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Symmetrical Structure Allowing the Selective Multiple Release of a Defined Quantity of Peptide from a Single Bead of Polymeric Support

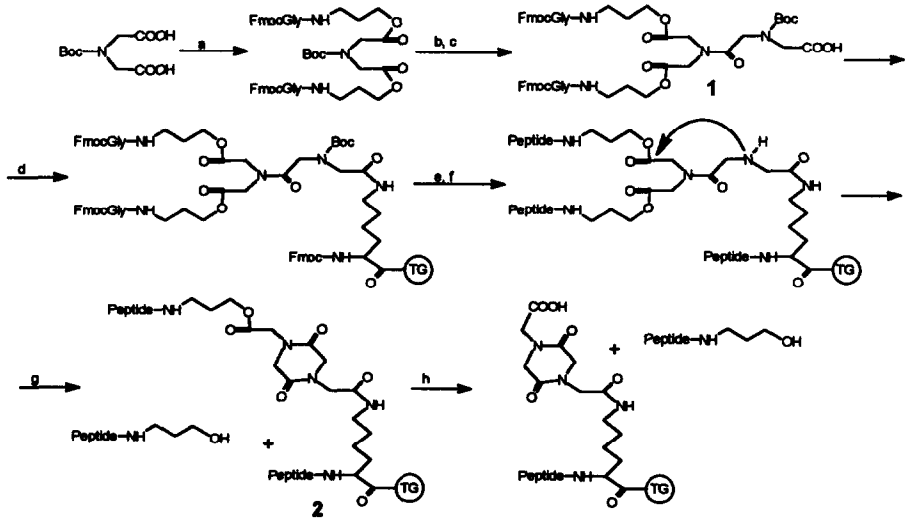
Petr Kočiš, Viktor Krchňák and Michal Lebl*

Selectide Corporation, 1580 E. Hanley Blvd., Tucson, AZ 85737, U.S.A.

Abstract: A linker based on the combination of two molecules of iminodiacetic acid that allows for the release of peptide molecule from the polymeric bead in two equimolar portions in two distinct steps was designed and synthesized and its predicted properties were verified.

The methodology of synthesizing libraries of peptides (or non-peptide structures[1]) and testing for activity on solid phase support[2,3] was significantly extended by the application of selectively cleavable linkers. These linkers allow for the screening based on the determination of biological activity in solution.[4,5] The originally described linker combination[4] is based on a spontaneous formation of diketopiperazine structure[6] in the first step of the release and alkaline hydrolysis or ammonolysis[7] as the second stage release mechanism. The originally described linker allows the release of peptides of the same structure in both steps,[4] however, synthesis of the linker is complicated and employs expensive selectively protected derivatives of amino acids. Our search for a simpler and more convenient linker which exhibits the same features resulted in structure 1 illustrated in Scheme 1.

Scheme 1



a) Fmoc-Gly-NH-$(CH_2)_3$-OH/DIC/DMAP; b) TFA; c) Boc-N$(CH_2$-CO$)_2$O; d) Fmoc-Lys-TG/DIC/HOBt; e) Peptide synthesis; f) TFA; g) Buffer pH 8.5; h) 0.05% NaOH or NH_3 (gas)

This structure can be attached to the polymeric carrier by activation of a carboxyl group (see Scheme 1, path d). Coupling to diaminocarboxylic acid (e.g., lysine) affords another amino group on which the peptide (or non-peptide) structure can be synthesized. Peptide synthesized directly on the lysine side chain is permanently linked to the bead and serves as the "reference" for structural determination. It can be either identical to the peptides synthesized on the branches of the cleavable linker, or it can be a peptide coding for the non-peptide structure on the releasable linker.[1] In this case the N^{α}-amino group of lysine must be protected by an orthogonally cleavable protecting group.

After the peptide (or non-peptide) synthesis on all points of attachment, the constructed library is deprotected and the Boc group protecting the second molecule of iminodiacetic acid is removed. This structure is stable in acidic pH (3-4), but removal of the proton from the secondary amino group (pH 7-9) leads to the rapid spontaneous formation of diketopiperazine structure (2 in Scheme 1) and release of the first part of peptide as hydroxypropylamide (overnight incubation in buffer pH 7-9, 50-100 µl per well). The release of the second part of the peptide is achieved by the application of higher pH (3-12h in 10-50 µl 0.05-0.2% NaOH per well) or by exposure to gaseous ammonia (12-24h incubation in desiccator).

Synthesis of the multicleavable linker is schematically shown in Scheme 1. N-Protected iminodiacetic acid is esterified by hydroxypropylamide of Fmoc-Gly using carbodiimide condensation catalyzed by dimethylaminopyridine. The Boc group is removed by trifluoroacetic acid and the aminogroup is acylated by anhydride derived from protected iminodiacetic acid. The linker was attached to the polymeric carrier by a carbodiimide reaction. To follow the release kinetics, we have coupled tryptophan to both arms and exposed the construct to the conditions of both releases. The traces (Figure 1) illustrate a rapid and quantitative release in both steps. The described linker is now used routinely in the preparation of multireleasable libraries.

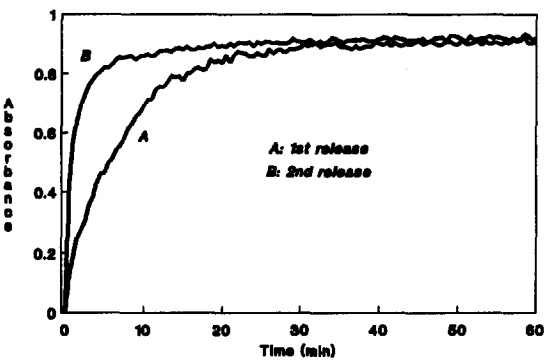


Fig. 1. Kinetics of the first (A) and second (B) release of peptide Trp-Gly-NH-$(CH_2)_3$-OH

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