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Rapid Dual Activation Approach in a Micro-flow Reactor for the Synthesis of Urethane-Protected α-Amino Acid N-Carboxy Anhydrides and Its Mechanistic Insight

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ARTICLE

Micro-Flow Rapid Dual Activation Approach for the Urethane-Protected α-Amino Acid *N*-Carboxy Anhydride Synthesis

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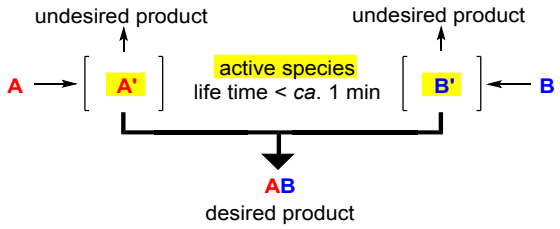
Abstract: This study demonstrated the rapid dual activation (10 s, 20 °C) of a combination of α-amino acid *N*-carboxyanhydride and alkyl chloroformate in the synthesis of urethane-protected α-amino acid *N*-carboxyanhydride in a micro-flow reactor. The key to success was the combined use of two amines that activated both substrates with proper timing. Three amines, *i*-Pr_2NEt, Me_2NBn, or *N*-ethylmorpholine were used with pyridine in accordance with the steric bulkiness of a side chain in amino acid *N*-carboxyanhydride. A variety of 16 urethane-protected α-amino acid *N*-carboxyanhydrides were synthesized in high yields. The role of amines was investigated based on measurements of time-dependent (0.5 to 10 s) decrease of α-amino acid *N*-carboxyanhydrides and alkyl chloroformate in the presence of amine *via* flash mixing technology using a micro-flow reactor. It was suggested that in situ generated acylpyridinium cation was highly active and less prone to causing undesired decompositions compared with acylammonium cation examined in this study. Thus, even at a very low concentration, acylpyridinium cation facilitated the desired coupling reaction.

Introduction

A synthetic approach that allows the dual activation of both substrates **A** and **B** is powerful because it enables a coupling of substrates that would be either impossible or inefficient *via* other approaches (Scheme 1). A number of reactions have been reported using either bifunctional or synergistic catalysts that activate both substrates.[1] With the dual activation approach, the suppression of undesired reactions from active species **A'** and **B'** is highly important.[2] In particular, when the lifetimes of **A'** and **B'** are less than 1 min, undesired reactions could occur before solutions are completely mixed, which increases the difficulty in obtaining satisfactory results.[3] Rapid dual activation of both substrates *via* flash mixing (< several milli seconds) in a micro-flow reactor[4,5] is an attractive approach to overcoming this difficulty. In 2014, Nagaki and Yoshida reported pioneering work in rapid dual activation. In that study, highly active aryllithium and benzyne were rapidly generated and coupled, while undesired reactions were suppressed *via* flash mixing technology using a micro-flow reactor.[6] As far as we could ascertain, that study and our previous report (described hereafter) remain the only examples of a rapid dual activation approach. In order to further increase the usefulness of this approach, an expansion of its applications is highly important.

Urethane-protected α-amino acid *N*-carboxyanhydride (UNCA) **I** is a useful building block for peptide synthesis because it enables rapid and coupling agent-free peptide bond formation with the emission of only carbon dioxide.[7] In addition,various organic transformations have been reported such as reductions to corresponding *N*-protected β-amino alcohols,[8a] dimerizations,[8b,c] and reactions with either organometallic reagents[8d-f] or an organo-catalyst[8g] in the syntheses of a variety of *N*-protected amino acid derivatives. Conventional synthesis of UNCA includes the coupling of *N*-urethane-protected bis-trimethylsilyl amino acids with phosgene and the activation of bis-urethane-protected amino acids with thionyl chloride or oxalyl chloride (not shown).[8a,g,9] The former approach suffers from a narrow substrate scope, however, and the latter approach requires multiple synthetic steps. Therefore, a reaction of alkyl chloroformate **II** and α-amino acid *N*-carboxyanhydride (α-NCA) **III** in the presence of *N*-methylmorpholine (NMM) is the most frequently used approach for the synthesis of UNCA **I** (Scheme 2a).[10] However, this approach requires the careful control of temperature (-30 to 0 °C) and addition rate of NMM in order to avoid undesired reactions from **II** and **III** that decreases the utility of this approach. We recently reported a rapid dual activation of alkyl chloroformate **II** and β-NCA **IV** with NMM in a micro-flow reactor that successfully avoided the undesired reactions from



Scheme 1. Dual activation approach for the coupling of substrates **A** and **B**.

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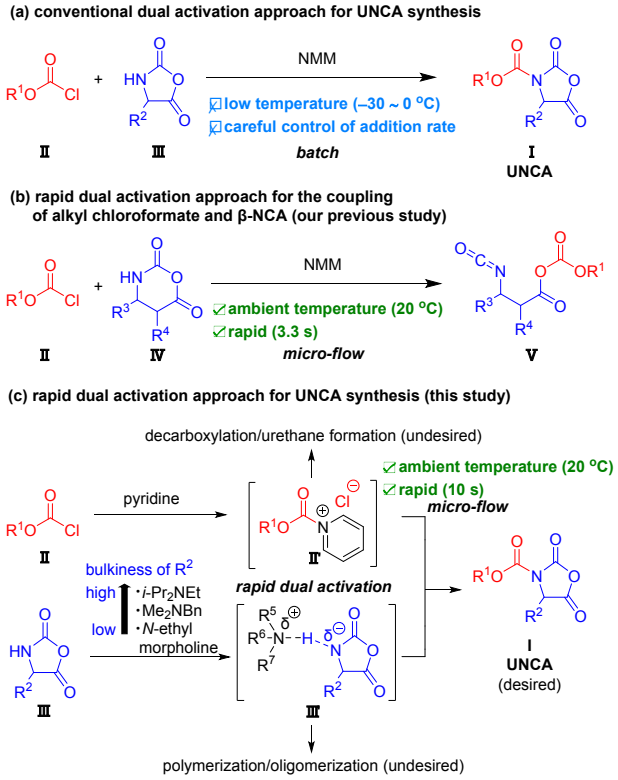


Scheme 2. (a) Conventional dual activation approach using NMM for synthesis of UNCA **I**. (b) Our previous rapid dual activation approach using NMM for the coupling of alkyl chloroformate **II** and β-NCA **IV** for synthesis of β-amino acid derivatives. (c) Rapid dual activation approach using three combinations of bases for synthesis of UNCA **I** (this study).

II and **IV**. The ring opening of β-NCA is reportedly easier than that of α-NCA,[11] and our developed approach afforded a β-amino acid scaffold **V** that contained isocyanate and mixed carbonic anhydride moieties (Scheme 2b).[12]

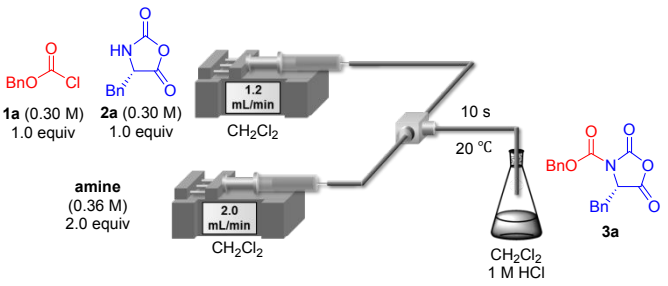
Herein, we report a rapid and mild synthesis of UNCA **I** from alkyl chloroformate **II** and α-NCA **III**[13] that is based on rapid dual activation in a micro-flow reactor (Scheme 2c). Typically in this reaction, α-NCA rapidly undergoes undesired oligomerization/polymerization,[14] and alkyl chloroformate decomposes *via* decarboxylation or urethane formation in the presence of amines.[15] Use of the rapid dual activation approach and the proper combination of two amines were both important in avoiding those undesired reactions. Interestingly, although NMM afforded the best results both in the most frequently used approach and in our previous approach (Schemes 2a and 2b), it afforded unsatisfactory results in the present approach (Scheme 2c). The role of the amines was investigated *via* the measurements of time-dependent decreases in α-NCAs and in alkyl chloroformates in the presence of the amines.

Results and Discussion

UNCA formation was examined using benzyl chloroformate (Cbz-Cl, **1a**) and α-phenylalanine-NCA[13a] (α-Phe-NCA, **2a**) in the presence of various amines (Table 1). The mixtures of **1a** (1.0 equiv) and **2a** (1.0 equiv) in CH_2Cl_2 and the amine (2.0 equiv) in CH_2Cl_2 were independently injected into a T-shaped mixer and reacted at 20 °C for 10 s. The resultant mixture containing Cbz-α-Phe-NCA (**3a**) was poured into a mixture of 1 M HCl and CH_2Cl_2. Following a simple aqueous workup, the yield of **3a** was determined *via* ^{1}H NMR analysis using an internal standard. Selected results appear in Table 1 (All the results are shown in the supporting information).

The use of weak bases such as pyridine (p*K*aH = 5.2) or *N*-methyimidazole (NMI, p*K*aH = 7.0) afforded **3a** in yields of 34% and 27%, respectively, with recovery of a large amount (68%) of unreacted **2a** (entries 1 and 2). It is conceivable that the basicity of the amines was insufficient for the activation of **2a** (p*K*aH = 11.3).[16] Interestingly, the use of NMM (p*K*aH = 7.4), which is used in the most conventional approach as well as in our previous approach (Schemes 2a and 2b), afforded **3a** in a moderate yield (56%) with a concomitant recovery (20%) of **2a** (entry 3). The use of *i*-Pr_2NEt—with its high basicity (p*K*aH = 11.4) and poor nucleophilicity— did not afford the desired **3a**, but did generate white precipitates, which probably was due to the undesired polymerization of **2a** (entry 4). We presumed that both the basicity (for activation of **2a**) and the nucleophilicity (for activation of **1a**) of the amines are indispensable for this reaction because it is well known that highly electrophilic acylammonium cations (acylAm$^+$) are generated from chloroformates and nucleophilic tertiary alkylamines.[17] As expected, the use of nucleophilic Me_2NBn with a medium level of basicity (p*K*aH = 8.9) afforded **3a** in a high yield (91%; entry 5); the use of either poorly nucleophilic *N*-ethylmorpholine (p*K*aH = 7.7) or Et_2NBn (p*K*aH = 9.5), however, afforded **3a** in lower yields (entry 3 vs. 6 and entry 5 vs. 7), although both have similar levels of basicity that compare to those of either NMM or Me_2NBn. Interestingly, the use of highly nucleophilic DMAP

Table 1. Examination of amines for micro-flow synthesis of **3a**



entry	amine (p*K*aH[a])	yield[b] (%)	
		3a	**2a**
1	pyridine (5.2)[18]	34	68
2	NMI (7.0)[19]	27	68
3	NMM (7.4)[18]	56	20
4	*i*-Pr_2NEt (11.4)[21]	0	0
5	Me_2NBn (8.9)[20]	91	0
6	*N*-ethylmorpholine (7.7)[20]	38	20
7	Et_2NBn (9.5)[20]	5	0
8	DMAP (9.7)[19]	36	0

[a]The p*K*a of conjugated acids in water. [b]Yields were determined by ^{1}H NMR analysis using 1,1,2-trichloroethane as an internal standard.

(p*K*aH = 9.7) afforded **3a** in a low yield (36%) with no recovery of **2a** (entry 8). The reasons for these results will be discussed subsequently.

In order to verify the above considerations, we investigated the time-dependent decrease of α-NCA **2a** in the presence of an amine (pyridine, NMI, NMM, Me_2NBn, DMAP, or *i*-Pr_2NEt) *via* flash mixing technology using a micro-flow reactor. The amine (1.0 equiv) and **2a** (1.0 equiv) were reacted at 20 °C for 0.5 to 10 s, and the unstable α-NCA was converted to the corresponding stable amide **4a** *via* reaction with an excess amount of isopropyl amine (*i*-$PrNH_2$, 5.0 equiv), as shown in Figure 1. The yield of **4a** was determined *via* ^{1}H NMR analysis using an internal standard. If the amine-mediated oligomerization/polymerization occurred, the yield of **4a** would decrease, and, thus, the time-dependent decrease of **2a** in the presence of the amine could be indirectly estimated. Not surprisingly, the use of highly basic *i*-Pr_2NEt (p*K*aH = 11.4) resulted in a time-dependent decrease in the yield of **4a**. The generation of white precipitates was observed, which probably was due to the polymerization of **2a**. A time-dependent decrease in the yield of **4a** was also observed when DMAP (p*K*aH = 9.7) was used as the amine. Since no precipitation was observed in this case, we speculated that soluble oligomers were generated from **2a**. We observed a DMAP-mediated rapid degradation of UNCA in our preliminary study; therefore, the unsatisfactory result (36%) observed in the synthesis of UNCA **3a** (Table 1, entry 8) is attributable to the undesired degradation and/or oligomerization of UNCA and NCA due to the high nucleophilicity of DMAP. A slight decrease (2-3% at 10 s) in the yield of **4a** was observed in the cases of both NMM (p*K*aH = 7.4) and Me_2NBn (p*K*aH = 8.9) with medium levels of basicity, but no decrease in the yields of **4a** were observed in the cases of the low basicities of either pyridine (p*K*aH = 5.2) or NMI (p*K*aH = 7.0). It is reasonable that the deprotonation of α-NCA **2a** was increased as the basicity of the amine increased, and both the undesired oligomerization/polymerization and the desired nucleophilic substitution against alkyl chloroformate were accelerated.

Next, the time-dependent decrease of Cbz-Cl (**1a**) in the presence of an amine (pyridine, NMI, NMM, Me_2NBn, DMAP, or *i*-Pr_2NEt) was investigated *via* flash mixing technology using a micro-flow reactor. An amine (1.0 equiv) and **1a** (1.0 equiv) were reacted at 20 °C for 0.5 to 10 s, and the unstable Cbz-Cl and/or acylAm$^+$ or acylpyridinium cation (acylPy$^+$) were converted to the corresponding stable carbamate **5a** *via* reaction with an excess amount of *i*-$PrNH_2$ (5.0 equiv), as shown in Figure 2. If the decomposition of acylAm$^+$ or acylPy$^+$ shown in Scheme 3 occurred, the yield of **5a** would decrease, which would allow the indirect estimation of the time-dependent decrease of **1a** in the presence of various amines. Rapid decreases in the yields of **5a** were observed in the cases of NMM, pyridine, or Me_2NBn. As previously described, alkyl chloroformate and either a nucleophilic tertiary alkylamine or pyridine and its derivative will form either acylAm$^+$ or acylPy$^+$. Both of these highly electrophilic species readily occur decarboxylation (Schemes 3a and 3b, red colored arrows).[15e-g] In addition, acylAm$^+$ readily occur urethane formation *via* dealkylation (Scheme 3b, blue colored arrow).[15a-d] Brunelle and coworkers analyzed the equilibrium between tertiary alkylamine + phenyl chloroformate (PhOCOCl) and the corresponding acylAm$^+$ *via* ^{1}H NMR in CD_2Cl_2 solvent.[22] The equilibrium lies significantly to the acylAm$^+$ (K_{eq} > 50,000) when

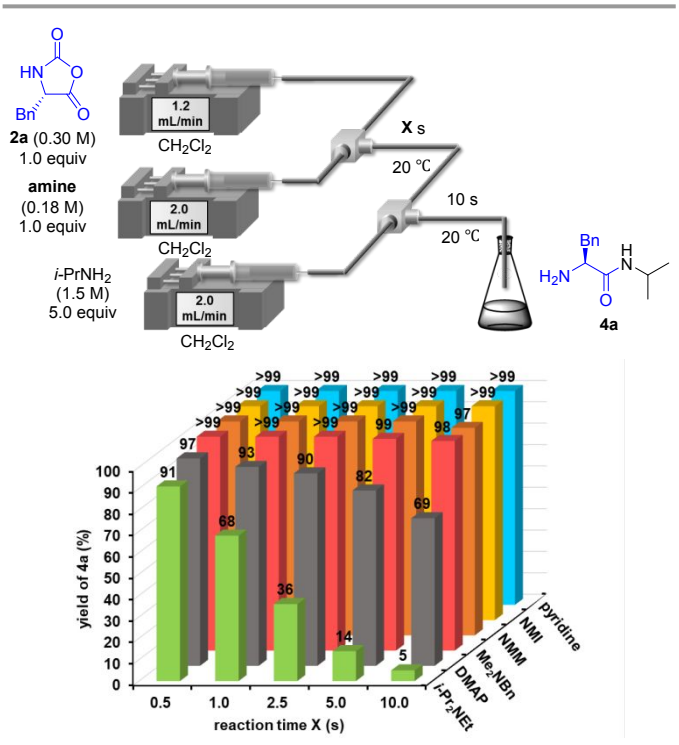


Figure 1. Time-dependent decrease of α-NCA **2a** in the presence amine (pyridine, NMI, NMM, Me_2NBn, DMAP, or *i*-Pr_2NEt) that was estimated from the yield of amide **4a**.

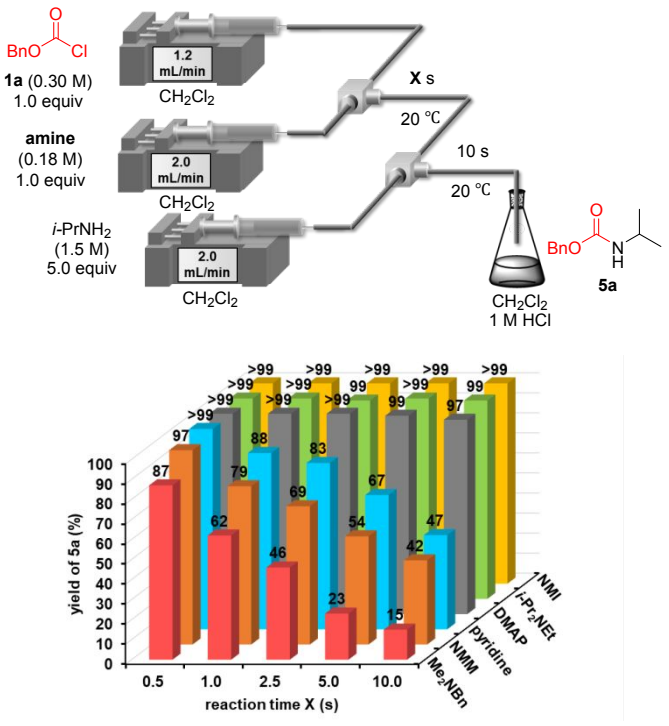
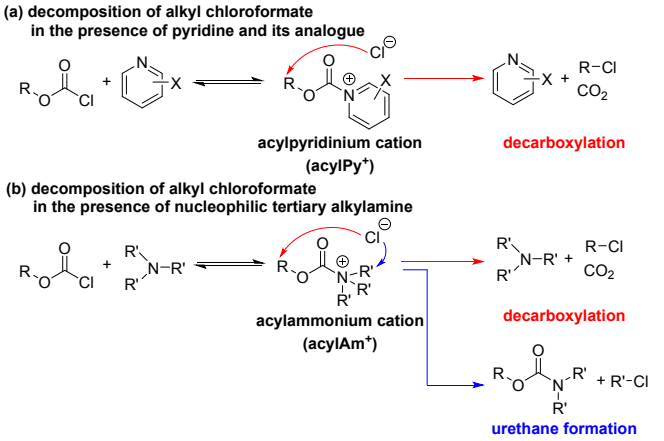
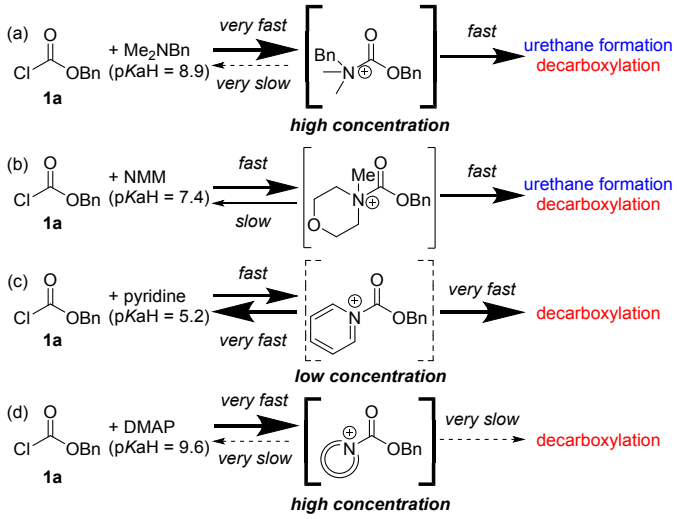


Figure 2. Time-dependent decrease of Cbz-Cl (**1a**) in the presence of amine (pyridine, NMI, NMM, Me_2NBn, DMAP, or *i*-Pr_2NEt) that was estimated from the yield of carbamate **5a**.



Scheme 3. Reported decomposition mechanisms of alkyl chloroformates in the presence of (a) pyridine and its analog *via* acylpyridinium cataion (acylPy$^+$) and (b) nucleophilic tertiary alkylamine *via* acylammonium cation (acylAm$^+$).



Scheme 4. Plausible decomposition mechanisms of Cbz-Cl in the presence of Me_2NBn (a), NMM (b), pyridine (c), and DMAP (d).

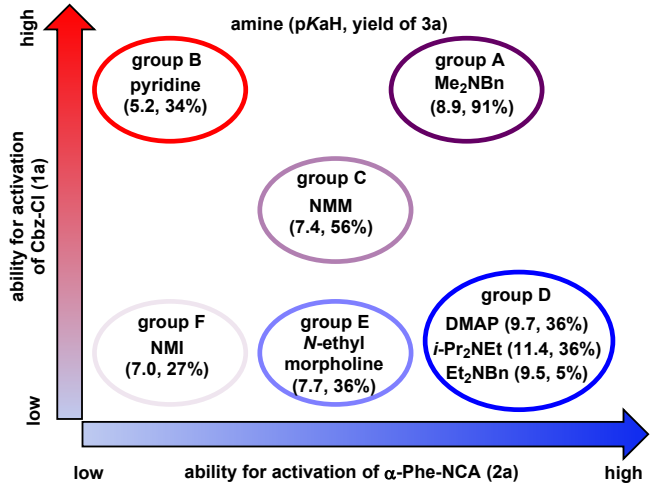
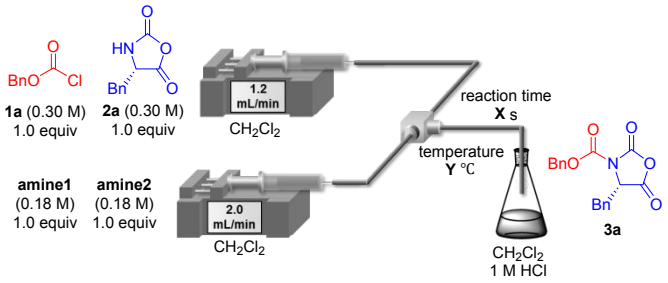


Figure 3. Estimated abilities of the used amines for activation of Cbz-Cl (**1a**) and α-Phe-NCA (**2a**). The p*K*aH and yield of **3a** in Table 1 were indicated in parentheses following the name of amines.

the amine has at least one methyl (= less bulky) group. It is reasonable that Me_2NBn containing two methyl groups and NMM containing one methyl group would readily form the corresponding acylAm$^+$ that leads to urethane formation and/or decarboxylation (Schemes 4a and 4b). Unexpectedly, an obvious time-dependent decrease in the yield of **5a** was observed in the presence of pyridine. Brunelle and coworkers reported that the equilibrium between pyridine + PhOCOCl and acylPy$^+$ (K_{eq} < 0.001) lies significantly to the pyridine + PhOCOCl in CD_2Cl_2 solvent.[22] According to the Brunelle report, the concentration of acylMe$_2$NBn$^+$ (generated from Me_2NBn and PhOCOCl**)** is estimated to be more than 5 × 10^7 times greater than that of acylPy$^+$ (generated from pyridine and PhOCOCl). Nevertheless, the estimated decomposition rate of **1a** in the presence of Me_2NBn was only *ca.* 10 times greater than that of pyridine (for details, see supporting information). This may be attributable to the leaving ability (= lower p*K*aH) of pyridine, which is higher than that of Me_2NBn (= higher p*K*aH). Based on these results, we speculated that the following two points are plausible: 1) the reaction of pyridine with **1a** is sufficiently fast, but the reverse reaction is ultra fast (thus, K_{eq} is very small); and, 2) acylPy$^+$ is extremely electrophilic. Therefore, although the concentration of acylPy$^+$ was very small, the time-dependent decrease of **1a** was obvious (Scheme 4c). Since DMAP has a high level of nucleophilicity[23] and generates a stabilized acylDMAP$^+$,[24] its concentration should be higher than that of acylPy$^+$,[22] but no significant decomposition of **1a** was observed. This is probably due to the high stability of acylDMAP$^+$ (Scheme 4d). Only a slight, or no, decomposition of **1a** was observed in the presence of either NMI or *i*-Pr_2NEt probably due to their insufficient levels of nucleophilicity (Figure 2). The estimated abilities of the amines used in the activations of Cbz-Cl (**1a**) and α-Phe-NCA (**2a**) are summarized in Figure 3. NMI has a low level of ability to activate both **1a** and **2a** (group F), whereas NMM (group C) and Me_2NBn (group A) have medium-to-high levels of ability to activate **1a** and **2a**, which resulted in medium-to-high yields. Pyridine has a good level of ability to activate **1a** but a low level of for the activation of **2a** (group B), whereas DMAP, *i*-Pr_2NEt and Et_2NBn are just the opposite—good activation for **2a** but low for **1a** (group D). *N*-Ethylmorpholine has a medium level of ability for the activation of **2a**, but shows poor activation of **1a** (group E).

In order to further improve the yield of **3a**, we examined combinations of the two amines in groups A, B, and D (Table 2). Combining pyridine with *i*-Pr_2NEt improved the yield of **3a** (92%, entry 1) compared with the sole use of pyridine (34%) or *i*-Pr_2NEt (0%), which both properly activated **1a** and **2a**, respectively. Unexpectedly, the use of *i*-Pr_2NEt with Me_2NBn afforded **3a** in a lower yield (84%) compared with the use of *i*-Pr_2NEt with pyridine (92%, entry 1 vs. 2). The desired coupling reaction of **2a** with acylMe$_2$NBn$^+$ or acylPy$^+$ competes with the undesired reactions (urethane formation and/or decarboxylation) from acylMe$_2$NBn$^+$ or acylPy$^+$. Of the two, acylMe$_2$NBn$^+$ could promote the undesired reactions to a greater extent. It is possible that the desired coupling between bulky acylMe$_2$NBn$^+$ and the bulky complex of *i*-Pr_2NEt-NCA were somewhat slower compared with the corresponding reaction of

Table 2. Examination of combinations of amines for micro-flow synthesis of **3a**

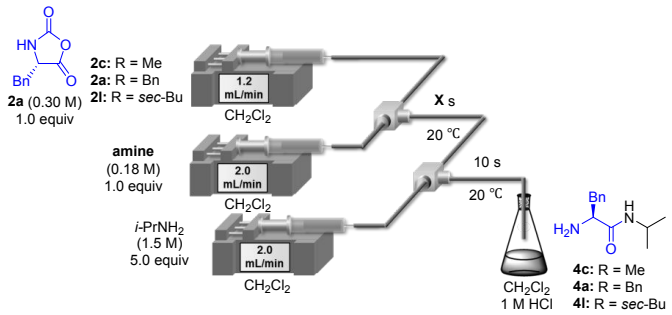


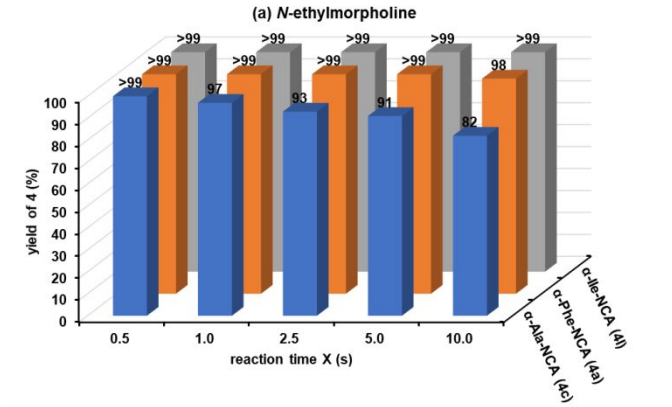
entry	amine1	amine2	X (s)	Y (°C)	yield[a] (%)	
					3a	2a
1	*i*-Pr_2NEt	pyridine	10	20	92	0
2	*i*-Pr_2NEt	Me_2NBn	10	20	84	0
3	Me_2NBn	pyridine	10	20	94-96[b] (74)[c]	2-3
4	Et_2NBn	pyridine	10	20	98	0
5	Me_2NBn	pyridine	10	0	84	5
6	Me_2NBn	pyridine	10	40	94	<1
7	Me_2NBn	pyridine	5	20	94	<1
8	Me_2NBn	pyridine	2.5	20	85	11
9[d]	Me_2NBn	pyridine	10	20	80	<1
10[e]	Me_2NBn	pyridine	10	20	93	3
11	Me_2NBn	pyridine	10	20	(66)[c],[f]	
12[g]	Me_2NBn	pyridine	10	20	71-81[b]	<1

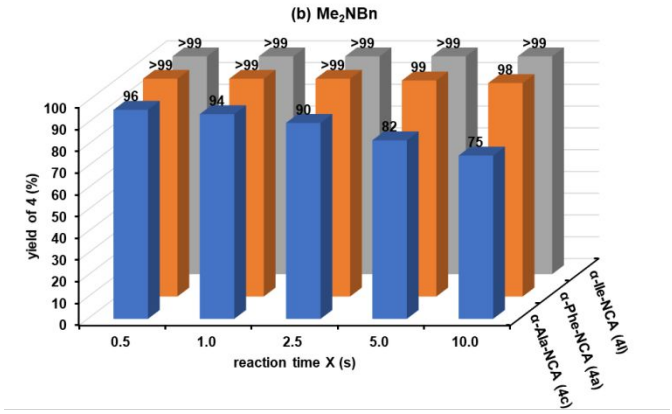
[a]The yields were determined by ^{1}H NMR analysis using 1,1,2-trichloroethane as an internal standard. [b]Three independent experiments were carried out. [c]Isolated yield. [d]MeCN was used as a solvent. [e]Flow rate is 4.0 and 2.4 mL/min. [f]Collection time was 680 s for the gram-scale synthesis. [g]Mixing was performed using a magnetic stirrer (1,000 rpm).

less bulky acylPy$^+$. The use of Me_2NBn with pyridine afforded **3a** in a higher yield (entry 3, 94-96%). Under these conditions, Me_2NBn with its medium level of basicity (p*K*aH = 8.9) is expected to activate **2a**, and the undesired polymerization of **2a** should be suppressed due to the mild basicity of Me_2NBn. Both Me_2NBn and pyridine were expected to activate **1a**, but the generation of acylMe_2NBn$^+$ could cause undesired reactions, as in the case of entry 2. Therefore, a combination of poorly nucleophilic Et_2NBn with a medium level of basicity (p*K*aH = 9.5) and pyridine was examined (entry 4) to avoid the undesired generation of acylAm$^+$. As expected, the highest yield (98%) was observed. Although this combination returned the best result, the removal of hydrophobic Et_2NBn *via* simple aqueous workup was somewhat problematic; in addition, the cost of Et_2NBn is higher than that of Me_2NBn. Therefore, we decided to use a combination of Me_2NBn and pyridine in the following examinations. Other combinations of various amines were also investigated, but no improvement in yield was observed (for details, see supporting information). The reaction temperature, time, solvent, and flow rate were optimized. A lower temperature (0 °C) resulted in an incomplete conversion of **2a** (entry 5), and a higher temperature (40 °C) did not improve the yield (entry 6). When the reaction time was shortened to 5 s, the yield was not changed, but a further reduction in the reaction time (2.5 s) resulted in an 11% recovery of **2a** (entries 7 and 8). The use of MeCN as a solvent decreased the yield (entry 9), and the use of THF, Et_2O, or 1,4-dioxane as a solvent caused clogs in the micro-flow reactor. The productivity of the developed process could be improved by increasing the flow rates without a significant decrease in the yield (entry 10). Gram-scale synthesis using the conditions of entry 10 afforded **3a** without a severe decrease in yield (entry 11; 1.7 g, 66% isolated yield, productivity: 9.2 g/h). In order to verify the importance of micro-flow conditions, comparative batch conditions were examined. 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 3 vs. 12). The observed yields from batch conditions were obviously lower than that from micro-flow conditions. In addition, sufficient care should be taken because the reaction is exothermic; therefore, large-scale syntheses using a batch reactor is not recommended.

We speculated that the activation of α-NCAs by the amine should be influenced by the steric bulkiness of side chains in α-NCAs. In order to estimate the reactivity of α-NCAs with different steric hindrance, the time-dependent (0.5 to 10 s) decreases of α-Ala-NCA (**2c**), α-Phe-NCA (**2a**), and α-Ile-NCA (**2l**) in the presence of *N*-ethylmorpholine, Me_2NBn, or *i*-Pr_2NEt were investigated (Figure 5). As was the case in the previous experiments (Figure 1), the unstable α-NCAs were converted to the corresponding stable amides **4**. The decrease in **2** was indirectly estimated *via* the NMR yield of **4**. As expected, the decomposition rates of **2** became higher (**2c** > **2a** > **2l**) as the steric bulkiness of side chains decreased. Based on these results, we assumed that the use of an amine with high basicity, such as *i*-Pr_2NEt, would be suitable combination of Me_2NBn (p*K*aH= 8.9) and pyridine was examined (method B) for the activation of α-NCA with a less bulky side chain; and, the combination of *N*-ethylmorpholine (p*K*aH = 7.7) and pyridine was examined (method C) for the activation of α-Gly-NCA (**3b**) and α-Ala-NCA (**3c**). As expected, method C afforded an excellent yield (>99%) in the synthesis of the least bulky **3b**; method B, however, resulted in a significant decrease in yield (35%). In the case of **3c**, method B afforded the best yield (83%) among all the examined methods. Method B afforded high to excellent yields (71-98%) for the syntheses of NCAs **3a** and **3d-3g** with bulky side chains. On the other hand, method A afforded good-to-excellent yields (64-97%) for the syntheses of NCAs **3h-3n** with more bulky side chains. The developed approach allowed the syntheses of α-NCAs **3f**, **3h**, **3m**, and **3n** containing acid-labile functional groups, but **3o** was an exception. The use of fluorenylmethyloxycarbonyl chloride (Fmoc-Cl) **1c** and allyl chloroformate (Alloc-Cl) **1d** instead of Cbz-Cl (**1a**) also afforded the desired **3p** and **3q** in high yields (85-96%). It should be noted that racemization did not proceed even in the cases of **3a**, **3c**, **3d**, **3e**, and **3f** that were derived from racemizable amino acids. In particular, highly racemizable **3e** was obtained without detectable racemization. The developed approach enabled rapid (10 s), mild (20 °C), and high yielding syntheses of structurally diverse UNCAs.







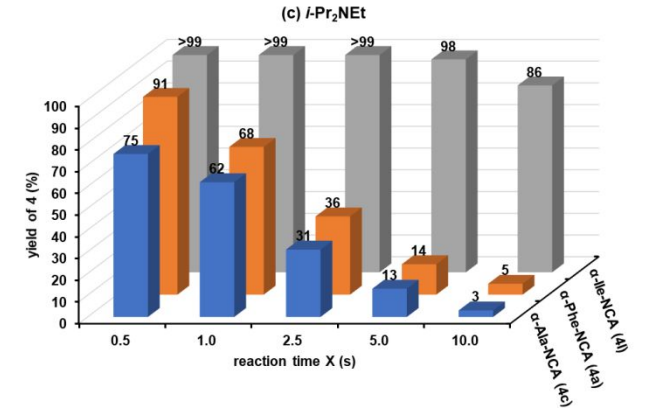


Figure 4. Comparison of time-dependent decrease of α-Ala-NCA (**2c**), α-Phe-NCA (**2a**), and α-Ile-NCA (**2l**) in the presence of *N*-ethylmorpholine (a), Me_2NBn (b), or *i*-Pr_2NEt (c) that was estimated by the yield of amide **4**.

Finally, we applied the developed approach to the syntheses of dipeptides **6a** and **6b**. Both Cbz- and Fmoc-protected

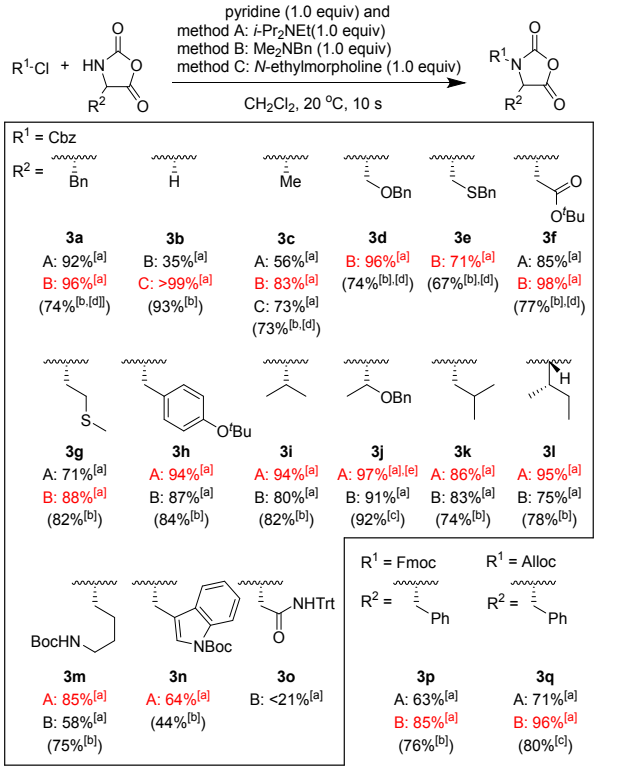
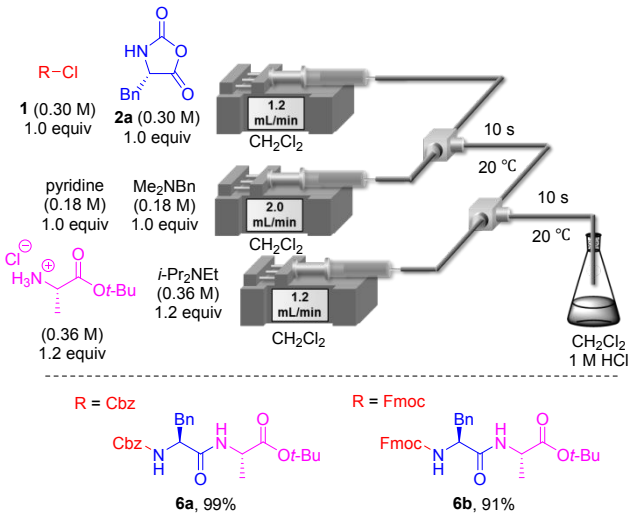


Figure 5. Substrate scope of our developed rapid dual activation approach. [a]Yields were determined by ^1H NMR analysis using 1,1,2-trichloroethane. [b]The product was isolated by recrystallization following the red-colored method. [c]The product was isolated by washing with hexane following the red-colored method. [d]Racemization was not detected by HPLC-UV analysis. [e]Reaction time was 30 s.



Scheme 5. Synthesis of dipeptide **6a**, **6b**

dipeptides were useful as building blocks in peptide syntheses, which were readily obtained in-high-to-excellent yields (91-99%) in a one-flow process (Scheme 5).

Conclusion

We successfully demonstrated rapid (10 s), mild (20 °C), and high yielding syntheses of 16 structurally diverse UNCAs. The

rapid dual activation approach using a micro-flow reactor and the proper use of two amines suppressed the undesired urethane formation/decarboxylation of acylAm$^+$ and acylPy$^+$, and the oligomerization/polymerization of NCAs. Although NMM afforded the best results both in the most frequently used conventional approach as well as in our previous approach, it afforded unsatisfactory results in the present approach. Instead, a combination of pyridine and a tertiary amine afforded the best results. Three combinations (method A: *i*-Pr_2NEt and pyridine; method B: Me_2NBn and pyridine; method C: *N*-ethylmorpholine and pyridine) were used depending on the bulkiness of the side chains in NCAs. In order to obtain mechanistic insight, time-dependent decreases of alkyl chloroformate and NCA in the presence of various amines were investigated *via* flash mixing technology using a micro-flow reactor. As a result, it was suggested that in situ generated acylPy$^+$ was highly active and less prone to cause undesired decompositions compared with acylAm$^+$ examined in this study. Thus, regardless of its very low concentration, acylPy$^+$ facilitated the desired coupling reaction with NCAs. In addition, sequential UNCA formation and amide bond formation in a one-flow process afforded useful Cbz- and Fmoc-protected dipeptides in high yields. The obtained results should expand the application of the rapid dual activation approach and afforded valuable insights for the chemistry of NCAs and amine-mediated acylations.

Conflicts of interest

There are no conflicts to declare.

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