Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplementary Information for

Logical Stimuli-Triggered Delivery of Small Molecules from Hydrogel Biomaterials

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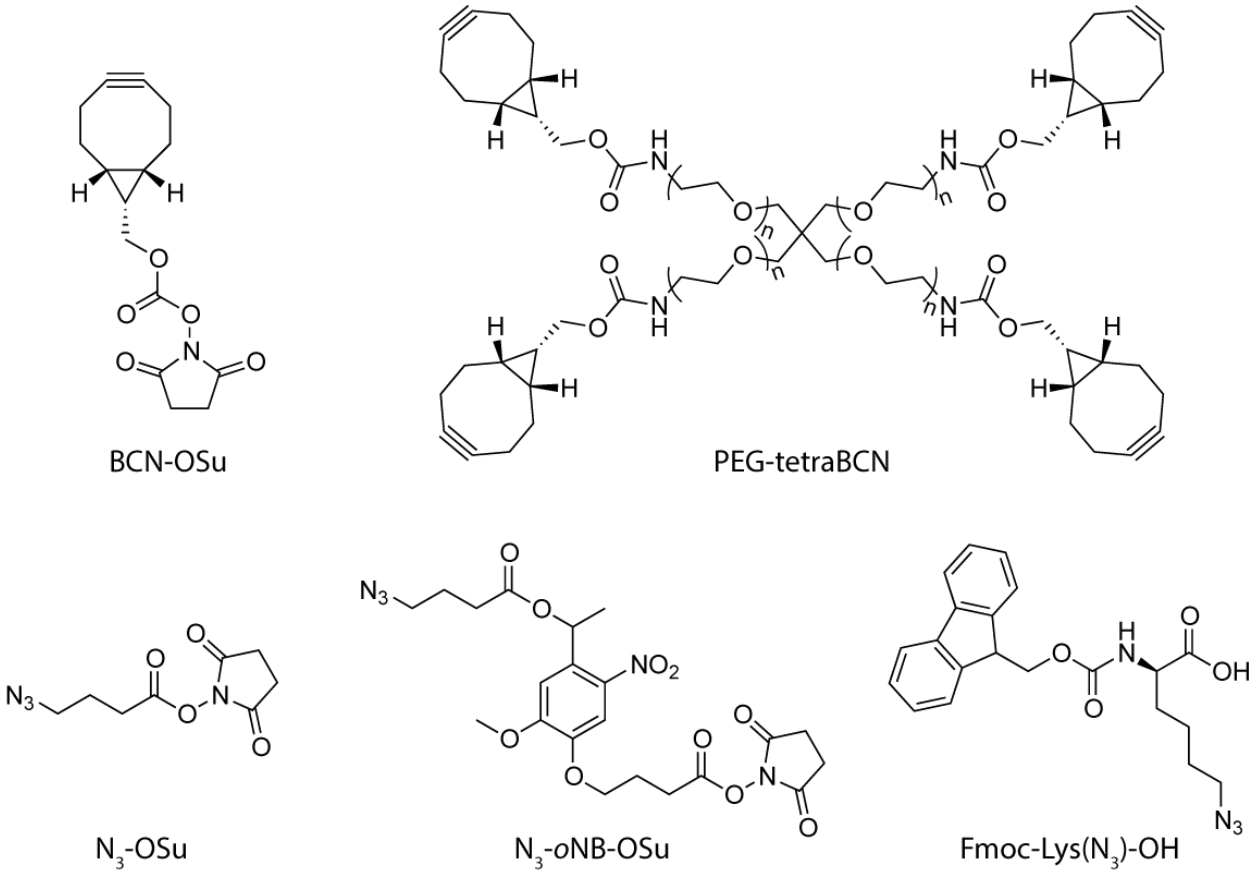
General Synthetic Information

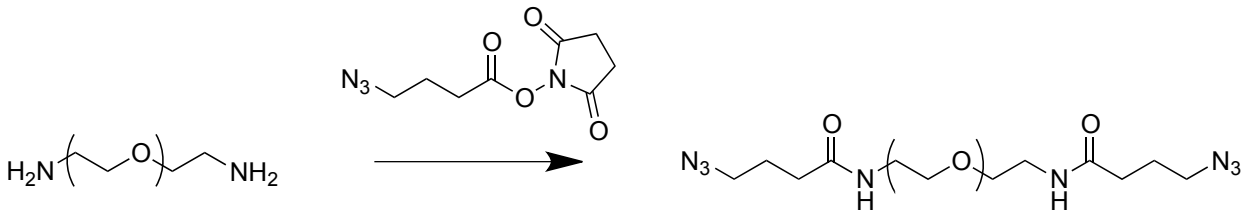
Chemical reagents and solvents were purchased from either Sigma-Aldrich or Fisher Scientific and used without further purification. Distilled water (dH_2O) was obtained from a U.S. Filter Corporation Reverse Osmosis system equipped with a desalination membrane. All chemical reactions were performed under inert nitrogen atmosphere in flame-dried glassware and were stirred with Teflon-coated magnetic stir bars. Solvent was removed under reduced pressure with a Buchi Rotovap R-3 by using either V-700 vacuum pump or Welch 1400 high vacuum pump. All peptides were synthesized using Fmoc-based, microwave-assisted, solid-phase methodologies on a CEM Liberty 1. All peptides were purified by semi-preparative reverse-phase high pressure liquid chromatography (RP-HPLC) performed on a Dionex Ultimate 3000 equipped with an RS multiple variable wavelength detector, an automated fraction collector, and a C18 column. Peptide characterization was performed by Matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) analysis on a Bruker AutoFlex II. Lyophilization was performed on a Labconco FreeZone 2.5 Plus freeze-dryer equipped with Labconco rotary vane 117 vacuum pump. A Lumen Dynamics OmniCure S1500 Spot UV curing system was used for photochemical cleavage reactions, where light intensity was determined using a Cole-Palmer radiometer (Series 9811-50, $\lambda = 365$ nm). Fluorescence measurements were performed using a SpectraMax M5 spectrometer. Fluorescence imaging was performed on a Nikon Eclipse TE2000-U fluorescent microscope or a Typhoon FLA9000 fluorescent gel scanner.

Synthesis of Previously Reported Compounds Used in this Work



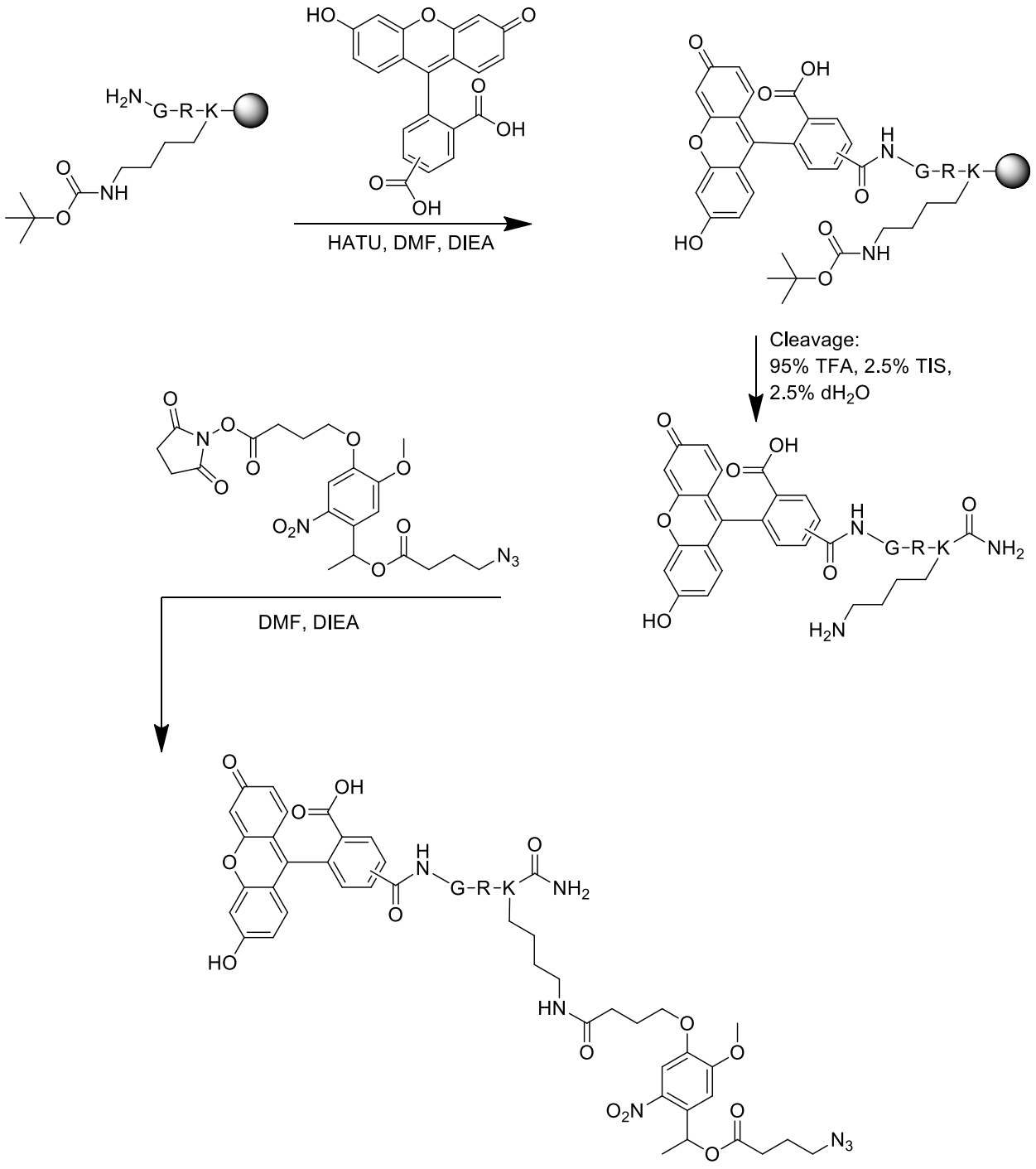
(1R,8S,9s)-bicyclo[6.1.0]non-4-yn-9-ylmethyl (2,5-dioxopyrrolidin-1-yl) carbonate (BCN-OSu), poly(ethylene glycol) tetrabicyclononyne (PEG-tetraBCN, M_n ~ 20,000 Da), 2,5-dioxopyrrolidin-1-yl 4-azidobutanoate (N_3-OSu), 2,5-dioxopyrrolidin-1-yl 4-(4-(1-((4-azidobutanoyl)oxy)ethyl)-2-methoxy-5-nitrophenoxy)butanoate (N_3-*o*NB-OSu), (*R*)-2-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-6-azidohexanoic acid (Fmoc-Lys(N_3)-OH), and recombinant MMP-8 were synthesized as previously reported[1–3].

Method S1: Synthesis of PEG-diazide (N_3-PEG-N_3)



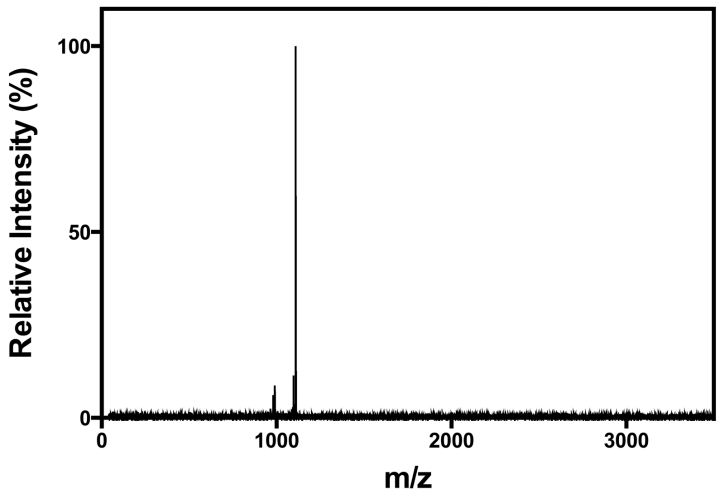
Linear poly(ethylene glycol) diamine (M_n ~ 3,500 Da, 1 g, 0.57 mmol NH_2, 1x, Jenkem) and N_3-OSu (194 mg, 0.86 mmol, 1.5x) were dissolved in dimethylformamide (5 mL). *N*,*N*-Diisopropylethylamine (398 µL, 294 mg, 2.28 mmol, 4x) was added to the mixture, and the reaction was stirred overnight, diluted in water (15 mL), dialyzed (MWCO ~ 2 kDa, SpectraPor), and lyophilized to yield a white powder (1.00 g, quantitative yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.75 (m, 4H), 3.65-3.61 (m, 318H), 3.28 (m, 4H), 2.35 (m, 4H), 1.86 (m, 4H). Functionalization was found to be >95% by comparing integral values for hydrogens introduced upon azide coupling (δ 3.28, 2.35, 1.86) with those from the PEG backbone (δ 3.60-3.42).

Method S2: Synthesis of FAM-P

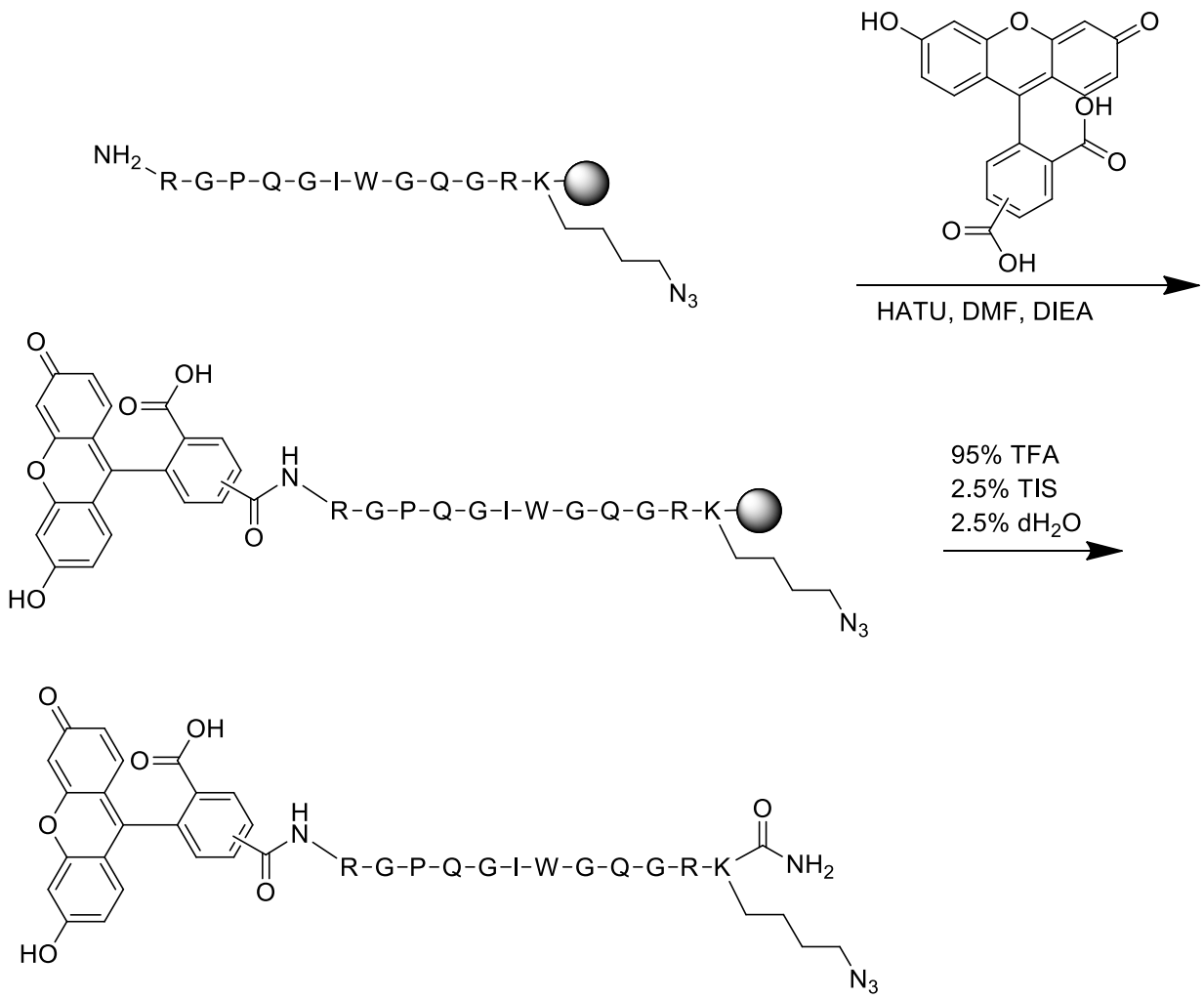


The base peptide H-GRK(Boc)-NH_2 was synthesized (0.25 mmol scale) on rink amide resin (ChemPep, loading capacity = 0.8 mmol/g) *via* standard microwave-assisted Fmoc solid-phase methodologies with HBTU activation. Microwave-assisted coupling of 5,6-Carboxyfluorescein (4x, 377 mg, Fisher) was conducted at 60 °C and 25W for 30 min on resin with HATU (3.9x, 371 mg) dissolved in minimal DMF containing DIEA (8x, 258.3 mg). Resin was treated with trifluoroacetic acid/triisopropylsilane/water (95:2.5:2.5) for 2 h, and the crude peptide was

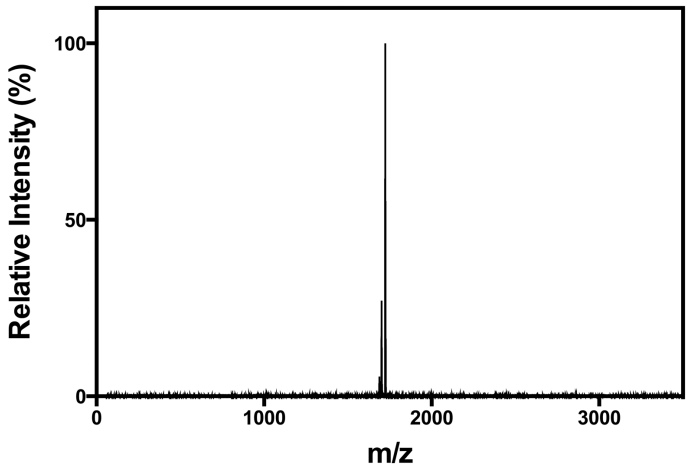
precipitated in and washed (2x) with ice-cold diethyl ether. The crude peptide was purified by RP-HPLC using a 55-min linear gradient (5–100% of acetonitrile and 0.1% trifluoroacetic acid) and lyophilized to give the intermediate (FAM-GRK-NH_2) as a fluffy, yellow solid. N_3-*o*NB-OSu (53 mg, 0.105 mmol, 1.3x) was dissolved in minimal DMF containing DIEA (40 mg, 0.31 mmol, 4x) and added to the peptide to react overnight. The peptide was purified by RP-HPLC using a 55-min linear gradient (5–100% of acetonitrile and 0.1% trifluoroacetic acid) and lyophilized to give the product (FAM-GRK(*o*NB-N_3)-NH_2, denoted FAM-P) as a fluffy, yellow solid (11.0 mg, 0.01 mmol) with a yield of 4%. Peptide purity was confirmed with analytical RP-HPLC and matrix-assisted laser desorption-ionization time-of-flight mass spectrometry using α-cyano-4-hydroxycinnamic acid matrix: MALDI-TOF: calculated for $C_{52}H_{60}N_{12}O_{16}^{+}$ $[M + {}^{1}H]^{+}$, 1109.1; found 1109.3.



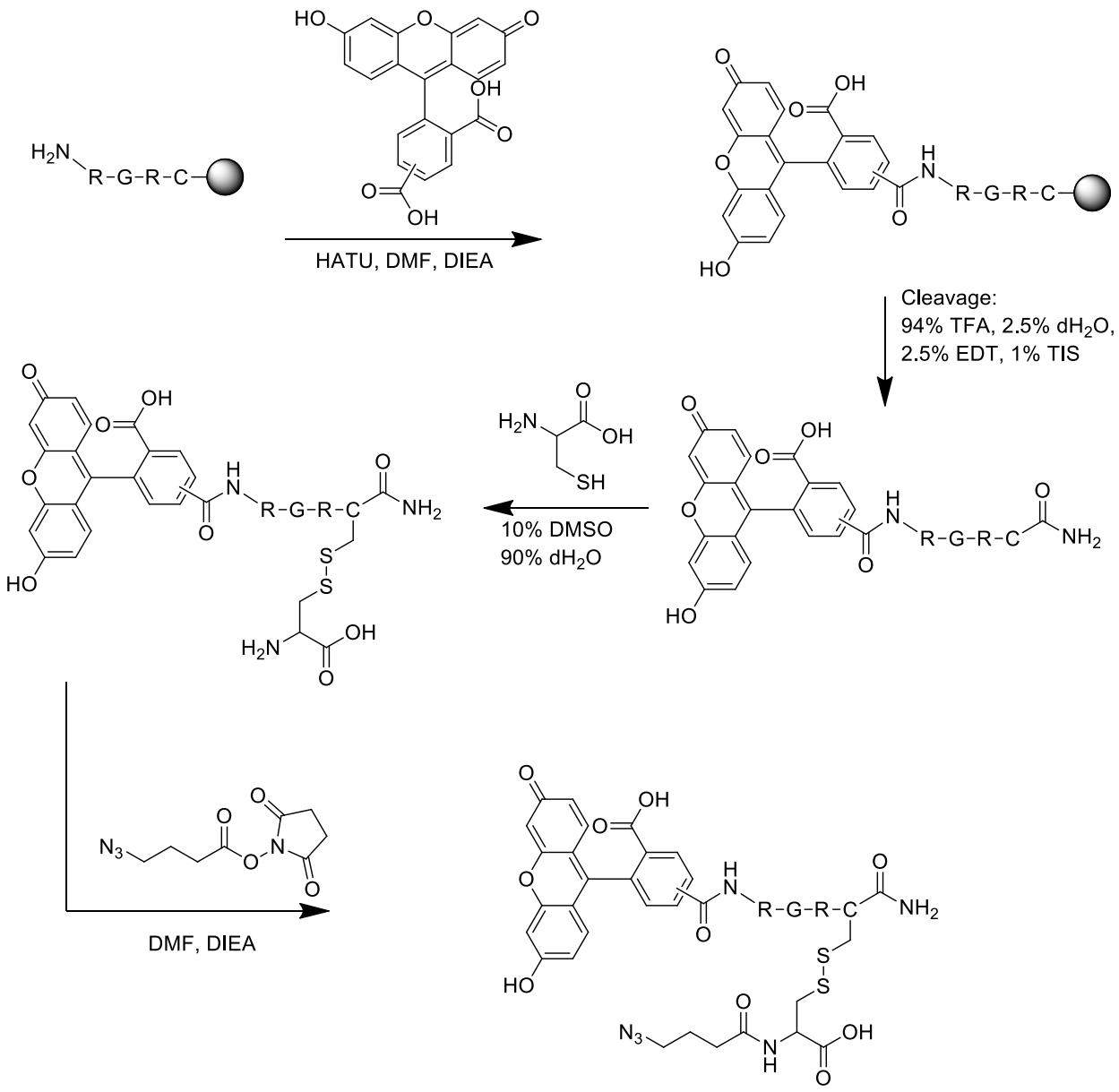
Method S3: Synthesis of FAM-E



The base peptide H-RGPQGIWGQGRK(N_3)-NH_2 was synthesized (0.25 mmol scale) on rink amide resin (ChemPep, loading capacity = 0.8 mmol/g) *via* standard microwave-assisted Fmoc solid-phase methodologies with HBTU activation. 5,6-Carboxyfluorescein (4x, 376 mg, Fisher) was coupled at room temperature twice for 2 h on resin with HATU (3.9x, 370 mg) dissolved in minimal DMF containing DIEA (8x, 258 mg). Resin was treated with trifluoroacetic acid/triisopropylsilane/water (95:2.5:2.5) for 2 h, and the crude peptide was precipitated in and washed (2x) with ice-cold diethyl ether. The crude peptide was purified using semi-preparative reversed-phase high-performance liquid chromatography (RP-HPLC) using a 55-min linear gradient (5–100% of acetonitrile and 0.1% trifluoroacetic acid) and lyophilized to give the product (FAM-RGPQGIWGQGRK(N_3)-NH_2, denoted FAM-E) as a fluffy, yellow solid (56.2 mg, 32.6 μmol, 13% overall). Peptide purity was confirmed with analytical RP-HPLC and matrix-assisted laser desorption-ionization time-of-flight mass spectrometry using α-cyano-4-hydroxycinnamic acid matrix: MALDI-TOF: calculated for $C_{79}H_{103}N_{25}O_{20}^{+}$ $[M + {}^{1}H]^{+}$, 1722.8; found 1722.6.

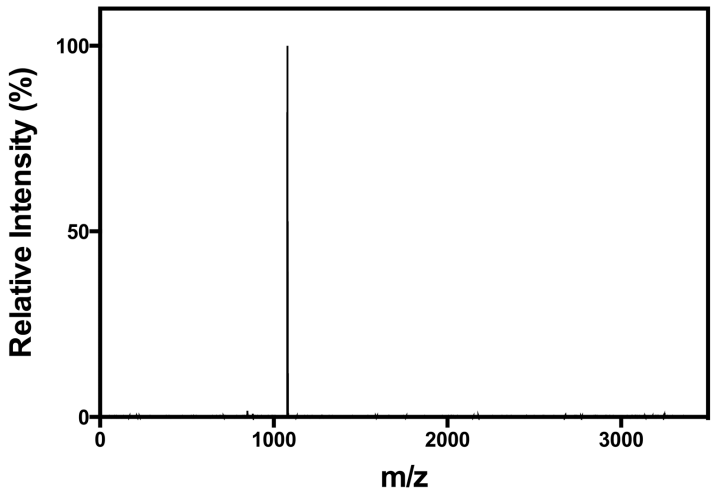

Relative Intensity (%)
100
50
0
0
1000
2000
3000
m/z

Method S4: Synthesis of FAM-R

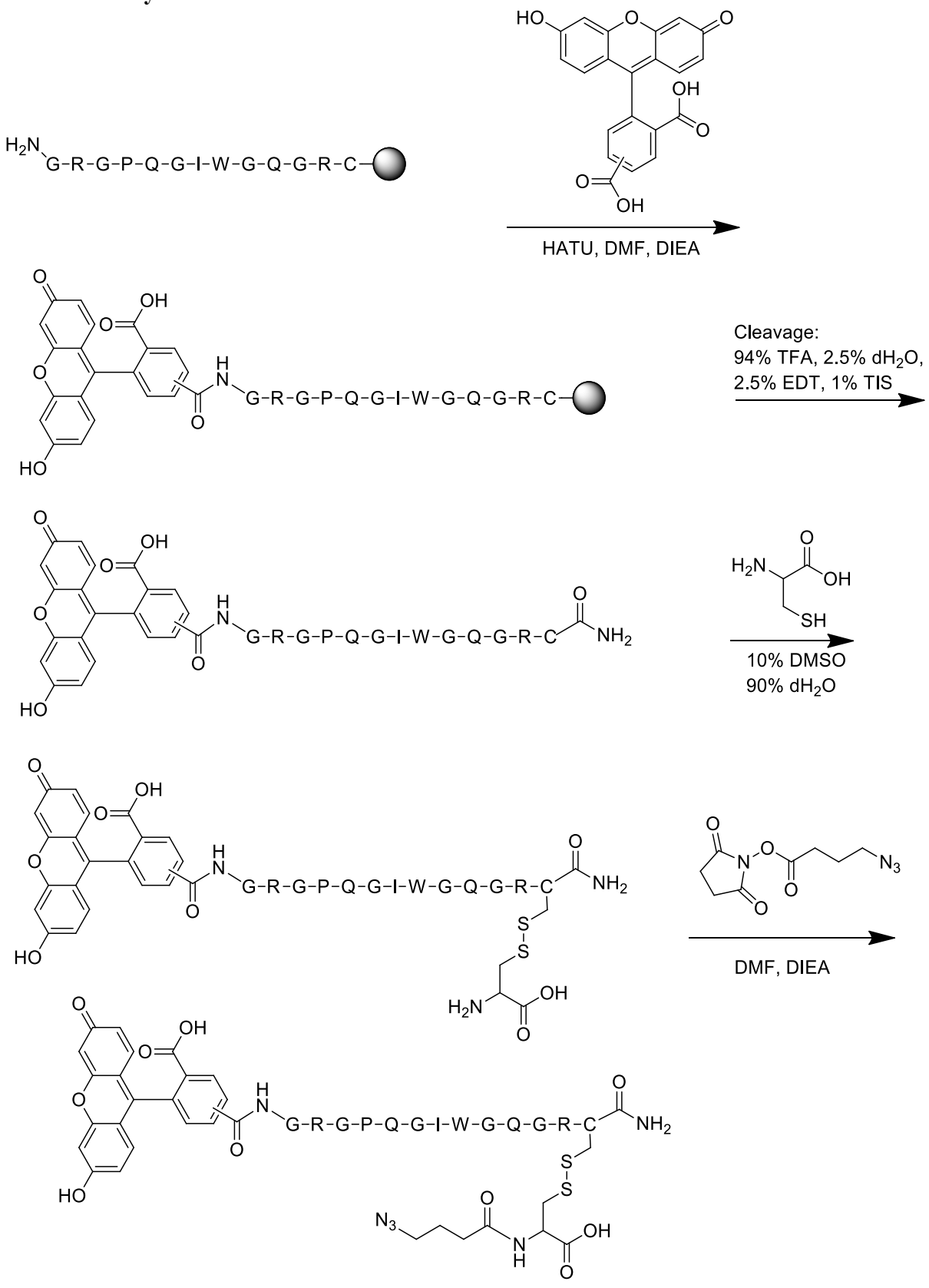


The base peptide H-RGRC-NH_2 was synthesized (0.25 mmol scale) on rink amide resin (ChemPep, loading capacity = 0.8 mmol/g) *via* standard microwave-assisted Fmoc solid-phase methodologies with HBTU activation. Microwave-assisted coupling of 5,6-Carboxyfluorescein (4x, 377 mg, Fisher) was conducted at 60 °C and 25 W for 30 min on resin with HATU (3.9x, 371 mg) dissolved in minimal DMF containing DIEA (8x, 258.3 mg). Resin was treated with trifluoroacetic acid/1,2-ethanedithiol/water/triisopropylsilane (94:2.5:2.5:1) for 2 h, and the crude peptide was precipitated in and washed (2x) with ice-cold diethyl ether. The crude peptide was purified by RP-HPLC using a 42-min linear gradient (20–100% of acetonitrile and 0.1% trifluoroacetic acid) and lyophilized to give the intermediate (FAM-RGRC-NH_2) as a fluffy, yellow solid. Cysteine (606 mg, 5 mmol, 20x) was codissolved with intermediate peptide in a

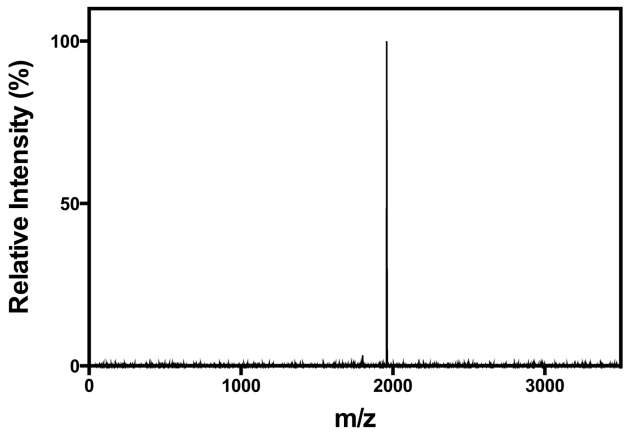
dH_2O/DMSO (90:10) solution (50 mL) and agitated for 24 h. Additional cysteine (606 mg, 5 mmol, 20x) was added to solution and reacted for 24 h while agitating at room temperature. Product was vacuum filtered and washed with dH_2O. The filtrate was frozen, lyophilized, and purified by RP-HPLC using a 42-min linear gradient (20–100% of acetonitrile and 0.1% trifluoroacetic acid); lyophilization afforded the purified intermediate (FAM-RGRC(C)-NH_2 linked *via* a cysteine-cysteine disulfide bridge) as a fluffy, yellow solid (104.4 mg, 0.10795 mmol). N_3-OSu (29.32 mg, 0.13 mmol, 1.2x) was coupled overnight with the peptide in minimal DMF containing DIEA (55.8 mg, 0.43 mmol, 4x). The crude peptide was purified by RP-HPLC using a 42-min linear gradient (20–100% of acetonitrile and 0.1% trifluoroacetic acid) and lyophilized to give the product (FAM-RGRC(C-N_3)-NH_2 linked *via* a cysteine-cysteine disulfide bridge, denoted FAM-R) as a fluffy, yellow solid (83.2 mg, 0.077 mmol) with a good overall yield (29.6%). Peptide purity was confirmed with analytical RP-HPLC and matrix-assisted laser desorption-ionization time-of-flight mass spectrometry using α-cyano-4-hydroxycinnamic acid matrix: MALDI-TOF: calculated for $C_{45}H_{56}N_{15}O_{13}S_2^+$ $[M + {}^1H]^+$, 1079.2; found 1079.3.



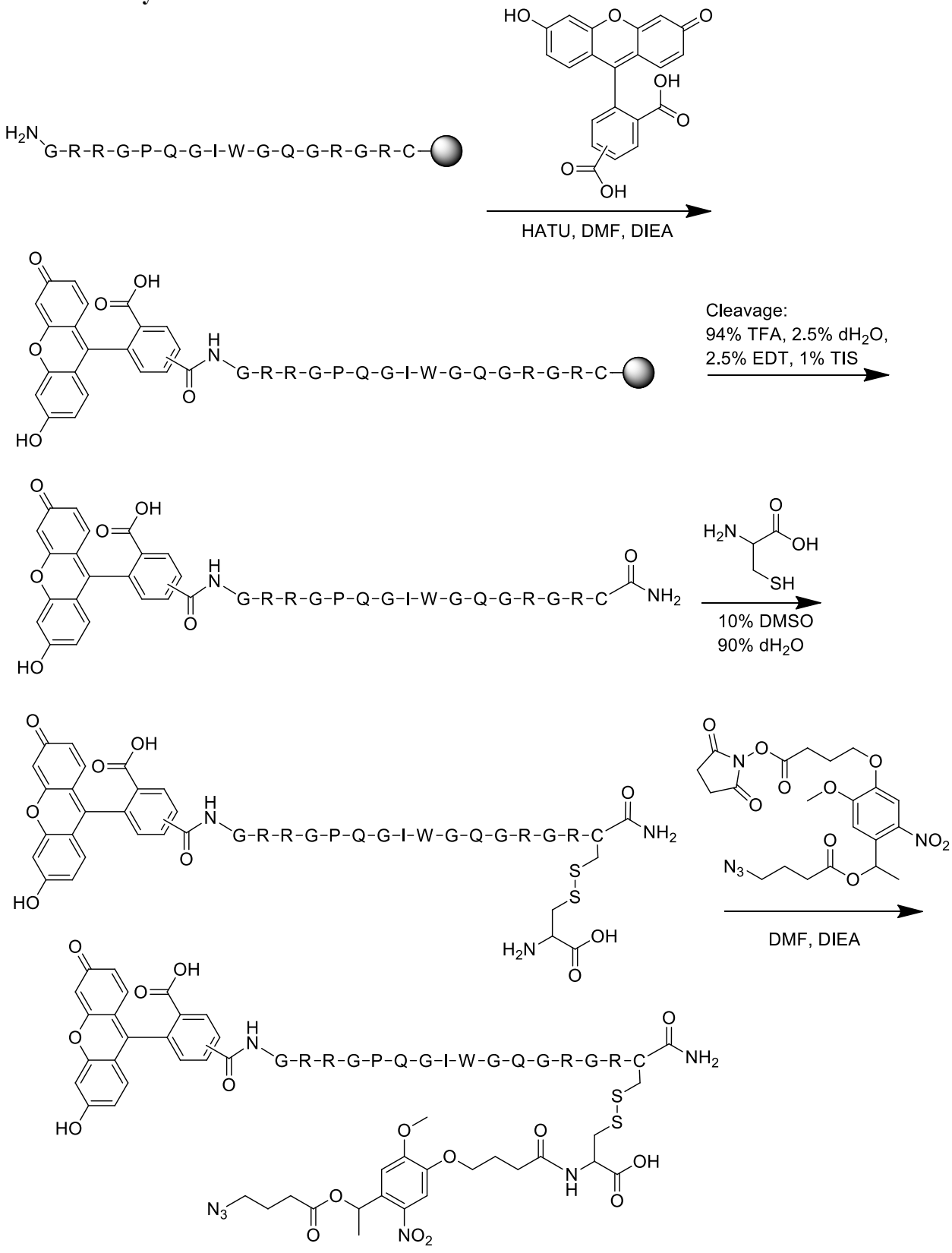
Method S5: Synthesis of FAM-EVR



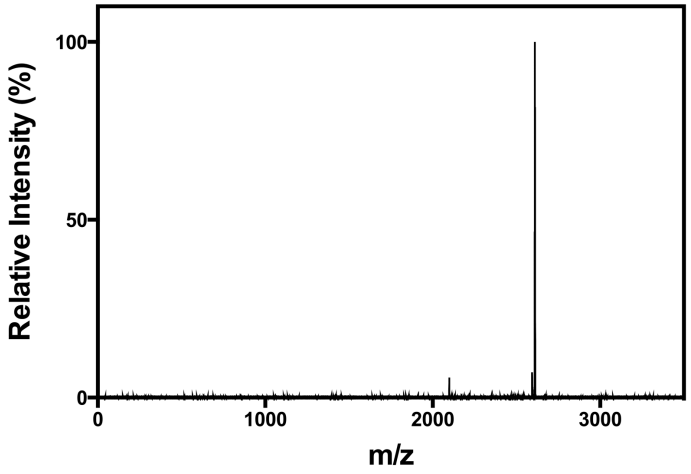
The base peptide H-GRGPQGIWGQGRC-NH_2 was synthesized (0.25 mmol scale) on rink amide resin (ChemPep, loading capacity = 0.8 mmol/g) *via* standard microwave-assisted Fmoc solid-phase methodologies with HBTU activation. Coupling of 5,6-Carboxyfluorescein (4x, 376 mg, Fisher) was conducted at room temperature overnight on resin with HATU (3.9x, 370 mg) dissolved in minimal DMF containing DIEA (8x, 258 mg). Resin was treated with trifluoroacetic acid/1,2-ethanedithiol/water/triisopropylsilane (94:2.5:2.5:1) for 2 h, and the crude peptide was precipitated in and washed (2x) with ice-cold diethyl ether. The crude peptide was purified by RP-HPLC using a 55-min linear gradient (5–100% of acetonitrile and 0.1% trifluoroacetic acid) and lyophilized to give the intermediate (FAM-GRGPQGIWGQGRC-NH_2) as a fluffy, yellow solid. Cysteine (605.8 mg, 5 mmol, 20x) was codissolved with intermediate peptide in a dH_2O/DMSO (90:10) solution (50 mL) and agitated for 24 h. Product was vacuum filtered and washed with dH_2O. The filtrate was frozen, lyophilized, and purified by RP-HPLC using a 42-min linear gradient (20–100% of acetonitrile and 0.1% trifluoroacetic acid) and lyophilized to give the intermediate (FAM-GRGPQGIWGQGRC(C)-NH_2 linked *via* a cysteine-cysteine disulfide bridge) as a fluffy, yellow solid. N_3-OSu (13.6 mg, 0.06 mmol, 2x) was reacted overnight with the peptide in minimal DMF containing DIEA (15.5 mg, 0.12 mmol, 4x). The peptide was purified by RP-HPLC using a 42-min linear gradient (20–100% of acetonitrile and 0.1% trifluoroacetic acid) and lyophilized to give the product (FAM-GRGPQGIWGQGRC(C-N_3)-NH_2 linked *via* a cysteine-cysteine disulfide bridge, denoted FAM-EvR) as a fluffy, yellow solid (21.7 mg, 11.1 μmol) with an overall yield of 4.4%. Peptide purity was confirmed with analytical RP-HPLC and matrix-assisted laser desorption-ionization time-of-flight mass spectrometry using α-cyano-4-hydroxycinnamic acid/2,5-dihydroxybenzoic acid (2:1) matrix: MALDI-TOF: calculated for $C_{85}H_{111}N_{27}O_{24}S_2^+$ $[M + {}^1H]^+$, 1959.1; found 1959.5.



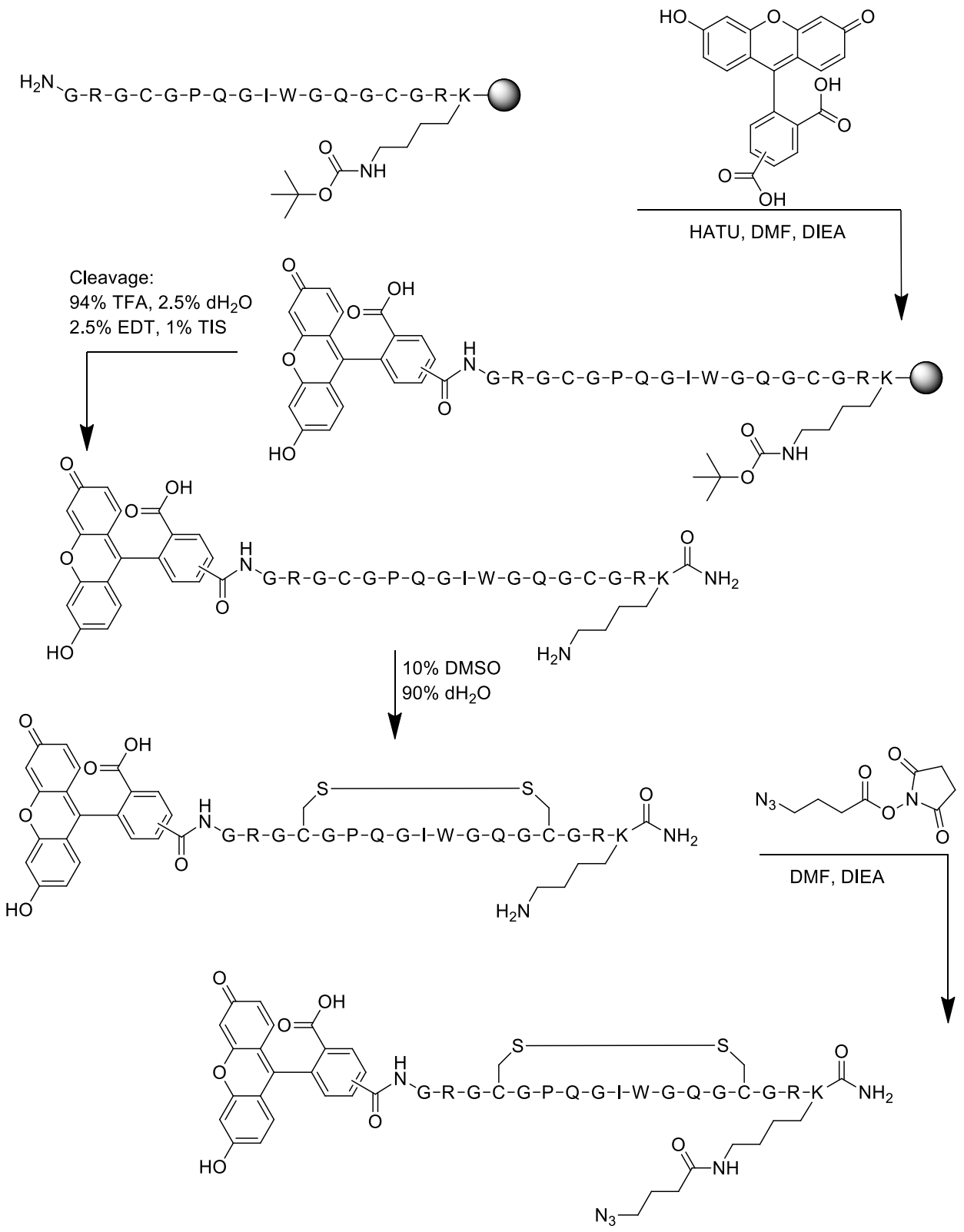
Method S6: Synthesis of FAM-EVRVP



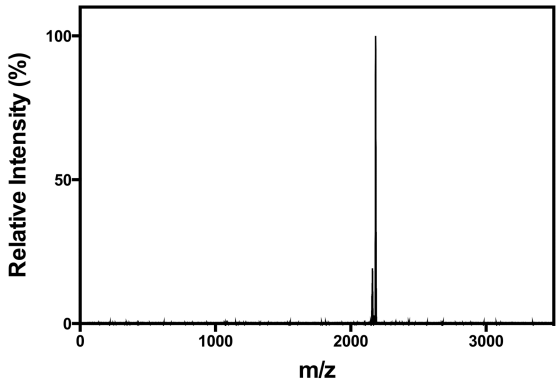
The base peptide H-GRRGPQGIWGQGRGRC-NH_2 was synthesized (0.25 mmol scale) on rink amide resin (ChemPep, loading capacity = 0.8 mmol/g) *via* standard microwave-assisted Fmoc solid-phase methodologies with HBTU activation. Microwave-assisted coupling of 5,6-Carboxyfluorescein (4x, 376 mg, Fisher) was conducted at 60 °C and 25W for 30 min on resin with HATU (3.9x, 370 mg) dissolved in minimal DMF containing DIEA (8x, 258 mg). Resin was treated with trifluoroacetic acid/1,2-ethanedithiol/water/triisopropylsilane (94:2.5:2.5:1) for 2 h, and the crude peptide was precipitated in and washed (2x) with ice-cold diethyl ether. The crude peptide was purified by RP-HPLC using a 55-min linear gradient (5–100% of acetonitrile and 0.1% trifluoroacetic acid) and lyophilized to give the intermediate (FAM-GRRGPQGIWGQGRGRC-NH_2) as a fluffy, yellow solid. Cysteine (606 mg, 5 mmol, 20x) was codissolved with the intermediate peptide in a dH_2O/DMSO (90:10) solution (50 mL) and agitated for 24 h. Product was vacuum filtered and washed with dH_2O. The filtrate was frozen, lyophilized, and purified by RP-HPLC using a 42-min linear gradient (20–100% of acetonitrile and 0.1% trifluoroacetic acid) and lyophilized to give the intermediate (FAM-GRRGPQGIWGQGRGRC(C)-NH_2 linked *via* a cysteine-cysteine disulfide bridge) as a fluffy, yellow solid. N_3-*o*NB-OSu (28.4 mg, 0.056 mmol, 1.3x) was dissolved in minimal DMF containing DIEA (22.2 mg, 0.172 mmol, 4x) and added to peptide to react overnight. The peptide was purified by RP-HPLC using a 42-min linear gradient (20–100% of acetonitrile and 0.1% trifluoroacetic acid) and lyophilized to give the product (FAM-GRRGPQGIWGQGRGRC(C-*o*NB-N_3)-NH_2 linked *via* a cysteine-cysteine disulfide bridge, denoted FAM-EvRvP) as a fluffy, yellow solid (60.0 mg, 23 μmol) with an overall yield of 9.2%. Peptide purity was confirmed with analytical RP-HPLC and matrix-assisted laser desorption-ionization time-of-flight mass spectrometry using α-cyano-4-hydroxycinnamic acid/2,5-dihydroxybenzoic acid (2:1) matrix: MALDI-TOF: calculated for $C_{112}H_{153}N_{37}O_{33}S_2^+$ $[M + {}^1H]^+$, 2609.8; found 2609.9.



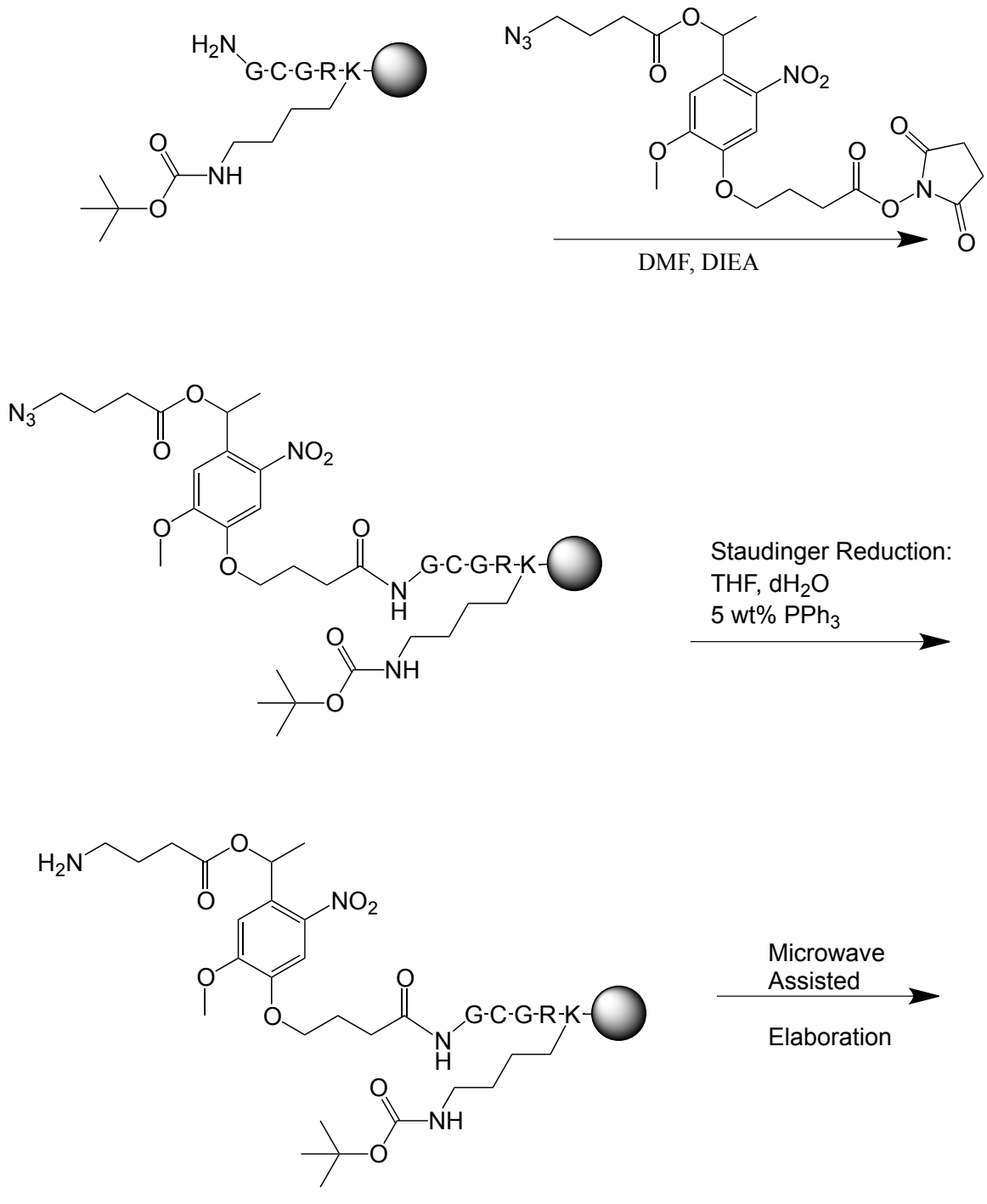
Method S7: Synthesis of FAM-EΛR

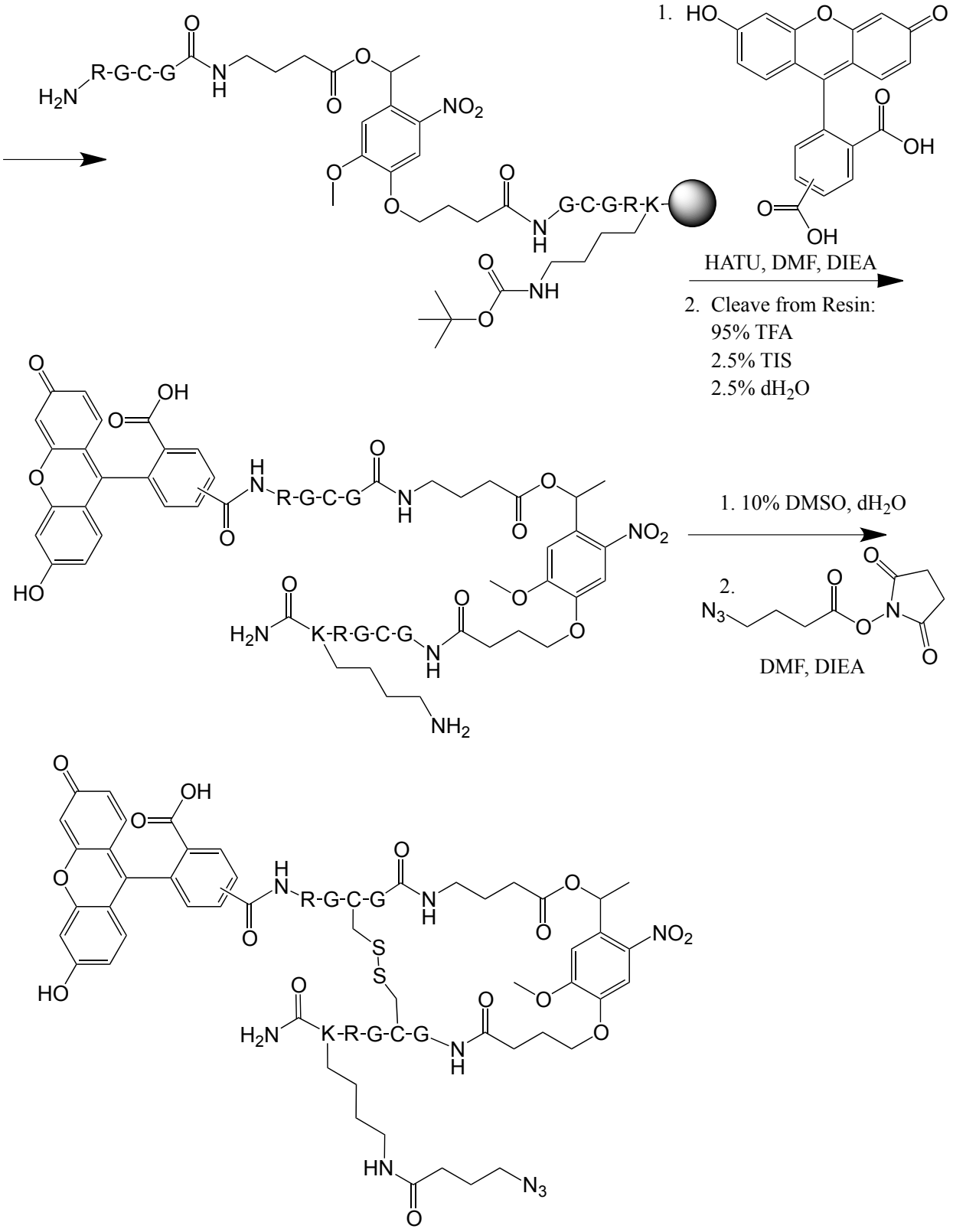


The base peptide H-GRGCGPQGIWGQGCGRK-NH_2 was synthesized (0.25 mmol scale) on rink amide resin (ChemPep, loading capacity = 0.8 mmol/g) *via* standard microwave-assisted Fmoc solid-phase methodologies with HBTU activation. Microwave-assisted coupling of 5,6-Carboxyfluorescein (282.2 mg, 0.75 mmol, 4x Fisher) was conducted at 60 °C and 25W for 30 min on resin with HATU (280.9 mg, 0.73 mmol, 2.95x) dissolved in minimal DMF containing DIEA (193.9 mg, 1.5 mmol, 6x). Resin was treated with trifluoroacetic acid/1,2-ethanedithiol/water/triisopropylsilane (94:2.5:2.5:1) for 2 h, and the crude peptide was precipitated in and washed (2x) with ice-cold diethyl ether. The crude peptide was purified by RP-HPLC using a 55-min linear gradient (5–100% of acetonitrile and 0.1% trifluoroacetic acid) and lyophilized to give the intermediate (FAM-GRGCGPQGIWGQGCGRK-NH_2) as a fluffy, yellow solid. The purified peptide (<0.5 mM) was dissolved in a dH_2O/DMSO (90:10) solution (50 mL) and agitated for 24 h. Product was concentrated, lyophilized, and subsequently purified by RP-HPLC using a 55-min linear gradient (5–100% of acetonitrile and 0.1% trifluoroacetic acid). Lyophilization afforded the purified intermediate (FAM-GRGCGPQGIWGQGCGRK-NH_2 cyclized *via* cysteine-cysteine disulfide bridge) as a fluffy, yellow solid. N_3-OSu (2.65 mg, 0.012 mmol, 1.2x) was coupled overnight with the peptide in minimal DMF containing DIEA (4.65 mg, 0.04 mmol, 4x). The peptide was purified by RP-HPLC using a 55-min linear gradient (5–100% of acetonitrile and 0.1% trifluoroacetic acid) and lyophilized to give the product (FAM-GRGCGPQGIWGQGCGRK(N_3)-NH_2 cyclized *via* cysteine-cysteine disulfide bridge, denoted FAM-E∧R) as a fluffy, yellow solid (5.7 mg, 2.6 μmol) with an overall yield of 1.1%. Peptide purity was confirmed with analytical RP-HPLC and matrix-assisted laser desorption-ionization time-of-flight mass spectrometry using α-cyano-4-hydroxycinnamic acid matrix: MALDI-TOF: calculated for $C_{95}H_{127}N_{31}O_{33}S_2^+$ $[M + {}^1H]^+$, 2183.3; found 2183.2.

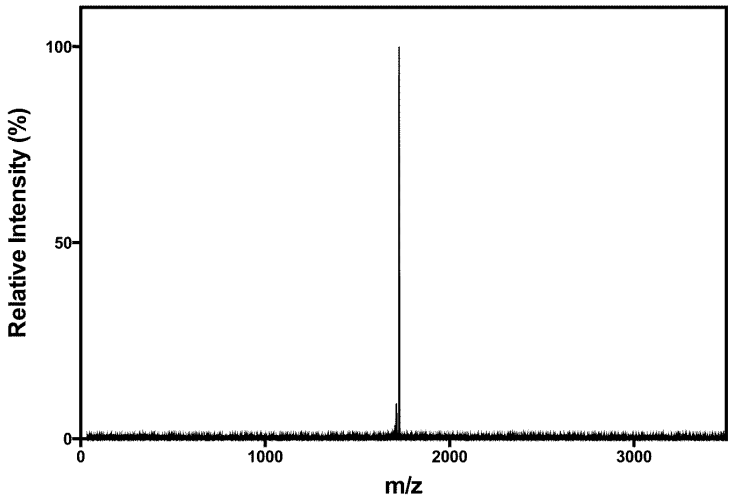


Method S8: Synthesis of FAM-PAR

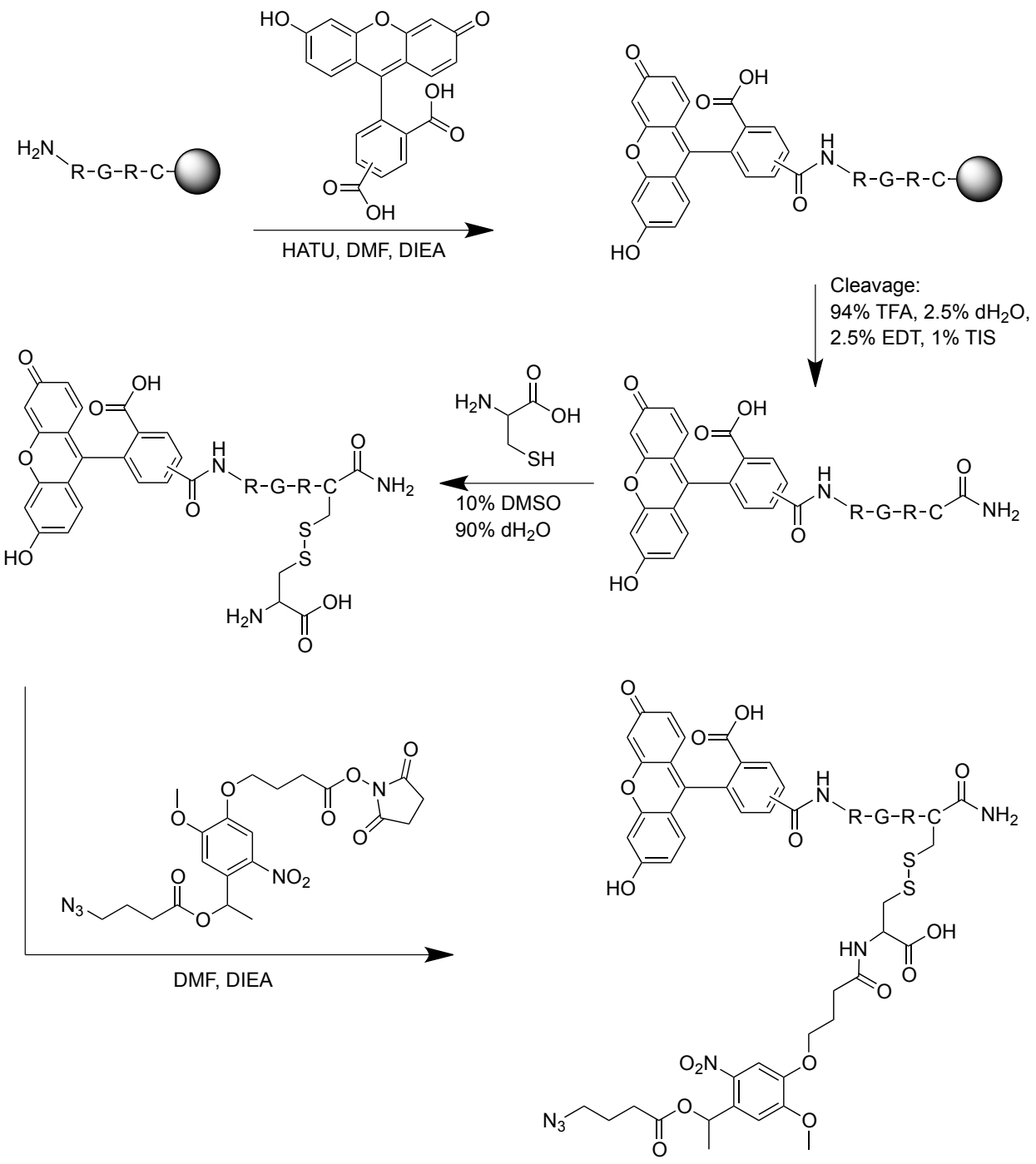



1.
HATU, DMF, DIEA
2. Cleave from Resin:
95% TFA
2.5% TIS
2.5% dH2O
1. 10% DMSO, dH2O
2.
DMF, DIEA

The base peptide H-GCGRK-NH_2 was synthesized (0.25 mmol scale) on rink amide resin (ChemPep, loading capacity = 0.8 mmol/g) *via* standard microwave-assisted Fmoc solid-phase methodologies with HBTU activation. N_3-*o*NB-OSu (329.55 mg, 0.325 mmol, 1.3x) was dissolved in minimal DMF containing DIEA (129.2 mg, 1.0 mmol, 4x) and added to peptide on resin to react overnight at room temperature. The peptide's N-terminal azide was reduced by Staudinger reduction[13]: the resin was first rinsed with a solution of tetrahydrafuran/dH_2O (90:10, 3 x 20 mL), followed by overnight reaction in a solution of triphenylphospine (5 wt%, 1.5 g, Sigma) in tetrahydrafuran/dH_2O (90:10, 30 mL). Peptide was rinsed with DMF (3 x 10 mL) and DCM (3 x 10 mL), and standard microwave-assisted Fmoc solid-phase methodologies with HBTU activation was used to elaborate the peptide to form the peptide H-RGCG-*o*NB-GCGRK-NH_2. 5,6-Carboxyfluorescein (377 mg, 1.0 mmol, 4x Fisher) was coupled at room temperature overnight on resin with HATU (371 mg, 0.98 mmol, 3.9x) dissolved in minimal DMF containing DIEA (8x, 258.3 mg). Resin was treated with trifluoroacetic acid/water/triisopropylsilane (95:2.5:2.5) for 2 h, and the crude peptide was precipitated in and washed (2x) with ice-cold diethyl ether. The crude peptide was purified by RP-HPLC using a 42-min linear gradient (20–100% of acetonitrile and 0.1% trifluoroacetic acid) and lyophilized to give the intermediate (FAM-RGCG-*o*NB-GCGRK-NH_2) as a fluffy, yellow solid. The purified peptide (<0.5 mM) was dissolved in a dH_2O/DMSO (90:10) solution (35 mL) and agitated for 24 h, then frozen and lyophilized. Intermediate product was purified by RP-HPLC using a 42-min linear gradient (20–100% of acetonitrile and 0.1% trifluoroacetic acid) and lyophilized to give the intermediate (FAM-RGCG-*o*NB-GCGRK-NH_2 cyclized *via* cysteine-cysteine disulfide bridge) as a fluffy, yellow solid. N_3-OSu (6.2 mg, 0.0274 mmol, 1.2x) was coupled overnight with the peptide in minimal DMF containing DIEA (10.9 mg, 14 µL, 0.084 mmol, 4x). The peptide was purified by RP-HPLC using a 42-min linear gradient (20–100% of acetonitrile and 0.1% trifluoroacetic acid) and lyophilized to give the product (FAM-RGCG-*o*NB-GCGRK(N_3)-NH_2 cyclized *via* cysteine-cysteine disulfide bridge, denoted FAM-P∧R) as a fluffy, yellow solid (1.33 mg, 0.77 µmol) with an overall yield of 0.15%. Peptide purity was confirmed with analytical RP-HPLC and matrix-assisted laser desorption-ionization time-of-flight mass spectrometry using α-cyano-4-hydroxycinnamic acid matrix: MALDI-TOF: calculated for $C_{74}H_{96}N_{22}O_{23}S_2^+$ $[M + {}^1H]^+$, 1725.8; found 1726.0.



Method S9: Synthesis of FAM-RVP



The base peptide H-RGRC-NH_2 was synthesized (0.25 mmol scale) on rink amide resin (ChemPep, loading capacity = 0.8 mmol/g) *via* standard microwave-assisted Fmoc solid-phase methodologies with HBTU activation. Microwave-assisted coupling of 5,6-Carboxyfluorescein (4x, 377 mg, Fisher) was conducted at 60 °C and 25W for 30 min on resin with HATU (3.9x, 371 mg) dissolved in minimal DMF containing DIEA (8x, 258.3 mg). Resin was treated with

trifluoroacetic acid/1,2-ethanedithiol/water/triisopropylsilane (94:2.5:2.5:1) for 2 h, and the crude peptide was precipitated in and washed (2x) with ice-cold diethyl ether. The crude peptide was purified by RP-HPLC using a 42-min linear gradient (20–100% of acetonitrile and 0.1% trifluoroacetic acid) and lyophilized to give the intermediate (FAM-RGRC-NH_2) as a fluffy, yellow solid. Cysteine (606 mg, 5 mmol, 20x) was codissolved with intermediate peptide in a dH_2O/DMSO (90:10) solution (50 mL) and agitated for 24 h. Additional cysteine (606 mg, 5 mmol, 20x) was added to solution and reacted for 24 h while agitating at room temperature. Product was vacuum filtered and washed with dH_2O. The filtrate was frozen, lyophilized, and purified by RP-HPLC using a 42-min linear gradient (20–100% of acetonitrile and 0.1% trifluoroacetic acid); lyophilization afforded the purified intermediate (FAM-RGRC(C)-NH_2 linked *via* a cysteine-cysteine disulfide bridge) as a fluffy, yellow solid (105 mg, 0.108 mmol). N_3-*o*NB-OSu (68 mg, 0.13 mmol, 1.2x) was coupled overnight with the peptide in minimal DMF containing DIEA (55.8 mg, 0.43 mmol, 4x). The crude peptide was purified by RP-HPLC using a 42-min linear gradient (20–100% of acetonitrile and 0.1% trifluoroacetic acid) and lyophilized to give the product (FAM-RGRC(C-*o*NB-N_3)-NH_2 linked *via* a cysteine-cysteine disulfide bridge, denoted FAM-RvP) as a fluffy, yellow solid (86.7 mg, 0.064 mmol) with a good overall yield (25.4%). Peptide purity was confirmed with analytical RP-HPLC and matrix-assisted laser desorption-ionization time-of-flight mass spectrometry using α-cyano-4-hydroxycinnamic acid matrix: MALDI-TOF: calculated for $C_{58}H_{71}N_{16}O_{19}S_2^+$ $[M + {}^1H]^+$, 1360.4; found 1359.5.

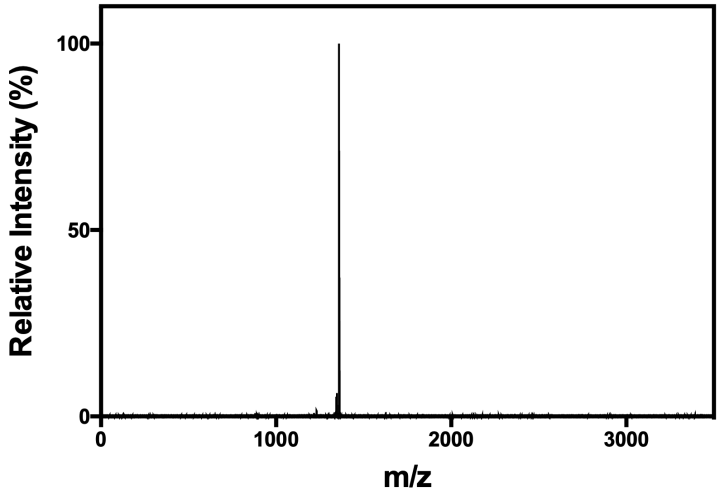
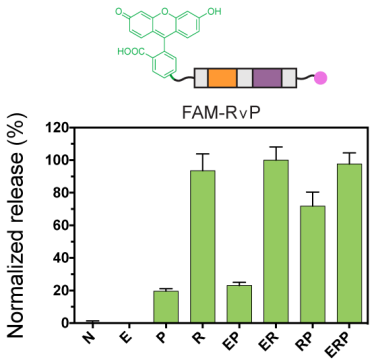


Figure S1: Small Molecule Release from Gels Containing FAM-PΛR Pendant



FAM is selectively released from gels for conditions involving either light OR reductant. X-axis labels indicate material treatment conditions (N indicates no treatment, E is MMP enzyme, R is a chemical reductant, P is UV light). The extent of release was normalized between 0% (corresponding to N) and 100% (in treatment with highest release) for each pendant. Green bars signify conditions expected to result in release; red bars indicate conditions expected not to yield release. Error bars correspond to ±1 standard deviation about the mean with propagated uncertainties for n = 3 experimental replicates.

References

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2. DeForest, C. A. & Tirrell, D. A. A photoreversible protein-patterning approach for guiding stem cell fate in three-dimensional gels. *Nat. Mater.* **14,** 523–531 (2015).
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