TECHNICAL NOTE

Determination of the Amino Acid Substitution Level *via* an Fmoc Assay

This procedure is used to quantitate the support loading (degree of substitution) of the *C*-terminal Fmoc-amino acid which has been esterified to a solid support (see the Technical Note entitled *Esterification of Hydroxymethyl-Functionalized Supports*). The procedure deblocks the solid support by removal of the N^{α}-Fmoc group of the attached amino acid with base solution (30% piperidine in DMF). The resulting solution is then measured spectrophotometrically to determine the extent of Fmoc groups removed. The degree of substitution is then calculated from the resulting absorbance reading.

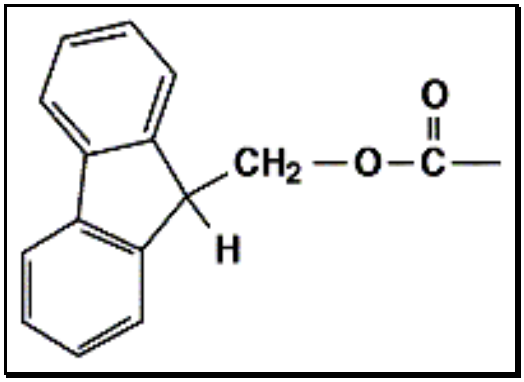

Figure 1. Structure of the Fmoc Protecting Group

Required Materials

- UV-Vis spectrophotometer
- 1.0-cm quartz cuvette
- 1.0 mL calibrated pipette
- Absolute ethanol- 100% (non-denatured)
- 30% piperidine in DMF
- Scintillation vial

Procedure

1. Weigh 10 mg $\pm$ 0.1 mg of esterified solid support into a scintillation vial.
2. Add 0.5 mL of 30% piperidine in DMF to the support sample and cap the vial tightly.
3. Mix and let stand for 30 minutes.
4. Add 19.5 mL of absolute ethanol. Mix and allow the support to settle for 5 minutes.
5. Pipette an aliquot of the ethanolic supernatant into a 1.0-cm quartz cuvette.
6. Place the sample cuvette into a UV-Vis spectrophotometer. Use absolute ethanol as the blank to zero the absorbance of the spectrophotometer.
7. Record the absorbance of the sample at 300 nm.
8. Calculate the support substitution using the following equation, based on an extinction coefficient at 300 nm of 6566:

$$\text{Substitution level (mmol/g)} = 3.05 \times A_{300}/m$$

Where: m = mass of support (mg)
A_{300} = Absorbance at 300 nm