Total Chemical Synthesis of a Glycoprotein by Native Chemical Ligation

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

1. **Fmoc-Gly-OH (4 equiv)**
   - PyBOP (3 equiv)
   - DIEA (9 equiv)
   - DMF, –20 °C, 8 h to r.t.

2. **SPPS using N-Fmoc-AA (11–23)**
   - DCC/HOBt in NMP

3. **N-Fmoc-Thr(Ac3-α-D-GalNAc) (5 equiv)**
   - DIC (10 equiv), HOBt (10 equiv)
   - DMF (30 min premix), 30 min

4. **SPPS using N-Fmoc-AA (2–9)**
   - DCC/HOBt in NMP

5. **N-Boc-Asp(OtBu)-OH (5 equiv)**
   - DIC (10 equiv), HOBt (10 equiv)
   - DMF, 30 min

6. **ICH2CN, DIEA, NMP, 24 h**

7. **BnSH, THF, 24 h**

8. **Reagent K (TFA (82.5%), phenol (5%), H2O (5%), thioanisole (5%), ethanedithiol (2.5%), 4 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 anti-microbial 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-methylation and then cleaved with a thiol nucleophile. This general synthetic approach permits access to unprecedented quantities of homogeneous glycoproteins.