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A SEMI-SYNTHETIC APPROACH TO OLEFINIC ANALOGS OF AMINO ACID ONE (MeBMT) IN CYCLOSPORIN A

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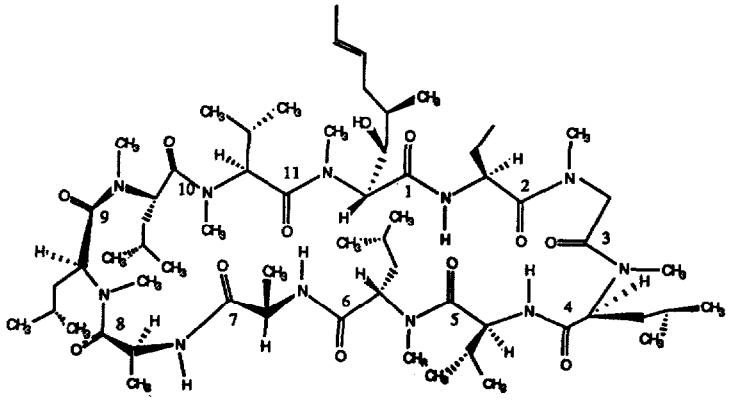
Abstract: The syntheses of four olefinic analogs of Cyclosporin A at amino acid one (MeBMT), a *residue critical for the Cyclophilin binding domain, are reported. The synthetic process is a rapid,* general, semi-synthetic sequence involving oxidative degradation of the olefinic sidechain followed by a Wittig olefination step.

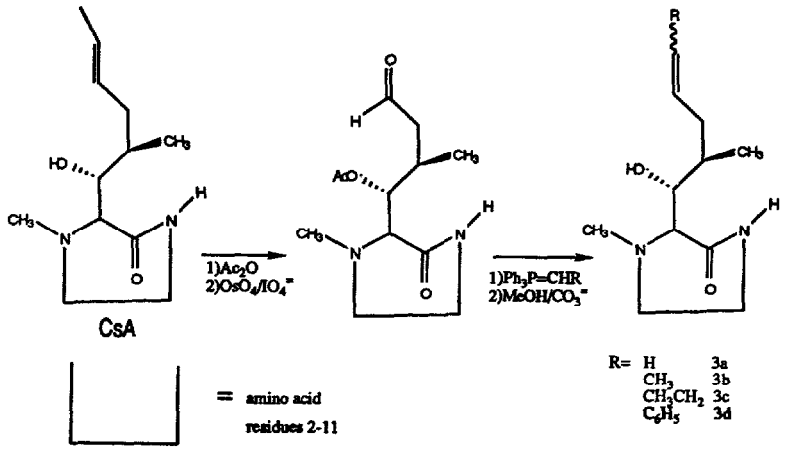
Cyclosporin A (CsA) - a cyclic, neutral, hydrophobic undecapeptide - is one of a small class of pharmacologically active agents with selective immunosuppressive activity.[1] The immunosuppressive activity of CsA is exhibited in the selective inhibition of clonal expansion of helper and cytotoxic T-cells, with only partial inhibition of the suppressor T-cells. As a result of this selectivity, CsA is currently used clinically for transplantation of kidney, bone marrow, liver, pancreas, heart, lung, and corneas.[2] In addition, CsA has been shown to have clinical indications for various autoimmune diseases.[3] The ultimate clinical use of CsA is limited, however, primarily due to nephro- and hepatotoxicity.[4]



Cyclosporin A (CsA)

The immunosuppressive activity of CsA may be linked to its ability to bind to a small (approx. 17 kD) cytosolic protein that has been designated cyclophilin.[5] More recently, cyclophilin has been shown to be the same as peptidyl-prolyl *cis-trans* isomerase (PPIase), a ubiquitous protein found in most cell types and in a wide variety of species.[6] Classical structure-activity-relationship (SAR) studies, limited primarily to naturally occurring and to *de novo* synthesized CsA analogs, have indicated that amino acid residues 1, 2, 3, 10, and 11 of CsA are critically involved in the cyclophilin binding domain[5], while residues 4-9 appear to be required for the proper tertiary structure (the "scaffolding domain").[7] These binding affinities to cyclophilin have been correlated to the immunological activity and aprotic solution structure of CsA analogs, except for analogs modified at residue 6 which appear to display anomalous behavior.[5b] Thus, both of these domains are required for the immunological selectivity and potency of CsA. However, it is the binding domain that most directly interacts with cyclophilin and rational modifications of CsA in this region should allow construction of a model of the interactions between CsA and cyclophilin in the binding domain. Thus, new cyclosporin analogs modified at residues 1, 2, 3, 10, or 11 would aid in mapping the binding domain of CsA and might prove to be useful clinical agents. We report herein the semi-synthesis of a series of olefinic analogs of CsA modified at residue 1.

Since amino acid 1 of CsA contains the only carbon-carbon double bond and hydroxyl group in the molecule, the synthetic strategy shown in scheme 1 was developed.



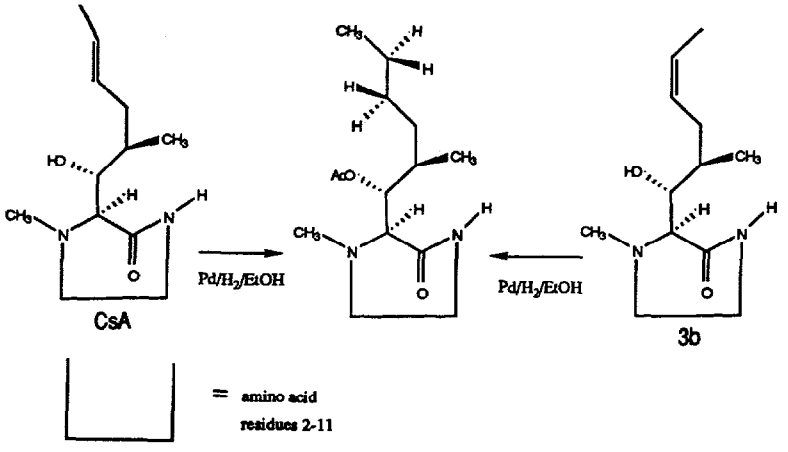
SCHEME I

Formation of the protected aldehyde was readily accomplished by acylation of the C-3 hydroxyl group of residue one with either acetic or (4-bromophenoxy)-acetic anhydride (pyr./DMAP/24°C: 97-100%) followed by treatment with sodium metaperiodate and catalytic osmium tetroxide (3 dioxane/1 H_2O: 86-90%). In agreement with work reported by Wenger *et al.*, attempts at acylation with various acyl halides were unsuccessful.[1]

Olefination and hydrolysis of the ester was readily accomplished in a two step process. The appropriate Wittig ylides (20 equiv.) were formed via Corey's dimsyl sodium procedure[8] and added to the aldehyde in THF at 0°C (0°C/2 hrs) to give the olefins. Purification (reverse phase HPLC) of the

crude product gave the olefins in the following yields: 3a: 34-40%; (Z)-3b: 40-43%; (Z)-3c: 32-39%; (Z)-3d: 19-21% and (E)-3d: 10-13% (Z:E=6:4). Various alternative approaches to the olefination were attempted. Direct oxidation of CsA leads to a diastereomeric mixture of tetrahydrofuran hemi-acetals, which gave reduced yields upon attempted methylenation. In addition, methylenation with methylide generated either by reaction of the phosphonium salt with ^{n}BuLi or under salt-free conditions gave a non-homogeneous reaction (-78 to 0°C) with decreased yields.[9] Deprotection was readily accomplished by treatment of the ester analogs with catalytic potassium carbonate and methanol under anhydrous conditions (reflux/4 hrs) to give the analogs (72 to 78%).

The structures of these olefin analogs were verified by ^{1}H- and ^{13}C-NMR spectroscopy (C_6D_6) as well as by FAB mass spectroscopy. The proton spectrum of the aldehyde-acetate showed an aldehyde resonance at δ9.94 (1H, dd, J=3.8, 0.5 Hz)[10] and an acetate methyl signal at δ1.96. The products from olefination did not show an aldehyde signal, but did show the appropriate vinyl C-H resonances (3a: δ5.96, 5.20, 5.07; (Z)3b: δ5.69, 5.47; (Z)3c: δ5.52, 5.54; (E)3d: δ6.53, 6.37 J=15.7 Hz; and (Z)3d: δ6.73, 6.03 J=12.2 Hz). It is also of interest to note that the introduction of variations in the olefinic site does not appear to significantly modify the conformation of the peptide, as indicted by the similarity of the CsA and olefinic analog amide N-H resonances.[11] Since it was possible that the conditions of the Wittig reaction could racemize the αprotons of the amino acid residues, we needed to prove unambiguously that epimerization had not occurred. In order to achieve this, a sample of CsA (as received) and our semi-synthetic olefinic isomer of CsA, "Z"-CsA (3b), were each hydrogenated (H_2/EtOH/Pd) to give the dihydro-product (Scheme II). Since these compounds were expected to be olefinic isomers of each other, they should result in the same product: the products of the reduction were shown to be identical by RP-HPLC, FAB mass spectroscopy, as well as by ^{1}H- and ^{13}C-NMR spectroscopy.



SCHEME II

We are currently studying the biological activities of these analogs and will report these results and full details of the synthesis of the analogs in a full paper.

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9 The formation of nonhomogeneous reaction mixtures from reactions of CsA with strong bases has been noted by Seebach *et al.* (D. Seebach: *Angew. Chem. Int. Ed. Engl.* 1988, 27:1624-1654) and the addition of LiX has been shown to improve both the solubility of the poly-anion and the yields of anion reactions: Addition of LiCl did not improve our Wittig reactions in either manner.

10 The protons alpha to the aldehyde are diastereotopic and observed the coupling constants suggest that the aldehyde does not freely rotate.

11 The amide N-H resonances are extremely sensitive indicators of the conformation of the peptide: in CsA there are only 4 N-H resonances (no rotomers) in benzene at 24°C but in other analogs rotomers have been observed: See reference 7 and J. D. Aebi, D. Guillaume, B. E. Dunlap, and D. H. Rich: *J. Med. Chem.* 1988, 32:9, 1805-1815.

12 Preliminary results from mouse thymocyte proliferation assays indicate that 3a, b, and c are equipotent with CsA and that (E)-3d is approximately 400 times less potent while (Z)-3d is 10^3 fold less potent.

13 After this work had been completed, it was brought to our attention that Sandoz had developed a similar process (ozonation instead of osymlation/periodate cleavage) and reported compounds 3b, c, and d in European Patent 0296122. We thank the reviewer for pointing this out to us.

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