1b infected patients receiving amantadine alongside SOC (pegylated interferon and ribavirin for 48 weeks).[256] Convincingly, engineering this mutation into Leu20-containing, rimantadine susceptible strains (genotype 1b, 2a) conferred resistance both *in vitro* and in culture, providing the first point mutation definition of a p7–drug interaction and supporting the binding of adamantanes to a peripheral site.[63]

In the same study, docking of nonylated imino sugars was predicted to differ markedly from that of adamantanes, whereby these molecules were predicted to intercalate between p7 protomers and so potentially prevent the formation of the channel complex.[63] One key interaction was predicted to occur between the imino sugar head group and Phe25. This residue is highly conserved across most HCV isolates with the exception of some genotype 3a (also some 4) sequences, where Ala is present. Genotype 3a p7 was shown to be less sensitive to imino sugars than other HCV strains,[64] and this was shown to correlate with the disruption/preservation of stable oligomers in the presence of drug by native PAGE, compared with sensitive p7 proteins.[63] Introduction of Phe25Ala into susceptible strains again conferred resistance to this inhibitor class, with no cross-resistance to adamantanes, confirming an entirely distinct mode of action. As discussed above, this mutation also caused a hyper-active channel phenotype *in vitro* when transferred into genotype 1b/2a proteins, consistent with a potential gating role for Phe25 in these p7 proteins,[48] but also showing that genotype 3a p7 likely utilises an alternative bulky gating residue. Neither Leu20Phe nor Phe25Ala imbued any significant fitness cost to the virus in culture when introduced into either a 1b or a 2a background, although infectious particle production was reduced by between 30 and 60%. Of course, the consequences of this *in vivo* are unknown, yet this indicates that the genetic barrier to escape from prototype inhibitors may be low.

Critically, the distinct modes of action and lack of cross-resistance between adamantane and imino sugar resistant-variants indicate that compounds targeting these sites could potentially be used in drug combinations.[63] It is important to point out that the mutations identified in this study are likely to represent only one of several sequence changes that may occur to effect resistance within the context of varying p7 sequences. Accordingly, other p7 sequence changes have been observed in patient amantadine studies[257] and even minor variations between 1b p7 sequences around the predicted binding site appear to reduce markedly amantadine sensitivity *in vitro*.[247] Hence the p7 sequence context must be borne in mind when interpreting inhibitor studies, and also for the future development of improved molecules. However, as discussed in previous sections for other viroporins, prototype p7 inhibitors serve to illustrate the presence of druggable sites that could be exploited as drug targets. Rimantadine inhibits all p7 sequences tested to date, albeit with moderate potency,[64,255] and the only exception to imino sugar susceptibility has been genotype 3a.[63] Hence improved molecules targeting these sites may be selected from screens or could be selected by rational design, whereby more efficient binding modes and stabilising contacts lead to improved potency. However, rational design will necessitate atomic models of the channel