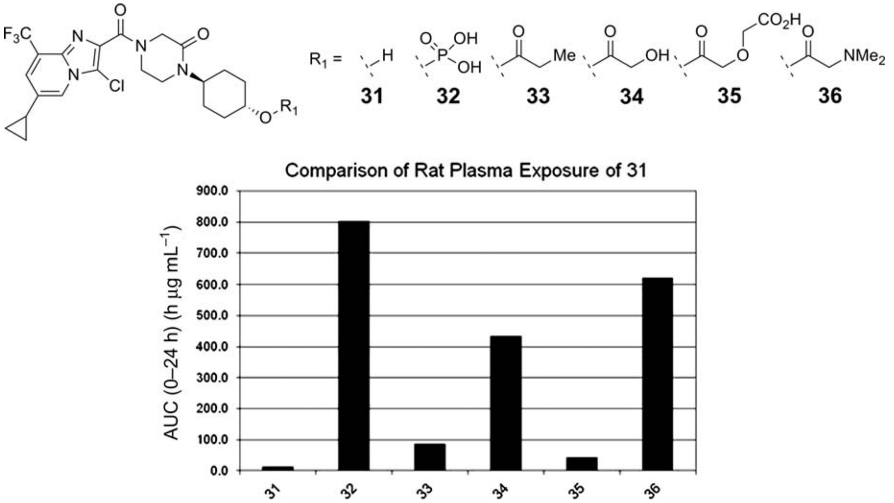

Figure 5.10 Plasma drug exposures of 31 in rat following p.o. administration of 300 mg kg^{-1} of 31 or prodrugs 32–36.

A diverse set of esters were examined to determine the effects of polarity and ionic character on high-dose oral PK. The prodrugs **32**–**36** were dosed at 300 mg kg^{-1} (p.o.) in rats and the plasma concentrations of **31** were determined (Figure 5.10).[37] The phosphate prodrug **32** outperformed the ester prodrugs, achieving the highest plasma exposures of **31** with no evidence of systemic exposure for the uncleaved prodrug. The phosphate prodrug also formed a stable, crystalline solid and was selected for all forthcoming *in vivo* studies.

The high plasma exposure achieved with prodrug **32** enabled the progression of **31** into rat 7 day safety studies. Unfortunately, an adverse cardiovascular finding was identified that led to its termination. In addition, *in vitro* resistance passaging studies identified several single-point mutations in the NS4B protein (positions 94 and 105) that render the HCV replicons partially resistant to **31**.[38] A backup effort was initiated to address these limitations: we aimed to eliminate the need for a prodrug and to improve the viral resistance profile. A significant number of analogs (>1500) had already been prepared and profiled in the imidazopyridine series and, therefore, we chose to pursue new chemical directions.

Recalling that the pyrazolopyridine **8** possessed slightly improved potency relative to the imidazopyridine **7** (Figure 5.4), we re-examined isosteric replacements to the imidazopyridine core in combination with highly optimized tail fragments. Our initial aim was the syntheses of the pyrazolo[1,5-*a*]pyridine **37**, *N*-methylbenzimidazole **38**, and benzofuran **39** cores in combination with the 4-hydroxycyclohexylpiperazinone (**37**–**39a**) and the spirocyclic ketal (**37-39b**) tail fragments (Table 5.3).[37] Stable genotype 1b replicons bearing the H94N mutation in NS4B were created to allow the development of SARs. The three cores (**37**–**39**) with either of the tail subunits (**a** or **b**) showed comparable or improved potency relative to the imidazopyridine core (**24** and **31**). Activity