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Artificial model for cystathionine β-synthase: construction of a catalytic cycle with a pyridoxal model compound having an ionophore function

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Abstract—Catalytic transformation of serine-*O*-carbonate to *S*-aryl cysteine derivatives was successfully achieved in the presence of Li^+ by the use of a pyridoxal model compound having an ionophore function, which is the first example mimicking cystathionine β-synthase, artificially.

1. Introduction

Pyridoxal 5′-phosphate (PLP, **1**) and pyridoxamine 5′-phosphate (PMP, **2**) are important coenzymes related with the biosynthetic and metabolic reactions of α-amino acids. A number of reactions mediated by these coenzymes are known.[1] β-Replacement reaction of the serine hydroxyl group with a nucleophile is one such reaction mediated by pyridoxal, and is a biologically important reaction for biosynthesis of amino acids. Tryptophan synthase and cystathionine β-synthase are typical examples, in which the nucleophiles are indole and homocysteine, respectively (Figure 2). In order to clarify the mechanisms of the biological reactions, and from the viewpoint of synthetic organic chemistry, a number of studies mimicking the biological reactions mediated by a pyridoxal–pyridoxamine system have been conducted.[2] In fact, pyridoxal model studies mimicking tryptophan synthase have also been reported,[3] but, to the best of our knowledge, that mimicking cystathionine β-synthase has not. Cystathionine β-synthase is a biologically important enzyme, working as a homocysteine scavenger,[4] deficiency of which causes homocystinuria, and is known to be related with cardiovascular diseases, neural tube defects and Altzheimer's disease.[5] Cystathionine β-synthase is known to require two cofactors, pyridoxal and heme. Although the role of pyridoxal is apparent, that of heme has not been clarified, but it may be a regulatory role.[6]

In previous papers, we reported a novel type of pyridoxal model compound having an ionophore side-chain at the C-3 hydroxyl group, which had not been modified in previous model compounds, and its application to the synthesis of α,α-dialkyl amino esters by the α-alkylation.[7,8] In these studies, it was found that aldimine, prepared from **3** and an amino ester, can capture Li^+ specifically as shown in Figure 1, and, as a consequence, the α-hydrogen is activated.[7] This interesting feature of **3** was expected to be of use for other reactions mediated by PLP (**1**). As the β-replacement reaction appears to be useful for the synthesis of various β-modified amino acids by employing various kinds of nucleophiles, we, at first, applied our model compound **3** to the β-replacement reaction employing thiols as a nucleophile, and successfully achieved the reaction

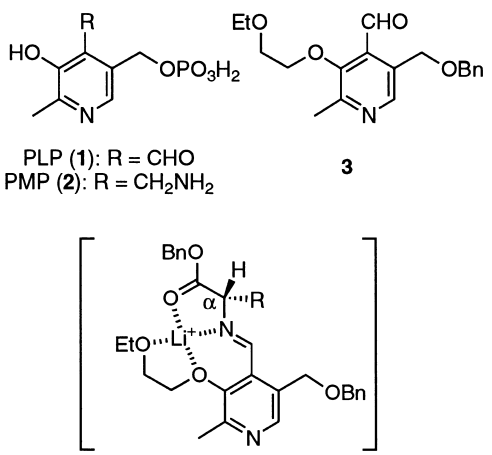

Figure 1. Structures of pyridoxal, pyridoxamine and model compound **3**.

Keywords: amino acids and derivatives; coenzymes; pyridines; substitution.

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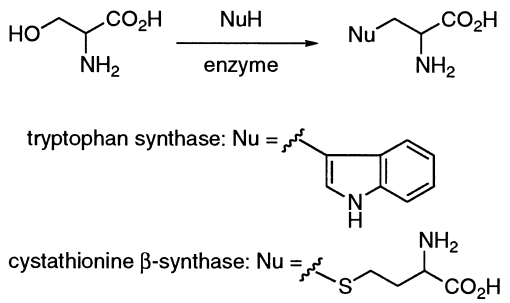
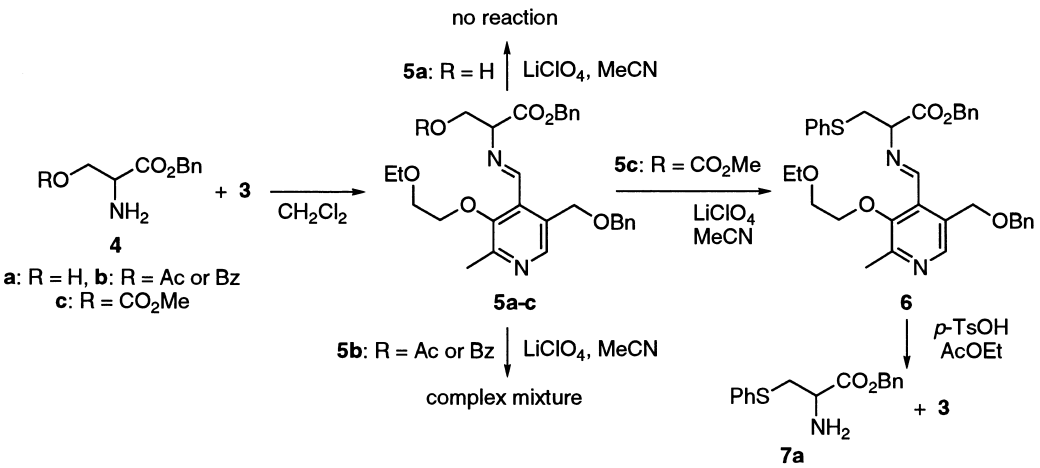


Figure 2. β-Replacement of reaction of Ser in a biological system.

under catalytic conditions. Herein we describe details of the results.[9]

2. Results and discussion

At first, the reaction was examined under stoichiometric conditions (Scheme 1). Namely, aldimine **5a**, which had been quantitatively prepared from **3** and serine ester **4a** only by mixing in CH_2Cl_2 at room temperature, was treated with thiophenol in the presence of lithium perchlorate ($LiClO_4$) in acetonitrile. However, no reaction took place, and only the starting aldimine **5a** was recovered. As this appears to be



Scheme 1.

due to the low eliminating ability of the hydroxyl group and/or an effect of the chelation with the hydroxyl group rather than with the ester moiety as shown in Figure 3, serine-*O*-acylates **4b** (acetate and benzoate) were examined in order to increase the eliminating ability of the hydroxyl group and to lower the chelation ability. However, as acyl migration of **4b** took place easily, it was difficult to handle. In addition, although the starting material **5b**, as expected, disappeared quickly on the TLC, a complex mixture was formed. In these reactions, acetic acid or benzoic acid was formed by the elimination, which was expected to interfere with the reaction. Eventually, we selected serine-*O*-carbonate **4c** as a starting material, because the expected by-products were methanol and carbon dioxide, both of which were neutral and expected not to affect the reaction. In this case, the reaction of **5c** smoothly proceeded to give the desired aldimine **6** in almost quantitative yield. To obtain cysteine derivative **7a**, the aldimine **6** was subjected to hydrolysis under conventional acidic conditions. However, despite extensive effort, hydrolysis of the aldimine **6** resulted in poor yield, giving unidentified products and *S*-phenylcysteine (**7a**) in 23% yield when *p*-toluenesulfonic acid was employed. Although the reason is not clear, the nucleophilic character of the neighboring sulfur atom of **6** might interfere with the hydrolysis.

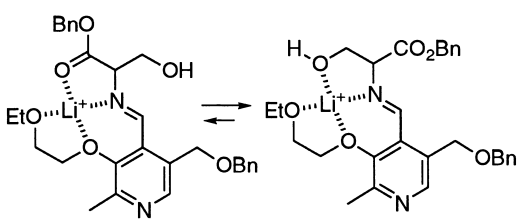
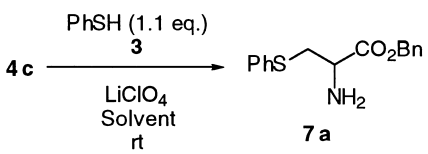


Figure 3. Possible chelation structure of **5a** with Li.

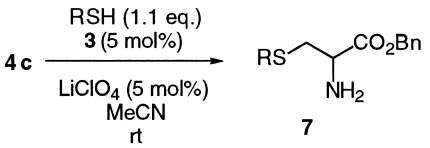
Table 1. β-Replacement reaction of **4c** with thiophenol catalyzed by pyridoxal **3**



Entry	**3** (mol%)	$LiClO_4$ (mol%)	Solvent	Time (h)	Yield (%) of **7a**	
					Based on **4c**	Based on **3**
1	1	1	MeCN	38	76	7600
2	5	5	MeCN	5	93	1860
3	10	10	MeCN	2	87	870
4	5	5	CH_2Cl_2	18	80	1600
5	5	5	AcOEt	27	88	1760
6	5	5	THF	27	30	600

From the results described above, the *O*-carbonate function was revealed to work as a good leaving group without affecting the β-replacement reaction. The unexpected resistance of the aldimine **6** towards hydrolysis was one problem which remained. It occurred to us that, if imine

Table 2. β-Replacement reaction of **4c** with thiols catalyzed by pyridoxal **3**



Entry	R	Time (h)	Product 7	Yield (%) of 7	
				Based on **4c**	Based on **3**
1	Ph	5	**a**	93	1860
2	4-MeC_6H_4	5	**b**	86	1720
3	4-$MeOC_6H_4$	5	**c**	90	1800
4	4-$O_2NC_6H_4$	3	**d**	92	1840
5	Bn	19	**e**	61	1220
6	Et	22	**f**	41	820
7	$HO(CH_2)_3$	72	**g**	30	600
8	$HS(CH_2)_3$[a]	25	**h**[b]	28	560

[a] Thiol (0.6 equiv.) was employed.
[b] Both thiol groups were alkylated.

exchange reaction between the aldimine **6** and the starting serine derivative **4c** took place, we could desirably make this reaction procedure catalytic. Based on this hypothesis, we examined the reaction employing a catalytic amount of **3** and $LiClO_4$, and the results are summarized in Table 1. The reaction smoothly took place at room temperature only by mixing serine-*O*-carbonate **4c** and thiophenol, in the presence of a catalytic amount of model compound **3** and $LiClO_4$ in acetonitrile, to give *S*-phenylcysteine **7a**.[10] It was found that the reaction proceeds in the presence of only 1 mol% of **3** to give **7a** in 76% yield based on **4c** (entry 1). In this case, the yield based on **3** was 7600%, which simultaneously means that one molecule of **3** converts 76 molecules of serine derivative **4c** to the corresponding cysteine derivative **7a**. However, from a practical viewpoint, the best yield based on **4c** was obtained when 5 mol% of **3** was used (entry 2). Increasing the amount of **3** shortened the reaction time, but lowered the yield as well (entry 3). The solvent effect was also examined as summarized in entries 4–6. Although the reaction proceeded in the solvents shown, acetonitrile was found to be the best choice. The low yield in THF could be attributable to solvation of Li^+ with THF (entry 6).

Under the conditions of entry 2 in Table 1, aromatic thiols reacted with **4c** to afford *S*-aryl cysteines **7a–d** in good yields (entries 1–4 in Table 2). As the reactions of various 4-substituted thiophenols took place, it is noteworthy that the thiophenol with an electron-withdrawing group is a good substrate for the reaction (entry 4 vs entries 1–3). In contrast, the reactions with alkane thiols proceeded, but required longer reaction time, and the yields were lower than those for aromatic thiols (entries 5–8).

In our previous report dealing with α-alkylation of α-amino esters employing the same model compound **3**, a combination of **3** with Li^+ was found to be essentially important for the reaction to proceed, and the chelation structure including Li^+ was also revealed by ^1H NMR.[7] From the results, it was easily assumed that Li^+ plays a crucial role in the present reaction as well. In order to confirm the role of Li^+, we studied the effect of metal ions on the elimination procedure of the carbonate moiety using ^1H NMR. In the presence of Li^+, the aldimine **5c** smoothly disappeared, while two olefinic hydrogens appeared, indicating the formation of unsaturated species **8** as expected. As is obvious from Figure 4, this reaction specifically proceeded only in the presence of Li^+, and did not proceed smoothly in the presence of other metal ions or in the absence of Li^+. These results clearly suggest that specific chelation of Li^+ between the iminoester moiety and the ethoxyethoxy group plays an important role similar to our previous results.[7]

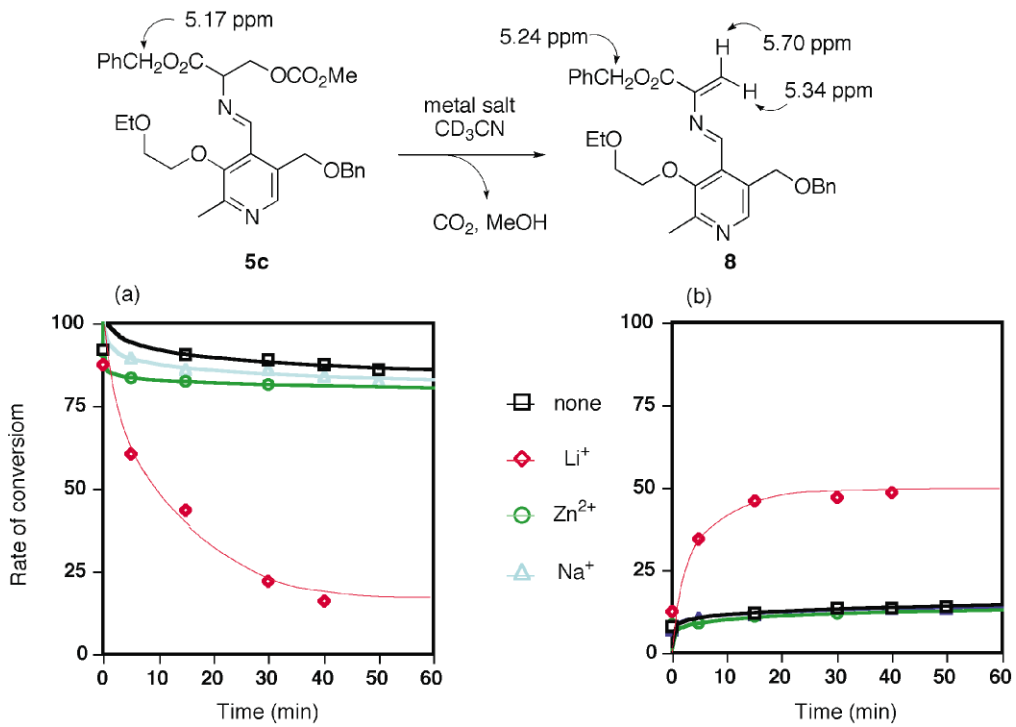


Figure 4. Elimination of aldimine **5c** in the absence and presence of metal ions.

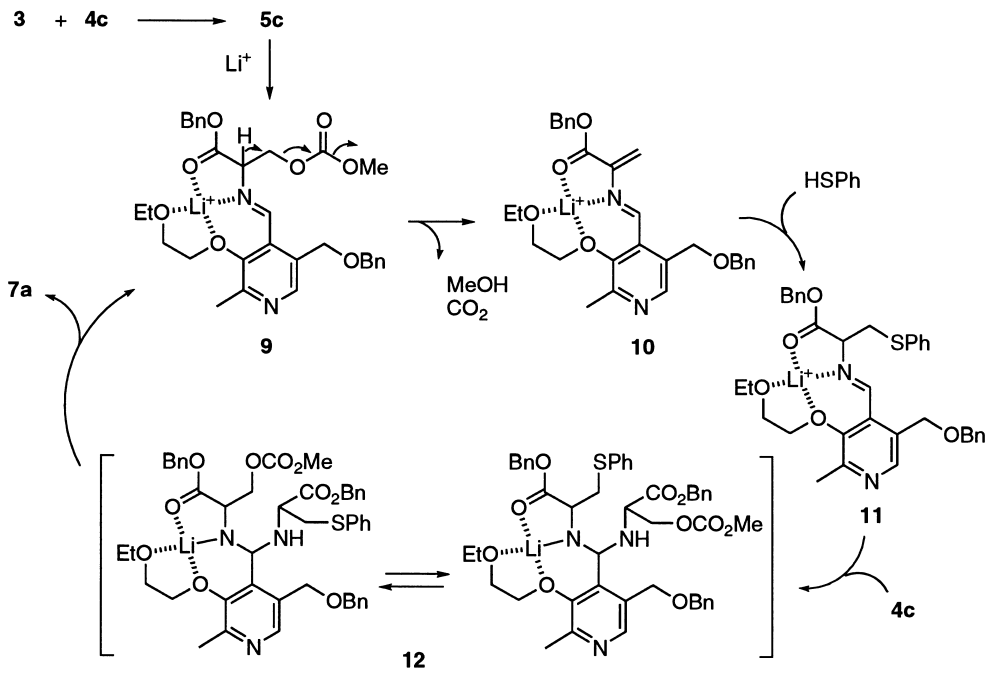


Figure 5. Possible mechanism for β-replacement reaction of **4c** catalyzed by a combination of Li^+ and **3**.

From the results described above, a possible reaction mechanism is proposed in Figure 5. The first step should be that pyridoxal derivative **3** reacts with **4c** to give the aldimine **5c**, which would capture Li^+ specifically to give complex **9**. This phenomenon would induce restriction of the conformation of the iminoester moiety in the same plane as that of the pyridine ring, and consequently would activate the α-hydrogen. As a result, elimination of the methyl carbonate moiety is thought to take place as the next step, which would produce carbon dioxide, methanol and unsaturated species **10**. This unsaturated compound **10** appears to be highly electrophilic, which would induce 1,4-addition of thiol to give cysteine aldimine **11**. Subsequent aldimine exchange reaction of **11** with **4c** via **12** would give **7a** and **9**, forming a catalytic cycle for the β-replacement reaction. In general, alkane thiols are more nucleophilic than aromatic thiols because of the electron-donating property of an alkyl group, from which, the lower reactivity of alkane thiols observed in the present experiments appears to be strange. As aromatic thiols are more acidic than alkane thiols, formation of more nucleophilic thiolate from thiol and/or a protonation step to an enolate species through the 1,4-addition procedure of thiol could be important to complete the catalytic cycle. This is also supported by the fact that the reaction with the most acidic thiol, 4-nitrothiophenol, proceeded most smoothly (Table 2, entry 4).

In conclusion, β-replacement reaction of serine derivative **4c** with aromatic thiols was successfully achieved under catalytic conditions, which is the first artificial model mimicking cystathionine β-synthase, and would serve as a useful method for the preparation of *S*-aryl substituted cysteines. Although the present model compound **3** cannot catalyze the β-replacement reaction with alkane thiols sufficiently, further effort to improve the model compound **3** to achieve the reaction with alkane thiols will be described in the following article.

3. Experimental

3.1. General

Infrared spectra were measured on a JASCO FT/IR-200 Fourier-transfer infrared spectrometer. ^{1}H NMR spectra were measured on a JEOL EX-270 (270 MHz) spectrometer and tetramethylsilane (TMS) was used as an internal standard. ^{13}C NMR spectra were measured on the same instrument (67.8 MHz) with $CDCl_3$ as an internal standard (77.0 ppm). Low and high resolution mass spectra (EI-MS and HR-MS) were obtained by use of a JEOL D-300 mass spectrometer. For silica gel column chromatography, E. Merck Kieselgel 60 (0.063–0.200 mm) was used.

3.1.1. Stoichiometric β-replacement reaction of serine-*O*-carbonate 4c. Aldimine **5c** was prepared from serine derivative **4c** and pyridoxal **3** by the same method described previously,[7] and the resultant aldimine **5c** was immediately employed for the next reaction without purification. β-Replacement reaction of **5c** with thiophenol was carried out as follows. To a stirred acetonitrile solution (5 mL) of **5c**, which had been prepared from serine-*O*-carbonate **4c** (110 mg, 0.43 mmol) and pyridoxal **3** (142 mg, 0.43 mmol), were added thiophenol (49 μL, 0.47 mmol) and $LiClO_4$ (2.3 mg, 0.022 mmol). After being stirred at room temperature for 2 h, the reaction mixture was diluted with ethyl acetate, washed with H_2O and saturated NaCl solution, and dried over Na_2SO_4. Concentration under reduced pressure afforded crude product of **6**, which was hydrolyzed without purification according to the same procedure as described previously,[7] and purified by silica gel column chromatography (ethyl acetate/hexane=1:1) to give *S*-phenylcysteine **7a** (28.4 mg, 23%). ^{1}H NMR data for aldimines **5c** and **6** taken in $CDCl_3$ are as follows. **5c**, δ: 1.21 (3H, t, *J*=7 Hz, OCH_2CH_3), 2.56 (3H, s, C2-Me), 3.53 (2H, q, *J*=7 Hz, OCH_2CH_3), 3.62–3.65 and 3.93–3.97 (each 2H, m, OCH_2CH_2O), 3.74 (3H, s, OMe), 4.35 (1H, dd, *J*=4.5,

8 Hz, α-H), 4.49 (1H, dd, *J*=8, 11 Hz), 4.51 (2H, s, OCH_2Ph), 4.73 (1H, dd, *J*=4.5, 8 Hz), 4.81 (2H, s, C5-CH_2), 5.18 (2H, AB type, *J*=12 Hz, CO_2CH_2Ph), 7.27–7.37 (10H, m, aromatic H), 8.56 (1H, s, C6-H), 8.77 (1H, s, imine H); **6**, δ: 1.15 (3H, t, *J*=7 Hz, OCH_2CH_3), 2.56 (3H, s, C2-Me), 3.31 (1H, dd, *J*=8.5, 14 Hz, CH_2SPh), 3.48 (2H, q, *J*=7 Hz, OCH_2CH_3), 3.63 (1H, dd, *J*=4.5, 14 Hz, CH_2SPh), 3.58–3.62 and 3.86–4.01 (each 2H, m, OCH_2CH_2O), 4.15 (1H, dd, *J*=4.5, 8.5 Hz, α-H), 4.52 (2H, s, OCH_2Ph), 4.82 (2H, s, C5-CH_2), 5.15 (2H, AB type, *J*=12.5 Hz, CO_2CH_2-Ph), 7.18–7.37 (15H, m, aromatic H), 8.57 (1H, s, C6-H), 8.68 (1H, s, imine H).

3.2. General procedure for catalytic β-replacement reaction

Under a nitrogen atmosphere, an acetonitrile solution of **3** (0.1 M, 0.1 mL, 0.01 mmol) and an acetonitrile solution of $LiClO_4$ (0.1 M, 0.1 mL, 0.01 mmol) were added to a stirred solution of serine-*O*-carbonate **4c** (50.6 mg, 0.20 mmol) and thiol (0.22 mmol) in acetonitrile (2 mL) at room temperature, and the whole was stirred at room temperature until **4c** disappeared on TLC (see Table 1). After concentration under reduced pressure, the resultant residue was purified by silica gel column chromatography (ethyl acetate/hexane=1:1) to afford *S*-substituted cysteines **7**. The yields are summarized in Table 1. Spectral properties of the products **7a**–**h** are as follows.

3.2.1. Benzyl 2-amino-3-phenylthiopropanoate (7a). A colorless oil. IR ν_{max} (KBr): 3373, 3060, 1737 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 3.16 (1H, dd, *J*=7, 14 Hz, SCH_2), 3.35 (1H, dd, *J*=5, 14 Hz, SCH_2), 3.68 (1H, dd, *J*=5, 7 Hz, $CH(NH_2)CO$), 4.95–5.13 (2H, AB type, *J*=12 Hz, CH_2Ph), 7.17–7.41 (10H, m, Aromatic H). ^{13}C NMR ($CDCl_3$) δ_C: 39.4, 53.9, 66.9, 126.8, 128.1, 128.3, 128.4, 128.5, 129.0, 130.5, 135.3, 173.5. EI-MS *m/z*: 287 (M^+, 38.7), 152 (M^+-CO_2Bn, 73.3), 91 (Bn^+, 100). High-resolution MS calcd for $C_{16}H_{17}NO_2S$ (M^+): 287.0980. Found: 287.0969.

3.2.2. Benzyl 2-amino-3-(*p*-tolylthio)propanoate (7b). A colorless oil. IR ν_{max} (KBr): 3376, 3030, 2925, 1737 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 2.31 (3H, s, Ar-CH_3), 3.11 (1H, dd, *J*=7, 14 Hz, SCH_2), 3.38 (1H, dd, *J*=5, 14 Hz, SCH_2), 3.64 (1H, dd, *J*=5, 7 Hz, $CH(NH_2)CO$), 4.95–5.11 (2H, AB type, *J*=12 Hz, CH_2Ph), 7.06–7.10 (2H, m, aromatic), 7.25–7.36 (7H, m, aromatic H). ^{13}C NMR ($CDCl_3$) δ_C: 21.0, 40.1, 53.9, 66.9, 128.3, 128.4, 128.6, 129.8, 131.1, 131.4, 135.4, 137.1, 173.6. FAB-MS *m/z*: 302 ($M+H^+$). High-resolution MS calcd for $C_{17}H_{20}NO_2S$ ($M+H^+$): 302.1215. Found: 302.1211.

3.2.3. Benzyl 2-amino-3-(4-methoxyphenylthio)propanoate (7c). A colorless oil. IR ν_{max} (KBr): 3373, 3033, 1737 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 3.07 (1H, dd, *J*=7, 14 Hz, SCH_2), 3.23 (1H, dd, *J*=5, 14 Hz, SCH_2), 3.62 (1H, dd, *J*=5, 7 Hz, $CH(NH_2)CO$), 3.77 (3H, s, OCH_3), 4.94–5.10 (2H, AB type, *J*=12 Hz, CH_2Ph), 6.80–6.83 (2H, m, aromatic H), 7.25–7.39 (7H, m, aromatic H). ^{13}C NMR ($CDCl_3$) δ_C: 41.2, 53.9, 55.3, 66.9, 114.7, 124.9, 128.2, 128.4, 128.5, 134.2, 135.4, 159.4, 173.6. EI-MS *m/z*: 317 (M^+, 80.3), 182 (M^+-CO_2Bn, 37.7), 91 (Bn^+, 100). High-resolution MS calcd for $C_{17}H_{19}NO_3S$ (M^+): 317.1085. Found: 317.1094.

3.2.4. Benzyl 2-amino-3-(4-nitrophenylthio)propanoate (7d). A colorless oil. IR ν_{max} (KBr): 3381, 3065, 1736, 1579, 1510, 1338 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 3.26 (1H, dd, *J*=7, 14 Hz, SCH_2), 3.47 (1H, dd, *J*=5, 14 Hz, SCH_2), 3.80 (1H, dd, *J*=5, 7 Hz, $CH(NH_2)CO$), 5.15 (2H, s, CH_2Ph), 7.31–7.42 (7H, m, aromatic), 8.10–8.11 (2H, m, aromatic H). ^{13}C NMR ($CDCl_3$) δ_C: 37.3, 53.9, 67.3, 123.9, 127.1, 128.3, 128.6, 128.7, 135.0, 145.4, 146.0, 173.1. FAB-MS *m/z*: 333 ($M+H^+$). High-resolution MS calcd for $C_{16}H_{17}N_2O_4S$ ($M+H^+$): 333.0909. Found: 333.0911.

3.2.5. Benzyl 2-amino-3-benzylthiopropanoate (7e). A colorless oil. IR ν_{max} (KBr): 3371, 3061, 1736, 1496 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 2.66 (1H, dd, *J*=7, 14 Hz, SCH_2), 2.83 (1H, dd, *J*=5, 14 Hz, SCH_2), 3.63 (1H, dd, *J*=5, 7 Hz, $CH(NH_2)CO$), 3.70 (2H, s, $PhCH_2S$), 5.15 (2H, s, CH_2Ph), 7.20–7.36 (10H, m, aromatic H). ^{13}C NMR ($CDCl_3$) δ_C: 36.4, 36.6, 54.1, 66.9, 127.1, 128.3, 128.4, 128.5, 128.6, 128.9, 135.4, 137.8, 173.8. EI-MS *m/z*: 301 (M^+, 40.1), 210 (M^+-Bn, 57.8), 166 (M^+-CO_2Bn, 78.3), 91 (Bn^+, 100). High-resolution MS calcd for $C_{17}H_{19}NO_2S$ (M^+): 301.1136. Found: 301.1139.

3.2.6. Benzyl 2-amino-3-ethylthiopropanoate (7f). A colorless oil. IR ν_{max} (KBr): 3370, 3033, 2965, 1737 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.22 (3H, t, *J*=7 Hz, CH_2CH_3), 2.52 (2H, q, *J*=7 Hz, CH_2CH_3), 2.80 (1H, dd, *J*=7, 14 Hz, SCH_2), 2.96 (1H, dd, *J*=5, 14 Hz, SCH_2), 3.68 (1H, dd, *J*=5, 7 Hz, $CH(NH_2)CO$), 5.17 (2H, s, CH_2Ph), 7.30–7.37 (5H, m, aromatic H). ^{13}C NMR ($CDCl_3$) δ_C: 14.7, 26.5, 36.8, 54.2, 66.9, 128.3, 128.4, 128.6, 135.4, 174.0. FAB-MS *m/z*: 240 ($M+H^+$). High-resolution MS calcd for $C_{12}H_{18}NO_2S$ ($M+H^+$): 240.1058. Found: 240.1058.

3.2.7. Benzyl 2-amino-3-(3-hydroxypropylthio)propanoate (7g). A colorless oil. IR ν_{max} (KBr): 3370, 3033, 2965, 1737 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.80 (2H, qn, *J*=7 Hz, $HOCH_2CH_2CH_2S$), 2.65 (2H, t, *J*=7 Hz, $HOCH_2CH_2CH_2S$), 2.90 (1H, dd, *J*=7, 14 Hz, SCH_2), 2.98 (1H, dd, *J*=5, 14 Hz, SCH_2), 3.72 (2H, t, *J*=7 Hz, $HOCH_2CH_2CH_2S$), 3.70–3.74 (1H, m, $CH(NH_2)CO$), 5.18 (2H, s, CH_2Ph), 7.30–7.37 (5H, m, aromatic H). ^{13}C NMR ($CDCl_3$) δ_C: 26.5, 36.8, 38.9, 54.2, 56.0, 66.9, 128.3, 128.4, 128.6, 135.4, 174.0. EI-MS *m/z*: 269 (M^+, 4.2), 91 (Bn^+, 100). High-resolution MS calcd for $C_{13}H_{19}NO_3S$ (M^+): 269.1085. Found: 269.1095.

3.2.8. Dibenzyl 2,10-diamino-4,8-dithiaundecanedioate (7h). A colorless oil. IR ν_{max} (KBr): 3364, 3033, 2948, 1734 cm^{-1}. ^1H NMR ($CDCl_3$) δ: 1.79 (2H, qn, *J*=7 Hz, $SCH_2CH_2CH_2S$), 2.57 (4H, t, *J*=7 Hz, $SCH_2CH_2CH_2S$), 2.78 (2H, dd, *J*=7, 14 Hz, SCH_2), 2.90 (2H, dd, *J*=5, 14 Hz, SCH_2), 3.68 (2H, dd, *J*=7, 5 Hz, $CH(NH_2)CO$), 5.17 (4H, s, CH_2Ph), 7.30–7.42 (10H, m, aromatic H). ^{13}C NMR ($CDCl_3$) δ_C: 20.9, 26.5, 36.8, 54.2, 66.9, 128.3, 128.4, 128.6, 135.4, 174.0. EI-MS *m/z*: 462 (M^+, 0.1), 91 (Bn^+, 100). High-resolution MS calcd for $C_{23}H_{30}N_2O_4S_2$ (M^+): 462.1647. Found: 462.1637.

3.3. ^{1}H NMR Analysis of the reaction in the absence and presence of metal ion

A solution of **3** (17 mg, 0.05 mmol) and serine-*O*-carbonate **4c** (12.7 mg, 0.05 mmol) in CH_2Cl_2 (2 mL) was stirred at room temperature for 5 min. After being concentrated under reduced pressure, the reaction mixture was azeotropically evaporated with benzene for three times. The resultant residue was dissolved in deuterated acetonitrile (0.5 mL). A solution of metal salt (0.1 M, $LiClO_4$, $Zn(OAc)_2$ or $NaClO_4$ in deuterated acetonitrile, 25 μL, 0.0025 mmol) was added to the above solution, ^{1}H NMR spectrum of which was taken from time to time. Disappearance of **5c** and appearance of **8** are summarized in Figure 4.

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10. Although we have employed optically active serine-*O*-carbonate **4c**, optical activity of the product **7a** was completely lost.