Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Electronic Supplementary Information for

Controlling the Liberation Rate of the In Situ Release of a Chemical Fuel for the Operationally Autonomous Motions of Molecular Machines

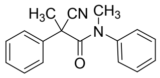
*Chiara Biagini, Giorgio Capocasa, Daniele Del Giudice, Valerio Cataldi, Luigi Mandolini and Stefano Di Stefano**

Dipartimento di Chimica, Università di Roma "La Sapienza", and Istituto CNR per i Sistemi Biologici (ISB-CNR), Sezione Meccanismi di Reazione, P.le A. Moro 5, 00185 Roma, Italy.
E-mail: stefano.distefano@uniroma1.it

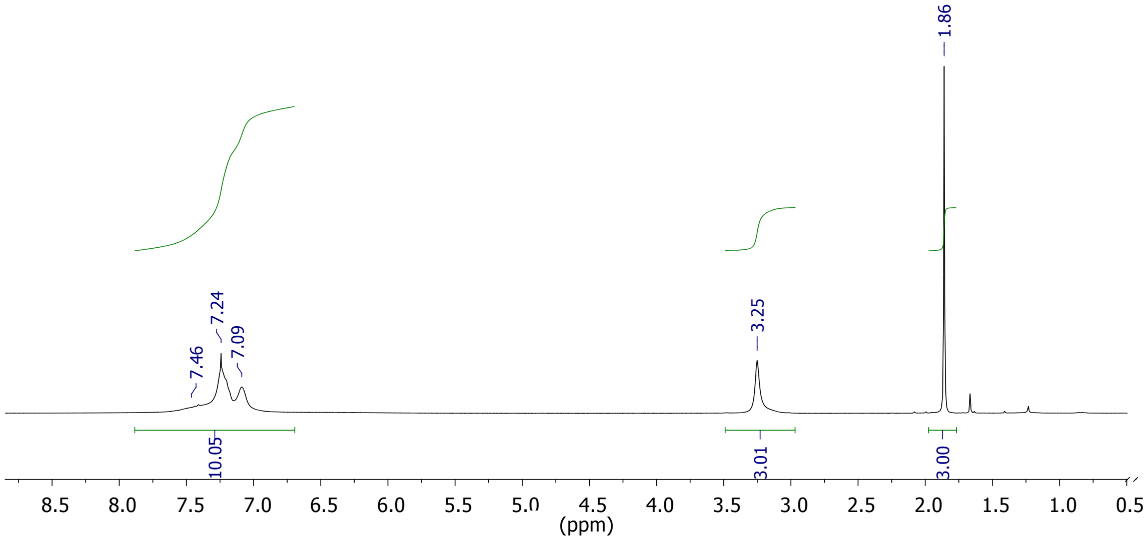
Contents

^{1}H and ^{13}C NMR spectra of all *N*-methylanilide compounds recorded at room temperature are characterized by broad signals due to the partially hindered rotation of the C-N amide bond. However, the purity of all *N*-methylanilide compounds was > 99 % as shown by GC analyses (*vide infra*).

^{1}H NMR spectrum ($CDCl_3$, 300MHz, 25°C) of **5a**

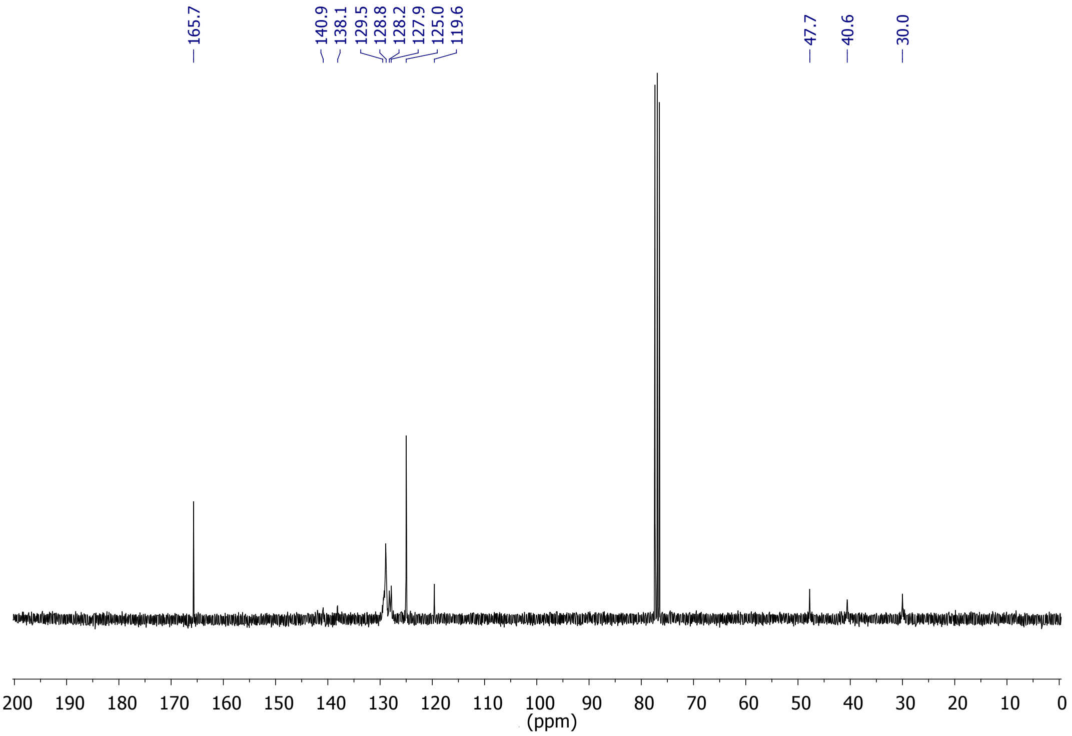


5a



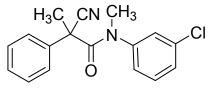
δ (ppm): 7.46-7.09 (*m*, 10H, Ar-*H*), 3.25 (*bs*, 3H, -NCH_3), 1.86 (*s*, 3H, -CH_3)

^{13}C NMR spectrum ($CDCl_3$, 75 MHz, 25°C) of **5a.**

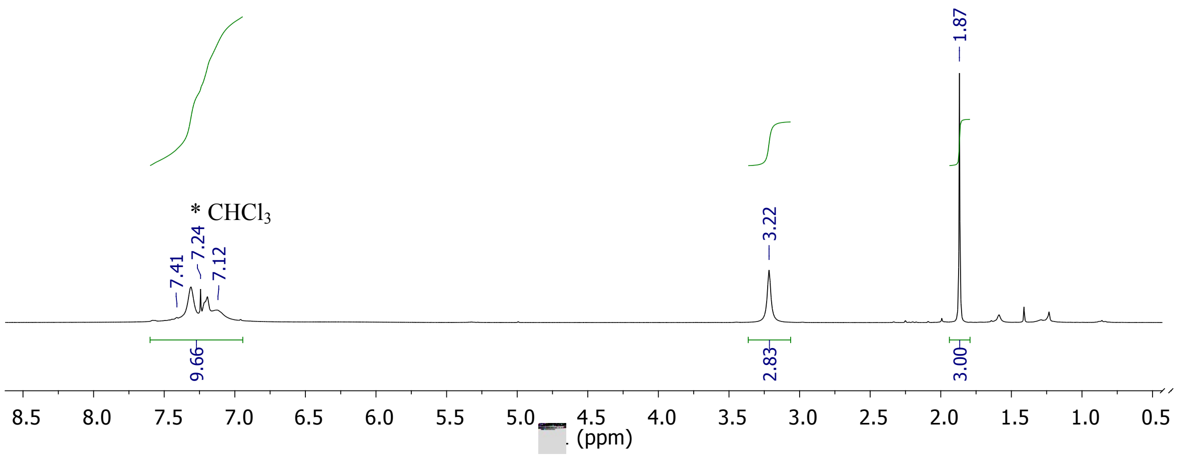


δ (ppm): 165.7, 140.9, 138.1, 129.5-128.8, 128.2, 127.9, 125.0, 119.6, 47.7, 40.6, 30.0

^{1}H NMR spectrum ($CDCl_3$, 300MHz, 25°C) of **5b**.

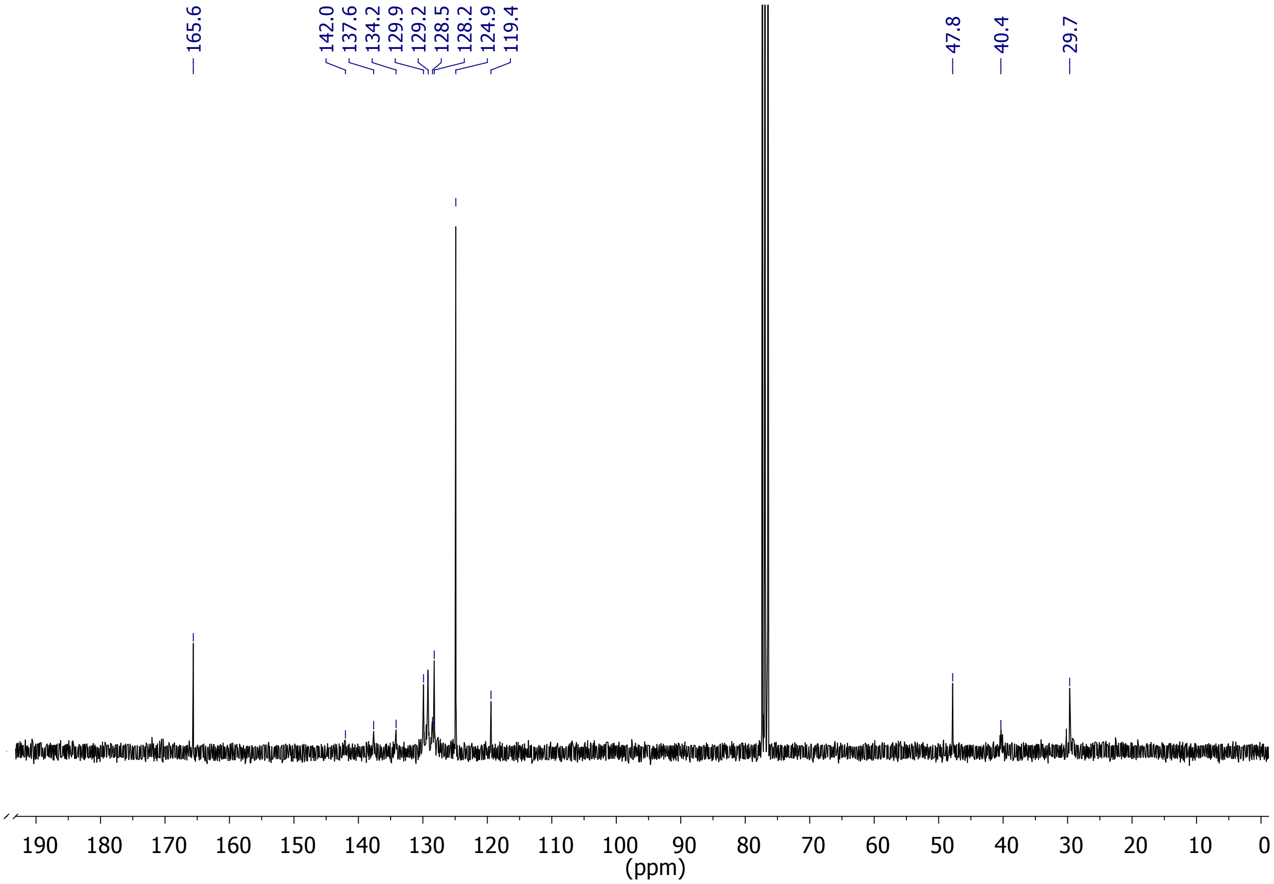


5b



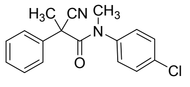
δ (ppm): 7.41-7.12 (m, 9H, Ar-*H*), 3.22 (bs, 3H, $-NCH_3$), 1.87 (s, 3H, $-CH_3$)

^{13}C NMR spectrum ($CDCl_3$, 75 MHz, 25°C) of **5b**.

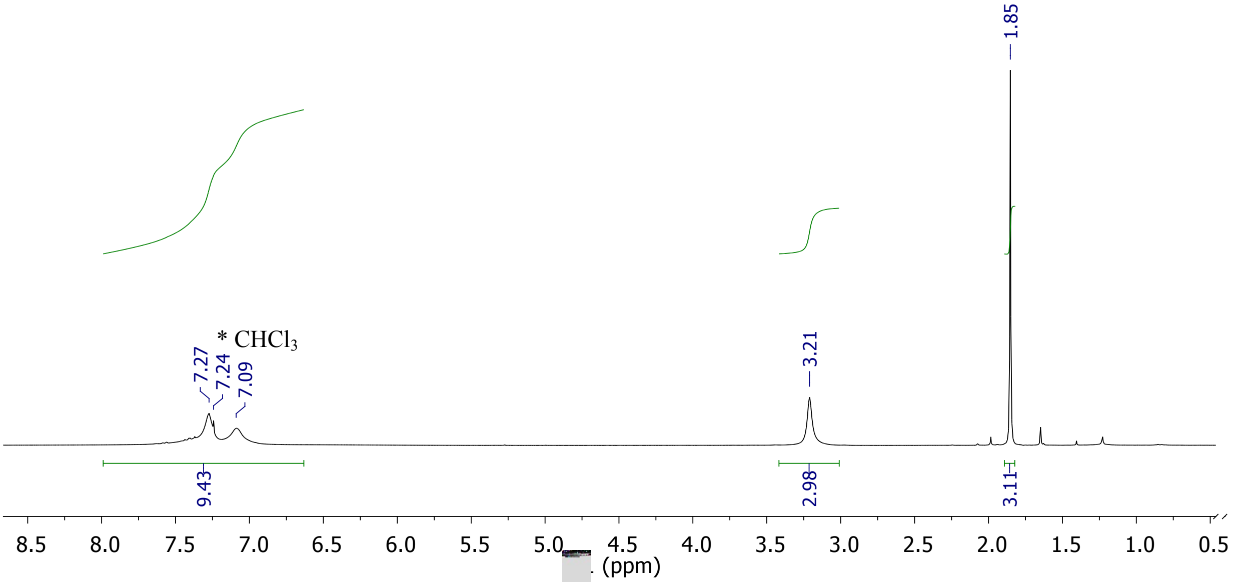


δ (ppm): 165.6, 142.0, 137.6, 134.2, 129.9-129.2, 128.5, 128.2, 124.9, 119.4, 47.8, 40.4, 29.7

^{1}H NMR spectrum (CDCl$_3$, 300MHz, 25°C) of **5c**.

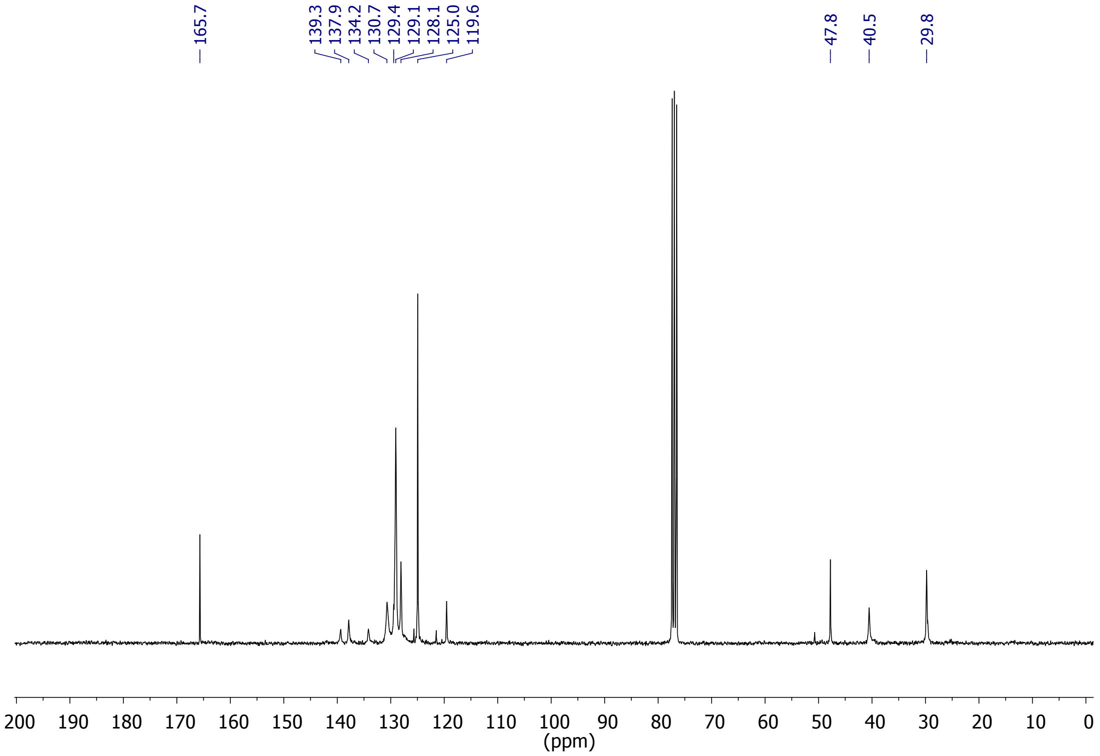


5c



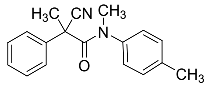
δ (ppm): 7.27-7.09 (*m*, 9H, Ar-*H*), 3.21 (bs, 3H, -NC*H*$_3$), 1.85 (s, 3H, -C*H*$_3$)

^{13}C NMR spectrum (CDCl$_3$, 75 MHz, 25°C) of **5c**.

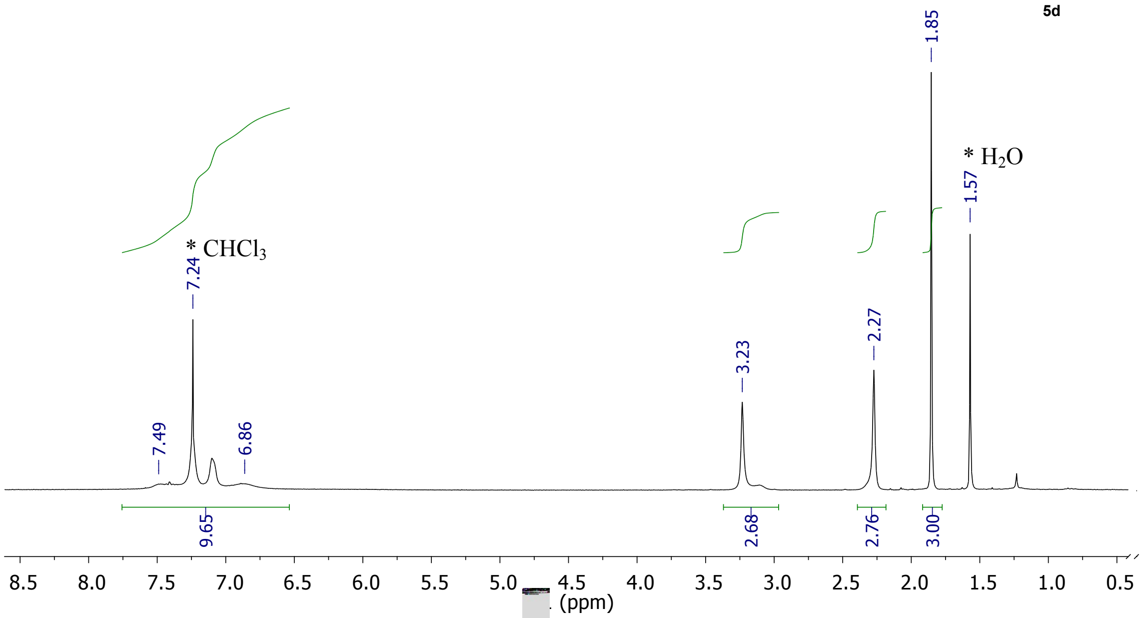


δ (ppm): 165.7, 139.3, 137.9, 134.2, 130.7, 129.4, 129.1, 128.1, 125.0, 119.6, 47.8, 40.5, 29.8

^{1}H NMR spectrum ($CDCl_3$, 300MHz, 25°C) of **5d**.

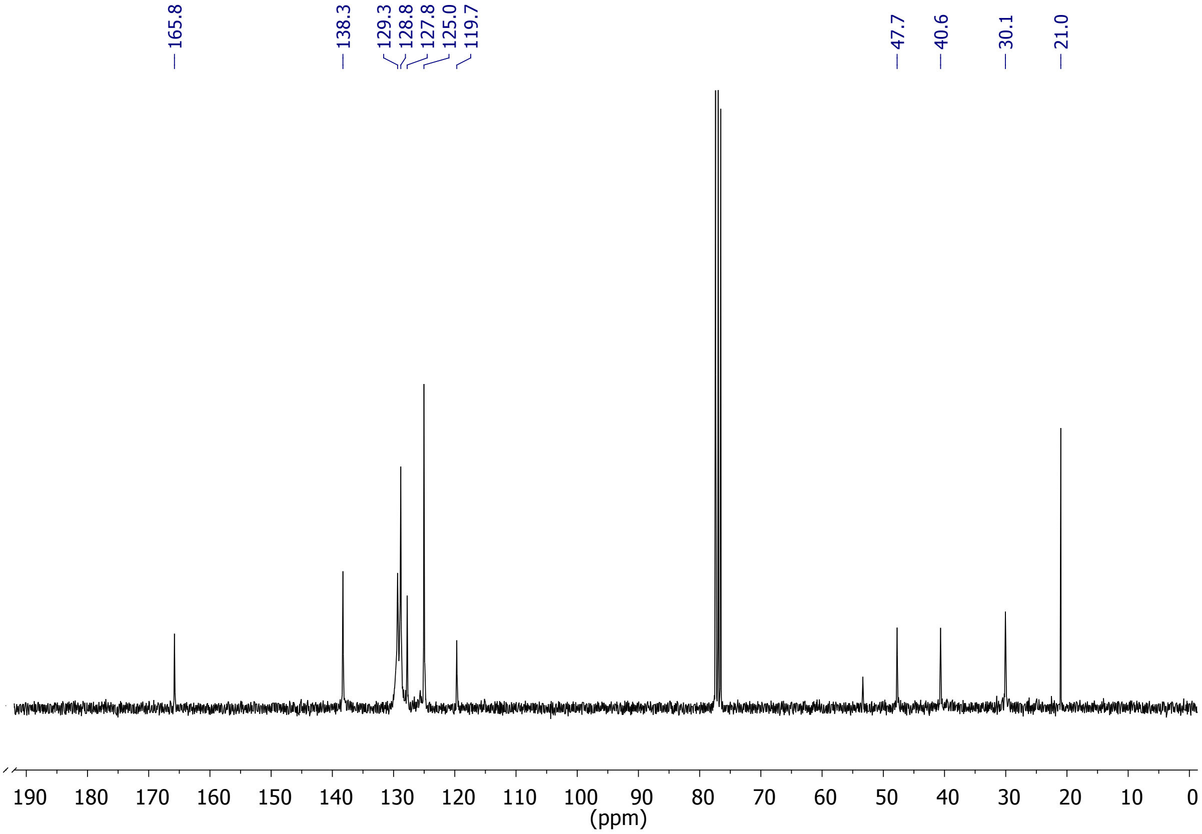


5d

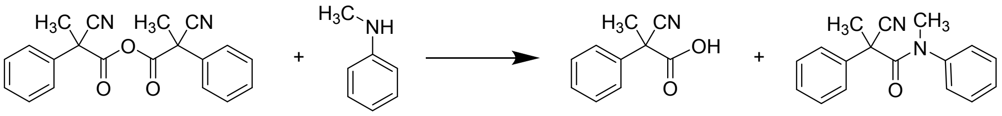


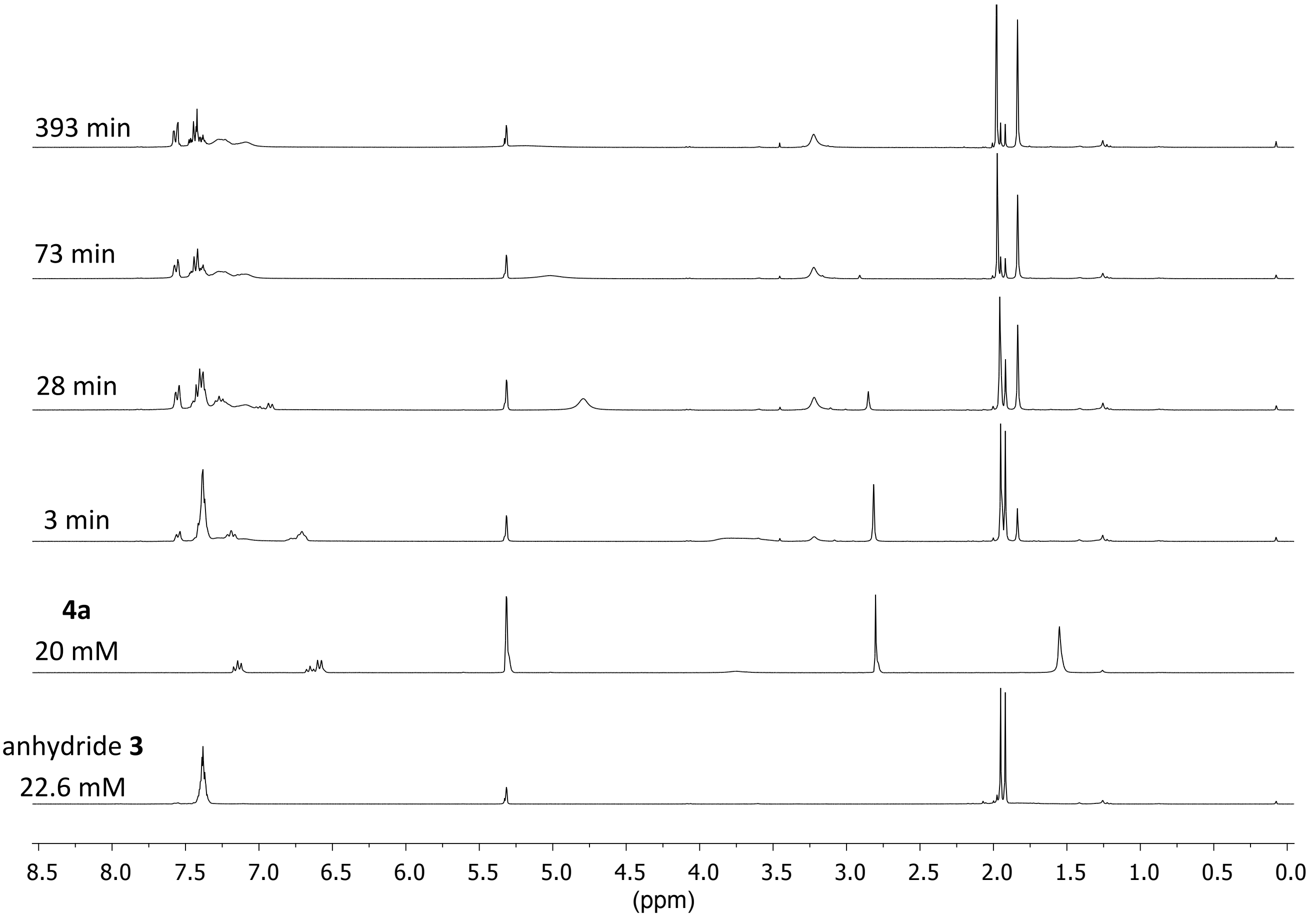
δ (ppm): 7.49-6.86 (*m*, 9H, Ar-*H*), 3.23 (s, 3H, -NC*H₃*), 2.27 (s, 3H, Ar-C*H₃*), 1.85 (s, 3H, -C*H₃*)

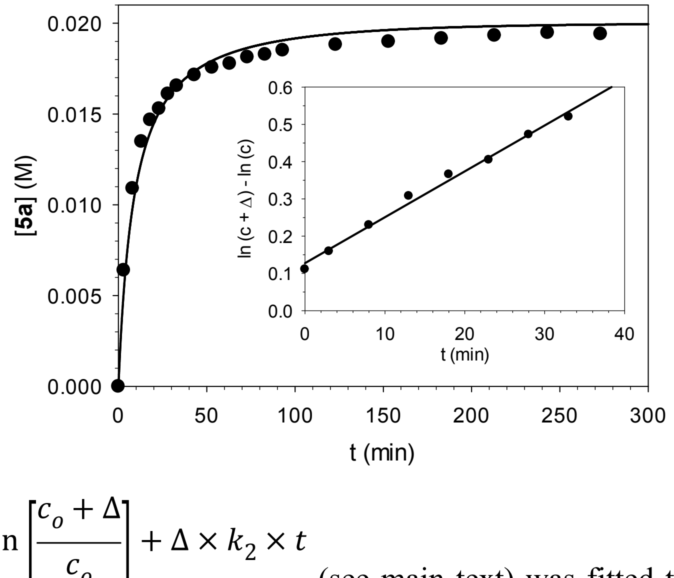
^{13}C NMR spectrum ($CDCl_3$, 75 MHz, 25°C) of **5d**.



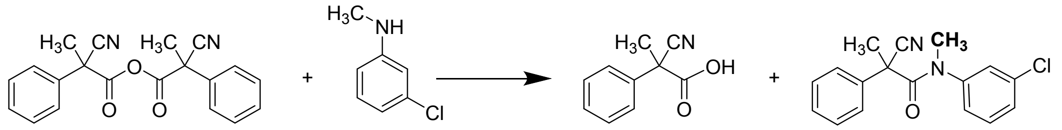
δ (ppm): 165.8, 138.3, 129.3-128.8, 127.8, 125.0, 119.7, 47.7, 40.6, 30.1, 21.0

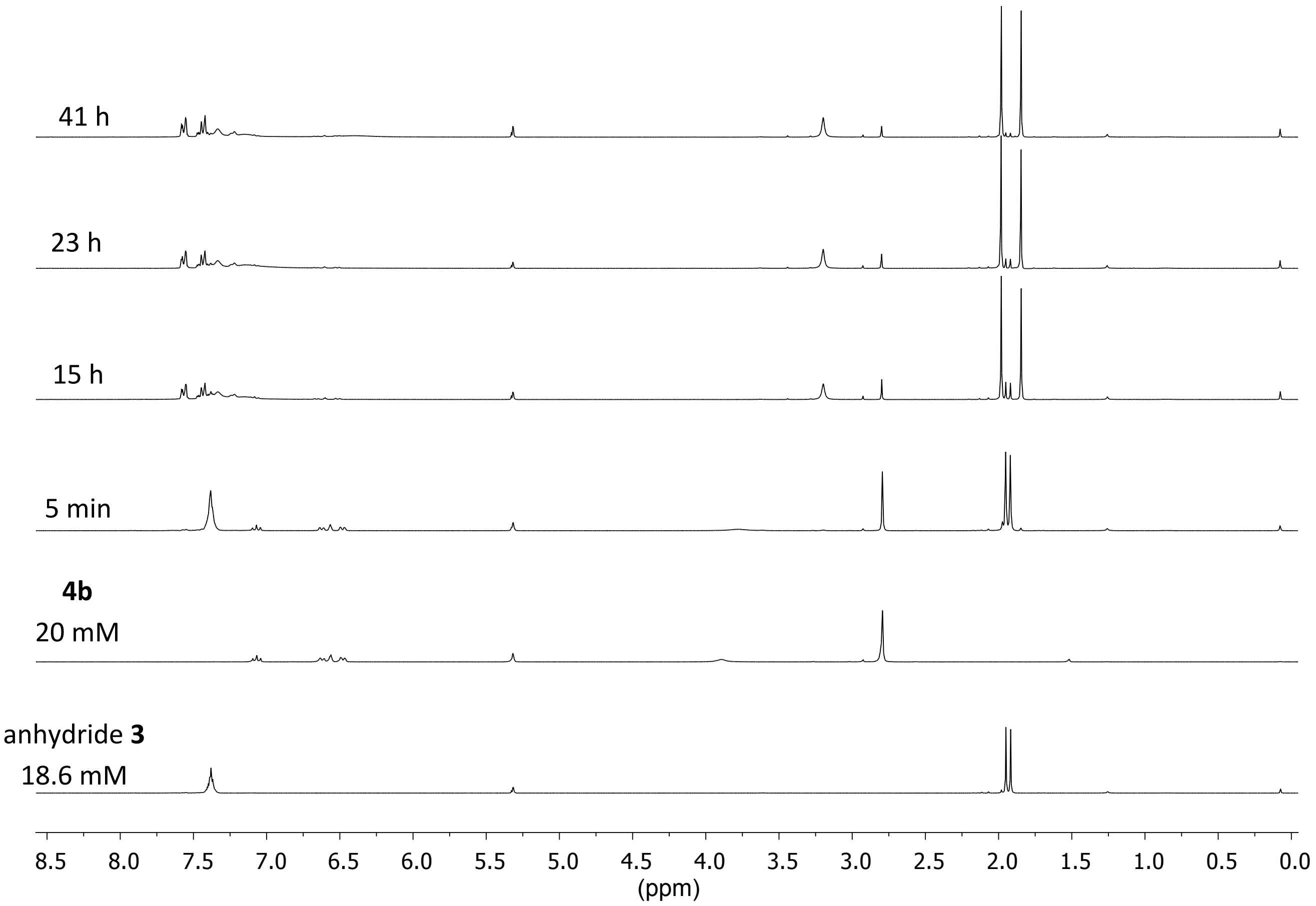


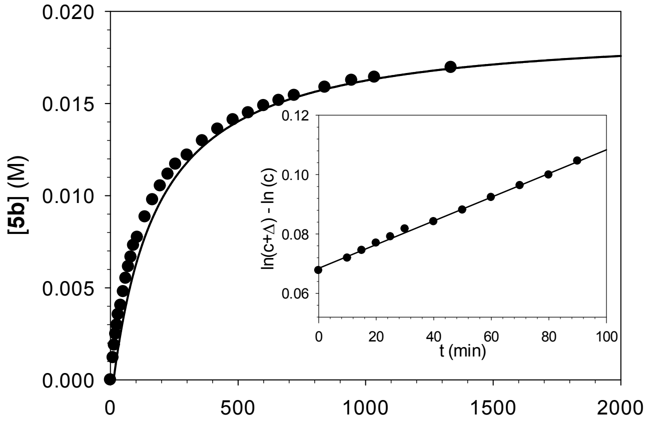




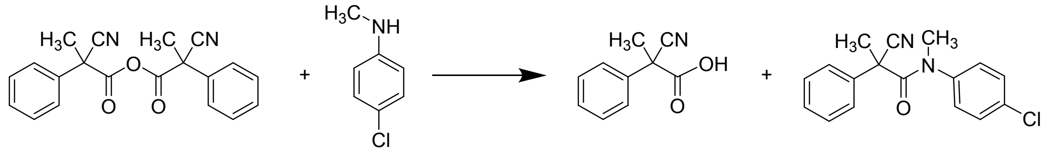
Equation $\ln\left[\frac{c+\Delta}{c}\right]=\ln\left[\frac{c_o+\Delta}{c_o}\right]+\Delta\times k_2\times t$ (see main text) was fitted to the data shown in the inset, to find the value of k_2 (slope = 0.013 min^{-1} = 0.00022 s^{-1}, R^2 = 0.992, Δ = 0.0026 M, k_2 = 0.085 $\mathrm{M}^{-1}\mathrm{s}^{-1}$). In the equation, c_o and c refer to the concentration of aniline **4a** at time 0 and t respectively and $\Delta = [anhydride]_o - [aniline]_o$ (see note S1). The second-order curve was calculated and drawn using such k_2 value.

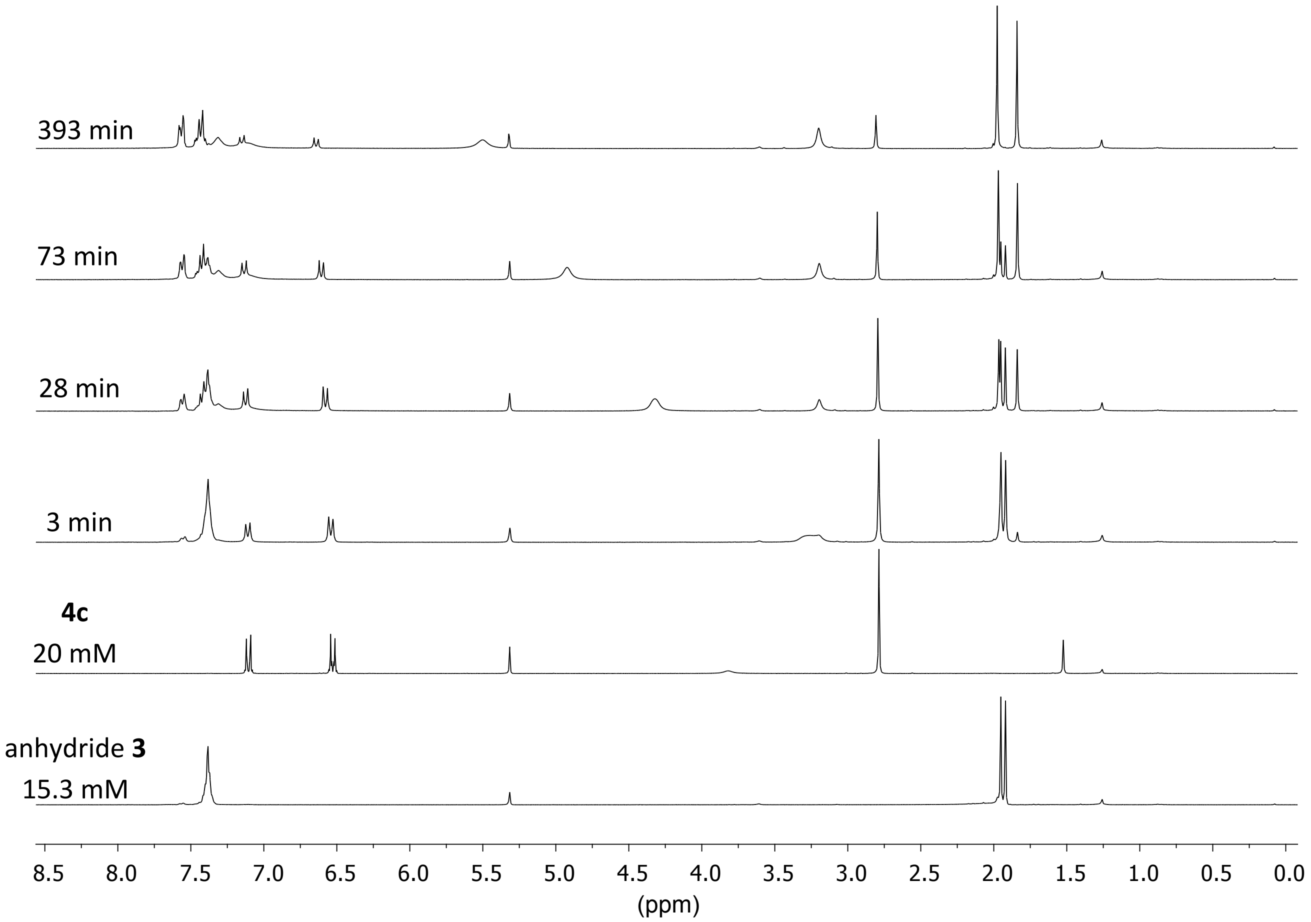


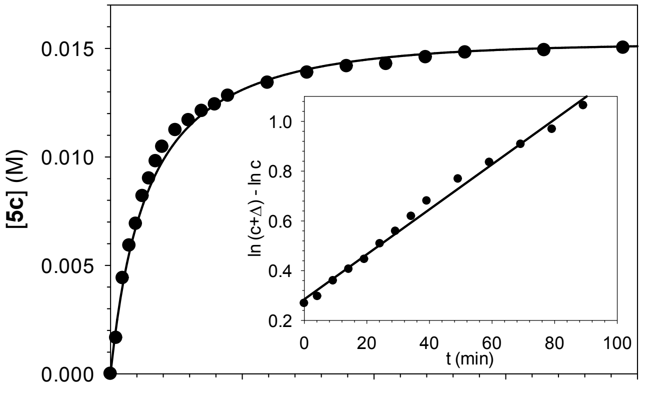




Equation $\ln\left[\frac{c+\Delta}{c}\right] = \ln\left[\frac{c_o+\Delta}{c_o}\right] + \Delta \times k_2 \times t$ (see main text) was fitted to the data shown in the inset to find the value of k_2 (slope = 0.00039 min^{-1} = 6.5×10^{-6} s^{-1}, R^2 = 0.998, Δ = 0.0014 M, k_2 = 0.0046 M^{-1}s^{-1}). In the equation, c_o and c refer to the concentration of anhydride **3** at time 0 and t respectively and $\Delta = [aniline]_o - [anhydride]_o$ (see note S1). The second-order curve was calculated and drawn using such k_2 value.

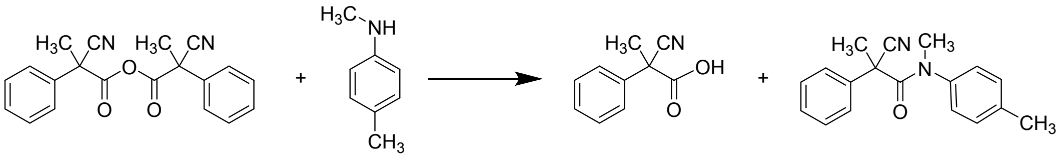


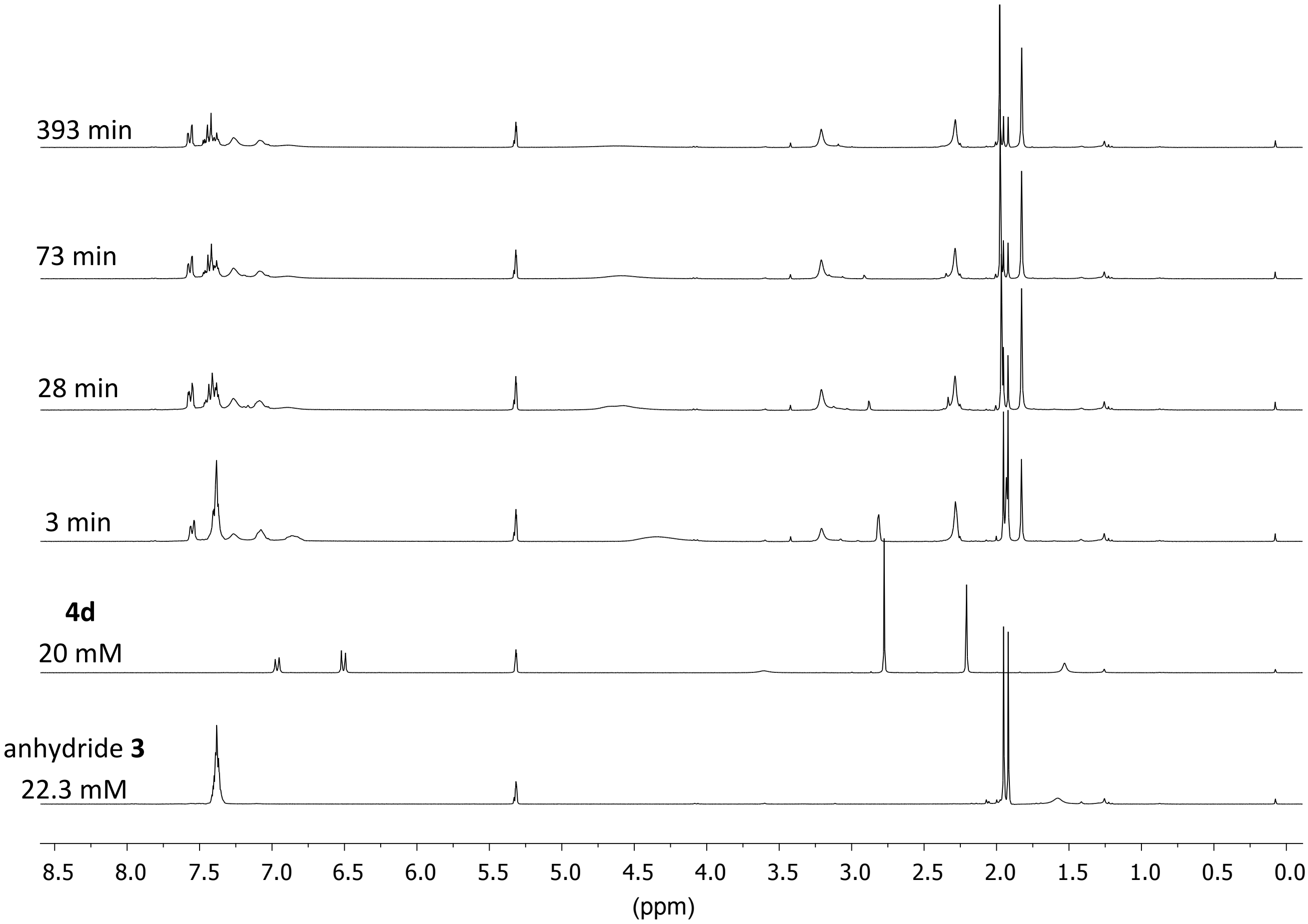


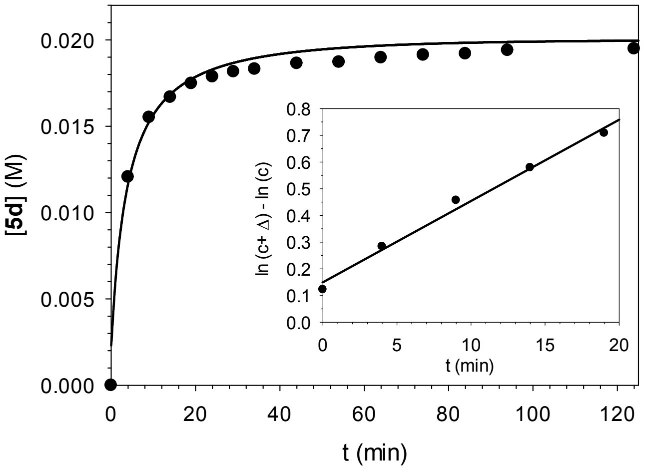


$$\ln\left[\frac{c+\Delta}{c}\right] = \ln\left[\frac{c_o+\Delta}{c_o}\right] + \Delta \times k_2 \times t$$

Equation (see main text) was fitted to the data shown in the inset to find the value of k_2 (slope 0.0090 min^{-1} = 1.5×10^{-4} s^{-1}, $R^2 = 0.992$, $\Delta = 0.0047$ M, $k_2 = 0.032$ $M^{-1}s^{-1}$). In the equation, c_o and c refer to the concentration of anhydride **3** at time 0 and t respectively and $\Delta = [aniline]_o - [anhydride]_o$ (see note S1). The second-order curve was calculated and drawn using such k_2 value.







Equation $\ln\left[\frac{c+\Delta}{c}\right] = \ln\left[\frac{c_o+\Delta}{c_o}\right] + \Delta \times k_2 \times t$ (main text) was fitted to the data shown in the inset to find the value of k_2 (slope = 0.030 min^{-1} = 5.0×10^{-4} s^{-1}, R^2 = 0.989, Δ = 0.0023 M, k_2 = 0.22 $\text{M}^{-1}\text{s}^{-1}$). In the equation, c_o and c refer to the concentration of aniline **4d** at time 0 and t respectively and

$\Delta = [anhydride]_o - [aniline]_o$ (see note S1). The second-order curve was calculated and drawn using such k_2 value.

In the following two pages ^{1}H NMR monitoring of the reaction between **C1** and excess **1** is reported in order to show that as long as excess acid **1** is present along the reaction path, the presence of the protonated catenane paired to $RCO_2^- \cdots HO_2CR$ is revealed by the insurgence of a complex pattern of signals from 0.2 to 0.8 ppm. The two experiments were already reported in the SI of J. A. Berrocal, C. Biagini, L. Mandolini and S. Di Stefano, *Angew. Chem. Int. Ed.* 2016, **55**, 6997-7001.

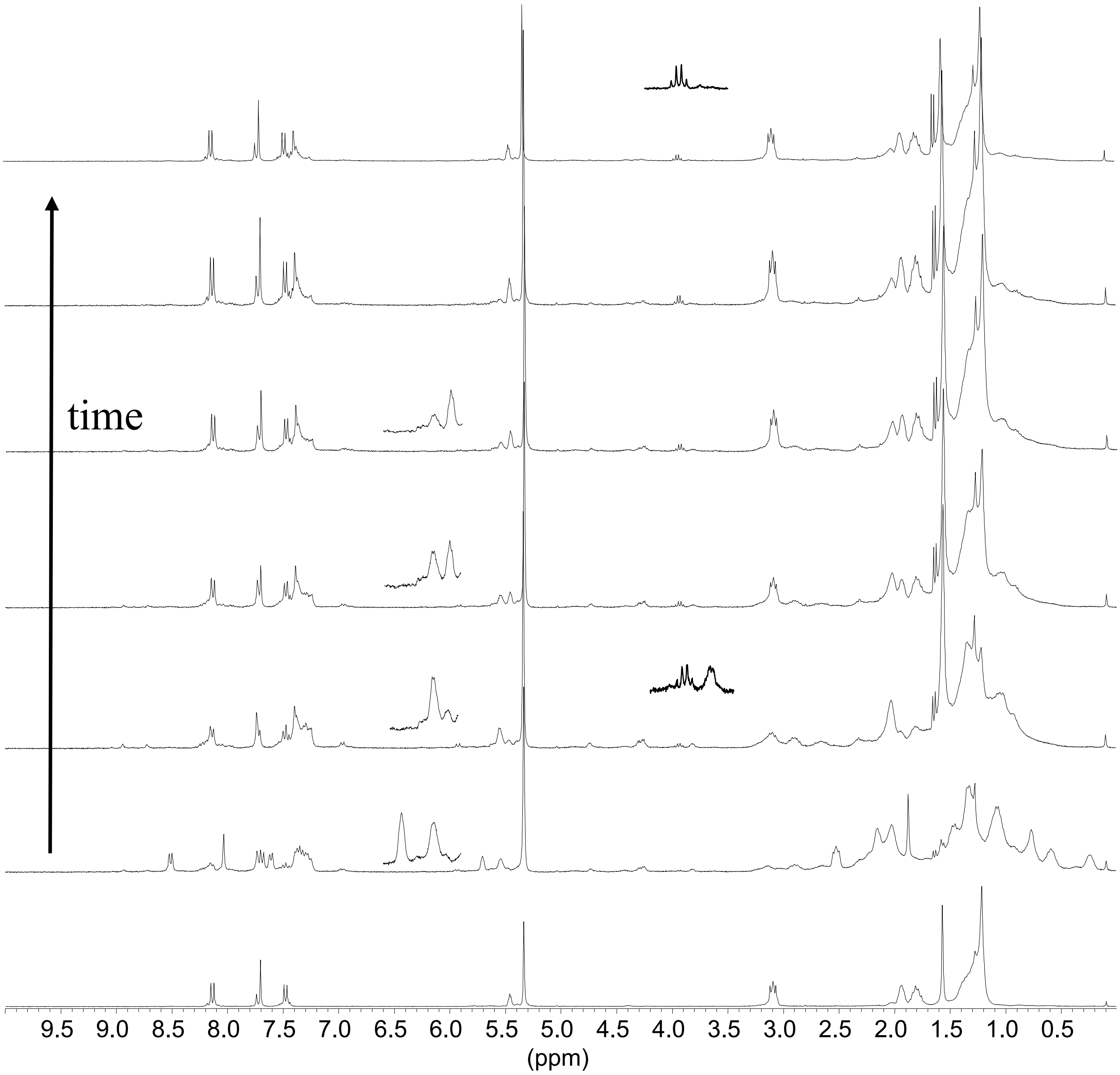


Fig. S1. ^{1}H NMR monitoring from bottom to top of the reaction between **C1** (2 mM) and fuel **1** (4 mM) in CD_2Cl_2. Data from ref S1. The bottom trace is the spectrum of **C1**. The second trace from bottom is the first spectrum taken after the addition of **1**. In the presence of excess of **1**, the structured pattern of signals from 0.2 to 0.8 ppm is indicative of the presence of the $RCO_2^- \cdots HO_2CR$ ion paired to the protonated catenane. Such signals persist as long as excess **1** is present.

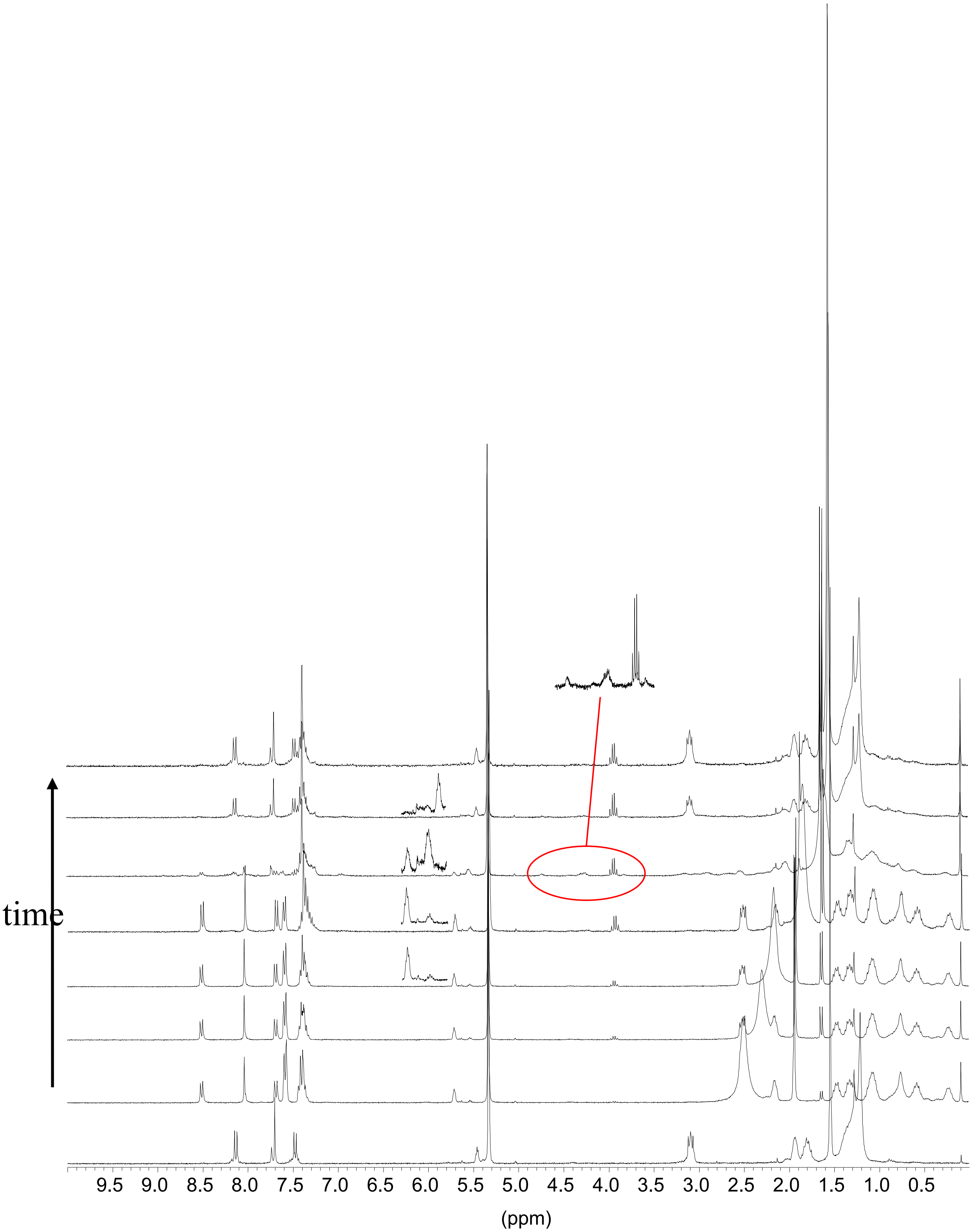


Fig. S2. ^{1}H NMR monitoring from bottom to top of the reaction between **C1** (0.5 mM) and fuel **1** (5 mM) in CD_2Cl_2. Data from ref S1. The bottom trace is the spectrum of **C1**. In the presence of excess of **1**, the structured pattern of signals from 0.2 to 0.8 ppm is indicative of the presence of the RCO_2^{-}•••HO_2CR ion paired to the protonated catenane.

GC-FID analyses were carried on an HP CP-3800 equipped with a capillary methylsilicone column (30 m x 0.25 mm x 25 µm) Chrompack CP-Sil 5 CB with the following method: (0-5)min: isotherm at T= 60°C; (5-17)min: temperature ramp of 15°C/min until T = 240°C; (17-40)min: isotherm at T = 240°C. Pressure = 12.0 psi; T(injector) = 250°C; T(FID)= 250°C; split 1/10.

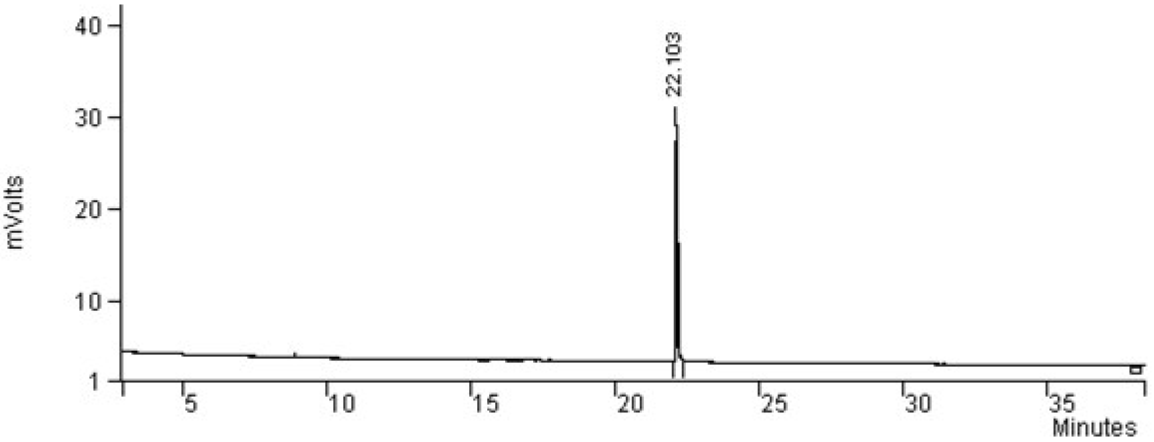


Fig. S3. Chromatogram of a pure sample of **5a**. RT = 22.103 min.

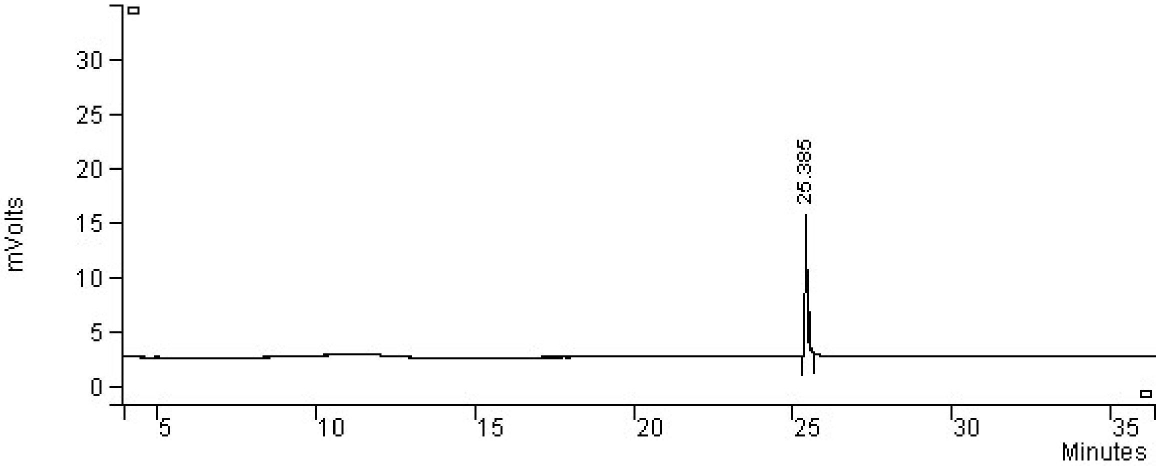


Fig. S4. Chromatogram of a pure sample of **5b**. RT = 25.385 min.

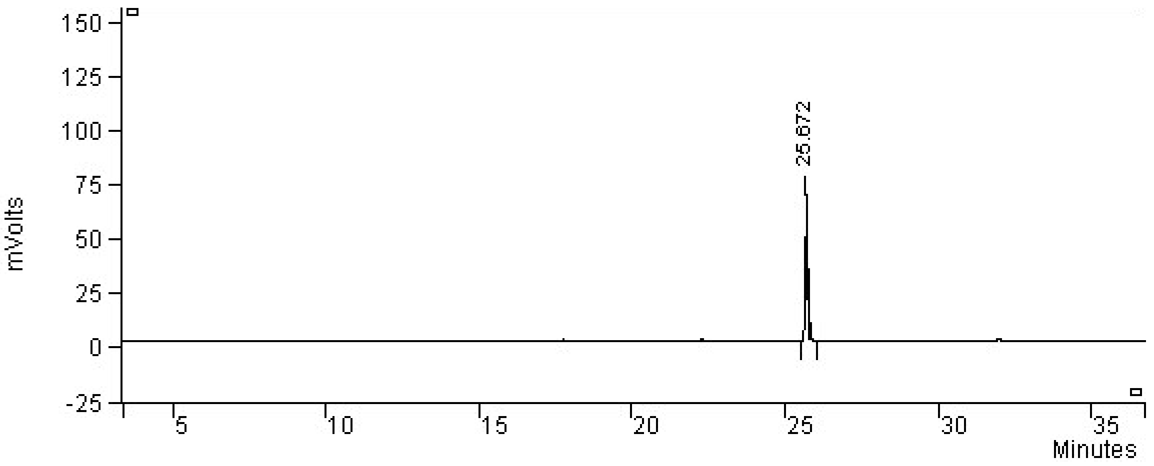


Fig. S5. Chromatogram of a pure sample of **5c**. RT = 25.672 min.

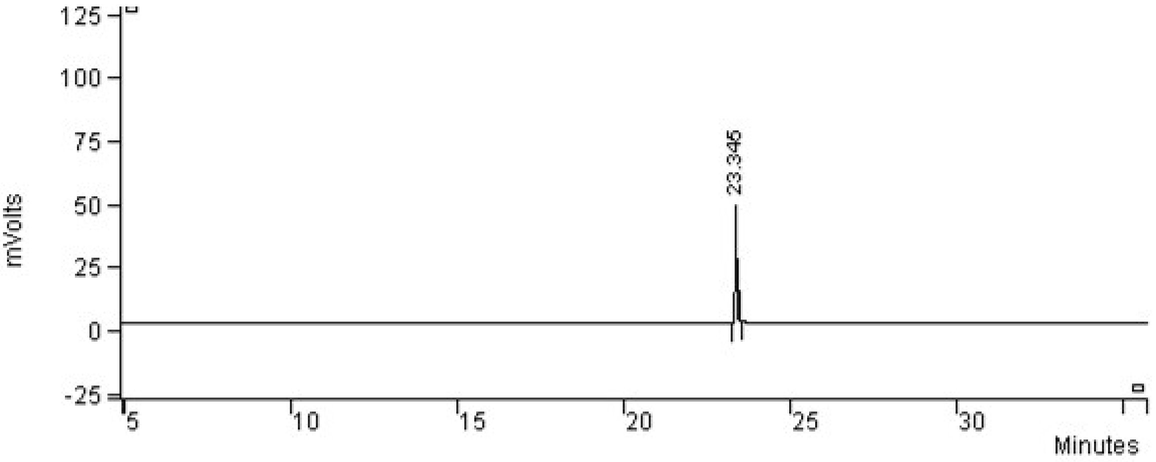


Fig. S6. Chromatogram of a pure sample of **5d**. RT = 23.345 min.

Notes and References

Note S1: c_0 and c are defined as the concentration of the limiting reagent (either anhydride **3** or anilines **4a-d**) at time 0 and t respectively; Δ is defined as the difference between the initial concentration of the excess reactant and that of the limiting reagent.

Ref S1: J. A. Berrocal, C. Biagini, L. Mandolini and S. Di Stefano, *Angew. Chem. Int. Ed.* 2016, **55**, 6997-7001.