4.2 HF Cleavage and Deprotection:

The crude peptide is detached from the resin by treatment with HF, concurrent with removal of the Ser^3 side chain Bzl protecting group. The peptide resin is treated in multiple batches, with an HF cocktail that consists of HF and anisole in the ratio of 5:1 (v/v).

The reaction is carried out for 60 min at -10 °C to -5 °C. Upon completion of the cleavage process, HF is removed by evaporation with a nitrogen flow. The crude peptide is precipitated by addition of diethyl ether, the crude peptide/spent resin mixture is isolated by filtration and washed with ether. The washed crude peptide is then scraped off the filter and transferred to a drying tray. The crude peptide in the tray is placed inside a fume hood for a minimum of 20 minutes to evaporate excess solvent.
The crude product is extracted from the spent resin with 90% acetic acid (HOAc)/water (2x) and then with neat TFA (1x). The combined crude peptide solution was shell frozen and lyophilized to yield the crude PPL2190 peptide.

After lyophilization, the crude peptide is analyzed by Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) for purity and by Liquid Chromatography/Mass Spectrometry (LC/MS) for identity.

4.3