Ste24, the S. cerevisiae analogue of human Rce1, is a unique metalloprotease with an unexplored mechanism of action at the molecular level, despite recent publication of its 3D crystal structure. It differs from the human enzyme in that it catalyzes two sequential cleavage reactions at two distinct sites within the same peptide molecule. The only bona fide Ste24 substrate is the precursor to the α-factor mating pheromones, although genetic analysis indicates the presence of additional substrates. The first cleavage step involves the removal of the three C-terminal amino acids adjacent to the isoprenylated cysteine residue, and the second involves a site-specific cleavage reaction 7 residues downstream of the N-terminus of α-factor precursor. This work describes the synthesis and NMR characterization of a 33mer α-factor precursor analogue with an Abz-Dnp donor-quencher pair positioned on the N-terminus and K11, respectively, of the peptide. This will be used in conjunction with structure guided mutagenesis for studying the kinetics of the second Ste24 catalyzed cleavage step. This overall synthetic strategy can also be used for the preparation of other types of biophysical probes designed to study Ste24 and other proteins that contain C-terminal prenylcysteine methyl esters.