

## **Supporting Information for:**

# **CO<sub>2</sub> Offgas as a Mechanistic Probe and Scale-up Tool in N-Acylation Using Mixed Anhydrides from Amino Acids and Isobutyl Chloroformate**

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## Experimental Section

GC operating parameters are given in Table 1.

**Table 1: GC Conditions**

Parameter	Value
Column pressure, psig	30
Carrier gas	He
Split ratio	50:1
Injection time, min	0.5
Oven Temperature, °C	40 (isothermal program)
Detector temperature, °C	270

HPLC Method details are given in Table 2.

**Table 2: HPLC Method Details**

Parameter	Value
Column	Waters Symmetry, C-18, 4.6 × 150 mm
Column temperature	Ambient
Mobile phase	Acetonitrile/0.1% trifluoroacetic acid in water (60:40)
Flow rate, mL/min	1
Detection wavelength, nm	226

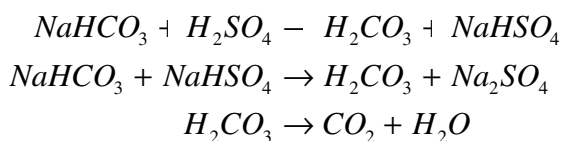
Sources and purities of materials are given in Table 3.

**Table 3 : Sources and Purities of Materials**

Material	Source	Purity
BOC-L-3-(2-naphthyl)alanine ( <b>1</b> )	Synthetec	99+%
Isobutyl chloroformate	Aldrich	98%
N,N,-Dimethylbenzylamine	Aldrich	99+%
N-Benzylmethylamine ( <b>2</b> )	Aldrich	97%
Toluene	Aldrich	99.8%

## Validation of Offgas Measurement Methodology

The reliability of the offgas measurement was first demonstrated using a reaction forming a known amount of gas during addition of a liquid, namely, the neutralization of sodium bicarbonate with sulfuric acid:



The stoichiometric reactions indicate that one mole of CO<sub>2</sub> is formed per mole of NaHCO<sub>3</sub> charged, and that one mole of sulfuric acid is needed to neutralize two moles of sodium bicarbonate. Using a dosing pump, a 60% stoichiometric excess of H<sub>2</sub>SO<sub>4</sub> (*viz.*, 0.245 mole) as a 23 wt% solution was added to the vessel containing 0.306 mol of NaHCO<sub>3</sub> at 25 °C, with N<sub>2</sub> purge gas flowing in the headspace, but without sparging. Sparging with N<sub>2</sub> was begun after the CO<sub>2</sub> evolution attained a low value (Figure 1). The Figure shows that the moles of CO<sub>2</sub> evolved, based on the cumulative volume of CO<sub>2</sub> calculated using the procedure given above, is within excellent agreement of the expected value (*i.e.*, 1.004 *vs.* 1). The offgas volume determination method was thus deemed reliable, but with sparging necessary to close a mass balance above 95%.

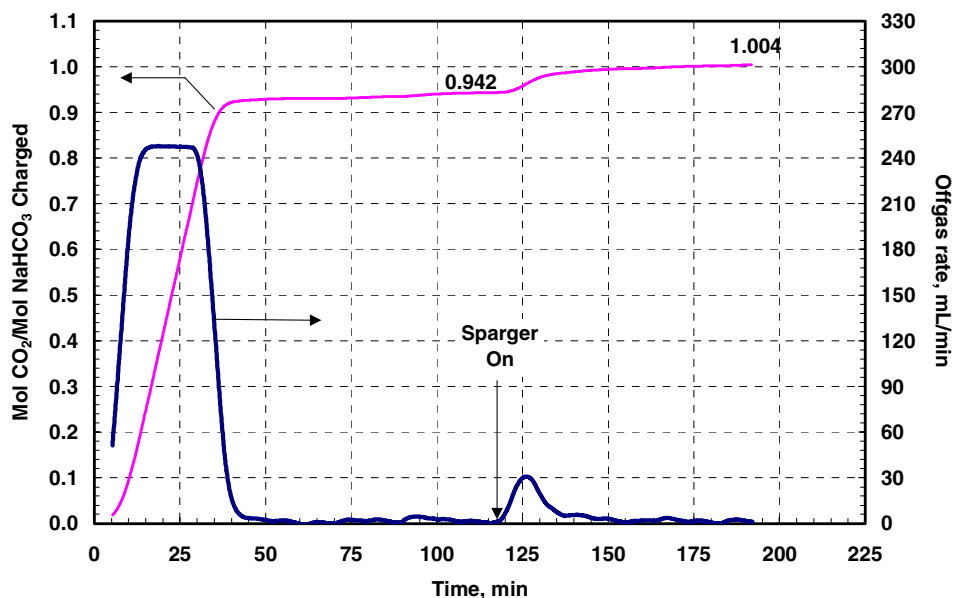
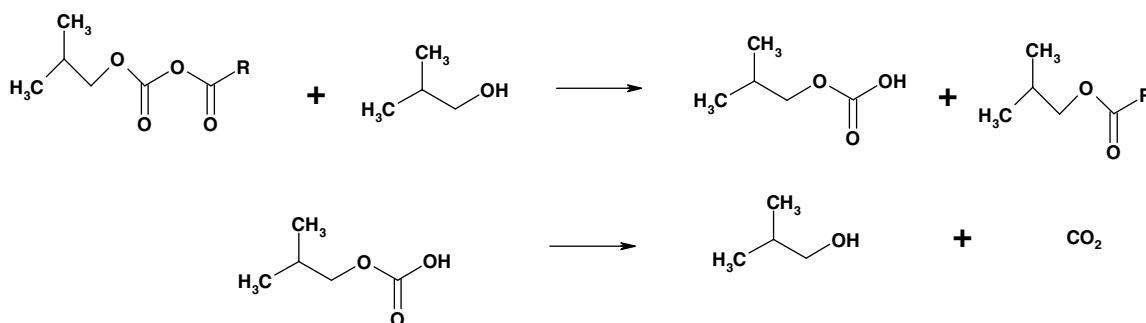


Figure 1: Validation of CO<sub>2</sub> offgas volume measurement.

## Check on Methodology

Two CO<sub>2</sub>-forming pathways are possible before addition of amine **2** that can potentially interfere with the use of CO<sub>2</sub> offgas as a mechanistic probe for *sym*- anhydride formation. We show below that neither pathway is operative under the conditions employed.

First, isobutyl alcohol, produced as a by-product upon *asym*-anhydride formation, could react with the *asym*-anhydride to give an ester, with CO<sub>2</sub> formed due to decomposition of the by-product carbonic acid:



**Scheme 1 : Possible reaction between *asym*-anhydride and *i*-BuOH.**

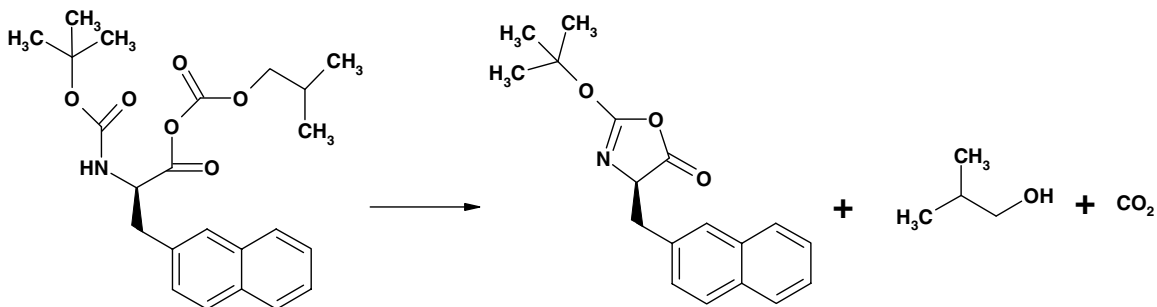
To determine if ester formation occurred at the conditions used here, an experiment was performed in which the reverse addition was performed as in Part I, followed by isobutyl alcohol addition.

A 500-mL Erlenmeyer flask was charged with 51.2 g (0.162 mol) of **1** in 113 g (131 mL) of toluene. N,N,-dimethylbenzylamine (23.8 g, 1.08 equiv) was added dropwise using an addition funnel, which was subsequently rinsed with 5.0 g (5.8 mL) of toluene. The MP10 vessel was charged with isobutyl chloroformate (23.56 g, 1.06 equiv) and toluene (337 g, or 390 mL), and the mixture was cooled at  $-12\text{ }^{\circ}\text{C}$ . The **1** anion solution (190 g) was then added at that temperature over a 40-min period, and the transfer line was rinsed with 15 g (17 mL) of toluene. Afterward, 14.8 g of isobutyl alcohol were added over 20 min, and the same amount was subsequently added over the same period, giving a total of 0.378 moles or more than a 100% stoichiometric excess relative to **1**.

No CO<sub>2</sub> formation was observed after alcohol addition at  $-12\text{ }^{\circ}\text{C}$  and subsequent warming to  $0\text{ }^{\circ}\text{C}$ . Thus, ester formation is negligible at these conditions. HPLC analyses of the reaction mixture also did not show peaks other than those corresponding to **1**, **3**,

and toluene unless the mixture was heated to about room temperature. The negligibly low degree of ester formation is consistent with the excellent mass balance closures (>96%) obtained in the other experiments with **1** and **3** assumed to be the only products formed in significant amounts.

Second, an oxazolinone could form from the *asym*-anhydride, resulting in CO<sub>2</sub> production (Scheme 2) [1] however, the *t*-butyloxycarbonyl protecting group on the nitrogen atom is known to prevent oxazolinone formation [2]. The formation of CO<sub>2</sub> before addition of **2** is thus indicative solely of symmetric anhydride formation.



**Scheme 2: Possible rearrangement of *asym*-anhydride.**

## References

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- [1] Chaudhary, A.; Girgis, M.J.; Prashad, M.; Hu, B.; Har, D.; Repič, O.; and Blacklock, T.J. Tetrahedron Lett., 2003, 44, 5543-5546.
- [2] Bodanszky, M.; Klausner, Y.S.; and Ondetti, M.A., Eds., Peptide Synthesis, John Wiley and Sons, 1976; pp 140.