Rapid Synthesis of the epi-Biotin Sulfone via Tandem S,N-Carbonyl Migration/aza-Michael/Spirocyclization and Haller–Bauer Reaction

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ABSTRACT: A synthesis of 2-epi-biotin sulfone was accomplished from commercially available L-cysteine. The synthesis features an unprecedented tandem S,N-carbonyl migration/aza-Michael/spirocyclization reaction from an L-cysteine-derived enone with aq. ammonia, in which three new sigma bonds and two rings are formed. In addition, the synthesis includes a highly diastereoselective late-stage Haller–Bauer reaction of sulfone for direct introduction of the carbon side chain.

INTRODUCTION

Stereoselective syntheses of molecules, both natural and designed, employing cascade reactions have emerged in recent years. Cascade reactions which involve the formation of multiple bonds or multiple transformations in a one-pot operation are often associated with an environmentally benign, atom-economical, and efficient process. A well-designed cascade and its execution are effective solutions to access desired biologically important natural products. In this context, considerable effort has been invested to explore various strategies. The development of cascades to provide specific biologically important molecules of unique architecture and stereocontrol presents a remarkable challenge.

(+)-Biotin (1, Figure 1), known as vitamin H, has long attracted intense attention from the synthetic community because of its important biological function in the human diet and animal strength. In addition, it is associated with an essential part of the metabolic cycle, resulting in catalytic fixation of carbon dioxide in the biosynthesis of organic molecules. From the pharmaceutical point of view, it is used as an additive and as an avidin complex in the field of drug delivery; biotinylation has allowed genomic and postgenomic eras to detect and isolate the protein complement of cells. The scarcity of efficient fermentation methods for biotin has drawn the attention of organic chemists toward its synthesis. The supply of (+)-biotin (1) required across the world has entirely relied on the synthetic method.

Additionally, biotin–(strept)avidin systems have been widely used for various applications including immunossay, diagnostics, and localization. However, major difficulties using biotinylation in the purification of protein are due to the strong affinity of biotin for avidin. Therefore, the synthesis of biotin analogues having a weak affinity for (strept)avidin is highly desirable.

To date, a number of synthetic approaches involving various strategies for the control of three contiguous stereogenic centers are reported. However, to the best of our knowledge, the Goldberg and Sternbach approach developed by Hoffman–La Roche, which was established about 60 years ago, is considered to be the most efficient and commercial approach. The Goldberg and Sternbach approach has been thoroughly modified for several years. With our ongoing interest and endeavors in the synthesis of biologically active compounds, we were interested in exploring an efficient synthesis of (+)-biotin.

Figure 1. Structures of (+)-biotin and 2-epi-biotin.
Of the several approaches reported toward (+)-biotin synthesis, cysteine\(^{12}\) has attracted a great deal of attention because it possesses requisite stereochemistry and ready availability. On the basis of Seki’s pioneering findings\(^{13}\) (Scheme 1), the utility of S,N-carbonyl migration of amide has provided a powerful protocol to effect one-pot C–N and S–C bond formation to access the cis-[S–S]-fused ring system of the (+)-biotin skeleton. This has inspired us to develop the elegant synthesis of (+)-biotin, utilizing an aza-Michael reaction followed by S,N-carbonyl migration to generate the cis-[S–S]-fused ring system of the (+)-biotin skeleton with excellent stereocontrol. The proposed hypothesis using aza-Michael and S,N-carbonyl migration in the efficient syntheses of (+)-biotin (1) is shown in Scheme 1.

**RESULTS AND DISCUSSION**

The enone 6 appeared to be an ideal scaffold to study the aza-Michael reaction. The retrosynthetic analysis of (+)-biotin indicated that the stereogenic centers of 1 would be established by the aza-Michael reaction of enone 6. The enone 6 can be obtained from aldol product 7 by a base-promoted elimination reaction. In turn, aldol adduct 7 can be accessed by aldol reaction of known \(\text{\(\alpha\)-amino aldehyde}}\) 8 with cyclohexanone. The \(\alpha\)-amino aldehyde 8 could be derived from commercially available \(\text{l-cysteine}}\) (9, Scheme 2). The side chain of 1 could be constructed through oxidative cleavage of enol-acetate 4, and in turn 1 could be assembled from 4 by using S,N-carbonyl migration and cyclization.

In this study, the synthesis of (+)-biotin (1) commenced with the synthesis of \(\alpha\)-amino aldehyde 8 in 4 steps from \(\text{l-cysteine}}\) (9) by following a known procedure.\(^{13}\) The direct diastereoselective aldol reaction\(^{4a,14b}\) of \((R)\)-amino aldehyde 8 was carried out with 2 mol equiv of cyclohexanone as a donor in the presence of 20 mol % of \((S)\)-proline at 0 °C to rt in CHCl\(_3\)–DMSO as the solvent to afford a mixture of \((\text{anti-syn})\)-aldol product 7 as a diastereomeric mixture in good yield (Scheme 3).

The \textit{anti} selectivity of the direct proline-catalyzed aldol reaction may be accounted for by the Houk–List model proposed for cyclic ketone. Intermolecular hydrogen bonding between the cyclic enamine intermediate and \(\alpha\)-amino aldehyde plays a critical role in providing \textit{anti}-7 stereoselectively.\(^{13b,c}\) A mixture of \textit{anti}-7 and \textit{syn}-7 could be used for the subsequent transformation. The diastereomeric mixture of aldol product 7 was subjected to mesylation using mesyl chloride and excess triethylamine to afford corresponding enone 6. The stereochemistry of enone 6 was confirmed by two-dimensional (2D) NOESY data, which supported the E configuration of olefin (Supporting Information).

The next task was the installation of a vicinal diamine moiety. We decided to introduce the second nitrogen by performing aza-Michael reaction\(^{16a}\) on enone 6. Various amines and catalysts were screened to achieve this transformation (Table 1). Thus, treatment of enone 6 with different amines (benzylamine/dibenzylamine/N-benzyl hydroxylamine)\(^{16b}\) in the presence of catalysts (amberlyst-15/CeCl\(_3\)/H\(_2\)O, NaI, SiO\(_2\))\(^{16b,c}\) was unsuccessful. After careful screening, treatment of enone 6 with TMSN\(_3\) and AcOH and catalyzed by triethylamine\(^{16d}\) did effect the aza-Michael adduct 12, however in poor yield. Interestingly, enone 6 and aza-Michael adduct 12 appeared at the same \(R\)\(_f\) rendering the purification a difficult task. After this disappointment, it was thought that aq. ammonia could be a better choice to access the aza-Michael adduct, based on significant rate acceleration of the aza-Michael reaction in water which was reported by Ranu and co-workers.\(^{16c}\) Aza-Michael reaction of enone 6 with aq. NH\(_3\) solution in ethanol at 140 °C in sealed tube was performed. Gratifyingly, a one-pot tandem S,N-carbonyl migration/aza-Michael reaction followed by an elimination was achieved to afford the aza-Michael adduct 12, which was shown to be a key intermediate for total synthesis of (+)-biotin (1).

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**Scheme 1. Key Inspiration and Crucial S,N-Carbonyl Migration Reaction Involved in the Earlier Total Synthesis of Biotin and Our Hypothesis**

**Scheme 2. Retrosynthetic Analysis**

**Scheme 3. Synthesis of Enone 6 and NOESY Correlation**
Michael/spirocyclization reaction allowed a facile entry to the requisite core of the biotin skeleton.

During the initial screening (entries 1–6) of various amines with enone 6, the usual formation of the aza-Michael adduct was expected. However, unusual spiroketone 13 was observed in 65% yield as a single diastereomer (Table 1). The resultant high degree of diastereoselectivity may be due to the facial selectivity and the rigid framework of enone 6 (for the proposed mechanism, see the Supporting Information). The cis stereochemistry of vicinal diamine was confirmed by 1H NMR coupling constants, $J = 8.0$ Hz, for product 13 (Supporting Information).

The introduction of a carbon side chain of biotin (1) was the next task of our investigation. Earlier, we successfully demonstrated the Baeyer–Villiger oxidation 17a of cyclic ketones and oxidative cleavage of cyclohexene derivatives using ozonolysis employed in the side-chain construction of biotin.17b,c Accordingly, the initial choice was the Baeyer–Villiger oxidation of ketone 13 to obtain the desired lactone. The reaction of ketone 13 with $m$-CPBA led to the formation of sulfone 14 in 83% yield as a yellow solid, and the desired lactone could not be isolated in various conditions. The structure and relative stereochemistry of sulfone 14 were confirmed by single-crystal X-ray crystallography, wherein it was found to possess the desired cis-vicinal diamine moiety (Scheme 4).

In order to construct the side chain of biotin (1), it was thought that thioacetate (16, Scheme 4) could be an ideal precursor to perform Baeyer–Villiger oxidation or oxidative cleavage via formation of enol-acetate 18. As the reactive sulfide was protected as its thioacetate, it was expected that the acetate group might remain intact in oxidation reaction conditions. Accordingly, the reductive cleavage 18 of spirokettle 13 with tributyltin hydride and AIBN as a radical initiator in refluxing toluene afforded thiohemiacetal 15 in 74% yield (dr = 9:1). Further, the chemoselective acetylation of 15 was carried out to access desired thioacetate 16 in 67% yield with dr = 7:3. Subsequently, a diastereomeric mixture of thioacetate 16 was subjected to acid-catalyzed enol acetate formation by using catalytic perchloric acid and acetic anhydride, which led to the formation of $N$-acetate 17, and desired enol acetate 18 was not formed, even after keeping the reaction for a prolonged period. The Baeyer–Villiger oxidation of ketone 16 with $m$-CPBA was also unsuccessful.

After disappointing results in the oxidation of thioacetate substrate 16, the Haller–Bauer reaction of sulfoxide 20 and sulfone 21 was planned (Scheme 5). It was envisioned that the base-induced cleavage Haller–Bauer reaction20a of ketone 20 should directly provide a biotin precursor. The reaction was tested by bases like NaOH, KOH, and NaNH$_2$; however, unfortunately, the reaction did not take place even after refluxing 20 in MeOH or toluene for a prolonged period of 48 h, and the starting material ketone 20 remained unreacted. Gratifyingly, it was understood that the oxidation of sulfoxide 20

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**Table 1. Aza-Michael Reaction of Enone 6**

| entry | conditions                  | temp (°C) | yield (%) |
|-------|-----------------------------|-----------|-----------|
| 1     | BnNH$_2$, EtOH              | 0–rt      | –         |
| 2     | BnNH$_2$, amberlyst-15, solvent free | rt | –         |
| 3     | BnNHOH, CH$_3$Cl            | 0–rt      | –         |
| 4     | Bn$_2$NH, CeCl$_2$·7H$_2$O, NaI, SiO$_2$ | 40 | –         |
| 5     | Na$_2$O$_2$, AcOH, THF      | trace     | trace     |
| 6     | TMSN$_3$, AcOH, Et$_3$N     | rt        | trace of 12 |
| 7     | aq. NH$_3$ (30%), EtOH      | 140       | 65 of 13  |

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**Scheme 4. Synthesis of Thioacetate 16**

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**Scheme 5. Synthesis of 2-epi-Biotin Sulfone and Optical Rotation**

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Optimum yields were realized when the temperature was prolonged the required reaction time. Accordingly, for quick sulfone skeleton, the next task was reduction of highly stable sulfone where the transformation is executed under mild conditions. The transformation is executed under mild conditions. Under this condition, excellent results could be obtained using KOH−tert-butyl alcohol system with a sulfone 21. Optimum yields were realized when the temperature was near room temperature, and lower temperatures (−10 to 0 °C) prolonged the required reaction time. Accordingly, for quick access to acid 22 through KOH−tert-butyl alcohol mediated Haller−Bauer cleavage, reaction was performed to furnish the acid 22 with dr = 12:1. We believe that this is the first example of KOH−tert-butyl alcohol mediated Haller−Bauer reaction of sulfone where the transformation is executed under mild conditions.

Having successfully introduced the side chain of the biotin sulfone skeleton, the next task was reduction of highly stable sulfone 22 to sulfide. Sulfone 22 was O-benzylated to obtain 23 in 75% yield over two steps (dr = 12:1). The absolute and relative stereochemistry of the side chain was established by comparison of specific rotation of O-benzyl ester 23 with known O-benzyl derivative 24, which was reported by Oh.23 It was found that the spectral as well as specific rotation data of 23 were significantly different, as shown in Scheme S.

The stereocenter at C(2) on the thiopeptide ring was found to be trans with respect to the stereocenter at C(3) and C(4). The O-benzyl ester 23 could be converted to hydroxyl-sulfide by sulfone reduction using LiAlH₄.22 Finally, the chemoselective oxidation of the resultant primary hydroxyl sulfide can be converted to the corresponding N,N-benzyl 2-epi-biotin derivative via the stepwise Swern oxidation and PDC oxidation reaction sequence. The N,N-benzyl 2-epi-biotin derivative, upon the known debenzylation conditions, would lead to 2-epi-biotin. Hence, the present route constitutes an attempt toward the synthesis of 2-epi-biotin (2).

### CONCLUSIONS

In summary, the synthesis of N,N’-dibenzyl 2-epi-biotin sulfone 22 using L-cysteine has been achieved. A direct proline-catalyzed aldol reaction, tandem S,N-carbonyl migration/aza-Michael/spirocyclization reaction, and late-stage Haller−Bauer reaction are the key steps in the synthesis. The tandem S,N-carbonyl migration/aza-Michael/spirocyclization reaction to access the required cis-[5−5] fused ring system of the (+)-biotin skeleton was one of the key findings of this work. The direct, flexible, and versatile introduction of a side chain at C(2) of 21 to form 22 opened an avenue for the synthesis of biotin analogues. Especially by varying the bases at the Haller−Bauer reaction step, we can enable the synthesis of biotin analogues. The work in this direction is in progress in our laboratory and will be communicated in due course.

### EXPERIMENTAL SECTION

General. All reactions were carried out in oven-dried glassware under a positive pressure of argon or nitrogen unless otherwise mentioned with magnetic stirring. Air-sensitive reagents and solutions were transferred via syringe or cannula and were introduced to the apparatus via a rubber septa. All reagents and solvents were used as received from the manufacturer. Solvents were dried over CaH₂ or sodium.

Analytical TLC was carried out using precoated silica gel plates (Merck silica gel 60 F₂₅₄), and visualization was accomplished with either UV light, with iodine adsorbed on silica gel, or by immersion in ethanolic solution of phosphomolybdic acid (PMA), p-anisaldehyde, 2,4-DNP, KMnO₄, or ninhydrin solution followed by heating with a heat gun for ~15 s. Merck’s flash silica gel (230–400 mesh) was used for column chromatography. IR spectra were recorded on a PerkinElmer 1615 FT infrared spectrophotometer using a NaCl cell. Melting points of solids were measured on a Buchi melting point apparatus. Optical rotation values were recorded on a P-2000 polarimeter at 589 nm. HRMS (ESI) were recorded on an ORBITRAP mass analyzer (Thermo Scientific, Q Exactive). Mass spectra were measured with ESI ionization in an MSQ LCMS mass spectrometer. ¹H and ¹³C NMR spectra were recorded using a Bruker Advance (200, 400, and 500 MHz) spectrometer. Chemical shifts are reported in ppm relative to residual CHCl₃ (δ = 7.26) in CDCl₃ for ¹H NMR and CDCl₃ (δ = 77.0) for ¹³C in the ¹³C NMR spectra. Structural assignments were made with additional information from gCOSY, gNOESY, gHSQC, and gHMBC experiments.

(R)-3-Benzyl-4-((R)-oxo-(S)-2-oxocyclohexyl)methylthiazolidin-2-one (7). Pyridine (0.70 mL, 8.8 mmol), TFA (0.66 mL, 8.8 mmol), and DCC (10.9 g, 52 mmol) in toluene (20 mL) were successively added to a solution of 11 (10.0 g, 44 mmol) in DMSO (22 mL) at 25 °C, and the mixture was stirred at 45 °C for 5 h. Toluene (100 mL) was added to the mixture, which was then cooled in an ice bath and filtered. The filtrate was washed with brine and water, while the aqueous layer was extracted with EtOAc. The extracts were combined, dried over anhydrous Na₂SO₄ and filtered, and the solvent was evaporated to afford 8 (8.9 g, 90%) as a viscous oil. The spectral data of aldehyde 8 matched well with the reported information.13 The obtained crude aldehyde 8 was directly used for the next step without further purification. To a stirred solution of α-amino aldehyde 8 (4.0 g, 18.09 mmol) and cyclohexanone (3.77 mL, 36.18 mmol) in solvent CHCl₃−DMSO (3:1, 40 mL) was added 20 mol % of (S)-proline (0.41 g) at 0 °C. The reaction was stirred for 48 h at room temperature and monitored by TLC. After completion of the reaction, the solvent was removed in vacuo. The resulting residue was taken up in EtOAc (40 mL) and stirred with 10% NaHCO₃ solution (10 mL). The organic layer was separated and washed with brine solution, dried over anhydrous Na₂SO₄, filtered, and evaporated in vacuo. The crude residue was purified by flash chromatography on silica gel (230–400 mesh) with EtOAc−PE (40:60) to give the aldehyde product 7 (4.0 g, 70% yield) as a yellow viscous oil with diastereoselectivity (anti/syn) = 3:1 determined by ¹H NMR analysis. The anti-7 stereoselectively may be confirmed by the proposed Houk−List model (Figure 2) for closely related proline-catalyzed direct aldol reaction of cyclohexanone and Garner’s aldehyde reported in the literature.

![Figure 2. Houk−List model for diastereoselective aldol reaction.](image-url)
1.96 (m, 3H), 1.61 (m, 2H). 13C{1H} NMR (125 MHz, CDCl 3) mixture of α, δ: 1.68 (m, 3H).

2. 1H NMR (500 MHz, CDCl 3): δ 7.37–7.30 (m, 5H), 4.86 (d, 1 = 15.3 Hz, 1H), 4.77 (br s, 1H), 4.30 (s, 1H), 4.03 (d, 1 = 15.3 Hz, 1H), 3.72–3.70 (m, 1H), 3.63–3.59 (m, 1H), 3.00 (dd, 1 = 6.4, 1.4 Hz, 1H), 3.36 (m, 1H), 3.00 (dd, 1 = 6.4, 1.4 Hz, 1H), 2.73 (dd, 1 = 5.3, 1.4 Hz, 1H), 2.07–1.98 (m, 2H), 1.91–1.85 (m, 2H), 1.60–1.55 (m, 3H), 1.40–1.37 (m, 2H). 31C{1H} NMR (125 MHz, CDCl 3) mixture of diastereomers was observed: δ 161.5, 136.6, 128.8 (2C), 128.0 (2C), 127.8, 81.0, 55.6, 54.0, 45.7, 44.8, 38.6, 26.9, 26.0, 24.1. LCMS (ESI): m/z [M + H]+ calculated for C 65H 59N O 7S: 3344.1247, found: 3344.065.

3. (S)-1-Benzyltetrahydrospiro[4H-cyclohexene-1,4'-thiino[3,4-d]imidazol-2(3H)-one (15). A flame-dried round-bottomed flask equipped with a reflux condenser, a solution of keto-sulfide 13 (200 mg, 0.63 mmol) in toluene (7 mL) was taken, and tri-n-butyltin hydride (0.25 mL, 0.94 mmol) was added following by 10 mg (0.06 mmol) of azobis(isobutyronitrile). The reaction mixture was refluxed for 8 h.

4. After that, the reaction mixture was cooled slowly to room temperature. The solvent was evaporated in vacuo. The residue was purified by silica gel (230–400 mesh) column chromatography using EtOAc–PE (40:60) to afford thiocaracemate 15 (150 mg, 74%) as a viscous liquid in nonseparable diastereomers (dr = 9:1) determined by 1H NMR analysis. Rf: 0.5 (EtOAc–PE = 60:40). IR (CHCl 3): δmax 3360, 1702, 1689, 1594, 1439, 1038 cm−1. [α]D +11.09 (c 1.0, CHCl 3). 1H NMR (500 MHz, CDCl 3) mixture of diastereomers was observed: δ 7.37–7.30 (m, 5H), 4.86 (d, 1 = 15.3 Hz, 1H), 4.77 (br s, 1H), 4.30 (s, 1H), 4.03 (d, 1 = 15.3 Hz, 1H), 3.72–3.70 (m, 1H), 3.63–3.59 (m, 1H), 3.00 (dd, 1 = 6.4, 1.4 Hz, 1H), 2.73 (dd, 1 = 5.3, 1.4 Hz, 1H), 2.07–1.98 (m, 2H), 1.91–1.85 (m, 2H), 1.60–1.55 (m, 3H), 1.40–1.37 (m, 2H). 13C{1H} NMR (125 MHz, CDCl 3) mixture of diastereomers was observed: 161.5, 136.6, 128.8 (2C), 128.0 (2C), 127.8, 81.0, 55.6, 54.0, 45.7, 44.8, 38.6, 26.9, 26.0, 24.1. LCMS (ESI): m/z [M + H]+ calculated for C 65H 59N O 7S: 3344.1247, found: 3344.065.
(230–400 mesh) column chromatography using EtOAc–PE (50:50) to give (115 mg, 67%) pure thio-acetate 16 as a viscous oil in nonseparable diasteromers (dr = 7:3) by 1H NMR analysis. Rf 0.5 (EtOAc–PE = 40:60). IR (CHCl3): $\nu_{\text{max}}$ 3381, 1723, 1608, 1447, 1128, 1128, 755 cm$^{-1}$. $\delta$ –2.21 (c 1.0, CHCl3). 1H NMR (400 MHz, CDCl3) mixture of diastereomers was observed: $\delta$ 7.36–7.27 (m, 5H), 5.31 (br s, 1H), 5.01 (d, $J = 15.3$ Hz, 1H), 4.83 (d, $J = 15.3$ Hz, 1H), 3.94–3.90 (m, 2H), 3.71 (t, $J = 7.9$ Hz, 1H), 3.60–3.56 (m, 1H), 3.38 (dd, $J = 4.3$, 14.0 Hz, 1H), 2.95–2.83 (m, 2H), 2.58–2.51 (m, 1H), 2.36 (s, 3H), 1.97–1.87 (m, 2H), 1.76–1.68 (m, 2H). 13C{1H} NMR (100 MHz, CDCl3) mixture of diastereomers was observed: $\delta$ 212.0, 211.6, 195.0, 194.3, 161.1, 161.5, 137.0, 136.8, 128.6, 128.1, 128.0, 127.5, 56.1, 55.3, 54.8, 53.5, 51.2, 50.9, 44.9, 42.3, 31.6, 31.4, 30.7, 29.6, 28.2, 27.9, 26.8, 26.6, 24.7, 24.4. HRMS (ESI): $m/z$ [M + H]+ calc for C$_{36}$H$_{38}$N$_2$O$_7$S: 607.1886, found 607.1900.

(3a$^S$,6a$R$)-1,3-Dibenzyttetrahydropiro(cyclohexane-1,4-thieno)[3,4-dijmidazol-2,2-1′H]-dione 5′,5′-dioxide (21). To a stirred solution of sulfone ketone 20 (100 mg, 0.24 mmol) in dry dichloromethane (5 mL) at 0 °C was added a meta-chloroperbenzoic acid (127 mg, 0.72 mmol, 70% w/w) portionwise. The reaction was stirred for 16 h at room temperature and quenched with aqueous sodium bicarbonate. The reaction mixture was partitioned between dichloromethane and brine and extracted using dichloromethane (20 mL × 3). The combined organic layers were washed with brine and dried over anhydrous Na$_2$SO$_4$ filtered, and concentrated in vacuo. The residue was purified by silica gel column chromatography using EtOAc–PE (50:50) to give a sulfone 21 (90 mg, 83%) as a colorless syrup. Rf 0.5 (EtOAc/PE = 30:70). IR (CHCl3): $\nu_{\text{max}}$ 2930, 1702, 1689, 1549, 1449, 1038, 777 cm$^{-1}$. $\alpha$ –90.86 (c 1.0, CHCl3). 1H NMR (400 MHz, CDCl3): $\delta$ 7.35–7.24 (m, 10H), 4.86 (d, $J = 10.1$ Hz, 1H), 4.80 (d, $J = 15.0$ Hz, 1H), 4.65 (d, $J = 15.0$ Hz, 1H), 4.20 (d, $J = 15.0$ Hz, 1H), 4.06–4.00 (m, 1H), 3.97 (d, $J = 15.0$ Hz, 1H), 3.19–3.08 (m, 2H), 2.65–2.63 (m, 2H), 2.40–2.28 (m, 2H), 2.13–2.07 (m, 1H), 1.89–1.85 (m, 2H), 1.49–1.42 (m, 1H). 13C{1H} NMR (100 MHz, CDCl3): $\delta$ 201.8, 160.5, 136.5, 135.4, 129.0 (2C), 128.7 (4C), 128.4 (2C), 128.3, 127.9, 74.7, 57.0, 51.3, 50.5, 48.0, 47.6, 41.6, 29.3, 25.7, 19.7. HRMS (ESI): $m/z$ [M + H]+ calc for C$_{41}$H$_{36}$N$_2$O$_8$: 649.1697, found: 649.1699.

5-([3a$^S$,6a$R$]-1,3-Dibenzyttetrahydropiro(cyclohexane-1,4-thieno)[3,4-dijimidazol-4-yl]pentanoic Acid (22). To a ketone sulfone 21 (100 mg, 0.22 mmol) in t-butyl alcohol (5 mL) was added 38 mg (0.68 mmol) of powdered potassium hydroxide at room temperature. The reaction was monitored by TLC, and after completion of the reaction, the solvent was evaporated in vacuo. While cooling with ice water, the mixture was carefully acidified to pH 1 with 2 N aq. HCl and then extracted with EtOAc. The extracts were washed twice with water, dried over anhydrous Na$_2$SO$_4$ and filtered. Concentrations of the organic layer in vacuo furnished acid 22 (94 mg, crude) as a viscous oil in nonseparable diasteromers (dr = 12:1) by 1H NMR analysis. Rf 0.5 (EtOAc–PE = 90:10). IR (CHCl3): $\nu_{\text{max}}$ 3422, 2866, 1720, 1690, 1502, 1330 cm$^{-1}$. $\alpha$ +12.58 (c 1.0, CHCl3). 1H NMR (400 MHz, CDCl3) mixture of diastereomers was observed: $\delta$ 7.41–7.27 (m, 10H), 4.72 (dd, $J = 6.7$, 15.3 Hz, 2H), 4.42 (dd, $J = 15.3$ Hz, 1H), 4.28 (dd, $J = 15.3$ Hz, 1H), 4.14–4.08 (m, 1H), 3.77 (dd, $J = 5.5$, 9.2 Hz, 1H), 3.17–3.03 (m, 3H), 2.58–2.29 (m, 2H), 1.91–1.83 (m, 1H), 1.66 (br s, 1H), 1.35–1.22 (m, 2H). 13C{1H} NMR (100 MHz, CDCl3) mixture of diastereomers was observed: $\delta$ 178.7, 159.0, 136.1, 135.8, 135.3, 129.8, 129.1 (3C), 128.3 (2C), 127.7 (2C), 63.4, 59.4, 53.0, 51.2, 47.4, 47.2, 33.4, 27.1, 26.0, 24.1. HRMS (ESI): $m/z$ [M + H]+ calc for C$_{23}$H$_{23}$N$_2$O$_2$: 347.1797, found: 457.1795.

Benzyl 5-([3a$^S$,6a$R$]-1,3-Dibenzyttetrahydropiro(cyclohexane-1,4-thieno)[3,4-dijimidazol-4-yl]pentanoate (23). To a stirred solution of epi-biotin sulfone 22 (100 mg, 0.21 mmol) in dry dimethylformamide (5 mL) at room temperature was
added sodium hydride (10 mg, 0.43 mmol) portionwise over 3 min. The resulting mixture was stirred for 30 min at room temperature. After cooling the mixture to room temperature, benzyl bromide (0.04 mL, 0.32 mmol) was added. The resulting mixture was stirred for 16 h at room temperature. While cooling with ice-water, water was added to quench the reaction, and the reaction mixture was diluted with ethyl acetate. The mixture was partitioned between ethyl acetate and brine and extracted using ethyl acetate (10 mL × 3). The combined organic layers were washed with brine, dried over anhydrous Na2SO4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (elucent 30:70 EtOAc–PE) to give the sulfone ester 23 as a thick syrup (100 mg, 75% over two steps) in nonseparable diastereomers (dr = 12:1) by 1H NMR analysis. Rf: 0.5 (EtOAc–PE = 20:80). IR (CHCl3): νmax 3031, 2942, 1700, 1496, 1449, 1357, 1311, 1235, 1142, 1111, 1076, 740, 698 cm−1. [α] + 26.05 (c 2.0, CHCl3). 1H NMR (500 MHz, CDCl3) mixture of diastereomers was observed: δ 7.42–7.27 (m, 15H), 5.15 (s, 2H), 4.72 (dd, J = 3.6, 15.6 Hz, 2H), 4.37 (d, J = 15.6 Hz, 1H), 4.29 (d, J = 14.9 Hz, 1H), 4.13–4.08 (m, 1H), 3.75–3.74 (m, 1H), 3.16 (dd, J = 7.1, 13.5 Hz, 1H), 3.06–2.97 (m, 2H), 2.31 (t, J = 7.1 Hz, 2H), 1.91–1.83 (m, 1H), 1.57–1.49 (m, 4H), 1.23–1.20 (m, 1H). 13C{1H} NMR (125 MHz, CDCl3) mixture of diastereomers was observed: δ 172.9, 158.9, 136.2, 135.9, 129.0 (2C), 128.6 (2C), 128.3, 128.2 (4C), 128.0 (4C), 127.7 (3C), 66.2, 63.4, 59.3, 53.0, 51.2, 47.3, 47.2, 33.6, 27.1, 26.1, 24.4. HRMS (ESI): m/z [M + H]+ calcd for C31H35N2O5S: 547.2261, found: 547.2263.

### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.2c01030.

Proposed mechanism for compounds 13 and 22, characterization of compounds including copies of 1H and 13C NMR spectra for compounds 6, 7, 13–17, and 20–23, and 2D NMR spectra of 6 (PDF)

X-ray crystal data and structural refinement for compound 14 (CIF)

### Accession Codes

CCDC 2024029 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/data_request/cif, or by emailing data_request@ccdc.cam.ac.uk, or by contacting The Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +4 1223 336033.

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### Notes

The authors declare no competing financial interest.

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