TECHNICAL NOTE

Guanidinium Formation during *in situ* Activation of Amino Acids by Uronium-Salts

For many years, the stand-alone coupling agent BOP*, in the presence of base, was successfully used in both Fmoc/*t*Bu and Boc/Bzl methods for *in situ* activation of amino acids for the assembly of peptide sequences.[1] The disadvantage of BOP is the generation of a known carginogen, HMPA. Knorr, et al.[2] reported that the uronium analogs of BOP, TBTU and HBTU, which do not release carcinogenic by-products, gave much lower rates of racemization when compared to BOP.

Gausepohl, et al.[3] reported a loss of reactive amino groups when TBTU or HBTU was added to the resin prior to the amino acid to be activated. This was attributed to the reaction of the tetramethyluronium in the activator with the amino groups on the resin.

A similar phenomenon was observed during HBTU-mediated cyclization of a peptide.[4] The desired homodetic peptide was not obtained, and subsequent analysis indicated that the product was the tetramethylguanidinium derivative of the linear sequence.

In-house studies with HATU have shown similar results.

To prevent the undesired guanidinium formation capping reaction, the following is suggested:

1. Use no greater than a 1:1 molar ratio of uronium agent to amino acid .

2. In cyclization and other slow coupling reactions, use PyAOP as a substitute for HATU. PyAOP is as effective as HATU with the benefit that guanidinium formation is avoided.[5]

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*The abbreviations used are: BOP, benzotriazol-1-oxy-tris-(dimethylamino) hexafluorophosphate, HMPA, hexamethylphosphoramide, TBTU, 2-1*H*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate, HBTU, 2-1*H*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate,
HATU, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-b] pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide,
PyAOP, 7-azabenzotriazol-1-yloxytris (pyrrolidino) phosphonium hexafluorophosphate

References

1. Castro, B., Domoy, J.R., Evin, G., and Selve, C. *Tetrahedron Lett*., **14**, 1219-1222 (1975).

2. Knorr, R., Trzeciak, A., Bannwarth, W., and Gillessen, D. *Tetrahedron Lett*., **30**, 15, 1927-1930 (1989).

3. Gausepohl, H., Pieles, U., and Frank, R.W. (1992) In *Peptides: Chemistry and Biology* (J.A. Smith and J.E. Rivier, Eds.), ESCOM, Leiden, 523-524.

4. Story, S.C., and Aldrich, J.V. *Int. J. Peptide Protein Res*. **43,** 292-296 (1994).

5. Albericio, F., Cases, M., Alsina, J., Triolo, S.A., Carpino, L.A., and Kates, S.A. *Tetrahedron Lett*., **38**, 4853 (1997).

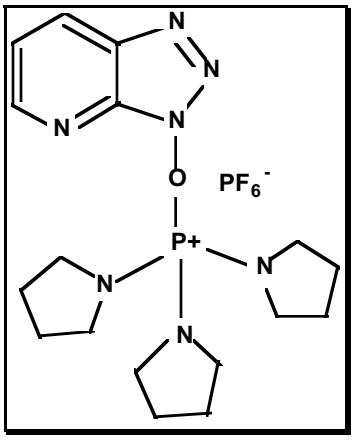

Fig. 1: The Structure of PyAOP (MW = 520)