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NMR Chemical Shielding in Cyclosarcosyl

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ABSTRACT

NMR chemical shifts have a role to play when determining the structure of proteins by NMR methods through their dependence on the local conformation. Large inequivalences are seen in the ^{1}H NMR chemical shifts of the CαH in the cyclosarcosyls making these rigid ring systems ideal for studying the neighbouring group effect of a peptide linkage. NMR chemical shielding calculations are presented demonstrating that with an appropriate electronic model and basis set the CαH inequivalence can be rationalised. However, in the case of cyclotrisarcosyl the inequivalence is best understood in terms of a steric effect owing to the close proximity of the inner Hα. While for cyclotetrasarcosyl, although significant distant, non-bonded, effects of a peptide are seen, the vast majority of the large, greater than 2 ppm, inequivalence is attributable to the local conformation between adjacent sarcosine.

Keywords: NMR chemical shielding

Introduction

Chemical shifts of resonances arising from nuclei in a protein reflect their local environment and from the earliest studies were recognised as being invaluable structural probes.[1,2] From a structural point of view the important conformation dependent shifts or secondary chemical shifts are those arising from neighbouring group effects.[3,4] Neighbouring group effects are through space perturbations of the local electronic structure of a nucleus by the group in question without changes in the bonding electron density. At the time it was hoped these secondary chemical shifts could be used to determine the solution structure of proteins. Larger secondary chemical shifts were recognised as being produced by the magnetic anisotropy of aromatic rings, so called ring currents, and these could be calculated with a fair accuracy.[5,6] Along with aromatic rings the other principal neighbouring group present in a protein is the peptide group which can be expected to have a significant effect on backbone resonances. Unfortunately, structural approaches based on chemical shifts were greatly hindered by the inability of semi-classical methods to calculate accurately electric field and magnetic anisotropy effects arising from peptide groups.[7] Little further work was put into developing these ideas since around the same time NOE methods were demonstrated to be a more powerful tool, enabling the construction of a three-dimensional structure for a protein in solution.[8]

Modern approaches to the use of chemical shifts in structure determination have largely overcome the problems arising from the semi-empirical methods by utilising mixed procedures based on sequence homology and the calculation of chemical shifts using protein co-ordinates.[9] A number of reviews describe in detail the range of methods available and their associated programs.[9,10] One of the most successful programs appears to be SHIFTX2, with a correlation coefficient of 0.974 and rms error of 0.12 ppm for $^{1}H\alpha$.[11] In addition, computational approaches have been developed for the rapid prediction of protein structures based on NMR chemical shifts and molecular dynamics.[12] Although still very much a specialised area, NMR chemical shifts remain invaluable in the study of intrinsically disordered proteins [13,14] and as an additional complementary technique in the refinement of NMR structures.[15]

Methylene groups such as those associated with the $C\alpha$ carbon of glycine or sarcosine are of particular interest when investigating conformational effects because the through bond interactions on the two methylene hydrogens will be similar. The chemical shift inequivalence considered here is the difference in the ^{1}H NMR chemical shift seen for these two $C\alpha$ hydrogens. Large $^{1}H\alpha$ chemical shift inequivalences are seen in a number of rigid cyclosarcosyls, for example, cyclotrisarcosyl (1.37 ppm) and cyclotetrasarcosyl (2.03 and 0.71 ppm in $CDCl_3$).[16] These cannot be replicated by semi-empirical methods for calculating NMR chemical shifts, even the more recent programs fail to give such large differences. Similarly sequence based methods substituting the sarcosine by a glycine, but maintaining the peptide torsion angles, fail to predict a large chemical shift inequivalence presumably because proteins determined by X-ray crystallography either do not have an appropriate set of torsion angles or, if they do, also have an inherent flexibility like the cyclic glycyl structures. In this respect the cyclic sarcosyl peptides may be seen as a rather unique set of structures having little bearing on the wider applicability of using chemical shifts in protein structure prediction. On the other hand, N-substituted glycine oligomers, of which sarcosine is perhaps the simplest, form the class of compounds known as peptoids [17]. Peptoids can show biological activity and are an active research area [18]. Given the N-substitution, it might be expected that larger methylene group inequivalences will be seen for rigid cyclic peptoids and thus relevant in their conformational analysis. An example of this being so is cyclo-$[(cis)Nme_2\text{-}(trans)Nme_2]_2$ {Nme = N-methoxyethyl glycine} [19] which can thought of as having a structural similarity to cyclooctasarcosyl. Here a methylene inequivalence of 1.64 ppm can be identified.

In the original work [7] the possibility of steric effects [20] arising from transannular interactions in these rigid constrained cyclic structures was overlooked. Subsequently, large ^{1}H NMR chemical shift inequivalence, in excess of 2 ppm were reported for the $H\alpha$ resonances in N,N'-[Dimethyl-(2,2'-dithiobisacetyl)] ethylenediamine.[21] Furthermore, ab initio calculations of the $^{1}H\alpha$ NMR chemical shifts at DFT using B3LYP functionals and 6-31G* basis set demonstrated that a chemical shift inequivalence of the order of 2 ppm can be replicated. The authors carried out a fragment approach combined with the ab initio calculations to establish the contributions from the peptide group anisotropy and steric effects. They found 3-21G* performed as well as the larger 6-31G* basis set. This is perhaps surprising since accurate ^{1}H NMR chemical shifts are known to require the use of extended basis sets with polarisation which make them more demanding on computation time.[22] For example, in the calculation of the chemical shifts for sarcosine itself DFT/B3LYP/6-311++G** was used.[23] Although sometimes smaller basis sets, such as 6-31(d) are reported as having small errors, 0.3 ppm for ^{1}H, this is of the same order as the secondary shifts themselves.[24] Even larger basis sets DFT/B3LYP/6-311G(d)[25] show errors, 0.5-1.00

ppm similar in magnitude to the secondary shifts. Large chemical shielding differences of over 2 ppm have also been calculated for the protons of the N-methyl group in the trans conformation of N-methyl acetamide[26] suggesting the chemical shift inequivalence seen in the cyclosarcosyls is an intra-residue effect and therefore gives no long range structural information. Thus, the precise conformational features that give rise to large Cα methylene group ^{1}H NMR chemical shift inequivalences in peptides is unclear. Nevertheless, the idea remains that the conformation places the peptide group in a particular orientation with respect to the methylene group thereby causing the observed chemical shift inequivalence by a through space neighbouring group effect. The aims of the current work are twofold. First, to establish the origin of the large ^{1}Hα NMR chemical shift inequivalences seen in cyclosarcosyl compounds, specifically cyclotri and cyclotetrasarcosyl and to see whether steric effects contribute. Second, whether replicating the ^{1}Hα chemical shift inequivalence requires larger basis sets.

Methods

Experimental values for the ^{1}H NMR chemical shifts of cyclo- tri and tetrasarcosyl were obtained from solution NMR reports. [7,16] Geometry optimisation and NMR chemical shielding calculations were carried out using Gaussian 03 [27] with the X-ray co-ordinates as starting structures. [28,29] Cyclotrisarcosyl was used first to explore the dependence of the calculated ^{1}Hα NMR chemical shift inequivalence on the electronic model and basis set used. Thus, the lowest energy conformation from the cyclotrisarcosyl X-ray starting structure was optimised at the HF, DFT and MP2 level of theory using a range of basis sets, STO-3G to 6-311++G(3pd,3df). Vibrational frequencies for the optimised structures were determined to ensure the structures were minima as shown by the absence of imaginary frequencies. The NMR chemical shielding was then determined using DFT/B3LYP/6-311++G(3df,3dp). When considering the effect of the basis set on the NMR chemical shielding for a given optimised structure DFT/B3LYP/STO-3G to 6-311++G(3pd,3df) were used. Vibrational corrections were not carried out since extensive calculations have shown that these are better treated as a simple scaling and systematic error.[30] The scaling will introduce an error of about 5% while the systematic error is removed by looking only at the chemical shielding differences. As only the ^{1}Hα NMR chemical shift inequivalence was of interest referencing of the chemical shielding was not generally carried out. However, the calculated NMR chemical shielding for the methylene hydrogens are given in the Supplementary Information and lay in the range 25.99 – 30.53 ppm. In comparison the NMR chemical shielding for the reference compound tetramethylsilane with both the structure optimisation and NMR calculation at DFT/B3LYP/6-311++G(3df,3dp) was 31.68 ppm. Within the formalism of the ab initio calculations reported here errors (differences between the actual and exact calculated chemical shielding) will arise because of the neglect of configuration interaction, intrinsic error, and the use of an incomplete basis set. The sum of the intrinsic error and basis set limit error is the apparent error. [31] As is the common practise, it is Implicitly assumed that the apparent errors tend to cancel when looking at the chemical shielding difference between two hydrogens that have a very similar local bonding connectivity.

In order, to determine the contributions made to the NMR chemical shift inequivalence from differing peptide groups in cyclotrisarcosyl, a fragment approach was taken whereby a sarcosyl residue was removed from the structure and replaced by terminating hydrogens. The steric component to the chemical shift inequivalence could then be determined from the quantum mechanical calculations as the residual difference when the peptide group contribution was removed. First the structure was optimised at DFT/B3LYP/6-

31G with the NMR chemical shifts determined at DFT/B3LYP/6-311++G(3d,3p). For fragment 1, the distant peptide group {CO-N(Me)} was removed and a hydrogen added to each of the remaining CH_2 groups so retaining any transannular interactions. No optimisation of the new structure was carried out. The two terminal CH_3 were then replaced by a hydrogen each, fragment 2, to remove the two transannular interactions between the sarcosyl methylene hydrogens. Fragment 3 was created by replacing only one of the terminal CH_3 by a hydrogen hence leaving one transannular interaction.

Steric contributions to the chemical shifts were also calculated using the semi-classical approach through the equation (1)

$$\Delta\delta = -150\sum_i \cos\theta_i \exp\left(-2.671 r_i\right) \qquad (1)$$

where θ_i is the angle between CH bond and other interacting proton, and r_i is the distance between the interacting protons[20] along with the DFT/B3LYP/6-311G optimised structures to determine the angle and distance.

The optimised structure for cyclotetrasarcosyl was found first using DFT at 6-311++G(3df,3dp) and then the NMR chemical shielding calculated using different basis sets 3-21G to 6-311++(3pd,3df).

Calculations were carried out for a variety of solvents (benzene, diethyl ether, chloroform, dichloromethane and water) with cyclotetrasarcosyl using the IEF-PCM model for all the solvents where both the geometry optimisation and NMR chemical shielding calculations were based on DFT/B3LYP/6-311++G(df,pd). A similar fragment approach to cyclotrisarcosyl was adopted for cyclotetrasarcosyl. Sets of fragments were formed by removing one, two or three peptide groups. Owing to the symmetry of the molecule only one cis or trans group had to be removed for the single peptide group removal and a dipeptide fragment including a methylene group type of either A or B for the two peptide group removal. (Type A and B are defined in the Results section). Tripeptide removal left a cis or trans peptide group with either a modified B or A type methylene group respectively. Hydrogens were then added to the methylene groups left after removing the CO-NMe group thereby terminating the molecule with two methyl groups. To a first approximation we can assume that the through bond effect of the replacement by hydrogen is isotropic and thus not contribute to an inequivalence in the NMR chemical shielding of the original methylene hydrogens. This being so we can use the chemical shielding seen for these hydrogens as an indicator of the adjacent and same residue chemical shielding contributions. No geometry optimisation was carried out for any of the fragments to ensure the same peptide conformations were adopted. The single trans peptide was used as the starting conformation for trans N,N' dimethyl acetamide whose geometry was then optimised and NMR chemical shielding calculated at DFT/B3LYP/6-11++ G(3d,3p).

Results and Discussion

Cyclotrisarcosyl

Cyclotrisarcosyl has been found by X-ray [29] and NMR [16] to have C_3 symmetry characterised by a very rigid all cis conformation. The experimental ^{1}H NMR chemical shift inequivalence for the Cα hydrogens is 1.37 ppm (doublets at 4.94 ppm and 3.57 ppm) .[16]

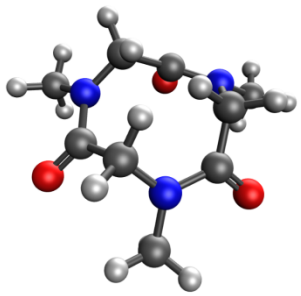

Figure 1. Structure of cyclotrisarcosyl

Ring inversion, as seen by coalescence in the NMR spectrum, only takes place at a temperature of 166°C implying a ring inversion energy of 84.1 kJ mol^{-1}.[16] Previous calculations at DFT/B3LYP/6-311++G(d,p) have explored the potential energy surface for this compound and determined the complete ring inversion process requires 87.4 kJ mol^{-1}, [32] confirming the rigid nature of the cyclic structure. A direct comparison of the structures calculated here with the earlier work is hindered by the absence of any absolute energies in the earlier paper. However, the close similarity between the calculated structure for the ground state cis-cis-cis conformation with all the CO pointing up at the same theory level as Alvarez et al [32] and the X-ray crystal structure is strong evidence for the consistency of the calculations (Supplemental Information). Owing to the rigidity of the molecule, cyclotrisarcosyl is an excellent model system for examining the effect of structure optimisation and the electronic model/basis set on the NMR Cα methylene proton inequivalence. One indicator of the likely effect of the changing structures caused by the level of theory and basis sets used is the $H\alpha_{1,2}CCO$ torsional angle since it describes the orientation of the Cα hydrogens with respect to the carbonyl group. Here the two torsional angles vary from DFT/B3LYP/6-31G (-152.12°, -34.53°) to MP2/6-31G (-150.21°, -32.29°) with DFT/B3LYP/6-311++(3df,3pd) (-151.11° – 34.03°) lying between. The calculated $^1H\alpha$ NMR chemical shift inequivalence determined using DFT/B3LYP/6-311++G(3df,3dp) for the C_3 symmetrised structures determined using these electronic models and basis set are shown in Figure 2

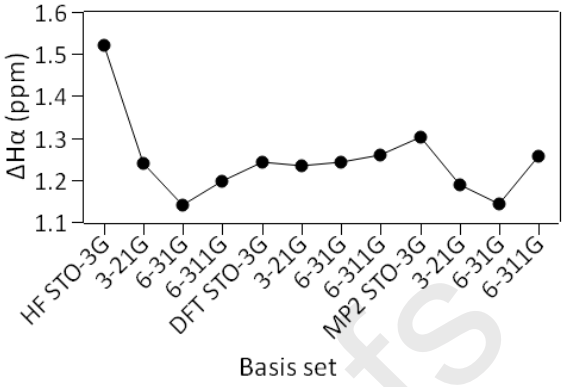

Figure 2. NMR chemical shifts calculated at DFT/B3LYP/6-311++G(3df,3dp) with the structure determined for a C_3 symmetrised cyclotrisarcosyl molecule using the stated electronic model and basis set. The line is drawn to guide the eye.

Clearly the small changes in the overall structure, as reflected in the torsional angle variation, cause a spread in the calculated methylene hydrogen ^1H NMR chemical shift inequivalence. However, overall, the splitting calculated is rather insensitive to the structure optimisation which is probably simply a reflection of the rigidity of the molecule. Omitting the HF/STO-3G structure, the calculated value for the splitting though at 1.22 ± 0.05 ppm is somewhat smaller than the observed splitting of 1.37 ppm.

Turning now to the effect of the basis set on the chemical shift inequivalence using the C_3 symmetrised structure optimised at DFT/B3LYP/6-311++G(3df,3pd), Figure 3. Inequivalences close to the experimental value can only be obtained when using (df, pd) diffuse and high angular momentum orbitals. This contrasts with results reported elsewhere.[21] Any possible contribution arising from the solvent was investigated by repeating the calculation in chloroform, first optimising the C_3 symmetrised structure at DFT/B3LYP/6-311++G(3df,3pd). Although a change in the $H\alpha_{1,2}CCO$ torsional angles from (-151.11° – 34.03°) to (-150.67°, -33.70°) was seen only small differences were observed in the calculated chemical shifts, Figure 4. Showing

that the effect of the chloroform solvent is rather small.

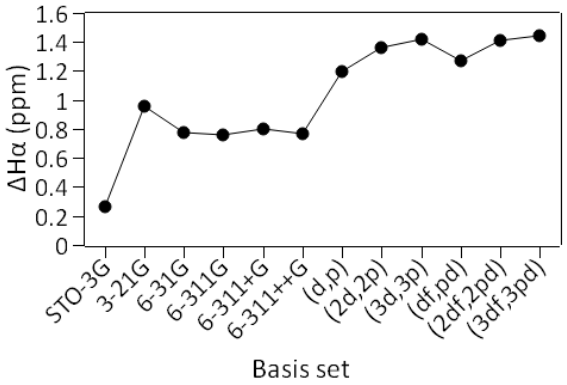

Figure 3. NMR chemical shifts calculated using DFT/B3LYP with the stated basis set. Structure determined for a C_3 symmetrised cyclotrisarcosyl molecule at DFT/B3LYP/6-311++G(3df,3pd). The line is drawn to guide the eye.

Clearly, it is possible to reproduce CαH splittings of the correct magnitude with errors of -0.07 ppm for 6-311++ (df,pd) to + 0.10 ppm for 6-311++ (3df,3pd). Satisfactory agreement was also seen between the calculated (4.79 ppm, 3.34 ppm) and experimental chemical shifts (4.94 ppm, 3.57 ppm). A conclusion which is hardly surprising given the extensive literature on NMR chemical shielding calculations. Yet, as noted by others, there remains the question of how to choose the appropriate electronic model and size of basis set if accurate calculation of the chemical shift inequivalence is required.

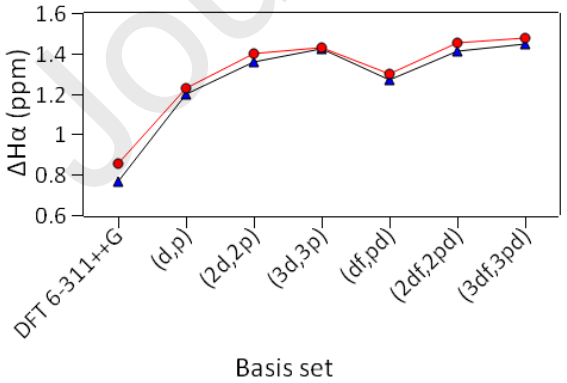

Figure 4. Comparison of the NMR chemical shifts in vacuum (●) and in chloroform (▲) calculated at DFT/B3LYP with the stated basis set for structures determined for a C_3 symmetrised cyclotrisarcosyl molecule at DFT/B3LYP/6-311++G(3df,3pd). The lines are drawn to guide the eye.

In the light of possible steric effects an attempt was made to determine the relative contributions of the peptide groups and transannular steric interactions by a fragment approach. In the favoured conformation for cyclotrisarcosyl the transannular interactions are between the CαH themselves, with the N-Me groups pointing away from the ring. The calculated NMR chemical shift inequivalences are shown in Table 1.

Table 1. ^{1}H NMR chemical shift differences between the two Cα methylene hydrogen for the original complete cyclotrisarcosyl molecule and the three fragments derived from it.

Fragment	Δ (ppm)
Original	1.3466
1	1.3735
2	0.1349
3	0.6507

Removing the distant peptide group, fragment 1, causes only a small change in the calculated inequivalence, of less than 0.03 ppm, which is perhaps not surprising as it lies about 4 Å distant. What is striking though is that removing both transannular interactions, fragment 2, almost eliminates the NMR chemical shift inequivalence while removing one, fragment 3 just about halves the inequivalence. This would suggest the two neighbouring peptide groups have no significant net contribution to the inequivalence of the CαH and that virtually all the experimental chemical shift inequivalence arises from a steric effect and not the peptide groups. On reflection this is consistent with the small transannular distance of only 1.95 Å between the methylene hydrogens pointing into the ring. Using the formula for steric effect on ^{1}H nuclei proposed by Cheney [17] it is possible to calculate a steric interaction of 0.56 ppm for each Hα pair. Since there are two

interactions the total steric shift would be 1.12 ppm, very close to the experimental value. The basis set dependence is thus indicating that a large basis set is required to accurately reproduce steric interactions.

Cyclotetrasarcosyl

The crystal structure for cyclotetrasarcosyl shows a rigid ring conformation with the CαH hydrogens held in significantly different environments like cyclotrisarcosyl but unlike cyclotrisarcosyl which has all cis peptide groups, cyclotetrasarcosyl has alternating trans (t) and cis (c) peptide groups $t_d(1)$ $c_d(2)$ $t_u(3)$ $c_u(4)$ with the trans and cis peptides having CO pointing up (u) and down (d). The two types of methylene group environments present are A: $t_{d,u}(1,3)$ $c_{d,u}(2,4)$ and B: $c_{d,u}(2,4)t_{u,d}(3,1)$

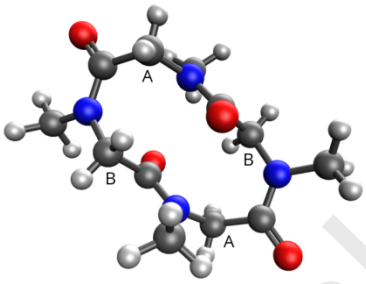

Figure 5. Structure of cyclotetrasarcosyl showing the labelling of the two types A and B of inequivalent methylene groups.

In the ^{1}H NMR spectrum two sets of resonances are seen for the CαH protons with chemical shift differences of 2.03 (doublets at 5.30 ppm, 3.27 ppm) and 0.71 ppm (doublets at 4.36 ppm, 3.65 ppm) in chloroform. These have been assigned to the CαH labelled A and B respectively in the structure.[7] Chemical exchange is seen in the ^{1}H NMR spectrum of cyclotetrasarcosyl beginning at 155°C and ending at 200°C in a single resonance suggesting the energy barrier to ring inversion is comparable if not higher to that in cyclotrisarcosyl.[16] In the light of the calculations on cyclotrisarcosyl geometry optimisation was carried out using DFT/B3LYP at 6-311++G(3df,3dp). ^{1}H NMR chemical shieldings were then calculated using DFT/ B3LYP with different basis sets as shown in Figure 6.

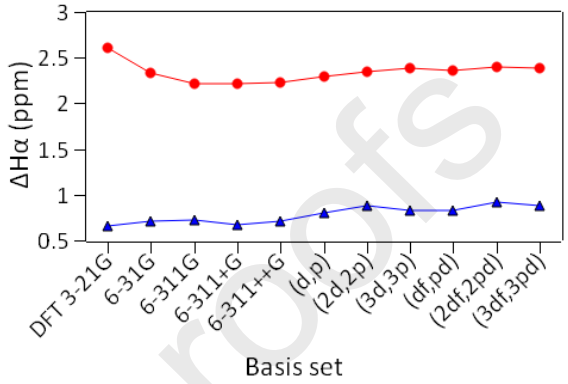

Figure 6. NMR chemical shifts calculated using DFT/B3LYP with the stated basis set, type A (●) and type B (▲). Structure determined for cyclotetrasarcosyl at DFT/B3LYP/6-311++G(3df,3dp). The lines are drawn to guide the eye.

In contrast to the earlier calculations for cyclotrisarcosyl, the experimental NMR data for cyclotetrasarcosyl can be reproduced by even very limited basis sets such as 6-31G perhaps suggesting the lack of steric effects. Although the exact agreement will depend on the actual basis set used, nevertheless it could be obtained with errors of + 0.35 ppm for the larger 2.03 ppm inequivalence and +0.16 ppm for the smaller 0.71 ppm inequivalence at 6-311++G(3df,3dp). Similarly, good agreement was seen between the calculated (A: 5.49 ppm, 3.10 ppm B: 4.36 ppm, 3.48 ppm) and experimental (A: 5.30 ppm, 3.27 ppm B: 4.36 ppm, 3.65 ppm) chemical shifts using the same basis set. Comparatively large errors are seen, of the order of 10%, but it must be borne in mind that these correspond to only 1% or so of the absolute shielding.

Previous work has shown that the smaller chemical shift inequivalence seen in cycloterasarcosyl may have a contribution from a solvent effect whereas the larger splitting is insensitive to the solvent as reflected in the

dependence on the solvent relative permittivity.[7] Geometry optimisation brought about only a small variation in the cyclotetrasarcosyl conformations: a range of ±1° in the peptide group torsion angles ∠ $OCNC_{Me}$ and ±1.5° in the torsion angles between the peptide groups and the CαH. The results shown in Figure 7 for the NMR chemical shift inequivalence qualitatively reproduce the experimental data with the larger splitting {experimental: 2.0-2.2 ppm}[7] showing only a small variation with the solvent reaction field while a more marked variation is seen for the smaller splitting {experimental: 0.7-0.2 ppm}.

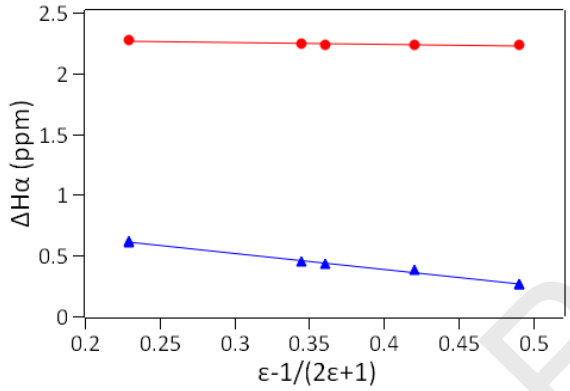

Figure 7. Variation in the CαH methylene ^{1}H NMR chemical shift inequivalence as a function of the solvent reaction field. Regression lines shown, (Type A : ● R^2 = 0.664 Type B: ▲R^2 = 0.993)

In order, to determine whether the CαH chemical shift inequivalence arose from neighbouring or distant peptide groups, fragments of the original cyclotetrasarcosyl were created. NMR shielding calculations were made at DFT/6-311++G(3d,3p) and are shown in Table 2. Overall, the chemical shift inequivalence can be thought of as arising from adjacent peptide groups through the backbone torsion angles adopted, distant peptide groups and steric effects. Inspection of the optimised structure for cyclotetrasarcosyl, indicates the only possibility of a steric interaction through a transannular interaction is between the inner pair of hydrogens from methylene groups A and B that are 2.11 Å apart. The angle for the inner A type hydrogen is 121.2° while that for the inner B type is 102.9°. Hence steric contributions of -0.28 ppm and -0.11 ppm respectively can be expected for the A and B type methylene groups.

Table 2. ^{1}H NMR chemical shielding differences (Δ) in ppm for the Cα methylene hydrogens.

Fragment	$t_d(1)c_d(2)$ A	$c_d(2)t_u(3)$ B	$t_u(3)c_u(4)$ A	$c_u(4)t_d(1)$ B
Original	2.3862	0.8262	2.3862	0.8262
No $t_d(1)$	-0.2787*	0.4172	2.5744	0.9518*
No $c_u(4)$	2.512	0.8568	2.4434*	0.2972*
No $c_u(4)t_d(1)$	-0.2498*	0.4634	2.7558*	-
No $t_u(3)c_u(4)$	2.6723	1.1262*	-	-0.2678*
Only $t_d(1)$	2.8226*	-	-	-0.2196*
Only $c_d(2)$	0.0412*	0.687*	-	-

* Indicates terminal methyl group.

Steric shifts are also possible between the CαH and the hydrogens of the N-methyl groups however, with the typical closest distances of 2.4 Å and angles θ in the range 60° - 90° these are predicted to be small, around 0.1 ppm, and thus not readily identifiable by the fragment approach used below. Therefore, the earlier failure to calculate the observed chemical shift inequivalence in cyclotetrasarcosyl cannot be attributed to the omission of steric effects.

Adjacent peptide group effects form the basis for identifying the type of secondary structure in a protein backbone while distant peptide groups can in principle inform on the tertiary structure. Distant peptide groups can make a substantial contribution to the splitting, as seen for the methylene groups B, with 0.41 ppm from $t_d(1)$ on methylene $c_d(2)t_u(3)$ at a distance of 2.94 Å between the nitrogen and the closer methylene proton. Yet, the large reduction of 0.65 ppm in the NMR shielding seen for the type B methylene group $c_u(4)t_d(1)$ when $c_u(4)$ is removed is an adjacent residue effect. Furthermore, for methylene group A much of the CαH chemical shift inequivalence arises from the orientation of the trans peptide group

formed by the nitrogen of the same residue, 2.3 Å distant. Compare the value seen for the A-type methylene group $t_d(1)c_d(2)$ in the no $t_d(1)$ and no $c_u(4)t_d(1)$ fragments with the original splitting. A similar observation has been noted about Z amide conformations.[21] All fragments that maintain the nitrogen trans peptide group show splittings in excess of 2.4 ppm. Thus the 2 ppm or so chemical shift inequivalence seen in cyclotetrasarcosyl must arise because of the specific peptide torsion angles seen for the CαH with respect to the peptide group formed by the nitrogen of that residue. In particular, one hydrogen lies parallel to the CO bond direction ($\angle$ $H_{a1}C_aNC_{Me}$= -180°, $\angle$ $OCNC_{Me}$ = 178°) while the other is effectively perpendicular to it ($\angle$ $H_{a2}C_aNC_{Me}$= -61°). Confirmation of this interpretation is provided by the ^{1}H NMR shielding that has been calculated for trans N-methyl acetamide[26], Table 8 in the reference. In the trans conformation the N-methyl adopts a geometry with H_c and H_g in similar positions to the N terminus cyclotetrasarcosyl trans fragment. ^{1}H NMR shielding of 26.9 (H_c) and 29.1(H_g) were calculated at DFT/B3LYP/ 6-31G(d,p) giving a methylene hydrogen chemical shift inequivalence of 2.2 ppm a similar value, in excess of 2 ppm, to that calculated for the cyclotetrasarcosyl fragments above. Furthermore, calculations using DFT/B3LYP/6-311++ G(3d,3p) on N,N' dimethyl acetamide for both geometry optimisation and NMR shielding reveal a conformation where two of the methyl protons adopt the same conformation as in cyclotetrasarcosyl ($\angle$ $H_{a1}C_aNC_{Me}$= -180°, $\angle$ $H_{a2}C_aNC_{Me}$= -61.6° $\angle$ $OCNC_{Me}$ = 177.7°) and the NMR shielding is found to be 26.8 ppm and 29.7 ppm respectively, giving a splitting of 2.9 ppm. Significantly the unusual shielding is associated with deshielding of the proton Hα1, where the CH bond lies parallel to the CO and 2.3 Å distant. In terms of the Ramachandran plot the ϕ, ψ values for the trans peptide group are +/-118° and -/+ 69°, while Hα2 can be placed in an equivalent position, as would be required for any L- amino acid other than glycine, with ϕ = -120°. Inspection of a database of ^{1}Hα chemical shifts (RefDB[33]) indicates that extreme deshielding such as seen in cyclotetrasarcosyl is rare. Nevertheless, it has been observed. On example being the GABA receptor associated protein GABARAP where Ala 108 (in the sequence of the protein studied by NMR there are an extra two residues hence appears as Ala 110) has an observed Hα chemical shift of 6.5 ppm.[33] In comparison, the ^{1}H chemical shift predicted for this residue by SHIFTX2[11] is only 5.77 ppm, a discrepancy of 0.71 ppm. Examination of Ala 108 in the crystal structure of 1GNU[34] reveals ϕ = -106° and hence the possibility of a significant peptide group shift. Confirmation of a significant deshielding seen when ϕ = -106° is provided by DFT/B3LYP/6-311++ G(3d,3p) calculations on N,N' dimethyl acetamide where the calculated deshielding is 1.95 ppm for the hydrogen corresponding to the Hα in alanine. Thus, a large chemical shift inequivalence for the CαH of 2 ppm or so in a protein or peptoid is indicative of a trans peptide group with ϕ = +/-118° and ψ = -/+ 69°

Conclusions

Shielding calculations were carried out on cyclo tri- and tetrasarcosyl with the aim of understanding the origin of the large CαH chemical shift inequivalence seen in the ^{1}H NMR spectra of these cyclic molecules. Structural calculations on cyclotrisarcosyl confirmed that the cic-cis-cis uuu conformer to be the lowest energy one, holding the methylene hydrogens in quite distinct environments. While it was possible to calculate the observed CαH NMR chemical shift inequivalence in cyclotrisarcosyl , large basis sets with diffuse and polarisable orbitals (3df, 3pd) were required. This is understandable in the light of the results from a fragment approach that demonstrated the importance of steric effects in the shielding rather than the neighbouring peptide groups. Indeed, the CαH NMR chemical shift inequivalence could be calculated quite accurately using the simple formula of Cheney. In the case of cyclotetrasarcosyl the lowest energy conformer is essentially identical to the

crystal structure. However, in contrast to cyclotrisarcosyl only moderately sized basis sets were required to calculate the observed CαH chemical shift inequivalence. While a fragment approach showed the importance of the neighbouring group effect of the peptide group and comparative absence of steric effects. More precisely, the large chemical shift inequivalence for the CαH could be attributed to a specific conformation, $\phi \approx$ -120°, of a trans N-terminus peptide group that places one CαH bond parallel with the CO bond. Although the CαH chemical shift inequivalence observed in cyclotetrasarcosyl is large and cannot be replicated by semi-empirical methods, it is more of a curiosity because large deshieldings are rare in the ^{1}H NMR spectra of proteins and the correct conformations would be identified by a sequence/shift homology in the absence of accurate calculated chemical shifts using semi-empirical methods.

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References

[1] H. Sternlicht, D. Wilson, Magnetic Resonance Studies of Macromolecules. I. Aromatic-Methyl Interactions and Helical Structure Effects in Lysozyme Biochemistry 6 (1967) 2881-2892.

[2] C.C. McDonald, W.D. Phillips, Proton magnetic resonance spectra of proteins in random-coil configurations J. Am. Chem. Soc. 91 (1969) 1513-1521.

[3] S.J Perkins, K. Wüthrich, Ring current effects in the conformation dependent NMR chemical shifts of aliphatic protons in the basic pancreatic trypsin inhibitor Biochem. Biophys. Acta 576 (1979) 409-423.

[4] I.D. Campbell, C.M. Dobson, R.J.P Williams, Assignment of the ^{1}H n.m.r. spectra of proteins Proc. Roy. Soc. A 345 (1975) *345*, 23-40.

[5] K. Wüthrich, NMR in Biological Research, North-Holland, Amsterdam, **1976**.

[6] S.J. Perkins, L.N. Johnson, D.C. Phillips, R.A. Dwek, Conformational changes, dynamics and assignments in ^{1}H NMR studies of proteins using ring current calculations Hen egg white lysozyme FEBS Letters 82 (1977) 17-22.

[7] N.J. Clayden, R.J.P Williams, Peptide Group Shifts J. Magn. Reson. 49 (1982) 383-396.

[8] K. Wüthrich, NOBEL LECTURE: NMR Studies of Structure and Function of Biological Macromolecules *Bioscience Rep.* 23 (2003) 119-168.

[9] M.V. Berjanskii, D.S. Wishart, Unraveling the meaning of chemical shifts in protein NMR *BBA - Proteins and Proteomics* 1865 (2017) 1564-1576.

[10] S. Nerli, A.C. McShan, N.G. Sgourakis, Chemical shift-based methods in NMR structure determination Prog. Nucl. Magn. Reson. Spectrosc. 106-107 (2018) 1-25.

[11] B. Han, Y. Liu, S. Ginzinger, D.S. Wishart, SHIFTX2: significantly improved protein chemical shift prediction *J. Biomol. NMR* 50 (2011) 43-57.

[12] A. Cavalli, X. Salvatella, C.M Dobson, M. Vendruscolo, Protein structure determination from NMR chemical shifts *PNAS* 104 (2007) 9615-9620.

[13] J. Kragelj, V. Ozenne, M. Blackledge, M.R. Jensen, Conformational propensities of intrinsically disordered proteins from NMR chemical shifts ChemPhysChem 14 (2013) 3034-3045.

[14] W.M. Borcherds, G.W. Daughdrill, Using NMR Chemical Shifts to Determine Residue-Specific Secondary Structure Populations for Intrinsically Disordered Proteins Methods Enzymol. 611 (2018) 101-136.

[15] D.S. Wishart D.A. Case, Use of chemical shifts in macromolecular structure determination. Methods Enzymol. 338 (2001) 3-34.

[16] J. Dale, K. Titlestad, Cyclic oligopeptides of sarcosine (N-methylglycine) Chem. Commun. (1969) 656-659.

[17] R.J. Simon, R.S. Kania, R.N. Zuckermann, C.K. Marlowe Peptoids: a modular approach to drug discovery PNAS 89 (1992) 9367-9371

[18] J. Sun, R. N. Zuckermann, Peptoid Polymers: A Highly Designable Bioinspired Material ACS Nano 7 (2013) 4715–4732

[19] R. Schettini, C. Costabile, G. Della Sala, V. Iuliano, C. Tedesco, I. Izzo, F. De Riccardis, Cation-Induced Molecular Switching Based on Reversible Modulation of Peptoid Conformational States J. Org. Chem. 83 (2018) 12648−12663

[20] B.V. Cheney, Magnetic deshielding of protons due to intramolecular steric interactions with proximate hydrogens J. Am. Chem. Soc. 90 (1968) 5386-5390.

[21] R.B. Maharajh, J.P. Snyder, J.F. Britten, R.A. Bell, Synthesis, high-field NMR, X-ray structure, and conformational analysis of a 10-membered diamide disulfide ring Can. J. Chem. 75 (1997) 140-161.

[22] D. Flaig, M. Maurer, M. Hanni, K. Braunger, L. Kick, M. Thubauville, C. Ochsenfeld, Benchmarking Hydrogen and Carbon NMR Chemical Shifts at HF, DFT, and MP2 Levels J. Chem. Theory Comput. 10 (2014) 572-578.

[23] Z. Atieh, A.R. Allouche, A. Lazariev, D. Van Ormondt, D. Graveron-Demilly, M. Aubert-Frécon, DFT calculations of ^{1}H chemical shifts, simulated and experimental NMR spectra for sarcosine Chem. Phys. Lett. 492 (2010) 297-301.

[24] Q. Go, S. Yokojima, T. Kohno, T. Ishida, D.G. Fedorov, K. Kitaura, M. Fujihira, S. Nakamura, Ab initio NMR chemical shift calculations on proteins using fragment molecular orbitals with electrostatic environment Chem. Phys. Lett. 445 (2007) 331-339.

[25] A. Frank, H.M. Möller, T.E. Exner, Toward the Quantum Chemical Calculation of NMR Chemical Shifts of Proteins. 2. Level of Theory, Basis Set, and Solvents Model Dependence J. Chem. Theory Comput. 8 (2012) 1480-1492.

[26] G. Cuevas, V. Renugopalakrishnan, G. Madrida, A.T. Hagler, Density function studies of peptides Part I. Vibrational frequencies including isotopic effects and NMR chemical shifts of N-methylacetamide, a peptide model, from density function and MP2 calculations Phys. Chem. Chem. Phys. 4 (2002) 1490-1499.

[27] M.J. Frisch, G.W. Trucks, H.B Schlegel, G.E. Scuseria, M.A. Robb, J.R. Cheeseman, J.A. Montgomery Jr., T. Vreven, K.N. Kudin, J.C. Burant, J.M. Millam, S.S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G.A. Petersson, H. Nakatuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J.E. Knox, H.P. Hratchian, J.B. Cross, C. Adamo, J. Jaramillo, R. Gomperts, R.E. Stratmann, O. Yazyev, A.J. Austin, R. Cammi, C. Pomelli, J.W. Ochterski, P.Y. Ayala, K. Morokuma, G.A. Voth, P. Salvador, J.J. Dannenberg, V.G. Zakrzewski, S. Dapprich, A.D. Daniels, M.C. Strain, O. Farkas, D.K. Malick, A.D. Rabuck, K. Raghavachari, J.B. Foresman, J.V. Ortiz, Q. Cui, A.G. Baboul, S. Clifford, J. Cioslowski, B.B Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R.L. Martin, D.J. Fox, T. Keith, M.A. Al-Laham, C.Y. Peng, A. Nanaykkara, M. Challacombe, P.M.W. Gill, B. Johnson, W. Chen, M.W. Wong, C. Gonzalez, J.A. Pople Gaussian 03, Revision B. 05 Gaussian,, Pittsburgh PA 2003.

[28] P. Groth, Crystal Conformation of Cyclotrisarcosyl at -160 degrees C Acta Chem. Scand. 30 (1976) 838-840.

[29] P. Groth, Crystal Structure of Cyclotetrasarcosyl. Acta Chem. Scand. 24 (1970) 780-790.

[30] Y-H. Ng, R.P.A. Bettens Improving on equilibrium isotropic nuclear shielding constants Intl. J. Quant. Chem. 117 (2017) 15-23

[31] T. H. Dunning, Jr., K. A. Peterson, and D. E. Woon, in The Encyclopedia of Computational Chemistry, edited by P. v. R. Schleyer, N. L. Allinger, T. Clark, J. Gasteiger, P. A. Kollman, H. F. Schaefer III, and P. R. Schreiner Wiley, Chichester, 1998.

[32]M.A. Alvarez, E.J. Saavedra, M.S. Olivella, F.D. Suvire, M.A. Zamora, R.D. Enriz, Theoretical study of the conformational energy hypersurface of cyclotrisarcosyl Cent. Eur. J. Chem. 10 (2012) 248-255.

[33] H. Zhang, S. Neal, D.S. Wishart, RefDB: a database of uniformly referenced protein chemical shifts J. Biomol. NMR 25 (2003) 173-195.

[34] H.M. Berman, J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, P.E. Bourne , The Protein Data Bank. Nucleic Acids Research 28 (2000) 235-242.

GRAPHICAL ABSTRACT

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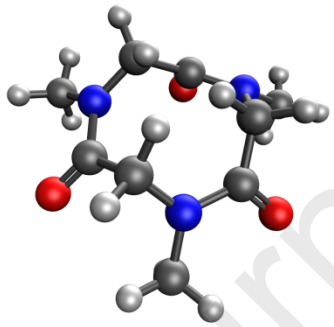
TITLE

NMR Chemical Shielding in Cyclosarcosyl

TEXT

Cyclotri and tetra sarcosyl have rigid structures that allow the effect of the conformation on NMR chemical shielding to be examined. Semi-classical methods commonly used in protein structure determination fail to predict the large chemical shift inequivalences seen for these molecules. DFT methods, however, can and with modest sized basis sets. In so doing the importance of the local conformation is shown.

GRAPHICAL ABSTRACT FIGURE



Supplementary Information

1. Comparison between the cyclotrisarcosyl crystal structure obtained by X-ray diffraction and the calculated structure

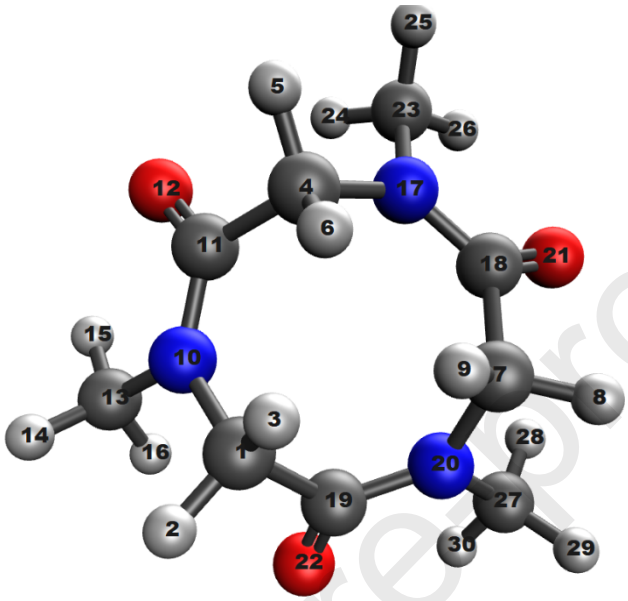


Table S1. Structure C_3 symmetry optimised using calculations at DFT B3LYP 6-311++G(3df, 3dp) X-ray crystal structure from [25]: Bond lengths			
Interatomic distances (Å)			
Bond	Experimental	Calculated	Difference
O12-C11	1.233, 1.232, 1.232	1.222	0.011, 0.010, 0.010
N10-C1	1.448, 1.462, 1.466	1.461	0.013, 0.001, 0.005
N10-C13	1.462, 1.465, 1.476	1.464	0.002, 0.001, 0.012
N10-C11	1.358, 1.352, 1.357	1.365	0.007, 0.013, 0.008
C11-C4	1.518, 1.518, 1.533	1.538	0.020, 0.020, 0.005

Table S2. Structure C_3 symmetry optimised using calculations at DFT B3LYP 6-311++G(3df, 3dp)

X-ray crystal structure from [25]: Bond angles

	Angles (°)		
Bond angle	Experimental	Calculated	Difference
O12-C11-C1	118.6, 118.6, 118.5	118.1	0.5, 0.5, 0.4
O12-C11-N10	122.3, 122.3, 122.0	122.6	0.3, 0.3, 0.6
C4-C11-N10	119.1, 119.2, 119.6	119.2	0.1, 0.0, 0.4
C1-N10-C13	115.0, 115.8, 117.9	115.6	0.6, 0.2, 2.3
C11-N10-C13	117.4, 116.8, 119.0	118.9	1.5, 2.1, 0.1
C1-N10-C11	125.9, 125.7, 124.4	125.5	0.4, 0.2, 1.1

Table S3. Structure C_3 symmetry optimised using calculations at DFT B3LYP 6-311++G(3df, 3dp)

X-ray crystal structure from [25]: Torsion angles

	Angles (°)		
Torsion angle	Experimental	Calculated	Difference
N10-C11-C4-N17	91.0, 99.7, 107.8	96.2	5.2, 3.5, 11.6
C1-N10-C11-C4	7.3, -9.9-11.9	1.4	5.9, 8.5, 10.5
C19-C1-N10-C11	-102.8, -86.8, -91.7	-97.8	5.0, 11.0, 6.1

2. Comparison between the cyclotetrasarcosyl crystal structure obtained by X-ray diffraction and the calculated structure

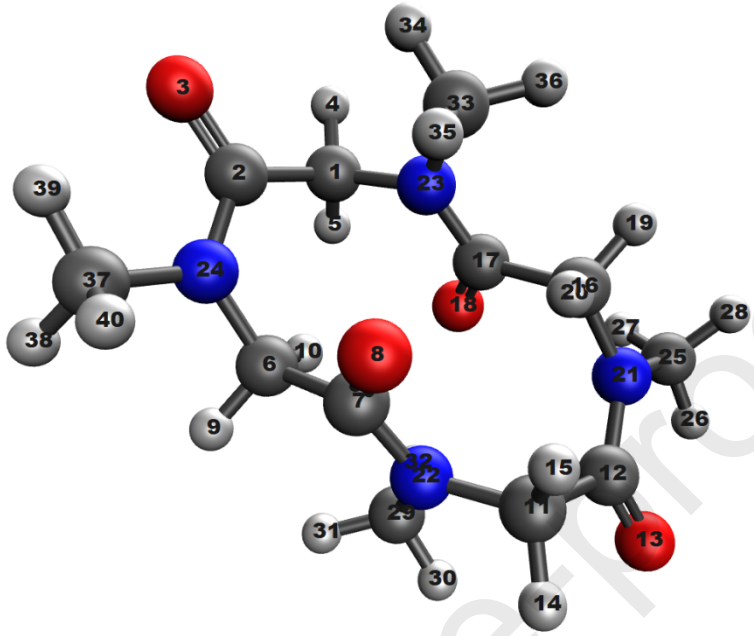


Table S4. Structure optimised using calculations at DFT B3LYP 6-311++G(3df, 3dp)

Experimental: X-ray crystal structure from [26]: Bond lengths

Interatomic distances (Å)			
Bond	Experimental	Calculated	Difference
C17-O18	1.215	1.223	0.008
C17-N23	1.364	1.361	0.003
N23-C33	1.471	1.464	0.007
N23-C1	1.461	1.460	0.001
C1-C2	1.530	1.542	0.012
C2-O3	1.235	1.221	0.014
C2-N24	1.352	1.367	0.015
N24-C37	1.462	1.459	0.003
N24-C6	1.454	1.445	0.009
C6-C7	1.531	1.543	0.012

Table S5. Structure optimised using calculations at DFT B3LYP 6-311++G(3df, 3dp)

X-ray crystal structure from [25]: Bond angles

Angles (°)			
Bond angle	Experimental	Calculated	Difference
O18-C17-N23	122.8	123.1	0.3
C17-N23-C33	124.3	123.4	0.9
C17-N23-C1	120.1	119.7	0.4
C33-N23-C1	115.6	116.6	1
N23-C1-C2	111.2	112.3	1.1
C1-C2-N24	119.1	118.8	0.3
C1-C2-O3	119.2	118.7	0.5
O3-C2-N24	121.7	122.5	0.8
C2-N24-C6	123.9	124.5	0.6
C2-N24-C37	119.8	119.1	0.7
C37-N24-C6	116.3	116.2	0.1
N24-C6-C7	112.1	113.5	1.4
C6-C7-N22	114.6	115.7	1.1
C6-C7-O8	122.6	121.1	1.5

Table S6. Structure optimised using calculations at DFT B3LYP 6-311++G(3df, 3dp)

X-ray crystal structure from [25]: Torsion angles

	Angles (°)		
Torsion angle	Experimental	Calculated	Difference
C16-C17-N23-C33	8.1	5.5	2.6
C1-C2-N24-C37	173.5	173.2	0.3
C16-C17-N23-C1	-170.6	-167.8	2.8
C1-C2-N24-C5	-5.4	-1.9	3.5
C17-N23-C1-C2	120.8	118.6	2.2
N23-C1-C2-N24	-65.5	-69.6	4.1
C2-N24-C6-C7	93.6	91.0	2.6
N24-C6-C7-N22	-169.5	-170.8	1.3
O18-C17-N23-C33	-175.0	-177.7	2.7
O3-C2-N24-C37	-5.7	-5.9	0.2
C33-N23-C1-C2	-58.1	-55.1	3
C37-N24-C6-C7*	-151.0?	-84.2	
N23-C1-C2-O3	113.7	109.5	4.2
N24-C6-C7-O8	68.7?	6.1	

* Labelled incorrectly in reference, C6-N2-C4-C1' [26]

? Inconsistent values, appear to be incorrect.

3. Absolute calculated NMR shielding and the chemical shift inequivalence (Δ) for cyclotrisarcosyl in Figure 2.

Numbering according to structure 1 above. $\Delta = \sigma(H2) - \sigma(H3)$. Units are in ppm

Theory	Basis set	H2	H3	Δ
HF	STO-3 G	28.4592	26.9383	1.5209
	3-21 G	28.5134	27.274	1.2394
	6-31 G	28.4682	27.3277	1.1405
	6-311 G	28.5624	27.3651	1.1973
DFT	STO-3 G	27.5454	26.3033	1.2421
	3-21 G	28.0604	26.8266	1.2338
	6-31 G	28.0278	26.7869	1.2409
	6-311 G	28.1459	26.8854	1.2605
MP2	STO-3 G	27.56	26.2586	1.3014
	3-21 G	27.9359	26.7476	1.1883
	6-31 G	27.8016	26.6596	1.142
	6-311 G	27.9372	26.6804	1.2568
Exp				1.37

4. Absolute calculated NMR shielding and the chemical shift inequivalence (Δ) for cyclotrisarcosyl in Figure 3.

Structure optimised at DFT/B3LYP/6-311++G(3df,3dp)

Numbering according to structure 1 above. $\Delta = \sigma(H2) - \sigma(H3)$. Units are in ppm

Basis set	H2	H3	Δ
STO-3G	30.1232	29.8557	0.2675
3-21G	29.7565	28.7997	0.9568
6-31G	29.6033	28.8304	0.7729
6-311G	29.6297	28.8675	0.7622
6-311+G	29.5542	28.7512	0.803
6-311++G	29.5584	28.7938	0.7646
(d,p)	28.7571	27.5619	1.1952
(2d,2p)	28.4379	27.0776	1.3603
(3d,3p)	28.3915	26.9708	1.4207
(df,pd)	28.6508	27.3836	1.2672
(2df,2pd)	28.3756	26.9646	1.411
(3df,3pd)	28.3317	26.8866	1.4451
Exp			1.37

5. Absolute calculated NMR shielding and the chemical shift inequivalence (Δ) for cyclotrisarcosyl in Figure 4.

Numbering according to structure 1 above. $\Delta = \sigma(H2) - \sigma(H3)$. Units in ppm

Vacuum			
Basis set	H2	H3	Δ
DFT 6-311++ G	29.5584	28.7938	0.7646
(d,p)	28.7571	27.5619	1.1952
(2d,2p)	28.4379	27.0776	1.3603
(3d,3p)	28.3915	26.9708	1.4207
(df,pd)	28.6508	27.3836	1.2672
(2df,2pd)	28.3756	26.9646	1.411
(3df,3pd)	28.3317	26.8866	1.4451
Exp.			1.37

Chloroform			
Basis set	H2	H3	Δ
DFT 6-311++ G	29.3609	28.5045	0.8564
(d,p)	28.5865	27.3577	1.2288
(2d,2p)	28.2746	26.8765	1.3981
(3d,3p)	28.1956	26.7656	1.43
(df,pd)	28.4771	27.1765	1.3006
(2df,2pd)	28.2185	26.7668	1.4517
(3df,3pd)	28.1593	26.6849	1.4744
Exp.			1.37

6. Absolute calculated NMR shielding and the chemical shift inequivalence (Δ) for cyclotetrasarcosyl in Figure 6.

Numbering according to structure 2 above. $\Delta 1 = \sigma(H4) - \sigma(H5)$, $\Delta 2 = \sigma(H9) - \sigma(H10)$. Units in ppm

Basis set	H4	H5	H9	H10	Δ1	Δ2
DFT 3-21 G	30.3828	27.7709	29.6261	28.9645	2.6119	0.6616
6-31 G	30.0341	27.6968	29.5989	28.8843	2.3373	0.7146
6-311 G	29.8749	27.6562	29.6093	28.8842	2.2187	0.7251
6-311+ G	29.8016	27.5918	29.5169	28.8843	2.2098	0.6326
6-311++ G	29.8288	27.6002	29.523	28.8157	2.2286	0.7073
(d,p)	29.0077	26.7112	28.7088	27.9105	2.2965	0.7983
(2d,2p)	28.6914	26.3431	28.3161	27.4406	2.3483	0.8755
(3d,3p)	28.6444	26.2582	28.2291	27.4029	2.3862	0.8262
(df,pd)	28.9182	26.5581	28.6003	27.7706	2.3601	0.8297

(2df,2pd)	28.647	26.2527	28.2727	27.3541	2.3943	0.9186
(3df,3pd)	28.5762	26.1924	28.1981	27.3224	2.3838	0.8757
Exp.					2.03	0.71

7. Absolute calculated NMR shielding and the chemical shift inequivalence (Δ) for cyclotetrasarcosyl in Figure 7.

Numbering according to structure 2 above. $\Delta 1 = \sigma(H4) - \sigma(H5)$, $\Delta 2 = \sigma(H9) - \sigma(H10)$. Units in ppm

Solvent	H4	H5	H9	H10	Δ1	Δ2
Benzene	28.7585	26.482	28.3884	27.7715	2.2765	0.6169
Diethylether	28.7046	26.4615	28.2669	27.8124	2.2431	0.4545
Chloroform	28.6973	26.4614	28.2434	27.811	2.2359	0.4324
Dichloromethane	28.6778	26.4477	28.1712	27.7914	2.2301	0.3798
Water	28.6519	26.4129	28.0433	27.7784	2.239	0.2649

Highlights

- Steric effects dominate the $^{1}H\alpha$ chemical shift inequivalence in cyclotrisarcosyl.
- $^{1}H\alpha$ inequivalence in cyclotetrasarcosyl arises from the adjacent peptide group conformation
- Distant non bonded peptide groups contribute relatively little to the $^{1}H\alpha$ inequivalence

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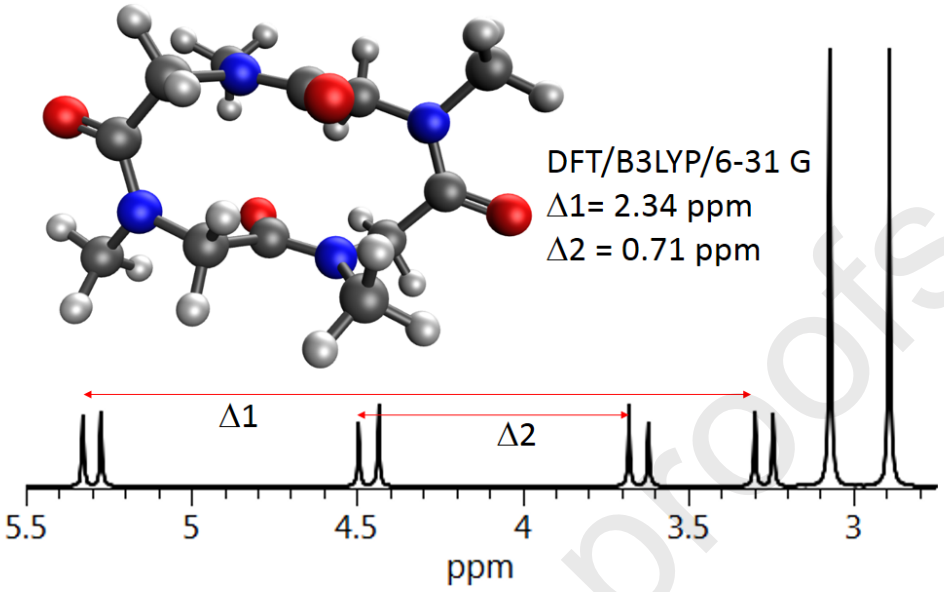
Conceptualization Methodology Validation Formal analysis Investigation Writing - Original Draft Writing - Review & Editing Visualization



Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: