Pergamon

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Tetrahedron Letters 44 (2003) 5445–5448

TETRAHEDRON LETTERS

New selenyl linker for solid-phase synthesis of dehydropeptides

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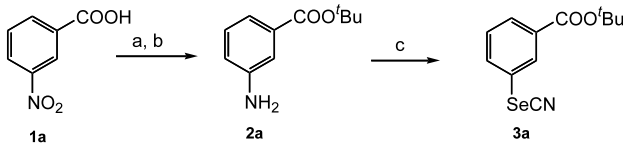
Received 13 March 2003; revised 12 May 2003; accepted 23 May 2003

Abstract—A novel linker possessing selenocyanate and masked carboxylic acid was developed for the solid-phase synthesis of dehydropeptides. This linker was used to demonstrate the synthesis of the model compound of RGD-conjugated dehydropeptide.

Dehydropeptides, containing α,β-unsaturated amino acid (or dehydroamino acid) moiety, are typical abnormal peptides.[1] Their structures are rigid and reactive because they have a double bond conjugated with a peptide linkage. In a previous study on the design of artificial peptides, the introduction of dehydroamino acid residues into normal peptides induced folded conformations based on structural factors.[2] A number of dehydropeptides are bioactive substances isolated from natural resources. Compounds typical of this class include AM-toxin,[3,4] kahalalide F[5] and Sch20561.[6] Dehydropeptides are also known as fairly reactive Michael acceptors that react readily with 'soft' nucleophiles, such as thiols or amines of biological molecules. This reactivity is thought to be one of the molecular mechanisms underlying the biological activities of dehydropeptides.[1] However, the instability of these chemical properties makes it difficult to synthesize such molecules, especially in the case of solid-phase synthesis.

Recently, the authors synthesized AM-toxin II using a solid-phase method.[7] That synthesis utilized commercially available nitrophenyl selenocyanate[8] as an activatable linker to immobilize a precursor of dehydroamino acid. The linker was introduced onto a starting substrate, instead of onto a polystyrene-based resin, followed by immobilization on a commercially available resin through activation of a carboxylic acid. The construction of an unstable double bond was realized simultaneously with oxidative cleavage from the solid phase in the final stage of synthesis.[9] However, to derive carboxylic acid from the nitro group requires a multi-step reaction (reduction to amine, then reaction with succinic anhydride to give hemi-amide), and a cyclic imide would be formed by a side reaction during the activation of the carboxylic acid for immobilization.[10]

To avoid these problems, we designed a new selenyl linker. Our purposes required an aryl selenocyanate with a direct carboxylic acid precursor.[11] Thus, we selected acid treatment to expose the carboxylic acid, whose condition is orthogonal to Fmoc (N-terminal) and allyl (C-terminal) deprotection. The typical synthesis of a seleno linker is depicted in Scheme 1. The synthesis was started with a nitrobenzoic acid **1**, which



Scheme 1. Synthesis of seleno linker. Keys: (a) Boc_2O, DMAP/*tert*-BuOH; (b) $H_2Pd(C)$/EtOH (two steps 90%); (c) $NaNO_2$, dil. HCl then KSeCN (50%).

Keywords: dehydropeptide; solid-phase synthesis; selenyl linker; RGD-conjugate.

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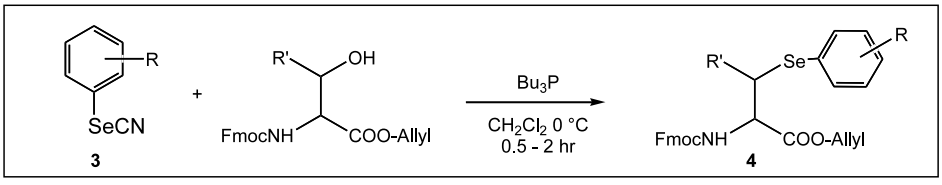
doi:10.1016/S0040-4039(03)01309-1

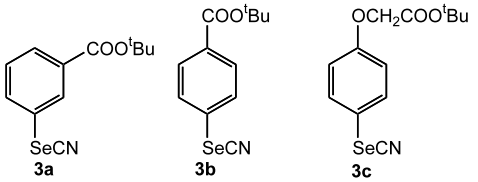
was derived into the corresponding *tert*-butyl ester. The nitroaryl ester was reduced to give the consequent aniline **2**. A subsequent Sandmeyer reaction[12] with potassium selenocyanate in aqueous media gave an aryl selenocyanate, the desired seleno linker **3**. N,C-Protected serine or threonine was reacted with the selenocyanate (**3**) and tributyl phosphine in CH_2Cl_2 at 0°C to give the corresponding selenoether[13] **4** (see table in Fig. 1). According to the result, the *m*-substituted selenocyanate **3a** provided the desired selenoether **4a** in acceptable yield. A byproduct, dehydroamino acid, is presumed to form via a Mitsunobu intermediate [**A**] by six-membered ring transition state.[14] The *tert*-butyl esters of selenoethers **4** could very much be directly converted into the corresponding carboxylic acids in quantitative yield by the action of 95% TFA, compared to the *o*-nitrophenyl selenoether for the synthesis of AM-toxin.[7] Therefore, the selenoether **4a** was selected for the precursor of side-chain immobilized amino acid for solid-phase synthesis of dehydropeptides.

Solid-phase synthesis using *tert*-butyl 3-selenocyanatobenzoate (**3a**) was demonstrated by the following synthesis of RGD-conjugated dehydropeptide. The RGD sequence is the common recognition motif for the integrin family of receptors, which are involved in cell–cell and cell–matrix adhesion,[15] and it was reported that β-turn conformation was important for the binding.[16] To the seleno ether **4a**, deprotection was achieved by the action of 95% TFA in quantitative yield. The resultant benzoic acid was immobilized to a commercially available aminoethyl resin (Calbiochem-Novabiochem, NovaSyn™ TG amino resin; amino content 0.29 mmol/g) with HATU and DIEA. The amount of seleno amino acid to load on solid support was determined by Fmoc quantitation[17,18] (0.14 mmol/g). This amount was the basis on which the molar scale was calculated for the following reactions.[19] After the C-terminal deprotection by palladium chemistry (20 mol% $Pd(PPh_3)_4$ and excess dimedone in THF), the C-terminal condensation was achieved by phenylalanyl alanine methyl ester hydrochloride and HATU-DIEA to give a protected tripeptide on the solid support **6**. This was followed by N-terminal elongation using Pioneer™ peptide synthesizer (Applied Biosystems Inc.). Automatic synthesis was performed by successive coupling with Fmoc-Asp(^{t}Bu)-OH, Fmoc-Gly-OH and Fmoc-Arg(Pfb)-OH by repeating the HATU chemistry using built-in protocols.[20] After the capping reaction, the acetylated hexapeptide was obtained on solid support **7**.

Finally, oxidative cleavage from the resin via selenoxide was performed by treatment with hydrogen peroxide in THF to give the desired RGD-conjugated dehydropeptide[11] **8** with concomitant formation of a double bond, which was easily purified by simple chromatography on silica gel. The yield (77%) was calculated on the basis of the loading amount determined for the starting immobilized amino acid. The final reaction was carried out without loss of any protecting groups for further chemical modification after appropriate deprotection (Scheme 2).

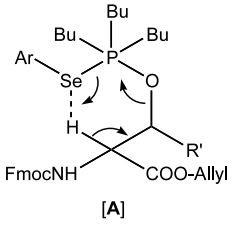
In conclusion, we have demonstrated the potentiality of the newly developed strategy to synthesize dehydropeptides, as well as the potentiality of our new selenium-containing linker. We successfully synthesized the model dehydropeptide by both manual (C-terminal) and automatic (N-terminal) procedures. We consider that this new synthetic methodology will also be quite useful in the preparation of unsaturated compounds using solid-phase chemistry.





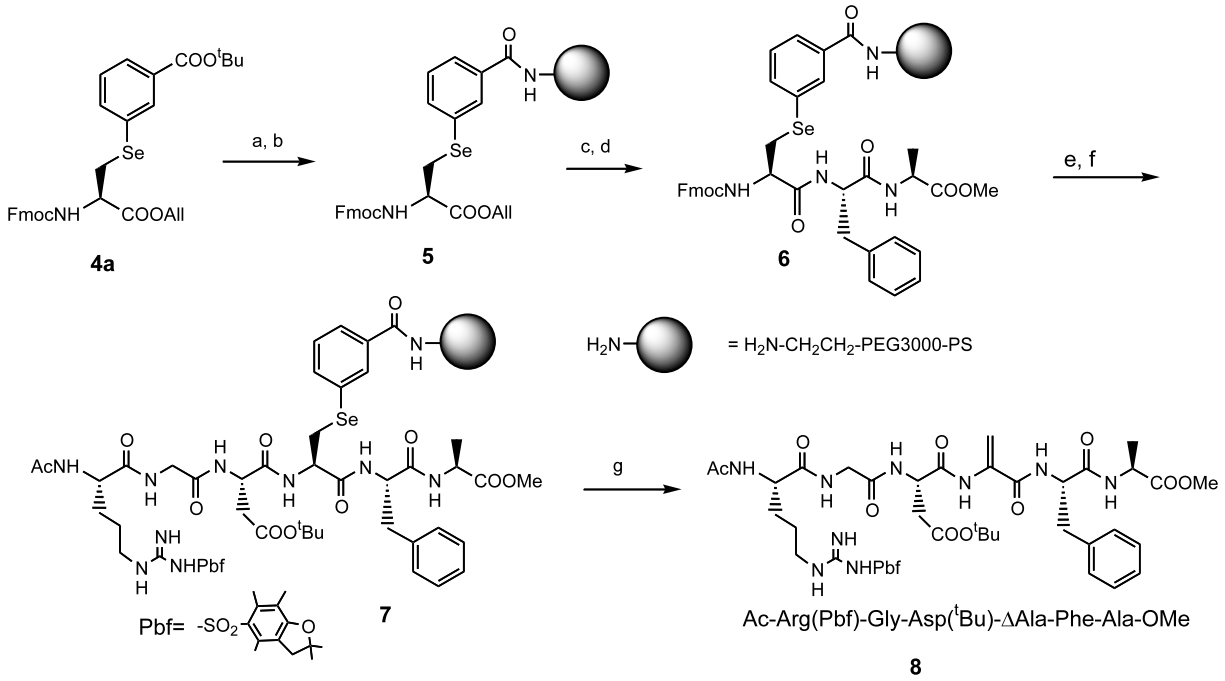
Yield of Selenoether **4**	**3a**	**3b**	**3c**
Fmoc-L-Ser-O-Allyl	74 %	11 %	n.t.*
Fmoc-L-Thr-O-Allyl	0 %†	0 %†	0 %†
Fmoc-D-allo-Thr-O-Allyl	55 %	0 %†	14 %†

*: not tested; †: dehydroamino acid was obtained in 60~80 % yield



[**A**]

Figure 1. Synthesis of selenoether.



Scheme 2. Synthesis of model dehydropeptide. *Reagents and conditions*: (a) TFA; (b) H_2N-resin, HATU, DIEA; (c) $Pd(PPh_3)_4$, dimedone/THF; (d) HCl Phe-Ala-OMe, HATU, DIEA/DMF; (e) Fmoc-Asp(^{t}Bu), Fmoc-Gly, Fmoc-Arg(Pbf)/HATU-DIEA by Pioneer™ Peptide Synthesizer; (f) Ac_2O–DIEA/DMF; (g) H_2O_2 aq./THF overnight.

Acknowledgements

The authors thank Professor Shigeru Nishiyama of Keio University for the HRMS measurements of our products.

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13. Selected data of the key compounds. Compound **4a**: mp 37–38°C; HRMS (EI) calcd for $C_{12}H_{14}NO_2{}^{80}Se$ m/z 284.0188, found m/z 284.0187; IR (film): 1714, 1569, 1369, 1302, 1163, 1127, 1012, 848, 748 cm^{-1}; ^1H NMR δ ($CDCl_3$): 1.61 (9H, s) 7.48 (1H, t, $J=8.0$ Hz), 7.82 (1H, d, $J=8.0$ Hz), 8.05 (1H, d, $J=8.0$ Hz), 8.23 (1H, s). Compound **5a**: $[\alpha]_D^{23}$ 25.2° (c 1.00, $CHCl_3$); HRMS (EI) calcd for $C_{32}H_{34}NO_6{}^{78}Se$ m/z 606.1558, found m/z 606.1584; IR (film): 3342, 1713br, 1507, 1450, 1413, 1368, 1300, 1126, 989, 936, 848, 743 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.57 (9H, s), 3.40 (1H, dd, $J=4.9$, 13.6 Hz), 3.47 (1H, dd, $J=4.6$, 13.6 Hz), 4.20 (1H, t, $J=7.0$ Hz), 4.33 (3H, complex), 4.53 (1H, dd, $J=5.5$, 12.8 Hz), 4.77 (1H, dist. dt, $J=4.6$, 7.9 Hz), 5.22 (1H, br. d, $J=10.3$ Hz), 5.27 (1H, br. d, $J=19.0$ Hz), 5.63 (1H, br. d, $J=7.9$ Hz), 5.80 (1H, complex), 7.25–7.35 (3H, arom.), 7.40 (2H, arom.), 7.56 (2H, arom.), 7.70 (1H. arom.), 7.70 (2H, arom.), 7.86 (1H, arom.), 8.17 (1H, s, arom.). Compound **9**: MALDI TOF-MS calcd for $C_{47}H_{67}N_9O_{13}S{\cdot}Na$ $(M+Na)^+$ m/z 1020.45, found m/z 1020.32; $[\alpha]_D^{22}$ −14.3° (c 1.00, $CHCl_3$);

^{1}H NMR ($CDCl_3$, selected) δ 1.40 (3H, s), 1.41 (3H, d, overlapped), 1.41 (3H, s), 1.42 (3H, s), 1.45 (9H, s), 2.08 (3H, s), 2.49 (3H, s), 2.56 (3H, s), 2.94 (3H, s), 3.69 (3H, s), 5.24 (1H, br.s), 6.33 (1H, br.s), 7.15–7.25 (5H, arom.). This compound was unstable during SiO_2 chromatography (MeOH–$CHCl_3$ 1:9) by formation of an MeOH adduct.

14. The detailed reaction mechanism and selectivity will be discussed elsewhere.
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18. Loading yield (52%) was calculated based on the amount of Fmoc-Ser-OAll, and it was in harmony with weight-based yield (51%). However, Keiser ninhydrin test[17] indicated very low concentration of remaining reactive amino groups (2.8 μmol/g). Thus, the authors did not carry out any capping reaction, and the following reactions were carried out using this loading amount.
19. The peptide elongation was carried out at 0.03 mmol scale.
20. Using 'extended cycle', Fmoc deprotection 5 min, coupling 60 min.