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Glutamic acid as a new linker for attachment of alcohols to solid support

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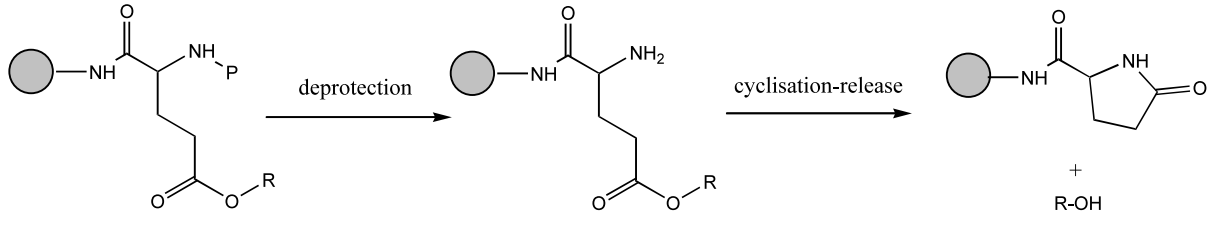
Abstract—We took advantage of pyroglutamic cyclization of glutamic acid to investigate the use of this amino acid as a linker for the attachment of alcohols. As a first example, we reported the solid-phase synthesis of dimers on a 3,3′-diaminodiphenylmethanol.

The use of solid-phase synthesis for the generation of diverse libraries of small organic compounds have been extensively used in the search of leading compounds for drug discovery.[1,2] As part of a project to synthesize on solid support libraries of dimers possessing an alcohol functionality, we focused our efforts towards the design of a new linker generation for such alcohols.[3] Numerous linkers have been already reported for the alcohol attachments;[4] nevertheless they have to be cleaved in well defined conditions dictating the protecting conditions strategy. A safety catch system, involving first activation of the linker and cleavage in a second step, appeared to be efficient for versatile library strategy.

Atrash et al.[5] have described a safety catch linker strategy for carboxylic acid based on diketopiperazine formation. To design a new linker,[6] we took advantage of another well know side reaction of peptide synthesis, namely pyroglutamic cyclization. The strategy we have followed is presented in Scheme 1. The alcohol moiety is attached to the glutamic acid side chain through an ester linkage. The α-amino group is protected as an urethane. At the end of the solid-phase synthesis, removal of the urethane protection followed by basic treatment, induced the γ-lactam cyclization and subsequent release of the alcohol function. The pyroglutamic moiety remains on the solid support with the desired product into solution.

To validate this approach, we used this strategy to synthesize dimers of a 3,3′-diaminodiphenylmethanol (Scheme 2). As a first proof of concept, we decided to use Boc-protected amino acids for dimer elongation and the orthogonal Fmoc protection of the linker amino group.

Fmoc-Glu(O*t*Bu)-OH was coupled to the polystyrene MBHA resin (0.8 mmol/g) using BOP as coupling reagent in presence of DIEPA in dimethylformamide.



Scheme 1. Stategy of glutamic acid linker cleavage.

Keywords: glutamic acid; linker; solid-phase synthesis; combinatorial chemistry.

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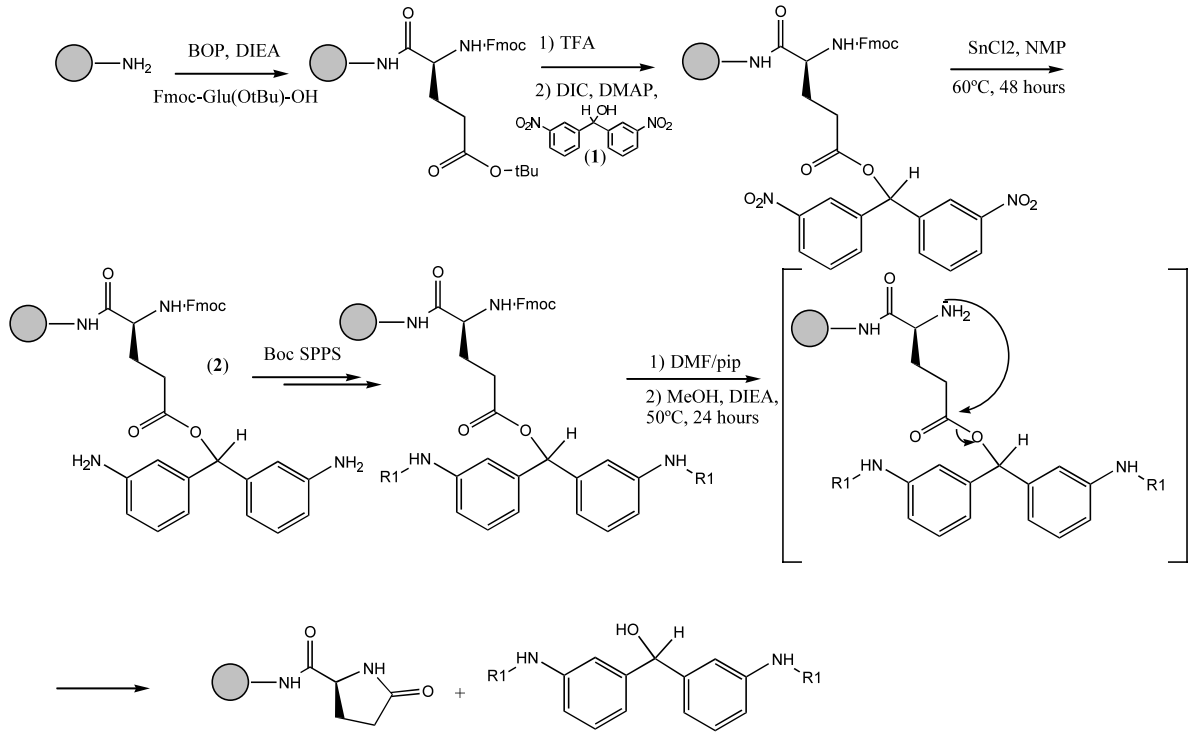


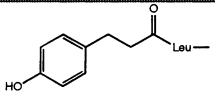
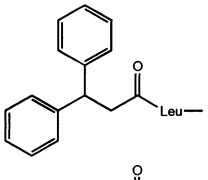
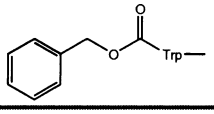
Scheme 2. Synthesis of 3,3′-diaminodiphenylmethanol dimers using the glutamic acid linker.

After capping the unreacted amino functions using acetic anhydride, O*t*Bu side-chain protection of glutamic acid was removed in acidic conditions using TFA/DCM 1/1 mixture to generate the free carboxylic acid on the resin. Diaminoalcohol moiety is anchored on solid support as 3,3′-dinitrophenylmethanol (**1**). This compound is directly obtained from reduction of commercially available dinitrobenzophenone using sodium borohydride ($NaBH_4$) in methanol for 60 min (88% yield). Loading of **1** on the resin was performed by diisopropylcarbodiimide (DIC) in the presence of dimethylaminopyridine (DMAP) in dichloromethane for 12 h. Nitro functions were reduced into the corresponding amino groups using tin(II) chloride ($SnCl_2$) in *N*-methyl pyrrolidone (NMP) at 50°C for 2 days to yield resin **2**.

The synthesis was performed simultaneously on the two amino function of **2** using standard Boc-strategy. BOP/DIEA were used for coupling steps and *N*-Boc protections were removed using TFA/DCM 1/1 solution. Nihydrine test[7] was used for the monitoring of coupling reactions.

The cleavage from the resin was performed in two steps. First, the Fmoc protecting group of the amino function of glutamic acid was removed by DMF/piperidine (80/20) solution treatment for 20 min. Then the resin was quickly washed with DMF, methanol and DCM to minimize premature lactam formation in basic conditions. Afterward, the cyclization was done in methanol/DIEA 95/5 at 60°C for 12 h. Resin was removed by

Table 1. Yield and purity of dimers **3**–**5**

Dimer	R1	Yield[a] (%)	Purity[b] (%)
(3)		65	72
(4)		79	92
(5)		80	82

[a] Percentage calculated on the basis of theoretical resin loading. [b] Percentage calculated from reversed-phase HPLC spectra at 220 nM.

filtration and solvent was concentrated under vacuum. Dimers **3**–**5** were obtained with at least 65% yield calculated from resin **2** theoretical loading and with purity ranging from 72 to 92%[8] (Table 1). Compounds were characterized by mass spectrometry ES+ on a Micromass Platform II spectrometer.

In conclusion, we have developed a new linker strategy based on lactam formation of glutamic acid to anchor alcohols on solid support. The supported linker is easily and quickly prepared from commercially available protected glutamic acid and MBHA resin. The alcohol is removed from the resin by a two-step treatment initiated with the amino group deprotection of glutamic acid followed by pyroglutamic cyclization. Our strategy has been successfully applied to the synthesis of dimers using Boc SPPS and Fmoc protection of the amino group of glutamic acid. Nevertheless, the principle of glutamic linker is versatile enough to be applied to other synthetic conditions by using appropriate *N*-protection of glutamic acid (Alloc, Boc,…).

The application of this strategy to synphase™ lanterns for the generation of libraries of alcohols as well as the evaluation of other protecting groups for glutamic acid is under investigation.

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6. While the writing of this paper, we were advised of a poster communication presented at the 27th European Peptide Symposium in Sorrento (Italy) August 31, September 6, 2002, by T. Wakamiya et al. about the use of glutamic acid linker.
7. Kaiser, E.; Collescot, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1967**, *40*, 2164–2167.
8. Purity was estimated by HPLC analysis at 220 nm based on peak areas.