Pergamon

Tetrahedron Letters 41 (2000) 10003–10007

TETRAHEDRON LETTERS

A novel lipophilic glyoxylic acid derivative for the lipidation of peptides using salt-free hydrazone ligation

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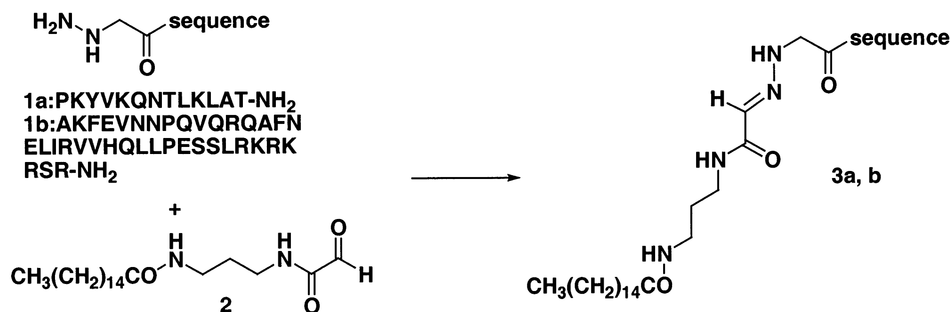
Received 11 August 2000; accepted 10 October 2000

Abstract

A lipophilic vector, composed of a palmitoyl and glyoxylyl group linked by a 1,3-diaminopropane moiety, was found to react rapidly and almost quantitatively with N-terminal α-hydrazino acetyl peptides to give the corresponding hydrazones. The ligation was performed in salt-free conditions, using a 95/5 mixture of 2-methyl-propan-2-ol/water. © 2000 Elsevier Science Ltd. All rights reserved.

Lipopeptides, owing to their ability to cross passively the cell membrane[1] or biological barriers,[2] are unique tools for the intracellular delivery of bioactive peptides. Mixtures of lipopeptides have been used successfully for the delivery of antigens into the cytoplasmic processing pathway of antigen presenting cells for induction of virus-specific cytotoxic T cells, with potential application in the field of clinically acceptable vaccine formulations. To speed up the access to these interesting compounds and to circumvent the difficulties associated with the conventional solid phase synthesis, we have designed a novel methodology for the synthesis of lipopeptides which involves the anchoring of the lipophilic vector **2** to the pre-purified hydrazino acetyl peptide **1** through the formation of a hydrazone bond (Scheme 1).



Scheme 1.

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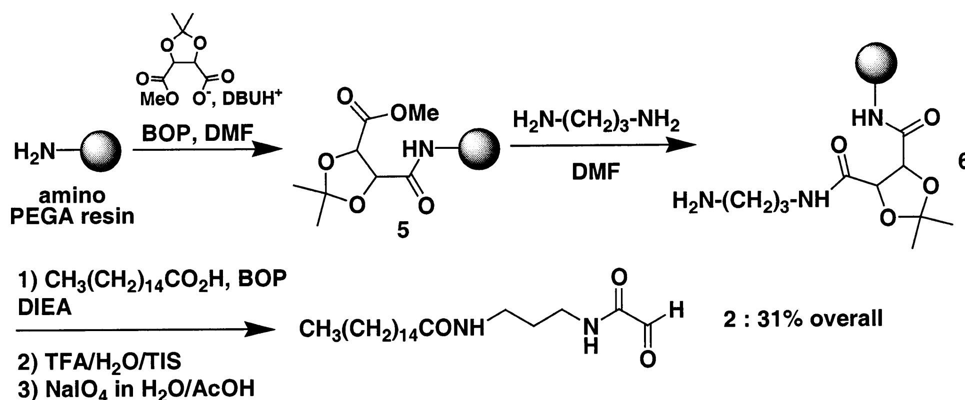
PII: S0040-4039(00)01784-6

α-Hydrazino acetyl peptides **1** are easily attainable using standard Fmoc/*tert*-butyl solid phase peptide synthesis and $(Boc)_2N$–$N(Boc)CH_2CO_2H$ **4** as the α-hydrazino acetyl precursor.[3] Compound **4** can be coupled to the peptidyl-resin using either *O*-(benzotriazol-1-yl)-*N*,*N*,*N′*,*N′*-tetramethyluronium hexafluorophosphate (HBTU)/*N*-hydroxybenzotriazole (HOBt)[4] or benzotriazole-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) activation.[5] The chemistry related to the preparation, reactivity, and coupling of compound **4** will be reported in detail elsewhere.[6]

The lipophilic vector **2**, composed of a palmitoyl and glyoxylyl group linked by a 1,3-diaminopropane moiety, was synthesized as described in Scheme 2 using our (+)-dimethyl-2,3-*O*-isopropylidene-D-tartrate based linker.[7] Briefly, (+)-dimethyl-2,3-*O*-isopropylidene-D-tartrate was partially saponified with water and DBU, and anchored to amino PEGA resin using BOP in situ activation. The second ester moiety was displaced by 1,3-diamino-propane to give resin **6**, whose terminal amino group was acylated with palmitic acid using BOP activation. Unmasking of the 1,2-diol with concentrated TFA was followed by several washings with a 3/2/1 (by vol.) mixture of 2-methyl-propan-2-ol/H_2O/AcOH to remove the excess of palmitic acid, which was found to stick tightly to the resin. The same solvent mixture was used for the solid phase periodic oxidation, which permitted both the formation of the α-oxo-aldehyde moiety and the separation of the product from the solid support. Compound **2** was extracted from the polymer by washing with 2-methyl-propan-2-ol/MeOH at 50°C, and was isolated with a 31% overall yield following purification.



Scheme 2.

Peptide **1a**, derived from the $HA^{307-319}$ sequence, was selected for the ligation studies. The ligations were first performed in a pH 6.0 citrate–phosphate buffer diluted with DMSO, which is a good solvent for lipopeptides and in general peptides which are prone to aggregation. DMSO was also found to speed up Schiff base formation.[8] The RP-HPLC yield was found to be modest in 25% DMSO, after 5 h at rt (Fig. 1). Unexpectedly, increasing the DMSO concentration to 50% resulted in lower yields. The replacement of DMSO by 2-methyl-propan-2-ol led to much better conversions. Indeed, ligation in 30 and 70% of 2-methyl-propan-2-ol resulted in 66 and 86% yield respectively after 5 h at rt. Finally, reaction in a 95/5 mixture of 2-methyl-propan-2-ol/water *without buffer* resulted in efficient hydrazone formation since lipopeptide **3a** represented 95% of the crude product over the same period. Lipopeptide **3a** was isolated with a 74% yield following RP-HPLC purification using a pH 2 water–acetonitrile linear gradient. An aliquot was solubilized in an 8/1 mixture of acetic acid/water to check the stability of the hydrazone link in acid media. The level of decomposition of **3a** reached only 10% after 5 h at rt, showing that the hydrazone link is quite resistant to acid hydrolysis.

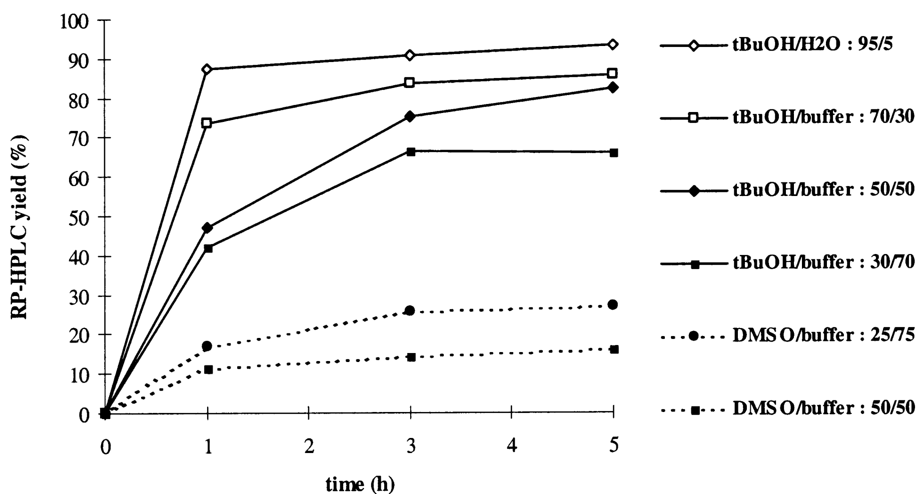


Figure 1. Reaction of HA$^{307-319}$ hydrazinopeptide **1a** with vector **2** in stoichiometric ratio. Influence of the solvent on hydrazone formation

The application of the methodology to the synthesis of large lipopeptides was undertaken using hydrazinopeptide **1b**, whose sequence is derived from the C-terminal 95–135 portion of interferon-γ.[9] Peptide **1b** reacted rapidly with the lipophilic vector **2** in a 95/5 mixture of 2-methyl-propan-2-ol/water at rt. Fig. 2(b) corresponds to the crude reaction mixture after 5 h of reaction. Lyophilization and RP-HPLC purification yielded 66% of hydrazone **3b**.

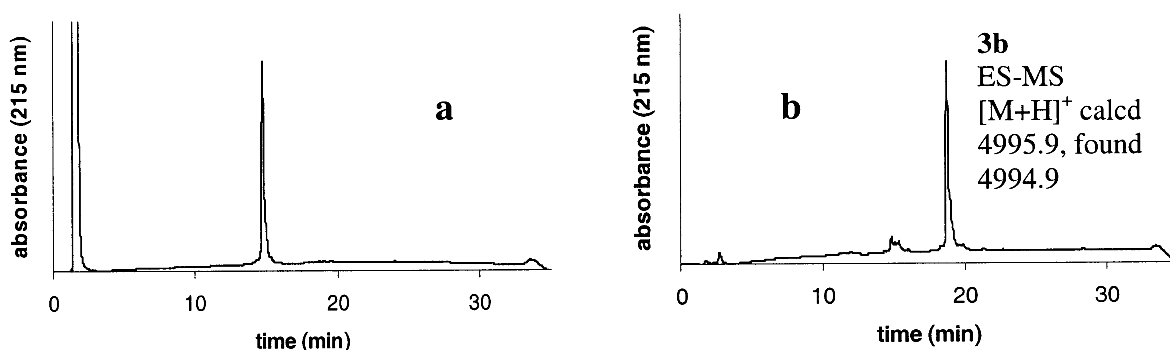
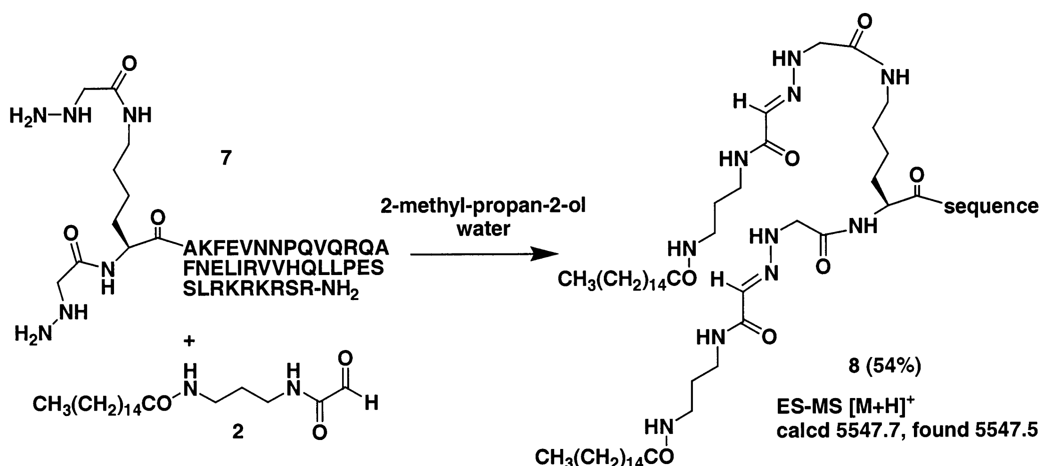


Figure 2. Ligation of peptide **1b** with vector **2**. (a) Peptide **1b**, (b) crude ligation mixture after 5 h at rt

We have also examined the application of this ligation chemistry to the synthesis of peptides modified by a double lipidic arm. Such peptide derivatives are of great interest, since the lipophilic tail allows a stable insertion into biological membranes.[10] Peptide **7** (Scheme 3), an analog of peptide **1b** derivatized at the N-terminus by two α-hydrazino acetyl moieties, was reacted with vector **2** as described above. Again, the reaction was found to be complete after 5 h at rt, and lipopeptide **8** was isolated with a 54% yield following RP-HPLC purification.



Scheme 3.

In conclusion, the ligation chemistry described in this letter permits the synthesis of large lipopeptides of high purity, as a consequence of the pre-purification of the α-hydrazino acetyl precursors and of the mildness of hydrazone formation. Interestingly, the salt-free process, the high yield of hydrazone formation and the use of stoichiometric amounts of vector **2** allows the direct lyophilization of the reaction mixture and the use of the crude lipopeptide in the bioassays.

Acknowledgements

We thank Bernadette Coddeville and Guy Ricart for the ES-MS studies (Université des Sciences et Technologies de Lille). We gratefully acknowledge financial support from Institut Pasteur de Lille, Agence Nationale de Recherche sur le SIDA (A.N.R.S.), Centre National de la Recherche Scientifique (C.N.R.S.) and Université de Lille 2. Dominique Bonnet holds a C.N.R.S.-Région Nord-Pas-de-Calais fellowship.

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