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Technical Support Information Bulletin 1202

Phosphoamino Acids

Phosphorylation of serine, threonine or tyrosine residues in peptides and proteins is one of the important regulatory mechanisms. Phosphorylation and dephosphorylation is effected by enzymes classified as kinases and phosphatases. Phosphorylated peptides are useful in studying the kinetics of these enzymes and for studying the binding sites of signal proteins.

Phosphorylated serine, threonine and tyrosine derivatives are available that allow phosphorylated peptides to be prepared by solid phase peptide synthesis. AAPPTec offers Fmoc-Tyr(PO_3H_2)-OH (AFY120), Fmoc-Tyr(PO_3Bzl_2)-OH (AFY125), Fmoc-Tyr(HPO_3Bzl)-OH (AFY115), Fmoc-Ser(HPO_3Bzl)-OH (AFS111) and Fmoc-Thr(HPO_3Bzl)-OH (AFT115).

Fmoc-Tyr(PO_3H_2)-OH is the most cost effective derivative for introducing a phosphotyrosine residue, but the unprotected phosphate group can cause difficulty coupling. Using additional diisopropylethylamine (DIPEA) with uronium coupling reagents often overcomes these difficulties. The unprotected phosphate group forms piperidine salts during Fmoc-removal. This piperidine reacts with the activated amino acids during coupling and can result in incomplete coupling. Exchanging the piperidine with a tertiary amine overcomes this problem.

To avoid the problems produced by the unprotected phosphate group, Fmoc-Tyr(PO_3Bzl_2)-OH can be used. The benzyl protecting groups can be removed in 1 to 2 hours with 95% trifluoroacetic acid (TFA) and are generally removed when the peptide is cleaved from the resin. Benzylation of sensitive residues may occur. Including ethanedithiol (EDT) in the cleavage mixture will minimize this.

The second benzyl group in Tyr(PO_3Bzl_2) is more labile than the first[1] and may come off during lengthy syntheses. Fmoc-Tyr(HPO_3Bzl)-OH is more stable, but requires additional DIPEA in uronium couplings. The partially protected phosphate group can form piperidine salts during Fmoc deprotection. As with Fmoc-Tyr(PO_3H_2)-OH, this problem can be overcome by exchanging piperidine with a tertiary amine.

Fmoc-Ser(HPO_3Bzl)-OH and Fmoc-Thr(HPO_3Bzl)-OH are the only phosphorylated serine and threonine derivatives that are sufficiently stable for use in solid phase peptide synthesis. Like Fmoc-Tyr(HPO_3Bzl)-OH, these building blocks require additional DIPEA during coupling and form piperidine salts during Fmoc deprotection. If there are multiple Ser(HPO_3Bzl), Thr(HPO_3Bzl) or Tyr(HPO_3Bzl) residues in the peptide, it may be necessary to exchange the piperidine for a tertiary amine.

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[1] Z. Tian, C. Gu, R.W. Roeske, M. Zhou, R.L. Van Etten, *Int. J. Pept. Protein Res.*, **1993**, *42*, 155-158.

Coupling Fmoc-Tyr(PO_3H_2)-OH

1. Remove the N-terminal Fmoc from the peptide-resin using standard procedures (AAPPTec Technical Support Bulletin 1140).

2. Dissolve Fmoc-Tyr(PO_3H_2)-OH (5 equiv. relative to resin substitution), and HATU or HCTU (5 equiv. relative to resin substitution) in a minimum amount of DMF.

3. Add DIPEA (20 equiv. relative to resin substitution), mix, and immediately add to the resin.

4. Mix at room temperature for 1 to 2 hours.

5. Perform a Kaiser test to check the coupling. If the coupling is not complete, filter the resin, wash the resin several times with DMF, and repeat the coupling. If the coupling is complete, proceed with the synthesis.

Coupling Fmoc-Tyr(HPO_3Bzl)-OH, Fmoc-Ser(HPO_3Bzl)-OH or Fmoc-Thr(HPO_3Bzl)-OH

1. Remove the N-terminal Fmoc from the peptide-resin using standard procedures (AAPPTec Technical Support Bulletin 1140).

2. Dissolve Fmoc-Tyr(PO_3H_2)-OH (5 equiv. relative to resin substitution), and HATU or HCTU (5 equiv. relative to resin substitution) in a minimum amount of DMF.

3. Add DIPEA (15 equiv. relative to resin substitution), mix, and immediately add to the resin.

4. Mix at room temperature for 1 to 2 hours.

5. Perform a Kaiser test to check the coupling. If the coupling is not complete, filter the resin, wash the resin several times with DMF, and repeat the coupling. If the coupling is complete, proceed with the synthesis.

Piperidine-DIPEA Exchange Solution

1. Mix DIPEA (20 equiv. relative to phosphate content of the resin-peptide) in enough DMF to slurry the resin.

2. Carefully add TFA (18 equiv. relative to phosphate content of the resin-peptide).

Piperidine-DIPEA Exchange

1. Prepare two portions of the Piperidine-DIPEA Exchange Solution.

2. Wash the peptide-resin twice with DMF.

3. Add one portion of the Exchange Solution, mix and filter.

4. Add the second portion of Exchange Solution, mix and filter.

5. Wash the resin twice with DMF.