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Kinetics of Amide Formation through Carbodiimide/ *N*-Hydroxybenzotriazole (HOBt) Couplings

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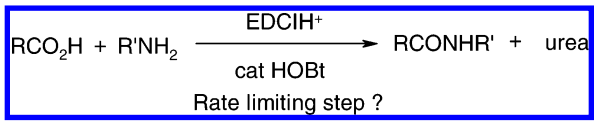
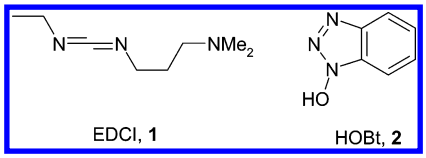


The kinetics of formation of amide, **4**, from the corresponding carboxylic acid by reaction with the isopropyl ester of methionine (MIPE), mediated by carbodiimide EDCI, **1**, and HOBt, **2**, have been studied in 1-methyl-2-pyrrolidinone (NMP) using reaction calorimetry. The reaction rates have been found to be independent of the concentration of HOBt, showing that the rate-determining step is the reaction between the carboxylic acid and EDCI to give the corresponding *O*-acylisourea. The pH dependence of the observed rate constants for *O*-acylisourea formation is consistent with a second-order reaction between doubly protonated EDCI ($EDCIH_2^{2+}$, **6**) and the carboxylate group. The observed rate constants fall sharply at high pH, as the fraction of EDCI as $EDCIH_2^{2+}$ continues to fall strongly, whereas the carboxylic acid group is already fully ionized. The rate constant, k_P, for reaction between the carboxylate group of acid, **3**, and $EDCIH_2^{2+}$ has a value of $k_P = 4.1 \times 10^4$ M^{-1} s^{-1} at 20 °C, some 10^5 times higher than similar rate constants measured in water. The subsequent catalytic cycle, involving reaction of *O*-acylisourea with HOBt to give HOBt ester, which then reacts with the amine to give the amide with regeneration of HOBt, determines the product distribution. In the case of the amino acid, **3**, reaction of the *O*-acylisourea with MIPE to give amide, **4**, is increasingly favored at higher pH values over that with the less basic internal aromatic amine of **3** to give the diamide **5**.

Introduction

The formation of amide bonds by condensation of amines with carboxylic acids in the presence of carbodiimides, such as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDCI, **1**), continues to be one of the most common synthetic procedures.[1] To optimize these couplings, additives such as *N*-hydroxybenzotriazole (HOBt, **2**) are widely used for the generation of active esters capable of efficient acylation of amino groups, especially in the case of amino acids and peptides.[2,3]



A typical reaction scheme for the coupling of acid RCO_2H with amine $R'NH_2$ to form the amide, RCONHR′, is shown in Scheme 1.

The reaction is, therefore, catalytic in HOBt but stoichiometric in EDCI, which is converted to the corresponding urea (eq 2). The overall stoichiometry of the reaction therefore involves reaction of the acid, amine, and EDCI to produce the amide and the EDCI-derived urea.

Little is known about the kinetics of the reactions contained within the scheme, apart from that between EDCI and various carboxylic acids, which has been studied in aqueous solution.[4] In particular, we wished to investigate the role of HOBt in determining the overall rates of the product formation. In the present paper, we report the results of a study of formation of the amide, isopropyl *N*-{5-amino-2-[2-(4-fluorophenyl)ethyl]-benzoyl}-L-methioninate, **4** (Scheme 2).

There are several areas of interest: the reaction rates, the reaction order with respect to EDCI and HOBt, the influence of added base, and, in the former case, the reaction selectivity

(1) Shelkov, K.; Nahmany, N.; Melman, A. *Org. Biomol. Chem.* **2004**, *2*, 397.

(2) Bodanszky, M. *Peptide Chemistry: A Practical Textbook*; Springer: New York, 1988.

(3) Wang, S. S.; Tam, B. S.; Wang, B. S.; Merrifield, B. *Int. J. Pept. Protein Res.* **1981**, *19*, 459.

(4) Williams, A.; Ibrahim, I. T. *J. Am. Chem. Soc.* **1981**, *103*, 7090.

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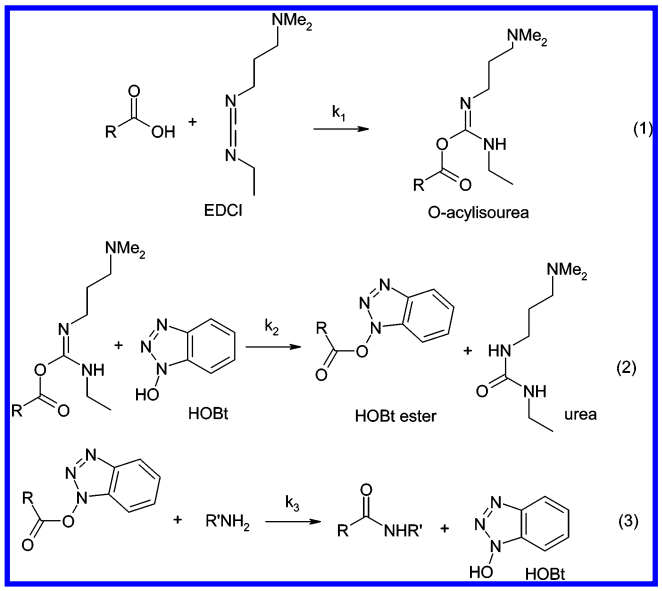
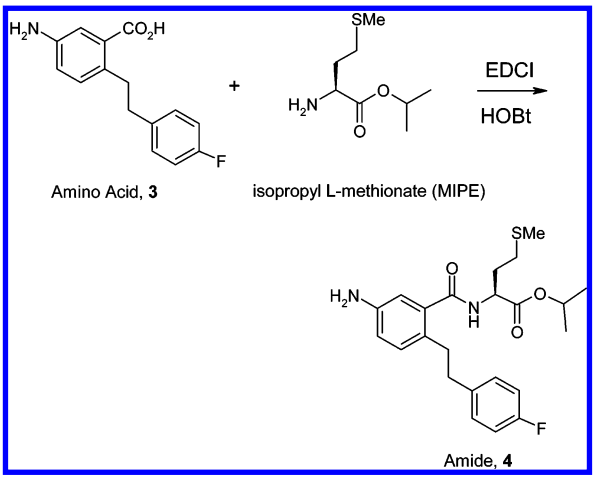
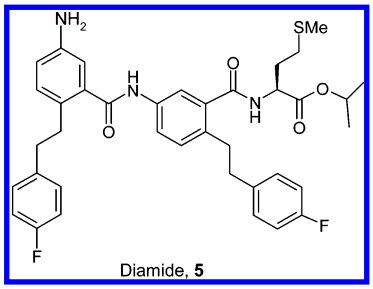
SCHEME 1. Typical Reaction for Coupling Acid with Amine in the Presence of EDCI/HOBt



SCHEME 2. Formation of Amide 4



with respect to product formation. Thus, the amine R′NH_2 in eq 3 may be either isopropyl L-methioninate (MIPE), which would give rise to the desired amide, isopropyl *N*-{5-amino-2-[2-(4-fluorophenyl)ethyl]benzoyl}-L-methioninate, **4**, or the amino function of a second molecule of the starting amino acid, 5-amino-2-[2-(4-fluorophenyl)ethyl]benzoic acid, **3**, leading to an unwanted 2:1 adduct, isopropyl *N*-{5-({5-amino-2-[2-(4-fluorophenyl)ethyl]benzoyl}amino)-2-(4-fluorophenyl)ethyl]benzoyl}-L-methioninate, **5**.



Results and Discussion

Kinetic Measurements. Reaction rates were measured at 19.6 °C in 1-methyl-2-pyrrolidinone (NMP) as solvent. Amide formation is strongly exothermic ($\Delta H = -135$ kJ mol^{-1}), and this proved to be particularly convenient for a calorimetric investigation. The work was carried out in an Omnical Ultralow Super CRC calorimeter, using an external circulating bath (Presto bath with Baysilone KT3 oil) to maintain the reaction temperature at 19.6 °C. In a typical reaction procedure, acid (1.45 mmol) in 1.5 mL of NMP (3.5 rel vol) was added to an NMP solution (1.5 mL, 3.5 rel vol) of amine hydrochloride (1.1 molar equiv), HOBt·H_2O (0.4 molar equiv), EDCI·HCl (1.1 molar equiv), water (0.16 mL, 0.24 rel vol), and base (3.2 molar equiv) at 19.6 °C. The power output was recorded every 10 s, and a sample was taken for HPLC analysis when the power output had returned to baseline level. The progressive and total heat output was calculated using baseline-to-baseline extrapolation in MS Excel.

A typical heat-output profile is shown in Figure 1. In all cases, there is an apparent initial lag in the rate of heat generation, due to the finite response time of the calorimeter. It was shown in separate experiments that the measured heat output for the "instantaneous" reaction between HCl and NaOH required approximately 6 min to reach 99% completion, and for all measurements, data accumulated over the first 6−10 min were neglected for the purposes of kinetic analysis. The total heat output of the reaction is unaffected by the instrument response time. The concentrations of reactants at any time, *t*, during the reactions were determined from the known initial concentrations combined with the fraction of reaction (equivalent to the fraction of the total heat output) that had occurred at time *t*, and these were then used to determine the second-order rate constants.

Influence of HOBt Concentration. Reactions of amino acid, **3**, with MIPE·HCl (1.1 mol), *N*-methylmorpholine (NMM, 3.2 mol), and EDCI·HCl (1.1 mol) were measured at various levels of HOBt: 1.0 molar equiv, 0.4 molar equiv, 0.1 molar equiv, and 0 mol. The results are shown in Figure 2 (1.0 molar equiv of HOBt data has been omitted for clarity, but coincides with that for 0.4 and 0.1 molar equiv).

It is immediately clear from the data presented that the reaction rates and heat outputs are identical for HOBt levels between 0.1 and 1.0 molar equiv. For reactions with no added HOBt, the rate is very similar, but the total heat output is considerably lower. When all data sets are replotted as a percentage of total heat output against time (Figure 3), it can be seen that the reaction profiles are identical, independent of the level (or indeed the presence) of HOBt.

HPLC analysis of the reaction mixtures showed, however, that whereas, in the presence of HOBt, high yields of the desired amide were produced (>80%), the yield of the amide was only 14% in the absence of HOBt, and high levels of amino acid **3** and its decomposition products were obtained.

We conclude from these results that the kinetics of the reactions are controlled by the reaction between the acid and EDCI to give the *O*-acylisourea (rate-determining step), but that the product distribution is controlled by the catalytic step involving formation of the HOBt ester and its subsequent reaction with the relevant amine.

Kinetics of the EDCI-Mediated Reactions of Amino Acid, 3. The reaction between EDCI and various nucleophiles, including carboxylic acids, has been subject to a detailed study in aqueous solution.[4] It has been shown that the rate-determining step for carboxylic acid addition is the reaction between the carboxylate anion and doubly protonated EDCI (**6**), shown by NMR studies in water and dimethylsulfoxide to comprise a mix-

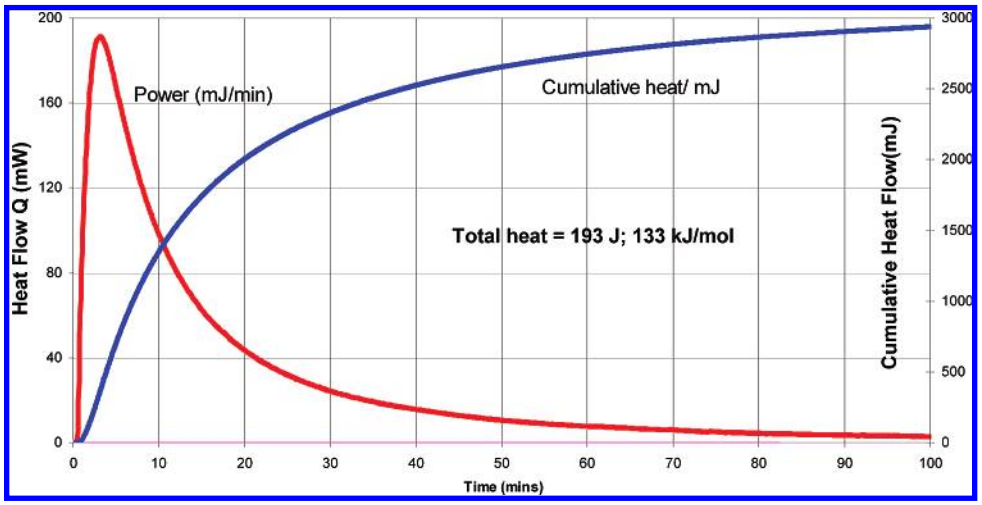


FIGURE 1. Calorimetric trace for reaction of amino acid **3** at 20 °C with 0.4 mol HOBt and 3.2 molar equiv of *N*-methylmorpholine as base.

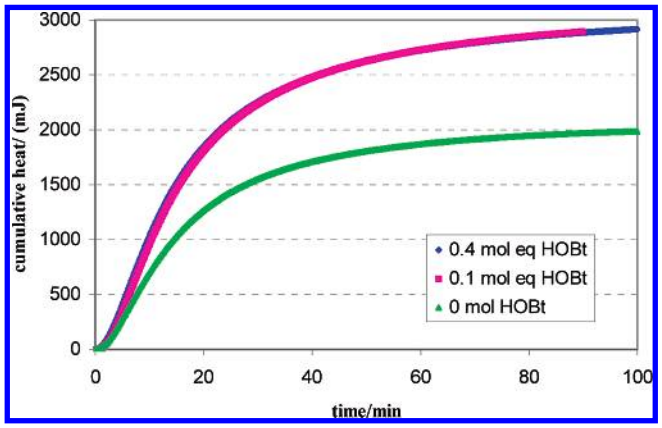


FIGURE 2. Influence of HOBt concentration on reaction of amino acid **3**.

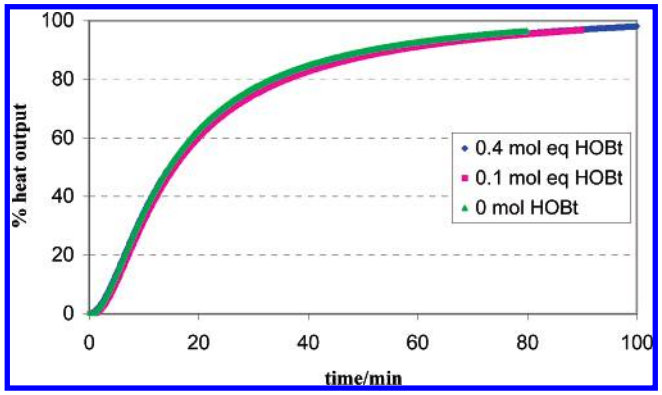
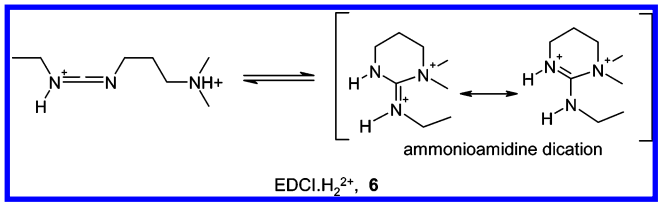


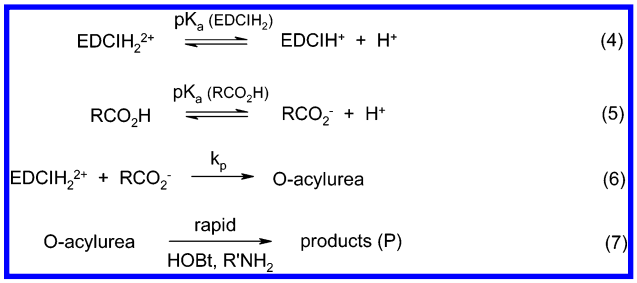
FIGURE 3. Time dependence of percentage heat output for reaction of amino acid **3**.

ture of an open chain and a cyclic form in rapid equilibrium.[5,6]



The kinetics may, therefore, be represented by Scheme 3, in

SCHEME 3



which $EDCIH^+$ represents monoprotonated EDCI (protonated on the alkylamino group).

It follows that the rate law for the reaction is given by eqs 8 and 9, where $[EDCI]_t$ and $[RCO_2H]_t$ represent the total concentrations of EDCI and RCO_2H in their various protonated and tautomeric forms, and k_e is the observed second-order rate constant.

$$\frac{d[P]}{dt} = -\frac{d[RCO_2]_t}{dt} = -\frac{d[EDCI]_t}{dt} = k_e[RCO_2H]_t[EDCI]_t \quad (8)$$

where

$$k_e = \frac{k_p}{\left\{1 + \frac{[H^+]}{K_{a(RCO_2H)}}\right\}\left\{1 + \frac{K_{a(EDCIH_2)}}{[H^+]}\right\}} \quad (9)$$

In eq 9, the two terms in the denominator arise from the fractions, respectively, of RCO_2H as RCO_2^- and of EDCI as $EDCIH_2^{2+}$.

It follows from eqs 8 and 9 that the rate constant, k_e, should show a bell-shaped variation with pH and reach a maximum when the pH is halfway between the pK_a values of $EDCIH_2^{2+}$ and RCO_2H, that is, at pH = (pK_a(RCO_2H) + pK_a($EDCIH_2$))/2. For EDCI and acetic acid in water, pK_a values of 3.1 and 4.7, respectively, this corresponds to a rate maximum at pH 3.9, with a rapid fall off in rate at either high (pH > 4.7) or low (pH < 3.1) pH values because of increasingly reduced levels

(5) Yavari, I.; Roberts, J. D. *J. Org. Chem.* **1978**, *43*, 4689.
(6) Tenforde, T.; Fawwaz, R. A.; Freeman, N. K. *J. Org. Chem.* **1972**, *37*, 3372.

TABLE 1. Second-Order Rate Constants for Reaction of Amino Acid 3 with EDCI in NMP[a]

[**3**] (M)	[EDCI·HCl] (M)	HOBt (molar equiv)	$10^3 k_e$ (M^{-1} s^{-1})[b]
0.291	0.320	1.0	4.3_0
0.291	0.320	0.4	4.5_0
0.145	0.160	0.4	4.6_3
0.291	0.320	0.1	4.4_5
0.291	0.320	0	4.5_0

[a] T = 19.6 °C; *N*-methylmorpholine (3.2 molar equiv) as base; MIPE·HCl (1.1 molar equiv); water 3.2 vol %. [b] The k_e values are ±5%.

of $EDCIH_2^{2+}$ and RCO_2^-, respectively. In NMP, pK_a values for carboxylic acids are much higher than those in water (e.g., pK_a(benzoic) ~ 11.5, in pure NMP,[7] cf. 4.2 in water), but those for protonated amines remain relatively unchanged (see below). The presence of water (3.2 vol %) in the present system (required to stabilize the HOBt) will reduce the pK_a of amino acid, **3**, to a value somewhat below 11.5, but nevertheless eqs 8 and 9 predict that there should be a substantial pH window in which the rate of product formation remains high.

Influence of pH. The rates of reaction and product distributions were measured in different buffer systems, prepared by the addition of the following bases to the methioninate (MIPE) hydrochloride: *N*,*N*′-dimethylaniline (DMeA) (pK_a(H_2O) = 5.12), *N*-methylmorpholine (NMM) (pK_a(H_2O) = 7.41), tributylamine (Bu_3N) (pK_a(H_2O) = 9.75), *N*-methylpiperidine (NMeP) (pK_a(H_2O) = 10.08), triethylamine (Et_3N) (pK_a(H_2O = 10.62), and diazobicyclo(5.4.0)undecane (DBU) (pK_a(H_2O) = 13.28).[8]

Reactions were initially carried out with *N*-methylmorpholine as base with several levels of HOBt and two different initial concentrations of amino acid, **3**. In all cases, reactions showed excellent second-order kinetics in accordance with eq 8, and the observed rate constants are recorded in Table 1. The constant values confirm the independence of the reaction rate on the level of HOBt.

Rate constants were then measured in the different base solutions, with HOBt fixed at 0.4 molar equiv. The pK_a values of the various tertiary amines in NMP, required for calculation of the solution pH values, have in most cases not been reported. Extensive compilations in dipolar aprotic solvents, however, show that pK_a value of a tertiary amine is constant within ±0.2 units in NMP, dimethylformamide (DMF), and dimethylsulfoxide (DMSO)[7,9−11] and so may be used interchangeably. Where values are not reported in any of the solvents (NMM, NMeP, DBU, and EDCI), values were estimated by subtracting 1.5 units from the aqueous values, this being the typical reduction in pK_a observed on transferring tertiary amines from water to NMP, DMF, or DMSO. The pK_a values for primary amines in these solvents are closer to their aqueous values, typically about 0.4 units lower than in water; thus the pK_a for protonated MIPE has been taken as 6.8. The pH values for the various reactions were then calculated from the simple equilibrium between MIPE hydrochloride and the added base. Observed rate constants are listed in Table 2.

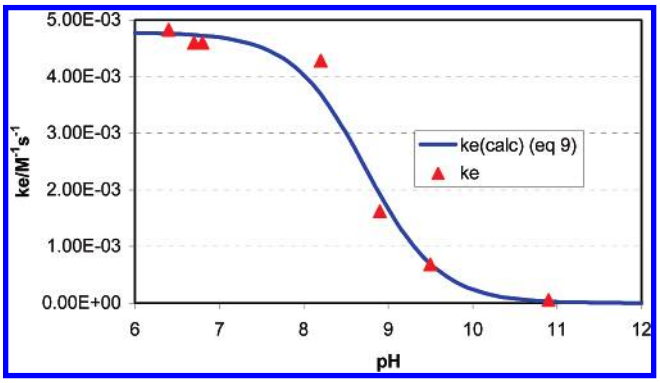


FIGURE 4. Amino acid **3**: pH dependence of EDCI reaction rate.

TABLE 2. Second-Order Rate Constants for Reaction of Met Sat Acid with EDCI in NMP[a]

base	molar equiv of base	pK_a (NMP)[b]	pH[b]	$10^3 k_e$ (M^{-1} s^{-1})
DMA	3.2	3.8	4.8	*c*
NMM	1.3	5.9	6.4	4.8_3
NMM	2.1	5.9	6.7	4.6_0
NMM	3.2	5.9	6.8	4.6
Bu_3N	2.1	8.2	8.2	4.2_8
NMeP	3.2	8.6	8.9	1.6_2
Et_3N	3.2	9.2	9.5	0.6_9[d]
DBU	3.2	11.8	10.9	0.06_0[e]

[a] T = 19.6 °C; MIPE·HCl (1.1 molar equiv); water 3.2 vol %. [b] See text. [c] Time scale similar to NMM reactions, but with significant deviation from second-order kinetics and high levels of diamide **5** and HOBt ester in product. [d] Reaction terminated after 4 h at 65% completion. [e] Reaction terminated after 3 h at 17% completion.

Analysis of the results in Table 2 according to eq 9 is shown in Figure 4, in which the calculated (best-fit) line corresponds to $k_P = 4.07 \times 10^4$ M^{-1} s^{-1} and pK_a (**3**) (NMP) = 8.75. The k_P value is not sensitive to the estimated pK_a values of the various bases, provided that EDCI does not behave in a very different manner to the other bases.

The rate constant, k_P, is some 10^5 times that for carboxylic acids in water,[4] in keeping with the lower solvation of the carboxylate anion in this medium. It is important to note, however, that the maximum *observed* rate constant, k_e, is very similar to that for the reaction between acetate and EDCI in water ($k_e = 8 \times 10^{-3}$ M^{-1} s^{-1} (25 °C); cf. k_e(NMP) = 4.7×10^{-3} M^{-1} s^{-1} (20 °C)), despite the much higher intrinsic reactivity of the benzoate in NMP. This highlights the importance of both the available concentrations of the reacting species (population) and their intrinsic reactivity in determining the overall reaction rates. Thus the lower reactivity of the carboxylate in water compared with NMP is compensated for by the fact that relatively high levels of the reactive species (RCO_2^- and $EDCIH_2^{2+}$) can coexist in water because of the similarity of the pK_a values for carboxylic acids and $EDCIH_2^{2+}$. The pK_a value of acid, **3**, in the present system, pK_a = 8.75, is considerably higher than that in water (pK_a ~ 3.1) but significantly lower than that, for example, of benzoic acid in pure NMP (pK_a ~ 11.5). The lower value relative to that expected in pure NMP is attributable to the presence of some water (3.2 vol %), combined with relatively high levels of RN_3H^+ in the reactions; it has been shown that carboxylate anions are subject to strong stabilization by H-bond formation

(7) Izutsu, K. *Dissociation Constants in Dipolar Aprotic Solvents*; Blackwell: Oxford, 1990.
(8) The pK_a values in water are from the database ACD/pK_a DB and referenced therein.
(9) Kolthoff, I. M.; Chantooni, M. K. *Anal. Chem.* **1970**, *42*, 1622.
(10) Bordwell, F. G. *Acc. Chem. Res.* **1988**, *21*, 456.
(11) Kolthoff, M. K.; Chantooni, M. K.; Bhowmik, S. *J. Am. Chem. Soc.* **1968**, *90*, 23.

TABLE 3. Amide 4 and Diamide 5 Yields[a]

base	molar equiv of base	% methionine free base	amide **4** % yield	diamide **5** % yield
DMA	3.2	1.0	6.6[b]	15.4
NMM	1.3	29	82	12.1
NMM	2.1	41.7	86.6	6.8
NMM	3.2	49.3	89.6	4.6
Bu_3N	2.1	96.1	92.5	3.1
NMeP	3.2	99.1	93.5	2.2
Et_3N	3.2	99.8	91.6[c]	2.1
DBU	3.2	100	(94)[d]	(0)[d]

[a] Solution yields (HPLC) based on acid consumed. [b] High level (42%) HOBt ester. [c] Reaction terminated after 4 h at 65% completion. [d] Reaction terminated after 3 h at 17% completion.

with suitable donors in NMP and related solvents, such as dimethylformamide and dimethylsulfoxide.[11,12]

pH Dependence of Product Distribution. Although it is clear from the results above that the overall kinetics of the amide coupling reaction are determined by the rate of formation of the *O*-acylisourea, the product distribution is controlled by the subsequent formation of the HOBt ester and its reaction with the amine (eqs 2 and 3, Scheme 1). In particular, at low pH values, protonation of the methioninate amine group will reduce the rate of the desired reaction relative to that of coupling with the less basic amino group of acid, **3** (to give diamide **5**), and ultimately severely inhibit product formation. This is confirmed by the yields of amide and diamide **5** in the various base solutions given in Table 3.

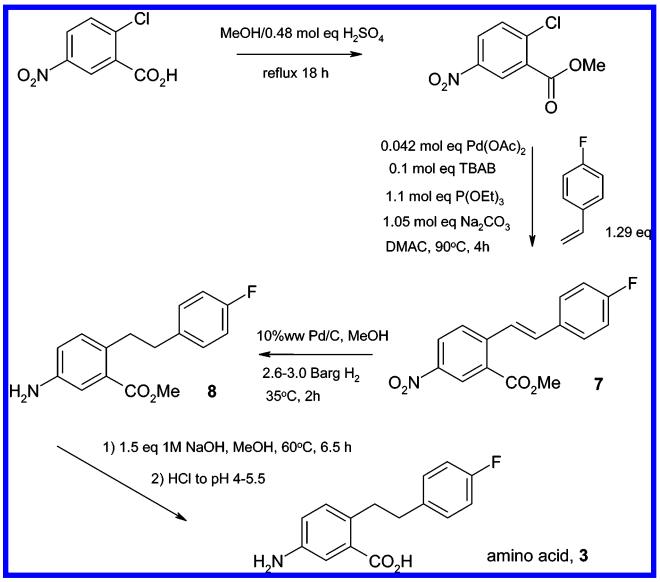
Further work[13] has shown that the conclusions from this work are not unique to the current system. Thus reactions involving a simple benzoic acid, rather than the present amino acid, again show reaction rates independent of HOBt levels, high yields of amide (>90%) in the presence of HOBt, but very poor conversion to amide (<50%) in the absence of HOBt. As in the present case, reaction rates fall off at high pH, but the product yields do not show a dependence on pH because there is no internal amino group to compete for the HOBt ester.

Experimental Section

5-Amino-2-[2-(4-fluorophenyl)ethyl]benzoic acid (**3**) was prepared from 2-chloro-5-nitrobenzoic acid, via methyl 2[(*E*)-2-(4-fluorophenyl)vinyl]-5-nitrobenzoate (**7**) and methyl 5-amino-2-[2-(4-fluorophenyl)ethyl]benzoate (**8**), according to Scheme 4.

Preparation of Methyl 2[(*E*)-2-(4-fluorophenyl)vinyl]-5-nitrobenzoate (7). A mixture of methyl 5-nitro-2-chlorobenzoate (31.0 kg, 1.00 molar equiv), sodium carbonate (16.0 kg, 1.05 molar equiv), tetra-*n*-butylammonium bromide (4.65 kg, 0.10 molar equiv), 4-fluorostyrene (22.0 kg, 1.25 molar equiv), and *N*,*N*-dimethylacetamide (110 kg) was stirred at ambient temperature under a nitrogen atmosphere. Palladium chloride (1.075 kg, 0.040 molar equiv) and triethyl phosphite (1.025 kg, 0.043 molar equiv) were added, and the mixture was heated at 90 °C for 4 h. The hot mixture was diluted with toluene (82.5 kg) and filtered to remove catalyst and other insoluble material; the filter cake was washed with hot toluene (34.5 kg). The total filtrate was concentrated by distillation under reduced pressure until all of the toluene had been removed. The residue was diluted with methanol (159 kg) and cooled and stirred at −5 °C for 30 min. The crystalline product was collected by filtration and washed with cold methanol (49 kg). The methanol-wet solid (60 kg) was used directly in the next stage without further drying. The yield of nitrostilbene **7** was about 90%: ^{1}H NMR (DMSO-d_6, 400 MHz, 300 K) δ 8.56 (d, J = 2.1 Hz, 1H), 8.39 (dd, J = 8.8, 2.6 Hz, 1H), 8.14 (d, J = 8.8 Hz, 1H), 7.84 (d, J = 16.4 Hz, 1H), 7.72−7.66 (m, 2H), 7.47 (d, J = 16.8 Hz, 1H), 7.31−7.24 (m, 2H), 3.94 (s, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz, 300 K) δ 165.5, 162.4 (d, J_{FC} = 241.1 Hz), 145.7, 144.0, 134.0, 132.9 (d, J_{FCCCC} = 3.0 Hz), 129.3 (d, J_{FCCC} = 8.3 Hz, 2C), 129.2, 128.0, 126.4, 125.3, 124.4 (d, J_{FCCCCC} = 2.2 Hz), 115.9 (d, J_{FCC} = 21.8 Hz, 2C), 52.8; IR ν_{max} (KBr disc) 1728, 1585, 1505, 1331, 1227 cm^{-1}.

SCHEME 4. Synthetic Route to Amino Acid 3

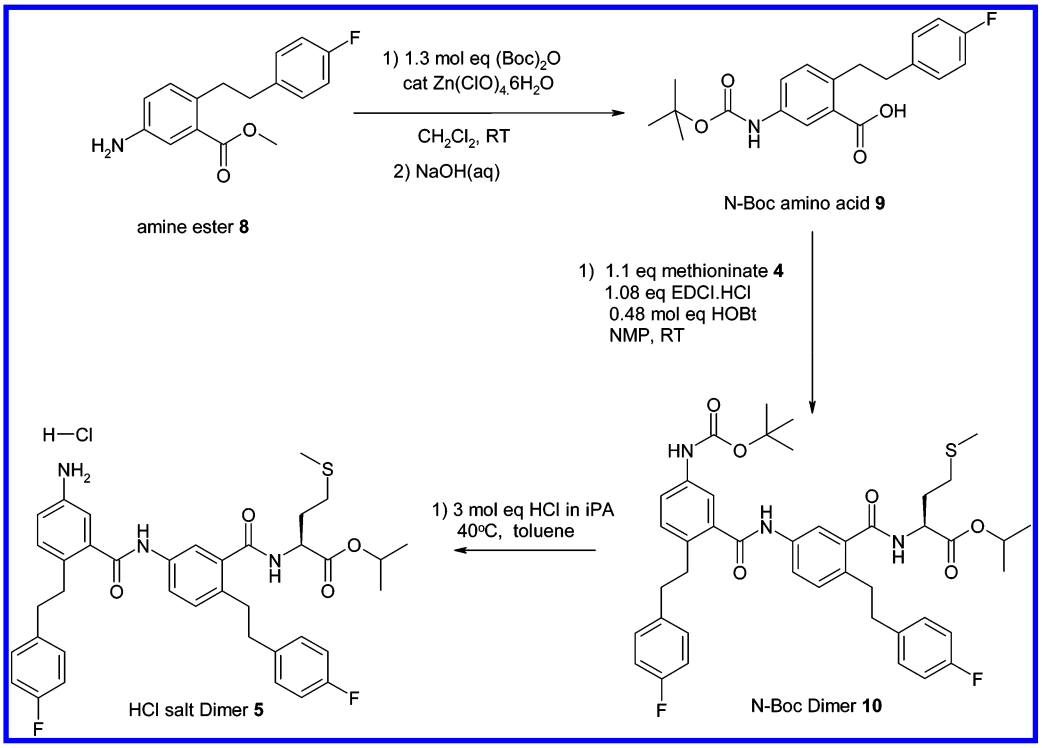


Preparation of Methyl 5-amino-2-[2-(4-fluorophenyl)ethyl]-benzoate] (8). Methanol (216 kg) was added to a mixture of a nitrostilbene (7) (60 kg of methanol-wet solid containing an estimated 39 kg at 100% strength, 1.00 molar equiv) and 10% palladium on carbon-wet paste (0.78 kg, 0.24 mol %). The mixture was stirred and heated at 35 °C under a hydrogen atmosphere (3.0 bar gauge) for 2 h. When the reaction was complete, the mixture was heated to 50 °C and passed through a filter aid to remove the catalyst; the filter cake was washed with methanol (26 kg). The total filtrate was diluted with water (56.7 kg), cooled to −5 °C with stirring, and maintained at −5 °C for 30 min. The crystalline product was collected by filtration, washed with a mixture of methanol (23 kg) and water (28.7 kg), and dried at ambient temperature and pressure. The yield of aminoester **8** (mean of four batches) was 31.8 kg (88%): ^{1}H NMR (DMSO-d_6, 400 MHz, 300 K) δ 7.24−7.16 (m, 2H), 7.11−7.04 (m, 3H), 6.95 (d, J = 8.4 Hz, 1H), 6.69 (dd, J = 8.3, 2.6 Hz, 1H), 5.16 (br s, 2H), 3.80 (s, 3H), 2.99−2.92 (m, 2H), 2.76−2.68 (m, 2H); MS (ES) m/z 274.1 (M + H)$^+$.

Preparation of 5-Amino-2-[2-(4-fluorophenyl)ethyl]benzoic acid (3). A mixture of aminoester **8** (73.3 kg, 1.00 molar equiv), methanol (220 L), and 47% w/w sodium hydroxide solution (34.5 kg, 1.50 molar equiv) was stirred and heated at 60 °C for 6.5 h. The solution was cooled to room temperature and adjusted to pH 5 using 1 M aqueous hydrochloric acid (402 L, about 1.50 molar equiv). The precipitated solid was collected by filtration, washed with water (2 × 147 L), and dried in vacuo at 40 °C. The yield of acid **3** (mean of three batches) was 65.0 kg (93%): ^{1}H NMR (DMSO-d_6, 400 MHz, 300 K) δ 7.25−7.17 (m, 2H), 7.11−7.02 (m, 3H), 6.90 (d, J = 8.2 Hz, 1H), 6.64 (dd, J = 8.1, 2.6 Hz, 1H), 3.33 (br s, 1H), 3.01−2.93 (m, 2H), 2.77−2.69 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz, 300 K) δ 169.2, 160.5 (d, J_{FC} = 241.1 Hz), 146.6, 138.2 (d, J_{FCCCC} = 3.0 Hz), 131.4, 130.4, 129.9 (d, J_{FCCC} = 7.9 Hz, 2C), 129.3, 117.2, 115.5, 114.8 (d, J_{FCC} = 21.1 Hz, 2C), 37.0, 35.5; MS (ES) m/z 260.1 (M + H)$^+$, 301 (MH + CH_3CN)$^+$.

(12) Bordwell, F. G.; Branca, J. C.; Hughes, D. L.; Olmstead, W. N. *J. Org. Chem.* **1980**, *45*, 3305.
(13) Chan, L. C. Unpublished results.

SCHEME 5. Synthetic Route to Dimer 5



Preparation of Isopropyl *N*-{5-Amino-2-[2-(4-fluorophenyl)-ethyl]benzoyl}-L-methioninate (4). A mixture of L-methionine isopropyl ester hydrochloride (135.3 kg, 1.10 molar equiv), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (113.9 kg, 1.10 molar equiv), and *N*-methylpyrrolidin-2-one (NMP) (420 L) was stirred at about 20 °C. *N*-Methylmorpholine (27.3 kg, 0.50 molar equiv) was added to the mixture followed by a solution of 1-hydroxybenzotriazole (29.2 kg, 0.40 molar equiv) in *N*-methylmorpholine (87.6 kg, 1.60 molar equiv) and water (29.1 kg) and a wash of NMP (35 L). A solution of the amino acid **3** (140.0 kg, 1.00 molar equiv) in NMP (350 L) was added over 2 h, keeping the temperature at about 20 °C, and this was followed by a wash of NMP (35 L). The mixture was maintained at 20 °C for a further 8 h to complete the reaction. Water (420 L) was added, and after stirring for 1 h at 20 °C, the product began to crystallize. The mixture was warmed to 40 °C, and more water (980 L) was added over 2 h. After stirring the mixture at 40 °C for 1 h, the solid was collected by filtration, washed with water (2 × 420 L), and dried in vacuo at 40 °C. The yield of amine **4** (mean of four batches) was 208.7 kg (89%): ^{1}H NMR (DMSO-d_6, 400 MHz, 300 K) δ 8.56 (d, J = 7.7 Hz, 1H), 7.23−7.16 (m, 2H), 7.08−7.01 (m, 2H), 6.86 (d, J = 7.9 Hz, 1H), 6.60 (d, J = 2.1 Hz, 1H), 6.52 (dd, J = 8.1, 2.4 Hz, 1H), 5.06 (br s, 2H), 4.97 (septet, J = 6.3 Hz, 1H), 2.84−2.66 (m, 4H), 2.64−2.51 (m, 2H), 2.04 (s, 3H), 1.99 (q, J = 6.4 Hz, 2H), 1.20 (d, J = 6.3 Hz, 3H), 1.17 (d, J = 6.2 Hz, 3H); ^{13}C NMR (DMSO-d_6, 125 MHz, 300 K) δ 171.4, 170.2, 160.5 (d, J_{FC} = 240.7 Hz), 146.4, 138.2 (d, J_{FCCCC} = 2.8 Hz), 136.7, 130.2, 130.0 (d, J_{FCCC} = 8.9 Hz, 2C), 125.9, 114.8, 114.7 (d, J_{FCC} = 20.7 Hz, 2C), 112.9, 67.9, 51.4, 36.8, 34.4, 30.0, 29.8, 21.5, 21.4, 14.5; MS (ES) m/z 433.2 $(M + H)^+$.

Preparation of Isopropyl *N*-{5-({5-Amino-2-[2-(4-fluorophenyl)-ethyl]benzoyl}amino)-2-[2-(4-fluorophenyl)ethyl]benzoyl}-L-methioninate hydrochloride (5). N-Boc amino ester **8** was hydrolyzed with base to N-Boc amino acid **9**, which was then coupled with methioninate **4** in the presence of EDCI·HCl and HOBt in *N*-methylpyrrolidinone to give N-Boc dimer **10**. The *tert*-butoxy group was then removed under acid conditions to give hydrochloride salt of dimer **5**. The overall reaction is shown in Scheme 5.

Preparation of 5-[(*tert*-Butoxycarbonyl)amino]-2-[2-(4-fluorophenyl)ethylbenzoic acid (9). To a mixture of methyl 5-amino-2-[2-(4-fluorophenyl)ethyl]benzoate (**8**) (11 g, 1.0 molar equiv) and di-*tert*-butyldicarbonate (11.40 g, 1.30 molar equiv) in dichloromethane (70 mL) was added zinc perchlorate hexahydrate (0.9 g, 0.06 molar equiv), washed in with 10 mL of dichloromethane. The mixture was stirred at ambient temperature overnight and then heated to 32 °C for 4 h to complete the reaction. The mixture was cooled to ambient temperature, and water (80 mL) was added, followed by sodium hydroxide (0.75 mL, 50% w/w, 0.35 molar equiv) so pH 12 was achieved. The mixture was stirred for 10 min, and the lower aqueous phase was separated and discarded.

The organic solution was then concentrated under reduced vacuo on a rotary evaporator to give an oil (19 g). The oil was redissolved in methanol (100 mL), and water (80 mL) was added, followed by sodium hydroxide (10 mL, 50% w/w, 4.7 molar equiv). The mixture was heated to 75 °C and was held at 75 °C for 1 h. The mixture was then cooled to ambient temperature and left to stir overnight. Water (80 mL) was added and the mixture cooled to 12 °C, before acidification with concentrated hydrochloric acid (15 mL, 4.33 molar equiv) to pH 1. The mixture was stirred for 10 min, then filtered, and the solid washed with water (80 mL). The product was dried in vacuo at 40 °C. The yield of benzoic acid **9** was 13.94 g (93%): ^{1}H NMR (DMSO-d_6, 400 MHz, 300 K) δ 9.41 (s, 1H), 8.00 (d, J = 2.0 Hz, 1H), 7.47 (dd, J = 8.3, 2.4 Hz, 1H), 7.27−7.17 (m, 2H), 7.14 (d, J = 8.3 Hz, 1H), 7.11−7.03 (m, 2H), 3.37 (br s, 1H), 3.13−3.03 (m, 2H), 2.82−2.71 (m, 2H), 1.47 (s, 9H); ^{13}C NMR ((DMSO-d_6, 100 MHz, 300 K) δ 168.7, 160.6 (d, J_{FC} = 240.7 Hz), 152.8, 137.9 (d, J_{FCCCC} = 3.0 Hz), 137.6, 135.9, 131.2, 130.4, 130.0 (d, J_{FCCC} = 8.3 Hz, 2C), 121.3, 119.8, 114.8 (d, J_{FCC} = 21.1 Hz, 2C), 79.1, 36.6, 35.4, 28.1 (3C); MS (ES) m/z 382 $(MH + Na)^+$.

Preparation of Isopropyl *N*-{5({5-[(*tert*-Butoxycarbonyl)-amino]-2-[2-(4-fluorophenyl)ethyl]benzoyl}amino)-2-[2-(4-fluorophenyl)ethyl]benzoyl}-L-methioninate (10). N-Boc amino acid **9** (11.63 g, 1.0 molar equiv) was added in portions to a mixture of methioninate **4** (15.54 g, 98.4% w/w, 1.10 molar equiv), EDCI·HCl (6.72 g, 1.08 molar equiv), and HOBt (20% w/w solution; 10.50 mL, 0.48 molar equiv) in *N*-methylpyrrolidin-2-one (42 mL),

keeping the reaction temperature at 20 °C. The mixture was stirred at ambient temperature overnight. Water (100 mL) and ethyl acetate (100 mL) were added, and the mixture was stirred for 10 min. The mixture was allowed to settle, and the lower aqueous phase was separated off and discarded. The organic phase was washed with more water (2 × 100 mL) and then concentrated under reduced vacuo on a rotary evaporator to give a solid. The solid was then recrystallized from methyl-*tert*-butyl ether (100 mL). The product (22.6 g, 90%) yield was isolated: ^{1}H NMR (DMSO-d_6, 400 MHz, 300 K) δ 10.43 (s, 1H), 9.46 (s, 1H), 8.78 (d, J = 7.5 Hz, 1H), 7.83−7.78 (m, 1H), 7.72 (dd, J = 8.3, 1.9 Hz, 1H), 7.67−7.61 (m, 1H), 7.39 (dd, J = 8.3, 2.0 Hz, 1H), 7.29−7.12 (m, 6H), 7.12−6.99 (m, 4H), 4.93 (septet, J = 6.3 Hz, 1H), 4.58−4.88 (m, 1H), 3.00−2.76 (m, 8H), 2.68−2.53 (m, 2H), 2.11−1.94 (m, 5H), 1.48 (s, 9H), 1.21 (d, J = 6.3 Hz, 3H), 1.18 (d, J = 6.3 Hz, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz, 300 K) δ 171.3, 169.5 (2C), 167.9, 160.5 (d, J_{FC} = 241.0 Hz, 2C), 152.8, 137.8 (d, J_{FCCCC} = 3.0 Hz), 137.7 (d, J_{FCCCC} = 3.0 Hz), 137.4, 137.1, 136.9 (d, J_{FCC} = 21.9 Hz, 2C), 134.1, 132.3, 130.1, 130.0 (d, J_{FCCC} = 7.9 Hz, 2C), 129.9 (d, J_{FCCC} = 7.9 Hz, 2), 120.4, 119.2, 118.7, 116.7, 114.9 (d, J_{FCC} = 21.1 Hz, 2C), 114.8 (d, J_{FCC} = 21.1 Hz, 2C), 79.2, 68.1, 51.5, 36.3 (2C), 34.5, 34.4, 30.1, 29.8, 28.1, 21.5, 21.4, 14.5; MS (ES) m/z 774 $(M + H)^+$, 718 (minus *tert*-butyl).

Preparation of Isopropyl *N*-{5-({5-Amino-2-[2-(4-fluorophenyl)-ethyl]benzoyl}amino)-2-[2-(4-fluorophenyl)ethyl]benzoyl}-L-methioninate hydrochloride (11). Boc dimer **10** (21.50 g, 1.0 molar equiv) in toluene (60 mL) was heated to 40 °C. To this solution was added HCl in isopropanol (5 M, 16 mL, 2.87 molar equiv). The solution was stirred at 40 °C for 1.5 h and then cooled to ambient temperature. An aqueous solution of potassium bicarbonate (9 g, 3.3 molar equiv) in water (80 mL) was added to the mixture. The organic phase was separated off and dried over anhydrous magnesium sulfate. The solution was filtered and concentrated under reduced vacuo on the rotary evaporator to give an oil (18 g).

The oil was redissolved in diethyl ether (100 mL), and HCl in isopropanol (5 M, 5.50 mL, 0.98 molar equiv) was added. The mixture was stirred at ambient temperature overnight. The solid was filtered off and dried in vacuo at 40 °C. The yield of hydrochloride salt of dimer **5** was 14.1 g (74%): ^{1}H NMR (DMSO-d_6, 400 MHz, 300 K) δ 10.63 (s, 1H), 8.79 (d, J = 7.7 Hz, 1H), 7.80 (d, J = 2.3 Hz, 1H), 7.71 (dd, J = 8.4, 2.0 Hz, 1H), 7.41−7.35 (m, 1H), 7.34−7.29 (m, 1H), 7.27−7.14 (m, 5H), 7.11−7.00 (m, 4H), 4.93 (septet, J = 6.3 Hz, 1H), 4.57−4.49 (m, 1H), 4.57−4.49 (m, 1H), 3.50 (br s, 2H), 3.04−2.74 (m, 8H), 2.67−2.52 (m, 2H), 2.07−1.95 (m, 5H), 1.21 (d, J = 6.3 Hz, 3H), 1.18 (d, J = 6.2 Hz, 3H); ^{13}C NMR (DMSO-d_6, 100 MHz, 300 K) δ 171.3, 169.5, 166.9, 160.7 (d, J_{FC} = 241.1 Hz, 2C), 138.0, 137.8 (d, J_{FCCCC} = 3.0 Hz), 137.3 (d, J_{FCCCC} = 3.0 Hz), 136.9, 136.7, 134.4 (2C), 131.2, 130.1 (2C), 130.5 (d, J_{FCCC} = 7.9 Hz, 2C), 130.0 (d, J_{FCCC} = 7.9 Hz, 2C), 123.4, 120.9, 120.5, 118.7, 114.9 (d, J_{FCC} = 21.1 Hz, 2C), 114.8 (d, J_{FCC} = 21.1 Hz, 2C), 68.1, 51.5, 36.2, 35.9, 34.5, 30.0, 29.8, 21.5, 21.4, 14.5.

HPLC Method	
column	Genesis, C-18, 15 cm × 3.0 mm i.d., 3 μm
temperature	40 °C
wavelength	265 nm
flow	1.0 mL/min
sample injection volume	10 μL
run time	16 min
post time	2.0 min

Gradient

time (min)	% water	% CH_3CN	1% trifluoroacetic acid
0	60	30	10
12	20	70	10
14	20	70	10
16	60	30	10

Sample Preparation:
Take 50 μL of reaction mixture into a 5 mL volumetric flask. Dilute to volume with methanol.

component	rt (min)
HOBt	1.00
amino acid **3**	1.48
amide **4**	4.7
HOBt ester	6.0
diamide **5**	8.5

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Supporting Information Available: ^{1}H, ^{13}C NMR and LCMS spectra of compounds **7**, **8**, **3**, **4**, **9**, **10**, and **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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