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SUPPLEMENTARY INFORMATION

A double-triggered self-immolative spacer for increased selectivity of molecular release

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The Supporting Information reports on:

1. Physicochemical experiments

1.1 Experimental procedures

1.1.1 UV-Vis absorption

UV-Vis absorption spectra were recorded in 1 cm × 1 cm quartz cuvettes (Hellma) on a diode array UV-Vis spectrophotometer (UV-Vis Cary 300, Agilent Technologies, Santa Clara, CA) at 298 K. Molar absorption coefficients were extracted while checking the validity of the Beer-Lambert law.

1.1.2 Steady-state fluorescence emission

Corrected fluorescence spectra upon one-photon excitation were recorded with a Photon Technology International QuantaMaster QM-1 spectrofluorimeter (PTI, Monmouth Junction, NJ). Solutions for fluorescence measurements were adjusted to concentrations such that the absorption maximum was around 0.15 at the excitation wavelength.

1.1.3 Irradiation experiments

Irradiations relying on one-photon excitation were performed at 298 K in CH_3CN:H_2O (4:1 v:v). Two different series of irradiation experiments have been performed:

- In the preparative experiments analyzed by UPLC/MS (gradient: 80 to 100% CH_3CN/H_2O), illumination was applied with 3 TLC 6W lamps (VL-6.LC 24W) emitting at 365 nm. Irradiation experiments were performed on 20 mL solutions of 1.75 mM carbamates in a 25 mL container under constant stirring. The aliquots for analysis were taken up and were subsequently stored in the dark and submitted to analyses following the evolution of the peak area of the photoreleased products as a function of the illumination duration;

- In the cuvette experiments, excitations were performed using a light-emitting diode (PE-2, CoolLED, Andover, United Kingdom; emission at 365±25 nm) and/or the 75 W xenon lamp of the spectrofluorometer at several slit widths. Irradiation experiments continuously followed by fluorescence emission were performed on 50 µL solutions of 20 µM carbamates in 0.2×1 cm^2 quartz fluorescence cuvettes (Hellma) under constant stirring.

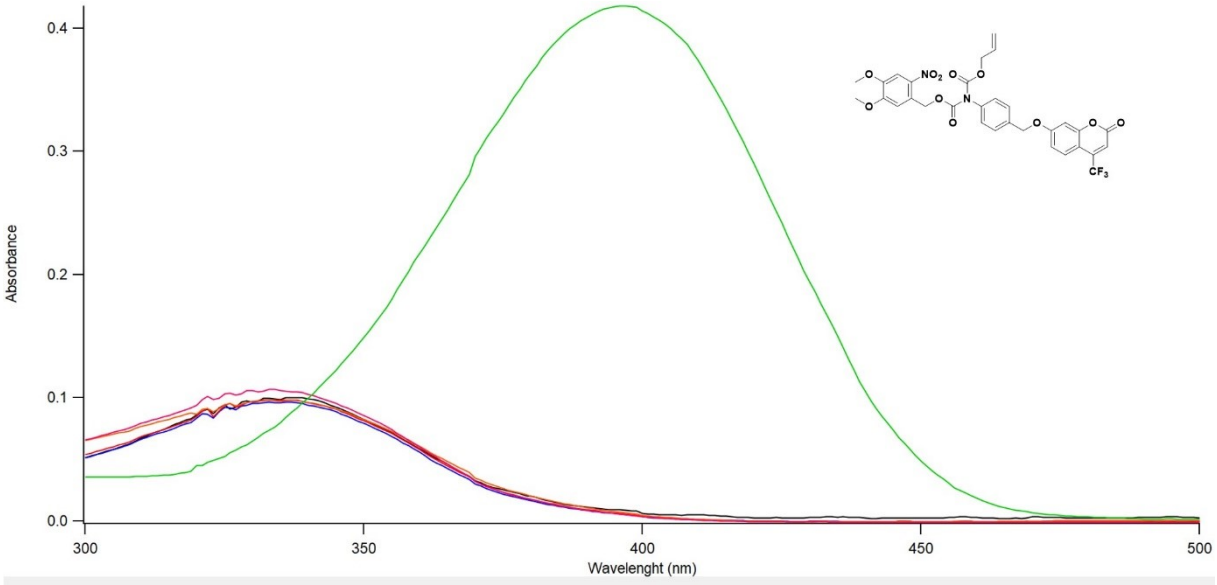
1.1.4 UPLC coupled to mass spectrometry

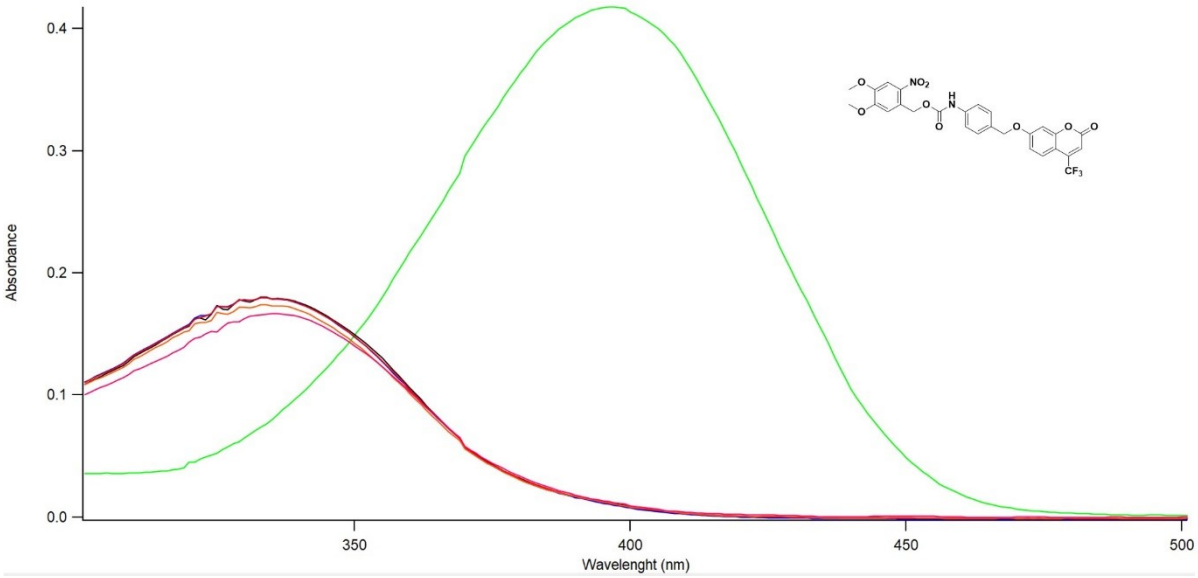
UPLC-MS analyses were obtained on a Waters ACQUITY UPLC H-Class System with a BEH C18 pre-column 2.1×5 mm and column 2.1×50 mm, 1.7 µm coupled to a SQ Detector 2 (Waters) at a flow rate of 0.6 ml/min with a mobile phase composed of two different solvents A and B, where A denotes for water containing 0.1% formic acid, and B acetonitrile containing 0.1% formic acid. Initially, the column contained a mobile phase consisting of 95% A and 5% B. These proportions were maintained for 0.2 min, then the proportion of B was fixed to 80%. After this step the gradient evolved to 10% A/90% B within 2.3min, was increased to 100% B for 0.5 min and went down to 95% A/5% B during the last minute.

1.2 Stability of the carbamates 7, 8 and 9 against hydrolysis

The stability of the carbamates **7**, **8** and **9** against hydrolysis was assessed by following the time evolution of the absorbance of 50 µM solutions of **7**, **8** and **9** in CH_3CN/pH=8 Briton Robinson buffer[1]

(1:1 v:v) kept in the dark over 24 h. Figure 1S displays the results. It shows that none of the three carbamates **7**, **8** and **9** released the coumarin **11** in the absence of trigger over 24 h.





[1] C. Frugoni. Tampone universale di Britton e Robinson a forza ionica costante.*Gazz. Chim. Ital*. **1957**, *87*, 403-407.

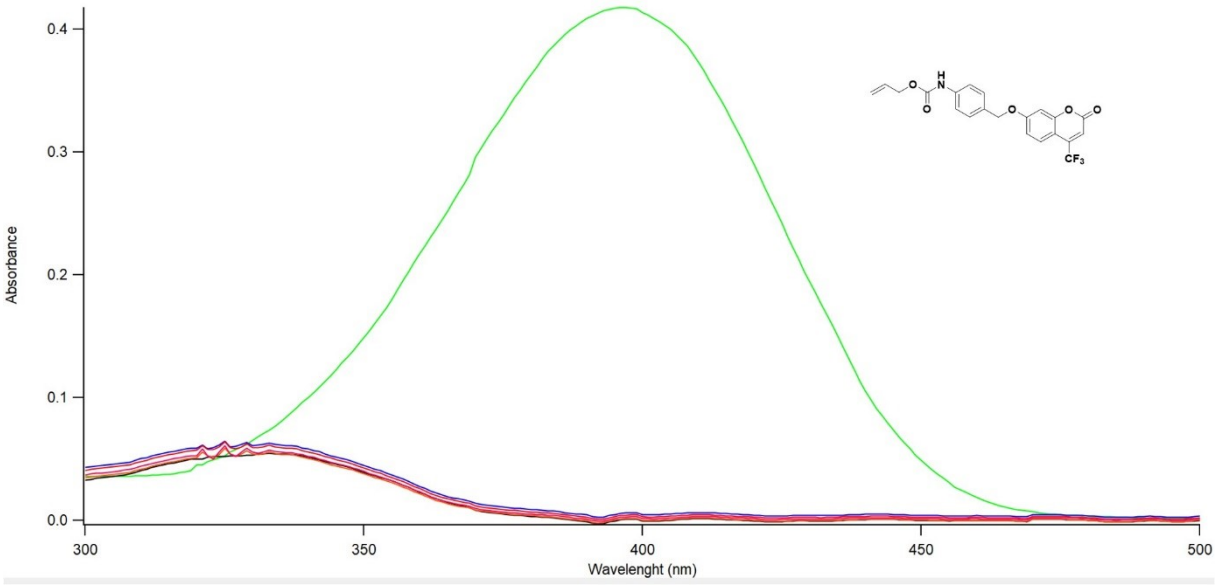
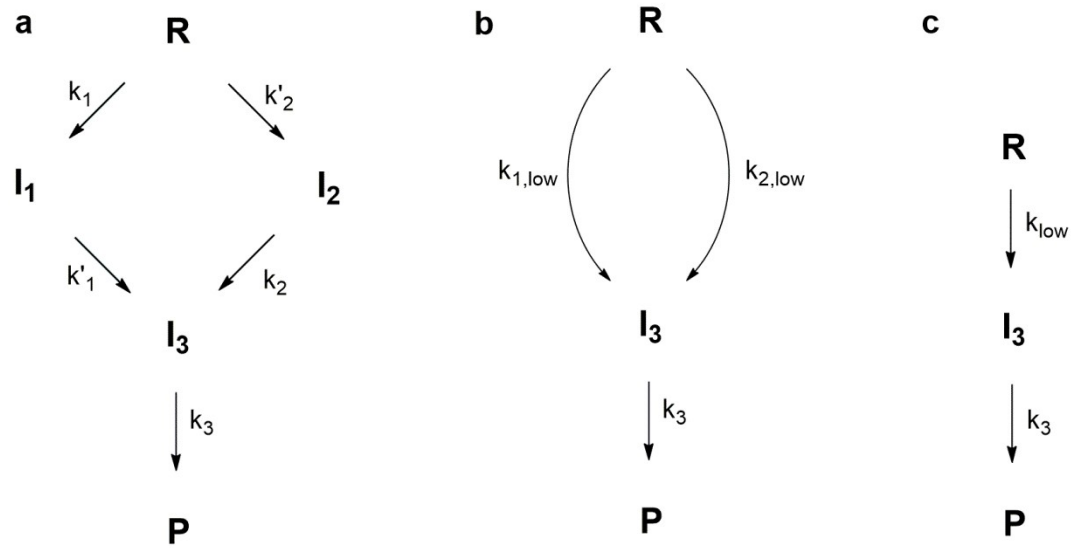


Figure 1S. Time evolution of the absorbance of 50 μM solutions of **7** (top), **8** (middle), and **9** (bottom) in CH_3CN/pH=8 Briton Robinson buffer (1:1 v:v) kept in the dark over 24 h. The absorption spectrum of the coumarin **11** is shown as a reference in green, t=0h in black, t=1h in blue, t=2h I, red, t=6h in orange and t=24h in pink.

1.3 Kinetic analysis of triggered disassembly of the dicarbamate 7 and of the monocarbamates 8 and 9

1.3.1 Theoretical model

The kinetic analysis of **7** disassembly in the presence of the triggers Pd(II) and hν relies on Scheme 2 of the Main Text, which recapitulates the steps occurring at timescales above the few seconds range. It has been schematized in Scheme 1Sa. To analyze the kinetic data, this model has been reduced by removing the fastest steps of the **R** ✂ $\mathbf{I_1}$ ✂ $\mathbf{I_3}$ and **R** ✂ $\mathbf{I_2}$ ✂ $\mathbf{I_3}$ branches so as to retain only the slowest steps respectively associated to the rate constants $k_{1,low}$ and $k_{2,low}$ (Scheme 1Sb). Since the rate constants k_1 and k_2 (respectively k'_1 and k'_2) are expected to have similar values since they rely on the same trigger and type of reaction, $k_{1,low} = k_1$ and $k_{2,low} = k_2$ or $k_{1,low} = k'_1$ and $k_{2,low} = k'_2$. This reduced model is equivalent to the two-steps model displayed in Scheme 1Sc with, $k_{low} = k_{1,low} + k_{2,low}$.



*Scheme 1S. Kinetic models for analyzing the kinetics of **7** disassembly in the presence of the triggers Pd(II) and h ν.* **a**: Starting model involving **R**, $\mathbf{I_1}$, $\mathbf{I_2}$, $\mathbf{I_3}$, and **P** for **7**, **8**, **9**, **10**, and **11** respectively. k_1 and k_2 (respectively k'_1 and k'_2) are the rate constants associated to Pd(II) (respectively hν) triggering; **b**: Reduced model retrieving the fastest steps in the **R** ✂ $\mathbf{I_1}$ ✂ $\mathbf{I_3}$ and **R** ✂ $\mathbf{I_2}$ ✂ $\mathbf{I_3}$ branches so as to retain only the lowest steps respectively associated to the rate constants $k_{1,low}$ and $k_{2,low}$; **c**: Same as **b** after writing $k_{low} = k_{1,low} + k_{2,low}$.

The set of differential equations governing the evolution of the concentration in the species shown in Scheme 1S is given in Eqs.(1-3):

$$\frac{dR}{dt} = -k_{low}R \tag{1}$$

$$\frac{dI_3}{dt} = k_{low}R - k_3 I_3 \tag{2}$$

$$\frac{dP}{dt} = k_3 I_3 \tag{3}$$

with the additional conservation law

$$R_0 = R + I_3 + P \tag{4}$$

since the system is closed.

In the most general case, the rate constants k_{low} and k_3 are different and the solutions of Eqs.(1-3) are given in Eqs.(5-7):

$$\frac{R(t)}{R_0} = e^{-k_{low}t} \tag{5}$$

$$\frac{I_3(t)}{R_0} = \frac{k_{low}}{k_3 - k_{low}}\left(e^{-k_{low}t} - e^{-k_3 t}\right) \tag{6}$$

$$\frac{P(t)}{R_0} = 1 - \frac{k_3 e^{-k_{low}t} - k_{low} e^{-k_3 t}}{k_3 - k_{low}} \tag{7}$$

By assuming that the coumarin **11** is the only species significantly generating fluorescence under the adopted experimental conditions, Eq.(7) yields

$$I_F(t) = Q_P R_0 \left(1 - \frac{k_3 e^{-k_{low}t} - k_{low} e^{-k_3 t}}{k_3 - k_{low}}\right) \quad (8)$$

for the time dependence of the fluorescence signal from the mixture.

In the presence of only one trigger (Pd(II) or hν), the **7** disassembly stops at the level of the intermediates $\mathbf{I_1}$ and $\mathbf{I_2}$. Then the kinetic analysis relies on a single reaction **R** ✂ $\mathbf{I_1}$ or **R** ✂ $\mathbf{I_1}$ for the Pd(II) or hν triggers respectively and

$$\frac{R(t)}{R_0} = e^{-k_1 t} \quad (8)$$

$$\frac{I_1(t)}{R_0} = 1 - e^{-k_1 t} \quad (9)$$

with the Pd(II) trigger whereas

$$\frac{R(t)}{R_0} = e^{-k_2' t} \quad (10)$$

$$\frac{I_2(t)}{R_0} = 1 - e^{-k_2' t} \quad (11)$$

with the hν one.

1.3.2 Kinetics during the preparative experiments

Figures 2S-4S display the time evolutions of the UPLC chromatograms from 1.75 mM solutions of **7, 8** or **9** submitted to Pd(II) ($Pd(OAc)_2$ 2 mol%, TPPTS 4 mol%, $HNEt_2$ 2.2 eq) (Figure 2S), hν (365 nm) (Figure 3S), and Pd(II) ($Pd(OAc)_2$ 2 mol%, TPPTS 4 mol%, $HNEt_2$ 2.2 eq) + hν (365 nm) (Figure 4S).

Time	Concentration of **8** (µM)	Concentration of **11** (µM)
0	1.75	0.00
10min	1.74	0.01
30min	1.71	0.04
1h	1.66	0.09
2h	1.57	0.18
6h	1.12	0.63
24h	0.25	1.50

*Table 1: Temporal evolution of concentrations for compounds **8** and **11** from **8** upon hν (365 nm).*

Time	Concentration of	Concentration of

	9 (µM)	**11** (µM)
0	1.75	0.00
10min	0.55	1.20
30min	0.00	1.75

*Table 2: Temporal evolution of concentrations for compounds **8** and **11** from **8** upon Pd(II) ($Pd(OAc)_2$ 2 mol%, TPPTS 4 mol%, $HNEt_2$ 2.2 eq).*

Time	Concentration of **7** (µM)	Concentration of **8** (µM)	Concentration of **11** (µM)
0	1.75	0.00	0.00
10min	1.27	0.48	0.00
30min	1.24	0.47	0.04
1h	1.21	0.48	0.06
2h	0.00	1.55	0.20
6h	0.00	0.94	0.81
24h	0.00	0.00	1.75

*Table 3: Temporal evolution of concentrations for compounds **7**, **8** and **11** from **7** submitted to Pd(II) ($Pd(OAc)_2$ 2 mol%, TPPTS 4 mol%, $HNEt_2$ 2.2 eq) + hν (365 nm). <u>Compound **9** was not observed on ULPC chromatograms.</u>*

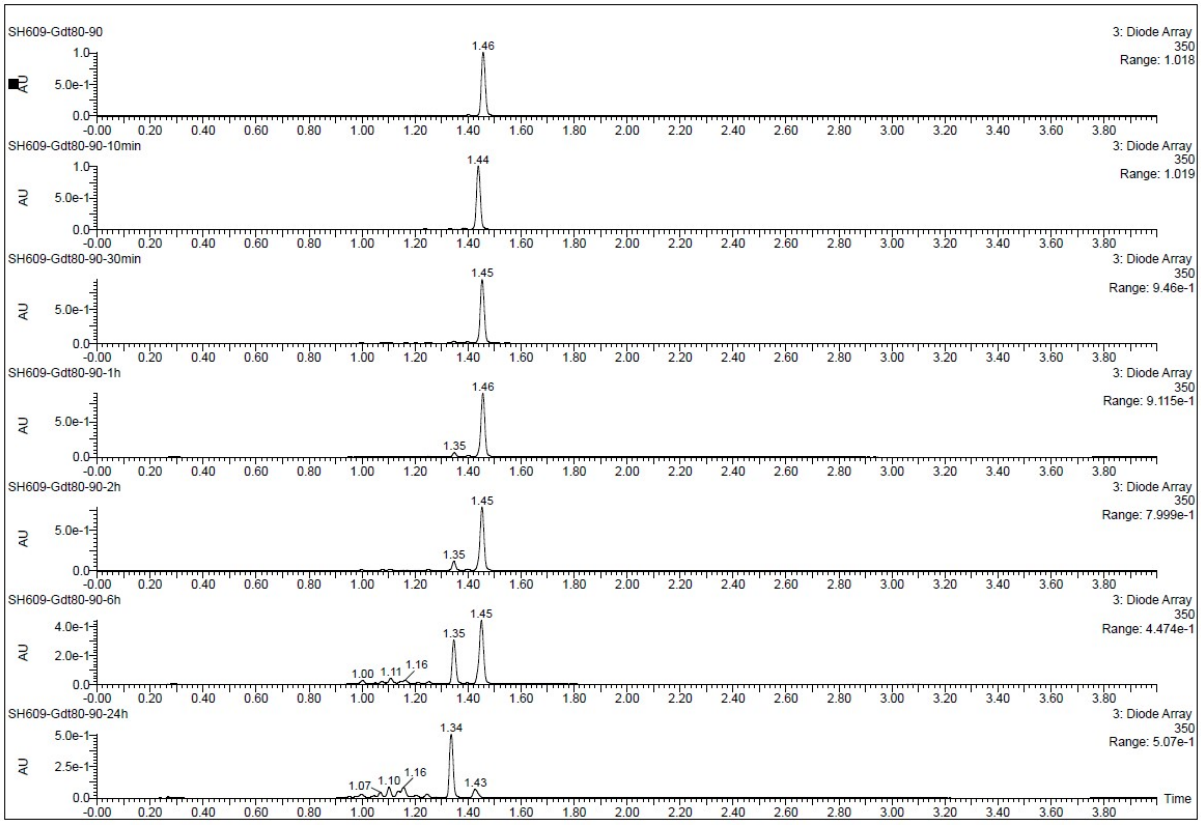


*Figure 2S: Time evolution of the UPLC chromatograms of 1.75 mM solutions of **8** submitted to hν (365 nm).* Dates (from top to bottom): initial time, 10 min, 30 min, 1 h, 2 h, 6 h and 24 h. Mass spectra confirmed peaks at 1.44-1.46 and 1.34-1.35 min to correspond to compounds **8** and **11** respectively. Despite an unexpected shift in retention time, we found $[M-H]^-$ = 227, which was coherent to the release of coumarin **11**. Solvent: CH_3CN/H_2O (4:1 v:v); T = 293 K.

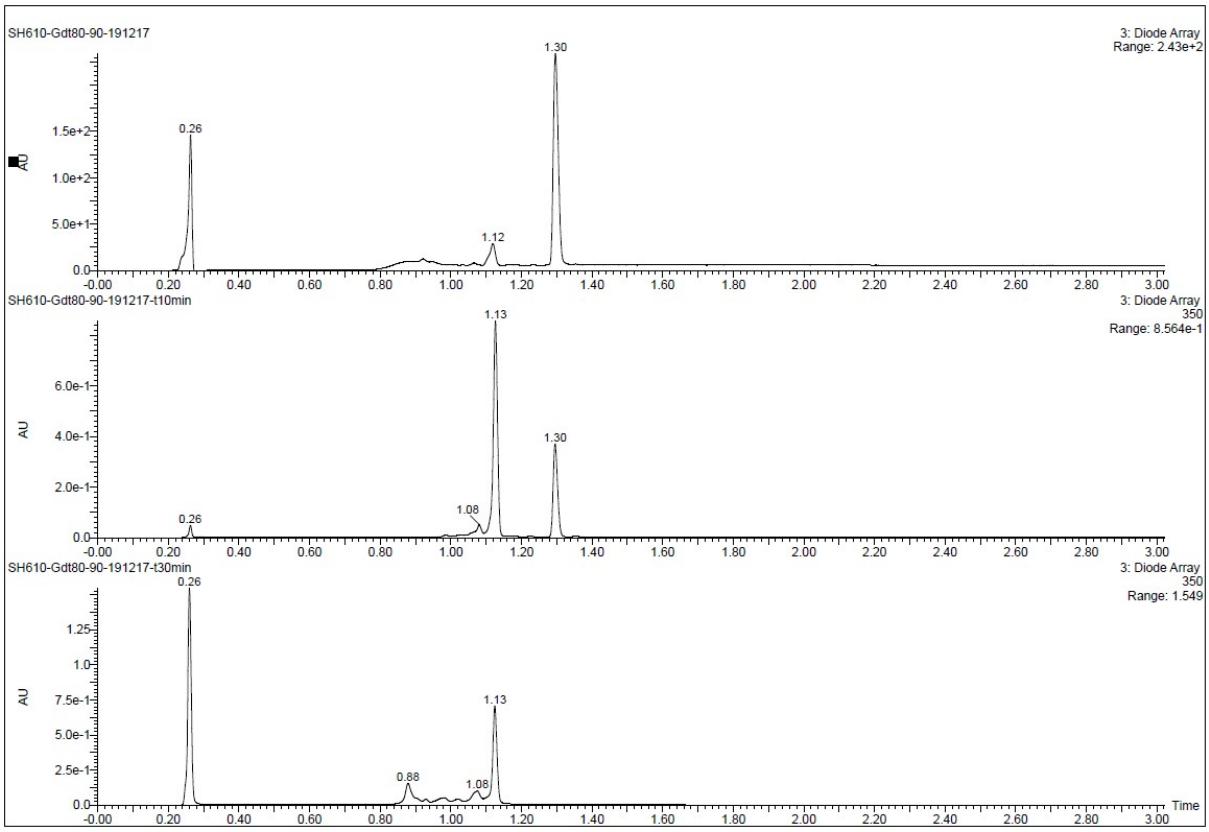


*Figure 3S: Time evolution of the UPLC chromatograms of 1.75 mM solutions of **9** submitted to Pd(II) ($Pd(OAc)_2$ 2 mol%, TPPTS 4 mol%, $HNEt_2$ 2.2 eq).* Dates (from top to bottom): initial time, 10 min, and 30 min. Mass spectra confirmed peaks at 1.30 and 1.13 min to correspond to compounds **9** and **11** respectively. Solvent: CH_3CN/H_2O (4:1 v:v); T = 293 K.

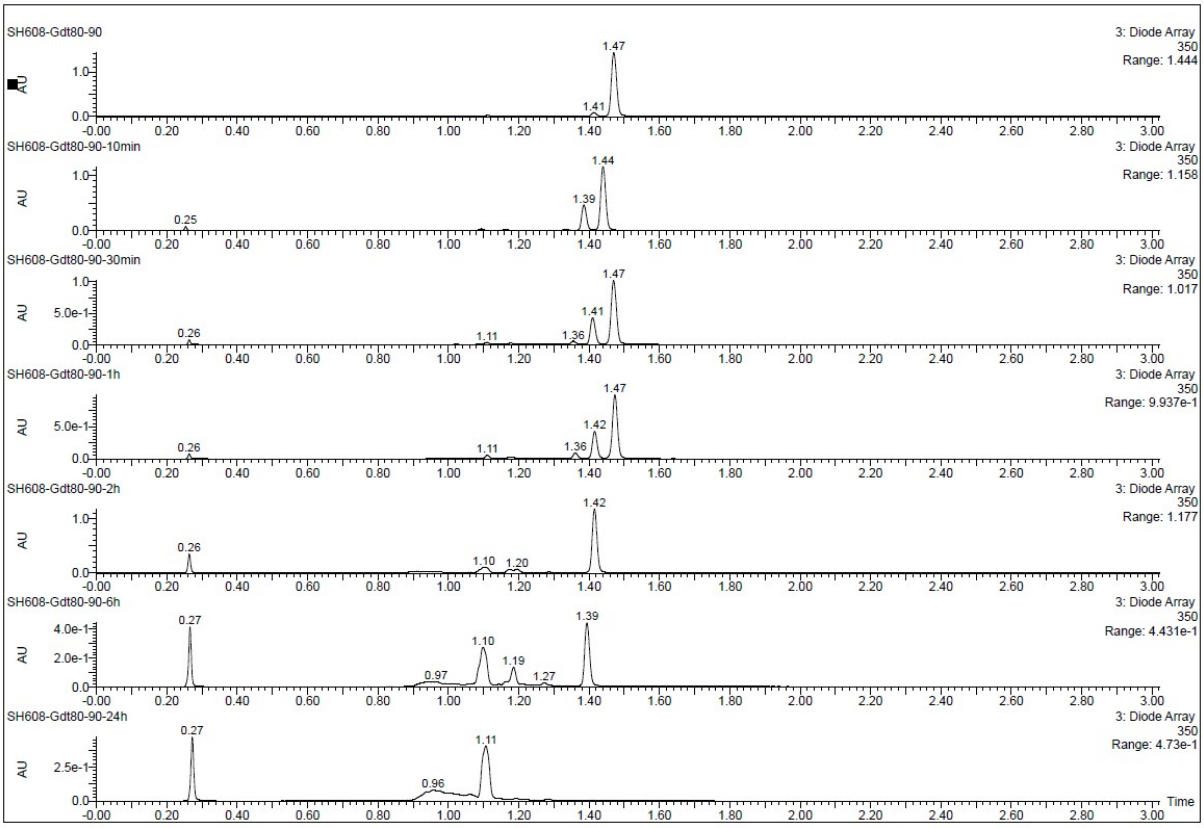


*Figure 4S: Time evolution of the UPLC chromatograms of 1.75 mM solutions of **7** submitted to Pd(II) ($Pd(OAc)_2$ 2 mol%, TPPTS 4 mol%, $HNEt_2$ 2.2 eq) + hν (365 nm).* Dates (from top to bottom): initial time, 10 min, 30 min, 1 h, 2 h, 6 h and 24 h. Mass spectra confirmed peaks at 1.44-1.47, 1.39-1.42 and 1.10-1.11 min to correspond to compounds **7**, **8** and **11** respectively. Solvent: CH_3CN/H_2O (4:1 v:v); T = 293 K.

1.3.3 Kinetics during the cuvette experiments

In view of possible biological applications in addition to the experiment reported at 298 K in the Main Text, we also analyzed the liberation of the coumarin **11** from dual activation of **7** at 310 K. We recorded the temporal evolution of the fluorescence intensity from a **7** solution submitted to both Pd(II) and continuous illumination at 365 nm. Figure 5S displays the temporal evolution of the fluorescence emission reporting on the release of the coumarin **11** upon continuously illuminating a 20 µM solution of the precursor **7** containing Pd(II) at 310 K.

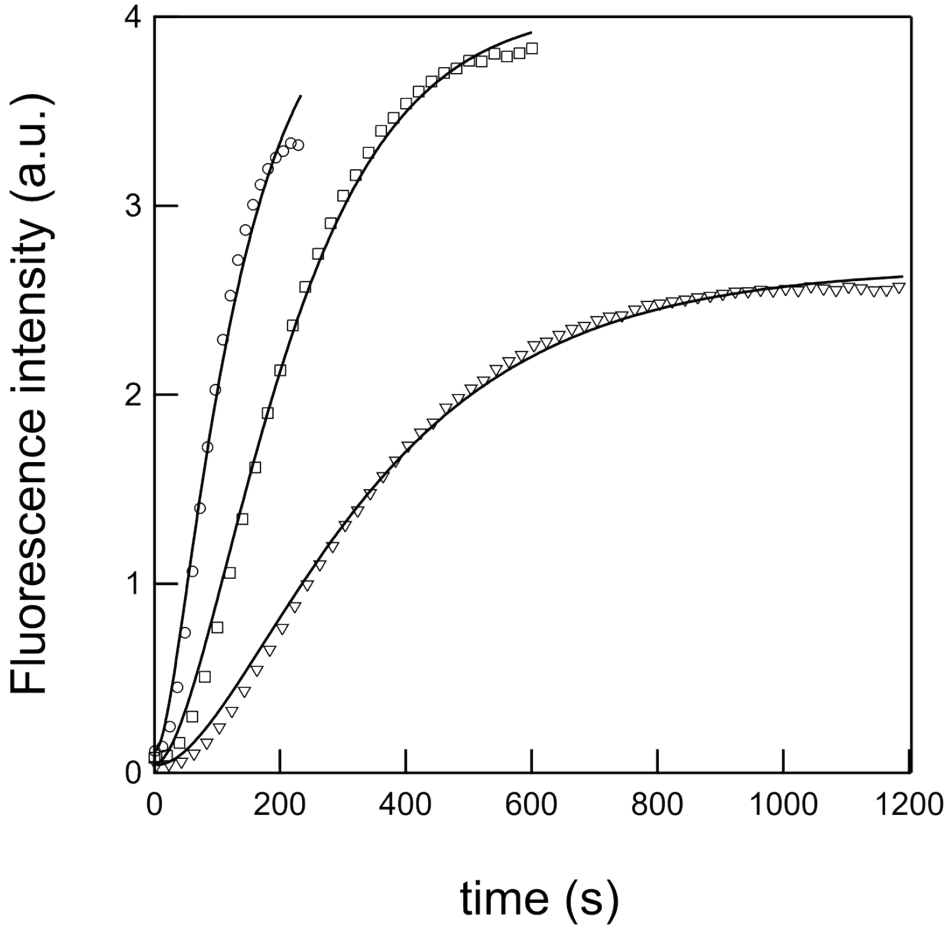


*Figure 5S. Temporal evolution of the fluorescence emission (λ_{em}=500 nm) upon illuminating at λ_{exc}=365 nm a 20 μM **7** solution containing $Pd(OAc)_2$ 2 mol%, TPPTS 4 mol%, $HNEt_2$ 2.2 eq at various light intensities in the ratios 1 (circles), 0.4 (squares), 0.2 (triangles). Markers: experimental data; solid line: fits with Eq.(8). Solvent: CH_3CN:H_2O (4:1 v:v). T = 310 K.*

The time evolution of the fluorescence signal was analyzed upon adopting the reduced kinetic scheme shown in Scheme 1S. We obtained satisfactory fits by constraining k_{low} to be proportional to the light intensity (as expected from a photochemical step) and k_3 = 8.4 10^{-3}± 10^{-3} s^{-1}.

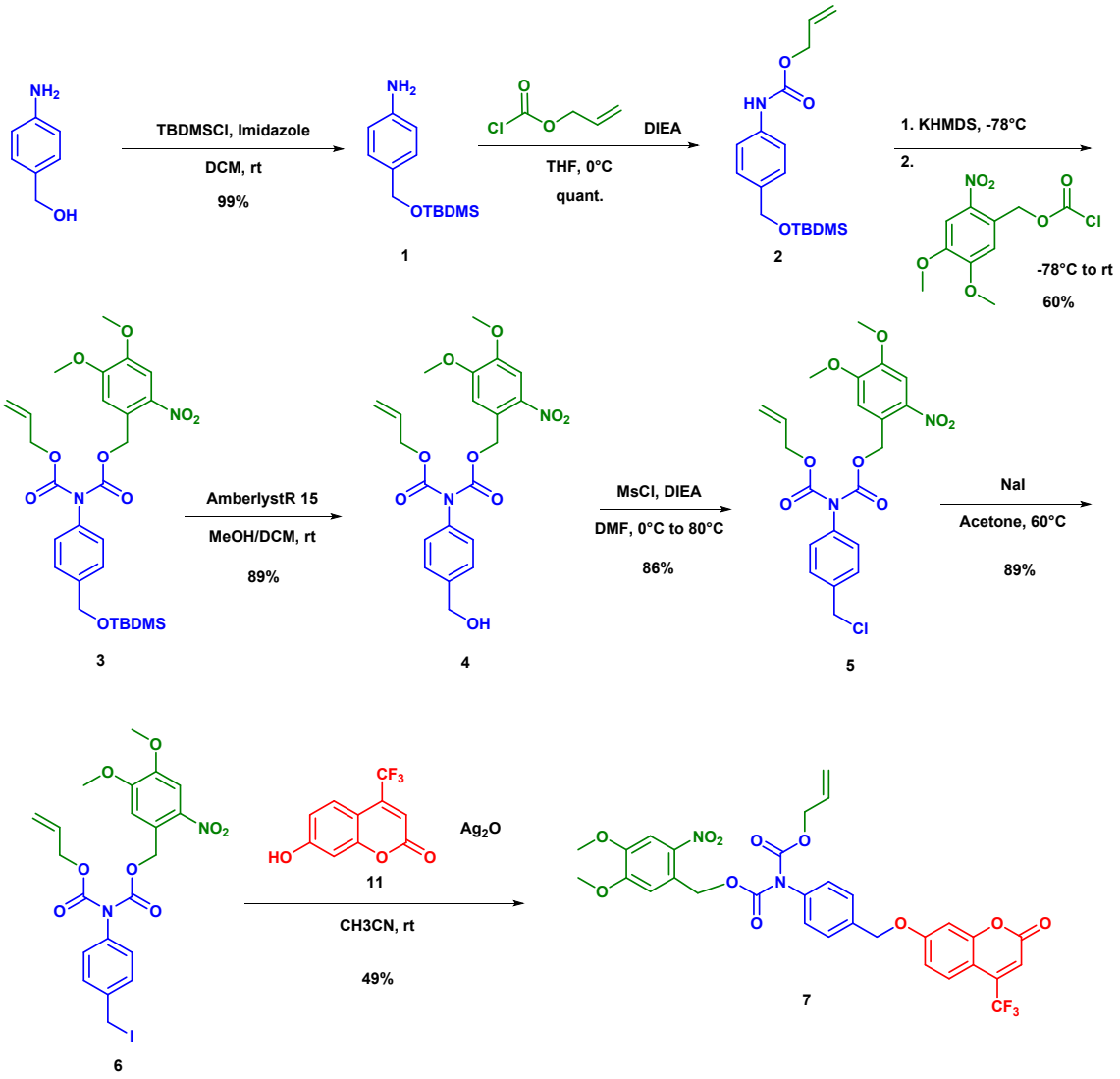


*Figure 6S: Synthetic scheme of N,N'-bis-carbamate **7***

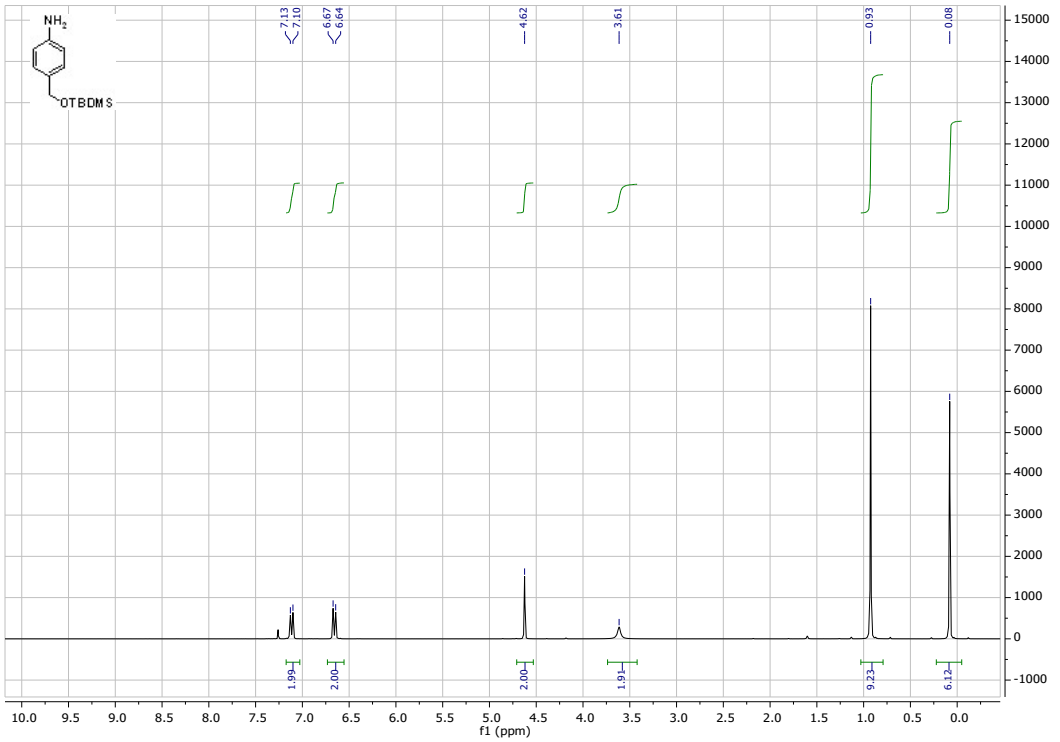


*Figure 7S: ^{1}H NMR spectrum of compound **1***

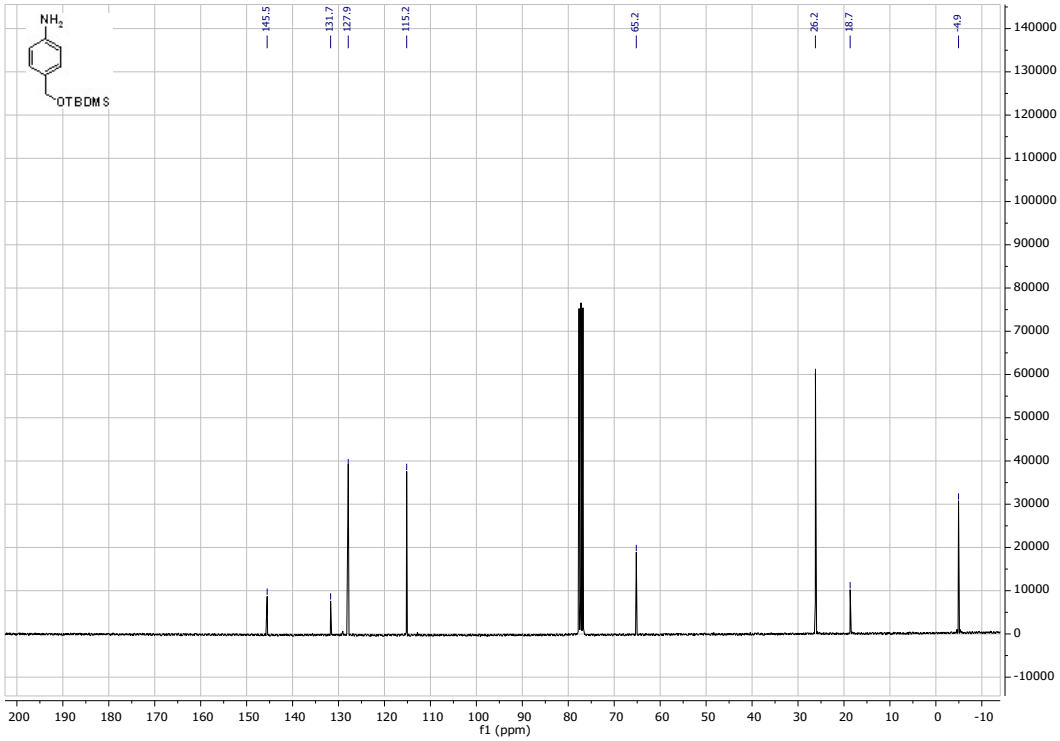


*Figure 8S: ^{13}C NMR spectrum of compound **1***

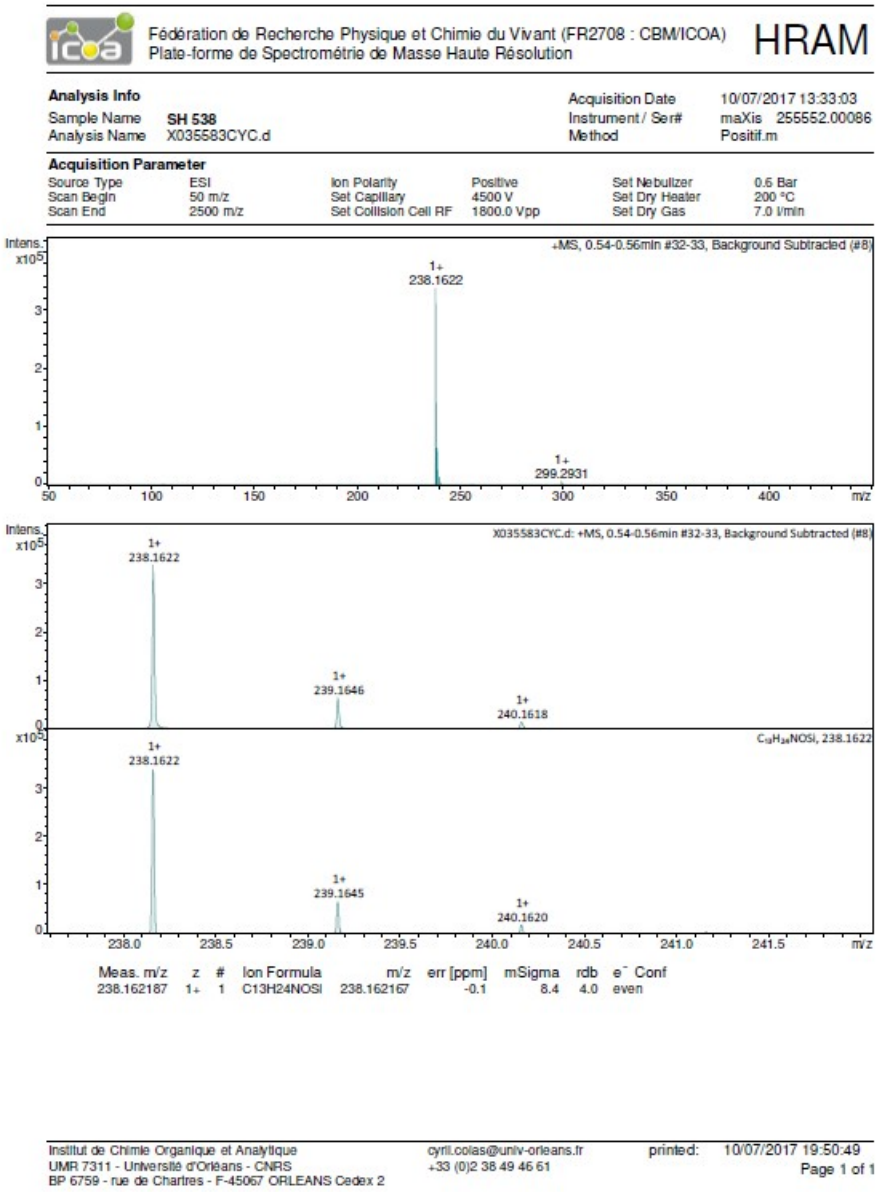


*Figure 9S: HRMS spectrum of compound **1***

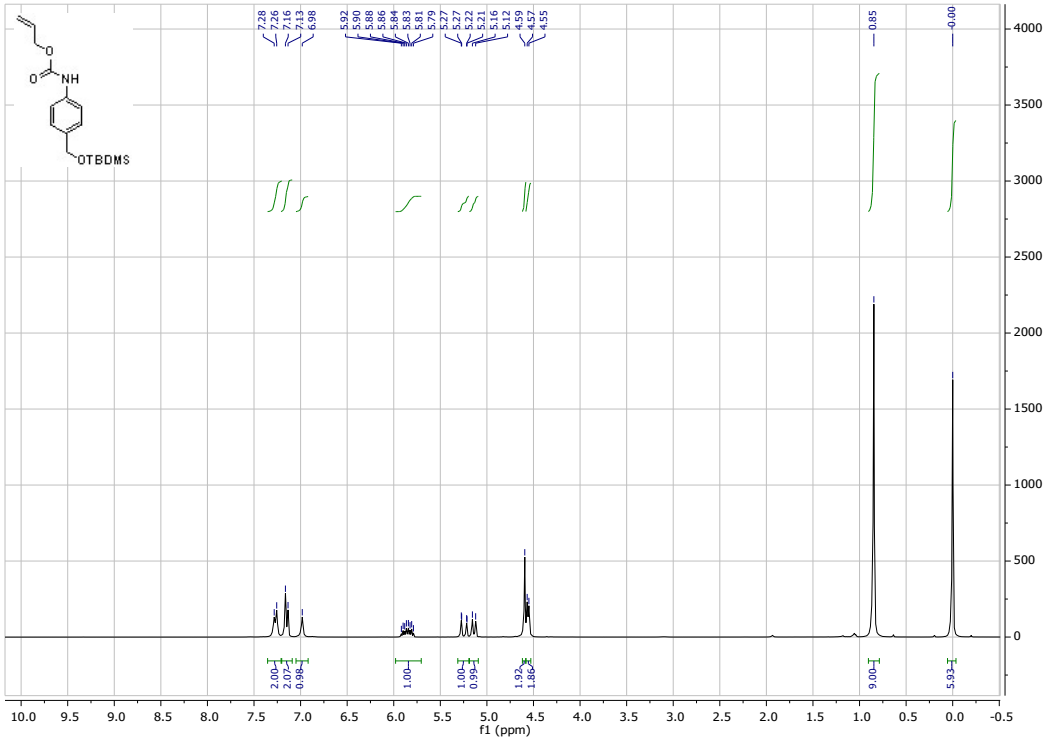


*Figure 10S: ^{1}H NMR spectrum of compound **2***

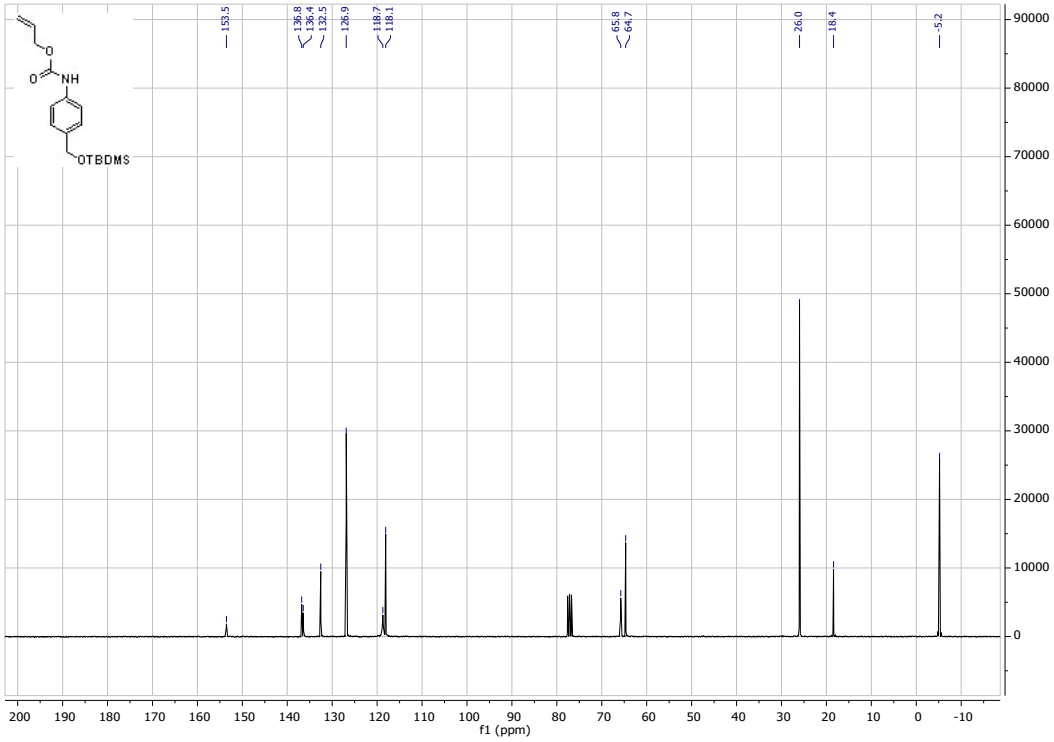


*Figure 11S: ^{13}C NMR spectrum of compound **2***

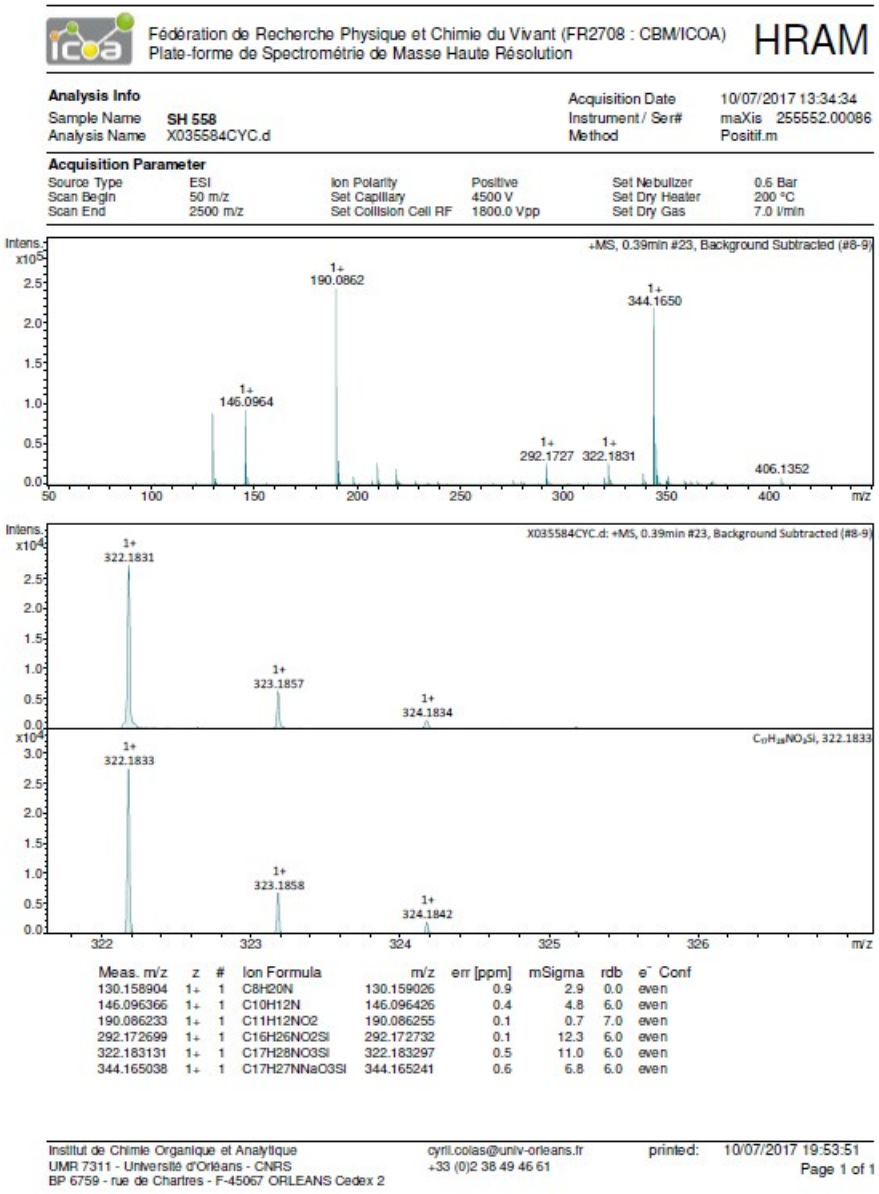


*Figure 12S: HRMS spectrum of compound **2***

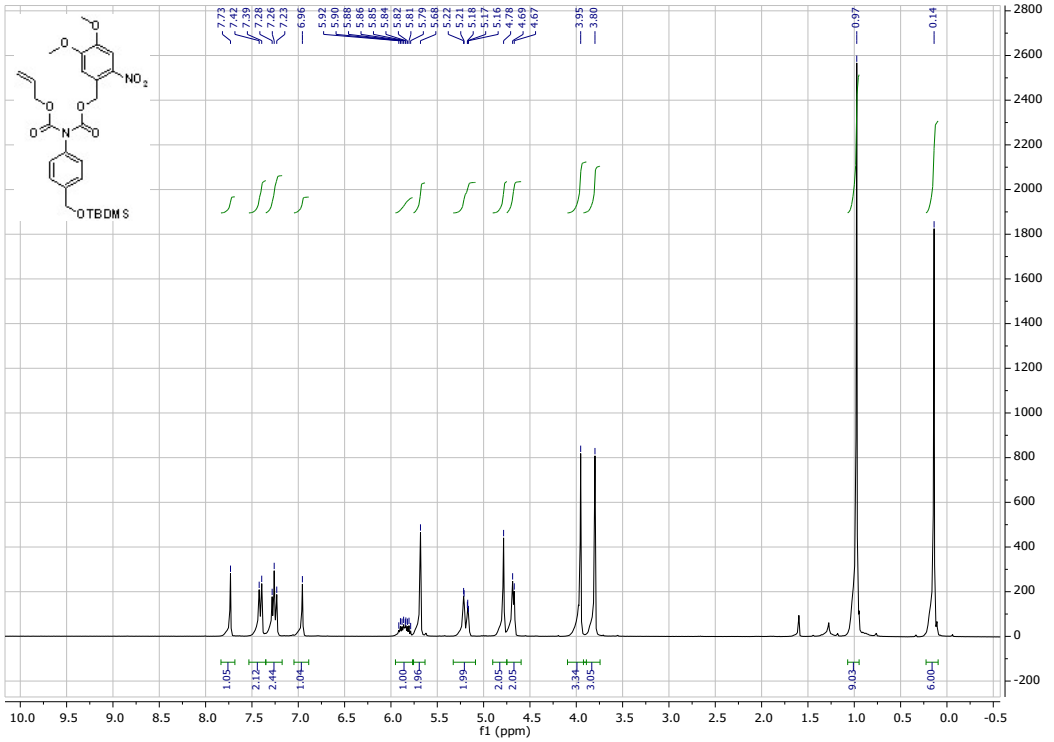


*Figure 13S: ^{1}H NMR spectrum of compound **3***

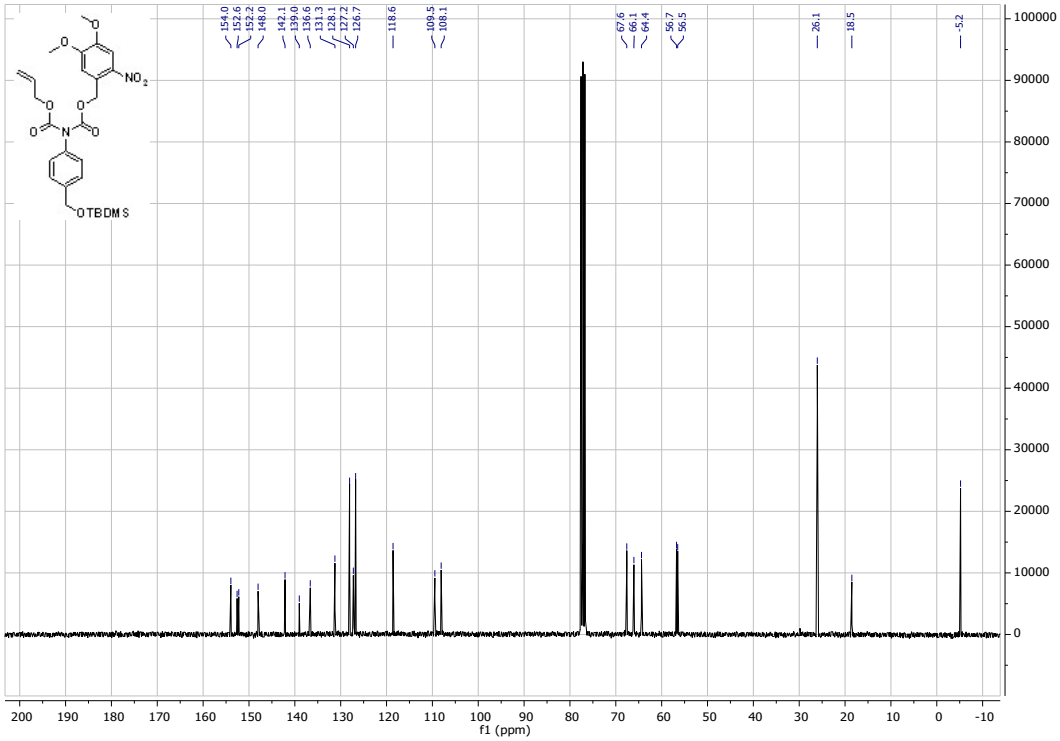


*Figure 14S: ^{13}C NMR spectrum of compound **3***

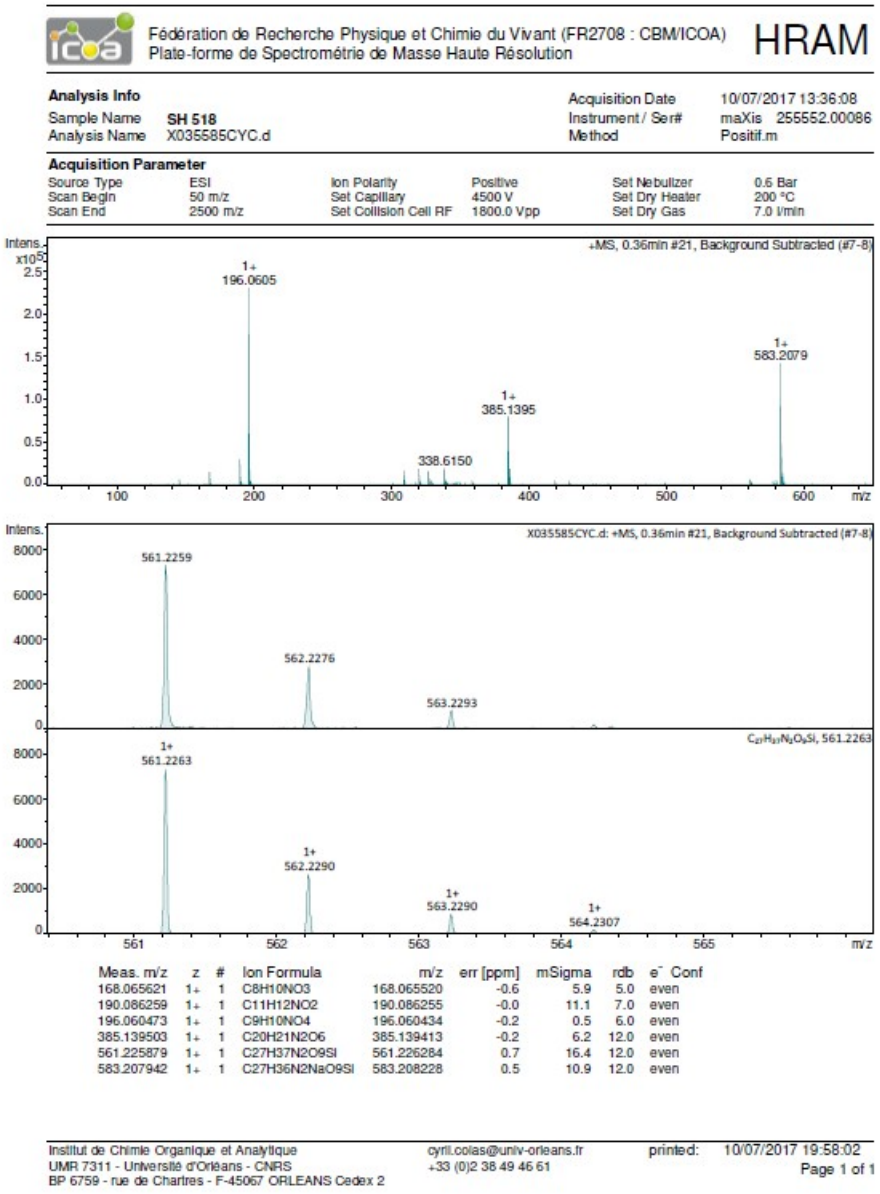


*Figure 15S: HRMS spectrum of compound **3***

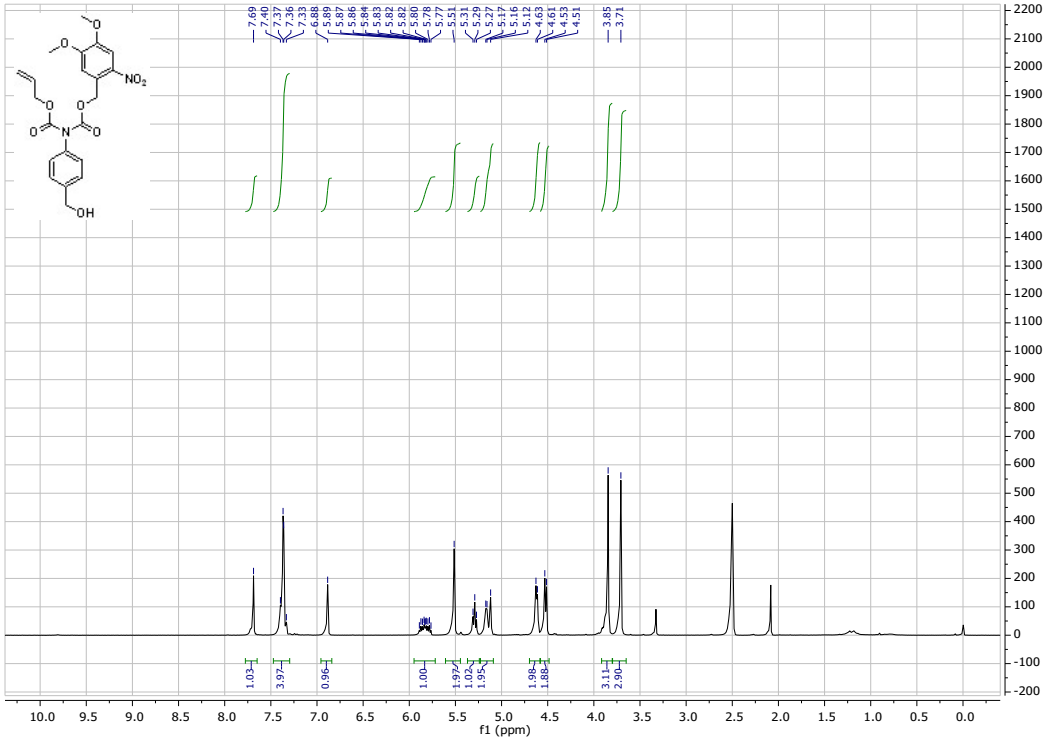


*Figure 16S: [1]H NMR spectrum of compound **4***

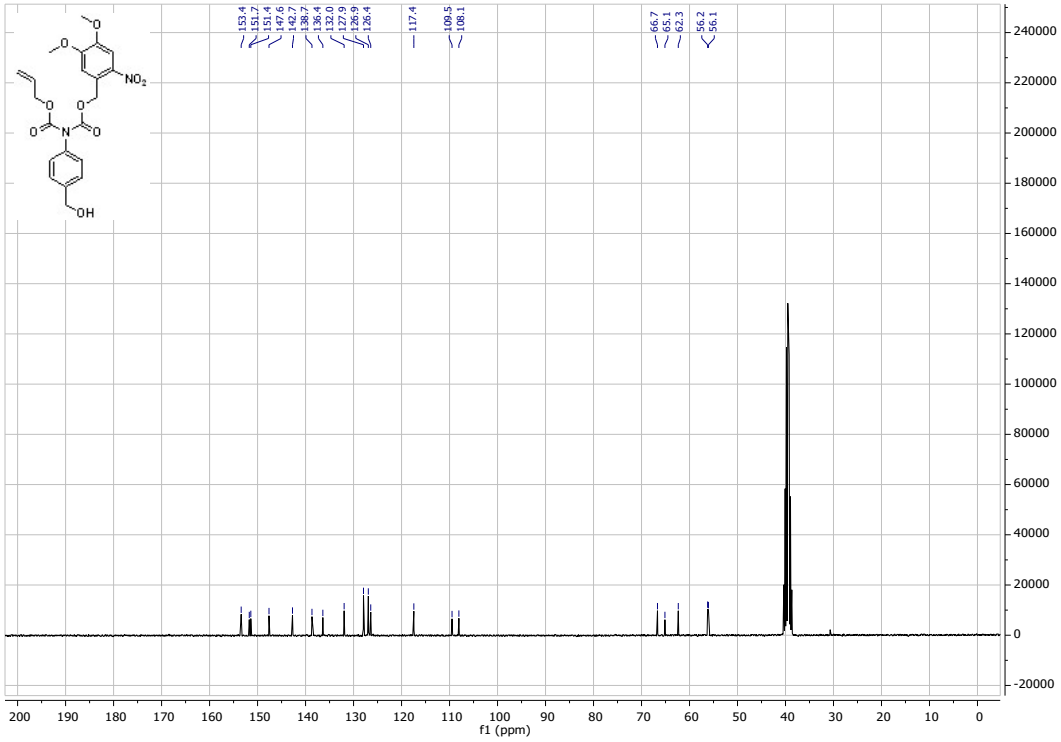


*Figure 17S: [13]C NMR spectrum of compound **4***

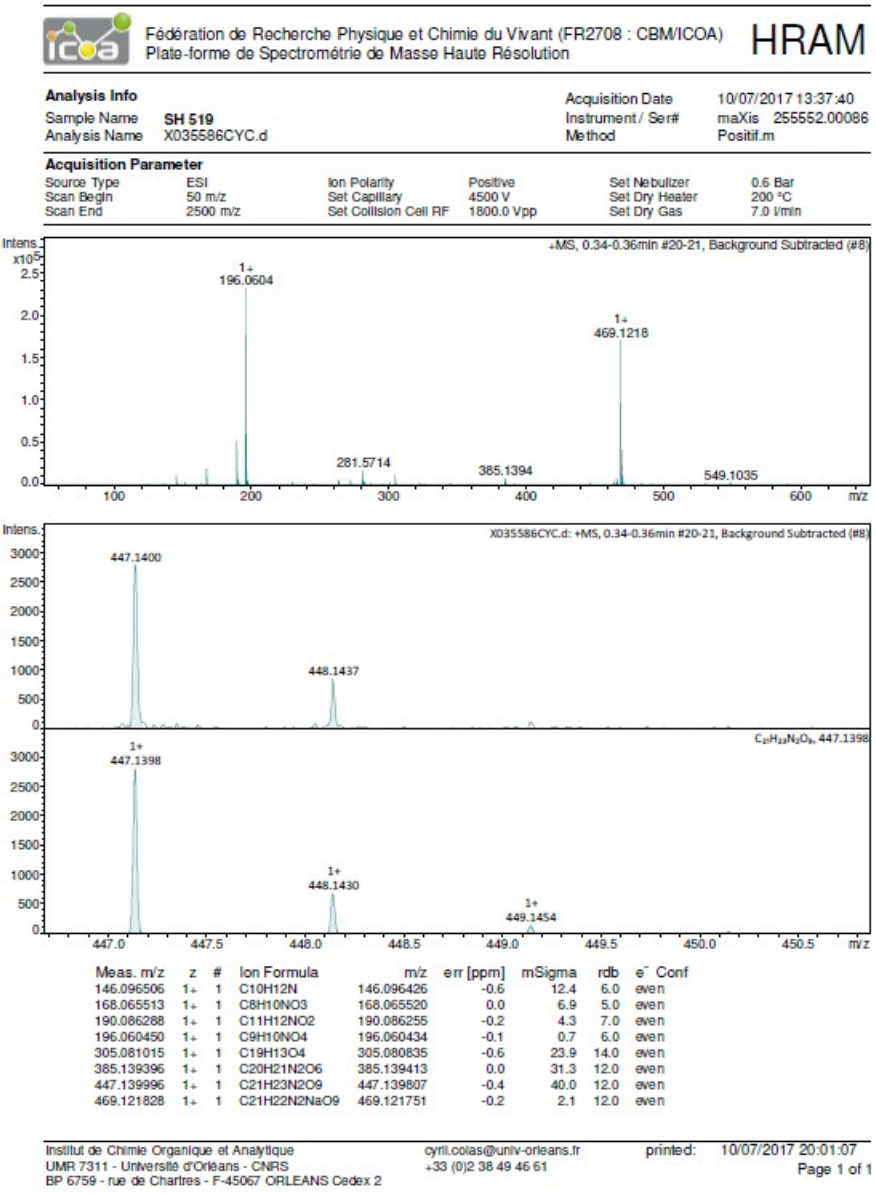


*Figure 18S: HRMS spectrum of compound **4***

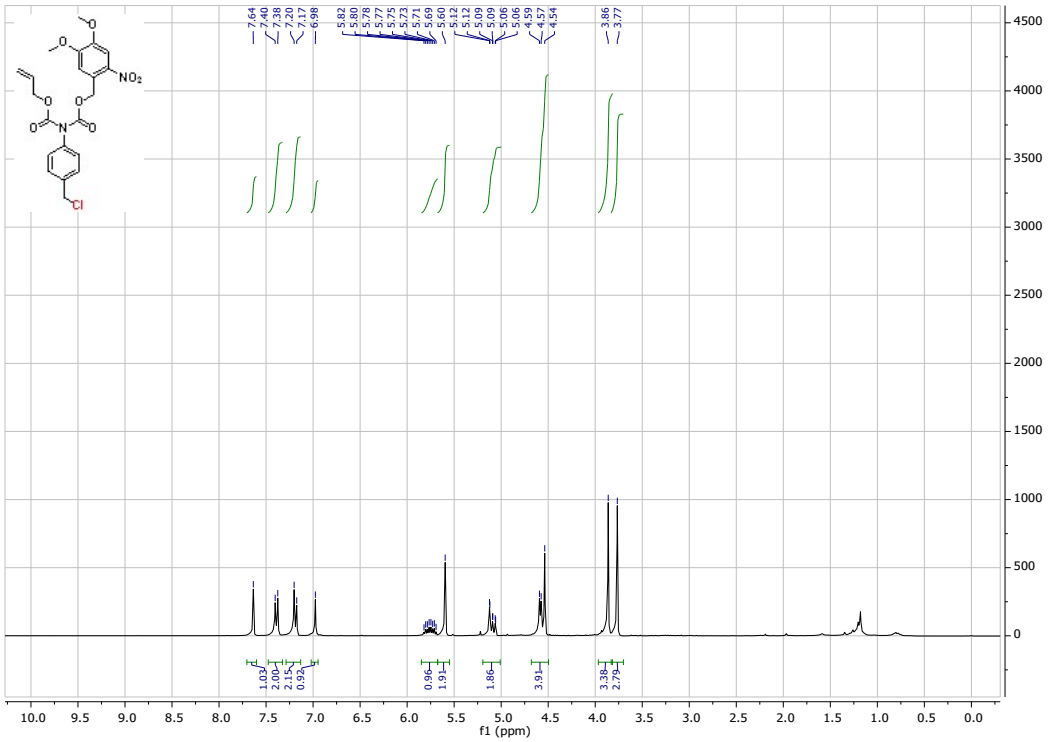


*Figure 19S: [1]H NMR spectrum of compound **5***

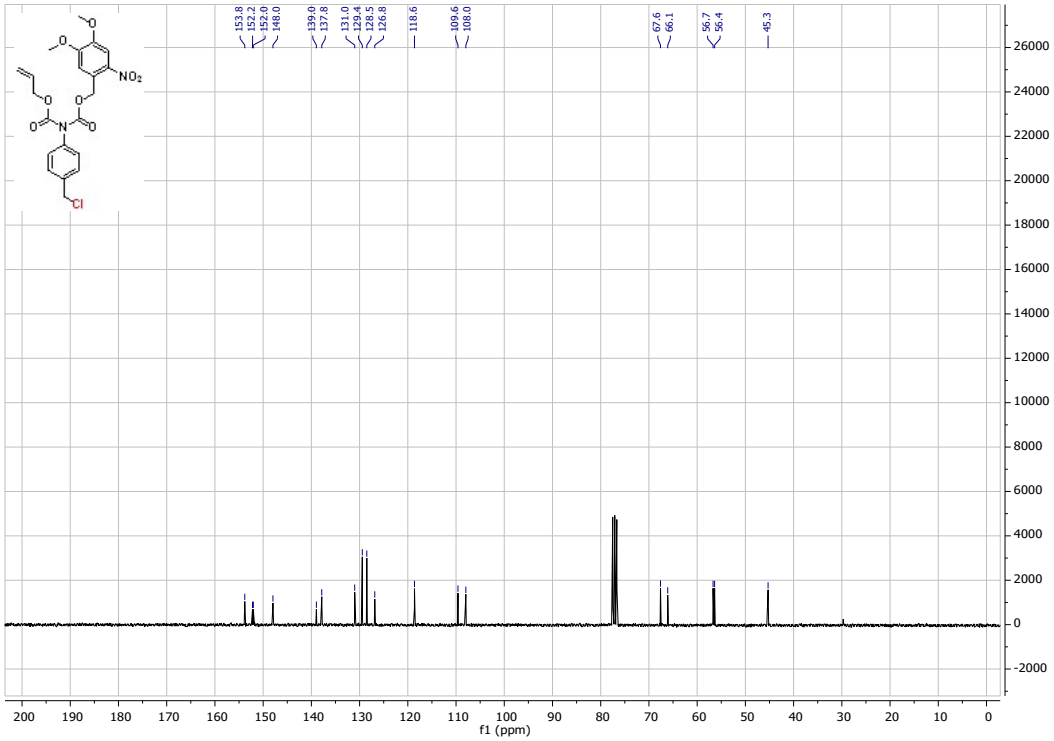


*Figure 20S: [13]C NMR spectrum of compound **5***

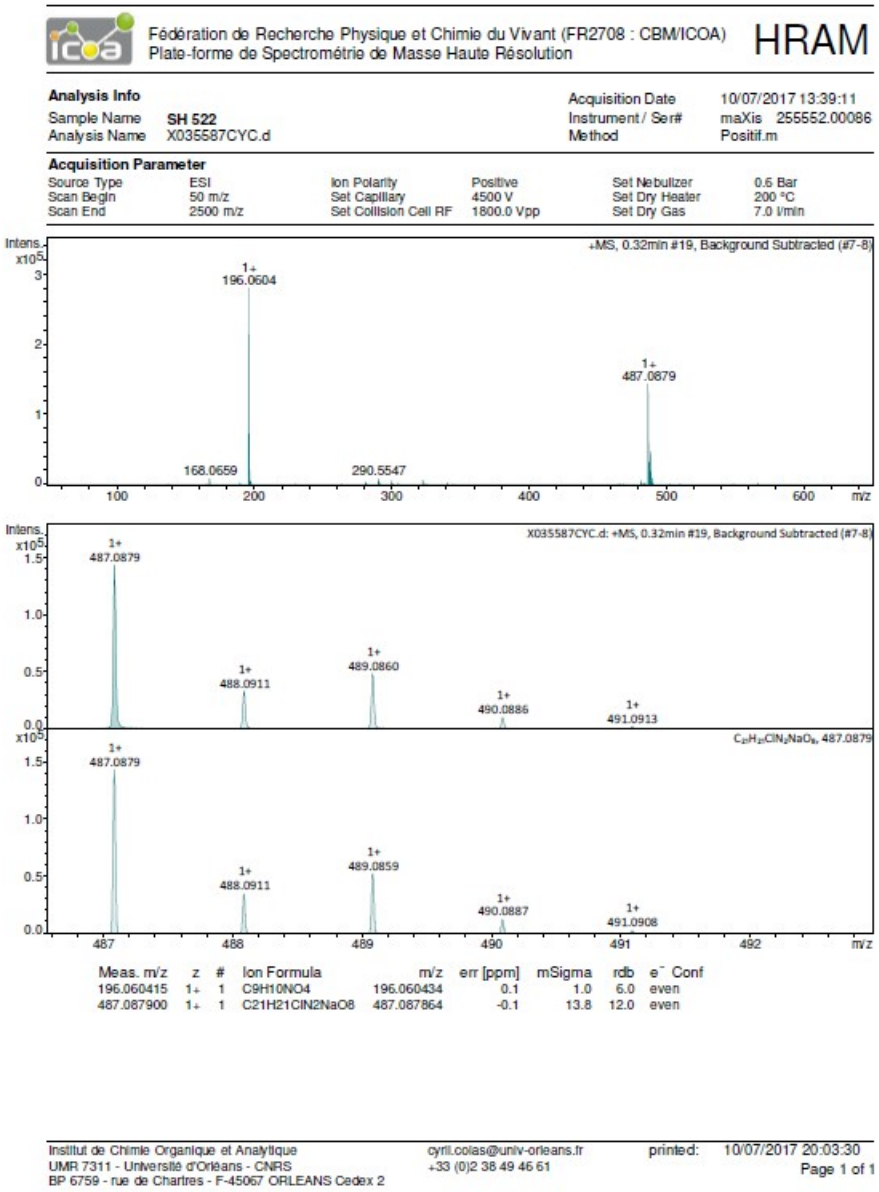


*Figure 21S: HRMS spectrum of compound **5***

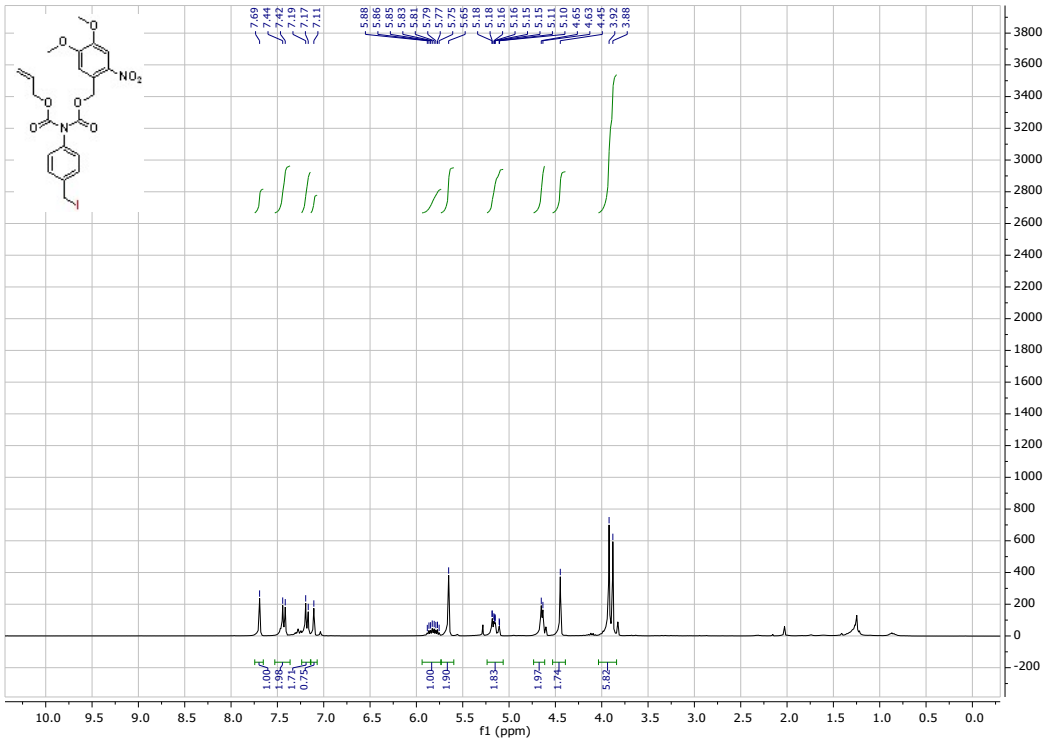


*Figure 22S: [1]H NMR spectrum of compound **6***

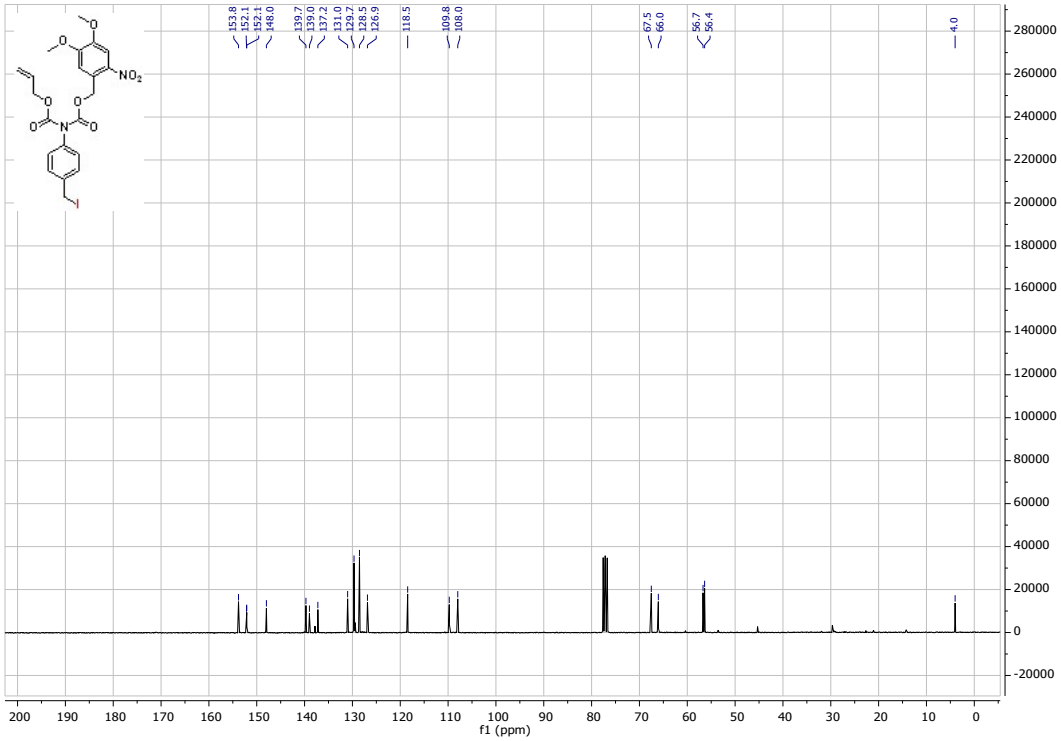


*Figure 23S: [13]C NMR spectrum of compound **6***

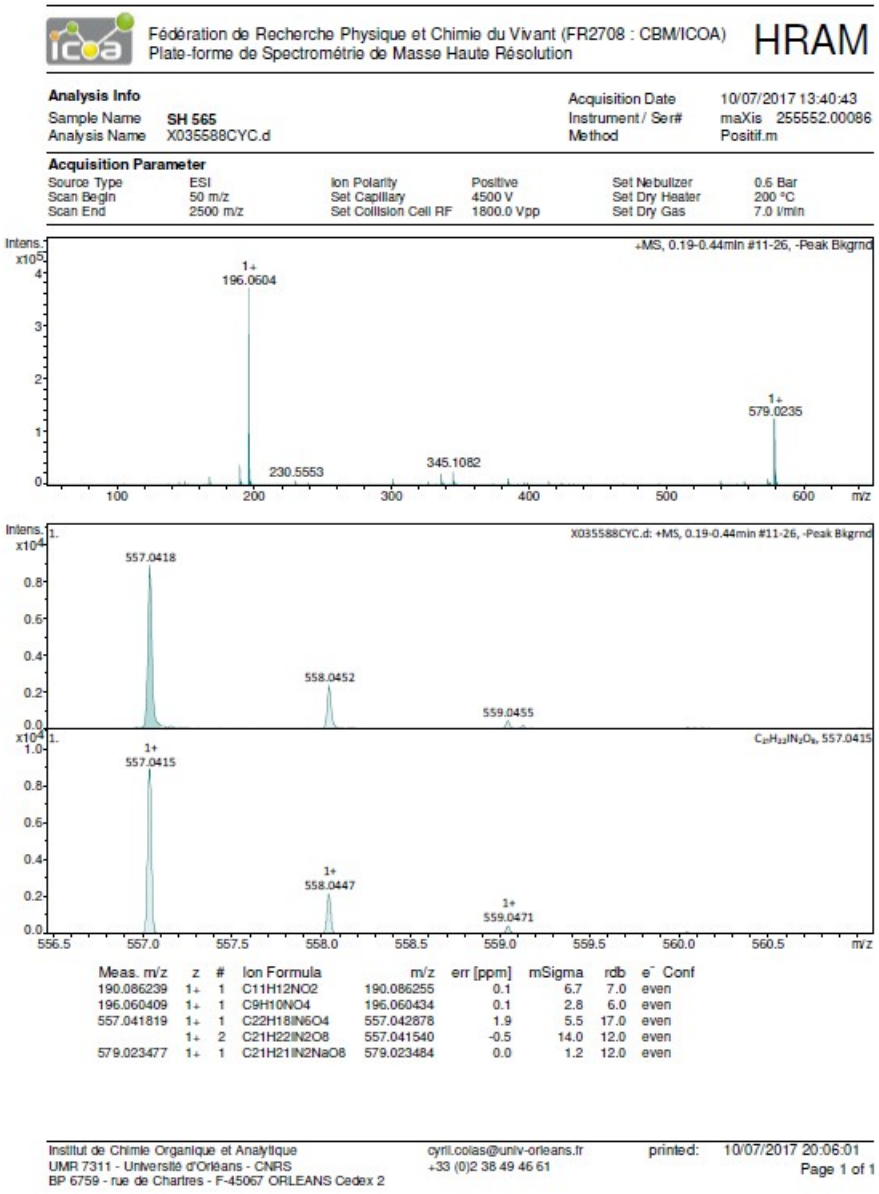


*Figure 24S: HRMS spectrum of compound **6***

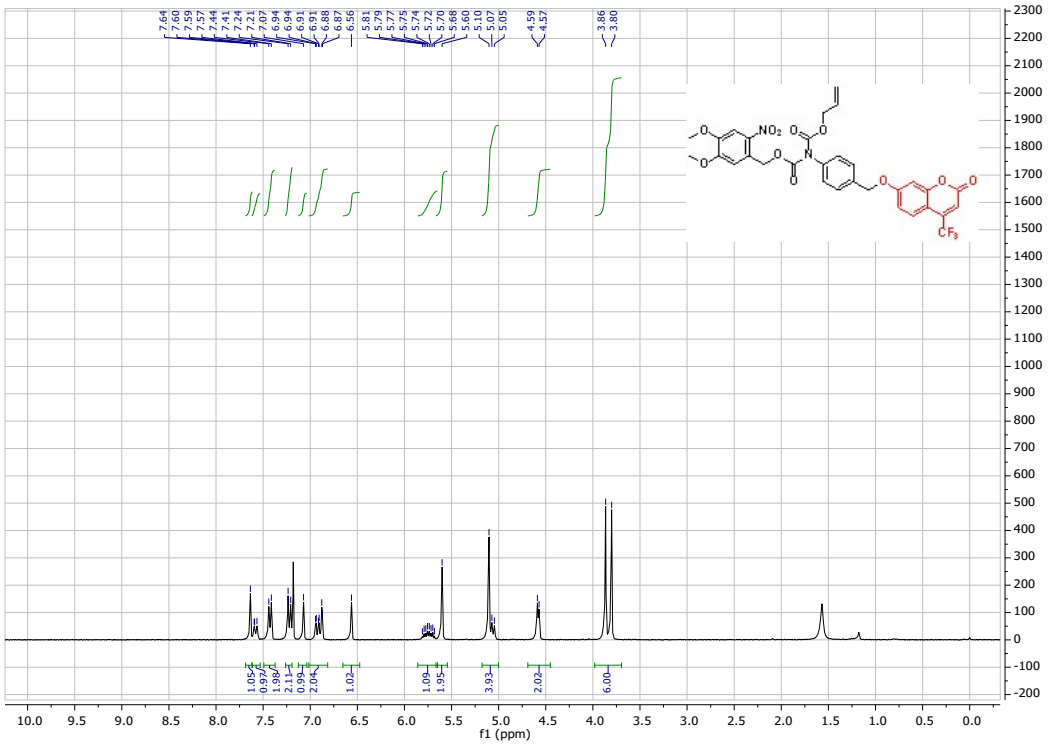


*Figure 25S: [1]H NMR spectrum of compound **7***

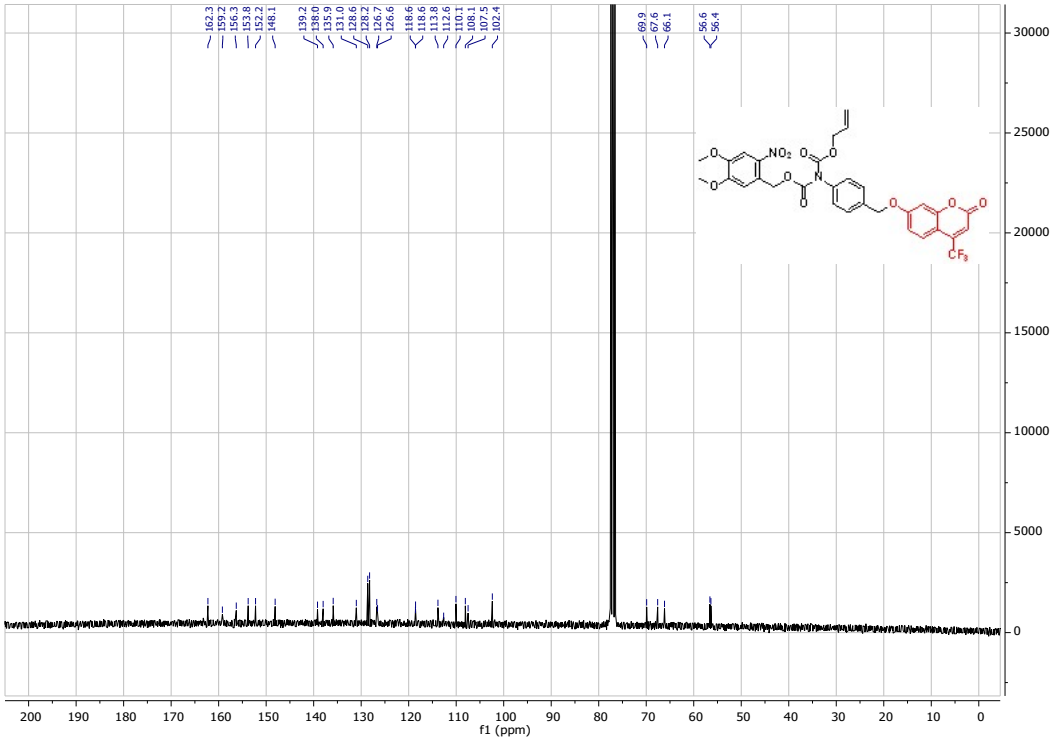


*Figure 26S: [13]C NMR spectrum of compound **7***

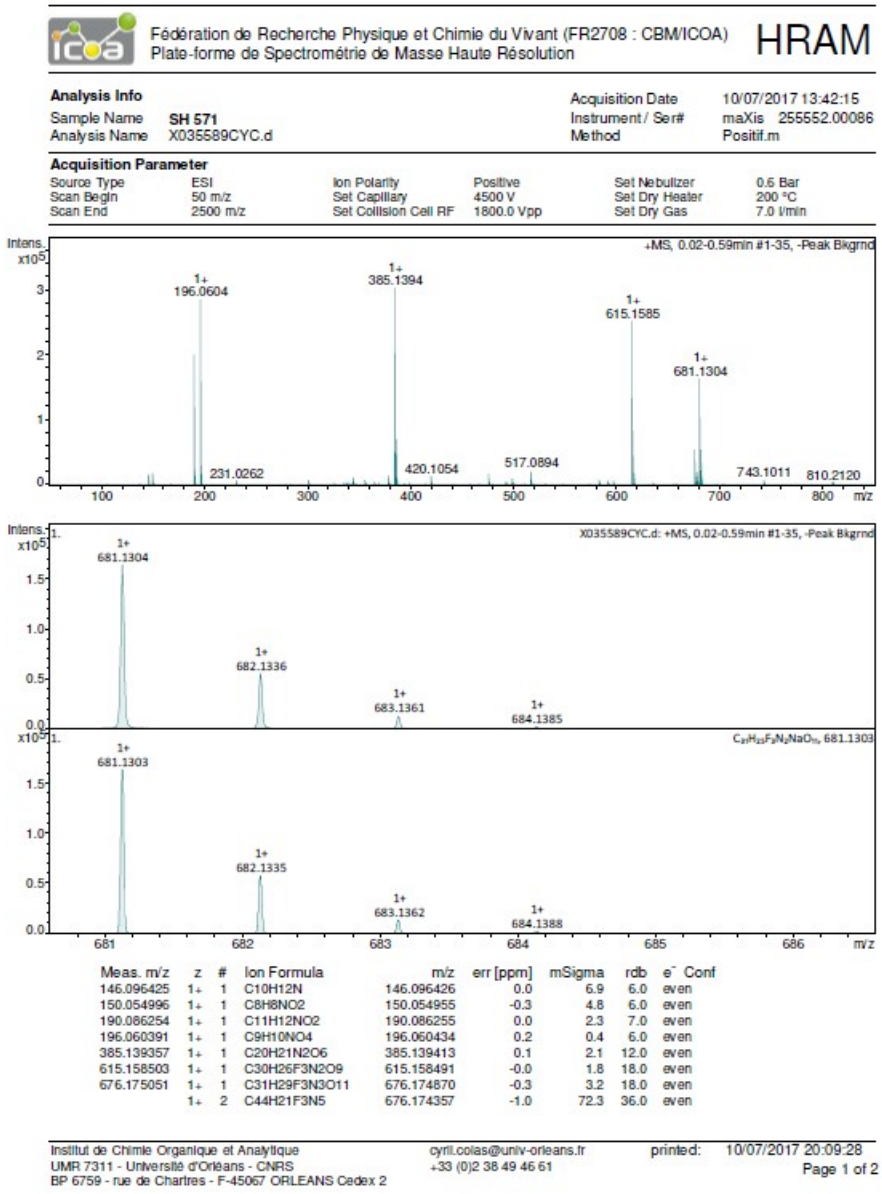


*Figure 27S: HRMS spectrum of compound **7***

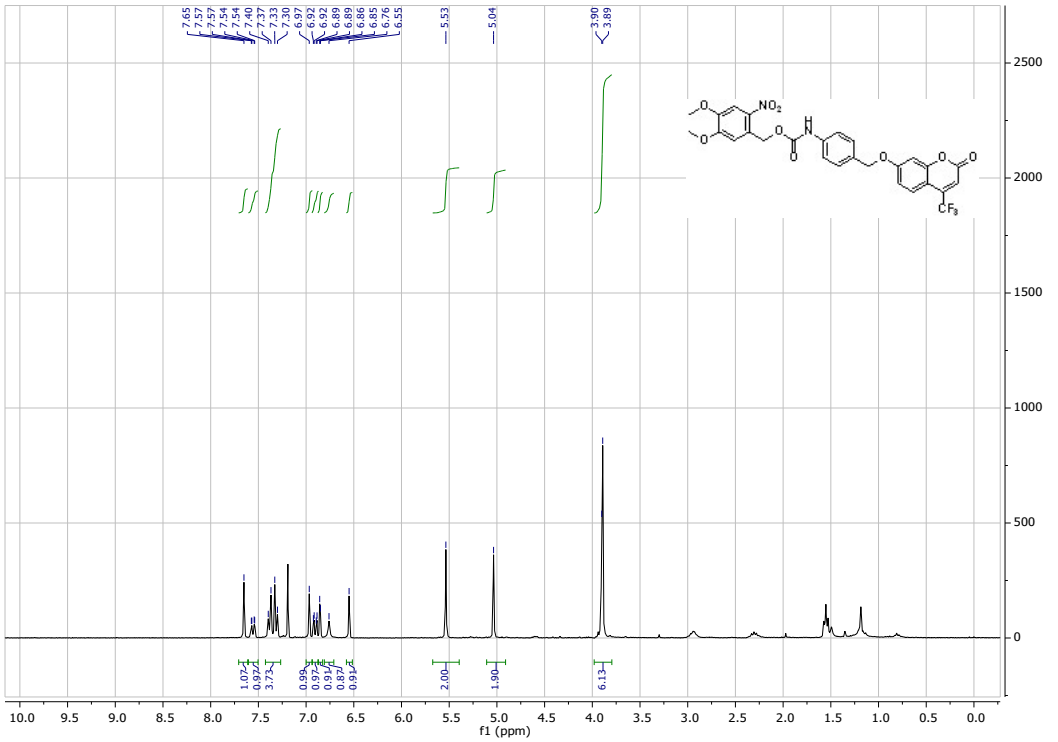


*Figure 28S: [1]H NMR spectrum of compound **8***

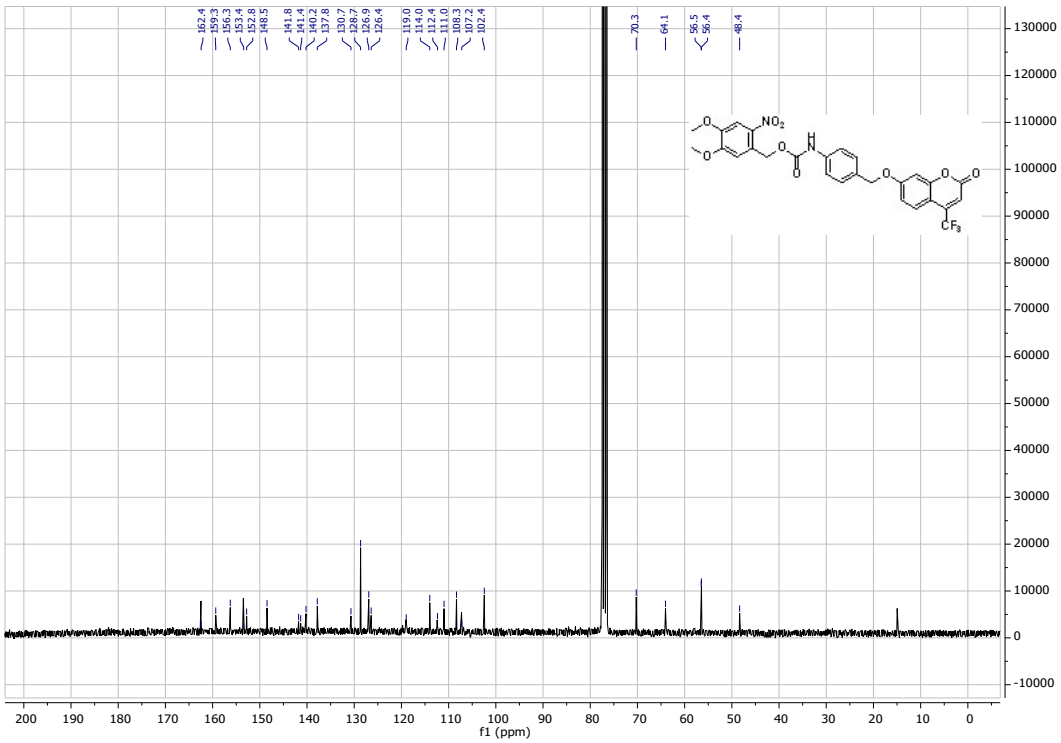


*Figure 29S: [13]C NMR spectrum of compound **8***

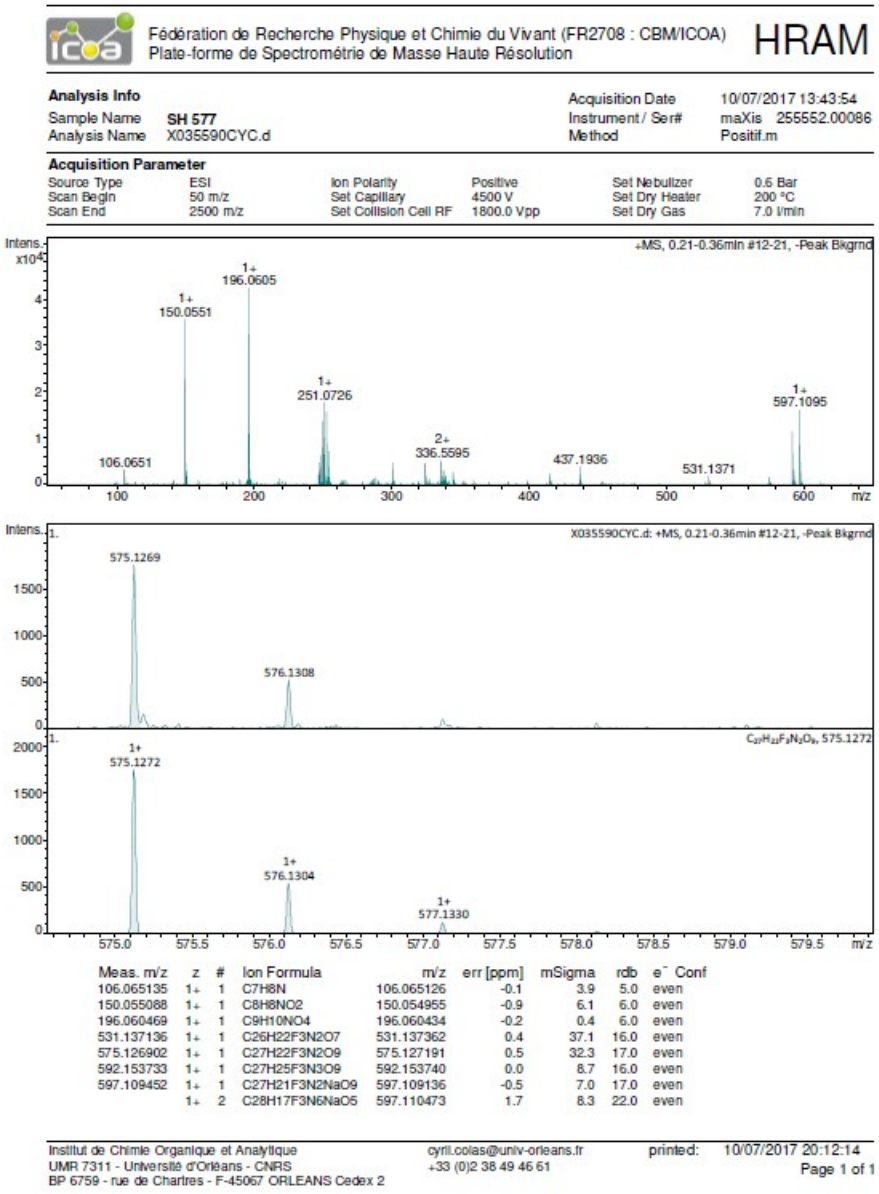


*Figure 30S: HRMS spectrum of compound **8***

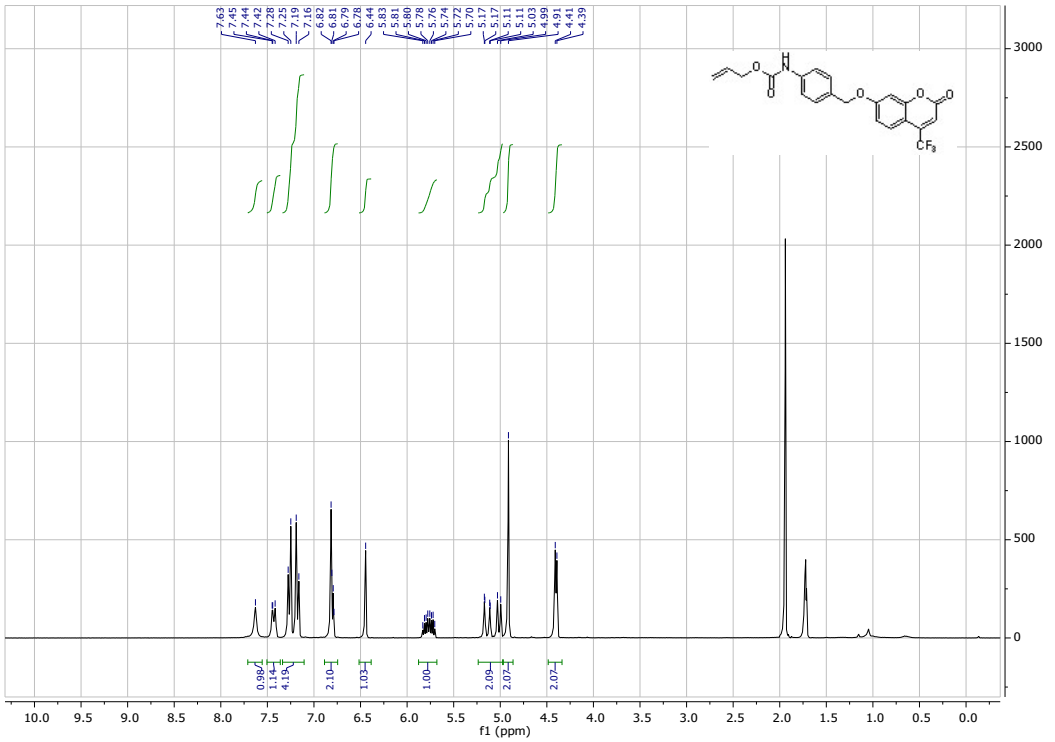


*Figure 31S: [1]H NMR spectrum of compound **9***

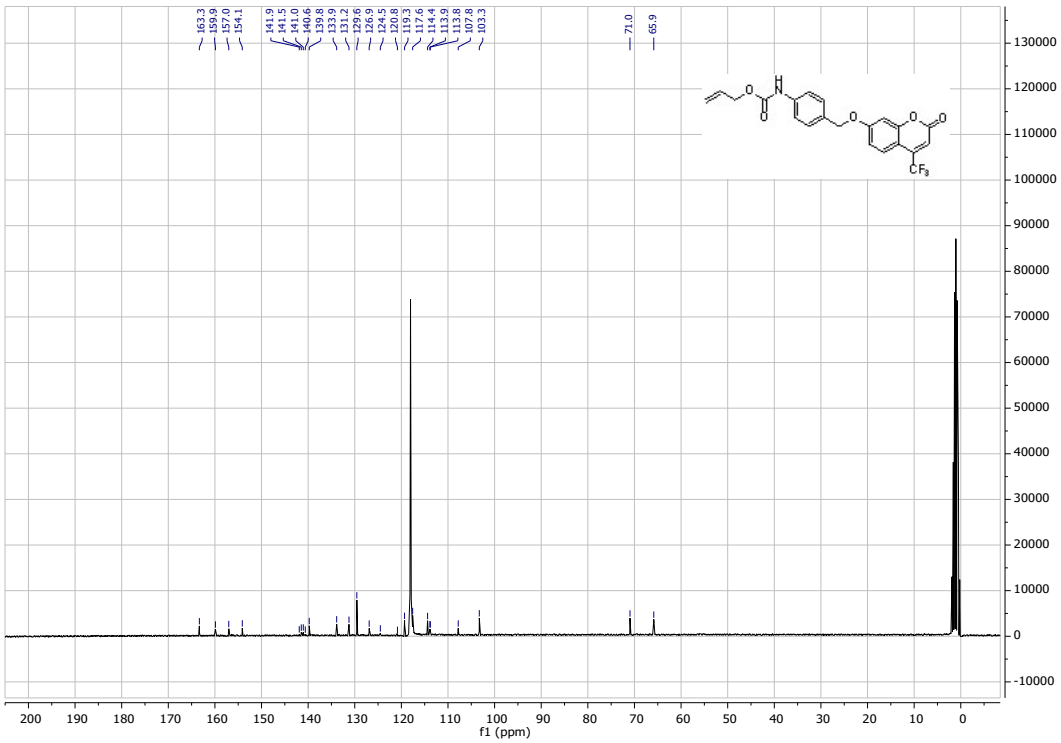


*Figure 32S: [13]C NMR spectrum of compound **9***

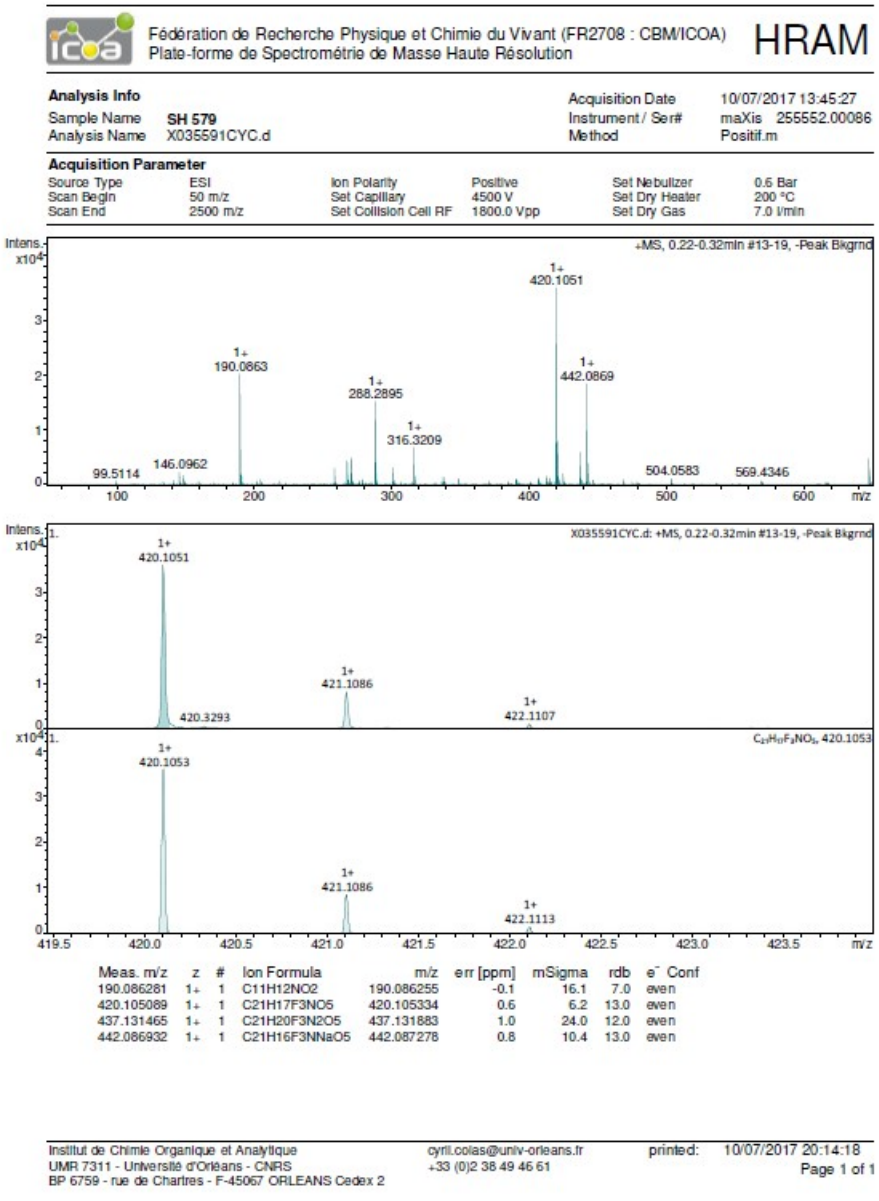


*Figure 33S: HRMS spectrum of compound **9***