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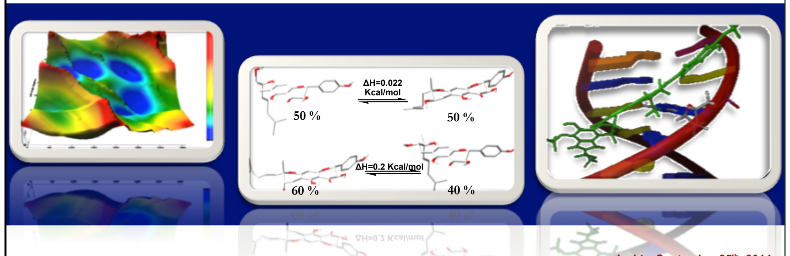
Quantum chemical calculation of NMR parameters in the stereochemical determination of organic compounds



Ischia, September 25th 2014

Solving the relative configuration of organic molecules is essential in organic and bioorganic chemistry

For:
- *undertaking total synthesis of natural compounds*
- *conformational and structure activity relationship studies*
- *understanding the biological mechanism of action at the molecular level*

The configurational analysis of highly *flexible carbon chains* is usually complicated by the lack of information on the conformational behaviour

***Problems arise from*:**
- presence of multiple conformer equilibria
- large contributions to NOE intensity derived from ***minor populations***

Consolidated Approaches
- Application of Nuclear Overhauser Effect (NOE) in combination with computational techniques
- Simple NMR studies of a flexible system transformed in a rigid one (e.g.: the Rychnovsky method for 1,3-diols)

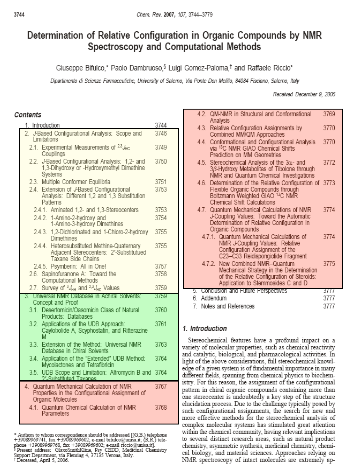
Determining relative configuration on flexible carbon chains by NMR spectroscopy

Recent Approaches

- **The *J*-based approach (M. Murata, *et al. J. Org. Chem* 1999) and its modifications**
- **Conformational analysis and Boltzmann-averaged ^{13}C NMR chemical shift calculations by ab initio methods (G. Bifulco, *et al. Chem. Eur. J.* 2002; *Pure and Appl. Chem.* 2003)**
- **Quantum mechanical calculations of NMR *J* coupling values (G. Bifulco, *et al Organic Letters* 2004, 6(6), 1025-1028; *J. Org. Chem.* 2010, 75 (6), 1982–1991)**

Other Perspectives

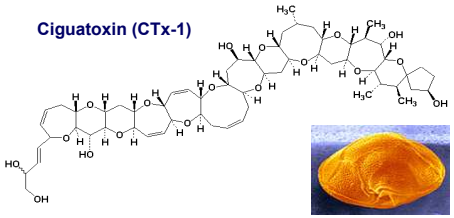
- **UDB *(U*niversal NMR *Data*Base)** (Kishi. Y. et al *Org. Lett.* **1999**, *1*, 2177)



Ciguatera Fish Poisoning

**"The name may be difficult to remember.
But if you get this disease, you'll never forget it."**

Ciguatoxin (CTx-1)



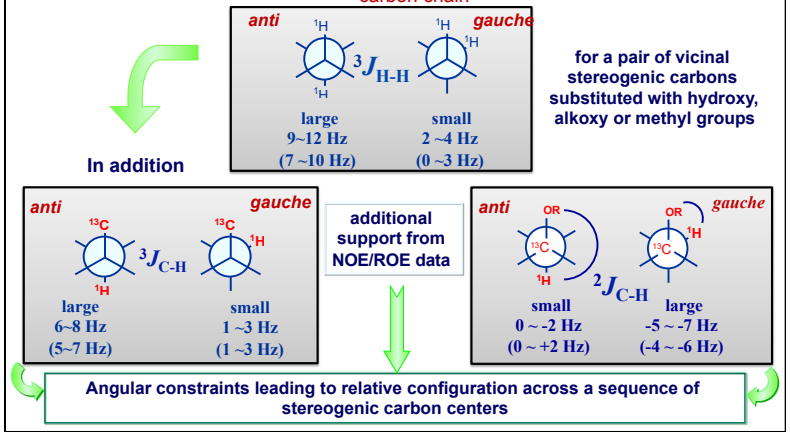
Ciguatera fish
- ❖ is endemic in all tropical area where coral reef fishes are a food source
- ❖ results from the eating of reef fish affected with ciguatoxin

CTx-1
- ❖ is produced by the dinoflagellate *Gambierdiscus toxicus* which colonizes coral beds
- ❖ has been also isolated from the flesh and viscera of ciguatoxic fish

Ciguatera symptoms were first described in the 1500's by the Spanish explorers to Cuba and were attributed to the ingestion of a small snail which they called *cigua*.

Fundaments of the *J*-based approach of configurational analysis

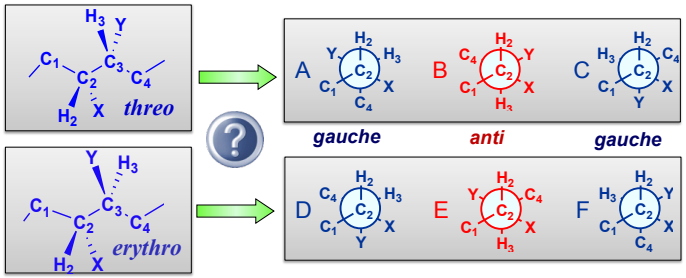
Homo- and hetero-nuclear spin-coupling constants can provide reliable stereochemical information on the major staggered conformation in a flexible carbon chain



Relative Configuration Assignment in Oxygenated C_2 Subunits by *J*-based Configuration Analysis (Murata, *et al.*)

for any pair of vicinal stereogenic carbons substituted with **hydroxy**, **alkoxy** or **methyl** groups:

- ➘ six possible staggered conformers with ***threo*** and ***erytro*** arrangements
- ➘ determination of the dominant rotamer and of its relative configuration can be achieved through angular constrains inferred by measuring $^3J_{H,H}$ and $^{2,3}J_{C,H}$ values and NOE



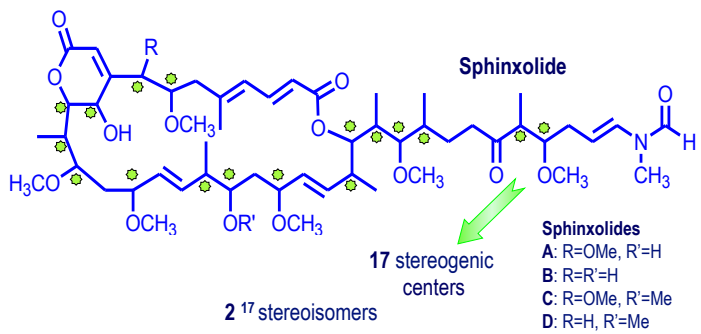
Relative Configuration Assignment in Oxygenated C_2 Subunits by *J*-based Configuration Analysis (Murata, *et al.*)

- ➘ by the expected $^3J_{HH}$ and $^{2,3}J_{CH}$ coupling pattern of the six staggered rotamers with ***threo*** (***syn***) and ***erythro*** (***anti***) geometries

threo (syn)	A	B	C
$^3J_{H2H3}$	small	large	small
$^3J_{H2C4}$	large	small	small
$^3J_{H3C1}$	large	small	small
X = Me, Y = OR			
$^3J_{CxH3}$	small	small	large
$^2J_{C3H2}$	large	large	small
X = OR, Y = OR			
$^2J_{C2H3}$	large	large	small
$^2J_{C3H2}$	large	large	small

erythro (anti)	A	B	C
$^3J_{H2H3}$	small	large	small
$^3J_{H2C4}$	small	small	large
$^3J_{H3C1}$	large	small	small
X = Me, Y = OR			
$^3J_{CxH3}$	small	small	large
$^2J_{C3H2}$	small	large	large
X = OR, Y = OR			
$^2J_{C2H3}$	large	large	small
$^2J_{C3H2}$	small	large	large

Sphinxolides: a family of potent anti-tumor marine macrolides acting on cell microfilaments



Guella,G.; Mancini, I.; Chiasera, G.; Pietra, F.. *Helv. Chem. Acta* **1989**, *72*, 237-46.
D'Auria, M. V.; Gomez Paloma, L.; Minale, L.; Zampella, A.; Verbist ,J. F.; Roussakis, C.; Debitus, C. *Tetrahedron* **1993**, *49*, 8657-645.
Minale, L.; D'Auria, M. V.; Zampella, A.; Gomez Paloma, L. *Korean J. Med. Chem.* **1996**, *6*, 135-140.

Measuring $^{2,3}J_{C,H}$

HETLOC: w_1-half filtered TOCSY
$^{2,3}J_{C,H}$ values with signs, dislocation along w_2 of a doublet cross peak split by $^{1}J_{C,H}$

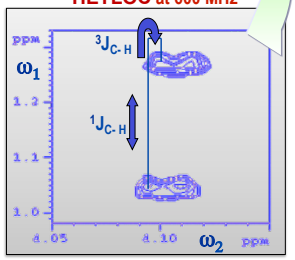
PS-HMBC
$^{2,3}J_{C,H}$ absolute values by the intensity ratio of the cross peak magnitudes respect to a common proton *e.g.* $^{2,3}J_{Cb,H}$

A reference $^{2,3}J_{C,H}$ value is required

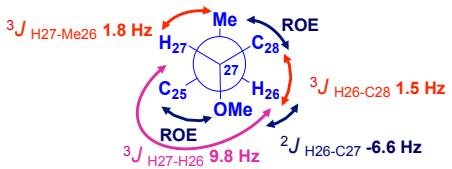
Integration of cross peak volumes

$I_{Ca,H}/I_{Cb,H} = \sin^2(\pi J_{Ca,H} D)/\sin^2(\pi J_{Cb,H} D)$

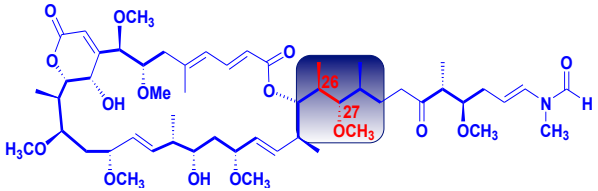
HETLOC at 600 MHz



Sphinxolide: relative configuration at C27-C26

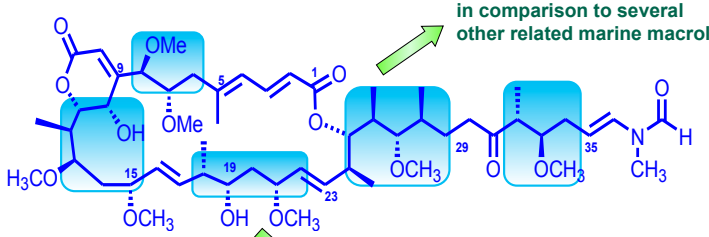


4 $^{1}J_{H-H}$ and $^{2,3}J_{C-H}$ + 2 ROE convergent data



Relative stereochemical assignments in five stereoclusters of sphinxolide

in comparison to several other related marine macrolides



by synthetic model

2^{17} stereoisomers → 2^{5} stereoisomers → 2^{4} stereoisomers

Bassarello, C.; Bifulco, G.; Zampella , A.; D'Auria, M. V.; Riccio, R.; Gomez Paloma, L. *Eur. J. Org. Chem.* **2001**, *1*, 39-44.

Limits of the *J*-based configurational analysis protocol …

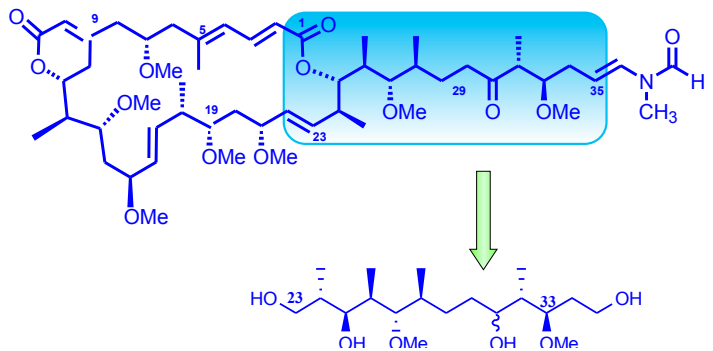
- Limited literature data that correlate the size of heteronuclear $^{2,3}J_{CH}$ values to molecular structures
- Pattern small/large
- The identical small/large *J* coupling pattern in C2 fragments with protons in *anti* arrangement

…….. and current developments

Quantum mechanical calculation of coupling constants

- ✓ powerful and versatile calculation packages: Gaussian 09 software package
- ✓ good reliability and efficiency in the *J* and $^{13}C/^{1}H$ calculation through different DFT theory levels and medium size basis sets

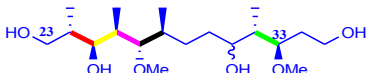
Stereochemical analysis in the side chain of Reidispongiolide A by quantum mechanical calculation of coupling constants



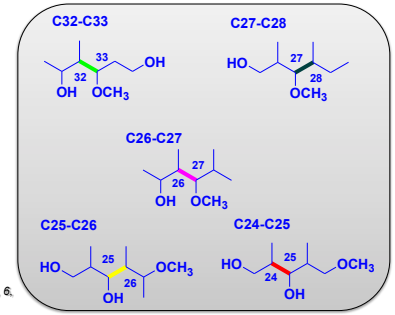
Zampella, A.; Bassarello, C.; Bifulco, G.; Gomez Paloma, L.; D'Auria, M. V. *Eur. J. Org. Chem.* **2002**, *5*, 785-790.
Zampella, A.; Sepe, V.; D'Orsi, R.; Bifulco, G.; Bassarello, C.; D'Auria, M. V. *Tetrahedron: Asymmetry* **2003**, *14*, 1787-1798

The basics of configurational analysis by quantum mechanical calculation of coupling constants

Model compound under investigation
7 stereogenic centers



6^5 staggered rotamers

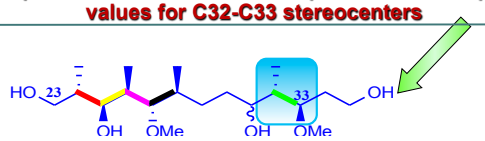


The complexity of the problem can be reduced by breaking down the carbon framework into 5 individual C_2 fragments

Analysis of 30 relative spatial relationships across two adiacent stereogenic centers

Bifulco, G.; Bassarello, C.; Riccio, R.; Gomez-Paloma, L. *Org. Lett.* **2004**, *6*, 1025-1028.

Comparison of calculated and experimental *J* coupling values for C32-C33 stereocenters



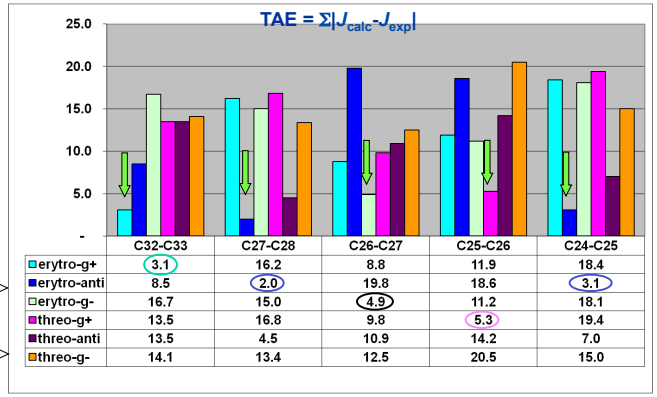
Calculated and exp *J* values at the C32-C33 bond

	erythro			*threo*			
							exp
$^{3}J_{H32-OH}$							2.6
$^{2}J_{H32-C33}$	***-4.7***	-1.5	0.9	0.4	-3.4	-3.5	-5.4
$^{3}J_{H32-C34}$	***4.4***	5.2	1.1	2.9	1.4	5.1	4.8
$^{3}J_{H33-C31}$	***1.9***	1.0	6.1	3.7	0.8	5.6	0.5
$^{3}J_{H33-Me32}$	***4.8***	7.5	3.6	3.7	3.1	1.0	4.4
$\Sigma\lvert J_{calc}-J_{exp}\rvert$	***3.1***	8.5	16.7	13.5	13.5	14.1	

-Geometry optimization: DFT/MPW1PW91/6-31G(d)
-Property calculation: SP DFT/MPW1PW91/6-31G(d,p)

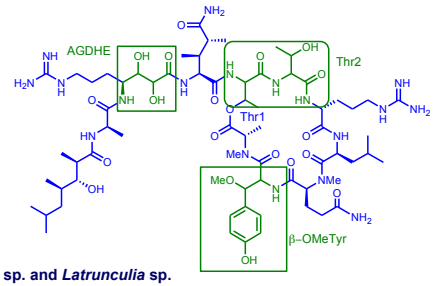
Cimino, P.; Gomez-Paloma, L.; Duca, D.; Riccio, R.; Bifulco, G. *Mag. Res. Chem.* **2004**, *42*, S26–S33.

Conformational and configurational assignment across all the individual C_2 fragments through minimum values of Total Absolute Error (TAE)



	C32-C33	C27-C28	C26-C27	C25-C26	C24-C25
erytro-g+	3.1	16.2	8.8	11.9	18.4
erytro-anti	8.5	2.0	19.8	18.6	3.1
erytro-g-	16.7	15.0	4.9	11.2	18.1
threo-g+	13.5	16.8	9.8	5.3	19.4
threo-anti	13.5	4.5	10.9	14.2	7.0
threo-g-	14.1	13.4	12.5	20.5	15.0

First Application of the QM-J: method to a "real" and complex case



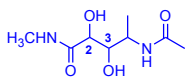
Callipeltin A

isolated from *Callipelta* sp. and *Latrunculia* sp.

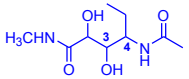
Activity: antiviral & anti HIV-1
Strong inhibition of the cardiac sodium/calcium pump
Potential regulator of the miocardium cantractility

ISOLATION: Zampella, A.; D'Auria, M. V.; Gomez-Paloma, L.; Casapullo, A.; Minale, L.; Debitus, C.; Henin, Y. *J. Am. Chem. Soc.* **1996**, *118*, 6202–6209.
I REVISION: Zampella, A. Randazzo, A.; Borbone, N.; Luciani, S. ; Trevisi, L. C. Debitus, M. V. D'Auria, *Tetrahedron Lett.* **2002**, *43*, 6163–6166.
II REVISION: Bassarello, C.; Zampella, A.; Monti, M. C.; Gomez-Paloma, L.; D'Auria, M. V.; Riccio, R.; Bifulco, G. *Eur. J. Org. Chem.* **2006**, 604-609.

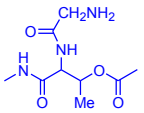
Molecular fragments of the five C_2 systems of callipeltin A



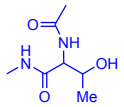
$AGDHE_{2,3}$



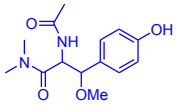
$AGDHE_{3,4}$



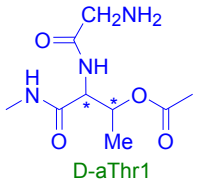
D-*a*Thr1



D-*a*Thr2



β-OMeTyr

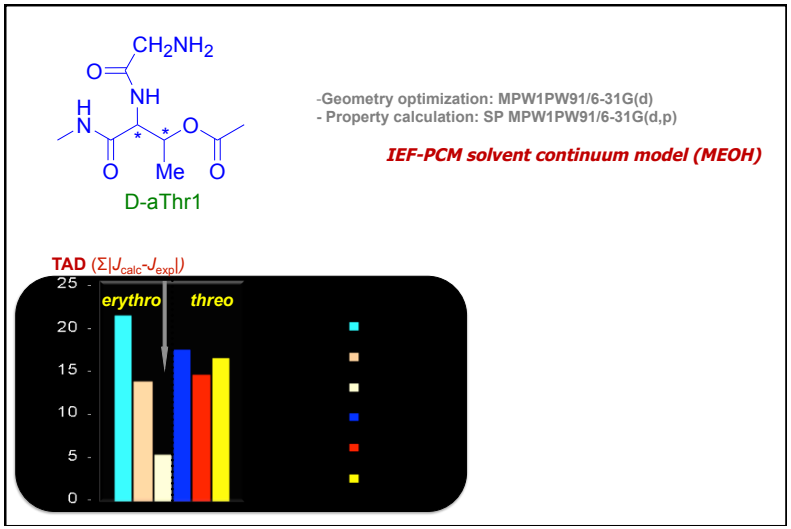


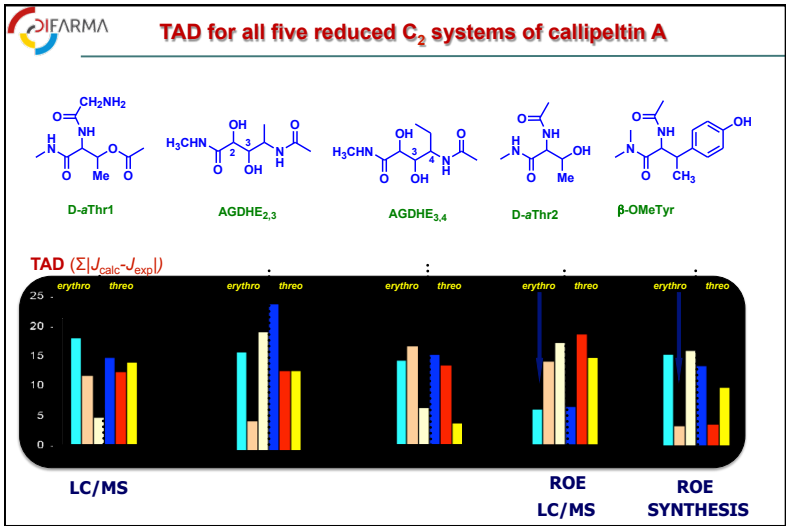
D-aThr1

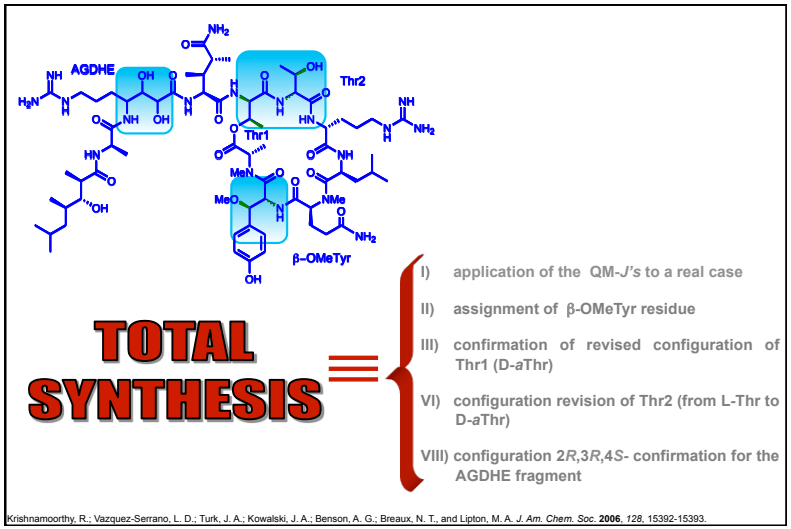
-Geometry optimization: MPW1PW91/6-31G(d)
- Property calculation: SP MPW1PW91/6-31G(d,p)

***IEF-PCM solvent continuum model* (MEOH)**

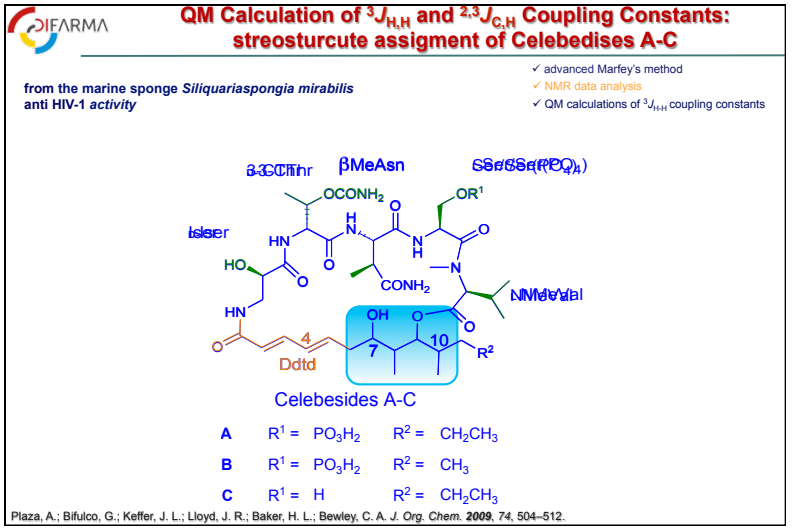
D-aThr1	*erythro*			*threo*			expt
(calc)	g+	anti	g-	g+	anti	g-	
$^3J_{H2-H3}$	3.7	8.6	2.6	4.5	9.2	1.3	2.8
$^2J_{H2-C3}$	0.9	-4.4	-4.9	-4.5	-4.2	0.6	-5.7
$^3J_{H2-Me}$	0.7	2.3	4.0	0.3	2.7	1.9	5.0
$^2J_{H3-C2}$	-1.4	-2.2	0.3	-0.6	-1.8	0.6	-1.4
$^3J_{H3-C=O}$	7.2	2.4	1.4	7.4	1.0	2.1	2.0
TAD ($\Sigma\|J_{calc}-J_{exp}\|$)	**17.0**	**11.0**	**4.3**	**13.8**	**11.6**	**13.0**	







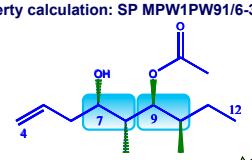
Krishnamoorthy, R.; Vazquez-Serrano, L. D.; Turk, J. A.; Kowalski, J. A.; Benson, A. G.; Breaux, N. T., and Lipton, M. A. *J. Am. Chem. Soc.* **2006**, *128*, 15392-15393.



Plaza, A.; Bifulco, G.; Keffer, J. L.; Lloyd, J. R.; Baker, H. L.; Bewley, C. A. *J. Org. Chem.* **2009**, *74*, 504–512.

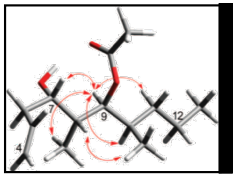
QM Calculation of $^{3}J_{H,H}$ and $^{2,3}J_{C,H}$ Coupling Constants: streosturcute assigment of Celebedises A-C

✓Geometry optimization: MPW1PW91/6-31G(d)
✓Property calculation: SP MPW1PW91/6-31G(d,p)



7S,8R*,9S*,10R**

Confirmed by key ROE correlations of the all molecule



celebeside A

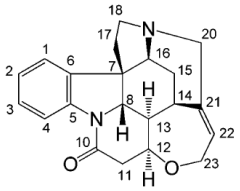
	Calculated						Exp
	threo			*erythro*			
C7-C8	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**	
H7-Me8	2.7	4.0	4.3	2.9	4.2	5.1	5.5
H7-C9	4.9	0.4	2.1	5.6	1.5	0.1	3.1
H8-C6	4.4	1.7	0.5	0.7	5.2	5.7	2.6
H8-H7	2.7	7.5	2.2	2.4	4.1	6.3	1
$^{2,3}J_{C,H}$	8.0	11.7	5.5	8.4	8.7	11.8	
C8-C9	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	
H8-C9	-0.4	-4.8	-3.8	-4.1	-0.7	-5.2	-7.2
H9-C7	5.2	5.5	1.8	2.3	2.3	1.2	3.0
H9-Me8	1.5	3.6	2.7	1.0	6.5	5.1	1.3
H9-H8	2.0	2.5	9.0	9.2	1.2	5.1	10.6
$^{2,3}J_{C,H}$	17.8	13.3	7.0	5.5	21.9	13.6	
C9-C10	**C1**	**C2**	**C3**	**C4**	**C5**	**C6**	
H9-Me10	5.0	0.4	2.5	6.6	4.7	2.9	5.3
H9-C11	2.6	6.2	4.4	0.3	2.2	4.7	4.0
H10-C9	2.3	-2.0	-5.7	-2.1	-5.7	2.3	1.3
H10-H9	2.6	4.4	2.3	4.8	2.3	2.7	1.1
$^{2,3}J_{C,H}$	4.2	11.7	11.3	12.1	10.7	8.7	

[a]Total absolute deviation ($\Sigma|J_{exp} - J_{calc}|$) values.

Plaza, A.; Bifulco, G.; Keffer, J. L.; Lloyd, J. R.; Baker, H. L.; Bewley, C. A. *J. Org. Chem.* ***2009***, *74*, 504–512.

Quantum Chemical Calculations of $^{1}J_{CC}$ Coupling Constants for the Stereochemical Determination of Organic Compounds

✓sensitivity advances in state of art cryogenic NMR probes
✓number of efficient pulse sequences such as *J*-modulated ADEQUATE
✓feasibility of collecting a significant set of $^{1}J_{CC}$ and $^{n}J_{CC}$ data even for relatively mass-limited samples
✓these parameters are relatively easily measured in an overnight NMR experiment using few milligrams of material



✓for strychnine a mean absolute error (**MAE**) of **0.84 Hz** and a **correlation coefficient** of **0.9986** was observed in a comparison of experimental and calculated $^{1}J_{CC}$ and $^{3}J_{CC}$ NMR data

Bifulco, G.; Riccio, R.; Martin, G., E.; Buevich, A. V.; Williamson, R.T. *Org. Lett.*, **2013**, *15*, 654–657.

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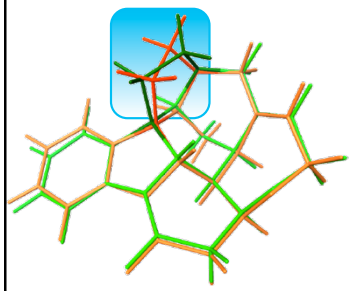

Lowest energy conformation (green, **1a**) and higher energy conformation (orange, **1b**) with RMSD of only 0.524 A ° between them

$^{1}J_{CC}$	exptl, (Hz)	**1a**	**1b**
C1–C2	58.1	59.1	59.2
C2–C3	58.9	59.9	59.8
C3–C4	59.9	59.0	59.1
C4–C5	64.9	66.9	66.8
C1–C6	65.7	65.9	66.0
C7–C8	33.9	34.1	35.2
C10–C11	50.1	49.6	49.7
C11–C12	37.6	36.5	36.8
C12–C13	36.8	35.3	34.5
C8–C13	32.4	32.4	32.6
C13–C14	32.4	32.3	32.6
C14–C15	32.6	32.3	32.3
C7–C16	33.7	33.4	29.9
C15–C16	33.3	32.9	33.8
C17–C18	34.0	32.6	29.9
C20–C21	41.6	40.2	39.1
C14–C21	37.6	37.2	37.3
C21–C22	71.8	73.9	74.4
C22–C23	45.6	45.2	45.3
C7–C17	32.1	31.6	38.0
C5–C6	60.7	61.4	61.7
C6–C7	43.3	43.3	42.4
MAE, (Hz)		0.75	1.47

1b is 4.01 kcal/mol less stable than **1a** (~0.11% of **1b** at 25 C to the conformational equilibrium)

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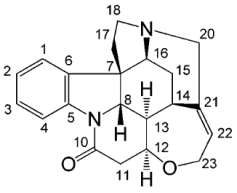
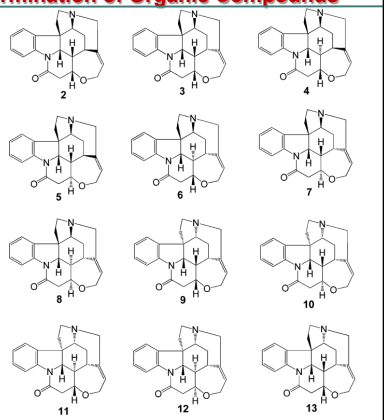


molecular modeling investigation filter



✓ Conformational search at the empirical level
✓ geometry- and energy optimized at the B3LYP/6-31G(d) level of theory

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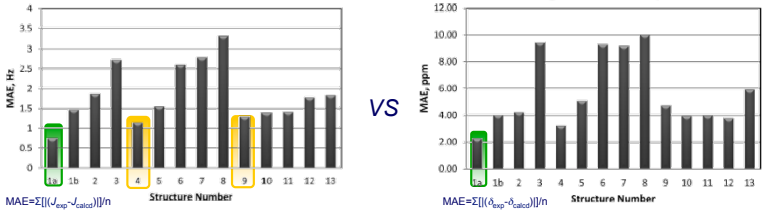

QM calculations of $^1J_{C\text{-}C}$ coupling constants (B3LYP/6-311+G(d,p). The Fermi contact (FC) contributions were calculated by adding tights polarization functions for the s and d orbitals to the original 6-311+G(d,p) basis set, which was accomplished in Gaussian09 with the "mixed" keyword)

QM calculations of ^{13}C NMR chemical shifts (B3LYP/6-31G(d) optimized geometries(GIAO method at the MPWPW91/6-31G(d,p))

- The use of $^1J_{C\text{-}C}$ coupling constants as important and self-consistent parameters for the determination of the conformational and configurational features of complex organic molecules.

✓The experimental measurement of $^1J_{C\text{-}C}$ coupling constants is also much easier experimentally than the measurement of $^nJ_{C\text{-}C}$ coupling constants.

✓The use of $^1J_{C\text{-}C}$ coupling constants to define stereochemistry should prove to be a powerful tool that can be interfaced and combined with other currently available spectroscopic methods to corroborate and confirm the relative stereochemical assignment of unknown molecules

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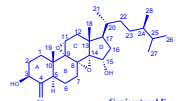
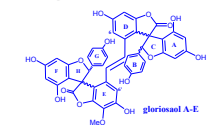
Quantum mechanical methods as a support in the determination of the relative configuration of organic molecules

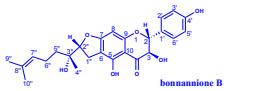
- QM calculation of ^1H /^{13}C chemical shift (DFT methods)

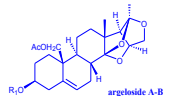
Comparison of the experimental data of the compound with unkwnown configuration with the calculated results for all the possible stereoisomers

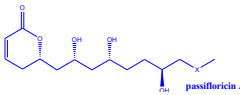
Barone, G.; Duca, D.; Silvestri, A.; Gomez-Paloma, L.; Riccio, R.; Bifulco, G. *Chem. Eur. J.* **2002**, *8*, 3240-3245.
Bifulco, G.; Dambruoso, P.; Gomez-Paloma, L.; Riccio, R. *Chem. Rev. (Washington, DC, U. S.)* **2007**, *107*, 3744-3779.
Di Micco, S.; Chini, M. G.; Riccio, R.; Bifulco, G. *Eur. J. Org. Chem.* **2010**, *8*, 1411-1434.

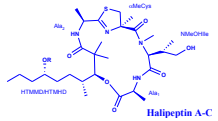

Conicasterol F
Chini, M. G.; Jones, C. R.; Zampella, A. , D'Auria, M. V.; Renga, B.; Fiorucci, S.; Butts C. P.; Bifulco G. *J. Org. Chem.* **2012**, *77*, 1489-1496. *77*, 1489-1496.


gloriosaol A-E
a) Bassarello, C.; Bifulco, G.; Montoro, P.; Skhirtladze, A.; Kemertelidze, E.; Pizza, C.; Piacente, S. *Tetrahedron* **2007**, *63*, 148–154. b) Bassarello, C.; Bifulco, G.; Montoro, P.; Skhirtladze, A.; Kemertelidze, E.; Pizza, C.; Piacente, S. *J. Agric. Food Chem.* **2007**, *55*, 6636–6642

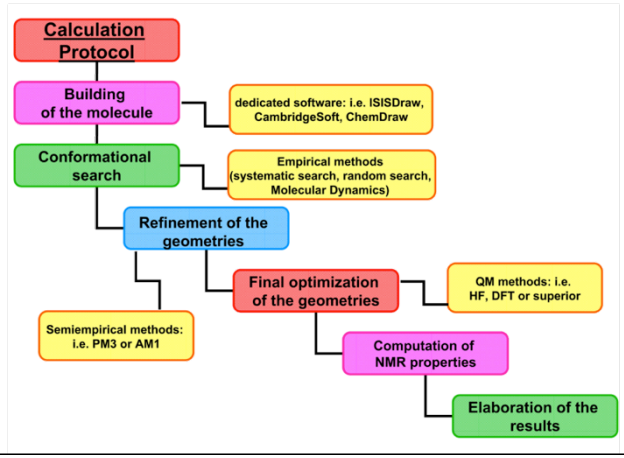

bonnannione B
Rosselli, S.; Bruno, M.; Maggio, A.; Bellone, G.; Formisano, C.; Mattia, C. A.; Di Micco, S.; Bifulco, G. *Eur. J. Org. Chem.* **2007**, 2504–2510.


argeloside A-B
Plaza, A.; Bifulco, G.; Hamed, A.I.; Pizza, C.; Piacente, S. *Tetrahedron Letters* **2003**, *44*, 8553–8558


passifloricin A
Bifulco, G.; Gomez-Paloma, L.; Riccio, R. *Tetrahedron Letters* **2003**, *44*, 7137–7141


Halipeptin A-C
Della Monica,C.; Randazzo, A.; Bifulco, G.; Cimino, P.; Aquino,M.;Izzo, I.; De Riccardis, F.; Gomez-Paloma, L. *Tetrahedron Letters* **2002**, *43*, 5707–5710.

Calculation Protocol for Rigid/Flexible Molecular Frameworks



Quantum mechanical methods as a support in the determination of the relative configuration of organic molecules

^{13}C Nuclear shielding as a sensitive probe of the molecular environment

Theoretical Methods for Shielding Computation

1. Solution of Schroedinger equation in the absence of a magnetic field
2. Application of a magnetic moment and a static external magnetic field
3. Transformation of the zero order and first order density matrices are then used to give the diamagnetic and paramagnetic shielding terms, respectively

Common Approaches for the Chemical Shift Calculation

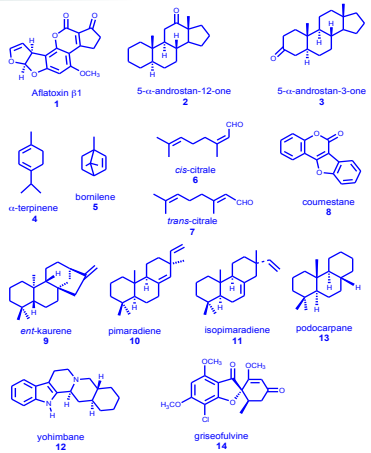
- GIAO – Gauge Including Atomic Orbital method
- IGLO – Individualized Gauge for Localized Orbitals method
- LORG – Localized ORbital Local Origin method
- CSGT – Continuos Set of Gauge Transformation method

These approaches have been often limited to small molecules due to large computational costs

Validation of the Calculation Protocol in 14 Model Compounds

^{13}C NMR chemical shifts calculations, employing either HF or DFT approaches, displayed good to excellent agreements with experimental data

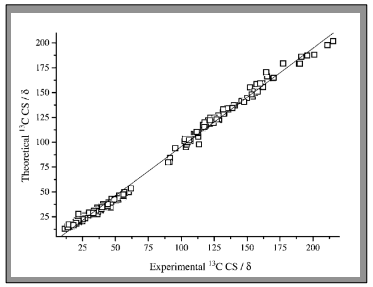
expecially for **low polar compounds** whose NMR spectra were typically recorded in **D-chloroform solution**



Barone, G.; Gomez-Paloma, L.; Duca, D.; Silvestri, A.; Riccio, R.; Bifulco, G. *Chem Eur. J* **2002** 8, 3233-3239.

Validation of the Calculation Protocol in 14 Model Compounds

Correlation plot of calculated vs. experimental ^{13}C NMR chemical shifts, at HF level, for the species 1-14



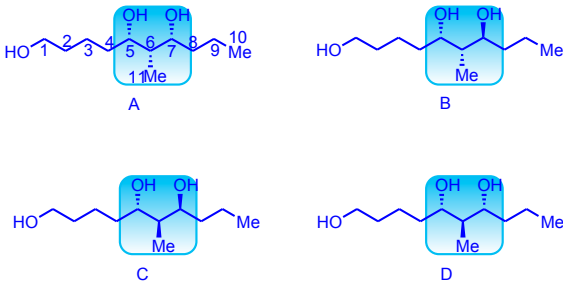
Intercept, slope, correlation coefficient (**r**) and corresponding HF and B3LYP energy, obtained by linear fits of calculated vs. experimental ^{13}C NMR C.S. plots for the species **1-14**.

Species	Theory	Intercept	Slope	r	Energy (a.u.)
1	HF	-5.650	1.008	0.995(5)	-1099.9125
2	HF B3LYP	-2.080 3.530	0.933 0.934	0.998(0) 0.999(8)	-811.8504 -817.3365
3	HF	-2.060	0.936	0.998(2)	-811.8499
4	HF	-1.252	0.996	0.999(5)	-387.9785
5	HF	-2.068	0.985	0.997(1)	-387.9604
6	HF	0.758	0.955	0.999(7)	-462.8238
7	HF	0.556	0.956	0.999(7)	-462.8236
8	HF B3LYP	4.285 2.443	0.950 0.934	0.989(2) 0.996(4)	-797.3028 -802.1093
9	HF	-3.278	0.974	0.994(7)	-775.9779
10	HF	-3.568	0.981	0.997(1)	-775.9683
11	HF	-3.594	0.992	0.995(5)	-775.9673
12	HF	-5.551	1.029	0.997(8)	-842.3934
13	HF	3.985	0.747	0.995(8)	-661.2445
14	HF	-5.810	1.011	0.997(4)	-1562.2763
average	HF	-3.265	0.990	0.997(6)	---

Barone, G.; Gomez-Paloma, L.; Duca, D.; Silvestri, A.; Riccio, R.; Bifulco, G. *Chem Eur. J* **2002** 8, 3233-3239.

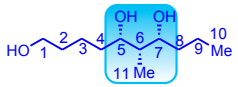
From Rigid Compounds with Known Stereochemistry to Flexible Compounds with Unknown Stereochemistry....

Geometry optimization and ^{13}C NMR chemical shift calculation by ab initio methods as a support to stereochemical investigation of low-polar open chain compounds



Synthetic model stereoisomers: Kobayashi, Y.; Tan, C.-H.; Kishi, Y. *J. Am. Chem. Soc.* **2001**, *123*, 2076-2078

Stereoisomer A: values of the GIAO calculated ^{13}C NMR c.s. (ppm relative to TMS) for the conformers A1-A8 and their Boltzmann weighted average

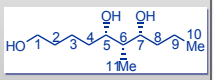


^{13}C atom	A1	A2	A3	A4	A5	A6	A7	A8	average
1	57.1	56.8	56.3	56.9	56.1	56.9	56.8	56.8	57.0
2	29.9	29.6	31.0	29.7	32.2	29.7	32.7	32.7	29.8
3	21.8	21.6	20.8	21.8	22.4	21.8	19.8	19.2	21.7
4	33.7	34.6	33.2	34.5	34.8	34.1	33.3	34.4	34.1
5	67.7	69.8	68.8	62.4	62.9	61.9	61.3	61.8	68.6
6	39.2	39.3	35.2	42.9	41.4	38.0	36.8	33.0	39.1
7	69.8	67.8	67.5	64.9	63.9	65.9	65.4	63.1	68.8
8	35.7	34.8	35.0	33.9	34.3	31.3	33.4	33.5	35.3
9	19.5	19.7	19.7	19.2	19.6	19.8	18.8	14.5	19.6
10	15.2	15.5	15.5	15.4	15.4	15.4	15.5	15.5	15.4
11	6.8	6.7	5.9	10.6	9.9	11.0	10.0	10.3	6.7
ΔG° (kJ/mol)	0	0.3833	7.0520	13.2298	15.5639	14.5767	17.9479	19.7043	
%	51.94	44.50	3.02	0.25	0.10	0.14	0.04	0.01	

^{13}C atom	average
1	57.0
2	29.8
3	21.7
4	34.1
5	68.6
6	39.1
7	68.8
8	35.3
9	19.6
10	15.4
11	6.7

Stereoisomer **A**

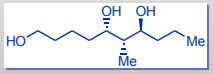
values of GIAO ^{13}C c.s. (ppm relative to TMS) calcd. for the Boltzmann average of conformers **A1-A8**



^{13}C atom	average
1	56.9
2	30.2
3	21.7
4	33.8
5	62.5
6	38.6
7	68.6
8	34.9
9	19.3
10	15.4
11	14.1

Stereoisomer **B**

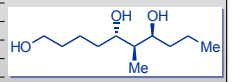
values of GIAO ^{13}C c.s. (ppm relative to TMS) calcd. for the Boltzmann average of conformers **B1-B8**



^{13}C atom	average
1	56.2
2	31.5
3	21.7
4	33.5
5	68.8
6	38.8
7	62.2
8	34.4
9	19.5
10	15.5
11	14.7

Stereoisomer **C**

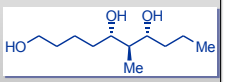
values of GIAO ^{13}C c.s. (ppm relative to TMS) calcd. for the Boltzmann average of conformers **C1-C13**



^{13}C atom	average
1	56.9
2	30.1
3	21.2
4	33.5
5	66.4
6	40.1
7	67.4
8	35.3
9	19.1
10	15.4
11	15.3

Stereoisomer **D**

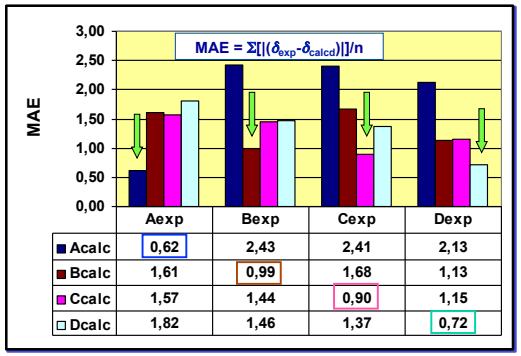
values of GIAO ^{13}C c.s. (ppm relative to TMS) calcd. for the Boltzmann average of conformers **D1-D10**



Barone, G.; Duca, D.; Silvestri, A.; Gomez Paloma, L.; Riccio, R.; Bifulco, G. *Chem Eur. J* **2002**, 8, 3240-3245.

Mean Absolute Error (MAE) for the ^{13}C NMR calculated chemical shifts of the four stereoisomers A-D

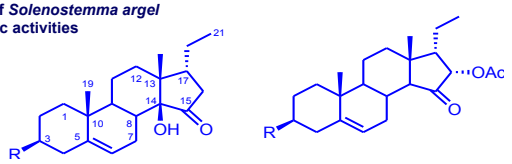
summation through n of the absolute error values (difference of the absolute values between corresponding experimental and calculated ^{13}C chemical shifts), normalized to the number of the carbon atoms considered

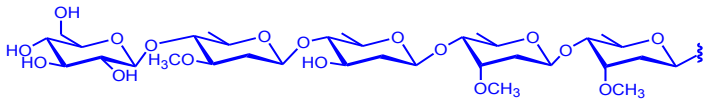


	Aexp	Bexp	Cexp	Dexp
Acalc	0,62	2,43	2,41	2,13
Bcalc	1,61	0,99	1,68	1,13
Ccalc	1,57	1,44	0,90	1,15
Dcalc	1,82	1,46	1,37	0,72

Stemmosides C and D: structural elucidation and configurational study by a combined NMR-quantum mechanical strategy

from the pericarps of *Solenostemma argel*
antitumor & cytotoxic activities



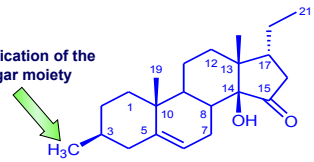


β-D-Glu **β-D-Ole** **β-D-Can** **β-D-Cym** **β-D-Cym**

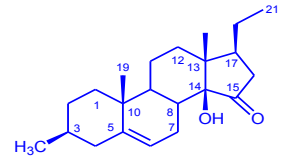
Plaza, A.; Piacente, S.; Perrone, A.; Hamed, A.; Pizza, C.; Bifulco, Giuseppe *Tetrahedron* **2004**, 60, 12201-12209.

Stemmosides C and D: structural elucidation and configurational study by a combined NMR-quantum mechanical strategy

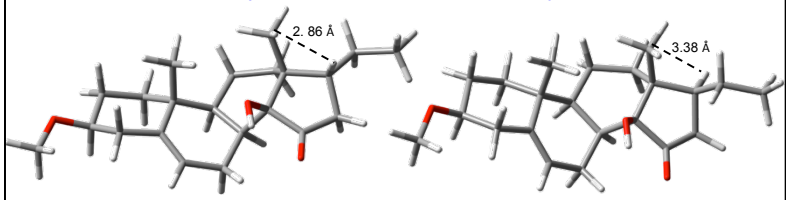
Simplification of the sugar moiety



1a



1b



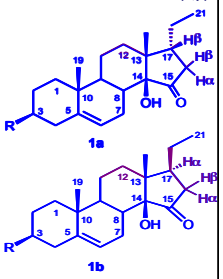
1a **1b**

Plaza, A.; Piacente, S.; Perrone, A.; Hamed, A.; Pizza, C.; Bifulco, Giuseppe *Tetrahedron* **2004**, 60, 12201-12209.

^{13}C NMR of stemmoside C (1) and calculated GIAO ^{13}C NMR c.s.(δ) of stereoiomers 1a and 1b, and Δδ for 1a e 1b.

atom	1	1a	1b	Δδ 1a	Δδ 1b
C-8	38.0	36.1	35.4	1.9	2.6
C-11	21.9	22.9	23.1	-1.0	-1.3
C-12	29.3	29.5	38.0	-0.2	-8.7
C-13	45.7	46.2	45.1	-0.5	0.6
C-14	82.5	79.9	80.0	2.6	2.5
C-16	40.0	39.6	39.0	0.4	1.0
C-17	43.5	42.1	44.6	1.4	-1.1
C-18	15.3	15.4	15.3	-0.1	0.0
C-20	22.4	24.2	31.1	-1.8	-8.7
C-21	13.4	15.1	15.4	-1.7	-2.0

-Geometry optimization: MPW1PW91/6-31G(d)
- ^{13}C and ^{1}H : GIAO/ SP MPW1PW91/6-31G(d,p)



Key Chemical shifts ^{1}H NMR of 1, and calculated c.s. GIAO ^{1}H NMR for the steroisomers 1a and 1b, and Δδ for 1a and 1 b.

atom	1	1a	1b	Δδ 1a	Δδ 1b
H16α	1.77	1.74	2.33	0.03	-0.56
H16β	2.62	2.60	2.09	0.02	0.53
H17	2.17	2.28	1.33	-0.11	0.84

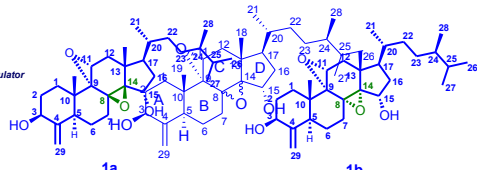
Comaprison between $^{3}J_{HH}$ (Hz) experimental and (1) calculated values (stereoisomerers 1a and 1b) of ring D (ONIOM calculation using the MPW1PW91 functional and the 3-21G (low level, rings A–B) and 6-31G(d,p) (high level, ring C–D))

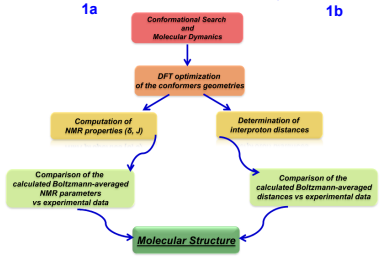
	1	1a	1b
H16α-H17	9.0	7.7	9.7
H16β-H17	9.0	8.9	3.3

Plaza, A.; Piacente, S.; Perrone, A.; Hamed, A.; Pizza, C.; Bifulco, G. *Tetrahedron* **2004**, 60, 12201-12209.

Stereochemical determination of conicasterol F by QM/NMR + Quantitative ROE

isolated from *Theonella swinhoei*
human nuclear receptor (FXR) modulator

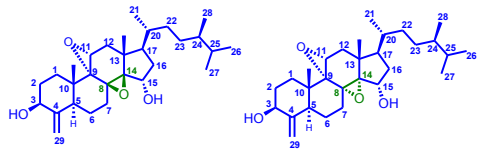


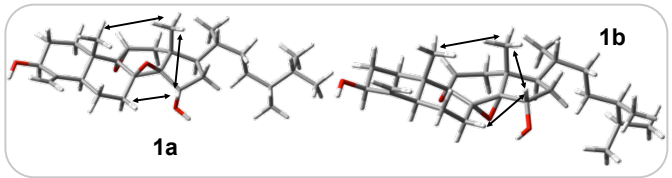


a) Bifulco, G .; Dambruoso, P.; Gomez-Paloma, L.; Riccio. R. *Chem. Rev.* **2007**, *107*, 3744-3779; b) Butts, C. P.; Jones, C. R.; Towers, E. C.; Flynn, J. L.; Appleby, L.; Barron, N. *Org. Biomol. Chem.* **2011**, *9*, 177–184; c) Butts, C. P.; Jones, C. R.; Harvey, J. N. *Chem. Commun.* **2011**, *47*, 1193–1195; d) Butts, C. P.; Jones, C. R.; Harvey, J. N. *Beilstein J. Org. Chem.* **2011**, *7*, 145–150; e) Chini, M. G.; Jones, C. R.; Zampella, A .; D'Auria, M. V.; Renga, B.; Fiorucci, S.; Butts C. P.; Bifulco G . *J. Org. Chem.* **2012**, 77, 1489-1496.

Stereochemical determination of conicasterol F by QM/NMR + Quantitative ROE

❖ Step 1-2 Conformational search/DFT optimization MPW1PW91 level and basis set 6-31g(d)





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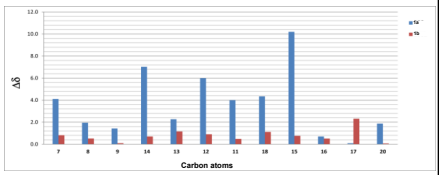
Stereochemical determination of conicasterol F by QM/NMR + Quantitative ROE

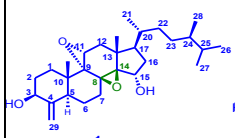
❖ Step 3. ^{13}C chemical shifts calculation at DFT/MPW1PW91 level and basis set 6-31g(d,p)

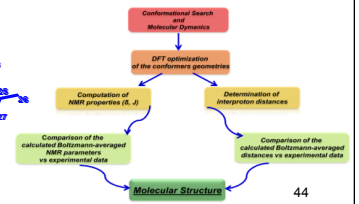
		1a		1b	
Carbon	δ_{exp}	δ_{calc}	$^{a}\|\Delta\delta\|$,ppm	δ_{calc}	$^{a}\|\Delta\delta\|$,ppm
7	27.3	31.4	4.1	28.1	0.8
8	60.8	62.8	2.0	61.3	0.5
9	61.5	62.9	1.4	61.6	0.1
14	79.8	72.8	7.0	79.1	0.7
13	44.9	42.6	2.3	46.1	1.2
12	35.7	41.5	5.8	36.4	0.7
11	50.1	54.1	4.0	49.6	0.5
18	14.8	19.1	4.3	15.9	1.1
15	65.0	75.3	10.3	64.3	0.7
16	40.9	40.2	0.7	41.4	0.5
17	50.6	50.5	0.1	48.1	2.5
20	35.0	33.2	1.8	35.0	0.0
^{b}MAE			3.7		0.8

$^{a}|\Delta\delta| = |\delta_{exp} - \delta_{calc}|$, absolute differences for experimental versus calculated NMR chemical shifts.

$^{b}MAE = \sum|\delta_{exp} - \delta_{calc}|/n$.







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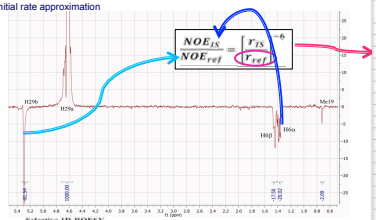
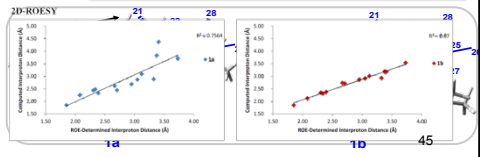
Stereochemical determination of conicasterol F by QM/NMR + Quantitative ROE

✓1D-DPFGSE (double pulsed field gradient spin-echo) sequences (1D-Selective ROESY, 600 MHz, C_5D_5N)
✓Initial rate approximation



Proton		Exp.	1a		1b	
		R_{ROE}(Å)	R_{calc}(Å)	ABS % error[a]	R_{calc}(Å)	ABS % error[a]
H29a	**H29b**	1.85	1.85		1.85	
H7β	H15β	2.73	2.45	**11.4%**	2.71	**0.5%**
H11β	H12α	3.07	2.87	6.9%	2.92	5.1%
H11β	Me19	3.43	3.83	10.4%	3.20	7.4%
H11β	H1β	2.09	2.26	7.4%	2.12	1.7%
H15β	H16α	2.94	2.69	9.3%	2.87	2.5%
H15β	H16β	2.38	2.48	4.0%	2.32	2.9%
Me18	H15β	3.42	4.37	**21.7%**	3.18	**7.5%**
Me18	H11β	3.67	3.71	1.2%	3.54	3.7%
Me18	H12β	3.12	2.89	8.1%	2.93	6.3%
Me18	H16β	3.02	3.09	2.2%	3.01	0.4%
Me18	H20	2.70	2.61	3.1%	2.74	1.6%
Me18	Me19	3.31	4.00	**17.2%**	3.28	**1.0%**
H29a	H6β	2.34	2.33	0.4%	2.38	1.7%
H29a	H6α	2.39	2.44	2.0%	2.35	1.8%
[b]MAE				**7.5%**		**3.1%**
STD				**6.2%**		**2.5%**

[a]% error|= $|r_{calc}-r_{ROE}|/r_{calc}$, absolute differences for calculated versus ROE derived distances/calculated distances
[b]MAE=$\sum$[%error|]/n.



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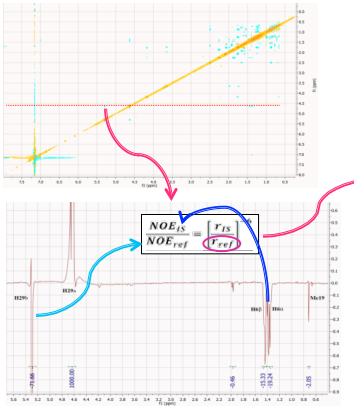
Stereochemical determination of conicasterol F by QM/NMR + Quantitative ROE



Proton		Exp.	1a		1b	
		R_{ROE}(Å)	R_{calc}(Å)	ABS % error[a]	R_{calc}(Å)	ABS % error[a]
H29a	**H29b**	**1.85**	**1.85**		**1.85**	
H7β	H15β	2.70	2.45	**10.4%**	2.71	**0.4%**
H11β	H12α	3.05	2.87	6.3%	2.92	4.5%
H11β	Me19	3.38	3.83	11.9%	3.20	5.6%
H11β	H1β	2.08	2.26	7.8%	2.12	2.1%
H15β	H16α	2.95	2.69	9.6%	2.87	2.8%
H15β	H16β	2.34	2.48	5.9%	2.32	0.8%
Me18	H15β	3.41	4.37	**22.0%**	3.18	**7.1%**
Me18	H11β	3.73	3.71	0.5%	3.54	5.5%
Me18	H12β	3.32	2.89	15.2%	2.93	13.3%
Me18	H16β	3.12	3.09	1.0%	3.01	3.6%
Me18	H20	2.67	2.61	2.0%	2.74	2.7%
H29a	H6α	2.39	2.33	2.9%	2.38	0.7%
H29a	H6β	2.30	2.44	5.6%	2.35	1.9%
[b]MAE				**7.7%**		**3.9%**
STD				**6.2%**		**3.5%**

[a]% error|= $|r_{calc}-r_{ROE}|/r_{calc}$, absolute differences for calculated versus ROE derived distances/calculated distances
[b]MAE=$\sum$[%error|]/n.

2D vs 1D

ADVANTAGES:
Possible to observe more distances
Less setup required

DISADVANTAGES:
Significantly longer experiment time for comparable accuracy
Prone to artefacts e.g. t_1 noise

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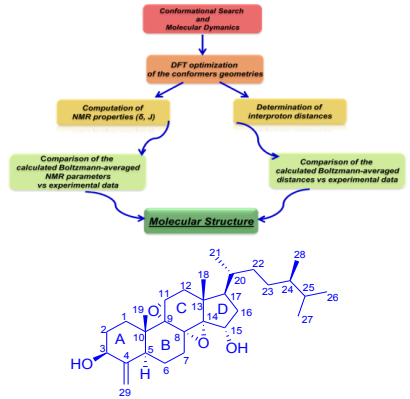

Conicasterol F

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