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# Enhanced Stereoselectivity of a Cu(II) Complex Chiral Auxiliary in the Synthesis of Fmoc-L-γ-carboxyglutamic Acid

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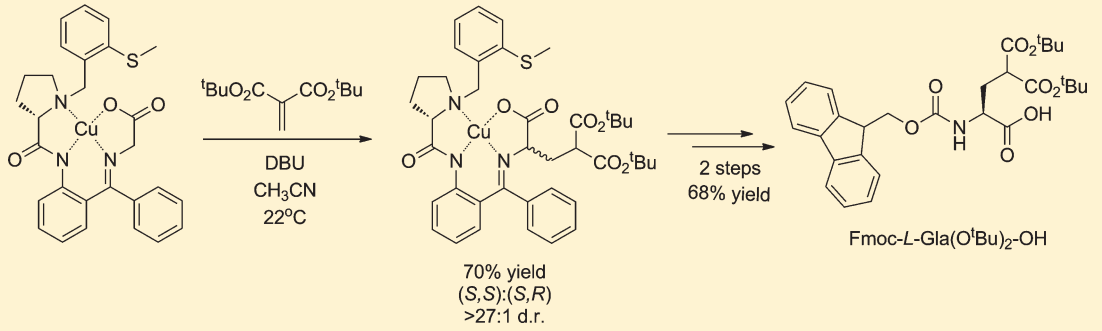
Supporting Information

**ABSTRACT:**




L-γ-Carboxyglutamic acid (Gla) is an uncommon amino acid that binds avidly to mineral surfaces and metal ions. Herein, we report the synthesis of *N*-α-Fmoc-L-γ-carboxyglutamic acid γ,γ′-*tert*-butyl ester (Fmoc-Gla($O^tBu$)$_2$-OH), a suitably protected analogue for Fmoc-based solid-phase peptide synthesis. The residue was synthesized using a novel chiral Cu(II) complex, whose structure-based design was inspired by the blue copper protein rusticyanin. The five-coordinate complex is formed by Shiff base formation between glycine and the novel ligand (*S*)-2-(*N*-(2-methylthio)benzylprolyl)aminobenzophenone in the presence of copper. Michael addition of di-*tert*-butyl methylenemalonate to the α-carbon of the glycine portion of the complex occurs in a diastereoselective fashion. The resulting (*S*,*S*)-complex diastereomer can be easily purified by chromatography. Metal complex decomposition followed by Fmoc protection affords the enantiomerically pure amino acid. With the use of this novel chiral complex, the asymmetric synthesis of Fmoc-Gla($O^tBu$)$_2$-OH was completed in nine steps from thiosalicylic acid in 14.5% overall yield.

## INTRODUCTION

L-γ-Carboxyglutamic acid (Gla) occurs in natural proteins as a result of post-translational modification of glutamic acid residues and serves both functional and structural roles.[1] For example, in the blood coagulation protein prothrombin, a host of Gla residues in the *N*-terminal domain of the protein bind calcium ions with high affinity resulting in a conformational change that enables phospholipid binding and ultimate coagulant activity.[2,3] The mineral-binding, Gla-containing domains of these proteins have been mimicked by integration of the Gla residue into synthetic peptides.[4,5] For example, we, as well as Murphy and co-workers, have shown that peptides containing Gla residues can undergo calcium-mediated binding to surfaces such as hydroxyapatite with high avidity[4,6,7] and can possibly be used as tools to better understand apatite remodeling.[8] This uncommon amino acid is typically incorporated into peptides synthetically, rather than enzymatically, using solid-phase peptide synthesis (SPPS). Syntheses are quite costly due to the commercial expense of the suitably protected analogue of the Gla amino acid. This has served as motivation to develop synthetic approaches to this residue that are amenable to academic laboratories. Asymmetric routes[9,10] have greatly improved the yields of the amino acid in comparison to past racemic syntheses;[11−13] however, reducing the number of steps required to access the fully protected Gla analogue (*S*)-**1** necessary for Fmoc-based SPPS still poses a challenge, Figure 1.

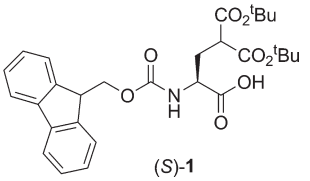



**Figure 1.** Fmoc-protected L-γ-carboxyglutamic acid.

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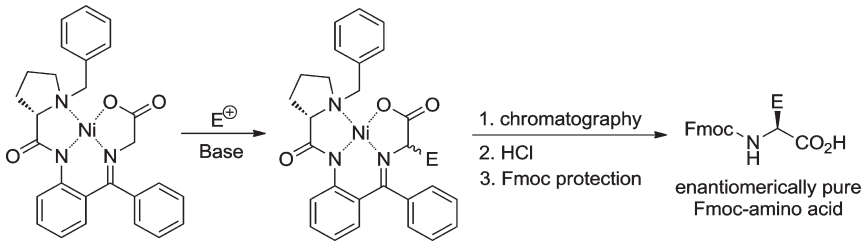



**Figure 2.** General route for preparation of enantiomerically pure Fmoc-amino acids starting from a chiral nucleophilic glycine equivalent.

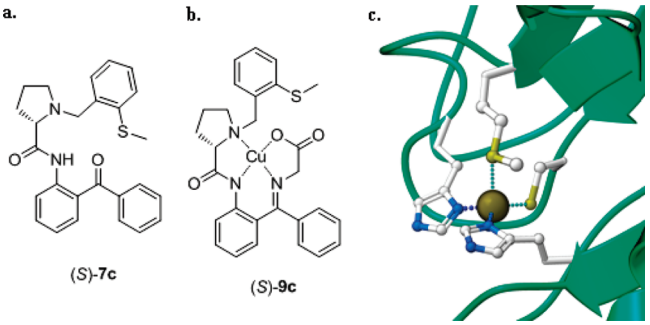


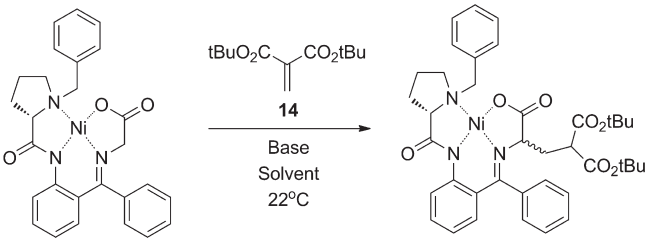

**Figure 3.** (a) Novel thioether-containing chiral ligand (*S*)-**7c**. (b) Structure of the chiral BPB metal complex containing an ortho-substituted benzyl group. (c) Highlighted residues bound to Cu(II) in the protein rusticyanin (PDB: 1RCY); note the axial methionine ligation.

One common approach for asymmetric amino acid synthesis is the use of chiral nucleophilic glycine equivalents. This method allows vast structural diversity at the amino acid side chain. Addition reactions to the Ni(II) complex of the Schiff base of glycine and (*S*)-BPB (Figure 2) generally give rise to product mixtures with a high excess of the diastereomer containing the (*S*)-amino acid.[14−16] Isolation of the major diastereomer through chromatography, followed by decomposition of the metal complex, results in enantiomerically pure amino acid and recovery (>80%) of the chiral ligand.

These chiral templates have been used to gain access to a variety of glutamic acid[17−21] and proline[22,23] derivatives in a highly stereoselective fashion through Michael additions of activated olefins to the glycine enolate. An attractive benefit of using the BPB complexes for Michael additions is the mild conditions under which the reactions can be performed. Early transformations using excesses of sodium methoxide or triethylamine as a base provided moderate yields and diastereoselectivities.[17,22] More recently developed conditions employing catalytic amounts (15 mol %) of DBU as a base could provide near-quantitative yields of a single product diastereomer within minutes at ambient temperature.[24−28] However, as will be shown, the previously reported four-coordinate, square planar Ni complex[16] in Figure 2 proved to be ineffective for the synthesis of (*S*)-**1**. Using previously reported and optimized reaction conditions afforded a mixture of diastereomers that could not be separated by conventional normal-phase chromatography. Thus, both poor selectivity and poor chromatographic resolution resulted in the inefficient preparation of (*S*)-**1** using this common chiral auxiliary.

Inspired by the axial binding interactions between methionine and copper in blue copper proteins, we set out to improve upon the results of others[29] by designing a novel chiral complex that contains an additional axial thioether ligand, which can better shield the top face of the coordination plane toward an incoming electrophile. This proported five-coordinate, square-pyramidal complex could effectively increase the ratio of diastereomers formed in the Michael addition reaction and, in addition, allow better resolution and subsequent separation of diastereomers. Herein, we synthesize the novel thioether-containing ligand (*S*)-**7c** (Figure 3a) and demonstrate the increased diastereoselectivity of the corresponding Cu(II)-containing glycine Schiff base (*S*)-**9c** (Figure 3b) using di-*tert*-butyl methylenemalonate as a Michael acceptor in our synthesis of $\gamma$-carboxyglutamic acid.

**Table 1. Solvent and Base Effect on the Addition of 14 to BPB-Ni-Gly**



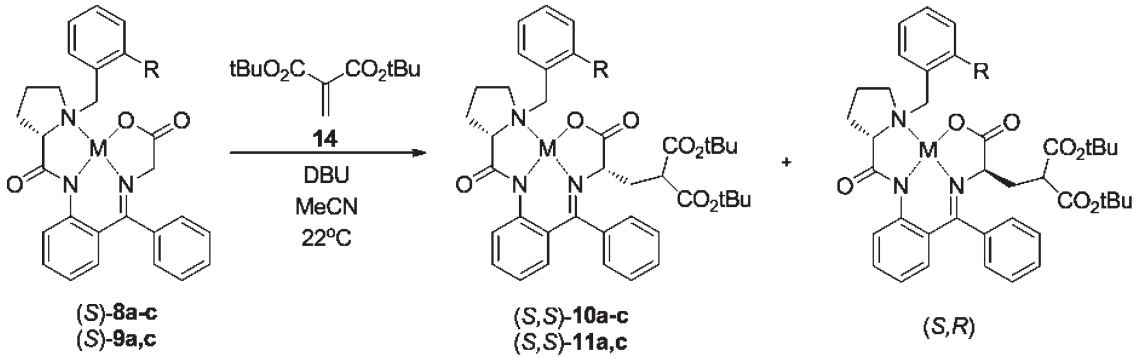

| entry | base | solvent | time (min) | yield[a] (%) | ee[b] (%) |
|---|---|---|---|---|---|
| 1 | DBU[c] | DMF | 10 | 70 | 80 |
| 2 | DBU[c] | MeCN | 10 | 75 | 82 |
| 3 | $NEt_3$[d] | 1:1 MeCN:DMF | 60 | 48 | 71 |
| 4 | $NH(iPr)_2$[d] | 1:1 MeCN:DMF | 60 | 41 | 58 |
| 5 | $NEt(iPr)_2$[d] | 1:1 MeCN:DMF | 60 | 25 | 70 |
| 6 | KOH[e] | DCM | 60 | 42 | 51 |

[a] Combined yield of major (*S*,*S*) and minor (*S*,*R*) diastereomers. [b] Enantiomeric excess of the (*S*)-amino acid based on Marfey's assay of the amino acid after decomposition of the metal complex diastereomers. [c] 0.15 equiv of base. [d] 3.0 equiv of base. [e] 10 equiv of base.

## RESULTS AND DISCUSSION

Our need for a more selective chiral glycine equivalent was realized during the optimization of reaction conditions for the Michael addition reaction employing the known Ni(II) complex of the Schiff base of glycine and (*S*)-2-(*N*-benzylprolyl)aminobenzophenone (BPB)[30,31] shown in Table 1. Initially, Michael additions of the glycinate (BPB-Ni-Gly) to di-*tert*-butyl methylenemalonate **14**[32] were optimized around conditions described in the literature.[21,22,33] The selectivity of the addition reaction was assessed by decomposing the resulting metal complex to free the amino acid, whose enantiomeric excess was measured using Marfey's reagent (Table 1, note b). The diastereomeric ratio of the products favoring the (*S*,*S*) configuration was consistent with reports discussed earlier. A slightly higher yield was obtained by

**Table 2. Michael Additions to Ni(II) Complexes (*S*)-8a−c and Cu(II) Complexes (*S*)-9a,c**




| entry | complex | product | metal (M) | R | yield[a] (%) | ee[b] (%) |
|---|---|---|---|---|---|---|
| 1 | (*S*)-**8a** | (*S*,*S*)-**10a** | Ni | H | 75 | 82 |
| 2 | (*S*)-**9a** | (*S*,*S*)-**11a** | Cu | H | 64 | 49 |
| 3 | (*S*)-**8b** | (*S*,*S*)-**10b** | Ni | Cl | 94 | 85 |
| 4 | (*S*)-**8c** | (*S*,*S*)-**10c** | Ni | SMe | 81 | 90 |
| 5 | (*S*)-**9c** | (*S*,*S*)-**11c** | Cu | SMe | 70 | 93 |
| 6[c] | (*S*)-**9c** | (*S*,*S*)-**11c** | Cu | SMe | 67 | >99 |

[a] Combined yield of major (*S*,*S*) and minor (*S*,*R*) diastereomers. [b] Enantiomeric excess of the (*S*)-amino acid based on Marfey's assay of the amino acid after decomposition of the metal complex diastereomers. [c] Isolated yield of (*S*,*S*)-**11c** from product mixture (entry 5).

exchanging DMF with $CH_3CN$ as the reaction solvent (Table 1, entries 1 and 2). In addition, the reaction proceeded more slowly when DBU was exchanged for other amine and alkaline bases, and resulted in lower yields and selectivity (Table 1, entries 3−6). To our dismay, regardless of the reaction conditions, the product diastereomers were inseparable by chromatography on silica gel using typical solvent systems.[14,34] Owing to the fact that this method of amino acid synthesis relies on the stereodiscriminating ability of the complex[35] in the reaction and separation of products to obtain a high excess of enantiomerically pure amino acid, we began to examine methods to improve both of these features en route to the synthesis of Gla residue (*S*)-**1**.

Efforts by others have been made to improve the stereoselectivity of addition reactions through substitutions on the *N*-benzyl portion of the chiral complex.[36,37] Saghiyan and co-workers reported that 3,4-dimethyl-, 3,4-dichloro-, and 2-chloro-substituted benzyl analogues of the Ni(II) complex gave better selectivity due to increased shielding of the coordination plane.[29]

Crystal structure analysis of the unsubstituted complex revealed that the benzyl group (Table 2, R = H, M = Ni) centers its aromatic ring directly above the metal in the coordination plane.[38] In comparison, when a chloro group is placed in the ortho position of the benzyl ring (Table 2, R = Cl, M = Ni), the aromatic ring moves off the metal atom and the Cl is positioned directly above the nickel.[39] Moreover, the sum of the Van der Waals radii of the Cl and Ni atoms is greater than the Ni−Cl atomic distance of 3.149 Å, implying a distinct axial interaction between the nickel and the chlorine.[39] This interaction was postulated to shield the top face of the complex toward an approaching electrophile, thus increasing the selectivity of the Michael addition. This crystallographic observation suggested to us that other bonafide axial ligand−metal interactions could be designed that enhance diastereoselectivity. Such axial interactions can be found in some blue copper proteins such as rustacyanin (Figure 3c), **1**where sulfur coordination (from methionine) to a central Cu atom occurs above the coordination plane formed from a set of trigonal side-chain ligands at a Cu−S atomic distance of 2.885 Å.[40] We investigated the possibility of mimicking this naturally occurring complex by replacing the 2-chloro substituent on BPB with a methylthio-substituent and swapping the nickel with copper (Table 2, R = SMe, M = Cu) to form a purported five-coordinate pyramidal complex. The axial sulfur donor should enhance the effective shielding of the coordination plane and increase the stereodiscriminating ability of the complex, and as a result, increase selectivity.

We began by preparing both the Ni(II) and Cu(II) complexes containing methylsulfide and chloro ortho-substituted benzyl groups to assess the ligating potential of the thioether and gauge the importance of swapping the nickel with copper. Based on the known propensity of thioethers to coordinate very weakly[41] or not at all[42] axially to Ni(II), we did not expect the thioether to coordinate to the Ni center, making it necessary to swap this metal with Cu. Lastly, the unsubstituted benzyl complexes were prepared to complete the comparative study.

Complexes of the Schiff base of glycine and 2-benzyl-substituted ligands based on (*S*)-2-(*N*-Benzylprolyl)-aminobenzophenone (BPB) were synthesized according to Scheme 1 starting from the aryl chloride. *N*-Benzylation of (*S*)-proline provided compounds (*S*)-**6a**−**c**, which were amidated with 2-aminobenzophenone to give chiral ligands (*S*)-**7a**−**c**. Metal complexes (*S*)-**8a**−**c**, (*S*)-**9a**, and (*S*)-**9c** were prepared using the corresponding ligands, metal salts, and glycine to form the Schiff base complex. Starting compound **5c** was prepared by alkylating,[43,44] reducing,[45,46] and chlorinating[47] thiosalicylic acid (Scheme 2).

Evaluation of the chiral metal complexes was performed (Table 2) using the optimal conditions that were established earlier (Table 1, entry 2). As expected, the Cu(II)-containing complex (*S*)-**9a** is less diastereoselective than the analogous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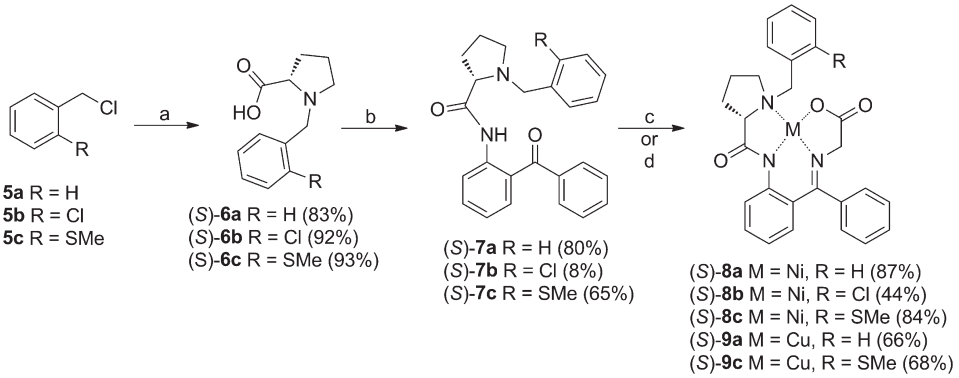
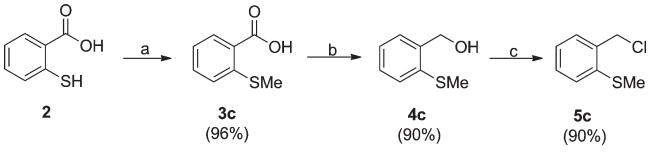
**Scheme 1**[a]




[a] Key: (a) (*S*)-Proline, NaOMe, MeOH, 45 °C, 16 h; (b) *N*-MeIm, MsCl, 2-aminobenzophenone, DCM, 48 °C 16 h; (c) glycine, $Ni(NO_3)_2 \cdot 6H_2O$, NaOMe, MeOH, 55 °C, 90 min; (d) glycine, $CuSO_4 \cdot 5H_2O$, KOH, MeOH, rt, 1 h.

**Scheme 2**[a]




[a] Key: (a) iodomethane, $K_2CO_3$, acetone, rt, 2 h; (b) $LiAlH_4$, THF, rt, 3 h; (c) concd HCl, toluene, rt, 90 min.

Ni(II) containing complex (*S*)-**8a** because of the weaker ligand−metal coordination and distortion of the coordination plane in the Cu(II) complex versus the Ni(II) complex.[34] Improved selectivity and yield was observed using the 2-Cl-benzyl-substituted Ni(II) complex (*S*)-**8b**, providing separable diastereomers in a combined yield of 94%. Further improvement in selectivity occurred using the 2-methylthio-benzyl-substituted Ni(II) complex (*S*)-**8c**, albeit in lower yield than (*S*)-**8b**. This is somewhat surprising, since we did not expect the thioether to coordinate to the nickel. In fact, the crystal structure of (*S*)-**8c** in Figure 4 shows that this is indeed the case. Here, the benzylic moiety assumes a conformation that directs the thioether away from the metal center; however, we cannot rule out any Ni-thioether interaction that may be occurring in solution. At this time, the reason for the slight increase in diastereoselectivity is not yet known.

Finally, the Cu(II) complex (*S*)-**9c** outperformed all others in terms of selectivity, resulting in an 11% increase in ee compared to the known Ni complex (*S*)-**8a**. Unlike the inseparable mixture of diastereomers resulting from the Michael addition to (*S*)-**8a**, the mixture of diastereomers from the reaction of (*S*)-**9c** were easily separated by chromatography ($\Delta R_f$ = 0.1, 3:1 hexanes/acetone) to allow isolation of the desired isomer (*S*,*S*)-**11c** in high stereochemical purity (Table 2, entry 6).

Although we were unable to obtain a crystal structure of (*S*)-**9c**, UV spectroscopic data (see Supporting Information), taken together with the increase in diastereoselectivity, supports axial coordination of the methyl sulfide similar to the coordination in complexes described by Belle and co-workers.[48] A UV difference spectrum was generated using the complexes (*S*)-**9c** and (*S*)-**9a** in $CH_3CN$ and shows maxima at 309 ($\Delta\varepsilon$ = −211) and 422 nm ($\Delta\varepsilon$ = 135), a shoulder at 335 nm ($\Delta\varepsilon$ = −761), and a broad band at 658 nm ($\Delta\varepsilon$ = 24). These features in the range of 300−450 nm and at 658 nm are consistent with sulfur−copper charge transfer bands and a copper d-d transition, respectively, and are consistent with assignments made for other compounds reported in the literature.[49]

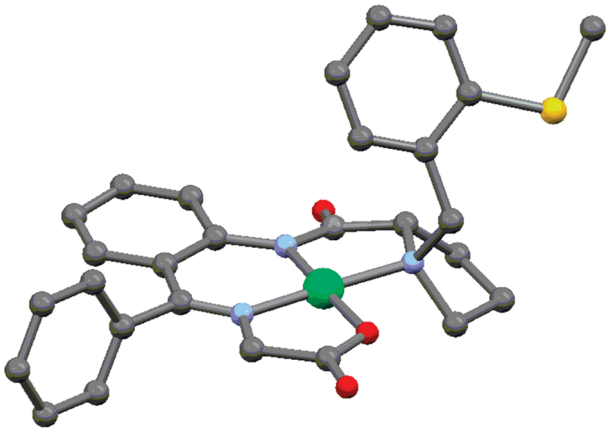
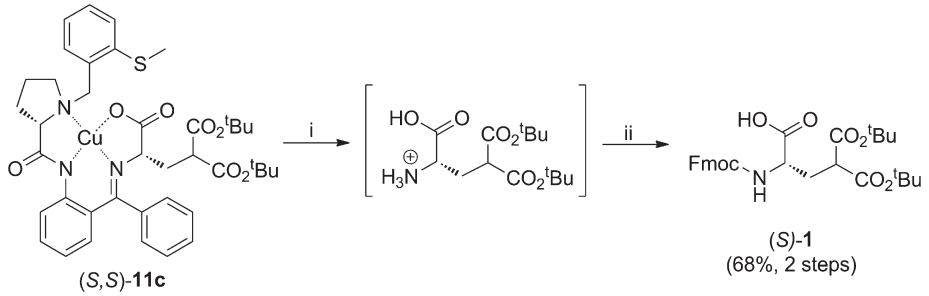


**Figure 4.** Crystal structure of Ni(II) complex (*S*)-**8c** showing the noncoordinated thioether. Second symmetry unique molecule and hydrogen atoms omitted for clarity. Atoms refined anisotropically but depicted with arbitrary radii. Crystal data: $C_{28}H_{27}N_3NiO_3S$, monoclinic, $P2_1$, $a$ = 10.428(5) Å, $b$ = 8.062(4) Å, $c$ = 29.581 Å, $\beta$ = 96.108(8)°, V = 2473(2) Å$^3$, $Z$ = 4, $D_{calc}$ = 1.462 g/cm$^3$, 32913 reflections collected, 12271 unique, $R_{int}$ = 0.0825, 651 parameters, $R_f$ = 0.0674, wR2 = 0.1302, Flack parameter = −0.028(14), CCDC 795141.

In addition, axial coordination of the thioether may also be responsible for the difference in the color of the solid compounds, changing from a brown color for (*S*)-**9a** to a green color for (*S*)-**9c**. Comparing the selectivity of complexes (*S*)-**9a** and (*S*)-**9c**, the ratio of diastereomeric products increases from 3:1 to >27:1, respectively. We attribute this large difference to a more effective shielding of the coordination plane via the addition of intramolecular axial ligand coordination to the metal.

With high diastereoselectivity and facile separation methods in hand, the synthesis of (*S*)-**1** was finished by optimizing metal complex decomposition and Fmoc-protection conditions. Typically, isolation of the amino acid from the metal complex is accomplished by decomposing the compound in a heated mixture of methanol and aqueous HCl.[14] However, for the Gla

**Scheme 3**[a]




[a] Key: (i) 1.5 M aq HCl, THF, rt, 7 h; (ii) EDTA, Fmoc-OSu, $NaHCO_3$, 1:1 MeCN/$H_2O$, 0 °C to rt, 24 h.

residue, milder conditions were sought to ensure that the acid-labile *tert*-butyl esters would remain intact. It was found that stirring any of the complexes ((*S*,*S*)-**10a**−**c** or (*S*,*S*)-**11a**,**c**) in THF with a minimal amount of dilute aqueous HCl decomposed the complex and preserved the t-butyl protecting groups. Concentrating the reaction mixture allowed for recovery of the ligands (*S*)-**7a**−**c** by filtration or phase separation. To complete the synthesis of (*S*)-**1**, (*S*,*S*)-**11c** was decomposed, Scheme 3. The filtrate was neutralized after ligand isolation and the soluble amino acid directly Fmoc-protected. From (*S*,*S*)-**11c**, the yield of (*S*)-**1** was 68% over two steps.

## CONCLUSION

We have successfully completed a facile chiral synthesis of Fmoc-Gla($O^tBu$)$_2$-OH in nine steps from thiosalicylic acid in 14.5% overall yield. We have designed the novel chiral ligand (*S*)-**7c**, whose glycine Schiff base metal complexes show increased diastereoselectivity in the Michael addition reaction of di-*tert*-butyl methylenemalonate over previously reported ligands. Most notably, the Cu(II) complex (*S*)-**9c** allowed us to complete a highly stereoselective route to (*S*)-**1** in three-steps with facile separation of diastereomers. The increase in selectivity is most likely due to an intramolecular axial coordination of a thioether to the Cu(II) center of a BPB system, a design inspired by the blue copper protein rusticyanin.

## EXPERIMENTAL SECTION

**Synthesis of (*S*)-1 from Metal Complexes 10a−c or 11a, c.** In a typical procedure, 1.5 M HCl (3.33 mL, 5 mmol) was added to a solution of the Ni(II) or Cu(II) complex of γ-carboxyglutamic acid (1 mmol) dissolved in THF (13 mL). The reaction was stirred for 7 h or until no more metal complex was present (TLC) and was then concentrated under vacuum to 1/6 the original volume. In the case of **10a** or **11a**, the precipitated ligand was removed from the aqueous portion by filtration and was washed with $H_2O$ (2 × 1 mL). For **10b**−**c** and **11c**, the biphasic mixture was transferred to a separatory funnel and allowed to separate. The dense oily residue was removed, and the aqueous portion was diluted with 2 mL of $H_2O$. In both cases, the aqueous portion was transferred to a clean flask, and solid $NaHCO_3$ (336 mg, 4 mmol) was carefully added with stirring to neutralize the solution, followed by $Na_2EDTA·H_2O$ (372 mg, 1 mmol), and was stirred for 5 min. Additional solid $NaHCO_3$ (336 mg, 4 mmol) was added, followed by a solution of Fmoc-OSu (337 mg, 1 mmol) in $CH_3CN$ (5 mL). The reaction was stirred for 24 h under Ar, concentrated in vacuo to 1/2 its original volume, adjusted to pH 3 with 10% citric acid, and extracted with EtOAc (3 × 15 mL). Combined organic extracts were washed with brine, dried with $MgSO_4$, concentrated, and purified on silica gel using an automated flash chromatography system employing a gradient of acetone in hexanes; product elutes at ∼20% acetone to yield 357 mg of (*S*)-**1** (0.68 mmol, 68% over two steps) as a waxy solid: $[\alpha]^{20}_D$ = −8.6 (*c* 0.29, MeOH) [lit.[50] $[\alpha]^{20}_D$ = −8.6 (*c* 0.173, MeOH)]; $^1$H NMR (400 MHz, $CDCl_3$) δ = 7.77 (m, 2H, ArH-4 and ArH-4′), 7.63 (m, 2H, ArH-1 and ArH-1′), 7.41 (m, 2H, ArH-3 and ArH-3′), 7.33 (m, 2H, ArH-2 and ArH-2′), 5.61 (N*H*), 4.42 (m, 2H, CHC$H_2$O), 4.33 (m, 1H, C*H*C$H_2$O), 4.24 (m, 1H, α-H), 3.38 (m, 1H, γ-H), 2.49 (m, 1H, β-H), 2.21 (m, 1H, β′-H), 1.48 (s, 18H, C($CH_3$)$_3$); $^{13}$C NMR (100 MHz, $CDCl_3$): δ = 173.7 (C-1), 168.5 (C-5), 168.2 (C-5′), 156.2 (C-8), 144.0 (C-11), 143.9 (C-11′), 141.3 (C-12 and C12′) 127.7 (C-14 and C-14′), 127.1 (C-13 and C-13′), 125.3 (C-15), 125.2 (C-15′), 120.0 (C-16 and C-16′), 82.0 (C-6 and C-6′), 67.1 (C-9), 52.6 (C-2), 50.9 (C-4), 47.2 (C-10), 31.2 (C-3), 27.9 (C-7 and C-7′); ESI-MS calcd for $C_{29}H_{34}NO_8Na$ ($M + Na^+$) 548.2, found 548.2.

**2-(Methylthio)benzoic Acid (3c).** Thiosalicylic acid (1.54 g, 10 mmol) was dissolved in acetone (81 mL), and $K_2CO_3$ (4.14 g, 30 mmol) was added. Iodomethane (685 μL, 11 mmol) was added dropwise and the mixture stirred for 1 h at room temperature, after which time solvent was removed in vacuo and the residue dissolved in water (40 mL) and cooled to 0 °C. With stirring, the pH of the solution was adjusted to ∼1 with 2 M HCl. The precipitated solid was filtered, washed with cold $H_2O$, and allowed to air-dry overnight to afford 1.61 g of the acid **3c** (9.6 mmol, 96%) as a white solid: mp 168−170 °C (lit.[51] mp 169 °C); $^1$H NMR (400 MHz, $CD_3OD$) δ = 7.94 (dd, 1H, *J* = 7.8, 1.5 Hz, ArH-4), 7.46 (ddd, 1H, *J* = 8.1, 7.3, 1.6 Hz, ArH-2), 7.32 (d, 1H, *J* = 8.1 Hz, ArH-1), 7.13 (m, 1H, ArH-3), 2.38 (s, 3H, S$CH_3$); $^{13}$C NMR (100 MHz, $CD_3OD$) δ = 168.3 (C-7), 143.3 (C-1), 132.1 (C-5), 131.1 (C-3), 127.0 (C-2), 124.1 (C-6), 122.9 (C-4), 14.0 (C-8); ESI-MS calcd for $C_8H_8O_2S$ ($MH^-$) 167.0, found 167.0.

**2-(Methylthio)benzyl Alcohol (4c).** Acid **3c** (16.64 g, 99 mmol) was dissolved in THF (225 mL) and added dropwise via addition funnel to a slurry of $LiAlH_4$ (7.14 g, 178 mmol) in THF (275 mL) under an Ar atmosphere. The mixture was stirred at room temperature for 3 h, after which time 1 M HCl (370 mL) was carefully added. The aqueous mixture was extracted 3 × 250 mL with ether. Combined organics were washed 2 × 100 mL with 1 M NaOH and 1 × 100 mL $H_2O$, dried with $MgSO_4$, and concentrated to give 13.7 g of the alcohol **4c** (89.1 mmol, 90%) as an oil: $^1$H NMR (400 MHz, $CDCl_3$) δ = 7.42 (d, 1H, *J* = 7.2 Hz, ArH-4), 7.30−7.23 (m, 2H, ArH-2 and ArH-1), 7.18 (ddd, 1H, *J* = 7.5, 5.9, 2.8 Hz, ArH-3), 4.74 (s, 2H, C$H_2$OH), 2.48 (s, 3H, S$CH_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) δ = 139.1 (C-2), 136.4 (C-1), 128.0 (C-5), 127.6 (C-3), 126.2 (C-6), 125.3 (C-4), 62.9 (C-7), 15.9 (C-8); APCI-MS **4c** ($C_8H_{10}OS$) readily ionized to a benzylic cation through loss of OH; calcd for $C_8H_9S$ ($M^+$) 137.0, found 137.0.

**2-(Methylthio)benzyl Chloride (5c).** Alcohol **4c** (1.23 g, 8 mmol) was dissolved in toluene (2.85 mL) and cooled to 0 °C. Concentrated HCl (1.73 mL, 20.8 mmol) was added dropwise at 0 °C, and the biphasic mixture was vigorously stirred to form an emulsion. Stirring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was continued for 2 h at room temperature. The biphasic mixture was then diluted with 6.4 mL of toluene, transferred to a separatory funnel, and allowed to separate, and the aqueous portion was removed. The organic portion was washed with 3 mL of $H_2O$ and stirred with solid $NaHCO_3$ (0.223 g, 2.66 mmol) for 15 min. The toluene solution was decanted and concentrated in vacuo to give 1.23 g of the chloride **5c** (7.2 mmol, 90%) as an oil: $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ = 7.38 (d, 1H, $J$ = 7.2 Hz, ArH-4), 7.32−7.27 (m, 2H, ArH-2 and ArH-1), 7.16 (ddd, 1H, $J$ = 7.6, 5.7, 2.9 Hz, ArH-3), 4.74 (s, 2H, C$H_2$Cl), 2.50 (s, 3H, SC$H_3$); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ = 138.3 (C-2), 135.5 (C-1), 130.0 (C-5), 129.2 (C-3), 127.0 (C-6), 125.5 (C-4), 44.4 (C-7), 16.3 (C-8); APCI-MS **5c** ($C_8H_9ClS$) readily ionized to a benzylic cation through loss of Cl; calcd for $C_8H_9S$ ($M^+$) 137.0, found 137.0.

**(*S*)-*N*-(2-(Methylthio)benzyl)pyrrolidine-2-carboxylic Acid (6c).** In a round-bottomed flask equipped with a reflux condenser was added the benzyl chloride **5c** (5.14 g, 30 mmol) dropwise via syringe through the condenser to a stirring solution of (*S*)-proline (3.45 g, 30 mmol) and NaOMe (3.24 g, 60 mmol) in MeOH (30 mL) at 48 °C. After the mixture was stirred overnight at 48 °C, the reaction was allowed to cool to room temperature, and concd HCl (30 mmol) was added via syringe to achieve a pH of ∼5. $CHCl_3$ (30 mL) was added and the mixture stirred for 1 h, after which time the precipitate was filtered over Celite and washed with $CHCl_3$. The filtrate was concentrated, and the residue was purified on silica gel using an automated flash chromatography system employing a shallow gradient of methanol in $CH_2Cl_2$; product elutes at ∼20% methanol to yield 5.57 g of the acid **6c** (22.2 mmol, 74%) as a waxy solid: $[\alpha]^{20}_D$ = −16.9 (*c* 1.0, MeOH); $^1$H NMR (400 MHz, $CD_3OD$) $\delta$ = 7.47 (d, 1H, $J$ = 7.9 Hz, ArH-1), 7.45−7.39 (m, 2H, ArH-3 and ArH-4), 7.23 (m, 1H, ArH-2), 4.48 (s, 2H, C$H_2$Ph), 3.95 (dd, 1H, $J$ = 9.3, 5.0 Hz, α-H), 3.59 (m, 1H, $\delta$-H), 3.22 (m, 1H, $\delta'$-H), 2.54 (s, 3H, C$H_3$), 2.43 (m, 1H, $\beta'$-H), 2.21−2.04 (m, 2H, $\beta$-H and $\gamma'$-H), 1.90 (m, 1H, $\gamma$-H); $^{13}$C NMR (100 MHz, $CD_3OD$) $\delta$ = 171.6 (C-1), 139.2 (C-8), 131.6 (C-12), 130.5 (C-9), 128.9 (C-7), 127.9 (C-10), 125.8 (C-11), 68.6 (C-2), 55.6 (C-6), 54.4 (C-5), 28.6 (C-3), 23.1 (C-4), 15.4 (C-13); ESI-MS calcd for $C_{13}H_{17}NO_2S$ ($MH^-$) 250.1, found 250.1.

**(*S*)-*N*-(2-Benzoylphenyl)-1-(2-(methylthio)benzyl)pyrrolidine-2-carboxamide (MeS-BPB) (7c).** *N*-Methylimidazole (1.55 mL, 19.6 mmol) was added via syringe to a solution of benzylproline **6c** (2.24 g, 8.91 mmol) in $CH_2Cl_2$ (22 mL) under Ar atmosphere. The solution was cooled to 0 °C, and methanesulfonyl chloride (693 $\mu$L, 8.91 mmol) was added dropwise via syringe, after which the solution was warmed to room temperature and a solution of 2-aminobenzophenone (1.58 g, 8.02 mmol) in $CH_2Cl_2$ (8 mL) was added dropwise via addition funnel. The reaction flask was fitted with a reflux condenser, heated to 45 °C and stirred overnight under Ar, allowed to cool to room temperature, quenched with satd aq $NH_4Cl$ (30 mL), and extracted with $CH_2Cl_2$ (3 × 50 mL). Organic extracts were concentrated, and the residue was purified on silica gel using an automated flash chromatography system employing a gradient of ethyl acetate in hexanes; product elutes at ∼40% ethyl acetate to yield 2.24 g of carboxamide **7c** (5.21 mmol, 65% based on 2-aminobenzophenone) as a yellow oil: $[\alpha]^{20}_D$ = −120.9 (*c* 1.0, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ = 10.99 (s, NH), 8.29 (d, 1H, $J$ = 8.3 Hz, ArH-5), 7.75 (dd, 2H, $J$ = 8.3, 1.2 Hz, ArH-9 and ArH-9′), 7.58 (m, 1H, ArH-11), 7.53−7.43 (m, 5H, ArH-6, ArH-2, ArH-4, ArH-10, and ArH-10′), 7.17−7.03 (m, 3H, ArH-8, ArH-3, and ArH-1), 6.96 (m, 1H, ArH-7), 3.84 (d, 1H, $J$ = 13.5 Hz, C*H*HPh), 3.84 (d, 1H, $J$ = 13.5 Hz, CH*H*Ph), 3.34 (dd, 1H, $J$ = 10.1, 4.6 Hz, α-H), 3.22 (m, 1H, $\delta$-H), 2.45 (m, 1H, $\delta'$-H), 2.31 (s, 3H, C$H_3$), 2.23 (m, 1H, $\beta$-H), 1.86 (m, 1H, $\beta'$-H), 1.78−1.67 (m, 2H, $\gamma$-H and $\gamma'$-H); $^{13}$C NMR (100 MHz, $CDCl_3$): $\delta$ = 197.3 (C-17), 174.1 (C-1), 138.3 (C-11), 138.2 (C-18), 137.6 (C-8), 135.9 (C-7), 132.7 (C-13), 132.5 (C-21), 131.8 (C-24), 129.9 (C-19), 129.6 (C-10), 128.2 (C-20), 127.6 (C-15), 126.5 (C-16), 125.1 (C-9), 124.6 (C-14), 122.5 (C-23), 121.8 (C-12), 68.3 (C-2), 57.2 (C-6), 54.1 (C-5), 30.8 (C-3), 24.0 (C-4), 15.5 (C-22); APCI-MS calcd for $C_{26}H_{26}N_2O_2S$ ($MH^+$) 431.2, found 431.1.

**Ni(II) Complex of Schiff Base of (*S*)-MeS-BPB and Glycine (MeS-BPB-Ni-Gly) (8c).** In a round-bottomed flask fitted with an addition funnel were added glycine (803 mg, 10.7 mmol) and Ni-$(NO_3)_2\cdot 6H_2O$ (1.87 g, 6.42 mmol) to a solution of benzophenone **7c** (2.3 g, 5.35 mmol) in MeOH (16.5 mL) and the mixture heated to 55 °C. A 2.5 M solution of NaOMe (4.42 g, 81.85 mmol) in MeOH was added dropwise via the addition funnel at 55 °C, after which time the flask was fitted with a reflux condenser and stirred for 90 min. The reaction was allowed to cool to room temperature, poured into 30 mL of 10% citric acid, extracted with $CH_2Cl_2$ (3 × 50 mL), concentrated, and purified on silica gel using an automated flash chromatography system employing a gradient of acetone in $CH_2Cl_2$; product elutes at ∼80% acetone to yield 2.46 g of complex **8c** (4.52 mmol, 84%) as a red solid: $[\alpha]^{20}_D$ = +2474.0 (*c* 0.1, $CHCl_3$); $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ = 8.06 (d, 1H, $J$ = 7.4 Hz, ArH-4), 7.95 (d, 1H, $J$ = 8.6 Hz, ArH-5) 7.57−7.48 (m, 3H, ArH-11, ArH-10, and ArH-12), 7.28 (m, 1H, ArH-2), 7.26−7.20 (m, 2H, ArH-1 and ArH-3), 7.16 (ddd, 1H, $J$ = 8.6, 7.1, 1.3 Hz, ArH-6), 7.1 (d, 1H, $J$ = 7.1 Hz, ArH-13), 6.97 (m, 1H, ArH-9), 6.80 (dd, 1H, $J$ = 8.2, 1.5 Hz, ArH-8), 6.70 (m, 1H, ArH-7), 4.50 (d, 1H, $J$ = 13.0 Hz, C*H*HPh), 3.80 (d, 1H, $J$ = 13.0 Hz, CH*H*Ph), 3.80 (d, 1H, $J$ = 20.1 Hz, Gly α-H), 3.67 (d, 1H, $J$ = 20.1 Hz, Gly α′-H), 3.62−3.43 (m, 3H, Pro α-H, $\gamma$-H, and $\delta$-H), 2.78 (m, 1H, $\beta$-H), 2.56 (m, 1H, $\beta'$-H), 2.50 (s, 3H, C$H_3$), 2.17−2.05 (m, 2H, $\gamma'$-H and $\delta'$-H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ = 180.3 (C-1), 177.1 (C-25), 171.0 (C-17), 142.4 (C-11), 140.0 (C-27), 134.5 (C-18), 133.1 (C-8), 132.8 (C-15), 131.8 (C-13), 131.3 (C-7), 129.8 (C-10), 129.7 (C-21), 129.5 (C-20), 129.2 (C-22), 126.9 (C-26), 126.2 (C-23), 125.6 (C-19), 125.5 (C-16), 125.3 (C-9), 124.5 (C-12), 120.8 (C-14), 70.2 (C-2), 61.2 (C-24), 60.4 (C-6), 57.8 (C-5), 30.4 (C-3), 23.8 (C-4), 16.5 (C-28); HRMS-MALDI-TOF calcd for $C_{28}H_{28}N_3O_3SNi$ ($MH^+$) 544.120, found 544.117.

**Cu(II) Complex of Schiff Base of (*S*)-MeS-BPB and Glycine (MeS-BPB-Cu-Gly) (9c).** Glycine (2.5 g, 33.3 mmol) and Cu-$SO_4\cdot 5H_2O$ (3.32 g, 13.3 mmol) were added to a solution of benzophenone **7c** (2.87 g, 6.67 mmol) in MeOH (24 mL) at room temperature. A 4.6 M solution of KOH (2.61 g, 46.7 mmol) in MeOH was added to the mixture in one portion. The mixture was stirred for 1 h at room temperature, poured into 30 mL of 10% citric acid, extracted with $CH_2Cl_2$ (3 × 50 mL), concentrated, and purified on silica gel using an automated flash chromatography system employing a gradient of acetone in $CH_2Cl_2$; product elutes at ∼80% acetone to yield 2.50 g of complex **9c** (4.56 mmol, 68%) as a green solid: $[\alpha]^{20}_D$ = −639.4 (*c* 0.1, $CHCl_3$); HRMS-MALDI-TOF calcd for $C_{28}H_{28}N_3O_3SCu$ ($MH^+$) 549.115, found 549.117.

**Preparation of 10a−c and 11a,c.** In a typical procedure, the metal complex (**8a−c** or **9a,c**, 1 equiv) was dissolved in $CH_3CN$, and DBU (0.15 equiv) was added at room temperature. Freshly prepared di-*tert*-butyl methylenemalonate (1 equiv) was added dropwise via syringe and stirred for 30 min. The reaction mixture was poured into 10% citric acid, extracted with $CH_2Cl_2$ (3×), concentrated, and purified on silica gel using an automated flash chromatography system employing a gradient of acetone in hexanes; product elutes at ∼50% acetone to yield red (**10a−c**), brown (**11a**), and green (**11c**) solids. Combined yield of both diastereomers is shown for **10a−c**, **11a**, and **11c**; however, only spectral data for the major diastereomer (*S*,*S*) is listed.

**Ni(II) Complex of Schiff Base of (*S*)-BPB and $\gamma$-Carboxyglutamic Acid (BPB-Ni-Gla) (10a).** Results: yield 75%; $^1$H NMR (400 MHz, $CDCl_3$) $\delta$ = 8.19 (d, 1H, $J$ = 8.1 Hz, ArH-4), 8.06 (d, 2H, $J$ = 7.1 Hz, ArH-1 and ArH-1′), 7.52−7.47 (m, 3H, ArH-10, ArH-11, and ArH-9), 7.34 (m, 2H, ArH-2 and ArH-2′), 7.23−7.09 (m, 4H, ArH-12, ArH-3, ArH-8, and ArH-5), 6.67−6.59 (m, 2H, ArH-6 and ArH-7), 4.41 (d, 1H, $J$ = 12.6 Hz, C*H*HPh), 3.90 (m, 1H, Gla $\gamma$-H), 3.84 (dd, 1H,

$J$ = 10.4, 5.2 Hz, Gla α-H), 3.74 (m, 1H, Pro γ-H), 3.61−3.52 (m, 2H, Pro δ-H and CH*H*Ph), 3.46 (dd, 1H, $J$ = 10.9, 5.8 Hz, Pro α-H), 2.97 (m, 1H, Gla β-H), 2.84 (m, 1H, Pro β-H), 2.53 (m, 1H, Pro β′-H), 2.24−2.05 (m, 3H, Pro γ′-H, Gla β′-H, and Pro δ′-H), 1.39 (s, 9H, C($CH_3$)$_3$), 1.35 (s, 9H, C($CH_3$)$_3$′); $^{13}$C NMR (100 MHz, $CDCl_3$) δ = 180.3 (C-1), 178.1 (C-25), 171.3 (C-17), 167.9 (C-28), 167.7 (C-28′), 142.4 (C-11), 133.5 (C-15), 133.4 (C-18), 133.3 (C-7), 132.1 (C-13), 131.5 (C-8), 129.6 (C-21), 129.1 (C-20), 128.8 (C-22), 128.7 (C-9), 128.6 (C-10), 127.8 (C-23), 126.9 (C-19), 126.1 (C-16), 123.5 (C-12), 120.5 (C-14), 81.8 (C-29), 81.5 (C-29′), 70.4 (C-2), 68.8 (C-24), 63.0 (C-6), 57.3 (C-5), 49.8 (C-27), 33.6 (C-26), 30.7 (C-3), 27.8 (C-30 and C-30′), 23.7 (C-4); HRMS-MALDI-TOF calcd for $C_{39}H_{45}N_3O_7NiNa$ ($M + Na^+$) 748.251, found 748.257.

**Ni(II) Complex of Schiff Base of (*S*)-Cl-BPB and γ-Carboxyglutamic Acid (Cl-BPB-Ni-Gla) (10b).** Results: yield 94%; $^1$H NMR (400 MHz, $CDCl_3$) δ = 8.19 (dd, 1H, $J$ = 7.6, 1.5 Hz, ArH-4), 8.07 (d, 1H, $J$ = 8.7 Hz, ArH-5), 7.52−7.47 (m, 3H, ArH-11, ArH-12, and ArH-10), 7.34−7.25 (m, 2H, ArH-3 and ArH-1), 7.23 (m, 1H, ArH-13), 7.18−7.08 (m, 3H, ArH-2, ArH-9, and ArH-6), 6.68−6.61 (m, 2H, ArH-7 and ArH-8), 4.45 (d, 1H, $J$ = 12.9 Hz, C*H*HPh), 3.93 (m, 1H, Gla γ-H), 3.85−3.78 (m, 2H, CH*H*Ph and Gla α-H), 3.72 (m, 1H, Pro γ-H), 3.57−3.45 (m, 2H, Pro α-H and Pro δ-H), 3.07 (m, 1H, Pro β-H), 2.93 (m, 1H, Gla β-H), 2.63 (m, 1H, Pro β′-H), 2.24 (m, 1H, Pro γ′-H), 2.15−2.04 (m, 2H, Pro δ′-H and Gla β′-H), 1.39 (s, 9H, C($CH_3$)$_3$), 1.34 (s, 9H, C($CH_3$)$_3$′); $^{13}$C NMR (100 MHz, $CDCl_3$) δ = 179.4 (C-1), 178.0 (C-25), 171.3 (C-17), 167.9 (C-30), 167.6 (C-30′), 142.1 (C-11), 135.6 (C-27), 133.9 (C-8), 133.4 (C-15), 133.3 (C-18), 132.1 (C-13), 131.2 (C-7), 130.4 (C-10), 130.2 (C-26), 129.6 (C-21), 129.1 (C-20), 128.7 (C-22), 127.7 (C-19), 127.0 (C-9), 126.9 (C-23), 126.4 (C-16), 123.3 (C-12), 120.6 (C-14), 81.9 (C-31), 81.5 (C-31′), 70.8 (C-2), 68.7 (C-24), 59.8 (C-6), 57.3 (C-5), 49.7 (C-29), 33.6 (C-28), 30.3 (C-3), 27.7 (C-32 and C32′), 23.5 (C-4); HRMS-MALDI-TOF calcd for $C_{39}H_{44}ClN_3O_7NiNa$ ($M + Na^+$) 782.212, found 782.217.

**Ni(II) Complex of Schiff Base of (*S*)-MeS-BPB and γ-Barboxyglutamic Acid (MeS-BPB-Ni-Gla) (10c).** Results: yield 81%; $^1$H NMR (400 MHz, $CDCl_3$) δ = 7.90 (d, 1H, $J$ = 7.4 Hz, ArH-4), 7.84 (d, 1H, $J$ = 8.6 Hz, ArH-5), 7.53−7.46 (m, 3H, ArH-11, ArH-10, and ArH-12), 7.24−7.04 (m, 6H, ArH-2, ArH-9, ArH-3, ArH-1, ArH-13, and ArH-6), 6.68−6.58 (m, 2H, ArH-7 and ArH-8), 4.34 (d, 1H, $J$ = 13.0 Hz, C*H*HPh), 3.89 (dd, 1H, $J$ = 7.1, 5.5 Hz, Gla γ-H), 3.85−3.71 (m, 2H, Gla α-H and Pro γ-H), 3.65 (d, 1H, $J$ = 13.0 Hz, CH*H*Ph), 3.54 (dd, 1H, $J$ = 10.7, 6.8 Hz, Pro α-H), 3.45 (dd, 1H, $J$ = 9.8, 7.0 Hz, Pro δ-H), 3.13 (m, 1H, Pro β-H), 3.01 (m, 1H, Gla β-H), 2.67 (m, 1H, Pro β′-H), 2.45 (s, 3H, $CH_3$), 2.27 (m, 1H, Pro γ′-H), 2.16−2.00 (m, 2H, Gla β′-H and Pro δ′-H), 1.40 (s, 9H, C($CH_3$)$_3$), 1.33 (s, 9H, C($CH_3$)$_3$′); $^{13}$C NMR (100 MHz, $CDCl_3$) δ = 179.2 (C-1), 177.8 (C-25), 170.6 (C-17), 167.9 (C-31), 167.5 (C-31′), 142.3 (C-11), 139.8 (C-27), 133.2 (C-18), 133.0 (C-15), 132.9 (C-8), 131.8 (C-13), 131.2 (C-7), 129.6 (C-10), 129.5 (C-21), 129.1 (C-20), 128.6 (C-22), 127.7 (C-23), 126.8 (C-19), 126.7 (C-16), 126.5 (C-26), 125.0 (C-9), 123.7 (C-12), 120.4 (C-14), 81.8 (C-32), 81.3 (C-32′), 71.1 (C-2), 68.6 (C-24), 60.8 (C-6), 57.7 (C-5), 49.7 (C-30), 33.6 (C-29), 30.3 (C-3), 27.7 (C-33 and C33′), 23.7 (C-4), 16.3 (C-28); HRMS-MALDI-TOF calcd for $C_{40}H_{48}N_3O_7SNi$ ($MH^+$) 772.257, found 772.259.

**Cu(II) Complex of Schiff Base of (*S*)-BPB and γ-Carboxyglutamic Acid (BPB-Cu-Gla) (11a).** Results: yield 64%; HRMS-MALDI-TOF calcd for $C_{39}H_{45}N_3O_7CuNa$ ($M + Na^+$) 753.245, found 753.245.

**Cu(II) Complex of Schiff Base of (*S*)-MeS-BPB and γ-Carboxyglutamic Acid (MeS-BPB-Cu-Gla) (11c).** Results: yield 70%; $[\alpha]^{20}_D$ = −1322.4 (*c* 0.1, $CHCl_3$, pure (*S*,*S*) diastereomer); HRMS-MALDI-TOF calcd for $C_{40}H_{48}N_3O_7SCu$ ($MH^+$) 777.251, found 777.247.

## ■ ASSOCIATED CONTENT

**Supporting Information.** General methods and experimental procedures for compounds (*S*)-**6a,b**, (*S*)-**7a,b**, (*S*)-**8a,b**, (*S*)-**9a**, and **14**; $^1$H and $^{13}$C NMR spectra for all compounds except **2**, (*S*)-**9a**, (*S*)-**9c**, **11a**, and **11c**; UV difference spectrum of (*S*)-**9c** and (*S*)-**9a**; MS data for all compounds; chiral HPLC chromatograms for compounds (*S*)-**7b,c**, (*S*)-**8b,c**, (*S*)-**9a**, and (*S*)-**9c**; crystallographic data (ORTEP file and CIF) for compound (*S*)-**8c**. This material is available free of charge via the Internet at http://pubs.acs.org.

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## ■ ABBREVIATIONS

BPB, (*S*)-2-(*N*-benzylprolyl)aminobenzophenone; MeCN, acetonitrile; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DMF, dimethylformamide; $NEt_3$, triethylamine; $NH(iPr)_2$, diisopropylamine; [$NEt(iPr)_2$], diisopropylethylamine; DCM, dichloromethane; *N*-MeIm, *N*-methylimidazole; MsCl, methanesulfonyl chloride; THF, tetrahydrofuran; EDTA, ethylenediaminetetraacetic acid; Fmoc-OSu, *N*-(9-fluorenylmethoxycarbonyloxy)succinimide; Fmoc-Gla-$(OtBu)_2$-OH, *N*-α-(9-fluorenylmethyloxycarbonyl)-γ-carboxy-L-glutamic acid γ,γ′-di-*tert*-butyl ester.

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