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Structure–Activity Relationships

Synthesis of Firefly Luciferin Analogues and Evaluation of the Luminescent Properties

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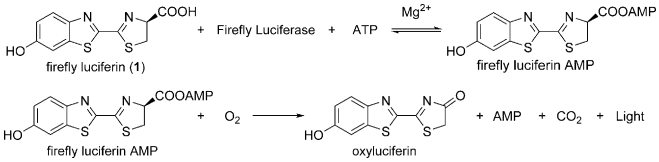
Abstract: Five new firefly luciferin (**1**) analogues were synthesized and their light emission properties were examined. Modifications of the thiazoline moiety in **1** were employed to produce analogues containing acyclic amino acid side chains (**2**–**4**) and heterocyclic rings derived from amino acids (**5** and **6**) linked to the benzothiazole moiety. Although methyl esters of all of the synthetic derivatives exhibited chemiluminescence activity, only carboluciferin (**6**), possessing a pyrroline-substituted benzothiazole structure, had bioluminescence (BL) activity (λ_{max} = 547 nm). Results of bioluminescence studies with AMP-carboluciferin (AMP = adenosine monophosphate) and AMP-firefly luciferin showed that the nature of the thiazoline mimicking moiety affected the adenylation step of the luciferin–luciferase reaction required for production of potent BL. In addition, BL of **6** in living mice differed from that of **1** in that its luminescence decay rate was slower.

Introduction

Bioluminescence (BL)-mediated imaging is a powerful technique used in both biological and related investigations.[1] Among the various processes used for this purpose, the luciferin–luciferase reaction (LLR) has advantageous features related to its emission properties because it does not require excitation by irradiation with an external light source. BL production in the LLR system is a consequence of luciferase-catalyzed transformation of firefly luciferin[2] (**1**) in the presence of Mg^{2+}, adenosine triphosphate (ATP), and O_2 that generates the excited state of oxyluciferin. Excited oxyluciferin then emits yellow–green light (λ_{max} = 553–559 nm)[3] with a high quantum efficiency (ϕ_{BL} = 41 %;[4] Scheme 1).[5] Because of its high efficiency and the availability of firefly luciferase and luciferin, the LLR system has been widely used in biological studies.[6]



Scheme 1. Four-step BL pathway for LLR-promoted BL involving enzyme binding, adenylation, oxidative decarboxylation, and light emission.

The mechanistic pathway for the reaction catalyzed by firefly luciferase, leading to BL, has been fully elucidated. In addition, many investigations have been carried out to expand the substrate scope of this process.[7] The results of these studies showed that the generation of BL required the use of substrates that, similar to **1**, had an α-amino acid center with the D-absolute configuration.[8] In particular, aminoluciferin, in which the phenolic hydroxyl group of the parent substrate was replaced by an NH_2 group, was found to have BL activity.[9] In an earlier study, we demonstrated that the emission wavelength maxima for BL by analogues of **1** were dependent on the nature of the benzothiazole ring. For instance, incorporation of a more extensively conjugated chain in this moiety induces a redshift in the luminescence wavelength.[10]

Importantly, analogues of **1** that display redshifted BL are suitable for use in noninvasive, whole-body imaging systems

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because they generate near-IR light that is more capable of penetrating thick tissues than short-wavelength light arising from the LLR. The investigation described below was guided by the goal of expanding the substrate range of the luciferase-promoted reaction and to produce new substances that efficiently produced BL. In these effort, substances were prepared and explored in which groups of the thiazoline moiety of **1** were replaced with those derived biosynthetically from D-cysteine.[5] The results of earlier studies of luciferin analogues in which the sulfur atom of the thiazoline ring was replaced by an selenium atom, and NH and Me_2C groups, have been reported.[11] Herein, several novel analogues are described in which the thiazoline ring in **1** is replaced by glycine, D-alanine, D-serine side chains (**2**–**4**; Figure 1), D-oxazoline, and D-pyrroline rings (**5** and **6**; Figure 1). All compounds were evaluated to explain the role of the thiazoline moiety in BL. The results of BL studies showed that the nature of the thiazoline mimicking moiety affected the adenylation step of the LLR required for the production of potent BL. Moreover, BL of one of the newly prepared derivatives in living mice was shown to differ from that of **1** in that its decay rate was lower.

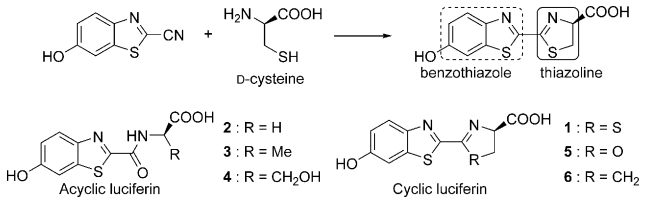
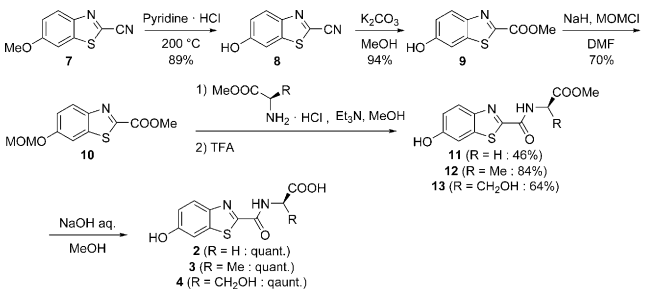


Figure 1. Firefly luciferin (**1**) is biosynthetically produced by the condensation of D-cycteine and 2-cyano-6-hydroxybenzothiazole. Luciferin analogues **2**–**6** derived from amino acids prepared in this study.

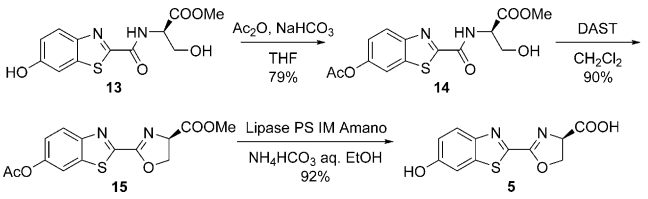
Results and Discussion

The route used for the synthesis of the benzothiazoylamino acids **2**–**4** was initiated by the pyridine hydrochloride promoted thermal transformation of 2-cyano-6-methoxybenzothiazole (**7**) to form phenol **8**[12] (Scheme 2). Conversion of the nitrile group in **8** into the corresponding methyl ester in **9**,[13] by treatment with MeOH/K_2CO_3, was followed by MOM protection of the phenolic hydroxyl group to give **10**. Coupling reactions of **10** with acid hydrochlorides of glycine, D-alanine, and D-serine, followed by removal of the MOM group in each amide by using TFA, formed the respective ester derivatives **11**–**13**. Finally, treatment of **11**–**13** with a solution of NaOH in methanol generated the respective benzothiazoylamino acids **2**–**4**.[14]



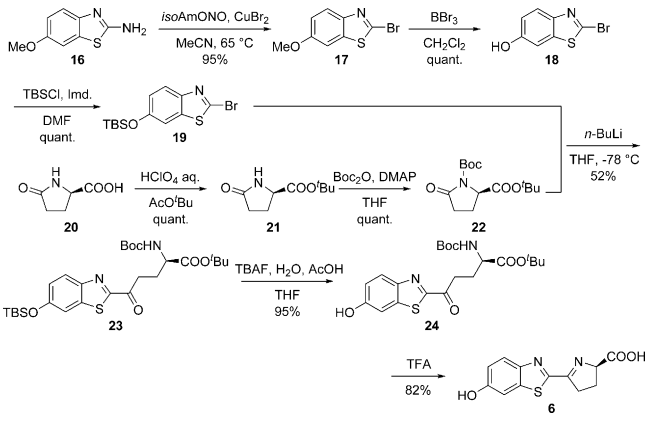
Scheme 2. Synthesis of benzothiazoylamino acids **2**–**4**. MOM = methoxymethyl, TFA = trifluoroacetic acid.

Preparation of the oxazoline benzothiazole **5** began with selective acetylation of the phenolic hydroxyl group of the serine-derived intermediate **13** (Scheme 3). This process produced monoacetate **14** in only low yield because of competitive esterification of the primary alcohol group in **13**. Importantly, the direct cyclization reaction of unprotected phenol **13** did not take place cleanly. Treatment of **14** with DAST promoted the desired cyclization to generate oxazoline **15**, which underwent sequential lipase-promoted deacetylation and methyl ester hydrolysis to form the oxazoline ring-containing luciferin analogue **5**.



Scheme 3. Synthesis of oxazoline benzothiazole **5**. DAST = diethylaminosulfur trifluoride.

In the pathway for the synthesis of carboluciferin (**6**; Scheme 4), bromide **17**[15] was generated through Sandmeyer reaction of a commercially available aminothiazolidine **16**. Removal of the methoxy group in **17** gave the corresponding phenol **18**, which was protected by using TBSCl to give the phenylsilyl ether **19**. To generate the pyrrolidinone **22**, which was used in a coupling reaction with **19**, the pyrrolidione carboxylate **20** was transformed into *tert*-butyl ester **21**.[16] Protection of **21** with Boc gave **22**,[16] which reacted with the lithium organometallic derivative of **19**, produced by treatment with *n*BuLi, to generate the glutamate-linked benzothioazole **23**.



Scheme 4. Synthesis of carboluciferin (**6**). *iso*AmONO = isoamyl nitrite, TBSCl = *tert*-butyldimethylsilyl chloride, Boc = *tert*-butyloxycarbonyl, DMAP = 4-dimethylaminopyridine, TBAF = tetrabutylammonium fluoride.

TBAF-mediated desilylation of **23** formed the free phenol **24**, which then underwent TFA-promoted cyclization to produce linked pyrroline benzothiazole **6**.

Luciferase-promoted BL was evaluated[7] by using 50 mM solutions of the luciferin analogues **2**–**6** in potassium phosphate buffer (pH 6.0). The results showed that only **6** produced a BL response, whereas benzothiazoylamino acids **2**–**4** and oxazoline **5** analogues did not (Figure 2 and Table S1 in the Supporting Information). The total photon yield of the luciferase-catalyzed reaction of **6** (1.52×10^5) was 0.56% of the process with **1** as a substrate (2.72×10^7). In addition, the maximum emission wavelength of **6** BL was blueshifted ($\lambda_{max}=547$ nm) relative to that of **1**. To assess the binding affinity of synthetic luciferin **6** to luciferase, the Michaelis constant (K_m, defined as the concentration at half of the maximum reaction rate) was determined.[10,17] The K_m value for **6** ((82.5 ± 7.2) μM) was smaller than that of **1** ((116 ± 22.0) μM; Figure S1 in the Supporting Information), which showed that the binding affinity of **6** to luciferase was higher than that of **1**. The K_m value of **1** was different from that previously reported because of the low ATP-Mg^{2+} concentrations (see the Experimental Section).

Next, the chemiluminescence properties of methyl esters of the synthetic derivatives were examined.[18] For this purpose, *t*BuOK (80 μL) in DMSO (100 mM) was added to solutions of the individual luciferin analogues in DMSO (1 μM, 20 μL) at room temperature. Light emission was monitored for 60 s at sampling intervals of 0.1 s. The results showed that the methyl esters of **2**–**6** generated chemiluminescence with nearly identical efficiencies (Figure S2 in the Supporting Information).

The results appear to show that the structures of the groups mimicking the thiazoline ring in **1** influence the BL yield as a consequence of their effect on binding to and/or reacting with the enzyme. Information was gained to show why **2**–**5** did not display BL activities, despite having chemiluminescence activities. For this purpose, the K_i values of these substances as inhibitors of the luciferase-catalyzed reaction of **1** were determined by means of Lineweaver–Burk analysis (Table S1 and Figure S3 in the Supporting Information).[19] L-Firefly luciferin (L-**1**) was utilized as a positive control because it was known to inhibit BL similar to that of **1**.[5c] The results demonstrated that alanine derivative **3** and oxazoline analogue **5** bound to luciferase, whereas related substances **2** and **4** did not. On the basis of these observations, the cyclic structure of the thiazoline moiety was important for the recognition of firefly luciferase (alanine derivative **3** could not be explained). We propose that the thiazoline moiety in **1** is a key structural requirement for substances that participate in the LLR leading to BL (Figure 2).

The BL activity of AMP-carboluciferin (AMP = adenosine monophosphate) was explored next to clarify why the BL activity of **6** was lower than that of **1**. AMP-carboluciferin and AMP-firefly luciferin were prepared by using a procedure reported in the literature,[7,11c] and purified by means of HPLC (Figure S4 in the Supporting Information). The luciferase solution used was diluted 10-fold because the photon emission of AMP-firefly luciferin exceeded the limit of the luminometer. The BL activity of AMP-carboluciferin (4.75×10^7) was 13000 times greater than that of **6** (3.54×10^3). In contrast, the bioluminescent activity of AMP-firefly luciferin (2.11×10^8) was only 285 times greater than that of **1** (7.42×10^5; Figure 3). These observations suggested that the low BL activity of **6** was a consequence of the low efficiency of the adenylation step in the LLR pathway.

In the final phase of this investigation, the ability of **6** to display BL in CAG-ffLuc-cp156 transgenic mice, developed in our previous research, was explored.[20] Compounds **1** and **6** (5 mM; 100 μL in sterile water) were independently injected into the abdominal cavities of mice ($n=3$). The results of in vivo imaging showing the change in BL with time are displayed in Figure 4. Carboluciferin (**6**) displayed BL in a mouse that increased with time and peaked at 2400 s following injec-

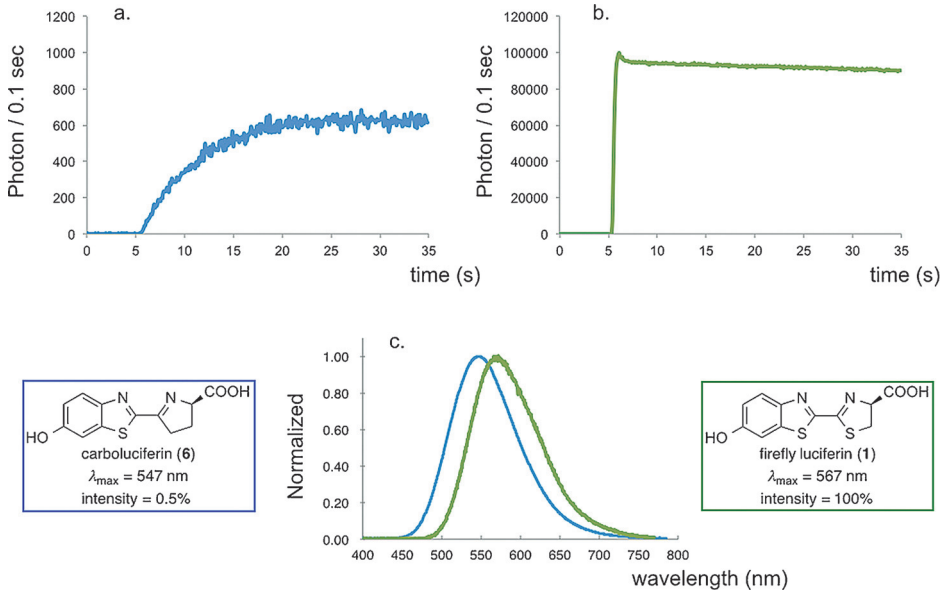


Figure 2. Change in the BL properties of a) **6** (blue) and b) **1** (green) with time; c) BL spectra. Compounds **3**–**5** did not show BL.

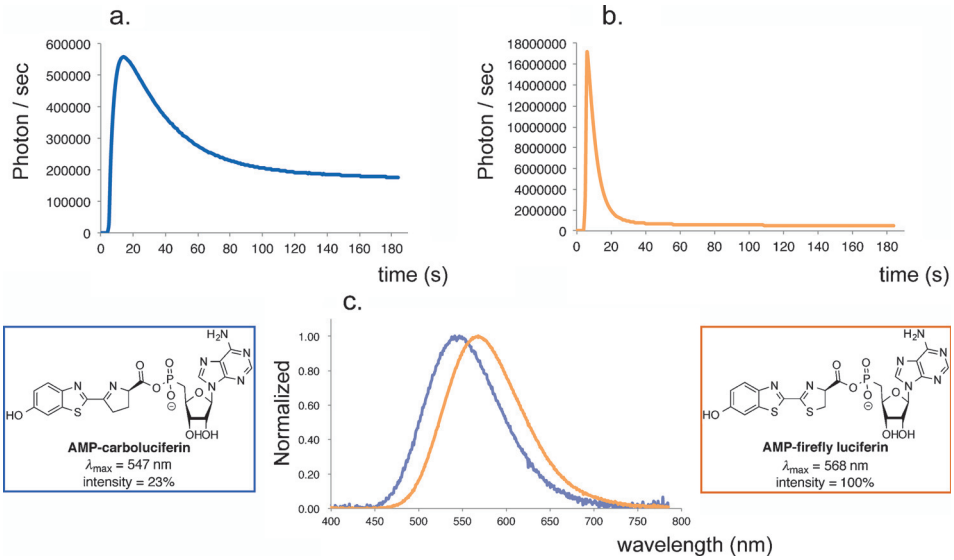


Figure 3. Change in BL activities of AMP-carboluciferin (a) and AMP-firefly luciferin (b); c) BL spectra.

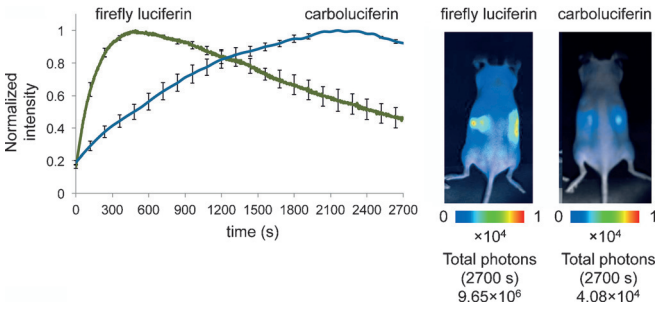


Figure 4. In vivo imaging showing changes in BL as a function of time following injection of mice with **6** (blue) and **1** (green).

tion. In comparison, BL from **1** increased more rapidly with time and reached a maximum at 300 s after injection.

Conclusion

We synthesized new luciferin analogues containing acyclic D-amino acid side chains (**2**–**4**) and their cyclic derivatives derived from D-amino acid (**5** and **6**) linked to the benzothiazole ring system of the parent compound. Evaluation of these substances showed that **6** had only 0.5% of the luciferase-promoted BL activity of **1**, and that the benzothiazoylamino acids and oxazoline luciferin analogues did not display BL in the presence of this enzyme. Observations made in an additional study showed that ester derivatives of these substances were chemiluminescent. An evaluation of BL of AMP-carboluciferin and AMP-firefly luciferin revealed that the activity of the former substance was 13000 times larger than that of **6**, whereas the activity of AMP-firefly luciferin was only 285 times larger than that of **1**. These results indicated that the thiazoline-mimicking moiety in **6** affected the adenylation step of LLR, whereas the benzothiazole moiety played an important role in determining the luminescence intensity and wavelength. Finally, longer lived BL was displayed by **6** than that of **1** following injection into live mice. The observations made in this investigation provide information that could contribute to the design of substrates that undergo improved luciferase-promoted BL.

Experimental Section

General

IR spectra were recorded on a JASCO Model FT/IR-4200 spectrophotometer. ^{1}H and ^{13}C NMR spectra were obtained on JEOL JNM AL-400, JEOL JNM ECX-400, and JEOL JNM α-400 spectrometers as solutions in $CDCl_3$ with tetramethylsilane as the internal standard. HRMS results were obtained on a Waters LCT Premier XE (ESI) spectrometer. Preparative and analytical TLC were carried out on silica-gel plates (Kieselgel 60 F254, E. Merck AG, Germany), and UV ($\lambda =$ 254 nm) light and 5% phosphomolybdic acid in ethanol were used as developers. Kanto Chemical silica gel 60N (spherical, neutral, 63–210 mm) was used for column chromatography. All reactions were carried out under an argon atmosphere. When necessary, solvents were dried prior to use. Anhydrous solvents were purchased from Kanto Chemical Co. Inc. and stored over 4 Å molecular sieves under an argon atmosphere.

Determination of luminescent activities

A 1 mg mL^{-1} stock solution of commercially available luciferase (Promega (QuantiLum recombinant *Photinus pyralis* luciferase, E1701) and Sigma (L9506)) in 50 mM Tris-HCl buffer (pH 8.0) containing 10% glycerol was prepared and stored at −80 °C. Prior to use, the luciferase stock solution was diluted 100-fold with 50 mM potassium phosphate buffer (pH 6.0) containing 35% glycerol. The diluted luciferase solution was cooled on ice until required. ATP-Mg^{2+} was purchased from Nacalai Tesque, and buffer chemicals were obtained from Wako Chemicals or Kanto Chemicals. A stock solution of the synthesized analogue (5 mM) in 50 mM potassium phosphate buffer (pH 6.0) was prepared and stored at −80 °C. Deionized water (Millipore, Milli-RX-12a) was used to prepare aqueous assay solutions. The pH values of the solutions were determined

with a Horiba F-23 pH meter. Chemiluminescence and BL intensities were measured by using ATTO AB-2200 or AB-2700 luminometers (Hamamatsu, R4220 photomultiplier tube). Chemiluminescence and BL wavelength spectra were recorded on an ATTO AB-1850 spectrophotometer.

Chemiluminescence activity

Methyl ester derivatives were synthesized to evaluate chemiluminescence. Chemiluminescence measurements were made by adding a solution of *t*BuOK (80 μL) in DMSO (100 mM) into solutions of the methyl esters of the luciferin analogues in DMSO (1 μM, 20 μL) at room temperature. Light emission was monitored for 60 s at sampling intervals of 0.1 s. Emission intensities were expressed as the light count per 0.1 s. Spectra were recorded from $\lambda = 400$ to 750 nm in 1 nm increments.

BL activity

Solutions for BL measurements were prepared by mixing the luciferin derivatives (20 μL; 100 μM), firefly luciferase solution (20 μL; 0.01 mg mL^{-1}), and potassium phosphate buffer (20 μL; 500 mM, pH 8). The enzymatic reactions were initiated by adding ATP-Mg^{2+} (40 μL; 200 μM) to these solutions at room temperature. A low ATP-Mg^{2+} concentration was used because high concentrations gave a strong background signal. Light emission was monitored for 30 s at sampling intervals of 0.1 s. Measurements of emission intensities and wavelengths were made by using the same techniques as those employed for chemiluminescence measurements.

The K_m values of the luciferase-catalyzed reactions of **1** and **6** were determined by using the initial maximum light intensities to estimate initial velocities. Reactions were initiated by injection of 200 μM ATP-Mg^{2+} into a mixture of 100 mM potassium phosphate buffer containing 1–500 μM **1** and **6** and 20 μg mL^{-1} firefly luciferase (*P. pylalis*, Promega Corp). For the K_i determinations, reactions were initiated by the injection of 200 μM ATP-Mg^{2+} into a mixture of 100 mM potassium phosphate buffer containing 1–500 μM of luciferin, 1–500 μM of analogues, and 20 μg mL^{-1} firefly luciferase.

Following a procedure reported in the literature,[7,11c] we prepared and purified AMP-firefly luciferin and AMP-carboluciferin immediately prior to their use. For this purpose, a solution of dicyclohexylcarbodiimide (DCC; 20 mg, 0.097 mmol) in DMSO (0.8 mL) was added to a solution of **1** or **6** (1 mg, 3.81 μmol) and (−)-adenosine-5′-monophosphoric acid (AMP; free acid, Oriental Yeast Co.; 10 mg, 0.029 mmol) in DMSO (0.5 mL) under an argon atmosphere. The mixture was stirred vigorously for 10 min at room temperature and then acetone (1.5 mL) was added to quench the reaction. The white precipitate formed was separated by centrifugation and the supernatant was discarded. The precipitates were suspended in ice-cold acetone (1 mL) and then centrifuged. The washing operation was repeated. The twice-washed precipitates were dissolved in distilled water containing 0.05% (v/v) TFA (0.5 mL). Acetone, dissolved in the solution, was removed under reduced pressure and the resulting aqueous solution was promptly subjected to HPLC purification immediately prior to use (Figure S4 in the Supporting Information). To evaluate their bioluminescent activities, luciferase solution (20 μL; 1 μg mL^{-1}) was added to a solution of the adenylated substrates (20 μL; 100 μM), potassium phosphate buffer (20 μL; 500 mM, pH 8.0), and water (40 μL). Light emission was monitored for 180 s at sampling intervals of 1 s. Emission intensities were expressed in light counts per second (cps).

In vivo imaging

For BL measurements, 37-week-old CAG-ffLuc-cp156 transgenic mice were grown. A mouse was injected with a mixture of somnopentyl (30 mg kg^{-1}) and luciferin (5 mM, 100 μL in sterile water) into its abdominal cavity. Immediately after injection, the scans were performed with an in vivo imaging system (LumazoneFA, Roper Japan). Light emission was monitored for 2700 s at exposure times of 0.5 (**1**) and 60 s (**6**). The experimental procedures and housing conditions for animals were approved by the Institutes Animal Experiments Committee at RIKEN and all animals were cared for and treated in accordance with the Institutional Guidelines for Experiments Using Animals. Synthesis of new compounds NMR spectra of new compounds are shown in the Supporting Information.

Methyl 6-(methoxymethoxy)benzo[d]thiazole-2-carboxylate (10)

NaH (60 mg, 1.50 mmol, 60% dispersion in mineral oil) and MOMCl (0.51 mL, 6.75 mmol) were added to a stirred solution of **9** (284 mg, 1.35 mmol) in DMF (5 mL). The resulting mixture was stirred at room temperature for 30 min, diluted with H_2O (10 mL), and extracted with $CHCl_3$. The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was subjected to column chromatography on silica gel (EtOAc/hexane, 1/2) to give **10** (207.5 mg, 61%) as a yellow oil. ^{1}H NMR ($CDCl_3$): $\delta = 8.08$ (d, $J = 9.2$ Hz, 1H; Ar), 7.57 (d, $J = 2.5$ Hz, 1H; Ar), 7.23 (dd, $J = 2.5$, 9.2 Hz, 1H; Ar), 5.23 (s, 2H; OCH_2OCH_3), 4.03 (s, 3H; $COOCH_3$), 3.48 ppm (s, 3H; OCH_2OCH_3); ^{13}C NMR ($CDCl_3$): $\delta = 161.2$ ($COOCH_3$), 157.2 (Ar), 155.9 (Ar), 148.5 (Ar), 138.5 (Ar), 126.2 (Ar), 118.7 (Ar), 106.9 (Ar), 94.8 (OCH_2OCH_3), 56.3 (OCH_2OCH_3), 53.6 ppm ($COOCH_3$); IR (film): $\tilde{\nu} = 1743$ cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{11}H_{12}NO_4S$ [$M^+ + H$]: 254.0487; found: 254.0486.

Methyl 2-(6-hydroxybenzo[*d*]thiazole-2-carboxamido)acetate (11)

Glycine methyl ester hydrochloride (286 mg, 2.28 mmol) and Et_3N (0.63 mL, 4.56 mmol) were added to a stirred solution of **10** (57.7 mg, 0.228 mmol) in MeOH (4 mL). The mixture was stirred at room temperature for 19 h, diluted with a saturated aqueous solution of NH_4Cl (20 mL), and extracted with $CHCl_3$. The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was dissolved in TFA (2 mL). After being stirred for 30 min., the mixture was concentrated in vacuo. The residue was subjected to column chromatography on silica gel (EtOAc/hexane, 1/1) to give **11** (28.1 mg, 46%) as a yellow powder. M.p. 215–217 °C; ^{1}H NMR ([D_6]acetone): $\delta = 8.48$ (br, 1H; N*H*), 7.92 (d, $J = 9.0$ Hz, 1H; Ar), 7.53 (d, $J = 2.5$ Hz, 1H; Ar), 7.16 (dd, $J = 2.5$, 9.0 Hz, 1H; Ar), 4.23 (d, $J = 6.3$ Hz, 2H; CH_2), 3.73 ppm (s, 3H; $COOCH_3$); ^{13}C NMR ([D_6]acetone): $\delta = 170.5$ (*C*ONH), 161.1 ($COOCH_3$), 158.2 (Ar), 157.4 (Ar), 147.7 (Ar), 139.5 (Ar), 126.0 (Ar), 118.2 (Ar), 107.7 (Ar), 52.4 ($COOCH_3$), 41.8 ppm (CH_2); IR (KBr): $\tilde{\nu} = 3265$, 1734, 1654 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{11}H_{11}N_2O_4S$ [$M^+ + H$]: 267.0440; found: 267.0437.

2-(6-Hydroxybenzo[*d*]thiazole-2-carboxamido)acetic acid (2)

A 25 mM aqueous solution of NaOH (5 mL) was added to a stirred solution of **11** (12.0 mg, 0.0432 mmol) in MeOH (1 mL). The resulting mixture was stirred at room temperature for 25 min, diluted with 2 M HCl (0.1 mL), and extracted with EtOAc. The organic layers

were combined, washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo to give **2** (11.3 mg, quant) as a white powder. M.p. 210–212 °C; ^{1}H NMR (CD_3OD): $\delta = 7.93$ (d, $J = 9.3$ Hz, 1H; Ar), 7.36 (d, $J = 2.4$ Hz, 1H; Ar), 7.07 (dd, $J = 2.4$, 9.3 Hz, 1H; Ar), 4.15 ppm (s, 2H; CH_2); ^{13}C NMR (CD_3OD): $\delta = 172.5$ (*C*ONH), 162.6 (*C*OOH), 160.4 (Ar), 158.7 (Ar), 148.2 (Ar), 140.0 (Ar), 126.3 (Ar), 118.3 (Ar), 107.4 (Ar), 42.0 ppm (CH_2); IR (KBr): $\tilde{\nu} = 3379$, 3093, 1736, 1644 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{10}H_9N_2O_4S$ [$M^+ + H$]: 253.0283; found: 253.0291.

(*R*)-2-(6-Hydroxybenzo[*d*]thiazole-2-carboxamido)propanoic acid (3)

Compound **10** (69.6 mg, 0.274 mmol) in MeOH (4 mL) was treated with D-alanine methyl ester hydrochloride (279 mg, 2.00 mmol) and Et_3N (0.55 mL, 4.00 mmol) in the same procedure as that described for **11** to give **12** (60.2 mg, 84%) as a white powder. M.p. 172–175 °C; $[\alpha]_D^{25} = -15.6$ ($c = 1.00$, CH_3OH); ^{1}H NMR (CD_3OD): $\delta =$ 7.90 (d, $J = 9.0$ Hz, 1H; Ar), 7.33 (d, $J = 2.5$ Hz, 1H; Ar), 7.06 (dd, $J =$ 2.5, 9.0 Hz, 1H; Ar), 4.65 (q, $J = 7.2$ Hz, 1H; C*H*CH_3), 3.77 (s, 3H; COOCH_3), 1.53 ppm (d, $J = 7.2$ Hz, 3H; CHCH_3); ^{13}C NMR (CD_3OD): $\delta = 174.1$ (*C*ONH), 162.0 (*C*OOCH$_3$), 160.0 (Ar), 159.4 (Ar), 147.9 (Ar), 140.0 (Ar), 126.2 (Ar), 118.7 (Ar), 107.5 (Ar), 53.0 (COO*C*H$_3$), 49.8 (*C*HCH$_3$), 17.5 ppm (*C*H*C*H$_3$); IR (KBr): $\tilde{\nu} = 3286$, 1733, 1652 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{12}H_{13}N_2O_4S$ [$M^+ + H$]: 281.0596; found: 281.0601.

Alkaline hydrolysis of **12** (17.3 mg, 0.0624 mmol), as in the case of **2**, provided **3** (11.3 mg, quant) as a white powder. M.p. 174–176 °C; $[\alpha]_D^{25} = -24.4$ ($c = 1.00$, CH_3OH); ^{1}H NMR (CD_3OD): $\delta = 7.93$ (d, $J =$ 9.3 Hz, 1H; Ar), 7.35 (d, $J = 2.4$ Hz, 1H; Ar), 7.07 (dd, $J = 2.4$, 9.3 Hz, 1H; Ar), 4.62 (q, $J = 7.3$ Hz, 1H; C*H*CH_3), 1.56 ppm (d, $J = 7.3$ Hz, 3H; CHCH_3); ^{13}C NMR (CD_3OD): $\delta = 173.0$ (*C*ONH), 161.8 (*C*OOH), 160.6 (Ar), 158.7 (Ar), 148.2 (Ar), 139.9 (Ar), 126.2 (Ar), 118.3 (Ar), 107.4 (Ar), 49.8 (*C*HCH$_3$), 17.8 ppm (*C*H*C*H$_3$); IR (KBr): $\tilde{\nu} = 3388$, 2942, 1729, 1652 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{11}H_{11}N_2O_4S$ [$M^+ + H$]: 267.0440; found: 267.0420.

(*R*)-3-Hydroxy-2-(6-hydroxybenzo[*d*]thiazole-2-carboxamido)-propanoic acid (4)

Compound **10** (232 mg, 0.916 mmol) in MeOH (10 mL) was treated with D-serine methyl ester hydrochloride (1.01 g, 6.39 mmol) and Et_3N (1.8 mL, 12.8 mmol) in the same procedure as that described for **11** to give **13** (161 mg, 64%) as a white powder. M.p. 181–183 °C; $[\alpha]_D^{25} = -4.7$ ($c = 1.00$, CH_3OH); ^{1}H NMR (CD_3OD): $\delta = 7.91$ (d, $J = 9.0$ Hz, 1H; Ar), 7.34 (d, $J = 2.5$ Hz, 1H; Ar), 7.07 (dd, $J = 2.5$, 9.0 Hz, 1H; Ar), 4.74 (t, $J = 4.0$ Hz, 1H; C*H*CH_2OH), 4.06 (dd, $J = 4.0$, 11.4 Hz, 1H; CHCH_2OH), 3.97 (dd, $J = 4.0$, 11.4 Hz, 1H; CHCH_2OH), 3.80 ppm (s, 3H; COOCH_3); ^{13}C NMR (CD_3OD): $\delta = 171.8$ (*C*ONH), 162.1 (*C*OOCH$_3$), 159.8 (Ar), 159.6 (Ar), 147.8 (Ar), 140.1 (Ar), 126.2 (Ar), 117.6 (Ar), 107.6 (Ar), 62.8, (CH*C*H$_2$OH), 56.4 (*C*HCH$_2$OH), 53.1 ppm (COO*C*H$_3$); IR (KBr): $\tilde{\nu} = 3373$, 3262, 1749, 1653 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{12}H_{13}N_2O_5S$ [$M^+ + H$]: 297.0545; found: 297.0569.

Alkaline hydrolysis of **13** (12.8 mg, 0.0462 mmol), as in the case of **2**, provided **4** (10.1 mg, quant) as a white powder. M.p. 171–172 °C; $[\alpha]_D^{25} = -8.9$ ($c = 1.00$, CH_3OH); ^{1}H NMR (CD_3OD): $\delta = 7.94$ (d, $J =$ 9.3 Hz, 1H; Ar), 7.36 (d, $J = 2.4$ Hz, 1H; Ar), 7.08 (dd, $J = 9.3$, 2.4 Hz, 1H; Ar), 4.69 (t, $J = 3.9$ Hz, 1H; C*H*CH_2OH), 4.07 (dd, $J = 3.9$, 11.2 Hz, 1H; CHCH_2OH), 3.99 ppm (dd, $J = 3.9$, 11.2 Hz, 1H; CHCH_2OH); ^{13}C NMR (CD_3OD): $\delta = 172.9$ (*C*ONH), 161.9 (*C*OOH), 160.4 (Ar), 158.8 (Ar), 148.1 (Ar), 140.0 (Ar), 126.3 (Ar), 118.4 (Ar), 107.5 (Ar), 62.8 (CH*C*H$_2$OH), 56.4 ppm (*C*HCH$_2$OH); IR(KBr): $\tilde{\nu} = 3388$, 2942, 1729, 1652 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{11}H_{11}N_2O_5S$ [$M^+ + H$]: 283.0389; found: 283.0379.

Methyl (*R*)-2-(6-acetoxybenzo[*d*]thiazole-2-carboxamido)-3-hydroxypropanoate (14)

Acetic anhydride (0.11 mL, 1.02 mmol) and $NaHCO_3$ (103.0 mg, 1.23 mmol) were added to a stirred solution of **13** (132.1 mg, 0.407 mmol) in THF (10 mL). The mixture was stirred at room temperature for 1 h, diluted with a saturated aqueous solution of NH_4Cl (30 mL), and extracted with $CHCl_3$. The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was subjected to column chromatography on silica gel (EtOAc/hexane, 1/1) to give **14** (109.4 mg, 79%) as a white powder. M.p. 138–141 °C; $[\alpha]_D^{25} = -16.6$ ($c = 1.00$, $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 8.26$ (d, $J = 7.8$ Hz, 1H; N*H*), 8.02 (d, $J =$ 8.8 Hz, 1H; Ar), 7.68 (d, $J = 2.0$ Hz, 1H; Ar), 7.26 (dd, $J = 2.0$, 8.8 Hz, 1H; Ar), 4.87 (ddd, $J = 3.9$, 7.8 Hz, 1H; C*H*CH_2OH), 4.16 (dd, $J = 3.9$, 11.7 Hz, 1H; CHCH_2OH), 4.08 (dd, $J = 3.9$, 11.7 Hz, 1H; CHCH_2OH), 3.82 (s, 3H; COOCH_3), 2.35 ppm (s, 3H; ArOCOCH_3); ^{13}C NMR ($CDCl_3$): $\delta = 170.4$ (*C*ONH), 169.5 (ArO*C*OCH$_3$), 163.1 (*C*OOCH$_3$), 160.0 (Ar), 150.1 (Ar), 149.5 (Ar), 137.8 (Ar), 125.2 (Ar), 121.9 (Ar), 115.1 (Ar), 63.1 (CHCH_2OH), 55.1 (*C*HCH$_2$OH), 53.1 (COO*C*H$_3$), 21.3 ppm (ArOCO*C*H$_3$); IR (KBr): $\tilde{\nu} = 3402$, 1752, 1743, 1653 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{14}H_{15}N_2O_6S$ [$M^+ + H$]: 339.0626; found: 339.0651.

Methyl (*R*)-2-(6-acetoxybenzo[d]thiazol-2-yl)-4,5-dihydrooxazole-4-carboxylate (15)

DAST (0.42 mL, 3.17 mmol) was added to a stirred solution of **14** (109.4 mg, 0.323 mmol) in CH_2Cl_2 (110 mL). The resulting mixture was stirred at −80 °C for 20 min, diluted sequentially with saturated aqueous solutions of $NaHCO_3$ (30 mL) and NH_4Cl (50 mL), and extracted with $CHCl_3$. The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was subjected to column chromatography on silica gel (EtOAc/hexane, 1/1) to give **15** (95.2 mg, 90%) as a white powder. M.p. 168–172 °C; $[\alpha]_D^{25} = -14.1$ ($c = 1.00$, $CHCl_3$); ^{1}H NMR ($CDCl_3$): $\delta = 8.16$ (d, $J = 9.0$ Hz, 1H; Ar), 7.72 (d, $J = 2.2$ Hz, 1H; Ar), 7.28 (dd, $J = 2.2$, 9.0 Hz, 1H; Ar), 5.07 (dd, $J = 8.3$, 10.8 Hz, 1H; C*H*CH_2O), 4.87 (dd, $J = 8.3$, 8.8 Hz, 1H; CHCH_2OH), 4.77 (dd, $J = 8.8$, 10.8 Hz, 1H; CHCH_2OH), 3.84 (s, 3H; COOCH_3), 2.51 ppm (s, 3H; ArOCOCH_3); ^{13}C NMR ($CDCl_3$): $\delta = 170.6$ (N*C*O), 169.4 (ArO*C*OCH$_3$), 161.1 (*C*OOCH$_3$), 155.1 (Ar), 151.1 (Ar), 149.8 (Ar), 137.0 (Ar), 125.7 (Ar), 121.9 (Ar), 114.7 (Ar), 71.1 (NCH*C*H$_2$O), 68.9 (N*C*HCH$_2$O), 53.1 (COO*C*H$_3$), 21.3 ppm (ArOCO*C*H$_3$); IR (KBr): $\tilde{\nu} = 1740$, 1731, 1635 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{14}H_{13}N_2O_5S$ [$M^+ + H$]: 321.0545; found: 321.0521.

(*R*)-2-(6-Hydroxybenzo[*d*]thiazol-2-yl)-4,5-dihydrooxazole-4-carboxylic acid (5)

A 100 mM aqueous solution of NH_4HCO_3 (10 mL) was added to a stirred solution of **15** (32.0 mg, 0.0992 mmol) and Lipase PS IM Amano (200 mg) in isopropyl ether (10 mL). The resulting mixture was stirred at 50 °C for 6 h and concentrated in vacuo. The residue was filtered to give **5** (95.2 mg, 91%) as a brown powder. M.p. 140–143 °C; $[\alpha]_D^{25} = -2.89$ ($c = 1.00$, CH_3OH); ^{1}H NMR (CD_3OD): $\delta =$ 7.89 (d, $J = 8.8$ Hz, 1H; Ar), 7.34 (d, $J = 2.4$ Hz, 1H; Ar), 7.07 (dd, $J =$ 2.4, 8.8 Hz, 1H; Ar), 4.88 (t, $J = 8.8$ Hz, 1H; C*H*CH_2O), 4.75–4.68 ppm (m, 2H; NCHCH_2O); ^{13}C NMR (CD_3OD): $\delta = 177.5$ (N*C*O), 161.3 (*C*OOH), 159.0 (Ar), 153.1 (Ar), 147.7 (Ar), 139.0 (Ar), 125.8 (Ar),

118.5 (Ar), 107.2 (Ar), 73.6 (NCHCH$_2$O), 72.1 ppm (NCHCH$_2$O); IR (KBr): $\tilde{\nu}$ = 3287, 2960, 1729, 1647 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{11}H_9N_2O_4S$ [M^+ + H]: 265.0265; found: 265.0285.

Methyl 2-(6-hydroxybenzo[*d*]thiazol-2-yl)-4,5-dihydrooxazole-4-carboxylate (25)

$NaHCO_3$ (10.5 mg, 0.125 mmol) was added to a stirred solution of **15** (13.4 mg, 0.0416 mmol) in MeOH (5 mL). The resulting mixture was stirred at room temperature for 2 h, diluted with a saturated aqueous solution of NH_4Cl (30 mL), and extracted with EtOAc. The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was subjected to column chromatography on silica gel (EtOAc/hexane, 1/1) to give **25** (10.4 mg, 90%) as white needlelike crystals. M.p. 155–156 °C; $[\alpha]_D^{25} = -2.98$ (c = 1.00, $CHCl_3$); ^{1}H NMR (CD_3OD): δ = 7.91 (d, J = 9.0 Hz, 1H; Ar), 7.36 (d, J = 2.5 Hz, 1H; Ar), 7.09 (dd, J = 2.5, 9.0 Hz, 1H; Ar), 5.07 (dd, J = 8.0, 10.3 Hz, 1H; C*H*CH$_2$O), 4.87–4.75 (m, 2H; NCHC*H*$_2$O), 3.82 ppm (s, 3H; COOC*H*$_3$); ^{13}C NMR (CD_3OD): δ = 172.3 (N*C*O), 162.8 (*C*OOCH$_3$), 159.2 (Ar), 152.3 (Ar), 148.0 (Ar), 139.2 (Ar), 126.0 (Ar), 118.6 (Ar), 107.2 (Ar), 72.4 (NCHCH$_2$O), 69.6 ppm (N*C*HCH$_2$O); IR (KBr): $\tilde{\nu}$ = 3260, 1743, 1620 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{12}H_{10}N_2O_4S$ [M^+ + H]: 278.0361; found: 279.0442.

2-Bromobenzo[*d*]thiazol-6-ol (18)

A 1.0 M solution of BBr_3 in CH_2Cl_2 (4 mL) was added to a stirred solution of **17** (555 mg, 2.28 mmol) in CH_2Cl_2 (10 mL). The resulting mixture was stirred at room temperature for 2 h, diluted with cold H_2O (30 mL), and extracted with $CHCl_3$. The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was subjected to column chromatography on silica gel (EtOAc/hexane, 1/3) to give **18** (521 mg, 95%) as a brown powder. M.p. 200–202 °C; ^{1}H NMR (CD_3OD): δ = 7.72 (d, J = 8.8 Hz, 1H; Ar), 7.26 (d, J = 2.4 Hz, 1H; Ar), 6.97 ppm (dd, J = 2.4, 8.8 Hz, 1H; Ar); ^{13}C NMR (CD_3OD): δ = 157.9 (Ar), 147.0 (Ar), 139.8 (Ar), 136.2 (Ar), 123.8 (Ar), 117.4 (Ar), 107.2 ppm (Ar); IR (KBr): $\tilde{\nu}$ = 3118, 1598 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_7H_5{}^{79}BrNOS$ [M^+ + H]: 229.9275; found: 229.9303.

2-Bromo-6-(*tert*-butyldimethylsilyloxy)benzo[*d*]thiazole (19)

Imidazole (1.41 g, 20.7 mmol) was added to a stirred solution of **18** (794 mg, 3.45 mmol) and TBSCl (1.75 g, 11.6 mmol) in DMF (42 mL). The resulting mixture was stirred at room temperature for 24 h, diluted with a saturated aqueous solution of NH_4Cl (50 mL), and extracted with $CHCl_3$. The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was subjected to column chromatography on silica gel (EtOAc/hexane, 1/3) to give **19** (1.21 g, quant.) as a brown oil. ^{1}H NMR ($CDCl_3$): δ = 7.79 (d, J = 8.8 Hz, 1H; Ar), 7.19 (d, J = 2.5 Hz, 1H; Ar), 6.94 (dd, J = 2.5, 8.8 Hz, 1H; Ar), 0.97 (s, 9H; Si(CH$_3$)$_2$C(C*H*$_3$)$_3$), 0.20 ppm (s, 6H; Si(C*H*$_3$)$_2$C(CH$_3$)$_3$); ^{13}C NMR ($CDCl_3$): δ = 154.1 (Ar), 147.3 (Ar), 138.4 (Ar), 135.9 (Ar), 123.2 (Ar), 120.3 (Ar), 111.1 (Ar), 25.7 (Si(CH$_3$)$_2$C(*C*H$_3$)$_3$), 18.2 (Si(CH$_3$)$_2$*C*(CH$_3$)$_3$), −4.4 ppm (Si(*C*H$_3$)$_2$C(CH$_3$)$_3$); IR (film): $\tilde{\nu}$ = 1597 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{13}H_{19}{}^{79}BrNOSSi$ [M^+ + H]: 344.0140; found: 344.0168.

(*R*)-*tert*-Butyl-2-(*tert*-butoxycarbonylamino)-5-[6-(*tert*-butyldimethylsilyloxy)benzo[*d*]thiazol-2-yl]-5-oxopentanoate (23)

A 2.7 M solution of *n*BuLi in hexane (0.63 mL) was added to a stirred solution of **19** (528 mg, 1.53 mmol) in dry THF (15.3 mL). The resulting mixture was stirred at −78 °C for 10 min and diluted by the addition of **22** (558 mg, 1.96 mmol) in dry THF (10 mL) at −78 °C. The resulting mixture was stirred at −40 °C for 2 h, diluted with a saturated aqueous solution of $NaHCO_3$ (50 mL), and extracted with $CHCl_3$. The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was subjected to column chromatography on silica gel (EtOAc/hexane, 1/5) and gel permeation chromatography (AIGEL-1H, $CHCl_3$, flow rate 3.5 mL min^{-1}, UV detection at λ = 254 nm) to give **23** (491 mg, 52%) as a yellow oil. $[\alpha]_D^{25} = -7.16$ (c = 1.00, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ = 7.99 (d, J = 9.0 Hz, 1H; Ar), 7.34 (d, J = 2.2 Hz, 1H; Ar), 7.07 (dd, J = 2.2, 9.0 Hz, 1H; Ar), 5.18 (d, J = 8.8 Hz, 1H; N*H*), 4.30 (d, J = 6.0 Hz, 1H; ArCOCH$_2$CH$_2$C*H*), 3.31 (m, 2H; ArCOCH$_2$C*H*$_2$), 2.30 (m, 1H; ArCOC*H*$_2$CH$_2$), 2.11 (m, 1H; ArCOC*H*$_2$CH$_2$), 1.47 (s, 9H; NHCOOC(C*H*$_3$)$_3$), 1.40 (s, 9H; COOC(C*H*$_3$)$_3$), 1.00 (s, 9H; Si(CH$_3$)$_2$C(C*H*$_3$)$_3$), 0.24 ppm (s, 6H; Si(C*H*$_3$)$_2$C(CH$_3$)$_3$); ^{13}C NMR ($CDCl_3$): δ = 194.3 (Ar*C*OCH$_2$), 171.6 (*C*O*O*t*B*u), 164.0 (NH*C*OO), 156.1 (Ar), 155.5 (Ar), 148.6 (Ar), 139.1 (Ar), 126.2 (Ar), 121.7 (Ar), 111.9 (Ar), 82.3 (COO*C*(CH$_3$)$_3$), 79.9 (NHCOO*C*(CH$_3$)$_3$), 53.6 (NH*C*HCOO*t*Bu), 34.5 (ArCOCH$_2$*C*H$_2$), 28.4 (COOC(*C*H$_3$)$_3$), 28.1 (NHCOOC(*C*H$_3$)$_3$), 27.2 (ArCOCH$_2$*C*H$_2$), 25.7 (Si(CH$_3$)$_2$C(*C*H$_3$)$_3$), 18.4 (Si(CH$_3$)$_2$*C*(CH$_3$)$_3$), −4.2 ppm (Si(*C*H$_3$)$_2$C(CH$_3$)$_3$); IR (film): $\tilde{\nu}$ = 2930, 1717, 1710, 1703 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{27}H_{44}N_2O_6SSi$ [M^+ + H]: 551.2611; found: 551.2599.

(*R*)-*tert*-Butyl-2-(*tert*-butoxycarbonylamino)-5-(6-hydroxybenzo[*d*]thiazol-2-yl)-5-oxopentanoate (24)

A solution of 1.0 M TBAF (0.3 mL) in THF (1 mL), AcOH (0.057 mL), and H_2O (0.018 mL) were added to a stirred solution of **23** (133 mg, 0.242 mmol) in THF (3 mL). The resulting mixture was stirred at room temperature for 1 h, diluted with a saturated aqueous solution of NH_4Cl (20 mL), and extracted with EtOAc. The organic layers were combined, washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was subjected to column chromatography on silica gel (EtOAc/hexane, 1/1) to give **24** (100 mg, 95%) as a yellow powder. M.p. 180–181 °C; $[\alpha]_D^{25} = -17.2$ (c = 1.00, CH_3OH); ^{1}H NMR (CD_3OD): δ = 7.89 (d, J = 9.0 Hz, 1H; Ar), 7.30 (d, J = 2.2 Hz, 1H; Ar), 7.09 (dd, J = 2.2, 9.0 Hz, 1H; Ar), 5.35 (d, J = 8.1 Hz, 1H; N*H*), 4.29 (d, J = 5.6 Hz, 1H; ArCOCH$_2$CH$_2$C*H*), 3.29 (m, 2H; ArCOCH$_2$C*H*$_2$), 2.28 (m, 1H; ArCOC*H*$_2$CH$_2$), 2.10 (m, 1H; ArCOC*H*$_2$CH$_2$), 1.46 (s, 9H; NHCOOC(C*H*$_3$)$_3$), 1.43 ppm (s, 9H; COOC(C*H*$_3$)$_3$); ^{13}C NMR (CD_3OD): δ = 194.1 (Ar*C*OCH$_2$), 171.6 (*C*O*O*t*B*u), 162.9 (NH*C*OO), 157.1 (Ar), 156.0 (Ar), 147.7 (Ar), 139.4 (Ar), 126.4 (Ar), 117.8 (Ar), 106.9 (Ar), 82.8 (COO*C*(CH$_3$)$_3$), 80.6 (NHCOO*C*(CH$_3$)$_3$), 53.8 (NH*C*HCOO*t*Bu), 34.6 (ArCOCH$_2$*C*H$_2$), 28.5 (COOC(*C*H$_3$)$_3$), 28.1(NHCOOC(*C*H$_3$)$_3$), 27.1 ppm (ArCOCH$_2$*C*H$_2$); IR (KBr): $\tilde{\nu}$ = 3322, 1729, 1700 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{21}H_{29}N_2O_6S$ [M^+ + H]: 437.1746; found: 437.1732.

(*R*)-5-(6-Hydroxybenzo[*d*]thiazol-2-yl)-3,4-dihydro-2*H*-pyrrole-2-carboxylic acid (6)

A solution of **24** (20.8 mg, 0.0476 mmol) in TFA (1 mL) was stirred at room temperature for 24 h. The resulting mixture was concentrated in vacuo. The residue was subjected to reverse-phase preparative column chromatography on silica gel (H_2O/MeOH, 1/1) to give **6** (10.3 mg, 82%) as yellow platelike crystals. M.p. 82–84 °C (dec); $[\alpha]_D^{25} = -26.5$ (c = 1.00, CH_3OH); ^{1}H NMR ([D$_6$]DMSO): δ = 7.93 (d, J = 9.2 Hz, 1H; Ar), 7.41 (d, J = 2.5 Hz, 1H; Ar), 7.03 (dd, J = 2.5, 9.2 Hz, 1H; Ar), 4.87 (dd, J = 6.7, 8.5 Hz, 1H; N*C*HCOOH), 3.17–3.08 (m, 2H; ArCNCH$_2$C*H*$_2$), 2.34 (m, 1H; ArCNC*H*$_2$CH$_2$), 2.13 ppm (m, 1H; ArCNCH$_2$C*H*$_2$); ^{13}C NMR (CD_3OD): δ = 175.2 (COOH), 175.0 (Ar*C*N), 159.8 (Ar), 159.3 (Ar), 148.8 (Ar), 139.6 (Ar), 126.2 (Ar), 118.5 (Ar),

107.5 (Ar), 75.3 (NCHCOOH), 36.7 ($ArCNCH_2CH_2$), 27.7 ppm ($ArCNCH_2CH_2$); IR (film): $\tilde{\nu}=3088$, 2924, 1702, 1677, 1597 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{12}H_{11}N_2O_3S$ $[M^+ + H]$: 263.0490; found: 263.0490.

Methyl 5-(6-hydroxybenzo[*d*]thiazol-2-yl)-3,4-dihydro-2*H*-pyrrole-2-carboxylate (26)

A solution of 0.6 mM $TMSCH_2N_2$ in hexane (0.08 mL) was added to a stirred solution of **6** (10.0 mg, 0.0381 mmol) in MeOH (3 mL). The resulting mixture was stirred at room temperature for 12 h and concentrated in vacuo. The residue was subjected to preparative column chromatography on silica gel (EtOAc/hexane, 1/1) to give **26** (3.20 mg, 33%) as a white powder. M.p. 237–238 °C (dec); $[\alpha]_D^{25}=-7.20$ ($c=1.00$, $CHCl_3$); ^1H NMR ($[D_6]$DMSO): $\delta=7.93$ (d, $J=8.8$ Hz, 1H; Ar), 7.41 (d, $J=2.5$ Hz, 1H; Ar), 7.03 (dd, $J=2.5$, 8.8 Hz, 1H; Ar), 4.97 (dd, $J=6.7$, 8.5 Hz, 1H; N*CH*$COOCH_3$), 3.71 (s, 3H; NCHCOO*CH*$_3$), 3.22–3.08 (m, 2H; $ArCNCH_2CH_2$), 2.35 (m, 1H; $ArCNCH_2CH_2$), 2.14 ppm (m, 1H; $ArCNCH_2CH_2$); ^{13}C NMR (CD_3OD): $\delta=181.7$ ($COOCH_3$), 181.3 (ArCN), 168.7 (Ar), 166.6 (Ar), 156.2 (Ar), 146.6 (Ar), 134.2 (Ar), 126.2 (Ar), 116.2 (Ar), 83.6 (NCHCOOCH_3), 61.5 (NCHCOOCH_3), 44.8 ($ArCNCH_2CH_2$), 35.7 ppm ($ArCNCH_2CH_2$); IR (KBr): $\tilde{\nu}=3088$, 1745, 1640 cm^{-1}; HRMS (ESI): *m/z* calcd for $C_{13}H_{13}N_2O_3S$ $[M^+ + H]$: 277.0647; found: 277.0649.

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