Technical Support
Information Bulletin 1176

Ring Closing Methathesis on Resin

Ring closing methathesis is an efficient method for forming a hydrocarbon bridging structure in peptides. This is the crucial step in forming stapled peptides[1] and hydrocarbon analogs of cystine[2]. The immediate product from ring closing metathesis contains a hydrocarbon double bond. The double bond can be susceptible to oxidation or acid catalyzed side reactions but adds rigidity to the hydrocarbon bridge. Hydrogenating the double bond creates a saturated hydrocarbon bridge[3] that is unlikely to form side products, but it also makes the bridge structure more flexible.

Peptide Methathesis on Resin[1]

1. Dissolve 0.17 equiv (based on peptide-resin) of Grubb's catalyst in enough 1,2-dichloroethane to make a 10 mM solution.
2. Degas the catalyst solution and add it to the peptide-resin.
3. Shake the mixture at room temperature for 2 hours.
4. Filter off the catalyst.
5. Repeat Steps 1-4 once.
6. The reaction can be monitored by removing a few beads, cleaving the peptide and analyzing if by HPLC. The metathesized peptide elutes before the unmetathesized peptide.
7. Wash the resin 3 times with 1,2-dichloroethane and 3 times with DCM.
8. The peptide can be cleaved with standard cleavage procedures or the hydrocarbon double bond in the staple can be reduced on resin.

Olefin Hydrogenation on Resin[1]

1. Prepare a solution of 10 equiv (based on peptide resin) 2,4,6-tri-isopropylbenzenesulphonyl hydrazine and 20 equiv (basede on peptide resin) of piperdine in NMP to make approximately 0.7 M in the hydrazine.
2. Add the solution to the peptide resin and seal the reaction flask.
3. Place the sealed flask in a 47 °C waterbath for 2 hours.
4. Filter the resin.
5. Repeat steps 1-4 four more times.

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[1] Schafmeister CE, Po J, Verdine GL., *J. Am. Chem. Soc.* **2000**, *122,* 5891-5891 (Supporting Information, p5.)
[2] Hossain MA, Rosengren KJ, Zhang S, Bathgate RA, Tregear GW, van Lierop BJ, Robinson AJ, Wade JD. *Org Biomol Chem*. **2009**, *7*, 1547-53
[3] Berezowska I, Chung NN, Lemieux C, Wilkes BC, Schiller PW. *J Med Chem.* **2007**, *50*, 1414-7.

6. The reaction can be monitored by removing a few beads, cleaving the peptide and analyzing if by HPLC. The hydrogenated peptide elutes between the unhydrogenated peptide and the unmetathesized peptide.
7. Wash the resin 3 times with NMP and 3 times with DCM.
8. The peptide can be cleaved following standard cleavage protocols.