In particular, amantadine, which was initially shown to inhibit genotype 1b p7 channels at low micromolar concentrations *in vitro*,[37] was required at much higher concentrations to inhibit genotype 1a p7 peptides *in vitro*[243] and appeared ineffective in cell culture at standard concentrations, even against genotype 1b chimeric viruses (up to $\sim$50 μM).[64,243] By contrast, 50 μM nonyl-imino sugars were able to cause up to 80% reductions in secreted infectivity in one-step assays and could eliminate HCV from sequential supernatant passage.[243] However, in assessing the susceptibility of multiple HCV sub/genotypes both in culture and *in vitro*, it became clear that p7 inhibitors exerted their effects in a p7 sequence-dependent fashion, with p7 displaying subtle resistance behaviour even to very similar compounds, generating specific patterns for each isolate tested.[64] For example, whilst rimantadine has been shown to inhibit most strains tested,[64,255] both genotype 2a JFH-1 and 1a H77 p7 appeared highly resistant to amantadine, despite the obvious similarity between the two compounds.[64] In addition, genotype 3a p7 appeared resistant to the nonylated imino sugar *N*-nonyldeoxynojirimycin (*N*N-DNJ; see Table 9.2).[64] Although effective *in vitro* against various isolates, HMA toxicity prevented its effects in culture from being characterised. Such genotype-dependent patterns of susceptibility were reproduced in studies assessing proton conductance of channels present within isolated cellular membranes;[40] it also became apparent that cellular uptake/turnover of certain compounds likely affected their cell culture efficacy as activity against channels in purified vesicles was greater compared with intact cells; this may be related to hepatic metabolic turnover *in vivo*.

Genotype-dependent differences in compound susceptibility have subsequently been levied by some as a criticism of p7 inhibitors as a whole. However, from a medicinal chemistry perspective, they point to specific drug–protein interactions that may be defined through resistance and so permit mapping of inhibitor binding sites and the potential definition of compound modes of action. Early clues to the action of adamantanes came from the partial amantadine resistance phenotype for a genotype 1b p7 mutant where a series of C-terminal leucines was changed to alanine.[241] However, like the amantadine-resistant JFH-1 HCV, this mutant retained sensitivity to rimantadine, as is the case for all HCV genotypes to date to a greater or lesser extent (*e.g.* genotype 1a H77 is less sensitive).[64,255] Interaction of these C-terminal leucine residues with amantadine was also shown in ssNMR studies,[246] and together this suggested that adamantanes might bind to a peripheral, membrane-exposed site that potentially stabilises the closed form of channel complexes, as proposed for M2.[80,127] This notion gathered considerable momentum when, in the absence of a channel structure, *de novo* molecular models of the p7 channel complex were employed as templates for *in silico* compound docking studies.[63] These predicted adamantane interactions at a peripheral cavity, which comprised several of the C-terminal leucines and was formed by residues from both adjacent protomers and the N-terminal TMD. Intriguingly, the predicted site contained Leu20, which was previously observed to undergo a non-synonymous Leu20Phe change in non-responsive genotype