

Baker's Yeast Reduction of PEG-Linked Acetoacetate

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Abstract: Bioreduction of PEG-acetoacetate **1** is achieved using dry baker's yeast in toluene with a small amount of added water. After detachment from the polymer under basic conditions, the corresponding (*S*)-(+)-3-hydroxybutanoic acid **3** was esterified with Me₃SiCl and ethanol and ethyl (*S*)-(+)-3-hydroxybutanoate **4** was isolated with 97% e.e. and 70% overall yield.

Keywords: Baker's yeast, bioreduction, organic solvent, PEG.

1. INTRODUCTION

Polymer-supported chemistry has become increasingly important in organic synthesis owing to the many advantages it offers in comparison with solution chemistry, such as, for example, the easy removal of reagent excess and reaction soluble by-products [1]. However, solid-phase synthesis suffers from the drawback of the heterogeneity of the process. This disadvantage can be circumvented by the use of soluble polymer supports, which allows supported reaction to be performed in full solution conditions [2]. In case of enzyme-catalysed reactions the solubility properties of the polymeric matrix are particularly important. Poly(ethylene glycol) (PEG) fulfils these solubility requirements since it is largely soluble both in water as well as in most organic solvents. In addition, it is easy to handle, inexpensive and commercially available, and carries terminal functionalities useful for a reversible anchoring of the molecule to be reacted. Recently, an interesting application of a catalytic modification of a PEG-supported substrate, where the enzyme was immobilised on a resin, has been reported [3]. In our case, this soluble supported process seems especially suitable because a troublesome removal of the final product can be expected in yeast-based stereoselective reductions.

Reduction of prochiral carbonyl groups by baker's yeast (*Saccharomyces cerevisiae*) is a well-known process and β -ketoesters are unquestionably the compounds of reference [4]. As a standard compound, ethyl acetoacetate has been reported to undergo reduction by baker's yeast to the corresponding ethyl 3-hydroxybutanoate, with the (*S*) configuration in 58-98% e.e. under various reaction conditions (aqueous solution [5,6], organic solvents [7,8], and immobilised yeast [9]).

In this paper we report the reduction of PEG-acetoacetate (**1**) by dry baker's yeast both in water and in organic solvent.

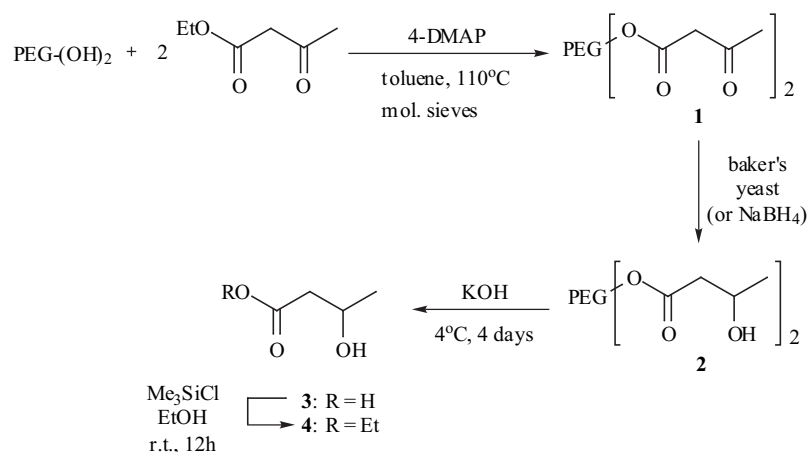
2. RESULTS AND DISCUSSION

Among the commercially available polyethylene glycols, a 3 kDa bifunctional PEG was chosen as support, since its

chemico-physical features allow easy manipulation and isolation of the product. The substrate was anchored to the polymer by a transesterification procedure [10] without any linker. Subsequently, PEG-acetoacetate (**1**) [11] (Scheme 1) was reduced with dry baker's yeast in water as well as in organic solvent, in order to study the bioreduction of the supported acetoacetate under the same reaction conditions reported in the literature [7] for baker's yeast reduction of ethyl acetoacetate. Reduction with sodium borohydride [12] in the absence of solvent [13] was also performed to compare ¹H NMR data with those of bioreduction. In order to determine the e.e. of the reduction product, and the enantioselectivity of the reductase responsible for the reduction [14], the reduction product was detached from the polymeric support by treatment with potassium hydroxide [15] at 4 °C for 4 days. Acidification of the medium and continuous extraction with ether afforded the (*S*)-(+)-3-hydroxybutanoic acid (**3**) (82% yield), which was quantitatively esterified to the corresponding ethyl ester (**4**) using trimethylsilyl chloride in ethanol [16]. Other methods aimed at detaching the product from the polymer, such as transesterification under basic conditions (NEt₃/EtOH [17]; NEt₃/EtOH/toluene; Na/MeOH [10]) failed, while acidic transesterification afforded the hydroxyester (**4**) in only 18% yield. In all cases the reactions were monitored by ¹H NMR spectroscopy, using the signal of the methylene group linked to the acetoacetate moiety (PEG-OCH₂CH₂OCOCH₂COCH₃) as the internal standard (4.29 ppm).

Prior to the choice of dry baker's yeast for the bioreduction of (**1**), attempts were made to perform the same reduction using raw baker's yeast in water (300 g/l) under non-fermenting conditions. However, as already reported by Janda and co-workers for another substrate linked to PEG [18], no reduction product was obtained, and the substrate (**1**) was recovered unchanged after 15 days. The bioreduction of PEG-acetoacetate (**1**) therefore was performed using dry baker's yeast under two different solvent conditions, namely in water and toluene, the latter with a small amount of water added. The amount of water was critical. In fact, as shown by Smallridge and co-workers for bioreductions of ethyl acetoacetate carried out in several organic solvents [7], it is the water/yeast ratio which critically affects the reaction yield, with 0.8 ml of water/g of yeast the minimum ratio required. It must be underlined that the same ratio also holds

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Scheme 1.

for the bioreduction of (1) in water (1.0 g, 0.7 meq of substrate (1)), 1.6 ml of water and 2 g of dry baker's yeast) with no organic solvent added, where PEG itself might work as a co-solvent. After 7 days and 62% conversion, PEG-(S)-3-hydroxybutanoate (2) was obtained with 94% e.e. and 77% yield.

When the bioreaction was performed in toluene (0.8 ml of water/g of dry baker's yeast), the reaction was much faster: after 48 hours and 90% conversion, PEG-(S)-3-hydroxybutanoate (2) was obtained with 97% e.e. and 88% yield [19]. The reaction went to completion (100% conversion) after 7 days, while the enantiomeric excess remained constant [20].

Under the same conditions, the unlinked ethyl acetoacetate was reduced in a 53% yield [7], confirming the advantages conferred by the presence of PEG.

Other PEG-ketoesters, such as piruvate and levulinate, have been investigated to explore the versatility of our approach. However, from preliminary investigations, the bioreduction of the α -ketoester seems affected by some detachment of the product from the support, while the γ -ketoester exhibits a low degree of conversion.

In conclusion, this paper reports an example of an enantioselective bioreduction of a substrate linked to a soluble polymer mediated by dry baker's yeast. This reaction can be carried out in water and in organic solvent, provided the amount of water is small. In all cases the configuration of the product is (S) and the enantiomeric excess reached up to 97%.

Further studies on the bioreduction of PEG-ketoesters are in progress to better understand the role of the polymer, which does not seem to be solely of support, as indicated by its inefficiency in the presence of raw baker's yeast in water. We could reasonably speculate that the long polymeric chains of support hamper the usual interaction with yeast's enzymes, since a larger amount of water generates a bulky hydration shell around the PEG.

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- [11] (PEG)_{n-2} (OCH₂CH₂OCOCH₂COCH₃)₂ (1): 97% mass recovery; ¹H NMR, 400 MHz, CDCl₃ (δ): 1.94 (0.3 H, s, CH₃ of the enol form), 2.26 (2.7 H, s, CH₃), 3.47 (1.8 H, s, CH₂), 3.50-3.70 (bs, OCH₂ of (PEG)_{n-2} fragment), 4.29 (2H, t, PEG-OCH₂CH₂-OCO), 5.02 (0.1 H, s, =CH); ESI-MS (Electron Spray Ionisation): mass increments between native and functionalised PEG (1) calculated (84 Th) and measured (84.0-84.1 Th) for the two charge cluster are in complete accordance.
- [12] To the polymer (1) (1.0 g, 0.7 meq) NaBH₄ (60 mg, 1.67 mmol) was added, the solid mixture was stirred overnight, a buffer solution (pH 5, 5.0 ml) was added and the mixture was extracted with CH₂Cl₂ (3 x 50 ml), the combined organic layers were dried over anhydrous Na₂SO₄. The solution was concentrated to ca. 10 ml. Standard precipitation procedure afforded the polymer (2) (0.95 g, 95% mass recovery).
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- [19] (PEG)_{n-2}(OCH₂CH₂OCOCH₂CH(OH)CH₃)₂ (**2**): ¹H NMR, 400 MHz, CDCl₃ (δ): 1.21 (3H, d, J 6.2, CH₃), 2.47, 2.48 (2H, AB part of an ABX system, J_{AB} 16.3, CH₂CH(OH)), 3.50-3.70 (bs, OCH₂ of (PEG)_{n-2} fragment), 4.19 (1H, m, CHOH), 4.29 (2H, q, J 4.6, PEG-OCH₂CH₂-OCO); ¹³C NMR, 400 MHz, CDCl₃ (δ): 172.6 (s, COO), 70.6 (t, OCH₂ of (PEG)_{n-2} fragment), 68.9 (t, CH₂OCO), 64.2 (d, CHOH), 63.5 (t, PEG-OCH₂), 43.2 (t, CH₂CH(OH)), 22.5 (q, CH₃). ESI-MS (Electron Spray Ionisation): mass increments between native and functionalised PEG-3-hydroxybutanoate (**2**) calculated (86 Th) and measured (86.5±0.2 Th) for the two charge cluster are in accordance.
- [20] PEG-acetoacetate (**1**) (2.7 g, 1.8 meq) was dissolved in 54 ml of dry toluene, 4.3 ml of water and 5.4 g of dry baker's yeast (Type II, purchased from Sigma) were added. After stirring for 7 days, the solvent was separated, CH₂Cl₂ (125 ml) was added and the mixture was sonicated for 30 min. The yeast was filtered off, washed with CH₂Cl₂, and resonicated with CH₂Cl₂ (75 ml). The combined organic layers were concentrated to ca. 10 ml and a standard precipitation procedure was applied. PEG-(S)-3-hydroxybutanoate (**2**) (2.36 g, 88% mass recovery, 97% e.e., 100% conversion, determined by ¹H NMR analysis) was recovered. [α]_D²⁵ = +2.4 (c 2.6, CHCl₃), [α]_D²⁵ = +1.0 (c 2.1, MeOH); CD: [ψ]₂₁₁ = -21 (2.1 w/v %, MeOH); for an authentic sample of (S)-(+)-(**4**): [ψ]₂₁₁ = -261 (0.6 w/v %, MeOH), [θ]₂₁₁ = -344 (0.048 M, MeOH) with 85% e.e.