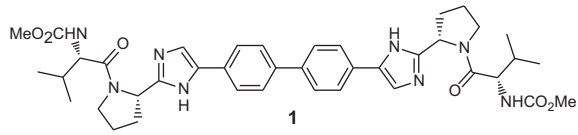

Figure 1.1 Daclatasvir.

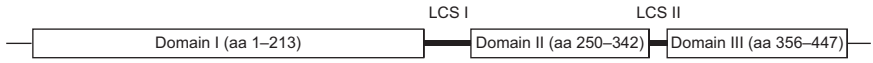

Figure 1.2 NS5A protein organization.

biochemical pharmacology of the NS5A protein, along with the discovery, the mode of action and the clinical characterization of a potent class of NS5A inhibitors, are discussed in this chapter.

1.2 The HCV NS5A Protein

The HCV RNA genome encodes a ~3000 amino acid polypeptide that is processed by both viral and cellular proteases into structural proteins (Core, E1 and E2), an ion channel (p7) and non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B).[2] The non-structural proteins are responsible for replication of the viral genome and for the assembly of the viral particle from the structural proteins, with the assistance of host factors. HCV NS5A is a 447 residue peptide that is comprised of three domains, which are interlinked with short fragments designated as low-complexity sequences (LCSs) (Figure 1.2).[3] Various studies have demonstrated that NS5A is an RNA-binding protein, although the specific elements of the protein that are establishing the biologically relevant interactions with ribonucleic acid still need to be identified.[4–6] For example, one study has indicated that all three domains of NS5A exhibit RNA-binding properties, albeit with differential affinities, whereas a different study showed that the Domain I/LCS I peptide fragment exhibited RNA-binding affinity that is comparable to that of the full-length NS5A protein, supported by the observation that the RNA-binding property of NS5A is abolished if Domain I is deleted.[4,5] Whatever their specific RNA-binding properties may be, all three domains contribute to genome replication, while Domain III plays a key role in viral particle assembly.[7,8] In addition, all three domains play a diverse set of regulatory roles in modulating host–virus interactions so as to facilitate the establishment of an environment conducive to successful viral replication.

Domain I of NS5A is a Zn^{2+}-binding moiety with an amphipathic α-helix at its N-terminal that is believed to anchor the protein to cellular membranes. X-ray structural studies by two independent groups on similar amino acid constructs of Domain I, both lacking the amphipathic α-helix motif, revealed that the protein crystallizes as a homodimer (Figure 1.3).[9,10] Interestingly, although the monomeric units in the two X-ray studies were highly structurally