TECHNICAL NOTE

Viewing Detector Traces on the 9050Plus PepSynthesizer During HATU-Mediated Synthesis

The use of HOAt, (1-hydroxy-7-aza-benzotriazole) and its uronium* salt, HATU, (*O-(*7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate), shown in Figure 1, results in improved coupling efficiency during solution and solid phase methods of peptide assembly [1,2].

With the 9050Plus PepSynthesizer, HOAt-promoted couplings using DIPCDI- or -OPfp ester-mediated chemistries are readily monitored at 365 nm by the built-in detector system. However, HATU produces a bright yellow color when dissolved in a solution of a tertiary amine base in DMF during the pre-activation and dissolution of the amino acid. The absorbance at 365 nm of the resulting solution, when it is circulated through the column, results in off-scale detector readings.

For example, during synthesis of the ACP [65-74] decamer using HATU and Diisopropylethylamine (DIEA) with the standard 365 nm filters and aperture plates, the absorbance during coupling of Asp^{70} for both the pre-column (top) and post-column (middle) detector traces was off-scale (see Figure 2).

Changing the pre-column detector filter and aperture plate to 436 nm and 280 nm respectively, prior to synthesis of the same peptide resulted in the Asp^{70} detector traces shown in Figure 3. *Note: Recirculation of the coupling solution through the column can be viewed with the 9050Plus.*

If a return to DIPCDI or -OPfp activation is required, it is not necessary to return the filter and aperture plate on the pre-column detector to the 365 nm package. The 365 nm package on the post-column detector can be used to view both the deprotection and coupling steps (see Figure 4). Only the pre-column detector filter and aperture plate should be changed when HATU activation chemistry is used. Changing the post-column package results in the loss of the monitoring of the Fmoc-deprotection absorbance. Filter and aperture plate changes are easily performed. Call Technical Support at (800)-899-5858 x 7778 for more details.

ORDERING INFORMATION

GEN600039 HATU filter set

REFERENCES

1. Carpino, Louis A. 1993. 1-Hydroxy benzotriazole. An Efficient Peptide Coupling Additive. J. Am. Chem. Soc., 115, 4397-4398.
2. Carpino, Louis A., El-Faham, Ayman, Minor, Charles A., and Albericio, Fernando. 1994. Advantageous Applications of Azabenzotriazole (Triazolopyridine)-based Coupling Reagents to Solid-phase Peptide Synthesis. J. Chem. Soc., Chem. Comm., 201-203.
3. Abdelmoty, I., Albericio, F., Carpino, L.A.,. Foxman, B.M , and Kates, S.A. 1994. Structural studies of reagents for peptide bond formation: Crystal and molecular structures of HBTU and HATU. Letters in Peptide Science, 1:57-67.

**** Note: To maintain consistency of historical nomenclature, we continue to define HATU and HBTU as uronium salts. Please see reference #3 for further details.***

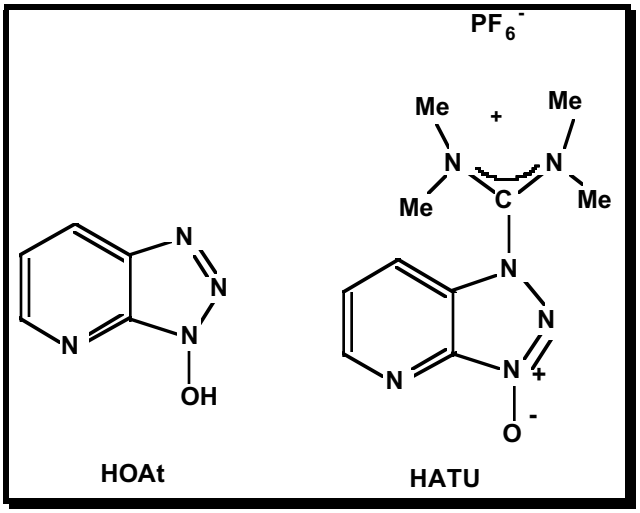

Figure 1. The chemical structures of HOAt and HATU

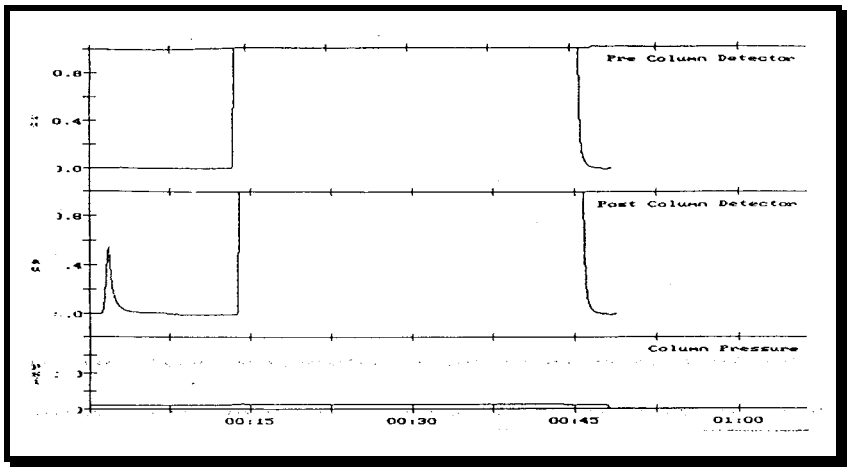

Figure 2. HATU-mediated synthesis monitored with standard 365 nm filter set.

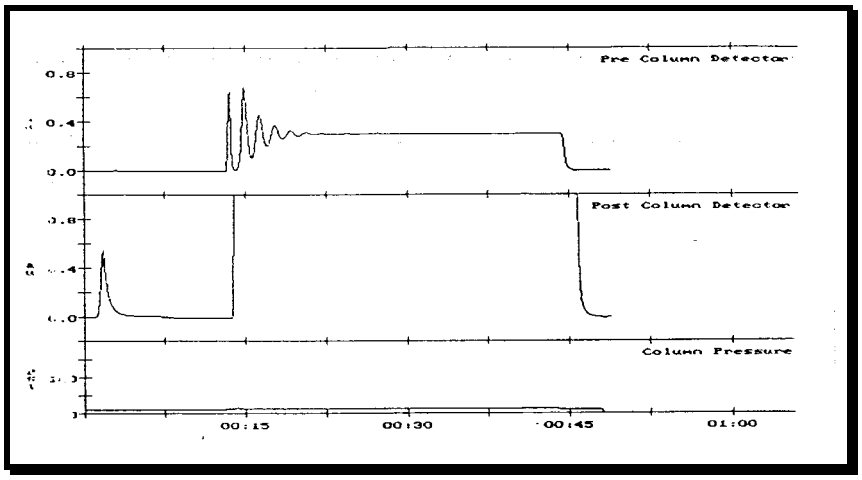

Figure 3. HATU-mediated synthesis monitored with HATU filter set

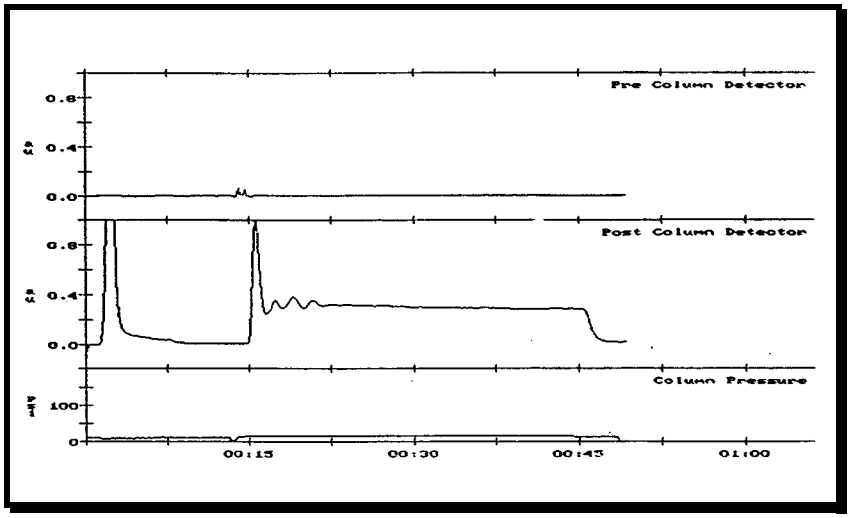

Figure 4. OPfp-ester mediated synthesis monitored with the HATU filter set