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Single-Step, Rapid, and Mild Synthesis of β-Amino Acid *N*-Carboxy Anhydrides Using Micro-Flow Technology

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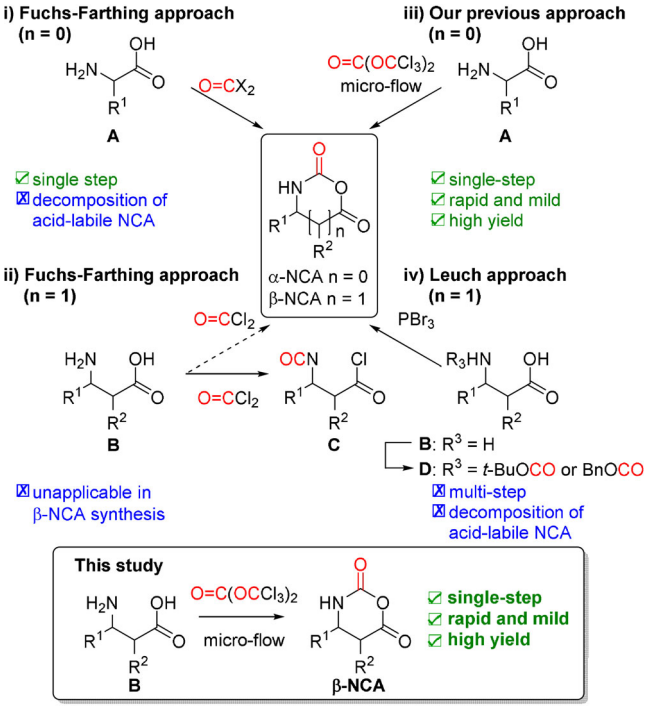
Dedicated to the memory of Prof. Jun-ichi Yoshida

Abstract: β-Amino acid *N*-carboxy anhydrides (β-NCAs) are rarely used in the synthesis of β-peptides, which is due mainly to the poor availability of these potentially useful substrates. Herein, we describe the heretofore challenging synthesis of β-NCAs via a single-step, rapid, and mild formation using pH flash switching and flash dilution, which are aspects of micro-flow technology. We synthesized 15 β-NCAs in good to excellent yields that included acid-labile β-NCAs that cannot be readily synthesized using the conventional Leuchs approach. Scaled-up synthesis using this process can be readily achieved via continuous operation.

Introduction

β-Peptides have garnered much attention due to properties that include the formation of stable secondary structures and good stability against enzymatic degradation as well as their potential applications for drugs, drug carriers, and catalysts for organic transformations.[1] α-Amino acid *N*-carboxy anhydrides (α-NCAs, Scheme 1, $n=0$) are important building blocks in the synthesis of α-peptides[2] and polypeptides,[3] whereas β-NCAs (Scheme 1, $n=1$) have not been frequently used for the synthesis of β-peptides despite their potential utility.[4] One of the prime reasons for their avoidance has been the poor availability of β-NCAs due to their slower cyclization rate and lower stability by comparison with α-NCAs.[4f] Basically both α-NCAs and β-NCAs readily undergo polymerization under basic conditions. In particular, even the moisture in ambient air can induce the spontaneous polymerization of β-NCAs due to their extreme instability.



Scheme 1. Previously reported synthetic approaches i)–iv) for α-NCAs ($n=0$) and β-NCAs ($n=1$), and the developed approach for β-NCAs (this study).

The Fuchs-Farthing approach is the most frequently used for preparing α-NCAs from α-amino acids **A** (Scheme 1–i),[5] although this approach cannot produce acid-labile α-NCAs and often generates undesired ring-opening products.[6] On the other hand, the Fuchs-Farthing approach does not afford β-NCAs from β-amino acids **B** in satisfactory yields (0.3%), but does afford the undesired isocyanate **C** (Scheme 1–ii).[4a,f] Therefore, the Leuchs approach,[7] which involves the PBr_3-mediated cyclization of carbamate-protected β-amino acids **D**[4a,f,8] has been used for the synthesis of β-NCA (Scheme 1–iv).[9] Recently, the gram scale synthesis of a β-NCA from β-phe-

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nylalanine was reported.[10] The Leuchs approach, however, requires multiple synthetic steps, and a protecting group, but this approach cannot produce acid-labile β-NCAs. Roberts and co-workers have reported that a pure sample of β-NCA could not be prepared via the Leuchs approach.[1e]

Micro-flow technologies[11] enable the precise control of both a short reaction time (< 1 sec) and the reaction temperature.[12] The generation of turbulence flow and short diffusion length in a micro-flow reactor enables control of a short reaction time. The large surface-to-volume ratio of a micro-flow reactor enables rapid heat transfer for temperature control. We have reported efficient micro-flow peptide synthesis.[13] In addition, we recently reported a rapid, mild, and high-yielding synthesis of α-NCAs using flash pH switching and flash dilution in a micro-flow reactor (Scheme 1–iii).[14] The developed approach enabled a single-step synthesis of acid-labile α-NCAs. Herein, we report the challenging synthesis of β-NCAs via a single-step, protecting-group-free process using the corresponding β-amino acids based on our developed flash pH switching and flash dilution in a micro-flow reactor.

Results and Discussion

The details of a working hypothesis[14] for β-NCA formation based on micro-flow technologies are shown in Figure 1. β-Amino acid sodium salts **1**[15] and a base in H_2O along with a solution of phosgene in MeCN are independently injected into a V-shape mixer, which has a mixing efficiency that is higher than that of a T-shape mixer.[14] We expected the desired cyclization to occur rapidly between **1** (1.0 equiv) and the phosgene (> 1.0 equiv) under basic conditions and to generate HCl (1.0 equiv) that would be trapped by the base (> 1.0 equiv) and cause the reaction mixture to remain basic (step 1). The unreacted phosgene is then decomposed by water to generate HCl (step 2). After the base is completely protonated by HCl, the reaction mixture becomes acidic (step 3). This pH flash switching from basic to acidic avoids the undesired polymerization and/or hydrolysis of β-NCA. The obtained mixture is rapidly diluted by injecting ethyl acetate to a T-shape mixer. This flash dilution avoids the undesired decomposition of acid-labile functional groups.

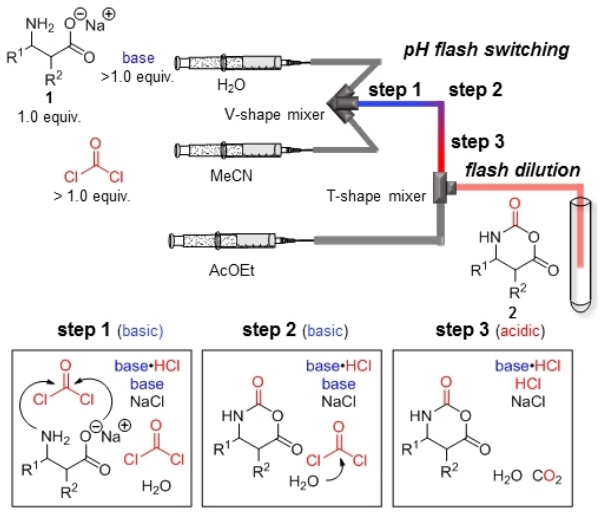


Figure 1. Working hypothesis for the rapid and mild formation of β-NCAs based on pH flash switching and flash dilution.

β-NCA formation was examined using sodium salts of β-phenylalanine (**1a**, racemic mixture), *N*-methyl morpholine (NMM), and the safely handled triphosgene as a phosgene equivalent in accordance with our previously reported procedure.[14] The resultant mixture was collected and was followed by an aqueous work-up to remove the hydrochloric salts of NMM and **1a**. As previously described, even moisture in the air induced spontaneous polymerization of β-NCAs, and, thus, spectrum measurements were carried out immediately following the synthesis. Initially, the concentration of **1a**, and the amount (equiv) of triphosgene and NMM were examined (Figure 2).

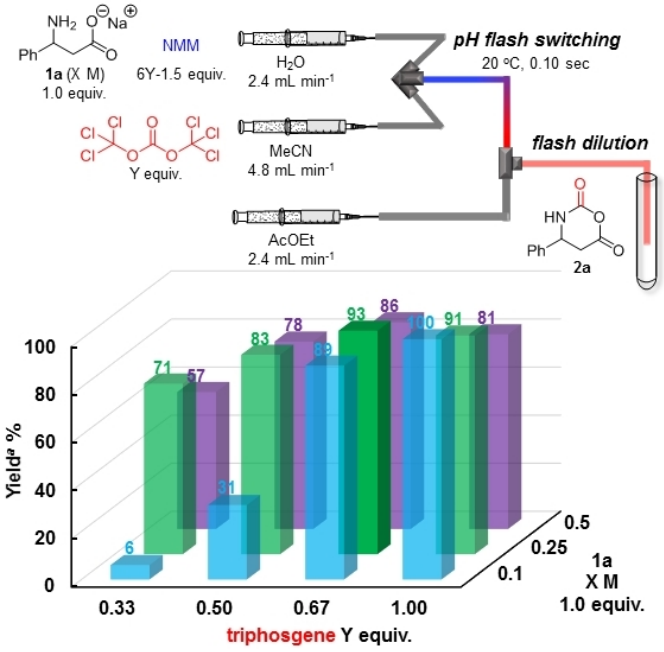
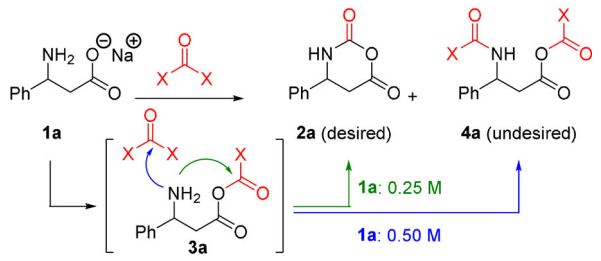


Figure 2. Examination of the concentration of **1a** and the amount of triphosgene in the micro-flow synthesis of β-NCA **2a**. [a] Yields were determined by ^{1}H NMR analysis using 1,1,2-trichloroethane as an internal standard.

The use of a smaller amount of triphosgene (Y = 0.33 or 0.50 equiv) resulted in lower yields compared with the use of a larger amount of triphosgene (Y = 0.67 or 1.00 equiv) probably due to the insufficient conversion of **1a**. The use of a higher concentration solution of **1a** (X = 0.50 M) resulted in lower yields compared with the use of a lower concentration solution of **1a** (X = 0.25 M) within the examined range of Y (0.33 to 1.00 equiv). We speculated that the overreaction of an intermediate **3a** with phosgene, or its equivalent, occurred under these higher concentration conditions (X = 0.50 M) to generate the undesired **4a** (Scheme 2). The combinations of X = 0.25 M (1.0 equiv) and Y = 0.67 equiv and X = 0.10 M (1.0 equiv) and Y = 1.0 equiv afforded excellent yields. We used the former conditions in the following investigations because of their

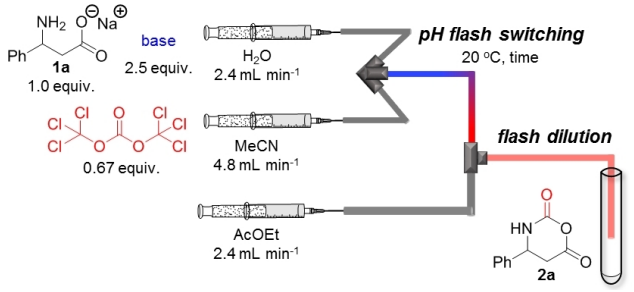


Scheme 2. Plausible reason for the decreased yield of β-NCA **2a** under conditions employing 0.5 M solution of **1a**.

higher productivity due to the use of a higher concentration solution of **1a** as well as to the decreased amounts of triphosgene and NMM that were used.

The reaction time and base were examined (Table 1). The decreased yield (86%) was observed when the reaction was carried out in 0.02 sec probably due to insufficient conversion of

Table 1. Examination of time and base.

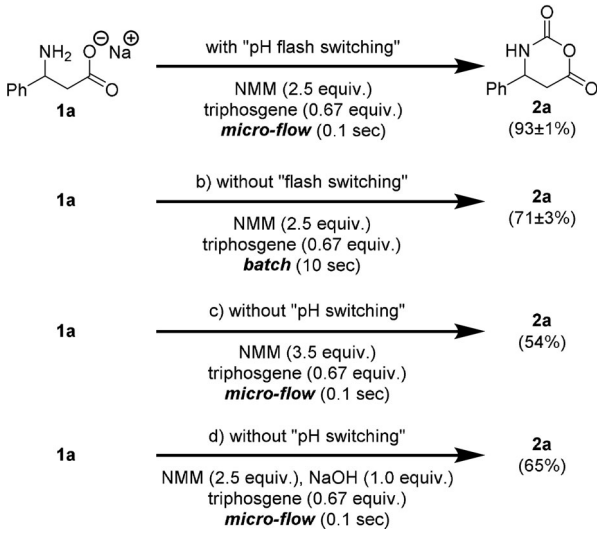


Entry	*t* [sec]	Base (pKa of conjugate acid)	Yield[a]
1	0.02	NMM (7.4[16])	86
2	0.10	NMM (7.4[16])	93±1%
3	0.20	NMM (7.4[16])	93
4	0.10	*N*-methylpiperidine (10.1[16])	73
5	0.10	*N*-ethylmorphorine (7.7[16])	65
6	0.10	pyridine (5.2[17])	54

[a] Yields were determined by ^{1}H NMR analysis with 1,1,2-trichloroethane as an internal standard.

1a (entry 1). The 0.10 sec conditions (entry 2) and 0.20 sec conditions (entry 3) afforded the same yield (93%), thus 0.10 sec is sufficient to complete NCA formation. Triethylamine and *N*,*N*-diisopropylethylamine could not be used as a base due to their insufficient solubility against water. The use of a stronger base, *N*-methylpiperidine, resulted in a decreased yield (entry 3 vs. 4) probably due to a base-mediated side reaction such as the previously described isocyanate formation. The use of a more sterically hindered base such as *N*-ethylmorpholine resulted in a decreased yield (entry 3 vs. 5). Reportedly, NMM can form corresponding acylammonium cation through its nucleophilic acyl substitution.[18] Therefore, it is conceivable that a less sterically hindered NMM could attack triphosgene to form a highly active acylammonium cation,[19] whereas a more sterically hindered *N*-ethylmorpholine could not attack, and, thus, the reaction would become slow. The use of a highly nucleophilic and less-basic amine such as pyridine also resulted in a decreased yield probably due to its insufficient ability to trap the in situ-generated HCl (entry 6). The conditions shown in entry 2 were optimal, and the average yield from three independent experiments was 93±1%. The result was highly reproducible. Gram-scale (2.3 g) preparation of β-NCA **2a** was successfully achieved by continuous running for 21.3 min.

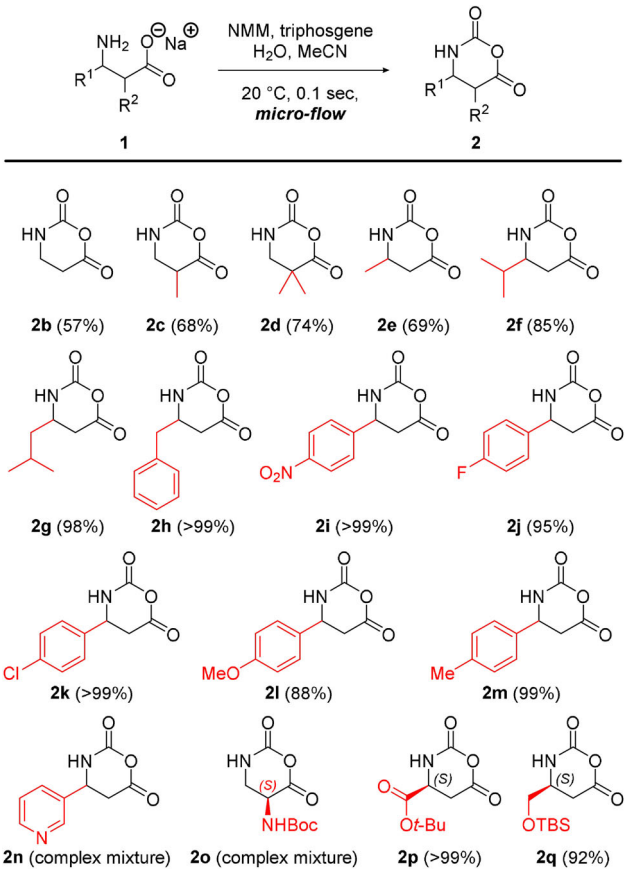
In order to verify the importance of "pH flash switching," comparative batch reactions were carried out under the same reaction conditions with the exception of reaction time (10 s), because it was impossible to perform the batch reaction in 0.1 sec (Scheme 3b). Average yield from three independent ex-



Scheme 3. Verification of the necessity of pH flash switching in the synthesis of β-NCA **2a**.

periments was 71±3%. The batch conditions afforded reduced yields with lower reproducibility. In order to verify the importance of "pH switching," the developed micro-flow reaction was carried out with an additional 1.0 equiv of NMM or NaOH (Scheme 3c and 3d). The medium was kept basic throughout the reaction under these conditions. Not surprisingly, undesired polymerization occurred and the yields were reduced (54% and 65%, respectively). These results clearly indicated the importance of the "pH flash switching" that was enabled via micro-flow, rapid-mixing technology in this reaction. In addition, this reaction involves the generation of toxic phosgene gas, and, therefore, it would be dangerous to carry out the batch reaction on a large scale. However, this reaction can be safely performed using a micro-flow reactor.

The substrate scope was examined using the optimized reaction conditions (Scheme 4). β-Alanine-derived NCA **2b** was obtained in a 57% yield. This is an acceptable result because the synthesis of NCA **2b** is a challenging task due to its extreme instability, as Roumestant and co-workers previously reported.[20] Less lipophilic NCAs such as **2c**, **2d**, and **2e** were obtained in acceptable to good yields (68, 74, and 69%, respectively). Interestingly, our developed approach tended to



Scheme 4. Substrate scope of the developed micro-flow β-NCA formation. Isolated yields appear in parentheses. Boc, *tert*-butoxycarbonyl; TBS, *tert*-butyldimethylsilyl.

afford high yields in the case of lipophilic NCAs containing sterically bulky side chains, which contrasts with the Leuchs approach that tends to afford lower yields in the case of NCAs with sterically bulky side chains.[4f] Our approach afforded NCAs **2f**–**2h** containing sterically bulky alkyl side chains in high to excellent yields (85, 98, and >99%, respectively). NCAs **2i**–**2m** containing substituted phenyl groups were obtained in high to excellent yields (88%->99%) irrespective of the electron-donating or -withdrawing nature of the substituents. On the other hand, an NCA **2n** containing a pyridine ring was not obtained but a complex mixture was generated due to pyridine ring-induced undesired reactions. An NCA **2o** containing an NHBoc side chain was not obtained, which was probably due to an undesired cyclization that generated the corresponding oxazolone. It should be noted that NCAs **2p** and **2q** containing either an acid-labile *tert*-butyl ester or a TBS ether were obtained in high to excellent yields. Syntheses of these acid-labile NCAs are difficult using the Leuchs approach. These results clearly indicate the mildness of our developed conditions.

Conclusions

A single-step, rapid, and mild formation of synthetically challenging β-NCAs was successfully achieved. The key to success was our originally developed "pH flash switching" and "flash dilution" processes that were enabled by micro-flow technology. We achieved good to excellent yields in the synthesis of 15 β-NCAs, which included acid-labile NCA that cannot be readily synthesized using the conventional Leuchs approach. It should be noted that the developed reaction can be safely and reproducibly performed only by using micro-flow technology. Scaled-up synthesis can be readily achieved via continuous running. The developed approach will accelerate the use of β-NCAs in β-peptide synthesis.

Experimental Section

General procedure: Micro-flow β-NCA formation

A solution of amino acid sodium salt (0.250 M, 1.00 equiv.), *N*-methyl morpholine (0.630 M, 2.52 equiv.) in H_2O (flow rate: 2.40 mL min^{-1}) and a solution of triphosgene (0.250 M, 0.670 equiv.) in MeCN (flow rate: 4.80 mL min^{-1}) was introduced into a V-shape mixer at 20 °C using the syringe pumps. The resultant mixture was passed through the reaction tube 1 (inner diameter: 0.250 mm, length: 244 mm, volume: 12.0 μL, reaction time: 0.100 s) at the same temperature. Then, the resultant mixture and EtOAc (flow rate: 2.40 mL min^{-1}) were introduced to the T-shape mixer at 20 °C with the syringe pumps. The resultant mixture was passed through the reaction tube 2 (inner diameter: 0.800 mm, length: 298 mm, volume: 150 μL, reaction time: 0.940 s) at the same temperature. After being eluted for 20 s to reach a steady state, the resultant mixture was poured into EtOAc (40 mL) for 100 s at 0 °C. The aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, filtered and concentrated in vacuo at 25 °C *(caution: β-NCAs gradually decomposed).*

β-Phenylalanine-NCA (2a)

Purification method: β-NCA **2a** was obtained without further purification. The reaction mixture was collected for 1275 sec. 2.32 g, 12.1 mmol, 95%. White solid; mp 97–99 °C; IR (ATR): $\tilde{\nu}$ = 3230, 3148. 1791, 1734, 1383, 1332, 1115, 1023, 977 cm^{-1}; ^{1}H NMR (500 MHz, CD_3CN): δ = 7.42–7.39 (m, 2H), 7.37–7.33 (m, 3H), 6.94 (brs, 1H), 4.79–4.76 (m, 1H), 3.04 (dd, J = 5.5, 16.5 Hz, 1H), 2.88 ppm (dd, J = 8.0, 16.5 Hz, 1H); ^{13}C NMR (125 MHz, CD_3CN): δ = 166.2, 150.4, 139.7, 129.9, 129.5, 127.0, 51.0, 37.2 ppm; HRMS (ESI-TOF): calcd for $[C_6H_9NO_3 + Na]^+$ 214.0475, found 214.0476.

β-Alanine-NCA (2b)

Purification method: β-NCA **2b** was obtained without further purification. 65.9 mg, 0.57 mmol, 57%. White solid; 84–86 °C decomp.; IR (ATR): $\tilde{\nu}$ = 3254, 3150, 2929, 1798, 1705, 1344, 1060, 749 cm^{-1}; ^{1}H NMR (500 MHz, CD_3CN): δ = 6.49 (brs, 1H), 3.33–3.30 (m, 2H), 2.71 ppm (t, J = 7.0 Hz, 2H); ^{13}C NMR (125 MHz, CD_3CN): δ = 167.3, 150.8, 35.6, 29.1 ppm; HRMS (ESI-TOF): calcd for $[C_5H_7NO_3 + Na]^+$ 138.0162, found 138.0161.

α-Methyl-β-alanine-NCA (2c)

Purification method: β-NCA **2c** was obtained without further purification. 88.1 mg, 0.68 mmol, 68%. White solid; mp 112–114 °C; IR (ATR): $\tilde{\nu}$ = 3280, 2338, 1792, 1734, 1642, 1354, 1077, 973, 598 cm^{-1}; ^{1}H NMR (400 MHz, CD_3CN): δ = 6.43 (brs, 1H), 3.36–3.30 (m, 1H), 3.11–3.05 (m, 1H), 2.89–2.80 (m, 1H), 1.19 ppm (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, CD_3CN): δ = 170.4, 150.8, 42.0, 34.5, 12.5 ppm;

HRMS (ESI-TOF): calcd for $[C_5H_7NO_3+Na]^+$ 152.0318, found 152.0317.

α-Dimethyl-β-alanine-NCA (2d)

Purification method: β-NCA **2d** was obtained without further purification. 106.2 mg, 0.74 mmol, 74%. White solid; mp 110–112 °C; IR (ATR): $\tilde{\nu}$ = 3258, 3162, 2987, 1794, 1733, 1338, 1051, 961, 697 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.06 (brs, 1H), 3.22 (d, J = 2.0 Hz, 2H), 1.36 ppm (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 170.5, 151.0, 47.7, 37.0, 22.6 ppm; HRMS (ESI-TOF): calcd for $[C_6H_9NO_3+Na]^+$ 166.0475, found 166.0474.

β-Homoalanine-NCA (2e)

Purification method: β-NCA **2e** was obtained without further purification. The reaction mixture was collected for 63 sec. 56.4 mg, 0.44 mmol, 69%. White solid; 92–95 °C decomp.; IR (ATR): $\tilde{\nu}$ = 3154, 2975, 1807, 1714, 1386, 1331, 1106, 995, 595 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 6.95 (brs, 1H), 3.79–3.78 (m, 1H), 2.87 (dd, J = 4.0, 16.0 Hz, 1H), 2.53 (dd, J = 9.5, 16.0 Hz, 1H), 1.34 ppm (d, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 164.5, 150.4, 42.9, 36.2, 20.7 ppm. ^1H NMR and ^{13}C NMR spectra of DL-β-homoalanine-NCA were identical to the previously reported spectra of (*S*)-β-homoalanine-NCA.[4f]

β-Homovaline-NCA (2f)

Purification method: β-NCA **2f** was obtained without further purification. 133.6 mg, 0.85 mmol, 85%. White solid, mp 69–72 °C; IR (ATR): $\tilde{\nu}$ = 3224, 2972, 2941, 1791, 1723, 1396, 1336, 1069, 966 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.19 (brs, 1H), 3.45–3.41(m, 1H), 2.82 (dd, J = 5.0, 16.0 Hz, 1H), 2.64 (dd, J = 8.0, 16.0 Hz, 1H), 1.87–1.81 (m, 1H), 1.00 ppm (t, J = 7.5 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 165.1, 150.9, 52.4, 32.1, 31.6, 17.9, 17.8 ppm. ^1H NMR and ^{13}C NMR spectra of DL-β-homovaline-NCA were identical to the previously reported spectra of (*S*)-β-homovaline-NCA.[4f]

β-Homoleucine-NCA (2g)

Purification method: β-NCA **2g** was obtained without further purification. The reaction mixture was collected for 76 sec. 127.5 mg, 0.74 mmol, 98%. White solid; mp 63–65 °C; IR (ATR): $\tilde{\nu}$ = 3270, 2959, 1797, 1721, 1389, 1329, 1120, 1012, 977 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.29 (s, 1H), 3.69 (brs, 1H), 2.88 (dd, J = 4.5, 16.0 Hz, 1H), 2.55 (dd, J = 8.0, 16.0 Hz, 1H), 1.78–1.70 (m, 1H), 1.57–1.52 (m, 1H), 1.39–1.35 (m, 1H), 0.95 ppm (d, J = 4.5 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 164.8, 150.7, 45.7, 43.8, 34.7, 24.2, 22.5, 22.0 ppm. ^1H NMR and ^{13}C NMR spectra of DL-β-homoleucine-NCA were identical to the previously reported spectra of (*S*)-β-homoleucine-NCA.[4f]

β-Homophenylalanine-NCA (2h)

Purification method: β-NCA **2h** was obtained without further purification. 208.6 mg, 1.02 mmol, >99%. White solid; mp 97–99 °C; IR (ATR): $\tilde{\nu}$ = 3306, 1794, 1721, 1387, 1341, 1087, 982, 745, 695 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ = 7.35–7.17 (m, 5H), 6.77 (brs, 1H), 3.86–3.83 (m, 1H), 2.91–2.79 (m, 3H), 2.61 ppm (dd, J = 8.0, 16.5 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 164.4, 150.1, 134.8, 129.3, 129.2, 127.7, 48.1, 41.2, 33.7 ppm. ^1H NMR and ^{13}C NMR spectra of DL-β-homophenylalanine-NCA were identical to the previously reported spectra of (*S*)-β-homophenylalanine-NCA.[4f]

p-Nitrophenyl-β-alanine-NCA (2i)

Purification method: β-NCA **2i** was obtained without further purification. 261.0 mg, 1.11 mmol, >99%. Yellow oil; IR (neat): $\tilde{\nu}$ = 3300, 1801, 1748, 1520, 1350, 1079, 977 cm^{-1}; ^1H NMR (500 MHz, CD_3CN): δ = 8.19 (d, J = 8.5 Hz, 2H), 7.56 (d, J = 8.5 Hz, 2H), 7.04 (brs, 1H), 4.95–4.91 (m, 1H), 3.12 (dd, J = 5.5, 16.5 Hz, 1H), 2.91 ppm (dd, J = 8.0, 16.5 Hz, 1H); ^{13}C NMR (125 MHz, CD_3CN): δ = 165.5, 150.1, 148.8, 146.9, 128.3, 124.9, 50.6, 36.6 ppm; HRMS (ESI-TOF): calcd for $[C_{11}H_{11}NO_4+Na]^+$ 259.0325, found 259.0328.

p-Fluorophenyl-β-alanine-NCA (2j)

Purification method: β-NCA **2j** was obtained without further purification. 199.3 mg, 0.95 mmol, 95%. White solid; mp 85–87 °C; IR (ATR): $\tilde{\nu}$ = 3243, 3155, 1792, 1737, 1511, 1323, 1121, 834, 517 cm^{-1}; ^1H NMR (500 MHz, CD_3CN): δ = 7.37–7.34 (m, 2H), 7.15–7.11 (m, 2H), 6.96 (brs, 1H), 4.79–4.76 (m, 1H), 3.05–3.00 (m, 1H), 2.87 ppm (dd, J = 8.5, 16.5 Hz, 1H); ^{13}C NMR (125 MHz, CD_3CN): δ = 166.1, 163.4 (d, $J_{C\text{-}F}$ = 243.9 Hz), 150.3, 135.8 (d, $J_{C\text{-}F}$ = 2.8 Hz), 129.2 (d, $J_{C\text{-}F}$ = 8.5 Hz), 116.6 (d, $J_{C\text{-}F}$ = 21.8 Hz), 50.5, 37.2 ppm; HRMS (ESI-TOF): calcd for $[C_{10}H_8FNO_3+Na]^+$ 232.0380, found 232.0382.

p-Chlorophenyl-β-alanine-NCA (2k)

Purification method: β-NCA **2k** was obtained without further purification. 231.1 mg, 1.02 mmol, >99%. White solid, mp 117–120 °C, IR (ATR): $\tilde{\nu}$ = 3240, 3150, 2258, 1793, 1739, 1371, 1326, 1119, 972 cm^{-1}; ^1H NMR (500 MHz, CD_3CN): δ = 7.39 (d, J = 8.5 Hz, 2H), 7.32 (d, J = 8.5 Hz, 2H), 6.96 (brs, 1H), 4.79–4.76 (m, 1H), 3.04 (dd, J = 5.5, 16.5 Hz, 1H), 2.86 ppm (dd, J = 8.5, 16.5 Hz, 1H); ^{13}C NMR (125 MHz, CD_3CN): δ = 165.9, 150.3, 138.6, 134.7, 129.9, 128.9, 50.5, 37.0 ppm; HRMS (ESI-TOF): calcd for $[C_{10}H_8ClNO_3+Na]^+$ 248.0085, found 248.0087.

p-Methoxyphenyl-β-alanine-NCA (2l)

Purification method: β-NCA **2l** was obtained without further purification. 193.6 mg, 0.99 mmol, 88%. White solid; mp 117–119 °C; IR (ATR): $\tilde{\nu}$ = 3221, 3146, 1799, 1739, 1614, 1378, 1174, 823, 598 cm^{-1}; ^1H NMR (500 MHz, CD_3CN): δ = 7.26–7.23 (m, 2H), 6.94–6.91 (m, 2H), 6.87 (brs, 1H), 4.72–4.69 (m, 1H), 3.75 (s, 3H), 2.98 (dd, J = 5.0, 16.0 Hz, 1H), 2.86 ppm (dd, J = 8.5, 16.0 Hz, 1H); ^{13}C NMR (125 MHz, CD_3CN): δ = 166.4, 160.7, 150.4, 131.5, 128.4, 115.1, 55.8, 50.6, 37.3 ppm; HRMS (ESI-TOF): calcd for $[C_{11}H_{11}NO_4+Na]^+$ 244.0580, found 244.0579.

p-Tolyl-β-alanine-NCA (2m)

Purification method: β-NCA **2m** was obtained without further purification. 202.6 mg, 0.99 mmol, 99%. White solid; mp 119–120 °C; IR (ATR): $\tilde{\nu}$ = 3253, 3165, 2933, 1792, 1741, 1340, 1079 cm^{-1}; ^1H NMR (500 MHz, CD_3CN): δ = 7.21 (s, 4H), 6.92 (brs, 1H), 4.74–4.70 (m, 1H), 3.00 (dd, J = 5.5, 16.5 Hz, 1H), 2.85 (dd, J = 8.5, 16.5 Hz, 1H), 2.31 ppm (s, 3H); ^{13}C NMR (125 MHz, CD_3CN): δ = 166.3, 150.5, 139.4, 136.7, 130.5, 126.9, 50.8, 37.3, 21.0 ppm; HRMS (ESI-TOF): calcd for $[C_6H_9NO_3+Na]^+$ 228.0631, found 228.0632.

α-*O*-*tert*Butyl-L-β-aspartate-NCA (2p)

Purification method: β-NCA **2p** was obtained without further purification. 225.1 mg, 1.05 mmol, >99%. White solid; mp 84–86 °C; IR (ATR): $\tilde{\nu}$ = 3231, 3167, 2985, 1801, 1759, 1720, 1366, 1090 cm^{-1}; ^1H NMR (500 MHz, CD_3CN): δ = 6.82 (brs, 1H), 4.10–4.07 (m, 1H), 3.02 (dd, J = 6.5, 16.5 Hz, 1H), 2.86 (dd, J = 4.5, 16.5 Hz, 1H),

1.42 ppm (s, 9H); ^{13}C NMR (125 MHz, CD_3CN): $\delta = 169.7$, 165.4, 149.8, 84.2, 50.3, 31.8, 27.9 ppm; HRMS (ESI-TOF): calcd for $[C_9H_{13}NO_5 + Na]^+$ 238.0686, found 238.0683; $[\alpha]_D^{29} = +40.12$ ($c = 1.00$, CH_3CN).

*O-tert*Butyldimethylsilyl-L-β-homoserine-NCA (2q)

Purification method: β-NCA **2q** was obtained without further purification. The reaction mixture was collected for 25 sec. 59.9 mg, 0.23 mmol, 92%. White amorphous solid, IR (neat): $\tilde{\nu} = 3380$, 2938, 1798, 1746, 1651, 1404, 1110, 1038 cm^{-1}; ^{1}H NMR (400 MHz, CD_3CN): $\delta = 6.40$ (brs, 1H), 3.69 (dd, $J = 2.8$, 10.8 Hz, 1H), 3.62 (dd, $J = 2.8$, 8.8 Hz, 1H), 2.88 (dd, $J = 6.8$, 16.4 Hz, 1H), 2.60 (m, 1H), 0.87 (s, 9H), 0.05 (s, 3H), 0.04 ppm (s, 3H); ^{13}C NMR (100 MHz, CD_3CN): $\delta = 167.0$, 151.0, 67.2, 49.1, 31.5, 26.0, 18.8, −5.6 ppm; HRMS (ESI-TOF): calcd for $[C_{11}H_{21}NO_4Si + Na]^+$ 282.1132, found 282.1133; $[\alpha]_D^{31} = +20.20$ ($c = 1.00$, CH_3CN).

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Conflict of interest

The authors declare no conflict of interest.

Keywords: acylation · anhydrides · flow chemistry · β-amino acids

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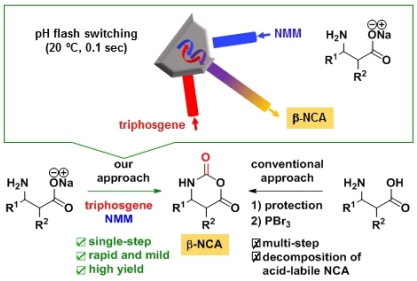
FULL PAPER

A challenging synthesis of β-amino acid *N*-carboxy anhydrides (β-NCAs) via a single-step, rapid, and mild formation using pH flash switching and flash dilution, in a micro-flow reactor has been achieved. Fifteen β-NCAs were synthesized in good to excellent yields that included acid-labile β-NCAs that cannot be readily synthesized using the conventional Leuchs approach. Scaled-up synthesis can be readily achieved through continuous operation.



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Single-Step, Rapid, and Mild Synthesis of β-Amino Acid *N*-Carboxy Anhydrides Using Micro-Flow Technology