patient population, thereby improving the efficacy of HCV therapy by reducing the likelihood of developing resistance and rapid viral rebound.[73]

Even though this generation of benzothiadiazine derivatives was successfully optimized to provide analogs with exquisite cell-based potency in gt1a and 1b replicons and some analogs (*e.g.*, **48**) exhibited favorable pharmacokinetic profiles in preclinical species, others suffered from extremely poor aqueous solubility, complicating formulation development and compromising further *in vivo* evaluation. A strategy was therefore developed to address this issue, which entailed replacing the ring nitrogen of analogs such as **48** with a quaternary carbon center to reduce planarity. This core modification was expected to minimize π-stacking of molecules in the solid state, reducing crystallinity and thus improving solubility. The relationship between topology and increased sp^3 character of molecules and improved solubility and ADME properties is nowadays a well-established paradigm within the medicinal chemistry community.[74] Encouraging initial results were obtained with more synthetically accessible achiral vinylogous acid derivatives such as **51** (Figure 8.12) that demonstrated the viability of this approach in terms of gt1a and 1b replicon potency, pharmacokinetic profile in rats (low clearance and high bioavailability) and plasma-to-liver distribution (maximum liver/plasma ratio = 48 at 6 h).[75a]

Chemistry was subsequently developed to access synthetically more challenging unsymmetric derivatives and investigate substitution patterns that would provide optimal interactions within the benzothiadiazine binding pocket and also antiviral potency in the replicon assays. The effort produced racemic and subsequently enantiomerically pure *R*-isomers of analogs **52** (A-837093) and **53** (A-848837) that exhibited excellent single-digit nanomolar potency.[75b] The corresponding *S*-distomers were 500–2500-fold less potent in biochemical assays. In the presence of human serum, EC_{50} values shifted 14–17-fold, compared with >50-fold for aza analogs such as **50**. While the effect on solubility of introducing a quaternary carbon center in the scaffold was somewhat ambiguous depending on pH and solvent, a significant decrease in melting point was observed for the free form of **53**, suggesting weaker association of molecules within the crystalline lattice.

Both analogs **52** and **53** were profiled *in vivo*. In the rat, isoamyl analog **52** suffered from a short elimination half-life (1.2 h). A cannulated bile duct experiment indicated that biliary excretion was the major route of excretion and hydroxylated derivative **54** was identified as the main metabolite in this species.[75b] The sodium salt of *tert*-butyl analog **53**, on the other hand, displayed an improved i.v. profile with a 3–4-fold improvement in clearance and elimination half-life in rats (4.5 h). Prolonged exposure was observed following oral administration, presumably as a consequence of the low solubility of the precipitated form of the neutralized species in the acidic environment of the stomach. The liver distribution was similar for both compounds, with liver/plasma ratio ~20 at 12 h in rats. In higher species, *in vivo* parameters correlated well for both compounds with *in vitro* clearance data obtained in microsomes and hepatocytes. Overall, analog **53** is more potent than **52** and both compounds exhibited attractive pharmacokinetic profiles (**53** featuring