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Peptide Bond Formation via N^{α}-Protected Diacyldiselenides

U. Vathsala[1] · L. Roopesh Kumar[1] · N. R. Sagar[1] · M. Mahesh[2] · P. Venkata Ramana[2] · Vommina V. Sureshbabu[1]

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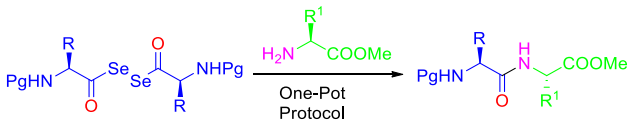
Abstract

A simple, straightforward, for the peptide bond formation employing corresponding carboxylic acids and amines derived from amino acids via N^{α}-protected diacyldiselenide is delineated. The key step of the synthesis is the in situ generation of N^{α}-protected diacyldiselenide using $NaBH_2Se_3$ as selenating reagent, followed by trapping with an amino acid ester leading to the peptide. The formation of N^{α}-protected diacyldiselenide was confirmed through TLC and HRMS analysis using crude sample. The reaction is clean and all the products were obtained in moderate to good yields, including for sterically hindered amino acids. The protocol is free from racemisation, compatible with Fmoc, Cbz and Boc groups.

Graphical Abstract



Keywords Deselenization · Diacyldiselenides · Peptides · Selenating reagent

Introduction

The amide bond construction is one of the most significant synthetic transformations in organic and peptide chemistry (Pattabiraman and Bode 2011; Sewald and Jakubke 2002; Greenberg et al. 2000; Funabashi et al. 2010; Simonovic and Steitz 2009; Fischbach and Walsh 2006). Amide bonds are not limited to biological systems and are indeed present in an enormous array of molecules, including major marketed drugs such as valsartan (blockade of angiotensin-II receptors), lisinopril (inhibitor of angiotensin converting enzyme), chloramphenical (against bacterial infections), cyclosporine (calcineurin inhibitor immunosuppressant), and somatostatin (growth hormone–inhibiting hormone) etc (Greenberg et al. 2000; Gasparo and Whitebread 1995; Patchett 1993). Several chemical methods are reported in the literature for the synthesis of amides within small molecules, large polypeptides and proteins are obtained through native chemical ligation (Dawson et al. 1994; Kent 2009; Hemantha et al. 2012; Prabhu et al. 2015). Under neutral and acidic conditions, the lower pKa value of selenol (5.3) in selenocysteine (Sec) compared to thiol (8.3) in cysteine (Cys), which makes Sec as significantly more potent nucleophile i.e., selenolate (RSe^-) is more nucleophilic than its analogous thiolate (RS^-). In addition, the reduction potential of diselenide bond is less than that of the corresponding disulfide (McGrath and Raines 2011; Huber and Criddle 1967; Gowd et al. 2012; Hondal et al. 2013). Recently, Koppenol and colleagues proved that the reaction rates of selenium as a nucleophile and as an electrophile are 2–3 and 4 orders of magnitude higher, respectively, than those of sulfur (Steinmann et al. 2010). The chemistry of native chemicalligation towards synthesizing native backbone

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✉ Vommina V. Sureshbabu
sureshbabuvommina@rediffmail.com; hariccb@hotmail.com

[1] #109, Peptide Research Laboratory, Department of Studies in Chemistry, Central College Campus, Bangalore University, Dr. B. R. AmbedkarVeedhi, Bangalore 560 001, India

[2] Department of Chemistry, Sri Krishnadevaraya University, Ananthapuramu, Andhra Pradesh 515 003, India

proteins by the assembly of two or more unprotected peptide fragment is fully exploited (Vázquez and Seitz 2014; Thapa et al. 2014; Malins and Payne 2014). However, selenoester mediated native chemical ligation (NCL) is a fast ligation process over thioester mediated NCL to provide longer proteins (Durek and Alewood 2011). Efficiently, C-terminal peptide thioester react with N-terminal peptide containing selenocysteine resulting in selenoester intermediate, which then subsequently rearranges to give a native amide bond (Mitchell et al. 2015).

The ligation–desulfurization technologies lead to the synthetic proteins. The rate of ligation reactions are very slow at sterically hindered C-terminal thioesters and significant thioester hydrolysis take place (Mitchell et al. 2015). Consequently, NCL routes have been explored between selenocysteine (Sec)/diselenide/selenol-derived amino acids and thioesters (Rasale et al. 2014; Malins and Payne 2012; Hondal 2005; Gieselman et al. 2001; Lara et al. 2014). Recently, Carlo Siciliano et al. have demonstrated the utility of selenoesters in peptide synthesis (Andrea et al. 2017). In view of these developments, herein we established a traceless and novel synthesis of dipeptides via N^{α}-protected diacyldiselenides.

Protocols for synthesizing diacyldiselenides have been reported (Niyomura et al. 1999; Nishiyama et al. 1989; Ishihara and Kato 1972; Jensen et al. 1972; Koketsu et al. 2002). These methods have certain adverse like the slender availability of the precursor, use of swank reagents and the multiple steps for preparation. Our group reported a few routes for the synthesis of amides, including peptides by employing different functionalities (Madhu et al. 2013; Ananda and Sureshbabu 2000; Tantry et al. 2003; Krishnamurty et al. 2015). In this letter, we report a peptide bond formation reaction starting from corresponding carboxylic acid and amine via N^{α}-protected diacyldiselenides.

Results and Discussion

Initially, the selenating reagent was prepared using the protocol, which was previously reported by us (Nageswararao et al. 2015). Briefly, 1.0 mmol of $NaBH_4$ was treated with 3.0 mmol of selenium powder in THF at 0 °C under nitrogen atmosphere and stirred till the liberation of hydrogen gas. After 10 min, the reaction turns black to reddish brown colour indicating the formation of sodium selenoborate ($NaBH_2Se_3$).

Next, we started to investigate the protocol for peptide bond formation by the coupling of N^{α}-protected amino acids with amino acid esters. In a typical experiment, a solution of Fmoc-Ala-OH **1a** in dry THF was treated with *N*-methylpiperidine and isopropyl chloroformate at − 15 °C for 20 min to the corresponding mixed anhydride. The reaction mixture was stirred for 10 min, filtered through vacuum and then the filtrate was added dropwise into a freshly prepared sodium selenoborate solution ($NaBH_2Se_3$, **2**). Then oxidative dimerization was carried out by following the reported protocol (Mali and Gopi 2014) in THF–H_2O (3:1) solution about 12 h in the presence of I_2 at 0 °C, which lead to the less conversion to the corresponding (Fmoc-Ala-CO-Se-)$_2$ **3a** (about 60% yield; Table 1, entry 1) as confirmed through TLC and HRMS analysis. To improve the yield of **3a**, the reaction was studied using various oxidants. About 58% of yield was obtained when reaction was carried out in the presence of I_2/O_2 at r.t. for 8 h (Table 1, entry 2) (Ishihara et al. 1977). However, when the reaction was studied at 0 °C for 1.5 h in the presence of I_2/KI lead to **3a** with yield about 86% (Table 1, entry 3) (Koketsu et al. 2002). Hence, the optimized protocol was selected to be carboxylic acid (1.0 equiv.), $NaBH_2Se_3$ (1.0 equiv.) and I_2/KI (0.2 equiv.) in THF, at 0 °C for 1.5 h. But, we were unable to isolate **3a** during the purification through column chromatography. In the next step, N^{α}-protected diacyldiselenides **3a** was readily trapped with amino acid ester **4a** in about 10–15 min lead to the crude dipeptide. A simple work up followed by column chromatography gave the desired product **5a** in 84% yield (Scheme 1).

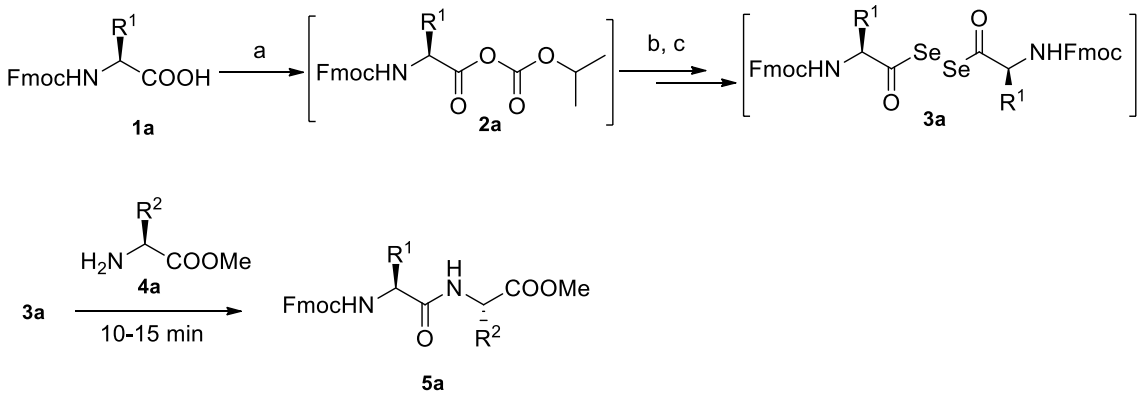
To further explore the scope of this reaction, various N^{α}-protected amino acids were coupled with amino acid esters derived from simple as well as sterically hindered amino acids in dry THF in about 10–15 min to afford the corresponding dipeptides in moderate to good yields (Table 2). All products are obtained with high purity and characterized by HRMS, ^{1}H and ^{13}C NMR analysis.

During the coupling of substrates, the possibility of racemization was studied through chiral HPLC. Initially, the prepared diastereomeric products Fmoc-(L)-Phg-Phe-OMe **5e** and Fmoc-(D)-Phg-Phe-OMe **5f** were analysed. Both have been appeared as single peaks at different retention times R_t = 8.17 min and R_t = 6.19 min respectively. Further, intentionally prepared equimolar mixture of 5e and 5f showed distinct peaks at R_t = 8.22 min and R_t = 6.19 min. From these results, it is confirmed that the present protocol is free from racemization.

Table 1 Optimization of reaction conditions

Entry	Oxidants	Solvents	Temp (°C)	Time (h)	Yield **3a** (%)[a]
1	I_2	THF:H_2O	0	12	60
2	I_2/O_2	THF	r.t	8	58
3	I_2/KI (0.2 equiv.)	THF	0	1.5	86

[a]Yield corresponding to the crude product



Scheme 1 Synthesis of dipeptides via N^{α}-protected diacyldiselenides **3a**

Conclusion

We demonstrated a new protocol for the synthesis of peptides employing N^{α}-protected diacyldiselenide and amino acid esters in dry THF. The protocol showed tolerance towards all the common urethane protecting groups. The products are formed in good yields with high purity in short duration.

Experimental Section

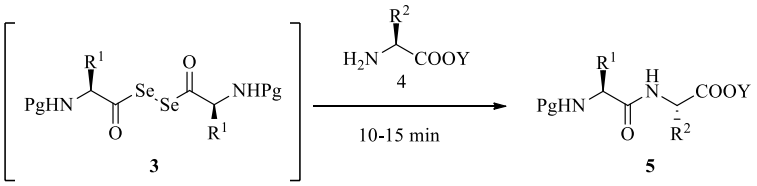
Materials and Methods

All the chemicals and reagents were procured from Sigma-Aldrich Chemicals, USA. All the solvents were dried and distilled prior to use. Reactions were monitored using Merck aluminum TLC sheets (Silica gel 60 F_{254}), the chromatograms were visualized either by UV light or exposing in an iodine chamber. Silica gel (100–200 mesh) was used for the column chromatography using mixtures of ethyl acetate and hexane as eluents. HRMS spectra were recorded in a Micromass Q-TOF mass spectrometer using electronspray ionization mode. ^{1}H and ^{13}C NMR spectra were recorded on JEOL ECX-400P (400, 100 MHz) spectrometer.

General Procedure for the Synthesis of Peptide

To a solution of N^{α}-protected amino acid (1.0 mmol) in THF (5.0 mL), *N*-methylpiperidine (1.2 mmol) and isopropyl chloroformate (1.2 mmol) were added at − 15 °C and stirred for 20 min. The reaction mixture was filtered and added to the freshly prepared $NaBH_2Se_3$ dropwise, and stirred for 15 min. Then I_2/KI (0.2 mmol) in THF was added to the reaction mixture, stirred for 1.5 h, followed by filtration to obtain diacyldiselenides. Without purification of diacyldiselenide amino acid ester was added at 0 °C. The reaction was allowed to stir till the completion of the reaction (TLC analysis). The reaction mixture was extracted into EtOAc and washed with 5% citric acid (10 mL), 5% Na_2CO_3 (10 mL), water and brine solution and dried over anhydrous Na_2SO_4. The solvent was evaporated and crude product was purified by column chromatography on silica gel adopting ethyl acetate and hexane as eluents.

Table 2 Synthesis of dipeptides via N^{α}-protected diacyldiselenides



Pg = Fmoc, Boc, Cbz
R^1, R^2 = amino acid side chain
Y = Me, Et

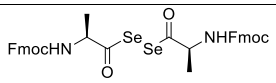
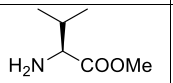
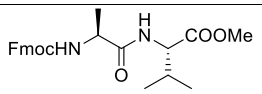
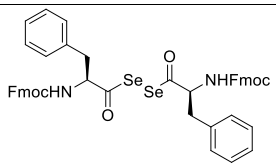
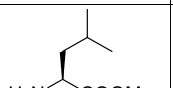
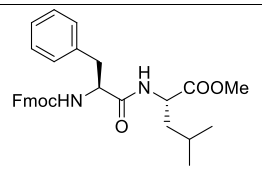
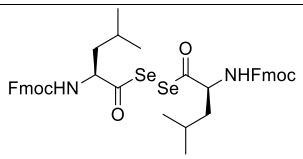
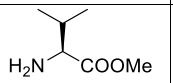
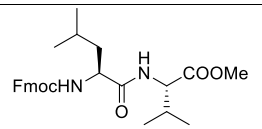
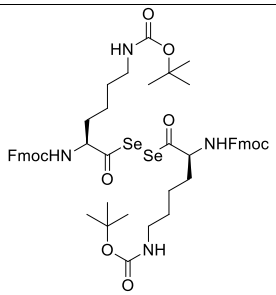
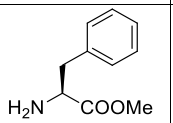
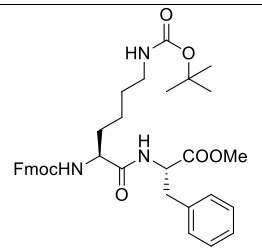
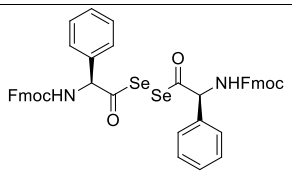
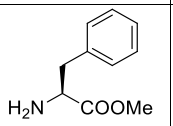
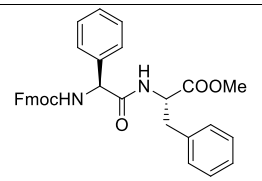
Entry	Diacyldiselenide	Amino acid ester	Peptide	Yield[a]
a				84
b				77
c				70
d				81
e				75

Table 2 (continued)

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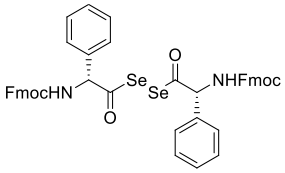
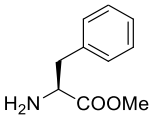
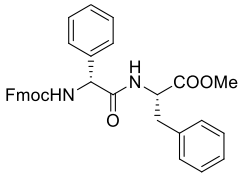
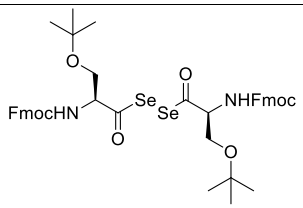
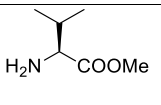
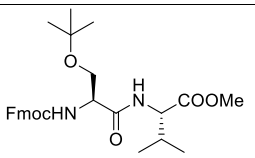
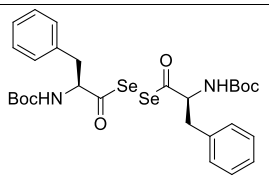
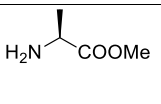
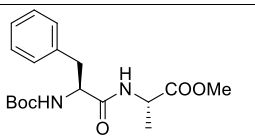
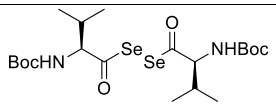
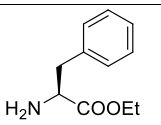
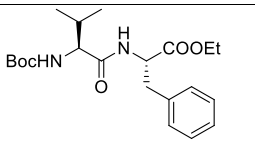
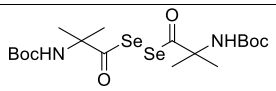
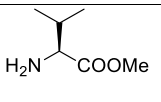
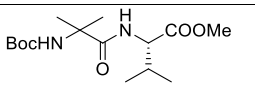
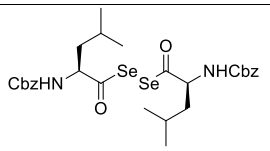
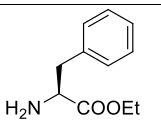
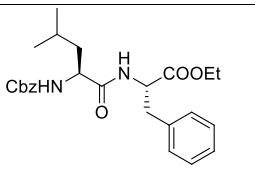
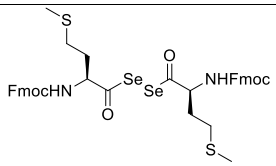
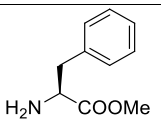
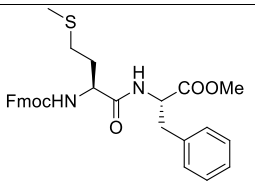
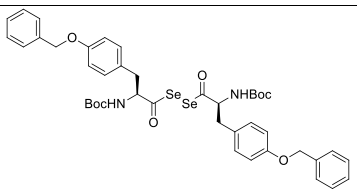
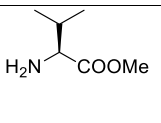
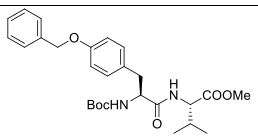
f				72
g				79
h				76
i				77
j				60
k				79
l				66
m				69

[a]Yields (%) corresponding to the isolated pure dipeptides

Compliance with Ethical Standards

Conflict of interest The authors declare that this article content has no conflicts of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent The article does not contain any studies in patients by any of the authors.

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