{-1}), thrombin (0.05 U mL^{-1}) and chymotrypsin inhibitor (CHST) and trypsin inhibitor (TI)

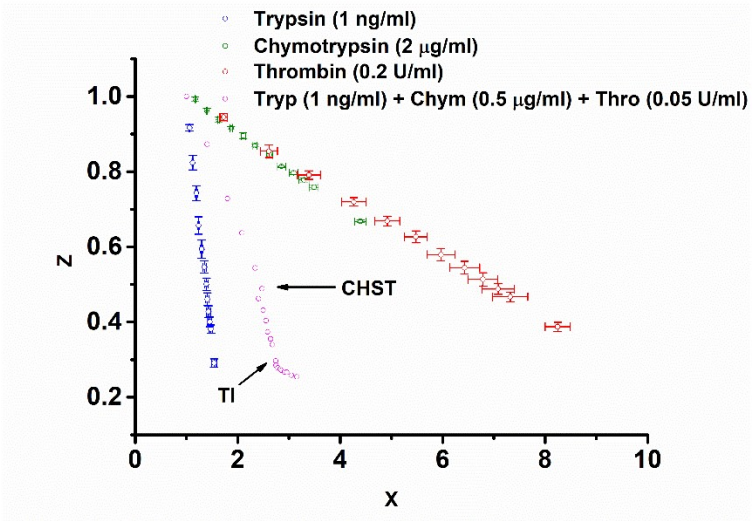
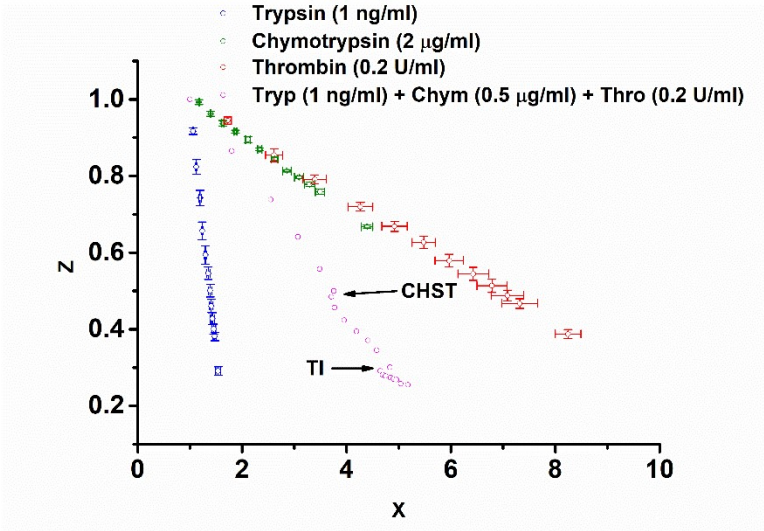


Figure S33: Fluorescence emission response of the probe **1** within the time, in the co-presence of trypsin (1 ng mL^{-1}), chymotrypsin (0.5 μg mL^{-1}), thrombin (0.2 U mL^{-1}) and chymotrypsin inhibitor (CHST) and trypsin inhibitor (TI)



2.6. Tables with data

Table S1: Time-dependent fluorescence response of the probe **1** – blank sample (addition of 1 mM HCl). (see Figure 6A)

Time [min]	DEAC Average	DEAC St. Dev.	Fl Average	Fl St. Dev.	RhB Average	RhB St. Dev.
0	18.76	0.58	75.02	2.04	180.05	3.15
2	18.68	0.41	74.62	1.24	179.54	1.50
5	18.82	0.61	75.30	1.46	179.29	1.22
8	18.88	0.57	74.74	1.56	178.22	1.37
12	18.97	0.78	74.45	1.37	178.09	1.75
16	18.98	0.72	75.07	1.67	178.56	1.91
20	18.82	0.97	75.52	2.12	178.89	2.34
25	18.98	0.89	75.42	1.45	178.69	2.29
30	19.04	0.81	75.96	2.21	179.37	1.77
35	19.53	0.95	76.28	2.44	179.54	2.59
40	19.45	1.06	76.39	2.04	179.07	1.85
45	19.59	0.96	76.97	2.15	179.47	2.28
75	19.93	1.60	78.35	3.34	180.30	2.59

Preparation: Probe **1** in 10 µL DMSO (0.5 mM) dissolved in Tris buffer (480 µL) (0 min), then addition of 1 mM HCl (10 µL) (0–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}/Slit_{EMS}$=10/10 nm. All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S2: Time-dependent fluorescence response of the probe **1** – blank sample (addition of 0.9% saline). (See Figure 6B)

Time [min]	DEAC Average	DEAC St. Dev.	Fl Average	Fl St. Dev.	RhB Average	RhB St. Dev.
0	18.97	0.69	75.84	2.83	181.75	7.04
2	18.98	0.52	74.70	2.06	180.16	4.95
5	18.87	0.50	74.65	1.63	179.81	4.46
8	18.98	0.68	74.65	1.84	180.20	5.16
12	19.05	0.61	75.40	2.03	180.81	4.99
16	19.05	0.62	75.37	1.95	179.91	4.66
20	19.24	0.65	75.07	1.96	180.65	4.85
25	19.36	0.52	75.78	2.01	181.00	4.69
30	19.03	0.71	75.61	1.91	181.28	5.75
35	19.19	0.43	76.01	1.86	181.41	4.99
40	19.36	0.68	76.14	1.63	180.73	5.13
45	19.34	0.61	76.35	2.12	181.74	5.26
75	19.46	0.65	77.00	2.00	182.84	5.52

Preparation: Probe **1** in 10 µL DMSO (0.5 mM) dissolved in Tris buffer (480 µL) (0 min), then addition of 0.9% saline (w/V) (10 µL) (0–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}/Slit_{EMS}$=10/10 nm. All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S3: Time-dependent fluorescence response of the probe **1** – detection of trypsin (c=1 ng/mL). (see Figure 7A)

Time [min]	DEAC Average	DEAC St. Dev.	Fl Average	Fl St. Dev.	RhB Average	RhB St. Dev.
0	19.51	0.33	73.09	1.38	175.78	4.50
2	22.77	0.55	80.36	0.63	177.20	4.76
5	27.00	0.95	90.11	1.18	178.51	4.34
8	31.97	0.98	100.40	1.96	179.26	3.57
12	37.78	1.95	114.32	3.08	180.47	3.27
16	43.99	1.87	126.94	3.36	181.19	4.35
20	50.03	1.38	138.74	2.42	182.12	4.10
25	56.69	1.84	152.86	3.49	183.94	3.68
30	63.07	2.36	167.61	4.62	185.49	3.67
35	69.26	2.60	181.36	5.10	186.33	3.47
40	75.68	2.78	194.86	5.26	187.54	2.87
45	81.51	3.18	206.88	5.27	189.51	3.14
75	115.07	4.69	280.13	9.88	196.02	3.26

Preparation: Probe **1** in 10 µL DMSO (0.5 mM) dissolved in Tris buffer (480 µL) (0 min), then addition of 1 mM HCl (10 µL) with trypsin (0.5 ng) (0–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}/Slit_{EMS}$=10/10 nm. All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S4: Time-dependent fluorescence response of the probe **1** – detection of trypsin (c=0.5 ng/mL). (see Figure S16)

Time [min]	DEAC Average	DEAC St. Dev.	Fl Average	Fl St. Dev.	RhB Average	RhB St. Dev.
0	19.60	0.66	75.49	1.33	182.56	2.78
2	20.80	0.90	78.08	2.02	182.15	2.86
5	23.21	1.03	83.61	2.09	182.52	3.46
8	25.60	1.24	88.96	2.51	182.63	2.77
12	29.17	1.61	96.80	4.26	182.37	3.68
16	32.56	2.03	103.62	3.82	183.72	3.63
20	36.00	2.09	111.44	4.24	183.88	4.79
25	40.44	1.94	121.32	4.47	184.71	3.43
30	44.72	2.13	130.40	5.30	185.41	3.65
35	48.86	3.17	138.18	7.02	186.86	4.95
40	52.33	3.31	146.75	7.94	186.83	3.91
45	56.53	3.58	155.58	8.08	187.62	4.27
75	78.98	5.27	204.40	11.86	192.04	4.86

Preparation Probe **1** in 10 µL DMSO (0.5 mM) dissolved in Tris buffer (480 µL) (0 min), then addition of 1 mM HCl (10 µL) with trypsin (0.25 ng) (0–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}/Slit_{EMS}$=10/10 nm. All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S5: Time-dependent fluorescence response of the probe **1** – detection of trypsin (c=0.25 ng/mL). (see Figure S17)

Time [min]	DEAC Average	DEAC St. Dev.	Fl Average	Fl St. Dev.	RhB Average	RhB St. Dev.
0	20.48	0.52	75.25	1.78	183.80	1.10
2	21.21	0.19	76.71	2.61	181.96	1.78
5	22.65	0.39	79.36	3.99	181.37	2.04
8	24.12	0.88	82.89	4.51	181.12	1.55
12	25.63	1.29	86.55	5.43	181.72	2.08
16	27.67	2.18	90.52	6.49	182.59	1.50
20	29.42	2.38	94.66	6.97	182.39	1.03
25	31.56	2.86	98.99	8.06	182.78	1.30
30	33.72	3.60	104.29	9.36	183.43	1.29
35	35.79	3.85	108.30	10.03	183.30	1.37
40	37.73	4.52	112.52	11.88	183.44	1.27
45	40.03	5.44	117.18	12.34	184.24	0.65
75	52.58	8.97	143.57	19.62	186.74	1.80

Preparation: Probe **1** in 10 µL DMSO (0.5 mM) dissolved in Tris buffer (480 µL) (0 min), then addition of 1 mM HCl (10 µL) with trypsin (0.125 ng) (0–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}$/$Slit_{EMS}$=10/10 nm. All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S6: Time-dependent fluorescence response of the probe **1** – detection of trypsin (c=0.125 ng/mL). (see Figure S18)

Time [min]	DEAC Average	DEAC St. Dev.	Fl Average	Fl St. Dev.	RhB Average	RhB St. Dev.
0	20.83	1.02	75.97	1.94	180.89	3.61
2	21.11	1.26	76.13	1.27	179.42	3.03
5	21.84	1.29	78.27	1.84	179.13	3.48
8	22.31	1.33	79.60	2.18	179.31	2.87
12	23.24	1.49	81.51	2.45	179.29	3.23
16	24.10	1.98	83.75	2.83	180.85	3.71
20	25.12	1.87	85.72	2.86	181.03	3.61
25	26.45	2.27	88.38	3.50	180.68	3.52
30	27.43	2.56	90.64	4.03	181.50	3.39
35	28.38	2.85	93.08	4.60	181.16	4.26
40	29.50	3.03	95.24	4.49	181.73	3.65
45	30.56	3.13	97.00	4.78	181.45	3.73
75	37.20	4.49	110.68	7.40	183.45	3.88

Preparation: Probe **1** in 10 µL DMSO (0.5 mM) dissolved in Tris buffer (480 µL) (0 min), then addition of 1 mM HCl (10 µL) with trypsin (0.0625 ng) (0–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}$/$Slit_{EMS}$=10/10 nm. All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S7: Time-dependent fluorescence response of the probe **1** – detection of trypsin (c=0.0625 ng/mL). (see Figure S19)

Time [min]	DEAC Average	DEAC St. Dev.	Fl Average	Fl St. Dev.	RhB Average	RhB St. Dev.
0	19.37	0.08	77.00	0.19	187.27	0.88
2	19.10	0.13	76.17	0.33	183.99	1.49
5	19.54	0.22	77.28	0.36	183.33	1.57
8	19.91	0.36	78.06	0.61	183.00	1.56
12	20.40	0.28	79.29	0.71	183.41	1.56
16	20.94	0.50	80.05	0.94	183.46	1.14
20	21.28	0.42	81.06	1.17	183.75	1.58
25	21.96	0.26	82.37	1.23	183.60	1.08
30	22.59	0.48	83.62	0.89	183.95	1.46
35	23.09	0.29	85.30	1.24	183.62	1.85
40	23.56	0.49	86.27	1.62	183.89	1.70
45	24.52	0.65	87.83	1.71	184.94	1.92
75	27.63	0.96	95.54	2.98	185.44	1.68

Preparation: Probe **1** in 10 µL DMSO (0.5 mM) dissolved in Tris buffer (480 µL) (0 min), then addition of 1 mM HCl (10 µL) with trypsin (0.03125 ng) (0–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}$/$Slit_{EMS}$=10/10 nm. All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S8: Time-dependent fluorescence response of the probe **1** – detection of chymotrypsin (c= 2 µg/mL). (see Figure S21)

Time [min]	DEAC Average	DEAC St. Dev.	Fl Average	Fl St. Dev.	RhB Average	RhB St. Dev.
0	20.49	0.06	74.73	0.20	183.67	1.57
2	24.10	0.23	74.63	0.34	182.67	0.75
5	29.59	0.18	76.55	0.44	182.54	0.50
8	35.32	0.20	78.74	0.28	182.18	1.24
12	41.49	0.65	80.38	0.11	181.79	0.67
16	47.95	1.15	82.61	0.25	182.20	1.32
20	54.50	1.20	84.42	0.42	181.50	0.86
25	63.09	2.06	87.10	0.63	181.94	1.03
30	70.65	2.24	89.42	0.51	180.41	1.41
35	78.19	2.59	91.56	0.65	180.63	1.06
40	85.67	3.00	94.05	0.67	181.25	1.32
45	92.73	2.97	96.16	1.02	180.63	1.19
75	132.08	2.84	108.62	0.03	179.96	1.96

Preparation: Probe **1** in 10 µL DMSO (0.5 mM) dissolved in Tris buffer (480 µL) (0 min), then addition of 1 mM HCl (10 µL) with chymotrypsin (1 µg) (0–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}$/$Slit_{EMS}$=10/10 nm. All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S9: Time-dependent fluorescence response of the probe **1** – detection of chymotrypsin (c= 1 µg/mL). (see figure S22)

Time [min]	DEAC Average	DEAC St. Dev.	Fl Average	Fl St. Dev.	RhB Average	RhB St. Dev.
0	21.10	0.46	69.93	1.65	182.49	2.47
2	22.83	0.55	69.58	1.63	180.04	2.97
5	25.97	1.13	70.83	1.76	181.07	1.85
8	29.25	1.12	72.10	1.94	180.35	2.98
12	33.33	1.44	73.49	2.11	180.09	2.36
16	37.42	1.68	74.56	2.00	180.58	1.74
20	41.24	1.67	75.65	1.73	180.63	2.75
25	46.58	2.29	77.88	2.05	180.16	2.16
30	51.23	2.11	79.52	1.86	180.28	3.16
35	55.89	2.71	80.62	1.72	180.78	2.40
40	60.62	3.08	82.34	2.12	181.09	2.32
45	64.79	3.29	83.88	2.17	180.23	2.68
75	92.18	5.82	92.56	1.96	181.47	2.96

Preparation: Probe **1** in 10 µL DMSO (0.5 mM) dissolved in Tris buffer (480 µL) (0 min), then addition of 1 mM HCl (10 µL) with chymotrypsin (0.5 µg) (0–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}/Slit_{EMS}$=10/10 nm. All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S10: Time-dependent fluorescence response of the probe **1** – detection of chymotrypsin (c= 0.5 µg/mL). (see Figure S23)

Time [min]	DEAC Average	DEAC St. Dev.	Fl Average	Fl St. Dev.	RhB Average	RhB St. Dev.
0	20.67	0.50	73.68	3.14	184.93	5.56
2	21.80	0.36	73.25	2.05	182.79	4.28
5	23.30	0.69	74.61	1.58	182.49	4.30
8	24.85	0.78	74.84	1.72	182.59	3.93
12	27.15	0.81	75.09	1.57	182.38	4.15
16	29.31	0.87	76.44	2.20	182.56	3.89
20	31.35	1.07	76.95	1.91	182.96	3.82
25	33.99	1.11	77.87	2.01	181.61	3.79
30	36.34	1.26	78.42	1.63	182.26	3.52
35	38.81	1.19	79.51	1.92	182.00	2.86
40	41.19	1.64	80.88	2.13	181.54	3.07
45	43.96	1.37	81.09	1.69	182.30	3.45
75	58.72	2.60	86.27	1.85	181.34	3.38

Preparation: Probe **1** in 10 µL DMSO (0.5 mM) dissolved in Tris buffer (480 µL) (0 min), then addition of 1 mM HCl (10 µL) with chymotrypsin (0.25 µg) (0–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}/Slit_{EMS}$=10/10 nm. All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S11: Time-dependent fluorescence response of the probe **1** – detection of chymotrypsin (c= 0.25 µg/mL). (see Figure S24).

Time [min]	DEAC Average	DEAC St. Dev.	Fl Average	Fl St. Dev.	RhB Average	RhB St. Dev.
0	20.54	0.48	71.14	2.06	181.85	1.83
2	20.76	0.45	70.82	2.03	178.09	3.87
5	21.48	0.35	70.85	2.12	177.76	3.09
8	22.24	0.65	71.73	1.88	177.78	3.39
12	23.29	0.64	72.20	1.98	176.79	4.14
16	24.50	0.50	72.49	1.74	177.53	3.50
20	25.24	0.64	73.25	2.02	176.54	3.06
25	26.51	0.48	73.54	1.96	177.34	3.67
30	27.98	1.00	73.57	2.15	177.26	3.64
35	28.85	1.01	74.00	1.64	177.50	4.06
40	29.98	0.99	74.88	1.89	177.34	3.51
45	31.26	0.69	75.54	1.91	177.12	3.51
75	38.05	0.72	77.91	1.53	178.04	3.43

Preparation: Probe **1** in 10 µL DMSO (0.5 mM) dissolved in Tris buffer (480 µL) (0 min), then addition of 1 mM HCl (10 µL) with chymotrypsin (0.125 µg) (0–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}$/$Slit_{EMS}$=10/10 nm. All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S12: Time-dependent fluorescence response of the probe **1** – detection of chymotrypsin (c= 0.125 µg/mL). (see Figure 7B)

Time [min]	DEAC Average	DEAC St. Dev.	Fl Average	Fl St. Dev.	RhB Average	RhB St. Dev.
0	20.50	0.47	74.54	2.47	185.61	4.65
2	20.99	0.64	72.92	2.54	185.00	5.60
5	21.43	0.46	73.50	2.22	184.60	5.26
8	21.77	0.53	73.81	2.34	184.86	5.30
12	22.14	0.74	73.98	2.42	184.31	5.10
16	23.05	0.56	74.25	2.33	185.06	5.18
20	23.45	0.66	74.77	2.30	184.24	5.25
25	24.20	0.80	75.01	2.47	183.22	4.84
30	25.10	1.05	75.47	1.92	183.68	4.85
35	25.67	0.96	76.05	2.26	183.72	4.34
40	26.23	0.77	75.99	1.80	184.31	4.11
45	27.08	1.27	76.74	2.21	183.41	4.43
75	31.90	1.92	78.07	2.09	182.97	3.60

Preparation: Probe **1** in 10 µL DMSO (0.5 mM) dissolved in Tris buffer (480 µL) (0 min), then addition of 1 mM HCl (10 µL) with chymotrypsin (0.0625 µg) (0–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}$/$Slit_{EMS}$=10/10 nm. All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S13: Time-dependent fluorescence response of the probe **1** – detection of thrombin (c= 0.2 U/mL). (see Figure S26)

Time [min]	DEAC Average	DEAC St. Dev.	Fl Average	Fl St. Dev.	RhB Average	RhB St. Dev.
0	19.59	0.66	77.57	2.33	190.23	4.41
2	35.22	1.08	80.98	1.73	187.66	5.14
5	57.86	2.71	87.82	1.63	184.04	5.09
8	80.21	5.28	93.71	2.44	181.85	4.87
12	108.44	6.66	100.71	3.19	177.76	5.07
16	133.45	8.52	107.43	3.75	176.14	4.61
20	156.17	9.80	112.86	4.04	173.43	4.62
25	180.57	13.06	119.80	5.50	170.03	4.56
30	204.06	14.76	125.67	5.72	167.74	4.33
35	224.63	16.53	130.98	5.74	164.82	4.32
40	243.70	17.90	136.17	5.87	162.78	4.23
45	259.72	19.42	140.45	5.85	160.74	3.18
75	332.20	21.67	159.47	7.23	151.38	3.04

Preparation: Probe **1** in 10 µL DMSO (0.5 mM) dissolved in Tris buffer (480 µL) (0 min), then addition of 0.9% saline (w/V) (10 µL) with thrombin (0.1 U) (0–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}$/$Slit_{EMS}$=10/10 nm. All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S14: Time-dependent fluorescence response of the probe **1** – detection of thrombin (c= 0.1 U/mL). (see Figure S27)

Time [min]	DEAC Average	DEAC St. Dev.	Fl Average	Fl St. Dev.	RhB Average	RhB St. Dev.
0	19.33	0.53	77.12	3.67	185.21	7.93
2	27.89	0.75	79.04	3.70	184.28	6.83
5	40.97	0.70	82.93	3.52	182.88	6.78
8	54.20	1.33	86.70	3.05	181.00	6.67
12	69.87	1.79	90.74	3.25	179.46	6.13
16	85.26	1.86	95.12	3.66	178.62	7.03
20	98.89	2.04	98.94	3.62	176.36	6.70
25	115.57	2.70	103.27	3.21	174.01	6.95
30	131.88	2.91	107.97	3.35	172.83	6.92
35	146.51	2.46	111.70	3.24	171.08	6.44
40	160.57	3.64	115.87	3.74	169.27	6.50
45	174.52	3.39	119.73	3.38	168.16	6.43
75	242.44	4.19	137.68	2.90	160.03	5.80

Preparation: Probe **1** in 10 µL DMSO (0.5 mM) dissolved in Tris buffer (480 µL) (0 min), then addition of 0.9% saline (w/V) (10 µL) with thrombin (0.05 U) (0–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}$/$Slit_{EMS}$=10/10 nm. All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S15: Time-dependent fluorescence response of the probe **1** – detection of thrombin (c= 0.05 U/mL). (see Figure S28)

Time [min]	DEAC Average	DEAC St. Dev.	Fl Average	Fl St. Dev.	RhB Average	RhB St. Dev.
0	18.81	0.09	74.37	1.72	182.15	2.60
2	22.90	0.25	74.93	1.65	182.09	1.64
5	29.14	0.47	76.89	1.61	180.65	2.34
8	36.38	0.70	78.60	1.32	179.54	2.22
12	44.84	1.10	81.42	1.29	177.99	2.52
16	53.60	1.76	83.44	0.62	177.17	1.71
20	61.00	2.59	85.89	1.24	177.06	2.89
25	70.70	3.29	87.86	1.27	175.94	2.60
30	80.29	4.31	90.65	1.05	174.59	3.10
35	88.76	4.27	92.80	0.83	173.71	2.03
40	97.15	5.40	95.35	1.65	172.67	2.67
45	105.69	6.43	97.87	1.55	171.83	2.10
75	150.46	9.41	109.40	2.53	166.62	3.14

Preparation: Probe **1** in 10 µL DMSO (0.5 mM) dissolved in Tris buffer (480 µL) (0 min), then addition of 0.9% saline (w/V) (10 µL) with thrombin (0.025 U) (0–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}$/$Slit_{EMS}$=10/10 nm. All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S16: Time-dependent fluorescence response of the probe **1** – detection of thrombin (c= 0.025 U/mL). (see Figure S29)

Time [min]	DEAC Average	DEAC St. Dev.	Fl Average	Fl St. Dev.	RhB Average	RhB St. Dev.
0	19.09	0.25	75.45	1.35	183.83	3.49
2	21.49	0.26	75.81	1.50	182.34	3.41
5	25.64	1.01	76.99	0.85	181.55	3.48
8	29.87	1.56	78.78	0.46	181.39	3.60
12	35.86	2.80	80.72	0.59	180.28	2.85
16	40.90	3.42	82.28	0.33	179.74	3.24
20	46.67	4.17	83.54	0.96	180.20	3.34
25	53.03	5.31	85.68	0.73	179.56	3.68
30	59.68	5.93	88.06	1.33	178.36	3.73
35	66.69	6.99	89.47	1.57	177.90	3.77
40	73.42	8.19	91.42	2.15	177.50	3.76
45	79.47	8.66	93.05	2.21	176.95	3.48
75	115.86	12.61	103.22	3.15	173.23	4.01

Preparation: Probe **1** in 10 µL DMSO (0.5 mM) dissolved in Tris buffer (480 µL) (0 min), then addition of 0.9% saline (w/V) (10 µL) with thrombin (0.0125 U) (0–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}$/$Slit_{EMS}$=10/10 nm. All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S17: Time-dependent fluorescence response of the probe **1** – detection of thrombin (c= 0.0125 U/mL). (see Figure 7C)

Time [min]	DEAC Average	DEAC St. Dev.	Fl Average	Fl St. Dev.	RhB Average	RhB St. Dev.
0	18.71	0.49	74.77	1.95	183.20	5.50
2	19.33	0.56	73.76	1.69	179.09	4.04
5	21.06	0.45	74.61	1.45	178.88	4.56
8	23.09	1.12	75.41	0.91	178.66	4.73
12	25.36	0.92	76.22	1.01	178.42	3.87
16	28.07	0.89	77.44	0.91	178.76	4.22
20	30.54	0.71	77.82	1.08	178.97	4.59
25	33.41	1.19	79.03	0.55	178.56	4.32
30	36.82	1.43	79.57	0.54	177.73	3.70
35	39.15	2.07	80.77	0.82	178.00	3.55
40	42.06	2.13	81.57	0.79	177.54	3.97
45	44.67	3.06	82.02	0.75	177.41	4.21
75	61.07	4.79	87.16	1.64	175.97	3.06

Preparation: Probe **1** in 10 µL DMSO (0.5 mM) dissolved in Tris buffer (480 µL) (0 min), then addition of 0.9% saline (w/V) (10 µL) with thrombin (6.25 mU) (0–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}/Slit_{EMS}$=10/10 nm. All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S18: Ratios of fluorescence intensities of the probe **1** at times 75 min and 0 min. (see Figure 8)

Protease	Conc. [/mL]	DEAC(I_{75min}/I_{0min})/ Fl(I_{75min}/I_{0min})	DEAC/Fl St. Dev.	RhB(I_{75min}/I_{0min})/ DEAC(I_{75min}/I_{0min})	RhB/DEAC St. Dev.
/	0	1.02	0.03	0.95	0.05
Tryp	0.0625 ng	1.15	0.01	0.69	0.02
Tryp	0.125 ng	1.22	0.05	0.57	0.05
Tryp	0.25 ng	1.34	0.09	0.41	0.06
Tryp	0.5 ng	1.49	0.04	0.26	0.01
Tryp	1 ng	1.54	0.04	0.19	<0.01
Chym	0.125 µg	1.49	0.09	0.64	0.03
Chym	0.25 µg	1.69	0.03	0.53	0.01
Chym	0.5 µg	2.42	0.09	0.35	0.01
Chym	1 µg	3.30	0.11	0.23	0.01
Chym	2 µg	4.39	0.11	0.15	<0.01
Thrombin	0.0125 U	2.80	0.17	0.30	0.02
Thrombin	0.025 U	4.43	0.34	0.16	0.02
Thrombin	0.05 U	5.43	0.15	0.11	0.01
Thrombin	0.1 U	7.02	0.09	0.07	<0.01
Thrombin	0.2 U	8.25	0.24	0.05	<0.01

X_{75}/Y_{75} and X_0/Y_0 – ratio of fluorescence intensities of different fluorophores at times 75 min and 0 min; All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S19: Ratios of fluorescence intensities of the probe **1** – detection of trypsin (1 ng/mL) and chymotrypsin (0.5 μg/mL). (see Figure 9A)

Time [min]	DEAC(I_{75min}/I_{0min})/ Fl(I_{75min}/I_{0min})	DEAC/Fl St. Dev.	RhB(I_{75min}/I_{0min})/ DEAC(I_{75min}/I_{0min})	RhB/DEAC St. Dev.
0	1.000	0.000	1.000	0.000
2	1.109	0.007	0.828	0.003
5	1.238	0.014	0.669	0.006
8	1.331	0.008	0.563	0.010
12	1.458	0.015	0.461	0.010
16	1.539	0.007	0.397	0.014
20	1.608	0.007	0.357	0.014
25	1.666	0.021	0.330	0.009
30	1.717	0.022	0.314	0.010
35	1.775	0.036	0.300	0.010
40	1.830	0.040	0.287	0.008
45	1.877	0.042	0.277	0.011
75	2.169	0.073	0.232	0.009

Preparation: Probe **1** in 10 μL DMSO (0.5 mM) dissolved in Tris buffer (470 μL) (0 min), then addition of 1 mM HCl (10 μL) with trypsin (0.5 ng) and 1 mM HCl (10 μL) with chymotrypsin (0.25 μg) (0–16 min). Then addition of Tris buffer (10 μL) with trypsin inhibitor (5 ng) (16–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}/Slit_{EMS}$=10/10 nm. X_t/Y_t and X_0/Y_0 – ratio of fluorescence intensities of different fluorophores at times t (0–75 min) and 0 min; All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S20: Ratios of fluorescence intensities of the probe **1** – detection of trypsin (1 ng/mL) and chymotrypsin (0.125 μg/mL). (see Figure 9B)

Time [min]	DEAC(I_{75min}/I_{0min})/ Fl(I_{75min}/I_{0min})	DEAC/Fl St. Dev.	RhB(I_{75min}/I_{0min})/ DEAC(I_{75min}/I_{0min})	RhB/DEAC St. Dev.
0	1.000	0.000	1.000	0.000
2	1.069	0.022	0.856	0.028
5	1.154	0.012	0.707	0.002
8	1.228	0.003	0.593	0.009
12	1.314	0.012	0.489	0.013
16	1.372	0.011	0.422	0.014
20	1.399	0.006	0.389	0.009
25	1.443	0.028	0.366	0.008
30	1.430	0.010	0.364	0.005
35	1.457	0.012	0.354	0.006
40	1.483	0.010	0.348	0.005
45	1.483	0.016	0.345	0.005
75	1.555	0.015	0.323	0.004

Preparation: Probe **1** in 10 μL DMSO (0.5 mM) dissolved in Tris buffer (470 μL) (0 min), then addition of 1 mM HCl (10 μL) with trypsin (0.5 ng) and 1 mM HCl (10 μL) with chymotrypsin (0.0625 μg) (0–16 min). Then addition of Tris buffer (10 μL) with trypsin inhibitor (5 ng) (16–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}/Slit_{EMS}$=10/10 nm. X_t/Y_t and X_0/Y_0 – ratio of fluorescence intensities of different fluorophores at times t (0–75 min) and 0 min; All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S21: Ratios of fluorescence intensities of the probe **1** – detection of trypsin (0.125 ng/mL) and chymotrypsin (0.5 μg/mL). (see Figure 9C)

Time [min]	DEAC(I_{75min}/I_{0min})/ Fl(I_{75min}/I_{0min})	DEAC/Fl St. Dev.	RhB(I_{75min}/I_{0min})/ DEAC(I_{75min}/I_{0min})	RhB/DEAC St. Dev.
0	1.000	0.000	1.000	0.000
2	1.058	0.013	0.925	0.010
5	1.135	0.015	0.834	0.008
8	1.204	0.025	0.764	0.017
12	1.303	0.009	0.683	0.010
16	1.392	0.034	0.621	0.020
20	1.375	0.019	0.632	0.017
25	1.385	0.025	0.618	0.016
30	1.371	0.030	0.611	0.018
35	1.389	0.026	0.595	0.015
40	1.385	0.020	0.581	0.014
45	1.389	0.026	0.568	0.017
75	1.401	0.017	0.501	0.008

Preparation: Probe **1** in 10 μL DMSO (0.5 mM) dissolved in Tris buffer (470 μL) (0 min), then addition of 1 mM HCl (10 μL) with trypsin (0.0625 ng) and 1 mM HCl (10 μL) with chymotrypsin (0.25 μg) (0–16 min). Then addition of DMSO (10 μL) with chymostatin (30 μg) (16–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}/Slit_{EMS}$=10/10 nm. X_t/Y_t and X_0/Y_0 – ratio of fluorescence intensities of different fluorophores at times t (0–75 min) and 0 min; All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S22: Ratios of fluorescence intensities of the probe **1** – detection of trypsin (0.125 ng/mL) and chymotrypsin (0.125 μg/mL). (see Figure 9D)

Time [min]	DEAC(I_{75min}/I_{0min})/ Fl(I_{75min}/I_{0min})	DEAC/Fl St. Dev.	RhB(I_{75min}/I_{0min})/ DEAC(I_{75min}/I_{0min})	RhB/DEAC St. Dev.
0	1.000	0.000	1.000	0.000
2	0.996	0.002	0.976	0.003
5	1.026	0.010	0.921	0.005
8	1.051	0.002	0.878	0.006
12	1.092	0.009	0.820	0.008
16	1.142	0.004	0.772	0.004
20	1.145	0.008	0.771	0.008
25	1.140	0.022	0.760	0.017
30	1.153	0.005	0.738	0.013
35	1.161	0.006	0.723	0.012
40	1.163	0.021	0.702	0.012
45	1.163	0.010	0.691	0.016
75	1.189	0.001	0.612	0.008

Preparation Probe **1** in 10 μL DMSO (0.5 mM) dissolved in Tris buffer (470 μL) (0 min), then addition of 1 mM HCl (10 μL) with trypsin (0.0625 ng) and 1 mM HCl (10 μL) with chymotrypsin (0.0625 μg) (0–16 min). Then addition of DMSO (10 μL) with chymostatin (30 μg) (16–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}/Slit_{EMS}$=10/10 nm. X_t/Y_t and X_0/Y_0 – ratio of fluorescence intensities of different fluorophores at times t (0–75 min) and 0 min; All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S23: Ratios of fluorescence intensities of the probe **1** – detection of trypsin (0.125 ng/mL) and chymotrypsin (0.125 μg/mL). (see Figure 9E)

Time [min]	DEAC(I_{75min}/I_{0min})/ Fl(I_{75min}/I_{0min})	DEAC/Fl St. Dev.	RhB(I_{75min}/I_{0min})/ DEAC(I_{75min}/I_{0min})	RhB/DEAC St. Dev.
0	1.000	0.000	1.000	0.000
2	1.069	0.022	0.856	0.028
5	1.154	0.012	0.707	0.002
8	1.228	0.003	0.593	0.009
12	1.314	0.012	0.489	0.013
16	1.372	0.011	0.422	0.014
20	1.399	0.006	0.389	0.009
25	1.443	0.028	0.366	0.008
30	1.430	0.010	0.364	0.005
35	1.457	0.012	0.354	0.006
40	1.483	0.010	0.348	0.005
45	1.483	0.016	0.345	0.005
75	1.555	0.015	0.323	0.004

Preparation: Probe **1** in 10 μL DMSO (0.5 mM) dissolved in Tris buffer (470 μL) (0 min), then addition of 1 mM HCl (10 μL) with trypsin (0.0625 ng) and 1 mM HCl (10 μL) with chymotrypsin (0.0625 μg) (0–16 min). Then addition of Tris buffer (10 μL) with trypsin inhibitor (5 ng) (16–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}/Slit_{EMS}$=10/10 nm. X_t/Y_t and X_0/Y_0 – ratio of fluorescence intensities of different fluorophores at times t (0–75 min) and 0 min; All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S24: Ratios of fluorescence intensities of the probe **1** – detection of trypsin (1 ng/mL) and thrombin (0.2 U/mL). (see Figure 10A)

Time [min]	DEAC(I_{75min}/I_{0min})/ Fl(I_{75min}/I_{0min})	DEAC/Fl St. Dev.	RhB(I_{75min}/I_{0min})/ Fl(I_{75min}/I_{0min})	RhB/Fl St. Dev.
0	1.000	0.000	1.000	0.000
2	1.787	0.044	0.855	0.012
5	2.614	0.094	0.714	0.013
8	3.139	0.092	0.635	0.012
12	3.659	0.090	0.550	0.012
16	4.051	0.097	0.493	0.008
20	4.375	0.134	0.446	0.012
25	4.740	0.140	0.418	0.011
30	5.090	0.172	0.395	0.011
35	5.357	0.172	0.376	0.008
40	5.588	0.171	0.361	0.007
45	5.811	0.163	0.349	0.006
75	6.606	0.176	0.303	0.004

Preparation: Probe **1** in 10 μL DMSO (0.5 mM) dissolved in Tris buffer (470 μL) (0 min), then addition of 1 mM HCl (10 μL) with trypsin (0.5 ng) and 0.9% saline (w/V) (10 μL) with thrombin (0.1 U) (0–16 min). Then addition of Tris-HCl buffer (10 μL) with trypsin inhibitor (5 ng) (16–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}/Slit_{EMS}$=10/10 nm. X_t/Y_t and X_0/Y_0 – ratio of fluorescence intensities of different fluorophores at times t (0–75 min) and 0 min; All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S25: Ratios of fluorescence intensities of the probe **1** – detection of trypsin (1 ng/mL) and thrombin (0.0125 U/mL). (see Figure 10B)

Time [min]	DEAC(I_{75min}/I_{0min})/ Fl(I_{75min}/I_{0min})	DEAC/Fl St. Dev.	RhB(I_{75min}/I_{0min})/ Fl(I_{75min}/I_{0min})	RhB/Fl St. Dev.
0	1.000	0.000	1.000	0.000
2	1.136	0.003	0.897	0.016
5	1.279	0.007	0.774	0.023
8	1.389	0.019	0.690	0.029
12	1.519	0.043	0.608	0.026
16	1.596	0.031	0.547	0.030
20	1.669	0.017	0.501	0.028
25	1.721	0.012	0.478	0.025
30	1.794	0.034	0.470	0.028
35	1.873	0.046	0.463	0.027
40	1.918	0.034	0.455	0.025
45	1.991	0.053	0.453	0.026
75	2.346	0.098	0.428	0.025

Preparation: Probe **1** in 10 µL DMSO (0.5 mM) dissolved in Tris buffer (470 µL) (0 min), then addition of 1 mM HCl (10 µL) with trypsin (0.5 ng)and 0.9% saline (w/V) (10 µL) with thrombin (6.25 mU) (0–16 min). Then addition of Tris buffer (10 µL) with trypsin inhibitor (5 ng) (16–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}/Slit_{EMS}$=10/10 nm. X_t/Y_t and X_0/Y_0 – ratio of fluorescence intensities of different fluorophores at times t (0-75 min) and 0 min; All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S26: Ratios of fluorescence intensities of the probe **1** – detection of trypsin (0.5 ng/mL) and thrombin (0.2 U/mL). (see Figure 10C)

Time [min]	DEAC(I_{75min}/I_{0min})/ Fl(I_{75min}/I_{0min})	DEAC/Fl St. Dev.	RhB(I_{75min}/I_{0min})/ Fl(I_{75min}/I_{0min})	RhB/Fl St. Dev.
0	1.000	0.000	1.000	0.000
2	1.778	0.038	0.886	0.011
5	2.666	0.035	0.762	0.011
8	3.212	0.032	0.679	0.016
12	3.795	0.056	0.595	0.019
16	4.169	0.077	0.537	0.019
20	4.533	0.113	0.483	0.015
25	4.915	0.135	0.449	0.016
30	5.236	0.144	0.421	0.017
35	5.484	0.131	0.401	0.013
40	5.721	0.142	0.381	0.011
45	5.936	0.165	0.368	0.012
75	6.675	0.136	0.312	0.009

Preparation: Probe **1** in 10 µL DMSO (0.5 mM) dissolved in Tris buffer (470 µL) (0 min), then addition of 1 mM HCl (10 µL) with trypsin (0.25 ng) and 0.9% saline (w/V) (10 µL) with thrombin (0.1 U) (0–16 min). Then addition of Tris buffer (10 µL) with trypsin inhibitor (5 ng) (16–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}/Slit_{EMS}$=10/10 nm. X_t/Y_t and X_0/Y_0 – ratio of fluorescence intensities of different fluorophores at times t (0–75 min) and 0 min; All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S27: Ratios of fluorescence intensities of the probe **1** – detection of trypsin (0.5 ng/mL) and thrombin (0.0125 U/mL). (see Figure 10D)

Time [min]	DEAC(I_{75min}/I_{0min})/ Fl(I_{75min}/I_{0min})	DEAC/Fl St. Dev.	RhB(I_{75min}/I_{0min})/ Fl(I_{75min}/I_{0min})	RhB/Fl St. Dev.
0	1.000	0.000	1.000	0.000
2	1.104	0.008	0.958	0.005
5	1.224	0.022	0.886	0.005
8	1.331	0.018	0.829	0.002
12	1.441	0.030	0.759	0.004
16	1.552	0.038	0.699	0.006
20	1.636	0.048	0.651	0.008
25	1.736	0.047	0.633	0.009
30	1.837	0.038	0.621	0.014
35	1.928	0.067	0.612	0.019
40	2.005	0.073	0.602	0.020
45	2.114	0.085	0.591	0.021
75	2.601	0.141	0.550	0.028

Preparation: Probe **1** in 10 µL DMSO (0.5 mM) dissolved in Tris buffer (470 µL) (0 min), then addition of 1 mM HCl (10 µL) with trypsin (0.25 ng) and 0.9% saline (w/V) (10 µL) with thrombin (6.25 mU) (0–16 min). Then addition of Tris buffer (10 µL) with trypsin inhibitor (5 ng) (16–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm; $Slit_{EXC}/Slit_{EMS}$=10/10 nm. X_t/Y_t and X_0/Y_0 – ratio of fluorescence intensities of different fluorophores at times t (0–75 min) and 0 min; All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S28: Time-dependent fluorescence response of the probe **1** – detection of chymotrypsin (c= 2 µg/mL) and thrombin (c= 0.2 U/mL). (see Figure 11A)

Time [min]	DEAC Average	DEAC St. Dev.
0	20.74	0.46
2	39.13	2.90
5	63.20	7.38
8	81.57	11.16
12	102.32	16.04
16	119.46	20.56
20	128.30	22.35
25	134.76	22.56
30	141.83	22.29
35	148.48	22.66
40	154.96	21.73
45	161.24	22.04
75	196.60	20.23

Preparation: Probe **1** in 10 µL DMSO (0.5 mM) dissolved in Tris buffer (470 µL) (0 min), then addition of 1 mM HCl (10 µL) with chymotrypsin (1 µg) and 0.9% saline (w/V) (10 µL) with thrombin (0.1 U) (0–16 min). Then addition of Tris buffer (10 µL) with dabigatran (0.25 µg) (16–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm; $Slit_{EXC}/Slit_{EMS}$=10/10 nm. All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S29: Time-dependent fluorescence response of the probe **1** – detection of chymotrypsin (c= 2 µg/mL) and thrombin (c= 0.05 U/mL) (see Figure 11C)

Time [min]	DEAC Average	DEAC St. Dev.
0	20.90	0.24
2	30.57	1.26
5	42.38	2.11
8	51.91	2.74
12	62.17	3.53
16	71.89	3.94
20	78.51	4.14
25	85.42	4.48
30	92.11	4.12
35	98.87	3.65
40	10.74	4.65
45	11.57	4.35
75	147.80	4.75

Preparation: Probe **1** in 10 µL DMSO (0.5 mM) dissolved in Tris buffer (470 µL) (0 min), then addition of 1 mM HCl (10 µL) with chymotrypsin (1 µg) and 0.9% saline (w/V) (10 µL) with thrombin (0.025 U) (0–16 min). Then addition of Tris buffer (10 µL) with dabigatran (0.25 µg) (16–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}$/$Slit_{EMS}$=10/10 nm. All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S30: Time-dependent fluorescence response of the probe **1** – detection of chymotrypsin (c= 0.5 µg/mL) and thrombin (c= 0.2 U/mL). (see Figure 11B)

Time [min]	DEAC Average	DEAC St. Dev.
0	20.50	0.35
2	32.48	0.93
5	48.30	1.24
8	63.86	2.37
12	82.39	3.44
16	99.16	4.54
20	103.80	4.86
25	106.43	5.28
30	108.66	5.86
35	110.47	5.76
40	113.66	6.45
45	114.59	6.48
75	126.15	6.77

Preparation: Probe **1** in 10 µL DMSO (0.5 mM) dissolved in Tris buffer (470 µL) (0 min), then addition of 1 mM HCl (10 µL) with chymotrypsin (0.25 µg) and 0.9% saline (w/V) (10 µL) with thrombin (0.1 U) (0–16 min). Then addition of Tris buffer (10 µL) with dabigatran (0.25 µg) (16–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}$/$Slit_{EMS}$=10/10 nm. All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S31: Time-dependent fluorescence response of the probe **1** – detection of chymotrypsin (c= 0.5 µg/mL) and thrombin (c= 0.05 U/mL). (see Figure 11D)

Time [min]	DEAC Average	DEAC St. Dev.
0	20.25	0.33
2	26.14	0.49
5	36.08	0.87
8	44.95	0.78
12	57.34	1.00
16	67.56	1.39
20	71.34	2.21
25	72.99	2.15
30	74.70	2.37
35	76.06	3.03
40	78.35	2.61
45	80.02	2.98
75	90.65	3.68

Preparation: Probe **1** in 10 µL DMSO (0.5 mM) dissolved in Tris buffer (470 µL) (0 min), then addition of 1 mM HCl (10 µL) with chymotrypsin (0.25 µg) and 0.9% saline (w/V) (10 µL) with thrombin (0.025 U) (0–16 min). Then addition of Tris buffer (10 µL) with dabigatran (0.25 µg) (16–75 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}$/$Slit_{EMS}$=10/10 nm. All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S32: Ratios of fluorescence intensities of the probe **1** – detection of trypsin (1 ng/mL), chymotrypsin (2 μg/mL) and thrombin (0.2 U/mL). (see Figure 12A)

Time [min]	$(DEAC_t/Fl_t)/(DEAC_0/Fl_0)$	DEAC/Fl St. Dev.	$(RhB_t/Fl_t)/(RhB_0/Fl_0)$	RHB/Fl St. Dev.
0	1	0	1	0
2	1.87	0.05	0.82	0.01
5	2.49	0.11	0.68	0.01
8	2.82	0.13	0.59	0.02
12	3.04	0.15	0.51	0.02
16	3.16	0.17	0.45	0.02
20	3.24	0.19	0.41	0.02
25	3.31	0.18	0.39	0.02
30	3.44	0.19	0.37	0.02
35	3.54	0.19	0.36	0.02
40	3.64	0.19	0.35	0.02
45	3.74	0.17	0.34	0.02
60	4.01	0.19	0.33	0.02
65	3.73	0.19	0.35	0.02
70	3.73	0.20	0.35	0.02
75	3.72	0.18	0.35	0.02
80	3.75	0.19	0.34	0.02
85	3.76	0.19	0.34	0.02
90	3.77	0.20	0.34	0.02
105	3.80	0.20	0.33	0.02
120	3.84	0.20	0.33	0.02

Preparation: Probe **1** in 10 μL DMSO (0.5 mM) dissolved in Tris buffer (460 μL) (0 min), then addition of 1 mM HCl (10 μL) with trypsin (0.5 ng), chymotrypsin (1 μg) and 0.9% saline (w/V) (10 μL) with thrombin (0.1 U) (0–16 min). Then addition of Tris buffer (10 μL) with trypsin inhibitor (5 ng) (16–60 min). Then addition of DMSO (10 μL) with chymostatin (30 μg) (60–120 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}/Slit_{EMS}$=10/10 nm. X_t/Y_t and X_0/Y_0 – ratio of fluorescence intensities of different fluorophores at times t (0-75 min) and 0 min; All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S33: Ratios of fluorescence intensities of the probe **1** – detection of trypsin (1 ng/mL), chymotrypsin (2 μg/mL) and thrombin (0.2 U/mL). (see Figure 12D)

Time [min]	$(DEAC_t/Fl_t)/(DEAC_0/Fl_0)$	DEAC/Fl St. Dev.	$(RhB_t/Fl_t)/(RhB_0/Fl_0)$	RHB/Fl St. Dev.
0	1	0	1	0
2	1.79	0.06	0.84	0.01
5	2.35	0.10	0.72	0.01
8	2.61	0.13	0.63	0.01
12	2.86	0.13	0.55	0.01
16	2.96	0.15	0.49	0.01
20	2.82	0.09	0.49	0.01
25	2.78	0.08	0.47	0.00
30	2.81	0.08	0.45	0.00
35	2.85	0.11	0.43	0.01
40	2.90	0.11	0.42	0.01
45	2.93	0.13	0.40	0.01
60	2.96	0.14	0.36	0.02
65	2.82	0.14	0.33	0.01
70	2.84	0.13	0.33	0.01
75	2.86	0.13	0.33	0.01
80	2.89	0.14	0.32	0.01
85	2.91	0.15	0.32	0.01
90	2.93	0.16	0.32	0.01
105	2.99	0.17	0.31	0.01
120	3.04	0.17	0.31	0.01

Preparation: Probe **1** in 10 μL DMSO (0.5 mM) dissolved in Tris buffer (460 μL) (0 min), then addition of 1 mM HCl (10 μL) with trypsin (0.5 ng), chymotrypsin (1 μg) and 0.9% saline (w/V) (10 μL) with thrombin (0.1 U) (0–16 min). Then addition of DMSO (10 μL) with chymostatin (30 μg) (16–60 min). Then addition of Tris buffer (10 μL) with trypsin inhibitor (5 ng) (60–120 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}/Slit_{EMS}$=10/10 nm. X_t/Y_t and X_0/Y_0 – ratio of fluorescence intensities of different fluorophores at times t (0–75 min) and 0 min; All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S34: Ratios of fluorescence intensities of the probe **1** – detection of trypsin (0.5 ng/mL), chymotrypsin (2 μg/mL) and thrombin (0.2 U/mL). (see Figure 12E)

Time [min]	$(DEAC_t/Fl_t)/(DEAC_0/Fl_0)$	DEAC/Fl St. Dev.	$(RhB_t/Fl_t)/(RhB_0/Fl_0)$	RHB/Fl St. Dev.
0	1	0	1	0
2	1.82	0.10	0.88	0.01
5	2.47	0.15	0.77	0.02
8	2.83	0.17	0.70	0.02
12	3.17	0.18	0.62	0.01
16	3.37	0.20	0.57	0.01
20	3.21	0.16	0.57	0.01
25	3.22	0.20	0.54	0.01
30	3.26	0.18	0.52	0.02
35	3.29	0.18	0.51	0.02
40	3.30	0.16	0.49	0.02
45	3.34	0.16	0.47	0.02
60	3.38	0.14	0.43	0.02
65	3.39	0.19	0.40	0.02
70	3.41	0.17	0.40	0.02
75	3.44	0.20	0.39	0.02
80	3.46	0.21	0.39	0.02
85	3.50	0.22	0.39	0.02
90	3.50	0.21	0.38	0.02
105	3.58	0.22	0.37	0.02
120	3.68	0.23	0.37	0.02

Preparation: Probe **1** in 10 μL DMSO (0.5 mM) dissolved in Tris buffer (460 μL) (0 min), then addition of 1 mM HCl (10 μL) with trypsin (0.25 ng), chymotrypsin (1 μg) and 0.9% saline (w/V) (10 μL) with thrombin (0.1 U) (0–16 min). Then addition of DMSO (10 μL) with chymostatin (30 μg) (16–60 min). Then addition of Tris buffer (10 μL) with trypsin inhibitor (5 ng) (60–120 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}/Slit_{EMS}$=10/10 nm. X_t/Y_t and X_0/Y_0 – ratio of fluorescence intensities of different fluorophores at times t (0–75 min) and 0 min; All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S35: Ratios of fluorescence intensities of the probe **1** – detection of trypsin (0.5 ng/mL), chymotrypsin (0.5 μg/mL) and thrombin (0.05 U/mL). (see Figure 12F)

Time [min]	$(DEAC_t/Fl_t)/(DEAC_0/Fl_0)$	DEAC/Fl St. Dev.	$(RhB_t/Fl_t)/(RhB_0/Fl_0)$	RHB/Fl St. Dev.
0	1	0	1	0
2	1.30	0.05	0.92	0.01
5	1.61	0.05	0.83	0.01
8	1.88	0.06	0.77	0.01
12	2.13	0.11	0.69	0.02
16	2.34	0.13	0.63	0.02
20	2.31	0.12	0.62	0.02
25	2.41	0.11	0.60	0.01
30	2.51	0.13	0.58	0.01
35	2.61	0.15	0.55	0.01
40	2.70	0.17	0.54	0.01
45	2.77	0.21	0.52	0.02
60	2.99	0.28	0.47	0.02
65	3.09	0.33	0.44	0.01
70	3.16	0.34	0.44	0.01
75	3.21	0.36	0.43	0.01
80	3.28	0.38	0.42	0.01
85	3.36	0.40	0.42	0.01
90	3.42	0.40	0.41	0.01
105	3.56	0.44	0.40	0.01
120	3.74	0.48	0.39	0.01

Preparation: Probe **1** in 10 μL DMSO (0.5 mM) dissolved in Tris buffer (460 μL) (0 min), then addition of 1 mM HCl (10 μL) with trypsin (0.25 ng), chymotrypsin (0.25 μg) and 0.9% saline (w/V) (10 μL) with thrombin (0.025 U) (0–16 min). Then addition of DMSO (10 μL) with chymostatin (30 μg) (16–60 min). Then addition of Tris buffer (10 μL) with trypsin inhibitor (5 ng) (60–120 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}/Slit_{EMS}$=10/10 nm. X_t/Y_t and X_0/Y_0 – ratio of fluorescence intensities of different fluorophores at times t (0–75 min) and 0 min; All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S36 Ratios of fluorescence intensities of the probe **1** – detection of trypsin (1 ng/mL), chymotrypsin (0.5 μg/mL) and thrombin (0.05 U/mL). (see Figure 12B)

Time [min]	$(DEAC_t/Fl_t)/(DEAC_0/Fl_0)$	DEAC/Fl St. Dev.	$(RhB_t/Fl_t)/(RhB_0/Fl_0)$	RHB/Fl St. Dev.
0	1	0	1	0
2	1.38	0.01	0.88	0.01
5	1.76	0.01	0.76	0.01
8	2.01	0.02	0.66	0.02
12	2.20	0.01	0.58	0.01
16	2.30	0.01	0.51	0.01
20	2.37	0.02	0.47	0.01
25	2.48	0.04	0.45	0.00
30	2.60	0.06	0.44	0.01
35	2.71	0.07	0.43	0.01
40	2.79	0.08	0.42	0.00
45	2.86	0.09	0.41	0.01
60	3.08	0.11	0.40	0.00
65	3.14	0.12	0.39	0.00
70	3.16	0.12	0.39	0.01
75	3.19	0.14	0.39	0.01
80	3.20	0.12	0.39	0.01
85	3.22	0.13	0.39	0.01
90	3.26	0.12	0.39	0.01
105	3.33	0.12	0.38	0.01
120	3.38	0.11	0.38	0.01

Preparation: Probe **1** in 10 μL DMSO (0.5 mM) dissolved in Tris buffer (460 μL) (0 min), then addition of 1 mM HCl (10 μL) with trypsin (0.5 ng), chymotrypsin (0.25 μg) and 0.9% saline (w/V) (10 μL) with thrombin (0.025 U) (0–16 min). Then addition of Tris buffer (10 μL) with trypsin inhibitor (5 ng) (16–60 min). Then addition of Tris buffer (10 μL) with dabigatran (0.25 μg) (60–120 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}/Slit_{EMS}$=10/10 nm. X_t/Y_t and X_0/Y_0 – ratio of fluorescence intensities of different fluorophores at times t (0–75 min) and 0 min; All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.

Table S37: Ratios of fluorescence intensities of the probe **1** – detection of trypsin (1 ng/mL), chymotrypsin (0.5 μg/mL) and thrombin (0.2 U/mL). (see Figure 12C)

Time [min]	$(DEAC_t/Fl_t)/(DEAC_0/Fl_0)$	DEAC/Fl St. Dev.	$(RhB_t/Fl_t)/(RhB_0/Fl_0)$	RHB/Fl St. Dev.
0	1	0	1	0
2	1.77	0.06	0.83	0.01
5	2.46	0.10	0.69	0.01
8	2.92	0.09	0.61	0.01
12	3.22	0.13	0.52	0.00
16	3.42	0.18	0.46	0.00
20	3.36	0.16	0.44	0.01
25	3.36	0.19	0.43	0.01
30	3.36	0.20	0.43	0.01
35	3.37	0.18	0.42	0.01
40	3.37	0.19	0.42	0.01
45	3.38	0.20	0.41	0.01
60	3.41	0.19	0.40	0.01
65	3.15	0.16	0.43	0.01
70	3.12	0.16	0.43	0.01
75	3.12	0.19	0.42	0.01
80	3.12	0.16	0.42	0.01
85	3.10	0.16	0.41	0.01
90	3.11	0.18	0.41	0.01
105	3.06	0.17	0.40	0.01
120	3.05	0.18	0.40	0.01

Preparation: Probe **1** in 10 μL DMSO (0.5 mM) dissolved in Tris buffer (460 μL) (0 min), then addition of 1 mM HCl (10 μL) with trypsin (0.5 ng), chymotrypsin (0.25 μg) and 0.9% saline (w/V) (10 μL) with thrombin (0.1 U) (0–16 min). Then addition of Tris buffer (10 μL) with dabigatran (0.25 μg) (16–60 min). Then addition of DMSO (10 μL) with chymostatin (30 μg) (60–120 min); Incubation: T=37 °C; Excitation: λ=425 nm; Emissions: λ_{DEAC}=475 nm, λ_{FL}=526 nm, λ_{RhB}=590 nm; $Slit_{EXC}/Slit_{EMS}$=10/10 nm. X_t/Y_t and X_0/Y_0 – ratio of fluorescence intensities of different fluorophores at times t (0–75 min) and 0 min; All measurements were performed in three parallels. The corresponding average values and standard deviations are reported.