to design novel molecules with improved properties. Macrocyclization is a well-established approach to improving potency (both intrinsic and cell-based) of inhibitors and enhancing the drug-like properties of molecules.[45] This approach has already borne fruit in the structure-based design of successful HCV protease drug candidates, as described in chapter 10 of this book. In the particular case of the Tibotec NS5B inhibitors, focus was on exploring carboxylic acid bio-isosteres, introducing PK-enhancing substituents in solvent-exposed areas while avoiding zwitterionic species and exploiting conformational rigidification to improve potency. These objectives were partially fulfilled with inhibitors in which a 13-atom bridge was installed that linked the C6 position of the indole scaffold to the *ortho*-position of an aromatic ring at the C2 position (**30**). This 20-membered ring macrocycle, containing an acylsulfamide link to the indole core, exhibited potency comparable to that of previously described indole-*N*-acetamides (*e.g.*, **14**) and tetracyclic derivatives (*e.g.*, **21**) with $IC_{50} = 0.11\ \mu M$ and $EC_{50} = 0.19\ \mu M$. This class of derivatives had good distribution to the liver in rats; however, early analogs suffered from suboptimal PK properties in rodents, such as short half-life ($t_{\frac{1}{2}} = 1$ h) and low bioavailability (14%).[44a]

These deficiencies were addressed in a subsequent round of design which led to the discovery of **29**. In this phase of the optimization process, pre-orientation of the C2 indole substituent in the bioactive conformation using previously described tetracyclic analogs was exploited to provide analogs with increased overall rigidity. The introduction of a bridge in the tetracyclic indole series proved to be beneficial, as a twofold improvement in cell-based potency was generally observed compared with previous macrocycles. The structural features present in the bridge had a strong impact on the *in vivo* preclinical profiles of these molecules. Compound **29** (TMC647055), with an unsaturated amide bridge, provided the best overall profile. The compound inhibited the 1a/1b replicons with $EC_{50} = 74$–166 nM, depending on assay readout (luciferase or qRT-PCR),[46] and had improved clearance, liver distribution (liver/plasma = 46 in rats) and bioavailability (66% in rats). The X-ray structure of a macrocyclic analog bound to NS5B revealed the presence of additional protein residues interacting with the molecule compared with previous structures, providing a rationale for the improved intrinsic potency.[44c] Despite the deviation of these molecules from what are generally considered drug-like features (*e.g.*, high molecular weight and clog*P*), the macrocyclic structures designed by the Tibotec group feature improved PK behavior relative to some previously described series of inhibitors. The antiviral activity of TMC647055 in 5 day monotherapy was studied in gt1a/1b HCV patients. Doses of 500 or 1000 mg bid produced median maximum decrease in HCV RNA of $3.3\log_{10}$ and $3.4\log_{10}$ in 1b patients. In 1a genotypes, the effect was dose dependent ($1.4\log_{10}$ and $2.4\log_{10}$ reductions in HCV RNA depending on the dose). These results support further development of TMC647055 for the treatment of hepatitis C.[46]

In summary, thumb pocket 1 allosteric NS5B inhibitors, discovered jointly by Japan Tobacco and Boehringer Ingelheim, have evolved over the years from benzimidazole-5-carboxylic acids, which exhibited modest potency in cell