SYNTHESIS AND BIOLOGICAL PROPERTIES OF NOVEL CYCLOSPORINE ANALOGS

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Cyclosporin A (CsA) is an immunosuppressive drug used to prevent graft rejection in human organ transplantations. CsA suppresses the cellular immune response through inhibition of the expression of interleukin-2 from T cells. The major cytosolic receptors of CsA are cyclophilins (CyPs), which are peptidyl-prolyl *cis/trans* isomerases (PPIase). It was shown recently that the structure of a CsA-CyP complex is consistant with a two-domain function of CsA, comprising a CyP-binding domain (residues 9-3) and an exposed effector domain (residues 4-8), which is thought to interact with calcineurin (CaN), a protein phosphatase 2B. The CsA-CyP complex interacts with CaN and inhibits its phosphatase activity, which inhibits translocation of a nuclear factor of activated T-cell required for the expression of IL-2 gene transcription, thus inhibiting T-cell activation.

In order to search for new target proteins in some immune-related cells, two photoaffinity labeling CsA analogs were developed. The construction of functionality at the 6- and 8-positions in CsA is the key step to accomplish the synthesis of these analogs. Further, to evaluate the effect of multiple substitutions on biological properties, new multi-substituted CsA analogs

modified near the effector domain were synthesized. Surprisingly, the PPIase-binding and immunosuppressive activities of [D-MeAla^3,Phe^7,D-Ser^8]CsA were greater than predicted from the activities of the corresponding singly- or bi-substituted derivatives, [Phe^7]-, [D-MeAla^3,Phe^7]-, and [Phe^7,D-Ser^8]CsA. These results are partially explained by conformational effects. NMR studies of the corresponding CsA analogs in DMSO were carried out to evaluate effect of substitution on ring conformation.

Finally, previous several investigators have demonstrated that CsA can inhibit replication of the human immunodeficiency virus-1 (HIV-1) in the early stage of infection, that HIV-1 gag protein Pr55^{gag} binds strongly to CyP, and that CsA can efficiently disrupt the gag-CyP interaction. To test whether non-immunosuppressive analogs inhibit HIV replication, I designed and synthesized a series of non-immunosuppressive CsA analogs modified at the 1-, 4- or 6-positions, [MeLeu(OH)^1]-, [MeLeu(OH)^1,MeAla^6]-, and [MeLeu(OH)^1,MeAla^{4,6}]CsA. The non-immunosuppressive, tight-binding inhibitor of PPIase was obtained and shown to inhibit early stage HIV replication.

APPROVED: _____

Professor Daniel H. Rich

DATE: 6/9/94

A dissertation entitled

SYNTHESIS AND BIOLOGICAL PROPERTIES OF

NOVEL CYCLOSPORINE ANALOGS

submitted to the Graduate School of the
University of Wisconsin-Madison
in partial fulfillment of the requirements for the
degree of Doctor of Philosophy

by

MING-KUAN HU

Degree to be awarded: December 19___ May 19___ August 19 94

Approved by Dissertation Readers:

Major Professor — June 7, 1994 — Date of Examination

Dean, Graduate School

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A thesis submitted in partial fulfillment of

the requirements for the degree of

Doctor of Philosophy

(Pharmacy)

at the

UNIVERSITY OF WISCONSIN-MADISON

1994

To my parents, wife, son and daughter.

Acknowledgements

I would like to express my gratitude to my advisor Professor Daniel H. Rich for his encouragement, guidence and critical advice during the course of this work.

I would like to thank Dr. Alison Badger (SmithKline Beecham Pharmaceuticals) and Dr. Malkovsky, Steven Bartz, and Eric Hohenwalter (Medical Immunology Department) for their execution of the biological activity test.

The assistance, helpful discussions and friendship of the numerous memebers of Rich's group, especially Chris Lynch, Aldoph Bonsdalt, Sejin Lim and Dr. Petr Kuzmic are appreciated.

I acknowledge with gratitude for the financial support from the National Defense Medical Center in Taiwan, Republic of China (1990-1994).

With deepest thanks, I acknowledge the constant support of my wife, Pao-Chen, which made this work possible.

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ABBREVIATIONS

Nomenclature and symbols of amino acids and peptides generally follow the recommendations of the IUPAC-IUB Joint Commission of Biological Nomenclature [*Pure Appl. Chem.* 1984, 56, 595-624]. All amino acids are of the L-configuration, unless otherwise noted. The commonly used abbreviations are listed below; others, which are used more rarely are defined in the text.

AcOH	acetic acid
Boc	tert-butyloxycarbonyl
BOP	benzotriazo-1-yl-oxytris-(dimethylamino)phosphonium hexafluorophosphate
BOP-Cl	N,N-bis-(2-oxo-3-oxazolidinyl)phosphinic chloride
Bu	butyl
tBu	tert-butyl
Bzl	benzyl
CaN	calcineurin
Cbz	benzyloxycarbonyl
CsA	cyclosporin A
CyP	cyclophilin
Daz	4-(1-Azi-2,2,2-trifluoroethyl)benzoyl
DCC	dicylohexylcarbodiimide

DCM	dichloromethane
DIEA	diisopropylethylamine
DMAP	4-dimethylamino-pyridine
EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
e. e.	enantiomeric excess
equiv	equivalent
Et	ethyl
EtOAc	ethyl acetate
FABMS	fast atom bombartment mass spectrum
Fmoc	[(9-fluorenylmethyl)oxy]carbonyl
g	gram
h	hour
HIV	human immunodeficiency virus
HOBt	1-hydroxybenzotriazole
HR-FABMS	high resolution-FABMS
Ki	inhibition constant
M	mole
Me	methyl
MeBmt	(4R)-4-[(2'E)-butenyl]-4,N-dimethyl-L-threonine
min	minutes
mg	milligram

ml	milliliter
mM	millimolar
mp	melting point
MS	mass spectrum
NMM	N-methylmorpholine
NMR	nuclear magnetic resonances
NOE	nuclear overhauser effect
Paloc	[((3-pyridyl)allyl)oxy]carbonyl
PBMCs	peripheral blood mononuclear cells
PHA	phytohemagglutinin
PMA/iono	phorbol 12-myristate 13-acetate / ionomycin
PPIase	peptidy prolyl *cis-trans* isomerase
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography

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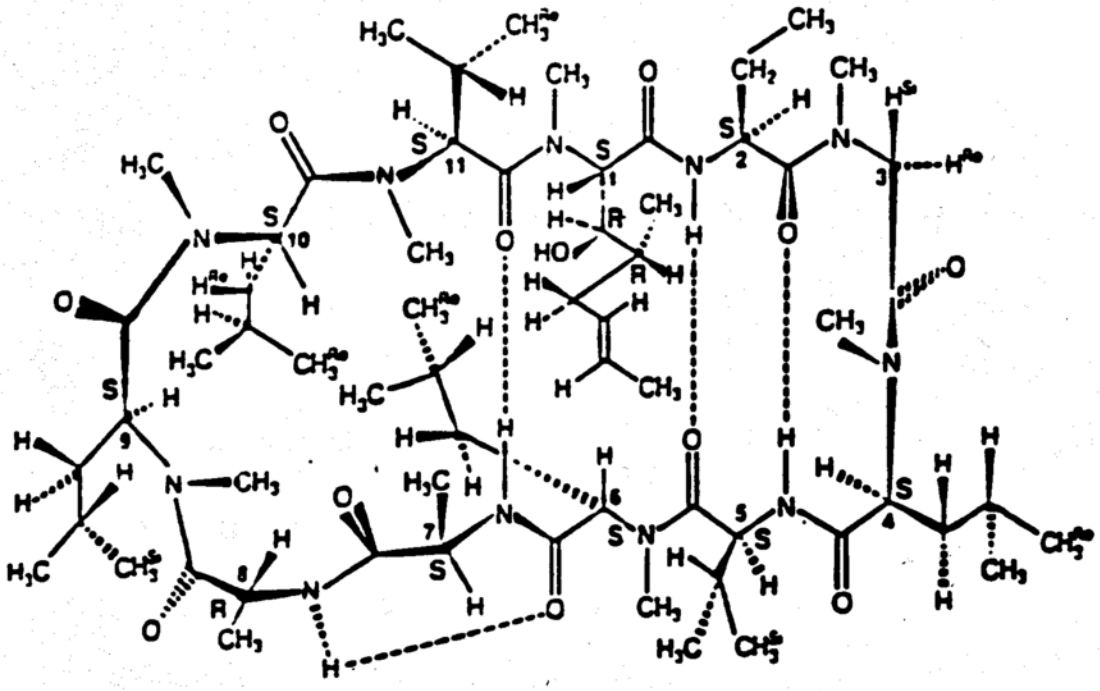
Chapter I. Introduction

A. *General*

Cyclosporin A (CsA, Figure **1.1**),[1-3] a secondary fungal metabolite from *Polypocladium inflatum gams*, is currently an important immunosuppressive drug, which was first marketed in 1983 with the trade name Sandimmune® for preventing graft rejection during bone marrow and tissue transplantation.[4,5] CsA, a cyclic undecapeptide cyclo(-MeBmt^{1}-Abu^{2}-Sar^{3}-MeLeu^{4}-Val^{5}-MeLeu^{6}-Ala^{7}-(D)-Ala^{8}-MeLeu^{9}-MeLeu^{10}-MeVal^{11}-), contains seven N-methyl amino acids and a novel amino acid, (4R)-4-[(2'E)-butenyl]-4,N-dimethyl-(L)-threonine (abbreviated as MeBmt, **1.2**) in the 1-position.[6] The first synthesis of enantiomerically pure MeBmt and the total synthesis of CsA were accomplished by Wenger in 1983 and 1984 at Sandoz,[7,8] thus opening the way to investigating the structure-biological activity relationships (SAR) of CsA.[9-19] Important advances during the last several years have included the determination of the X-ray structure of crystalline CsA,[20] the nuclear magnetic resonance (NMR) structure of CsA in organic solvents,[21] the identification of its cytosolic binding protein cyclophilin (CyP),[22] and the observation that CyP is identical to the enzyme peptidyl-prolyl isomerase (PPIase),[23,24,25] of which CsA is a potent inhibitor of the isomerase activity.[26,27] Furthermore, the observation of bound CsA-CyP

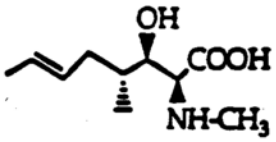


MeLeu$_{10}$—MeVal$_{11}$—MeBmt$_1$—Abu$_2$—Sar$_3$

|

MeLeu$_9$—D-Ala$_8$—Ala$_7$—MeLeu$_6$—Val$_5$—MeLeu$_4$

Figure 1.1. Structure of cyclosporin A (CsA, **1.1**) corresponding to the conformation observed in the crystal; MeBmt (**1.2**): (4R)-4-[(2'E)-butenyl]-4,N-dimethyl-L-threonine.



1.2

complex by NMR identified a number of intermolecular NOEs between ligand and receptor.[28,29] This suggested that CsA binds to a hydrophobic pocket of the CyP through amino acid residues 9-11 and 1-3.[30] Therefore, these residues are the binding domain of CsA. In contrast, CsA residues 4-8 show no NOE interactions with CyP and are proposed to be solvent-exposed. [31,32] An important observation concerning the molecular mechanism of action of CsA in early 1991 established that calcineurin (CaN), also referred to as protein phosphatase 2B, a calmodulin-dependent serine/threonine protein phosphatase,[33,34] binds as a common target protein to both CsA-CyP and FK506-FKBP,[35,36] another complex derived from a chemically unrelated immunosuppressive drug, FK506,[37,38] and its binding protein, FK506-binding protein (FKBP).[39,40] The binding of the complexes to CaN inhibits certain Ca^{2+}-dependent cellular processes, among which are T-cell receptor (TCR)-mediated signal transduction and interleukin-2 (IL-2) release.[41,42,43]

B. *Isolation and Structure Elucidation of CsA*

1. *Isolation of CsA and its Analogues*

Cyclosporines are produced by fungi of the genus *Tolypocladium* in submerged cultures.[1,44] CsA is the main component of a group of cyclic peptides in normal fermentation broths. CsA easily dissolves in most organic solvents but is poorly soluble in water. Extensive chromatographic

Table 1.1 Naturally occurring cyclosporines and their bioactivities

Name	Amino acid in CsA	Pos.	amino acid in analogue	Immuno-suppression[a]
CsA	Abu	2		+++
CsB	Abu	2	Ala	+
CsC	Abu	2	Thr	++
CsD	Abu	2	Val	+
CsE	MeVal	11	Val	(+)
CsF	MeBmt	1	(3'-deoxy)MeBmt	(+)
CsG	Abu	2	Nva	+++
CsH	MeVal	11	D-MeVal	–
CsI	Abu^{2},$MeLeu^{10}$	2,10	Val^{2},Leu^{10}	(+)
CsK	$MeBmt^{1}$,Abu^{2}	1,2	(3'-deoxy)$MeBmt^{1}$,Val^{2}	(+)
CsL	MeBmt	1	Bmt	+
CsM	Abu^{2},Val^{5}	2,5	Nva^{2},Nva^{5}	++
CsN	Abu^{2},$MeLeu^{10}$	2,10	Nva^{2},Leu^{10}	+
CsO	$MeBmt^{1}$,Abu^{2}	1,2	$MeLeu^{1}$,Nva^{2}	(+)
CsP	$MeBmt^{1}$,Abu^{2}	1,2	Bmt^{1},Thr^{2}	+
CsQ	MeLeu	4	Val	(+)
CsR	$MeLeu^{6}$,$MeLeu^{10}$	6,10	Leu^{6},Leu^{10}	(+)
CsS	Abu^{2},$MeLeu^{4}$	2,4	Thr^{2},Val^{4}	(+)
CsT	MeLeu	10	Leu	++
CsU	MeLeu	6	Leu	+
CsV	Ala	7	Abu	++
CsW	Abu^{2},$MeVal^{11}$	2,11	Thr^{2},Val^{11}	(+)
CsX	Abu^{2},$MeLeu^{9}$	2,9	Nva^{2},Leu^{9}	+
CsY	Abu^{2},$MeLeu^{6}$	2,6	Nva^{2},Leu^{6}	+
CsZ	$MeBmt^{1}$	1	L-2-methyl-octanoic acid(=MeAoc)	(+)

[a] +++: highly active; ++: effective; +: fairly active; (+)/-: weak/inactive

separations yielded 25 cyclosporines named CsA through CsZ (Table 1.1).[45,46] The most common variation is in position 2, Abu ((L)-2-aminobutyric acid). Replacements include alanine, threonine, valine or norvaline. Some metabolites are derived from a lower degree of N-methylation. Replacement of MeBmt by (3'-deoxy)MeBmt, MeLeu, or (L)-2-methylamino-octanoic acid (MeAoc) has also been observed. No natural analogs with variation at positions 3 and 8 have yet been found. Some of the observed variations resulted in considerable conformational changes (as determined by NMR or X-ray analysis), which in most cases had profound effects on the biological activity of these metabolites.

2. *Structure Determination*

The structure of CsA was elucidated by a combination of chemical degradation[47] and X-ray crystal structure analysis.[48] Microtitration and electrophoretic mobility indicated a neutral compound while IR- and NMR-spectroscopy suggested the presence of amide groups, a substituted double bond, and alcoholic hydroxyl group that was detected as an acetyl derivative. Hydrolysis of CsA yielded 4 moles of N-methyl-(L)-leucine, 1 mole each of sarcosine, N-methyl-(L)-valine, (L)-valine, (L)-2-aminobutyric acid, racemic alanine (2 moles) and an unknown amino acid consisting of 9 carbon atoms. At that time this amino acid could not be isolated from CsA hydrolysis. After

catalytic hydrogenation and hydrolysis a bistrifluoroacetyl-dihydro derivative could be obtained and was assigned as 3-hydroxy-4-methyl-2-methylamino-6-octenoic acid. The configuration of this amino acid was elucidated by X-ray analysis of an iodo-derivative of CsA. Confirmation of (4R)-4((E)-2-butenyl)-4,N-dimethyl-(L)-threonine as the structure of MeBmt became possible only after total synthesis (see Scheme **1.1**, page 34). The sequence of amino acids in CsA was later assigned and confirmed by X-ray crystal structure determination and by two dimensional nuclear magnetic resonance studies.[20,49]

In summary, CsA is a neutral cyclic peptide composed of eleven amino acids, all having an (L)-configuration, except for the (D)-Ala in position 8 and the non-chiral sarcosine (Sar) in position 3. Seven amino acids in CsA are N-methylated (positions 1, 3, 4, 6, 9, 10, 11). The unusual amino acid in position 1, MeBmt, is highly characteristic for CsA and most of its natural analogs.

3. *Conformation of CsA in the Crystal*

The conformation of CsA that was observed in the crystal is shown in Figure **1.2** (see Appendix **I**, panel **A**; page 223)[20]. In the crystal structure, CsA assumes a rather rigid conformation in which it can be divided into two overlapping domains. The first consists of residues 11-3 and 4-7 which form two strands of an antiparallel β-sheet joined by a type βII′ turn[49,50,51] at residues 3 and 4. There are hydrogen bonds between Abu[2] NH and Val[5] CO,

Val[5] NH and Abu[2] CO, and Ala NH and MeVal[11] CO stabilizing the rigid structure of CsA in the crystal form. Notably, all N-methyl groups (at residues 11, 1, 3, 4, and 6) in this domain are directed outside the macrocyclic ring. The second domain consists of residues 7-11, which form an irregular loop stabilized by a hydrogen bond from D-Ala[8] NH and MeLeu[6] CO and a number of van der Waals contacts, including several to the N-methyl group of MeVal[11], which is directed into the center of the macrocycle. All amide bonds in the structure are *trans*, except that between MeLeu[9] and MeLeu[10], which is *cis*. The four intramolecular H-bonds, whose positions are forced by the presence of six other N-methyl groups, and the *cis* amide bond contribute significantly to the rigidity of the CsA skeleton. The side chain of MeBmt[1] is folded into the ply of the β-pleated sheet and held together by the carbon chain of MeLeu at residues 4 and 6, allowing the molecule to adopt a globular compact shape.

4. *Conformation of CsA in Aprotic Solvents*

In organic solvents, such as $CDCl_3$, CD_2Cl_2 and C_6D_6, only one conformation of CsA is populated to the extent of about 95%, as determined by integration of the N-methyl resonances. Kessler and co-workers[21] performed molecular dynamics (MD) calculations to calculate the most populated structure in aprotic solvents. Overall, the NMR structure of CsA in

aprotic solvents as shown in Figure **1.2** (see Appendix **I**, panel **B**; page 223)[21] is similar to the crystal structure. In particular, the four hydrogen bonds identified in the crystal are preserved, the amide bond between MeLeu[9] and MeLeu[10] is *cis*, and the side chain of MeBmt[1] is folded back on the molecule.

5. *The Solution Conformation of CsA in LiCl-THF*

The dramatic effects of inorganic salts, notably lithium salts, on the solubility in non-polar organic solvents, such as THF, and on the reactivity of amides and peptides under aprotic conditions have been described extensively.[52,53] This effect was exploited for synthetic purposes; for instance, for the generation and alkylation of peptide enolates[54,55] or for improved peptide solubility and coupling.[56] Further, the complexation of a peptide with lithium salts in THF may lead to a new conformation of the peptide backbone. In the case of CsA, the structure in THF in the presence of 31 equiv of LiCl displays an extremely different conformation compared to that in aprotic solvents.[57,58] The four intramolecular H-bonds are all annihilated in the complex, and the *cis* configuration of the peptide bond between MeLeu[9] and MeLeu[10] is changed to *trans* as shown in Figure **3** (see Appendix **I**, structure **3B**; page 224).[21] The LiCl/THF conformation is similar to the one found when CsA is complexed to CyP as shown Figure **3** (see Appendix **I**, structure **3C**; page 224).[28,29] The results are in accord with the kinetic results of

the inhibition of the PPIase activity of CyP with CsA/LiCl, in which the conformer with the *trans* amide bond between residues 9 and 10 shows a stronger and faster inhibition of the PPIase activity of CyP as compared to CsA in THF.[26,27] This indicates that the LiCl/THF system presents CsA to CyP in a conformation that is similar to the one found in the CsA/CyP complex.

6. *The Solution Conformation of CsA bound to CyP*

The complexation of CsA and CyP has been determined by heteronuclear three-dimensional NMR spectroscopy[28,61] and X-ray analysis.[59,60] Strikingly, the kinetic[26,27] and structural data[29] established that the *cis* amide bond of MeLeu[10]-MeLeu[10] of CsA becomes *trans* when bound to CyP. The structure of CyP changes very little after complexing with CsA, whereas the conformation of CsA when bound to CyP as shown in Figure **1.3** (see Appendix **I**, structure **3C**; page 224) is very different from either the crystal[20] or chloroform-solution structures.[21] In contrast to free CsA, the bound molecule has no elements of regular secondary structure. In addition, there are no intramolecular hydrogen bonds. Of the seven N-methyl groups, only two, those of MeVal[11] and MeLeu[9], are solvent-exposed. The remaining N-methyls are directed into the macrocycle and make a number of intramolecular van der Waals contacts.

The structure of the CsA/CyP complex is stabilized by several hydrophobic interactions (see Figure **1.4** in Appendix **I**, page 225).[60] The side chains of CyP

residues Trp 121, Gln 63, Ile 57, Leu 122, Phe 113, His 126, Ala101, Ala 103 and Thr 73 form a hydrophobic pocket that interacts with the hydrophobic surface of CsA residues 9 through 11 and 1 through 3.[61] Several hydrogen bonds (between MeLeu^{10} CO and Arg 55 ηNH, MeLeu^{9} CO and Trp 121 εNH, as well as Abu^{2} NH and Asn 102 CO) also stabilize the structure of the complex. According to the NMR structure reported by Fesik and co-workers,[61] the MeBmt^{1} hydroxyl group forms a hydrogen bond to the MeLeu^{4} carbonyl group and not to the Asn 102 carbonyl of CyP as proposed by previous observations.[32,62] This intramolecular hydrogen bond may be important for stabilizing the conformation of CsA and cannot be used to explain the importance of the MeBmt hydroxyl group for CyP-binding and immunosuppressive activity as prevously thought.[32,62] In summary, residues 9-11 and 1-3 portions which show strong intermolecular interactions with CyP, constitute the binding domain of CsA. In contrast, residues 4 through 8 show only intramolecular NOEs, indicating that they do not interact with the protein and presumably these residues are believed to constitute the region of CsA that is in contact with CaN. These models offer a good explanation for the inhibitory and immunosuppressive properties of a variety of CsA analogs.

C. *Pharmacological Properties of CsA*

The pharmacological spectrum of CsA has many facets. Because of its potent inhibition of the antibody- and cell-mediated immune response, CsA is now being used as the mainstay in clinical immunosuppression and has significantly improved the survival of kidney, liver, and heart allografts. It is also opening the way for the transplantation of other organs such as lung, skin, nerves and blood vessels.[63,64] In contrast to classical immunosuppressants, CsA exerts a specific action on lymphocytes and does not interfere with the functions of phagocytes or haemopoietic stem cells. It is neither mutagenic nor lymphocytotoxic, as its action is reversible. Nephrotoxicity is its major side effect, but this can largely be minimized by drug combinations, especially in the early stages, or by dose reduction during the maintenance phase.[65,66,67]

CsA has been reported to be effective against several types of autoimmune diseases. Promising therapeutic effects have been observed in the clinic in the treatment of psoriasis[68] and a number of autoimmune disorders,[69] including type I diabetes mellitus,[70] multiple sclerosis and rheumatoid arthritis. Further, *in vitro* and *in vivo* experiments have shown CsA to possess antiparasitic activity against malaria,[71] schistosomiasis,[72,73] leishmaniasis[74] and coccidiosis.[75]

Besides being an immunosuppressive drug, CsA can reverse multiple drug resistance (MDR) experimentally both *in vitro* and *in vivo*.[76] CsA has P-

glycoprotein binding activity but less specific membrane effects.[77] Inhibition of protein kinase C may be involved in its reversal of MDR.[78] A number of non-immunosuppressive analogs of CsA have also been shown to reverse MDR. Some are more active than the parent compound. The use of non-immunosuppressive derivatives may allow a full test of the hypothesis that reversal of multidrug resistance is a useful clinical strategy.

More importantly, CsA possesses anti-HIV properties. During the last few years, several investigators demonstrated that CsA suppresses the replication of HIV-1 *in vitro* and helps prevent the virus from infecting and killing CD4 T lymphocytes.[79-82] Recent studies indicate that the mechanism underlying the action of CsA on HIV infection may be more complex than that of merely blocking cellular activation in related T cells.[83] It has been demonstrated *in vitro* that CsA interferes with the binding of cellular cyclophilin A (CyPA) and B (CyPB) to the HIV gag protein. This interaction may interfere with HIV replication.[84] For a further understanding of the mechanism of action of CsA on T cells, the development of non-immunosuppressive CsA derivatives may prove to be more appropriate than CsA for testing in HIV-infected individuals.[85] The administration of non-immunosuppressive CsA analogs in the early course of infection may act to block immune activation and to avoid severe immunosuppression.

D. *Mechanism of Action of CsA*

1. *Biological Action of CsA in T Cells*

CsA was initially identified by screening for materials with antibiotic properties that were produced by fungi. It was later shown to be a powerful inhibitor of T-cell activation, induced by a mitogen or by a mixed lymphocyte reaction.[2,86] In 1977, Borel and co-workers observed that it was non-toxic to cells at concentrations that completely blocked T-cell activation, and that it did not block the proliferation of other cell types, suggesting that it might make a useful immnosuppressive agent. This prediction was accurate and opened the way to search for the mechanism of action of the drug.

In a simple model of T-cell activation, T cells can be stimulated to proliferate and to function through their surface T cell antigen receptor (TCR) interacting with an antigen presenting cell in the presence of specific extracellular stimuli. TCR-dependent T cell activation leads to the induction of an ordered cascade of biochemical signals that results in gene activation. Studies of CsA's effects on T-cell activation indicate that it acts within several hours of the activation. If the drug is given more than six hours after the activation stimulus, there is little effect on T-cell proliferation induced in resting T cells by either mitogen or antigen. This indicates that CsA might be interfering with the critical molecules that orchestrate later events in T-cell proliferation and activation.[87,88,89] Further, inhibition of T-cell proliferation

mediated by either CsA or FK506 can be partially reversed by the addition of exogenous IL-2,[90] suggesting that the block in T-cell function is proximal to the production of IL-2. In mast cells, CsA and FK506 block degranulation as well as the transcriptional activation of several cytokine genes, such as IL-3 and IL-5,[91,92,93] and also the genes involved in leukotriene synthesis. A common requirement for each of these events is an increase in the concentration of intracellular Ca^{2+}. The cytokine genes that are blocked in mast cells are largely the same as those blocked in T cells, suggesting an effect on a regulatory protein common to mast cells and T cells.

Since CsA and FK506 were known to block the induction of cytokine gene transcription at an early stage of antigen-induced helper T-cell activation (G_0 to G_1 transition of the cell cycle),[94-97] a logical place to look for a molecular structure that received signals from the antigen receptor was among the proteins binding to the IL-2 enhancer. These proteins appear to function cooperatively to activate transcription of the IL-2 gene. Potential candidates, which are essential for IL-2 gene expression, are NF-AT (a nuclear factor of activated T cells)[98] and NF-κB (a nuclear factor of immunoglobulin κ light chain in B cells),[99] which were both reported as being affected in their IL-2 promoter binding activity by CsA and FK506 (Figure 1.5, page 18).[100] In all probability, DNA binding of both transcription factors depends on protein modification and nuclear translocation of cytoplasmic precursors prior to

their participation in the formation of a functional transcription complex. NF-κB in nonstimulated cells is bound as an inactive precursor to its inhibitor I-κB. Upon T-cell activation, I-κB becomes phosphorylated and subsequently releases NF-κB from the cytoplasm into the nucleus.[101]

2. *Intracellular Binding Molecule for CsA*

The search of the mechanism of action of CsA was spurred by the observation that its intracellular binding protein, cyclophilin (CyP),[22] was identical to *cis-trans* peptidyl-prolyl isomerase[23,24,25] and that the activity of the isomerase was blocked by the binding of CsA.[23,26] This observation was soon followed by the discovery of a binding protein for FK506,[35,36] FK506-binding protein (FKBP).[37,38] These cytosolic receptors have become known as immunophilins. Interestingly, FKBP is also a *cis-trans* peptidyl-prolyl isomerase and binding of the drug blocks its isomerase activity. Remarkably, despite the fact that both CyP and FKBP are isomerases, they share no sequence homology and the drugs do not cross-inhibit isomerase activity.[39,40] This suggested that the isomerase activity was not important in mediating the action of the immunosuppressive drugs. Since each drug blocks only one isomerase, cells treated with one drug should have fully functional isomerases remaining, yet either drug can block T-cell activation. Since the isomerases were known to lack absolute specificity,[102,103] it might have been

expected that they would compensate for one another. This suggested that the drugs somehow have a dominant action and induce a gain in function of their cognate immunophilin.

Studies on yeast found that deletion of the major intracellular receptor for CsA was not lethal, although the organism failed to grow in the presence of the drug.[104,105] This observation suggested that a unique entity was formed through the interaction of the drug and its receptor (the CsA-CyP or FK506-FKBP complex). This led to the observation that CsA-CyP and FK506-FKBP complexes, neither CsA nor FK506 alone, bind competitively to a different protein, later identified as calcineurin (CaN).[35,36] Calcineurin, the Ca^{2+}/calmodulin-dependent protein phosphatase also termed phosphatase 2B, is a heterodimer composed of two subunits.[106,107] Calcineurin A (CaNA) is a 59 kDa catalytic subunit with a binding site for calmodulin and calcineurin B (CaNB), a 19 kDa regulatory subunit. In the presence of Ca^{2+}, calmodulin binds to and activates CaN, perhaps explaining the calcium requirements of CsA- and FK506-sensitive pathways. *In vitro*, the complexes of CsA-CyP and FK506-FKBP inhibit the phosphatase activity of CaN.[36,108] This establishes CaN as the common biological target for the actions of both CsA and FK506. The interaction of these complexes with CaN appears to represent a "gain of function" by CsA or FK506 under normal cellular conditions.

3. *The Role of CsA-CyP on the Nuclear Translocation of NF-AT*

The biochemical, pharmacological and genetic studies reviewed above have led to the consensus that CaN phosphatase activity is critical for propagating the calcium-dependent T cell signaling pathway. Currently, considerable efforts in many laboratories are focusing on identifying phosphoprotein substrates of CaN in T cells. One potential substrate is the transcription factor NF-AT. As previously described (page 14), NF-AT is one of many transcription factors required for antigen-induced IL-2 gene expression.[42] NF-AT is a multimeric complex composed of a nuclear component ($NF\text{-}AT_n$), whose synthesis is induced by signals dependent on protein kinase C and a pre-existing component ($NF\text{-}AT_p$) that translocates from the cytoplasm to the nucleus in a Ca^{2+}-dependent dephosphorylation process. CsA inhibits the translocation of $NF\text{-}AT_p$ to the nucleus, suggesting that $NF\text{-}AT_p$ could be a calcineurin substrate.[109,110] $NF\text{-}AT_p$ has recently been found to be a phosphoprotein and a CaN substrate. CsA blocks dephosphorylation of $NF\text{-}AT_p$ by inhibiting CaN.[111]

The investigations of the mechanism of action of CsA at the molecular and cellular levels have established that the drug blocks induction of NF-AT by interfering with the calcium-dependent appearance of $NF\text{-}AT_p$ in the nucleus. $NF\text{-}AT_p$ is a direct substrate of CaN which can be inhibited by the CsA-CyP complex. Therefore, inhibiting the dephosphorylation of $NF\text{-}AT_p$ by

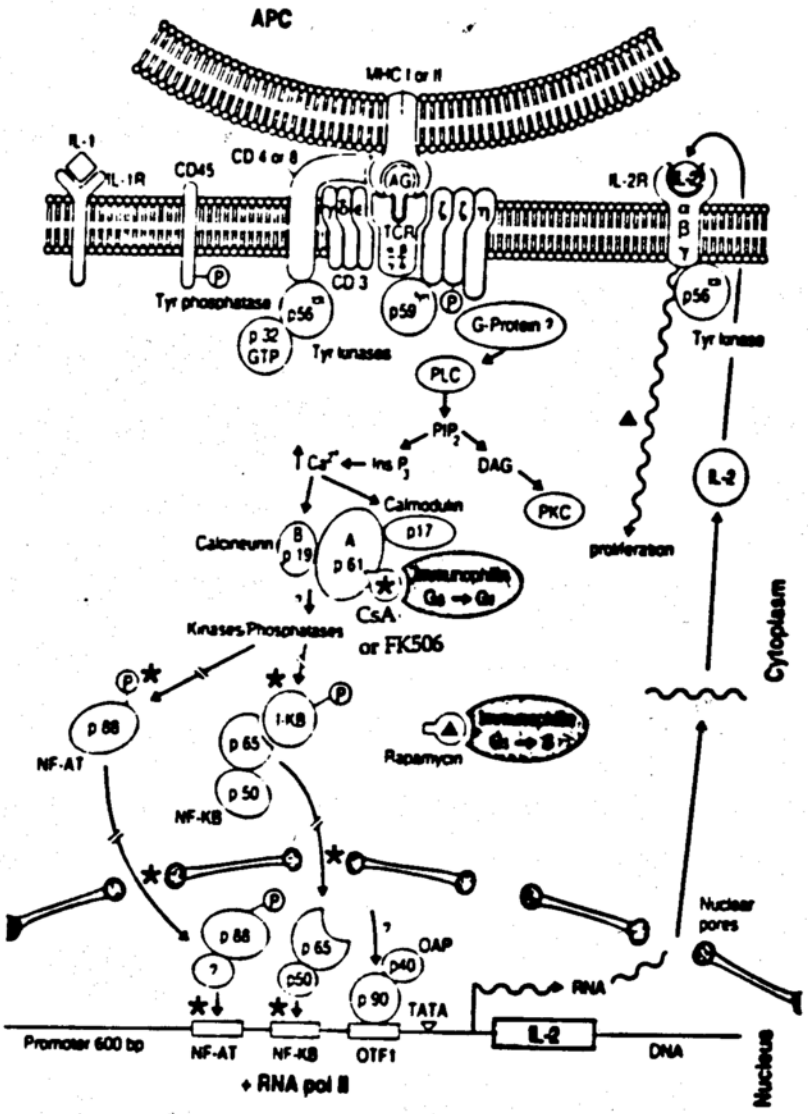


Figure 1.5. CsA and FK506 both interfere, by binding to their respective immunophilins, with the function of intracellular molecules that transmit Ca++-associated signals between the T-cell receptor (TCR) and the activation of lymphokine gene (IL-2) in the nucleus. Transcriptional regulation of IL-2 gene expression is moduated by the combination of transcription factors (e.g. NF-AT, NF-κB, OTF1) interacting with their corresponding recognition sites at the IL-2 promoter. These DNA/protein complexes, together with RNA polymerase II (RNA pol II), result in the antigen-inducible transcription of IL-2 gene. CsA and FK506 interfere with the G_0 to G_1 transition of the cell cycle (ref. 100).

the drug/immunophilin/calcineurin complex is thought to mediate the CsA inhibition of IL-2 gene induction. In future studies, purification and molecular cloning of NF-AT_p will allow a more detailed understanding of the mechanism by which NF-AT_p is modified during T cell activation, and the mechanism of action of immunosuppressive drugs.

E. *Structure and Biological Activity Relationships of CsA*

Chemical and pharmacological studies of naturally occurring cyclosporines[46] and numerous specially modified analogs[9-19] have impressively characterized much of the structure and activity relationships (SAR) of CsA for immunosuppressive activity.[9-19] As shown in Table **1.1**, most naturally occurring CsA analogs possess weaker immunosuppressive activity than CsA, with the exception of CsG ([Nva^2]CsA), which has been evaluated further as a clinical candidate.[112] Some general SARs have emerged for CsA. Desmethylation of any of the N-methylated amino acids in CsA decreased the drug's activity. The residues 11, 1 and 2 appear to be critical for maintenance of immunosuppression. Earlier studies from the Sandoz group[11-13] have focused on the following areas: 1) the importance of the unusual amino acid MeBmt; 2) the effects of varying the amino acid residues adjacent to MeBmt (i.e. positions 2, 3 and 11); and 3) the structural and conformational relevance of residues 7-11 of CsA. According to the study of a

large number of CsA analogs, the Sandoz group has pointed out that the biological activity is associated with a large portion of the three-dimensional structure, including residues 10, 11, 1, 2 and 3. In further studies, a group at Merck[9,10] investigated the modification of positions 4, 5, 6 and 9 with alanine or N-methylalanine. Their results suggested a more extended, active portion of CsA as compared to that of the Sandoz group previously described. The immunosuppressive potencies of most CsA analogs studied primarily by the Sandoz, Merck and Rich groups[15-17] are listed in Table **1.2**. The reported activities are designated by different rating systems. A simple qualitative rating on a scale of plus one to three or five (depending on the publication shown in the footnotes of the Table) was used by the Sandoz group. Quantitative values were reported by the Merck and Rich groups. It also should be noted that immunosuppressive activities are obtained by different assay methods employed by different groups (see footnotes in Table **1.2**). Nevertheless, from the data presented in Table **1.2**, several trends are apparent:

1. Residue 1, MeBmt, is critically important and essential for maximal biological activity of CsA. Modifications of the double bond (entries 1-7, 11, 12, 18-23), hydroxyl group (entries 9, 13, 34), or removal of the non-polar portion of the carbon side chain (entries 24-26) dramatically reduce the immunosuppressive activity. Epimerization of C-4 as in [(4S)-MeBmt1] (entry

14) or removal of the 4R-methyl group (entry 15) also reduces the activity. With an additional methyl group at C-4, analogue [$MeBm_2t^1$] (entry 28) showed 100-fold weaker CyP-binding but still retains strong immunosuppressive activity. The weaker affinity for CyP-binding was recently attributed to the close contact between residue $MeBm_2t$ and the active site Ala 103 of CyP, which causes small conformational changes in both protein and drug.[172] These changes are postulated to cause tighter binding of residue $MeLeu^6$ to CaN and thereby produce higher affinity of the CyP-[$MeBm_2t$]CsA complex for the protein. Removal of the N-methyl group, as in the naturally occurring CsL (entry 30), decreases immunosuppressive activity.

2. Position 2 (Abu) tolerates some variation in structure. The Sandoz group found that natural analogs [Thr^2]- and [Nva^2]-CsA (entries 47, 51) still maintain potent immunosuppressive activity, but [Ala^2] and [Ser^2] analogs with smaller side chains are significantly less active (entries 48, 49). The Merck group found that fluorination of the side chain at this position only produced less potent analogs (entries 53-56).

3. Modifications of position 3 (Sar), which participates in the type II' ß-turn structure found in the solid and solution state of CsA, provided some interesting analogs. [(D)-Pro^3]-, [(D)-$MeAla^3$]- and [(D)-$MePhe^3$]CsA analogs (entries 59, 61, 63) display almost the same conformation as CsA, but [Pro^3]

and [MeAla^3] analogs (entries 60, 62) were found to lose the type II' ß-turn due to the steric hindrance between the N-methyl of MeLeu^4 and the alkyl substituent at α-carbon of Sar^3. All these analogs, except [(D)-MeAla^3], were significantly less active than CsA. This suggests that at this position CsA forms a tight fit against its binding pocket and this rigidity seems to be necessary for the biological activity.

4. According to an investigation of the X-ray structure of a monomeric CyP-CsA crystal complex, the residues MeLeu^4 and MeLeu^6 of CsA are thought to interact specifically with CaN.[60] Modifications in the side chain in either the 4- (entries 64-72) or 6-position, e.g. [MeAla^6], [MeVal^6] and [MeAbu^6] (entries 74, 75, 77), dramatically decrease immunosuppressive activity. A desmethylated analogue, such as [Leu^6], or an aromatic modification, like [MePhe^6] (entries 79, 80), also show poor activity. These results suggest that the orientation and hydrophobic structure of the isobutyl side chain at MeLeu^4 and MeLeu^6 act as a conformational lock for binding to and inactivating CaN.

5. Very few modifications at Positions 5 (Val) and 7 (Ala) of CsA have been investigated. The [Ala^5] (entry 73) and naturally occurring [Abu^7] analogs show poor activities. The replacement of [Ala^7] by [(D)-Ala^7] (entry 82) may induce conformational disturbance in the loop and reduce the activity.

6. At position 8 (D-Ala), Sandoz developed a series of active

semisynthetic CsA analogs such as [D-Ser^8]CsA, [D-Ser^8]CsC, [D-Ser^8]CsD and [D-Ser^8]CsG (entries 91-94), which establish that this residue can tolerate some structural variations without destroying the activities. Some cysteine derivatives (entries 97, 98) still maintain good activity. In order to study the binding of CsA to its target protein, CsA analogs such as [(D)-Dab^8], [(D)-Dap^8] and [(D)-Lys^8] (entries 89, 90, 84) were made with a derivatizable side chain. These analogs have reduced activity, probably due to the charge of the sidechain amine group. SDZ IMM 125 (entry 101), derived from [(D)-Ser^8]CsA[113] by alkylation of the hydroxyl O-group, has all the *in vitro* properties of a powerful immunosuppressant and because of its reduced side effects in animal studies, it may become a clinical candidate.[115] Position 8 is located between the CyP-binding domain and the effector domain of CsA, possibly not interacting with either protein. Therefore, most analogs modified at position 8 have little or no effect on the biological activity.

7. Position 11 (MeVal) is very sensitive to any structural variation. [MeAla^{11}] and [MeLeu^{11}] analogs (entries 108, 109) are inactive, suggesting that this position forms a very tight fit against the receptor pocket. Increased or reduced steric bulk of the carbon chain at position 11 probably prevents efficient binding to the receptor. When the pro-R methyl group of MeVal is extended by an extra carbon (entry 110), an inactive analog results. Elongation of the pro-S methyl group (entry 109) is somewhat less detrimental to activity.

Epimerization of position 11 yields [(D)-MeVal[11]]CsA (entry 105), which is unrecognized by CyP and totally inactive as an immunosuppressive agent due to the massive change in the analog's conformation.[19]

8. Most naturally occurring bis-modified CsA metabolites, varied mainly at residues 9, 10, 11, 1 or 2 by de-methylation (entries 36-45), are devoid of immunosuppressive activity.[46] These residues are present in the CyP-binding domain, which seems to be sensitive to changes in the cyclic peptide conformation. Recently, a structure-based approach was used to introduce a conformational constraint into the bound structure of CsA. Tricyclic CsA analog (entry 114) described by Schreiber and co-workers showed slightly greater affinity for CyP and immunosuppressive activity as compared to CsA.

Table 1.2. Structure Activities of Selected Cyclosporine Analogs

Entry CsA Analogue	CyP binding (% of CsA) or Ki(nM)	Immuno-suppressive (% of CsA)	IL-2 release inhibition (% of CsA)	Sourcei
(CsA)	100	100	100	n
Sidechain modified in MeBmt				
1. $CH(OH)CH(CH_3)CH_2SCH_3$	178	9.5[a]		s
2. $CH(OH)CH(CH_3)CH_2S(O)CH_3$	2.7	< 0.1[a]		s
3. $CH(OH)CH(CH_3)CH_2S(O_2)CH_3$	4	< 0.1[a]		s
4. $CH(OH)CH(CH_3)CH_2CH_2SCH_3$	12	< 0.1[a]		s
5. $CH(OH)CH(CH_3)CH_2$ S-Phenyl	18	18[a]		s
6. $CH(OH)CH(CH_3)CH_2COOH$	3	< 0.1[a]		s
7. $CH(OH)CH(CH_3)CH_2CH_2OH$	16	< 0.1[a]		s
8. $CH(OH)CH(CH_3)CH_2CH_2CH_2CH_3$	36	40[h], 26[b]	48	ss, s
9. $CH(OOCCH_3)CH(CH_3)CH_2$-(trans)$CH=CHCH_3$	< 1	8[b]	12	s
10. $CH(OOCH)CH(CH_3)CH_2$-(trans)$CH=CHCH_3$	< 1	16[b]	19	s
11. $CH(OH)CH(CH_3)CH_2CH_2OCH_3$	6	12[b]	8	s

Table 1.2. Continued

Entry	CsA Analogue	CyP binding (% of CsA) or Ki(nM)	Immuno-suppressive (% of CsA)	IL-2 release inhibition (% of CsA)	Source
12.	$CH(OH)CH(CH_3)CH_2CH_2OEt$	6	5[b]	11	s
13.	$C(O)CH(CH_3)(CH_2)_3CH_3$	< 1	< 1[b]	1.4	
14.	[(4S)-MeBmt[1]]		2-4[c]		s
15.	$CH(OH)CH_2CH_2CH{=}CHCH_3$ [MeBth[1]]		10-13[c]		s
16.	$CH(OH)CH_2CH(CH_3)CH{=}CHCH_3$ [5-Me MeBth[1]]		10-15[c]		s
17.	$C(3S\text{-}OH)(CH_3)CH_2CH_2CH{=}C(CH_3)_2$		<< 1[c]		s
18.	$C(CH_3)(3R\text{-}OH)CH_2CH_2CH{=}C(CH_3)_2$		<< 1[c]		s
19.	$CH(OH)CH(CH_3)CH_2OCH_2Ph$ [MeBOmt[1]]		20-25[c]		s
20.	$CH(OH)CH(CH_3)CH_2OCH_2CH_3$ [MeEOmt[1]]		7-10[c]		s
21.	$CH(OH)CH(CH_3)CH_2OCH_3$ [MeMOmt[1]]		2-4[c]		s
22.	$CH(OH)CH(CH_3)CH_2OH$ [MeHOmt[1]]		2[c]		s
23.	$CH(OH)CH(CH_3)CH_2OCH_2Ph$(p-benzoyl)		5[c]		s
24.	[MeThr[1]]		< 0.1[h]		s
25.	[MeAbu[1]]		<<0.1[h]		s
26.	[Me(3-OH)Leu[1]]		< 1[h]		s
27.	[N-Me-2-Amino octanoic acid[1]]CsA (CsZ)		_[1]		n

Table 1.2. Continued

Entry	CsA Analogue	CyP binding (% of CsA) or Ki(nM)	Immuno-suppressive (% of CsA)	IL-2 release inhibition (% of CsA)	Source
28.	$CH(OH)C(CH_3)_2CH_2CH=CHCH_3$ [$MeBm_2t^1$]	540 nM[k]	30[i], 50[k]		s
29.	$CH(OH)CH(CH_3)CH_2C=C\text{-}CH_3$ [$MeByt^1$]		10[i]		s
30.	Bmt^1 [CsL]		+[e]		n
31.	$CH(OOCH)CH(CH_3)CH_2$ (epoxy-CH-CH)CH_3	< 1[b]	1[b]	1[b]	s
32.	$CH(OH)CH(Me)CH_2CH_2OH$	16[b]	< 1[d]	2[b]	s
33.	tetrahydrofuran alcohol[b]	1.3[b]	< 1[b]	< 1[b]	s
34.	[3'-deoxy-MeBmt] [CsF]		(+)[e]		n
35.	[MeCyclohexylala^1]CsA		_[l]		ss
	Bis-substituted analogs				
36.	[(3'-deoxy)$MeBmt^1$,Val^2] [CsK]		_[l]		n
37.	[$MeLeu^1$,Nva^2] [CsO]		(+)[e]		n
38.	[Bmt^1,Thr^2] [CsP]		+[e]		n
39.	[Thr^2,Val^4] [CsS]		(+)[e]		n
40.	[Nva^2,Val^5] [CsM]		++[e]		n
41.	[Nva^2,Leu^6] [CsY]		+[e]		n
42.	[Nva^2,Leu^9] [CsX]		+[e]		n
43.	[Nva^2,Leu^{10}] [CsN]		+[e]		n
44.	[Val^2,Leu^{10}] [Cs I]		(+)[e]		n
45.	[Thr^2,Val^{11}] [CsW]		(+)[e]		n
46.	[MeAla^6,dihydro$MeBmt^1$]	10[a]	< 0.1[a]		s

Table 1.2. Continued

Entry CsA Analogue	CyP binding (% of CsA) or Ki(nM)	Immuno-suppressive (% of CsA)	IL-2 release inhibition (% of CsA)	Source
2-position analogs				
47. Thr [CsC]		+++[d]		n
48. Ser		++[d]		s
49. Ala [CsB]		++[d]		n
50. Val [CsD]		++[d]		n
51. Nva [CsG]	23	+++[d], 40[b]	50	n
52. S-CH_3-Cys	2.5	8[b]	11	n
53. 4,4-Difluoro-Abu	26	59[b]		s
54. 4-Fluoro-Abu	12	32[b]		s
55. 5-Fluoro-Nva	2	8[b]		s
56. 4-Fluoro-Nva		1[b]		s
57. S(O)CH_3-Cys	< 0.1[a]	< 0.1[a]		s
58. Allyl-Gly		++[l]		ss
3-position analogs				
59. (D)-Pro		+[d]		s
60. (L)-Pro		+[d]		s
61. (D)-MeAla	82	++[d], 50[g], 61[a]		ss, s
62. (L)-MeAla		+[d], 5[g]		ss, s
63. (D)-MePhe		+[d]		ss
4-position analogs				
64. Val [CsQ]		+[e]		n
sidechain modifications				
65. CH_3	6	< 2[b]	1	s

Table 1.2. Continued

Entry CsA Analogue	CyP binding (% of CsA) or Ki(nM)	Immuno-suppressive (% of CsA)	IL-2 release inhibition (% of CsA)	Source
66. $CH(CH_3)_2$	0.5[t]	130[t]	2500[t]	s
67. CH_2CH_3	1.2[t]	65[t]	163[t]	s
68. CH_2-cyclopropyl	2.8[t]	5.0[t]	3.5[t]	s
69. $CH_2C(CH_2)(CH_3)$	1.8[t]	1.6[t]	4.0[t]	s
70. $CH_2CH_2CH(CH_3)_2$	1.9[t]	3.5[t]	9.0[t]	s
71. $CH_2CF(CH_3)_2$	2.1[t]	25[t]	7.0[t]	s
72. $CH_2C(OH)(CH_3)_2$	0.9[t]	130[t]	110[t]	ss
5-position analogs				
73. Ala	10	19[b]	14	s
6-position analogs				
74. MeAla	51[a], 48[b]	0.4[a], 1[b]	1	s
75. MeVal	5[a], 4[b]	0.2[a], 0.4[b]		s
76. MeNva	43[a], 52[b]	45.8[a], 49[b]		s
77. MeAbu	78[a],	7[a]		s
78. MeIle	5[a,b]	1[a,b]		s
79. MePhe	31[a], 16[b]	11[a], 13[b]		s
80. Leu [CsU]		+[e]		n
7-position analogs				
81. ABu [CsV]		++[e]		n
82. (D)-Ala		+[d]		s

Table 1.2. Continued

Entry CsA Analogue	CyP binding (% of CsA) or Ki(nM)	Immuno-suppressive (% of CsA)	IL-2 release inhibition (% of CsA)	Source
8-position analogs				
83. Des-(D)Ala		+[d]		S
84. (D)-Lys				S
85. (ε-Alloc)-(D)-Lys		20[f]		S
86. (ε-Boc)-(D)-Lys		10[f]		S
87. (ε-Aipps)-(D)-Lys		10[f]		S
88. (D)-Dab(Boc)	2.4nM	14		S
89. (D)-Dab	4 nM	30[k]		S
90. (D)-Dap	4 nM	10[k]		S
91. (D)-Ser		+++[l]		SS
92. [(D)-Ser[8]]CsC		++[l]		SS
93. [(D)-Ser[8]]CsD		+++[l]		SS
94. [(D)-Ser[8]]CsG		+++[l]		SS
95. [2-Deutero-3-fluoro-(D)-Ala]		80.7[m]		SS
96. [Δ-Ala]		30[m]		SS
97. [S-Me-(D)Cys]		85.4[m]		SS
98. [S-CH_2CH_2OH-(D)Cys]		80.8[m]		SS
99. [S(O)-Me (D)Cys]		5.6[m]		SS
100. [β-Chloro-(D)Ala]		++[q]		SS
101. [O-CH_2CH_2OH-(D)Ser] [SDZ IMM 125]	100[n]	100[n]	100[n]	SS

Table 1.2. Continued

Entry CsA Analogue	CyP binding (% of CsA) or Ki(nM)	Immuno-suppressive (% of CsA)	IL-2 release inhibition (% of CsA)	Source
<u>9-position analog</u>				
102. MeAla	7[b]	10[a], 3[b]	10[b]	s
<u>10-position analogs</u>				
103. MeAla	4[b]	7[a,ab]	8[b]	s
104. Leu [CsT]		++[d]		n
<u>11-position analogs</u>				
105. (D)-MeVal [CsH]	< 0.1[r]	–[e]		n
106. Val		(+)[e]		n
107. MeAla	8.5[b]	11[b]	15[b]	s
108. MeLeu	< 1[b]	< 1[b]	< 1[b]	s
109. MeIle		++[d]		s
110. aMeIle		+[d]		s
<u>Miscellaneous</u>				
111. 3,4-Lactam		5[d]		s
112. des(8,9,10)-γ-Abu		+[d]		s
113. des(8,9,10)-γ-MeAbu		+[d]		s
114. Tricyclic CsA[P]	2±0.5 nM	2 nM[P]		s
115. [Thr[2], Leu[5], Leu[10]]CsA (FR901459)	33[u]	33[u]		n

a. PMA/iono -induced murine T cell proliferation assay; data from ref. 10.

b. Either mixed lymphocyte reaction inhibition or PMA/iono -stimulated inhibition and detailed structures see ref. 9.

c. Con A-stimulated murine thymocytes inhibition; data from ref. 15 and 16.

d. Ratings based on a scale of one to three, where "+" : little or no activity, "++": average activity, "+++": strong activity; data from ref. 18.

e. Ratings based on a scale of one to five, where "_": no activity, "(+)": slight activity, "+": moderate activity, "++": good activity, "+++": strong activity; data from ref. 46.

f. Dunlap, B.; Tung, R. D.; Rich, D. H. unpublish data.

g. Data from ref. 118.

h. Con A-stimulated murine thymocytes inhibition; data from ref. 17.

i. Con A-stimulated murine thymocytes inhibition; data from ref. 15.

j. "n": natural, "ss": semi-synthesis, "s": synthesis.

k. Data from ref. 14.

l. Ratings based on a scale of one to three, where "+" : little or no activity, "++": average activity, "+++": strong activity; data from ref. 113.

m. PMA/iono -induced murine T cell proliferation assay; data from ref. 114.

n. potency reported similar to CsA; data from ref. 115.

P. The activity was based on IC_{50} (4 nM for CsA) and the detailed structure of the tricyclic CsA was shown in ref. 116.

q. Data from ref. 117.

r. Shown as Percentage (%) of IC_{50} of CsA; data from ref. 19.

t. Results expressed as relative IC_{50} derivative/IC_{50} CsA; data from ref. 173.

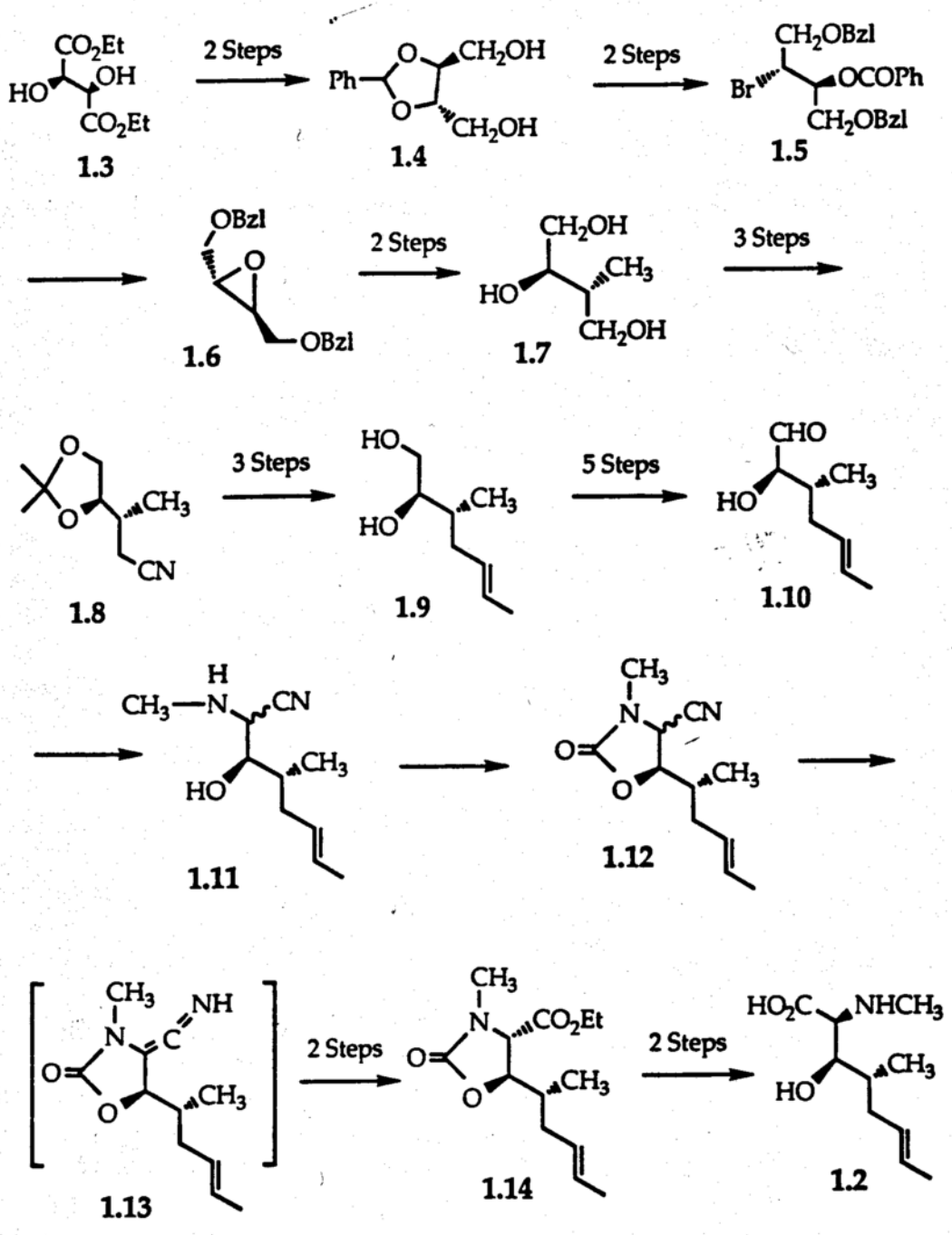
u. Data from ref. 174.

F. *Chemistry of CsA*

1. *Synthesis of MeBmt*

The first total synthesis of the unusual amino acid MeBmt was reported by Wenger in 1983.[7] The challenge for a synthesis of MeBmt is the building of the three contiguous asymmetric centers. The synthetic approach is illustrated in Scheme **1.1**. Starting from (R,R)-(+)-tartaric acid, an optically active triol **1.7** was obtained after a series of operations. The key step in the formation of the triol is the introduction of the methyl group by reacting the (S,S)-epoxide **1.6** with methyllithium cuprate, which proceeds in excellent yield (89%). This provided the correct centers for C3 and C4 found in MeBmt. In a second operation, the vicinal hydroxyl groups were selectively protected as ketal **1.8**, followed by oxidation of the primary alcohol to the aldehyde. A Wittig olefination under Schlosser conditions and removal of the acetonide gave the trans olefinic glycol **1.9**. After a series of protection/deprotection steps and an oxidation, the unprotected aldehyde **1.10** was obtained. The final operation began with a Strecker reaction, followed by formation of the oxazolidinone as a mixture of diastereomers **1.12**. Ethanolysis of the nitrile through the ketene imine intermediate **1.13** set the correct center at C_2. Acidic hydrolysis followed by basic conditions afforded enantiomerically pure MeBmt (**1.2**).

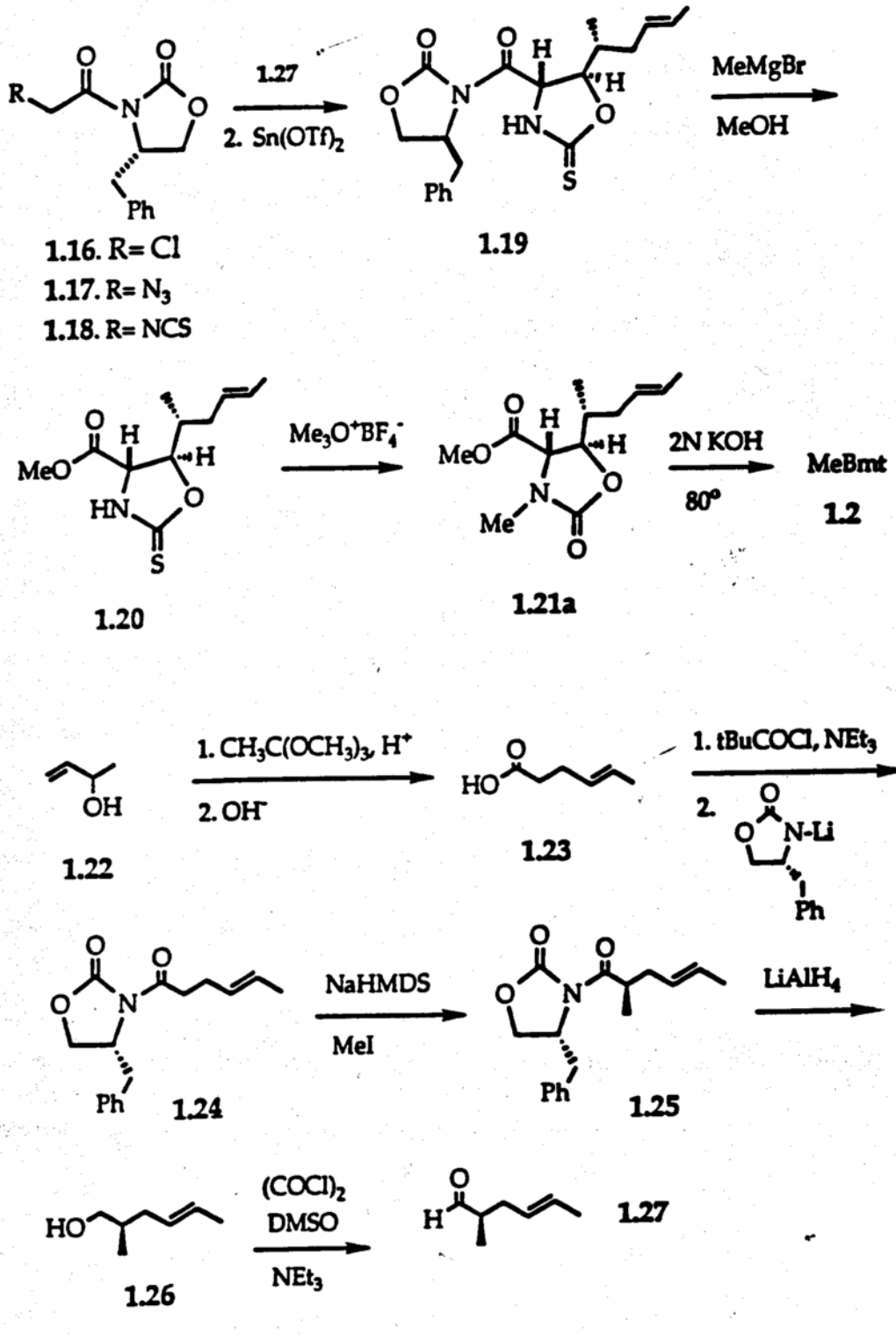
The accomplishment of the finely designed stereospecific synthesis of



Scheme 1.1. Wenger's MeBmt Synthesis

MeBmt opened the way for the total synthesis of CsA and its derivatives. CsA analogs could now be prepared to study the structure-activity relationships and to pursue immunochemical investigations. Due to the synthetic challenge and length of synthesis requiring 24 steps, several other approaches have also been reported.

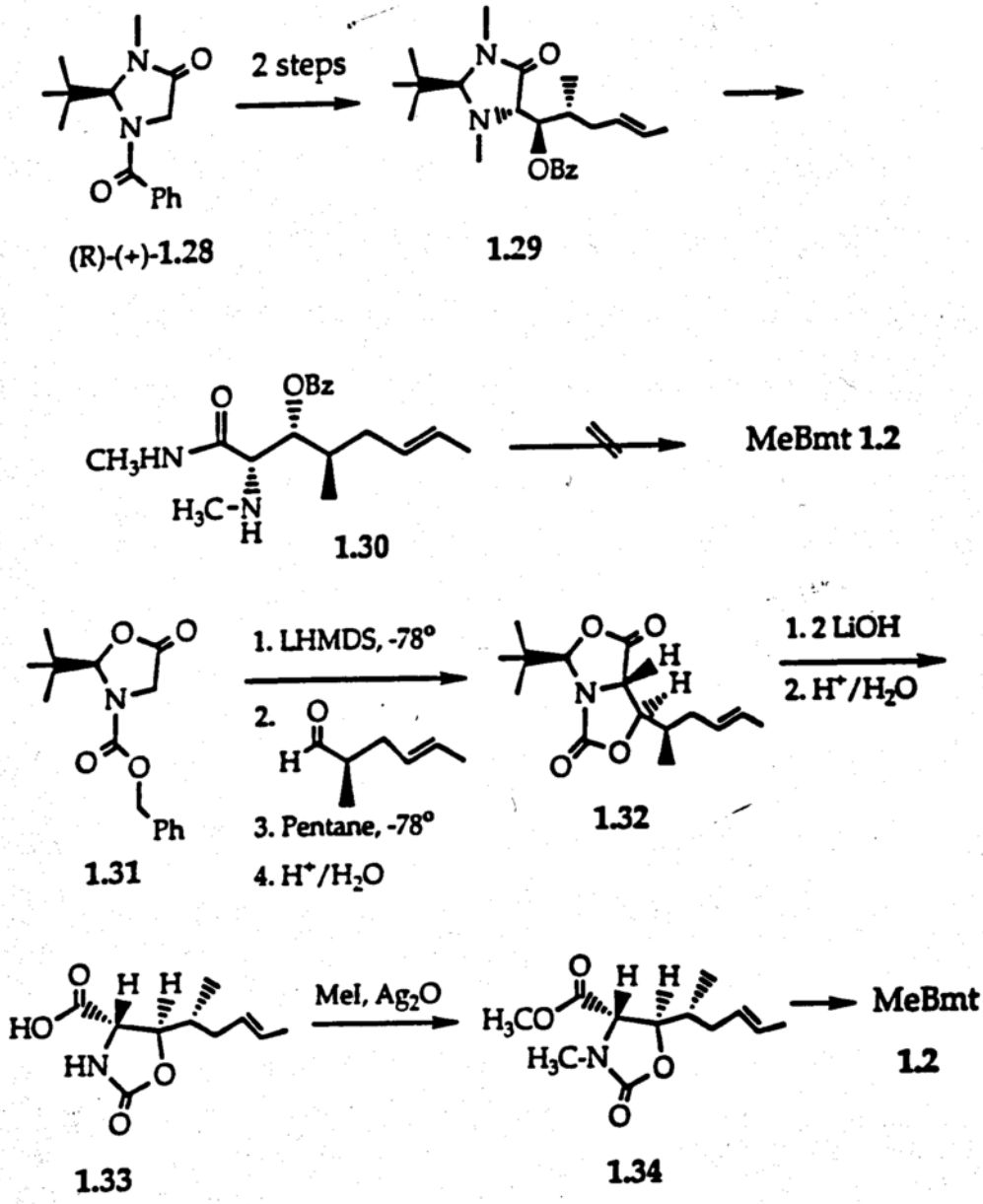
Evans and Weber utilized an oxazolidinone chiral auxiliary for absolute stereochemical control in the asymmetric glycine enolate aldol reaction (Scheme **1.2**).[119] The chiral glycine synthon was prepared from the corresponding isothiocyanate **1.18**, which was obtained through a series of steps from chloroacetate **1.16**. The critical chiral hexenyl aldehyde **1.27** was prepared starting from a Claisen rearrangement between butenol **1.22** and trimethyl orthoacetate. The resulting hexenoic acid **1.23** was incorporated into a oxazolidinone chiral auxiliary followed by methylation of the corresponding sodium enolate to yield the oxazolidinone **1.24**. Reductive cleavage of the chiral auxiliary and Swern oxidation give the desired aldehyde **1.27**. The stannous triflate-mediated aldol reaction of the isothiocyanate **1.18** and aldehyde **1.27** afforded the desired *threo* aldol adduct as the *trans*-thiooxazolidinone **1.19** (> 90% e.e). The simultaneous cleavage of the chiral auxiliary and transesterification of the aldol adduct was performed using methylmagnesium bromide and methanol to give the methyl ester **1.20**. The introduction of the N-methyl group was achieved by



Scheme 1.2. Evans and Weber Synthesis of MeBmt (ref. 119)

treatment of **1.20** with Meerwein's reagent to afford N-methyl oxazolidinone **1.21a**. Basic hydrolysis of the oxazolidinone **1.21a** by Wenger's procedure produced enantiomerically pure MeBmt (**1.2**).

Seebach and co-workers described a general synthesis the *threo* β-hydroxy α-amino acids, which utilized chiral glycine enolate precursors based on imidazolidinones, such as (R)-(+)-Cbz-BMI (**1.28**) shown in Scheme **1.3**.[120] Although this methodology successfully afforded *threo* β-hydroxy α-amino acids in high enantiomeric purity, the rather drastic conditions required for the final hydrolysis of **1.29** have limited its use only to the preparation of amino acids that do not contain acid-sensitive substituents. This problem was later solved by using oxazolidinones as chiral glycine building blocks.[121] The approach is shown in Scheme **1.3**. The aldol reaction of the chiral oxazolidinone **1.31** and the known aldehyde **1.27** was performed in pentane under optimized reaction conditions (LHMDS, -100 °C, 20 min). The expected aldol adducts (epimeric mixture) were obtained only as minor products. The major product, the bicyclic carbamate **1.32**, was first regarded as a troublesome and unwanted byproduct, but it turned out to be "just right" in view of the goal of synthesizing MeBmt. Simple hydrolysis using LiOH in THF/H_2O afforded the carboxylic acid **1.33** quantitatively. N-methylation was performed using MeI/Ag_2O in DMF. The product **1.34**, a known precursor of MeBmt, was then converted to the final amino acid following Wenger's

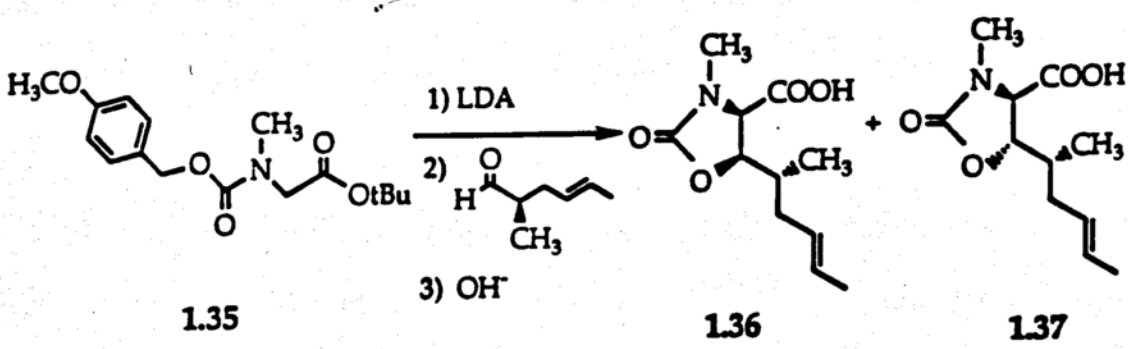


Scheme 1.3. Seebach's Synthesis of MeBmt (ref. 120, 121)

procedure.

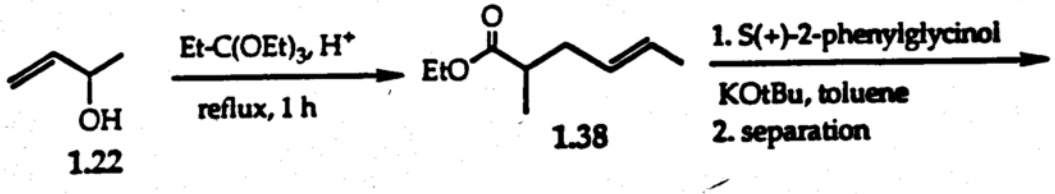
Rich and co-workers reported a MeBmt synthesis by an aldol reaction between the chiral aldehyde and N-methylglycine (sarcosine).[122] This method was developed by using Shanzer's diastereoselective synthesis of *threo*-α-amino-β-hydroxy acids.[123] As shown in Scheme **1.4**, reaction of aldehyde **1.27** and the lithium enolate of **1.35** at room temperature, followed by heating the reaction solution under reflux with ethanolic KOH, gave a crude product containing two *trans*-oxazolidinones **1.36** and **1.37** in a ratio of 47:53. The unwanted starting material is easily removed by an acid wash. After resolving the mixture by ephedrine crystallizations, the desired diastereomer **1.36** was obtained. It was converted to pure MeBmt by Wenger's basic hydrolysis procedure. Rich and Deyo also described an economical route towards the chiral aldehyde **1.27** (Scheme **1.4**).[124] The unsaturated ester **1.38** was obtained via an orthoester Claisen rearrangement between 1-buten-3-ol (**1.22**) and triethyl orthopropionate. Direct coupling of the ester to the chiral auxiliary L-2-phenylglycinol under basic KOtBu gave the desired amide **1.39b** after separation of the two diastereomeric products. Hydrolysis followed by reduction using $LiAlH_4$, gave alcohol **1.40**. Swern oxidation of the alcohol gave the desired chiral aldehyde **1.27**.

Although many effective approaches towards MeBmt have been reported, several groups continue to work on this problem. For instance, another

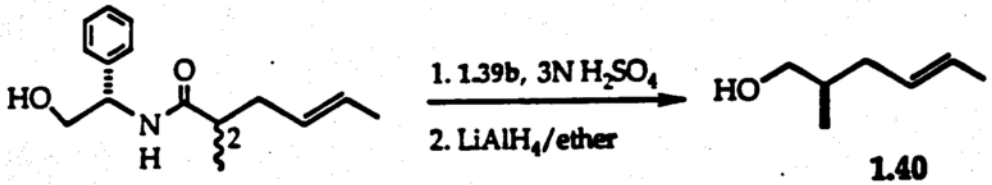


1.35 1.36 1.37

1) Resolution
2) aq. KOH
Reflux
MeBmt 1.2

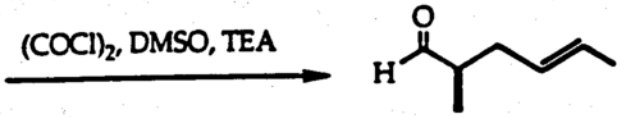


1.22 1.38



1.39a. (2S)
1.39b. (2R)

1.40



1.27

Scheme 1.4. Rich's MeBmt Synthesis (ref. 122, 124)

MeBmt synthesis based on Shanzer's methodology for diastereoselective synthesis of *erythro* β-hydroxy α-amino acids was reported by Schmidt and Siegel.[125] Togni and co-workers[126] described a short route to MeBmt by an asymmetric homogeneous transition metal-catalyzed reaction based on Hayashi and Ito's report.[127] Rao's[128] and Rich's[129] groups independently reported a linear MeBmt synthesis involving the Sharpless asymmetric epoxidation. Rapoport's group[130] described a facile method suitable for large-scale preparation of MeBmt and its analogs starting from (D)-Ser. All these approaches provided enantiomerically pure MeBmt that can be readily applied to the total synthesis of CsA analogs.

2. *Synthesis of CsA*

a. *General Strategy*

The total synthesis of the cyclo-undecapeptide CsA was first reported by Wenger in 1984.[8] The strategy followed a route in which the peptide was built up in the direction of the arrows in Figure **1.6** by using the sequence of steps, indicated numerically. The point of cyclization was chosen at the peptide bond between Ala[7] and (D)-Ala[8] for the following two reasons: 1) both amino acids are without an N-methyl group, which presents an easier bond formation as compared to bond formation involving N-methyl amino acids; 2) intramolecular H-bonds might be present in the linear undecapeptide,

which stabilize the linear undecapeptide in a folded conformation thus facilitating ring closure. For the synthesis of the linear undecapeptide, a technique of fragment coupling between the tetrapeptide (residues 8-11) and the heptapeptide (residues 1-7) was chosen. The heptapeptide fragment was prepared by a fragment coupling of the dipeptide (residues 2-3) and tetrapeptide (residues 4-7), followed by introducing the amino acid MeBmt at the end of the synthesis. This sequence had two obvious advantages: 1) fragment coupling onto the sarcosine (residue 3) prevented the possibility of racemization; 2) the number of steps after the introduction of the precious MeBmt was minimized. The undecapeptide could be cyclized to CsA after removal of N- and C-terminal protecting groups.

Further discussion on the synthesis of this peptide will be presented in the following sections.

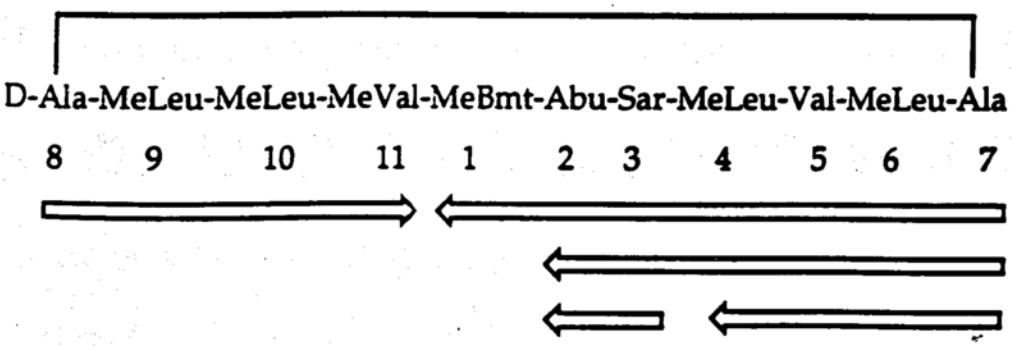


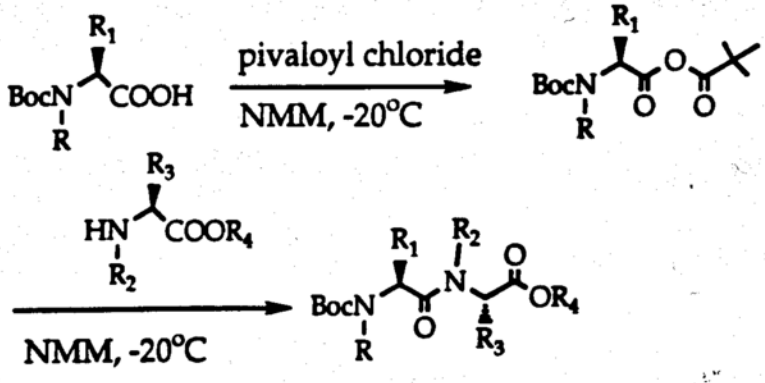
Figure 1.6. Wenger's Strategy for Synthesis of Cyclosporin A

b. *Synthesis of the Peptide Fragments* of CsA

Wenger applied a variation of the mixed pivalic anhydride method reported by Zaoral[131] to minimize racemization and epimerization during coupling reactions for the synthesis of a series of peptide fragments in CsA. To modify this method for N-methyl amino acids derivatives, Wenger allowed for slow anhydride formation with pivaloyl chloride at -20 °C in chloroform in the presence of 2 equivalents of tertiary base such as N-methylmorpholine before adding the O-protected amino acid or peptide to be coupled as free base (Scheme **1.5**). Application of this mixed pivalic anhydride method allowed the ready synthesis of 8-11 tetrapeptide and 1-7 hexapeptide of CsA, with the exception that dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) were employed in the final 1 + 6 coupling.[8] The hexapeptide was made from the fragment coupling of dipeptide (residue 2-3) and tetrapeptide (residue 4-7). The tetrapeptide H-MeLeu-Val-MeLeu-Ala-OBzl was synthesized in the conventional fashion from the right to the left (C to N terminal, see Figure **1.6**) to avoid racemization of the N-methyl amino acids. In contrast, the 8-11 tetrapeptide was built up in a highly unconventional fashion from the left to the right also indicated in Figure **1.6**. This tetrapeptide could not be made in the reverse direction starting with H-MeVal-OBzl, due to instant diketo-piperazine formation of the dipeptide ester H-MeLeu-MeVal-OBzl.[132] Wenger's route also suffered from some

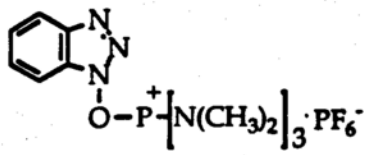
epimerization of residues 10 and 11 due to the N- to C-terminal coupling strategy.

Very interesting results were obtained for the fragment coupling of the



Scheme 1.5. Wenger's Synthesis of Peptides using Pivaloyl Chloride

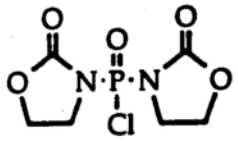
tetrapeptide (Boc-(D)-Ala-MeLeu-MeLeu-MeVal-OH, residues 8-11) and the heptapeptide (H-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl, residues 1-7). Using pivaloyl chloride at -20 °C, the unexpected undecapeptide, in which (L)-MeVal in position 11 was epimerized to (D)-MeVal, was the major product, which was isolated in 44% yield. In contrast, use of Castro's BOP reagent ($BtOP^+(NMe_2)_3 \cdot PF_6^-$, **1.41**) as the coupling reagent afforded the desired undecapeptide (with the retention of (L)-MeVal in position 11) in 73% yield.



1.41

When the BOP reagent was applied to the coupling with the tetrapeptide containing (D)-MeVal, surprisingly, only product with (L)-MeVal in position 11 of the undecapeptide was formed in 47% yield. No explanation for these stereoselective fragment couplings has been proposed.

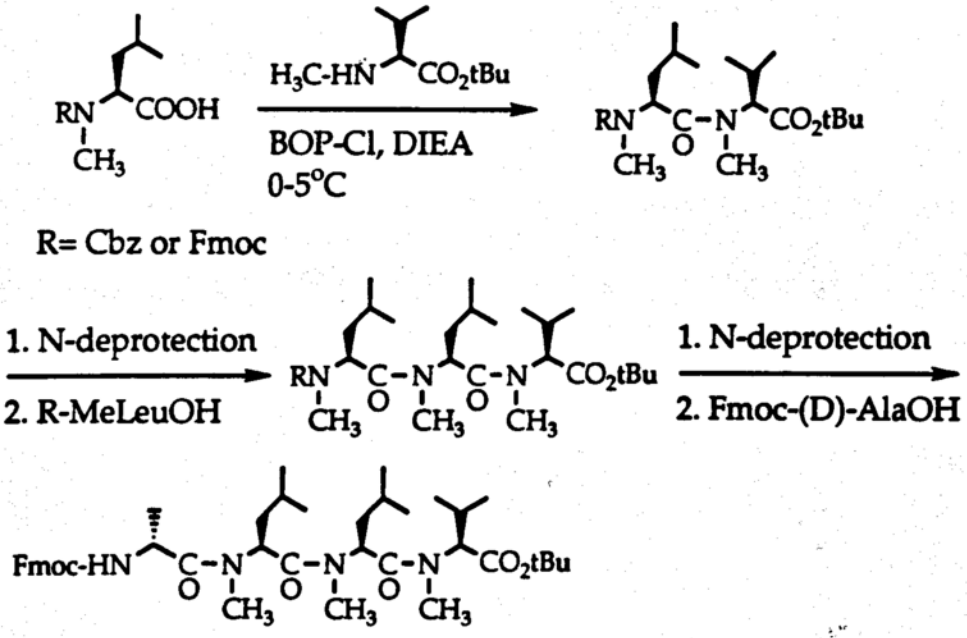
Rich and Tung developed an efficient method for synthesizing the CsA 2-7 hexapeptide[133] and the 8-11 tetrapeptide[134] fragments by using bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl, **1.42**)[151] as the coupling reagent.



1.42

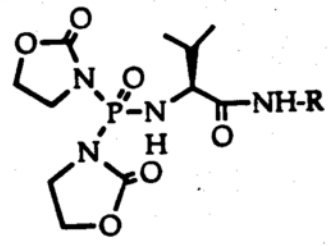
The BOP-Cl method allowed reactions at easily obtained temperatures (0-10 °C) and with shorter reaction times (4-20 h), and avoided epimerizations of MeLeu. The successful synthesis of the tetrapeptide (residues 8-11) with BOP-Cl was performed by choosing the tert-butyl ester as carboxylate protecting group and proceeded in the usual C to N direction, using the non-acid labile 9-fluorenylmethoxycarbonyl (Fmoc) or benzyloxycarbonyl (Cbz) groups for the N-terminal protection (Scheme **1.6**). Cbz protection provided more stable intermediates suitable for larger scale synthesis.

The utilization of BOP-Cl for the synthesis of the 2-7 hexapeptide fragment encountered a few unexpected difficulties. A poorer yield (compared with



Scheme 1.6. Synthesis of Tetrapeptide (Residues 8-11) by BOP-Cl Method (ref. 134)

Wenger's pivaloyl chloride method) was usually obtained for the coupling between H-Val-MeLeu-Ala-OBzl and Boc-MeLeuOH. This was probably due to the hindrance between the bulky side chains (isopropyl and isobutyl groups) of the substrates and the reaction of BOP-Cl with primary amine, yielding phosphinamidates (**1.43**) as a side product.



1.43

c. *Approaches towards Cyclization of CsA Precursors*

According to Wenger's methods, cyclization was first attempted by the azide method due to its resistance to racemization.[135] First, the linear Boc-protected undecapeptide benzyl ester was transamidated to a hydrazide which, after deprotection of the N-terminal Boc-group, was converted to the active azide leaving group by treatment with nitrous acid. Cyclization of the resultant azide was performed in dilute solution (4×10^{-3} M) with diisopropylethylamine at -20 °C to give the desired CsA in only 15% yield. Wenger found that a better yield could be achieved by using the fully deprotected undecapeptide (removal of C-terminal benzyl ester with aqueous NaOH and N-terminal Boc by TFA at -20 °C) directly in the cyclization. Castro's BOP reagent[136], DCC/pentafluorophenyl (Pfp) complex and propylphosphonic anhydride are all suitable for the final cyclization with the free undecapeptide in a dilute solution (~ 1×10^{-4} M) in the presence of a tertiary base to give a yield of 50-65% at room temperature.

In addition, Rich's group also developed a similar method to accomplish the cyclization reaction.[133] The linear undecapeptide, derived from 4 + 7 coupling, was obtained in the N-Fmoc and C-benzyl protected form that could be deprotected simultaneously under aqueous NaOH in ethanol at 0 °C. The resultant free undecapeptide was cyclized by Wenger's procedure to afford cyclosporines in yields similar to those obtained by the high dilution method.

Chapter II. Research Plan

As stated on page 13, one of the early actions of CsA thought to be involved in the mechanism of immunosuppression is the inhibition of lymphokine IL-2 messenger RNA synthesis. Several studies have suggested the involvement of a receptor mediated event in this inhibition. This process was thought originally to result from the binding of the drug to cyclophilin (CyP). Subsequently, CyP was discovered to be a peptidyl-prolyl *cis-trans* isomerase (PPIase).[23,24] Later, the structure-activity data of CsA analogs demonstrated that inhibition of CyP's PPIase activity does not always correlate with immunosuppression. Most notably, the weakly immunosupressive analogue [MeAla^6]CsA (see Table **1.2**, entry 74) strongly inhibits CyP whereas the moderately immunosuppressive analogue [MeBm$_2$t^1]CsA (Table **1.2**, entry 28) poorly inhibits CyP. These exceptions may be explained by the recent discovery that the complex formed between CsA and CyP inhibits the calmodulin-dependent phosphatase, calcineurin (CaN). These results also suggest that other binding proteins may exist which exhibit properties more consistent with the immunosuppressive or other biological actions of the drug. Recently, the Rich group utilized a biologically active [(D)-Lys^8]CsA analog, to which N-(3-(4-azido-3-^{125}I-iodophenyl)propionyl)-succimide (A^{125}IPPS)[137] has been coupled, to investigate if other proteins exist in immune competent cell types.[138]

I began my research by building functionalities at positions 6 and 8 of CsA, which are presumably either near or at the effector domain (residues 4-8) of the bound CsA, that could be used to incorporate a suitable photoaffinity labeling agent. By using this type of photoaffinity labeling CsA analog, it would be possible to search for new target proteins in immune-related cells. This remains an important objective because a detailed understanding of the mechanism of action of CsA for all its broad biological activities, including its adverse effects, remains elusive.

Although CsA has been regarded as the prototype of a new generation of immunosuppressive drug, efforts to discover new analogs or other agents of a different structure continue. The goal of these efforts is to overcome the adverse effects of CsA and to improve the pharmacokinetic profile of CsA, especially the absorption, distribution and metabolism in the clinical use of the drug. Over the last decade, a wide variety of CsA analogs have been synthesized and evaluated for biological-activity relationships, but very few analogs with multiple substitutions at residues 3-8 of CsA were developed. Most naturally occurring bis-substituted CsA metabolites (see Table 1.2, pages 25-31) were modified at residues 9, 10, 11, 1 and 2 and are devoid of immunosuppressive effects. Thus, the lack of convincing data that single site analogs accurately predict activity of multiply substituted analogs, encouraged me to design and synthesize multiply substituted analogs modified either near or at the effector domain of enzyme-bound CsA.

As my work progressed, several investigators[79-82] independently demonstrated that CsA can inhibit replication of the human immunodeficiency virus-I (HIV-I) by an unknown mechanism. Several explanations for the antiviral effect of CsA have been proposed, but all were based on assuming an immunosuppressive effect of CsA. *In vitro* CsA was supposed to help prevent the virus from infecting and killing CD4 T lymphocytes in the early stage of infection. As previously described (page 15), two target proteins, a peptidyl-prolyl *cis-trans* isomerase CyP and a protein phosphatase CaN, are involved in the biological actions of cyclosporines. It was not clear, however, whether CyP acts mainly by presenting CsA to its target molecules, or whether CyP has an intrinsic biological activity directly relevant for HIV replication. A recent report described that gag protein Pr55gag binds strongly to cyclophilin A (CyPA) and CsA can efficiently disrupt the gag-CyPA interaction. This suggested that a gag-CyPA interaction may play an important role in acquired immunodeficiency syndrome (AIDS) immunopathology.[84] Since Rich's group previously reported[17] that several non-immunosuppressive CsA analogs are tight-binding inhibitors of CyP, we decided to test if non-immunosuppressive CsA analogs would inhibit HIV replication. We reasoned that if non-immunosuppressive CsA analogs could inhibit replication, then the immune system would not have to be suppressed by the normal action of CsA. Therefore, there was a need for novel non-immunosuppressive CsA analogs that could be used to explore

the biological effect on the replication of HIV-1.

In summary, I started my project with the following specific aims:

1. Develop an orthogonal strategy towards the synthesis of CsA analogs suitable for photoaffinity labeling studies.

2. Design and synthesize multiply substituted CsA analogs and determine their CyP-binding affinity and immunosuppressive activities.

3. Synthesize novel non-immunosuppressive CsA analogs and evaluate their activities on HIV replication.

Chapter III. Synthesis of Suitable Precursors for Photoaffinity Labeling of CsA

Progress in the development of chemically efficient methods for the total synthesis of CsA and its analogs has been made in Rich's laboratories during the past several years.[15-17,123,133,134] The overall strategy is similar to that reported by Wenger,[8] with the exception that BOP-Cl instead of pivaloyl mixed anhydride has been used to synthesize the 2-7 hexapeptide and 8-11 tetrapeptide fragments by the usual C to N terminal extension with high efficiency (Figure 3.1). The use of benzyl ester protection of the carboxyl terminal at residue 7 and N-terminal Fmoc group at residue 8 in the linear undecapeptide is especially advantageous because both groups could be removed simultaneously under basic conditions and the resulting free undecapeptide cyclized cleanly using high dilution techniques.[139]

As outlined in Figure 3.1, the temporary protection of the side-chain amino group at 6-position, MeLys, is dependent upon the whole deprotection/coupling strategy to access the desired [MeLys^6]CsA analogs. A 3-(3'-Pyridyl)-allyloxycarbonyl (Paloc) group[140] was chosen as a suitable ε-amino protecting group of MeLys^6 to be consistent with the current N/C-terminal strategy (Fmoc/Bzl). The Paloc group is stable under acidic (TFA) or basic (e.g. 0.2N NaOH/EtOH) conditions[140] and readily removed under neutral conditions (various palladium(0) complexes). I found that

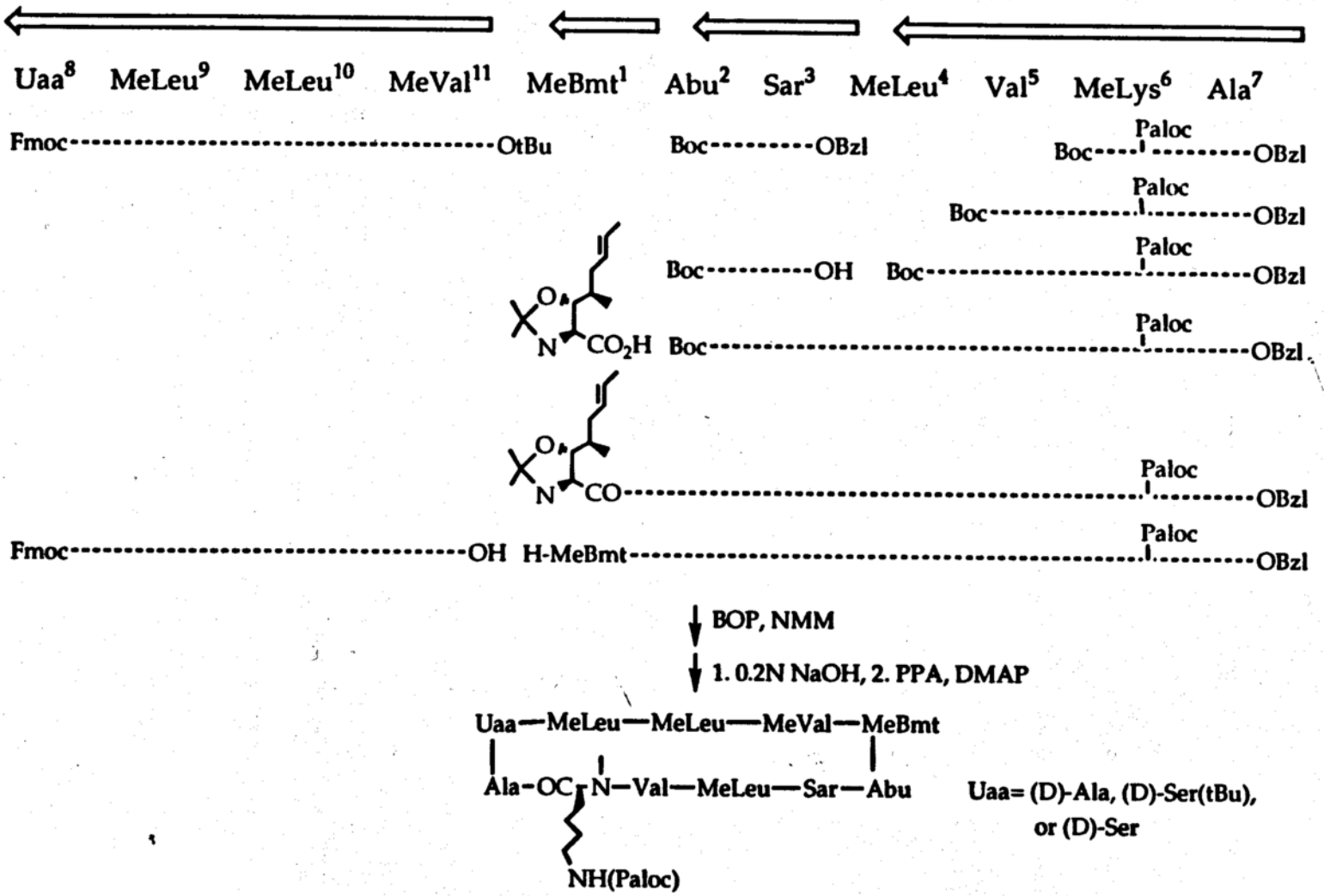
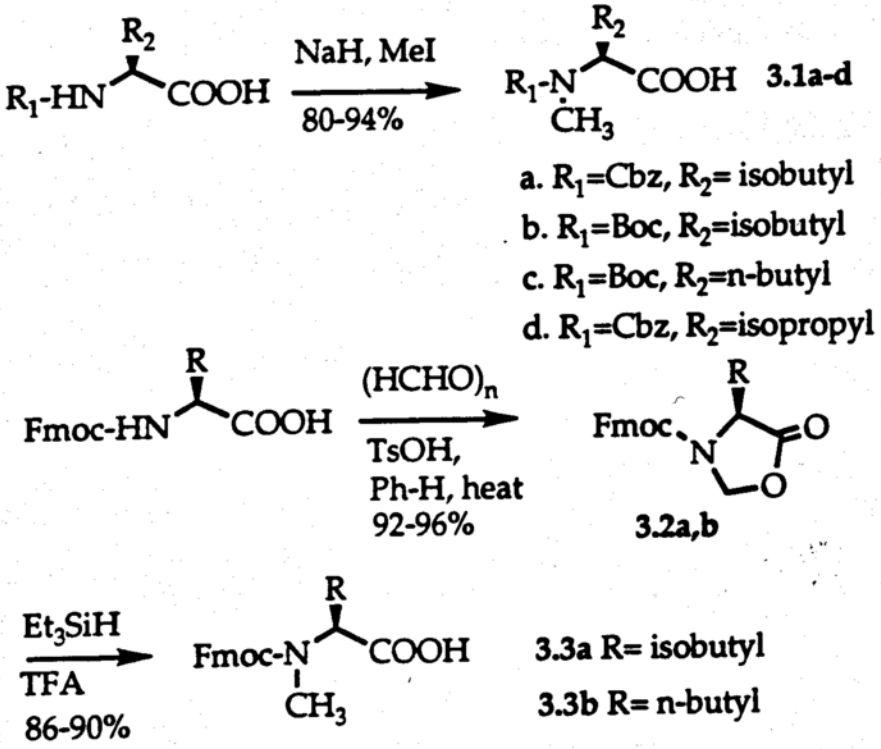


Figure 3.1. Strategy for Synthesis of [MeLys[6]]CsA Derivatives

Pd^{II} complex/Bu_3SnH would work well without disturbing other functional groups in the CsA analogs that I planned to make.

A. *Synthesis of N-Methyl Amino Acids*

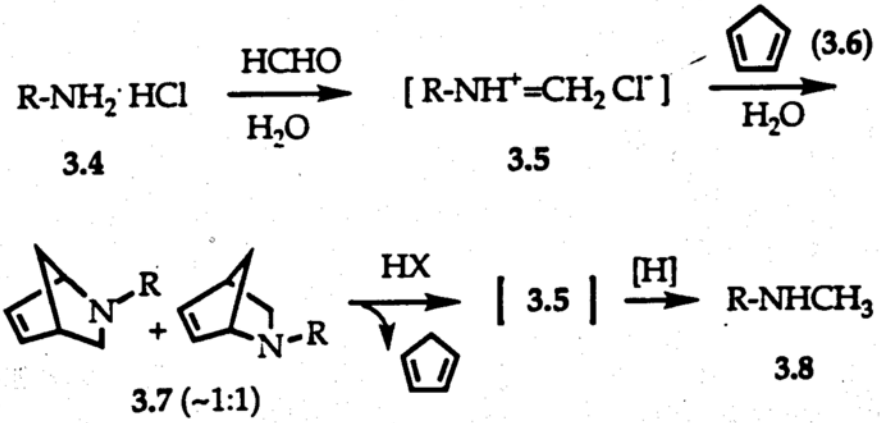
Most of the N-methyl amino acids needed for the synthesis of CsA analogs were prepared according to the procedure of McDermott and Benoiton[141] in which various amino acids protected with base-stable groups such as Boc and Cbz at the N-terminus were treated with NaH/MeI in anhydrous THF at room temperature for one day. This approach easily gave pure N-methyl amino acids (< 1% racemization) suitable for peptide synthesis without ester formation. Thus, Cbz-MeLeuOH, Boc-MeLeuOH, Boc-MeNleOH and Cbz-MeValOH (**3.1a-d**) were obtained in 80-94% yields (Scheme **3.1**) and used as amino acid residues in the designed CsA analogs. In terms of preparation of N-methyl amino acids with a base-labile group such as Fmoc at the N-terminal position, the procedure developed by Freidinger and co-workers[142] was applied and the synthesis of Fmoc-MeLeuOH or Fmoc-MeNleOH is shown in Scheme **3.1**. Fmoc-amino acids were condensed with paraformaldehyde under acid catalysis to form oxazolidinones **3.2a,b** in 92-96% yields. The intermediates **3.2a,b** were readily reduced with triethylsilane and trifluoroacetic acid to give the desired N^{α}-Fmoc-N^{α}-methyl amino acid derivatives **3.3a,b**.



Scheme 3.1. Synthesis of N-Methyl Amino Acids

In order to obtain analogs with [Nα-MeLys] replacing [MeLeu] at position 6 in CsA, an Nα-methyllysine derivative **3.14** was needed. It was known that methylation of amino acids by using NaH/MeI cannot be applied to amino acids such as lysine and histidine. Also, selective formation of the desired oxazolidinone by reaction of Nα-Fmoc-Nε-Cbz-Lys with paraformaldehyde could not be accomplished.[142] Three approaches to Nα-

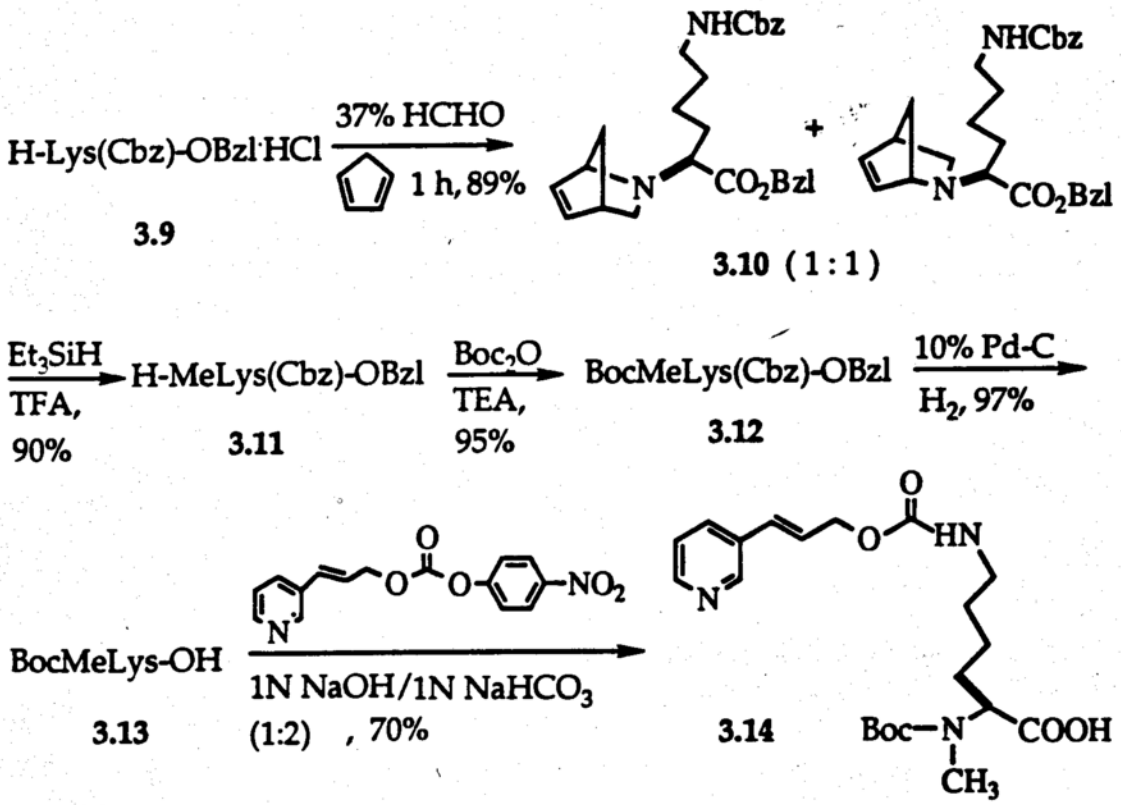
methyllysine[143] derivatives have been investigated and these serve to point out the limitations as well as the versatility of the methods. One of the strategies, developed by Grieco[144], is based on the fact that iminium ions **3.5** generated in situ from primary alkylamines **3.4** (c.f. amino acids) and formaldehyde in water can undergo a facile cyclocondensation with cyclopentadiene (**3.6**) at room temperature to give the 2-azanorbornene derivative **3.7**. The N-methylated amine derivative **3.8** can be obtained during the retro aza Diels-Alder process of the 2-azanorbornene **3.7** when the resultant iminium salt **3.5** is being trapped by reduction (Scheme **3.2**). The synthesis of suitable Nα-Me-lysine derivative **3.14** is shown in Scheme **3.3**.



Scheme 3.2. Grieco's synthesis of N-methyl amines (ref. 144)

Exposure of an aqueous suspension of the hydrochloride salt **3.9** to cyclopentadiene and formaldehyde gave the 2-azanorborn-5-ene derivatives

3.10 as a 1:1 mixture of diastereomers in 89% yield. Subsequent treatment of the azanorbornene with trifluoroacetic acid/triethylsilane for 24 hr gave benzyl Nε-Cbz-Nα-methyllysine ester 3.11[145] (Figure 3.2). After protection of the N-terminus of 3.11 with a Boc group, both Cbz and benzyl groups of the resulting fully-protected ester 3.12 were simultaneously removed by catalytic reduction (H_2, Pd-C) to provide Nα-Boc-Nα-methyllysine (3.13).



Scheme 3.3. Synthesis of Nα-Methyl-Nα-(tert-butyloxycarbonyl)-Nε-[3-(3'-pyridyl)allyloxycarbonyl]-L-lysine (3.14)

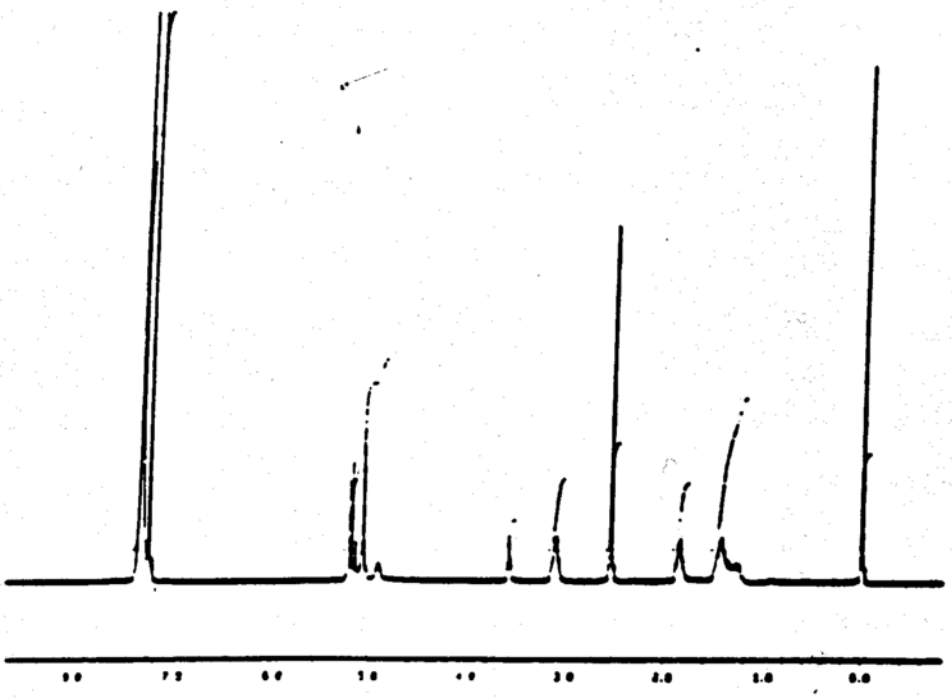


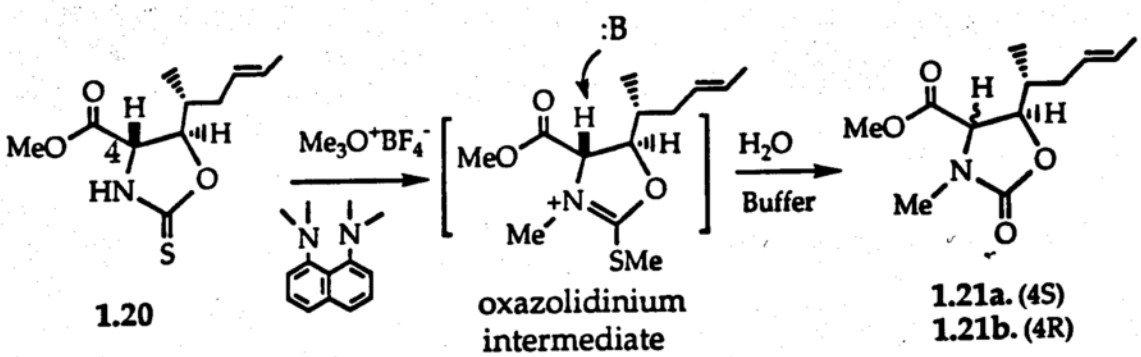
Figure 3.2. ^1H NMR ($CDCl_3$) spectrum of N^{ε}-Benzyloxycarbonyl-N^{α}-methyllysine benzyl ester (**3.11**).

A suitable protecting group, (3-pyridyl)allyloxycarbonyl (Paloc) which derived from 3-(3'-pyridyl)allyl-(4''-nitrophenyl)carbonate (**3.18**, see Scheme 3.5, page 61) was then added to the ε-amino group of BocMeLys (**3.13**) under basic conditions (1N NaOH + 1N $NaHCO_3$)[146] to give the desired N^{α}-Boc-N^{α}-methyl-N^{ε}-[3-(3'-pyridyl)allyloxycarbonyl]-lysine (**3.14**).

B. *Synthesis of MeBmt*

MeBmt can be synthesized by the different approaches described in

Schemes 1.1 ~ 1.4. In the course of this work, the precious amino acid was prepared by using the method of Weber and Evans (see Scheme 1.2, page 36),[119] whereas the critical chiral aldehyde (2R,4E)-2-methyl-4-hexenal (1.27), required for Evans' asymmetric glycine enolate reaction, was obtained via the method of Deyo and Rich (see Scheme 1.4, page 40).[124] Following the procedure in Scheme 1.2, the aldol adduct 1.19 was obtained in 56-62% yields in my hands. Transesterification of 1.19 to the corresponding methyl ester was readily accomplished using MeMgBr and MeOH (82% yield). Introduction of the N-methyl group as needed for MeBmt was achieved by using Meerwein's reagent $Me_3{}^+OBF_4{}^-$ (0 °C, 5 h) to give N-methyl oxazolidinone 1.21a in relatively low yield (35-50%). In this reaction, a minor (4R)-epimer 1.21b was usually obtained in 5-10% yield. The occurrence of epimerization at the C_4-center was probably due to deprotonation of the acidic proton by basic 1,8-bis(dimethylamino)naphthalene as shown in Equation 3.1.



(Equation 3.1)

In the final step, **1.21a** was hydrolyzed (2N KOH, 75-80 °C) and purified to give the desired MeBmt (**1.2**) in 82-90% yield. The NMR and melting point data were identical with those reported by Evans.[119] The chiral aldehyde **1.27** was obtained in 5 steps (overall yield 28-32%) starting from 1-buten-3-ol (**1.22**) (Scheme **1.4**). The NMR spectrum (in D_2O) of MeBmt is shown in Figure **3.3.**

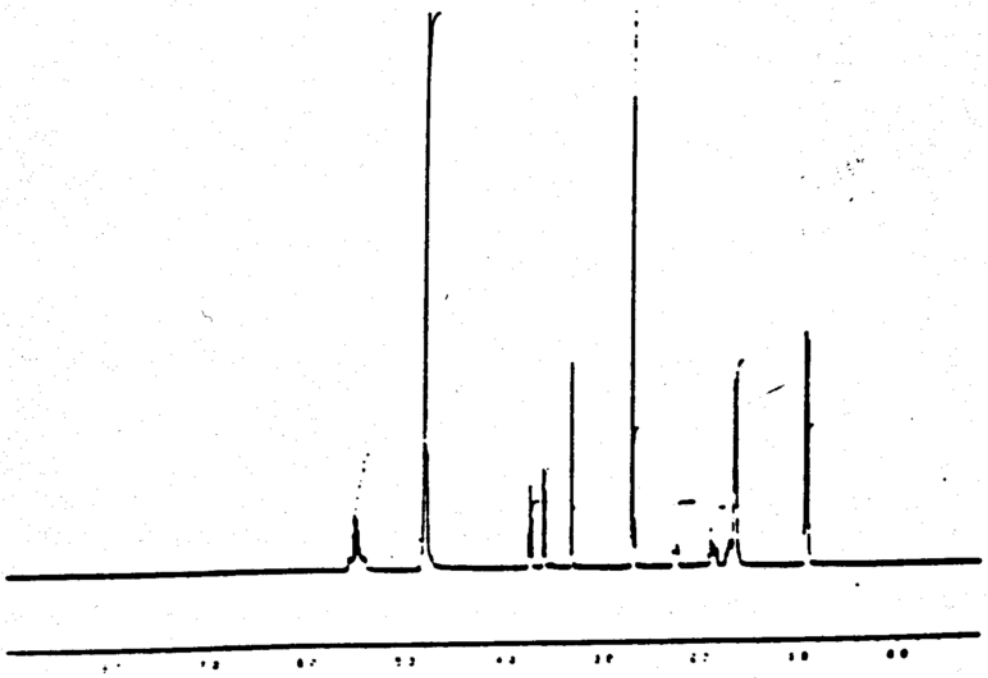
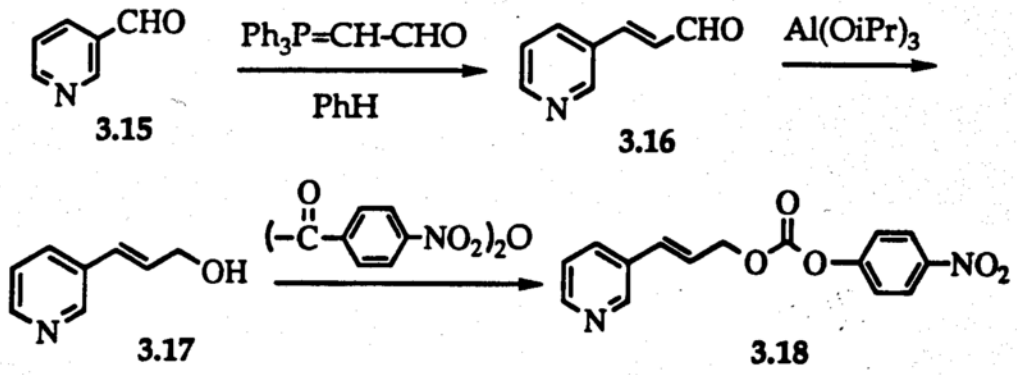


Figure 3.3. ^1H NMR (D_2O) spectrum of MeBmt (**1.2**).

C. *Synthesis of 3-(3'-Pyridyl)allyl-(4''-nitrophenyl)carbonate (Paloc-ONp)*

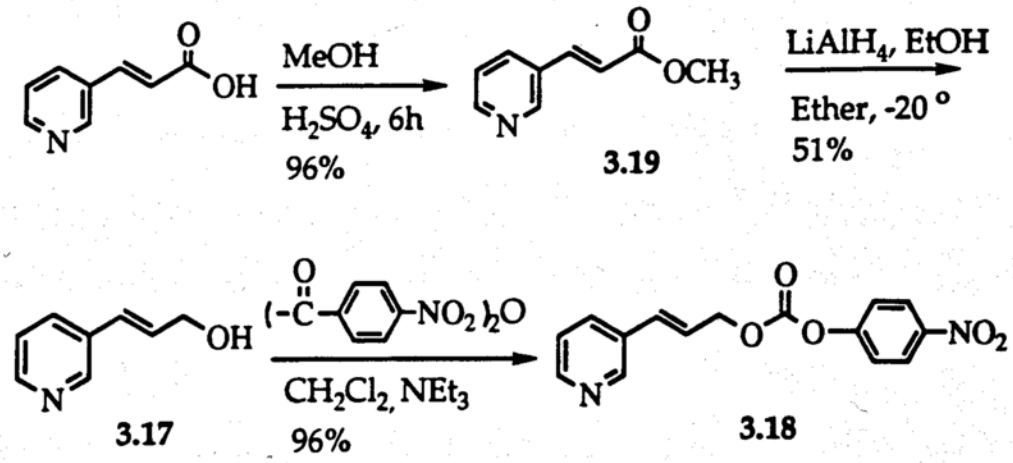
A suitable reagent for the attachment of the Paloc group to N$^{\alpha}$-MeLys derivative **3.14** is 3-(3'-pyridyl)allyl-(4''-nitrophenyl)carbonate (**3.18**) . The

synthesis of the carbonate was reported earlier by Kunz.[140] The critical 3-pyridylallyl alcohol (**3.17**) was obtained from the corresponding aldehyde **3.16**, which could be prepared by Wittig reaction of 3-formylpyridine (**3.15**) and a triphenyl phosphine ylide, Ph_3P=CH-CHO (Scheme 3.4). However, the



Scheme 3.4. Kunz synthesis of 3-(3'-pyridyl)allyl-(4"-nitrophenyl)-carbonate (Paloc-ONP) (ref. 140)

synthesis of alcohol **3.17** was also achieved from the corresponding methyl ester **3.19** in 51% yield by using $LiAlH_4$ in the presence of 2 equiv of EtOH[181] (Scheme 3.5). The resulting $LiAl(OEt)_2H_2$ decreases the undesired 1,4-reduction of the unsaturated allylic group, which occurs by using $LiAlH_4$ alone. The alcohol **3.17** was treated with bis(4-nitrophenyl)carbonate in the presence of triethylamine to afford the desired carbonate **3.18** (Figure 3.4) in excellent yield (96%) as a source of the Paloc group in **3.14**.



Scheme 3.5. Synthesis of Paloc-ONP starting from Pyridylacrylic acid

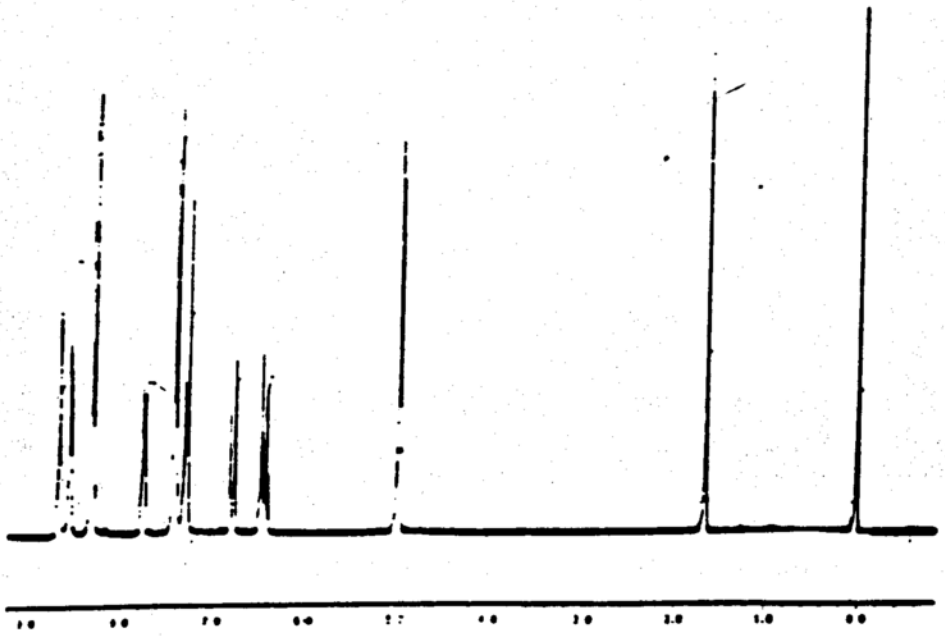
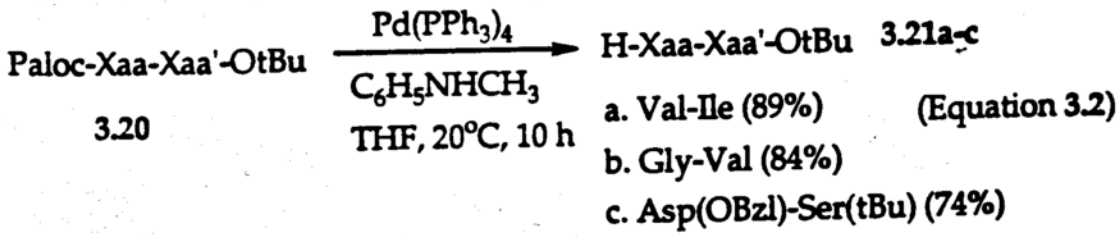


Figure 3.4. ^1H NMR ($CDCl_3$) spectrum of 3-(3'-pyridyl)allyl-(4-nitrophenyl) carbonate (3.18).

D. *Selective Incorporation-Deprotection of the Paloc Group in Peptide Synthesis*

The Paloc group combines the advantages of stability under various conditions, cleavability under neutral conditions, and solubility in water.[140] It is stable to treatment with trifluoroacetic acid so that the tert-butyl ester or ether can be removed selectively, and it is stable under basic (e.g. 0.2N NaOH solution) conditions so that the N^{α}-Fmoc group and benzyl ester can be simultaneously cleaved in the penultimate step of the synthesis of CsA analogs. Moreover, it is also stable under the conditions of the rhodium(I)-catalyzed cleavage of the allyl ester. Nevertheless, like the allylic ester and allyloxycarbonyl (Aloc) group, it can be removed from the blocked amino function under mild conditions by palladium(0), as well as other soluble palladium complexes, as catalysts and morpholine, pyrrolidine, and hydride donors, formate, tributyltin hydride, or borohydride as nucleophiles to effect an allylic exchange reaction.[147] According to Kunz[140], the Paloc group in the various N^{α}-Paloc dipeptides **3.20** can be removed under $Pd(PPh_3)_4$/N-methylaniline conditions (Equation 3.2), which leave benzyl and tert-butyl

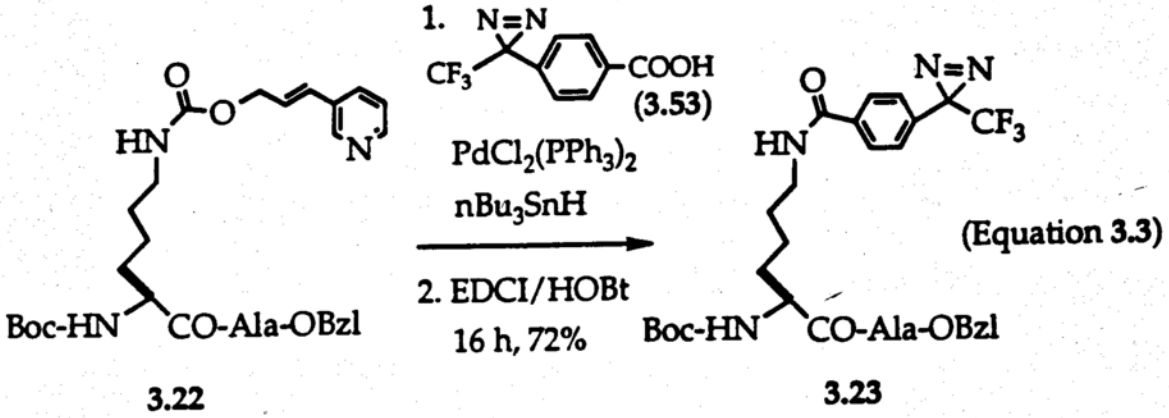


(Equation 3.2)

protecting groups intact (e.g. 3.21c).[140] Following a similar method, I tried deprotecting the substrate BocLys(Paloc)-Ala-OBzl with $Pd(PPh_3)_4$/N-methylaniline, $Pd(PPh_3)_4$/N-methylaniline/PPh_3, or $Pd(PPh_3)_4$/piperidine, separately. Surprisingly, no desired Paloc-deprotected product was obtained under these conditions at room temperature in 2 days. Further, using $HCOOH/nBuNH_2$ as a basic scavenger in the presence of $Pd(PPh_3)_4/PPh_3$, the Paloc group was removed slowly in 3 days, but the desired product was difficult to isolate by flash chromatography. Guibé and co-workers[147] found that N-allyloxycarbonyl (Aloc) amines or amino acids can be readily converted to free amino compounds by palladium(II)-catalyzed hydrostannolytic cleavage with tributyltin hydride in the presence of a proton donor (e.g. acetic acid, p-nitrophenol, or water). Although the removal of the N^{α}-Aloc protecting group could be accomplished using Bu_3SnH and a catalytic amount of $PdCl_2(PPh_3)_2$ in wet methylene chloride, the resultant amine proved to be difficult to isolate and purify.[148] Directly utilizing the N-protected amino acid as the proton source for the removal of N^{α}-Aloc, followed by addition of a coupling reagent, provides *in situ* the desired peptide without isolation of the intermediate amine.

Following this procedent, I tried the deprotection reaction by using $PdCl_2(PPh_3)_2/Bu_3SnH$ and a photoaffinity labeling reagent, diazirinylbenzoic

acid 3.53[149] (Daz, see page 78), as the proton source for the removal of the Paloc group in the dipeptide BocLys(Paloc)-Ala-OBzl (Equation 3.3). The desired product, Boc-Lys(Daz)-Ala-OBzl (3.23), was readily isolated by flash chromatography in 72% yield. It proved that the Paloc group, which is more acid-stable than the Aloc group, can be used as an additional temporary amino-protecting group in the orthogonal strategy for the synthesis of complicated peptides.



3.22 3.23 (Equation 3.3)

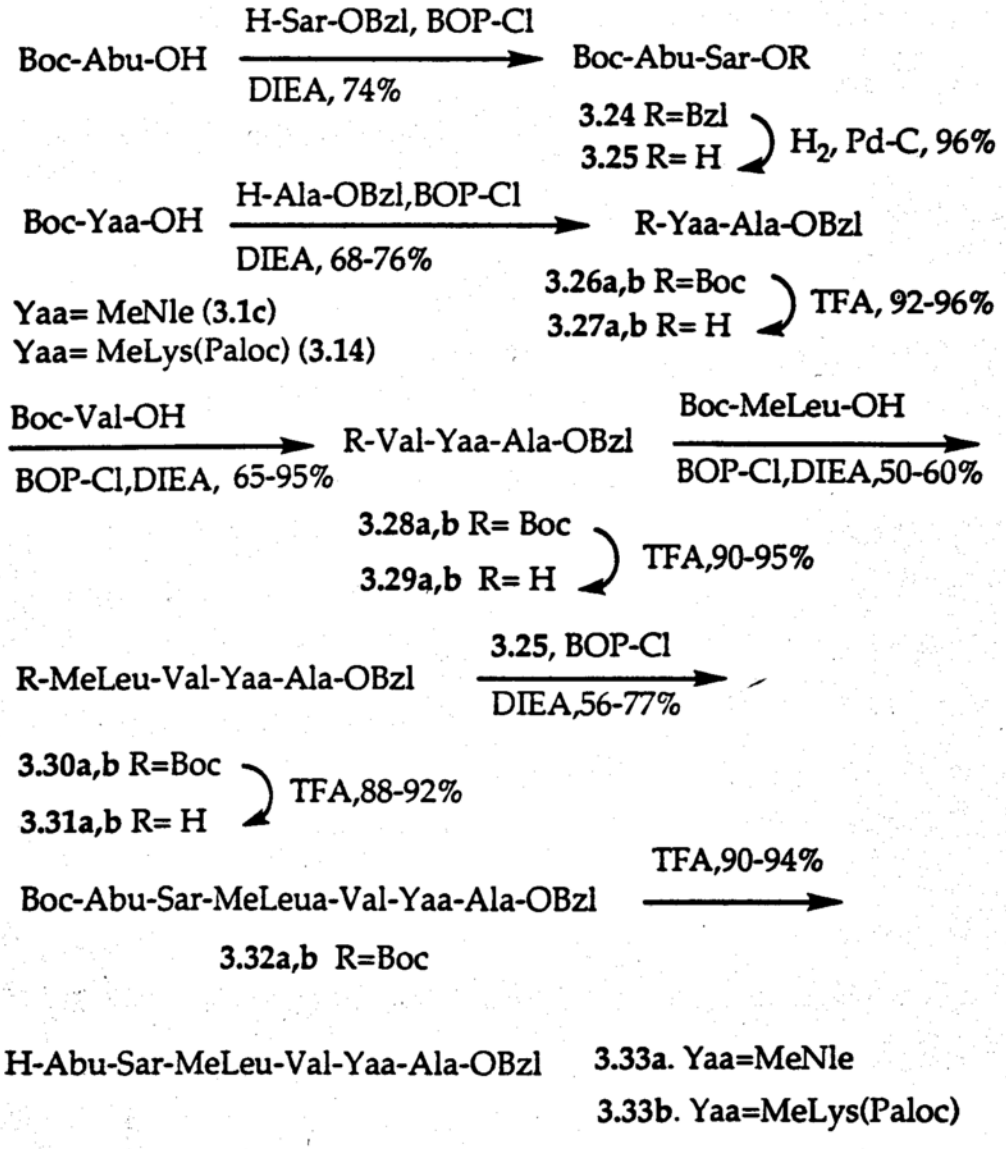
E. *Synthesis of 1-7 CsA Heptapeptide Analogues, H-MeBmt-Abu-Sar-MeLeu-Val-Yaa-Ala-OBzl.*

1. *Synthesis of 2-7 Hexapeptide Fragments*

The synthesis of the 2-7 CsA hexapeptide analogues 3.33a,b, which were modified in the 6-position, was carried out by using Wenger's method[8] except that bis(2-oxo-3-oxazolidinyl)phosphinic chloride (BOP-Cl)[133] was employed for coupling N-methyl amino acids, instead of pivalic anhydride.

[131] The hexapeptide fragments were synthesized by C to N extension and employed a 4 + 2 fragment coupling strategy. Tung and Rich employed the special coupling agent, BOP-Cl, to synthesize the 2-7 CsA hexapeptide with high efficiency and in moderately high yield with essentially no racemization.[133] As shown in Scheme 3.6, the 2-3 dipeptide fragment Boc-Abu-Sar-OH (**3.25**) was prepared via the BOP-Cl coupling method (67-74% yield), followed by the removal of the benzyl protecting group with 10% Pd-C/H_2 in MeOH at room temperature (96% yield). The 4-7 tetrapeptide fragments **3.31a,b** were synthesized starting with the C-terminal residue H-Ala-OBzl, which was coupled to Boc-MeNleOH or Boc-MeLys(Paloc)OH (**3.14**) to give dipeptides **3.26a,b** in 74-84% yields. After removal of the N-terminal Boc group with TFA, the resulting aminopeptides **3.27a,b** were condensed with BocValOH via BOP-Cl activation to afford tripeptides **3.28a,b**. Aminotripeptides **3.29a,b**, Boc-protected tetrapeptides **3.30a,b**, and aminotetrapeptides **3.31a,b** were prepared by following similar procedures for TFA-treated deprotection and BOP-Cl mediated coupling and were isolated in yields of 90-95%, 52-60%, and 88-95% respectively. Dipeptide **3.25** and tetrapeptides **3.31a,b** were condensed by using the BOP-Cl strategy described above to provide hexapeptides **3.32a,b** in 82-90% yields. Likewise, the Boc-protecting groups of the hexapeptides **3.32a,b** were removed with TFA at -16 °C and neutralized with $NaHCO_3$ to give the aminohexapeptides

3.33a,b in 87-98% yields, which were readily used in the further reactions.



Scheme 3.6. Synthesis of CsA 2-7Analogous Fragments **3.33a,b**

The optical rotations and yields of the BOP-Cl mediated couplings for the synthesis of fully protected peptide fragments are shown in Table 3.1, while those of the related amino-peptides and acid fragments are summarized in Table 3.2.

The 3 + 1 coupling leading to the tetrapeptide analogue 3.30a was the lowest yielding step. It seemed that the reaction was sensitive to the bulky

Table 3.1. BOP-Cl coupling[a] of N-protected amino acids with segments of the 2-7 analogous peptides

entry	compound	product sequence[b]	$[\alpha]_D$(c, $CHCl_3$)	yield(%)
1	3.24	BocAbu-SarOBzl	-4.2°(1.0)	74
2	3.26a	BocMeNle-AlaOBzlc	-4.0°(0.3)	68
3	3.26b	BocLys(Paloc)-AlaOBzl	-47.1°(2.2)	76
4	3.28a	BocVal-MeNleAlaOBzl	-69.5°(0.55)	65
5	3.28b	BocVal-MeLys(Paloc)AlaOBzl	-54.6°(2.0)	95
6	3.30a	BocMeLeu-ValMeNleAlaOBzl	-86.0°(0.35)	50
7	3.30b	BocMeLeu-ValMeLys(Paloc)AlaOBzl	-92.0°(1.0)	60
8	3.30c	FmocMeLeu-ValMeNleAlaOBzld	-89.6°(0.57)	56
9	3.32a	BocAbuSar-MeLeuValMeNleAlaOBzl	-118.7°(1.5)	47-56
10	3.32b	BocAbuSar-MeLeuValMeLys(Paloc)AlaOBzl	-108.2°(1.1)	77

[a] BOP-Cl, DIEA; in situ activation of the amino acid as described in ref. 133. [b] The line (-) indicates site of new peptide bond formed from acid-amine coupling. [c] BOP-Cl, DIEA; preactivation of the amino acid prior to addition of the amine peptide. d FmocMeLeuCl was formed from oxalyl chloride/DMF and used in coupling.

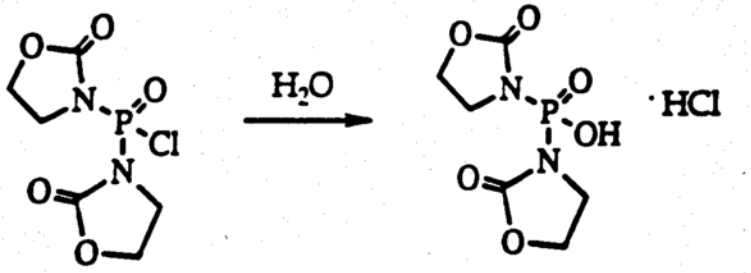
side chains of the substrates. Previous data from Rich's group had shown that the steric bulk of the protecting groups could definitely affect the yield of the 3 + 1 coupling reaction.[133] Not much improvement in yield was obtained by using Fmoc-MeLeuCl as a reactive acylating agent in the 3 + 1 coupling (Table 3.1, 3.30c), although Carpino's group has reported success in by using Fmoc-acyl chloride as a highly reactive agent for amino acids couplings.[150] Preactivation of an N-protected amino acid with BOP-Cl before the amine component was added only provided a slightly higher yield (Table 3.1, 3.26a).

Table 3.2. Optical rotations and yields of aminopeptide or acid fragments

entry	compound	product sequence	$[\alpha]_D$(c, $CHCl_3$)	yield(%)
1	3.25	BocAbuSar-OH	-5.3°(1.0)	96
2	3.27a	H-MeNleAlaOBzl	-36.7°(0.7)	92
3	3.27b	H-MeLys(Paloc)AlaOBzl	-33.0°(1.2)	98
4	3.29a	H-ValMeNleAlaOBzl	-49.3°(0.3)	89
5	3.29b	H-ValMeLys(Paloc)AlaOBzl	-43.9°(2.0)	99
6	3.31a	H-MeLeuValMeNleAlaOBzl	-79.1°(1.2)	91
7	3.31b	H-MeLeuValMeLys(Paloc)AlaOBzl	-80.9°(1.1)	85
8	3.33a	H-AbuSarMeLeuValMeNleAlaOBzl	-81.5°(0.8)	92
9	3.33b	H-AbuSarMeLeuValMeLys(Paloc)AlaOBzl	-88.7°(1.1)	96

Recent experiences by Rich et al. and other groups had indicated that the yields of products obtained from BOP-Cl mediated reactions could be

dependent upon the source of the coupling reagent.[133] BOP-Cl purchased from Chemical Dynamics gave better results in coupling reactions than that purchased elsewhere.[133] I found that utilizing fresh commercial BOP-Cl gave much better yields. The reason may be that BOP-Cl is not very stable to prolonged storage even below 0 °C. Presumably it degrades slowly to give BOP·HCl salt when traces of moisture are present (Equation 3.4).



1.42

(Equation 3.4)

2. *Synthesis of 1-7 CsA Heptapeptide Fragments*

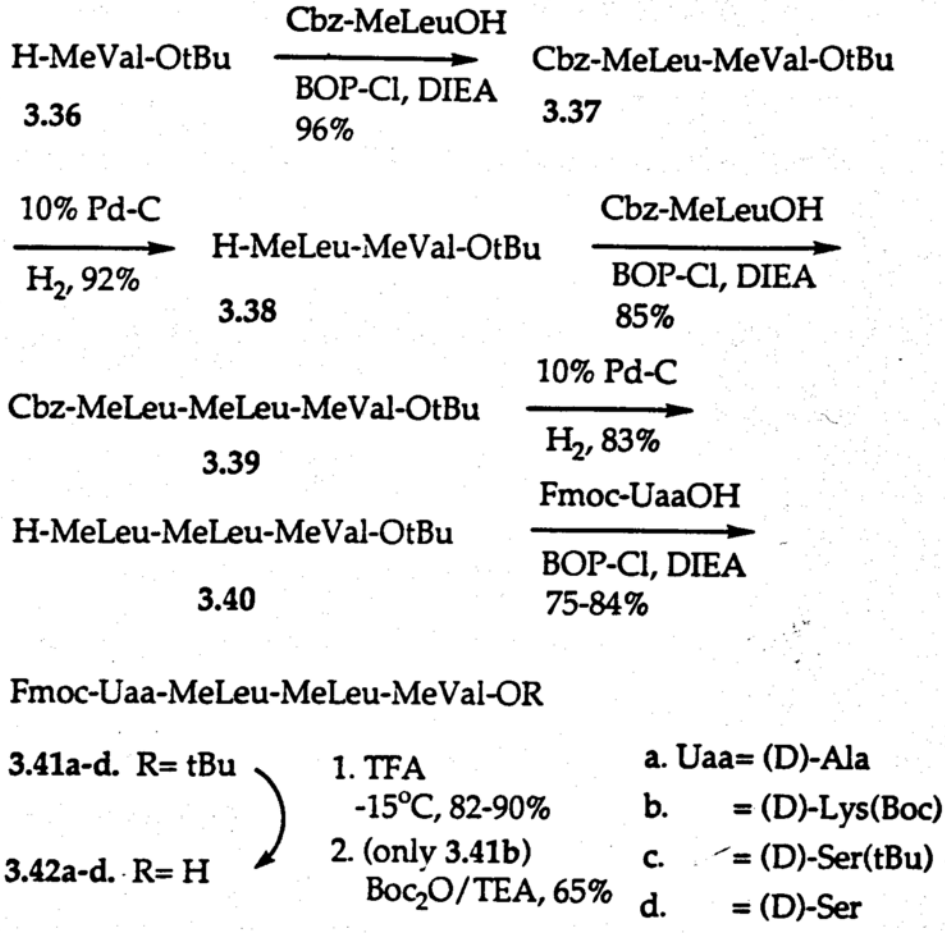
The unusual amino acid MeBmt **1.2** (Scheme 3.8, page 76) was incorporated as an isopropylidene derivative. The acetonide protecting group was introduced into MeBmt in over 90% yield by refluxing the amino acid in freshly distilled acetone for 24 h. This protecting group had the advantage of avoiding epimerization of MeBmt during peptide-bond formation. Thus, the resulting amino hexapeptides **3.33a,b** were condensed with the acetonide-protected MeBmt (**1.2a**) using the mixed carbodiimide method (DCC/HOBt) in the presence of N-methylmorpholine. The acetonide-protected heptapeptides **3.34a,b** were obtained with yields in the range of 62-92%. These heptapeptides

appeared to adopt more than one conformation in $CDCl_3$ as observed by NMR.

The acetonide group of **3.34a,b** was removed by use of 1N HCl in methanol at room temperature. Wenger's reported conditions (1 equiv of 1N HCl/MeOH) usually required longer reaction times (over 24 h) to drive the reaction to completion. According to Rich's report,[133] the deprotection was usually complete within 16 h in high yields (> 79%) when an excess of 1N HCl/MeOH was used, which limited the occurrence of transesterification (methyl ester formation) that usually occurred with longer reaction times. The heptapeptides **3.35a,b** showed multiple conformations in $CDCl_3$ by NMR.

F. *Synthesis of 8-11 CsA Tetrapeptide Analogues, Fmoc-Uaa-MeLeu-MeLeu-MeVal-OH .*

Synthesis of the 8-11 CsA tetrapeptide (Fmoc-(D)-Ala-MeLeu-MeLeu-MeVal, **3.42a**) and its analogues **3.42b-d** followed directly from methods previously reported for the synthesis of the CsA 8-11 fragment (Scheme **3.7**).[134] These methods have as their basis BOP-Cl activation and benzyloxycarbonyl (Cbz) amino protection utilized in a series of highly efficient couplings of N-protected amino acids to N-methylamino tert-butyl ester segments of the 8-11 peptides. This strategy proved successful and relied largely on the choice of protecting groups. In contrast to Wenger's method, in



Scheme 3.7. Synthesis of CsA 8-11 Analogous Fragments **3.42a-d**

which the construction of the 8-11 tetrapeptide fragment was by the N to C terminal extension, the usual C to N extension with coupling of the Cbz protected intermediates as well as hydrogenolytic removal of N-protection was chosen. As shown in Scheme 3.7, dipeptide **3.37** was obtained by the usual BOP-Cl mediated reaction in 96% yield. After catalytic hydrogenolysis of the Cbz group of **3.37**, the resultant dipeptide **3.38** was condensed with Cbz-

MeLeu-OH to give tripeptide **3.39** in 85% yield. The protected tetrapeptides **3.41a-d** were produced by the coupling of deprotected tripeptide **3.40** to a variety of Fmoc-(D)-amino acids in 75-94% yields. TFA treatment of **3.41a,b,d** and reprotection (only for **3.41b**) of the ε-amino group gave the desired Nα-Fmoc tetrapeptide acids **3.42a-d**. Deprotection of tetrapeptide **3.41c** gave two products, **3.42c** (38% yield), which still contained the tert-butyl group at the side chain of residue (D)-Ser, and **3.42d** (56% yield) in which the tert-butyl group at (D)-Ser was removed. The optical rotations and yields of these tetrapeptides are shown in Table 3.3.

Table 3.3. Optical rotations and yields of residues 8-11 analogous peptides[a] and acids

entry	compound	product sequence[b]	$[\alpha]_D$(c, $CHCl_3$)	yield(%)
1	**3.41a**	Fmoc(D)Ala-MeLeuMeLeuMeValOtBu	-99.6°(1.2)	94
2	**3.41b**	Fmoc(D)Lys(Boc)-MeLeuMeLeuMeValOtBu	-78.8°(1.0)	95
3	**3.41c**	Fmoc(D)Ser(tBu)-MeLeuMeLeuMeValOtBu	-86.3°(1.5)	88
4	**3.41d**	Fmoc(D)Ser-MeLeuMeLeuMeValOtBu	-131°(0.8)	75
5	**3.42a**	Fmoc(D)AlaMeLeuMeLeuMeVal-OH	-104.5°(0.66)	82
6	**3.42b**	Fmoc(D)Lys(Boc)MeLeuMeLeuMeVal-OH	-67.2°(1.1)	65
7	**3.42c**	Fmoc(D)Ser(tBu)MeLeuMeLeuMeVal-OH	-158°(0.05)	38
8	**3.42d**	Fmoc(D)SerMeLeuMeLeuMeVal-OH	-100.6°(0.31)	90

[a] BOP-Cl, DIEA; in situ activation of the amino acid as described in ref. 134.
[b] The line(-) indicates site of new bond formed from acid-amine starting materials or deprotection steps.

The synthesis of the critical Cbz-MeVal-OtBu via the standard acid catalyzed reaction of Cbz-MeVal-OH with isobutylene resulted in poor yields. An alternative method that employed silver cyanide to assist esterification of the stable Fmoc-MeVal-Cl proved to be useful.[134] I found that the desired Cbz-MeVal-OtBu was obtained in 97% yield from the corresponding acid (Equation 3.5) according to Castro's strategy by using a catalytic amount of DMAP, triethylamine, and Boc_2O in $tBuOH/CH_2Cl_2$.[152] This method is quite efficient and can be carried out on a large scale with no risk of racemization.

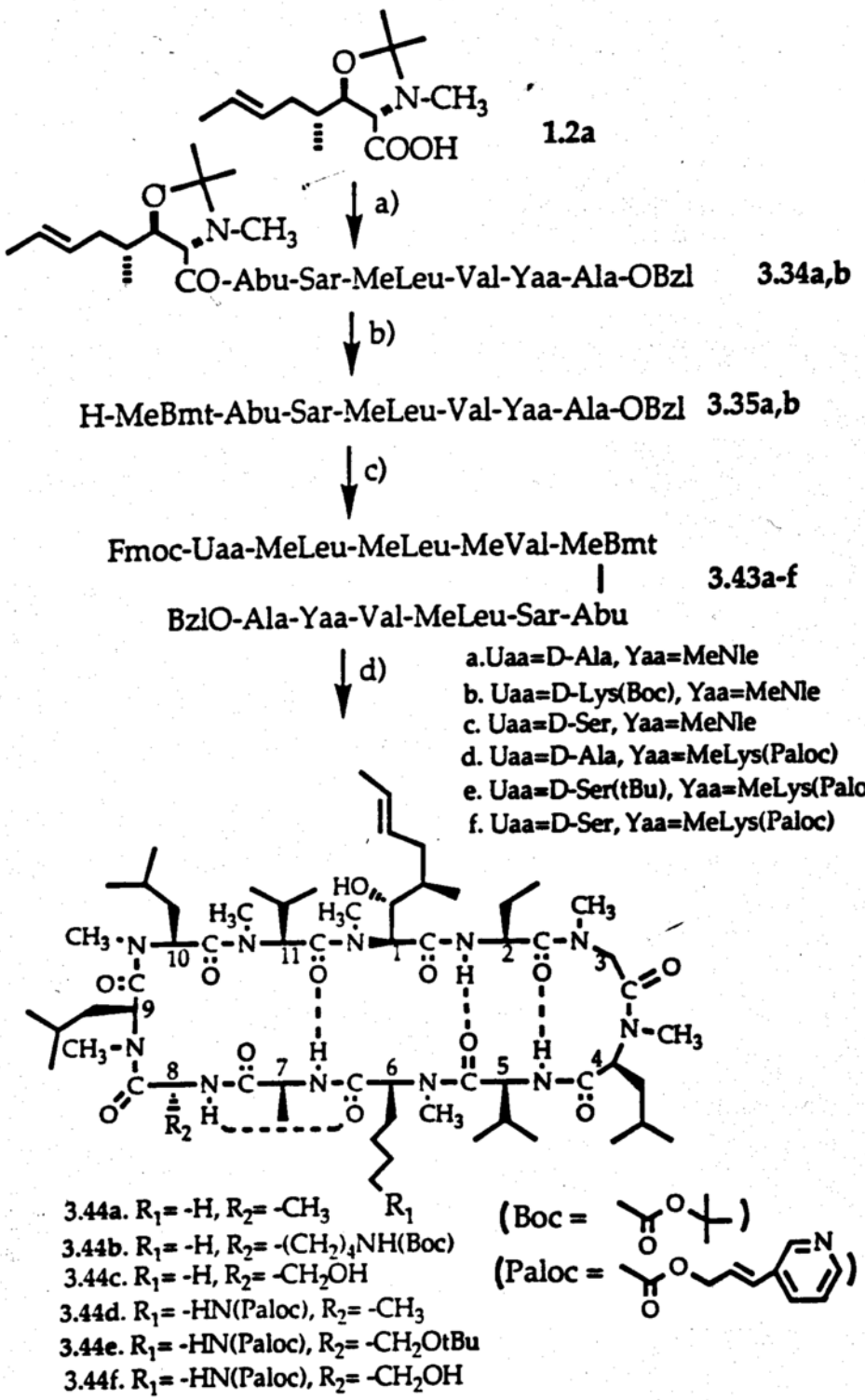
$$\text{Cbz-MeVal-OH} \xrightarrow[\text{TEA, } BOC_2O \text{ (2 eq)}\\ tBuOH/CH_2Cl_2 \text{ (1:1)}]{\text{DMAP (0.1 eq)}} \text{Cbz-MeVal-OtBu} \quad \text{(Equation 3.5)}$$

G. *Fragment Coupling and Final Cyclization*

The linear undecapeptides **3.43a-e** were synthesized according to Wenger's reported procedure.[8] The deprotected heptapeptides were coupled with the tetrapeptides in the presence of 1.5 equiv of BOP reagent **1.41** and 2 equiv of N-methylmorpholine in methylene chloride at room temperature for 2.5-3 days (Scheme 3.8). The desired undecapeptides **3.43a-e** were isolated by flash chromatography (10-50% acetone/hexane) in 34-62% yields (Table 3.4), which were lower than the yield (72%) reported by Wenger for the synthesis of CsA.

The final cyclization was also carried out according to the method described

by Wenger.[8] Saponification of the benzyl ester of the fully protected undecapeptides **3.43a-e** (Scheme 3.8) using ethanolic/aqueous NaOH, was accompanied by rapid removal of the N-terminal Fmoc protecting group. The reactions were usually completed in 5-12 h depending on the starting materials. As expected, the N$^{\varepsilon}$-Paloc group at [MeLys^{6}] (**3.43c-e**) and N$^{\varepsilon}$-Boc at [(D)-Lys^{8}] (**3.43b**) remained intact under the basic (0.2N NaOH/EtOH) conditions. After workup, the crude deprotected undecapeptides were directly cyclized by using propylphosphonic anhydride (50% w/w in CH_2Cl_2) and DMAP in a dilute solution (~ 2 x 10^{-4} M). Cyclization was usually complete in 2 days. The desired CsA analogues **3.44a-e** were isolated by flash chromatography (usually 10-40% acetone/hexane as eluent except **3.44e** which needed a higher ratio (20-60%) of acetone in hexane as co-solvents). The yields for the cyclization reactions were usually in the range of 38-74% (Table **3.4**). The lower yield (32%) of the product **3.44e** was expected due to the existence of the primary hydroxyl group at (D)-Ser^{8} residue. The ^{1}H NMR spectra of these CsA analogs are shown in Figures **3.5 ~ 3.10** (see Appendix II, pages 228 ~ 233).



[a]a). H-Abu-Sar-MeLeu-Val-Yaa-Ala-OBzl (3.33a,b), DCC, HOBt, NMM, 20 h, 63-96%. b). aq. HCl, CH_3OH, 16 h, r.t. 80-96%. c). 3.42a-d, BOP, NMM, 3 days, 36-58%. d). (1). 0.2N NaOH, EtOH, (2). $(Pr\text{-}PO_2)_3$, DMAP, 2 days, 32-59%.

Scheme 3.8. Synthesis of CsA Analogs 3.44a-f

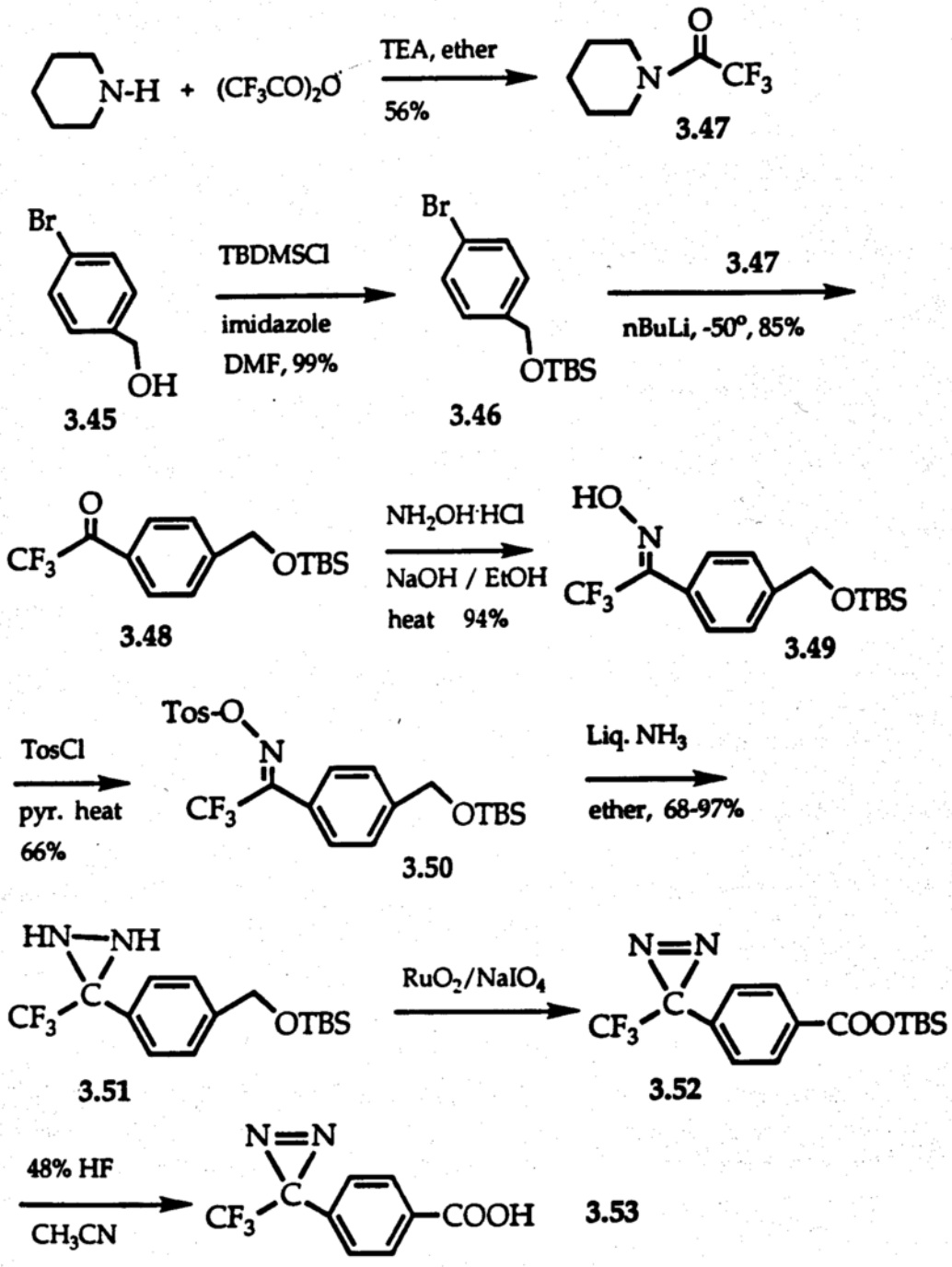
Table 3.4. Physical properties of CsA analogs **3.44a-f, 3.54** and their linear undecapeptide intermediates **3.43a-f.**

entry	compound	structure[b]	R_f (%)[a]	$[\alpha]_D$ (c, $CHCl_3$)	yield(%)
1	**3.43a**	[MeNle^6]	0.55(60)	-148.0°(0.6)	61
2	**3.43b**	[MeNle^6,(D)-Lys(Boc)^8]	0.29(40)	-82.1°(1.9)	40
3	**3.43c**	[MeNle^6,(D)-S^8]	0.45(50)	-168.8°(0.08)	36
4	**3.43d**	[MeLys(Paloc)6]	0.34(66)	-95.0°(0.02)	36
5	**3.43e**	[MeLys(Paloc)^6,(D)-S(tBu)^8]	0.33(66)	-155.8°(0.8)	40
6	**3.43f**	[MeLys(Paloc)^6,(D)-S^8]	0.54(80)	-106.4°(0.7)	50
7	**3.44a**	[MeNle^6]	0.43(50)	-172.5°(0.04)	46
8	**3.44b**	[MeNle^6,(D)-Lys(Boc)^8]	0.31(50)	-145.0°(0.02)	85
9	**3.44c**	[MeNle^6,(D)-S^8]	0.39(60)	-250.0°(0.05)	71
10	**3.44d**	[MeLys(Paloc)^6]	0.26(65)	-140.0°(0.03)	59
11	**3.44e**	[MeLys(Paloc)^6,(D)-S(tBu)^8]	0.49(65)	-216.0°(0.1)	50
12	**3.44f**	[MeLys(Paloc)^6,(D)-S^8]	0.29(70)	-184.0°(0.1)	47
13	**3.54**	[MeNle^6,(D)-Lys^8]	0.54[c]	-187.5°(0.04)	82

[a] TLC (% acetone/hexane). [b] Abbreviated symbol: S=Ser. [c] Eluent : (CH_2Cl_2/MeOH/NH_4OH, 88:8:4).

H. *Synthesis of 4-(1-Azi-2,2,2-trifluoroethyl)benzoic Acid*

The synthesis of 4-(1-Azi-2,2,2-trifluoroethyl)benzoic acid **3.53**, which can be incorporated into the sidechain amino or hydroxyl group of CsA analogs [(D)-Ser^8,MeLys(Paloc)^6]CsA(**3.44e**) or [(D)-Lys^8,MeNle^6]CsA (**3.54**), is shown in



Scheme 3.9. Synthesis of Photoaffinity Labeling Agent 3.53

Scheme 3.9.[149] Benzyl alcohol **3.45** was protected by silylation (compound **3.46**), and converted to the α,α,α-trifluoroacetobenzyl silyl ether **3.48** in 85% yield in a one-pot procedure upon treatment with n-butyllithium under careful temperature control followed by treatment with N-trifluoroacetyl piperidine **3.47**, which was readily prepared via trifluoroacetylation of piperidine in TEA/ether. Elaboration of the ketone **3.48** to generate the diaziridine **3.51** via the oxime **3.49** and tosyl oxime **3.50** was effected by sequential treatment with hydroxylamine, tosyl chloride, and liquid ammonia in 60% yield overall. Intermediate **3.51** possessed the requisite function for the formation of the desired diazirine **3.53.** The subsequent oxidation of diaziridinyl silyl ether **3.51** with silver oxide was ineffective. An alternative reagent, ruthenium dioxide in excess of sodium metahyperiodate,[153] was sought and used to successfully oxdize both benzyl methylene to the corresponding silyl benzoate, and the diaziridine to the diazirine, to furnish the desired silyl diazirinylbenzoate **3.52** (72%) under mild conditions. The final desilylation of this silyl benzoate with 48% hydrofluoride solution gave the desired diazirinylbenzoic acid **3.53** (NMR spectrum shown in Figure 3.11) in 82% yield.

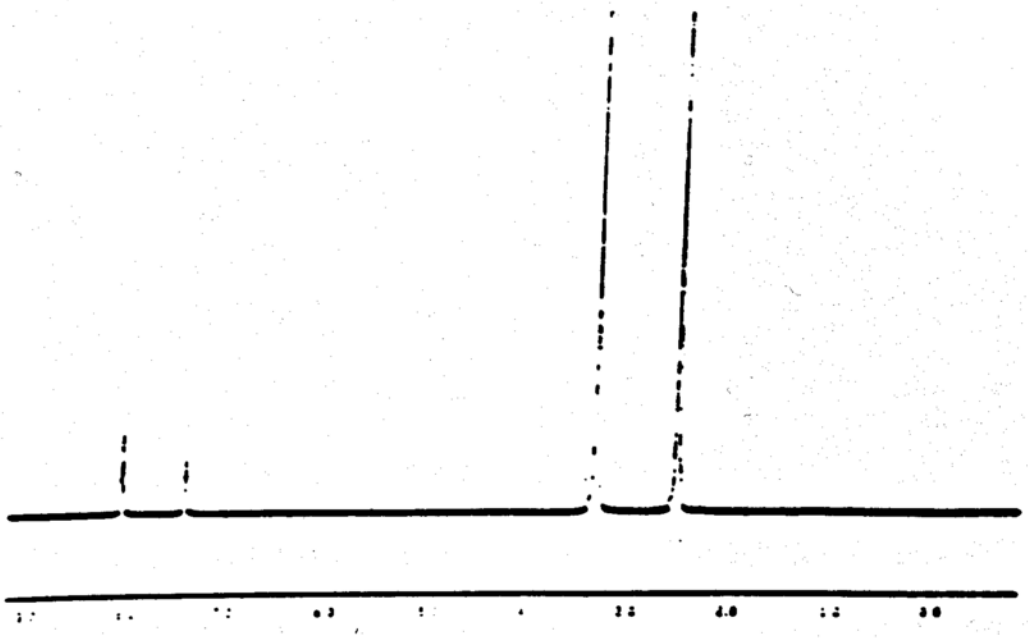
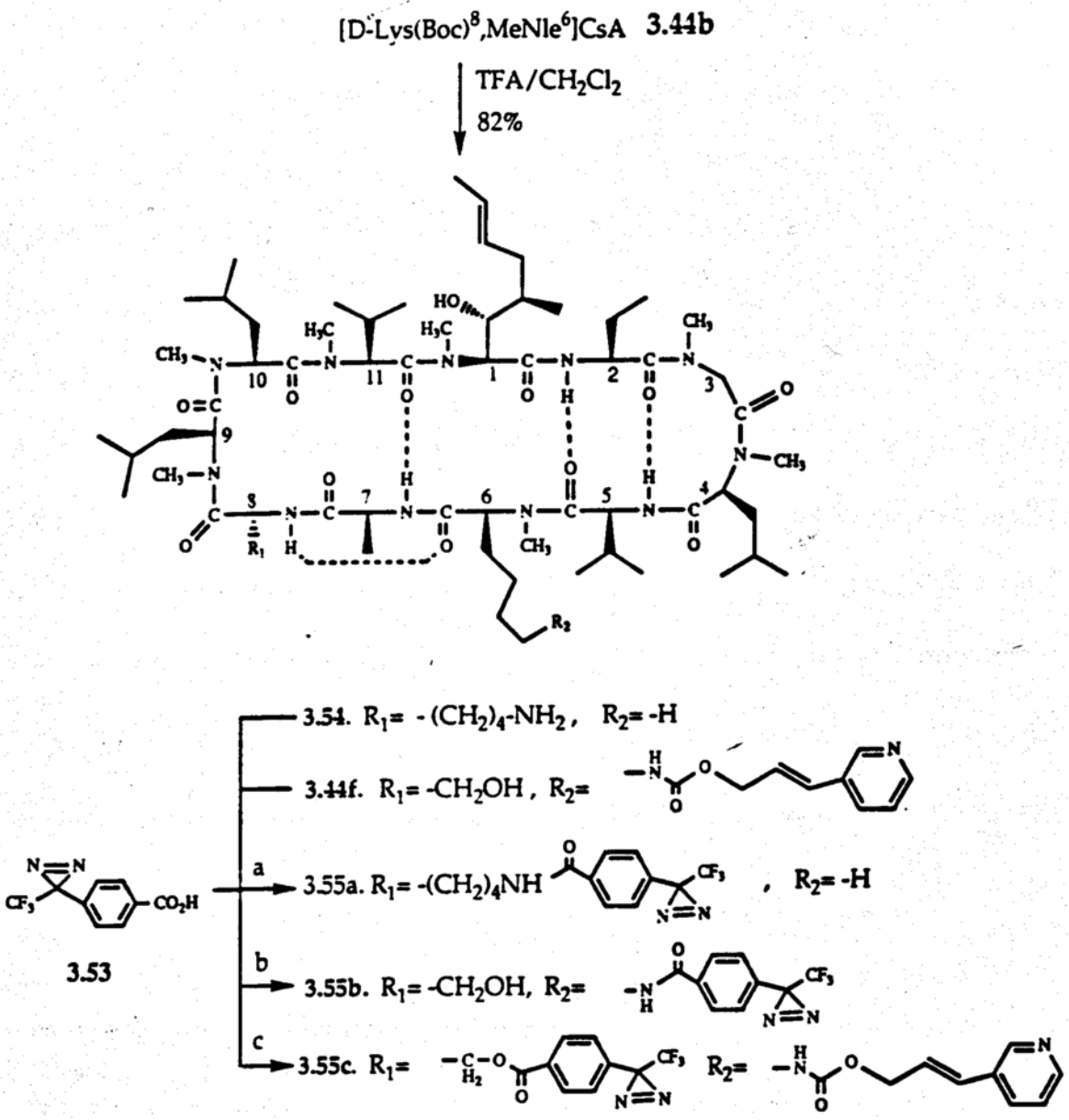


Figure 3.11. ^{1}H NMR (DMSO-d6) spectrum of 4-(1-Azi-2,2,2-trifluoroethyl)-benzoic acid (3.53).

I. *Synthesis of Photoaffinity Labeling CsA Analogues*

[(D)-Lys^8,MeNle^6]CsA (3.54), obtained from deprotection of the corresponding analogue 3.44b under TFA conditions (Scheme 3.10), was coupled to the diazirinylbenzoic acid 3.53 with 1.5 equiv of EDCI and 1-HOBt at room temperature. The desired [(D)-Lys(Daz)^8,MeNle^6]CsA (3.55a) was easily isolated by flash chromatography (15-40 % acetone/hexane) in 97% yield. Under 30 mol% $PdCl_2(PPh_3)_2$, excess of Bu_3SnH, and the diazirinyl-



a. 3.54, EDCI/HOBt, 97%. b. 3.44f, $PdCl_2(PPh_3)_2$, nBu_3SnH, then EDCI/HOBt, 72%. c. 3.44f, EDCI/DMAP, 70%.

Scheme 3.10. Synthesis of Photoaffinity Labeling CsA Analogs 3.55a-c

benzoic acid **3.53** as a proton source, the Paloc group in [(D)-Ser^8,MeLys(Paloc)^6]-CsA (**3.44f**) was removed and the resulting amine intermediate was converted into [(D)-Ser^8, MeLys(Daz)^6]CsA (**3.55b**) *in situ* in the presence of EDCI and 1-HOBt at room temperature. The desired photoaffinity labeling product was easily isolated by flash chromatography in 72% yield. The photoaffinity labeling group can also be incorporated into the 8-position of [(D)-Ser^8,MeLys(Paloc)^6]-CsA with 1.5 equiv of EDCI and 30 mol% of DMAP for 6 h at room temperature. The desired product [(D)-Ser(Daz)^8,MeLys(Paloc)^6]CsA (**3.55c**) was obtained after chromatography (10-40% acetone/hexane) in excellent yield (97%). The ^{1}H-NMR spectra of analogs **3.54** and **3.55a-c** are shown in Figures **3.12 ~ 3.15** (see Appendix **II**, pages 234 ~ 237). The chemical shifts of amide protons and α-proton of amino acids residues in **3.44a-f, 3.54** analogs are summarized in Table **3.5** and **3.6** and compared with CsA.

Table 3.5. Chemical Shifts of Protons in CsA and CsA Analogs **3.44a-f, 3.54**

Compound	NH				N-CH$_3$						
	2	5	7	8	1	3	4	6	9	10	11
CsA	7.96	7.48	7.68	7.17	3.51	3.40	3.11	3.25	3.12	2.70	2.71
3.44a	8.00	7.44	7.60	7.11	3.55	3.37	3.09	3.28	3.12	2.69	2.70
3.44b	8.00	7.47	7.65	7.04	3.55	3.38	3.06	3.28	3.12	2.69	2.70
3.44c	8.00	7.46	7.73	7.31	3.55	3.38	3.11	3.27	3.15	2.70	2.70
3.44d	8.00	7.51	7.60	7.03	3.52	3.38	3.11	3.27	3.12	2.69	2.71
3.44e	8.16	7.49	7.70	6.88	3.48	3.36	3.11	3.25	3.19	2.68	2.70
3.44f	7.92	7.56	7.63	6.90	3.55	3.38	3.12	3.26	3.24	2.72	2.72
3.54	7.98	7.46	7.73	7.04	3.55	3.37	3.05	3.26	3.12	2.68	2.69

Table 3.6. Chemical Shifts of Protons in CsA and CsA Analogs (continued)

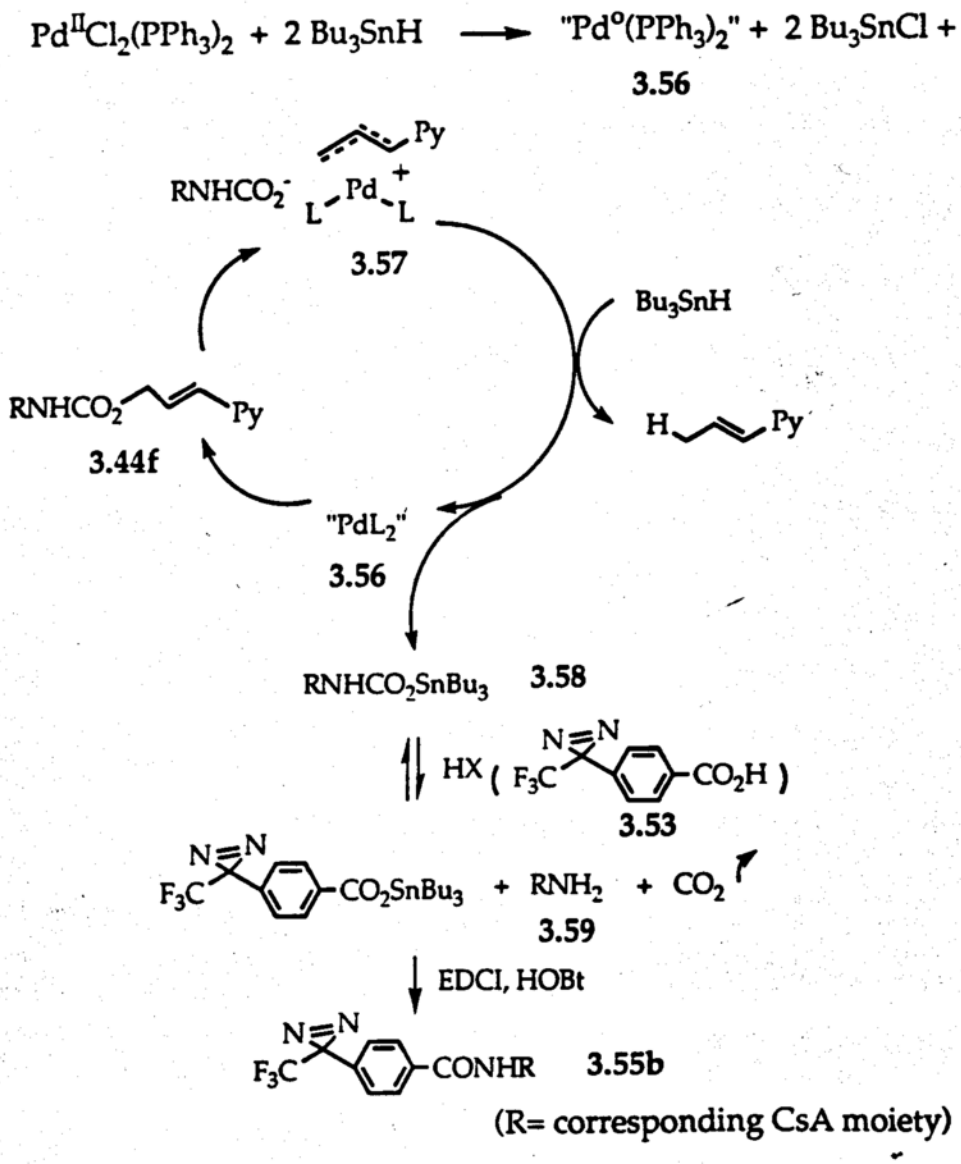
Compound	α-H												Cβ-H
	1	2	3		4	5	6	7	8	9	10	11	1
			re-H	si-H									
CsA	5.47	5.03	3.23	4.76	5.34	4.66	5.02	4.52	4.83	5.70	5.10	5.14	3.82
3.44a	5.60	5.01	3.18	4.74	5.27	4.59	4.87	4.61	4.80	5.69	5.05	5.15	3.62
3.44b	5.63	5.04	3.19	4.75	5.29	4.55	4.87	4.59	4.81	5.68	5.16	5.17	3.64
3.44c	5.60	5.06	3.19	4.74	5.29	4.55	4.92	4.59	4.86	5.68	5.00	5.17	3.68
3.44d	5.65	5.04	3.19	4.74	5.27	4.53	4.98	4.56	4.88	5.63	4.92	5.17	3.68
3.44e	5.72	5.04	3.19	4.71	5.30	4.56	4.93	4.59	5.08	5.68	5.00	5.12	3.66
3.44f	5.70	5.06	3.18	4.74	5.25	4.55	4.48	5.28	5.28	5.66	5.00	5.22	3.66
3.54	5.61	5.03	3.18	4.75	5.29	4.60	4.87	4.55	4.82	5.68	5.05	5.16	3.63

J. *Discussion*

1. *Chemistry of Palladium-catalyzed Paloc Deprotection*

As the photoaffinity labeling CsA analogue **3.55b** demonstrates, the successful palladium-catalyzed hydrostannolytic cleavage of Paloc group followed by the coupling of the resultant amine to acid *in situ* can be employed as an efficient three-dimensional orthogonality for the synthesis of complicated peptides with special functionalities. The probable reaction pathways for the deprotection of pyridylallyl carbamates as well as for the competitive amide formation *in situ* are outlined in Scheme **3.11.** As generally assumed for most palladium-catalyzed allylic alkylation reactions,[147] the true catalytic species is believed to be the coordinatively unsaturated bis(triphenylphosphine)palladium(0) complex **3.56,** which is

formed upon reduction by tributyltin hydride (nBu_3SnH) of dichlorobis-(triphenylphosphine)palladium(II). In the catalytic cycle, $Pd^0(PPh_3)_2$ reacts with the pyridylallyl carbamate **3.44f** to give the π-pyridylallylpalladium(II)



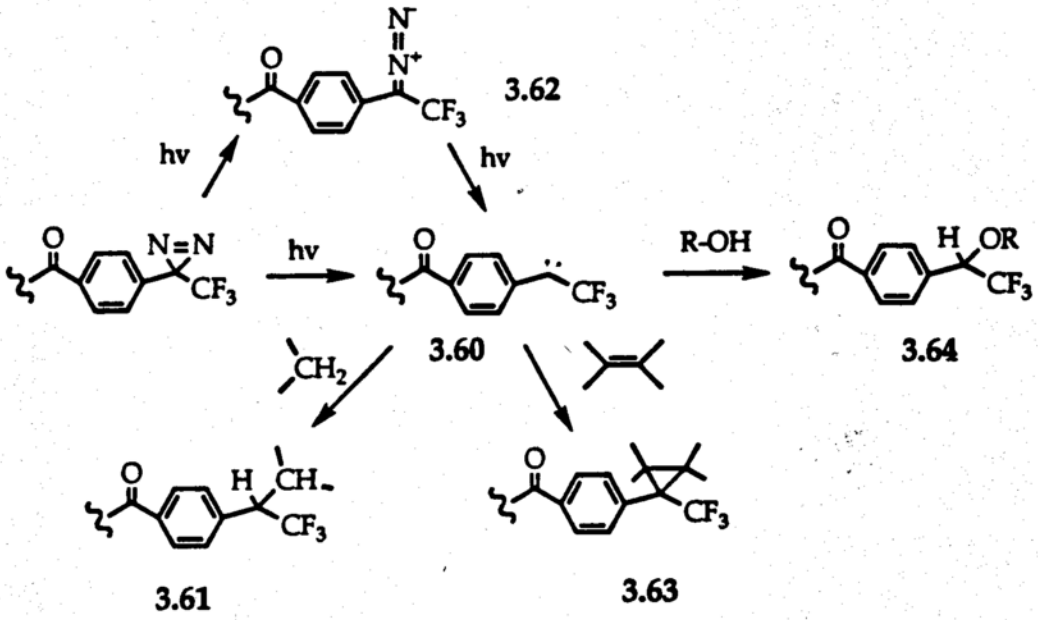
Scheme 3.11. Probable reaction pathways for the deprotection of pyridylallyl carbamate as well as for the competitive amide formation *in situ*.

complex **3.57**, which further reacts with tributyltin hydride to produce the tributyltin carbamate **3.58** and pyridylpropene with regeneration of the catalyst **3.56**. With the proton source (e.g. diazirinylbenzoic acid **3.53**), protonolysis and decarboxylation of **3.58** gives the free amino compound **3.59** and tributylstannous benzoate, which can couple together *in situ* under EDCI/HOBt conditions to give the desired amide **3.55b** without further isolation. The use of Paloc as a temporary amino-protecting group and the highly efficient deprotection-coupling *in situ* method described above can be valuable for the synthesis of complicated peptides imbedded with special functionalities, such as photoaffinity labeling groups and fluorescent moieties, which will be used for biochemical or biological investigations.

2. *The Structural Properties of Aryldiazirine Functionality*

While several types of photoaffinity labeling reagents have been described in the literature,[154] the diazirinyl benzoic acid **3.53** showed several advantageous features. It is small, stable in the dark, and susceptible to light of a wavelength (355 nm) to generate a highly reactive carbene **3.60** which can efficiently insert into a C-H bond (**3.61**, Scheme **3.12**). Due to the electron-withdrawing effect of the trifluoromethyl group, the diazo-isomer **3.62** is so strongly stabilized that it can be considered as unreactive under the normal (physiological) conditions of labeling. Reactions of the singlet carbene **3.60**

with various functional groups frequently occurring in biomolecules are also shown in Scheme 3.12.[155]



Scheme 3.12. Reactions of photoreactive diazirinyl group (ref. 154)

3. *The Biological Properties of Synthetic CsA Derivatives*

The CyP-binding ability and immunosuppressive activity of some of these CsA analogs were also determined. An improved spectrophotometric assay[27] of peptidyl prolyl *cis-trans* isomerase was applied to quantify inhibition of CyP by CsA analogs, whereas the immunosuppressive activity of these CsA analogs was determined by Dr. Alison Badger (SmithKline Beecham Pharmaceuticals) according to procedures as previously described.[14] The

Table 3.7. Structure, PPIase inhibition and immunosuppressive properties of CsA analogs

CsA Analogue	Amino Acid Residue		PPIase (Ki, nM)	Immunosuppression(IC_{50}, nM)	IL-2 release (IC_{50}, nM)
	R_1 at -HN 6 CO-	R_2 at -HN 8 CO-			
CsA	CH_3, CH_3	CH_3	6±1	100	15
[MeNle^6] (3.44a)	CH_3, $(CH_2)_3$	CH_3	22±7	ND	ND
[MeLys(Paloc)^6] (3.44d)	NH(Paloc), $(CH_2)_4$	CH_3	3±2	>>1000	>800
[MeLys(Paloc)^6, D-Ser(OtBu)^8] (3.44e)	NH(Paloc), $(CH_2)_4$	$O-C(CH_3)_3$	3±1	ND	ND
[MeLys(Paloc)^6, D-Ser^8] (3.44f)	NH(Paloc), $(CH_2)_4$	OH	7±2	>600	200

CyP-binding ability of CsA or CsA analogs **3.44a,d-f** was evaluated with inhibition constants presented in Table **3.7**. As expected, modifications at 6- or 8-position in these analogs maintain stronger CyP-binding abilities (e.g. analogs **3.44d,e**, Ki=3±2 nM vs Ki=6±1 nM for CsA). But the extension of the side chain at residue 6 of these analogs showed negligible or weak immunosuppressive activities, which were evaluated in terms of inhibition of Con A-stimulated BDF1 mouse spleen cell activation and PMA/ionomycin stimulated IL-2 release in Jurkat cells (Table **3.7**). Some analogs modified at the 6-position with shorter side chains were already known to be relatively inactive for immunosuppression (for instance, [MeAla^6]-, [MeAbu^6]-, and MeVal^6]-CsA).[10] These results were in agreement with the postulate that the side chain of MeLeu^6 in CsA fits tightly in the binding pocket of the target protein.[60]

With the photoreactive diazirinyl functionality at the 6- or 8-position, the photoaffinity labeling CsA analogs **3.55a-c** can be used to explore possible target proteins other than calcineurin or specific binding sites of CsA in immune-related cells.

Chapter IV. Discovery of Multiply Substituted CsA Analogs as Strong Immunosuppressive Agents

The classical approaches to peptide structure-function analysis have involved single and multiple substitutions of amino acid residues for other "normal" amino acid residues. Although there are several limitations of such studies for rational design, they are generally needed to gain insight into those amino acid residues which are critical for the biological activity. Single site modifications have been employed impressively in the pursuit of potent and highly selective peptide hormones or neurotransmitters, such as agonists or antagonists of oxytocin,[169] vasopressin,[170] and luteinizing hormone-releasing hormone (LHRH).[171]

CsA has been regarded as the prototype of a new generation of immunosuppressive agents. Efforts to discover new analogs or other agents of a differing structure continue today toward the goal of obtaining new drugs with fewer clinical side effects.[156]

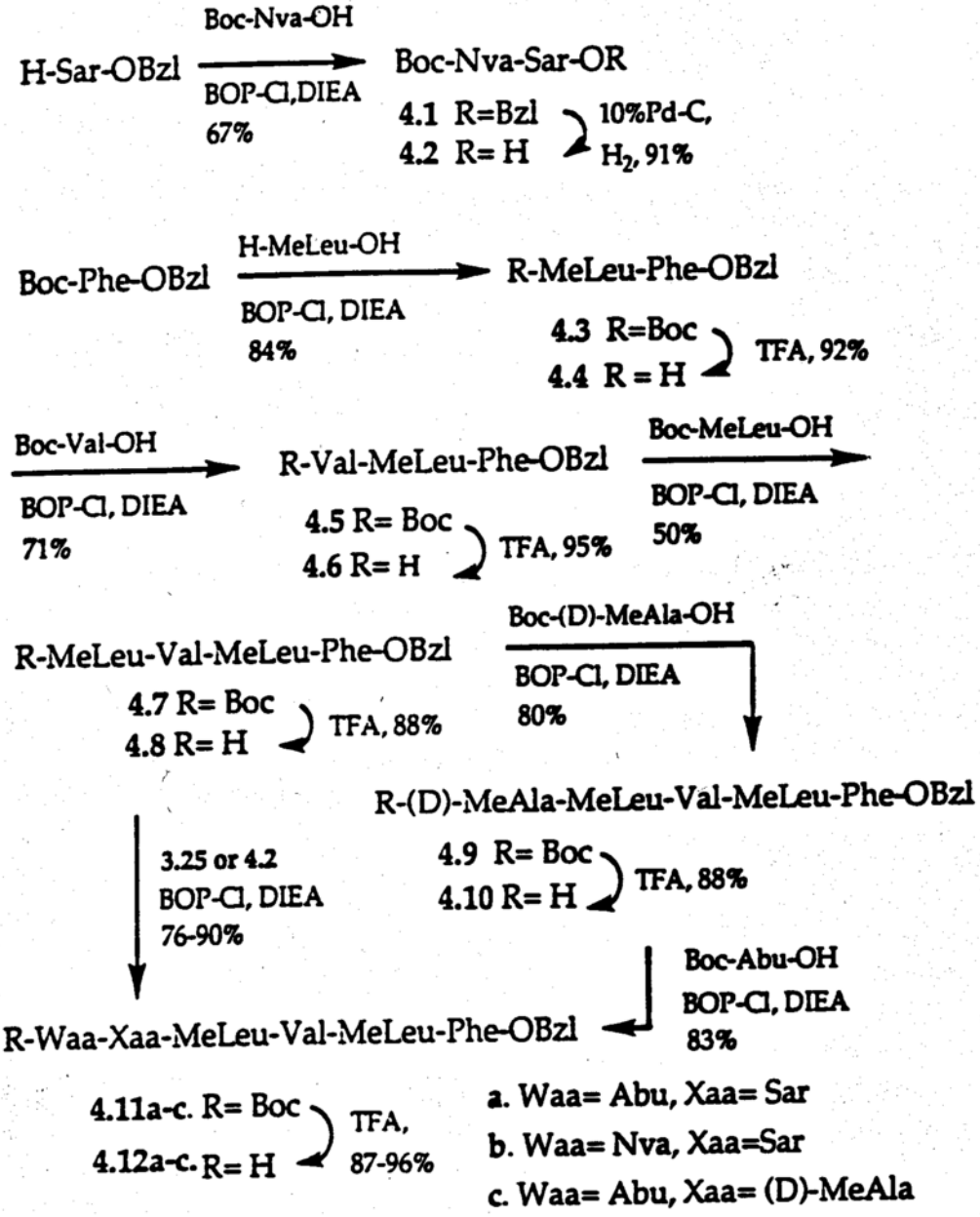
Biophysical studies of CsA as previously described (see Section D, Chapter I), have demonstrated that the solvent-exposed surface (at residues 4-8 of CsA) of the CsA-CyP complex binds to a complex involving CaN and calmodulin in the presence of calcium.[36] It is possible that the immunosuppressive activity is mediated by the solvent-exposed surface, or the so-called effector region of the bound drug.[60,157] Interestingly, the effector region is only present

in the bimolecular complex, since neither CsA nor CyP alone binds to activated CaN.[36] The presentation of the effector domain of CsA allowed explanation of the biochemical and biological data of a wide range of CsA analogs. As previously discussed (see Table 1.2, Chapter I), very few multiply substituted CsA analogs have been synthesized for the investigation of the biological activity of CsA. Taken together, these results encouraged me to design multiply substituted CsA analogs, modified at residues 2-8, which were presumably either near or at the effector region of the bound CsA.

The synthesis and immunosuppressive activity of the new CsA analogs **4.16a-e**, which replaced Ala^7 with Phe^7 and simultaneously modified the 2-, 3-, or 8-position with other amino acids, are described.

A. *Synthesis of 2-7 Hexapeptide Fragments, H-Waa-Xaa-MeLeu-Val-MeLeu-Phe-OBzl*

The synthesis of the 2-7 hexapeptide fragments **4.12a-c** was usually carried out by employing 4 + 2 fragment coupling (Scheme 4.1). Dipeptide **4.1** was routinely obtained by BOP-Cl mediated coupling in 67% yield. Catalytic hydrogenation of **4.1** gave dipeptide **4.2** with a free carboxylic group. The synthesis of the tetrapeptide **4.8** was started from the C-terminal residue H-Phe-OBzl, which was condensed with Boc-MeLeuOH utilizing BOP-Cl/DIEA for 12 h to give Boc-protected dipeptide **4.3** in 84% yield. After removal of the Boc protecting group with TFA, the resultant dipeptide **4.4** was



Scheme 4.1. Synthesis of CsA 2-7Analogous Fragments 4.12a-c

coupled with Boc-ValOH under the same BOP-Cl conditions. The Boc-protected tripeptide **4.5** was isolated by flash chromatography (10-30% EtOAc/hexane) in 71% yield.

Removal of the N-Boc protecting group by TFA followed by neutralization with $NaHCO_3$ yielded free tripeptide **4.6** in 95% yield. The desired tetrapeptide **4.8** was then obtained by coupling the tripeptide **4.6** with Boc-MeLeuOH followed by the removal of N-Boc group using TFA. The hexapeptides **4.11a,b** were prepared in 76-90% yields by coupling of the dipeptide **3.25** or **4.2** with the tetrapeptide **4.8** via the BOP-Cl method. Another hexapeptide **4.11c** was built by stepwise addition of the corresponding amino acids to the tetrapeptide **4.8**. The N-Boc protecting groups of the resultant hexapeptides **4.11a-c** were removed under similar TFA conditions to give the desired hexapeptides **4.12a-c** in 87-96% yields, which were readily used in further extension reactions. The yields and optical rotations for the BOP-Cl mediated coupling reactions and TFA-mediated deprotections are summarized in Table **4.1** and **4.2**. The formation of tetrapeptide **4.7** (via 3 + 1 fragment coupling) showed the lowest yield among these coupling results.

Table 4.1. BOP-Cl coupling[a] of N-protected amino acids with segments of the 2-7 peptides

entry	compound	product sequence[b]	$[\alpha]_D$ (c, $CHCl_3$)	yield(%)
1	4.1	BocNva-SarOBzl	-8.6°(0.8)	67
2	4.3	BocMeLeu-PheOBzl	-75.1°(0.67)	84
3	4.5	BocVal-MeLeuPheOBzl	-74.0°(0.55)	71
4	4.7	BocMeLeu-ValMeLeuPheOBzl	-60.4°(0.7)	50
5	4.9	Boc(D)MeAla-MeLeuValMeLeuPheOBzl	-63.9°(1.0)	80
6	4.11a	BocAbuSar-MeLeuValMeLeuPheOBzl	-105.0°(0.7)	85-90
7	4.11b	BocNvaSar-MeLeuValMeLeuPheOBzl	-97.6°(1.2)	76
8	4.11c	BocAbu-(D)MeAlaMeLeuValMeLeuPheOBzl	-71.2°(2.0)	75-83

[a] BOP-Cl, DIEA; in situ activation of the amino acid as described in ref. 133.

[b] The line (-) indicates site of new peptide bond formed from acid-amine coupling.

Table 4.2. Optical rotations and yields of aminopeptide or acid fragments

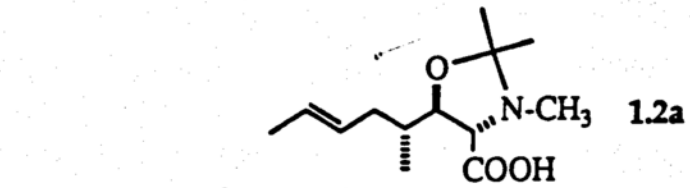
entry	compound	product sequence	$[\alpha]_D$ (c, $CHCl_3$)	yield(%)
1	4.2	BocNvaSar-OH	-4.3°(1.4)	91
2	4.4	H-MeLeuPheOBzl	-22.3°(0.35)	96
3	4.6	H-ValMeLeuPheOBzl	-60.0°(0.3)	97
4	4.8	H-MeLeuValMeLeuPheOBzl	-98.9°(1.1)	87
5	4.10	H-(D)MeAlaMeLeuValMeLeuPheOBzl	-87.1°(1.4)	96
6	4.12a	H-AbuSarMeLeuValMeLeuPheOBzl	-98.7°(0.54)	96
7	4.12b	H-NvaSarMeLeuValMeLeuPheOBzl	-107.7°(1.0)	98
8	4.12c	H-Abu(D)MeAlaMeLeuValMeLeuPheOBzl	-76.0°(0.27)	96

B. *Synthesis of CsA 1-7 Heptapeptide Analogous Fragments, H-MeBmt-Waa-Xaa-MeLeu-Val-MeLeu-Phe-OBzl*

The synthesis of aminoheptapeptides **4.14a-c** employed DCC coupling of acetonide-protected MeBmt (**1.2a**) to hexapeptides **4.12a-c**, with subsequent deprotection in dilute aqueous HCl in methanol (Scheme **4.2**). The protected heptapeptides **4.13a-c** were obtained in relatively low yields (49-68%), while acetonide deprotection gave **4.14a-c** in 79-81% yields.

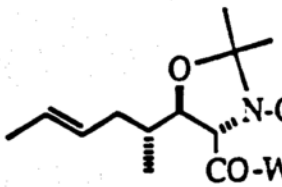
C. *Fragment Coupling and Cyclization*

The remaining 4 + 7 coupling (Scheme **4.2**) was still the most challenging step in the overall synthesis of the CsA analogs. Wenger's method, utilizing Castro's BOP reagent, was employed in the coupling, although it seldom gave better than 50% yield of linear undecapeptides.[8] In my hands, the undecapeptides **4.15a-e** were obtained by coupling of heptapeptides **4.14a-c** with N-protected tetrapeptides **3.42a,d** in moderate 42-52% yields after 3 days reaction. A major impurity usually appeared in 10-15% (believed to be the diastereomer resulting from racemization of MeVal at the 11-position).[133] The final cyclization was also carried out according to methods described by Wenger.[8] Saponification of the C-terminal benzyl ester and concomitant removal of the N-terminal Fmoc group of **4.15a-e** using ethanolic/0.2N $NaOH_{(aq)}$ at 0-5 °C for 12 h gave crude deprotected undecapeptide intermediates. After workup, the free undecapeptides were



1.2a

4.12a-c



DCC, HOBT
NMM, 49-68 %

CO-Waa-Xaa-MeLeu-Val-MeLeu-Phe-OBzl

aq. HCl, MeOH 4.13a-c
79-81%

H-MeBmt-Waa-Xaa-MeLeu-Val-MeLeu-Phe-OBzl

4.14a-c

3.42a,d

BOP, NMM,
42-52%

Fmoc-Uaa-MeLeu-MeLeu-MeVal-MeBmt
|
BzlO-Phe-MeLeu-Val-MeLeu-Xaa-Waa 4.15a-e

1. aq. NaOH, EtOH
2. $(Pr\text{-}PO_2)_3$, DMAP
30-70%

Uaa-MeLeu-MeLeu-MeVal-MeBmt
| |
Phe-MeLeu-Val-MeLeu-Xaa-Waa 4.16a-e

4.16a : Uaa=(D)-Ala, Waa=Abu, Xaa=Sar
4.16b : Uaa=(D)-Ala, Waa=Nva, Xaa=Sar
4.16c : Uaa=(D)-Ala, Waa=Abu, Xaa=(D)-MeAla
4.16d : Uaa=(D)-Ser, Waa=Abu, Xaa=Sar
4.16e : Uaa=(D)-Ser, Waa=Abu, Xaa=(D)-MeAla

Scheme 4.2. Synthesis of CsA Analogues **4.16a-e**

directly cyclized, employing propylphosphonic anhydride/4-(dimethylamino)-pyridine, in a dilute solution ($2x10^{-4}$ M of CH_2Cl_2) for 2 days at room temperature. The desired CsA analogs **4.16a-c** were readily isolated by flash chromatography (10-50% acetone/distilled hexane) in 57-70% yields, whereas with a primary hydroxyl group at (D)-Ser^8 and/or (D)-MeAla^3, compounds **4.16d,e** were usually obtained in lower yields (30-32%) in the final cyclization. One reason for this may be that in the rigid ßII' turn region of CsA, Sar^3 was replaced with the more hindered (D)-MeAla^3, thus disfavoring a driving force for the cyclization process. Another reason is that the

Table 4.3. Physical properties of CsA analogs **4.16a-e** and their undecapeptide intermediates **4.15a-e**.

entry	compound	structure[b]	R_f (%)[a]	$[\alpha]_D$ (c, $CHCl_3$)	yield(%)
1	**4.15a**	[F^7]	0.47(50)	-137.8°(0.52)	42
2	**4.15b**	[Nva^2,F^7]	0.57(50)	-144.6°(0.5)	52
3	**4.15c**	[(D)-MeA^3,F^7]	0.68(55)	-124.5°(0.8)	45
4	**4.15d**	[F^7,(D)-S^8]	0.50(50)	-110.0°(0.02)	42
5	**4.15e**	[(D)-MeA^3,F^7,(D)-S^8]	0.58(50)	-70.9°(0.11)	53
6	**4.16a**	[F^7]	0.41(45)	-170.0°(0.03)	70
7	**4.16b**	[Nva^2,F^7]	0.46(50)	-146.0°(0.05)	49
8	**4.16c**	[(D)-MeA^3,F^7]	0.62(60)	-216.0°(0.05)	30
9	**4.16d**	[F^7,(D)-S^8]	0.38(50)	-240.0°(0.02)	38
10	**4.16e**	[(D)-MeA^3,F^7,(D)-S^8]	0.38(40)	-180.0°(0.04)	31

[a] TLC (% acetone/hexcane). [b] Abbreviated symbol: A=Ala, F=Phe, S=Ser.

unwanted acylation of the primary hydroxyl group of (D)-Ser[8] readily occurs under the basic conditions. The physical properties of CsA analogs **4.16a-e** and their undecapeptide intermediates **4.15a-e** are summarized in Table **4.3**. The ^{1}H NMR spectra of these CsA analogs are shown in Figures **4.1 ~ 4.5** (see Appendix II, pages 238 ~ 242).

D. *Results of Conformational Analysis by NMR*

Solution NMR studies of the conformations of the CsA analogs **4.16a-e** were carried out by using 1D and 2D NMR methods. Each analogue is present dominantly as a single conformation (>90%) in chloroform. NMR analysis indicates that the chemical shifts of the amide protons, the N-methyl protons, the α protons, and the carbonyl carbons are very similar to those of CsA for all the five [Phe[7]]CsA analogues (Table **4.4-4.6**), indicating that they adopt a similar conformation in chloroform solution. In comparison with the 1D ^{1}H NMR spectrum of CsA in chloroform, the [Phe[7]] analogues do show some characteristic differences: 1) The chemical shifts of the four amide protons of five [Phe[7]]CsA analogues are somewhat different from those of CsA. With D-Ser at 8-position, the amide protons at residue 7 in compounds **4.16d,e** are shifted downfield (7.88 vs. 7.68 ppm). For the amide proton at residue 8, an upfield shift (7.03 vs. 7.17 ppm) is observed for analogs **4.16a-c**, whereas a downfield shift (7.46-7.47 vs. 7.17 ppm) is present in the analogues **4.16d,e.** 2) The α-protons show a downfield shift (4.71-4.74 vs. 4.52 ppm) at residue 7 and

also display a downfield shift (5.66-5.76 ppm vs. 5.47 ppm) at residue 1 in these [Phe^7]CsA analogs. These major spectral differences seemed to be the result of the structural modification in residues 7 and 8. The phenyl group in residue 7 contributes to the shielding effect on the amide proton in residue 8, whereas the β-hydroxy group in residue 8 might hydrogen-bond with the amide protons at residues 7 and 8, hence causing the observed downfield shifts. 3) A 2D correlation spectroscopy experiment established that an upfield doublet that resonates near 0.54-0.60 ppm arises from one of the two methyl groups of the Val^5 isopropyl side chain, which, in the case of CsA, is found close to 0.82 ppm. The marked difference is due to the shielding effect of the phenyl group at residue 7 in which the dominant conformation of the aromatic ring is close and parallel to the methyl group at Val^5, while this orientation contributes to the deshielding effect on the α protons at residues 1 and 7.

Table 4.4. Chemical Shifts of Protons in CsA and CsA Analogues **4.16a-e**

Compound	NH				N-CH$_3$						
	2	5	7	8	1	3	4	6	9	10	11
CsA	7.96	7.48	7.68	7.17	3.51	3.40	3.11	3.25	3.12	2.70	2.71
4.16a	7.83	7.53	7.61	7.03	3.54	3.39	3.09	3.15	3.13	2.71	2.74
4.16b	7.77	7.56	7.61	7.03	3.55	3.38	3.09	3.16	3.12	2.72	2.74
4.16c	8.00	7.55	7.62	7.03	3.55	3.24	3.07	3.17	3.13	2.70	2.75
4.16d	7.98	7.51	7.88	7.47	3.55	3.45	3.09	3.27	3.19	2.72	2.75
4.16e	7.96	7.52	7.88	7.46	3.56	3.27	3.07	3.26	3.18	2.71	2.75

Table 4.5. Chemical Shifts of Protons in CsA and CsA Analogues (continued)

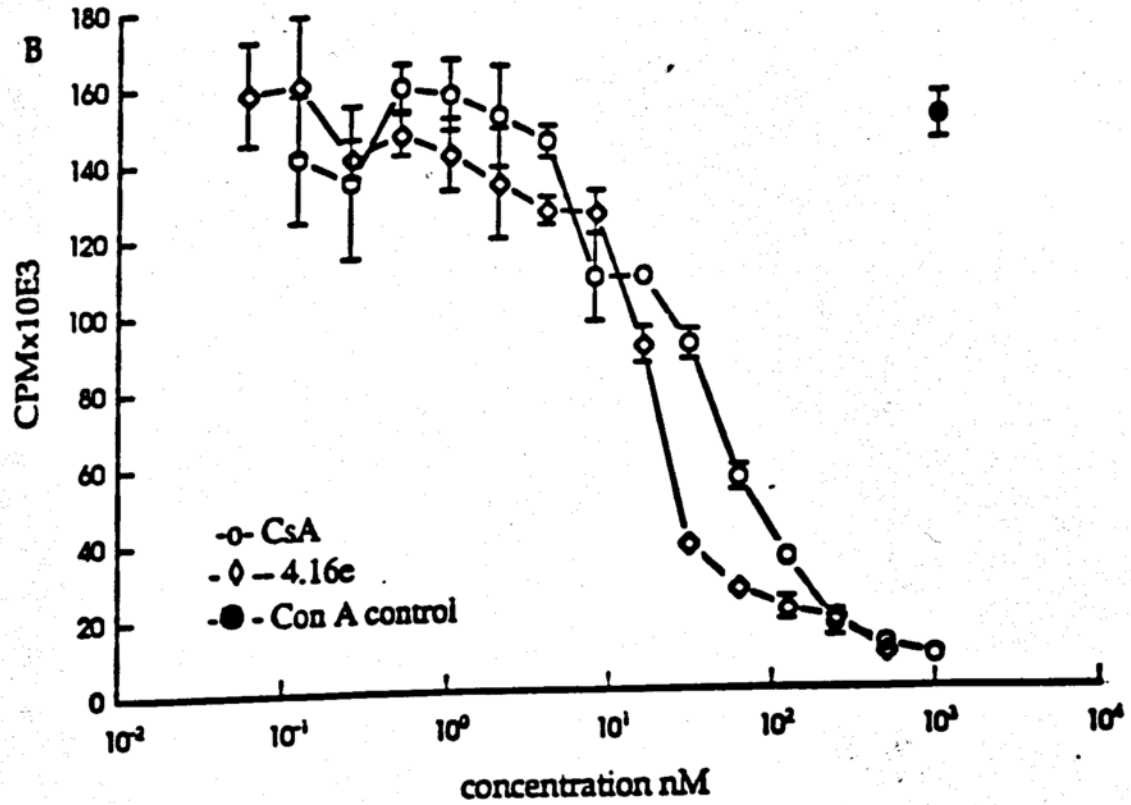
Compound	α-H												Cβ-H
	1	2	3		4	5	6	7	8	9	10	11	1
			re-H	si-H									
CsA	5.47	5.03	3.23	4.76	5.34	4.66	5.02	4.52	4.83	5.70	5.10	5.14	3.82
4.16a	5.72	5.05	3.22	4.73	5.29	4.51	5.09	4.72	4.85	5.68	5.12	5.18	3.81
4.16b	5.74	5.12	3.21	4.72	5.31	4.50	5.07	4.71	4.85	5.68	5.14	5.18	3.80
4.16c	5.76	5.03		4.94	5.25	4.48	5.07	4.73	4.85	5.68	5.11	5.17	3.76
4.16d	5.66	5.02	3.21	4.73	5.14	4.55	5.05	4.71	4.87	5.69	5.08	5.17	3.88
4.16e	5.73	5.06		4.94	5.26	4.51	5.05	4.74	4.86	5.68	5.08	5.16	3.82

Table 4.6. Chemical shifts of carbonyls in CsA and CsA Analogues **4.16a-e**

Compound	Carbonyl of amino acid										
	1	2	3	4	5	6	7	8	9	10	11
CsA	169.7	173.0	169.4	173.1	170.4	170.9	170.4	172.9	169.8	169.5	172.9
4.16a	170.3	173.6	171.2	169.9	173.9	172.1	170.8	173.3	170.3	170.0	173.2
4.16b	170.3	173.7	171.2	169.9	174.0	172.1	170.8	173.3	173.4	170.0	173.2
4.16c	170.3	175.5	172.2	169.8	174.6	173.3	170.8	173.7	170.3	170.1	173.4
4.16d	170.2	173.7	171.6	170.1	173.9	172.4	171.2	173.5	170.7	170.1	172.6
4.16e	170.2	175.4	172.3	170.2	174.5	172.4	171.4	173.7	170.7	170.0	173.4

E. *Kinetic Studies and Biological Evaluation.*

Compounds **4.16a-e** were monitored for their abilities to inhibit PPIase activity of CyP according to the assay procedures previously described by the Rich group.[26,27] **4.16c-e** showed a similar or even stronger PPIase inhibition constant (Ki=3 ~ 6± 2 nM) than that of CsA (Ki=6±1 nM), whereas compounds **4.16a,b** showed weak binding to CyP (Ki= 33±7 nM and 140±20 nM, respectively). *In vitro* immunosuppressive activity of products **4.16a-e** was



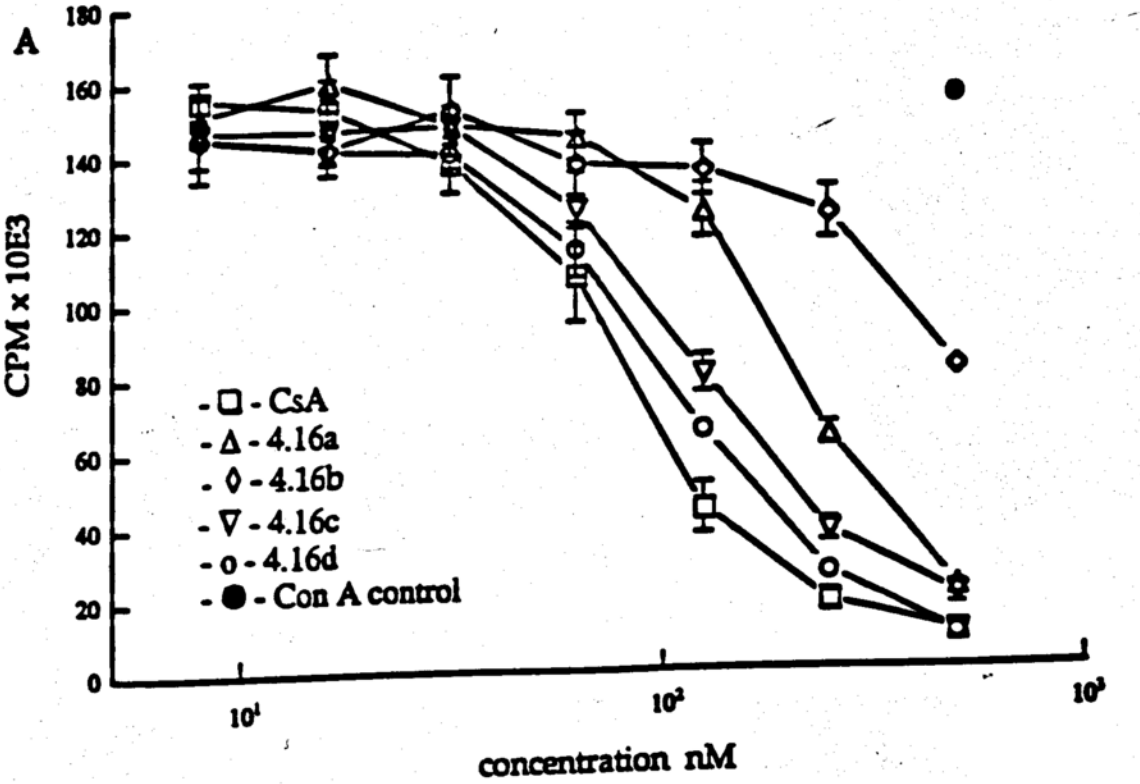


Figure 4.6. Effect of CsA and Analogs 4.16a-d (A) and 4.16e (B) on proliferation of BDF1 mouse cells stimulated with optimal Con A.

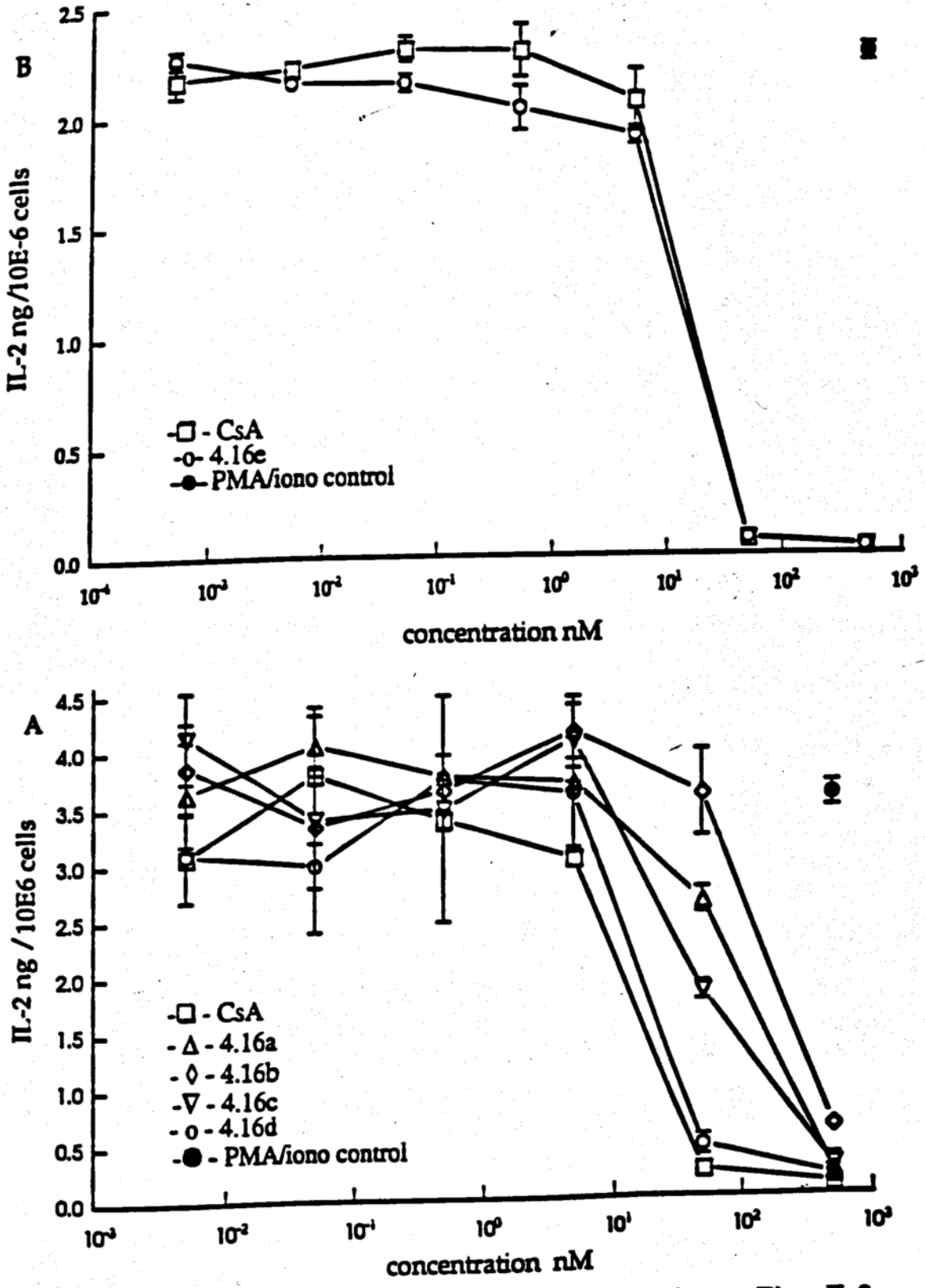


Figure 4.7. Effect of CsA and analogs 4.16a-d (A) and 4.16e (B) on IL-2 production by PMA/ionomycin-stimulated Jurkat cells.

determined by Dr. Alison Badger (SKB Pharmaceuticals) using the inhibition of concanavalin A (Con A) stimulated BDF1 mouse spleen cells (Figure 4.6) and of IL-2 release stimulated with PMA/ionomycin in Jurkat cells (Figure 4.7) as previously described[14]. These results are summarized in Table 4.7, and indicated that compounds **4.16a-e** show a good correlation between PPIase inhibition and immunosuppressive activity with multiply-substituted modifications at residues 2-8 of CsA.

F. *Discussion*

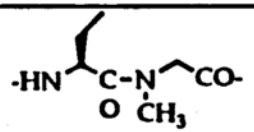
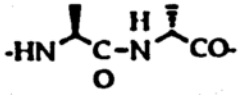
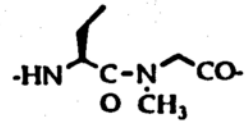
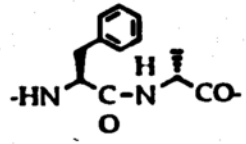
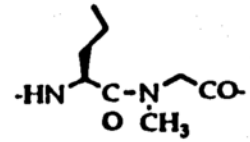
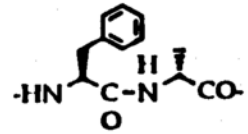
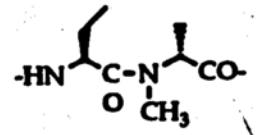
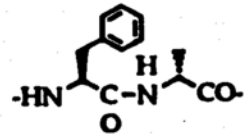
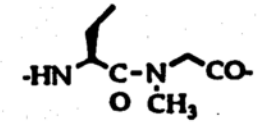
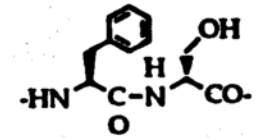
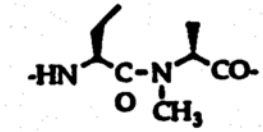
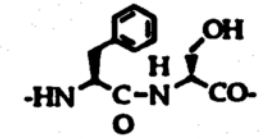
Some corresponding singly-modified CsA analogues have been studied for CyP-binding or immunosuppressive activity. For instance, [(D)-MeAla[3]]CsA,[9,12] which preserves the peptide ring conformation with a right handed type-II' ß- turn at residue 3 and 4 of CsA,[50] still showed 81% of CyP binding and 61% of IL-2 release inhibition of CsA. Modification at the 2-position also afforded analogues which retained immunosuppressive activity, such as the natural metabolites [Thr[2]]CsA (CsC) and [Nva[2]]CsA (CsG).[11,12,13] In fact, [Nva[2]]CsA has received considerable attention as a potential second generation cyclosporine with diminished nephrotoxicity. (D)-Ala at position 8 could be successfully substituted with (D)-Ser or (D)-MeSAla without loss of immunosuppressive activity. [(D)-Ser[8]]CsA which was obtained by precursor directed biosynthesis exhibited strong immunosuppressive activity.[113]

In this study, [Phe[7]]CsA analogs **4.16a-e**, derived from the modification

between residues 2-8 of CsA, still maintained a good correlation between the immunosuppressive activity and their affinity for CyP, which has been established by several studies.[9,10,19] The results are shown in Table 4.7, and indicate that replacement of Ala^7 with aromatic Phe^7 to give [Phe^7]CsA (**4.16a**) only decreased both CyP-binding ability and immunosuppressive activity.

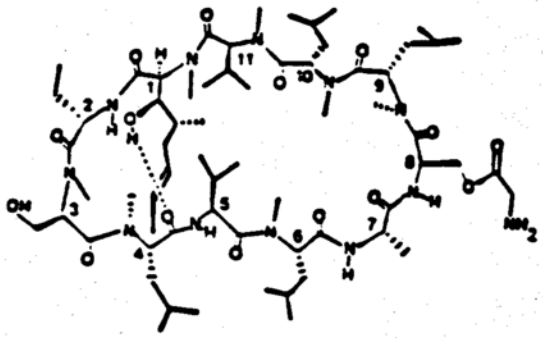
Extension of the side chain at residue 2 (Abu) or 3 (Sar) with a methyl group, respectively, in [Phe^7]CsA gave dramatic effects on biological activity. Substitution of Abu^2 by Nva^2 to give [Nva^2,Phe^7] (**4.16b**) diminished both CyP affinity and immunosuppressive effect in spite of the fact that Nva^2 is a good substitution in CsA itself (see CsG, Table 1.1; page 4). In contrast, replacement of Sar^3 with (D)-MeAla^3 gave the [(D)-MeAla^3,Phe^7]CsA (**4.16c**), which regains good CyP-binding affinity (6±2 nM) and has 67% the immunosuppressive activity of CsA. These results show that multiple substitutions of CsA can lead to retention of or regaining of the biological activities associated with CsA. When a polar hydroxy group is added at the 8-position, [(D)-Ser^8,Phe^7]CsA (**4.16d**) is a potent immunosuppressant (83% of CsA) and also binds tighter to CyP. It was expected that the free hydroxyl group at position-8 would contribute the solvating effect of **4.16d** when binding to CyP. Most importantly, simultaneous modification of CsA with (D)-MeAla^3, Phe^7 and (D)-Ser^8 results in **4.16e**, which binds more efficiently than CsA to CyP (3±1.5 nM vs. 6±1 nM) and fully maintains the

Table 4.7. Structure, PPIase inhibition and immunosuppressive properties of CsA analogs

CsA Analogue	Amino Acid Residue				PPIase (Ki, nM)	Immunosuppression(IC_{50}, nM)	IL-2 release (IC_{50}, nM)
	2	3	7	8			
CsA					6±1	100	15
[Phe^7] (4.16a)					33±7	230	130
[Nva^2,Phe^7] (4.16b)					140±20	600	200
[D-MeAla^3,Phe^7] (4.16c)					6±2	150	60
[D-Ser^8,Phe^7] (4.16d)					6±2	120	21
[D-MeAla^3,Phe^7, D-Ser^8] (4.16e)					3±1.5	50±10	15

strong immunosuppressive activity of CsA in terms of inhibition of BDF-1 spleen cell proliferation and IL-2 release in Jurkat cells (Figure 4.6, 4.7 and Table 4.7).

The origin of these remarkable structure-activity relationships may be due to conformational effects, Recently, Wenger and co-workers[167] determined the conformations of [(D)-MeSer^3,(D)-Ser(O-Gly)^8]CsA (4.17), a water soluble CsA derivative containing a (D)-MeSer residue in position 3 and a solubilizing (D)-Ser-O-glycine ester residue in position 8. The conformations were determined in DMSO and in water by use of NMR. In these polar solvents the conformations of 4.17 are essentially identical to each other and to the structure found in the CyP-CsA complex with respect to the peptide ring system. This result confirmed the prediction of Rich and co-workers[168]



4.17

that dissolution of CsA in water induces the "bioactive conformation", as opposed to the "bioactive conformation" being induced as CsA binds to CyP.[177,178]

Wenger's results further showed that the conformation of CsA analog **4.17** in DMSO is identical, within experimental error, to the conformation of **4.17** in water. This is a remarkable observation since CsA in DMSO adopts multiple conformations (see Figure 4.8, panel A). Therefore, it seemed reasonable that examining the conformations of my non-water soluble analogs in DMSO-d6 should provide a reasonable estimate of the aqueous conformation of each analog. According to this strategy, the spectrum of [(D)-MeAla^3,Phe^7,(D)-Ser^8]CsA (**4.16e**) was determined in DMSO-d6 and is shown in Figure **4.8** (panel B), where it is compared with the conformation of analogue **4.17** reported by Wenger (Figure **4.8**, panel C).[167] The data are consistent with both analogs having similar conformations in this solvent. These results suggest that the modifications at position 3 with (D)-MeAla and/or at position 8 with (D)-Ser contribute to conformational stability of the analogs [(D)-MeAla^3,Phe^7,(D)-Ser^8]CsA (**4.16e**) and Wenger's compound **4.17**. I then determined the DMSO conformation of [(D)-MeAla^3,Phe^7]CsA (**4.16c**) (Figure **4.9**, panel A) and found that this compound also existed predominantly in a single conformation. From these data, I concluded that the (D)-amino acid in position-3 is primarily responsible for restricting the conformation of these CsA analogs to one major conformation. The 8-position modification ((D)-Ala → (D)-Ser, etc) serves to help solublize the derivatives. This was confirmed when I determined the NMR of the [(D)-Ser^8,Phe^7]CsA derivative (**4.16d**), which adopted multiple conformations in

DMSO (Figure 4.9, panel B), as does CsA itself.

The increased conformational stability of CsA substituted with (D)-amino acids in the 3-position may contribute to the biological activities of the resulting analogs by stabilizing the "bioactive conformation" for immunosuppressive activity. The increased activity likely results from the increased concentration of the "bioactive conformation" of CsA. Likewise, the improved CyP-binding ability of **4.16e** might be due to the compound existing in the CyP-binding conformation in the polar solvent (e.g. where the assay was done). It also suggested that multiple substitutions at residues 3-8 of CsA that improve potencies in both PPIase inhibitions and immunosuppressive properties are related in part to the compensating increase in CyP and CaN bindings seen with compound **4.16e** compared to the structurally related compounds **4.16a,c,d**. However, since [(D)-MeAla^3,Phe^7]CsA (**4.16c**) does not regain the same activity as [(D)-MeAla^3,Phe^7,(D)-Ser^8]CsA (**4.16e**), the additional 8-position modification in same way further increases activity. These results suggest that the biological properties of multiply modified CsA derivatives cannot be readily predicted from the biological properties of singly substituted CsA analogs. My results suggest that it will be necessary to synthesize all relative CsA derivatives to characterize the biology of CsA analogs.

In summary, these findings demonstrate that modifications near the effector domain of CsA in positions 3, 7, and 8 with multiple substitutions

like analogue **4.16e** appear to significantly improve immunosuppressive activity compared to the analogs **4.16a,c,d**. Substitution at the 3-position with a (D)-amino acid stabilizes the "bioactive conformation" for target protein binding. Further, the compensatory effects of multiple replacements in CsA on PPIase inhibition and immunosuppressive activity are not yet predictable from the biology of the corresponding singly substituted derivatives with respect to the 3-, 7- and 8-positions.

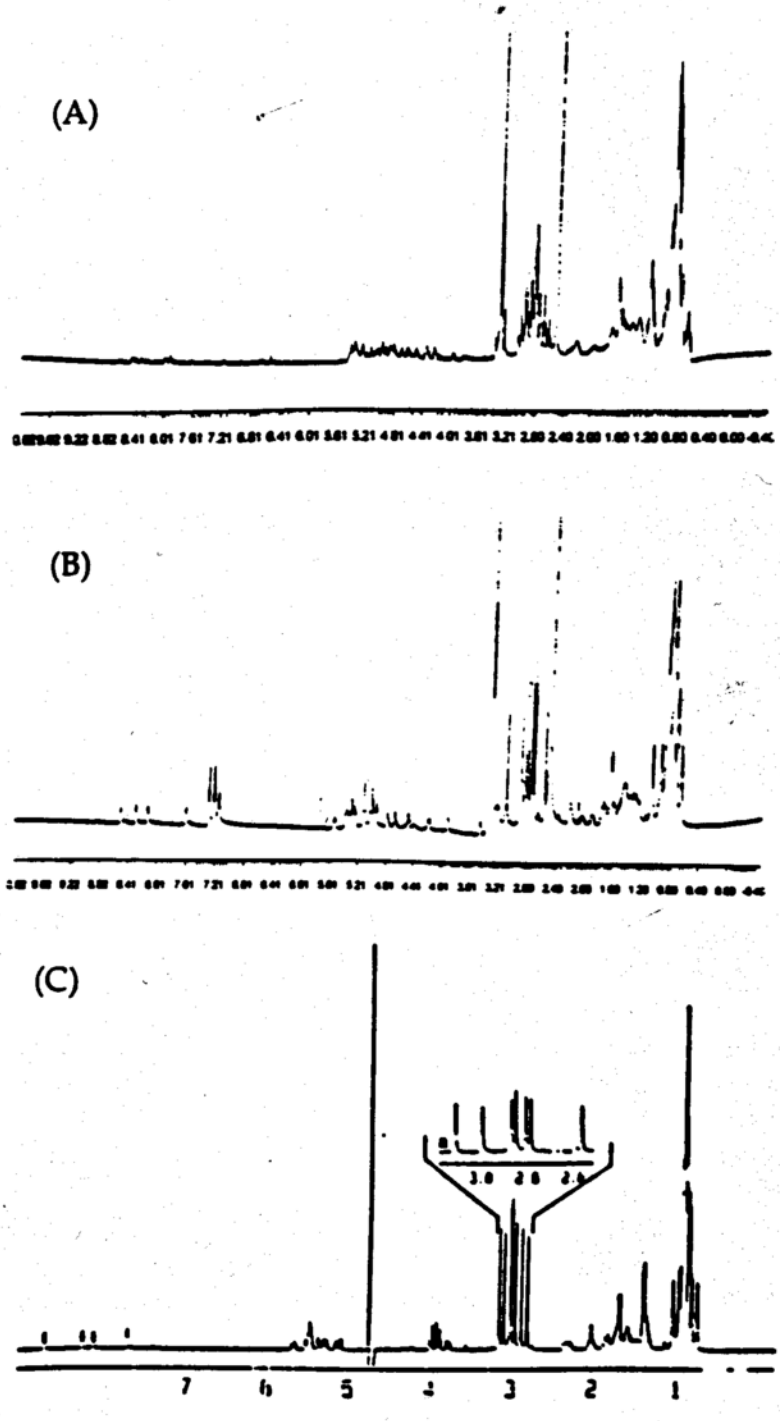


Figure 4.8. ^{1}H NMR spectra of CsA (panel A) and [(D)-MeAla^{3},Phe^{7},(D)-Ser^{8}]CsA (**4.16e**) in DMSO-d6 at 500 MHz (panel B), and [(D)-MeSer^{3},(D)-Ser(O-Gly)^{8}]CsA·HCl (**4.17**)[167] in water (panel C) and DMSO-d6 (at insert a, the N-methyl region of the NMR spectrum is shown) at 400 MHz.

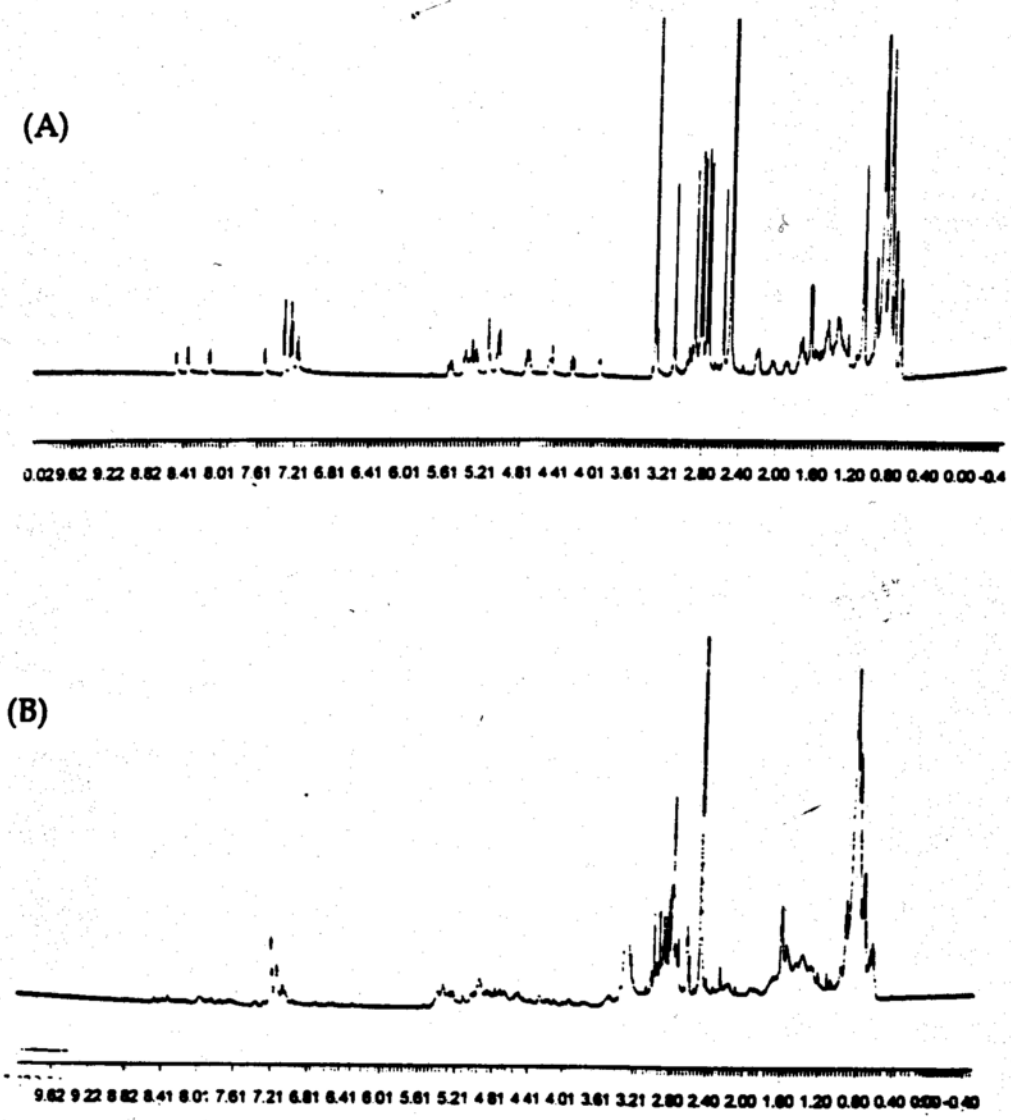


Figure 4.9. ^{1}H NMR spectra of [(D)-MeAla^{3},Phe^{7}]CsA (**4.16c**, panel A) and [(D)-Ser^{8},Phe^{7}]CsA (**4.16d**) in DMSO-d6 at 500 MHz (panel B).

Chapter V. Synthesis of Non-immunosuppressive CsA Analogs as Anti-HIV Agents

Several investigators[79-82] have demonstrated that CsA inhibits replication of the human immunodeficiency virus (HIV) and cell growth in chronically infected cells *in vitro*, especially when it was administrated early during infection.[79] Although the mechanism of this action of CsA is still unknown, it was conceivable that blocking immune activation of HIV before severe immunosuppression set in would have a beneficial effect.[158] Since Rich's group previously reported[17] the synthesis of several non-immunosuppressive CsA analogs that are tight-binding inhibitors of CyP, it was of interest to know if non-immunosuppressive CsA derivatives also would inhibit HIV replication. The reason is that if non-immunosuppressive CsA analogs could inhibit HIV replication, then the immune system would not have to be suppressed by the normal action of CsA. Thus, the undesired side effects of immunosuppression, especially the risk of malignant lymphoma associated with long-term immunosuppression could be avoided. A recent report established that CsA can block the binding of HIV-1 gag protein to cyclophilin A (CyPA).[84] This implied that a gag-CyPA interaction may play an important role in the HIV-1 life cycle, which may be relevant to acquired immunodeficiency syndrome (AIDS) immunopathology. Taken together, it was proposed that the anti-viral effect of CsA might be due to

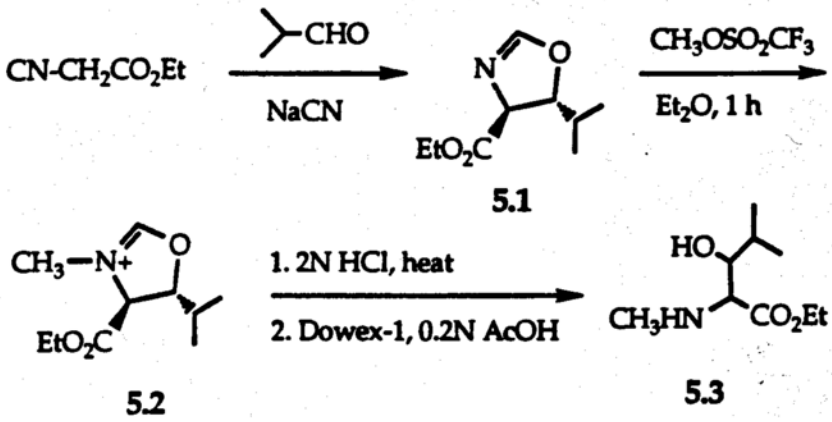
mechanisms that do not involve immunosuppression.

In order to design non-immunosuppressive CsA analogs that can be tested for the effect on HIV-1 replication without causing immunosuppression, selective modifications of the effector domain (residues 4-8) in CsA would separate the CyP-binding properties of CsA from the immunosuppressive properties of CsA. According to Rich's report, the CsA analogue [MeLeu(OH)^1]CsA (**5.21a**), which has a shorter side chain in the 1-position than is found in CsA, showed negligible immunosuppressive activity, yet it retained the conformation of the cyclic ring system in chloroform.[17] These results, which were reported long before either the enzyme-bound conformation of CsA had been discovered or before the CsA-CyP-CaN mechanism had been discovered, had to be reported. These encouraged us to synthesize new analogs **5.21b-d** based on [MeLeu(OH)^1]CsA with modifications at the effector domain of the bound CsA and to investigate their anti-HIV properties and relative CyP-binding abilities.

A. *Synthesis of β-Hydroxy-N-methylleucine (MeLeu(OH))*

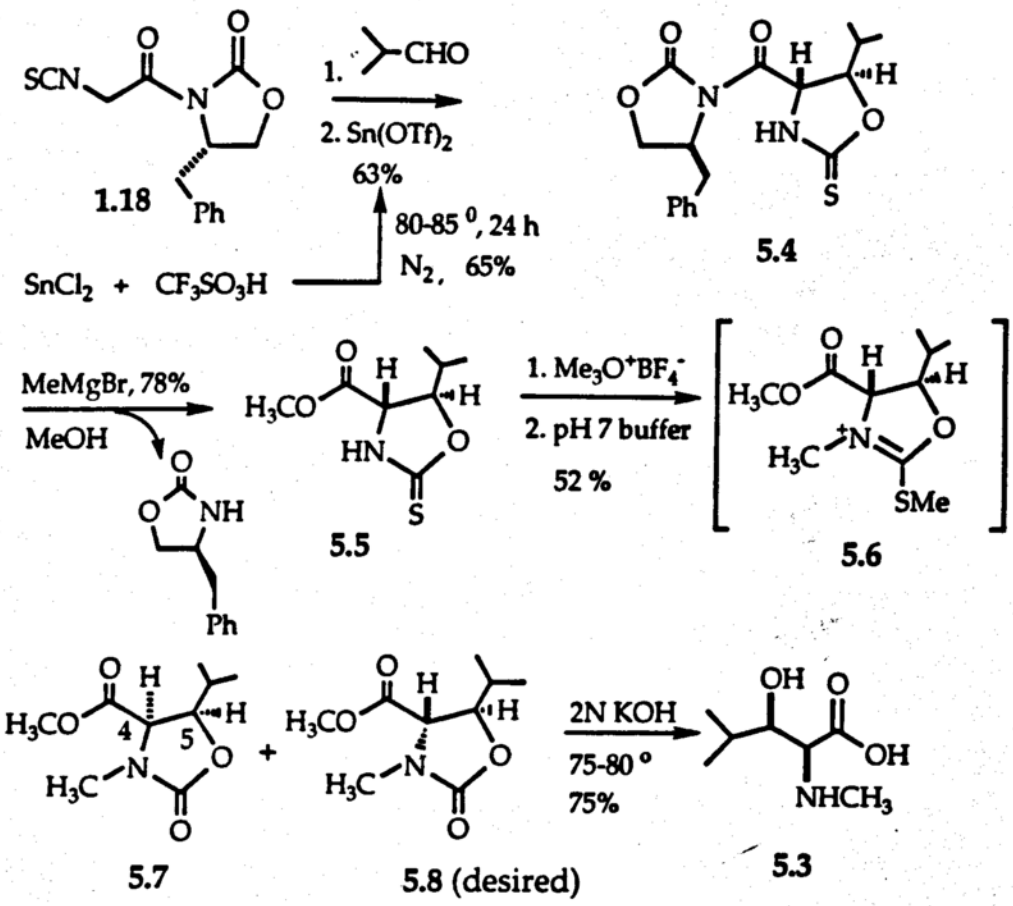
The synthesis of MeLeu(OH) has been reported by Rich's group,[17] in which the procedure developed by Schöllkopf[166] for the synthesis of β-hydroxy amino acids (Scheme **5.1**) was adapted. The reaction of isocyanoacetate with isobutyraldehyde in the presence of NaCN gave the thermodynamically stable *trans*-oxazoline **5.1** as the major product. The *trans*-oxazoline was treated

with methyl triflate at room temperature to give the N-methyl imidate **5.2**. Hydrolysis of **5.2** with dilute HCl followed by ion-exchange chromatography of the amino acid gave (±)-threo-β-hydroxy-N-methylleucine (**5.3**).



Scheme 5.1. Synthesis of (±)-threo-β-Hydroxy-N-methylleucine (ref. 17)

At the same time, an elegant asymmetric glycine enolate reaction was developed by Evans and Weber for the synthesis of MeBmt (see Scheme **1.2**, page 35)[119] and other chiral amino acids. The approach was also applied to prepare MeLeu(OH). In Scheme **5.2**, I followed this reaction sequence starting from the chiral glycine synthon isothiocyanate **1.18**, which was obtained from the corresponding chloroacetate **1.16** and followed by azide replacement (forming **1.17**) in 56% yield. The isothiocyanate chiral auxiliary **1.18** was condensed with isobutyraldehyde under stannous triflate mediated aldol reaction (-78 °C for 4 h) to give the aldol adduct **5.4** in 63% yield (> 90% e.e). Transesterification of **5.4** with a solution of magnesium methoxide in methanol at room temperature for 3 min gave methyl ester **5.5** in 78-82%



Scheme 5.2 : Synthesis of (2S,3R)-3-Hydroxy-N-methylleucine (**5.3**) by Evans Method

yield. The bis-methylation with trimethoxonium tetrafluoroborate did not go well in my hands. The yield of the reaction was still low (52% as compared to 76% of Evans in MeBmt synthesis) even when the commercially available Meerwein's reagent was repurified. Two epimers **5.7** and **5.8** were usually obtained in a ratio of 1:5, which was not found by Evans for the MeBmt synthesis. The occurrence of the kinetically favored minor product **5.7** may be due to abstraction of the labile 4-proton in the intermediate **5.6**

under basic conditions with proton sponge as previously described in Equation 3.1 (page 59). The ^{1}H NMR spectra of epimers 5.7/5.8 are shown in in Figure 5.1 and compared in Table 5.1. Hydrolysis of the desired *trans*-oxazolidinone 5.8 with 2N KOH under reflux gave the pure β-hydroxy-N-methylleucine (5.3, Figure 5.2) after chromatographic purification over Sephadex LH-20.

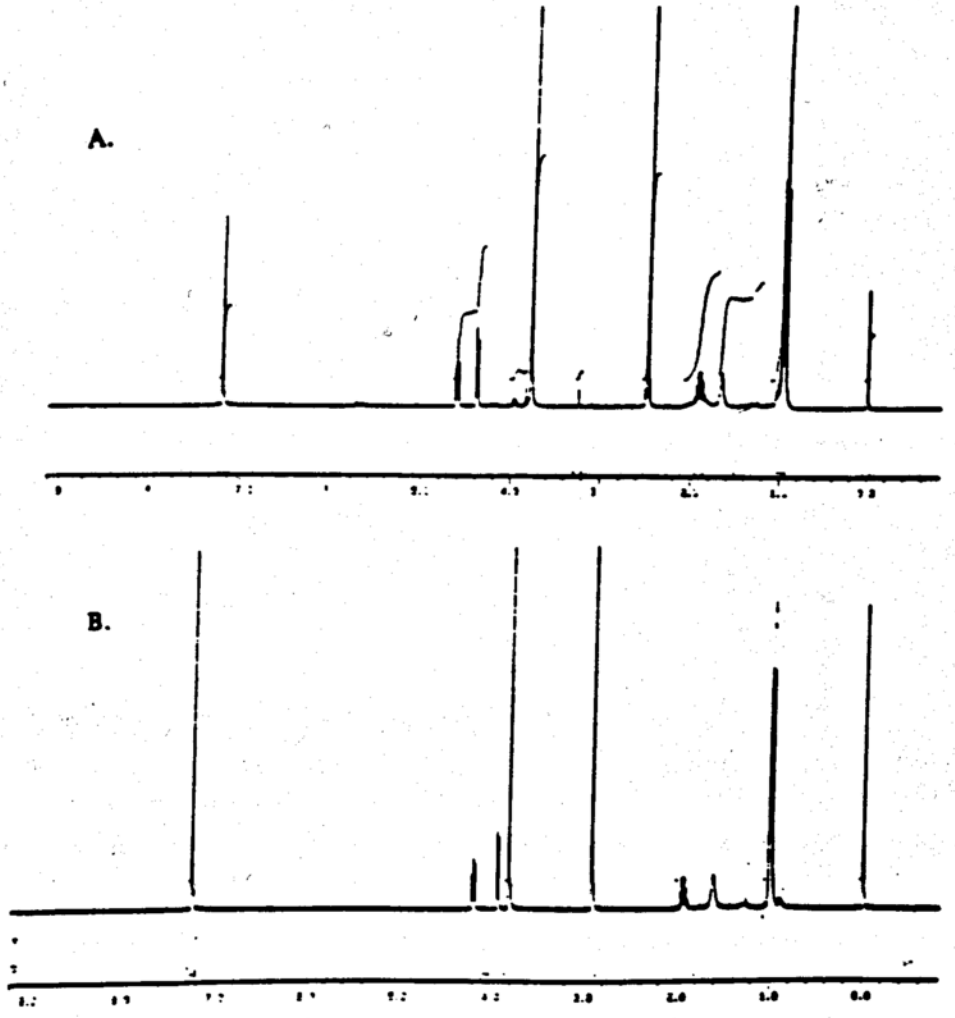


Figure 5.1. ^{1}H NMR spectra of oxazolidinone eipmers 5.7 (A) and 5.8 (B); the chemical shifts are compiled in Table 5.1.

Table 5.1. Chemical Shifts of Oxazolidinone Diastereomers 5.7 & 5.8

Oxazolidinone	Chemical shift δ (coupling constant J)			
	H-4	H-5	N-Me	O-Me
erythro-5.7	4.39 (d, J=6.7)	4.61 (t, J=6.7)	2.48	3.78
threo-5.8	3.94 (d, J=4.8)	4.21 (dd, J=4.8, 6.7)	2.92	3.82

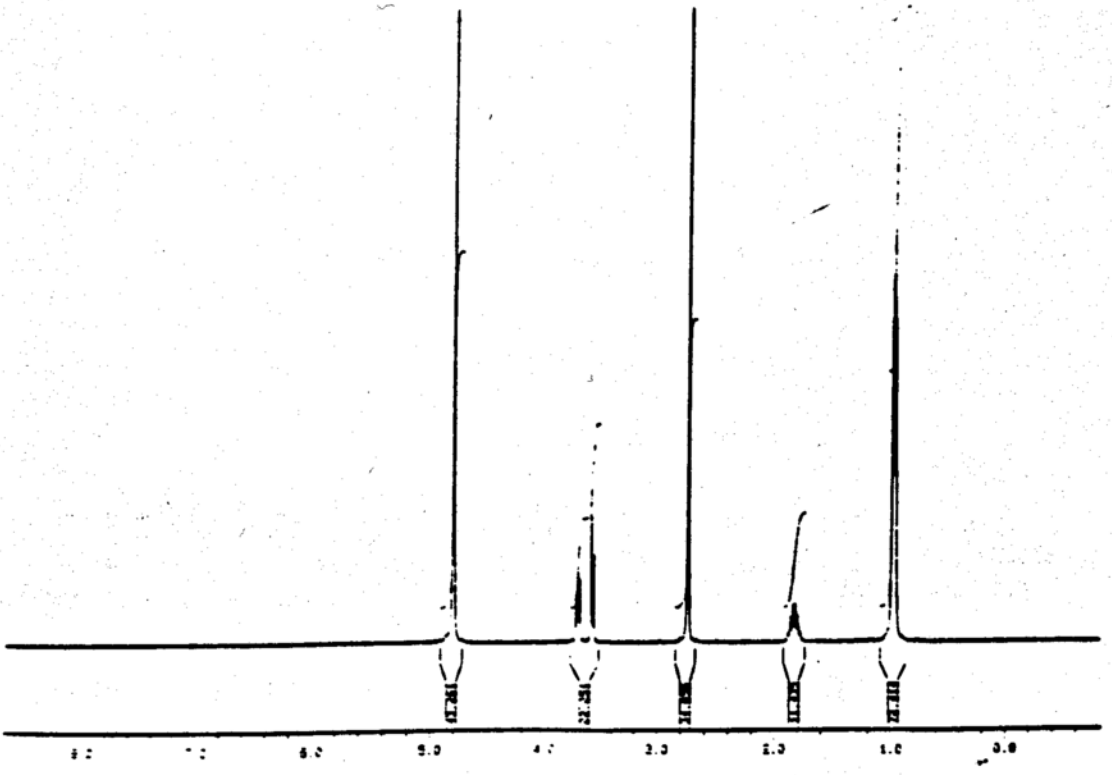
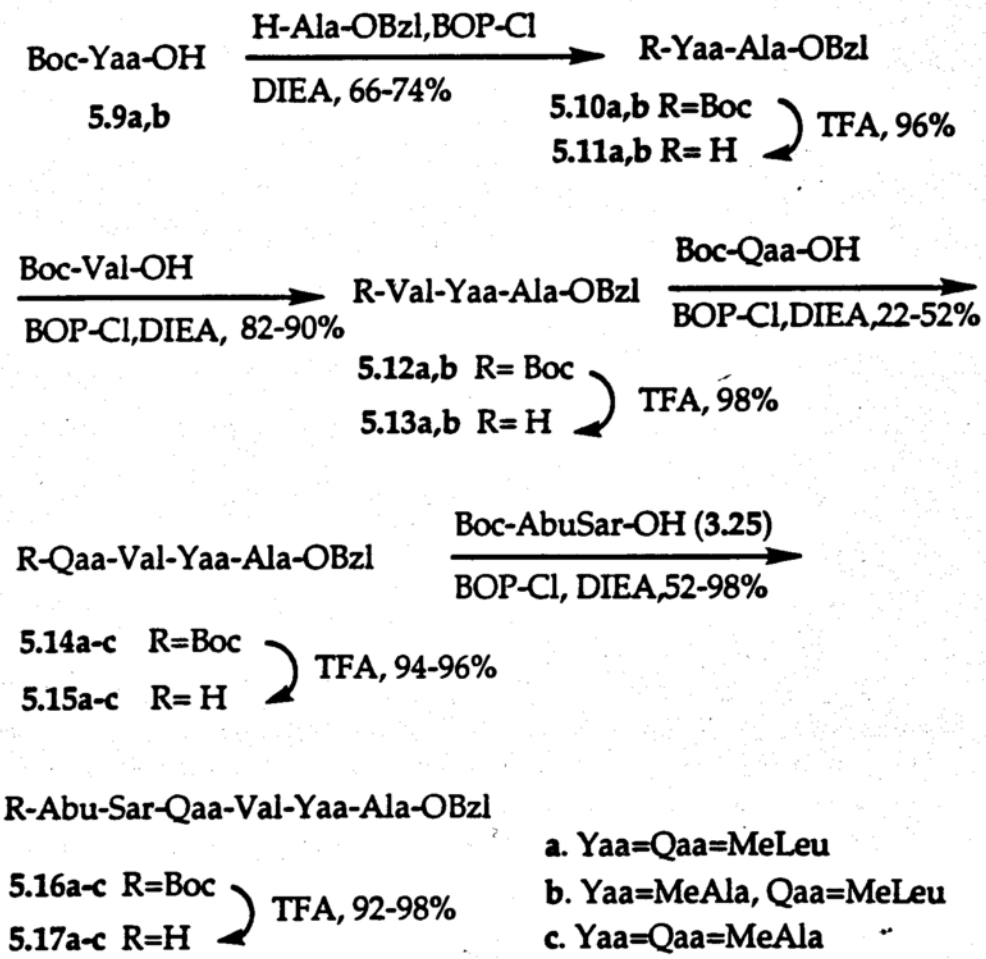


Figure 5.2. ^{1}H NMR spectrum of β-Hydroxy-N-methylleucine (5.3).

B. *Synthesis of [MeLeu(OH)1]CsA Analogs*

The synthesis of [MeLeu(OH)1]CsA analogs modified at residues 3-8 was carried out as described in Rich's original report.[133] The tetrapeptides **5.14a-c** were constructed starting from H-Ala-OBzl and adding the appropriate amino acids step by step in a series of coupling-deprotection procedures (Scheme 5.3). After deprotection of N-terminal Boc group, the resultant tetrapeptides



Scheme 5.3. Synthesis of CsA 2-7 Analogous Fragments **5.17a-c**

5.15a-c were condensed with Boc-AbuSar-OH (**3.25**) using the BOP-Cl/DIEA method to provide the hexapeptides **5.16a-c**. After removal of the N-Boc group with TFA, the corresponding amino-hexapeptides **5.17a-c** were obtained and quickly used for further reactions. The optical rotations and yields of the peptide fragments **5.10** ~ **5.17** for BOP-Cl mediated couplings and N-deprotections are summarized in Table **5.2** and **5.3**. The available hexapeptides were then acylated with the acetonide-protected MeLeu(OH) (**5.3**) using DCC/HOBt method to give desired protected heptapeptides

Table 5.2. BOP-Cl coupling[a] of N-protected amino acids with segments of the 2-7 peptides

entry	compound	product sequence[b]	$[\alpha]_D$(c, $CHCl_3$)	yield(%)
1	5.10a	BocMeLeu-AlaOBzl	-67.0°(1.0)[c]	74
2	5.10b	BocMeAla-AlaOBzl	-65.2°(1.5)	66
3	5.12a	BocVal-MeLeuAlaOBzl	-97.2°(1.0)[c]	76
4	5.12b	BocVal-MeAlaAlaOBzl	-86.0°(1.7)	90
5	5.14a	BocMeLeu-ValMeLeuAlaOBzl	-126.7°(1.0)[c]	95
6	5.14b	BocMeLeu-ValMeAlaAlaOBzl	-136.0°(0.82)	24
7	5.14c	BocMeAla-ValMeAlaAlaOBzl	-116.0°(0.9)	22
8	5.16a	BocAbuSar-MeLeuValMeLeuAlaOBzl	-128.1°(1.0)	56
9	5.16b	BocAbuSar-MeLeuValMeAlaAlaOBzl	-124.8°(0.5)	92
10	5.16c	BocAbuSar-MeAlaValMeAlaAlaOBzl	-128.0°(0.7)	98

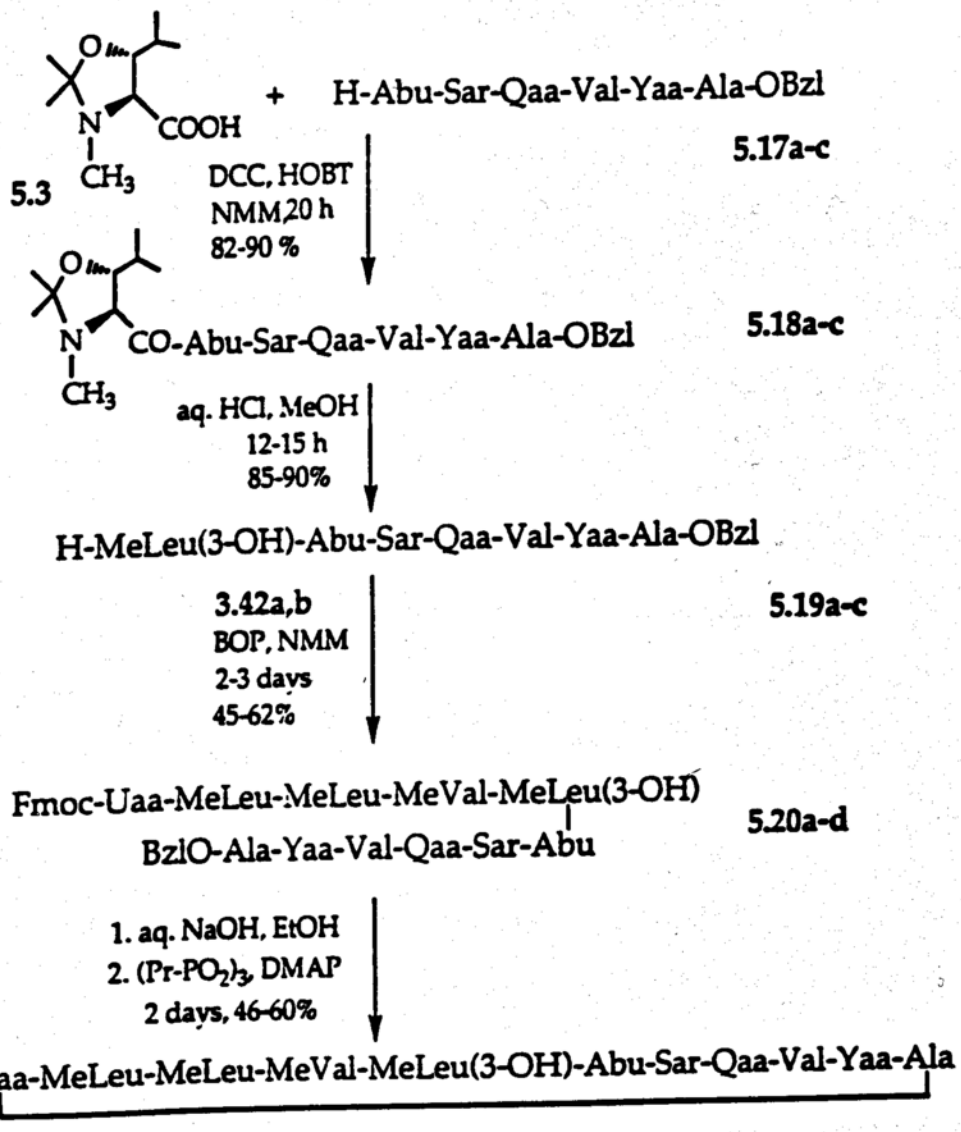
[a] BOP-Cl, DIEA; in situ activation of the amino acid as described in ref. 133. [b] The line (-) indicates site of new peptide bond formed from acid-amine coupling. [c] Data from ref. 133.

Table 5.3. Optical rotations and yields of aminopeptide fragments

entry	compound	product sequence	$[\alpha]_D$(c, $CHCl_3$)	yield(%)
1	**5.11a**	H-MeLeuAlaOBzl	-44.5°(1.0)[a]	96
2	**5.11b**	H-MeAlaAlaOBzl	-24.8°(0.66)	96
3	**5.13a**	H-ValMeLeuAlaOBzl	-102.0°(1.0)[a]	98
4	**5.13b**	H-ValMeAlaAlaOBzl	-45.4°(1.2)	98
5	**5.15a**	H-MeLeuValMeLeuAlaOBzl	-130.9°(1.0)	99
6	**5.15b**	H-MeLeuValMeAlaAlaOBzl	-101.0°(1.1)	94
7	**5.15c**	H-MeAlaValMeAlaAlaOBzl	-112.0°(0.95)	96
8	**5.17a**	H-AbuSarMeLeuValMeLeuAlaOBzl	-108.9°(1.27)	92
9	**5.17b**	H-AbuSarMeLeuValMeAlaAlaOBzl	-126.7°(0.12)	96
10	**5.17c**	H-AbuSarMeAlaValMeAlaAlaOBzl	-102.2°(0.9)	98

[a] Data from ref. 133.

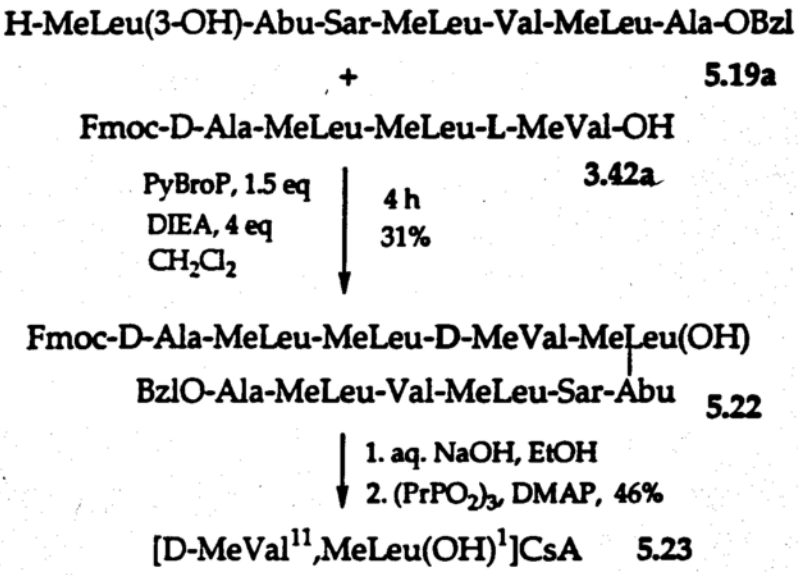
heptapeptides **5.18a-c** (82-90%) as shown in Scheme **5.4**. These heptapeptides appear as two major conformers in $CDCl_3$ by NMR due to the N-methyl amide conformers. Removal of the acetonide protecting group of the heptapeptides **5.18a-c** was performed using 1M HCl in methanol for 15 h. The resultant amino-heptapeptides **5.19a-c** were purified by flash chromatography (85-90%). For the coupling of the available tetrapeptides **3.42a,b** and heptapeptides **5.19a-c**, Castro's BOP reagent (**1.41**) and N-methyl morpholine were employed to achieve these linkages. The undecapeptides **5.20a-d** were obtained usually in relatively low yields 45-62% as compared to the 73% yield reported by Wenger in the case of CsA synthesis.[8]



5.21a: Uaa=D-Ala, Qaa=Yaa=MeLeu
5.21b: Uaa=D-Ala, Qaa=MeLeu, Yaa=MeAla
5.21c: Uaa=D-Ala, Qaa=Yaa=MeAla
5.21d: Uaa=D-Lys(Boc), Qaa=Yaa=MeLeu

Scheme 5.4. Synthesis of [MeLeu(OH)^1]CsA Analogs 5.21a-d

Recently, a type of pyrrolidinophosphonium complexes abbreviated as PyBroP (bromotripyrrolidino-phosphonium hexafluorophosphate, **5.27**), PyCloP, and PyBoP were reported as coupling reagents for peptide synthesis.[159] According to Castro's report, N-methyl amino acids could be coupled efficiently by using PyBroP/DIEA. Because of the low yield I was getting for the 4 + 7 coupling for the synthesis of undecapeptides, I tried to use PyBroP (1.5 equiv) in the coupling of the tetrapeptide **3.42a** (1.5 equiv) and the heptapeptide **5.19a** in the presence of DIEA (4 equiv) as shown in Scheme 5.5.



Scheme 5.5. PyBroP Mediated 4 + 7 Coupling Reaction

PyBroP indeed drove the coupling reaction to completion in 4 h. However, PyBroP also gave multiple spots by TLC and did not improve the yield of product (only 32%). The unexpected result was that the epimerized

undecapeptide **5.22** (with (D)-configuration at residue MeVal), which showed a higher R_f as compared to the corresponding **5.20a**, was obtained as the major product. Racemization was presumably due to the formation of hydrobromide during the activation of the carboxylic group of tetrapeptide **3.42a** with PyBroP, which could cause C-terminal residue [MeVal[11]] to epimerize.[175] A similar result has been reported by Wenger[8] (described in Scheme **5.6**), in which the mixed pivalic anhydride method (using pivaloyl chloride/N-methylmorpholine), gave the (MeVal) configuration-inverted undecapeptide Boc-(D)-Ala-MeLeu-MeLeu-(D)-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl (**5.26**) when coupling the tetrapeptide Boc-(D)-Ala-MeLeu-MeLeu-L-MeVal-OH (**5.24**) with the corresponding heptapeptide **5.25**. In both cases, epimerization may be due to high halide concentrations in the reaction media.

H-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl **5.24**

+

Boc-D-Ala-MeLeu-MeLeu-L-MeVal-OH **5.25**

$(CH_3)_3COCl$, NMM, 2 eq | 4 days, 44%

Boc-D-Ala-MeLeu-MeLeu-D-MeVal-MeBmt
|
BzlO-Ala-MeLeu-Val-MeLeu-Sar-Abu **5.26**

Scheme 5.6. Wenger's Report of Inverted Conversion in 4 + 7 Coupling (ref. 8)

Although it took 3 days to complete the coupling reaction, the BOP reagent is still preferable for the 4 + 7 fragment coupling in the synthesis of CsA analogs, since racemization of MeVal is minimized. In order to complete the final cyclization, the N-Fmoc and C-Bzl protecting groups of undecapeptides **5.20a-d** and **5.22** were removed simultaneously by reaction with 0.2N of aqueous NaOH in ethanol for 5-12 h (Scheme **5.4** and **5.5**). After workup, the crude, fully-deprotected undecapeptides were cyclized, using propyl-phosphonic anhydride (1.5 equiv) and DMAP (6 equiv), in a dilute solution (~2 x 10^{-4} M) for 2 days to give CsA analogs **5.21a-d** and **5.23** in 37-60% yields.

Table 5.4. Physical properties of CsA analogs **5.21a-d**, **5.23** and their linear undecapeptide intermediates **5.20a-d**, **5.22**.

entry	compound	structure[b]	R_f (%)[a]	$[\alpha]_D$ (c, $CHCl_3$)	yield(%)
1	**5.20a**	[MeL(OH)^1]	0.53(50)	-102.1°(0.73)	62
2	**5.20b**	[MeL(OH)^1,MeA^6]	0.49(50)	-122.0°(0.2)	56
3	**5.20c**	[MeL(OH)^1,MeA^{4,6}]	0.67(60)	-146.7°(0.02)	34
4	**5.20d**	[MeL(OH)^1,D-Lys(Boc)^8]	0.31(50)	-133.1°(1.9)	62
5	**5.22**	[MeL(OH)^1,D-MeVal^{11}]	0.71(40)	-103.2°(2.5)	32
6	**5.21a**	[MeL(OH)^1]	0.49(50)	-200.0°(0.04)	41
7	**5.21b**	[MeL(OH)^1,MeA^6]	0.34(40)	-215.0°(0.2)	56
8	**5.21c**	[MeL(OH)^1,MeA^{4,6}]	0.53(60)	-247.5°(0.05)	69
9	**5.21d**	[MeL(OH)^1,D-Lys(Boc)^8]	0.47(50)	-182.5°(0.04)	74
10	**5.23**	[MeL(OH)^1,D-MeVal^{11}]	0.54(50)	-162.5°(0.04)	46

[a] TLC (% acetone/hexane). [b] Abbreviated symbol: A=Ala, L=Leu.

The physical properties of these CsA analogs and their linear undecapeptide intermediates are summarized in Table 5.4.

C. NMR Results

Solution NMR studies of the conformations of the CsA analogs **5.21a-d** (Figures 5.3 ~ 5.7, see Appendix II, pages 243 ~ 247) were carried out since these spectra are highly diagnostic of the CsA ring system. Each analogue is present as essentially one single conformer (> 95%) in $CHCl_3$, respectively. The comparisons of chemical shifts for these analogs with CsA are summarized in

Table 5.5. Chemical Shifts of Protons in CsA and CsA Analogues **5.21a-d**

Compound	NH				N-CH3						
	2	5	7	8	1	3	4	6	9	10	11
CsA	7.96	7.48	7.68	7.17	3.51	3.40	3.11	3.25	3.12	2.70	2.71
5.21a	8.29	7.61	7.86	7.33	3.49	3.42	3.09	3.28	3.15	2.67	2.68
5.21b	8.42	7.35	8.13	7.05	3.56	3.38	3.07	3.32	3.13	2.69	2.70
5.21c	8.42	7.30	8.12	7.06	3.55	3.37	3.07	3.31	3.16	2.69	2.70
5.21d	8.28	7.62	7.90	7.34	3.49	3.42	3.09	3.29	3.14	2.66	2.67

Table 5.6. Chemical Shifts of Protons in CsA and CsA Analogues (continued)

Compound	α-H												Cβ-H
	1	2	3		4	5	6	7	8	9	10	11	1
			re-H	si-H									
CsA	5.47	5.03	3.23	4.76	5.34	4.66	5.02	4.52	4.83	5.70	5.10	5.14	3.82
5.21a	5.26	5.06	3.17	4.70	5.33	4.64	4.97	4.47	4.84	5.68	5.10	5.11	3.87
5.21b	5.57	4.97	3.18	4.73	5.32	4.72	5.08	4.64	4.81	5.62	5.04	5.07	3.40
5.21c	5.57	4.97	3.14	4.72	5.31	4.75	5.08	4.62	4.81	5.62	5.04	5.07	3.44
5.21d	5.27	4.97	3.18	4.70	5.33	4.63	5.12	4.48	4.82	5.69	5.09	5.08	3.86

Table 5.5 and 5.6. The four amide protons in **5.13a,d** shift downfield around 0.14~0.32 ppm. Amide protons at residues 5 and 8 are upfield shifted in **5.21c**, whereas downfield shifts at residues 2 and 7 are observed. The chemical shifts in N-methyl protons of **5.21a-d** are quite similar to those in CsA. The α-proton at 1-position in **5.21a,d** is upfield shifted (5.26~5.27 vs 5.47 ppm). In terms of the chemical shift of the β-proton at the 1-position, an upfield shift (3.44 vs 3.82 ppm) is observed in **5.21c**. Other α-protons in the series of [MeLeu(OH)[1]]CsA analogs are also similar to CsA. With a (D)-configuration amino acid in the 11-position, the NMR spectrum of CsA analogue **5.23**, like [(D)-MeVal[11]]CsA (CsH),[176] was complicated (multiple conformers) in $CDCl_3$ at room temperature.

D. *Biological Properties*

1. *PPIase Inhibition and Immunosuppressive Activity*

The CyP-binding affinity of compounds **5.21a-c** was determined by an established chymotrypsin-coupled spectroscopic PPIase assay which was previously described.[26,27] The relative immunosuppressive effect of CsA and its analogs were determined by Steven Bartz, Eric Hohenwalter and Dr. Miroslav Malkovsky (Medical Microbiology Department). In the initial set of experiments, CsA and the CsA analogs were tested for their immunosuppressive activity by assaying their ability to inhibit the

proliferation of PHA activated blood mononuclear cells (PBMCs).[160] PBMCs were treated with CsA or a CsA analogue at the time of PHA activation. The concentrations, which inhibit proliferation by fifty percent (IC_{50}), are reported in Table 5.7. As expected, [MeLeu(OH)^1]CsA (5.21a), which is obtained by replacement of MeBmt by MeLeu(OH) in the 1-position,

Table 5.7. CyP-binding and inhibition of proliferation of PHA-activated human PBMCs by CsA or CsA Analogs

Compound	CyP-binding[b] (Ki, nM)	Proliferation[a] (IC_{50}, nM)
CsA	6±1	4.2
[MeAla^6]CsA	16±2	> 8.6 x 10^3
[MeLeu(OH)^1]CsA(5.21a)	11±3	1.1 x 10^3
[MeLeu(OH)^1,MeAla^{4,6}]CsA(5.21c)	35±13	> 9.3 x 10^3
[D-MeVal^{11},MeLeu(OH)^1]CsA(5.23)	—	> 8.6 x 10^3
[MeBm$_2$t^1]CsA	1%[c]	33

[a] PBMCs (1 x 10^5 cells/well) were stimulated with 1 µg/ml PHA-P (Sigma) in the presence of various concentrations of CsA or CsA analogs. The cultures were pulsed with 0.5 µCi ^{3}H-thymidine / well on day three and harvested on day 4. Results are expressed as the concentration which inhibited proliferation by 50%. Analogs 5.21c and 5.23 augmented proliferation of PHA-activated PBMCs at 10 µg/ml (8.6-9.3 µM).

[b] Determined on inhibition of PPIase activity of CyP.

[c] Data obtained compared to CsA as 100%, ref. 10.

displays negligible immunosuppressive activity (IC_{50}= 1.1 x 10^3 nM or 0.4% activity of CsA) but shows potent CyP-binding ability (Ki=11±3 nM). Similarly, replacement of MeLeu by MeAla in the 4- and 6-positions of CsA could separate the CyP-binding ability and the immunosuppressive activities. Thus, [MeAla^6]CsA[10] and [MeLeu(OH)^1, MeAla^{4,6}]CsA (5.21c) are potent inhibitors of CyP (Ki=16±2 and 35±13 nM, respectively, *versus* CsA, Ki=7 nM) but both show no detectable immunosuppressive activity at concentration up to 10 μg/ml.

These biological data are consistent with the X-ray structure of CsA bound to CyP in which the 1-, 4-, and 6-amino acid side chains are exposed to solvent and are positioned for interaction with CaN.[60] It is important to point out that analogs with no binding to CyP or no inhibition of CaN have no immunosuppressive activity. As expected, [(D)-MeVal^{11}, MeLeu(OH)^1]CsA (5.23), the 11-position epimer of [MeLeu(OH)^1]CsA (5.21a), has no detectable inhibition of CyP (similar to the naturally occurring [(D)-MeVal^{11}]CsA)[19] and no immunosuppressive activity even at 10 μg/ml (Ki > 8.6 x 10^3 nM). [MeBm$_2$t^1]CsA is an immunosuppressive CsA derivative that has greatly diminished binding to CyP (Ki=670 nM).[10,14] It was found that the CyP-[MeBm$_2$t^1]CsA complex effectively binds to CaN.[172] It is noteworthy that at the highest tested concentration (10 μg/ml), both [(D)-MeVal^{11}, MeLeu(OH)^1]CsA (5.23) and [MeLeu(OH)^1, MeAla^{4,6}]CsA (5.21c) augmented

the PHA-induced DNA synthesis response of human PBMCs (data not shown). The observed IC_{50}'s of [MeLeu(OH)^1]CsA, [MeBm$_2$t^1]CsA, and CsA are in agreement with the values reported elsewhere.[14,17]

2. *Anti-HIV properties*

The evaluation of the anti-HIV properties of CsA analogs were accomplished by Dr. Miroslav Malkovsky's group using several experimental models. The work was carried out by Steven Bartz and Eric Hohenwalter. CsA and the CsA analogs were initially tested for their ability to inhibit the cytopathic effect (CPE) in newly infected CEMx174 cells.[161] CEMx174 cells were treated with CsA or the CsA analogue at the time of infection with HIV_{LAI}. As reported by others,[79,80] we observed that CsA was able to inhibit CPE. In addition, [MeLeu(OH)^1]CsA (**5.21a**), [MeAla^6]CsA, and [MeLeu(OH)^1,MeAla^{4,6}]CsA (**5.21c**) were able to inhibit CPE. The non-immunosuppressive analogue [(D)-MeVal^{11}, MeLeu(OH)^1]CsA (**5.23**) and the immunosuppressive analogue [MeBm$_2$t^1]CsA were not effective at inhibiting CPE. To quantitate the inhibitory effect of CsA and the CsA analogs on the replication of HIV, the amount of HIV p24 in cell-free supernatants from drug-treated or control untreated HIV-infected CEMx174 cultures were assayed by ELISA. CEMx174 cells were infected for two hours with HIV_{LAI} and the inoculum washed off prior to treatment with CsA or CsA analogs. We found that the ability of the analogs to inhibit the replication of HIV did not

correlate with their immunosuppressive activity (Figure 5.8). In two representative experiments (Figure 5.8A and 5.8B), CsA inhibited p24 production by 96% and > 99%, [MeLeu(OH)^1]CsA (5.21a) by 96% and 99%, [MeLeu(OH)^1,MeAla^{4,6}]CsA (5.21c) by 95% and 96% , and [MeAla^6]CsA by 92% and 97%, respectively (values for a final drug concentration of 10 μg/ml). [MeBm$_2$t^1]CsA, which has reduced binding to CyP, was less effective at inhibiting p24 production (54% and 58% inhibition at 10 μg/ml). [(D)-MeVal^{11}, MeLeu(OH)^1]CsA (5.23), which does not bind to CyP, was not effective at inhibiting HIV replication. The possibility that the inhibition of CPE and p24 production observed with CsA or CsA analogs was due to the drugs decreasing CD4 expression seems unlikely since the treatment of CEMx174 cells with 10 μg/ml of drug for two days did not reduce surface expression of CD4 (figure 5.8C).[162] In addition, the decrease in CPE and p24 production was not due to a toxic effect of the drugs because treatment with 10 μg/ml of CsA or CsA analogue had no effect on proliferation of CEMx174 cells (Figure 5.8D).[163]

We also wanted to determine if CsA, or any of the analogs could decrease the amount of virus produced from a cell line chronically infected with HIV (Figure 5.9). We used the HCEM cell line which is chronically infected with HIV_{LAI} and continuously produces large quantities of virus.[164] After HCEM cells were treated with 10 μg/ml of CsA or CsA analogs for two days, we found

that only CsA could inhibit the production of HIV from HCEM cells [66% inhibition compared to the ethanol (dilute) control]. In some experiments, weak inhibition of p24 production was observed in [MeBm$_2$t^1]CsA treated persistently infected cells; however, in other experiments no inhibition was observed. CsA and CsA analogs did not effect DNA synthesis in HCEM cells indicating that the decrease in virus production was not due to a decrease in proliferation of the HCEM cells (data not shown).

Our results suggest that CsA can inhibit HIV replication by potentially two distinct mechanisms. The first mechanism of inhibiting HIV replication can also be mediated by non-immunosuppressive analogs of CsA in newly infected cells. The mechanism may require the ability of CsA or the CsA analogs to bind to CyP, but appears to be independent of the ability to inhibit CaN. In contrast, only CsA itself was able to inhibit the production of HIV from the persistently infected HCEM cells, suggesting that the anti-viral CsA analogs can inhibit a preintegration, but not a postintegration, step in the HIV life cycle. Our findings are compatible with the observation of 1) Luban and co-workers[84] showing that HIV-1 p55^{gag} binds to CyP and that this binding is sensitive to CsA, and 2) Schmidt's group[65] demonstrating that the binding of NF-κB to the NF-κB site in the HIV-1 LTR is sensitive to CsA. It remains to be seen whether or not the non-immunosuppressive derivatives of CsA would be active in these systems. Further, the combination of antiviral and co-stimulatory properties of [MeLeu(OH)^1, MaAla^{4,6}]CsA (**5.21c**) makes this

molecule a unique lead compound for development of drugs suitable for AIDS treatment.

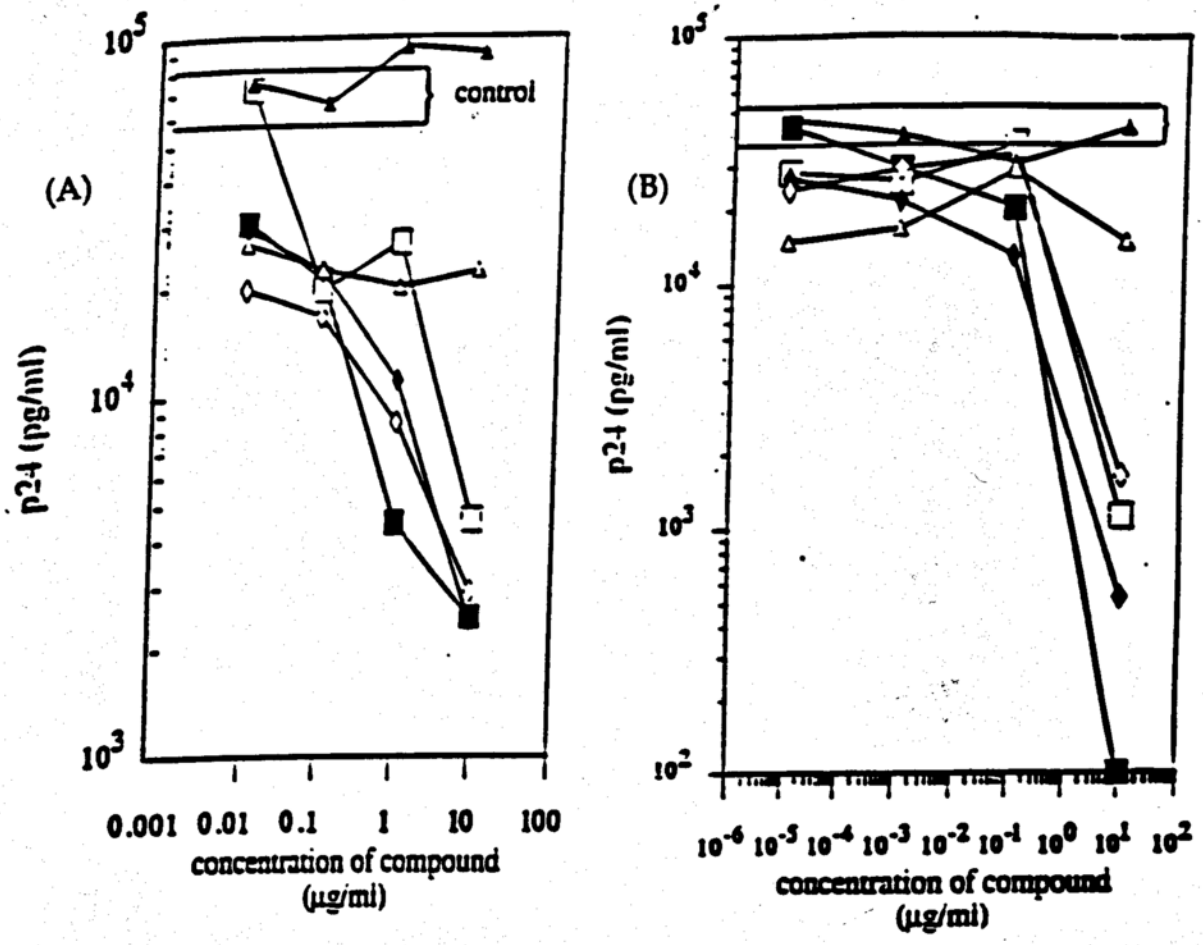


Figure 5.8. Inhibition of HIV-1 p24 production in freshly infected CEMx174 cells by CsA and non-immunosuppressive analogs of CsA. (A and B) CsA - closed squares; [MeAla^6]CsA - open squares; [MeLeu(OH)^1]CsA (5.21a) - closed diamonds; [MeLeu(OH)^1, MeAla^{4,6}]CsA (5.21c) - open diamonds; [D-MeVal^{11}, Me)Leu(OH)^1]CsA (5.23) - closed triangles; [MeBm$_2$t]CsA - open triangles. The replication of HIV in CEMx174 cells was determined by measuring the amount of p24 released into the culture supernatant on day three. CEMx174 cells were infected with HIV-1_{LAI} for 2 h and the inoculum was washed off prior to plating the cells in the presence of various concentration of CsA or CsA analogs. On day three, cell-free supernatants were collected and the amount of p24 in the supernatant was quantitated by ELISA (Coulter Corp.). Control: ethanol (dilute) = 80,468 pg/ml or 35,390 pg/ml (Exp. A or B); media only = 57,588 pg/ml or 52,132 pg/ml (Exp. A or B).

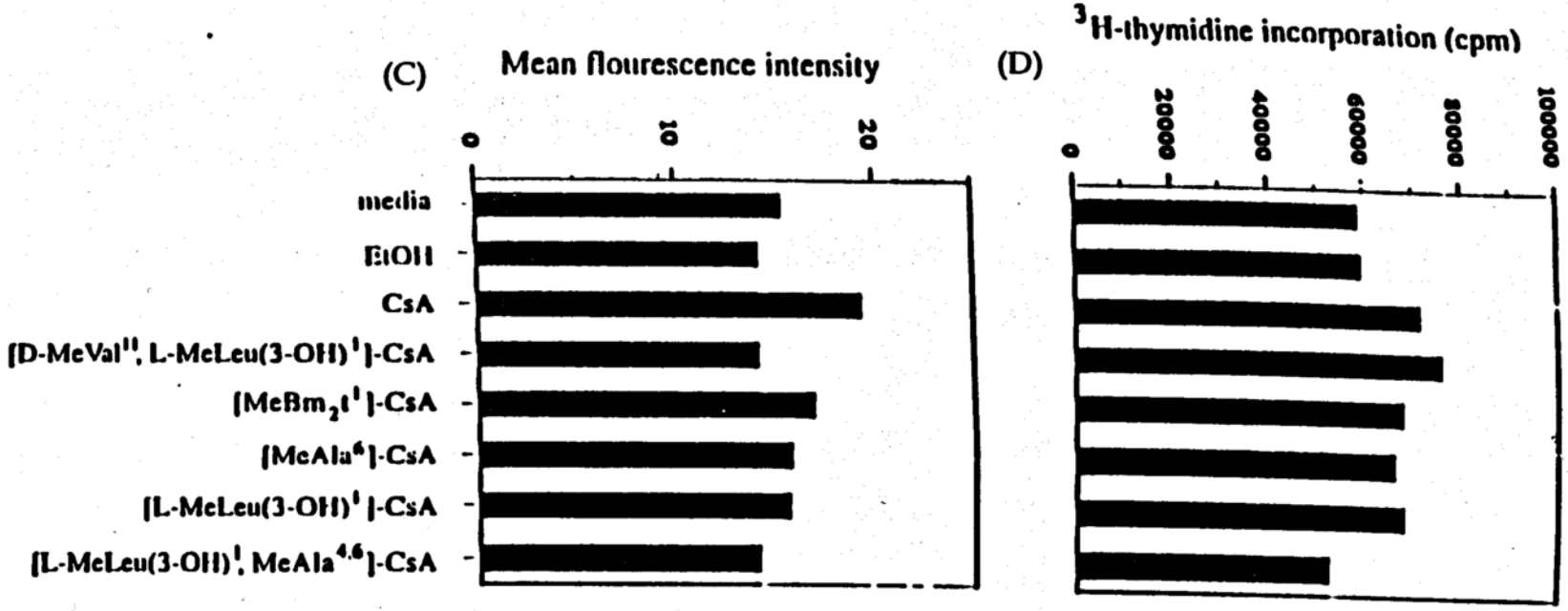


Figure 5.8 (continued). The inhibition of p24 production observed with CsA and non-immunosuppressive analogs of CsA was not due to the drugs affecting the expression of CD4 on the surface of CEMx174 cells (C). CEMx174 cells were incubated for 2 days in the presence of 10 µg/ml of CsA or CsA analogs and the expression of CD4 was analyzed by flow cytometry using the anti-T4 mAb (Olympus). The inhibition of p24 production observed with CsA and the non-immunosuppressive analogs of CsA was not due to the drugs inhibiting the proliferation of CEMx174 cells (D). CEMx174 cells were incubated for 2 days in the presence of 10 µg/ml of CsA or CsA analogs and then pulsed for 4 h with 0.5 µCi of ^{3}H-thymidine. The amount of radioactive label incorporated was measured by scientllation counting.

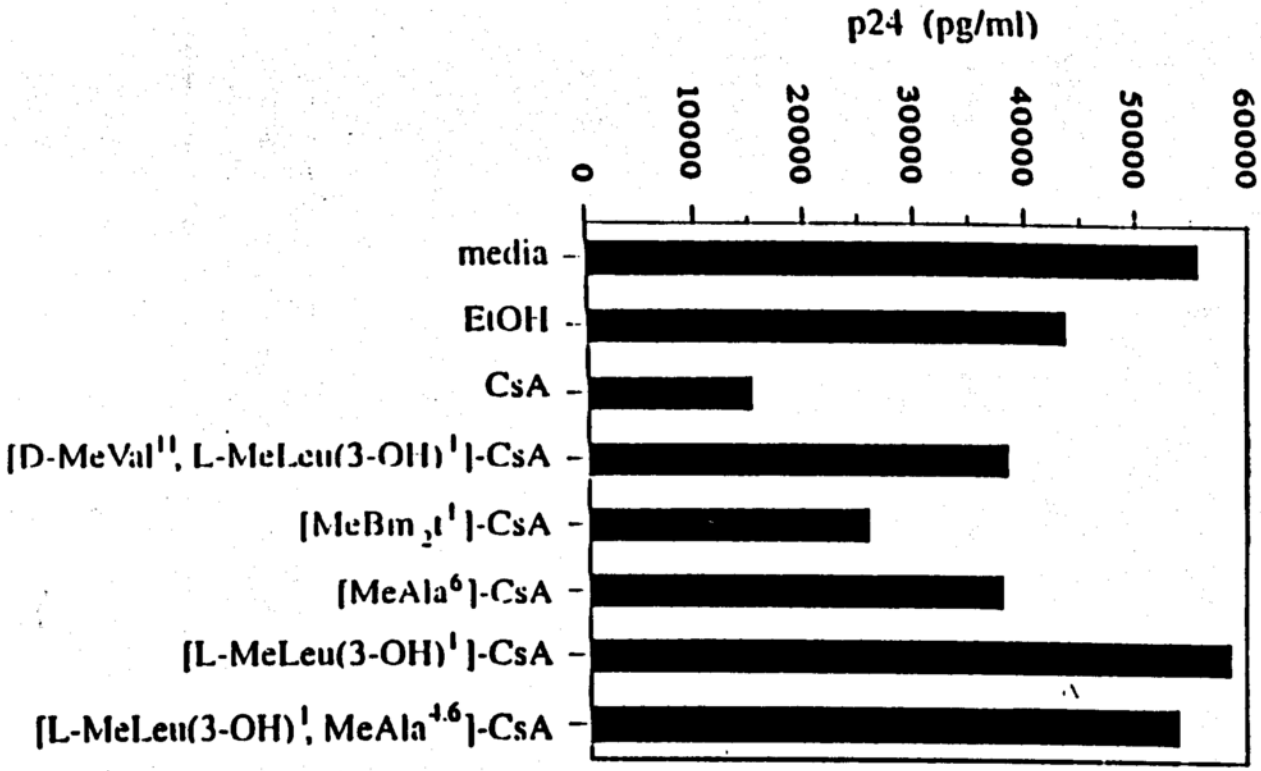


Figure 5.9. Inhibition of HIV-1 p24 production from chronically HIV-1_{LAI} infected HCEM cells by CsA. HCEM cells were incubated for 4 h in the presence of 10 µg/ml of CsA or CsA analogs before being washed and replated of 10 µg/ml of CsA or CsA analogs. After 2 days cell-free supernatants were collected and analyzed for p24 by ELISA.

Nebraska-Lincoln, Lincoln, NE). Silica gel chromatography was carried out under low pressure (5-15 psi) utilizing Merck grade 60 silica, 230-400 mesh. Thin layer chromatography (TLC) was run on Merck Kieselgel 60-F_{254} with fluorescent indicator visualized by ultraviolet (UV) or 5% phosphomolybdic acid in ethanol.

Dichloromethane and tetrahydrofuran were distilled from calcium hydride and sodium/benzophenone, respectively. n-Hexane was freshly distilled prior to use. Acetone (HPLC grade, Aldrich Chemical Co.) was stored over 4-Å molecular sieve for 24 h prior to use. Ethyl acetate was reagent grade (Baker, Phillipsburg, NJ). All other reagents and solvents were either ACS or HPLC grade and used without further purification.

B. *General Synthetic Procedures*

General Procedure A. Synthesis of Heptapeptides by Coupling of Acetonide-protected MeBmt or MeLeu(OH) with Hexapeptide Amine Analogs (H-Waa-Xaa-Qaa-Val-Yaa-Zaa-OBzl)

1. *Acetonide Formation of MeBmt or MeLeu(OH)*

A suspension of MeBmt or MeLeu(OH) (0.2 mmol, 1 equiv) in freshly distilled acetone (60 ml) was heated to reflux under N_2 for 24 h until an almost clear solution appeared. The solution was concentrated in vacuo to 1.5 ml which was directly used for the next coupling reaction without further purification.

Nebraska-Lincoln, Lincoln, NE). Silica gel chromatography was carried out under low pressure (5-15 psi) utilizing Merck grade 60 silica, 230-400 mesh. Thin layer chromatography (TLC) was run on Merck Kieselgel 60-F_{254} with fluorescent indicator visualized by ultraviolet (UV) or 5% phosphomolybdic acid in ethanol.

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2. *Coupling Reaction*

A solution of freshly prepared acetonide-protected amino acid (0.2 mmol, 1 equiv) in acetone (1.5 ml) was added 3 ml of THF, N-methylmorpholine (0.22 mmol, 1.1 equiv), 1-hydroxybenzotriazole (0.44 mmol, 2.2 equiv), and hexapeptide amine (0.22 mmol, 1.1 equiv). The resultant mixture was cooled to 0 °C and DCC (0.22 mmol, 1.1 equiv) was added. The mixture was allowed to warm up to room temperature and stirred under N_2 for 20 h, after which time the precipitated dicyclohexylurea (DCU) was removed by filtration and washed with small portion of CH_2Cl_2. The combined filtrate was washed with saturated $NaHCO_3$ solution and dried over $MgSO_4$. Concentration in vacuo and dissolving the residue in EtOAc yielded more DCU. The residue remaining after a second filtration and concentration in vacuo was purified by chromatography with 10-40% acetone in freshly distilled n-hexane to give the title compoumd.

General Procedure B. Removal of the N,O-Isopropylidene Protecting Group of Heptapeptides (Residue 1-7)

A solution of N,O-isopropylidene heptapeptide (0.156 mmol) in 3 ml of MeOH was stirred with 1N HCl aqueous solution (0.6 mmol, 4 equiv) at room temperature for 15 h. The reaction mixture was treated with $NaHCO_3$ (2 mmol) and concentrated in vacuo to a white solid. The residue was taken up

in 2% MeOH in CH_2Cl_2 and flash-chromatographed with 2-4% MeOH in CH_2Cl_2 to give the title compound.

General Procedure C. Synthesis of Linear Undecapeptides

To a solution of N-protected heptapeptide (residue 1-7) (0.1 mmol) and tetrapeptide amine (residue 8-11) (0.15mmol, 1.5 equiv) in CH_2Cl_2 (2 ml) was treated sequentially with N-methylmorpholine (0.2 mmol) and BOP reagent. The reaction mixture was sealed tightly and stirred at room temperature under N_2 for 3 days. The mixture was then diluted with CH_2Cl_2 (15 ml) and water (10 ml). The aqueous layer was extracted with additional CH_2Cl_2 (3x10 ml) and the combined organic layers were dried over $MgSO_4$ and concentrated in vacuo. The resultant residue was purified by flash chromatography on silica gel with 10-40% acetone in freshly distilled n-hexane to give a pure, fully-protected undecapeptide. Some impurities with higher R_f, possibly another undecapeptide epimer or unreacted substrates were usually isolated during the chromatographic process.

General Procedure D. Synthesis of Cyclosporine Analogs

1. *Removal of Fmoc and OBzl-ester Protecting Groups.*

A solution of the protected undecapeptide (0.05 mmol) in EtOH (2 ml)

was flushed with N_2 and cooled to 0 °C. The mixture was treated with 0.2N NaOH solution (0.5 ml) and stirred for 1.5 h; an additional portion of 0.2N NaOH solution (0.25 ml) was added and stirring was continued at 0 °C for 3.5-12 h. The reaction mixture was then neutralized to pH 6 with 0.2N HCl solution (0.75 ml) and washed with brine (10 ml) and CH_2Cl_2 (20 ml). The aqueous layer was then extracted with additional CH_2Cl_2 (4x10 ml). The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo to dryness to give a clear oil which was used directly for further reaction.

2. *Cyclization to Cyclosporine Analogs*

The oily residue (0.05 mmol) was dissolved in CH_2Cl_2 (200 ml) and treated sequentially with DMAP (0.25 mmol) and propyl phosphonic anhydride (a 50% w/v solution in CH_2Cl_2 from Fluka). The reaction mixture was stirred at room temperature under N_2 for 2 days, concentrated to 1-2 ml and applied directly to a silica gel column. Flash chromatography with 10-40% acetone in freshly distilled n-hexane gave a pure cyclic undecapeptide compound.

C. *Specific Experimental Procedures.*

1. *Synthesis of N^{α}-Methyl-N^{α}-(tert-butyloxycarbonyl)-Nε-[3-(3-pyridyl)-allyloxycarbonyl]-L-lysine (3.14)*

Methyl 3-(3'-pyridyl)-acrylate (3.19)

A suspension of 3-pyridylacrylic acid (12.5 gm, 83.7 mmol) in 120 ml of anhydrous MeOH was cooled at 0 °C and 120 ml of concentrated sulfuric acid was added slowly. The reaction mixture was refluxed under Dean-Stark apparatus under N_2 for 6 h. The resulting mixture was concentrated in vacuo, the oily residue was cooled and neutralized with concentrated NH_4OH to pH 10. The residue was dissolved in CH_2Cl_2 (3x150 ml) and the combined organic layers were dried over $MgSO_4$, concentrated in vacuo to give 13.3 gm (97%) of the title compound as a white powder: TLC R_f 0.38 (EtOAc/hexane, 2:1); mp 39-41 °C; FABMS: [M+] m/z 163; ^1H NMR (300 MHz, $CDCl_3$) δ 8.76 (d, J=1.6, 1H, H-2), 8.61 (dd, J=4.6, 1.4, 1H, H-6), 7.84 (dd, J=7.5, 1.4, 1H, H-5), 7.69 (d, J=16.5, 1H, CH=C), 7.36-7.31 (m, 1H, H-4), 6.52 (d, 1H, J=16.5 Hz, C=CH), 3.83 (s, 3H, O-CH_3).

3-(3'-Pyridyl)-2-propen-1-ol (3.17)

A solution of anhydrous EtOH (11.6 gm, 0.25 mol) in dry ether (25 ml) was added dropwise to a suspension of $LiAlH_4$ (4.85 gm, 0.125mol) in dry ether (150 ml) and stirred on an ice bath. After additional stirring for 30 min, the resultant suspension of $LiAl(EtO)_2H_2$ in ether was added dropwise to a solution of the ester **3.19** (12.25 gm, 75 mmol) in dry ether (180 ml) and stirred on an ice-salt bath below -10 °C. After additional stirring for 30 min, the ice-

salt bath was removed and the reaction mixture was stirred for 1h at room temperature. To the stirred mixture on an ice bath, 50 ml of aqueous $(NH_4)_2SO_4$ solution was carefully added. After additional stirring was continued for 1 h at room temperature, the reaction mixture was filtered with suction using Celite and the residue was washed with water and CH_2Cl_2 several times by turns. The combined filtrate was extracted with CH_2Cl_2 (4x150 ml). The combined organic layers were dried over $MgSO_4$ and evaporated. The residue was purified on a 70-gm silica gel (50-75% EtOAc/hexane & 1-3% MeOH/EtOAc as eluents) to give 3.8 gm (37.5%) of unsaturated alcohol **3.17**: TLC R_f 0.25 (EtOAc/hexane 2:1); ^1H NMR (300MHz, $CDCl_3$) δ 8.58 (d, 1H, J=1.9 Hz), 8.45 (dd, J=1.4, 4.6, 1H), 7.71 (dt, J=1.9, 5.9, 1H), 7.25 (dd, J= 4.6, 5.9, 1H), 6.62 (dt, J=16, 1H, HC=C), 6.44 (dt, J=16, 5.2, 1H, =CH-), 4.37 (dd, J=5.2, 1.5, 2H, -CH_2-), 2.37 (br s, 1H, OH); ^{13}C (DEPT) δ 148, 147, 133, 130, 124, 122, 63.

3-(3'-Pyridyl)allyl-(4''-nitrophenyl) Carbonate (3.18)

To a solution of allyl alcohol **3.17** (0.55 gm, 4.06 mmol) in 50 ml of CH_2Cl_2 was added 0.4 ml of triethylamine (4.46 mmol, 1.1 equiv) and bis-(4-nitrophenyl)carbonate (1.12 gm, 1.0 equiv). The reaction mixture was stirred at room temperature for 4 h and concentrated in vacuo. The residue was taken in 30 ml of EtOAc and washed with 5% $KHSO_4$, 5% $NaHCO_3$, and brine.

The organic layer was dried over $MgSO_4$ and concentrated to dryness to give, after purification by chromatography over 30-gm silica gel (20-50% EtOAc/hexane as eluent), 1.17 gm (96%) of the title compound: TLC R_f 056 (EtOAc/hexane/MeOH, 49:49:2); FABMS $[M+H]^+$ m/z 301; ^1H NMR (300 MHz, $CDCl_3$) δ 8.66 (br s, 1H, H-2), 8.54 (br s, 1H, H-6), 8.30 (dt, 2H, J=9.2, 2.2, 2H-3'), 7.75 (dt, 1H, J=7.9, 1.7, H-5), 7.42 (dt, 2H, J=9.2, 2.2, 2H-2'), 7.30 (dd, 1H, J=8.0, 5.0, H-4), 6.78 (d, 1H, J=16.0, CH=C), 6.44 (dt, J=16, 6.4, C=CH-), 4.97 (dd, 2H, J=6.4, 1.3, CH_2-O).

Nα-Methyl-Nε-benzyloxycarbonyl-L-lysine Benzyl Ester (3.11)

According to Grieco's reported procedure,[144] a homogeneous solution of H-Lys(Cbz)-OBzl·HCl (**3.9**, 2.19 gm, 5.4 mmol) in 12 ml of water was added with vigorous stirring cyclopentadiene (1.0 ml, 12.2 mmol) and an aqueous solution of 37% formaldehyde (0.48 ml, 6.0 mmol). After 1 h at room temperature, the heterogeneous reaction mixture was washed with hexane and neutralized with 5% $NaHCO_3$ solution. The product was isolated by extraction with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo to dryness to give crude 2-azanorbornene adduct **3.10** (2.17 gm, 89%) which was used directly in the further reaction.

To a solution of the above 2-azanorbornene adduct **3.10** (0.93 gm, 2.07 mmol) in 10 ml of chloroform was added 10 ml of cooled (0 °C) trifluoroacetic

acid and 1.0 ml of triethylsilane (6.2 mmol, 3 equiv). The reaction mixture was stirred under N_2 and at room temperature for 24 h. The resultant mixture was concentrated in vacuo and the residue was dissolved in 10 ml of chloroform, treated with 10% aqueous HCl solution, and washed with hexane/ether (1:1). The aqueous layer was neutralized with 5% $NaHCO_3$ solution to pH 6 and the product was isolated by extraction with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated in vacuo to give 0.82 gm (92%) of the amino ester **3.11** as a pure oil: TLC R_f 0.4 ($CHCl_3$/MeOH/NH_4OH, 96:3:1); MS(40 eV,m/z): 384 (M^+), 293; ^1H NMR (300 MHz, $CDCl_3$) δ7.38-7.34 (s, 5H, Aromatic H's), 5.20 (q_{AB}, 2H, O-CH_2), 5.14 (s, 2H, O-CH_2), 4.80 (br s, 1H, (C)^2-H), 3.12 (br s, 2H, N-CH_2), 2.70 (s, 3H, N-CH_3), 1.98 (br s, 2H, CH_2), 1.50-1.30 (m, 4H, CH_2CH_2).

N^{α}-Methyl-N^{α}-(tert-butyloxycarbonyl)-N^{ε}-(benzyloxycarbonyl)-L-Lysine Benzyl Ester (3.12)

To a solution of 1.0 gm of amino ester **3.11** (2.0 mmol) in 20 ml of dioxane/water (1:1) was treated with di-tert-butyl dicarbonate (1.31 gm, 6.0 mmol) and triethylamine (0.68 ml, 5.0 mmol) at 0 °C. The reaction mixture was stirred under N_2 at room temperature for 16 h. The resultant mixture was concentrated in vacuo and the residue was dissolved in EtOAc and washed with 10% $NaHSO_4$, 5% $NaHCO_3$, and brine. The combined organic

layers were dried over $MgSO_4$ and purified on 50-gm silica gel to give 1.05 gm (83%) of fully protected amino ester **3.12**: TLC R_f 0.9 (50% EtOAc/hexane); $[\alpha]_D$ -17.7° (c 1.4, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$), δ 7.38 (s, 5H, Aromatic H's), 7.34 (s, 5H, Aromatic H's), 5.24 (s, 2H, O-CH_2), 5.08 (s, 2H, O-CH_2); 3.62 (br s, 1H, (C)^2-H), 3.18 (br s, 2H, N-CH_2-), 2.75 (s, 3H, N-CH_3), 1.96 (br s, 2H, -CH_2), 1.54 (s, 9H, $C(CH_3)_3$), 1.5-1.2 (m, 4H, -CH_2CH_2).

Nα-Methyl-Nα-(tert-butyloxycarbonyl)-L-Lysine (3.13)

To a solution of 3.8 gm of fully protected lysine **3.12** (7.84 mmol) in 70 ml of MeOH was flushed with N_2 and treated with 970 mg of 10% Pd-C powder. The reaction mixture was influxed with H_2 for 2 h and filtrated through Celite. The filtrate was dried over $MgSO_4$ and concentrated in vacuo to give 1.99 gm (97%) of **3.13** as a white, foamy glass: TLC R_f 0.2 (10% MeOH/CH_2Cl_2) ; $[\alpha]_D$ -19.7° (c 1.25, MeOH); ^1H NMR (300 MHz, D_2O) δ 4.45-4.23 (br d, 1H, (C)^2-H), 3.00 (t, 2H, J=7.6, N-CH_2), 2.78 (s, 3H, N-CH_3), 1.92-1.61 (m, 4H, $(CH_2)_2$-), 1.43 (s, 9H, $C(CH_3)_3$), 1.38-1.30 (m, 1H, CH_2).

Nα-Methyl-Nα-(tert-butyloxycarbonyl)-Nε-[3-(3'-pyridyl)allyloxycarbonyl]-L-Lysine (3.14)

The N-methyllysine **3.13** (1.99 gm, 7.65 mmol) was dissolved in 8 ml of 1N NaOH solution and 16 ml of 1N $NaHCO_3$ solution. The carbonate **3.18** (2.3

gm, 7.65 mmol) in 20 ml of dioxane was added in one portion at 0 °C and stirring was continued at room temperature for 1 h. The resultant mixture was concentrated to remove dioxane and the alkaline solution remaining was extracted with EtOAc (2x15 ml). The extract was discarded whereas the aqueous layer was cooled and acidified to pH 4 with 2N HCl solution. The oily suspension was extracted with EtOAc (3x100 ml). The combined organic layer was washed with water and dried over Na_2SO_4. After evaporation and dryness, it gave 3.0 gm (70%) of the almost pure title compound: TLC R_f 0.26 (10% $MeOH/CH_2Cl_2$); $[\alpha]_D$ -16.6° (c 1.0, CH_3OH); FABMS m/z $[M+H]^+$ 422; ^1H NMR (300 MHz, $CDCl_3$, two conformers were observed at room temperature) δ 8.64 (br s, 1H, H-2), 8.43 (d, 1H, J=4.3, H-6), 7.82 (d, 1H, J=4.1, H-5), 7.35 (dd, 1H, J=4.9, 8.0, H-4), 6.63 (d, 1H, J=15.7, C=CH), 6.42 (dt, 1H, HC=C), 4.75 (d, 2H, J=5.3 Hz, CH_2-O), 3.27-3.09 (br d, 2H, N-CH_2-), 2.83 (br d, 3H, N-CH_3); 2.0-1.65 (m, 2H, CH_2), 1.57-1.20 (m, 4H, -CH_2CH_2-), 1.47 (d, 9H, $C(CH_3)_3$).

2. *Synthesis of β-Hydroxy-N-methylamino Acids: (4R)-4-((E)-2-butenyl)-4,N-dimethyl-L-threonine (MeBmt, **1.2**) and (2S,3R)-3-Hydroxy-N-methylleucine (MeLeu(OH), **5.3**)*

(1). *Synthesis of (2R,4E)-2-Methyl-4-hexenal (**1.27**)*

*Ethyl (4E)-2-Methyl-4-hexenoate (**1.38**)*

Following the procedure reported by Deyo *et al*,[124] a solution of 3-buten-2-ol (27 ml, 0.31 mol), $EtC(OEt)_3$ (230 ml, 1.15 mol) and propionic acid (1 gm, 13.5 mmol) in benzene (300 ml) was refluxed for 1 h. The benzene/ethanol azeotrope was slowly distilled over 15 h, followed by distillation to bp 100°C to remove the remaining solvent. The mixture was partitioned between ether (300 ml) and 0.5N HCl (100 ml). The organic layer was washed with 5% $NaHCO_3$ and dried over $MgSO_4$. After removal of ether and ethyl propionate (distilled to bp 100 °C at atmosphere pressure), the remaining was distilled at 75-85°C/25 mbar to give 38.2 gm (80%) of the title compound[124]: ^1H NMR (300 MHz, $CDCl_3$) δ 5.44-5.30 (m, 2H), 4.12 (q, J=7.5, 2H), 2.42 (sextet, J=6.8, 1H), 2.28 (quintet, J=5.7, 1H), 2.10 (quintet, J=7.5, 1H), 1.63 (d, J=5.6, 3H), 1.25 (t, J=7.5, 3H), 1.14 (d, J=6.0, 3H).

N-[(2R, 4E)-2-Methyl-4-hexenoyl]-S-phenylglycinol (1.39b)

Following the procedure reported by Deyo *et al*,[124] S(+)-2-Phenylglycinol (5 gm, 36 mmol) in 200 ml of toluene was treated with 4.0 gm (36 mmol, 1 equiv) of KOtBu. After 10 min, hexenoate **1.38** (5.7 gm, 36 mmol) was added to the stirred solution. The mixture was heated from room temperature to 105°C over 1 h, cooled to 0 °C, and quenched with 5% citric acid (50 ml). The mixture was then diluted with ether (500 ml) and washed with 1N HCl (120 ml), 5% $NaHCO_3$ (200 ml). The organic layer was dried over $MgSO_4$ and

concentrated to give 6.0 gm (68%) of 1:1 mixture of diastereomers which were separated by flash chromatography (20-50% EtOAc/hexane) to afford the title compound: $[\alpha]_D$ +34.0° (c 1.2, CH_2Cl_2) [lit.[124] $[\alpha]_D$ +38.0° (c 1.8, CH_2Cl_2); R_f 0.22 (50% EtOAc/hexane); ^1H NMR (300 MHz, $CDCl_3$) δ 7.38-7.25 (m, 5H), 6.26 (br d, J=6.4, 1H), 5.52-5.28 (m, 2H), 5.08-5.01 (dt, J=8.5, 5.0, 1H), 3.86 (d, J=5.0, 2H), 2.99 (br s, 1H), 2.36-2.04 (m, 3H), 1.61 (d, J=6.0, 3H), 1.16 (d, J=6.5, 3H); The diastereomer **1.39a** was obtained as a white solid: $[\alpha]_D$ +49.0° (c 1.2, CH_2Cl_2) [lit.[124] $[\alpha]_D$ +56.3° (c 1.0, CH_2Cl_2); R_f 0.36 (50% EtOAc/hexane); ^1H NMR (300 MHz, $CDCl_3$) δ 7.40-7.26 (m, 5H), 6.16 (br d, 1H), 5.59-5.35 (m, 2H), 5.07 (q, J=6.2, 1H), 5.59-5.35 (m, 2H), 2.87 (br s, 1H), 2.39-2.09 (m, 3H), 1.66 (dd, J=1.1, 5.9, 3H), 1.15 (d, J=6.7, 3H).

(2R, 4E)-2-Methyl-4-hexen-1-ol (1.40)

Following the procedure reported by Deyo *et al*,[124] a solution of amide **1.39b** (2.5 gm, 10.1 mmol) in 3N H_2SO_4 (150 ml, dioxane/water, 1:1) was heated at 70-80 °C for 3 h, diluted with water (150 ml). The aqueous solution was extracted with CH_2Cl_2 (3x120 ml). The combined organic layers were dried over $MgSO_4$ and concentrated to afford a hexenoic acid (1.2 gm, 93%) as a clear oil which was used directly in the subsequent reaction. A solution of the clear oil (1.08 gm, 8.42 mmol) in ether (10 ml) was added over a period of 10 min to a stirred suspension of $LiAlH_4$ (0.56 gm, 15 mmol) in ether (20 ml). After

stirring at room temperature for 1 h. The reaction was quenched with 0.5N H_2SO_4 (3 ml). The ether layer was washed with 1N $NaHCO_3$ (30 ml) and concentrated in vacuo. The resultant residue was distilled at 80 °C/25 mbar to give 0.76 gm (79%) of hexenol **1.40** as a clear oil: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.46 (m, 2H); 3.50 (m, 2H), 2.10-1.88 (m, 2H), 1.75-1.70 (m, 1H), 1.65 (d, J=5.8, 3H), 1.52 (br s, 1H), 0.91 (d, J=6.8, 3H). The corresponding acid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.56-5.32 (m, 2H), 2.49 (sextet, J=6.9, 1H), 2.42-2.31 (m, 1H), 2.17-2.08 (m, 1H), 1.67 (dd, J=1.1, 6.0, 3H), 1.17 (d, J=6.9, 3H).

(2R,4E)-2-Methyl-4-hexenal (1.27)

Following the procedure reported by Deyo *et al*,[124] a stirred solution of oxalyl chloride (1.66 ml, 19 mmol) in CH_2Cl_2 (40 ml) was treated with anhydrous DMSO (2.7 ml, 38 mmol) at -78 °C. A solution of alcohol **1.40** (1.05 gm, 9.24 mmol) in CH_2Cl_2 was added via syringe. The resultant suspension was stirred at 0 °C for 40 min and then NEt_3 (8.1 ml, 58 mmol) was added in one portion. The mixture was stirred at -30 °C for 1h. The organic layer was washed with 1N $KHSO_4$ (2 x 100 ml), brine, and dried over Na_2SO_4. The solvent was removed and the residue was distilled under 60 °C (~60 mbar) to give the aldehyde in 0.62 gm (60%): TLC R_f 0.62 (20% EtOAc/hexane); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.66 (s, 1H), 5.58-5.30 (m, 2H), 2.46-2.34 (m, 2H), 2.20-2.05 (m, 1H), 1.64 (d, J=6.0, 3H), 1.04 (d, J=6.7, 3H).

(2). *Synthesis of MeBmt*

The following reaction procedures for the synthesis of MeBmt were carried out according to Evans and Weber's method.[119]

(4S)-3-((4'S,5'R)-5'-((1"R,3"E)-1"--Methyl-3"-pentenyl)-2'-thioxo-4'-oxazolidinylcarbonyl)-4-(phenylmethyl)-2-oxazolidinone (1.19)

Stannous triflate (2.3 gm, 1.1 equiv) was quickly transferred to a flask purged with nitrogen. 5.8 ml of THF was added and a suspension was formed under -78 °C. To the resultant suspension was added N-ethylpiperidine (1.1 ml, 1.5 equiv), followed by a solution of (4S)-3-(isothiocyanacetyl)-4-(phenylmethyl)-2-oxazolidinone (**1.18**, 1.6 gm, 1.1 equiv) in 12 ml of THF at -78 °C via canula. The suspension dissolved. After the pale yellow solution was stirred at -78 °C for 1.5 h, the chiral aldehyde **1.27** (0.6 gm, 1.0 equiv) was added neat. The reaction solution was stirred for 4 h at -78 °C and then quenched by the addition of pH 7 phosphate buffer. The resultant suspension was filtered through Celite. The filtrate was diluted with CH_2Cl_2, washed with 1N $NaHSO_4$, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash chromatography (10-35% EtOAc/hexane) to give the title compound (1.26 gm, 61%) as an oil: R_f 0.39 (40% EtOAc/hexane); ^1H NMR (300 MHz, $CDCl_3$) δ 7.52 (s, 1H, NH), 7.42-7.15 (m, 5H), 5.54-5.39 (m, 3H, CH=CH, C_5'-H), 4.84-4.71 (m, 2H, C_4'-H & C_4-H), 4.42-4.32 (m, 2H, C_5-H_2),

3.20(dd, J=3.6, 13.5, 1H), 2.93 (dd, J=8.4, 13.5, 1H), 2.26-2.20 (m, 1H), 2.05-1.91 (m, 2H), 1.66 (d, J=6.0, 3H), 0.95 (d, J=6.5, 3H).

Methyl (4S,5R)-5-((1'R,3'E)-1'-methyl-3'-pentenyl)-2-thioxo-oxazolidine-4-carboxylate (1.20)

To a 0 °C solution of 2.73 gm (7.03 mmol) of aldol adduct **1.19** in 34 ml of methanol was added via canula a suspension formed by the addition of 2.56 ml (7.7 mmol, 3.0 M in ethyl ether) of methylmagnesium bromide to 25 ml of anhydrous methanol. After the solution was stirred for 5 min, it was quenched with pH 7 phosphate buffer and concentrated in vacuo. The residue was dissolved in 1N HCl, extratcted with CH_2Cl_2 (3x50 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. The resultant pale yellow oil was purified by flash chromatography (20-50% EtOAc/hexane) to give the title compound (1.36 gm, 79%) as a clear oil: R_f 0.38 (40% EtOAc/hexane); $[\alpha]_D$ +76° (c 0.1, $CHCl_3$) (lit.[119] $[\alpha]_D$ +80.6° (c 1.02, CH_2Cl_2); ^1H NMR (300MHz, $CDCl_3$) δ 7.62 (br s, 1H, NH), 5.58-5.32 (m, 2H, CH=CH), 4.88 (t, J=5.6, 1H), 4.31 (d, J=5.4, 1H), 3.83 (s, 3H), 2.27-2.20 (br m, 1H), 2.05-1.94 (m, 2H), 1.67 (d, J=6.2, 3H), 0.98 (d, J=6.7, 3H).

Methyl (4S,5R)-3-methyl-5-((1'R,3'E)-1'-methyl-3'-pentenyl)-2-oxazolidinone-4-carboxylate (1.21a)

To a 0 °C suspension of 1.67 gm (11.74 mmol, 2.1 equiv) of trimethyloxonium tetrafluoroborate and 1.32 gm (6.15 mmol, 1.1 equiv) of 1,8-bis(dimethylamino)naphthalene in 20 ml of CH_2Cl_2 was added via canula a 0 °C solution of 1.36 mg (5.59 mmol) of methyl carboxylate **1.20** in 10 ml of CH_2Cl_2. After the white suspension was stirred for 3 h, it was concentrated in vacuo at 0 °C. The residue was diluted with 34 ml of THF and 17 ml of pH 7 phosphate buffer was added. The resultant mixture was stirred at 0 °C for 1.5 h, poured into 220 ml of 1N $NaHSO_4$, and extracted with CH_2Cl_2 (3x150 ml). The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to give an oily residue which was purified by flash chromatography (20-40% EtOAc/hexane) to give the title compound (610 mg, 45%) as a clear oil: R_f 0.34 (40% EtOAc/hexane); $[\alpha]_D$ +31.0° (c 1.2, $CHCl_3$) [lit.[119] $[\alpha]_D$ +37.1° (c 1.51, CH_2Cl_2)] ; ^1H NMR (300 MHz, $CDCl_3$) δ 5.52-5.27 (m, 2H, CH=CH), 4.27 (dd, J=4.8, 6.2, 1H), 3.95 (d, J=4.7, 1H), 3.81 (s, 3H), 2.90 (s, 3H), 2.22-2.16 (m, 1H), 1.99-1.81 (m, 2H), 1.66 (dd, J=1.0, 5.0, 3H), 0.93 (d, J=6.7, 3H). A minor epimer **1.21b** with higher R_f value was isolated as an oil: R_f 0.38 (40% EtOAc/hexane); $[\alpha]_D$ +16.5° (c 0.8, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 5.46-5.31 (m, 2H), 4.69 (t, J=6.6, 1H), 4.40 (d, J=6.8, 1H), 3.78 (s, 3H), 2.48 (s, 3H), 2.20-2.08 (m, 1H), 1.91-1.77 (m, 2H), 1.64 (d, J=5.9, 3H), 0.89 (d, J=6.6, 3H).

*(4R)-4-((E)-2-butenyl)-4,N-dimethyl-L-threonine (MeBmt, **1.2**)*

A solution of methyl ester **1.21a** (340 mg, 1.41 mmol) in 3.1 ml of 2N KOH solution was heated at 75-80 °C for 15 h. The solution was allowed to cooled to room temperature and neutralized to pH 5 with 1N HCl. The resultant mixture was concentrated in vacuo and chromatographed (Sephadex LH-20, methanol) to give 220 mg (78%) of the pure MeBmt: mp and ^{1}H NMR data were identical to those reported by Evans and Weber: mp 241-243 °C; ^{1}H NMR (300 MHz, D_2O) δ 5.56-5.40 (m, 2H), 3.74 (t, J=6.0, 1H), 3.60 (d, J=5.9, 1H), 2.70 (s, 3H), 2.30-2.20 (br d, 1H), 1.90-1.80 (m, 2H), 1.62 (d, J=5.7, 3H), 0.91 (d, J=6.8, 3H); $[\alpha]_D$ +24.2° (c 0.5, 0.4N HCl) [lit.[119] $[\alpha]_D$ +17° (c 0.51, 0.4N HCl)].

(3). *Synthesis of (2S,3R)-3-Hydroxy-N-methylleucine (MeLeu(OH), 5.3)*

(4S)-3-((4'S,5'R)-5'-isopropyl-2'-thioxo-4'-oxazolidinylcarbonyl)-4-(phenylmethyl)-2-oxazolidinone (5.4)

Isobutyraldehyde (0.3 ml, 3.2 mmol) and isothiocyanate chiral auxiliary **1.18** (1.3 gm, 4.8 mmol) were condensed as described above for the homologue **1.19** to give 0.6 gm (54%) of the title compound as a foamy solid: TLC R_f 0.43 (50% EtOAc/hexane); $[\alpha]_D$ +158.1° (c 0.8, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.53 (s, 1H), 7.42-7.15 (m, 5H), 5.29 (t, J=5.0, 1H), 4.79 (dd, J=1.8, 4.8, 1H), 4.76-4.72 (m, 1H), 4.42-4.36 (m, 2H), 3.20 (dd, J=3.6, 13.5, 1H), 2.93 (dd, J=8.5, 13.6, 1H), 2.07 (m, 1H), 1.04 (d, J=1.67, 3H), 1.00 (d, J=6.8, 3H).

Methyl (4S,5R)-5-isopropyl-2-thioxo-oxazolidine-4-carboxylate (5.5)

Aldol adduct **5.4** (550 mg, 1.58 mmol) was hydrolyzed as described above for the homologue **1.20** to afford the title compound 240 mg (75%) as a clear oil: TLC R_f 0.57 (50% EtOAc/hexane); $[\alpha]_D$ -33.2° (c 1.26, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.50 (br s, 1H), 4.80 (t, J=5.7, 1H), 4.27 (d, J=5.6, 1H), 3.83 (s, 3H), 2.09 (m, 1H), 1.07 (d, J=6.8, 3H), 1.04 (d, J=6.7, 3H).

Methyl (4S,5R)-5-isopropyl-3-methyl-2-oxazolidinone-4-carboxylate (5.8)

Carboxylate **5.5** (700 mg, 3.45 mmol) was treated with Meerwein reagent as described above for the homologue **1.21a** to give 246 mg (35%) of the title compound as a clear oil: TLC R_f 0.24 (40% EtOAc/hexane); $[\alpha]_D$ -65.2° (c 1.2, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 4.20 (dd, J=4.9, 6.1, 1H), 3.94 (d, J=4.8, 1H), 3.82 (s, 3H), 2.92 (s, 3H), 1.99-1.89 (m, 1H), 1.01 (d, J=6.7, 3H), 1.00 (d, J=6.8, 3H); (4R)-Epimer **5.7** was obtained as a foamy solid (104 mg, 14%): TLC R_f 0.40 (40% EtOAc/hexane); $[\alpha]_D$ +129.6° (c 0.29, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 4.61 (t, J=6.7, 1H), 4.39 (d, J=6.8, 1H), 3.78 (s, 3H), 2.48 (s, 3H), 1.96-1.84 (m, 1H), 0.97 (t, J=7.3, 6H).

(2S,3R)-3-Hydroxy-N-methylleucine (5.3)

Methyl ester **5.8** (150 mg, 0.75 mmol) was hydrolyzed with 0.2N KOH as described above for the synthesis of the homologue MeBmt (**1.2**) to give, after

purification with Sephadex LH-20, 90 mg (75%) of the title compound as a white solid: TLC R_f 0.71 (nBuOH+AcOH+H_2O, 2:1:1); $[\alpha]_D$ -56.6° (c 0.3, MeOH); $[\alpha]_D$ -2.8° (c 1.0, 0.4N HCl) [lit.[119] $[\alpha]_D$ -1.6° (c 0.55, 0.4N HCl); ^1H NMR (300 MHz, D_2O) δ 3.69 (dd, J=5.0, 7.1, 1H), 3.6 (d, J=7.1, 1H), 2.73 (s, 3H), 1.88-1.76 (m, 1H), 0.99 (d, J=6.8, 3H), 0.96 (d, J=6.6, 3H).

3. *Synthesis of 4-[1-Azi-2,2,2-trifluoroethyl]benzoic acid (3.53)*

The following procedures for the synthesis of **3.53** were carried out according to Nassal's report[149] except where noted.

(4-Bromobenzyloxy)-tert-butyldimethylsilane (3.46).

A solution of 4-bromobenzyl alcohol **3.45** (7.5 gm, 40.1 mmol) and tert-butyldimethylsilyl chloride in 15 ml of DMF was treated with imidazole (7.05 gm, 0.1 mmol) under an ice bath. After the mixture was stirred at room temperature for 16 h. It was diluted with water and extracted with petroleum ether (3 x 50 ml). The combined organic extracts were washed with water (2 x 30 ml), dried over Na_2SO_4, and concentrated in vacuo to give 12.0 gm (98%) of the titile compound as a pale yellow oil (pure on TLC) which was used for the next reaction without further purification: TLC R_f 0.87 (50% CH_2Cl_2/hexane); MS (40 eV, m/z): 300 (M^+); ^1H NMR (300 MHz, $CDCl_3$) δ 7.40 & 7.16 (q, AA'BB' system, aromatic H's), 4.65 (s, 2H), 0.91 (s, 9H), 0.07 (s, 6H).

[4'-[(tert-Butyldimethylsiloxy)methyl]phenyl]-2,2,2-trifluoro-1-ethanone (3.48).

To a stirred solution of silyl ether **3.46** (6 gm, 20 mmol) in 100 ml of ether was dropwise added nBuLi (1.6 M in hexane, 15 ml, 22 mmol) at -30 °C under N_2. The mixture was allowed to warm up to 0 °C within 2 h and cooled down again to -50 °C. Then N-trifluoroacetyl piperidine **3.47** (prepared from piperidine and trifluoroacetyl anhydride) (3.68 g, 20 mmol) in 20 ml of ether was added. After 3 h at this temperature the mixture was treated with saturated NH_4Cl solution and the organic layer was washed with water (3 x 20 ml) and dried over Na_2SO_4. The solvent was removed in vacuo to give, after chromatography (25-35% CH_2Cl_2/hexane), 5.4 gm (85%) of the title compound as a colorless oil: TLC R_f 0.85 ($CHCl_3$); MS (40 eV, m/z): 318 (M+); ^1H NMR (300 MHz, $CDCl_3$) δ 8.05 & 7.51 (q, AA'BB' system, aromatic H's), 4.83 (s, 2H), 0.96 (s, 9H), 0.12 (s, 6H).

[4'-[(tert-Butyldimethylsiloxy)methyl]phenyl]-2,2,2-trifluoro-1-ethanone oxime (3.49).

To a solution of hydroxyamine·HCl (0.8 gm, 11.5 mmol) and sodium hydroxide (0.42 gm, 10 mmol) in 45 mmol of boiling ethanol was added a solution of trifluoromethyl ketone **3.48** (1.2 gm, 3.77 mmol) in 20 ml of ethanol. After the mixture was refluxed for 16 h (at 86-90 °C), the solvent was evaporated in vacuo. The resultant residue was partitioned between ether

and water. The organic phase was washed with 10% $KHSO_4$ (3 x 20 ml), water (3 x 20 ml), brine, and dried over Na_2SO_4. Then the sovent was removed in vacuo to give, after flash chromatography (2-8% MeOH / CH_2Cl_2 + hexane (1:1)), 1.1 gm (87%) of the title compound (*cis/trans* oxime) as a white solid: TLC R_f 0.67 ($CHCl_3$); m.p. 62-76 °C; MS (40 eV, m/z): 333 (M^+); ^1H NMR (300 MHz, $CDCl_3$) δ 7.36-7.20 (m, 4H), 4.64 (s, 2H), 0.84 (s. 9H), 0.04 (s, 6H).

[4'-[(tert-Butyldimethylsiloxy)methyl]phenyl]-2,2,2-trifluoro-1-ethanone O-(p-tolylsulfonyl) oxime (3.50).

A solution of oxime **3.49** (1.1 gm, 3.3 mmol) in 30 ml of pyridine was refluxed with p-toluenesulfonyl chloride (1.02 gm, 5.02 mmol). After 3 h the solvent was evaporated in vacuo and the residue was subjected to flash chromatography (30-60% CH_2Cl_2/hexane) to give 1.03 gm (66%) of the title compound as a colorless oil: TLC R_f 0.7 (30% CH_2Cl_2/hexane); ^1H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, 2H, parts of aromatic H's), 7.30-7.16 (m, 6H, aromatic H's), 4.66 (s, 2H), 2.38 (s, 3H), 0.84 (s, 9H), 0.02 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 129.9, 129.3, 128.5, 126.0, 64.2, 25.9, 21.8.

3-[4'-[(tert-Butyldimethylsiloxy)methyl]phenyl]-3-trifluoromethyl diaziridine (3.51).

A solution of O-tolylsulfonyl oxime **3.50** (1.0 gm, 2.1 mmol) in 15 ml of

ether was added to 2 ml of liquid ammonia in a thick-walled screw-up glass tube at dried ice bath. After stirring the mixture for 16 h at room temperature in the closed tube, the solution was cooled down to -40 °C, poured into a beaker to evaporate the remaining ammonia and concentrated in vacuo. The residue was partitioned between water and ether. The organic layer was dried over Na_2SO_4 and concentrated in vacuo to give, after flash chromatography (2-5% MeOH/CH_2Cl_2+hexane(1:1)), 0.69 gm (97%) of the title compound as a colorless oil: TLC R_f 0.3 ($CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.57 & 7.39 (q, AA'BB' system, aromatic H's), 4.75 (s, 2H), 2.77 (d, J=8.5, 1H, NH), 2.20 (d, J=8.5, 1H, NH), 0.94 (s, 9H), 0.10 (s, 6H); ^{13}C NMR (75 MHz, CDCl3) δ 143.8, 130.2, 128.0, 126.2, 64.4, 25.9, 18.4.

Tert-Butyldimethylsilyl 4-[1-Azi-2,2,2-trifluoroethyl]benzoate (3.52).

Following the same procedure reported by Baldwin *et al.*,[153] the diaziridine **3.51** (0.69 gm, 2.1 mmol) and sodium metahyperiodate (3.5 gm, 16.3 mmol) in a mixture of tetrachloromethane (6 ml), acetonitrile (6 ml), and water (8 ml) was treated with ruthenium dioxide (28 mg, 0.21 mmol), and stirred overnight at room temperature. The mixture was filtered through Celite, diluted with CH_2Cl_2 and water. The aqueous phase was washed with additional CH_2Cl_2. The combined organic extracts were washed with brine, dried over Na_2SO_4, and concentrated in vacuo to give 0.52 gm (72%) of the

title compound (pure on TLC): TLC R_f 0.13 (40% acetone/hexane); ^1H NMR (300 MHz, $CDCl_3$) δ 7.80 & 7.18 (q, AA'BB' system, aromatic H's), 0.92 (s, 9H), 0.28 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 130.4, 126.4, 25.6, -4.8 ; FABMS (3-NBA matrix): m/z $[M+H]^+$ 345.

4-[1-Azi-2,2,2-trifluoroethyl]benzoic acid (3.53).

A solution of diazirine **3.52** (0.3 gm, 0.87 mmol) in 25 ml of 24:1 CH_3CN/48% HF(aq) was stirred for 6 h at room temperature. The mixture was poured into water (25 ml) and EtOAc (50 ml). The aqueous phase was washed with additional EtOAc (3 x 25 ml). The combined organic extracts were washed with water (2 x 20 ml), dried over Na_2SO_4, and concentrated in vacuo to give 0.18 gm (90%) of the title compound (pure on TLC): m.p. 123-124 °C [lit.[149] m.p. 123-125 °C]; ^1H NMR (300 MHz, $CDCl_3$) δ 8.04 & 7.40 (q, AA'BB' system, aromatic H's); MS (40 eV, m/z): 231 ($[M+H]^+$, 8), 202 ($[M-N_2]^+$, 100); FABMS (3-NBA matrix): m/z $[M]^+$ 230.

4. *Synthesis of Cyclosporine Analogs*

[(4S,5R,1'R,3'E)-2,2,3-Trim ethyl-5-(1'-methyl-3'-pentenyl)-4-oxazolidinyl]-carbonyl]-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-norleucyl-L-Alanine Benzyl Ester (N,O-Isopropylidene-MeBmt-Abu-Sar-

MeLeu-Val-MeNle-Ala-OBzl) (3.34a)

The title compound was synthesized according to the general procedure A in 63% yield and obtained as a foamy solid: TLC R_f 0.42 (50% acetone/hexane); $[\alpha]_D$ -128.0° (c 0.15, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, at least two conformers at room temperature and major one is described) δ 7.86-6.42 (3d, J=6.8, 3H, H-N^2, H-N^5, H-N^7), 7.34 (br s, 5H, aromatic H's), 5.42 (m, 2H, CH=CH-C(5^1)), 5.15 (d, J=4.0, 2H, O-CH_2Ph), 5.24-2.80 (m, 9H, H-C(2^1), H-C(3^1), H-C(2^2), 2H-C(2^3), H-C(2^4), H-C(2^5), H-C(2^6), H-C(2^7)), 3.13, 3.00, 2.91, 2.29 (4s, 12H, CH_3-N^1, CH_3-N^3, CH_3-N^4, CH_3-N^6), 2.36-1.15 (m, 15H, H-C(4^1), 2H-C(5^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), H-C(3^5), 2H-C(3^6), 2H-C(4^6), 2H-C(5^6)), 1.63 (d, J=4.47, 3H, CH_3-C(7^1)), 1.33 (d, J=4.62, 3H, CH_3-C(2^7)), 1.34, 1.20 (2s, 6H, $2CH_3$ of isopropylidene), 0.98-0.81 (m, 21H, CH_3-C(4^1), CH_3-C(3^2), $2CH_3$-C(4^4), $2CH_3$-C(3^5), CH_3-C(5^6)); FABMS (DTT/DTE matrix): m/z $[M+H]^+$ 912.5; HR-FABMS exact mass calcd for $C_{49}H_{82}N_7O_9$ $[M+H]^+$ 912.6174, found 912.6178.

[(4S,5R,1'R,3'E)-2,2,3-Trim ethyl-5-(1'-methyl-3'-pentenyl)-4-oxazolidinyl]-carbonyl]-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N^{α}-Methyl-N^{ε}-[3-(3'-pyridyl)allyloxy-carbonyl]-L-lysyl-L-Alanine Benzyl Ester (N,O-Isopropylidene-MeBmt-Abu-Sar-MeLeu-Val-MeLys(Paloc)-Ala-OBzl) (3.34b)

The title compound was synthesized according to the general procedure A in 92% yield and obtained as a pale yellow oil: TLC R_f 0.30 (60%

acetone/hexane); $[\alpha]_D$ -51.5° (c 1.2, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.59 (br s, 1H, H-2 of pyirdine), 8.48 (d, 1H, H-6 of pyridine), 7.86, 6.63, 6.50 (3d, 3H, H-N^2, H-N^5, H-N^7), 7.69 (m, 1H, H-5 of pyridine), 7.33 (br d, J=3.51, 5H, aromatic H's), 7.23 (m br, 1H, H-4 of pyridine), 6.60 (br d, 1H, CH=C-C-O^6), 6.38 (m, 1H, -C=CH=C-O^6), 5.42 (m, 2H, CH=CH-C(5^1)), 5.17 (d, J=6.72, 2H, O-CH_2Ph), 4.72 (d, J=5.73, 2H, -C=C-CH_2-O^6), 5.20-2.80 (m, 12H, H-C(2^1), H-C(3^1), H-C(2^2), 2H-C(2^3), H-C(2^4), H-C(2^5), H-C(2^6), 2H-C(6^6), H-C(2^7), HN-C(6^6)), 3.16, 3.03, 2.91, 2.33 (4s, 12H, CH_3-N^1, CH_3-N^3, CH_3-N^4, CH_3-N^6), 2.45-1.20 (m, 15H, H-C(4^1), 2H-C(5^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), H-C(3^5), 2H-C(3^6), 2H-C(4^6)), 2H-C(5^6)), 1.63 (d, J=7.74, 3H, CH_3-C(7^1)), 1.35 (d, J=7.14, 3H, CH_3-C(2^7)), 1.34, 1.20 (2s, 6H, $2CH_3$ of isopropylidene), 0.96-0.82 (m, 18H, CH_3-C(4^1), CH_3-C(3^2), $2CH_3$-C(4^4), $2CH_3$-C(3^5)); FABMS (DTT/DTE matrix): m/z $[M+H]^+$ 1088.8; HR-FABMS exact mass calcd for $C_{58}H_{90}N_9O_{11}$ $[M+H]^+$ 1088.6760; found 1088.6728.

(2S, 3R, 4R, 6E)-3-Hydroxy-4-methyl-2-(methylamino)-6-octenoyl-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-norleucyl-L-Alanine Benzyl Ester (H-MeBmt-Abu-Sar-MeLeu-Val-MeNle-Ala-OBzl) (3.35a)

The title compound was synthesized according to the general procedure B in 80% yield and obtained as a foamy solid: TLC R_f 0.25 (10% MeOH/CH_2Cl_2); $[\alpha]_D$ -186.0° (c 0.2, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$ at least two conformers at

room temperature and major one is described) δ 7.80-6.80 (3d, J=7.9, 3H, H-N^2, H-N^5, H-N^7), 7.16 (br s, 5H, aromatic H's), 5.46 (m, 2H, CH=CH-$C(5^1)$), 5.26-2.80 (m, 13H, -OCH_2Ph, H-N^1, HO-$C(3^1)$, H-$C(2^1)$, H-$C(3^1)$, H-$C(2^2)$, 2H-$C(2^3)$, H-$C(2^4)$, H-$C(2^5)$, H-$C(2^6)$, H-$C(2^7)$), 3.28, 3.10, 3.02, 2.44 (4s, 12H, CH_3-N^1, CH_3-N^3, CH_3-N^4, CH_3-N^6), 2.44-1.20 (m, 15H, H-$C(4^1)$, 2H-$C(5^1)$, 2H-$C(3^2)$, 2H-$C(3^4)$, H-$C(4^4)$, H-$C(3^5)$, 2H-$C(3^6)$, 2H-$C(4^6)$, 2H-$C(5^6)$), 1.63 (d, J=4.47, 3H, CH_3-$C(7^1)$), 1.33 (d, J=4.62, 3H, CH_3-$C(2^7)$), 0.96-0.82 (m, 21H, CH_3-$C(4^1)$, CH_3-$C(3^2)$, $2CH_3$-$C(4^4)$, $2CH_3$-$C(3^5)$, CH_3-$C(5^6)$); FABMS (3-NBA matrix): m/z $[M+H]^+$ 872.7; HR-FABMS exact mass calcd for $C_{46}H_{78}N_7O_9$ $[M+H]^+$ 872.5861, found 872.5844.

(2S, 3R, 4R, 6E)-3-Hydroxy-4-methyl-2-(methylamino)-6-octenoyl-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N^{α}-Methyl-N^{ε}-[3-(3'-pyridyl)allyloxy-carbonyl]-L-lysyl-L-Alanine Benzyl Ester (H-MeBmt-Abu-Sar-MeLeu-Val-MeLys(Paloc)-Ala-OBzl) (3.35b)

The title compound was synthesized according to the general procedure B in 49% yield and obtained as a foamy solid: TLC R_f 0.41 (10% $MeOH/CH_2Cl_2$); $[\alpha]_D$ -116.2° (c 0.95, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$ at least two conformers at room temperature and major one is described) δ 8.59 (br s, 1H, H-2 of pyirdine), 8.47 (d, J=4.96, 1H, H-6 of pyridine), 7.84, 7.10, 6.80 (3d, 3H, H-N^2, H-N^5, H-N^7), 7.68 (m, 1H, H-5 of pyridine), 7.33 (br d, 5H, aromatic H's), 7.24 (m br, 1H, H-4 of pyridine), 6.60 (br d, J=15.54, 1H, -CH=C-C-O^6), 6.38 (m, 1H,

C=CH-C-O^6), 5.42 (m, 2H, CH=CH-C(5^1)), 5.18 (d, J=6.72, 2H, O-CH_2Ph), 4.70 (d, J=5.75, 2H, -C=C-CH_2-O^6), 5.60-2.80 (m, 12H, H-N^1, HO-C(3^1), H-C(2^1), H-C(3^1), H-C(2^2), 2H-C(2^3), H-C(2^4), H-C(2^5), H-C(2^6), 2H-C(6^6), H-C(2^7), HN-C(6^6)), 3.31, 3.10, 3.02, 2.42(4s, 12H, CH_3-N^1, CH_3-N^3, CH_3-N^4, CH_3-N^6), 2.45-1.20 (m, 15H, H-C(4^1), 2H-C(5^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), H-C(3^5), 2H-C(3^6), 2H-C(4^6)), 2H-C(5^6)), 1.63 (d, J=4.8, 3H, CH_3-C(7^1)), 1.35 (d, J=7.17, 3H, CH_3-C(2^7)), 1.05-0.80 (m, 18H, CH_3-C(4^1), CH_3-C(3^2), 2CH_3-C(4^4), 2CH_3-C(3^5)); FABMS (3-NBA matrix): m/z [M+H]+ 1048.7; HR-FABMS exact mass calcd for $C_{55}H_{86}N_9O_{11}$ [M+H]+ 1048.6447, found 1048.6453.

N-[[(9-Fluorenylmethyl)oxy]carbonyl]-D-Alanyl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-Valyl-[(2S,3R,4R,6E)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl] -L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-norleucyl-L-Alanine Benzyl Ester (Fmoc-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeNle-Ala-OBzl) (3.43a)

The pure undecapeptide was synthesized according to the general procedure C in 61% yield and obtained as a foamy solid: TLC R_f 0.55 (60% acetone/hexane); $[\alpha]_D$ -148° (c 0.6, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) spectrum available as supplemental material; FABMS (DTT/DTE matrix): m/z [M+H]+ 1532.8; HR-FABMS exact mass calcd for $C_{84}H_{130}N_{11}O_{15}$ [M+H]+ 1532.9748, found 1532.9685.

N^{α}-[[(9-Fluorenylmethyl)oxy]carbonyl]-N^{ε}-(tert-butyloxycarbonyl)-D-Lysyl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-Valyl-[(2S,3R,4R,6E)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl]-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-norleucyl-L-Alanine Benzyl Ester (Fmoc-D-Lys(BOC)-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeNle-Ala-OBzl) (3.43b)

The pure undecapeptide was synthesized according to the general procedure C in 40% yield and obtained as a foamy solid: TLC R_f 0.29 (40% acetone/hexane); $[\alpha]_D$ -82.1° (c 1.9, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) spectrum available as supplemental material; FABMS (3-NBA matrix): m/z $[M+H]^+$ 1690; HR-FABMS exact mass calcd for $C_{92}H_{145}N_{12}O_{17}[M+H]^+$ 1690.0851, found 1690.0786.

N-[[(9-Fluorenylmethyl)oxy]carbonyl]-D-Seryl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-Valyl-[(2S,3R,4R,6E)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl]-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-norleucyl-L-Alanine Benzyl Ester (Fmoc-D-Ser-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeNle-Ala-OBzl) (3.43c)

The pure undecapeptide was synthesized according to the general procedure C in 36% yield and obtained as a foamy solid: TLC R_f 0.44 (40% acetone/hexane); $[\alpha]_D$ -168.8° (c 1.9, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$)

spectrum available as supplemental material; FABMS (3-NBA matrix): m/z [M+H]+ 1548; HR-FABMS exact mass calcd for $C_{84}H_{130}N_{11}O_{16}$ [M+H]+ 1548.9697, found 1548. 9696.

N-[[(9-Fluorenylmethyl)oxy]carbonyl]-D-Alayl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-valyl-[(2S,3R,4R,6E)-3-Hydroxy-4-methyl-2-(methylamino)-6-octenoyl]-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N$^{\alpha}$-Methyl-N$^{\varepsilon}$-[3-(3'-pyridyl)allyloxy-carbonyl]-L-lysyl-L-Alanine Benzyl Ester (Fmoc-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLys(Paloc)-Ala-OBzl) (3.43d)

The pure undecapeptide was synthesized according to the general procedure C in 36% yield and obtained as a foamy solid: TLC R_f 0.34 (65% acetone/hexane); $[\alpha]_D$ -95.0° (c 0.02, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) spectrum available as supplemental material; FABMS (3-NBA/Gly + 1% TFA as matrix): m/z [M+H]+ 1709.2; HR-FABMS exact mass calcd for $C_{93}H_{138}N_{13}O_{17}$ [M+H]+ 1709.0337, found 1709.0296 .

N-[[(9-Fluorenylmethyl)oxy]carbonyl]-D-(O-tert-Butyl)-seryl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-valyl-[(2S,3R,4R,6E)-3-Hydroxy-4-methyl-2-(methylamino)-6-octenoyl]-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N$^{\alpha}$-Methyl-N$^{\varepsilon}$-[3-(3'-pyridyl)allyloxy-carbonyl]-L-lysyl-L-

Alanine Benzyl Ester (Fmoc-D-Ser(tBu)-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLys(Paloc)-Ala-OBzl) (3.43e)

The pure undecapeptide was synthesized according to the general procedure C in 40% yield and obtained as a foamy solid: TLC R_f 0.33 (66% acetone/hexane); $[\alpha]_D$ -155.8° (c 0.8, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) spectrum available as supplemental material; FABMS (3-NBA matrix): m/z $[M+H]^+$ 1781.0; HR-FABMS exact mass calcd for $C_{97}H_{146}N_{13}O_{18}$ $[M+H]^+$ 1781.0909, found 1781.0895.

N-[[(9-Fluorenylmethyl)oxy]carbonyl]-D-seryl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-valyl-[(2S,3R,4R,6E)-3-Hydroxy-4-methyl-2-(methylamino)-6-octenoyl]-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N$^{\alpha}$-Methyl-N$^{\varepsilon}$-[3-(3'-pyridyl)allyloxy-carbonyl]-L-lysyl-L-Alanine Benzyl Ester (Fmoc-D-Ser-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLys(Paloc)-Ala-OBzl) (3.43f)

The pure undecapeptide was synthesized according to the general procedure C in 53% yield and obtained as a foamy solid: TLC R_f 0.54 (80% acetone/hexane); $[\alpha]_D$ -106.4° (c 0.7, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) spectrum available as supplemental material; FABMS (DTT/DTE matrix): m/z $[M+H]^+$ 1724.9; HR-FABMS exact mass calcd for $C_{93}H_{138}N_{13}O_{18}$ $[M+H]^+$ 1725.0283, found 1725.0256.

Cyclo[(2S,3R,4R,6E)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl)-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-norleucyl-L-Alanyl-D-Alanyl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-valyl] ; [MeNle$^{(6)}$]Cyclosporine (3.44a)

The title compound was synthesized according to the general procedure D in 46% yield and obtained as a foamy solid: TLC R_f 0.43 (50% acetone/hexane) ; $[\alpha]_D$ -172.5° (c 0.04, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, J=9.84 1H, H-N^2), 7.60 (d, J=8.07, 1H, H-N^7), 7.44 (d, J=8.13, 1H, H-N^5), 7.11 (d, J=8.04, 1H, H-N^8), 5.69 (dd, J=10.59, 4.05, 1H, H-C(2^9)), 5.60 (d, J=5.13, 1H, H-C(2^1)), 5.36-5.29 (m, 2H, CH=CH-C(5^1), 5.27 (d, J=8.25, 1H, H-C(2^4)), 5.15 (d, J=11.1 1H, H-C(2^{11})), 5.05 (br s, 1H, H-C(2^{10})), 5.01 (br s, 1H, H-C(2^2), 4.87 (t, J=7.77, 1H, H-C(2^6)), 4.80 (quintet, J=6.96, 1H, H-C(2^8)), 4.74 (d, J=13.92, 1H, si-H-C(2^3)), 4.61 (quintet, J=7.85, 1H, H-C(2^7)), 4,59 (t, J=10.2, 1H, H-C(2^5)), 3.62 (br s, 1H, H-C(3^1)), 3.55 (s, 3H, CH_3-N^1), 3.37 (s, 3H, CH_3-N^3), 3.28 (s, 3H, CH_3-N^6), 3.17 (d, J=13.92, 1H, re-H-C(2^3)), 3.12 (s, 3H, CH_3-N^9), 3.09 (s, 3H, CH_3-N^4), 2.70 (s, 3H, CH_3-N^{11}), 2.69 (s, 3H, CH_3-N^{10}), 2.55 (br d, J=9.2, 1H, H-C(5^1)), 2.45 (m, 1H, H-C(3^5)), 2.25 (m, 1H, H-C(4^{10})), 2.10-1.15 (m, 19H, H-C(4^1), H-C(5^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), 2H-C(3^6), 2H-C(4^6), 2H-C(5^6), 2H-C(3^9), H-C(4^9), 2H-C(3^{10}), H-C(3^{11})), 1.61 (d, J=5.7, 3H, CH_3-C(7^1)), 1.35 (d, J=7.3, 3H, CH_3-C(2^7)), 1.24 (d, J=6.9, 3H, CH_3-C(2^8)), 1.10-0.82 (m, 36H, CH_3-C(3^2), 2CH_3-C(4^4), 2CH_3-C(3^5), CH_3-C(5^6), 2CH_3-C(4^9), 2CH_3-C(4^{10}), 2CH_3-C(3^{11})), 0.54 (d, J=5.85, 3H, CH_3-C(4^1));

FABMS (3-NBA+1% TFA matrix): m/z $[M+H]^+$ 1203; HR-FABMS exact mass calcd for $C_{62}H_{112}N_{11}O_{12}$ $[M+H]^+$ 1202.8492, found 1202.8469.

Cyclo[(2S,3R,4R,6E)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl)-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-norleucyl-L-Alanyl-N$^{\varepsilon}$-(tert-Butyloxycarbonyl)-D-lysyl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-valyl] ; *[D-Lys(BOC)$^{(8)}$,MeNle$^{(6)}$]Cyclosporine* ***(3.44b)***

The title compound was synthesized according to the general procedure D in 20% yield and obtained as a foamy solid: TLC R_f 0.31 (50% acetone/hexane) ; $[\alpha]_D$ -145° (c 0.02, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, J=10.0, 1H, H-N^2), 7.65 (d, J=7.7, 1H, H-N^7), 7.47 (d, J=7.8, 1H, H-N^5), 7.04 (d, J=8.1, 1H, H-N^8), 5.68 (m, 1H, H-C(2^9)), 5.63 (d, J=5.0, 1H, H-C(2^1)), 5.36-5.30 (m, 2H, CH=CH-C(5^1); 5.29 (m, 1H, H-C(2^4)), 5.16 (d, J=11.1, 1H, H-C(2^{11})), 5.06 (br s, 1H, H-C(2^{10})), 5.04 (br s, 1H, H-C(2^2)), 4.87 (m, 1H, H-C(2^6)), 4.81 (m, 1H, H-C(2^8)), 4.75 (d, J=13.9, 1H, si-H-C(2^3)), 4.62 (d, J=7.1, 1H, H-N$^{\varepsilon-8}$), 4.59 (m, 1H, H-C(2^7)), 4.55 (m, 1H, H-C(2^5)), 3.64 (br s, 1H, H-C(3^1)), 3.55 (s, 3H, CH_3-N^1), 3.38 (s, 3H, CH_3-N^3), 3.28 (s, 3H, CH_3-N^6), 3.19 (d, J=13.9, 1H, re-H-C(2^3)), 3.12 (s, 3H, CH_3-N^9), 3.10-3.04 (br m, 2H, 2H-C(6^8)), 3.06 (s, 3H, CH_3-N^4), 2.70 (s, 3H, CH_3-N^{11}), 2.69 (s, 3H, CH_3-N^{10}), 2.54 (m, 1H, H-C(5^1)), 2.47 (m, 1H, H-C(3^5)), 2.29 (m, 1H, H-C(4^{10})), 2.10-1.24 (m, 25H, H-C(4^1), H-C(5^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), 2H-C(3^6), 2H-C(4^6), 2H-C(5^6), 2H-C(3^8), 2H-C(4^8), 2H-C(5^8), 2H-C(3^9), H-C(4^9), 2H-

C(3^{10}), H-C(3^{11})), 1.62 (d, J=5.45, 3H, CH_3-C(7^1)), 1.43 (s, 9H, $(CH_3)_3$C-O^8), 1.38 (d, J=7.3, 3H, CH_3-C(2^7)), 1.10-0.82 (m, 36H, CH_3-C(3^2), 2CH_3-C(4^4), 2CH_3-C(3^5), CH_3-C(5^6), 2CH_3-C(4^9), 2CH_3-C(4^{10}), 2CH_3-C(3^{11})), 0.56 (d, J=6.1, 3H, CH_3-C(4^1)); FABMS (3-NBA+1% TFA matrix): m/z $[M+H]^+$ 1360; HR-FABMS exact mass calcd for $C_{70}H_{127}N_{12}O_{14}$ $[M+H]^+$ 1359.9595, found 1359.9588.

Cyclo[(2S,3R,4R,6E)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl)-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-norleucyl-L-Alanyl-D-Seryl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-valyl] ; *[D-Ser$^{(8)}$,MeNle$^{(6)}$]Cyclosporine (3.44c)*

The title compound was synthesized according to the general procedure D in 71% yield and obtained as a foamy solid: TLC R_f 0.36 (50% acetone/hexane); $[\alpha]_D$ -250° (c 0.05 , $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.00 (d, J=9.75, 1H, H-N^2), 7.73 (d, J=7.3, 1H, H-N^7), 7.46 (d, J=8.1, 1H, H-N^5), 7.31 (d, J=7.4, 1H, H-N^8), 5.68 (dd, J=4.6, 10.5, 1H, H-C(2^9)), 5.60 (d, J=5.3, 1H, H-C(2^1)), 5.34-5.32 (m, 2H, CH=CH-C(5^1)), 5.29 (dd, J=3.9, 11.6, 1H, H-C(2^4)), 5.17 (d, J=11.1, 1H, H-C(2^{11})), 5.06 (m, 1H, H-C(2^2)), 5.00 (m, 1H, H-C(2^{10})), 4.92-4.84 (m, 2H, H-C(2^6) & H-C(2^8)), 4.74 (d, J=13.9, 1H, si-H-C(2^3)), 4.59 (m, 1H, H-C(2^7)), 4.55 (d, J=6.87, 1H, H-C(2^5)), 3.75-3.65 (br m, 3H, H-C(3^1) & 2H-C(3^8)), 3.55 (s, 3H, CH_3-N^1), 3.38 (s, 3H, CH_3-N^3), 3.27 (s, 3H, CH_3-N^6), 3.22 (d, J=13.9, 1H, re-H-C(2^3)), 3.15 (s, 3H, CH_3-N^9), 3.11 (s, 3H, CH_3-N^4), 2.70 (s, 6H, CH_3-N^{11} & CH_3-N^{10}), 2.55-1.25 (m,

22H, H-C(4^1), 2H-C(5^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), H-C(3^5), 2H-C(3^6), 2H-C(4^6), 2H-C(5^6), 2H-C(3^9), H-C(4^9), 2H-C(3^{10}), H-C(4^{10}), H-C(3^{11})), 1.61 (d, J=4.62, 3H, CH_3-C(7^1)), 1.37 (d, J=7.3, 3H, CH_3-C(2^7)), 1.20-0.82 (m, 36H, CH_3-C(3^2), $2CH_3$-C(4^4), $2CH_3$-C(3^5), CH_3-C(5^6), $2CH_3$-C(4^9), $2CH_3$-C(4^{10}), $2CH_3$-C(3^{11})), 0.56 (d, J=5.7, 3H, CH_3-C(4^1)); FABMS (3-NBA/Gly/TFA matrix): m/z $[M+H]^+$ 1218.7; HR-FABMS: exact mass calcd for $C_{62}H_{112}N_{11}O_{13}$ $[M+H]^+$ 1218.8441, found 1218.8427.

Cyclo[((2S,3R,4R,6E)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl)-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-Nα-Methyl-Nε-([3-(3'-pyridyl)allyloxy-carbonyl]-L-lysyl-L-Alanyl-D-Alanyl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-valyl] ; *[MeLys(Paloc)(6)]Cyclosporine (3.44d)*

The title compound was synthesized according to the general procedure D in 59% yield and obtained as a foamy solid: TLC R_f 0.26 (65% acetone/hexane) ; $[\alpha]_D$ -140° (c 0.03, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.58 (s, 1H, H-2 of pyridine), 8.48 (d, J=3.8, 1H, H-6 of pyridine), 8.00 (d, J=9.8, 1H, H-N^2), 7.69 (d, J=7.9, 1H, H-4 of pyirdine), 7.60 (d, J=7.8, 1H, H-N^7), 7.51 (d, J=8.0, 1H, H-N^5), 7.24 (m, 1H, H-5 of pyridine), 7.03 (d, J=8.5, 1H, H-N^8), 6.58 (d, J=16.0, 1H, CH=C-C-O), 6.37 (m, 1H, C=CH-C-O), 5.79 (br t, 1H, HN-C(6^6)), 5.65 (d, J=8.95, 1H, H-C(2^1)), 5.63 (br s, 1H, H-C(2^9)), 5.35-5.32 (m, 2H, CH=CH-C(5^1)), 5.27 (dd, J=3.9, 3.9, 1H, H-C(2^4)), 5.17 (d, J=11, 1H, H-C(2^{11})), 5.04 (m, 3H, H-C(2^2)), 4.98

(m, 1H, H-C(2^6)), 4.92 (m, 1H, H-C(2^{10})), 4.88 (quintet, J=7.25, 1H, H-C(2^8)), 4.74 (d, J=13.95, 1H, si-H-C(2^3)), 4.68 (t, J=5.35, 2H, C=C-CH_2-O-), 4.56 (m, 1H, H-C(2^7)), 4.53 (dd, J=8.15, 8.15, 1H, H-C(2^5)), 4.35 (d, J=6.5, 1H, HO-C(3^1)), 3.68 (m, 1H, H-C(3^1)), 3.39-3.33, 3.02-2.98 (m, 2H, 2H-C(6^6)), 3.52 (s, 3H, CH_3-N^1), 3.38 (s, 3H, CH_3-N^3), 3.27 (s, 3H, CH_3-N^6), 3.19 (d, J=13.95, 1H, re-H-C(2^3)), 3.12 (s, 3H, CH_3-N^9), 3.11 (s, 3H, CH_3-N^4), 2.71 (s, 3H, CH_3-N^{11}), 2.69 (s, 3H, CH_3-N^{10}), 2.49 (br s, 1H, H-C(4^1)), 2.28-1.15 (m, 22H, H-C(4^1), 2H-C(5^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), H-C(3^5), 2H-C(3^6), 2H-C(4^6), 2H-C(5^6), 2H-C(3^9), H-C(4^9), 2H-C(3^{10}), H-C(4^{10}), H-C(3^{11})), 1.62 (d, J=4.75, 3H, CH_3-C(7^1)), 1.35 (d, J=4.47, 3H, CH_3-C(2^7)), 1.28 (d, J=6.8, 3H, CH_3-C(2^8)), 1.10-0.82 (m, 33H, CH_3-C(3^2), 2CH_3-C(4^4), 2CH_3-C(3^5), 2CH_3-C(4^9), 2CH_3-C(4^{10}), 2CH_3-C(3^{11})), 0.59 (d, J=6.1, 3H, CH_3-C(4^1)); FABMS (3-NBA matrix): m/z $[M+H]^+$ 1378.9; HR-FABMS exact mass calcd for $C_{71}H_{120}N_{13}O_{14}$ $[M+H]^+$ 1378.9078, found 1378.9088.

Cyclo[((2S,3R,4R,6E)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl)-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-Nα-Methyl-Nε-([3-(3'-pyridyl)allyloxy-carbonyl]-L-lysyl-L-Alanyl-D-(O-tert-Butyl)-seryl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-valyl] ;

[D-Ser(tBu)(8),MeLys(Paloc)(6)]Cyclosporine (3.44e)

The title compound was synthesized according to the general procedure D in 59% yield and obtained as a foamy solid: TLC R_f 0.49 (65% acetone/hexane);

$[\alpha]_D$ -216° (c 0.1, $CHCl_3$) ; ^1H NMR (500 MHz, $CDCl_3$) δ 8.60 (s, 1H, H-2 of pyridine), 8.49 (m, 1H, H-6 of pyridine), 8.16 (d, J=9.54 1H, H-N^2), 7.70 (m, 2H, H-N^7 & H-4 of pyirdine), 7.49 (d, J=8.1, 1H, H-N^5), 7.24 (m, 1H, H-5 of pyridine), 6.88 (d, J=8.04, 1H, H-N^8), 6.60 (d, J=15.51, 1H, CH=C-C-O), 6.37 (dt, J=15.5, 6.1, 1H, C=CH-C-O), 5.72-5.68 (m, 2H, H-C(2^1), H-C(2^9)), 5.57 (br t, 1H, HN-C(6^6)), 5.39-5.34 (m, 2H, CH=CH-C(5^1)), 5.30 (m, 1H, H-C(2^4)), 5.12 (d, J=11.0, 1H, H-C(2^{11})), 5.05-5.00 (m, 3H, H-C(2^{10}), H-C(2^2), H-C(2^8)), 4.93 (m, 1H, H-C(2^6)), 4.71 (d, J=13.5, 1H, si-H-C(2^3)), 4.56 (m, 1H, H-C(2^7)), 4.46 (d, J=7.2, 1H, H-C(2^5)), 3.66 (m, 1H, H-C(3^1)), 3.35-3.32 (br s, 2H, 2H-C(3^8)), 3.30-3.25, 3.08-3.05 (m,2H, 2H-C(6^6)), 3.48 (s, 3H, CH_3-N^1), 3.36 (s, 3H, CH_3-N^3), 3.25 (s, 3H, CH_3-N^6), 3.21 (d, J=13.5, 1H, re-H-C(2^3)), 3.15 (s, 3H, CH_3-N^9), 3.11 (s, 3H, CH_3-N^4), 2.70 (s, 3H, CH_3-N^{11}), 2.68 (s, 3H, CH_3-N^{10}), 2.49 (br s, 1H, H-C(4^1)), 2.43-1.14 (m, 21H, 2H-C(5^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), H-C(3^5), 2H-C(3^6), 2H-C(4^6), 2H-C(5^6), 2H-C(3^9), H-C(4^9), 2H-C(3^{10}), H-C(4^{10}), H-C(3^{11})), 1.62 (d, J=4.5, 3H, CH_3-C(7^1)), 1.34 (d, J=4.5, 3H, CH_3-C(2^7)), 1.12 (s, 9H, $(CH_3)_3$C-O^8), 1.08-0.84 (m, 33H, CH_3-C(3^2), 2CH_3-C(4^4), 2CH_3-C(3^5), 2CH_3-C(4^9), 2CH_3-C(4^{10}), 2CH_3-C(3^{11})), 0.59 (d, J=5.37, 3H, CH_3-C(4^1)); FABMS (3-NBA matrix): m/z $[M+H]^+$ 1451; HR-FABMS exact mass calcd for $C_{75}H_{128}N_{13}O_{15}$ $[M+H]^+$ 1450.9653, found 1450.9666.

Cyclo[(2S,3R,4R,6E)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl)-L-2-

Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-Nα-Methyl-Nε-([3-(3'-pyridyl)allyloxy-carbonyl]-L-lysyl-L-Alanyl-D-Seryl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-valyl] ; [D-Ser(8),MeLys(Paloc)(6)]Cyclosporine (3.44f)

The title compound was synthesized according to the general procedure D in 47% yield and obtained as a foamy solid: TLC R_f 0.29 (70% acetone/hexane); $[\alpha]_D$ -184° (c 0.1, $CHCl_3$) ; ^1H NMR (500 MHz, $CDCl_3$, two conformers exist at room temperature and major one is described) δ 8.59 (s, 1H, H^2 of pyridine), 8.47 (br s, 1H, H^6 of pyridine), 7.92 (d, J=9.8, 1H, H-C(2^2)), 7.70 (br d, 1H, H^4 of pyridine), 7.63 (d, J=7.5, 1H, H-C(2^7)), 7.56 (d, J=7.7, 1H, H-C(2^5)), 7.23 (m, 1H, H^5 of pyridine), 6.90 (d, J=8.3, 1H, H-C(2^8)), 6.60 (d, J=15.9, 1H, O-C-C=CH-), 6.40-6.32 (m, 1H, O-C-CH=C-), 5.84 (br s, 1H, HN-C(6^6)), 5.70 (br d, 1H, H-C(2^1)), 5.66 (br d, 1H, H-C(2^9)), 5.36-5.25 (m, 4H, CH=CH-C(5^1), H-C(2^8), H-C(2^4)), 5.24-5.20 (m, 1H, H-C(2^{11})), 5.06-4.92 (m, 3H, H-C(2^2), H-C(2^6), H-C(2^{10})), 4.75-4.70 (m, 3H, si-H-C(2^3), O-CH_2-C=C-), 4.56-4.44 (m, 2H, H-C(2^5), H-C(2^7)), 3.78-3.70 (m, 3H, 2H-C(3^8), H-C(3^1)), 3.55 (s, 3H, CH_3-N^1), 3.38 (s, 3H, CH_3-N^3), 3.34-3.32, 3.10-3.00 (br m, 2H, 2H-C(6^6)), 3.26 (s, 3H, CH_3-N^6), 3.24 (s, 3H, CH_3-N^9), 3.18 (d,J=15.9, 1H, re-H-C(2^3)), 3.12 (s, 3H, CH_3-N^4), 2.72 (s, 6H, CH_3-N^{10}, CH_3-N^{11}), 2.48-1.25 (m, 22H, H-C(4^1), 2H-C(5^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), H-C(3^5), 2H-C(3^6), 2H-C(4^6), 2H-C(5^6), 2H-C(3^9), H-C(4^9), 2H-C(3^{10}), H-C(4^{10}), H-C(3^{11})), 1.62 (d, J=4.0, 3H, CH_3-C(7^1)), 1.38 (d, J=7.3, 3H, CH_3-C(2^7)), 1.15-0.82 (m, 33H, CH_3-

C(3^2), 2CH_3-C(4^4), 2CH_3-C(3^5), 2CH_3-C(4^9), 2CH_3-C(4^{10}), 2CH_3-C(3^{11})), 0.59 (d, 5.6, CH_3-C(4^1)); FABMS (DTT/DTE matrix): m/z $[M+H]^+$ 1395.1; HR-FABMS exact mass calcd for $C_{71}H_{120}N_{13}O_{15}$ $[M+H]^+$ 1394.9027, found 1394.8993.

Cyclo[((2S,3R,4R,6E)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl)-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-norleucyl-L-Alanyl-D-Lysyl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-valyl] ; [D-Lys$^{(8)}$,MeNle$^{(6)}$]Cyclosporine (3.54)

[D-Lys(Boc)^8,MeNle^6]CsA (**3.44b**, 53 mg, 38.9 μmol) was treated with 0.1 ml of methylene chloride and 0.9 ml of TFA at -16 °C for 2 h. After neutralization with saturated $NaHCO_3$ solution and extraction with methylene chloride (3 x 10 mL), the combined organic layers were dried over anhydrous $MgSO_4$ to give, after concentration in vacuo, 39 mg (82%) of the title compound as a foamy solid: TLC R_f 0.25 (10% MeOH/$CHCl_3$); $[\alpha]_D$ -187.5° (c 0.035, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.98 (d, J=9.8, 1H, H-N^2), 7.73 (d, J=7.5, 1H, H-N^7), 7.26 (d, J=8.0, 1H, H-N^5), 7.04 (d, J=8.3, 1H, H-N^8), 5.68 (m, 1H, H-C(2^9)), 5.61 (d, J=5.0, 1H, H-C(2^1)), 5.35-5.31 (m, 2H, CH=CH-C(5^1)), 5.29 (dd, J=3.9, 11.7, 1H, H-C(2^4)), 5.16 (d, J=11.1, 1H, H-C(2^{11})), 5.06 (m, 1H, H-C(2^{10})), 5.03 (m, 1H, H-C(2^2)), 4.87 (t, J=8.1, 1H, H-C(2^6)), 4.82 (m, 1H, H-C(2^8)), 4.75 (d, J=14.0, 1H, si-H-C(2^3)), 4.60 (m, 1H, H-C(2^5)), 4.55 (m, 1H, H-C(2^7)), 3.63 (br s, 1H, H-C(3^1)), 3.55 (s, 3H, CH_3-N^1), 3.37 (s, 3H, CH_3-N^3), 3.26 (s, 3H, CH_3-N^6), 3.18 (d, J=13.90, 1H,

re-H-C(2^3)), 3.12 (s, 3H, CH_3-N^9), 3.05 (s, 3H, CH_3-N^4), 2.95-2.85 (br m, 2H, 2H-C(6^8)), 2.69 (s, 3H, CH_3-N^{11}), 2.68 (s, 3H, CH_3-N^{10}), 2.52-1.20 (m, 28H, H-C(4^1), 2H-C(5^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), H-C(3^5), 2H-C(3^6), 2H-C(4^6), 2H-C(5^6), 2H-C(3^8), 2H-C(4^8), 2H-C(5^8), 2H-C(3^9), H-C(4^9), 2H-C(3^{10}), H-C(4^{10}), H-C(3^{11})), 1.62 (d, J=4.9, 3H, CH_3-C(7^1)), 1.34 (d, J=8.5, 3H, CH_3-C(2^7)), 1.10-0.82 (m, 36H, CH_3-C(3^2), $2CH_3$-C(4^4), $2CH_3$-C(3^5), CH_3-C(5^6), $2CH_3$-C(4^9), $2CH_3$-C(4^{10}), $2CH_3$-C(3^{11})), 0.55 (d, J=5.3, 3H, CH_3-C(4^1)); FABMS (3-NBA matrix): m/z $[M+H]^+$ 1259.8; HR-FABMS exact mass calcd for $C_{65}H_{119}N_{12}O_{12}$ $[M+H]^+$ 1259.9074, found 1209.9056.

Cyclo[((2S,3R,4R,6E)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl)-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-norleucyl-L-Alanyl-D-(Nε-(4-(1-Diaziryl-2,2,2-trifluoroethyl)benzoyl))-Lysyl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-valyl] ;

[D-Lys(Daz)(8), MeNle(6)]Cyclosporine (3.55a)

A solution of CsA analogue **3.54** (15 mg, 11.9 μmol), diazirinylbenzoic acid **3.53** (3.0 mg, 13.0 μmol, 1.1 equiv), and 1-hydroxybenzotriazole (2.5 mg, 17.9 μmol, 1.5 equiv) in 0.5 ml of THF was stirred and treated with EDCI (3.45 mg, 17.9 μmol, 1.5 equiv) under 0 °C. After it was stirred at room temperature for 16 h, the reaction mixture was concentrated in vacuo and washed with water and CH_2Cl_2. The water layer was then extracted with additional CH_2Cl_2 (3 x 5

ml). The combined organic layers were dried over anhydrous $MgSO_4$ to give, after purification by flash chromatography (15-40% acetone/n-hexane), 17.1 mg (97%) of the title compound as a foamy solid: TLC R_f 0.56 (50% acetone/n-hexane); $[\alpha]_D$ -152.5° (c 0.04, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.94 (d, J=10.4, 1H, H-N^2), 7.93, 7.18 (2d, J=8.1, 4H, AA'BB' of aromatic H's), 7.69 (d, J=7.6, 1H, H-N^7), 7.50 (d, J=8.0, 1H, H-N^5), 7.12 (d, J=3.5, 1H, H-N^8), 7.10 (d, J=5.0, 1H, HN-C(6^6)), 5.68 (dd, J=10.0, 3.4, 1H, H-C(2^9)), 5.61 (d, J=5.1, 1H, H-C(2^1)), 5.35-5.30 (m, 2H, CH=CH-C(5^1)), 5.29-5.26 (m, 1H, H-C(2^4)), 5.17 (d, J=11.2, 1H, H-C(2^{11})), 5.06 (m, 1H, H-C(2^2)), 5.04 (m, 1H, H-C(2^{10})), 4.87 (m, 1H, H-C(2^6)), 4.83 (m, 1H, H-C(2^8)), 4.75 (d, J=13.9, 1H, si-H-C(2^3)), 4.53 (dd, J=9.0, 8.3 , 1H, H-C(2^5)), 4.48 (quintet, J=7.5, 1H, H-C(2^7)), 3.63 (m, 1H, H-C(3^1)), 3.54-3.52, 3.39-3.37 (m, 2H, 2H-C(6^6)), 3.55 (s, 3H, CH_3-N^1), 3.38 (s, 3H, CH_3-N^3), 3.15 (s, 3H, CH_3-N^6), 3.17 (d, J=13.90, 1H, re-H-C(2^3)), 3.13 (s, 3H, CH_3-N^9), 3.05 (s, 3H, CH_3-N^4), 2.70 (s, 3H, CH_3-N^{11}), 2.69 (s, 3H, CH_3-N^{10}), 2.55-1.20 (m, 28H, H-C(4^1), 2H-C(5^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), H-C(3^5), 2H-C(3^6), 2H-C(4^6), 2H-C(5^6), 2H-C(3^8), 2H-C(4^8), 2H-C(5^8), 2H-C(3^9), H-C(4^9), 2H-C(3^{10}), H-C(4^{10}), H-C(3^{11})); 1.63 (d, J=5.8, 3H, CH_3-C(7^1)), 1.37 (d, J=7.5, 3H, CH_3-C(2^7)), 1.15-0.82 (m, 36H, CH_3-C(3^2), 2CH_3-C(4^4), 2CH_3-C(3^5), CH_3-C(5^6), 2CH_3-C(4^9), 2CH_3-C(4^{10}), 2CH_3-C(3^{11})), 0.55 (d, J=6.0, 3H, CH_3-C(4^1)); FABMS (3-NBA/Gly + 1%TFA as matrix): m/z $[M+H]^+$ 1472; HR-FABMS exact mass calcd for $C_{74}H_{122}F_3N_{14}O_{13}$ $[M+H]^+$ 1471.9267, found 1471.9277.

Cyclo[((2S,3R,4R,6E)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl)-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-Nα-Methyl-Nε-(4-(1-Diaziryl-2,2,2-trifluoroethyl)benoyl)--L-lysyl-L-Alanyl-D-Seryl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-valyl] ;

[D-Ser(8),MeLys(Daz)(6)]Cyclosporine (3.55b)

To a solution of CsA analogue **3.44f** (6 mg, 4.3 μmol), diazirinylbenzoic acid **3.53** (2 mg, 8.6 μmol), and $(PPh_3)_2PdCl_2$ (1.0 mg, 1.4 μmol) in 0.4 ml of CH_2Cl_2/THF (3:1) was added dropwise tri-n-butyltin hydride (~ 20 μL) until the color of the reaction mixture turned dark orange. Then 1-hydroxybenzotriazole (1.3 mg, 9.5 μmol) was added, and the mixture was cooled to 0 °C. EDCI (1.8 mg, 9.5 μmol) was added and the mixture allowed to warm slowly to ambient temperature. After 17 h, the reaction mixture was condensed in vacuo and the residue was diluted with CH_2Cl_2, washed with diluted HCl (3 x 2 mL) and 5% $NaHCO_3$, and dried over $MgSO_4$. After the solution was concentrated in vacuo, the residue was purified by flash chromatography (10-60% acetone/hexane) to give 4.5 mg (72%) of the title compound as a foamy solid: TLC R_f 0.38 (50% acetone/hexane); $[\alpha]_D$ -195° (c 0.04, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.94 (d, J=9.7, 1H, H-N^2), 7.82, 7.24 (2d, J=8.3, 4H, AA'BB' of aromatic H's), 7.68 (d, J=7.8, 1H, H-N^7), 7.58 (d, J=8.0, 1H, H-N^5), 7.46 (d, J=7.7, 1H, H-N^8), 6.90 (br t, 1H, HN-C(6^6)), 5.64 (br d, J=5.6,

2H, H-C(2^9) & H-C(2^1)), 5.36-5.30 (m, 2H, CH=CH-C(5^1)), 5.22 (dd, J=3.4, 8.3, 1H, H-C(2^4)), 5.18 (d, J=11.1, 1H, H-C(2^{11})), 5.07-5.02 (m, 3H, H-C(2^{10}), H-C(2^2) & H-C(2^6)), 4.93 (m, 1H, H-C(2^8)), 4.74 (d, J=13.9, 1H, si-H-C(2^3)), 4.56 (t, J=7.4, 1H, H-C(2^5)), 4.50 (q, J=4.9, 1H, H-C(2^7)), 3.75-3.70 (m, 2H, 2H-C(3^8)), 3.68 (m, 1H, H-C(3^1)); 3.52, 3.37, 3.24, 3.18, 3.11, 2.71, 2.69 (7s, 21H, CH_3-N^1, CH_3-N^3, CH_3-N^6, CH_3-N^9, CH_3-N^4, CH_3-N^{11}, CH_3-N^{10}), 2.50-1.20 (m, 22H, H-C(4^1), 2H-C(5^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), H-C(3^5), 2H-C(3^6), 2H-C(4^6), 2H-C(5^6), 2H-C(3^9), H-C(4^9), 2H-C(3^{10}), H-C(4^{10}), H-C(3^{11})), 1.62 (d, J=4.2, 3H, CH_3-C(2^7)), 1.38 (d, J=7.3, 3H, CH_3-C(2^7)), 1.15-0.78 (m, 33H, CH_3-C(3^2), $2CH_3$-C(3^4), $2CH_3$-C(3^5), $2CH_3$-C(3^9), $2CH_3$-C(3^{10}), $2CH_3$-C(3^{11})); FABMS (3-NBA matrix): m/z $[M+H]^+$ 1445.8; FABMS (3-NBA/NaI as matrix): m/z $[M+Na]^+$ 1467.8; HR-FABMS: exact mass calcd for $C_{71}H_{115}N_{14}F_3O_{14}Na$ $[M+Na]^+$ 1467.8566, found 1467.8533.

Cyclo[((2S,3R,4R,6E)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl)-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-Nα-Methyl-Nε-([3-(3'-pyridyl)allyloxy-carbonyl]-L-lysyl-L-Alanyl-D-(O-(4-(1-Diaziryl-2,2,2-trifluoroethyl)benoyl))-seryl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-valyl] ; *[D-Ser(Daz)(8),MeLys(Paloc)(6)]Cyclosporine (3.55c)*

A solution of CsA analogue **3.44f** (27 mg, 19.4 μmol), diazirinylbenzoic acid **3.53** (5 mg, 21.7 μmol, 1.1 equiv), and 4-dimethylaminopyridine (0.95 mg, 7.7 μmol, 0.4 equiv) in 0.5 ml of methylene chloride and 0.2 ml of THF was

added with EDCI (8.1 mg, 42.4 μmol, 2.2 equiv) at 0 °C. The reaction mixture was stirred for 10 min and then the ice bath was removed. The solution was stirred for 9 h and concentrated in vacuo. The residue was diluted with CH_2Cl_2 and washed with water. The water layer was extracted with additional methylene chloride (3 x 5 ml). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated in vacuo. Then the residue was purified by flash chromatography (15-50% acetone/n-hexane) to give 22 mg (71%) of the title compound as a foamy solid: TLC R_f 0.49 (55% acetone/hexane); $[\alpha]_D$ -195° (c 0.04, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.53 (s, 1H, H-2 of pyridine), 8.41 (d, J=4.0, 1H, H-6 of pyridine), 8.04(d, J=8.4, 1H, H-N^2), 7.96, 7.20 (2d, J=8.3, 5H, AA'BB' of aromatic H's & H-N^8)), 7.68 (d, J=7.8, 1H, H-N^7), 7.62(d, J=7.4, 1H, H-4 of pyirdine), 7.52 (d, J=7.8, 1H, H-N^5), 7.17-7.13 (m, 1H, H-5 of pyridine), 6.54 (d, J=15.9, 1H, CH=C-C-O), 6.34 (dt, J=15.9, 6.3, 1H, C=CH-C-O), 5.81 (br t, 1H, HN-C(6^6)), 5.68-5.61 (m, 2H, H-C(2^1), H-C(2^9)), 5.33-5.30 (m, 2H, CH=CH-C(5^1)), 5.30-5.25 (m, 2H, H-C(2^4) & H-C(2^8)), 5.15 (d, J=11.0, 1H, H-C(2^{11})), 5.05-4.96 (m, 3H, H-C(2^2), H-C(2^{10})), 4.89 (m, 1H, H-C(2^6)), 4.73 (d, J=14.0, 1H, si-H-C(2^3)), 4.67 (m, 2H, O-CH_2-C=C), 4.60-4.50 (m, 3H, H-C(2^7), H-C(2^5), H-C(3^8)), 4.30 (d, J=6.6, 1H, HO-C(3^1)), 4.27-4.25 (m, 1H, H-C(3^8)), 3.71 (m, 1H, H-C(3^1)), 3.38-3.32, 3.03-2.96 (2m, 2H, 2H-C(6^6)), 3.50 (s, 3H, CH_3-N^1), 3.37(s, 3H, CH_3-N^3), 3.26 (s, 3H, CH_3-N^6), 3.24 (s, 3H, CH_3-N^9), 3.18 (d, J=14.0, 1H, re-H-C(2^3)), 3.11 (s, 3H, CH_3-N^4), 2.70 (s, 3H, CH_3-N^{11}), 2.66 (s, 3H, CH_3-N^{10}),

2.48-1.25 (m, 22H, H-C(4^1), 2H-C(5^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), H-C(3^5), 2H-C(3^6), 2H-C(4^6), 2H-C(5^6), 2H-C(3^9), H-C(4^9), 2H-C(3^{10}), H-C(4^{10}), H-C(3^{11})), 1.61 (d, J=4.8, 3H, CH_3-C(7^1)), 1.33 (d, J=7.3, 3H, CH_3-C(2^7)), 1.10-0.82 (m, 33H, CH_3-C(3^2), $2CH_3$-C(4^4), $2CH_3$-C(3^5), $2CH_3$-C(4^9), $2CH_3$-C(4^{10}), $2CH_3$-C(3^{11})), 0.59 (d, J=5.6, 3H, CH_3-C(4^1)); FABMS (3-NBA matrix): m/z $[M+H]^+$ 1606.9; HR-FABMS exact mass calcd for $C_{80}H_{123}F_3N_{15}O_{16}$ $[M+H]^+$ 1606.9224 found 1606.9234.

[(4S,5R,1'R,3'E)-2,2,3-Trim ethyl-5-(1'-methyl-3'-pentenyl)-4-oxazolidinyl]-carbonyl]-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-leucyl-L-Phenylalanine Benzyl Ester (N,O-Isopropylidene-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Phe-OBzl) (4.13a)

The title compound was synthesized according to the general procedure A in 68% yield and obtained as a foamy solid: TLC R_f 0.60 (50% acetone/hexane); $[\alpha]_D$ -100.8° (c 0.23, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.80, 6.55, 6.28 (3d, J=9.33, 3H, H-N^2, H-N^5, H-N^7), 7.30-6.98 (m, 10H, aromatic H's), 5.40 (m, 2H, CH=CH-C(5^1)), 5.12 (d, J=4.59, 2H, O-CH_2Ph), 5.20-2.80 (m, 11H, H-C(2^1), H-C(3^1), H-C(2^2), 2H-C(2^3), H-C(2^4), H-C(2^5), H-C(2^6), H-C(2^7), 2H-C(3^7)), 3.16, 2.91, 2.72, 2.29 (4s, 12H, CH_3-N^1, CH_3-N^3, CH_3-N^4, CH_3-N^6), 2.45-1.25 (m, 12H, H-C(4^1), 2H-C(5^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), H-C(3^5), 2H-C(3^6), H-C(4^6)), 1.64 (d, J=4.8, 3H, CH_3-C(7^1)), 1.35, 1.20(2s, 6H, $2CH_3$ of isopropylidene), 0.97-0.78 (m,

24H, CH_3-C(4^1), CH_3-C(3^2), $2CH_3$-C(4^4), $2CH_3$-C(3^5), $2CH_3$-C(4^6)); FABMS(3-NBA matrix): m/z $[M+H]^+$ 988.5; HR-FABMS exact mass calcd for $C_{55}H_{86}N_7O_9$ $[M+H]^+$ 988.6487, found 988.6464.

[(4S,5R,1'R,3'E)-2,2,3-Trim ethyl-5-(1'-methyl-3'-pentenyl)-4-oxazolidinyl]-carbonyl]-L-Norvalyl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-leucyl-L-Phenylalanine Benzyl Ester (N,O-Isopropylidene-MeBmt-Nva-Sar-MeLeu-Val-MeLeu-Phe-OBzl) ***(4.13b)***

The title compound was synthesized according to the general procedure A in 49% yield and obtained as a foamy solid: TLC R_f 0.50 (50% acetone/hexane); $[\alpha]_D$ -110.9° (c 0.72, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.90, 6.56, 6.30 (3d, J=9.00, 3H, H-N^2, H-N^5, H-N^7), 7.36-6.99 (m, 10H, aromatic H's), 5.40 (m, 2H, CH=CH-C(5^1)), 5.12 (d, J=4.89, 12.15, 2H, O-CH_2Ph), 5.08-2.80 (m, 9H, H-C(2^1), H-C(3^1), H-C(2^2), H-C(2^4), H-C(2^5), H-C(2^6), H-C(2^7), 2H-C(3^7)), 4.70, 3.70 (2d, J=16.11, 2H, 2H-C(2^3)), 3.16, 2.91, 2.72, 2.2.47 (4s, 12H, CH_3-N^1, CH_3-N^3, CH_3-N^4, CH_3-N^6), 2.35-1.33 (m, 14H, H-C(4^1), 2H-C(5^1), 2H-C(3^2), 2H-C(4^2), 2H-C(3^4), H-C(4^4), H-C(3^5), 2H-C(3^6), H-C(4^6)), 1.64 (d, J=4.65, 3H, CH_3-C(7^1)), 1.34, 1.19(2s, 6H, $2CH_3$ of isopropylidene), 0.95-0.78 (m, 24H, CH_3-C(4^1), CH_3-C(3^2), $2CH_3$-C(4^4), $2CH_3$-C(3^5), $2CH_3$-C(4^6)); FABMS (3-NBA matrix): m/z $[M+H]^+$ 1002.6; HR-FABMS exact mass calcd for $C_{56}H_{88}N_7O_9$ $[M+H]^+$ 1002.6643, found 1002.6638.

[(4S,5R,1'R,3'E)-2,2,3-Trim ethyl-5-(1'-methyl-3'-pentenyl)-4-oxazolidinyl]-carbonyl]-L-2-Aminobutyryl-N-Methyl-D-alanyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-leucyl-L-Phenylalanine Benzyl Ester (N,O-Isopropylidene-MeBmt-Abu-D-MeAla-MeLeu-Val-MeLeu-Phe-OBzl) (4.13c)

The title compound was synthesized according to the general procedure A in 67% yield and obtained as a foamy solid: TLC R_f 0.50 (40% acetone/hexane); $[\alpha]_D$ -51.8° (c 0.23, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.15-6.46 (3d, J=8.7, 3H, H-N^2, H-N^5, H-N^7), 7.32-6.96 (m, 10H, aromatic H), 5.40-5.30 (m, 2H, CH=CH-$C(5^1)$), 5.16 (d, J=4.62, 2H, O-CH_2Ph), 5.52-2.80 (m, 10H, H-$C(2^1)$, H-$C(3^1)$, H-$C(2^2)$, H-$C(2^3)$, H-$C(2^4)$, H-$C(2^5)$, H-$C(2^6)$, H-$C(2^7)$, 2H-$C(3^7)$), 3.03, 2.91, 2.80, 2.22 (4s, 12H, CH_3-N^1, CH_3-N^3, CH_3-N^4, CH_3-N^6), 2.30-1.20 (m, 12H, H-$C(4^1)$, 2H-$C(5^1)$, 2H-$C(3^2)$, 2H-$C(3^4)$, H-$C(4^4)$, H-$C(3^5)$, 2H-$C(3^6)$, H-$C(4^6)$), 1.63 (d, J=4.8, 3H, CH_3-$C(7^1)$), 1.23 (d, J=7.56, 3H, CH_3-$C(2^3)$), 1.35, 1.18 (2s, 6H, $2CH_3$ of isopropylidene), 0.98-0.75 (m, 24H, CH_3-$C(4^1)$, CH_3-$C(3^2)$, $2CH_3$-$C(4^4)$, $2CH_3$-$C(3^5)$, $2CH_3$-$C(4^6)$); FABMS (3-NBA matrix): m/z $[M+H]^+$ 1002.6; HR-FABMS exact mass calcd for $C_{56}H_{88}N_7O_9$ $[M+H]^+$ 1002.6644, found 1002.6654.

(2S, 3R, 4R, 6E)-3-Hydroxy-4-methyl-2-(methylamino)-6-octenoyl-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-leucyl-L-Phenylalanine Benzyl Ester (H-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Phe-OBzl) (4.14a)

The title compound was synthesized according to the general procedure B in 80% yield and obtained as a foamy solid: TLC R_f 0.46 (10% MeOH/CH_2Cl_2); $[\alpha]_D$ -151.3° (c 0.08, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, at least two conformers at room temperature and major one is described) δ 8.20-6.50 (3d, 3H, H-N^2, H-N^5, H-N^7), 7.40-7.20 (m, 10H, aromatic H's), 5.46 (m, 2H, CH=CH-C(5^1)), 5.12 (d, J=4.52, 2H, O-CH_2Ph), 5.20-2.80 (m, 13H, H-N^1, HO-C(3^1), H-C(2^1), H-C(3^1), H-C(2^2), 2H-C(2^3), H-C(2^4), H-C(2^5), H-C(2^6), H-C(2^7), 2H-C(3^7)), 3.11, 2.96, 2.88, 2.46 (4s, 12H, CH_3-N^1, CH_3-N^3, CH_3-N^4, CH_3-N^6), 2.42-1.20 (m, 12H, H-C(4^1), 2H-C(5^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), H-C(3^5), 2H-C(3^6), H-C(4^6)), 1.63 (br s, 3H, CH_3-C(7^1)), 1.10-0.78 (m, 24H, CH_3-C(4^1), CH_3-C(3^2), $2CH_3$-C(4^4), $2CH_3$-C(3^5), $2CH_3$-C(4^6)); FABMS (3-NBA matrix): m/z $[M+H]^+$ 948.5; HR-FABMS exact mass calcd for $C_{52}H_{82}N_7O_9$ $[M+H]^+$ 948.6174, found 948.6164.

(2S, 3R, 4R, 6E)-3-Hydroxy-4-methyl-2-(methylamino)-6-octenoyl-L-Norvalyl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-leucyl-L-Phenylalanine Benzyl Ester (H-MeBmt-Nva-Sar-MeLeu-Val-MeLeu-Phe-OBzl) ***(4.14b)***

The title compound was synthesized according to the general procedure B in 79% yield and obtained as a foamy solid: TLC R_f 0.51 (10% MeOH/CH_2Cl_2); $[\alpha]_D$ -108.1° (c 0.83, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, at least two conformers at room temperature and major one is described) δ 8.67, 6.75, 6.48 (3d, 3H, H-N^2, H-N^5, H-N^7), 7.35-7.01 (m, 10H, aromatic H's), 5.47-5.44 (m, 2H, CH=CH-

C(5^1)), 5.20-2.80 (m, 12H, O-CH_2Ph, H-N^1, HO-C(3^1), H-C(2^1), H-C(3^1), H-C(2^2), 2H-C(2^3), H-C(2^4), H-C(2^5), H-C(2^6), H-C(2^7), 2H-C(3^7)), 4.39, 3.90 (2d, J=15.63, 2H, 2H-C(2^3)), 3.21, 2.96, 2.87, 2.43 (4s, 12H, CH_3-N^1, CH_3-N^3, CH_3-N^4, CH_3-N^6), 2.40-1.26 (m, 14H, H-C(4^1), 2H-C(5^1), 2H-C(3^2), 2H-C(4^2), 2H-C(3^4), H-C(4^4), H-C(3^5), 2H-C(3^6), H-C(4^6)), 1.64 (d, J=4.11, 3H, CH_3-C(7^1)), 1.04-0.80 (m, 24H, CH_3-C(4^1), CH_3-C(3^2), 2CH_3-C(4^4), 2CH_3-C(3^5), 2CH_3-C(4^6)); FABMS (3-NBA matrix): m/z $[M+H]^+$ 962.7; HR-FABMS exact mass calcd for $C_{53}H_{84}N_7O_9$ $[M+H]^+$ 962.6331, found 962.6323.

(2S,3R,4R,6E)-3-Hydroxy-4-methyl-2-(methylamino)-6-octenoyl-L-2-Aminobutyryl-N-Methyl-D-alanyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-leucyl-L-Phenylalanine Benzyl Ester (H-MeBmt-Abu-D-MeAla-MeLeu-Val-MeLeu-Phe-OBzl) ***(4.14c)***

The title compound was synthesized according to the general procedure B in 81% yield and obtained as a foamy solid: TLC R_f 0.5 (10% MeOH/CH_2Cl_2); $[\alpha]_D$ -105.7° (c 0.08, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$ at least two conformers at room temperature and major one is described) δ 8.30-6.84 (3d, J=8.6, 3H, H-N^2, H-N^5, H-N^7), 7.32-7.00 (m, 10H, aromatic H's), 5.42-5.38 (m, 2H, CH=CH-C(5^1)), 5.08 (d, J=4.80, 2H, O-CH_2Ph), 5.30-2.90 (m, 12H, H-N^1, HO-C(3^1), H-C(2^1), H-C(3^1), H-C(2^2), H-C(2^3), H-C(2^4), H-C(2^5), H-C(2^6), H-C(2^7), 2H-C(3^7)), 3.13, 3.00, 2.93, 2.35 (4s, 12H, CH_3-N^1, CH_3-N^3, CH_3-N^4, CH_3-N^6), 2.30-1.20 (m,

12H, H-C(4^1), 2H-C(5^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), H-C(3^5), 2H-C(3^6), H-C(4^6)), 1.63 (d, J=4.41, 3H, CH_3-C(7^1)), 1.36 (d, J=7.17, 3H, CH_3-C(2^3)), 0.94-0.80 (m, 24H, CH_3-C(4^1), CH_3-C(3^2), 2CH_3-C(4^4), 2CH_3-C(3^5), 2CH_3-C(4^6)); FABMS (3-NBA matrix): m/z $[M+H]^+$ 962.7; HR-FABMS exact mass calcd for $C_{53}H_{84}N_7O_9$ $[M+H]^+$ 962.6381, found 962.6363.

N-[[(9-Fluorenylmethyl)oxy]carbonyl]-D-Alanyl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-Valyl-[(2S,3R,4R,6E)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl] -L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-leucyl-L-Phenylalanine Benzyl Ester (Fmoc-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Phe-OBzl) (4.15a)

The pure undecapeptide was synthesized according to the general procedure C in 42% yield and obtained as a foamy solid: TLC R_f 0.47 (50% acetone/hexane); $[\alpha]_D$ -137.8° (c 0.52, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) spectrum available as supplemental material; FABMS (3-NBA matrix): m/z $[M+H]^+$ 1609.0; HR-FABMS exact mass calcd for $C_{90}H_{134}N_{11}O_{15}$ $[M+H]^+$ 1609.0061, found 1609.0050.

N-[[(9-Fluorenylmethyl)oxy]carbonyl]-D-Alanyl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-Valyl-[(2S,3R,4R,6E)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl]-L-Norvalyl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-

N-Methyl-L-leucyl-L-Phenylalanine Benzyl Ester (Fmoc-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Nva-Sar-MeLeu-Val-MeLeu-Phe-OBzl) (4.15b)

The pure undecapeptide was synthesized according to the general procedure C in 52% yield and obtained as a foamy solid: TLC R_f 0.47 (50% acetone/hexane); $[\alpha]_D$ -144.6° (c 0.52, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) spectrum available as supplemental material; FABMS (3-NBA matrix): m/z $[M]^{+\cdot}$ 1622; HR-FABMS exact mass calcd for $C_{90}{}^{13}C_1H_{135}N_{11}O_{15}$ $[M^{+\cdot}\ {}^{13}C]$ 1623.0172, found 1623.0110 .

N-[[(9-Fluorenylmethyl)oxy]carbonyl]-D-Alanyl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-Valyl-[(2S,3R,4R,6E)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl] -L-2-Aminobutyryl-N-Methyl-D-alanyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-leucyl-L-Phenylalanine Benzyl Ester (Fmoc-D-Ala-MeLeu-MeLeu-MeVal-MeBmt-Abu-D-MeAla-MeLeu-Val-MeLeu-Phe-OBzl) (4.15c)

The pure undecapeptide was synthesized according to the general procedure C in 42% yield and obtained as a foamy solid: TLC R_f 0.68 (55% acetone/hexane); $[\alpha]_D$ -124.5° (c 0.8, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) spectrum available as supplemental material; FABMS (3-NBA/Gly + 1% TFA matrix): m/z $[M+H]^+$ 1623; HR-FABMS exact mass calcd for $C_{91}H_{136}N_{11}O_{15}$ $[M+H]^+$ 1623.0217, found 1623.0152.

N-[[(9-Fluorenylmethyl)oxy]carbonyl]-D-Seryl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-Valyl-[(2S,3R,4R,6E)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl] -L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-leucyl-L-Phenylalanine Benzyl Ester (Fmoc-D-Ser-MeLeu-MeLeu-MeVal-MeBmt-Abu-Sar-MeLeu-Val-MeLeu-Phe-OBzl) (4.15d)

The pure undecapeptide was synthesized according to the general procedure C in 20% yield and obtained as a foamy solid: TLC R_f 0.63 (50% acetone/hexane); $[\alpha]_D$ -110.0° (c 0.02, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) spectrum available as supplemental material; FABMS (3-NBA/Gly + 1% TFA matrix): m/z $[M+H]^+$ 1625.1; HR-FABMS exact mass calcd for $C_{90}H_{134}N_{11}O_{16}$ $[M+H]^+$ 1625.0010, found 1625.0067.

N-[[(9-Fluorenylmethyl)oxy]carbonyl]-D-Seryl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-Valyl-[(2S, 3R, 4R, 6E)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl] -L-2-Aminobutyryl-N-Methyl-D-alanyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-leucyl-L-Phenylalanine Benzyl Ester (Fmoc-D-Ser-MeLeu-MeLeu-MeVal-MeBmt-Abu-D-MeAla-MeLeu-Val-MeLeu-Phe-OBzl) (4.15e)

The pure undecapeptide was synthesized according to the general procedure C in 53% yield and obtained as a foamy solid: TLC R_f 0.58 (50% acetone/hexane); $[\alpha]_D$ -70.9° (c 0.11, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$)

spectrum available as supplemental material; FABMS (3-NBA/Gly/TFA as matrix): m/z $[M+H]^+$ 1639.1, HR-FABMS exact mass calcd for $C_{91}H_{136}N_{11}O_{16}$ $[M+H]^+$ 1639.0166, found 1639.0148.

Cyclo[(2S,3R,4R,6E)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl)-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-leucyl-L-Phenylalanyl-D-Alanyl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-valyl] ; [Phe(7)]Cyclosporine (4.16a)

The title compound was synthesized according to the general procedure D in 70% yield and obtained as a foamy solid: TLC R_f 0.41 (45% acetone/hexane); $[\alpha]_D$ -177.8 ° (c 0.03, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.83 (d, J=9.06, 1H, H-N^2), 7.61 (d, J=8.04, 1H, H-N^7), 7.53 (d, J=7.41, 1H, H-N^5), 7.20 (d, J=4.4, 4H, Aromatic H's^7), 7.13 (m, 1H, Aromatic H^7), 7.03 (d, J=7.56, 1H, H-N^8), 5.74 (d, J=6.35, 1H, H-C(2^1)), 5.68 (dd, J=10.80, 4.45, 1H, H-C(2^9)); 5.32-5.30 (m, 2H, CH=CH-C(5^1)), 5.29 (br s, 1H, H-C(2^4)), 5.18 (d, J=11.0, 1H, H-C(2^{11})), 5.12 (m, 1H, H-C(2^{10})), 5.09 (m, 1H, H-C(2^6)), 5.05 (m, 1H, H-C(2^2)), 4.85 (quintet, J=7.45, 1H, H-C(2^8)), 4.73 (d, J=14.0, 1H, si-H-C(2^3)),4.72 (m, 1H, H-C(2^7)), 4,51 (dd, J=10.35, 8.3, 1H, H-C(2^5)), 3.97 (br d, 1H, HO-C(3^1)), 3.80 (m, 1H, H-C(3^1)), 3.55 (s, 3H, CH_3-N^1); 3.47 (dd, J=14.2, 4.65, 1H, H-C(3^7)), 3.38 (s, 3H, CH_3-N^3), 3.22 (d, J=14.0, 1H, re-H-C(2^3)), 3.18 (s, 3H, CH_3-N^6), 3.14 (s, 3H, CH_3-N^9), 3.09 (s, 3H, CH_3-N^4), 2.88-2.81 (m, 1H, H-C(3^7)), 2.76 (s, 3H, CH_3-N^{11}), 2.73 (s, 3H, CH_3-N^{10}), 2.40

(br d, 1H, H-C(5^1)), 2.30 (m, 1H, H-C(3^5)), 2.08-1.10 (m, 17H, H-C(4^1), H-C(5^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), 2H-C(3^6), H-C(4^6), 2H-C(3^9), H-C(4^9), 2H-C(3^{10}), H-C(4^{10}), H-C(3^{11})), 1.63 (d, J=6.0, 3H, CH_3-C(7^1)), 1.24 (d, J=6.9, 3H, CH_3-C(2^8)); 1.04-0.84 (m, 36H, CH_3-C(3^2), $2CH_3$-C(4^4), CH_3-C(3^5), $2CH_3$-C(4^6), $2CH_3$-C(4^9), $2CH_3$-C(4^{10}), $2CH_3$-C(3^{11})), 0.72 (d, J=6.65, CH_3-C(4^1)), 0.60 (d, J=6.85, 3H, CH_3-C(3^5)); FABMS (3-NBA matrix): m/z $[M+H]^+$ 1278.9; HR-FABMS exact mass calcd for $C_{68}H_{116}N_{11}O_{12}$ $[M+H]^+$ 1278.8805, found 1278.8798.

Cyclo[((2S,3R,4R,6E)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl)-L-Norvalyl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-leucyl-L-Phenylalanyl-D-Alanyl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-valyl] ; [Nva(2),Phe(7)]Cyclosporine ***(4.16b)***

The title compound was synthesized according to the general procedure D in 49% yield and obtained as a foamy solid: TLC R_f 0.46 (50% acetone/hexane) ; $[\alpha]_D$ -146.0° (c 0.05 , $CHCl_3$) ; ^1H NMR (500 MHz, $CDCl_3$) δ 7.77 (d, J=9.5, 1H, H-N^2), 7.59 (d, J=7.85, 1H, H-N^7), 7.56 (d, J=8.2, 1H, H-N^5), 7.28-7.11 (m, 5H, Aromatic's-H^7), 7.03 (d, J=7.56, 1H, H-N^8), 5.74 (d, J=5.60, 1H, H-C(2^1)), 5.68 (dd, J=10.3, 4.3, 1H, H-C(2^9)), 5.33 (br s, 2H, CH=CH-C(5^1)), 5.31 (m, 1H, H-C(2^4)), 5.18 (d, J=10.95, 1H, H-C(2^{11})), 5.16-5.10 (m, 2H, H-C(2^{10}), H-C(2^2)), 5.07 (m, 1H, H-C(2^6)), 4.85 (quintet, J=7.05, 1H, H-C(2^8)), 4.73-4.70 (m, 2H, si-H-C(2^3), H-C(2^7)); 4,50 (t, J=8.4, 1H, H-C(2^5)), 4.07 (br d, 1H, HO-C(3^1)), 3.80 (br s, 1H, H-C(3^1)), 3.55,

3.38, 3.16, 3.12, 3.09, 2.75, 2.72 (7s, 21H, CH_3-N^1, CH_3-N^3, CH_3-N^4 , CH_3-N^6, CH_3-N^9, CH_3-N^{10}, CH_3-N^{11}), 3.48-3.42 (m, 1H, H-C(3^7)), 3.22 (d, J=13.9, 1H, re-H-C(2^3)), 3.00-2.80 (m, 1H, H-C(3^7)), 2.40-1.20 (m, 21H, 2H-C(5^1), H-C(4^1), 2H-C(3^2), 2H-C(4^2), 2H-C(3^4), H-C(4^4), H-C(3^5), 2H-C(3^6), H-C(4^6), 2H-C(3^9), H-C(4^9), 2H-C(3^{10}), H-C(4^{10}), H-C(3^{11})), 1.62 (d, J=5.7, 3H, CH_3-C(7^1)), 1.22 (d, J=6.4, 3H, CH_3-C(2^8)), 1.02-0.84 (m, 36H, CH_3-C(4^2), 2CH_3-C(4^4), CH_3-C(3^5), 2CH_3-C(4^6), 2CH_3-C(4^9), 2CH_3-C(4^{10}), 2CH_3-C(3^{11})), 0.70 (d, J=6.33, CH_3-C(4^1)), 0.60 (d, J=6.60, 3H, CH_3-C(3^5)); FABMS (3-NBA matrix): m/z $[M+H]^+$ 1293.0; HR-FABMS exact mass calcd for $C_{69}H_{118}N_{11}O_{12}$ $[M+H]^+$ 1292.8961, found 1292.8958 .

Cyclo[((2S,3R,4R,6E)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl)-L-2-Aminobutyryl-N-Methyl-D-alanyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-leucyl-L-Phenylalanyl-D-Alanyl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-valyl] ; *[D-MeAla(3),Phe(7)]Cyclosporine (4.16c)*

The title compound was synthesized according to the general procedure D in 30% yield and obtained as a foamy solid: TLC R_f 0.62 (60% acetone/hexane) ; $[\alpha]_D$ -216° (c 0.05, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.82 (d, J=9.66, 1H, H-N^2); 7.62 (d, J=7.92, 1H, H-N^7), 7.55 (d, J=7.98, 1H, H-N^5), 7.20 (d, J=4.3, 4H, Aromatic H's^7), 7.13 (m, 1H, Aromatic H^7), 7.03 (d, J=7.74, 1H, H-N^8), 5.76 (d, J=6.12, 1H, H-C(2^1)), 5.68 (dd, J=10.50, 4.25, 1H, H-C(2^9)), 5.33-5.29 (m, 2H, CH=CH-C(5^1)), 5.25 (dd, J=9.85, 4.20, 1H, H-C(2^4)), 5.17 (d, J=11.04, 1H, H-C(2^{11})),

5.11 (m, 1H, H-C(2^{10})), 5.08-5.02 (m, 2H, H-C(2^6), H-C(2^2)), 4.94 (q, J=7.29, 1H, H-C(2^3)), 4.85 (quintet, J=7.2, 1H, H-C(2^8)), 4.73 (m, 1H, H-C(2^7)), 4,48(dd, J=10.29, 8.16, 1H, H-C(2^5)), 4.11 (br s, 1H, HO-C(3^1)), 3.76 (m, 1H, H-C(3^1)), 3.55 (s, 3H, CH_3-N^1), 3.48 (dd, J=14.43, 4.44, 1H, H-C(3^7)), 3.24(s, 3H, CH_3-N^3), 3.17 (s, 3H, CH_3-N^6), 3.13 (s, 3H, CH_3-N^9), 3.07 (s, 3H, CH_3-N^4), 2.88-2.84 (dd, J=14.43, 8.94, 1H, H-C(3^7)), 2.74 (s, 3H, CH_3-N^{11}), 2.70 (s, 3H, CH_3-N^{10}), 2.41 (br d, 1H, H-C(5^1)), 2.33 (m, 1H, H-C(3^5)), 2.22-1.12 (m, 18H, H-C(4^1), H-C(5^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), 2H-C(3^6), H-C(4^6), 2H-C(3^9), H-C(4^9), 2H-C(3^{10}), H-C(4^{10}), H-C(3^{11})), 1.61 (d, J=3.63, 3H, CH_3-C(7^1)), 1.40 (d, J=7.20, 3H, CH_3-C(2^3)), 1.23 (d, J=7.05, 3H, CH_3-C(2^8)), 1.15-0.82 (m, 36H, CH_3-C(3^2), $2CH_3$-C(4^4), CH_3-C(3^5), $2CH_3$-C(4^6), $2CH_3$-C(4^9), $2CH_3$-C(4^{10}), $2CH_3$-C(3^{11})), 0.70 (d, J=6.27, CH_3-C(4^1)), 0.60 (d, J=6.69, 3H, CH_3-C(3^5)); FABMS (3-NBA matrix): m/z $[M+H]^+$ 1292.9; HR-FABMS exact mass calcd for $C_{69}H_{118}N_{11}O_{12}$ $[M+H]^+$ 1292.8961, found 1292.8936.

Cyclo[((2S,3R,4R,6E)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl)-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-leucyl-L-Phenylalanyl-D-Seryl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-valyl] ; [D-Ser(8),Phe(7)]Cyclosporine (4.16d)

The title compound was synthesized according to the general procedure D in 38% yield and obtained as a foamy solid: TLC R_f 0.39 (50% acetone/hexane); R_f 0.43 (10% MeOH/CH_2Cl_2); $[\alpha]_D$ -240° (c 0.02 , $CHCl_3$) ; ^1H NMR (500 MHz,

$CDCl_3$) δ 7.98 (d, J=9.5, 1H, H-N^2), 7.88 (d, J=7.7, 1H, H-N^7), 7.51 (d, J=9.3, 1H, H-N^5), 7.47 (d, J=7.8, 1H, H-N^8), 7.23-7.15 (m, 5H, Aromatic's H), 5.70-5.68 (m, 1H, H-C(2^9)), 5.66 (d, J=6.5, 1H, H-C(2^1)), 5.33-5.30 (br s, 2H, CH=CH-C(5^1)), 5.17 (d, J=11.0, 1H, H-C(2^{11})), 5.13-5.16 (m, 1H, H-C(2^4)), 5.08-5.01 (m, 3H, H-C(2^{10}) & H-C(2^2) & H-C(2^6)), 4.87 (m, 1H, H-C(2^8)), 4.73 (d, J=14.0, 1H, si-H-C(2^3)), 4.72-4.70 (m, 1H, H-C(2^7)), 4.55 (t, J=8.7, 1H, H-C(2^5)), 3.88 (q, J=6.3, 1H, H-C(3^1)), 3.76-3.72 (br d, 1H, 2H-C(3^8)), 3.55 (s, 3H, CH_3-N^1), 3.51 (br s, 1H, HO-C(3^1)), 3.46-3.40, 2.90-2.82 (2m, 2H, 2H-C(3^7), 3.45 (s, 3H, CH_3-N^3), 3.27 (s, 3H, CH_3-N^6), 3.21 (d, J=14.1, 1H, re-H-C(2^3)), 3.19 (s, 3H, CH_3-N^9), 3.09 (s, 3H, CH_3-N^4); 2.95 (br s, 1H, HO-C(3^8)), 2.75 (s, 3H, CH_3-N^{11}), 2.72 (s, 3H, CH_3-N^{10}), 2.40-1.20 (m, 19H, H-C(4^1), 2H-C(5^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), H-C(3^5), 2H-C(3^6), H-C(4^6), 2H-C(3^9), H-C(4^9), 2H-C(3^{10}), H-C(4^{10}), H-C(3^{11})), 1.62 (d, J=4.65, 3H, CH_3-C(7^1)), 1.04-0.76 (m, 39H, CH_3-C(3^2), 2CH_3-C(4^4), 2CH_3-C(3^5), 2CH_3-C(4^6), 2CH_3-C(4^9), 2CH_3-C(4^{10}), 2CH_3-C(3^{11})), 0.54 (d, J=6.7, 3H, CH_3-C(4^1)). FABMS (3-NBA matrix) : m/z $[M+H]^+$ 1294.7; HR-FABMS: exact mass calcd for $C_{68}H_{116}N_{11}O_{13}$ $[M+H]^+$ 1294.8754, found 1294.8767.

Cyclo[((2S,3R,4R,6E)-3-hydroxy-4-methyl-2-(methylamino)-6-octenoyl)-2-Aminobtyryl-N-Methyl-D-alanyl-Methyl-L-leucyl-L-Valyl-N-Methyl-L-leucyl-L-Phenylalanyl-D-Seryl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-valyl] ; *[D-MeAla(3),Phe(7),D-Ser(8)]Cyclosporine* ***(4.16e)***

The title compound was synthesized according to the general procedure D in 31% yield and obtained as a foamy solid: TLC R_f 0.38 (40% acetone/hexane); $[\alpha]_D$ -180.0 ° (c 0.04 , $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 7.96 (d, J=9.7, 1H, H-N^2), 7.88 (d, J=7.6, 1H, H-N^7), 7.52 (d, J=8.4, 1H, H-N^5), 7.46 (d, J=7.2, 1H, H-N^8), 7.23-7.12 (m, 5H, Aromatic H's), 5.73 (d, J=6.8, 1H, H-C(2^1)), 5.68 (dd, J=6.5, 4.4, 1H, H-C(2^9)), 5.32-5.26 (m, 3H, CH=CH-C(5^1) & H-C(2^4)), 5.16 (d, J=11, 1H, H-C(2^{11})), 5.08 (m, 1H, H-C(2^{10})), 5.06-5.03 (m, 2H, H-C(2^2) & H-C(2^3)), 4.94 (q, J=7.4, 1H, H-C(2^3)), 4.86 (m, 1H, H-C(2^8)), 4.74 (m, 1H, H-C(2^7)), 4.51 (m, 1H, H-C(2^5)), 3.82 (m, 1H, H-C(3^1)), 3.73 (br s, 2H, 2H-C(3^8)), 3.58 (br d, 1H, HO-C(3^1)), 3.52-3.48 & 2.89-2.48 (2m, 2H, 2H-C(3^7)), 3.56 (s, 3H, CH_3-N^1), 3.27 (s, 3H, CH_3-N^3), 3.26 (s, 3H, CH_3-N^6), 3.18 (s, 3H, CH_3-N^9), 3.07 (s, 3H, CH_3-N^4), 2.98 (br s, 1H, HO-C(3^8)), 2.75 (s, 3H, CH_3-N^{11}), 2.71 (s, 3H, CH_3-N^{10}), 2.48-1.15 (m, 19H, H-C(4^1), 2H-C(5^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), H-C(3^5), 2H-C(3^6), H-C(4^6), 2H-C(3^9), H-C(4^9), 2H-C(3^{10}), H-C(4^{10}), H-C(3^{11})), 1.62 (d, J=3.8, 3H, CH_3-C(7^1)), 1.42 (d, J=7.3, 3H, CH_3-C(2^3)), 1.04-0.74 (m, 39H, CH_3-C(3^2), 2CH_3-C(4^4), 2CH_3-C(3^5), 2CH_3-C(4^6), 2CH_3-C(4^9), 2CH_3-C(4^{10}), 2CH_3-C(3^{11})), 0.54 (d, J=6.9, 3H, CH_3-C(4^1)); FABMS (Gly/3-NBA/TFA matrix): m/z $[M+H]^+$ 1309.2; HR-FABMS: exact mass calcd for $C_{69}H_{118}N_{11}O_{13}$ $[M+H]^+$ 1308.8910, found 1308.8857.

[(4S,5R)-2,2,3-Trimethyl-5-isopropyl-4-(oxazolidinyl)-carbonyl]-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-leucyl-L-

Alanine Benzyl Ester (N,O-Isopropylidene-Me(3-OH)Leu-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl) (5.18a)

The title compound was synthesized according to the general procedure A in 79% yield and obtained as a foamy solid: TLC R_f 0.36 (40% acetone/hexane); $[\alpha]_D$ -119.6° (c 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.00-6.42 (3d, J=9.2, 3H, H-N^2, H-N^5, H-N^7), 7.33 (br s, 5H, aromatic H's), 5.12 (m, 2H, O-CH_2Ph), 5.20-2.80 (m, 9H, H-C(2^1), H-C(3^1), H-C(2^2), 2H-C(2^3), H-C(2^4), H-C(2^5), H-C(2^6), H-C(2^7)), 3.18, 3.02, 2.92, 2.30 (4s, 12H, CH_3-N^1, CH_3-N^3, CH_3-N^4, CH_3-N^6), 2.36-1.24 (m, 10H, H-C(4^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), H-C(3^5), 2H-C(3^6), H-C(4^6)), 1.37 (d, J=7.2, CH_3-C(2^7)), 1.35, 1.18 (2s, 6H, $2CH_3$ of isopropylidene), 1.00-0.83 (m, 27H, $2CH_3$-C(4^1), CH_3-C(3^2), $2CH_3$-C(4^4), $2CH_3$-C(3^5), $2CH_3$-C(4^6)); FABMS (3-NBA matrix) m/z $[M+H]^+$ 872; HR-FABMS exact mass calcd for $C_{46}H_{78}N_7O_9$ $[M+H]^+$ 872.5861, found 872.5863.

[(4S,5R)-2,2,3-Trimethyl-5-isopropyl-4-(oxazolidinyl)-carbonyl]-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-alanyl-L-Alanine Benzyl Ester (N,O-Isopropylidene-Me(3-OH)Leu-Abu-Sar-MeLeu-Val-MeAla-Ala-OBzl) (5.18b)

The title compound was synthesized according to the general procedure B in 89% yield and obtained as a foamy solid: TLC R_f 0.33 (40% acetone/hexane); $[\alpha]_D$ -112.9° (c 0.45, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ

7.82 (d, 1H), 6.64 (d, 1H), 6.52 (d, 1H), 7.34 (br s, 5H), 5.20-3.40 (m, 11H, O-CH_2-Ph, H-C(2^1), H-C(3^1), H-C(2^2), 2H-C(2^3), H-C(2^4), H-C(2^5), H-C(2^6), H-C(2^7)), 3.18, 3.08, 2.87, 2.36 (4s, 12H, 4 CH_3-N), 2.10-1.40 (m, 7H, H-C(4^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), H-C(3^5)), 1.40-1.15 (m, 12H, CH_3-C(2^6), CH_3-C(2^7), $2CH_3$ of isopropylidene), 1.05-0.83 (m, 21H, $2CH_3$-C(4^1), CH_3-C(3^2), $2CH_3$-C(4^4), $2CH_3$-C(3^5)), FABMS (3-NBA matrix): m/z $[M+H]^+$ 830.5; HR-FABMS: exact mass calcd for $C_{43}H_{72}N_7O_9$ $[M+H]^+$ 830.5391, found 830.5386.

[(4S,5R)-2,2,3-Trimethyl-5-isopropyl-4-(oxazolidinyl)-carbonyl]-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-alanyl-L-Valyl-N-Methyl-L-alanyl-L-Alanine Benzyl Ester (N,O-Isopropylidene-Me(3-OH)Leu-Abu-Sar-MeAla-Val-MeAla-Ala-OBzl) (5.18c)

The title compound was synthesized according to the general procedure B in 55% yield and obtained as a foamy solid: TLC R_f 0.59 (70% acetone/hexane); $[\alpha]_D$ -72.5° (c 0.45, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, at least two conformers at room temperature and major one is described) δ 7.82, 6.64, 6.52 (3d, 4H, H-N^2, H-N^5, H-N^7), 7.34 (br s, 5H, aromatic H's), 5.20-3.40 (m, 9H, OCH_2Ph, H-C(2^1), H-C(3^1), H-C(2^2), 2H-C(3^2), H-C(2^4), H-C(2^5)), 3.18, 3.08, 2.87, 2.36 (4s, 12H, CH_3-N^1, CH_3-N^3, CH_3-N^4, CH_3-N^6), 2.10-1.20 (m, 13H, $2CH_3$ of isopropylidene, H-C(4^1), 2H-C(3^2), 2H-C(3^4), H-C(3^5), H-C(4^4)), 1.05-0.83 (m, 21H, $2CH_3$-C(4^1), CH_3-C(3^2), $2CH_3$-C(4^4), $2CH_3$-C(3^5)).

(2S,3R)-3-Hydroxy-N-methyl-leucyl-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-leucyl-L-Alanine Benzyl Ester (H-Me(3-OH)Leu-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl) (5.19a)

The title compound was synthesized according to the general procedure B in 92% yield and obtained as a foamy glass: TLC R_f 0.46 (10% MeOH/CH_2Cl_2); $[\alpha]_D$ -135.1° (c 2.2, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, at least two conformers at room temperature and major one is described) δ 8.10-6.70 (3d, J=9.2, 3H, H-N^2, H-N^5, H-N^7), 7.34 (br s, 5H, aromatic H's), 5.15 (m, 2H, O-CH_2Ph), 5.28-2.80 (m, 11H, H-N^1, HO-C(3^1), H-C(2^1), H-C(3^1), H-C(2^2), 2H-C(2^3), H-C(2^4), H-C(2^5), H-C(2^6), H-C(2^7)), 3.35, 3.08, 2.98, 2.42 (4s, 12H, CH_3-N^1, CH_3-N^3, CH_3-N^4, CH_3-N^6), 2.40-1.40 (m, 10H, H-C(4^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), H-C(3^5), 2H-C(3^6), H-C(4^6)), 1.37 (d, J=7.3, CH_3-C(2^7)), 1.08-0.80 (m, 27H, 2CH_3-C(4^1), CH_3-C(3^2), 2CH_3-C(4^4), 2CH_3-C(3^5), 2CH_3-C(4^6)); FABMS (3-NBA matrix) m/z $[M+H]^+$ 872; HR-FABMS exact mass calcd for $C_{46}H_{78}N_7O_9$ $[M+H]^+$ 872.5861, found 872.5863. ; FABMS (GSI/Gly matrix) m/z $[M+H]^+$ 832.5; HR-FABMS exact mass calcd for $C_{43}H_{74}N_7O_9$ $[M+H]^+$ 832.5548, found 832.5563.

(2S,3R)-3-Hydroxy-N-methyl-leucyl-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-alanyl-L-Alanine Benzyl Ester (H-Me(3-OH)Leu-Abu-Sar-MeLeu-Val-MeAla-Ala-OBzl) (5.19b)

The title compound was synthesized according to the general procedure B

in 92% yield and obtained as a foamy solid: TLC R_f 0.46 (10% MeOH/CH_2Cl_2); $[\alpha]_D$ -124.5° (c 0.22, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, at least two conformers at room temperature and major one is described) δ 8.18, 8.02, 7.60 (3d, 3H, 3 H-N), 5.28-3.51 (m, 11H, O-CH_2-Ph, H-C(2^1), H-C(3^1), H-C(2^2), 2H-C(2^3), H-C(2^4), H-C(2^5), H-C(2^6), H-C(2^7)), 3.34, 3.03, 2.88, 2.40 (4s, 12H, 4 CH_3-N), 2.18-1.35 (m, 7H, H-C(4^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), H-C(3^5)), 1.35-1.25 (m, 6H, CH_3-C(2^6), CH_3-C(2^7)), 1.05-0.83 (m, 21H, 2CH_3-C(4^1), CH_3-C(3^2), 2CH_3-C(4^4), 2CH_3-C(3^5)), FABMS (3-NBA matrix): m/z $[M+H]^+$ 790.5 ; HR-FABMS : exact mass calcd for $C_{40}H_{68}N_7O_9$ $[M+H]^+$ 790.5078, found 790.5062.

(2S,3R)-3-Hydroxy-N-methyl-leucyl-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-alanyl-L-Valyl-N-Methyl-L-alanyl-L-Alanine Benzyl Ester (H-Me(3-OH)Leu-Abu-Sar-MeAla-Val-MeAla-Ala-OBzl) (5.19c)

The title compound was synthesized according to the general procedure B in 62% yield and obtained as a foamy glass: TLC R_f 0.36 (10% MeOH/CH_2Cl_2); $[\alpha]_D$ -85° (c 0.67, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$, at least two conformers at room temperature and major one is described) δ 8.18, 8.02, 7.60 (3d, 3H, H-N^2, H-N^5, H-N^7), 7.34 (br s, 5H, aromatic H's), 5.28-3.51 (m, 9H, OCH_2Ph, H-C(2^1), H-C(3^1), H-C(2^2), 2H-C(3^2), H-C(2^4), H-C(2^5)), 3.34, 3.03, 2.88, 2.40 (4s, 12H, CH_3-N^1, CH_3-N^3, CH_3-N^4, CH_3-N^6), 2.18-1.26 (m, 7H, H-C(4^1), 2H-C(3^2), 2H-C(3^4), H-C(3^5), H-C(4^4)), 1.05-0.80 (m, 21H, 2CH_3-C(4^1), CH_3-C(3^2), 2CH_3-C(4^4), 2CH_3-

C(3^5)); FABMS (3-NBA/Gly/TFA matrix): m/z $[M+H]^+$ 748.4; HR-FABMS: exact mass calcd for $C_{37}H_{62}N_7O_9$ $[M+H]^+$ 748.4609, found 748.4606.

[[(9-Fluorenylmethyl)oxy]carbonyl]-D-Alanyl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-Valyl-[(2S,3R)-3-hydroxy-N-methyl-leucyl]-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-leucyl-L-Alanine Benzyl Ester (Fmoc-D-Ala-MeLeu-MeLeu-MeVal-Me(3-OH)Leu-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl) (5.20a)

The pure undecapeptide was synthesized according to the general procedure C in 62% yield and obtained as a foamy solid: TLC R_f 0.53 (50% acetone/hexane); $[\alpha]_D$ -102.1° (c 0.73, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) spectrum available as supplemental material; FABMS (3-NBA matrix): m/z $[M+H]^+$ 1492.9; HR-FABMS: exact mass calcd for $C_{81}H_{126}N_{11}O_{15}$ $[M+H]^+$ 1492.9434, found 1492.9383.

[[(9-Fluorenylmethyl)oxy]carbonyl]-D-Alanyl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-Valyl-[(2S,3R)-3-hydroxy-N-methyl-leucyl]-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-alanyl-L-Alanine Benzyl Ester (Fmoc-D-Ala-MeLeu-MeLeu-MeVal-Me(3-OH)Leu-Abu-Sar-MeLeu-Val-MeAla-Ala-OBzl) (5.20b)

The pure undecapeptide was synthesized according to the general

procedure C in 56% yield and obtained as a foamy solid: TLC R_f 0.49 (50% acetone/hexane); $[\alpha]_D$ -122.0° (c 0.19, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) spectrum available as supplemental material; FABMS (3-NBA matrix): m/z $[M+H]^+$ 1450.9; HR-FABMS: exact mass calcd for $C_{78}H_{120}N_{11}O_{15}$ $[M+H]^+$ 1450.8965, found 1450.8903.

[[(9-Fluorenylmethyl)oxy]carbonyl]-D-Alanyl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-Valyl-[(2S,3R)-3-hydroxy-N-methyl-leucyl]-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-alanyl-L-Valyl-N-Methyl-L-alanyl-L-Alanine Benzyl Ester (Fmoc-D-Ala-MeLeu-MeLeu-MeVal-Me(3-OH)Leu-Abu-Sar-MeAla-Val-MeAla-Ala-OBzl) (5.20c)

The pure undecapeptide was synthesized according to the general procedure C in 34% yield and obtained as a foamy solid: TLC R_f 0.67 (60% acetone/hexane); $[\alpha]_D$ -146.7° (c 0.015, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) spectrum available as supplemental material; FABMS (3-NBA/Gly/TFA as matrix): m/z $[M+H]^+$ 1408.6; HR-FABMS: exact mass calcd for $C_{75}H_{114}N_{11}O_{15}$ $[M+H]^+$ 1408.8496, found 1408.8435.

N^{α}-[[(9-Fluorenylmethyl)oxy]carbonyl]-N^{ε}-(tert-butyloxycarbonyl)-D-Lysyl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-Valyl-[(2S, 3R)-3-hydroxy-N-methyl-leucyl]-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-

Methyl-L-leucyl-L-Alanine Benzyl Ester (Fmoc-D-Lys(BOC)-MeLeu-MeLeu-MeVal-Me(3-OH)Leu-Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl) (5.20d)

The pure undecapeptide was synthesized according to the general procedure C in 63% yield and obtained as a foamy solid: TLC R_f 0.31 (50% acetone/hexane); $[\alpha]_D$ -133.1° (c 1.9, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) spectrum available as supplemental material; FABMS (DTT/DTE matrix): m/z $[M+H]^+$ 1650; HR-FABMS exact mass calcd for $C_{89}H_{141}N_{12}O_{17}[M+H]^+$ 1650.0538, found 1650.0508.

Cyclo[(2S,3R)-3-hydroxy-N-methyl-leucyl-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-leucyl-L-Alanyl-D-Alanyl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-valyl] ; [Me(3-OH)Leu$^{(1)}$]Cyclosporine (5.21a)

The title compound was synthesized according to the general procedure D in 41% yield and obtained as a foamy solid: TLC R_f 0.49 (50% acetone/hexane); $[\alpha]_D$ -200.0° (c 0.04, $CHCl_3$) ; ^1H NMR (500 MHz, $CDCl_3$) δ 8.29 (d, J=9.66, 1H, H-N^2), 7.86 (d, J=7.02, 1H, H-N^7), 7.61 (d, J=8.25, 1H, H-N^5), 7.33 (d, J=7.77, 1H, H-N^8), 5.68 (m, 1H, H-C(2^9)), 5.33 (m, 1H, H-C(2^4)), 5.26 (d, J=8.25, 1H, H-C(2^1)), 5.09 (m, 1H, H-C(2^{10})), 5.08 (d, J=10.9, 1H, H-C(2^{11})), 5.06 (br s, 1H, H-C(2^6)), 4.97 (q, J=7.68, 1H, H-C(2^2)), 4.84 (quintet, J=7.35, 1H, H-C(2^8)), 4.70 (d, J=13.95, 1H, si-H-C(2^3)), 4.64 (t, J=9.39, 1H, H-C(2^5)), 4.47 (quintet, J=7.02, 1H, H-C(2^7)); 3.87 (m,

1H, H-C(3^1)), 3.49 (s, 3H, CH_3-N^1), 3.42 (s, 3H, CH_3-N^3), 3.28 (s, 3H, CH_3-N^6), 3.17 (d, J=13.95, 1H, re-H-C(2^3)), 3.15 (s, 3H, CH_3-N^9), 3.09 (s, 3H, CH_3-N^4), 2.68 (s, 3H, CH_3-N^{11}), 2.67 (s, 3H, CH_3-N^{10}), 2.44 (m, 1H, H-C(3^5)); 2.17-1.16 (m, 16H, H-C(4^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), 2H-C(3^6), H-C(4^6), 2H-C(3^9), H-C(4^9), 2H-C(3^{10}), H-C(4^{10}), H-C(3^{11})), 1.33 (d, J=7.14, 3H, CH_3-C(2^7)); 1.25 (d, J=4.4, 3H, CH_3-C(2^8)), 1.06-0.82 (m, 39H, CH_3-C(4^1), CH_3-C(3^2), $2CH_3$-C(4^4), $2CH_3$-C(3^5), $2CH_3$-C(4^6), $2CH_3$-C(4^9), $2CH_3$-C(4^{10}), CH_3-C(3^{11})), 0.76 (d, J=6.54, 3H, CH_3-C(3^{11})), 0.72 (d,J=6.51, 3H, CH_3-C(4^1)); FABMS (3-NBA matrix): m/z $[M+H]^+$ 1162.8; HR-FABMS: exact mass calcd for $C_{59}H_{108}N_{11}O_{12}$ $[M+H]^+$ 1162.8179, found 1162.8169.

Cyclo[(2S,3R)-3-hydroxy-N-methyl-leucyl-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-alanyl-L-Alanyl-D-Alanyl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-valyl] ;

[Me(3-OH)Leu(1),MeAla(6)]Cyclosporine (5.21b)

The title compound was synthesized according to the general procedure D in 56% yield and obtained as a foamy solid: TLC R_f 0.34 (40% acetone/hexane); $[\alpha]_D$ -215.0° (c 0.2, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.42 (d, J=9.8, 1H, H-N^2), 8.13 (d, J=8.3, 1H, H-N^7), 7.35 (d, J=8.5, 1H, H-N^5), 7.05 (d, J=8.1, 1H, H-N^8), 5.63 (m, 1H, H-C(2^9)), 5.57 (d, J=4.8, 1H, H-C(2^1)), 5.31 (m, 1H, H-C(2^4)), 5.08 (m, 1H, H-C(2^6)), 5.07 (d, J=11.0, 1H, H-C(2^{11})), 5.04 (m, 1H, H-C(2^{10})), 4.97 (q, J=7.8,

1H, H-C(2^2)), 4.81 (m, 1H, H-C(2^8)), 4.73 (d, J=15.9, 1H, si-H-C(2^3)), 4.72 (m, 1H, H-C(2^5)), 4.67 (d, J=6.0, 1H, HO-C(3^1)), 4.62 (m, 1H, H-C(2^7)), 3.56 (s, 3H, CH_3-N^1), 3.44 (br m, 1H, H-C(3^1)), 3.38 (s, 3H, CH_3-N^3), 3.32 (s, 3H, CH_3-N^6), 3.18 (d, J=15.9, 1H, re-H-C(2^3)), 3.13 (s, 3H, CH_3-N^9), 3.07 (s, 3H, CH_3-N^4), 2.70 (s, 3H, CH_3-N^{11}), 2.69 (s, 3H, CH_3-N^{10}), 2.40-1.25 (m, 14H, H-C(4^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), H-C(3^5), 2H-C(3^9), H-C(4^9), 2H-C(3^{10}), H-C(4^{10}), H-C(3^{11})), 1.46 (d, J=6.0, 3H, CH_3-C(2^6)), 1.31 (d, J=7.2, 3H, CH_3-C(2^7)), 1.26 (d, J=5.4, 3H, CH_3-C(2^8)), 1.15-0.83 (m, 36H, CH_3-C(4^1), CH_3-C(3^2), $2CH_3$-C(4^4), $2CH_3$-C(3^5), $2CH_3$-C(4^9), $2CH_3$-C(4^{10}), $2CH_3$-C(3^{11})), 0.50 (d, J=6.5, 3H, CH_3-C(4^1)); FABMS (3-NBA matrix): m/z $[M+H]^+$ 1120.8; HR-FABMS: exact mass calcd for $C_{56}H_{102}N_{11}O_{12}$ $[M+H]^+$ 1120.7709, found 1120.7688.

Cyclo[(2S,3R)-3-hydroxy-N-methyl-leucyl-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-Alanyl-L-Valyl-N-Methyl-L-Alanyl-L-Alanyl-D-Alanyl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-valyl] ; [Me(3-OH)Leu$^{(1)}$,MeAla$^{(4)}$, MeAla$^{(6)}$]Cyclosporine (5.21c)

The title compound was synthesized according to the general procedure D in 69% yield and obtained as a foamy solid: TLC R_f 0.53 (60% acetone/hexane); $[\alpha]_D$ -247.5° (c, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.41 (d, J=10.2, 1H, H-N^2), 8.12 (d, J=8.7, 1H, H-N^7), 7.30 (d, J=8.7, 1H, H-N^5), 7.06 (d, J=8.2, 1H, H-N^8), 5.62 (dd, J=4.4, 9.7, 1H, H-C(2^9)), 5.57 (d, J=4.8, 1H, H-C(2^1)), 5.31 (q, J=7.1, 1H, H-

C(2^4)), 5.10-5.06 (m, 1H, H-C(2^6)), 5.07 (d, J=10.7, 1H, H-C(2^{11})), 5.06-5.01 (m, 1H, H-C(2^{10})), 4.97 (q, J=9.0, 1H, H-C(2^2)), 4.81 (quintet, J=7.3, 1H, H-C(2^8)), 4.76-4.73 (m, 1H, H-C(2^5)), 4.72 (d, J=13.6, 1H, si-H-C(2^3)), 4.62 (quintet, J=7.9, 1H, H-C(2^7)), 4.56 (d, J=7.1 1H, HO-C(3^1)), 3.87 (m, 1H, H-C(3^1)), 3.55 (s, 3H, CH_3-N^1), 3.37 (s, 3H, CH_3-N^3), 3.31 (s, 3H, CH_3-N^6), 3.14 (d, J=13.6, 1H, re-H-C(2^3)), 3.16 (s, 3H, CH_3-N^9), 3.07 (s, 3H, CH_3-N^4), 2.70 (s, 3H, CH_3-N^{11}), 2.69 (s, 3H, CH_3-N^{10}), 2.38-1.25 (m, 11H, H-C(4^1), 2H-C(3^2), H-C(3^5), 2H-C(3^9), H-C(4^9), 2H-C(3^{10}), H-C(4^{10}), H-C(3^{11})), 1.46 (d, J=7.2, 3H, CH_3-C(2^6)), 1.41 (d, J=7.3, 3H, CH_3-C(2^4)), 1.31 (d, J=7.2, 3H, CH_3-C(2^7)), 1.25 (d, J=6.2, 3H, CH_3-C(2^8)), 1.08-0.82 (m, 30H, CH_3-C(4^1), CH_3-C(3^2), 2CH_3-C(3^5), 2CH_3-C(4^9), 2CH_3-C(4^{10}), 2CH_3-C(3^{11})), 0.72 (d, J=6.51, 3H, CH_3-C(4^1)); FABMS (3-NBA/Gly/TFA matrix): m/z $[M+H]^+$ 1078.6; HR-FABMS exact mass calcd for $C_{53}H_{96}N_{11}O_{12}$ $[M+H]^+$ 1078.7240, found 1078.7205.

Cyclo[(2S,3R)-3-hydroxy-N-methyl-leucyl-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-leucyl-L-Alanyl-N$^{\varepsilon}$-(tert-butyloxycarbonyl)-D-lysyl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-L-valyl] ; *[D-Lys(BOC)$^{(8)}$,Me(3-OH)Leu$^{(1)}$]Cyclosporine (5.21d)*

The title compound was synthesized according to general procedure D in 74% yield and obtained as a white solid: TLC R_f 0.48 (50% acetone/hexane); $[\alpha]_D$ -182.5° (c 0.04, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 8.28 (d, J=9.60, 1H, H-

N^2), 7.90 (d, J=7.05 1H, H-N^7), 7.62 (d, J=8.55, 1H, H-N^5), 7.34 (br s, 1H, H-Nε-^{8}), 7.22 (d, J=8.30, 1H, H-N^8), 5.69 (dd, J=10.85, 4.45, 1H, H-C(2^9)), 5.33 (dd, J=11.95, 3.9, 1H, H-C(2^4)), 5.27 (d, J=8.45, 1H, H-C(2^1)), 5.12 (m, 1H, H-C(2^6)), 5.09 (d, J=10.9, 1H, H-C(2^{10})), 5.08 (br s, 1H, H-C(2^{11})), 4.97 (q, J=7.68, 1H, H-C(2^2)), 4.82 (quintet, J=7.35, 1H, H-C(2^8)), 4.70 (d, J=13.75, 1H, si-H-C(2^3)), 4.63 (t, J=9.39, 1H, H-C(2^5)), 4.48 (quintet, J=7.02, 1H, H-C(2^7)), 3.86 (m, 1H, H-C(3^1)), 3.49 (s, 3H, CH_3-N^1), 3.42 (s, 3H, CH_3-N^3), 3.29 (s, 3H, CH_3-N^6), 3.18 (d, J=13.75, 1H, re-H-C(2^3)), 3.14 (s, 3H, CH_3-N^9), 3.09 (s, 3H, CH_3-N^4), 3.04-3.00 (br m, 2H, 2H-C(6^8), 2.67 (s, 3H, CH_3-N^{11}); 2.66 (s, 3H, CH_3-N^{10}), 2.45 (m, 1H, H-C(3^5)), 2.14-1.18 (m, 22H, H-C(4^1), 2H-C(3^2), 2H-C(3^4), H-C(4^4), 2H-C(3^6), H-C(4^6), 2H-C(3^8), 2H-C(4^8), 2H-C(5^8), 2H-C(3^9), H-C(4^9), 2H-C(3^{10}), H-C(4^{10}), H-C(3^{11})), 1.42 (s, 9H, $(CH_3)_3$C-O^8), 1.34 (d, J=7.20, 3H, CH_3-C(2^7)), 1.06-0.83 (m, 39H, CH_3-C(4^1), CH_3-C(3^2), 2CH_3-C(4^4), 2CH_3-C(3^5), 2CH_3-C(4^6), 2CH_3-C(4^9), 2CH_3-C(4^{10}), CH_3-C(3^{11})), 0.77 (d, J=6.55, 3H, CH_3-C(3^{11})), 0.72 (d,J=6.55, 3H, CH_3-C(4^1)); FABMS (3-NBA/Gly + 1% TFA matrix): m/z $[M+H]^+$ 1319; HR-FABMS exact mass calcd for $C_{67}H_{123}N_{12}O_{14}$ $[M+H]^+$ 1319.9282, found 1319.9272.

[[(9-Fluorenylmethyl)oxy]carbonyl]-D-Alanyl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-D-Valyl-[(2S,3R)-3-hydroxy-N-methyl-leucyl]-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-leucyl-L-Alanine Benzyl Ester (Fmoc-D-Ala-MeLeu-MeLeu-D-MeVal-Me(3-OH)Leu-

Abu-Sar-MeLeu-Val-MeLeu-Ala-OBzl) ***(5.22)***

To a solution of heptapeptide **5.19a** (67 mg, 0.08 mmol) and tetrapeptide **3.42a** (82 mg, 0.12 mmol) in 1 ml of CH_2Cl_2 was added PyBroP (57 mg, 0.12 mmol) and DIEA (60 µl, 0.32 mmol) under an ice bath. The reaction mixture was stirred for 4 h, diluted with ethyl acetate (5 ml), and then washed with water (1 ml), 10% $KHSO_4$, 5% $NaHCO_3$, and brine. The organic layer was dried over anhydrous $MgSO_4$ and concentrated in vacuo. The resultant residue was purified by flash chromatography (10-40% acetone/hexane) to give 37 mg (32%) of the title compound as a foamy solid. TLC R_f 0.71 (40% acetone/hexane); $[\alpha]_D$ -103.1° (c 2.5, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) spectrum available as supplemental material; FABMS (DTT/DTE as matrix): m/z $[M+H]^+$ 1492; HR-FABMS: exact mass calcd for $C_{81}H_{126}N_{11}O_{15}$ $[M+H]^+$ 1492.9438, found 1492.9393.

Cyclo[(2S,3R)-3-hydroxy-N-methyl-leucyl-L-2-Aminobutyryl-Sarcosyl-N-Methyl-L-leucyl-L-Valyl-N-Methyl-L-leucyl-L-Alanyl-D-Alanyl-N-Methyl-L-leucyl-N-Methyl-L-leucyl-N-Methyl-D-valyl] ; *[D-MeVal^{11},Me(3-OH)Leu$^{(1)}$]Cyclosporine* ***(5.23)***

The title compound was synthesized according to the general procedure D in 46% yield and obtained as a foamy solid: TLC R_f 0.54 (50% acetone/hexane) ; $[\alpha]_D$ -162.5° (c 0.04, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) showed multiple

conformations in $CDCl_3$ at room temperature; Spectrum available as supplementary material; FABMS (3-NBA as matrix): m/z $[M+H]^+$ 1162.8; HR-FABMS exact mass calcd for $C_{59}H_{108}N_{11}O_{12}$ $[M+H]^+$ 1162.8179, found 1162.8145.

D. *Cyclophilin-Binding Assays*

1. *Materials*

CsA was purified from a commercial preparation (Sandmmune) by silica gel flash chromatography and checked by NMR analysis. The peptide substrate Suc-Ala-Ala-Pro-Phe-pNA was purchased from Sigma Chemical Co. and used without further purification. The synthetic CsA analogs were purified by flash chromatography and were dried in P_2O_5 in vacuo. THF was distilled over sodium-benzophenone. LiCl was dried by heating to 250 °C in vacuo. All other materials were of reagent grade and used without further purification. Recombinant human cyclophilin (neutral form)[179] was generous gift from Dr. Thomas Holzman (Abbott Laboratory).

2. *Peptidyl Prolyl Isomerase (PPIase) Assays*

A stock solution of CsA or CsA analogue (1 mM) was prepared in THF and serially diluted to give a secondary stock solution 10 times more concentrated than the concentration desired in the enzyme assay (between 5 nM and 1 μM). The substrate Suc-Ala-Ala-Pro-Phe-pNA was dissolved in 0.47

M LiCl in THF. The population of the *cis* Ala-Pro conformer of the substrate was determined by the previously reported method (typically 40% *cis*)[27]. A stock solution of cyclophilin (5 μM, 40 μL) was added to the thermally equilibrated buffer at 0 °C (1 mL, 50 mM Hepes-NaOH, 100 mM NACl, pH 8.0 at 0 °C). A serially diluted solution of CsA or CsA analog (final concentration: 100 μM- 50 nM) was then added and the components were mixed. The resultant aliquots of the enzyme-inhibitor mixture were withdrawn (each time 890 μL) at 4 h preincubation and assayed for PPIase inhibition after addition of the substrate (10 μL) and chymotrypsin (100 μL) as described elsewhere.[26,27] Absorbance readings were collected on a CARY 14 spectrophotometer interfaced to an IBM computer by use of commercial data acquisition software (OLIS; On Line Instrument Systems, Jefferson, GA).

3. *Determination of Initial Velocities*

The initial velocities in PPIase assays were determined by nonlinear least-squares fit of time-absorbance data according to the method described by the Rich group.[26] The enzymatic rate equation for a tight-binding inhibitor is then simply expressed as in Equation 6.1, where $[S]_o$ represents the *cis* isomer of the substrate and $[E]_f$ is the fraction of free enzyme corrected for tight-binding, defined in the auxiliary Equation 6.2. The apparent inhibition constant K_i^{app} in Equation 6.2 is a useful measure of inhibitory potency for

"tight" inhibitors, which has been described in detail by Cha[180] to optimize the kinetic mechanism of inhibition.

$$V = K_{cat} E_f \frac{[S]_o}{[S]_o + K_m} \qquad \text{(Equation 6.1)}$$

$$E_f = \frac{1}{2}\{[E]_o - [I]_o - K_i^{app} + \sqrt{\{[E]_o - [I]_o - K_i^{app}\}^2 + 4K_i^{app}[E]_o}\} \qquad \text{(Equation 6.2)}$$

Chapter VII. References

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160. PBMCs were obtained from healthy donors by density centrifugation over histopaque (Sigma, St. Louis, MO). PBMCs were incubated at 1 x 10^5 cells per well in complete medium (RPMI-1640/10% iron-enriched calf serum/L-glutamine/penicillin-streptomycin) with 1 μg/ml phytohemagglutinin (PHA) (Sigma) with or without varying concentrations of CsA or CsA analog. After three days the cells were pulsed with 0.5 μCi of ^{3}H-thymidine, harvested after 18 hr, and the amount of radioactivity incorporated was determined by scintillation counting as described in detailed previously bescribed in : Malkovsky, M.; Asherson, G. L.; Stockinger, B. & Watkins, M. C. *Nature*, **1982**, 300, 652.
161. CEMx174 cells at 5 x 10^5 were infected for 2 hr at 37°C with cell free HIV-1_{LAI} at approximately 300 ng/ml p24. The inoculum was washed off and the cells were plated at 5 x 10^4 cells/well in various concentrations of CsA or CsA analog. After three days post infection cell free supernatants was collected. The amount of p24 in the cell free supernatants was determined by using a commercially available ELISA kit (Coulter Corp., Hialeah, FL) according to the manufacturers instructions.
162. To determine the level of CD4 expression, 1 x 10^5 CEMx174 cells, which had been grown in the presence of 10 μg/ml of CsA or CsA analog for two days, were incubated with FITC conjugated anti-T4 (Olympus Immunochemicals, Lake Success, NY) for 30 min on ice, washed with PBS and analyzed using a FACScan (Becton Dickinson, Mountain View, CA) as described in detail elsewhere - Fisch, P.; Malkovsky, M.; Kovats, S.; Sturn, E.; Braakman, E.; Klein, B. S.; Voss, S. D.; Morrissey, L. W.; Demars,

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163. CEMx174 cells were incubated at 5 x 10^4 cells per well in complete media with 10 μg/ml of CsA or CsA analog. After two days, the cells were pulsed with 0.5 μCi of ^{3}H-thymidine and harvested four hours later.

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Appendix I.

Stereographic and schematic presentations of the conformations of cyclosporine A.

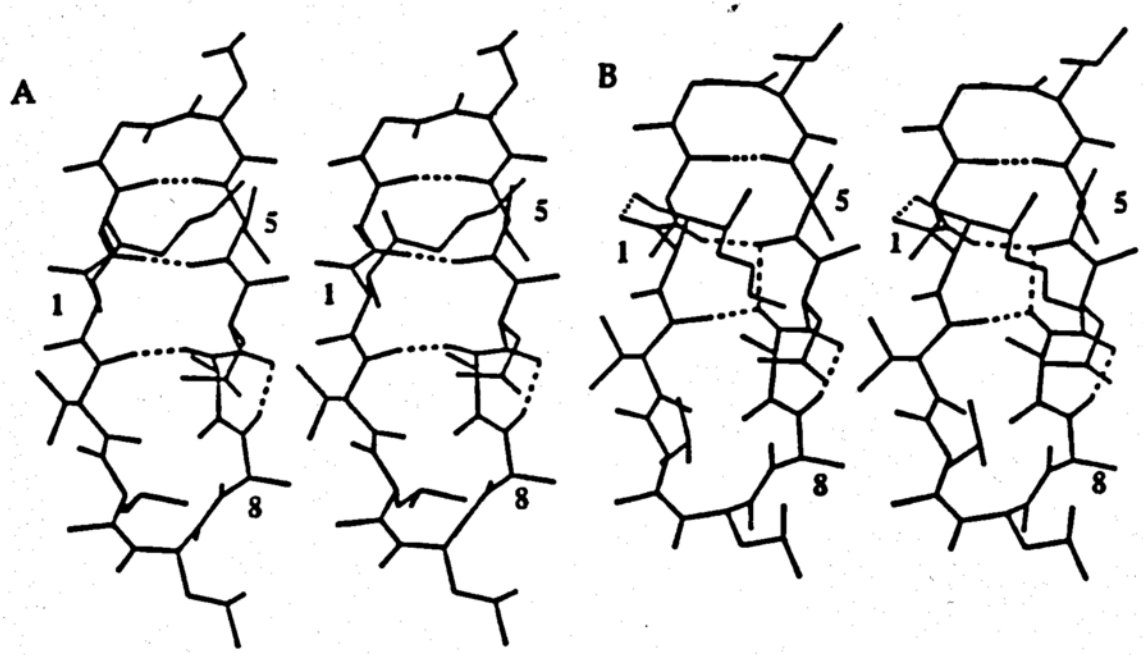

Figure 1.2. Panel A, X-ray structure of CsA in single crystals (ref. 20); Panel B, NMR structure of CsA in chloroform solution (ref. 21). Stereoviews are shown of the bonds connecting all heavy atoms.

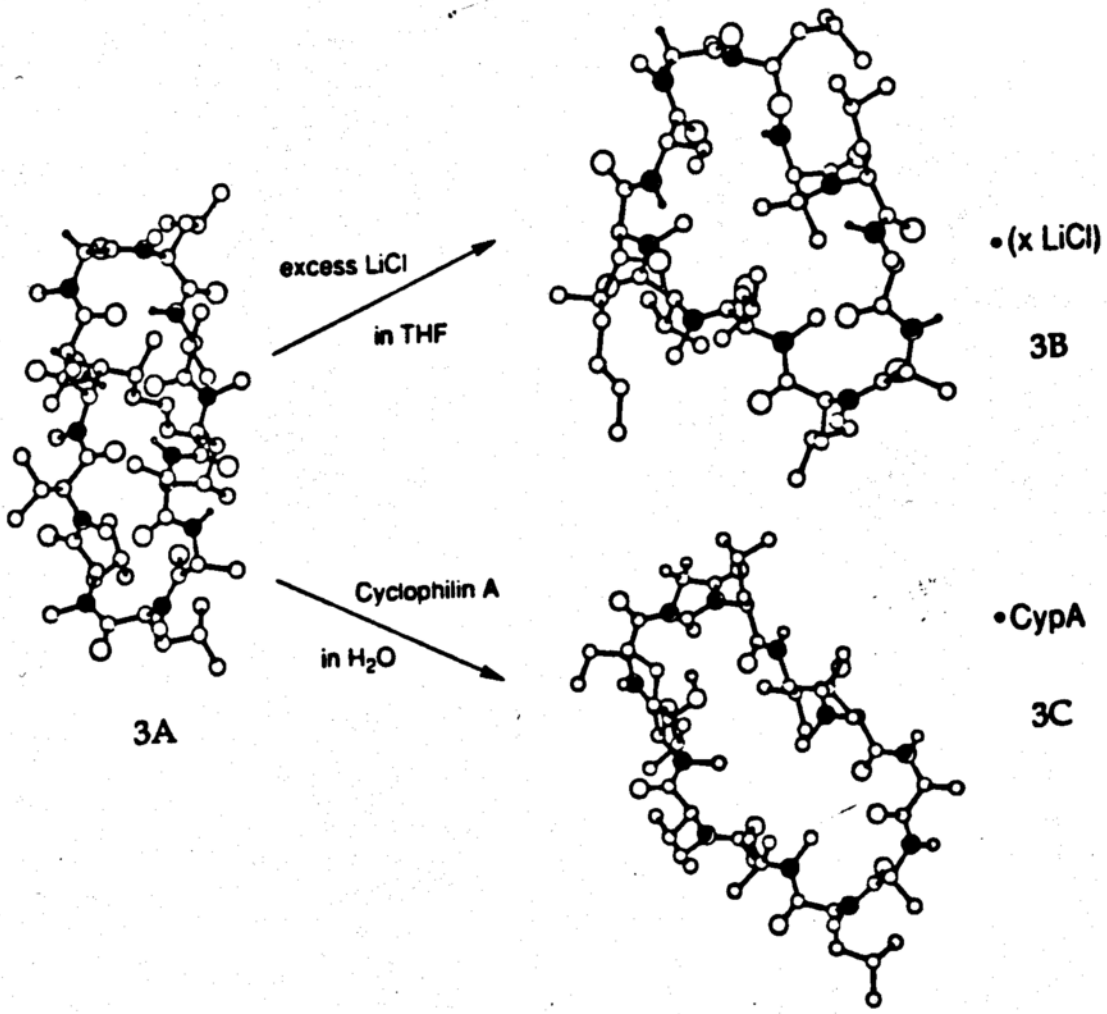

Figure 1.3. Conformation of CsA.[21,28,29] In an organic solvent such as THF or $CHCl_3$ (3A), in THF/LiCl (3B), and in the complex with its binding protein CyP (3C).

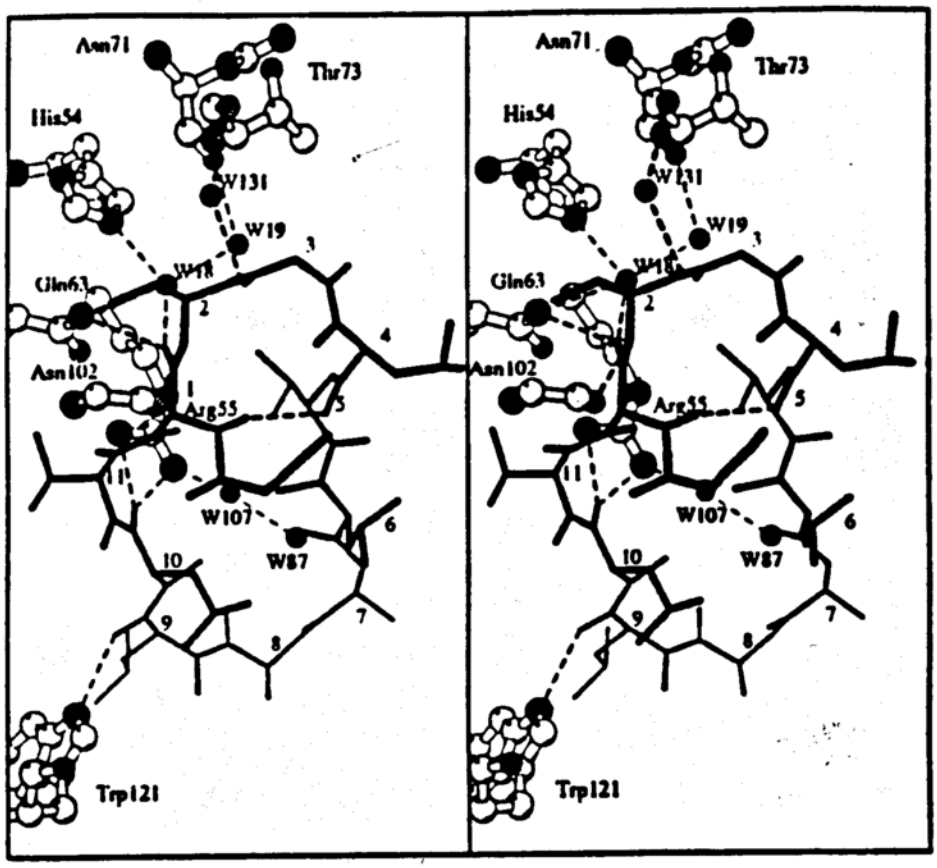

Figure 1.4. Stereoview of the contacts[60] (shown by dashed lines) between CsA and CyP (partial structure); W: water molecule.

Appendix II.

The ^1H NMR spectra (obtained in $CDCl_3$ by Bruker-Am500 Spectrometer) of CsA analogs, [MeNle^6]CsA(**3.44a**), [MeNle^6,D-Lys(Boc)^8]CsA(**3.44b**), [MeNle^6,D-Ser^8]CsA(**3.44c**), [MeLys(Paloc)^6]CsA(**3.44d**), [MeLys(Paloc)^6,D-Ser(tBu)^8]CsA(**3.44e**), [MeLys(Paloc)^6,D-Ser^8]CsA(**3.44f**), [MeNle^6,D-Lys^8]CsA(**3.54**), [MeNle^6,D-Lys(Daz)^8]CsA(**3.55a**), [MeLys(Daz)^6,D-Ser^8]CsA(**3.55b**), [MeLys(Paloc)^6,D-Ser(Daz)^8]CsA(**3.55c**), [Phe^7]CsA(**4.16a**), [Nva^2,Phe^7]CsA(**4.16b**), [D-MeAla^3,Phe^7]CsA(**4.16c**), [Phe^7,D-Ser^8]CsA (**4.16d**), [D-MeAla^3,Phe^7,D-Ser^8]CsA(**4.16e**), [MeLeu(OH)^1]CsA(**5.21a**), [[MeLeu(OH)^1,MeAla^6]CsA(**5.21 b**), [MeLeu(OH)^1,MeAla^{4,6}]CsA(**5.21c**), [MeLeu(OH)^1,D-Lys(Boc)^8]CsA(**5.21d**), and [D-MeVal^{11},MeLeu(OH)^1]CsA(**5.23**) , all of which were synthesized in this course of work are presented in photoreduced form in this appendix, and the spectrum of CsA (Figure **1.1**) is also attached for comparison.

Figure 1.1. Cyclosporin A (CsA, 1.1)

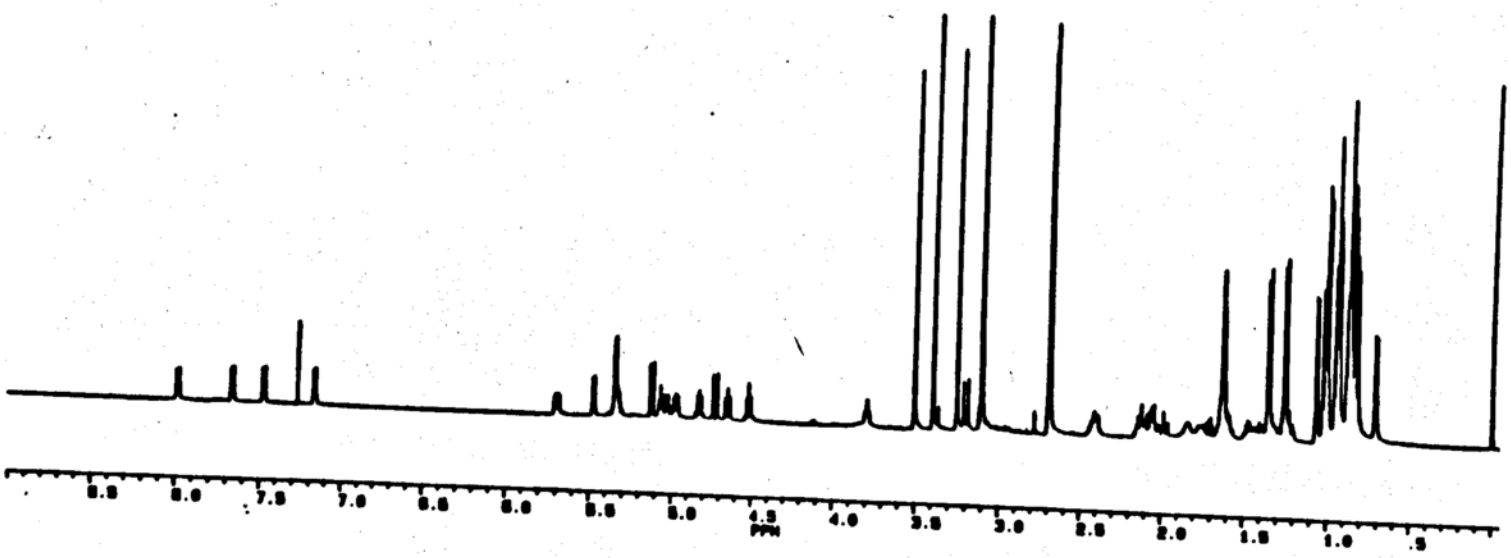

Figure 3.5. [MeNle^6]CsA (3.44a)

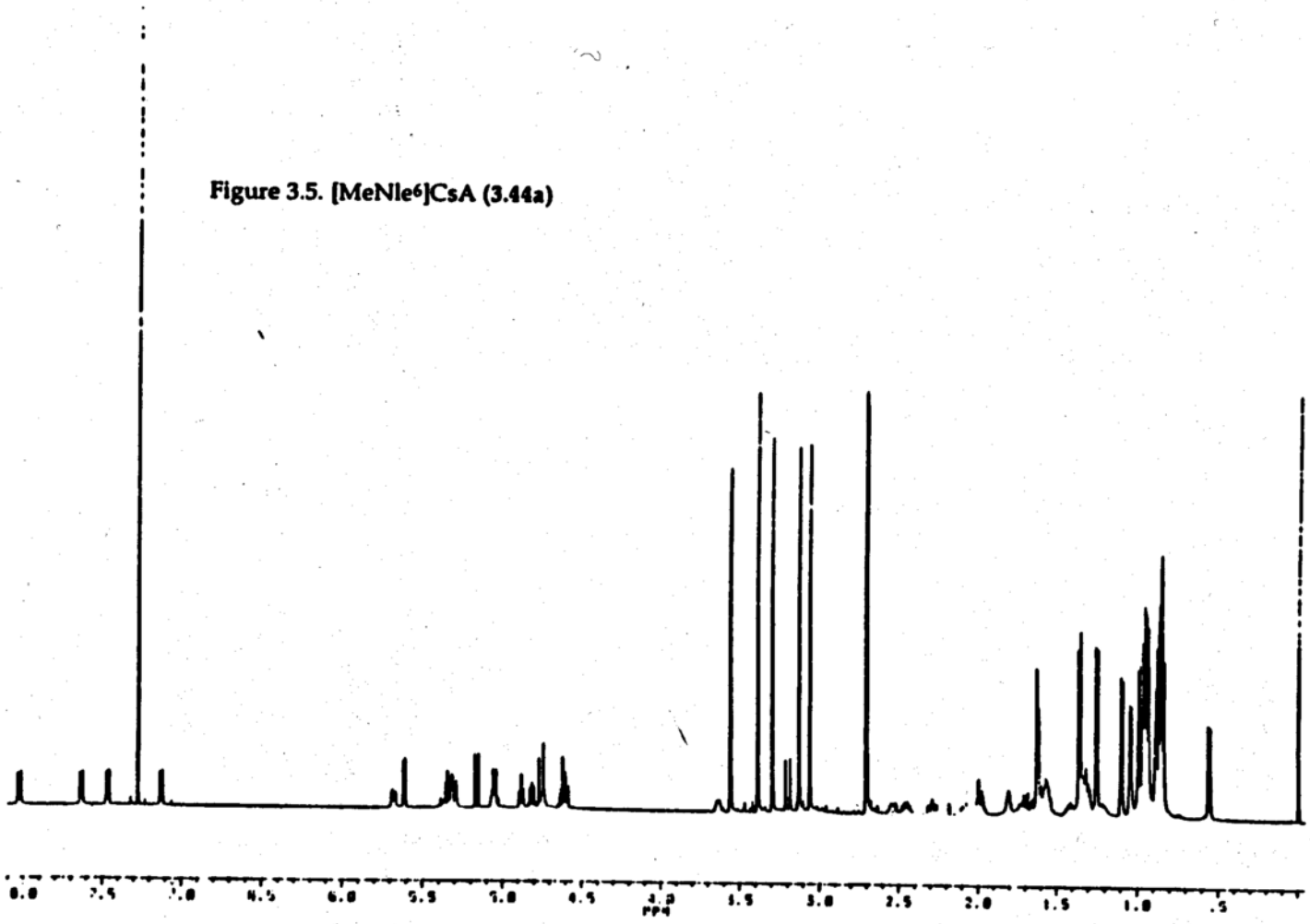

Figure 3.6. [MeNle^{6},D-Lys(Boc)^{8}]CsA (3.44b)

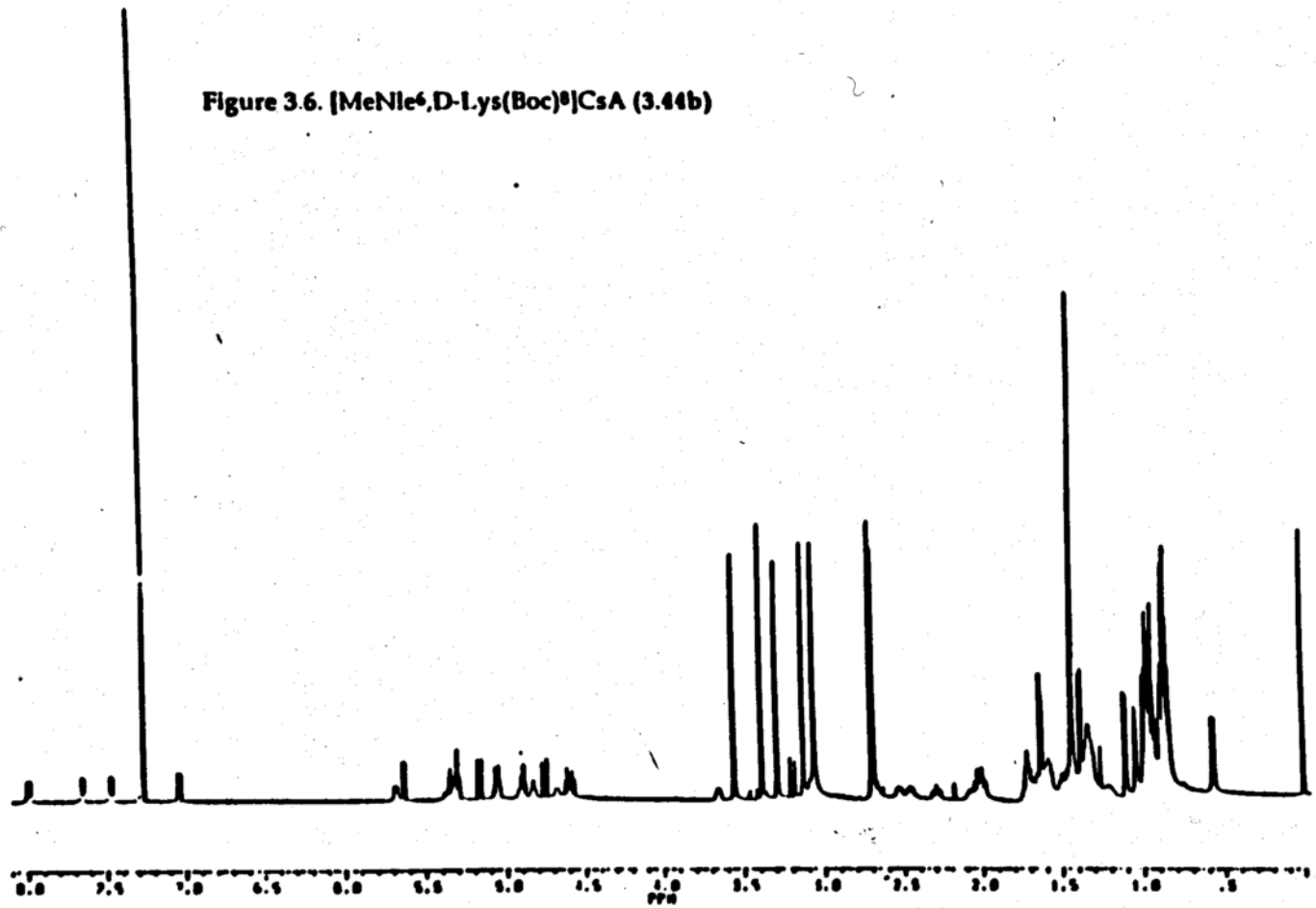

Figure 3.7. [MeNle^{6}, D-Ser^{8}]CsA (3.44c)

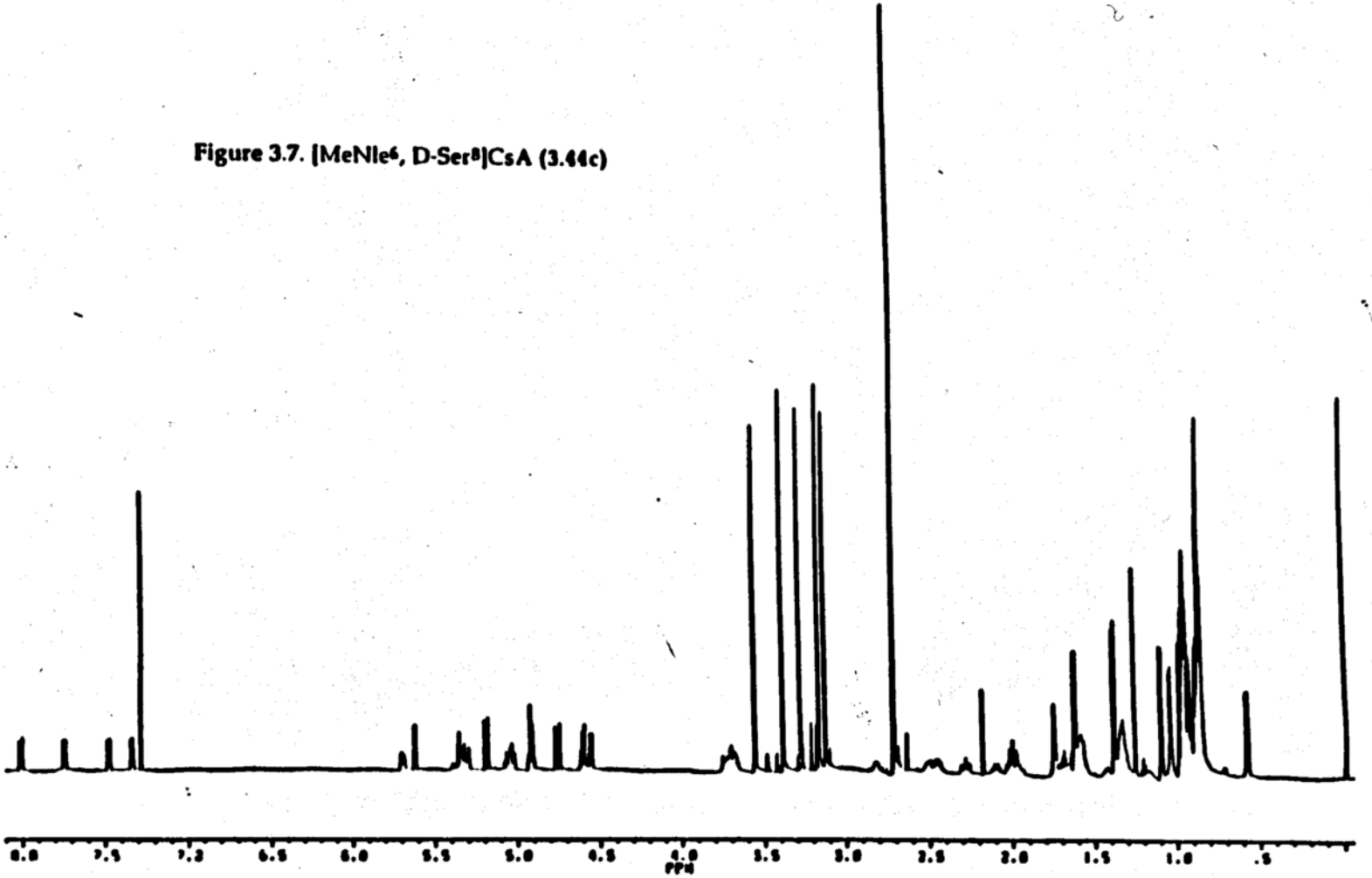

Figure 3.8. [MeLys(Paloc)6]CsA (3.44d)

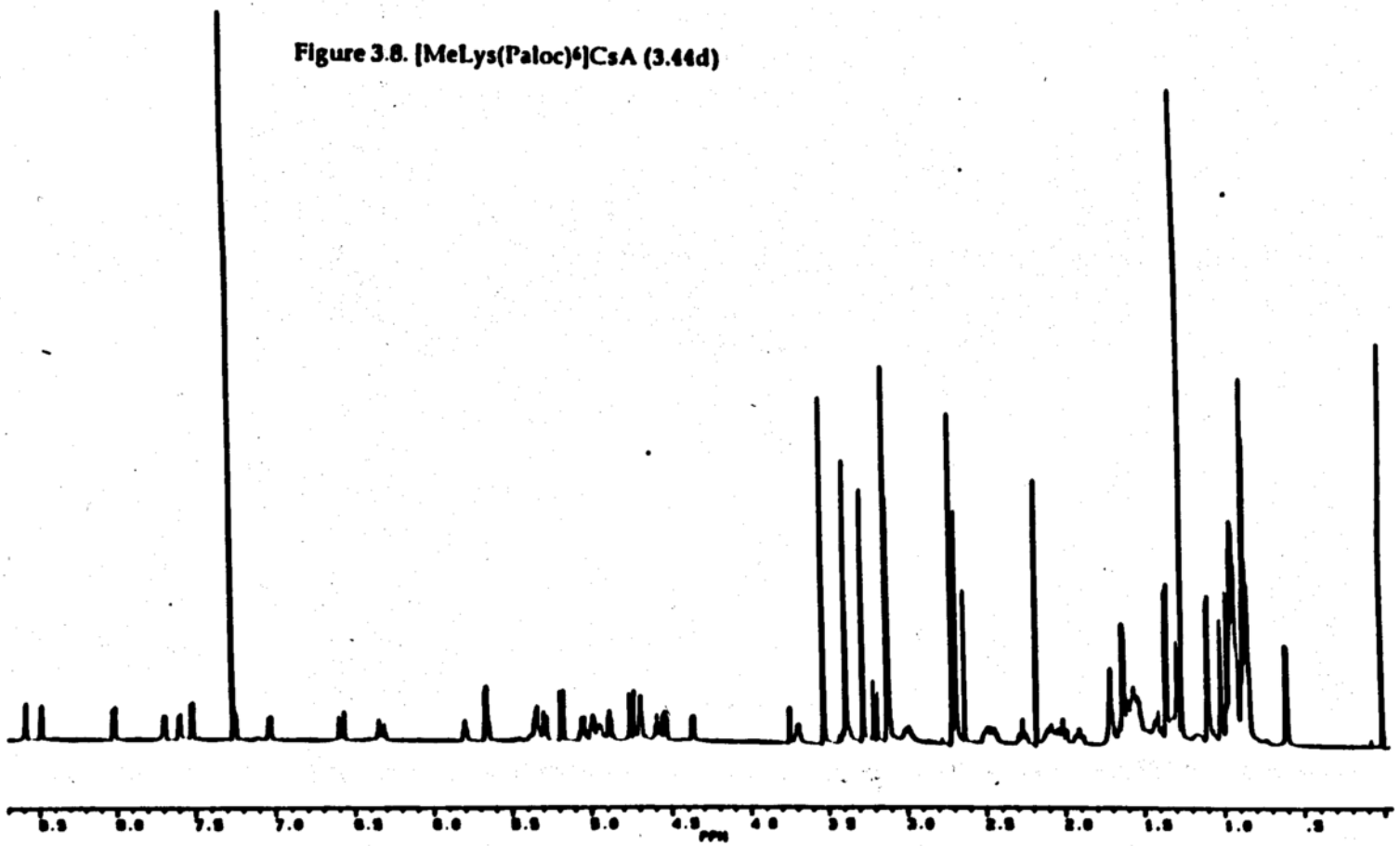

Figure 3.9. [MeLys(Paloc)^{6},D-Ser(tBu)^{8}]CsA (3.44e)

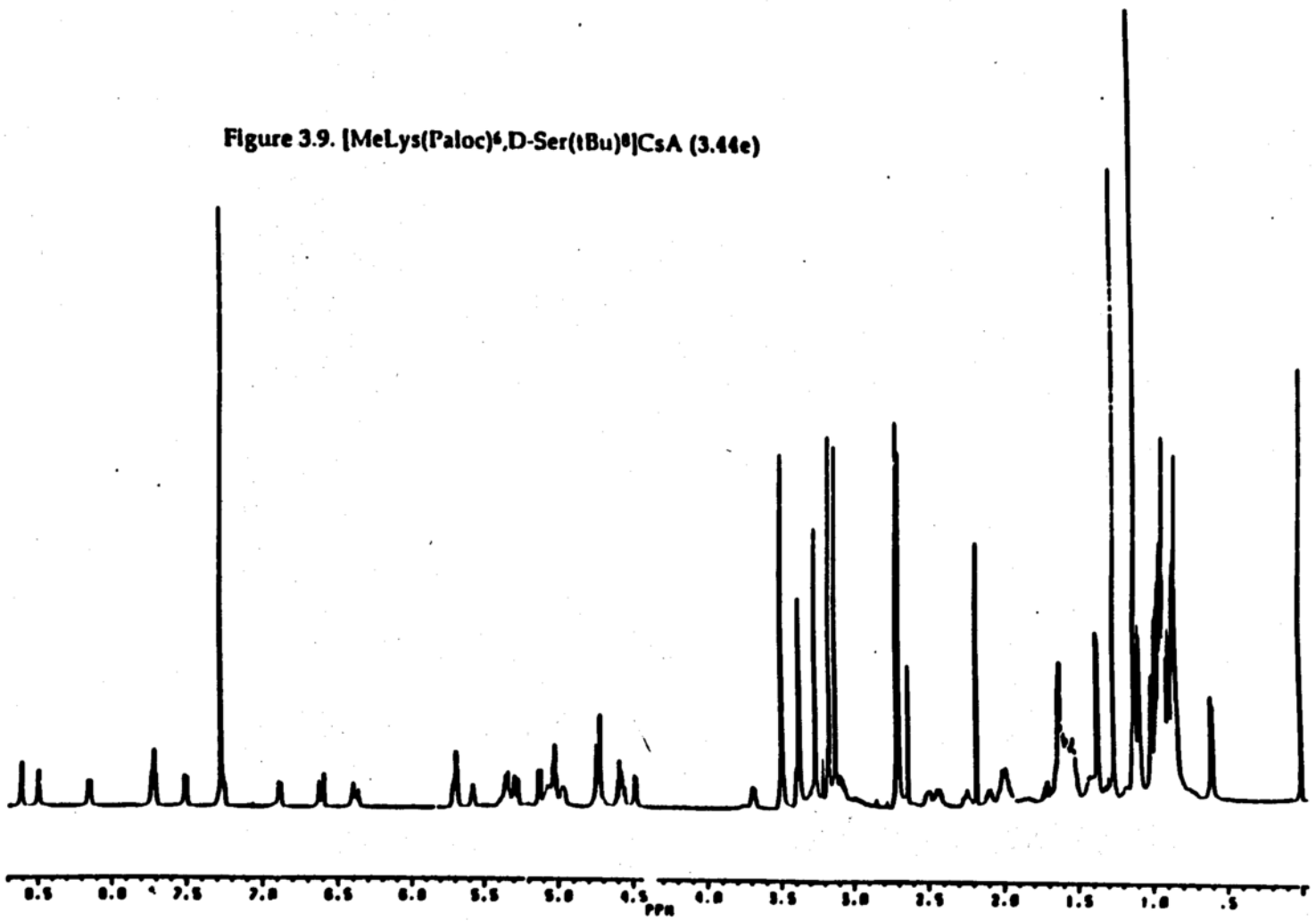

Figure 3.10. [MeLys(Paloc)^{6},D-Ser^{8}]CsA (3.44f)

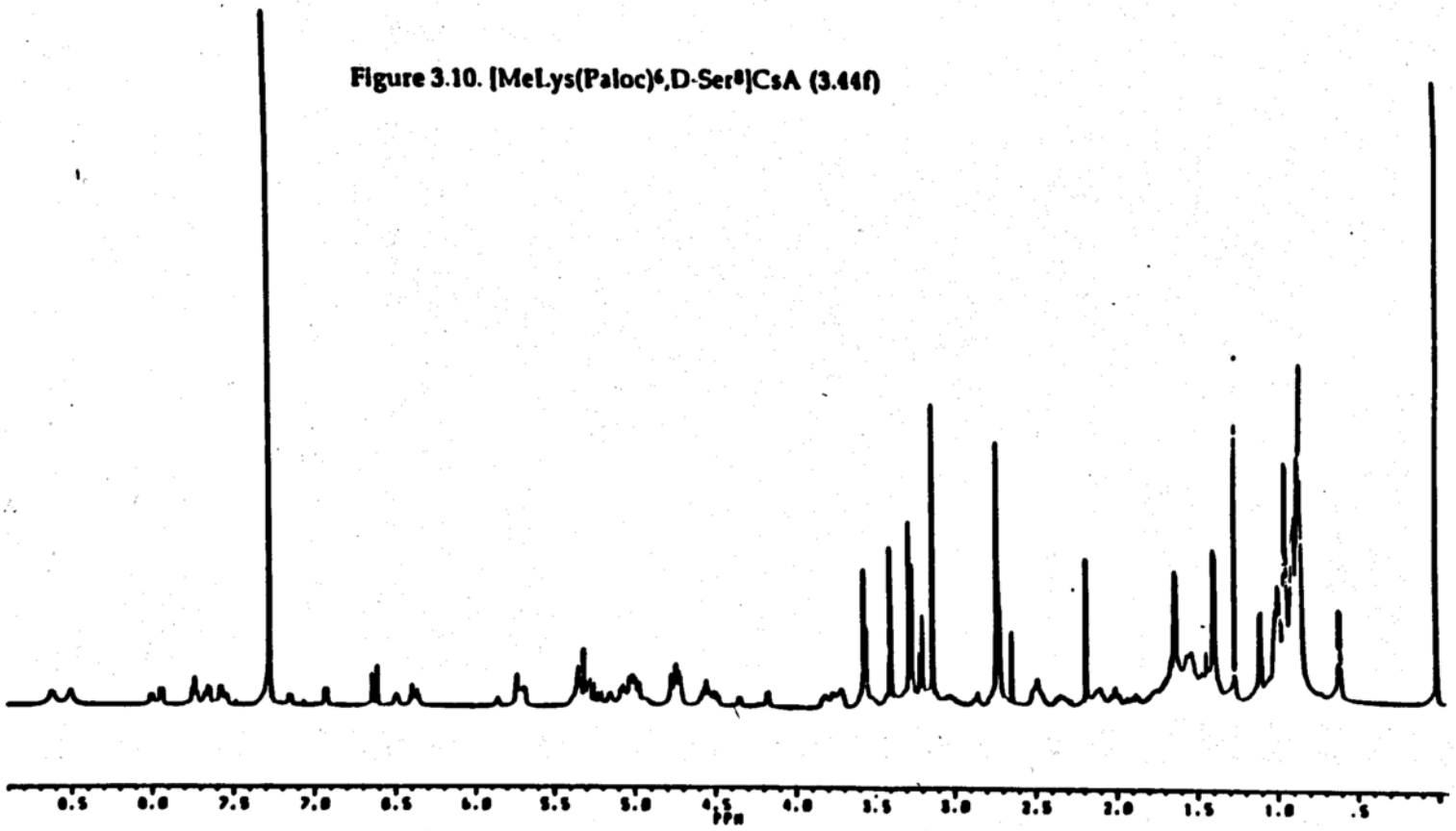

Figure 3.12. [MeNle^{6},D-Lys^{8}]CsA (3.54)

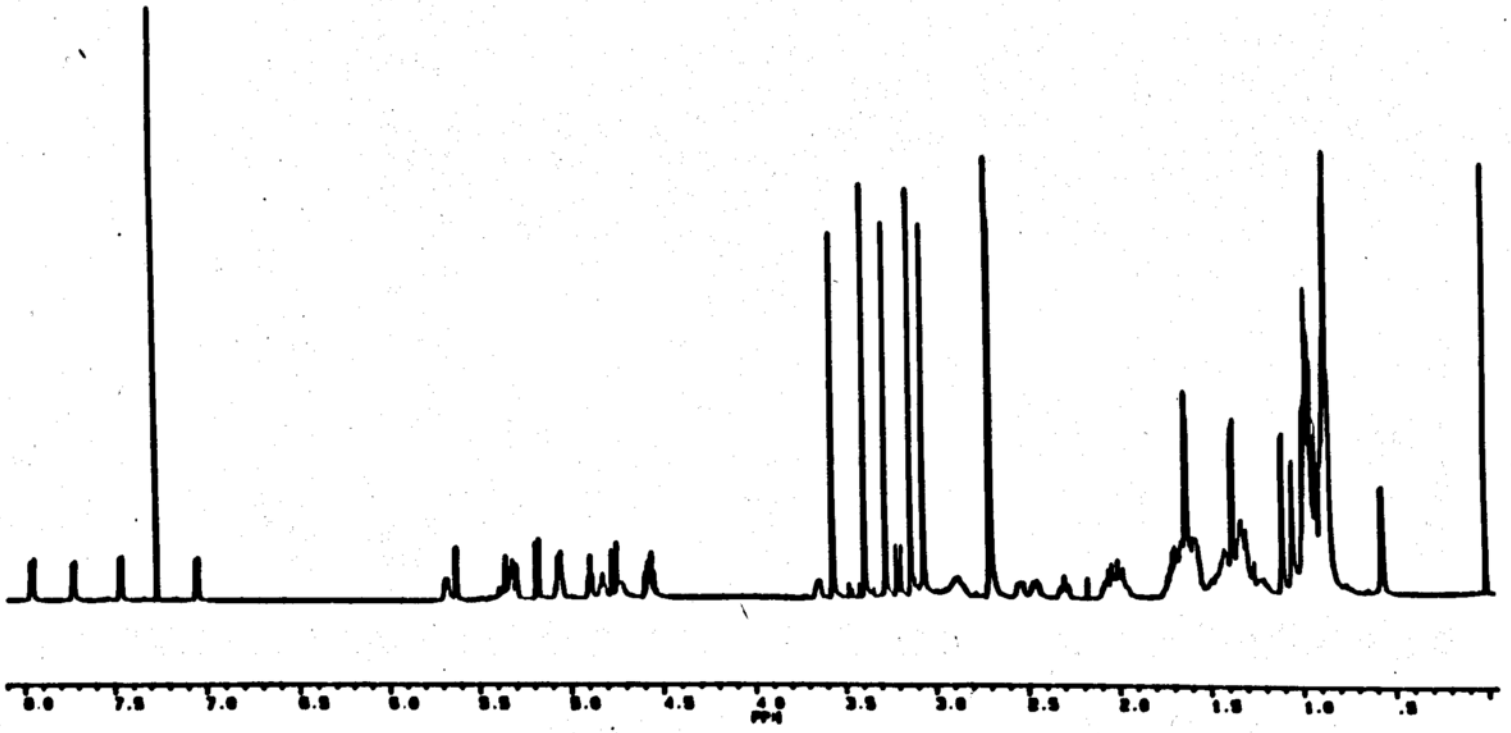

Figure 3.13. [MeNle^{6},D-Lys(Daz)^{8}]CsA (3.55a)

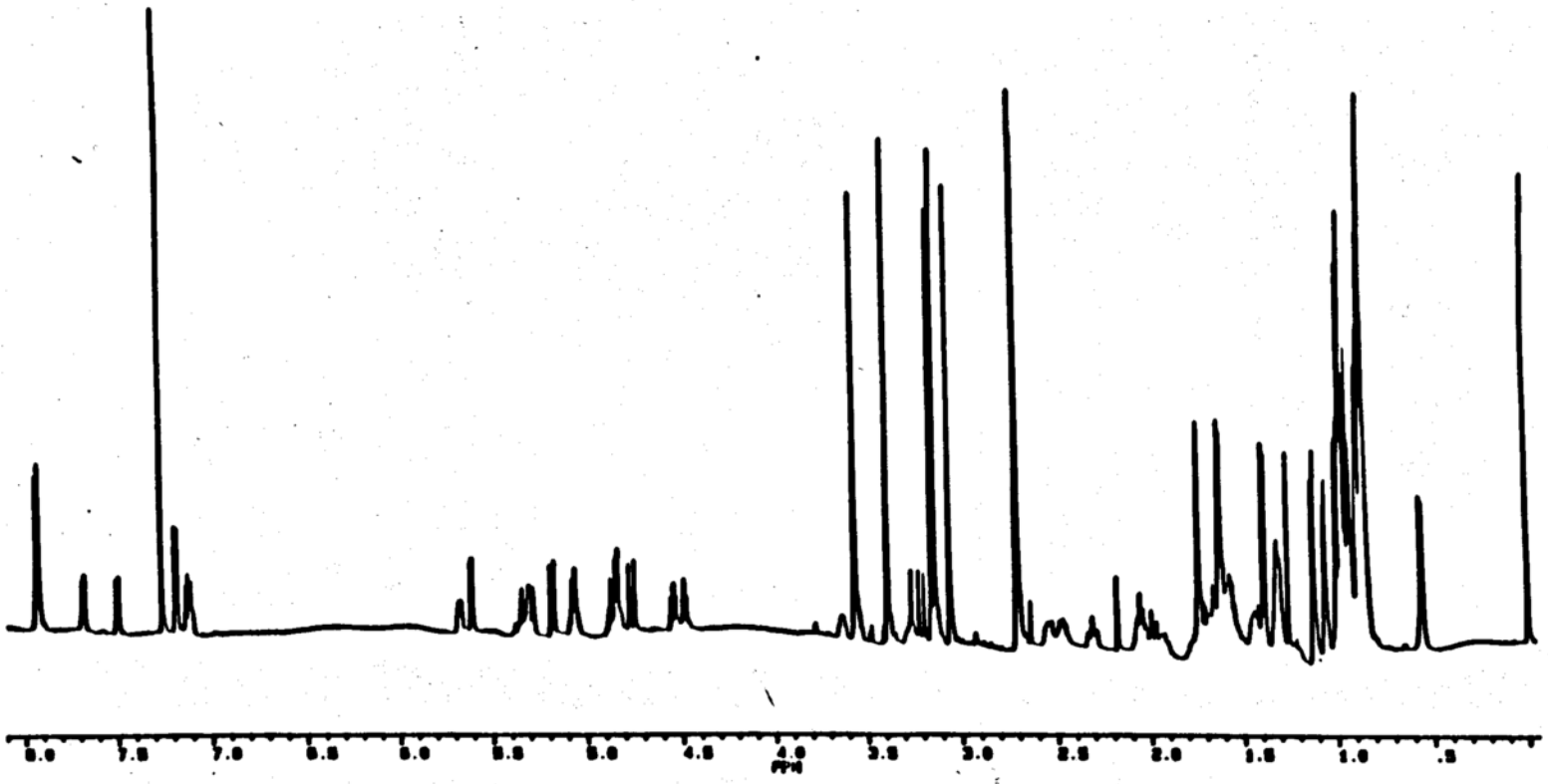

Figure 3.14. [MeLys(Daz)^{6},D-Ser^{8}]CsA (3.55b)

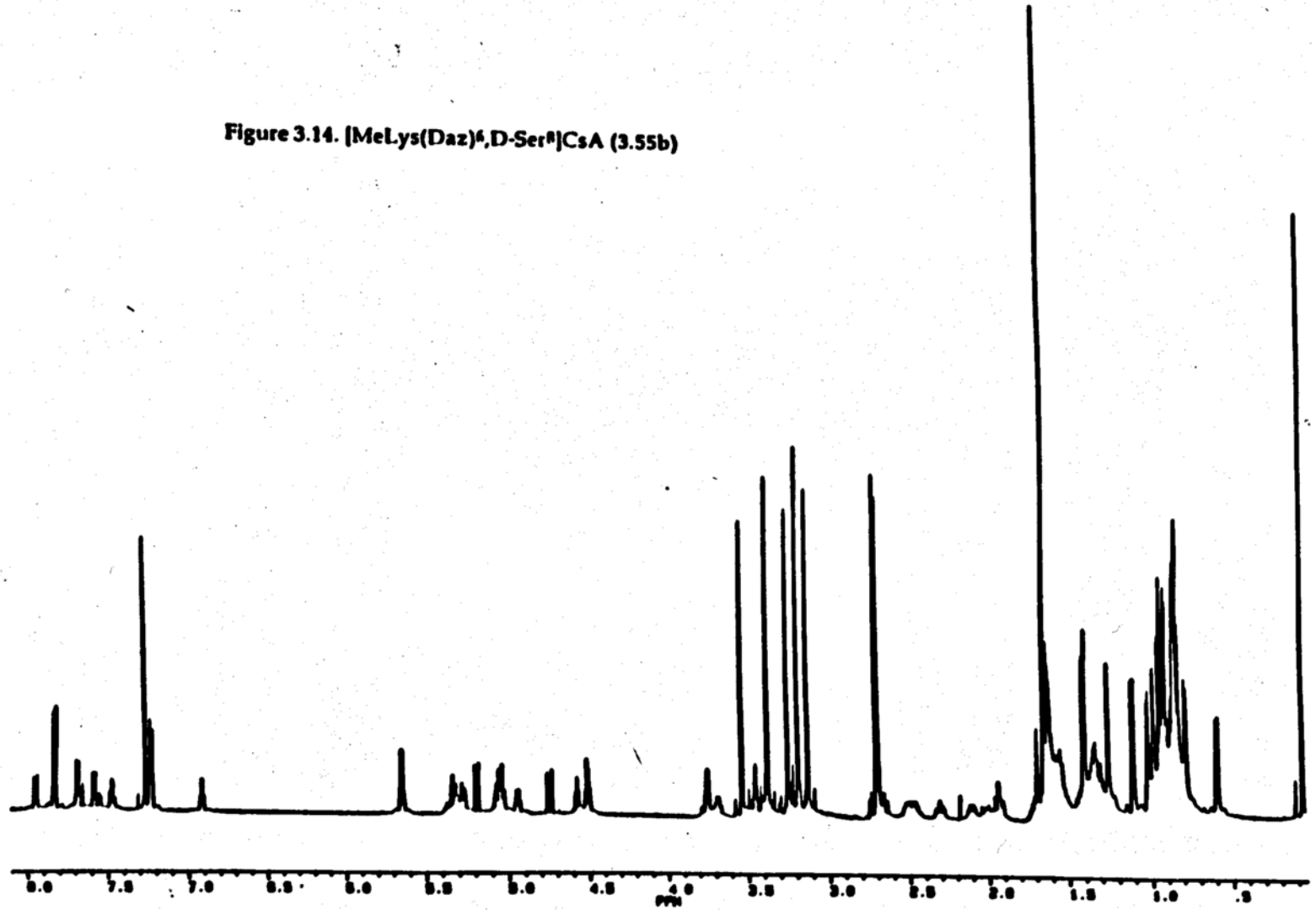

Figure 3.15. [MeLys(Paloc)^{6},D-Ser(Daz)^{8}]CsA (3.55c)

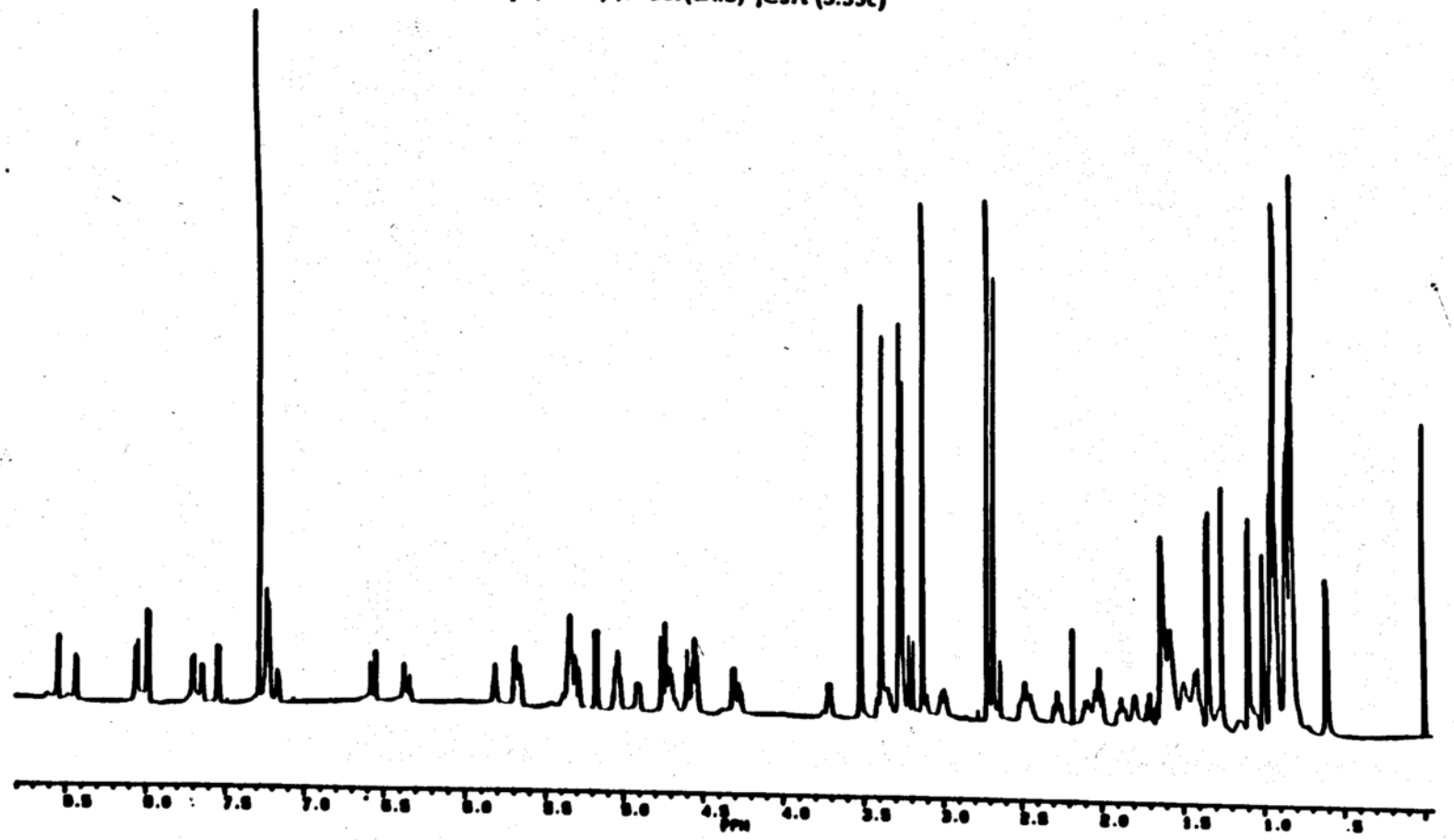

Figure 4.1. [Phe^7]CsA (4.16a)

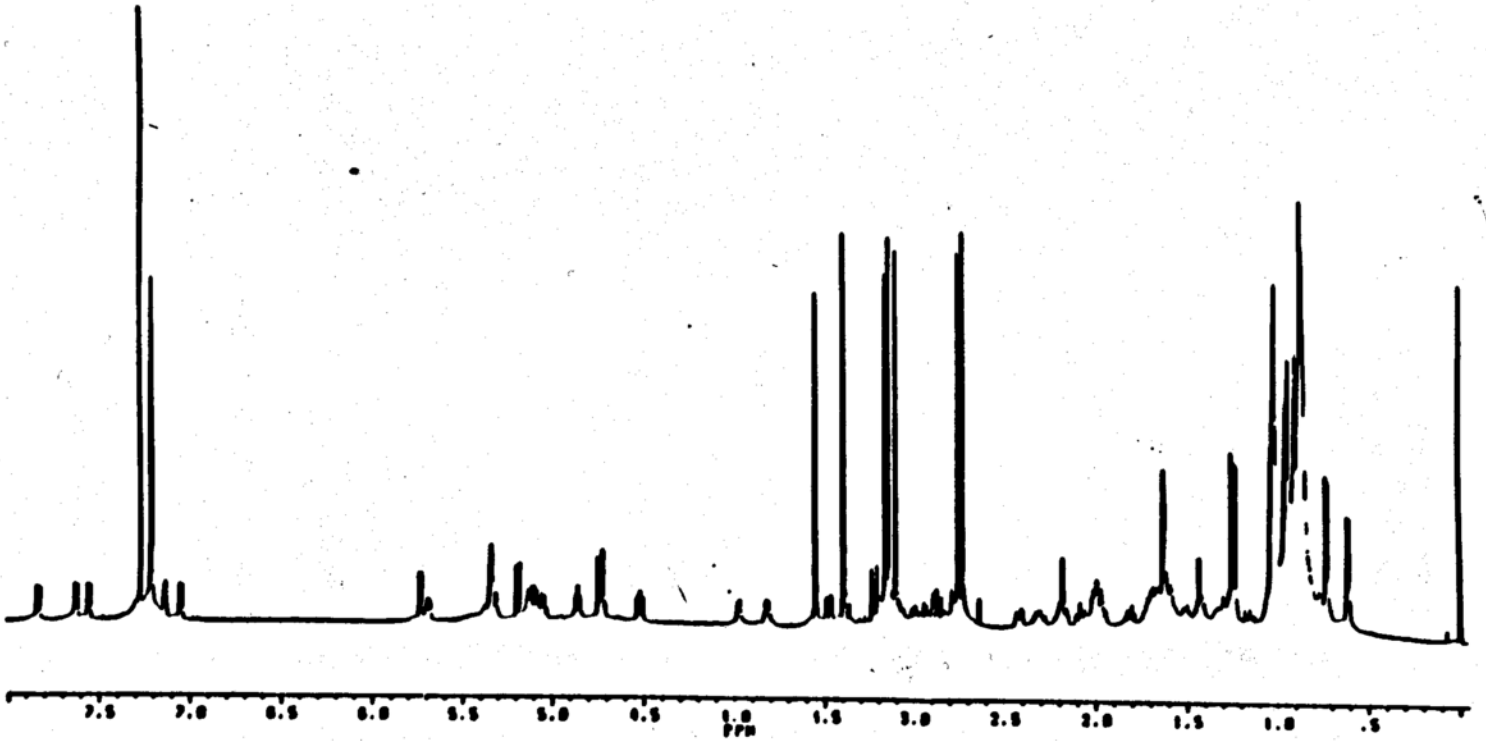

Figure 4.2. [Nva^2,Phe^7]CsA (4.16b)

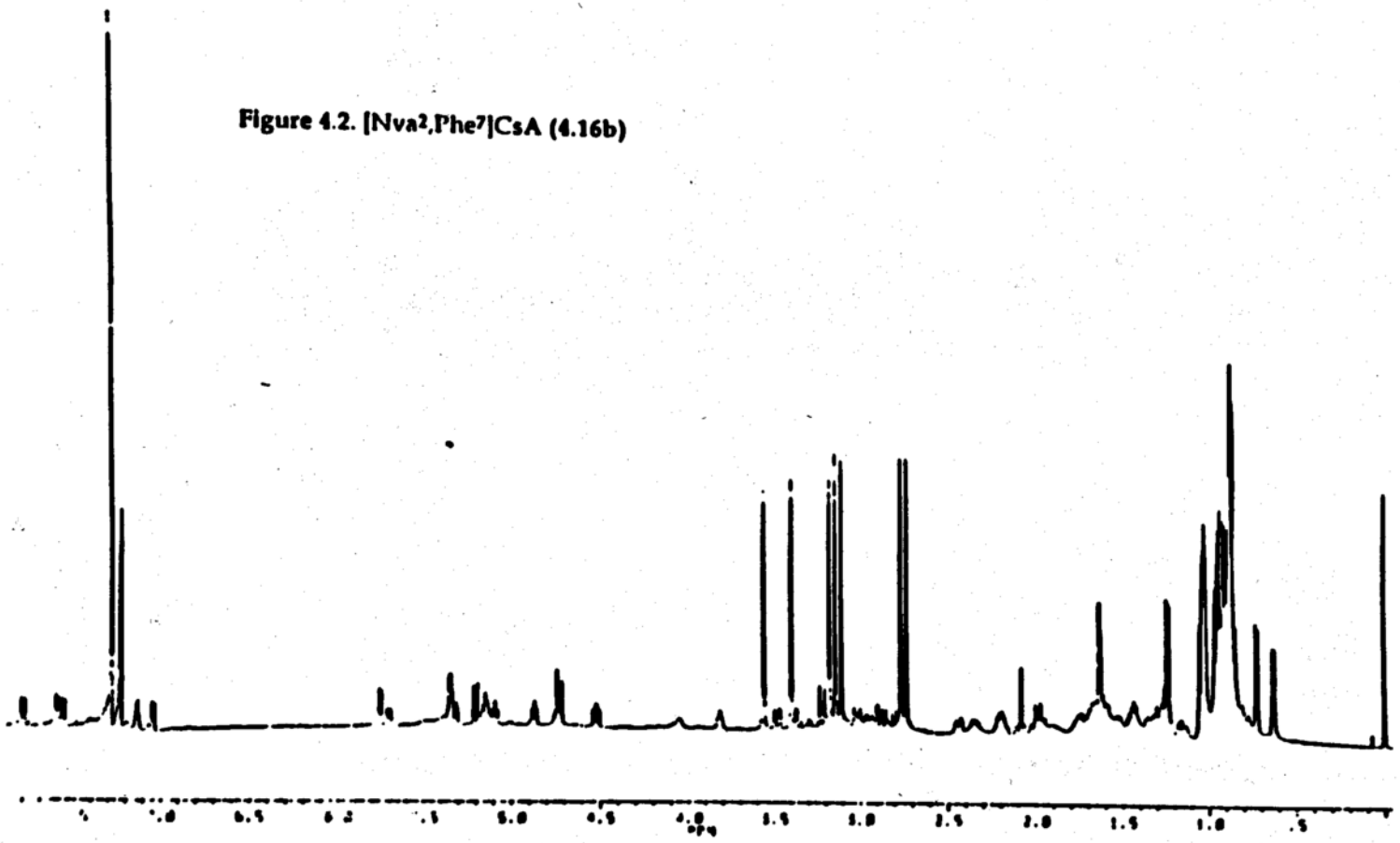

Figure 4.3. [D-MeAla^3,Phe^7]CsA (4.16c)

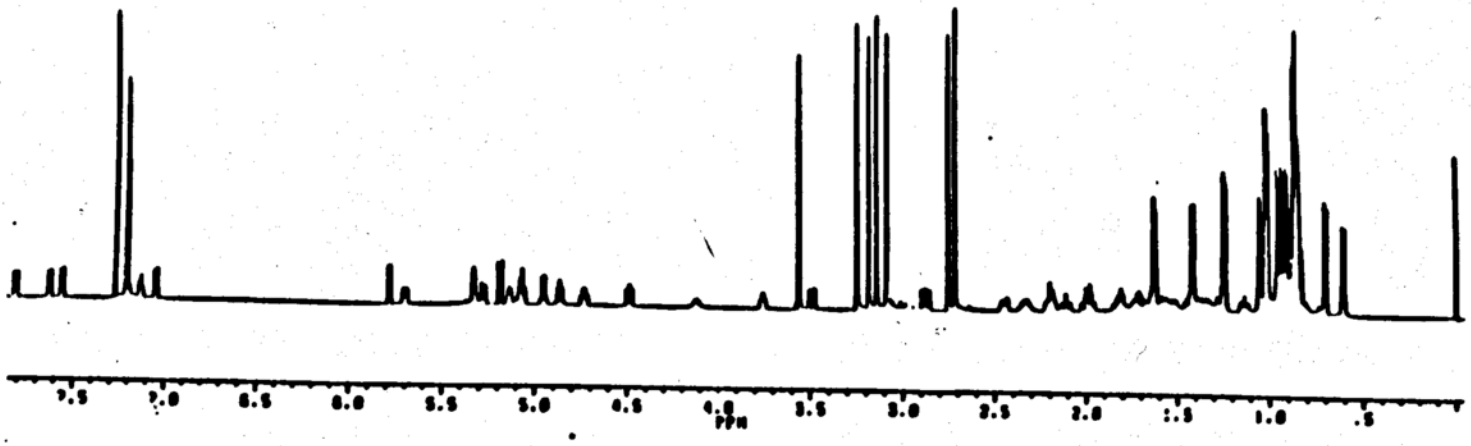

Figure 4.4. [Phe^{7},D-Ser^{8}]CsA (4.16d)

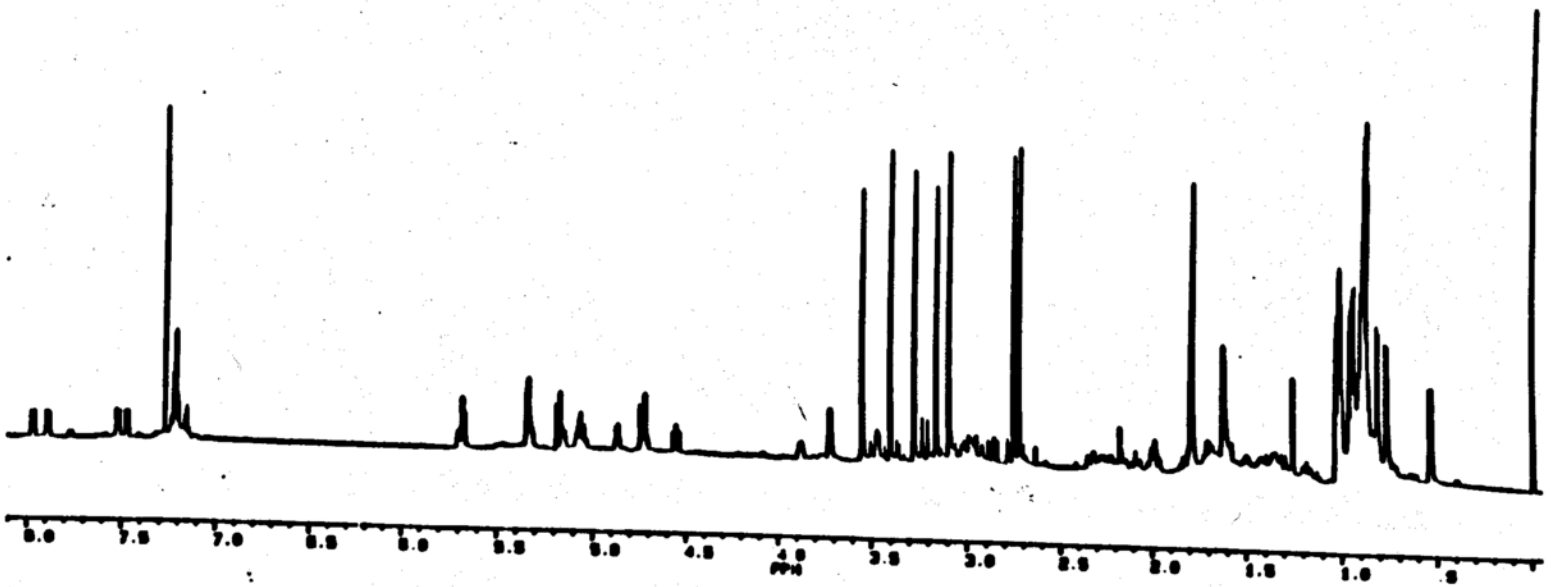

Figure 4.5. [D-MeAla^3,Phe^7,D-Ser^8]CsA (4.16e)

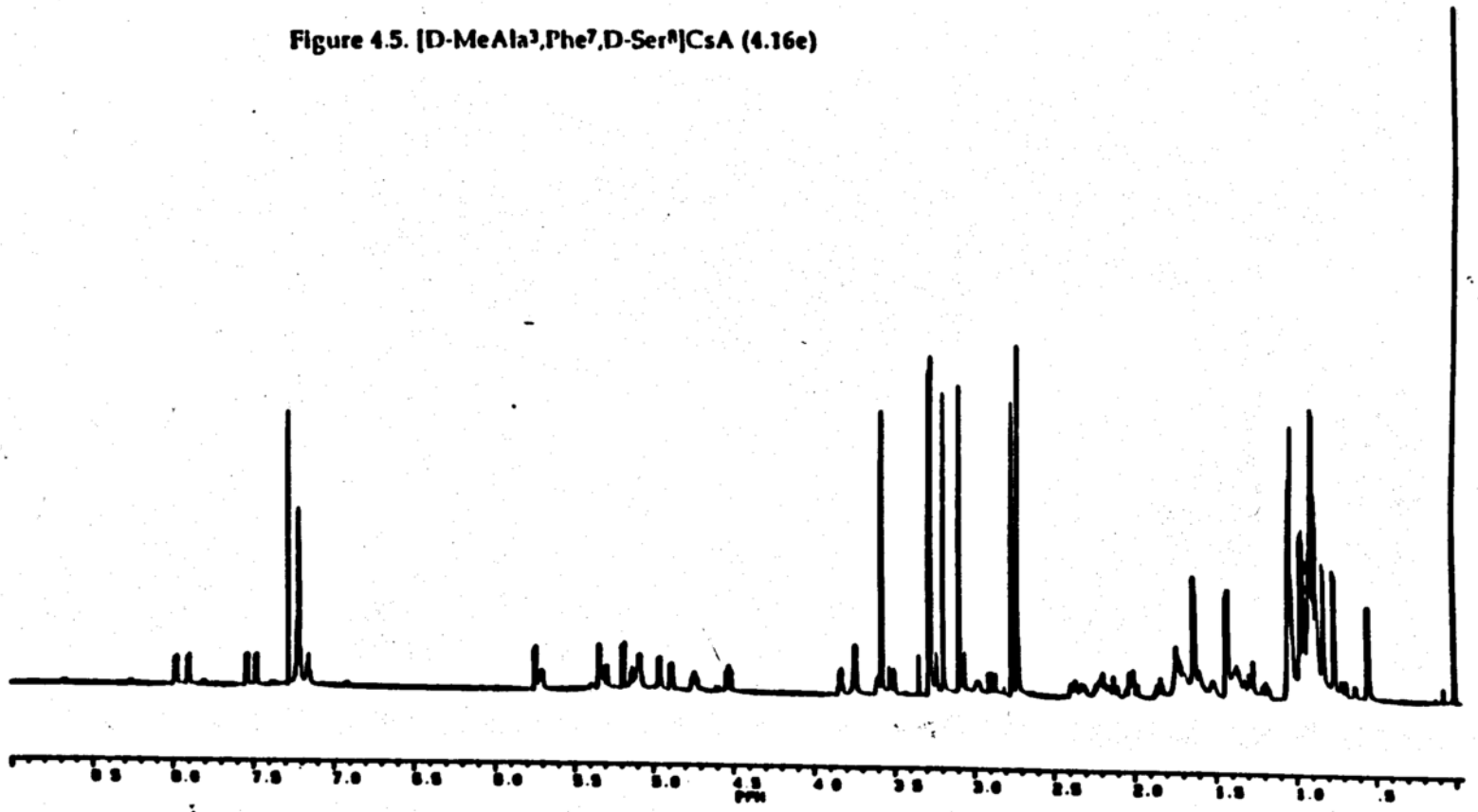

Figure 5.3. [MeLeu(OH)^{1}]CsA (5.21a)

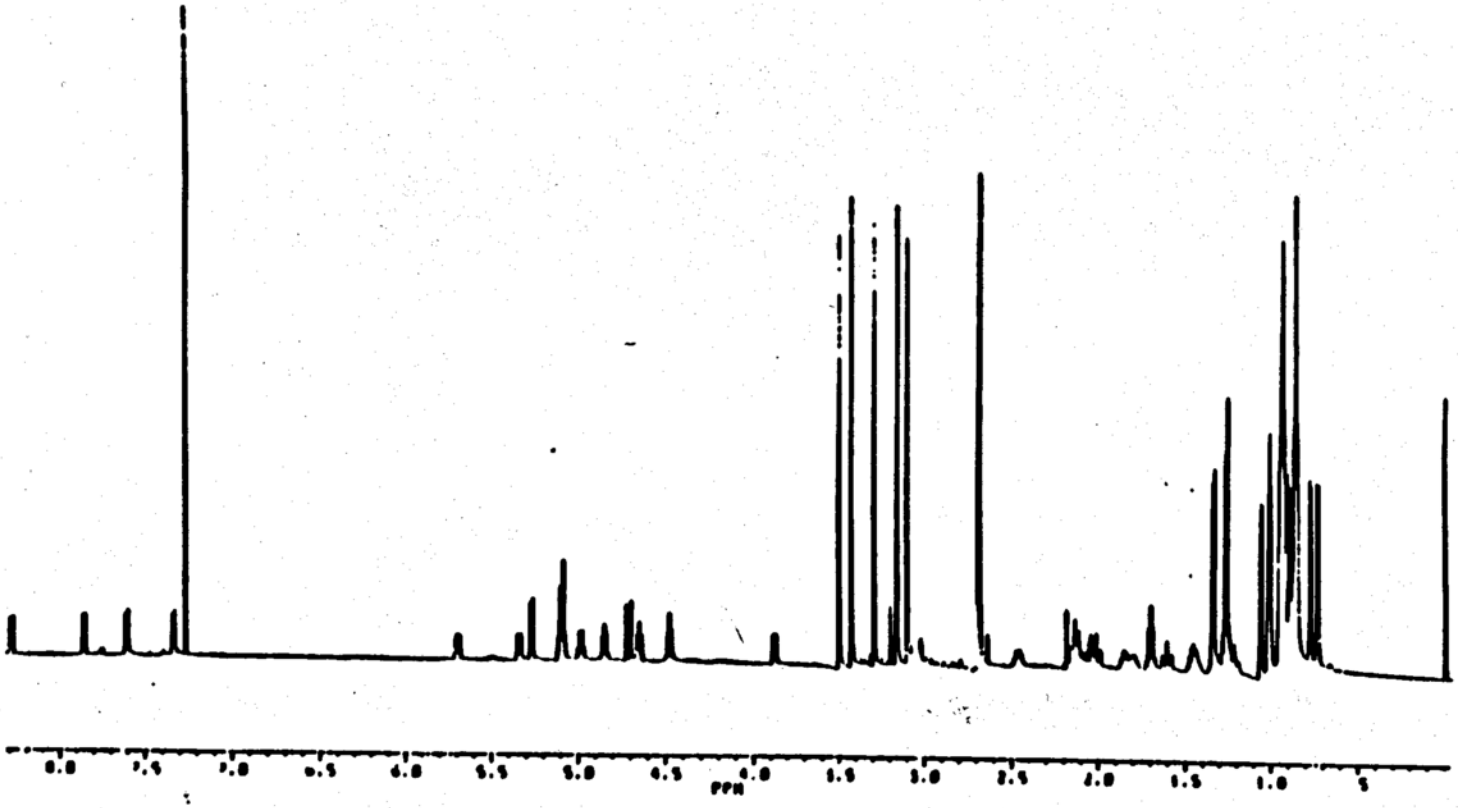

Figure 5.4. [MeLeu(OH)^1,MeAla^6]CsA (5.21b)

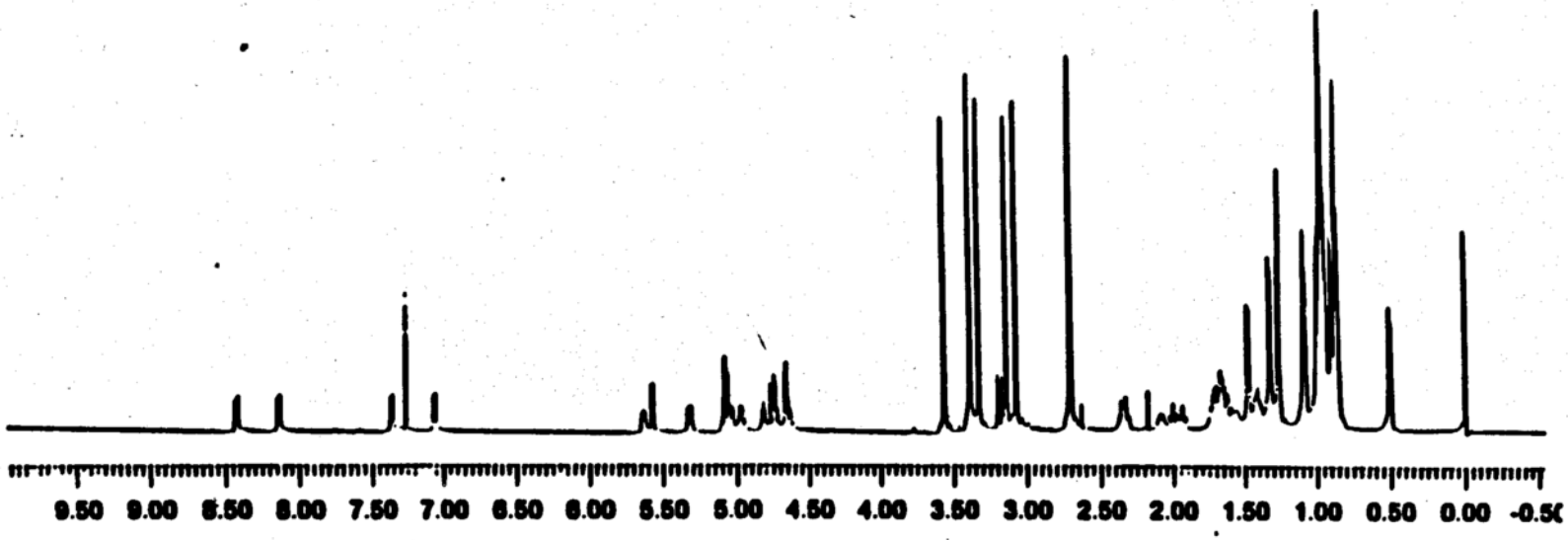

Figure 5.5. [MeLeu(OH)^{1},MeAla^{4,6}]CsA (5.21c)

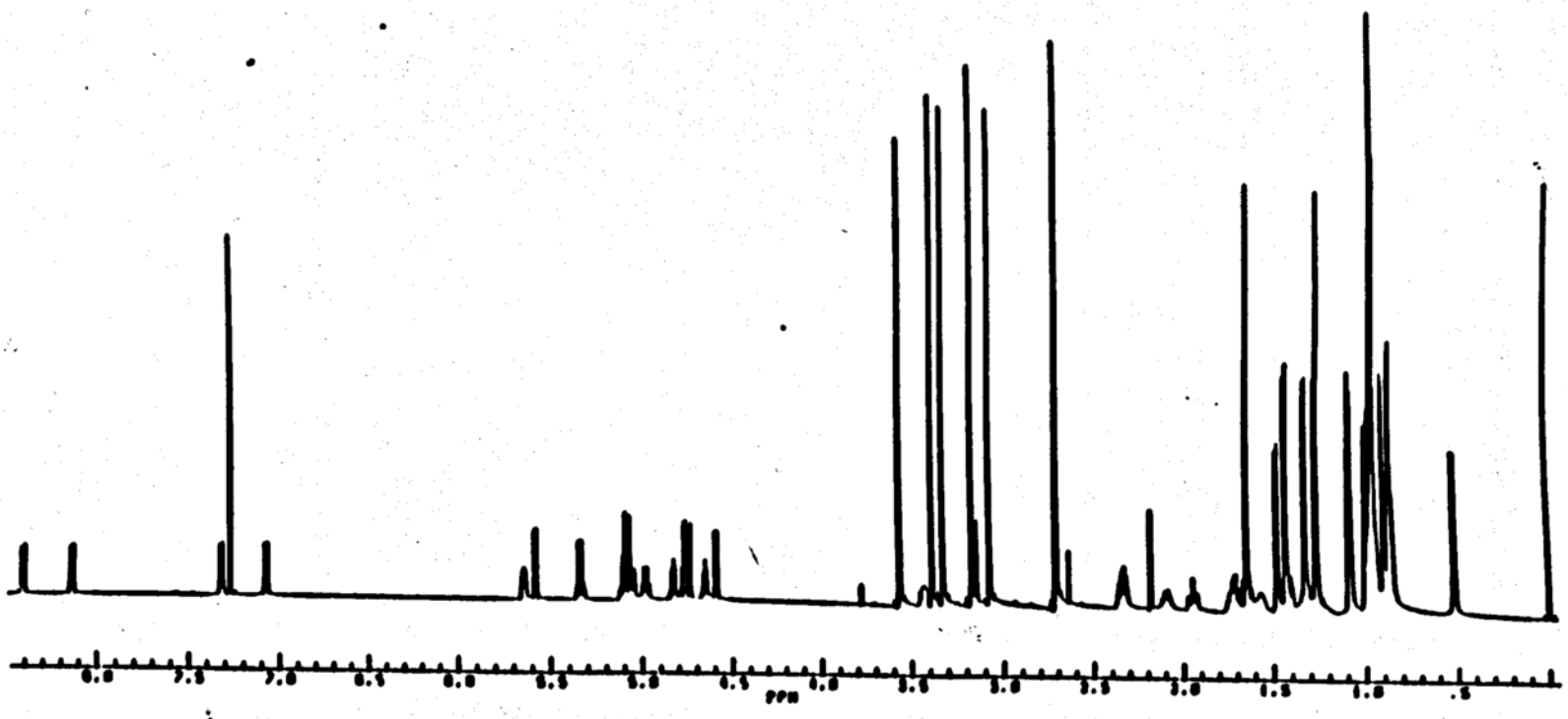

Figure 5.6. [MeLeu(OH)^{1},D-Lys(Boc)^{8}]CsA (5.21d)

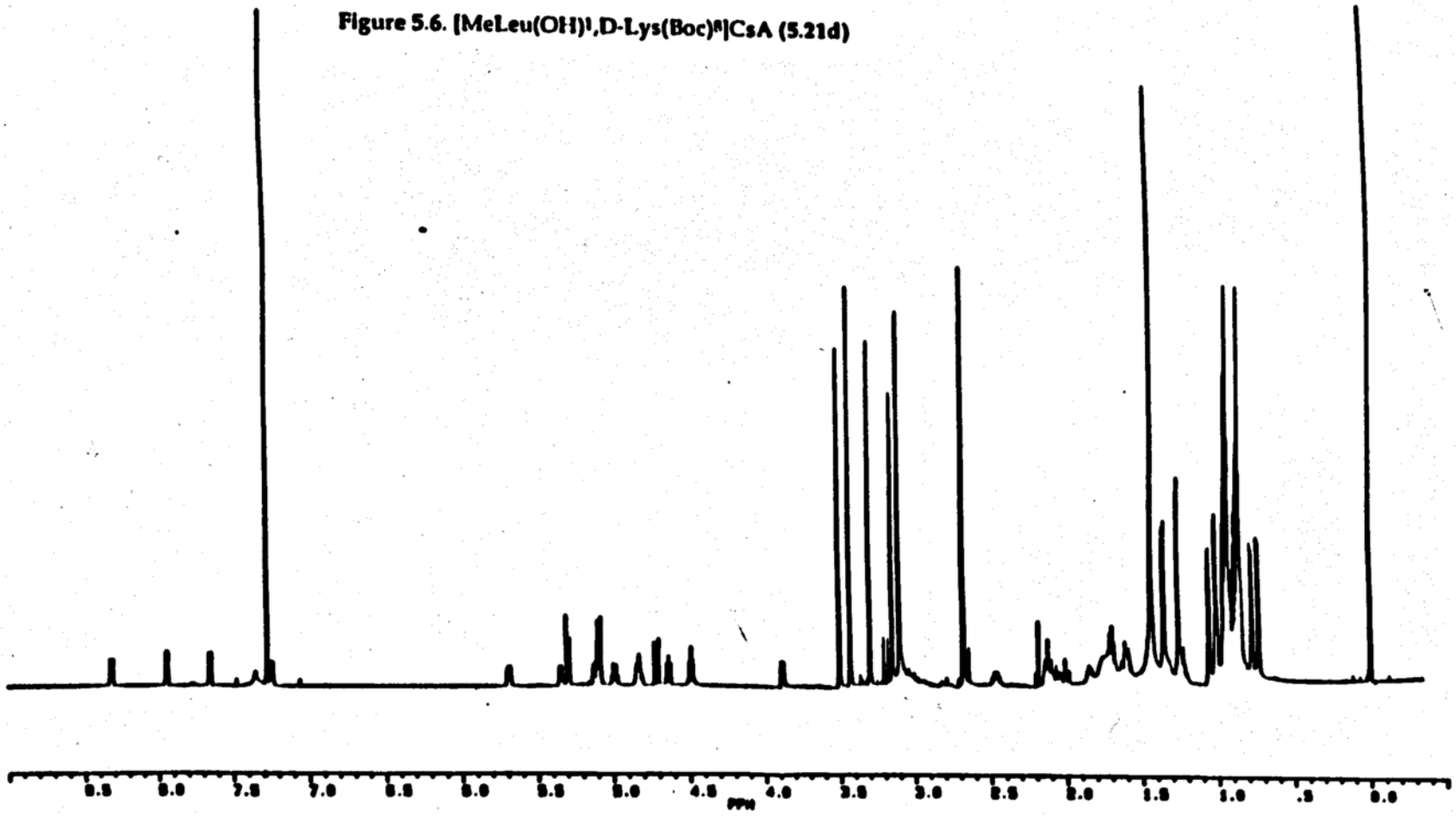

Figure 5.7. [D-MeVal[11],MeLeu(OH)[1]]CsA (5.23)