A third member of the guanosine class of phosphoramidate prodrug HCV polymerase inhibitors is represented by PSI-353661 (**24**, Figure 12.13).[59] Like BMS-986094, PSI-353661 also possesses the double prodrug feature where the C6-position of the purine base is substituted with a methoxy group. PSI-353661 is differentiated from BMS-98094 and IDX-184 by the presence of a 2′-fluoro substituent, the phosphoramidate substitution and the fact that it is a single phosphoramidate diastereomer. Like the other guanosine prodrugs, it demonstrated a favorable *in vitro* stability profile and ability to produce high levels of intracellular triphosphate in hepatocytes.[65] A study using radio-labeled prodrug was able to show a favorable 3.5:1 and 4.5:1 liver to plasma ratio of triphosphate and triphosphate precursor metabolites at 1 and 6 h, respectively, after oral administration, thus supporting the liver-targeting potential of the prodrug.[59] PSI-353661 has not yet entered clinical development.

Another unique prodrug construct that was used to leverage the potential of the 2′-fluoro-2′-*C*-methylguanosine triphosphate is the 3′,5′-cyclic phosphate. Although 3′,5′-cyclic phosphates had been investigated for the delivery of nucleoside 5′-monophosphates, none were shown to be effective in a human clinical setting.[66–68] The first 3′,5′-cyclic phosphate prodrug to show clinical proof of concept in the delivery of a nucleotide was PSI-352938 (**25**, Figure 12.13).[69,70] PSI-352938 is a 3′,5′-cyclic phosphate of 2′-fluoro-2′-*C*-methyl-6-ethoxy-4-aminopurine and consequently employs the double prodrug approach by masking the C6 position of the purine base. Mechanistic studies showed that the metabolic pathway for release of the cyclic phosphate promoiety employed a CYP3A4-mediated oxidative cleavage of the isopropyl ester followed by phosphodiesterase cleavage of the 3′ phosphate–oxygen bond.[71] It was shown that once the 5′-monophosphate was revealed, then cleavage of the C6-ethoxyl group proceeded *via* an ADAL-1 mediated hydrolysis to give the 2′-fluoro-2′-*C*-methylguanosine 5′-monophosphate. Liver targeting of PSI-352938 was enabled by the existence of the CYP3A4 oxidative cleavage of the isopropyl phosphate ester, since CYP enzymes are highly concentrated in the liver. In both rat and dog, high liver levels of the active nucleoside triphosphate were detected after oral dosing. However, unlike with the phosphoramidate prodrug approach, rapid first-pass metabolism was not observed with 3′,5′-cyclic phosphate prodrugs.[69] When administered orally to animals or humans, PSI-352938 exhibited high circulating levels of intact prodrug.[72] In a Phase 1 7-day multiple ascending dose human clinical study in HCV-infected patients, doses of PSI-352938 at 100, 200 and 300 mg qd resulted in a mean viral load decline of 4.31, 4.65 and 3.94$\log_{10}$ IU mL–1, respectively and with no adverse side effects.[73] These results demonstrated the first clinical proof of concept study using a 3′,5′-cyclic phosphate prodrug strategy to deliver a nucleotide. A 14-day study combining PSI-352938 with PSI-7977 to assess the potential for an interferon-free regimen also resulted in dramatic declines in viral load (4.6–5.5$\log_{10}$ IU mL^{-1}).[74] However, a subsequent extended human clinical study resulted in ALT elevations in patients taking the drug, leading to the suspension of clinical trials. No mechanistic rationale was