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Tetrahedron Letters 45 (2004) 99–102
Tetrahedron Letters

Synthesis of an olefin-containing cyclic peptide using the solid-phase Horner–Emmons reaction

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Received 2 October 2003; revised 16 October 2003; accepted 20 October 2003

Abstract—Linear and cyclic olefin peptides containing the substrate sequence for human T-cell leukemia virus type-1 (HTLV-1) were efficiently synthesized on a solid support using the Horner–Emmons reaction. The precursor peptide aldehyde was prepared by oxidation of the corresponding peptide alcohol with Dess–Martin periodinane. The oxidation reaction proceeded quantitatively on a cross-linked ethoxylate acrylate resin (CLEAR) support instead of a polystyrene-based support. Cyclization on the solid support was achieved via an amide bond formation mediated by EDC/HOAt to yield a single major product. The linear olefin peptide was cleaved by HTLV-1 protease at the scissile site, whereas the cyclic olefin peptide functions as a competitive inhibitor rather than a substrate.

Olefin-containing peptides have been applied mainly for the preparation of cyclic peptide analogues, and for the preparation of peptide mimetics with (*E*)-alkene amide bond replacements. The former cyclization reaction is employed for the restriction of conformational flexibilities, which will influence the biological activities of the corresponding linear precursor, more or less.[1] One of the most frequently used reactions for this approach is the ruthenium-catalyzed ring-closing metathesis reaction of Grubbs.[2] Although the reaction can be achieved on solid support, the reaction efficiency is remarkably affected by secondary structures of the precursor peptides.[3] In the latter application of olefin peptides to the preparation of peptide bond mimetics, (*E*)-alkene dipeptide isosteres were usually synthesized by a solution-phase method,[4] and thus few applications to the solid-phase synthesis of peptide mimetic inhibitors have been reported.[5] In our ongoing effort to develop human T-cell leukemia virus type 1 (HTLV-1) protease inhibitors,[6] convenient solid-phase procedures are being developed to prepare substrate and inhibitor libraries based on linear and cyclic olefin-containing peptides. Herein, we report the solid-phase synthesis of a cyclic olefin-containing peptide using the Horner–Emmons reaction, and effects of the cyclization on the cleavage by HTLV-1 protease.

To incorporate an olefin bond into the peptide backbone, we selected the Horner–Emmons reaction on a solid support, which had been successfully applied to the preparation of small compound libraries.[7] The necessary aldehyde could be obtained by oxidation of the corresponding alcohol using Dess–Martin periodinane, a mild and convenient oxidizing reagent.[8] We selected the alcohol derivative as a suitable precursor since the necessary peptide alcohol can be constructed easily by Fmoc-based solid-phase peptide synthesis (SPPS). As the target compounds, we selected olefin peptides containing the substrate sequence for HTLV-1 protease to evaluate the effects of cyclization on the substrate cleavage.

Based on the above principle, we prepared olefin-containing peptides on a solid support according to the

Abbreviations: AM, aminomethyl; DCC, dicyclohexyl carbodiimide; DIEA, diisopropylethylamine; DIPCDI, diisopropylcarbodiimide; DPPA, diphenylphosphoryl azide; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodi imide; HATU, 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo-[4,5-*b*]pyridinium hexafuluorophosphate 3-oxide; HBTU, 1-[bis(dimethylamino)methylene]-1*H*-benzotriazolium hexafuluorophosphate 3-oxide; HOAt, 7-aza-1-hydroxybenzotriazole; HOBt, 1-hydroxy benzotriazole; MBHA, 4-methylbenzhydryl amine; PyBroP, benzotriazol-1-yl-N-oxy-tris(pyrrolidino) phosphonium hexafluorophosphate; TFA, trifluoroacetic acid.

Keywords: Olefin peptide; Cyclic peptide; Solid phase synthesis; Horner–Emmons reaction; HTLV-1 protease.

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doi:10.1016/j.tetlet.2003.10.093

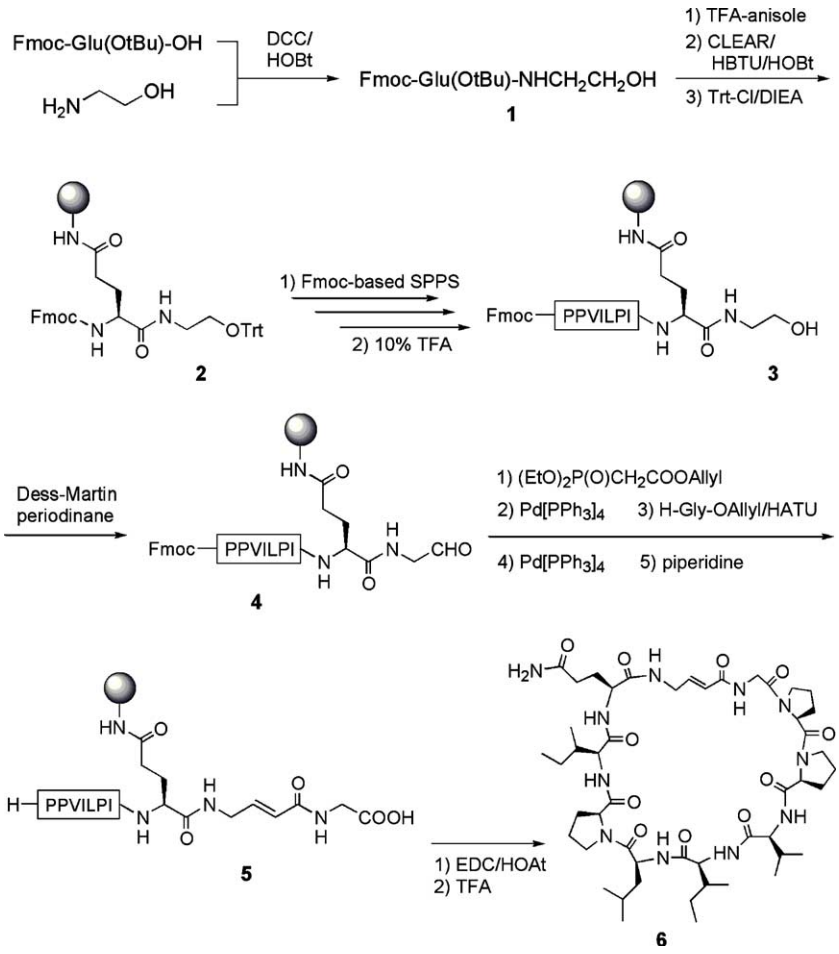
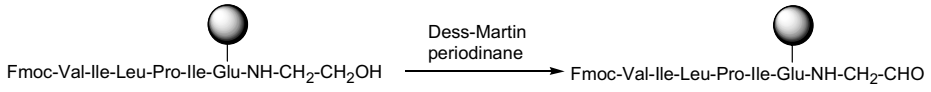


Figure 1. Synthetic scheme for the cyclic olefin peptide.

route shown in Figure 1. Coupling of Fmoc-Glu(O*t*-Bu)-OH with ethanol amine using DCC/HOBt furnished the alcohol derivative **1**, which was then treated with TFA–anisole to remove the side chain *t*-Bu group. The product was linked to a solid support through the side-chain γ-carboxyl group of Glu. The hydroxyl group was then protected with a trityl group to yield the starting resin **2**. As the solid support, three commercially available resins (MBHA-polystyrene, AM-polystyrene, and CLEAR[9]) were selected to find the most suitable conditions for the solid-phase synthesis of olefin peptides. Fmoc-protected amino acids were introduced successively by carbodiimide-mediated coupling in the presence of HOBt. The terminal trityl group was then removed by brief treatment with 10% TFA to yield the peptide alcohol resin **3**.[10]

Prior to the olefin bond formation, we examined the reaction conditions to convert peptide alcohol to aldehyde on the solid support using the intermediate hexapeptide alcohol resin[11] (Table 1). Solid-phase oxidation using Dess–Martin periodinane proceeded sluggishly even at 40 °C when the polystyrene-based support was employed. A prolonged reaction at the elevated temperature lowered the yield due to the partial cleavage of the peptide chain, which is presumably mediated by

Table 1.



Dess–Martin (equiv)	MBHA-PS resin (40 °C in DCM)				AM-PS resin (40 °C in DCM)				CLEAR (25 °C in DCM)			
	1 h	3 h	6 h	12 h	1 h	3 h	6 h	12 h	1 h	3 h	6 h	12 h
2		No	No	No	No	5	Trace	No	10	15	25	25
5	No	No	Trace	No		21	14	4	30	80	75	50
10	No	No	Trace	16			10	Trace	30	85		
15									90	90		

AcOH derived from the periodinane. In contrast, a nearly quantitative conversion to the aldehyde was achieved on the CLEAR support within 3 h, probably due to the high swelling properties of CLEAR. The reaction can be conducted at room temperature without the loss of peptide chain, which makes the application of an acid-labile linker feasible. Thus, we used a TFA-labile 4-(2,4-dimethoxyphenyl-Fmoc-aminoethyl) phenoxyacetyl linker for CLEAR support instead of a TFA-stable MBHA or AM linker for polystyrene resin. The presence of a resin-bound aldehyde group was detected by a specific color test using 4-amino-3-hydraino-5-mercapto-1,2,4-triazole (Purpald).[12]

According to the above results, the peptide alcohol on CLEAR support was treated with Dess–Martin periodinane (15 equiv) at 25 °C for 3 h. The resulting peptide aldehyde resin was then reacted with allyl diethylphosphono acetate (20 equiv) in the presence of lithium bis(trimethylsilyl) amide $[(CH_3)_3Si]_2NLi$, 1.0 M in THF, 4 equiv) at 25 °C for 3 h. The product has an N-terminal Fmoc group and C-terminal allyl group, and thus the olefin peptide chain can be elongated in both directions, selectively. The C-terminal allyl group was removed by treatment with tetrakis(triphenylphosphine)palladium(0), $[(C_6H_5)_3P]_4Pd$. To the resulting resin, H-Gly-OAllyl was coupled using HATU. The N-terminal Fmoc group and C-terminal allyl group were then removed by treatment with $[(C_6H_5)_3P]_4Pd$ and piperidine, respectively, to yield the linear olefin peptide resin **5**.

Finally, the linear olefin peptide was cyclized on solid support by an amide bond formation. Among the coupling reagents examined (DPPA, PyBroP, HATU, and the carbodiimide/additive system), the EDC/HOAt system efficiently yielded a cyclized product suppressing the formation of the dimer and polymers.

The progress of a series of solid-phase reactions was monitored with an analytical HPLC after cleavage from the resin with TFA. As shown in Figure 2, each product showed a single major peak on HPLC,[13] which clearly showed that a nearly quantitative conversion was achieved at each step to give a cyclized product as a single major product after muti-step reactions on solid support (overall yield calculated from the starting resin; 11.4%). When an alternative cyclization involving an olefin bond formation was employed,[14] the desired cyclic olefin peptide was also obtained; but the product, after cleavage from the resin, gave a complex mixture. Thus, the cyclization via an amide bond formation was more efficient than that via an olefin bond formation.

Digestion of the linear and cyclic olefin peptides was examined qualitatively using the auto-digestion resistant HTLV-1 protease mutant.[15] The cleavage reaction was monitored with an analytical HPLC according to a published procedure.[6,15] After 18 h incubation with the protease, the linear olefin peptide derived from **5** was cleaved quantitatively at the scissile Leu-Pro site to yield the expected fragment peptide on HPLC, whereas substantially all of the cyclic olefin peptide **6** was kept intact. In addition, the cleavage level of the linear olefin peptide decreased to 38% after 18 h incubation with the protease in the presence of the cyclic olefin peptide. These preliminary results strongly suggest that the cyclic olefin peptide **6** functions as a competitive inhibitor of the digestion of substrate by the HTLV-1 protease.

In conclusion, we have demonstrated that linear and cyclic olefin-containing peptides could be efficiently prepared by a combination of Dess–Martin oxidation and the Horner–Emmons reaction on a solid support. Application of this method to the preparation of inhibitors containing (*E*)-alkene dipeptide isostere is currently in progress.

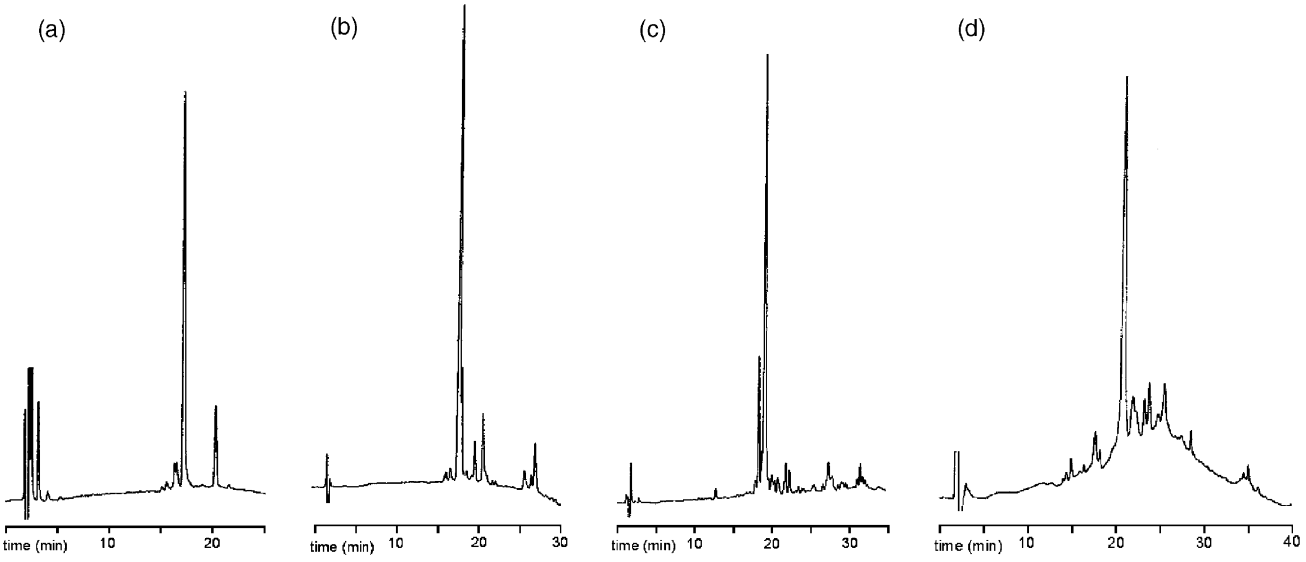


Figure 2. HPLC profiles of the crude products: (a) peptide alcohol from **3**; (b) peptide aldehyde from **4**; (c) linear olefin peptide from **5**; (d) cyclic olefin peptide **6**.

References and Notes

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10. Typical experimental procedures using 4-(2,4-dimethoxy phenyl-Fmoc-aminoethyl) phenoxyacetyl CLEAR are as follows: Fmoc-Glu-$NHCH_2CH_2OH$ (1.2 equiv), HBTU (2.0 equiv), HOBt (2.0 equiv), and DIEA (2 equiv) were added to the swollen resin (0.36 mmol/g) in CH_2Cl_2, and the mixture was agitated at 25 °C for 3 h. Kaiser ninhydrin test of the resulting resin was negative. Four equivalents each of Trt-Cl and DIEA were added to the resin in CH_2Cl_2, and the mixture was agitated at 25 °C for 30 min. After washing the resin with CH_2Cl_2, the same reaction was repeated. Peptide chain elongation was conducted using 5 equiv each of Fmoc-amino acid, DCC, and HOBt in DMF for 40 min/cycle. Cleavage of the C-terminal Trt group was carried out by duplicated 10 min treatment with 10% TFA/CH_2Cl_2 in the presence of triisopropylsilane.
11. Conversion yields (%) listed in Table 1 were estimated using an analytical HPLC of the crude products cleaved from the corresponding resins with TFA.
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13. HPLC was carried out using a cosmosil 5C18 AR column (4.6×150 mm) eluted with a gradient of CH_3CN in 0.1% aq TFA at a flow rate of 1.0 mL/min. Compound **3**: m/z $[M+Na]^+$ 1163.53 (calcd 1163.65 for $C_{60}H_{88}N_{10}O_{12}Na$). HPLC retention time 17.80 min (CH_3CN 30–70%, 45 min). Compound **4**: m/z $[M+Na]^+$ 1162.78 (calcd 1162.37 for $C_{60}H_{86}N_{10}O_{12}Na$). HPLC retention time 17.43 min (CH_3CN 30–70%, 45 min). Compound **5**: m/z $[M+H]^+$ 1016.26 (calcd 1016.61 for $C_{49}H_{82}N_{11}O_{12}$). HPLC retention time 19.01 min (CH_3CN 15–50%, 60 min). Compound **6**: m/z $[M+H]^+$ 998.40 (calcd 998.60 for $C_{49}H_{80}N_{11}O_{11}$). HPLC retention time 20.63 min (CH_3CN 15–70%, 45 min).
14. The necessary linear precursor, $(EtO)_2P(O)CH_2CO$-Gly-Pro-Pro-Val-Ile-Leu-Pro-Ile-Glu(CLEAR)-$NHCH_2CHO$, was prepared by the successive condensations of Fmoc-Gly-OH and diethylphosphono acetic acid to H-Pro-ProVal-Ile-Leu-Pro-Ile-Glu(CLEAR)-$NHCH_2CH_2O$Trt followed by detritylation and Dess–Martin oxidation.
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