Total Chemical Synthesis of a Glycoprotein by Native Chemical Ligation

Synthesis of glycopeptide-thioester using the alkanesulfonamide “safety-catch” linker:

- **a)** Fmoc-Gly-OH (4 equiv)
  
  PyBOP (3 equiv)
  
  DIEA (9 equiv)
  
  DMF, –20 °C, 8 h to r.t.
  
- **b)** SPPS using N-Fmoc-AA (11–23)
  
  DCC/HOBt in NMP
  
- **c)** N-Fmoc-Thr(α-D-GalNAc) (5 equiv)
  
  DIC (10 equiv), HOBt (10 equiv)
  
  DMF (30 min premix), 30 min
  
- **d)** SPPS using N-Fmoc-AA (2–9)
  
  DCC/HOBt in NMP
  
- **e)** N-Boc-Asp(OtBu)-OH (5 equiv)
  
  DIC (10 equiv), HOBt (10 equiv)
  
  DMF, 30 min
  
- **f)** ICH₂CN, DIEA, NMP, 24 h

Significance: The authors have developed a new approach for the synthesis of unprotected thioesters by using Fmoc-based solid-phase peptide synthesis and have demonstrated its utility in the total synthesis of a glycosylated protein, the antimicrobial O-linked glycoprotein diptericin, by the native chemical ligation method. This method utilizes an alkanesulfonamide ‘safety-catch’ linker, which circumvented the problems associated with the incompatibility of glycosidic linkages with Boc chemistry and of thioesters with Fmoc chemistry.

Comment: The C-terminal residue of the peptide is attached to the resin through an acid- and base-stable N-acyl sulfonamide linkage. After peptide synthesis, the sulfonamide is activated by cyano-ethylation and then cleaved with a thiol nucleophile. This general synthetic approach permits access to unprecedented quantities of homogeneous glycoproteins.