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Efficient Amidation from Carboxylic Acids and Azides via Selenocarboxylates: Application to the Coupling of Amino Acids and Peptides with Azides

Xinghua Wu[†] and Longqin Hu*[,†,‡]

Department of Pharmaceutical Chemistry, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, New Jersey 08854, and The Cancer Institute of New Jersey, New Brunswick, New Jersey 08901

longhu@rutgers.edu

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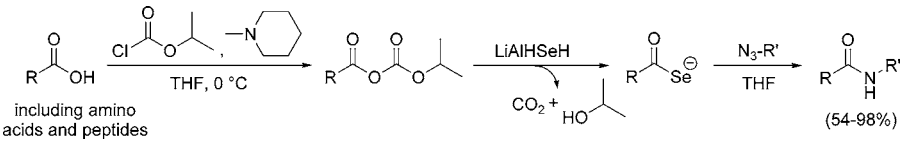

A facile one-pot procedure for the coupling of carboxylic acid and azide via selenocarboxylate and selenatriazoline has been developed and successfully applied to the coupling of amino acids and peptides with azides. Selenocarboxylates are readily prepared by the reaction of the activated carboxylic acids with LiAlHSeH under mild conditions. The selenocarboxylates formed in situ are used to react directly with azides to form the corresponding amides via a selenatriazoline intermediate. Excellent yields were obtained for electron-deficient azides, and moderate to good yields were obtained for electron-rich azides. The selenocarboxylate/azide amidation reaction is clean and chemoselective. It provides an attractive alternative method to the conventional acylation of amines when an amide bond needs to be formed without going through an amine intermediate.

Introduction

The carboxamide is among the most common functionalities in synthetic organic compounds and natural products. Many methods have been developed for amide bond formation with most involving the reaction between an activated carboxylic acid as an electrophile and a free amine as a nucleophile.[1] However, there are situations where free amines cannot be used because of either structural instability or the presence of functional groups that are incompatible with amines. Azides are neither basic nor nucleophilic and have been used to directly form amide bonds in several strategies including the Staudinger-type ligation involving the acylation of an iminophosphorane,[2−7] the amidation of a carboxylic acid with an azide in the presence of a trialkyl phosphine,[8−10] the Williams thio acid/azide amidation,[11,12] and the selenocarboxylate/azide amidation.[13−15] Recently, the modified Staudinger ligation,[6,7] which forms an amide bond starting from an azide and a phosphine-linked ester/thio ester in the form of R′COO−$C_{n=1,2}$−PR_2 or R′COS−$C_{n=1,2}$−PR_2, has been successfully used in N-glycosylation,[16−21] peptide

* To whom correspondence should be addressed. Phone: 732-445-5291. Fax: 732-445-6312.
[†] Rutgers, The State University of New Jersey.
[‡] The Cancer Institute of New Jersey.

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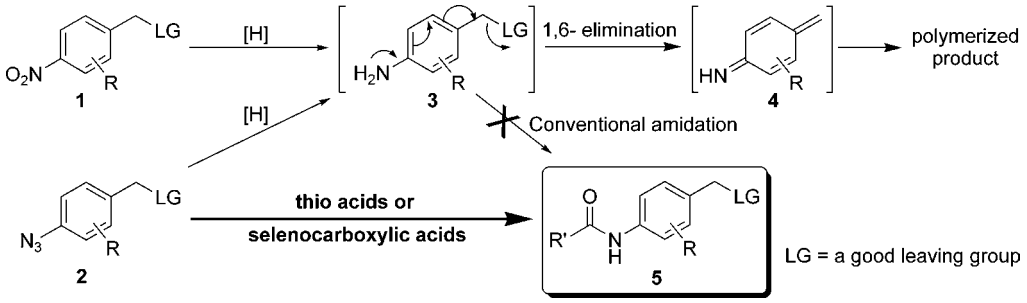
SCHEME 1. Direct Amidation from Azides Avoiding 1,6-Elimination from 4-Aminobenzyl Derivatives



ligation,[22−25] and lactamization.[26] The reaction involves a five- or six-membered intramolecular transacylation of an iminophosphorane, the intermediate of the Staudinger reaction,[27] followed by hydrolysis to form an amide. The Staudinger ligation favors aliphatic azides, whereas for aromatic azides, especially those with electron-withdrawing substituents, the reactions are very slow and may fail.[28] When the Staudinger-type ligation is incomplete or fails, the iminophosphorane intermediate is converted to the corresponding amine by hydrolysis in an aqueous medium or during aqueous workup without the possibility of recovering the azide starting material.

The amidation reaction of thio acids with azides has recently attracted broad attention. Although acetylation of organic azides with thioacetic acid was first reported in 1980,[29] it was postulated as a conventional nucleophilic acylation reaction between thioacetic acid and the amine formed via the in situ reduction of the azide by the adventitious hydrogen sulfide, which is regenerated during the acylation reaction.[30] However, recent results from the Williams' group suggested a new mechanism that involves the formation of a thiatriazoline intermediate followed by a retro-[3+2] cycloaddition to form the amide product.[12] Two mechanistic pathways were proposed for the formation of the thiatriazoline intermediate: for electron-deficient azides, the thiatriazoline is formed via a stepwise linear coupling of the thio acid with the azide followed by intramolecular cyclization, and for electron-rich azides, the thiatriazoline is formed via a concerted intermolecular [3+2] cycloaddition reaction between the thio acid and the azide. Several applications and modifications of this Williams thio acid/azide amidation have been reported.[31−35]

In our research, reduction of compounds **1** and **2** in the form of 4-nitro or 4-azidobenzyl-LG (LG = a good leaving group) failed to give the desired amine intermediate **3** that is needed to couple to an amino acid or peptide via the conventional amidation procedures. Instead, the amine intermediate **3** underwent a spontaneous 1,6-elimination to form quinonimine methide **4** followed by polymerization as shown in Scheme 1.[36] Thus, the spontaneous 1,6-elimination precludes the use of conventional amidation procedures that require the amine intermediate **3**. In this case, the Williams thio acid/azide amidation would provide an attractive alternative. However, the thio acid/azide amidation gives low yields for electron-rich and sterically hindered azides and often requires high reactant concentration and temperature to achieve a satisfactory conversion rate.[12] This prompted us to develop a new amidation method that can be carried out under mild conditions and is applicable to amino acids and peptides.

Selenium shares some of the same chemical properties as sulfur, but the larger and more easily polarizable selenium atom makes it more nucleophilic than sulfur. For example, selenophenolate was found to react with methyl iodide ~7 times faster than thiophenolate in a S_N2 displacement reaction.[37] We thought that selenocarboxylic acids ought to be more reactive than thio acids and potentially facilitate the amide formation with azides. Indeed, a sterically hindered 2-azidopiperidine derivative reacted with selenoacetic acid to form the corresponding acetamide in 75% yield after 12 h in refluxing chloroform in the presence of 2,6-lutidine, whereas it was entirely unreactive toward thioacetic acid under the same reaction conditions.[13] Although selenocarboxylic acids are known to be unstable and readily oxidized to diacyl diselenides upon exposure to air, their corresponding alkali metal and trialkylammonium salts are relatively stable, especially for aromatic selenocarboxylates. We recently published two communications on the amidation reaction of various aromatic azides with selenocarboxylates generated from diacyl selenides or diacyl diselenides.[14,15] The reactions were found to be highly chemoselective and compatible with a variety of

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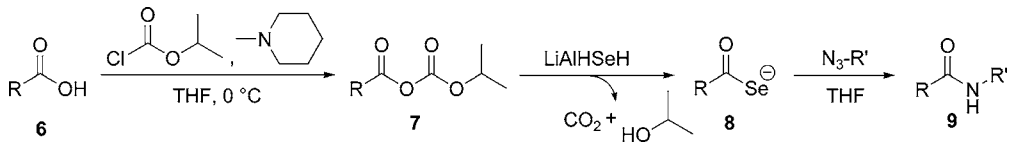
SCHEME 2. Three-Step One-Pot Synthesis of Amides from Azides and Carboxylic Acids



functional groups, including hydroxyl, carbonyl, carboxyl, and cyano. The reactions work very efficiently with electron-deficient azides. For the less reactive electron-rich azides, mild heating was required to produce satisfactory reaction rates.[15] The amidation reactions could also be carried out under aqueous conditions without significant loss of yields. However, what was limiting the application of selenocarboxylate/azide amidation was the lack of a practical method to prepare selenocarboxylates. The known methods of preparing selenocarboxylates include the treatment of trimethylsilyl selenocarboxylates with alkali metal fluorides;[38,39] the reaction of acyl chlorides with alkali metal selenides;[40] the reaction of diacyl selenides or diacyl diselenides with alkali metal hydroxide,[41] alkali metal methoxide,[42] or piperidine;[43]and the reaction of carboxylic acids with Woollin's reagent, $[PhP(=Se)Se]_2$.[44] However, all of the above methods for the preparation of selenocarboxylates have disadvantages: trimethylsilyl selenocarboxylic esters are highly moisture sensitive; alkali metal selenides are poorly soluble in organic solvents; and starting from diacyl selenides or diacyl diselenides is not economical as half of the carboxylate equivalents are wasted. Although Woollin's reagent can directly convert carboxylic acids to the corresponding selenocarboxylic acids in refluxing toluene, such high reaction temperature would cause racemization of chiral centers in amino acids and peptides. Herein, we report a practical method for the preparation of various selenocarboxylates under much milder conditions and a general one-pot amidation procedure that is applicable to amino acids and peptides starting from carboxylic acids and azides via selenocarboxylate and selenatriazoline intermediates.

Results and Discussion

Preparation of Selenocarboxylates and a General One-Pot Amidation Procedure. We found that Ishihara selenating reagent, LiAlHSeH, smoothly converted mixed anhydrides to the corresponding selenocarboxylates under very mild conditions as shown in Scheme 2. The preparation of LiAlHSeH entails treating $LiAlH_4$ with a stoichiometric amount of selenium in THF at 0 °C to give a gray LiAlHSeH suspension.[45] LiAlHSeH reacted readily with the freshly prepared mixed anhydride at 0 °C to give the corresponding selenocarboxylate which can be used directly for the amidation reaction. Thus, we developed the following general one-pot procedure for the selenocarboxylate/azide amidation reaction. First, the carboxylic acid is activated with isopropyl chloroformate in THF at 0 °C in the presence of *N*-methylpiperidine to form the corresponding mixed anhydride. Then, the mixed anhydride solution is added to the freshly prepared suspension of LiAlHSeH in THF via cannula under a nitrogen atmosphere. The reaction starts immediately with the release of CO_2 to form the corresponding selenocarboxylate in situ. A solution of the azide in organic solvent is then added to the above selenocarboxylate solution via a syringe. The reaction is monitored using TLC and/or LC-MS and worked up after the disappearance of the starting azide, or no further change of the reaction mixture is observed. Isopropyl chloroformate was found to be a better choice over other common chloroformates (e.g., methyl chloroformate, ethyl chloroformate, and isobutyl chloroformate) as the resulting mixed anhydride, acyl isopropyl carbonate, is more stable and relatively more resistant to hydrolysis.

Application of the One-Pot Selenocarboxylate/Azide Amidation to Amino Acids and Short Peptides. As the electron-deficient aromatic azides react more readily with selenocarboxylates,[14,15] we selected 4-cyanophenyl azide, a representative electron-deficient aromatic azide, to demonstrate the general applicability of our one-pot amidation procedure to various protected amino acids and short peptides. As shown in Table 1, various amino acids all gave excellent yields (~90%) of the desired amide products (entries 1−8) with the exception of Fmoc-glutamine which gave only 70% isolated yield because of partial dehydration (entry 9). For dipeptides, Boc-Leu-Trp-OH and Boc-Ser(Ac)-Phe-OH, and the tripeptide, Boc-Asn-Leu-Trp-OH, the desired amidation products were obtained in 83−92% yields without significant racemization (entries 10−12, Table 1). Furthermore, common amino protecting groups such as Boc, Fmoc, Z, acetyl, and OBzl are well tolerated under the current conditions. The high yields of the desired amide products demonstrate that the carboxylic acids were effectively converted to the corresponding selenocarboxylates in our one-pot amidation procedure.

THF is the solvent of choice for the preparation of LiAlHSeH. The reaction of $LiAlH_4$ with selenium did not occur in CH_2Cl_2. For the amidation step (step 3), both protic and aprotic solvents could be used as cosolvents. Overall, we found that the selenocarboxylate/azide amidation proceeded faster in polar solvents. As shown in Table 2, in comparison with the reaction in neat THF (entry 1), mixing with polar organic solvents including acetone, methanol, and acetonitrile slightly accelerated the reaction but there were no significant effects on the overall yields (entries 2−4). Increasing the ratio of acetonitrile from 25% to 50% shortened the reaction time slightly from 1.5 to 1.0 h (entries 4 vs 5, Table 2). However, further increasing the concentration of acetonitrile to 75% caused slight reaction retardation (entries 5 vs 6, Table 2). This could be because of the decreased solubility of the highly lipophilic azide in the presence of too much polar solvent leading to heterogeneity of the reaction mixture. It should also be noted that the presence of water did not adversely affect the amidation reaction as we reported previously,[15] and a good overall yield of 89% was

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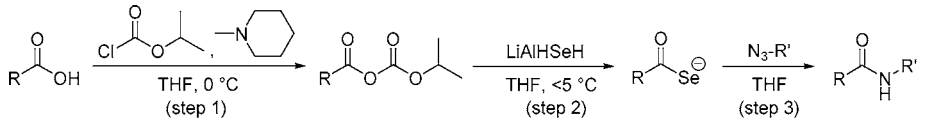
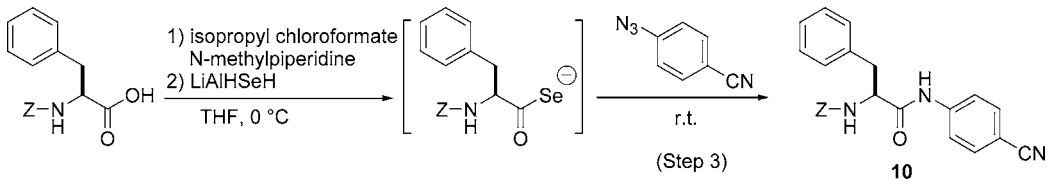
TABLE 1. Three-Step Amidation of 4-Cyanophenyl Azide with Amino Acids and Peptides[a]



Entry	Amino acid or peptide	Amide product	Yield (%)[b]
1	Z-Phe-OH	Z-Phe-NH-C6H4-CN **10**	85
2	Z-Ser(Bzl)-OH	Z-Ser(Bzl)-NH-C6H4-CN **11**	89
3	Z-Gln-OH	Z-Gln-NH-C6H4-CN **12**	90
4	Z-Met-OH	Z-Met-NH-C6H4-CN **13**	88
5	Boc-Glu(OBzl)-OH	Boc-Glu(OBzl)-NH-C6H4-CN **14**	89
6	Boc-Pro-OH	Boc-Pro-NH-C6H4-CN **15**	90
7	Boc-Val-OH	Boc-Val-NH-C6H4-CN **16**	89
8	Fmoc-Gln-OH	Fmoc-Gln-NH-C6H4-CN **17**	70
9	Fmoc-Trp-OH	Fmoc-Trp-NH-C6H4-CN **18**	90
10	Boc-Leu-Trp-OH	Boc-Leu-Trp-NH-C6H4-CN **19**	87
11	Boc-Ser(Ac)-Phe-OH	Boc-Ser(Ac)-Phe-NH-C6H4-CN **20**	92
12	Boc-Asn-Leu-Trp-OH	Boc-Asn-Leu-Trp-NH-C6H4-CN **21**	83

[a] General conditions: amino acid or peptide (1.1 mmol), isobutyl chloroformate (1.1 mmol), and *N*-methylpiperidine (1.1 mmol), THF, 0 °C, 20 min; LiAlHSeH (1.1 mmol), 5 °C, 30 min; 4-cyanophenyl azide (1.0 mmol), THF, room temperature, 3 h. [b] Isolated yields based on azides as the limiting reagents.

TABLE 2. Effect of Solvents on Reaction Time and Yield of the Amidation Reaction[a]



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Entry	Solvent system used in step 3	Reaction time (h)	Yield of **10** (%)[b]
1	THF	3	85
2	THF/CH_3COCH_3 (3:1, v/v)	1.5	87
3	THF/MeOH (3:1, v/v)	1.0	88
4	THF/CH_3CN (3:1, v/v)	1.5	89
5	THF/CH_3CN (1:1, v/v)	1.0	92
6	THF/CH_3CN (1:3, v/v)	2.0	91
7	THF/H_2O (3:1, v/v)	1.5	89

[a] General conditions: *Z*-Phe-OH (1.1 mmol), isobutyl chloroformate (1.1 mmol), and *N*-methylpiperidine (1.1 mmol), THF, 0 °C, 20 min; LiAlHSeH (1.1 mmol); 4-cyanophenyl azide (1.0 mmol), room temperature. [b] Isolated yields based on azides as the limiting reagents.

obtained when a mixed solvent of THF and water (3:1, v/v) was used as the solvent in the third amidation step (entry 7, Table 2).

Determination of Amino Acid Racemization during the Three-Step Amidation Process. To determine whether our conditions caused racemization of the amino acids during the one-pot procedure, we hydrolyzed compound **10** with 6 N HCl followed by derivatization with *o*-phthalaldehyde (OPA) and N^{α}-*tert*-butyloxycarbonyl-L-cysteine (NBC). The resulting isoindole derivatives were analyzed by reversed-phase HPLC and compared with L-phenylalanine and D-phenylalanine isoindole derivatives. NBC was found to give better separation of the isoindole derivatives of D- and L-phenylalanines over the other often used reagent N^{α}-acetyl-L-cysteine (NAC)[46] presumably due

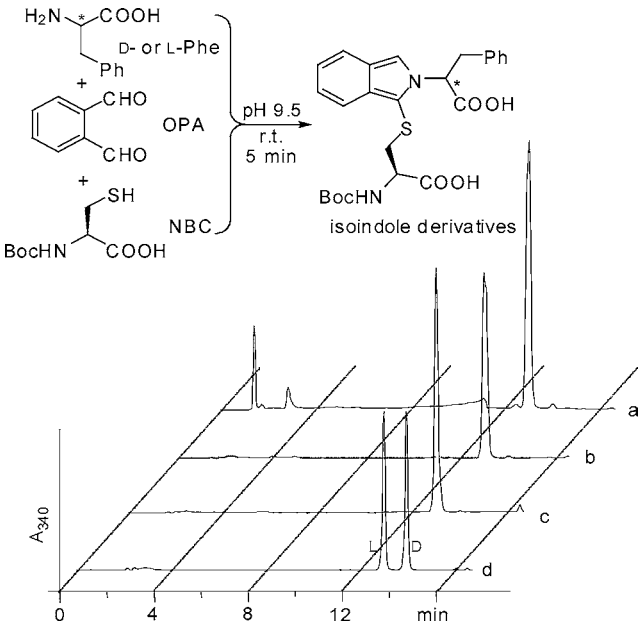


FIGURE 1. OPA/NBC derivatization and subsequent HPLC analysis of D- and L-phenylalaninamides after acid hydrolysis. The chromatograms shown are (a) N^{α}-*Z*-*N*′-(4-cyanophenyl)-L-phenylalaninamide **10**, (b) N^{α}-*Z*-L-phenylalanine control, (c) N^{α}-*Z*-*N*′-benzyl-L-phenylalaninamide control, and (d) DL-phenylalanine standards.

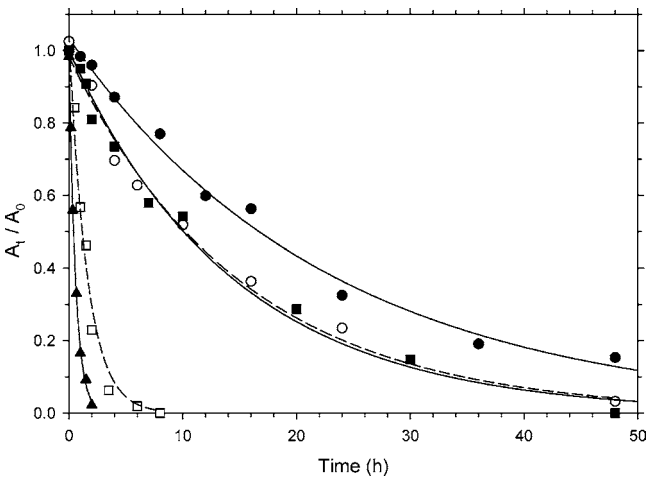


FIGURE 2. Stabilities of benzeneselenocarboxylate in THF at room temperature (−●−) and 55 °C (--○--), in acetonitrile at room temperature (−■−) and 55 °C (--□--), and in DMSO/EtOAc (1:1 v/v) at room temperature (−▲−) as monitored by conversion to *N*-(*p*-toluenesulfonyl)benzamide in an HPLC assay.

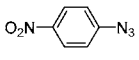
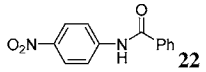
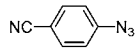
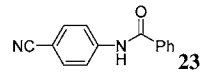
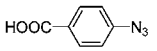
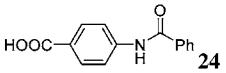
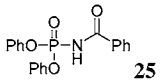
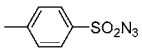
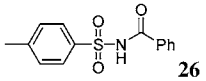
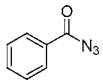
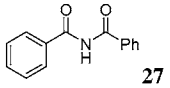
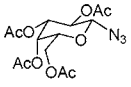
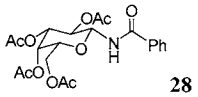
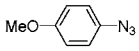
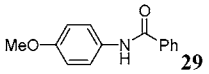
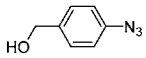
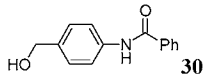
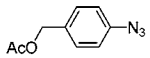
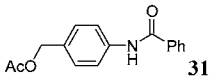
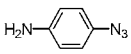
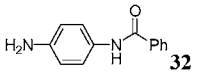
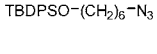
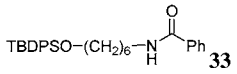
to the increased bulkiness of the *t*-Boc group as compared to acetyl. As shown in Figure 1, the resulting isoindole derivatives of D- and L-phenylalanine standards were baseline separated with retention times of 13.55 and 12.59 min, respectively, in our reversed-phase HPLC system (chromatogram d, Figure 1). Using this analytical method, we found that amide **10** prepared using our one-pot, three-step amidation process contains a small amount (1.7%) of the D-isomer (chromatogram a, Figure 1), and the control N^{α}-*Z*-*N*′-benzyl-L-phenylalaninamide sample prepared using the conventional direct coupling of the mixed anhydride with benzyl amine contains about 1.5% of the D-isomer (chromatogram c, Figure 1). These results indicate that any racemization during our one-pot, three-step amidation process is the result of the first step of carboxylate activation, i.e., the formation of a mixed anhydride, rather than of the last two steps of selenocarboxylate generation and amidation. This is consistent with an earlier report that conventional amidation methods using mixed anhydrides could lead to a small amount of racemization during peptide synthesis.[47]

Stability of the Selenocarboxylates. We previously demonstrated that the stability of the selenocarboxylate affected the overall yields of amidation for electron-rich azides, although it did not present a problem for the faster amidation reactions with electron-deficient azides.[14] We were able to improve the amidation yields for the less reactive electron-rich azides by increasing the half-life of the selenocarboxylate.[15] Therefore, the stability of selenocarboxylate under the current conditions was also determined. We used the same model substrate, benzeneselenocarboxylate, to compare its stability under the current conditions using THF as the solvent (conditions A) to that under previous conditions using acetonitrile (conditions B) and DMSO/EtOAc (conditions C) as the solvents. The stability of benzeneselenocarboxylate was measured by monitoring the amide formation between benzeneselenocarboxylate and 4-toluenesulfonyl azide under the present reaction conditions. The amide product, *N*-(4-toluenesulfonyl)benzamide, was then analyzed to determine the amount of benzeneselenocarboxylate that remained in solution via an HPLC assay. To ensure fast and quantitative consumption of all benzeneselenocarboxylate that remained in solution at a given time point, 2 equiv of 4-toluenesulfonyl azide was used. Complete and quantitative amidation was achieved in less than 5 min under these conditions as monitored by HPLC. Such fast product formation allowed us to accurately measure the amount of benzeneselenocarboxylate during our stability study. Figure 2 illustrates the stability profiles of benzeneselenocarboxylate in THF under our current conditions as compared to our earlier conditions in acetonitrile[15] and in 50% DMSO/EtOAc.[14] The half-life of benzeneselenocarboxylate was found to be 16 h in THF at 25 °C ($k_{obs} = 3.1 \times 10^{-5}$ s^{-1}) as compared to 11 h in acetontrile[15] ($k_{obs} = 1.8 \times 10^{-5}$ s^{-1}) and 25 min in DMSO/EtOAc[14] ($k_{obs} = 4.6 \times 10^{-4}$ s^{-1}) at 25 °C. When the temperature was raised to 55 °C, the half-life of benzeneselenocarboxylate was around 10 h in THF ($k_{obs} = 1.9 \times 10^{-5}$ s^{-1}) as compared to 1.4 h in acetonitrile ($k_{obs} = 1.4 \times 10^{-4}$ s^{-1}). The longer half-life of the selenocarboxylate under our present conditions might be due to the presence of trace amounts of reducing agents in the reaction mixture, which probably delayed the oxidation/decomposition of selenocarboxylates caused by a trace amount of oxygen present in the solvent. This, thus, benefited the amidation reactions that require longer time and mild heating.

Effects of Azides on the Amidation Reaction. After we confirmed the increased stability of benzeneselenocarboxylate under our new reaction conditions, a variety of azides were used to explore the effects of this improved stability on the amidation reaction and for comparison with previous amidation conditions. Similar to what we reported previously, electron-deficient azides were more reactive than electron-rich azides (Table 3). For electron-deficient azides, 1.1 equiv of benzeneselenocarboxylate was sufficient to give excellent conversion to the corresponding

(46) Chernobrovkin, M. G.; Anan'eva, I. A.; Shapovalova, E. N.; Shpigun, O. A. *J. Anal. Chem.* **2004**, *59*, 64−72.
(47) Benoiton, N. L.; Lee, Y. C.; Steinaur, R.; Chen, F. M. *Int. J. Pept. Protein Res.* **1992**, *40*, 559−566.

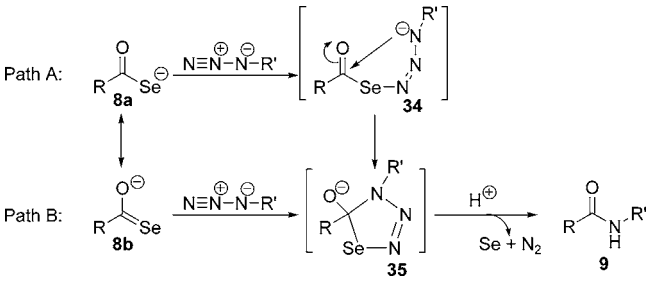
TABLE 3. Amidation of Benzeneselenocarboxylate with Azides under Different Conditions

Entry	Azide	Amide product	Conditions[a]	Selenocarboxylate (equiv)	T (°C)	Yield[b] (%)
1		 **22**	A	1.1	25	96
			B	1.2	25	95
			C	2.0	25	98
2		 **23**	A	1.1	25	94
			B	1.2	25	96
			C	2.0	25	98
3		 **24**	A	1.1	25	91
			B	1.2	25	89
			C	2.0	25	87
4		 **25**	A	1.1	25	95
			B	1.2	25	88
5		 **26**	A	1.1	25	98
			B	1.2	25	96
6		 **27**	A	2.0	55	81
			B	2.0	55	51
7		 **28**	A	2.0	55	71
8		 **29**	A	2.0	55	63
			B	2.0	55	65
			C	2.0	55	7
9		 **30**	A	2.0	55	70
			B	2.0	55	56
			C	2.0	55	44
10		 **31**	A	2.0	55	76
			B	2.0	55	68
			C	2.0	55	70
11		 **32**	A	2.0	55	54
			B	2.0	55	54
12		 **33**	A	2.0	55	62

[a] General conditions: (A) in THF with benzeneselenocarboxylate prepared by treating the mixed anhydride with LiAlHSeH; (B) in acetonitrile with benzeneselenocarboxylate prepared by treating diacyl diselenide with 1 equiv of piperidine in the presence of 1 equiv of diisopropylethylamine (ref 16); (C) in DMSO/EtOAc (1:1, v/v) with benzeneselenocarboxylate prepared by treating dibenzoyl selenide with 1 equiv of KOMe (ref 15). [b] Isolated yields based on azides as the limiting reagents.

amides with isolated yields of 81−98% (conditions A, entries 1−6, Table 3). For electron-rich azides that are less reactive, 2.0 equiv of bezeneselenocarboxylate and mild heating were used to obtain good yields (conditions A, entries 7−12, Table 3). We previously demonstrated that the yield of selenocarboxylate/azide amidation correlated well with the stability and solubility of the selenocarboxylate in the reaction medium.[14,15] For highly reactive electron-deficient azides, the reactions were complete in a very short time. Thus, the increased half-life of selenocarboxylate did not seem to offer any benefits for electron-deficient azides as similar overall yields were obtained under the present conditions (A) as compared to the previous reported conditions of B and C (entries 1−5, Table 3). For benzoyl azide, the desired amide **27** was obtained in 81% yield under the present conditions (A) as compared to the previous 51% yield using conditions B[15] (entry 6, Table 3). For 2,3,4,6-tetra-*O*-acetyl-*β*-D-galactopyranosyl azide, the desired product **28** was obtained in 71% yield (entry 7, Table 3). 6-[(*tert*-Butyldiphenylsilyl)oxy]hexyl azide, representing an aliphatic azide, also reacted with selenocarboxylate to form the desired amide **33** in 62% yield under our current conditions (A, entry 12, Table 3). For the less reactive 4-methoxyphenyl azide, the corresponding amide **29** was obtained in 63% yield under our current conditions (A) compared to the previous 65% yield under conditions B[15] and only 7% yield under conditions C[14] (entry 8, Table 3). Similarly, 4-azido-benzyl alcohol gave the desired

SCHEME 3. Proposed Mechanisms of Selenocarboxylate/Azide Amidation



product **30** with an improved yield of 70% compared to the previous 56%[15]and 44%[14] yields under conditions B and C, respectively (entry 9, Table 3). Even the more electron-rich 4-aminophenyl azide reacted with the selenocarboxylate affording 54% yield of *N*-(4-aminophenyl)benzamide **32** under both conditions A and B. These observations are consistent with our earlier findings that the more electron-rich azides would give poorer amidation yields. The successful amidation of 4-azidobenzyl acetate to 4-benzamidobenzyl acetate **31** in 68−76% yield (entry 10, Table 3) suggests that the amidation does not involve in situ reduction of the azide to the corresponding amine prior to amide bond formation. Otherwise, the reduction would have led to the formation of polymerized quinonimine methide instead of the desired amide as discussed earlier and depicted in Scheme 1. Under our current conditions, we were able to apply mild heating to accelerate the amidation process for the less reactive azides and to improve the amidation yields. Although both conditions A and B showed comparable yields for both electron-deficient and electron-rich azides, the easy adaption of conditions A to the one-pot procedure starting directly from carboxylic acids provides a straightforward, practical, and efficient route to the selenocarboxylate/azide amidation reaction that is applicable to amino acids and peptides.

Mechanisms of Selenocarboxylate/Azide Amidation. Our experimental results clearly demonstrated that the yields of selenocarboxylate/azide amidation depend primarily upon the electronic properties of the azides. Electron-deficient azides react with selenocarboxylates much faster and give better yields than electron-rich azides. This is in apparent contrast to the structure−activity relationship expected of the "in situ reduction and acylation" mechanism where electron-rich azides would give more nucleophilic amines and the more nucleophilic amines should facilitate the nucleophilic acylation and afford higher yields than electron-deficient azides. Furthermore, when benzeneselenocarboxylate was incubated with 4-cyanoaniline, there was no detectable amide formed whereas incubation of benzeneselenocarboxylate with 4-cyanophenyl azide gave 94% yield of the amide. In addition, we were able to successfully synthesize 4-benzamidobenzyl acetate **31** from 4-azidobenzyl acetate, suggesting that there was no in situ reduction of the azide otherwise the spontaneous decomposition of 4-aminobenzyl acetate would lead to the polymerized quinonimine methide instead of the amide product. On the basis of careful mechanistic studies, two pathways leading to the formation of a thiatriazoline intermediate have been identified for the Williams thio acid/azide amidation.[12] Depending on the electronic properties of the azide, a thiatriazoline intermediate forms via a stepwise or concerted mechanism. By analogy, selenocarboxylate/azide amidation likely proceeds through the corresponding selenatriazoline intermediate **35**. As shown in Scheme 3, a stepwise mechanism, path A, could be operative. Bimolecular union of the terminal nitrogen of the electron-deficient azide with selenium of the selenocarboxylate followed by an intramolecular cyclization form selenatriazoline intermediate **35**. Alternatively, a concerted [3+2] cycloaddition, path B, would give the selenatriazoline intermediate **35** before decomposition of **35** via a retro-[3+2] cycloaddition to the amide product **9**. The observed solvent effects and electronic effects of the azide substrates are consistent with these mechanisms. The amidation reaction proceeded faster in polar solvents, indicating the involvement of a polar transition state that can be stabilized by polar solvents. Electron-withdrawing groups on azides help stabilize the transition state **34** by delocalizing the negative charge on the nitrogen and facilitate the amidation reaction through the path A stepwise mechanism. For the electron-rich azides, the amidation reaction may proceed through the path B concerted [3+2] cyclization mechanism and is much slower and requires a longer reaction time at an elevated temperature just like the coupling of thio acids with azides.

Conclusion

Selenocarboxylate/azide amidation provides an attractive alternative method to the conventional nucleophilic acylation when an amide bond needs to be formed without going through an amine intermediate. The reaction is highly chemoselective and clean and can be carried out under aqueous conditions. The reaction also represents a traceless coupling between a carboxylic acid and an azide. Selenocarboxylate/azide amidation is complementary to the Staudinger ligation, as the Staudinger ligation favors electron-rich azides and selenocarboxylate/azide amidation works more effectively on electron-deficient azides. However, the lack of practical preparation of selenocarboxylates limited the application of this type of amidation reaction. Here, we developed a convenient one-pot procedure to form selenocarboxylates by reacting the mixed anhydrides of carboxylic acids with LiAlHSeH under mild conditions. It is effective over a range of carboxylic acids, including amino acids and peptides. It overcomes disadvantages of other methods of generating selenocarboxylates, such as the high temperature required when Woollin's reagent[44] is used and the waste of the carboxylate equivalent when a diacyl diselenide or diacyl selenide is used. This easy-to-handle procedure to form selenocarboxylates directly from carboxylic acids facilitates the selenocarboxylate/azide amidation and makes this type of amidation reaction practical. It should also benefit other reactions related to selenocarboxylates. In the selenocarboxylate/azide amidation reaction, excellent yields are obtained for electron-deficient azides and significantly improved yields are achieved for the less reactive azides because of the dramatically increased stability of selenocarboxylates under the present conditions.

Experimental Section

Preparation of LiAlHSeH. LiAlHSeH was prepared essentially following the literature procedure.[45] Briefly, to a suspension of $LiAlH_4$ (44 mg, 1.1 mmol) in anhydrous THF (10 mL) was added selenium powder (88 mg, 1.1 mmol) in one portion at 0 °C. The mixture was stirred at 0 °C for 20 min under a nitrogen atmosphere and ready to be used.

General Amidation Procedure. To a solution of carboxylic acid (1.1 mmol) and *N*-methylpiperidine (0.134 mL, 1.1 mmol) in THF (10 mL) was added a 1.0 M solution of isopropylchloroformate in toluene (1.1 mL, 1.1 mmol) at 0 °C under a nitrogen atmosphere. The resulting mixture was stirred for 20 min at 0 °C. Then, the

obtained mixed anhydride solution was slowly added into the prepared LiAlHSeH solution via cannulation over a period of 5 min. The reaction mixture was stirred for an additional 30 min below 5 °C under a nitrogen atmosphere. Then, a solution of azide (1.0 mmol) in THF (1 mL) was added into the above selenocarboxylate solution via a syringe. For electron-deficient azides, the amidation reaction was carried out at 0 °C to room temperature. For electron-rich azides, 0.5 mmol of the azide was used and the reaction was carried out at 55 °C. When TLC and/or LC-MS showed the disappearance of the starting azide or no further change of the reaction mixture, the reaction mixture was filtered through a Celite pad that was then rinsed with EtOAc (3 × 25 mL). The combined organic phase was washed with 5% $NaHCO_3$, water, and brine and dried over anhydrous Na_2SO_4. After removal of Na_2SO_4 through filtration, the filtrate was treated with activated charcoal. The activated charcoal was filtered off, and the filtrate was then concentrated to dryness. The crude product was purified by flash column chromatography (FCC) on silica gel. Yields and physical and spectroscopic data of all amides are consistent with their structures.

***N*α-Benzyloxycarbonyl-*N*′-(4-cyanophenyl)-L-phenylalaninamide (10).** The product (340 mg) was obtained after FCC (CH_2Cl_2/MeOH, 10:1) in 85% isolated yield. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.80 (brs, 1H, −NH), 7.16−7.48 (m, 14H), 5.82 (d, −NH, J = 8.0 Hz), 5.07 (s, 2H), 4.66 (q, 1H, J = 8.0 Hz), 3.13 (dd, 2H, J = 6.6 Hz, J = 7.4 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 170.2, 156.7, 141.5, 136.0, 135.8, 133.2, 129.3, 129.0, 128.7, 128.5, 128.1, 127.9, 127.4, 119.8, 107.3, 67.6, 57.4, 38.4. MS (ESI$^+$): *m*/*z* (intensity), 400.2 (MH$^+$, 100%), 441.2 (MH$^+$ + CH_3CN, 15%).

***O*³-Benzyl-*N*α-benzyloxycarbonyl-*N*′-(4-cyanophenyl)-L-serinamide (11).** The product (384 mg) was obtained after FCC (CH_2Cl_2/MeOH, 50:1) in 89% isolated yield. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.85 (brs, 1H, −NH), 7.45−7.55 (m, 4H), 7.23−7.32 (m, 10H), 5.92 (d, 1H, J = 6.8 Hz), 5.11 (s, 2H), 4.47−4.60 (m, 3H), 3.92−3.97 (m, 1H), 3.62−3.70 (m, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 16.8.7, 156.3, 141.4, 136.9, 135.7, 133.0, 128.5, 128.4, 128.2, 128.1, 127.9, 127.7, 119.6, 118.7, 107.1, 73.5, 69.3, 67.3, 55.0. MS (ESI$^+$): *m*/*z* (intensity), 430.2 (MH$^+$, 100%), 452.2 (M + Na$^+$, 20%).

***N*α-Benzyloxycarbonyl-*N*′-(4-cyanophenyl)-L-glutaminamide (12).** The product (342 mg) was obtained after FCC (CH_2Cl_2/MeOH, 20:1) in 90% isolated yield. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 10.55 (s, 1H), 7.70−7.85 (m, 4H), 7.36 (brs, 5H), 6.85 (s, 1H), 5.04 (s, 2H), 4.11−4.20 (m, 1H), 2.15−2.21 (m, 2H), 1.70−1.99 (m, 2H). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 174.2, 172.4, 156.8, 143.9, 137.7, 134.0, 129.1, 128.9, 128.5, 120.1, 119.8, 105.9, 66.3, 56.1, 32.1, 28.0. MS (ESI$^+$): *m*/*z* (intensity), 381.2 (MH$^+$, 100%), 444.4 (M + Na$^+$ + CH_3CN, 15%).

***N*α-Benzyloxycarbonyl-*N*′-(4-cyanophenyl)-L-methioninamide (13).** The product (337 mg) was obtained after FCC (CH_2Cl_2/MeOH, 40:1) in 88% isolated yield. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 9.02 (brs, 1H, −NH), 7.48−7.61 (m, 4H), 7.35 (brs, 5H), 5.78 (d, J = 8.0 Hz), 5.14 (s, 2H), 4.51−4.62 (m, 1H), 2.62 (t, 2H, J = 6.6 Hz), 1.98−2.26 (m, 5H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 170.3, 156.9, 141.7, 135.8, 133.3, 128.8, 128.6, 128.1, 119.8, 118.8, 107.4, 67.7, 54.9, 30.9, 30.3, 15.4. MS (ESI$^+$): *m*/*z* (intensity), 384.1 (MH$^+$, 100%).

***O*⁵-Benzyl-*N*α-benzyloxycarbonyl-*N*′-(4-cyanophenyl)-L-glutamic Amide (14).** The product (394 mg) was obtained after FCC (CH_2Cl_2/MeOH, 50:1) in 90% isolated yield. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 9.30 (brs, 1H, −NH), 7.51−7.64 (m, 4H), 7.36 (brs, 5H), 5.61 (d, −NH, J = 6.6 Hz), 5.13 (s, 2H), 4.37−4.40 (m, 1H), 2.45−2.65 (m, 2H), 1.98−2.29 (m, 2H), 1.49 (s, 9H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 173.3, 170.7, 156.5, 142.0, 135.6, 133.2, 128.7, 128.5, 128.3, 119.7, 118.9, 107.2, 81.0, 66.9, 54.7, 30.7, 28.4, 27.5. MS (ESI$^+$): *m*/*z* (intensity), 438.2 (MH$^+$, 100%).

***N*α-*tert*-Butyloxycarbonyl-*N*′-(4-cyanophenyl)-L-prolinamide (15).** The product (285 mg) was obtained after FCC (CH_2Cl_2/MeOH, 50:1) in 89% isolated yield. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 10.05 (brs, 1H, −NH), 7.38−7.52 (m, 4H), 4.50 (brs, −NH), 3.41−3.53 (m, 2H), 1.91−2.28 (m, 4H), 1.49 (s, 9H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 171.2, 156.3, 142.6, 133.0, 119.4, 119.0, 106.3, 81.2, 60.7, 47.4, 28.5, 28.3, 24.6. MS (ESI$^+$): *m*/*z* (intensity), 316.2 (MH$^+$, 40%), 334.2 (MH$^+$ + H_2O, 100%).

***N*α-*tert*-Butyloxycarbonyl-*N*′-(4-cyanophenyl)-L-valinamide (16).** The product (282 mg) was obtained after FCC (CH_2Cl_2/MeOH, 50:1) in 89% isolated yield. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 9.35 (brs, 1H, −NH), 7.39−7.52 (m, 4H), 5.40 (d, −NH), 4.09−4.16 (m, 1H), 2.06−2.16 (m, 1H), 1.43 (s, 9H), 1.01 (d, 6H, J = 7.0 Hz). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 171.5, 156.8, 142.0, 133.0, 119.5, 118.9, 106.9, 80.9, 61.2, 30.8, 28.4, 19.6, 18.5. MS (ESI$^+$): *m*/*z* (intensity), 318.2 (MH$^+$, 100%).

***N*α-(9-Fluorenyloxycarbonyl)-*N*′-(4-cyanophenyl)-L-glutaminamide (17).** The product (328 mg) was obtained after FCC (CH_2Cl_2/MeOH, 20:1) in 70% isolated yield. ^{1}H NMR (DMSO-d_6, 200 MHz): δ 10.54 (s, 1H, −NH), 7.72−7.91 (m, 8H), 7.30−7.46 (m, 4H), 6.83 (s, 1H, −NH), 4.09−4.32 (m, 4H), 2.17−2.21 (m, 2H), 1.83−1.99 (m, 2H). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 173.4, 171.7, 156.1, 143.8, 143.1, 140.7, 133.3, 127.7, 127.1, 125.3, 120.1, 119.3, 119.1, 105.1, 65.8, 55.4, 46.6, 31.5, 27.3. MS (ESI$^+$): *m*/*z* (intensity), 469.2 (MH$^+$, 100%), 491.2 (M + Na$^+$, 50%).

***N*α-(9-Fluorenyloxycarbonyl)-*N*′-(4-cyanophenyl)-L-tryptophanamide (18).** The product (458 mg) was obtained after FCC (hexane/CH_2Cl_2/MeOH, 10:10:1) in 87% isolated yield. ^{1}H NMR ($CDCl_3$, 200 MHz): δ 8.38 (brs, 1H, −NH), 8.10 (s, 1H, NH), 7.78 (d, 2H, J = 7.4 Hz), 7.28−7.61 (m, 11H), 7.17 (t, 2H, J = 8.0 Hz), 7.05 (t, 2H, J = 8.0 Hz), 6.95 (brs, 1H, NH), 4.68 (m, 1H), 4.34 (d, 2H, J = 6.8 Hz), 4.17 (t, 1H, J = 7.4 Hz), 3.26−3.31 (m, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 170.4, 156.5, 143.5, 143.4, 141.3, 136.1, 133.0, 127.8, 127.1, 127.0, 124.9, 123.2, 122.5, 120.1, 120.0, 119.7, 118.7, 118.4, 111.4, 109.9, 107.1, 67.4, 56.4, 46.9, 28.2. MS (ESI$^+$): *m*/*z* (intensity), 527.2 (MH$^+$, 100%), 549.2 (M + Na$^+$, 20%).

***N*α-*tert*-Butyloxycarbonyl-L-leucyl-*N*′-(4-cyanophenyl)-L-tryptophanamide (19).** The product (450 mg) was obtained after FCC (CH_2Cl_2/MeOH, 20:1) in 87% isolated yield. ^{1}H NMR (CD_3OD, 200 MHz): δ 7.59 (d, 2H, J = 8.4 Hz), δ 7.43−7.50 (m, 3H), 7.21 (d, 1H, J = 8.0 Hz), 7.01 (s, 1H), 6.85−6.97 (m, 2H), 4.67 (m, 1H), 3.95 (t, 1H), 3.22−3.25 (m, 2H), 1.40−1.60 (m, 1H), 1.35 (t, 2H, J = 6.6 Hz), 1.25 (s, 9H), 0.80 (dd, 6H, J = 8.2 Hz). ^{13}C NMR (CD_3OD, 50 MHz): δ 175.7, 172.7, 158.2, 143.8, 138.0, 134.0, 128.7, 124.7, 122.5, 121.2, 119.9, 119.8, 119.3, 112.3, 110.2, 107.8, 80.9, 56.1, 55.1, 41.6, 28.6, 25.8, 23.3, 21.8. MS (ESI$^+$): *m*/*z* (intensity), 540.3 (M + Na$^+$, 100%), 581.3 (M + Na$^+$ + CH_3CN, 37%).

***O*³-Acetyl-*N*α-*tert*-butyloxycarbonyl-D,L-seryl-*N*′-(4-cyanophenyl)-L-phenylalaninamide (20).** The product (454 mg) was obtained after FCC (CH_2Cl_2/MeOH, 20:1) in 92% isolated yield. ^{1}H NMR (CD_3OD, 200 MHz): δ 7.68 (d, 2H, J = 8.4 Hz), 7.55 (d, 2H, J = 8.4 Hz), 7.14 (brs, 5H), 4.67 (dd, 1H, J = 8.4 Hz, J = 8.4 Hz), 4.14−4.20 (m, 1H), 4.00−4.09 (m, 2H), 2.88−3.18 (m, 2H), 1.89 (s, 3H), 1.36 (s, 9H). ^{13}C NMR (CD_3OD, 50 MHz): δ 172.2, 171.8, 157.6, 143.7, 137.9, 137.7, 134.1, 130.3, 129.5, 127.9, 121.2, 119.7, 107.8, 81.0, 64.7, 56.5, 55.1, 38.9, 28.6, 20.7. MS (ESI$^+$): *m*/*z* (intensity), 517.3 (M + Na$^+$, 100%), 558.2 (M + Na$^+$ + CH_3CN, 40%).

***N*α-*tert*-Butyloxycarbonyl-L-asparaginyl-L-leucyl-*N*′-(4-cyanophenyl)-L-tryptophanamide (21).** The product (523 mg) was obtained after FCC (CH_2Cl_2/MeOH, 20:1) in 83% isolated yield. ^{1}H NMR (CD_3OD, 200 MHz): δ 7.78 (d, 2H, J = 8.8 Hz), 7.52 (d, 3H, J = 8.8 Hz), 7.34 (d, 1H, J = 8.8 Hz), 7.05 (s, 1H), 6.85−7.00 (m, 2H), 4.59−4.68 (m, 1H), 4.30−4.37 (m, 1H), 4.05 (t, 1H, J = 7.2 Hz), 3.19−3.4 (m, 2H), 2.48−3.13 (m, 2H), 1.47−1.60 (m, 3H), 1.33 (s, 9H), 0.70 (dd, 6H, J = 8.8 Hz). ^{13}C NMR (CD_3OD, 50 MHz): δ 175.0, 174.9, 172.9, 157.5, 144.0, 138.0, 134.0, 128.7, 124.6, 122.4, 121.3, 119.8, 119.3, 112.3, 111.3, 107.8, 80.9,

56.8, 54.5, 52.3, 40.8, 37.8, 28.6, 28.2, 25.6, 23.9, 21.6. MS (ESI^+): *m/z* (intensity), 632.3 (MH^+, 10%), 654.3 ($M + Na^+$, 100%).

***N*-(4-Nitrophenyl)benzamide (22).**[48] The product (237 mg) was obtained after FCC (hexane/EtOAc, 5:1) in 98% isolated yield. ^1H NMR (DMSO-d_6, 200 MHz): δ 10.82 (s, −NH), 8.27 (d, 2H, J = 7.4 Hz), 8.08 (d, 2H, J = 8.4 Hz), 7.99 (d, 2H, J = 8.4 Hz), 7.52−7.65 (m, 3H). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 167.1, 146.3, 143.3, 135.0, 132.9, 129.3, 128.7, 125.5, 120.6. MS (ESI^+): *m/z* (intensity), 243.1 (MH^+, 100%), 284.1 ($MH^+ + CH_3CN$, 60%).

***N*-(4-Cyanophenyl)benzamide (23).**[49] The product (209 mg) was obtained after FCC (hexane/EtOAc, 4:1) in 94% isolated yield. ^1H NMR ($CDCl_3$, 200 MHz): δ 8.13 (s, 1H, −NH), 7.70−7.92 (m, 4H), 7.48−7.70 (m, 5H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 165.9, 142.1, 134.2, 133.5, 132.6, 129.1, 127.2, 120.0, 118.9, 107.5. MS (ESI^+): *m/z* (intensity), 223.1 (MH^+, 100%), 264.1 ($MH^+ + CH_3CN$, 70%).

4-Benzamidobenzoic Acid (24).[50] The product (210 mg) was obtained after FCC (hexane/EtOAc, 1:1) in 87% isolated yield. ^1H NMR (DMSO-d_6, 200 MHz): δ 10.5 (s, −COOH), 7.95 (brs, 7H), 7.53−7.56 (m, 3H). ^{13}C NMR (DMSO-d_6, 50 MHz): δ 167.0, 166.0, 143.3, 134.7, 131.8, 130.2, 128.4, 127.8, 125.5, 119.5. MS (ESI^-): *m/z* (intensity), 240.1 ($M - H^-$, 100%), 258.1 ($M - H^- + H_2O$, 10%).

Diphenyl *N*-Benzoylphosphoramidate (25). The product (337 mg) was obtained after FCC (hexane/EtOAc, 2:1) in 95% isolated yield. ^1H NMR ($CDCl_3$, 200 MHz): δ 9.67 (brs, −NH), 7.98 (d, 2H, J = 8.6 Hz), 7.56 (t, 1H, J = 7.8 Hz), 7.39 (dd, 2H, J = 8.0 Hz, J = 7.6 Hz), 7.08−7.27 (m, 10H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 167.7, 150.2 (d, J = 6.8 Hz), 133.0, 132.4 (d, J = 11 Hz), 129.8, 128.6, 125.7, 120.7, 120.6. MS (ESI^+): *m/z* (intensity), 354.1 ($M + H^+$, 90%), 417.1 ($M + Na^+ + CH_3CN$, 85%).

***N*-(4-Toluenesulfonyl)benzamide (26).**[51] The product (270 mg) was obtained after FCC (hexane/CH_2Cl_2/MeOH, 10:10:1) in 98% isolated yield. ^1H NMR ($CDCl_3$, 200 MHz): δ 9.52 (brs, −NH), 8.05 (d, 2H, J = 8.4 Hz), 7.83 (d, 2H, J = 7.4 Hz), 7.32−7.58 (m, 5H), 2.43 (s, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 164.4, 145.2, 135.4, 133.4, 131.1, 129.68, 128.8, 128.7, 127.9, 21.7. MS (ESI^-): *m/z* (intensity), 274.1 ($M - H^+$, 100%).

***N*-Benzoylbenzamide (27).**[52] The product (91 mg) was obtained after FCC (hexane/EtOAc, 1:1) in 81% isolated yield. ^1H NMR ($CDCl_3$, 200 MHz): δ 9.26 (brs, −NH), δ 7.84 (dd, 4H, J = 8.0 Hz, J = 1.4 Hz), δ 7.40−7.60 (m, 6H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 166.7, 133.3, 133.0, 128.7, 127.9. MS (ESI^+): *m/z* (intensity), 226.1 ($M + H^+$, 100%), 289.1 ($M + Na^+ + CH_3CN$, 85%).

***N*-(2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl)benzamide (28).** The product (160 mg) was obtained after FCC (hexane/EtOAc, 4:1) in 71% isolated yield. ^1H NMR ($CDCl_3$, 200 MHz): δ 7.77 (d, 2H, J = 6.6 Hz), 7.40−7.54 (m, 3H), 5.44−5.49 (m, 2H), 5.22−5.26 (m, 2H), 4.09−4.17 (m, 3H), 2.15 (s, 3H), 2.04 (s, 3H), 2.01 (s, 6H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 171.8, 170.5, 170.1, 169.9, 167.2, 133.0, 132.4, 128.8, 127.3, 79.3, 72.4, 70.9, 68.7, 67.4, 61.2, 20.9, 20.8, 20.7, 20.6. MS (ESI^+): *m/z* (intensity), 452.2 ($M + H^+$, 45%), 474.2 ($M + Na^+$, 100%), 515.2 ($M + Na^+ + CH_3CN$, 25%).

***N*-(4-Methoxyphenyl)benzamide (29).**[53] The product (72 mg) was obtained after FCC (hexane/EtOAc, 4:1) in 63% isolated yield (95% yield based on the recovery). ^1H NMR (acetone-d_6, 200 MHz): δ 9.40 (brs, NH), 7.97 (dd, 2H, J = 7.8 Hz, J = 1.4 Hz), 7.72 (d, 2H, J = 8.6 Hz), 7.42−7.59 (m, 3H), 6.91 (dd, 2H, J = 6.8 Hz, J = 2.4 Hz), 3.78 (s, 3H, $-OCH_3$). ^{13}C NMR (acetone-d_6, 50 MHz): δ 165.7, 156.9, 136.3, 133.3, 131.9, 129.0, 125.0, 122.4, 114.4, 55.5. MS (ESI^+): *m/z* (intensity), 228.1 (MH^+, 100%), 269.1 ($MH^+ + CH_3CN$, 5%).

***N*-(4-Hydroxymethylphenyl)benzamide (30).** The product (80 mg) was obtained after FCC (hexane/EtOAc, 2:1) in 70% isolated yield (95% yield based on the recovery). ^1H NMR (CD_3OD, 200 MHz): δ 7.94 (dd, 2H, J = 8.4 Hz, J = 2.0 Hz), 7.67 (dd, 2H, J = 7.8 Hz, J = 1.8 Hz), 7.47−7.60 (m, 3H), 7.37 (d, 2H, J = 8.4 Hz), 4.61 (s, 2H, $-CH_2OH$). ^{13}C NMR (CD_3OD, 50 MHz): δ 168.9, 139.0, 136.3, 132.9, 129.6, 128.6, 128.5, 122.2, 64.9. MS (ESI^+): *m/z* (intensity), 228.1 (MH^+, 100%), 269.1 ($MH^+ + CH_3CN$, 10%).

4-Benzamidobenzyl Acetate (31). The product (94 mg) was obtained after FCC (hexane/EtOAc, 2:1) in 70% isolated yield (97% yield based on the recovery). ^1H NMR ($CDCl_3$, 200 MHz): δ 8.27 (brs, 1H, −NH), δ 7.86 (d, 2H, J = 8.0 Hz), 7.67 (d, 2H, J = 8.4 Hz), 7.39−7.54 (m, 3H), 7.34 (d, 2H, J = 8.4 Hz), 5.08 (s, 2H), 2.10 (s, 3H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 171.1, 166.1, 138.2, 134.9, 132.1, 131.9, 129.3, 128.8, 127.2, 120.5, 66.0, 21.1. MS (ESI^+): *m/z* (intensity), 270.1 (MH^+, 100%), 311.1 ($MH^+ + CH_3CN$, 30%).

***N*-(4-Aminophenyl)benzamide (32).**[54] The product (57 mg) was obtained after FCC (hexane/EtOAc, 2:1) in 54% isolated yield (96% yield based on the recovery). ^1H NMR (CD_3OD, 200 MHz): δ 7.89 (dd, 2H, J = 8.0 Hz, J = 1.8 Hz), 7.42−7.52 (m, 3H), 7.37 (dd, 2H, J = 7.0 Hz, J = 2.0 Hz), 6.73 (dd, 2H, J = 8.0 Hz, J = 1.8 Hz). ^{13}C NMR (CD_3OD, 50 MHz): δ 168.6, 145.9, 136.3, 132.6, 130.4, 129.5, 128.4, 124.2, 116.6. MS (ESI^+): *m/z* (intensity), 213.1 (MH^+, 80%), 254.1 ($MH^+ + CH_3CN$, 100%).

***N*-{6-[(*tert*-Butyldiphenylsilyl)oxy]hexyl}benzamide (33).** The product (143 mg) was obtained after FCC (hexane/EtOAc, 4:1) in 62% isolated yield. ^1H NMR ($CDCl_3$, 200 MHz): δ 7.75 (dd, 2H, J = 7.2 Hz, J = 1.4 Hz), δ 7.61−7.71 (m, 3H), 7.24−7.50 (m, 10H), 6.2 (brs, −NH), 3.65 (t, 2H, J = 6.4 Hz), 3.41 (dd, 2H, J = 7.0 Hz, J = 7.2 Hz), 1.51−1.61 (m, 4H), 1.35−1.47 (m, 4H),1.04 (s, 9H). ^{13}C NMR ($CDCl_3$, 50 MHz): δ 167.5, 135.2, 134.8, 134.0, 131.3, 129.5, 128.5, 127.5, 126.8, 63.7, 40.0, 32.4, 29.6, 26.8, 26.7, 25.5, 19.2. MS (ESI^+): *m/z* (intensity), 482.3 ($M + Na^+$, 100%), 523.3 ($M + Na^+ + CH_3CN$, 10%).

Stability Studies. Aliquots of freshly prepared benzeneselenocarboxylate in solvents were incubated at room temperature and at 55 °C under a nitrogen atmosphere. The amount of benzeneselenocarboxylate was measured at different time intervals by the reaction with 2 equiv of 4-toluenesulfonyl azide for 1 h. The reaction mixtures were separated on a Shimadzu 2010 LCMS system using a Chromolith SpeedROD RP-18e column (50 × 4.6 mm) at 1 mL/min with a 10-min gradient of 10−90% acetonitrile containing 0.1% formic acid. The *N*-4-toluenesulfonylbenzamide was quantitated based on UV absorption at 220 nm, and its structure was confirmed by the mass spectrometry detector. The relative peak area of *N*-4-toluenesulfonylbenzamide represents the amount of benzeneselenocarboxylate that remained in the solution at a given time point and was plotted against the time of incubation prior to the addition of 4-toluenesulfonyl azide. The data were fitted using the single, two-parameter exponential decay equation in Sigma Plot to obtain the pseudo first-order rate constant and calculate the $t_{1/2}$ value.

Acid Hydrolysis of Amides. Solutions (10 μL) of N^α-benzyloxycarbonyl-*N*′-(4-cyanophenyl)-L-phenylalaninamide **10** (0.03 mM), N^α-benzyloxycarbonyl-*N*′-benzyl-L-phenylalaninamide (0.03 mM), and N^α-benzyloxycarbonyl-phenylalanine (0.03 mM) were placed separately in a 6 × 50 mm test tube. The samples were then dried

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using a speed-vac at room temperature. The test tubes containing amides were placed inside an acid hydrolysis vessel, and 200 μL of 6 N HCl was added. The vessel was flushed three times with nitrogen and then heated under a vacuum at 110 ± 2 °C in a convection oven for 24 h. The test tubes were dried using a speed-vac for 30 min prior to derivatization.

OPA/NBC Derivatization and HPLC Analysis of Amino Acids. The OPA/NBC reagent solutions were freshly prepared by dissolving 11 mg of *o*-phthaldialdehyde (OPA) and 20 mg of N^{α}-*tert*-butyloxycarbonyl-L-cysteine (NBC) in 1 mL of methanol, each. For derivatization, 50 μL of 0.1 M $Na_2B_4O_7$ (pH 9.6), 50 μL of deionized distilled water, 20 μL of OPA solution, and 20 μL of NBC solution were added to each of the test tubes containing hydrolyzed samples. The resulting solution was vortexed for 5 min and then stored at −20 °C prior to HPLC analysis.

A 20 μL solution of each sample was injected into an HPLC system using a C_{18} column (150 × 4.6 mm, 3 μm) and aqueous acetonitrile containing 0.1% trifluoroacetic acid (TFA) as the mobile phase at a flow rate of 1 mL/min and a detection wavelength of 340 nm. The elution started with an isocratic step of 40% acetonitrile for 5 min followed by a gradient step from 40−80% acetonitrile in 25 min. The relative amount of D- and L-amino acids was determined by comparing the relative peak areas of OPA/NBC-derived isoindoles corresponding to the D- and L-amino acids.

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Supporting Information Available: General methods and ^{1}H NMR and ^{13}C NMR spectra of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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