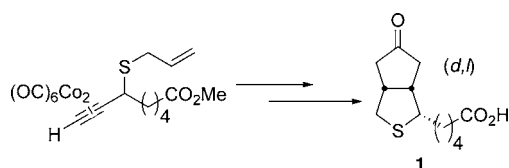# Synthesis of a Ketone Analogue of Biotin via the Intramolecular Pauson–Khand Reaction

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ABSTRACT

We report an improved synthesis of 5-(5-oxohexahydrocyclopenta[c]thiophen-1-yl)pentanoic acid (ketone biotin, 1) based on the intramolecular Pauson–Khand cyclization. The synthesis proceeds in eight steps and in 2.7% overall yield from cyclohexene.

Site-specific conjugation of small-molecule probes to proteins of interest in living cells is a powerful technique for investigating protein function, interactions, and trafficking.[1] We recently reported the synthesis of a ketone analogue of biotin, "ketone biotin" (**1**, Figure 1), that is ligated site

material and proceeds in 0.66% overall yield, so a more efficient synthesis of **1** is desirable. Herein, we report a more concise synthesis that affords **1** in eight steps and 2.7% overall yield, a 4-fold improvement over the previous synthesis.

Figure 1. Biotin and ketone biotin.

specifically to a 15-amino acid acceptor peptide (AP) by the *Escherichia coli* enzyme biotin ligase (BirA).[2] Through this ketone handle, AP-tagged cell surface proteins can be labeled with hydrazide- or hydroxylamine-bearing biophysical probes in a two-step process (Figure 2). The reported synthesis of **1** requires 12 steps from commercially available starting
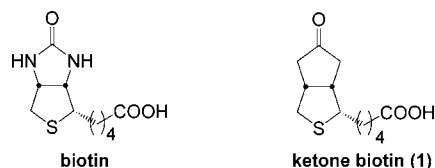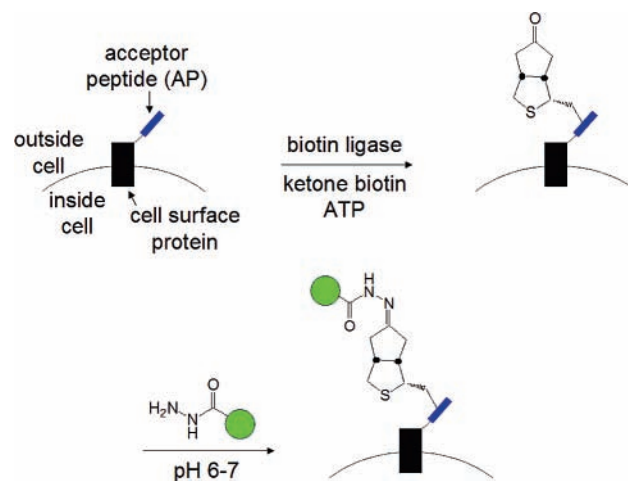

Figure 2. Labeling of cell surface proteins (black rectangle) linked to an acceptor peptide (blue rectangle) with ketone biotin and hydrazide-bearing biophysical probes (green circle).[2]

(1) Chen, I.; Ting, A. Y. *Curr. Opin. Biotechnol.* **2005**, *16*, 35 and references therein.
(2) Chen, I.; Howarth, M.; Lin, W.; Ting, A. Y. *Nat. Methods* **2005**, *2*, 99.

Our approach to ketone biotin (**1**) centers around the intramolecular Pauson−Khand reaction, a [2+2+1] cyclo-addition which has proven to be an effective technique for accessing bicyclo-[3,3,0]-oct-5-en-7-ones from 1,6-enynes,[3] including allyl propargyl sulfides.[4,5] We envisioned building the substituted allyl propargyl sulfide **3** from methyl 6-oxohexanoate **4** by addition of acetylene, followed by displacement of the resulting alcohol with allyl mercaptan. After complexation of the alkyne with octacarbonyldicobalt, a Pauson−Khand reaction should give α,β-unsaturated bicyclic ketone **2** (Scheme 1). Reduction of the alkene, followed by

Scheme 1. Retrosynthetic Analysis of **1**
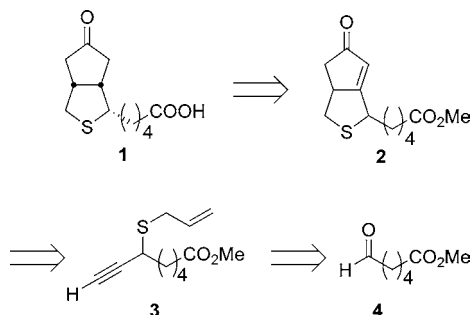
hydrolysis of the methyl ester to the free acid, should afford ketone biotin (**1**).

The synthesis of **1** (Scheme 2) proceeded in eight steps. Methyl 6-oxohexanoate (**4**) was synthesized from commercially available cyclohexene in one step in 98% yield according to literature procedure.[6] Addition of *in situ* generated lithium trimethylsilylacetylene to **4** under anhydrous conditions led to the formation of the known propargyl alcohol **5**.[7] Attempts to synthesize only the desired *R* enantiomer of **5** using Carreira addition of trimethylsilyl-acetylene to **4**[8] led to almost exclusive formation of aldol self-condensation products, possibly due to the lack of a substituent at the α position of **4**. Removal of the trimethyl-silyl protecting group with potassium carbonate afforded **6** in 66% yield from **4**. Following literature precedent,[4] we next formed the hexacarbonyldicobalt complex of **6** by reaction with octacarbonyldicobalt in dichloromethane. Complexation of the alcohol to BF$_3$·Et$_2$O, followed by nucleophilic displacement with allyl mercaptan, formed the Pauson−Khand cyclization precursor **7**, which was not isolated due to concerns about air stability.

When run at 70 °C in toluene, the Pauson−Khand reaction was found to proceed in 55% yield in three steps from **6**;
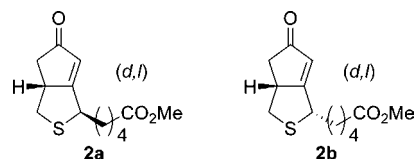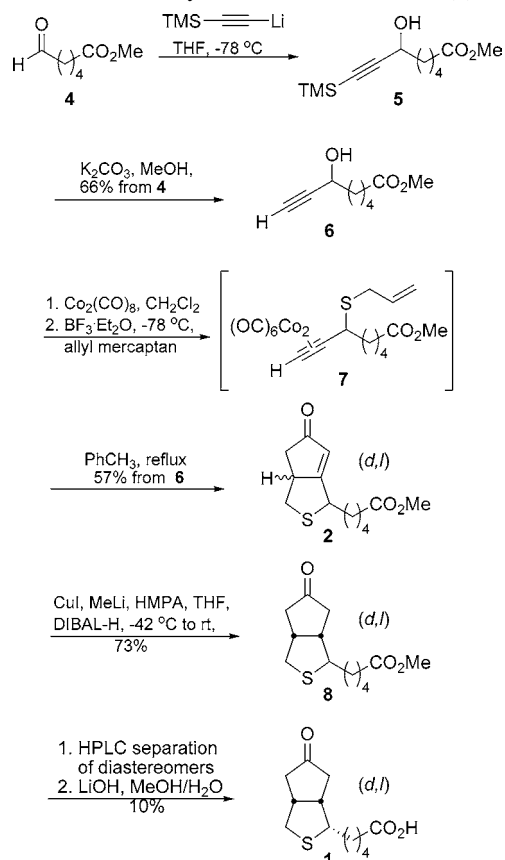
Scheme 2. Synthesis of Ketone Biotin (**1**)

Figure 3. Major (**2a**) and minor (**2b**) products of the Pauson−Khand reaction.

however, the product formed was a mixture of diastereomers **2a** and **2b** (Figure 3), with the desired diastereomer **2b** forming only 25% of the total product as determined by ^{1}H NMR analysis. The cobaltacyclic Pauson−Khand intermediate that places the valeric acid side chain on the more sterically congested concave face of the bicyclic ring structure is disfavored relative to the intermediate where the side chain is on the convex face, leading to preferential formation of product **2a** (see Figure S1, Supporting Information).[9] It was found that carrying out the reaction in refluxing toluene rather than at 70 °C gave a 57% total yield of cyclization products, and the proportion of **2b** increased to 35% of the total product formed.

(3) Pauson−Khand reaction: (a) Schore, N. E.; Croudace, M. C. *J. Org. Chem.* **1981**, *46*, 5436. (b) Pauson, P. L. *Tetrahedron* **1985**, *41*, 5855. (c) Geis, O.; Schmalz, H.-G. *Angew. Chem., Int. Ed.* **1998**, *37*, 911. (d) Brummond, K. M.; Kent, J. L. *Tetrahedron* **2000**, *56*, 3263.

(4) Stumpf, A.; Jeong, N.; Sunghee, H. *Synlett* **1997**, 205.

(5) Castro, J.; Moyano, A.; Pericàs, M. A.; Riera, A. *J. Org. Chem.* **1998**, *63*, 3346.

(6) Schreiber, S. L.; Claus, R. E.; Reagan, J. *Tetrahedron Lett.* **1982**, *23*, 3867.

Hess, C.; Phillips, R. S. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2821.

(7) Hess, C.; Phillips, R. S. *J. Chem. Soc., Perkin Trans. 1* **2000**, 2821.

(8) Anand, N. K.; Carreira, E. M. *J. Am. Chem. Soc.* **2001**, *123*, 9687.

(9) Mukai, C.; Kim, J. S.; Uchiyama, M.; Sakamoto, S.; Hanaoka, M. *J. Chem. Soc., Perkin Trans. 1* **1998**, 2903.

Reducing the double bond of **2** proved more difficult than we had anticipated. Attempts at reduction using Pd/C or PtO$_2$ failed, even at high catalyst loadings and elevated temperatures and H$_2$ pressures. In light of this, we turned to copper-mediated 1,4-reduction. A procedure in which **2** was reacted with an *in situ* generated copper hydride complex proved to be effective, giving the reduced product **8** cleanly in 73% yield.[10] The diastereomers were separated by preparative HPLC, and the methyl ester was deprotected with lithium hydroxide to afford **1**. The low overall yield for this pair of steps (10%) reflects the fact that only 35% of the diastereomeric mixture was the desired diastereomer.

This synthesis of ketone biotin provides a route to the protein-labeling reagent **1** that is four steps shorter and 4-fold higher in yield. Moreover, there is precedent in the literature for intramolecular Pauson−Khand reactions on 1,6-enynes with nitrogen or oxygen groups present between the double and triple bonds.[3] This should provide expedient access to oxygenated, nitrogenated, or carbocyclic analogues of **1** (Scheme 3). The previously published synthetic route[2] cannot access these analogues because it makes use of the reduced acidity of the α-carbons of sulfoxides to install the side chain. Such analogues of ketone biotin should be useful in our

(10) (a) Tsuda, T.; Hayashi, T.; Satomi, H.; Kawamoto, T.; Saesuga, T. *J. Org. Chem.* **1986**, *51*, 537. (b) Dahnke, K. R.; Paquette, L. A. *Organic Syntheses Collection*; Wiley: New York, 1998; Vol.9, p 396.

Scheme 3. Possible Use of the Pauson−Khand Reaction to Synthesize Analogues of **1**
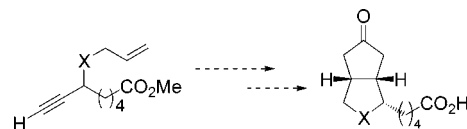
continued efforts to probe the active site geometry and chemistry for both *E. coli* BirA and the high-affinity biotin-binding protein streptavidin.

Supporting Information Available: Figure S1, experimental procedures, and spectral data for new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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