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## Coupling Reagents

### Carbodiimides

Dicyclohexylcarbodiimide (DCC) and diisopropylcarbodiimide (DIC) are commonly used to prepare amides, esters and acid anhydrides from carboxylic acids. These reagents can also convert primary amides to nitriles, which can be useful in organic synthesis but is a troublesome side reaction of asparagine and glutamine residues in peptide synthesis. Dicyclohexylurea, the byproduct formed from DCC, is nearly insoluble in most organic solvents and precipitates from the reaction mixture as the reaction progresses. Hence DCC is very useful in solution phase reactions, but is not appropriate for reactions on resin. DIC is used instead in solid phase synthesis since the urea byproduct is more soluble and will remain in solution. In certain applications, such as modifying proteins, ethyl-(N',N'-dimethylamino)propylcarbodiimide hydrochloride (EDC) is used. This carbodiimide reagent and its urea by-product are water soluble, so the byproduct and any excess reagent are removed by aqueous extraction.

Carbodiimide activation of amino acid derivatives often causes a partial racemization of the amino acid. In peptide synthesis, adding an equivalent of 1-hydroxybenzotriazole (HOBt) minimizes this problem. The OBt esters that form as intermediates couple with primary amines with little racemization, although certain residues such as histidine may be troublesome. Coupling an amino acid derivative to a hydroxy-functionalized resin requires a catalytic amount of 4-(N,N-dimethylamino)pyridine (DMAP). The basic DMAP can produce undesirable levels of racemization, so no more than 0.15 equivalents should be used.

### Phosphonium-Based Reagents

To avoid the racemization and side reactions that can occur with carbodiimide reagents, many alternative reagents were developed to generate OBt esters *in situ*. (Benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) is one of the first reagents developed.<sup>1</sup> BOP does not generate asparagine and glutamine dehydration byproducts and racemization is minimal. BOP is also useful for preparing esters under mild conditions.<sup>2</sup> It must be handled with caution as highly carcinogenic hexamethylphosphoramide is formed as a byproduct in coupling reactions.

(Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate couples amino acids as efficiently as BOP, but the by-products are less hazardous. Coupling reactions are rapid, being nearly complete within a few minutes. (Benzotriazol-1-yloxy)tripyrrolidino-phosphonium hexafluorophosphate may be used in place of BOP in peptide synthesis without loss of coupling efficiency.

<sup>1</sup> Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, 1219-1222.

<sup>2</sup> a) Kim, M. H.; Patel, D. V. *Tetrahedron Lett.* **1994**, 35, 5603-5606; b) Sliedregt, K. M.; Schouten, A.; Kroon, J.; Liskamp, R. M. J. *Tetrahedron Lett.* **1996**, 37, 4237-4240.

Bromotripyrrolidinophosphonium hexafluorophosphate is a more reactive coupling reagent. It is especially useful in difficult coupling, such as coupling N-methylamino acids or  $\alpha,\alpha$ -dialkylglycines, where other coupling reagents are inefficient.

### **Aminium-Based Reagents**

Two other popular coupling reagents are O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) and O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU). As their names reflect, these reagents were believed to have a uronium structure, but crystal and solution structure studies revealed that these reagents actually have aminium structure.<sup>3</sup> Both are very efficient peptide coupling reagents with little racemization. Coupling reactions are complete in as little as six minutes and when HOBt is added, racemization can be reduced to insignificant levels.<sup>4</sup> This makes these the reagents of choice in critical applications. TBTU was very effective, for instance, in key macrocyclization and coupling steps in the total synthesis of the macrocyclic peptide cyclotheonamide B.<sup>5</sup>

These reagents should in equal molar amounts relative to the carboxylic acid component of the coupling reaction. Excess HBTU and TBTU can react with the unprotected N-terminal of the peptide and form a guanidinium moiety that blocks further elongation of the peptide.

O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HATU) is similar to HBTU, but reacts faster with less epimerization during coupling. HATU is preferred to HBTU in most rapid coupling protocols. HATU is utilized in the same manner as HBTU. As with HBTU, HATU should not be used in excess because it can react with the unprotected N-terminal and block further chain elongation.

O-(6-Chlorobenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HCTU) remains colorless through long synthesis sequences and presumably has greater stability. It is reported to be less allergenic than other coupling reagents, but nonetheless it should be handled cautiously. DiFenza and Rovero have reported that HCTU showed reduced rates of racemization compared to BOP.<sup>6</sup>

O-(3,4-Dihydro-4-oxo-1,2,3-benzotriazine-3-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate TDBTU is a coupling reagent that causes very little epimerization. In the coupling of peptide fragments to form SK&F 107647, TDBTU was shown to produce significantly less epimerization than PyBOP, HBTU, HATU, and many other common coupling reagents.<sup>7</sup> TDBTU was utilized in the large scale synthesis of over 2 kg of SK&F 107647.<sup>7</sup>

### **Other Coupling Reagents**

3-(Diethylphosphoryloxy)-1,2,3-benzotriazin-4(3H)-one (DEPBT) is a coupling reagent that causes very little epimerization during coupling<sup>8</sup>. It is especially useful for coupling easily

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<sup>3</sup> Abdelmoty, I.; Albericio, F.; Carpino, L. A.; Foxman, B. M.; Kates, S. A. *Lett. Pept. Sci.* **1994**, *1*, 57-67.

<sup>4</sup> Knorr, R.; Trzeciak, A.; Bannwarth, W.; Gillessen, D. *Tetrahedron Lett.* **1989**, *30*, 1927-1930.

<sup>5</sup> Bastiaans, H. M. M.; Van der Baan, J. L.; Ottenheijm, H. C. J. *J. Org. Chem.* **1997**, *62*, 3880-3889.

<sup>6</sup> DiFoena, A. and Rovero, P. "Racemization studies on a novel Cl-HOBt-based coupling reagents" Presented at the European Peptide Symposium, August 2002.

<sup>7</sup> Hiebl, J., et al. *J. Peptide Res.* **1999**, *54*, 54-65.

<sup>8</sup> Li, H.; Jiang, X.; Ye, Y.; Fan, C.; Romoff, T.; Goodman, M. *Org. Lett.* **1999**, *1*, 91-93

epimerized amino acids such as arylglycines.<sup>9</sup> DEPBT was also shown to be a superior reagent for head-to-tail cyclization of linear peptides.<sup>10</sup>

Carbonyldiimidazole (CDI) is useful for forming amides, esters and thioesters. It is not commonly used in routine peptide synthesis, but is quite useful for coupling peptide fragments to form large peptides and small proteins. One unique application of CDI is the preparation of urea dipeptides.<sup>11</sup>

### **Standard DIC/HOBt Coupling**

1. Remove the N-terminal protecting group by standard deprotection protocols.
2. Suspend the resin in dichloromethane (DCM, 10 mL per gram resin)
3. Dissolve 5 equivalents (based on resin substitution) in DMF (approximately 1 mL per gram of amino acid derivative).
4. Dissolve 5.5 equivalents (based on resin substitution) of HOBt in DMF (minimum volume necessary for complete solution).
5. Add the amino acid solution and the HOBt solution to the resin suspension.
6. Shake the mixture at room temperature under inert gas. Monitor the reaction using the ninhydrin test. When the ninhydrin test is negative, filter and wash the resin three times with DMF, three times with DIC, then three times with either methanol or DCM. If the ninhydrin test is not negative within four hours, repeat the coupling procedure.

### **Coupling with EDC**

1. Dissolve the N-protected amino acid and the amino acid ester to be coupled in dichloromethane (DCM).
2. Cool the mixture in an ice bath.
3. Add 1.2 equivalents of EDC and stir the mixture.
4. When the reaction is complete, wash the mixture with water to remove excess EDC and urea by-product.
5. Dry the organic phase over sodium sulfate, filter, and evaporate to obtain the crude product.

### **Coupling with BOP Reagent<sup>12</sup>**

1. Remove the N-protecting group using standard deprotection protocols.
2. Dissolve 2.0 equivalents (based on resin substitution) of the protected amino acid in DMF (5 mL/g of resin) and add to the resin.
3. Add 2.0 equivalents (based on resin substitution) of 1.0 M BOP solution and 4.0 equivalents (based on resin substitution) of diisopropylethylamine (DIPEA). 2.0 equivalents (based on resin substitution) of 0.5 M HOBt solution in DMF can be added to suppress racemization.
4. Mix for 10-60 minutes until the Kaiser test is negative.

### **Coupling with Benzotriazole-1-yloxy-tris-pyrrolidinophosphonium Hexafluorophosphate<sup>13</sup>**

<sup>9</sup> Boger, D.L.; Kim, S.H.; Miyazaki, S.; Strittmatter, H.; Weng, J.-H.; Mori, Y.; Rogel, O.; Castle, S.L.; McAtee, J.J. *J. Am. Chem. Soc.* **2000**, *122*, 7416-7417.

<sup>10</sup> Tang, Y.-C.; Xie, H.-B.; Tian, G.-L.; Ye, Y.-H. *J. Peptide Res.* **2002**, *60*, 95-103.

<sup>11</sup> Peña, M., et al. *J. Org. Chem.* **1997**, *62*, 6420-6023.

<sup>12</sup> Rovero, P.; Quartara, L.; Fabbri, G. *Tetrahedron Lett.* **1991**, *32*, 2639-2642.

<sup>13</sup> Based on the procedure in Hoeg-Jensen, T.; Jakobsen, M. H.; Holm, A. *Tetrahedron Lett.* **1991**, *32*, 6387-6390.

1. Remove the N-protecting group using standard deprotection protocols.
2. Dissolve 1.1 equivalents (based on resin substitution) of the protected amino acid in DMF (5 mL/g of resin) and add to the resin.
3. Add 1.1 equivalents (based on resin substitution) of 1.0 M PyBOP solution and 2.2 equivalents (based on resin substitution) of diisopropylethylamine (DIPEA). 1.1 equivalents (based on resin substitution) of 0.5 M HOBt solution in DMF can be added to suppress racemization.
4. Mix for 10-60 minutes until the Kaiser test is negative.

#### **Coupling N-Methyl Amino Acids with Bromo-tris-pyrrolidino-phosphonium hexafluorophosphate<sup>14</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.

#### **Coupling with HBTU or TBTU**

1. Remove the N-protecting group using standard deprotection protocols.
2. Dissolve 2.0 equivalents (based on resin substitution) of the protected amino acid in DMF (5 mL/g of resin) and add to the resin.
3. Add 2.0 equivalents (based on resin substitution) of 1.0 M HBTU solution and 4.0 equivalents (based on resin substitution) of diisopropylethylamine (DIPEA). 2.0 equivalents (based on resin substitution) of 0.5 M HOBt solution in DMF can be added to suppress racemization.
4. Mix for 10-60 minutes until the Kaiser test is negative.
5. Filter and wash the resin with DMF.

#### **Coupling with TSTU in Aqueous Solvent Mixtures<sup>15</sup>**

1. Dissolve the acid in a 2:2:1 mixture of DMF/dioxane/water.
2. Add 3 equivalents of diisopropylethylamine and 1.3 equivalents of TSTU.
3. After the formation of the -OSu ester is complete, add 1.5 equivalents of the amine.
4. After the reaction is complete, the solvents are removed and the crude product is isolated.

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<sup>14</sup> Based on procedure of Coste, J.; Frérot, E.; Jouin, P.; Castro, B. *Tetrahedron Lett.* **1991**, 32, 1967-1970.

<sup>15</sup> Bannwarth, W.; Knorr, R. *Tetrahedron Lett.* **1991**, 32, 1157-1160.