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Use of *N*-trifluoroacetyl-protected amino acid chlorides in peptide coupling reactions with virtually complete preservation of stereochemistry

Paul A. Jass,[a,†] Victor W. Rosso,[a] Saibaba Racha,[b] Nachimuthu Soundararajan,[a] John J. Venit,[a] Andrew Rusowicz,[a] Shankar Swaminathan,[a] Julia Livshitz[a] and Edward J. Delaney[a,*]

[a]*Bristol-Myers Squibb Company, Process Research and Development, P.O. Box 191, New Brunswick, NJ 0890, USA*
[b]*Bristol-Myers Squibb Company, P.O. Box 4755, Mail Stop: N-6, Syracuse, NY 13221-4755, USA*

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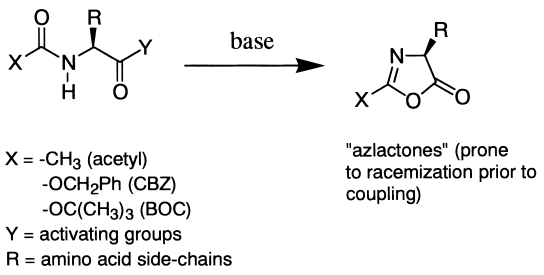
Abstract—The use of protected amino acid chlorides for peptide coupling reactions has long been avoided due to the extensive racemization that commonly occurs during either the acid chloride formation or the coupling reaction itself. Conditions are described which allow *N*-trifluoroacetyl-protected amino acid chlorides to be generated in high purity and with high retention of stereochemical integrity. Control of temperature is the predominant factor in controlling racemization, and rapid formation of acid chlorides under low temperature can be conveniently achieved using Vilsmeier reagent. Stereochemical integrity is further maintained when coupling of *N*-trifluoroacetyl acid chlorides is carried out with amino acid esters under Schotten–Baumann conditions using specific controls on pH, temperature, and agitation. Second order rate constants for coupling and the azlactone formation associated with racemization were measured to be 4260 and 3.6 L/mol s, respectively. This high rate differential allows for the reaction to be run with a minimum excess of amine ester, and makes it suitable for continuous processing. The applicability of the preferred coupling conditions to a range of amino acid couplings is described.

1. Introduction

Peptide bond formation is among the most important and widely studied chemical transformations in synthetic organic chemistry. Racemization of the amine-protected amino acid partner is the predominant problem in coupling two amino acids, and this issue has traditionally been addressed by using urethane-based protecting groups, Boc, Cbz, and Fmoc being among the most common. Simple amide acids, such as *N*-acetyl, are well known to racemize at an unacceptable rate upon activation of the carboxylic acid function in the presence of base. The mechanism for racemization is widely accepted to occur via an azlactone intermediate (Scheme 1).[1]

In contrast, urethane-protecting groups have been shown to be much less prone to azlactone formation and subsequent scrambling of the chiral center. Nonetheless, appreciable racemization can still occur, and is typically suppressed by use of the intermediate activated esters (e.g. –OBt, or –OSu) formed in conjunction with carbodiimides (DCC or EDAC).



Scheme 1.

Use of the trifluoroacetyl group as an amine protecting group is merely footnoted in most textbooks on peptide synthesis, as early efforts to prepare *N*-trifluoroacetyl amino acids employing trifluoroacetic anhydride were plagued by racemization, and the use of other groups, such as Boc and Cbz, proved to be acceptable alternatives. In spite of later reports of a much cleaner method to prepare *N*-trifluoroacetyl-protected amino acids using ethyl trifluoroacetate,[2] and in spite of the ease which with TFA may be removed (dilute base), *N*-trifluoroacetyl protection is rarely used in the preparation of peptides and peptide mimetic drugs.

Keywords: peptide coupling; *N*-trifluoroacetyl protection; acid chloride formation; amide formation; Schotten–Baumann; azlactone formation; kinetic rate of coupling; kinetic rate of racemization.

* Corresponding author. Tel.: +1-732-519-3092; fax: +1-732-519-2531; e-mail: edward.delaney@bms.com

† Present address: Cambrex Pharma, Cambrex Charles City, Inc., 1205 11th Street, Charles City, IA 50616-3466, USA.

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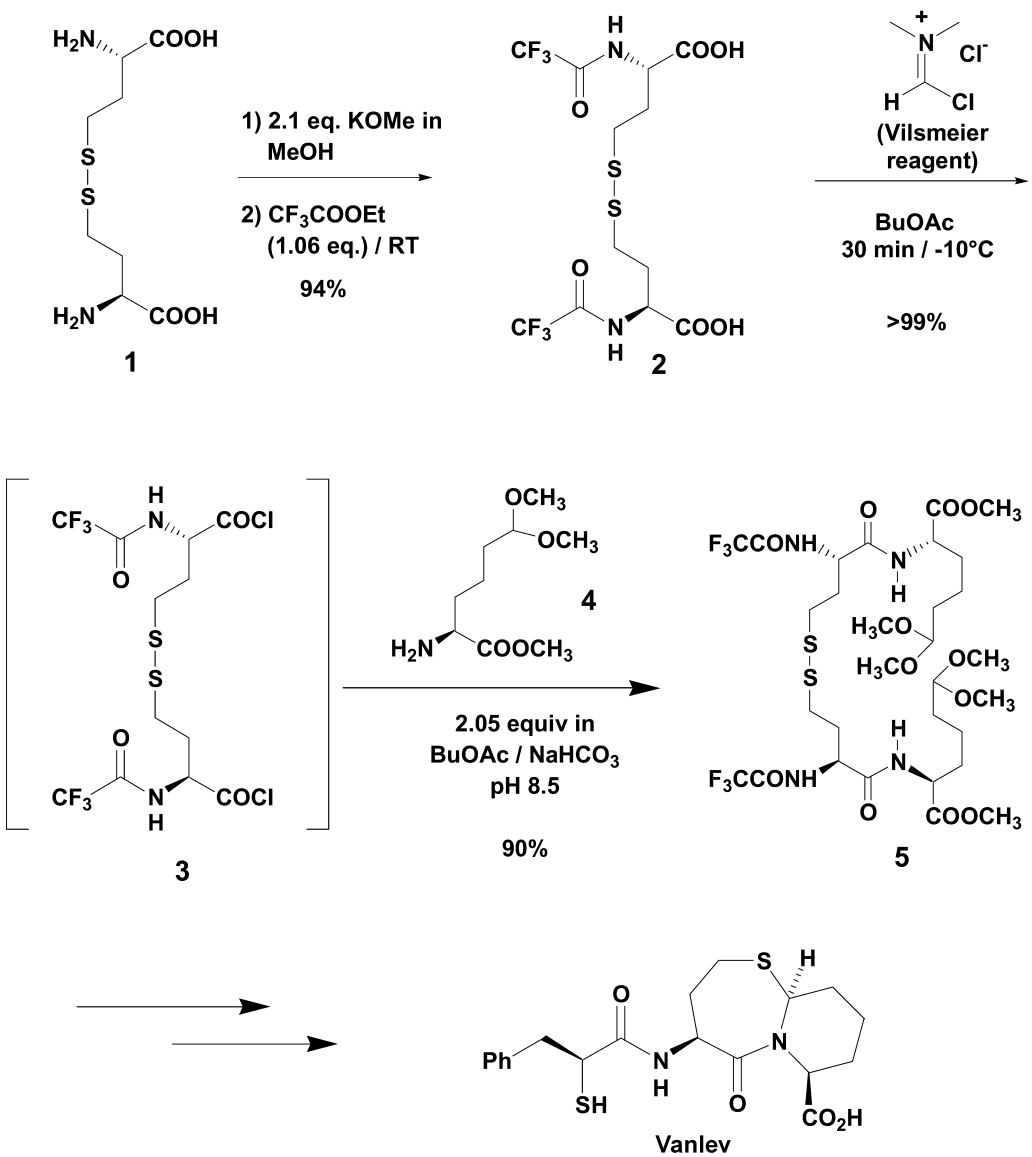
Many attempts were made during the 1950s and early 1960s to prepare N-protected amino acid chlorides and employ them in peptide couplings. However, with the exception of glycine, this approach has universally been dismissed as untenable due to the high propensity of N-protected amino acid chlorides to racemize.[3] Nonetheless, the use of chiral TFA-protected amino acid chlorides in Friedel–Crafts acylations has been reported to proceed with high retention of stereochemical integrity.[4] At the same time, the generation of Fmoc-protected amino acid chlorides and their use in peptide couplings under Schotten–Baumann conditions has also been reported to proceed with high retention of stereochemical integrity.[5] These reports suggested that *N*-trifluoroacetyl amino acid chlorides may be similarly useful in peptide couplings.

In the course of process development studies, we sought (1) an amino acid protecting group that would be inexpensive and more readily removed for our purpose than existing agents, and (2) coupling conditions that would provide a high rate of conversion while minimizing the molar excesses of intermediates normally required to force coupling to completion. In the work described herein, we report general techniques by which *N*-trifluoroacetyl chlorides may be effectively prepared and used in peptide coupling reactions with very little loss of stereochemical integrity. This method has been demonstrated on a variety of amino acid substrates, and in many cases should prove a superior approach at commercial scale, where significant advantages of cost, simplicity, and atom-economy may be recognized over long-standing methods.

2. Results and discussion

2.1. *N*-Trifluoroacetylation of amino acids

Slight modifications were made to extend the methods of Steglich[2a] and Curphy[2b] for converting amino acids to their respective *N*-trifluoroacetyl derivatives. General conditions developed involve the generation of the desired amino acid potassium salt in methanol, and subsequent treatment with



Scheme 2.

methyl trifluoroacetate. The aminolysis reaction can be brought to completion at 5°C within 2 h when 1.2–2 equiv. of TFA ester are used in the presence of as little as 0.1 equiv. excess of potassium methoxide. For convenience, the amino acid is suspended in dry methanol, and 1.1 equiv. of potassium methoxide solution (or 2.1 equiv. in the case of amine diacids) are added to insure potassium salt formation and catalysis of the reaction. Potassium and dipotassium salts of the amino acids studied were found to be much more soluble in this system than their respective sodium or triethylamine salts, and dramatic improvements in reaction time were obtained in the case of amine diacids, such as aspartic acid, glutamic acid, and homocystine. Several representative examples of the recommended conditions are provided in Section 4.

2.2. Preparation of *N*-trifluoroacetyl amino acid chlorides

In the course of developing a process for the anti-hypertensive agent, Vanlev (Scheme 2), we sought coupling conditions that would allow for the diacid **1** to be reacted with amino ester **4** to provide isolated intermediate **5** in high yield with minimal loss of stereochemical integrity.

The intermediacy of acid chlorides for this purpose showed promise over traditional coupling methods in screening studies, and was deemed worthy of exploration in spite our initial skepticism. With further development we found that **2** could be effectively activated with Vilsmeier reagent at low temperature to cleanly provide the intermediate acid chloride, and that coupling of the latter with **2** under Schotten–Baumann conditions (vide infra) provided **3** in high yield with high preservation of stereochemical integrity. Since amino ester **4** was costly to produce, the prospect of achieving rapid reaction completion with slight excesses of amine while maintaining high retention of stereochemistry motivated us to develop a rugged process around this finding.

While the extensive racemization that accompanies the use of protected amino acid chlorides in peptide couplings is well documented, no systematic investigations appear to have been done to determine the point at which racemization occurs, or the factors that control it. The use of TFA-protected acid chloride substrates in Friedel–Crafts reactions has been reported in many cases[4] to proceed with high retention of enantiomeric integrity, suggesting that racemization is not so much a problem during acid chloride formation as it is during coupling. At least one GC method for the determination of chiral purity has been reported based on the reaction of *N*-TFA protected amino acid chlorides with chiral primary amines.[6] Finally, a report from the early days of peptide methodology exploration recommended widespread use of *N*-trifluoroacetyl protection based on a case where *N*-TFA-L-Phe acid chloride coupling with aniline was found to proceed with retention of stereochemistry![7] In light of these past reports and the success observed in coupling **1** with **4**, it appeared that the general preparation and use of *N*-TFA-protected acid chlorides in peptide couplings was merely dependent on specific techniques that, once understood, could be applied broadly.

Table 1. Levels of diastereomeric dipeptide formed under various coupling conditions

Reagent (solvent)	Temperature (°C)	Sampling time (h)[a,b]	Example 1, TFA-Met-Phe-OMe D,L diastereomer (area% by GC)	Example 2, TFA-Ile-Phe-OMe D,L diastereomer (area% by GC)
$SOCl_2$ (toluene)	80	4	5.3	2.4
		5	7.0	2.3
		9	12.4	12.9
CO_2Cl_2 / cat. DMF (CH_2Cl_2)	25	1.5	1.8	1.7
		2.5	2.7	2.7
		24.0	24.4	28.0
Vilsmeier (BuOAc)	5	4	0.9	2.8
		5	1.0	3.3
		9	1.3	3.7
		27	2.7	6.2
Vilsmeier (BuOAc)	0	4.5	0.53	0.31
		6	0.82	0.43
		23	1.08	1.19
Vilsmeier (BuOAc)	−10	4	<0.2	<0.2
		7	<0.2	0.22
		27	0.22	0.27
Vilsmeier (CH_2Cl_2)	−10	3	0.35	–
		23	0.42	–
Vilsmeier (CH_2Cl_2)	−15	1	<0.2	–
		3.25	<0.2	–
		21	0.43	–

[a] Times shown are from initiation of acid chloride reaction to point of sampling for coupling reaction; actual completion of acid chloride formation was confirmed within 30–60 min.

[b] TFA-L-Met and TFA-L-Ile acid chloride samples tested by quenching into 0°C BuOAc containing 3.5 equiv. of L-Phe-OMe; dipeptide derivatives were then analyzed for diastereomer content by GC.

To test the sensitivity of TFA acid chlorides to racemization under the conditions of their formation, two standard *N*-TFA protected amino acids (TFA-L-Met and TFA-L-Ile) were subjected to various conditions in order to convert them to their respective acid chlorides. Once formed, the stability of the acid chlorides to racemization was monitored by withdrawing samples over time and subjecting them to a rapid coupling with a 3-fold excess of L-phenylalanine methyl ester at about 0°C. The resulting dipeptide samples were then analyzed by GC for diastereomeric purity. Variables investigated were (1) reagent/solvent combinations, (2) temperature and (3) time. The results of this study are shown in Table 1.

Considerable racemization was observed for acid chlorides formed under most experimental conditions, but clearly, the key variable for controlling racemization is temperature. Toward that end, use of Vilsmeier reagent at or below −10°C provided a reasonably rapid reaction while conferring excellent control over racemization. Moreover, stability was demonstrated for at least 24 h when completed reaction mixtures were maintained at that temperature. While Vilsmeier reagent is relatively insoluble in organic solvents, it reacts readily through its soluble fraction. Various protected amino acids tested were reacted completely in either ethyl or butyl acetate within 30–60 min, even when reactions were carried out at or slightly below −10°C. Dichloromethane also appeared to be a suitable solvent with temperatures at or below −10°C, although greater care is required to control the reaction exotherm owing to the better solubility of Vilsmeier reagent in dichloromethane relative to that observed with the ester solvents.

When using Vilsmeier reagent to effect activation, the formation of DMF/HCl complex was observed as a precipitate or as a second liquid phase. This finding initially suggested that the formation and segregation of DMF/HCl complex may play a role in stabilizing the acid chloride product by suppressing an acid-catalyzed racemization pathway. To test the relative importance of DMF/HCl segregation to racemization, several independent experiments were conducted using the Vilsmeier/dichloromethane condition at about 5°C in order to achieve maximum solubility of all components while maintaining concentration as close as possible to the original condition (Table 2). In addition to a control experiment in which MTBE was added to insure full solubility and availability of the DMF/HCl complex, identically completed activations were spiked with 4 and 9 extra equivalents of HCl introduced as MTBE solutions. Interestingly, the presence of added HCl had a minimal impact in both cases, as the acid chloride solutions retained their stability relative to the control. These findings clearly ruled out acidity as a significant contributing factor to acid chloride racemization.

DMF was found to be an unsuitable solvent for the activation step, as racemization rates were shown to be quite high in its presence. Reactions completed using Vilsmeier reagent in BuOAc at −10°C were also found to reform significant levels of free acid when DMF was added. These findings suggest an appreciable reverse rate for the Vilsmeier activation step, and implicate activated intermediate **7** (Scheme 3) as a species which is likely present at low concentration, but which possesses a much faster racemization rate relative to the acid chloride. Toward that end, in tying up free DMF, the HCl generated during the activation step helps suppress reverse reaction and plays an important role in stabilizing the acid chloride product.

2.3. *N*-Trifluoroacetyl amino acid chloride coupling with amino esters under Schotten–Baumann conditions

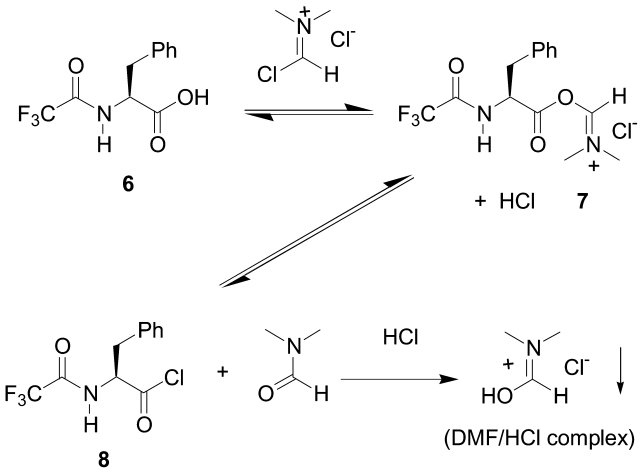
Racemization during coupling is widely held to occur via azlactone intermediates which form most readily under basic conditions. It should come as no surprise then that peptide couplings are most successfully performed under conditions that maximize stability of the activated acid moiety while allowing the amine partner to exist as free base under as close to neutral pH as possible. For this reason, *N*-methyl morpholine (pKa ~7.4) has been cited to be a preferred base for neutralizing the acidic counterion generated from mixed anhydride couplings, and for freeing the amine coupling partner for reaction.[8] In the course of

Table 2. Effect of acidity and reversibility on racemization during activation

Alterations to standard activation procedure	Tempeature (°C)	Time (h)[a,b]	TFA-Met-Phe-OMe D,L diastereomer (area% by GC)
CH_2Cl_2 solvent, MTBE added (control)	5	4	0.89
		6	0.97
		27	1.15
MTBE containing four additional equivalents HCl added to $MeCl_2$	5	4	0.57
		6	1.00
		27	0.97
MTBE containing nine additional equivalents HCl added to CH_2Cl_2	5	4	0.54
		6	0.74
		27	0.75
DMF as solvent	−10	3	15.0
		23	24.3

[a] Times shown are from initiation of acid chloride reaction to point of sampling for coupling reaction; actual completion of acid chloride formation was confirmed within 30–60 min.

[b] TFA-L-Met and TFA-L-Ile acid chloride samples tested by quenching into 0°C BuOAc containing 3.5 equiv. of L-Phe-Ome; dipeptide derivatives were then analyzed for diastereomer content by GC.



Scheme 3.

developing an economical process for Vanlev, we explored the applicability of the Schotten–Baumann technique to the coupling of the acid chloride of bis-*N*-TFA protected homocystine **1** with the acetal amine ester **2** (Scheme 2). Done properly, this reaction was found to proceed very cleanly, with very little hydrolysis, and virtually no loss of stereochemical integrity. We subsequently studied this coupling with an eye toward generality in peptide couplings. Keys to the method are temperature control (0–5°C), controlled addition of the acid chloride mixture, the maintenance of pH between 7.0 and 8.0, and highly efficient mixing. For this system, a narrow pH range was found to be necessary; with significantly higher pH, azlactone formation, racemization, and acid chloride hydrolysis competed more effectively with coupling, while at significantly lower pH, protonation of the amine ester inhibited both its transfer to the organic compartment and the coupling reaction itself. Optimally, a buffered environment (K_2HPO_4) is used to maintain the pH in the aqueous layer, and pH is maintained throughout addition of the acid chloride by simultaneous addition of aqueous sodium hydroxide. This control is most efficiently achieved using a feedback loop from a pH meter to control the flow of aqueous base. Relatively constant pH can also be insured by employing a large excess of aqueous sodium bicarbonate as a buffer to the aqueous phase. Control of racemization is virtually equivalent under these more convenient conditions. Amine esters are typically introduced into the procedure as their hydrochloride salts, and sufficient base is included at the onset to effect liberation of the free base. Since many amine esters are prone to dimer and diketopiperazine formation over time, extensive hold times of the free base should be avoided prior to coupling.

Thorough mixing is needed to insure full availability of the amine ester present in the aqueous phase to the organic phase, and the avoidance of localized acid chloride hydrolysis and azlactone formation when the reaction is carried out on large scale. This aspect can be particularly important, as some of the amine esters measured possess partition coefficients that favor the aqueous phase over the organic in ratios of 5 to 1 or greater. Controlled direct addition of the TFA-protected acid chloride mixture has also been found to be helpful in conjunction with thorough mixing to minimize the competing hydrolysis, and to allow the pH to be maintained in a stable condition with the concomitant addition of 1N sodium hydroxide. Maintenance of the aqueous bicarbonate buffer between pH 7.0 and 8.0 helps minimize hydrolysis. Most importantly, however, it also serves as a superior surrogate for weak tertiary amines in neutralizing the acid generated, since the competing protonation of the amine coupling partner that typically occurs with tertiary amines is eliminated.

2.4. Kinetic study to determine rates of coupling, azlactone formation, and hydrolysis

While the conditions cited work optimally for the Vanlev intermediate, a kinetic study was undertaken on a more general model reaction (*N*-TFA-L-Phe acid chloride with L-Phe-OMe) to gain a fundamental understanding of the relative rates of the reaction and side-reactions involved, which could then be extrapolated to other closely-related couplings. In preparation for this study, an authentic sample of the corresponding *N*-TFA-L-Phe azlactone was prepared and confirmed to be stable to the HPLC conditions by which the rates would be measured.

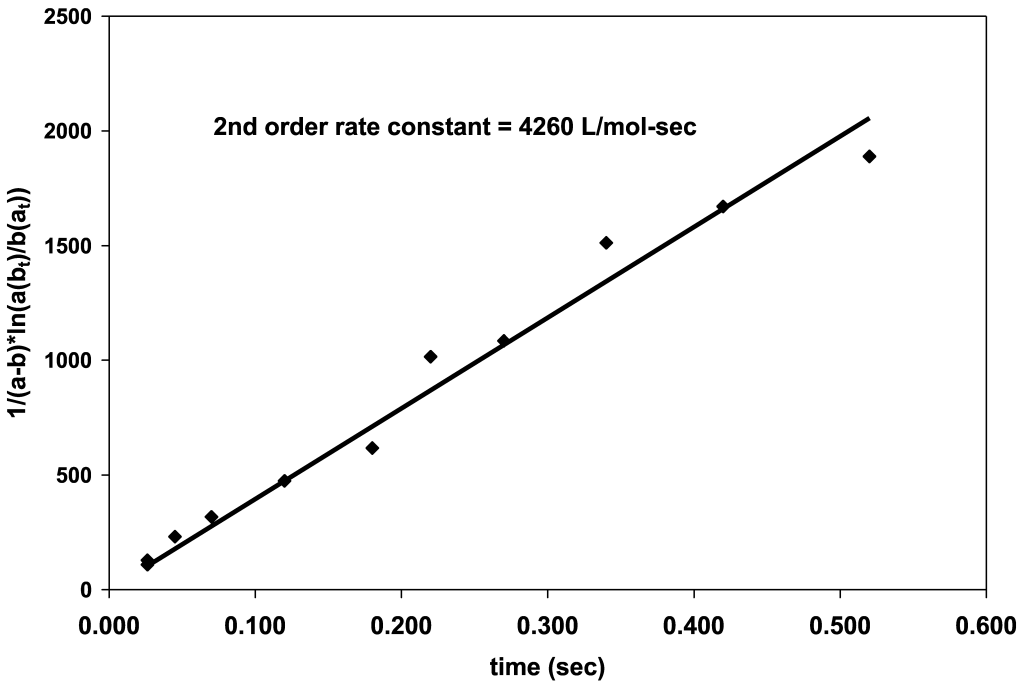
Coupling of TFA protected L-Phe acid chloride with L-Phe methyl ester was measured at 5°C under dilute conditions to possess a second order rate constant of 4260 L/mol s (Fig. 1), and a half-life back-calculated on the basis of the normal concentration to be 0.7 ms. The competing rate of azlactone formation was estimated at 3.6 L/mol s based on the amount of azlactone competitively formed as determined by HPLC (corresponding half-life of 0.8 s).[‡] A secondary study of the rate of azlactone formation in the presence of diisopropylethylamine measured 4.1 L/mol s (Fig. 2). These results were very much in line with results from HPLC that indicate very little azlactone formation during coupling (<0.4%). In contrast, the pseudo first-order acid chloride hydrolysis at pH 8.5 was roughly measured to be about 0.04/s (half-life of 17 s). This finding is also consistent with the relatively small amount of free acid formed by acid chloride hydrolysis under the Schotten–Baumann conditions employed.

Beyond the excellent control of azlactone formation and hydrolysis provided under these conditions, the extremely rapid rate of coupling provides two key advantages. First, it allows for the reaction to be completed rapidly and efficiently with a minimal excess of amine ester (e.g. equivalent excesses of 5% or less). Second, it makes the procedure highly amenable to the engineering of a continuous process.

2.5. Scope and limitations

In order to examine the range of applicability of this method, the TFA derivatives of several additional amino acids were converted to their respective acid chlorides, and

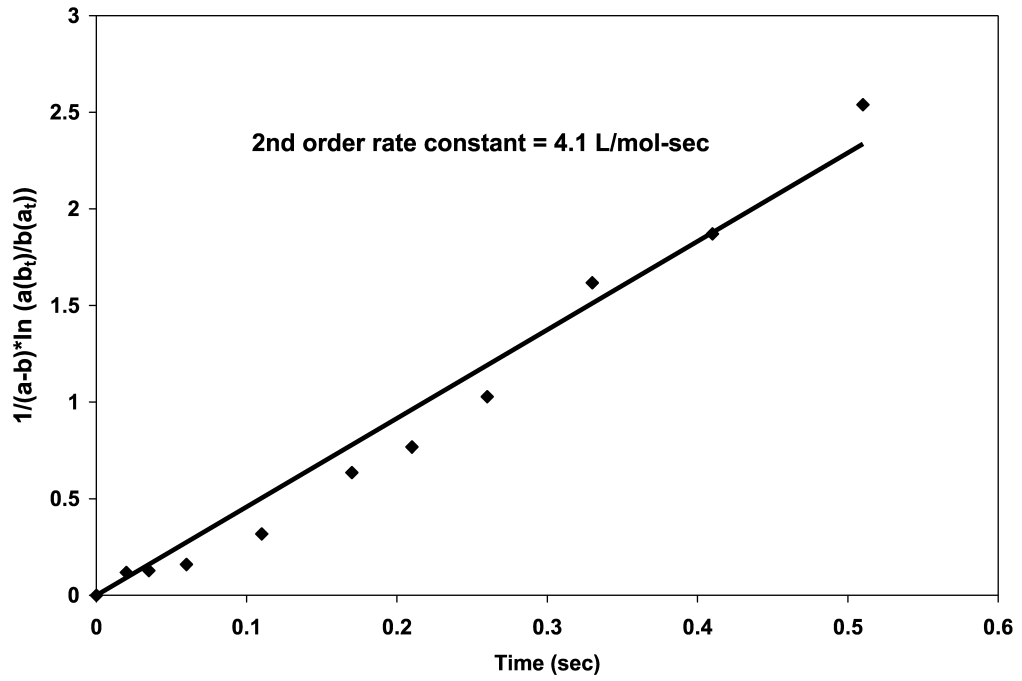
[‡] These are simplified half-live estimations, based on the assumptions of having both substrates being present in the organic compartment, instant mixing, and perfect temperature maintenance. While this condition obviously does not exist throughout the course of reaction, it is most closely held during reaction initiation, and the rates are provided to give a sense of feeling for the overall reaction speed.



a, b = initial concentration of amine, acid chloride, respectively
a_t, b_t = concentrations of amine, acid chloride, respectively, at time t

Figure 1. Measurement of second-order rate constant for reaction of *N*-TFA-L-phenylalanyl acid chloride with L-phenylalanine methyl ester (coupling).

coupling with L-phenylalanine ethyl ester was carried out under Schotten–Baumann conditions. Rapid coupling completion was achieved in all cases. While the data shown in Table 3 verify that the coupling method is generally applicable to other amino acids, we have found minor losses of stereochemical integrity (<0.5% wrong diastereomer) when variables such as buffer concentration and choice of base are varied. Most important of these variables, however, is the temperature control during acid chloride formation and during the hold time prior to its addition to the coupling mixture. While yields obtained for the coupling partners shown in Table 3 are generally very



a, b = initial concentration of amine, acid chloride, respectively
a_t, b_t = concentrations of amine, acid chloride, respectively, at time t

Figure 2. Measurement of second-order rate constant for reaction of *N*-TFA-L-phenylalanyl acid chloride with diisopropylethylamine (azlactone formation).

Table 3. Coupling of various TFA-amino acid chlorides with L-phenylalanine methyl ester

Coupled products	Yield (%)	D,L diastereomer (%)	Method
TFA-*N*-L-Met-L-Phe-OMe	92	<0.2	4
TFA-*N*-L-Ile-L-Phe-OMe	98	<0.2	1
TFA-*N*-L-Pro-L-Phe-OMe	44	<0.2	3
TFA-*N*-L-Phe-L-Phe-OMe	91	<0.2	1
TFA-*N*-L-Leu-L-Phe-OMe	95	<0.2	3
Bis-TFA-*N*,*N*-L-Lys-L-Phe-OMe	89	<0.2	2
TFA-*N*-L-Tyr-L-Phe-OMe	74	<0.2	2

good, coupling with TFA proline was complicated by side reactions which substantially lowered the yield, illustrating the fact that optimization of coupling conditions may be warranted with specific individual cases.

3. Conclusions

These studies conclusively demonstrate the viability of carrying out acid chloride-mediated peptide couplings in conjunction with *N*-trifluoroacetyl protection, without appreciable scrambling of stereochemistry. Use of Vilsmeier reagent at low temperature (−10°C or below) in ethyl acetate, butyl acetate, or dichloromethane provides a special condition allowing for *N*-TFA-protected amino acids to be efficiently converted to their respective acid chlorides with high retention of stereochemistry. While other means of forming the acid chloride can also be used with adequate temperature control, the rapid and clean nature of the Vilsmeier/low temperature technique provides an advantage in providing stability of the acid chloride toward racemization prior to coupling. Subsequent use of the Schotten–Baumann technique, in conjunction with excellent agitation and pH control during reaction, provides a mild set of conditions under which coupling can take place with very little loss of stereochemical integrity. Once peptide coupling is completed, the protecting trifluoroacetyl group can be readily removed using methods well documented in the chemical literature, including base-assisted hydrolyses sufficiently mild as to be compatible with the remote presence of a methyl ester.[9] With these specific conditions, the use of TFA protection becomes highly competitive with other more commonly used amine protecting groups. In fact, the coupling conditions described in Scheme 2 were the basis of a process by which we ultimately made Vanlev intermediate **5** in high yield and purity at >100 kg input scale. Furthermore, with further optimization, the method could conceivably be used in repeat cycles to prepare polypeptides in a highly efficient, continuous manner.

4. Experimental

All NMR spectra were obtained in DMSO-d_6 or CD_3CN using a 300 MHz spectrometer. Uncorrected melting points were determined on a capillary melting point apparatus. HPLC assays to measure acid chloride formation (via MeOH quench) and coupling were performed by injecting 5 μL samples onto a YMC ODS-A 4.6×50 mm column, and eluting with 75/25 0.1% aqueous phosphoric acid/acetonitrile mixture for 1 min, followed by a linear gradient over 4 min to 15/85 0.1% aqueous phosphoric acid/acetonitrile, finishing with a 1 min elution with the later. Flow rate was 2 mL/min and detection was at 210 nm. All compounds were linear in the analyzed range and each component was corrected for molar extinction coefficient. Vilsmeier reagent used in all studies was purchased from commercial sources.

Methods used to determine diastereomeric purity (Table 3) were as follows.

Method 1. Isocratic HPLC, 1 mL/min 60% 0.01 M KH_2PO_4 (pH 3.0), HP ODS Hypersil 125 mm×4 mm column, detection at 220 nm.

Method 2. Isocratic HPLC, 1 mL/min, 80% water/20% methanol, Astec Chirobiotic T, 250 mm×4.6 mm column, detection at 220 nm.

Method 3. Hewlett–Packard 6890 GC, Helium flow 47 cm/s, 220°C Hewlett Packard HP-5 (5%diphenyl–95%dimethylsiloxane) column, 25 m×0.32 mm i.d.×0.17 μm capillary column, FID detector.

Method 4. Hewlett–Packard 6890 GC, He flow 47 cm/s, 230°C Hewlett–Packard HP-5 (5% diphenyl–95% dimethylsiloxane) column, 25 m×0.32 mm i.d.×0.17 μm capillary column, FID detector.

4.1. General procedure for TFA protection of amino acids

The amino acid substrate (10 mmol) was dissolved in 2.3 mL of 4.4 M (1.0 equiv.) potassium methoxide in methanol. The solution was then reacted with 1.2 mL ethyl trifluoroacetate (2.0 equiv.) at 40°C until complete (usually <0.5% remaining starting material) by HPLC assay. The solution was cooled and quenched into 4.1 mL of 2.5 M aqueous HCl and extracted with 8 mL of ethyl acetate. The organic phase was washed once with 10 wt% brine in 1 M HCl, and concentrated to a residue. The residue was then crystallized from a solvent combination, or converted directly to its acid chloride as described below. In cases where it was difficult to isolate the TFA protected amino acids,[10] the crude material obtained was telescoped directly into the coupling step. Reaction yields are typically close to quantitative, and the yields reported below are unoptimized.

4.1.1. *N*-Trifluoroacetyl-L-phenylalanine. Isolated yield: 84%. Colorless solid (1:1 heptane/toluene). Mp: 90–91°C; IR (cm^{-1}, KBr): 1711, 1562, 1214, 1178, 1168, 758, 702; ^1H NMR (DMSO-d_6) δ: 3.00 (αβq, 1H), 3.23 (αβq, 1H), 4.54 (αβq, 1H), 7.27 (m, 5H), 8.83 (d, NH), 13.0 (br, 1H); ^{19}F NMR (DMSO-d_6), δ: −75.3 (s, CF_3); ^{13}C NMR (DMSO-d_6), δ: 35.7, 54.1, 126.7, 128.3, 129.1, 137.4, 156.4, 171.5; MS *m*/*z* $M-H^-$ 260, $M+COCF_3^-$ 374. Anal: calcd for $C_{11}H_{10}NO_3F_3$+3.56% H_2O measured by KF: C, 48.78; H, 4.14; N, 5.17; F, 21.05. Found: C, 48.63; H, 4.04; N, 5.13; F, 21.05.

4.1.2. L-Phenylalanine, *N*-trifluoroacetyl. Isolated yield: 70%. Colorless solid (1:2 ethyl acetate/heptane). Mp: 91–92°C; IR (cm^{-1}, KBr): 1714, 1559, 1212, 1178, 757, 700; ^1H NMR (CD_3CN) δ: 3.05 ($\alpha\beta q$, 1H), 3.25 ($\alpha\beta q$, 1H), 4.68 ($\alpha\beta q$, 1H), 7.26 (m, 5H), 7.72 (d, 1H); ^{19}F NMR, δ: −74.2 (s, CF_3); ^{13}C NMR ($CDCl_3$) δ: 37.1, 53.3, 117.3q, 127.8, 128.9, 129.3, 134.3, 156.5q, 174.0; MS *m/z* $M-H^-$ 260, $M+COCF_3^-$ 374. Anal. calcd for $C_{11}H_{10}NO_3F_3$: C 49.45; H, 4.02; N, 5.24; F, 19.94. Found: C, 49.32; H, 4.12; N, 5.04; F, 20.15.

4.1.3. L-Methionine, *N*-trifluoroacetyl. Isolated yield: 81%. Colorless solid (CH_2Cl_2/toluene/heptane 1:2). Mp: 61°C; IR (cm^{-1}, KBr): 1710, 1746, 1567, 1244, 1224, 1183, 1158, 727, 691; ^1H NMR (CD_3CN) δ: 2.08 (s, 3H), 2.18 (m, 2H), 2.62 (m, 2H) 4.58 (m, 1H) 7.8 (br, 1H); ^{19}F NMR, δ: −74.1 (s, CF_3); ^{13}C NMR ($CDCl_3$) δ: 15.2, 29.7, 30.1, 51.9, 115 (q), 157.4 (q), 174.3; MS *m/z* $M+NH_4^-$=263. Anal. calcd for $C_7H_{10}NO_3F_3S$ +3.34% H_2O measured by KF: C, 33.14; H, 4.35; N, 5.52; S, 12.64; F, 22.47. Found: C, 32.99; H, 4.34; N, 5.39; S, 13.14; F 22.43.

4.1.4. L-Lysine, N2,N6-bis(trifluoroacetyl). Isolated yield: 60%. Colorless solid (from chloroform after passing through alumina column with THF). Mp: 113–115°C; IR (cm^{-1} KBr): 1740, 1705, 1560, 1230, 1170, 735; ^1H NMR (CD_3CN) δ: 1.32 (m, 2H), 1.48 (m, 2H), 1.82 ($\alpha\beta q$, 2H), 3.29 (q, 1H), 4.38 (m, 1H), 7.6 (s, NH), 7.8 (d, NH), 8.95 (br, COOH); ^{19}F NMR, δ: −75.1 (s, CF_3), −75.4 (s, CF_3); ^{13}C NMR (CD_3CN) δ: 23.5, 28.8, 30.9, 40.1, 53.7, 116.4 (q), 158.1 (q), 172.7. Anal. calcd for $C_{10}H_{12}N_2O_4F_6$+3.34% H_2O: C, 35.51; H, 3.58; N, 8.28; F, 33.70. Found: C, 35.44; H, 3.59; N, 8.13; F, 32.79.

4.1.5. L-Tyrosine, *N*-trifluoroacetyl. (Note: required 2 equiv. KOMe) Isolated yield: 86%. Colorless solid (from MTBE/hexane 1:1). Mp 194–195°C; IR (cm^{-1}, KBr): 1694, 1560, 1516, 1250, 1210, 1185, 1152, 678; ^1H NMR (CD_3CN) δ: 3.05 ($\alpha\beta q$, 2H), 4.60 ($\alpha\beta q$, 1H), 6.72 (d, 2H) 7.05 (d, 2H) 7.65 (d, NH); ^{19}F NMR, δ: −75.4 (s, CF_3), −75.4 (s, CF_3); ^{13}C NMR (CD_3CN) δ: 36, 55, 116, 117q, 128, 131, 135, 148, 155q, 173; MS *m/z* $M-H^-$=276. Anal. calcd for $C_{11}H_{10}NO_4F_3$+3.34% H_2O: C, 47.66; H, 3.64; N, 5.05. Found: C, 47.65; H, 3.74; N, 4.97.

4.1.6. L-Leucine, *N*-trifluoroacetyl. Isolated yield: 93% (crude, yellow oil). IR (cm^{-1}, KBr): 1706, 1562, 1183, 1260, 1209, 1168, 732, 702, 922; ^1H NMR ($CDCl_3$) δ: 0.96 (d, 6H), 1.31 (m, 1H), 1.78 (m, 2H), 4.64 (m, 1H) 7.2 (d, NH), 9.8 (brs, OH); ^{19}F NMR, δ: −76.4 (s, CF_3); ^{13}C NMR ($CDCl_3$) δ: 21.5, 22.6, 24.8, 40.6, 51.2, 117.5 (q), 157.1 (q), 175.7; MS *m/z* $M+NH_4^-$=245.

4.1.7. L-Isoluecine, *N*-trifluoroacetyl. Isolated yield: 61% (waxy solid). Mp 61°C; IR (cm^{-1}, KBr): 1731, 1567, 1470, 1393, 1372, 1163, 732; ^1H NMR ($CDCl_3$) δ: 0.97 (d, 3H), 0.99 (d, 3H), 1.29 (m, 2H), 2.05 (m, 1H) 4.6 (m, 1H), 7.2 (d, NH), 11.0 (brs, OH); ^{19}F NMR, δ: −76.3 (s, CF_3); ^{13}C NMR ($CDCl_3$) δ: 11.3, 15.1, 24.9, 37.5, 56.9, 115.2, (q, J=87 Hz), 157.3 (q, J=39 Hz), 174.7; MS *m/z* $M+NH_4^-$=245.

4.1.8. L-Proline, *N*-trifluoroacetyl. Isolated yield: 73% (crude oil). IR (cm^{-1}, KBr): 1700, 1454, 1352, 758, 722, 702; ^1H NMR ($CDCl_3$) δ: 2.1 (m, 2H), 2.3 (m, 2H), 3.8 (m, 1H), 4.6 (m, 1H) 10.7 (brs, OH); ^{19}F NMR, δ: −73.3 (s, CF_3); ^{13}C NMR ($CDCl_3$) δ: 24.8, 28.3, 47.3, 60.1, 115.8q, 156.1q, 174.9. MS *m/z* $M-H^-$=210.

4.2. General procedure for acid chloride formation

To a 250-mL flask, set up for cooling to −10 to −15°C and equipped with an overhead agitator and a nitrogen inlet, was added TFA protected (L)-amino acid (20.4 mmol) and *n*-BuOAc (50 mL). The stirred slurry was cooled to −10 to −12°C. With vigorous agitation, Vilsmeier reagent was charged in three equal portions (2 g each) with 0.5 h stir time between each addition (6 g total, 46.9 mmol, 2.3 equiv.). Typically, the acid chloride preparation was complete after 3 h from the initial charge as determined by HPLC analysis. The acid chloride was maintained at −10 to −15°C prior to use in the subsequent coupling reaction.

4.3. General procedure for amine ester coupling

To a 250-mL round-bottomed flask was charged (L)-phenylalanine ethyl ester·HCl (4.9 g, 1.05 equiv.) followed by sodium bicarbonate (2.1 g, 1.2 equiv.) and 50 mL of pH 8 buffer solution (prepared by mixing 250 mL 0.1 M KH_2PO_4+230 mL 0.1 M NaOH and adjusting to 500 mL with water). The aqueous mixture was cooled to 1–4°C (Note: Cooling below 0°C resulted in a thick slurry). While maintaining its temperature below −10°C, the acid chloride solution was added drop wise via cannula (nitrogen pressure). The reaction temperature was maintained between 1 and 4°C using an ice bath. In addition, the reaction pH was controlled between 7.0 and 8.0 by concurrent charge of 1 M sodium hydroxide. This is most effectively achieved using a pH meter and automated feedback control of the addition rate. Alternatively, 5 equiv. of $NaHCO_3$ can be used in the same volume as above. HPLC analysis typically indicated reaction completion immediately following acid chloride addition completion. The phases were then separated and the organic phase was washed twice with 50 mL of water. The rich organic phase was concentrated to minimal volume under nitrogen at 40°C. The product was then crystallized from 5 mL/g butyl acetate and 5–20 mL/g heptane and collected by vacuum filtration. In cases where the TFA-protected amino acid starting material was not crystallized, but telescoped directly into the coupling, solvent was exchanged to butyl acetate until <0.001% methanol was detected by GC. The N-protected amino acid was then dissolved in 10 mL/g ethyl acetate/butyl acetate, (3:1) converted to acid chloride, and coupled with the same protocol as above.

4.3.1. L-Phenylalanine, *N*-L-phenylalanyl, *N*-trifluoroacetyl-, ethyl ester. Yield: 91%. Colorless solid (from butyl acetate/heptane, 1:3). Mp: 135–136°C; IR (cm^{-1}, KBr): 1716, 1654, 1547, 1186, 785, 702; ^1H NMR ($CDCl_3$) δ: 1.3 (t, 3H), 3.05 (m, 2H), 3.15 (m, 2H), 4.18 (m, 2H), 4.55 (m, 1H), 4.63 m 1H, 5.75 d 1H, 6.90 d 2H, 7.12 d 1H, 7.12 (m, 8H); ^{19}F NMR, δ: −78.37 (s, CF_3); ^{13}C NMR δ: 35.1, 93.6, 15.8q, 128.4, 129.7, 130.5, 134.6, 156.3q, 164.8, 168.8; MS *m/z* $M-H^-$=437, $M+NH_4^-$=454. Anal. calcd for $C_{22}H_{23}N_2O_4F_3$: C, 60.55; H, 5.31; N, 6.42; F, 13.06. Found: C, 60.57; H, 5.27; N, 6.10; F, 13.14.

4.3.2. L-Phenylalanine, *N*-L-lysyl, N2,N6-bis(trifluoroacetyl)-, ethyl ester. Yield: 89%. Colorless solid (from butyl acetate/heptane, 1:2). Mp:150–152°C; IR (cm^{-1}, KBr): 1705, 1649, 1183, 702; ^1H NMR ($CDCl_3$) δ: 1.28 (t, 3H), 2.03 (m, 2H), 1.78 (m, 1H) 1.82 (αβq, 2H), 3.13 (q, 2H), 3.26 (q, 2H), 4.30 (q, 2H), 4.38 (q, 1H), 4.75 (q, 1H), 6.12 (br, NH), 6.73 (br, NH), 7.18 (m, 2H), 7.28 (m 3H); ^{19}F NMR, δ: −73.64 (s, CF_3), −73.96 (s, CF_3); ^{13}C NMR (DMSO-d_6) δ: 13.9, 22.7, 27.8, 30.7, 36.5, 38.7, 53.0, 53.8, 60.5, 114.2, 118.3, 126.6, 128.2, 129.1, 137.0, 157.1, 170.4, 171.2; MS $M-H^-$=514, $M+NH_4^-$=531. Anal. calcd for $C_{21}H_{25}N_3O_5F_6$: C, 49.13; H, 4.91; N, 8.18; F, 22.20. Found: C, 49.20; H, 4.81; N, 8.16; F 22.11.

4.3.3. L-Phenylalanine, *N*-L-methionyl, *N*-trifluoroacetyl-, ethyl ester. Yield: 92%. Colorless solid (from butyl acetate/heptane, 1:4. Mp: 103–104°C; IR (cm^{-1}, KBr): 1736, 1705, 1649, 1538, 1378, 1209, 1183, 707; ^1H NMR ($CDCl_3$) δ: 1.24 (t, 3H), 2.05 (m, SCH_3), 2.62 (αβ, 2H), 3.18 (αβ, 2H), 4.21 (q, 2H), 4.62 (q, 1H), 4.82 (q, 1H), 6.60 (d, NH), 7.16 (1H, ϕH), 7.17 (br, NH), 7.28(m, 4ϕH); ^{19}F NMR, δ: −76.33 (s, CF_3); ^{13}C NMR (DMSO-d_6) δ: 13.9, 14.6, 29.6, 30.9, 36.5, 52.4, 53.8, 60.6, 115.3q, 126.6, 128.2, 129.1, 137.0, 156q, 169.9, 171.2; MS *m/z* $M-H^-$=421, $M+NH_4^-$=438. Anal. calcd for $C_{18}H_{23}N_2O_4F_3S$: C, 51.42; H, 5.51; N, 6.66; F, 13.56; S, 7.63. Found: C, 51.49; H, 5.47; N, 6.65; F, 13.81; S, 7.85.

4.3.4. L-Phenylalanine, *N*-L-tyrosyl, *N*-trifluoroacetyl-, ethyl ester. Yield: 74%. Colorless solid (from butyl acetate/heptane, 1:2). Mp: 190–192°C; IR (cm^{-1}, KBr): 1665, 1726, 1723, 1521, 1220, 1194, 1185. ^1H NMR ($CDCl_3$) δ: 1.27 (t, 3H), 3.05 (m, 4H), 4.18 (q, 2H), 4.49 (αβ, 1H), 4.63 (αβ, 1H), 4.68 (s, ϕOH), 5.75 (αβ, 1H), 6.88 (d, 2ϕH), 7.05 (d, 2ϕH), 7.12 (d, 1NH), 7.26 (s, 5H); ^{19}F NMR, δ: −73.64 (s, CF_3); ^{13}C NMR (DMSO-d_6) δ: 13.9, 36.1, 36.8, 53.9, 54.8, 60.6, 114.9, 115.9q, 126.6, 127.3, 128.2, 129.1, 130.0, 137.0, 155.9, 157.2q, 170.14, 171.2; MS *m/z* $M-H^-$=453, $M+NH_4^-$=470. Anal. calcd for $C_{22}H_{23}N_2O_5F_3$: C, 58.41; H, 5.12; N, 6.19; F, 12.60. Found: C, 58.51; H, 5.11; N, 6.02; F, 12.36.

4.3.5. L-Phenylalanine, *N*-L-leucyl, *N*-trifluoroacetyl-, ethyl ester. Telescoped yield: 95%. Colorless solid (from butyl acetate/heptane, 1:4). Mp: 139–140°C; IR (cm^{-1}, KBr): 1726, 1657, 1555, 1220, 1200, 1180, 1160, 702; ^1H NMR ($CDCl_3$) δ: 0.96 (d, 6H), 1.18 (t, 3H), 1.61 (m, 3H) 3.18 (αβq, 2H), 4.13 (q, 2H), 4.23 (q, 1H), 4.87 (q, 1H), 6.17 (br, NH), 6.83 (br, NH), 7.23 (s, 5H); ^{19}F NMR, δ: 73.42 (s, CF_3), −73.46 (s, CF_3), 95:5 ratio; ^{13}C NMR (CD_3CN) δ: 13.9, 23.0, 23.4, 24.6, 38.0, 42.2, 52.1, 53.3, 61.8, 127.8, 128.6, 129.2, 136.4, 156q, 172, 173; MS *m/z* $M-H^-$=403, $M+NH_4^-$=420. Anal. calcd for $C_{19}H_{25}N_2O_4F_3$: C, 56.71; H, 6.26; N, 6.96; F, 14.16. Found: C, 56.77, H, 6.40; N, 6.91; F, 14.36.

4.3.6. L-Phenylalanine, *N*-L-prolyl, *N*-trifluoroacetyl, ethyl ester. Telescoped yield: 44%. Colorless solid (from butyl acetate/heptane, 1:4). Mp: 108–110°C; IR (cm^{-1}, KBr): 1751, 1701, 1650, 1552, 1230, 1210, 1180, 1155, 700: ^1H NMR ($CDCl_3$) δ: 1.26 (t, 3H), 1.98 (m, 2H), 2.08 (m, 1H) 3.13 (αβq, 2H), 3.68 (q, 2H), 4.18 (q, 2H), 4.53 (q, 1H), 4.82 (q, 1H), 6.67 (br, NH), 7.14 (br, NH), 7.26 (s, 5H); ^{19}F NMR, δ: −71.1 (s, CF_3), −70.2 (s, CF_3), 69.7:29.3 ratio; ^{13}C NMR (DMSO-d_6) δ: 13.9, 19.8, 24.3, 28.7 m, 36.5, 47.0, 53.7, 60.6, 98.2q, 126.5, 128.2m 128.9, 138.2, 154.0q, 170.1, 172.0; MS *m/z* $M-H^-$=387, $M+NH_4^-$=404. Anal. calcd for $C_{18}H_{21}N_2O_4F_3$: C, 55.96; H, 5.48; N, 7.25; F, 14.75. Found: C, 55.99; H, 5.46; N, 7.25; F, 14.85.

4.3.7. L-Phenylalanine, *N*-L-isoleucyl, *N*-trifluoroacetyl, ethyl ester. Telescoped yield: 98%. Colorless solid (from butyl acetate/heptane, 1:5). Mp: 166–168°C; IR (cm^{-1}, KBr): 1741, 17.02, 1654, 1550, 1194, 707; ^1H NMR ($CDCl_3$) δ: 0.93 (m, 6H), 1.18 (m, 1H), 1.27 (t, 3H), 1.47 (m, 1H), 1.86 (m, 1H), 3.14 (t, 2H), 4.19 (q, 2H), 4.30 (t, 1H), 4.86 (q, 1H), 6.19 (d, NH), 7.08 (m, 2ϕH, 1NH), 7.30 (m, 3ϕH); ^{19}F NMR, δ: 73.28 (s, CF_3); ^{13}C NMR ($CDCl_3$) δ: 10.2, 13.8, 14.8, 24.4, 35.4, 36.6, 53.5, 57.4, 60.5, 114.8q, 126.5, 128.1, 129.1, 137.0, 157.2q, 169.7, 171.0; MS *m/z* $M-H^-$=402. Anal. calcd for $C_{19}H_{25}N_2O_4F_3$: C, 56.71; H, 6.26; N, 6.96; F, 14.16. Found: C, 56.78; H, 6.36; N, 6.91; F, 14.44.

4.4. General procedure for kinetic studies

Measurement of reaction rates was performed at 5°C using a Chemical/Freeze Quench Apparatus (Update Instruments System 1000, Madison WI). Diluted reactant streams were directed through a Wiskind grid mixer via syringe using a Model 1019 Syringe Ram at identical, precisely controlled flow rates. Reactions were rapidly quenched by combining the reaction stream with a 0.25 M methanolic HCl stream in a secondary in-line grid mixer. Altering the tube length between reactant mixing and quench mixing in repeated experiments allowed samples representing partially completed reactions to be collected in conjunction with precise time measurements. Using the above apparatus, residence time between reaction start and quench could be controlled to as little as 20 ms. Quenched samples obtained from a continuous flow through the system were then analyzed by HPLC. The HPLC method involved 5 μL sample injections onto a YMC ODS-A 4.6×50 mm eluted with a gradient running at 75/25 0.1% aqueous phosphoric acid/acetonitrile for 1 min, followed by a 4 min linear ramp to 15/85 0.1% aqueous phosphoric acid/acetonitrile, and ending with a 1 min hold at this level. Flow rate was 2 mL/min and detection was at 210 nm. Retention times for the components of interest were 3.6 min for TFA-L-Phe, 4.6 min for TFA-L-Phe ethyl ester, 6.1 min for TFA-L-Phe-L-Phe-OEt, and 5.5 min for azlactone.

Authentic samples of the components of additional interest to the kinetic studies were prepared as follows:

4.4.1. *N*-Trifluoroacetyl-L-phenylalanine methyl ester. *N*-Trifluoroacetyl-L-phenylalanine (0.83 g, 3.18 mmol, 1.0 equiv.) was stirred in 8.38 mL of dry ethyl acetate at −5°C. Vilsmeier reagent (490 mg, 3.83 mmol, 1.2 equiv.) was charged and the slurry was stirred 1 h. The reaction mixture was quenched with 50 mL of methanol. MTBE (50 mL) was added and the mixture was extracted once with 50 mL saturated potassium carbonate. The organic phase was concentrated to an oil and triturated with heptane to afford white crystalline solid 0.44 g (50 M%) with comparable properties to material previously reported;[11] mp

50–52°C; IR (KBr) 1752, 1711, 1578, 1557, 1332, 1275, 1178, 1163, 758, 707; ^{1}H NMR ($CDCl_3$) δ: 3.19 (ab q, 2H), 3.78 (s, 3H), 4.88 (ab q, 1H), 6.91 (d, NH), 7.09 (m, 2H), 7.29 (m, 3H); ^{19}F NMR, δ: −76.4; ^{13}C NMR ($CDCl_3$) 37.1, 52.7, 53.5, 115.8q, 127.5, 128.7, 129.1, 134.5, 156.7, 170.4.

4.4.2. 5(4*H*)-Oxazolone, 4-(phenylmethyl)-2-(trifluoromethyl). Preparation of this material (azlactone) was analogous to that described by Weygand.[12] *N*-Trifluoroacetyl-L-phenylalanine (1.3 g, 5.0 mmol, 1.0 equiv.) was stirred in 14 mL of dry ethyl acetate at 0°C. Vilsmeier reagent (1300 mg, 10 mmol, 2.0 equiv.) was charged and the slurry was stirred for 1 h. After reaction completion was confirmed by HPLC, the solution was quenched into 2.6 mL DIPEA (15 mmol, 3.0 equiv.) in 15 mL of ethyl acetate. After stirring 5 min the reaction mixture was washed with 5 mL of 1N HCl, 5 mL water, 5 mL 0.5 M potassium phosphate, dibasic, 5 mL 1N HCl and concentrated to afford 1.15 g (>99%) of a light yellow oil: IR (cm^{-1}, KBr): 3037, 1803, 1649; ^{1}H NMR (CD_3CN) δ: 4.04 (d, 2H), 6.35 (t, 1H) 7.38 (m, 5H); 19F NMR, δ: −78.37 (s, CF_3); ^{13}C NMR δ: 35.1, 118.3, 122.4q, 128.4, 129.7, 130.5, 134.6, 164.8, 168.8. ms $M-H^+$=242.

4.5. Measurement of acid chloride/amino ester coupling rate

N-Trifluoroacetyl-L-phenylalanine (0.798 g, 3.05 mmol, 1.0 equiv.) was dissolved in 8.6 mL of dry ethyl acetate at 0°C. Vilsmeier reagent (470 mg, 3.67 mmol, 1.2 equiv.) was charged and the slurry was stirred. After 45 min, the solution was diluted to a volume of 66 mL with dry ethyl acetate (0.046 M). This acid chloride solution was then further diluted 50-fold with dry ethyl acetate chilled to 5°C to provide a 0.00092 M solution of acid chloride (reactant B). The amine ester solution was prepared by mixing 3.66 g L-phenylalanine ethyl ester HCl salt (15.95 mmol) with 25 mL ethyl acetate and 25 mL 1 M K_2HPO_4. The phases were separated and the aqueous was extracted twice with 25 mL ethyl acetate. The organic phases were combined and adjusted to a volume of 100 mL with dry ethyl acetate (0.16 M). The amine solution was then diluted 50-fold with dry ethyl acetate and chilled to 5°C to provide a 0.0035 M amine ester solution (reactant A). The acid chloride solution (reactant B) and amine ester in ethyl acetate (reactant A) were then used as inputs to rapid quench experiments as described above. Remaining acid chloride at any given time was indirectly measured as *N*-TFA-L-phenylalanine methyl ester relative to coupled product as determined by HPLC. Values of A and B at each time point were used along with initial A and B concentrations to calculate the *y* axis values used in Figure 1, from which the second-order rate constant of 3900 L/M s was determined.

4.6. Measurement of rate of *N*-TFA-L-Phe acid chloride reaction with diisoproplyethylamine (azlactone formation)

TFA protected phenylalanine (0.45 g, 1.72 mmol, 1.0 equiv.) was stirred in 4.9 mL of dry ethyl acetate at 0°C, 270 mg of Vilsmeier reagent (2.10 mmol, 1.0 equiv.) was charged, and the slurry was stirred. After the acid chloride formation reached completion as detected by HPLC (45 min), the solution of acid chloride (reactant B) was diluted to a volume of 37 mL with dry ethyl acetate. The acid chloride solution (0.046 M) and diisopropylethylamine (0.17 M, 3.7 equiv.) in ethyl acetate (reactant A) were then used as inputs to rapid quench experiments as described above. Remaining acid chloride at any given time was indirectly measured as *N*-TFA-L-phenylalanine methyl ester relative to azlactone as determined by HPLC. Values of A and B at each time point were used along with initial A and B concentrations to calculate the y axis values used in Figure 2, from which the second-order rate constant of 4.8 L/M s was determined.

4.7. Measurement of *N*-TFA-L-phenylalanine acid chloride hydrolysis rate at pH 8.5

N-Trifluoroacetyl-L-phenylalanine (1.10 g, 4.2 mmol, 1.0 equiv.) was stirred in 11.8 mL of dry ethyl acetate at 0°C. Vilsmeier reagent (720 mg, 5.6 mmol, 1.2 equiv.) was charged and the slurry was stirred until complete acid chloride formation was achieved by HPLC assay. A 100 μL sample of the acid chloride solution (0.0035 mmol) was charged to a vigorously stirred solution of 1 mL aqueous pH 8.5 buffer (0.07 M K_2CO_3, 0.175 M $KHCO_3$) and 1 mL of ethyl acetate. After 9 and 15 s the sample was quenched with 1 mL of 0.1 M aqueous solution of ethyl phenylalanine hydrochloride (0.01 mmol), stirred 1 min and diluted with 10 mL of 1:1 acetonitrile:2% aqueous phosphoric acid. The quenched solutions were then analyzed by HPLC, showing fractions of 28 and 42% *N*-TFA-L-Phe relative to *N*-TFA-L-Phe-L-Phe-OEt (representive remaining acid chloride at the time of quench). At constant pH 8.5, these data calculate to a first-order rate constant of 0.04/s.

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