that, for the Rice dimer, the amino acids decorate the positively charged groove of the protein and not the similarly charged back side of the dimer, whereas for the Love dimer, a ribbon pattern surrounding the structure is observed. It was claimed that this cross-linking result is more consistent with Rice's dimer. Moreover, others have hypothesized that the groove in the Rice dimer may serve as an 'RNA railway system' that connects different functional states that the RNA has to traverse, along with providing a role of protecting the viral RNA from cellular factors that degrade exogenous RNA.[12] Whatever the case may be, the fact that highly potent NS5A inhibitors with resistance mutations that map to Domain I constitute a dimeric pharmacophore that complements the symmetrical features revealed by the X-ray studies and supported by biochemical studies, is unlikely to be coincidental (see below).

Unlike Domain I, Domains II and III are disordered proteins that lack secondary structural elements.[13,14] It is hypothesized that disordered proteins have an extended surface area that promotes simultaneous interactions with multiple proteins and/or an interaction with RNA, which could be a reflection of the multifunctional nature of these domains, the details of which still need to be delineated.[15]

## 1.3 The Discovery of HCV NS5A Replication Complex Inhibitors

HCV replicons, cell-based assay systems that support the autonomous replication of the subgenomic and genomic HCV, have played a central role in the HCV drug discovery field since the introduction of the first genotype 1b (G-1b) system in 1999, most notably in creating opportunities to exploit the potential of viral targets devoid of enzymatic functions.[16,17] As part of a campaign directed at identifying inhibitors of HCV that act by novel mechanisms to disrupt replication, scientists at Bristol-Myers Squibb (BMS) devised a unique, dual-replicon assay system that was used to conduct a phenotype-based, high-throughput screening (HTS) campaign.[18] Specifically, this assay system utilized a mixture of a G-1b HCV replicon and a replicon of a closely related virus, bovine viral diarrhea virus (BVDV), in the same well. The two replicons had the same Huh-7 cellular background but orthogonal activity reporters – a FRET assay based on NS3 protease activity for HCV and a luciferase expression assay for BDVD. In addition, cell toxicity was assessed in the same well using a standard Alamar Blue assay. It is noteworthy that since a luciferase enzyme assay is more sensitive than a FRET assay, this reporter combination placed a stringent criterion for the identification of HCV-specific inhibitors. The BMS compound collection was screened with this dual replicon assay system and initial hits that had either cytotoxic properties or poor HCV specificity, as reflected by a <10-fold potency spread between HCV and BVDV inhibitory activities, were discarded. Counter-screening of the resultant hit set with NS3 protease, NS3 helicase and NS5B polymerase enzymatic assays afforded a thiazolidinone chemotype, exemplified by carbamate **2**, as a novel class of HCV