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# Resonance driven regioselective demethylation of berberine. Microwave assisted synthesis of berberrubine and its assessment as fluorescent chemosensor for alkanes



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# ABSTRACT

Berberrubine has been synthesized by microwave assisted selective demethylation of berberine. The high selectivity observed in this reaction has been explained and justified by means of computational calculations using Density Functional Theory (DFT) and Natural Resonance Theory (NRT). The existence of two resonant structures of berberrubine is the driving force of regioselective demethylation. Berberrubine is a chemosensor of alkanes, and may have practical applications in petrochemical analysis as a 'mass' detector because fluorescent response of saturated hydrocarbons does not depend on hydrocarbon chain length. Berberrubine operates via dipole-induced dipole interactions. Likewise, it has two fluorescent forms in acidic and basic media, which correspond to a keto-enol tautomerism. The fluorescent signal for berberrubine and the amplification of berberrubine-alkane signals by heating can be rationalized from the predominance of enol form when berberrubine is adsorbed onto silica gel.

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# 1. Introduction

In the field of hydrocarbon conversion, it is necessary to combine the use of progressively heavier hydrocarbon-containing feedstocks with the development of sustainable processes that meet environmental standards more and more stringent and restrictive.

Development of economical and environmental friendly techniques for characterizing heavy hydrocarbon-containing feedstocks is crucial for anticipating their behaviour in refining optimization, both in terms of the quality of the obtained final product as with regard to the fine-tuning of the process variables that contribute to its environmental impact. For this, it is necessary to have a detailed knowledge of the composition of chemical families, which constitute these heavy products. Hydrocarbon-containing products consist of an important proportion of saturated hydrocarbons, which have structures of increasing chain length and boiling point. A rapid and accurate determination of these compounds as a group, i.e. Saturates, and other hydrocarbon types in heavy petroleum products has not been fully solved yet.<sup>1</sup>

Despite the interest of this problem and the emergence in the development of systems in the field of fluorescent sensing,<sup>2</sup> little research has been conducted for detecting and quantitatively determining these compounds in such matrices.

In a previous work, berberine cation was proposed as a chemosensor for alkanes, based on non-specific interactions.<sup>3,4</sup> When berberine is in presence of an alkane, and the system is irradiated with appropriate UV light, a fluorescent signal is produced, which depends on the alkane concentration and on the alkane length. Computational results suggested that enhancements in fluorescent signal are consequence of the interaction between the cationic fluorophore and the alkanes (or the hydrocarbon chain in other

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low-polarity analytes), which isolates the fluorophore in an apolar microenvironment. This protects it from polar nonradiative decays.

A model was proposed<sup>3,5</sup> for this ion-induced dipole interaction, which accounted for experimental results. As the intensity enhancement will also be proportional to the interaction energy, we have, for an ionic probe P and analyte i (alkane)

$$U_{\mathrm{P},i} = -1 / \left(4\pi\varepsilon_0^2\right) \left(Z_P^2 e^2 \alpha_i\right) / \left(2r^4\right) \tag{1}$$

where *r* is the average probe-analyte intermolecular distance,  $Z_P$  is the charge of the fluorescent probe,  $\varepsilon_0$  is the dielectric permittivity of the medium, and  $\alpha_i$  is the polarizability of *i*.

At a given temperature and concentration, the enhancement of intensity is linearly dependent of  $\alpha_i$  of the neutral molecule surrounding the fluorophore, since the remaining magnitudes must be practically constant on passing from one alkane to another.

The possibility of using dipolar probes (*DP*) for sensing alkanes was also suggested in another work,<sup>4</sup> using the Debye equation for modelling the corresponding dipole-induced dipole interactions.<sup>6</sup> In the case of polarizable analytes with no permanent dipole moments, i.e., alkanes, the equation for the interaction energy becomes:

$$U_{\text{DP},i} = -1 / \left( 4\pi \varepsilon_0^2 \right) \left( \mu_{DP}^2 \alpha_i \right) / \left( r^6 \right)$$
<sup>(2)</sup>

where  $\alpha_i$  is the polarizability of *i* and  $\mu_{DP}$  is the corresponding permanent dipole moment.

We report in this paper that dipolar probe berberrubine, a berberine-derivative, provides increases in its fluorescence emission in the presence of alkanes, behaving as a quantitative chemosensor for them. In this way, non-absorbing alkanes can be quantitatively detected.

Berberrubine shows different properties than berberine. They are described here, and applied to determine saturated hydrocarbons in petroleum products in a rapid way with low solvent consumption.

Berberrubine has been obtained from berberine by solvent-free, microwave-based synthesis. The origin on the selectivity of this demethylation process has also been analysed by means of computational calculations.

## 2. Results and discussion

#### 2.1. Berberrubine synthesis

Berberrubine was reported to be obtained by direct microwave irradiation (5 min) in a simple, straightforward way under solvent and catalyst-free conditions, according to Das and Srinivas<sup>7</sup> although working power was not specified in that work. Under the described conditions, we were unable to reproduce their results probably by using a different power. Berberrubine was finally synthesized by direct microwave irradiation under different conditions of temperature, time, and power, which are detailed in Table 1.

 Table 1

 Catalyst and solvent-free, microwave-assisted synthesis of berberrubine from berberine

Entry	Temperature (°C)	Time (min.)	Power (W)	Conversion (%)	Yield (%)
1	180	20	300	a	a
2	200	15	300	a	a
3	130	5/10/5 <sup>b</sup>	300	47	n.m.
4	130/180	5/10 <sup>c</sup>	300	100	85.3

n.m.: not measured.

<sup>a</sup> Berberrubine was not obtained.

<sup>b</sup> Reaction carried out in three steps.

<sup>c</sup> Reaction carried out in two steps.

Berberine chloride (100 mg, 0.287 mmol) was introduced in a vial, and vacuum was made. After selecting conditions of maximum temperature, time, and power, reaction was carried out in one step (entry 1 and 2, Table 1), two steps (entry 4, Table 1) or three steps (entry 3, Table 1).

Selected conditions were those of entry 4 (two steps, 100% conversion): i) sample was irradiated 5 min at 300 W up to 130  $^{\circ}$ C, then allowed to cool to room temperature; and ii) 10 min-irradiation at 300 W to 180  $^{\circ}$ C. The ketonic form of berberrubine was obtained as a reddish solid (yield: 85.3%).

Characterization of reaction products and monitoring of conversion of berberine into berberrubine were done by <sup>1</sup>H NMR spectroscopy (Fig. 1). Furthermore, assignment of the signals were verified by comparison with the NMR spectra simulated at GIAO-B3LYP/6-31+G\* level of theory (See Supplementary data for further details).

# 2.2. DFT study

In order to shed some light on the high selectivity observed in the demethylation of berberine to form berberrubine, we have performed a computational study of this process. Formally, this transformation consists of a  $S_N2$  reaction on a methyl group, in which the nucleophile is the chloride counterion and the leaving group can be berberrubine **2** or, alternatively, thalifendine **3**. Since there are two possible methyl groups capable of reacting with chloride, formation of both demethylated compounds was considered (Fig. 2). Moreover, the non-standard reaction conditions (namely high temperature and low pressure) were also considered in the study.

Our results show that the activation barrier associated with the formation of berberrubine is lower than that of thalifendine (ca. 3 kcal/mol). In both cases, the high activation barriers computed agree well with the harsh experimental reaction conditions required. In addition, the polarity increases going from the reactant to the possible transition structures, thus explaining the suitability of microwave irradiation as heating source<sup>8,9</sup> Besides, berberrubine formation is more exergonic than the formation of thalifendine. Therefore, generation of berberrubine is both kinetically and thermodynamically favoured.

NRT analysis of the final products show that berberrubine has two possible resonance forms, being the neutral structure the most relevant one (>90% of the computed relative weight). On the other

H<sub>2</sub>O



Fig. 1. The ketonic form of berberrubine obtained by microwave irradiation. Signal assigned <sup>1</sup>H NMR.



Fig. 2. Activation and reaction Gibbs free energies level of theory at 298 K, 1 atm and 498 K 0.01 atm (in parentheses) associated with the formation of berberrubine 2 and thalifendine 3 computed at B3LYP-D3/6-31G\*. Relevant resonance structures of products obtained via NRT analysis are also shown. Energies are in kcal/mol and distances are in Å.

hand, thalifendine only presents a zwitterionic structure. The existence of an additional resonant structure in berberrubine (**2b**) compared to thalifendine implies a higher stabilization of the former, which in turn can be assumed to be the driving force of the demethylation reaction.

Moreover, the computed NICS<sup>10</sup> values on the critical ring point of the electron density for both stationary points are quite similar and therefore, no explanation of the observed selectivity can be extracted from these data (See Supplementary data for further details).

In conclusion, these computational results are in good agreement with the excellent selectivity observed in the demethylation of berberine under the studied reaction conditions.

## 2.3. Fluorescence response analysis

When berberrubine (impregnated onto a silica gel plate<sup>11</sup>) is in presence of alkanes, and the system is heated at 65 °C during 30 min and excited at 365 nm, we have found that fluorescent signals are produced (emission collected at 400 nm). However, unlike the case of berberine where the response is proportional to the polarizability of the alkane, fluorescent response of berberrubine is almost constant with the number of carbon atoms in the hydrocarbon chain (Fig. 3A). Likewise, for a given alkane, a linear dependence of response with sample load has been obtained (Fig. 3B). Although, berberrubine gives a moderate level of fluorescent signal in its interaction with alkanes (for  $C_{24}$  is a 33% of the berberine response), the absolute value of signal is measurable, repeatable, and can be used in combination with chromatographic techniques as, for example, HPTLC with fluorescence scanning densitometry. Methods for detection of saturated hydrocarbons and also lipids were developed<sup>12–14</sup> using this technique in combination with berberine.

Fig. 4 shows that fluorescence signals corresponding to saturated hydrocarbons in synthetic mixture-1 increase with sample load. Signals were obtained using the berberrubine system under the described conditions. Scanning densitometry is a rapid measurement. Detection can be performed at will on a desired zone of the plate.

It is the combination of the peculiar behaviour of berberrubine with regard to the alkane chain length and an adequate level of signal that makes it especially useful for a practical application in petrochemistry. For practical purposes, the fact that the fluorescent response is almost constant for alkanes of different number of carbon atoms makes that berberrubine can be used as a mass detector calibrant in the analysis of mixtures of alkanes of different chain length.

Indeed, unknown samples containing alkanes, such as those found in heavy petroleum products, may be analysed by calibrating using the mass pattern of a single alkane or a mixture of alkanes. This is of interest for petrochemical analysis because calibration steps are usually tedious and time-consuming. They include preparative isolation of saturated hydrocarbons and use of this whole separated fraction as a calibrant. This should be done every time for each petroleum product or set of representative samples of a product type in order to obtain a quantitative analysis.

Our results indicate that a pure alkane or mixture of alkanes can be used as a single calibrant in the berberrubine-chemosensing system for determination of saturates in heavy petroleum products.

Percentage of saturated hydrocarbons has been determined in different heavy petroleum products. Fig. 5 shows results from a correlation study between Saturates wt% obtained from using reference values (*Y*-axis) and Saturates wt% obtained from using berberrubine-induced fluorescence (*X*-axis).

Reference values are from well-established techniques in petroleum industry. Studied petroleum products include the



**Fig. 3. A**: Fluorescent chromatographic responses (area counts) of alkanes with different number of C atoms using berberrubine-impregnated HPTLC silica gel plate (applied mass: 3 µg). **B**: Fluorescent chromatographic response (area counts) of different sample loads of saturated hydrocarbons using berberine (fled symbols) or berberrubine (non filled symbols) impregnated HPTLC silica gel plates.  $\blacktriangle$ : C<sub>28</sub>;  $\blacksquare$ : C<sub>24</sub>;  $\blacksquare$ : C<sub>24</sub>;  $\blacksquare$ : C<sub>18</sub>;  $\diamond$ : C<sub>32</sub>;  $\land$ : C<sub>28</sub>;  $\blacksquare$ : C<sub>24</sub>.



**Fig. 4.** HPTLC chromatogram of synthetic mixture-1. Fluorescent scanning densitometry ( $\lambda_{exc}$ =365 nm;  $\lambda_{em}$ >400 nm). Applied mass: 2 µg, 4 µg, and 6 µg.



**Fig. 5.** Determination of Saturates (wt %) in heavy petroleum products. Δ: Lubricating oil; •: Refining products; o: Bitumes; \*: Vacuum gas oil; +: Synthetic mixture. Y-axis: values from reference techniques (see text). X-axis: values from berberrubine sensing.

following types (the reference technique used for each type of sample is given in brackets): i) a lubricating oil (Iatroscan<sup>15</sup>); ii) 13 bitumes (Iatroscan); iii) eight vacuum gas oils (ASTM D2007<sup>16</sup>); iv) two refining products (HPLC<sup>17</sup>); and v) two synthetic mixtures (true values from known composition).

For obtaining Saturates wt % from berberrubine-induced fluorescence, a simple calibration was done using points from C<sub>24</sub>, C<sub>28</sub> and C<sub>32</sub>, with sample loads between 1 and 5 µg. Calibration curve and regression data were  $y=-33.05 x^2+544.63 x+306.85$ ( $r^2=0.9822$ ).

Results from Fig. 5 show that weight percentages of saturates obtained by HPTLC calibration procedure are coherent with the reference values. Plot gives a linear regression (for n=48;  $r^2=0.974$ ) with a slope of 0.954 (±0.046) and intercept of 0.513 (±1.745).

An amplification of fluorescent signal for berberrubine-alkane interaction can be obtained in two ways. As in the case of berberine,<sup>4</sup> detection sensitivity can be enhanced somewhat by increasing concentration of berberrubine from 60 to 120 ppm. On the other hand, unlike berberine, sensitivity has been additionally improved by heating the silica gel plate where detection has been performed.

As mentioned, the starting level of fluorescent signal for dipolar probes is low. In the case of berberrubine, the reason for an adequate emission level may be related to its special spectroscopic properties. Our results support the formation of a keto-enol equilibrium for berberrubine (in its C-7), depending on the pH (Fig. 6).

In this work, UV–vis absorption and steady-state fluorescence emission spectra of berberrubine were recorded in solution (in methanol, in acid and basic media). It was reported that UV–vis absorption spectra of berberrubine depends on pH.<sup>18</sup> As our results show (Fig. 7), the ketone or the enol is obtained in basic or acidic medium, respectively.

They have remarkable differences in their fluorescence spectra (Fig. 8). Although berberrubine is fluorescent in both media, its emission in acidic solution is higher and shifted to longer wavelengths (from 465 to 540 nm) because of the higher aromaticity of the enol. On the other hand, the fluorescence spectrum of berberrubine in methanol has two regions, which correspond to each tautomeric form, although the maximum of this spectrum is in the zone of enol (540 nm). Fluorescence intensity for the methanolic solution at these wavelengths is lower than that of acidic solution.

Excitation and emission fluorescence spectra of berberrubine were also obtained by direct measurement on silica gel plates (Fig. 9). Spectra were corrected with the background of the empty



Fig. 6. Keto-enol tautomerism of berberrubine in acidic media.



**Fig. 7.** UV–vis absorption spectra of berberrubine in (...) acidic medium, (-) basic medium. Berberrubine concentration of methanolic solution was 13 mgL<sup>-1</sup>. Acidic and basic media were obtained from the methanolic solution by adding 0.05 mL of HCl and 0.05 mL of NaOH.



**Fig. 8.** Fluorescence emission spectra ( $\lambda_{exc}$ =360 nm; 5 nm slits) of berberrubine in (—) acidic medium, (...) basic medium, and (- - -) methanol. Berberrubine concentration of methanolic solution was 13 mgL<sup>-1</sup>. Acidic and basic media were obtained from the methanolic solution by adding 0.05 mL of HCl and 0.05 mL of NaOH.

silica plate. Similar results were obtained by subtracting or by dividing by intensity background signal. Emission spectrum (Fig. 9B) shows a unique maximum at 523 nm, which suggest a preponderance of the enol form, at room temperature, when berberrubine is adsorbed onto silica gel. From  $\lambda_{em}$ >400 nm, the enol form provides higher fluorescence intensity than the keto one.

The enol form implies an increase of the probability of proton donor-acceptor interactions with OH groups on the silica gel



**Fig. 9.** Corrected fluorescence spectra of berberrubine on a silica gel plate. **A**: excitation spectrum ( $\lambda_{em}$ =530 nm). **B**: emission spectrum ( $\lambda_{exc}$ =360 nm). Spectra were obtained at room temperature (—) or heating the plate (60 °C) and leaving cool at room temperature (…).

surface, which may limit the freedom of orientation of berberrubine to interact with alkane molecules. This may explain that the fluorescent response of berberrubine is almost constant with alkane chain length, in contrast to the results obtained in the case of berberine, and also the lower response of berberrubine with regard to berberine.

Spectra measured after heating the plate and then leaving it cool at room temperature, show an increase in fluorescence intensity throughout the whole range of emission wavelengths with regard to those recorded at room temperature. These results are in agreement with those of peak areas using the densitometer. These properties make that berberrubine may also be considered an acidbase fluorescent probe.



Fig. 10. Most relevant MO associated with the absorption and emission of berberrubine computed at CAM-B3LYP(PCM, methanol) level of theory.

Furthermore, additional TD-DFT calculations at CAM-B3LYP(PCM,methanol) level of theory have been carried out in order to identify the main transitions involved on the absorptionemission processes of berberrubine in methanol. Our calculations on the fully optimized structure show the existence of two absorption bands. The main one is associated with the allowed HOMO+LUMO+1 ( $\pi$ + $\pi$ ) transition with a computed wavelength of 365 nm with oscillator strength (f) of 0.53. On the other hand, the secondary band is associated with the HOMO+LUMO ( $\pi$ + $\pi$ ) transition and have a computed wavelength of 438 nm with oscillator strength (f) of 0.17. Both computed values are close to those observed experimentally (vide supra) thus pointing out the reliability of the chosen computational method (Fig. 10).

In order to simulate the emission spectra, full optimization of the geometry on the excited state at CAM-B3LYP(PCM,methanol) level of theory was carried out. TDDFT calculations on this geometry show that the emission band corresponds to a  $\pi \rightarrow \pi$  (LUMO $\rightarrow$ HOMO) transition with a computed wavelength of 560 nm and an oscillator strength (*f*) of 0.10, value, which also agree with the experimental results.

#### 3. Conclusions

Computational results are in good agreement with the excellent selectivity observed in the demethylation of berberine obtained by solvent-free, microwave-assisted synthesis. DFT calculations explains that the observed selectivity depends on electron delocalization capability. Moreover, TDDFT calculations were performed showing that the main absorption bands corresponds to  $\pi \rightarrow \pi$  (HOMO>LUMO+1) and (HOMO>LUMO) transitions, whereas the emission band corresponds mainly to a  $\pi \rightarrow \pi$  (LUMO>HOMO) transition.

Berberrubine has interesting properties. It behaves as a fluorescent chemosensor of alkanes with an almost constant response regardless of alkane chain length. In addition to provide a fluorescent response via dipole-induced dipole interaction between alkanes and berberrubine, the existence of a keto-enol tautomerism for this compound helps to explain the constant response of different alkanes per mass unit. Therefore, a rapid, and quantitative determination of saturated hydrocarbons in heavy petroleum conversion products is possible using a minimum amount of solvent, and avoiding the tedious calibration steps used in other techniques.

#### 4. Experimental section

### 4.1. General

4.1.1. *Fluorophore.* Berberrubine (CAS number: 15401-69-1) was obtained from berberine chloride (CAS number: 633-65-8), from Sigma–Aldrich, Steinheim, Germany.

4.1.2. Samples and standards. n-Tetracosane,  $C_{24}$  ( $\geq$ 99%; [646-31-1] CAS); n-octacosane,  $C_{28}$  ( $\geq$ 98%; [630-02-4] CAS) and n-

dotriacontane,  $C_{32}$  ( $\geq$ 96%; [544-85-4] CAS) from Sigma–Aldrich Inc., St. Louis, MO, were used as standards.

Twenty four petroleum-derived samples (1 lubricant oil, 13 bitumes, two refining products and eight vacuum gas oils) were provided by TOTAL RM. Samples were heated before sampling during 30 min at 150 °C for bitumes and refining products and 80 °C for the lubricant oil and vacuum gas oils.

Two synthetic mixtures have been prepared for modelling in a simple way a heavy petroleum product. Mixtures consist of known proportions of several alkanes and a heavy aromatic model compound, violanthrone-79 or perylene-66. Synthetic mixture-1 composition: 69.9 wt% of alkanes (9.3 wt%  $C_{24}$ ; 36.6 wt%  $C_{28}$ ; 24.1 wt%  $C_{32}$ ), and 30.1 wt% violanthrone-79. Synthetic mixture-2 composition: 31.4 wt% of alkanes (9.4 wt%  $C_{24}$ ; 11.7 wt%  $C_{28}$ ; 10.2 wt%  $C_{32}$ ), and 57.7 wt% perylene-66.

4.1.3. Microwave reactions and products characterization. A CEM Discovery equipment from Vertex (NC, USA) was used for microwave irradiation reactions. Products were characterized by <sup>1</sup>H NMR spectroscopy, using a Brucker Avance 500 Mhz apparatus, in DMSO- $d_6$  (Uvasol<sup>®</sup>, Merck). Chemical shifts ( $\delta$ ) are in ppm with regard to internal reference trimethylsilane.

4.1.4. Chromatographic HPTLC plates. HPTLC silica gel plates  $(10 \times 20 \text{ cm}, \text{ pore size } 60 \text{ Å}, \text{ layer thickness } 0.20 \text{ mm})$  were used from Merck (Darmstadt, Germany).

4.1.5. Sample application. Standards and the lubricant oil were dissolved in HPLC-grade *n*-heptane (99.1%, from Panreac, Barcelona, Spain). Bitumes, vacuum gas oils, refining products and two synthetic mixtures were dissolved in stabiliser-free tetrahydrofuran (THF, 99.9%, from Panreac, Barcelona, Spain).

Standards and samples were applied using the Automatic TLC Sampler 4 (Camag, Müttenz, Switzerland), as 4 mm bands. Typically, up to 30 samples were applied on the same plate with a distance of 2.2 mm between tracks. One track was always kept free of application, as blank run.

The first application position was 10 mm (x coordinate), and the distance from the lower edge of the plate was 10 mm (y coordinate).

Typical application volume for standards and samples were between 1 and 10  $\mu L$  The concentration was 1 mg mL^{-1}

4.1.6. Chromatographic elution. An automated multiple development (AMD2) system (Camag, Müttenz, Switzerland) was used in isocratic mode with HPLC-grade n-heptane (99.1%, from Panreac, Barcelona, Spain) as mobile phase over 30 mm total migration distance. Duration of run is 10 min. Reproducibility of migration distance has been always better than  $\pm 0.45$  mm.

4.1.7. Fluorescence scanning densitometry. A TLC Scanner 3 (Camag, Müttenz, Switzerland) was used in fluorescence mode. HPTLC silica gel plates were post-impregnated with a solution of

the berberrubine in MeOH (60  $mgL^{-1}$ ) by dipping using a Camag Chromatogram Immersion Device III. In order to amplify fluorescent signal, the plate was subsequently dried 30 min at 65 °C.

Fluorescence mode using a mercury lamp was used by excitation at 365 nm, and induced fluorescence was collected at wavelengths longer than 400 nm Camag WinCats software was used for controlling ATS4. AMD2 and TLC Scanner 3. and also for data acquisition and treatment.

4.1.8. Computational methods. All the calculations were obtained with the GAUSSIAN09 suite of programs.<sup>19</sup> Electron correlation was partially taken into account by using the hybrid functional B3LYP<sup>20</sup> and the standard 6-31G\* or 6-31+G\* basis sets.<sup>21</sup> Dispersion effects were computed by means of Grimme's D3 correction method.<sup>22</sup> Thermal correction of Gibbs free energies were computed at the B3LYP/6-31G\* level of theory at 298 and 498 K and were not scaled. All stationary points were characterized by harmonic analysis. Reactants and cycloadducts have positive definite Hessian matrices. Transition structures show only one negative eigenvalue in their diagonalysed force constant matrices, and their associate eigenvectors were confirmed to correspond to the motion along the reaction coordinate under consideration.

Natural Bond Orbital (NBO)<sup>23</sup> analysis of all the stationary points was also performed. In this method the full density matrix is partitioned into localized one centre (core and lone pair) and two center orbitals describing a Lewis-type structure. Within this framework, we used  $NRT^{24,25}$  to measure the relative weight of the contributing Lewis structures to the wave function.

Nucleus-independent chemical shifts (NICS) as defined by Schlever<sup>26</sup> were computed by using the gauge invariant atomic orbital<sup>27</sup> (GIAO) approach at the GIAO-B3LYP/6-31+G\*//B3LYP/6-31G\* level.

Simulation of absorption and emission spectra were carried out using the coulombic-attenuating method developed by Handy et al.<sup>28</sup> named CAM-B3LYP, which was successfully optimized for vertical excitations and optimization of excited states within the time dependent TDDFT theory.<sup>29</sup>

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## Supplementary data

Supplementary data related to this article can be found at http:// dx.doi.org/10.1016/j.tet.2015.06.098.

#### **References and notes**

- 1. Hsu, C. S. Analytical Advances for Hydrocarbon Research; Kluwer Academic/Plenum: New York, 2003.
- 2. (a) Thompson, R. B. Fluorescence Sensors and Biosensors; Taylor and Francis: Boca Raton, 2006; (b) Valeur, B.; Brochon, J. C. (eds) in Fluorescence in Sensing Applications, in New Trend in Fluorescence Spectroscopy. Applications to Chemical and Life Sciences. Part 3. Ed by Wolfbeis, O., Springer Series on Fluorescence. Methods and Applications. Springer: Berlin, 2001.
- 3. Cossío, F. P.; Arrieta, A.; Cebolla, V. L.; Membrado, L.; Garriga, R.; Vela, J.; Domingo, M. P. Org. Lett. 2000, 2, 2311-2313.
- Gálvez, E. M.; Matt, M.; Cebolla, V. L.; Fernandes, F.; Membrado, L.; Cossío, F. P.; Garriga, R.; Vela, J.; Guermouche, M. H. Anal. Chem. 2006, 78, 3699-3705.
- 5. Cebolla, V. L.; Mateos, E.; Garriga, R.; Membrado, L.; Cossío, F. P.; Gálvez, E. M.; Matt, M.; Delgado-Camón, A. ChemPhysChem 2012, 13, 291–299.
- Debye, P. Phys. Z. 1920, 21, 178-187; 1921, 22, 302-308.
- Das, B.; Srinivas, K. V. N. S. Synth. Commun. 2002, 32, 3027-3029.
- 8.. Microwave can effectively interact with polar molecules, which can absorb enough energy to overcome high activation barriers unreachable by other heting sources
- 9. De Cózar, A.; Millán, M. C.; Cebrián, C.; Prieto, P.; Díaz-Ortiz, A.; de la Hoz, A.; Cossío, F. P. Org. Biomol. Chem. 2010, 8, 1000-1009.
- Schleyer, P. v. R.; Manoharan, M.; Wang, Z. X.; Kiran, B.; Jiao, H.; Puchta, R.; Hommes, N. J. R. v. E. Org. Lett. 2001, 3, 2465–2468.
- 11.. This phenomenon was reported to take place in solution (see, e.g., Ref. 4) and, to a greater extent, on silica gel plates, which provide a rigid support that enhances the fluorescent response and a chromatographic system to separate alkanes from other compounds in petrochemical samples. HPTLC is especially adequate for non-volatile, complex mixtures because, after elution, the whole sample is scanned and quantified using a fluorescence scanning densitometer.
- Matt, M.; Gálvez, E. M.; Cebolla, V. L.; Membrado, L.; Bacaud, R.; Pessayre, S. J. ep. Sci. 2003, 26, 1665–1674.
- 13. Jarne, C.; Cebolla, V. L.; Domingo, M. P.; Domínguez, A.; Delgado-Camón, A.; Garriga, R.; Galbán, J.; Membrado, L.; Gálvez, E. M.; Cossío, F. P. J. Chromatogr., A 2011, 1218, 2668-2675.
- 14. Megyesi, M.; Biczok, L. J. Phys. Chem. B 2007, 111, 5635-5639.
- 15. Vela, J.; Cebolla, V. L.; Membrado, L.; André, s. J. M. J. Chromatogr. Sci. 1995, 33, 417-425.
- 16. Drews, A. W. Manual on Hydrocarbon Analysis, 4th ed.; American Society for Testing of Materials: Philadelphia, PA, 1989; ASTM D2007.
- 17. IP 391 Standard. Standard Methods for Analysis and Testing of Petroleum and Related Products 1992; Institute of Petroleum: London, 1992, Vol. 2.
- 18. Gasparec, Z.; Komorski-Lovrić, Š.; Lovrić, M. Can. J. Chem. 1982, 60, 970-975.
- 19. Frisch, M. J., et al. Gaussian09, Revision A.02; Gaussian: Wallingford CT, 2009; (full reference in the Supporting data).
- 20. Becke, A. D. J. Chem. Phys. 1993, 98, 5648-5650.
- 21. Hehre, W. J.; Radom, L.; Schleyer, P. V. R.; Popple, J. A. Ab Initio Molecular Orbital Theory; Wiley: New York, 1986; pp 70-80; and references cited therein.
- 22. Kruse, H.; Goerik, L.; Grimme, S. J. Org. Chem. 2012, 77, 10824–10834.
- Foster, J. P.; Weinhold, F. J. Am. Chem. Soc. **1980**, 102, 7711–7218.
   Glendening, E. D.; Weinhold, F. J. Comput. Chem. **1998**, 19, 593–609.
- 25. Glendening, E. D.; Weinhold, F. J. Comput. Chem. 1998, 19, 610-627. 26. Schleyer, P. v. R.; Maerker, C.; Dransfeld, A.; Jiao, H.; Hommes, N. J. R. v. E. J. Am.
- Chem. Soc. 1996, 118, 6317-6318. 27
- Wolinski, K.; Hilton, J. F.; Pulay, P. J. Am. Chem. Soc. 1990, 112, 8251-8260. 28. Yanai, T.; Tew, D. P.; Handy, N. C. Chem. Phys. Lett. 2004, 393, 51-57.
- 29. Charaf-Eddin, A.; Planchat, A.; Mennucci, B.; Adamo, C.; Jacquemin, D. J. Chem. Theor. Comput. 2013, 9, 2749–2760.