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Review

Semi-synthesis of cyclosporins ☆

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ABSTRACT

Background: Since its isolation in 1970, and discovery of its potent inhibitory activity on T-cell proliferation, cyclosporin A (CsA) has been shown to play a significant role in diverse fields of biology. Furthermore, chemical modification of CsA has led to analogs with distinct biological activities associated with its protein receptor family, cyclophilins.
Scope of review: This review systematically collates the synthetic chemistry performed at each of the eleven amino acids, and provides examples of the utility of such transformations. The various modifications of CsA are traced from early, modest chemistry performed at the unique Bmt residue, through the remarkable use of a polyanion enolate that can be stereoselectively manipulated, and onto application of more recently developed olefin metathesis chemistry to prepare new CsA derivatives with unexpected biological activity.
Major conclusions: The myriad biological activities of CsA and its synthetic derivatives have inspired the development of new approaches to modify the CsA ring. In turn, these new CsA derivatives have served as tools in the discovery of new roles for cyclophilins.
General significance: This review provides information on the types of cyclosporin derivatives that are available to the many biologists working in this field, and should be of value to the medicinal chemist trying to discover drugs based on CsA. This article is part of a Special Issue entitled Proline-directed foldases: Cell signaling catalysts and drug targets.

1. Introduction

1.1. Structure of Cyclosporins

Cyclosporin (aka cyclosporin A, cyclosporine, cyclosporine A, ciclosporin, ciclosporin A, or CsA, **1**) is a cyclic undecapeptide first isolated from the fungus *Tolypocladium inflatum* in 1970 [1]. Its importance to medicinal chemistry has been eloquently stated by Jean Bernard: "Ciclosporin, the object of so many hopes, the source of so much promise for the future, stands at the crossroads of two great lines of research that were opened up a century ago by Pasteur: the study of drugs and agents for combating infection, and the study of the natural defenses, of immunity." [2] Throughout the literature, the structure of CsA, first elucidated in 1976 [3–5], is typically drawn as some variant of that shown in Fig. 1, with each amino acid numbered 1 through 11 in a clockwise manner starting at 12 o'clock. Ten of the eleven amino acids are known, or derivatives of known amino acids, with several having branched lipophilic sidechains; however, the amino acid at position one, Bmt, or (4*R*)-4-[(*E*)-2-butenyl]-4-methyl-L-threonine is previously unknown. All of the chiral amino acids have the natural L configuration except for [D-Ala][8], and seven of the 11 amide nitrogens are capped with a methyl group.

Cyclosporin adopts different conformations depending on the polarity of its environment [6]. In crystalline form, and in non-polar solvents such as chloroform or THF, the hydrogens on the four uncapped amide nitrogens form intramolecular hydrogen bonds with the carbonyl oxygens of other amides within the molecule (represented as dashed lines in Fig. 1). In this conformation residues 1–6 adopt an antiparallel β-pleated sheet conformation, while residues 7–11 form an open loop featuring a *cis* amide bond between [MeLeu][9] and [MeLeu][10] with the result that the compound maximally presents its hydrophobic side chains for interaction with solvent [7]. It is notable that this stable conformation is achieved by CsA and close analogs; however, many synthetic derivatives of CsA disrupt this internal hydrogen bonding network and exist as multiple conformers in nonpolar solvent. Examples include derivatives that possess an L-substituent at [Sar][3] [8,9], and derivatives that are *N*-substituted at the [Val][5] nitrogen [10]. This has a consequence on the biological activity of CsA analogs that will be discussed.

In polar solvent such as DMSO, water, or THF charged with LiCl, this intramolecular hydrogen bonding network is disrupted, which results in significant changes to the conformation of the molecule. The amide bond between [MeLeu][9] and [MeLeu][10] now adopts a *trans* conformation [11], and the [MeBmt][1]/[Abu][2] amide bond becomes exposed and available for hydrogen bonding. CsA resides in this conformation

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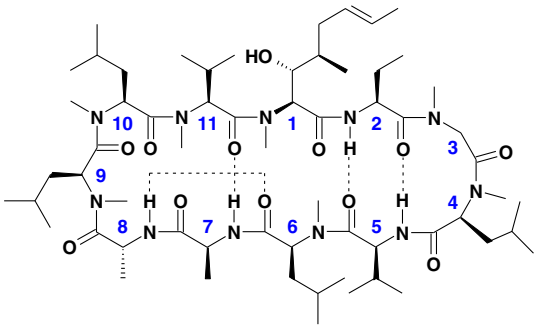

Fig. 1. The structure of cyclosporin A.

when bound to cyclophilin. In addition, it has been shown that temperature can influence CsA conformational structure in polar solvent [12].

Beyond cyclosporin A, there are, to date, at least 37 additional structurally similar cyclic undecapeptides that are considered part of the cyclosporin family [13–17]. The discovery and isolation of each of these cyclosporins has been reviewed elsewhere [13,14,18–21].

1.2. Biological activity of cyclosporins

Cyclosporins exhibit a broad spectrum of biological activities which can be applied toward the treatment of a wide variety of diseases [13]. CsA is a powerful immunosuppressant, and is the active ingredient of drugs used to prevent organ transplant rejection (Sandimmune® and Neoral®), dry eye disease (Restatis®), and atopic dermatitis in companion animals (Atopica®). In addition, CsA has been investigated in several other autoimmune disorders including psoriasis [22], endogenous uveitis [23], autoimmune urticaria [24], rheumatoid arthritis [25], myasthenia gravis [26], aplastic anemia [27], nephrotic syndrome [28], and Crohn's disease [29].

CsA exerts its immunosuppressive activity by binding to two proteins sequentially to form a ternary complex [30–33]. The first of these is a cyclophilin (Cyp), which is a *cis-trans* proline isomerase, of which there now at least 20 known paralogs in human, with the most predominant being cyclophilin A (CypA) [34]. The binary CsA–CypA complex is a potent inhibitor of the phosphatase activity expressed by calcineurin (CN), a calcium-dependent serine/threonine phosphatase that promotes the synthesis of T cell lymphokines such as interleukin-2 (IL-2) [35,36]. Thus, CN inhibition ultimately suppresses immune response [35,36].

X-ray crystallography has revealed which residues of CsA bind to CypA and CN, respectively [30,32]. Fig. 2 shows that residues 9, 10, 11, 1, and 2 form the 'cyclophilin binding domain' that binds to CypA, while residues 4, 5, 6, and 7 comprise the 'calcineurin binding domain' that binds to CN. Residues 3 and 8 are at interfaces between these two binding domains, and can potentially have an impact of both CypA and CN binding.

In addition to the immune diseases discussed above that can be addressed by CN inhibition, cyclosporin derivatives that only inhibit Cyp, without effects on CN, have attracted a great deal of recent attention due to their activity against human immunodeficiency virus 1 (HIV-1) [37] and HCV [38–41]. Reports describing a role for CypD inhibition as an approach to protect mitochondria has led to studies of CsA and non-CN inhibiting CsA derivatives in both acute and chronic degenerative diseases [42,43]. In such cases in which Cyp inhibition alone is sought, it is preferred to use a drug that does not bind CN and is therefore non-immunosuppressive, particularly when combating a viral disease in which functional immune response is needed. Such selective inhibition of Cyps without inhibition of CN can be achieved by careful modification of the CsA scaffold.

The purpose of this review is to provide a summary of synthetic organic chemistry that has been used to prepare cyclosporin analogs through semi-synthesis. To that end, this review will navigate around the circumference of cyclosporin, exploring key synthetic chemistry that has been developed at each of its 11 amino acids, one at a time, starting with MeBmt.

2. Semi-synthesis of cyclosporins

2.1. [MeBmt]^1

The amino acid at the cyclosporin 1-position, methyl (4*R*)-4-[(*E*)-2-butenyl]-4-methyl-L-threonine (MeBmt, Fig. 3), is the largest amino acid on the cyclosporin scaffold, and is the only one to possess a heteroatom and a C═C double bond on the sidechain. Consequently, MeBmt has been synthetically derivitized more than any other amino acid on cyclosporin. Reactions of MeBmt can be subdivided between reactions of 3′-OH, reactions of the C6′═C7′ double bond, and reactions of the terminal carbon C8′.

2.1.1. Reactions of 3′-OH

The MeBmt 3′-OH is the only heteroatom substituent on any of the 11 amino acids of cyclosporin, and can undergo a variety of reactions including acylation (intramolecular or intermolecular), etherification (intramolecular or intermolecular), oxidation, and substitution. These transformations are summarized in Fig. 4.

Treating CsA with a strong acid such as methanesulfonic acid induces an intramolecular acyl transfer from the 2-nitrogen to the 3-oxygen (Scheme 1) [3,44–47]. The resulting equilibrium lies toward the ester product, isocyclosporin (isoCsA), since the 2-nitrogen is protonated and rendered non-nucleophilic in acidic medium.

This migration becomes particularly important when performing chemical modifications of CsA, since evaporation of acidic CsA solutions can not only isomerize CsA to isoCsA, but can also cleave the ring between the [Val]^{11} and [MeBmt]^1 residues, and degrade the resulting

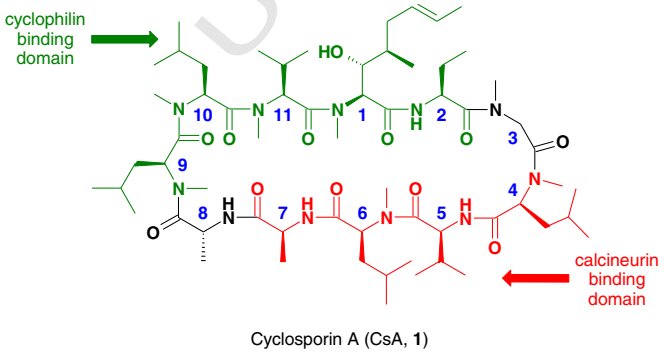

Fig. 2. Binding domains of cyclosporin A.

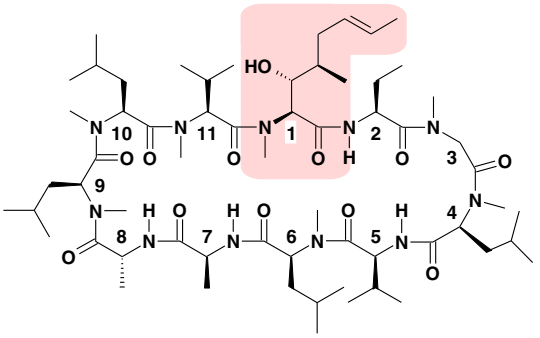

Fig. 3. [MeBmt]^1.

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