Mechanically Triggered Carbon Monoxide Release with Turn-On Aggregation-Induced Emission

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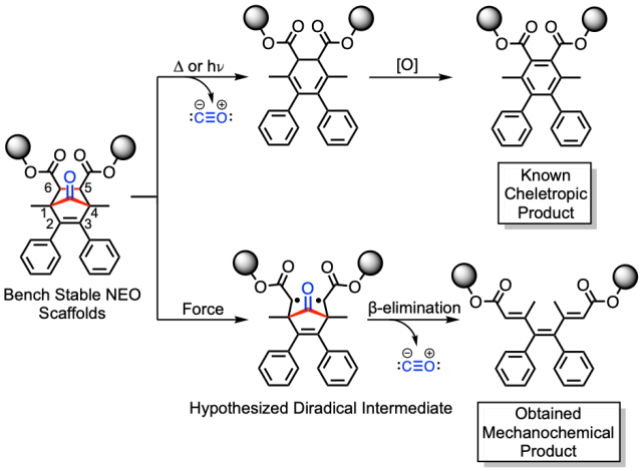
ABSTRACT: Polymers that release functional small molecules under mechanical stress potentially serve as next-generation materials for catalysis, sensing, and mechanochemical dynamic therapy. To further expand the function of mechanoresponsive materials, the discovery of chemistries capable of small molecule release are highly desirable. In this report, we detail a non-scissile bifunctional mechanophore (i.e., dual mechano-activated properties) based on a unique mechanochemical reaction involving norborn-2-en-7-one (NEO). One property is the release of carbon monoxide (CO) upon pulsed solution ultrasonication. A release efficiency of 58% is observed at high molecular weights (M_n = 158.8 kDa), equating to ~154 molecules of CO released per chain. The second property is the bright cyan emission from the macromolecular product in its aggregated state, resulting in a turn-on fluorescence readout coincident with CO release. This report not only demonstrates a unique strategy for the release of small molecule in a non-scissile way, but also guides future design of force-responsive aggregation-induced emission (AIE) luminogens.

Carbon monoxide (CO) is one of the deadliest chemicals in the world, sending nearly 50,000 people to the hospital per year in the US.[1] In contrast, it is also widely acknowledged as an important signaling molecule akin to hydrogen sulfide (H_2S) and nitric oxide (NO) with essential physiological roles.[2] Moreover, CO has been developed as a potential therapeutic agent due to its cytoprotective,[3] antibacterial,[4] anti-inflammatory,[5] and anticancer effects.[6] As early clinical trials that administer CO through inhalators reported safety concerns and difficulties in delivering precise amounts of CO to patients, the development of safer alternatives for CO delivery are needed.[7] CO-releasing molecules (CO-RMs) serve as a promising strategy to overcome these limitations, as they allow for the controlled release of CO under external stimuli such as light,[8] reactive oxygen species (ROS),[9] and enzymes.[10] Although ongoing research in organic based CO-RMs have eliminated the use of potentially toxic metals while providing improved synthetic tunability,[11] the discovery of alternative chemistries and stimuli for the release of CO will further help advance CO-associated biological studies and therapeutics.

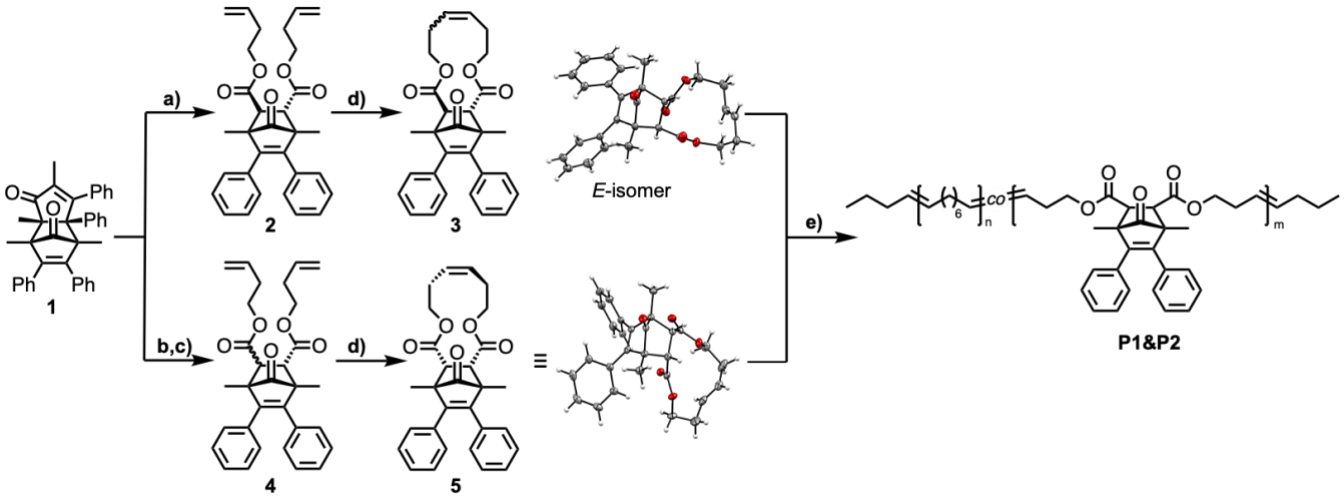
Polymer mechanochemistry is a potentially useful platform for the controlled release of small molecules for therapeutic applications,[12-14] as ultrasound has the unique ability to penetrate deep within biological tissues to achieve mechanochemical transformations noninvasively with precise spatial and temporal control.[15] On top of this, multimechanophore (MMP) materials that release large amounts of cargo in a highly tunable fashion are particularly attractive.[16-21] The mechanically triggered CO release was investigated before using diphenyl cyclopropenone motif with no success.[33] We envisioned that bench-stable norborn-2-en-7-one (NEO)[22] typically used for the cheletropic extrusion of CO,[9,23] is a putative mechanophore for the release of CO when tethered to a polymer at the 5, 6 positions (**Scheme 1**). As illustrated in **Scheme 1**, mechanical force transduced from the polymer backbone is hypothesized to selectively cleave the C_5-C_6 bond, resulting in a diradical intermediate. A subsequent β-elimination leads to the release of CO without backbone scission, thus allowing for multiple activations (i.e., multiple CO-release) to occur per chain. Herein, we report our findings of a CO-releasing mechanophore with concomitant turn-on aggregation-induced emission (AIE) via a fundamentally unique mechanochemical transformation.

Scheme 1. Known and Postulated CO Release Pathways from the Norborn-2-En-7-One (NEO) Scaffold[a]



[a]Red bonds indicate bonds broken upon applied mechanical stimuli. Blue bonds indicate the small molecule released.

Scheme 2. Synthesis of P1 and P2 Containing NEO Mechanophores



a) (3E)-di(but-3-en-1-yl) fumarate (2 equiv), reflux in toluene, 12 h (76%); b) maleic acid (2 equiv), toluene, reflux, 12 h; c) 3-buten-1-ol (3 equiv), EDC (2.2 equiv), DMAP (0.1 equiv) THF, rt, 18 h (52% over 2 steps); d) second generation Grubbs catalyst (0.05 equiv), DCM (5mM), reflux, 12h (**5** was isolated from mixture with 44% yield; **3** was obtained as E/Z mixture with 57% yield); e) Polymerizations performed in $CHCl_3$ at 60 ºC for 20 h at [NEO]:[COE] = 1:1; $[olefin]_0$/ $[G2]_0$ = 4000; $[olefin]_0$ = 1.5 M.

Ring opening metathesis polymerization (ROMP) was chosen as our polymerization strategy for the incorporation of NEO into a MMP system. As described in previous reports, mechanophores with different isomeric configurations lead to unique products and exhibit large difference in activation percentages (Φ).[24-26] Thus, we individually designed and prepared *cis*- and *trans*-NEO containing isomers (**Scheme 2**). Starting from cyclopentadienone dimer **1**, Diels-Alder cycloreversion/cycloaddition in the presence of a fumarate derivative followed by ring-closing metathesis (RCM) afforded macrocycle **3** (*E*:*Z* = 9:1) in 43% yield. Following a similar strategy but using maleic acid in place of the fumarate derivative, **5** was obtained in 23% yield over 3 steps. Surprisingly, epimerization was observed during esterification leading to a mixture of *cis*- and *trans*- product (*cis*: *trans* = 5:1). Following the RCM reaction, macrocycle **5** was isolated (confirmed by single crystal X-ray diffraction) from other diastereomers in moderate yield (44%). Subsequent ROMP of **3** or **5** with comonomer (*Z*)-cyclooctene (COE) and chain transfer agent (*Z*)-oct-4-ene produced **P1** and **P2**, respectively, with molecular weights (M_n) ranging from 6.2 to 158.8 kDa and NEO incorporations of 44.1-52.6 mol%.

The mechanochemical reactivity of **P2** (M_n = 158.8 kDa) was evaluated in dilute THF solutions using pulsed ultrasonication (1 s on/1 s off, -10 °C, 20 kHz, 8.8 W/cm^2). Over 240 min of sonication, six new resonance peaks emerged and intensified in the ^{1}H NMR of the resulting material **SP2** at δ = 6.9, 5.8, 5.6, 4.3, 2.4, and 2.2 ppm with a concomitant decrease of all corresponding NEO resonances (**Figure 1**). The microstructure of **SP2** was determined *via* 2D NMR analysis (**Figure S10-12**) and was consistent with the formation of a single isomer, as evidenced by the two sharp singlet resonances around δ = 5.9 and 2.2 ppm. While multiple isomers are conceivable based on the hypothesized diradical intermediate and its presumed isomerization pathways,[24,25,27] formation of the sole (*E*,*Z*,*E*) isomer was confirmed from ROESY and ^{13}C-gated decoupling experiments (**Figure S14-17**). From peak assignments, it was determined that 58% of the NEO mechanophores were activated after 240 min of ultrasonication based on integration in ^{1}H NMR (**Figure S7**), which is in good agreement with quantitative ^{13}C NMR analysis (**Figure S9**). Low molecular weight **P2** (M_n = 6.2 kDa) showed no activation under the same sonication conditions, indicating the mechanical nature of this transformation (**Figure S13**).

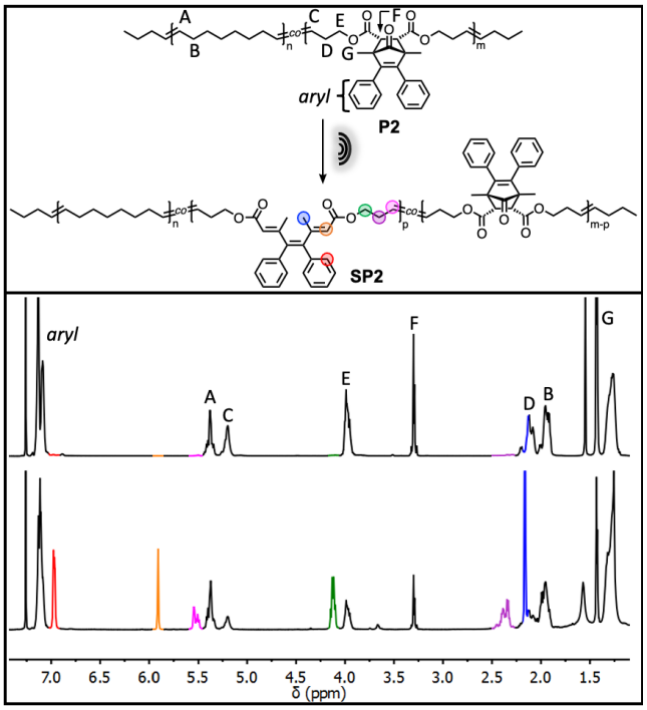


Figure 1: Stacked ^{1}H NMR transformation of **P2** to **SP2** in $CDCl_3$ upon sonication. NMR assignments of resonances in **SP2** are color coded in the lower spectrum.

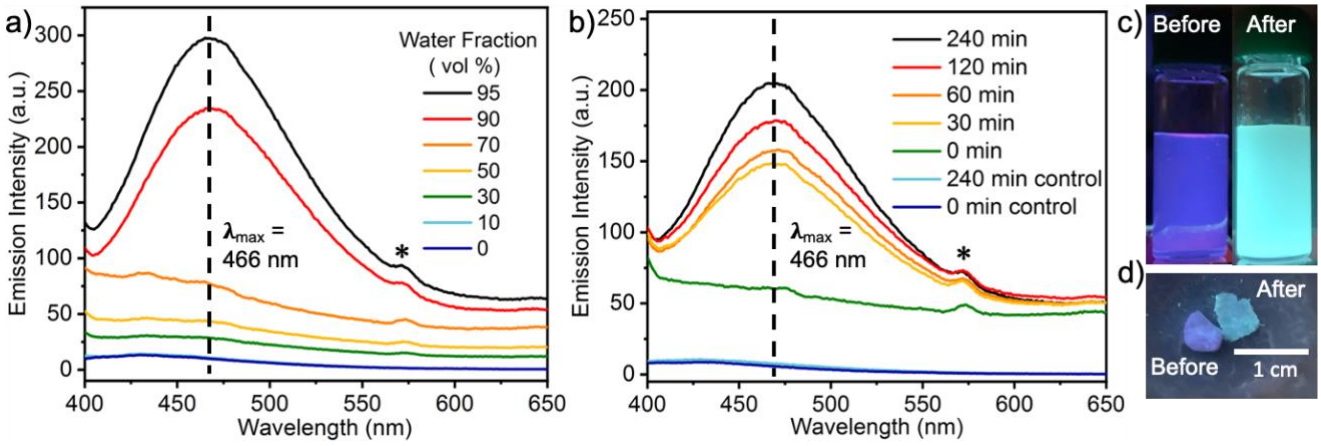


Figure 2: (a) Fluorescence spectra of **SP2** in water/THF mixtures at different water fractions, f_w, (λ_{ex} = 350 nm). (b) Fluorescence spectra change during the sonication of **P2** (M_n = 158.8 kDa, polymer suspension was prepared in THF-water mixture with f_w = 90% at 0.05 mg/mL, λ_{ex} = 350 nm). Control experiments were done using pure THF solution of **P2** at 0.05 mg/mL. (c) Photograph of polymer suspension before and after ultrasonication under 365 nm UV. Purple color was from the scattering light of the UV-lamp. (d) Solid-state mechanoactivation of **P2**. Photographs were taken under 365 nm UV irradiation. The emission peak labeled * originated from light-scattering efftects.

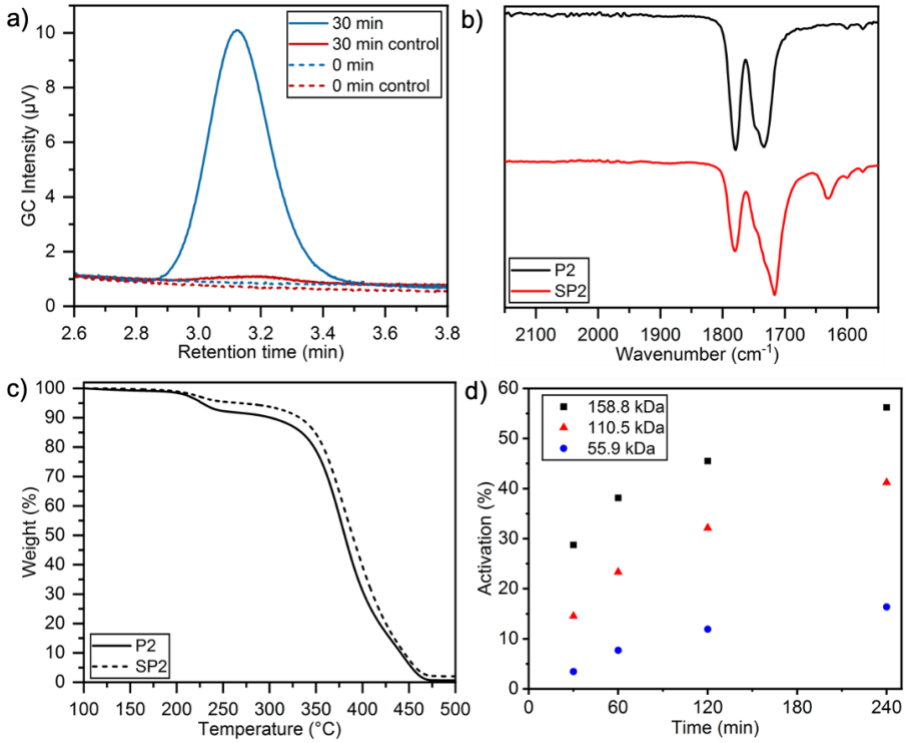


Figure 3: a) CO detection of **P2** (M_n = 129.7 kDa) and control polymer (M_n = 6.2 kDa) upon sonication using GC-TCD. b) IR spectra of **P2** and **SP2**(Φ = 58%). c) TGA analysis of **P2** and **SP2.** d) Activation% (Φ) of **P2** with different M_n upon sonication. Φ was determined from the average of two trials.

Significantly lower mechanochemical activation (Φ = 12%) was observed for **P1** of a similar molecular weight (M_n = 143.7 kDa, 46.8% NEO incorporation) after 240 min of sonication. While the main product obtained matched with that of **P2**, two smaller resonances appeared in the conjugated olefin region around δ = 5.9 ppm, indicating the generation of other stereoisomers (**Figure S18**). Due to the overall low Φ, we were unable to identify these minor products. Studies to determine the structure of these isomers as well as the origin of stereoselectivity and mechanosensitivity between the *cis*- and *trans*-NEO are ongoing.

Based on the determined structure of **SP2**, we reasoned by analogy to tetraphenylethene (TPE), one of the most common AIE luminogens, that the diphenylethene motif in the macromolecular product exhibits similar photophysical properties.[28] Different from typical aromatic luminophores that suffer from aggregation-caused quenching (ACQ) effects, AIE luminogens show dramatically enhanced emission profiles in the aggregated state.[29] To our delight, under 365

nm UV irradiation, aggregates of **SP2** showed cyan fluorescence when suspended in THF-water mixtures(**Figure 2c**). Comparatively, in pure THF solutions, minimal emissions were observed (**Figure S25**). To gain a better understanding of the photoluminescence property of **SP2**, the water fraction (f_w, vol% water) in these THF-water suspensions were varied. Rising of the baseline and tailing of the spectra were observed in the emission spectrum when increasing f_w from 0 to 70% due to the light-scattering effect of the formed nanoaggregates (**Figure 2a**).[30] Maximum emission intensities were observed at f_w = 95% with a maximum emission wavelength (λ_{max}) of 466 nm. Aliquots from sonication experiments showed a continuous increase of emission when monitored over 240 min, indicating an accumulation of activated *cis*-NEO with time (**Figure 2b**). Fluorescence of **SP2** was also measured at different concentrations and in the solid state, giving a similar λ_{max} (**Figure S22**). Solid state activation of **P2** was also achieved by compression and shear of **P2** (M_n = 120.0 kDa) in a mortar by a pestle for 2 minutes and showed a similar cyan emission upon 365nm UV irradiation (**Figure 2d**).

To confirm the release of CO from **P2**, gas chromatography (GC), infrared spectroscopy (IR) and thermal gravimetric analysis (TGA) were conducted (**Figure 3**). A GC equipped with a thermal conductivity detector (TCD) was first injected with a CO standard, as shown in **Figure S27**, and the retention time (t_R) of CO was determined to be around 3.1 min. After sonication of **P2** (M_n = 129.7 kDa) for 30 min, an aliquot of the headspace was injected and showed the appearance of a peak with t_R = 3.1 min, confirming the release of CO. Contrary, negligible CO was detected when low molecular weight **P2** (M_n = 6.2 kDa) was sonicated. Infrared (IR) spectroscopy analysis of **SP2** vs **P2** revealed a new band (1631 cm^{-1}) and a small shift of the ester carbonyl band (from 1734 to 1717 cm^{-1}) due to the generated conjugated alkenes as well as the unsaturated esters. Reduction of the bridge carbonyl stretching band (1779 cm^{-1})[22] intensity after 240 min of sonication further validated the CO release. Lastly, differences in the weight loss between **P2** and **SP2** (6.8 and 3.6 wt%, respectively) at the first onset of degradation (T_D = 206 ºC), were in relatively good agreement with predicted values (4.7 and 2.9 %, respectively) for the mass loss of CO at 43.2 mol% incorporation and Φ = 38%.

As the ability to control the amount of CO released in a tunable fashion is an important characteristic of CO-RMs, various molecular weight **P2** were synthesized and the kinetics of activation were investigated. Sonication experiments were monitored by ^{1}H NMR and size-exclusion chromatography (SEC) analysis (**Table S3**) and showed an increase of Φ from 16% to 58% over the molecular weight range after 240 min (**Figure 3d**). This molecular weight dependent activation profile is in good agreement with other MMPs[31,32] and provides further proof of the mechanical nature of the mechanophore. SEC analysis showed a continuous decrease in molecular weight during sonication, indicating the competition between NEO activation and random backbone scission (**Figure S30**). For **P2** of 55.9 kDa, 110.5 kDa, and 158.8 kDa, it was determined that about 19, 83, and 154 CO molecules are released on average per chain, respectively (See SI for more details).

In summary, we have demonstrated a new non-scissile MMP system that releases CO with turn-on AIE. A triene motif was generated upon mechanical force from a NEO scaffold as a distinctive product that has never been accessed before under other stimuli (heat, light, etc.). In addition, the NEO mechanophore represents the first example of mechanically induced AIE *via* covalent polymer mechanochemistry. We foresee that the NEO mechanophore will not only open exciting biological and biomedical opportunities, but also guide the design of novel force-responsive AIE luminogens for damage detection and bioimaging applications.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.
Synthesis and characterization of compounds (PDF)
Crystallographic data for compound **3** (*E*-isomer) and **5** (CIF)

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Notes

The authors declare no competing financial interest.

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