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Kinetics

Continuous Flow Synthesis of ACE Inhibitors From N-Substituted L-Alanine Derivatives

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Abstract: A strategy for the continuous flow synthesis of angiotensin converting enzyme (ACE) inhibitors is described. An optimization effort guided by in situ IR analysis resulted in a general amide coupling approach facilitated by *N*-carboxyanhydride (NCA) activation that was further characterized by reaction kinetics analysis in batch. The three-step continuous process was demonstrated by synthesizing 8 different ACE inhibitors in up to 88% yield with throughputs in the range of $\approx$0.5 g h^{-1}, all while avoiding both isolation of reactive intermediates and process intensive reaction conditions. The process was further developed by preparing enalapril, a World Health Organization (WHO) essential medicine, in an industrially relevant flow platform that scaled throughput to $\approx$1 g h^{-1}.

ACE inhibitors are a safe and effective treatment for hypertension.[1] Despite the structural similarities shared between these active pharmaceutical ingredients (APIs) (Figure 1 A), a number of different synthetic approaches have been pursued.[2] Herein, we report a synthetic plan that consolidates the preparation of these essential medicines to a single three-step continuous process.

The first ACE inhibitor was described by E.R. Squibb and Sons Pharmaceuticals in 1977 with the disclosure of captopril **1**.[3] This successful development initiated intense campaigns in structure–activity relationship optimization that yielded a range of potent and structurally diverse ACE inhibitors.[4] The presence of an N-substituted L-alanine fragment proved to be a robust structural motif present in many effective ACE inhibitors.

As shown in Figure 1 B, two representative examples of ACE inhibitor syntheses highlight improvement opportunities in the manufacturing processes. In 2005, Lupin Ltd. reported the treatment of fragment **14** with PCl_5 to yield the corresponding acid chloride **15**.[2] Isolation of this activated intermediate required careful handling the stoichiometric $POCl_3$ byproduct and was followed by amide coupling with **16**. The sequence concluded with a process intensive catalytic hydrogenolysis step to afford perindopril **6**. Warner-Lambert's approach instead relied on the activation of **18** with highly toxic phosgene to produce *N*-carboxyanhydride **19**.[5] Again, the activated intermediate was isolated before amide coupling with **20**.

As an alternative to these batch syntheses, we envisioned a continuous flow approach that would unite these structurally related products under a single synthetic paradigm while also mitigating process intensity. Specifically, we aimed to develop a rapid, scalable, and safe synthesis of ACE inhibitors starting from well-established N-substituted L-alanine derivatives **14** and **20** as a part of our ongoing interest in this class of APIs.[6] Mild activation with *N*,*N*-carbonyldiimidazole (CDI) would produce competent NCA amide coupling partners (Figure 1 C) and subsequent coupling with various *t*Bu-protected amino acid derivatives would be followed by acidic deprotection to reveal the API.

Continuous flow tactics for amide coupling have emerged as a preferable alternative to traditional batch approaches.[7] Notable advantages of these works include precisely controlled activation and amination conditions wherein deleterious pathways, such as racemization, may be avoided. Though a number of examples have been demonstrated in microscale reactors, these approaches benefit from development in inherently scalable reactors.

Our development began by evaluating the amide coupling of **18** by acid chloride activation in flow (Scheme 1). As an alternative to toxic and otherwise hash chlorinating reagents such as PCl_5, a highly soluble analogue of the Vilsmeir reagent **22** was prepared from diethyl formamide and phthalyl chloride. Activation of fragment **18** with the chlorinating reagent was followed by addition of tetrahydroisoquinoline **23**. Quenching of this stream with aqueous base and partitioning with a membrane phase separator afforded quinapril-OBn **24** in up to 69% yield. However, persistent production of insoluble material attributed to hydrochloride salts prior to the aqueous quench prevented sustained performance of the system and this route was ultimately abandoned.

As shown in Figure 2, activation of **18** by NCA was subsequently investigated. NCAs have found utility in polypeptide synthesis and their preparation has recently been embodied as an efficient continuous process, but general application to stepwise peptide coupling reactions has been limited by a propensity for undesired oligomerization.[8] It was initially suspected that in situ IR analysis would be an ideal analytical tool to monitor the unique carbonyl moieties formed upon NCA cyclization. As expected, two strong IR signals at 1783 and 1854 cm^{-1} appeared upon treatment of **18** with 1.0 equivalent

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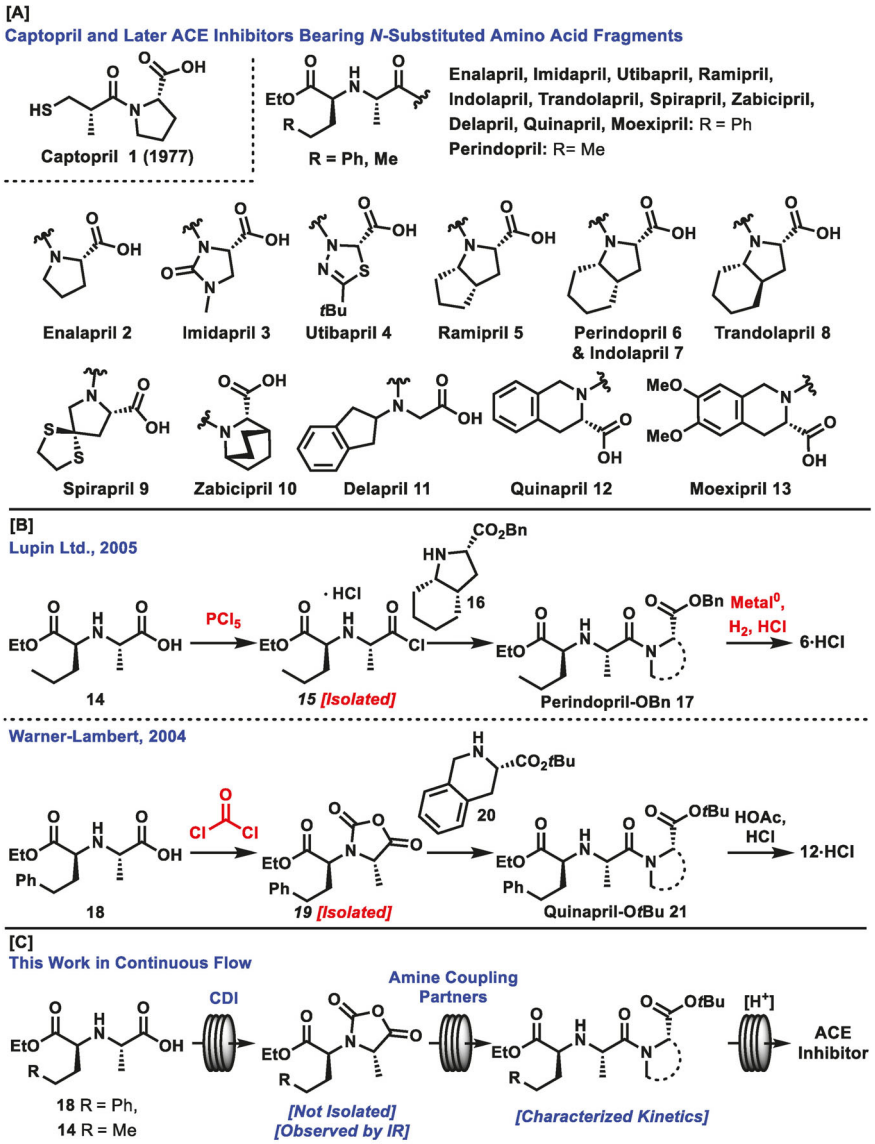
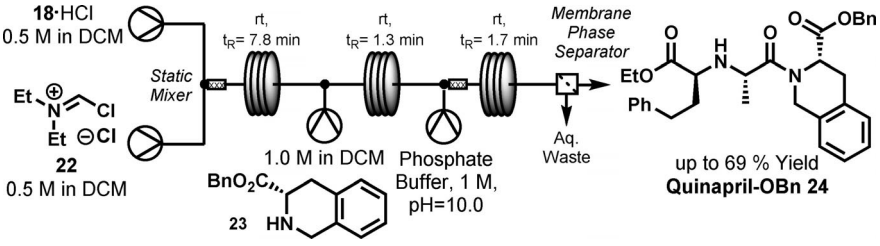


Figure 1. (A) Captopril and several related ACE inhibitors bearing a similar N-substituted L-alanine fragment. (B) Two common process strategies for activation of N-substituted L-alanine fragments in the production of perindopril **6** and quinapril **12**. (C) Continuous flow approach described herein.



Scheme 1. Initial investigation into amide coupling of **18** and **23** facilitated by acid chloride activation with iminium chloride **22**.

of CDI at room temperature under batch conditions. The NCA signals stabilized within 9 minutes, indicating that **19** could be formed under mild conditions in reaction times relevant to implementing a continuous process. In the same reaction vessel, addition of **20** resulted in decay of the anhydride signals. It is also worth noting that the carbon dioxide generated as a by-product of amide coupling was detected at 2340 cm^{-1}. Most importantly, both ^1H NMR and HPLC-MS analysis showed that the reaction sequence was not impeded by oligomerization of **18**.

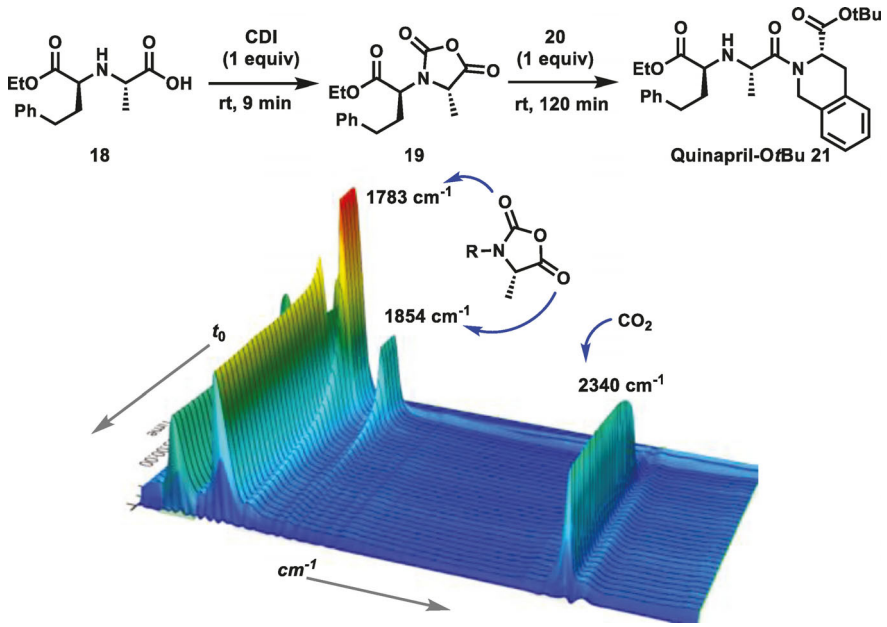
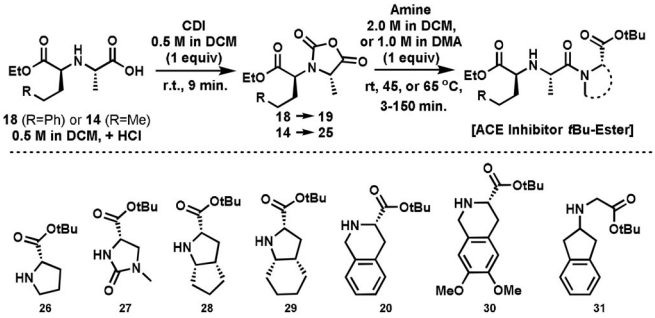


Figure 2. Preliminary experiment showing utility of in situ IR analysis in the amide coupling of **18** and **20**.

We subsequently turned our attention towards evaluating the scope of ACE inhibitors accessible via NCA activation in batch in order to outline potential residence time regimes in flow. As shown in Table 1, a range of *t*Bu-protected ACE inhibitors were accessible upon treatment of **19** and **25** with different coupling partners. In situ IR monitoring was employed throughout these trials in order to determine how reaction times varied across the range of substrates. In general, secondary amines constrained in five membered rings reacted with the highest rate and were isolated in the highest yields (Table 1, entries 1–6). Oxoimidazolidine **27** was a notable exception to this trend (entry 2) and did not react under standard conditions presumably due to poorer nucleophilicity. Reactivity was achieved by generating the potassium salt of **27** after addition of 1.0 equivalent of potassium *tert*-pentoxide, which necessitated a solvent switch of the amine solution from DCM to DMA in order to maintain homogeneity. In the case of indolapril **35**, reaction times could be shortened from 17 to 6 minutes by heating to 45 °C albeit with a loss of 16% isolated yield. (entries 4 and 5). In moving to tetrahydroisoquinoline derived **20** and **30** (entries 7–10), reaction times at room temperature were extended up to 120 minutes while still maintaining good yields. These lengthy reaction times were both reduced to 60 minutes by applying gentle heat with a slight loss of isolated yields. Acyclic amino indane **31** proved to react particularly slowly at room temperature, taking over 8 hours to reach full consumption of **19**. Further heating to 65 °C as well as addition of 20 mol% DMAP resulted in a more rapid amide coupling while maintaining good isolated yield of delapril **38** (entry 8).

We were able to gain kinetic understanding of the one-pot procedure by calibration of the IR signal with a sample of **18**. It was shown that the observed IR signal intensity correlated linearly with molar concentration under relevant reaction concentrations by examining the 1783 and 1854 cm^{-1} carbonyl frequencies (see the Supporting Information). The reactions of amines **26**, **28**, **29**, **20**, and **30** with **19** generated by reaction

Table 1. Amide coupling of N-substituted L-alanine fragments **18** and **14** with amino acid derivatives in batch.



Entry[a]	Coupling Partners	ACE Inhibitor-O*t*Bu	*T* [°C]	*t* [min][b]	*Y* [%][c]
1	**18** + **26**	enalapril **32**	23	3	99
2[d]	**18** + **27**	imidapril **33**	23	3	79
3	**18** + **28**	ramipril **34**	23	4	96
4	**18** + **29**	indolapril **35**	23	17	99
5	**18** + **29**	indolapril **35**	45	6	83
6	**14** + **29**	perindopril **36**	23	16	92
7	**18** + **20**	quinapril **21**	23	120	92
8	**18** + **20**	quinapril **21**	45	55	90
9	**18** + **30**	moexipril **37**	23	118	94
10	**18** + **30**	moexipril **37**	45	60	89
11[e]	**18** + **31**	delapril **38**	65	150	88

[a] All trials performed on 0.89 mmol scale. [b] Reaction endpoints judged by in situ IR analysis of NCA carbonyl signals. [c] Yields refer to isolated and spectroscopically pure compounds. [d] Amine was 1 M in DMA containing 1.0 equivalent of potassium *tert*-pentoxide (2 M in THF). [e] Amine solution contained 20 mol% DMAP.

of **18** with CDI were then evaluated on 1.0 mmol scale at ambient temperature. Molar reaction kinetics data was generated by normalizing the reaction times and converting IR signal intensity to molar concentration (Figure 3). Further processing revealed linear initial rates within the first minute of reaction (Figure 3 inset). Initial rate values within this time were derived from linear regression analysis, allowing for direct comparison of reaction efficiency (see the Supporting Information). The most rapid coupling was observed during formation of enalapril **32**. The initial rates analysis was also able to capture a slight loss in coupling efficiency in moving from ramipril **34** to indolapril **35**, which is likely caused by the increased steric effects of perhydroindole **29**. Lastly, a dramatic decrease in amide coupling rate was observed for tetrahydroisoquinolines **20** and **30**.

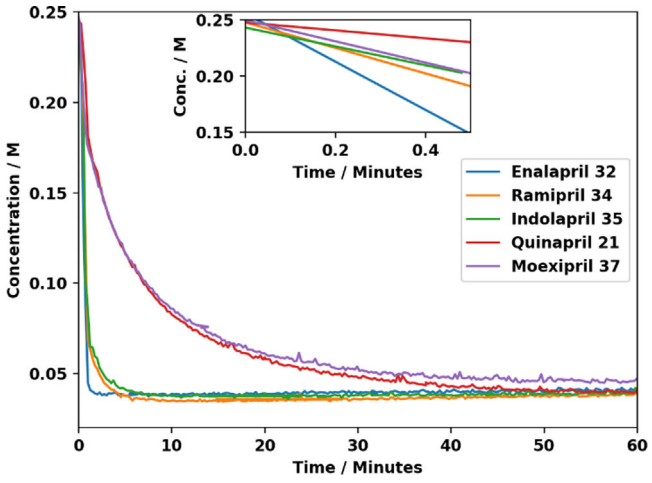
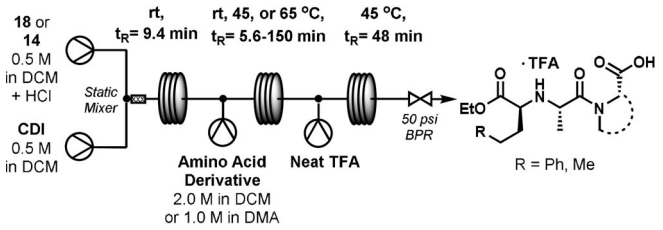


Figure 3. Amide coupling reaction kinetics with linear initial reaction rate inset. All trials were performed on 1.0 mmol scale at ambient temperature in batch by recirculating through a flow IR cell.

With the activation and amide coupling well understood, we sought to incorporate ester deprotection as a telescoped three step continuous flow process. A residence time study of the formation of NCA **19** enabled by calibrated flow IR analysis was performed in a preliminary experiment aimed at evaluating the translation of batch kinetics observation to flow (see the Supporting Information). After passing streams of **18** and CDI through a helical-type static mixer it was found that >96% yield of **19** could be achieved in ≈9.5 minutes of residence time, a value in agreement with observations made in batch. A simple tubular reactor for the fully telescoped process was then constructed from perfluoroalkoxy (PFA) polymer tubing, T-mixers, a back-pressure regulator, and a water bath for heating (Table 2). Amide coupling residence times were varied in order to accommodate the range of reaction times observed during reaction scope exploration. This aspect was not detrimental to ease of operation due to the simplicity of this continuous tubular reactor. The three-step continuous flow process resulted in good isolated yields of ACE inhibitor products starting from either **18** or **14**. Similar to the batch amide coupling results, amine substrates bearing five-membered rings were isolated in the highest yield except for imidapril **3** (Entries 1–5). In moving to quinapril **12**, moexipril **13**, and delapril **11**, yields were generally lower. The throughput of this compact benchtop reactor was ≈500 mg h^{-1}. In the case of enalapril **2**, for example, 517.8 mg of the API was isolated from a one-hour collection.

Table 2. Telescoped continuous synthesis of ACE inhibitors from **18** or **20**.



Entry[a]	Coupling Partners	ACE Inhibitor·TFA	Coupling T [°C]	Coupling t_R [min]	Y [%][b]
1	**18**+**26**	enalapril **2**	23	5.6	88
2[c]	**18**+**27**	imidapril **3**	23	5.6	74
3	**18**+**28**	ramipril **5**	23	5.6	83
4	**18**+**29**	indolapril **7**	23	16.7	82
5	**14**+**29**	perindopril **6**	23	16.7	84
6	**18**+**20**	quinapril **12**	45	45.0	86
7	**18**+**30**	moexipril **13**	45	45.0	81
8[d]	**18**+**31**	delapril **11**	65	150.0	80

[a] Continuous flow experiments were equilibrated for 3 t_R before collecting the crude reaction stream for 60 minutes. [b] Yields refer to isolated compounds after off-line purification. [c] Amine was 1 M in DMA containing 1.0 equivalent of potassium *tert*-pentoxide (2 M in THF). [d] Amine solution contained 20 mol% DMAP.

The scalability of this approach was demonstrated by performing the synthesis of enalapril **2** using a Corning Advanced-Flow Reactor platform. The molar flow rate was scaled to twice that of the benchtop reactor (Table 2, entry 1) and the system utilized 8 Low-Flow reactor plates connected in series followed by a G1 plate for a total internal volume of 12.6 mL and a total residence time of ≈47 minutes (see the Supporting Information). By taking advantage of this platform, the throughput of **2** was raised to ≈1 g h^{-1} (≈8.8 kg yr^{-1}) while maintaining a similar yield to the custom bench-top reactor (86%). Due to the well characterized thermal and mass transport properties of this system, it may be feasible to increase the throughput of this process by scaling the reactor plate volumes without need for further reaction optimization.[9]

Minimizing formation of therapeutically inactive diketopiperazine (DKP) impurities during amide coupling, protecting group removal, and isolation procedures has motivated a number of process optimization efforts.[10] Thus, avoiding this complication was a significant guiding principle of this work. Applied reaction temperature was the greatest contributing factor to DKPs observed during this study. Temperatures above 50 °C often resulted in significant DKP formation as detected by LCMS analysis of crude reaction mixtures. Even though total residence times may be reduced by thermal acceleration, eliminating DKP formation was seen as a more important target. A notable exception to this trend was delapril. Both the *t*Bu-

ester **38** and final API **11** were more thermally stable than the other ACE inhibitors.

In summary, a general strategy for synthesizing ACE inhibitors in a fully telescoped three-step continuous flow process has been established. We initially discovered that an NCA facilitated amide coupling approach was more suitable for a continuous process than acid chloride activation. Development of the NCA approach was expedited by the use of calibrated in situ IR analysis and greater understanding of kinetic behavior was gained by initial rates analysis. A key feature of this approach is the unification of the synthetic process of this class of important APIs. Future work will focus on select ACE inhibitors that do not bear the commonly employed N-substituted L-alanine fragment exploited in this strategy.

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Conflict of interest

The authors declare no conflict of interest.

Keywords: ACE inhibitors · amides · anhydrides · kinetics · multistep continuous flow synthesis

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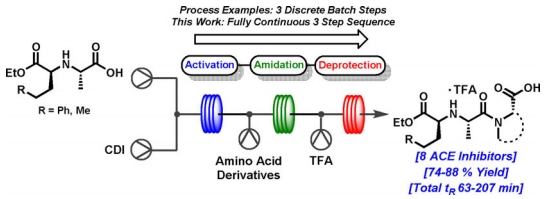

An "ACE" in the hole: A range of angiotensin converting enzyme inhibitors were synthesized in continuous flow using a single synthetic approach in good overall yields (see scheme). Utilization of in situ IR analysis facilitated a rapid development process and provided kinetic insight to the key amide coupling step across the set of important active pharmaceutical ingredients.