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HCV replication in the whole cell replicon assay; however, it was also shown that the uridine triphosphate was a potent inhibitor of the HCV polymerase and that it had a long intracellular half-life. The long intracellular half-life was expected potentially to translate into a once-a-day dosing regimen. Further study revealed that the uridine nucleoside was not a substrate for deoxycytidine kinase needed to convert the uridine nucleoside to the 5′-monophosphate, but that the uridine 5′-monophosphate (**18**) was converted to the active triphosphate by mono- and diphosphate kinases.[45,46] To overcome this non-productive monophosphorylation step and take advantage of first-pass metabolism for liver targeting, a phosphoramidate prodrug strategy was implemented. Execution of this strategy ultimately led to a series of 2′-fluoro-2′-*C*-methyluridine 5′-phosphoramidates that demonstrated potent anti-HCV activity in the whole cell replicon assay, produced high levels of active triphosphate in primary human, rat, dog and monkey hepatocytes and high liver levels of triphosphate when administered orally to rats, dogs and monkeys.[44] Care was also taken in the choice of promoiety substituents to minimize any potential toxicity, as there were concerns regarding the nature of the phenolic substituent and its ultimate release upon prodrug decomposition. The diastereomeric mixture phosphoramidate prodrug having isopropylalanate and phenol substituents, PSI-7851, was chosen as the clinical development candidate and in a Phase 1 human clinical study demonstrated proof of concept by reducing HCV RNA levels in a dose-dependent manner with a maximum mean change of $-1.95 \log_{10}$ IU mL^{-1} after qd dosing at 400 mg over 3 days.[13,47] It was also observed that little circulating prodrug was present, indicating rapid conversion of the prodrug and, coupled with the efficacy data, supported the objective of achieving a high liver-to-plasma ratio. Subsequent clinical studies with the more potent pure *S*p diastereomer PSI-7977 (GS-7977, sofosbuvir) (**19**, Figure 12.11) combined with ribavirin produced a sustained virological response (SVR) in HCV genotype 2- and 3-infected patients after 12 weeks of therapy.[4,48] The metabolic pathway for release of the promoiety was elucidated and involved carboxylesterase or cathepsin A hydrolysis of the terminal amino acid ester followed by loss of the phenol and final removal of the amino acid by histidine triad nucleotide-binding protein 1 (HINT-1).[47] Sofosbuvir is currently in Phase 3 clinical studies for the treatment of HCV in all patient populations.

The development of 2′-fluoro-2′-*C*-methyl- and 2′-*C*-methylguanosine nucleotides for the treatment of HCV infection also has benefited from the use of a prodrug strategy employing phosphoramidate technology.[4] In addition, several of these guanosine nucleotide derivatives relied on a double prodrug approach that combines a phosphoramidate promoiety with an enzymatically cleavable C6 substituent on a 2-aminopurine that functions as a masked guanine base. The purpose of the C6-purine substituent is to mask the polar nature of the base unit and increase lipophilicity to improve transport across biological membranes. This strategy of masking the nature of a guanine base has been used previously in the nucleoside field, for example, in the case of abacavir.[49–51] With regard to the C6-substituted purine HCV nucleotide prodrugs, studies demonstrated that the metabolic pathway proceeded first via