Since submitting R01 AI161245-01 on 13 Aug 2020, we have synthesized and assayed more than 509 new compounds, generated 135 new X-ray structures, and carried out additional assays that support the likelihood of achieving our Target Product Profile (TPP) for an orally bioavailable SARS-CoV-2 Mpro inhibitor.

**Aim 1:** Our main synthetic effort (189 new compounds) has been focused on the *aminopyridine* lead series, achieving >50-fold improvement in biochemical IC50, antiviral activity IC50 <5 µM with cytotoxicity CC50 >100 µM, 50-fold selectivity over host proteases, and measurable oral bioavailability in rats. The figure below reports progress on a high ligand efficiency P1-P2 scaffold, achieving IC50 < 300 nM before integrating P1’ and P4 substituents. SAR (not shown) in P1’ and P4 suggests potency goals of <50 nM are readily achievable, with a compound (MAT-POS-53907a1c-3) integrating the P1’ and P4 substituents reported in Figure 7 of the proposal demonstrating a biochemical IC50 of 58 nM with near-additive SAR. In addition, SAR around the P1-P2 scaffold suggests metabolic and PK goals will be attainable with further rounds of med chem.

**Aim 2:** For the *quinolone* series, biochemical potency has progressed to <1 µM, with low oral exposure achieved in rat PK.

**Aim 3:** The *benzotriazole* series has progressed biochemical IC50 from 12.5 µM to <500 nM.

**Modifications to TPP goals:** We have observed a smaller drop-off than initially expected from enzyme to cell activity—likely resulting from the non-peptidomimetic nature of our lead compounds—and have therefore relaxed the criteria for biochemical potency to <50 nM. Further benchmarking for rapidly generating a first-in-class agent also led us to reduce the hurdle for initial solubility to >10 µM, although formulation to higher solubility remains desirable.