

# Use of Standard Addition to Quantify In Situ FTIR Reaction Data

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Cite This: *J. Org. Chem.* 2021, 86, 2012–2016



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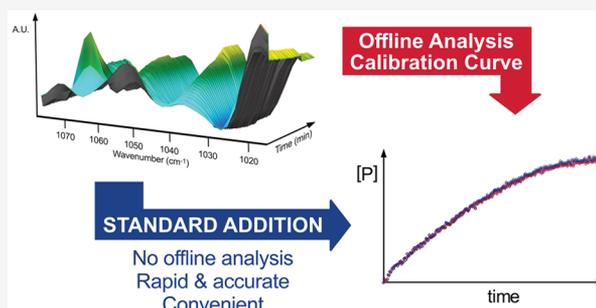


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**ABSTRACT:** FTIR spectroscopy is a common in situ reaction monitoring technique used in modern academic and industrial environments. The FTIR signals collected during the course of a reaction are proportional to the concentration of the reaction components but not intrinsically quantitative. To make FTIR data quantitative, precalibration or offline analyses of reaction samples are required, which diminishes the unique benefits of in situ reaction monitoring techniques. Herein, we report the use of standard addition as a convenient method to obtain quantitative FTIR data.



Spectroscopic in situ reaction monitoring techniques have several advantages when compared with offline analyses of reaction samples: sampling and quenching of the reaction are not required, unstable intermediates can be quantified, dozens of data points can be acquired, and the results can be obtained almost instantaneously.<sup>1</sup> In situ FTIR has these advantages, but, in order to provide quantitative concentration profiles, it has to be calibrated.<sup>2</sup> Calibration curves can be used, but they are tedious to generate and can be inaccurate if the matrices of the calibration solutions and the reaction mixture differ significantly. Calibration curves are best used to monitor the same reaction several times, for example, in process control.<sup>3</sup> However, calibration curves are not practical when monitoring diverse reactions, reactions run under different conditions (i.e., in mechanistic studies), or reactions with complicated matrices. In these cases, it is more convenient to estimate the background and the sensitivity in the linear dynamic range for each reaction using two points with a known concentration of the analyte. Usually, the concentrations of all of the reaction components at the beginning of the reaction are known, but not at the end, as the reaction might not proceed to completion or could generate side products. Consequently, the reaction must be sampled at least once, often at the end of the reaction, to quantify the concentration of the analyte using an offline technique.<sup>4</sup> The need for sampling and offline analysis lessens the intrinsic benefits of in situ FTIR spectroscopy. Alternatively, standard addition can determine the concentration of an analyte without sampling and offline analysis. Standard addition has been successfully used to determine by ATR-FTIR the concentration of analytes in static samples.<sup>5</sup> Herein, we report the use of standard additions to quantify the concentration of reaction components without the need for sampling and offline analyses.

We selected the organocatalytic  $\alpha$ -selenylation of aldehydes to demonstrate the effectiveness of a standard addition to in

situ calibrate FTIR data. The selenylation reaction was described by Melchiorre and Córdova,<sup>6,7</sup> and its mechanism was studied by Blackmond.<sup>8</sup> We reacted *N*-(phenylseleno)phthalimide (**2**) with an excess of isovaleraldehyde (**1**) and AcOH in a vial fitted with the FTIR probe and initiated the reaction by adding the Jørgensen–Hayashi type catalyst **3** (Figure 1). To show the versatility of the method, we carried out two different standard addition calibrations: with a reactant, the *N*-(phenylseleno)phthalimide (**2**), and with a product, the phthalimide (**5**).

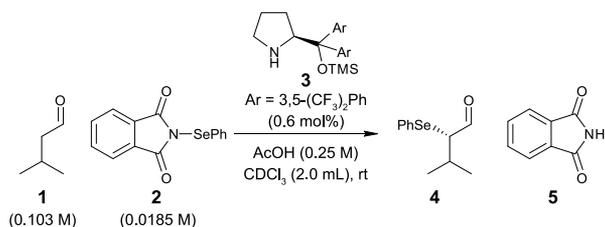
When monitoring the reaction by following the signal corresponding to a reactant, we started collecting spectra before adding the reactant in two portions, which constitute the standard additions. We collected consecutive FTIR spectra from before the addition of the stock solution of *N*-(phenylseleno)phthalimide (**2**) to the reaction mixture until the end of the reaction (Figure 1a). We applied the second derivative<sup>9</sup> to the spectra to increase the resolution of the peaks (Figure 1b). Finally, we measured the signal height relative to zero at 862 cm<sup>-1</sup>, which corresponds to the *N*-(phenylseleno)phthalimide (**2**) (Figure 1c) and adjusted the signal height before the addition of *N*-(phenylseleno)phthalimide (**2**) to 0 AU (Figure 1d).

The reaction profile in Figure 1d is a relative reaction profile (with arbitrary units on the Y-axis) that must be calibrated to become a reaction profile in absolute terms (with concen-

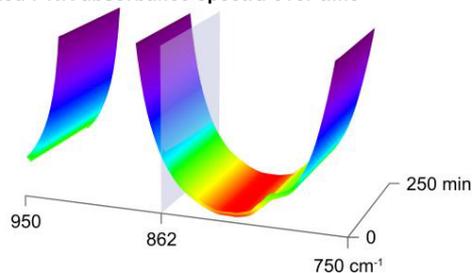
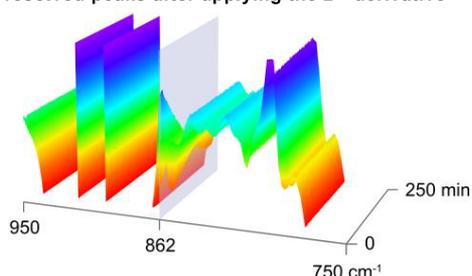
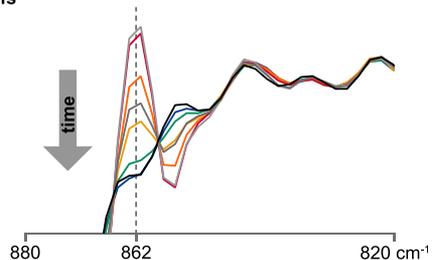
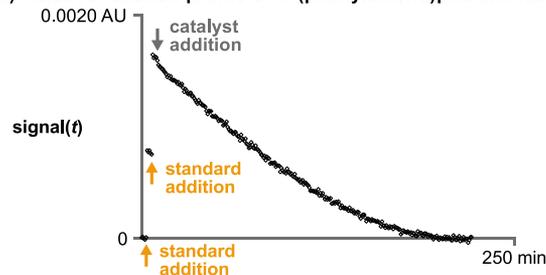
Received: November 10, 2020

Published: December 24, 2020





a) Stacked FTIR absorbance spectra over time

b) More resolved peaks after applying the 2<sup>nd</sup> derivativec) *N*-(Phenylseleno)phthalimide peak at 862 cm<sup>-1</sup> selected for analysisd) Relative reaction profile of *N*-(phenylseleno)phthalimide

**Figure 1.** Treatment of FTIR data to extract a relative temporal reaction profile of disappearance of *N*-(phenylseleno)phthalimide (2).

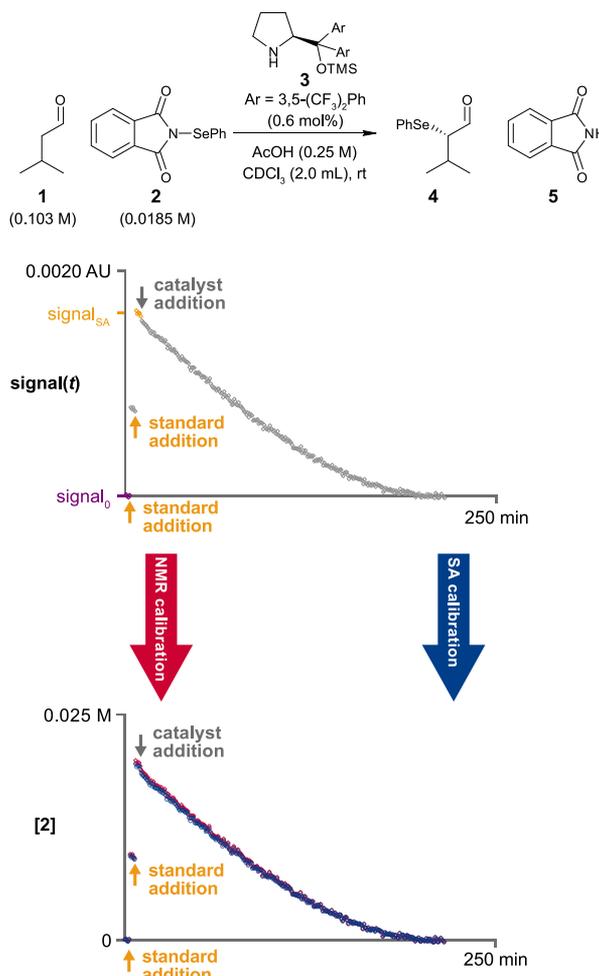
tration units on the *Y*-axis). We calibrated the entire reaction profile using eq 1:<sup>10</sup>

$$[2]_t = k \times \text{signal}(t); \quad k = \frac{n_{SA}}{\text{signal}_{SA} \times V_{SA}} \quad (1)$$

where *k* represents the inverse of the response factor, *signal*(*t*) represents the signal at time *t* (AU), *n*<sub>SA</sub> represents the amount of the standard added (mmol), *signal*<sub>SA</sub> represents the signal

after the standard addition (AU), and *V*<sub>SA</sub> represents the volume after the standard addition (mL).

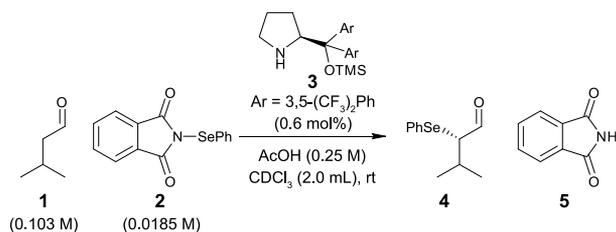
We also calibrated the data using a traditional offline analysis technique, quantitative NMR of an end-point sample, for comparison. Gratifyingly, the quantitative FTIR data we generated by standard addition calibration of the reactant 2 (blue circles, Figure 2) and offline quantitative NMR calibration (red diamonds, Figure 2) overlaid perfectly.



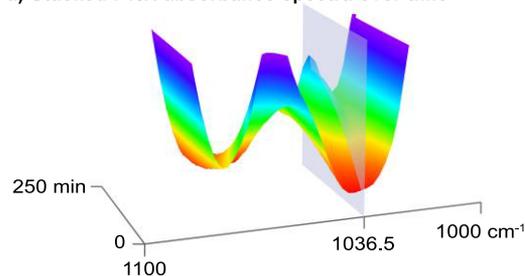
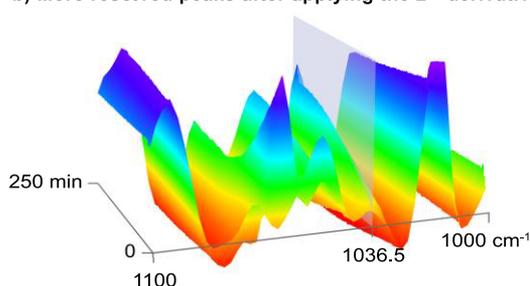
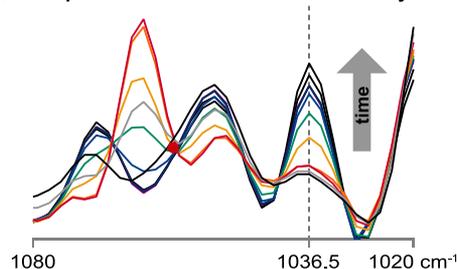
**Figure 2.** FTIR data calibrated by standard addition of the reagent 2 (blue) perfectly overlays with the data calibrated by NMR offline analysis (red).

In some reactions, it may be necessary to follow the progress of the reaction by monitoring the signal of a product. In these cases, the calibration by standard addition of the product can be performed at the end of the reaction. As an example, we calibrated the reaction profile by the addition of the product 5, phthalimide, in two portions at the end of the reaction. We collected consecutive FTIR spectra from the beginning of the reaction (Figure 3a), and we applied the second derivative to increase the resolution of the peaks (Figure 3b). We measured the signal height of the peak at 1036 cm<sup>-1</sup> (Figure 3c) relative to a single point baseline<sup>11</sup> (1058 cm<sup>-1</sup>, red dot) and adjusted the signal height before the catalyst addition to 0 AU (Figure 3d).

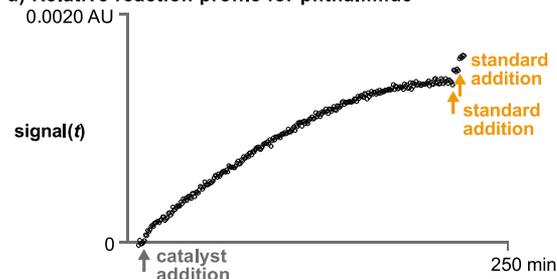
As in the previous example, the relative reaction profile (with arbitrary units on the *Y*-axis) in Figure 3d had to be calibrated to become a reaction profile in absolute terms (with



a) Stacked FTIR absorbance spectra over time

b) More resolved peaks after applying the 2<sup>nd</sup> derivativec) Phthalimide peak at 1036 cm<sup>-1</sup> selected for analysis

d) Relative reaction profile for phthalimide



**Figure 3.** Treatment of FTIR data to extract a relative temporal reaction profile of appearance of phthalimide (5).

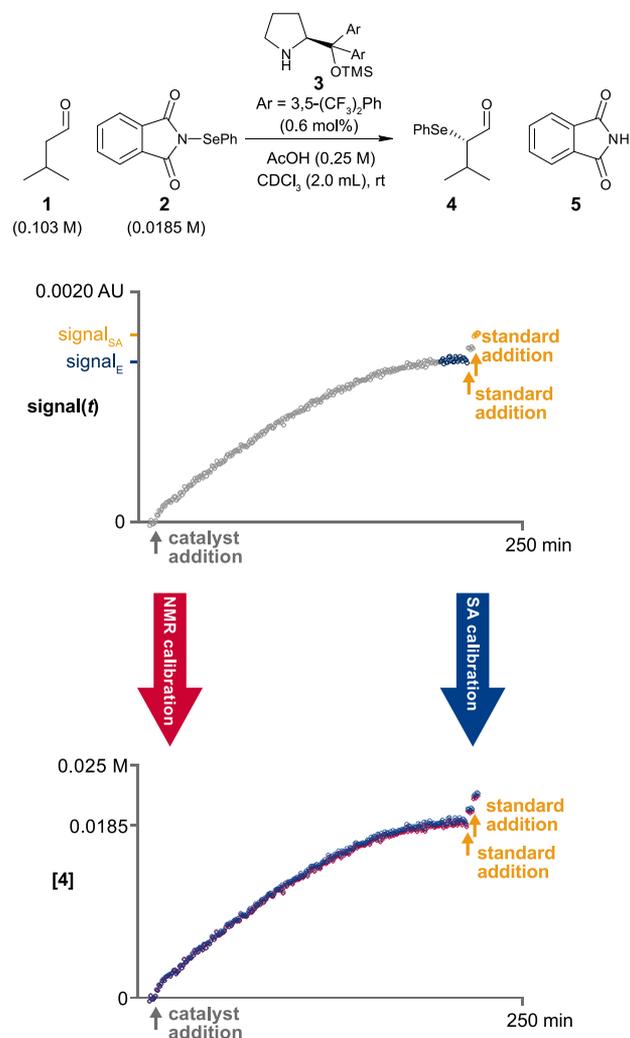
concentration units in the Y-axis). To calibrate the entire profile using the standard addition of one of the products of the reaction, we used eq 2.<sup>10</sup>

$$[4]_t = k \times \text{signal}(t);$$

$$k = \frac{n_{\text{SA}}}{\text{signal}_{\text{SA}} \times V_{\text{SA}} - \text{signal}_{\text{E}} \times V_{\text{E}}} \quad (2)$$

*k* represents the inverse of the response factor, signal(*t*) represents the signal at time *t* (AU), *n*<sub>SA</sub> represents the amount of the standard added (mmol), signal<sub>SA</sub> represents the signal after the standard addition (AU), *V*<sub>SA</sub> represents the volume after the standard addition (mL), signal<sub>E</sub> represents the signal at the end of the reaction (AU), and *V*<sub>E</sub> represents the volume of the reaction mixture (mL)

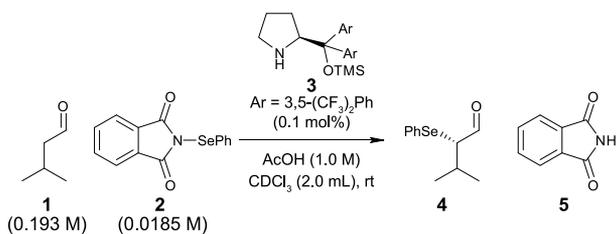
As in the first example, we also calibrated the data using quantitative NMR of an end-point sample, for comparison. The temporal concentration profiles generated using the calibration with standard addition of the product 5 (blue circles, Figure 4) and offline quantitative NMR calibration (red diamonds, Figure 4) overlaid perfectly.



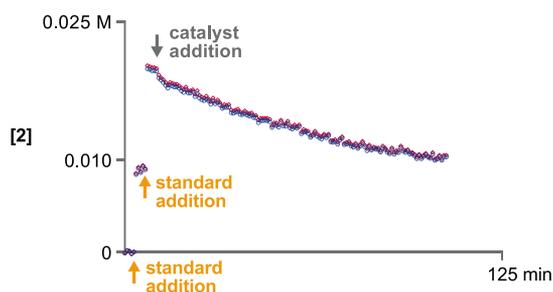
**Figure 4.** FTIR data calibrated by standard addition of the product 5 (blue) overlays with the data calibrated by NMR offline analysis (red).

Calibrating FTIR data is more important when the reaction does not arrive at completion because the variation of the signal during the reaction cannot be assumed to be 100%. To further test the calibration method by standard addition of reactants and products, we monitored the reaction carried out with a much smaller catalyst loading. This reaction did not achieve full conversion and better showed the need for a calibration method to obtain concentration profiles. In this reaction, the temporal reaction profiles obtained with an offline

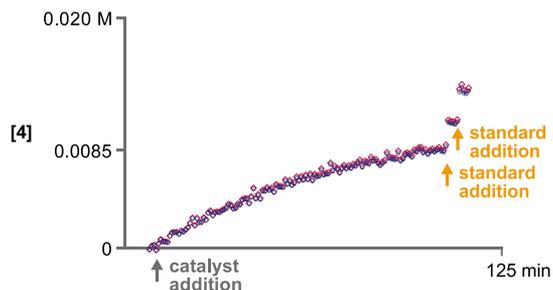
quantitative NMR calibration (red diamonds, Figure 5) matched the profiles obtained by calibration by standard addition of a reactant (blue circles, Figure 5a) and by standard addition of a product (blue circles, Figure 5b).



a) Calibration by standard addition of a reactant (2)



b) Calibration by standard addition of a product (5)



**Figure 5.** Reaction profiles obtained with calibration by standard addition of the reactant 2 (blue), by standard addition of product 5 (blue), and by offline quantitative NMR (red) match.

We have described the use of the standard addition method to calibrate in situ FTIR data. The method is rapid, accurate, and adequate for complicated matrices and does not require calibration curves or sampling and offline analyses. This more convenient method to obtain temporal concentration profiles from in situ FTIR data should facilitate the mechanistic study of a variety of reactions. Other reaction monitoring techniques, such as in situ Raman and UV–vis spectroscopy, could also benefit from the use of this method. The application of orthogonal techniques, such as verifying analyte concentration by HPLC or qNMR, is still good practice and is required during the initial investigations of a new reaction to ensure the chosen signals adequately represent the reaction of interest.

## EXPERIMENTAL SECTION

**General Experimental.** Unless otherwise specified, reagents and solvents were used as purchased from Merck, Alfa-Aesar, and Fisher Scientific.  $\text{CDCl}_3$  was dried over 4 Å activated molecular sieves and stored under nitrogen prior to use. The amine catalyst, (*S*)- $\alpha,\alpha$ -bis[3,5-bis(trifluoromethyl)phenyl]-2-pyrrolidinemethanol trimethylsilyl ether, was purified by flash column chromatography (100%  $\text{CH}_2\text{Cl}_2$ ) to remove any deprotected alcohol. *N*-(Phenylseleno)phthalimide (NPSp) was recrystallized from  $\text{CHCl}_3$ , washed with cold

isopropyl alcohol, and stored at  $-18^\circ\text{C}$  under nitrogen. The recrystallized NPSp was found to be 98% pure by NMR, and this has been accounted for in all concentration calculations. Isovaleraldehyde was freshly distilled under a vacuum prior to use.

All FTIR spectra were taken with a Mettler Toledo ReactIR 15 equipped with a  $\text{LN}_2$  MCT detector and a 9.5 mm AgX Fiber DiComp probe at  $4\text{ cm}^{-1}$  resolution. The spectra were recorded every 45 s, and each one was comprised of 122 scans. The FTIR data underwent second derivative processing using the standard function in the Mettler-Toledo iCIR software. This function applies a 7-point Savitzky–Golay filter and an inversion. The *N*-(phenylseleno)phthalimide (NPSp) was monitored using the height of the signal at  $862\text{ cm}^{-1}$  relative to zero. The height of the signal at  $1036\text{ cm}^{-1}$  relative to a single point baseline at  $1058\text{ cm}^{-1}$  was used to monitor the concentration of phthalimide (NHP). A minimum of 5 data points before and after each addition were taken to ensure a reliable average. The NPSp and NHP were each added in two portions, and the average of these two calibrations was reported. The average temperatures during the reactions were recorded by the FTIR probe.

All NMR spectra were recorded on a Bruker AVIII HD 400 MHz spectrometer with a BBO prodigy probe. The  $^1\text{H}$  NMR chemical shifts ( $\delta$ ) are quoted in ppm relative to residual solvent peaks (for  $\text{CDCl}_3$ , given in ppm: 7.26). Quantitative  $^1\text{H}$  NMR (qNMR) was performed with a  $d_1 = 25\text{ s}$  to ensure all species had fully relaxed. The analysis of reaction yield by qNMR used 1,1,2,2-tetrachloroethane as the internal standard.

**Complete Organocatalytic  $\alpha$ -Selenylation of Isovaleraldehyde.** A vial fitted with a septum, the ReactIR probe, and a stirrer bar was charged successively with stock solutions of isovaleraldehyde (500  $\mu\text{L}$ , 0.206 mmol, 5.6 equiv,  $\text{CDCl}_3$ ), acetic acid (500  $\mu\text{L}$ , 0.503 mmol,  $\text{CDCl}_3$ ), and 1,1,2,2-tetrachloroethane (400  $\mu\text{L}$ , 0.041 mmol,  $\text{CDCl}_3$ ). Five consecutive FTIR spectra were taken before a stock solution of *N*-(phenylseleno)phthalimide (500  $\mu\text{L}$ , 0.037 mmol, 1.0 equiv,  $\text{CDCl}_3$ ) was added in two portions (200  $\mu\text{L}$ , 300  $\mu\text{L}$ ). Five consecutive FTIR spectra were taken after both additions before a stock solution of (*S*)- $\alpha,\alpha$ -bis[3,5-bis(trifluoromethyl)phenyl]-2-pyrrolidinemethanol trimethylsilyl ether (100  $\mu\text{L}$ ,  $2.1 \times 10^{-4}$  mmol, 0.6 mol %,  $\text{CDCl}_3$ ) was added to initiate the reaction. FTIR spectra were recorded consecutively until the reaction appeared to end by visual analysis of the data. The stock solution of phthalimide (500  $\mu\text{L}$ , 0.017 mmol,  $\text{CDCl}_3$ ) was added in two portions (200  $\mu\text{L}$ , 300  $\mu\text{L}$ ). Immediately after the second addition, a 0.6 mL aliquot of the reaction was taken, frozen in liquid  $\text{N}_2$ , and analyzed by qNMR. The spectroscopic data of the reduced product, 3-methyl-2-(phenylselenenyl)butanol, matched that from the literature.<sup>6</sup>

**Incomplete Organocatalytic  $\alpha$ -Selenylation of Isovaleraldehyde.** A vial fitted with a septum, the ReactIR probe, and a stirrer bar was charged successively with stock solutions of isovaleraldehyde (500  $\mu\text{L}$ , 0.385 mmol, 10 equiv,  $\text{CDCl}_3$ ), acetic acid (500  $\mu\text{L}$ , 1.997 mmol,  $\text{CDCl}_3$ ), and 1,1,2,2-tetrachloroethane (400  $\mu\text{L}$ , 0.041 mmol,  $\text{CDCl}_3$ ). Five consecutive FTIR spectra were taken before a stock solution of *N*-(phenylseleno)phthalimide (500  $\mu\text{L}$ , 0.037 mmol, 1.0 equiv,  $\text{CDCl}_3$ ) was added in two portions (200  $\mu\text{L}$ , 300  $\mu\text{L}$ ). Five consecutive FTIR spectra were taken after each addition before a stock solution of (*S*)- $\alpha,\alpha$ -bis[3,5-bis(trifluoromethyl)phenyl]-2-pyrrolidinemethanol trimethylsilyl ether (100  $\mu\text{L}$ ,  $3.8 \times 10^{-5}$  mmol, 0.1 mol %,  $\text{CDCl}_3$ ) was added to initiate the reaction. FTIR spectra were recorded consecutively until the reaction appeared to stall by visual analysis of the data. The stock solution of phthalimide (500  $\mu\text{L}$ , 0.017 mmol,  $\text{CDCl}_3$ ) was added in two portions (200  $\mu\text{L}$ , 300  $\mu\text{L}$ ). Immediately after the second addition, a 0.6 mL aliquot of the reaction was taken, frozen in liquid  $\text{N}_2$ , and analyzed by qNMR. The spectroscopic data of the reduced product, 3-methyl-2-(phenylselenenyl)butanol, matched that from the literature.<sup>6</sup>

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.joc.0c02684>.

Experimental details, tabulated data, and mathematical derivations (PDF)

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<https://pubs.acs.org/10.1021/acs.joc.0c02684>

### Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

The research leading to these results has received funding from the EPSRC projects EP/S005315/1 and EP/R513131/1.

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- (10) See [Supporting Information](#).
- (11) For the product, the signal height was measured relative to a single point baseline to mitigate the change in the signal caused by nearby peaks and to ensure the signal height was proportional to the concentration of the product. For the reactant, the signal height was proportional to the concentration of reactant without baseline correction.