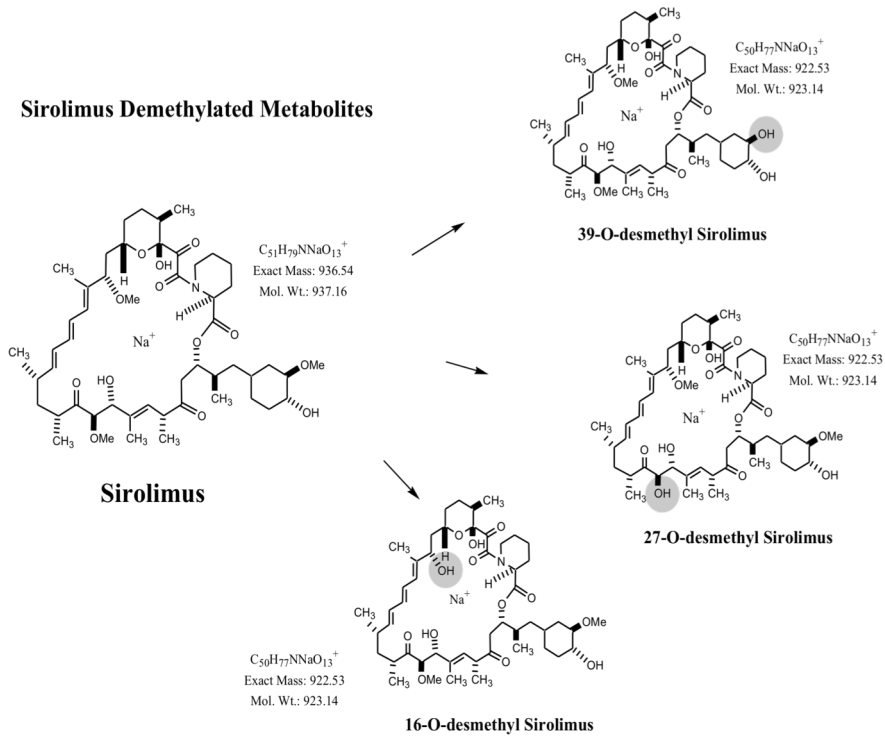
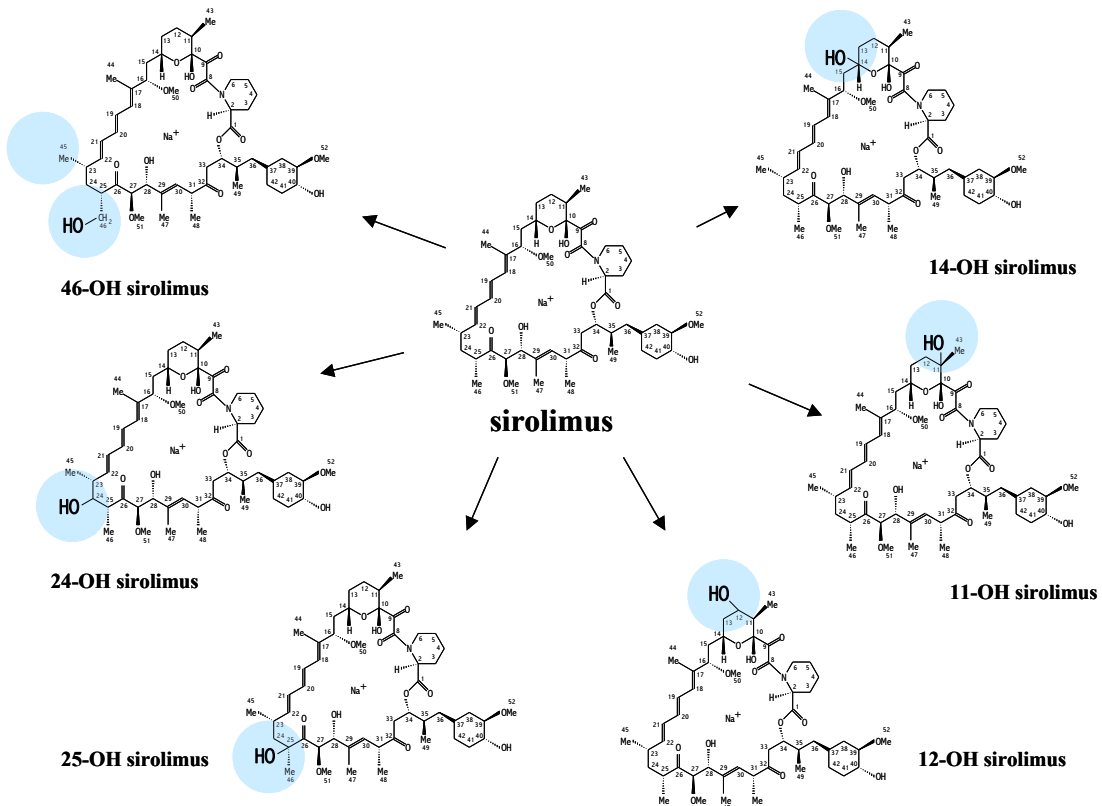


1.15 Purified and Certified Immunosuppressant Metabolites

Sirolimus Demethylated Metabolites



Sirolimus hydroxylated metabolites



Sirolimus

Atom numbering follows the IUPAC nomenclature

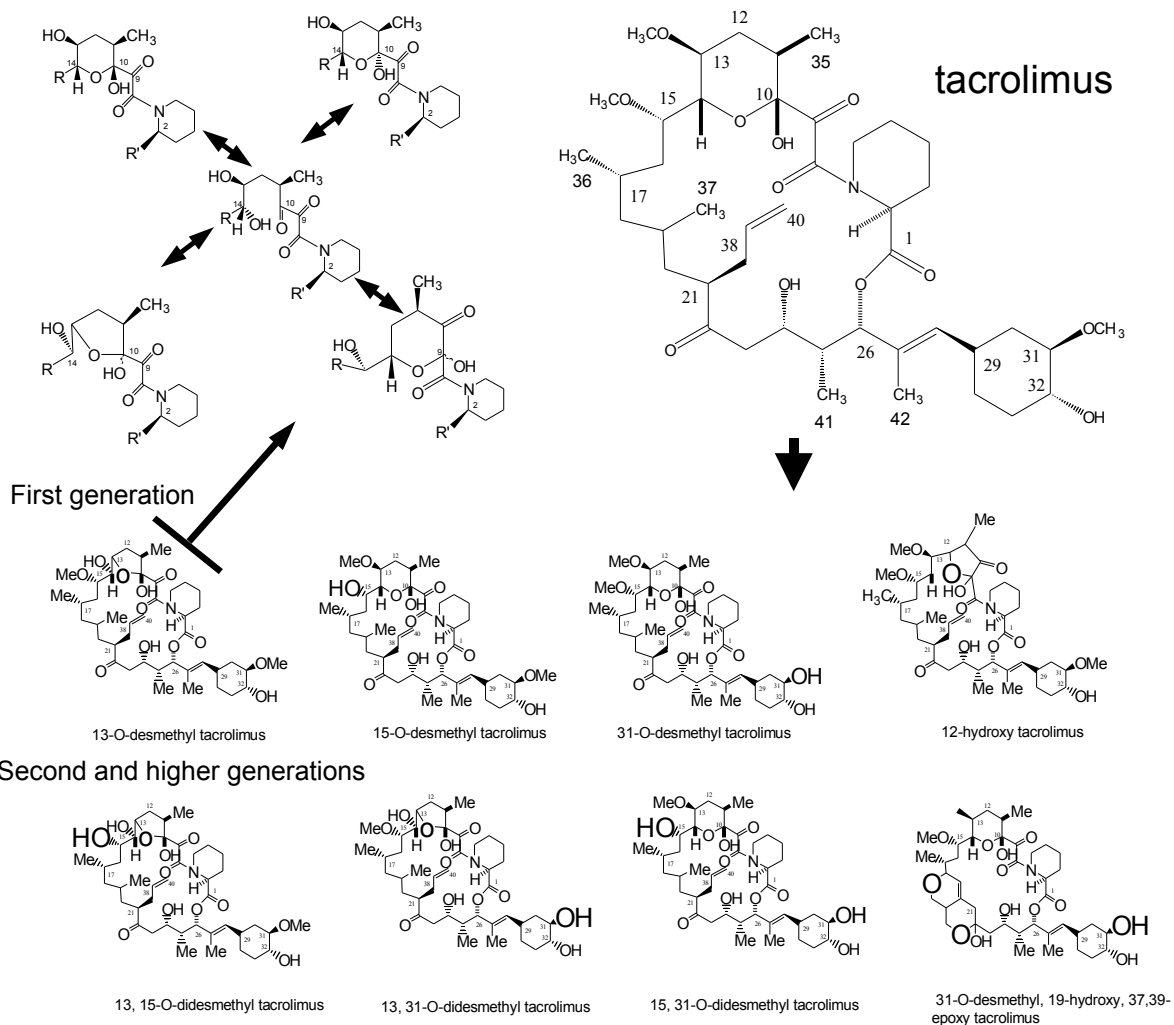
All 10-50 µg/ batch

- 12-hydroxy sirolimus
 - 24-hydroxy sirolimus
 - 25-hydroxy sirolimus
 - 46-hydroxy sirolimus
 - Piperidine hydroxy sirolimus
 - 49-hydroxy sirolimus
 - 16-O-desmethyl sirolimus
 - 27-O-desmethyl sirolimus
 - 39-O-desmethyl sirolimus
 - Selected didesmethyl sirolimus
- Metabolites

Publications:

1. Christians U, Sattler M, Schiebel HM, Kruse C, Radeke HH, Linck A, Sewing KF. Isolation of two immunosuppressive metabolites after in vitro metabolism of rapamycin. *Drug Metab Dispos* 1992, 20: 186-191
2. Sattler M, Guengerich FP, Yun CH, Christians U, Sewing KF, Cytochrome P4503A enzymes are responsible for biotransformation of FK506 and rapamycin in man and rat. *Drug Metab Dispos* 1992; 20: 753-761
3. Streit F, Christians U, Schiebel HM, Napoli KL, Ernst L, Linck A, Kahan BD, Sewing KF. Sensitive and specific quantification of sirolimus (rapamycin) and its metabolites in blood of kidney graft recipients by HPLC/electrospray-mass spectrometry. *Clin Chem* 1996; 42: 1417-1425
4. Streit F, Christians U, Schiebel HM, Meyer A, Sewing KF. Structural identification of four metabolites of the macrolide immunosuppressant sirolimus after in vitro metabolism by electrospray-MS/MS. *Drug Metab Dispos* 1996; 24: 1272-1278
5. Lampen A, Zhang Y, Hackbarth I, Benet LZ, Sewing KF, Christians U. Metabolism and transport of the macrolide immunosuppressant sirolimus in the small intestine. *J Exp Pharmacol Ther* 1998; 285: 1104-1112.
6. Jacobsen W, Serkova N, Hausen B, Morris RE, Benet LZ, Christians U. Comparison of the in vitro metabolism of the immunosuppressants sirolimus and RAD. *Transplant Proc* 2001; 33: 514-515
7. Filler G, Bendrick-Peart J, Strom T, Zhang YL, Johnson G, Christians U. Characterization of sirolimus metabolites in pediatric solid organ transplant recipients. *Pediatr Transplant*. 2009, 13; 44-53

1.15. Purified and Certified Immunosuppressant Metabolites (continued)



Structure of tacrolimus (A), major metabolic pathways (B), and structural isomers of 13-O-demethyl tacrolimus (C).

Tacrolimus

Atom numbering follows the IUPAC nomenclature

- 13-O-desmethyl tacrolimus: 250-500 µg
- 15-O-desmethyl tacrolimus: 50-100 µg
- 31-O-desmethyl tacrolimus: 50-100 µg
- 12-hydroxy tacrolimus: 10-25 µg
- 13,31-di-O-desmethyl tacrolimus: 10-25 µg
- 15,31-di-O-desmethyl tacrolimus: 10-25 µg
- 13,15-di-O-desmethyl tacrolimus: 10-25 µg
- 31-O-desmethyl and ring formation between C19, C36 and C37: 10-25 µg

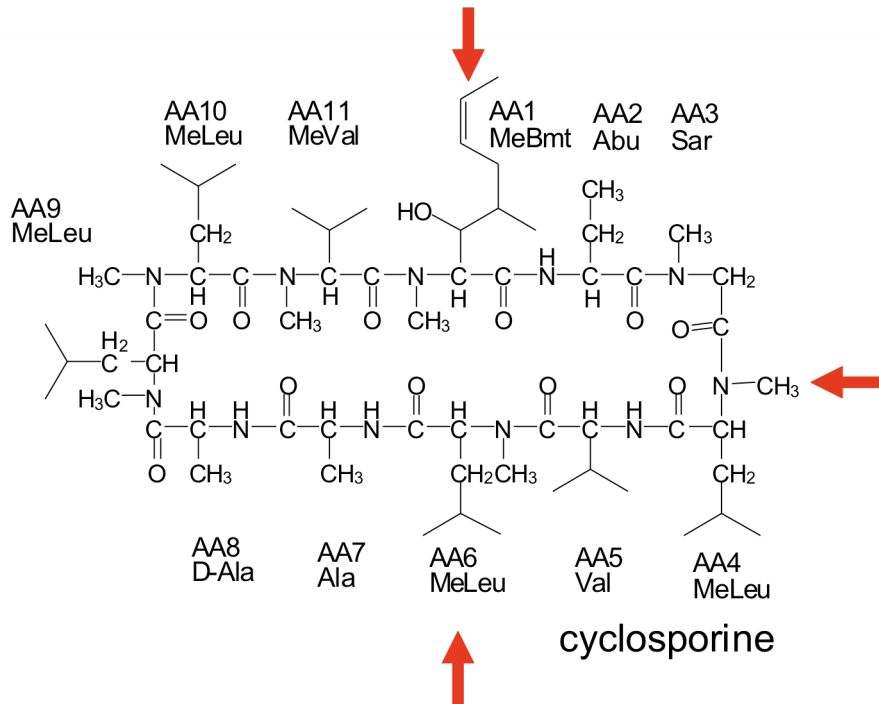
Publications:

1. Christians U, Radeke HH, Kownatzki R, Schottmann R, Sewing KF. Isolation of an immunosuppressive metabolite of FK506. *Clin Biochem* 1991; 24: 271-275
2. Christians U, Braun F, Schmidt M, Kosian N, Schiebel HM, Ernst L, Winkler M, Kruse C, Linck A, Sewing KF. Specific and sensitive measurement of FK506 and its metabolites in blood and urine of liver graft recipients. *Clin Chem* 1992; 38: 2025-2032
3. Sattler M, Guengerich FP, Yun CH, Christians U, Sewing KF, Cytochrome P4503A enzymes are responsible for biotransformation of FK506 and rapamycin in man and rat. *Drug Metab Dispos* 1992; 20: 753-761
4. Schüler W, Christians U, Schmieder P, Schiebel HM, Holze I, Sewing KF, Kessler H. Structural identification of 13-demethyl-FK506 and its isomers generated by in-vitro metabolism of FK506 using human liver microsomes. *Helv Chim Acta* 1993; 76: 2288-2302
5. Lampen A, Christians U, Guengerich FP, Watkins P, Kolars JC, Bader A, Dralle H, Hackbarth I, Sewing KF. Metabolism of the immunosuppressant tacrolimus in the small intestine: cytochrome P450, drug interactions and interindividual variability. *Drug Metab Dispos* 1995; 23: 1315-1324



1.15. Purified and Certified Immunosuppressant Metabolites (continued)

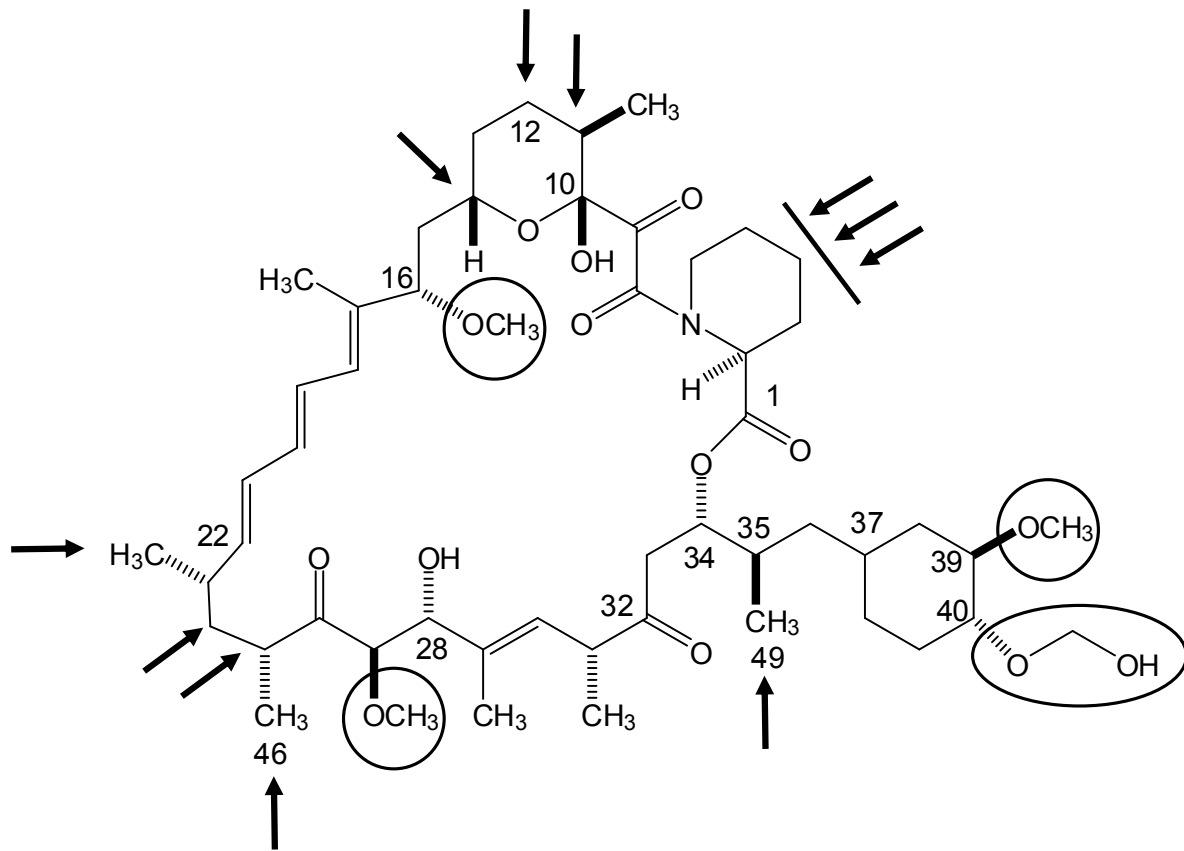
Major Metabolism Sites of Cyclosporine



Publications:

- Christians U, Strohmeyer S, Kownatzki R, Schiebel HM, Bleck JS, Greipel J, Kohlhaw K, Schottmann R, Sewing KF. Investigations on the metabolic pathways of cyclosporine: I. Excretion of cyclosporine and its metabolites in human bile- Isolation of 12 new cyclosporine metabolites. *Xenobiotica*, 21 (1991) 1185-1198
- Christians U, Strohmeyer S, Kownatzki R, Schiebel HM, Bleck J, Kohlhaw K, Schottmann R, Sewing KF. Investigations on the metabolic pathways of cyclosporine: Elucidation of the metabolic pathway in vitro by human liver microsomes. *Xenobiotica*, 21 (1991) 1199-1210
- Christians U, Sewing KF. Cyclosporin metabolism in transplant patients. *Pharmacol Ther*, 57 (1993) 291-345
- Christians U, Sewing KF. Alternative cyclosporine metabolic pathways and toxicity. *Clin Biochem*, 28 (1995) 547-559
- Lampen A, Christians U, Bader A, Hackbarth I, Sewing KF. Drug interactions and interindividual variability of cyclosporin metabolism in the small intestine. *Pharmacology* 52 (1996) 159-168

1.15. Purified and Certified Immunosuppressant Metabolites (continued)



Everolimus

Numbering follows the IUPAC nomenclature, the arrows indicate hydroxylation positions, circles O-dealkylation.

All 10-50 µg/ batch

- 12-hydroxy everolimus
- 24-hydroxy everolimus
- 25-hydroxy everolimus
- 46-hydroxy everolimus
- Piperidine hydroxy everolimus
- 49-hydroxy everolimus
- 16-O-desmethyl everolimus
- 27-O-desmethyl everolimus
- 39-O-desmethyl everolimus
- Selected didesmethyl everolimus metabolites
- Selected dihydroxy everolimus metabolites
- seco everolimus and other selected degradation products

Publications

1. Jacobsen W, Serkova N, Hausen B, Morris RE, Benet LZ, Christians U. Comparison of the in vitro metabolism of the immunosuppressants sirolimus and RAD. *Transplant Proc* 2001; 33: 514-515
2. Strom T, Haschke M, Boyd J, Roberts M, Arabshahi L, Marbach P, Christians U. Cross reactivity of the major everolimus metabolites with the Innofluor Certican immunoassay for therapeutic drug monitoring of everolimus. *Ther Drug Monit* 2007 29:743-749.
3. Strom T, Haschke M, Bendrick-Peart J, Boyd J, Roberts M, Arabshahi L, Marbach P, Christians U. Everolimus metabolite patterns in the blood of kidney transplant patients. *Ther Drug Monit* 2007; 29: 592-599