Synthesis of Peptide Isocyanates and Isothiocyanates

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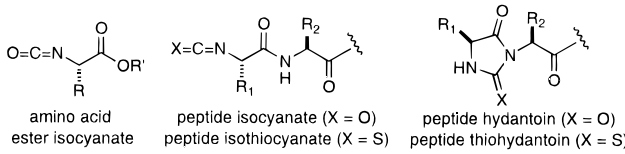
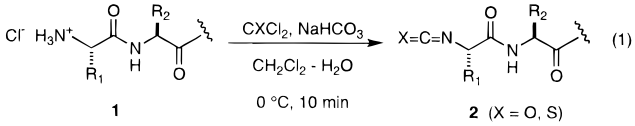
Peptide isocyanates are a hitherto unreported class of compounds that we required as building blocks for the synthesis of artificial β-sheets.[1] In 1950, Goldschmidt and Wick reported that amino acid ester hydrochlorides could readily be converted to the corresponding amino acid ester isocyanates by refluxing in toluene while sparging with gaseous phosgene.[2] When they attempted to convert a peptide (glycylgylcine ethyl ester hydrochloride) to its isocyanate, the peptide hydantoin was formed. The authors postulated that the peptide isocyanate formed and subsequently cyclized to the hydantoin. These studies raised the question that the isocyanates might be inherently unstable and unsuitable for use as synthetic building blocks. Because the reaction conditions used by Goldschmidt and Wick involved high temperatures (110 °C) and the liberation of hydrogen chloride, we decided to investigate whether peptide isocyanates could be prepared using milder reaction conditions.



In 1992, we reported that amino acid ester hydrochlorides could readily be converted to amino acid ester isocyanates by treatment with a solution of phosgene in toluene and pyridine in methylene chloride at 0 °C.[3] The commercial availability (Fluka) and ease of handling of a phosgene solution rendered these reaction conditions particularly attractive. Because free hydrogen chloride is not formed, these conditions are compatible with acid-sensitive functionality, such as *tert*-butyl ether groups, which cannot tolerate the conditions of Goldschmidt and Wick. When the hydrochloride salts of peptides were subjected to these reaction conditions, the corresponding peptide isocyanates were generated, along with variable quantities of the peptide hydantoins. Upon further investigation, we discovered that modified Schotten−Baumann conditions afforded the isocyanates reproducibly and in satisfactory yield, while minimizing the formation of hydantoins and other side products.[4] Using these conditions, we have also prepared peptide isothiocyanates, a second unreported class of compounds.[5]

Results

Addition of a solution of phosgene in toluene to a solution of a peptide (as the hydrochloride salt, **1**) in an ice-cooled mixture of methylene chloride and saturated aqueous sodium bicarbonate solution affords the peptide isocyanate **2** (eq 1). In a typical reaction procedure, 2.0



(1)

equiv of phosgene is used and the reaction is complete after 10 min of rapid stirring. Using these reaction conditions, we have prepared a variety of peptide isocyanates (Table 1). Cases studied include dipeptides (Table 1, entries 1−4), tripeptides (Table 1, entries 5 and 6), and monopeptide amides (Table 1, entries 7, 8). To achieve a satisfactory yield of phenylalanine methylamide isocyanate (entry 7), 1.1 equiv of phosgene was used (vide infra). Amino acid ester isocyanates can also be prepared by this method (Table 1, entry 9). (Because the procedure is so convenient and sodium bicarbonate is nontoxic and environmentally benign, we now consider it preferable to our earlier method of preparing amino acid ester isocyanates using pyridine.[3]) When thiophosgene is used in place of a solution of phosgene in toluene, the corresponding peptide isothiocyanates are formed (Table 1, entries 10 and 11).

Triphosgene ($Cl_3COCO_2CCl_3$) is a popular alternative to phosgene.[6,7] Use of triphosgene instead of phosgene (0.7 mmol/mmol peptide hydrochloride, Table 1, entries 12 and 13) gave isocyanates **2a** and **2b** in yields similar to those obtained using phosgene. The isocyanates produced using triphosgene were contaminated with ca. 10% of unreacted triphosgene, as evidenced by a peak at 1828 cm^{-1} in the infrared spectrum. We prefer to use phosgene, because excess phosgene is removed upon evaporation of the solvent, while unreacted triphosgene is not volatile and is reactive toward nucleophiles (1 mmol of triphosgene can react with 12 mmol of an amine). Although phosgene is toxic, its solution is easily and safely dispensed by syringe in a fume hood, and over a dozen co-workers in our laboratories have used this reagent hundreds of times without incident.

The peptide isocyanates and isothiocyanates that are produced by this procedure are generally formed in near-quantitative mass recovery and in ca. 60−100% purity, as indicated by ^{1}H NMR spectroscopic analysis. Whereas amino acid ester isocyanates are sufficiently volatile that they are conveniently purified by Kugelrohr distillation,[3] peptide isocyanates and isothiocyanates are not. In lieu of purification, we chose to trap these compounds with *N*-ethylaniline as urea and thiourea derivatives **3**, which were readily purified to analytical purity (eq 2). Thus, the yields reported in Table 1 are the isolated yields of

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(2) (a) Goldschmidt, S.; Wick, M. *Z. Naturforsch. B* **1950**, *5*, 170. (b) Goldschmidt, S.; Wick, M. *Liebigs Ann. Chem.* **1952**, *575*, 217.

(3) Nowick, J. S.; Powell, N. A.; Nguyen, T. M.; Noronha, G. *J. Org. Chem.* **1992**, *57*, 7364.

(4) For related examples, see: (a) Gazzard, E. G.; Greenshields, J. N.; Phillipson, J. M. Br. Patent 1 152 877, 1969; *Chem. Abstr.* **1969**, *71*, 48896. (b) Botta, A.; Krimm, H. Ger. Patent 1 913 273, 1970; *Chem. Abstr.* **1970**, *73*, 120074.

(5) One reported attempt to prepare a peptide isothiocyanate afforded the corresponding peptide thiohydantoin: Böhme, H.; Martin, F.; Strahl, J. *Arch. Pharm. (Weinheim, Ger.)* **1980**, *313*, 10.

(6) Eckert, H.; Forster, B. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 894.

(7) Majer, P.; Randad, R. S. *J. Org. Chem.* **1994**, *59*, 1937.

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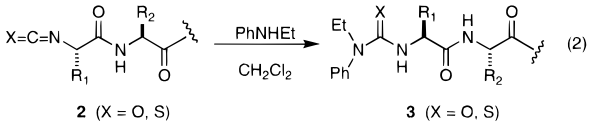
purified ureas and thioureas **3** formed from the corresponding peptide hydrochlorides.

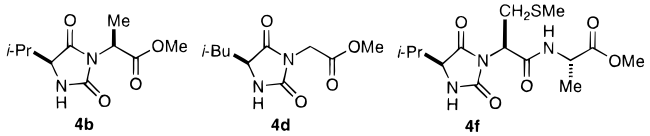


2 (X = O, S) **3** (X = O, S)

The peptide isocyanates are moderately stable to short-term storage at ambient temperature. Valylalanine methyl ester isocyanate (**2b**) underwent ca. 50% decomposition upon storage, either neat or as a solution in $CDCl_3$, for 1 week at 20 °C. The predominant decomposition product was identified as hydantoin **4b** (vide infra). Under similar conditions, phenylalanylleucine methyl ester isocyanate (**2a**) underwent ca. 10% decomposition. The instability of these compounds contrasts the stability of amino acid ester isocyanates[3] (e.g., **2i**), which were found to be indefinitely stable if protected from moisture in airtight vials.

Discussion

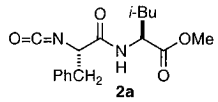
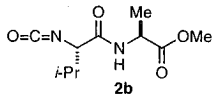
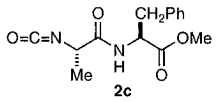
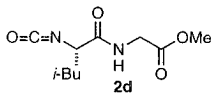
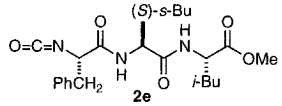
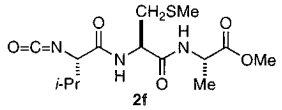
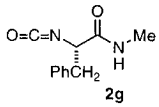
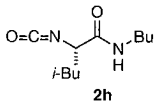
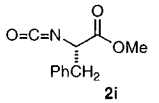
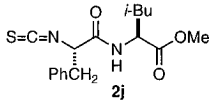
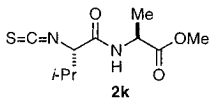
Two pathways were identified that diminished the yields of the peptide isocyanates and resulted in the formation of side products: hydantoin formation and *N*-acylation of amide groups by phosgene. Valylalanine methyl ester hydrochloride, leucylglycine methyl ester hydrochloride, and valyl-*S*-methylcystylalanine afforded ca. 20% of hydantoins **4b**, **4d**, and **4f** in addition to isocyanates **2b**, **2d**, and **2f** (Table 1, entries 2, 4, and 6). At present, it is not clear why these cases form hydantoin and others do not. In optimizing the reaction conditions to minimize hydantoin formation, the effect of a number of factors upon the amount of hydantoin formed were investigated, including the number of equivalents of phosgene added, the volumes of methylene chloride and sodium bicarbonate solution used, the temperature (0 °C vs ambient), the reaction time, the rate of stirring, and the manner of addition of the phosgene. No definite correlations were observed, although higher temperatures, excessive stirring rates, and stirring while the phosgene is added appeared to result in slight increases in the amount of hydantoin formed. Eventually, we chose to standardize the conditions used for generation of the isocyanates by using 2.0 equiv of phosgene, adding the phosgene in one portion to an ice-cooled solution of peptide hydrochloride in equal volumes of methylene chloride and saturated aqueous sodium bicarbonate without stirring, and then stirring at 600 rpm with a mechanical stirrer for 10 min (reaction conditions A, Table 1). Although these conditions gave consistent and reproducible yields of isocyanates and side products, we have also successfully generated isocyanates with magnetic stirring and with smaller or larger quantities of phosgene with only minor variations in results.



4b **4d** **4f**

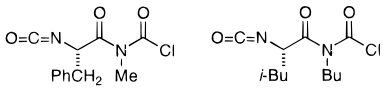
Peptides bearing sterically unhindered amide groups formed *N*-acylation side products in addition to the desired isocyanates. Phenylalanine methylamide **1g** was the worst offender, forming *N*-acylated isocyanate **5g** as the predominant product upon treatment with 2.0 equiv of phosgene. Reduction of the amount of phosgene to 1.1 equiv helped alleviate this problem, affording the desired isocyanate **2g** in 49% yield (Table 1, entry 7). Leucine butylamide **1h** formed the corresponding isocyanate in 71% yield and generated only 16% of *N*-acylation product **5h** when 2.0 equiv of phosgene was used (Table 1, entry 8). No *N*-acylation product was detected upon treatment

Table 1. Conversion of Peptides to Isocyanates and Isothiocyanates

entry	reactant	reaction conditions[a]	product	% yield[b]
1	phenylalanylleucine methyl ester hydrochloride **1a**	A	 **2a**	93
2	valylalanine methyl ester hydrochloride **1b**	A	 **2b**	69
3	alanylphenylalanine methyl ester hydrochloride **1c**	A	 **2c**	79
4	leucylglycine methyl ester hydrochloride **1d**	A	 **2d**	50
5	phenylalanylisoleucylleucine methyl ester hydrochloride **1e**	A	 **2e**	81
6	valyl-*S*-methylcystylalanine methyl ester hydrochloride **1f**	A	 **2f**	69
7	phenylalanine methylamide hydrochloride **1g**	B	 **2g**	49
8	leucine butylamide hydrochloride **1h**	A	 **2h**	71
9	phenylalanine methyl ester hydrochloride **1i**	A	 **2i**	93
10	**1a**	C	 **2j**	94
11	**1b**	C	 **2k**	62
12	**1a**	D	**2a**	91
13	**1b**	D	**2b**	67

[a] Reaction conditions: A, 2.0 equiv of $COCl_2$ solution in toluene; B, 1.1 equiv of $COCl_2$ solution in toluene; C, 2.0 equiv of $CSCl_2$; D, 0.7 equiv of $Cl_3COCO_2CCl_3$ (triphosgene). [b] Yields are based upon quantity of analytically pure ureas **3a**−**i** and thioureas **3j**−**k** obtained upon trapping of the unpurified products with *N*-ethylaniline. (See text for details).

of leucylglycine methyl ester **1d** with 2.0 equiv of phosgene (Table 1, entry 4). Apparently, phosgene only acylates unhindered amides (e.g., methyl and butyl) under the reaction conditions used for generation of the isocyanates.



5g **5h**

Amino acids and peptides can epimerize during activation and coupling. Although a number of mechanisms for epimerization upon treatment with phosgene can also be envisioned, we have not observed epimerization under the reaction conditions described in this paper. In our previous report of a method for preparing amino acid ester isocyanates, we determined that the isocyanates produced were enantiomerically pure (>99% ee) by reacting the isocyanates with enantiomerically pure (*R*)-1-phenylethylamine and looking for diastereomeric products by ^{1}H NMR spectroscopy.[3] In the current study, epimerization would generate diastereomeric isocyanates and diastereomeric urea trapping products. These isomers would likely exhibit ^{1}H NMR resonances that are distinct from those of unepimerized products. Throughout our studies of the side products produced, and in using peptide isocyanates in the synthesis of artificial β-sheets,[1] we have not detected epimers. (Epimerization was, however, detected upon preparation of large quantities of a peptide isocyanate for another project using different reaction conditions.[8]) These experiences suggest that peptide isocyanate and isothiocyanate formation proceeds with little or no epimerization under the reaction conditions described in this paper.

Conclusions

These studies offer a practical method for the preparation of peptide isocyanates and peptide isothiocyanates, two classes of compounds that have not previously been reported. The yields of these compounds are generally good, in spite of the side reactions that can occur. Because the isocyanates cannot easily be purified, it would still be desirable to improve this method to allow the generation of isocyanates without side products. The clean reactions of isocyanates and isothiocyanates with nucleophiles, such as amines and alcohols, render these compounds particularly attractive as synthetic building blocks for combinatorial chemistry and the creation of unnatural biopolymers, two areas that are undergoing rapid developments.[9] Other reactions of potential interest include condensation with carboxylic acids to form amides[2,10] and cycloaddition reactions to form β-lactams[11] and other heterocycles.[12] In our own laboratories, we will continue to report the application of peptide isocyanates to the synthesis of artificial β-sheets, and we will describe any further improvements in peptide isocyanate formation therein.

Experimental Section

General Procedures. Dipeptide methyl ester hydrochlorides **1a**−**c** were prepared by coupling of Cbz-protected amino acids and amino acid methyl ester hydrochlorides (using either DCC or the nitrophenyl ester method), followed by hydrogenolysis in 2 M hydrochloric acid in methanol.[13] Leucylglycine methyl ester hydrochloride (**1d**) was prepared by hydrogenolysis of Cbz-protected leucylglycine methyl ester (Sigma) in 2 M hydrochloric acid in methanol. Tripeptide methyl ester hydrochlorides **1e** and **1f** were prepared by solid-phase synthesis of the Cbz-protected tripeptides on Merrifield resin, cleavage from the resin as the methyl esters (treatment with methanol, dimethylformamide, and triethylamine), and hydrogenolysis in 2 M hydrochloric acid in methanol.[14] Monopeptide amides **1g** and **1h** were prepared by aminolysis of the corresponding amino acid ester hydrochlorides with methylamine or butylamine in methanol. Phosgene was obtained from Fluka as a 20% (1.93 M) solution in toluene. Thiophosgene and triphosgene were obtained from Aldrich. Methylene chloride used in trapping the peptide isocyanates and isothiocyanates was dried by distillation from CaH_2. *N*-Ethylaniline was distilled under vacuum and stored under nitrogen. High-resolution mass spectra (HRMS) were obtained by fast-atom bombardment (FAB) of samples in *m*-nitrobenzyl alcohol with Cs^+ ions ($\sigma = \pm 1$ mmu). Combustion analyses were performed by Desert Analytics, Tucson, AZ.

General Procedure for the Preparation of Peptide Isocyanates and Isothiocyanates. [CAUTION: PHOSGENE AND THIOPHOSGENE ARE VOLATILE AND HIGHLY TOXIC—USE HOOD.] A 25-mL, one-necked, round-bottomed flask, equipped with a mechanical stirrer, was charged with 0.50 mmol of hydrochloride salt **1**, 5 mL of CH_2Cl_2, and 5 mL of saturated aqueous $NaHCO_3$. The biphasic mixture was cooled

(8) Epimerization was detected when multigram quantities of phenylalanylphenylalanine methyl ester hydrochloride were converted to the corresponding isocyanate by treatment with 13 equiv of phosgene in an ice-cooled mixture of aqueous sodium bicarbonate and methylene chloride. The phenylalanylphenylalanine methyl ester isocyanate was trapped with an amine, the products of reaction were separated by preparative HPLC, and epimeric ureas were identified by ^{1}H NMR spectroscopy. Subsequent studies involving the preparation of this isocyanate have not resulted in the detection of epimers. When this isocyanate was prepared under the reaction conditions described in this paper, digested with HCl to form phenylalanine hydrochloride, and then analyzed by polarimetry, no loss of optical activity was detected. Further studies are required to corroborate this anomalous result and to determine whether factors such as large excesses of phosgene or large-scale reactions lead to epimerization.

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(11) (a) Perelman, M.; Mizsak, S. A. *J. Am. Chem. Soc.* **1962**, *84*, 4988. (b) Opitz, G.; Koch, J. *Angew. Chem., Int. Ed. Engl.* **1963**, *2*, 152.

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(14) Stewart, J. M.; Young, J. D. *Solid Phase Peptide Synthesis*, 2nd ed.; Pierce Chemical Company: Rockford, IL, 1984.

to 0 °C in an ice bath while stirring for ca. 10 min at 600 rpm. Stirring was stopped, the layers were allowed to separate, and phosgene (520 μL of a 1.93 M solution in toluene, 1.00 mmol), thiophosgene (76 μL, 1.00 mmol), or triphosgene (a solution of 100 mg in 1 mL of methylene chloride, 0.34 mmol) was added in a single portion via syringe to the lower (organic) phase. Stirring was resumed immediately, and the ice-cooled reaction mixture was stirred for 10 min at 600 rpm. The layers were then separated, the aqueous phase was extracted with three 5-mL portions of CH_2Cl_2, and the combined organic layers were dried over Na_2SO_4, filtered, and concentrated by rotary evaporation. The crude isocyanate or isothiocyanate **2** was further freed of solvent under vacuum (0.1 mmHg), weighed, characterized by ^{1}H NMR and IR spectroscopy, and then trapped with *N*-ethylaniline as described in the following procedure.

General Procedure for Trapping Peptide Isocyanates and Isothiocyanates with *N*-Ethylaniline. A 25-mL, one-necked, round-bottomed flask fitted with a nitrogen inlet adapter and containing peptide isocyanate or isothiocyanate **2** from the above procedure was evacuated and filled with nitrogen three times. Methylene chloride (5 mL) was added, the isocyanate or isothiocyanate was allowed to dissolve, and *N*-ethylaniline (125 μL, 0.99 mmol) was added in a single portion. After 20 h, the reaction mixture was concentrated by rotary evaporation, and the residue was purified by column chromatography on silica gel (EtOAc−CH_2Cl_2 or EtOAc−$CHCl_3$) or recrystallization (EtOAc−hexanes) to afford pure urea or thiourea **3**.

Phenylalanylleucine Methyl Ester Isocyanate (2a) and Urea 3a (PhN(Et)CONH-(*S*)-CH(CH_2Ph)CONH-(*S*)-CH(*i*-Bu)CO_2CH_3). Reaction of phenylalanylleucine methyl ester hydrochloride (**1a**, 164 mg, 0.499 mmol) with phosgene (520 μL of a 1.93 M solution in toluene, 1.00 mmol) yielded 159 mg (100%) of crude isocyanate **2a** as a white solid: IR ($CHCl_3$) 3417, 2266, 1741, 1684 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34 (t, *J* = 7.2 Hz, 2 H), 7.29 (t, *J* = 6.9 Hz, 1 H), 7.25 (d, *J* = 8.0 Hz, 2 H), 6.62 (d, *J* = 8.1 Hz, 1 H), 4.61 (td, *J* = 8.4, 5.3 Hz, 1 H), 4.39 (dd, *J* = 8.5, 4.2 Hz, 1 H), 3.73 (s, 3 H), 3.35 (dd, ABX pattern, J_{AB} = 13.9 Hz, J_{AX} = 4.1 Hz, 1 H), 3.01 (dd, ABX pattern, J_{AB} = 13.9 Hz, J_{BX} = 8.5 Hz, 1 H), 1.65−1.60 (m, 1 H), 1.55−1.48 (m, 2 H), 0.923 (d, *J* = 6.1 Hz, 3 H), 0.917 (d, *J* = 6.1 Hz, 3 H).

Reaction of 159 mg of **2a** with *N*-ethylaniline (125 μL, 0.99 mmol) yielded 204 mg (93% from **1a**) of **3a** as white crystals from ethyl acetate−hexanes: mp 98−100 °C; IR ($CHCl_3$) 3427, 1741, 1674, 1643 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37−7.30 (m, 3 H), 7.23−7.20 (m, 3 H) 7.04−7.02 (m, 2 H), 6.98 (d, *J* = 7.1 Hz, 2 H), 6.91 (d, *J* = 7.6 Hz, 1 H), 4.58 (q, *J* = 7.2 Hz, 1 H), 4.53−4.49 (m, 1 H), 4.45 (d, *J* = 7.4 Hz, 1 H), 3.71 (s, 3 H), 3.73−3.63 (m, 2 H), 3.02 (dd, ABX pattern, J_{AB} = 14.0 Hz, J_{AX} = 6.0 Hz, 1 H), 2.90 (dd, ABX pattern, J_{AB} = 14.0 Hz, J_{BX} = 8.0 Hz, 1 H), 1.62−1.50 (m, 3 H), 1.06 (t, *J* = 7.0 Hz, 3 H), 0.90 (d, *J* = 5.9 Hz, 3 H), 0.88 (d, *J* = 5.9 Hz, 3 H); HRMS *m*/*e* for $C_{25}H_{34}N_3O_4$ (M + H)$^+$ calcd 440.2549, found 440.2547. Anal. Calcd for $C_{25}H_{33}N_3O_4$: C, 68.31; H, 7.57; N, 9.56. Found: C, 68.47; H, 7.48; N, 9.53.

Phenylalanylleucine Methyl Ester Isocyanate (2a) and Urea 3a (PhN(Et)CONH-(*S*)-CH(CH_2Ph)CONH-(*S*)-CH(*i*-Bu)CO_2CH_3) (Using Triphosgene). Reaction of phenylalanylleucine methyl ester hydrochloride (**1a**, 164 mg, 0.499 mmol) with triphosgene (a solution of 104 mg in 1 mL of methylene chloride, 0.35 mmol) yielded 162 mg (102%) of crude isocyanate **2a** as a colorless viscous oil that crystallized upon standing.

Reaction of 162 mg of **2a** with *N*-ethylaniline (125 μL, 0.99 mmol) yielded 199 mg (91% from **1a**) of **3a** as a colorless glassy solid.

Valylalanine Methyl Ester Isocyanate (2b) and Urea 3b (PhN(Et)CONH-(*S*)-CH(*i*-Pr)CONH-(*S*)-CH(CH_3)CO_2CH_3). Reaction of valylalanine methyl ester hydrochloride (**1b**, 118 mg, 0.494 mmol) with phosgene (520 μL of a 1.93 M solution in toluene, 1.00 mmol) yielded 102 mg (90%) of crude isocyanate **2b** as a colorless oil. ^{1}H NMR analysis of this product revealed 16% of hydantoin **4b** as an impurity. **2b**: IR ($CHCl_3$) 3415, 2262, 1743, 1684 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.80 (br s, 1 H), 4.61 (appar q, *J* = 6.9 Hz, 1 H), 4.04 (br d, *J* = 1.7 Hz, 1 H), 3.78 (s, 3 H), 2.42−2.35 (m, 1 H), 1.44 (d, *J* = 7.1 Hz, 3 H), 1.08 (d, *J* = 6.8 Hz, 3 H), 0.91 (d, *J* = 6.7 Hz, 3 H). **4b** (isolated by column chromatography of crude **3b**): IR ($CHCl_3$) 3460, 1780, 1747, 1720 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.46 (s, 1 H), 4.75 (q, *J* = 7.3 Hz, 1 H), 3.97 (dd, *J* = 3.4, 0.9 Hz, 1 H), 3.74 (s, 3 H), 2.30−2.24 (m, 1 H), 1.62 (d, *J* = 7.4 Hz, 3 H), 1.05 (d, *J* = 7.0 Hz, 3 H), 0.96 (d, *J* = 6.8 Hz, 3 H); HRMS *m*/*e* for $C_{10}H_{17}N_2O_4$ (M + H)$^+$ calcd 229.1188, found 229.1195.

Reaction of 102 mg of **2b** with *N*-ethylaniline (125 μL, 0.99 mmol) yielded 119 mg (69% from **1b**) of **3b** as a colorless viscous oil: IR ($CHCl_3$) 3427, 1741, 1672, 1651 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 (t, *J* = 7.7 Hz, 2 H), 7.34 (t, *J* = 7.4 Hz, 1 H), 7.29 (d, *J* = 7.3 Hz, 2 H), 7.04 (d, *J* = 7.0 Hz, 1 H), 4.66 (d, *J* = 8.6 Hz, 1 H), 4.51 (quintet, *J* = 7.2 Hz, 1 H), 4.22 (dd, *J* = 8.5, 6.5 Hz, 1 H), 3.75−3.70 (m, 2 H), 3.72 (s, 3 H), 1.98 (octet, *J* = 6.7 Hz, 1 H), 1.39 (d, *J* = 7.2 Hz, 3 H), 1.10 (t, *J* = 7.1 Hz, 3 H), 0.92 (d, *J* = 6.8 Hz, 3 H), 0.77 (d, *J* = 6.8 Hz, 3 H); HRMS *m*/*e* for $C_{18}H_{28}N_3O_4$ (M + H)$^+$ calcd 350.2080, found 350.2085. Anal. Calcd for $C_{18}H_{27}N_3O_4$: C, 61.87; H, 7.79; N, 12.03. Found: C, 61.65; H, 7.80; N, 11.75.

Valylalanine Methyl Ester Isocyanate (2b) and Urea 3b (PhN(Et)CONH-(*S*)-CH(*i*-Pr)CONH-(*S*)-CH(CH_3)CO_2CH_3) (Using Triphosgene). Reaction of valylalanine methyl ester hydrochloride (**1b**, 123 mg, 0.515 mmol) with triphosgene (a solution of 112 mg in 1 mL of methylene chloride, 0.38 mmol) yielded 112 mg (95%) of crude isocyanate **2b** as a colorless oily solid. ^{1}H NMR analysis of this product revealed 8% of hydantoin **4b** and 15% of an additional impurity. Additional impurity (identified by examination of the spectra of the crude product): ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.76 (s, 3 H), 1.01 (d, *J* = 6.6 Hz, 3 H), 1.00 (d, *J* = 6.7 Hz, 3 H).

Reaction of 112 mg of **2b** with *N*-ethylaniline (125 μL, 0.99 mmol) yielded 120 mg (67% from **1b**) of **3b** as a colorless viscous oil.

Alanylphenylalanine Methyl Ester Isocyanate (2c) and Urea 3c (PhN(Et)CONH-(*S*)-CH(CH_3)CONH-(*S*)-CH(CH_2Ph)-CO_2CH_3). Reaction of alanylphenylalanine methyl ester hydrochloride (**1c**, 143 mg, 0.499 mmol) with phosgene (520 μL of a 1.93 M solution in toluene, 1.00 mmol) yielded 133 mg (97%) of crude isocyanate **2c** as a pale tan oil: IR ($CHCl_3$) 3415, 2268, 2241, 1743, 1686 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.32−7.26 (m, 3 H), 7.09 (d, *J* = 7.1 Hz, 2 H), 6.64 (d, *J* = 7.3 Hz, 1 H), 4.85 (appar q, *J* = 6.6 Hz, 1 H), 4.11 (q, *J* = 7.0 Hz, 1 H), 3.75 (s, 3 H), 3.19 (dd, ABX pattern, J_{AB} = 13.9 Hz, J_{AX} = 5.7 Hz, 1 H), 3.09 (dd, ABX pattern, J_{AB} = 13.9 Hz, J_{BX} = 6.3 Hz, 1 H), 1.48 (d, *J* = 6.9 Hz, 3 H).

Reaction of 133 mg of **2c** with *N*-ethylaniline (125 μL, 0.99 mmol) yielded 157 mg (79% from **1c**) of **3c** as a colorless viscous oil: IR ($CHCl_3$) 3427, 1738, 1676, 1645 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46−7.12 (m, 11 H), 4.79 (appar q, *J* = 6.8 Hz, 1 H) 4.58 (d, *J* = 7.5 Hz, 1 H), 4.45 (quintet, *J* = 7.2 Hz, 1 H), 3.78−3.68 (m, 2 H), 3.68 (s, 3 H), 3.14 (dd, ABX pattern, J_{AB} = 13.9 Hz, J_{AX} = 5.8 Hz, 1 H), 3.03 (dd, ABX pattern, J_{AB} = 13.9 Hz, J_{BX} = 7.0 Hz, 1 H), 1.20 (d, *J* = 7.0 Hz, 3 H), 1.09 (t, *J* = 7.1 Hz, 3 H); HRMS *m*/*e* for $C_{22}H_{28}N_3O_4$ (M + H)$^+$ calcd 398.2080, found 398.2086. An analytically pure sample was obtained by repeated chromatography: Anal. Calcd for $C_{22}H_{27}N_3O_4$: C, 66.48; H, 6.85; N, 10.57. Found: C, 66.38; H, 6.71; N, 10.64.

Leucylglycine Methyl Ester Isocyanate (2d) and Urea 3d (PhN(Et)CONH-(*S*)-CH(*i*-Bu)CONHC$H_2CO_2CH_3$). Reaction of leucylglycine methyl ester hydrochloride (**1d**, 119 mg, 0.499 mmol) with phosgene (520 μL of a 1.93 M solution in toluene, 1.00 mmol) yielded 105 mg (92%) of crude isocyanate **2d** as a colorless oil. ^{1}H NMR analysis of this product revealed 25% of hydantoin **4d** as an impurity. **2d**: IR ($CHCl_3$) 3427, 2268, 1749, 1687 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.81 (br s, 1 H), 4.17 (dd, *J* = 8.3, 5.7 Hz, 1 H), 4.07 (d, *J* = 5.2 Hz, 2 H), 3.78 (s, 3 H), 1.87−1.80 (m, 1 H), 1.77−1.72 (m, 2 H), 0.99 (d, *J* = 6.6 Hz, 3 H), 0.97 (d, *J* = 6.5 Hz, 3 H). **4d** (identified by examination of the spectra of the crude product): IR ($CHCl_3$) 1782, 1726 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$, partial data) δ 6.32 (br s, 1 H), 4.25 (s, 2 H), 3.76 (s, 3 H), 1.62−1.58 (m, 2 H), 1.00 (d, *J* = 6.6 Hz, 3 H).

Reaction of 105 mg of **2d** with *N*-ethylaniline (125 μL, 0.99 mmol) yielded 87 mg (50% from **1d**) of **3d** as a pale yellow oily solid: IR ($CHCl_3$) 3429, 1745, 1674, 1651 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (t, *J* = 7.4 Hz, 2 H), 7.36 (d, *J* = 7.3 Hz, 1 H), 7.32−7.23 (m, 3 H), 4.49−4.38 (m, 2 H), 4.06 (dd, ABX pattern, J_{AB} = 18.2 Hz, J_{AX} = 5.5 Hz, 1 H), 3.95 (dd, ABX pattern, J_{AB} = 18.1 Hz, J_{BX} = 5.4 Hz, 1 H), 3.74 (s, 3 H), 3.77−3.67 (m, 2 H), 1.64−1.50 (m, 2 H), 1.38−1.22 (m, 1 H), 1.10 (t, *J* = 7.1 Hz, 3 H), 0.90 (d, *J* = 4.7 Hz, 3 H), 0.88 (d, *J* = 5.2 Hz, 3 H); HRMS *m*/*e* for $C_{18}H_{28}N_3O_4$ (M + H)$^+$ calcd 350.2080, found

350.2074. Anal. Calcd for $C_{18}H_{27}N_3O_4$: C, 61.87; H, 7.79; N, 12.03. Found: C, 61.47; H, 7.63; N, 11.79.

Phenylalanylisoleucylleucine Methyl Ester Isocyanate (2e) and Urea 3e (PhN(Et)CONH-(*S*)-CH(CH_2Ph)CONH-(*S*)-CH((*S*)-*s*-Bu)CONH(*S*)-CH(*i*-Bu)CO_2CH_3). Reaction of phenylalanylisoleucylleucine methyl ester hydrochloride (**1e**, 217 mg, 0.491 mmol) with phosgene (520 μL of a 1.93 M solution in toluene, 1.00 mmol) in 10 mL of CH_2Cl_2 and 10 mL of saturated aqueous $NaHCO_3$[15] yielded 191 mg (90%) of crude isocyanate **2e** as a white solid: IR ($CHCl_3$) 3413, 2266, 2240 (sh), 1741, 1672 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.34−7.27 (m, 3 H), 7.23 (d, J = 7.1 Hz, 2 H), 7.18 (d, J = 8.7 Hz, 1 H), 6.80 (d, J = 7.5 Hz, 1 H), 4.56 (appar q, J = 8.2 Hz, 1 H), 4.40 (t, J = 8.2 Hz, 1 H), 4.35 (dd, J = 8.7, 3.7 Hz, 1 H), 3.73 (s, 3 H), 3.30 (dd, ABX pattern, J_{AB} = 13.8 Hz, J_{AX} = 3.6 Hz, 1 H), 2.96 (dd, ABX pattern, J_{AB} = 13.8 Hz, J_{BX} = 8.7 Hz, 1 H), 1.87−1.77 (m, 1 H), 1.71−1.60 (m, 2 H), 1.62−1.54 (m, 1 H), 1.52−1.42 (m, 1 H), 1.13−1.02 (m, 1 H), 0.93−0.88 (m, 12 H).

Reaction of 191 mg of **2e** with *N*-ethylaniline (125 μL, 0.99 mmol) yielded 221 mg (81% from **1e**) of **3e** as white crystals from ethyl acetate−hexanes: mp 118−120 °C; IR ($CHCl_3$) 3423, 3307, 1741, 1664 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38−7.32 (m, 3 H), 7.22−7.17 (m, 3 H), 6.97−6.93 (m, 4 H), 6.83 (d, J = 8.1 Hz, 1 H), 6.67 (d, J = 8.1 Hz, 1 H), 4.60−4.56 (m, 1 H), 4.46 (dt, J = 8.1, 5.9 Hz, 1 H), 4.38 (d, J = 6.1 Hz, 1 H), 4.29 (dd, J = 8.6, 5.4 Hz, 1 H), 3.74−3.67 (m, 1 H), 3.72 (s, 3 H), 3.64−3.57 (m, 1 H), 3.06 (dd, ABX pattern, J_{AB} = 14.1 Hz, J_{AX} = 5.5 Hz, 1 H), 2.88 (dd, ABX pattern, J_{AB} = 14.1 Hz, J_{BX} = 8.4 Hz, 1 H), 2.07−1.97 (m, 1 H), 1.68−1.60 (m, 3 H), 1.45−1.36 (m, 1 H), 1.09−1.01 (m, 1 H), 1.04 (t, J = 7.1 Hz, 3 H), 0.96 (d, J = 6.1 Hz, 3 H), 0.93 (d, J = 6.1 Hz, 3 H), 0.90 (t, J = 7.4 Hz, 3 H), 0.86 (d, J = 6.8 Hz, 3 H); HRMS m/e for $C_{31}H_{45}N_4O_5$ (M + H)$^+$ calcd 553.3390, found 553.3399. Anal. Calcd for $C_{31}H_{44}N_4O_5$: C, 67.37; H, 8.02; N, 10.14. Found: C, 67.08; H, 7.95; N, 9.95.

Valyl-*S*-methylcystylalanine Methyl Ester Isocyanate (2f) and Urea 3f (PhN(Et)CONH-(*S*)-CH(*i*-Pr)CONH-(*S*)-CH(CH_2SCH_3)CONH-(*S*)-CH(CH_3)CO_2CH_3). Reaction of valyl-*S*-methylcystylalanine methyl ester hydrochloride (**1f**, 178 mg, 0.500 mmol) with phosgene (520 μL of a 1.93 M solution in toluene, 1.00 mmol) yielded 159 mg (92%) of crude isocyanate **2f** as a pale yellow solid. ^{1}H NMR analysis of this product revealed 19% of hydantoin **4f** as an impurity. **2f**: IR ($CHCl_3$) 3405, 3320, 2262, 1741, 1670 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (d, J = 8.6 Hz, 1 H), 7.24 (d, J = 7.4 Hz, 1 H), 4.63 (q, J = 7.0 Hz, 1 H), 4.53 (quintet, J = 7.2 Hz, 1 H), 4.06 (d, J = 3.4 Hz, 1 H), 3.76 (s, 3 H), 2.95−2.80 (m, 2 H), 2.40−2.25 (m, 1 H), 2.19 (s, 3 H), 1.43 (d, J = 7.2 Hz, 3 H), 1.07 (d, J = 6.8 Hz, 3 H), 0.91 (d, J = 6.7 Hz, 3 H). **4f** (identified by examination of the spectra of the crude product): IR ($CHCl_3$) 1770, 1718 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$, partial data) δ 6.97 (s, 1 H), 4.76 (dd, J = 10.5, 5.6 Hz, 1 H), 3.74 (s, 3 H), 2.11 (s, 3 H), 1.40 (d, J = 7.0 Hz, 3 H), 1.09 (d, J = 6.8 Hz, 3 H), 0.99 (d, J = 6.8 Hz, 3 H).

Reaction of 159 mg of **2f** with *N*-ethylaniline (125 μL, 0.99 mmol) yielded 161 mg (69% from **1f**) of **3f** as a colorless glassy solid: IR ($CHCl_3$) 3415, 3305, 1741, 1659 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52−7.46 (m, 3 H), 7.40 (t, J = 7.4 Hz, 1 H), 7.30 (d, J = 7.4 Hz, 2 H), 7.03 (d, J = 7.9 Hz, 1 H), 4.60 (dt, J = 7.7, 5.0 Hz, 1 H), 4.55 (quintet, J = 7.3 Hz, 1 H), 4.47 (d, J = 6.1 Hz, 1 H), 4.06 (appar t, J = 5.8 Hz, 1 H), 3.83−3.76 (m, 1 H), 3.74 (s, 3 H), 3.72−3.64 (m, 1 H), 3.06 (dd, ABX pattern, J_{AB} = 14.0 Hz, J_{AX} = 5.4 Hz, 1 H), 2.85 (dd, ABX pattern, J_{AB} = 14.0 Hz, J_{BX} =6.2 Hz, 1 H), 2.20−2.13 (m, 1 H), 2.17 (s, 3 H), 1.46 (d, J = 7.2 Hz, 3 H), 1.12 (t, J = 7.1 Hz, 3 H), 0.86 (d, J = 6.8 Hz, 3 H), 0.67 (d, J = 6.7 Hz, 3 H); HRMS m/e for $C_{22}H_{35}N_4O_5S$ (M + H)$^+$ calcd 467.2328, found 467.2330. An analytically pure sample was obtained by repeated chromatography: Anal. Calcd for $C_{22}H_{34}N_4O_5S$: C, 56.63; H, 7.34; N, 12.01. Found: C, 56.85; H, 7.30; N, 11.87.

Phenylalanine Methylamide Isocyanate (2g) and Urea 3g (PhN(Et)CONH-(*S*)-CH(CH_2Ph)CONHCH$_3$). Reaction of phenylalanine methylamide hydrochloride (**1g**, 107 mg, 0.498 mmol) with phosgene (275 μL of a 1.93 M solution in toluene, 0.531 mmol) yielded 96 mg (94%) of crude isocyanate **2g** as a colorless waxy solid. ^{1}H NMR analysis of this product revealed 6 mol % of *N*-acylation product **5g** and a number of other impurities comprising ca. 25%. **2g**: IR ($CHCl_3$) 3444, 2268, 1677 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36−7.22 (m, 5 H), 6.29 (br s, 1 H), 4.33 (dd, J = 8.6, 4.4 Hz, 1 H), 3.37 (dd, ABX pattern, J_{AB} = 13.8 Hz, J_{AX} = 4.1 Hz, 1 H), 2.97 (dd, ABX pattern, J_{AB} = 13.7 Hz, J_{BX} = 9.0 Hz, 1 H), 2.83 (d, J = 4.6 Hz, 3 H). **5g** (identified by examination of the spectra of the crude product): IR ($CHCl_3$) 2241, 1751, 1718 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40−7.16 (m, 5 H), 5.11 (dd, J = 8.7, 3.5 Hz, 1 H), 3.47 (s, 3 H), 3.21 (dd, ABX pattern, J_{AB} = 13.6 Hz, J_{AX} = 3.4 Hz, 1 H), 2.82 (dd, ABX pattern, J_{AB} = 13.6 Hz, J_{BX} = 8.7 Hz, 1 H).[16]

Reaction of 96 mg of **2g** with *N*-ethylaniline (125 μL, 0.99 mmol) yielded 79 mg (49% from **1g**) of **3g** as a colorless oil after purification by preparative reversed-phase HPLC (C_{18} column, 80:20 methanol−water): IR ($CHCl_3$) 3444, 1668 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.38−7.31 (m, 3 H), 7.25−7.20 (m, 3 H), 7.04−6.90 (m, 4 H), 6.63 (appar q, J = 4.8 Hz, 1 H), 4.58−4.50 (m, 2 H), 3.73−3.65 (m, 1 H), 3.65−3.57 (m, 1 H), 3.00 (dd, ABX pattern, J_{AB} = 13.9 Hz, J_{AX} = 5.9 Hz, 1 H), 2.88 (dd, ABX pattern, J_{AB} = 13.9 Hz, J_{BX} = 7.5 Hz, 1 H), 2.71 (d, J = 4.8 Hz, 3 H), 1.03 (t, J = 7.1 Hz, 3 H); HRMS m/e for $C_{19}H_{24}N_3O_2$ (M + H)$^+$ calcd 326.1868, found 326.1874. Anal. Calcd for $C_{19}H_{23}N_3O_2$: C, 70.13; H, 7.12; N, 12.91. Found: C, 70.26; H, 7.07; N, 12.75.

Leucine Butylamide Isocyanate (2h) and Urea 3h (PhN(Et)CONH-(*S*)-CH(*i*-Bu)CONHBu). Reaction of leucine butylamide hydrochloride (**1h**, 110 mg, 0.494 mmol) with phosgene (520 μL of a 1.93 M solution in toluene, 1.00 mmol) yielded 106 mg (101%) of crude isocyanate **2h** as a colorless oil. ^{1}H NMR analysis of this product revealed 16 mol % of *N*-acylation product **5h** as an impurity. **2h**: IR ($CHCl_3$) 3435, 2266, 1676 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 6.28 (br s, 1 H), 4.09 (dd, J = 10.1, 3.9 Hz, 1 H), 3.28 (q, J = 6.8 Hz, 2 H), 1.87−1.65 (m, 3 H), 1.55−1.48 (m, 2 H), 1.40−1.32 (m, 2 H), 0.98 (d, J = 8.9 Hz, 3 H), 0.96 (d, J = 8.9 Hz, 3 H), 0.94 (t, J = 7.3 Hz, 3 H). **5h** (identified by examination of the spectra of the crude product): ^{1}H NMR (500 MHz, $CDCl_3$, partial data) δ 4.88 (dd, J = 9.8, 3.6 Hz, 1 H), 3.97 (ddd, J = 15.8, 9.9, 5.7 Hz, 1 H), 3.92 (ddd, J = 15.7, 10.1, 5.8 Hz, 1 H).

Reaction of 104 mg of **2h** with *N*-ethylaniline (125 μL, 0.99 mmol) yielded 116 mg (71% from **1h**) of **3h** as a colorless oil: IR ($CHCl_3$) 3435, 1666, 1645 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (t, J = 7.6 Hz, 2 H), 7.34 (t, J = 7.4 Hz, 1 H), 7.22 (d, J = 7.7 Hz, 2 H), 6.72 (br s, 1 H), 4.47 (d, J = 8.1 Hz, 1 H), 4.30 (td, J = 8.3, 5.8 Hz, 1 H), 3.77−3.65 (m, 2 H), 3.28−3.15 (m, 2 H), 1.56−1.44 (m, 4 H), 1.38−1.28 (m, 3 H), 1.10 (t, J = 7.1 Hz, 3 H), 0.92 (t, J = 7.3 Hz, 3 H), 0.89 (d, J = 6.5 Hz, 3 H), 0.87 (d, J = 6.6 Hz, 3 H); HRMS m/e for $C_{19}H_{32}N_3O_2$ (M + H)$^+$, calcd 334.2494, found 334.2495. Anal. Calcd for $C_{19}H_{31}N_3O_2$: C, 68.43; H, 9.37; N, 12.60. Found: C, 68.22; H, 9.37; N, 12.46.

Phenylalanine Methyl Ester Isocyanate (2i)[3] and Urea 3i[9l] (PhN(Et)CONH-(*S*)-CH(CH_2Ph)CO_2CH_3). Reaction of phenylalanine methyl ester hydrochloride (**1i**, 111 mg, 0.515 mmol) with phosgene (520 μL of a 1.93 M solution in toluene, 1.00 mmol) yielded 103 mg (98%) of crude isocyanate **2i** as a colorless oil.[3]

Reaction of 103 mg of **2i** with *N*-ethylaniline (125 μL, 0.99 mmol) yielded 156 mg (93% from **1i**) of **3i** as a colorless oil.[9l]

Phenylalanylleucine Methyl Ester Isothiocyanate (2j) and Thiourea 3j (PhN(Et)CSNH(*S*)-CH(CH_2Ph)CONH(*S*)-CH(*i*-Bu)CO_2CH_3). Reaction of phenylalanylleucine methyl ester hydrochloride (**1a**, 165 mg, 0.502 mmol) with thiophosgene (76 μL, 1.00 mmol) yielded 160 mg (95%) of crude isothiocyanate **2j** as a pale tan oil: IR ($CHCl_3$) 3417, 2056, 1741, 1684 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37−7.22 (m, 5 H), 6.58 (d, J = 8.3 Hz, 1 H), 4.64 (dd, J = 7.2, 4.2 Hz, 1 H), 4.58 (td, J = 8.6, 5.5 Hz, 1 H), 3.72 (s, 3 H), 3.35 (dd, ABX pattern, J_{AB} = 13.8, J_{AX} = 4.2 Hz, 1 H), 3.19 (dd, ABX pattern, J_{AB} = 13.9, J_{BX} = 7.4

(15) A larger volume of methylene chloride was required to dissolve this peptide.

(16) *N*-acylation product **5g** was further characterized by trapping with *N*-ethylaniline to form PhN(Et)CONHCH(CH_2Ph)CON(Me)CON(Et)Ph, which was isolated by column chromatography: IR ($CHCl_3$) 3435, 1716, 1674, 1657 (sh) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48−7.19 (m, 11 H), 7.09 (d, J = 7.0 Hz, 2 H), 6.97 (d, J = 7.4 Hz, 2 H), 5.12 (br s, 1 H), 4.57 (d, J = 8.5 Hz, 1 H), 4.05−3.85 (m, 1 H), 3.78−3.57 (m, 3 H), 3.40−3.25 (m, 1 H), 2.63 (br s, 3 H), 2.62−2.50 (m, 1 H), 1.28 (t, J = 7.1 Hz, 3 H), 1.03 (t, J = 7.1 Hz, 3 H); HRMS m/e for $C_{28}H_{33}N_4O_3$ (M + H)$^+$, calcd 473.2552, found 473.2556. Anal Calcd for $C_{28}H_{32}N_4O_3$: C, 71.16; H, 6.83; N, 11.86. Found: C, 70.80; H, 6.67; N, 11.69.

Hz, 1 H), 1.65−1.35 (m, 3 H), 0.90 (d, J = 6.3 Hz, 3 H), 0.88 (d, J = 6.4 Hz, 3 H).

Reaction of 160 mg of **2j** with *N*-ethylaniline (125 μL, 0.99 mmol) yielded 215 mg (94% from **1a**) of **3j** as a pale yellow oil: IR ($CHCl_3$) 3415, 3381, 1741, 1678 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 7.43−7.36 (m, 3 H), 7.25−7.19 (m, 3 H), 7.09 (dd, J = 7.5, 1.8 Hz, 2 H), 6.94 (br d, J = 5.2 Hz, 2 H), 6.54 (d, J = 7.8 Hz, 1 H), 5.58 (d, J = 7.5 Hz, 1 H), 5.31 (q, J = 7.2 Hz, 1 H), 4.47 (td, J = 8.3, 5.1 Hz, 1 H), 4.25−4.10 (m, 2 H), 3.70 (s, 3 H), 3.07 (dd, ABX pattern, J_{AB} = 14.1 Hz, J_{AX} = 6.9 Hz, 1 H), 3.00 (dd, ABX pattern, J_{AB} = 14.1 Hz, J_{BX} = 7.1 Hz, 1 H), 1.60−1.45 (m, 3 H), 1.15 (t, J = 7.1 Hz, 3 H), 0.89 (d, J = 6.3 Hz, 3 H), 0.87 (d, J = 6.2 Hz, 3 H); HRMS m/e for $C_{25}H_{32}N_3O_3S$ $(M - H)^+$ calcd 454.2164, found 454.2175. Anal. Calcd for $C_{25}H_{33}N_3O_3S$: C, 65.91; H, 7.30; N, 9.22; S, 7.04. Found: C, 65.57; H, 7.24; N, 9.02; S, 6.68.

Valylalanine Methyl Ester Isothiocyanate (2k) and Thiourea 3k (PhN(Et)CSNH-(*S*)-CH(*i*-Pr)CONH-(*S*)-CH-(CH_3)CO_2CH_3). Reaction of valylalanine methyl ester hydrochloride (**1b**, 124 mg, 0.519 mmol) with thiophosgene (76 μL, 1.00 mmol) yielded 101 mg (80%) of crude isothiocyanate **2k** as a pale orange oil. ^1H NMR analysis of this product revealed 12% of an impurity. **2k**: IR ($CHCl_3$) 3415, 2108, 2056, 1743, 1682 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.91 (d, J = 6.7 Hz, 1 H), 4.60 (quintet, J = 7.2 Hz, 1 H), 4.29 (d, J = 3.4 Hz, 1 H), 3.78 (s, 3 H), 2.55−2.47 (m, 1 H), 1.46 (d, J = 7.2 Hz, 3 H), 1.13 (d, J = 6.8 Hz, 3 H), 0.96 (d, J = 6.7 Hz, 3 H). Impurity (identified by examination of the spectra of the crude product): ^1H NMR (500 MHz, $CDCl_3$, partial data) δ 8.35 (br s, 1 H), 4.45 (br s, 1 H), 4.25 (br s, 1 H), 3.72 (s, 3 H), 2.38 (br s, 1 H), 1.09 (br d, J = 4.4 Hz, 3 H).

Reaction of 101 mg of **2k** with *N*-ethylaniline (125 μL, 0.99 mmol) yielded 118 mg (62% from **1b**) of **3k** as a white foamy solid: mp 98−100 °C; IR ($CHCl_3$) 3426, 3388, 1741, 1678 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.52 (t, J = 7.5 Hz, 2 H), 7.43 (t, J = 7.3 Hz, 1 H), 7.22 (d, J = 7.3 Hz, 2 H), 6.62 (d, J = 7.1 Hz, 1 H), 5.70 (d, J = 8.2 Hz, 1 H), 4.91 (dd, J = 8.1, 6.2 Hz, 1 H), 4.48 (quintet, J = 7.2 Hz, 1 H), 4.24 (appar q, J = 7.1 Hz, 2 H), 3.72 (s, 3 H), 2.16−1.98 (m, 1 H), 1.41 (d, J = 7.2 Hz, 3 H), 1.20 (t, J = 7.1 Hz, 3 H), 0.87 (d, J = 6.8 Hz, 3 H), 0.79 (d, J = 6.9 Hz, 3 H); HRMS m/e for $C_{18}H_{28}N_3O_3S$ $(M + H)^+$ calcd 366.1851, found 366.1853. Anal. Calcd for $C_{18}H_{27}N_3O_3S$: C, 59.15; H, 7.45; N, 11.50; S, 8.77. Found: C, 59.41; H, 7.29; N, 11.25; S, 8.92.

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