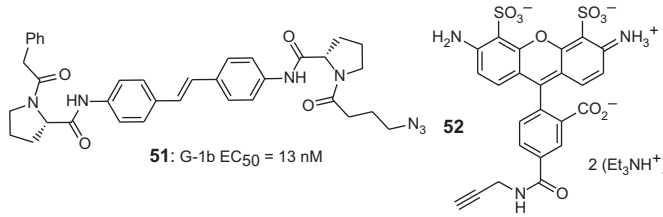

Figure 1.15 Click chemistry substrates for NS5A co-localization study.

inhibitor (**51**) and an alkyne-containing fluorophore (**52**) demonstrated the colocalization of an NS5A replication complex inhibitor with the NS5A protein (Figure 1.15).[91]

The unusually high *in vitro* and *in vivo* inhibitory potency of the NS5A replication complex inhibitor class, coupled with the absence of clearly defined functions of the NS5A protein and of inhibitor binding data for the purified protein, has catalyzed numerous mode of action studies. Although considerable advances have been made, much remains to be discovered in order to illuminate further the complex set of processes that NS5A appears to orchestrate during the HCV replication cycle.

1.7 Conclusion

The complex but ill-defined role of NS5A in the HCV replication cycle continues to drive the curiosity of investigators across academia and industry. Although NS5A was not among the initial targets of choice in the HCV drug discovery campaigns that started over 15 years ago, the pioneering work by Bristol-Myers Squibb scientists in this field has culminated in the discovery of daclatasvir (**1**). The key to success included devising a unique dual-assay screening system that identified a single hit from a collection of over one million compounds; defining aspects of the instability of one of the lead compounds that uncovered significantly active dimeric degradants; simplifying the dimeric pharmacophore into a progressible lead; and successfully optimizing a chemotype with a molecular footprint that is outside of what is considered to be traditional drug-like space. Daclatasvir (**1**) established clinical proof-of-concept for the HCV NS5A protein as a therapeutic target and set a potency benchmark for the HCV field. In addition, as part of a DAA combination, it demonstrated for the first time that a PEG-IFN/RBV-free regimen could cure HCV infection. The clinical validation of NS5A has made it an attractive target for therapeutic intervention, as evidenced by the significant number of patent filings and the growing number of NS5A-targeting compounds entering clinical trials. It is anticipated that NS5A replication complex inhibitors will become integral components of the more effective HCV combination therapies that are expected to emerge in the near future. The genesis of Bristol-Myers Squibb's NS5A drug discovery effort that enabled this successful endeavor is a clear testament to the