Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 3973–3979

BIOORGANIC & MEDICINAL CHEMISTRY

Stereoselectivity in Reactions of Amino Acids Catalyzed by Pyridoxal Derivatives Carrying Rigidly-Attached Chirally-Mounted Basic Groups—Transamination, Racemization, Decarboxylation, Retro-Aldol Reaction, and Aldol Condensation

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Received 26 February 2002; accepted 7 July 2002

Abstract—A tetrahydroquinoline ring was used to mount the critical functional groups of pyridoxal, and also two examples of rigidly held chirally mounted basic groups. They were able to selectively catalyze decarboxylation, aldol reaction, and retro-aldol reaction of amino acids rather than transamination, and with stereoselectivity. In the aldol reaction of glycine with acetaldehyde to synthesize threonine and allo-threonine, one of the catalysts reversed its stereoselectivity when the basic group was protonated. The observed stereoselectivities were all consistent with prediction.

Introduction

Pyridoxal 5-phosphate is a central coenzyme for amino acid metabolism in diverse biological systems.[1] The reactions it can catalyze include transamination, racemization, aldol and retroaldol reactions, and decarboxylation (Fig. 1). In all these reactions, the key step is the formation of a Schiff base between an amino acid and pyridoxal, whose pyridine nitrogen is then protonated. The protonated pyridine ring can serve as an electron sink and thereby activate the C–H or C–C bond on the α carbon of the amino acid. Loss of the α proton results in transamination if the 4′ carbon of pyridoxal is then protonated, or racemization if the α carbon is reprotonated from the opposite side. Cleavage of the C–C bond on the α carbon leads to retro-aldol reaction, where the leaving group is an aldehyde, or decarboxylation where the leaving group is carbon dioxide.[2]

The different reaction pathways use the same coenzyme and substrate, and the enzyme directs the selectivity among these possible paths. Thus treatment of amino acids with simple free pyridoxal, without the enzyme, always affords a variety of possible reaction products, among which the transamination product is the most prominent.[3] Since the transamination with an amino acid converts pyridoxal to pyridoxamine, which can no longer react with an amino acid,[4] transamination actually kills the catalyst and thereby suppresses all other pyridoxal-dependent reactions.

The fact that transamination severely suppresses other pyridoxal-dependent reactions proves to be a considerable problem in the construction of models of pyridoxal-dependent enzymes. In fact, most of the reported pyridoxal models were designed for transamination only,[5,6] whereas models for other pyridoxal-dependent reactions usually were found to be associated with very low catalytic turnovers and yields. Therefore, blocking the transamination process is important in order to develop effective pyridoxal catalysts for amino acid reactions other than transamination.

In a recent study we described a catalyst **1** with a rigidly mounted base designed to perform proton transfer at the C-α proton on the amino acid unit in a pyridoxal Schiff base, but in which we hoped to suppress proton transfer to the 4′ position of the pyridoxal unit, a process that kills the catalyst.[7] The preference was found to be as large as 11 times — **1** can selectively catalyze racemization of an amino acid in preference to transamination about 11 times better than does simple free

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0968-0896/02/$ - see front matter
PII: S0968-0896(02)00334-6

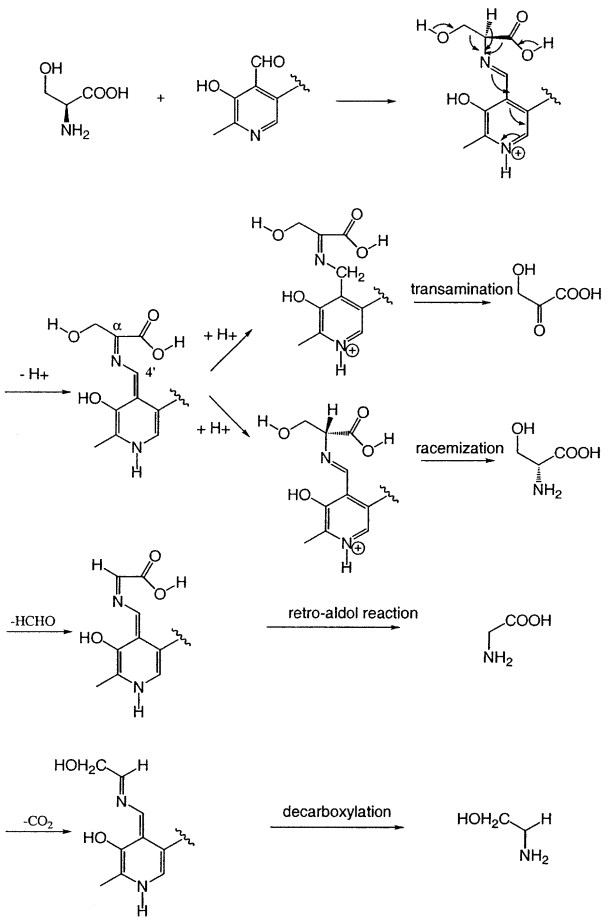
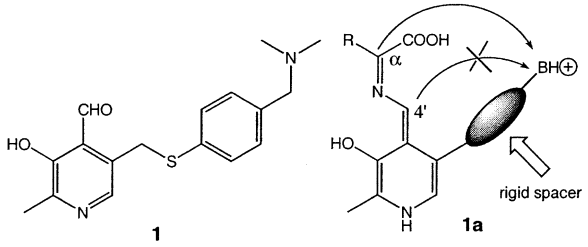
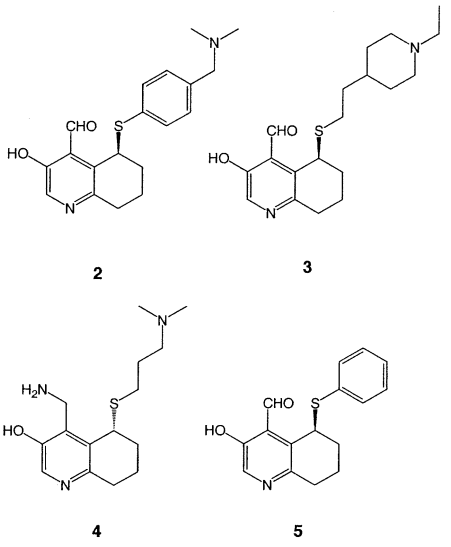


Figure 1. Serine can undergo transamination, racemization, the retro-aldol reaction, and decarboxylation catalyzed by pyridoxal and its derivatives.

pyridoxal. With the rigidly mounted base (**1a**) we thus achieved good regioselectivity in pyridoxal catalysis.



We wanted to know if the same rigid-base approach could be used to make pyridoxal catalysts for amino acids reactions other than racemization. These reactions include decarboxylation, aldol reaction and retro-aldol reaction. Since chiral amino acid products are usually formed in these reactions with enzymes, we also wanted to see if we could achieve some optical induction with our catalysts. Therefore, we designed the catalysts **2** and **3** in which rigid amine side chains are linked to a tetrahydroquinoline backbone. CPK models of **2** and **3** indicate that the basic side chain is able to reach only the α carbon, and also from only one face of the catalyst. We had made pyridoxamine catalyst **4** earlier, carrying a chirally-mounted flexible basic arm. It performed a stereoselective transamination, forming an amino acid as the product in 93:7 D to L ratio.[8]



Results and Discussion

Synthesis

The synthesis of the catalysts begins with glutaric anhydride. Using the previously published procedure,[8] we obtained the chiral alcohol **6** (over 99% pure in chirality as determined by proton NMR) in 3.1% yield over 13 steps. Then by attaching the appropriate amine side chain to **6** through *S*-alkylation, we were able to obtain the final chiral pyridoxal catalysts **2** and **3** in an overall yield of ca. 0.6 and 0.2% over 18 steps (Fig. 2). Compound **5** was also synthesized as a control.

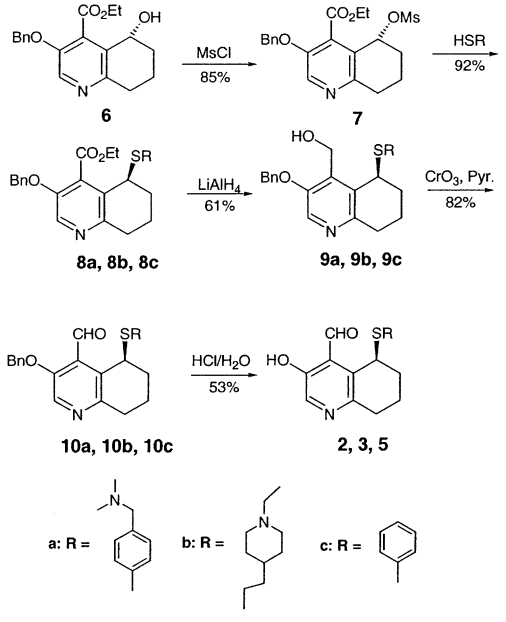


Figure 2. The synthesis of compounds **2**, **3**, and **5**.

Transamination and racemization

We examined transamination of L-alanine with our new catalysts, to see if the rigid geometry would suppress transamination. Therefore, we used the standard spectrophotometric method developed by Martell[9] to monitor the rate of aldimine to ketimine conversion. The results are summarized in Table 1.

As Table 1 shows, catalysts **1**, **2**, **3**, and **5** all have a smaller aldimine to ketimine transformation rate compared with pyridoxal. The rate suppression is not large, but at least the pyridoxal catalysts with rigid base chains do not show the 20∼80-fold rate enhancement for transamination that we observed previously when flexible base chains were used.[5] The rigid base chains cannot reach the 4′ carbon of the pyridoxal; they therefore cannot accelerate the proton shift between this carbon and the α carbon of the amino acid unit in the Schiff base, as required for transamination. The fact that **1**, **2**, **3**, and **5** have even smaller transamination rates than does pyridoxal may reflect a steric effect on proton transfer exerted by the side chains of **1**, **2**, **3**, and **5**.

Racemization of amino acid products by pyridoxal catalysts is another problem seen with the models of pyridoxal-enzyme when optical induction is one of the goals. In the present study, we used an HPLC method to examine the racemization activities of catalysts **1**, **2**, **3**, and **5** with L-alanine. The results are also shown in Table 1.

Catalyst **1** — whose basic group can remove and deliver protons on both faces of the amino acid — racemizes the amino acids 8.2 times as rapidly as does simple free pyridoxal. This current result agrees with our previous finding.[7] In comparison, catalysts **2**, **3**, and **5** do not have a significantly better racemization activity than does simple free pyridoxal. This is consistent with the CPK models indicating that the basic side chain of these catalysts cannot reach *both* faces of the Schiff base formed between the catalyst and an amino acid. The basic side chain does not increase the transamination or racemization activity since it can reach and protonate or deprotonate only the α carbon of the amino acid unit in the Schiff base, and on only one face.

Table 1. Transamination and racemization activities of the pyridoxal enzyme models with L-alanine

Compd	Transamination		Racemization	
	k_{trans} ($\times 10^{-5}$ min^{-1})[b]	$k_{relative}$	k_{rac} ($\times 10^{-2}$ min^{-1})[a]	$k_{relative}$
Pyridoxal	5.1±0.5	1.00	2.42±0.08	1.00
1	41.8±1.3	0.64	1.56±0.06	8.2
2	6.9±0.7	0.74	1.78±0.03	1.4
3	5.8±0.4	0.75	1.82±0.05	1.1
5	5.3±0.3	0.82	1.98±0.07	1.0

[a]Pseudo-first order rate constant for conversion of aldimine to ketimine in transamination at 60.0 °C in H_2O at pH 9.0. The reaction solution is 5.0×10^{-5} M in pyridoxal derivative, 5.0×10^{-5} M in $CuCl_2$, and 1.35 M in L-alanine.

[b]Pseudo-first order rate constant for conversion of L-alanine to D-alanine at 30.0 °C in ethanol/H_2O (7:3, v:v) at pH 9.0. The reaction solution is 1.0×10^{-3} M in pyridoxal derivative, 1.0×10^{-3} M in $CuCl_2$,and 1.0×10^{-2} M in L-alanine.

Decarboxylation

Decarboxylation of an amino acid is an important reaction, catalyzed by a pyridoxal-dependent decarboxylase, that affords an amine as the product.[10] We wanted to see if our chiral pyridoxal catalysts could achieve good optical induction in the synthesis of amines from amino acids. However, as found earlier in this lab, treatment of α-alkyl amino acids with pyridoxal affords only ketone and pyridoxamine as products by a transamination-dependent decarboxylation process.[11] Therefore, we studied the pyridoxal-dependent decarboxylation of an aminomalonic acid, a process that has been well investigated before with simple free pyridoxal.[12]

We used α-benzyl aminomalonic acid (**11**, Fig. 3). At pH 9.0 (0.02 M CHES buffer in 7:3/v:v water–ethanol solution), the spontaneous decarboxylation of α-benzyl aminomalonic acid to form phenylalanine **12** at 60.0 °C follows first-order kinetics with a rate constant of $(1.0\pm0.4)\times10^{-5}$ min^{-1} as determined by HPLC. Addition of a catalytic amount (0.2 equiv) of pyridoxal greatly increases the rate of decarboxylation. The pseudo-first order rate constant at 60.0 °C is $(7.3\pm0.6)\times10^{-4}$ min^{-1}, 72 times larger than the spontaneous decarboxylation rate constant.[13]

The other pyridoxal catalysts, **1**, **2**, **3**, and **5** (0.2 equivalents) also catalyze the decarboxylation of α-benzyl aminomalonic acid **11**. The corresponding pseudo-first order rate constants are $(6.8\pm0.5)\times10^{-4}$ min^{-1}, $(5.2\pm0.3)\times10^{-4}$ min^{-1}, $(4.9\pm0.7)\times10^{-4}$ min^{-1}, and $(5.6\pm0.3)\times10^{-4}$ min^{-1}, respectively. These values are not significantly different from that for simple free pyridoxal, presumably because the rate-determining step in decarboxylation, the breaking of the C–C bond, is not significantly affected by the basic side chains.

Examination of the amount of L- and D-phenylalanine produced from the catalyzed decarboxylation of **11** reveals the stereoselectivity of the reaction. Unfortunately, the ee% value we found for the decarboxylation with catalyst **2** is only (22±5)% at pH 9.0, L-phenylalanine **12** being excess.[14] At pH 8.0, the ee% value with catalyst **2** is a little higher (42±6)% (L). The fact that L-phenylalanine is the favored product is in agreement with the proposed mechanism (Fig. 3), in which the basic side chain is indeed able to deliver a proton to the decarboxylation intermediate in a stereoselective fashion. Intermolecular protonation may be competing.

Catalyst **3** shows even poorer optical induction in decarboxylation of **11**. The ee% value at pH 9.0 is lower than 10% and hard to measure. At pH 8.0 the ee% value is measurable, 17±4%. In comparison, the ee% value measured with catalyst **1** or **5** is undetectable, certainly smaller than 10%. Catalyst **1** is of course achiral and must produce a racemate, and catalyst **5** apparently does not use a simple steric effect to induce chiral protonation from the solvent.

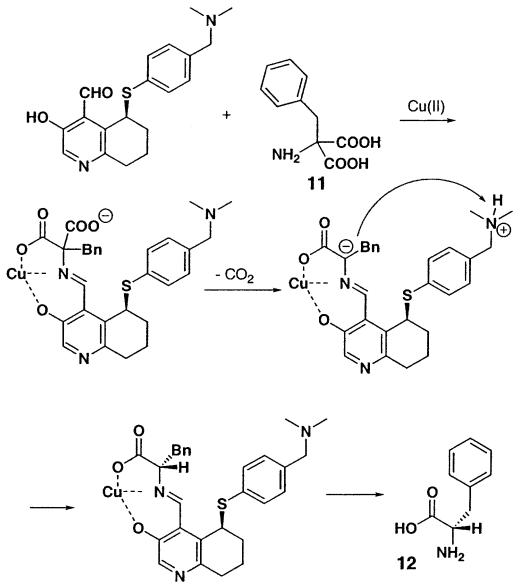


Figure 3. Stereoselective formation of phenylalanine **12** by decarboxylation of **11** with catalyst **2**.

Retro-aldol reaction

Retro-aldol reaction of serine or its derivatives catalyzed by pyridoxal shares the same mechanism for pyridoxal-dependent decarboxylation except that the leaving species is an aldehyde instead of carbon dioxide. Non-enzymatic retro-aldol reaction with pyridoxal has been well studied before.[15] Here we are mainly interested to see if our catalysts could achieve any optical induction in the process.

We used 1-methyl-2-phenylserine **13**. Treatment of this compound with 0.20 equivalents of pyridoxal yields L- and D-alanine as the products, along with benzaldehyde (Fig. 4). The pseudo-first order rate constant is $(1.4\pm0.3)\times10^{-4}$ min^{-1} at pH 7.5.[16] In comparison, the pseudo-first order rate constants for catalysts **1**, **2**, **3**, and **5** under the same reaction condition are $(1.2\pm0.1)\times10^{-4}$ min^{-1}, $(9.0\pm1.2)\times10^{-5}$ min^{-1}, $(7.4\pm0.9)\times10^{-5}$ min^{-1}, and $(1.1\pm0.3)\times10^{-4}$ min^{-1}. These values are not significantly different from that for simple free pyridoxal; apparently the rate-determining breaking of the C–C bond is not catalyzed by the (protonated) base groups.

Examination of the L- and D-alanine production of this retro-aldol reaction reveals that catalyst **2** gives an ee% value of 59 ± 3 at pH 7.5, L-alanine being the favored product. Catalyst **3** gives a lower ee% value, 36 ± 4 under the same reaction condition. It should be mentioned that with catalyst **1** or **5**, negligible ee% values

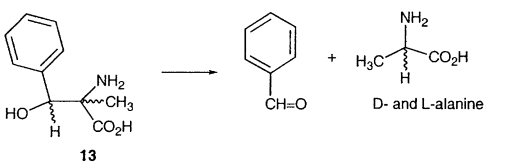


Figure 4. The retro-aldol reaction of **13** to afford L- and D-alanine and benzaldehyde.

are seen for this reaction. Therefore, the fact that catalyst **2** and **3** can lead to some extent of optical selectivity shows the ability of their chirally-mounted (protonated) basic side chains to transfer protons. However, the ee% values obtained are not very high; improving the induced chirality is a goal for future work.

Aldol reaction

Pyridoxal-catalyzed aldol reaction of an amino acid with an aldehyde or ketone is biologically important, as it affords new C–C bonds. This reaction occurs not only with enzymes, but also in simple model systems. For example, treatment of a mixture of glycine and acetaldehyde with simple free pyridoxal gives significant amount of threonine and *allo*-threonine as the products.[17]

Geometric control of a pyridoxal-catalyzed aldol condensation is an interesting target in pyridoxal model studies. For instance, Kuzuhara et al. synthesized a chiral pyridoxal-like pyridinophane with an 'ansa chain' across the face of the pyridine ring. The ee% values they achieved with this catalyst in threonine and *allo*-threonine synthesis were about 70 and 45.[18] Murakami et al., on the other hand, used pyridoxal imbedded in chiral micelles. They obtained ee% values of about 20–50 in the aldol condensation of glycine with benzaldehyde.[19] We also synthesized a chiral cyclophane derivative of pyridoxal that has amino groups oriented specifically over one face of the cofactor.[20] The ee% values we obtained for glycine-acetaldehyde condensation ranged from 20 to 70. An interesting dependence of enantioinduction on pH, that is reversal of the optical selectivity between low pH and high pH, was also observed in the study.[20]

We have now used chiral catalysts **2**, **3**, and **5** in an aldol condensation, the synthesis of threonine and allo-threonine from glycine and acetaldehyde. The results are summarized in Table 2. From the results, it can be seen that for most of the cases L-threonine and L-allo-threonine are the favored product over their D-enantiomers. The highest ee% value is 37.3%, which corresponds to a ratio of 2.2:1 in L-to D-enantiomer. The origin of the selectivity in favor of the L-enantiomer can be attributed to the steric effects exerted by the side chain, as shown in Figure 5. The side chain blocks one of the sides of the Schiff base from attack by acetaldehyde.

Interestingly, it was observed that at low pH (Table 2), catalyst **2** gave D-threonine and D-*allo*-threonine as the favored products. The highest ee% value here is 62.9%, which corresponds to a ratio of 4.4:1 for D- versus L-enantiomers. The basic side chain of **2** is protonated under the acidic reaction conditions, but not at high pH; the ammonium ion of **2** can interact with acetaldehyde, resulting in the stereochemistry observed (Fig. 5). The same pH effect was not observed for catalyst **3** or **5**. Of course, with **5**, only the steric blocking that led to the formation of the L enantiomers is possible. The more basic **3** is protonated at all the pH's of Table 2, but the BH+ is not as acidic as with **2**, and more crowded. Thus it cannot hydrogen bond to the acetaldehyde and steer the reaction to the hindered face.

Table 2. Enantioinductions in the catalyzed synthesis of threonine and *allo*-threonine from glycine and acetaldehyde[a]

Catalyst	ee%	pH = 9.0	pH = 8.0	pH = 7.0	pH = 6.0	pH = 5.0	pH = 4.0
2	Threonine	37.3 L	25.2 L	12.1 L	13.6 D	48.4 D	62.9 D
	allo-Threonine	22.6 L	16.7 L	8.4 L	—[b]	27.3 D	43.5 D
3	Threonine	26.4 L	18.3 L	10.0 L	—[b]	—[b]	—[b]
	allo-Threonine	19.8 L	12.7 L	7.1 L	—[b]	—[b]	—[b]
5	Threonine	17.0 L	9.5	—[b]	—[b]	—[b]	—[b]
	allo-Threonine	13.9 L	—[b]	—[b]	—[b]	—[b]	—[b]

[a]Reactions were performed at 40 °C in 7:3/v:v ethanol/water. The pH of the reaction solution was adjusted with HCl/NaOH. The solution was 0.0025 M in glycine, 0.0025 M in the catalyst, 0.020 M in acetaldehyde, and 0.0025 M in $CuCl_2$. Conversion of the reaction was set as 10% to avoid equilibration.
[b]The ee% value was too small to be accurately measured.

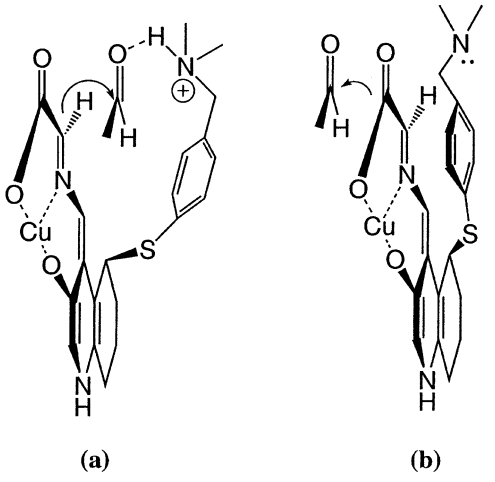


Figure 5. The aldol reaction between glycine and acetaldehyde catalyzed by catalyst **2** affords an excess of D-threonine and D-allo-threonine at low pH (a), but L-threonine and L-allo-threonine at higher pH (b).

Conclusions

Replacing the flexible chirally-mounted basic group of our previous catalyst **4** with the rigid chirally-mounted bases in catalysts **2** and **3** has steered the reactions of amino acids away from transamination or racemization, and toward decarboxylation, retroaldol reaction, and aldol reaction. Furthermore, the chirally mounted rigid bases are able to protonate the newly created amino acid α-carbon chiral centers with some enantioselectivity. In the aldol condensation of glycine with acetaldehyde the rigid sidechains of catalysts **2**, **3**, and **5** steer the acetaldehyde to the unhindered face of the glycine anion, but when **2** is protonated it uses that proton to reverse the stereochemical preference. The trends are all reasonable, and the need is simply for even larger enantiomeric excesses in order that these be useful synthetic procedures.

Experimental

General

Solvents, inorganic salts, and organic reagents were purchased from commercial sources and used without further purification unless otherwise mentioned. Merck pre-coated 0.25 mm silica plates containing a 254 nm fluorescence indicator were used for analytical thin-layer chromatography. Flash chromatography was performed on 230–400 mesh silica (Silica Gel 60) from EM Science. Analytical HPLC was run on a HP1090 liquid chromatography (series II) equipped with a DR5 pumping system, a temperature controlled autosampler, and a diode-array UV–vis detector. Rainin Microsorb™ C-18 reverse-phase analytical columns (particle size 5 μm, 4.6×150 mm) were used as solid phase. NMR spectra were obtained on a Bruker DPX 300 or 400 spectrometer. UV–vis spectra were taken on a Varian Cary IE UV–vis spectrometer. CI MS spectra were taken on a Nermag R-10-10 instrument.

Kinetics

The rate of transamination reaction was monitored with a UV–vis instrument by following the decrease of absorption at 400 nm. The rates of racemization, decarboxylation, retro-aldol reaction, and aldol reaction were monitored with HPLC by detecting the formation of the amino acid product. For this purpose, a derivatizing solution (OPA/NBC) of 0.2 M *o*-phthalaldehyde and 0.2 M *N*-Boc-cysteine in methanol as well as an aqueous buffer solution (KHP) of 1.0 M pH 8.0 K_2HPO_4 were prepared. The amino acid solutions were derivatized in the autosampler of HPLC by drawing 3 μL KHP, 3 μL OPA/NBC, 12 μL reaction mixture, and 1.5 μL air into the sample loop and mixing 19.5 μL for 20 cycles. A mixture of 55% methanol and 45% acetate buffer (0.2 M, pH 4.5) was used as eluent. The reaction was monitored at 344 nm, bandwidth 4 with the baseline taken at 450 nm, bandwidth 40. The retention time is 30.2 min for L-alanine, and 37.9 min for D-alanine. For aldol reactions, because there are four amino acid products in total, we chose to use *N*-acetylcysteine instead of *N*-Boc-cysteine to achieve a better separation. The retention times are 16.1 min for D-threonine, 17.5 min for L-threonine, 18.6 min for glycine, 29.4 min for D-allo-thronine, and 31.2 min for L-allo-threonine.

3-Benzyloxy-5-(4-dimethylaminomethyl-phenylsulfanyl)-5,6,7,8-tetrahydro-quinoline-4-carboxylic acid ethyl ester (8a). 100 mg of 3-benzyloxy-5-methane-sulfonyloxy-5,6,7,8-tetrahydro-quinoline-4-carboxylic acid ethyl ester (**7**) was dissolved in 10 mL of methanol, to which 100 mg of 4-dimethylaminomethyl-benzenethiol was added. After addition of 1 mL of triethylamine, the reaction mixture was stirred at room temperature under argon for 4 h. The solution was then concentrated in vacuo, and the remaining oil was purified by silica flash

chromatography (10–30% MeOH/$CHCl_3$). ^1H NMR ($CDCl_3$, 300 MHz) 8.30 (1H, s), 7.40 (9H, m), 5.30 (2H, s), 4.70 (m, 1H), 4.40 (2H, m), 3.45 (2H, s), 1.80–3.10 (12H, m), 1.30 (3H, s). CI MS 477 (M + 1).

[3-Benzyloxy-5-(4-dimethylaminomethyl-phenylsulfanyl)-5,6,7,8-tetrahydro-quinoline-4-yl]methanol (9a). 29 mg of **8a** was dissolved in 2 mL of dry ether. 10 of mg of $LiAlH_4$ was then added at 0 °C. The reaction mixture was stirred under argon at room temperature for 50 min, which was quenched by addition of 2 mL of ethyl acetate. The mixture was extracted with 10 mL of ethyl acetate twice, and the extracts were concentrated in vacuo. The product was purified by silica flash chromatography (10–50% MeOH/$CHCl_3$). ^1H NMR ($CDCl_3$) 8.30 (1H, s), 7.40 (9H, m), 5.22 (2H, d), 5.00 (2H, m), 4.70 (1H, m), 3.45 (2H, s), 1.80–3.10 (12H, m). CI MS 435 (M + 1).

3-Benzyloxy-5-(4-dimethylaminomethyl-phenylsulfanyl)-5,6,7,8-tetrahydro-quinoline-4-carbaldehyde (10a). 0.1 mL of pyridine was dissolved in 2 mL of dry methylene chloride and 60 mg CrO_3 was added to the solution. The resulting slurry was stirred for 60 min at room temperature. This slurry was then treated with 43 mg of **9a** dissolved in 2 mL of CH_2Cl_2. The reaction mixture was stirred for 15 min, after which the slurry was passed through a small column of Celite. No further purification was conducted on this compound as it decomposes quickly in air. CI MS: 433 (M + 1).

5-(4-dimethylaminomethyl-phenylsulfanyl)-3-hydroxy-5,6,7,8-tetrahydro-quinoline-4-carbaldehyde (2). 20 mg of **10a** was dissolved in 4 mL of 18% HCl aqueous solution. The reaction was refluxed at 150 °C under argon for 1.5 h. The reaction mixture was then cooled to room temperature, neutralized with $NaHCO_3$ aqueous solution, and extracted with ethyl acetate twice. The extracts were concentrated in vacuo, and the remaining oil was purified by silica flash chromatography (10–50% MeOH/$CHCl_3$). The final product is a yellow solid. ^1H NMR (CD_3CN): 10.40 (1H, s), 7.95 (1H, s), 7.46 (2H, d, $J = 8.1$ Hz), 7.28 (2H, d, $J = 8.1$ Hz), 4.65 (1H, m), 3.38 (2H, s), 2.60 (3H, m), 2.18 (6H, s), 1.78 (3H, m). CI MS: 343 (M + 1).

(3). With a similar route, we obtained **3** as a yellow solid. ^1H NMR (CD_3CN) 10.60 (1H, s), 8.32 (1H, s), 4.75 (1H, m), 3.05–1.30 (27H, m). CI MS: 349 (M + 1).

(5). Similarly to **2** and **3**, **5** was obtained as a yellowish brown solid. ^1H NMR (CD_3CN): 10.53 (1H, s), 8.32 (1H, s), 7.40 (5H, m), 4.71 (1H, m), 2.70–1.65 (6H, m). CI MS: 286 (M + 1).

Benzyl aminomalonic acid (11). This compound was synthesized from diethyl aminomalonate, which was protected with BOC anhydride (94% yield), alkylated with benzyl bromide (92% yield), deprotected with trifluoroacetic acid (quantitative), and finally hydrolyzed with stoichiometric sodium hydroxide (quantitative). The product was purified with reverse phase chromatography and obtained as a white solid. ^1H NMR (D_2O): 7.30 (5H, m), 3.15 (2H, s). ^{13}C NMR (D_2O): 180, 138, 131, 129, 127, 69, 42. MS FAB+ (glycerol): 254 (M + 1), 277 (M + 23).

1-Methyl-2-phenyl-serine (13). This compound, as a mixture of diasteromers, was synthesized from ethyl benzoylacetate, which was mono-alkylated with methyl iodide (86% yield), and then brominated with bromine (quantitative). The bromide was replaced by azide (97% yield), and the azide was reduced with triphenylphosphine to amine (75% yield). The ketone group in the compound was then reduced with sodium borohydride (quantitative), and the final product was obtained from hydrolysis with stoichiometric sodium hydroxide (quantitative). This product was purified with reverse phase chromatography and obtained as a white solid. ^1H NMR (D_2O) 7.25 (5H, m), 4.85–5.00 (1H, ss), 1.10–1.60 (3H, ss). MS FAB+ (glycerol): 218 (M + 23). MS FAB−(glycerol): 194 (M−1).

References and Notes

1. (a) John, R. A. *Biochim. Biophys. Acta* **1995**, *1248*, 81. (b) Hayashi, H. *J. Biochem.* **1995**, *118*, 463. (c) Schneider, G.; Kack, H.; Lindqvist, Y. *Structure* **1998**, *8*, R1.
2. Martell, A. E. *Acc. Chem. Res.* **1989**, *22*, 115.
3. Metzler, D. E.; Snell, E. E. *J. Am. Chem. Soc.* **1952**, *74*, 979.
4. Of course pyridoxamine can then react with an α-keto acid to regenerate pyridoxal via transamination. However, in aqueous solution excessive amounts of keto acid must be added in order to achieve transamination with simple free pyridoxamine. No keto acids were added in our studies reported here.
5. (a) Breslow, R.; Hammond, M.; Lauer, M. *J. Am. Chem. Soc.* **1980**, *102*, 421. (b) Zimmerman, S. C.; Czarnik, A. W.; Breslow, R. *J. Am. Chem. Soc.* **1983**, *105*, 1694. (c) Breslow, R.; Czarnik, A. W.; Lauer, M.; Leppkes, R.; Winkler, J.; Zimmerman, S. *J. Am. Chem. Soc.* **1986**, *108*, 1969. (d) Breslow, R.; Chmielewski, J.; Foley, D.; Johnson, B.; Kumabe, N.; Varney, M.; Mehra, R. *Tetrahedron* **1988**, *44*, 5515. (f) Breslow, R.; Canary, J. W.; Varney, M.; Waddell, S. T.; Yang, D. *J. Am. Chem. Soc.* **1990**, *112*, 5212. (g) Fasella, E.; Dong, S. D.; Breslow, R. *Bioorg. Med. Chem.* **1999**, *7*, 709.
6. (a) Kikuchi, J.-I.; Zhang, Z.-Y.; Murakami, Y. *J. Am. Chem. Soc.* **1995**, *117*, 5383. (b) Kuang, H.; Distefano, M. D. *J. Am. Chem. Soc.* **1998**, *120*, 1072. (c) Shogren-Knaak, M. A.; Imperiali, B. *Bioorg. Med. Chem.* **1998**, *7*, 1993.
7. Liu, L.; Breslow, R. *Tetrahedron Lett.* **2001**, *42*, 2775.
8. Zimmerman, S. C.; Breslow, R. *J. Am. Chem. Soc.* **1984**, *106*, 1490.
9. Matsushima, Y.; Martell, A. E. *J. Am. Chem. Soc.* **1967**, *89*, 1331.
10. Kalyankar, G. D.; Snell, E. E. *Biochemistry* **1962**, *1*, 594.
11. Koh, J. PhD thesis, Department of Chemistry. Columbia University, 1994.
12. (a) Thanassi, J. W. *Biochemistry* **1970**, *9*, 525. (a) Thanassi, J. W. *Biochemistry* **1971**, *11*, 2909. (a) Thanassi, J. W. *Biochemistry* **1973**, *12*, 5109.
13. The detailed reaction conditions: pH 9.0, 60.0 °C, 0.0015 M in α-benzyl aminomalonic acid **11**, 0.0003 M in pyridoxal or its analogue, 0.0003 M in $CuCl_2$, and 0.02 M in CHES buffer (ethanol/water = 7/3). We conducted the decarboxylation reaction under basic conditions, under acidic conditions the reaction intermediate undergoes C-4′ protonation, affording pyridoxamine and keto acid as products. For details, see Zabinski, R. F.; Toney, M. D. *J. Am. Chem. Soc.* **2001**, *123*, 193.

14. Measurement of ee% was conducted after 2% conversion. Increasing the reaction time lowers the ee% value because of racemization.
15. (a) Metzler, D. E.; Snell, E. E. *J. Biol. Chem.* **1952**, *198*, 353. (b) Marcello, J. A.; Martell, A. E.; Abbot, E. H. *J. Chem. Soc. Chem. Commun.* **1975**, 16. (c) Tatsumoto, K.; Martell, A. E. *J. Am. Chem. Soc.* **1978**, *100*, 5549. (d) Marcello, J. A.; Martell, A. E. *J. Am. Chem. Soc.* **1982**, *104*, 1087.
16. The reaction conditions: 40.0 °C, pH 7.5, 0.005 M in 1-methyl-2-phenyl-serine 13, 0.001M in the catalyst, 0.001M in $CuCl_2$. The solvent is 7:3/v:v water/ethanol. The pH of the reaction solution was adjusted with HCl/NaOH.
17. Metzler, D. E.; Longnecker, J. B.; Snell, E. E. *J. Am. Chem. Soc.* **1953**, *75*, 2786.
18. (a) Kuzuhara, H.; Watanabe, N.; Ando, M. *J. Chem. Soc. Chem. Commun.* **1987**, 95. (b) Ando, M.; Watanabe, J.; Kuzuhara, H. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 88.
19. Murakami, Y.; Kikuchi, J.-I.; Miyajima, T.; Hisaeda, Y. *Chem. Lett.* **1994**, 55.
20. Koh, J. T.; Delaude, L.; Breslow, R. *J. Am. Chem. Soc.* **1994**, *116*, 1123.