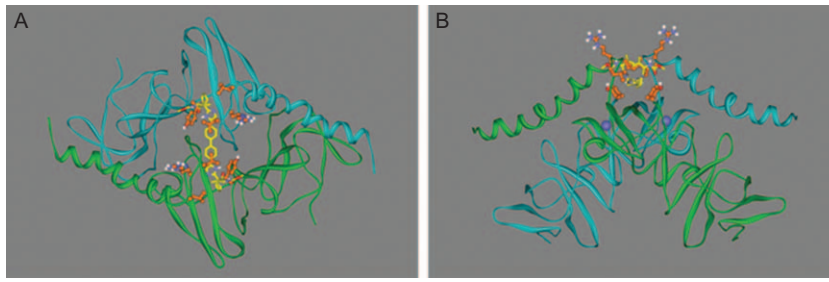

Figure 1.13 Model of daclatasvir (**1**) bound within the NS5A Domain I dimer, with alternative views. The monomers are colored either blue or green and the carbons of daclatasvir are yellow. The position and color of the carbon atoms of the residues associated with primary clinical resistance mutations (amino acids 28, 30, 31, 93) are noted in orange.

Additional evidence that further corroborated the importance of the N-terminal region of Domain I of NS5A as a potential site of interaction for inhibitors of interest was obtained from chimeric replicon studies conducted using compounds with differing inhibitory activities.[19] For example, compound **3**, which inhibits a G-1b replicon with an EC_{50} of 0.018 μM but is devoid of activity in a G-1a replicon ($EC_{50} > 10$ μM), exhibited an enhanced inhibitory activity ($EC_{50} = 0.032$ μM) when tested in a hybrid G-1a replicon in which the first 76 amino acids of its NS5A protein were replaced with the corresponding G-1b sequence. (The difference in the G-1b EC_{50} reported for **3** in different sections (i.e., 18 nM *versus* 6 nM) is a result of assay variations between studies.) Conversely, replacement of the first 76 amino acids of the NS5A region of a G-1b replicon with the corresponding G-1a sequence resulted in a decrease in the inhibitory potency of **3** (EC_{50} of >10 μM). The fact that the inhibitor sensitivity domain of NS5A overlaps with the region that resistance mutations map to is consistent with the direct engagement of NS5A with inhibitors.

A diastereomeric pair of biotin-tagged analogs with differential inhibitory potencies was used in an NS5A pull-down experiment to provide evidence for a direct and specific binding interaction between NS5A and its putative inhibitors.[1] Specifically, NS5A was selectively pulled down when a G-1b replicon was incubated with the biotinylated inhibitor **50a** ($EC_{50} = 33$ nM) (Figure 1.14), lysed and passed over streptavidin–agarose beads, but not when the replicon was lysed prior to treatment with the biotinylated compound. This result signifies that a specific conformation of NS5A is needed for a productive interaction with an inhibitor and is accessible only in a cellular context, presumably in the virus replication complex, which is consistent with a report that NS5A inhibitors failed to bind to the isolated protein.[89] More importantly, in control experiments conducted in parallel, it was observed that little HCV NS5A was pulled down by the inactive stereoisomer **50b** ($EC_{50} > 10$ μM). Moreover, only NS5A, and not NS3 or NS5B, was detected in the bound