Novel Oxidation of Cyclosporin A: Preparation of Cyclosporin Methyl Vinyl Ketone (Cs-MVK)

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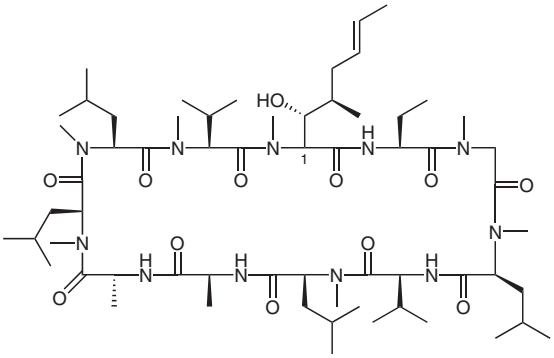
Abstract: Cyclosporin A (CsA) was converted into cyclosporin methyl vinyl ketone (Cs-MVK) by either a biocatalytic method utilizing 1-hydroxybenzotriazole-mediated laccase oxidation or by a chemical oxidation using *t*-butyl hydroperoxide and potassium periodate as co-oxidants. Cs-MVK is a novel, versatile synthetic intermediate that can be used for the preparation of many novel cyclosporin analogues possessing therapeutic potential as immunosuppressive agents.

Key words: cyclosporin A, cyclosporin methyl vinyl ketone, laccase oxidation, allylic oxidation, immunosuppression

Cyclosporin A (**1**; Figure 1), a naturally occurring cyclic peptide isolated from the fungus *Tolypocladium inflatum*,[1] possesses potent immunosuppressive activity and continues to be an important first-line agent prescribed for the prevention of organ transplantation rejection.[2,3] In addition, cyclosporin A and its analogues have demonstrated potential utility in other therapeutic areas, such as psoriasis, asthma and other autoimmune diseases.[3] The reactivity of a variety of biocatalysts on CsA was explored in our laboratory with the goal of introducing functionality onto the CsA scaffold that could lead to novel and medicinally useful CsA analogues.

It has been well established that the side chain of the first amino acid of cyclosporin A [*N*,4-dimethyl-4(*R*)-[2(*E*)-butenyl]-L-threonine (MeBmt, **2**); Figure 2] plays an essential role in the immunosuppressive activity of CsA.[4] The presence of the *trans* carbon–carbon double bond and the hydroxy substituent on the MeBmt side chain makes this a readily accessible part of the molecule for synthetic modification. Therefore, many medicinal chemistry efforts have focused on modification of the side chain of this amino acid.[4–7]

One of the interesting reactions that was found to take place during the biocatalyst screening strategy was the 1-hydroxybenzotriazole (HOBt)-mediated laccase oxidation of CsA **1**. Laccase (EC 1.10.3.2) is characterized as a multi-copper oxidase that can catalyze the oxidation of a range of reducing substances with concomitant reduction of O_2.[8a] The use of laccase in synthesis is not well-known. One of the few published reports indicates that a laccase mediator-assisted oxidation is useful for the transformation of a benzyl methyl group or an allylic alcohol into the corresponding alcohol and/or aldehyde, respectively.[8b]



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cyclosporin A (CsA) is abbreviated as

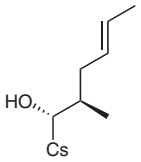

Figure 1 Cyclosporin A (CsA)

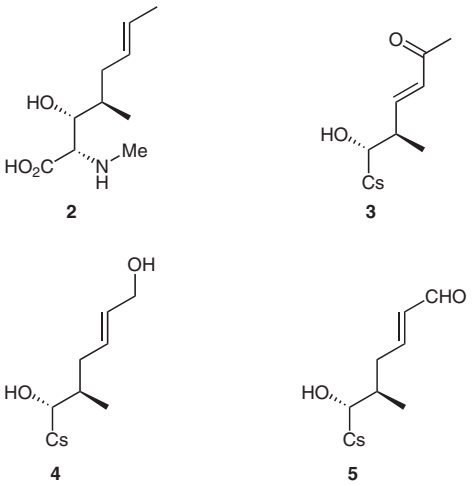

Figure 2 MeBmt (**2**), Cs-MVK (**3**), and other cyclosporin analogues

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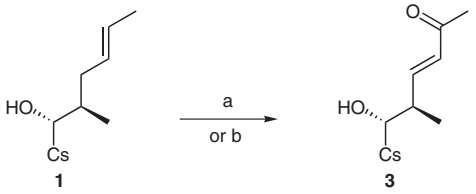
Application of the laccase mediator-assisted oxidation conditions to CsA **1** (Scheme 1) afforded the unexpected cyclosporin methyl vinyl ketone **3** (Cs-MVK) as the major product, instead of the expected products from the allylic oxidation of the terminal methyl group to a Cs-alcohol **4** or aldehyde **5**[9] shown in Figure 2, which were only observed in trace amounts. Cs-MVK **3** is a novel CsA derivative that has neither been reported previously in the literature as an oxidative metabolite from human liver metabolism nor as a reaction product from biocatalytic or chemical reaction.

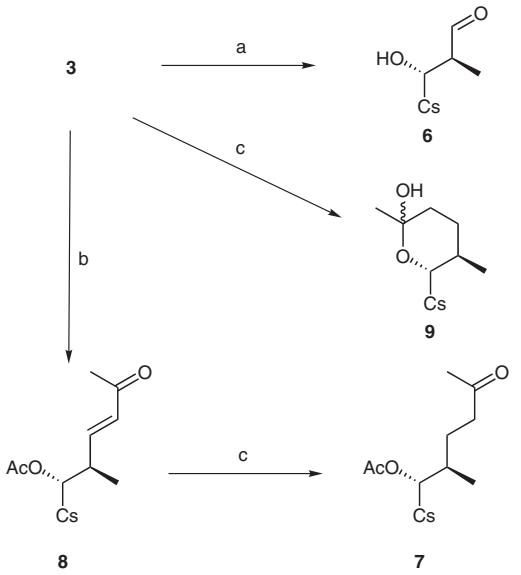


Scheme 1 *Reagents and conditions*: (a) laccase, HOBt, methyl *t*-butyl ether, buffer pH 5.6 (biocatalytic method); (b) 70% *t*-butyl hydroperoxide, KIO_4, 18-crown-6, acetone, benzene, H_2O, r.t., 3 d (chemical method).

The novel structure and potential synthetic utility of Cs-MVK **3** prompted us to look for a more convenient chemical method for the preparation of this compound. A survey of the chemistry literature indicated that co-oxidants have been used successfully to effect oxidation of allylic methyl groups. 1-Hydroxyphthalimide/benzoyl peroxide $(PhCOO)_2$,[10] sodium periodate ($NaIO_4$)/*tert*-butyl hydroperoxide (*t*-BuOOH),[11] and chromium trioxide (CrO_3)/*t*-BuOOH[12] have been successfully applied in the oxidation of the allylic carbon atoms on steroids. However, when CsA **1** was treated under these reaction conditions, only a trace (<5% yield) of the desired Cs-MVK **3** was observed along with unreacted CsA.

Attempts to drive the reaction to completion at higher reaction temperatures led to decomposition of the desired product that formed (Table 1, entries 1 and 3). Treatment of CsA with $NaIO_4$/*t*-BuOOH at room temperature in the presence of a phase-transfer catalyst ($BnNEt_3Cl$, Table 1, entry 4), however, provided a modest improvement (10% yield). Variation of the co-oxidizing agents led to the finding that potassium periodate (KIO_4)/*t*-BuOOH with 18-crown-6 as the phase-transfer catalyst in a solvent system of benzene–acetone–water (1:1:1) for three days at room temperature was optimal (Scheme 1, Table 1, entry 5). This reaction was run several times on a 5 g scale under the conditions described (Table 1, entry 5) to provide the desired product in yields consistently in the 50–70% range.[9]

The precise mechanism of the oxidation of cyclosporin A by the HOBt-mediated laccase oxidation or under the KIO_4/*t*-BuOOH/18-crown-6 system remains unclear; however, the synthetic utility of the novel Cs-MVK **3** was readily demonstrated (Scheme 2). The novel Cs-aldehyde **6** was prepared in excellent yield by ozonolysis of Cs-MVK **3** followed by reductive workup. Acetylation of the secondary alcohol of Cs-MVK **3** followed by catalytic hydrogenation of the double bond afforded the saturated Cs-ketone **7**. Direct hydrogenation of compound **3** resulted in the formation of the stable Cs-cyclic hemi-ketal **9**.

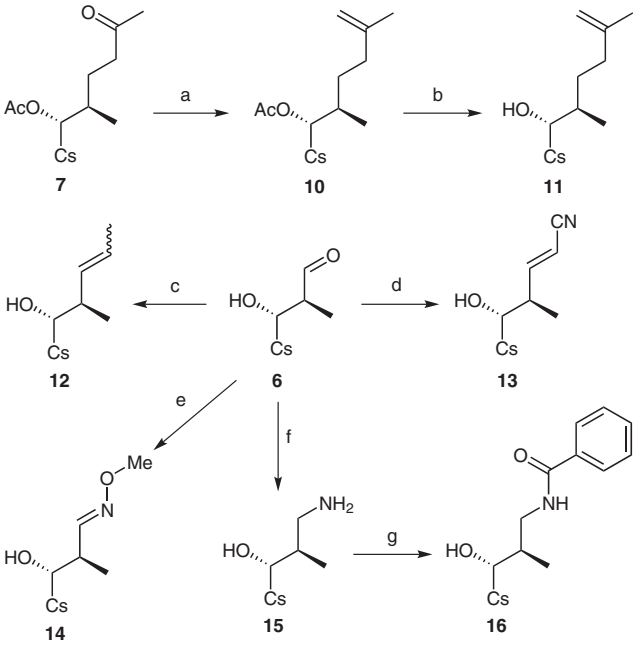


Scheme 2 Reagents and conditions: (a) O_3, CH_2Cl_2, –78 °C, 20 min, then Me_2S, –78 °C to r.t., 90%; (b) Ac_2O, pyridine, DMAP, CH_2Cl_2, r.t., 95%; (c) H_2 (30 psi), 10% Pd/C, MeOH, r.t., 85%.

Both Cs-aldehyde **6** and Cs-ketone **7** were further transformed into novel cyclosporin analogues as outlined in Scheme 3. Treatment of Cs-ketone **7** with ten equivalents of Wittig reagent prepared from methyltriphenylphosphonium bromide and sodium bis(trimethylsilyl)amide, provided Cs-alkene **10**, which was deacylated under mild basic conditions (potassium carbonate/methanol) to give the novel analogue **11**.

Table 1 Oxidation of Cyclosporin A **1** to Cs-MVK **3**

Entry	Co-oxidants	Conditions	Yield of **3** (%)
1	1-hydroxyphthalimide/$(PhCO_2)_2$	acetone, reflux, 24 h	<5
2	$NaIO_4$/*t*-BuOOH	acetone, r.t., 3 d	<5
3	$NaIO_4$/*t*-BuOOH	acetone, 50 °C, 12 h	decomposed
4	$NaIO_4$/*t*-BuOOH	$BnNEt_3Cl$, benzene, acetone, H_2O, r.t., 3 d	10
5	KIO_4/*t*-BuOOH	18-crown-6, benzene, acetone, H_2O, r.t., 3 d	50–70



Scheme 3 *Reagents and conditions*: (a) CH_3PPh_3Br, NaHMDS, THF, 0 °C, 30 min, 12%; (b) K_2CO_3, MeOH, r.t., 12 h, 35%; (c) $MeCH_2PPh_3Br$, NaHMDS, THF, 0 °C, 30 min, 30%; (d) $(EtO)_2P(O)CH_2CN$, NaHMDS, THF, 0 °C, 30 min, 49%; (e) $MeONH_2$·HCl, pyridine, MeOH, r.t., 20%; (f) NH_4OAc, $NaBH_3CN$, HOAc, MeOH, r.t., 20%; (g) benzoyl chloride, pyridine, CH_2Cl_2, r.t., 26%.

Similarly, a mixture of *cis*/*trans*-alkenes **12** resulted from a Wittig reaction between the Cs-aldehyde **6** and an excess of ethylidene triphenylphosphorane generated in situ. Horner–Emmons reaction of Cs-aldehyde **6** and diethyl cyanomethylphosphonate using sodium bis(trimethylsilyl)amide as base afforded Cs-α,β-unsaturated nitrile **13** exclusively as the *trans*-alkene.

In addition, treatment of Cs-aldehyde **6** with *O*-methylhydroxylamine gave the oxime **14**, and reductive amination of the same aldehyde led to the preparation of a novel Cs-amine derivative **15**, which was benzoylated to give the Cs-amide **16**.

Novel cyclosporin analogs were tested for activity in a one-way murine mixed lymphocyte reaction (MLR) assay. The MLR assay is designed to measure ^{3}H-thymidine uptake by murine splenocytes that are undergoing cell proliferation in an immune response to allogeneic stimulation.[13] The Cs-MVK **3** and the Cs-cyclic hemi-ketal **9** retained significant IC_{50} values (310 and 160 ng/mL, respectively) but attenuated inhibition of ^{3}H-thymidine uptake relative to CsA **1** (IC_{50} values of 14 and 16 ng/mL, respectively), which is run as a positive control in the assay.

In conclusion, an HOBt-mediated laccase oxidation of CsA **1** produced the novel derivative Cs-MVK **3** in good yield. Efforts undertaken to identify a chemical oxidation method with which to synthesize Cs-MVK **3** led to identification of a co-oxidizing system, (KIO_4)/*t*-BuOOH with 18-crown-6 in benzene–acetone–water (1:1:1) that afforded the desired product in good yield. Cs-MVK **3** has been shown to be a synthetically useful intermediate that was transformed into several new cyclosporin analogues via standard methodology. The discovery of this chemistry provides a facile approach to a variety of novel cyclosporin derivatives from commercially available CsA that were not readily accessible prior to the existence of this methodology.

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(9) Biocatalytic Method: Cyclosporin A (1.0 g) and 1-hydroxybenzotriazole (500 mg) were dissolved in *tert*-butanol (70 mL) in a 500 mL reaction vessel equipped with a stir bar. Sodium citrate/sodium phosphate buffer (80 mM, 250 mL, pH 5.6) was added while stirring, resulting in a thick white suspension. Laccase C (1.8 g, ASA Spezialenzyme) was added as a solution in 35.5 mL of the same buffer, turning the reaction mixture slightly yellow in appearance. The reaction was mechanically stirred enough to create a vortex, open to ambient atmosphere at room temperature for a period of 20 h, after which time the reaction mixture became orange in appearance. After removing a portion of the *tert*-butanol via rotavapor, the orange reaction mixture was loaded onto a pre-conditioned VARIAN Bond-Elut® C8 solid-phase extraction cartridge (60 cc, 10 g of sorbent). After a wash with water, the cyclosporin-related products were eluted using acetonitrile. The acetonitrile eluate was concentrated in vacuo, and the residue was transferred to a tared scintillation vial and dried in vacuo inside a Savant dryer to provide 913 mg of crude product as tan solids. The solids were re-dissolved in a minimal volume of acetonitrile and purified by reversed-phase semi-prep chromatography to provide 551 mg of CsA-MVK.
Chemical Method: Cyclosporin A (5 g, 4.2 mmol) was dissolved in acetone (25 mL), benzene (25 mL) and H_2O (25 mL). *tert*-Butyl hydroperoxide (31.25 mL of 70% aqueous solution, 258 mmol), potassium periodate (6.5 g, 28.3 mmol), and 18-crown-6 (4.38 g, 16.5 mmol) were added to the reaction mixture at room temperature. The resulting mixture was stirred vigorously at room temperature under N_2

atmosphere for 3 d. Organic solvents were removed from the reaction mixture in vacuo. The remaining mixture was poured into ice-water (1 L) and extracted twice with a mixture of EtOAc–hexanes (200 mL, 1:1). The combined extracts were stirred in a 10% sodium sulfite solution for 2 h. The organic layer was separated, dried over Na_2SO_4 and concentrated. The crude product was purified by either preparative or semi-preparative HPLC, using acetonitrile (containing 0.05% TFA)/water (containing 0.05% TFA) solvent system, to provide CsA-MVK (2.5–3.5 g, 50–70%) as light-yellow solid.

Analytical Data of **3**: ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.03 (d, *J* = 9.9 Hz, 1 H), 7.79 (d, *J* = 7.8 Hz, 1 H), 7.44 (d, *J* = 8.0 Hz, 1 H), 7.13 (d, *J* = 8.0 Hz, 1 H), 6.89 (dd, *J* = 16.1, 7.6 Hz, 1 H), 6.06 (d, *J* = 16.1 Hz, 1 H), 5.71 (dd, *J* = 11.0, 3.8 Hz, 1 H), 5.65 (br s, 1 H), 5.22 (dd, *J* = 11.5, 3.8 Hz, 1 H), 5.10 (d, *J* = 11.0 Hz, 2 H), 5.05 (dd, *J* = 15.7, 9.1 Hz, 1 H), 4.96 (dd, *J* = 10.1, 5.7 Hz, 1 H), 4.85 (q, *J* = 7.2 Hz, 1 H), 4.73 (d, *J* = 14.1 Hz, 1 H), 4.65 (q, *J* = 8.7 Hz, 1 H), 4.55 (q, *J* = 7.4 Hz, 1 H), 4.04 (br s, 2 H), 3.52 (s, 3 H), 3.39 (s, 3 H), 3.31 (s, 3 H), 3.20 (d, *J* = 13.9 Hz, 1 H), 3.12 (s, 3 H), 3.11 (s, 3 H), 2.72 (s, 3 H), 2.68 (s, 3 H), 2.54–2.34 (m, 3 H), 2.26 (s, 3 H), 2.20–1.76 (m, 11 H), 1.75–1.35 (m, 6 H), 1.32 (d, *J* = 7.3 Hz, 3 H), 1.26 (d, *J* = 7.3 Hz, 3 H), 1.10–0.81 (m, 39 H); ^{13}C NMR (90 MHz, $CDCl_3$): δ = 198.6, 174.7, 174.1 (2 C), 173.8, 171.7, 171.6, 171.3, 170.6, 170.5, 170.3, 170.2, 148.9, 131.8, 74.9, 59.6, 58.2, 57.8, 55.7 (2 C), 55.2, 50.6, 48.9, 48.6, 48.3, 45.3, 40.7, 39.7, 39.5, 39.2, 38.0, 36.2, 35.0, 31.7, 31.6, 31.3, 30.1 (2 C), 29.8, 29.6, 27.5, 25.3, 25.1, 24.9 (2 C), 24.6, 24.0 (4 C), 23.8, 23.6, 22.0 (2 C), 21.3, 20.8, 20.0, 18.8 (2 C), 18.6, 18.4, 16.1; MS (ESI): *m*/*z* = 1216.

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(13) The murine system uses the H2 disparate inbred mouse strains: Balb/c ($H2^d$) and C57B1/6 ($H2^b$). Splenocytes of the C57B1/6 mice are γ-irradiated so as to act as stimulators of an immune response from the splenocytes from the Balb/c mice. IC_{50} values are the concentration of test compound that inhibit ^{3}H-thymidine uptake by 50% relative to control cells and are determined from 7 point concentration-response curves using GraphPad software.