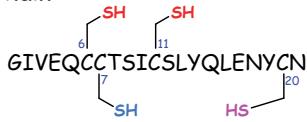


# Human Insulin Synthesis

There are many kinds of proteins and bioactive peptides with multiple disulfide bonds. TRC has technology for site-selective disulfide bond formation to synthesize multiple-disulfide peptides with high yield and high purity. Here is an example of synthesis of human insulin (HI) with three disulfide bonds.

## Issues in synthesis of HI

### A-Chain

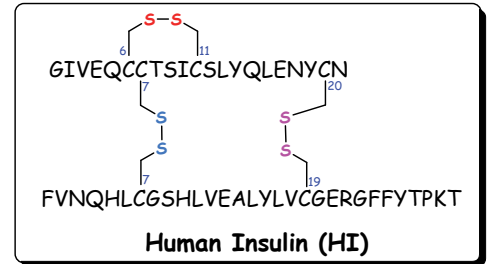


### B-Chain



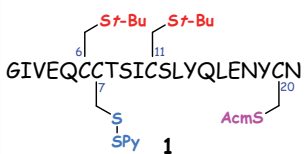
*low yield...*

Due to disulfide bond isomers and polymers

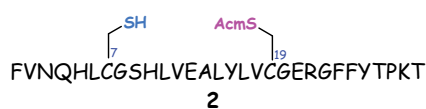


## Site-selective disulfide bond formation of HI

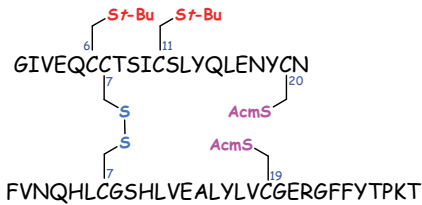
### A-Chain



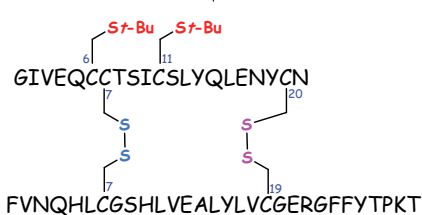
### B-Chain



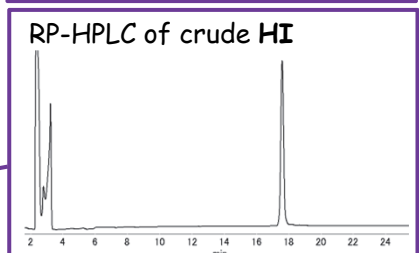
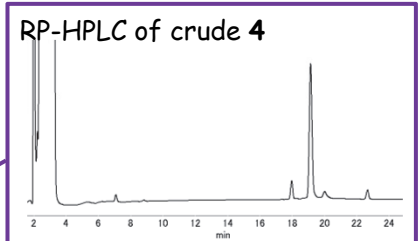
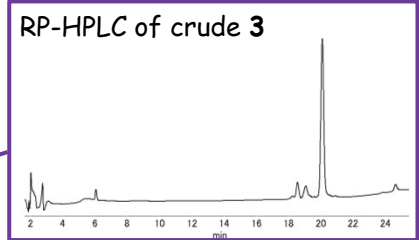
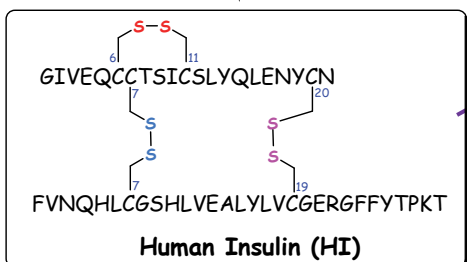
disulfide bond (A7-B7) formation



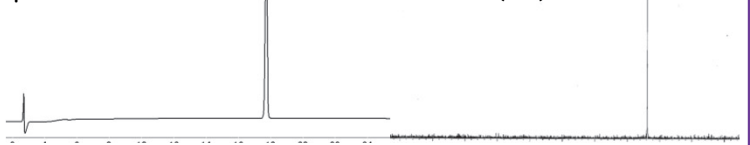
disulfide bond (A20-B19) formation



disulfide bond (A6-A11) formation



RP-HPLC of purified HI



MALDI-TOF-MS

calcd. for (M+H)<sup>+</sup>: 5804.6  
found (M+H)<sup>+</sup>: 5804.6

Three disulfide bonds in HI were formed in a site-selective manner with high yield (33%) and high purity (>98%).