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Spirostrain-Accelerated Chemiexcitation of Dioxetanes Yields Unprecedented Detection Sensitivity in Chemiluminescence Bioassays

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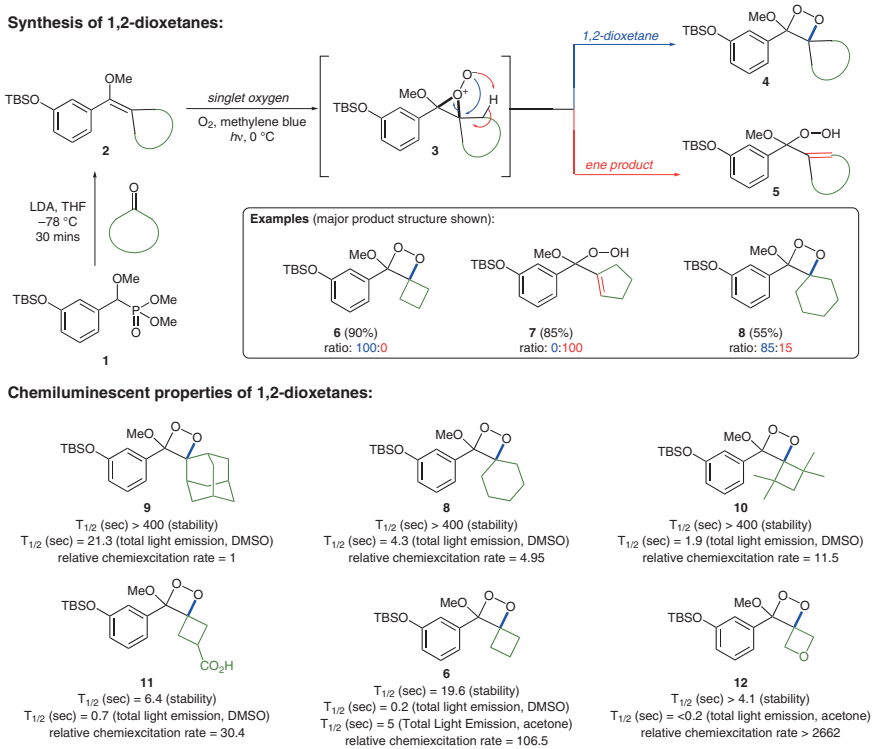
Spiro-Substituted Dioxetane-Based Chemical Probes with Enhanced Detection and Sensitivity Limits



Significance: The design of chemical probes to track biological processes in cellular assays is a critical research area with the speed of detection and sensitivity of such molecules is key to accurately tracking enzymatic activity. Chemiluminescence is a phenomenon that involves light produced from a chemical reaction and can be sub-divided into 'stable glow-type' that produces a stable long light emission profile with low intensity and 'fast flash-type', which occurs rapidly with a high intensity signal. The current report describes the synthesis and evaluation of a series of substituted phenoxy-1,2-dioxetanes as chemiluminescent luminophores with the incorporation of a spiro-cyclobutyl substituent shown to significantly accelerate chemiexcitation with an exponential increase in both the detection time and sensitivity.

Comment: The chemiexcitation of these probes occurs through electron transfer from the phenoxide to the dioxetane leading to both O–O and C–C cleavage that generates the excited benzoate that emits visible light. The adamantly-phenoxy-1,2-dioxetane (**9**) is established as the benchmark for chemiluminescent cell imaging with the EWG incorporated at the *ortho* position to prevent water-mediated quenching and increase light-emission intensity (D. Shabat and co-workers *ACS Cent. Sci.* **2017**, *3*, 349). Spiro-strain-release was shown to accelerate the chemiexcitation rate (see **6**, **12**) with the cyclobutyl-based motif (**6**) utilized in a cellular assay for the detection of the enzyme β-gal and shown to be 125-fold more sensitive than **9**.

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Category

Synthesis of Heterocycles

Key words

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oxetanes
cyclobutanes
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