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Synthesis of a 'twisted' transition-state analogue of biotin

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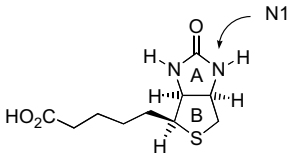
Abstract—A facile, racemic synthesis of a 'twisted' transition-state analogue of biotin is described. A key reaction is the electronically assisted ring-closure of the sulfur containing ring by displacement of an in situ generated mesylate by a suitably positioned 4-methoxybenzyl sulfide. The crystal structure of tricyclic compound **6** shows the AB ring system to indeed be twisted. The 'twist' was introduced to examine the possible involvement of sulfur participation in biotin biochemistry.

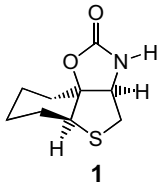
The question of whether sulfur participates in biotin biochemistry via a transannular interaction has attracted the interest of several research groups over the years.[1,2] The idea is that such an interaction could (a) increase the nucleophilicity of N1 thereby facilitating attack by N1 onto the 'CO_2 equivalent'; either carboxyphosphate or CO_2 itself and (b) avoid the formation of a ureide anion intermediate. Despite considerable effort to find experimental evidence for such an interaction, the general consensus is that it probably doesn't exist.[3]



biotin

We recently reported a molecular orbital (MO) study that suggested that during the first half-reaction of biotin-dependent enzyme-catalyzed carboxylations, sulfur could enhance the nucleophilicity of N1 via a combined through-bond/through-space interaction between sulfur and the urea.[4] This interaction could occur if, in the transition-state for biotin carboxylation, the enzyme stabilized a conformation of biotin that broke the symmetry of the bicyclic portion of the molecule by pulling the sulfur off of the centerline of ring A. This distortion, or 'twist', results in net overlap, in the form of a 2-orbital/4 electron destabilizing interaction, between sulfur and the ureido moiety.

To find experimental evidence for this through-bond/through-space interaction, what was needed was a molecule whose AB ring structure was permanently twisted in its ground state. Examination of hand held models and some preliminary modeling suggested that incorporation of a six-membered ring as in **1** below would induce the appropriate twist. As **1** has structural characteristics of potential interest to a wider audience, we wish to report an efficient synthesis of racemic **1**. An analysis of the crystal structure of the thiooxazolidone precursor **6** shows the molecule to indeed be twisted.



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The synthesis of **1** was fashioned after Volkmann's synthesis of biotin.[5] Hence, the oxazolidone ring would be formed via the addition of the anion of ethyl isothiocyanatoacetate to a suitably α-thio-functionalized

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cyclohexanone. Unmasking of the thiol and suitable manipulation of the ester would hopefully lead to closure of ring-B, assuming addition of the isothiocyanate to the ketone would yield at least some of the desired diastereomer. The choice of a suitable protecting group for the thiol then became the main issue.

We eventually settled on the use of a benzyl group, more out of convenience than foresight, but the choice proved to be a useful one. We initially synthesized α-*S*-benzylcyclohexanone **2a** via displacement of bromine from α-bromocyclohexanone with the anion of benzyl mercaptan. However we found the synthesis of α-bromocyclohexanone troublesome, and as shown in Scheme 1 we adopted the methodology of Scholz,[6] wherein α-*S*-benzylcyclohexanone is prepared in one step from the enolate of cyclohexanone and the thiolating agent *S*-benzyl 4-methylbenzenethiosulfonate **3a**. Reagent **3a** is prepared from potassium thiotosylate and benzyl chloride in DMF. With a convenient preparation of a key starting material in hand, the synthesis proceeded as outline in Scheme 1.

Treatment of ethyl isothiocyanatoacetate with an equivalent of lithium bis-(trimethylsilyl)amide in THF at −78 °C generated the anion, which added smoothly to α-*S*-benzylcyclohexanone to give a 60:40 separable mixture of stereoisomers, the major one proving eventually to be the desired diastereomer **4a**. The ester group of **4a** was reduced with $NaBH_4$ in THF/MeOH (9:1) at room temperature over a period of several hours to give alcohol **5a** in moderate yield. Treatment of **5a** with a slight excess of methanesulfonyl chloride in pyridine as solvent yielded the tricyclic thiooxazolidone **6** directly, without isolation of the mesylate, again in moderate yield, over a period of several hours.[7]

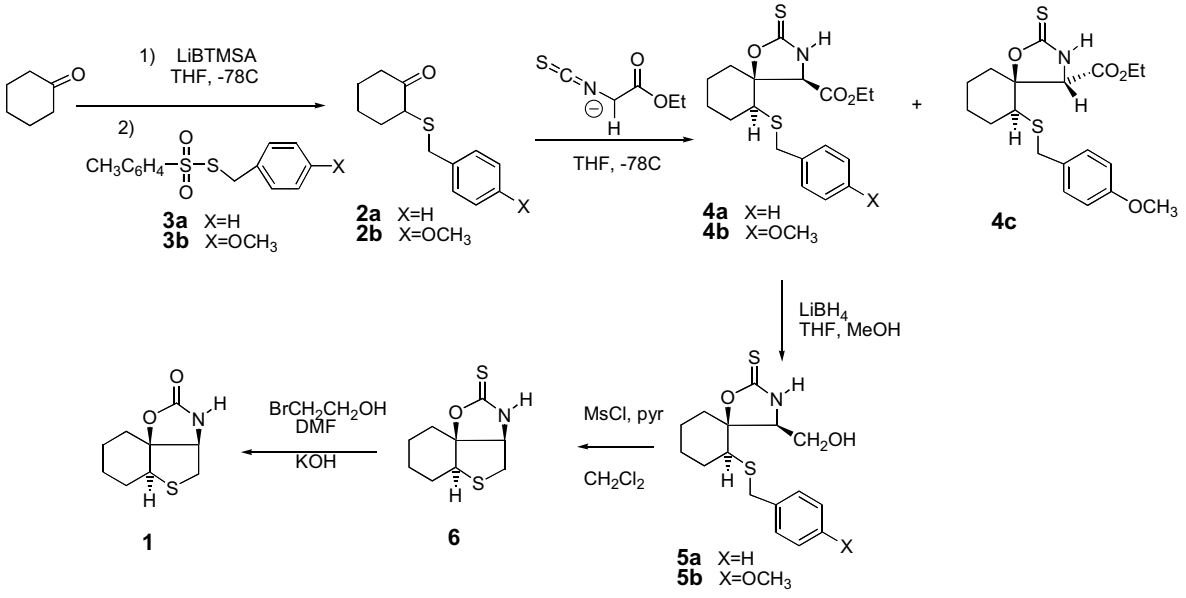
We were delighted at this one step ring closure, but overall yields were low. Hand held models of the alcohol **5a** showed that the sulfide is ideally positioned to displace the mesylate as soon as it is formed. It seemed reasonable that an electron donating group on the aromatic group would accelerate this intramolecular ring closure. We therefore decided to run through the synthesis again with a methoxy group in the para position.

The requisite thiolating agent **3b** was obtained by stirring 4-methoxybenzyl chloride with potassium thiotosylate in DMF for 12 h at room temperature. Treatment of the enolate of cyclohexanone with **3b** yielded **2b**, which had the advantage over **2a** in that it crystallized from ether, thus avoiding a chromatographic step.[8] The anion of ethyl isothiocyanatoacetate added to ketone **2b** again yielding a 60:40 mixture of diastereomers.[9] Fortuitously, the major desired isomer **4b** crystallized from an ether solution of the crude worked-up reaction in 50% overall yield. The remaining 10% can be recovered by column chromatography.

The stereochemistry of the minor isomer was eventually shown by X-ray analysis of a single crystal to be **4c** as indicated in Scheme 1. This result shows that the isothiocyanate added with complete facial selectivity to the hydrogen side of ketone **2b**.

Reduction of ester **4b** with $NaBH_4$ again seemed sluggish, but fresh $LiBH_4$ in THF/MeOH (9:0) reduced the ester cleanly to the alcohol **5b** within minutes.[10] Treatment of **5b** in pyridine with a slight excess of methanesulfonyl chloride gave the desired ring-closed product **6** cleanly within 10 min, thus confirming our hunch that the 4-methoxy group would accelerate the reaction.[11]

The sulfur/oxygen exchange was again taken from Volkmann.[5] Heating thiooxazolidone **6** with 2-bromoethanol in DMF at 100 °C for 2 h, followed by addition of 6 M KOH and stirring at 50 °C for an additional 2 h



Scheme 1.

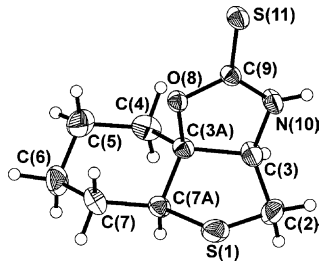


Figure 1. Crystal structure of thiooxazolidone **6**.

yielded **1** in variable yields ranging from 40–100%.[13] Starting material and product are conveniently separated on silica gel using CH_2Cl_2:acetone (20:1) as eluent. Yields based on recovered starting material were greater than 90%.

We were unable to grow suitable crystals of **1**, but crystals of thiooxazolidone **6** (mp = 223 °C) were obtained via slow evaporation of chloroform at room temperature. As shown in Figure 1, X-ray analysis[12] confirmed the overall structure and revealed the precise nature of the twist.

A key indicator of the twist is the dihedral angle N(10)–C(3)–C(3A)–O(8) = −13° obtained from the X-ray data. The equivalent dihedral in biotin[17] (N(1)–C(4)–C(3)–N(3)) is essentially 0°. In dethiobiotin[18] it is +22° and in oxybiotin[19] it is −12°. It has been suggested that a possible role for sulfur in biotin is to maintain the planarity of the 2-imidazolidone ring[18] for the purpose of preventing N1-carboxybiotin from spontaneously decarboxylating.[20] Compound **6** then has sulfur in ring-B but is twisted (presumably **1** is as well), which satisfies our definition[4] of a transition-state analogue for the carboxylation of biotin. We are interested to know whether that twist translates into unique chemistry at nitrogen.

A consequence of the twist can be appreciated from a comparison of the S(1)–O(8), S(1)–C(9), and S(1)–N(10) bond distances obtained from the X-ray data of **6** (3.1043, 3.3905, 3.5289 Å, respectively) with the corresponding distances obtained from the energy minimized structure (6-31G*) of the nontwisted bicyclic analogue that lacks the six-membered ring (3.2920, 3.3605, 3.6501 Å, respectively). The S(1)–O(8) and S(1)–N(10) distances are similar in the nontwisted structure. The same distances in the X-ray structure show that S(1) has moved closer to the oxygen and further from nitrogen. Also, S(1) has moved closer to the thiocarbonyl carbon; 3.5289 Å versus 3.6541 Å for the nontwisted structure. The N–C–C–O dihedral angle in the nontwisted structure is essentially 0° as in biotin. Therefore, the effect of adding the six-membered ring is to twist the AB ring portion of the molecule (−13° dihedral) moving the sulfur in ring-B up and over to the left relative to ring-A.

Assuming the structures of **1** and **6** are similar, we are currently in the process of studying **1** and some related compounds to determine what effect the 'twist' has in terms of chemical reactivity at nitrogen, and these results will be reported shortly. As mentioned, the overall goal is to examine the possibility that sulfur could participate in biotin biochemistry via a combined through-bond/through-space interaction between sulfur and N1 that operates at the transition-state for carboxylation.[4]

Acknowledgements

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8. Synthesis of **2b**: Lithium bis(trimethylsilyl)amide (17 mL) was added to a stirred solution of cyclohexanone (1.5 g, 17 mmol) in dry THF (20 mL) at −78 °C under argon. After 5 min, a solution of toluene-4-thiosulfonic acid *S*-(4-methoxybenzyl)ester **3b** (2.0 g, 16 mmol) in dry THF (10 mL) was added to the enolate over a period of 2–3 min. Stirring was continued at −78 °C for 5 min, the ice bath was removed, and after an additional 5 min, the reaction was quenched with 15 mL of saturated NH_4Cl. The quenched reaction mixture was transferred to a separatory funnel, diluted with EtOAc (75 mL) and the aqueous layer drained off. The organic layer was washed with 10% HCl (3 × 25 mL), saturated $NaHCO_3$ (1 × 30 mL) and brine (1 × 25 mL), dried with $MgSO_4$, filtered and evaporated to dryness. The crude oil was taken up in 3–4 mL of ether and placed in the fridge over night to yield 1 g of white crystalline product. Mp 60 °C; ^{1}H NMR (270 MHz, $CDCl_3$) δ = 7.24 (d, 2H), 6.84 (d, 2H), 3.81 (s, 3H), 3.63 (s, 2H), 3.74 (t, 1H), 3.0 (m, 1H), 2.24 (m, 1H), 2.10 (m, 1H), 1.95 (m, 2H), 1.70 (m, 3H); ^{13}C NMR (270 MHz, $CDCl_3$) δ = 208.12, 158.78, 130.30, 129.62, 113.95, 55.32, 50.77, 37.77, 35.12, 32.81, 26.94, 21.93; IR ($CHCl_3$) 2940(s), 1697(s), 1610(s), 1511(s) cm^{-1}.
9. Synthesis of **4b**: A THF (20 mL) solution of ethyl isothiocyanatoacetate (3.0 g, 20 mmol) was added to a dry THF (20 mL) solution of lithium bis(trimethylsilyl)amide (20 mL) at −78 °C over a period of 2 min. To the resultant anion was added compound **2b** (5.1 g, 20 mmol) dissolved in dry THF (20 mL) over a period of 5 min. The reaction was stirred for 10 min at −78 °C, the ice bath was removed, stirred for an additional 15 min, and the reaction quenched with saturated NH_4Cl. The quenched reaction mixture was poured into a separatory funnel and diluted with EtOAc (200 mL). The aqueous

layer was drained off, and the organic layer was washed with 10% HCl (3 × 35 mL), saturated $NaHCO_3$ (1 × 35 mL) and brine (1 × 35 mL). Organic layer was dried ($MgSO_4$), filtered, and concentrated down to a volume of about 30 mL and placed in the fridge for 12 h. The excess EtOAc was pipetted off to yield 2.3 g of crystalline **4b**. An additional 1.3 g of crystalline **4b** was obtained from the EtOAc layer by concentrating and seeding it. Compound **4b** recrystallizes from EtOAc. Isolated yield 50%. Mp 153 °C. ^{1}H NMR (270 MHz, $CDCl_3$) $\delta = 7.71$ (s, 1H), 7.22 (m, 2H), 6.82 (m, 2H), 4.21 (s, 1H), 4.20 (m, 2H), 3.84 (d, 1H), 3.80 (s, 3H), 3.69 (d, 1H), 3.10 (dd, 1H), 2.25 (m, 1H), 1.85 (m, 6H), 1.28 (t, 3H); ^{13}C NMR ($CDCl_3$) $\delta = 187.77$, 167.06, 158.73, 130.25, 129.55, 113.89, 95.04, 66.56, 62.39, 55.28, 47.69, 39.96, 34.23, 30.11, 25.44, 21.57, 13.99; IR ($CHCl_3$) 3445, 2989, 2942, 1733, 1495; IR ($CHCl_3$) 3446(m), 2942(m), 1735(s), 1494(s), 1251(s) cm^{-1}.

10. Synthesis of **5b**: Thiooxazolidone ester **4b** (2.3 g, 5.8 mmol) was dissolved in a 9:1 mixture of /MeOH (20 mL). To this solution was added solid $LiBH_4$ (500 mg) and the resulting (heterogeneous) solution was stirred for 15 min. (TLC, 2:1 hexane:EtOAc) showed reaction to be complete after 5–6 min. The reaction was quenched by drop-wise addition of 10% HCl (10 mL) over a period of 30 min. The reaction was then partitioned between ethyl acetate (100 mL) and water (50 mL). The organic layer was washed with saturated bicarbonate (25 mL) then brine (25 mL), dried over $MgSO_4$, filtered and evaporated to dryness. Recrystallization from EtOAc yields 2.0 g (95% yield) of a white solid, mp 145 °C; ^{1}H NMR (270 MHz, $CDCl_3$) $\delta = 8.33$ (s, 1H, NH), 7.29 (m, 2H), 6.87 (m, 2H), 3.95 (m, 5H), 3.84 (s, 3H), 3.14 (t, 1H), 2.85 (s, 1H), 2.24 (m, 1H), 1.95 (m, 1H), 1.75 (m, 4H), 1.40 (m, 2H); ^{13}C NMR (d_6-DMSO) δ 187.17, 158.76, 130.75, 130.46, 114.34, 92.01, 66.07, 50.10, 55.60, 45.77, 35.21, 31.38, 23.43, 22.37; IR ($CHCl_3$) 3441(m), 3355(m), 2944(s), 1610(w), 1511(s), 1178(s) cm^{-1}.
11. Synthesis of compound **6**: Thiooxazolidone alcohol **5b** (0.95 g, 2.9 mmol) was dissolved in pyridine (5 mL) and to this solution was added methanesulfonyl chloride (350 mg, 3.0 mmol). The reaction is stirred at room temperature for 2 h. TLC in hexane/EtOAc (1:1) shows lose of starting material at rf = 0.4 and the appearance of product at rf = 0.3 (iodine). The reaction was quenched by the addition of EtOAc (50 mL) followed by 10% HCl (100 mL). The organic layer was washed with saturated bicarbonate (1 × 25 mL) and brine (1 × 30 mL). The organic layer is dried ($MgSO_4$) filtered and evaporated to yield a solid. The product recrystallizes from EtOAc: mp 223 °C; ^{1}H NMR (270 MHz, $CDCl_3$) $\delta = 8.80$ (s, 1H, NH), 4.19 (s, 1H, NCH), 2.95 (m, 3H, SCH's), 2.25 (d, 1H), 2.0 (m, 4H), 1.80 (m, 1H), 1.60 (m, 1H) 1.40 (m, 1H); ^{13}C NMR ($CDCl_3$) $\delta = 188.57$, 99.72, 66.02, 57.34, 40.50, 33.62, 25.30, 24.93, 20.99; IR ($CHCl_3$) 3446(w), 3185(w), 2946(m), 1494(s), 1365(w), 1290(w), 1186(s) cm^{-1}.
12. Crystals of **6** were grown by slow evaporation of chloroform at room temperature. Single crystals were coated with Paratone-N oil, mounted using a glass fiber and frozen in the cold nitrogen stream of the goniometer. A hemisphere of data was collected on a Bruger AXS P4/SMART 1000 diffractometer using ω and θ scans with a scan width of 0.3° and 30 s exposure times. The dector distance was 5 cm. The data were reduced (SAINT)[14] and corrected for absorption (SADABS).[15] The structure was solved by direct methods and refined by full-matrix least squares on F^2(SHELXTL).[16] All nonhydrogen atoms were refined anisotropically. Hydrogen atoms were found in Fourier difference maps and refined isotropically.
13. Synthesis of compound **1**: 2-Bromoethanol (100 mg) was added to a DMF (5 mL) solution of thioanalogue **6** (65 mg, 0.3 mmol) and the resultant mixture was heated to 100 °C in an oil bath for 2 h. The reaction was allowed to cool to 50 °C, a 6 M solution of KOH (1 mL) was added and the reaction stirred at that temperature for an additional 2 h. After cooling to room temperature, the reaction was diluted with EtOAc (50 mL) and washed with water (3 × 20 mL) and brine (1 × 20 mL). The aqueous washings were re-extracted with EtOAc (25 mL), the EtOAc layer was washed with water (1 × 20 mL) followed by brine (1 × 20 mL). The two organic layers were combined, dried ($MgSO_4$) filtered and evaporated to yield a solid. TLC of the organic layer in 20:1 CH_2Cl_2:acetone shows starting material (rf = 0.6) and product (rf = 0.3). Column chromatography of the crude reaction mixture with the above solvent mixture and isolation of the lower spot gave product in a yield ranging from 40–100% (always >95% based on recovered starting material), which was re-crystallized (white filaments) from $CHCl_3$. Mp 228 °C; ^{1}H NMR (270 MHz, $CDCl_3$) $\delta = 6.2$ (s, 1H, NH), 3.97 (d, 1H, NCH), 2.88 (2m, 2H, SCH's), 2.7 (d, 1H, SCH), 2.3 (d, 1H), 1.9 (m, 4H), 1.7 (m, 1H), 1.4 (m, 2H); ^{13}C NMR ($CDCl_3$) $\delta = 159.06$, 91.83, 62.53, 57.12, 40.80, 34.13, 25.42, 24.99, 21.10; IR ($CHCl_3$) 3461(m), 3259(w), 2944(s), 1752(s), 1402(w), 1371(w) cm^{-1}.
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