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Note

# Chemical synthesis and crystallization of the dipeptide AcPheIleNH$_2$ in TTAB/heptane/octanol reversed micelles

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**Abstract**

The chemical synthesis of the dipeptide acetyl phenyl isoleucinamide (AcPheIleNH$_2$) in tetradecyl trimethyl ammonium bromide (TTAB)/heptane/octanol/carbonate buffer reversed micelles is described. The co-existence of the surfactant bounded minute water pools within the bulk organic solvent enables the simultaneous solubilization of the polar (IleNH$_2$) and apolar (AcPheOEt) substrates, thus enabling the synthesis to take place at the micellar interface. The synthesis was favored by increasing the micellar interface via an increase in water content and surfactant concentration. The best dipeptide yield (87%) was obtained at 32 °C, with the largest concentrations of TTAB (200 mM) and water (1100 mM) tested. The low solubility of the dipeptide in the micellar media further led to the formation and growth of needle-like crystals during synthesis, thus enabling the removal of product from solution.


*Keywords:* Reversed micelles; Chemical synthesis; Dipeptide; Crystallization

## 1. Introduction

Under specific conditions, surfactant molecules aggregate in non-polar solvents with their polar groups directed towards the interior of the aggregate and their hydrophobic moieties extended into the bulk apolar solvent. These so-called reversed micelles can solubilize certain amounts of water, yielding a homogeneous and optically transparent solution. The size of the nanoscopic droplets thus formed can be easily changed by varying the water content (usually defined by the parameter $W_0$ = [water]/[surfactant]) of the system. The ability to selectively solubilize polar and apolar compounds and the large contact area provided by the interface are two of the most interesting features of reversed micelles. Reversed micelles have been used as "nanoreactors" in numerous applications, ranging from enzymatic [1,2] and chemical catalysis [3–5], protein separation and refolding [6,7], synthesis of nanoparticles and quantum dots [8–10], drug delivery [11,12] and analytical methodologies [13,14].

The synthesis of a peptide (amide) bond involves the condensation of an amine group of one amino acid and a carboxyl group of another. However, given the large free energy difference between hydrolyzed and condensed amino acids, equilibrium is mostly shifted towards hydrolysis. Thus, reversed micelles, with their characteristic low water content (typically <5 M), provide favorable thermodynamic conditions for the enzymatic formation of peptide bonds [15–17].

In this article we demonstrate that peptide bond synthesis can also be accomplished chemically (no enzyme) in reversed micelles at moderate temperatures (5–35 °C), albeit at a much lower rate. Specifically, the chemical synthesis of the dipeptide acetyl phenyl isoleucinamide (**III**, AcPheIleNH$_2$) in tetradecyl trimethyl ammonium bromide (TTAB) reversed micelles dispersed in a heptane/octanol solvent mixture is reported (Scheme 1). In this system the polar acceptor isoleucinamide (**II**, IleNH$_2$) is solubilized in the water pool whereas the apolar acyl donor phenylalanine ethyl ester (**I**, AcPheOEt) is found in the bulk solvent. The dipeptide synthesis takes place at the micellar interface (Scheme 1). Substrate AcPheOEt can also be hydrolyzed and transesterified with the co-solvent octanol (**IV**) to yield AcPheOH (**V**) and AcPheO(CH$_2$)$_7$CH$_3$ (**VI**) side products, respectively (Scheme 1). Another important characteristic

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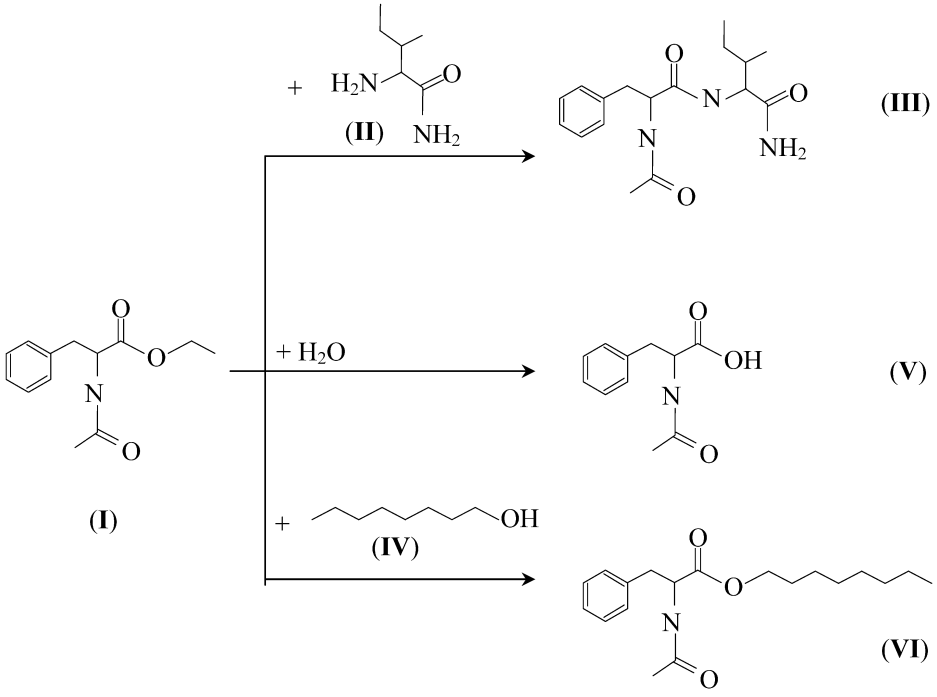


Scheme 1.

of this system is the low solubility of the dipeptide in the reaction media. The concomitant crystallization during the reaction, together with the low water content of the media, thus favors the synthesis of the dipeptide by shifting the equilibrium towards the product side [17].

## 2. Materials and methods

### 2.1. Chemicals

TTAB, AcPheOEt and $IleNH_2$ were obtained from Sigma. AcPheOH was purchased from Bachem. *n*-Heptane (95% purity) was obtained from Labscan, 1-octanol (purity >95%) was from Merck. Analytical grade acetonitrile and methanol were obtained from Riedel-de-Haen (Selze, Germany).

### 2.2. Analytical determinations

The course of reaction was followed by reverse-phase HPLC with a 20 cm LiCrosphere 100, RP-18 column (Merck Darmstadt, Germany). The transesterification product, $AcPheO(CH_2)_7CH_3$, was quantified using isocratic elution with 70% acetonitrile in 20 mM sodium phosphate at a final pH of 6.5. AcPheOEt and AcPheOH were separated and quantified in the same column using isocratic elution with 35% acetonitrile in 20 mM sodium phosphate at a final pH of 3.5. Although the dipeptide also eluted as an isolated peak, the total amount formed (soluble + solid) was estimated by mass balance using the determined concentrations of the ester substrate and of the hydrolysis and transesterification products. Eluent flow rate was 1 ml $min^{-1}$ in both cases. The compounds were detected by UV at 220 nm. Samples (200 µl) were dissolved in 800 µl methanol prior to injection (20 µl).

### 2.3. Synthesis reactions

Micellar systems with the following conditions were used for the dipeptide synthesis: 90/10% (v/v) heptane/octanol; 0.3 M carbonate buffer pH 10; 9 mM $IleNH_2$ and 3 mM AcPheOEt. These conditions had been previously optimized for the enzymatic synthesis of $AcPheIleNH_2$ [17]. All concentrations are reported to the total volume of the system with exception of buffer molarity which is referred to the volume of the aqueous pool. The solutions were prepared by injection of appropriate volumes of carbonate buffer containing $IleNH_2$ in a heptane/octanol mixture containing TTAB. The TTAB concentration and the water content, $W_0$, were varied in the range 50–200 mM and 3.5–5.5, respectively. The reaction was started by the addition of AcPheOEt dissolved in the heptane/octanol mixture. Reaction mixtures (15 ml) were placed in 25 ml closed glass vessels which were subsequently immersed in a temperature controlled bath. Aliquots (200 µl) were withdrawn at certain time intervals and dissolved in 800 µl methanol prior to HPLC analysis.

## 3. Results and discussion

The time course of the chemical dipeptide synthesis at 32 °C is shown in Fig. 1. The reaction is very slow, but proceeds up to the complete consumption of the limiting ester substrate (>99% conversion) with a dipeptide yield higher than 87%. The substrate ester not converted to dipeptide (13%) was directed towards the formation of the hydrolysis (10%) and transesterification side-products (3%). The reaction rates are four orders of magnitude lower than those reported in the literature for the $\alpha$-chymotrypsin catalyzed synthesis in an equivalent reversed micellar system [17]. The dipeptide could also be