Automated Disulfide Bond Formation on the Pioneer™ Peptide Synthesis System

Disulfide bridges play a crucial role in the folding and structural stabilization of many important extracellular peptide and protein molecules, including hormones, enzymes, growth factors, toxins, and immunoglobulins.[1]

Disulfide bonds are formed by the oxidation of the side-chain sulfhydryl of Cysteine residues. When more than two Cysteines are present in a peptide, formation of the proper disulfide bonds can be directed by the use of alternate side-chain protection strategies.

There are two basic methods for the formation of disulfide bonds: solid-phase (i.e. on support) or solution phase.

Solution Phase Formation of Disulfides

Since disulfides can form either intramolecularly, to form cyclic peptides, or intermolecularly, to form cross-linked polymers, this can make formation of cyclic peptides problematic in solution. Concentrations must be kept very low, to promote the internal bond formation. The process is generally facilitated by the use of thiol-disulfide exchange conditions that ultimately favor correct pairing or folding into thermodynamically stable conditions.[2]

Solid-Phase Formation of Disulfides

For solid-phase formation of disulfide bonds to occur, it is necessary to deprotect the Cysteine sulfhydryls without cleaving the peptide from the support. It is therefore necessary to use a Cysteine side-chain protection group that is removed with something other than TFA. *Bis*-acetamidomethyl (Acm) is the most commonly used protecting group. Metal-assisted removal of the Acm is generally accomplished by use of Mercury (II) Acetate or Thallium (III) Trifluoroacetate. After formation of the disulfide bond, the cyclic peptide is then cleaved and fully deprotected with TFA.

Automated Disulfide Bond Formation

A method has been developed for automated intramolecular disulfide bond formation on the Pioneer™ Peptide Synthesis System.[3] The methodology consists of modification of the allyl deblock scheme, whereby thallium(III) trifluoroacetate in dimethylformamide is used in place of the palladium (0) reagent, and an oxidation occurs instead of the reduction.

No actual changes are made to the allyl deblock cycle; only the reagents are changed (see Table 1). However, the thallium(III) trifluoroacetate must be added to the CA1 derivative of the chemical table (the OH derivatives or equivalent).

Protecting group	Amino Acid Tube	Act 3	Aux 3
Allyl	$Pd(PPh_3)_4$	Allyl Diluent	Allyl wash
Acm	$Tl(OTf)_3$	DMF	DMF

Table 1. ***Comparison of Reagents: Allyl versus Acm***

Table 2 is an example of a notebook that has been set up to run a cyclic peptide (Oxytocin) on a Pioneer™ Peptide Synthesis System. The procedure is as follows:

1. Open a new notebook.
2. Choose the desired protocol and chemistry files (the modified one with the thallium). Select Final Fmoc off if so desired.
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 diluent, which in this case is just DMF).
4. Enter in the sequence. Type **1** (for CA1) at the *N*-terminal.
5. Go to the synthesis view. Edit the cycles and derivatives such that CA1 uses an Allyl Deblock Cycle to dissolve thallium (III) trifluoroacetate.
   NOTE: An allyl deblock cycle will automatically draw from Act 3 regardless of what is selected.
6. Submit the notebook, and continue as normal.

AA	Cycle	Act.	Derivative
Gly (G)	Standard	Act 1/2	Fmoc--Gly-OH
Leu (L)	Standard	Act 1/2	Fmoc-L-Leu-OH
Pro (P)	Standard	Act 1/2	Fmoc-L-Pro-OH
Cys (C)	Standard	Act 1/2	Fmoc-L-Cys(Acm)-OH
Asn (N)	Standard	Act 1/2	Fmoc-L-Asn(Trt)-OH
Gln(Q)	Standard	Act 1/2	Fmoc-L-Gln(Trt)-OH
Ile (I)	Standard	Act 1/2	Fmoc-L-Ile-OH
Tyr (Y)	Standard	Act 1/2	Fmoc-L-Tyr(tBu)-OH
Cys (C)	Standard	Act 1/2	Fmoc-L-Cys(Acm)-OH
CA1(1)	Allyl Deblock	Act 3	Thallium (III) trifluoroacetate

Table 2. ***Notebook Settings for Cyclic Peptide***

The allyl deblock cycle will recycle the thallium solution for two hours. During this time, the Acm groups are removed and the disulfide bond is formed as the sulfhydryls oxidize.

References:

1. Andreu, D., F. Albericio, N.A. Solé, M.C. Munson, M. Ferrer, and G. Barany. 1994. From ***Methods in Molecular Biology, Vol. 35: Peptide Synthesis Protocols*** (M.W. Pennington and B.M. Dunn, Eds.), Humana Press, Totowa, NJ, pp. 91-169.

2. Andreu, D., and E. Nicolas. 2000. . ***Solid-Phase Synthesis. A Practical Guide*** (Kates, S.A. and F. Albericio, Eds.). Marcel Dekker, Inc., New York, pp. 365-375.

3. Schmidt, M.A., R.R. Wilhelm, and A. Srinivasan. 1999. Poster presented at the 16th American Peptide Symposium (Minneapolis).