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Enantioselective Biotransformations of Racemic and Meso Pyrrolidine-2,5-dicarboxamides and Their Application in Organic Synthesis

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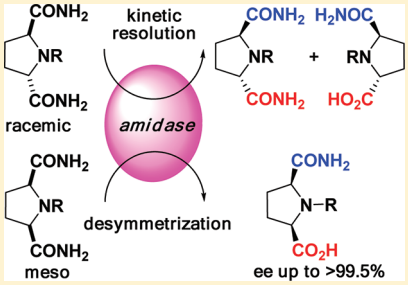
Supporting Information

ABSTRACT: In this paper, we report the amidase-catalyzed hydrolysis of pyrrolidine-2,5-dicarboxamides and their application in organic synthesis. Catalyzed by *Rhodococcus erythropolis* AJ270, an amidase containing microbial whole cell catalyst, racemic *trans*-pyrrolidine-2,5-carboxamide was kinetically resolved into (2*S*,5*S*)-pyrrolidine-2,5-dicarboxamide and (2*R*,5*R*)-5-carbamoylpyrrolidine-2-carboxylic acid in high yields and excellent enantioselectivity. Biocatalytic desymmetrization of meso *cis*-pyrrolidinedicarboxamide afforded enantiomerically pure (2*R*,5*S*)-5-carbamoylpyrrolidine-2-carboxylic acid in an almost quantitative yield. In both kinetic resolution and desymmetrization, the amidase always exhibited excellent 2*R*-enantioselectivity, although its catalytic efficiency was influenced dramatically by the steric effect of the substituent on the nitrogen atom of pyrrolidine ring. The synthetic potential of biotransformation was demonstrated by the scalable preparation of (2*R*,5*R*)- and (2*R*,5*S*)-5-carbamoylpyrrolidine-2-carboxylic acids and their conversions to aza-nucleoside analogues and druglike pyrroline-fused diazepin-11(5*H*)-one compounds.



INTRODUCTION

Chiral 2,5-disubstituted pyrrolidine derivatives are a very important type of compound in organic chemistry. First, they constitute the core structure of natural products[1] and synthetic compounds of pharmaceutical interest.[2] Gerrardine, for example, is a pyrrolidine alkaloid isolated from both *Cassipourea gerrardii*[3a] and *Cassipourea guianensis*,[3b] while (2*R*-*trans*)-2-butyl-5-heptylpyrrolidine, a potent σ receptor ligand, was found in the culture broth of *Streptomyces longispororuber*.[4] (2*S*,5*S*)-Pyrrolidine-2,5-dicarboxylic acid, an interesting C_2-symmetric *N*-heterocyclic compound, is a marine natural product first isolated from red alga *Schizmenia dubyi* in 1975.[5] Second, both trans- and cis-2,5-functionalized pyrrolidines serve as unique building blocks in the synthesis of natural products and bioactive compounds such as pyrrolizidines and indolizidines.[6] In addition, enantiopure 2,5-substitued pyrrolidines are widely used as chrial auxiliaries[7] and ligands[8] in asymmetric synthesis. Moreover, chiral pyrrolidine2,5-dicarboxylic acid has been used as an organocatalyst in enamine catalysis albeit the chiral induction needs further improvement.[9]

Because of their importance in organic and medicinal chemistry, chiral 2,5-disubstituted pyrrolidine derivatives have attracted continuous attention from synthetic chemists. Although various synthetic methods have been developed,[10] general approaches to enantiomerically pure pyrrolidine stereoisomers bearing two functional groups at the 2,5-positions, which are versatile and invaluable synthetic intermediates, remain largely unexplored and challenging. Synthesis of (2*S*,5*S*)-2,5-pyrrolidinedicarboxylic acid, for instance, comprises mainly multistep chemical transformations starting from chiral materials such as *S*-pyroglutamate[11] and *S*-proline derivatives,[12] *meso*-dimethyl 2,5-dibromoadipate,[13] chiral epoxide,[14] and amino acid.[15] They suffer from tedious chemical manipulations, using expensive chemical reagents and providing low overall yields. In 1987, Achiwa[16] reported biocatalytic kinetic resolution of racemic dimethyl *trans*-pyrrolidine-2,5-dicarboxylates using pig liver esterase (PLE). The reaction gave unfortunately very low conversion and enantioselectivity. PLE-catalyzed desymmetrization of dimethyl cis-1-benzylpyrrolidine-2,5-carboxylate was reported.[17] Although high enantioselectivity was achieved under controlled conditions, the chemical conversion was appallingly low. Recently, PLE-catalyzed desymmtrization of dimethyl *cis*-1-(*tert*-butoxycarbonyl)pyrrolidine-2,5-carboxylate has been reported to take place in 36 h, giving (2*S*,5*R*)-1-(*tert*-butoxycarbonyl)-5-methoxycarbonyl)pyrrolidine-2-carboxylic acid in 75% yield and >99% ee.[18]

Amidases [3.5.1.4] are a type of hydrolytic enzyme catalyzing the conversion of primary amides into carboxylic acids with the release of ammonia.[19] To date, a large number of amidases and microorganisms that contain amidases have been reported, and

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some of them have been used extensively in the kinetic resolution of racemic amides.[20] *Rhodococcus erythropolis* AJ270,[21] a nitrile hydratase and amidase-containing microbial whole cell catalyst, for instance, has been shown to catalyze enantioselective biotransformation of a large number of structurally varied racemic amides including amino,[22] hydroxyl and alkoxy,[23] alkenyl,[24] alkynyl,[25] allenyl,[25] and azido[26] bearing amides, cyclopropanecarboxamide,[27] and various heterocyclic amides[28−31] to produce highly enantiopure carboxylic acid and amide products. Surprisingly, biocatalytic enantioselective of dicarboxamides, including racemic, prochiral,[32] and meso ones,[33] remains largely unexplored. On the basis of easy availability of racemic and meso dicarboxamide compounds, high catalytic efficiency, and enantioselectivity of the amidase in *Rhodococcus erythropolis* AJ270, we envisioned that the amidase-catalyzed enantioselective hydrolysis of racemic and meso pyrrolidine-2,5-dicarboxamides[33] would provide a unique method for the synthesis of enantioenriched 2,5-functionalized pyrrolidine stereoisomers. It is worth emphasizing that, being different from kinetic resolution and desymmetrization of a diester which afford only a monoester of diacid, amidase-catalyzed reactions of diamides produce carbamoyl-substituted acids that are more versatile and useful in organic synthesis. We report herein the efficient and practical *Rhodococcus erythropolis* AJ270 whole cell-catalyzed biotransformation of both racemic and meso pyrrolidine-2,5-dicarboxamides.[33] The synthetic potential of the resulting enantiopure 5-carbamoylpyrrolidine-2-carboxylic acid is also demonstrated by the synthesis of druglike compounds.

RESULTS AND DISCUSSION

We initiated our study by examining the biocatalytic kinetic resolution of racemic *trans*-pyrrolidine-2,5-dicarboxamides **1a** and **1b**. To facilitate the isolation of products, acids and diacids were converted into their methyl esters **3** and **4**, respectively, using CH_2N_2. In the case of the reaction of **1c**, the substrate contains no substituent on pyrrolidine nitrogen (R = H), and the acid product was transformed into its corresponding benzyl ester using benzyl bromide as an alkylation reagent. It should be pointed out that, under the basic reaction conditions, benzylation also occurred on pyrrolidine nitrogen to afford the final products **2a** and **3c** (R = R′ = Bn) (Scheme 1). As summarized in Table 1, under very mild conditions such as in neutral aqueous potassium phosphate buffer at 30 °C, *Rhodococcus erythropolis* AJ270 whole cell catalyst was able to catalyze the hydrolysis of the testing racemic *trans*-pyrrolidine-2,5-dicarboxamides in a highly enantioselective manner. The reaction velocity, however, was governed dramatically by the substituent on the heterocyclic ring. For example, *N*-benzyl-substituted *trans*-pyrrolidine-2,5-dicarboxamide substrate **1a** underwent a slower biotransformation, with 50% of the substrate being converted within 39 h (entry 1, Table 1). Doubling the biocatalyst loading led noticeably to the shortening of the reaction period (entry 2, Table 1). In both cases, enantiopure (2*S*,5*S*)-1-benzylpyrrolidine-2,5-dicarboxamide **2a** and (2*R*,5*R*)-methyl 1-benzyl-5-carbamoylpyrrolidine-2-carboxylate **3a** were produced in excellent yields. In addition to the isolation of monocarboxylate product **3a**, a small amount of enantiopure (2*R*,5*R*)-dimethyl 1-benzylpyrrolidine-2,5-dicarboxylate **4a** was also obtained, indicating the enzymatic activity of the amidase toward initially formed (2*R*,5*R*)-1-benzyl-5-carbamoylpyrrolidine-2-carboxylic acid (entries 1 and 2, Table 1), albeit in low catalytic efficiency. Replacement of the benzyl substituent with an allyl group on the pyrrolidine ring resulted in the enhancement of reaction rate. A 7 h incubation of substrate **1b** with microbial cells under identical conditions thus gave rise to (2*S*,5*S*)-1-allylpyrrolidine-2,5-dicarboxamide **2b**, (2*R*,5*R*)-methyl 1-allyl-5-carbamoylpyrrolidine-2-carboxylate **3b**, and (2*R*,5*R*)-dimethyl 1-allylpyrrolidine-2,5-dicarboxylate **4b** in yields of 45%, 36%, and 10%, respectively (entry 3, Table 1). Single enantiomers **3b** and **4b** were obtained, whereas the enantiomeric excess value of **2b** decreased from >99.5% to 43.1% when the conversion of **1b** was low in a shorter reaction time (entry 4, Table 1), a reflection of a typical kinetic resolution process. Biocatalytic kinetic resolution of **1c**, a substrate devoid of any *N*-substituent, proceeded very rapidly. After interaction with biocatalyst in 10−15 min, enantiopure products **2a** and **3c** were obtained in high yields. In such a short reaction period, no diacid product **4c** was observed (entries 5 and 6, Table 1). It should be noted that use of DMSO as a cosolvent improved the solubility of substrates in buffer and therefore facilitated the biotransformation. The enantioselectivity of enzymatic reaction was, however, not affected at all (entries 5 and 6, Table 1). Biocatalytic kinetic resolution of **1c** was also readily scalable. This was exemplified by a 10 mmol scale reaction which produced enantiopure (2*S*,5*S*)-pyrrolidine-2,5-dicarboxamide **2d** in 47% yield and enantioenriched (2*R*,5*R*)-5-carbamoylpyrrolidine-2-carboxylic acid **3d** in 52% with 87.1% ee. Enantiomeric purity of **3d** was improved to >99.5% ee after recrysttalization in a mixture of water and methanol (entry 7, Table 1).

All resulting dicarboxamides **2** and 5-carbomylpyrrolidine-2-carboxylates **3** were easily hydrolyzed chemically under acidic conditions, allowing the preparation of both antipodes of pyrrolidine-2,5-dicarboxylic acids. Demonstrated in Scheme 2, for instance, is the chemical transformation of (2*S*,5*S*)-**2a** and (2*R*,5*R*)-**3c** into (2*S*,5*S*)-**4a** and (2*R*,5*R*)-**4a**, respectively. Products were isolated in high yields albeit partial racemization was observed in both cases.

In order to synthesize other diastereomers of 5-carbamoylpyrrolidine-2-carboxylic acid derivatives and to explore the enzymatic activity of the amidase in *Rhodococcus erythropolis* AJ270 in catalyzing desymmetrization reactions, hydrolysis of *cis*-pyrrolidine-2,5-dicarboxamides was studied (Scheme 3). In comparison to racemic *trans*-1-benzylpyrrolidine-2,5-dicarboxamide **1a**, the meso substrate *cis*-1-benzylpyrrolidine-2,5-dicarboxamide **5a** underwent a very sluggish biocatalytic hydrolysis. It took 4 days to produce (2*R*,5*S*)-methyl 1-benzyl-5-carbamoylpyrrolidine-2-carboxylate **6a** in 15% yield, with a large amount of starting material (75%) being intact (entry 1, Table 2). A slow reaction rate was also observed in the

Scheme 1. Biocatalytic Kinetic Resolution of Racemic *trans*-Pyrrolidine-2,5-dicarboxamides

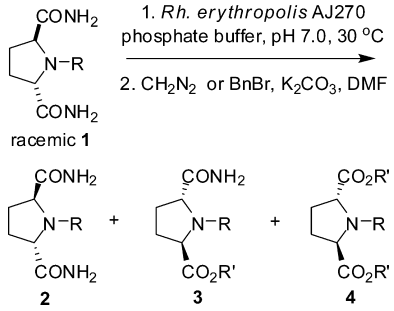

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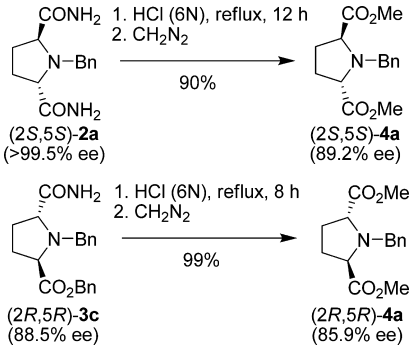
Table 1. Enantioselective Biotransformations of Racemic Dicarboxamides 1[a]

entry	1	R	time	2 (yield, %)[b] (ee, %)[c]	3 (R′) (yield, %)[b] (ee %)[c]	4 (R′) (yield, %)[b] (ee, %)[c]
1	**1a**	Bn	39 h	**2a** (49) (>99.5)	**3a** (Me) (39) (>99.5)	**4a** (Me) (6) (>99.5)
2	**1a**	Bn	15 h[d]	**2a** (49) (>99.5)	**3a** (Me) (32) (>99.5)	**4a** (Me) (12) (>99.5)
3	**1b**	allyl	7 h	**2b** (45) (>99.5)	**3b** (Me) (36) (96.8)	**4b** (Me) (10) (93.3)
4	**1b**	allyl	3.5 h	**2b** (61) (43.1)	**3b** (Me) (27) (>99.5)	**4b** (Me) (8) (>99.5)
5	**1c**	H	10 min	**2a** (45) (>99.5)	**3c** (Bn) (36) (93.5)	**4c** (N.O.)[e]
6[f]	**1c**	H	15 min	**2a** (45) (>99.5)	**3c** (Bn) (44) (93.0)	**4c** (N.O.)[e]
7[g]	**1c**	H	1 h	**2d** (47) (>99.5)	**3d** (H) (52) (87.1) (>99.5%)[h]	**4d** (N.O.)[e]

[a]Substrate (1 mmol) was incubated with *Rhodococcus erythropolis* AJ270 cells (2 g wet weight) in potassium phosphate buffer (0.1 M, pH 7.0, 50 mL) at 30 °C. DMSO (2.5 mL) was used as a cosolvent. [b]Isolated yield. [c]Determined by chiral HPLC analysis. [d]*Rhodococcus erythropolis* AJ270 cells (4 g wet weight) were used. [e]N.O. = not observed. [f]**1c** (3 mmol) was used in the absence of DMSO. [g]**1c** (10 mmol) was used. [h]After recrystallization.

Scheme 2. Chemical Hydrolysis of 2a and 3c



Scheme 3. Biocatalytic Desymmetrization of Meso Dicarboxamides 5

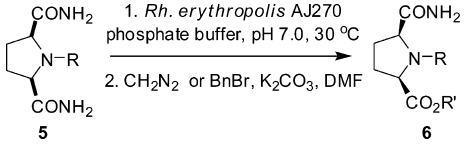


Table 2. Biocatalytic Desymmetrization of Meso Dicarboxamides 5[a]

entry	5	R	time (h)	6 (R′) (yield, %)[b] (ee %)[c]
1[d]	**5a**	Bn	96	**6a** (Me) (15) (>99.5)
2[e]	**5b**	allyl	96	**6b** (Me) (60) (95.2)
3	**5c**	H	1	**6c**[f] (Bn) (96) (94.8)
4[g]	**5c**	H	1	**6c**[f] (Bn) (90) (>99.5)
5[g,h]	**5c**	H	12	**6d** (H) (94) (>99.5)

[a]Substrate **5** (1 mmol) was incubated with *Rhodococcus erythropolis* AJ270 cells (2 g wet weight) in potassium phosphate buffer (0.1 M, pH 7.0, 50 mL) at 30 °C. DMSO (2.5 mL) was used as a cosolvent. [b]Isolated yield. [c]Determined by chiral HPLC analysis. [d]Starting material **5a** (75%) was recovered. [e]Starting material **5b** (25%) was recovered. [f]Benzylation also occurred on heterocyclic nitrogen. [g]DMSO was not used. [h]Substrate (20 g) in 250 mL of buffer was used.

hydrolysis of *N*-allyl-substituted *cis*-pyrrolidine-2,5-dicarboxamide **5b**, which afforded 60% of (2*R*,5*S*)-methyl 1-allyl-5-carbamoylpyrrolidine-2-carboxylate **6b** along with the recovery of reactant **5b** in 25% (entry 2, Table 2). In both cases, the amidase exhibited excellent enantioselectivity as enantiomeric excess values of **6a** and **6b** reached >99.5% and 95.2%, respectively (entries 1 and 2, Table 2). To our delight, amidase-catalyzed desymmetrization of *cis*-pyrrolidine-2,5-dicarboxamide **5c** proceeded efficiently.[33] Quantitative conversion of **5c** was effected within 1 h, and (2*R*,5*S*)-benzyl 1-benzyl-5-carbamoylpyrrolidine-2-carboxylate **6c** was isolated in 96% with 94.8% ee (entry 3, Table 2). In the absence of DMSO as a cosolvent, enantiopure **6c** (>99.5% ee) was produced in 90% yield (entry 4, Table 2). The amidase in *Rhodococcus erythropolis* AJ270 was robust, and it was able to tolerate high concentrations of substrate **5c** and product **6d**. In a scaled-up biocatalytic reaction, for example, 20 g of meso dicarboxamide **5c** was transformed almost quantitatively into a single enantiomeric product (2*R*,5*S*)-5-carbamoylpyrrolidine-2-carboxylic acid **6d** (R = R′ H) within 12 h[33] (entry 5, Table 2).

The outcomes of biocatalytic hydrolysis of racemic *trans*- and meso *cis*-pyrrolidine-2,5-dicarboxamide compounds showed clearly that the amidase within *Rhodococcus erythropolis* AJ270 displayed higher enzymatic activity against trans-configured dicarboxamides **1** than the cis-isomers **5**. This is most likely attributable to the steric effect of the substrates, as two cis-positioned amido groups in **5** may mutually prevent the interaction from reaching the active site of the amidase, whereas trans-orientation of two amido groups in **1** does not pose steric encumbrance to the substrate−enzyme interaction. The sensitivity of the amidase toward the steric feature of the substrates is in agreement to previous observations.[20a−c] It has been hypothesized that the amidase in *Rhodococcus erythropolis* AJ270 may comprise a deep-buried and size-limited active site.[27d−f]

The structure of all products was established on the basis of spectroscopic data and microanalysis. The absolute configuration of biocatalytic kinetic resolution products was assigned on the basis of the comparison of the optical rotation of (2*R*,5*R*)-dimethyl 1-benzylpyrrolidine-2,5-dicarboxylate **4a** with that of authentic sample reported in literature.[16] Since **4a** was derived from **3a**, compound **3a** therefore has the same stereochemistry as **4a**. The absolute configuration of biocatalytic desymmetrization products **6** was determined by X-ray diffraction analysis of the single-crystal structure of the salt of (2*R*,5*S*)-5-carbamoylpyrrolidine-2-carboxylic acid **6c** with HCl, which was cultivated conveniently from crystallization by means of diffusion of diethyl ether into a mixture of **6c** in aqueous HCl and ethanol.[33] It is interesting to address that the amidase within *Rhodococcus erythropolis* AJ270 shows 2*R*-enantioselectivity against both *trans*- and *cis*-pyrrolidine-2,5-dicarboxamides.

Functionalized enantiopure pyrrolidine derivatives produced from biotransformation of *trans*- and *cis*-pyrrolidine-2,5-dicarboxamides are conceivably invaluable chiral building blocks for the synthesis of natural products and druglike compounds. Starting from (2*R*,5*S*)-5-carbamoylpyrrolidine-2-carboxylic acid **6d**, we[33] have previously synthesized a pair of

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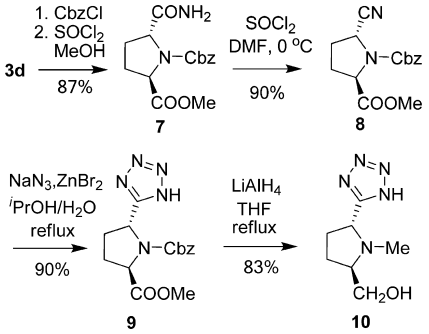
enantiomers of aza-sugar containing nucleoside analogues in which the anomeric carbon is substituted by a tetrazole moiety, compounds of pharmaceutical interest.[34] Now having enantioenriched (2*R*,5*R*)-5-carbamoylpyrrolidine-2-carboxylic acid **3d** in hand, we attempted the synthesis of compound **10**, a diastereomer of tetrazole-bearing aza-nucleoside analogues. As depicted in Scheme 4, *N*-protection with CbzCl followed by

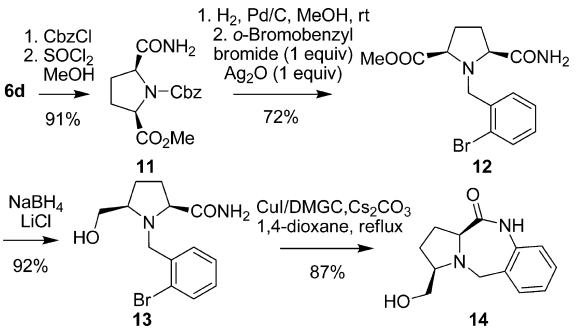
Scheme 4. Synthesis of Aza-nucleoside Diastereomer 10



esterification via acyl chloride led to the intermediate **7** in 87%. High-yielding dehydration of amide **7** produced nitrile **8**, which underwent 1,3-dipolar cycloaddition with azide to afford tetrazole product **9** effieciently. Treatment of **9** with $LiAlH_4$ led to simultaneous reduction of the ester and *N*-Cbz groups, furnishing the desired product **10** in a good yield (Scheme 4).

To explore the synthetic potential of enantiopure **6d**, available in tens of grams from biocatalytic desymmetrization of *cis*-pyrrolidine-2,5-carboxamide **5c** (entry 5, Table 2), we conducted the synthesis of the druglike pyrrole-fused benzo[1,4]diazepinone derivative **14** (Scheme 5). Biotransformation

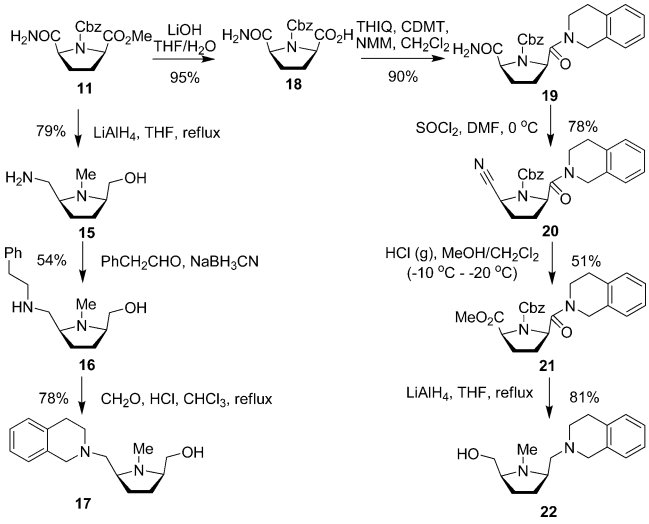
Scheme 5. Synthesis of Bicyclic Ring Products 14



product **6d** was first converted into **11** in excellent yield simply following the same procedure for the transformation of **3d** to **7**. The *N*-Cbz group was removed easily by catalytic hydrogenolysis and subsequent *N*-benzylation with *o*-bromobenzyl bromide in the presence of Ag_2O afforded intermediate **12** in 72% yield. Selective reduction of the ester group was achieved using a combination of $NaBH_4$ and LiCl[35] in a mixture of ethanol and THF (1:1), yielding compound **13** in an excellent yield. A CuI-catalyzed intramolecular cross-coupling reaction[36] resulted in the effective construction of (3*R*,11a*S*)-3-(hydrixymethyl)-2,3,10,11a-tetrahydro-1*H*-benzo[*e*]pyrrolo[1,2-*a*][1,4]diazepin-11(5*H*)-one **14** in good yield.

Considering the cis configuration of 2,5-functional groups and their facile interconversions, we also envisioned the concise and enantiodivergent construction of both antipodes of aza-sugar containing nucleoside analogues, compounds of significance in the study of anticancer and antivirus agents.[33,34] As illustrated in Scheme 6, compound **11** underwent exhaustive

Scheme 6. Synthesis of Both Antipodes of Aza-nucleotide Analogues 17 and 22



reduction with $LiAlH_4$ to afford amino alcohol **15** in 79% yield. Reductive amination with 2-phenylacetaldehyde, which gave **16**, and consecutive Pictet–Spengler reaction led conveniently to the formation of **17**. Its enantiomer **22** was then synthesized through the intermediate **19** which was resulted from the high-yielding ester hydrolysis of **7** and subsequent amide formation upon the treatment with 1,2,3,4-tetrahydroisoquinoline (THIQ) in the presence of chlorodimethoxytriazine (CDMT) and *N*-methylmorpholine (NMM).[37] Conversion of primary amide **19** into methyl ester **21** via nitrile **20** was effected under mild conditions, and the final reduction of both ester and amide functionalities with $LiAlH_4$ afforded the desired product **22** (Scheme 6).

CONCLUSION

In summary, we have showed that the amidase-catalyzed hydrolysis of pyrrolidine-2,5-dicarboxamides provides unique and practical synthetic routes to highly enantiopure functionalized pyrrolidine derivatives under very mild conditions. In the presence of *Rhodococcus erythropolis* AJ270, an amidase containing microbial whole cell catalyst, kinetic resolution of racemic *trans*-pyrrolidine-2,5-dicarboxamides give (2*S*,5*S*)-pyrrolidine-2,5-dicarboxamides and (2*R*,5*R*)-5-carbamoylpyrrolidine-2-carboxylic acids or (2*R*,5*R*)-pyrrolidine-2,5-dicarboxylic acids in excellent yields, while desymmetrization of meso *cis*-pyrrolidine-2,5-dicarboxamides produced nearly quantitatively (2*R*,5*S*)-5-carbamoylpyrrolidine-2-carboxylic acids. In both reactions, although the amidase exhibited the substrate- or *N*-substituent-dependent catalytic efficiency, it always displayed excellent 2*R*-enantioselectivity, giving products of >99.5% ee. The resulting chiral pyrrololidine derivatives, which are not readily available by other synthetic methods, are versatile building blocks in organic synthesis. Their synthetic applications have been demonstrated by the construction of tetrazole-substituted pyrrolidine and pyrroline-fused diazepin-11(5*H*)-one compounds. We have also shown the practical enantiodivergent preparation of a pair of enantiomers of aza-

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sugar containing nucleoside analogs from a common intermediate (2*R*,5*S*)-5-carbamoylpyrrolidine-2-carboxylic acid. Further exploration of the application of amidase and its catalytic mechanism is being actively pursued in this laboratory and the results will be reported in due course.

EXPERIMENTAL SECTION

Starting pyrrolidine-2,5-dicarboxamides **1** and **5** were synthesized from chemical hydrolysis of pyrrolidine-2,5-dicarbonitriles using concentrated H_2SO_4.[38] Dicarbonitriles in turn were prepared from the simple Strecker reaction using succinaldehyde bis-sodium bisulfite and amines according to a method reported in the literature.[39]

(±)-*trans*-1-Benzylpyrrolidine-2,5-dicarboxamide (1a): 1.8 g, 74%; mp 218−219 °C; IR (KBr) ν 3414, 1670, 1628 cm^{-1}; ^{1}H NMR (300 MHz/DMSO-d_6) δ 7.32−7.21 (m, 5 H), 7.17 (br, s, 2 H), 6.88 (br, s, 2 H), 3.81 (d, J = 13.5 Hz, 1 H), 3.69 (d, J = 13.5 Hz, 1 H), 3.56−3.54 (m, 2 H), 2.23−2.09 (m, 2 H), 1.71−1.66 (m, 2 H); ^{13}C NMR (75 MHz/DMSO-d_6) δ 176.4, 139.6, 129.0, 128.6, 127.3, 64.5, 53.5, 29.4; MS (ESI) m/z 248.2 (M + 1, 100), 270.2 (M + 23, 92). Anal. Calcd for $C_{13}H_{17}N_3O_2$: C, 63.14; H, 6.93; N, 16.99. Found: C, 62.94; H, 6.92; N, 17.05.

(±)-*trans*-1-Allylpyrrolidine-2,5-dicarboxamide (1b): 1.1 g, 57%; mp 223−224 °C; IR (KBr) ν 3413, 3378, 3200, 1660, 1633, 1459, 1415 cm^{-1}; ^{1}H NMR (300 MHz/DMSO-d_6) δ 7.23 (br, s, 2 H), 6.87 (br, s, 2 H), 5.97−5.84 (m, 1 H), 5.15−5.01 (m, 2 H), 3.55−3.53 (m, 2 H), 3.28 (dd, J = 13.6, 7.2 Hz, 1 H), 3.15 (dd, J = 13.5, 5.7 Hz, 1 H), 2.21−2.07 (m, 2 H), 1.69−1.59 (m, 2 H); ^{13}C NMR (75 MHz/DMSO-d_6) δ 176.0, 136.4, 116.5, 64.3, 52.5, 28.9; MS (ESI) m/z 198.1 (M + 1, 100), 220.2 (M + 23, 17). Anal. Calcd for $C_9H_{15}N_3O_2$: C, 54.81; H, 7.67; N, 21.30. Found: C, 54.75; H, 7.71; N, 21.36.

(±)-*trans*-Pyrrolidine-2,5-dicarboxamide (1c): 279 mg, 89%; mp 189−190 °C; IR (KBr) ν 3401, 3317, 3171, 1703, 1628 cm^{-1}; ^{1}H NMR (300 M Hz/D_2O) δ 3.86−3.82 (m, 2 H), 2.21−2.10 (m, 2 H), 1.85−1.72 (m, 2 H); ^{13}C NMR (75 MHz/D_2O) δ 180.0, 60.3, 30.4; MS (ESI) m/z 113.19 (M − 44, 100), 158.3 (M + 1, 20). Anal. Calcd for $C_6H_{11}N_3O_2$: 158.0930 (M + 1)$^+$. Found: 158.0925.

***cis*-1-Benzylpyrrolidine-2,5-dicarboxamide (5a):** 12.1 g, 84%; mp 156−157 °C; IR (KBr) ν 3384, 3174, 1693, 1663 cm^{-1}; ^{1}H NMR (300 M Hz/DMSO-d_6) δ 7.82 (br, s, 2 H), 7.33−7.21 (m, 5 H), 7.03 (br, s, 2 H), 3.73 (s, 2 H), 3.24−3.21 (m, 2 H), 2.03−1.91 (m, 2 H), 1.75−1.62 (m, 2 H); ^{13}C NMR (75 MHz/DMSO-d_6) δ 176.1, 137.2, 129.4, 128.0, 127.1, 66.6, 57.1, 29.9; MS(ESI) m/z 248.2 (M + 1, 22), 270.2 (M + 23, 100), 286.2 (M + 39, 21.2). Anal. Calcd for $C_{13}H_{17}N_3O_2$: C, 63.14; H, 6.93; N, 16.99. Found: C, 63.44; H, 6.97; N, 17.11.

***cis*-1-Allylpyrrolidine-2,5-dicarboxamide (5b):** 1.2 g, 61%; mp 148−149 °C; IR (KBr) ν 3367, 3166, 1697, 1667 cm^{-1}; ^{1}H NMR (300 M Hz/DMSO-d_6) δ 7.78 (br, s, 2 H), 7.07 (m, 2 H), 5.98−5.84 (m, 1 H), 5.18−5.06 (m, 2 H), 3.18−3.13 (m, 4 H), 2.09−1.96 (m, 2 H), 1.72−1.61 (m, 2 H); ^{13}C NMR (75 MHz/DMSO-d_6) δ 176.2, 134.7, 117.9, 66.9, 56.9, 29.9; MS (ESI) m/z 198.2 (M + 1, 22), 220.2 (M + 23, 100). Anal. Calcd for $C_9H_{15}N_3O_2$, C, 54.81; H, 7.67; N, 21.30. Found: C, 54.73; H, 7.76; N, 20.92.

***cis*-Pyrrolidine-2,5-dicarboxamide (5c).** 280 mg, 91%; mp 220−221 °C; IR(KBr) ν 3356, 3209, 1643 cm^{-1}; ^{1}H NMR (300 M Hz/DMSO-d_6) δ 8.09 (br, s, 1 H), 7.77 (br, s, 1 H), 4.21 (t, 2 H, J = 5.8 Hz), 2.39−2.26 (m, 2 H), 1.93−1.78 (m, 2 H); ^{13}C NMR (75 MHz/DMSO-d_6) δ 169.3, 59.3, 29.6; MS (ESI) m/z 158.1 (M + 1, 100), 180.1 (M + 23, 95). Anal. Calcd for [M + H] $C_6H_{12}N_3O_2$: 158.09295 [M + H]. Found: 158.09246.

General Procedure for the Biotransformation of Pyrrolidine-2,5-dicarboxamides. To an Erlenmeyer flask (150 mL) with a screw cap were added *Rhodococcus erythropolis* AJ270 cells[21,23a] (2 g wet weight) and potassium phosphate buffer (0.1 M, pH 7.0, 50 mL), and the resting cells were activated at 30 °C for 0.5 h with orbital shaking. Racemic *trans*-pyrrolidine-2,5-dicarboxamides **1** or meso *cis*-pyrrolidine-2,5-dicarboxamides **5**, which may be dissolved in DMSO (2.5 mL) (see Tables 1 and 2), were added in one portion to the flask, and the mixture was incubated at 30 °C using an orbital shaker (200 rpm). The reaction, monitored by TLC or HPLC, was quenched after a specified period of time (see Tables 1 and 2) by removing the biomass through a Celite pad filtration, and the filtrate was freeze-dried. For biotransformation of **1a−c**, the residue was mixed with methanol (2 mL), and a freshly prepared solution of CH_2N_2 in diethyl ether (10 mL) was added while the solution was kept cool with an ice−water bath. The mixture was stirred overnight. Water (15 mL) was added, and the resulting mixture was extracted with ethyl acetate (8 × 50 mL). The combined organic layer was dried with anhydrous Na_2SO_4. After removal of solvent, the residue was chromatographed using a silica gel column eluted with a mixture of petroleum ether and ethyl acetate (12:1) to give diester product **4**, a mixture of petroleum ether and ethyl acetate (1: 2) to give monoester product **3**, and then a mixture of ethyl acetate and methanol (5:1) to give dicarboxamide product **2**. The aqueous phase from extraction was freeze-dried again, and the residue was chromatographed using a silica gel column eluted with a mixture of ethyl acetate and methanol (5:1) to give pure dicarboxamide product **2**. For biotransformation of **5a** or **5b**, the residue was mixed with methanol (2 mL), and a freshly prepared solution of CH_2N_2 in diethyl ether (10 mL) was added while the mixture was kept cool with an ice−water bath. The mixture was stirred overnight. Water (10 mL) was added, and the resulting mixture was extracted with ethyl acetate (3 × 10 mL) and dried with anhydrous Na_2SO_4. After removal of solvent, the residue was chromatographed using a silica gel column eluted with ethyl acetate to give product **6a** or **6b**. For the biotransformation of **5c**, the residue was mixed with DMF (2 mL), BnBr (1 mL), and K_2CO_3 (1.38 g, 10 mmol). After the mixture was stirred for 24 h, water (10 mL) was added, and the resulting mixture was extracted with ethyl acetate (3 × 100 mL). The combined organic layer was washed with brine (3 × 2 mL) and dried with anhydrous Na_2SO_4. After removal of solvent, the residue was chromatographed using a silica gel column with ethyl acetate as eluent to give pure product **6c**.

Procedure for Large-Scale Biocatalytic Kinetic Resolution of 1c. *Rhodococcus erythropolis* AJ270 cells[21,23a] (2 g wet weight) were suspended in aqueous potassium phosphate buffer (0.1 M, pH 7.0, 50 mL) in an Erlenmeyer flask (150 mL) with a screw cap and activated at 30 °C for 0.5 h with orbital shaking. Dicarboxamide **1c** (1.57 g, 10 mmol) was added in one portion to the flask, and the resulting mixture was incubated at 30 °C using an orbital shaker (200 rpm). The reaction, monitored by HPLC, was quenched after 1 h by removing the biomass by filtration through a Celite pad. After removal of solvent, the residue was loaded to an ion-exchange column (Dowex, 50WX8 200−400 mesh) and eluted with pure water until pH was around 7.0. Elution with ammonia solution (1%) gave a mixture of **2d** and **3d**. The mixture was chromatographed on a C-18 reverse phase column eluted with water to afford enantioenriched products **2d** (740 mg, 47%) and **3d** (826 mg, 52%).

Procedure for Large-Scale Biocatalytic Desymmetrization of 5c. *Rhodococcus erythropolis* AJ270 cells[21,23a] (2 g wet weight) were suspended in aqueous potassium phosphate buffer (0.1 M, pH 7.0, 250 mL) in an Erlenmeyer flask (500 mL) with a screw cap and activated at 30 °C for 0.5 h with orbital shaking. Dicarboxamide **5c** (20 g) was added in one portion to the flask, and the resulting mixture was incubated at 30 °C using an orbital shaker (200 rpm). The reaction, monitored by HPLC, was quenched after 12 h by removing the biomass by filtration through a Celite pad. The filtrate was concentrated to 100 mL using a rotary evaporator under reduced pressure, and product **6d** (8.0 g) precipitated from solution. The mother liquid was subjected to an ion-exchange column and eluted with aqueous ammonia solution (1%) to give another portion of product **6d** (11.0 g).

All biotransformation products were fully characterized, and characterization data are as follows.

(2*S*,5*S*)-1-Benzylpyrrolidine-2,5-dicarboxamide (2a): 121 mg, 49%; mp 235 °C; $[\alpha]^{25}_D$ −48.0 (c 0.5, CH_3OH); ee >99.5% (HPLC with ADH). Spectroscopic data identical to those of racemic compound **1a** were obtained.

(2*S*,5*S*)-1-Allylpyrrolidine-2,5-dicarboxamide (2b): 88.7 mg, 45%; mp 220−221 °C; $[\alpha]^{25}_D$ −58.8 (c 0.5, CH_3OH); ee >99.5%

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(HPLC with ADH). Spectroscopic data identical to those of racemic **1b** were obtained.

(2*S*,5*S*)-Pyrrolidine-2,5-dicarboxamide (2d): 738 mg, 47%; mp 170 °C; $[\alpha]^{25}_{D}$ −111.3 (*c* 0.75, H_2O). Spectroscopic data identical to those of **1c** were obtained. To determine the enantiomeric excess (ee) value and the absolute configuration of product **2d**, a transformation of **2d** into **2a** was conducted. Thus, a mixture of **2d** (75 mg), K_2CO_3 (0.7 g), and benzyl bromide (0.5 mL) in dry DMF (1 mL) was stirred at ambient temperature for 24 h. The mixture was submitted to a short silica column and eluted with ethyl acetate to remove side product and then with a mixture of ethyl acetate and methanol (20:1−10:1) to gave **2a** with ee >99.5% (HPLC analysis with a Daicel ADH column).

(2*R*,5*R*)-Methyl 1-benzyl-5-carbamoylpyrrolidine-2-carboxylate (3a): 84 mg, 32%; mp 140−141 °C; $[\alpha]^{25}_{D}$ +121.5 (*c* 4.0, $CHCl_3$); ee >99.5% (HPLC with ADH); IR (KBr) ν 3446, 1734, 1685, 1658 cm^{-1}; ^1H NMR (300 MHz/$CDCl_3$) δ 7.36−7.21 (m, 5 H), 6.91 (br, s, 1 H), 5.50 (br, s, 1 H), 3.92 (d, 1 H, *J* = 13.1 Hz), 3.84 (d, 1 H, *J* = 13.1 Hz), 3.80−3.72 (m, 2 H), 3.69 (s, 3 H), 2.58−2.51 (m, 1 H), 2.13−2.03 (m, 1 H), 2.00−1.85 (m, 2 H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 177.7, 173.5, 138.0, 128.74, 128.66, 127.6, 65.9, 62.9, 54.3, 51.4, 29.4, 28.6; MS (ESI) *m*/*z* 263.2 (M + 1, 100%), 285.2 (M + 23, 64.3), 301.2 (M + 39, 4.9). Anal. Calcd for $C_{14}H_{18}N_2O_3$: C, 64.10; H, 6.92; N, 10.68. Found: C, 63.85; H, 6.90; N, 10.94.

(2*R*,5*R*)-Methyl 1-allyl-5-carbamoylpyrrolidine-2-carboxylate (3b): 57 mg, 27%; mp 104−105 °C; $[\alpha]^{25}_{D}$ = +78.0 (*c*1.0, $CHCl_3$); ee >99.5% (HPLC with ADH); IR(KBr) ν 3377, 3190, 1728, 1654 cm^{-1}; ^1H NMR (300 MHz/$CDCl_3$) δ 6.91 (br, s, 1 H), 5.89−5.78 (br, s, 1 H, m, 1 H), 5.20−5.10 (m, 2 H), 3.93 (d, *J* = 7.5 Hz, 1 H), 3.69 (s, 3 H), 3.61 (dd, *J* = 10.8, 3.3 Hz, 1 H), 3.36−3.34 (m, 2 H), 2.54−2.47 (m, 1 H), 2.16−2.09 (m, 1 H), 1.96−1.87 (m, 2 H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 178.2, 173.5, 134.8, 118.0, 65.4, 63.3, 53.1, 51.4, 29.3, 28.6; MS (ESI) *m*/*z* 213.2 (M + 1, 100), 235.2 (M + 23, 6.5). Anal. Calcd for $C_{10}H_{16}N_2O_3$: C, 56.59; H, 7.60; N, 13.20. Found: C, 56.57; H, 7.61; N, 12.94.

(2*R*,5*R*)-Benzyl 1-benzyl-5-carbamoylpyrrolidine-2-carboxylate (3c): 446 mg, 44%; mp 104−105 °C; $[\alpha]^{25}_{D}$ = +156.0 (*c* 1.0, $CHCl_3$); ee 93.0% (HPLC with ADH); IR (KBr) ν 3396, 3172, 1726, 1657, 1161 cm^{-1}; ^1H NMR (300 MHz/$CDCl_3$) δ 7.41−7.10 (m, 10 H), 6.96 (br, s, 1 H), 5.49 (br, s, 1H), 5.18 (d, *J* = 12.0 Hz, 1 H), 5.08 (d, *J* = 12.0 Hz, 1 H), 3.91−3.72 (m, 4 H), 2.61−2.47 (m, 1 H), 2.15−1.85 (m, 3 H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 177.9, 172.9, 137.9, 135.6, 128.8, 128.6, 128.5, 127.5, 66.3, 65.8, 62.9, 54.1, 29.4, 28.6; MS (ESI) *m*/*z* 339.1 (M + 1, 100), 361.2 (M + 23, 82). Anal. Calcd for $C_{20}H_{22}N_2O_3$: C, 70.99; H, 6.55; N, 8.28. Found: C, 70.60; H, 6.60; N, 8.37.

(2*R*,5*R*)-5-Carbamoylpyrrolidine-2-carboxylic acid (3d): 822 mg, 52%; mp 251 °C (sublimation); $[\alpha]^{25}_{D}$ = +95.3 (*c* 1.0, H_2O); IR (KBr) ν 3396, 3340, 3129, 3050, 1692, 1689, 1657, 1613, 1405 cm^{-1}; ^1H NMR (300 MHz/D_2O) δ 4.43 (t, *J* = 7.3 Hz, 1 H), 4.20 (t, *J* = 7.3 Hz, 1 H), 2.41−2.28 (m, 2 H), 2.13−1.98 (m, 2 H); ^{13}C NMR (75 MHz/D_2O) δ 173.8, 171.4, 61.9, 60.1, 29.6, 28.8; MS (ESI) *m*/*z* 339.1 (M + 1, 100), 361.2 (M + 23, 82). Anal. Calcd for $C_6H_{11}N_2O_3$: 159.0769. Found: 159.0760. To determine the ee value and the absolute configuration of product **3d**, transformation of **3d** into **3c** was conducted. Thus, a mixture of **3d** (75 mg), K_2CO_3 (0.7 g), and benzyl bromide (0.5 mL) in dry DMF (1 mL) was stirred at ambient temperature for 24 h. Water (10 mL) was then added, and the mixture was extracted with ethyl acetate (3 × 10 mL). The combined organic layer was washed with brine (3 × 10 mL) and dried with anhydrous Na_2SO_4. After removal of organic solvent, the residue was chromatographed on a silica gel column eluted with ethyl acetate to give **3c** with ee 87.1% (HPLC analysis with a Daicel ADH column). Enantiomeric purity of **3d** was improved to >99.5% ee after recrysttalization in a mixture of water and methanol

(2*R*,5*R*)-Dimethyl 1-benzylpyrrolidine-2,5-dicarboxylate (4a): 33 mg, 12%; oil; $[\alpha]^{25}_{D}$ = +58.7 (*c* 1.5, $CHCl_3$); ee >99.5% (HPLC with ODH); IR (KBr) ν 1737 cm^{-1}; ^1H NMR (300 MHz/$CDCl_3$) δ 7.31−7.21 (m, 5 H), 3.97 (d, *J* = 12.9 Hz, 1 H), 3.86−3.82 (m, 2 H), 3.79 (d, *J* = 13.1 Hz, 1 H), 3.64 (s, 6 H), 2.38−2.24 (m, 2 H), 1.99−1.88 (m, 2 H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 174.7, 138.5, 129.0, 128.2, 127.2, 63.4, 54.1, 51.5, 28.4; MS (ESI) *m*/*z* 278.2 (M + 1, 100), 300.2 (M + 23, 8.0). Anal. Calcd for $C_{15}H_{19}NO_4$: 277.1314. Found: 277.1318.

(2*R*,5*R*)-Dimethyl 1-allylpyrrolidine-2,5-dicarboxylate (4b): 23 mg, 10%; oil; $[\alpha]^{25}_{D}$ = +72.0 (*c* 1.5, $CHCl_3$); ee 93.3% (HPLC with OJH); IR ν (KBr) 1738 cm^{-1}; ^1H NMR (300 MHz/$CDCl_3$) δ 5.96−5.82 (m, 1 H), 5.18−5.05 (m, 2 H), 3.89−3.85 (m, 2 H), 3.70 (s, 6 H), 3.45−3.33 (m, 2 H), 2.36−2.28 (m, 2 H), 1.94−1.89 (m, 2 H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 174.7, 135.3, 117.7, 63.6, 53.5, 51.6, 28.4; MS (EI) *m*/*z* 227 (M^+, 3), 168 (100). Anal. Calcd for $C_{11}H_{17}NO_4$: 227.1158. Found: 227.1161.

(2*R*,5*S*)-Methyl 1-benzyl-5-carbamoylpyrrolidine-2-carboxylate (6a): 39 mg, 15%; oil; $[\alpha]^{25}_{D}$ +10.7 (*c* 3.0, C_6H_6); ee >99.5% (HPLC with ADH); IR (KBr) ν 3415, 1736, 1678 cm^{-1}; ^1H NMR (300 MHz/$CDCl_3$) δ 7.97 (br, s, 1 H), 7.33−7.25 (m, 5 H), 5.57 (br, s, 1 H), 3.89 (d, *J* = 13.2 Hz, 1 H), 3.82 (d, *J* = 13.2 Hz, 1 H), 3.63−3.51 (m, 2 H), 3.58 (m, 3 H), 2.20−1.85 (m, 4 H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 177.9, 175.7, 137.2, 129.3, 128.5, 127.6, 67.7, 66.2, 59.0, 52.0, 30.7, 30.4; MS (ESI) *m*/*z* 263.2 (M + 1, 60), 285.2 (M + 23, 100). Anal. Calcd for: $C_{14}H_{18}N_2O_3$, C, 64.10; H, 6.92; N, 10.68; Found: C, 64.25; H, 6.87; N, 10.92.

(2*R*,5*S*)-Methyl 1-allyl-5-carbamoylpyrrolidine-2-carboxylate (6b): 127 mg, 60%; oil; $[\alpha]^{25}_{D}$ +6.0 (*c* 4, CH_2Cl_2); ee 95.2% (HPLC with ADH); IR (KBr) ν 3415, 1738, 1681 cm^{-1}; ^1H NMR (400 MHz/$CDCl_3$) δ 7.99 (br, s, 1 H), 5.87−5.78 (m, 1 H), 5.45 (br, s, 1 H), 5.20−5.12 (m, 2 H), 3.73 (s, 3 H), 3.57 (t, 1 H, *J* = 7.6 Hz), 3.45−3.42 (m, 1 H), 3.32 (d, 2 H, *J* = 6.8 Hz), 2.20−2.12 (m, 2 H), 2.07−2.01 (m, 1 H), 1.92−1.86 (m, 1 H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 178.1, 175.8, 133.8, 118.7, 67.0, 65.7, 57.2, 52.1, 30.7, 30.4; MS (ESI) *m*/*z* 213.2 (M + 1, 43), 235.2 (M + 23, 100). Anal. Calcd for $C_{10}H_{16}N_2O_3$: C, 56.59; H, 7.60; N, 13.20. Found: C, 56.62; H, 7.68; N, 13.16.

(2*R*,5*S*)-Benzyl 1-benzyl-5-carbamoylpyrrolidine-2-carboxylate (6c): 304 mg, 90%; mp 58−59 °C; $[\alpha]^{25}_{D}$ +19.2 (*c* 1, CH_2Cl_2); ee >99.5% (HPLC analysis with a Diacel ADH column); IR (KBr) ν 3422, 3369, 1740, 1663 cm^{-1}; ^1H NMR (300 MHz/$CDCl_3$) δ 7.89 (br, s, 1 H), 7.37−7.22 (m, 10 H), 5.90 (br, s, 1 H), 4.98 (s, 2 H), 3.88 (d, *J* = 13.2 Hz, 1 H), 3.79 (d, *J* = 13.2 Hz, 1 H), 3.63 (t, 1 H, *J* = 7.4 Hz), 3.54−3.50 (m, 1 H), 2.19−1.88 (m, 4 H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 177.1, 174.1, 136.4, 134.7, 128.5, 127.8, 127.6, 127.57, 127.3, 126.8, 67.1, 65.9, 65.5, 58.2, 29.8, 29.5; MS (ESI) *m*/*z* 339 (M + 1, 86), 361 (M + 23, 100). Anal. Calcd for $C_{20}H_{22}N_2O_3$: C, 70.99; H, 6.55; N, 8.28. Found: C, 70.65; H, 6.71; N, 8.53. Slow vapor diffusion of diethyl ether into a mixture of **6c** (15.7 mg), concentrated hydrochloric acid (15 μL) and ethanol (0.5 mL) at 5 °C for 2 days led to the formation of an X-ray-quality single crystal of **6c**·HCl.[33]

(2*R*,5*S*)-5-Carbamoylpyrrolidine-2-carboxylic acid (6d): 19.0 g, 94%; mp 233−234 °C; $[\alpha]^{25}_{D}$ −9.6 (*c* 1, H_2O); IR (KBr) ν 3308, 3153, 2361, 1696, 1642, 1576 cm^{-1}; ^1H NMR (300 MHz/D_2O) δ 4.34 (t, *J* = 7.2 Hz, 1 H), 4.11 (t, *J* = 6.3 Hz, 1 H), 2.39−2.25 (m, 2 H), 2.06−1.91 (m, 2 H); ^{13}C NMR (75 MHz/D_2O) δ 173.8, 171.4, 61.8, 60.31, 29.8, 29.2; MS (ESI) *m*/*z* 159 (M + 1, 7), 181 (M + 23, 100). Anal. Calcd for $C_6H_{10}N_2O_3$: C, 45.57; H, 6.37; N, 17.71. Found: C, 45.54; H, 6.53; N, 17.78. To determine the enantiomeric excess (ee) value and the absolute configuration of product **6d**, transformation of **6d** into **6c** was conducted. Thus, a mixture of **6d** (158 mg), K_2CO_3 (1.38 g), and benzyl bromide (1 mL) in dry DMF (2 mL) was stirred at ambient temperature for 24 h. Water (10 mL) was then added, and the mixture was extracted with ethyl acetate (3 × 10 mL). The combined organic layer was washed with brine (3 × 10 mL) and dried with anhydrous Na_2SO_4. After removal of organic solvent, the residue was chromatographed on a silica gel column eluted with ethyl acetate to give **6c** [ee >99.5% (HPLC analysis with a Daicel ADH column)].

General Procedure for Chemical Hydrolysis of 2a and 3c. A solution of (2*S*,5*S*)-**2a** (50 mg, 0.2 mmol, ee >99.5%, HPLC analysis with a Daicel ADH column) or (2*R*,5*R*)-**3c** (67.6 mg, 0.2 mmol, ee 88.5%, HPLC analysis with a Daicel ADH column) in hydrochloric acid (6 N, 8 mL) was refluxed for 12 or 8 h. The solvent was removed under vacuum, and water in the residue was further removed by azotropic distillation with methanol (3 × 3 mL) using a rotary

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evaporator. The resulting residue was mixed with methanol (2 mL), and then CH_2N_2 (5 mL) was added dropwise at ambient temperature.After the mixture was stirred overnight, water (2 mL) was added, and the mixture was extracted with ethyl aceate (3 × 10 mL). The combined organic layer was dried with anhydrous sodium sulfate. After removal of solvent, the residue was purified through a silica gel column eluted with a mixture of petroleum ether and ethyl acetate (6:1) to give pure (2*S*,5*S*)-**4a** (50 mg, 90%) with 89.2% ee (HPLC analysis with a Daicel ODH column) or (2*R*,5*R*)-**4a** (55 mg, 99%) with 85.9% ee (HPLC analysis with a Daicel ODH column).

(2*S*,5*S*)-Dimethyl 1-benzylpyrrolidine-2,5-dicarboxylate (4a): $[\alpha]^{25}_{D}$ = −40.0 (*c* 1.5, $CHCl_3$); ee >89.2% (HPLC with ODH). Identical spectroscopic data as that of (2*R*,5*R*)-**4a** were obtained.

Synthesis of 7. To a mixture of **3d** (1.19 g, 7.5 mmol) in ethanol (20 mL) and saturated $NaHCO_3$ aqueous solution (20 mL) was added dropwise CbzCl (3 mL) at room temperature with stirring. The resulting mixture was allowed to stire overnight. The reaction was quenched by addition of hydrochloric acid (2 N) until the pH was adjusted to around 7.0. Under reduced pressure, the solvent was completely removed. The residue was mixed with methanol (50 mL) and $SOCl_2$ (4 mL) at ambient temperature. After the misture was stirred for another 4 h at ambient temperature, saturated $NaHCO_3$ aqueous solution (100 mL) was added. The mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layer was washed with brine (3 × 10 mL) and dried with anhydrous Na_2SO_4. After removal of organic solvent, the residue was chromatographed on a silica gel column eluted with ethyl acetate to give product **7** (2.0 g, 87%): oil; $[\alpha]^{25}_{D}$ +30 (*c* 1, $CHCl_3$); IR (KBr) ν 3421, 1743, 1698, 1683 cm^{-1}; ^1H NMR (400 MHz/DMSO-d_6, 405 K) δ 7.34−7.28 (m, 5 H), 6.76 (br, s, 2 H), 5.04 (t, *J* = 14.0 Hz, 2 H), 4.43 (dd, *J* = 8.8, 1.7 Hz, 1 H), 4.33−4.30 (m, 1 H), 3.58 (s, 3 H), 2.32−2.13 (m, 2 H), 1.92−1.86 (m, 2 H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 175.2, 174.5, 172.9, 172.7, 1584.9, 154.7, 136.2, 136.0, 128.0, 127.9, 127.7, 127.5, 67.4, 60.6, 60.1, 59.7, 52.3, 52.1, 29.5, 29.3, 28.0, 27.6; MS (ESI) *m*/*z* 329.4 (M + 23, 100). Anal. Calcd for $C_{15}H_{19}N_2O_5$: 307.1294. Found: 307.1291.

Synthesis of 8. To a solution of **7** (1.6 g, 5.2 mmol) in dry DMF (10 mL) was added $SOCl_2$ (1.1 mL) at 0 °C. After being stirred for another 1 h at room temperature, the reaction was quenched by the addition of saturated $NaHCO_3$ aqueous solution (10 mL). The mixture was extracted with ethyl acetate (3 × 30 mL). The combined organic layer was washed with brine (3 × 10 mL) and dried with anhydrous Na_2SO_4. After removal of organic solvent, the residue was chromatographed on a silica gel column eluted with a mixture of ethyl acetate and petroleum ether (1:2) to give compound **8** (1.3 g, 90%): oil; $[\alpha]^{25}_{D}$ +31.3 (*c* 1.3, $CHCl_3$); IR (KBr) ν 1747, 1717, 1407, 1351 cm^{-1}; ^1H NMR (300 MHz/DMSO-d_6) δ 7.41−7.27 (m, 5 H), 5.20−4.94 (m, 3 H), 4.55−4.46 (m, 1 H), 3.65 (s, 1.2 H), 3.56 (s, 1.7 H), 2.44−2.31 (m, 2 H), 2.26−2.08 (m, 2 H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 171.8, 153.5, 135.6, 135.57, 128.59, 128.51, 128.3, 128.0, 118.5, 118.3, 68.3, 68.0, 59.0, 58.8, 52.6, 52.4, 48.1, 47.5, 29.9, 29.7, 28.9, 28.6; MS (ESI) *m*/*z* 311.5 (M + 23, 100). Anal. Calcd for $C_{15}H_{17}N_2O_4$: 289.1188. Found: 289.1188.

Synthesis of 9. A mixture of **8** (576 mg, 2 mmol), NaN_3 (260 mg,4 mmol), and $ZnBr_2$ (225 mg,1 mmol) in water (6 mL) and 2-propanol (3 mL) was refluxed for 4 h. After the mixture was cooled to room temperature, hydrochloric acid (1 N, 10 mL) was added. The mixture was extracted with ethyl acetate (3 × 20 mL). The combined organic layer was washed with brine (3 × 4 mL) and dried with anhydrous Na_2SO_4. After removal of organic solvent, the residue was chromatographed on a silica gel column eluted with a mixture of ethyl acetate and methanol (1:2) to give compound **9** (596 mg, 90%): mp 136−137 °C; $[\alpha]^{25}_{D}$ +28.0 (*c* 1.0, MeOH); IR (KBr) ν 1746, 1713, 1412, 1353, 1202 cm^{-1}; ^1H NMR (400 MHz/DMSO-d_6, 405 K) δ 7.23−7.13 (m, 5 H), 5.42−5.40 (m, 1 H), 4.92 (s, 2 H), 4.48 (d, *J* = 6.7 Hz, 1 H), 3.60 (s, 3 H), 2.42−2.20 (m, 2 H), 1.95−1.81 (m, 2 H); ^{13}C NMR (75 MHz/DMSO-d_6) δ 172.6, 172.3, 153.6, 153.2, 136.6, 136.4, 128.3, 128.1, 127.8, 127.3, 126.5, 66.3, 65.8, 59.3, 58.8, 53.3, 53.0, 52.0, 31.2, 30.0, 28.4, 27.1; MS (ESI) *m*/*z* 330.5 (M − 1, 100). Anal. Calcd for $C_{15}H_{18}N_5O_4$: 332.1359. Found: 332.1355.

Synthesis of 10. Under argon protection, a mixture of **9** (397 mg, 1.2 mmol) and $LiAlH_4$ (456 mg, 12 mmol) in dry THF (10 mL) was refluxed for 24 h. While the mixture was kept at −20 °C, NaOH aqueous solution (2 N, 1 mL) was slowly injected through a syringe. The resulting mixture was filtrated through a Celite pad and washed thoroughly with a mixture of CH_2Cl_2 and MeOH (20:1). After removal of solvent, the residue was mixed with hydrochloric acid (1 N) with pH value of the mixture being adjusted to around 2.0. The resulting mixture was subjected to an ion-exchange column (Dowex, 50WX8 100−200 mesh) and then eluted with pure water. When the pH of the fraction was around 7, the column was eluted with aqueous ammonia solution (1%). After removal of solvent, product **10** was obtained. **10** (182 mg, 83%): mp 194−195 °C; $[\alpha]^{25}_{D}$ +16.0 (*c* 1.0, H_2O); IR (KBr) ν 3412, 3155, 1575, 1461, 1408, 1089 cm^{-1}; ^1H NMR (600 MHz/D_2O) δ 5.00 (t, *J* = 12.6 Hz, 1 H), 3.87−3.86 (m, 2 H), 3.58−3.55 (m, 1 H), 2.54−2.34 (m, 3 H), 2.41 (s, 3 H), 2.06−2.00 (m, 2 H); ^{13}C NMR (75 MHz/D_2O) δ 158.0, 67.0, 60.7, 59.3, 35.7, 27.4, 25.5; MS (ESI) *m*/*z* 184.5 (M + 1, 100). Anal. Calcd for $C_7H_{14}N_5O_1$: 184.1198. Found: 184.1194.

Synthesis of 11. To a stirred mixture of **6d** (4.0 g, 25 mmol) in ethanol (20 mL) and saturated aqueous $NaHCO_3$ solution (20 mL) was added dropwise CbzCl (6 mL) at room temperature. The resulting mixture was allowed to stir overnight at room temperature. After concentration by removing ethanol using a rotary evaporator under reduced pressure, the resulting aqueous solution (about 15 mL) was neutralized with hydrochloric acid (2 N) to pH 7.0. The solvent was removed under vacuum, and water in the residue was further removed by azotropic distillation with methanol (3 × 30 mL) using a rotary evaporator. The resulting residue was mixed with methanol (100 mL) and then $SOCl_2$ (4 mL) at room temperature. After being stirred for another 4 h, the reaction mixture was poured slowly into a saturated aqueous solution of $NaHCO_3$ (200 mL), and the resultant mixture was extracted with ethyl acetate (3 × 100 mL). The combined organic layer was washed with brine (3 × 20 mL) and dried with anhydrous sodium sulfate. The solvent was removed and the residue was chromatographed on a silca gel column eluted with ethyl acetate to give pure product **11** (6.96 g, 91%): mp 104−105 °C; $[\alpha]^{25}_{D}$ +46.6 (*c* 0.6, $CHCl_3$); IR (KBr) ν 3435, 3401, 1734, 1700, 1683, 1415, 1356, 1213, 1117, 1004 cm^{-1}; ^1H NMR (300 MHz/DMSO-d_6, 375 K) δ 7.35−7.25 (m, 5 H), 6.97 (br, s, 2 H), 5.09 (d, *J* = 12.6 Hz, 1 H), 5.05 (d, *J* = 12.6 Hz, 1 H), 4.42 (t, *J* = 7.2 Hz, 1 H), 4.18 (dd, 1 H, *J* = 3.6, 8.3 Hz), 3.66 (s, 3 H), 2.31−2.17 (m, 2 H), 1.98−1.87 (m, 2 H); ^{13}C NMR (75 MHz/DMSO-d_6) δ 175.0, 174.8, 174.3, 174.0, 154.4, 154.1, 136.9, 136.7, 128.8, 128.4, 128.3, 127.7, 127.6, 67.2, 67.1, 62.5, 62.1, 60.5, 60.1, 53.1, 53.0, 30.6, 29.7,29.6, 28.8; MS (ESI) *m*/*z* 307.35 (M + 1, 20), 329.29 (M + 23, 100). Anal.Calcd for $C_{15}H_{18}N_2O_5$: C, 58.82; H, 5.92; N, 9.15. Found: C, 58.87; H, 5.99; N, 9.10.

Synthesis of 12. To a flask charged with Pd/C catalyst (10%, 300 mg) was added a solution of **11** (3.06 g, 10 mmol) in methanol (50 mL). The mixture was allowed to stir at room temperature for 4 h under hydrogen atmosphere with a H_2 balloon. After removal of catalyst by filtration, the filtrate was concentrated to 20 mL. Ag_2O (2.32 g, 10 mmol) and 1-bromo-2-(bromomethyl)benzene (2.5 g, 10 mmol) were added, and the resulting mixture was stirred at room temperature for 2.5 h. Ethyl acetate (10 mL) was added, and the reaction mixture was filtrated to remove solid residue. The filtrate was concentrated under vacuum, and the residue was chromatographed on a silica gel column eluted with ethyl acetate to afford pure **12** (2.46 g, 72%): mp 110−111 °C; $[\alpha]^{25}_{D}$ −8.0 (*c* 2.0, $CHCl_3$); IR (KBr) ν 3419, 1737, 1678 cm^{-1}; ^1H NMR (300 MHz/$CDCl_3$) δ 7.91 (br, s, 1 H), 7.47 −7.05 (m, 5 H), 5.80 (br, 1 H), 3.94 (d, *J* = 13.5 Hz, 1 H), 3.91 (d, *J* = 13.5 Hz, 1 H), 3.60−3.47 (m, 2 H), 3.49 (s, 3 H), 2.20−1.97 (m, 3 H), 1.90−1.77 (m, 1 H); ^{13}C NMR (75 MHz/$CDCl_3$) 177.7, 175.3, 136.5, 133.0, 131.8, 129.4, 127.5, 125.0, 67.8, 66.6, 58.8, 52.0, 30.7, 30.4. MS (ESI) *m*/*z* 341.3, 343.1 (M + 1, 20), 363.2, 365.2 (M + 23, 100). Anal. Calcd for $C_{14}H_{17}BrN_2O_3$: C, 49.28; H, 5.02; N, 8.21. Found: C, 49.32; H, 5.20, N, 8.07.

Synthesis of 13. To a solution of **12** (342 mg, 1 mmol) in a mixture of THF and ethanol (10 mL, 1:1) were added $NaBH_4$ (80 mg, 2.1 mmol) and LiCl (80 mg, 1.9 mmol). After the mixture was stirred

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at room temperature for 12 h, the reaction was quenched by adding a saturated aqueous $NaHCO_3$ solution (10 mL). The mixture was extracted with ethyl acetate (3 × 10 mL), and the combined organic layer was dried with anhydrous sodium sulfate. The solvent was removed using a rotavapor and the residue was chromatographed on a silica gel column eluted with ethyl acetate to yield pure **13** (287 mg, 92%): mp 114−115 °C; $[\alpha]^{25}_D$ +14.2 (*c* 1.5, $CHCl_3$); IR(KBr) ν 3405, 3318, 1669 cm^{-1}; ^1H NMR (300 MHz/DMSO-*d6*) δ 7.58−7.17 (m 5 H), 6.95 (br, s, 1 H), 4.67 (t, 1 H, *J* = 5.1 Hz), 3.95 (d, *J* = 13.5 Hz, 1 H), 3.78 (d, *J* = 13.5 Hz, 1 H), 3.41−3.37 (m, 2 H), 3.21−3.17 (m, 1 H), 2.94 (br, s, 1 H), 2.05−1.96 (m, 1 H), 1.86−1.58 (m, 3 H).^{13}C NMR (75 MHz/DMSO-d_6) δ 176.6, 137.8, 132.5, 132.0, 129.1, 127.5, 124.0, 67.2, 66.8, 62.7, 57.9, 29.2, 27.4; MS (ESI) *m*/*z* 313.3, 315.2 (M + 1, 50), 335.2, 337.2 (M + 23, 100). Anal. Calcd for $C_{13}H_{17}BrN_2O_2$: C, 49.85; H, 5.47; N, 8.94. Found: C, 49.79; H, 5.57; N, 8.58.

Synthesis of 14. Under argon protection, a mixture of **13** (157 mg, 0.5 mmol), CuI (38.2 mg, 0.2 mmol), *N*,*N*-dimethylglycine hydrochloric acid (DMGC) (56 mg, 0.4 mmol), and Cs_2CO_3 (326 mg, 1 mmol) in dry 1,4-dioxane (15 mL) was refluxed for 12 h. Water (10 mL) was added, and the resulting mixture was extracted with ethyl acetate (3 × 10 mL). The combined organic layer was dried with anhydrous sodium sulfate. The solvent was removed using a rotavapor, and the residue was chromatographed on a silica gel column eluted with ethyl acetate to produce **14** (101 mg, 87%): oil; $[\alpha]^{25}_D$ +22 (*c* 1.0, $CHCl_3$); IR (KBr) ν 3417, 1455, 1424 cm^{-1}; ^1H NMR (300 MHz/DMSO-d_6) δ 9.73 (s, 0.5 H), 7.18−6.96 (m, 4 H), 4.63 (t, 0.3 H, *J* = 5.1 Hz), 4.28 (d, *J* = 15.3 Hz, 1 H), 3.92 (d, *J* = 15.3 Hz, 1 H), 3.53−3.48 (m, 1 H), 3.39−3.28 (m 2 H), 2.77−2.72 (m, 1 H), 2.23−2.14 (m, 1 H), 1.81−1.76 (m, 2 H), 1.51−1.43 (m, 1 H). ^{13}C NMR (75 MHz/DMSO-d_6) δ 172.3, 136.9, 136.7, 129.3, 127.73, 127.68, 127.1, 123.0, 122.9, 120.8, 120.7, 67.0, 66.9, 65.8, 65.7, 63.8, 63.7, 56.9, 56.8, 27.3, 24.1; MS (EI) *m*/*z* 232 (M^+). Anal. Calcd for $C_{13}H_{16}N_2O_2$: 232.1212. Found: 232.1214.

Synthesis of 15. A mixture of **11** (1.53 g, 5 mmol) and $LiAlH_4$ (1.9 g, 50 mmol) in dry THF (50 mL) was refluxed for 24 h. After the mixture was cooled to room temperature, aqueous NaOH (2 N) was added, and the resulting mixture was filtrated through a Celite pad. The filter cake was washed thoroughly with a mixture of ethyl acetate and methanol (20:1). After removal of solvent, the residue was loaded to an ion-exchange column (Dowex, 50WX8 100−200 mesh) and eluted with pure water until the pH was around 7.0. Elution with ammonia solution (1−5%) gave product **15** (568 mg, 79%): oil; $[\alpha]^{25}_D$ −18 (*c* 1.0, $CHCl_3$); IR (KBr) ν 3403, 2952, 2875, 1637, 1569 cm^{-1}; ^1H NMR (300 MHz/$CDCl_3$) δ 3.64−3.59 (m, 1 H), 3.42−3.37 (m, 1 H), 2.77−2.64 (m, 2 H), 2.58−2.42 (m, 2 H), 2.30 (s, 3 H), 1.82−1.69 (m, 6 H), 1.59−1.50 (m, 1 H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 68.7, 67.5, 61.7, 44.5, 39.3, 27.2, 26.1; MS (ESI) *m*/*z* 145.1 (M + 1, 100). Anal. Calcd for $C_7H_{17}N_2O$: 145.1341. Found: 145.1336.

Synthesis of 16. To a solution of **15** (90 mg, 0.625 mmol) in dichloromethane (4 mL) were added 2-phenylacetaldehyde (72 mg, 0.6 mmol) and $NaBH_3CN$ (110 mg, 1.9 mmol), and the resulting mixture was stirred at room temperature for 24 h. The organic phase was washed with aqueous HCl (2 N) (3 × 5 mL), and product was transferred into aqueous solution. The combined aqueous solution was basified with $NaHCO_3$ to pH around 8.0, and was extracted with ethyl acetate (3 × 10 mL). After removal of solvent, the residue was subjected to a silica gel column coated with $C_{18}H_{38}$ using a mixture of methanol and water (4:6) as an eluent. Product **16** (84 mg, 54%) was obtained. **16**: oil; $[\alpha]^{25}_D$ −24.6 (*c* 1.4, $CHCl_3$); IR (KBr) ν 3420, 1628, 1605 cm^{-1}; ^1H NMR (300 MHz/$CDCl_3$) δ 7.32−7.19 (m, 5 H), 3.63−3.58 (m, 1 H), 3.39−3.35 (m, 1 H), 2.93−2.71 (m, 4 H), 2.64−2.54 (m, 3 H), 2.35−2.32 (m, 1 H), 2.28 (s, 3 H), 1.88−1.72 (m, 3 H), 1.59−1.50 (m, 1 H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 139.9, 128.7, 128.5, 126.2, 67.5, 66.6, 61.5, 53.2, 51.7, 39.7, 36.2; MS (ESI) *m*/*z* 249.5 (M + 1, 100). Anal. Calcd for $C_{15}H_{25}N_2O$: 249.1967. Found: 249.1962.

Synthesis of 17. A mixture of **16** (120 mg, 0.48 mmol), formaldehyde (35%, 1.2 mL), concentrated hydrochloric acid (2.4 mL), and chloroform (2.4 mL) was refluxed for 24 h. After being cooled to room temperature, the mixture was basified with saturated aqueous $NaHCO_3$ to pH 8.0 and extracted with dichloromethane (3 × 10 mL). The combined organic layer was dried with anhydrous sodium sulfate. After removal of solvent, the residue was chromatographed on a C-18 reversed-phase column eluted with a mixture of methanol and water (1:1) to yield product **17** (100 mg, 78%): oil; $[\alpha]^{25}_D$ −50.7 (*c* 1.5, $CHCl_3$); IR (KBr) ν 3424, 2943, 1641, 1457, 1036 cm^{-1}; ^1H NMR (300 MHz/DCl) δ 6.95−6.80 (m, 4 H), 4.31 (dd, *J* = 15.0, 7.8 Hz, 1 H), 4.11 (dd, *J* = 14.9, 5.4 Hz, 1 H), 3.79−3.73 (m, 1 H), 3.67−3.34 (m, 6 H), 3.24−3.13 (m, 1 H), 3.02−2.88 (m, 1 H), 2.81−2.68 (m, 1 H), 2.76 (s, 1.5 H), 2.74 (s, 1.5 H), 2.35−2.23 (m, 1 H), 2.02−1.90 (m, 1 H), 1.80−1.58 (m, 2 H); ^{13}C NMR (75 MHz/D_2O) δ133.6, 133.2, 128.7, 126.8, 126.7, 126.1, 68.7, 65.4, 61.9, 59.4, 55.3, 50.5, 39.7, 28.9, 27.1, 25.9; MS (ESI) *m*/*z* 261.4 (M + 1, 100). Anal. Calcd for $C_{16}H_{25}N_2O$: 261.1967. Found: 261.1961.

Synthesis of 18. A mixture of **11** (306 mg, 1 mmol), LiOH (80 mg, 2 mmol), THF (4 mL), and water (4 mL) was stirred at room temperature for 2 h. The mixture was acidified with hydrochloric acid (10%) to pH 2.0. THF was removed using a rotavapor, and white solids precipitated from solution. After the mixture was cooled in an ice−water bath, filtration and washing with cold water afforded pure product **18** (277 mg, 95%): mp 198−199 °C; IR (KBr) ν 3374, 1712 cm^{-1}; ^1H NMR (300 MHz/DMSO-d_6) δ 13.55 (br, s, 1 H), 7.88 (br, s, 0.5 H), 7.86 (br, s, 0.5 H), 7.60 (br, s, 0.5 H), 7.58 (br, s, 0.5 H), 7.39−7.33 (m, 5 H), 5.15−5.03 (m, 2 H), 4.41−4.24 (m, 2 H), 2.36−2.21 (m, 2 H), 1.92−1.85 (m, 2 H); ^{13}C NMR (75 MHz/DMSO-d_6) δ 175.3, 175.1, 174.9, 174.7, 153.6, 153.5, 136.41, 136.36, 128.3, 127.8, 127.1, 127.0, 66.51, 66.46, 61.5, 61.0, 60.5, 60.0, 30.2, 29.3, 28.2; MS (ESI) *m*/*z* 293.3 (M + 1, 10), 315.3 (M + 23, 100). Anal. Calcd for $C_{14}H_{16}N_2O_5$: C, 57.53; H, 5.52; N, 9.58. Found: C, 57.75; H, 5.51; N, 9.58.

Synthesis of 19. To a suspension of **18** (1.46 g, 5 mmol) in dichloromethane (10 mL) were added 2-chloro-4,6-dimethoxytriazine (CDMT) (1.05 g, 6 mmol) and *N*-methylmorpholine (NMM) (1.52 g, 15 mmol). After the mixture was stirred at room temperature for 1 h, tetrahydroisoquinoline (732 mg, 5.5 mmol) was added, and the resulting mixture was stirred overnight. The reaction was quenched by adding hydrochloric acid (1 N, 10 mL), and was extracted with dichloromethane (3 × 10 mL). The combined organic phase was dried with anhydrous sodium sulfate. After removal of the solvent, the residue was chromatographed on a silica gel column eluted with ethyl acetate to give product **19** (1.83 g, 90%) as a glassy solid: mp 77−78 °C; $[\alpha]^{25}_D$ +22.6 (*c* 0.26, $CHCl_3$); IR (KBr) ν 3421, 1579, 1446, 1410, 1106, 1057, 1034 cm^{-1}; ^1H NMR (300 MHz/DMSO-d_6, 363 K) δ 8.51 (br, s, 1 H), 7.32−7.17 (m, 9 H), 6.71 (br, s, 1 H), 5.09−4.91 (m, 3 H), 4.67 (br, s, 2 H), 4.20−4.16 (m, 1 H), 3.73−3.69 (m, 2 H), 2.80 (br, s, 2 H), 2.39−2.17 (m, 2 H), 2.01−1.76 (m, 2 H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 175.62, 175.16, 172.03, 171.87, 171.83, 154.44, 153.80, 136.06, 135.84, 135.61, 134.75, 134.67, 133.71, 133.62, 132.68, 132.49, 131.59, 131.45, 128.96, 128.51, 128.38, 128.32, 128.30, 128.18, 128.13, 128.08, 127.95, 127.79, 127.75, 127.72, 127.3, 126.9, 126.8, 126.6, 126.0, 125.96, 67.9, 67.7, 67.6, 62.2, 61.98, 58.9, 58.7, 58.2, 47.1, 47.0, 45.0, 44.8, 43.3, 43.1, 40.8, 30.8, 30.7, 30.1, 29.4, 29.1, 28.7, 28.6, 28.4, 28.2; MS (ESI) *m*/*z* 408.5 (M + 1, 60), 430.5 (M + 23, 100). Anal. Calcd for $C_{23}H_{26}N_3O_4$: 408.1923. Found: 408.1909.

Synthesis of 20. To a well-stirred solution of **19** (1.63 g, 4 mmol) in dry DMF at 0 °C was added dropwise $SOCl_2$ (1 mL). The reaction mixture was allowed to stire at room temperature for another 1 h. The reaction was quenched by adding water (20 mL) followed by extraction with ethyl acetate (3 × 20 mL). The organic layers were combined and washed with saturated aqueous $NaHCO_3$ (3 × 4 mL). After drying with anhydrous sodium sulfate and removal of solvent, the residue was chromatographed on a silica gel column eluted with a mixture of petroleum ether and ethyl acetate (1:1) to yield **20** (1.21 g, 78%): mp 175−176 °C; $[\alpha]^{25}_D$ −16.0 (*c* 1, $CHCl_3$); IR (KBr) ν 1712, 1644 cm^{-1}; ^1H NMR (300 MHz/DMSO-d_6, 375 K) δ 7.26−7.15 (m, 9 H), 5.07 (br, s, 2 H), 4.92 (dd, *J* = 7.5, 5.7 Hz, 1 H), 4.84 (dd, *J* = 7.4, 5.1 Hz, 1 H), 4.70 (d, *J* = 16.2 Hz, 1 H), 4.60 (d, *J* = 16.2 Hz, 1 H), 3.72−3.63 (m, 2 H), 2.80 (br, s, 2 H), 2.45−2.18 (m, 3 H), 1.98−1.93 (m, 1 H); ^{13}C NMR (75 MHz/$CDCl_3$) δ 169.53, 169.48, 153.4, 153.3, 135.7, 135.6, 135.5, 135.0, 134.9, 133.9, 133.7, 133.2, 133.1,

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131.9, 131.8, 129.1, 129.05, 128.6, 128.4, 128.3, 128.25, 128.17, 128.12, 127.97, 127.1, 126.8, 126.7, 126.5, 126.0, 125.9, 118.1, 117.7, 68.2, 67.9, 57.8, 57.5, 57.2, 48.2, 47.6, 47.2, 47.1, 44.7, 44.65, 43.3, 43.0, 40.4, 30.6, 30.5, 29.8, 29.6, 29.5, 29.2, 28.7, 28.6, 28.4, 28.3; MS (ESI) m/z 390.4 (M + 1, 50), 412.5 (M + 23, 100). Anal. Calcd for $C_{23}H_{23}N_3O_3$: C, 70.93; H, 5.95; N, 10.79. Found: C, 70.68; H, 5.90; N, 10.78.

Synthesis of 21. To a solution of **20** (155 mg, 0.4 mmol) in a mixture of dichloromethane (5 mL) and methanol (10 mL) at −10 °C was bubbled into dry HCl gas for 1 h, and the reaction mixture was kept at −20 °C overnight. The mixture was added dropwise into hydrochloric acid (6 N, 10 mL) at 0 °C, and then was neutralized with saturated aqueous $NaHCO_3$ solution to pH 7.0. After extraction with ethyl acetate (3 × 20 mL), drying with anhydrous sodium sulfate, and removal of solvent under vacuum, the residue was chromatographed on a silica gel column eluted with a mixture of petroleum ether and ethyl acetate (1:2) to furnish product **21** (86 mg, 51%): oil; $[\alpha]^{25}_D$ −34.0 (c 1, $CHCl_3$); IR (KBr) ν 1635, 1019 cm^{-1}; ^1H NMR (300 MHz/DMSO-d_6, 375 K) δ 7.34−7.13 (m, 9 H), 5.01 (s, 2 H), 4.89−4.84 (m, 1 H), 4.65 (s, 2 H), 4.46 (t, J = 6.72 Hz, 1 H), 3.73−3.57 (m, 2 H), 3.66 (s, 3 H), 2.95 (br, s, 1 H), 2.80−2.78 (m, 2 H), 2.30−2.12 (m, 3 H), 1.94−1.86 (m, 1 H); ^{13}C NMR (75 MHz/MeOD) δ 172.1, 171.8, 171.0, 170.8, 170.6, 154.8, 154.7, 136.3, 136.0, 135.8, 134.8, 134.6, 134.4, 134.2, 132.9, 132.8, 132.7, 132.6, 128.4, 128.3, 128.2, 128.1, 128.0, 127.9, 127.7, 127.6, 127.5, 127.45, 127.3, 126.6, 126.3, 126.2, 126.1, 125.84, 125.79, 67.4, 67.1, 60.2, 59.9, 58.7, 58.3, 57.9, 51.3, 51.2, 44.4, 44.3, 43.1, 42.8, 40.5, 29.3. 29.0, 28.9, 28.8, 28.6, 28.5, 28.2, 27.8, 27.75; MS (ESI) m/z 423.5 (M + 1, 25), 445.5 (M + 23, 100). Anal. Calcd for $C_{24}H_{27}N_2O_5$: 423.1920. Found: 423.1910.

Synthesis of 22. A mixture of **22** (42 mg, 0.1 mmol) and $LiAlH_4$ (60 mg, 1.58 mmol) in dry THF (4 mL) was refluxed for 24 h. After the mixture was cooled to room temperature, aqueous NaOH solution (2 N, 5 mL) was added, and the mixture was extracted with ethyl acetate (3 × 10 mL). The combined organic layer was dried with anhydrous sodium sulfate. After removal of solvent, the residue was chromatographed on a C-18 reversed-phase column eluted with a mixture of methanol and water (1:1) to afford product **22** (21 mg, 81%): $[\alpha]^{25}_D$ +54.7 (c 1.5, $CHCl_3$). Compound **22** gives spectroscopic data identical to those for **17**.

■ ASSOCIATED CONTENT

Supporting Information

Chiral HPLC analysis and ^1H and ^{13}C spectra of all products. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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