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Chemoselective acylation of hydrazinopeptides: a novel and mild method for the derivatization of peptides with sensitive fatty acids

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Abstract

N-terminal α-hydrazinoacetylpeptides were synthesized and chemoselectively acylated on the hydrazine moiety with various fatty acid succinimidyl esters or *N*-(cholesterylcarbonyloxy) succinimide. The acylation was performed in water/2-methyl-propane-2-ol mixtures buffered at pH 5.1. The mild reaction conditions allow the derivatization of peptides by sensitive fatty acids. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: lipopeptide; α-hydrazinoacetyl; acylation; fatty acids; hydrazinopeptide.

The modification of peptides by hydrophobic moieties, such as fatty acids, is now widely recognized as a means of enhancing their transport across biological membranes.[1] Among other properties, lipopeptides have found widespread use in both the targeting of intracellular receptors[2] and for the development of synthetic vaccines.[3] However, the synthesis of lipopeptides remains a difficult task. The recurrent solid phase synthesis of lipopeptides suffers from the necessity to perform a complex RP-HPLC purification following the cleavage and deprotection procedure. Few convergent strategies have been described but their scope is quite limited.[4] In addition, the available methods for the synthesis of lipopeptides do not allow the lipophilic moiety to be easily modulated. The solid phase approach, which requires a strong acid for the final cleavage and deprotection steps, does not allow the modification of peptides by unsaturated fatty acids or cholesterol derivatives. This point is crucial since the nature of the fatty acid is known to have a profound effect upon the interaction with the membrane and its alteration.[5] Thus, a novel method was developed for the synthesis of this important class of compounds, based upon the following criteria: (1) The reaction of a fully deprotected and purified peptide with activated fatty acids. (2) The necessity of very mild experimental conditions, compatible with sensitive fatty acids. (3) The stability of the linkage during the purification steps and in vivo. The chemoselective reaction of α-hydrazinoacetylpeptides **1** with activated fatty acids fulfills these specifications, and allows the synthesis of hydrazides **2** in good yield and high purity (Scheme 1).[6]

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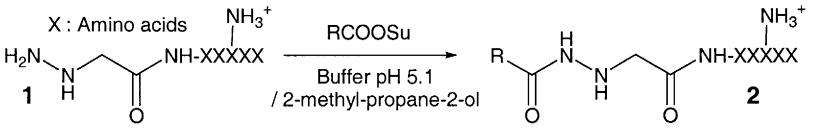
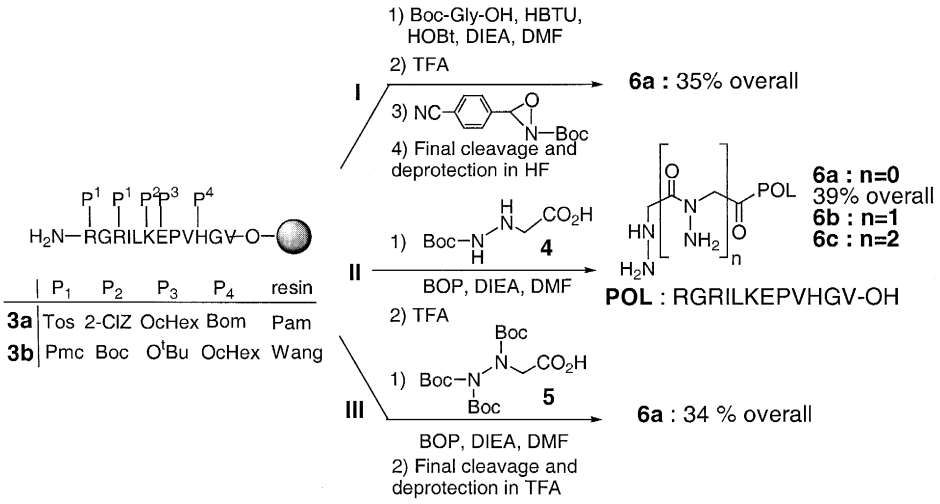


Scheme 1.

Three different syntheses of the model hydrazinopeptide **6a**, derived from the POL 476–484 sequence, were undertaken according to Scheme 2. In the first approach, Boc-Gly-OH was coupled to peptidyl resin **3a**, elaborated starting from Boc-L-Val-Pam resin using the Boc/benzyl strategy. Then, the α-amino group was deprotected and converted on the solid phase into the corresponding Boc-protected hydrazino moiety using our *N*-electrophilic amination procedure with *N*-Boc-3-(4-cyanophenyl)oxaziridine.[7] Hydrazinopeptide **6a** was isolated with a 35% yield following RP-HPLC purification, but was contaminated by 11% of the native peptide H-GRGRILKEPVHGV-OH, as the consequence of the partial instability of the N–N bond in liquid HF.[8] Alternatively, the α-hydrazinoacetyl moiety was found to be stable in TFA, so that further studies were performed using the Fmoc/*tert*-butyl strategy.



Scheme 2.

The solid phase *N*-electrophilic amination is a powerful tool, which avoids both the preparation and coupling of protected α-hydrazino acids. However, to favor a scale-up at reasonable cost, we took advantage of the commercial availability of α-hydrazinoacetic acid ethyl ester, and focused our attention on the coupling of Boc protected derivatives of α-hydrazinoacetic acid. Peptidyl resin **3b**, elaborated starting from Fmoc-L-Val-Wang resin, was acylated with **4** using in situ BOP activation. ES-MS analysis of the crude product revealed that the target compound **6a** was contaminated with significant amounts of peptide bearing 2–3 α-hydrazinoacetyl moieties (**6a**/**6b**/**6c**: 72/16/12). RP-HPLC purification led to a 39% yield of hydrazinopeptide **6a**, which was still contaminated by 8% of **6b**. This result corroborated the recent findings of Collet et al.,[9] who demonstrated that N^{β}-protection of α-hydrazinoacids did not suppress the nucleophilic character of N^{α}, and hence the polymerization of the activated α-hydrazinoesters. The problem was solved by using compound **5**, a fully protected derivative of α-hydrazinoacetic acid. In this latter case, hydrazinopeptide **6a** was isolated in a pure form with a 34% yield following RP-HPLC purification.

With the hydrazinopeptide **6a** in hand, we were faced with a rapid decomposition of the compound when stored in the lyophilized form following RP-HPLC purification with acetonitrile as the organic

mobile phase. This behavior was unexpected since 6-hydrazinonorleucine-containing peptides were found to be stable for months when purified under the same conditions. The decomposition occurred irrespective of the nature of the counter ion (chloride, trifluoroacetate). Alternatively, the organic solvent used for the RP-HPLC purification was found to be crucial. Decomposition always occurred when purification was performed using water–acetonitrile gradients, whereas α-hydrazinoacetylpeptides proved fully stable when acetonitrile was replaced by isopropanol.

The ability of hydrazinopeptide **6a** to be selectively acylated on the hydrazino moiety was then examined, and it was hypothesized that the low pK of the α-hydrazinoacetyl group[10] could allow the reaction to be performed at pH 5, thus permitting the protection of the ε-amino group of Lys[6] through protonation. In addition α-hydrazino acids are known to be regioselectively acylated at the N^{β} position, so that only one isomer could be expected.[11] Finally, a pH as low as 5 should prevent the imidazole ring of histidine from acting as an acylation catalyst and should diminish the hydrolysis of the active ester in the aqueous medium. The water/2-methyl-propane-2-ol solvent mixture was chosen since it allowed solubilization of peptides and the activated succinimidyl esters. The first experiments were performed with a 50 mM citrate/phosphate buffer at pH 5.2 using 1.1 equiv. of palmitic acid succinimidyl ester on a 1 mg scale. The hydrazino moiety was found to be selectively acylated, but unexpectedly, aggregation occurred when the reactions were performed on a larger scale. The problem was solved by diminishing the concentration of the buffer (25 mM). The best yields were obtained at pH 5.1. The RP-HPLC profile of the reaction mixture after 48 h at rt is shown in Fig. 1a. The minor and final eluting peak corresponded to peptide **6a** acylated both at the hydrazine and ε-amino groups. This side product is usually isolated with a 5% yield. As expected, the control experiment with the native peptide H-Gly-POL resulted in a low conversion and a poor selectivity (Fig. 1b). Scheme 3 lists some other selected examples. The synthesis of peptide **8d** is particularly noteworthy and highlights the mildness of the process, since the epoxy moiety of *cis*-9,10-epoxystearic acid is known to be very sensitive to acids. Indeed, purification of compound **8d** by RP-HPLC at pH 2 resulted in the opening of the epoxide. Peptide **8d** was saved by performing the purification at pH 7.2.

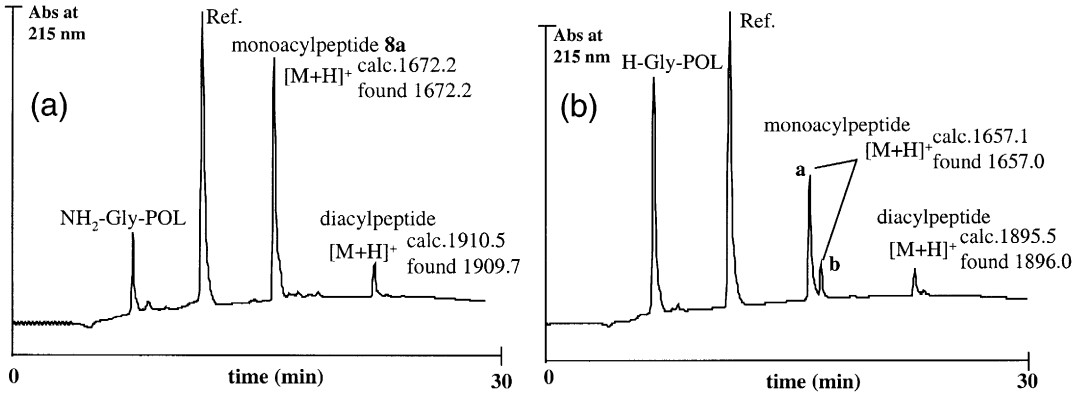
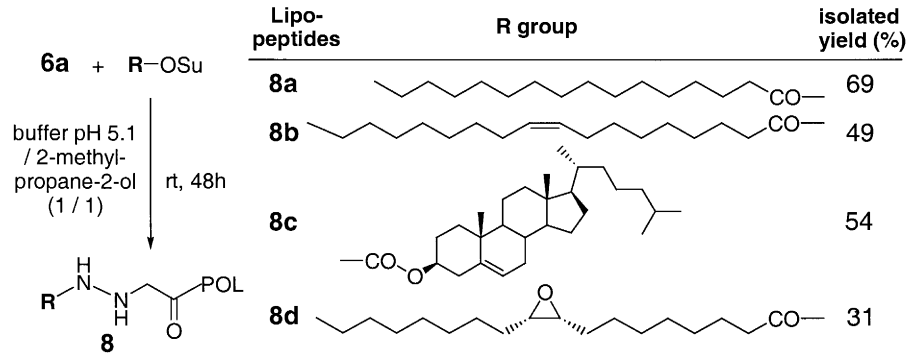


Fig. 1. RP-HPLC of the crude reaction mixtures after 48 h at rt, C3 Zorbax column (1 mL/min, 215 nm, linear gradient 0–100% acetonitrile in water in 30 min, 0.05% TFA). The compounds were analyzed by ES-MS; (a) H_2N-Gly-POL; (b) H-Gly-POL. Peaks a and b corresponded to peptides Palm-GRGRILKEPVHGV-OH and H-GRGRILK(Palm)EPVHGV-OH, respectively.[12]

In conclusion, we have described a novel strategy for the synthesis of lipopeptides, using very mild experimental conditions compatible with acid sensitive lipophilic moieties. This methodology will allow the parallel synthesis of various lipophilic derivatives of biologically active peptides for the rapid optimization of the cellular delivery.



Scheme 3.

Acknowledgements

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