Geometric Control of a Pyridoxal-Catalyzed Aldol Condensation†

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Abstract: A chiral cyclophane derivative of pyridoxal has been synthesized that has amino groups oriented specifically over one face of the cofactor. The compound catalyzes the formation of threonine and *allo*-threonine from glycine and acetaldehyde with enantioinductions that are a function of pH, reversing the optical selectivity between low pH and high pH. The stereochemical results are compared with those of structurally related pyridoxal cyclophanes that lack titratable catalytic groups. Explanations are advanced for this stereochemical reversal and for the otherwise surprising preference of most of these compounds to react on the more hindered face of the pyridoxal. Models indicate that the transamination intermediate is distorted by the transannular chain, and stereoelectronic arguments predict that this distortion should lead to reaction on the face that carries the chain, as observed. The stereochemical reversal with the attached (dimethylamino)alkyl group, as a function of pH, may reflect catalysis by the protonated form, but metal coordination by the basic form cannot be excluded.

Introduction

Pyridoxal phosphate and pyridoxamine phosphate are cofactors for myriad biochemical reactions involving amino acids.[1] For example, with amino acid substrates pyridoxal phosphate can participate in transamination, racemization, decarboxylation, α,β-elimination, and forward and reverse aldol condensations. Such reactions have been mimicked in model systems, but usually without the selectivity that enzymes impart.

We have described pyridoxal/pyridoxamine model systems in which the attachment of a hydrophobic binding group promoted selectivity for amino acids with hydrophobic side chains, such as phenyl or indole groups.[2-4] We have also focussed on the incorporation of basic sidearms to catalyze the proton transfers needed in transamination, and have mounted them asymmetrically so as to favor the production of one enantiomer of the amino acid produced by transamination.[3] With a rigidly mounted basic group we have been able to select in favor of racemization relative to transamination in another system.[5]

In a different approach to chiral induction during transamination, Kuzuhara synthesized an optically resolved pyridoxamine with an "ansa chain" across the face of the pyridine ring.[6] With the five-carbon chain in **1a**, the two isomers do not interconvert readily, although with one more carbon in the chain it can pass around the pyridine ring, racemizing the compound. Kuzuhara found that transamination by **1a** led to chiral induction in the product amino acids.

The results were surprising.[7] With a metal ion such as Zn^{2+}, the initial Schiff base with a keto acid will have structure **2**, and the amino acid stereochemistry in transamination (Figure 1) is set by the protonation of intermediate **3** on one face or the other. Kuzuhara found that preferential protonation occurred from the *same* face on which the ansa chain was located, contrary to simple ideas about steric hindrance to solvent proton approach. He also found that the enantioselectivity was greater with 0.5 equiv of Zn(II), not 1.0 equiv, so he proposed that a *dimer* was involved, with two pyridoxamine derivatives coordinated to the same Zn(II) and held near each other's unhindered face.[7] By this proposal protonation occurs from the face carrying the ansa chain since that face is not blocked by the other pyridoxamine species. We will return to this matter later and propose alternative explanations of this surprising result.

It seemed to us that such an ansa chain would be an ideal place on which to mount a basic sidearm, since it would then originate on the face of the pyridoxamine system, where the proton transfers must occur. In early unpublished work Chmielewski[8] and Paik[9] did synthesize some derivatives of **1a** carrying flexible basic groups and saw useful rate accelerations of transaminations. However, chiral induction was not examined. In this paper we will describe the synthesis of **4**, an optically resolved derivative of **1b** carrying basic catalytic groups on relatively rigid spacer arms. It catalyzes the aldol condensation of glycine and acetaldehyde to afford threonine and *allo*-threonine with an optical selectivity whose direction is a function of pH. At one pH there is selectivity because of effective steric blocking, while at another pH the selectivity is reversed, probably because of active catalysis by the side chain function.

The pyridoxal-catalyzed condensation of glycine with acetaldehyde to form threonine and *allo*-threonine—and the corresponding retroaldol reaction—has been examined previously by Metzler and Snell,[10,11] by Martell,[12,13] and by Kuzuhara,[14,15]

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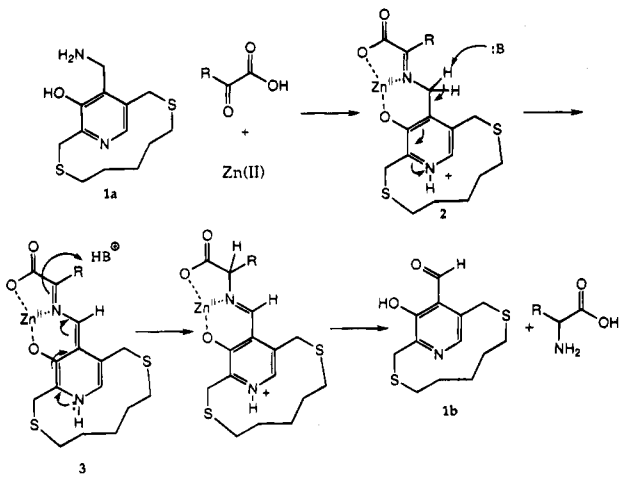

Figure 1. Conversion of a keto acid to an amino acid by pyridoxamine analog **1a**, which is converted to pyridoxal derivative **1b**.

the latter with some chiral induction, as will be discussed below. Condensations and their reversal with other aldehydes have also been examined by Metzler and Snell,[16] by Martell,[12,17] and by Murakami,[18] who induced chiral condensation of glycine with benzaldehyde in some micellar processes. These reactions are directly analogous to forward and reverse aldol condensations with glycine catalyzed by enzymes that use pyridoxal phosphate.[19]

Results and Discussion

The synthesis of **4** is outlined in Scheme 1. The dichloride **5**[6] was converted to **7** by reaction with the dithiolate ion generated from intermediate **8**. Then **7** was deprotected and oxidized to the pyridoxal **4**. The optical resolution was performed on intermediate **7**, using chiral HPLC on a Chiralcel OD column.[20] With repeated injections, 60−80 mg of resolved **7** was obtained as two fractions, one of them (−) 99.6% ee and the other (+) 92.0% ee. The purer (−) isomer was used for all subsequent chiral studies.

The absolute configuration of compound **9** had been determined by anomalous X-ray scattering methods previously,[21] so we related our resolved **7** to **9**. Various attempts to replace the thioketal group in **7** with hydrogen atoms were unsuccessful, but we were able to hydrolyze the thioketal of **7** to afford ketone **10** and reduce this to a mixture of diastereomeric alcohols **11a,b** (Scheme 2). We also resolved acetonide **12** and saw that its R enantiomer had essentially identical but opposite circular dichroism to that of our pure (−) enantiomer of thioketal **7**, our ketone **10**, and both diastereomeric alcohols **11a** and **11b** (Figure 2). Apparently the CD spectra are dominated by the interaction of the pyridine ring with the ansa chain—probably due to distortion of the pyridine ring out of planarity or an influence of the sulfur atoms that link the chain to the ring—and are not significantly affected by the substituents at the middle of the chain of **7**, **10**, or **11**.

Because the enantiomer of **12** that we examined could be hydrolyzed to (+)-**9**, that was known to have the *R* configuration, this means that our pure (−) enantiomer of **7** has the *S* configuration depicted in Figure 3. As a control compound, we also synthesized and resolved **14**, which lacks the dimethylamino groups. The acetonide intermediate **15** was resolved on a Chiralcel OD column;[20] its (−) isomer had a CD essentially identical to that of (−)-**7**, and opposite to that of (+)-**12**. Again we carried the *S* isomer through to the pyridoxal derivative **14**.

We used these pyridoxal derivatives to catalyze the condensation of glycine with acetaldehyde, forming threonine and *allo*-threonine (Figure 4). The enantiomers of threonine and *allo*-threonine were resolved and quantified by a modified method of that used by Nimura[22] and Buck,[23] forming diastereomeric isoindoles of the amino acids with *o*-phthalaldehyde and chiral thiols. Although the use of *N*-Boc-cysteine as a chiral thiol was found to be very efficient at resolving the enantiomers of both threonine and *allo*-threonine, the substrate glycine was found to interfere with this analysis. Derivatives made with *N*-acetylcysteine do not resolve the threonine isomers as efficiently but the overall method was found to give more consistent results than with *N*-Boc-cysteine derivatives.

Two general procedures were used. One involves the direct treatment of the reaction solution with the derivatizing agent, and the other first treats the reaction mixture with 0.1 N aqueous HCl prior to derivatization. The acid pretreatment method was found to give better results with reactions conducted under basic conditions where the solutions were sometimes found to be slightly turbid. Direct derivatization was generally used for reactions run at pH 7.0 or lower and was found to give sharper peaks. It should be stressed that the two methods gave the same results for the same reaction mixtures.

Reactions were conducted in 1:1 methanol/water with buffer with 1 equiv of catalyst, 1 equiv of glycine, 10 equiv of acetaldehyde, and 3.5 equiv of $Zn(NO_3)_2$. The pH values cited in the text refer to the pH of the buffer used in the reaction mixture. Conversions were taken to less than 15%, to avoid equilibration. The results are listed in Table 1.

As that table shows, with our catalyst **4** there is a complete reversal of optical selectivity as a function of pH. At high pH (10.0) the (*S*)-**4** produces *d*-threonine and *d-allo*-threonine, while at low pH (5.0) there is a strong preference for the formation of the *l* isomers. This is not seen with the Kuzuhara compound **1b**, not surprising since it has no titratable catalytic group on the face of the pyridine ring as **4** does.

Kuzuhara had reported that the aldol condensation of glycine with acetaldehyde catalyzed by **1b** occurred on the same face as was occupied by the ansa chain,[14,15] just as protonation at that carbon had occurred in the transamination process; our results confirm this for **1b**. We also see it for our compound **14** with an even bulkier ansa chain but without catalytic groups as are in **4**. Thus two points need explanation. (1) Why do catalysts **1b** and **14** direct the aldol condensations on the same face as is occupied by the ansa chain, even the very bulky ansa chain in **14**? (2) Why does catalyst **4** direct the condensation to the *opposite* face as carries the ansa chain at high pH and switch at low pH?

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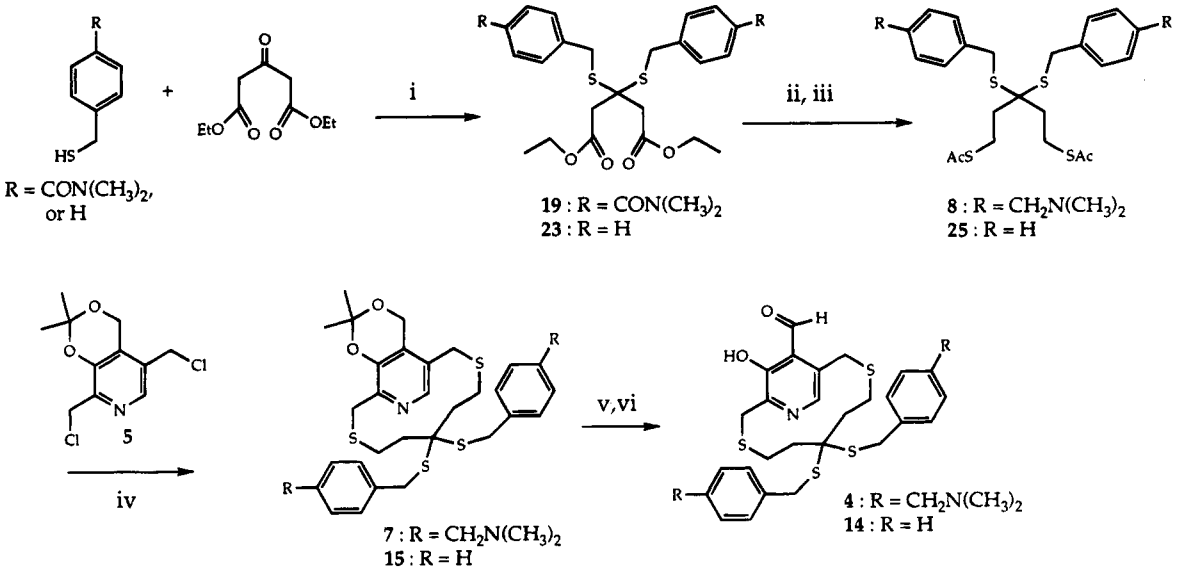
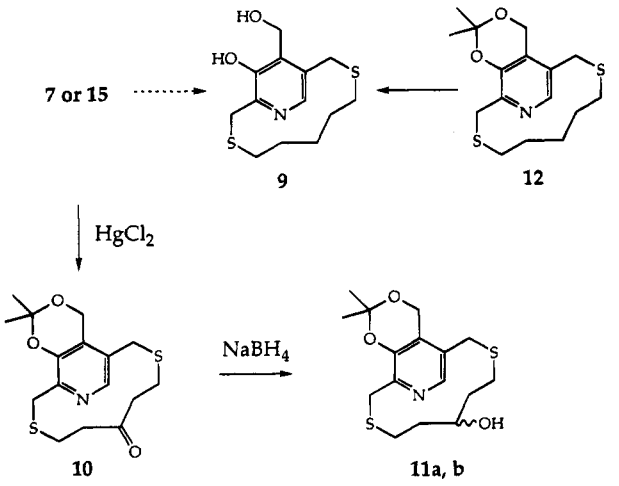
Scheme 1



[a] (i) $TiCl_4$, CH_2Cl_2; (ii) Red-Al, THF, 0 °C; (iii) 1. MsCl, $(i\text{-}Pr)_2NEt$, CH_2Cl_2, 2. HSAc, $(i\text{-}Pr)_2NEt$, DMA; (iv) 1. NH_3/MeOH, 2.5, NaH in THF, high dilution; (v) 1 N HCl, (vi) MnO_2, $CHCl_3$, Pyr.

Scheme 2



With respect to point 1, there seem to be two possibilities. One of them, invoked by Kuzuhara, is that **1b**—and perhaps others of the catalysts—can form dimers if two catalysts coordinate to the same Zn(II). If this happened, the pyridine rings might aggregate on their unhindered faces, leaving only the faces carrying the ansa chains accessible for reaction. In favor of this model is that the enantioselectivity for transamination with **1a** is apparently somewhat better when 0.5 equiv of Zn(II) are used, rather than 1 equivalent. Against it is the finding by Kuzuhara[15]—using the method of continuous variation—that the complex of Zn(II) with the glycine Schiff base of **1b** has a 1:1 stoichiometry. Most serious is our finding that we also see this preference with **1b** and with **14** even under our conditions in which 3.5 equiv of Zn(II) are used. Formation of a 2:1 complex of catalyst with Zn seems very unlikely under these conditions. The selectivity of **1b** is indeed somewhat better with 0.5 equiv of Zn(II), and this requires an explanation of its own, but it is still necessary to explain the direction of the selectivity when excess Zn(II) makes dimerization unlikely.

A second possibility is stereoelectronic. The ansa chain not only furnishes some bulk on the face of the pyridine ring, it may distort it. X-ray structures show some ring distortion even in the pyridoxine compounds themselves;[21,8] in the reactive intermediate **3** for addition to acetaldehyde the distortion should be easier since the driving force to retain planarity is less. If the ansa chain pulls the two sulfur atoms closer, this could lead to the situation shown in **16** (Figure 5). The pyridine nitrogen now has an unshared pair of electrons that should be preferentially pointed away from the ansa chain, and this could lead to electron density alternating above and below the pyridine plane, as shown, with the electron density highest on the *same* side as the ansa chain for the final carbon that adds to the acetaldehyde.[24] This would also explain the preferential protonation on that face in transaminations with **1a**.

A third related possibility is that the distortion from planarity invoked above in structure **16** may have subtle geometric effects—for instance on the geometry of Zn(II) coordination—that cause the face with the ansa chain to be more accessible sterically. We find that the glycine–acetaldehyde reaction using Ni(II) instead of Zn(II) shows significantly lower enantioinductions. The essence of these last two explanations is that the effect of the ansa chain is not to block one face of the pyridine ring, but is instead to distort the geometry of the reactive intermediate **3** away from strict planarity. Since the dimerization explanation seems to be excluded, such distortion and its consequences are the only obvious alternatives.

Based on the behavior of **1b** and of **14**, our most unusual result is the stereochemistry induced by **4** at high pH. Here the ansa chain and its substituents direct the condensation preferentially onto the opposite face. Models show that a dimethylamino group of **4** can coordinate onto the Zn(II) in the complex, and that when this occurs the benzylic methylene is partially blocking access to the same face of the glycine carbon. Not only is the stereochemical preference reversed, this is the only case in which the optical induction is greater for threonine than for *allo*-threonine.

With **1b** and with **14** in its likely conformation in aqueous methanol neither the ansa chain nor its substituents are really close enough to the glycine carbon to directly block approach by the glycine carbon. This explains why they do not block aldol condensation on the same face; the *preference* for the condensation on the same face with **1b** and **14** was discussed above.

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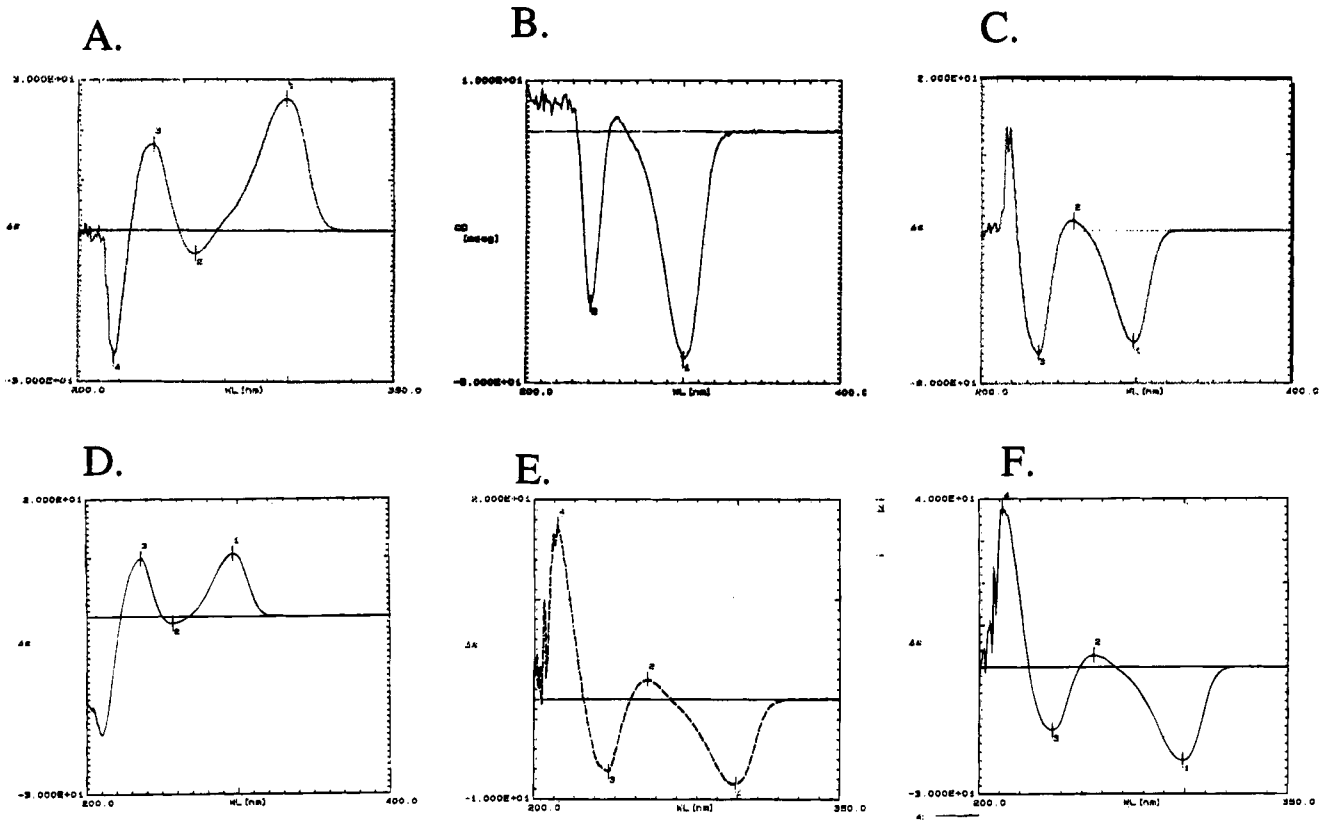
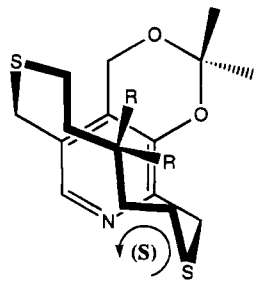


Figure 2. Circular dichoism spectra of A: (*R*)-**12**; B: (*S*)-(−)-**7**; C: (*S*)-(−)-**15**; D: (*R*)-**10**; E: (*S*)-(−)-**11a**; F: (*S*)-(−)-**11b**. See Experimental Section for conditions.



(S)-**7** : R = $SCH_2(C_6H_4)CH_2N(CH_3)_2$
(S)-**15** : R = $SCH_2(C_6H_5)$

Figure 3. The *S* configuration of two of our intermediates.

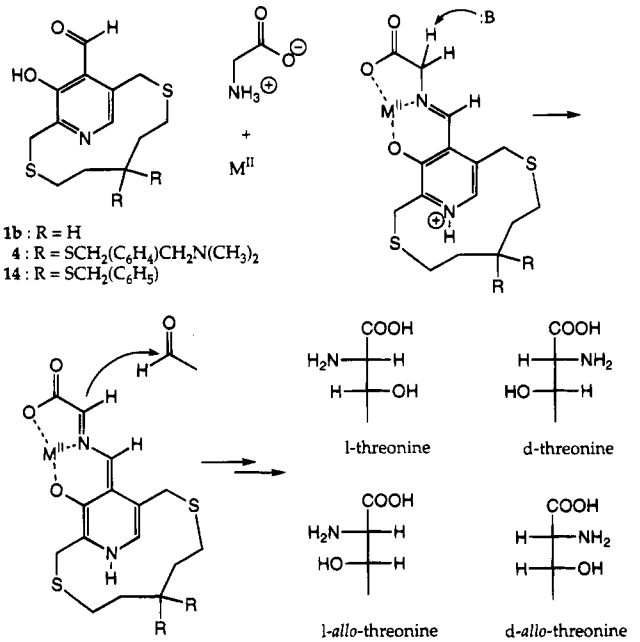


Figure 4. The condensation of glycine with acetaldehyde, catalyzed by some derivatives of pyridoxal, to form *d* and *l* threonine and *allo*-threonine.

The cause of the reversal with **4** at low pH is ambiguous. One possibility is that protonation of the dimethylamino group simply causes it to release from Zn(II) binding and allows aldol condensation on the same face as is occupied by the ansa chain for the reasons discussed above. However, models show that a protonated dimethylamino group of **4** can reach the developing oxyanion in the aldol condensation (Figure 6), and this would favor the observed stereochemistry. In favor of this is that our optical induction at pH 5.0 is somewhat greater than is that for **1b** at that pH under our conditions, but the advantage is too small to be taken seriously. Thus at the current time we suggest the mechanism of Figure 6 as one alternative, but feel that it is not yet established.

Experimental Section

General. Solvents and drying agents were purchased from Fisher Scientific Co. Ethyl ether and tetrahydrofuran (THF) were dried by distillation from Na (or K)/benzophenone. Benzene, methylene chloride, and acetonitrile were dried by distillation from CaH_2. Anhydrous dimethylacetamide (DMA), anhydrous dimethylformamide (DMF), anhydrous dimethyl sulfoxide (DMSO), and anhydrous pyridine were purchased from Aldrich Chemical Co. Deuterated solvents were obtained from Cambridge Isotope Laboratories.

dl-Threonine, *dl-allo*-threonine, *d*-threonine, *l*-threonine, *d-allo*-threonine, l-*allo*-threonine, and *N*-acetylcysteine were purchased from Sigma Co., and α-bromo-*p*-toluic acid was purchased from Janssen Chemical Co. Reagent gases were obtained from Matheson Co. All other chemicals were obtained from the Aldrich Chemical Co. unless otherwise noted.

Table 1. Enantioinductions in the Catalyzed Synthesized of Threonine and *allo*-Threonine from Glycine and Acetaldehyde[a]

catalyst	pH[b]	metal ion (equiv)	% ee[c] of *d*-threonine	% ee[c] of *d-allo*-threonine
(*S*)-**4**	10.0 (CHES)	Zn (3.5)	+48.8	+23.5
(*S*)-**4**	8.0 (HEPES)	Zn (3.5)	+30.3	−0.3
(*S*)-**4**	7.0 (PIPES)	Zn (3.5)	−40.8	−58.1
(*S*)-**4**	6.0 (MES)	Zn (3.5)	−52.1	−65.0
(*S*)-**4**	5.0 (NaOAc)	Zn (3.5)	−63.4	−75.3
(*S*)-**4**	10.0 (CHES)	Ni (3.5)	+14.6	+22.6
(*R*)-**1b**	10.0 (CHES)	Zn (3.5)	+37.5	+47.9
(*R*)-**1b**	7.0 (PIPES)	Zn (3.5)	+40.9	+46.5
(*R*)-**1b**	6.0 (MES)	Zn (3.5)	+44.6	+60.5
(*R*)-**1b**	5.0 (NaOAc)	Zn (3.5)	+54.7	+71.6
(*R*)-**1b**	10.0 (CHES)	Zn (0.5)	+57	+73
(*R*)-**1b**	8.0 (HEPES)	Zn (0.5)	+66	+79
(*R*)-**1b**	6.0 (MES)	Zn (0.5)	+63	+64
(*R*)-**1b**	10.0 (CHES)	Ni (3.5)	+6.8	+17.6
(*S*)-**14**	10.0 (CHES)	Zn (3.5)	−22.9	−40.4

[a] Reactions conducted at 35 °C in methanol/water mixtures at 1.0 mM glycine, 1.0 mM catalyst, and 10 mM acetaldehyde with 20.0 mM buffer, and with 3.5 mM or 0.5 mM Zn(II) or Ni(II), as indicated. [b] The pH values refer to the pH of the aqueous buffers incorporated into the reaction mixtures. CHES: 2-(cyclohexylamino)ethanesulfonic acid. HEPES: *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid). PIPES: Piperazine-*N*,*N*′-bis(2-ethanesulfonic acid). MES: 2-morpholinoethanesulfonic acid. [c] Positive values indicate an excess of the *d* isomer, negative values an excess of the *l* isomer.

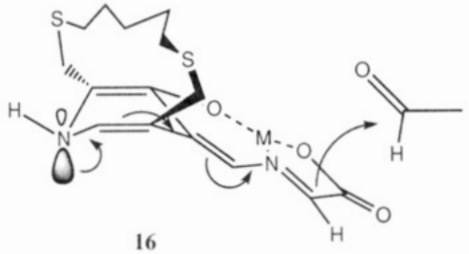


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Figure 5. A stereoelectronic explanation of the preferential reaction of many pyridoxal ansa compounds on their seemingly more hindered faces.

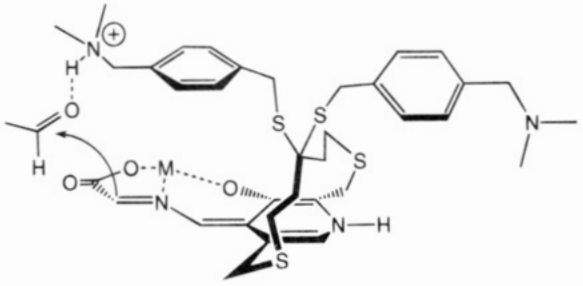

Figure 6. Catalysis by the protonated amine could explain the stereochemistry of threonine and *allo*-threonine synthesis by our catalyst **4** at low pH.

^{1}H-NMR spectra were measured on Varian VXR 200, 300, and 400 spectrometers, with tetramethylsilane as internal reference for $CDCl_3$ solutions. Residual solvent protons were used as references for spectra in D_2O, CD_3OD, DMSO-d_6. ^{13}C-NMR were taken on a Varian VXR 300 spectrometer at 75 MHz. All shifts are in ppm, all coupling constants in hertz. Circular dichroism was measured on a Jasco J720 spectropolarimeter. Polarimetry was performed on a Jasco DIP181 digital polarimeter. Capillary melting points were obtained on a Mel-Temp apparatus and values are reported uncorrected. Chemical ionization (CI) and electron impact (EI) mass spectra were obtained on a Nermag R-10-10-10 quadropole mass spectrometer. Chemical ionization was performed using either CH_4 or NH_3 gas. Fast atom bombardment (FAB) mass spectra were obtained on a JEOL DX303HF spectrometer using either glycerol or *p*-nitrobenzyl alcohol matrices.

Thin layer chromatography was performed on E. Merck precoated SiO_2 plates with fluorescent indicator. "Flash" silica chromatography was performed on E. Merck Kieselgel 60, 230−400 mesh silica. Silica flash chromatography of basic amines was sometimes performed with NH_3-saturated MeOH that was purchased from the Janssen Chemical Co. Such methanol-containing columns were first preequilibrated with the eluent.

Determination of Enantiomeric Purity of Threonine and *allo*-Threonine. In a typical reaction a solution consisting of 10 μL of 10 mM **4** (or **14**, or **1b**) in methanol, 10 μL of 10 mM glycine (aqueous), 10 μL of 35 mM $Zn(NO_3)_2$, 40 μL of methanol, and 20 μL of 0.1 M buffer in a 100 μL microvial was vortexed and treated with 10 μL of 10 mM acetaldehyde. The solution was then incubated at 35 °C in the HPLC autosampler. At various time points samples were removed, derivatized in the injector loop of the auto injector, and analyzed as outlined below. In derivatization a 5.0 μL aliquot was mixed with 5.0 μL of 0.10 N HCl for 6 min and then treated with 5.0 μL of derivativing agent followed by 5.0 μL of an aqueous solution that was 0.1 M in NaOH and 0.1 M in sodium borate. This solution was mixed for 12 min prior to injection.

Analyses were conducted with a Hewlett Packard 1090 (series II) liquid chromatograph with a DR5 ternary pumping system and a temperature-controlled auto injector. A Hewlett Packard 1046A fluorescence detector was used with excitation at 234 nm and emission at 443 nm. The derivatizing solution contained 15 mg of *N*-acetylcysteine (NAC) and 10 mg of *o*-phthalaldehyde (OPA) in 1.0 mL MeOH. A C-18 reverse phase 100 × 4.6 mm, Rainin Microsorb 5 μm column was used at 23.5 °C, and a gradient elution was done with MeOH and aqueous 0.02% triethylammonium acetate, pH 8.4 (15−30% in 25 min). The retention times (min) for the NAC/OPA derivatives were: *d*-threonine (15.1), *l*-threonine (16.4), glycine (17.2), *d-allo*-threonine (25.4), *l-allo*-threonine (26.0).

α-(Acetylthio)-*p*-toluic acid (17). To a rapidly stirred suspension of 20 g of $NaHCO_3$ (238 mmol) and 9.6 mL (135 mmol) of thioacetic acid in 250 mL of acetone was added 10 g (46.5 mmol) of α-bromo-*p*-toluic acid. The solution was stirred at ambient temperature for 24 h, concentrated in vacuo to 100 mL, and poured into 300 mL of 1 N HCl and 300 mL of brine. Concentrated HCl was added until the solution was strongly acidic, and it was extracted with 3 × 200 mL of ether. The combined organic extracts were dried with $MgSO_4$ and evaporated in vacuo. The resulting solid was redissolved in 200 mL of ether and precipitated with 40−60 mL of hexane. The ether was allowed to slowly evaporate to fully precipitate the thioacetate as white crystals. The crystals were washed with cold hexane and dried in vacuo to afford 9.2 g (43.7 mmol, 96% yield) of product, mp 154−155 °C (lit.[25] 144−145 °C); ^{1}H-NMR ($CDCl_3$, 200 MHz) 8.04 (2H, d, J = 8.1), 7.40 (2H, d, J = 8.1), 4.16 (2H, s), 2.37 (3H, s).

α-(Acetylthio)-*N*,*N*-dimethyltoluamide (18). To a rapidly stirred solution of 0.50 g (2.6 mmol) of α-(acetylthio)-*p*-toluic acid **17** in 50 mL of dry CH_2Cl_2 and 0.2 mL of DMF was added 0.45 mL (5.14 mmol) of oxalyl chloride dropwise. The solution was stirred 1 h under N_2 and the volatiles were removed in vacuo. The solids were redissolved in 30 mL of dry CH_2Cl_2 and dimethylamine hydrochloride (420 mg, 5.14 mmol) was added, followed by the dropwise addition of 0.5 mL of dry pyridine. The solution was stirred at ambient temperature for 12 h, poured into a mixture of 20 mL of brine and 20 mL of 1 N HCl and extracted with 3 × 20 mL of CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and evaporated in vacuo to afford 0.609 g (2.56 mmol, 99% yield) of the desired amide: ^{1}H-NMR ($CDCl_3$, 200 MHz) 7.32 (4H, s), 4.42 (2H, s), 3.09 (3H, s), 2.97 (3H, s), 2.35 (3H, s).

Diethyl 3,3-Bis[[4′-(dimethylcarbamoyl)benzyl]thio]glutarate (19). *All operations were carried out under an inert atmosphere to prevent oxidation of the free* thiol. α-(Thioacetyl)-*p*-toluic acid dimethylamide (**18**) (6.0 g, 25.3 mmol) was dissolved in 30 mL of MeOH. The solution was degassed by sparging with nitrogen for 20 min. Ammonia gas was added to the solution until saturated. The solution was allowed to stand for 1 h, sparged with nitrogen, and then evaporated in vacuo using a rotary evaporator equipped with a nitrogen inlet. The remaining oil was placed under vacuum and acetamide was sublimed away with gentle heating. The remaining oil was dissolved in 10 mL of dry CH_2-

(25) Okuno, H. Y.; Uoto, K.; Tomohiro, T.; Youinou, M-T. *J. Chem. Soc., Dalton Trans.* **1990**, 3375−3381.

Cl_2 and 1.4 mL (7.7 mmol) of diethyl 1,3-acetonedicarboxylate was added. The solution was cooled to −78 °C, and then 9.6 mL (87.5 mmol) of $TiCl_4$ was added. The solution was stirred at ambient temperature for 36 h, cooled to 0 °C, and 40 mL of CH_2Cl_2 was added followed by 40 mL of EtOAc with rapid stirring. The resulting slurry was poured into 200 mL of saturated $NaHCO_3$. The solution was made basic by adding 1 N NaOH and the solution was extracted with 3 × 100 mL EtOAc. The combined organic extracts were dried over $MgSO_4$ and concentrated in vacuo. The resulting oil was purified by silica flash chromatography with 10−40% acetone/CH_2Cl_2 to afford 3.5g (80% yield) of the desired thioketal (yields range from 45−90%): ^{1}H-NMR ($CDCl_3$, 200 MHz) 7.35 (8H, s), 2.85 (4H, q, J = 7.2), 3.97 (4H, s), 3.28 (4H, s), 3.11 (6H, br. s), 2.96 (6H, br. s), 1.29 (6H, t, J = 7.2); ^{13}C-NMR ($CDCl_3$, 75 MHz) 171.06, 168.75, 138.00, 135.27, 129.10, 127.38, 60.71, 58.89, 41.49, 39.46, 35.25, 34.06, 14.12; MS (FAB, PBA) 575 (M + 1).

3,3-Bis[[4′-(dimethylcarbamoyl)benzyl]thio]-1,5−pentanediol (20). The diester **19** (200 mg, 0.348 mmol) in 10 mL of dry Et_2O and 0.42 mL of MeOH was treated with 0.50 mL of $LiBH_4$ (2.0 M in THF). After 6 h an additional 0.6 mL of $LiBH_4$ solution was added and the solution was refluxed 8 h. The solution was poured into 50 mL of water and extracted with 4 × 25 mL of EtOAc. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The remaining oil was chromatographed on silica with 40−60% acetone/EtOAc to afford 63.3 mg (40% yield) of the desired diol: ^{1}H-NMR (CD_3OD, 200 MHz) 7.44 (4H, d, J = 8.6), 7.37 (4H, d, J = 8.4), 3.92 (4H, s), 3.80 (4H, t, J = 7.4), 3.09 (6H, br. s), 2.99 (6H, br. s), 2.02 (4H, t, J = 7.2).

3,3-Bis[[4′-[(dimethylamino)methyl]benzyl]thio]-1,5-pentanediol (21). To a rapidly stirred solution of the diester **19** (2.73 g, 4.75 mmol) dissolved in 100 mL dry THF at 0 °C was added 11.0 mL (37.4 mmol) of Red-Al, sodium bis(2−methoxyethoxy)aluminum hydride (3.4 M in toluene). The solution was stirred at 0 °C for 1.5 h, and then MeOH (1 mL) was added dropwise to quench the excess Red-Al. The solution was poured into 100 mL of 0.25 N NaOH and the resulting mixture was extracted with 5 × 60 mL of EtOAc. The combined organic extracts were dried over $MgS0_4$ and evaporated in vacuo. The resulting oil was purified by flash chromatography on silica with 10% NH_3 saturated MeOH/CH_2Cl_2 to afford 1.72 g (3.72 mmol, 78.3% yield) of the desired diol: ^{1}H-NMR ($CDCl_3$, 200 MHz) 7.26 (8H, 2), 3.85 (4H, s), 3.81 (4H, t, J = 5.9), 3.42 (4H, s), 2.24 (12H, s), 2.06 (4H, t, J = 5.9).

***S,S*-Diacetyl-3,3-bis[[4′-[(dimethylamino)methyl]benzylthio]-1,5-pentanedithiol (8).** Methanesulfonyl chloride (0.73 mL, 9.28 mmol) was added dropwise to a solution of the diol **21** (1.72 g, 3.70 mmol) and *N,N*-diisopropylethylamine (1.29 mL, 7.4 mmol) in 35 mL of CH_2Cl_2. The solution was stirred at ambient temperature for 1.5 h, and the solvent was carefully evaporated in vacuo without heating. The resulting oil was dissolved in 70 mL of dry, degassed DMA, and the solution was cooled to 0 °C while 5.6 g (74.2 mmol) of thioacetic acid and then 8.6 g (66.7 mmol) of *N,N*-diisopropylethylamine were added dropwise. The solution was kept under argon, and heated to 40 °C for 36 h, and then poured into 100 mL of saturated $NaHCO_3$ and 100 mL brine, made basic with 1 N NaOH and extracted with 4 × 60 mL of EtOAc. The combined organic extracts were dried over $MgSO_4$ and evaporated in vacuo. The resulting oil was chromatographed twice on silica, first with 3 to 8% Et_3N/acetone and then with 3% Et_3N/CH_2Cl_2, to afford 1.16 g (2.0 mmol, 54% yield) of product: ^{1}H-NMR ($CDCl_3$, 200 MHz) 7.34 (4H, d, J = 8.0), 7.26 (4H, d, J = 8.0), 3.91 (4H, s), 3.44 (4H, s), 3.06 (4H, m), 2.32 (6H, s), 2.26 (12H, s), 1.92 (4H, m).

17,17-Dimethyl-19*H*-*m*-dioxino[19,1-*c*]-6,6-bis [[4′-[(dimethylamino)methyl]benzyl]thio]-3,9-dithia-12-azabicyclo[9.2.2]pentadeca-11,13,14-triene (7). *All operations were carried out in the absence of oxygen to prevent oxidation of the free thiol.* A solution of the dithioacetate **8** (348 mg, 0.60 mmol) in 25 mL of MeOH was degassed by sparging for 15 min with nitrogen and then saturated with ammonia gas and allowed to stand for 1 h at ambient temperature. The solution was then sparged with argon and evaporated in vacuo using a rotary evaporator equipped with a nitrogen inlet. The remaining crude dithiol was placed under high vacuum and acetamide was sublimed off with gentle heating. Solutions of the dithiol in 10 mL of THF and of the dichloride **5**[6] in 10 mL of THF were added in separate syringes via syringe pump over a period of 24 h, to a suspension of 72 mg of NaH (60% dispersion in mineral oil), in refluxing THF. The solution was poured into 50 mL of saturated $NaHCO_3$ and 50 mL of brine and extracted with 4 × 100 mL of EtOAc. The combined organic extracts were dried over $MgSO_4$ and evaporated in vacuo. The resulting oil was chromatographed on silica with 3−10% NH_3 saturated MeOH/CH_2Cl_2 to afford 359 mg (394 μmol, 65.7% yield) of the desired macrocyclic ketal: ^{1}H-NMR ($CDCl_3$, 200 MHz) 7.75 (1H, s), 4.98 (1H, d, J = 16.3), 4.72 (1H, d, J = 16.3), 4.13 (1H, d, J = 12.2), 3.66 (1H, d, J = 12.2), 3.66 (2H, s), 3.60 (2H, 2), 3.57 (2H, d, J = 8.6), 3.47 (4H, s), 3.44 (2H, d), 2.26 (12H, s), 1.55 (3H, s), 1.53 (3H, s).

The enantiomers were separated by chiral HPLC on a Chiralcel OD cellulose tris[(3,5-dimethylphenyl)carbamate] column[20] (0.46 × 25 cm) using 20% 2-propanol, 0.2% diethylamine/hexane 1.0 mL/min. The high R_f fraction had $[\alpha]_D$ = −17°, and circular dichroism (CD) in CH_3CN of −45.12 at 300.0 nm and -33.97 mdeg at 241.0 nm with A =1.03 at 300 nm. The low R_f fraction had CD of +37.17 mdeg at 300.0 nm and +47.58 mdeg at 237.0 nm with A = 0.495 at 297 nm.

14-(Hydroxymethyl)-6,6-bis[[4′-[(dimethylamino)methyl]benzylthio]-3,9-dithia-12-azabicyclo[9.2.2]pentadeca-11,13,14-trien-15-ol (22). To 10 mg of acetonide **7** in 2 mL of water at 0 °C were added six drops of concentrated HCl. The solution was stirred for 6 h at ambient temperature and was then cooled to −10 °C. To the rapidly stirred solution was added 2 mL of NH_3-saturated MeOH. The volatiles were removed in vacuo and the residue was chromatographed on silica with 10% NH_3 saturated MeOH/CH_2Cl_2 to afford 3.5 mg of the desired diol (37% yield): ^{1}H-NMR ($CDCl_3$, 300 MHz) 7.78 (1H, s), 7.27 (8H, s), 4.98 (1H, d, J = 14.6), 4.76 (1H, d, J = 14.6), 3.71 (1H, d, J = 12.4), 4.34 (1H, d, J = 12.4), 3.65 (2H, br. s), 3.61 (2H, d, J = 13.4), 3.59 (2H, d, J = 12.3), 3.46 (2H, d, J = 13.3), 3.42 (2H, s), 3.37 (d, J = 12.6), 2.6−2.1 (m), 2.37 (2H, s), 2.25 (2H, s), 2.09−1.96 (1H, m), 1.60−1.72 (1H, m), 1.38−1.50 (1H, m), 1.05−1.14 (1H, m), 0.48−0.62 (1H, m); ^{13}C-NMR ($CDCl_3$, 75 MHz) 153.0, 145.9, 141.0, 137.1, 137.0, 136.1, 130.5, 129.8, 129.5, 129.2, 129.1, 128.7, 63.82, 63.75, 60.6, 45.2, 45.1, 40.0, 39.5, 34.3, 33.8, 31.8, 31.1, 24.7, 23.6.

15-(Hydroxy)-6,6-bis[[4′-[(dimethylamino)methyl]benzyl]thio]-3,9-dithia-12-azabycyclo[9.2.2]pentadeca-11,13,14-triene-14-carboxaldehyde (4). To a solution of 10 mg (15.8 μmol) of the diol **22** (derived from (−)-**7**) in 0.4 mL of pyridine and 1.5 mL of $CHCl_3$ was added γ-MnO_2 (25 mg, 0.28 mmol). The suspension was briefly sonicated and then heated to 35 °C for 40 min under nitrogen. It was filtered through a very small plug of silica and the solid was washed with 40% NH_3-saturated MeOH in CH_2Cl_2. The yellow solution was evaporated in vacuo and chromatographed on a 2 mL plug of silica with 10% NH_3-saturated MeOH in CH_2Cl_2. The lower R_f yellow fraction afforded 3.5 mg (5.6 μmol, 38% yield) of the desired pyridoxal catalyst. MS CI, (CH_4) 669 (M + C_2H_4); ^{1}H-NMR ($CDCl_3$, 300 MHz) 7.78 (1H, s) , 7.2−7.5 , 5.58 (1H, s), 5.01 (1H, d, J = 13), 5.84 (1H, d, J = 13), 4.26 (1H, d, J = 12), 4.84 (1H, d, J = 10).

17,17-Dimethyl-19*H*-*m*-dioxino[19,1-*c*]-6-oxo-3,9-dithia-12-azabicyclo[9.2.2]pentadeca-11,13,14-triene (10). The thioketal (+)-**15** (13.3 mg, 18.7 μmol) was dissolved in 200 μL of CH_3CN and 200 μL of H_2O. $CdCO_3$ (12 mg) was added and the suspension was rapidly stirred as 15 mg of $HgCl_2$ in 200 μL of CH_3CN was added dropwise to form a finely dispersed suspension. The mixture was heated 5 min at 50 °C, cooled to ambient temperature, and diluted with 2 mL of MeOH before 2 drops of 2-mercaptoethanol were added with vigorous stirring. The mixture was briefly sonicated and then filtered. The pellet was washed with MeOH and the filtrate was evaporated in vacuo. The resulting oil was separated on preparative TLC (0.5 mm silica) with 10% NH_3-saturated MeOH/CH_2Cl_2. The highest R_f, UV active fraction was isolated to afford 3.2 mg (47% yield) of the desired macrocyclic ketone. In a similar manner (+)-**7** was also converted to **10.** MS (CI, NH_3) 340 (M + 1); IR (neat) 1711 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 200 MHz) 7.82 (1H, s), 5.20 (1H, d, J = 16.4), 4.75 (1H, d, J = 16.4), 4.10 (1H, d, J = 12.4), 3.69 (1H, d, J = 14), 3.63 (1H, d, J = 12.6), 3.47 (1H, d, J = 14), 2.80−2.52 (4H, m), 2.07−2.96 (2H, m), 1.62 (3H, s), 1.60 (3H, s), 1.75−1.94 (1H, m); CD (0.109 mM, CH_3CN) 296.5 (Δε = +10.67), 256.5 (Δε = −1.13), 236.0 (Δε = +9.88).

17,17-Dimethyl-19*H*-*m*-dioxino[19,1-c]-6-hydroxy-3,9-dithia-12-azabicyclo[9.2.2]pentadeca-11,13,14-triene (11 a,b). To 3.2 mg (9.4 μmol) of the ketone **10** (derived from (−)-**7**) in 0.5 mL of dry EtOH

was added 1.8 mg (47 μmol) of $NaBH_4$. The solution was stirred 2 h at ambient temperature and then diluted with 2.0 mL of water, and 0.1 N HCl was added dropwise until *ca.* pH 4. The solution was then made basic with saturated $NaHCO_3$ (aqueous) and extracted with 4 × 2 mL of EtOAc. The combined organic layers were evaporated in vacuo and purified by preparative TLC (0.25 mm silica) with 5% NH_3-saturated MeOH to afford two diastereomeric alcohols. High R_f alcohol **11a** (1.95 mg, 60% yield): MS (CI, CH_4) 342 (M + 1); ^{1}H-NMR ($CDCl_3$, 400 MHz) 7.99 (1H, s), 7.4−7.3, 5.30 (1H, d, J = 16.3), 4.81 (1H, d, J = 16.3), 4.31 (1H, d, J = 12.8), 3.68 (1H, d, J = 13.7), 3.08−3.17 (1H, m), 2.58−2.76 (2H, m), 2.11−2.32 (2H, m), 1.62 (3H, s), 1.58 (3H, s), 0.80−0.98 (1H, m), 0.57−0.68 (1H, m); CD **11a** (0.193 mM, CH_3CN) 299.0 ($\Delta\epsilon$ = -8.509), 255.5 ($\Delta\epsilon$ = +1.945), 236.5 ($\Delta\epsilon$ = −8.509). Low R_f alcohol **11b** (0.62 mg, 19% yield): ^{1}H-NMR ($CDCl_3$, 400 MHz) 8.02 (1H, s), 7.20−7.33 , 5.20 (1H, d, J = 16.3), 4.81 (1H, d, J = 16.3), 4.17 (1H, d, J = 13.1), 3.70 (1H, d, J = 13.7), 3.51 (1H, d, J = 13.1), 3.50 (1H, d, J = 13.1), 2.84−2.93 (1H, m), 2.62−2.73 (2H, m), 2.1−2.3 (2H, m), 0.79−1.02 (2H, m), 0.28−0.38 (2H, m). CD (0.122 mM, CH_3CN) 299.0 ($\Delta\epsilon$ = −22.05), 255.5 ($\Delta\epsilon$ = +2.86), 235.5 ($\Delta\epsilon$ = −14.83).

Diethyl 2,2-Bis(benzylthio)glutarate (23). Titanium(IV) chloride (10.0 mL) was added dropwise to a solution of benzyl mercaptan (2.5 mL, 20 mmol) and diethyl 1,3-acetonedicarboxylate in 2.0 mL CH_2Cl_2 at −78 °C, and the solution was allowed to warm to ambient temperature. After 36 h at ambient temperature the solution was poured into a rapidly stirred mixture of 50 mL of ether and 100 mL of 2.5 N NaOH at 0 °C. The aqueous layer was extracted with 3 × 50 mL ether. The combined organic extracts were dried over Na_2SO_4 and concentrated in vacuo. The remaining oil was purified by silica flash chromatography with 10% EtOAc/hexane to afford 3.61 g (8.1 mmol, 91% yield) of the desired thioketal: ^{1}H-NMR ($CDCl_3$, 200 MHz) 7.40−7.18 (10H, m), 4.18 (4H, q, J = 7.2), 3.97 (4H, s), 3.18 (4H, s), 1.28 (6H, t, J = 7.0).

3,3-Bis(benzylthio)-1,5-pentanediol (24). A solution of 3.5 g (8.1 mmol) of the diester **23** in 50 mL of dry THF was treated with 16 mL of Red-Al (3.4 M in toluene) at 0 °C. The solution was stirred at 0 °C for 1 h, and then 3.0 mL of MeOH was added dropwise at 0 °C. The solution was poured into 50 mL of brine and the mixture was extracted with 2 × 50 mL of EtOAc. The combined organic extracts were dried over Na_2SO_4 and evaporated under reduced pressure. The remaining oil was purified by silica flash chromatography with 50% EtOAc/hexane to afford 2.07 g (7.3 mmol, 90% yield) of the desired diol: ^{1}H-NMR ($CDCl_3$, 200 MHz) 7.38−7.19 (10H, m), 3.89 (4H, s), 3.86 (4H, t, J = 6.0), 2.09 (4H, t, J = 6.0).

***S,S*-Diacetyl-2,2-bis(benzylthio)-1,5-pentanedithiol (25).** The diol **24** (257 mg, 0.90 mmol) and *N,N*-diisopropylethylamine (233 mg, 1.8 mmol) in 3.0 mL of dry CH_2Cl_2 were treated with 227 mg (1.98 mmol) of methanesulfonyl chloride. The reaction mixture was stirred at ambient temperature for 2 h and the volatiles were removed in vacuo. The remaining oil was redissolved in 10 mL of acetone with 1.1 g (9.0 mmol) of *N,N*-diisopropylethylamine and 685 mg (9.0 mmol) of thioacetic acid, and the solution was refluxed for 8 h under N_2. It was then poured into 100 mL of brine and 1 N NaOH (1:1) and extracted with 3 × 60 mL of EtOAc. The combined organic extracts were washed with 100 mL of 1.0 N HCl, dried over $MgSO_4$, and concentrated in vacuo. The resulting brown oil was purified by silica flash chromatography with 10−30% EtOAc/hexane, followed by flash chromatography with toluene to afford 133 mg (0.51 mmol, 57% yield) of the dithioacetate **25**: ^{1}H-NMR ($CDCl_3$, 200 MHz) 7.43−7.12 (10H, m), 3.92 (4H, s), 3.13−2.96 (4H, m), 2.32 (6H, s), 2.00−1.82 (4H, m).

17,17-Dimethyl-19*H*-*m*-dioxino[19,1-*c*]-6,6-bis(benzylthio)-3,9-dithia-12-azabicyclo[9.2.2]pentadeca-11,13,14-triene (15). A solution of dithioacetate **25** (233 mg, 0.50 mmol) in 15 mL of EtOH and 10 mL hexane. was degassed by sparging with N_2 for 20 min, and NH_3 (g) was passed through the solution until saturation. The solution was stirred for 1 h at ambient temperature, and the volatiles were removed in vacuo using a rotary evaporator equipped with a N_2 inlet. Acetamide was sublimed off under high vacuum with gentle heating. The dithiol was dissolved in 10 mL of dry THF. This solution and a solution of the dichloride **5**[6] (131.4 mg, 0.50 mmol) in 10 mL of THF were added in separate syringes via syringe pump over a period of 30 h into a solution of NaH (4.0 mmol) in 50 mL of THF at 60 °C. The solution was then poured into a mixture of 30 mL brine and 30 of mL saturated $NaHCO_3$ and extracted with 3 × 100 mL of EtOAc. The combined organic extracts were dried over $MgSO_4$ and then evaporated in vacuo. The remaining oil was then purified by silica flash chomatography using 5−30% EtOAc/CH_2Cl_2 to afford (66.5 mg, 23% yield) of the macrocyclic ketal **15**: ^{1}H-NMR ($CDCl_3$, 200 MHz) 7.93 (1H, s), 7.37−7.10 (10H, m), 5.17 (1H, d, J = 16.6), 4.75 (1H, d, J = 16.4), 4.20 (1H, d, J = 12.2), 3.70 (2H, s), 3.69 (1H, d, J = 13.2), 3.64 (2H, s), 3.59 (1H, d, J = 12.2), 3.47 (1H, d, J = 13.2), 2.55−2.32 (2H, m), 2.26−1.97 (2H, m), 1.58 (3H, s), 1.55 (3H, s), 1.55−1.13 (3H, m), 0.95−0.74 (1H, m).

The enantiomers were separated by chiral HPLC on a Chiralcel OD cellulose tris[(3,5-dimethylphenyl)carbamate] column[20] (0.46 × 25 cm) using 15% 2-propanol, 0.2% diethylamine/hexane 1.0 mL/min. High R_f isomer: specific rotation (CH_3CN) $[\alpha]_D$ = −96.3°; CD (0.16 mM, CH_3CN) 298.5 ($\Delta\epsilon$ = −14.55), 259.5 ($\Delta\epsilon$ = +1.34), 237.0 ($\Delta\epsilon$ = −15.97). Low R_f isomer: specific rotation (CH_3CN) $[\alpha]_D$ = +66.9°; CD (0.15 mM, CH_3CN) 298.5($\Delta\epsilon$ = +14.15), 259.0 ($\Delta\epsilon$ = −1.159), 237.0 ($\Delta\epsilon$ = +15.63).

14-(Hydroxymethyl)-6,6-bis(benzylthio)-3,9-dithia-12−azabicyclo-[9.2.2]pentadeca-11,13,14-trien-15-ol (26). The acetonide (−)-**15** (8.4 mg, 14.7 μmol) in 2.0 mL of THF was treated with 2.0 mL of 1.0 N HCl and stirred for 2 h at ambient temperature. Concentrated HCl (1.0 mL) was added and the mixture was stirred for an additional 3 h. The reaction mixture was then heated to 35 °C for 40 min. The mixture was cooled to 0 °C and then neutralized by slow dropwise addition of 5 M NaOH at 0 °C. The solution was then extracted with 4 × 20 mL EtOAc, the extracts were concentrated in vacuo, and the remaining oil was purified by silica flash chromatography (5−10% NH_3-saturated MeOH/CH_2Cl_2) to afford 5.7 mg (10.8 μmol, 73% yield) of the desired diol: ^{1}H-NMR ($CDCl_3$, 200 MHz) 7.91 (1H, s), 5.22 (1H, d, J = 14.2), 4.98 (1H, d, J = 14.3), 4.33 (1H, d, J = 12.2), 3.48−3.86 (6H, m), 2.76−2.49 (2H, m), 2.17−1.94 (2H, m), 1.55−1.19 (3H, m), 1.05−0.83 (1H, m). Specific rotation ($CHCl_3$) $[\alpha]_D$ = −139°.

15-Hydroxy-6,6-bis(benzylthio)-3,9-dithia-12-azabycyclo[9.2.2]-pentadeca-11,13,14-triene-14-carboxaldehyde (14). A solution of the diol (−)-**26** (5.7 mg, 10.8 μmol) in 1.0 mL of dry CH_2Cl_2 and 0.3 mL of pyridine was treated with 30 mg of γ-MnO_2. The reaction mixture was briefly sonicated and then heated to 35 °C under N_2 for 20 min. The reaction mixture was filtered through a 1 cm plug of silica which was then washed with 20% NH_3-saturated MeOH/CH_2Cl_2. The filtrate was refiltered through a 0.2 μm nylon membrane and then evaporated under reduced pressure. The remaining oil was purified by preparative TLC (0.1 mm E. Merck silica) with 5.0% MeOH, 0.1% Et_3N/CH_2Cl_2 to afford 1.88 mg (3.56 μmol, 33% yield) of the desired aldehyde as a yellow oil: MS (FAB, NBA) 669 (M + 1); ^{1}H-NMR ($CDCl_3$, 400 MHz) 10.49 (1H, s), 8.04 (1H, s), 4.36 (1H, d, J = 12.4), 4.24 (1H, d, J = 13.8), 3.75 (1H, d, J = 13.8), 3.67 (1H, d, J = 12.4), 3.50 (2H, s), 2.71−2.47 (2H, m), 2.10−2.20 (2H, m).

Resolution of (±)-14-Hydroxy-15-(hydroxymethyl)-14,15′-*O,O*-isopropylidene-2,8-dithia[9](2,5)pyridinophane (12). The intermediate **12** was synthesized according to the indications of Kuzuhara[6,26] and resolved by chiral HPLC on a Chiracel OD column[20] (0.46 × 25 cm) using 20% ethanol/0.1% diethylamine/hexane at 1 mL/min. The high R_f enantiomer was obtained in 90.9% ee, and the low R_f enantiomer in 99.8% ee. Low R_f isomer: CD (0.227 mM, CH_3CN) 299.0 ($\Delta\epsilon$ = +26.1), 255 ($\Delta\epsilon$ = −4.5), 236.0 nm ($\Delta\epsilon$ = +17.2).

(26) See also Ando, M.; Tachibana, Y.; Kuzuhara, H. *Bull. Chem. Soc. Jpn.* **1982**, *55*, 829−832 for a related procedure with more details.