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An efficient epimerization of biotin sulfone derivatives to 2-*epi*-biotin analogs

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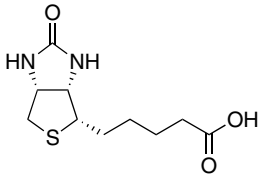
Abstract—Reduction of biotin sulfone derivatives leads to 2-*epi*-biotin analogs. The stereochemistry of the side chain at C_2 can be simply deduced from the diagnostic chemical shift pattern of the benzylic protons, rather than through the conventional analysis of the coupling constants using the empirical Karplus equation.

Biotin (**1**), also known as a vitamin H, is a water-soluble biocatalyst, which participates in the reversible fixation of carbon dioxide in the biosynthesis of organic molecules.[1] Its early discovery from egg yolk followed by isolation from beef liver and milk concentrates initiated a considerable effort toward the understanding of its important biological roles in human nutrition and animal health.[2] Moreover, the application of the biotin–(strept)avidin system has provided a useful tool for identification and purification of the protein complement of cells in the genomic and post-genomic era.[3] Since there are only a few naturally occurring proteins that bind to biotin, the cross-contamination during the protein purification using biotinylation is quite rare. One of the difficulties in protein purification using biotinylation is due to the fact that biotin has the strongest non-covalent interaction known in nature with avidin ($K_d = 10^{-15}$ M). This extremely high affinity of biotin for avidin raised some concern upon the recovery of protein–receptor covalent complexes. Several approaches have been tested to facilitate the release of biotinylated targets from (strept)avidin complexes, however use of biotin derivatives that possess decreased affinity for (strept)avidin has not been extensively exploited due to the lack of a general synthetic approach to biotin analogs.[4] To facilitate the recovery of the biotinylated target from (strept)avidin complexes, we have focused on two variables in biotin analog structures; (1) the oxidation state of the sulfur,[5] and (2) the stereochemistry of the valeryl chain. Herein, we report our preliminary findings regarding an efficient epimerization of the valeryl side chain of biotin derivatives through reduction of biotin sulfone derivatives.[6]



(+)-Biotin (1)

Commercially available (+)-biotin (**1**) was oxidized to biotin sulfone (**2**) in excellent yield using H_2O_2 in acetic acid[7] or in the presence of a large excess of Oxone® in methanol and water.[8] The resulting biotin sulfone (**2**) was first globally protected as the benzyl derivative **3**. The benzyl ester moiety in **3** was then reduced using lithium trimethoxy aluminum hydride[6a] and the subsequent reduction of sulfone alcohol **4** using lithium aluminum hydride gave alcohol **5** in modest yield. Alternatively, sulfone ester **3** was reduced directly to thiacyclopentane alcohol **5** in excellent yield.[6] To the best of our knowledge, there is no precedence for the epimerization of substituted thiacyclopentane dioxides by reduction protocols.[9] Thus, we initially assigned the stereochemistry of the C_2 substituent of **5** based on analogous biotin derivatives.[10] The coupling constant between H_2 and H_3 in the ^{1}H NMR spectrum of **5** was 5.7 Hz, which was in agreement with similar structures of biotin derivatives reported by Bates and Rosenblum.[11] However, there was some discrepancy in the chemical

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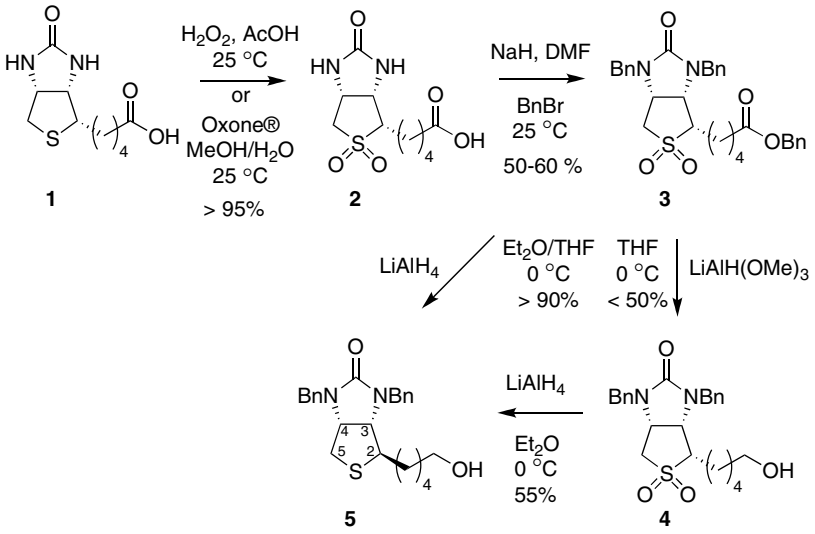
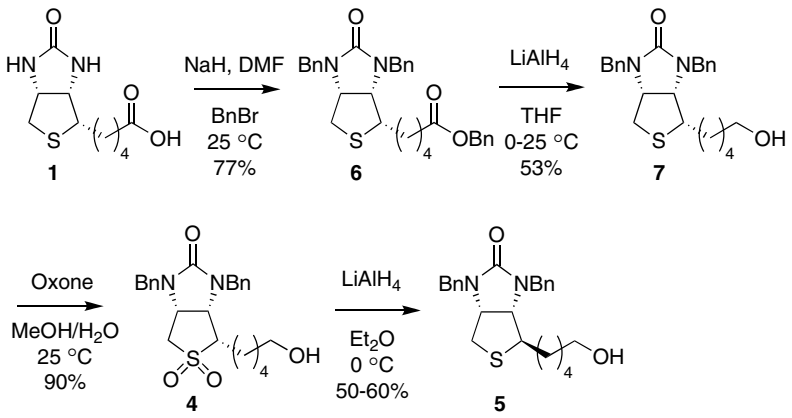


Scheme 1. Synthesis of **5** from biotin sulfone **2**.

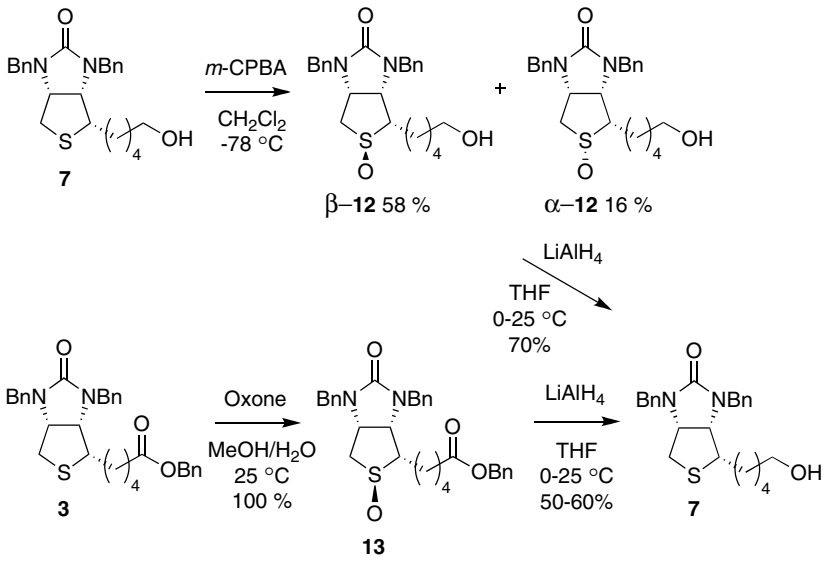
shifts of H_2, H_{5exo}, and H_{5endo} of **5** when compared to the *N*,*N*-benzyl protected biotin derivatives **9**[11a] and **11**[11b] (see Supplementary data) (Scheme 1).

In order to confirm the structure of **5**, an alternative synthetic sequence was pursued. Global protection of (+)-biotin (**1**) and the subsequent reduction of the ester moiety in **6** provided alcohol **7**. To our surprise, the NMR spectrum of the resulting biotin alcohol **7** did not match with that of **5**. The coupling constant between H_2 and H_3 in **7** was 5.6 Hz, however the chemical shifts of H_2, H_{5exo}, and H_{5endo} in **7** were strikingly similar to those of the known biotin derivatives **9**[11a] and **11**[11b] with the correct C_2 stereochemistry. We hypothesized that an epimerization was occurring during the reduction of sulfone **4**. This was confirmed by oxidation of sulfide alcohol **7** using Oxone®, which delivered the identical sulfone alcohol **4**. Finally reduction of biotin sulfone alcohol **4** using $LiAlH_4$ in diethyl ether once again gave alcohol **5** (Scheme 2).

The relative stereochemistry of substitution in hexahydrothienoimidazolones could be determined by application of the empirical generalized Karplus equation.[11] In biotin, the dihedral angle, calculated from the generalized Karplus equation, between the cis protons H_2–H_3 is approximately 45°, while the dihedral angle from the literature X-ray crystallographic data is 54°. Furthermore, the $^3J_{2,3}$, $^3J_{4,5endo}$, and $^3J_{4,5exo}$ coupling constants revealed that in all cases cis coupling constants were at least 1.5 Hz larger than the corresponding trans coupling constants. Thus, the trans coupling constants for **8**–**11** ranged from 1.66 to 4.56 Hz, while the cis coupling constants ranged from 4.64 to 6.08 Hz with an exception of $^3J_{3,4}$ (9.12–9.59 Hz). The preferred conformation of **5** is predicted as a twist-envelop with C_2 out of the plane formed by C_3, C_4, and C_5 to minimize eclipsing interactions between the C_{2exo} substituent, H_3, and H_4. Therefore, our $^3J_{2,3}$ coupling constant (5.6 Hz) of **5** initially led to the misinterpretation of the stereochemistry of **5**. The coupling constants based upon the empirical Karplus equation as a sole determinant of structural analysis of the hexahydrothioimidazolone ring thus should be interpreted with considerable caution where the *Cs* symmetrical envelope conformation of **5** is distorted to accommo-



Scheme 2. Synthesis of **7** from biotin benzyl ester **6**.



Scheme 3. Synthesis of **7** by reduction of sulfoxides.

date the eclipsing interactions between the substituent, H_4, and the side chain.[10]

In contrast to coupling constant analysis, the NMR interpretation based upon the chemical shift patterns is more straightforward in the determination of the stereochemistry of the side chain of the tetrahydrothiophene ring. As the previous NMR studies of biotin and related hexahydrothienoimidazolone derivatives showed, the chemical shifts of H_2, H_3, H_4, H_{5endo}, and H_{5exo} in **8**–**11** are relatively insensitive to the substitution at C_2. Although the chemical shift of H_2 in **5** comes between the H_{5endo} and H_{5exo}, this chemical shift pattern has been ruled out as a signature of the stereochemistry at C_2. The diagnostics of the stereochemistry of the side chain at C_2 comes from, as observed, the analysis of benzylic protons H_a and H_b (or $H_{a'}$ and $H_{b'}$) due to the inherent asymmetry of the hexahydrothienoimidazolone and preferred conformation of the benzyl groups. In compounds **5**, **8**,[11a] and **10**,[11b] which have a C_{2exo} substituent, the difference in chemical shift between H_a and $H_{a'}$ (or H_b and $H_{b'}$) is small (see Supplementary data). However, in **7**, **9**,[11a] and **11**[11b] the chemical shift difference between H_b and $H_{b'}$ is greater than that between H_a and $H_{a'}$ because the magnetic dissimilarity between H_b and $H_{b'}$ is enhanced by the proximity of H_b to the C_{2endo} substituent (see also NMR spectra of **6** in Supplementary data). Therefore, the stereochemistry at C_2 of the hexahydrothienoimidazolone can be determined through the diagnostic chemical shift pattern of the benzylic protons,[12] not through the analysis of the coupling constant using the empirical Karplus equation.

A facile epimerization at C_2 of the biotin sulfone derivatives upon reduction using $LiAlH_4$ implies a possible reduction mode of the sulfone moiety. Although the detailed mechanism of the epimerization needs further investigation,[13] a plausible intermediate such as an α-monoanion or an α,α′-dianion of the sulfone could be envisioned.[9] The epimerization observed at C_2 of the biotin sulfone derivatives upon reduction is unique to the sulfone moiety, as the related sulfoxides **12** and **13**, prepared either using *m*-CPBA or Oxone®, do not lead to the epimerization at C_2 (Scheme 3). A selective oxidation of alcohol moiety in **5** and **7** to carboxylic acid would furnish *N*,*N*-benzyl biotin derivatives,[14] which upon the known debenzylation conditions lead to biotin[15] and 2-*epi*-biotin.

In conclusion, we demonstrated that the reduction of biotin sulfone derivatives leads to 2-*epi*-biotin analogs and the stereochemistry of the side chain at C_2 can be deduced from the diagnostic chemical shift patterns of the benzylic protons, rather than by analysis of the coupling constants using the empirical Karplus equation. The binding affinity assay of the prepared sulfone, sulfoxides, and 2-*epi*-biotin derivatives to strept(avidin) is currently underway in our laboratory and our results will be reported in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.03.129.

References and notes

1. Uskokovic, M. R. In *Encyclopedia of Chemical Technology*, 3rd ed.; Kirk, R. E., Othmer, D. E., Eds.; Wiley: New York, 1984; Vol. 24, p 41.

2. For an excellent review, see: De Clercq, P. J. *Chem. Rev.* **1997**, *97*, 1755.
3. For examples, see: (a) van Werven, F. J.; Timmers, H. T. *Nucleic Acids Res.* **2006**, *34*, e33; (b) Nguyen, G. H.; Milea, J. S.; Rai, A.; Smith, C. L. *Biomol. Eng.* **2005**, *22*, 147; (c) Chen, I.; Ting, A. Y. *Curr. Opin. Biotechnol.* **2005**, *16*, 35; (d) de Boer, E.; Rodriguez, P.; Bonte, E.; Krijgsveld, J.; Katsantoni, E.; Heck, A.; Grosveld, F.; Strouboulis, J. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 7480, and references cited therein.
4. Desarnaud, F.; Marie, J.; Larguier, R.; Lombard, C.; Jard, S.; Bonnafous, J.-C. *J. Chromatogr.* **1992**, *603*, 95, and references cited therein.
5. For the relative avidin binding affinity of biotin and metabolites, see: (a) Zempleni, J.; Mock, D. M. *J. Nutr.* **1999**, *129*, 494S; (b) Finn, F. M.; Yamanouchi, K.; Titus, G.; Hofmann, K. *Bioorg. Chem.* **1995**, *23*, 152.
6. For reduction of biotin sulfone analogs and related examples, see: (a) Bates, H. A.; Smilowitz, L.; Lin, J. *J. Org. Chem.* **1985**, *50*, 899; (b) Kotake, H.; Inomata, K.; Murata, Y.; Kinoshita, H.; Katsuragawa, M. *Chem. Lett.* **1976**, 1073; (c) Martin, S. F.; Anderson, B. G.; Daniel, D.; Gaucher, A. *Tetrahedron* **1997**, *53*, 8997.
7. Sachon, E.; Tasseau, O.; Lavielle, S.; Sagan, S.; Bolbach, G. *Anal. Chem.* **2003**, *75*, 6536.
8. Trost, B. M.; Curran, D. P. *Tetrahedron Lett.* **1981**, *22*, 1287.
9. Webber, W. P.; Stromquist, P.; Ito, T. I. *Tetrahedron Lett.* **1974**, *15*, 2595.
10. Similar *N*,*N*′-*m*-bromobenzyl derivatives are known, but No NMR data was available for direct comparison (personal communication), see: Han, Q.; Lafontaine, J.; Bacheler, L. T.; Rayner, M. M.; Klabe, R. M.; Erickson-Viitanen, S.; Lam, P. Y. S. *Bioorg. Med. Chem. Lett.* **1996**, *12*, 1371.
11. (a) Bates, H. A.; Rosenblum, S. B. *J. Org. Chem.* **1986**, *51*, 3441; (b) Bates, H. A.; Rosenblum, S. B. *Tetrahedron* **1985**, *41*, 2331, and references cited therein.
12. For additional NMR data of 2-*epi*-biotin derivatives, see: (a) Chavan, S. P.; Chittiboyina, A. G.; Ramakrishna, G.; Tejwani, R. B.; Ravindranathan, T.; Kamat, S. K.; Rai, B.; Sivadasan, L.; Balakrishnan, K.; Ramalingam, S.; Deshpande, V. H. *Tetrahedron* **2005**, *61*, 9273; (b) Chen, F.-E.; Chen, X.-X.; Dai, H.-F.; Kuang, Y.-Y.; Xie, B.; Zhao, J.-F. *Adv. Synth. Catal.* **2005**, *347*, 549; (c) Moolenaar, M. J.; Speckamp, W. N.; Hiemstra, H.; Poetsch, E.; Casutt, M. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2391; (d) Turos, E.; Parvez, M.; Garigipati, R. S.; Weinreb, S. M. *J. Org. Chem.* **1988**, *53*, 1116.
13. Although we observed a partial epimerization of biotin sulfone using 1 N NaOH, decomposition was a major pathway upon a prolong exposure of other biotin sulfone derivatives under basic conditions, see: Sachon, E.; Tasseau, O.; Lavielle, S.; Sagan, S.; Bolbach, G. *Anal. Chem.* **2003**, *75*, 6536.
14. For a leading reference of oxidation of alcohol to carboxylic acid in the presence of sulfide moiety, see: Goud, P. M.; Sheri, A.; Desai, P. V.; Watkins, E. B.; Tekwani, B.; Sabnis, Y.; Gut, J.; Rosenthal, P. J.; Avery, M. A. *Med. Chem. Res.* **2005**, *14*, 74.
15. Chavan, S. P.; Tejwani, R.; Ravindranathan, T. *J. Org. Chem.* **2001**, *66*, 6197.