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Macrocyclization on Solid Support using Heck Reaction

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Abstract: A novel intramolecular macrocyclization reaction on solid support using Heck reaction was achieved. The Heck coupling of acrylic acid amide to 3-iodobenzylamine on solid support proceeds smoothly to yield a cyclic tetrapeptide derivative containing a new 3-substituted cinnamic acid template and Arg-Gly-Asp sequence. The macrocyclization reaction takes place more rapidly on solid support than in solution. © 1997 Elsevier Science Ltd.

Chemical synthesis on a solid support has been predominantly used to prepare biopolymers such as peptides or oligonucleotides. Recently, this technology has been extended beyond traditional biopolymers to the synthesis of general organic molecules to prepare chemical libraries of diverse functionalities in combinatorial organic synthesis[1]. For solid phase synthesis to be useful in generating diversity, however, the repertoire of carbon-carbon bond forming reactions that can be efficiently carried out on solid support should be increased. Here, we report a novel intramolecular macrocyclization reaction on solid support using Heck reaction.

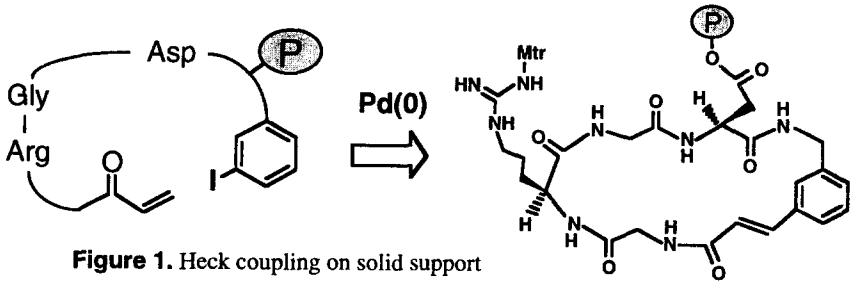

Figure 1. Heck coupling on solid support

We selected intramolecular macrocyclization as a suitable reaction that could be carried out efficiently on solid support rather than in solution because of the "pseudo-dilution" effect[2]. This specific effect due to the polymeric solid support has been used successfully for macrocyclization by disulfide bond formation in peptide chemistry[3]. In contrast to these solid phase results, infinite dilution is generally inevitable in solution phase reactions to obtain reasonable reaction efficiency of the monomeric product. For macrocyclization, we used Heck reaction[4], a palladium-mediated vinylation of organic halide, as a suitable carbon-carbon bond

forming reaction. The reaction does not require anhydrous or inert atmosphere conditions and proceeds readily at room temperature in the presence of phase transfer agent[5].

Based on the above approach, we synthesized a cyclic tetrapeptide derivative using the Heck coupling of acrylic acid amide to a 3-iodobenzyl amine moiety on solid support (Figure 1). The cyclic derivative contains a new 3-substituted cinnamic acid template to construct the rigid cyclic structure and Arg-Gly-Asp (RGD), a tripeptide sequence known to bind to the glycoprotein IIb/IIIa (GP IIb/IIIa)[6]. GPIIb/IIIa is a membrane protein expressed on the surface of activated platelets which binds to fibrinogen to cause platelet aggregation. Thus, RGD-containing derivatives with affinities for GPIIb/IIIa could be effective drug candidates to prevent the formation of platelet-rich clots[7].

Our synthetic scheme is outlined in Figure 2. Coupling of Fmoc-Asp(OtBu)-OH **1** with 3-iodobenzylamine by a 60 min reaction using CIP/HOAt[8] furnished the amide **2**, which was then treated with TFA-anisole to remove the side chain tBu group. The product was linked to a 2-chlorotrityl resin (Clt-resin)[9] through the side-chain β-carboxyl group of Asp to yield the starting resin [substitution; 0.224 mmol/g resin].

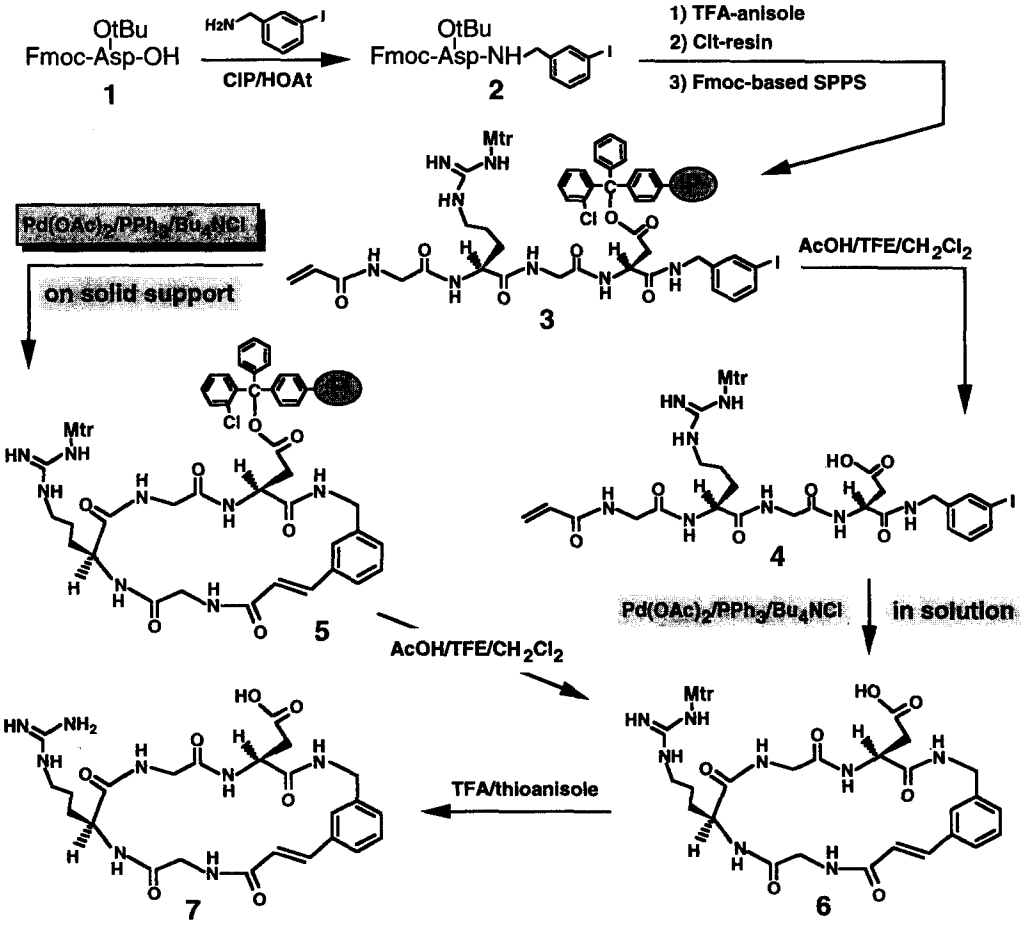

Figure 2. Synthetic scheme for cyclic tetrapeptide derivative **7**

To this resin, Fmoc-Gly-OH, Fmoc-Arg(Mtr)-OH, Fmoc-Gly-OH, and acrylic acid monomer were successively introduced by the standard Fmoc-based SPPS to yield the protected peptide resin **3**[10]. The quantitative incorporation of each amino acid was confirmed by amino acid analysis of the acid hydrolysate of **3**. Prior to the on-resin macrocyclization, an aliquot of product **3** was subjected to cleavage reaction to examine the purity of the linear precursor; the linear product **4** with a single main peak on analytical HPLC was obtained by treatment of **3** with a mixture of AcOH-TFE-CH_2Cl_2 (1:1:8, 25°C, 40 min) [11].

Palladium(0)-mediated macrocyclization of **3** employing $Pd(OAc)_2$ with Ph_3P and Bu_4NCl in a DMF/H_2O/Et_3N solvent system was carried out at 37°C for 4 h to provide **5**[12]. The cyclized peptide resin **5** was then treated with AcOH-TFE-CH_2Cl_2 (1:1:8, 25°C, 40 min) to cleave the protected cyclic tetrapeptide **6** from the resin support[11]. Without further purification, the cyclic product **6** was treated with TFA-thioanisole at 25°C for 3 h to remove the Mtr group from the Arg side chain. The deprotected product **7** was purified by preparative HPLC, and the homogeneous product was obtained in 30 % overall yield (calculated from the starting resin). The purified cyclic derivative **7** exhibited a single sharp peak on analytical HPLC and was shown to be a monomer by fast atom bombardment-mass spectrometry (FAB-MS)[13].

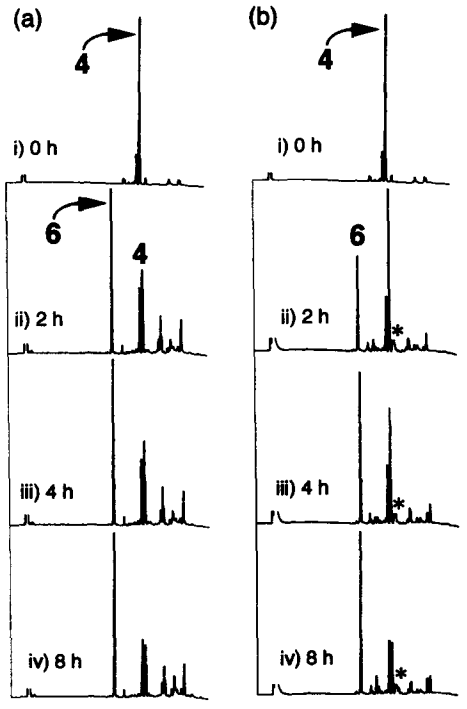

Figure 3.

HPLC profile of the cyclized products on solid support or in solution.
a) on solid support. b) in solution.
* derived from Ph_3P. HPLC on YMC AM302 [CH_3CN (20 % to 80 %, 30 min) in 0.1 % aq. TFA, 0.9 ml/min.]

These successful results on solid phase intramolecular macrocyclization prompted us to investigate cyclization efficiency on solid support in comparison with that in solution phase. The linear precursor **4** in DMF/H_2O/Et_3N was treated with $Pd(OAc)_2$/Ph_3P/Bu_4NCl under the same reaction conditions as described in the above solid phase reaction[12]. An aliquot was periodically taken from the reaction mixture and the progress of the reaction was examined by analytical HPLC. For comparison, the cyclization reactions on solid support were also conducted for 2, 4, and 8 h under the same conditions. Each resin was then treated with AcOH-TFE-CH_2Cl_2 (1:1:8, 25°C, 40 min) and the detached product was analyzed similarly using HPLC.

The intramolecular cyclization in solution proceeded in proportion to the reaction time, but was relatively slow. Most of the precursor **4** was converted to the cyclized product **6** after the 8 h reaction (Figure 3-b). In contrast, the same cyclization reaction occurred rapidly on solid support as shown in Figure 3-a. Most of the precursor was converted to the product within 2 h and the time course of the reaction was quite different with that of solution phase reaction. The results clearly showed that the Pd(0)-mediated intramolecular macrocyclization described in this paper is a unique reaction especially suitable for solid phase organic synthesis.

In conclusion, we have demonstrated that the Heck reaction can be used to prepare macrocyclic derivatives on solid support. The mild reaction conditions and high efficiency allow the application of this procedure to combinatorial library synthesis for designing high affinity ligands of GPIIb/IIIa.

Abbreviations: tBu=tert-butyl, CIP=2-chloro-1,3-dimethylimidazolidium hexafluorophosphate, DIPCDI =diisopropylcarbodiimide, Fmoc=fluoren-9-ylmethyloxycarbonyl, HOAt=1-hydroxy-7-azabenzotrizazole, HOBt=1-hydroxybenzotriazole, MALDI=matrix-assisted laser desorption ionization, TOF MS=time of flight mass spectrometry, Mtr=4-methoxy-2,3,6-trimethylbenzenesulfonyl, SPPS=solid phase peptide synthesis, TFA=trifluoroacetic acid, TFE=trifluoroethanol.

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10. N^{α}-Fmoc group was removed by 20 % piperidine/DMF (25°C, 20 min). Each coupling was conducted with Fmoc-amino acid, HOBt, and DIPCDI (2.5 equiv. each) in DMF at 25°C for 90 min. The coupling reaction was monitored by Kaiser ninhydrin test. Amino acid analysis after 12N HCl-propionic acid hydrolysis of **3**: Asp 0.97, Gly 1.98, Arg 1.00.
11. The product was identified by amino acid analysis, analytical HPLC and mass spectrometry. Compound **4**: Amino acid analysis; Asp 1.07, Gly 1.88, Arg 1.00. FAB-MS; *m/z* $[M+H]^+$ 885.2091 (Calcd. 885.2102 for $C_{34}H_{46}N_8O_{10}SI$). HPLC on a YMC AM302 (4.6 x 150 mm); retention time 17.59 min, 20 % to 80 % CH_3CN (30 min) in 0.1 % aq.TFA, 0.9 ml/min. Compound **6**: Amino acid analysis; Asp 1.00, Gly 2.05, Arg 0.88. MALDI TOF-MS; *m/z* $[M+H]^+$ 757.407 (Calcd. 757.297 for $C_{34}H_{45}N_8O_{10}S$). HPLC on a YMC AM302 (4.6 x 150 mm); retention time 13.60 min, 20 % to 80 % CH_3CN (30 min) in 0.1 % aq.TFA, 0.9 ml/min.
12. To the protected peptide resin **3**, $Pd(OAc)_2$, PPh_3, and Bu_4NCl (0.4 equiv. each) in DMF-H_2O-Et_3N (9:1:1, 8 ml/g resin) were added in one portion. The mixture was agitated at 37°C for 4 h. The resin was filtered, washed extensively with DMF and MeOH, and dried *in vacuo*. Amino acid analysis after 12N HCl-propionic acid hydrolysis of the product: Asp 1.00, Gly 1.94, Arg 0.95.
13. Amino acid analysis; Asp 1.00, Gly 2.03, Arg 0.96. FAB-MS; *m/z* $[M+H]^+$ 545.2469 (Calcd. 545.2472 for $C_{24}H_{33}N_8O_7$). HPLC on a YMC AM302 (4.6 x 150 mm); retention time 10.22 min, 10 % to 20 % CH_3CN (30 min) in 0.1 % aq.TFA, 0.9 ml/min. *trans*-configuration of the product **7** was defined by ^1H NMR analysis. Details of the configuration and conformational analysis of **7** will be published elsewhere.

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