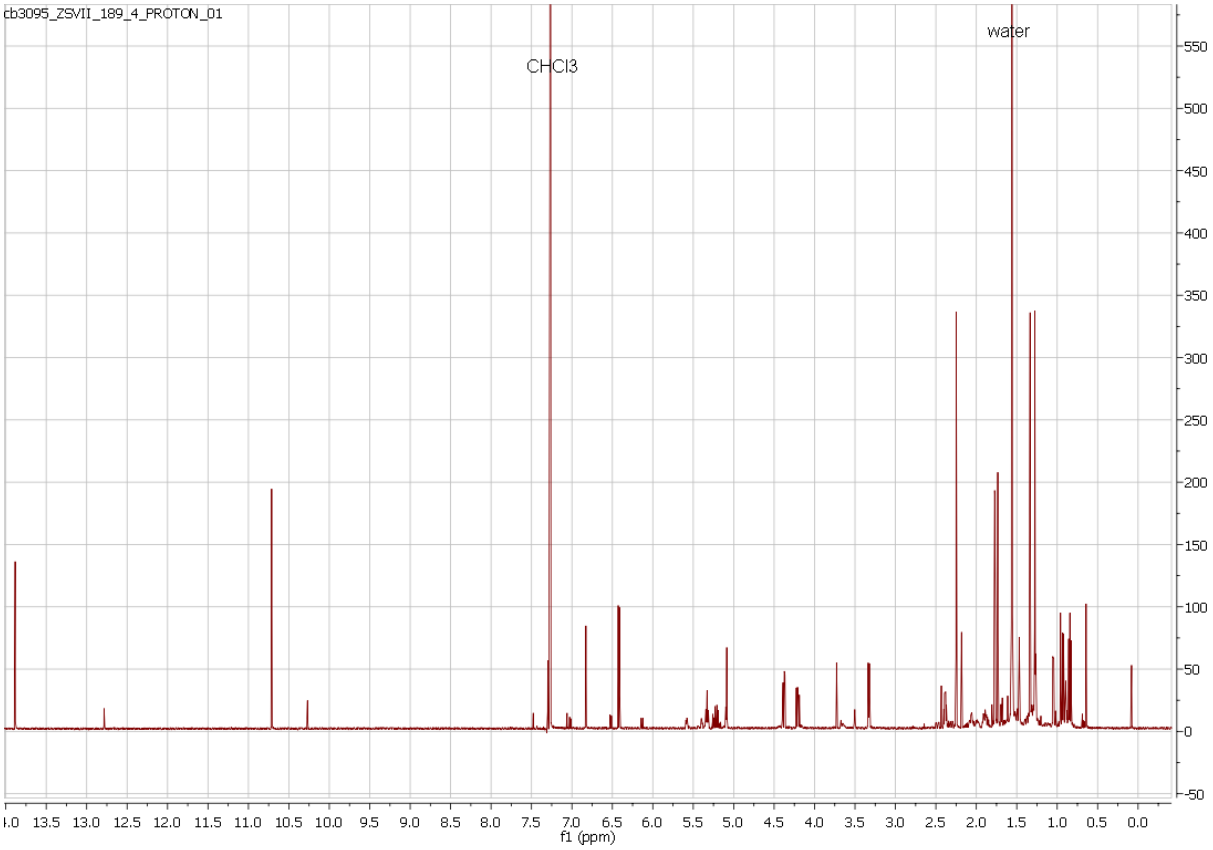
Supplementary Information

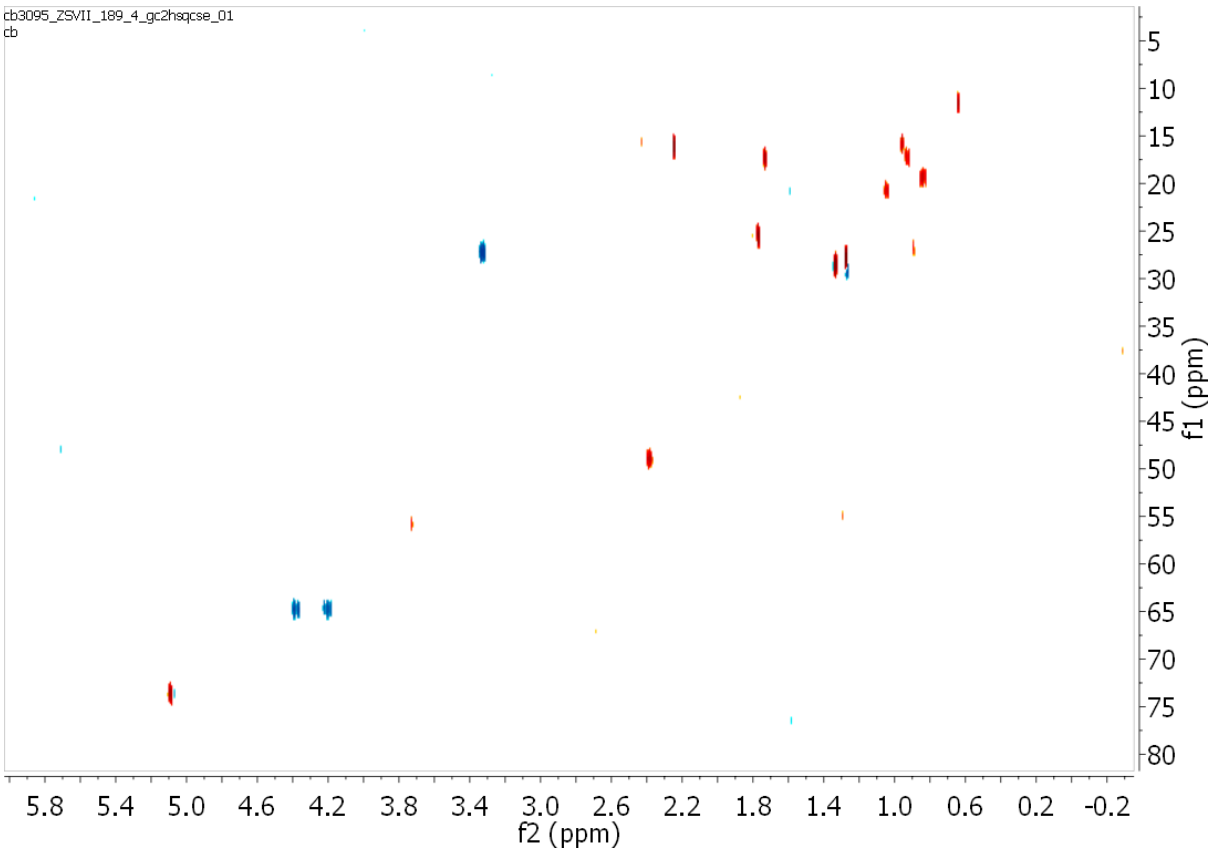
S1. Production and purification of arugosin C

Aspergillus variecolor(Aspergillus stellatus IMI 53749) was purchased from CABI as freeze-dried ampoule. The strain was recovered on Czapek-Dox agar dishes at 25°C. Single colonies were transferred to fresh Czapek-Dox agar dishes (10) and incubated for 14 days. Total mycelium and agar from three of above agar dishes were harvested and extracted with acetone (500 mL) for 3 hours. The solid was removed by filtration and two further rounds of extraction were carried out. The acetone extracts were combined and acetone was removed by rotary evaporation. The aqueous residue was extracted with ethyl acetate (3x 50 mL). The crude extract obtained after ethyl acetate was removed via rotary evaporation was then passed through a silica gel flash column, eluted with ethyl acetate:hexane (5:95 increased to 20:80). Fractions 4 and 5 were confirmed by NMR spectroscopy to contain arugosin C with some impurities (~10% by ^{1}H NMR integration). The sample was then further purified by preparative TLC, developed with ethyl acetate:hexane (40:60). The pure band was removed, eluted with ethyl acetate and used for NMR analysis after removal of solvent.

^{1}H NMR spectrum of the sample of arugosin C used for NOESY studies



HSQC spectrum of sample of arugosin C used for NOESY studies



S2. NOE method and analysis

Assuming that the molecule of interest is in the fast tumbling regime and that the Initial Rate Approximation holds true, the normalised NOE intensity between two spins I and S, η_{IS}, is proportional to the cross-relaxation rate, σ_{IS}, between these spins and the mixing time, τ_m, of the experiment (Equation 1). In turn, the cross-relaxation rate, σ_{IS}, between spins I and S is proportional to the internuclear distance between spins I and S (r_{IS}^{-6}) as described in Equation 2. A more complete description of these equations and their use in determining interproton distances can be found in references 1 and 2.

$$\eta_{IS}=\sigma_{IS}\tau_m \qquad \text{Equation (1)}$$

$$\sigma_{IS}=kr_{IS}^{-6} \qquad \text{Equation (2)}$$

where $k=\left(\frac{\mu_0}{4\pi}\right)\frac{\hbar^2\gamma^4}{10}\left(\frac{6\tau_c}{1+4\omega^2\tau_c^2}-\tau_c\right)$

Assuming that the values defining k (ω-Larmor frequency, τ_c-rotational correlation time, γ-gyromagnetic ratio) remain constant for each spin pair in a spectrum, the ratio of intensities of a pair of NOE signals, η_{I1S}: η_{I2S}, within that spectrum can thus be assumed to be proportional to the ratio of their internuclear distances (Equation 3). Thus, by measuring η_{I1S} and η_{I2S}, we only need to know one distance, e.g. r_{I1S}, in order to calculate the second distance, r_{I2S}.

$$\frac{\eta_{I1S}}{\eta_{I2S}}=\frac{r_{I1S}^{-6}}{r_{I2S}^{-6}} \qquad \text{Equation (3)}$$

In cases where multiple conformers exist to describe a flexible molecular system, the matter of internuclear distance determination becomes more challenging. A general outline of the treatment of flexible small molecules using NOE experiments for both conformational and population analysis can be found in reference 1.

If one assumes that flexible systems exhibiting multiple conformations, such as those detailed in this report, are interconverting rapidly on the NMR time-scale, then conformational exchange will lead to ensemble-averaging of the observed NOEs. The calculated populations of the contributing conformers and their respective interproton distances can be used to calculate these ensemble-averaged NOEs, which can subsequently be converted into effective distances for comparison with experimental values. To do so, the appropriate distance must be measured in each contributing conformer and then converted into an NOE value using the $n_{IS} = r_{IS}^{-6}$ relationship. These individual contributing NOE values must then be weighted according to the Boltzmann populations of the conformers, resulting in an "effective" NOE which can be converted back into an "effective" distance to be compared to the NOE-derived value.

(1) Neuhaus, D.; Williamson, M. P. Wiley. The Nuclear Overhauser Effect in Structural and Conformational Analysis; 2nd ed.; VCH: New York, 2000.
(2) Butts, C. P.; Jones, C. R.; Towers, E. C.; Flynn, J. L.; Appleby, L.; Barron, N. J. Org. Biomol. Chem. 2011, 9, 177-184.

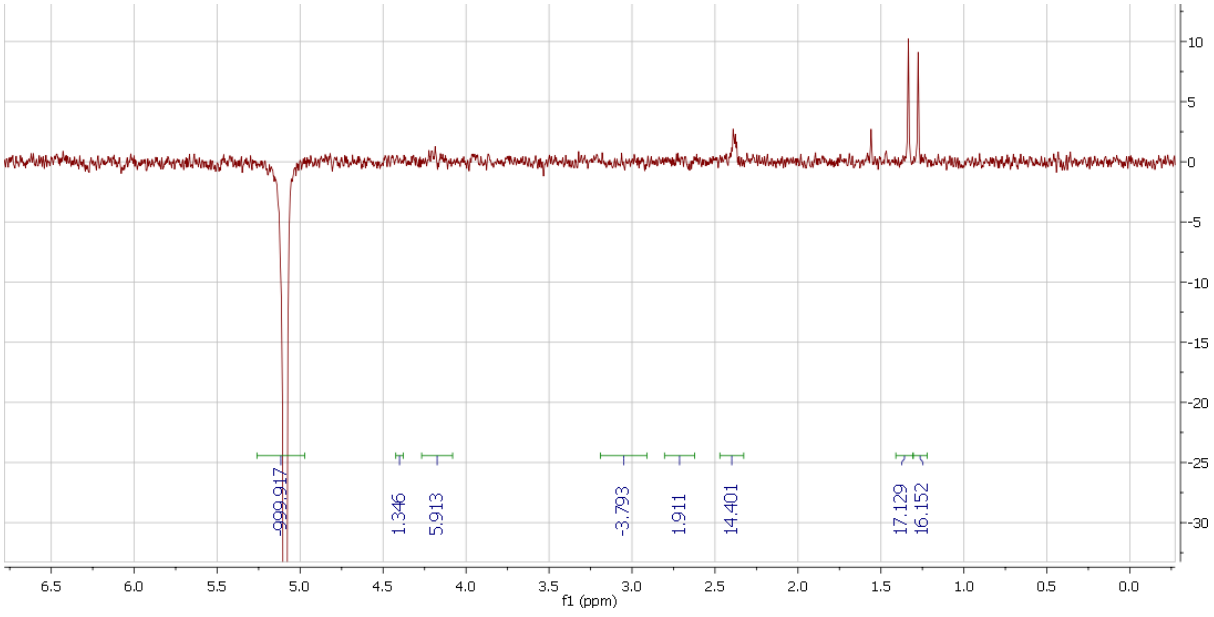
S3. NMR Experimental details

NMR samples were prepared in 5mm tubes with 0.7 mL $CDCl_3$ under air without degassing. NMR data were collected on a 500MHz Varian VNMRS DirectDrive spectrometer equipped with an indirect observe probe. 1D selective transient NOESY spectra (64k data points, 8 kHz sweep width, 500 ms mixing time, 4.096s acquisition time, 1s relaxation delay, 512 scans) were obtained using the Varian Chempack NOESY1D sequence which is based on the DPFGSENOE (double-pulse field gradient spin-echo NOE) excitation sculpted selective sequence reported by Stott *et al.*[3] and incorporates a zero-quantum filter element.[4]

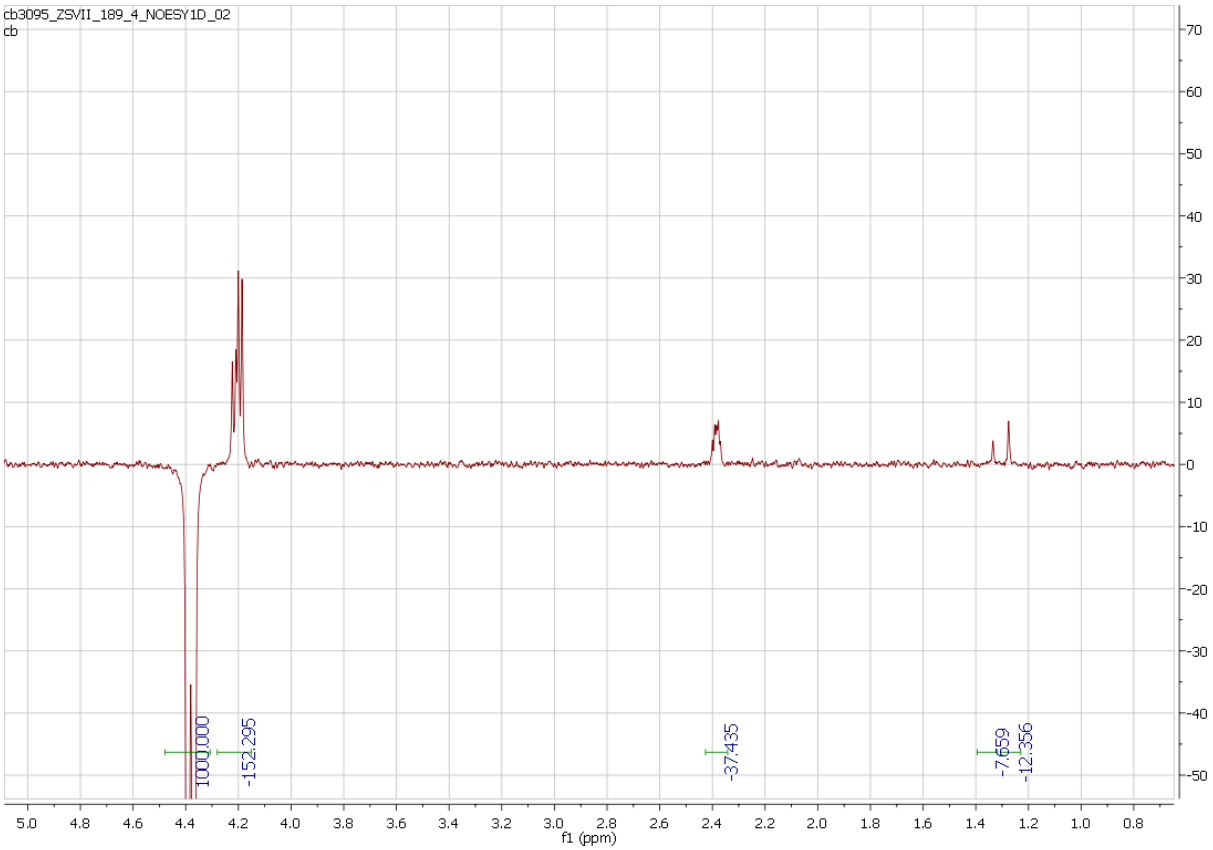
(3) Stott, K.; Keeler, J.; Van, Q. N.; Shaka, A. J. *J. Magn. Reson*. **1997**, *125*, 302-324.

(4) Thrippleton, M. J.; Keeler, J. *Angew. Chem. Int. Ed*. **2003**, *42*, 3938-3941.

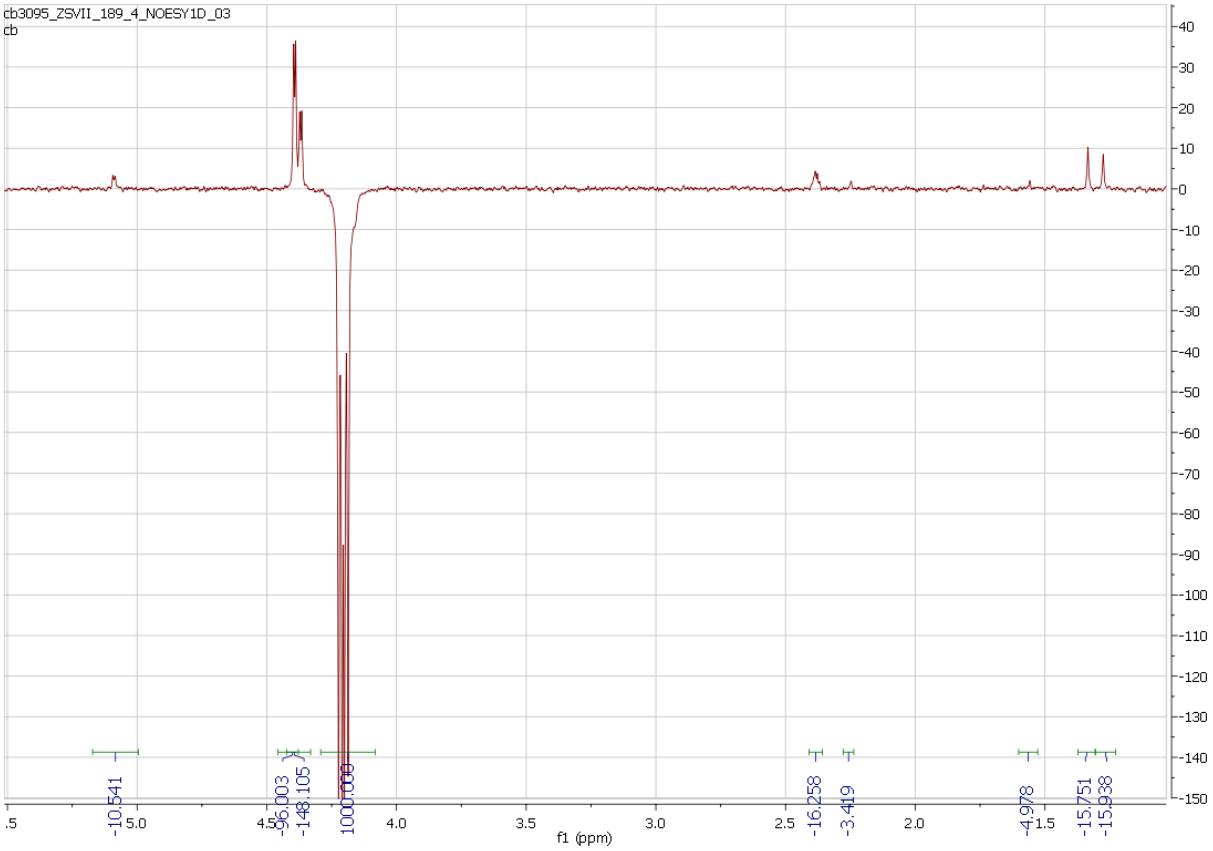
1D-NOESY spectrum of H25 of arugosin C



1D-NOESY spectrum of H19b of arugosin C



1D-NOESY spectrum of H19a of arugosin C



S4. Computational details

Conformational searches were conducted on truncated models of diastereomers **1** and **2** (C11 chain replaced with a methyl group) using the Spartan software package with the MMFF forcefield. From the resulting conformers found, geometries **a** and **b** were identified as the only unique conformers for the core arugosin skeleton (all remaining conformers were rotamers of the hydroxyisopropyl substituent). Subsequent geometry optimisations of **1a**, **1b**, **2a** and **2b** at the DFT B3LYP/6-31g* level were carried out using the Gaussian 09 software package and frequencies were computed to characterise the minima and derive statistical mechanical corrections to the electronic energies. Single point energies were then calculated for all structures in Gaussian, with B3LYP/6-31G* and a polarisable continuum model (IEF-PCM, parameters for chloroform solvent, ε=4.9). The B3LYP/6-31g* optimised geometries were optimized in vacuo at the DFT level using the 6-31G(d,p) basis set (Gaussian 09 software package) and then the optimized structures were used as inputs for the single-point GIAO ^{13}C chemical shift and J-coupling calculations performed in vacuo employing the MPW1PW91 functional combined with the 6-31G(d,p) basis set. The calculated values of chemical shifts were referenced to the theoretical tetramethylsilane ^{13}C chemical shift value (previously optimized at DFT level), computed at the same level of theory.

Figure S5. Plot of the sum of the mean absolute error (MAE) and standard deviation (STD) of interproton distances arising from fitting the population distribution of conformer **a** of both the *trans* (**1**) and *cis* (**2**) isomers to the experimental NOE-derived interproton distances (where % population of conformer **b** = (100 - % population of **a**)).

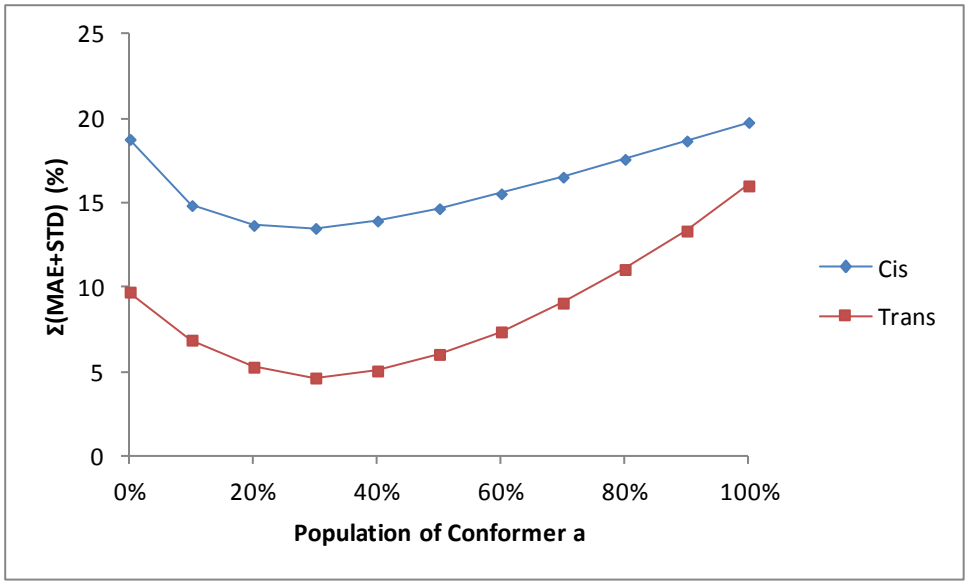


Table S6. Comparison between experimental ^{13}C chemical shifts in $CDCl_3$ and calculated ^{13}C chemical shifts *in vacuo* for C19, 20, 21 and 25 of conformers **a** and **b** of both isomers

	δ_{exp}	$\delta_{calc.}$							
		1a	**1b**	**Best fit 30% 1a : 70% 1b**	**Error (δ / ppm)**	**2a**	**2b**	**Best fit 20% 2a : 80% 2b**	**Error (δ / ppm)**
C19	64.2	62.7	65.2	64.5	0.3	65.7	61.8	62.6	1.6
C20	48.7	49.2	48.7	48.8	0.1	45.9	46.8	46.6	2.1
C21	69.8	69.1	69.4	69.3	0.5	71.0	69.2	73.8	0.7
C25	73.1	70.0	74.0	72.8	0.3	79.4	72.4	69.6	0.2
				MAE	0.3			MAE	1.2
				STD	0.1			STD	0.9

Figure S7. Plot of the sum of the mean absolute error (MAE) and standard deviation (STD) arising from fitting the population distribution of conformer **a** of both the *trans* (**1**) and *cis* (**2**) isomers to the experimental ^{13}C chemical shift data for C19, 20, 21 and 25 (where % population of conformer **b** = (100 - % population of **a**)).

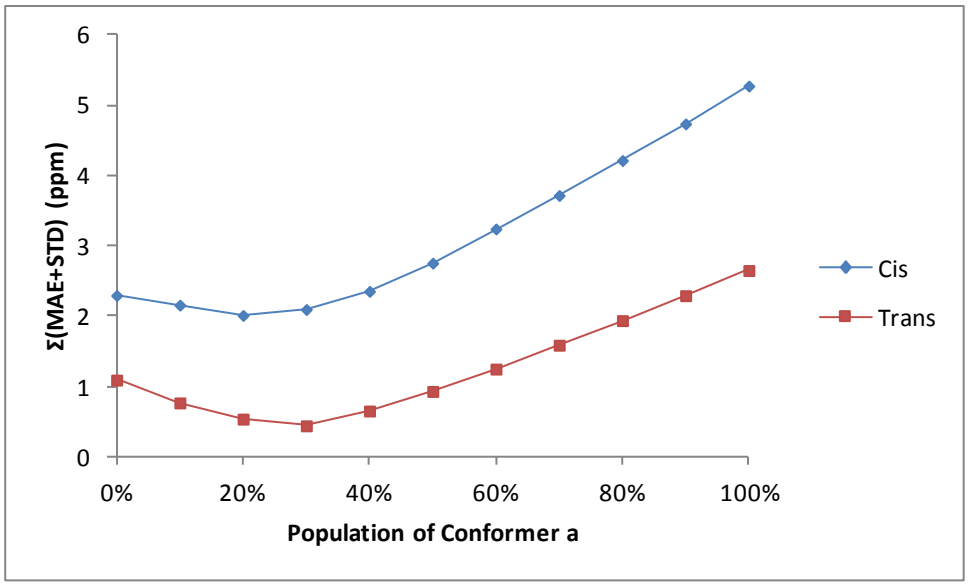


Table S8. $J_{H25,H20}$ coupling constants (using calculated J values from MPW1PW91 calculations) calculated for population distribution of conformer **a** in the *trans* (**1**) and *cis* (**2**) isomers.

	$J_{H25,H20}$ / Hz	
% population of conformer a	*trans* (**1**)	*cis* (**2**)
0%	6.1	4.4
10%	5.6	4.8
20%	**5.1**	**5.2**
30%	4.6	5.6
40%	4.1	6.0
50%	3.7	6.4
60%	3.2	6.8
70%	2.7	7.3
80%	2.2	7.7
90%	1.7	8.1
100%	1.2	8.5

Experimental $J_{H25,H20}$ = 5.0 Hz