



## Technical Support Information Bulletin 1177

### Solid Phase Peptide Synthesis With N-Methyl Amino Acids

Incorporating N-methyl amino acids into peptides can have many beneficial effects, such as improving oral bioavailability<sup>1</sup>, enzymatic stability<sup>2</sup>, selectivity<sup>3</sup>, and inhibit aggregation and increase water solubility.<sup>4</sup> N-Methyl amino acids have greater steric hinderance than their unmethylated analogs. Coupling to N-methyl amino acids requires special coupling conditions to achieve high yields. Coupling an N-methyl amino acid to a second N-methyl amino acid is especially difficult. Many methods for successfully coupling N-methyl amino acids have been reported. Pre-formed Fmoc-amino acid chlorides have been used<sup>5</sup> as well as Fmoc-amino acid chlorides form in situ with triphosgene.<sup>6</sup> HATU has been utilized with success,<sup>7</sup> but HBTU and HCTU are less effective. Other coupling reagents employed for N-methyl amino acid coupling include BOP-Cl,<sup>8</sup> PyBroP,<sup>9</sup> PyAOP and PyBOP/HOAt<sup>10</sup> Coupling to N-methyl amino acids should be monitored with bromophenol blue, as unprotected N-methyl amino acids produce little or no color change with ninhydrin or

#### HATU Coupling to N-Methyl Amino Acids

1. Swell the resin in DMF or NMP.
2. Dissolve 4 equivalents (based on the resin) of the Fmoc-amino acid in DMF or NMP (approximately 10 ml per gram of resin). Add 4 equivalents (based on resin) of HATU and 8 equivalents of diisopropylethylamine (DIEA).

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<sup>3</sup> Doedens, L.; Opperer, F.; Cai, M.; Beck, J.G.; Dedek, M.; Palmer, E.; Hruby, V.J.; Kessler, H. *J. Am. Chem. Soc.* **2010**, *132*, 8115-28.

<sup>4</sup> Bose, P.P.; Chatterjee, U.; Hubatsch, I.; Artursson, P.; Govender, T.; Kruger, H.G.; Bergh, M.; Johansson, J.; Arvidsson, P.I. *Bioorg. Med. Chem.* **2010**, *18*, 5896-902.

<sup>5</sup> a) Sewald, N. *Angew. Chemie, Int. Ed.* **2002**, *41*, 4661-3; b) DiGioia, M.L.; Leggio, A.; Siciliano, C.; Sindona, G. *J. Org. Chem.* **2003**, *68*, 7416-21; c) Tantry, S.J.; Venkataramanar, R.; Chennakrishnareddy, G.; Sureshbabu, V. V. *J. Org. Chem.* **2007**, *72*, 9360-3.

<sup>6</sup> a) Falb, E.; Yechezkel, T.; Salitra, Y.; Gilon, C. *J. Pept. Res.* **1999**, *53*, 507-17; b) Biron, E.; Lagonegger, D.; Hoyer, D.; Kessler, H. "Understanding Biology Using Peptides" S.E. Bloudelle (Ed.) American Peptide Society, **2005**, pp 423-4.

<sup>7</sup> Miller, S.C.; Scanlan, T.S. *J. Am. Chem. Soc.* **1997**, *119*, 2301-2.

<sup>8</sup> Diago-Meseguer, J.; Palomo-Coo, A.L.; Fernandez-Lizerbe, J.R.; Zugaza-Bilbao, A. *Synthesis*, **1980**, 547-50.

<sup>9</sup> a) Frérot E.; Coste, J.; Poncet, J.; Castro, B. *Tetrahedron Lett.* **1992**, *33*, 2815-6; b) Coste, J.; Frérot E.; Jouin, P. *J. Org. Chem.* **1994**, *59*, 2437-46.

<sup>10</sup> Teixidó, M.; Albericio, F.; Giralt, E. *J. Pept. Res.* **2005**, *65*, 153-66.

3. Mix the solution at room temperature for 5 minutes.
4. Add the solution of activated amino acid to the resin.
5. Shake the resin suspension at room temperature for one hour.
6. Test coupling with the bromothymol blue test. If coupling is incomplete, perform a second coupling.
7. Filter the resin and wash with fresh solvent.

#### Coupling N-Methyl Amino Acids with PyBroP<sup>11</sup>

1. Remove the N-protecting group from the resin peptide using standard procedures.
2. Suspend the resin in DCM (10 mL/gram resin).
3. Dissolve 2 equivalents (based on resin substitution) of the protected amino acid in DCM or DMF. Add the solution to the resin.
4. Add 2 equivalents (based on resin substitution) of PyBroP<sup>®</sup>. Cool the mixture to 0 °C.
5. Add 6 equivalents of diisopropylethylamine (DIPEA). Mix 1 minute cold and 1 hour at room temperature.
6. Filter the resin and wash with DCM.

#### Bromophenol Blue Test<sup>12</sup>

Test Solution: Dissolve 250 mg of bromophenol blue in 50 ml of DMF.

##### Test Procedure

1. Remove a small sample of resin and wash it with methanol.
2. Add 2 ml of the test solution.
3. Observe the color of the suspension.

If the color is yellow: Coupling is complete.

If the color is blue to green: Coupling is not complete. Recouple.

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<sup>11</sup> Based on procedure of Coste, J.; Frérot, E.; Jouin, P.; Castro, B. *Tetrahedron Lett.* **1991**, 32, 1967-1970.

<sup>12</sup> Based on procedure of Krchňák, V.; Vágner, J.; Safář, P.; Lebl, M. *Coll. Czech., Chem. Comm.* **1988**, 53, 2542-8.