microbial oxidation by *Sebekia benihana* significantly diminished the immunosuppressive potential by >100-fold while also increasing CypA binding affinity 1–2-fold.[96,109,110] Several additional CsA analogs bearing alternative groups at position 4 have been prepared,[96,104,109–113] although the three most distinct examples of abolishing immunosuppressive potential without loss of Cyp binding are the three cited above.

Replacing the amide proton at the nitrogen of [Val]^5 with an allyl or benzyl group was found to attenuate immunosuppressive potential by >100-fold while substantially maintaining CypA binding affinity.[29] Replacing [Val]^5 altogether with a pseudo-proline residue that cyclically tethered the amino acid side chain to the amide nitrogen introduced a *cis*-amide bond between the 4- and 5-residues and caused a greater reduction in immunosuppressive activity than Cyp binding.[115]

Amino acid 6, [MeLeu]^6, appears also to be crucial for CaN binding. Replacing [MeLeu]^6 with [MeVal] causes a 46-fold decrease in immunosuppressivity but only a threefold decrease in Cyp binding.[2] Replacement of [MeLeu]^6 with Ala, Abu, Ile or Phe were also shown to be more detrimental to immunosuppressive activity than to Cyp binding.[95]

Lastly, amino acid 7, [Ala]^7, also showed that it could be used to tune down immunosuppressive potential yet retain Cyp binding. Replacing [Ala]^7 with Val, Ser, Thr or Gly all caused significantly greater reductions in immunosuppressive activity than Cyp binding.[104]

Derivatives bearing modifications on the residues at the interface of Cyp and CaN binding domains, residues 3 and 8, yield more heterogeneous data regarding Cyp and CaN binding. A substituent on the α-face of [Sar]^3, resulting in a D-amino acid, typically retains and occasionally improves Cyp binding while also showing some attenuated immunosuppression.[79,95,96] Regarding the 8-position, [D-Ser]^{8}CsA derivatives have typically shown Cyp binding that parallels immunosuppressive activity,[120,121] whereas other compounds bearing alkylamines of varying length in place of the Ala methyl group showed a decrease in immunosuppressive potential without a significant reduction in Cyp binding as the chain length increased.[122] The D-stereochemistry of the 8-position substituent appears to be important: [dehydro-Ala]^{8}CsA, lacking a stereogenic center at the 2′-α-carbon, exhibits a 3.75-fold reduction in Cyp binding affinity, but is completely non-immunosuppressive.[111]

Beyond this, it is known that the MeBmt side chain drapes over the cavity of cyclosporine when bound to Cyp and consequently modifying this MeBmt side chain at the terminus that approaches CaN can influence CaN binding. [MeThiaBmt]^{1}CsA (**8**, Figure 11.8), which possesses a thiomethyl bioisosteric replacement of the $CH_3CH{=}CH{-}$MeBmt terminus, exhibits almost a twofold increase in Cyp binding affinity, yet only 9.5% of the immunosuppressive activity of CsA.[95] Other compounds possessing extended alkyl functionality beyond the 8′-position, as found in ISA247 (**9**, Figure 11.8) and related compounds, still possess immunosuppressive activity.[126–128]

Clearly, attenuation of CaN inhibition, and consequent immunosuppressive potential, can also be achieved by various combinations of the modifications