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IS THERE A SCAFFOLDING DOMAIN WITHIN THE STRUCTURE OF THE IMMUNOSUPPRESSIVE AGENT CYCLOSPORIN A (CsA)? STUDIES OF THE CYCLOPHILIN BINDING DOMAIN OF CsA

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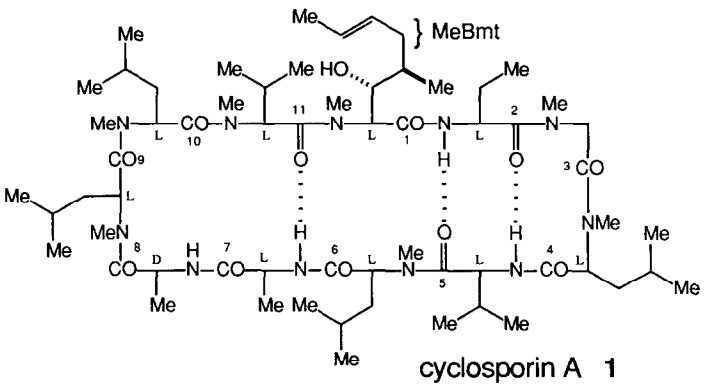
Abstract The synthesis of a peptide that approximates the critical binding/immunosuppressive domain within CsA but which is lacking any form of molecular scaffolding is described. The analysis of the conformation and cyclophilin binding properties of this synthetic analogue provides further evidence for the existence of a scaffolding domain within the natural product.

The search for more effective methods for allotransplantation has been a major focus of modern immunological research. The selective inactivation or removal of clones of host T cells that recognize donor MHC antigens remains an important challenge, as the remainder of the host T cell repertoire would thereby remain intact. In this way the problem of nonspecific immune suppression that accompanies current regimens of immunosuppressive agents would be abated. Although molecular and cell biological solutions to this problem are under active investigation,[1] the methods

Figure 1



cyclosporin A **1**

of organic chemistry *(immunoorganic chemistry)* hold considerable promise for new advances in this area. The discovery of the potent immunosuppressive agent and fungal product, cyclosporine A[2] (CsA) **1** is exemplary. Although it is not equipped with the absolute selectivity for the T cell subset outlined above,[3] CsA has nevertheless had a profound impact on the prevention of graft rejection by humans following bone-marrow and organ transplantations.

The unique molecular geometry of CsA serves as an important lead structure for the design of new immunosuppressive agents, hopefully with improved selectivity profiles. Information concerning the nature of CsA receptors and the detailed molecular mechanisms associated with

CsA biological activity are of enormous value to efforts aimed at the design of potent and selective immunomodulating substances. Evidence is accumulating that the immunosuppressive activity of CsA derivatives is linked to their ability to bind to a cytoplasmic protein termed cyclophilin.[4,5] These binding and biological studies suggest that CsA possesses a cyclophilin binding domain (N-MeLeu$_{10}$→Abu$_2$) that is held in an orientation suitable for interactions with the CsA receptor by a second domain that serves the role of a molecular "scaffolding" (Sar$_3$→N-MeLeu$_9$). If this hypothesis is correct, an important question follows: can replacement, or surrogate scaffolding be found that is reduced in size, perhaps with concommitant removal of the peptide linkages? Since the three-dimensional structure of cyclophilin is currently unknown,[6] the rational modification of CsA structure within the binding domain would appear to be difficult or impossible. However, with the X-ray crystal structure coordinates for CsA available in the Cambridge Crystallographic Data Base, a protocol for the design of replacement scaffolding can be easily imagined.

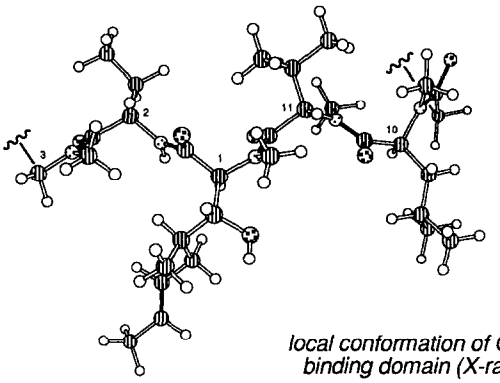
In this Letter, we describe the synthesis of a peptide that represents the complete binding/immunosuppressive domain within CsA but which is lacking any form of molecular scaffolding. The analysis of the conformation and cyclophilin binding properties of this synthetic analogue provides further evidence for the existence of a scaffolding domain within the natural product.

The solid state conformation of the CsA binding domain is depicted in Figure 2. NMR studies indicate that a closely related backbone conformation predominates in $CDCl_3$ solution to the extent of >95%.[7] Note that the Z-amide rotamer exists for each amide linkage with the exception of N-MeLeu$_9$ (partially clipped). The partial structure in the Figure (clipped from the X-ray structure) suggested the formulation of structure **11** as the target peptide of these studies. The convergent synthesis of tetrapeptide **11** is outlined in the scheme.

Efficient N-methylation[8] of N-Boc-L-α-aminobutyric acid **1**, was followed by conversion of the free acid to the corresponding dimethylamide *via* aminolysis of its mixed pivaloyl anhydride. The N-Boc group was subsequently removed upon treatment with trifluoroacetic acid, to give the crystalline ammonium salt **5** in good yield. N-Methyl (4R)-4-But-2E-1-yl-4-methyl-L-threonine (MeBmt) **2**, prepared in gram quantity according to the procedure of Evans and Weber,[9] was quantitatively protected as the dimethyloxazolidine **6**.[10] This acid underwent a DCC/HOBT mediated coupling reaction[11] with the N-methylated amine derived from ammonium salt **5**. Acid catalysed methanolysis liberated the dipeptide **9**. N-methylation[8] of N-Cbz-L-valine **3** and N-acetyl-L-leucine **4** afforded the corresponding amino acid derivatives in 97 and 53% yield, respectively. The N-methylated valine derivative was subsequently converted into the volatile amine **7** *via* sequential esterification and hydrogenation. Addition of the amine **7** to the mixed pivaloyl anhydride[12] of acid **8** gave a 56% yield of the corresponding dipeptide. Basic hydrolysis of the methyl ester afforded the acid **10**. Final coupling of the hydroxyamine **9** and the acid **10** was achieved by utilisation of the BOP reagent[10,13] in the presense of N-methylmorpholine. The desired tetrapeptide **11** was isolated yield of 75% after silica gel chromatography.

The ability of peptide **11** to bind to cyclophilin was evaluated in several systems. Displacement of [^{3}H]-CsA from cyclophilin was assayed with use of an LH-20 minicolumn[4] and an equilibrium cyclophilin affinity column procedure. Alternatively, changes in the intrinsic fluorescence of the single tryptophan in cyclophilin were determined and compared to the approximately two-fold enhancement seen with saturating concentrations of CsA ($K_d = 3 \times 10^{-8}$ M).

Figure 2

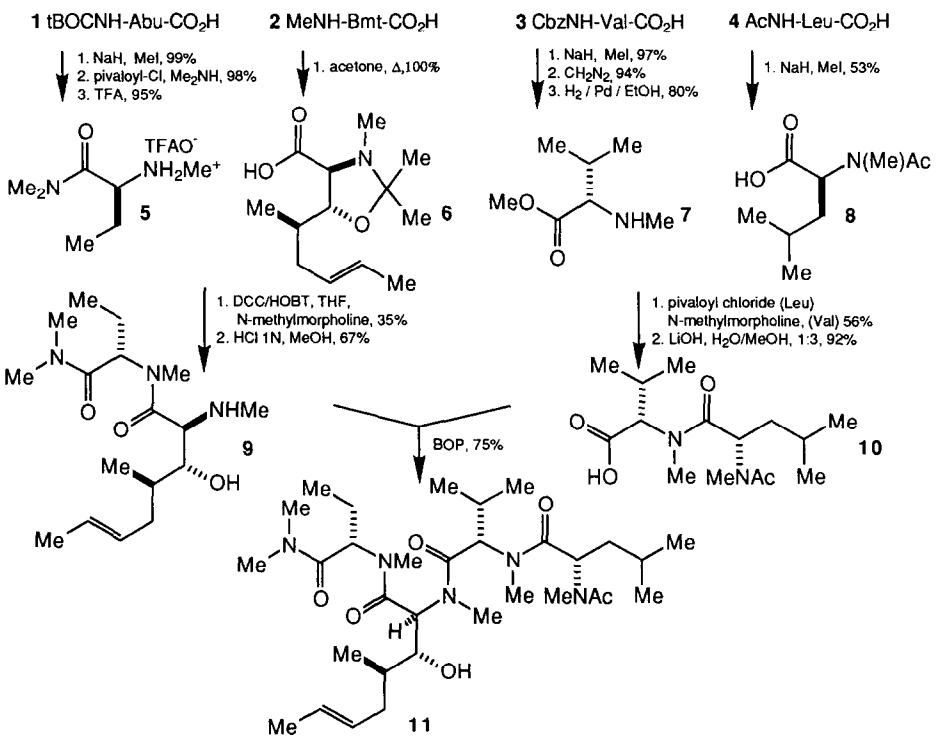


local conformation of CsA binding domain (X-ray)

*Peptide **11** did not displace [^{3}H]-CsA or enhance tryptophan fluorescence when present at 200 times the concentration of CsA required for a significant signal.*

Inspection of the ^{1}H NMR spectrum of **11** revealed a striking difference in the conformational properties of this compound, relative to the natural product. In contrast to CsA *(vide supra)*, the tetrapeptide **11** exists as a mixture of E/Z amide rotamers in $CDCl_3$ (at least three isomers are detected in ~ 3:1:1 ratio). These observations are fully consistent with modeling studies of **11** that were performed on MacroModel with the AMBER force field. A large number of conformational isomers (differing in side chain and amide conformations) were identified that were within several

Scheme



kcals/mol of total strain energy. These findings suggest that the ring constraint (via the additional peptide residues) in the natural product results in a considerable decrease in the conformational freedom of the peptide backbone relative to the acyclic peptide **11**. Taken together with the binding studies of **11** and CsA analogues,[5] *these results are consistent with the view that the peptide residues Sar$_3$→N-MeLeu$_9$ serve as a scaffolding domain within the natural product and function (vis-à-vis cyclophilin binding) to maintain a geometry of the binding domain that is complementary to the cyclophilin receptor site.*

In conclusion, our results indicate that the preparation of new cyclophilin ligands (immunosuppressive agents) based on a CsA model will require the design of dual domain agents equipped with both binding and scaffolding domains. We have recently utilized modeling methods to formulate several classes of target structures that feature the binding domain (or a portion thereof) held in the orientation found in CsA with assistance from simplified (relative to the natural product) scaffolding elements. The synthesis and evaluation (cyclophilin binding and immunosuppressive properties) of these molecules is ongoing in our laboratories.

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References and Footnotes

1. E.g., (a) Barnes, D. M. *Science* **1988**, *241*, 24. (b) Spangrude, G. J.; Heimfeld, S.; Weissman, I. L. *Science* **1988**, *241*, 58.
2. Wenger, R. M. *Angew. Chem. Int. Ed. Eng.* **1985**, *24*, 77.
3. CsA was shown to prevent clonal expansion of helper and cytotoxic T cells[a,b] while semi-selectvely sparing suppressor cells[c,d]: a) Larsson, E. *J. Immunol.* **1980**, *124*, 2828. b) Palacios, R. *J. Immunol.* **1982**, *128*, 337. c) Hess, A. D.; Tutschka, P. J. *J. Immunol.* **1980**, *124*, 2601. d) Hess, A. D.; Tutschka, P. J.; Santos, G. W. *J. Immunol.* **1981**, *126*, 961.
4. Handschumacher, R. E.; Harding, M. W.; Rice, J.; Drugge, R. J.; Speicher, D. W. *Science* **1984**, *226*, 544.
5. Quesniaux, V.F.G; Schreier, M.H; Wenger, R.M; Heistand, P.C; Harding, M.W; Van Regenmortel, M.H.V *Eur. J.Immun.* **1988**, *17*, 1359.
6. For recent progress, see: Dalgarno, D. C.; Harding, M. W.; Lazarides, A.; Handschumacher, R.E.; Armitage, I. M. *Biochemistry* **1986**, *25*, 6778.
7. Loosli, H.-R.; Kessler, H.; Oschkinat, H.; Weber, H.-P.; Petcher, T. J.; Widmer, A. *Helv. Chim. Acta* **1985**, *68*, 682.
8. McDermott, J.R; Benoiton, N.L *Can J. Chem.* **1973**, *55*, 1915.
9. Evans, D.A; Weber, A.E *J. Am. Chem. Soc.* **1986**, *108*, 6757.
10. Wenger, R.M *Helv. Chim. Acta.* **1984**, *67*, 502.
11. Konig, W, Geiger,R *Chem Ber.* **1970**, *103*, 788.
12. Zarral, M *Collect. Czech. Chem. Commun.* **1962**, *27*, 1273; Wenger, R.M *Helv. Chim. Acta.* **1983**, *66*, 2672
13. Castro, B; Dormoy, D.R; Evin, G; Selue, C; *Tetrahedron. Lett.*, **1975**,1219

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