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Using mixed anhydrides from amino acids and isobutyl chloroformate in *N*-acylations: a case study on the elucidation of mechanism of urethane formation and starting amino acid liberation using carbon dioxide as the probe

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Abstract—A case study on the elucidation of mechanism of urethane by-product formation and starting amino acid liberation during the conventional two-step isobutyl chloroformate mediated *N*-acylation is described using carbon dioxide offgas as the probe. The main reason for the urethane formation and starting amino acid liberation was found to be the formation of the symmetrical anhydride of the amino acid during the preparation of the mixed carboxylic–carbonic anhydride intermediate, as determined by quantifying the evolved carbon dioxide. New conditions were developed to minimize this side reaction.

Isobutyl chloroformate (IBCF)[1] is a well-known coupling agent in peptide synthesis[2–4] and has been found to be a reagent of choice for large scale syntheses.[5–8] Conventional conditions involve two steps: activation of the carboxyl group with IBCF in the presence of a tertiary amine base to form the mixed carboxylic–carbonic anhydride, and the reaction of this intermediate with the amine component.[9] One of the drawbacks with this methodology is a side reaction,[4,5,10–15] in some cases leading to the formation of urethane by-product and liberation of the starting amino acid. The starting amino acid detected at the end of the reaction is due to the side reaction and not to incomplete reaction, an after thought. Although the aminolysis at the undesired carbonyl in the mixed carboxylic–carbonic anhydride has been implicated as the cause of the urethane formation[5,10–15] and liberation of the starting amino acid, to the best of our knowledge there is no method known in the literature to ascertain the actual cause of this side reaction in the two-step procedure. Another possible pathway that could result in this side reaction is the symmetrical anhydride formation[12] in the first activation step of the amino acid with IBCF. In this paper we report the elucidation of the mechanism of urethane formation and the starting amino acid liberation by determining, and quantifying, the evolved carbon dioxide during the first step involving the preparation of the mixed carboxylic–carbonic anhydride intermediate, which shows that symmetrical anhydride formation is the main cause of this side reaction.

In a development program we needed to develop a large-scale synthesis of BOC-(*S*)-3-(2-naphthyl)alanyl-*N*-benzyl-*N*-methylamide (**2**) from BOC-L-3-(2-naphthyl)alanine (**1**). Synthesis of **2**, using conventional two-step conditions involving the addition of IBCF to a solution of BOC-L-3-(2-naphthyl)alanine (**1**) and a base (4-methylmorpholine or *N*,*N*-dimethylbenzylamine) in ethyl acetate or toluene at −12°C and then the addition of *N*-benzylmethylamine to the resulting mixed carboxylic–carbonic anhydride (**I**, Scheme 1) led to substantial amounts of urethane **3** and liberated starting acid **1**. This side reaction was also scale-dependent.[5] Urethane **3** did not present any purification problems since it was easily removed in the mother liquor during the isolation of crystalline (*S*)-3-(2-naphthyl)alanyl-*N*-benzyl-*N*-methylamide hydrochloride after the deprotection of **2** with HCl. However, multiple additions of IBCF and *N*-benzylmethylamine were necessary to recycle in situ the liberated starting material **1** and drive the reaction to amide **2** to completion.[5] The formation

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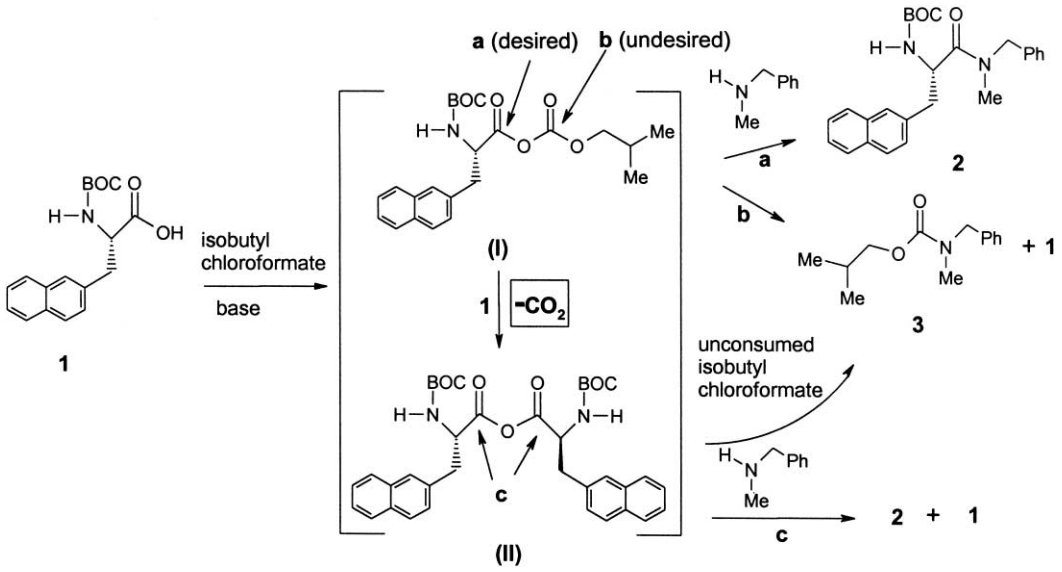

Scheme 1.

of the urethane by-product (**3**) could either be due to the aminolysis with *N*-benzylmethylamine at the undesired carbonyl[5,10–15] in the mixed carboxylic–carbonic anhydride intermediate (**I**) or because of the symmetrical anhydride (**II**) formation, leaving unconsumed IBCF in the reaction mixture that then reacts with *N*-benzylmethylamine. In both cases the reaction would also result in the liberation of the starting material **1**. To avoid the multiple additions of reagents and make the process more convenient for manufacturing, it became necessary for us to understand the mechanism of this side reaction. We have developed a novel method using the formation of CO_2 offgas as the probe for elucidating the mechanism of the formation of urethane **3** and liberation of the starting material **1**. This method was based on the rationale that any symmetrical anhydride (**II**) formation would be accompanied by the formation of an equivalent amount of CO_2 offgas during the preparation of mixed carboxylic–carbonic anhydride (**I**) from **1** by treatment with IBCF. This was because during the addition of IBCF to **1** and the base, initially formed **I** could react further with **1**, present in excess under conventional conditions, to afford the symmetrical anhydride (**II**), isobutanol, and CO_2, leaving some of isobutyl chloroformate unconsumed. Thus, if the CO_2 formation were observed during the preparation of mixed carboxylic–carbonic anhydride (**I**) (before amine addition) then the urethane **3** would form as a result of the reaction of unconsumed IBCF with *N*-benzylmethylamine. Otherwise, its origin must be aminolysis at the undesired carbonyl in **I**. Thus, by quantifying the amount of CO_2 formed during the preparation of **I**, one can also determine whether there was any aminolysis at the undesired carbonyl in **I**. This would establish whether only one or both effects are operating and to what extent. Although in situ Fourier-transform infrared spectroscopy[16] could be used to address this problem, application of this technique, which was tried initially, was complicated by several factors, including the necessity of synthesizing authentic samples of various species and the complexity of spectra due to various carbonyl group containing species and the possibility of overlapping peaks.

The addition of isobutyl chloroformate to a solution of **1** and *N*,*N*-dimethylbenzylamine in toluene led to a significant amount of CO_2 formation. Characterization and quantification of evolved CO_2 indicated the formation of 17.5% (Table 1) of symmetrical anhydride (**II**) along with **I**.[17] Further reaction of this reaction mixture with *N*-benzylmethylamine led to only minor (<2.0%)[18] aminolysis at the undesired carbonyl in **I**. These results were consistent with the amount of liberated amino acid **1** at the end of the reaction as determined by HPLC. Formation of substantial amounts of CO_2 in the first step clearly demonstrated that the formation of the urethane **3** and liberation of the starting material **1** was mainly due to the symmetrical anhydride (**II**) formation during the preparation of **I**. No corresponding isobutyl ester of **1** was detected suggesting that liberated isobutanol did not react with **I** to produce CO_2. *t*-Butyloxycarbonyl protecting group on nitrogen is known[19] to prevent the formation of oxazolinone that would have also resulted in CO_2 production from **I**. We reasoned that the symmetrical anhydride formation, and in turn the multiple additions of reagents to recycle in situ the liberated starting material **1**, could be avoided by reverting the order of addition of IBCF and the amino acid. One-stage conditions[20] involving the addition of isobutyl chloroformate to a solution of **1**, *N*-methylbenzylamine and base (4-methylmorpholine) in THF also led to the urethane formation and liberation of ~30% of **1**, again requiring multiple additions of isobutyl chloroformate to drive the reaction to completion. We have now developed a new procedure[21] that involves a reverse addition of **1** and *N*,*N*-dimethylbenzylamine in toluene to a solution of isobutyl chloroformate in toluene for the formation of **I**, followed by the addition of *N*-benzylmethylamine. These conditions led to completion of the reaction and avoided the multiple

Table 1. Comparison of conventional and new method

Conditions	Symmetrical anhydride (**II**) based on CO_2	Aminolysis[18] at undesired carbonyl in **I**
Conventional	17.5%	<2.0%
New	1.9%	<2.0%

additions of isobutyl chloroformate. Only 1.9% (Table 1) of symmetrical anhydride (**II**) formed under these conditions during the reaction of **1** with isobutyl chloroformate as determined by CO_2 analysis and confirmed by measuring liberated **1**. Such conditions involving the reverse addition of the amino acid to IBCF are not reported previously for the *N*-acylation (peptide coupling) and they represent a new methodology that was racemization-free. This newly developed reaction was successfully scaled-up in our pilot plant on a 63.0 kg scale of **1**.

In summary, a case study on the elucidation of mechanism of urethane by-product formation and starting amino acid liberation during the conventional two-step isobutyl chloroformate mediated *N*-acylation using carbon dioxide offgas as the probe is described. The formation of the symmetrical anhydride from the amino acid in the first step involving the preparation of the mixed carboxylic–carbonic anhydride intermediate, as determined by quantifying the evolved carbon dioxide, was found to be the main reason for the urethane formation and starting amino acid liberation in our case under conventional conditions. Only minor amounts of these two by-products resulted from the aminolysis at the undesired carbonyl group in the mixed carboxylic–carbonic anhydride intermediate in the second step. Based on this mechanistic understanding, a new protocol for the coupling of **1** with *N*-benzylmethylamine, involving a reverse addition of **1** and the base to isobutyl chloroformate, was developed that decreased the formation of symmetrical anhydride to <2%. This methodology will be useful to address this side reaction also in peptide synthesis.

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17. Reactions were carried out in a Mettler–Toledo RC1 reaction calorimeter equipped with a 1 L glass MP-10 vessel. 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 ID) and a thermal conductivity detector. Offgas volume was determined using a Ritter model TG05 wet test meter. The wet test meter was placed downstream of the GC sampling loop, and the cumulative volume was recorded using a datalogging system. During the experiment, which was always performed with nitrogen 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. The flow rate of the CO_2 offgas was determined by subtracting the N_2 purge gas flow rate determined earlier from the total offgas rate during the experiment. The cumulative CO_2 offgas volume was subsequently determined by numerical integration of the CO_2 flow rate. 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 validity of the offgas measurement was confirmed by first forming a known amount of CO_2 by the neutralization of sodium bicarbonate with sulfuric acid. (A manuscript describing the details of this methodology is in preparation).

18. Calculated based on the mass balance on **1** remaining at the end of the reaction and CO_2 formed in the first step (a manuscript with detailed methodology is under preparation).
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21. The Mettler–Toledo Labmax reactor, equipped with a 1 L glass vessel, pitched-blade impeller, RTD sensor, addition funnel, nitrogen inlet/outlet and distillation setup was charged with BOC-L-3-(2-naphthyl)alanine (**1**, 51.2 g, 162.3 mmol) and toluene (130.0 mL). The suspension was cooled to an internal temperature at 20±2°C and *N*,*N*-dimethylbenzylamine (29.64 g, 219.3 mmol) was added over a period of 10 min while maintaining the internal temperature at 20±2°C (jacket temperature 17±3°C). The addition funnel was washed with toluene (5.2 mL) and the wash was added to the reaction mixture. This solution was held for further use.

    The Mettler–Toledo Labmax reactor, equipped with a clean 1 L glass vessel, pitched-blade impeller, RTD sensor, addition funnel, nitrogen inlet/outlet and distillation setup was charged with toluene (390.0 mL) and isobutyl chloroformate (28.86 g, 211.3 mmol). The solution was cooled to an internal temperature at −12±2°C, and the above-prepared solution of BOC-L-3-(2-naphthyl)alanine (**1**) and *N*,*N*-dimethylbenzylamine in toluene was added over a period of 40 min while maintaining an internal temperature at −12±3°C (jacket temperature −15±3°C). The addition funnel was washed with toluene (2×5.2 mL) and added to the reaction mixture. The slurry was stirred at −12±2°C for an additional 30 min, and a solution of *N*-benzylmethylamine (24.6 g, 203.0 mmol) in toluene (15.6 mL) was added over a period of 30 min while maintaining an internal temperature at −12±2°C (jacket temperature −15±3°C). The addition funnel was washed with toluene (2×5.2 mL) and added to the reaction mixture. The reaction mixture was stirred at −12±2°C for 30 min and then warmed to an internal temperature at 21±2°C over a period of 35 min (the completion of the reaction was monitored by HPLC). The reaction mixture was quenched by addition of 1N H_2SO_4 (125.0 mL) and stirred for 10 min. The organic layer was separated and washed sequentially with water (125.0 mL), 5% aqueous sodium bicarbonate (125.0 mL), and water (125.0 mL). The organic layer was concentrated at a jacket temperature of 65±5°C in vacuo (150 mbar) to collect 340 mL (∼309.0 g) of solvent and obtain a of solution of BOC-(*S*)-3-(2-naphthyl)alanyl-*N*-benzyl-*N*-methylamide in toluene (**2**, 310.0 mL, 272.0 g; containing 68.0 g of **2**, assumed 100% yield) that was used crude in the next step.