Synthesis of terpyridine-modified peptides

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Abstract

The synthesis of peptides with a terpyridine chelating ligand attached to a lysine side chain is described. The main advantage of this methodology relies on the introduction of the terpyridine unit while the peptide is still in the solid phase, thus avoiding reactions in solution and laborious HPLC purifications of synthetic intermediates.

Introduction

Terpyridines are widespread used ligands that when tethered to peptides can be used to promote their dimerization by addition of a suitable metal ion. The general protocol to obtain this kind of peptide derivatives involves the purification of a thiol-containing peptide \(obtained after standard solid-phase peptide synthesis) and alkylation of the free thiol with 4'-methylbromo-2,2':6,'2"-terpyridine or similar alkylating agents. We found that a 5,5"-dimethyl-\[2,2':6',2"-terpyridine]-4'-carboxylic acid \(or other terpyridine derivatives having a carboxylic acid handle) can be selectively coupled to a resin-linked peptide bearing an orthogonally deprotected Lys side chain, using standard coupling reagentd, such as HATU. In this way, the terpyridine unit is incorporated while the peptide is still on the solid-phase, which is clearly advantageous from the experimental point of view.

Reagents

• Dimethylformamide \(DMF) peptide grade • 2-\(1H-7-Azabenzotriazol-1-yl)–1,1,3,3-tetramethyl uronium hexafluorophosphate methanaminium \(HATU) • Fmoc-Lys\(Mtt)-OH • N,N-Diisopropylethylamine \(DIEA) • Trifluoroacetic acid \(TFA) • Dichloromethane \(DCM) • Triisopropylsilane \(TIS) • 0.19 mmol/g loading Fmoc-PAL-PEG-PS resin from Applied Biosystems • Piperidine • Diethyl ether • 5,5"-dimethyl-\[2,2':6',2"-terpyridine]-4'-carboxylic acid or other analogs bearing a carboxilic acid.

Equipment

• RP-HPLC • HPLC columns • Electrospray ionization mass spectrometer • PS3 automatic peptide synthesizer • Peptide synthesis reaction vessel

Procedure

Peptides were synthesized using standard Fmoc/tBu solid-phase peptide synthesis protocol on a 0.1 mmol scale, using a Fmoc-PAL-PEG-PS resin with 0.19 mmol/g loading from Applied Biosystems. Key steps of the protocol are: Coupling of Fmoc-Lysine\(Mtt)-OH: 1. Place the dry resin in an appropriate reaction vessel \(0.1 mmol of the Fmoc-PAL-PEG-PS resin) 2. Fill the reactor with DMF until all resin beads are immersed. Leave the mixture for 10 min and remove the solvent by filtration. 3. Treatment of the resin with 20% piperidine in DMF for 10 min. Repeat twice. 4. Wash the resin with DMF \(10 mL, three times). 5. Mix 4 equiv of Fmoc-Lys\(Mtt)-OH \(0.4 mmol, 250 mg) and 4 equiv HATU \(0.4 mmol, 152 mg)

and shake the mixture for 30 seconds in 5 ml of DIEA 0.117 M in DMF, and then add the solution onto the resin. Keep the resulting mixture for 30 min with mild shaking. 6. Wash the resin with DMF \(10 mL, three times) and repeat the operation with DCM. 7. Dry the resin under an Argon stream. Deprotecion of Lys\(Mtt) side chain on the resin-bound peptide: 1. The above resin \(approx. 0.1 mmol) is treated with a mixture of TFA \(500 µL), triisopropylsilane \(100 µL) and CH2Cl2 \(9.4 ml) for 5 min. Remove the liquid by filtration and repeat the operation. 2. Wash resin with DCM \(10 mL, four times) Coupling of 5,5"-dimethyl-\[2,2':6',2"-terpyridine]-4'-carboxylic acid: 1. A mixture containing 2 equiv of 5,5"-dimethyl-\[2,2':6',2"-terpyridine]-4'-carboxylic acid \(0.2 mmol, 61 mg) and 2 equiv of HATU \(0.2 mmol, 76 mg) was stirred for 30 seconds in 2.5 ml of DIEA 0.117 M in DMF. The resulting solution was added onto the lysine-containing resin and the resulting mixture was kept under mild shaking for 30 min. 2. Wash resin with DMF \(10 mL, three times). Continuation of the solid phase synthesis: 1. Treatment of the resin with 20% piperidine in DMF for 10 min, repeat twice. 2. Continue with the standard solid phase synthesis procedures to assemble the rest of the peptide chain.

Timing

two-three days

Troubleshooting

1- The deprotection of Mtt group can cause problems if there are Lys\(Boc) residues in the peptide, due to the partial deprotection of the tert-butyloxycarbonyl group in acid medium. In this case, it is necessary to optimize the time of the deprotection step, or else replace the Mtt group for Alloc \(the deprotection step will be then carried out using Pd catalysis). 2- The terpyridine moiety can trap traces of iron cations that could be present in the medium. This can be easily detected because the purified peptide shows a pink color.

References

B. Cuenoud & A. Schepartz. A general scheme for incorporating nonnatural functionality into peptides. Tetrahedron Lett. 32, 3325-3328 \(1991) B. Cuenoud & A. Schepartz. Altered specificity of DNA-binding proteins with transition metal dimerization domains. Science 259, 510-513 \(1993). Marcel Heller and Ulrich S. Schubert. Functionalized 2,2’-Bipyridines and 2,2’:6’,2”-Terpyridines via Stille-Type Cross-Coupling Procedures. J. Org. Chem. 67, 8269-8272 \(2002).

Figures

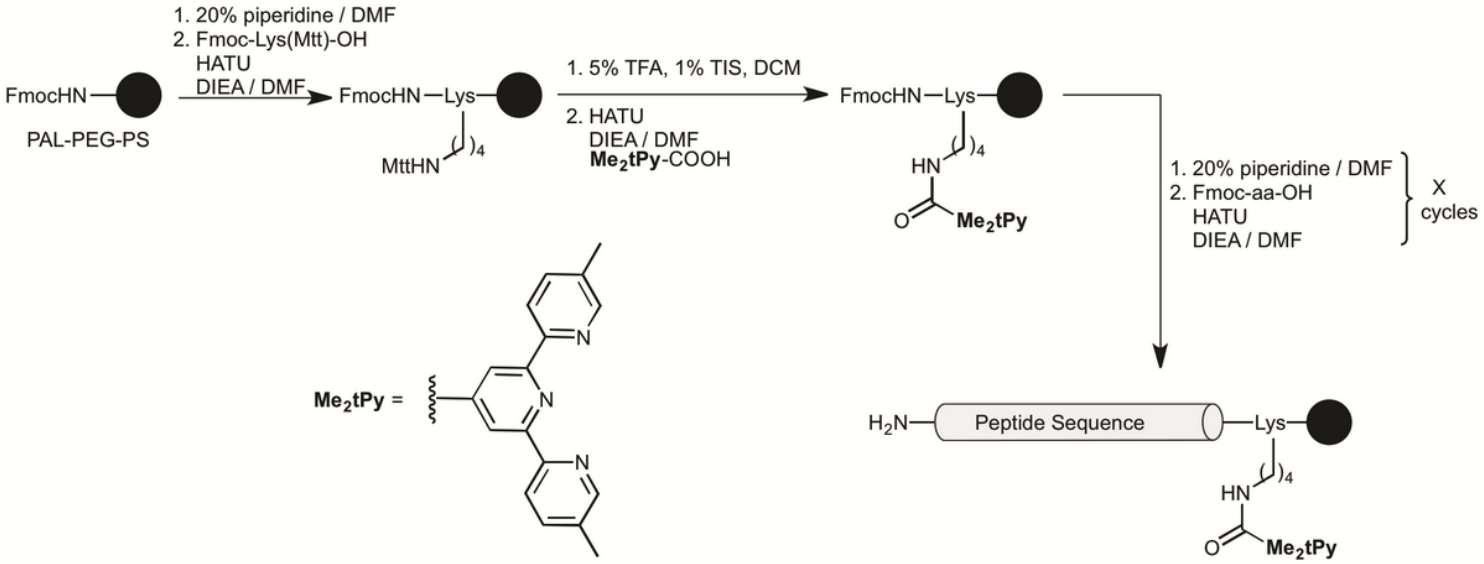

Figure 1

Figure Synthetic Scheme Scheme of the protocol for the orthogonal synthesis of terpyridine-modified peptides.