Development and Application of NMR-methods for Structural Investigations of Small Molecules and Proteins

Dissertation

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D7

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Tag der mündlichen Prüfung:

"Yo what the schnitzl..."

Adrock

Zusammenfassung

Die Aufklärung der relativen Stereochemie von organischen Kleinmolekülen ist eine große Herausforderung in der Chemie, da gleichzeitig die Konformation sowie Konfiguration bestimmt werden müssen. Während konventionelle NMR-parameter wie NOEs und ^{3}J-Kopplungen, die Informationen über Distanzen und Dihedralwinkel beinhalten, die Konfiguration von Stereozentren starrer Moleküle liefern, kann diese Vorgehensweise schwierig oder unmöglich werden bei flexiblen Molekülen oder Molekülen die Brüche in dem erforderlichen Protonennetzwerk aufweisen. Residuale dipolare Kopplugen (RDCs) haben bewiesen, dass sie Stereozentren sehr erfolgreich bestimmen können und dass dies auch für flexible Moleküle zutreffen kann. In den letzten Jahren wurden eine Reihe von neuen Orientierungsmedien für organische Lösungsmittel entwickelt, zu denen auch das PH-gel und PPH-gel zählen, die in dieser Arbeit vorgestellt werden. Diese Entwicklung erlaubte es, RDCs als wichtigen strukturbestimmeneden Parameter für Kleinmoleküle zu etablieren.

In der vorliegenden Arbeit werden konformationelle und konfigurationelle Studien einer Reihe von Kleinmolekülen und Naturstoffen präsentiert. Für das zyklische Depsipeptid Hormaomycin konnten RDCs konformationelle Unklarheiten aufklären. Weiters wurde die Konformation des zyklischen Octapeptides Hymenistatin in DMSO, $CDCl_3$ und THF bestimmt unter Einbeziehung von NOEs, J-Kopplungen und RDCs. Zusätzlich wurde die konfigurationelle Zuordnung der prochiralen Stereozentren dieses Moleküls mittels RDCs untersucht. Letztlich wurde noch die relative Konfiguration von ((-)-menthyl)-(5-oxo-5,6-dihydro-2H-pyran-2-yl)-diphtalate (DiaA) bestimmt. Dieses Molekül konnte weder kristallisiert werden, noch führten die koventionellen NMR-parameter zu einer Lösung.

Zwei neue paramagnetische "tags" wurden in unserem Arbeitskreis entwickelt und fanden Anwendung bei dem diamagnetischen Protein trigger factor. Mit Hilfe der zusätzlichen Orientierungstensoren konnte die Struktur genauer bestimmt werden. Zusätzlich wurde eine neue HSQC-Pulssequenz entwickelt, welche ein ^{13}C-^{13}C-stopfilter Element enthält. Dadurch wurde es möglich, Pseudokontakt Verschiebungen (PCS) und RDCs des nicht isotopen markierten Liganden suc-AAPF-pNA komplexiert mit dem ^{13}C-markierten Protein zu messen.

Abstract

The elucidation of the relative stereochemistry of asymmetric centers of organic molecules is an important challenge in chemistry since it requires the simultaneous determination of conformation and configuration. While the conventional NMR parameters like NOE and ^{3}J coupling constants, which provide internuclear distances and dihedral angles, yield the configuration of stereocenters in rigid compounds, this approach is difficult or impossible in cases where the molecule is flexible or the stereocenters are remote in the bonding network. Residual dipolar couplings (RDCs) have proven to be very efficient in the stereochemical assignment of moieties and hold the promise of defining the stereochemistry even in non-rigid molecules. Over the past few years a series of new alignment media compatible with organic solvents have been introduced, including the two polyacrlyamide-based DMSO-compatible alignment media (PH-gel and PPH-gel) described in this thesis, which established the use of RDCs within the small molecule community.

The present work provides information about conformational and configurational studies of a series of small molecules and natural products. For the cyclic depsipeptide hormaomycin RDCs could resolve conformational ambiguities in DMSO. The solvent dependent conformation of the cyclic octapeptide hymenistatin for DMSO, $CDCl_3$ and THF could be resolved by the combined use of NOEs, J couplings and RDCs. Furthermore the applicability for the use of RDCs in the configurational assignmnet of prochiral centers of this peptide has been addressed. Finally the relative configuration of the quite flexible ((-)-menthyl)-(5-oxo-5,6-dihydro-2H-pyran-2-yl)-diphtalate (DiaA), which could neither be crystallized nor solved by traditional NMR-parameters could be established with RDCs.

Two new paramagnetic tags have been developed in our group and applied to the diamagnetic protein trigger factor. The additional alignment tensors allowed us to improve the precision of the structure. Additionally a new HSQC experiment using a ^{13}C-^{13}C-stop-filter element has been introduced to measure pseudocontact shifts (PCS) and RDCs of the non-labeled ligand suc-AAPF-pNA in complex with ^{13}C-labeled trigger factor.

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Publication list

This thesis is based on the following papers:

Chapter 2

[1] P. Haberz, J. Farjon, C. Griesinger. A DMSO-compatible orienting medium: towards the investigation of th stereochemistry of natural products. *Angew. Chem. Int. Ed. Engl.*, **44**(3): 427-429, (2005).

[2] U. M. Reinscheid, J. Farjon, M. Radzom, P. Haberz, A. Zeeck, M. Blackledge, C. Griesinger. Effect of the solvent on the conformation of a depsipeptide: NMR-derived solution structure of hormaomycin in DMSO from residual dipolar couplings in a novel DMSO-compatible alignment medium. *Chembiochem*, **7**(2): 287-296, (2006).

Chapter 3

[3] P. Haberz, F. Rodriguez-Castaneda, J. Junker, S. Becker, A. Leonov, C. Griesinger. Two new chiral EDTA-based metal chelates for weak alignment of proteins in solution. *Org. Lett.*, **8**(7): 1275-1278, (2006).

[4] F. Rodriguez-Castaneda, P. Haberz, A. Leonov, C. Griesinger. Paramagnetic tagging of diamagnetic proteins for solution NMR. *Org. Lett.*, **44** Spec No: S10-16, (2006).

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Abbreviations

A

A_λ	absorption at wavelength λ
Ala	alanine (A)
AMPS	2-acrylamido-2-methyl-1-propanesulfonic acid
Ap	ampicillin
APS	ammonium persulphate
APTMAC	(3-acrylamidopropyl)trimethylammonium chloride
Arg	arginine (R)
Asn	asparagine (N)
Asp	aspartic acid (D)

B

B_0	magnetic field strength
BIS	N,N'-methylenbisacrylamide
BMRB	Biological Magnetic Resonance Bank

C

CaM	calmodulin
CD	circular dichroism
COSY	correlation spectroscopy

D

D_a	magnitude of the alignment tensor
DMAA	N,N-dimethylacrylamide

DMF dimethylformamide

DMSO dimethylsulfoxide

DQF double quantum filtered

DTPA diethylentriaminepentaacetic acid

DTT dithiothreitol

δ chemical shift

E

E. coli *Escherichia coli*

EDTA ethylene diamine tetraacetic acid

ESI electrospray ionization

F

FPLC fast protein liquid chromatography

G

Gln glutamine (Q)

Glu glutamic acid (E)

Gly glycine (G)

γ nuclear gyromagnetic ratio

H

h Planck's constant (=6.6260693 x 10^{-34} Js)

$\hbar$ reduced Planck's constant (=1.05457168 x 10^{-34} Js)

His histidine (H)

HMBC heteronuclear multiple bond correlation experiment

HPLC high pressure liquid chromatography

HSQC heteronuclear single-quantum coherence

I

Ile isoleucine (I)

INEPT insensitive nuclei enhancement by polarization transfer

IPAP in-phase / anti-phase

IPTG isopropyl-β-D-thiogalactopyranoside

IR inverted repeat

K

K_d	dissociation constant
kDa	kilo-Dalton (=10^3 g/mol)

L

LB	Luria Bertani
Leu	leucine (L)
Lys	lysine (K)

M

M	mol/l
Met	methionine (M)
MOPS	3-(N-morpholino)propane-sulfonic acid
MS	mass spectrometry
MW	molecular weight
MWCO	molecular weight cutoff

N

Ni-NTA	nickel-nitrilotriacetic acid
NMR	nuclear magnetic resonance
NOE	nuclear Overhauser effect
NOESY	nuclear Overhauser effect spectroscopy

O

OD	optical density

P

PAGE	polyacrylamide gel electrophoresis
PAN	poly(acetonitrile)
PCS	pseudocontact shift
PDMS	poly(dimethylsiloxane)
pdb	Protein Data Bank
PBLG	poly-γ-benzyl-L-glutamate
PELG	poly-γ-ethyl-L-glutamate
PH	Peter Haberz

Phe phenylalanine (F)

PMSF phenylmethylsulphonyl fluoride

PPH positive Peter Haberz

ppm parts per million ($=10^{-6}$)

Pro proline (P)

R

R Pearson's correlation factor

Rh rhombicity of the alignment tensor

RDC residual dipolar coupling

RMSD root mean square deviation

ROE Rotating frame Overhauser effect

ROESY Rotating frame Overhauser effect spectroscopy

S

SDS sodium dodecylsulfate

Ser serine (S)

suc-AAPF-pNA (*N*-

1

Theory

1.1 Residual dipolar couplings

Dipolar couplings of spin-$\frac{1}{2}$ nuclei are the dominant term in solid state NMR, but are averaged to zero for isotropically reorienting molecules in the liquid state. This allows high resolution spectra in liquid state NMR, but a wealth of information is lost, if dipolar couplings vanish. Therefore a series of alignment media (for details see 2.1 and 3.1) have been developed for liquid state NMR applications. They introduce a slight preferential orientation of the molecule in order to reintroduce a 'residual' dipolar coupling, that can be measured relatively easily by comparison of line splittings in the isotropic and in the aligned sample (for further details see 5.5.1.1). The alignment of a molecule can be described by an alignment tensor, which is a real symmetric traceless matrix (often also referred to as Saupe matrix [1]) and defined by its diagonal elements A_{zz}, A_{yy} and A_{xx}. Defining $|A_{zz}|>|A_{yy}|>|A_{xx}|$, the residual dipolar coupling between two coupled nuclei A and B can be described by Eqn.1.1, where θ denotes the polar angle between the interatomic vector A-B and the z-axis of the alignment tensor and ϕ is the angle of the projection of the interatomic vector onto the x-y plane relative to the x-axis (Fig.1.1).

$$D^{AB}(\theta,\phi) = D_a \left[\left(3\cos^2\theta - 1\right) + \frac{3}{2} Rh \sin^2\theta \cos 2\phi \right] \tag{1.1}$$

Eqn.1.1 finds its maximum for a θ angle of zero, which yields the relationship between the maximum dipolar coupling (D^{AB}_{max}) and the axial component of the alignment tensor (D_a) (Eqn.1.2).

$$D^{AB}_{max} = 2D_a \tag{1.2}$$

D_a is also referred to as magnitude of the alignment tensor and Rh is the rhombicity,

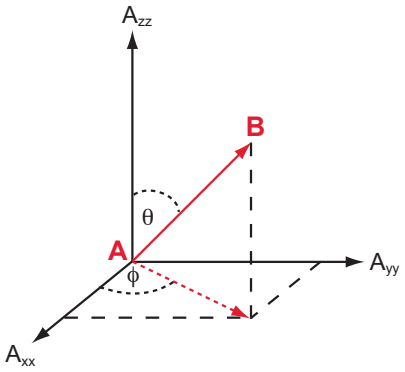

Figure 1.1: Interatomic vector A-B and its relationship to the principal axis system of the alignment tensor

reflecting the axial symmetry of the alignment tensor as can be seen in Fig.1.2. They are defined in Eqn.1.3 and Eqn.1.4. Note that the rhombicity reaches a maximum value of $\frac{2}{3}$ when A_{zz} = -A_{yy} and $A_{xx} = 0$.

$$D_a = \frac{1}{2} D^{AB}_{max} A_a \text{ and } Rh = \frac{A_r}{A_a} \tag{1.3}$$

$$A_a = \frac{3}{2} A_{zz} \text{ and } A_r = (A_{xx} - A_{yy}) \tag{1.4}$$

For a given value of D^{AB} there is an entire cone of solutions for θ and ϕ. Furthermore the alignment tensor is of 2^{nd} rank and the inverted cone is also part of the solution. For a better understanding three alignment tensors with a magnitude (D_a) of 3 Hz and different rhombicities have been calculated from Eqn.1.1 (Fig.1.2). The red and blue circles represent possible orientations for internuclear vectors, that start from the origin of the coordinate system of the principal axis for a 4 Hz and -2 Hz RDC. By definition the interatomic vector of two coupling nuclei in a molecule, that give rise to the RDC with the biggest absolute value, points along the A_{zz} axis and the

one with the biggest absolute value and opposite sign to A_{zz} along A_{yy}. The values for the RDCs along the principal axis system of the alignment tensors depicted in Fig.1.2 are given in Tab.1.1.

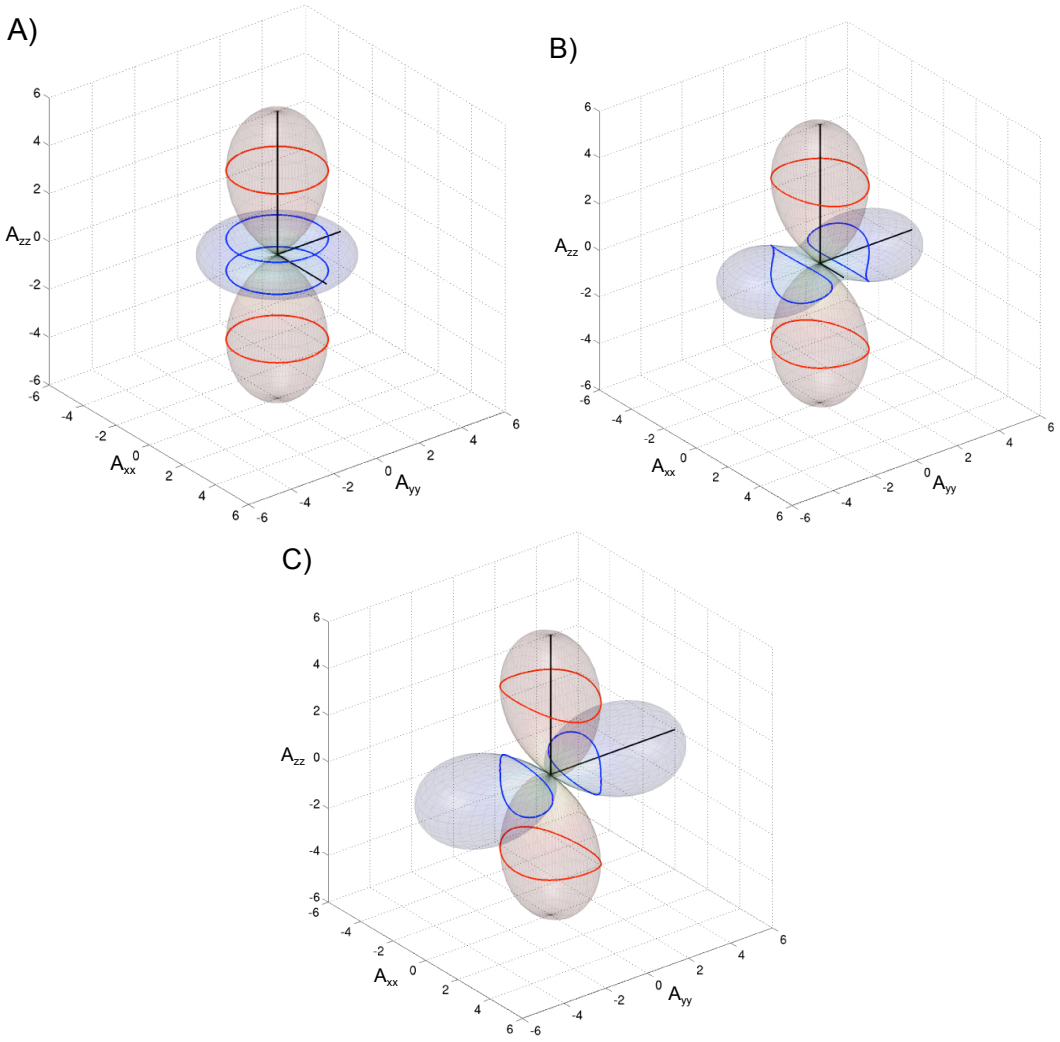

Figure 1.2: Graphical representation of the alignment tensor with a D_a of 3 Hz and different rhombicities . A) $Rh = 0$ B) $Rh = 1/3$ C) $Rh = 2/3$. Red and blue circles represent possible orientations of the internuclear vector, that starts from the origin of the coordinate system of the principal axis for a 4 Hz and -2 Hz RDC

1.2 Paramagnetic NMR

Molecules with an unpaired electron spin possess a considerable magnetic susceptibility, that is anisotropic and can be described by a magnetic susceptibility tensor (χ).

Table 1.1: Biggest RDC possible for the three principal axis of the alignment tensor with a fixed D_a and varying rhombicities.

$\boldsymbol{D_a}$	$\boldsymbol{Rh}$	$\mathbf{A}_{zz}$	$\mathbf{A}_{yy}$	$\mathbf{A}_{zz}$
3 Hz	0	6 Hz	-3 Hz	-3 Hz
3 Hz	1/3	6 Hz	-4.5 Hz	-1.5 Hz
3 Hz	2/3	6 Hz	-6 Hz	0 Hz

The interaction between a static magnetic field and this anisotropic susceptibility leads to a preferential orientation of the molecules. The energy of this interaction is given by Eqn.1.5 where $\vec{B_0}$ denotes the magnetic field vector.

$$E = -\frac{1}{2}\vec{B_0} \cdot \chi \cdot \vec{B_0} \tag{1.5}$$

As it is possible to derive a orientational probability distribution function from Eqn.1.5, the *Saupe* matrix can be rewritten in terms of the magnetic susceptibility tensor. Subsequently the residual dipolar coupling for a magnetically ordered molecule is formulated in Eqn.1.6

$$D^{AB} = -\frac{B_0^2}{15kT}\frac{\gamma_A\gamma_B h}{16\pi^3 r_{AB}^3}\left(\Delta\chi_{ax}\left(3\cos^2\theta - 1\right) + \frac{3}{2}\Delta\chi_{rh}\sin^2\theta\cos 2\phi\right) \tag{1.6}$$

B_0 is the magnetic field strength, γ_A and γ_A are the gyromagnetic ratios of the resonating nuclei A and B and $\Delta\chi_{ax}$ and $\Delta\chi_{rh}$ are the axial and rhombic components of the magnetic susceptibility tensor, that can be expressed via the principal components of the *Saupe* matrix (Eqn.1.7).

$$\Delta\chi_{ax} = \chi_{zz} - \frac{\chi_{xx} - \chi_{yy}}{2} \; and \; \Delta\chi_{rh} = \chi_{xx} - \chi_{yy} \tag{1.7}$$

As the dipolar interaction is proportional to the square of the magnetic field strength, it is preferable to use the highest field strength possible to increase the range of observed RDCs.

Furthermore, NMR resonances experience an extra contribution to the chemical shift in the presence of unpaired electrons (e.g. a lanthanide ion). This hyperfine shift is either called contact shift (δ^c), if the unpaired electron can delocalize on the

resonating nulcei, or pseudocontact shift (δ^{pc}) in case the electron magnetic moment is anisotropic (e.g. in a static magnetic field) and the number of chemical bonds is larger than four and do not include π bonds. The structural information contained in contact shifts is not easily accessible as it is hidden inside the particular mechanism of unpaired electron delocalization and require quantomechanical calculations. Therefore we will focus here on the pseudocontact shifts as they rely on the same alignment tensor as the RDCs. They are given by Eqn.1.8.

$$\delta^{pc} = \frac{1}{12\pi r_{AB}^3}\left(\Delta\chi_{ax}\left(3\cos^2\theta - 1\right) + \frac{3}{2}\Delta\chi_{rh}\sin^2\theta\cos 2\varphi\right) \tag{1.8}$$

Pseudocontact shifts provide a distance map of the metal ion (A) to the shifted nuclei (B), where $\Delta\chi_{ax}$ and $\Delta\chi_{rh}$ set the radius of the sphere where hyperfine shifts are measureable. Subsequently a value of pseudocontact shift corresponds to a whole sphere of possible coordinates with the metal ion being positioned at the center of the magnetic susceptibility tensor Fig.1.3.

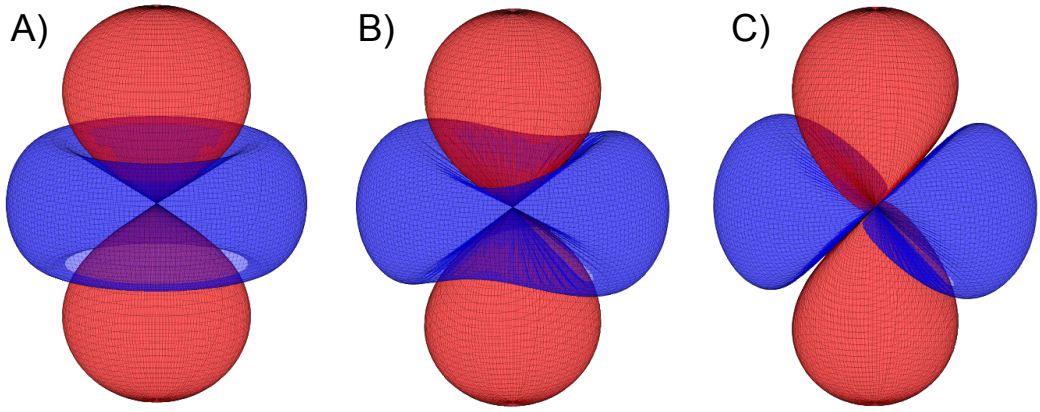

Figure 1.3: Graphical representation of the isopseudocontact shift surfaces with different rhombicities . A) $\Delta\chi_{rh} = 0$ B) $\Delta\chi_{rh} = {}^1/_3\ \Delta\chi_{ax}$ C) $\Delta\chi_{rh} = {}^2/_3\ \Delta\chi_{ax}$.

2

Determination of the conformation and configuration of small molecules with residual dipolar couplings

2.1 Introduction

The elucidation of the relative stereochemistry of asymmetric centers of organic molecules is an important challenge in chemistry since it requires the simultaneous determination of conformation and configuration. The classical NMR approach for the determination of stereochemistry of small molecules uses a combination of the nuclear overhauser effect (NOE) and scalar J couplings. Therefore a so called proton bonding network is required. If two protons are more than 5 Å apart and no $^3J_{HH}$ or heteronuclear long-range J_{XH} can be measured this method fails. That is why, residual dipolar couplings (RDCs), which do not rely on an uninterrupted proton bonding network gained more and more importance over the past few years. They rely on the weak alignment of molecules in solution and provide angular as well as distance information that are not contained in the NOE or J couplings. Originally RDCs have been used as restraints in the conformational refinement of biomolecules and various alignment media have been developed that are compatible with water. The most widely used media for water soluble compounds are bicelles [2, 3, 4], bacteria phages [5, 6] and polyacrylamide gels [7, 8], among others. The development of alignment media compatible with organic solvents and therefore feasible for small molecules and

natural products was carried out only over the past few years. These new alignment media can generally be divided in two groups: liquid crystalline phases and polymer gels.

At first liquid crystalline media like poly-γ-benzyl-L-glutamate (PBLG) or poly-γ-ethyl-L-glutamate (PELG) were successfully used to align small molecules in organic solvents [9, 10, 11]. However liquid crystalline media are restricted to very apolar solvents and a minimal concentration is necessary, which yields a minimal alignment of the molecules. This minimal orientation tends to be too strong already, which results in broadened resonances due to ^{1}H-^{1}H dipolar couplings and leads to problems in the analysis of standard spectra. This problem has recently been solved by two completely different approaches. The first one incorporates a Flip-Flop decoupling during detection of an HSQC [12]. Thus, unwanted homonuclear couplings are removed and chemical shifts as well as heteronuclear couplings are scaled for a better dispersion of the NMR resonances. The second one simply decreases the amount of alignment and therefore scales the RDCs by variable angle sample spinning (VASS) in a solid-state spectrometer [13]. Hereby the sample is spun at an angle close to the magic angle of 54.7° at which dipolar interactions would be averaged to zero.

At present the most widespread approach for the orientation of small molecules is the use of the second group of alignment media, the polymeric gels. Over the past few years a series of new cross-linked polymers have been introduced that are compatible with apolar and polar solvents. They are summarized along with their applications in

Table 2.1: Polymer based alignment media

Polymeric gel	**Compatible solvents**	**Investigated molecules**
Polystyrene (PS) [14, 15]	$CHCl_3$, CH_2Cl_2, dioxane, benzene	menthol, strychnine, norcamphor
Poly(dimethylsiloxane) (PDMS) [16]	$CHCl_3$, CH_2Cl_2, THF, benzene, n-hexane	spiroindene, cyclosporin A, hymenistatin
Poly(vinyl acetate) (PVAC) [17]	$CHCl_3$, dioxane, benzene, ethyl acetate, acetone, acetonitrile, methanol, DMSO	norcamphor, sphaeropsidin A
Negatively charged polyacrylamide (PH) [18]	DMSO, DMF, water	menthol, decasaccharide, hormaomycin, hymenistatin, DiaA, archazolid A
Positively charged polyacrylamide (PPH) [19]	DMSO, water	menthol, hymenistatin
Poly(acetonitrile) (PAN) [20]	DMSO, DMF	hymenistatin

Tab.2.1. Most of them can be easily synthesized and therefore uniquely architectured through variation of their monomer composition, polymerization conditions and gel diameter to any alignment strength.

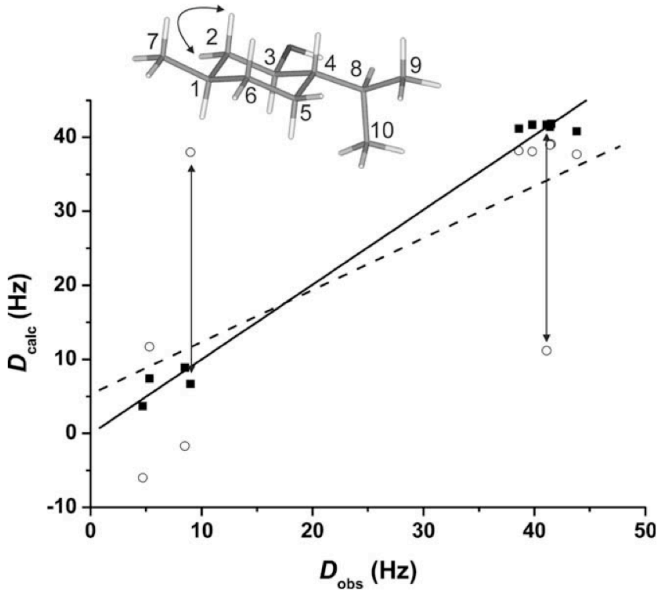

Figure 2.1: Superposition of the fitting curves for the observed (D_{obs}) and backcalculated (D_{calc}) of the $^1D_{CH}$ of (-)-menthol in PBLG/$CDCl_3$ when the diastereotopic protons at the C_2 position are exchanged. Dashed curve and circles are used for the wrong diastereotopic assignment. (This figure has been used with the permission of the authors of [11])

RDCs together with order matrix calculations have proven to be a very efficient tool in the determination of the conformation and relative configuration of small molecules and natural products. It could be shown for a series of relatively rigid molecules like dihydropyridone [9] and others [11, 21, 22, 23, 24] that RDCs could unambigously determine the relative stereochemistry. This could be achieved by either backcalculating the measured RDCs to a crystal structure or a structure derived from MD simulations with different configurations and subsequent comparison of the fitting quality between experimental and backcalculated RDCs. In cases of molecules, which are symmetric according to their internuclear vector orientations like in six-membered chair-like rings, where all axial substituents are roughly parallel and therefore have similar RDC values, this differentiation can be made by simple inspection

of the size of RDCs without the determination of an alignment tensor. Furthermore RDCs have been successfully exploited to probe the diastereotopicity of methylene protons of strychnine [25] and menthol [11]. As can be seen by the correlation plots of experimental versus backcalculated RDCs of (-)-menthol in PBLG/$CDCl_3$ (Fig.2.1), a wrong assignment of the methylene protons at the C_2 position yields a much worse fit.

The determination of the conformation for small molecules and natural products with RDCs is carried out in analogy with the conformational refinement of proteins. A set of distance restraints (NOEs or ROEs) as well as angular restraints (J couplings) is determined in addition to RDCs and subsequently used as restraints in simulating annealing protocols to refine the structure. It has been demonstrated that including RDCs into refinement protocols of cyclic natural products could improve the accuracy of their backbone structure [26], solve conformational ambiguities [27] and refine conformations for different solvents [28].

2.2 Results and discussion

2.2.1 Properties of the PH- and PPH-gel

The negatively charged PH-gel and the positively charged PPH-gel have been synthesized as described in 5.3. NMR-samples prepared according to this procedure exhibit a quadrupolar deuterium splitting of the solvent of up to 4.3 Hz (Fig.2.2). The two

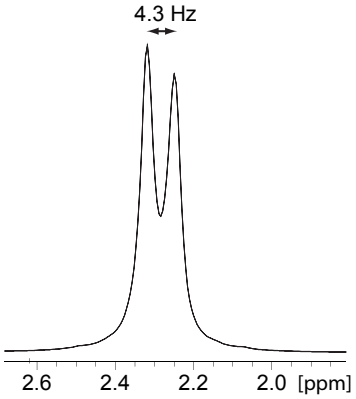

Figure 2.2: 400 MHz deuterium spectrum of d_6-DMSO in PH-gel

resonances have about the same intensities and lineshapes, which indicates a excellent homogeneity of the sample. As a first test molecule (+)-menthol was dissolved in DMSO and the solution was introduced with a PH- or PPH-gel stick into an NMR tube. After reswelling and equilibration of the gels, RDCs in the range from -0.8–4.8 Hz (PH-gel) and -0.6–5.3 Hz could be measured. The single value decomposition module (SVD) [29] of the program PALES [30] was used to fit the twelve experimental dipolar couplings (Tab.B.1, excluding the $^1D_{CH}$ of C6-H6eq due to an uninterpretable coupling pattern in the anisotropic HSQC spectrum) to a reference structure. We used the crystal structure of (+)-menthol for which the proton positions have been optimized by conjugate gradient minimization. A correlation factor R of 0.98 could be obtained for both RDC data sets (Fig.2.4). The RDCs derived from the PH-gel and PPH-gel are different due to different steric and electrostatic interactions of the solute with the polymer matrix [31, 32]. Therefore linearly independent alignment for the two gels can be obtained and essential structural information can be deduced. That is illustrated by the poor correlation between the experimental RDCs of (+)-menthol

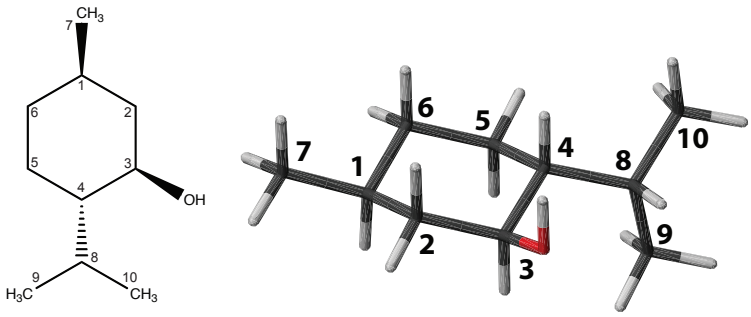

Figure 2.3: Formula (left) and structure (right) of (+)-menthol

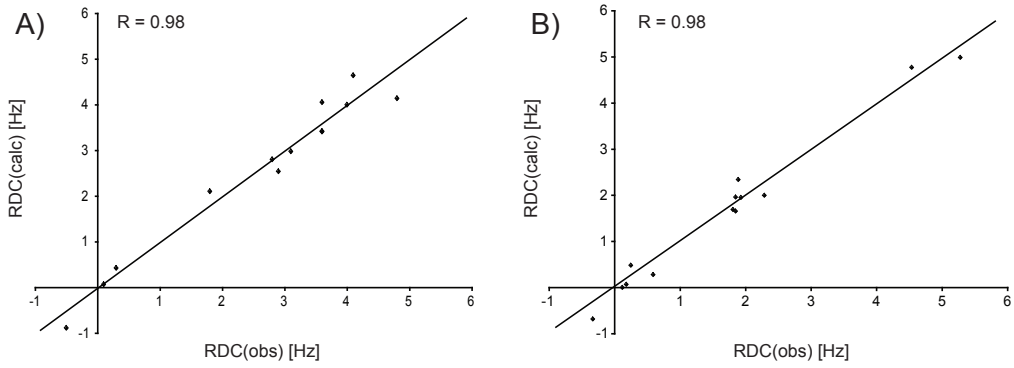

Figure 2.4: Correlation between observed (RDC(obs)) and backcalculated (RDC(calc)) dipolar couplings for (+)-menthol in A) PH-gel/DMSO and B) PPH-gel/DMSO

measured in the two gels (Fig.2.5 A)) and the comparison of the principal axis system of the alignment tensor in Fig.2.5 B). Additionally an intertensor 5D space angle of 23.8° could be determined.

PH- and PPH-gel are very versatile and have been applied to various molecule/solvent systems. For (+)-menthol in PH-gel/DMF dipolar couplings range from -0.7–2.6 Hz and a similar fit (R = 0.95) was obtained (see B.1). In a further application we investigated a decasaccharide with a molecular weight of 1804 g/mol. It was dissolved in D_2O, aligned in PH-gel and the measured $^1D_{CH}$ ranged from -22 to 28 Hz, a range in which they can be evaluated easily and accuratly. The cyclic peptides hormaomycin (MW = 1130 g/mol) and hymenistatin (MW = 894 g/mol) were oriented in PH-gel/DMSO and gave rise to RDCs from -22–35 Hz and -20–22 Hz, respectively. For an accordingly prepared PPH-gel/DMSO sample of hymenistatin RDCs range from -18

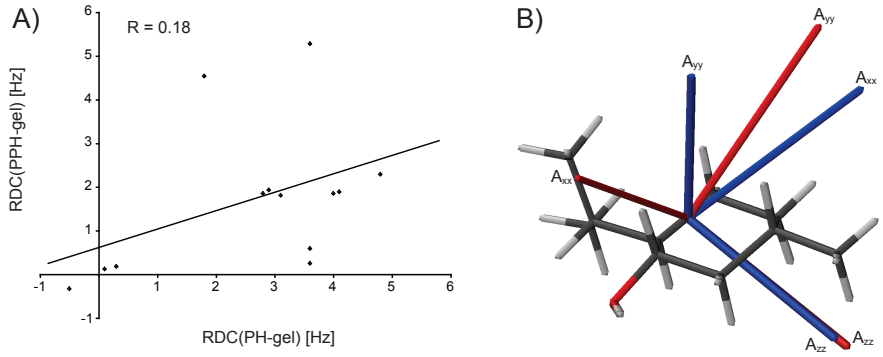

Figure 2.5: Independent alignments in PH- and PPH-gel. A) Correlation between the observed RDCs of (+)-menthol in PH- and PPH-gel. B) Comparison of the principal axis system of the alignment tensors for PH-gel (blue) and PPH-gel (red). The principal axis system is drawn within the molecular frame of (+)-menthol.

to 22 Hz. Finally ((-)-menthyl)-(5-oxo-5,6-dihydro-2H-pyran-2-yl)-diphtalate (DiaA) with a molecular weight of 400 g/mol derived from chemical synthesis was successfully aligned in PH-gel/DMSO and the extracted RDCs range from -11–8 Hz.

In contrast to liquid crystalline media (e.g. PBLG, PELG etc.), which can only be used in a certain temperature and concentration range [9], alignment media prepared through polymerization can be influenced easily in many different ways. First of all it is important to understand the mechanism of the alignment, which is schematically illustrated in Fig.2.6. On the left the isotropic case is pictured. The solute (blue bars) can adopt all possible orientation in the gel pores, which leads to an averaging of the dipolar couplings to zero. On the right side the anisotropic case is shown. Here the gel pores are radially compressed leading to an preferential orientation of the solute, which allows the measurement of RDCs. Therefore the way to scale the size of the dipolar coupling is to engineer the size and shape of the gel pores accordingly. The size can be easily changed by chemical means, simply varying the total monomer concentration and/or the concentration of the linker. The shape can be manipulated either by changing the diameter of the gelcylinder used for the polymerization or by changing the diameter of the NMR-tube. The latter effect is shown for the RDCs of (+)-menthol measured in a 3.5 mm PH-gel (Fig.2.7 A)) and PPH-gel (Fig.2.7 B)). Missing resonances can be explained by the different spectral quality of the

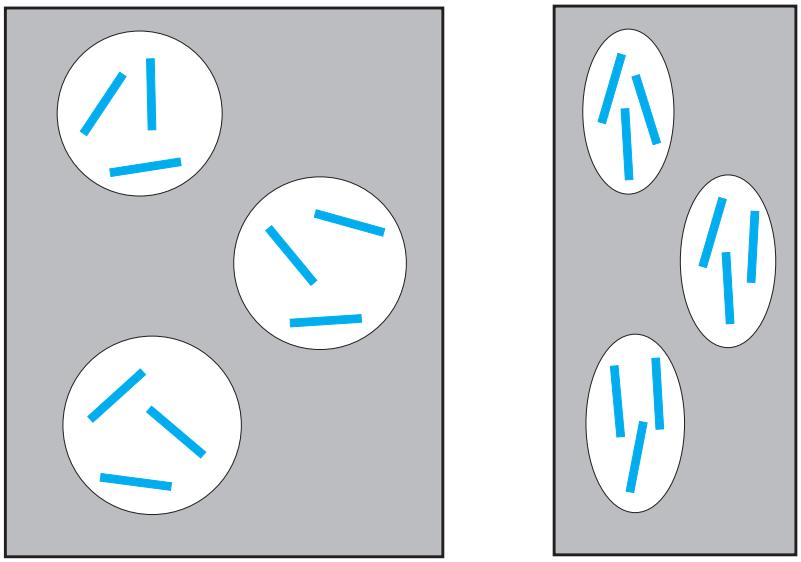

Figure 2.6: Cartoon of a polyacrylamide-based gel before (left) and after (right) radial compression. The gel-matrix is coloured in grey; the solute is denoted as blue bars and the gel pores are drawn as circles and ellipsoids respectively.

gel samples. Resonances only appear, if the RDCs could be extracted for all three diameters.

The quality of the NMR-spectra obtained in PH- and PPH-gel samples is illustrated in the following. The amount of alignment in PH-gel is largely reduced compared to PBLG as can be appreciated by the narrow ^{1}H-resonances for the PH-gel/DMSO (Fig.2.8) and PPH-gel/DMSO sample (Fig.2.9). The alignment in PBLG

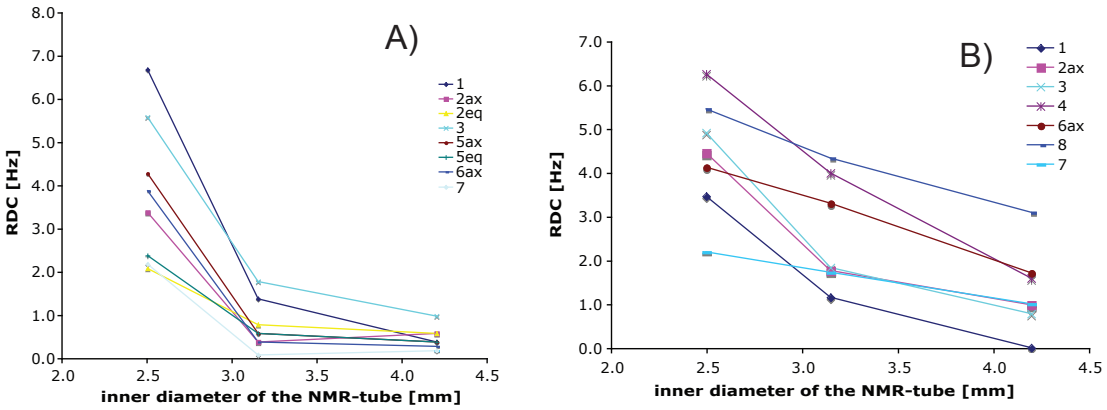

Figure 2.7: Scalability of 3.5 mm PH- (left) and 3.5 mm PPH-gel (right). Plots show the change of the measured RDC with respect to the inner diameter of the NMR-tube.

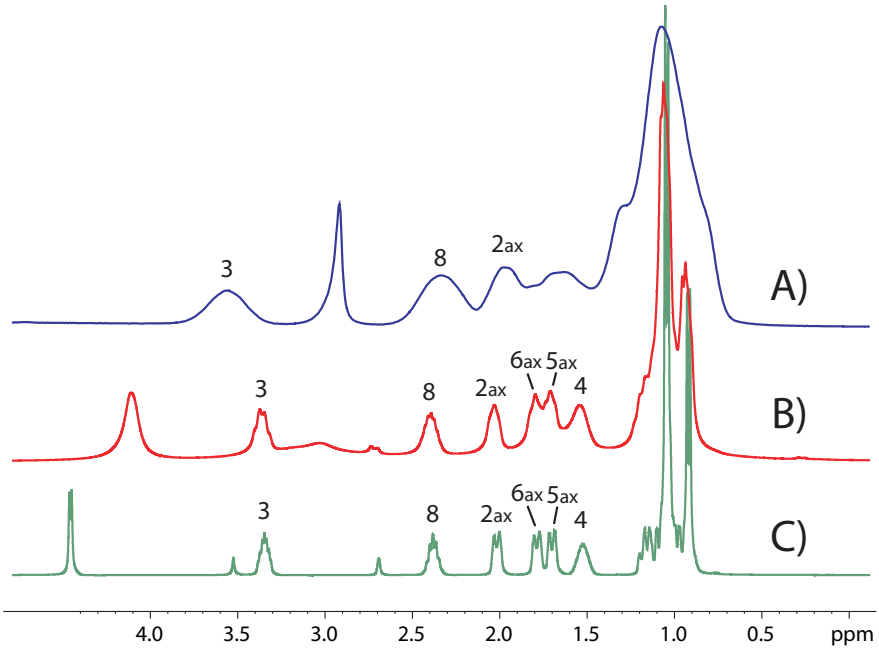

Figure 2.8: 400MHz ^{1}H-spectra of a 1 M (+)-menthol sample in A) PBLG/$CDCl_3$, B) in PH-gel/DMSO and C) in DMSO.

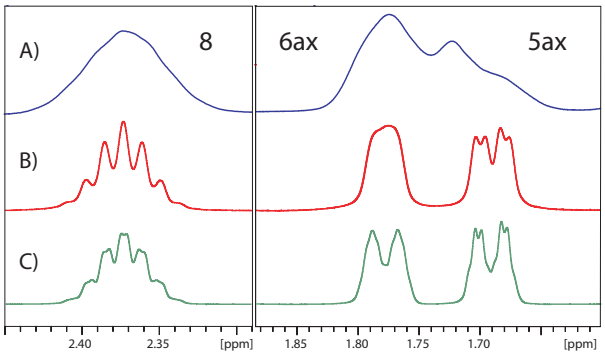

Figure 2.9: Comparison of excerpts of ^{1}H-spectra of a 1 M (+)-menthol sample in A) PBLG/$CDCl_3$, B) in PPH-gel/DMSO and C) in DMSO.

is too strong and therefore ^{1}H,^{1}H dipolar couplings cause extensive line broadening. That is why liquid crystalline media are only applicable to natural products with higher molecular weight if sophisticated NMR experiments [12, 13] are used.

On the other hand RDCs obtained from PH- and PPH-gel samples can easily be scaled as described above and subsequently are amenable to natural products of any size. The minimum concentration used to date for the two gels was 20 mM. The t_2-

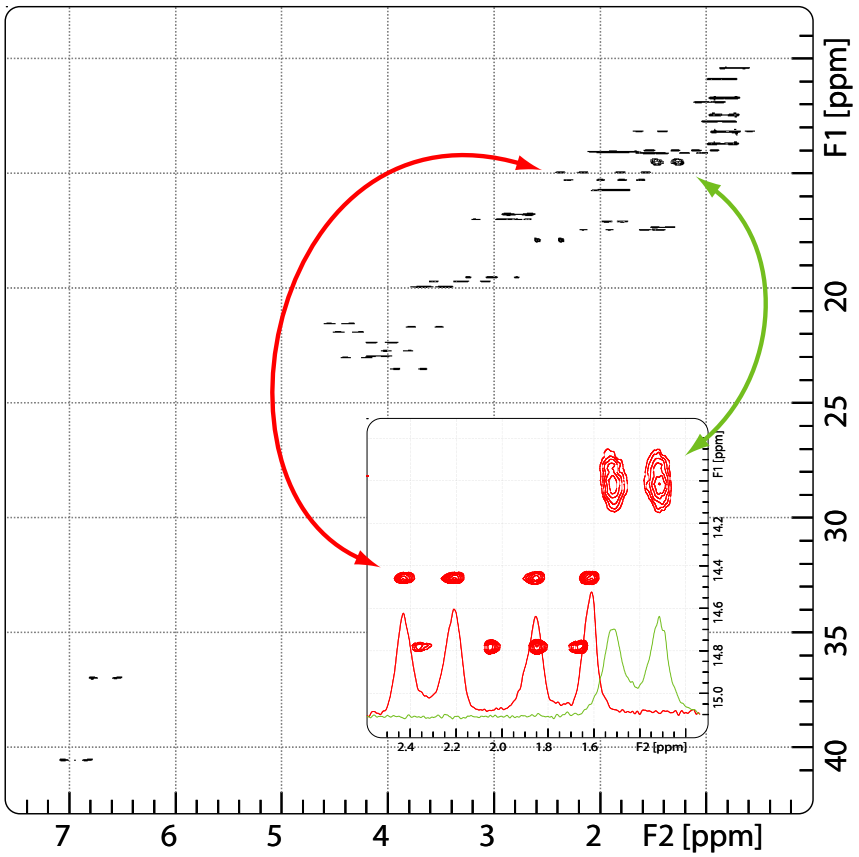

Figure 2.10: 600 MHz t_2-coupled ^{13}C-^{1}H spectrum of a 20 mM hymenistatin sample in PH-gel/DMSO. The insert shows traces of the Pro(1) C_β-H_β resonances (red) compared to the biggest PH-gel resonance (green). 8192 complex data points in t_2 and 1024 experiments in t_1 were aquired with 16 scans. The formula of hymenistatin can be found in Fig.2.19.

coupled ^{13}C-^{1}H spectra of those 20mM samples of hymenistatin at natural abundance that were acquired for 12 h (16 scans and 1024 experiments in t_1) are shown in Fig.2.10 and Fig.2.11. By looking at the trace of the biggest PH-gel resonance and the trace of the Pro(1) C_β-H_β resonances one can clearly see, that the signals have about the same intensity and therefore RDCs could be measured accurately. The biggest resonance arising from the PPH-gel in the 2D spectrum is about twice as intense as the hymenistatin resonances, due to the higher gel concentration needed to achieve alignment. Again RDCs could be measured accurately. The comparison of the two spectra shows, that the minimum solute concentration to get enough spectral resolution for the extraxtion of RDCs can be lower in the PH-gel.

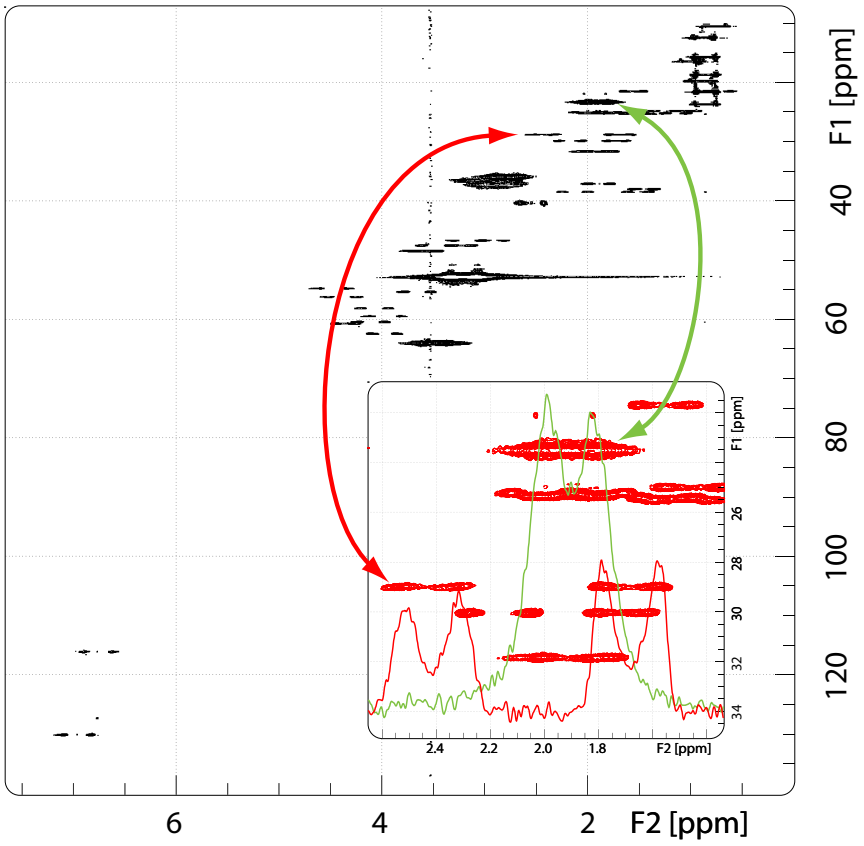

Figure 2.11: 600 MHz t_2-coupled ^{13}C-^{1}H spectrum of a 20 mM hymenistatin sample in PPH-gel/DMSO. The insert shows traces of the Pro(1) C_β-H_β resonances (red) compared to the biggest PPH-gel resonance (green). 8192 complex data points in t_2 and 1024 experiments in t_1 were aquired with 16 scans. The formula of hymenistatin can be found in Fig.2.19.

2.2.2 Conformational refinement of hormaomycin

2.2.2.1 Introduction

Hormaomycin is a secondary metabolite produced by *Streptomyces griseoflavus* (strain W-384) [33]. This depsipeptide contains (S)-isoleucine [(S)-Ile] as the only proteinogenic amino acid along with two units of (2S,3R)-3-methylphenylalanine [β-Me)Phe], one of R-*allo*-threonine [*a*-Thr] as well as two moieties of (1'R,2'R)-3-(2'-nitrocyclopropyl)alanine [(3-Ncp)Ala, the (2S)-diastereomer in the bulky side chain and the (2R) diastereomer in the macrocyclic ring of the molecule] and one residue of (2S,4R)-4-(Z)-propenylproline [(4-Pe)Pro] (Fig.2.12). The bulky side chain of hor-

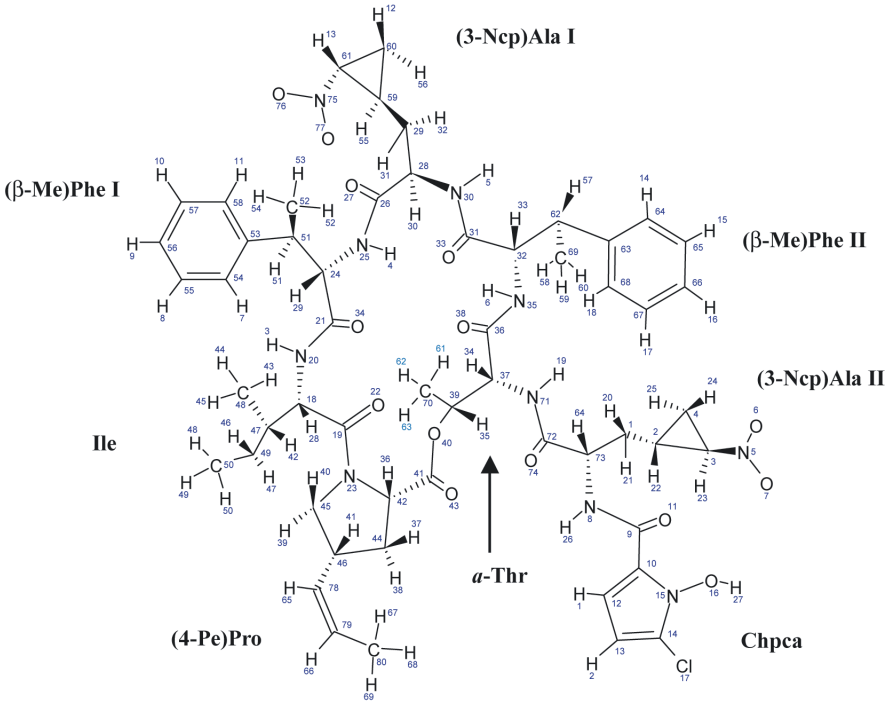

Figure 2.12: Molecular formula of hormaomycin with numbering used for calculations

maomycin is terminated by an amide bound 5-chloro-1-hydroxypyrrole-2-carboxylic acid [Chpca]. The latter three components have never been found in any natural product before. Besides challenging structural features, hormaomycin possesses an interesting spectrum of biological activities, including a marked influence on the secondary metabolite production of other streptomycetes, an exceptionally selective an-

tibiotic activity against coryneforme bacteria [33] and also an antimalarial activity [34].

The total synthesis became possible after the correct absolute configurations of all stereocenters especially of the two (3-Ncp)Ala moieties had been established [35, 36]. Recently a structure of hormaomycin in $CDCl_3$ [37] has been published. Also a crystal structure (50/50 mixture of hexylene glycol/H_2O buffered at pH = 8.0 with the addition of 0.1 M $MgCl_2$) has been obtained lately [38].

Since the conformation of the depsipeptide differs significantly in solution and in the crystal, its solvent dependence has been investigated here. DMSO was chosen as solvent, because its solvent properties are in between the crystallization medium and those of chloroform. RDCs were obtained in the PH-gel.

2.2.2.2 DMSO assignment

Spin systems were identified by DQF-COSY, TOCSY and ^{1}H,^{13}C-HMBC experiments (Tab.2.2). Smaller chemical shift dispersion within the set of HN (7.18–8.45 ppm) and H_α (3.95–4.91 ppm) protons compared with hormaomycin in $CDCl_3$ (HN: 6.54–9.13 ppm, H: 3.51–5.16 ppm) indicates a less rigid structure. Especially the missing long-range NOEs between aromatic protons of the distal Chpca and (β-Me)Phe I component further support this finding. A second conformation (using the same batch the ratios between minor and major resonances differed for $CDCl_3$ and DMSO) could be detected when dissolving the depsipeptide in DMSO independently of whether the sample was in the isotropic or anisotropic phase. The concentration of this minor conformer did not exceed 8 % relative to the major conformer and was not considered further.

2.2.2.3 NOE and J-coupling refined structures of hormaomycin

A total number of 85 NOEs together with 8 dihedral angle restraints were used for structure calculations. 900 structures were calculated using only the NOE and ^{3}J derived restraints, by using protocol 2 in 5.6.1.2, starting from 900 different structures calculated without restraints with protocol 1 (5.6.1.1). The resultant NJ ensemble

Table 2.2: Chemical shift assignments of hormaomycin in DMSO

Carbon, proton or group	σ_H [ppm]	σ_C [ppm]	**Carbon, proton or group**	σ_H [ppm]	σ_C [ppm]
Chpca C9	-	158.2	C10	-	116.2
C12-H1	6.70	108.5	C10	-	116.2
C14	-	108.5	C13-H2	6.10	102.1
(3-Ncp)Ala II					
C72	-	170.0	C73-H64	4.51	51.8
C1-H20/H21	1.80 (proS), 1.85 (proR)	32.3	C2-H22	1.98	22.6
C3-H23	4.52	58.6	C4-H25/H24	1.25 (proS), 1.93 (proR)	17.6
H26	8.23	-			
a-Thr					
C36	-	167.4	C37-H34	4.50	57.8
C39-H35	5.00	70.7	C70-H61/H62/H63	1.21	16.9
H19	8.40	-			
(4-Pe)Pro					
C41	-	170.7	C42-H36	3.95	59.0
C44-H38/H37	1.51 (proR), 2.27 (proS)	34.4	C46-H41	3.24	36.1
C45-H39/H40	3.16 (proS), 3.90 (proR)	51.1	C78-H65	5.33	128.5
C79-H66	5.54	126.6	C80-H67/H68/H69	1.65	12.8
Ile					
C19	-	169.8	C18-H28	4.49	53.9
C47-H42	1.89	35.5	C49-H47/H46	0.99 (proR), 1.42 (proS)	22.5
C50-H48/H49/H50	0.84	10.7	C48-H43/H44/H45	0.92	15.4
H3	7.18	-			
(β-Me)Phe I					
C21	-	169.5	C24-H29	4.20	58.9
C51-H51	3.55	38.4	C52-H52/H53/H54	1.23	13.3
C53	-	38.4	C58-H11 and C54/H7	7.23	127.3
C57-H10 and C55-H8	7.16	126.1	C56-H9	7.02	127.5
H4	8.42	-			
(3-Ncp)Ala					
C26	-	170.9	C28-H30	4.51	50.4
C29-H31/H32	0.68 (proS), 0.88 (proR)	33.1	C59-H55	1.21	21.9
C61-H13	4.00	58.2	C60-H56/H12	0.28 (proR), 1.28 (proS)	18.2
H5	8.45	-			
(β-Me)Phe II					
C31	-	169.2	C32-H33	4.91	56.0
C62-H57	2.89	43.8	C69-H58/H59/H60	1.01	18.1
C63	-	142.5	C64-H14 and C68-H18	7.12	127.6
C65-H15 and C67-H17	7.07	127.5	C66-H16	6.95	125.6
H6	7.81	-			

comprises the 40 lowest experimental energy structures (total experimental NOE and J-coupling energy term < 17.5 kcal mol^{-1} Å^{-2}, minimum energy 13.3 mol^{-1} Å^{-2}, 5.4 $\pm$ 0.9 restraint violations beyond 0.15 Å and 1.1 $\pm$ 0.2 beyond 0.3 Å). Two families can be resolved in this ensemble (representatives of which are shown in blue and red in Fig.2.13 A) and B)), family NJa comprising 23 structures and family NJb comprising 17 structures. Family NJa forms a tighter bundle (0.1 $\pm$ 0.1 Å RMSD). The average pair-wise RMSD over all atoms between members of the two different groups is 3.63 Å while the average intra-family is 1.34 Å for NJa and 2.94 Å for NJb. For the macrocyclic ring atoms, these numbers fall to 0.2 Å for NJa and 0.7 Å for NJb, and 1.4 Å average pairwise RMSD between members of the two different families. We can conclude that although both families are in agreement with the NOEs and J-couplings to essentially the same extent, the structure of the macrocyclic ring is significantly different. This structural difference occurs around the ψ dihedral angle of (β-Me)Phe II (Fig.2.14). There are therefore two conformations of the

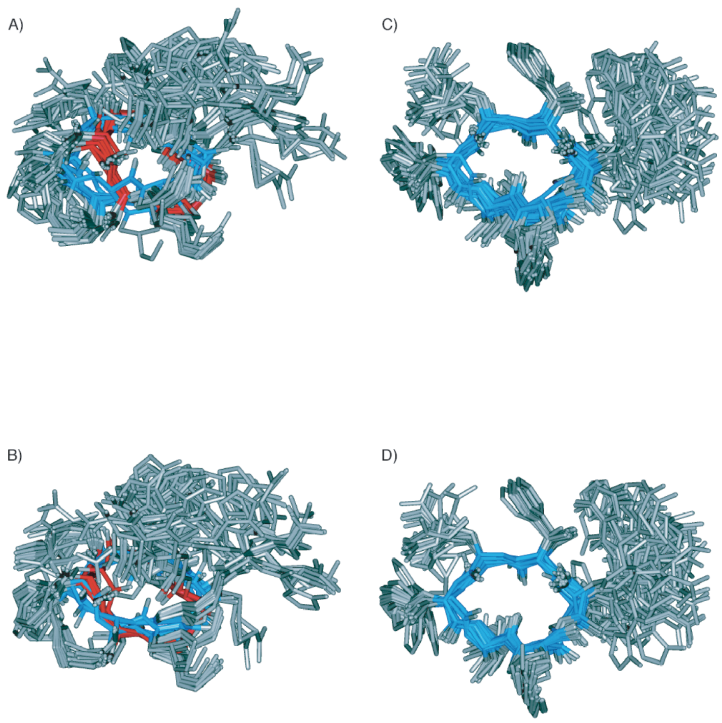

Figure 2.13: Comparison of the NOE and J-coupling (NJ) and the NOE/J-coupling and RDC (NJR) refined ensembles. A) 40 lowest energy structures from the NJ ensemble (all atom superposition). The two families of macrocyclic ring conformations are coloured red and blue. B) 40 lowest energy structures from the NJ ensemble (macrocyclic ring superposition). C) 40 lowest energy structures from the NJR ensemble (all atom superposition). D) 40 lowest energy structures from the NJR ensemble (macrocyclic ring superposition).

macrocycle that are essentially in equal agreement with the NOE and J-coupling restraints. This is not necessarily due to molecular flexibility, it is much more likely that the experimental data are not sufficient to define a single conformation of the macrocycle. In order to further refine this structural ambiguity we have introduced RDCs into the structure calculation. The orientational information present in these restraints is complementary to the distance and dihedral angle restraints used for the NJ ensemble. In order to facilitate the use of RDCs in structure calculation it is useful to have some initial idea of the molecular alignment tensor resulting from the partial alignment of the molecule. As this is difficult to predict without a known structure we have used the extensive conformational sampling present in the NOE/J-coupling (NJ) and the restraint-free (RF) ensembles to represent the available conformational

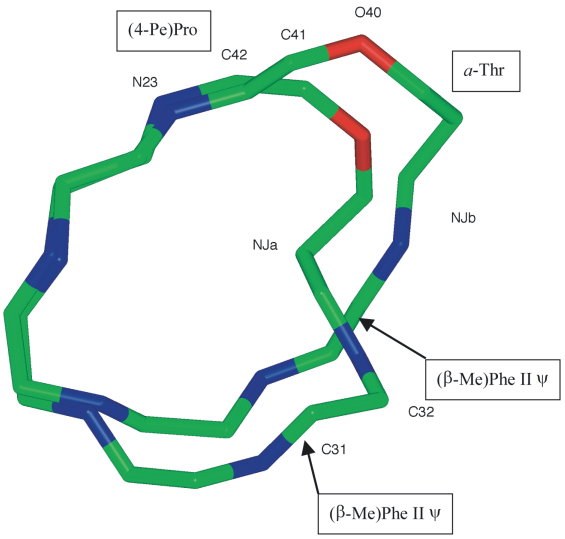

Figure 2.14: Representation of the two major families in the NOE/Jcoupling ensemble (NJ). Lowest energy members of the two families show major differences about the (β-Me)Phe II ψ dihedral angle

sampling for the molecule, and attempted to identify the alignment tensor that fits best to members of this ensemble of 900 structures. If the conformational sampling of this ensemble is sufficiently large this procedure should simultaneously identify the best-fitting conformers with respect to the RDCs, and determine the most appropriate alignment tensor. As the side-chains are potentially more flexible than the macrocyclic ring, we have restricted this analysis to the 16 RDCs present on the backbone.

The entire NOE/J coupling refined NJ ensemble (both families) was also used in the initial RDC analysis. Each of the 900 structures was used to fit the RDCs from the macrocyclic ring (4 NH RDCs and 12 CH RDCs). Although there is a very broad spectrum of fit quality, illustrating the diversity of the entire ensemble, a family of structures does actually fit these RDCs quite well (Fig.2.16 and Fig.2.15). The 40 structures that best-fit the macrocyclic ring RDCs all give very similar alignment tensor parameters (A_a = (8.71 $\pm$ 0.03) x 10^{-4}, A_r = (5.69 $\pm$ 0.03) x 10^{-4}), and are in fact very similar with respect to their RMSD. This sub-ensemble only contains members of family NJb, and none from family NJa, and therefore already demon-

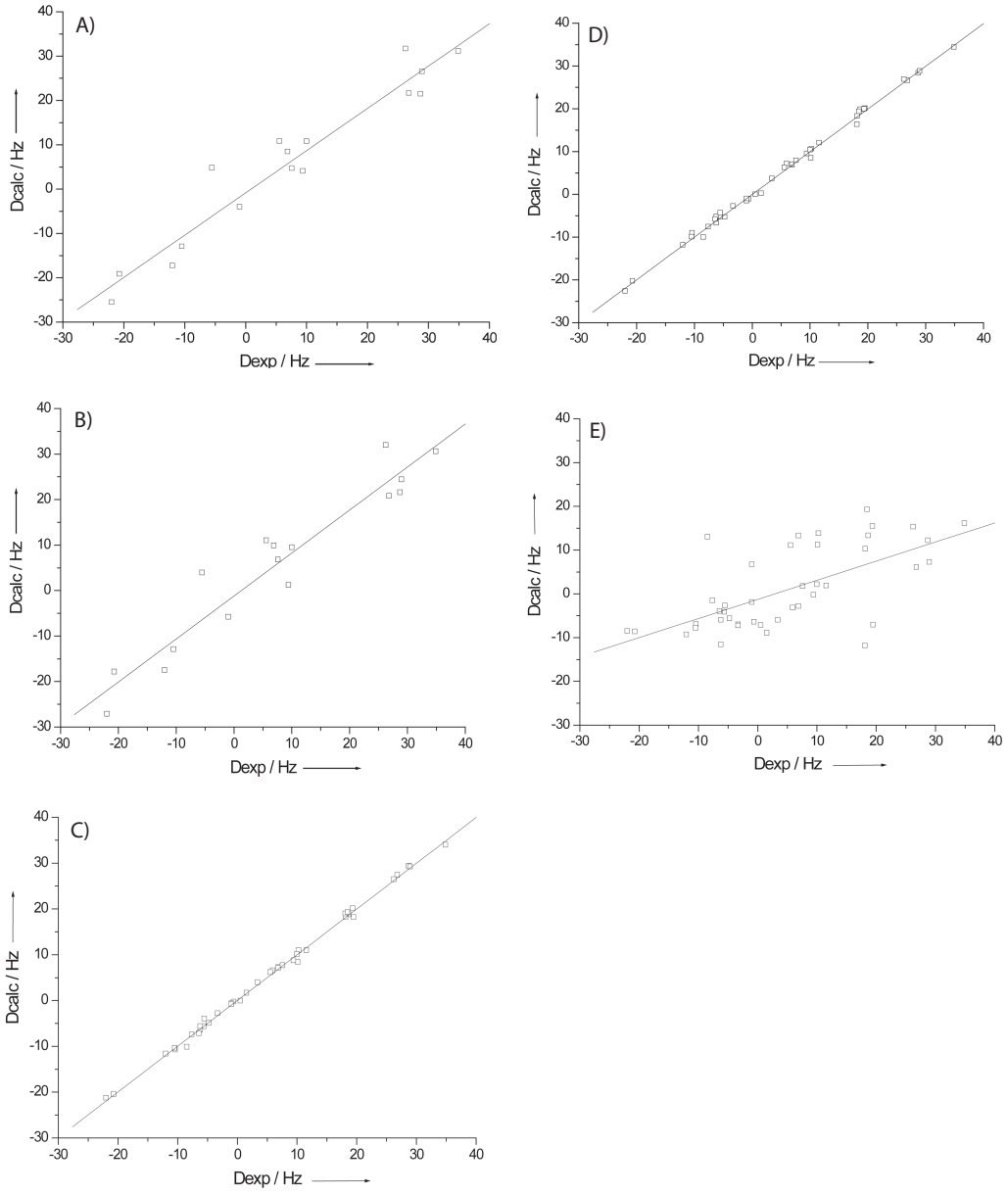

Figure 2.15: Experimental versus back-calculated RDCs for NJ1, NJ19 and NJR19. Comparison of the 16 RDCs of the macrocyclic ring for NJ1 (A) the equation of the fitting curve is Dcalc = 0.95*Dexp - 0.85 and for NJ19 (B) : D_{calc} = 0.95*Dexp - 1.2 and with the 42 RDCs for NJR19 (C) : Dcalc = 0.99*Dexp - 0.04, for NJR1 (D) : Dcalc = 0.99*Dexp - 0.02 and NJ19 (E) : Dcalc = 0.44*Dexp - 1.26.

strates the power of RDCs to resolve structural ambiguity resulting from NOE-based analysis. This stage of fitting RDC values over the structures obtained by NOE and J analysis allows to exclude the NJa family.

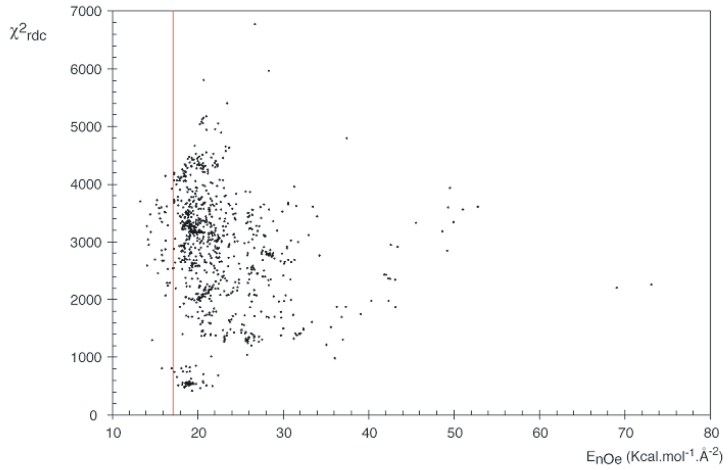

Figure 2.16: Structure selection to create the NJ ensemble based on NOE/J coupling terms with respect to the RDC fitting. Only RDCs originating from the macrocyclic core structure are used in the fitting procedure . Structures with experimental energies $E_{NOE} < 17$ kcal mol^{-1} Å^{-2} were selected for inclusion in the ensemble NJ. Notice that conformers that fulfill the NOE/J coupling restraints to a similar extent reproduce the macrocycle ring RDCs to very different degrees.

2.2.2.4 Refining the structure of hormaomycin using RDCs

The RDC derived structure of hormaomycin was determined using an identical protocol to that used for the NJ ensemble, with additional steps allowing for initial determination of the optimal orientation of the alignment tensor for the respective structure using the 16 macrocyclic ring RDCs. The eigenvalues A_a and A_r were tethered to the values determined as described above and the orientation was allowed to evolve freely throughout the calculation (protocol 3 in 5.6.1.3). During the 80 ps sampling period 42 RDCs (except those measured in the aromatic and methyl groups) were used with equal weighting. Again the 40 structures with the lowest combined experimental energy term were taken to form the final NJR ensemble (Fig.2.13 C) and D)). Not surprisingly the average RMSD of the macrocyclic ring coordinates of this ensemble is lower than those measured for the NJ ensemble (1.9 ± 0.3 Å for heavy atoms compared to 2.6 ± 0.7 Å and 0.32 ± 0.16 Å for the macrocyclic ring atoms compared to 0.60 ± 0.22 Å).

Table 2.3: Pairwise RMSDs of superimposed structures

	NJ1	**NJ19**	**NJ1**	**NJ19**
A) whole molecule				
NJR19	2.75	2.68	2.28	
NJR1	1.58	1.48		
NJR19	0.60			
Structures number 1 of family NJa	5.54	5.54	4.36	4.36
Structures number 19 of family NJa	5.69	5.66	4.49	4.49
B) macrocyclic ring NJR19	0.53	0.53	0.38	
NJR1	0.31	0.31		
NJR19	0.05			
Structures number 1 of family NJa	1.37	1.36	1.22	0.98
Structures number 19 of family NJa	1.38	1.37	1.23	0.99

The refinement procedure results in a structural ensemble (NJR) whose macrocycle ring conformation strongly resembles the NJb family determined from the NOE/J-coupling data only (Tab.2.3). This is not surprising, as the NJa family was found to agree less well with the RDCs than the NJb family. It therefore appears that the structural ambiguity present in the NJ ensemble was indeed due to a lack of sufficient restraints, and that the RDCs have provided the necessary complementary orientational information to clearly distinguish between the two families. The average pair-wise RMSD of the macrocyclic ring conformations of the NJR ensemble is 1.2 Å with respect to the NJa family and only 0.75 Å with respect to the NJb family. From the reduction of the RMSD, it is evident that the RDCs have defined both backbone and side-chain conformations significantly. In this case the average number of NOE violations above 0.15 Å is 9.1 ± 0.9 and 1.4 ± 0.6 beyond 0.3 Å. The total experimental NOE and J-coupling energy term is 19.6 ± 2.4 kcal mol^{-1} Å^{-2}, (minimum energy 15.8 kcal mol^{-1} Å^{-2}). Although the NJR ensemble is in slightly worse agreement with the NOE and J couplings, this effect is quite small and apparently not significant.

2.2.2.5 Comparison of refined structures

The pairwise RMSDs of the representatives structures of the ensembles (NJ, NJR and the family A of the NJ ensemble) are listed in Tab.2.3 A) and B). Except for the closely defined NJa family (RMSD of NJ19-NJ1 = 0.60 Å), the RMSD for the

Table 2.4: Different R and Q factors for different structures of hormaomycin comparing the RDCs both of the macrocyclic ring and of the side chains with the experimentally observed values

Refined structures	**R backbone**[a]	**all**[b]	**Q backbone**[a]	**all**[b]
NJR1	0.99	0.99	0.01	0.02
NJR19	0.99	0.99	0.01	0.02
NJR1	0.96	0.60	0.17	0.81
NJR19	0.96	0.64	0.16	0.84

[a] 16 RDCs used, [b] 42 RDCs used

whole molecule is higher than 1.5 Å. The latter can be explained by the different side chain conformations. In contrast, the RMSD of the macrocyclic ring is similar for the structure pairs between the NJR ensemble and family NJb.

Two structures of the NJb ensemble (NJ1 and NJ19) were analysed by comparing the calculated RDCs from the structure and the experimental ones. The 16 backbone RDCs are in good agreement (R = 0.96, Tab.2.4, Fig.2.15 A) and B)) with the back-calculated ones from these structures. However the entire 42 experimental RDCs (excluding methyl groups and the aromatic ortho and meta RDCs) do not fit very well with the ones calculated from the NOE structures (Tab.2.4, Fig.2.15 E)).

In order to show the benefit of using RDCs for structural refinement, the experimental RDCs were compared with the back calculated by using representative structures of the NJR ensemble (NJR1 and NJR19). For the structure NJR1 as well as NJR19, the fitting factor R is close to 1 when the 16 backbone RDCs are used,

Table 2.5: The most different dihedral angles in the bulky side chains of the two RDC structures NJR1 and NJR19

	NJR1	**NJR19**
NH (3-Ncp)Ala II)-H_α(*a*-Thr)	137°	-174°
ψ of (3-Ncp)Ala II	41°	-27°
N-CO-C-N (Chpca)	165°	-17°
N-C_α-C_α-C_α (3-Ncp)Ala II	61°	-89°

which indicates a perfect fit between the calculated structure and the RDC values that had been used to refine the structure (Tab.2.4). Moreover with 42 RDCs the fitting factors are again very good: R = 0.99 (Tab.2.4, Fig.2.15 C) and D)), even if the side-chains have different conformations. A similar fitting factor has been obtained for the other structures of the NJR ensemble (data not shown).

2.2.2.6 Flexibility of hormaomycin in DMSO

Within the NJR ensemble the most prominent structural differences are found in the bulky side chain starting at the C_α of *a*-Thr. Due to severe overlap only a limited number of ^{3}J couplings and NOE signals could be extracted unambiguously for this part of the molecule. The comparison with the NMR structure in $CDCl_3$ indicated a more disordered conformation ensemble because long range NOEs of the pyrrole ring protons are missing. A detailed analysis was undertaken using the RDC structures NJR1 and NJR19 as representatives. In the bulky side chain four dihedrals differ, two of which substantially (N-CO-C-N of Chpca and N-C_α-C_β-C_γ of (3-Ncp)Ala II (Tab.2.5). The first dihedral amounts to a value of 165° in the NJR1 structure and to -17° in NJR19, which constitutes a pyrrole ring, flip. Both positions can be stabilized by a hydrogen bond (Chpca (O)H and Chpca (C)O or (3-Ncp)Ala II (N)H and Chpca O(H)). The measured NOE between Chpca 3-H and (3-Ncp)Ala II NH (see Tab.B.4 in the appendix) is intermediate between the distances in the two structures (NJR1: 2.0 Å NJR19: 4.4 Å. The same holds for all proton pairs for which a reliable NOE could be measured and which have different distances in the two structures. The spatial proximity between the NH of (3-Ncp)Ala II and the NH of (β-Me)Phe II indicated by the measured NOE integrals is achieved by a different set of dihedrals in the two structures NJR19 and NJR1 (Tab.2.5). In the two structures there are two combinations of the ϕ dihedral of *a*-Thr and the ψ of (3-Ncp)Ala II which direct in both cases the NH of (3-Ncp)Ala II in the vicinity of the NH of the (β-Me)Phe II component. We therefore conclude that the bulky side chain of hormaomycin is sampling the conformational space with the concomitant change in several dihedral angles.

2.2.2.7 Structural characterization of the macrocyclic ring of the RDC structure NJR19

The structure of hormaomycin exhibits one type II' and one type III β-turn (Fig.3.3). The III β-turn comprises the four amino acids Ile (i), (4-Pe)Pro (i+1), *a*-Thr (i+2) and (β-Me)Phe II (i+3) while the II' β-turn comprises the amino acids (β-Me)Phe II (i), (3-Ncp)Ala I (I+1), (β-Me)Phe I (i+2) and Ile (i+3). For the two β-turns a C_α(i)-C_α(i+3) distance of 6.5 Å is found for the components Ile and (β-Me)Phe II. Assignment of the turns to their respective types is done based on the values of the observed dihedral angles of the residues i+1 and i+2 (Tab.2.6). These are indicative for type II' and III β-turns. For the identification of the two β-turns we took the general criterium that the distance between C_α(i) and C_α(i+3) is less than 7 Å [39]. There is no hydrogen bond between CO (Ile) and NH (*a*-Thr) in accordance with the observation that a CO(i)-HN(i+3) hydrogen bond is not necessary for the stabilization of a β-turn.

Backbone chirality plays an important role in defining the conformational space for β-turn formation [40]. L-Xaa-D-Yaa and D-Xaa-L-Yaa dyads have a high tendency to be in the corner positions of type II and type II' turns, respectively. Indeed, the type II' β-turn in hormaomycin is formed with the D-diastereomer of (3-Ncp)Ala I in the corner position followed by the L-diastereomer of (β-Me)Me I. In an idealised type III and type II' β-turn all four C_α atoms lie within one plane. In hormaomycin both turns are twisted when compared to the ideal structure of type III and II' β-turns.

Table 2.6: Dihedral angles of ideal β-turns of types III and II' of the components of NJR19

	ϕ(i+1)	ψ(i+1)	ϕ(i+2)	ψ(i+2)
ideal type III	-60°	-30°	-60°	-30°
ideal type II'	60°	-120°	-80°	0°
ideal type II	-60°	120°	80°	0°
(4-Pe)Pro, *a*-Thr	-66°	41°	-68°	-80°
(3-Ncp)Ala I, (β-Me)Phe I	116°	-133°	-83°	-7°

2.2.2.8 Comparison of the NMR solution structures of hormaomycin in $CDCl_3$ and DMSO, and of the crystal structure from hexylene glycol/H_2O (50/50)

The structures in the three solvents are characterized by two β-turns, which in DMSO and $CDCl_3$ are formed by the same amino acids supporting the observation that β-turns are rather stable [39]. The comparison of the dihedral angles of the macrocyclic ring clearly shows considerable similarities between the three structures (Tab.2.7; Fig.2.17). Nevertheless, the RMSD for the macrocyclic ring between the DMSO structure and the crystal structure is high (2.3 Å), in contrast to a low value between the DMSO and $CDCl_3$ structure (0.66 Å). The same components form two β-turns

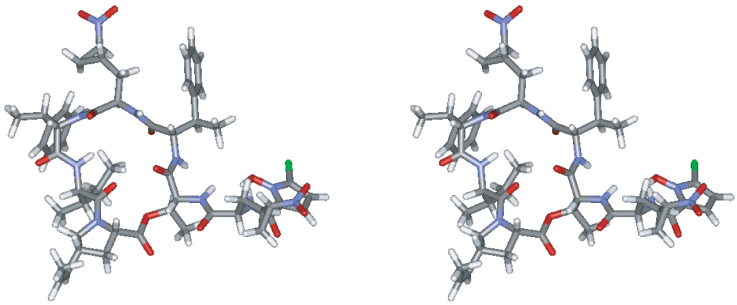

Figure 2.17: Stereoview of the RDC structure NJR19

in the DMSO and $CDCl_3$ structure resulting in the low RMSD for the macrocyclic ring. One of the turns is classified in both cases as type II' and the other is different: type III in DMSO and type II in $CDCl_3$. This is confirmed by an average fitting factor R = 0.80 between the back-calculated RDCs from the chloroform structure

Table 2.7: Dihedral angles of ideal β-turns of types III and II' of the components of NJR19

	ϕ			ψ			ω Ile to (β-Me)Phe I		
	DMSO	$CDCl_3$	crystal	DMSO	$CDCl_3$	crystal	DMSO	$CDCl_3$	crystal
(β-Me)Phe I (βII')	**-83**	**-90**	**-80**	**-7**	-47	**-15**	**180**	**173**	**173**
(3-Ncp)Ala I (βII')	**116**	69	**131**	**-133**	**-135**	-163	**178**	**-168**	**-179**
(β-Me)Phe II	**-98**	-67	**-103**	**126**	180	**133**	**170**	**166**	**172**
a-Thr (βIII) (OCCαCβO)	-80	**43**	**54**				**-173**	**170**	**180**
a-Thr (βIII) (HNCOCαCβ)				-57	170	-89			
Ester (βIII) (CαCβOCO)				-68	90	158			
Ester (βIII) (CβOCOCα)							143	**-173**	**168**
(4-Pe)Pro	**-66**	**-61**	**-58**				**174**	**-176**	**179**
(4-Pe)Pro (OCOCαN)				41	**143**	**152**			
Ile	**-128**	**-93**	**-110**	**160**	**152**	**167**			

Table 2.8: R factors for different structures of hormaomycin, comparing the calculated RDCs of the macrocyclic ring alone and of the ring plus the side chains with the values determined experimentally in DMSO

	R	
Structures	**backbone**[a]	**all**[b]
Chloroform	0.80	0.41
NJR19	0.74	0.52

[a] 16 RDCs used, [b] 42 RDCs used

and the experimental ones (Tab.2.8 and Fig.B.2 A) in the appendix). Nevertheless a poor fit (R = 0.40) is obtained with 42 RDCs (Tab.2.8 and Fig.B.2 B) in the appendix). In the crystal structure the positions of the amino acids i and i+3 are (3-Ncp)Ala I and (4-Pe)Pro which amounts to a rotation of the secondary structure by one residue as compared to the secondary structure found in $CDCl_3$ and DMSO (Fig.2.18). The respective distance between C_α,(i) and C_α,(i+3) is 6.7 Å. Assuming the same secondary structure in the crystal as in $CDCl_3$ and DMSO the distance of the C_α,(i) and C_α,(i+3) would be 7.3 Å which is beyond the allowed distance for a β-turn. Thus, the secondary structure is indeed rotated in the crystal compared to $CDCl_3$ and DMSO. Because of this rotation the pairwise RMSD of the crystal structure with both NMR structures is very large. Not surprisingly, for the same reason, the RDCs calculated from the crystal structure are in poor agreement (Tab.2.8 and Fig.B.2 A)and B) in the appendix) with the experimental RDCs.

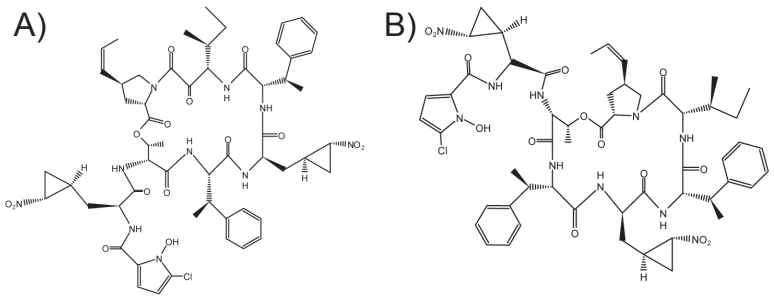

Figure 2.18: β-turns in the NMR solution structures. A) chloroform and DMSO and B) in the crystal.

These structural dissimilarities could be rationalized by the different ε values and different hydrogen bond characteristics of the three solvents: $CDCl_3$ is a weak donor and acceptor, DMSO is a strong acceptor, and hexylene glycol/H_2O is a strong donor and acceptor.

In $CDCl_3$ the conformation of the side chain is very well defined by long range NOEs between Chpca/(β-Me)Phe I and Chpca/(3-Ncp)Ala I [37]. The pyrrole ring of Chpca is in a stacking interaction with the phenyl ring of (β-Me)Phe II. This type of interaction does not occur in the DMSO structures indicating a flexible and/or under-determined bulky side chain. The crystal structure of the monomer would lead to the observation of ROEs between Chpca and (β-Me)Phe II, which are not visible neither in DMSO nor in $CDCl_3$ (see Tab.B.3 in the appendix). Furthermore the observed ROE signals in $CDCl_3$ between Chpca/(c-Me)Phe I and Chpca/(3-Ncp)Ala I could also not occur in the crystal structure which shows too long distances (see Tab.B.4 in the appendix).

Consequently, the RMSDs between the RDC structure NJR19 compared with the crystal structure and the NMR structure in $CDCl_3$ are substantial: 6.91 Å and 4.10 Å respectively. The RMSD between the DMSO and $CDCl_3$ structure reflects the higher flexibility of the bulky side chain, whereas the further increased value with the crystal structure indicates a substantial influence of the solvent and/or a methodological component.

2.2.3 Conformational and configurational refinement of hymenistatin

The aim of this project was to determine the conformation of hymenistatin in DMSO, $CDCl_3$ and THF and the configuration of its thirteen prochiral centers. The refinement protocols used are described in 5.6.2.

2.2.3.1 Introduction

Hymenistatin is a cyclo octapeptide, that can be synthesized [41] or extracted from *Hymeniacidon* sponge collected in the Pacific Ocean [42].The amino acid sequence has been determined to be cylco-(-Pro1-Pro2-Tyr3-Val4-Pro5-Leu6-Ile7-Ile8-) and its formula can be found in Fig.2.19. The substance shows an interesting cytostatic activity on murine lymphoblastic leukaemia. Due to its cyclic nature and solubility in DMSO, $CDCl_3$ and THF hymenistatin presents itself as an ideal test case for our new approach to probe conformation and configuration with RDCs.

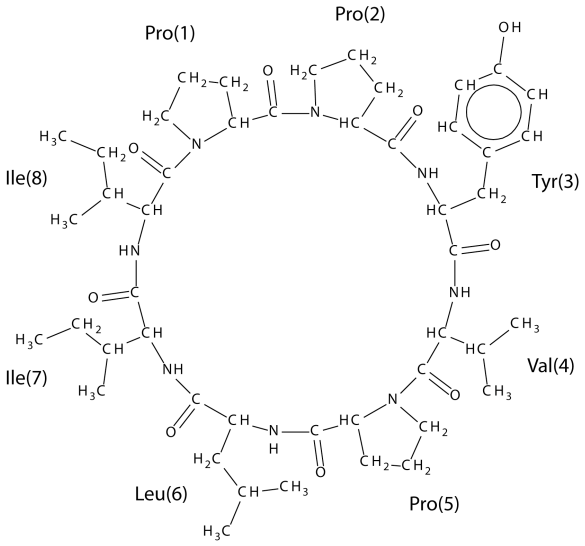

Figure 2.19: Formula of hymenistatin

2.2.3.2 Comparison of the five different sets of RDCS

For hymenistatin we could measure five different data sets of RDCs in three different solvents. The alignment medium/solvent combination together with their intertensor

Table 2.9: Comparison of the alignment tensors obtained for hymenistatin in five different alignment media and three different solvents

	Intertensor angle [°]				
alignment medium/ solvent	**PH-gel/ DMSO**	**PPH-gel/ DMSO**	**PAN-gel/ DMSO**	**PDMS-gel/ $CDCl_3$**	**PDMS-gel/ THF**
PH-gel/DMSO	0	53.2	76.6	84.0	87.6
PPH-gel/DMSO	53.2	0	117.6	46.4	59.8
PAN-gel/DMSO	76.6	117.6	0	125.8	105.6
PDMS-gel/DMSO	84.0	46.4	125.8	0	42.1
PDMS-gel/DMSO	87.6	59.8	105.6	42.1	0

5D space angles are summarized in Tab.2.9. All the intertensor angles are notably different from another and therefore the structural information, which can be deduced is independent. In Fig.2.20 the principal axis coordinate system of all five sets of RDCs are depicted and are drawn next to the molecular frame of the illustrated backbone structure of hymenistatin. They have been determined by fitting the measured backbone RDCs on a hymenistatin structure, that had been previously refined with the respective NOE and RDC dataset.

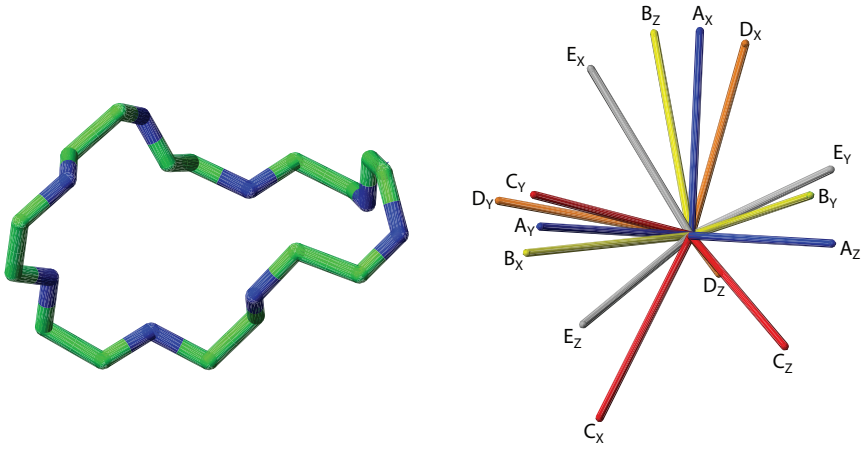

Figure 2.20: Comparison of the principal axis component system of the alignment tensors of A) PH-gel/DMSO B) PPH-gel/DMSO C) PAN-gel/DMSO D) PDMS-gel/$CDCl_3$ and E) PDMS-gel/THF drawn next to the molecular frame of hymenistatin

2.2.3.3 DMSO conformation

Each of the three RDC data sets for DMSO have been used individually to refine the conformation of hymenistatin as described in 5.6.2. The resulting twenty best

Table 2.10: Average correlation factors (R) of the refined RDC data set (bold) and the backbone RDCs of the omitted datasets

Refined data set	**R** [ ] PH-gel	**R** [ ] PPH-gel	**R** [ ] PAN-gel
PH-gel	**0.99**	0.90	0.97
PPH-gel	0.95	**0.99**	0.99
PAN-gel	0.96	0.95	**1.00**

energy structures for each set have then been cross-validated by back-calculating the backbone RDCs of the two data sets not included in the refinement onto those structures. The resulting average correlation factors (R) are listed in Tab.2.10 and indicate a good agreement of the backbone structures of hymenistatin refined with three different RDC data sets. The overall backbone conformation of hymenistatin in DMSO can be described as pseudo-β strand, extending from Pro2-Pro5 on one side and Leu6-Pro1 on the other side. The pseudo-β strand is not flat and extended, but adopts a 'twisted banana' conformation (Fig.2.21). The turns formed at each ends are a βVIa around Pro1-Pro2 and a βII around Pro5-Leu6. The average value for the according ϕ and ψ angles for the twenty best energy structures of the NOE and J-coupling (NJ) ensemble as well as the twenty best energy structures for the NOE, J-coupling and RDC (NJR) ensembles for the PH-gel, PPH-gel and PAN-gel are listed in Tab.2.11 along with the values of the ideal type β turn. These results are in accordance with a previously solved DMSO conformation of hymenistatin [41]. In Fig.2.21, the twenty lowest energy structures of NJ and NJR ensembles are superimposed. The RMSD to the mean of the backbone atoms is 0.185 Å.

2.2.3.4 Chloroform conformation

The overall backbone conformation of hymenistatin in $CDCl_3$ is similar to that found in DMSO (RMSD = 0.233 Å). There is a βVIa turn formed around Pro1-Pro2 for the twenty best energy structures found in the NJ ensemble as well as the NJR ensemble of $CDCl_3$ (Tab.2.12). The major difference in the conformation, however, is found around Leu6. While the NJ ensemble forms a βII turn around Pro5-Leu6, which compares quite well with the conformation in DMSO, two families of structures

Table 2.11: Dihedral angles [°] of ideal β turns of type II and VIa and the ones found for the hymenistatin in DMSO

	ϕ(i+1)	ψ(i+2)	ϕ(i+2)	ψ(i+2)
ideal type II	-60	120	80	0
ideal type VIa	-60	120	-90	0
Pro1-Pro2				
NOE/J	-70	155	-84	17
PH-gel	-53	171	-81	21
PPH-gel	-64	148	-89	21
PAN-gel	-54	145	-90	17
Pro5-Leu6				
NOE	-56	140	63	-1
PH-gel	-50	135	77	-14
PPH-gel	-55	140	66	-11
PAN-gel	-47	148	68	-18

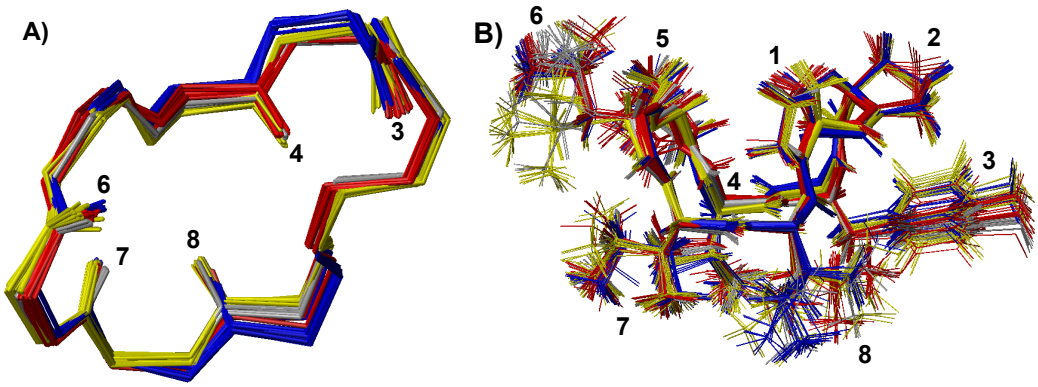

Figure 2.21: Conformation of hymenistatin in DMSO. The twenty best structures of the NJ ensemble (grey) and the NJR ensembles from PH-gel (blue), PPH-gel (red) and PAN-gel (yellow) have been superimposed. A) Backbone representation including amide protons, B) All atom representation in which the backbone atoms a drawn slightly thicker to guide the eye.

are found within the NJR ensemble (referred here as C61 and C62), which differ in the orientation of the amide bond of Leu6 (Fig.2.22). The population within the twenty best energy structures in the C61 and C62 subensembles is 5:15. The orientation of the Leu6 amide proton in the C61 conformation is very similar to that found in DMSO corresponding to a βII turn around Pro5-Leu6. For conformation C62, the NH-bond is tilted about 100° out of the ring compared to C61 and the ϕ

Table 2.12: Dihedral angles [°] of ideal β turns of type II and VIa and the ones found for the hymenistatin in $CDCl_3$

	ϕ(i+1)	ψ(i+2)	ϕ(i+2)	ψ(i+2)
ideal type II	-60	120	80	0
ideal type VIa	-60	120	-90	0
Pro1-Pro2				
NOE/J	-72	163	-72	1
PDMS-gel	-56	163	-86	18
Pro5-Leu6				
NOE/J	-42	148	74	-33
PDMS-gel (C61)	-45	142	61	-43
PDMS-gel (C62)	-45	-118	-48	-5

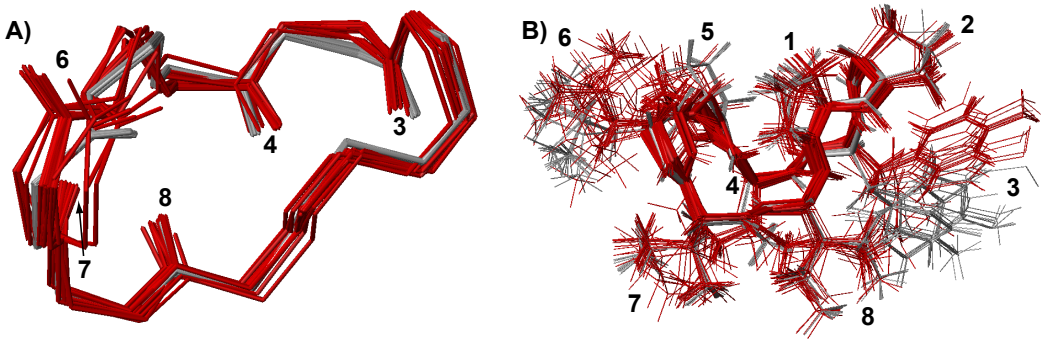

Figure 2.22: Conformation of hymenistatin in $CDCl_3$. The twenty best structures of the NJ ensemble (grey) and the NJR ensemble from PDMS-gel (red) have been superimposed. A) Backbone representation including amide protons, B) All atom representation in which the backbone atoms a drawn slightly thicker to guide the eye.

and ψ angles do not allow to interpret a standard β turn. Fig.2.23 shows the heavy atoms of Pro5 and Leu6 along with the Leu6 amide proton of NJ and NJR ensemble. The blue and green circles indicate the two subensembles C61 and C62. Within the less populated C61 structures the amide proton of Leu6 is more solvent exposed than for the structures of the subensemble C62, where it is better protected by the neighbouring side chains. For a detailed discussion of the solvent effect see 2.2.3.6. The two conformations found for the NJR ensemble do not agree with the chloroform conformation previously published *Konat et al.* [41]. The major difference is that the γ turn around Ile7 stabilized by the intramolecular H-bond between Ile8-NH

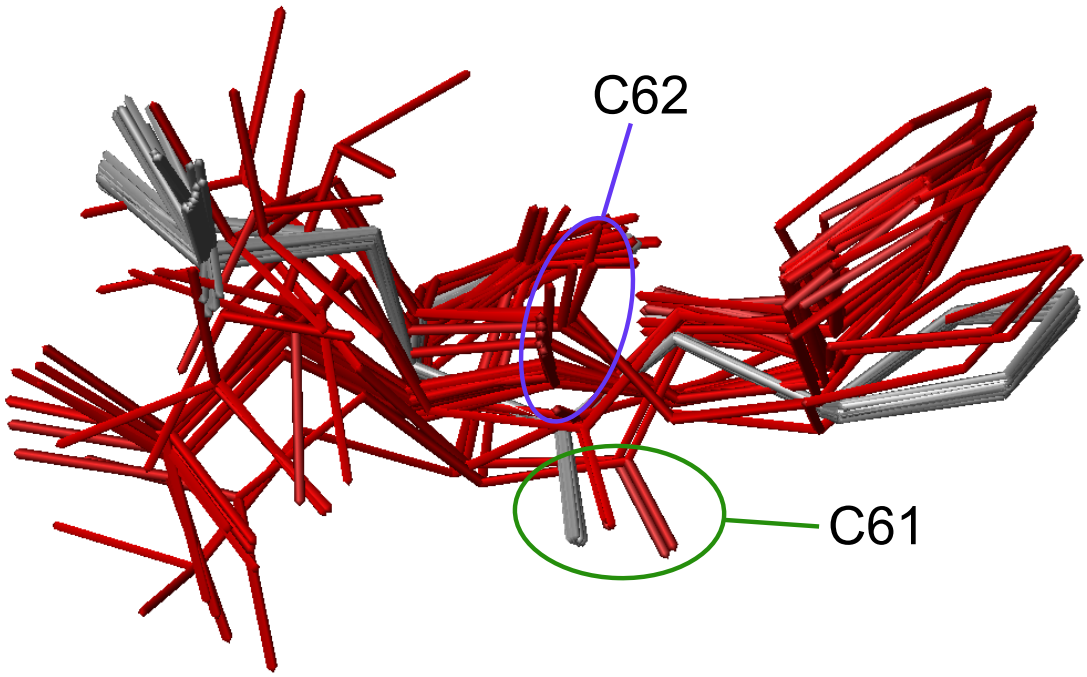

Figure 2.23: Pro5 and Leu6 of hymenistatin in $CDCl_3$. The structures of the NOE ensemble are drawn in grey and the for the NJR ensemble in red. The blue and green circles indicate the amide protons for the two subensembles C61 and C62 for the NJR ensemble

and Leu6-CO could not be observed in any of our lowest energy ensembles. In these conformations the Ile8-NH is forming a H-bond with Val4-CO. *Konat et al.* state, that the NOE distance-restraint violation during their restraint MD simulations are 62 pm with only three NOEs violated more than 100 pm. A closer inspection of the published NOE data lead to the fact, that actually six out of eight NOEs involving Ile8-NH are violated by more than 50 pm. Therefore we performed a calculation in XPLOR-NIH using the protocol described in 5.6.2 and the NOE data set from *Konat et al.*. Among the resulting twenty lowest energy structures, no NOE is violated and none of conformations exhibits a γ turn around Ile7, but formed a H-bond between Ile8-NH and Val4-CO, which is in accordance with our findings. In a second test calculation, we included the published ϕ and ψ as restraints to mimic the previously found chloroform conformation. All the twenty best energy structures of this simulation showed six NOE violations bigger than 50 pm of which five involved Ile8-NH.

Furthermore we found a solvent accessible surface for Ile8-NH of ∼1.1 Å^2, whereas all other amide protons are buried. In contrast to the conformations found in the NJR ensemble were Leu6-NH is the only solvent accessible amide proton (see Tab.2.14 below), which suits the ^{1}H-spectrum, where only this amide proton shows a solvent dependent high field shift. Therefore we can conclude, that in the correct chloroform conformation Ile8-NH is forming a intramolecular H-bond with Val4-CO.

2.2.3.5 THF conformation

The overall backbone conformation of hymenistatin in THF is again very similar to those found in DMSO and $CDCl_3$ RMSD = 0.295 Å and RMSD = 0.300 Å, respectively). A βVIa turn around Pro-Pro2 can be deduced from the ϕ and ψ angles listed in Tab.2.13 and the overall 'twisted banana' conformation is observed as shown in Fig.2.24. The difference in conformation arises around Leu6. In contrast to DMSO and $CDCl_3$, THF structures of the NJR ensemble are forming a βI turn around Pro5-Leu6 (Tab.2.13). In the NOE ensemble, the Leu6-NH is tilted about 80° out of the ring compared to the NJR ensemble and therefore no standard β turn can be assigned. It is important to note, that no NOE is violated for this 80° flip of Leu6-NH, which makes the increase of the precision of the structure apparent, when RDCs are used in the structural refinement.

Table 2.13: Dihedral angles [°] of ideal β turns of type II and VIa and the ones found for the hymenistatin in THF. The ϕ and ψ angles reflect the similarity of the NOE and NR ensembles for the β turn formed around Pro1-Pro2 and the difference for the one around Pro5-Leu6 (this conformational difference is in accordance with the NOE data)

	ϕ(i+1)	ψ(i+2)	ϕ(i+2)	ψ(i+2)
ideal type I	-60	-30	-90	0
ideal type VIa	-60	120	-90	0
Pro1-Pro2				
NOE	-80	170	-89	-1
PDMS-gel	-75	166	-83	16
Pro5-Leu6				
NOE	-72	80	-171	-49
PDMS-gel	-34	-15	-125	7

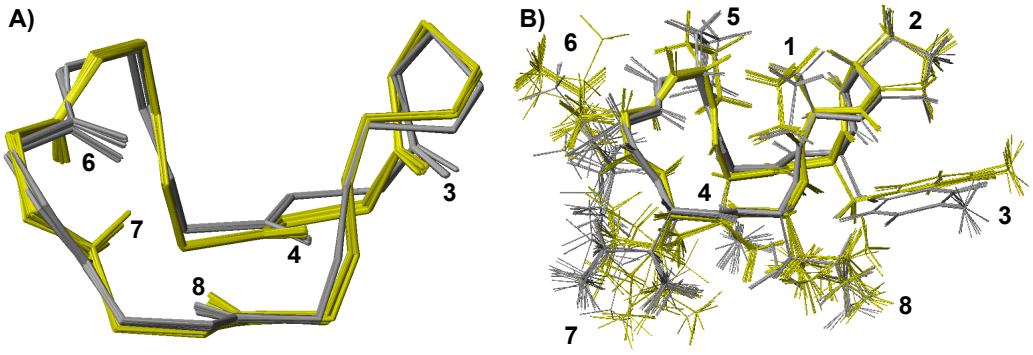

Figure 2.24: Conformation of hymenistatin in THF. The twenty best structures of the NJ ensemble (grey) and the NJR ensemble from PDMS-gel (yellow) have been superimposed. A) Backbone representation including amide protons, B) All atom representation in which the backbone atoms a drawn slightly thicker to guide the eye.

2.2.3.6 Comparison of the DMSO, $CDCl_3$ and THF conformations of hymenistatin

As noted previously, the overall conformations of hymenistatin in DMSO, $CDCl_3$ and THF are very similar, which can be seen in Fig.2.25 where the twenty best energy structures of each NJR ensembles of the PH-gel/DMSO, PDMS-gel/$CDCl_3$ and PDMS-gel/THF are superimposed (RMSD = 0.312Å). The major effect of the solvent on the conformation has been found for the orientation of the amide proton

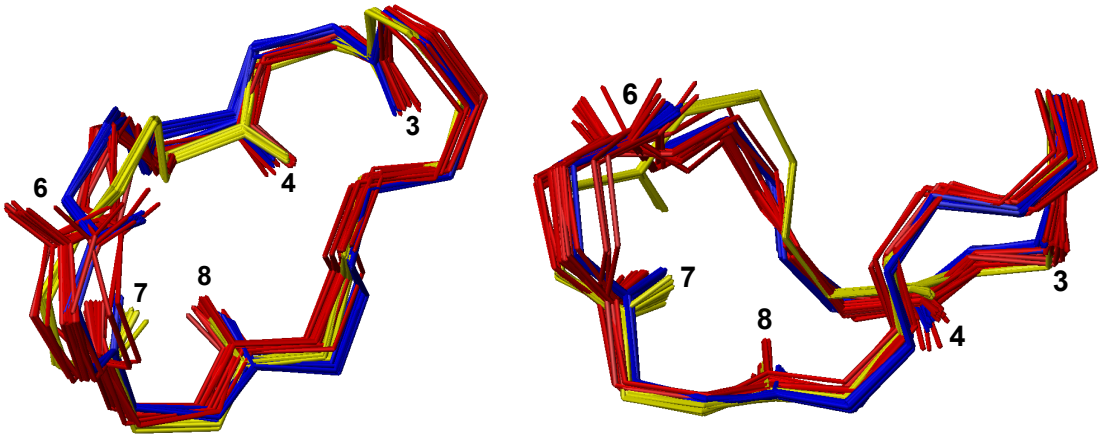

Figure 2.25: Two different views of the superposition of the twenty best energy backbone structures including amide protons of hymenistatin of the three NJR ensembles of PH-gel/DMSO (blue), $CDCl_3$ (red) and THF (yellow).

of Leu6. The solvent effect on this proton can be appreciated by the comparison of the ^{1}H-spectra of hymenistatin in the three different solvents (Fig.2.26). The amide

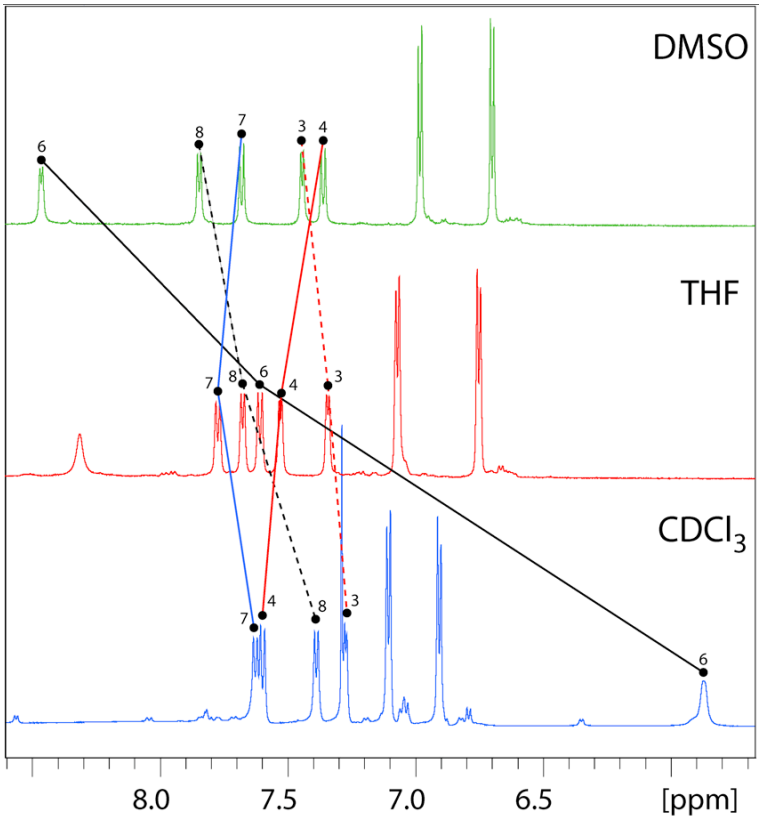

Figure 2.26: Comparison of the ^{1}H-spectra of the amide region of hymenistatin in DMSO, THF and $CDCl_3$ recorded on a 600 MHz spectrometer.

proton of Leu6 is very solvent exposed in the DMSO-conformation (Tab.2.14), forms intermolecular H-bonds and therefore unveils an downfield shift in the ^{1}H-spectrum compared to the other amide protons. In contrast, $CDCl_3$, which is less polar than DMSO, subsequently can not form intermolecular H-bonds with hymenistatin, which leads to an upfield shift in the ^{1}H-spectrum of the amide proton of Leu6 compared to the other amide protons. Furthermore the shifted amide resonance is broadened, which might reflect the conformational flexibility of this proton in $CDCl_3$. The solvent accessible surface of the two conformations found for the NJR ensemble of $CDCl_3$ are listed in Tab.2.14. The solvent accessibility for the highly-populated conformation C62 is significantly lower, which is reasonable as a conformation of hymenistatin in $CDCl_3$ where the protons are very solvent accessible is unfavourable due to the

Table 2.14: Solvent accessible surface of the Leu6 amide proton in the different NJR ensembles of hymenistatin. The surfaces have been calculated using the program Macromodel [43]

solvent **alignment medium**	**DMSO** **PH-gel**	**DMSO** **PPH-gel**	**DMSO** **PAN-gel**	**$CDCl_3$** **PDMS-gel**	**THF** **PDMS-gel**
solvent accessible surface [Å^2]	2.6 ± 0.3	3.6 ± 0.3	4.1 ± 0.5	3.3 ± 0.6 (C61) 1.2 ± 0.8 (C62)	0.0 ± 0.0

solvents lack to form intermolecular H-bonds. In THF no solvent effect could be observed on the ^{1}H chemical shift of the amide proton of Leu6 as all amide protons resonate in the same chemical shift region. This can be explained by the fact that no solvent accessible surface could be detected for the Leu6 amide proton in the THF conformation of the NJR ensemble.

2.2.3.7 Configuration of hymenistatin

Residual dipolar couplings are a very powerful tool to provide the correct assignment of diastereotopic protons as has been abundantly shown for compounds with rigid conformation [10, 11]. Therefore it was our aim to simultaneously determine the conformation and configuration of the thirteen prochiral centers of hymenistatin making combined use of NOEs, J-couplings and RDCs as restraints in a simulated annealing calculation. In our refinement protocol (described in 5.6.2), we used a special force field that allowed methylene protons to switch positions during the first high temperature stage of the calculation which is known as floating chirality [44]. In the second high temperature stage the geometries of the prochiral centers were fixed, before the structures were further refined in a cooling stage and powell minimization. The twenty lowest energy structure for the NJR ensembles of PH-gel/DMSO, PPH-gel/DMSO, PAN-gel/DMSO and PDMS-gel/$CDCl_3$ and the NR ensemble for PDMS-gel/$CDCl_3$, the two NJ ensembles for DMSO and $CDCl_3$ and the NOE ensemble of THF were then inspected, if the correct assignments of all thirteen prochiral centers could be achieved. A prochiral center was considered assigned, if 80% out of the twenty lowest energy structures converged to the same attribution.

Tab.2.15 lists all measured RDC data sets. Values in bold indicate that for these prochiral centers no assignment could be achieved, values with a grey background indicated that they could be stereospecifically assigned in the NJ ensembles or the NOE ensemble and for values with a yellow background the assignment from the NOE and NR ensemble differs.

There are various reasons, why the assignment of a prochiral center will fail. First of all, if the difference in the value of the two RDCs for a prochiral center is small compared to the range of the measured RDCs, the orientations of the internuclear vectors with respect to the alignment tensor are very similar and a differentiation becomes impossible.

Secondly, if the difference in the RDCs of a prochiral center is large and the assignment fails, the following considerations have be taken into account. Conformational flexibility of a residue will lead to an increased averaging of the RDC values compared to more rigid parts in the molecule on one hand and to multiple solutions in the refinement procedure due to the increased conformational space, that can be sampled by flexible moieties on the other hand. For example the RDCs of the prochiral center C_γ of Ile7 in the PAN-gel/DMSO data set are reasonably different and therefore one would expect to achieve a clear assignment for this moiety. Unfortunately this prochiral center is two rotatable bonds away from the more rigid backbone and multiple solutions for the orientation of the internuclear bond vectors are found during the structural refinement.

Thirdly, an unfavourable orientation of the alignment tensor can yield multiple solutions for a prochiral center even if the difference in the RDCs is large. This is often the case when prochiral centers in proline residues or the prochiral C_β of Tyr and Leu fail to show an assignment. This fact can best be explained by inspection of the prochiral C_γ of Pro3 for the NJR ensemble of the PPH-gel data set (Fig.2.27). This moiety has a pseudo-assignment, meaning although the prochiral assignment is mixed up, the RDC with the positive value has been attributed to the axial protons within the two conformations and the RDC with the negative to the equatorial protons. That happens because the axial protons have the same orientation with respect to

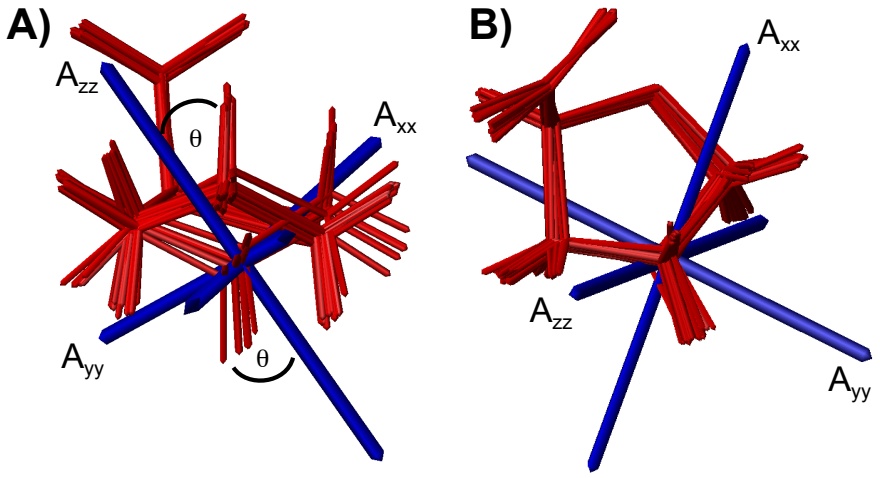

Figure 2.27: Pro3 of the NJR ensemble of PPH-gel along with the principal axis system of the alignment tensor

the alignment tensor (indicated by the θ angle in Fig.2.27 A)) on the one hand and the equatorial protons are able to fulfill different orientations on the cone of solutions around the A_{yy} principal axis on the other hand. The use of a second RDC data set is a possible solution for the diversity of prochiral assignments in the refinement procedure due to conformational flexibility and the unfavourable tensor orientations. The cone of solutions for one alignment tensor then reduces to the intersecting regions of the two cones of two alignment tensors, which drastically reduces the possible orientations of the internuclear vectors. Calculations with multiple RDC data sets are currently underway for DMSO and will hopefully resolve the configurational ambiguities for the DMSO configuration in the future.

Finally, some moieties like C_γ Pro5 fail to show an assignment in the NJR and NR ensembles, although the NJ or NOE attribution is provided. Surprisingly this moiety could only be assigned in the NJ ensemble of DMSO, but in non of its NJR ensembles even if no RDC was provided for the structural refinement. Closer inspection of the NOE data revealed, that an NOE between one H_γ and the H_α of Pro5 causes the difference. In the NOE ensemble Pro5 adopts a single conformation, while in the NJR ensemble this NOE is fulfilled with a pucker of C_γ or a tolerable NOE violation if a single conformation is found as the difference in the distance between the H_α and the two H_γ in a *trans*-proline is just 0.2 Å. For DMSO eight out of eleven

prochiral centers for which at least one RDC was extracted could be assigned with our structural refinement protocol. These are three additional attributions compared to the NJ-ensemble. The missing assignments concern the two C_γ moieties of Ile7 and Ile8 and the C_γ of Pro5, which have been discussed above. For chloroform, five out of ten possible assignments could be achieved, which is an increase of four attributions compared to the NJ ensemble. Again an unfavourable tensor orientation and/or the small difference of the RDC compared to the range of RDCs measured account for the failed assignments. For THF eight of the nine possible prochiral assignments could be achieved. That are two additional assignments compared to the NOE ensemble, but in contrast to the DMSO and $CDCl_3$ configuration the assignment for the two prochiral centers of Pro2-C_β and Pro5-C_δ differed in the NOE and NR ensemble. It is important to note, that no significant difference in the NOE violations could be observed upon closer inspection of those two moieties. To resolve this problem an E.COSY experiment to obtain $^3J_{HH}$ will be measured to obtain more insights in the conformation of these proline residues. It will be interesting to see in the future wether the NOE or NR ensemble resolved the configuration of those prochiral centers correctly.

Table 2.15: Residual dipolar couplings of hymenistatin. Values in bold represent prochiral centers where the configurational assignment could not be achieved. Values with a grey background have a prochiral assignment in the NJ ensemble or NOE ensemble, respectively. For values with a yellow background the assignment from the NOE ensemble differs from the NR ensemble

	PH-gel/ DMSO	**PPH-gel/ DMSO**	**PAN-gel/ DMSO**	**PDMS-gel/ $CDCl_3$**	**PDMS-gel/ THF**
Pro1					
HA	-6.4	20.9	40.7	-8.1	5.2
HB1	0.0	-8.8	3.7	-11.7	-4.8
HB2	14.1	-4.9	-9.2	23.0	5.8
HG1	-	-	-	**-12.0**	-
HG2	-	-	-	**-24.9**	-
HD2	-2.1	-	-20.9	-10.6	-11.7
HD1	-4.1	-	32.3	-14.0	4.6
Pro2					
HA	-13.0	-18.0	-22.4	-32.7	-13.9
HB1	-	-	-	-14.5	2.8
HB2	-	-	-	31.0	16.0
HG1	9.0	**-16.6**	10.5	**16.5**	**-1.1**
HG2	7.5	**15.9**	-14.1	**12.9**	**6.1**
HD1	-10.4	**-**	-	**21.6**	11.3
HD2	7.1	**-**	-	**10.6**	7.7
Tyr3					
HA	-8.6	8.3	37.1	-13.3	-0.5
HB1	-3.1	17.3	5.1	**23.6**	-2.8
HB2	-11.2	3.2	15.0	**-11.3**	15.3
Val4					
HA	-7.1	18.3	42.5	-	4.4
HB	-	-	-	-14.0	-
Pro5					
HA	6.6	0.7	-11.1	17.0	5.0
HB1	-	**3.6**	-20.6	34.9	12.5
HB2	-17.0	**-13.0**	-25.2	-13.2	-7.0
HG2	**-8.8**	**-**	**-**	-	-
HG1	**5.2**	**-**	**-**	-	-
HD2	13.2	-	-	-37.3	5.7
HD1	-5.4	-	-	32.9	-15.5
Leu6					
HA	21.8	18.5	-6.9	36.4	16.0
HB2	16.8	17.7	**-3.0**	**28.1**	13.2
HB1	-19.2	4.7	**18.7**	**-13.2**	-4.6
HG	-	4.7	12.8	-14.3	-0.7
Ile7					
HA	-20.4	7.4	23.6	-23.5	-9.4
HB	-16.3	4.2	20.6	-27.1	-9.8
HG12	-	**2.8**	**16.3**	-	-
HG11	**-8.1**	**6.5**	**-2.0**	-	-
Ile8					
HA	-5.3	21.6	33.9	-5.7	5.9
HB	-15.5	7.0	25.1	-14.9	-0.9
HG12	**-8.5**	**0.4**	**13.4**	-	-
HG11	**-10.1**	**6.1**	**1.0**	-	-

2.2.4 Determination of the relative configuration of diastereomers

2.2.4.1 Introduction

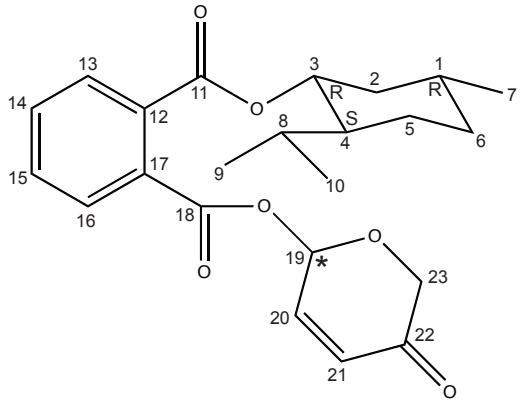

Figure 2.28: Formula of ((-)-menthyl)-(5-oxo-5,6-dihydro-2H-pyran-2-yl)-diphtalate (DiaA) with numbering. The unknown stereocenter is marked with an asterisk, stereocenters known are indicated with their correct configuration.

((-)-menthyl)-(5-oxo-5,6-dihydro-2H-pyran-2-yl)-diphtalate (DiaA) (Fig.2.28) presented itself to us as a very challenging test molecule for the elucidation of its relative configuration. This compound could neither be crystallized nor is it possible to elucidate its relative stereochemistry with traditional NMR parameters like NOE and J-couplings as the ester moieties form breaks in the H-bonding network. Generally one can divide this molecule into three subunits. A menthyl-subunit for which the configuration is known, an aromatic-subunit and a pyranyl-subunit with unknown stereochemistry at position 19. These three subunits are linked with two ester bonds each bearing three rotatable bonds providing DiaA with more flexibility than the previous examples of hymenistatin or menthol. Therefore the aim of this project is to reaveal by means of the "real case" DiaA, that it is possible to determine the relative configuration of a quite flexible small molecule.

2.2.4.2 Determination of the relative configuration

Two NOE ensembles for the R- and S-diastereomer were created using eighteen NOEs and 54 non-NOEs with the refinement protocol described in 5.6.3. The non-NOEs

were used to prevent the refinement to position protons in close proximity for which no NOEs were observed. Furthermore two sets of RDCs were measured in DMSO. One in PH-gel and the other in PAN-gel. Attempts to measure a third RDC data set in PPH-gel failed, because DiaA showed an accelerated degradation process in this gel compared to isotropic DMSO solution, which made the measurement of RDCs impossible. The RDCs derived from the PH-gel (listed in C.3.3.1) and the PAN-gel (listed in C.3.3.2) were fitted with the SVD module of PALES to every single structure of the NOE ensembles for the R- and the S-diastereomer. Correlation plots of the observed versus backcalculated RDCs for the best fitting structure of each ensemble can be found in Fig.2.29.

The correlation factors of 0.98 and 0.94 for the S-diastereomer compared to 0.91 and 0.93 for the R-diastereomer for the PAN-gel and PH-gel RDCs indicate that the S-configuration is the correct one. However, this molecule is flexible due to the six rotatable bonds connecting its three subunits so that NMR observables like NOEs and RDCs generally do not reflect the measurement of a single conformer, but rather a collection of interconverting structures. Therefore comparing the best fit to a single structure can only be an indication for the right configuration. To get a more dynamic picture we decided to fit the RDCs to an ensemble of four structures, that are randomly picked out of the NOE ensembles of the R- or S-diastereomer. Hereby the RDCs do not have to fulfill a single structure, but the ensemble of structures. The ten best overall Q-factors for both RDC data sets are listed in Tab.2.16. The significantly lower Q-factors for the NOE ensembles of the S-diastereomers again evidence the better correlation of the RDCs. Due to the sparse data, that can be obtained for this molecule it will be necessary to cross-validate these results in the future. The approach will be to generate an additional conformational ensemble for both diastereomers from quantum-chemical calculations using a solvent box to ensure the correctness of the NOE ensembles derived from NOEs and non-NOEs.

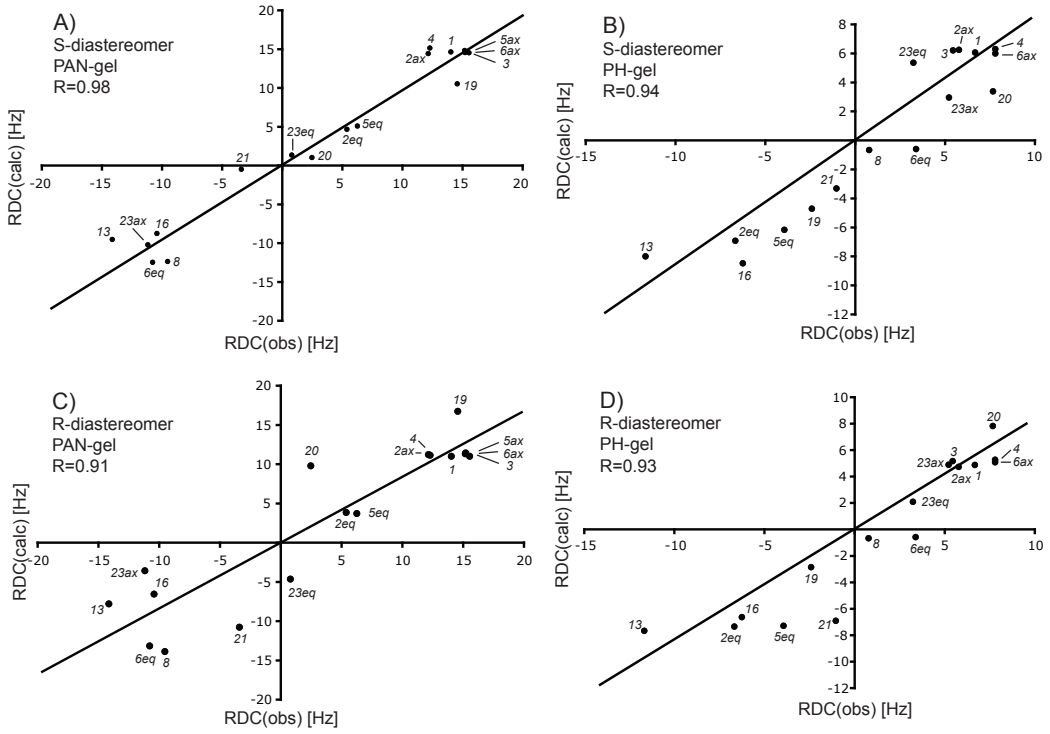

Figure 2.29: Correlation plots between the observed (RDC(obs)) and backcalculated RDCs (RDC(calc)) of DiaA. A) PAN-gel RDCs fitted on the structure of the S-diastereomer, B) PH-gel RDCs fitted on the structure of the S-diastereomer, A) PAN-gel RDCs fitted on the structure of the R-diastereomer and A) PH-gel RDCs fitted on the structure of the R-diastereomer.

Table 2.16: Best ten overall Q-factors for the PH- and PAN-gel RDCs for the R- and S-diastereomers of DiaA

	Q-factor [ ]	
ensemble	**R-diastereomer**	**S-diastereomer**
1	0.206	0.151
2	0.216	0.154
3	0.217	0.172
4	0.230	0.176
5	0.234	0.182
6	0.234	0.182
7	0.235	0.186
8	0.240	0.187
9	0.242	0.187
10	0.244	0.191

2.3 Conclusion and outlook

2.3.1 Properties of the PH- and PPH-gel

In summary, we have shown that reliable alignment can be obtained by using PH- and PPH-gel with DMSO and other solvents for complex organic molecules of various sizes. The magnitude of the dipolar couplings is in a range where they can be evaluated accurately since they are large enough to be measured with an acceptable spectral resolution since ^{1}H,^{1}H dipolar couplings do not cause extensive line broadening. The alignment of the two gels is independent and can be scaled by the use of NMR-tubes with different diameters.

2.3.2 Conformational refinement of hormaomycin

We have shown that the structure of hormaomycin in DMSO differs from the NMR structure determined in H_2O and the crystal structure. Especially the bulky side chain is flexible in DMSO which is not the case in chloroform. The secondary structure of hormaomycin adopting two β-turns is identical in DMSO and chloroform but differs from the crystal structure obtained from ethanol in which the two β-turns are rotated by one residue. The structure in DMSO could be refined using residual dipolar couplings obtained from the PH-gel. Without RDC refinement two major conformations for the macrocyclic ring were derived, whereas after refinement with RDCs from the backbone and the side chains, only one of the two macrocyclic ring conformations remained valid. Since there is a strong dependence of the conformation adopted on the solvent system in the absence of knowledge about interaction partners it remains unclear which conformation is the biologically active. The bulky sidechain is quite unstructured in the refined NJR ensemble. Future work will investigate the mobility of the bulky sidechain via measurements of RDCs in a second alignment medium and subsequent structure calculations.

2.3.3 Conformational and configurational refinement of hymenistatin

We could obtain five sets of RDCs in three different solvents. The RDC data sets were used to refine the conformation in DMSO, $CDCl_3$ and THF and we could show that the conformation changes locally at residue Leu6 in the different solvents. For DMSO the conformation previously published by *Konat et al.* could be reproduced, whereas the chloroform conformation differed significantly. Thorough analysis of the previously published NOE data and solvent accessible surfaces could confirm that Ile8-NH is forming a H-bond with Val4-CO and not with Leu6-CO as stated by *Konat et al.*. The conformation of hymenistatin in THF has been elucidated for the first time. The overall fold has been found to be similar to the conformation in DMSO and $CDCl_3$. A βVIa turn is formed around Pro1-Pro2 like in DMSO and $CDCl_3$, but around Pro5-Leu6 a βI turn was found, whereas in DMSO and $CDCl_3$ (C61) a βII and in $CDCl_3$ (C62) no turn could be assigned. RDCs proved to be a helpful tool to assign the configuration of the thirteen prochiral centers of hymenistin. Refinement protocols including one RDC data set lead to additional assignments of one to four prochiral moieties compared to structures only refined with NOE and J-couplings. Prochiral centers for which RDCs could be obtained sometimes failed to show configurational assignment due to conformational flexibility and/or an unfavourable tensor orientation. Therefore, future investigations will focus on refinement protocols with multiple RDC data sets to reduce orientational ambiguities of internuclear bondvectors.

2.3.4 Determination of the relative configuration of diastereomers

Two sets of RDCs could be extracted for DiaA, which have been fitted to NOE ensembles generated for both diastereomers. The single structure, that fulfilled both sets of RDCs best, exhibited a S-configuration for the unknown configuration of the stereocenter at position 19. To account for the flexibility of this molecule the RDCs were fitted on an ensemble of four structures randomly picked from the NOE ensembles of

each configuration. The overall Q-factors for both alignment media again indicated a better correlation for the S-configuration. Future work concerns quantum chemical calculations with a solvent box to obtain an independent conformational ensemble and cross-validate our findings.

3

Structural investigations of PPIase domain of trigger factor and its complex to suc-AAPF-pNA with paramagnetic tagging

The aim of our investigations was to test new paramagnetic tags, that have been developed in our group, and to obtain the structure of the complex between the artificial ligand suc-AAPF-pNA (*N*-succinyl-alanine-alanine-proline-phenylalanine-*p*-nitroanilide) and the PPIase domain of trigger factor from *Mycoplasma genitalium*. All the studies presented here were carried out on the central domain of trigger factor, which was found to be responsible for the PPIase activity of the protein.

3.1 Introduction

3.1.1 Biological background of trigger factor

A peptide chain folding into its native conformation undergoes many transitions. One of those, the *cis-trans* isomerization of the peptidyl-prolyl bond is catalyzed by the peptidyl-prolyl isomerases (PPIases) [45]. The *cis* and the *trans* conformation are separated by a high energetic barrier [46] leading to a slow interconversion between them. For proline, different from all other aminoacids, the cis-peptidyl bond plays a significant role in the peptide backbone, making the peptidyl-prolyl cis-trans isomerization a crucial step in the folding process of proteins. Supporting this assumption

are results showing the impact of PPIases on the rate of *in-Vitro* protein folding [47]. Three structurally unrelated subclasses of PPIases are identified so far depending, on their binding to different drugs. The FK506 binding proteins (FKBPs), the parvulins and the cyclophilins [45], of which parvulins and FKBPs adopt a similar fold of their PPIase domain (FKBP fold) [48], while cyclophilins are structurally unrelated. Another PPIase is the trigger factor, a 59 kDa modular protein with three domains, a N-terminal ribosome binding domain [49], a central PPIase domain and a C-terminal domain of unknown function [50]. It was initially found to be involved in the transport of secretory export proteins in *Escherichia coli* [51], were it seems to stabilize their partially unfolded states [52]. Trigger factor shows high affinity to unfolded proteins independent of proline residues and has the highest *in-Vitro* folding activity of known PPIases [53]. The current view of its function *in-Vivo* is therefore that trigger factor is a multifunctional enzyme having PPIase and chaperone activity [53]. Its importance is outlined by the fact that in the minimalistic genome [54] of the bacterium Mycoplasma genitalium, trigger factor is the only known enzyme having PPIase function so far [55]. Beside the evidence for the significant role of trigger factor, this singularity might lead to new treatments against the pathogenic bacterium. Recently the structure of the trigger factor PPIase domain from *Mycoplasma genitalium* was solved by NMR [56] and x-ray [57] and allowed explanations why the trigger factor despite its strong homology to the FKBP class does not bind FK506 [56].

3.1.2 Paramagnetic tagging

External alignment media are very popular for the determination of structures and dynamics, but there are certain aspects of biomolecular structures and dynamics that are difficult to address using these media and that have led to the introduction of paramagnetic alignment even of diamagnetic molecules. While metal binding proteins often self align for example using lanthanides instead of Ca^{2+}, Zn^{2+} or Mg^{2+}, proteins without metal binding sites need to be tagged in order to achieve sufficiently large alignment. Then not only dipolar couplings can be observed but also pseudocontact shifts that depend on the same magnetic susceptibility tensor as the dipolar couplings.

All contributions from self-alignment for the dipolar couplings can be subtracted by comparing the paramagnetic species with the spectrum of a diamagnetic species.

The first problem using external alignment media is the use of dipolar couplings for the characterization of complexes of small molecules and proteins with medium or low affinity. Small molecules tend to be rather hydrophobic and therefore are incompatible with many or all alignment media. Thus, so far, only sugar/protein complexes were successfully studied due to the large hydrophilicity of oligosaccharides [58].

Another aspect is the study of the structure of symmetrical homodimers [59]. While external alignment can only reveal the axis of symmetry as one of the three principal axes of the alignment tensor, incomplete labeling of a homodimer with a paramagnetic tag leads to two sets of signals that contain information about the relative orientation and contact surfaces of the homodimer. A third aspect is the study of domain motion, since it is enhanced using paramagnetic alignment as compared to external alignment [60, 61]. This is due to the fact that a reduction of the alignment tensor of the non-tagged domain versus the tagged domain can only result from interdomain motion. In contrast to external alignment media, for paramagnetic tagging, the alignment tensors for two domains can only be the same if they are static with respect to each other. However, identical alignment tensors induced by external alignment, must not be interpreted as a proof for lack of interdomain motion.

Approaches for the alignment of diamagnetic proteins are summarized in Fig.3.1. The approach that has been used is to fuse paramagnetic protein domains to align diamagnetic proteins. Pseudocontact shifts of only 0.05 ppm and RDCs below 1 Hz were obtained in a zinc finger protein where zinc had been replaced by Co^{2+} or Mn^{2+} [62]. A larger alignment was observed using EF hands loaded with Dy^{3+} and Tb^{3+} if the EF hand was attached to a peptide that inserts into a micelle (Fig.3.1 A)). The linker length had to be carefully optimized to observe dipolar couplings of the order of 8 Hz [63]. Pseudocontact shifts were not reported in this publication, maybe due to the large distance of the EF hand from the peptide. A similar approach was taken for diamagnetic proteins extending them by a cognate peptide of CaM and binding lanthanide (Tb^{3+}) loaded CaM to this construct (Fig.3.1 B)). Dipolar

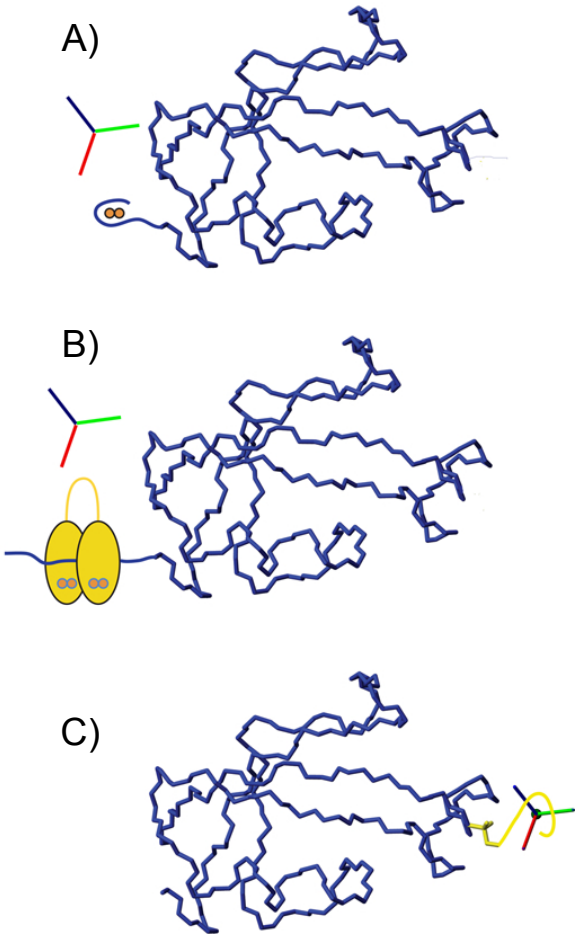

Figure 3.1: Different strategies for paramagnetic tagging. The metal is indicated by orange spheres: A) Tagging by a N- or C-terminal extension. Normally, the extension will be labeled in the same way as the protein of interest. B) Extension by a peptide that is recognized by a metal binding protein such as CaM. The metal binding protein need not be isotopically labeled. C) Attaching a paramagnetic tag to the protein of interest, preferably via a cysteine

couplings of up to 8 Hz and pseudocontact shifts of below 0.05 ppm were observed [64]. While the approach is very elegant, the increase in molecular weight of the complex by approximately 16 kDa called for the reduction of the paramagnetic tag. Also, the metal binding sites of CaM do not have exceedingly high affinities to lanthanides which makes the study of metal binding proteins with this technique impossible. Peptide tags with yet even less affinity to the metal have been developed in the groups of Imperiali and Schwalbe. A seventeen amino acid long peptide attached to the C- or N-terminus of the protein yields dipolar couplings and pseudocontact shifts (Fig.3.1 A)) after careful optimization of the linker length [65]. Different alignment tensors

were observed for different lanthanides as had been observed also for paramagnetic proteins [66].

Small paramagnetic tags that are attached to proteins via cysteines have been introduced in order to reduce the increase in the molecular weight of the protein upon tagging [60] (Fig.3.1 C)). The first chelator was based on DTPA which was attached to the N-terminus of a peptide that bound to CaM (Fig.3.2 A)). Loading of the DTPA with lanthanides could be achieved selectively due to the enormous affinity (10^{-21} M) of this compound to lanthanides. However, it turned out that DTPA exists in different diastereomeric forms that have different alignment tensors [67]. DTPA has been recently used for paramagnetic tagging to two cysteine residues via a double linker [68] (Fig.3.2 B)). However, as observed also for the DTPA alone, there are five

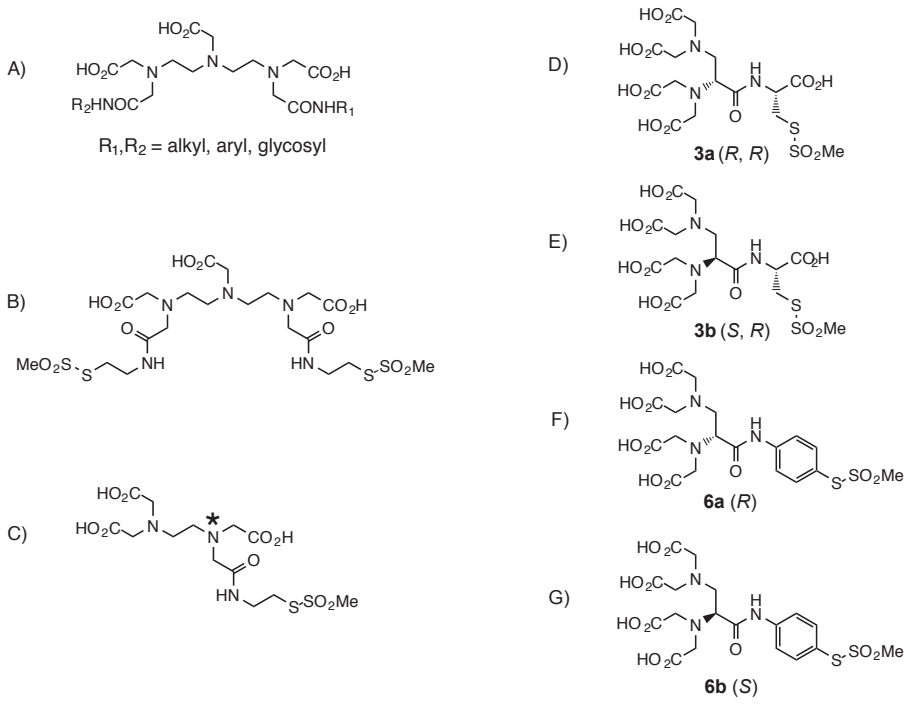

Figure 3.2: Different paramagnetic tags that have been used together with paramagnetic ions. A) DTPA amide, B) the linker used in Ref.[68], C) the linker used in Refs. [59, 62, 69, 70, 71], D) and E) diastereomeric linkers that adopt only one stereoisomer when loaded with lanthanides explored in [69, 72] F) and G) with reduced number of rotatory bonds used in [73]

paramagnetic species in slow exchange. They all lead to different dipolar couplings and different pseudocontact shifts which render the spectrum rather complicated. While in principle the different species can be assigned due to different populations of the diastereomers, it is not practical to work with these highly overlapped spectra. In addition, for resonances that are far away from the tag, the cross peaks for the different species overlap such that only an averaged dipolar coupling can be extracted which would be difficult to analyse.

EDTA derived tags have been used as alternative to DTPA since they are stereochemically less problematic. Several compounds have been proposed that are summarized in Fig.3.2(C-G). The compound in Fig.3.2 C) is easily sythesized from EDTA anhydride and can be linked to single, accessible cysteines in proteins. However, upon binding of lanthanides, the nitrogen atom indicated with a star in Fig.3.2 C) becomes chiral and therefore, at least two different alignment tensors are observed which yield very complicated spectra (vide infra) [69]. At acidic pH and using only bivalent ions (Co^{2+}), the exchange rate between the two chiral forms is fast enough so that only one set of signals is observed (Byrd R.A., personal communication); however, these conditions also loosen the binding of the metal to the tag which can cause other complications. Nevertheless, the orientation of homodimeric proteins could be studied conclusively by inducing pseudocontact shifts and dipolar couplings after substoichiometric addition of Co^{2+} to the homodimer. Then, three resonances are observed for each of the homodimers, the isotropic one when there is no loading, and two if one of the tags is loaded, namely from the loaded monomer and from the non-loaded monomer. Peaks from the doubly loaded homodimer were not observed at the low concentrations of the lanthanide used [70]. In addition, the same authors showed that paramagnetic tagging is useful for the improvement of large structures [71]. The paramagnetic tag in Fig.3.2 C) was investigated in a recent study [69] and tagged to trigger factor. A duplication of cross peaks was observed for this tag when loading the tag with Dy^{3+} (Fig.3.3) after full loading of the tag which was proven by the absence of the isotropic cross peaks in the HSQC spectrum. The reason for this behavior of the tag could be traced back to the chirality introduced at the nitrogen center

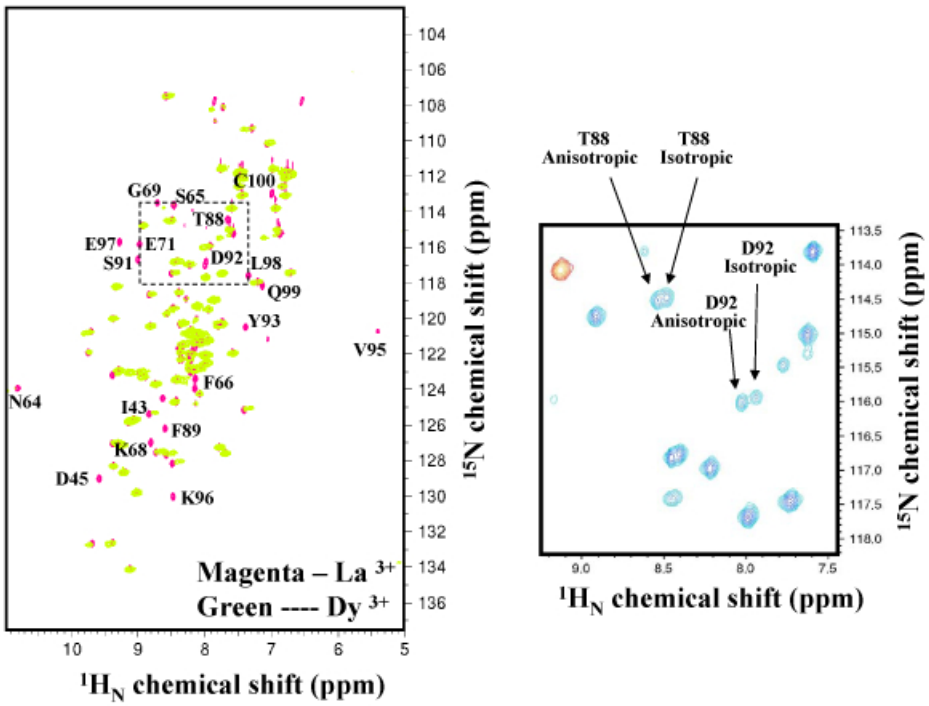

Figure 3.3: Doubling of resonances of a ^{15}N,^{1}H-HSQC spectrum of trigger factor S100C upon loading of the attached tag of Fig.3.2 C) with Dy^{3+}. The right spectrum is an expansion of the left spectrum

(Fig.3.4) by attaching the tag to a small chiral compound 2-phenyl-propaneamine which yielded only one set of signals in the absence of metal but two in the presence of La^{3+} [69]. Thus, the tag should be designed in a way that no additional stereocenter forms upon complexation with the lanthanide. The paramagnetic tags described in Figs.3.2(D-G) [69, 72, 73] do not form new stereocenters when tagged with lanthanides. They have dissociation constants in the 10^{-18} M range [72] and can therefore be used for the investigation of metal binding proteins [73]. They strongly align proteins at 800 MHz and lead to NH dipolar couplings exceeding 8 Hz. The tags are uniquely suited to align metal binding proteins. Apo-calmodulin (Apo-CaM) as depicted in Fig.3.5 was tagged with the compounds of Fig.3.2 F) and G). The

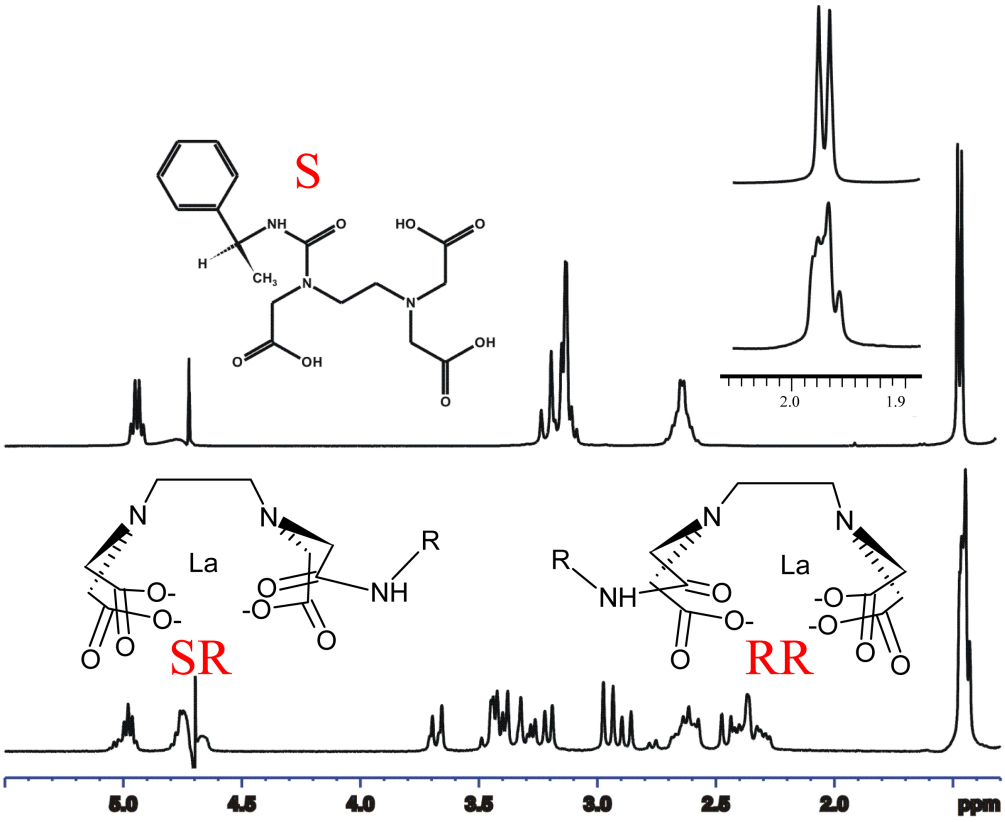

Figure 3.4: The top trace shows the spectrum of the tag of Fig.3.2 C) attached to the chiral compound 2-phenyl-propaneamine. There is only one set of signals. Binding of La^{3+} leads to doubling of the resonances indicating the formation of the chiral nitrogen centre

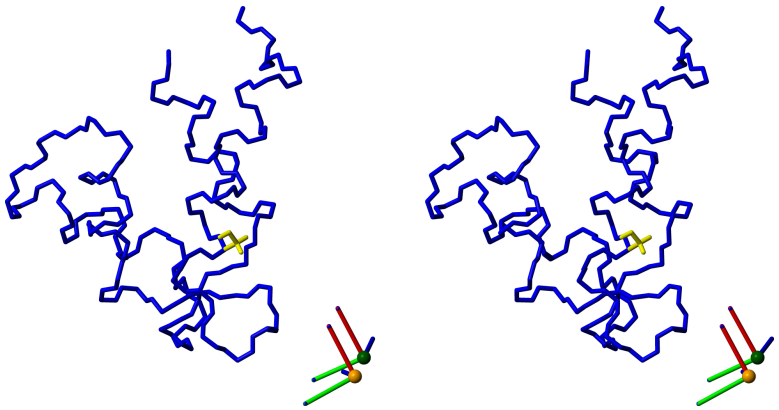

Figure 3.5: Apo-calmodulin (Apo-CaM) tagged with the tags of Fig.3.2 F) and G). The positions of the metals and the tensor orientations are indicated. The single cysteine residue is marked in yellow

observed tensor orientation as well as the metal positions are indicated in the figure. NH dipolar couplings up to 8 Hz were observed in this case at 800 MHz.

3.2 Results and discussion

3.2.1 Paramagnetic tags 6a and 6b

The tags **6a** and **6b** have been attached to the trigger factor mutant TFS100C via a covalent sulfide bond and loaded with Dy^{3+} as described in 5.2.4. An ω_1-coupled ^{15}N,^{1}H-HSQC spectrum of trigger factor is shown in Fig.3.6 for tag **6a** loaded with Dy^{3+}. The overview spectrum indicates the high quality of the sample. No isotropic peaks were detected and NH resonances even in close proximity to the tag site (C100) can be observed, as for example Q99. This is due to the fact that the metal is farther away from the protein backbone for **6a** and **6b** (16 Å) than for the tags **3a** and **3b** (13 Å). The assignment of the peaks is straight-forward, since the anisotropic peaks are shifted on a 45° diagonal (ppm scale) compared to the isotropic cross peaks (indicated in the insert of Fig.3.6). The previously published tags **3a** and **3b** and

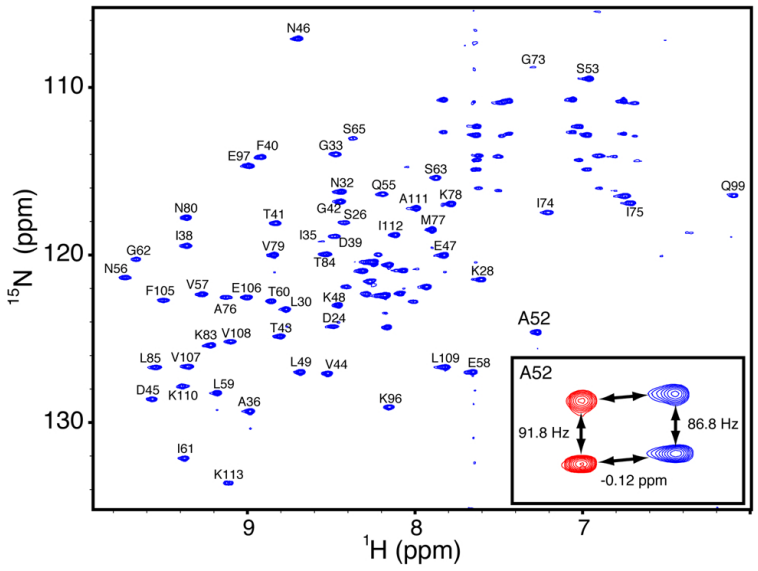

Figure 3.6: Anisotropic ^{15}N,^{1}H-IPAP-HSQC spectrum (high-field cross peak) in the ω_1-dimension. The spectrum only shows isotropic peaks indicating the total loading of the tag with the lanthanide. The insert shows A52 isotropic in red and anisotropic in blue (both cross peaks) with a dipolar coupling of 5 Hz and a pseudocontact shift of -0.12 ppm

the new tags **6a** and **6b** induce alignment tensors that have different orientation and locate the metal at different positions as indicated in Fig.3.7 and Tab.3.1. The size of the measured RDCs ranges from -6 Hz to 8 Hz for all four tags (see C.4.5). The expected increase of the size of the RDCs for the new tags **6a** and **6b** due to their shorter linker could therefore not be observed as the conformational averaging of the RDCs is probalbly dominated by the rotation around the disulphide bond.

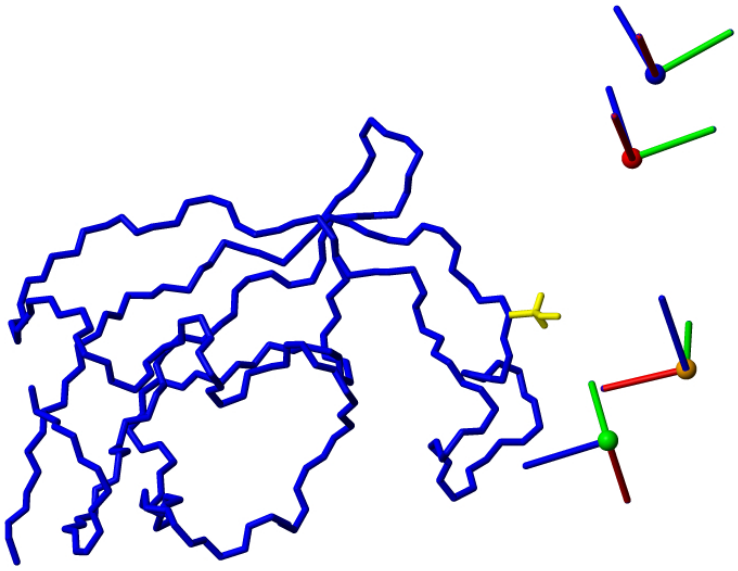

Figure 3.7: Trigger factor S100C mutant tagged with the tags shown in Fig.3.2(D-G). The metal positions (green for tag **3b**, orange for **3a**, red for **6b** and blue for **6a**) as well as the tensor orientations (A_{xx} = green, A_{yy} = blue and A_{zz} = red) are indicated.

Table 3.1: Axial (D_a-HN) and rhombic (Rh) components of the alignment tensors and angles between them achieved with four paramagnetic tags for trigger factor.

tag1/tag2	**angle** [°]	**tag**	D_a**-HN** [Hz]	***Rh***	**distance**[a] [Å]	**R(PCS)**[b] [ ]
3a/3b	69	**3a**	4.1	0.56	13	0.96
3a/6a	147	**3b**	4.4	0.38	13	0.86
3a/6b	157	**6a**	4.3	0.35	16	0.82
3b/6a	120	**6b**	4.1	0.47	16	0.86
3b/6b	119					
6a/6b	8					

[a]Distances from the tagged sulfur atom to the metal position.
[b]Correlation factors between the observed and backcalculated PCS

It has been shown for external alignment media, that it is possible to determine intradomain mobility [74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84] and that it is possible to define structures more precisely when more than one alignment medium is used [85]. Similarly, using different tags for the same binding site improves the precision of the structure determination of trigger factor. When the dipolar couplings induced by the four tags of Fig.3.2(D-G) were used for trigger factor, we obtained a structural ensemble with a RMSD to the mean structure in the backbone of only 0.202 Å (Fig.3.8) while we obtained a RMSD of 0.33 Å to the mean structure using only the dipolar couplings of the tag of Fig.3.2 F). A detailed description of the structural calculations can be found in 5.6.4.

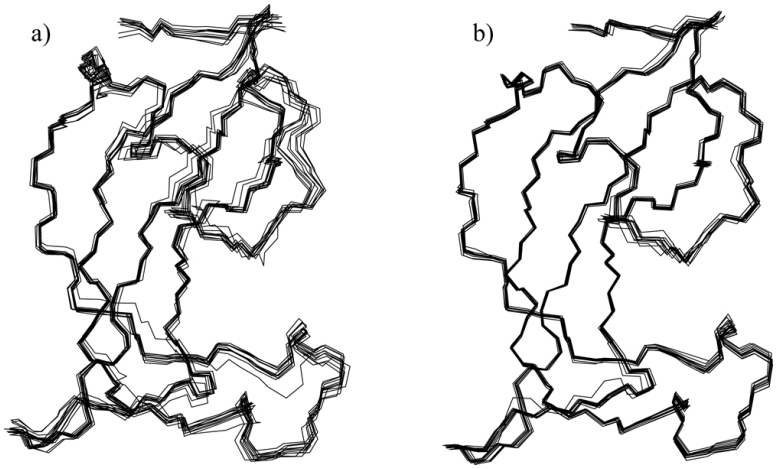

Figure 3.8: Comparison of the structures of trigger factor obtained. A) including the RDCs from from the tag of Fig.3.2 F) and B) all four tags shown in Fig.3.2(D-G). The improvement of the precision of the structure is obvious

3.2.2 Determination of the K_d

The trigger factor/suc-AAPF-pNA complex formation was monitored by recording a series of ^{15}N-^{1}H-HSQC spectra of a 0.3 mM ^{15}N-labeled NMR-sample of trigger factor with a stepwise increasing suc-AAPF-pNA concentration. suc-AAPF-pNA was added from a 20 mM stock solution (d_6-DMF/D_2O) and final suc-AAPF-pNA concentrations were 50, 100, 200, 300, 400, 500, 1000 and 2000 μM. The dissociation constants shown in Tab.3.2 were determined as described in 5.5.2. As example , the fitting curve of residue E71 is shown in Fig.3.9 . All other fitting curves can be found in the appendix (B.5.2). The K_d of the trigger factor/suc-AAPF-pNA complex has

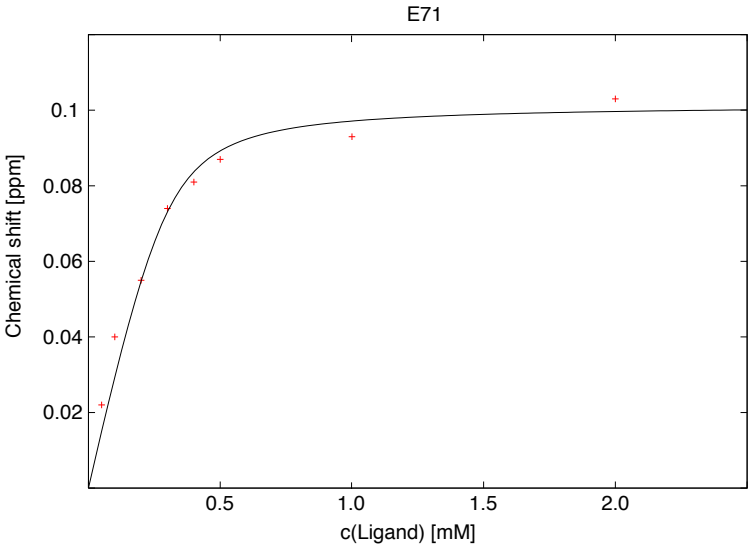

Figure 3.9: K_d fitting curve of residue E71 of trigger factor. For this residue a K_d of 0.033 ± 0.017 could be determined.

been investigated with a d_6-DMSO/D_2O stock solution before [56]. Results yielded a K_d of 0.1 mM, but an affinity of DMSO itself (K_d = 200 mM) to trigger factor was reported as well, which made a reinvestigation of this parameter necessary. The K_d found for the d_6-DMF/D_2O is about a factor of two smaller than the data previously published. This might be due to the lack of competition between the ligand and DMSO or more likely because of the inaccuracy of the K_d-determination by NMR. The errors for the K_d-determination via NMR titration usually range from 33% to 75% [86, 87].

Table 3.2: Experimental K_d-values for the ten residues exhibiting the biggest chemical shift differences upon ligand titration

Residue	**K_d [mM]**
L49	0.011
N56	0.097
N64	0.038
S65	0.027
F66	0.045
E71	0.033
T72	0.027
F105	0.000
E106	0.037
L109	0.052
Average	0.037 ± 0.026

3.2.3 ^{13}C-filtered HSQC pulse sequence

A review on the most general principles and filter elements used in NMR pulse sequences can be found in [88]. Protein-ligand complexes can be tackled by labeling either the ligand or the protein. Labeling the ligand may be preferred if the main interest lies with the ligand-bound conformation. Drawbacks of this approach are that only limited information on the protein partner is likely to be available, and that, unless the ligand can readily be prepared in the desired labeled form by biosynthetic means or by over-expression, synthesis may be technically challenging or excessively costly. In many cases, labeling the protein rather than the ligand, and performing isotope-filtered experiments is then the preferred option for a couple of reasons. Firstly, if the protein is obtained by overexpression, labeling is often reasonably straightforward and usually not prohibitively expensive. Secondly, provided the spectrum of the bound ligand is interpretable, much more information can be obtained from such a system, than if the protein is unlabeled. Thirdly, if the study is part of a drug discovery program, it is usually desired to obtain structural information on complexes with a number of different ligands, and this is most economically done if the ligands are unlabeled. The disadvantage of the labeled protein-approach is

that the spectral information of isotope-filtered experiments on the unlabeled bound ligand are rarely as 'clean' as that from labeled ligands using isotope-editing. Here we introduce a new ^{13}C-filtered HSQC pulse sequence (Fig.3.10). It is based on a standard sensitivity enhanced and gradient selective HSQC [89, 90] pulse sequence and extended by a $^{1}J_{CC}$-stop-filter (The sequence in BRUKER language can be found in the appendix B.5.3). Below, the filter mechanism is described in terms of product

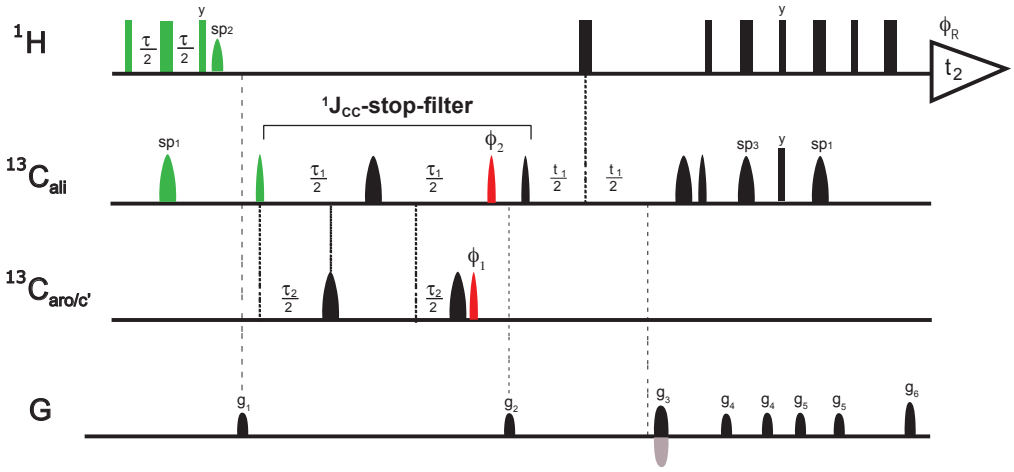

Figure 3.10: ^{13}C filtered pulse sequence. Narrow and thick bars represent 90° and 180° RF-pulses, respectively. Narrow and thick shaped bars correspond to 90° Q5 and 180° Q3 gaussian pulse cascades if not indicated differently. The pulse lengths were calibrated to the desired excitation bandwidth. sp1 is a 180° smoothed adiabatic pulse (60 kHz) with a pulse length of 500 μs ; sp2 is a 1 ms water flip-back pulse; sp3 is a 180° composite adiabatic pulse (60 kHz) with a pulse length of 2 ms. The pulse phases were along x if not indicated otherwise. Phase cycles were: ϕ_1 = x, -x, ϕ_1=ϕ_R = x, x, -x, -x. The delays were set to the reciprocal of the aliphatic $^{1}J_{CH}$ coupling for τ (7.14 ms), the aliphatic $^{1}J_{CC}$ coupling for τ_1 (28.57 ms) and the aromatic $^{1}J_{CC}$ coupling for τ_2 (18.18 ms). PFG durations and strengths were: g_1 = 1 ms at 30 G/cm, g_2 = 1 ms at -27.5 G/cm, g_3 = 1 ms at 40 G/cm, g_4 = 500 μs at 5.5 G/cm, g_5 = 1 ms at -2.5 G/cm, g_6 = 1 ms at 10.05 G/cm.

operator formalism. The first $90^{\circ}_{x}(H)$ pulse turns the equilibrium magnetization H_z into the transversal plane.

$$H_z + C_z \xrightarrow{90^{\circ}_{x}(H)} -H_y + C_z$$

The following two delays $\tau/2$ that are set to $1/(2\ ^1\mathrm{J}_{CH})$ and flank two 180° pulses on each nucleus, transfer the magnetization into antiphase magnetization.

$$-H_y + C_z \xrightarrow{\pi J_{CH}(\tau/2)H_zC_z\ -\ 180^\circ_x(H)\ ,\ 180^\circ_x(C)\ -\ \pi J_{CH}(\tau/2)H_zC_z} 2\ H_xC_z$$

A 90°_y(H) and 90°_x(C) complete the INEPT-transfer (Insensitive Nucleus Enhanced Polarization Transfer) and transform the proton antiphase magnetization onto the carbon.

$$2\ H_xC_z \xrightarrow{90^\circ_y(H)\ ,\ 90^\circ_x(C)} 2\ H_zC_y$$

At this stage one has to differentiate between magnetization of the unlabeled ligand and the labeled protein. For the unlabeled ligand the $^1\mathrm{J}_{CC}$-stop-filter has no effect as the natural abundance of ^{13}C is only 1.01 % and therefore the probability (P_{CC}) of two neighbouring ^{13}C atoms in a C-C_n moiety (with n being the number of neighbouring carbon atoms) is given by Eqn.3.1.

$$P_{CC} = n(1.01 \cdot 10^{-2})^2 \tag{3.1}$$

Subsequently the probability of two neighbouring ^{13}C atoms in the ligand is in the order of 10^{-4} and therefore the loss in magnetization can be neglected.

$$2\ H_zC_y \xrightarrow{\pi J_{CC'}(\tau_1/2)C_zC'_z\ -\ 180^\circ_x(C)\ -\ \pi J_{CC'}(\tau_1/2)H_zC_z} 2\ H_zC_y$$

The labeled protein evolves a $^1\mathrm{J}_{CC}$ coupling and therefore magnetization is transformed to -4 $H_zC_xC'_z$ with C' being the neighbouring carbon and τ set to $1/(2\ ^1\mathrm{J}_{CC})$. In the ^{13}C-filtered HSQC pulse sequence in Fig.3.10 magnetization evolving an aromatic or aliphatic $^1\mathrm{J}_{CC}$ coupling is filtered simultaneously by setting τ_1 and τ_2 accordingly.

$$2\ H_zC_y \xrightarrow{\pi J_{CC'}(\tau_1/2)C_zC'_z\ -\ 180^\circ_x(C)\ -\ \pi J_{CC'}(\tau_1/2)H_zC_z} -4\ H_zC_xC'_z$$

The following 90°_{x}(C) pulse concludes the $^{1}J_{CC}$-stop-filter and turns magnetization from the protein into unobservable multiple quantum coherence and magnetization arising from the unlabeled ligand onto the z-axis.

$$2\ H_zC_y \xrightarrow{90^{\circ}_{x}(C)} 2\ H_zC_z$$

$$-4\ H_zC_xC'_z \xrightarrow{90^{\circ}_{x}(C)} 4\ H_zC_xC'_y$$

A cartoon of the suppression pathway for magnetization that evolves an aromatic and aliphatic $^{1}J_{CC}$ coupling can be found in Fig.3.11 As one can clearly see from

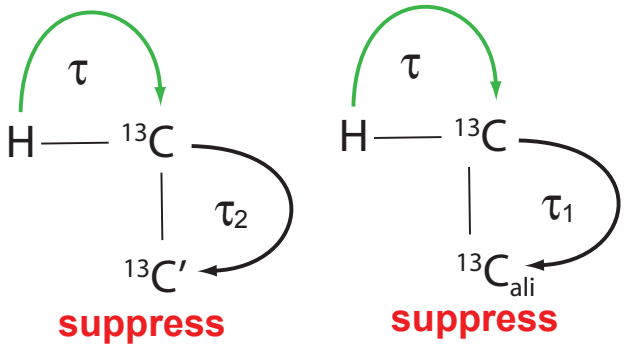

Figure 3.11: Scheme of the suppression pathway

the traces through the ^{13}C-filtered HSQC spectrum shown in Fig.3.12 RDCs of the ligand are detectable in the range of -2.8 to 1.1 Hz. The ^{13}C-filtered HSQC spectrum (Fig.B.7) could not be perfectly phased in the direct dimension (t_2), which lead to an opposite phase of the C_α-H_α and C_β-H_β resonances. That is most probably due to chemical shift evolution of the carbons during the filter element so that a pure phase at the start of the evolution time (t_1) is not present. For the extraction of RDCs the traces of the C_α-H_α and C_β-H_β resonances were phased seperately. Unfortunately only two out of four C_α-H_α and two out of six C_β-H_β resonances could be extracted due to the rather poor quality of the filtered HSQC spectrum, which can be found in Fig.B.7 and due to the signal overlap with resonances from the solution buffer and protein signals that "survived" the filter element. Due to the poor quality of filtered HSQC spectrum a discussion on the error of the measured RDCs has to be carried

out, which depends on a number of factors [91]. The two most important ones are the ratio of the signal line width to the dipolar splitting and the signal to noise ratio. The first factor can be neglected in our case as the line width at half-hight of our signal is about 6-7 times smaller than the $^1J_{CH}$ coupling. For the evaluation of the error that is introduced by the signal to noise ratio of the spectrum, the following test has been performed. To the isotropic and anisotropic trace of Ala1 C_α-H_α ten different traces with pure noise have been added and subsequently the RDC was extracted. The list of RDCs along with its mean value and standard deviation can be found in Tab.3.3.

Table 3.3: Evaluation of the error on the extracted RDC introduced by the signal to noise ratio

Trial	**RDC [Hz]**
1	-3.3
2	-2.6
3	-2.8
4	-2.1
5	-2.5
6	-2.5
7	-3.3
8	-2.2
9	-2.5
10	-2.0
Average	-2.6 $\pm$ 0.5

By incident, a two-fold excess of ligand has been added to trigger factor. This results in a decrease of the size of the measured RDCs by a factor of two as only the ligand bound to the aligned trigger factor gives rise to RDCs. H-N RDCs for trigger factor bound to the ligand range from -5.4 to 10.0 Hz at 900 MHz (data not shown). Taking into account the different gyromagnetic ratios of carbon and nitrogen, the different bond lengths as well as the fact that the ligand RDCs have been measured at 800 MHz, RDCs can be expected in the range of -8.6 to 15.8 Hz. The smallest RDC measured for the ligand has a value of -2.8 Hz and would be -5.6 Hz taking the concentration ratio into account. As only four RDCs could be obtained, which by

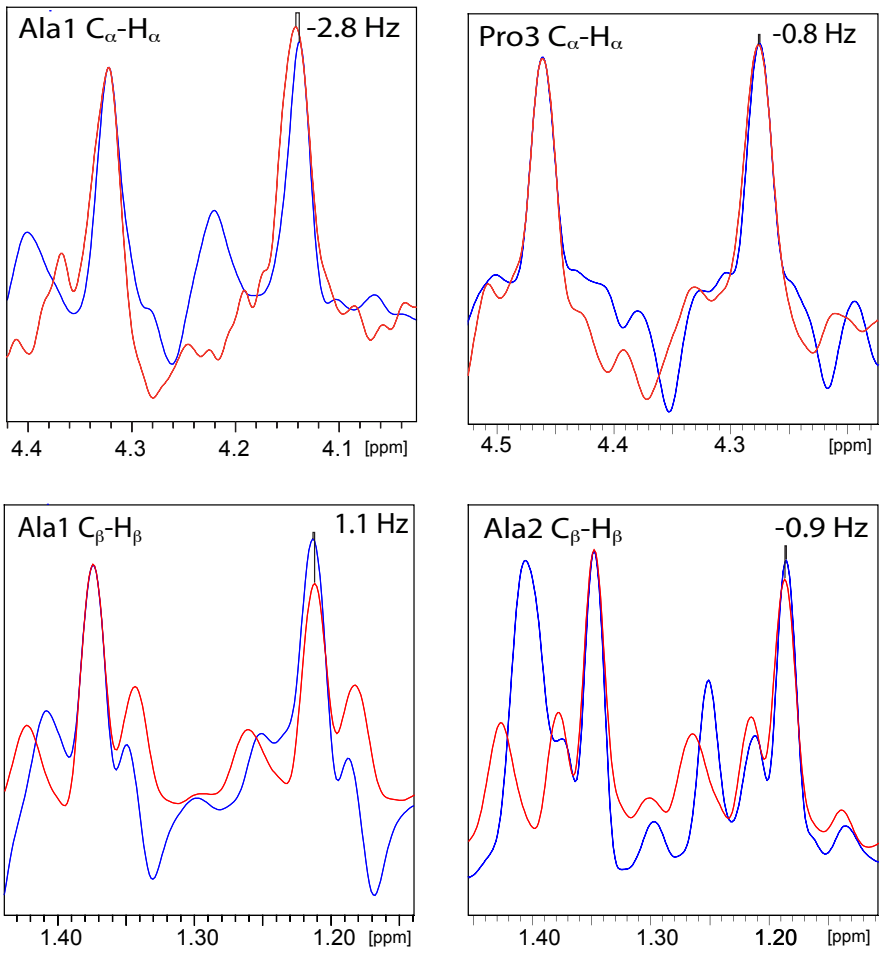

Figure 3.12: Traces trough the 800 MHz ^{13}C-filtered HSQC spectra of suc-AAPF-pNA bound to trigger factor. The isotropic trace is shown in blue and the anisotropic one in red.

no means samples all the conformational space one can conclude that the measured RDCs are in the correct range.

Ala1-H$_\alpha$ showed the biggest pseudocontact shift (PCS) with 0.011 ppm (Tab.3.4) of the extracted resonances (0.022 ppm taking the concentration ratio into account). For comparison the PCS of the ten residues exhibiting the biggest chemical shift upon ligand titration range from -0.135–0.237 ppm (see Tab.B.6). As the structure of the suc-AAPF-pNA bound to trigger factor is not known, the internuclear distances of the shifted nuclei to the metal ion are also unknown, which makes a conclusion about our measured PCS difficult. In Fig.3.13A) all amide protons exhibiting a positive

Table 3.4: Extracted pseudocontact shifts for suc-AAPF-pNA

	PCS [ppm]
Ala1-H$_\alpha$	0.011
Ala1-H$_\beta$	0.005
Ala2-H$_\beta$	0.001
Pro3-H$_\alpha$	0.000

PCS are drawn as red spheres and all negative ones as blue spheres. In Fig.3.13B) only PCS in the range of 0.00–0.04 ppm are indicated. By looking at the distribution of the PCS in Fig.3.13 and their trend (biggest PCS for Ala1 and smallest for Pro3) there is a slight indication, that in the complex structure of trigger factor/suc-AAPF-pNA the ligand is oriented as such that residue Ala1 is positioned deeper inside the binding pocket, where only positive PCS could be measured, and Phe4 towards the outside. Future investigations will focus on improving the ^{13}C-filtered pulse sequence to measure sufficient RDCs and PCS of the ligand to prove this binding mode.

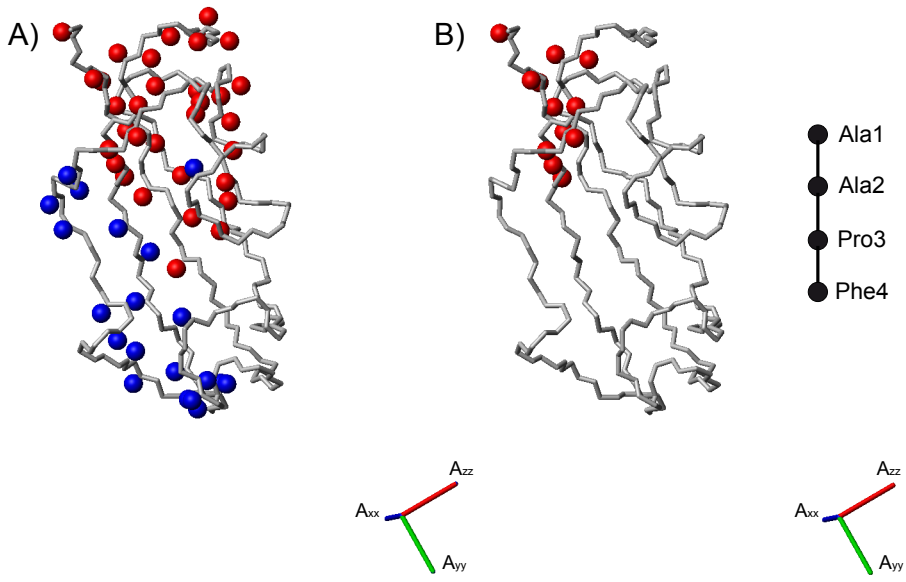

Figure 3.13: Backbone structure of trigger factor tagged with **6a** and loaded with Dy^{3+}. A) All amide protons showing a positive PCS are drawn as red spheres and negative ones as blue spheres. B) Only amide protons exhibiting a PCS in the range of 0.00–0.04 ppm are indicated.

3.3 Conclusion and Outlook

We developed two new tags, inducing new alignments that are linearly independent from those induced by previously published tags. In contrast to metal binding protein domains that normally have binding affinities only in the μM to nM range and considerably increase the molecular weight of the protein, paramagnetic tags based on EDTA have a small molecular weight that is comparable to an extension of the protein by only three amino acids. The tags are introduced at a single cysteine site that can be incorporated at any given protein position. For trigger factor the additional alignment tensors allowed us to improve the precision of the structure. The extremely large affinity of the tag to lanthanides allows to investigate proteins that have tight metal binding sites such as apo-CaM and is therefore a versatile tag for all kinds of proteins. Furthermore work towards the investigation of the protein/ligand complex trigger factor/suc-AAPF-pNA, that could not have been carried out with external alignment media due to unwanted interactions of the ligand, has been shown. A new ^{13}C-filtered pulse sequence has been introduced to measure RDCs of the non-labeled ligand in complex with the labeled protein. Future work will focus on improving the filter efficiency to accurately measure sufficient ligand RDCs and PCS, which will yield the complex structure by means of structural calculations.

4

Materials

4.1 Culture media and antibiotics

All culture media were prepared according to Table 4.1 followed by autoclave sterilisation. Before use, 50 μg/l of ampicillin and 34 μg/l of kanamycin was added to every medium. For agar plates 15 g agar per 1 l medium were added before autoclaving.

Table 4.1: Culture media and antibiotics

NAME	AMOUNT	CHEMICAL	REMARK
LB-medium	10 g	tryptone	
	5 g	yeast extract	
	10 g	NaCl	
	up to 1 l	H_2O	
M9-minimalmedium	6.8 g	Na_2HPO_4	
	3 g	KH_2PO_4	
	0.5 g	NaCl	
	1 g	NH_4Cl or $^{15}NH_4Cl$	
	4 g	glucose or $^{13}C_6$-glucose	
	2 ml, 1 M	$MgSO_4$	
	50 μl, 2 M	$CaCl_2$	
	0.03 g	thiaminechloride hydrochloride	
	10 ml	trace elements	
	up to 1 l	H_2O	
trace elements	0.6 g	$FeSO_4 \cdot 7\ H_2O$	
	0.094 g	$MnCl_2 \cdot 2\ H_2O$	
	0.08 g	$CoCl_2 \cdot 6\ H_2O$	
	0.07 g	$ZnSO_4 \cdot 7\ H_2O$	
	0.03 g	$CuCl_2 \cdot 2\ H_2O$	
	0.002 g	H_3BO_3	
	0.025 g	$(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$	
	up to 100 ml	H_2O	stir 10 min
	0.5 g	EDTA	stir over night
ampicillin stock solution	50 mg/ml	ampicillin sodium salt	steril filtrated, stored at -20 °C
kanamycin stock solution	34 mg/ml	kanamycin	steril filtrated, stored at -20 °C
IPTG stock solution	1 M	IPTG	steril filtrated, stored at -20 °C

4.2 Buffers and solutions

The buffers and solutions used for the methods and protein preparations described in this work are subsumed in Table 4.2.

Table 4.2: Buffers and solutions

METHOD	NAME	AMOUNT	CHEMICAL
Affinity chromatography	wash buffer	50 mM	NaPi pH 8.0
		300 mM	NaCl
		15 mM	βME
		0.5 mM	Pefabloc
		10 mM	imidazole
	elution buffer	50 mM	NaPi pH 8.0
		300 mM	NaCl
		15 mM	βME
		0.5 mM	Pefabloc
		250 mM	imidazole
Cell lysis	lysis buffer	50 mM	NaPi pH 8.0
		300 mM	NaCl
		15 mM	βME
		1 mg/ml	Lysozym
		0.5 mM	Pefabloc
Ion exchange	ion exchange buffer A	50 mM	NaPi pH 6.5
		5 mM	DTT
	ion exchange buffer A	50 mM	NaPi pH 6.5
		5 mM	DTT
		1 M	NaCl
NMR	NMR buffer	50 mM	MOPS pH7.0
		50 mM	NaCl
		10 %	D_2O (v/v)
SDS-PAGE	APS	10 %	(w/v) in H_2O
	destaining solution	100 ml	acetic acid
		900 ml	H_2O
	4 x protein loading buffer	1.7 g	SDS
		7.5 ml, 1 M	Tris/HCl pH 6.8
		23 ml	glycerol
		50 mg	bromphenol blue
		0.5 ml	β-mercaptoethanol
		up to 50 ml	H_2
	running buffer	1 g	SDS
		3.03 g	Tris
		14.4 g	glycine
		up to 1 l	H_2O
	staining solution	2.2 g	Coomassie Brilliant blue G250
		100 ml	acetic acid
		250 ml	isopropanol
		650 ml	H_2O
TEV-cleavage	TEV-buffer	50 mM	Tris/HCl pH 8.0
		1 mM	DTT
		0.5 mM	EDTA
		0.5 mM	PMSF
Pre-gel solution	PH-gel	365 mM	AMPS
		365 mM	DMAA
		20 mM	BIS
	PPH-gel	730 mM	AMPS
		730 mM	DMAA
		30 mM	BIS
Ligand titration	Ligand stock solution	20 mM	suc-AAPF-pNA
		1:1	NMR Buffer / 99 % d_6-DMF

4.3 Enzymes and Chemicals

Enzymes and chemicals used in this thesis can be found in Table 4.3.

Table 4.3: Enzymes and chemicals

CHEMICALS/ENZYMES	COMPANY
ammonium chloride (>98 % ^{15}N)	Cambridge Isotope Laboratories, Andover, USA
$^{13}C_6$-D-glucose (>98 % ^{13}C)	Spectra Stable Isotopes, Columbia, USA
99.9 % D_2O, 99.9 % d_6-DMSO, 99.9 % d_6-DMF, 99.8 % $CDCl_3$	Deutero GmbH, Germany
ammonium molybdate tetrahydrate, ascorbic acid, Coomassie Brillant Blue R-250, copper chloride dihydrate, iron (II) sulfate heptahydrate	Fluka, Neu-Ulm, Germany
DTT	Gerbu, Gaiberg, Germany
agarose, kanamycin sulphate	GibcoBRL, Karlsruhe, Germany
Bench Mark protein ladder	Invitrogen, Karlsruhe, Germany
acetic acid, α-D(+)-glucose monohydrate, ammonium acetate, ammonium chloride, ammonium hydrocarbonate, boric acid, disodium hydrogen phosphate, ethanol, glycerol, glycine, hydrochloric acid, manganese chloride dihydrate, magnesium sulfate heptahydrate, sodium chloride, sodium dihydrogen phosphate, sodium hydroxide, thiaminechloride hydrochloride, Tris	Merck, Darmstadt, Germany
Ni-NTA Agarose	Qiagen, Hilden, Germany
ampicillin sodium salt, APS, dipotassium hydrogen phosphate, EDTA, imidazole, magnesium chloride hexahydrate, MOPS, potassium dihydrogen phosphate, TEMED, Pefabloc, yeast extract	Roth, Karlsruhe, Germany
SDS	Serva, Heidelberg, Germany
suc-AAPF-pNA, 2-acrylamido-2-methyl-1-propanesulfonic acid, N,N-dimethylacrylamide, (3-acrylamidopropyl)trimethylammonium chloride solution 75 wt. % in H_2O, ammonium persulphate, N,N'-methylenbisacrylamide, poly-γ-ethyl-L-glutamate, poly-γ-benzyl-L-glutamate	Sigma-Aldrich, Schnellendorf, Germany

4.4 Equipment

In the following, laboratory instruments and consumables are tabulated.

Table 4.4: Equipment and supplier

COMMON NAME	IDENTIFIER/COMPANY
Balances	Sartorius B 3100 S, Sartorius, Göttingen, Germany Sartorius AC 210 S, Sartorius, Göttingen, Germany
Centrifuges	Beckmann-Coulter Avanti J-20 and J-301, rotors: JLA 8.100, JLA 9.100, JLA 16.250, JA 25.50 Ti, JA 30.50 Ti, Krefeld, Germany Eppendorf Centrifuge 5415D, Wesseling-Berzdorf, Germany Eppendorf Centrifuge 5804, Wesseling-Berzdorf, Germany Heraeus Biofuge primo, Kendro, Hanau, Germany
Concentrators	Centricon, YM-3, Amicon, Bedford, USA
Dialysis	Slide-A-Lyzer Dialysis Cassettes, MWCO 3500, 0.1-0.5 ml Capacity, Pierce Biotechnology, Inc., Rockford, IL, USA
Electrophoresis	Kodak Electrophoresis documentation and analysis system 120, Eastman Kodak Co., New York, NY, USA Power Pac 300, BioRad, München, Germany Polyacrylamide gel electrophoresis: Mini-PROTEAN 3 Cell, BioRad, München, Germany Agarose gel electrophoresis: Mini-Sub Cell GT, BioRad, München, Germany
-80 °C freezer	MDF-U71V Ultra-low temperature freezer, SANYO Electric Co., Ltd, Osaka, Japan
Filtering	sterile filter 0,20 μm, Sartorius, Göttingen, Germany
FPLC	Äkta prime, Amersham Pharmacia Biotech, Freiburg, Germany Disposable 10 ml polypropylene columns, Pierce Biotechnology, Inc., Rockford, IL, USA Frac-100, Amersham Pharmacia Biotech, Freiburg, Germany HiTrap SP-Sepharose, Amersham Pharmacia Biotech, Freiburg, Germany
Incubator	Infors Multitron HT, Einsbach, Germany Certomat R, B. Braun Biotech International, Melsungen, Germany
Lyophylisation	Christ Alpha 2-4, B. Braun Biotech International, Melsungen, Germany
NMR	AVANCE 400, Bruker, Karlsruhe, Germany AVANCE 600, Bruker, Karlsruhe, Germany DRX 600, Bruker, Karlsruhe, Germany AVANCE 700, Bruker, Karlsruhe, Germany DRX 800, Bruker, Karlsruhe, Germany AVANCE 900, Bruker, Karlsruhe, Germany Quality NMR Sample Tubes 5 mm, Norell, Inc., Landisville, NJ, USA Shigemi NMR tube 5 mm, Shigemi Corp., Tokyo, Japan
pH-Meter	PB11 PY-P10, Sartorius, Göttingen, Germany
Spectroscopy	UV/VIS-Spectrophotometer, Hewlett-Packard 8453, Böblingen, Germany Mass spectrometer, Water Micromass ZQ single quadrupole, Waters, Saint-Quentin, France
Ultrasound	SONOPLUS HD 2200, Bandelin, Berlin, Germany

4.5 Software

The software used for processing and analysing NMR spectra, for calculating and analysing structures and for preparing figures is summarized in Table 4.5.

Table 4.5: Software

PROGRAM	REFERENCE/ORIGIN
InsightII	MSI 2000 release, San Diego, CA, USA
MOLMOL	[92]
Macromodel	[43]
NMRPipe/NMRDraw	[93]
NMRView 5.0.4	[94]
PALES	[30]
PROCHECK	[95]
PROCHECK-NMR	[96]
PyMOL	http://www.pymol.org, [97]
Sparky 3	T. D. Goddard and D. G. Kneller, University of California, San Francisco
XPLOR-NIH 2.9.7	[98]
SCULPTOR	[99]
X-WINNMR 3.5	Bruker, Karlsruhe, Germany
TOPSPIN 1.3	Bruker, Karlsruhe, Germany

5

Methods

Here, all general methods applied in this thesis are presented. Components of the required buffers and solutions can be found in Table 4.2.

5.1 Protein expression

5.1.1 Cultivation and storage of *E. coli*

Trigger factor was expressed in the *E. coli* strain BL21(DE3). Agar plates and all liquid nutrient media contained 50 μg/l of ampicillin and 34 μg/l of kanamycin. Cells were grown at 37 °C. Single colonies of *E. coli* BL21(DE3) harbouring the plasmid pJC$_{Tev}$TFS100C were grown on an agar plate and used to inoculate two 3 ml LB, which were incubated for 5h. They were then transferred into two 50 ml M9-minimal media overnight precultures containing the required nitrogen and carbon sources. The 100 ml overnight preculture was then used to inoculate a 1 l M9-minimal medium culture. Cell growth was monitored by measuring the OD_{600}. At an OD_{600} of 0.6-0.8 the protein expression was induced by addition of 1 M IPTG to a final concentration of 1 mM. The cells were harvested at an OD_{600} of 1.2-1.6 by centrifugation at 7500 x g and 4 °C for 15 min. The cell pellets were stored at -80 °C.

For long term storage of the *E. coli* strains, 350 μl stationary LB culture were mixed with 150 μl sterile 50 % glycerol and stored at -80 °C.

5.1.2 Cell lysis

For cell lysis cell pellets from 1 l cultures were thawed on ice and suspended in 30-40 ml lysis buffer. The suspension was sonicated 8 x 20 s on ice with 2 minute breaks. The lysate was centrifuged for 45 min at 48000 x g and 4 °C.

5.2 Protein methods

5.2.1 Nickel-nitrilotriacetic acid (Ni-NTA) agarose affinity chromatography

Overexpressed trigger factor contained a N-terminal His-tag and was purified via a Ni-NTA agarose column. 10 ml of resin were used per protein solution from 1 l of expression culture. The resin, equilibrated with wash buffer, was incubated with the supernatant from the cell lysis (see 5.1.2) for 1 h at 4 °C. After washing the column with 10 column volumes of wash buffer, bound proteins were eluted by increasing the imidazole concentration using 5 column volumes of elution buffer.

5.2.2 Ion exchange chromatography

Ion exchange chromatography was performed on a ÄKTA prime low pressure liquid chromatography system with HiTrap columns. Before each run, the HiTrap column was prepared by washing at first with 5 bed volumes of buffer A and then with 5 bed volumes of buffer B. Finally, the column was equilibrated with 5-10 bed volumes of buffer A. Samples were loaded at a flow rate of 1 ml/min onto the equilibrated column. Unbound protein was washed with 5-10 column volumes of buffer A. The elution was performed applying a linear gradient of 0-100 % buffer B at a flow rate of 1 ml/min and collecting 0.5 ml fractions. Protein elution was detected by measuring the absorption at 280 nm (A_{280}).

5.2.3 Cleavage with TEV-protease

The N-terminal His-tag was cleaved with the TEV-protease. At first, His-tagged trigger factor that was prepurified by affinity chromatography was dialysed at 4 °C

against 5 l TEV-buffer (see 5.2.1). Then the protein concentration was determined (see 5.4.1) and 1 mg of TEV-protease per 100 mg of protein was added. The cleavage reaction was performed for 24 h at room temperature. As the TEV-protease contains a His-tag it was removed via a second affinity chromatography step. After completed cleavage, the protein solution was dialysed against 5 l of wash buffer and applied to a 2 ml Ni-NTA Agarose column. The flow through and the first 5 ml from wash step were collected for further purification.

5.2.4 Tagging reaction

The protein solution was concentrated to a volume of 200 μl using a concentrator with MWCO 3000 Da. The sample was then washed twice by addition of 1.8 ml NMR-buffer and concentrated to 200 μl in the same concentrator. For the tagging reaction a 6 fold molar excess of the tag was dissolved in 1 mL NMR-buffer and added to the protein sample and subsequently shaken at room temperature for 1.5 h.

5.2.5 Concentrating of proteins

Protein solutions were concentrated by ultrafiltration using 0.5, 2 or 20 ml concentrators with the appropriate molecular weight cutoff (MWCO) of 3500 Da. The centrifugation was performed at 4 °C as recommended by the supplier (Table 4.4).

5.2.6 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

The discontinuous Tris-glycine buffer system as described by Laemmli [100] was used to separate proteins by molecular weight. The components of the stacking and separating gel are given in Table 5.1. All gels had a separating gel with an acrylamide concentration of 15 % and a 3 % acrylamide stacking gel. Protein samples were mixed with 4 x protein loading buffer and denatured at 100 °C for 5 min. Gels were run at 25 mA and stained with Coomassie blue R-250 by heating for 90 s in the microwave. For destaining, several 90 s heating steps in the microwave were performed and gels

were completely destained by shaking overnight. Finally, the SDS-gels were imaged and digitized for documentation.

Table 5.1: Composition of the 17.5 % SDS-gels

	Seperating gel	Stacking gel
Rotiphorese Gel 30	3 ml	250 μl
1 M or 2 M Tris/HCl pH 8.8	1.88 ml	-
1 M Tris/HCl pH 6.8	-	313 μl
H_2O	30 μl	1.88 ml
10 % SDS	50 μl	25 μl
TEMED	2.5 μl	2 μl
10 % APS	50 μl	25 μl

5.3 Preparation of the polyacrylamide-based alignment media

The deionized water as well as all solutions were degassed for at least 15-30 min to get rid of solubilized oxygen, that functions as an inhibitor during polymerization. This step is therefore very important to ensure the homogeneity as well as the quality of the produced gels. A general scheme of the radical polymerisation carried out is shown in Figure 5.1

5.3.1 Preparation of the negatively charged PH-gel

For 10 ml of pre-gel solution the amounts of the reactands are listed in Table 5.2. AMPS, DMAA and BIS were dissolved in 9 ml water. Sometimes it was necessary to heat the solution a bit to dissolve everything of BIS. After the solution cooled down

Table 5.2: Composition of the PH-gel pre-gel solution

2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS)	757 mg
N,N-dimethylacrylamide (DMAA)	377 μl
N,N'-methylenbisacrylamide (BIS)	30 mg
APS stock solution (0.015 g/l)	1ml

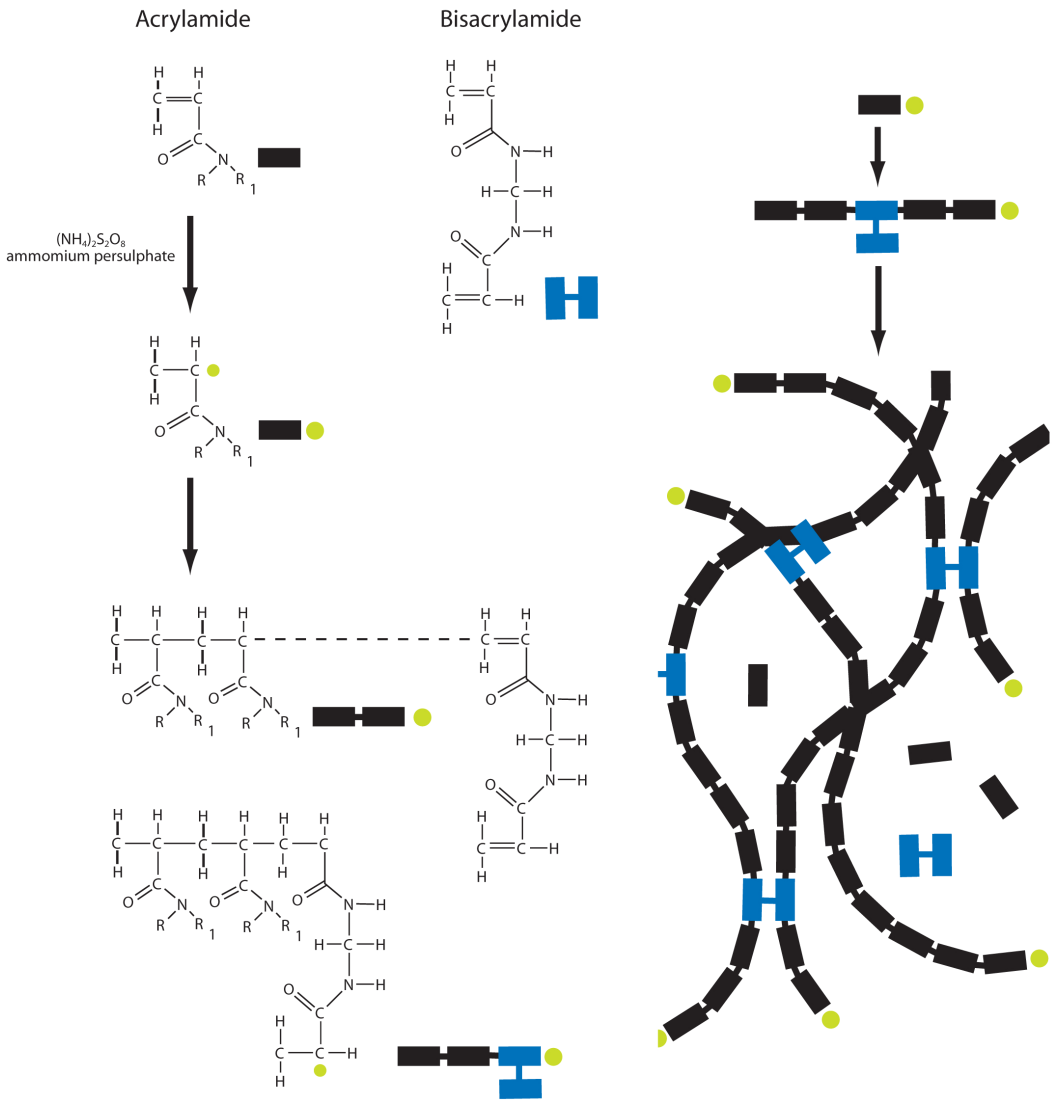

Figure 5.1: Radical polymerization of acrylamide to polyacrylamide gels

to room temperature 1 ml of APS stock solution was added and carefully mixed. The pre-gel solution was inserted into a gel cylinder with an inner diameter of 3.5 mm, 5.4 mm or 6.4 mm and polymerized for 2 h at 70 °C in a heatable dessicator. Therefore gels are later referred to as 3.5 mm, 5.4 mm or 6.4 mm gels. Extreme care has to be taken drilling the gels out of the cylinder, because they are very sensitive at this stage of polymerization. It can take up to 10 min to drill out one gel and it is favourable to keep its top end lubricated with water. The PH-gels were then washed once with NaOH solution (0.02 M) and at least 4 times with water, each time for several hours to remove not polymerized monomers and the starter APS. The swollen gels were now

cut with a scalpel in 3.7 cm pieces, which corresponds to a filling height of 500 μl in a 5 mm NMR-tube. Only the most homogeneous part of the gels are cut out, parts that bear bubbles or other deficiencies were discarded. The cut gels were pierced with a glass capillary (diameter 0.3-0.4 mm), which is used to position the gel later in the middle of the fill height in the NMR-tube. The drying was carried out at room temperature on a petri dish, that was covered with regular household foil to reduce friction between the gel and the glass surface. The gels were usually completely dried after 12-24 h. Drying at higher temperatures and reduced pressure also possible, but bears the risks, that the dry gels crack and subsequently can't be used for NMR measurements. The gels in different states during polymerization and the equipment used for it can be seen in Figure A.1. For NMR measurements the dried gels were put in a 5 mm NMR tube, that had been treated with Repelsilan, which renders the glass surface hydrophobic and therfore facilitates the equilibration of the gel. With the help of the capillary the gel can be placed in the middle of the fill height. Now one of the compatible solvents DMSO, DMF or water containing the compound of interest is added and NMR tube then sealed with a Shigemi plunger. If no Shigemi plunger is used, the gel can rip on top. After five days the gel has reswollen and is ready for use. If possible equilibration times of 7-10 days are recommended.

5.3.2 Preparation of the positively charged PPH-gel

Table 5.3: Composition of the PPH-gel pre-gel solution

(3-acrylamidopropyl)trimethylammonium chloride solution 75 wt. % (APTMAC)	2014 μl
N,N-dimethylacrylamide (DMAA)	754 μl
N,N'-methylenbisacrylamide (BIS)	45 mg
APS stock solution (0.015 g/l)	1ml

The procedure was exactly the same as for the PH-gel, except for the different components and amounts used, that are listed in Table 5.3. The first washing step was carried out with a 0.02 M HCl solution instead of NaOH.

5.4 Spectroscopic methods

5.4.1 Determination of protein concentration

The concentration of proteins in solution was determined by measuring the absorption at 280 nm (A_{280}).

$$A_\lambda = \epsilon_\lambda \cdot c \cdot d \tag{5.1}$$

A_λ:	absorption at wavelength λ
ϵ_λ:	molar extinktion coefficient at wavelength λ in $M^{-1}cm^{-1}$
c:	protein concentration (M)
d:	thickness of the cuvette (cm)

5.4.2 Electrospray ionization mass spectrometry (ESI-MS)

ESI-MS was performed after the final purification of the first preparation of each protein sample to confirm the identity by the molecular weight and verify the purity. All samples were transferred into 20 mM NH_4OAc by dialysis.

5.5 NMR spectroscopy

All NMR experiments were acquired at 400, 600, 700, 800 or 900 MHz on Bruker AVANCE 400, 600, 700, and 900 or DRX 600 and 800 spectrometers running under X-WINNMR 3.5 or TOPSPIN 1.3. All NMR spectra were processed using X-WINNMR 3.5, TOPSPIN 1.3 or NMRPipe/NMRDraw [93] and analysed using NMRView 5.0.4 [94] or Sparky 3 (T. D. Goddard and D. G. Kneller, University of California, San Francisco) or TOPSPIN 1.3.

5.5.1 Residual dipolar couplings (RDCs)

Anisotropic media for measurement of RDCs were prepared as described in A.1. RDCs were back-calculated from X-ray or NMR structures using singular value decomposition (SVD) as implemented in PALES [101]. The Pearson's correlation factor

(R) and the quality or Q-factor were used to evaluate the agreement between a structure and the observed RDCs. The Q-factor [102] was determined as:

$$\frac{rms(^1\mathrm{D}_{HN}^{measured} -^1 \mathrm{D}_{HN}^{calculated})}{rms(^1\mathrm{D}_{HN}^{measured})} \tag{5.2}$$

5.5.1.1 Measurement

^{15}N-^{1}H splittings for trigger factor were measured under isotropic and anisotropic conditions using 2D IPAP-^{15}N-^{1}H HSQC experiments [103]. For all other samples ^{15}N-^{1}H and ^{13}C-^{1}H splittings were measured under isotropic and anisotropic conditions using sensitivity enhanced gradient selective HSQC [104, 105, 106] or CH_2-TROSY HSQC [107, 108] experiments. RDCs were extracted by subtraction of the $^1\mathrm{J}_{NH}$ or $^1\mathrm{J}_{CH}$ scalar coupling measured for the isotropic sample [109].

5.5.2 Determination of protein-ligand dissociation constants

Chemical shift changes observed in a series of ^{15}N-^{1}H-HSQC spectra during the titration of a ligand to a protein can be used to calculate the dissociation constant K_d if the exchange rate is much faster than the difference between the chemical shifts of the free and bound protein. In this case of fast exchange on the NMR time scale, the K_d is larger than 10^{-3} M.

Considering a complex with 1:1 stoichiometry composed of a protein (P) which binds a ligand (L), the complex formation and dissociation is described as follows: and K_d

$$P + L \underset{k_{on}}{\overset{k_{off}}{\rightleftharpoons}} PL$$

is defined as:

$$K_d = \frac{k_{on}}{k_{off}} = \frac{[P][L]}{[PL]} = \frac{([P]_0 - X_{PL}[P]_0)([L]_0 - X_{PL}[P]_0)}{X_{PL}[P]_0} \tag{5.3}$$

with $[P]_0 = [P] + [PL]$ and $X_{PL} + X_P = 1$.

k_{off}: rate of dissociation

k_{on}: rate of association

$[P]_0$: total or starting concentration of the protein

[P]: concentration of the protein

$[L]_0$: total or starting concentration of the ligand

[L]: concentration of the ligand

[PL]: concentration of the complex

X_P: fraction of the free protein

X_{PL}: fraction of the bound protein

The observed chemical shift δ_{obs} can be written as:

$$\delta_{obs} = X_P\delta_P + X_{PL}\delta_{PL} \tag{5.4}$$

and with $r = \frac{[L]_0}{[P]_0}$ the following equation can be derived:

$$\delta_{obs} = \delta_P + (\delta_{PL} - \delta_P)\frac{(K_d + (1+r)[P]_0) - \sqrt{(K_d + (1+r)[P]_0)^2 - 4[P]_0^2 r}}{2[P]_0} \tag{5.5}$$

δ_P: chemical shift of the protein

δ_{PL}: chemical shift of the complex

Fitting the experimental data to equation 5.5 yields the K_d [110].

5.6 Structure calculation

5.6.1 Hormaomycin

All NMR restraints and the SCULPTOR input file (has to be modified accordingly for the three protocols) can be found in the appendix (C.1 on page 125 and D.1 on page 165)

5.6.1.1 Restraint-free ensemble (protocol 1)

In this protocol no experimental restraints were used. An initial molecular model was first minimized with a gradient criterion of less than 0.5 kcal/mol. The molecule is then heated up to a temperature of 1000K over a period of 5 ps, in steps of 1 fs. The molecule evolves at this temperature for 80 ps (80000 steps), thus sampling conformational space extensively. This is followed by a 3 ps cooling period to 100 K and energy minimisation. This protocol was repeated 500 times and the resulting conformers were placed in the RF (restraint free) ensemble.

5.6.1.2 NOE- and J coupling-refined ensemble (NJ) (protocol 2)

Pseudo-atoms were used for the methyl protons and unresolved methylene protons. Distance restraints derived from NOE cross peaks integrals were applied as biharmonic restraints with lower and upper bounds of 2.0–2.5, 2.0–2.8, 2.0–3.5, 2.0–4.0 and 2.0–5.0 Å, respectively. The timing of this protocol is identical to that described for protocol 1. The only difference is that during the 80 ps (80000 steps), sampling period NOE and J-coupling-derived dihedral angle terms are raised from their initial weighting (0.2 kcal mol^{-1} $Å^{-2}$ and 1.0 kcal mol^{-1} deg^{-2}) to their final weighting of (20.0 kcal mol^{-1} $Å^{-2}$ and 1000 kcal mol^{-1} deg^{-2}) respectively. This protocol was repeated 900 times and the resulting lowest energy conformers were placed in the NJ (NOE/J-coupling) ensemble, which consists of two families, NJa and NJb. For further characterization two structures of the NJb family were arbitrarily chosen and designated NJ1 and NJ19. We always checked that the other structures in the same ensemble would have shown the same behavior as these two representative structures.

5.6.1.3 NOE/J coupling/RDC ensemble (NJR) (protocol 3)

The determination of molecular structures using RDC restraints applied the following protocols that were required to optimize the treatment of the alignment tensor. The parameters Aa and Ar are restrained to be close to predetermined values using a harmonic potential function. Starting structures are taken from the NJ ensemble derived

from step 2. The molecular coordinates are initially fixed, while the orientation of the alignment tensor evolves under the influence of RDCs measured from sites present in the macrocyclic ring of hormaomycin. This step is composed of a sampling period of 4 ps at 300 K, followed by 3 ps at 200 K and conjugate gradient minimization. For each structure, the most appropriate orientation for the tensor is thus defined. The molecule is then released, and the tensor orientation and molecular structure evolve under the influence of 42 RDC restraints. Remaining parameters are identical to those described in protocols 2 and 3. This protocol was repeated 900 times and the resulting lowest energy conformers were placed in the NJR (NOE/J-coupling/RDC) ensemble. For further characterization two representatives structures were arbitrarily selected : structures NJR1 and NJR19 and similarly to the NJ structures all the conclusions were found to be independent of this selection.

5.6.2 Hymenistatin

All NMR restraints and the XPLOR-NIH input file (has to be modified accordingly for the three protocols) can be found in the appendix (C.2 on page 129 and D.2 on page 173).

The aim of the structural calculations of hymenistatin was to determine its conformation as well as the configuration of the thirteen prochiral centers at the same time. The experimental restraints used in the structural calculations of hymenistatin in the three solvents DMSO, $CDCl_3$ and THF are summarized in Tab.5.4. At

Table 5.4: Experimental restraints of hymenistatin for the structural calculations in XPLOR-NIH

	DMSO	**$CDCl_3$**	**THF**
NOEs	113	78	136
Dihedrals	6	2	-
RDCs/PH-gel	35	-	-
RDCs/PPH-gel	29	-	-
RDCs/PAN-gel	32	-	-
RDCs/PDMS-gel	-	36	34

first a restraint-free ensemble was generated to sample the conformational space and scramble up the assignment of the prochiral centers. Therefore a modified simulating annealing protocol was used which consists of four stages. The variation of the force constant are summarized in Tab.5.5. In the first high temperature stage (40000 steps, 120 ps, 2000 K) all force constants attached to experimental restraints, parameters (angles, dihedrals and impropers) fixing the geometry of the prochiral centers in the forcefield (FC) and non-bonded energies (VdW) were set to zero. Subsequently the methylene protons of the prochiral centers could switch positions during the first high temperature stage, which is also often referred to as floating chirality. During the second high temperature stage (40000 steps, 120 ps, 2000 K) the force constants fixing the correct geometries of the prochiral centers were brought up, which finished the floating chirality. This was followed by a cooling stage (4000 steps, 12 ps), which started at 2000 K and reached a final temperature of 100 K. The resulting structure was powell minimized for 4000 steps. This protocol was looped 700 times and the resulting structures were placed in the restraint-free (RF) ensemble. The structures

Table 5.5: Scaling factors for different restraints during the four major stages in the structure calculations of restraint-free ensemble

	1st high temperature dynamics [kcal/mol]	**2nd high temperature dynamics** [kcal/mol]	**Cooling stage** [kcal/mol]	**Final minimization** [kcal/mol]
VDW	0.002	0.002	0.003 → 4	1
NOE	0	0	0	0
Dihedrals	5 → 200	200	200	200
$^1\mathbf{D}_{NH}$	0	0	0	0
$^1\mathbf{D}_{CH}$	0	0	0	0
FC	0	25 → 500	500	500

from the RF ensemble were then taken as starting structures for the refinement with NOE and J-coupling (NJ). The timings and temperatures for the simulated annealing were indentical to those from the restraint-free protocol. In addition the force constants for the NOEs and dihedral restraints were increased during the first high temperature stage (see Tab.5.6). The resulting structures were subsequently put in the NJ ensemble. For the conformational refinement with RDCs it was necessary to know the magnitude (D_a) and rhombicity (Rh) of the alignment tensor. These

Table 5.6: Scaling factors for different restraints during the four major stages in the structure calculations of NOE and J-coupling ensemble

	1st high temperature dynamics [kcal/mol]	**2nd high temperature dynamics** [kcal/mol]	**Cooling stage** [kcal/mol]	**Final minimization** [kcal/mol]
VDW	0.002	0.002	0.003 → 4	1
NOE	1 → 50	50	50	50
Dihedrals	5 → 200	200	200	200
$^1\mathbf{D}_{NH}$	0	0	0	0
$^1\mathbf{D}_{CH}$	0	0	0	0
FC	0	25 → 500	500	500

parameters were derived by fitting the backbone RDCs to the structures of NJ ensemble. The average tensor values of the ten best fits, which were among the lowest energy structures were then taken for the NOE, J-coupling and RDC (NJR) refinement. The same protocol as for the NOE and J-coupling refinement was used taking the structures from the NJ ensemble as starting structures, but this time the module *sani* in XPLOR-NIH was used to introduce the RDCs in the first high temperature stage of the simulated annealing protocol (Tab.5.7). The resulting structures were subsequently placed in the NJR ensemble.

Table 5.7: Scaling factors for different restraints during the four major stages in the structure calculations of NOE, J-coupling and RDC ensemble

	1st high temperature dynamics [kcal/mol]	**2nd high temperature dynamics** [kcal/mol]	**Cooling stage** [kcal/mol]	**Final minimization** [kcal/mol]
VDW	0.002	0.002	0.003 → 4	1
NOE	1 → 50	50	50	50
Dihedrals	5 → 200	200	200	200
$^1\mathbf{D}_{NH}$	0.1 → 1	1	1	1
$^1\mathbf{D}_{CH}$	0.1 → 0.5	0.5	0.5	0.5
FC	0	25 → 500	500	500

5.6.3 DiaA

All NMR restraints and the XPLOR-NIH input file (has to be modified accordingly for the three protocols) can be found in the appendix (C.3 on page 138 and D.3 on page 184).

For the structure calculations in XPLOR-NIH of DiaA a parameter set had to be generated, as XPLOR-NIH only provides parameter sets for standard biomolecules like proteins, RNA and DNA. Therefore the parameters of the R- and S-diastereomer of DiaA have been generated by the program XPLO2D [111] from a pdb-file. These parameters were then used to generate two restraint-free ensembles of 500 structures for each diastereomer. The weighting factors during the different stages of the structure calculation protocol are summarized in Tab.5.8. At first an initial powell minimization was carried out for 50 steps. Followed by a high temperature stage consisting of 8000 steps (4 ps) at 2000 K. In the cooling stage the temperature was gradually reduced to a final temperature of 100 K (4000 steps, 4 ps). The calculation was then concluded by a final powell minimization of 1000 steps. This protocol was looped 500 times and the resulting structures were put in the restraint-free (RF) ensembles for the two diastereomers. The structures of the RF ensembles were used as starting

Table 5.8: Scaling factors for different restraints during the four major stages in the structure calculations of the restraint-free ensemble

	Initial minimization [kcal/mol]	**High temperature dynamics** [kcal/mol]	**Cooling stage** [kcal/mol]	**Final minimization** [kcal/mol]
VDW	0.002	0.002	$0.003 \rightarrow 4$	1
NOE	0	0	0	0

structures for the generation of the NOE ensembles. The timing of this calculation is equal to the one from the RF ensemble and the scaling factors are listed in Tab.5.9. The distance restraints used consisted of eighteen NOEs and 54 non-NOEs interconnecting the three subunits of DiaA, the menthyl part, the aromatic part and the pyranone part. The resulting 500 structures for each diastereomer were placed in the according NOE ensemble.

5.6.4 Trigger factor

The following restraints were used for structural calculations with the CNS program package [112]: 1024 NOEs, 35 hydrogen bonds, 68 $^{3}J_{HN-H\alpha}$ coupling constants, 66 dihedral angle restraints, 54 RDCs (Fig.3.8(a)) and 200 RDCs (Fig.3.8(b)) respec-

Table 5.9: Scaling factors for different restraints during the four major stages in the structure calculations of the NOE ensemble

	Initial minimization [kcal/mol]	**High temperature dynamics** [kcal/mol]	**Cooling stage** [kcal/mol]	**Final minimization** [kcal/mol]
VDW	0.002	0.002	0.003 → 4	1
NOE	0	1 → 50	50	50

tively and are listed in the appendix. All restraints were taken from [69] except the RDC data for tag **6a** and **6b**. The calculations started from a previously solved NMR-structure (PDB-ID 1HXV). Prior to the dynamics calculations the structure has been minimized in 2000 steps powell minimization. The actual dynamics has been done in three distinct stages followed by a final minimization. At first a 500 steps (7.5 ps) high temperature dynamics stage at 10000 K has been performed. It was followed by a 1000 steps (15 ps) cool down torsion angle dynamics stage reaching a final temperature of 0 K. A second cooling loop was done in cartesian coordinates, starting with 2000 K, for 5000 steps (25 ps) reaching a final temperature of 0 K. The resulting structure was powell minimized with 2000 steps. The scaling factors are summarized in Tab.5.10. All NMR restraints and CNS input files can be found in

Table 5.10: Scaling factors for different restraints during the four major stages in the structure calculations

	High temperature dynamics [kcal/mol]	**1st cooling stage** [kcal/mol]	**2nd cooling stage** [kcal/mol]	**Final minimization** [kcal/mol]
VDW	0.1	0.1 → 1	1 → 4	1
NOE	150	150	150	100
$^3\mathbf{J}_{HN-H\alpha}$	2	2	2	2
Dihedrals	10	200	200	400
RDCs	0.01	0.1 → 1	1 → 6.5	6.5

the appendix (C.4 and D.4)

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A

Appendix: Sample preparation

A.1 Preparation of the polyacrylamide-based alignment media

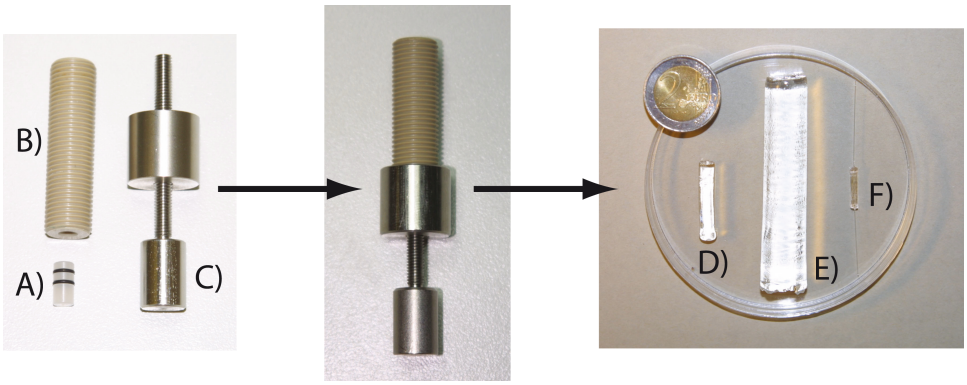


Figure A.1: Photograph of the equipment used for the polymerization and of the PH-gel in the different states during preparation. A) 5.4 mm piston B) 5.4 mm gel cylinder C) piston driver D) gel after polymerization E) gel after washing in water F) gel dried on a glass capillary.

A.2 Trigger factor

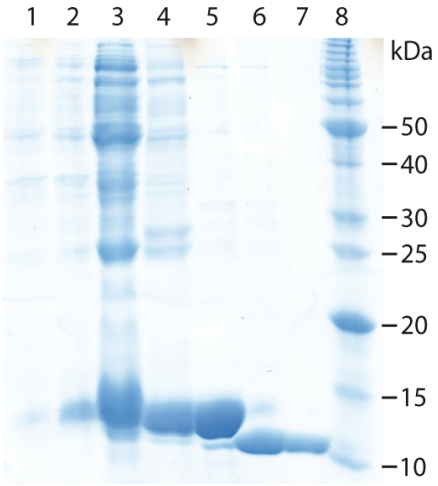


Figure A.2: Coomassie blue-stained SDS-PAGE documenting the expression and purification of trigger factor in *E. coli* BL21(DE3). Lane 1, before induction. Lane 2, after induction. Lane 3, supernatant proteins of the sonicated cells. Lane 4, after Ni-NTA column. Lane 5 after ion exchange chromatography. Lane 6, after cleavage of the 6xHistag. Lane 7, flow through of the Ni-NTA column. Lane 8, molecular weight standard.

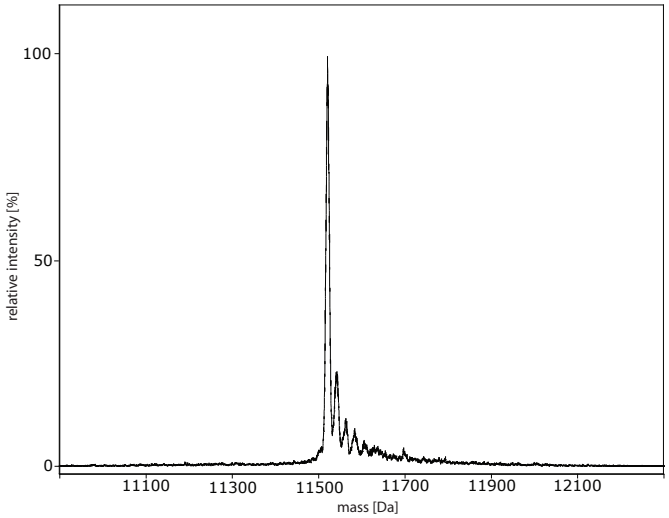


Figure A.3: ESI-MS spectrum of ^{15}N labeled trigger factor after purification. The detected molecular mass is 11505.42± 0.63, what deviates 0.02% from the expected mass.

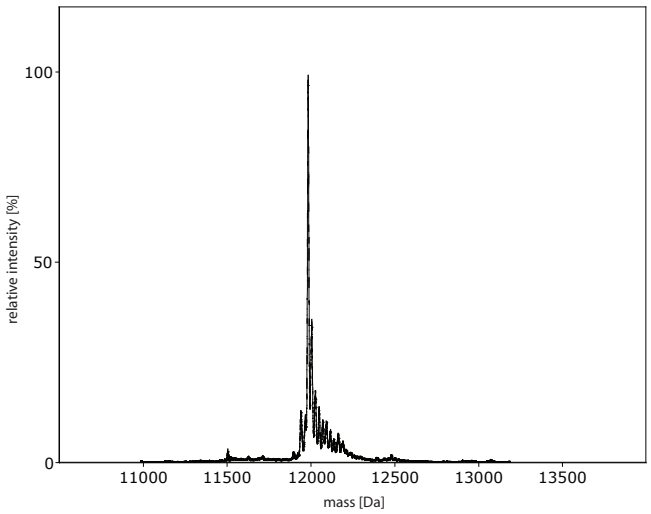


Figure A.4: ESI-MS spectrum of ^{15}N trigger factor tagged with 6. The detected molecular mass is 11986.15± 0.35 , what deviates 0.01% from the mass of tagged ^{15}N labeled trigger factor + K^+.

B

Appendix: NMR data

B.1 Menthol

Table B.1: Experimental ^{1}H-^{13}C residual dipolar couplings of (+)-menthol in PH-gel/DMSO and PPH-gel/DMSO and PH-gel/DMF recorded on a 400 MHz spectrometer at 298 K. The value in brackets is according to Fig.2.4 the backcalculated $^{1}D_{CC}$ dipolar couplings [11].

	PH-gel/DMSO	PPH-gel/DMSO	PH-gel/DMF
C1-H1	4.1	1.9	2.5
C2-H2eq	2.8	1.9	1.4
C2-H2ax	3.6	0.6	1.8
C3-H3	4.8	2.3	2.5
C4-H4	4.0	1.9	1.5
C5-H5eq	3.1	1.8	1.2
C5-H5ax	3.6	0.3	2.6
C6-H6ax	3.6	5.3	2.1
C7-H7	1.8 (-0.5)	1.2 (-0.3)	0.6 (-0.2)
C8-H8	1.8	4.5	1.4
C9-H9	-0.5 (0.1)	-0.4 (0.1)	-0.7 (0.2)
C10-H10	-0.8 (0.3)	-0.6 (0.2)	-0.6 (0.2)

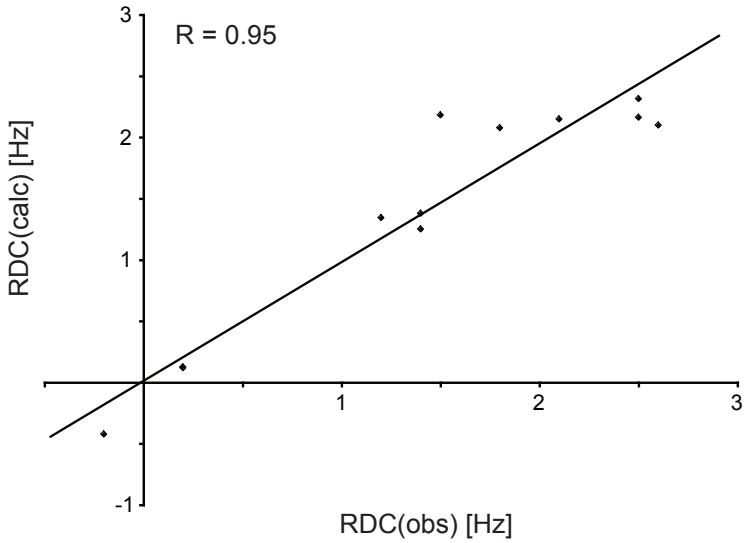


Figure B.1: Correlation between observed (RDC(obs)) and backcalculated (RDC(calc)) dipolar couplings for (+)-menthol in PH-gel/DMF

B.2 Hormaomycin

Table B.2: Distances and NOE intensities of proton pairs of the bulky side chain of NJR1 and NJR19 (in bold are the restraints that have been used)

	NJR1	**NJR19**	**NOE-derived distances**
H1-H26	2.0	4.4	**<4.0**
H1-H6	3.7	8.1	no NOE measured
H26-H6	3.9	4.1	**<4.0**
H26-H22	3.5	3.7	**<4.0**
H26-H24	3.2	5.8	<5.0
H26-H25	2.0	5.9	<5.0
H26-H19	3.0	2.1	<4.0
H26-H20 (overlay)	3.8	2.8	**<3.5**
H26-H64	3.1	3.1	<3.5
H26-H58/59/60	4.2	3.0	<4.0
H19-H6	2.7	2.4	<2.8

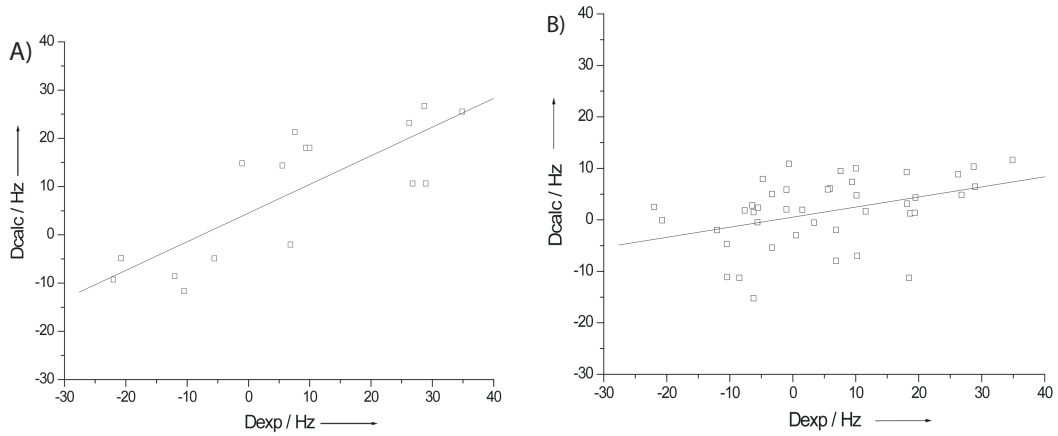


Figure B.2: Correlation between experimental RDCs derived from PH-gel/DMSO and the ones calculated from the chloroform structure of hormaomycin. A) RDCs from the backbone of the macrocyclic ring are used. The equation of the fitting curve is Dcalc = 0.59*Dexp + 4.50 B) when 42 RDCs are used: Dcalc = 0.20*Dexp + 0.54

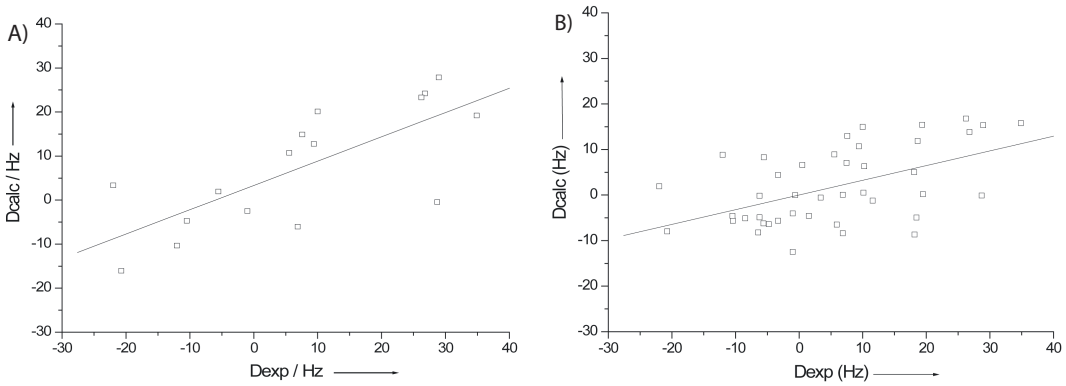


Figure B.3: Fitting between experimental RDCs and the ones calculated from the crystal structure of hormaomycin. A) Dcalc = 0.55*Dexp + 3.32 is obtained with RDCs from the backbone of the macrocyclic ring. B) Dcalc = 0.32*Dexp + 0.03 is obtained with 42 RDCs.

Table B.3: Distances between protons of Chpca with protons of (β-Me)Phe II

	Distance [Å]
H1-H57	2.44
H1-H14/H18	2.34
H1-H58/59/60	4.26
H2-H57	4.54
H2-H14/H18	3.65
H2-H58/59/60	5.97

Table B.4: Distances in the crystal structure for proton pairs that show ROE signals in $CDCl_3$

	Distance [Å]
Chpca with (β-Me)Phe I	
H2-H51	13.7
H1-aromatic protons	11.0
H1-CH3	11.5
Chpca with (3-Ncp)Ala I	
H2-H13	8.7
H2-H31	8.5
H1-H31	7.1

B.3 Hymenistatin

B.3.1 NMR spectra

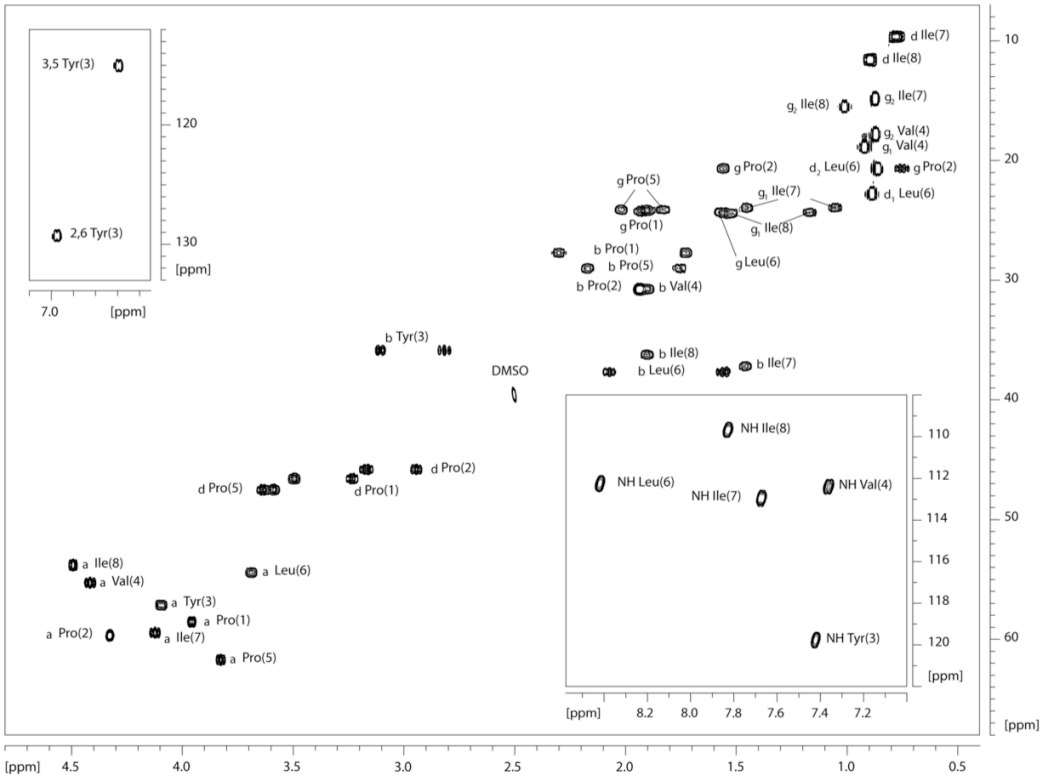


Figure B.4: Assigned 600MHz ^{13}C,^{1}H HSQC spectrum of 20 mM hymenistatin sample in DMSO. The formula of hymenistatin can be found in Fig.2.19 on page 32.

B.4 DiaA

B.4.1 NMR-spectra

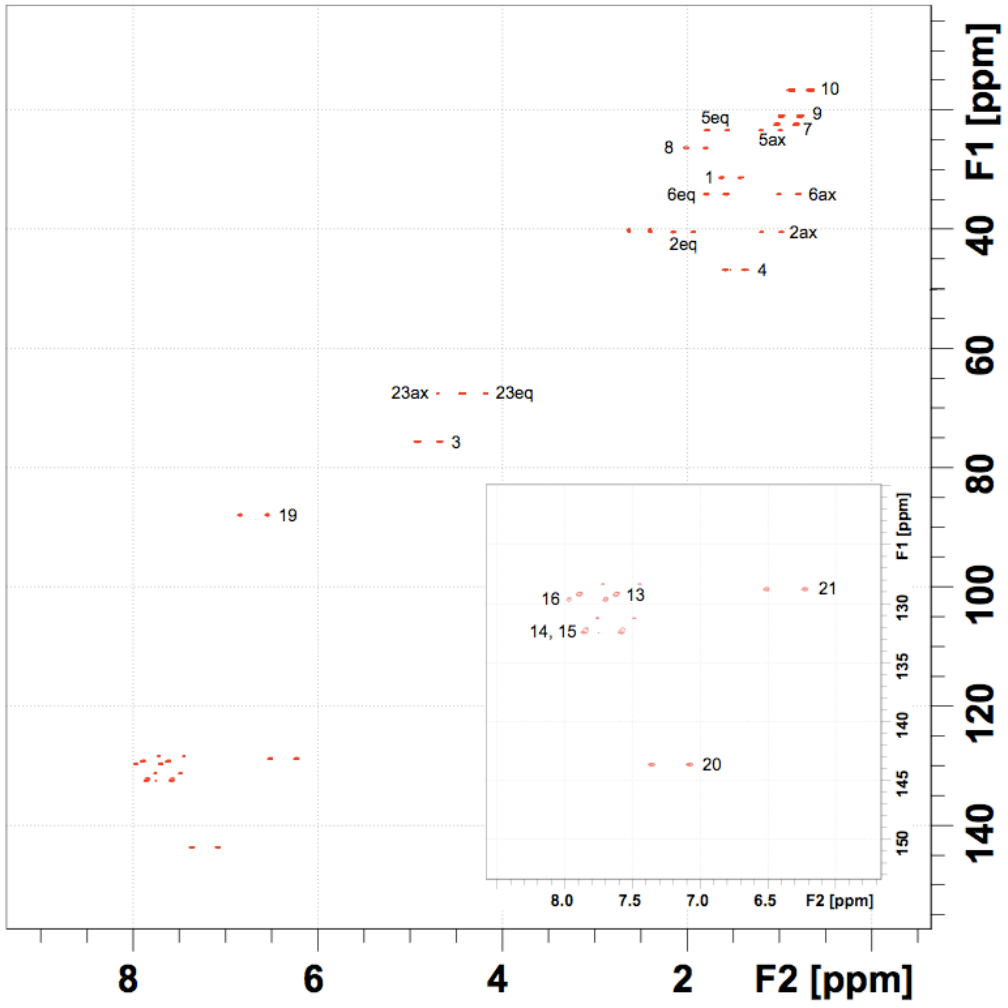


Figure B.5: Assigned 600 MHz t_2-coupled ^{13}C, ^{1}H HSQC of DiaA. The insert shows the aromatic region. The formula of DiaA can be found in Fig.2.28 on page 46.

B.5 Trigger factor

B.5.1 Pseudocontact shifts

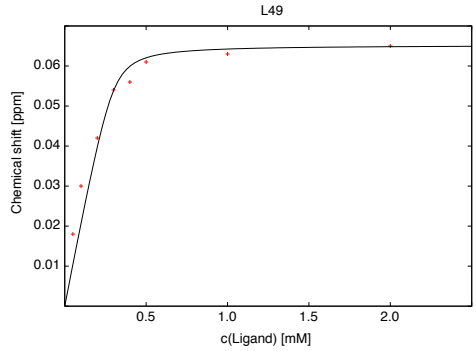
Table B.5: Pseudocontactshifts of trigger factor tagged with **6a** and loaded with Dy^{3+}

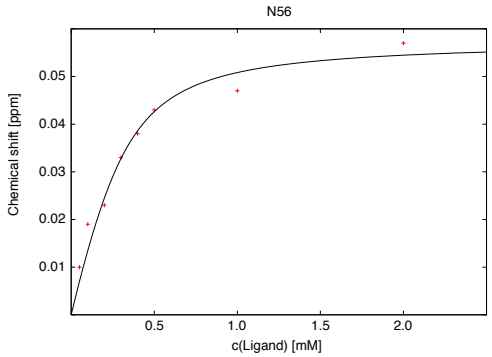
Residue	PCS [ppm]	Residue	PCS [ppm]	Residue	PCS [ppm]
L30	0.066	L49	-0.131	K78	0.061
A31	0.071	A52	-0.198	V79	0.043
N32	0.081	S53	-0.149	N80	0.042
G33	0.063	Q55	-0.040	K83	0.100
D34	0.051	N56	-0.002	T84	0.163
I35	0.028	V57	-0.011	L85	0.195
A36	0.034	E58	-0.019	K96	-0.394
I38	0.014	L59	0.017	E97	-0.455
D39	0.021	T60	0.011	L98	-0.775
F40	-0.013	I61	0.061	Q99	0.080
T41	-0.003	G62	0.120	E106	0.019
G42	-0.095	S63	0.055	V107	0.093
I43	-0.121	S65	-0.011	V108	0.047
V44	-0.161	E71	0.376	L109	0.056
D45	-0.188	I74	0.290	K110	0.034
N46	-0.032	I75	0.208	A111	0.023
E47	-0.075	A76	0.111	I112	0.018
K48	-0.050	M77	0.102	K113	0.018

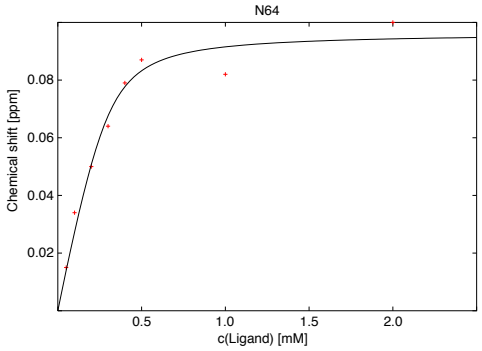
Table B.6: Pseudocontactshifts of trigger factor tagged with **6b** and loaded with Dy^{3+}

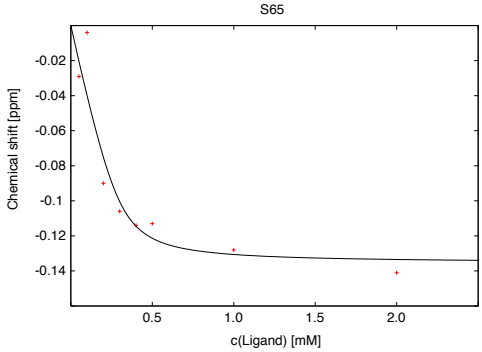
Residue	PCS [ppm]	Residue	PCS [ppm]	Residue	PCS [ppm]
L30	0.007	S53	-0.121	N80	0.008
N32	-0.003	Q55	-0.053	K83	0.058
G33	-0.016	N56	-0.028	T84	0.143
I35	-0.021	V57	-0.046	L85	0.155
A36	-0.027	E58	-0.049	K96	-0.345
I38	-0.031	L59	-0.041	E97	-0.313
D39	-0.010	T60	-0.058	L98	-0.630
F40	-0.029	I61	-0.020	Q99	-1.073
T41	-0.010	G62	-0.055	F105	0.093
G42	-0.065	S63	-0.061	E106	0.024
I43	-0.046	S65	-0.122	V107	0.053
V44	-0.064	E71	-0.017	V108	0.011
D45	-0.034	I74	-0.015	L109	0.020
N46	0.083	I75	-0.023	K110	-0.004
E47	0.017	A76	0.011	A111	-0.010
K48	0.006	M77	0.009	I112	-0.019
L49	-0.075	K78	0.020	K113	-0.031
A52	-0.150	V79	0.005		

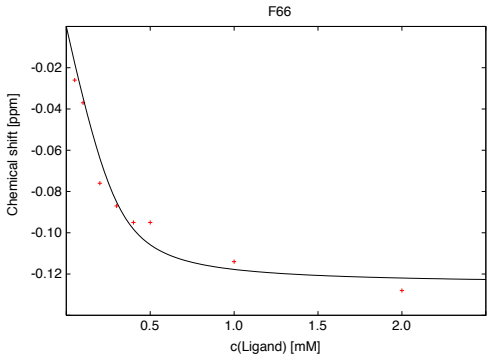
B.5.2 K_d determination

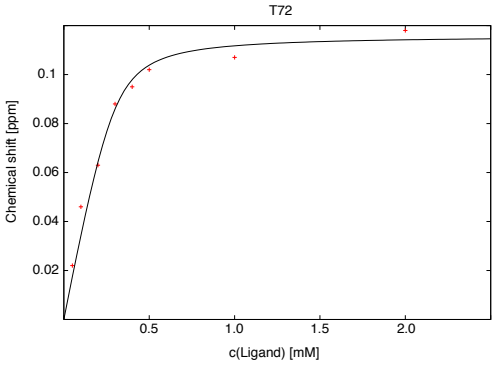


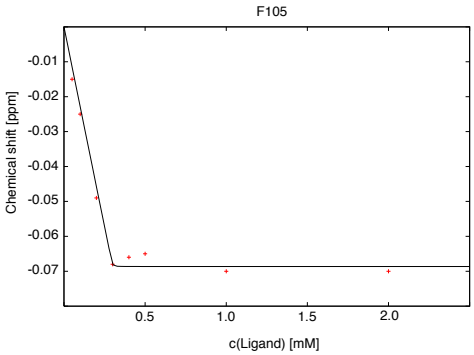












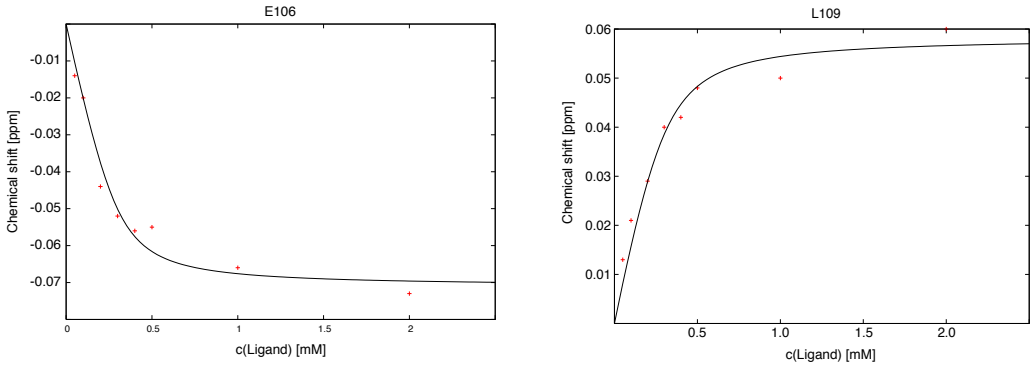


Figure B.6: K_d fitting curves of the ten residues exhibiting the biggest chemical shift upon ligand titration of trigger factor

B.5.3 ^{13}C-filtered pulse sequence

```
;13C filter
;hsqcetgpsisp2.2
;avance-version (02/12/09)
;HSQC
;2D H-1/X correlation via double inept transfer
;   using sensitivity improvement
;phase sensitive using Echo/Antiecho-TPPI gradient selection
;with decoupling during acquisition
;using trim pulses in inept transfer
;using shaped pulses for all 180degree pulses on f2 - channel
;with gradients in back-inept
;
;A.G. Palmer III, J. Cavanagh, P.E. Wright & M. Rance, J. Magn.
;   Reson. 93, 151-170 (1991)
;L.E. Kay, P. Keifer & T. Saarinen, J. Am. Chem. Soc. 114,
;   10663-5 (1992)
;J. Schleucher, M. Schwendinger, M. Sattler, P. Schmidt, O. Schedletzky,
;   S.J. Glaser, O.W. Sorensen & C. Griesinger, J. Biomol. NMR 4,
;   301-306 (1994)


#include <Avance.incl>
#include <Grad.incl>
#include <Delay.incl>



"p2=p1*2"

"d0=3u"

"d4=1s/(cnst2*4)"

"d11=30m"

"d5=1s/(cnst3*4)"
"d6=1s/(cnst4*4)"
"d9=d5+p12/2"
"d7=d6-d9-p10"
"d8=d6-p12-p10-5u-5u"
"DELTA=p16+d16+p2+d0*2-4u"
"DELTA1=p16+d16+8u"
"DELTA2=d4-larger(p2,p14)/2-4u"
```

```
"DELTA3=d24-cnst17*p24/2-p19-d16-4u"
"DELTA4=d4-larger(p2,p14)/2-p16-d16-4u"


1 ze
  d11 pl12:f2
2 d1 do:f2
3 (p1 ph1)
  DELTA2 pl0:f2
  4u
  (center (p2 ph1) (p14:sp3 ph6):f2 )
  4u
  DELTA2 pl2:f2 UNBLKGRAD
  (p1 ph2)
  4u pl0:f1
  (p9:sp9 ph1:r):f1
  4u
  p17:gp5
  d17 pl1:f1
  (p13:sp13 ph1):f2
  d9
  (p10:sp10 ph10):f2
  d7
  (p11:sp11 ph11):f2
  d8
  (p10:sp10 ph10):f2
  5u
  (p12:sp12 ph12):f2
  10u
  (p15:sp14 ph13):f2
  5u
  p18:gp6
  d17
  (p13:sp13 ph3):f2
  d0
  (p2 ph7)
  d0
  p16:gp1*EA
  d16 pl0:f2
  (p11:sp11 ph11):f2
  4u
  DELTA pl2:f2
  (p15:sp14 ph4):f2
  5u
  (p1 ph1)
  4u
  p19:gp3
  d16
  DELTA3 pl0:f2
  (center (p2 ph1) (p24:sp7 ph9:r):f2 )
  4u
  DELTA3 pl2:f2
  p19:gp3
  d16
  (center (p1 ph2) (p3 ph5):f2 )
  4u
  p16:gp4
  d16
  DELTA4 pl0:f2
  (center (p2 ph1) (p14:sp3 ph1):f2 )
  4u
  DELTA4
  p16:gp4
  d16
  (p1 ph1)
  DELTA1
  (p2 ph1)
  4u
```

```
  p16:gp2
  d16 pl12:f2
  4u BLKGRAD
  go=2 ph31 cpd2:f2
  d1 do:f2 mc #0 to 2
     F1EA(igrad EA & ip5*2, id0 & ip3*2 & ip6*2 & ip31*2)
exit


ph1=0
ph2=1
ph3=0 ;2
ph4=0 ;0 2 2
ph5=1 ;1 3 3
ph6=0
ph7=0 ;0 2 2
ph8=0 ;0 2 2
ph9=0
ph10=0
ph11=0
ph12=0 2
ph13=0 0 2 2
ph31=0 0 2 2


;pl0 : 120dB
;pl1 : f1 channel - power level for pulse (default)
;pl2 : f2 channel - power level for pulse (default)
;pl3 : f3 channel - power level for pulse (default)
;pl12: f2 channel - power level for CPD/BB decoupling
;sp3: f2 channel - shaped pulse (180degree inversion)
;spnam3: Crp60,0.5,20.1
;sp7: f2 channel - shaped pulse (180degree refocussing)
;sp9: powerlevel for water flipback
;spnam7: Crp60comp.4
;p1 : f1 channel -  90 degree high power pulse
;p2 : f1 channel - 180 degree high power pulse
;p3 : f2 channel -  90 degree high power pulse
;p14: f2 channel - 180 degree shaped pulse for inversion
;     = 500usec for Crp60,0.5,20.1
;p9: waterflipback puls
;p10: 180 degree Q3 for arom, carbonyl
;p11: 180 degree Q3 for aliph
;p12: 90 degree Q5 for arom, carbonyl
;p13: 90 degree Q5 for aliph
;p15: 90 degree Q5tr for aliph
;p16: homospoil/gradient pulse                         [1 msec]
;p19: gradient pulse 2                                 [500 usec]
;p22: f3 channel - 180 degree high power pulse
;p24: f2 channel - 180 degree shaped pulse for refocussing
;     = 2msec for Crp60comp.4
;p28: f1 channel - trim pulse
;d0 : incremented delay (2D)                           [3 usec]
;d1 : relaxation delay; 1-5 * T1
;d4 : 1/(4J)XH
;d11: delay for disk I/O                               [30 msec]
;d16: delay for homospoil/gradient recovery
;d24: 1/(8J)XH for all multiplicities
;     1/(4J)XH for XH
;cnst2: = J(XH)
;cnst3: = J(CC) arom., carbonyl
;cnst4: = J(CC) aliph.
;cnst17: = -0.5 for Crp60comp.4
;in0: 1/(2 * SW(X)) = DW(X)
;nd0: 2
;NS: 1 * n
;DS: >= 16
;td1: number of experiments
```

```
;FnMODE: echo-antiecho
;cpd2: decoupling according to sequence defined by cpdprg2
;pcpd2: f2 channel - 90 degree pulse for decoupling sequence


;use gradient ratio:  gp 1 : gp 2 : gp 3 : gp 4
;                       80 : 20.1 :   11 :   -5    for C-13
;                       80 :  8.1 :   11 :   -5    for N-15

;for z-only gradients:
;gpz1: 80%
;gpz2: 20.1% for C-13, 8.1% for N-15
;gpz3: 11%
;gpz4: -5%

;use gradient files:
;gpnam1: SINE.100
;gpnam2: SINE.100
;gpnam3: SINE.100
;gpnam4: SINE.100


;cnst17: Factor to compensate for coupling evolution during a pulse
;       (usually +1). A positive factor indicates that coupling
;       evolution continues during the pulse, whereas a negative
;       factor is necessary if the coupling is (partially) refocussed.



;$Id: hsqcetgpsisp2.2,v 1.1 2003/01/02 16:31:23 ber Exp $
```

B.5.4 NMR spectra

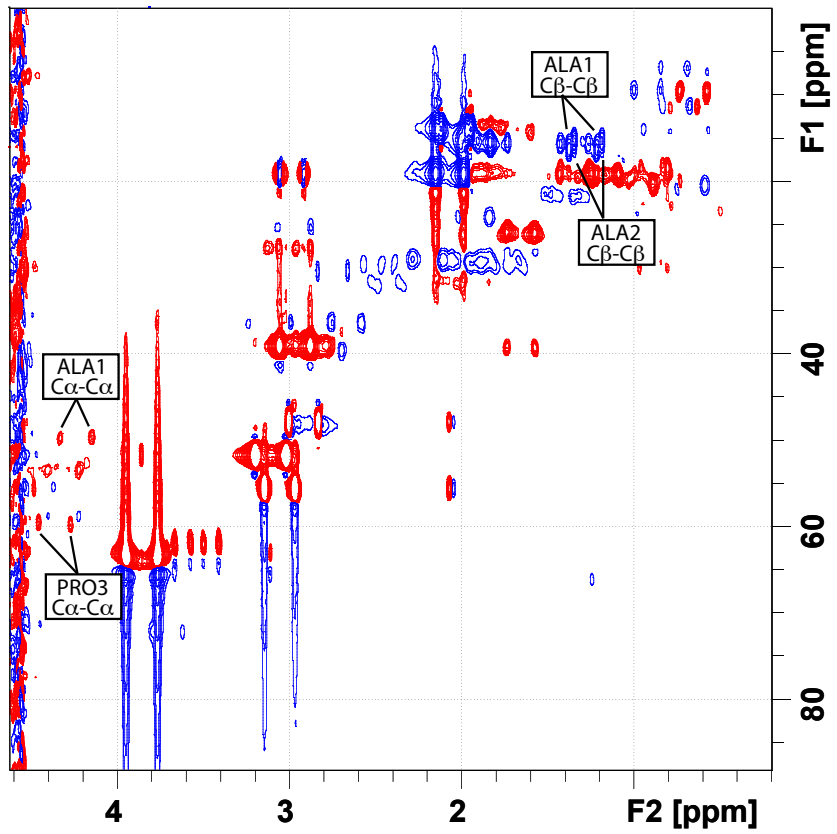


Figure B.7: Expansion of an 800 MHz ^{13}C-filtered HSQC spectrum of a 2 mM suc-AAPF-pNA bound to 1 mM of trigger factor tagged with **6a** and loaded with Dy^{3+}. The traces of the indicated residues can be found in Fig.3.12. 2048 complex data points in t_2 and 256 experiments in t_1 were aquired with 128 scans.

C

Appendix: NMR restraints

C.1 Hormaomycin

C.1.1 NOEs, Dihedrals and Residual dipolar couplings

```
!BIOSYM restraint 1
!
!
#chiral
19:MON_1:C73 S
19:MON_1:C2 R
19:MON_1:C3 R
19:MON_1:C37 R
19:MON_1:C32 S
19:MON_1:C62 R
19:MON_1:C28 R
19:MON_1:C59 R
19:MON_1:C61 R
19:MON_1:C24 S
19:MON_1:C51 R
19:MON_1:C18 S
19:MON_1:C47 S
19:MON_1:C46 R
19:MON_1:C42 S
19:MON_1:C39 R
!
!
#distance
19:MON_1:H26        19:MON_1:H6 2.0 4.0 20.00 20.00 50.0
19:MON_1:H26        19:MON_1:H1 2.0 4.0 20.00 20.00 50.0
19:MON_1:H26        19:MON_1:H22 2.0 4.0 20.00 20.00 50.0
19:MON_1:H26        19:MON_1:H20 2.0 3.5 20.00 20.00 50.0
!
19:MON_1:H6        19:MON_1:H14 2.0 4.0 20.00 20.00 50.0
19:MON_1:H6        19:MON_1:H35 2.0 2.8 20.00 20.00 50.0
19:MON_1:H6        19:MON_1:H33 2.0 4.0 20.00 20.00 50.0
19:MON_1:H6        19:MON_1:H57 2.0 3.5 20.00 20.00 50.0
!
19:MON_1:H7        19:MON_1:H29 2.0 2.8 20.00 20.00 50.0
19:MON_1:H7        19:MON_1:H13 2.0 4.0 20.00 20.00 50.0
19:MON_1:H7        19:MON_1:H51 2.0 2.5 20.00 20.00 50.0
!
19:MON_1:H14        19:MON_1:H33 2.0 2.8 20.00 20.00 50.0
19:MON_1:H14        19:MON_1:H57 2.0 2.5 20.00 20.00 50.0
!
!
19:MON_1:H1        19:MON_1:H2 2.0 2.8 20.00 20.00 50.0
```

```
!
19:MON_1:H66        19:MON_1:H65 2.0 2.5 20.00 20.00 50.0
19:MON_1:H66        19:MON_1:H39 2.0 5.0 20.00 20.00 50.0
19:MON_1:H66        19:MON_1:H41 2.0 5.0 20.00 20.00 50.0
19:MON_1:H66        19:MON_1:H40 2.0 5.0 20.00 20.00 50.0
19:MON_1:H66         19:MON_1:H37 2.0 5.0 20.00 20.00 50.0
19:MON_1:H66         19:MON_1:PE3 2.0 2.8 20.00 20.00 50.0
19:MON_1:H66         19:MON_1:H38 2.0 4.0 20.00 20.00 50.0
!
19:MON_1:H65         19:MON_1:H39 2.0 3.5 20.00 20.00 50.0
19:MON_1:H65         19:MON_1:H41 2.0 3.5 20.00 20.00 50.0
19:MON_1:H65         19:MON_1:H40 2.0 3.5 20.00 20.00 50.0
19:MON_1:H65         19:MON_1:H37 2.0 3.5 20.00 20.00 50.0
19:MON_1:H65         19:MON_1:PE3 2.0 4.0 20.00 20.00 50.0
19:MON_1:H65         19:MON_1:H38 2.0 2.5 20.00 20.00 50.0
!
!
19:MON_1:H35        19:MON_1:H57 2.0 5.0 20.00 20.00 50.0
!
19:MON_1:H33        19:MON_1:H57 2.0 3.5 20.00 20.00 50.0
!
19:MON_1:H29        19:MON_1:H51 2.0 2.5 20.00 20.00 50.0
19:MON_1:H29        19:MON_1:AL11 2.0 5.0 20.00 20.00 50.0
!
19:MON_1:H13        19:MON_1:AL11 2.0 4.0 20.00 20.00 50.0
19:MON_1:H13        19:MON_1:AL12 2.0 4.0 20.00 20.00 50.0
19:MON_1:H13        19:MON_1:AL11 2.0 3.5 20.00 20.00 50.0
!
19:MON_1:H36        19:MON_1:H41 2.0 2.8 20.00 20.00 50.0
19:MON_1:H36        19:MON_1:H40 2.0 5.0 20.00 20.00 50.0
19:MON_1:H36        19:MON_1:H37 2.0 2.5 20.00 20.00 50.0
19:MON_1:H36        19:MON_1:PE3 2.0 4.0 20.00 20.00 50.0
19:MON_1:H36        19:MON_1:H38 2.0 3.5 20.00 20.00 50.0
19:MON_1:H36        19:MON_1:ILE1 2.0 5.0 20.00 20.00 50.0
!
19:MON_1:H39        19:MON_1:H41 2.0 2.8 20.00 20.00 50.0
19:MON_1:H39        19:MON_1:H37 2.0 5.0 20.00 20.00 50.0
19:MON_1:H39        19:MON_1:H42 2.0 4.0 20.00 20.00 50.0
19:MON_1:H39        19:MON_1:PE3 2.0 5.0 20.00 20.00 50.0
19:MON_1:H39        19:MON_1:H38 2.0 4.0 20.00 20.00 50.0
19:MON_1:H39        19:MON_1:ILE1 2.0 4.0 20.00 20.00 50.0
!
19:MON_1:H41        19:MON_1:H40 2.0 2.5 20.00 20.00 50.0
19:MON_1:H41        19:MON_1:H37 2.0 2.8 20.00 20.00 50.0
19:MON_1:H41        19:MON_1:PE3 2.0 3.5 20.00 20.00 50.0
19:MON_1:H41        19:MON_1:H38 2.0 4.0 20.00 20.00 50.0
!
19:MON_1:H40        19:MON_1:H37 2.0 4.0 20.00 20.00 50.0
19:MON_1:H40        19:MON_1:H42 2.0 4.0 20.00 20.00 50.0
19:MON_1:H40        19:MON_1:PE3 2.0 5.0 20.00 20.00 50.0
19:MON_1:H40        19:MON_1:H38 2.0 4.0 20.00 20.00 50.0
19:MON_1:H40        19:MON_1:ILE1 2.0 4.0 20.00 20.00 50.0
!
19:MON_1:H37        19:MON_1:PE3 2.0 4.0 20.00 20.00 50.0
!
19:MON_1:H22        19:MON_1:H21 2.0 3.5 20.00 20.00 50.0
19:MON_1:H22        19:MON_1:H20 2.0 4.0 20.00 20.00 50.0
19:MON_1:H22        19:MON_1:AL22 2.0 3.5 20.00 20.00 50.0
!
19:MON_1:H42        19:MON_1:ILE2 2.0 4.0 20.00 20.00 50.0
19:MON_1:H42        19:MON_1:ILE1 2.0 2.8 20.00 20.00 50.0
19:MON_1:H42        19:MON_1:ILE3 2.0 3.5 20.00 20.00 50.0
!
19:MON_1:ILE2       19:MON_1:ILE1 2.0 4.0 20.00 20.00 50.0
19:MON_1:ILE2       19:MON_1:ILE3 2.0 2.8 20.00 20.00 50.0
!
19:MON_1:AL11       19:MON_1:AL12 2.0 4.0 20.00 20.00 50.0
!
```

```
19:MON_1:H43       19:MON_1:H48 2.0 2.8 20.00 20.00 50.0
!
!
19:MON_1:H6        19:MON_1:H5 2.0 4.0 20.00 20.00 50.0
19:MON_1:H4        19:MON_1:H3 2.0 4.0 20.00 20.00 50.0
19:MON_1:H19        19:MON_1:H6 2.0 2.5 20.00 20.00 50.0
19:MON_1:H26        19:MON_1:H19 2.0 4.0 20.00 20.00 50.0
!
19:MON_1:H6        19:MON_1:H34 2.0 3.5 20.00 20.00 50.0
19:MON_1:H5        19:MON_1:H33 2.0 2.5 20.00 20.00 50.0
19:MON_1:H3        19:MON_1:H29 2.0 4.0 20.00 20.00 50.0
19:MON_1:H19        19:MON_1:H35 2.0 2.8 20.00 20.00 50.0
!
19:MON_1:H26        19:MON_1:H20 2.0 4.0 20.00 20.00 50.0
19:MON_1:H26        19:MON_1:H21 2.0 4.0 20.00 20.00 50.0
!
19:MON_1:H3        19:MON_1:H28 2.0 2.8 20.00 20.00 50.0
19:MON_1:H3        19:MON_1:H42 2.0 4.0 20.00 20.00 50.0
!
19:MON_1:H4        19:MON_1:H29 2.0 3.5 20.00 20.00 50.0
19:MON_1:H4        19:MON_1:H51 2.0 4.0 20.00 20.00 50.0
!
19:MON_1:H5        19:MON_1:H30 2.0 3.5 20.00 20.00 50.0
19:MON_1:H5        19:MON_1:H31 2.0 4.0 20.00 20.00 50.0
19:MON_1:H5        19:MON_1:H32 2.0 4.0 20.00 20.00 50.0
!
19:MON_1:H57        19:MON_1:H6 2.0 3.5 20.00 20.00 50.0
19:MON_1:H33        19:MON_1:H6 2.0 4.0 20.00 20.00 50.0
!
!
!
!
!
#NMR_dihedral
!
19:MON_1:H20        19:MON_1:C1         19:MON_1:C2        19:MON_1:H22          -80        -40 50 50 500
19:MON_1:H42        19:MON_1:C47        19:MON_1:C18        19:MON_1:C19          40   80 50 50 500
19:MON_1:H29        19:MON_1:C24        19:MON_1:C51        19:MON_1:H51          40   80 50 50 500
19:MON_1:H34        19:MON_1:C37        19:MON_1:C39        19:MON_1:H35         170       -170    100     100
500
19:MON_1:H33        19:MON_1:C32        19:MON_1:C62        19:MON_1:H57         120       -120 50 50 500
19:MON_1:H36        19:MON_1:C42        19:MON_1:C44        19:MON_1:H37         -50        -10 50 50 500
19:MON_1:H36        19:MON_1:C42        19:MON_1:C44        19:MON_1:H38        -179       -140 50 50 500
!
19:MON_1:H42        19:MON_1:C47        19:MON_1:C49        19:MON_1:C50         120       -120 50 50 500
!
! trans
19:MON_1:C42        19:MON_1:N23        19:MON_1:C19        19:MON_1:C18          170   -170 100 100 500
19:MON_1:C28        19:MON_1:N30        19:MON_1:C31        19:MON_1:C32          170   -170 100 100 500
!
19:MON_1:C18        19:MON_1:N20        19:MON_1:C21        19:MON_1:C24          170   -170 100 100 500
!
19:MON_1:C24        19:MON_1:N25        19:MON_1:C26        19:MON_1:C28          170   -170 100 100 500
19:MON_1:C32        19:MON_1:N35        19:MON_1:C36        19:MON_1:C37          170   -170 100 100 500
19:MON_1:C37        19:MON_1:N71        19:MON_1:C72        19:MON_1:C73          170   -170 100 100 500
!
!
! 4-Pe Pro propenyl configuration cis
19:MON_1:H65        19:MON_1:C78        19:MON_1:C79        19:MON_1:H66         -20   20 100 100 500
!
! Ala-Ncp I ring configuration: cis H12-H13
19:MON_1:H12        19:MON_1:C60        19:MON_1:C61        19:MON_1:H13         -20   20 100    100 500
!
! Ala-Ncp II ring configuration: cis H23-H25
19:MON_1:H23        19:MON_1:C3        19:MON_1:C4        19:MON_1:H25          -20   20 100    100 500
!
!
! ring Phe Me I
```

```
19:MON_1:C53        19:MON_1:C58        19:MON_1:C57        19:MON_1:H10         180  180 500 500 500
19:MON_1:H11        19:MON_1:C58        19:MON_1:C57        19:MON_1:H10           0    0 500 500 500
19:MON_1:C58        19:MON_1:C57        19:MON_1:C56        19:MON_1:H9          180   180 500 500 500
19:MON_1:C57        19:MON_1:C56        19:MON_1:C55        19:MON_1:H8          180   180 500 500 500
19:MON_1:C56        19:MON_1:C55        19:MON_1:C54        19:MON_1:H7          180   180 500 500 500
19:MON_1:H8         19:MON_1:C55        19:MON_1:C54        19:MON_1:H7            0        0 500 500 500
19:MON_1:H7         19:MON_1:C54        19:MON_1:C53        19:MON_1:C58         180   180 500 500 500
19:MON_1:H7         19:MON_1:C54        19:MON_1:C53        19:MON_1:C51           0     0 500 500 500
19:MON_1:H11        19:MON_1:C58        19:MON_1:C53        19:MON_1:C51          0     0 500 500 500



! ring Phe Me II
19:MON_1:C68        19:MON_1:C67        19:MON_1:C66        19:MON_1:H16          180   180 500 500 500
19:MON_1:C67        19:MON_1:C68        19:MON_1:C63        19:MON_1:C62          180   180 500 500 500
19:MON_1:C62        19:MON_1:C63        19:MON_1:C64        19:MON_1:C65          180   180 500 500 500
19:MON_1:C63        19:MON_1:C64        19:MON_1:C65        19:MON_1:C66            0     0 500 500 500
19:MON_1:C64        19:MON_1:C65        19:MON_1:C66        19:MON_1:H16          180   180 500 500 500
19:MON_1:C62        19:MON_1:C63        19:MON_1:C64        19:MON_1:H14            0     0 500 500 500
19:MON_1:H14        19:MON_1:C64        19:MON_1:C65        19:MON_1:C66          180   180 500 500 500



! ring Chpca
19:MON_1:C13         19:MON_1:C12        19:MON_1:C10         19:MON_1:C9          180      180 500 500
500
19:MON_1:H1         19:MON_1:C12        19:MON_1:C13         19:MON_1:H2           0          0 500 500 500
19:MON_1:C10        19:MON_1:C12        19:MON_1:C13         19:MON_1:H2         180 180 500 500 500
19:MON_1:C14        19:MON_1:C13        19:MON_1:C12         19:MON_1:H1         180 180 500 500 500
19:MON_1:C13        19:MON_1:C14        19:MON_1:N15         19:MON_1:C10          0          0 500 500 500
19:MON_1:CL17        19:MON_1:C14        19:MON_1:N15         19:MON_1:O16         0          0 500 500 500
19:MON_1:CL17        19:MON_1:C14        19:MON_1:C13         19:MON_1:C12        180 180     500 500 500
19:MON_1:CL17        19:MON_1:C14        19:MON_1:N15         19:MON_1:C10        180 180     500 500 500



!
#dipnh1
!
!
19:MON_1:N8    19:MON_1:H26                  5.5   1.0       1.00    0
19:MON_1:N30    19:MON_1:H5      11.9   1.0       1.00    0
19:MON_1:N20    19:MON_1:H3      -7.0   1.0       1.00    0
19:MON_1:N71   19:MON_1:H19         7.6   1.0      1.00    0
19:MON_1:N25   19:MON_1:H4        20.6   1.0      1.00    0
19:MON_1:N35   19:MON_1:H6        10.4   1.0      1.00    0
!
#dipch1
!
!
19:MON_1:C73     19:MON_1:H64      0.6         1.0     1.00    0
19:MON_1:C1    19:MON_1:H20      -3.2       1.0     1.00    0
19:MON_1:C1    19:MON_1:H21      10.3       1.0     1.00    0
19:MON_1:C2    19:MON_1:H22      18.7       1.0     1.00    0
19:MON_1:C3    19:MON_1:H23      7.0       1.0     1.00    0
19:MON_1:C4    19:MON_1:H24     -10.2       1.0     1.00    0
19:MON_1:C4    19:MON_1:H25      8.4       1.0     1.00    0
!
19:MON_1:C28     19:MON_1:H30     28.8     1.0     1.00    0
19:MON_1:C29    19:MON_1:H32     18.2     1.0     1.00    0
19:MON_1:C29    19:MON_1:H31      -0.5       1.0     1.00    0
19:MON_1:C59    19:MON_1:H55      6           1.0     1.00    0
19:MON_1:C61    19:MON_1:H13      18.2       1.0     1.00    0
19:MON_1:C3    19:MON_1:H23      7.0       1.0     1.00    0
19:MON_1:C60    19:MON_1:H56     -6.1       1.0     1.00    0
19:MON_1:C60    19:MON_1:H12      19.6       1.0     1.00    0
!
19:MON_1:C18     19:MON_1:H28     -0.9       1.0     1.00    0
```

```
19:MON_1:C47    19:MON_1:H42     -6.1      1.0     1.00    0
19:MON_1:C49    19:MON_1:H46     -4.7       1.0     1.00    0
19:MON_1:C49    19:MON_1:H47      1.6          1.0     1.00    0
!
19:MON_1:C37     19:MON_1:H34     26.9     1.0     1.00    0
19:MON_1:C39    19:MON_1:H35     29.0      1.0        1.00    0
!
19:MON_1:C42    19:MON_1:H36      9.5       1.0     1.00    0
19:MON_1:C44    19:MON_1:H38      -5.5       1.0     1.00    0
19:MON_1:C44    19:MON_1:H37       5.6      1.0     1.00    0
19:MON_1:C46    19:MON_1:H41      10.1    1.0     1.00    0
19:MON_1:C45    19:MON_1:H40      -21.9    1.0     1.00    0
19:MON_1:C45    19:MON_1:H39      7.7     1.0     1.00    0
19:MON_1:C78    19:MON_1:H65      11.6   1.0     1.00    0
19:MON_1:C79    19:MON_1:H66      -10.3    1.0     1.00    0
!
19:MON_1:C12    19:MON_1:H1                -0.9    1.0     1.00    0
19:MON_1:C13    19:MON_1:H2         3.50     1.0     1.00    0
!
19:MON_1:C24    19:MON_1:H29      35.0       1.0     1.00    0
19:MON_1:C51    19:MON_1:H51      -6.3       1.0     1.00    0
19:MON_1:C54    19:MON_1:H7                9.5    1.0     1.00    0
19:MON_1:C58    19:MON_1:H11      9.5    1.0     1.00    0
19:MON_1:C55    19:MON_1:H8         0.4     1.0     1.00    0
19:MON_1:C57    19:MON_1:H10     -21.4   1.0     1.00    0
19:MON_1:C56    19:MON_1:H9        18.5    1.0     1.00    0
!
19:MON_1:C32    19:MON_1:H33      26.3       1.0     1.00    0
19:MON_1:C62    19:MON_1:H57      19.5       1.0     1.00    0
19:MON_1:C68    19:MON_1:H18               17.5    1.0     1.00    0
19:MON_1:C64    19:MON_1:H14      17.5    1.0     1.00    0
19:MON_1:C67    19:MON_1:H17         18.5     1.0     1.00    0
19:MON_1:C65    19:MON_1:H15     18.5   1.0     1.00    0
19:MON_1:C66    19:MON_1:H16        -3.2    1.0     1.00    0
!
#dipcc1
!
!19:MON_1:C50    19:MON_1:C49      0.5       1.0     1.00    0
!19:MON_1:C48    19:MON_1:C47      -1.8      1.0     1.00    0
!19:MON_1:C69    19:MON_1:C62      0.3      1.0     1.00    0
!19:MON_1:C52    19:MON_1:C51      2.8      1.0     1.00    0
!19:MON_1:C80    19:MON_1:C79       0.50     1.0     1.00    0
!19:MON_1:C70    19:MON_1:C39      3.9       1.0     1.00    0
```

C.2 Hymenistatin

C.2.1 NOEs (DMSO)

```
assign (residue 2 and name HA) (residue 2 and name HB1) 2.4 .5 .5
assign (residue 2 and name HA) (residue 2 and name HB2) 2.7 .5 1.0
assign (residue 2 and name HA) (residue 2 and name HD1) 3.3 .5 1.0
assign (residue 2 and name HA) (residue 3 and name HD2) 4.2 .5 1.0
assign (residue 2 and name HB1) (residue 2 and name HB2) 1.9 .5 .5
assign (residue 2 and name HD2) (residue 2 and name HD1) 1.8 .5 1.0
assign (residue 2 and name HD2) (residue 1 and name HN) 3.8 .5 1.0
assign (residue 3 and name HA) (residue 2 and name HA) 2.3 .5 .5
assign (residue 3 and name HA) (residue 2 and name HB1) 2.9 .5 .5
assign (residue 3 and name HA) (residue 2 and name HB2) 2.8 .5 .5
assign (residue 3 and name HA) (residue 3 and name HD1) 3.5 .5 1.0
assign (residue 3 and name HA) (residue 6 and name HA) 3.1 .5 1.0
assign (residue 3 and name HD1) (residue 3 and name HD2) 1.8 .5 .5
assign (residue 3 and name HD2) (residue 3 and name HG2) 2.5 .5 1.0
assign (residue 4 and name HD*) (residue 3 and name HD2) 3.4 .5 1.0
assign (residue 4 and name HD*) (residue 4 and name HA) 2.7 .5 .5
```

```
assign (residue 4 and name HD*) (residue 4 and name HB1) 2.7 .5 1.5
assign (residue 4 and name HD*) (residue 4 and name HB2) 2.6 .5 1.5
assign (residue 4 and name HD*) (residue 1 and name HG2*) 3.5 .5 1.5
assign (residue 4 and name HE*) (residue 3 and name HD2) 2.8 .5 1.5
!assign (residue 4 and name HE*) (residue 6 and name HD2) 2.8 .5 1.5
assign (residue 4 and name HN) (residue 2 and name HA) 2.6 .5 .5
assign (residue 4 and name HN) (residue 3 and name HA) 3.3 .5 1.0
assign (residue 4 and name HN) (residue 3 and name HD2) 2.9 .5 .5
assign (residue 4 and name HN) (residue 3 and name HG2) 3.2 .5 1.0
assign (residue 4 and name HN) (residue 4 and name HD*) 2.9 .5 1.0
assign (residue 4 and name HN) (residue 4 and name HB1) 3.3 .5 .5
assign (residue 4 and name HN) (residue 4 and name HB2) 2.7 .5 .5
assign (residue 4 and name HB1) (residue 4 and name HB2) 1.8 .5 .5
assign (residue 4 and name HB2) (residue 1 and name HG2*) 3.3 .5 1.0
assign (residue 5 and name HN) (residue 2 and name HA) 2.9 .5 .5
assign (residue 5 and name HN) (residue 4 and name HD*) 3.7 .5 1.5
assign (residue 5 and name HN) (residue 4 and name HA) 3.4 .5 1.0
assign (residue 5 and name HN) (residue 4 and name HB1) 3.1 .5 1.0
assign (residue 5 and name HN) (residue 4 and name HB2) 2.5 .5 0.5
assign (residue 5 and name HN) (residue 6 and name HA) 4.0 .5 1.0
assign (residue 5 and name HN) (residue 6 and name HD1) 3.8 .5 1.0
assign (residue 5 and name HN) (residue 1 and name HG2*) 3.5 .5 1.0
!assign (residue 5 and name HA) (residue 4 and name HA) 2.9 .5 0.5
assign (residue 5 and name HA) (residue 6 and name HA) 3.6 .5 1.0
assign (residue 5 and name HA) (residue 6 and name HD2) 2.3 .5 .5
assign (residue 5 and name HA) (residue 6 and name HD1) 2.2 .5 .5
assign (residue 6 and name HA) (residue 2 and name HB1) 3.0 .5 1.0
assign (residue 6 and name HA) (residue 2 and name HD1) 2.9 .5 1.0
assign (residue 6 and name HA) (residue 6 and name HB1) 2.4 .5 .5
assign (residue 6 and name HA) (residue 6 and name HG1) 3.1 .5 .5
assign (residue 6 and name HB1) (residue 6 and name HB2) 2.0 .5 .5
assign (residue 6 and name HD2) (residue 6 and name HB2) 2.9 .5 1.0
assign (residue 6 and name HD1) (residue 6 and name HB2) 3.2 .5 1.0
assign (residue 6 and name HD1) (residue 6 and name HG1) 2.6 .5 1.0
assign (residue 7 and name HN) (residue 2 and name HB1) 3.5 .5 1.0
assign (residue 7 and name HN) (residue 2 and name HD1) 3.7 .5 1.5
!assign (residue 7 and name HN) (residue 5 and name HN) 3.2 .5 1.0
assign (residue 7 and name HN) (residue 6 and name HA) 2.3 .5 .5
assign (residue 7 and name HN) (residue 6 and name HB1) 3.7 .5 .5
assign (residue 7 and name HN) (residue 6 and name HB2) 3.3 .5 1.0
assign (residue 7 and name HN) (residue 7 and name HB2) 2.9 .5 .5
assign (residue 7 and name HN) (residue 8 and name HN) 2.9 .5 .5
assign (residue 8 and name HN) (residue 2 and name HD1) 3.3 .5 .5
!assign (residue 8 and name HN) (residue 4 and name HN) 3.0 .5 1.0
assign (residue 8 and name HN) (residue 5 and name HN) 3.4 .5 1.0
assign (residue 8 and name HN) (residue 6 and name HA) 3.7 .5 1.0
assign (residue 8 and name HN) (residue 7 and name HB2) 3.2 .5 .5
assign (residue 8 and name HN) (residue 8 and name HG11) 3.0 .5 1.0
assign (residue 8 and name HA) (residue 8 and name HD1*) 3.1 .5 1.0
assign (residue 8 and name HA) (residue 8 and name HG11) 2.8 .5 1.5
assign (residue 1 and name HN) (residue 2 and name HA) 4.0 .5 1.5
assign (residue 1 and name HN) (residue 2 and name HD2) 3.8 .5 1.0
assign (residue 1 and name HN) (residue 2 and name HD1) 3.1 .5 .5
assign (residue 1 and name HN) (residue 4 and name HN) 3.9 .5 1.0
assign (residue 1 and name HN) (residue 5 and name HN) 2.9 .5 1.0
assign (residue 1 and name HN) (residue 8 and name HN) 2.2 .5 .5
assign (residue 1 and name HN) (residue 8 and name HA) 3.4 .5 1.0
assign (residue 1 and name HN) (residue 1 and name HG2*) 3.2 .5 1.0
assign (residue 1 and name HA) (residue 2 and name HD2) 2.4 .5 .5
assign (residue 1 and name HA) (residue 2 and name HD1) 2.7 .5 .5
assign (residue 1 and name HA) (residue 2 and name HD2) 2.4 .5 .5
assign (residue 1 and name HA) (residue 1 and name HG11) 3.2 .5 1.0
assign (residue 1 and name HA) (residue 1 and name HG2*) 2.9 .5 .5
assign (residue 2 and name HD2) (residue 2 and name HB1) 4.1 .5 1.0
assign (residue 2 and name HD2) (residue 2 and name HB2) 3.3 .5 .5
assign (residue 2 and name HD2) ( (residue 1 and name HB) or (residue 5 and name HB) or
 (residue 3 and name HB*) or (residue 2 and name HG*) ) 2.6 .5 1.0
assign (residue 2 and name HD1) (residue 2 and name HB2) 3.6 .5 1.0
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assign (residue 2 and name HD1) ( (residue 1 and name HB) or (residue 5 and name HB)
 or (residue 3 and name HB*) or (residue 2 and name HG*) ) 2.6 .5 1.0
assign (residue 3 and name HA) (residue 3 and name HG2) 3.5 .5 1.0
assign (residue 4 and name HN) (residue 4 and name HA) 3.1 .5 1.0
assign (residue 5 and name HN) (residue 5 and name HA) 2.9 .5 1.0
assign (residue 6 and name HG1) (residue 6 and name HG2) 1.8 .5 1.0
assign (residue 8 and name HN) (residue 8 and name HA) 3.0 .5 1.0
assign (residue 1 and name HN) (residue 1 and name HA) 3.0 .5 1.0
assign (residue 3 and name HD1) ((residue 7 and name HB1) or (residue 1 and name HG1*)
 or (residue 7 and name HG) or (residue 3 and name HG*)) 2.5 .5 1.0
assign (residue 3 and name HD1) ( (residue 1 and name HB) or (residue 5 and name HB)
 or (residue 3 and name HB*) or (residue 2 and name HG*) ) 2.8 .5 1.0
assign (residue 3 and name HD2) ( (residue 7 and name HB1) or (residue 1 and name HG1*)
 or (residue 7 and name HG) or (residue 3 and name HG*) ) 2.9 .5 .5
assign (residue 3 and name HD2) ( (residue 1 and name HB) or (residue 5 and name HB)
 or (residue 3 and name HB*) or (residue 2 and name HG*) ) 3.4 .5 .5
assign (residue 4 and name HB1) ( (residue 7 and name HD1*) or (residue 7 and name HD2*)
 or (residue 5 and name HG1*) or (residue 5 and name HG2*) or (residue 8 and name HG2*)
 or (residue 1 and name HD1*) ) 3.1 .5 1.0
assign (residue 4 and name HB2) ( (residue 7 and name HD1*) or (residue 7 and name HD2*)
 or (residue 5 and name HG1*) or (residue 5 and name HG2*) or (residue 8 and name HG2*)
 or (residue 1 and name HD1*) ) 3.1 .5 1.0
assign (residue 4 and name HB2) ( (residue 1 and name HB) or (residue 5 and name HB)
 or (residue 3 and name HB*) or (residue 2 and name HG*) ) 3.5 .5 1.0
assign (residue 5 and name HN) ( (residue 1 and name HB) or (residue 5 and name HB)
 or (residue 3 and name HB*) or (residue 2 and name HG*) ) 2.6 .5 .5
assign (residue 5 and name HA) ( (residue 7 and name HD1*) or (residue 7 and name HD2*)
 or (residue 5 and name HG1*) or (residue 5 and name HG2*) or (residue 8 and name HG2*)
 or (residue 1 and name HD1*) ) 2.7 .5 1.0
assign (residue 6 and name HG*) ( (residue 8 and name HB) or (residue 8 and name HG1*) ) 2.9 .5 1.0
assign (residue 7 and name HN) ( (residue 1 and name HB) or (residue 5 and name HB)
 or (residue 3 and name HB*) or (residue 2 and name HG*) ) 3.7 .5 1.5
assign (residue 7 and name HN) ( (residue 7 and name HB1) or (residue 1 and name HG1*)
 or (residue 7 and name HG) or (residue 3 and name HG*) ) 2.9 .5 .5
assign (residue 7 and name HN) ( (residue 7 and name HD1*) or (residue 7 and name HD2*)
 or (residue 5 and name HG1*) or (residue 5 and name HG2*) or (residue 8 and name HG2*)
 or (residue 1 and name HD1*) ) 3.4 .5 1.0
assign (residue 7 and name HB2) ( (residue 8 and name HB) or (residue 8 and name HG1*) )  2.9 .5 1.0
assign (residue 3 and name HG1) (residue 3 and name HG2) 1.9 .5 .5
assign ( (residue 7 and name HB1) or (residue 1 and name HG1*) or (residue 7 and name HG)
 or (residue 3 and name HG*) ) (residue 1 and name HG*) 3.0 .5 1.0
assign (residue 8 and name HN) ( (residue 7 and name HB1) or (residue 1 and name HG1*)
 or (residue 7 and name HG) or (residue 3 and name HG*) ) 3.4 .5 1.0
assign (residue 8 and name HN) ( (residue 8 and name HB) or (residue 8 and name HG1*) )  2.6 .5 .5
assign (residue 8 and name HN) ( (residue 1 and name HB) or (residue 5 and name HB) or
 (residue 3 and name HB*) or (residue 2 and name HG*) )  3.1 .5 1.5
assign (residue 8 and name HA) ( (residue 7 and name HD1*) or (residue 7 and name HD2*)
 or (residue 5 and name HG1*) or (residue 5 and name HG2*) or (residue 8 and name HG2*)
 or (residue 1 and name HD1*) ) 2.8 .5 1.0
assign ( (residue 8 and name HB) or (residue 8 and name HG1*) ) ( (residue 7 and name HD1*)
 or (residue 7 and name HD2*) or (residue 5 and name HG1*) or (residue 5 and name HG2*) or
 (residue 8 and name HG2*) or (residue 1 and name HD1*) ) 2.2 .5 1.0
assign (residue 1 and name HN) ( (residue 7 and name HB1) or (residue 1 and name HG1*)
 or (residue 7 and name HG) or (residue 3 and name HG*) ) 3.4 .5 .5
assign (residue 1 and name HN) ( (residue 7 and name HD1*) or (residue 7 and name HD2*)
 or (residue 5 and name HG1*) or (residue 5 and name HG2*) or (residue 8 and name HG2*)
 or (residue 1 and name HD1*) ) 3.2 .5 1.0
assign (residue 1 and name HN) ( (residue 8 and name HB) or (residue 8 and name HG1*) )  2.5 .5 .5
assign (residue 1 and name HN) ( (residue 1 and name HB) or (residue 5 and name HB)
 or (residue 3 and name HB*) or (residue 2 and name HG*) )  2.4 .5 .5
assign (residue 1 and name HA) ( (residue 7 and name HB1) or (residue 1 and name HG1*)
 or (residue 7 and name HG) or (residue 3 and name HG*) ) 2.9 .5 .5
assign ( (residue 1 and name HB) or (residue 5 and name HB) or (residue 3 and name HB*)
 or (residue 2 and name HG*) ) ( (residue 7 and name HB1) or (residue 1 and name HG1*)
 or (residue 7 and name HG) or (residue 3 and name HG*) ) 2.1 .5 .5
assign ( (residue 1 and name HB) or (residue 5 and name HB) or (residue 3 and name HB*)
 or (residue 2 and name HG*) ) ( (residue 8 and name HB) or (residue 8 and name HG1*) ) 2.4 .5 1.0
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C.2.2 NOEs ($CDCl_3$)

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assign (residue 6 and name HD1) (residue 5 and name HN) 4.5 .5 .5
assign (residue 2 and name HD2) (residue 1 and name HN) 4.3 .5 .5
assign (residue 6 and name HA) (residue 5 and name HN) 4.3 .5 .5
assign (residue 6 and name HA) (residue 8 and name HN) 4.2 .5 .5
assign (residue 8 and name HD1*) (residue 5 and name HN) 4.1 .5 1.0
assign (residue 8 and name HD1*) (residue 1 and name HN) 4.1 .5 1.0
assign (residue 2 and name HA) (residue 1 and name HN) 3.9 .5 .5
assign (residue 8 and name HD1*) (residue 8 and name HN) 3.7 .5 1.0
assign (residue 7 and name HN) (residue 8 and name HN) 3.7 .5 .5
!assign (residue 2 and name HB2) (residue 4 and name HA) 3.6 .5 .5
assign (residue 7 and name HG) (residue 7 and name HN) 3.6 .5 .5
assign (residue 1 and name HG1*) (residue 1 and name HN) 3.6 .5 .5
assign (residue 1 and name HG2*) (residue 5 and name HN) 3.6 .5 .5
assign (residue 8 and name HA) (residue 1 and name HN) 3.6 .5 1.0
assign (residue 2 and name HD1) (residue 8 and name HN) 3.5 .5 .5
assign (residue 7 and name HB1) (residue 8 and name HN) 3.5 .5 .5
assign (residue 3 and name HA) (residue 4 and name HN) 3.5 .5 .5
assign (residue 4 and name HA) (residue 5 and name HN) 3.5 .5 .5
assign (residue 2 and name HD1) (residue 2 and name HA) 3.5 .5 .5
assign (residue 3 and name HD1) (residue 4 and name HN) 3.5 .5 .5
assign (residue 7 and name HA) (residue 8 and name HN) 3.4 .5 .5
assign (residue 1 and name HB) (residue 5 and name HN) 3.4 .5 .5
assign ((residue 5 and name HG1*) or (residue 5 and name HG2*)) (residue 6 and name HD1) 3.4 .5 1.0
assign (residue 1 and name HG2*) (residue 1 and name HN) 3.3 .5 1.0
assign (residue 1 and name HB) (residue 1 and name HN) 3.3 .5 .5
assign (residue 2 and name HA) (residue 4 and name HA) 3.3 .5 .5
assign (residue 2 and name HB2) (residue 2 and name HD2) 3.3 .5 .5
assign (residue 1 and name HG1*) (residue 5 and name HN) 3.3 .5 .5
assign (residue 7 and name HD1*) (residue 7 and name HA) 3.2 .5 1.0
!assign (residue 3 and name HB1) (residue 4 and name HA) 3.2 .5 .5
assign (residue 7 and name HB2) (residue 7 and name HN) 3.1 .5 .5
assign (residue 8 and name HG1*) (residue 8 and name HN) 3.1 .5 .5
assign (residue 8 and name HG2*) (residue 1 and name HA) 3.1 .5 1.0
!assign (residue 6 and name HA) (residue 5 and name HA) 3.0 .5 .5
assign (residue 2 and name HD1) (residue 1 and name HN) 3.0 .5 .5
assign (residue 8 and name HG1*) (residue 8 and name HN) 3.0 .5 .5
assign (residue 8 and name HA) (residue 8 and name HN) 3.0 .5 .5
assign (residue 3 and name HD2) (residue 4 and name HN) 3.0 .5 .5
assign (residue 1 and name HG1*) (residue 1 and name HN) 3.0 .5 .5
assign (residue 8 and name HG2*) (residue 1 and name HN) 3.0 .5 1.0
assign (residue 7 and name HB2) (residue 8 and name HN) 3.0 .5 .5
assign ((residue 5 and name HG1*) or (residue 5 and name HG2*)) (residue 5 and name HN) 2.9 .5 1.0
assign (residue 8 and name HG1*) (residue 8 and name HA) 2.9 .5 .5
assign (residue 1 and name HG1*) (residue 1 and name HA) 2.9 .5 .5
assign (residue 4 and name HN) (residue 5 and name HN) 2.9 .5 .5
assign (residue 8 and name HB) (residue 8 and name HN) 2.9 .5 .5
assign (residue 4 and name HA) (residue 4 and name HN) 2.9 .5 .5
assign (residue 8 and name HG2*) (residue 8 and name HN) 2.9 .5 1.0
assign (residue 5 and name HA) (residue 5 and name HN) 2.9 .5 .5
assign (residue 1 and name HA) (residue 1 and name HN) 2.9 .5 .5
assign (residue 8 and name HD1) (residue 8 and name HA) 2.9 .5 .5
assign (residue 2 and name HA) (residue 5 and name HN) 2.8 .5 .5
assign (residue 2 and name HA) (residue 4 and name HN) 2.8 .5 .5
assign (residue 1 and name HG1*) (residue 1 and name HA) 2.8 .5 .5
assign (residue 5 and name HB) (residue 5 and name HA) 2.8 .5 .5
assign (residue 8 and name HG1*) (residue 8 and name HA) 2.8 .5 .5
assign (residue 2 and name HB2) (residue 3 and name HA) 2.7 .5 .5
assign ((residue 5 and name HG1*) or (residue 5 and name HG2*)) (residue 6 and name HD2) 2.7 .5 1.0
assign (residue 5 and name HB) (residue 5 and name HN) 2.7 .5 .5
assign (residue 7 and name HA) (residue 7 and name HN) 2.7 .5 .5
assign (residue 8 and name HB) (residue 1 and name HN) 2.7 .5 .5
assign (residue 6 and name HA) (residue 2 and name HA) 2.7 .5 .5
assign (residue 4 and name HB2) (residue 4 and name HN) 2.6 .5 .5
assign (residue 1 and name HN) (residue 8 and name HN) 2.6 .5 .5
assign (residue 8 and name HB) (residue 8 and name HA) 2.6 .5 .5
assign (residue 6 and name HA) (residue 7 and name HN) 2.6 .5 .5
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assign (residue 7 and name HD2) (residue 7 and name HA) 2.6 .5 .5
assign (residue 1 and name HB) (residue 1 and name HA) 2.5 .5 .5
assign (residue 2 and name HD1) (residue 1 and name HA) 2.5 .5 .5
assign (residue 5 and name HB) (residue 1 and name HN) 2.5 .5 .5
assign (residue 8 and name HG2*) (residue 8 and name HA) 2.5 .5 1.0
assign (residue 4 and name HB2) (residue 5 and name HN) 2.4 .5 .5
assign (residue 3 and name HB1) (residue 3 and name HA) 2.4 .5 .5
assign (residue 1 and name HG2*) (residue 1 and name HA) 2.4 .5 1.0
assign (residue 6 and name HB1) (residue 6 and name HA) 2.3 .5 .5
assign (residue 6 and name HD2) (residue 5 and name HA) 2.3 .5 .5
assign (residue 3 and name HB2) (residue 3 and name HA) 2.2 .5 .5
assign (residue 4 and name HB1) (residue 4 and name HA) 2.2 .5 .5
assign (residue 2 and name HA) (residue 3 and name HA) 2.2 .5 .5
assign (residue 2 and name HD2) (residue 1 and name HA) 2.1 .5 .5
assign (residue 6 and name HD1) (residue 5 and name HA) 2.1 .5 .5
assign (residue 6 and name HD2) (residue 6 and name HD1) 1.9 .5 .5
assign (residue 4 and name HB2) (residue 4 and name HB1) 1.8 .5 .5
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C.2.3 NOEs (THF)

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assign (residue 8 and name HG1*) (residue 1 and name HN) 4.6 .5 .5
assign (residue 5 and name HA) (residue 4 and name HD*) 4.5 .5 1.0
assign (residue 3 and name HD1) (residue 2 and name HA) 4.2 .5 .5
assign (residue 2 and name HD1) (residue 7 and name HN) 4.0 .5 .5
assign (residue 2 and name HA) (residue 1 and name HN) 4.0 .5 .5
assign (residue 2 and name HD1) (residue 7 and name HA) 4.0 .5 1.0
assign (residue 3 and name HB2) (residue 4 and name HN) 3.9 .5 .5
assign (residue 8 and name HA) (residue 7 and name HN) 3.9 .5 .5
assign (residue 6 and name HD2) (residue 7 and name HN) 3.9 .5 .5
assign (residue 4 and name HB1) (residue 4 and name HE*) 3.9 .5 1.0
assign (residue 4 and name HE*) (residue 4 and name HN) 3.9 .5 1.0
assign (residue 1 and name HG2*) (residue 8 and name HN) 3.9 .5 1.5
assign (residue 1 and name HG1*) (residue 1 and name HN) 3.8 .5 .5
assign (residue 3 and name HG*) (residue 3 and name HA) 3.8 .5 .5
assign (residue 8 and name HA) (residue 1 and name HA) 3.8 .5 .5
assign (residue 7 and name HD1*) (residue 1 and name HN) 3.8 .5 1.5
assign (residue 1 and name HG1*) (residue 4 and name HN) 3.8 .5 .5
assign (residue 1 and name HG1*) (residue 5 and name HN) 3.8 .5 .5
assign (residue 5 and name HG1*) (residue 7 and name HN) 3.8 .5 1.5
assign (residue 1 and name HG1*) (residue 4 and name HD*) 3.7 .5 1.0
assign (residue 3 and name HD1) (residue 4 and name HN) 3.7 .5 .5
assign (residue 2 and name HB1) (residue 7 and name HN) 3.7 .5 .5
assign (residue 2 and name HG*) (residue 7 and name HN) 3.7 .5 .5
assign (residue 3 and name HD1) (residue 2 and name HD2) 3.7 .5 1.3
assign (residue 2 and name HD2) (residue 1 and name HN) 3.6 .5 .5
assign (residue 5 and name HG1*) (residue 5 and name HN) 3.6 .5 1.5
assign (residue 6 and name HA) (residue 8 and name HN) 3.6 .5 .5
assign (residue 8 and name HA) (residue 1 and name HN) 3.6 .5 .5
assign (residue 3 and name HD2) (residue 2 and name HA) 3.6 .5 .5
assign (residue 7 and name HD1*) (residue 8 and name HN) 3.6 .5 1.5
assign (residue 6 and name HB2) (residue 7 and name HN) 3.6 .5 .5
assign (residue 4 and name HB2) (residue 5 and name HN) 3.6 .5 .5
assign (residue 3 and name HB1) (residue 4 and name HN) 3.6 .5 .5
assign (residue 3 and name HD2) (residue 3 and name HA) 3.6 .5 .5
assign (residue 5 and name HG1*) (residue 1 and name HN) 3.5 .5 1.5
assign (residue 7 and name HB1) (residue 7 and name HN) 3.5 .5 .5
assign (residue 6 and name HB1) (residue 7 and name HN) 3.5 .5 .5
assign (residue 7 and name HG) (residue 6 and name HA) 3.5 .5 .5
assign (residue 6 and name HD1) (residue 4 and name HA) 3.5 .5 1.0
assign (residue 1 and name HG2*) (residue 8 and name HA) 3.5 .5 1.5
assign (residue 1 and name HG1*) (residue 1 and name HN) 3.5 .5 .5
assign (residue 1 and name HG2*) (residue 2 and name HA) 3.5 .5 1.5
assign (residue 3 and name HD1) (residue 4 and name HE*) 3.5 .5 1.0
assign (residue 3 and name HD1) (residue 4 and name HD*) 3.4 .5 1.0
assign (residue 3 and name HD1) (residue 3 and name HA) 3.4 .5 .5
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assign (residue 2 and name HD1) (residue 8 and name HN) 3.4 .5 .5
assign (residue 7 and name HN) (residue 8 and name HN) 3.4 1.0 .5
assign (residue 2 and name HD1) (residue 8 and name HA) 3.4 .5 .5
assign (residue 1 and name HG2*) (residue 4 and name HE*) 3.4 .5 1.5
assign (residue 1 and name HG2*) (residue 5 and name HN) 3.4 .5 1.5
assign (residue 5 and name HG1*) (residue 8 and name HN) 3.4 .5 1.5
assign (residue 1 and name HG2*) (residue 2 and name HD2) 3.4 .5 1.5
assign (residue 7 and name HD2*) (residue 7 and name HN) 3.4 .5 1.5
assign (residue 1 and name HB) (residue 4 and name HD*) 3.4 .5 1.0
assign (residue 1 and name HG2*) (residue 1 and name HN) 3.3 .5 1.5
assign (residue 7 and name HB1) (residue 8 and name HN) 3.3 .5 .5
assign (residue 7 and name HA) (residue 8 and name HN) 3.3 .5 .5
assign (residue 2 and name HA) (residue 4 and name HE*) 3.3 .5 1.0
assign (residue 3 and name HB2) (residue 3 and name HD2) 3.2 .5 .5
assign (residue 2 and name HD1) (residue 2 and name HA) 3.2 .5 .5
assign (residue 2 and name HD2) (residue 2 and name HA) 3.2 .5 .5
assign (residue 6 and name HA) (residue 3 and name HA) 3.2 .5 .5
assign (residue 3 and name HG*) (residue 4 and name HN) 3.1 .5 .5
assign (residue 7 and name HB2) (residue 8 and name HN) 3.1 .5 .5
assign (residue 4 and name HA) (residue 5 and name HN) 3.1 .5 .5
assign (residue 5 and name HG1*) (residue 6 and name HD1) 3.1 .5 1.5
assign (residue 2 and name HG*) (residue 2 and name HA) 3.1 .5 .5
assign (residue 8 and name HG1*) (residue 8 and name HN) 3.1 .5 .5
assign (residue 1 and name HD1*) (residue 1 and name HA) 3.0 .5 1.5
assign (residue 5 and name HB) (residue 4 and name HB1) 3.0 .5 1.0
assign (residue 2 and name HD1) (residue 1 and name HN) 3.0 .5 .5
assign (residue 7 and name HB2) (residue 7 and name HN) 3.0 .5 .5
assign (residue 1 and name HG2*) (residue 4 and name HD*) 3.0 .5 1.5
assign (residue 1 and name HG2*) (residue 4 and name HB1) 2.9 .5 1.5
assign (residue 8 and name HA) (residue 8 and name HN) 2.9 .5 .5
assign (residue 3 and name HD2) (residue 4 and name HE*) 2.9 .5 1.0
assign (residue 7 and name HD1*) (residue 7 and name HA) 2.8 .5 1.5
assign (residue 3 and name HG*) (residue 3 and name HB1) 2.8 .5 .5
assign (residue 1 and name HA) (residue 1 and name HN) 2.8 .5 .5
assign (residue 1 and name HG1*) (residue 1 and name HA) 2.8 .5 .5
assign (residue 7 and name HB2) (residue 7 and name HA) 2.8 .5 .5
assign (residue 2 and name HG*) (residue 2 and name HD1) 2.8 .5 .5
assign (residue 5 and name HA) (residue 5 and name HN) 2.8 .5 .5
assign (residue 3 and name HD2) (residue 4 and name HN) 2.8 .5 .5
assign (residue 1 and name HG1*) (residue 1 and name HA) 2.7 .5 .5
assign (residue 2 and name HA) (residue 5 and name HN) 2.7 .5 .5
assign (residue 2 and name HB1) (residue 2 and name HD1) 2.7 .5 .5
assign (residue 8 and name HD1*) (residue 8 and name HA) 2.7 .5 1.5
assign (residue 4 and name HD*) (residue 4 and name HN) 2.7 .5 1.0
assign (residue 5 and name HG2*) (residue 4 and name HB2) 2.7 .5 1.5
assign (residue 5 and name HG1*) (residue 6 and name HD2) 2.7 .5 1.5
assign (residue 1 and name HG1*) (residue 1 and name HB) 2.7 .5 .5
assign (residue 3 and name HB2) (residue 3 and name HD1) 2.7 .5 1.0
assign (residue 4 and name HN) (residue 5 and name HN) 2.6 .5 .5
assign (residue 3 and name HG*) (residue 3 and name HD1) 2.6 .5 .5
assign (residue 2 and name HB2) (residue 2 and name HA) 2.6 .5 .5
assign (residue 2 and name HB1) (residue 3 and name HA) 2.6 .5 .5
assign (residue 8 and name HG1*) (residue 8 and name HA) 2.6 .5 .5
assign (residue 2 and name HA) (residue 4 and name HN) 2.6 .5 .5
assign (residue 8 and name HB) (residue 8 and name HN) 2.6 .5 .5
assign (residue 3 and name HG*) (residue 3 and name HD2) 2.6 .5 .5
assign (residue 5 and name HG2*) (residue 4 and name HB1) 2.6 .5 1.5
assign (residue 5 and name HB) (residue 5 and name HN) 2.6 .5 .5
assign (residue 4 and name HB1) (residue 5 and name HN) 2.5 .5 1.0
assign (residue 5 and name HB) (residue 5 and name HA) 2.5 .5 .5
assign (residue 1 and name HG1*) (residue 1 and name HB) 2.5 .5 .5
assign (residue 1 and name HN) (residue 8 and name HN) 2.5 .5 .5
assign (residue 6 and name HA) (residue 2 and name HA) 2.5 .5 .5
assign (residue 2 and name HB2) (residue 3 and name HA) 2.5 .5 .5
assign (residue 4 and name HB1) (residue 4 and name HN) 2.5 .5 1.0
assign (residue 4 and name HA) (residue 4 and name HD*) 2.5 .5 1.0
assign (residue 7 and name HD1*) (residue 7 and name HB2) 2.4 .5 1.5
assign (residue 1 and name HG2*) (residue 1 and name HA) 2.4 .5 1.5
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assign (residue 3 and name HB1) (residue 3 and name HA) 2.4 .5 .5
assign (residue 2 and name HG*) (residue 2 and name HD1) 2.4 .5 .5
assign (residue 5 and name HG2*) (residue 5 and name HA) 2.4 .5 1.5
assign (residue 1 and name HB) (residue 1 and name HA) 2.4 .5 .5
assign (residue 1 and name HB) (residue 1 and name HN) 2.4 .5 .5
assign (residue 8 and name HG2*) (residue 8 and name HA) 2.4 .5 1.5
assign (residue 2 and name HD1) (residue 1 and name HA) 2.4 .5 .5
assign (residue 7 and name HD2*) (residue 7 and name HA) 2.4 .5 1.5
assign (residue 8 and name HB) (residue 8 and name HA) 2.3 .5 .5
assign (residue 4 and name HB2) (residue 4 and name HD*) 2.3 .5 1.0
assign (residue 3 and name HG*) (residue 3 and name HD1) 2.3 .5 .5
assign (residue 3 and name HG*) (residue 3 and name HD2) 2.3 .5 .5
assign (residue 7 and name HB1) (residue 7 and name HA) 2.3 .5 .5
assign (residue 5 and name HG1*) (residue 5 and name HA) 2.3 .5 1.5
assign (residue 4 and name HB1) (residue 4 and name HD*) 2.3 .5 1.0
assign (residue 2 and name HB1) (residue 2 and name HA) 2.2 .5 .5
assign (residue 3 and name HB2) (residue 3 and name HA) 2.2 .5 .5
assign (residue 6 and name HD2) (residue 5 and name HA) 2.1 .5 .5
assign (residue 2 and name HD2) (residue 1 and name HA) 2.1 .5 .5
assign (residue 2 and name HA) (residue 3 and name HA) 2.0 .5 .5
assign (residue 6 and name HD1) (residue 5 and name HA) 2.0 .5 .5
assign (residue 4 and name HB1) (residue 4 and name HB2) 1.8 .5 .5
assign (residue 2 and name HB2) (residue 2 and name HB1) 1.8 .5 .5
```

C.2.4 Dihedrals (DMSO)

```
assign (resid   3 and name HD1 )   (resid   3 and name CD )
       (resid   3 and name CG )   (resid   3 and name HG2 )  1.0  180.0 10.0 2

assign (resid   4 and name HA )   (resid   4 and name CA )
       (resid   4 and name CB )   (resid   4 and name HB2 )  1.0  180.0 10.0 2

assign (resid   5 and name HA )   (resid   5 and name CA )
       (resid   5 and name CB )   (resid   5 and name HB )  1.0  180.0 10.0 2

assign (resid   7 and name HA )   (resid   7 and name CA )
       (resid   7 and name CB )   (resid   7 and name HB2 )  1.0  180.0 10.0 2

assign (resid   8 and name HA )   (resid   8 and name CA )
       (resid   8 and name CB )   (resid   8 and name HB )  1.0  180.0 10.0 2
```

C.2.5 Dihedrals ($CDCl_3$)

```
assign (resid   4 and name HA )   (resid   4 and name CA )
       (resid   4 and name CB )   (resid   4 and name HB2 )  1.0  180.0 10.0 2

assign (resid   5 and name HA )   (resid   5 and name CA )
       (resid   5 and name CB )   (resid   5 and name HB )  1.0  180.0 10.0 2
```

C.2.6 Residual dipolar couplings

C.2.6.1 PH-gel/DMSO

```
   2    PRO     CA     2    PRO     HA     -6.390     2.000 0.50
   2    PRO     CB     2    PRO     HB1     0.020     2.000 0.50
   2    PRO     CB     2    PRO     HB2    14.050     2.000 0.50
   2    PRO     CD     2    PRO     HD2    -2.090     2.000 0.50
   2    PRO     CD     2    PRO     HD1    -4.050     2.000 0.50
   3    PRO     CA     3    PRO     HA    -13.020     2.000 0.50
```

```
3    PRO     CG     3    PRO     HG1     9.020     2.000 0.50
3    PRO     CG     3    PRO     HG2     7.480     2.000 0.50
3    PRO     CD     3    PRO     HD1   -10.380     2.000 0.50
3    PRO     CD     3    PRO     HD2     7.080     2.000 0.50
4    TYR     CA     4    TYR     HA     -8.560     2.000 0.50
4    TYR     CB     4    TYR     HB1    -3.080     2.000 0.50
4    TYR     CB     4    TYR     HB2   -11.180     2.000 0.50
5    VAL     CA     5    VAL     HA     -7.090     2.000 0.50
6    PRO     CA     6    PRO     HA      6.640     2.000 0.50
6    PRO     CB     6    PRO     HB2   -17.030     2.000 0.50
6    PRO     CG     6    PRO     HG2    -8.770     2.000 0.50
6    PRO     CG     6    PRO     HG1     5.160     2.000 0.50
6    PRO     CD     6    PRO     HD2    13.160     2.000 0.50
6    PRO     CD     6    PRO     HD1    -5.410     2.000 0.50
7    LEU     CA     7    LEU     HA     21.800     2.000 0.50
7    LEU     CB     7    LEU     HB2    16.780     2.000 0.50
7    LEU     CB     7    LEU     HB1   -19.190     2.000 0.50
8    ILE     CA     8    ILE     HA    -20.380     2.000 0.50
8    ILE     CB     8    ILE     HB    -16.320     2.000 0.50
8    ILE     CG1    8    ILE     HG11   -8.090     2.000 0.50
1    ILE     CA     1    ILE     HA     -5.290     2.000 0.50
1    ILE     CB     1    ILE     HB    -15.540     2.000 0.50
1    ILE     CG1    1    ILE     HG12   -8.510     2.000 0.50
1    ILE     CG1    1    ILE     HG11  -10.080     2.000 0.50
4    TYR      N     4    TYR     HN     -3.990     1.000 1.00
5    VAL      N     5    VAL     HN     -4.330     1.000 1.00
7    LEU      N     7    LEU     HN      6.170     1.000 1.00
8    ILE      N     8    ILE     HN     -9.900     1.000 1.00
1    ILE      N     1    ILE     HN     -4.780     1.000 1.00
```

C.2.6.2 PPH-gel/DMSO

```
2    PRO     CA     2    PRO     HA     20.860     2.000 0.50
2    PRO     CB     2    PRO     HB1    -8.800     2.000 0.50
2    PRO     CB     2    PRO     HB2    -4.890     2.000 0.50
3    PRO     CA     3    PRO     HA    -17.950     2.000 0.50
3    PRO     CG     3    PRO     HG1   -16.580     2.000 0.50
3    PRO     CG     3    PRO     HG2    15.870     2.000 0.50
4    TYR     CA     4    TYR     HA      8.250     2.000 0.50
4    TYR     CB     4    TYR     HB1    17.260     2.000 0.50
4    TYR     CB     4    TYR     HB2     3.180     2.000 0.50
4    TYR     CE1    4    TYR     HE1     4.780     2.000 0.50
4    TYR     CD2    4    TYR     HD2     4.250     2.000 0.50
5    VAL     CA     5    VAL     HA     18.290     2.000 0.50
6    PRO     CA     6    PRO     HA      0.700     2.000 0.50
6    PRO     CB     6    PRO     HB1     3.560     2.000 0.50
6    PRO     CB     6    PRO     HB2   -12.950     2.000 0.50
7    LEU     CA     7    LEU     HA     18.540     2.000 0.50
7    LEU     CB     7    LEU     HB2    17.660     2.000 0.50
7    LEU     CB     7    LEU     HB1     4.710     2.000 0.50
7    LEU     CG     7    LEU     HG      4.650     2.000 0.50
8    ILE     CA     8    ILE     HA      7.440     2.000 0.50
8    ILE     CB     8    ILE     HB      4.170     2.000 0.50
8    ILE     CG1    8    ILE     HG12    2.760     2.000 0.50
8    ILE     CG1    8    ILE     HG11    6.540     2.000 0.50
1    ILE     CA     1    ILE     HA     21.640     2.000 0.50
1    ILE     CB     1    ILE     HB      6.990     2.000 0.50
1    ILE     CG1    1    ILE     HG12    0.380     2.000 0.50
1    ILE     CG1    1    ILE     HG11    6.080     2.000 0.50
4    TYR      N     4    TYR     HN     11.010     1.000 1.00
5    VAL      N     5    VAL     HN     14.350     1.000 1.00
8    ILE      N     8    ILE     HN      4.700     1.000 1.00
1    ILE      N     1    ILE     HN     11.300     1.000 1.00
```

C.2.6.3 PAN/DMSO

```
2    PRO     CA     2    PRO     HA     40.700     2.000 0.50
2    PRO     CB     2    PRO     HB1     3.700     2.000 0.50
2    PRO     CB     2    PRO     HB2    -9.200     2.000 0.50
2    PRO     CD     2    PRO     HD1   -20.900     2.000 0.50
2    PRO     CD     2    PRO     HD2    32.300     2.000 0.50
3    PRO     CA     3    PRO     HA    -22.400     2.000 0.50
3    PRO     CD     3    PRO     HD1    10.500     2.000 0.50
3    PRO     CD     3    PRO     HD2   -14.100     2.000 0.50
4    TYR     CA     4    TYR     HA     37.100     2.000 0.50
4    TYR     CB     4    TYR     HB1     5.100     2.000 0.50
4    TYR     CB     4    TYR     HB2    15.000     2.000 0.50
5    VAL     CA     5    VAL     HA     42.500     2.000 0.50
6    PRO     CA     6    PRO     HA    -11.100     2.000 0.50
6    PRO     CB     6    PRO     HB1   -20.600     2.000 0.50
6    PRO     CB     6    PRO     HB2   -25.200     2.000 0.50
7    LEU     CA     7    LEU     HA     -6.900     2.000 0.50
7    LEU     CB     7    LEU     HB2    -3.000     2.000 0.50
7    LEU     CB     7    LEU     HB1    18.700     2.000 0.50
7    LEU     CG     7    LEU     HG     12.800     2.000 0.50
8    ILE     CA     8    ILE     HA     23.600     2.000 0.50
8    ILE     CB     8    ILE     HB     20.600     2.000 0.50
8    ILE     CG1    8    ILE     HG11   16.300     2.000 0.50
8    ILE     CG1    8    ILE     HG12   -2.000     2.000 0.50
1    ILE     CA     1    ILE     HA     33.900     2.000 0.50
1    ILE     CB     1    ILE     HB     25.100     2.000 0.50
1    ILE     CG1    1    ILE     HG11   13.400     2.000 0.50
1    ILE     CG1    1    ILE     HG12    1.000     2.000 0.50
4    TYR      N     4    TYR     HN     17.400     1.000 1.00
5    VAL      N     5    VAL     HN     13.900     1.000 1.00
7    LEU      N     7    LEU     HN     -6.000     1.000 1.00
8    ILE      N     8    ILE     HN     14.800     1.000 1.00
1    ILE      N     1    ILE     HN     17.000     1.000 1.00
```

C.2.6.4 PDMS/$CDCl_3$

```
2    PRO     CA     2    PRO     HA     -8.100     2.000 0.50
2    PRO     CB     2    PRO     HB1   -11.700     2.000 0.50
2    PRO     CB     2    PRO     HB2    23.000     2.000 0.50
2    PRO     CD     2    PRO     HD2   -10.600     2.000 0.50
2    PRO     CD     2    PRO     HD1   -14.000     2.000 0.50
2    PRO     CG     2    PRO     HG1   -12.000     2.000 0.50
2    PRO     CG     2    PRO     HG2   -24.900     2.000 0.50
3    PRO     CA     3    PRO     HA    -32.700     2.000 0.50
3    PRO     CD     3    PRO     HD1    21.600     2.000 0.50
3    PRO     CD     3    PRO     HD2    10.600     2.000 0.50
3    PRO     CB     3    PRO     HB1   -14.500     2.000 0.50
3    PRO     CB     3    PRO     HB2    31.000     2.000 0.50
3    PRO     CG     3    PRO     HG1    16.500     2.000 0.50
3    PRO     CG     3    PRO     HG2    12.900     2.000 0.50
4    TYR     CA     4    TYR     HA    -13.300     2.000 0.50
4    TYR     CB     4    TYR     HB1    23.600     2.000 0.50
4    TYR     CB     4    TYR     HB2   -11.300     2.000 0.50
5    VAL     CA     5    VAL     HA    -12.300     2.000 0.50
5    VAL     CB     5    VAL     HB    -14.000     2.000 0.50
6    PRO     CA     6    PRO     HA     17.000     2.000 0.50
6    PRO     CD     6    PRO     HD1   -37.300     2.000 0.50
6    PRO     CD     6    PRO     HD2    32.900     2.000 0.50
6    PRO     CB     6    PRO     HB1    34.900     2.000 0.50
6    PRO     CB     6    PRO     HB2   -13.200     2.000 0.50
7    LEU     CA     7    LEU     HA     36.400     2.000 0.50
7    LEU     CB     7    LEU     HB2    28.100     2.000 0.50
7    LEU     CB     7    LEU     HB1   -13.200     2.000 0.50
7    LEU     CG     7    LEU     HG    -14.300     2.000 0.50
8    ILE     CA     8    ILE     HA    -23.500     2.000 0.50
```

```
8    ILE     CB     8    ILE     HB    -27.100     2.000 0.50
1    ILE     CA     1    ILE     HA     -5.700     2.000 0.50
1    ILE     CB     1    ILE     HB    -14.900     2.000 0.50
4    TYR      N     4    TYR     HN     -9.400     1.000 1.00
5    VAL      N     5    VAL     HN     -2.200     1.000 1.00
8    ILE      N     8    ILE     HN    -10.600     1.000 1.00
1    ILE      N     1    ILE     HN     -6.200     1.000 1.00
```

C.2.6.5 PDMS/THF

```
2    PRO     CA     2    PRO     HA      5.200     2.000 0.50
2    PRO     CB     2    PRO     HB1    -4.800     2.000 0.50
2    PRO     CB     2    PRO     HB2     5.800     2.000 0.50
2    PRO     CD     2    PRO     HD1   -11.700     2.000 0.50
2    PRO     CD     2    PRO     HD2     4.600     2.000 0.50
3    PRO     CA     3    PRO     HA    -13.900     2.000 0.50
3    PRO     CD     3    PRO     HD1    11.300     2.000 0.50
3    PRO     CD     3    PRO     HD2     7.700     2.000 0.50
3    PRO     CB     3    PRO     HB2     2.800     2.000 0.50
3    PRO     CB     3    PRO     HB1    16.000     2.000 0.50
3    PRO     CG     3    PRO     HG1    -1.100     2.000 0.50
3    PRO     CG     3    PRO     HG2     6.100     2.000 0.50
4    TYR     CA     4    TYR     HA     -0.500     2.000 0.50
4    TYR     CB     4    TYR     HB1    -2.800     2.000 0.50
4    TYR     CB     4    TYR     HB2    15.300     2.000 0.50
5    VAL     CA     5    VAL     HA      4.400     2.000 0.50
6    PRO     CA     6    PRO     HA      5.000     2.000 0.50
6    PRO     CD     6    PRO     HD1     5.700     2.000 0.50
6    PRO     CD     6    PRO     HD2   -15.500     2.000 0.50
6    PRO     CB     6    PRO     HB1    12.500     2.000 0.50
6    PRO     CB     6    PRO     HB2    -7.000     2.000 0.50
7    LEU     CA     7    LEU     HA     16.000     2.000 0.50
7    LEU     CB     7    LEU     HB2    13.200     2.000 0.50
7    LEU     CB     7    LEU     HB1    -4.600     2.000 0.50
7    LEU     CG     7    LEU     HG     -0.700     2.000 0.50
8    ILE     CA     8    ILE     HA     -9.400     2.000 0.50
8    ILE     CB     8    ILE     HB     -9.800     2.000 0.50
1    ILE     CA     1    ILE     HA      5.900     2.000 0.50
1    ILE     CB     1    ILE     HB     -0.900     2.000 0.50
4    TYR      N     4    TYR     HN      1.100     1.000 1.00
5    VAL      N     5    VAL     HN      2.500     1.000 1.00
7    LEU      N     7    LEU     HN      2.400     1.000 1.00
8    ILE      N     8    ILE     HN     -1.800     1.000 1.00
1    ILE      N     1    ILE     HN      2.100     1.000 1.00
```

C.3 DiaA

C.3.1 NOEs

```
assign ( name H4 )  ( name H9 ) 4.0 1.0 1.0
assign ( name H4 )  ( name H19 ) 4.0 1.0 1.0
assign ( name H4 )  ( name H11 ) 4.0 1.0 1.0
assign ( name H4 )  ( name H8 ) 4.0 1.0 1.0
assign ( name H6 )  ( name H9 ) 4.0 1.0 1.0
assign ( name H6 )  ( name H8 ) 4.0 1.0 1.0
assign ( name H26 )  ( name H9 ) 4.0 1.0 1.0
assign ( name H26 )  ( name H19 ) 4.0 1.0 1.0
assign ( name H26 )  ( name H11 ) 4.0 1.0 1.0
assign ( name H26 )  ( name H8 ) 4.0 1.0 1.0
assign ( name H5 )  ( name H9 ) 4.0 1.0 1.0
assign ( name H28 )  ( name H8 ) 4.0 1.0 1.0
```

```
assign ( name H28 )  ( name H1 ) 4.0 1.0 1.0
assign ( name H1 )  ( name H26 ) 4.0 1.0 1.0
assign ( name H28 )  ( name H19 ) 4.0 1.0 1.0
assign ( name H28 )  ( name H11 ) 4.0 1.0 1.0
assign ( name H28 )  ( name H9 ) 4.0 1.0 1.0
assign ( name H28 )  (( name H20 ) or ( name H21 ) or ( name H22 )) 4.0 1.0 2.0
```

C.3.2 non-NOEs

```
assign ( name H5 )  ( name H7 ) 5.0 1.0 6.0
assign ( name H5 )  ( name H8 ) 5.0 1.0 6.0
assign ( name H5 )  ( name H10 ) 5.0 1.0 6.0
assign ( name H5 )  ( name H11 ) 5.0 1.0 6.0
assign ( name H5 )  ( name H12 ) 5.0 1.0 6.0
assign ( name H5 )  ( name H13 ) 5.0 1.0 6.0
assign ( name H5 )  ( name H14 ) 5.0 1.0 6.0
assign ( name H5 )  ( name H15 ) 5.0 1.0 6.0
assign ( name H5 )  ( name H19 ) 5.0 1.0 6.0
assign ( name H5 )  (( name H16 ) or ( name H17 ) or ( name H18 )) 5.0 1.0 6.0
assign ( name H5 )  (( name H20 ) or ( name H21 ) or ( name H22 )) 5.0 1.0 6.0
assign ( name H5 )  (( name H23 ) or ( name H24 ) or ( name H25 )) 5.0 1.0 6.0

assign ( name H6 )  ( name H7 ) 5.0 1.0 6.0
assign ( name H6 )  ( name H10 ) 5.0 1.0 6.0
assign ( name H6 )  ( name H11 ) 5.0 1.0 6.0
assign ( name H6 )  ( name H12 ) 5.0 1.0 6.0
assign ( name H6 )  ( name H13 ) 5.0 1.0 6.0
assign ( name H6 )  ( name H14 ) 5.0 1.0 6.0
assign ( name H6 )  ( name H15 ) 5.0 1.0 6.0
assign ( name H6 )  ( name H19 ) 5.0 1.0 6.0
assign ( name H6 )  (( name H16 ) or ( name H17 ) or ( name H18 )) 5.0 1.0 6.0
assign ( name H6 )  (( name H20 ) or ( name H21 ) or ( name H22 )) 5.0 1.0 6.0
assign ( name H6 )  (( name H23 ) or ( name H24 ) or ( name H25 )) 5.0 1.0 6.0

assign ( name H26 )  ( name H7 ) 5.0 1.0 6.0
assign ( name H26 )  ( name H10 ) 5.0 1.0 6.0
assign ( name H26 )  ( name H12 ) 5.0 1.0 6.0
assign ( name H26 )  ( name H13 ) 5.0 1.0 6.0
assign ( name H26 )  ( name H14 ) 5.0 1.0 6.0
assign ( name H26 )  ( name H15 ) 5.0 1.0 6.0
assign ( name H26 )  (( name H16 ) or ( name H17 ) or ( name H18 )) 5.0 1.0 6.0
assign ( name H26 )  (( name H20 ) or ( name H21 ) or ( name H22 )) 5.0 1.0 6.0
assign ( name H26 )  (( name H23 ) or ( name H24 ) or ( name H25 )) 5.0 1.0 6.0

assign ( name H27 )  ( name H7 ) 5.0 1.0 6.0
assign ( name H27 )  ( name H8 ) 5.0 1.0 6.0
assign ( name H27 )  ( name H9 ) 5.0 1.0 6.0
assign ( name H27 )  ( name H10 ) 5.0 1.0 6.0
assign ( name H27 )  ( name H11 ) 5.0 1.0 6.0
assign ( name H27 )  ( name H12 ) 5.0 1.0 6.0
assign ( name H27 )  ( name H13 ) 5.0 1.0 6.0
assign ( name H27 )  ( name H14 ) 5.0 1.0 6.0
assign ( name H27 )  ( name H15 ) 5.0 1.0 6.0
assign ( name H27 )  ( name H19 ) 5.0 1.0 6.0
assign ( name H27 )  (( name H16 ) or ( name H17 ) or ( name H18 )) 5.0 1.0 6.0
assign ( name H27 )  (( name H20 ) or ( name H21 ) or ( name H22 )) 5.0 1.0 6.0
assign ( name H27 )  (( name H23 ) or ( name H24 ) or ( name H25 )) 5.0 1.0 6.0

assign ( name H28 )  ( name H7 ) 5.0 1.0 6.0
assign ( name H28 )  ( name H10 ) 5.0 1.0 6.0
assign ( name H28 )  ( name H12 ) 5.0 1.0 6.0
assign ( name H28 )  ( name H13 ) 5.0 1.0 6.0
assign ( name H28 )  ( name H14 ) 5.0 1.0 6.0
assign ( name H28 )  ( name H15 ) 5.0 1.0 6.0
assign ( name H28 )  (( name H16 ) or ( name H17 ) or ( name H18 )) 5.0 1.0 6.0
```

```
assign ( name H28 )  (( name H20 ) or ( name H21 ) or ( name H22 )) 5.0 1.0 6.0
assign ( name H28 )  (( name H23 ) or ( name H24 ) or ( name H25 )) 5.0 1.0 6.0
```

C.3.3 Residual dipolar couplings

C.3.3.1 PH-gel/DMSO

```
   1    MET     C1     1    MET     H7     6.630     1.000 1.00
   1    MET     C2     1    MET     H9    -6.720     1.000 1.00
   1    MET     C2     1    MET     H8     5.730     1.000 1.00
   1    MET     C3     1    MET     H10    5.400     1.000 1.00
   1    MET     C4     1    MET     H11    7.750     1.000 1.00
   1    MET     C5     1    MET     H12   -3.990     1.000 1.00
   1    MET     C6     1    MET     H14    3.340     1.000 1.00
   1    MET     C6     1    MET     H15    7.750     1.000 1.00
   1    MET     C8     1    MET     H19    0.730     1.000 1.00
   1    MET     C13    1    MET     H4   -11.710     1.000 1.00
   1    MET     C16    1    MET     H1    -6.300     1.000 1.00
   1    MET     C19    1    MET     H26   -2.460     1.000 1.00
   1    MET     C20    1    MET     H6     7.620     1.000 1.00
   1    MET     C21    1    MET     H5    -1.090     1.000 1.00
   1    MET     C23    1    MET     H28    5.170     1.000 1.00
   1    MET     C23    1    MET     H27    3.190     1.000 1.00
```

C.3.3.2 PAN/DMSO

```
   1    MET     C1     1    MET     H7    13.960     1.000 1.00
   1    MET     C2     1    MET     H9     5.310     1.000 1.00
   1    MET     C2     1    MET     H8    12.080     1.000 1.00
   1    MET     C3     1    MET     H10   15.130     1.000 1.00
   1    MET     C4     1    MET     H11   12.210     1.000 1.00
   1    MET     C5     1    MET     H12    6.190     1.000 1.00
   1    MET     C5     1    MET     H13   15.470     1.000 1.00
   1    MET     C6     1    MET     H14  -10.860     1.000 1.00
   1    MET     C6     1    MET     H15   15.110     1.000 1.00
   1    MET     C8     1    MET     H19   -9.610     1.000 1.00
   1    MET     C13    1    MET     H4   -14.220     1.000 1.00
   1    MET     C16    1    MET     H1   -10.500     1.000 1.00
   1    MET     C19    1    MET     H26   14.490     1.000 1.00
   1    MET     C20    1    MET     H6     2.390     1.000 1.00
   1    MET     C21    1    MET     H5    -3.470     1.000 1.00
   1    MET     C23    1    MET     H28  -11.250     1.000 1.00
   1    MET     C23    1    MET     H27    0.720     1.000 1.00
```

C.4 Triggerfactor

C.4.1 NOEs

```
! noes for protein with hydrogens
! an assumed half-width of 0.1 ppm
! frequencies binned to allow quantitative measurement:
! vweak  < 4.2, upper limit 6   (pkVol < 400212.18)
! weak   < 3.6, upper limit 5   (pkVol < 1009185.58)
! medium < 3,   upper limit 3.6 (pkVol < 3013411.99)
! strong < 2.5, upper limit 2.7 (pkVol < 8998000.00)

! Non-ambiguous Restraints:
! ========================
```

```
! Contact order window (i,i+1) and larger
! Intra (i,i): 0
! Sequential (i,i+1): 309
! Local (i,i+2..4): 94
! Non Local (i,i+5..): 196
assign (resid  35 and name HN) (resid  34 and name HB#) 3.6 3.6 1.4
assign (resid  35 and name HN) (resid  34 and name HN) 4.0 4.0 1.6
assign (resid  23 and name HN) (resid  24 and name HN) 4.0 4.0 1.6
assign (resid  24 and name HN) (resid  23 and name HA) 3.0 3.0 0.8
assign (resid  24 and name HN) (resid  25 and name HN) 4.0 4.0 1.6
assign (resid  26 and name HN) (resid  25 and name HN) 3.6 3.6 1.4
assign (resid  30 and name HN) (resid  29 and name HN) 4.0 4.0 1.6
assign (resid  31 and name HN) (resid  30 and name HN) 4.2 4.2 1.6
assign (resid  31 and name HN) (resid  32 and name HN) 4.0 4.0 1.6
assign (resid  33 and name HN) (resid  34 and name HN) 3.6 3.6 1.4
assign (resid  35 and name HN) (resid  36 and name HN) 3.6 3.6 1.4
assign (resid  37 and name HN) (resid  36 and name HN) 4.0 4.0 1.6
assign (resid  37 and name HN) (resid  38 and name HN) 4.0 4.0 1.6
assign (resid  38 and name HN) (resid  39 and name HN) 4.2 4.2 1.6
assign (resid  39 and name HN) (resid  40 and name HN) 4.0 4.0 1.6
assign (resid  41 and name HN) (resid  42 and name HN) 4.0 4.0 1.6
assign (resid  45 and name HN) (resid  46 and name HN) 3.6 3.6 1.4
assign (resid  46 and name HN) (resid  47 and name HN) 4.0 4.0 1.6
assign (resid  48 and name HN) (resid  47 and name HN) 4.2 4.2 1.6
assign (resid  49 and name HN) (resid  48 and name HN) 4.0 4.0 1.6
assign (resid  53 and name HN) (resid  52 and name HN) 2.5 2.5 0.4
assign (resid  53 and name HN) (resid  54 and name HN) 4.0 4.0 1.6
assign (resid  54 and name HN) (resid  55 and name HN) 4.0 4.0 1.6
assign (resid  55 and name HN) (resid  56 and name HN) 4.0 4.0 1.6
assign (resid  56 and name HN) (resid  57 and name HN) 3.6 3.6 1.4
assign (resid  57 and name HN) (resid  58 and name HN) 4.0 4.0 1.6
assign (resid  58 and name HN) (resid  59 and name HN) 4.0 4.0 1.6
assign (resid  59 and name HN) (resid  60 and name HN) 4.0 4.0 1.6
assign (resid  60 and name HN) (resid  61 and name HN) 4.0 4.0 1.6
assign (resid  61 and name HN) (resid  62 and name HN) 4.0 4.0 1.6
assign (resid  62 and name HN) (resid  63 and name HN) 3.0 3.0 0.8
assign (resid  63 and name HN) (resid  64 and name HN) 3.0 3.0 0.8
assign (resid  64 and name HN) (resid  65 and name HN) 4.0 4.0 1.6
assign (resid  65 and name HN) (resid  66 and name HN) 3.0 3.0 0.8
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assign (resid  68 and name HN) (resid  67 and name HN) 4.0 4.0 1.6
assign (resid  68 and name HN) (resid  69 and name HN) 4.0 4.0 1.6
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assign (resid  87 and name HN) (resid  88 and name HN) 4.0 4.0 1.6
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assign (resid  92 and name HN) (resid  91 and name HN) 3.6 3.6 1.4
assign (resid  92 and name HN) (resid  93 and name HN) 3.6 3.6 1.4
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assign (resid  96 and name HN) (resid  97 and name HN) 4.0 4.0 1.6
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assign (resid  98 and name HN) (resid  99 and name HN) 2.5 2.5 0.4
assign (resid  99 and name HN) (resid 100 and name HN) 3.6 3.6 1.4
assign (resid 100 and name HN) (resid 101 and name HN) 3.0 3.0 0.8
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assign (resid 104 and name HN) (resid 105 and name HN) 4.0 4.0 1.6
assign (resid 108 and name HN) (resid 109 and name HN) 4.0 4.0 1.6
assign (resid 109 and name HN) (resid  80 and name HN) 4.2 4.2 1.6
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assign (resid 110 and name HN) (resid 109 and name HN) 4.2 4.2 1.6
assign (resid 110 and name HN) (resid 111 and name HN) 3.6 3.6 1.4
assign (resid 112 and name HN) (resid 111 and name HN) 3.6 3.6 1.4
assign (resid 112 and name HN) (resid 113 and name HN) 4.0 4.0 1.6
assign (resid  27 and name HN) (resid  26 and name HA) 3.0 3.0 0.8
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assign (resid  35 and name HN) (resid  34 and name HA) 2.5 2.5 0.4
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assign (resid  37 and name HN) (resid  36 and name HA) 3.0 3.0 0.8
assign (resid  38 and name HN) (resid  37 and name HA) 3.0 3.0 0.8
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assign (resid  43 and name HN) (resid  42 and name HA#) 4.0 4.0 1.6
assign (resid  44 and name HN) (resid  43 and name HA) 2.5 2.5 0.4
assign (resid  45 and name HN) (resid  44 and name HA) 2.5 2.5 0.4
assign (resid  46 and name HN) (resid  45 and name HA) 2.5 2.5 0.4
assign (resid  47 and name HN) (resid  46 and name HA) 3.0 3.0 0.8
assign (resid  48 and name HN) (resid  47 and name HA) 2.5 2.5 0.4
assign (resid  49 and name HN) (resid  48 and name HA) 2.5 2.5 0.4
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assign (resid  56 and name HN) (resid  55 and name HA) 2.5 2.5 0.4
assign (resid  57 and name HN) (resid  56 and name HA) 3.0 3.0 0.8
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assign (resid  75 and name HN) (resid  74 and name HA) 4.0 4.0 1.6
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assign (resid  89 and name HN) (resid  88 and name HA) 2.5 2.5 0.4
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assign (resid 100 and name HN) (resid  99 and name HA) 3.0 3.0 0.8
assign (resid 101 and name HN) (resid 100 and name HA) 3.0 3.0 0.8
assign (resid 104 and name HN) (resid 103 and name HA) 2.5 2.5 0.4
assign (resid 105 and name HN) (resid 104 and name HA) 2.5 2.5 0.4
assign (resid 108 and name HN) (resid 107 and name HA) 3.0 3.0 0.8
assign (resid 109 and name HN) (resid 108 and name HA) 2.5 2.5 0.4
assign (resid 110 and name HN) (resid 109 and name HA) 3.6 3.6 1.4
assign (resid 111 and name HN) (resid 110 and name HA) 4.2 4.2 1.6
assign (resid 112 and name HN) (resid 111 and name HA) 2.5 2.5 0.4
assign (resid  26 and name HN) (resid  25 and name HB) 4.0 4.0 1.6
assign (resid  28 and name HN) (resid  27 and name HB#) 4.0 4.0 1.6
assign (resid  32 and name HN) (resid  31 and name HB#) 2.5 2.5 0.4
assign (resid  33 and name HN) (resid  34 and name HB#) 3.6 3.6 1.4
assign (resid  33 and name HN) (resid  32 and name HB#) 4.0 4.0 1.6
assign (resid  40 and name HN) (resid  39 and name HB#) 3.6 3.6 1.4
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assign (resid  41 and name HN) (resid  40 and name HB#) 3.6 3.6 1.4
assign (resid  42 and name HN) (resid  41 and name HB) 4.0 4.0 1.6
assign (resid  44 and name HN) (resid  43 and name HB) 3.0 3.0 0.8
assign (resid  45 and name HN) (resid  44 and name HB) 4.0 4.0 1.6
assign (resid  46 and name HN) (resid  45 and name HB#) 4.0 4.0 1.6
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assign (resid  48 and name HN) (resid  46 and name HB#) 4.2 4.2 1.6
assign (resid  53 and name HN) (resid  52 and name HB#) 3.0 3.0 0.8
assign (resid  54 and name HN) (resid  53 and name HB#) 4.0 4.0 1.6
assign (resid  55 and name HN) (resid  54 and name HB#) 2.5 2.5 0.4
assign (resid  56 and name HN) (resid  39 and name HB#) 4.2 4.2 1.6
assign (resid  57 and name HN) (resid  56 and name HB#) 4.0 4.0 1.6
assign (resid  58 and name HN) (resid  57 and name HB#) 4.0 4.0 1.6
assign (resid  61 and name HN) (resid  60 and name HB) 4.0 4.0 1.6
assign (resid  65 and name HN) (resid  64 and name HB#) 3.6 3.6 1.4
assign (resid  66 and name HN) (resid  65 and name HB#) 3.6 3.6 1.4
assign (resid  71 and name HN) (resid  70 and name HB#) 3.0 3.0 0.8
assign (resid  72 and name HN) (resid  71 and name HB#) 3.0 3.0 0.8
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assign (resid  85 and name HN) (resid  84 and name HB) 4.0 4.0 1.6
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assign (resid  93 and name HN) (resid  92 and name HB#) 4.0 4.0 1.6
assign (resid 105 and name HN) (resid 104 and name HG2#) 3.0 3.0 0.8
assign (resid 108 and name HN) (resid 107 and name HB) 4.0 4.0 1.6
assign (resid 109 and name HN) (resid 108 and name HB) 4.0 4.0 1.6
assign (resid 110 and name HN) (resid 109 and name HB#) 4.2 4.2 1.6
assign (resid 111 and name HN) (resid 110 and name HB#) 3.6 3.6 1.4
assign (resid 112 and name HN) (resid 111 and name HB#) 2.5 2.5 0.4
assign (resid 113 and name HN) (resid 112 and name HG2#) 2.5 2.5 0.4
assign (resid  30 and name HN) (resid  77 and name HN) 4.2 4.2 1.6
assign (resid  30 and name HN) (resid  76 and name HN) 4.2 4.2 1.6
assign (resid  36 and name HN) (resid  59 and name HN) 4.2 4.2 1.6
assign (resid  37 and name HN) (resid 111 and name HN) 4.2 4.2 1.6
assign (resid  39 and name HN) (resid 108 and name HN) 4.2 4.2 1.6
assign (resid  40 and name HN) (resid  56 and name HN) 4.2 4.2 1.6
assign (resid  61 and name HN) (resid  34 and name HN) 4.2 4.2 1.6
assign (resid  35 and name HN) (resid 113 and name HN) 4.2 4.2 1.6
assign (resid  40 and name HN) (resid  41 and name HN) 3.6 3.6 1.4
assign (resid  41 and name HN) (resid 108 and name HN) 4.2 4.2 1.6
assign (resid  43 and name HN) (resid 104 and name HN) 4.2 4.2 1.6
assign (resid  40 and name HN) (resid  54 and name HN) 4.2 4.2 1.6
assign (resid  42 and name HN) (resid  54 and name HN) 4.2 4.2 1.6
assign (resid  42 and name HN) (resid 105 and name HD#) 4.2 4.2 1.6
assign (resid  53 and name HN) (resid  42 and name HN) 4.2 4.2 1.6
assign (resid  43 and name HN) (resid 105 and name HD#) 4.2 4.2 1.6
assign (resid  95 and name HN) (resid  94 and name HA) 4.2 4.2 1.6
assign (resid  62 and name HN) (resid  64 and name HN) 4.2 4.2 1.6
assign (resid  66 and name HN) (resid  64 and name HN) 4.2 4.2 1.6
assign (resid  80 and name HN) (resid  81 and name HN) 3.6 3.6 1.4
assign (resid  82 and name HN) (resid  83 and name HN) 4.0 4.0 1.6
assign (resid  83 and name HN) (resid  84 and name HN) 4.0 4.0 1.6
assign (resid  84 and name HN) (resid  83 and name HA) 2.5 2.5 0.4
assign (resid  82 and name HN) (resid  81 and name HA) 3.0 3.0 0.8
assign (resid  83 and name HN) (resid  82 and name HA) 3.0 3.0 0.8
assign (resid  79 and name HN) (resid  78 and name HA) 3.6 3.6 1.4
assign (resid  77 and name HN) (resid  78 and name HN) 4.0 4.0 1.6
assign (resid  81 and name HN) (resid  80 and name HA) 3.0 3.0 0.8
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assign (resid  72 and name HN) (resid  69 and name HA#) 4.2 4.2 1.6
assign (resid  75 and name HN) (resid  72 and name HA) 4.2 4.2 1.6
assign (resid  73 and name HN) (resid  71 and name HA) 4.2 4.2 1.6
assign (resid  74 and name HN) (resid  71 and name HA) 4.2 4.2 1.6
assign (resid  75 and name HN) (resid  73 and name HA#) 4.2 4.2 1.6
assign (resid  93 and name HN) (resid  91 and name HA) 4.2 4.2 1.6
assign (resid  98 and name HN) (resid  96 and name HA) 4.2 4.2 1.6
```

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assign (resid  99 and name HN) (resid  96 and name HA) 4.2 4.2 1.6
assign (resid  99 and name HN) (resid  97 and name HA) 4.2 4.2 1.6
assign (resid 101 and name HN) (resid 100 and name HB#) 3.6 3.6 1.4
assign (resid 101 and name HN) (resid  99 and name HA) 4.2 4.2 1.6
assign (resid  97 and name HN) (resid  99 and name HN) 4.2 4.2 1.6
assign (resid  98 and name HN) (resid 100 and name HN) 4.2 4.2 1.6
assign (resid 101 and name HN) (resid  99 and name HN) 4.2 4.2 1.6
assign (resid 101 and name HN) (resid  89 and name HN) 4.2 4.2 1.6
assign (resid  89 and name HN) (resid 100 and name HN) 4.2 4.2 1.6
assign (resid  89 and name HN) (resid 103 and name HN) 4.2 4.2 1.6
assign (resid 103 and name HN) (resid  87 and name HN) 4.2 4.2 1.6
assign (resid 105 and name HN) (resid  87 and name HN) 4.2 4.2 1.6
assign (resid  57 and name HN) (resid  40 and name HE#) 4.2 4.2 1.6
assign (resid  58 and name HN) (resid  57 and name HD#) 3.6 3.6 1.4
assign (resid  59 and name HN) (resid  57 and name HD#) 4.2 4.2 1.6
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assign (resid  74 and name HN) (resid  72 and name HN) 4.2 4.2 1.6
assign (resid  73 and name HN) (resid  75 and name HN) 4.2 4.2 1.6
assign (resid  70 and name HN) (resid  72 and name HN) 4.2 4.2 1.6
assign (resid  73 and name HN) (resid  71 and name HN) 4.2 4.2 1.6
assign (resid  78 and name HN) (resid  81 and name HN) 4.2 4.2 1.6
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assign (resid  59 and name HN) (resid  35 and name HA) 4.2 4.2 1.6
assign (resid  65 and name HN) (resid  64 and name HA) 3.0 3.0 0.8
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assign (resid  74 and name HN) (resid  75 and name HA) 4.0 4.0 1.6
assign (resid  24 and name HN) (resid  23 and name HB) 3.6 3.6 1.4
assign (resid  24 and name HN) (resid  23 and name HG1#) 3.6 3.6 1.4
assign (resid  26 and name HN) (resid  25 and name HG1#) 4.0 4.0 1.6
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assign (resid  39 and name HN) (resid  38 and name HG1#) 4.0 4.0 1.6
assign (resid  42 and name HN) (resid  41 and name HG2#) 3.6 3.6 1.4
assign (resid  56 and name HN) (resid  55 and name HG#) 4.0 4.0 1.6
assign (resid  56 and name HN) (resid  55 and name HB#) 3.6 3.6 1.4
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assign (resid  52 and name HN) (resid  50 and name HA) 4.2 4.2 1.6
assign (resid  52 and name HN) (resid  53 and name HB#) 4.0 4.0 1.6
assign (resid  52 and name HN) (resid  51 and name HA) 4.0 4.0 1.6
assign (resid  53 and name HN) (resid  51 and name HA) 4.2 4.2 1.6
assign (resid  46 and name HN) (resid  44 and name HA) 4.2 4.2 1.6
assign (resid  47 and name HN) (resid  44 and name HA) 4.2 4.2 1.6
assign (resid  47 and name HN) (resid  45 and name HA) 4.2 4.2 1.6
assign (resid  70 and name HN) (resid  68 and name HA) 4.2 4.2 1.6
assign (resid  73 and name HN) (resid  72 and name HB) 3.6 3.6 1.4
assign (resid  73 and name HN) (resid  69 and name HA#) 4.2 4.2 1.6
assign (resid 106 and name HN) (resid 105 and name HN) 4.0 4.0 1.6
assign (resid 107 and name HN) (resid 106 and name HN) 4.0 4.0 1.6
assign (resid 110 and name HN) (resid  39 and name HN) 4.2 4.2 1.6
assign (resid 101 and name HN) (resid  89 and name HD#) 4.2 4.2 1.6
assign (resid  95 and name HN) (resid  93 and name HE#) 4.2 4.2 1.6
assign (resid  41 and name HN) (resid  40 and name HD#) 4.0 4.0 1.6
assign (resid  42 and name HN) (resid 105 and name HE#) 4.2 4.2 1.6
assign (resid  54 and name HN) (resid  40 and name HE#) 4.2 4.2 1.6
assign (resid  54 and name HN) (resid  40 and name HA) 4.2 4.2 1.6
assign (resid  52 and name HN) (resid  49 and name HN) 4.2 4.2 1.6
assign (resid  71 and name HN) (resid  70 and name HD#) 4.0 4.0 1.6
assign (resid  81 and name HN) (resid 109 and name HN) 4.2 4.2 1.6
assign (resid  86 and name HN) (resid  87 and name HN) 4.0 4.0 1.6
assign (resid 107 and name HN) (resid  83 and name HN) 4.2 4.2 1.6
assign (resid 108 and name HN) (resid  40 and name HA) 4.2 4.2 1.6
```

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assign (resid 108 and name HN) (resid 107 and name HN) 4.0 4.0 1.6
assign (resid  55 and name HN) (resid  39 and name HA) 4.2 4.2 1.6
assign (resid  55 and name HN) (resid  39 and name HB#) 4.2 4.2 1.6
assign (resid  23 and name HN) (resid  22 and name HA) 3.6 3.6 1.4
assign (resid  28 and name HN) (resid  26 and name HA) 4.2 4.2 1.6
assign (resid  30 and name HN) (resid  29 and name HA) 3.0 3.0 0.8
assign (resid  30 and name HN) (resid  76 and name HA) 4.2 4.2 1.6
assign (resid  30 and name HN) (resid  77 and name HB#) 4.2 4.2 1.6
assign (resid  30 and name HN) (resid  61 and name HD1#) 4.2 4.2 1.6
assign (resid  31 and name HN) (resid  34 and name HB#) 4.2 4.2 1.6
assign (resid  31 and name HN) (resid  30 and name HD2#) 3.6 3.6 1.4
assign (resid  31 and name HN) (resid  30 and name HD1#) 3.6 3.6 1.4
assign (resid  31 and name HN) (resid  61 and name HD1#) 4.2 4.2 1.6
assign (resid  32 and name HN) (resid  75 and name HB) 4.2 4.2 1.6
assign (resid  32 and name HN) (resid  75 and name HG2#) 4.2 4.2 1.6
assign (resid  32 and name HN) (resid  75 and name HD1#) 4.2 4.2 1.6
assign (resid  33 and name HN) (resid  62 and name HA#) 4.2 4.2 1.6
assign (resid  33 and name HN) (resid  61 and name HG2#) 4.2 4.2 1.6
assign (resid  34 and name HN) (resid  61 and name HG1#) 4.2 4.2 1.6
assign (resid  34 and name HN) (resid  61 and name HA) 4.2 4.2 1.6
assign (resid  34 and name HN) (resid  32 and name HA) 4.2 4.2 1.6
assign (resid  34 and name HN) (resid  30 and name HD2#) 4.2 4.2 1.6
assign (resid  34 and name HN) (resid  30 and name HD1#) 4.2 4.2 1.6
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assign (resid  36 and name HN) (resid  59 and name HG) 4.2 4.2 1.6
assign (resid  36 and name HN) (resid  35 and name HD1#) 4.0 4.0 1.6
assign (resid  37 and name HN) (resid 110 and name HB#) 4.2 4.2 1.6
assign (resid  39 and name HN) (resid  38 and name HD1#) 3.6 3.6 1.4
assign (resid  39 and name HN) (resid 108 and name HB) 4.2 4.2 1.6
assign (resid  39 and name HN) (resid 109 and name HA) 4.2 4.2 1.6
assign (resid  39 and name HN) (resid  55 and name HA) 4.2 4.2 1.6
assign (resid  39 and name HN) (resid 107 and name HA) 4.2 4.2 1.6
assign (resid  40 and name HN) (resid  55 and name HA) 4.2 4.2 1.6
assign (resid  40 and name HN) (resid 106 and name HG#) 4.2 4.2 1.6
assign (resid  40 and name HN) (resid 108 and name HB) 4.2 4.2 1.6
assign (resid  40 and name HN) (resid  38 and name HG1#) 4.2 4.2 1.6
assign (resid  41 and name HN) (resid 106 and name HB#) 4.2 4.2 1.6
assign (resid  41 and name HN) (resid 107 and name HG2#) 4.2 4.2 1.6
assign (resid  41 and name HN) (resid  43 and name HD1#) 4.2 4.2 1.6
assign (resid  41 and name HN) (resid 107 and name HA) 4.2 4.2 1.6
assign (resid  41 and name HN) (resid 105 and name HA) 4.2 4.2 1.6
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assign (resid  42 and name HN) (resid 103 and name HG2#) 4.2 4.2 1.6
assign (resid  43 and name HN) (resid 105 and name HA) 4.2 4.2 1.6
assign (resid  43 and name HN) (resid 103 and name HG2#) 4.2 4.2 1.6
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assign (resid  45 and name HN) (resid  44 and name HG2#) 3.0 3.0 0.8
assign (resid  46 and name HN) (resid  43 and name HG2#) 4.2 4.2 1.6
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assign (resid  47 and name HN) (resid  43 and name HG2#) 4.2 4.2 1.6
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assign (resid  48 and name HN) (resid  47 and name HG#) 3.6 3.6 1.4
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assign (resid  54 and name HN) (resid  39 and name HA) 4.2 4.2 1.6
assign (resid  56 and name HN) (resid  39 and name HA) 4.2 4.2 1.6
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assign (resid  57 and name HN) (resid  39 and name HA) 4.2 4.2 1.6
assign (resid  57 and name HN) (resid  38 and name HG1#) 4.2 4.2 1.6
assign (resid  57 and name HN) (resid  54 and name HB#) 4.2 4.2 1.6
assign (resid  57 and name HN) (resid  38 and name HD1#) 4.2 4.2 1.6
assign (resid  57 and name HN) (resid  37 and name HG2#) 4.2 4.2 1.6
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assign (resid  58 and name HN) (resid  37 and name HG2#) 4.2 4.2 1.6
assign (resid  59 and name HN) (resid  58 and name HG#) 4.0 4.0 1.6
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assign (resid  59 and name HN) (resid  35 and name HG1#) 4.2 4.2 1.6
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assign (resid  60 and name HN) (resid  59 and name HG) 3.0 3.0 0.8
assign (resid  60 and name HN) (resid  59 and name HB#) 4.0 4.0 1.6
assign (resid  61 and name HN) (resid  35 and name HG1#) 4.2 4.2 1.6
assign (resid  61 and name HN) (resid  60 and name HG2#) 3.0 3.0 0.8
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assign (resid  62 and name HN) (resid  61 and name HG2#) 3.0 3.0 0.8
assign (resid  62 and name HN) (resid  61 and name HD1#) 4.0 4.0 1.6
assign (resid  63 and name HN) (resid  60 and name HG2#) 4.2 4.2 1.6
assign (resid  65 and name HN) (resid  59 and name HD2#) 4.2 4.2 1.6
assign (resid  66 and name HN) (resid  59 and name HD2#) 4.2 4.2 1.6
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assign (resid  69 and name HN) (resid  68 and name HG#) 4.0 4.0 1.6
assign (resid  68 and name HN) (resid  67 and name HG1#) 4.0 4.0 1.6
assign (resid  68 and name HN) (resid  67 and name HG2#) 4.0 4.0 1.6
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assign (resid  70 and name HN) (resid  87 and name HD2#) 4.2 4.2 1.6
assign (resid  70 and name HN) (resid  67 and name HG1#) 4.2 4.2 1.6
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assign (resid  73 and name HN) (resid  72 and name HG2#) 4.0 4.0 1.6
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assign (resid  73 and name HN) (resid  85 and name HD1#) 4.2 4.2 1.6
assign (resid  73 and name HN) (resid  85 and name HD2#) 4.2 4.2 1.6
assign (resid  74 and name HN) (resid  75 and name HG2#) 4.0 4.0 1.6
assign (resid  74 and name HN) (resid  85 and name HD1#) 4.2 4.2 1.6
assign (resid  75 and name HN) (resid  74 and name HB#) 3.6 3.6 1.4
assign (resid  76 and name HN) (resid  30 and name HB#) 4.2 4.2 1.6
assign (resid  76 and name HN) (resid  75 and name HG2#) 3.0 3.0 0.8
assign (resid  76 and name HN) (resid  75 and name HD1#) 4.0 4.0 1.6
assign (resid  77 and name HN) (resid  29 and name HA) 4.2 4.2 1.6
assign (resid  77 and name HN) (resid  30 and name HB#) 4.2 4.2 1.6
assign (resid  77 and name HN) (resid  30 and name HD2#) 4.2 4.2 1.6
assign (resid  77 and name HN) (resid 109 and name HD1#) 4.2 4.2 1.6
assign (resid  78 and name HN) (resid 109 and name HD1#) 4.2 4.2 1.6
assign (resid  78 and name HN) (resid  77 and name HB#) 3.0 3.0 0.8
assign (resid  78 and name HN) (resid 109 and name HB#) 4.2 4.2 1.6
assign (resid  79 and name HN) (resid  78 and name HG#) 3.6 3.6 1.4
assign (resid  79 and name HN) (resid 109 and name HD1#) 4.2 4.2 1.6
assign (resid  80 and name HN) (resid 110 and name HA) 4.2 4.2 1.6
assign (resid  80 and name HN) (resid  79 and name HG1#) 2.5 2.5 0.4
assign (resid  80 and name HN) (resid 109 and name HD1#) 4.2 4.2 1.6
assign (resid  81 and name HN) (resid 108 and name HA) 4.2 4.2 1.6
assign (resid  81 and name HN) (resid  80 and name HB#) 4.0 4.0 1.6
assign (resid  81 and name HN) (resid  79 and name HA) 4.2 4.2 1.6
assign (resid  81 and name HN) (resid 109 and name HD1#) 4.2 4.2 1.6
assign (resid  84 and name HN) (resid  83 and name HD#) 4.0 4.0 1.6
assign (resid  84 and name HN) (resid  83 and name HB#) 3.6 3.6 1.4
assign (resid  85 and name HN) (resid  84 and name HG2#) 3.0 3.0 0.8
assign (resid  85 and name HN) (resid 105 and name HB#) 4.2 4.2 1.6
assign (resid  85 and name HN) (resid 106 and name HA) 4.2 4.2 1.6
assign (resid  86 and name HN) (resid  84 and name HG2#) 4.2 4.2 1.6
assign (resid  86 and name HN) (resid  85 and name HD1#) 3.6 3.6 1.4
assign (resid  87 and name HN) (resid 103 and name HG1#) 4.2 4.2 1.6
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assign (resid  87 and name HN) (resid  86 and name HA) 2.5 2.5 0.4
assign (resid  88 and name HN) (resid  87 and name HB#) 3.0 3.0 0.8
assign (resid  88 and name HN) (resid  87 and name HD2#) 3.6 3.6 1.4
assign (resid  88 and name HN) (resid  87 and name HD1#) 4.0 4.0 1.6
assign (resid  88 and name HN) (resid 103 and name HG1#) 4.2 4.2 1.6
assign (resid  89 and name HN) (resid 101 and name HB#) 4.2 4.2 1.6
assign (resid  89 and name HN) (resid  88 and name HG2#) 3.0 3.0 0.8
assign (resid  89 and name HN) (resid 103 and name HG1#) 4.2 4.2 1.6
assign (resid  89 and name HN) (resid  98 and name HA) 4.2 4.2 1.6
assign (resid  91 and name HN) (resid  90 and name HA) 3.0 3.0 0.8
assign (resid  91 and name HN) (resid  90 and name HB#) 4.0 4.0 1.6
assign (resid  92 and name HN) (resid  90 and name HB#) 4.2 4.2 1.6
assign (resid  92 and name HN) (resid  93 and name HB#) 4.0 4.0 1.6
assign (resid  92 and name HN) (resid  91 and name HB#) 4.0 4.0 1.6
assign (resid  93 and name HN) (resid  91 and name HB#) 4.2 4.2 1.6
assign (resid  93 and name HN) (resid  99 and name HA) 4.2 4.2 1.6
assign (resid  93 and name HN) (resid  99 and name HG#) 4.2 4.2 1.6
assign (resid  93 and name HN) (resid  90 and name HB#) 4.2 4.2 1.6
assign (resid  98 and name HN) (resid  99 and name HB#) 4.0 4.0 1.6
assign (resid  93 and name HN) (resid  99 and name HB#) 4.2 4.2 1.6
assign (resid  96 and name HN) (resid  95 and name HG1#) 3.0 3.0 0.8
assign (resid  96 and name HN) (resid  95 and name HG2#) 3.6 3.6 1.4
assign (resid  97 and name HN) (resid  96 and name HB#) 3.0 3.0 0.8
assign (resid  97 and name HN) (resid  96 and name HD#) 4.0 4.0 1.6
assign (resid  97 and name HN) (resid  95 and name HG1#) 4.2 4.2 1.6
assign (resid  97 and name HN) (resid  95 and name HG2#) 4.2 4.2 1.6
assign (resid  98 and name HN) (resid  99 and name HG#) 3.6 3.6 1.4
assign (resid  98 and name HN) (resid  97 and name HG#) 3.6 3.6 1.4
assign (resid  98 and name HN) (resid  97 and name HB#) 3.0 3.0 0.8
assign (resid  98 and name HN) (resid  95 and name HB) 4.2 4.2 1.6
assign (resid  99 and name HN) (resid  93 and name HB#) 4.2 4.2 1.6
assign (resid  99 and name HN) (resid  98 and name HB#) 4.0 4.0 1.6
assign (resid  99 and name HN) (resid  98 and name HG) 4.0 4.0 1.6
assign (resid  99 and name HN) (resid  98 and name HD1#) 4.0 4.0 1.6
assign (resid  99 and name HN) (resid  95 and name HG2#) 4.2 4.2 1.6
assign (resid 100 and name HN) (resid  91 and name HA) 4.2 4.2 1.6
assign (resid 100 and name HN) (resid  89 and name HB#) 4.2 4.2 1.6
assign (resid 100 and name HN) (resid  99 and name HG#) 4.0 4.0 1.6
assign (resid 100 and name HN) (resid  99 and name HB#) 3.6 3.6 1.4
assign (resid 100 and name HN) (resid 101 and name HG#) 4.0 4.0 1.6
assign (resid 101 and name HN) (resid 102 and name HD#) 4.0 4.0 1.6
assign (resid 101 and name HN) (resid  89 and name HB#) 4.2 4.2 1.6
assign (resid 103 and name HN) (resid 102 and name HA) 2.5 2.5 0.4
assign (resid 103 and name HN) (resid  88 and name HB) 4.2 4.2 1.6
assign (resid 103 and name HN) (resid 101 and name HB#) 4.2 4.2 1.6
assign (resid 103 and name HN) (resid 102 and name HB#) 4.0 4.0 1.6
assign (resid 103 and name HN) (resid 101 and name HG#) 4.2 4.2 1.6
assign (resid 104 and name HN) (resid 103 and name HB) 3.0 3.0 0.8
assign (resid 104 and name HN) (resid  43 and name HG2#) 4.2 4.2 1.6
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assign (resid 105 and name HN) (resid  85 and name HD2#) 4.2 4.2 1.6
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assign (resid 105 and name HN) (resid  85 and name HG) 4.2 4.2 1.6
assign (resid 105 and name HN) (resid  85 and name HB#) 4.2 4.2 1.6
assign (resid 105 and name HN) (resid  85 and name HA) 4.2 4.2 1.6
assign (resid 105 and name HN) (resid  86 and name HA) 4.2 4.2 1.6
assign (resid 105 and name HN) (resid  84 and name HA) 4.2 4.2 1.6
assign (resid 107 and name HN) (resid  84 and name HA) 4.2 4.2 1.6
assign (resid 107 and name HN) (resid  82 and name HA) 4.2 4.2 1.6
assign (resid 107 and name HN) (resid 106 and name HA) 2.5 2.5 0.4
assign (resid 107 and name HN) (resid 106 and name HG#) 4.0 4.0 1.6
assign (resid 107 and name HN) (resid 106 and name HB#) 3.6 3.6 1.4
assign (resid 107 and name HN) (resid  83 and name HB#) 4.2 4.2 1.6
assign (resid 107 and name HN) (resid  83 and name HD#) 4.2 4.2 1.6
assign (resid 107 and name HN) (resid  84 and name HG2#) 4.2 4.2 1.6
assign (resid 108 and name HN) (resid  39 and name HB#) 4.2 4.2 1.6
assign (resid 108 and name HN) (resid  40 and name HB#) 4.2 4.2 1.6
assign (resid 108 and name HN) (resid 107 and name HG1#) 4.0 4.0 1.6
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assign (resid 109 and name HN) (resid 107 and name HB) 4.2 4.2 1.6
assign (resid 109 and name HN) (resid  79 and name HA) 4.2 4.2 1.6
assign (resid 109 and name HN) (resid  80 and name HA) 4.2 4.2 1.6
assign (resid 109 and name HN) (resid  82 and name HA) 4.2 4.2 1.6
assign (resid 110 and name HN) (resid  38 and name HA) 4.2 4.2 1.6
assign (resid 111 and name HN) (resid  36 and name HA) 4.2 4.2 1.6
assign (resid 110 and name HN) (resid 108 and name HB) 4.2 4.2 1.6
assign (resid 109 and name HN) (resid  82 and name HG#) 4.2 4.2 1.6
assign (resid 109 and name HN) (resid 108 and name HG1#) 3.0 3.0 0.8
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assign (resid 110 and name HN) (resid  37 and name HG1#) 4.2 4.2 1.6
assign (resid 110 and name HN) (resid 108 and name HG1#) 4.2 4.2 1.6
assign (resid 110 and name HN) (resid 109 and name HD1#) 4.0 4.0 1.6
assign (resid 112 and name HN) (resid  37 and name HB) 4.2 4.2 1.6
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assign (resid 111 and name HN) (resid  37 and name HD1#) 4.2 4.2 1.6
assign (resid 113 and name HN) (resid 112 and name HA) 3.0 3.0 0.8
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assign (resid 113 and name HN) (resid  35 and name HG1#) 4.2 4.2 1.6
assign (resid 113 and name HN) (resid  35 and name HD1#) 4.2 4.2 1.6
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assign (resid  81 and name HN) (resid 109 and name HA) 4.2 4.2 1.6
assign (resid  38 and name HN) (resid  57 and name HB#) 4.2 4.2 1.6
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assign (resid  82 and name HN) (resid  81 and name HB#) 3.6 3.6 1.4
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assign (resid  83 and name HN) (resid 107 and name HB) 4.2 4.2 1.6
assign (resid  83 and name HN) (resid  82 and name HG#) 3.0 3.0 0.8
assign (resid  83 and name HN) (resid 107 and name HG2#) 4.2 4.2 1.6
assign (resid  83 and name HN) (resid 107 and name HG1#) 4.2 4.2 1.6
assign (resid 106 and name HN) (resid  42 and name HA#) 4.2 4.2 1.6
assign (resid 106 and name HN) (resid  41 and name HB) 4.2 4.2 1.6
assign (resid 106 and name HN) (resid  41 and name HG2#) 4.2 4.2 1.6
assign (resid 106 and name HN) (resid 104 and name HG2#) 4.2 4.2 1.6
assign (resid 106 and name HN) (resid  43 and name HD##) 4.2 4.2 1.6
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assign (resid  25 and name HN) (resid  24 and name HA) 3.0 3.0 0.8
assign (resid  25 and name HN) (resid  24 and name HB#) 3.6 3.6 1.4
assign (resid  41 and name HN) (resid 106 and name HN) 4.2 4.2 1.6
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assign (resid  73 and name HN) (resid  72 and name HA) 3.0 3.0 0.8
assign (resid  74 and name HN) (resid  72 and name HA) 4.2 4.2 1.6
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assign (resid  29 and name HN) (resid  26 and name HN) 4.2 4.2 1.6
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assign (resid  54 and name HN) (resid  40 and name HD#) 4.2 4.2 1.6
assign (resid  67 and name HN) (resid  66 and name HD#) 4.0 4.0 1.6
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assign (resid  67 and name HN) (resid  66 and name HB#) 3.6 3.6 1.4
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assign (resid  72 and name HN) (resid  69 and name HN) 4.2 4.2 1.6
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assign (resid  29 and name HN) (resid  27 and name HB#) 4.2 4.2 1.6
assign (resid  29 and name HN) (resid  28 and name HB#) 4.0 4.0 1.6
assign (resid  29 and name HN) (resid  26 and name HB#) 4.2 4.2 1.6
assign (resid  29 and name HN) (resid  26 and name HA) 4.2 4.2 1.6
assign (resid  29 and name HN) (resid  27 and name HA) 4.2 4.2 1.6
assign (resid  25 and name HN) (resid  26 and name HA) 3.6 3.6 1.4
assign (resid  23 and name HN) (resid  24 and name HB#) 4.0 4.0 1.6
assign (resid  22 and name HN) (resid  21 and name HA) 4.2 4.2 1.6
assign (resid  22 and name HN) (resid  21 and name HG2#) 4.2 4.2 1.6
assign (resid  52 and name HN) (resid  42 and name HA#) 4.2 4.2 1.6
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assign (resid  52 and name HN) (resid  51 and name HN) 4.2 4.2 1.6
assign (resid  27 and name HN) (resid  22 and name HA) 4.2 4.2 1.6
assign (resid  28 and name HN) (resid  25 and name HA) 4.2 4.2 1.6
assign (resid  32 and name HN) (resid  31 and name HA) 2.5 2.5 0.4
assign (resid  51 and name HN) (resid  50 and name HB#) 4.2 4.2 1.6
assign (resid  51 and name HN) (resid  50 and name HA) 4.2 4.2 1.6
assign (resid  98 and name HN) (resid  95 and name HG1#) 4.2 4.2 1.6
assign (resid 105 and name HN) (resid 104 and name HB) 4.0 4.0 1.6
assign (resid  43 and name HN) (resid 104 and name HB) 4.2 4.2 1.6
assign (resid  26 and name HN) (resid  28 and name HN) 4.2 4.2 1.6
assign (resid  21 and name HN) (resid  22 and name HA) 4.2 4.2 1.6
assign (resid 104 and name HN) (resid  44 and name HG2#) 4.2 4.2 1.6
assign (resid  49 and name HN) (resid  43 and name HA) 4.2 4.2 1.6
assign (resid  83 and name HN) (resid 108 and name HB) 4.2 4.2 1.6
assign (resid  83 and name HN) (resid 108 and name HA) 4.2 4.2 1.6
assign (resid  36 and name HN) (resid  35 and name HG1#) 4.0 4.0 1.6
assign (resid  53 and name HN) (resid  41 and name HA) 4.2 4.2 1.6
assign (resid  99 and name HE2#) (resid  96 and name HA) 4.2 4.2 1.6
assign (resid  56 and name HD2#) (resid  55 and name HB#) 4.0 4.0 1.6
assign (resid  80 and name HD2#) (resid 108 and name HG2#) 4.2 4.2 1.6
assign (resid  44 and name HN) (resid  43 and name HG1#) 4.0 4.0 1.6
assign (resid  57 and name HN) (resid  37 and name HA) 4.2 4.2 1.6
assign (resid  81 and name HN) (resid 109 and name HG) 4.2 4.2 1.6
assign (resid  80 and name HN) (resid 109 and name HB#) 4.2 4.2 1.6
assign (resid  42 and name HN) (resid  49 and name HN) 4.2 4.2 1.6
assign (resid  60 and name HN) (resid  35 and name HA) 4.2 4.2 1.6
assign (resid 106 and name HN) (resid 105 and name HB#) 4.0 4.0 1.6
assign (resid 106 and name HN) (resid  40 and name HB#) 4.2 4.2 1.6
assign (resid 106 and name HN) (resid 105 and name HA) 2.5 2.5 0.4
assign (resid 106 and name HN) (resid  40 and name HA) 4.2 4.2 1.6
assign (resid 106 and name HN) (resid 105 and name HD#) 3.6 3.6 1.4
assign (resid  37 and name HN) (resid 111 and name HA) 4.2 4.2 1.6
assign (resid  36 and name HN) (resid  60 and name HA) 4.2 4.2 1.6
assign (resid  45 and name HN) (resid 103 and name HA) 4.2 4.2 1.6
assign (resid 103 and name HN) (resid  88 and name HA) 4.2 4.2 1.6
assign (resid  85 and name HN) (resid 104 and name HA) 4.2 4.2 1.6
assign (resid  38 and name HN) (resid  57 and name HN) 4.2 4.2 1.6
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assign (resid 108 and name HN) (resid  38 and name HA) 4.2 4.2 1.6
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assign (resid 104 and name HN) (resid  43 and name HG1#) 4.2 4.2 1.6
assign (resid  70 and name HN) (resid  73 and name HN) 4.2 4.2 1.6
assign (resid  49 and name HN) (resid  43 and name HG2#) 4.2 4.2 1.6
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assign (resid  68 and name HN) (resid  67 and name HA) 3.0 3.0 0.8
assign (resid  59 and name HN) (resid  35 and name HG2#) 4.2 4.2 1.6
assign (resid  33 and name HN) (resid  61 and name HN) 4.2 4.2 1.6
assign (resid  76 and name HN) (resid  31 and name HA) 4.2 4.2 1.6
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!assign (resid 100 and name HA) (resid  88 and name HG2#) 4.0 4.0 0.5
assign (resid  60 and name HB) (resid  63 and name HB#) 4.0 4.0 0.5
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assign (resid 103 and name HG1#) (resid  67 and name HD1#) 4.0 4.0 0.5
assign (resid  85 and name HD1#) (resid  83 and name HE#) 4.0 4.0 0.5
assign (resid  35 and name HG2#) (resid  58 and name HA) 4.0 4.0 0.5
assign (resid  35 and name HD1#) (resid  60 and name HA) 4.0 4.0 0.5
assign (resid  58 and name HG#) (resid  37 and name HG2#) 4.0 4.0 0.5
assign (resid  38 and name HA) (resid 109 and name HD2#) 4.0 4.0 0.5
assign (resid  45 and name HB#) (resid  44 and name HG2#) 4.0 4.0 0.4
assign (resid 103 and name HA) (resid  44 and name HG2#) 4.0 4.0 0.5
assign (resid  98 and name HD1#) (resid 101 and name HB#) 4.0 4.0 0.5
assign (resid  67 and name HD1#) (resid  90 and name HG#) 4.0 4.0 0.0
assign (resid  67 and name HG2#) (resid  89 and name HA) 4.0 4.0 0.0
assign (resid  31 and name HA) (resid  76 and name HA) 4.0 4.0 0.5
assign (resid  38 and name HA) (resid  37 and name HG1#) 4.0 4.0 0.4
assign (resid  39 and name HA) (resid  54 and name HA) 4.0 4.0 1.0
assign (resid  39 and name HA) (resid  56 and name HA) 4.0 4.0 0.5
assign (resid  41 and name HB) (resid  43 and name HG1#) 4.0 4.0 0.5
assign (resid  41 and name HA) (resid  43 and name HG1#) 4.0 4.0 0.5
assign (resid  41 and name HB) (resid  43 and name HD1#) 4.0 4.0 0.5
assign (resid  40 and name HA) (resid  39 and name HB#) 4.0 4.0 0.4
assign (resid  40 and name HA) (resid 108 and name HB) 4.0 4.0 0.5
assign (resid  45 and name HB#) (resid  47 and name HB#) 4.0 4.0 0.5
assign (resid  52 and name HB#) (resid  98 and name HD1#) 4.0 4.0 0.5
assign (resid  53 and name HB#) (resid  41 and name HG2#) 4.0 4.0 0.5
assign (resid  68 and name HA) (resid  67 and name HG1#) 4.0 4.0 0.4
assign (resid  70 and name HB#) (resid  67 and name HG1#) 4.0 4.0 0.5
assign (resid  72 and name HA) (resid  71 and name HB#) 4.0 4.0 0.4
assign (resid  82 and name HA) (resid  81 and name HA) 4.0 4.0 0.4
assign (resid  82 and name HA) (resid  81 and name HB#) 4.0 4.0 0.4
assign (resid  82 and name HA) (resid  83 and name HA) 4.0 4.0 0.4
assign (resid  84 and name HA) (resid 107 and name HG2#) 4.0 4.0 0.5
assign (resid  86 and name HA) (resid  85 and name HD2#) 4.0 4.0 0.4
assign (resid  86 and name HA) (resid 103 and name HG1#) 4.0 4.0 0.5
assign (resid  89 and name HB#) (resid  67 and name HD1#) 4.0 4.0 0.5
assign (resid  90 and name HB#) (resid  67 and name HG2#) 4.0 4.0 0.5
assign (resid  97 and name HA) (resid  95 and name HG1#) 4.0 4.0 0.5
assign (resid  97 and name HA) (resid  98 and name HD1#) 4.0 4.0 0.4
assign (resid  97 and name HA) (resid  95 and name HG2#) 4.0 4.0 0.5
assign (resid 109 and name HD2#) (resid  74 and name HD1#) 4.0 4.0 0.5

assign (resid  66 and name HE2) (resid  70 and name HE2) 4.2 4.2 1.6
assign (resid  70 and name HE1) (resid  38 and name HG2#) 4.2 4.2 1.6
assign (resid  70 and name HE2) (resid  87 and name HD2#) 4.2 4.2 1.6
assign (resid  70 and name HE1) (resid 107 and name HG2#) 4.2 4.2 1.6
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assign (resid  70 and name HE2) (resid  67 and name HG12) 4.2 4.2 1.6
assign (resid  70 and name HE1) (resid  74 and name HG) 4.2 4.2 1.6
assign (resid  70 and name HE1) (resid  74 and name HD1#) 4.2 4.2 1.6
assign (resid  93 and name HD2) (resid  98 and name HG) 4.2 4.2 1.6
assign (resid  93 and name HD1) (resid  67 and name HG2#) 4.2 4.2 1.6
assign (resid  93 and name HD2) (resid  98 and name HB2) 4.2 4.2 1.6
assign (resid  93 and name HD2) (resid  95 and name HB) 4.2 4.2 1.6
assign (resid  93 and name HD1) (resid  90 and name HD2) 4.2 4.2 1.6
assign (resid  93 and name HD2) (resid  98 and name HA) 4.2 4.2 1.6
assign (resid 105 and name HD#) (resid 103 and name HG2#) 4.2 4.2 1.6
assign (resid 105 and name HD#) (resid 103 and name HG1#) 4.2 4.2 1.6
assign (resid 105 and name HE#) (resid 103 and name HG1#) 4.2 4.2 1.6
assign (resid 105 and name HZ) (resid 103 and name HG2#) 4.2 4.2 1.6
assign (resid 105 and name HZ) (resid 103 and name HG1#) 4.2 4.2 1.6
assign (resid 105 and name HD2) (resid  87 and name HG) 4.2 4.2 1.6
assign (resid 105 and name HD2) (resid  87 and name HB3) 4.2 4.2 1.6
assign (resid 105 and name HD1) (resid 103 and name HB) 4.2 4.2 1.6
assign (resid 105 and name HD2) (resid  87 and name HB2) 4.2 4.2 1.6
assign (resid 105 and name HE2) (resid  87 and name HD2#) 4.2 4.2 1.6
assign (resid 105 and name HE2) (resid 107 and name HG2#) 4.2 4.2 1.6
assign (resid 105 and name HE2) (resid  87 and name HG) 4.2 4.2 1.6
assign (resid 105 and name HE1) (resid  52 and name HB#) 4.2 4.2 1.6
assign (resid  40 and name HD1) (resid 107 and name HG1#) 4.2 4.2 1.6
assign (resid  40 and name HD2) (resid 107 and name HG2#) 4.2 4.2 1.6
assign (resid  40 and name HD2) (resid  54 and name HB#) 4.2 4.2 1.6
assign (resid  40 and name HD1) (resid  38 and name HG13) 4.2 4.2 1.6
assign (resid  40 and name HD1) (resid  38 and name HG12) 4.2 4.2 1.6
assign (resid  40 and name HD1) (resid  38 and name HB) 4.2 4.2 1.6
assign (resid  40 and name HD1) (resid  57 and name HB#) 4.2 4.2 1.6
assign (resid  40 and name HD#) (resid  70 and name HE2) 4.2 4.2 1.6
assign (resid  40 and name HD1) (resid  57 and name HD2) 4.2 4.2 1.6
assign (resid  40 and name HE1) (resid  66 and name HE2) 4.2 4.2 1.6
assign (resid  40 and name HZ) (resid  57 and name HD2) 4.2 4.2 1.6
assign (resid  66 and name HE2) (resid  40 and name HZ) 4.2 4.2 1.6
assign (resid  66 and name HE2) (resid  40 and name HE1) 4.2 4.2 1.6
assign (resid  66 and name HE1) (resid  57 and name HE1) 4.2 4.2 1.6
assign (resid  66 and name HE1) (resid  57 and name HD1) 4.2 4.2 1.6
assign (resid  66 and name HE2) (resid  40 and name HD1) 4.2 4.2 1.6
assign (resid  70 and name HD2) (resid 105 and name HD2) 4.2 4.2 1.6
assign (resid  70 and name HD2) (resid 105 and name HE2) 4.2 4.2 1.6
assign (resid  89 and name HD1) (resid 105 and name HE1) 4.2 4.2 1.6
assign (resid 105 and name HE2) (resid  70 and name HD2) 4.2 4.2 1.6
assign (resid 105 and name HD1) (resid  42 and name HA2) 4.2 4.2 1.6
assign (resid 105 and name HD1) (resid  42 and name HA1) 4.2 4.2 1.6
assign (resid  57 and name HD1) (resid  59 and name HB2) 4.2 4.2 1.6
assign (resid  40 and name HE1) (resid  57 and name HB#) 4.2 4.2 1.6
assign (resid  66 and name HD1) (resid  59 and name HD1#) 4.2 4.2 1.6
assign (resid  40 and name HZ) (resid  54 and name HB#) 4.2 4.2 1.6
assign (resid  40 and name HE2) (resid  54 and name HB#) 4.2 4.2 1.6
assign (resid  70 and name HD2) (resid  87 and name HD2#) 4.2 4.2 1.6
assign (resid  70 and name HD2) (resid  67 and name HG12) 4.2 4.2 1.6
assign (resid  70 and name HD1) (resid  74 and name HG) 4.2 4.2 1.6
assign (resid  70 and name HD1) (resid  74 and name HD1#) 4.2 4.2 1.6
assign (resid  70 and name HD2) (resid  67 and name HD11) 4.2 4.2 1.6
assign (resid  57 and name HD#) (resid  38 and name HG12) 4.2 4.2 1.6
assign (resid  57 and name HD1) (resid  59 and name HG) 4.2 4.2 1.6
assign (resid  57 and name HD2) (resid  54 and name HB#) 4.2 4.2 1.6
assign (resid 105 and name HZ) (resid  87 and name HG) 4.2 4.2 1.6
assign (resid 105 and name HZ) (resid  52 and name HB#) 4.2 4.2 1.6
assign (resid  66 and name HE1) (resid  38 and name HD1#) 4.2 4.2 1.6
assign (resid  66 and name HE1) (resid  59 and name HD1#) 4.2 4.2 1.6
assign (resid  57 and name HE1) (resid  59 and name HB2) 4.2 4.2 1.6
assign (resid  57 and name HE1) (resid  59 and name HG) 4.2 4.2 1.6
assign (resid  57 and name HE2) (resid  54 and name HB#) 4.2 4.2 1.6
assign (resid  57 and name HE1) (resid  59 and name HD1#) 4.2 4.2 1.6
assign (resid  93 and name HE1) (resid  90 and name HD#) 4.2 4.2 1.6
assign (resid  89 and name HD2) (resid  67 and name HD1#) 4.2 4.2 1.6
assign (resid  93 and name HE1) (resid  98 and name HD2#) 4.2 4.2 1.6
```

```
assign (resid  93 and name HE2) (resid  98 and name HG) 4.2 4.2 1.6
assign (resid  93 and name HE2) (resid  98 and name HB2) 4.2 4.2 1.6
assign (resid  57 and name HD1) (resid  59 and name HD1#) 4.2 4.2 1.6
assign (resid  57 and name HE2) (resid  40 and name HZ) 4.2 4.2 1.6
assign (resid  89 and name HE1) (resid 103 and name HG1#) 4.2 4.2 1.6
assign (resid  89 and name HE1) (resid  52 and name HB#) 4.2 4.2 1.6
assign (resid  70 and name HD1) (resid  38 and name HG2#) 4.2 4.2 1.6
assign (resid  89 and name HE1) (resid 103 and name HG2#) 4.2 4.2 1.6
assign (resid  89 and name HD1) (resid 103 and name HG1#) 4.2 4.2 1.6
assign (resid  89 and name HD1) (resid 103 and name HG2#) 4.2 4.2 1.6
assign (resid  89 and name HD1) (resid 103 and name HA) 4.2 4.2 1.6
assign (resid  70 and name HD1) (resid  71 and name HA) 2.5 2.5 0.4
assign (resid  89 and name HD1) (resid  98 and name HD2#) 4.2 4.2 1.6
assign (resid  89 and name HE1) (resid  98 and name HD2#) 4.2 4.2 1.6
assign (resid  93 and name HE2) (resid  95 and name HB) 4.2 4.2 1.6
assign (resid  89 and name HD1) (resid  98 and name HA) 4.2 4.2 1.6
assign (resid  66 and name HD1) (resid  38 and name HD1#) 4.2 4.2 1.6
assign (resid  40 and name HD2) (resid  52 and name HA) 4.2 4.2 1.6
assign (resid  40 and name HE2) (resid  52 and name HA) 4.2 4.2 1.6
assign (resid  89 and name HE2) (resid  67 and name HD1#) 4.2 4.2 1.6
assign (resid  89 and name HZ) (resid  52 and name HB#) 4.2 4.2 1.6
assign (resid  57 and name HE1) (resid  59 and name HA) 4.2 4.2 1.6
assign (resid  57 and name HD1) (resid  59 and name HA) 4.2 4.2 1.6
assign (resid  89 and name HE1) (resid  98 and name HD1#) 4.2 4.2 1.6
assign (resid  57 and name HD1) (resid  38 and name HD1#) 4.2 4.2 1.6
assign (resid  57 and name HE1) (resid  38 and name HD1#) 4.2 4.2 1.6
assign (resid 105 and name HD2) (resid  87 and name HD2#) 4.2 4.2 1.6
assign (resid  40 and name HD1) (resid  38 and name HD1#) 4.2 4.2 1.6
assign (resid  40 and name HE1) (resid  38 and name HD1#) 4.2 4.2 1.6
assign (resid 105 and name HE2) (resid  70 and name HE2) 4.2 4.2 1.6
assign (resid 105 and name HE2) (resid  87 and name HD1#) 4.2 4.2 1.6
assign (resid 105 and name HD2) (resid  87 and name HD1#) 4.2 4.2 1.6
assign (resid  93 and name HD1) (resid  98 and name HD2#) 4.2 4.2 1.6
assign (resid  70 and name HZ) (resid 105 and name HB3) 4.2 4.2 1.6
assign (resid  70 and name HZ) (resid 105 and name HB2) 4.2 4.2 1.6
assign (resid  66 and name HD#) (resid  70 and name HB#) 4.2 4.2 1.6
assign (resid  70 and name HD1) (resid 107 and name HG2#) 4.2 4.2 1.6
assign (resid  40 and name HE1) (resid  38 and name HG13) 4.2 4.2 1.6
assign (resid 105 and name HD2) (resid  70 and name HE2) 4.2 4.2 1.6
assign (resid  93 and name HD2) (resid  98 and name HD1#) 4.2 4.2 1.6
assign (resid 105 and name HZ) (resid  98 and name HD1#) 4.2 4.2 1.6
assign (resid 105 and name HZ) (resid  98 and name HD2#) 4.2 4.2 1.6
assign (resid 105 and name HZ) (resid  87 and name HD2#) 4.2 4.2 1.6
assign (resid  89 and name HD1) (resid  98 and name HD1#) 4.2 4.2 1.6
assign (resid  89 and name HD2) (resid  67 and name HG23) 4.2 4.2 1.6
assign (resid  89 and name HE2) (resid  67 and name HG23) 4.2 4.2 1.6
assign (resid  89 and name HD1) (resid  52 and name HB#) 4.2 4.2 1.6
assign (resid 105 and name HD2) (resid 107 and name HG2#) 4.2 4.2 1.6
assign (resid  66 and name HE1) (resid  70 and name HE1) 4.2 4.2 1.6
```

C.4.2 Hydrogen bonds

```
assign (resid 34 and name HN) (resid 61 and name O) 2.0 0.7 0.5
assign (resid 34 and name N) (resid 61 and name O) 3.0 0.7 0.5
assign (resid 34 and name O) (resid 61 and name HN) 2.0 0.7 0.5
assign (resid 34 and name O) (resid 61 and name N) 3.0 0.7 0.5
assign (resid 36 and name HN) (resid 59 and name O) 2.0 0.7 0.5
assign (resid 36 and name N) (resid 59 and name O) 3.0 0.7 0.5
assign (resid 36 and name O) (resid 59 and name HN) 2.0 0.7 0.5
assign (resid 36 and name O) (resid 59 and name N) 3.0 0.7 0.5
assign (resid 38 and name HN) (resid 57 and name O) 2.0 0.7 0.5
assign (resid 38 and name N) (resid 57 and name O) 3.0 0.7 0.5
assign (resid 38 and name O) (resid 57 and name HN) 2.0 0.7 0.5
assign (resid 38 and name O) (resid 57 and name N) 3.0 0.7 0.5
assign (resid 40 and name HN) (resid 54 and name O) 2.0 0.7 0.5
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assign (resid 40 and name N) (resid 54 and name O) 3.0 0.7 0.5
assign (resid 44 and name HN) (resid 47 and name O) 2.0 0.7 0.5
assign (resid 44 and name N) (resid 47 and name O) 3.0 0.7 0.5
assign (resid 44 and name O) (resid 47 and name HN) 2.0 0.7 0.5
assign (resid 44 and name O) (resid 47 and name N) 3.0 0.7 0.5
assign (resid 35 and name HN) (resid 112 and name O) 2.0 0.7 0.5
assign (resid 35 and name N) (resid 112 and name O) 3.0 0.7 0.5
assign (resid 35 and name O) (resid 112 and name HN) 2.0 0.7 0.5
assign (resid 35 and name O) (resid 112 and name N) 3.0 0.7 0.5
assign (resid 37 and name HN) (resid 110 and name O) 2.0 0.7 0.5
assign (resid 37 and name N) (resid 110 and name O) 3.0 0.7 0.5
assign (resid 37 and name O) (resid 110 and name HN) 2.0 0.7 0.5
assign (resid 37 and name O) (resid 110 and name N) 3.0 0.7 0.5
assign (resid 39 and name HN) (resid 108 and name O) 2.0 0.7 0.5
assign (resid 39 and name N) (resid 108 and name O) 3.0 0.7 0.5
assign (resid 39 and name O) (resid 108 and name HN) 2.0 0.7 0.5
assign (resid 39 and name O) (resid 108 and name N) 3.0 0.7 0.5
assign (resid 41 and name HN) (resid 106 and name O) 2.0 0.7 0.5
assign (resid 41 and name N) (resid 106 and name O) 3.0 0.7 0.5
assign (resid 41 and name O) (resid 106 and name HN) 2.0 0.7 0.5
assign (resid 41 and name O) (resid 106 and name N) 3.0 0.7 0.5
assign (resid 43 and name HN) (resid 104 and name O) 2.0 0.7 0.5
assign (resid 43 and name N) (resid 104 and name O) 3.0 0.7 0.5
assign (resid 43 and name O) (resid 104 and name HN) 2.0 0.7 0.5
assign (resid 43 and name O) (resid 104 and name N) 3.0 0.7 0.5
assign (resid 101 and name HN) (resid 89 and name O) 2.0 0.7 0.5
assign (resid 101 and name N) (resid 89 and name O) 3.0 0.7 0.5
assign (resid 101 and name O) (resid 89 and name HN) 2.0 0.7 0.5
assign (resid 101 and name O) (resid 89 and name N) 3.0 0.7 0.5
assign (resid 103 and name HN) (resid 87 and name O) 2.0 0.7 0.5
assign (resid 103 and name N) (resid 87 and name O) 3.0 0.7 0.5
assign (resid 103 and name O) (resid 87 and name HN) 2.0 0.7 0.5
assign (resid 103 and name O) (resid 87 and name N) 3.0 0.7 0.5
assign (resid 105 and name HN) (resid 85 and name O) 2.0 0.7 0.5
assign (resid 105 and name N) (resid 85 and name O) 3.0 0.7 0.5
assign (resid 105 and name O) (resid 85 and name HN) 2.0 0.7 0.5
assign (resid 105 and name O) (resid 85 and name N) 3.0 0.7 0.5
assign (resid 107 and name HN) (resid 83 and name O) 2.0 0.7 0.5
assign (resid 107 and name N) (resid 83 and name O) 3.0 0.7 0.5
assign (resid 107 and name O) (resid 83 and name HN) 2.0 0.7 0.5
assign (resid 107 and name O) (resid 83 and name N) 3.0 0.7 0.5
assign (resid 109 and name HN) (resid 81 and name O) 2.0 0.7 0.5
assign (resid 109 and name N) (resid 81 and name O) 3.0 0.7 0.5
assign (resid 109 and name O) (resid 81 and name HN) 2.0 0.7 0.5
assign (resid 109 and name O) (resid 81 and name N) 3.0 0.7 0.5
assign (resid 74 and name HN) (resid 70 and name O) 2.0 0.7 0.5
assign (resid 74 and name N) (resid 70 and name O) 3.0 0.7 0.5
assign (resid 75 and name HN) (resid 71 and name O) 2.0 0.7 0.5
assign (resid 75 and name N) (resid 71 and name O) 3.0 0.7 0.5
assign (resid 76 and name HN) (resid 72 and name O) 3.0 0.7 0.5
assign (resid 76 and name H) (resid 72 and name O) 2.0 0.7 0.5
assign (resid 77 and name HN) (resid 73 and name O) 3.0 0.7 0.5
assign (resid 77 and name H) (resid 73 and name O) 2.0 0.7 0.5
assign (resid 95 and name O) (resid 98 and name HN) 2.0 0.7 0.5
assign (resid 95 and name O) (resid 98 and name N) 3.0 0.7 0.5
assign (resid 96 and name O) (resid 99 and name HN) 2.0 0.7 0.5
assign (resid 96 and name O) (resid 99 and name N) 3.0 0.7 0.5
assign (resid 97 and name O) (resid 100 and name HN) 2.0 0.7 0.5
assign (resid 97 and name O) (resid 100 and name N) 3.0 0.7 0.5
```

C.4.3 $^3J_{HN-H\alpha}$ coupling constants

```
 assign (resid  14 and name hn ) (resid  14 and name n )
        (resid  14 and name ca ) (resid  14 and name ha ) 6.3 2.1
 assign (resid  16 and name hn ) (resid  16 and name n )
```

```
       (resid  16 and name ca ) (resid  16 and name ha ) 3.3 2.1
assign (resid  17 and name hn ) (resid  17 and name n )
       (resid  17 and name ca ) (resid  17 and name ha ) 5.5 2.1
assign (resid  19 and name hn ) (resid  19 and name n )
       (resid  19 and name ca ) (resid  19 and name ha ) 5.1 2.1
assign (resid  23 and name hn ) (resid  23 and name n )
       (resid  23 and name ca ) (resid  23 and name ha ) 6.8 2.1
assign (resid  24 and name hn ) (resid  24 and name n )
       (resid  24 and name ca ) (resid  24 and name ha ) 5.6 2.1
assign (resid  25 and name hn ) (resid  25 and name n )
       (resid  25 and name ca ) (resid  25 and name ha ) 6.3 2.1
assign (resid  26 and name hn ) (resid  26 and name n )
       (resid  26 and name ca ) (resid  26 and name ha ) 1.3 2.1
assign (resid  27 and name hn ) (resid  27 and name n )
       (resid  27 and name ca ) (resid  27 and name ha ) 3.1 2.1
assign (resid  28 and name hn ) (resid  28 and name n )
       (resid  28 and name ca ) (resid  28 and name ha ) 3.7 2.1
assign (resid  29 and name hn ) (resid  29 and name n )
       (resid  29 and name ca ) (resid  29 and name ha ) 3.2 2.1
assign (resid  31 and name hn ) (resid  31 and name n )
       (resid  31 and name ca ) (resid  31 and name ha ) 8.3 2.1
assign (resid  34 and name hn ) (resid  34 and name n )
       (resid  34 and name ca ) (resid  34 and name ha ) 5.4 2.1
assign (resid  35 and name hn ) (resid  35 and name n )
       (resid  35 and name ca ) (resid  35 and name ha ) 8.2 2.1
assign (resid  36 and name hn ) (resid  36 and name n )
       (resid  36 and name ca ) (resid  36 and name ha ) 7.8 2.1
assign (resid  37 and name hn ) (resid  37 and name n )
       (resid  37 and name ca ) (resid  37 and name ha ) 8.1 2.1
assign (resid  38 and name hn ) (resid  38 and name n )
       (resid  38 and name ca ) (resid  38 and name ha ) 10.1 2.1
assign (resid  39 and name hn ) (resid  39 and name n )
       (resid  39 and name ca ) (resid  39 and name ha ) 8.8 2.1
assign (resid  40 and name hn ) (resid  40 and name n )
       (resid  40 and name ca ) (resid  40 and name ha ) 6.2 2.1
assign (resid  41 and name hn ) (resid  41 and name n )
       (resid  41 and name ca ) (resid  41 and name ha ) 8.9 2.1
assign (resid  43 and name hn ) (resid  43 and name n )
       (resid  43 and name ca ) (resid  43 and name ha ) 8.8 2.1
assign (resid  44 and name hn ) (resid  44 and name n )
       (resid  44 and name ca ) (resid  44 and name ha ) 8.1 2.1
assign (resid  45 and name hn ) (resid  45 and name n )
       (resid  45 and name ca ) (resid  45 and name ha ) 3.6 2.1
assign (resid  46 and name hn ) (resid  46 and name n )
       (resid  46 and name ca ) (resid  46 and name ha ) 6.0 2.1
assign (resid  47 and name hn ) (resid  47 and name n )
       (resid  47 and name ca ) (resid  47 and name ha ) 8.2 2.1
assign (resid  48 and name hn ) (resid  48 and name n )
       (resid  48 and name ca ) (resid  48 and name ha ) 3.9 2.1
assign (resid  49 and name hn ) (resid  49 and name n )
       (resid  49 and name ca ) (resid  49 and name ha ) 8.2 2.1
assign (resid  52 and name hn ) (resid  52 and name n )
       (resid  52 and name ca ) (resid  52 and name ha ) 9.5 2.1
assign (resid  53 and name hn ) (resid  53 and name n )
       (resid  53 and name ca ) (resid  53 and name ha ) 8.5 2.1
assign (resid  54 and name hn ) (resid  54 and name n )
       (resid  54 and name ca ) (resid  54 and name ha ) 4.6 2.1
assign (resid  55 and name hn ) (resid  55 and name n )
       (resid  55 and name ca ) (resid  55 and name ha ) 8.7 2.1
assign (resid  57 and name hn ) (resid  57 and name n )
       (resid  57 and name ca ) (resid  57 and name ha ) 6.6 2.1
assign (resid  58 and name hn ) (resid  58 and name n )
       (resid  58 and name ca ) (resid  58 and name ha ) 7.3 2.1
assign (resid  59 and name hn ) (resid  59 and name n )
       (resid  59 and name ca ) (resid  59 and name ha ) 8.0 2.1
assign (resid  60 and name hn ) (resid  60 and name n )
       (resid  60 and name ca ) (resid  60 and name ha ) 5.6 2.1
assign (resid  63 and name hn ) (resid  63 and name n )
```

```
       (resid  63 and name ca ) (resid  63 and name ha ) 5.8 2.1
assign (resid  65 and name hn ) (resid  65 and name n )
       (resid  65 and name ca ) (resid  65 and name ha ) 6.6 2.1
assign (resid  66 and name hn ) (resid  66 and name n )
       (resid  66 and name ca ) (resid  66 and name ha ) 7.4 2.1
assign (resid  74 and name hn ) (resid  74 and name n )
       (resid  74 and name ca ) (resid  74 and name ha ) 6.0 2.1
assign (resid  77 and name hn ) (resid  77 and name n )
       (resid  77 and name ca ) (resid  77 and name ha ) 4.1 2.1
assign (resid  78 and name hn ) (resid  78 and name n )
       (resid  78 and name ca ) (resid  78 and name ha ) 9.0 2.1
assign (resid  80 and name hn ) (resid  80 and name n )
       (resid  80 and name ca ) (resid  80 and name ha ) 3.6 2.1
assign (resid  81 and name hn ) (resid  81 and name n )
       (resid  81 and name ca ) (resid  81 and name ha ) 6.4 2.1
assign (resid  82 and name hn ) (resid  82 and name n )
       (resid  82 and name ca ) (resid  82 and name ha ) 8.0 2.1
assign (resid  83 and name hn ) (resid  83 and name n )
       (resid  83 and name ca ) (resid  83 and name ha ) 7.5 2.1
assign (resid  84 and name hn ) (resid  84 and name n )
       (resid  84 and name ca ) (resid  84 and name ha ) 8.7 2.1
assign (resid  85 and name hn ) (resid  85 and name n )
       (resid  85 and name ca ) (resid  85 and name ha ) 8.4 2.1
assign (resid  86 and name hn ) (resid  86 and name n )
       (resid  86 and name ca ) (resid  86 and name ha ) 8.3 2.1
assign (resid  87 and name hn ) (resid  87 and name n )
       (resid  87 and name ca ) (resid  87 and name ha ) 9.1 2.1
assign (resid  88 and name hn ) (resid  88 and name n )
       (resid  88 and name ca ) (resid  88 and name ha ) 7.6 2.1
assign (resid  89 and name hn ) (resid  89 and name n )
       (resid  89 and name ca ) (resid  89 and name ha ) 3.1 2.1
assign (resid  95 and name hn ) (resid  95 and name n )
       (resid  95 and name ca ) (resid  95 and name ha ) 7.3 2.1
assign (resid 104 and name hn ) (resid 104 and name n )
       (resid 104 and name ca ) (resid 104 and name ha ) 5.3 2.1
assign (resid 105 and name hn ) (resid 105 and name n )
       (resid 105 and name ca ) (resid 105 and name ha ) 7.5 2.1
assign (resid 106 and name hn ) (resid 106 and name n )
       (resid 106 and name ca ) (resid 106 and name ha ) 6.0 2.1
assign (resid 107 and name hn ) (resid 107 and name n )
       (resid 107 and name ca ) (resid 107 and name ha ) 8.7 2.1
assign (resid 108 and name hn ) (resid 108 and name n )
       (resid 108 and name ca ) (resid 108 and name ha ) 8.7 2.1
assign (resid 109 and name hn ) (resid 109 and name n )
       (resid 109 and name ca ) (resid 109 and name ha ) 6.6 2.1
assign (resid 110 and name hn ) (resid 110 and name n )
       (resid 110 and name ca ) (resid 110 and name ha ) 6.2 2.1
assign (resid 111 and name hn ) (resid 111 and name n )
       (resid 111 and name ca ) (resid 111 and name ha ) 4.7 2.1
assign (resid 112 and name hn ) (resid 112 and name n )
       (resid 112 and name ca ) (resid 112 and name ha ) 8.6 2.1
assign (resid 113 and name hn ) (resid 113 and name n )
       (resid 113 and name ca ) (resid 113 and name ha ) 7.3 2.1


assign (resid  33 and name hn ) (resid  33 and name n )
       (resid  33 and name ca ) (resid  33 and name ha1 )
       (resid  33 and name hn ) (resid  33 and name n )
       (resid  33 and name ca ) (resid  33 and name ha2 ) 5.95 1.04 2.1 2.1
assign (resid  42 and name hn ) (resid  42 and name n )
       (resid  42 and name ca ) (resid  42 and name ha1 )
       (resid  42 and name hn ) (resid  42 and name n )
       (resid  42 and name ca ) (resid  42 and name ha2 ) 4.5 16.5 10 10
assign (resid  62 and name hn ) (resid  62 and name n )
       (resid  62 and name ca ) (resid  62 and name ha1 )
       (resid  62 and name hn ) (resid  62 and name n )
       (resid  62 and name ca ) (resid  62 and name ha2 ) 8.9 5.00 2.1 2.1
```

C.4.4 Dihedral angles

```
assign (resid 29 and name c) (resid 30 and name n) (resid 30 and name ca) (resid 30 and name c)  1.0 -65.0 20.0 2
assign (resid 30 and name c) (resid 31 and name n) (resid 31 and name ca) (resid 31 and name c)  1.0 -100.0 40.0 2
assign (resid 31 and name c) (resid 32 and name n) (resid 32 and name ca) (resid 32 and name c)  1.0 -65.0 20.0 2
assign (resid 32 and name c) (resid 33 and name n) (resid 33 and name ca) (resid 33 and name c)  1.0 80.0 20.0 2
assign (resid 33 and name c) (resid 34 and name n) (resid 34 and name ca) (resid 34 and name c)  1.0 -85.0 40.0 2
assign (resid 34 and name c) (resid 35 and name n) (resid 35 and name ca) (resid 35 and name c)  1.0 -100.0 40.0 2
assign (resid 35 and name c) (resid 36 and name n) (resid 36 and name ca) (resid 36 and name c)  1.0 -100.0 40.0 2
assign (resid 36 and name c) (resid 37 and name n) (resid 37 and name ca) (resid 37 and name c)  1.0 -100.0 40.0 2
assign (resid 37 and name c) (resid 38 and name n) (resid 38 and name ca) (resid 38 and name c)  1.0 -100.0 40.0 2
assign (resid 38 and name c) (resid 39 and name n) (resid 39 and name ca) (resid 39 and name c)  1.0 -100.0 40.0 2
assign (resid 39 and name c) (resid 40 and name n) (resid 40 and name ca) (resid 40 and name c)  1.0 -100.0 40.0 2
assign (resid 40 and name c) (resid 41 and name n) (resid 41 and name ca) (resid 41 and name c)  1.0 -100.0 40.0 2
assign (resid 41 and name c) (resid 42 and name n) (resid 42 and name ca) (resid 42 and name c)  1.0 -100.0 40.0 2
assign (resid 42 and name c) (resid 43 and name n) (resid 43 and name ca) (resid 43 and name c)  1.0 -100.0 40.0 2
assign (resid 43 and name c) (resid 44 and name n) (resid 44 and name ca) (resid 44 and name c)  1.0 -100.0 40.0 2
assign (resid 44 and name c) (resid 45 and name n) (resid 45 and name ca) (resid 45 and name c)  1.0 60.0 20.0 2
assign (resid 45 and name c) (resid 46 and name n) (resid 46 and name ca) (resid 46 and name c)  1.0 80.0 40.0 2
assign (resid 46 and name c) (resid 47 and name n) (resid 47 and name ca) (resid 47 and name c)  1.0 -100.0 40.0 2
assign (resid 47 and name c) (resid 48 and name n) (resid 48 and name ca) (resid 48 and name c)  1.0 -100.0 40.0 2
assign (resid 48 and name c) (resid 49 and name n) (resid 49 and name ca) (resid 49 and name c)  1.0 -100.0 40.0 2
assign (resid 49 and name c) (resid 50 and name n) (resid 50 and name ca) (resid 50 and name c)  1.0 -80.0 40.0 2
assign (resid 50 and name c) (resid 51 and name n) (resid 51 and name ca) (resid 51 and name c)  1.0 -65.0 40.0 2
assign (resid 51 and name c) (resid 52 and name n) (resid 52 and name ca) (resid 52 and name c)  1.0 -100.0 40.0 2
assign (resid 52 and name c) (resid 53 and name n) (resid 53 and name ca) (resid 53 and name c)  1.0 -100.0 40.0 2
assign (resid 53 and name c) (resid 54 and name n) (resid 54 and name ca) (resid 54 and name c)  1.0 -100.0 40.0 2
assign (resid 54 and name c) (resid 55 and name n) (resid 55 and name ca) (resid 55 and name c)  1.0 -100.0 40.0 2
assign (resid 55 and name c) (resid 56 and name n) (resid 56 and name ca) (resid 56 and name c)  1.0 -100.0 40.0 2
assign (resid 56 and name c) (resid 57 and name n) (resid 57 and name ca) (resid 57 and name c)  1.0 -100.0 40.0 2
assign (resid 57 and name c) (resid 58 and name n) (resid 58 and name ca) (resid 58 and name c)  1.0 -100.0 40.0 2
assign (resid 58 and name c) (resid 59 and name n) (resid 59 and name ca) (resid 59 and name c)  1.0 -100.0 40.0 2
assign (resid 59 and name c) (resid 60 and name n) (resid 60 and name ca) (resid 60 and name c)  1.0 -100.0 40.0 2
assign (resid 60 and name c) (resid 61 and name n) (resid 61 and name ca) (resid 61 and name c)  1.0 -65.0 40.0 2
assign (resid 61 and name c) (resid 62 and name n) (resid 62 and name ca) (resid 62 and name c)  1.0 120.0 20.0 2
assign (resid 62 and name c) (resid 63 and name n) (resid 63 and name ca) (resid 63 and name c)  1.0 -90.0 40.0 2
assign (resid 63 and name c) (resid 64 and name n) (resid 64 and name ca) (resid 64 and name c)  1.0 60.0 40.0 2
assign (resid 64 and name c) (resid 65 and name n) (resid 65 and name ca) (resid 65 and name c)  1.0 -90.0 40.0 2
assign (resid 65 and name c) (resid 66 and name n) (resid 66 and name ca) (resid 66 and name c)  1.0 -90.0 40.0 2
assign (resid 66 and name c) (resid 67 and name n) (resid 67 and name ca) (resid 67 and name c)  1.0 -65.0 20.0 2
assign (resid 67 and name c) (resid 68 and name n) (resid 68 and name ca) (resid 68 and name c)  1.0 -65.0 20.0 2
assign (resid 69 and name c) (resid 70 and name n) (resid 70 and name ca) (resid 70 and name c)  1.0 -65.0 20.0 2
assign (resid 70 and name c) (resid 71 and name n) (resid 71 and name ca) (resid 71 and name c)  1.0 -65.0 20.0 2
assign (resid 71 and name c) (resid 72 and name n) (resid 72 and name ca) (resid 72 and name c)  1.0 -65.0 20.0 2
assign (resid 74 and name c) (resid 75 and name n) (resid 75 and name ca) (resid 75 and name c)  1.0 -65.0 20.0 2
assign (resid 75 and name c) (resid 76 and name n) (resid 76 and name ca) (resid 76 and name c)  1.0 -65.0 20.0 2
assign (resid 76 and name c) (resid 77 and name n) (resid 77 and name ca) (resid 77 and name c)  1.0 -100.0 40.0 2
assign (resid 77 and name c) (resid 78 and name n) (resid 78 and name ca) (resid 78 and name c)  1.0 -100.0 40.0 2
assign (resid 78 and name c) (resid 79 and name n) (resid 79 and name ca) (resid 79 and name c)  1.0 -100.0 40.0 2
assign (resid 79 and name c) (resid 80 and name n) (resid 80 and name ca) (resid 80 and name c)  1.0 60.0 40.0 2
assign (resid 81 and name c) (resid 82 and name n) (resid 82 and name ca) (resid 82 and name c)  1.0 -100.0 20.0 2
assign (resid 82 and name c) (resid 83 and name n) (resid 83 and name ca) (resid 83 and name c)  1.0 -100.0 20.0 2
assign (resid 83 and name c) (resid 84 and name n) (resid 84 and name ca) (resid 84 and name c)  1.0 -100.0 20.0 2
assign (resid 84 and name c) (resid 85 and name n) (resid 85 and name ca) (resid 85 and name c)  1.0 -100.0 20.0 2
assign (resid 85 and name c) (resid 86 and name n) (resid 86 and name ca) (resid 86 and name c)  1.0 -100.0 20.0 2
assign (resid 86 and name c) (resid 87 and name n) (resid 87 and name ca) (resid 87 and name c)  1.0 -100.0 20.0 2
assign (resid 87 and name c) (resid 88 and name n) (resid 88 and name ca) (resid 88 and name c)  1.0 -100.0 20.0 2
assign (resid 90 and name c) (resid 91 and name n) (resid 91 and name ca) (resid 91 and name c)  1.0 -65.0 20.0 2
assign (resid 91 and name c) (resid 92 and name n) (resid 92 and name ca) (resid 92 and name c)  1.0 -65.0 20.0 2
assign (resid 92 and name c) (resid 93 and name n) (resid 93 and name ca) (resid 93 and name c)  1.0 -65.0 20.0 2
assign (resid 93 and name c) (resid 94 and name n) (resid 94 and name ca) (resid 94 and name c)  1.0 -65.0 20.0 2
assign (resid 94 and name c) (resid 95 and name n) (resid 95 and name ca) (resid 95 and name c)  1.0 -100.0 20.0 2
assign (resid 95 and name c) (resid 96 and name n) (resid 96 and name ca) (resid 96 and name c)  1.0 -65.0 20.0 2
assign (resid 96 and name c) (resid 97 and name n) (resid 97 and name ca) (resid 97 and name c)  1.0 -65.0 20.0 2
assign (resid 97 and name c) (resid 98 and name n) (resid 98 and name ca) (resid 98 and name c)  1.0 -100.0 40.0 2
assign (resid 110 and name c) (resid 111 and name n) (resid 111 and name ca) (resid 111 and name c)  1.0 -100.0 40.0 2
assign (resid 111 and name c) (resid 112 and name n) (resid 112 and name ca) (resid 112 and name c)  1.0 -100.0 40.0 2
assign (resid 112 and name c) (resid 113 and name n) (resid 113 and name ca) (resid 113 and name c)  1.0 -100.0 40.0 2
```

C.4.5 Residual dipolar couplings

C.4.5.1 Tag 3a loaded with Dy^{3+}

30	LEU	N	30	LEU	HN	3.900	1.000 1.00
31	ALA	N	31	ALA	HN	-3.400	1.000 1.00
32	ASN	N	32	ASN	HN	-3.900	1.000 1.00
33	GLY	N	33	GLY	HN	0.900	1.000 1.00
34	ASP	N	34	ASP	HN	-5.200	1.000 1.00
35	ILE	N	35	ILE	HN	3.900	1.000 1.00
36	ALA	N	36	ALA	HN	0.300	1.000 1.00
37	ILE	N	37	ILE	HN	0.500	1.000 1.00
38	ILE	N	38	ILE	HN	-4.700	1.000 1.00

```
 39    ASP      N    39    ASP     HN    -6.600     1.000 1.00
 40    PHE      N    40    PHE     HN    -5.800     1.000 1.00
 41    THR      N    41    THR     HN    -5.500     1.000 1.00
 42    GLY      N    42    GLY     HN    -5.600     1.000 1.00
 43    ILE      N    43    ILE     HN     0.900     1.000 1.00
 44    VAL      N    44    VAL     HN     2.200     1.000 1.00
 46    ASN      N    46    ASN     HN     4.100     1.000 1.00
 47    LYS      N    47    LYS     HN     4.800     1.000 1.00
 52    ALA      N    52    ALA     HN     5.600     1.000 1.00
 53    SER      N    53    SER     HN    -4.30     1.000 1.00
 54    ALA      N    54    ALA     HN    -6.600     1.000 1.00
 55    GLN      N    55    GLN     HN    -5.400     1.000 1.00
 56    ASN      N    56    ASN     HN    -5.200     1.000 1.00
 57    TYR      N    57    TYR     HN    -4.300     1.000 1.00
 58    GLU      N    58    GLU     HN    -4.600     1.000 1.00
 59    LEU      N    59    LEU     HN    -1.400     1.000 1.00
 60    THR      N    60    THR     HN    -2.900     1.000 1.00
 61    ILE      N    61    ILE     HN     0.200     1.000 1.00
 62    GLY      N    62    GLY     HN     3.400     1.000 1.00
 63    SER      N    63    SER     HN     5.100     1.000 1.00
 73    GLY      N    73    GLY     HN    -3.300     1.000 1.00
 74    LEU      N    74    LEU     HN    -0.400     1.000 1.00
 75    ILE      N    75    ILE     HN     0.300     1.000 1.00
 76    ALA      N    76    ALA     HN    -5.200     1.000 1.00
 77    MET      N    77    MET     HN    -1.700     1.000 1.00
 78    LYS      N    78    LYS     HN     0.400     1.000 1.00
 79    VAL      N    79    VAL     HN     2.700     1.000 1.00
 80    ASN      N    80    ASN     HN    -4.300     1.000 1.00
 82    LYS      N    82    LYS     HN    -3.000     1.000 1.00
 83    LYS      N    83    LYS     HN    -6.600     1.000 1.00
 86    ALA      N    86    ALA     HN     0.500     1.000 1.00
 89    PHE      N    89    PHE     HN     4.300     1.000 1.00
103    VAL      N   103    VAL     HN     6.200     1.000 1.00
104    THR      N   104    THR     HN     3.200     1.000 1.00
106    GLU      N   106    GLU     HN    -3.800     1.000 1.00
107    VAL      N   107    VAL     HN    -6.200     1.000 1.00
108    VAL      N   108    VAL     HN    -5.700     1.000 1.00
109    LEU      N   109    LEU     HN    -5.900     1.000 1.00
110    LYS      N   110    LYS     HN     0.900     1.000 1.00
111    ALA      N   111    ALA     HN     0.600     1.000 1.00
112    ILE      N   112    ILE     HN     0.400     1.000 1.00
113    LYS      N   113    LYS     HN     0.200     1.000 1.00
```

C.4.5.2 Tag 3b loaded with Dy^{3+}

```
 30    LEU      N    30    LEU     HN     7.930     1.000 1.00
 32    ASN      N    32    ASN     HN    -5.550     1.000 1.00
 36    ALA      N    36    ALA     HN    -3.960     1.000 1.00
 40    PHE      N    40    PHE     HN    -5.550     1.000 1.00
 41    THR      N    41    THR     HN    -4.360     1.000 1.00
 42    GLY      N    42    GLY     HN    -5.550     1.000 1.00
 44    VAL      N    44    VAL     HN    -6.730     1.000 1.00
 47    LYS      N    47    LYS     HN    -2.380     1.000 1.00
 48    LYS      N    48    LYS     HN     0.800     1.000 1.00
 49    LEU      N    49    LEU     HN    -0.400     1.000 1.00
 52    ALA      N    52    ALA     HN     5.940     1.000 1.00
 53    SER      N    53    SER     HN    -1.590     1.000 1.00
 55    GLN      N    55    GLN     HN     -3.170     1.000 1.00
 56    ASN      N    56    ASN     HN     3.170     1.000 1.00
 57    TYR      N    57    TYR     HN     1.590     1.000 1.00
 58    GLU      N    58    GLU     HN    -1.580     1.000 1.00
 59    LEU      N    59    LEU     HN     0.400     1.000 1.00
 60    THR      N    60    THR     HN     0.000     1.000 1.00
 61    ILE      N    61    ILE     HN    -1.190     1.000 1.00
 73    GLY      N    73    GLY     HN    0.800     1.000 1.00
 75    ILE      N    75    ILE     HN    -3.170     1.000 1.00
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```
 76    ALA      N    76    ALA     HN    -3.170     1.000 1.00
 77    MET      N    77    MET     HN     1.590     1.000 1.00
 78    LYS      N    78    LYS     HN     6.340     1.000 1.00
 79    VAL      N    79    VAL     HN     5.950     1.000 1.00
 80    ASN      N    80    ASN     HN     0.000     1.000 1.00
 84    THR      N    84    THR     HN    -3.170     1.000 1.00
 85    LEU      N    85    LEU     HN    -5.940     1.000 1.00
 86    ALA      N    86    ALA     HN    -0.790     1.000 1.00
 93    VAL      N    93    VAL     HN    -3.170     1.000 1.00
 95    VAL      N    95    VAL     HN    -0.800     1.000 1.00
 96    LYS      N    96    LYS     HN    -3.960     1.000 1.00
 98    LEU      N    98    LEU     HN    -1.980     1.000 1.00
105    PHE      N   105    PHE     HN    -5.150     1.000 1.00
107    VAL      N   107    VAL     HN    -6.340     1.000 1.00
109    LEU      N   109    LEU     HN    -1.980     1.000 1.00
110    LYS      N   110    LYS     HN    -1.580     1.000 1.00
111    ALA      N   111    ALA     HN    -2.780     1.000 1.00
112    ILE      N   112    ILE     HN    -1.580     1.000 1.00
113    LYS      N   113    LYS     HN    -3.960     1.000 1.00
```

C.4.5.3 Tag 6a loaded with Dy^{3+}

```
 30    LEU      N    30    LEU     HN    -3.140     1.000 1.00
 32    ASN      N    32    ASN     HN     5.700     1.000 1.00
 33    GLY      N    33    GLY     HN    -3.560     1.000 1.00
 34    ASP      N    34    ASP     HN     8.160     1.000 1.00
 35    ILE      N    35    ILE     HN    -4.110     1.000 1.00
 36    ALA      N    36    ALA     HN    -3.940     1.000 1.00
 38    ILE      N    38    ILE     HN    -0.240     1.000 1.00
 39    ASP      N    39    ASP     HN     5.180     1.000 1.00
 40    PHE      N    40    PHE     HN     6.770     1.000 1.00
 41    THR      N    41    THR     HN     4.680     1.000 1.00
 42    GLY      N    42    GLY     HN     7.890     1.000 1.00
 43    ILE      N    43    ILE     HN     1.020     1.000 1.00
 44    VAL      N    44    VAL     HN     1.860     1.000 1.00
 45    ASP      N    45    ASP     HN     0.750     1.000 1.00
 46    ASN      N    46    ASN     HN    -2.420     1.000 1.00
 47    LYS      N    47    LYS     HN    -3.880     1.000 1.00
 48    LYS      N    48    LYS     HN    -3.690     1.000 1.00
 49    LEU      N    49    LEU     HN     1.640     1.000 1.00
 52    ALA      N    52    ALA     HN    -5.840     1.000 1.00
 53    SER      N    53    SER     HN     4.740     1.000 1.00
 55    GLN      N    55    GLN     HN     4.560     1.000 1.00
 56    ASN      N    56    ASN     HN     3.870     1.000 1.00
 57    TYR      N    57    TYR     HN     4.600     1.000 1.00
 58    GLU      N    58    GLU     HN     4.200     1.000 1.00
 59    LEU      N    59    LEU     HN    -3.390     1.000 1.00
 60    THR      N    60    THR     HN    -1.830     1.000 1.00
 61    ILE      N    61    ILE     HN    -1.120     1.000 1.00
 62    GLY      N    62    GLY     HN    -3.670     1.000 1.00
 63    SER      N    63    SER     HN    -4.670     1.000 1.00
 65    SER      N    65    SER     HN    -4.120     1.000 1.00
 71    GLU      N    71    GLU     HN     1.350     1.000 1.00
 74    LEU      N    74    LEU     HN    -2.8300     1.000 1.00
 75    ILE      N    75    ILE     HN    -1.820     1.000 1.00
 76    ALA      N    76    ALA     HN     3.490     1.000 1.00
 77    MET      N    77    MET     HN    -2.010     1.000 1.00
 78    LYS      N    78    LYS     HN     0.160     1.000 1.00
 79    VAL      N    79    VAL     HN    -2.670     1.000 1.00
 80    ASN      N    80    ASN     HN    -0.740     1.000 1.00
 83    LYS      N    83    LYS     HN     6.360     1.000 1.00
 84    THR      N    84    THR     HN     4.640     1.000 1.00
 85    LEU      N    85    LEU     HN     5.890     1.000 1.00
 96    LYS      N    96    LYS     HN     8.530     1.000 1.00
 97    ILE      N    97    ILE     HN     7.800     1.000 1.00
 98    LEU      N    98    LEU     HN     3.560     1.000 1.00
```

105	PHE	N	105	PHE	HN	2.520	1.000 1.00
106	GLU	N	106	GLU	HN	3.930	1.000 1.00
107	VAL	N	107	VAL	HN	8.260	1.000 1.00
108	VAL	N	108	VAL	HN	5.350	1.000 1.00
109	LEU	N	109	LEU	HN	4.620	1.000 1.00
110	LYS	N	110	LYS	HN	-3.280	1.000 1.00
111	ALA	N	111	ALA	HN	-3.200	1.000 1.00
112	ILE	N	112	ILE	HN	-3.350	1.000 1.00
113	LYS	N	113	LYS	HN	-1.660	1.000 1.00

C.4.5.4 Tag 6b loaded with Dy^{3+}

30	LEU	N	30	LEU	HN	-4.250	1.000 1.00
31	ALA	N	31	ALA	HN	0.000	1.000 1.00
32	ASN	N	32	ASN	HN	6.360	1.000 1.00
33	GLY	N	33	GLY	HN	-2.100	1.000 1.00
35	ILE	N	35	ILE	HN	-0.590	1.000 1.00
36	ALA	N	36	ALA	HN	-3.220	1.000 1.00
38	ILE	N	38	ILE	HN	0.120	1.000 1.00
39	ASP	N	39	ASP	HN	4.830	1.000 1.00
40	PHE	N	40	PHE	HN	6.490	1.000 1.00
41	THR	N	41	THR	HN	5.480	1.000 1.00
42	GLY	N	42	GLY	HN	7.580	1.000 1.00
43	ILE	N	43	ILE	HN	0.800	1.000 1.00
44	VAL	N	44	VAL	HN	0.050	1.000 1.00
45	ASP	N	45	ASP	HN	-1.000	1.000 1.00
46	ASN	N	46	ASN	HN	-2.150	1.000 1.00
47	LYS	N	47	LYS	HN	-5.350	1.000 1.00
48	LYS	N	48	LYS	HN	-5.610	1.000 1.00
49	LEU	N	49	LEU	HN	0.820	1.000 1.00
52	ALA	N	52	ALA	HN	-5.020	1.000 1.00
53	SER	N	53	SER	HN	4.300	1.000 1.00
55	GLN	N	55	GLN	HN	4.650	1.000 1.00
56	ASN	N	56	ASN	HN	3.870	1.000 1.00
57	TYR	N	57	TYR	HN	4.820	1.000 1.00
58	GLU	N	58	GLU	HN	4.450	1.000 1.00
59	LEU	N	59	LEU	HN	-1.900	1.000 1.00
60	THR	N	60	THR	HN	-0.590	1.000 1.00
61	ILE	N	61	ILE	HN	-2.030	1.000 1.00
62	GLY	N	62	GLY	HN	-3.480	1.000 1.00
63	SER	N	63	SER	HN	-5.940	1.000 1.00
65	SER	N	65	SER	HN	-1.830	1.000 1.00
73	GLY	N	73	GLY	HN	-1.130	1.000 1.00
74	LEU	N	74	LEU	HN	-2.4200	1.000 1.00
75	ILE	N	75	ILE	HN	-1.580	1.000 1.00
76	ALA	N	76	ALA	HN	3.180	1.000 1.00
77	MET	N	77	MET	HN	-2.030	1.000 1.00
78	LYS	N	78	LYS	HN	0.200	1.000 1.00
79	VAL	N	79	VAL	HN	-2.700	1.000 1.00
80	ASN	N	80	ASN	HN	0.050	1.000 1.00
83	LYS	N	83	LYS	HN	6.610	1.000 1.00
84	THR	N	84	THR	HN	5.670	1.000 1.00
85	LEU	N	85	LEU	HN	4.620	1.000 1.00
96	LYS	N	96	LYS	HN	6.930	1.000 1.00
97	ILE	N	97	ILE	HN	7.860	1.000 1.00
98	LEU	N	98	LEU	HN	3.110	1.000 1.00
99	GLN	N	99	GLN	HN	1.750	1.000 1.00
105	PHE	N	105	PHE	HN	3.810	1.000 1.00
106	GLU	N	106	GLU	HN	3.680	1.000 1.00
107	VAL	N	107	VAL	HN	7.780	1.000 1.00
108	VAL	N	108	VAL	HN	4.700	1.000 1.00
109	LEU	N	109	LEU	HN	4.720	1.000 1.00
110	LYS	N	110	LYS	HN	-2.010	1.000 1.00
111	ALA	N	111	ALA	HN	-2.350	1.000 1.00
112	ILE	N	112	ILE	HN	-2.630	1.000 1.00
113	LYS	N	113	LYS	HN	-1.710	1.000 1.00

—♦—

D

Appendix: Structure calculation input files

D.1 SCULPTOR input file for hormaomycin

```
        set dsl output off
        reduce
        overlap =      0.0
        igrpck  =     -1
        itrap   =      1
        dseed   =    1707
!
        begin templ="mon.car"
     *    add-automatic bond torsion valence out-of-plane
!
      fkchir = 100
      set dielectric= 46.7*r
!
!
        method = 2
        nocrash
!        inertia
        deltgrad   =    0.00001
        ormass     =     1000.0
        eigvmass   =    10000.0
!
        gh         =  267669000
        gn         =  -27125000
        gc         =   67302700
        csaval     =   170.0
        rnhcst
        rnh0       =    1.04
        rchcst
        rch0       =    1.08
        rcncst
        rcn0       =    1.33
        rcccst
        rcc0       =    1.53
!
        damin      =    -0.0
        dascale    =     20.0
        dbmin      =    -0.0
        dbscale    =     20.0
        order
        noeigvrst
        daamin     =     0.00
```

```
        daamax     =    30.00
        daascale   =   100000
        dramin     =     1.00
        dramax     =    20.00
        drascale   =   100000
        dabmin     =    12.50
        dabmax     =    14.50
        dabscale   =   100000
        drbmin     =     6.40
        drbmax     =     7.20
        drbscale   =   100000
!
      assign the torsion name T1 to
     * molecule 19 atom C70, atom C39, atom C37, atom H34
!
      assign the torsion name I1 to
     * molecule 19 atom O22, atom C19, atom C18, atom H28
!
      assign the torsion name P1 to
     * molecule 19 atom H29, atom C24, atom C51, atom H51
!
      assign the torsion name P2 to
     * molecule 19 atom H33, atom C32, atom C62, atom H57
!
      assign the torsion name I2 to
     * molecule 19 atom H28, atom C18, atom C47, atom H42
!
      assign the torsion name T2 to
     * molecule 19 atom H34, atom C37, atom N71, atom H19
!
!       Main loop to generate multiple structures
!
        nstructures  =   1000
        xstructures  =  1
        ystructure   =   2
        pframe       =   1
        set output filename  number pstructure
        set orput filename  number pstructure
stlp    continue
        pstructure   =   1
!
        intermolecular interaction is off among
     *  molecule 1 to 19
!
        iframe       =   1
        read coordinate="mon.car"
!

!
!       Phase 1 -- Minimization
!
stly    continue
!
        fact = 0.950
!
        constrain nothing
!
!
        set nonbond normal
!
!
!
!        cutoff              =     6.0000000
!        cutdis              =     5.00000000
!        swtdis              =      1.500000
!
!
```

```
!
!
        cutoff              =    7.5000000
        cutdis              =     6.000000
        swtdis              =     1.500000
!
!
        fix nothing
!
!
!
!
!
        fix residues
     *  molecule  1
     *  molecule  2
     *  molecule  3
     *  molecule  4
     *  molecule  5
     *  molecule  6
     *  molecule  7
     *  molecule  8
     *  molecule  9
     *  molecule 10
     *  molecule 11
     *  molecule 12
     *  molecule 13
     *  molecule 14
     *  molecule 15
     *  molecule 16
     *  molecule 17
     *  molecule 18
!
!
!
        checkor
pror
!
!
!
        constrain nothing
!
!
!
!
        scale nothing
        fkchir              =        1.0
        scale bond            by     1.0
!        scale 1-4             by     0.5
        scale theta           by     1.0
        scale phi             by     1.0
        scale out-of-plane    by     1.0
        cscale              =        1.0
        dscale              =        1.0
        fscale              =        1.0
        scale   nonbond term  by     1.0
        scale_noe           =        0.0
        fkchir  =  fkchir   *       100.0
        scale_dnh1 =                 0.10
        scale_dch1 =                 0.10
        scale_dcc1 =                 0.10
        scale_dcn1 =                 0.10
        scale_dnh2 =                 0.10
        scale_dch2 =                 0.100
        scale_dcc2 =                 0.100
        scale_dcn2 =                 0.100
!
!
```

```
!
pror
        checkor
!
!
        minimize using steepest descents
     *    no morse
     *    no cross
     *      for 300 cycles
     *      until the maximum derivative is less than 0.0100
!
!
        minimize using conjugate gradients
     *    no morse
     *    no cross
     *      for 300 cycles
     *      until the maximum derivative is less than 0.0100
!
!
!
!
!       Phase 2 -- Minimization
!
!
!
        temp = 1000.0000
        demax               = 80000000000.000000
!
!
        initialize dynamics at temp  K for 1000 steps of 1.0 fs
     *    no morse
     *    no cross
     *      write averages every 1000 steps
     *      write history every 1000  steps
        print energy summary
        checkor
!
        iframe = 1
!
loo2    resume dynamics at temp  K for 1000 steps of 1.0 fs
     *    no morse
     *    no cross
        if status .ne. 0.0 then nexx
        scale_dnh1 = scale_dnh1 * 1.0798
        scale_dch1 = scale_dch1 * 1.0798
        scale_dcc1 = scale_dcc1 * 1.0798
        scale_dcn1 = scale_dcn1 * 1.0798
        scale_dnh2 = scale_dnh2 * 1.0798
        scale_dch2 = scale_dch2 * 1.0798
        scale_dcc2 = scale_dcc2 * 1.0798
        scale_dcn2 = scale_dcn2 * 1.0798
!
! scale nonbond term    by     1.2589254
!
        iframe = iframe + 1
!
        print energy summary
        checkor
        if iframe .le. 30 then loo2
!
!
!
        scale_dnh1 = 1.0
        scale_dch1 = 1.00
        scale_dcc1 = 1.00
        scale_dcn1 = 1.0
        scale_dnh2 = 1.00
        scale_dch2 = 1.00
```

```
        scale_dcc2 = 1.00
        scale_dcn2 = 1.00
!
       resume dynamics at temp  K for 1000 steps of 1.0 fs
     *    no morse
     *    no cross
        if status .ne. 0.0 then nexx
!
!
desc    resume dynamics at temp  K for  800 steps of 1.0 fs
     *    no morse
     *    no cross
        if status .ne. 0.0 then nexx
        print energy summary
        temp = fact*temp
        if temp .gt. 100 then desc
!
!
!
        minimize using steepest descents
     *    no morse
     *    no cross
     *      for 300 cycles
     *      until the maximum derivative is less than 0.0100
!
!
        minimize using conjugate gradients
     *    no morse
     *    no cross
     *      for 1000 cycles
     *      until the maximum derivative is less than 0.0100
!
!
!
!
        print noe_dist  violations exceeding  0.100000
print distance violations
print dihedral violations
!
!
        archive as file number pframe
pframe = pframe + 1
!
        fix nothing
!
!
!
        fix residues
     *  molecule  1
     *  molecule  2
     *  molecule  3
     *  molecule  4
     *  molecule 11
     *  molecule 12
     *  molecule 13
     *  molecule 14
     *  molecule 15
     *  molecule 16
     *  molecule 17
     *  molecule 18
!
        fixed atom list generation
     *  add all
     *  molecule  5 residue DIPA 1 atom O1
     *  add all
     *  molecule  6 residue DAA  1 atom A1
     *  add all
     *  molecule  7  residue DRA  1 atom A1
```

```
     *  add all
     *  molecule  8 residue DIPB 1 atom O1
     *  add all
     *  molecule  9 residue DAB  1 atom A1
     *  add all
     *  molecule  10 residue DRB  1 atom A1
!
!
        checkor
pror
!
!
!
        constrain nothing
        constrain using "alldist.rstrnt"
!
        print noe_dist  violations exceeding  0.100000
print distance violations
print dihedral violations
!
!
        scale nothing
        fkchir               =        1.0
        scale bond             by     1.0
!        scale 1-4              by     0.5
        scale theta            by     1.0
        scale phi              by     1.0
        scale out-of-plane     by     1.0
        cscale               =        0.010
        dscale               =        0.010
        fscale               =        0.010
        scale   nonbond term   by     1.0
        scale_noe            =        0.0
        fkchir  =  fkchir    *       100.0
        scale_dnh1 =                  0.10
        scale_dch1 =                  0.10
        scale_dcc1 =                  0.10
        scale_dcn1 =                  0.10
        scale_dnh2 =                  0.100
        scale_dch2 =                  0.100
        scale_dcc2 =                  0.100
        scale_dcn2 =                  0.100
!
!
!
pror
        checkor
!
!
        minimize using steepest descents
     *    no morse
     *    no cross
     *      for 300 cycles
     *      until the maximum derivative is less than 0.0100
!
!
        minimize using conjugate gradients
     *    no morse
     *    no cross
     *      for 300 cycles
     *      until the maximum derivative is less than 0.0100
!
!
!
!
!       Phase 2 -- Minimization
!
!
```

```
!
        temp = 1000.0000
        demax               = 80000000000.000000
!
!
        initialize dynamics at temp  K for 5000 steps of 1.0 fs
     *    no morse
     *    no cross
     *      write averages every 1000 steps
     *      write history every 1000  steps
        print energy summary
        checkor
!
        iframe = 1
!
loo1    resume dynamics at temp  K for 2500 steps of 1.0 fs
     *    no morse
     *    no cross
        if status .ne. 0.0 then nexx
cscale = cscale * 1.166
fscale = fscale * 1.166
dscale = dscale * 1.166
        scale_dnh1 = scale_dnh1 * 1.0798
        scale_dch1 = scale_dch1 * 1.0798
        scale_dcc1 = scale_dcc1 * 1.0798
        scale_dcn1 = scale_dcn1 * 1.0798
        scale_dnh2 = scale_dnh2 * 1.0798
        scale_dch2 = scale_dch2 * 1.0798
        scale_dcc2 = scale_dcc2 * 1.0798
        scale_dcn2 = scale_dcn2 * 1.0798
!
! scale nonbond term    by     1.2589254
!
        iframe = iframe + 1
!
        print energy summary
        checkor
        if iframe .le. 30 then loo1
!
!
!
        scale_dnh1 = 0.0
        scale_dch1 = 1.00
        scale_dcc1 = 1.00
        scale_dcn1 = 1.0
        scale_dnh2 = 0.00
        scale_dch2 = 1.00
        scale_dcc2 = 1.00
        scale_dcn2 = 1.00
cscale = 1.0
fscale = 1.0
dscale = 1.0
!
       resume dynamics at temp  K for 5000 steps of 1.0 fs
     *    no morse
     *    no cross
        if status .ne. 0.0 then nexx
!
!
des3    resume dynamics at temp  K for  800 steps of 1.0 fs
     *    no morse
     *    no cross
        if status .ne. 0.0 then nexx
        print energy summary
        temp = fact*temp
        if temp .gt. 100 then des3
!
!
```

```
!
        minimize using steepest descents
     *    no morse
     *    no cross
     *      for 300 cycles
     *      until the maximum derivative is less than 0.0100
!
!
        minimize using conjugate gradients
     *    no morse
     *    no cross
     *      for 1000 cycles
     *      until the maximum derivative is less than 0.0100
!
!
!
        print noe_dist  violations exceeding  0.100000
print distance violations
print dihedral violations
!
!
!
!
        pror
!
!        checkor
!
        savor
        archive as file number pframe
pframe = pframe + 1
nexx    continue
!
        scale nothing
        fkchir               =        1.0
        scale bond             by     1.0
!        scale 1-4              by     0.5
        scale theta            by     1.0
        scale phi              by     1.0
        scale out-of-plane     by     1.0
        cscale               =        1.0
        dscale               =        1.0
        fscale               =        1.0
        scale   nonbond term   by     1.0
        scale_noe            =        0.0
        fkchir  =  fkchir    *       100.0
        scale_dnh1 =                  1.00
        scale_dch1 =                  1.00
        scale_dcc1 =                  1.00
        scale_dcn1 =                  1.00
        scale_dnh2 =                  1.00
        scale_dch2 =                  1.00
        scale_dcc2 =                  1.00
        scale_dcn2 =                  1.00
        print energy summary
!
!
!
        pstructure   = pstructure + 1
        if pstructure .lt. ystructures then stly
!
!
!
        xstructure   = xstructure + 1
        if xstructure .lt. nstructures then stlp
!
!
        end
```

D.2 XPLOR-NIH input file for hymenistatin

```
!******************************************************************************
!   SA.INP

! First round simulated annealing to include NOE, dihedral and direct
! coupling restraints (optional). Written by MC 2001 (After Nilges, see below).
!
! Hbonds defined with a bond length and angle via a patch
!
! RDCs added 2003. See psfgen.inp for notes on seting up for RDCs.


!           ++++++++ Notes on input structures +++++++++

! The following are the remarks from the original version from Nilges.

! Simulated annealing protocol for NMR structure determination.
! The ing structure for this protocol can be any structure
! with a reasonable geometry, such as randomly assigned torsion
! angles or extended strands.

! Author: Michael Nilges
!
! Please cite the following papers when using this protocol:
!
! 1. Nilges, M., Clore, G.M., and Gronenborn, A.M.  (1988).
! Determination of three-dimensional structures of proteins from
! interproton distance data by dynamical simulated annealing
! from a random array of atoms,  FEBS Lett. 239, 129-136.
! 2. Nilges, M., Kuszewski, J., and Brunger, A.T. (1991).
! In: Computational Aspects of the Study of Biological
! Macromolecules by NMR, (J.C. Hoch, ed.).  New York: Plenum Press.
!

! A "regularised random structure" can be used as an input structures
! such as that derived from generate_.inp. Otherwise a structure
! from a previous run can be used. What works well is the regularised
! average structure from a previous structure family.
! Well defined structures such as a crystal structure or a homology
! model can of course also be used.
!
!++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++




!============================ Restraint Potentials ==================================


!======================= Run options and set-up ================================
!
! The following section selects which restraint potentials are to be used.
! The NOE and dihedral restrain potential are always used. The proton and
! carbon potentials and ramachandran potentials are not used here. Direct
! J-coupling and RDC refinement are optional.

! Turn this on for direct coupling-constant refinement.
!

evaluate ($couplings = 1.0)

! Turn this on for residual dipolar coupling refinement.
!
evaluate ($rdcs = 1.0)
```

```
!================== Force constants and floating chiralities ==================================

! The improper maintaining peptide bond planarity is set to 500 Kcal/radˆ2
! to avoid cis-trans flipping of the peptide bonds at high temps.  Note that
! the real value is 8-10 Kcal/radˆ2!!  In later protocols this value is lowered
!

evaluate ($kpept=500)


! The force constants on Hbond length and angles. These should generally
! not be changed. Values of 50, 12 can be used for initial structures, but
! 14, 4 is recommended for refined structures.
!
evaluate ($khb_bond=14)  ! force constant on Hbond length
evaluate ($khb_angl=4) ! force constant on Hbond angle


! Uses optional floating chirality. Set $float = 1 for general floating chirality
! for all metylene groups.  Otherwise there is specific floating chirality
! for glycine and leucine which can be set with a patch.

evaluate ($float = 0.0)

! Floating proline cis-pro isomerisation can be used. Set $cispro = 1.0
! for floating isomerism, then define a CIPP patch for all prolines which
! should float. Otherwise, if $cispro = 0.0, all prolines will stay trans
! except for those patched with CIPP, which will remain cis.

evaluate ($cispro = 0.0)


!======================= Input and Output Files ======================================
!
! Next the name and number of the output structure family
!
! evaluate ($directory = "./pph_dmso/all_rdc/")  !! NOTE: "./" is allowed
! evaluate ($outfamily = "pphall")
! evaluate ($endcount= 20 )


!============================= Structure ======================================
!
! Next is the molecular structure file (from psfgen.inp)

structure
                @/md3/peha/hymenistatin/xplor/new/MAIN/parameters/Hymenistatin.psf
                @/md3/peha/hymenistatin/xplor/new/MAIN/parameters/axis_new_500.psf
              !   @axis_new_600.psf
                end

!================================ Run Set-up ====================================

!
! A seed for the random number generator can be set. This will change
! the randomisation of atom velocities.
!
set seed=7478978723259 end

!
! The following are the conditions for the molecular dynamics steps
! The defaults work well for most proteins
!
! Temperature for high-temp dynamics and initial SA temperature.
!
evaluate ($init_t = 2000 )
```

```
! Number of steps in high temp dymanics

evaluate ($high_100steps= 4000 )

! Total number of steps, the final temperature and the
! temperature step in the SA cooling.

evaluate ($cool_steps = 4000 )

    evaluate ($final_t = 100)      { K }
    evaluate ($tempstep = 50)      { K }

! Number of steps in final minimisation

evaluate ($minimise_steps = 2000 )

!============================== Parameters ==================================
!

! The parameters. These are from the general SA (or DG) set, modified
! with a few fixes. This script will communicate with these files (for
! the floating chirality and peptide bond force constant etc.) and other
! parameter files will probably not work.

! The extra parameters for Hbond patches are read first, then the
! general parameters.

evaluate ($kmene = 25)
parameter
!     @XP:/library/hbond.pro
!     @XP:/library/parallhdg.pro
    @/md3/peha/hymenistatin/xplor/new/MAIN/parameters/parallhdg_vf.pro
    @/md3/peha/hymenistatin/xplor/new/MAIN/parameters/par_axis.pro !peha
end

!============================== Patches ==================================
!
! All the extra patches for floating proline pucker, Hbonds etc. are in
! this file.


topology
! peha     @XP:/library/extra_patches.pro
end

! Insert all patches for floating proline pucker, proline isomerism
! here.

! Apply floating pucker for all prolines

! patch fpro reference=1=( residue 5   ) end
! patch fpro reference=1=( residue 10  ) end

!       patches specifying cis peptide bond 9-10

! patch cipp reference=+=( resid 10 ) reference=-=( resid 9 ) end
! patch cipp reference=+=( resid 5  ) reference=-=( resid 4 ) end

!        patch LTOD reference=NIL=( residue 4 ) end
!        patch LTOD reference=NIL=( residue 9 ) end

!        patch PEPT reference=-=( residue 10 ) reference=+=( residue 1 ) end



!!============================== H-Bonds ==================================

! Read the Hbond patches
```

```
! @Restraints/HBonds.tbl

!!============================ Restraints ==================================

!     ++++++++ NOE Restraints ++++++++++


        set message on echo on end

noe

! Estimate greater than the actual number of NOEs.

nres=10000

! Read NOE restraint files

class int     @/md3/peha/hymenistatin/xplor/new/MAIN/restraints/DMSO_NOE_peha_all.tbl


!  Averaging and Scales for each class. Averaging has to be consistent
! with the choice of psuedo-atom.

averaging int sum

scale int 0.0


! Parameters for NOE effective energy term (should not need changing).

ceiling=1000
potential  * soft
sqoffset   * 0.0
sqconstant * 1.0
sqexponent * 2
soexponent * 1
asymptote  * 0.1     ! Initial value, modified later.
rswitch    * 0.5
end



!     ++++++++ Dihedral Restraints ++++++++++

! Read the dihedral restraint files

restraints dihedral
             reset
             nass = 100
             scale = 0
    @/md3/peha/hymenistatin/xplor/new/MAIN/restraints/DMSO_dihe.tbl
end


!     ++++++++ J-Coupling restraints ++++++++++

! Only used if $couplings = 1. Use the Karplus coefficients of
! your choice. Note that gly restraints are given separately.

if($couplings=1.0) then

couplings
  nrestraints = 300
  potential harmonic

  class phi
  degen 1
```

```
  force 1.0
  coefficients 6.98 -1.38 1.72 -60.0
 ! @j_nh_ca.tbl

  class gly
  degen 1
  force 1.0
  coefficients 6.98 -1.38 1.72 60.0
!  @@j3hnha_gly.tbl
end

end if

 if($rdcs=1.0) then


   sani
     reset
     nres=1200

           class CH_a
potential harmonic
force 0.000
coefficients 0.00 13.2 0.36
          @/md3/peha/hymenistatin/xplor/new/MAIN/restraints/DMSO_PPH_rdc_oNH_500.tbl


           class CH_b
potential harmonic
force 0.000
coefficients 0.00 6.6 0.36
@/md3/peha/hymenistatin/xplor/new/MAIN/restraints/DMSO_PPH_rdc_NH_500.tbl


!           class CH_g
! potential harmonic
! force 1.000
! coefficients 0.00 -16.8 0.54
! @@DipolCoup/xp_rdc_cghg_jk.txt


!      class CH_d
! potential harmonic
! force 1.000
! coefficients 0.00 -18.0 0.55
! @@DipolCoup/xp_rdc_cdhd_jk.txt


!      class C_am
! potential harmonic
! force 1.000
! coefficients 0.00 -18.0 0.55
! @@DipolCoup/xp_rdc_camethyl_jk.txt

!      class C_gm
! potential harmonic
! force 1.000
! coefficients 0.00 -18 0.55
! @@DipolCoup/xp_rdc_cgmethyl_jk.txt

!      class C_dm
! potential harmonic
! force 1.000
! coefficients 0.00 -18 0.55
! @@DipolCoup/xp_rdc_cdmethyl_jk.txt

    end
end if
```

```
!========================== Disulphide Bridges ============================

! Disulphide bridges are eplaced by psuedo-noes. There is a
! constraints interaction statement below which turns off the
! S-S bond interaction. The bridges should be defined in the psf file
! (see psfgen.inp).

!       This distance restraint is used in place of the cyclising peptide bond
!    during dynamics.

noe
  class dis
!peha     assign(resid 1 and name N)(resid 10 and name C) 1.43 0.1 0.1
  averaging dis center
  scale dis 10
end

!++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++

! Nothing below this line should need to be changed

!++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++


flags exclude * include bonds angle impr vdw  cdih end

if ($couplings = 1.0 ) then
  flags include coup end
end if

if ($rdcs = 1.0 ) then
  flags include sani end
end if

! Friction coefficient for MD heatbath, in 1/ps.

vector do (fbeta=10) (all)

! Uniform heavy masses to speed molecular dynamics.*}

vector do (mass=100) (all)

vector do (vx=maxwell($init_t)) (all)
vector do (vy=maxwell($init_t)) (all)
vector do (vz=maxwell($init_t)) (all)


! Parameters for the repulsive energy term.

parameter
         nbonds
repel=1.                    ! Initial value for repel, modified later.
rexp=2 irexp=2 rcon=1.
nbxmod=3
wmin=0.01
cutnb=4.5 ctonnb=2.99 ctofnb=3.
tolerance=0.5
   end
end

!============================================================================

! Read the  structure and copy the coordinates into the buffer.

coor @/md3/peha/hymenistatin/xplor/new/MAIN/dmso_noe/start_structures/start100.pdb  ! peha end
```

```
coor @/md3/peha/hymenistatin/xplor/new/MAIN/parameters/axis500.pdb       ! peha end
!coor @axis600.pdb       ! peha end

coor copy end

set message on echo on end

! evaluate ($count = 0)
! while ($count < $endcount ) loop main

! evaluate ($count=$count+1)



! Setup the names for the violations files


! Swap the coordinates from the buffer to the main coordinate set, then
! copy them back to the buffer. This means the  structure coordinates
! are reloaded for each run.

    coor swap end
    coor copy end

if($rdcs = 1.0) then
    constraints
fix=(resname ANI)
    end
end if



!====================== High-temperature dynamics ========================

flags exclude * include bonds angle impr vdw cdih noe sani end

constraints
         interaction (all) (all) weights * 1 vdw 0.002 end
         interaction (resid 500) (not resid 500) weights * 1  vdw 0.002 end
         interaction (resid 600) (not resid 600) weights * 1  vdw 0.002 end
         interaction (resid 600) (resid 500) weights * 0 end
end   !peha
noe
scale int 0
end


! The dynamics are divided into two parts of two-thirds one-third
  evaluate ($ncycle = 100)
  evaluate ($ini_sani = 0.01)
  evaluate ($fin_sani = 1.0)
  evaluate ($sani_fac = ($fin_sani/$ini_sani)^(1/$ncycle))
  evaluate ($ksani = $ini_sani)
  evaluate ($ini_sani1 = 0.01)
  evaluate ($fin_sani1 = 0.5)
  evaluate ($sani1_fac = ($fin_sani1/$ini_sani1)^(1/$ncycle))
  evaluate ($ksani1 = $ini_sani1)
  evaluate ($ini_noe = 1.)
  evaluate ($fin_noe = 50.)
  evaluate ($noe_fac = ($fin_noe/$ini_noe)^(1./$ncycle))
  evaluate ($knoe = $ini_noe)
  evaluate ($ini_dihe = 5.)
  evaluate ($fin_dihe = 200.)
  evaluate ($dihe_fac = ($fin_dihe/$ini_dihe)^(1./$ncycle))
  evaluate ($kdihe = $ini_dihe)

        evaluate ($i_cool = 0)
        while ($i_cool < $ncycle) loop hot2
```

```
        evaluate ($i_cool=$i_cool+1)

        evaluate ($ksani=$ksani*$sani_fac)
        evaluate ($ksani1=$ksani1*$sani1_fac)
        evaluate ($knoe=$knoe*$noe_fac)
        evaluate ($kdihe=$kdihe*$dihe_fac)
evaluate ($nstep1=int($high_100steps * 2. / 3. ) )
evaluate ($nstep2=int($high_100steps * 1. / 3. ) )

sani
force  $ksani1 class CH_a
end

sani
force  $ksani class CH_b
end

noe
scale int $knoe
end

rest dihe
scale $kdihe
end

dynamics  verlet
    nstep=$nstep1   timestep=0.003    iasvel=current   tcoupling=true
    tbath=$init_t  nprint=$nstep2  iprfrq=0
end

end loop hot2


!========= Tilt the NOE asymptote and increase weights on geometry =======

noe asymptote * 1.0  end

constraints
         interaction (all) (all) weights * 1 vdw 0.0001 end
         interaction (resid 500) (not resid 500) weights * 1  vdw 0.002 end
         interaction (resid 600) (not resid 600) weights * 1  vdw 0.002 end
         interaction (resid 600) (resid 500) weights * 0 end
end   !peha


dynamics  verlet
    nstep=$nstep2   timestep=0.003    iasvel=current   tcoupling=true
    tbath=$init_t  nprint=$nstep2  iprfrq=0
end

!====================== High-temperature dynamics 2=======================

flags exclude * include bonds angle impr vdw cdih noe sani end

constraints
         interaction (all) (all) weights * 1 vdw 0.001 end
         interaction (resid 500) (not resid 500) weights * 1  vdw 0.002 end
         interaction (resid 600) (not resid 600) weights * 1  vdw 0.002 end
         interaction (resid 600) (resid 500) weights * 0 end
end   !peha



! The dynamics are divided into two parts of two-thirds one-third
  evaluate ($ncycle = 100)
  evaluate ($ini_mene = 25.)
  evaluate ($fin_mene = 500.)
  evaluate ($mene_fac = ($fin_mene/$ini_mene)^(1./$ncycle))
```

```
  evaluate ($kmene = $ini_mene)


        evaluate ($i_cool = 0)
        while ($i_cool < $ncycle) loop hot3
        evaluate ($i_cool=$i_cool+1)


evaluate ($kmene=$kmene*$mene_fac)
evaluate ($nstep1=int($high_100steps * 2. / 3. ) )
evaluate ($nstep2=int($high_100steps * 1. / 3. ) )

parameter
reset
@/md3/peha/hymenistatin/xplor/new/MAIN/parameters/parallhdg_vf.pro @/md3/peha/hymenistatin/xplor/new/MAIN/parameters/pa
end

dynamics  verlet
    nstep=$nstep1   timestep=0.003    iasvel=current   tcoupling=true
    tbath=$init_t  nprint=$nstep2  iprfrq=0
end

end loop hot3


!========= Tilt the NOE asymptote and increase weights on geometry =======

noe asymptote * 1.0  end

constraints
         interaction (all) (all) weights * 1 vdw 0.0001 end
         interaction (resid 500) (not resid 500) weights * 1  vdw 0.002 end
         interaction (resid 600) (not resid 600) weights * 1  vdw 0.002 end
         interaction (resid 600) (resid 500) weights * 0 end
end   !peha


dynamics  verlet
    nstep=$nstep2   timestep=0.003    iasvel=current   tcoupling=true
    tbath=$init_t  nprint=$nstep2  iprfrq=0
end


!========================== Cooling loop ==============================
flags exclude * include bonds angle impr vdw cdih noe sani end
!restraints dihedral   scale=200.   end
coupling   force=1.0    end


evaluate ($ncycle = ($init_t-$final_t)/$tempstep)
evaluate ($nstep = int($cool_steps/$ncycle))

evaluate ($ini_rad  = 0.9)        evaluate ($fin_rad  = 0.75)
evaluate ($ini_con=  0.003)       evaluate ($fin_con=  4.0)

evaluate ($bath  = $init_t)
evaluate ($k_vdw = $ini_con)
evaluate ($k_vdwfact = ($fin_con/$ini_con)^(1/$ncycle))
evaluate ($radius=    $ini_rad)
evaluate ($radfact = ($fin_rad/$ini_rad)^(1/$ncycle))

evaluate ($i_cool = 0)


while ($i_cool < $ncycle) loop cool
    evaluate ($i_cool=$i_cool+1)

    evaluate ($bath  = $bath  - $tempstep)
```

```
    evaluate ($k_vdw=min($fin_con,$k_vdw*$k_vdwfact))
    evaluate ($radius=max($fin_rad,$radius*$radfact))

    parameter  nbonds repel=$radius   end end


    constraints
              interaction (all) (all) weights * 1 vdw $k_vdw end
              interaction (resid 500) (not resid 500) weights * 1  vdw 0.002 end
              interaction (resid 600) (not resid 600) weights * 1  vdw 0.002 end
              interaction (resid 600) (resid 500) weights * 0 end
    end



    dynamics  verlet
nstep=$nstep time=0.002 iasvel=current firstt=$bath
tcoup=true tbath=$bath nprint=$nstep iprfrq=0
    end




    evaluate ($critical=$temp/$bath) ! Abort condition. Should never be violated

!     if ($critical >  10. ) then
! display  ****&&&& rerun job with smaller timestep (i.e., 0.003)
! stop
!     end if

end loop cool

!========================== Final minimization setup ==============================

constraints
              interaction (all) (all) weights * 1 end
              interaction (resid 500) (not resid 500) weights * 1  vdw 0.002 end
              interaction (resid 600) (not resid 600) weights * 1  vdw 0.002 end
              interaction (resid 600) (resid 500) weights * 0 end
end   !peha



parameter
    nbonds
repel=0.80
rexp=2 irexp=2 rcon=1.
nbxmod=3
wmin=0.01
cutnb=6.0 ctonnb=2.99 ctofnb=3.
tolerance=1.5
    end
end

!========================== Final minimization ============================

minimize powell nstep=$minimise_steps drop=10.0 nprint=$minimise_steps end


! ======================== Calculate Violations ====================


! Setup the names for the violations files

evaluate($printfile = "/md3/peha/hymenistatin/xplor/new/MAIN/pph/sani_J/structures/high_100.viol")
set print-file = $printfile end

set message on echo on end
```

```
{* NOE Data Analysis *}
   print threshold = 0.20 noe
   evaluate ($noe2 = $violations)
   evaluate ($rms_noe = $result)


{* CDIH Data Analysis *}
   print threshold = 3.0 cdih
   evaluate ($cdih3 = $violations)
   evaluate ($rms_cdih = $result)


{* BOND Data Analysis *}
   print thres=0.05 bond
   evaluate ($bond5=$violations)
   evaluate ($rms_bond = $result)

{* ANGLE Data Analysis *}
   print thres=5.0 angle
   evaluate ($angle5=$violations)
   evaluate ($rms_angle = $result)

{* IMPROPER Data Analysis *}
   print thres=5.0 improper
   evaluate ($improper5=$violations)
   evaluate ($rms_improper = $result)

{* J-coupling constant analysis *}

if ($couplings = 1.0 ) then
   couplings print threshold 0.0 class phi end
   evaluate ($rms_coup_phi = $result)
   evaluate ($viol_coup_phi = $violations)

   couplings print threshold 0.0 class gly end
   evaluate ($rms_coup_gly = $result)
   evaluate ($viol_coup_gly = $violations)

   evaluate ($viol_coup = $viol_coup_gly + $viol_coup_phi)

end if

if ($couplings = 0.0 ) then
   evaluate ($rms_coup_phi = 0.0)
   evaluate ($rms_coup_gly = 0.0)
   evaluate ($viol_coup = 0.0)
   evaluate($COUP = off)
end if

if ($rdcs = 1.0 ) then
    sani print threshold 0.0 end end
   evaluate ($rms_rdc = $result)
   evaluate ($viol_rdc = $violations)
end if

if ($rdcs = 0.0 ) then
   evaluate ($rms_rdc = 0.0)
   evaluate ($viol_rdc = 0.0)
   evaluate($SANI = off)
end if


{* ENERGY Data Analysis *}
   energy end
```

```
! ================== Write out the final structure ===============


remarks ============================================================
remarks  Violations :    noe  cdih  coup   rdcs  bonds  angles  improp
remarks             :  $noe2[I5] $cdih3[I5] $viol_coup[I5]  $viol_rdc[I5]  $bond5[I5]   $angle5[I5]   $improper5[I5]
remarks  RMSD rest. :    noe    cdih  coup (phi)  coup (gly)    rdcs
remarks             : $rms_noe[F6.3]  $rms_cdih[F6.3]      $rms_coup_phi[F6.3]      $rms_coup_gly[F6.3]  $rms_rdc[F6.3]
remarks  RMSD geom. :  bonds  angles  improp
remarks             : $rms_bond[F6.3]  $rms_angle[F6.3]  $rms_improper[F6.3]
remarks ============================================================
remarks Energies
remarks
remarks overall = $ener
remarks noe  = $NOE
remarks cdih = $CDIH
remarks vdw  = $VDW
remarks bon  = $BOND
remarks ang  = $ANGL
remarks imp  = $IMPR
remarks coup = $COUP
remarks sani = $SANI
remarks ============================================================

close $printfile end
set print=OUTPUT end

! Put together the output filenames

evaluate ($filename="/md3/peha/hymenistatin/xplor/new/MAIN/pph/sani_J/structures/high_100.pdb")

   write coordinates output =$filename end

!end loop main

stop
```

D.3 XPLOR-NIH input file for DiaA

```
!============================== Structure ======================================
!
! Next is the molecular structure file (from psfgen.inp)

structure
@/md3/peha/DiaA/restraints/pyr_S.psf
              !  @axis_new_500.psf
              !   @axis_new_600.psf
                end

!================================ Run Set-up ===================================

!
set seed=7478978723259 end

!
! The following are the conditions for the molecular dynamics steps
! The defaults work well for most proteins
!
! Temperature for high-temp dynamics and initial SA temperature.
!
```

```
evaluate ($init_t = 2000 )

! Number of steps in high temp dymanics

evaluate ($high_10steps= 400 )

! Total number of steps, the final temperature and the
! temperature step in the SA cooling.

evaluate ($cool_steps = 4000 )

    evaluate ($final_t = 100)      { K }
    evaluate ($tempstep = 50)      { K }

! Number of steps in final minimisation

evaluate ($minimise_steps = 1000 )

!============================== Parameters ====================================
!

parameter @/md3/peha/DiaA/restraints/pyr_S.par
  !  @par_axis_3.pro !peha
end


!!============================ Restraints ==================================

!     ++++++++ NOE Restraints ++++++++++


        set message on echo on end

noe

! Estimate greater than the actual number of NOEs.

nres=10000

! Read NOE restraint files

clas gut
  nres 500
  pote gut square
  scale gut 0
  @/md3/peha/DiaA/restraints/NOE_DiaA_new_020906.tbl
  clas mittel
  pote mittel square
  scale mittel 0
  @/md3/peha/DiaA/restraints/non_NOEs_DiaA1.tbl
  clas ambi
  pote ambi square
  scale ambi 10
 ! @NOE_DiaA_ambi.tbl


!  Averaging and Scales for each class. Averaging has to be consistent
! with the choice of psuedo-atom.

! averaging int sum

! scale int 50.0


! Parameters for NOE effective energy term (should not need changing).

ceiling=1000
potential  * soft
```

```
sqoffset   * 0.0
sqconstant * 1.0
sqexponent * 2
soexponent * 1
asymptote  * 0.1     ! Initial value, modified later.
rswitch    * 0.5
end


!++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++

! Nothing below this line should need to be changed

!++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++++


flags exclude * include bonds angle impr vdw noe dihe cdihe end

if ($couplings = 1.0 ) then
  flags include coup end
end if

if ($rdcs = 1.0 ) then
  flags include tenso end
end if

! Friction coefficient for MD heatbath, in 1/ps.

vector do (fbeta=10) (all)

! Uniform heavy masses to speed molecular dynamics.*}

vector do (mass=100) (all)



! Parameters for the repulsive energy term.

parameter
         nbonds
repel=1.                    ! Initial value for repel, modified later.
rexp=2 irexp=2 rcon=1.
nbxmod=3
wmin=0.01
cutnb=4.5 ctonnb=2.99 ctofnb=3.
tolerance=0.5
   end
end

!============================================================================

! Read the start10 structure and copy the coordinates into the buffer.

coor @/md3/peha/DiaA/xplo2d/S_pyr/nores_S/start10.pdb  ! peha end
!coor @axis500.pdb       ! peha end
!coor @axis600.pdb       ! peha end

coor copy end

set message on echo on end

! evaluate ($count = 0)
! while ($count < $endcount ) loop main

! evaluate ($count=$count+1)
```

```
! Setup the names for the violations files


! Swap the coordinates from the buffer to the main coordinate set, then
! copy them back to the buffer. This means the start10 structure coordinates
! are reloaded for each run.

    coor swap end
    coor copy end

if($rdcs = 1.0) then
    constraints
fix=(resname ANI)
    end
end if


!======================Initial minimization ============================

restraints dihedral   scale=5.   end
coupling   force =0.1 end
noe asymptote * 0.1  end
parameter  nbonds repel=1.   end end


! Note: this statement means that the S-S bond in disulphides will
! be ignored.


constraints
         interaction (all) (all) weights * 1 vdw 0.002 end
         interaction (resid 500) (not resid 500) weights * 1  vdw 0.002 end
         interaction (resid 600) (not resid 600) weights * 1  vdw 0.002 end
         interaction (resid 600) (resid 500) weights * 0 end
end   !peha


minimize powell nstep=50 drop=10.  nprint=50 end


!==================== High-temperature dynamics ========================

flags exclude * include bonds angle impr vdw dihe noe tenso cdihe end

constraints
         interaction (all) (all) weights * 1 vdw 0.002 end
         interaction (resid 500) (not resid 500) weights * 1  vdw 0.002 end
         interaction (resid 600) (not resid 600) weights * 1  vdw 0.002 end
         interaction (resid 600) (resid 500) weights * 0 end
end   !peha
noe
scale gut 0
end


! The dynamics are divided into two parts of two-thirds one-third
  evaluate ($ncycle = 20)
  evaluate ($ini_tenso = 0.001)
  evaluate ($fin_tenso = 1.0)
  evaluate ($tens_fac = ($fin_tenso/$ini_tenso)^(1/$ncycle))
  evaluate ($ktenso = $ini_tenso)
  evaluate ($ini_noe = 1)
  evaluate ($fin_noe = 50)
  evaluate ($noe_fac = ($fin_noe/$ini_noe)^(1/$ncycle))
  evaluate ($knoe = $ini_noe)

        evaluate ($i_cool = 0)
        while ($i_cool < $ncycle) loop cool
```

```
        evaluate ($i_cool=$i_cool+1)

        evaluate ($ktenso=$ktenso*$tens_fac)
evaluate ($knoe=$knoe*$noe_fac)
evaluate ($nstep1=int($high_10steps * 2. / 3. ) )
evaluate ($nstep2=int($high_10steps * 1. / 3. ) )

tenso
force $ktenso class CH_a
end

noe
scale gut $knoe
end

noe
scale mittel $knoe
end


dynamics  verlet
    nstep=$nstep1   timestep=0.00005   iasvel=maxwell   firstt=$init_t
    tcoupling=true  tbath=$init_t    nprint=50  iprfrq=0
end
        end loop cool



!========= Tilt the NOE asymptote and increase weights on geometry =======

noe asymptote * 1.0  end

constraints
         interaction (all) (all) weights * 1 vdw 0.002 end
         interaction (resid 500) (not resid 500) weights * 1  vdw 0.002 end
         interaction (resid 600) (not resid 600) weights * 1  vdw 0.002 end
         interaction (resid 600) (resid 500) weights * 0 end
end   !peha


dynamics  verlet
    nstep=$nstep2   timestep=0.0005    iasvel=current   tcoupling=true
    tbath=$init_t  nprint=$nstep2  iprfrq=0
end


!============================ Cooling loop ================================

restraints dihedral   scale=200.   end
coupling   force=1.0    end


evaluate ($ncycle = ($init_t-$final_t)/$tempstep)
evaluate ($nstep = int($cool_steps/$ncycle))

evaluate ($ini_rad  = 0.9)        evaluate ($fin_rad  = 0.75)
evaluate ($ini_con=  0.003)       evaluate ($fin_con=  4.0)

evaluate ($bath  = $init_t)
evaluate ($k_vdw = $ini_con)
evaluate ($k_vdwfact = ($fin_con/$ini_con)^(1/$ncycle))
evaluate ($radius=    $ini_rad)
evaluate ($radfact = ($fin_rad/$ini_rad)^(1/$ncycle))

evaluate ($i_cool = 0)


while ($i_cool < $ncycle) loop cool
```

```
    evaluate ($i_cool=$i_cool+1)

    evaluate ($bath  = $bath  - $tempstep)
    evaluate ($k_vdw=min($fin_con,$k_vdw*$k_vdwfact))
    evaluate ($radius=max($fin_rad,$radius*$radfact))

    parameter  nbonds repel=$radius   end end


    constraints
              interaction (all) (all) weights * 1 vdw $k_vdw end
              interaction (resid 500) (not resid 500) weights * 1  vdw 0.002 end
              interaction (resid 600) (not resid 600) weights * 1  vdw 0.002 end
              interaction (resid 600) (resid 500) weights * 0 end
    end



    dynamics  verlet
nstep=$nstep time=0.001 iasvel=current firstt=$bath
tcoup=true tbath=$bath nprint=$nstep iprfrq=0
    end



    evaluate ($critical=$temp/$bath) ! Abort condition. Should never be violated

!     if ($critical >  10. ) then
! display  ****&&&& rerun job with smaller timestep (i.e., 0.003)
! stop
!     end if

end loop cool

!========================== Final minimization setup ==============================

constraints
              interaction (all) (all) weights * 1 end
              interaction (resid 500) (not resid 500) weights * 1  vdw 0.002 end
              interaction (resid 600) (not resid 600) weights * 1  vdw 0.002 end
              interaction (resid 600) (resid 500) weights * 0 end
end   !peha



parameter
    nbonds
repel=0.80
rexp=2 irexp=2 rcon=1.
nbxmod=3
wmin=0.01
cutnb=6.0 ctonnb=2.99 ctofnb=3.
tolerance=1.5
    end
end

!========================== Final minimization ============================

minimize powell nstep=$minimise_steps drop=10.0 nprint=$minimise_steps end


! ======================== Calculate Violations ====================


! Setup the names for the violations files

evaluate($printfile = "/md3/peha/DiaA/pyr_S/non_NOE1/structures/high_10.viol")
```

```
set print-file = $printfile end


{* NOE Data Analysis *}
   print threshold = 0.20 noe
   evaluate ($noe2 = $violations)
   evaluate ($rms_noe = $result)


{* DIHE Data Analysis *}
   print threshold = 0.0 dihe
   evaluate ($dihe3 = $violations)
   evaluate ($rms_dihe = $result)


{* BOND Data Analysis *}
   print thres=0.05 bond
   evaluate ($bond5=$violations)
   evaluate ($rms_bond = $result)

{* ANGLE Data Analysis *}
   print thres=5.0 angle
   evaluate ($angle5=$violations)
   evaluate ($rms_angle = $result)

{* IMPROPER Data Analysis *}
   print thres=5.0 improper
   evaluate ($improper5=$violations)
   evaluate ($rms_improper = $result)

{* J-coupling constant analysis *}

if ($couplings = 1.0 ) then
   couplings print threshold 0.0 class phi end
   evaluate ($rms_coup_phi = $result)
   evaluate ($viol_coup_phi = $violations)

   couplings print threshold 0.0 class gly end
   evaluate ($rms_coup_gly = $result)
   evaluate ($viol_coup_gly = $violations)

   evaluate ($viol_coup = $viol_coup_gly + $viol_coup_phi)

end if

if ($couplings = 0.0 ) then
   evaluate ($rms_coup_phi = 0.0)
   evaluate ($rms_coup_gly = 0.0)
   evaluate ($viol_coup = 0.0)
   evaluate($COUP = off)
end if

if ($rdcs = 1.0 ) then
    tenso print threshold 0.0 end end
   evaluate ($rms_rdc = $result)
   evaluate ($viol_rdc = $violations)
end if

if ($rdcs = 0.0 ) then
   evaluate ($rms_rdc = 0.0)
   evaluate ($viol_rdc = 0.0)
   evaluate($TENS = off)
end if


{* ENERGY Data Analysis *}
   energy end
```

```
! ================== Write out the final structure ================


remarks ============================================================
remarks  Violations :    noe  dihe  coup   rdcs  bonds  angles  improp
remarks             :  $noe2[I5] $dihe3[I5] $viol_coup[I5]  $viol_rdc[I5]  $bond5[I5]   $angle5[I5]   $improper5[I5]
remarks  RMSD rest. :    noe    dihe  coup (phi)  coup (gly)    rdcs
remarks             : $rms_noe[F6.3]  $rms_dihe[F6.3]      $rms_coup_phi[F6.3]      $rms_coup_gly[F6.3]  $rms_rdc[F6.3]
remarks  RMSD geom. :  bonds  angles  improp
remarks             : $rms_bond[F6.3]  $rms_angle[F6.3]  $rms_improper[F6.3]
remarks ============================================================
remarks Energies
remarks
remarks overall = $ener
remarks noe  = $NOE
remarks dihe = $DIHE
remarks vdw  = $VDW
remarks bon  = $BOND
remarks ang  = $ANGL
remarks imp  = $IMPR
remarks coup = $COUP
remarks tenso = $TENS
remarks ============================================================

close $printfile end
set print=OUTPUT end

! Put together the output filenames

   evaluate ($filename= "/md3/peha/DiaA/pyr_S/non_NOE1/structures/high_10.pdb")

   write coordinates output =$filename end

!end loop main

stop
```

D.4 CNS input file for trigger factor

The following input file can be used with up to four different sets of RDCs.

```
{+ file: anneal.inp +}
{+ directory: nmr_calc +}
{+ description: dynamical annealing with NOEs, coupling constants,
                chemical shift restraints starting from extended
                strands or pre-folded structures. +}
{+ authors: Gregory Warren, Michael Nilges, John Kuszewski,
    Marius Clore and Axel Brunger +}
{+ copyright: Yale University +}

{+ reference: Clore GM, Gronenborn AM, Tjandra N, Direct structure refinement
              against residual dipolar couplings in the presence of rhombicity
              of unknown magnitude., J. Magn. Reson., 131, In press, (1998) +}
{+ reference: Clore GM, Gronenborn AM, Bax A, A robust method for determining
              the magnitude of the fully asymmetric alignment tensor of
              oriented macromolecules in the absence of structural
              information., J. Magn. Reson., In press (1998) +}
{+ reference: Garrett DS, Kuszewski J, Hancock TJ, Lodi PJ, Vuister GW,
              Gronenborn AM, Clore GM, The impact of direct refinement against
              three-bond HN-C alpha H coupling constants on protein structure
              determination by NMR., J. Magn. Reson. Ser. B, 104(1),
              99-103, (1994) May +}
```

```
{+ reference: Kuszewski J, Qin J, Gronenborn AM, Clore GM, The impact of direct
              refinement against 13C alpha and 13C beta chemical shifts on
              protein structure determination by NMR., J. Magn. Reson. Ser. B,
              106(1), 92-6, (1995) Jan +}
{+ reference: Kuszewski J, Gronenborn AM, Clore GM, The impact of direct
              refinement against proton chemical shifts on protein structure
              determination by NMR., J. Magn. Reson. Ser. B, 107(3), 293-7,
              (1995) Jun +}
{+ reference: Kuszewski J, Gronenborn AM, Clore GM, A potential involving
              multiple proton chemical-shift restraints for
              nonstereospecifically assigned methyl and methylene protons.
              J. Magn. Reson. Ser. B, 112(1), 79-81, (1996) Jul. +}
{+ reference: Nilges M, Gronenborn AM, Brunger AT, Clore GM, Determination
              of three-dimensional structures of proteins by simulated
              annealing with interproton distance restraints: application
              to crambin, potato carboxypeptidase inhibitor and barley
              serine proteinase inhibitor 2. Protein Engineering 2,
              27-38, (1988) +}
{+ reference: Nilges M, Clore GM, Gronenborn AM,  Determination of
              three-dimensional structures of proteins from interproton
              distance data by dynamical simulated annealing from a random
              array of atoms. FEBS LEtt. 239, 129-136. (1988) +}
{+ reference: Rice LM, Brunger AT, Torsion Angle Dynamics: Reduced Variable
              Conformational Sampling Enhances Crystallographic Structure
              Refinement., Proteins, 19, 277-290 (1994) +}
{+ reference: Stein EG, Rice LM, Brunger AT, Torsion angle molecular
              dynamics: a new efficient tool for NMR structure calculation.,
              J. Mag. Res. Ser. B 124, 154-164 (1997) +}
{+ reference: Tjandra N, Garrett DS, Gronenborn AM, Bax A, Clore GM, Defining
              long range order in NMR structure determination from the
              dependence of heteronuclear relaxation times on rotational
              diffusion anisotropy. Nature Struct. Biol., 4(6), 443-9,
              (1997) June +}
{+ reference: Tjandra N, Omichinski JG, Gronenborn AM, Clore GM, Bax A, Use of
              dipolar 1H-15N and 1H-13C couplings in the structure
              determination of magnetically oriented macromolecules in
              solution. Nature Struct. Biol., 4(9), 732-8, (1997) Sept +}

! Data taken from: Qin J, Clore GM, Kennedy WP, Kuszewski J, Gronenborn AM,
!                   The solution structure of human thioredoxin complexed with
!                   its target from Ref-1 reveals peptide chain reversal.,
!                   Structure, 4(5), 613-620, 1996 May 15.

{- Guidelines for using this file:
   - all strings must be quoted by double-quotes
   - logical variables (true/false) are not quoted
   - do not remove any evaluate statements from the file -}

{- begin block parameter definition -} define(

{====================== molecular structure ========================}

{* parameter file(s) *}
{===>} par.1="CNS_TOPPAR:protein-allhdg.param";
{===>} par.2="CNS_TOPPAR:axis.param";
{===>} par.3="";
{===>} par.4="";
{===>} par.5="";

{* structure file(s) *}
{===>} struct.1="tgf_start2.mtf";
{===>} struct.2="axis_500.mtf";
{===>} struct.3="axis_501.mtf";
{===>} struct.4="axis_700.mtf";
{===>} struct.5="axis_800.mtf";

{* input coordinate file(s) *}
{===>} pdb.in.file.1="resultsanilast_3.pdb";
```

```
{===>} pdb.in.file.2="axis.pdb";
{===>} pdb.in.file.3="";
{===>} pdb.in.file.4="";
{===>} pdb.in.file.5="";

{========================== atom selection ==========================}

{* input "backbone" selection criteria for average structure generation *}
{* for protein      (name n or name ca or name c)
   for nucleic acid (name O5' or name C5' or name C4' or name C3'
                     or name O3' or name P) *}
{===>} pdb.atom.select=(name n or name ca or name c);

{====================== refinement parameters ======================}

{* type of molecular dynamics for hot phase *}
{+ choice: "torsion" "cartesian" +}
{===>} md.type.hot="torsion";

{* type of molecular dynamics for cool phase *}
{+ choice: "torsion" "cartesian" +}
{===>} md.type.cool="torsion";

{* refine using different initial velocities or coordinates
   (enter base name in "input coordinate files" field) *}
{+ choice: "veloc" "coord" +}
{===>} md.type.initial="veloc";

{* seed for random number generator *}
{* change to get different initial velocities *}
{===>} md.seed=182364;

{* select whether the number of structures will be either trial or
   accepted structures and whether to print only the trial, accepted,
   both sets of structures. *}
{+ list: The printing format is as follows:
         trial = pdb.out.name + _#.pdb , accepted = pdb.out.name + a_#.pdb +}

{* are the number of structures to be trials or accepted? *}
{+ choice: "trial" "accept" +}
{===>} flg.trial.struc="trial";
{* number of trial or accepted structures *}
{===>} pdb.end.count=200;

{* print accepted structures *}
{+ choice: true false +}
{===>} flg.print.accept=false;
{* print trial structures *}
{+ choice: true false +}
{===>} flg.print.trial=true;

{* calculate an average structure for either the trial or
   accepted structure.  If calculate accepted average is false then
   an average for the trial structures will be calculated. *}

{* calculate an average structure? *}
{+ choice: true false +}
{===>} flg.calc.ave.struct=false;
{* calculate an average structure for the accepted structures? *}
{+ choice: true false +}
{===>} flg.calc.ave.accpt=false;
{* minimize average coordinates? *}
{+ choice: true false +}
{===>} flg.min.ave.coor=false;

{=================== torsion dynamics parameters ===================}

{* maximum unbranched chain length *}
```

```
{* increase for long stretches of polyalanine or for nucleic acids *}
{===>} md.torsion.maxlength=50;

{* maximum number of distinct bodies *}
{===>} md.torsion.maxtree=8;

{* maximum number of bonds to an atom *}
{===>} md.torsion.maxbond=6;

{========== parameters for high temperature annealing stage ==========}

{* temperature (proteins: 50000, dna/rna: 20000) *}
{===>} md.hot.temp=10000;
{* number of steps (proteins: 1000, dna/rna: 4000) *}
{===>} md.hot.step=500;
{* scale factor to reduce van der Waals (repel) energy term *}
{===>} md.hot.vdw=0.1;
{* scale factor for NOE energy term *}
{===>} md.hot.noe=150;
{* scale factor for dihedral angle energy term (proteins: 100, dna/rna: 5) *}
{===>} md.hot.cdih=10;
{* molecular dynamics timestep *}
{===>} md.hot.ss=0.015;

{======== parameters for the first slow-cool annealing stage =========}

{* temperature (cartesian: 1000, torsion: [proteins: 50000, dna/rna: 20000]) *}
{===>} md.cool.temp=10000;
{* number of steps *}
{===>} md.cool.step=1000;
{* scale factor for final van der Waals (repel) energy term
   (cartesian: 4.0, torsion: 1.0) *}
{===>} md.cool.vdw=1.0;
{* scale factor for NOE energy term *}
{===>} md.cool.noe=150;
{* scale factor for dihedral angle energy term *}
{===>} md.cool.cdih=200;
{* molecular dynamics timestep (cartesian: 0.005, torsion: 0.015) *}
{===>} md.cool.ss=0.015;
{* slow-cool annealing temperature step (cartesian: 25, torsion: 250) *}
{===>} md.cool.tmpstp=100;

{========= parameters for a second slow-cool annealing stage ==========}

{* cartesian slow-cooling annealing stage to be used only with torsion
   slow-cool annealing stage *}
{* this stage is only necessary when the macromolecule is a protein
   greater than 160 residues or in some cases for nucleic acids *}

{* use cartesian cooling stage? *}
{+ choice: true false +}
{===>} md.cart.flag=true;
{* temperature *}
{===>} md.cart.temp=2000;
{* number of steps *}
{===>} md.cart.step=5000;
{* scale factor for initial van der Waals (repel) energy term *}
{===>} md.cart.vdw.init=1.0;
{* scale factor for final van der Waals (repel) energy term *}
{===>} md.cart.vdw.finl=4.0;
{* scale factor for NOE energy term *}
{===>} md.cart.noe=150;
{* scale factor for dihedral angle energy term *}
{===>} md.cart.cdih=200;
{* molecular dynamics timestep *}
{===>} md.cart.ss=0.005;
{* slow-cool annealing temperature step *}
{===>} md.cart.tmpstp=25;
```

```
{=============== parameters for final minimization stage ==============}

{* scale factor for NOE energy term *}
{===>} md.pow.noe=100;
{* scale factor for dihedral angle energy term *}
{===>} md.pow.cdih=400;
{* number of minimization steps *}
{===>} md.pow.step=200;
{* number of cycles of minimization *}
{===>} md.pow.cycl=10;

{============================== noe data ==============================}

{- Important - if you do not have a particular data set then
   set the file name to null ("") -}

{* NOE distance restraints files. *}

{* restraint set 1 file *}
{===>} nmr.noe.file.1="15nnoe.tbl";
{* restraint set 2 file *}
{===>} nmr.noe.file.2="aliphnoe.tbl";
{* restraint set 3 file *}
{===>} nmr.noe.file.3="aromnoe.tbl";
{* restraint set 4 file *}
!!{===>} nmr.noe.file.4="ambiguous.tbl";
{* restraint set 5 file *}
{===>} nmr.noe.file.5="";

{* NOE averaging modes *}

{* restraint set 1 *}
{+ choice: "sum" "cent" "R-6" "R-3" "symm" +}
{===>} nmr.noe.ave.mode.1="sum";
{* restraint set 2 *}
{+ choice: "sum" "cent" "R-6" "R-3" "symm" +}
{===>} nmr.noe.ave.mode.2="sum";
{* restraint set 3 *}
{+ choice: "sum" "cent" "R-6" "R-3" "symm" +}
{===>} nmr.noe.ave.mode.3="sum";
{* restraint set 4 *}
{+ choice: "sum" "cent" "R-6" "R-3" "symm" +}
{===>} nmr.noe.ave.mode.4="sum";
{* restraint set 5 *}
{+ choice: "sum" "cent" "R-6" "R-3" "symm" +}
{===>} nmr.noe.ave.mode.5="sum";

{======================== hydrogen bond data ========================}

{* hydrogen-bond distance restraints file. *}
{===>} nmr.noe.hbnd.file="hbonds.tbl";

{* enter hydrogen-bond distance averaging mode *}
{+ choice: "sum" "cent" "R-6" "R-3" "symm" +}
{===>} nmr.noe.ave.mode.hbnd="R-3";

{======================= 3-bond J-coupling data =======================}
{* the default setup is for the phi dihedral *}

{* Class 1 *}

{* 3-bond J-coupling non-glycine restraints file *}
{===>} nmr.jcoup.file.1="jcoup.tbl";
{* 3-bond J-coupling non-glycine potential *}
{+ choice: "harmonic" "square" "multiple" +}
{===>} nmr.jcoup.pot.1="harmonic";
{* 3-bond J-coupling non-glycine force value *}
```

```
{===>} nmr.jcoup.force.1.1=2;
{* 3-bond j-coupling multiple class force second value *}
{===>} nmr.jcoup.force.2.1=0;
{* 3-bond j-coupling Karplus coefficients *}
{* the default values are for phi *}
{===>} nmr.jcoup.coef.1.1=6.98;
{===>} nmr.jcoup.coef.2.1=-1.38;
{===>} nmr.jcoup.coef.3.1=1.72;
{===>} nmr.jcoup.coef.4.1=-60.0;

{* Class 2 *}

{* 3-bond j-coupling glycine restraints files *}
{===>} nmr.jcoup.file.2="jcoup2.tbl";
{* 3-bond J-coupling glycine potential *}
{* The potential for the glycine class must be multiple *}
{+ choice: "harmonic" "square" "multiple" +}
{===>} nmr.jcoup.pot.2="multiple";
{* 3-bond J-coupling first force value *}
{===>} nmr.jcoup.force.1.2=1;
{* 3-bond j-coupling glycine or multiple force second value *}
{===>} nmr.jcoup.force.2.2=0;
{* 3-bond j-coupling Karplus coefficients *}
{* the default values are for glycine phi *}
{===>} nmr.jcoup.coef.1.2=6.98;
{===>} nmr.jcoup.coef.2.2=-1.38;
{===>} nmr.jcoup.coef.3.2=1.72;
{===>} nmr.jcoup.coef.4.2=0.0;

{================ 1-bond heteronuclear J-coupling data ================}

{* Class 1 *}

{* 1-bond heteronuclear j-coupling file *}
{===>} nmr.oneb.file.1="";
{* 1-bond heteronuclear j-coupling potential *}
{+ choice: "harmonic" "square" +}
{===>} nmr.oneb.pot.1="harmonic";
{* 1-bond heteronuclear j-coupling force value *}
{===>} nmr.oneb.force.1=1.0;

{=============== alpha/beta carbon chemical shift data ================}

{* Class 1 *}

{* carbon, alpha and beta, chemical shift restraints file *}
{===>} nmr.carb.file.1="";
{* carbon, alpha and beta, chemical shift restraint potential *}
{+ choice: "harmonic" "square" +}
{===>} nmr.carb.pot.1="harmonic";
{* carbon, alpha and beta, chemical shift restraint force value *}
{===>} nmr.carb.force.1=0.5;

{===================== proton chemical shift data =====================}

{* Class 1 *}

{* class 1 proton chemical shift restraints file *}
{===>} nmr.prot.file.1="";
{* class 1 proton chemical shift potential *}
{+ choice: "harmonic" "square" "multiple" +}
{===>} nmr.prot.pot.1="harmonic";
{* class 1 proton chemical shift force value *}
{===>} nmr.prot.force.1.1=7.5;
{* 2nd class 1 proton chemical shift force value for multi *}
{===>} nmr.prot.force.2.1=0;
{* class 1 proton chemical shift violation cutoff threshold *}
{===>} nmr.prot.thresh.1=0.3;
```

```
{* Class 2 *}

{* class 2 proton chemical shift restraints file *}
{===>} nmr.prot.file.2="";
{* class 2 proton chemical shift potential *}
{+ choice: "harmonic" "square" "multiple" +}
{===>} nmr.prot.pot.2="harmonic";
{* class 2 proton chemical shift force value *}
{===>} nmr.prot.force.1.2=7.5;
{* 2nd class 2 proton chemical shift force value for multi *}
{===>} nmr.prot.force.2.2=0;
{* class 2 proton chemical shift violation cutoff threshold *}
{===>} nmr.prot.thresh.2=0.3;

{* Class 3 *}

{* class 3 proton chemical shift restraints file *}
{===>} nmr.prot.file.3="";
{* class 3 proton chemical shift potential *}
{+ choice: "harmonic" "square" "multiple" +}
{===>} nmr.prot.pot.3="harmonic";
{* class 3 proton chemical shift force value *}
{===>} nmr.prot.force.1.3=7.5;
{* 2nd class 3 proton chemical shift force value for multi *}
{===>} nmr.prot.force.2.3=0;
{* class 3 proton chemical shift violation cutoff threshold *}
{===>} nmr.prot.thresh.3=0.3;

{* Class 4 *}

{* class 4 proton chemical shift restraints file *}
{===>} nmr.prot.file.4="";
{* class 4 proton chemical shift potential *}
{+ choice: "harmonic" "square" "multiple" +}
{===>} nmr.prot.pot.4="multiple";
{* class 4 proton chemical shift force value *}
{===>} nmr.prot.force.1.4=7.5;
{* 2nd class 4 proton chemical shift force value for multi *}
{===>} nmr.prot.force.2.4=0;
{* class 4 proton chemical shift violation cutoff threshold *}
{===>} nmr.prot.thresh.4=0.3;

{================ diffusion anisotropy restraint data =================}

{* fixed or harmonically restrained external axis *}
{+ choice: "fixed" "harm" +}
{===>} nmr.dani.axis="harm";

{* Class 1 *}

{* diffusion anisotropy restraints file *}
{===>} nmr.dani.file.1="";
{* diffusion anisotropy potential *}
{+ choice: "harmonic" "square" +}
{===>} nmr.dani.pot.1="harmonic";
{* diffusion anisotropy initial force value *}
{===>} nmr.dani.force.init.1=0.01;
{* diffusion anisotropy final force value *}
{===>} nmr.dani.force.finl.1=1.0;
{* diffusion anisotropy coefficients *}
{* coef: <Tc> <anis> <rhombicity> <wh> <wn> *}

{* Tc = 1/2(Dx+Dy+Dz) in <ns> *}
{===>} nmr.dani.coef.1.1=7.35;
{* anis = Dz/0.5*(Dx+Dy) *}
{===>} nmr.dani.coef.2.1=2.69;
{* rhombicity = 1.5*(Dy-Dx)/(Dz-0.5*(Dy+Dx)) *}
```

```
{===>} nmr.dani.coef.3.1=1.78;
{* wH in <MHz> *}
{===>} nmr.dani.coef.4.1=600.13;
{* wN in <MHz> *}
{===>} nmr.dani.coef.5.1=60.82;

{============= susceptability anisotropy restraint data ===============}

{* fixed or harmonically restrained external axis *}
{+ choice: "fixed" "harm" +}
{===>} nmr.sani.axis="harm";

{* Class 1 *}

{* susceptability anisotropy restraints file *}
{===>} nmr.sani.file.1="test2.tbl";
{* susceptability anisotropy potential *}
{+ choice: "harmonic" "square" +}
{===>} nmr.sani.pot.1="harmonic";
{* susceptability anisotropy initial force value *}
{===>} nmr.sani.force.init.1=0.01;
{* susceptability anisotropy final force value *}
{===>} nmr.sani.force.finl.1=6.5;
{* susceptability anisotropy coefficients *}
{* coef: <DFS> <axial > <rhombicity>;
   a0+a1*(3*cos(theta)^2-1)+a2*(3/2)*sin(theta)^2*cos(2*phi) *}

{* DFS = a0 *}
{===>} nmr.sani.coef.1.1=-0.0601;
{* axial = a0-a1-3/2*a2 *}
{===>} nmr.sani.coef.2.1=4.147;
{* rhombicity = a2/a1 *}
{===>} nmr.sani.coef.3.1=0.488;

{* Class 2 *}

{* susceptability anisotropy restraints file *}
{===>} nmr.sani.file.2="tgf_pk2_sani_last.tab";
{* susceptability anisotropy potential *}
{+ choice: "harmonic" "square" +}
{===>} nmr.sani.pot.2="harmonic";
{* susceptability anisotropy initial force value *}
{===>} nmr.sani.force.init.2=0.01;
{* susceptability anisotropy final force value *}
{===>} nmr.sani.force.finl.2=6.5;
{* susceptability anisotropy coefficients *}
{* coef: <DFS> <axial > <rhombicity>;
   a0+a1*(3*cos(theta)^2-1)+a2*(3/2)*sin(theta)^2*cos(2*phi) *}

{* DFS = a0 *}
{===>} nmr.sani.coef.1.2=-0.0601;
{* axial = a0-a1-3/2*a2 *}
{===>} nmr.sani.coef.2.2=4.041;
{* rhombicity = a2/a1 *}
{===>} nmr.sani.coef.3.2=0.416;

{* Class 3 *}

{* susceptability anisotropy restraints file *}
{===>} nmr.sani.file.3="rdc_43D_Dy.tbl";
{* susceptability anisotropy potential *}
{+ choice: "harmonic" "square" +}
{===>} nmr.sani.pot.3="harmonic";
{* susceptability anisotropy initial force value *}
{===>} nmr.sani.force.init.3=0.01;
{* susceptability anisotropy final force value *}
{===>} nmr.sani.force.finl.3=6.5;
{* susceptability anisotropy coefficients *}
```

```
{* coef: <DFS> <axial > <rhombicity>;
   a0+a1*(3*cos(theta)^2-1)+a2*(3/2)*sin(theta)^2*cos(2*phi) *}

{* DFS = a0 *}
{===>} nmr.sani.coef.1.3=-0.0601;
{* axial = a0-a1-3/2*a2 *}
{===>} nmr.sani.coef.2.3=4.245;
{* rhombicity = a2/a1 *}
{===>} nmr.sani.coef.3.3=0.353;

{* Class 4 *}

{* susceptability anisotropy restraints file *}
{===>} nmr.sani.file.4="rdc_43L_Dy.tbl";
{* susceptability anisotropy potential *}
{+ choice: "harmonic" "square" +}
{===>} nmr.sani.pot.4="harmonic";
{* susceptability anisotropy initial force value *}
{===>} nmr.sani.force.init.4=0.01;
{* susceptability anisotropy final force value *}
{===>} nmr.sani.force.finl.4=6.5;
{* susceptability anisotropy coefficients *}
{* coef: <DFS> <axial > <rhombicity>;
   a0+a1*(3*cos(theta)^2-1)+a2*(3/2)*sin(theta)^2*cos(2*phi) *}

{* DFS = a0 *}
{===>} nmr.sani.coef.1.4=-0.0601;
{* axial = a0-a1-3/2*a2 *}
{===>} nmr.sani.coef.2.4=4.106;
{* rhombicity = a2/a1 *}
{===>} nmr.sani.coef.3.4=0.426;

{======================== other restraint data ========================}

{* dihedral angle restraints file *}
{* Note: the restraint file MUST NOT contain restraints
         dihedral or end *}
{===>} nmr.cdih.file="dihedral.tbl";

{* DNA-RNA base planarity restraints file *}
{* Note: include weights as $pscale in the restraint file *}
{===>} nmr.plan.file="";
{* input planarity scale factor - this will be written into $pscale *}
{===>} nmr.plan.scale=150;

{* NCS-restraints file *}
{* example is in inputs/xtal_data/eg1_ncs_restrain.dat *}
{===>} nmr.ncs.file="";

{======================== input/output files ==========================}

{* base name for input coordinate files *}
{===>} pdb.in.name="";

{* base name for output coordinate files *}
{===>} pdb.out.name="TGFall4";

{=============================================================================}
{         things below this line do not normally need to be changed         }
{         except for the torsion angle topology setup if you have           }
{         molecules other than protein or nucleic acid                      }
{=============================================================================}
flg.cv.flag=false;
flg.cv.noe=false;
flg.cv.coup=false;
flg.cv.cdih=false;
flg.dgsa.flag=false;
nmr.cv.numpart=10;
```

```
 ) {- end block parameter definition -}

!checkversion 1.0

evaluate ($log_level=silent)
!evaluate ($log_level=verbose)

structure
   if  (&struct.1 # "") then
      @@&struct.1
   end if
   if  (&struct.2 # "") then
      @@&struct.2
   end if
   if  (&struct.3 # "") then
      @@&struct.3
   end if
   if  (&struct.4 # "") then
      @@&struct.4
   end if
   if  (&struct.5 # "") then
      @@&struct.5
   end if
end

if ( &BLANK%pdb.in.file.1 = false ) then
   coor @@&pdb.in.file.1
end if
if ( &BLANK%pdb.in.file.2 = false ) then
   coor @@&pdb.in.file.2
end if
if ( &BLANK%pdb.in.file.3 = false ) then
   coor @@&pdb.in.file.3
end if

parameter
   if (&par.1 # "") then
      @@&par.1
   end if
   if (&par.2 # "") then
      @@&par.2
   end if
   if (&par.3 # "") then
      @@&par.3
   end if
   if (&par.4 # "") then
      @@&par.4
   end if
   if (&par.5 # "") then
      @@&par.5
   end if
end

if ( $log_level = verbose ) then
  set message=normal echo=on end
else
  set message=off echo=off end
end if

parameter
   nbonds
      repel=0.80
      rexp=2 irexp=2 rcon=1.
      nbxmod=3
      wmin=0.01
      cutnb=6.0 ctonnb=2.99 ctofnb=3.
      tolerance=1.5
```

```
   end
end

{- Read experimental data -}

   @CNS_NMRMODULE:readdata ( nmr=&nmr;
                             flag=&flg;
                             output=$nmr; )

{- Read and store the number of NMR restraints -}

   @CNS_NMRMODULE:restraintnumber ( num=$num; )

!!read in vector angles

!vean
!  nres = 6000
!  class allvean @dipo2.tbl
!  set echo on message on end
1end

!! define prochiral methyl groups


aria
   analyse_restraints
      equivalent
         initialize
         methyle
      end
   end
end



{- Set mass values -}

do (fbeta=10) (all)
do (mass=100) (all)

evaluate ($nmr.trial.count = 0)    {- Initialize current structure number   -}
evaluate ($nmr.accept.count = 0)   {- Initialize number accepted            -}
evaluate ($nmr.counter  = 0)
evaluate ($nmr.prev.counter = -1)

!! vean stuff - initially adapted from jens meiler - changed!!

         evaluate ($ini_cen = 0.1)
         evaluate ($fin_cen =  2.0)
         evaluate ($ini_bor = 0.1)
         evaluate ($fin_bor = 8.0)

!! end of vean stuff


@CNS_NMRMODULE:initave  ( ave=$ave;
                          ave2=$ave2;
                          cv=$cv;
                          ener1=$ener1;
                          ener2=$ener2;
                          flag=&flg;
                          nmr.prot=&nmr.prot; )

{- Zero the force constant of disulfide bonds. -}
parameter
   bonds ( name SG ) ( name SG ) 0. TOKEN
end
```

```
{- define a distance restraints for each disulfide bond, i.e.,
   treat it as if it were an NOE. -}
for $ss_rm_id_1 in id ( name SG ) loop STRM
  for $ss_rm_id_2 in id ( name SG and
  bondedto ( id $ss_rm_id_1 )  ) loop STR2
    if ($ss_rm_id_1 > $ss_rm_id_2) then
      pick bond ( id $ss_rm_id_1 ) ( id $ss_rm_id_2 ) equil
      evaluate ($ss_bond=$result)
      noe
         assign ( id $ss_rm_id_1 ) ( id $ss_rm_id_2 ) $ss_bond 0.1 0.1
      end
    end if
  end loop STR2
end loop STRM

{- Count the number of residues and determine molecule type -}
identify (store9) (tag)
evaluate ($nmr.rsn.num = $SELECT)
identify (store9) ( tag and ( resn THY or resn CYT or resn GUA or
                              resn ADE or resn URI ))
evaluate ($nmr.nucl.num = $SELECT)

{- Improve geometry for torsion angle molecular dynamics -}
evaluate ($flag_tad=false)
if ( &md.type.hot = "torsion" ) then
   if ($nmr.nucl.num > 0) then
      flag exclude * include bond angl impr dihed vdw end
      minimize powell nstep=2000 drop=10.  nprint=100 end
   else
      flag exclude * include bond angl impr vdw end
      minimize powell nstep=2000 drop=10.  nprint=100 end
   end if
   evaluate ($flag_tad=true)
end if

if ( &md.type.cool="torsion") then
   evaluate ($flag_tad=true)
end if

if (&nmr.dani.axis = "harm") then
   do (harmonic=20.0) (resid 500 and name OO)
   do (harmonic=0.0) (resid 500 and name Z )
   do (harmonic=0.0) (resid 500 and name X )
   do (harmonic=0.0) (resid 500 and name Y )
   do (harmonic=0.0) (not (resid 500))
   restraints harmonic exponent=2 end
elseif (&nmr.sani.axis = "harm") then
   do (harmonic=20.0) (resid 500 and name OO)
   do (harmonic=0.0) (resid 500 and name Z )
   do (harmonic=0.0) (resid 500 and name X )
   do (harmonic=0.0) (resid 500 and name Y )
   do (harmonic=0.0) (not (resid 500))
   do (harmonic=20.0) (resid 501 and name OO)
   do (harmonic=0.0) (resid 501 and name Z )
   do (harmonic=0.0) (resid 501 and name X )
   do (harmonic=0.0) (resid 501 and name Y )
   do (harmonic=0.0) (not (resid 501))
   restraints harmonic exponent=2 end
end if

do (refx=x) ( all )
do (refy=y) ( all )
do (refz=z) ( all )

set seed=&md.seed end

{- Begin protocol to generate structures -- loop until done -}
while (&pdb.end.count > $nmr.counter) loop main
```

```
{- Set parameter values -}
parameter
   nbonds
      repel=0.80
      rexp=2 irexp=2 rcon=1.
      nbxmod=3
      wmin=0.01
      cutnb=6.0 ctonnb=2.99 ctofnb=3.
      tolerance=1.5
   end
end

evaluate ($nmr.trial.count = $nmr.trial.count + 1)

if (&md.type.initial = "coord") then
   if ($nmr.trial.count < &pdb.end.count) then
      evaluate ($coor_count = $nmr.trial.count)
      evaluate ($coor_count_init=0.)
   else
      evaluate ($coor_count_init=$coor_count_init+1)
      evaluate ($coor_count = $coor_count_init)
      if ($coor_count_init > &pdb.end.count ) then
         evaluate ($coor_count = 1)
      end if
   end if
   set remarks=reset end
   evaluate ($in_file = &pdb.in.name + "_" + encode($coor_count) + ".pdb")
   coor @@$in_file
else
   do (x=refx) ( all )
   do (y=refy) ( all )
   do (z=refz) ( all )
end if

if (&nmr.dani.axis = "fixed" ) then
   fix
      select=(resname ANI)
   end
elseif (&nmr.sani.axis = "fixed" ) then
   fix
      select=(resname ANI)
   end
end if

do ( vx = maxwell(0.5) ) ( all )
do ( vy = maxwell(0.5) ) ( all )
do ( vz = maxwell(0.5) ) ( all )

flags exclude *
      include bond angle dihe impr vdw
              noe cdih coup oneb carb ncs dani
              sani vean harm end

{- scaling of nmr restraint data during hot dynamics -}
@CNS_NMRMODULE:scalehot ( md=&md;
                          nmr=&nmr;
                          input.noe.scale=&md.hot.noe;
                          input.cdih.scale=&md.hot.cdih; )

evaluate( $k_bor = $fin_bor )
vean class allvean force = $k_bor 0 end

{- Zero the force constant of disulfide bonds. -}
parameter
   bonds ( name SG ) ( name SG ) 0. TOKEN
end
```

```
   if ($flag_tad=true) then

      {- initialize torsion dynamics topology for this iteration -}

      dyna torsion
         topology
            maxlength=&md.torsion.maxlength
            maxtree=&md.torsion.maxtree
            maxbond=&md.torsion.maxbond
            {- All dihedrals w/ (force constant > 23) will be locked -}
            {- This keeps planar groups planar -}
            kdihmax = 23.
            @CNS_TOPPAR:torsionmdmods
         end
      end
   end if

{- High temperature dynamics -}

   if ( &md.type.hot = "torsion" ) then

      igroup
     interaction (chemical h* ) (all) weights * 1 vdw 0. elec 0. end
     interaction (not chemical h* ) (not chemical h*) weights * 1 vdw &md.hot.vdw
     end
      end

      dyna torsion
         cmperiodic=500
         vscaling = false
         tcoupling = true
         timestep = &md.hot.ss
         nstep = &md.hot.step
         nprint = 50
         temperature = &md.hot.temp
      end
   else
      evalutate ($md.hot.nstep1=int(&md.hot.step* 2. / 3. ))
      evalutate ($md.hot.nstep2=int(&md.hot.step* 1. / 3. ))
      noe asymptote * 0.1  end
      parameter  nbonds repel=1.   end end
      igroup
     interaction (chemical h* ) (all) weights * 1 vdw 0. elec 0. end
     interaction (not chemical h* ) (not chemical h*) weights * 1 angl 0.4 impr 0.1
                 vdw &md.hot.vdw end
      end

      dynamics cartesian
         cmperiodic=500
         vscaling = true
         tcoupling=false
         timestep=&md.hot.ss
         nstep=$md.hot.nstep1
         nprint=50
         temperature=&md.hot.temp
      end

      noe asymptote * 1.0  end
      igroup
     interaction (chemical h* ) (all) weights * 1 vdw 0. elec 0. end
     interaction (not chemical h* ) (not chemical h*) weights * 1 vdw &md.hot.vdw end
      end

      dynamics cartesian
         cmperiodic=500
         vscaling = true
         tcoupling=false
         timestep=&md.hot.ss
```

```
      nstep=$md.hot.nstep2
      nprint=50
      temperature=&md.hot.temp
   end

   end if

{- The first slow-cooling with torsion angle dynamics -}

   flags include plan end

   {- Increase the disulfide bond force constants to their full strength -}
   parameter
      bonds ( name SG ) ( name SG ) 1000. TOKEN
   end

   evaluate ($final_t = 0)

   evaluate ($ncycle = int((&md.cool.temp-$final_t)/&md.cool.tmpstp))
   evaluate ($nstep = int(&md.cool.step/$ncycle))

   evaluate ($ini_vdw =  &md.hot.vdw)
   evaluate ($fin_vdw =  &md.cool.vdw)
   evaluate ($vdw_step = ($fin_vdw-$ini_vdw)/$ncycle)

   if (&md.type.cool = "cartesian") then

      evaluate ($vdw_step = (&md.cool.vdw/&md.hot.vdw)^(1/$ncycle))
      evaluate ($ini_rad  = 0.9)
      evaluate ($fin_rad  = 0.8)
      evaluate ($rad_step = ($ini_rad-$fin_rad)/$ncycle)
      evaluate ($radius=    $ini_rad)

      do (vx=maxwell(&md.cool.temp)) ( all )
      do (vy=maxwell(&md.cool.temp)) ( all )
      do (vz=maxwell(&md.cool.temp)) ( all )

   end if

   {- set up nmr restraint scaling -}

   evaluate ($kdani.inter.flag=false)
   evaluate ($ksani.inter.flag=false)
   evaluate ($kdani.cart.flag=false)
   evaluate ($ksani.cart.flag=false)
   if (&md.cart.flag=true) then
      evaluate ($kdani.inter.flag=true)
      evaluate ($ksani.inter.flag=true)
      @CNS_NMRMODULE:scalecoolsetup ( kdani=$kdani;
                                      ksani=$ksani;
                                      nmr=&nmr;
                                      input.noe.scale=&md.cool.noe;
                                      input.cdih.scale=&md.cool.cdih;
                                      input.ncycle=$ncycle; )
      evaluate ($kdani.cart.flag=true)
      evaluate ($ksani.cart.flag=true)
   else
      @CNS_NMRMODULE:scalecoolsetup ( kdani=$kdani;
                                      ksani=$ksani;
                                      nmr=&nmr;
                                      input.noe.scale=&md.cool.noe;
                                      input.cdih.scale=&md.cool.cdih;
                                      input.ncycle=$ncycle; )
   end if

   evaluate ($k_cen = $ini_cen)
   evaluate ($cen_fac = ($fin_cen/$ini_cen)^(1/$ncycle))
```

```
   evaluate ($bath  = &md.cool.temp)
   evaluate ($k_vdw = $ini_vdw)

   evaluate ($i_cool = 0)
   while ($i_cool <= $ncycle) loop cool
      evaluate ($i_cool = $i_cool + 1)

!vean - now the wall is erected

  evaluate( $k_cen  = $k_cen * $cen_fac )
  vean class allvean force = $k_bor $k_cen end


!end of vean

!flip leucine methyl groups

!aria flip (bondedto ((resid 30 or resid 49 or resid 74 or resid 85 or resid 87 or resid 98 or
!resid 109) and (name cd1 or name cd2)) and name h*) end

!flip valine methyl groups

!aria flip (bondedto ((resid 44 or resid 79 or resid 95 or resid 103 or resid 107) and
!(name cg1 or name cg2)) and name h*) end


      igroup
         interaction (chemical h*) (all) weights * 1 vdw 0. elec 0. end
         interaction (not chemical h*) (not chemical h*) weights * 1 vdw $k_vdw end
      end

      if ( &md.type.cool = "torsion" ) then
    dynamics  torsion
       cmremove=true
       vscaling = true
       tcoup = false
       timestep = &md.cool.ss
       nstep = $nstep
       nprint = $nstep
       temperature = $bath
    end
      else
    dynamics  cartesian
       cmremove=true
      vscaling = true
       tcoup = false
       timestep = &md.cool.ss
       nstep = $nstep
        nprint = $nstep
       temperature = $bath
    end
      end if

      if (&md.type.cool = "cartesian") then
      evaluate ($radius=max($fin_rad,$radius-$rad_step))
     parameter  nbonds repel=$radius   end end
         evaluate ($k_vdw=min($fin_vdw,$k_vdw*$vdw_step))
      else
         evaluate ($k_vdw= $k_vdw + $vdw_step)
      end if
      evaluate ($bath  = $bath  - &md.cool.tmpstp)



      @CNS_NMRMODULE:scalecool ( kdani=$kdani;
                                 ksani=$ksani;
```

```
                                nmr=&nmr; )

   end loop cool

{- A second slow-cooling with cartesian dyanmics -}

   evaluate ($flag_cart=false)
   if (&md.cart.flag=true) then
      if (&md.type.cool = "torsion") then

         evaluate ($flag_cart=true)

         dynamics torsion
         topology
            reset
         end
      end

         evaluate ($cart_nucl_flag=false)
         if ($nmr.nucl.num > 0) then
            evaluate ($cart_nucl_flag=true)
            parameter
               nbonds
                  repel=0
                  nbxmod=5
                  wmin=0.01
                  tolerance=0.5
                  cutnb=11.5 ctonnb=9.5 ctofnb=10.5
                  rdie vswitch switch
               end
            end
            flags include elec end
         end if

      evaluate ($ncycle=int((&md.cart.temp-$final_t)/&md.cart.tmpstp))
      evaluate ($nstep=int(&md.cart.step/$ncycle))

      evaluate ($vdw_step=(&md.cart.vdw.finl/&md.cart.vdw.init)^(1/$ncycle))
      evaluate ($ini_rad=0.9)
      evaluate ($fin_rad=0.8)
      evaluate ($rad_step=($ini_rad-$fin_rad)/$ncycle)
      evaluate ($radius=$ini_rad)


         @CNS_NMRMODULE:scalecoolsetup ( kdani=$kdani;
                                         ksani=$ksani;
                                         nmr=&nmr;
                                         input.noe.scale=&md.cart.noe;
                                         input.cdih.scale=&md.cart.cdih;
                                         input.ncycle=$ncycle; )

      do (vx=maxwell(&md.cart.temp)) ( all )
      do (vy=maxwell(&md.cart.temp)) ( all )
      do (vz=maxwell(&md.cart.temp)) ( all )

      evaluate ($bath=&md.cart.temp)
      evaluate ($k_vdw=&md.cart.vdw.init)

      evaluate ($i_cool = 0)
      while ($i_cool <= $ncycle) loop cart
         evaluate ($i_cool = $i_cool + 1)

         igroup
            interaction (chemical h*) (all) weights * 1 vdw 0. elec 0. end
            interaction (not chemical h*) (not chemical h*) weights * 1 vdw $k_vdw
            end
         end
```

```
         dynamics  cartesian
            vscaling = true
            tcoup = false
            timestep = &md.cart.ss
            nstep = $nstep
            nprint = $nstep
            temperature = $bath
         end

            if ($cart_nucl_flag=false) then
            evaluate ($radius=max($fin_rad,$radius-$rad_step))
            parameter  nbonds repel=$radius   end end
         end if
         evaluate ($k_vdw=min(&md.cart.vdw.finl,$k_vdw*$vdw_step))
         evaluate ($bath=$bath-&md.cart.tmpstp)

!! vean
             evaluate( $k_cen  = $k_cen * $cen_fac)

!! vean end

            @CNS_NMRMODULE:scalecool ( kdani=$kdani;
                                       ksani=$ksani;
                                       nmr=&nmr; )

      end loop cart

      end if
   end if

   {- reset torsion angle topology -}
   if ( $flag_tad=true ) then
      if ($flag_cart=false) then
         dynamics torsion
            topology
               reset
            end
         end
      end if
   end if


{- Final minimization -}

   {- turn on proton chemical shifts -}

   flags include prot end

   noe
      scale * &md.pow.noe
   end

   restraints dihedral
      scale = &md.pow.cdih
   end

   igroup interaction ( all ) ( all ) weights * 1 end end

   evaluate ($count=0 )
   evaluate ($nmr.min.num=0.)
   while (&md.pow.cycl > $count) loop pmini

      evaluate ($count=$count + 1)
      minimize powell nstep=&md.pow.step drop=10.0 nprint=25 end
      evaluate ($nmr.min.num=$nmr.min.num + $mini_cycles)
```

```
   end loop pmini

   {- translate the geometric center of the structure to the origin -}
   if ($num.dani > 0. ) then
   elseif ($num.sani > 0. ) then
   else
      show ave ( x ) ( all )
      evaluate ($geom_x=-$result)
      show ave ( y ) ( all )
      evaluate ($geom_y=-$result)
      show ave ( z ) ( all )
      evaluate ($geom_z=-$result)
      coor translate vector=( $geom_x $geom_y $geom_z ) selection=( all ) end
   end if

   @CNS_NMRMODULE:printaccept ( ave=$ave;
                                ave2=$ave2;
                                cv=$cv;
                                ener1=$ener1;
                                ener2=$ener2;
                                flag=&flg;
                                md=&md;
                                nmr=&nmr;
                                num=$num;
                                output=$nmr;
                                pdb=&pdb;  )

end loop main

   @CNS_NMRMODULE:calcave ( ave=$ave;
                            ave2=$ave2;
                            cv=$cv;
                            ener1=$ener1;
                            ener2=$ener2;
                            flag=&flg;
                            md=&md;
                            nmr=&nmr;
                            num=$num;
                            output=$nmr;
                            pdb=&pdb;  )


stop
```

Curriculum Vitae

Personal data

Name	Peter Haberz
Date of birth	April 10^{th}, 1979
Place of birth	Graz, Austria
Citizenship	Austrian

Education and studies

1985-1991	Primary and grammar School, Graz, Austria
06/1997	General qualification for university entrance
10/1997	Beginning of chemistry studies at the Karl-Franzens-University Graz, Austria
03/2002-10/2002	Student at The University of Queensland Brisbane, Australia
05/2003	Final examination in chemistry at the Karl-Franzens-University Graz, Austria
03/2002-5/2003	Diploma thesis with the title "Synthesis and complexation studies of bridged Calixarens" under the supervision of Prof. K. Wentrup and Prof. G. Kollenz
10/2003-03/2007	PhD. thesis with the title "Development and Application of NMR-Methods for Structural Investigations of Small Molecules and Proteins" in the department of NMR-based structural biology of Prof. Dr. Christian Griesinger at the Max Planck Institute for Biophysical Chemistry in Göttingen, Germany

Lebenslauf

Persönliche Daten

Name	Peter Haberz
Geburtstag	10.04.1979
Geburtsort	Graz
Staatsangehörigkeit	österreichisch

Schulbildung

1986-1990	Volkschule Eisteich, Graz
1990-1997	Akademisches Gymnasium, Graz
06/1997	Allgemeine Hochschulreife

Studium

11/1998	Beginn des Diplomstudienganges Chemie an der Karl-Franzens-Universität Graz
03/2002-10/2002	Student an der University of Queensland Brisbane, Australien
05/2003	Diplomprüfung
03/2002-05/2003	Anfertigung der Diplomarbeit mit dem Titel ,,Synthese und Komplexierungseigenschaften überbrückter Calixarene" am Lehrstuhl für Chemie von Prof. Dr. Gert Kollenz und am Department of Chemistry an der University of Queensland
10/2003-03/2007	Anfertigung der vorliegenden Arbeit mit dem Titel ,,Entwicklung und Anwendung von NMR-Methoden zur strukturellen Aufklärung von Klein- und Biomolekülen" in der Abteilung NMR-basierte Strukturbiologie von Prof. Dr. Christian Griesinger am Max Planck Institut für Biophysikalische Chemie in Göttingen