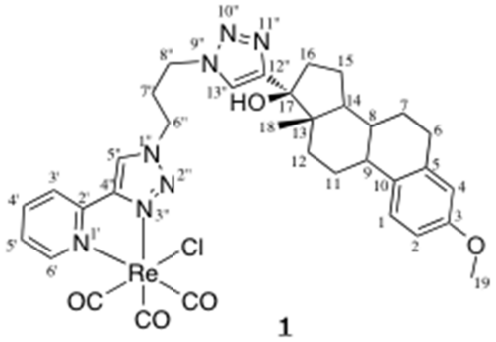
Electronic Supplementary Material (ESI) for Analyst

Supporting information

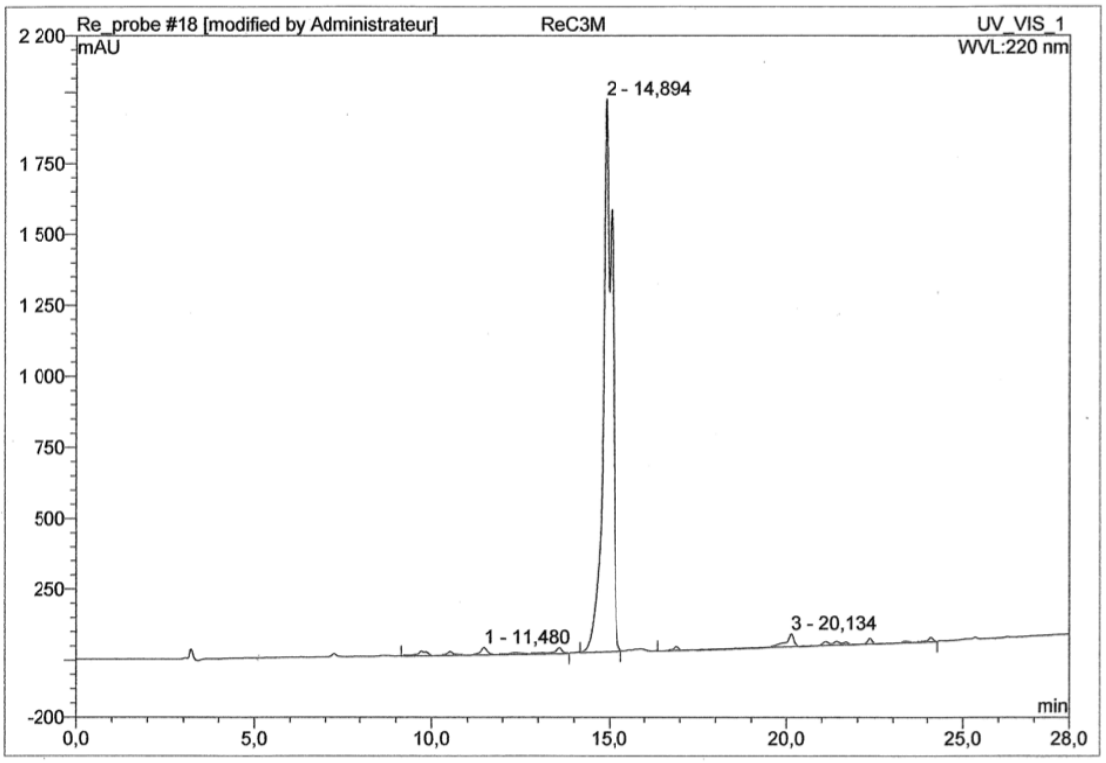
Content:



1

1. Characterizations of 1

HPLC purity was checked with a Dionex C18 analytical column and the chromatogram is shown below.



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Type
1	11,48	n.a.	23,978	16,265	2,70	n.a.	BMB*
2	14,89	n.a.	1945,453	574,084	95,38	n.a.	BMB*
3	20,13	n.a.	42,877	11,556	1,92	n.a.	BMB*
Total:			2012,309	601,905	100,00	0,000	

Figure S1. HPLC chromatogram. Solvent was a CH_3CN:H_2O (0.1 % TFA) mixture from 2:3 to 1:0 in 30 minutes, retention times of the two diastereoisomers of **1** were 14.89 and 15.01 minutes (7:3 for CH_3CN:H_2O). Wavelength of irradiation: 220 nm.

FTIR spectra

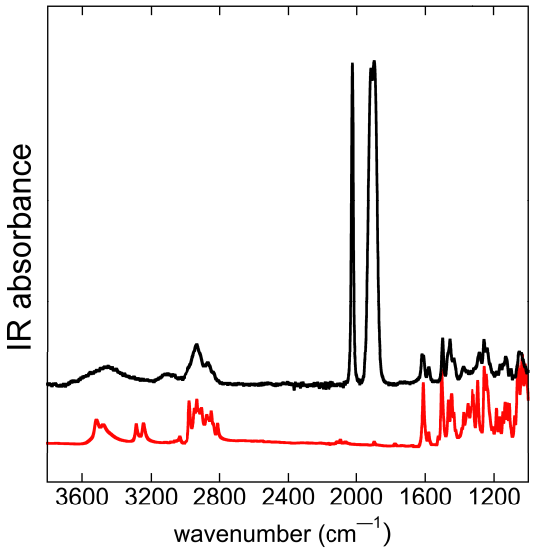


Figure S2. FTIR spectra recorded on an ATR sampling accessory of mestranol (red trace) and **1** (black trace). The triple bond stretching vibration in mestranol (around 2100 cm^{-1}) is very weak compared with the CO stretchings in **1**. Spectra have been scaled to present the same absorption for the C-H stretching vibrations.

2. Effect of the temperature on the cellular uptake of 1 and Re(CO)$_3$Cl-pyta-C$_{12}$N$_3$[1]

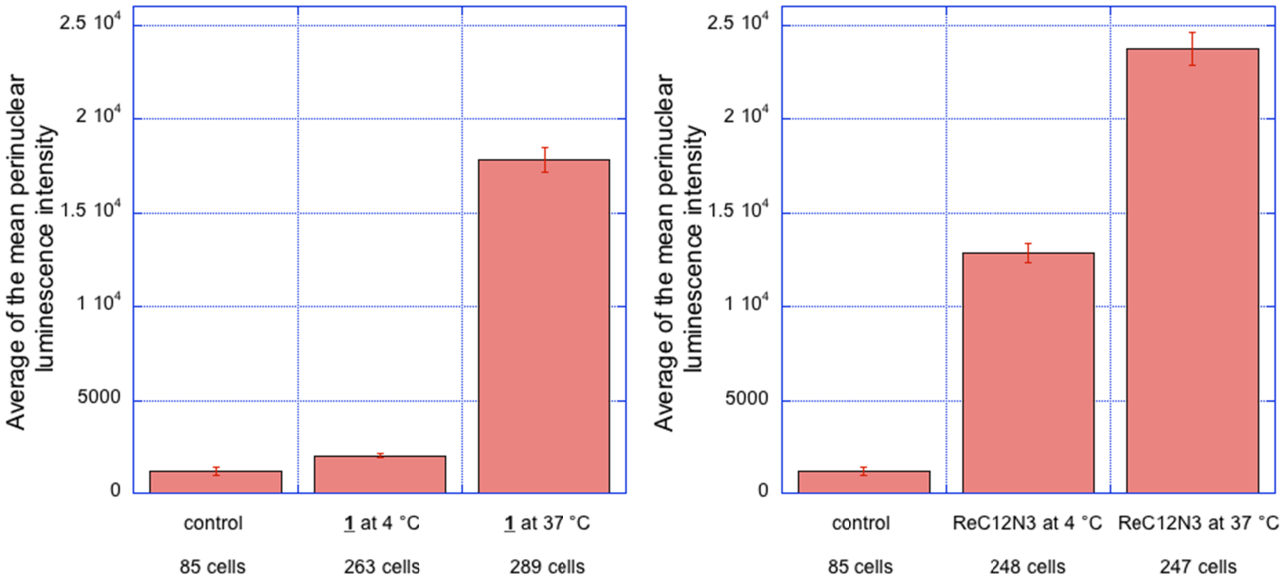


Figure S3. Average of the mean perinuclear luminescence for collections of control cells and cells incubated at 25 µM for 1 h at 4 °C or at 37 °C (MDA-MB-231 cell line). **Left:** uptake of **1**. **Right:** uptake of Re(CO)$_3$Cl-pyta-C$_{12}$N$_3$ previously described.[1] For each condition the number of cells used is specified. Luminescence signals of **1** and Re(CO)$_3$Cl-pyta-C$_{12}$N$_3$ are identical and were monitored using the same filter set (see section 3.6) and the same conditions of acquisition. Mean perinuclear luminescence corrected for the background before averaging. Standard error calculated with a Student coefficient for a two-sided confidence interval of 90% and for the number of cells of the collection.

3. Synchrotron radiation FTIR spectromicroscopy (SR-FTIR-SM)

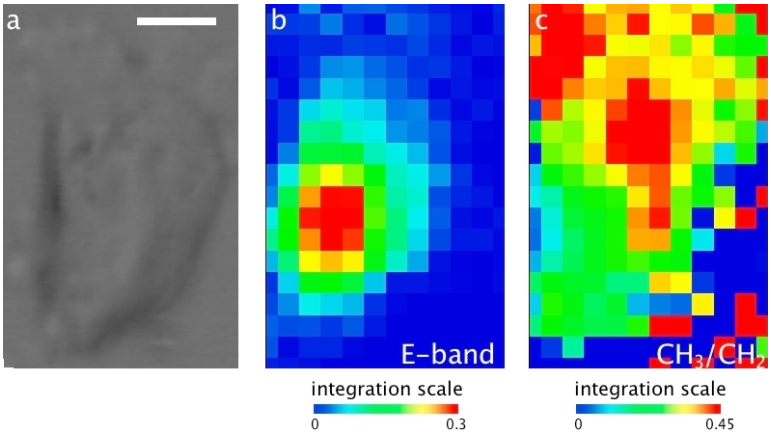


Figure S4. Same single MCF-7 cell as in Figure 2, incubated with **1** (25 µM, 1 h). (a) Bright field image, scale bar: 10 µm. **Mapping of the integral of IR bands of interest** (pixel size 3 x 3 μm^2): (b) E-band (1940-1879 cm^{-1}), (c) CH_3/CH_2 ratio (using CH_3 asymmetric stretching band from 2986 to 2948 cm^{-1} and CH_2 asymmetric stretching from 2948 to 2897 cm^{-1}). The red spots out of the cell in (c) are due to a division by very small values of the CH_2 band and thus are not relevant.

4. Confocal Raman microspectroscopy and imaging

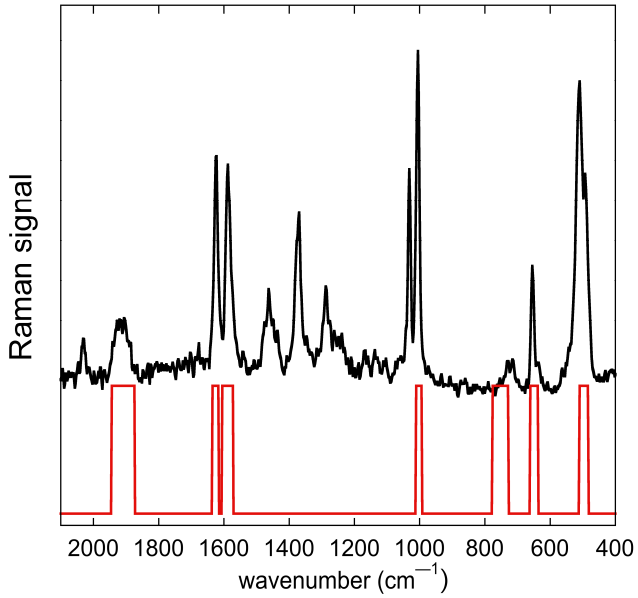


Figure S5. Raman spectra of **1** at solid state (l_{ex} = 532 nm, T_{acq}= 2 s). The red mask shows the selected bands used for the HCA analysis. The band at 1915 cm^{-1} is assigned to CO vibration mode of **1**. The bands at 1624 and 1587 cm^{-1} are assigned to C=C and C=N vibrational modes respectively. The bands at lower wavenumber correspond to the ring breathing vibrational modes of **1**.

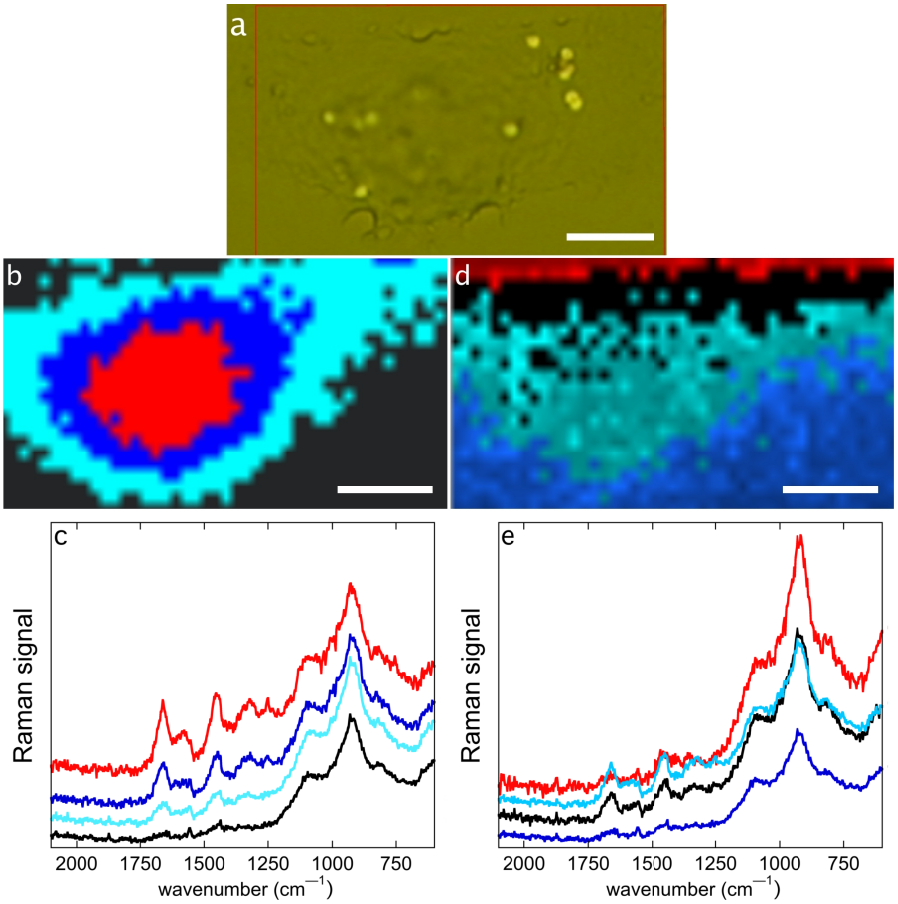


Figure S6. Control MDA-MB-231 breast cancer cell. The same dataset is processed with two different HCA masks. Scale bars: 6 µm. (a) Bright field image, the red frame corresponds to the measured area, (b) and (c) results of the HCA analysis with a mask defined on two domains characteristic of proteins and lipids (1600-1700 cm^{-1} and 2800-3000 cm^{-1}): (b) chemical map of lipid and protein distribution (gives the general form of the cell), (c) cluster average spectra from HCA results.
(d) and (e) results of a second HCA analysis on the mask corresponding to **1** defined in Fig. S5: (e) cluster average spectra from HCA results. All the spectra determined by HCA are very similar and revealed mainly the contribution of the glass and some bands assigned to organic material present in the cell, as proteins (band at around 1660 cm^{-1}).
No band of the carbonyl metal complex was detected in (c) or (e), as expected.

5. Synchrotron-based multiple beam FTIR imaging

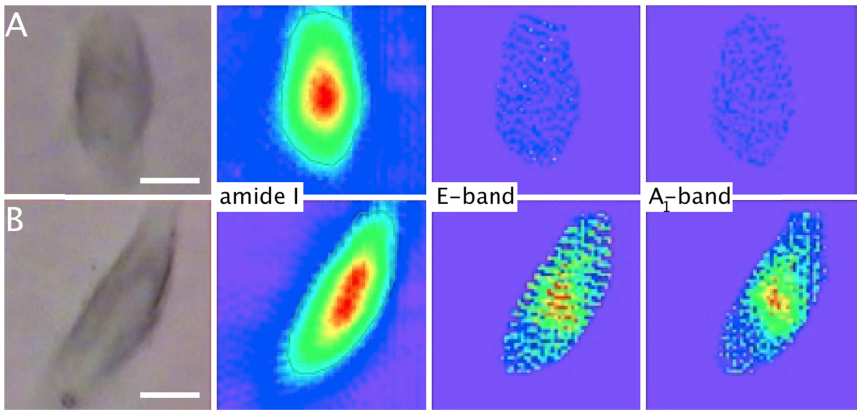


Figure S7. MDA-MB-231 cells. Visible and FT-IR images based on the integration of specific absorption bands. Scale bars: 10 µm. **A.** A single MDA-MB-231 control cell. **B.** A single MDA-MB-231 cell incubated with **1** (25 µM, 1 h). Constant scaling of the contour colors has been applied for each band, with red/purple equivalent to high/low absorption intensity.

1. S. Clède, F. Lambert, C. Sandt, Z. Gueroui, M. Refregiers, M.-A. Plamont, P. Dumas, A. Vessieres and C. Policar, *Chem Commun*, 2012, **48**, 7729-7731.