Conversion of Commercial Peptides into “Clickable” Derivatives for Labeling and Conjugate Synthesis

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Method Article

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Abstract

Introduction

Peptides conjugated to labels as well as to other biomolecules are essential tools in biomedical research.1,2 We have developed a convenient method for conversion of commercially available basic peptides into protected derivatives that can be readily used in synthesis of conjugates between the peptide and different labels/signals or other biomolecules. To achieve this we have converted the peptides into alkyne and azide derivatives at the carboxyterminus so that the peptide can be further reacted in a site selective fashion by 1,3-dipolar cycloaddition chemistry \(click chemistry). 3,4 The method involves protection of aminofunctions in the peptide as trifluoroacetamides followed by coupling to either propargylamine or azidoethylamine. The protection can then be kept or optionally removed before use in subsequent click reactions.

Reagents

Reagents • Peptide PKKKRKVG \(1) was purchased from Anaspec Inc \(San Jose, USA) • Methanol was purchased from VWR \(52542-25) • Ethyl trifluoroacetate was purchased from Fluka \(91710) • Triethylamine was purchased from Merck \(27468-1) • Acetonitrile \(HPLC grade) was purchased from VWR \(21497-25) • HBTU \(2-\(1H-Benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) was purchased from IRIS BioTech GmbH, Germany \(RL-1030) • N-methylmorpholine was purchased from Sigma-Aldrich \(M5,655-7) • 2-azidoethylamine was prepared according to know procedure5 • Propargylamine was purchased from Fluka \(81825) • Dimethylformamide \(DMF) was purchased from VWR \(17134-25) and stored over molecular sieves \(4Å) • Conc. ammonia \(25%) was purchased from Merck \(35432-25)

Equipment

HPLC-system with reversed-phase \(RP-18) column

Procedure

CRITICAL The C-terminal amino acid should be a glycine \(or an amino acid or linker that is not chiral on the carbon adjacent to the carboxyl) to avoid racemisation during coupling to the azido or alkyne amines. Trifluoroacetyl protection. Synthesis of compound 2 1] Add 25 mg of peptide PKKKRKVG \(1) to 10mL round-bottomed flask containing a Teflon-coated magnetic stirrer bar. 2] Transfer 5 mL of methanol to the flask. 3] Put the flask to the ice-bath and turn on stirring. 4] Add dropwise, 0.3 mL of ethyl trifluoroacetate \(by use of a 0.5-1 mL syringe). 5] Add 0.1 mL of triethylamine dropwise \(by use of a 0.5-1 mL syringe). 6] Close the flask with the glass stopper and keep stirring for a total of 3 days. 7] Remove the solvent under reduced pressure on a rotatory evaporator. 8] Purify and analyze products by HPLC at 50 ºC on a Vydac RP-C18 column \(218TP510, 10x250 mm,) using a flow rate of 4.0 mL/min and first a linear

gradient from 15% to 35% aqueous acetonitrile in 40 minutes followed by 35 to 70% acetonitrile in 10 minutes \(with 0.1 % trifluoroacetic acid in all solutions). Synthesis of TFA protected clickable peptides 3a or 3b 1] Add 0.18mg HBTU to a 1.5 mL eppendorf tube. 2] Add 0.43mg N-methylmorpholine to the tube. 3] Add 25 µL DMF to the tube. 4] Add 3 mg of peptide 2 to the tube. 5] Close the tube and seal it. 6] Keep the tube shaking on a vortex for 30 min. 7] Add 25 µL of a THF solution \(made from stock) containing 2-azidoethylamine \(0.18 mg) or propargylamine \(0.12 mg) using an auto pipette. 8] Keep shaking for 2h. 9] Lyophilize the solution. 10] Dissolved the crude mixture in the HPLC starting buffer. 11] Purify and analyze products by HPLC at 50 ºC on a Vydac RP-C18 column \(218TP510, 10x250 mm,) using a flow rate of 4.0 mL/min and first a linear gradient from 15% to 35% aqueous acetonitrile in 40 minutes followed by 35 to 70% acetonitrile in 10 minutes \(with 0.1 % trifluoroacetic acid in all solutions). Fractions containing product were collected and the acetonitrile was removed under reduced pressure whereupon the remaining solution was lyophilized. Synthesis of unprotected clickable peptides 4a or 4b 1] Add 0.18 mg of HBTU to a 1.5 mL eppendorf tube. 2] Add 0.43 mg N-methylmorpholine to the tube. 3] Add 25 mL DMF to the tube. 4] Add 3 mg of peptide 2 to the tube. 5] Close the tube and seal it. 6] Keep the tube shaking on a vortex for 30 min. 7] Add 25 mL of a THF solution \(made from stock) containing 2-azidoethylamine \(0.18 mg) or propargylamine \(0.12 mg) using an automated pipette. 8] Keep shaking for 2h. 9] Transfer the solution to a 10mL glass vial. 10] Add 2 mL of conc. ammonia using a disposable syringe. 11] Seal the glass vial and keep at 55 ºC overnight. 12] Lyophilize the crude peptide mixture. 13] Purify and analyze products by HPLC at 50 ºC on a Vydac RP-C18 column \(218TP510, 10x250 mm,) using a flow rate of 4.0 mL/min and first a linear gradient from 15% to 35% aqueous acetonitrile in 40 minutes followed by 35 to 70% acetonitrile in 10 minutes \(with 0.1 % trifluoroacetic acid in all solutions). Fractions containing product were collected and the acetonitrile was removed under reduced pressure whereupon the remaining solution was lyophilized.

Timing

Part 1. 3 days \(plus HPLC purification) Part 2. 1 day \(plus HPLC-purification)

Anticipated Results

Typically the reactions in both steps give virtually quantitative conversion. Analytical data: Peptide 2: Rt = 45.7 min, ESI-TOF MS \(m+1) m/z = Calculated: 1420.55 Found: 1420.55 Peptide 3a, Rt = 47.3 min; ESI-TOF MS \(m+1) m/z = Calculated: 1457.58 Found: 1457.58; Peptide 3b, Rt = 47.4 min. ESI-TOF MS \(m-1) m/z = Calculated: 1486.59 Found: 1486.68 Peptide 4a: Rt = 9.9 min, ESI-TOF MS \(m-1) m/z = Calculated: 975.66 Found: 975.65; Peptide 4b: Rt = 9.4 min. ESI-TOF MS \(m+1) m/z = Calculated: 1008.69 Found: 1008.69

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Figures

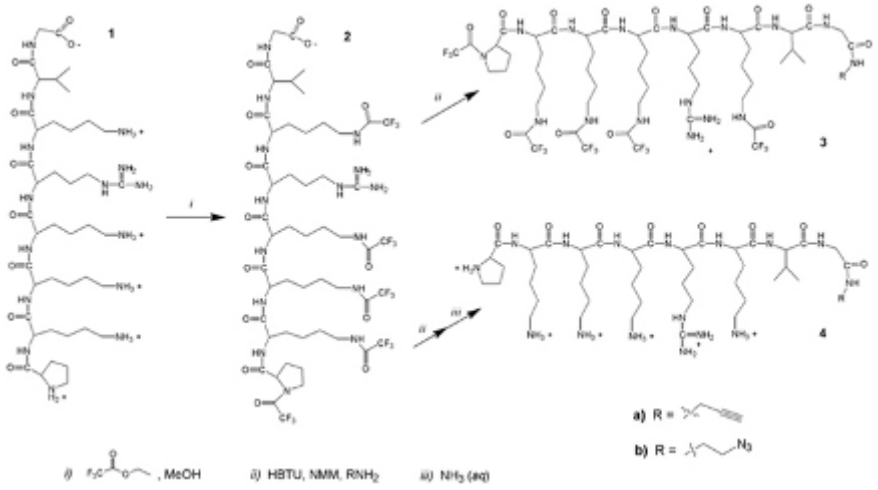

Figure 1

Scheme Synthesis of peptide alkyne and azido derivatives soluble in water or organic solvents

Figure 2