TECHNICAL NOTE

Use of Allyl-based Protecting Groups for the Automated Synthesis of Cyclic and Branched-Chain Peptides on the Pioneer™ Peptide Synthesis System

Methods have been developed for the fully automated synthesis of cyclic and branched-chain peptides, and with protocols that enable the selective removal of allyl groups in a manner that is compatible with other protecting groups (Fmoc, Boc, *t*Bu), sensitive amino acids (Met, Trp), and side-chain modifications (Tyr(SO_3H) etc.).[1]

Allyl-based protecting groups have been used extensively in organic synthesis and have recently been applied to DNA, carbohydrate, and peptide synthesis.[2] The mild conditions used to remove the allyl groups are compatible with classical Fmoc/*t*Bu methods for solid-phase peptide synthesis. [3,4]

Initially, the potential of allyl chemistry was recognized in solid-phase synthesis as a handle to attach the growing peptide to the resin. The peptide was cleaved from the resin by treatment with Pd (0) in THF in the presence of a nucleophile such as morpholine. [5,6]

Furthermore, N^{α}-Fmoc groups in combination with *C*-terminal allyl esters were used as an alternative method for the preparation of *O*-glycopeptides in solution. [7,8]

The use of allyl protection of amino acids such as lysine, aspartic, and glutamic acid in combination with the classical Fmoc and *t*Bu strategy provides a third level of orthogonality that allows the synthesis of cyclic and branched- chain peptides.

Removal of the Allyl Group
Optimal conditions for the removal of the allyl group are attained with tetrakistriphenyl phosphine palladium(0) [$PdP(Ph_3)_4$] in a solution of chloroform ($CHCl_3$) containing 5% acetic acid (HOAc) and 2.5% *N*-methylmorpholine (NMM).

Protocol for Automated Allyl Removal
Regular cycles (Fast, standard or extended) are used for chain elongation with Fmoc/*t*Bu and Fmoc/allyl amino acids on PEG-PS supports. The allyl deblock cycle is used to remove the allyl-based protecting group as follows:

1. Act 3 delivers the solution containing 5% HOAc and 2.5% NMM in $CHCl_3$ to the amino acid vial to dissolve the palladium catalyst.
2. The allyl cleavage solution is then recycled through the column for 2 hours.
3. The column is washed with a solution of 0.5 % DIEA and 0.5% sodium diethyldithiocarbamate in DMF (in the Aux 3 bottle) to remove trace metal ions and prepare the support for the next cycle.

Automated Synthesis of Cyclic Peptides
Table 1 is an example of a notebook that has been set up to run a cyclic peptide (Tachykinin Peptide Antagonist) on a Pioneer™ Peptide Synthesis System. The procedure is as follows:

1. Open a new notebook.
2. Choose the desired protocol and chemistry. Final cycle is **Fmoc On** (since there will be no Fmoc to remove once it has been cyclized, there is no need to do a final deblock), and the first amino acid is **on the support**.
3. Select Properties and enter in the parameters. For activation, choose either Act 1/2, Act 1 or Act 2 (Act 3 is reserved for the allyl deblock solution).
4. Enter in the sequence, with CA2 and CA1 at the *N*-terminal (CA1 for the allyl deblock cycle and CA2 for the cyclization).
5. Edit the cycle and derivatives such that CA1 uses an Allyl Deblock Cycle to dissolve Palladium, and CA2 uses an extended cycle to “cyclize”. There is no need to worry about the activator for the palladium: it will automatically deliver from Act 3.

AA	Cycle	Act.	Derivative
Asp(D)			
Arg(R)	Standard	Act 1/2	Fmoc-L-Arg(Pbf)-OH
Trp(W)	Standard	Act 1/2	Fmoc-D-Trp-OH
Trp(W)	Standard	Act 1/2	Fmoc-D-Trp-OH
Val(V)	Standard	Act 1/2	Fmoc-L-Val-OH
Trp(W)	Standard	Act 1/2	Fmoc-D-Trp-OH
Thr(T)	Standard	Act 1/2	Fmoc-L-Thr(tBu)-OH
CA1(1)	Allyl Deblock	Act 3	Palladium(0)
CA2(2)	Extended	Act 2	Cyclize

Table 1. Notebook settings for a Cyclic Peptide

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NOTE: We recommend PyAOP be used as the activator for the cyclization[9].

If PyAOP is to be used as the activator for the entire peptide, place the 0.5 M PyAOP solution in Act1, and the 1.0 M DIEA solution in Act2. Place an empty tube in the rack in the final position, so that the activator can be recycled to perform the cyclization reaction.

If PyAOP is to be used only for the cyclization reaction, weigh out the PyAOP, place it dry in the vial in place of an amino acid, and select an Act2 (i.e. DIEA only) activation scheme.

Automated Synthesis of Branched Peptides
The strategy shown in Figure 1 is used to synthesize a branched peptide.

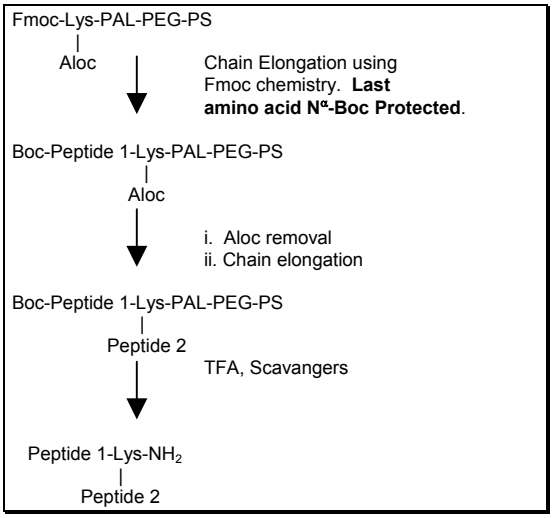

Figure 1. Branched Chain Strategy

Note: You must edit your chemistry file to add the N^{α}-Boc protected amino acids. See the *Workstation Getting Started Guide* for more information.

The procedure for setting up the notebook is similar to that in the section on Cyclic Peptides. Refer to Table 2.

AA	Cycle	Act.	Derivative
Lys(K)	Standard	Act 1/2	Fmoc-L-Lys(Aloc)OH
Val(V)	Standard	Act 1/2	Fmoc-L-Val-OH
Glu(E)	Standard	Act 1/2	Fmoc-L-Glu(OtBu)OH
Glu(E)	Standard	Act 1/2	Fmoc-L-Glu(OtBu)-OH
Leu(L)	Standard	Act 1/2	Fmoc-L-Leu-OH
Phe(F)	Standard	Act 1/2	Fmoc-L-Phe-OH
Gly(G)	Standard	Act 1/2	Fmoc-Gly-OH
Lys(K)	Standard	Act 1/2	Fmoc-L-Lys(Boc)-OH
Asn(N)	Standard	Act 1/2	Fmoc-L-Asn(trt)-OH
Ala(A)	Standard	Act 1/2	tBoc-L-Ala-OH
CA1(1)	Allyl Deblock	Act 3	Palladium(0)
Leu(L)	Standard	Act 1/2	Fmoc-L-Leu-OH
Phe(F)	Standard	Act 1/2	Fmoc-L-Phe-OH
Gly(G)	Standard	Act 1/2	Fmoc-Gly-OH
Gly(G)	Standard	Act 1/2	Fmoc-Gly-OH
Tyr(Y)	Standard	Act 1/2	Fmoc-L-Tyr(OtBu)-OH

Table 2. Notebook settings for a Branched Peptide

References

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8. Freidrich-Bochnistschek, S., H. Waldmann, and H. Kunz. 1989. *J. Org. Chem*. **54**:751-756.
9. For more information, see the Applied Biosystems Technical Note entitled: *Guanidinium Formation during in situ Activation of Amino Acids by Uronium-Salts*