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Micro-flow synthesis of β-amino acid derivatives *via* a rapid dual activation approach†

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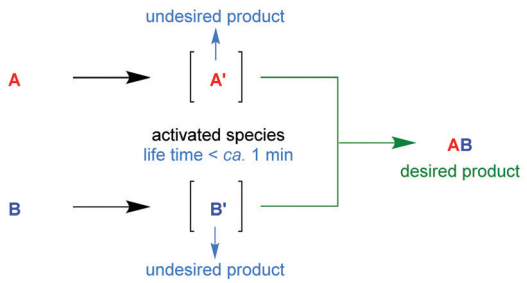
Rapid dual activation (≲3.3 s) of both β-amino acid *N*-carboxy anhydride and alkyl chloroformate for the synthesis of a β-amino acid-derived scaffold was demonstrated. The key to success was the use of rapid mixing enabled by using a micro-flow reactor. The one-flow synthesis of 22 β-amino acid derivatives was achieved.

Dual activation is an attractive synthetic approach (Scheme 1), because it enables challenging coupling reactions that are impossible or insufficient using other approaches. Elegant bifunctional and synergistic catalysts have been developed as dual activators of both substrates **A** and **B**.[1] One of the important challenges in developing a dual activation approach is the avoidance of undesired reactions from activated species **A**′ and **B**′.[2] In particular, if the lifetime of the active species **A**′ and **B**′ is insufficient compared to the mixing time of the solutions (*ca.* <1 min),[3] it would be difficult to obtain satisfactory results, because undesired reactions from **A**′ and **B**′ occur concomitantly with their generation during mixing. In order to avoid these undesired reactions, a rapid dual activation of substrates, and their immediate use for the subsequent coupling reactions would be a valuable aid in developing dual activation approaches.

β-Amino acid derivatives are attractive as drug candidates,[4] asymmetric catalysts in organic transformation,[5] and organogels for surface modification.[6] They are usually synthesized by stepwise chemical modifications of amino- and/or carboxyl groups in β-amino acids.[7] β-Amino acid-derived scaffolds containing multiple-reaction sites that allow selective and sequential chemical modifications are useful for the rapid synthesis of β-amino acid derivatives. In 1966, Iwakura and coworkers reported the preparation of β-amino acid-derived scaffold **I** containing both isocyanate and acyl chloride moieties (Scheme 2a) and its use in a one-pot, three-component coupling reaction to synthesize β-amino acid derivatives **II**.[8] Mormann and coworkers also reported derivatization and polymerization based on **I**.[9] Despite the usefulness of **I**, its preparation requires treatment with toxic gases such as phosgene and HCl, the expenditure of time (*ca.* 1 day), and heating conditions (65–70 °C). This complicates the synthesis of **I** containing acid labile R^1 and/or R^2. The development of a mild and rapid synthesis of β-amino acid-derived scaffolds containing multiple reaction centers remains to be an important pursuit.



Scheme 1 Dual activation approach for the coupling of substrates **A** and **B**, and the risk of undesired reactions from activated species **A**′ and **B**′.

Micro-flow technology[10] allows precise control for a short amount of time by rapid mixing (milli-seconds)[11] as a result of shortening of the diffusion length. Recently, we reported the one-step micro-flow synthesis of β-amino acid *N*-carboxy anhydrides (β-NCAs) **IV**.[12] Herein, we report the rapid and mild synthesis of a β-amino acid-derived scaffold **VI** containing isocyanate and mixed carbonic anhydride moieties, and its use in the synthesis of various β-amino acid derivatives. The key to success was the rapid generation of active species **IV**′ and **V**′ and their immediate use in a subsequent coupling reaction, which avoided the undesired oligomerization/polymerization of **IV**′ and the decomposition of **V**′. This accomplishment was realized *via* precise control of the reaction time (≲3.3 s) using micro-flow technology.

We examined the rapid dual activation of β-NCA (**1a**, racemic mixture) and benzyl chloroformate (CbzCl) for the synthesis of

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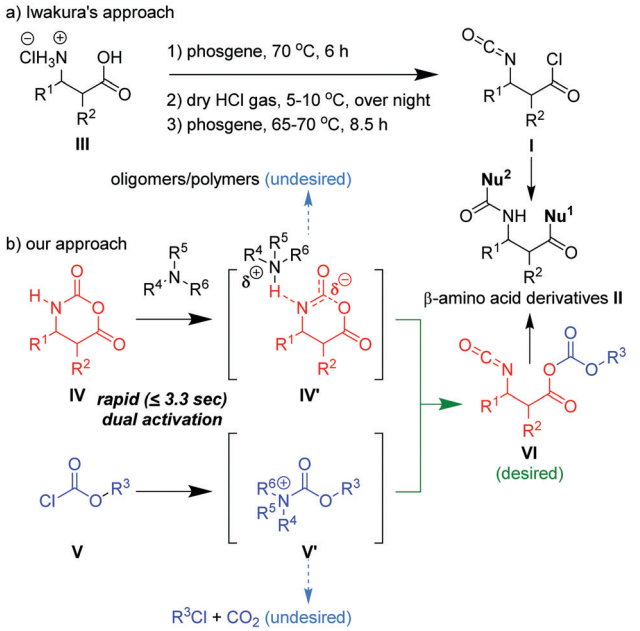
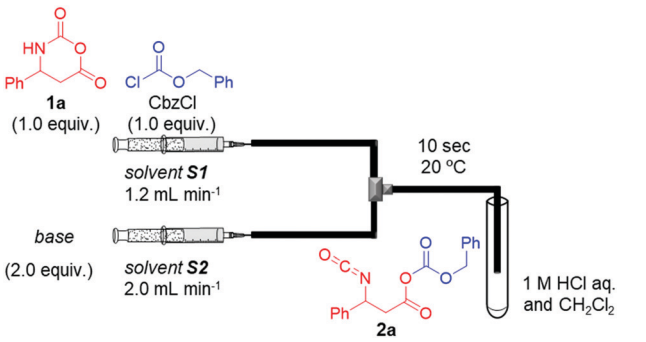


Scheme 2 (a) Reported preparation of β-amino acid-derived scaffold **I** containing isocyanate and acyl chloride moieties. (b) Preparation of β-amino acid-derived scaffold **VI** containing isocyanate and mixed carbonic anhydride moieties *via* the rapid dual activation approach reported in this study.

β-phenylalanine-derived scaffold **2a**. Bases, reaction time and solvents were examined, as shown in Table 1. We connected a T-shape mixer and Teflon® tubing and immersed them in a water bath (20 °C). Then, β-NCA **1a** (1.00 equiv.) and CbzCl (1.00 equiv.) in solvent **S1** and a base (2.00 equiv.) in solvent **S2** were independently injected into the T-shape micromixer. The resultant mixture was poured into a mixture of 1 M HCl aq. and CH_2Cl_2. Following a simple aqueous workup, the yield was determined *via* ^{1}H NMR analysis. No reaction of **1a** occurred in the absence of a base (entry 1). Insufficient yields occurred with the sole use of either pyridine or i-Pr_2NEt (entries 2 and 3), whereas an excellent yield was observed when both pyridine and i-Pr_2NEt were used in combination (entry 4). It is well known that pyridine reacts with acyl chloride to form a highly electrophilic acylpyridinium cation, but the nucleophilic substitution of i-Pr_2NEt against acyl chloride is slow.[17] Therefore, it is reasonable that low yields were observed (entries 2 and 3), because pyridine, which is a nucleophilic and weak base, could not activate **1a**, while, i-Pr_2NEt, which is a less nucleophilic strong base, could not sufficiently activate CbzCl. However, the combined use of pyridine and i-Pr_2NEt dually activated both substrates, which produced an excellent yield (entry 4). From a practical point of view, the dual activation of both substrates by a sole base would be desirable. Examination of various bases revealed that the sole use of Et_3N or *N*-ethylmorpholine afforded **2a** in good yields (entries 5 and 6). It is conceivable that these bases could react with CbzCl to form acylammonium cations due to their decreased steric bulkiness by comparison with that of i-Pr_2NEt. In addition, each was sufficiently basic to activate **1a**. The use of *N*-methylpiperidine, *N*,*N*-dimethylaminopyridine (DMAP), and Me_2NBn resulted in the generation of a complex mixture probably due to an undesired

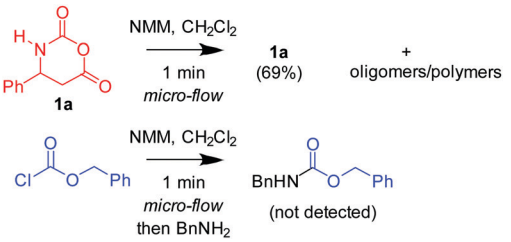
Table 1 Rapid dual activation of **1a** and benzyl chloroformate for the synthesis of **2a**



Entry	Base	Solvent S1	Solvent S2	Yield[b] (%)
1	—	CH_2Cl_2	CH_2Cl_2	n.r.[c]
2	Pyridine (pK_a = 5.2)[13]	CH_2Cl_2	CH_2Cl_2	n.r.[c]
3	i-Pr_2NEt (pK_a = 11.4)[14]	CH_2Cl_2	CH_2Cl_2	28
4	i-Pr_2NEt + pyridine[a]	CH_2Cl_2	CH_2Cl_2	99
5	Et_3N (pK_a = 10.7)[13]	CH_2Cl_2	CH_2Cl_2	85
6	*N*-Ethylmorphorine (pK_a = 7.7)[13]	CH_2Cl_2	CH_2Cl_2	70
7	*N*-Methylpiperidine (pK_a = 10.1)[15]	CH_2Cl_2	CH_2Cl_2	—[d]
8	DMAP (pK_a = 9.6)[14]	CH_2Cl_2	CH_2Cl_2	—[d]
9	Me_2NBn (pK_a = 8.9)[15]	CH_2Cl_2	CH_2Cl_2	—[d]
10	NMI (pK_a = 7.0)[16]	CH_2Cl_2	CH_2Cl_2	n.r.[c]
11	NMM (pK_a = 7.4)[13]	CH_2Cl_2	CH_2Cl_2	>99[g] (>99[e])
12	NMM	THF	CH_2Cl_2	96
13	NMM	THF	THF	—[f]
14	NMM	MeCN	CH_2Cl_2	73
15	NMM	MeCN	MeCN	62
16	NMM	CH_2Cl_2	CH_2Cl_2	68–89[gh]

[a] 1.0 + 1.0 equiv. [b] Yields were determined *via* ^{1}H NMR analysis using 1,1,2-tirichloroethane as an internal standard. [c] No reaction of **1a** occurred. [d] A complex mixture was generated. [e] Isolated yields. [f] The reactor was clogged. [g] Three independent experiments were carried out. [h] Batch conditions included 1000 rpm.

oligomerization/polymerization of **1a** (entries 7–9), whereas the use of NMI resulted in no reaction (entry 10). We speculated that the use of a strong base (pK_a > 8 and 9), with the noted exception of i-Pr_2NEt, induced a very rapid oligomerization/polymerization of **1a**, but a weak base (pK_a ≤ 7) could not activate **1a**. It seemed that the basicity of i-Pr_2NEt (pK_a = 11.4) was sufficient, but its bulkiness avoided the undesired reactions with **1a** (entries 3 and 4). To our delight, the sole use of *N*-methylmorphorine (NMM), which has medium levels of both nucleophilicity and basicity, quantitatively afforded **2a** (entry 11). The use of either THF or MeCN instead of CH_2Cl_2 resulted in a decrease in yields or clog generation (entries 12–15). In order to verify the importance of the microflow conditions, comparative batch conditions were used. Although the reaction mixture was vigorously stirred during the experiment, reproducible results were not observed due to batch-to-batch differences in the mixing efficiency (entry 16). Although base-induced rapid oligomerization/polymerization of **1**[18] and rapid decarboxylation of acylammonium cation generated from alkyl chloroformate[19] are known to occur, we experimentally confirmed the instability of the activated species (Scheme 3). The reaction of **1a** with NMM in the absence of CbzCl for 1 min induced oligomerization/polymerization and 69% of **1a** was



Scheme 3 Reaction of **1a** or CbzCl with NMM in the absence of a coupling partner in order to confirm the instability of the generated activated species.

recovered. The reaction of CbzCl with NMM in the absence of **1a** for 1 min and a subsequent nucleophilic addition using benzylamine[20] was performed. The amide was not detected. The results indicated rapid decarboxylation of the *in situ* generated acylammonium cation. Thus, rapid activation of **1a** and CbzCl and their immediate use for the coupling were important in order to avoid these undesired reactions.

β-Amino acid-derived scaffold **2** has isocyanate and mixed carbonic anhydride moieties that allow further chemical modifications. We carried out *in situ* generation of **2**[21] and the following reaction with various nucleophiles using the two electrophilic reaction sites of **2** in a collection flask (Fig. 1). β-NCA **1** (1.0 equiv.), isobutyl chloroformate (1.0 equiv.),[22] and NMM (2.0 equiv.) were reacted to generate **2** *in situ*, which was reacted with various nucleophiles (2.0 equiv.) without isolation to obtain β-amino acid derivatives **3**. Desired derivatives **3a–3d** containing an alkyl or a phenyl side chain were obtained in high yields. It should be noted that **3e** and **3f** containing acid-labile *tert*-butyl ester or silyl ether moieties that cannot be synthesized by Iwakura's approach were obtained in high yields. Various primary and secondary amines including α- and β-amino acids could be used as nucleophiles and the desired derivatives **3g–3k** were obtained in excellent yields, whereas the use of bulky diisopropyl amine as a nucleophile did not afford the desired product **3l**.

In order to expand the structural diversity of the β-amino acid derivatives obtained by the developed approach, we examined the sequential introduction of two different nucleophiles into *in situ* generated **2b** (Fig. 2). β-NCA **1** (1.0 equiv.), isobutyl chloroformate (1.0 equiv.), and NMM (2.0 equiv.) were reacted to generate **2b** *in situ*. The first nucleophile (**Nu**^{1}, 1 equiv.) was reacted with **2b** in the presence of a catalytic amount of DMAP (0.1 equiv.) that preferentially activated the mixed carbonic anhydride moiety to generate a highly active acyl pyridinium cation (for details, see ESI†). The second nucleophile (**Nu**^{2}, 1 equiv.) was reacted in the presence of a stoichiometric amount of i-Pr$_2$NEt (1 equiv.)[23] in the collection flask to afford the desired products **4** and **5**. The sequential introduction of various primary and secondary amines including α- and β-amino acids and subsequent 1-octanethiol afforded the desired products **4a–4e** in good yields.[24] The use of either a benzyl mercaptan or a thiophenol instead of 1-octanethiol also afforded the desired products **4f** and **4g** in good yields. The sequential introduction of different amines was examined because β-amino acid derivatives containing both urea and amide moieties are attractive as drug candidates and organogels.[6,7a,d] The desired products **4h** and **4i** were obtained in good to excellent yields. We carried out dihydrouracil synthesis because they are frequently found in bioactive compounds.[25] The desired products **5a** and **5b** were

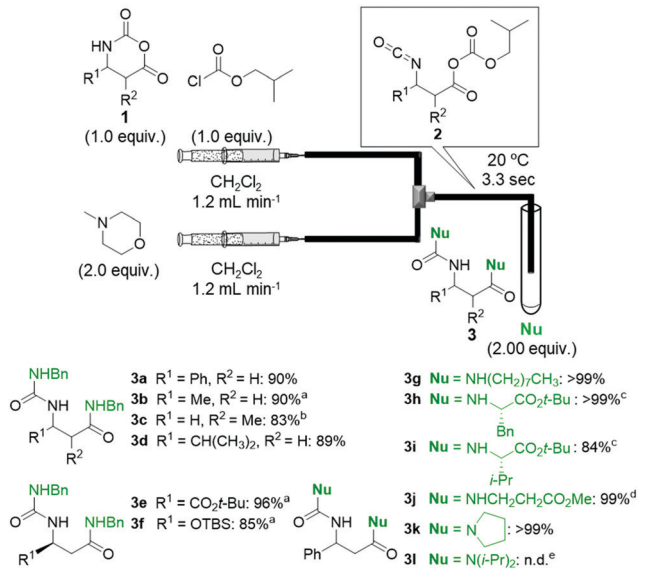


Fig. 1 Introduction of a nucleophile against *in situ* generated **2**. [a] THF was used to dissolve **1** and isobutyl chloroformate. [b] CH_2Cl_2/MeCN (v/v = 1/1) was used to dissolve **1** and isobutyl chloroformate. [c] α-Amino acid *tert*-butyl ester hydrochloride was used as Nu. [d] β-Alanine methyl ester hydrochloride was used as Nu. [e] The desired product was not detected.

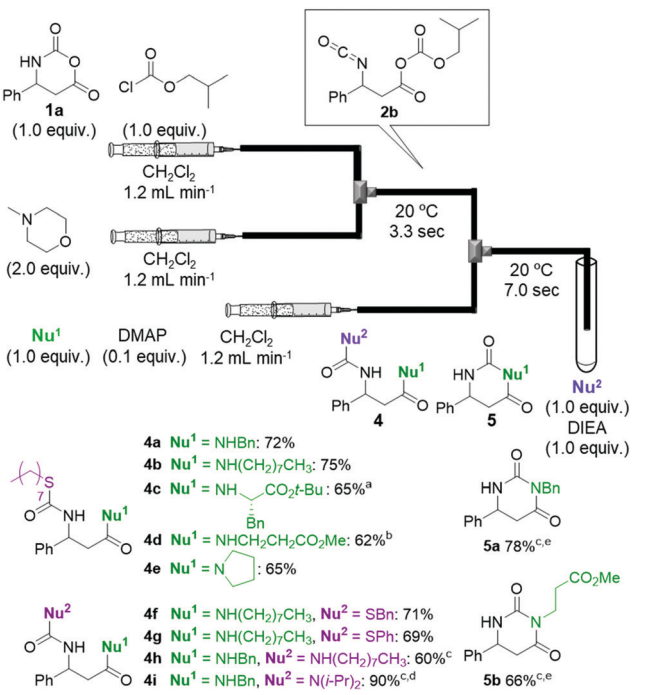


Fig. 2 Introduction of different nucleophiles against *in situ* generated **2**. [a] α-Amino acid *tert*-butyl ester hydrochloride was used as Nu. [b] β-Alanine methyl ester hydrochloride was used as **Nu**^{1}. [c] i-Pr$_2$NEt (1.0 equiv.) was not used. [d] 2.0 equiv. of diisopropylamine was used as **Nu**^{2}. [e] The cyclization was carried out at 100 °C for 2 h in toluene.

obtained in good yields without using a second nucleophile in the collection flask.

We demonstrated the mild and rapid synthesis of β-amino acid-derived scaffold **2** containing isocyanate and mixed carbonic anhydride moieties. The key to success was a ''rapid dual activation'' ($\leq$3.3 s) of **1** and alkyl chloroformate in the presence of NMM and their immediate use for a subsequent coupling reaction with the avoidance of undesired reactions of the activated species. This could only be realized by the use of rapid mixing that was enabled by using a micro-flow reactor. These 22 structurally diverse β-amino acid derivatives are attractive as drug candidates, and their one-flow synthesis was demonstrated. Again, we emphasize that the developed reaction can be safely and reproducibly performed only *via* the use of micro-flow technology. The developed methodology should be useful for developing various dual activation approaches and be valuable for accelerating drug, catalyst, and material discovery based on β-amino acid derivatives.

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Conflicts of interest

There are no conflicts to declare.

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21 We examined substrate scope with the reaction time of 3.3 s. Because yield was not changed by reducing the reaction time from 10 s to 3.3 s in our preliminary examination. Details, see ESI†.

22 Inexpensive, readily available, and stable isobutyl chloroformate was used instead of CbzCl for investigation of substrate scope. We experimentally confirmed instability of acylammonium cation generated from isobutyl chloroformate. Details, see ESI†.

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