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Syntheses, bioactivities and conformations of peptidomimetics containing 2,3-methanoamino acids

Ho, Kwok-Kan, Ph.D.

Rice University, 1994
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SYNTHESES, BIOACTIVITIES AND CONFORMATIONS OF PEPTIDOMIMETICS CONTAINING 2,3-METHANOAMINO ACIDS

by

Kwok-Kan Ho

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE DOCTOR OF PHILOSOPHY

APPROVED, THESIS COMMITTEE

K. Burgess, Associate Professor, Chair Chemistry

R. J. Parry, Professor Chemistry

E. Nikonowicz, Assistant Professor Biochemistry and Cell Biology

Houston, Texas
May, 1994
ABSTRACT

Syntheses, Bioactivities and Conformations of Peptidomimetics Containing 2,3-Methanoamino Acids

by

Kwok-Kan Ho

Asymmetric syntheses of E- and Z-2,3-methanomethionine (ie E- and Z-cyclo-Met), protected Z-2,3-methanogarginine, and Z-2,3-methanoaspartic acid derivatives have been developed. These functionalized 2,3-methanoamino acids were prepared from a common intermediate: 1-[(tert-butoxy)carbonyl]-2-oxo-3-oxa-bicyclo[3.1.0]hexane.

Peptidomimetics containing some of these 2,3-methanoamino acids mentioned above were synthesized via a solid phase approach. The Met2 in neuropeptide Phe-Met-Arg-Phe-NH2 (FMRF-NH2, one letter code for amino acids) was systematically replaced by each of the isomers of cyclo-Met giving four peptidomimetics, namely F{2S,3R-cyclo-M}RF-NH2, F{2R,3S-cyclo-M}RF-NH2, F{2S,3S-cyclo-M}RF-NH2 and F{2R,3R-cyclo-M}RF-NH2. A peptidomimetic containing two 2,3-methanoamino acids substituted in the 2 and 4 positions (ie F{2S,3S-cyclo-M}R{2R,3R-cyclo-F}·NH2) was also prepared. Generally, 2,3-methanoamino acids are compatible with solid phase chemistry, although they are more sterically hindered than their corresponding natural amino acids.

Anti-opiate activities, and proteolytic stabilities of the peptidomimetics were studied. The peptidomimetics were more active (in vivo) than the FMRF-NH2 but bind less strongly to the appropriate receptor sites. This observation seems to be related to the enhanced bio-availability of the peptidomimetics, because the F{E-cyclo-M}RF-NH2
analogs were much more proteolytically stable than the parent peptide with respect to leucine aminopeptidase digestion.

Solution conformations of the peptidomimetics were investigated by NMR. Incorporation of 2,3-methanoamino acids into peptides induced NMR observable conformational rigidity. This was evident from: (i) interresidue ROE crosspeaks centered at the methanologs; (ii) unusual chemical shifts and temperature coefficients of some NH signals; and, (iii) line broadening for F{2S,3S-cyclo-M\}R{2R,3R-cyclo-F}-NH\textsubscript{2} in the \textsuperscript{1}H-NMR spectrum.

The NMR data was correlated with the structures obtained from quenched molecular dynamics (QMD), which was performed by using a set of empirical parameters. Structures obtained from the QMD studies gave good correlation with the experimental data. Defined secondary structure (γ-turn) was observed for peptidomimetic F{2S,3S-cyclo-M}RF-NH\textsubscript{2}. 
ACKNOWLEDGEMENTS

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List of Abbreviations

cyclo- 2,3-methano-
Acc 1-aminocyclopropane-1-carboxylic acid
CCK cholecystokinin
DPPA diphenylphosphoryl azide
CSF cerebral spinal fluid
Boc tert-butoxycarbonyl-
Fmoc 9-fluorenlymethoxycarbonyl-
DCC 1,3-dicyclohexylcarbodiimide
EDC 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
MBHA resin p-methylbenzydrylamine resin
BOP benzotriazolyloxy-tris(dimethylaminophosphonium)
         hexafluorophosphate
RP-HPLC reverse phase-high performance liquid chromatography
HOBt N-hydroxybenzotriazole
Mtr 4-methoxy-2,3,6-trimethylbenzene sulfonyl-
Rink resin 4-(2',4'-dimethoxyphenyl-Fmoc-aminomethyl)-phenoxy resin
DQF-COSY double quantum filter correlation spectroscopy
NOESY nuclear Overhauser enhancement spectroscopy
ROESY rotating-frame Overhauser enhancement spectroscopy
HOHAHA homonuclear Hartmann-Hahn
SD steepest decent
ABNR adopted basis Newton-Raphson
Chapter 1. Reviews on Asymmetric Syntheses of 2,3-Methanoamino Acids

1.1 Introduction

This chapter is a summary of the asymmetric syntheses of 2,3-methanoamino acids. As this is a review, there is no experimental data for this section.

2,3-Methanoamino acids, also previously referred to as "methanologs", "cyclopropyl amino acids" and "cyclopropylogs", may be structurally related to natural amino acids in two ways, as exemplified by arginine in Figure 1.1. Joining the Cα and Cβ carbons of the Arg with a methylene group (ie the cyclo-Arg) gives "2,3-methanologine". Alternatively, a cyclopropane ring can be formed by joining Cα and Cγ with a bond (ie the cyclo-Arg') giving an arginine analog with one carbon less in the side chain. This work is concentrated on the first type of compound.

Figure 1.1. Types of 2,3-methanoamino acids exemplified with Arg analogs.
1.1.1 Applications of Optically Active 2,3-Methanoamino Acids

Enantioenriched 2,3-methanoamino acids have been used as mechanistic probes, and as enzyme inhibitors. For instance, selectively deuterated Acc (1-aminocyclopropane-1-carboxylic acid) was used to investigate the biosynthesis of ethene in plants,\(^1\) and of ammonia and 2-ketobutyrate in *Pseudomonas*.\(^2\) Some cyclopropane-based peptidomimetics of substrates for zinc metalloproteins have been shown to act as suicide enzyme inhibitors via ring cleavage mediated by the Lewis acidic zinc center, and concomitant alkylation of a residue in the enzyme active site.\(^3\),\(^4\) Cyclo-*m*-Tyr analogs were shown to be competitive inhibitors of pig liver L-aromatic amino acid decarboxylase against D-*m*-tyrosine.\(^5\)

\[
\begin{align*}
\text{Acc} & : & \text{H}_2\text{N} & \text{ COOH} \\
\text{dimethyl-Acc} & : & \text{H}_2\text{N} & \text{ COOH} \\
\text{trimethyl-Acc} & : & \text{H}_3\text{C} & \text{ CH}_3
\end{align*}
\]

\[
\begin{align*}
\text{cyclo-*m*-Tyr} & : & \text{H}_2\text{N} & \text{ COOH} \\
\text{Z-cyclo-Phe} & : & \text{H}_2\text{N} & \text{ COOH} \\
\text{E-cyclo-Phe} & : & \text{H}_2\text{N} & \text{ COOH}
\end{align*}
\]

Perhaps the most exciting applications of 2,3-methanoamino acids are for syntheses and design of conformationally constrained peptidomimetics.\(^6\)\textsuperscript{15} Peptide analogs from 2,3-methanoamino acids are relatively rigid, a characteristic which influences the biological properties. Incorporation of a methanolog could be rationally applied to almost any bioactive peptide.
Some examples of this type of research have already emerged. For instance, replacement of Phe by cyclo-Phe gave tasteless analogs of aspartame (Asp-Phe-OMe), although Asp-Acc-O(n-Pr) was found to be 250-300 times sweeter than sucrose.\textsuperscript{15,17} Conversely, some dimethyl-Acc and trimethyl-Acc aspartame analogs [Asp-(Me)\textsubscript{n}Acc-OMe] gave a bitter taste.\textsuperscript{14}

Neuropeptides containing 2,3-methanoamino acids also have been studied. Substitution of Phe with cyclo-Phe in [D-Ala\textsuperscript{2}, Leu\textsuperscript{5}]-enkephalin gave analogs with lower affinity for the opioid receptors in mouse vas deferens and guinea pig ileum.\textsuperscript{18-21} These peptidomimetics bound less strongly to the receptors than the [D-Ala\textsuperscript{2}, Leu\textsuperscript{5}]-enkephalin, but they are potentially useful for their \(\delta\)-receptor selectivity.\textsuperscript{22} These enkephalin analogs had exceptional proteolytic stability towards carboxypeptidase digestion.\textsuperscript{18} This implies substitution of methanologs for protein amino acids generally can provide proteolytically stable materials. Finally, CCK (26-33) analogs containing Z-cyclo-Phe or E-cyclo-Phe show different selectivities for the CCK-A or CCK-B receptors, although they were less tightly bound than the parent peptide.\textsuperscript{23}

Incorporation of 2,3-methanoamino acids into peptides provides access to alternate their conformations, and in turn their bioactivities as exemplified above. However, the number of proven cases is few in comparison with the vast potential for this type of peptidomimetic.

1.1.2 Naturally Occurring 2,3-Methanoamino Acids

Several 2,3-methanoamino acids occur naturally (Table 1.1). Isolation from these sources is inconvenient for the production of 2,3-methanoamino acids on a large scale. The significance of such isolation studies is therefore mainly connected with work designed to elucidate the biosynthetic origins of methanologs.
Table 1.1. Naturally occurring 2,3-methanoamino acids.

<table>
<thead>
<tr>
<th>amino acid</th>
<th>natural source</th>
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<tr>
<td>H₂N[bracket]COOH&lt;br&gt;Acc</td>
<td>intermediate in the conversion of Met to ethene in plants¹,²⁴,²⁵</td>
</tr>
<tr>
<td>H₂N₆[bracket]COOH&lt;br&gt;C₂H₅&lt;sub&gt;coronamic acid&lt;/sub&gt;</td>
<td>isolated after hydrolysis of bacterial toxin coronatine in Pseudomonas coronafaciens²⁶</td>
</tr>
<tr>
<td>H₂N₆[bracket]COOH&lt;br&gt;CH₃&lt;sub&gt;norcoronamic acid&lt;/sub&gt;</td>
<td>isolated after hydrolysis of bacterial toxin norcoronatine in Pseudomonas syringae²⁷</td>
</tr>
<tr>
<td>H₂N[bracket]COOH&lt;br&gt;H₂C₂&lt;sub&gt;allo-coronamic acid&lt;sup&gt;a&lt;/sup&gt;&lt;/sub&gt;</td>
<td>substrate for 1-butene biosynthesis in plants²⁸</td>
</tr>
<tr>
<td>HOOC&lt;sub&gt;camosadine&lt;/sub&gt;</td>
<td>produced by the red algae, Grateloupia carmasa²⁹,³⁰</td>
</tr>
</tbody>
</table>

<sup>a</sup> This compound has not been isolated from plant source.

1.2 Syntheses of 2,3-Methanoamino Acids by Fractional Recrystallizations or Kinetic Resolutions

Biological studies generally require optically pure compounds because trace stereoisomeric impurities can skew bioassays. Progress in the development of
methanolog-containing peptidomimetics has been retarded by the lack of efficient asymmetric syntheses.

The earliest synthetic routes to optically active 2,3-methanoamino acids involved preparation of racemic materials, followed by resolution via fractional recrystallization of diastereomeric salts or derivatives. These approaches have been reviewed by Stammer\textsuperscript{31} and Alami\textsuperscript{32}

**Scheme 1.1. Methods for preparation of racemic 2,3-methanoamino acids.**

EWG = electron withdrawing groups, e.g. -COOR, -CN, -NC, -NCPh\textsubscript{2} etc

Generally, racemic cyclopropane intermediates have been formed by (Scheme 1.1) : (i) reaction of a glycine or malonate derivative with a dielectrophile such as 1,2-dibromoethane; (ii) Michael additions of dimethyl sulfoxonium methylide; or, (iii) 1,3-dipolar additions of diazomethane to an alkene. Quinine and ephedrine salts have been
used to resolve 2,3-methanoamino acid derivatives.\textsuperscript{5} Alternatively, some functional
groups have been covalently united to facilitate separation, \textit{eg} compound \textbf{1}.\textsuperscript{11}

\[ \text{R} = \text{Me or Et} \]

\textbf{1}

Biocatalytic resolutions are potentially useful for preparations of some optically
active 2,3-methanoamino acids. Nevertheless, the only reported enzymatic resolution of a
2,3-methanoamino acid is of \textit{N}-chloroacetyl norcoronamic acid \textbf{2} resolved by porcine
kidney acylase \textbf{1}.\textsuperscript{11} However, intermediates for syntheses of 2,3-methanologs, \textit{ie} the
tetrahydropyranyl derivative \textbf{3} and cyclopropane diester \textbf{4}, were resolved by esterase
30,000.\textsuperscript{33,34} At 60\% conversion, 90\% \textit{ee} of tetrahydropyranyl \textbf{3}, and the optically
enriched diester \textbf{4} (no \textit{ee} reported) were observed. Similarly, when dioxolane \textbf{5} was
resolved by carboxyl esterase NP, an optical yield of 91\% \textit{ee} was obtained at 60\%
conversion.\textsuperscript{35}
1.3 Asymmetric Syntheses Based on Diastereoselective Reactions

Diastereoselective reactions mediated by chiral auxiliaries have been explored to prepare 2,3-methanoamino acids. Such reactions may be grouped in the following categories: (i) dialkylation of chiral glycine equivalents with dielectrophiles; (ii) Michael additions of sulfoxonium ylides; (iii) 1,3-dipolar additions of diazomethane to chiral dehydroamino acids; (iv) diastereoselective addition of chiral nucleophiles; and (iv) rhodium catalyzed cyclopropanations using chiral diazo compounds.

The synthetic challenge for preparation of 3-substituted 2,3-methanoamino acids is to set up both C2 and C3 chiral centers at once. Reaction of a glycine equivalent with a dielectrophile was effective to control the diastereoselectivity of the C2 carbon, but less diastereoiinduction was observed for the C3 chiral center. Michael addition of sulfoxonium ylide, or 1,3-dipolar addition of diazomethane to a chiral dehydroamino acid involved the addition of a methylene synthon to either the Si or Re face of an alkene. This face selectivity determined the enantiomeric purity of the 2,3-methanoamino acid, and the E- or Z-stereochemistry of the 3-substituent was predetermined by the dehydroamino acid component. For the rhodium catalyzed cyclopropanation, the stereomeric outcome is more difficult to predict, but a rhodium carbene transition state has been proposed.\textsuperscript{36,37}

1.3.1 Dialkylation of Chiral Glycine Equivalents with Dielectrophiles

Schollkopf's bis-lactim ethers have been used to make a few 2,3-methanoamino acids, eg allo-coronamic acid, Scheme 1.2.\textsuperscript{38} Thus, bis-lactim ether 6 was reacted with trans-1,4-dichloro-2-butene to give vinyl cyclopropane 7 containing 85% of the major isomer, and 15% of the other three possible diastereoisomers combined. Separation of the diastereomeric mixture is required at this stage. Diimide reduction of 7 followed by
hydrolysis afforded *allo*-coronamic acid. *Cyclo-Phe*\textsuperscript{23} and deuteriated Acc\textsuperscript{39} were also prepared using similar approaches.

**Scheme 1.2.** Synthesis of *allo*-coronamic acid via Schollkopf's bis-lactim ether method.

Syntheses of this type require separation of diastereoisomers. This inconvenience, coupled with the cost of the reagents, constitutes a significant obstacle to preparation of multi-gram quantities of the desired products.

Seebach's chiral imidazolidinone auxiliaries seem to be even less suitable than Schollkopf's system for the preparation of 2,3-methanoamino acids. This assertion is based on the fact that attempted preparation of selectively deuteriated Acc using auxiliary 8 failed (Scheme 1.3).\textsuperscript{40} The first alkylation with methyl bromoacetate afforded 9 with good *trans* selectivity relative to the *tert*-butyl group. However, the second alkylation of compound 9 occurred in a stereorandom fashion, so a *racemic* mixture of deuteriated Acc 11 was obtained after hydrolysis of cyclopropane 10.
Scheme 1.3. Synthesis of deuteriated Acc via Seebach's imidazolidinone auxiliary.

Husson et al have developed syntheses of 2,3-methanoamino acids based on N-cyanomethyl-1,3-oxazolidine 13.\textsuperscript{41,42} This oxazolidine was reacted with racemic epibromohydrin 12 to give all four possible isomers of cyclopropane 14 in a ratio of 44:37:12:7 (Scheme 1.4). Partial chromatographic separation of these gave two pairs of compounds, each pair contained two isomers with the hydroxyl side chain either cis or trans to the amino function. Lactonization of the trans isomer provided 15, which was then separated from the uncyclized cyclopropane 16 by a second column chromatography. Compounds 15 and 16 were then converted to the 2,3-methanohomoserines 17 and 18, respectively, then to allo-coronamic acid\textsuperscript{43} and carnosadine.\textsuperscript{44}
Scheme 1.4. Synthesis of 2,3-methanohomoserine via Husson's auxiliary.

Extensive chromatography was required for this approach because the two chiral centers were introduced in a near stereorandom fashion. Consequently, this route is impractical for large scale preparations of 2,3-methanamino acids.

1.3.2 Michael Additions of Sulfoxonium Ylides to Chiral Dehydroamino Acids

Excellent diastereoselectivity has been obtained by using a diphenyltetrahydro-oxazinone derivative 19 to direct Michael additions of [(diethylamino)phenyl]oxosulfonium methyldie giving the bicyclic oxazinone 20 (Scheme 1.5).\(^\text{45}\) Reaction occurred to the same face as the diphenyl substituents of the oxazinone, presumably due to \(\pi\)-stacking between the phenyl group of the ylide and the diphenyl...
substituents of the oxazinone. The phenylcyclopropyl lactone system 20 could not be deprotected under reductive conditions since the benzylic cyclopropane bond is easily cleaved in this way. Consequently, removal of the chiral auxiliary was achieved by an oxidative procedure employing lead tetraacetate. An overall yield of 28% was obtained for \textit{E-cyclo-Phe} from 20.\textsuperscript{46}

\textbf{Scheme 1.5.} Synthesis of \textit{E-cyclo-Phe} by diphenyltetrahydrooxazinone auxiliary.

In syntheses related to that shown in Scheme 1.5, alkyl-substituted 2,3-methanoamino acids were prepared by using similar addition reactions but with a reductive cleavage step to remove the auxiliary (eq 1.1).\textsuperscript{45} Thus, deblocking the cyclopropyl lactone 23 with Li/NH\textsubscript{3} gave about 60% yield of the corresponding \textit{E-2,3-methanoamino acid} 24 (eq 1). Only the \textit{E}-isomers were accessible using this approach; the corresponding \textit{Z}-isomers have not been prepared by an analogous method.
Asymmetric Michael additions also shown promise for syntheses of other 2,3-methanoamino acids. Crich's group synthesized the tryptophan-based auxiliary 25 as a Michael acceptor for sulfoxonium ylide addition.\textsuperscript{47} Reaction of the tricyclic compound 25 with the ylide gave the tetracyclic derivative 26 in 56% yield (Scheme 1.6) via cyclopropanation on the convex face of the alkene. Reaction of the tetracycle with TFA led to ring opening, and afforded the protected \textit{Z-cyclo-Trp} analog 27. Unfortunately, attempted removal of the indole protecting group mediated ring opening of the cyclopropane, so the free 2,3-methanotryptophan could not be obtained.

\textbf{Scheme 1.6.} Synthesis of \textit{Z-cyclo-Trp} analog from an auxiliary prepared from Trp.
1.3.3 1,3-Dipolar Additions of Diazomethane to Dehydroamino Acid Auxilaries

Diketopiperazine\textsuperscript{48,49} and hydroxypinanone\textsuperscript{50} derived auxilaries in 1,3-dipolar additions of diazomethane have been explored for the syntheses of 3-substituted 2,3-methanoamino acids. For instance, a Z-cyclo-Phe was prepared by the addition of diazomethane to diketopiperazine 28 (Scheme 1.7), a reaction which apparently proceeds with >95 \% diastereoselectivity.\textsuperscript{48} Hydrolysis of the auxiliary, followed by removal of proline, afforded the Z-cyclo-Phe in 44 \% overall yield from 28. Allo-coronamic acid was prepared using an analogous method.\textsuperscript{49}

Scheme 1.7. Synthesis of Z-cyclo-Phe from diketopiperazine auxiliary.

![Scheme 1.7. Synthesis of Z-cyclo-Phe from diketopiperazine auxiliary.](image)

Inferior yields and diastereoselectivity were observed for diazomethane addition to pinanone auxiliary 30 in the preparation of cyclopropane derivative 31 (Scheme 1.8).\textsuperscript{50} This reaction type was attempted for two derivatives of 30 (R = Me or R = Et). When R = Me, 35 \% yield of the major isomer 31 was obtained after chromatographic separation of a mixture containing the other diastereoisomer and starting material 30. Hydrolytic removal of the pinanone auxiliary afforded the allo-norcoronamic acid. Since separation of the tricyclic derivative 31 for R = Et was not possible, optically pure allo-coronamic could not be prepared via this route.
Scheme 1.8. Synthesis of *allo*-norcoronamic acid and *allo*-coronamic acid via 2-hydroxy-3-pinanone auxiliary.

\[
\begin{align*}
\text{O} & \quad \text{N} & \quad \text{O} \\
\text{30} & \quad \text{CH}_2\text{N}_2 & \quad \text{31} \\
\text{O} & \quad \text{N} & \quad \text{O} \\
\text{HCl} & \quad \text{HOOC} & \quad \text{31} \\
\text{R} = \text{Me}, \text{allo}-\text{norcoronamic acid} & \quad \text{R} = \text{Et}, \text{allo}-\text{coronamic acid}
\end{align*}
\]

1.3.4 Diastereoselective Addition of a Chiral Nucleophile

Diastereoselective addition of 4,5-diphenyloxazolidine-2-one 33 to Michael acceptor 2-chloro-2-cyclo-propylideneacetate 32 gave the cyclopropyl derivative 34 with almost complete stereoselectivity (Scheme 1.9). Separation of the two *trans* cyclopropane diastereomers, reductive dehalogenation, then azide transfer yielded compound 35. Successive treatment of the azide with base and acid furnished an α-keto acid 36. Decarboxylation of this α-keto acid under oxidative conditions, followed by hydrogenolytic removal of the auxiliary afforded methanolog 37. Various amino acids including the coronamic acids (R = Et), norcoronamic acids (R = Me), and 2,3-methanohomoserine derivatives (R = CH₂OMe, CH₂OBz and CH₂OH) were prepared by this method.
Scheme 1.9. Synthesis of 2,3-methanoamino acids via 4,5-diphenyloxazolidine-2-one auxiliary.

1.3.5 Rhodium Catalyzed Cyclopropanation

A pantolactone ester has been used to direct a rhodium catalyzed cyclopropanation for synthesis of a cyclo-Phe stereoisomer. Indeed, asymmetric cyclopropanation using vinyldiazomethane derivatives of (R)-(−)-pantolactone 38 (Scheme 1.10) constitutes possibly the most practical synthesis of 2R,3R-cyclo-Phe published to date.\textsuperscript{36,37} The vinylcyclopropane derivative 39 was obtained in \textit{de} $>98\%$ after a single recrystallization. Oxidative cleavage of the benzylidene, Curtius rearrangement of the resulting carboxylic acid, and hydrolysis of the chiral auxiliary gave the 2R,3R-cyclo-Phe.
Scheme 1.10. Synthesis of $2R,3R$-cyclo-Phe via rhodium catalyzed cyclopropanation.

Preparation of the $2S,3S$-cyclo-Phe by the approach outlined in Scheme 1.10 is difficult because $(S)$-$(+)$-pantolactone is not commercially available. However, an variant of the key step has been performed using a chiral rhodium catalyst from $(S)$-$N$-benzenesulfonylprolinate 41 to give an enantioselective cyclopropanation (eq 1.2). $^{52}$
Thus, cyclization of vinyl diazomethane derivatives 40 gave the 2S,3S-vinylcyclopropane analog 42 with 90% ee. This route potentially could be modified to give the 2S,3S-cyclo-Phe, although this has not yet been reported.

One copper catalyzed cyclopropanation was recently reported to prepare γ-lactone 45 (eq 1.3), but poor enantioselectivity (ca 32% ee) was obtained. Lactone 45 has been used as a key intermediate in preparations of some 2,3-methanoamino acids (vide infra).

1.4 Use of Optically Pure Starting Material from A Chiral Pool

Starting materials from the chiral pool also have been used to prepare 2,3-methanoamino acids. Allo-norcoronamic acid was synthesized from reacting optically active dibromopropane 46 with ethyl isocyanoacetate 47. High degree of anti diastereoselection for the alkyl and carbonyl group were observed (eq 1.4). However, the dibromopropane prepared from (S)-2-bromopropionic acid had only 92% ee.

Pirrung and co-worker developed the first asymmetric synthesis of one isomer of 2,3-methanohomoserine (Scheme 1.11). The source of chirality was obtained from the
optically active epichlorohydrin prepared from D-mannitol. The reactions involved
selective attack of the malonate anion at C3 of epichlorohydrin 48. Cyclopropanation
followed by lactonalization in the presence of excess base afforded 49. Ammonolysis, and
temporary protection of the hydroxy function by acetylation yielded 50. Hofmann
rearrangement, and removal of protecting groups gave 2,3-methanohomoserine 52.
Although most of the optical purity was transferred from the epichlorohydrin to the lactone
49, low yield was observed (ca 30 %) for this reaction. This synthesis is also
inconvenient because many steps are required for preparation of the optically active
epichlorohydrin from mannitol.

**Scheme 1.11.** Syntheses of 2,3-methanohomoserine from epichlorohydrin.

Norcoronamic acid was synthesized from commercial available optically active 3-
hydroxy-2-methyl-propionate 53 (Scheme 1.12). Conversion of 53 to an imine, followed
by cyclization gave cyclopropane 56. Hydrolyses of the imine and the nitrile afforded the
norcoronamic acid.
**Scheme 1.12.** Synthesis of norcoronamic acid from 3-hydroxy-2-methyl-propionate.

\[
\text{HO} \xrightarrow{(i) \text{TBSCI}} \text{TBSO} \xrightarrow{(ii) \text{DIBAL}} \text{CHO} \xrightarrow{(i) \text{TMSCN, ZnI}_2} \text{(iii) \text{n-Bu}_4\text{NF}} \text{(iv) \text{TsCl}} \text{(v) LiCl} \text{(iv)\text{TsCl}} \text{(v) LiCl} \text{\text{CHO}}
\]

\[
\text{Cl} \xrightarrow{(i) \text{(COCl)}_2, \text{DMSO, NEt}_3} \text{H}_3\text{C} \xrightarrow{(i) \text{HCl}} \text{HOOC} \xrightarrow{(ii) \text{NaOH}} \text{NH}_2
\]

1.5 Conclusion

Many of the syntheses discussed up to now involved difficult fractional recrystallizations, imperfect diastereoselectivity, and/or costly or otherwise inconvenient starting materials. Except for a few cases, nearly all of them are only suitable for alkyl or aryl substituted methanologs. In our work, we sought to use material from the chiral pool to prepare 2,3-methanoamino acids, particularly those with functionalized side chains. Chapter 2 will discuss the preparations of several functionalized 2,3-methanoamino acids analogs based on this approach.
Chapter 2. Asymmetric Syntheses of $E$- and $Z$-cyclo-Met, Boc-$Z$-cyclo-Arg(Ts), and Boc-$Z$-cyclo-Asp-O-$t$-Bu

2.1 Introduction

Asymmetric syntheses of $E$- and $Z$-cyclo-Met, protected derivatives of $Z$-cyclo-Arg, and $Z$-cyclo-Asp are presented in this chapter. These 2,3-methanoamino acids are side chain functionalized, and each of them may exist in four stereochemically distinct isomeric forms (e.g., Figure 2.1).

Figure 2.1. Four stereoisomers of cyclo-Met.

\[
\begin{align*}
\text{HO}_2\text{C} & \text{NH}_2 \\
\text{Z-2S,3R-cyclo-Met} & \text{Z-2R,3S-cyclo-Met} \\
\text{HO}_2\text{C} & \text{NH}_2 \\
\text{E-2R,3R-cyclo-Met} & \text{E-2S,3S-cyclo-Met} \\
\end{align*}
\]

Retrosynthetic analyses of these methanologs converge to 2,3-methanohomoserine (Figure 2.2), which in turn can be synthesized from the 1-[(tert-butoxy)carbonyl]-2-oxo-3-oxa-bicyclo[3.1.0]hexane 45. The general strategy conceived then executed in this work involved selective conversion of the ester or the lactone function of 45 to an amine derivative, then transformation of the hydroxy group produced by opening the lactone to the side chain functionalities of the corresponding amino acids. The syntheses described in
the following subsections will illustrate the use of lactone 45 to prepare several 2,3-methanoamino acids.

**Figure 2.2.** Intermediates for syntheses of side chain functionalized 2,3-methanologs.

![](image)

2,3-methanohomoserine

### 2.2 Syntheses of 1-[(tert-Butoxy)carbonyl]-2-oxo-3-oxa-bicyclo[3.1.0]hexane 45

Pirring and co-workers had shown that near optically pure (R)-epichlorohydrin 48 (prepared from D-mannitol) reacts with dimethyl malonate to give a *IS,5R*-bicyclic lactone 49 with 93.4 % ee in 36 % yield (Figure 2.3).\(^{54}\)

**Figure 2.3.** The configuration of lactone 49 is governed by the regioselectivity of the first nucleophilic attack on the epichlorohydrin 48.
The slight loss of stereochemical fidelity observed in this process is a consequence of direct displacement of the chloride competing with attack at the epoxide followed by a Payne rearrangement, the latter being the major pathway (Figure 2.3).

In the present work, the triflate 57 was chosen as a starting material. Reaction of this with di-tert-butyl malonate gave lactone 1R,5R-45 in 48 % yield (eq 2.1), with most of the optical activity transferred from the glycidal triflate (92 % ee) to the lactone (91 % ee). The reaction proceeds via direct displacement of triflate followed by ring opening of the epoxide. This observation is consistent with the literature for reaction of substrate 57 with oxygen nucleophiles. Both enantiomers of the glycidol precursor may be purchased or conveniently prepared, therefore, the 1S,5S-45 also was prepared via the same route.

\[
\text{O} \quad \text{OTf} + \quad \text{tBuO}_2\text{C} - \text{CO}_2\text{tBu} \quad \text{NaH, C}_6\text{H}_6, 15\text{-crown-5} \quad \text{tBuO}_2\text{C} - \text{CO}_2\text{H} \quad \text{eq 2.1}
\]

25 °C (48 %)  \quad 1R,5R-45, 91 % ee

2.3 Syntheses of Z-cyclo-Met

Transformation of lactone 1R,5R-45 into alcohol 60 (Scheme 2.1) is directly analogous to steps used in a literature preparation of model compounds for investigations of ethene biosynthesis. First, the lactone was treated with aqueous ammonia, and the resulting alcohol was protected by acylation to give amide 58. Hofmann rearrangement of the amide, and base hydrolysis of the acetate group afforded alcohol 60. Mosher's derivatization method indicated the alcohol 60 had an enantiomeric excess of greater than 95 % after recrystallization. Mesylation of this alcohol, reaction with thiomethoxide, and TFA removal of protecting groups gave Z-cyclo-Met. Interestingly, some similar reactions attempted in the corresponding synthesis of E-cyclo-Met were unsuccessful (vide infra).
**Scheme 2.1. Synthesis of Z-cyclo-Met.**

\[ \text{tBuO}_2\text{C}-\text{NHBOc} \xrightarrow{\text{(i) NH}_4\text{OH}(aq), 25 ^\circ\text{C}} \text{tBuO}_2\text{C}-\text{CONH}_2 \quad \text{Pb(OAc)}_4, \text{t-BuOH, reflux} \]

\[ \text{tBuO}_2\text{C}-\text{NHBoc} \xrightarrow{\text{(i) Ac}_2\text{O, cat DMAP, NEt}_3, \text{CH}_2\text{Cl}_2} \] (85%)

\[ \text{K}_2\text{CO}_3, \text{MeOH, 70 ^\circ\text{C}} \xrightarrow{(83\%)} \text{tBuO}_2\text{C}-\text{NHBoc} \xrightarrow{\text{(i) MsCl, NEt}_3, \text{CH}_2\text{Cl}_2} \]

\[ \text{K}_2\text{CO}_3, \text{MeOH, 70 ^\circ\text{C}} \xrightarrow{(83\%)} \text{tBuO}_2\text{C}-\text{NHBoc} \xrightarrow{\text{(ii) NaSMe, DMF, 25 ^\circ\text{C}}} \] (81%)

\[ \text{tBuO}_2\text{C}-\text{NHBOc} \xrightarrow{\text{(i) CF}_3\text{CO}_2\text{H, CH}_2\text{Cl}_2} \text{HO}_2\text{C}-\text{NH}_2 \xrightarrow{\text{(ii) Dowex-H}^+} \] (80%)

\[ \text{Z-2S,3R-cyclo-Met} \]

An alternative to the approach depicted in Scheme 2.1 would be to resolve one of the intermediates in the corresponding racemic synthesis; this would be economical since both enantiomers of Z-cyclo-Met are required for future work. Racemic lactone 60 was readily obtained in 61% yield via the reaction of di-t-butyl malonate with (±)-epibromohydrin (see Appendix 1). Several biocatalytic resolutions of racemic intermediates in Scheme 2.1 were explored.

\[ \text{tBuO}_2\text{C}-\text{NHBOc} \xrightarrow{\text{AcO-CH}_2} \text{tBuO}_2\text{C}-\text{NHBoc} \quad \text{Lipase P} \]

54% ee at 53% conversion

\[ \text{tBuO}_2\text{C}-\text{NHBOc} \]

(eq 2.2)

Enzyme mediated hydrolysis of racemic 59 or acetylation of racemic 60 was studied using lipase from *Pseudomonas AK, Pseudomonas K*₁₀, *Candida cylindracea*, procine pancreatic lipase, lipase P and papain. The most encouraging result was obtained for
acylation of alcohol 60 mediated by Lipase P from Amano (eq 2.2), but enantioselection in this and related reactions was below acceptable levels for a practical asymmetric synthesis.

2.4 Syntheses of E-cyclo-Met

Scheme 2.2 describes the route eventually developed to E-cyclo-Met. The initial step in the synthesis was to introduce the α-amino functionality. Consequently, treatment of lactone 1R,5R-45 with trifluoroacetic acid was used to hydrolyze the t-butyl ester, providing a carboxylic acid.

Scheme 2.2. Synthesis of E-cyclo-Met.

1R,5R-45

(i) CF$_3$CO$_2$H, CH$_2$Cl$_2$
(ii) (PhO)$_2$PON$_3$NET$_3$,

$t$-BuOH (83 %)

62

(i) NH$_4$OH$_{(aq)}$, 25 °C
(ii) CH$_2$(CH$_2$)$_4$COCl,

NET$_3$, DMAP

63

CONH$_2$

TSO$_2$P(NO$_2$)$_2$,

py, reflux

(62 % from 62)

64

65

Candida cylindracea
pH 7.5 buffer (87 %)

BocHN

66

CN

OH

Me$_2$SO,

NET$_3$, CH$_2$Cl$_2$

(88 % from 65)

67

K$_2$CO$_3$, MeOH, 0 °C

(89 %)

68

(i) (MeO)$_2$SO$_2$, NaOH, MeOH, 0 °C
(ii) 6M HCl, reflux (52 %)

H$_2$N

E-2R,3R-cyclo-Met

CO$_2$H

SMe
Curtius rearrangement of the carboxylic acid in the presence of t-BuOH gave Boc protected amino lactone 62. The major challenge in this sequence was then encountered: to open lactone 62 and manipulate the products in such a way that the two functionalized tethers will not reform a cyclic system. This was achieved by reacting lactone 62 with aqueous ammonium hydroxide, and esterifying the resulting alcohol to give the hexanoate 63. A long chain ester was deliberately chosen for this step to facilitate deprotection later in the sequence (vide infra). The amide group of compound 63 was then dehydrated to a nitrile 64, thus avoiding ring closure in subsequent steps. Manipulation of the alcohol function into a leaving group in amide 69, without the dehydration step, failed because of the ring closure reaction (Scheme 2.3). The reaction presumably proceeded via the intermediate 70, but we were unable to isolate it.

Scheme 2.3. Ring closure of amide derivatives 69.
Acidic hydrolysis of ester 64 was inappropriate since the Boc-protecting group also would also be removed, and it was necessary to shield the amine during subsequent steps in the synthesis. Unfortunately, hexanoate 64 and the corresponding acetate (prepared by an analogous sequence, not shown) decomposed when exposed to several of the mildly basic conditions which might otherwise be used to cleave esters. Consequently, hydrolysis mediated by the lipase from *Candida cylindracea* was used to achieve the desired reaction at near neutral pH. Hexanoate 64 reacted more rapidly under these conditions and gave superior yields than the corresponding acetate. Incidentally, there was no evidence for kinetic resolution of 64 at approximately 50% conversion in this enzymatic hydrolysis. The enantiomeric excess of the alcohol 65 was determined via a Eu(fod)$_3$ chiral shift experiment on the corresponding Mosher’s ester prepared from R-(+)-MTPA.

The next step in the synthesis of *E-cyclo-Met* was introduction of the sulfur functionality, but a problem arose. Mesylate 66 (prepared without difficulty) was reacted with thiomethoxide under a variety of conditions but gave primarily the cyclopropane-cleavage product 71 (eq 2.3).

\[
\begin{align*}
76 & \quad \xrightarrow{\text{NaSMe, DMF}} \quad 71 \\
\text{(eq 2.3)}
\end{align*}
\]

Failure of the desired transformation was surprising given that the similar synthesis of sulfide 61 via displacement of a mesylate had been successful in the preparation of *Z-cyclo-Met*. Decreased steric hindrance and increased electrophilicity at the α-carbon of substrate 66 makes this site more susceptible to nucleophilic attack, and this perhaps accounts for these observations. Fortunately, thioacetate displaced mesylate from 66 in an $S_{N2}$ sense affording the thioester 67 without formation of significant by-products (Scheme 2.2). Base-mediated cleavage of this thioester gave a good yield of the corresponding
alcohol, a notable observation since alkaline hydrolysis of ester 64 was impractical. Finally, E-cyclo-Met was obtained via methylation and exhaustive acidic hydrolysis of the intermediate thiol 68.

2.5 Syntheses of Boc-Z-cyclo-Orn-Ot-Bu and Boc-Z-cyclo-Arg(Ts)³²

An enantiomer of 2,3-methanohomoserine derivative (i.e. IR,2S-60) was prepared via the same procedures as Scheme 2.1. Then, the side chain was homologated and manipulated to include amine and guanidine functionalities (Scheme 2.4).

Scheme 2.4. Synthesis of Boc-Z-cyclo-Arg(Ts).

\[
\begin{align*}
&\text{IR,2S-60} \\
\text{MsCl, NEt}_3, \text{CH}_2\text{Cl}_2 \quad (i) &\rightarrow \quad \text{CN} \quad \text{72} \\
&\text{KCN, Bu}_3\text{NCN, H}_2\text{O} \quad \text{C}_6\text{H}_6, \text{65 °C} \quad (62 \%, \text{2 steps}) \\
&\text{300 psi H}_2, \text{Raney-Ni} \quad \text{MeOH, NH}_4\text{OH} \quad (100 \%) \\
&\text{Boc-Z-cyclo-Orn-Ot-Bu} \\
\text{TsN=C(SMe)_2, xylenes} \quad 140 \text{ °C} \quad (63 \%) \\
&\text{NH}_3, \text{AgNO}_3 \quad \text{MeCN} \quad (100 \%) \\
\text{73} \\
\end{align*}
\]

\[
\begin{align*}
&\text{74} \\
\text{TFA, CH}_2\text{Cl}_2 \quad (i) &\rightarrow \quad \text{74} \\
&\text{(Boc)}_2\text{O, NaOH} \quad \text{t-BuOH} \quad (60 \%, \text{2 steps}) \\
&\text{Boc-Z-cyclo-Arg(Ts)}
\end{align*}
\]
Alcohol \textit{IR,2S-60} was mesylated then transformed into nitrile 72 using phase transfer conditions. Stammer and co-workers have reported that hydrogenation of \textit{N}-benzylxoxycarbonyl protected \textit{Z-cyclo-Phe} gives only reductive cleavage of the cyclopropane ring (1 atm. of H\textsubscript{2}, 5\% Pd/C cat.).\textsuperscript{63} Consequently, reduction of nitrile 72 via catalytic hydrogenation at elevated pressures may have given ring-cleavage products. In fact, these concerns were unfounded; the nitrile was reduced under such conditions without the formation of detectable amounts of ring-cleavage products. This sequence gave \textit{Boc-Z-cyclo-Orn-Ot-Bu}.

Introduction of guanidine functionality into the side chain was necessary for conversion of \textit{Boc-Z-cyclo-Orn-Ot-Bu} into a protected form of \textit{cyclo-Arg}. This was achieved by forming the methylmercapto derivative 73, then treating this with ammonia to produce the \textit{N}-tosyl protected guanidine group in the standard way.\textsuperscript{64,65} Presumably, treatment of compound 74 with HF-based systems would remove the \textit{tert}-butyloxycarbonyl and tosyl protecting groups liberating the free amino acid analog, \textit{cyclo-Arg}.\textsuperscript{66,67} For the purposes of this project, however, compound 74 was treated with trifluoroacetic acid to remove both the \textit{tert}-butyloxycarbonyl groups (conditions to remove one of these protecting groups selectively could not be identified). The \textit{Nα}-terminus was then protected using di-\textit{tert}-butyl dicarbonate to give \textit{Boc-Z-cyclo-Arg(Ts)} with only the \textit{C}-terminus exposed. This product is in the correct form for introduction into a peptidomimetic using the classical Boc-protection/HF cleavage scheme.\textsuperscript{68}

### 2.6 Synthesis of \textit{Boc-Z-cyclo-Asp-Ot-Bu}

Oxidation of the 2,3-methanohomoserine derivative \textit{IR,2S-60} by ruthenium chloride and sodium periodate gave the \textit{Boc-Z-cyclo-Asp-Ot-Bu} in 51\% yield (eq 2.4). Attempted removal of the \textit{t}-Bu and Boc protecting groups (TFA, 0\°C to 25\°C)
cause decomposition of Z-cyclo-Asp, presumably induced by amine assisted ring opening (eq 2.5).

\[
\begin{align*}
\text{tBuO}_2\text{C} & \quad \text{NHBOc} & \quad \text{RuCl}_3 \cdot 5\text{H}_2\text{O}, \text{NaIO}_4, \quad \text{CCl}_4, \text{CH}_3\text{CN}, \text{H}_2\text{O} & \quad \text{tBuO}_2\text{C} & \quad \text{NHBOc} & \quad \text{TFA} & \quad \text{HOOC}_n\text{NH}_2 \\
1R,2S-60 & & & & \text{Boc-Z-cyclo-Asp-Ot-Bu} & \rightarrow & \text{Z-cyclo-Asp} & \\
\end{align*}
\]

(eq 2.4)

Z-cyclo-Asp

\[
\begin{align*}
\text{HOOC} & \quad \text{NH}_2 & \quad \text{ring opened products} \\
\text{HOOC} & \quad \text{NH}_2 & \quad \text{75} & \quad \text{76} & \quad \text{77} \\
\end{align*}
\]

(eq 2.5)

(eq 2.6)

Similar cyclopropane ring opening reaction was reported for compound 76 (eq 2.6).\textsuperscript{69} However, one racemic synthesis of E- and Z-cyclo-Asp was found in literature (Scheme 2.5), with no ring opening product reported.\textsuperscript{70} This result is somewhat surprising because of the fragile nature of the cyclo-Asp observed in our laboratory.
2.7 Attempted Syntheses of E-cyclo-Arg and Asp Derivatives

Attempted syntheses of E-cyclo-Arg by nucleophilic displacement of the mesylate 66 were not successful. Cyanide or nitromethane anion were used under the conditions as shown below:

(i) KCN, Bu₄NCN in solvent benzene/H₂O, t-BuOH, or CH₃CN at 55 °C;
(ii) CH₃NO₂/MeOH with NaOH at 0°C;
(iii) CH₃NO₂/MeOH with NaOMe at 25 °C; or
(iv) CH₃NO₂ with CsCO₃ at 60 °C.

The first three conditions (i to iii) gave a mixture of alkenes, and no reaction was observed for condition forth (iv) (Scheme 2.6). The formation of alkenes might result from the fragile cyclopropane ring opening under the basic medium.
**Scheme 2.6.** Attempted syntheses of \( E \)-cyclo-Arg via nucleophilic displacement of mesylate 66.

\[
\text{BocNH} \bigg\updownarrow \text{CN} \quad \text{KCN, Bu}_4 \text{NCN}, \quad \text{Solvents:} \quad \text{benzene} / \text{H}_2 \text{O}; \quad \text{t-BuOH}; \quad \text{or} \quad \text{CH}_3 \text{CN} \rightarrow \quad \text{BocNH} \bigg\updownarrow \text{CN} \quad 81
\]

\[
\text{BocNH} \bigg\updownarrow \text{CN} \quad \text{OMs} \quad \text{CH}_2 \text{NO}_2, \text{MeOH} \quad \text{Base: NaOH or NaOMe} \rightarrow \quad \text{BocNH} \bigg\updownarrow \text{CN} \quad \text{CH}_2 \text{NO}_2 \quad 82
\]

\[
\text{BocNH} \bigg\updownarrow \text{CN} \quad \text{OMs} \quad \text{CsCO}_3, \text{CH}_3 \text{NO}_2 \rightarrow \quad \text{BocNH} \bigg\updownarrow \text{CN} \quad \text{CH}_2 \text{NO}_2 \quad 82
\]

Similar failures were observed for attempted oxidation of the nitrile alcohol 65 and the amide alcohol 84 under Swern,\(^{71}\) pyridinium dichromate,\(^{72}\) or ruthenium chloride conditions\(^{73}\) (Scheme 2.7); again, a mixture of alkenes was observed.

**Scheme 2.7.** Attempted syntheses of \( E \)-cyclo-Asp via oxidation.

\[
\text{BocNH} \bigg\updownarrow \text{CN} \quad \text{OMs} \quad (\text{COCl})_2, \text{DMSO, CH}_2 \text{Cl}_2, \text{-60 °C, and } \text{R} = \text{H; or} \quad \text{Py}_2 \text{Cr}_2 \text{O}_7, \text{CH}_2 \text{Cl}_2, \text{25 °C, and } \text{R} = \text{OH} \rightarrow \quad \text{BocNH} \bigg\updownarrow \text{CN} \quad \text{R} \quad 83
\]

\[
\text{BocNH} \bigg\downarrow \text{CONR}^1 \text{R}^2 \quad \text{OMs} \quad \text{Py}_2 \text{Cr}_2 \text{O}_7, \text{CH}_2 \text{Cl}_2, \text{25 °C, and } \text{R} = \text{OH; or} \quad \text{RuCl}_3, \text{NaIO}_4, \text{CCl}_4/\text{CH}_3 \text{CN/H}_2 \text{O, 25 °C and } \text{R=OH} \rightarrow \quad \text{BocNH} \bigg\downarrow \text{CN} \quad \text{R} \quad 85
\]

\( R^1 = \text{H}, \ R^2 = \text{Me}; \quad R^1 = \text{H}, \ R_2 = \text{H} \)
2.8 Conclusions

Since both enantiomers of glycidol, from which triflate 57 is derived, are commercially available, the syntheses shown in Scheme 2.1 and 2.2 were each performed using both enantiomers of lactone 45, hence samples of both optical isomers of Z- and E-cyclo-Met were obtained. One of the enantiomers was prepared for the Boc-Z-cyclo-Arg(Ts) and the Boc-Z-cyclo-Asp-Ot-Bu, and this sequence can be repeated with 1S,2R-60 to obtain the other antipodes. These syntheses illustrated the versatility of the lactone 45 in syntheses of 2,3-methanoamino acids. This approach significantly reduces the synthetic effort required for making those amino acids because a common intermediate was used. However, there are three major weaknesses of these sequences: (i) the commercially available glycidols generally are not optically pure (ca 92 % ee); (ii) the formation of lactone 45 from triflate 57 is difficult to scale up with good yield; and, (iii) E-isomers of the cyclo-Arg and cyclo-Asp derivatives cannot be synthesized. The first two weaknesses were overcome by a procedure developed by another graduate student in our laboratory (Chun-Yen Ke) using mannitol as starting material to synthesize the lactone 45,74 and the intermediates obtained from this procedure were use to synthesize some E-isomers of 2,3-methanoamino acids including cyclo-Arg, cyclo-Glu and cyclo-Gln analogs (developed by Dongyeol Lim).75
Chapter 3. Solid Phase Syntheses, and Anti-opiate Activities of FMRF-NH₂ Peptidomimetics Containing 2,3-Methanoamino Acids

3.1 Introduction

3.1.1 Background on Neuropeptide Phe-Met-Arg-Phe-NH₂

The neuropeptide Phe-Met-Arg-Phe-NH₂ (FMRF-NH₂, one letter code for amino acids) was first isolated from mollusks, and subsequently reported to exert anti-opiate effects in variety of mammalian test systems.⁷⁶ While FMRF-NH₂ has not been found in mammals, the octapeptide Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH₂ (named as neuropeptide FF, or NPFF) was initially detected using FMRF-NH₂ antiserum,⁷⁷ then isolated from bovine brain.⁷⁸

The following observations indicate NPFF is an endogenous antiopiate peptide, counteracting certain actions of opiates and endogenous opiate peptides: (i) NPFF is localized in brain regions which are rich in endogenous opioids;⁷⁹ (ii) morphine infusion causes FMRF-NH₂-like immunoreactivity to be released in rat brain;⁷⁶ and, (iii) NPFF and other FMRF-NH₂-related peptides antagonize analgesic effects of morphine and certain endogenous opiate peptides.⁷⁶,⁷⁸,⁸⁰,⁸¹ These antiopiate properties appear to be mediated through receptors which are specific to NPFF, rather than through opiate receptors.⁸²

There is a growing body of evidence that production of NPFF may be activated by chronic opiate exposure, and is associated with opiate tolerance and dependence. First, levels of NPFF in CSF (cerebral spinal fluid) are markedly higher in opiate tolerant/dependent rats than in opiate-naïve controls.⁸³ Second, large amounts of NPFF are released when naloxone is introduced in the spinal cord of morphine dependent rats.⁷⁸ Third, low doses of NPFF precipitate abstinence syndrome when injected into the third
ventricle of morphine dependent rats, and higher doses induced a morphine-reversible quasi-abstinence syndrome when injected via the same route in opiate naive rats.\textsuperscript{84} Furthermore, pretreatment with IgG from NPFF antiserum prevented naloxone precipitated abstinence syndrome in morphine-dependent rats.\textsuperscript{84,85}

Immunoneutralization of -RF-NH\textsubscript{2} peptides can selectively restore morphine sensitivity in opiate-tolerant rats. Specifically, IgG from FMRF-NH\textsubscript{2} antiserum cross-reacts with NPFF and attenuates acute morphine tolerance,\textsuperscript{76} and IgG from NPFF-selective antiserum has the same effect.\textsuperscript{86} In one set of experiments, injection of IgG from NPFF antiserum into the third ventricle restored analgesic sensitivity (6 \( \mu \)g of morphine, intracerebroventricular (i.c.v.) administered 60 min after the end of the IgG injection and monitored via radiant heat tail flick) in morphine tolerant rats, whereas the same treatment with IgG from NPFF antiserum did not change the analgesic response to i.c.v. morphine in opiate-naive rats.

To date, very few NPFF analogs have been prepared for studies of their physiological and pathological functions, but interesting results have been obtained from desaminoYFLFQPQRFNH\textsubscript{2} (daY8Ra).\textsuperscript{87} This synthetic peptide differs from NPFF in two ways. First R-NH\textsubscript{2} replaces RF-NH\textsubscript{2}, a modification intended to reduce receptor activation; \textit{cf} similar substitutions in other peptides with amidated C-termi (eg gastrin/CCK and bombesin) have transformed agonists into antagonists.\textsuperscript{88-90} Secondly, the presence of desaminotyrosine (daY) at the N-terminus gives enhanced stability towards N-peptidases and increased lypophilic character which, in turn, may enhance its binding affinity. It has been demonstrated that FMRF-NH\textsubscript{2}-analogs with similar N-terminal modifications can have increased affinity for FMRF-NH\textsubscript{2} receptors.\textsuperscript{91} Experimentally, daY8Ra (600 ng, third ventricle, i.c.v. injection) was observed to attenuate the quasi-abstinence syndrome subsequently precipitated by NPFF (10 \( \mu \)g i.c.v.). This suggests daY8Ra does have antagonist activity against NPFF.\textsuperscript{87} Pretreatment of the same dose of
daY8Ra also attenuated abstinence syndrome precipitated by 10 μg of naloxone (i.e. c.v.). Conversely, pretreatment with NPFF or with NPFF modified at the N-terminal only (i.e. daY9Fa) failed to attenuate subsequent naloxone precipitated abstinence. The latter observations imply the C-terminal modification is critical for NPFF antagonist activity.87

More systematic studied of structure-activity relationships for NPFF receptors were reported by using a number of FMRF-NH2 analogs.92 The results based on competitive binding assay suggested that changing the Arg or Phe residues to other amino acids in FMRF-NH2 reduced binding to the NPFF receptors. In contrast, substitution at the first and second positions of FMRF-NH2 were generally tolerated, with the most potent analogs being PMRF-NH2, FFRF-NH2, and FWRF-NH2. In another study, it had shown that the C-terminal amide appeared to be necessary for good affinity.82

3.1.2 Aims

Replacement of the natural amino acids in FMRF-NH2 by 2,3-methanoamino acids may have a drastic effect on the conformational properties of the peptide. The peptidomimetics so obtained are more constrained than the natural peptides because of the steric effects imposed by the cyclopropane ring. If a conformationally conained analog is found to strongly bind to the receptor(s), its solution structure may map the steric and electronic requirements for such binding. Consequently, the Met2 in FMRF-NH2 was systematically replaced by each of the isomers of cyclo-Met giving four peptidomimetics, namely F{2S,3R-cyclo-M}RF-NH2, F{2R,3S-cyclo-M}RF-NH2, F{2S,3S-cyclo-M}RF-NH2 and F{2R,3R-cyclo-M}RF-NH2. A peptidomimetic containing two 2,3-methanoamino acids substituted in the 2 and 4 positions (i.e. F{2S,3S-cyclo-M}R{2R,3R-cyclo-F}-NH2) was also prepared. Compatibility of 2,3-methanoamino acids with solid phase syntheses are discussed, and the anti-opiate activities of the above peptidomimetics are summarized at the end of this Chapter.
3.2 Syntheses of Peptidomimetics

3.2.1 Solution Phase Syntheses of Peptidomimetics Containing 2,3-Methanoamino Acids

Peptidomimetics containing 1-aminocyclopropane-1-carboxylic acid (Acc) were synthesized by various groups via a solution phase approach. Although Acc is more sterically hindered than natural amino acids, it gives satisfactory yields in solution phase couplings using DCC\textsuperscript{93} or EDC\textsuperscript{94} as activating reagents, or via the mixed anhydride method.\textsuperscript{21,63} For substituted 2,3-methanoamino acids, norcoronamic acid and $\beta,\beta'$-dimethylated-Acc analogs were incorporated into Asp-(2,3-methanologs)-O(nPr) dipeptide via the $N$-hydroxysuccinamide activated ester.\textsuperscript{14} $E$- and $Z$-cyclo-Phe containing enkephalin\textsuperscript{21,63} and CCK(30-33)\textsuperscript{23} analogs were also synthesized using $iso$-butylchloroformate as a coupling reagents in solution. Thus, before our attempts to incorporate 2,3-methanoamino acids into peptides by the solid phase approach, only solution phase syntheses had been reported.

Figure 3.1. Amino acids incorporated via solution phase approach.

\[
\begin{align*}
\text{Acc} & : \text{H}_2\text{N} & \begin{array}{c}
\text{COOH} \\
\end{array} \\
\text{norcoronamic acid} & : \text{H}_2\text{N} & \begin{array}{c}
\text{COOH} \\
\end{array} \\
& : \text{H}_2\text{N} & \begin{array}{c}
\text{COOH} \\
\end{array} \\
& & \begin{array}{c}
\beta' \text{Me} \\
\end{array} \\
& & \begin{array}{c}
\beta \text{Me} \\
\end{array} \\
& & \begin{array}{c}
\beta, \beta'-\text{dimethylated-Acc} \\
\end{array}
\end{align*}
\]

3.2.2 Syntheses of Phe-{$2S,3R$-cyclo-Met}-Arg-Phe-NH$_2$, and Phe-{$2S,3R$-cyclo-Met}-Arg-Phe-NH$_2$ via The Boc Approach

F-{$2S,3R$-cyclo-M}RF-NH$_2$ and F-{$2R,3S$-cyclo-M}RF-NH$_2$ were synthesized by using Boc chemistry\textsuperscript{68} on MBHA resin (Figure 3.2). A tosyl protecting group was used to mask the Arg side chain, and BOP was used as the coupling reagent.\textsuperscript{95} Three equivalents (relative to the resin) of natural amino acids were used for each coupling.
cycle, and the cycles were repeated until a negative ninhydrin test (Kaiser test) was obtained. For the precious Boc protected Z-cyclo-Met (both the 2S,3R-, or 2R,3S-isomers), 1.1 equivalents were used, and the reaction was allowed to proceed for 12 h.

**Figure 3.2.** Boc synthesis of F{2S,3R-cyclo-M}RF-NH₂.³

³ The horizontal lines of this scheme represent the covalent linkages between two residues (ie the amide bonds in the case of two amino acid residues). Each vertical line represents an amino acid residue. The diagonal line stands for the covalent linkage between the side chain of the amino acid, and the protecting group (ie toluenesulfonyl group was used as side chain protection for the guanidino side chain of Arginine).
Racemization observed for natural amino acids in prolonged activation time is not a concern for 2,3-methanologs, since the latter do not have α-protons. The peptide was cleaved from the resin by HF and purified by RP-HPLC to give 3% yield of the desired peptidomimetics. The loss of yield may be caused by poor handling technique on the cleavage step which was performed by a commercial laboratory (Immuno-dynamics, Inc., CA).

3.2.3 Syntheses of Phe-{2S,3S-cyclo-Met}-Arg-Phe-NH₂, and Phe-{2R,3R-cyclo-Met}-Arg-Phe-NH₂ via The Fmoc Approach

For F-{2S,3S-cyclo-M}-RF-NH₂ and F-{2R,3R-cyclo-M}-RF-NH₂, Fmoc amino protection, Rink’s amide resin, Mtr shielding of the Arg side chain, and BOP/HOBt coupling steps were used (Figure 3.3). Three equivalents (relative to the resin) of the natural amino acids were used in each coupling cycle, and a negative ninhydrin test was observed within 45 min. However, only 1.1 equivalents of the precious Fmoc protected E-cyclo-Met were used; after 2 h coupling time, the sequence therefore was end-capped (ie capping the unreacted amine by acetic anhydride). The peptide was cleaved from the resin using trifluoroacetic acid and purified by RP-HPLC. The overall yield of this sequence was 70%. This indicates that although the E-cyclo-Met (both the 2S,3S- or 2R,3R-isomers) are more sterically hindered than natural methionine, they are compatible with the solid phase approach. In contrast, α-methylated amino acids such as Aib (aminoisobutyric acid) usually give poor yield in solid phase syntheses. The natural peptide FMRF-NH₂ was synthesized by the same method with an overall yield of 89%.
3.2.4 Synthesis of Leu-\{\pm\}-Z-cyclo-Phe\}-NH\_2

Cyclo-Phe are more sterically hindered than cyclo-Met because of the bulkier aromatic ring. To test the coupling efficiency for the more hindered compound, a diastereomeric mixture of Leu-\{\pm\}-Z-cyclo-Phe was synthesized via BOP coupling (Figure 3.4). The required racemic Z-cyclo-Phe was prepared via Stammer's procedures (compound prepared by B. Pal). Rink's amide resin was coupled with 1.2 equivalents (relative to the resin) of the Fmoc-cyclo-Phe, followed by two couplings of Fmoc-Leu (3 equivalents).
**Figure 3.4.** Incorporation of (±)-Z-cyclo-Phe into model dipeptide.

After preparative RP-HPLC, three compounds were isolated. Two of them were the diastereoisomers of Leu-{(±)-Z-cyclo-Phe} dipeptides with a combined yield of 34%. The other compound was (±)-Z-cyclo-Phe-NH₂, *i.e.* uncoupled starting material, in 39% yield. The total coupling efficiency of the Fmoc-cyclo-Phe to the resin was 73% determined by total yield of the three compounds, while the more hindered amino terminus had only 34% (*i.e.* the total yield of the dipeptide diastereoisomers) coupling efficiency after two cycles. This indicates that multiple cycles are required to increase the coupling yield on the hindered side of the cyclo-Phe analogs, but it is possible to synthesize cyclo-Phe containing peptidomimetics via the solid phase approach.

### 3.2.5 Synthesis of Phe-{2S,3S-cyclo-Met}-{2R,3R-cyclo-Phe}-NH₂

F{2S,3S-cyclo-M}R{2R,3R-cyclo-F}-NH₂ was synthesized using an Fmoc approach (Figure 3.5). The optically active 2R,3R-cyclo-Phe was synthesized by rhodium catalysed cyclopropanation developed by Davies *et al.* (compound prepared by B. Pal).³⁶ The Rink-cyclo-Phe resin was coupled through six cycles of treatment with Fmoc-Arg(Mtr) to give a negative ninhydrin test. An overall yield of this sequence was 43%, which is impressive since two 2,3-methanoamino acids were incorporated.
3.2.6 Conclusions from Solid Phase Syntheses of the Peptidomimetics

Five peptidomimetics containing 2,3-methanoamino acids were synthesized. The 2,3-methanoamino acids are generally compatible with the solid phase approach. Multiple cycles were required for coupling of highly hindered analogs such as cyclo-Phe. With careful monitoring of the reaction by the ninhydrin test, several peptidomimetics of tetrapeptides containing one or two 2,3-methanoamino acids may be prepared in good to moderate yield via Fmoc chemistry.

3.3 Summary of Anti-opiate Activities, and Proteolytic Stability of Peptidomimetics Containing 2,3-Methanomethionine

Receptor binding studies were performed by Dr. Kemal Payza (NIMD, St. Elizabeths, DC), and in vivo activities of the peptide/peptidomimetics were studied by Professor David H. Malin (University of Houston-Clear Lake). Our group (K.-K. Ho) performed the leucine aminopeptidase digestions of the peptidomimetics. The results are summarized below.
3.3.1 Receptor Binding Studies of Phe-(cyclo-Met)-Arg-Phe-NH₂

Rat spinal cord membranes were incubated to equilibrium with [¹²⁵I]-Y8Fa ([¹²⁵I]-YLFQPQRF-NH₂), and various concentrations of FMRF-NH₂, or the peptidomimetics of FMRF-NH₂ containing 2,3-methanomethionine.¹⁰⁰,¹⁰¹ All four isomers of F-{cyclo-M}-RF-NH₂ bound to the NPFF receptor at higher concentrations, and displayed less affinity than FMRF-NH₂ (Table 3.1). Consequently, the relative binding of these peptidomimetics to NPFF receptors does not account for their enhanced potency in pharmacological studies (vide infra).

Observations that confirm these peptidomimetics were not simply acting as competitive ligands for the µ-opiate receptor binding are as follows. Morphine and naloxone were shown to very effectively displace the ligand ³H-DHM (³H-dihydromorphine) from this receptor (IC₅₀ values of 2.2 and 3.0 nM, respectively), but all the peptidomimetics competed for binding only at the highest concentration tested (10 µM; IC₅₀ values were 9 and 8 µM, respectively). The F-{cyclo-M}-RF-NH₂ peptidomimetics therefore were bound more weakly to µ-receptors than to NPFF receptors.

Table 3.1. Binding potencies of FMRF-NH₂, and the four peptidomimetics F-{cyclo-M}-RF-NH₂.

<table>
<thead>
<tr>
<th>Peptide/peptidomimetics</th>
<th>IC₅₀ (nM)ᵃ</th>
<th>Kᵢ (nM)ᵇ</th>
<th>n_Hᶜ</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMRF-NH₂</td>
<td>2.20</td>
<td>1.40</td>
<td>1.00</td>
</tr>
<tr>
<td>F-{2S,3R-cyclo-M}-RF-NH₂</td>
<td>122.00</td>
<td>76.00</td>
<td>0.83</td>
</tr>
<tr>
<td>F-{2R,3S-cyclo-M}-RF-NH₂</td>
<td>426.00</td>
<td>266.00</td>
<td>1.00</td>
</tr>
<tr>
<td>F-{2R,3R-cyclo-M}-RF-NH₂</td>
<td>86.70</td>
<td>43.00</td>
<td>0.88</td>
</tr>
<tr>
<td>F-{2S,3S-cyclo-M}-RF-NH₂</td>
<td>127.00</td>
<td>64.00</td>
<td>0.94</td>
</tr>
</tbody>
</table>

ᵃ IC₅₀ is the concentration of the peptide or peptidomimetics that inhibits 50% of specific [¹²⁵I]-Y8Fa binding. ᵇ Kᵢ = IC₅₀/(1+L/K_d), L is the concentration of [¹²⁵I]-Y8Fa, and K_d is equal to 0.08.¹⁰⁰,¹⁰¹ ᶜ n_H is the Hill coefficient.
3.3.2 *In Vivo* Bioactivities of Phe-\{(cyclo-Met)\}-Arg-Phe-NH₂

All four stereoisomers of F-\{cyclo-M\}-RF-NH₂ were tested for activity with respect to precipitating opiate abstinence syndrome.\(^{100,101}\) Rat was implanted subcutaneously with one Alzet 2ML1 osmotic minipump filled with a solution of morphine in isotonic saline. The subjects were infused for 7 d with 0.27 mg/kg/h morphine sulfate. Each rat received a third ventricle injection of FMRF-NH₂ or one of its cyclopropylcys; six different doses were used in each case. The rats were then observed under "blind" conditions for 20 min on a standard checklist of opiate abstinence signs (teeth chatter, writhes, wet shakes, etc).

Two peptidomimetics of FMRF-NH₂ prepared from the two enantiomers of Z-cyclo-Met were evaluated (Figure 3.6 a).\(^{100}\) Only the highest dose (25 μg) of FMRF-NH₂ precipitated withdrawal. However, both the cyclopropane-based peptidomimetics proved to be approximately 50 times as potent as FMRF-NH₂ with respect to precipitating opiate abstinence syndrome (Figure 3.6 a). The peptidomimetics containing both enantiomers of *E*-cyclo-Met were also tested (Figure 3.6 b).\(^{102}\) F-\{2R,3R-cyclo-M\}-RF-NH₂ proved to be as active as the two cyclopropane-based peptidomimetics from Z-cyclo-Met (*ie* 50x more active than FMRF-NH₂), but F-\{2S,3S-cyclo-M\}-RF-NH₂ was approximately 8x as potent.
Figure 3.6. Overall morphine abstinence signs over 20 min by third ventrical injection of six different doses (0.25, 0.50, 3.0, 6.0, 15.0, 25.0 μg) of (a) FMRF-NH₂, F-\{Z-cyclo-M\}-RF-NH₂; and (b) FMRF-NH₂ and F-\{E-cyclo-M\}-RF-NH₂. Each data point represents the mean (M) and standard error of mean (SEM) of two or three rats.

3.3.3 Leu-Aminopeptidase Digestion of Phe-\{E-cyclo-Met\}-Arg-Phe-NH₂

The peptide FMRF-NH₂, and the two peptidomimetics containing E-cyclo-Met were incubated with Leu-aminopeptidase in phosphate buffer (pH 8).\(^{102}\) Commercially available Leu-aminopeptidase was chosen because it digests peptide with a free amino
terminus efficiently. The amount of intact peptide/peptidomimetics at different time intervals was quantitated by RP-HPLC (anthranilic acid as internal standard). The $t_{1/2}$ of the molecules was determined by measuring the time required for the area of the substrate peak to decreased to half.

The $t_{1/2}$ for FMRF-NH$_2$ under these conditions was 0.38 h. The peptidomimetics containing \textit{E-cyclo-Met} were more proteolytic stable than the natural peptide. Thus, the F-\textit{\{2R,3R-cyclo-M\}}-RF-NH$_2$ and F-\textit{\{2S,3S-cyclo-M\}}-RF-NH$_2$ had $t_{1/2}$ of >60 h and 16 h, respectively, under identical conditions.

3.3.4 Conclusions on the Anti-opiate Activities and Proteolytic Stability Section

The cyclopropane-based peptidomimetics were more active (\textit{in vivo}) than FMRF-NH$_2$ but bind less strongly to the appropriate receptor sites. This observation seems to be related to the enhanced bio-availability of the peptidomimetics: these particular peptidomimetics are bound less strongly to the NPFF receptor, but exhibit potent pharmacological effects since they are present in the animal for relatively long half lives before proteolytic degradation.
Chapter 4. Solution Conformations of Peptidomimetics Containing 2,3-Methanoamino Acids: Part I NMR

4.1 Introduction

There are only a few amino acid surrogates that facilitate subtle restrictions of conformational flexibility without drastically changing the overall steric and electronic properties of a peptide framework. One of the classic example is α-methyl amino acids. These compounds are accessible via contemporary asymmetric syntheses and biocatalytic resolutions, and they have profound, though not completely understood, effects on φ,ψ, and χ angles of this residue. The simplest derivative in this series, 2-amino isobutyric acid or Aib, is well known to promote formation of helices and tight turns. Less well studied are the isomers of these compounds, the β-methyl amino acids. These are less easily prepared in stereochemically pure form, however, the β-methyl amino acids have been used to define the χ1 and χ2 conformers in cyclic peptides.

3-Substituted methanologs have four possible stereoisomers, and each will have distinct effects on the conformation of a peptidomimetic. Certain fundamental characteristics of these compounds are immediately evident. The side chain χ1 angle is locked at ca 0° for Z-methanoamino acids, and at ca ±150° for their E-isomers. The methylene group in a 2,3-methanoamino acid serves as both Cα and Cβ substituents,
which may constrain the $\phi$, $\psi$ and $\chi_2$ angles like the $\alpha$-methyl- and $\beta$-methyl-amino acids. Configurations at C3 also must have pronounced effects on rotation about bonds to C$\alpha$: $\psi$ angles will be more restricted for E-methanoamino acids, whereas local conformations about Z-isomers will be governed by serious perturbations of the allowed $\phi$ angles.

4.1.1 Aims

NMR determinations of solution structures of the peptide FMRF-NH$_2$, and peptidomimetics F(2S,3S-cyclo-M)RF-NH$_2$, F(2R,3R-cyclo-M)RF-NH$_2$, and F(2S,3S-cyclo-M)R(2R-3R-cyclo-F)-NH$_2$ are discussed in this chapter, and supporting computational models are discussed in the next.

Phe-Met-Arg-Phe-NH$_2$

Phe-2S,3S-cyclo-Met-Arg-Phe-NH$_2$

Phe-2R,3R-cyclo-Met-Arg-Phe-NH$_2$

Phe-2S,3S-cyclo-Met-Arg-2R-3R-cyclo-Phe-NH$_2$

Attempts were made to understand the conformational properties of these cyclopropane surrogates in solution, and to investigate the feasibility of using 2,3-methanoamino acids to constrain the neuropeptide FMRF-NH$_2$. Several issues were addressed: (i) conformational rigidity induced by incorporating 2,3-methanoamino acids into a linear peptide; (ii)
available conformational space for the 2,3-methanoamino acid residues; and, (iii) solution conformations of the peptidomimetics containing the 2,3-methanoamino acids. To achieve these goals, conformational constraints of the peptidomimetics were obtained from the NMR studies (this chapter), and the available conformational space were mapped using quenched molecular dynamics (QMD, next chapter).\textsuperscript{113,114} Low energy structures obtained from the QMD were correlated with the NMR data.

4.2 Background on NMR Techniques for Conformational Analyses of Peptides and Peptidomimetics

A combination of NMR techniques was used to study the conformations of the peptide/peptidomimetics. Table 4.1 summarizes the functions and data obtained from various experiments.\textsuperscript{115} Details of individual techniques are discussed in the following sections.

<table>
<thead>
<tr>
<th>experiments</th>
<th>observed data</th>
<th>function(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DQF-COSY</td>
<td>$J^3$-coupling crosspeaks</td>
<td>elucidation of through-bond connectivities</td>
</tr>
<tr>
<td>ROESY</td>
<td>dipolar coupling</td>
<td>sequential assignments and protons close contacts</td>
</tr>
<tr>
<td>variable temperature $^1$H-NMR</td>
<td>temperature coefficients</td>
<td>identification of hydrogen-bonded or solvent shielded amide protons</td>
</tr>
<tr>
<td>1D $^1$H-NMR</td>
<td>$J^3$-coupling constants</td>
<td>calculation of dihedral angles by Karplus equation</td>
</tr>
</tbody>
</table>
4.2.1 DQF-COSY

The first step of the NMR study involved chemical shift assignments of each proton in the peptide/peptidomimetics.\textsuperscript{115} Through-bond connectivities of protons were elucidated by DQF-COSY.\textsuperscript{116} DQF-COSY was used in this study instead of COSY because it has the advantages of (i) suppression of diagonal singlet, and (ii) reduction of dominant diagonal peaks.\textsuperscript{116} Therefore, crosspeak resolution around the diagonal area is enhanced. Three bond spin-spin coupling (Figure 4.1) between two protons appears as a crosspeak in the spectrum. Connecting these crosspeaks will give the sequences of individual spins successively joined by three bonds. This is exemplified by the assignment of protons of the Arg\textsuperscript{3} residue in FMRF-NH\textsubscript{2}. Joining the crosspeaks of the Arg\textsuperscript{3} amide NH to $\alpha$, $\alpha$ to $\beta$, $\beta$ to $\gamma$, $\gamma$ to $\delta$, followed by $\delta$ to NH$\epsilon$ as shown in Figure 4.2 identified the chemical shifts of these protons. Similar through-bond connectivities were made for Phe\textsuperscript{1}, Met\textsuperscript{2}, and Phe\textsuperscript{4} (not shown).

Figure 4.1. $J^3$-Coupling between amide NH and $\alpha$ proton.
Figure 4.2. DQF-COSY spectrum of FMRF-NH₂ showing the through-bond connectivities of the Arg³ residue.
4.2.2 ROESY

4.2.2.1 NMR Experiments to Identify Distance Constraints for Peptides

Estimates of distances between protons may be obtained by using either NOESY\textsuperscript{117} or ROESY\textsuperscript{118} experiments. The NOE build-up between two protons is seen as a crosspeak in the 2D spectrum. The intensity of this peak initially increases at a rate proportional to the inverse six power of the distance ($r^{-6}$) between the two protons, and has a detection limit of $< 5 \, \text{Å}$.\textsuperscript{115} For a molecule with $\omega\tau_c$ ($\omega$ is the spectrometer frequency in rad s$^{-1}$, and $\tau_c$ is the rotational correlation time) close to unity, the NOE build-up rate in NOESY approaches zero (Figure 4.3).\textsuperscript{119} Hence, NOESY experiments are unsuitable for small molecules (eg the tetrapeptide FMRF-NH$_2$), which typically have the $\omega\tau_c$ close to 1. Conversely, ROESY developed by Bothner-By \textit{et al} allows for the observation of NOE (ie ROE crosspeaks) for small molecules.\textsuperscript{118} In this experiment, the ROE build-up does not vanish for any value of $\omega\tau_c$, hence the ROE build-up remains relatively strong when $\omega\tau_c$ equal to 1. This enhanced sensitivity facilitates observation of ROE crosspeaks for such small molecules.

\textbf{Figure 4.3.} Dependent of maximum 2D NOE (N) and ROE (R) intensity on $\omega\tau_c$.\textsuperscript{119}
4.2.2.2 ROESY Artifacts

ROESY experiments are vulnerable to two main types of artifacts (i.e., crosspeaks that are not caused by dipolar transfer between proximal protons) viz COSY/HOHAHA type transfer, and "false" transverse NOE enhancements.\(^{120-122}\)

4.2.2.2.1 COSY/HOHAHA Artifacts

COSY/HOHAHA crosspeaks are a result of magnetization transfer via spin-spin coupling between two protons. ROESY spectra in the absorption mode (i.e., displaying only the real part of the Fourier transformed signals) facilitate identification of these artifacts since peaks generated by $J$-coupling were shown to have same phase as the diagonal peaks, while the genuine transverse NOE enhancements have opposite phase properties.\(^{120,121}\)

COSY/HOHAHA artifacts are sensitive to spin-lock field strength, flip angle during spin-lock period, and carrier frequency in ROESY experiments. A small spin-lock field strength (ca 2 kHz in this study) and a small flip angle (ca 30° pulse in this study) should be used to minimized this $J$-coupling process as suggested by Kessler et al.\(^{120}\)

The importance of carrier frequency\(^{121}\) selection was exemplified in the ROESY experiments for F(2S,3S-cyclo-M)RF-NH$_2$. The carrier frequency was positioned at 6.29 ppm (Figure 4.4), or 5.54 ppm (Figure 4.5). In the first experiment, a strong crosspeak for Phe$^4$NH-$\alpha$ (Figure 4.4 a) was shown to have same phase as the diagonal (Figure 4.4 b). This suggested that the crosspeak was generated by $J$-coupling between the Phe$^4$NH and Phe$^4$$\alpha$ protons.

![J-Coupling observed between Phe$^4$NH and Phe$^4$$\alpha$ at carrier frequency = 6.29 ppm](image_url)
When the position of the carrier frequency was changed to 5.54 ppm, this
crosspeak became weaker (Figure 4.5 a), and had opposite phase properties relative to the
diagonal (Figure 4.5 b), therefore it is a genuine transverse NOE enhancement.

**Figure 4.4.** Portion of ROESY spectrum of F\{2S,3S-cyclo-M\}RF-NH$_2$ with a
carrier frequency positioned at 6.29 ppm.

![diagram](image1)

**Figure 4.5.** Portion of ROESY spectrum of F\{2S,3S-cyclo-M\}RF-NH$_2$ with a
carrier frequency positioned at 5.54 ppm.

![diagram](image2)
4.2.2.2 "False" Transverse NOE Enhancements

"False" transverse NOE enhancement is caused by a two step pathway as shown in Figure 4.6.\textsuperscript{122} \textit{J}-Coupling between Phe\textsubscript{4}NH and Phe\textsubscript{4}α protons followed by through-space transverse NOE transfer between Phe\textsubscript{4}α and terminal CONHH protons (or \textit{vice versa}) generated a weak crosspeak between Phe\textsubscript{4}NH-CONHH (Figure 4.4 a), which was a "false" close proximity between Phe\textsubscript{4}NH-CONHH. This signal was removed by changing the position of the carrier frequency from 6.29 ppm to 5.54 ppm (Figure 4.5 a) since removal of the \textit{J}-coupling between Phe\textsubscript{4}NH and Phe\textsubscript{4}α protons terminated this energy transfer process.

**Figure 4.6.** "False" transverse NOE generated by spin-spin coupling followed by dipolar magnetization transfer for F\{2S,3S-cyclo-M\}RF-NH\textsubscript{2}.

4.2.3 Sequential Assignments

Sequential connectivities of amino acid residues in a peptide is usually achieved by connecting the ROE crosspeaks of the backbone protons.\textsuperscript{115} For FMRF-NH\textsubscript{2}, a continuous pathway of Phe\textsubscript{1}NH\textsubscript{3}-α, Met\textsubscript{2}NH-Phe\textsubscript{1}α, Met\textsubscript{2}NH-α, Arg\textsubscript{3}NH-Met\textsubscript{2}α, Phe\textsubscript{4}NH-Arg\textsubscript{3}α, Phe\textsubscript{4}NH-α, followed by CONHH-Phe\textsubscript{4}α crosspeaks (Figure 4.7) was observed, which shows the specific sequence of Phe\textsubscript{1}-Met\textsubscript{2}-Arg\textsubscript{3}-Phe\textsubscript{4}-NH\textsubscript{2}.
Figure 4.7. (a) Sequential connectivities observed for FMRF-NH$_2$. (b) ROESY spectrum of FMRF-NH$_2$ showing the sequential connectivities.
4.2.4 Temperature Coefficients of Amide Protons

Temperature coefficients of protons may be measured by variable temperature $^1$H-NMR experiments. Proton chemical shifts are plotted against the temperatures, and the slopes obtained are "temperature coefficients" (usually expressed in units of ppb K$^{-1}$). Amide protons with temperature coefficient $\leq \pm 3.00$ ppb K$^{-1}$ in DMSO are generally regarded as solvent shielded or hydrogen bonded. Variable temperature experiments were performed with a temperature range of 25 to 65 °C in this study.

4.3 Chemical Shift Assignments for FMRF-NH$_2$, F$\{2S,3S$-cyclo-M$\}$RF-NH$_2$, F$\{2R,3R$-cyclo-M$\}$RF-NH$_2$, and F$\{2S,3S$-cyclo-M$\}$R$\{2R$-3R$-cyclo-F$\}$-NH$_2$

4.3.1 Phe-Met-Arg-Phe-NH$_2$

Chemical shift assignments for FMRF-NH$_2$ were achieved by the method discussed in the previous section. Table 4.2 lists the chemical shifts, coupling constants and temperature coefficients of various protons.
Table 4.2. Chemical shifts, coupling constants and temperature coefficients of various protons in FMRF-NH₂.

<table>
<thead>
<tr>
<th></th>
<th>δ (ppm)</th>
<th>( J^3 ) (Hz)</th>
<th>NH-( \alpha )</th>
<th>( \alpha-\beta )</th>
<th>temperature coefficient (ppb K(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phe(^1)</td>
<td>NH</td>
<td>8.11</td>
<td>br singlet</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( \alpha )</td>
<td>4.07</td>
<td>( \ldots ) ( \ldots )</td>
<td>( \ldots ) ( \ldots )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( \beta )</td>
<td>3.05</td>
<td>( \ldots ) ( \ldots )</td>
<td>( \ldots ) ( \ldots )</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( \beta )</td>
<td>2.89</td>
<td>7.68</td>
<td>( \ldots ) ( \ldots )</td>
<td></td>
</tr>
<tr>
<td>Met(^2)</td>
<td>NH</td>
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<td>8.1</td>
<td>-3.68</td>
<td></td>
</tr>
<tr>
<td></td>
<td>( \alpha )</td>
<td>4.41</td>
<td>( \ldots ) ( \ldots )</td>
<td>( \ldots ) ( \ldots )</td>
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<tr>
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</tr>
<tr>
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<td>( \gamma )</td>
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<tr>
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<td>( \beta )</td>
<td>1.61</td>
<td>( \ldots ) ( \ldots )</td>
<td>( \ldots ) ( \ldots )</td>
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</tr>
<tr>
<td></td>
<td>( \beta )</td>
<td>1.50</td>
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<tr>
<td></td>
<td>( \gamma )</td>
<td>1.41</td>
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<td>( \ldots ) ( \ldots )</td>
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</tr>
<tr>
<td></td>
<td>( \gamma )</td>
<td>1.41</td>
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<td>( \ldots ) ( \ldots )</td>
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<tr>
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<td>( \ldots ) ( \ldots )</td>
<td>( \ldots ) ( \ldots )</td>
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<td>NH( \epsilon )</td>
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</tr>
<tr>
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<td>8.1</td>
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</tr>
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<td>( \alpha )</td>
<td>4.44</td>
<td>( \ldots ) ( \ldots )</td>
<td>( \ldots ) ( \ldots )</td>
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<td></td>
<td>( \beta )</td>
<td>2.98</td>
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</tr>
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<td>( \beta )</td>
<td>2.80</td>
<td>8.7</td>
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<td></td>
</tr>
</tbody>
</table>

\(^a\) Proton with coincident chemical shift with other proton.
4.3.2 Phe-(2S,3S-cyclo-Met)-Arg-Phe-NH$_2$

Sequential connectivities of F{2S,3S-cyclo-M}RF-NH$_2$ (Figure 4.8a) were shown in the ROESY spectrum (Figure 4.8b). The connections were broken by 2S,3S-cyclo-Met$^2$ residue because this analog does not have an $\alpha$ proton, and therefore there can be no crosspeaks for Met$^2\text{NH-}\alpha$ and Arg$^3\text{NH-Met}^2\alpha$ which are usually found in natural amino acids. Stereospecific assignments of the $\beta'$ protons for the 2S,3S-cyclo-Met$^2$ were not possible because one of the $\beta'$ protons overlapped with the $\beta$ proton (Figure 4.9). Assigned chemical shifts are listed in Table 4.3.

**Figure 4.8.** (a) Sequential crosspeaks observed for F{2S,3S-cyclo-M}RF-NH$_2$.
(b) Amide-aliphatic region of the ROESY spectrum showing the sequential connectivities.
Figure 4.9. Aliphatic-aliphatic region of the ROESY spectrum for F\{2S,3S-cyclo-M\}RF-NH₂ showing the overlapping signals between one of the β' protons and the β proton.

2S,3S-cyclo-Met²
Table 4.3. Chemical shifts, coupling constants and temperature coefficients of various protons in F(2S,3S-cyclo-M)RF-NH₂.

<table>
<thead>
<tr>
<th>amino acid</th>
<th>δ (ppm)</th>
<th>$J^3$ (Hz)</th>
<th>temperature coefficient (ppb K⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NH</td>
<td></td>
<td>NH-α</td>
</tr>
<tr>
<td>Phe¹</td>
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<td></td>
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</tr>
<tr>
<td></td>
<td>NH</td>
<td>8.17</td>
<td>br singlet</td>
</tr>
<tr>
<td></td>
<td>α</td>
<td>3.92</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>3.14</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>2.90</td>
<td>8.6</td>
</tr>
<tr>
<td>2S,3S-cyclo-M²</td>
<td>NH</td>
<td>9.12</td>
<td>singlet</td>
</tr>
<tr>
<td></td>
<td>α</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>1.38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>βᵃ</td>
<td>1.38</td>
<td></td>
</tr>
<tr>
<td></td>
<td>βᵃ</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td></td>
<td>γ</td>
<td>2.65</td>
<td></td>
</tr>
<tr>
<td></td>
<td>γ</td>
<td>2.56</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ε</td>
<td>2.06</td>
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</tr>
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<td>Arg³</td>
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<td>8.0</td>
</tr>
<tr>
<td></td>
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<td>4.31</td>
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</tr>
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<td></td>
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<td>3.00</td>
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</tr>
<tr>
<td></td>
<td>δ</td>
<td>3.00</td>
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</tr>
<tr>
<td></td>
<td>NHε</td>
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</tr>
<tr>
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<td>α</td>
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</tr>
<tr>
<td></td>
<td>β</td>
<td>2.96</td>
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</tr>
<tr>
<td></td>
<td>β</td>
<td>2.80</td>
<td>8.8</td>
</tr>
</tbody>
</table>

ᵃ Stereospecific assignment is not possible due to overlapping of β and β' protons (1.38 ppm). ᵇ Proton with coincident chemical shift with other proton.
4.3.3 Phe-{2R,3R-cyclo-Met}-{Arg-Phe-NH₂}

Similar ROESY connectivities were observed for F{2R,3R-cyclo-M}RF-NH₂ (Figure 4.10 a and b) as compared with F{2S,3S-cyclo-M}RF-NH₂. However, stereospecific assignments of the 2R,3R-cyclo-Met² β'cis and β'trans protons for F{2R,3R-cyclo-M}RF-NH₂ is possible by comparing the intensities of the ROE crosspeaks between the β proton and the two β' protons (Figure 4.11 a). A strong or a weak crosspeak was observed for 2R,3R-cyclo-Met²β-β'cis and 2R,3R-cyclo-Met²β-β'trans protons, respectively (Figure 4.11 b). These assignments were further confirmed by the J₃-coupling constants between the β-β'cis (9.6 Hz) and β-β'trans (7.6 Hz) protons. Table 4.3 lists the proton chemical shifts, vicinal coupling constants, and amide temperature coefficients for the assigned protons.
Figure 4.10. (a) Sequential crosspeaks observed for F[2R,3R-cyclo-M]RF-NH$_2$. (b) Amide-aliphatic region of the ROESY spectrum showing the sequential connectivities.
Figure 4.11. (a) Stereospecific assignments of $2R,3R$-cyclo-Met$^2$ $\beta'_\text{cis}$ and $\beta'_\text{trans}$ protons for $\text{F}(2R,3R$-cyclo-M)RF-NH$_2$. (b) Aliphatic-aliphatic region of the ROESY spectrum showing the weak and the strong crosspeaks for $2R,3R$-cyclo-Met$^2$$\beta-\beta'_\text{cis}$ and $2R,3R$-cyclo-Met$^2$$\beta-\beta'_\text{trans}$, respectively.

![Diagram of stereospecific assignments and ROESY spectrum]

strong ROE, $J^3 = 9.6$ Hz  \hspace{1cm} weak ROE, $J^3 = 7.6$ Hz

$2R,3R$-cyclo-Met$^2$
Table 4.4. Chemical shifts, coupling constants and temperature coefficients of various protons in F(2R,3R-cyclo-M)RF-NH$_2$.

<table>
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<th>amino acid</th>
<th>δ (ppm)</th>
<th>$J^3$ (Hz)</th>
<th>temperature coefficient (ppb K$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NH-α</td>
<td>α-β</td>
</tr>
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<td>br singlet</td>
</tr>
<tr>
<td></td>
<td>α</td>
<td>3.93</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β</td>
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<td>6.8</td>
</tr>
<tr>
<td></td>
<td>β</td>
<td>2.88</td>
<td>8.8</td>
</tr>
<tr>
<td>2R,3R-cyclo-Met$^2$</td>
<td>NH</td>
<td>8.91</td>
<td>singlet</td>
</tr>
<tr>
<td></td>
<td>α</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>β</td>
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</tr>
<tr>
<td></td>
<td>β$^{\text{trans}}$</td>
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</tr>
<tr>
<td></td>
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<td>ε</td>
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</tr>
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<td></td>
<td>β</td>
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<td>5.6 / 8.8$^b$</td>
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<tr>
<td></td>
<td>β$^1$</td>
<td>1.52</td>
<td>5.6 / 8.8$^b$</td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td></td>
<td>γ</td>
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<td></td>
<td>β$^1$</td>
<td>2.80</td>
<td>8.8</td>
</tr>
</tbody>
</table>

$^a$ Cis or trans relative to amino terminus. $^b$ Coupling constants estimated from the multiplets of α proton, and assignment of these coupling constants to a particular β proton is not possible because the β protons appear as a broad multiplets.
4.3.4 Phe-\{2S,3S-cyclo-Met\}-Arg-\{2R,3R-cyclo-Phe\}-NH$_2$

Two broken connectivities caused by the two 2,3-methanoamino acids were observed in the ROESY spectrum for \( \text{F}\{2S,3S-cyclo-M\}\text{R}\{2R,3R-cyclo-F\}-\text{NH}_2 \) (Figure 4.12 a and b). Stereospecific assignments of the \( \beta' \) protons for the \( 2S,3S-cyclo-Met \) were not possible because the signals of one of the \( \beta' \) protons overlapped with the \( \beta \) proton (Figure 4.13 b). However, the \( 2R,3R-cyclo-Phe^4 \) \( \beta'_{\text{cis}} \) and \( \beta'_{\text{trans}} \) (Figure 4.13 a) protons were assigned by a strong ROE observed for \( 2R,3R-cyclo-Phe^4 \beta-\beta_{\text{trans}} \) (Figure 4.13 b). Table 4.5 shows the chemical shift assignments for various protons.
Figure 4.12. (a) Sequential crosspeaks observed for F\(2S,3S\text{-cyclo-M}\)R\(2R,3R\text{-cyclo-F}\)NH\(_2\). (b) Amide-aliphatic region of the ROESY spectrum showing sequential connectivities.
Figure 4.13. (a) Stereospecific assignments of $2R,3R$-cyclo-Phe$^4\beta'_{\text{cis}}$ and $\beta'_{\text{trans}}$ protons in F{2S,3S-cyclo-M}R{2R,3R-cyclo-F}-NH$_2$. (b) Aliphatic-aliphatic region of the ROESY spectrum showing the strong crosspeaks for $2R,3R$-cyclo-Phe$^4\beta'_{\text{trans}}$. 

$$2R,3R$$-cyclo-Phe$^4$
Table 4.5. Chemical shifts, coupling constants and temperature coefficients of various protons in F(2S,3S-cyclo-M)R(2R,3R-cyclo-F)-NH₂.

<table>
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<th>Amino Acid</th>
<th>δ (ppm)</th>
<th>J₃ (Hz)</th>
<th>Temperature Coefficient (ppb K⁻¹)</th>
</tr>
</thead>
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<tr>
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<td>3.96</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>β</td>
<td>3.14</td>
<td>6.0</td>
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</tr>
<tr>
<td>β'</td>
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<td>8.0</td>
<td></td>
</tr>
<tr>
<td>2S,3S-cyclo-Met²</td>
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</tr>
<tr>
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</tr>
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<td>α</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>β</td>
<td>1.34</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>β'</td>
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<tr>
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<td></td>
</tr>
<tr>
<td>γ'</td>
<td>2.52</td>
<td>-</td>
<td></td>
</tr>
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</tr>
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<td></td>
</tr>
<tr>
<td>β'</td>
<td>1.34</td>
<td>-</td>
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<tr>
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<td>1.08</td>
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<td></td>
</tr>
<tr>
<td>δ</td>
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<td>-</td>
<td></td>
</tr>
<tr>
<td>δ'</td>
<td>2.88</td>
<td>-</td>
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<tr>
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<td>2R,3R-cyclo-Phe⁴</td>
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<td>singlet</td>
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</tr>
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<td>α</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>β</td>
<td>2.78</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>β''trans b</td>
<td>1.71</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>β''cis b</td>
<td>1.56</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

¹ Stereospecific assignment is not possible due to overlapping of β and β' protons (1.34 ppm). ² Cis or trans to amino terminus. ³ Proton with chemical shift coincident with another proton.
4.4 Distance Constraints Observed for FMRF-NH$_2$, F\{2S,3S-cyclo-M\}RF-NH$_2$, F\{2R,3R-cyclo-M\}RF-NH$_2$, and F\{2S,3S-cyclo-M\}R\{2R-3R-cyclo-F\}-NH$_2$

4.4.1 Phe-Met-Arg-Phe-NH$_2$

It is well known that short linear peptides undergo rapid conformational averaging in solution,\(^{126}\) therefore, a mixture of conformations exists instead of one or several highly populated conformers. This mixture of conformers is termed as "random coil structure".\(^{127}\) For a linear tetrapeptide like FMRF-NH$_2$, one would expect a random coil structure in solution. The ROESY spectrum of FMRF-NH$_2$ (Figure 4.7 b) is consistent with this because the spectrum showed only backbone sequential and short range intraresidue crosspeaks, and no interresidue ROE crosspeaks were observed. This is due to the rapid equilibrium between one conformer to the others prohibited the significant build-up of relatively long range ROE crosspeaks.

All the amide protons of FMRF-NH$_2$ had temperature coefficients > 3 ppb K$^{-1}$ (Table 4.2), which is evidence that no significant population of hydrogen-bonded amide conformer exists.

4.4.2 Phe-2S,3S-cyclo-Met-Arg-Phe-NH$_2$

Substitution of Met$^2$ in FMRF-NH$_2$ with 2S,3S-cyclo-Met$^2$ gave peptidomimetic F\{2S,3S-cyclo-M\}RF-NH$_2$, and induced two interresidue ROEs (Figure 4.14). Crosspeaks of 2S,3S-cyclo-Met$^2$NH$^3$NH and Phe$^1$aromatic-H-cyclo-Met$^2$$^2$$^2$H and/or $^\beta$ were observed in the ROESY experiment (Figure 4.8 b). Apart from these two ROE crosspeaks, a low temperature coefficient (-2.20 ppb K$^{-1}$) was observed for Arg$^3$NH (Table 4.3), and any proposed conformers should have an Arg$^3$NH which can account for the observed low temperature coefficient.
The intensities of the ROESY crosspeaks were assigned as VS (very strong), S (strong), M (medium), W (weak), and VW (very weak) by counting number of contours (Table 4.6). The crosspeaks intensities of the backbone sequential ROEs, and the two interresidue ROEs were obtained as distance constraints (Table 4.7).

**Figure 4.14.** Peptidomimetic F(2S,3S-cyclo-M)RF-NH$_2$ showing the two interresidue close contacts, and a low temperature coefficient for the Arg$^3$ amide proton.

![Diagram of peptidomimetic structure](image)

**Table 4.6.** Assignments of ROE crosspeaks intensities by counting number of contours.

<table>
<thead>
<tr>
<th>assignments</th>
<th>number of contours</th>
</tr>
</thead>
<tbody>
<tr>
<td>VS (very strong)</td>
<td>&gt; 4</td>
</tr>
<tr>
<td>S (strong)</td>
<td>4</td>
</tr>
<tr>
<td>M (medium)</td>
<td>3</td>
</tr>
<tr>
<td>W (weak)</td>
<td>2</td>
</tr>
<tr>
<td>VW (very weak)</td>
<td>1</td>
</tr>
</tbody>
</table>
Table 4.7. Distance constraints observed for F(2S,3S-cyclo-M)RF-NH$_2$.

<table>
<thead>
<tr>
<th>constraints</th>
<th>number of contours</th>
<th>intensities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phe$^1$aromaticH-2S,3S-cyclo-Met$^2$$^β/β'$$</td>
<td>1</td>
<td>VW</td>
</tr>
<tr>
<td>2S,3S-cyclo-Met$^2$NH-Arg$^3$NH</td>
<td>2</td>
<td>W</td>
</tr>
<tr>
<td>Phe$^1$$^α$-2S,3S-cyclo-Met$^2$NH</td>
<td>4</td>
<td>S</td>
</tr>
<tr>
<td>Arg$^3$NH-α</td>
<td>3</td>
<td>M</td>
</tr>
<tr>
<td>Arg$^3$$^α$-Phe$^4$NH</td>
<td>4</td>
<td>S</td>
</tr>
<tr>
<td>Phe$^4$NH-Phe$^4$$^α$</td>
<td>3</td>
<td>M</td>
</tr>
<tr>
<td>Phe$^4$$^α$-CONNH</td>
<td>4</td>
<td>S</td>
</tr>
</tbody>
</table>

4.4.3 Phe-2R,3R-cyclo-Met-Arg-Phe-NH$_2$

Two interresidue ROE's centered at 2R,3R-cyclo-Met$^2$ were found for F(2R,3R-cyclo-M)RF-NH$_2$ (Figure 4.15). Close contacts between 2R,3R-cyclo-Met$^2$NH-Arg$^3$NH and Phe$^1$aromaticH-2R,3R-cyclo-Met$^2$$^β_{cis}$ were observed (Figure 4.10 b). The intensities of the backbone sequential and interresidue crosspeaks are summarized in Table 4.8. Low temperature coefficients for the 2R,3R-cyclo-Met$^2$NH (-2.18 ppb K$^{-1}$) and Arg$^3$NH (-2.80 ppb K$^{-1}$) were also observed, indicating that these two protons may be involved in hydrogen bonding or be solvent shielded.

The interresidue ROE observed for F(2R,3R-cyclo-M)RF-NH$_2$ are very similar to F(2S,3S-cyclo-M)RF-NH$_2$, both having close contacts between the cyclo-Met$^2$NH-Arg$^3$NH and Phe$^1$aromaticH-cyclo-Met$^2$$^β$ and/or $β'$. However, two major differences were found between the NMR data for these two peptidomimetics. First, F(2S,3S-cyclo-M)RF-NH$_2$ has a weaker (ie weak intensity) crosspeak for the cyclo-Met$^2$NH-Arg$^3$NH close contact than F(2R,3R-cyclo-M)RF-NH$_2$ (ie medium intensity). Second, the low temperature coefficient for 2R,3R-cyclo-Met$^2$NH (-2.18 ppb K$^{-1}$) in F(2R,3R-cyclo-M)RF-NH$_2$ indicated H-bonded or solvent shielded
properties, while a relatively high temperature coefficient was found for the 2S,3S-cyclo-Met²NH (-3.89 ppb K⁻¹) in F{2S,3S-cyclo-M}RF-NH₂. These differences suggest that the two peptidomimetics may adopt different conformations.

**Figure 4.15.** Peptidomimetics F{2R,3R-cyclo-M}RF-NH₂ showing the two interresidue close contacts, and the amide protons giving low temperature coefficients.

![Diagram](image)

**Table 4.8.** Distance constraints for F{2R,3R-cyclo-M}RF-NH₂.

<table>
<thead>
<tr>
<th>constraints</th>
<th>number of contours</th>
<th>intensities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phe¹aromaticH(s)</td>
<td>2</td>
<td>W</td>
</tr>
<tr>
<td>2R,3R-cyclo-Met²β'cis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2R,3R-cyclo-Met²NH-Arg³NH</td>
<td>3</td>
<td>M</td>
</tr>
<tr>
<td>2R,3R-cyclo-Met²NH-Phe¹α</td>
<td>5</td>
<td>VS</td>
</tr>
<tr>
<td>Arg³NH-α</td>
<td>3</td>
<td>M</td>
</tr>
<tr>
<td>Phe⁴NH-Arg³α</td>
<td>4</td>
<td>S</td>
</tr>
<tr>
<td>Phe⁴NH-α</td>
<td>3</td>
<td>M</td>
</tr>
<tr>
<td>CONH-NH-Phe⁴α</td>
<td>4</td>
<td>S</td>
</tr>
</tbody>
</table>
4.4.4 Phe-{2S,3S-cyclo-Met}-Arg-{2R,3R-cyclo-Phe}-NH$_2$

Two natural amino acids were substituted by 2,3-methanoamino acids in F{2S,3S-cyclo-M}R{2R,3R-cyclo-F}-NH$_2$. These substitutions induced five interresidue side chain to side chain crosspeaks (Figure 4.16). The intensities of these and the backbone sequential crosspeaks were summarized in Table 4.9. The absence of NH to NH ROE crosspeaks, together with the high temperature coefficients (i.e., non-hydrogen bonded) observed for 2S,3S-cyclo-Met$^2$ (-3.80 ppb K$^{-1}$), Arg$^3$ (-6.10 bpb K$^{-1}$), and 2R,3R-cyclo-Phe$^4$ (-5.40 bpb K$^{-1}$) excluded tight turn structures as major conformers.

Figure 4.16. Interresidue side chain to side chain ROEs observed for F{2S,3S-cyclo-M}R{2R,3R-cyclo-F}-NH$_2$. 

![Diagram of the molecular structure showing the interactions between Phe, Met, and Arg residues.](image-url)
Table 4.9. Distance constraints for F\{2S,3S-cyclo-M\}R\{2R,3R-cyclo-F\}-NH$_2$.

<table>
<thead>
<tr>
<th>constraints</th>
<th>number of contours</th>
<th>intensities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phe$^1$β-Arg$^3$βs</td>
<td>2</td>
<td>W</td>
</tr>
<tr>
<td>Phe$^1$aromaticH-</td>
<td>2</td>
<td>W</td>
</tr>
<tr>
<td>2S,3S-cyclo-Met$^2$β/β'</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2R,3R-cyclo-Phe$^4$aromaticH-Arg$^3$βs</td>
<td>1</td>
<td>VW</td>
</tr>
<tr>
<td>2R,3R-cyclo-Phe$^4$aromaticH-Arg$^3$γs</td>
<td>2</td>
<td>W</td>
</tr>
<tr>
<td>2R,3R-cyclo-Phe$^4$aromaticH-Arg$^3$βs</td>
<td>1</td>
<td>VW</td>
</tr>
<tr>
<td>Phe$^1$α-</td>
<td>4</td>
<td>S</td>
</tr>
<tr>
<td>2S,3S-cyclo-Met$^2$NH</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg$^3$NH-α</td>
<td>1</td>
<td>VW</td>
</tr>
<tr>
<td>2R,3R-cyclo-Phe$^4$NH-Arg$^3$α</td>
<td>3</td>
<td>M</td>
</tr>
</tbody>
</table>

4.5 Upfield Chemical Shifts Observed for Amide Protons with Low Temperature Coefficients

The temperature coefficients and chemical shifts of the amide protons for the peptide FMRF-NH$_2$ and peptidomimetics F\{2S,3S-cyclo-M\}RF-NH$_2$, F\{2R,3R-cyclo-M\}RF-NH$_2$, and F\{2S,3S-cyclo-M\}R\{2R,3R-cyclo-F\}-NH$_2$ are tabulated in Table 4.10. Three of the amide protons were found to have temperature coefficients less than 3 ppb K$^{-1}$ (shown as italics in Table 4.10) indicating H-bonded or solvent shielded properties.
Table 4.10. Chemical shifts (at 25 °C) and temperature coefficients of amide protons for (a) FMRF-NH$_2$, (b) F(2S,3S-cyclo-M)RF-NH$_2$, (c) F(2R,3R-cyclo-M)RF-NH$_2$, and (d) F(2S,3S-cyclo-M)R(2R,3R-cyclo-F)-NH$_2$

<table>
<thead>
<tr>
<th>residues</th>
<th>peptide/peptidomimetics</th>
<th>chemical shifts in ppm (temperature coefficients in ppb K$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>a</td>
</tr>
<tr>
<td>Met$^2$/cyclo-Met$^2$NH</td>
<td></td>
<td>8.69 (-3.68)</td>
</tr>
<tr>
<td>Arg$^3$NH</td>
<td></td>
<td>8.20 (-4.61)</td>
</tr>
<tr>
<td>Phe$^4$/cyclo-Phe$^4$NH</td>
<td></td>
<td>7.95 (-4.49)</td>
</tr>
</tbody>
</table>

The 2S,3S-cyclo-Met$^2$NH in F(2S,3S-cyclo-M)RF-NH$_2$ had a temperature coefficient of -2.20 ppb K$^{-1}$, and the 2R,3R-cyclo-Met$^2$NH and the Arg$^3$NH in F(2R,3R-cyclo-M)RF-NH$_2$ had values of -2.18 and -2.80 ppb K$^{-1}$, respectively. Coincidentally, the amide protons with low temperature coefficients also experienced upfield chemical shifts at 25 °C (Table 4.10). The chemical shift of the 2R,3R-cyclo-Met$^2$NH (8.90 ppm) is the lowest of the three peptidomimetics containing 2,3-methanomethionine (ca 9.12 ppm for F(2S,3S-cyclo-M)RF-NH$_2$, and 9.09 for F(2S,3S-cyclo-M)R(2R,3R-cyclo-F)-NH$_2$). Similarly, an upfield chemical shift was observed for the Arg$^3$NH of F(2S,3S-cyclo-M)RF-NH$_2$. This proton had the lowest temperature coefficient when compared with Arg$^2$NH in other peptide or peptidomimetics, or with other intramolecular amide protons (i.e., 2S,3S-cyclo-Met$^2$NH or Phe$^4$NH in F(2S,3S-cyclo-M)RF-NH$_2$).

Hydrogen bonded amide protons usually experience an upfield shift in hydrogen bonding solvents (e.g., DMSO, H$_2$O). This is presumably a consequence of the intramolecular H-bonding involving the amide proton being weaker than the intermolecular H-bonding between the solvent exposed NH and the solvent. Shielding the amide protons from the relatively electronegative solvent may effect similar upfield shifts. Therefore, the
upfield shift for low temperature coefficient amide protons implies H-bonding or solvent shielding, and is consistent with the temperature coefficients observed in this study.

4.6 Conformational Rigidity Induced by Incorporation of 2,3-Methanoamino Acids to Linear Peptides

Short linear peptides such as the tetrapeptide FMRF-NH$_2$ undergo rapid conformational averaging in solution. Replacing the natural methionine with cyclo-Met analogs (ie F[2S,3S-cyclo-M]RF-NH$_2$ and F[2R,3R-cyclo-M]RF-NH$_2$) imposes a significant effect on this averaging process. Interresidue ROE crosspeaks were observed centered at these cyclo-Met$^2$ analogs suggesting that the conformational averaging process was reduced around this residue.

Replacement of two natural amino acids with two 2,3-methanoamno acids as in F[2S,3S-cyclo-M]R[2R,3R-cyclo-F]-NH$_2$ had a drastic effect on the linewidths of the $^1$H-NMR spectrum (Figure 4.17). Significant peak broadening was observed for the NMR spectrum of F[2S,3S-cyclo-M]R[2R,3R-cyclo-F]-NH$_2$ at 25 °C. The doublet Arg$^3$NH signal in FMRF-NH$_2$, F[2S,3S-cyclo-M]RF-NH$_2$, or F[2R,3R-cyclo-M]RF-NH$_2$ became a broad singlet in F[2S,3S-cyclo-M]R[2R,3R-cyclo-F]-NH$_2$. Also, the multiplets for the Phe$^1$$\alpha$ and Arg$^3$$\alpha$ in FMRF-NH$_2$, F[2S,3S-cyclo-M]RF-NH$_2$, or F[2R,3R-cyclo-M]RF-NH$_2$ became two broad signals in F[2S,3S-cyclo-M]R[2R,3R-cyclo-F]-NH$_2$. The broadening of these peaks suggests that F[2S,3S-cyclo-M]R[2R,3R-cyclo-F]-NH$_2$ may contain several conformers, which equilibrate slowly at 25 °C. This slow equilibration was likely caused by the conformational rigidity imposed by the two cyclopropane ring.
When the temperature was raised from 25 to 55 °C, the Arg\textsuperscript{3}NH, Phe\textsuperscript{4}α and Arg\textsuperscript{3}α signals in F{2S,3S-cyclo-M}R{2R,3R-cyclo-F}-NH\textsubscript{2} sharpened (ie Arg\textsuperscript{3}NH changed form a broad singlet to doublets, and the Phe\textsuperscript{4}α and Arg\textsuperscript{3}α became sharper multiplets). At this higher temperature (ie 55 °C), faster equilibration of the relatively rigid conformers induced the sharpening (ie decrease of linewidth) of the signals. Additionally, conformational rigidity of F{2S,3S-cyclo-M}R{2R,3R-cyclo-F}-NH\textsubscript{2} was further supported by the observation of extensive side chain to side chain ROEs. Therefore, it may conclude that the incorporation of 2,3-methanoamino acids into linear peptides does induce NMR observable conformational rigidity.

4.7 Conclusion

Incorporation of 2,3-methanoamino acids to linear peptide induced conformational rigidity, and the rigidity was observed by: (i) interresidue ROE crosspeaks centered at the methanologs; (ii) line broadening for F{2S,3S-cyclo-M}R{2R,3R-cyclo-F}-NH\textsubscript{2} in the \textsuperscript{1}H-NMR spectrum. Distance constraints for peptidomimetics F{2S,3S-cyclo-M}RF-NH\textsubscript{2}, F{2R,3R-cyclo-M}RF-NH\textsubscript{2}, and F{2S,3S-cyclo-M}R{2R,3R-cyclo-F}-NH\textsubscript{2} were obtained from this NMR study. The NMR data for the peptidomimetics clearly shows some conformational biases for these molecules that cannot be discerned for FMRF-NH\textsubscript{2} in solution. This was evident from unusual chemical shifts and temperature coefficients of some NH signals, and from some interresidue crosspeaks.
Chapter 5. Solution Conformations of Peptidomimetics Containing 2,3-Methanoamino Acids: Part II Molecular Modeling

5.1 Introduction

Molecular modeling studies presented in this Chapter involve: (i) parameterization of 2,3-methanoamino acids; (ii) $\phi, \psi$ grid searches for 2,3-methanoamino acids derivatives Ac-Acc-NHMe, Ac-2R,3R-cyclo-Phe-NHMe, Ac-2S,3S-cyclo-Met-NHMe, and Ac-2R,3R-cyclo-Met-NHMe; (iii) quenched molecular dynamics (QMD) for FMRF-NH$_2$, F[2S,3S-cyclo-M]RF-NH$_2$, F[2R,3R-cyclo-M]RF-NH$_2$, and F[2S,3S-cyclo-M]R[2R,3R-cyclo-F]-NH$_2$.

\[
\begin{align*}
\text{Ac-Acc-NHMe} & & \text{Ac-2R,3R-cyclo-Phe-NHMe} \\
\text{Ac-2R,3R-cyclo-Met-NHMe} & & \text{Ac-2S,3S-cyclo-Met-NHMe} \\
\end{align*}
\]

Phe-Met-Arg-Phe-NH$_2$
Phe-2S,3S-cyclo-Met-Arg-Phe-NH$_2$
Phe-2R,3R-cyclo-Met-Arg-Phe-NH$_2$
Phe-2S,3S-cyclo-Met-Arg-2R-3R-cyclo-Phe-NH$_2$
5.2 Secondary Structures of Peptides and Proteins

Geometries of some peptides and protein secondary structures are listed in Table 5.1. These structures will be referred to later in this Chapter. A steric contour diagram (Figure 5.1) for an alanine residue in a polypeptide chain with the locations of \( \phi, \psi \) dihedrals for various secondary structures is also included for comparison.  

Table 5.1. Standard geometry of some secondary structures found in peptides and proteins. 

<table>
<thead>
<tr>
<th>structure</th>
<th>i or ( i+1^a )</th>
<th>i+2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \phi )</td>
<td>( \psi )</td>
</tr>
<tr>
<td>( \alpha )-helix (( \alpha_L ))</td>
<td>-58</td>
<td>-47</td>
</tr>
<tr>
<td>( \alpha )-helix (( \alpha_R ))</td>
<td>58</td>
<td>47</td>
</tr>
<tr>
<td>( 3_{10} )-helix</td>
<td>-49</td>
<td>-26</td>
</tr>
<tr>
<td>inverse ( \gamma )-turn</td>
<td>-70 to -85</td>
<td>60 to 70</td>
</tr>
<tr>
<td>( \beta )-turn: type I</td>
<td>-60</td>
<td>-30</td>
</tr>
<tr>
<td>type I'</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td>type II</td>
<td>-60</td>
<td>120</td>
</tr>
<tr>
<td>type II'</td>
<td>60</td>
<td>-120</td>
</tr>
<tr>
<td>type III</td>
<td>-60</td>
<td>-30</td>
</tr>
<tr>
<td>type III'</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td>type VIa(cis)</td>
<td>-60</td>
<td>120</td>
</tr>
<tr>
<td>type VIb (cis)</td>
<td>-120</td>
<td>120</td>
</tr>
</tbody>
</table>

\( a \) i +1 for \( \beta \)-turn and inverse \( \gamma \)-turn, and i for the rest of the structures. i is the first amino acid residue involved in the particular structure.
Figure 5.1. Steric contour diagram for an alanine residue in a polypeptide chain. Dark zones show normal contact radii for various atom pairs, and light zones show some of the shortest contact radii observed in crystal structures. Symbols denote right hand $\alpha$ helix ($\alpha_R$), left hand $\alpha$ helix ($\alpha_L$), parallel ($\uparrow\uparrow$), antiparallel ($\uparrow\downarrow$) pleated sheet, polyglycine II (II), and collagen (C).\textsuperscript{130}

5.3 Force Field Parameterization

The CHARMM (version 20; version 19 default parameters) modeling package was used for the molecular simulations performed in this work.\textsuperscript{131,132} It was first necessary to parameterize the 2,3-methanoamino acids because CHARMM does not contain parameters for the methanologs.

Data for the parameterization process was obtained from crystallographic data\textsuperscript{9,11} and from CHARMM defaults. Specifically, equilibrium bond lengths, bond angles, and improper dihedrals were derived from crystallographic coordinates; force constants, non-bonded parameters, and atomic charges were adapted from the CHARMM defaults for
natural amino acids. Cyclopropane carbons were assumed to be the same as quaternary, methylene, and methine carbons already described in the CHARMM 19 carbon types. Extended atom representations of the non-polar hydrogens were used. A detailed list of the parameters are provided in Appendix 4.

5.4 Ramachandran Plots

5.4.1 Method

Ramachandran plots\textsuperscript{133} of Ac-Acc-NHMe, Ac-2\textit{R},3\textit{R}-cyclo-Phe-NHMe, Ac-2\textit{S},3\textit{S}-cyclo-Met-NHMe, and Ac-2\textit{R},3\textit{R}-cyclo-Met-NHMe were made to gauge the efficacy of the parameter sets generated. Ramachandran plot in this study represents the energy contours diagram obtained by varying two dihedral angles. In the case of Ac-2\textit{S},3\textit{S}-cyclo-Met-NHMe and Ac-2\textit{R},3\textit{R}-cyclo-Met-NHMe, "mirror image coordinates" were used for these enantiomers, otherwise the starting conformers have different energies due to dissimilar side chain conformations. All of the compounds were entered as extended conformations, and subsequently minimized in 1000 steps using the adopted basis Newton-Raphson method ($\varepsilon_0 = 1$, corresponding to vacuum). Conformational energies were then calculated by systematic variation of the $\phi$ and $\psi$ torsions in 20° intervals, followed by the 1000 steps of adopted basis Newton-Raphson minimization. A grid of 18 x 18 energy points in the $\phi,\psi$ plane was thereby generated. No constraints were applied to the side chain torsions during minimization. The 10 lowest energy contours were plotted at 1 kcal mol\textsuperscript{-1} intervals to give the desired Ramachandran plots (Figure 5.2 a to d). The calculations were repeated at $\varepsilon_0 = 45$ representing the dielectric constant of DMSO (Figure 5.2 e to h).
Figure 5.2. Ramachandran plots of 2,3-methanoamino acid derivatives; (a) to (d) in vacuum and (e) to (h) in DMSO.

a Ac-Acc-NHMe in vacuum

b Ac-2S,3S-cyclo-Met-NHMe

c Ac-2R,3R-cyclo-Met-NHMe

d Ac-2R,3R-cyclo-Phe-NHMe

e Ac-Acc-NHMe in DMSO

f Ac-2S,3S-cyclo-Met-NHMe

g Ac-2R,3R-cyclo-Met-NHMe

h Ac-2R,3R-cyclo-Phe-NHMe
5.4.2 Results and Discussions

As expected, C7 conformations ($\phi \sim +80^\circ$, $\psi \sim -80^\circ$, or $\phi \sim -80^\circ$, $\psi \sim 80^\circ$) were observed as the lowest energy conformations for all of the derivatives in vacuum (Figure 5.2 a to d). The $\alpha_R$, $\alpha_L$, 310-helix, and bridge ($\psi = 0^\circ$) regions were covered within the 10 kcal mol$^{-1}$ contours for Ac-Acc-NHMe (Figure 5.2 a), and Ac-$2R,3R$-cyclo-Phe-NHMe (Figure 5.2 d). The $\phi$, $\psi$ maps of Ac-$2S,3S$-cyclo-Met-NHMe (Figure 5.2 b), and Ac-$2R,3R$-cyclo-Met-NHMe (Figure 5.2 c) were identical to each other after 180$^\circ$ rotation of the contours, therefore the Ramachandran plots shown in Figure 5.2 b and c can be reflected in the diagonal running from -180$^\circ$, -180$^\circ$ to +180$^\circ$, +180$^\circ$. This is because the two molecules are symmetrical to each other, and identical energy should be obtained for one conformer and its mirror image. Unlike Ac-Acc-NHMe and Ac-$2R,3R$-cyclo-Phe-NHMe, the $\alpha_R$ region was marginally covered by the 10 kcal mol$^{-1}$ contours for Ac-$2R,3R$-cyclo-Met-NHMe (Figure 5.2 c), while the $\alpha_L$ region was marginally covered for Ac-$2S,3S$-cyclo-Phe-NHMe.

Figure 5.3. C7 conformation observed for 2,3-methanoamino acid derivatives.

The result of the Ac-Acc-NHMe calculation was consistent with parameterization/grid searches for 2,3-methanoamino acids previously reported by Barone and Stammer.\textsuperscript{9,134,135} Minor discrepancies between the two studies were observed, but this is expected since Barone's and Stammer's calculations used a different force field and
fixed atom geometries. The CHARMM package uses flexible atom geometries so that bonds may distort slightly when close contacts occur.

The predominance of low energy C7 conformers is caused by the large electrostatic interactions between the CQk-1 to NHk+1 atoms. This interaction was attenuated in DMSO (Figure 5.2 e to h). The 10 kcal mol⁻¹ contours covered a much broader conformational space as compared to the vacuum calculations, although the C7 conformation was still the lowest energy conformation. It is difficult to predict the favored conformers in DMSO based on these Ramachandran plots, because the energy contours nearly cover the whole \( \phi \), \( \psi \) plane.¹²

5.5 Quenched Molecular Dynamics

5.5.1 Methods

Quenched molecular dynamics (QMD)¹¹³,¹¹⁴ simulations (Figure 5.4) were performed using the newly generated parameters for the unusual amino acids (vide supra). In this technique, a large number of conformations was produced at high temperature (ca 1000 K). This elevated temperature allowed the molecule to explore a number of conformations that are separated by energy barriers which would be crossed very infrequently at room temperature. The high temperature structures were then quenched (ie removal of thermal energy) by two energy minimization techniques, namely steepest decent (SD), and adopted basis Newton-Raphson method (ABNR).¹³⁶ Steepest decent algorithm was used to preliminary eliminated some steric conflicts, and thorough minimization of the structures was achieved by the more convergent ABNR method.

The detail implementation of the method is as follow: FMRF-NH₂, F[2S,3S-cyclo-M]RF-NH₂, F[2R,3R-cyclo-M]RF-NH₂, and F[2S,3S-cyclo-M]R[2R,3R-cyclo-F]NH₂ were built in extended conformations with positive charges on the amino terminus and the \( \text{Arg}^3 \) guanidine side chain. These starting conformers were
minimized using 1000 steps of adopted basis Newton-Raphson method, and a dielectric constant of 45 representing DMSO. These minimized structures were then subjected to dynamics simulations. Throughout, the equations of motion were integrated using the Verlet algorithm with a time step of 1 fs (femto second), and SHAKE algorithm was used to constrain all bond lengths containing polar hydrogens. Each peptide was heated to 1000 K over 10 ps (pico second) by increasing the temperature by 10 K every 0.1 ps. Gaussian distributions of velocities were assigned to the atoms during the heating process. The peptide was equilibrated for 10 ps at 1000 K, during which time a ±13 K temperature constraint was applied to the system. Molecular dynamics production runs were then performed in the microcanonical (NVE) ensemble for a total time of 600 ps.\textsuperscript{132,136} The total energy of the system during the dynamics production runs was monitored (Section 5.5.2), and the trajectories were saved every 1 ps. A total of 600 structures was produced. Each of the structures was thoroughly minimized using 1000 steps of steepest decent (SD) followed by adopted basis Newton-Raphson until a RMS energy derivative of ≤ 0.001 kcal mol\textsuperscript{-1} Å\textsuperscript{-1} was obtained. During minimization procedure a harmonic potential (force constant of 50 kcal mol\textsuperscript{-1} rad\textsuperscript{-2} was applied to each of the \( \phi \) dihedrals of the peptide and peptidomimetics to eliminate some cis-peptide bonds which were a direct consequence of the force field and high temperature used, and did not suggest any physical peptide bond isomerization.\textsuperscript{113,114} Histograms of energies vs number of structures were plotted for the 600 structures (Section 5.5.4) after minimization.

Structures within ≤ 4 kcal mol\textsuperscript{-1} of the global minimum were selected for further analyses. Previous studies, specifically of tuftsin (Thr-Lys-Pro-Arg) and Met-enkephalin (Tyr-Gly-Gly-Phe-Met), have shown that a 3 to 7 kcal mol\textsuperscript{-1} energy cutoff was sufficient to select the structures which gave reasonable agreement with the NMR data.\textsuperscript{113,114} By analogy, it was assumed here that a 4 kcal mol\textsuperscript{-1} cutoff would select the most relevant structures for NMR comparison. The selected low energy structures were sorted into
families based on the RMS deviation of each Cα-CO fragments. Structures with RMS deviation ≤ 0.6 Å were grouped in the same families. Throughout this study, the family with the lowest energy conformer detected was labeled F1, and subsequent families were numbered according to the energies of the lowest energy structures in each. Previous studies have shown that including more backbone atoms in the fitting procedure did not significantly alter the results. The coordinates of the lowest energy structures of each family were extracted, and protons were built onto the heavy atoms using standard geometries. Finally, the interproton distances were calculated from these coordinates for comparisons of the simulated structures with the ROE data obtained in the NMR studies.
Figure 5.4. Procedures for quenched molecular dynamics.

[peptide/peptidomimetic]$^{2+}$ in extended conformation

↓

initial minimization

$\varepsilon_0 = 45$ (DMSO)

↓

SHAKE bonds

↓

heating: 0 to 1000 K in 10 ps

equilibration: 1000 K for 10 ps

dynamics simulation: 600 ps

(structures were saved every 1 ps)

↓

600 high temperature structures

↓

minimizations: 1000 steps SD + ABNR

until RMS deviation $\leq 0.001$ kcal mol$^{-1}$ Å$^{-1}$

↓

structures with energy $\leq 4$ kcal mol$^{-1}$ from minimum energy were selected

↓

sorted to families based on RMS deviation of $\text{C}^\alpha-\text{C}O$ of each residues

structures with RMS deviation $\leq 0.6$ were sorted to the same family

↓

lowest energy structure of each family was compared with NMR data
5.5.2 Constant Total Energy During Dynamics Production Runs

The total energy of the systems were monitored to ensure that constant temperatures were maintained during dynamics simulations. Figure 5.5 shows the total energy for the first 100 ps during dynamics simulations for the peptide and peptidomimetics. For all of the systems, the total energy first increased gradually during the heating process (0 to 10 ps), then fluctuated in a narrow range for equilibration (10 to 20 ps), and remained relatively constant during the dynamics production run (20 to 600 ps). The constant total energy during the dynamic production run indicated that a constant temperature was maintained.

Figure 5.5. Plots of total energy vs time for the dynamics simulations (only the first 100 ps are shown). (a) FMRF-NH₂, (b) F{2S,3S-cyclo-M}RF-NH₂, (c) F{2R,3R-cyclo-M}RF-NH₂, and (d) F{2S,3S-cyclo-M}R{2R,3R-cyclo-F}-NH₂.
5.5.3 $\phi$, $\psi$ Scatter plots for High Temperature Structures

Collected data for the 600 high energy conformers generated in each of the QMD studies (see Section 5.5.1) were revealing. These data are here presented as $\phi$, $\psi$ scatter plots wherein each point represents the conformation about the specified amino acid residue for one of the collection of structures downloaded. Figure 5.6 a to h shows such dot plots for FMRF-NH$_2$, and F[2S,3S-cyclo-M]R[2R,3R-cyclo-F]-NH$_2$, respectively, and Figure 5.7 a to h shows similar plots for Phe-[2S,3S-cyclo-M]RF-NH$_2$, and F[2R,3R-cyclo-M]RF-NH$_2$, respectively.

Distributions of $\phi$ torsions for all the natural amino acids in these target compounds were heavily bias towards negative values (eg Figure 5.6 b). This reflects the intrinsic steric effects imposed by the C$\alpha$ chiral center of natural amino acids, as can be seen from the Newman projections shown below.

Both negative and positive $\phi$ were equally populated in cyclo-Met$^2$ as is shown in Figures 5.6 f, 5.7 b, and 5.7 f, in striking contrast to the natural amino acids. This bias can also be explained using Newman projections. The only difference between the two C$\alpha$ "side chain" substituents of a 3-substituted 2,3-methanoamino acid is the 3-substituent. This substituent is rigidly oriented away from the N-C$\alpha$ bond, hence it has insignificant steric effects on the $\phi$ torsion.
By changing the side chain stereochemistry from E as for cyclo-Met\(^2\) to Z as for 2R,3R-cyclo-Phe\(^4\), a narrowing of negative $\phi$ region was observed (Figure 5.6 h). This is because the 3-substituent (i.e., the aromatic ring) was now on the same side of the $\phi$ torsion, and the bulky aromatic ring partially shielded the negative $\phi$ region as shown by the Newman projections below.

Scatter plots for 2S,3S-cyclo-Met\(^2\) (Figure 5.7 b) and 2R,3R-cyclo-Met\(^2\) (Figure 5.7 f) reveal that $\psi$ values for the former amino acid are more positive (or less negative) than the corresponding torsions for the latter stereoisomer. This is consistent with the following reasoning from Newman projections. For 2S,3S-cyclo-Met\(^2\), interactions of the cyclopropane 3-substituent between either the peptide fragment on the C-terminus (for negative $\psi$ torsions) or the carbonyl oxygen (for positive $\psi$ torsions), are
critical. These interactions tend to compress negative $\psi$ angles and expand positive $\psi$ values, as indicated below.

\[-ve \psi \text{ compressed} \quad +ve \psi \text{ expanded}\]

Exactly the inverse of the situation shown above is true for \textbf{2R,3R-cyclo-Met}. For this derivative, negative $\psi$ torsions are expanded, and positive ones are compressed, as shown below.

\[-ve \psi \text{ expanded} \quad +ve \psi \text{ compressed}\]
Figure 5.6. $\phi$, $\psi$ Scatter plots for the amino acid residues of FMRF-NH$_2$ (a to d), and $\text{F}[2S,3S$-$\text{cyclo-M}]\text{R}[2R,3R$-$\text{cyclo-F}]-\text{NH}_2$ (e to h) at 1000K.

Figure 5.7. $\phi$, $\psi$ Scatter plots for the amino acid residues of F(2S,3S-cyclo-M)RF-NH$_2$ (a to d), and F(2R,3R-cyclo-M)RF-NH$_2$ (e to h) at 1000K.
5.5.4 Gaussian Energy Histograms for Minimized Structures

Histograms of energies vs number of structures were plotted for the 600 structures after the molecular mechanics minimizations; Gaussian distributions were obtained for all the molecules (Figure 5.8) with no discrete high or low energy extremes. More degeneracy for the medium energy structures were observed.

Figure 5.8. Gaussian energy histograms for the QMD studies of (a) FMRF-NH₂, (b) F{2S,3S-cyclo-M}RF-NH₂, (c) F{2R,3R-cyclo-M}RF-NH₂, and (d) F{2S,3S-cyclo-M}R{2R,3R-cyclo-F}-NH₂.
5.5.5 $\phi, \psi$ Scatter Plots for Minimized Structures

Scatter plots generated for the 600 structures after molecular mechanics minimization were also informative. Figure 5.9 a to h show these plots for FMRF-NH$_2$, and F[2S,3S-cyclo-M]R[2R,3R-cyclo-F]-NH$_2$, respectively, and Figure 5.10 a to h show plots for F[2S,3S-cyclo-M]RF-NH$_2$, and F[2R,3R-cyclo-M]RF-NH$_2$, respectively.

Distribution of the natural amino acid conformers converges, as expected, to areas including $\phi, \psi$ torsion regions of most of the common elements of secondary structure (i.e. $\alpha$-helices, turn regions, $\beta$-sheets). Thus the torsions concentrate in the negative $\phi$ region ($\textit{vide supra}$), and in $\psi$ regions between approximately 60 to 180° and -70 to 0°. The $\phi, \psi$ scatter plots sampled most of the conformational space available for natural amino acids (Section 5.2), and resembled the $\phi, \psi$ distribution plot obtained from high resolution X-ray crystallography (Figure 5.11).$^{137}$ Successful sampling of the favorable $\phi, \psi$ conformational space by the scatter plots for the natural amino acids implies the same $\phi, \psi$ distribution plots for 2,3-methanoamino acids should give reasonable predictions of the available conformational space for such analogs.

The $\phi, \psi$ scatter plots for 2S,3S-cyclo-Met (Figure 5.9 f and Figure 5.10 b), 2R,3R-cyclo-Met (Figure 5.10 f), and 2R,3R-cyclo-Phe (Figure 5.10 h) show that the available conformational space is separated to four major regions. The $\phi$ torsions took very narrow values centered at +70° or -70°, while the $\psi$ torsions were more flexible, having values from approximately +20° to +160° and -20 to -160°. Consequently, these four plateaus covered the $\gamma$-turn, inverse-$\gamma$-turn, $\alpha_R$-helix, $\alpha_L$-helix, and $3_{10}$-helix regions. The change of stereochemistry from 2S,3S- to 2R,3R-cyclo-Met mediated an upward shift of $\psi$ angles (i.e. $\psi$ decrease for 2R,3R-cyclo-Met$^2$). This implies that fine tuning of the $\psi$ torsion by changing the absolute stereochemistry of the $E$-2,3-methanoamino acids is possible.
Figure 5.9. $\phi, \psi$ Scatter plots for FMRF-NH$_2$ (a to d), and F{2S,3S-cyclo-M}R{2R,3R-cyclo-F}-NH$_2$ (e to h) after molecular mechanics minimizations.

- **a** Phe$^1$
- **b** Met$^2$
- **c** Arg$^3$
- **d** Phe$^4$
- **e** Phe$^1$
- **f** 2S,3S-cyclo-Met$^2$
- **g** Arg$^3$
- **h** 2R,3R-cyclo-Phe$^4$
Figure 5.10. $\phi, \psi$ Scatter plots for $F\{2S,3S\text{-cyclo-M}\}RF\text{-NH}_2$ (a to d), and $F\{2R,3R\text{-cyclo-M}\}RF\text{-NH}_2$ (e to h) after molecular mechanics minimizations.

a Phe$^1$

b 2$S,3S$-cyclo-Met$^2$

c Arg$^3$

d Phe$^4$

e Phe$^1$

f 2$R,3R$-cyclo-Met$^2$

g Arg$^3$

h Phe$^4$
Figure 5.11. Plot of $\phi$, $\psi$ dihedral angles for approximately 1000 non-glycine residues in eight protein whose structures have been determined by X-ray crystallography.\textsuperscript{137}

5.5.6 $\phi$, $\psi$ Scatter Plots for Structures within 4 kcal mol$^{-1}$ Cutoff

Scatter plots of the lowest energy structures (i.e., within 4 kcal mol$^{-1}$ of the lowest energy structure overall) are shown in Figure 5.12 (a to h FMRF-NH$_2$ and F{2S,3S-cyclo-M}R{2R,3R-cyclo-F}-NH$_2$, respectively) and Figure 5.13 (a to h F{2S,3S-cyclo-M}RF-NH$_2$ and F{2R,3R-cyclo-M}RF-NH$_2$, respectively). As expected, the natural amino acid residues have low energy structures concentrated in the negative $\phi$ region of conformational space. The two positive $\psi$ quadrants of 2S,3S-cyclo-Met\textsuperscript{2} in F{2S,3S-cyclo-M}R{2R,3R-cyclo-F}-NH$_2$ are eliminated when applying the energy cutoff (Figure 5.12 f), and the remaining two quadrants are equally populated. For the rest of the 2,3-methanoamino acids (Figure 5.12 h, Figure 5.13 b, and Figure 5.13 f), a statistical preference for the positive $\phi$/negative $\psi$ quadrant is observed; however, relative energies of these structures within the allowed 4 kcal mol$^{-1}$ band were not a factor in these plots.
Figure 5.12. $\phi, \psi$ Scatter plots for FMRF-NH$_2$ (a to d), and F(2S,3S-cyclo-M)RF-NH$_2$ (e to h) after molecular mechanics minimizations.

(a) Phe$^1$

(b) Met$^2$

(c) Arg$^3$

(d) Phe$^4$

(e) Phe$^1$

(f) 2S,3S-cyclo-Met$^2$

(g) Arg$^3$

(h) 2R,3R-cyclo-Phe$^4$
Figure 5.13. $\phi$, $\psi$ Scatter plots for F[2S,3S-cyclo-M]RF-NH$_2$ (a to d), and F[2R,3R-cyclo-M]RF-NH$_2$ (e to h) after molecular mechanics minimizations.
5.5.7 Characteristics of Families Obtained from QMD

Structures within the 4 kcal mol\(^{-1}\) cutoff were selected and sorted into families based on the backbone RMS deviations. The lowest energy structure in each family was chosen as the representative structure for the corresponding family, and the characteristics of this structure is discussed.

5.5.7.1 Phe-Met-Arg-Phe-NH\(_2\)

Results for the QMD study of FMRF-NH\(_2\) were as follows. There were a total of 32 structures in the lowest 4 kcal mol\(^{-1}\) of the energy distribution (Table 5.2). These were grouped into six families, but four of these (i.e., F2, F4, F5, and F6) had only one or two members. The more populated families F1 and F3 contained 20 and 7 members, respectively. The lowest energy structure overall was part of the most populated family (F1 by definition, see Section 5.5.1); this had all the NHs oriented in one direction and all the CQs oriented in the opposite direction (Figure 5.14). The charged guanidine side chain of Arg\(^3\) was on the same side of the molecule as the hydrophobic side chains, therefore this structure is not strictly "amphiphilic", but it is closely related. No hydrogen bonding was observed for this structure. For family F3, the minimum energy conformer had a hydrogen bond between the Arg\(^3\) guanidine NH and the Phe\(^1\)CQ forming a 14-member ring. The two aromatic rings of Phe\(^1\) and Phe\(^4\) in this conformer were positioned near to each other, although not so close that any form of \(\pi\)-stacking was apparent (Figure 5.15).
Table 5.2. Structural characteristics of the low energy conformers from each of the families generated in the QMD study of FMRF-NH$_2$.

<table>
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<th>residue</th>
<th>dihedral angle</th>
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<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
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<td>-60</td>
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</table>

| no. of members in family | 20 | 2 | 7 | 1 | 1 | 1 |
| lowest energy (kcal mol$^{-1}$)$^a$ | -24.5 | -22.1 | -22.1 | -21.3 | -20.8 | -20.6 |

$^a$ Lowest energy structure in each family.
Figure 5.14. Lowest energy structure in family F1 for FMRF-NH$_2$.

Figure 5.15. Lowest energy structure in family F3 for FMRF-NH$_2$. 
5.5.7.2  Phe-{2S,3S-cyclo-Met}-Arg-Phe-NH₂

A total of 62 structures were within 4 kcal mol⁻¹ of the minimum energy conformer generated in the QMD study of F{2S,3S-cyclo-M}RF-NH₂. The major family, F1, contained 39 members and the lowest energy structure overall (Table 5.3). This low energy structure was stabilized by four hydrogen bonds (Phe¹NH to Phe⁴CO, Arg³NH to Phe¹CO, Phe⁴NH to Phe¹CO, and Arg³ guanidine NH to Phe¹CO). The hydrogen bond between Arg³NH and Phe¹CO generated a γ-turn structure centered at 2S,3S-cyclo-Met², and the Phe⁴NH to Phe¹CO gave a β-turn type structure (Figure 5.16). A similar γ-turn structure was also found in the lowest energy conformers of families F2 and F4.

Figure 5.16. Lowest energy conformer generated in the QMD study of F{2S,3S-cyclo-M}RF-NH₂ (from family F1).
**Table 5.3.** Structural characteristics of the low energy conformers from each of the families generated in the QMD study of F(2S,3S-cyclo-M)RF-NH₂.

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<th>dihedral</th>
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<th>F2</th>
<th>F3</th>
<th>F4</th>
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</table>

| no. of members in family | 39 | 4  | 2  | 4  | 4  | 5  |
| lowest energy (kcal mol⁻¹) | -17.7 | -16.3 | -15.2 | -15.0 | -14.8 | -14.7 |
5.5.7.3  **Phe-{2R,3R-cyclo-Met}-Arg-Phe-NH₂**

The QMD study of F{2R,3R-cyclo-M}RF-NH₂ gave significantly different results compared with the other peptidomimetics insofar as there was not one, but two major families (Table 5.4). Thus, there were 91 conformers within 4 kcal mol⁻¹ of the lowest energy structure overall, and 29 of them were grouped in the same family as this low energy structure. This structure was stabilized by two hydrogen bonds (Figure 5.17): one between Phe⁴NH and Phe¹CQ forming a 10 member ring, and the other was between Phe¹NH and Phe⁴CQ. The φ, ψ torsions of the 2R,3R-cyclo-Met² and Arg³ residues were 63°, -91° and -74°, -26° respectively, which are reasonably close to the expected values for i+1 and i+2 residues in a type II' β turn structure.¹²⁹ The other major family contains 28 members (F3). In the lowest energy conformer from this family, a hydrogen bond between the Phe⁴NH and the Phe¹CQ generated a structure very similar to a type II' β-turn (Figure 5.18). For the minor families, F2 (4 members) and F4 (7 members), the low energy conformers were inverse γ-turn structures centered at the 2R,3R-cyclo-Met² (i.e. hydrogen bonding between Arg³NH and the Phe¹CQ), while a γ-turn structure was the minimum in F5 (7 members).
Table 5.4. Structural characteristics of the lowest energy conformer of F\{2R,3R-cyclo-M\}RF-NH₂ from the QMD study.

<table>
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<th>residue</th>
<th>dihedral angle</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
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<tbody>
<tr>
<td>Phe&lt;sup&gt;1&lt;/sup&gt;</td>
<td>(\psi)</td>
<td>136</td>
<td>141</td>
<td>-56</td>
<td>144</td>
<td>-58</td>
<td>-86</td>
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<tr>
<td></td>
<td>(\chi_1)</td>
<td>64</td>
<td>-62</td>
<td>-171</td>
<td>-178</td>
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<td>-178</td>
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<tr>
<td></td>
<td>(\chi_2)</td>
<td>-71</td>
<td>93</td>
<td>-94</td>
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<td>62</td>
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<td>2R,3R-cyclo-Met&lt;sup&gt;2&lt;/sup&gt;</td>
<td>(\phi)</td>
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<td>-70</td>
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<tr>
<td></td>
<td>(\psi)</td>
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<td>-94</td>
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<td>-152</td>
<td>-153</td>
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<tr>
<td></td>
<td>(\chi_2)</td>
<td>179</td>
<td>178</td>
<td>67</td>
<td>178</td>
<td>177</td>
<td>64</td>
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<td>-155</td>
<td>-135</td>
<td>-85</td>
<td>-70</td>
<td>-115</td>
</tr>
<tr>
<td></td>
<td>(\psi)</td>
<td>-26</td>
<td>-24</td>
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<td>122</td>
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<td>-79</td>
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<tr>
<td></td>
<td>(\chi_2)</td>
<td>-122</td>
<td>-177</td>
<td>-161</td>
<td>171</td>
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<td>-67</td>
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<td>(\phi)</td>
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<td>-81</td>
<td>-90</td>
<td>-93</td>
<td>-83</td>
<td>-124</td>
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<tr>
<td></td>
<td>(\psi)</td>
<td>124</td>
<td>127</td>
<td>-56</td>
<td>-45</td>
<td>118</td>
<td>123</td>
</tr>
<tr>
<td></td>
<td>(\chi_1)</td>
<td>-54</td>
<td>-57</td>
<td>-50</td>
<td>-60</td>
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<tr>
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<td>(\chi_2)</td>
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<td>-61</td>
<td>-78</td>
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<td>110</td>
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<td>29</td>
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<td>28</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
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<td>-15.4</td>
<td>-15.0</td>
<td>-14.9</td>
<td>-14.6</td>
<td>-14.3</td>
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</tbody>
</table>
Figure 5.17. Lowest energy conformer generated in the QMD study of F{2R,3R-cyclo-M}RF-NH₂ (from family F1); this structure is a type II' β-turn.

Figure 5.18. The low energy conformer from family F3 in the QMD study of F{2R,3R-cyclo-M}RF-NH₂ has a structure very similar to a type II' β-turn.
5.5.7.4 Phe-{2S,3S-cyclo-Met}-Arg-{2R,3R-cyclo-Phe}-NH$_2$

Low energy structures of F{2S,3S-cyclo-M}R{2R,3R-cyclo-F}-NH$_2$ were sorted into four families with a total of 36 structures. Two major families F1 and F2 were isolated with 8 and 19 members, respectively (Table 5.5). The lowest energy structure in family F1 (Figure 5.19) had no hydrogen bonding. The side chains and the NH protons were oriented in one direction, while all the CQs were pointing in the opposite direction.

The lowest energy structure of family F2 had one hydrogen bond between the Phe$^4$NH and the Phe$^1$CQ giving a type I β-turn structure (Figure 5.20). Four minor families were also obtained with 1 to 4 members, the characteristics of the more populated F3 and F4 are shown (Table 5.5). The lowest energy structure of family F3 had a hydrogen bond between the Arg$^3$ guanidine NH and the Phe$^1$CQ, while the lowest energy structure of family F4 had a γ-turn structure hydrogen bonded between the Arg$^3$NH and Phe$^1$CQ.
Table 5.5. Structural characteristics of the low energy conformers from each of the families generated in the QMD study of F{2S,3S-cyclo-M}R{2R,3R-cyclo-F}-NH$_2$.

<table>
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<th>residue</th>
<th>dihedral angle</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
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<td>Phe$^1$</td>
<td>$\psi$</td>
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<td>114</td>
<td>-54</td>
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</tr>
<tr>
<td></td>
<td>$\chi 1$</td>
<td>-173</td>
<td>-162</td>
<td>-175</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>$\chi 2$</td>
<td>60</td>
<td>85</td>
<td>52</td>
<td>-71</td>
</tr>
<tr>
<td>2S,3S-cyclo-Met$^2$</td>
<td>$\phi$</td>
<td>-56</td>
<td>-63</td>
<td>81</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>$\psi$</td>
<td>-54</td>
<td>-34</td>
<td>-34</td>
<td>-78</td>
</tr>
<tr>
<td></td>
<td>$\chi 1$</td>
<td>151</td>
<td>151</td>
<td>157</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td>$\chi 2$</td>
<td>-64</td>
<td>-178</td>
<td>-68</td>
<td>62</td>
</tr>
<tr>
<td>Arg$^3$</td>
<td>$\phi$</td>
<td>-78</td>
<td>-89</td>
<td>-70</td>
<td>-141</td>
</tr>
<tr>
<td></td>
<td>$\psi$</td>
<td>-36</td>
<td>22</td>
<td>-28</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>$\chi 1$</td>
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<td>-67</td>
<td>-51</td>
<td>-168</td>
</tr>
<tr>
<td></td>
<td>$\chi 2$</td>
<td>179</td>
<td>67</td>
<td>-58</td>
<td>73</td>
</tr>
<tr>
<td>2R,3R-cyclo-Phe$^4$</td>
<td>$\phi$</td>
<td>-61</td>
<td>78</td>
<td>74</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>$\psi$</td>
<td>-67</td>
<td>-85</td>
<td>80</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>$\chi 1$</td>
<td>-4</td>
<td>-3</td>
<td>-3</td>
<td>-5</td>
</tr>
<tr>
<td></td>
<td>$\chi 2$</td>
<td>-92</td>
<td>78</td>
<td>100</td>
<td>-84</td>
</tr>
</tbody>
</table>

| no. of members in family | 8 | 19 | 4 | 2 |
| lowest energy (kcal mol$^{-1}$) | -11.2 | -9.9 | -8.5 | -8.2 |
Figure 5.19. Lowest energy structures in families F1 generated in the QMD study of F\{2S,3S-cyclo-M\}R\{2R,3R-cyclo-F\}-NH$_2$.

Figure 5.20. Lowest energy structure in family F2 for F\{2S,3S-cyclo-M\}R\{2R,3R-cyclo-F\}-NH$_2$ showing a type I $\beta$ turn structure.
5.5.8 Comparison of NMR Distance Constraints with Low Energy Structures Obtained from QMD

Distance constraints obtained from the NMR studies (Chapter 4) were compared with the lowest energy structures in each family. The best fit structures, together with the preferred $\phi$, $\psi$ torsions for individual 2,3-methanoamino acids are proposed.

5.5.8.1 Phe-Met-Arg-Phe-NH$_2$

Rapid conformational averaging of short linear peptides in solution causes close contacts to be of a transient nature, hence they are not observed by NMR.$^{119,126,127,138}$ This is true for FMRF-NH$_2$ in DMSO; no discernible interresidue crosspeaks arose in the ROESY spectra of this molecule, and QMD studies produced no clear preference for one conformer.

5.5.8.2 Phe-{2S,3S-cyclo-Met}-Arg-Phe-NH$_2$$^{139}$

Qualitative fit of the observed ROE crosspeaks with interproton distances for the lowest energy structure from each family of F{2S,3S-cyclo-M}RF-NH$_2$ is given in Table 5.6. The observed strong Phe$^1$$\alpha$-2S,3S-cyclo-Met$^2$$NH$, weak 2S,3S-cyclo-Met$^2$$NH$-Arg$^2$$NH$, and medium Arg$^2$$NH$-Arg$^2$$\alpha$ ROEs match well with the F1 minimum energy structure wherein the calculated distances for these contacts are 2.05, 4.08, 2.92 Å, respectively. The hydrogen bond between the Phe$^1$ carbonyl oxygen and the Arg$^2$$NH$ proton explains the observation of low temperature coefficient for the Arg$^2$$NH$, and formed a $\gamma$-turn structure (Figure 5.21) centered at the 2S,3S-cyclo-Met$^2$. Furthermore, the aromatic side chain of Phe$^1$ was within 5 Å of the 2S,3S-cyclo-Met$^2$$\beta$ and/or $\beta'$ protons, consistent with the weak ROE observed (vide supra). For the other families, the corresponding distance was greater than 5 Å. Less agreement was observed for the ROE crosspeak intensity and the interproton distance for the Arg$^2$$\alpha$- Phe$^4$$NH$, presumably
because Phe⁴ plays no role in the putative turn structure. Close correspondence with the
NMR data and the low energy conformer from F1 suggested that F(2S,3S-cyclo-
M)RF-NH₂ has a relatively high bias to fold into a γ-turn structure centered at the 2,3-
methanolog. This γ-turn structure was induced by the 2,3-methanolog since no such
conformational preference was observed for the parent peptide FMRF-NH₂.

Figure 5.21. Truncated version of the γ-turn region centered at cyclo-Met², for
F(2S,3S-cyclo-M)RF-NH₂. The double headed arrow is intended to indicate proximity
of the cyclopropane ring and the Phe¹ aromatic nucleus. The contact at 2.27 Å corresponds
to a H-bond between the Arg³NH and the Phe¹CO.
Table 5.6: Qualitative fit of ROE crosspeak intensities with the corresponding interproton distances for low energy structures in each family generated for F125 S-cyclo-M1RF-NH\(_2\) in QMD.

<table>
<thead>
<tr>
<th>contact/characteristic</th>
<th>ROE intensity</th>
<th>distance calculated in the QMD study (Å)</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phe(\alpha)-25S-cyclo-Met(\beta)-NH(_2)</td>
<td>S 2.05 3.45 3.56 2.06 2.43</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25S-cyclo-Met(\beta)-NH(_2)-Arg(\alpha)-3NH</td>
<td>W 4.08 3.61 3.70 3.88 4.31</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg(\alpha)-3NH-(\alpha)-Phe(\alpha)</td>
<td>M 2.92 2.87 2.85 3.31 2.88 2.92 3.39</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arg(\alpha)-Phe(\alpha)-Phe(\alpha)</td>
<td>S 3.44 3.48 1.89 3.51 2.89 3.56 3.93</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phe(\alpha)-CONH</td>
<td>M 2.89 2.26 2.92 1.90 2.15 &gt;5.00 3.56 5.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(\Phi) of 25S-cyclo-Met(\beta)</td>
<td>- 64.82 84.61 74.112 75.75 -68.90</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Phe(\alpha)-aromaticH</td>
<td>- 2.15 3.55 3.57 &gt;5.00 &gt;5.00</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>25S-cyclo-Met(\beta)cs</td>
<td>W 3.62 &gt;5.00 &gt;5.00 &gt;5.00 &gt;5.00 &gt;5.00</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(\Phi) of 25S-cyclo-Met(\beta)cs</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\(\Phi\) number in family energy (kcal mol\(^{-1}\))

- 39 4 -16.3 -15.0 -14.8
- 4 4 -4 4 -4 4
5.5.8.3 Phe-{2R,3R-cyclo-Met}-Arg-Phe-NH$_2$

Comparison of ROE data with the QMD data for F{2R,3R-cyclo-M}RF-NH$_2$
showed a relatively good match for the lowest energy conformer of family F1. The lowest
energy structures from the other families (ie F2 to F6) did not accommodate a close contact
between 2R,3R-cyclo-Met$^2$β$_{cis}$-Phe$^1$aromaticH (Table 5.7). However, two
discrepancies were found between the NMR data and the F1 lowest energy structure.
First, the interproton distance of 4.21 Å between the 2R,3R-cyclo-Met$^2$NH and
Arg$^3$NH was too long to generate a medium intensity crosspeak. Second, there was no
obvious structural basis for the low temperature coefficient observed for 2R,3R-cyclo-
Met$^2$NH (-2.18 ppb K$^{-1}$), because no hydrogen bond or hydrophobic shielding was
observed for this proton (Figure 5.17). The Phe$^1$ aromatic ring is more likely to shield this
proton, but the closest aromatic carbon is 4.46 Å away from the 2R,3R-cyclo-Met$^2$NH
proton.

An alternative approach was used to select the best fit structures for F{2R,3R-
cyclo-M}-RF-NH$_2$ due to the problems identified above. Consequently, the two inter-
residue ROEs (2R,3R-cyclo-Met$^2$NH-Arg$^3$NH and 2R,3R-cyclo-Met$^2$β$_{cis}$-
Phe$^1$aromaticH) were used as the selection criteria. These two ROEs were chosen because
these were relatively long range, and were not observed in FMRF-NH$_2$ thereby better
characterizing the structure. Constraints of 4 Å (medium) and 5 Å (weak) for 2R,3R-
cyclo-Met$^2$NH-Arg$^3$NH and 2R,3R-cyclo-Met$^2$β$_{cis}$-Phe$^1$aromaticH respectively
were set as the distance cutoffs, hence conformers with 2R,3R-cyclo-Met$^2$NH-Arg$^3$NH
≤ 4 Å and 2R,3R-cyclo-Met$^2$β$_{cis}$-Phe$^1$aromaticH ≤ 5 Å were sifted from the pool of 91
conformers within 4 kcal mol$^{-1}$ of the minimum energy structure. Eight conformers were
found to fit these criteria, and data for these is shown in Table 5.8. Six out of the eight
adopted negative φ and negative ψ (average value of φ = -71° and ψ = -80°) for the
2R,3R-cyclo-Met$^2$, including the lowest energy structure of this group, F1(44). Figure
5.22 shows the structure of conformer F1(44). The close proximity of the Phe\(^1\) aromatic ring and the \textbf{2R,3R-cyclo-Met\(^2\)NH\(^\_\)} (the distance of the NH to the closest aromatic carbon is 2.75 Å) explains why this particular proton should be shielded from the solvent and would have a low temperature coefficient (-2.18 ppb K\(^{-1}\)). It seems that the conformers of \(\phi \sim -71^\circ\) and \(\psi \sim -80^\circ\) at \textbf{2R,3R-cyclo-Met\(^2\)} gave the best fit to the experimental data.

**Figure 5.22.** The lowest energy conformer \{F1(44)\} of the 8 best fit conformers for F\{2R,3R-cyclo-M\}RF-NH\(_2\) chosen on the basis of distance constraints. The double arrow is intended to indicate proximity between the Phe\(^1\) aromatic ring and the \textbf{2R,3R-cyclo-Met\(^2\)NH\(^\_\).}
Table 5.7. Qualitative fit of ROE crosspeak intensities with the corresponding interproton distances for low energy structures in each family generated for F{2R,3R-cyclo-M}RF-NH₂ in QMD.

<table>
<thead>
<tr>
<th>contact/characteristic</th>
<th>ROE intensity</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
</tr>
</thead>
<tbody>
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<td>2R,3R-cyclo-Met²NH-Arg³NH</td>
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<td>3.79</td>
<td>4.20</td>
<td>4.02</td>
<td>4.16</td>
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<tr>
<td>2R,3R-cyclo-Met²β₉cis⁻</td>
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<td>3.37</td>
<td>&gt;5.00</td>
<td>&gt;5.00</td>
<td>&gt;5.00</td>
<td>&gt;5.00</td>
<td>&gt;5.00</td>
</tr>
<tr>
<td>Phe¹aromaticH</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2R,3R-cyclo-Met²NH-Phe¹α</td>
<td>VS</td>
<td>2.06</td>
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<td>3.56</td>
<td>2.11</td>
<td>3.56</td>
<td>3.55</td>
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<td>Arg³NH-α</td>
<td>M</td>
<td>2.88</td>
<td>2.88</td>
<td>2.93</td>
<td>2.90</td>
<td>2.86</td>
<td>2.95</td>
</tr>
<tr>
<td>Phe⁴NH-Arg³α</td>
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<td>3.44</td>
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<td>2.89</td>
<td>2.90</td>
<td>2.92</td>
<td>2.90</td>
<td>2.95</td>
</tr>
<tr>
<td>CONHH-Phe⁴α</td>
<td>S</td>
<td>2.15</td>
<td>2.13</td>
<td>3.58</td>
<td>3.55</td>
<td>2.08</td>
<td>2.14</td>
</tr>
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<td>63,-91</td>
<td>-77,68</td>
<td>67,-94</td>
<td>-70,81</td>
<td>67,-90</td>
<td>53,46</td>
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<td>-</td>
<td>29</td>
<td>4</td>
<td>28</td>
<td>7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>energy (kcal mol⁻¹)</td>
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<td>-16.4</td>
<td>-15.4</td>
<td>-15.0</td>
<td>-14.9</td>
<td>-14.6</td>
<td>-14.3</td>
</tr>
</tbody>
</table>
Table 5.8. Qualitative fit of ROE crosspeak intensities with the corresponding interproton distances for best fit structures generated for F{2R,3R-cyclo-M}RF-NH₂ in QMD.

<table>
<thead>
<tr>
<th>contact/characteristic</th>
<th>ROE intensity</th>
<th>distance calculated in the QMD study (Å)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F1(44)</td>
<td>F5(88)</td>
</tr>
<tr>
<td>2R,3R-cyclo-Met²NH-Arg³NH</td>
<td>M</td>
<td>2.86</td>
</tr>
<tr>
<td>2R,3R-cyclo-Met²β&lt;sub&gt;cis&lt;/sub&gt;-Phe¹aromaticH</td>
<td>W</td>
<td>3.21</td>
</tr>
<tr>
<td>2R,3R-cyclo-Met²NH-Phe¹α</td>
<td>VS</td>
<td>3.53</td>
</tr>
<tr>
<td>Arg³NH-α</td>
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<td>2.84</td>
</tr>
<tr>
<td>Phe&lt;sup&gt;4&lt;/sup&gt;NH-Arg³α</td>
<td>S</td>
<td>3.48</td>
</tr>
<tr>
<td>Phe&lt;sup&gt;4&lt;/sup&gt;NH-α</td>
<td>M</td>
<td>2.91</td>
</tr>
<tr>
<td>CONNH-Phe²α</td>
<td>S</td>
<td>2.11</td>
</tr>
<tr>
<td>φ, ψ of 2R,3R-cyclo-Met²</td>
<td>-</td>
<td>-71, -70</td>
</tr>
<tr>
<td>energy (kcal mol⁻¹)</td>
<td>-</td>
<td>-15.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> The number represents the identity of the structure in the corresponding family.
5.5.8.4 Phe-\{2S,3S-cyclo-Met\}-Arg-\{2R,3R-cyclo-Phe\}-NH₂

Eight ROE crosspeaks consisting of the backbone and interresidue side chain to side chain ROEs were selected to compare with the representative structures of families for F\{2S,3S-cyclo-M\}R\{2R,3R-cyclo-F\}-NH₂ (Table 5.9). These crosspeaks were chosen because these were relatively long range ROE crosspeaks than the intraresidue ROEs, and should better characterize the overall conformation. The representative structure of family F1 shows best fit to the ROE data among the four families (families F1 to F4). One violation was found for the 2R,3R-cyclo-Phe⁴aromaticH-Arg³γ (weak ROE observed for a QMD distance > 5 Å). However, the proximity of the side chains in the F1 lowest energy structure (Figure 5.18) is consistent with the observation of side chain to side chain ROEs. No hydrogen bond was observed for this structure which is consistent with the high temperature coefficients for the amide protons measured from the NMR experiments. Matching the NMR data with all 36 structures within the cutoff, provided three structures with interproton distances < 5 Å for all the eight corresponding ROE crosspeaks (Table 5.9, F1(22), F1(28), and F1(31)). All these structures were members of F1 indicating that the F1 type structure agreed well with the solution NMR data.

Replacing the Phe⁴ of F\{2S,3S-cyclo-M\}RF-NH₂ with 2R,3R-cyclo-Phe (ie F\{2S,3S-cyclo-M\}R\{2R,3R-cyclo-F\}-NH₂) destroyed the γ-turn structure centered at the 2S,3S-cyclo-Met² (Figure 5.23). Instead, the 2S,3S-cyclo-Met² in F\{2S,3S-cyclo-M\}R\{2R,3R-cyclo-F\}-NH₂ adopted φ, ψ values of approximately -60° and -45° (average values of structures 22, 28, and 31 of F1), respectively. It should be noted that the 2S,3S-cyclo-Met² for the γ-turn structure had φ, ψ values of 64°, -82°. Incorporation of the 2R,3R-cyclo-Phe⁴ changed these values to -60°, -45°.
Table 5.9. Qualitative fit of ROE crosspeak intensities with the corresponding interproton distances for low energy structures in each family, and best fit structures generated for F{2S,3S-cyclo-M}R{2R,3R-cyclo-F}-NH₂ in QMD

<table>
<thead>
<tr>
<th>contact/characteristic</th>
<th>ROE intensity</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F1(22)ᵃ</th>
<th>F1(28)</th>
<th>F1(31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phe₁α-2S,3S-cyclo-Met²NH</td>
<td>S</td>
<td>2.19</td>
<td>2.12</td>
<td>3.57</td>
<td>3.43</td>
<td>2.10</td>
<td>2.19</td>
<td>2.26</td>
</tr>
<tr>
<td>Arg³NH-α</td>
<td>VW</td>
<td>2.88</td>
<td>2.92</td>
<td>2.85</td>
<td>2.92</td>
<td>2.84</td>
<td>2.89</td>
<td>2.86</td>
</tr>
<tr>
<td>2R,3R-cyclo-Phe⁴NH-Arg³α</td>
<td>M</td>
<td>3.50</td>
<td>3.02</td>
<td>3.46</td>
<td>2.02</td>
<td>3.45</td>
<td>3.50</td>
<td>3.46</td>
</tr>
<tr>
<td>Phe₁aromaticH-</td>
<td>W</td>
<td>4.72</td>
<td>3.78</td>
<td>3.71</td>
<td>4.05</td>
<td>4.70</td>
<td>4.70</td>
<td>4.94</td>
</tr>
<tr>
<td>2S,3S-cyclo-Met²β and/or β'</td>
<td>W</td>
<td>4.92</td>
<td>&gt; 5.00</td>
<td>&gt; 5.00</td>
<td>&gt; 5.00</td>
<td>4.28</td>
<td>4.89</td>
<td>4.52</td>
</tr>
<tr>
<td>Phe₁β-Arg³βs</td>
<td>VW</td>
<td>3.72</td>
<td>&gt; 5.00</td>
<td>&gt; 5.00</td>
<td>&gt; 5.00</td>
<td>4.00</td>
<td>3.71</td>
<td>4.13</td>
</tr>
<tr>
<td>2R,3R-cyclo-Phe⁴aromaticH-Arg³βs</td>
<td>W</td>
<td>&gt;5.00</td>
<td>&gt; 5.00</td>
<td>&gt; 5.00</td>
<td>3.32</td>
<td>3.61</td>
<td>3.43</td>
<td>3.61</td>
</tr>
<tr>
<td>2R,3R-cyclo-Phe⁴aromaticH-Arg³δs</td>
<td>VW</td>
<td>3.94</td>
<td>&gt; 5.00</td>
<td>&gt; 5.00</td>
<td>3.60</td>
<td>3.54</td>
<td>3.06</td>
<td>3.79</td>
</tr>
<tr>
<td>φ, ψ of 2S,3S-cyclo-Met²</td>
<td>-</td>
<td>-56, -54</td>
<td>-63, -34</td>
<td>81, -34</td>
<td>73, -78</td>
<td>-64, -41</td>
<td>-54, -55</td>
<td>-61, -45</td>
</tr>
<tr>
<td>φ, ψ of 2R,3R-cyclo-Phe⁴</td>
<td>-</td>
<td>-61, -67</td>
<td>78, -85</td>
<td>74, 80</td>
<td>69, 83</td>
<td>-58, 99</td>
<td>-61, -72</td>
<td>-58, -63</td>
</tr>
<tr>
<td>number in family</td>
<td>-</td>
<td>8</td>
<td>19</td>
<td>4</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>energy (kcal mol⁻¹)</td>
<td>-</td>
<td>-11.2</td>
<td>-9.9</td>
<td>-8.5</td>
<td>-8.2</td>
<td>-8.9</td>
<td>-7.5</td>
<td>-9.6</td>
</tr>
</tbody>
</table>

ᵃ Members of F1 and the number represents the identity of the structure.
Figure 5.23. Truncated lowest energy structure of 
F-{2S,3S-cyclo-M}R{2R,3R-cyclo-F}-NH$_2$ showing that the Arg$^3$NH pointing 
away from the Phe$^1$CQ.
5.6 Conclusions

Ramachandran plots based on the empirical force-field parameters developed for the 2,3-methanoamino acids, and applied using electrostatic effects simulating $\varepsilon_0 = 1$, gave the expected C7-conformers and little other information. Conversely, the QMD technique illustrated conformational trends for the 2,3-methanoamino acids at a simulated high temperature, and after minimization. Four major regions were available for the 2,3-methanologs, with $\phi$ constrained to narrow values centered at $\pm70^\circ$. These data were consistent with the steric effects that might be predicted for the presence of the 2,3-methano-bridge (ie the extra carbon constituting the cyclopropane ring). The cyclopropane ring offsets the bias of L-amino acids for negative $\phi$ conformers, hence there is no clear preference for positive or negative $\phi$ values for the 2,3-methanoamino acids. Fine tuning of the $\psi$ torsion may be possible by changing the absolute stereochemistry of the E-2,3-methanoamino acids, since $\psi$ values may be shifted to more positive or more negative values by appropriate choice of the E-cyclo-Met isomer.

It would be inappropriate to suggest that there is one predominant rigid conformer for a tetrapeptide, or a peptidomimetic containing one to two 2,3-methanoamino acid(s).\textsuperscript{119,126,127} However, this study has shown that the methanolog in F(2S,3S-cyclo-M)RF-NH$_2$ tends to impose a preference for a $\gamma$-turn centered at the constrained residue. This conformational bias is not seen in FMRF-NH$_2$. The peptidomimetic almost certainly has some flexibility, particularly at the termini removed from the constraining effect of the 2,3-methanoamino acid, so the solution state behavior of this molecule is more accurately represented by interconversions between families of similar conformers than it ever could be by a single conformational model. Formation of a $\gamma$-turn for F(2S,3S-cyclo-M)RF-NH$_2$ must represent a somewhat favorable match between the conformational constraints imposed by the 2,3-methanoamino acid and of the accessible
conformational space for the remainder of the molecule, since most of the important families generated in the QMD study have this structural element.

Apparently the match of the conformational bias of the peptide backbone with the local effects for F\{2R,3R-cyclo-M\}RF-NH\textsubscript{2} is not as good as for the F\{2S,3S-cyclo-M\}RF-NH\textsubscript{2}. This molecule has more conformational rigidity than FMRF-NH\textsubscript{2} (from NMR), but there is no clear preference for any readily identified element of secondary structure. Conformations with $\phi \sim -71^\circ$ and $\psi \sim -80^\circ$ at the 2R,3R-cyclo-Met\textsuperscript{2} residue seem to be preferred.

The $\gamma$-turn structure observed in F\{2S,3S-cyclo-M\}RF-NH\textsubscript{2} became less favourable by replacing the Phe\textsuperscript{4} with 2R,3R-cyclo-Phe as for F\{2S,3S-cyclo-M\}R\{2R,3R-cyclo-F\}-NH\textsubscript{2}. For this peptidomimetic, the low energy conformers with all the side chains oriented to one direction (from QMD) agreed well with the observed NMR data, and suggested the propensity of this type of structures in solution.

The present work shows that 2,3-methanoamino acids impose conformational rigidity in peptidomimetics of peptides that are random coil conformers in solution, and is the first study to identify a series of explicit characteristics of these conformational effects. Many such studies will be required to delineate the scope of all methanologs with respect to manipulations of secondary structures. The present research is important because it illustrates a viable approach to the problem, laying the foundations for subsequent work. Consequently, we are extremely optimistic with regard to the potential for correlating the solution structures of methanolog-containing peptidomimetics in general, and adjusting them to attain enhanced bioactivities.
Appendix 1. Experimental for Chapter 2

General procedures: Melting points were uncorrected. High field NMR spectra were recorded on a Bruker AF300 (1H at 300 MHz, 13C at 75.4 MHz), or a Bruker AC250 (1H at 250 MHz, 13C at 62.9 MHz). 1H chemical shifts are reported in δ ppm relative to CHCl3 (7.25 ppm) as internal standard, and 13C chemical shifts are reported in ppm relative to DCl (77.0 ppm) unless specified otherwise. Multiplicities in 1H NMR are reported as (br) broad, (s) singlet, (d) doublet, (t) triplet, (q) quartet, and (m) multiplet. The carbon multiplicities are listed as (C) quaternary, (CH) methine, (CH2) methylene, and (CH3) methyl assigned via DEPT sequence experiments. Infrared (IR) spectra were recorded on a Nicolet 205 FTIR spectrometer. Low resolution (EI), and high resolution (EI) mass spectra were determined on a Finnigan 3300 mass spectrometer, and a CAC21/110 C high resolution mass spectrometer, respectively. FABMS spectra were obtained from the mass spectrometer facilities either at University of Texas (Medical School at Houston) or Texas A & M University. Optical rotations were determined on a Jasco DIP-370 digital polarimeter.

Thin layer chromatography was performed on silica gel 60 F254 plates from Whatman. Flash chromatography was performed on SP Silica Gel 60 (230-600 mesh ASTM). Optically active [( trifluoromethanesulfonyl)oxy]methyl]oxirane (57)55 was prepared from optically active glycidol purchased from Aldrich. Lipase from Candida cylindracea was purchased from Sigma. DMF was stored over 4Å molecular sieves for a week before use, benzene was distilled immediately before use from sodium benzophenone ketyl, pyridine was distilled from KOH pellets, CH2Cl2 and t-BuOH were distilled from CaH2. Other chemicals were purchased from commercial suppliers and used as received.

Spectra are shown in the order of 1H-NMR, IR, 13C-NMR, DEPT, LR-MS, and HR-MS unless specified.
(+)-1-(tert-Butyloxycarbonyl)-2-oxo-3-oxabicyclo[3.1.0]hexane (45)

Di-tert-butyl malonate (9.00 g, 41.61 mmol, 1.00 equiv) was added dropwise to a well-stirred solution of sodium hydride (2.20 g, 45.77 mmol, 1.10 equiv), tetrabutylammonium iodide (0.15 g, 0.42 mmol, 0.01 equiv), and DMF (166 mL) under nitrogen, and the resulting mixture was stirred at 25 °C for 15 min. The reaction was then heated to 80 °C, followed by dropwise addition of epibromohydrin (5.70 g, 41.61 mmol, 1.00 equiv). After being heated at 80 °C for 12 h, the mixture was poured into H2O (200 mL), and extracted with diethyl ether (3 x 150 mL). The combined organic layers were dried (anhydrous Na2SO4), the solvent was evaporated, and the residue was recrystallized from EtOAc/hexane. Racemic 45 (5.00 g, 61 %) was obtained as colorless crystals.

literature

Rf 0.35 (20 % acetone/hexane)

1H NMR (250 MHz, CDCl3)
δ 4.34 (dd, J=9.45, 4.78 Hz, 1H), 4.14 (d, J=9.38 Hz, 1H), 2.65 (m, 1H), 1.99 (dd, J=7.98, 4.63 Hz, 1H), 1.48 (s, 9H), 1.29 (t, J=5.02 Hz, 1H).
$^{13}$C NMR (62.9 MHz, CDCl$_3$)

$\delta$ 170.7 (C), 165.5 (C), 168.9 (C), 82.7 (C), 66.8 (CH$_2$), 29.9 (C), 27.9 (CH/CH$_3$), 27.5 (CH/CH$_3$), 20.3 (CH$_2$)
(±)-1-(tert-Butoxycarbonyl)-2-oxo-3-oxabicyclo[3.1.0]hexane (45)
(1R,5R)-(-)-1-(tert-Butylocarbonyl)-2-oxo-3-oxabicyclo[3.1.0]hexane  (45)

\[
\text{t-BuO}_2\text{C} \quad \overset{\text{O}}{\bigcirc} \quad \text{O}
\]

Di-tert-butyl malonate (7.49 g, 34.61 mmol, 1.00 equiv) was added dropwise to a well-stirred mixture of sodium hydride (1.99 g, 41.53 mmol, 1.20 equiv) and 15-crown-5 (0.25 mL, 1.25 mmol, 0.03 equiv) in dry benzene (87 mL) at 25 °C under nitrogen. The solution was stirred at 25 °C for 15 min, and (S)-(+)−

\{[(trifluoromethanesulfonyl)oxy]methyl\}oxirane 57 (8.56 g, 41.53 mmol, 1.20 equiv, 92 % ee) was added via syringe pump (ca 0.1 mL/min). After the reaction had been stirred for 12 h, the benzene was evaporated under reduced pressure. Water (80 mL) was added to the resulting residue, and the solution was extracted with EtOAc (3 x 80 mL). The combined organic layers were dried and removal of the solvent gave an oil which was purified by flash chromatography (5 % to 10 % acetone/hexane) to give compound 1R,5R-45 as colorless crystals (3.27 g, 48 %, 91 % ee). The enantiomeric excess of this material was determined by chiral shift experiment using Eu(hfc)₃ monitoring the \((\text{CH}_3)_3\text{CO}\) signal.

mp 66-67 °C

\([\alpha]_D^{25} -105.5^\circ\) (c = 1.30, CH₂Cl₂, compound prepared from D-mannitol had \([\alpha]_D^{25} = -135.1^\circ\), the value observed in the present is lower than that calculated by taking 100 % ee as equivalent to -135°. This observation may probably due to solvent impurities)
Eu(hfc)$_3$ Chiral shift Experiment of the racemic and ($IR$,5$R$)-(-)-1-((trans-\text{butoxycarbonyl})-2-oxo-3-oxabicyclo[3.1.0]hexane (45)
$(1R,2R)$-(+)-$\textit{tert}$-Butyl 2-(acetoxymethyl)-1-carbamoyl-cyclopropane-1-carboxylate (58)

$(1R,5R)$-(-)-1-($\textit{tert}$-Butoxycarbonyl)-2-oxo-3-oxabicyclo[3.1.0]hexane 45 (3.05 g, 15.39 mmol, 1.00 equiv) was added to a vigorously stirred ammonium hydroxide solution (14.8 M, 154 mL). After the mixture had been stirred overnight at 25 °C, the aqueous ammonium hydroxide was evaporated to dryness. A mixture of triethylamine (2.34 g, 23.09 mmol, 1.50 equiv), and DMAP (catalytic amount) in CH$_2$Cl$_2$ (77 mL) was added to the residue, followed by dropwise addition of acetic anhydride (2.36 g, 23.09 mmol, 1.50 equiv). After being stirred for 2 h, the solution was evaporated, water (50 mL) was added to the resulting residue, and the solution was extracted with EtOAc (3 x 50 mL). The combined organic layers was dried and evaporated. A yellow oil was obtained, and purified by flash chromatography (10 % to 30 % acetone/hexane) to give 58 (3.35 g, 85 %) as colorless solids.

mp 88-89 °C

$R_f$ 0.24 (20 % acetone/hexane)

$^1$H NMR (250 MHz, CDCl$_3$)

δ 8.33 (br s, 1H), 5.74 (br s, 1H), 4.42 (dd, J=11.94, 6.01 Hz, 1H), 4.12 (dd, J=11.74, 8.56 Hz, 1H), 2.10 (m, 1H), 2.04 (s, 3H), 1.86 (dd, J=7.87, 4.20 Hz, 1H), 1.70 (dd, J=9.38, 4.19 Hz, 1H), 1.44 (s, 9H)
$^{13}$C NMR (62.9 MHz, CDCl$_3$)

$\delta$ 171.0 (C), 170.9 (C), 168.9 (C), 82.6 (C), 62.0 (CH$_2$), 31.8 (C), 30.8 (CH/CH$_3$), 27.9 (CH/CH$_3$), 20.9 (CH/CH$_3$), 19.7 (CH$_2$)

IR (CHBr$_3$), cm$^{-1}$

3484, 3347, 1708, 1670, 1568

MS (EI, 70eV), m/e (%) 

258 (0.4, M+1), 201 (25), 159 (43), 141 (100), 102 (55), 57 (43)

$[\alpha]_D^{25} +14.5^\circ$ (c = 1.01, CHCl$_3$)

Anal calcd for C$_{12}$H$_{19}$NO$_5$: C, 56.02; H, 7.44; N, 5.44. Found: C, 56.16; H, 7.43; N, 5.30.
(1R,2R)-(+)-tert-Butyl 2-(acetoxymethyl)-1-carbamoyl-cyclopropane-1-carboxylate (58)
\((1R,2R)-(+)-\text{tert-Butyl 2-(acetoxy methyl)-1-carbamoyl-cyclopropane-1-carboxylate (58)\)
(1R,2R)-(+)-tert-Butyl 2-(acetoxyethyl)-1-carbamoyl-cyclopropane-1-carboxylate (58)
(IS,2R)-(-)-tert-Butyl 2-(acetoxyethyl)-1-[N-(tert-butoxycarbonyl)amino]-cyclopropane-1-carboxylate (59)

A solution of (IR,2R)-(+) -tert-butyl 2-(acetoxyethyl)-1-carbamoyle-cyclopropane-1-carboxylate 58 (2.44 g, 9.49 mmol, 1.00 equiv) and t-BuOH (47 mL) was heated to 70 °C under nitrogen. Lead tetraacetate (8.41 g, 18.97 mmol, 2.00 equiv) was added in one portion, and the mixture was heated at reflux for 2 h. After cooling to 25 °C, Et₂O (30 mL) followed by NaHCO₃ (2 g) were added, and the mixture was stirred for 10 min. The mixture was filtered through a short pack of silica, and the filtrate was evaporated. Residue was purified by flash chromatography (10 % to 20 % EtOAc/ hexane) to give 59 as colorless solids (2.61 g, 83 %).

mp 66-67 °C

Rf 0.46 (20% acetone/hexane)

¹H NMR (250 MHz, CDCl₃)
δ 5.30 (br s, 1H), 4.28 (m, 1H), 4.03 (m, 1H), 2.07 (s, 3H), 1.95 (m, 1H), 1.70 (m, 1H), 1.43-1.45 (2 overlapping s, 18H), 1.02 (m, 1H)

¹³C NMR (62.9 MHz, CDCl₃)
δ 171.2 (C), 171.1 (C), 156.2 (C), 81.5 (C), 79.8 (C), 63.3 (CH₂), 38.8 (C), 28.2 (CH/CH₃), 27.9 (CH/CH₃), 25.5 (CH/CH₃), 21.0 (CH/CH₃), 20.4 (CH₂)
IR (CHBr₃), cm⁻¹
3418, 1727, 1486, 1368, 1243, 1145, 1030

MS (EI, 70eV), m/e (%)
330 (0.1, M+1), 217 (66), 200 (30), 113 (74), 57 (100)

[α]D²⁵ -5.6° (c = 1.31, CHCl₃)

Anal calcd for C₁₆H₂₇NO₆:  C, 58.34; H, 8.26; N, 4.25. Found: C, 58.43; H, 8.22; N, 4.19
(1S,2R)-(-)-tert-Butyl 2-(acetoxyethyl)-1-[N-(tert-butoxycarbonyl)amino]-cyclopropane-1-carboxylate (59)
\((1S,2R)-(-)-\text{tert-Butyl 2-(acetoxyethyl)-1-}[N-(\text{tert-butoxycarbonyl)amino}]\text{-cyclopropane-1-carboxylate (59)}\)
(1S,2R)-(-)-tert-Butyl 2-(acetoxyethyl)-1-[N-(tert-butoxycarbonyl)amino]-cyclopropane-1-carboxylate (59)
(IS,2R)-(−)-tert-Butyl 1-[N-(tert-butoxycarbonyl)amino]-2-(hydroxymethyl)-cyclopropane-1-carboxylate (60)

Potassium carbonate (0.93 g, 6.74 mmol, 2.00 equiv) was added to a well-stirred solution of 59 (1.11 g, 3.37 mmol, 1.00 equiv) in MeOH (34 mL). After the mixture had been heated at 70 °C for 17 h, the MeOH was evaporated, water (50 mL) was added to the residue, and the resulting solution was extracted with EtOAc (3 x 50 mL). The combined organic layers were dried and the solvent was evaporated. The crude product was purified by flash chromatography (10 % to 25 % EtOAc/hexane) and recrystallized from EtOAc/hexane to give 60 as colorless crystals (0.81 g, 83 %). The optical purity was determined by converting the alcohol to the corresponding Mosher’s ester using R-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetic acid and DCC. A diastereomeric excess of ≥95 % was measured by 19F-nmr.

\[\text{mp 106-107}^\circ\]

\[R_f\] 0.23 (25 % EtOAc/hexane)

\[^{1}H\text{ NMR (300 MHz, CDCl}_3\]\n
δ 5.03 (br s, 1H), 3.95 (m, 1H), 3.17 (m, 1H), 2.22 (m, 1H), 1.46-1.43 (2 overlapped s, 19H), 0.70 (m, 1H)
$^{13}\text{C}$ NMR (62.9 MHz, CDCl$_3$) 
\[ \delta \] 171.2 (C), 158.1 (C), 81.6 (C), 80.8 (C), 61.6 (CH$_2$), 38.8 (C), 30.5 (CH/CH$_3$), 28.2 (CH/CH$_3$), 27.9 (CH/CH$_3$), 18.8 (CH$_2$)

IR (CHBr$_3$), cm$^{-1}$
3421, 1708, 1492, 1368, 1290, 1251, 1142

MS (EI, 70eV) m/e (%) 
288 (0.04, M+1), 175 (100.), 158 (26), 100 (71), 57 (87)

[\alpha]_D^{25} \text{ -41.0}^\circ \text{ (c = 1.25, CHCl$_3$)}

Anal calcd for C$_{14}$H$_{25}$NO$_5$: C, 58.52; H, 8.77; N, 4.87. Found: C, 58.74; H, 8.73; N, 4.77.
(1S,2R)-(−)-tert-Butyl 1-[N-(tert-butoxycarbonyl)amino]-2-
(hydroxymethyl)-cyclopropane-1-carboxylate (60)
(1S,2R)-(-)-tert-Butyl 1-[N-(tert-butoxycarbonyl)amino]-2-(hydroxymethyl)-cyclopropane-1-carboxylate (60)
\((1S,2R)-(-)-\text{tert-Butyl 1-[N-(\text{tert-butoxycarbonyl)amino}]2-}
\text{(hydroxymethyl)-cyclopropane-1-carboxylate (60)}\)
$^{19}\text{F-NMR}$ experiment of the racemic and $(1S,2R)$-(-)-tert-butyl 1-[$N$-($\text{tert}$-butoxycarbonyl)amino]-2-($\text{hydroxymethyl}$)-cyclopropane-1-carboxylate (60)
(2S,3R)-(-)-N-(tert-Butoxycarbonyl)-O-tert-butyl-2,3-methanomethionine (61)

(1S,2R)-(-)-tert-Butyl 1-N-(tert-butoxycarbonyl)amino)-2-(hydroxymethyl)-cyclopropane-1-carboxylate 60 (0.36 g, 1.25 mmol, 1.00 equiv) was dissolved in CH$_2$Cl$_2$ (7 mL) under nitrogen and the solution was cooled to 0 °C. Triethylamine (0.29 g, 2.50 mmol, 2.00 equiv) was added, followed by dropwise addition of methanesulfonyl chloride (0.25 g, 2.50 mmol, 2.00 equiv). After the solution had been stirred at 0 °C for 5 min and at 25 °C for 1 h, the solvent was evaporated. Water (10 mL) was added to the residue and the solution was extracted with EtOAc (3 x 10 mL). The combined organic layers were dried and the solvent was evaporated to give crude mesylate. The residue was redissolved in DMF (6 mL) under nitrogen, cooled to 25 °C in a water bath, and sodium thiomethoxide (0.22 g, 3.13 mmol, 2.50 equiv) was added in one portion. After the mixture had been stirred in the water bath for 45 min, the mixture was poured into water (15 mL), and the solution was extracted with EtOAc (3 x 15 mL). The combined organic layers were dried, and the solvent was evaporated to yield an oil which was purified by flash chromatography (5 % to 10 % EtOAc/ hexane) to give 61 as colorless solids (0.32 g, 81 %).

mp 94-95 °C

$R_f$ 0.68 (25 % EtOAc/ hexane)
$^1$H NMR (250 MHz, CDCl$_3$)
δ 5.16 (br s, 1H), 2.62 (m, 2H), 2.14 (s, 3H), 1.90 (m, 1H), 1.70 (m, 1H), 1.44-1.42 (2 overlapping s, 18H), 0.98 (m, 1H)

$^{13}$C NMR (62.9 MHz, CDCl$_3$)
δ 171.4 (C), 156.3 (C), 81.4 (C), 79.9 (C), 40.0 (C), 33.2 (CH$_2$), 28.3 (CH/CH$_3$), 28.0 (CH/CH$_3$), 26.5 (CH/CH$_3$), 23.2 (CH$_2$), 15.3 (CH/CH$_3$)

IR (CHBr$_3$), cm$^{-1}$
3418, 1716, 1484, 1367, 1290, 1244, 1143, 1073

MS (EI, 70eV) m/e (%)
318 (0.1, M+1), 317 (0.02 %, M+), 188 (100)

$[\alpha]_{D}^{25}$ -20.7° (c = 1.15, CHCl$_3$)

Anal. calcd for C$_{15}$H$_{27}$NO$_4$S: C, 56.76; H, 8.57; N, 4.41; S, 10.10. Found: C, 56.35; H, 8.31; N, 4.34; S 9.99.
(2S,3R)-(-)-N-(tert-Butoxycarbonyl)-O-tert-butyl-2,3-methanomethionine

(61)
(2S,3R)-(−)-N-(tert-Butoxycarbonyl)-O-tert-butyl-2,3-methanomethionine (61)
(2S,3R)-(-)-N-(tert-Butoxycarbonyl)-O-tert-butyl-2,3-methanomethionine

(61)
(2S,3R)-(−)-2,3-Methanomethionine, Z-(−)-cyclo-Met

![Chemical Structure](image)

(2S,3R)-(−)-N-(tert-Butoxycarbonyl)-O-tert-butyl-2,3-methano-methionine 61. (0.152 g, 0.477 mmol) was dissolved in CH₂Cl₂ (2 mL) and cooled to 0 °C. Trifluoroacetic acid (0.67 mL) was added dropwise, the mixture was allowed to warm to 25 °C and was stirred for 1 h at this temperature. After evaporation of the solvent, the residue was purified by ion-exchange chromatography (Dowex 50x8-100, H⁺ form, eluted with 2M ammonium hydroxide solution) to give a pale yellow solid. This was recrystallized from EtOH to give Z-(−)-cyclo-Met as colorless crystals (0.061 g, 80 %). A sample was purified by HPLC using a C₁₈ column (4.6 x 250 mm, 5 μm, eluted with A: 0.01 % TFA in H₂O; B: 0.01 % TFA in acetonitrile, 0-20 % B in 30 min). After lyophilization of the product fractions, Z-cyclo-Met.CF₃COOH was isolated as colorless crystals. The diastereotopic Hβ' cis and Hβ' trans (relative to the amino group) protons were assigned via difference NOE experiment irradiated at 2.13 ppm.

**literature**


**mp** 195-196 °C (decomposed)

**Rf** 0.49 (t-BuOH/H₂O/acetic acid, 12/5/3)
$^1$H NMR (300 MHz, D$_2$O, reference D$_2$O= 4.8 ppm)

$\delta$ 2.75 (d, J=7.66 Hz, 2H), 2.19 (s, 3H), 2.13 (m, 1H), 1.78 (dd, J=9.77, 6.53 Hz, 1H, H$_2^\beta$trans relative to amino group), 1.30 (t, J=7.06, 1H, H$_2^\beta$cis relative to amino group)

$^{13}$C NMR (75.4 MHz, D$_2$O, reference MeOH= 49.9 ppm)

$\delta$ 175.8 (C), 40.9 (C), 32.0 (CH$_2$), 24.0 (CH/CH$_3$), 19.30 (CH$_2$), 15.1 (CH/CH$_3$)

$[\alpha]_D^{25}$ -22.2° (c = 0.33, H$_2$O)

FABMS(Glycerol), m/e

162.1(M+1), 114.0(CF$_3$COOH$^+$)
$^1$H-NMR spectrum and difference NOE spectrum (irradiated at 2.13 ppm) of (2S,3R)-(-)-2,3-methanomethionine, Z-(-)-cyclo-Met
(2S,3R)-(-)-2,3-Methanomethionine, Z-(−)-cyclo-Met
FAB-MS and HETCOR spectra of (2S,3R)-(-)-2,3-methanomethionine, Z-(-)-cyclo-Met
The enantiomers of compound 45, 58 to 61, and (±)-Z-cyclo-Met were prepared in identical fashion.

\((IS,5S)\)-\((+)-1-(tert-Butoxycarbonyl)-2-oxo-3-oxabicyclo[3.1.0]hexane\) \((+)\)-\((45). \ [\alpha]_D^{25} +104.6^\circ \ (c = 1.30, \ CH_2Cl_2).\n
\((IS,2S)\)-\((-)-tert-Butyl\) \(2-(acetoxyethyl)-1-carbamoyl-cyclopropane-1-carboxylate\) \((-)(58). \ [\alpha]_D^{25} -13.9^\circ \ (c = 1.01, \ CHCl_3).\n
\((IR,2S)\)-\((+)-tert-Butyl\) \(2-(acetoxyethyl)-1-[N-(tert-butoxycarbonyl)amino]-cyclopropane-1-carboxylate\) \((+)(59). \ [\alpha]_D^{25} +5.6^\circ \ (c = 1.31, \ CHCl_3).\n
\((IR,2S)\)-\((+)-tert-Butyl\) \(1-[N-(tert-butoxycarbonyl)amino]-2-(hydroxymethyl)-cyclopropane-1-carboxylate\) \((+)(60). \ [\alpha]_D^{25} +40.6^\circ \ (c = 1.25, \ CHCl_3).\n
\((2R,3S)\)-\((+)-N-(tert-Butoxycarbonyl)-O-tert-butyl-2,3-methanomethionine\) \((+)(61). \ [\alpha]_D^{25} +21.0^\circ \ (c = 1.15, \ CHCl_3).\n
\((2R,3S)\)-\((+)-2,3-Methanomethionine. \ [\alpha]_D^{25} +22.2^\circ \ (c = 0.33, \ H_2O).\)
(1R,5R)-(−)-1-[N-(tert-Butoxycarbonyl)amino]-2-oxo-3-oxabicyclo[3.1.0]hexane (62)

A solution of trifluoroacetic acid (7.5 mL) in CH₂Cl₂ (22.5 mL) was added to 1R,5R-45 (2.48 g, 12.51 mmol, 1.00 equiv) and stirred at 25 °C for 80 min. The solution was evaporated, and the residue was dried under vacuum. The crude carboxylic acid was used without further purification. The acid was redissolved in t-BuOH (42 mL) and triethylamine (2.53 g, 25.02 mmol, 2.00 equiv) under nitrogen, diphenylphosphoryl azide (4.13 g, 15.04 mmol, 1.20 equiv) was added and the solution was refluxed for 17 h. The solvent was evaporated, the residue was redissolved in EtOAc (150 mL), and the solution was filtered. The organic filtrate was washed with saturated NaCl solution (80 mL), dried and evaporated. The resulting pale yellow solid obtained was purified by flash chromatography (20 % to 33 % acetone/hexane) to give 62 as colorless solid (2.21 g, 83 %).

literature

¹H NMR (250 MHz, CDCl₃)
δ 5.29 (br s, 1H), 4.52 (m, 1H), 4.14 (d, J=9.35 Hz, 1H), 2.42 (m, 1H), 1.51 (m, 1H), 1.44 (s, 9H), 1.21 (m, 1H)
$^{13}$C NMR (62.9 MHz, CDCl$_3$)

$\delta$ 174.6 (C), 155.5 (C), 80.9 (C), 68.2 (CH$_2$), 38.2 (C), 28.2 (CH/CH$_3$), 23.9 (CH/CH$_3$), 18.3 (CH$_2$)

$[\alpha]_D^{25}$ -30.9° (c = 0.43, CHCl$_3$).
(IR,SR)-(−)-1-[N-({tert-Butoxycarbonyl)amino]-2-oxo-3-oxabicyclo[3.1.0]hexane (62)
(IR,2R)-(−)-1-[N-(tert-Butoxycarbonyl)amino]-2-[(hexanoyloxy)methyl]-cyclopropane-1-carbonitrile (64)

(IR,5R)-(−)-1-[N-(tert-Butoxycarbonyl)amino]-2-oxo-3-oxabicyclo[3.1.0]hexane 62 (1.30 g, 6.09 mmol) was added to a vigorously stirred solution of NH₄OH (14.8 M, 60 mL). After the mixture had been stirred at 25 °C for 5 h, the NH₄OH was evaporated. The residue was suspended in a solution of CH₂Cl₂ (30 mL) with a catalytic amount of DMAP and triethylamine (0.92 g, 1.27 mL, 9.14 mmol) under nitrogen. The mixture was cooled to 0 °C and hexanoyl chloride (0.98 g, 0.96 mmol) was added dropwise. After stirring at 0 °C for 15 min, the temperature was raised to 25 °C and the mixture was stirred for 3 h. The solvent was evaporated, Et₂O (50 mL) was added to the residue, the solution was washed with saturated NaCl solution (50 mL), dried, and evaporated. Amide 63 was obtained as a solid and used without further purification. The amide was dissolved in pyridine (20 mL) under nitrogen and TsCl (1.74 g, 9.14 mmol) was added. After the solution was refluxed for 12 h, a dark brown solution was obtained. The pyridine was evaporated, water (100 mL) was added to the residue, the aqueous layer was extracted with EtOAc (100 + 50 mL), and the combined organic layers were dried and evaporated. The crude product was purified by flash chromatography (5 to 20 % acetone/hexane) to give 64 (1.18 g, 62 %) as an oil.

Rf 0.38 (20% acetone/hexane)
$^1$H NMR (250 MHz, CDCl$_3$)

$\delta$ 5.09 (br s, 1H), 4.35 (m, 1H), 4.07 (m, 1H), 2.35 (t, $J=7.43$ Hz, 2H), 1.88 (m, 1H), 1.63-1.31 (s overlapping with m, 17H), 0.89 (t, $J=6.70$ Hz, 3H)

$^{13}$C NMR (75.4 MHz, CDCl$_3$)

$\delta$ 173.5 (C), 154.6 (C), 118.2 (C), 81.4 (C), 63.0 (CH$_2$), 43.0 (C), 33.9 (CH$_2$), 31.1 (CH$_2$), 28.1 (CH/CH$_3$), 27.0 (CH/CH$_3$), 24.4 (CH$_2$), 22.2 (CH$_2$), 21.2 (CH$_2$), 13.8 (CH/CH$_3$)

IR (neat), cm$^{-1}$

3352, 2931, 2360, 1739, 1508, 1124, 1101

MS (EI, 70eV) m/e (%)

254 (66, M-56), 237 (9), 198 (8), 116 (37), 99 (38), 57 (100)

$[\alpha]_D^{25}$ -1.4$^\circ$ (c = 1.62, CHCl$_3$)

Anal calcd for C$_{16}$H$_{26}$N$_2$O$_4$: C, 61.91; H, 8.44; N, 9.03. Found: C, 62.13; H, 8.44; N, 9.26.
(IR,2R)-(-)-1-[N-(tert-Butoxycarbonyl)amino]-2-[(hexanoyloxy)methyl]-cyclopropane-1-carbonitrile (64)
(1R,2R)-(-)-1-[N-(tert-Butoxycarbonyl)amino]-2-[(hexanoxyloxy)methyl]-cyclopropane-1-carbonitrile (64)
(IR,2R)-(-)-1-[N-(tert-Butoxycarbonyl)amino]-2-[(hexanoyloxy)methyl]-cyclopropane-1-carbonitrile (64)
(1R,2R)-(+)·1-[N-(tert-Butoxycarbonyl)amino]-2-(hydroxymethyl)-cyclopropane-1-carbonitrile (65)

\[
\begin{align*}
\text{Boc} & \text{H} \\
\text{N} & \text{C} \\
\text{O} & \text{H}
\end{align*}
\]

(1R,2R)-(−)-1-[N-(tert-Butoxycarbonyl)amino]-2-[(hexanoyloxy)methyl]-cyclopropane-1-carbonitrile 64 (1.13 g, 3.64 mmol) was suspended in a vigorously stirred phosphate buffer (pH 7.5, 37 mL) at 25 °C, and lipase from Candida cylindracea (1.70 g, 1.50 mass equiv) was added. After the mixture had been stirred for 35 h, the enzyme was removed by filtration, water (50 mL) was added to the filtrate and the resulting solution was extracted with Et₂O (3 x 50 mL). The combined organic layers were dried and evaporated, leaving a residue which was purified by flash chromatography (15 to 33 % acetone/hexane) to give 65 as colorless crystals (0.57 g, 74 %, 91 % ee). The enantiomeric excess of this material was determined via a Eu(fod)₃ chiral shift experiment on the corresponding Mosher's ester prepared from R-(+)·MTPA.

\[ R_f 0.31 \text{ (33 % acetone/hexane)} \]

\[ ^1H \text{ NMR (250 MHz, CDCl}_3\text{)} \]
\( \delta \) 5.54 (br s, 1H), 4.02 (dd, J=11.55, 3.39 Hz, 1H), 3.51 (t, J=10.61, 1H), 2.51 (br s, 1H), 1.78 (m, 1H), 1.45 (s, 10H), 1.24 (m, 1H)

\[ ^{13}C \text{ NMR (62.9 MHz, CDCl}_3\text{)} \]
\( \delta \) 154.9 (C), 118.8 (C), 81.8 (C), 62.0 (CH₂), 43.5 (C), 31.2 (CH/CH₃), 28.1 (CH/CH₃), 20.5 (CH₂)
IR (neat), cm⁻¹
3331, 2239, 1704, 1512, 1363, 1281, 1164, 1100, 1037

MS (EI, 70 eV), m/e (%) 
212 (0.5, M⁺), 157 (21), 140 (14), 82 (25), 57 (100)

HRMS m/e calc'd for C₁₀H₁₆N₂O₃: 212.1161. Found 212.1154

[α]D²⁶ +2.5° (c = 1.12, CHCl₃).
(IR,2R)-(+)\(-[N-(\text{tert}-\text{Butoxycarbonyl})\text{amino}]\)-2-(\text{hydroxymethyl})-cyclopropane-1-carbonitrile (65)
(1R,2R)-(−)-1-[N-(tert-Butoxycarbonyl)amino]-2-(hydroxymethyl)-cyclopropane-1-carbonitrile (65)
(IR,2R)-(+)-1-[N-(tert-Butyloxycarbonyl)amino]-2-(hydroxymethyl)-cyclopropane-1-carbonitrile (65)
(IR,2R)-(+)-1-[N-(tert-Butoxycarbonyl)amino]-2-[(acetythio)methyl]-
cyclopropane-1-carbonitrile (67)

Methanesulfonfly chloride (0.326 g, 2.849 mmol, 1.20 equiv) was added dropwise
to a solution of 65 (0.504 g, 2.375 mmol) and triethylamine (0.228 g, 2.849 mmol, 1.20
equiv) in CH$_2$Cl$_2$ (12 mL) at 0 °C under nitrogen. After the addition, the reaction mixture
was warmed to 25 °C and stirred for 30 min. The CH$_2$Cl$_2$ was evaporated, water (20 mL)
was added to the residue, and the aqueous layer was extracted with EtOAc (3 x 20 mL).
The combined organic layers were dried and evaporated. Mesylate 66 was obtained as an
oil and used without further purification. The mesylate was dissolved in acetonitrile (12
mL), cesium carbonate (0.845 g, 2.593 mmol, 1.10 equiv) was added followed by
thioacetic acid (0.197 g, 2.593 mmol, 1.10 equiv), and the mixture was heated at 65 °C for
15 min. After cooling to 25 °C, water (20 mL) was added, and the resulting solution was
extracted with EtOAc (3 x 20 mL). The combined organic layers were dried and
evaporated, leaving a residue which was purified by flash chromatography (10 to 20 %
acetone/hexane) to give 67 as an oil (0.561 g, 88 % from 65).

$R_f$ 0.55 (33 % acetone/hexane)

$^1$H NMR (250 MHz, CDCl$_3$)
δ 5.04 (br s, 1H), 3.07 (br s, 2H), 2.36 (s, 3H), 1.74 (m, 1H), 1.47 (br s, 10H), 1.36
(m, 1H)
$^{13}$C NMR (62.9 MHz, CDCl$_3$)

δ 195.2 (C), 154.4 (C), 118.4 (C), 81.5 (C), 43.8 (C), 30.5 (CH/CH$_3$), 29.7 (CH$_2$), 28.6 (CH/CH$_3$), 28.2 (CH/CH$_3$), 23.7 (CH$_2$)

**IR (neat), cm$^{-1}$**

3370, 2924, 2854, 2361, 2344, 1648, 1137

**MS (EI, 70 eV), m/e (%)**

271 (0.9, M+1), 214 (70), 172 (33), 155 (64), 95 (97), 57 (100)

$[\alpha]_D^{25} +16.0^\circ$ (c = 1.12, CHCl$_3$)

Anal calcd for C$_{12}$H$_{18}$N$_2$O$_3$S: C, 53.31; H, 6.71; N, 10.36; S, 11.86. Found: C, 52.98; H, 6.64; N, 10.41; S, 11.56.
(IR,2R)-(+)-1-[N-(tert-Butoxycarbonyl)amino]-2-[(acetylthio)methyl]-cyclopropane-1-carbonitrile (67)
(IR,2R)-(+)-1-[N-(tert-Butoxycarbonyl)amino]-2-[(acethylthio)methyl]-cyclopropane-1-carbonitrile (67)
\((IR,2R)-(\pm)-1-[N-(tert-Butoxycarbonyl)amino]-2-[(acetythio)methyl]-cyclopropane-1-carbonitrile\) (67)
(1R,2R)-(+)·1-[N-(tert-Butyloxycarbonyl)amino]-2-(mercaptopemethyl)-cyclopropane-1-carbonitrile (68)

Potassium carbonate (0.529 g, 3.832 mmol) was added to a solution of 67 (0.518 g, 1.915 mmol) in MeOH (10 mL) at 0 °C. After the mixture had been stirred at 0 °C for 30 min, the MeOH was evaporated, water (20 mL) was added to the residue, and the solution was extracted with EtOAc (3 x 20 mL). The combined organic layers were dried and evaporated, leaving a residue which was purified by flash chromatography (15 to 30 % acetone/hexane) to give 68 as colorless crystals (0.338 g, 89 %).

Rf 0.20 (20 % acetone/hexane)

1H NMR (250 MHz, CDCl₃)
δ 5.46 (br s, 1H), 3.64 (m, 1H), 3.04 (d, J=10.8 Hz, 1H), 2.28 (m, 1H), 1.42 (s overlapped with m, 12H)

13C NMR (62 MHz, CDCl₃)
δ 155.3 (C), 102.6 (C), 80.2 (C), 46.5 (C), 30.9 (CH₂), 28.2 (CH/CH₃), 27.4 (CH/CH₃), 19.0 (CH₂)

IR (CHBr₃), cm⁻¹
3422, 3020, 2361, 1701, 1613, 1142
MS (EI, 70eV), \( m/e \) (%) 
229 (1, M+1), 228 (0.9, M\(^+\)), 172 (95), 127 (100), 57 (62) 

\([\alpha]_D^{26} +24.1^\circ\) (c = 1.115, CHCl\(_3\)) 

Anal calcd for C\(_{10}\)H\(_{16}\)N\(_2\)O\(_2\)S: C, 52.61; H, 7.06. Found: C, 52.19; H, 6.81.
(1R,2R)-(+)\cdot 1\cdot [N\cdot (\text{tert-Butoxycarbonyl})\cdot \text{amino}]\cdot 2\cdot (\text{mercaptomethyl})\cdot \text{cyclopropane-1-carbonitrile (68)}
(1R,2R)-(+)·1-[N-(tert-Butoxycarbonyl)amino]-2-(mercaptomethyl)-
cyclopropane-1-carbonitrile (68)
$(1R,2R)$-$(+)-1-[N-(\text{tert-Butoxycarbonyl})\text{amino}]-2-\text{(mercaptomethyl)}$-
cyclopropane-1-carbonitrile (68)
E-(2R,3R)-(+-)-2,3-Methanomethionine-trifluoroacetate, E-(+-)-cyclo-Met

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{CO}_2\text{H} \\
\text{SMe} & 
\end{align*}
\]

A solution of dimethylsulfate (0.456 g, 3.614 mmol, 2.50 equiv) in 50% NaOH(aq) (0.289 ml, 2.5 equiv) was added dropwise to a solution of 68 (0.330 g, 1.445 mmol, 1.00 equiv) in MeOH (7.3 mL) at 0 °C under nitrogen. After the reaction had been stirred at 0 °C for 30 min, water (20 mL) was added, and the solution was extracted with EtOAc (3 x 20 mL). The combined organic layers were dried and the solvent was evaporated, leaving a residue which was purified by flash chromatography (10 to 20% acetone/hexane) to give (1R,2R)-1-[N-(tert-butoxycarbonyl)amino]-2-[(methylthio)methyl]-cyclopropane-1-carbonitrile as a colorless solid. The nitrile was dissolved in 6M HCl (13 mL) and heated to reflux for 19 h. After the solution had been cooled to 25 °C, water (10 mL) was added and the solution was lyophilized. The lyophilized residue was purified by ion-exchange chromatography on a Dowex 50 x 8-100 column (H⁺ form, eluted with 2 M NH₄OH). After lyophilization of the product fractions, E-(+-)-cyclo-Met was obtained (0.105 g, 52%). A sample of the ion exchanged product was further purified by HPLC on a C₁₈ column (4.6 x 250 mm, 5μm, eluted with A: 0.01% TFA in H₂O; B: 0.01% TFA in acetonitrile, 0-20% B) to give colorless solids.

literature


\[ R_f \] 0.62 (14.8 M ammonium hydroxide/H₂O/1-propanol, 1/4/12)
$^1$H NMR (300 MHz, D$_2$O, ref CH$_3$OH= 3.70 ppm)

$\delta$ 3.28 (dd, J=13.95, 6.53 Hz, 1H), 3.13 (dd, J=13.95, 8.36 Hz, 1H), 2.51 (s, 3H), 2.36 (m, 1H), 1.97 (d, J=9.80 Hz, 2H)

$^{13}$C NMR (75.4 MHz, D$_2$O, ref CH$_3$OH= 49.9 ppm)

$\delta$ 172.6 (C), 39.5 (C), 31.4 (CH$_2$), 28.0 (CH/CH$_3$), 20.4 (CH$_2$), 15.2 (CH, CH$_3$)

FABMS (glycerol), m/e

162.1 (M+1), 114.0 (CF$_3$COOH$^+$)

$[\alpha]_b^{26}$ +19.0° (c = 0.29, H$_2$O)
E-(2R,3R)-(+)2,3-Methanomethionine-trifluoroacetate, E-(+)cyclo-Met
$E\cdot(2R,3R)\cdot(+)-2,3$-Methanomethionine-trifluoroacetate, $E\cdot(+)$-cyclo-Met
FAB-MS and HETCOR spectra of \(E-(2R,3R)-(+)\)-2,3-methanomethionine-trifluoroacetate, \(E-(+)\)-cyclo-Met
2-[(N-(tert-Butoxycarbonyl)amino)-2-(methylthio)pent-4-ene-1-nitrile (71)

\[ \text{BocHN} \quad \text{SMe} \quad \text{CH}_2 \]

Sodium thiomethoxide (0.0032 g, 0.046 mmol, 1.00 equiv) was added to a solution of the mesylate 66 (0.0133 g, 0.0458 mmol) in t-BuOH (0.2 mL) under nitrogen at 25 °C. After the mixture had been stirred for 30 min, the solvent was evaporated, and the residue was purified by flash chromatography (5 % acetone/hexane) to give 71 (0.0048 g, 43 %) as a colorless oil.

\[ R_f \ 0.26 \ (10 \% \ \text{acetone/hexane}) \]

\[^1\text{H} \ \text{NMR} \ (250 \text{ MHz, } \text{CDCl}_3)\]

\[ \delta \ 5.83 \ (m, 1H), \ 5.28 \ (m, 2H), \ 4.89 \ (\text{br s, } 1H), \ 2.92 \ (d, J=7.09 \text{ Hz, } 2H), \ 2.41 \ (s, 3H), \ 1.47 \ (s, 9H) \]

\[ \text{HRMS } m/e \ \text{calc'd for } \text{C}_{11}\text{H}_{18}\text{N}_2\text{O}_2\text{S}: \ 242.1089. \ \text{Found} \ 242.1089 \]
2-[(tert-Butoxycarbonyl)amino]-2-(methylthio)pent-4-ene-1-nitrile (71)
The enantiomers of compound 62 to 68, and (-)-E-cyclo-Met were prepared in identical fashion.

\((IS,SS)-(+)\)-1-[\(N\)-(tert-Butoxycarbonyl)amino]-2-oxo-3-oxabicyclo[3.1.0]hexane (+)-(62). \([\alpha]_D^{24} +31.5^\circ\) (c = 0.43, CHCl\(_3\)).

\((IS,2S)-(+)\)-1-[\(N\)-(tert-Butoxycarbonyl)amino]-2-(hexanoyloxy)methyl]-cyclopropane-1-carbonitrile (+)-(64). \([\alpha]_D^{25} +1.4^\circ\) (c = 1.62, CHCl\(_3\)).

\((IS,2S)-(\cdot)\)-1-[\(N\)-(tert-Butoxycarbonyl)amino]-2-(hydroxymethyl)-cyclopropane-1-carbonitrile (-)-(65). \([\alpha]_D^{25} -2.8^\circ\) (c = 1.12, CHCl\(_3\)).

\((IS,2S)-(\cdot)\)-1-[\(N\)-(tert-Butoxy)carbonyl]amino]-2-[(acetylthio)methyl]-cyclopropane-1-carbonitrile (-)-(67). \([\alpha]_D^{25} -16.1^\circ\) (c = 1.12, CHCl\(_3\)).

\((IS,2S)-(\cdot)\)-1-[\(N\)-(tert-Butoxy)carbonyl]amino]-2-(mercaptomethyl)-cyclopropane-1-carbonitrile (-)-(68). \([\alpha]_D^{25} -25.0^\circ\) (c = 1.12, CHCl\(_3\)).

\(E-(2S,3S)-(\cdot)\)-2,3-Methanomethionine-trifluoroacetic acid. \([\alpha]_D^{25} -18.9^\circ\) (c = 0.29, H\(_2\)O).
(1R,2R)-(+)-tert-Butyl 1-[N-(tert-butoxycarbonyl)amino]-2-(cyanomethyl)-
cyclopropane-1-carboxylate (72)

\[
\begin{array}{c}
\text{t-BuO}_2\text{C} \quad \text{NHBoc} \\
\text{CN}
\end{array}
\]

(1R,2S)-(+)-tert-Butyl 1-[N-(tert-butoxycarbonyl)amino]-2-(hydroxymethyl)-
cyclopropane-1-carboxylate \text{1R,2S-60} (0.31 g, 1.09 mmol, 1 equiv) was dissolved in
\text{CH}_2\text{Cl}_2 (5.4 mL) and triethylamine (0.17 g, 1.63 mmol, 1.5 equiv). The mixture was
cooled to 0 °C, and methanesulfonyl chloride (0.19 g, 1.63 mmol, 1.5 equiv) was added
dropwise. Reaction was stirred at 0 °C for 5 min, and 25 °C for 30 min. The solvent was
evaporated, \text{H}_2\text{O} (15 mL) was added and extracted with \text{EtOAc} (3 \times 15 mL). Combined
organic layers were dried and evaporated to obtain the crude mesylate. The crude mesylate
was redissolved in a mixture of \text{H}_2\text{O} (5.4 mL) and benzene (5.4 mL), followed by addition
of tetrabutylammonium cyanide (cat amount) and \text{KCN} (0.71 g, 10.89 mmol, 10 equiv).
The mixture was heated at 65 °C for 20 h. After cooling to 25 °C, \text{H}_2\text{O} (15 mL) was
added and extracted with \text{EtOAc} (3 \times 15 mL). Combined organic layers were dried and
evaporated. The residue was purified by column chromatography (10 to 25 %
\text{EtOAc/hexane}), and recrystallized (dissolved in 90 % \text{EtOH/H}_2\text{O} and saturated to cloud
point by \text{H}_2\text{O}) to give nitrile 72 as colourless solid (0.201 g, 62 %).

\text{mp 97-98 °C}

\text{R}_f 0.40 (25 % \text{EtOAc/hexane})
$^1$H NMR (300 MHz, CDCl$_3$)
δ 5.00 (br s, 1H), 2.71 (m, 1H), 2.44 (m, 1H), 2.05 (m, 1H), 1.44 (overlapping signals, 19 H), 1.02 (m, 1H)

$^{13}$C NMR (75.4 MHz, CDCl$_3$)
δ 170.4 (C), 156.2 (C), 118.4 (C), 82.1 (C), 38.9 (C), 28.1 (CH/CH$_3$), 27.7 (CH/CH$_3$), 22.2 (CH/CH$_3$), 21.3 (CH$_2$), 16.7 (CH$_2$)

IR (KBr), cm$^{-1}$
3345, 2979, 2360, 2331, 1716, 1501, 1369, 1251, 1153, 1050

FABMS (NBA), m/e (%)
297 (7, M+1), 289 (12), 246 (29), 185 (43), 154 (100), 137 (77)

Anal calcd for C$_{15}$H$_{24}$N$_2$O$_4$: C, 60.79; H, 8.16; N, 9.39. Found: C, 60.60; H, 8.16; N, 9.45.

$[\alpha]_D^{27} +49.1^\circ$ (c = 1.10, CHCl$_3$)
(IR,2R)-(+)\text{-}tert\text{-}Butyl 1\text{-}[N\text{-}(\text{tert\text{-}butoxycarbonyl})\text{amino}]\text{-}2\text{-}(\text{cyanomethyl})\text{-}cyclopropane\text{-}1\text{-}carboxylate (72)
\((IR,2R)-(+)-\text{tert-Butyl 1-}\left[N-(\text{tert-butoxycarbonyl}amino)\right]2-(\text{cyanomethyl})-\text{cyclopropane-1-carboxylate (72)}\)
(IR,2R)-(+)-tert-Butyl 1-[N-(tert-butoxycarbonyl)amino]-2-(cyanomethyl)-cyclopropane-1-carboxylate (72)
(2R,3S)-(+-)-N-(tert-Butoxycarbonyl)-O-tert-butyl-2,3-methanoornithine,
(2R,3S)-(+-)-Boc-cyclo-Orn-Ot-Bu

The nitrile 72 (0.10 g, 0.34 mmol, 1 equiv) was dissolved in a mixture of MeOH
(5.6 mL) and NH₄OH (14.8 M, 0.3 mL, 3.37 mmol, 10 equiv), and Raney Ni (0.5 mL of
50 % slurry) in a stainless steel bomb with a magnetic stirrer. The bomb was pressurized
with hydrogen gas (300 psi). The reaction was stirred at 25 °C for 8 h. The catalyst was
filtered, and washed with CH₂Cl₂ (20 mL). Evaporation of solvent, followed by vacuum
drying gave Boc-cyclo-Orn-Ot-Bu as a colourless oil (0.101 g, 100 %).

Rf 0.31 (33 % acetone/hexane)

¹H NMR (300 MHz, CDCl₃)
δ 5.69 (br s, 1H), 2.80 (m, 2H), 1.56 (m, 2H), 1.43-1.31 (overlapping signals, 20H),
0.80 (m, 1H)

¹³C NMR (75.4 MHz, CDCl₃)
δ 172.3 (C), 156.2 (C), 80.8 (C), 79.1 (C), 40.9 (CH₂), 38.2 (C), 31.1 (CH₂), 28.3
(CH₃), 28.0 (CH₃), 25.6 (CH), 22.2 (CH₂)

IR (CHBr₃), cm⁻¹
3426, 3020, 1713, 1488, 1391, 1367, 1290, 1249, 1143, 656
MS (EI, 70 eV), m/e (%)
301 (1.3, M+1*), 244 (5), 188 (29), 189 (29), 172 (30), 127 (62), 56 (100)

HRMS m/e calcld for C$_{15}$H$_{28}$N$_{2}$O$_{4}$: 300.2049. Found: 300.2048

[α]$_{D}^{26}$ +29.6° (c = 0.21, CHCl$_{3}$)
(2R,3S)-(+)\text{-}N\text{-}(\text{tert-Butoxycarbonyl})\text{-}O\text{-}\text{tert-butyl}\text{-}2,3\text{-}methanoornithine,

(2R,3S)-(+)\text{-}Boc\text{-}cyclo\text{-}Orn\text{-}Ot-Bu
(2R,3S)-(+-)N-(tert-Butoxycarbonyl)-O-tert-butyl-2,3-methanoornithine,
(2R,3S)-(+-)Boc-cyclo-Orn-Ot-Bu
(2R,3S)-(+)-N-(tert-Butoxycarbonyl)-O-tert-butyl-2,3-methanoornithine,
(2R,3S)-(+)-Boc-cyclo-Orn-Ot-Bu
(IR,2S)-(+) -tert-Butyl 1-(N-tert-butoxycarbonylamino)-2-[2-(methylmercapto-toluenesulfonylimido-methyl-amino)-ethyl]-cyclopropane-1-carboxylate (73)

A mixture of Boc-cyclo-Orn-Ot-Bu (0.10 g, 0.34 mmol, 1 equiv), S,S-dimethyl-N-[(p-toluenesulfonyl)-imino]dithiocarbodiimide (0.10 g, 0.371 mmol, 1.1 equiv), and xylenes (1.5 mL) was refluxed for 8 h. Evaporation of xylene, followed by column chromatography (5 to 33 % of EtOAc/hexane) gave 73 as a colorless oil (0.11g, 63 %).

$R_f$ 0.22 (33 % EtOAc/hexane)

$^1$H NMR (300 MHz, CDCl$_3$)
δ 8.26 (br s, 1H), 7.78 (d, J=8.01 Hz, 2H), 7.25 (d, overlapped with CHCl$_3$, 2H), 4.93 (br s, 1H), 3.42 (m, 2H), 2.43 (S, 3H), 2.38 (s, 3H), 2.03 (m, 1H), 1.63-1.25 (s overlapped with m, 22H), 0.83 (m, 1H)

$^{13}$C NMR (75.4 MHz, CDCl$_3$)
δ 171.5 (C), 169.3 (C), 156.2 (C), 142.7 (C), 139.7 (C), 129.3 (CH), 126.1 (CH), 81.5 (C), 79.9 (C), 43.8 (CH$_2$), 38.6 (C), 28.2 (CH$_2$), 24.6 (CH), 21.9 (CH$_2$), 21.5 (CH$_3$), 14.1 (CH$_3$)
IR (CHBr₃), cm⁻¹
3418, 3020, 2256, 1731, 1583, 1297, 1142, 1081, 693, 654

FABMS (thioglycerol), m/e (%)
528 (27, M+1), 472 (43), 416 (100), 374 (45), 262 (35)

HR-FABMS (thioglycerol)m/e calc'd for C₂₄H₃₈N₃O₆S₂ (M+1): 528.2202.
Found: 528.2206

[α]D²⁶ +20.9° (c = 0.47, CHCl₃)
(IR,2S)-(+)-tert-Butyl 1-(N-tert-butoxycarbonylamo)-2-[2-(methylmercapto-toluenesulfonylimido-methyl-amino)-ethyl]-cyclopropane-1-carboxylate (73)
(IR,2S)-(+)-tert-Butyl 1-(N-tert-butoxycarbonylamino)-2-[2-(methylmercapto-toluenesulfonylimido-methyl-amino)-ethyl]-cyclopropane-1-carboxylate (73)
(IR,2S)-(+)-tert-Butyl 1-(N-tert-butoxycarbonylamino)-2-[2-(methylmercapto-toluenesulfonylimido-methyl-amino)-ethyl]-cyclopropane-1-carboxylate (73)
(2R,3S)-(−)-N-α-(tert-Butoxycarbonyl)-N⁸-(p-toluenesulfonyl)-O-tert-butyl-2,3-methanoarginine, (2R,3S)-(−)-Boc-cyclo-Arg(Ts)-Ot-Bu (74)

The compound 73 (0.092 g, 0.174 mmol, 1 equiv) was dissolved in acetonitrile (4 mL), and cooled to 0 °C. The solution was then saturated with gaseous ammonia, followed by slow addition (ca 0.5 h) of a premixed solution of silver nitrate (0.033 g, 0.191 mmol, 1.1 equiv) in acetonitrile (1 mL). The mixture was warmed to 25 °C, and stirred for 2 h. The solution was then filtered to remove the AgSMe, and washed with MeOH (5 mL). The filtrate was evaporated, and the remaining residue was purified by column chromatography (15 to 33 % acetone/hexane), and recrystallized (dissolved in 90 % EtOH/H₂O and saturated to cloud point by H₂O) to give (2R,3S)-(−)-Boc-cyclo-Arg(Ts)-Ot-Bu (74) as colorless solid (0.087 g, 100 %).

mp 112-113 °C

RF 0.27 (33 % acetone/hexane)

¹H NMR (250 MHz, CDCl₃)
δ 7.75 (d, J=9.64 Hz, 2H), 7.21 (d, J=9.71 Hz, 2H), 6.84 (br s, 1), 5.07 (s, 1H), 3.29 (m, 2H), 2.37 (s, 3H), 1.68 (m, 2H), 1.43-1.39 (2 overlapping s, 20H), 0.62 (m, 1H)
\[ ^{13}\text{C} \text{ NMR (62.9 MHz, CDCl}_3\] 
\[ \delta 170.8 (\text{C}), 157.4 (\text{C}), 157.2 (\text{C}), 141.6 (\text{C}), 141.2 (\text{C}), 129.1 (\text{CH}), 125.8 (\text{CH}), 81.6 
\quad (\text{C}), 80.8 (\text{C}), 40.6 (\text{CH}_2), 39.6 (\text{C}), 28.3 (\text{CH}_2), 28.2 (\text{CH}), 27.9 (\text{CH/CH}_3), 24.4 
\quad (\text{CH/CH}_3), 21.4 (\text{CH}_2\text{CH}_3), 21.25 (\text{CH}_2)\]

**IR (CHBr}_3\), cm\(^{-1}\)**

3422, 3352, 3021, 1697, 1625, 1555, 1263, 1143, 1081, 654

**MS (EI, 70eV), m/e (%)**

496 (0.8, M\(^+\)), 214 (4), 155 (7), 98 (28), 56 (43), 41 (100), 39 (99)

**HR-FABMS (p-nitrobenzylalcohol)m/e calc'd for C\(_{23}\)H\(_{37}\)N\(_4\)O\(_6\)S (M+1\(^+\)):**

497.2434. **Found:** 497.2430

**Anal calc'd for C\(_{23}\)H\(_{36}\)N\(_4\)O\(_6\)S:** C, 55.62; H, 7.31; N, 11.28. **Found:** C, 55.46; H, 7.80; N, 10.48.

\[ [\alpha]_D^{26} -24.7^\circ \ (c = 0.51, \text{CHCl}_3)\]
(2R,3S)-(-)-N-α-(tert-Butoxycarbonyl)-N8-(p-toluenesulfonyl)-O-tert-butyl-2,3-methanoarginine, (2R,3S)-(-)-Boc-cyclo-Arg(Ts)-Ot-Bu (74)
(2R,3S)-(-)-N-α-(tert-Butoxycarbonyl)-N⁸-(p-toluenesulfonyl)-O-tert-butyl-2,3-methanoarginine, (2R,3S)-(-)-Boc-cyclo-Arg(Ts)-Ot-Bu (74)
(2R,3S)-(−)-N-α-(tert-Butoxycarbonyl)-N8-(p-toluenesulfonyl)-O-tert-butyl-2,3-methanoarginine, (2R,3S)-(−)-Boc-cyclo-Arg(Ts)-Ot-Bu (74)
(2R,3S)-(-)-N-α-(tert-Butoxycarbonyl)-N⁸-(p-toluenesulfonyl)-2,3-methanoarginine, (2R,3S)-(-)-Boc-cyclo-Arg(Ts)

Boc-cyclo-Arg(Ts)-Ot-Bu (74) (0.030 g, 0.060 mmol, 1 equiv) was dissolved in CH₂Cl₂ (0.3 mL), and cooled to 0 °C. Trifluoroacetic acid (TFA, 0.1 mL) was added slowly, and the mixture was stirred at 25 °C for 1 h. After evaporation of the solvent and the excess TFA, the residue was lyophilized in H₂O (1 mL). The lyophilized residue was redissolved in a mixture of t-BuOH (0.6 mL), aqueous 1 M NaOH (0.18 mL), and di-t-butyl-dicarbonate (0.04 g, 0.18 mmol, 3 equiv), and stirred at 25 °C for 18 h. The t-BuOH was evaporated, and H₂O (5 mL) was added. The aqueous was washed with hexane (5 mL), followed by addition of EtOAc (5 mL). The EtOAc/H₂O mixture was chilled in an ice bath, and acidified to pH = 2 by 1 M H₂SO₄. The EtOAc layer was separated, and the aqueous was further extracted with EtOAc (5 x 5 mL). The combined EtOAc layers were dried and evaporated. The residue was purified by column chromatography (90 % EtOAc/hexane acidified with acetic acid) to give (2R,3S)-(+)-Boc-cyclo-Arg(Ts) as colourless oil (0.16 g, 60 %).

Rf 0.45 (95 % EtOAc/hexane acetified with acetic acid)
$^1$H NMR (75.4 MHz, acetone-d$_6$)

$\delta$ 7.73 (d, $J$=8.08 Hz, 2H), 7.28 (d, $J$=8.01 Hz, 2H), 6.69 (overlapping signals, 3H), 3.36 (m, 2H), 2.37 (s, 3H), 1.73 (br s, 2H), 1.41 (overlapping signals, 11H), 0.87 (m, 1H)

$^{13}$C NMR (75.4 MHz, acetone-d$_6$)

$\delta$ 174.8 (C), 158.0 (2 overlapping C), 143.3 (C), 142.0 (C), 129.8 (CH/CH$_3$), 126.7 (CH/CH$_3$), 79.4 (C), 41.3 (CH$_2$), 38.6 (C), 28.6 (CH$_2$), 28.5 (CH/CH$_3$), 25.9 (C), 22.3 (CH$_2$), 21.3 (CH/CH$_3$)

FABMS (thioglycerol), $m/e$ (\%)

441 (9, M+1$^+$), 385 (100), 287 (47), 231 (38), 155 (15)

HR-FABMS (thioglycerol)$m/e$ calc'd for C$_{19}$H$_{29}$N$_4$O$_6$S (M+1$^+$): 441.1808.
Found: 441.1814

$[\alpha]_D^{27}$ +15.0$^\circ$ (c = 0.67, acetone)
2R,3S)-(−)-N-α-(tert-Butoxycarbonyl)-N⁸-(p-toluenesulfonyl)-2,3-methanoarginine, (2R,3S)-(−)-Boc-cyclo-Arg(Ts)
2R,3S)-(−)-N-α-(tert-Butoxycarbonyl)-N\textsubscript{G}-(p-toluenesulfonyl)-2,3-methanoarginine, (2R,3S)-(−)-Boc-cyclo-Arg(Ts)
$2R,3S$-(-)-$N$-$\alpha$-(tert-Butoxycarbonyl)$-N^8$-(p-toluenesulfonyl)-2,3-methanoarginine, $(2R,3S)$-(-)-Boc-cyclo-Arg(Ts)
(2R,3S)-(+)\text{-}N\text{-}\alpha\text{-}(\text{tert-Butoxycarbonyl})\text{-}O\text{-}\text{tert-butyl}-2,3\text{-}methanoaspartic acid, (2R,3S)-(+)\text{-}Boc\text{-cyclo-Asp-}O\text{-}t\text{-}Bu

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {\text{t\text{-}BuO}_2C_m};
\node (b) at (0,-0.5) {\text{NHBoc}};
\node (c) at (0.1,0) {\text{COOH}};
\draw (a) -- (b);
\end{tikzpicture}
\end{center}

(1R,2S)-(+)\text{-}\text{tert-Butyl 1}\text{-}N\text{-}(\text{tert-butoxycarbonyl} \text{amino})\text{-}2\text{-}(\text{hydroxymethyl})\text{-}\text{cyclopropane-1-carboxylate (1R,2S-60)} (0.10 \text{ g, 0.35 mmol, 1 equiv}) was dissolved in a mixture of \text{CCl}_4 (0.7 \text{ mL}), \text{CH}_3\text{CN} (0.7 \text{ mL}) and \text{H}_2\text{O} (1.0 \text{ mL}). Sodium metaperiodate (0.22 \text{ g, 1.04 mmol, 3 equiv}) and ruthenium chloride (1.6 mg, 8 \text{ \mu mol, 0.022 equiv}) was added. The mixture was stirred vigorously at 25 °C for 2 h. Water (5 \text{ mL}) and \text{EtOAc} (5 \text{ mL}) were added. The solution was chilled in an ice bath, acidified to pH = 2 by 1 M \text{H}_2\text{SO}_4, and extracted with \text{EtOAc} (5 \times 5 \text{ mL}). The combined organic layers were dried and evaporated, and residue was purified by column chromatography (5 to 20 \% acetone/hexane acidified with acetic acid) to give (2R,3S)-(+)\text{-}Boc\text{-cyclo-Asp-}O\text{-}t\text{-}Bu as yellow solid. The yellow solid was lyophilized from water to give off white solid (0.054 \text{ g, 51 \%}).

\textbf{R}_f 0.44 (33 \% acetone/hexane acidified with acetic acid)

\textbf{\textsuperscript{1}H NMR (300 MHz, CDCl}_3\text{)}
\begin{itemize}
\item \text{\delta} 8.98 (br s, 1H), 6.22 (br s, 1H), 5.29 (br s, 1H), 2.56 (m, 1H), 1.73 (m, 2H), 1.48-1.40 (2 overlapping s, 18H)
\end{itemize}

\textbf{\textsuperscript{13}C NMR (75.4 MHz, CDCl}_3\text{)}
\begin{itemize}
\item \text{\delta} 173.3 (2xC), 169.3 (C), 82.3 (2xC), 41.3 (C), 28.7 (CH), 21.7 (CH}_2\text{)}
\end{itemize}
IR \((\text{CHBr}_3)\), cm\(^{-1}\)

3391, 3206, 3020, 1716, 1504, 1369, 1295, 1254, 1142, 693, 653

FABMS (glycerol), \(m/e\) (%)

302 (0.3, M+1\(^+\)), 246 (29), 185 (100), 146 (40), 128 (15)

HR-FABMS (glycerol)\(m/e\) calc'd for \(\text{C}_{14}\text{H}_{24}\text{NO}_6\) (M+1\(^+\)): 302.1603. Found 302.1601

\([\alpha]_D^{27} +88.8^\circ\) \((c = 0.77, \text{CHCl}_3)\)
(2R,3S)-(+)-N-α-(tert-Butoxycarbonyl)-O-tert-butyl-2,3-methanoaspartic acid, (2R,3S)-(+)-Boc-cyclo-Asp-Or-Bu
(2R,3S)-(+)–N-α-(tert-Butyloxycarbonyl)-O-tert-buty1-2,3-methanoaspartic acid, (2R,3S)-(+)–Boc-cyclo-Asp-Or-Bu
(2R,3S)-(+)\text{-}N{\text{-}}\alpha{\text{-}}(\text{tert-Butoxycarbonyl})\text{-}O{\text{-}}\text{tert-butyl}-2,3\text{-}\text{methanoaspartic acid, } (2R,3S)-(+)\text{-}\text{Boc-cyclo-Asp-Ot-Bu}
(2R,3S)-(+)-N-α-(tert-Butoxycarbonyl)-O-tert-butyl-2,3-methanoaspartic acid, (2R,3S)-(+)-Boc-cyclo-Asp-Ot-Bu
Appendix 2. Experimental for Chapter 3

(2S,3R)-(-)-N-α-(tert-Butoxycarbonyl)-2,3-methanomethionine,

(2S,3R)-(-)-Boc-cyclo-Met

\[
\text{HO}_2\text{C} \quad \text{NH}_{\text{Boc}} \\
\text{SMe}
\]

2S,3R-cyclo-Met (0.059 g, 0.366 mmol, 1 equiv) was dissolved in a mixture of di-tert-butyl-dicarbonate (0.240 g, 1.098 mmol, 3 equiv), 1 M NaOH (0.73 mL), and t-BuOH (3 mL). The reaction was stirred at 25 °C for 10 h. Then H\text{2O} (10 mL) was added, and washed with hexane (8 mL). The aqueous layer was cooled to 0 °C, acidified to pH = 2 by 1 M H\text{2SO}_4, and extracted with EtOAc (4 x 10 mL). The combined organic layers were dried and evaporated. The residue was purified by column chromatography (25 % acetone/hexane acidified with acetic acid) to give (2S,3R)-Boc-cyclo-Met as colorless solid (0.063 g, 66 %).

\[R_f 0.29 \text{ (33 % acetone/hexane acidified with acetic acid)}\]

\(^1\text{H} \text{ NMR (250 MHz, CDCl}_3\)\]
\[\delta 5.32 \text{ (br s, J=7.40 Hz, 2H), 2.62 (m, 2H), 2.14 (s, 3H), 2.01 (m, 1H), 1.85 (m, 1H), 1.48-1.42 (m+s, 10H), 1.15 (m, 1H)}\]

\(^13\text{C} \text{ NMR (75.4 MHz, CDCl}_3\)\]
\[\delta 178.5 \text{ (C), 156.5 (C), 80.4 (C), 38.1 (C), 33.1 (CH}_2\text{), 27.9 (CH/CH}_3\text{), 27.4 (CH/CH}_3\text{), 24.3 (CH}_2\text{), 15.2 (CH/CH}_3\text{)}\]
**IR (CHBr₃), cm⁻¹**

3409, 3030, 1693, 1483, 1377, 1250, 1138, 653

\[ [\alpha]_D^{25} = -11.71° \] (c = 1.9, CHCl₃)
(2S,3R)-(-)-N-α-(tert-Butoxycarbonyl)-2,3-methanomethionine,
(2S,3R)-(−)-Boc-cyclo-Met
(2S,3R)-(-)-N-α-(tert-Butoxycarbonyl)-2,3-methanomethionine,
(2S,3R)-(-)-Boc-cyclo-Met
(2R,3S)-Boc-cyclo-Met. \([\alpha]_D^{25} +9.54^*\) (c = 1.3, CHCl₃)
Phe-{2S,3R-cyclo-Met}-Arg-Phe-NH₂

The peptide was prepared by stepwise couplings of N-tert-butyloxy carbonyl (Boc) amino acids derivatives on MBHA resin. Tosyl group was used as the side chain protection for Arg (ie Boc-Arg(Ts) was used). Manual peptide synthesis was carried out in a 30 mL vessel fitted with a coarse glass frit by using a manual wrist action shaker (Burrell Model 75) at room temperature. The MBHA resin (0.206 g, 0.128 mmol of 0.62 meq/g resin) was first swelled in CH₂Cl₂ for 5 min, and neutralized with 10 % solution of diisopropylethylamine (DIEA) in CH₂Cl₂. After successive washing of the neutralized resin with CH₂Cl₂ (4 x 1 min), MeOH (1 x 1 min), DMF (1 x 1 min), and CH₂Cl₂ (1 x 1 min), the resin was coupled with Boc-Phe¹. The Boc-Phe⁴ (0.102 g, 0.384 mmol, 3 equiv relative to resin) was premixed with BOP (0.170 g, 0.384 mmol, 3 equiv), DIEA (0.0877, 06784 mmol, 5.3 equiv) in 4 mM DMF. The mixture was added to the neutralized resin, shaken for 2 hr, and the amino acid bounded resin was resulted with a negative ninhydrin (Kaiser) test. Deprotection of the Boc group was effected by treating the resin with 50 % TFA in CH₂Cl₂ (20 min). The deprotected resin was first washed with CH₂Cl₂ (4 x 1 min) to remove the excess TFA, followed by the neutralization and washing cycles as the previous step. Successive amino acids (ie in the order of Arg³, 2S,3R-cyclo-Met², and
Phe<sup>1</sup>) were coupled and washed by the same procedures and reagents ratio except for the 2S,3R-cyclo-Met<sup>2</sup>. For this more precious amino acid, 1.1 equiv was used.

Cleavage of the peptide from the resin was done at Immuno-dynamics, Inc.. Dried resin was placed in a HF apparatus. Side chain protected group, as well as the peptide-resin bond were cleaved in a mixture of liquid HF (7 mL), anisole (0.6 mL), dimethyl sulfide (0.3 mL), 2-mercaptopyridine (3 mg), and 1,2-ethanediol (0.2 mL). The reaction was kept at -10 °C for 30 min, and warm to 0 °C for 35 min. After removal of HF under vacuum, the residue was washed with anhydrous ether, and dried. Acetic acid (5 % in water, <i>ca</i> 8 mL) was added to the dry residue and filtered. The filtrate was washed with ether (5 x 5 mL), and lyophilization of the aqueous afforded the crude peptide (9 mg). The crude peptide was purified by RF-HPLC (Vydac C18 column, 22 mm x 25 cm, 10 μm) with a linear gradient obtained by mixing solvent A (0.05 % TFA in water) and solvent B (0.05 % TFA in acetonitrile). The gradient was programmed to increase from 5 to 60 % B over 30 min with a flow rate of 6 mL min<sup>-1</sup>. The peak with a retention time of 21.80 min was collected and lyophilized. The desired peptide (TFA salt) was obtained as a colorless powder (3.1 mg, 3 %).

**Amino acid analysis (normalized for Phe), Phe 2.00, Arg 1.07**

**FABMS (glycerol), m/e 611.3 (M + 1)<sup>+</sup>**

**HR-FABMS (glycerol), m/e calcld for C<sub>30</sub>H<sub>43</sub>N<sub>8</sub>O<sub>4</sub>S: 611.3124. Found: 611.3127.**
Phe-(2S,3R-cyclo-Met)-Arg-Phe-NH₂
Phe-\{2R,3S-cyclo-Me\}-Arg-Phe-NH₂

F-\{2R,3S-cyclo-M\}-RF-NH₂ was synthesized by the same procedure as F-\{2S,3R-cyclo-M\}-RF-NH₂. MBHA resin of 0.093 mmol was used and the amount of reagents were adjusted accordingly. The peptide had a retention time of 22.47 min, and was isolated in a final yield of 2.2 mg (3%).

Amino acid analysis (normalized for Phe), Phe 2.00, Arg 1.02.

FABMS (glycerol), \(m/e\) 611.4, \((M + 1)^+\).

HR-FABMS (glycerol), \(m/e\) calcd for \(\text{C}_{30}\text{H}_{43}\text{N}_{8}\text{O}_{4}\text{S}\): 611.3124. Found: 611.3127.
Phe-(2R,3S-cyclo-Met)-Arg-Phe-NH₂
\(N\)-\(\alpha\)-(9-Fluorenylmethoxycarbonyl)-2,3-methanomethionine,

\((2S,3S)-(+)\)-Fmoc-cyclo-Met

\[
\text{FmocH} \begin{array}{c}
\text{CO}_2\text{H} \\
\text{SMe}
\end{array}
\]

Chlorotrimethylsilane (TMSCl, 0.092 g, 0.844 mmol) was added to a vigorously stirred suspension of \(2S,3S\)-cyclo-Met (0.068 g, 0.422 mmol) in CH\(_2\)Cl\(_2\) (2.1 mL), and the mixture was refluxed for 1 h. After cooling to 25 °C, the reaction was placed in an ice-bath, and \(N,N\)-diisopropylethylamine (0.175 g, 1.350 mmol) was added followed by 9-fluorenylmethyloxycarbonyl-\(N\)-hydroxysuccinimide (Fmoc-OSu, 0.157 g, 0.464 mmol). The reaction was allowed to proceed at 0 °C for 20 min, and 25 °C for 1.5 h. Aqueous solution of 2.5 % NaHCO\(_3\) (15 mL) was added, and then the aqueous was washed with Et\(_2\)O (3x15 mL). Combined Et\(_2\)O layers were back extracted with H\(_2\)O (2 x 10 mL). The NaHCO\(_3\) solution and the H\(_2\)O extracts were combined, acidified to pH = 2 by 1 M HCl, and extracted with EtOAc (3 x 15 mL). The combined organic layers were dried and evaporated. The residue was purified by flash chromatography (20 to 40 % acetone/hexane which was acidified with acetic acid by addition of ca 2 drops of acetic acid/20 mL eluent) to give Fmoc-\{(2S,3S-cyclo-Met]\ (0.0515 g, 32 %) as a colorless oil.

\(R_f\) 0.28 (33 % acetone/hexane acidified with acetic acid)
$^1$H NMR (250 MHz, CDCl$_3$)

$^1$H 7.74 (d, J=7.40 Hz, 2H), 7.57 (d, J=7.10 Hz, 2H), 7.41-7.27 (m, 4H), 5.45 (br s, 1H, 2 signals for rotamers), 4.43 (br s, 2H, 2 signals for rotamers), 4.21 (m, 1H), 2.82-2.17 (m, 2H, multiple signals for rotamers), 2.06 (m, 3H, multiple signals for rotamers), 1.90-1.24 (m, 3H, multiple signals for rotamers)

$^{13}$C NMR (75.4 MHz, CDCl$_3$)

$^1$C 176.1 (C), 156.5 (C), 143.7 (C), 141.3 (C), 127.7 (CH), 127.1 (CH), 125.0 (CH), 120.0 (CH), 67.0 (CH$_2$), 47.1 (CH/CH$_3$), 38.8 (C), 32.3 (CH/CH$_3$), 31.5 (CH$_2$), 24.5 (CH$_2$), 15.5 (CH, CH$_3$)

$[\alpha]_D^{26} +4.0^\circ$ (c = 1.6, DMF)
*N*-α-(9-Fluorenymethoxycarbonyl)-2,3-methanomethionine,

(2S,3S)-(+)-Fmoc-cyclo-Met
$N$-$\alpha$-(9-Fluorenylethoxycarbonyl)-2,3-methanomethionine,

$(2S,3S)$-$(+)$-Fmoc-cyclo-Met
Fmoc-\{2R,3R-cyclo-Met\} [\alpha]_D^{26} -2.9^\circ (c = 1.7, DMF)
Phe-{2S,3S-cyclo-Met}-Arg-Phe-NH₂

The peptide was prepared by stepwise couplings of 9-fluorenlymethoxycarbonyl (Fmoc) amino acids derivatives on Rink amide resin. 4-Methoxy-2,3,6-trimethylbenzene sulfonyl (Mtr) group was used as the side chain protection for Arg (ie Fmoc-Arg(Mtr) was used). Manual peptide synthesis was carried out in a 30 mL vessel fitted with a coarse glass frit by using a manual wrist action shaker (Burrell, Model 75), and reagents were added manually. All reactions were carried at 25 °C unless specified. A DMF washing cycle (10 x 1 min, ca 10 mL) was incorporated after each coupling and deprotection.

Rink amide resin (0.16 g of 0.47 mol g⁻¹ capacity) was first swelled in DMF (ca 10 mL) for 45 min, and the Fmoc protecting group was removed by shaking the resin with 20 % piperidine in DMF (2 times, 3 × 7 min). Coupling of Fmoc-Phe⁴ (0.087 g, 0.225 mmol, 3 equiv relative to the resin) was performed by first premixing the amino acids with NMM (0.034 g, 0.338 mmol, 4.5 equiv), HOBT (0.030 g, 0.225 mmol, 3 equiv ), and BOP (0.100 g, 0.225 mmol, 3 equiv) in DMF (5 mL), then the mixture was added to the resin, shaken for 45 min and washed. A negative ninhydrin test was observed after the 45 min reaction time. The same coupling cycle was repeated for Fmoc-Arg³(Mtr) (0.137 g, 0.225 mmol, 3 equiv). For the precious Fmoc-2S,3S-cyclo-Met² (0.032 g, 0.083 mmol), 1.1 equiv of the amino acid was used and the reaction was shaken for 2 h. The
resin was then end-capped using acetic anhydride (7 μL) and DMAP (10 mg) in DMF (2 mL) for 20 min, followed by removal of the Fmoc. The Fmoc-Phe 1 (0.087 g, 0.225 mmol, 3 equiv) was coupled and deprotected by the same procedure as the Fmoc-Phe 4 . The resin was then washed with CH₂Cl₂ (10 x 1 min), and dried under vacuum.

Cleavage of peptide from the resin was effected by the following procedures: the resin was cooled to 0°C in a round bottom flask with a stir bar, then a mixture of phenol (0.75 g), 1,2-ethanediol (0.25 mL), thioanisole (0.5 mL), deionized water (0.5 mL) and trifluoroacetic acid (10 mL) was pre-cooled to 0°C and added to the resin. The reaction was removed from the ice-bath and stirred at 25°C for 10 h. The resin was then filtered, washed with trifluoroacetic acid (5 mL) and CH₂Cl₂ (15 mL). The filtrate was evaporated to ca 2 mL. Deionized water (20 mL) was added and the aqueous was washed with Et₂O (5 x 10 mL). Crude peptide (52 mg) was obtained after lyophilization of the water layer. The crude peptide was further purified by preparative RP-HPLC (Vydac C18 column, 22 mm x 25 cm, 10 μm) with a linear gradient obtained by mixing solvent A (0.05 % TFA in water) and solvent B (0.05 % TFA in acetonitrile). The gradient was programmed to increase from 5 to 60 % B over 30 min with a flow rate of 6 mL min⁻¹. The peak with a retention time of 26.13 min was collected and lyophilized. The desired peptide (TFA salt) was obtained as a colorless powder (46 mg, 70 %).

Amino acid analysis (normalized for Phe), Phe 2.00, Arg 1.15.

FABMS (glycerol), m/e 611.3 (M + 1)⁺.

HR-FABMS (glycerol), m/e calcd for C₃₀H₄₃N₈O₄S: 611.3124. Found: 611.3120.
Phe-(2S,3S-cyclo-Met)-Arg-Phe-NH₂
Phe-{2R,3R-cyclo-Met}-Arg-Phe-NH$_2$

The peptidomimetic was prepared by using the same procedures as F-{2S,3S-cyclo-M}-RF-NH$_2$ except that 0.17 g of Rink amide resin (0.47 mol g$^{-1}$ capacity) was used. The desired peptide (TFA salt) was obtained as a colorless powder (48 mg, 73%) with a retention time of 26.45 min.

Amino acid analysis (normalized for Phe), Phe 2.00, Arg 1.15.

FABMS (glycerol), $m/e$ 611.3 (M + 1)$^+$. 

HR-FABMS (glycerol), $m/e$ calcd for C$_{30}$H$_{43}$N$_8$O$_4$S: 611.3124. Found: 611.3124.
Phe-{2R,3R-cyclo-Met}-Arg-Phe-NH$_2$
Phe-Met-Arg-Phe-NH₂

The peptidomimetic was prepared by using the same procedures as F-(2S,3S-cyclo-M)-RF-NH₂ except that 0.43 g of Rink amide resin (0.47 mol g⁻¹ capacity), and 2 equiv of amino acids were used. The desired peptide (TFA salt) was obtained as a colorless powder (147 mg, 89 %) with a retention time of 23.39 min.

Amino acid analysis (normalized for Phe), Phe 2.00, Arg 1.11, Met 0.88

FABMS (glycerol), m/z 599.3 (M + 1)⁺

HR-FABMS (glycerol), m/z calcd for C₂₉H₄₃N₈O₄S: 599.3128. Found: 599.3122
Phe-Met-Arg-Phe-NH₂
Leu-\{\pm-Z\text{-cyclo}-Phe\}-NH_2

![Chemical Structure Image]

The peptidomimetic was prepared by using the same procedures as F-\{2S,3S-cyclo-M\}-RF-NH_2 except the following modifications. Rink amide resin (0.113 g of 0.47 mol g\(^{-1}\) capacity), one coupling cycle of Fmoc-cyclo-Phe\(^2\) (1.2 equiv, 4 hr) followed by end-capping, and two coupling cycles of Fmoc-Leu\(^2\) (3 equiv, 1 hr) were used. The crude peptide was purified by preparative HPLC with the same gradient as F-\{2S,3S-cyclo-Met\}-RF-NH_2. Three peaks were collected with retention times of 16.63, 25.18 (fast) and 25.92 (slow) min. The 16.63 min peak isolated as (\pm\)-Z-cyclo-Phe-NH\(_2\) (5.9mg, 39 %), and the NMR sample of this compound decomposed upon standing overnight at 25 °C. The fast and slow peaks isolated as one of the two diastereoisomers of Leu-\{\pm-Z\text{-cyclo}-Phe\}-NH\(_2\) [Fast (2.5 mg, 12 %) and Slow (4.7 mg, 22 %)].

(\pm\)-Z-cyclo-Phe-NH\(_2\):

\(^1\)H NMR (300 MHz, D\(_2\)O, ref H\(_2\)O= 4.80 ppm)

\(\delta\) 7.46 (m, 5H), 3.28 (t, J=9.04 Hz, 1H), 2.08 (m, 2H)
Leu-(±-Z-cyclo-Phe)-NH$_2$, Fast:

$^1$H NMR (300 MHz, D$_2$O, ref H$_2$O = 4.80 ppm)

$\delta$ 7.38 (m, 2H), 7.28 (m, 2H), 3.76 (q, J=4.55, 10.50 Hz, 1H), 3.23 (t, J=8.89 Hz, 1H), 1.90 (q, J=6.32, 7.89 Hz, 1H), 1.77 (q, J=6.24, 9.68 Hz, 1H), 1.19 (m, 1H), 0.85-0.64 (m, 8H)

FABMS (glycerol), $m/e$ 290 (M + 1)$^+$

Leu-(±-Z-cyclo-Phe)-NH$_2$, Slow:

$^1$H NMR (300 MHz, 300 MHz, D$_2$O, ref H$_2$O = 4.80 ppm)

$\delta$ 7.44 (m, 2H), 7.32 (m, 2H), 3.77 (q, J=5.91, 8.93 Hz, 1H), 3.13 (t, J=8.71 Hz, 1H), 2.09 (q, J=6.32, 9.75 Hz, 1H), 1.86 (q, J=6.37, 8.24 Hz, 1H), 1.49 (m, 1H), 1.26 (m, 2H), 0.78-0.72 (2 overlapping d, 6H)

FABMS (glycerol), $m/e$ 290.3 (M + 1)$^+$
(±)-Z-cyclo-Phe-NH₂
Leu-\(\pm\)-cyclo-Phe)-NH\(_2\), Fast
Leu-\{\pm-Z\cdot\text{cyclo-Phe}\}-\text{NH}_2, \text{Slow}
Phe-{2S,3S-cyclo-Met}-Arg-{2R,3R-cyclo-Phe}-NH₂

The peptidomimetic was prepared by using the same procedures as F-{2S,3S-cyclo-M}-RF-NH₂, except the following modifications. Rink amide resin (0.098 g of 0.47 mol g⁻¹ capacity), one coupling cycle of cyclo-Phe⁴ (1.1 equiv) and cyclo-Met² (1.1 equiv), followed by end-capping, six coupling cycles of Fmoc-Arg³ (3 equiv) and two coupling cycles of Fmoc-Phe¹ (3 equiv) were used to drive a ninhydrin negative test. The crude peptide was purified by preparative HPLC. The gradient was programmed to increase from 5 to 60 % B over 40 min with a flow rate of 6 mL min⁻¹. The peak with a retention time of 29.04 min was collected. The desired peptide (TFA salt) was obtained as a colorless powder (17 mg, 43 %).

Amino acid analysis (normalized for Phe), Phe 1.00, Arg 1.06

FABMS (glycerol), m/e 623.4 (M + 1)⁺

HR-FABMS (glycerol), m/e calcd for C₃₁H₄₃N₇O₄S: 623.3128. Found 623.3125
Phe-(2S,3S-cyclo-Met)-Arg-(2R,3R-cyclo-Phe)-NH₂
General procedure for leucine aminopeptidase digestion

Sample (0.2 µmol) of FMRF-NH₂, F-{2S,3S-cyclo-M}-RF-NH₂, or F-{2R,3R-cyclo-M}-RF-NH₂ were incubated at 25 °C in 185 µL of phosphate buffer (pH = 8) with 0.05 mg leucine aminopeptidase (Sigma, St. Louis, MO). Anthranilic acid (0.16 µmol) was included as an internal standard. The amount of intact peptide at different time intervals was quantitated by RF-HPLC (Dynamax C18 column, 4.6 mm x 25 cm, 5 µm) with a linear gradient obtained by mixing solvent A (0.05 % TFA in water) and solvent B (0.05 % TFA in acetonitrile). The gradient was programmed to increase from 5 to 60 % B over 30 min with a flow rate of 1 mL min⁻¹. The detector was set at 215 nm. Relative peaks area were deduced through comparison with the internal standard. An exponential fit was calculated from the data points, and the half-life was calculated. The survival half-lives for FMRF-NH₂ is 0.38 h, F-{2S,3S-cyclo-M}-RF-NH₂ is 16 h, and F-{2R,3R-cyclo-M}-RF-NH₂ is 73 h.
Appendix 3. Experimental for Chapter 4

NMR Methods

The NMR spectra were recorded on a Varian XL-400 spectrometer (400 MHz). The peptide (10 mM) was dissolved in DMSO-$d_6$ (d, 99.9%) obtained from Cambridge Isotope Laboratories, and was distilled from CaSO$_4$ before use. DMSO-$d_6$ ($\delta = 2.49$ ppm) was used as an internal reference. One dimensional (1D) $^1$H-NMR spectra were recorded with spectral width of 5299.4 Hz, 52992 data points, 128 transients, and 5 s acquisition time. Vicinal coupling constants were measured from the 1D spectra at ambient temperature. Temperature coefficients of amide protons were measured by variable temperature 1D experiments of temperature ranging from 25 to 65 °C, and 10 °C increments with equilibration time of $\geq 8$ min after successive temperature change.

Two dimensional (2D) experiments were taken at ambient temperature with a spectral width of 5299.4 Hz. Through-bond connectivities were elucidated by DQF-COSY spectra, which were recorded with 3 s relaxation delay, 512 $t_1$ increments and 16 scans per $t_1$ increments with 2 K data points at $t_2$.

Sequential assignments and proton-proton close contacts were elucidated by ROESY spectra (absorption mode), which were recorded with a 2 s relaxation delay, 512 $t_1$ increments, and 32 scans per $t_1$ increments with 2 K data points at $t_2$. The spin-lock field was generated by a train of 30° pulses with a resulting spin-lock field strength of 2.0 kHz. The carrier frequency was varied to eliminate COSY, HOHAHA and "false" NOE artifacts. Generally, three carrier frequencies were chosen (ca 4.03, 5.54, and 6.29 ppm). Both DQF-COSY and ROESY data were zero-filled to 2 K x 2 K data sets, Gaussian transformed in both dimensions and symmetrized.

ROESY experiments with mixing time ranging from 75 to 450 ms were recorded to identify peaks caused by spin-diffusion.\textsuperscript{117} Build-up profile (ie plotting the ROE
intensities vs mixing times) for each crosspeak cannot be obtained due to inaccuracy in measuring the peak volume by the "vnmrsys" software. In general, ROE crosspeaks that were observed at both low (ca 100 ms) and high (ca 300 ms) mixing time were identified, and the intensities of these crosspeaks were assigned as VS (very strong, > 4 contours), S (medium, 4 contours), M (medium, 3 contours), W (weak, 2 contours), and VW (very weak, 1 contour) by counting number of contours (at 300 ms).

Chemical shifts of the amide and α protons for the peptidomimetics were monitored in concentration range from 2.0 mM to 10.0 mM. The chemical shifts in these concentrations remained relatively constant (Δδ ≤ 0.04 ppm), which indicates no significant aggregation occurred in the 10 mM solutions.

For F-(2S,3S-cyclo-M)-R-(2R,3R-cyclo-F)-NH₂, a 6 mM solution was used. The ROESY spectra were obtained by 64 scans per t₁ increments, and the rest of the acquisition and processing parameters remained the same. Dilution experiments were performed in concentration range from 2 mM to 6 mM.
$^1$H-NMR spectrum of Phe-Met-Arg-Phe-NH$_2$
Temperature coefficients of amide protons in Phe-Met-Arg-Phe-NH$_2$
DQF-COSY spectrum of Phe-Met-Arg-Phe-NH₂
ROESY spectrum (carrier frequency = 5.54 ppm, mixing time = 300 ms) of Phe-Met-Arg-Phe-NH₂
$^1$H-NMR spectrum of Phe-(2S,3S-cyclo-Met)-Arg-Phe-NH$_2$
Plot of chemical shifts of NH and Hα protons vs concentration for Phe-{2S,3S-cyclo-Met}·Arg·Phe·NH₂
Temperature coefficients of amide protons in Phe-\{2S,3S-cyclo-Met\}-Arg-Phe-NH₂

\begin{center}
\begin{tikzpicture}
\begin{axis}[
view={0}{90},
axis x line=middle,axis y line=middle,
xlabel={Temperature / K},
ylabel={Chemical Shifts / ppm},
xtick={290,300,310,320,330,340},
ytick={7.0,7.5,8.0,8.5,9.0,9.5},
]
\addplot[black,mark=square] coordinates {
(290,9.2) (300,9.0) (310,8.8) (320,8.6) (330,8.4) (340,8.2)
};
\addlegendentry{2S,3S-cyclo-Met²NH (-3.90 ppb K^{-1})}
\addplot[black,mark=triangle] coordinates {
(290,8.0) (300,7.8) (310,7.6) (320,7.4) (330,7.2) (340,7.0)
};
\addlegendentry{Phe⁴NH (-5.47 ppb K^{-1})}
\addplot[black,mark=diamond] coordinates {
(290,7.5) (300,7.3) (310,7.1) (320,6.9) (330,6.7) (340,6.5)
};
\addlegendentry{Arg³NH (-2.20 ppb K^{-1})}
\end{axis}
\end{tikzpicture}
\end{center}
DQF-COSY spectrum of Phe-(2S,3S-cyclo-Met)-Arg-Phe-NH₂
ROESY spectrum (carrier frequency = 5.54 ppm, mixing time = 300 ms) of Phe-{2S,3S-cyclo-Met}-Arg-Phe-NH₂
$^1$H-NMR spectrum of Phe\-(2R,3R-cyclo-Met)-Arg-Phe-NH$_2$
Plot of chemical shifts of NH and Hα protons vs concentration for Phe-{2R,3R-cyclo-Met}-Arg-Phe-NH₂
Temperature coefficients of amide protons in Phe-(2R,3R-cyclo-Met)-Arg-Phe-NH$_2$

\begin{align*}
\text{Chemical Shift / ppm} & \quad \text{Temperature / K} \\
\hline
2R,3R-cyclo-Met^2\text{NH} & \quad 290 \quad 300 \quad 310 \quad 320 \quad 330 \quad 340 \\
\text{Phe}^4\text{NH} & \quad 8.0 \\
\text{Arg}^3\text{NH} & \quad 7.5
\end{align*}

$2R,3R$-cyclo-Met$^2$NH
(-2.18 ppb K$^{-1}$)

Phe$^4$NH (-5.09 ppb K$^{-1}$)

Arg$^3$NH (-2.80 ppb K$^{-1}$)
DQF-COSY spectrum of Phe-(2R,3R-cyclo-Met)-Arg-Phe-NH₂
ROESY spectrum (carrier frequency = 5.54 ppm, mixing time = 300 ms) of Phe-{2R,3R-cyclo-Met}-Arg-Phe-NH₂
$^1$H-NMR spectrum of Phe-$\{2S,3S\text{-cyclo-Met}\}$-Arg-$\{2R,3R\text{-cyclo-Phe}\}$-$\text{NH}_2$
Plot of chemical shifts of NH and Hα protons vs concentration for Phe-\{2S,3S-cyclo-Met\}-Arg-\{2R,3R-cyclo-Phe\}-NH₂
Temperature coefficients of amide protons in 

Phe-{2S,3S-cyclo-Met}-Arg-{2R,3R-cyclo-Phe}-NH$_2$

![Graph showing temperature coefficients for different protons]
DQF-COSY spectrum of

Phe-{2S,3S-cyclo-Met}-Arg-{2R,3R-cyclo-Phe}-NH$_2$
ROESY spectrum (carrier frequency = 5.54 ppm, mixing time = 300 ms) of Phe-(2S,3S-cyclo-Met)-Arg-(2R,3R-cyclo-Phe)-NH₂
Appendix 4. Experimental for Chapter 5

This section provides comprehensive lists of topology and parameter files for 2,3-methanoamino acids in CHARMM text format. The input files for $\phi$, $\psi$ grid search and quenched molecular dynamics are also included.

**CHARMM topology files for 2,3-methanoamino acids**

* TOPOLOGY FILE FOR 2,3-METHANOAMINO ACIDS
*
MASS 17 CAA 12.01100
MASS 18 CA2E 14.02700
MASS 19 CB2E 4.02700
MASS 20 CB1E 13.01900
MASS 21 CA1E 13.01900

RESI ACC 0.00000 !1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID
GROU
ATOM N NH1 -0.35
ATOM H H 0.25
ATOM CA CAA 0.10
GROU
ATOM CB1 CA2E 0.00
GROU
ATOM CB2 CB2E 0.00
GROU
ATOM C C 0.55
ATOM O O -0.55
BOND N CA CA C C +N C O N H
BOND CA CB1 CB1 CB2 CB2 CA
DIHE -C N CA C N CA C +N CA C +N +CA
IMPH N -C CA H C CA +N O CA N C CB1
IMPH CA N C CB2
DONO H N
ACCE O C
IC -C CA *N H 0.0000 0.00 180.00 0.00 0.0000
IC -C N CA C 0.0000 0.00 180.00 0.00 0.0000
IC N CA C +N 0.0000 0.00 180.00 0.00 0.0000
IC +N CA *C O 0.0000 0.00 180.00 0.00 0.0000
IC CA C +N +CA 0.0000 0.00 180.00 0.00 0.0000
IC N C *CA CB1 0.0000 0.00 120.00 0.00 0.0000
IC N C *CA CB2 0.0000 0.00 -120.00 0.00 0.0000
RESI CYF3  0.00000 ! 2R,3R-cyclo-Phe

GROU
ATOM N  NH1  -0.35
ATOM H  H    0.25
ATOM CA  CAA  0.10
GROU
ATOM CB1  CA2E  0.00
GROU
ATOM CB2  CB1E  0.00
ATOM CG  C    0.00  CZ
ATOM CD1  CR1E  0.00  CE2
ATOM CD2  CR1E  0.00  CE1
GROU
ATOM CE1  CR1E  0.00
ATOM CE2  CR1E  0.00
ATOM CZ  CR1E  0.00
GROU
ATOM C  C    0.55
ATOM O  O    -0.55
BOND N  CA  CA  C  C  +N  C  O  N  H
BOND CA  CB1  CB1  CB2  CB2  CA  CB2  CG  CG  CD1
BOND CG  CD2  CD1  CE1  CD2  CE2  CE1  CZ  CE2  CZ
DIHE  -C  N  CA  C  N  CA  C  +N  CA  C  +N  +CA
DIHE  N  CA  CB2  CG  CA  CB2  CG  CD1
IMPH  N  -C  CA  H  C  CA  +N  O  CA  N  C  CB1
IMPH  CA  N  C  CB2  CB2  CA  CB1  CG
IMPH  CG  CD1  CE1  CZ  CD1  CE1  CZ  CE2  CE1  CZ  CE2  CD2
IMPH  CZ  CE2  CD2  CG  CE2  CD2  CG  CD1  CD2  CG  CD1  CE1
IMPH  CG  CD1  CD2  CB2
DONO  H  N
ACCE  O  C
IC  -C  CA  *N  H  0.0000  0.00  180.00  0.00  0.0000
IC  -C  N  CA  C  0.0000  0.00  180.00  0.00  0.0000
IC  N  CA  C  +N  0.0000  0.00  180.00  0.00  0.0000
IC  +N  CA  *C  O  0.0000  0.00  180.00  0.00  0.0000
IC  CA  C  +N  +CA  0.0000  0.00  180.00  0.00  0.0000
IC  N  C  *CA  CB1  0.0000  0.00  120.00  0.00  0.0000
IC  N  C  *CA  CB2  0.0000  0.00  120.00  0.00  0.0000
IC  CA  CB1  *CB2  CG  0.0000  0.00  120.00  0.00  0.0000
IC  N  CA  CB2  CG  0.0000  0.00  180.00  0.00  0.0000
IC  CA  CB2  CG  CD1  0.0000  0.00  90.00  0.00  0.0000
IC  CD1  CB2  *CG  CD2  0.0000  120.0  180.00  120.0  0.0000
IC  CD1  CG  CD2  CE2  0.0000  120.0  0.00  120.0  0.0000
IC  CD2  CG  CD1  CE1  0.0000  120.0  0.00  120.0  0.0000
IC  CG  CD1  CE1  CZ  0.0000  120.0  0.00  120.0  0.0000
RESI CYM4  0.00000  2S,3S-cyclo-Met
GROU
ATOM N NH1  -0.35
ATOM H H     0.25
ATOM CA CAA  0.10
GROU
ATOM CB1 CB2E 0.00
GROU
ATOM CB2 CA1E 0.00
GROU
ATOM CG CH2E  0.06
ATOM SD S     -0.12
ATOM CE CH3E  0.06
GROU
ATOM C C     0.55
ATOM O O     -0.55
BOND N CA CA C C +N C O N H
BOND CA CB1 CB1 CB2 CA CB2
BOND CB2 CG CG SD SD CE
DIHE -C N CA C N CA C +N CA C +N +CA
DIHE N CA CB2 CG CA CB2 CG SD CB2 CG SD CE
IMPH N -C CA H C CA +N O CA N C CB1
IMPH CA N C CB2 CB2 CA CB1 CG
DONO H N
ACCE O C
IC -C CA *N H  0.0000  0.00  180.00  0.00  0.0000
IC -C N CA C  0.0000  0.00  180.00  0.00  0.0000
IC N CA C +N  0.0000  0.00  180.00  0.00  0.0000
IC +N CA *C O  0.0000  0.00  180.00  0.00  0.0000
IC CA C +N +CA 0.0000  0.00  180.00  0.00  0.0000
IC N C *CA CB1 0.0000  0.00  120.00  0.00  0.0000
IC N C *CA CB2 0.0000  0.00  120.00  0.00  0.0000
IC CA CB1 *CB2 CG 0.0000  0.00  120.00  0.00  0.0000
IC N CA CB2 CG 0.0000  0.00  180.00  0.00  0.0000
IC CA CB2 CG SD 0.0000  0.00  180.00  0.00  0.0000
IC CB2 CG SD CE 0.0000  0.00  180.00  0.00  0.0000
RESI  CYM3  0.00000  ! 2R,3R-cyclo-Met
GROU
ATOM  N  NH1  -0.35
ATOM  H  H   0.25
ATOM  CA  CAA  0.10
GROU
ATOM  CB1  CB2E  0.00
GROU
ATOM  CB2  CA1E  0.00
GROU
ATOM  CG  CH2E  0.06
ATOM  SD  S   -0.12
ATOM  CE  CH3E  0.06
GROU
ATOM  C  C    0.55
ATOM  O  O   -0.55
BOND  N  CA  CA  C  C  +N  C  O  N  H
BOND  CA  CB1  CB1  CB2  CA  CB2
BOND  CB2  CG  CG  SD  SD  CE
DIHE  -C  N  CA  C  N  CA  C  +N  CA  C  +N  +CA
DIHE  N  CA  CB2  CG  CA  CB2  CG  SD  CB2  CG  SD  CE
IMPH  -C  CA  H  C  CA  +N  O  CA  N  C  CB1
IMPH  CA  N  C  CB2  CB2  CA  CB1  CG
DONO  H  N
ACCE  O  C
IC  -C  CA  *N  H  0.0000  0.00  180.00  0.00  0.0000
IC  -C  N  CA  C  0.0000  0.00  180.00  0.00  0.0000
IC  N  CA  C  +N  0.0000  0.00  180.00  0.00  0.0000
IC  +N  CA  *C  O  0.0000  0.00  180.00  0.00  0.0000
IC  CA  C  +N  +CA  0.0000  0.00  180.00  0.00  0.0000
IC  N  C  *CA  CB1  0.0000  0.00  120.00  0.00  0.0000
IC  N  C  *CA  CB2  0.0000  0.00  -120.00  0.00  0.0000
IC  CA  CB1  *CB2  CG  0.0000  0.00  120.00  0.00  0.0000
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IC  CA  CB2  CG  SD  0.0000  0.00  180.00  0.00  0.0000
IC  CB2  CG  SD  CE  0.0000  0.00  180.00  0.00  0.0000
2,3-Methanoamino acids parameters appended to CHARMM parameters file

(version 19)

*PARAMETER FOR 2,3-METHANOAMINO ACIDS
*THIS PARAMETERS DID NOT ACCOMMODATE THE π-EFFECT BETWEEN THE
*CYCLOPROPYL AND CARBONYL

BOND
CAA NH1 422.0 1.427
CAA NH2 422.0 1.45
C CAA 405.0 1.492
CAA CA2E 225.0 1.50
CA2E CB2E 225.0 1.50
CAA CB2E 225.0 1.50
CAA CB1E 225.0 1.50
CA2E CB1E 225.0 1.50
CB1E C 405.0 1.52
CB2E CA1E 225.0 1.50
CAA CA1E 225.0 1.50
CA1E CH2E 225.0 1.52

THETAS
C NH1 CAA 80.0 20.4
CAA NH1 H 35.0 120.0
CA2E CAA NH1 65.0 118.5
CB2E CAA NH1 65.0 118.5
C CAA NH1 70.0 117.0
CAA CA2E CB2E 45.0 60.0
CAA CB2E CA2E 45.0 60.0
CA2E CAA CB2E 45.0 60.0
C CAA CA2E 70.0 18.5
C CAA CB2E 70.0 118.5
CAA C NH1 20.0 117.5
CAA C O 85.0 121.5
CB1E CAA NH1 65.0 118.5
C CAA CB1E 70.0 118.5
CA2E CAA CB1E 45.0 60.0
CAA CA2E CB1E 45.0 60.0
CAA CB1E CA2E 45.0 60.0
CB1E C CR1E 70.0 121.5
C CB1E CAA 70.0 120.4
C CB1E CA2E 70.0 119.9
CAA CA1E CB2E 45.0 60.0
CAA CB2E CA1E 45.0 60.0
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**PHI**

**CYCLOPROPANE DIHEDRAL PARAMETERS**

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Input file for $\phi$, $\psi$ grid searches

*PHI PSI MAP
BOMB -1

!OPEN AND READ IN ACC TOPOLOGY
OPEN READ UNIT 12 CARD NAME D1:[HO.EXPT7]CY.RTF
READ RTF CARD UNIT 12
CLOSE UNIT 12

!READ THE SEQUENCE OF AMN-ACC-CBX
READ SEQUENCE CARD
*AMN-ACC-CBX SEQUENCE
*
3
AMN ACC CBX
GENERATE ACC1 SETUP

!OPEN AND READ PARAMETER FILE
OPEN READ UNIT 21 CARD NAME D1:[HO.EXPT7]CYPARAM.INP
READ PARAM CARD UNIT 21
CLOSE UNIT 21

!FILL PARAMETERS TO TABLE
IC PARAMETERS

!SEED THE THREE ATOMS
IC SEED 2 N 2 CA 2 C

!BUILD CARTESIAN COORDINATES FROM PARAMETERS
IC BUILD

!PRINT OUT CARTESIAN COORDINATE TO LOG FILE
PRINT COORDINATES

!OPEN UNIT TO WRITE FIRST MINIMIZED STRUCTURE TO PDB FILE
OPEN UNIT 20 WRITE FORM NAME D1:[HO.EXPT7]ACC.PDB

!DEFINE NBONDS LIST AND DIELECTRIC CONSTANT
NBONDS CDIE EPS 1.0 CUTNB 99.0 SWIT VSWIT

!FIRST MINIMIZATION TO ELIMINATE UNFAVOURABLE INTERACTIONS
MINI ABNR STEP 0.01 NSTEP 1000 NPRINT 100 INBFRQ 100 IHBFRQ 100 -
STRICT 0.05

!WRITE DATA TO PDB FILE
WRITE COOR PDB UNIT 20
*AMN-ACC-CBX
*
CLOSE UNIT 20
!BEGIN THE LOOP TO CALCULATE THE PHI-PSI PLOT IN 20 DEGREE STEP
!PARAMETER 1 IS USED TO STORE PHI
!PARAMETER 2 IS USED TO STORE PSI
!WRITE ENERGY TO .ENE FILE
OPEN WRITE UNIT 30 CARD NAME D1:[HO.EXPT7]ACC.ENE
SET 1 -180.0
SET 2 180.0

LABEL LOOP

CONS DIHE ACC1 1 C ACC1 2 N ACC1 2 CA ACC1 2 C FORCE 200.0 MIN @1
CONS DIHE ACC1 2 N ACC1 2 CA ACC1 2 C ACC1 3 N FORCE 200.0 MIN @2

MINI ABNR STEP 0.01 NSTEP 500 NPRINT 100 INBFRQ 0 IHBFRQ 100 STRICT-0.05
CONS CLDH

WRITE TITLE UNIT 30
* @1 @2 ?ENER ?ELEC ?ANGL ?DIHE ?BOND ?VDW
*

INCR 2 BY -20.
SET 2 @2.0
IF 2 GE -180. GOTO LOOP
SET 2 180.
INCR 1 BY 20.
SET 1 @1.0
IF 1 LE 180. GOTO LOOP

CLOSE UNIT 30

STOP
Input file for quenched molecular dynamics

RCH
*FMRF MM, PDB AND QMD
*
BOMB -1

!OPEN & READ TOPOLOGY FILE
!TOPOLOGY FILE WITH EXTENDED ATOM WAS USED
OPEN READ UNIT 02 CARD NAME D1:[HO]CYTOPH19.INP
READ RTF CARD UNIT 02
CLOSE UNIT 02

!READ SEQUENCE AND GENERATE SEGMENT FMRF
READ SEQUENCE CARD
*PHE MET ARG PHE CAMN
*
5
PHE MET ARG PHE CAMN
GENERATE FF SETUP

!OPEN & READ PARAMETER FILE
OPEN READ UNIT 10 CARD NAME D1:[HO.EXPT7]CYPARAM.INP
READ PARAMETER CARD UNIT 10
CLOSE UNIT 10

!FIND POSITION OF ALL ATOMS
IC PARAM
IC SEED 1 N 1 CA 1 C
IC BUILD
IC FILL
IC PURGE
PRINT IC
PRINT COOR

!MINIMIZE STRUCTURE TO ELIMINATE UNFAVOURABLE INTERACTION
NBONDS CDIE EPS 45.0 CUTNB 99.0 SWIT VSWIT
MINI ABNR STEP 0.01 NSTEP 1000 NPRINT 100 INBFREQ 100 IHBFREQ 100 -
STRICT 0.05

OPEN WRITE UNIT 55 FORM NAME D1:[HO.EXPT10]FFMM.PDB
WRITE COOR PDB UNIT 55
*PDB OF FMRF AFTER MM MINIMIZATION
*
CLOSE UNIT 55

!SHAKE ALL BONDS WITH HYDROGENS
SHAKE BONH
!!INITIALIZATION
!OPEN FILES FOR RST=RESTART STRUCTURES; DCD=STRUCTURES;
!DVL=VELOCITY & ENE=ENERGY
OPEN WRIT UNIT 31 CARD NAME D2:[HO.EXPT10]FFINT.RST
OPEN WRIT UNIT 32 FILE NAME D2:[HO.EXPT10]FFINT.DCD
OPEN WRIT UNIT 33 FILE NAME D2:[HO.EXPT10]FFINT.DVL
OPEN WRIT UNIT 34 CARD NAME D2:[HO.EXPT10]FFINT.ENE

DYNA STRT VERL NSTE 10000 TIME 0.001 CDIE EPS 45.0 -
   IPRFRQ 100 IHTFRQ 100 IEQFRQ 0 INBFREQ 50 IBHFRQ 0 -
   IUNREA -1 IUNWRI 31 IUNCRD 32 IUNVEL 33 KUNIT 34 -
   NPRINT 1000 NSAVC 1000 NSAVV 1000 -
   FIRSTT 0.0 FINALT 1000.0 TEMINC 10.0 -
   TWINDH 10.0 TWINDL -10.0 -
   IASORS 1 IASVEL 1 ICHECW 0

OPEN WRIT UNIT 41 CARD NAME D2:[HO.EXPT10]FFINT.CRD
WRIT COOR UNIT 41 CARD
*CORRDOINATES AFTER INITIALIZATION
*
CLOS UNIT 31
CLOS UNIT 34

!EQUILIBRATION
OPEN READ UNIT 30 CARD NAME D2:[HO.EXPT10]FFINT.RST
OPEN WRIT UNIT 31 CARD NAME D2:[HO.EXPT10]FFEQ.RST
OPEN WRIT UNIT 32 FILE NAME D2:[HO.EXPT10]FFEQ.DCD
OPEN WRIT UNIT 33 FILE NAME D2:[HO.EXPT10]FFEQ.DVL
OPEN WRIT UNIT 34 CARD NAME D2:[HO.EXPT10]FFEQ.ENE

DYNA REST VERL NSTE 10000 TIME 0.001 CDIE EPS 45.0 -
   IPRFRQ 100 IHTFRQ 100 IEQFRQ 0 INBFREQ 50 IBHFRQ 0 -
   IUNREA 30 IUNWRI 31 IUNCRD 32 IUNVEL 33 KUNIT 34 -
   NPRINT 1000 NSAVC 1000 NSAVV 1000 -
   FIRSTT 0.0 FINALT 1000.0 -
   TWINDH 13.0 TWINDL -13.0 -
   IASORS 0 ISCVEL 0 ICHECW 1

OPEN WRIT UNIT 41 CARD NAME D2:[HO.EXPT10]FFEQ.CRD
WRIT COOR UNIT 41 CARD
* COORDINATE AFTER EQUILIBRATION
*
CLOS UNIT 30
CLOS UNIT 31
CLOS UNIT 34

!!DYNAMIC RUN
OPEN READ UNIT 30 CARD NAME D2:[HO.EXPT10]FFEQ.RST
OPEN WRIT UNIT 31 CARD NAME D2:[HO.EXPT10]FFDY.RST
OPEN WRIT UNIT 32 FILE NAME D2:[HO.EXPT10]FFDY.DCD
OPEN WRIT UNIT 33 FILE NAME D2:[HO.EXPT10]FFDY.DVL
OPEN WRIT UNIT 34 CARD NAME D2:[HO.EXPT10]FFDY.ENE

DYNA REST VERL NSTE 600000 TIME 0.001 CDIE EPS 45.0 -
IPRFREQ 100 IHTFRQ 0 IEQFRQ 0 INBFREQ 50 IHBFREQ 0 -
IUNREA 30 IUNWRI 31 IUNCRD 32 IUNVEL 33 KUNIT 34 -
NPRINT 10000 NSAVC 1000 NSAVV 10000 -
FIRSTT 0.0 FINALT 1000.0 -
TWINDH 13.0 TWINDL -13.0 -
ICHECW 0

OPEN WRIT UNIT 41 CARD NAME D2:[HO.EXPT10]FFDY.CRD
WRIT COOR UNIT 41 CARD
* COORDINATES AFTER DYNAMICS RUN
*
CLOS UNIT 30
CLOS UNIT 31
CLOS UNIT 34

END
References


49. Alcaraz, C.; Herrero, A.; Marco, J. L.; Fernandez-Alvarez, E.; Bernabe, M.


Leyva, J. E.; Prasco, P. E.; Dougherty, T. M. Peptides 1990, 11, 277.

85. Rothman, R. B.; Xu, H.; Yang, H.-Y. T.; Long, J. B. in "Neurobiology of
opiates" Anti-opioid peptides in morphine tolerance and dependence: focus on NPFF;


Arcangeli, K. R.; Ludgate, K.; Moore, G. M.; Payza, K. Peptides 1991, 12,
1011.


90. Wang, L.; Coy, D. H.; Taylor, J. E.; Jiang, N.; Kim, S. H.; Moreau, J.; Huang,


88.


96. Atherton, E.; Sheppard, R. C. in Solid Phase Peptide Synthesis, a Practical

97. Wenschuh, H.; Beyermann, M.; Krause, E.; Carpino, L. A.; Bienert, M.
111. Huang, Z.; He, Y.-B.; Raynor, K.; Tallent, M.; Reisine, T.; Goodman, M. J. 

Protein Res.* **1993**, *41*, 347.

113. Pettitt, B. M.; Matsunaga, T.; Al-Obeidi, F.; Gehrig, C.; Hruby, V. J.; Karplus, 

*35*, 2870.


*103*, 3654.


129. Rose, G. D.; Gierasch, L. M.; Smith, J. A. in "Advances in Protein Chemistry" 

130. Cantor, C. R.; Schimmel, P. R. in "Biophysical Chemistry Part I: The 
Conformation of Biological Macromolecules"; Ed.; W. H. Freeman and Company: 


134. Barone, V.; Fraternali, F.; Cristinziano, P. L.; Lelj, F.; Rosa, A. Biopolymers 
1988, 27, 1673.


136. McCammon, J. A.; Harvey, S. C. in Dynamics of Proteins and Nucleic Acids; 

137. Richardson, J. S. in "Advances in Protein Chemistry"; C. B. Anfinsen, J. T. Edsall, 
