As mentioned previously, CsA residues 4, 5, 6 and 7 reside in the CaN binding domain; hence, structural modifications to these residues likely affect Cyp binding indirectly. At the 4-position, [4′-HOMeLeu]^{4}CsA has been measured to bind to Cyp comparably to CsA[96] or even twofold better than CsA,[109] while [MeVal]^{4}CsA and [MeIle]^{4}CsA have been shown to have comparable Cyp binding affinity to CsA itself.[70,72,104] Some modifications cause a drop in Cyp binding, such as the rigid [Pro]^{4}CsA, which has no detectable Cyp affinity within 20-fold of that of CsA.[104] Other examples of small alkyl groups, such as methyl, ethyl and CH_2-*c*-Pr, show Cyp affinity within threefold of CsA,[96,110,111] or greater affinity, as shown with *sec*-butyl.[112] Lastly, it is noted that the *N*-methyl group of MeLeu can be replaced with the more metabolically stable *N*-ethyl group without detriment to Cyp binding.[113]

Amino acid replacement at [Val]^5 generally causes a decrease in Cyp binding affinity, even for relatively modest changes such as [Ala]^{5}CsA or [4-F-Val]^{5}CsA.[95,104,114,115] Notably, however, alkylation of the nitrogen of [Val]^5 with a substituted allyl or benzyl group retains Cyp binding while diminishing CaN binding.[29]

Modifications at positions 6 and 7 rely on significant synthetic efforts and have been less well studied. Minor amino acid changes cause a minor decrease in Cyp binding affinity,[17,88,96] while introduction of larger or more rigid residues such as proline cause a greater decrease in binding affinity.[104,116,117] A 'tricyclic CsA' in which a bicyclic dipeptide mimic replaces [Ala]^7-[D-Ala]^8, shows threefold greater affinity for CypA and two- to threefold greater immunosuppressive activity than CsA.[118,119]

At the 8-position, we reach the interface between Cyp and CaN binding domains and can now potentially influence Cyp binding by a direct interaction between the modified side chain and Cyp rather than merely indirectly by conformational bias, as seen with modifications to residues 4–7. It has been shown that [D-Ser]^{8}CsA, [D-Thr]^{8}CsA, [D-Lys]^{8}CsA and [Boc-D-Lys]^{8}CsA each have a Cyp binding affinity identical with that of CsA.[96] In addition, [D-Gln]^{8}CsA and [D-Asn]^{8}CsA are also reported to have a Cyp binding activity identical with that of CsA, while the *N*-alkylated analogs [D-MeAla]^{8}CsA and [D-Pro]^{8}CsA show a 2.5- and 5-fold decrease in Cyp binding, respectively and interestingly [Sar]^{8}CsA shows an 8-fold decrease in Cyp binding, underscoring the importance of D-substitution at this residue.[104] Beyond this, sizeable side chains have been tethered to both [D-Ser]^{8}CsA[120,121] and [D-Lys]^{8}CsA[95,122] that can tune down CaN binding without an appreciable decrease in Cyp binding. As is the case with [Ala]^7, the nitrogen of [D-Ala]^8 has also been alkylated to prepare Cyp-binding conjugates.[123,124]

The 9-, 10- and 11-positions that reside in the Cyp binding domain are much more difficult to replace or modify synthetically and there are fewer literature reports of Cyp binding data for such compounds; nevertheless, it is clear that modification in this section of CsA can have a profound affect on Cyp binding. Both [MeAla]^{9}CsA and [MePhe]^{9}CsA have moderately reduced affinity for Cyp,[95,96] whereas [Pro]^{9}CsA has no detectable Cyp binding affinity.[104] At the 10-position, replacing [MeLeu]^{10} with MeAla or MePhe also reduces Cyp