A pendant peptide endows a sunscreen with water-resistance

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I. General

Amino-acid derivatives, Wang resin, and HOBt were from Chem-Impex International (Wood Dale, IL). Fmoc-Gly-2-chlorotrityl resin was from EMD Millipore (La Jolla, CA). Rhodamine Red™-X, succinimidyl ester, 5-isomer was from Thermo Fisher Scientific (Waltham, MA). DIC and 4-methylpiperidine were from Oakwood Chemical (Estill, SC). Collagen-coated plates were product A1142802 from Thermo Fisher Scientific and were coated with type I collagen from rat tail. Paper for cyanotyping was from Nature Print (www.natureprintpaper.com). Vitro-skin® was from IMS (Portland, ME). All other reagents were from Sigma–Aldrich (St. Louis, MO) and were used without further purification.

N,*N*-Dimethylformamide (DMF) was dried with a Glass Contour system from Pure Process Technology (Nashua, NH). In addition, DMF was passed through an isocyanate "scrubbing" column to remove any amines. Water was purified with an arium pro instrument from Sartorius (Goettingen, Germany).

All procedures were performed in air at ambient temperature (~22 °C) and pressure (1.0 atm) unless indicated otherwise.

II. Instrumentation

Solid-phase peptide synthesis was performed at the University of Wisconsin–Madison Biotechnology Center with a Prelude peptide synthesizer from Protein Technologies (Tucson, AZ) or with a Liberty Blue Peptide Synthesizer from CEM (Matthews, NC). Synthetic peptides were purified by HPLC using a Prominence instrument from Shimadzu (Kyoto, Japan) equipped with a VarioPrep 250/21 C18 column from Macherey–Nagel (Düren, Germany). Molecular mass was

determined by matrix-assisted laser desorption/ionization–time-of-flight (MALDI–TOF) mass spectrometry using an α-cyano-4-hydroxycinnamic acid matrix and a Bruker microflex LRF™ (Billerica, MA) or Voyager DE-Pro instrument from Thermo Fisher Scientific at the Biophysics Instrumentation Facility at the University of Wisconsin–Madison. Purity analyses were performed with an Acquity UPLC® H-Class system from Waters (Milford, MA) that was equipped with an Acquity photodiode array detector, Acquity quaternary solvent manager, Acquity sample manager with a flow-through needle, Acquity UPLC® BEH C18 column (2.1 × 50 mm, 1.7-μm particle size) and Empower 3 software. UV–vis spectra were obtained with a Cary 60 UV–vis spectrometer from Agilent (Santa Clara, CA) on solutions of analyte (10 μM) in methanol in a quartz cuvette having a 1-cm path length. UV absorbance was quantified with a Tecan Infinite M1000 plate reader (Männedorf, Switzerland). Fluorescence was quantified with a GE Healthcare LAS 4010 Imaging System (Marlborough, MA). Cyanotyping was performed with a UV lamp from a Gel Doc imager from Fotodyne (Hartland, WI).

III. Chemical and peptide synthesis

Sal-GlyOMe. A solution of salicylic acid (300.0 mg, 2.17 mmol), HATU (760.0 mg, 2.00 mmol), and DIEA (1.75 mL, 10.0 mmol) in DMF was allowed to stir for 15 min. HCl·GlyOMe (271.3 mg, 2.17 mmol) was added, and the resulting solution was stirred for 12 h. The reaction mixture was concentrated under reduced presure. Crude product was purified via silica gel chromatography, eluting with EtOAc (10% v/v) in hexanes to yield Sal-GlyOMe (0.224 g, 54%). HRMS–ESI (*m*/*z*): $[M - 1]^-$ calcd, 208.07; found, 208.07. ^{1}H NMR (400 MHz, $CDCl_3$, δ): 7.46 (dd, J = 8.0, 1.6 Hz, 1H), 7.38 (ddd, J = 8.6, 7.2, 1.6 Hz, 1H), 6.95 (dd, J = 8.4, 1.1 Hz, 1H), 6.84 (ddd, J = 8.2, 7.3, 1.2 Hz, 1H), 4.21 (d, J = 5.1 Hz, 2H), 3.80 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$, δ): 170.34, 170.09, 161.39, 134.59, 125.84, 118.87, 118.48, 113.68, 52.68, 41.25.

Fmoc-6-aminohexanoic acid. 6-Aminohexanoic acid (1.00 g, 7.62 mmol) was dissolved in a saturated aqueous solution of $NaHCO_3$ (50 mL). In a separate flask, Fmoc-OSu (2.82 g, 8.38 mmol) was dissolved in dioxane (50 mL). The two solutions were combined, and the solution became cloudy. The reaction mixture was stirred for 16 h, and then concentrated under reduced pressure. The residue was dissolved in EtOAc and washed with 1 N HCl and brine. The organic layer was dried over Na_2SO_4(s), decanted, and concentrated under reduced pressure. Crude product was purified via silica gel chromatography, eluting with 1% v/v acetic acid and 40% v/v EtOAc in hexanes to yield Fmoc-6-aminohexanoic acid (2.556 g, 95%) a white solid. HRMS–ESI (*m*/*z*): $[M + 1]^+$ calcd, 354.17; found, 354.17. ^{1}H NMR (400 MHz, MeOD, δ): 7.78 (d, J = 7.5 Hz, 2H), 7.63 (d, J = 7.5 Hz, 2H), 7.37 (t, J = 7.4 Hz, 2H), 7.33–7.26 (m, 2H), 4.33 (d, J = 6.8 Hz, 2H), 4.18 (t, J = 6.9 Hz, 1H), 3.08 (t, J = 7.0 Hz, 2H), 2.27 (t, J = 7.4 Hz, 2H), 1.60 (p, J = 7.5 Hz, 2H), 1.49 (p, J = 7.1 Hz, 2H), 1.33 (p, J = 10.1, 6.0 Hz, 2H). ^{13}C NMR (101 MHz, MeOD, δ): 157.49, 143.95, 141.20, 127.34, 126.71, 124.75, 119.50, 66.11, 47.13, 40.17, 33.71, 29.18, 25.97, 24.45.

Ac-Lys-(Ser-Gly)$_3$-(DPro-DPro-Gly)$_7$. An Fmoc-DPro-DPro-Gly-OH tripeptide was synthesized in solution as described previously.[1] Ac-Lys-(Ser-Gly)$_3$-(DPro-DPro-Gly)$_7$ was synthesized by two additions of Fmoc-DProOH monomer, followed by six segment condensations

of tripeptide, and then the remaining Fmoc-protected monomers on preloaded Fmoc-Gly-2-chlorotrityl resin (0.19 mmol/g). Fmoc-deprotection was achieved by treatment with piperidine (20% v/v) in DMF. The tripeptide or amino acid monomer (4 equiv) was converted to an active ester using HATU and NMM. Each residue was double-coupled between Fmoc-deprotection steps. Peptide was cleaved from the resin with 96.5:2.5:1.0 TFA/H_2O/TIPSH (5 mL), precipitated from diethyl ether at –80 °C, and isolated by centrifugation. Ac-Lys-(Ser-Gly)$_3$-(DPro-DPro-Gly)$_7$ was purified by preparative HPLC using a gradient of 10–50% v/v B over 50 min (A: H_2O containing 0.1% v/v TFA; B: acetonitrile containing 0.1% v/v TFA). MALDI (*m*/*z*): $[M + H]^+$ calcd, 2380.6; found, 2380.0. A 0.05-mmol scale synthesis afforded 18.2 mg (15%) of Ac-Lys-(Ser-Gly)$_3$-(D-Pro-DPro-Gly)$_7$ after purification.

Red-DCMP (which is Ac-Lys(Rhodamine Red™)-(Ser-Gly)$_3$-(DPro-DPro-Gly)$_7$). Ac-Lys-(Ser-Gly)$_3$-(DPro-DPro-Gly)$_7$ (3.4 mg, 1.43 μmol) was dissolved in 2.0 mL of DMSO. Rhodamine Red™-X, succinimidyl ester, 5-isomer (199.9 μL, 1.30 μmol) was added as a 5.0 mg/mL solution in DMSO. DIEA (200 μL, 1.14 mmol) was added dropwise. The reaction mixture was allowed to stir for 8 h, and then diluted with 7 mL of H_2O, frozen, and lyophilized. Red-DCMP was purified by preparative HPLC using a gradient of 65–95% v/v B over 55 min (A: H_2O containing 0.1% v/v TFA; B: methanol containing 0.1% v/v TFA) to yield 0.5 mg of peptide (11%). MALDI $[M + Na]^+$: calcd, 3056.42; found, 3056.36. Purity was assessed as >95% by UPLC.

Ac-Lys-(Ser-Gly)$_3$-(LPro-LPro-Gly)$_7$. An Fmoc-LPro-LPro-GlyOH tripeptide was synthesized in solution as described previously.[1] Ac-Lys-(Ser-Gly)$_3$-(LPro-LPro-Gly)$_7$ was synthesized by two additions of Fmoc-DProOH monomer, followed by six segment condensations of tripeptide, and then the remaining Fmoc-protected monomers on preloaded Fmoc-Gly-2-chlorotrityl resin (0.19 mmol/g). The tripeptide or amino acid monomer (4 equiv) was converted to an active ester using HATU and NMM. Each residue was double-coupled between Fmoc-deprotection steps. Peptide was cleaved from the resin with 96.5:2.5:1.0 TFA/H_2O/TIPSH (5 mL), precipitated from diethyl ether at –80 °C, and isolated by centrifugation. Ac-Lys-(Ser-Gly)$_3$-(LPro-LPro-Gly)$_7$ was purified by preparative HPLC using a gradient of 10–50% v/v B over 50 min (A: H_2O containing 0.1% v/v TFA; B: acetonitrile containing 0.1% v/v TFA). MALDI (*m*/*z*): MALDI $[M + Na]^+$: calcd, 2402.61; found, 2403.15. A 0.05-mmol scale synthesis afforded 12.0 mg (10%) of Ac-Lys-(Ser-Gly)$_3$-(LPro-LPro-Gly)$_7$ after purification.

Red-LCMP (which is Ac-Lys(Rhodamine Red™)-(Ser-Gly)$_3$-(LPro-LPro-Gly)$_7$). Ac-Lys-(Ser-Gly)$_3$-(LPro-LPro-Gly)$_7$ (6.4 mg, 2.68 μmol) was dissolved in 2.0 mL of DMSO. Rhodamine Red™-X, succinimidyl ester, 5-isomer (400 μL, 2.60 μmol) was added as a 5.0 mg/mL solution in DMSO. DIEA (2.2 μL, 13.0 μmol) was added dropwise. The reaction mixture was allowed to stir for 8 h, and then was diluted with 7 mL of H_2O, frozen, and lyophilized. The peptide was purified by preparative HPLC using a gradient of 65–95% v/v B over 55 min (A: H_2O containing 0.1% v/v TFA; B: methanol containing 0.1% v/v TFA) to yield 1.0 mg of Red-LCMP (12%). MALDI $[M + Na]^+$: calcd, 3056.42; found, 3056.16. Purity was assessed as >95% by UPLC.

Sal-LCMP (which is Sal-AHX-(Ser-Gly)$_3$-(LPro-LPro-Gly)$_7$). Sal-AHX-(Ser-Gly)$_3$-(Pro-Pro-Gly)$_7$ was synthesized by single amino-acid addition on preloaded Fmoc-Gly-Wang resin

(0.65 mmol/g). Fmoc-deprotection was achieved by treatment with 4-methylpiperidine (20% v/v) in DMF. The amino-acid monomer or small molecule (5 equiv) was converted to an active ester by using DIC and HOBt. The peptide was then cleaved from resin with 96.5:2.5:1.0 TFA/H_2O/TIPSH (5 mL), precipitated from diethyl ether at –80 °C, and isolated by centrifugation. The peptide was purified by preparative HPLC using a gradient of 10–50% v/v B over 50 min (A: H_2O containing 0.1% v/v TFA; B: acetonitrile containing 0.1% v/v TFA). MALDI (*m*/*z*): MALDI $[M + Na]^+$: calcd, 2463.15; found, 2463.48. A 0.25-mmol scale synthesis afforded 93.7 mg (15.2%) of Sal-LCMP after purification.

IV. Binding to collagen-coated wells

Collagen in the wells of a 96-well plate were treated with 16 μM solutions of either Red-LCMP or Red-DCMP. (Rhodamine Red™-X has excitation/emission maxima of ~560/580 nm.) Wells were agitated at 37 °C for 1 h. Solutions were then removed, and the fluorescence of the empty wells was measured with excitation at 560 nm and emission at 580 nm. For each wash, 50 μL of water was added to the wells and the wells were once again agitated at 37 °C for 1 h. Wells were emptied again and assessed with the plate reader. This procedure was repeated for each subsequent wash. The data are comprised of three experimental trials.

V. Cyanotyping

The collagen-coated wells of a 24-well plate were treated with 25 μL of 25 μM solutions of 2-ethylhexyl salicylate or Sal-LCMP in methanol or water, respectively. An additional well was treated with 25 μL of methanol. Wells were allowed to dry completely overnight. The treated wells were placed on cyanotyping paper and irradiated from above for 2 min. The paper was washed immediately with water to develop the image. Wells were then washed with water (3 × 200 μL), and the cyanotyping paper was replaced with new paper, and the procedure was repeated.

VI. Fluorescence assay of binding to Vitro-skin®

Vitro-skin® was hydrated according to the manufacturer's directions. Briefly, a mixture of water (298 g) and glycerin (52 g) was added to a 2.5-gallon sealable container to make a hydration chamber. Vitro-skin® was placed on racks in the hydration chamber for 24 h prior to use.

After hydration, Vitro-skin® was spotted with 5 μL of a solution of either Red-LCMP or Red-DCMP in water. The spots were allowed to dry. Spots were imaged with a GE Healthcare LAS 4010 Imaging System. The Vitro-skin® was washed under a stream of water. After washing, the Vitro-skin® was imaged again. The same procedure was repeated for each subsequent wash.

VII. UV assay of binding to Vitro-skin®

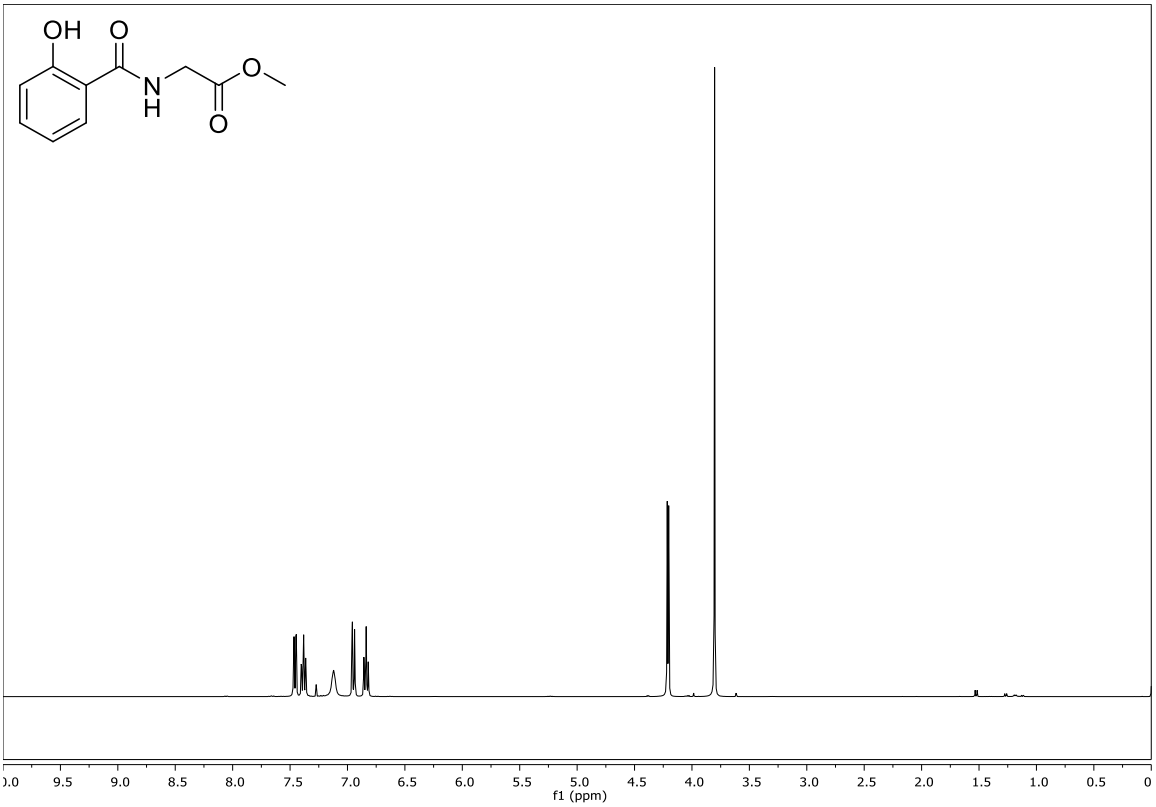
Vitro-skin® was cut into square pieces (6 cm × 6 cm) and hydrated according to the manufacturer's directions. After hydration, the surface was coated with 2-ethylhexyl salicylate or Sal-LCMP to a surface density of 133.5 nmol/cm^2. A vehicle control was treated with water and isopropanol. Samples are then dried for 15 min followed by 2 h in the hydration chamber. Each sample was

read in four different areas of the surface by using a UV–vis spectrometer with an insert that accommodates solid samples. The Vitro-skin® was then washed under a stream of water and dried for 15 min followed by 1 h in the hydration chamber. After rehydration, samples were read using UV–vis spectrometer. The same procedure was repeated for each subsequent wash. The data are comprised of three experimental trials.

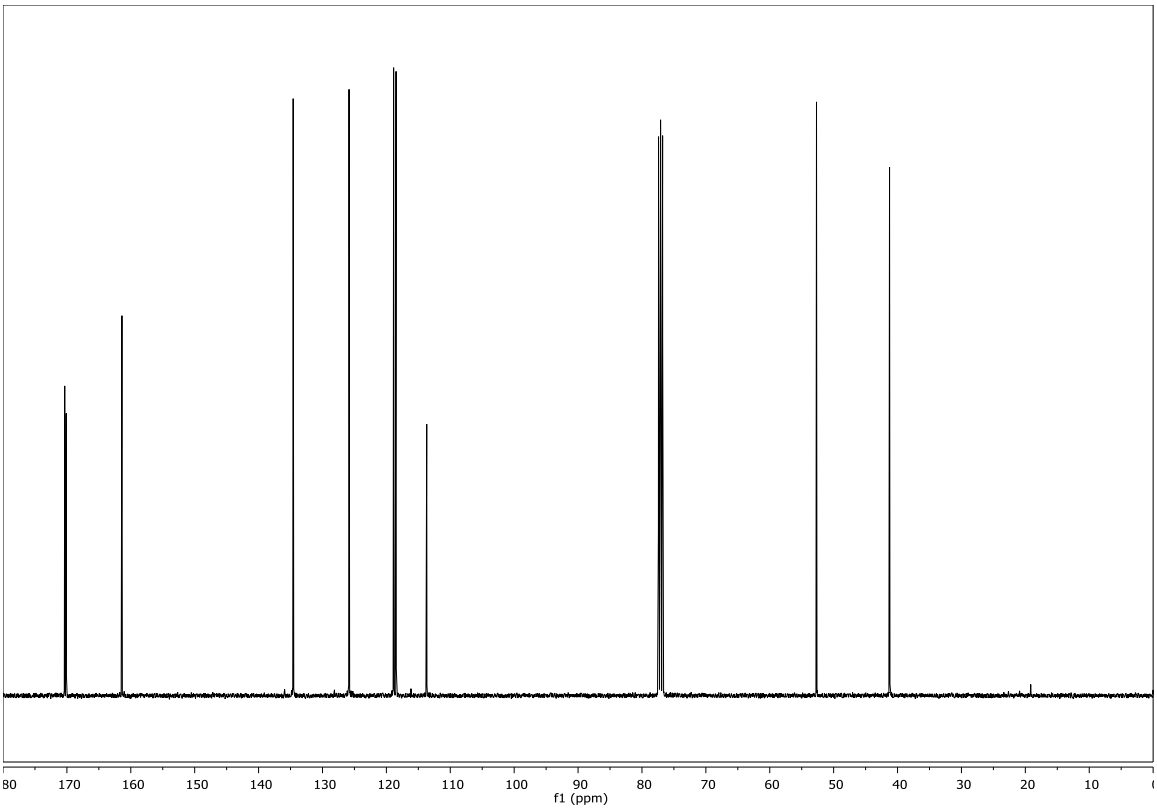
VIII. Reference

1. A. J. Ellison, B. VanVeller and R. T. Raines, *Peptide Sci*, 2015, **104**, 674-681.

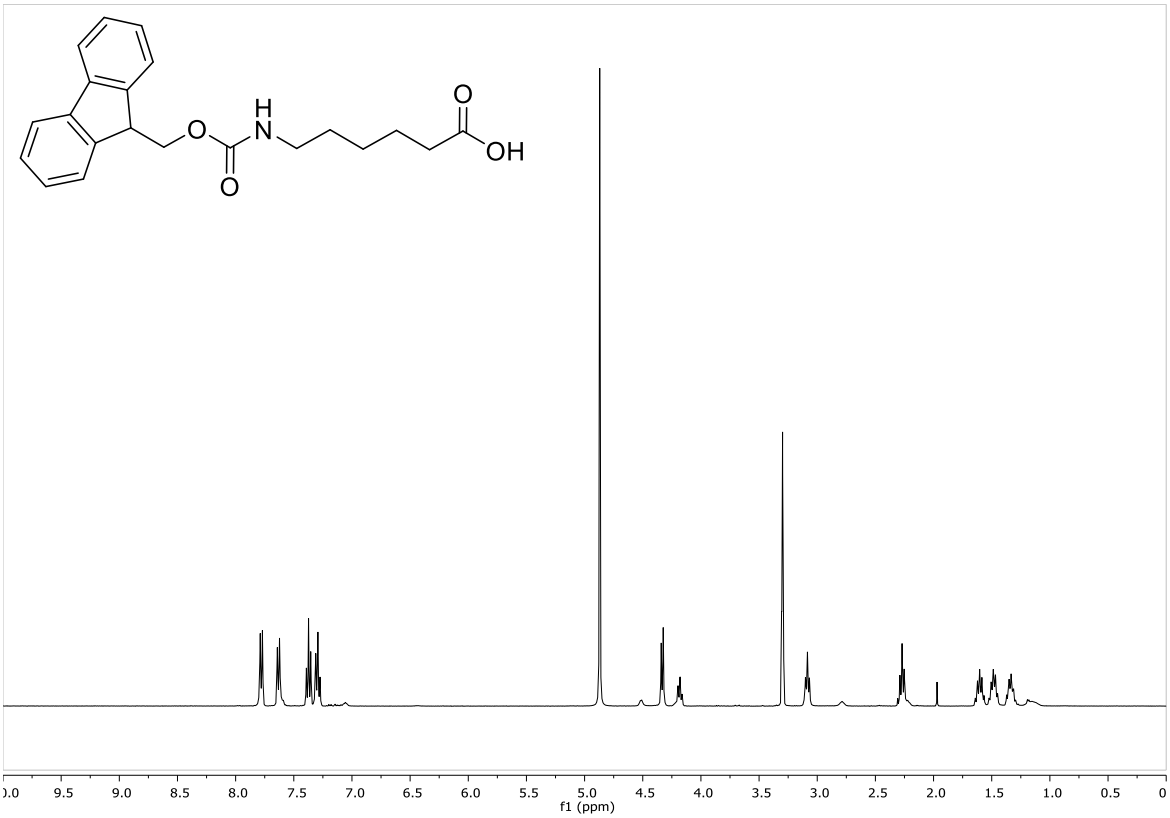
^{1}H NMR Spectrum of Sal-GlyOMe in $CDCl_3$ (400 MHz)



^{13}C NMR Spectrum of Sal-GlyOMe in $CDCl_3$ (101 MHz)



^{1}H NMR Spectrum of Fmoc-6-Aminohexanoic Acid in MeOD (400 MHz)



^{13}C NMR Spectrum of Fmoc-6-Aminohexanoic Acid in MeOD (101 MHz)