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CO_2 Offgas as a Mechanistic Probe and Scale-up Tool in N-Acylations Using Mixed Anhydrides from Amino Acids and Isobutyl Chloroformate

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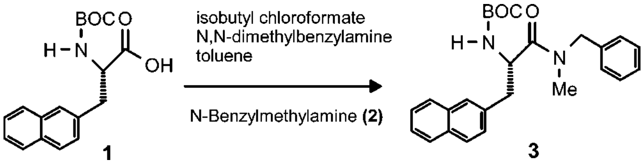
Abstract:

The reaction pathways of an N-acylation reaction, involving a mixed anhydride intermediate produced from reaction of an amino acid with isobutyl chloroformate, were analyzed using a novel technique involving the quantitation of CO_2 evolved during various parts of the process. Under the normal mode of addition, in which chloroformate is added to a carboxylate anion solution, the dominant mode of yield loss to undesired product was shown to be the formation of a symmetric anhydride, implying that byproduct urethane formation was almost exclusively due to reaction of unreacted chloroformate with amine. This result suggests that inferred high concentrations of carboxylate anion present with the normal addition mode should be minimized to decrease yield loss. This hypothesis was confirmed by demonstrating that symmetric anhydride formation is almost eliminated when the reverse addition (viz., addition of carboxylate anion solution to chloroformate) was carried out. These results, in conjunction with the relative temperature insensitivity of the pathways occurring after amine addition, were used to deduce that the higher amounts of remaining starting acid observed on scale-up were due to slower mixing at larger scales, resulting in greater symmetric anhydride yields. The reverse addition, although forming much lower symmetric anhydride amounts, is nevertheless a rapid reaction whose selectivity could be impacted adversely on scale-up due to slower mixing. The offgas methodology presented here can be used to quantify reaction selectivity as a function of scale and thus serve as a diagnostic tool for assessing mixing efficiency at larger scales.

Introduction

N-Acylation reactions, with mixed carboxylic−carbonic anhydrides intermediates produced by reaction of amino acids with isobutyl chloroformate, comprise key steps in the synthesis of a series of selective antagonists of the human NK-1 tachykinin receptor.[1] The first step in the synthesis of one such compound involves the coupling of BOC-L-3-(2-naphthyl)alanine (**1**) with *N*-benzylmethylamine (**2**) in the presence of isobutyl chloroformate and *N*,*N*,-dimethylbenzylamine (Scheme 1).

Scheme 1



The coupling is performed by first deprotonating amino acid **1** with *N*,*N*-dimethylbenzylamine to form a carboxylate anion. In typical preparations,[2,3] the coupling agent, isobutyl chloroformate in this case, is added to the reaction mixture and reacts with the carboxylate anion to form a mixed carboxylic−carbonic, or asymmetric, anhydride (Figure 1); this mode of addition is termed here the “normal addition mode”.

Undesired pathway *a* occurs via reaction of *asym*-anhydride with additional carboxylate anion to form a symmetric anhydride and a carbonate anion. The latter ultimately forms a carbonic acid, which decomposes to give isobutyl alcohol and CO_2. The formation of CO_2 before addition of **2** is thus indicative of symmetric anhydride formation, if no additional pathways forming CO_2 occur. Although two such additional pathways are possible, it is shown in the Supporting Information that their contributions are negligible under the present conditions. The formation of CO_2 before addition of **2** is thus indicative solely of *sym*-anhydride formation.

The desired reaction to form **3** involves reaction of *asym*-anhydride with the amine at the carboxylic acid carbonyl. The reaction byproduct is a carbonic acid which ultimately decomposes to give isobutyl alcohol and CO_2. Formation of the latter after addition of **2** is thus indicative of attack by the amine at the carboxylic acid carbonyl As this pathway gives the desired product, it is termed attack at the “desired” carbonyl in what follows.

Amine **2** can also react at the carbonic acid carbonyl by an analogous mechanism (undesired pathway *b*). In this case, referred to here as attack at the “undesired” carbonyl, the reaction products include a byproduct urethane and **1**. Although **1** formed via by the latter route can be converted to **3** by using additional amounts of *N*,*N*,-dimethylbenzylamine, isobutyl chloroformate, and **2**, this represents an inefficient mode of preparing **3**, as it requires multiple additions of reagents to consume the liberated **1**.

Attack by **2** at either carbonyl carbon atom of the symmetric anhydride (Figure 1) can also occur to give the desired amide **3** and liberate **1**. This pathway for **3** production is also inefficient because 2 moles of **1** are consumed per

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(1) Walpole, C. S. J.; Prashad, M.; Har, D.; U. S. Patent No. 6,107,293, 2000.

(2) Sole, N.; Torres, J. L.; Garcia Anton, J. M.; Valencia. G.; Reig, F. *Tetrahedron* **1986**, *42* (1), 193−198.

(3) Chen, F. M. F.; Lee, Y.; Steinauer, R.;Benoiton, N. L. *Can. J. Chem.* **1987**, *65*, 613−618.

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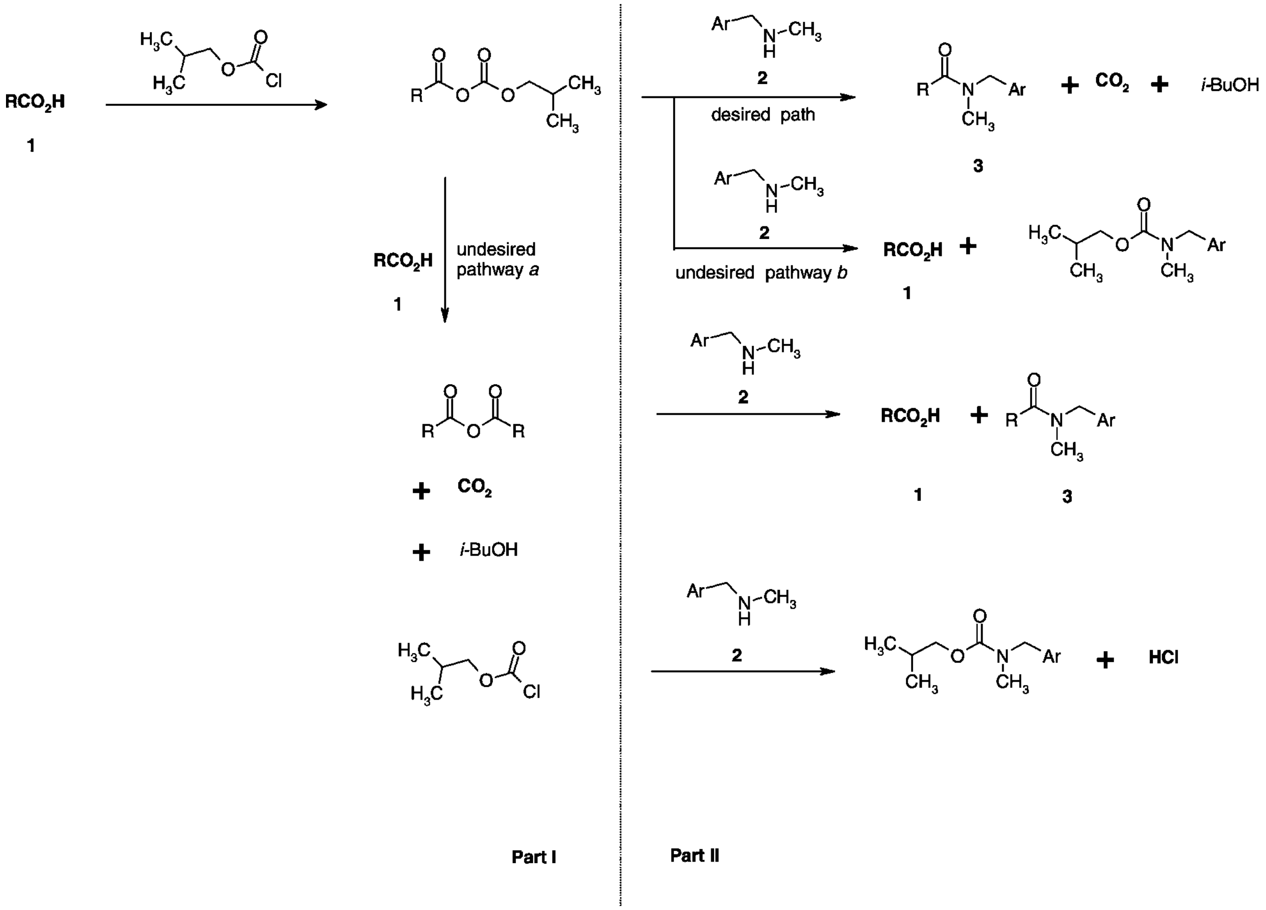

Figure 1. **Reaction scheme for entire process. Part I comprises formation of the carboxylate anion and reaction of the latter to form the anhydrides. Part II occurs after addition of amine 2.**

mole of **3** produced. The presence of **1** at the end of the reaction can thus occur by either of two undesired pathways, one of which (undesired path *b*) also forms a urethane byproduct. The urethane can also form from reaction of **2** with isobutyl chloroformate remaining from high consumption of **1** via undesired pathway *a*.

Previous investigations[2,3] have reported formation of the urethane byproduct and addressed key parameters impacting its yield (e.g, selection of solvent, coupling agent, and temperature); its formation pathways have not been delineated. Earlier work[4] has also shown that at larger scales, higher amounts of starting acid were detected at the end of the reaction and, by inference, lower amide yields. For example, at a reaction scale corresponding to 15.7 g of **1**, less than 3% of the **1** added initially was detected at the end of the reaction; in contrast, at a 400-g scale, 22% of the **1** added was detected. Additional amounts of reagents needed to be added to convert the remaining **1** to **3**. The reaction thus became more inefficient as the scale was increased. (In the latter work, *N*-methylmorpholine was used as the deprotonating base instead of *N*,*N*-dimethylbenzylamine, and ethyl acetate was the solvent, but neither change impacts the conclusions cited.) It would thus be instructive and useful to have a general means by which the contributions of the various reaction pathways can be quantified and, from this knowledge, to devise rational processing strategies to improve both selectivity and process robustness with increasing scale.

In this contribution, a novel methodology is presented in detail which allows quantitative determination of the contributions of the operative pathways; a preliminary account is given elsewhere.[5] This methodology comprises quantifying the CO_2 amount evolved during the various parts of the process in conjunction with HPLC analyses of the starting acid **1** and final product **3**. From such data, one can (a) determine the amount of *sym*- and *asym*-anhydride formed during the reaction of **1** with chloroformate before **2** addition and thus quantify the contribution of undesired path *a* and (b) distinguish between attack by **2** at the carboxylic acid carbonyl of the *asym* anhydride giving the desired product vs attack at the carbonic acid carbonyl (undesired path *b*). The contributions of the two urethane-forming pathways can also be established and thus allow rational development of conditions that give minimal byproduct yields. In addition, it was hoped that such quantitative insights could allow inference of the cause of the increasing amounts of **1** observed at the end of the reaction upon scale-up and thus provide guidance for improving process robustness.

Although HPLC analysis can in principle be used to quantify the amounts of *sym*- vs *asym*-anhydride formed, its

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(4) Prashad, M.; Prasad, K.; Repič, O.; Blacklock, T. J. *Org. Process Res. Dev.* **1999**, *3*, 409−415.

(5) Chaudhary, A.; Girgis, M. J.; Prashad, M.; Hu, B.; Har, D.; Repič, O.; Blacklock, T. J. *Tetrahedron Lett.* **2003**, *44*, 5543−5546.

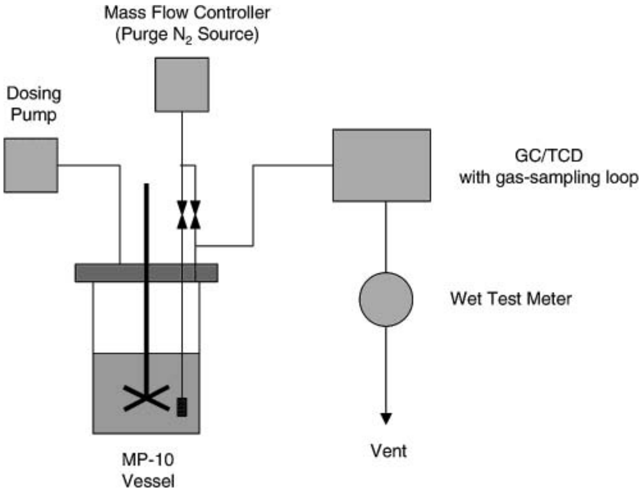

Figure 2. **Schematic diagram of apparatus.**

application is complicated by the possible instability of the anhydrides under HPLC analysis conditions and the need to synthesize and purify authentic samples of the intermediate anhydrides to serve as calibration standards for quantitative analysis. Using the CO_2 offgas technique, analysis of intermediates is obviated, and only HPLC analyses of starting acid **1** and product **3**, which are stable, are needed. This methodology also allows quantitative application to similar coupling reactions without the need to synthesize intermediates.

In situ Fourier transform infrared spectroscopy[6] could also be used to quantify the anhydride amounts. However, application of this method, which was tried at first, also requires synthesizing authentic samples of the anhydrides to identify their respective spectroscopic bands and to serve as calibration standards. Additional disadvantages include the complexity of the spectra due to the various carbonyl species present and the tedious calibrations required for quantitative analysis.

Experimental Section

Apparatus and Materials. Reaction experiments were performed using the apparatus shown schematically in Figure 2. The apparatus comprises a Mettler-Toledo RC1 reaction calorimeter equipped with a 1-L glass MP-10 vessel. The vessel inserts included a Hastelloy pitched-blade agitator and Hastelloy temperature and calibration probes. The jacket temperature was also monitored. Calorimeter operating principles are discussed elsewhere.[7] A dosing pump (Prominent G4) was used to meter liquids into the reaction vessel. Nitrogen gas was introduced either in the headspace or by sparging into the liquid with a dip tube fitted with a sintered metal filter element attached at the end of the tube to give fine bubbles. The nitrogen gas flow was metered with a Brooks model 5850i mass flow controller.

The composition of the offgas was determined by gas chromatography using a Hewlett-Packard 5890 GC equipped with a 5-μL sampling loop, an Agilent GasPro capillary column (60-m length, 0.25 mm i.d.) and a thermal conductivity detector. GC operating parameters are given in the Supporting Information.

Offgas volume was determined using a Ritter model TG05 wet test meter, which operates on the principle of gas displacement. The wet test meter was filled with a paraffinic white oil (Aldrich Mineral Oil, white, light, catalogue no. 33,077-9) in which N_2 and CO_2 are very sparingly soluble at atmospheric pressure; for example, at atmospheric pressure, the equilibrium mole fraction of N_2 and CO_2 in *n*-hexadecane are 0.0013 at 25 °C and 0.014 at 27 °C, respectively.[8,9] The wet test meter was placed downstream of the GC sampling loop, and the cumulative volume was recorded using a data-logging system.

HPLC analyses were performed on selected samples using a Hewlett-Packard series 1100 HPLC equipped with a diode array detector. Method details are given in the Supporting Information. All materials were used as received. Sources and purities of all materials are given in the Supporting Information.

Offgas Flow Volume and Flow Rate Determinations. During a gas-forming reaction, the wet test meter measures the cumulative volume of total gas flowing through it at ambient temperature and pressure, including N_2 purge gas as well as chemical offgas (i.e., the gas produced in the chemical reaction). To quantify the chemical offgas volume, it is necessary to decouple its flow rate from that of the purge gas. This decoupling was done as follows. First, after charging starting materials but before the start of the reaction, the flow rate of N_2 purge gas was set to a constant value (nominally 50 cm^3/min) with the mass flow controller. The cumulative volume was then determined with the wet test meter for at least 30 min. The actual purge gas flow rate was determined by numerical differentiation of the cumulative volume during this period. The numerical differentiation was carried out by determining the slope of a line, fitted by the method of least squares,[10] to at least 50 consecutive volume readings, each taken every 5 s.

During the experiment, which was always performed with N_2 purge gas flowing, the cumulative total gas volume was measured with the wet test meter. The flow rate of total offgas was determined by numerical differentiation as described above. The flow rate of the chemical offgas was determined by subtracting the purge gas flow rate determined earlier from the total offgas rate during the experiment. The cumulative offgas volume was subsequently determined by numerical integration of the offgas flow rate using the trapezoidal rule.[10] To allow rapid computation of these quantities, the procedure was automated by first inputting the purge and total offgas cumulative volumes into a relational database, and subsequently performing the calculations using a Microsoft Visual Basic computer program which read the input data from the database.

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(6) Landau, R. N.; McKenzie, P. F.; Forman, A. L.; Dauer, R. R.; Futran, M.; Epstein, A. D. *Process Control Qual.* **1995**, *7*, 133−142.

(7) Girgis, M. J.; Kiss, K.; Ziltener, C. A.; Prashad, M.; Har, D.; Yoskowitz, R. S.; Basso, B.; Repič, O.; Blacklock, T. J.; Landau, R. N. *Org. Process Res. Dev.* **1997**, *1*, 339−349.

(8) Tremper, K. K.; Prausnitz, J. M. *J. Chem. Eng. Data* **1976**, *21*, 295−299.

(9) Chai, C. P.; Paulaitis, M. E. *J. Chem. Eng. Data* **1981**, *26*, 277−279.

(10) Press, W. H.; Flannery, B. P.; Teukolsky, S. A.; Vettering, W. T. *Numerical Recipes The Art of Scientific Computing*; Cambridge University Press: New York, 1986; Chapter 14.

In the experiments performed here, the gas evolution occurred during periods in which liquid was added to the reactor. To prevent incorrectly counting the gas displaced by the liquid as evolved chemical offgas, it was necessary to subtract the volume of liquid added from the cumulative chemical offgas evolved. The reliability of the offgas measurement was demonstrated using a reaction forming a known amount of gas during addition of a liquid, namely, the neutralization of sodium bicarbonate with sulfuric acid; details are given in the Supporting Information.

Results and Discussion

Reaction Pathway Analysis. Part I reactions can be represented by the following chemical equations:

$$1 + DMBA + IBCF \rightarrow asym\text{-}anhydride + DMBA\text{-}HCl$$

$$1 + asym\text{-}anhydride \rightarrow sym\text{-}anhydride + i\text{-}BuOH + CO_2$$

where *DMBA*, *DMBA-HCl*, and *IBCF*, denote, respectively, *N*,*N*-dimethylbenzylamine, its hydrochloride salt, and isobutyl chloroformate. The chemical equations above indicate that 1 mol of CO_2 is produced per mol of *sym*-anhydride formed:

$$n_{sym} = n^{I}_{CO_2} \tag{1}$$

where n_i denotes the moles of component i and the superscript refers to Part I. The stoichiometry also implies that the molar amount of *asym*-anhydride formed equals the difference between the moles of **1** charged and the moles of **1** consumed to form *sym*-anhydride; because 2 mol of **1** are required to form 1 mol of *sym*-anhydride, the mass balance shows:

$$n_{asym} = n_1^{\,0} - 2n_{sym} = n_1^{\,0} - 2n^{I}_{CO_2} \tag{2}$$

where the 0 superscript denotes initial amount.

The contributions of the reaction pathways forming *asym*- and *sym*-anhydride can be expressed on a scale-independent basis as percentage yields:

$$y_{sym} = \frac{n_{sym}}{n_1^{\,0}} \times 100 = \frac{n^{I}_{CO_2}}{n_1^{\,0}} \times 100 \tag{3}$$

$$y_{asym} = \frac{n_{asym}}{n_1^{\,0}} \times 100 = \frac{n_1^{\,0} - 2n^{I}_{CO_2}}{n_1^{\,0}} \times 100 \tag{4}$$

Because 2 mol of **1** are required to form 1 mol of *sym*-anhydride, the sum of the percentage yields above do not sum to 100. Instead, twice the *sym* yield added to the *asym* yield gives 100.

The following chemical equations follow, respectively, from the pathways for Part II:

$$asym\text{-}anhydride + 2 \rightarrow 3 + CO_2 + i\text{-}BuOH$$

$$asym\text{-}anhydride + 2 \rightarrow 1 + urethane$$

$$sym\text{-}anhydride + 2 \rightarrow 3 + 1$$

$$IBCF + 2 \rightarrow urethane + HCl$$

The last reaction is due to unreacted IBCF as a consequence of **1** consumption to form *sym*-anhydride via undesired pathway *a* (Figure 1).

The contributions of the four reactions above can be determined from mass balances. Thus, the number of moles of **3** formed via attack at the desired carbonyl atom is given by:

$$n_3^{desired\ carbonyl} = n^{II}_{CO_2} \tag{5}$$

The contribution of the third pathway, representing the number of moles of **1** or **3** formed by reaction of *sym*-anhydride with **2**, can be calculated from the CO_2 amount formed in Part I:

$$n_1^{from\ sym-anhydride} = n_3^{from\ sym-anhydride} = n^{I}_{CO_2} \tag{6}$$

The contribution of the fourth pathway can be determined as follows. The number of moles of unreacted IBCF, and thus the moles of urethane $n^{sym}_{urethane}$ formed due to consumption of **1** to form *sym*-anhydride, can be calculated from the moles of *sym*-anhydride formed. Note, however, that the total amount of urethane $n^{A2rxn}_{urethane}$ formed by reaction of **2** and IBCF includes the amount formed due to the stoichiometric excess of chloroformate reacting with **2**:

$$n^{A2rxn}_{urethane} = n^{sym}_{urethane} + (n_{IBCF}^{\,0} - n_1^{\,0}) = n^{I}_{CO_2} + (n_{IBCF}^{\,0} - n_1^{\,0}) \tag{7}$$

Equations 5−7 indicate that the CO_2 amounts formed, in conjunction with the stoichiometry, suffice to determine the contributions of three of the four pathways above.

The contribution of the pathway pertaining to attack at the undesired carbonyl can be determined two ways. First, the molar amount of **1** formed by this pathway, which is the same as the molar amount of urethane formed by this pathway, is equal to the difference between the total molar amount of **1** obtained at the end of the reaction (which can be determined by HPLC) and the amount of **1** formed via *sym*-anhydride conversion. As the latter amount equals the molar amount of CO_2 formed in Part I, it follows that:

$$n_1^{undesired\ carbonyl} = n_{urethane}^{undesired\ carbonyl} = n_1 - n_1^{from\ sym-anhydride} = n_1 - n^{I}_{CO_2} \tag{8}$$

The undesired carbonyl pathway can also be expressed in terms of **3** molar amounts because 1 mol of **3** is formed per mol of **1** in the reaction of *sym*-anhydride with **2**. The contribution of *sym*-anhydride conversion can be expressed as the difference between the total molar amount of **3** formed and the molar amount of **3** formed by attack at the desired carbonyl. Thus, the contribution of the undesired carbonyl pathway can also be expressed as follows:

$$n_1^{undesired\ carbonyl} = n_{urethane}^{undesired\ carbonyl} = n_1 - (n_3 - n_3^{desired\ carbonyl}) = n_1 - (n_3 - n^{II}_{CO_2}) \tag{9}$$

The Part II results can also be expressed in terms of scale-independent percentage yields:

$$y_3^{desired\ carbonyl} = \frac{n_{CO_2}^{II}}{n_1^{\ 0}} \times 100 \qquad (10)$$

$$y_1^{undesired\ carbonyl} = y_{urethane}^{undesired\ carbonyl} = \frac{n_1^{undesired\ carbonyl}}{n_1^{\ 0}} \times 100 \qquad (11)$$

where $n_1^{undesired\ carbonyl}$ is given by either eq 8 or eq 9.

$$y_1^{sym} = y_3^{sym} = \frac{n_{CO_2}^{I}}{n_1^{\ 0}} \times 100 \qquad (12)$$

$$y_{urethane}^{A2\ rxn} = \frac{n_{CO_2}^{I} + (n_{IBCF}^{0} - n_1^{0})}{n_1^{0}} \times 100 \qquad (13)$$

Normal Addition Mode: Addition of IBCF to 1 Anion Solution. The pathways were first characterized quantitatively using the normal addition mode. **1** (51.22 g, 0.162 mol) and toluene (450.9 g, or 520 mL) were added to the MP10 vessel. At an agitation rate of 400 rpm, *N*,*N*,-dimethylbenzylamine (23.8 g, 0.176 mol, 1.08 equiv) was pumped into the vessel over 20 min at a 20 °C batch temperature. The transfer line (between the pump and vessel) was subsequently rinsed with toluene (3.9 g, 4.5 mL), which was added to the batch. The reaction mixture was cooled to −12 °C at 1 °C/min, and held at that temperature for 85 min.

Isobutyl chloroformate (23.5 g, 0.172 mol, 1.06 equiv) was subsequently added at a −12 °C batch temperature over a 40-min period, with gas evolution observed during the addition (Figure 3). The online GC analysis indicated that CO_2 was the sole reaction offgas component. The CO_2 rate and cumulative volume could therefore be determined by subtracting the contribution of the N_2 purge gas from the total flow. The CO_2 volume during Part I evolved was 688 cm^3, corresponding to 0.0285 mol at the 22 °C wet test meter temperature. Based on equations (3) and (4), the *sym*- and *asym*-anhydride yields are calculated to be 17.5% and 65.0%, respectively. Thus, a significant amount of *sym*-anhydride was formed using the normal addition mode illustrated in Figure 3.

The reaction mixture was held at −12 °C for 45 min, after which 37.7 g of a solution of **2** in toluene (prepared by adding 36.4 g of **2** to 20.2 g of toluene, giving 64.3 wt. % of **2**) was added over a 30-min period. The **2** added (0.200 mol) corresponds to 1.23 equiv. Additional CO_2 was evolved during this addition, the subsequent 30-min hold, the warm-up of the reaction mixture to 21 °C at 1 °C/min, and the H_2SO_4 addition (128 g of solution comprised of 7 g of concentrated H_2SO_4 in 121 g of water added over 12 min) to quench any remaining amines (Figure 3). Note that any HCl formed due to reaction between **2** and isobutyl chloroformate would be quenched by excess **2** in solution and thus not evolve as a gas.

Using eqs 10 and 12, the yields of **3** formed by *sym*-anhydride attack at the desired carbonyl and from reaction of *sym*-anhydride are calculated to be 66.7% and 17.5%, respectively. As twice the latter quantity added to 66.7 is close to 100%, it is inferred that the contribution of the pathway pertaining to attack at the undesired carbonyl is negligible. Calculation of the yield of **1** formed by the latter pathway using eqs 8 and 10 gives a value of 6.0%; a very small value (0.4%) is obtained if the calculation is done using eqs 9 and 10. These relatively small values and their large differences indicate that accurate calculation of the pathway involving **2** attack at the undesired carbonyl is difficult, primarily because it involves subtracting quantities that are similar in magnitude (e.g., n_{A1} and $n_{CO_2}^{I}$). Since the combined contributions of the other pathways are close to 100%, the main conclusion is that the contribution of the undesired carbonyl pathway is very small, and as a consequence, the amount of urethane formed due to attack at the undesired carbonyl is also small. Thus, the dominant pathway for urethane formation under the conditions used here is due to reaction of **2** with chloroformate, unconsumed due to *sym*-anhydride formation.

The foregoing results also imply that the main cause of yield loss with the normal addition mode at the scale employed here is the formation of *sym*-anhydride in Part I, and not due to attack at the undesired carbonyl by **2**. This finding suggests that high local **1** concentrations, which favor reaction of *asym*-anhydride with **1** anion to form *sym*-anhydride, should be avoided to obtain greater **3** yields. This hypothesis prompted us to change the addition mode to a reverse addition in Part I (viz., by dosing IBCF into **1** anion solution) with the expectation that the lower **1** anion amounts in the vessel should give less *sym*-anhydride formation.

Reverse Addition: Dosing of 1 Anion Solution into IBCF. A 500-mL Erlenmeyer flask was charged with 51.23 g (0.162 mol) of **1** in 112.95 g (131 mL) of toluene. *N*,*N*,-dimethylbenzylamine (23.8 g, 0.176 mol, 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 (23.55 g, 0.172 mol, 1.06 equiv) and toluene (337 g, 390 mL), and the mixture was cooled to −12 °C. The **1** anion solution (191.7 g) was then added at that temperature over a 40-min period, and the transfer line was rinsed with 10 g (11.6 mL) of toluene (Figure 4). CO_2 was again the sole offgas component detected, but as shown in Figure 3 and Table 1, the volume of CO_2 evolved was lower by almost a factor of about 9 in comparison to results from the experiment utilizing the normal addition mode.

After holding the reaction mixture for 40 min at −12 °C, 37.73 g of a solution comprising 36.4 g (0.200 mol, 1.2 equiv) of *N*-benzylmethylamine in 20.2 g of toluene was added over a 30-min period, with the transfer line subsequently rinsed with 9 g (10 mL) of toluene. After holding for 60 min, the reaction mixture was heated to 20 °C at 1 °C/min. Once the reaction mixture attained 20 °C and following a 10-min hold, a solution (128.2 g) comprised of 7 g of concentrated sulfuric acid in 121.2 g of water was added over 12 min, and the mixture was held for 1.5 h. CO_2 evolution was monitored throughout the experiment. As in the previous experiment, any HCl formed due to reaction

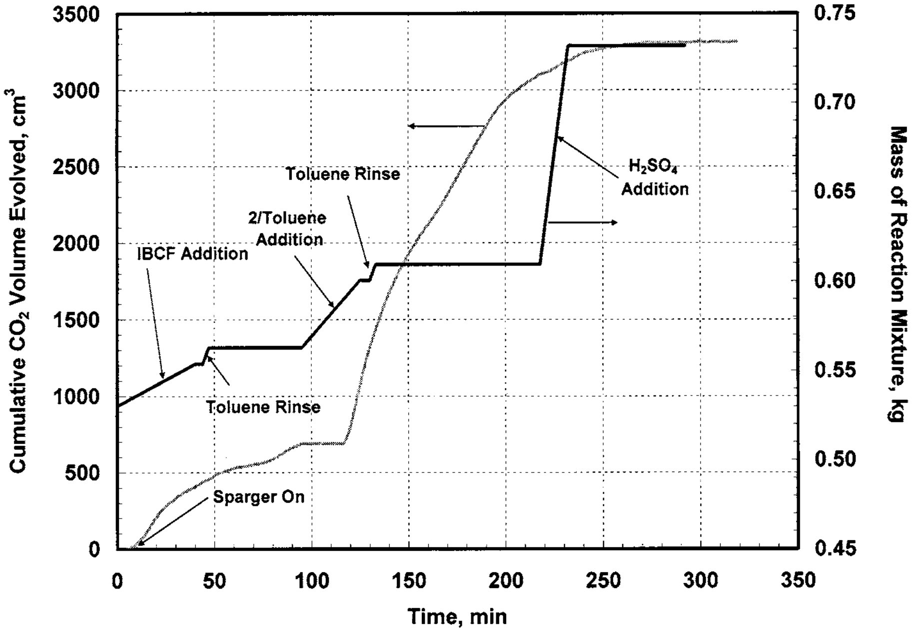

Figure 3. **Temporal profile of cumulative offgas volume and reaction mixture mass with reaction performed using the normal addition mode.**

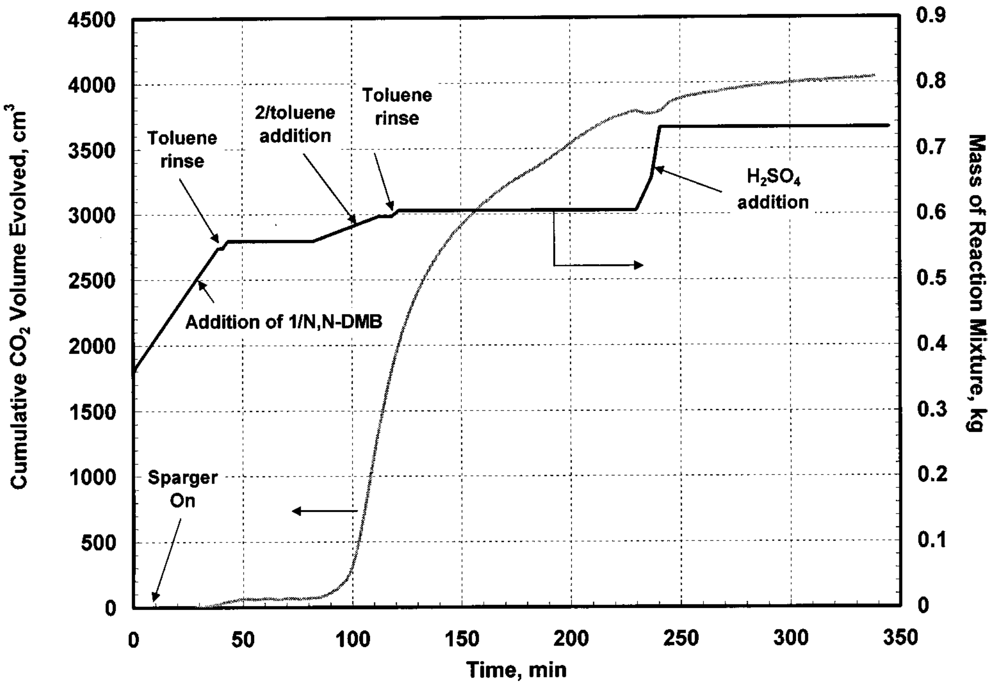

Figure 4. **Temporal profiles of cumulative CO_2 volume and reaction mixture in experiment with reverse addition.**

between **2** and isobutyl chloroformate would be quenched by excess **2** in solution.

Substantially more CO_2 was evolved upon addition of **2** in solution and subsequent warming and quenching with H_2SO_4. Table 1 also shows that the contribution of the undesired carbonyl pathway was again very small. This result is plausible, because Part II was carried out in the same way in both cases, and thus, the contribution of this pathway would not be expected to change. Furthermore, with the reverse addition, the contribution of the pathway forming **1** and **3** via the *sym*-anhydride is small as expected, since so little *sym*-anhydride is formed in Part I. The main source of yield loss under the normal addition mode (viz., *sym*-anhydride formation), is thus almost completely eliminated by the reverse addition. In fact, with the reverse addition, the last two lines of Table 1 imply that most of the urethane product is formed because of the stoichiometric excess of IBCF added.

Addition of 2 at Higher Temperature. In an attempt to induce reaction at the undesired carbonyl, an experiment was performed using the reverse addition but with subsequent **2** solution addition carried out at −2 °C, i.e., 10 °C higher than in previous experiments. As this higher temperature represents one-third of the adiabatic temperature rise of the

Table 1. **Comparison of pathway contributions using two modes of addition**

	normal addition	reverse addition
Part I		
y_{sym} (eq 3)	17.5%	1.89%
y_{asym} (eq 4)	65.0%	96.2%
Part II		
$y_3^{desired\ carbonyl}$ (eq 10)	66.7%	94.7%
$y_3^{undesired\ carbonyl} = y_{urethane}^{undesired\ carbonyl}$ (eq 11)	<2%	<2%
$y_1^{sym} = y_3^{sym} = y_{urethane}^{sym}$ (eq 12)	17.5%	1.89%
$y_{urethane}^{2\ rxn}$ (eq 13)	23.7%	8.74%

Table 2. **Comparison of process performance with reverse addition with 2 added at different temperatures**

temperature of **2** addition, °C	**−12 °C**	**−2 °C**
$y_3^{desired\ carbonyl}$ (eq 10)	94.7%	>99%
$y_3^{undesired\ carbonyl} = y_{urethane}^{undesired\ carbonyl}$ (eq 11)	<2%	<3%
$y_1^{sym} = y_3^{sym} = y_{urethane}^{sym}$ (eq 12)	1.89%	2.41%

reaction, it represents a reasonable estimate of the extent of local heating (i.e., a hot-spot formation) that could arise because of slow dispersion of **2** into the solution containing the anhydrides prepared in Part I.

A solution of deprotonated **1** in toluene was prepared as in the previous experiment and then added to the MP-10 vessel containing isobutyl chloroformate (23.56 g, 1.06 equiv) in toluene (335.3 g, 390 mL) at a −12 °C batch temperature. After rinsing the transfer line with toluene (11 g, 13 mL) and holding for 40 min, the batch was heated to −2 °C at 1 °C/min. A solution (37.73 g) of **2** in toluene (36.4 g of *N*-benzylmethylamine in 20.2 g of toluene) was added over 30 min (1.2 equiv of **2**). After a 1-h hold, the mixture was heated to 20 °C at 1 °C/min and quenched with H_2SO_4 as described above after a 30-min hold.

As shown in Table 2, the product yields were similar to those obtained with addition at −12 °C, suggesting that a −2 °C addition temperature is insufficiently high to cause attack by **2** at the undesired carbonyl.

Mechanistic and Scale-up Insights. The foregoing experiments thus indicate that the CO_2 offgas technique can delineate quantitatively the formation pathways for urethane byproduct formation. Although the pathway delineations were carried out for one particular N-acylation, the methodology is applicable to other reactions of the same type,[2,3] and should be especially useful to probe the impact of key process variables (e.g., temperature, solvent type) and other properties (e.g., steric hindrance of the coupling agent) on the pathways for byproduct formation.

The aforementioned experiments are also useful for inferring the cause of the higher amounts of **1** observed at the end of the reaction at larger scales. Bourne[11] has identified three mixing stages for single-phase fluids: (a) distributive, or macromixing, in which large fluid eddies exchange positions and mass, but with compositional uniformity occurring only at a scale greater than the eddy size; (b) dispersive, or mesomixing, in which larger eddies are reduced in size due to turbulent shear and finer-grained mixture forms, but with the mixture remaining highly segregated at the molecular scale; (c) diffusive, or micromixing, in which molecular diffusion occurs over short distances between finely dispersed structures to give a mixture that is randomized on the molecular scale. Good micromixing is thus a prerequisite to avoiding compositional inhomogeneities on the molecular scale (i.e., high local concentrations) that can impact selectivity of chemical reactions.

The macromixing time typically increases with increasing scale.[11] For example, when scaling up at constant power per unit volume in geometrically similar vessels, the macromixing time varies as the 2/3 power of the vessel diameter.[11] At constant power per unit volume, however, the micromixing time is not impacted significantly.[11] Thus, given that the stages occur mostly in series,[11] one should expect a longer time to achieve uniformity at the molecular scale with increasing scale (i.e. macromixing becomes the limiting mixing process).

We therefore infer that the yield loss of **3** occurring upon scale-up using the normal addition mode[4] is due to slower macromixing of isobutyl chloroformate with increasing scale. The slower macromixing is hypothesized to result in high local **1** concentrations over longer time periods, resulting in faster *sym*-anhydride formation, and thus giving greater yields of the latter as the scale is increased. The small contributions in all cases of the pathway involving attack of the wrong carbonyl, coupled with the yield insensitivity when adding **2** at −2 °C to simulate hot spot formation due to poor mixing, imply that the undesired carbonyl pathway is not a major cause of **3** yield loss on scale-up.

Although the reverse addition reduced high local **1** anion concentrations in the experiments performed on our scale, the temperature profiles during the reverse addition (Figure 5) are indicative of a rapid, addition-controlled reaction whose selectivity could be sensitive to the mixing rate, suggesting that yield loss could occur on scale-up with the reverse addition. The offgas methodology presented here could then be used to quantify the impact of the slower mixing expected upon scale-up on selectivity by measuring the CO_2 volume evolved during Part I as a function of scale. If it is found, for example, that higher CO_2 yields result at larger scales, slower mixing at the latter scale can be implicated. Furthermore, measures can be implemented to counter the impact of slower mixing, such as decreasing the addition rate, changing the feed location, adding at multiple points, and adding more dilute solutions. The effectiveness of these methods could be evaluated by measuring the CO_2 volume evolved during Part I. The offgas methodology could thus serve as valuable diagnostic tool that quantifies the

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(11) Bourne, J. R. Mixing in Single-phase Chemical Reactors. In *Mixing in the Process Industries*, 2nd ed.; Harnby, N., Edwards, M. F., Nienow, A. W., Eds.; Butterworth Heinman: Woburn, MA, 1992.

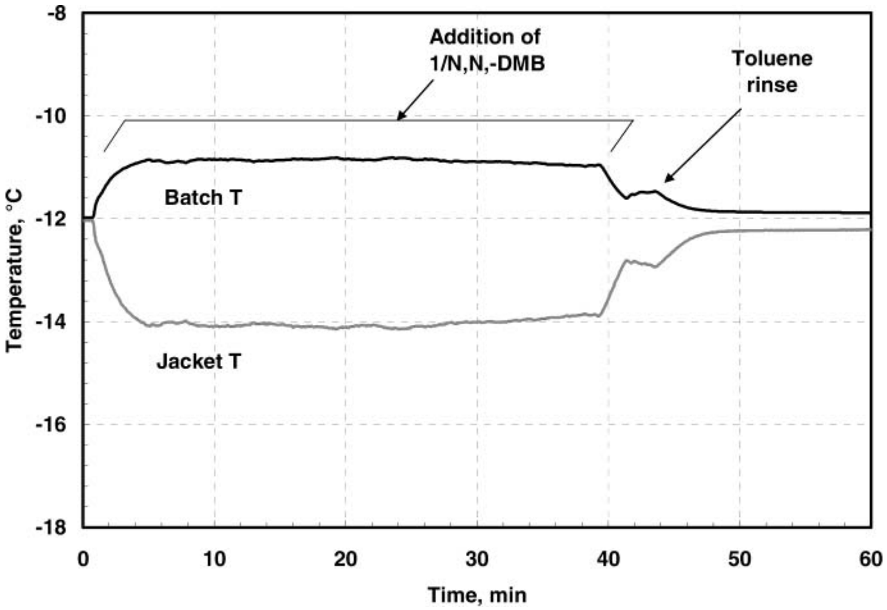

Figure 5. **Temporal temperature profiles in experiment with reverse addition.**

impact of mixing indirectly. Advantages of this methodology include its noninvasive nature (since it involves online analysis of a waste stream) and ease of implementation, since the integral nature of the offgas measurement reading merely requires reading the wet test meter at certain time intervals.

Conclusions

A methodology for quantifying the offgas produced during chemical reactions was demonstrated and applied to quantify the CO_2 evolved during various parts of an N-acylation reaction having a mixed anhydride intermediate produced from reaction of an amino acid with isobutyl chloroformate. The dominant pathway of yield loss under the normal addition mode at the conditions employed here was shown to involve *sym*-anhydride formation before **2** addition instead of attack of **2** at the undesired carbonyl of the *asym*-anhydride. Furthermore, the relatively high percentage yield (viz., 17.5%) of *sym*-anhydride formed in Part I, coupled with the reaction pathway analysis, suggests that high local **1** concentrations were present and that minimizing these is the key to decreasing yield loss.

This suggestion was corroborated in an experiment in which **3** yield loss was almost eliminated by reversing the mode of addition, viz., by adding carboxylate anion solution to isobutyl chloroformate to minimize the inferred high **1** local concentrations. With the reverse addition, the contribution to yield loss due to attack on the undesired carbonyl was also negligible, even when the **2** addition was performed at a 10 °C higher temperature.

These results were used to infer that the **3** yield losses observed on scale-up in earlier work were due to the slower macromixing on the larger scale, giving high local **1** concentrations that result in greater yields of *sym*-anhydride, and not to **2** attack at the undesired carbonyl.

The temperature profile during the reverse addition is indicative of a rapid, addition-controlled reaction whose selectivity could be sensitive to the slower mixing expected upon scale-up. The offgas methodology can be implemented easily to quantify the CO_2 yield formed in Part I as a function of scale and thus serve as a quantitative diagnostic for mixing rate.

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Supporting Information Available

GC method, HPLC method, sources and purities of material used, validation of methodology, and check on methodology. This material is available free of charge via the Internet at http://pubs.acs.org.

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