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Asymmetric catalysis of the Strecker amino acid synthesis by a cyclic dipeptide*

Review Article

M. S. Iyer, K. M. Gigstad, N. D. Namdev, and **M. Lipton**

Department of Chemistry, Purdue University, West Lafayette, Indiana, U.S.A.

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Summary. A novel cyclic dipeptide – *cyclo*[(*S*)-His-(*S*)-NorArg] – has been prepared which catalyzes an enantioselective version of the Strecker amino acid synthesis. The catalyst, when present in 2 mol % quantity in methanol solution, catalyzes the addition of hydrogen cyanide to *N*-alkylimines to afford α-amino nitriles in high yield and high enantiomeric excess. Furthermore, acid hydrolysis of *N*-benzhydryl-α-amino nitriles afforded the corresponding α-amino acids directly. This methodology affords a variety of arylglycines in exceptionally high enantiomeric excess, but aliphatic amino acids were obtained with low enantioselectivity. Current efforts are underway to expand the scope of this reaction, as well as to elucidate the mechanism of catalysis and the roles played by substrate and catalyst in determining the stereochemical outcome of the reaction.

Keywords: Amino acid – Catalysis – Strecker synthesis – Diketopiperazine

Introduction

In recent years, the use of unnatural α-amino acids has grown rapidly, due to their importance in site-directed mutagenesis (Ellman et al., 1991), peptide synthesis (Wallace et al., 1989) and the use of conformationally constrained peptides and peptide analogues (Hruby and Sharma, 1991). As the use of unnatural α-amino acids has grown, so too has the need for general methodology concerning their enantioselective synthesis. The last 20 years have seen an explosion of activity in this area, covered extensively in the reviews of Williams (1989) and Duthaler (1994). Nonetheless, at present there is

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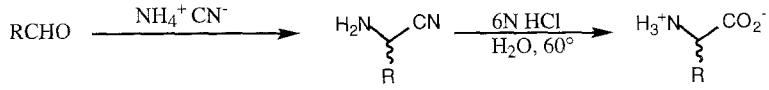
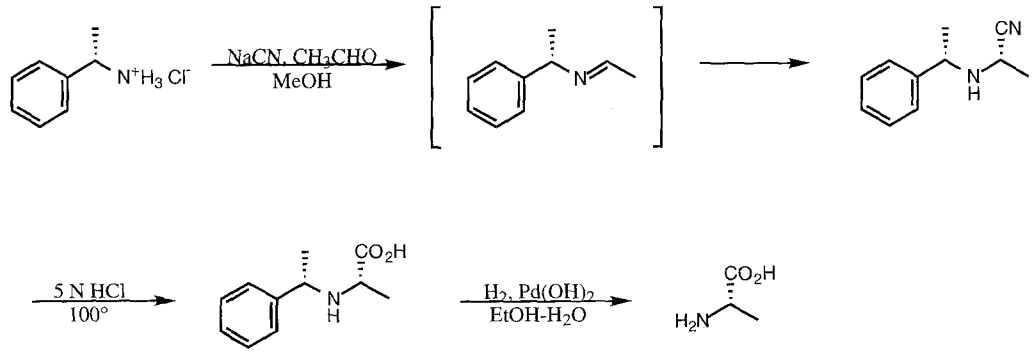
no single methodology capable of producing any α-amino acid in high enantiomeric purity.

In 1850, Strecker (1850) reported that, upon condensation with ammonium cyanide, carbonyl compounds such as aldehydes and ketones were converted to α-amino nitriles (the "Strecker synthesis"); subsequent hydrolysis of the nitrile functionality afforded α-amino acids (Scheme 1). This discovery represented the first *de novo* synthesis of α-amino acids and, nearly 150 years later, still finds frequent application in the synthesis of unnatural amino acids owing to its attractive generality, simplicity and low cost. It has proved especially useful in the synthesis of arylglycines, a class of α-amino acids difficult to synthesize by many other asymmetric methods (Williams, 1992). Although the mechanism of the Strecker synthesis has never been unambiguously proved, it is generally believed to proceed in two steps: condensation of the amine with carbonyl compound to form an imine, followed by nucleophilic addition of HCN to the imine (Walia et al., 1974).

The major limitation to the classic Strecker synthesis is its production of racemic products; obtaining chiral α-amino acids through the Strecker synthesis has traditionally been accomplished by resolving the racemic product. Beginning with the studies of Harada (1963), several researchers have attempted to render the Strecker synthesis enantioselective by substituting optically active amines for ammonia, thereby affording diastereoselective addition of HCN to the imine. In his studies, Harada formed an α-amino nitrile from (−)-α-methylbenzylamine, sodium cyanide and acetaldehyde and converted it to L-alanine by acid hydrolysis of the nitrile and hydrogenolysis of the benzylic amine (Scheme 2). This procedure has been modified to use pre-formed imines and HCN (Patel and Worsley, 1969) or BrCN (Phadtare et al., 1985), with largely similar results.



Scheme 1. The Strecker synthesis of α-amino acids

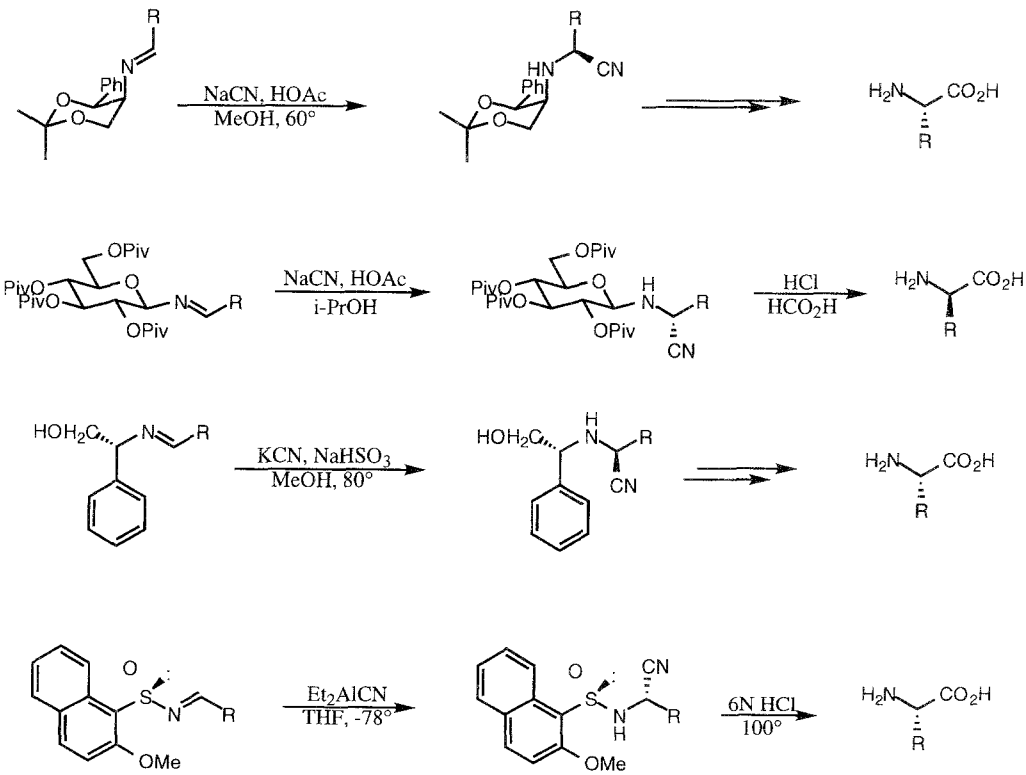


Scheme 2. Asymmetric synthesis of L-alanine using (−)-α-methylbenzylamine as a chiral auxiliary

More recently, other chiral auxiliaries have been used in the Strecker synthesis. Weinges (1980) has used 5-amino-4-phenyl-1,3-dioxanes, Kunz (1991) has used 2,3,4,6-tetra-*O*-pivaloyl-β-D-galactopyranosylamine, Inaba et al., (1992) and Chakraborty et al., (1991, 1995) have used phenylglycinol and Davis (1994, 1996) has used a chiral sulfinamine to obtain α-amino nitriles in diastereomeric excess (Scheme 3). Although several of these methods afford α-amino acids in high enantiomeric purity, the cost of using stoichiometric chiral auxiliaries, especially those which cannot be recovered, has hindered the adoption of these methods for large-scale synthesis of α-amino acids.

To avoid chiral auxiliaries one must instead use a chiral catalyst. Although chiral catalysts have been developed for a number of reactions, catalysis of the Strecker synthesis has been achieved in only a trivial sense. Ogata and Kawasaki (1971) demonstrated that protic solvents serve as catalysts in the addition of HCN to imines by studying the rate of addition of HCN to a preformed imine as a function of solvent composition. They found that the rate varied linearly with methanol concentration and that the kinetics were *third-order*, implying a termolecular transition state which they proposed to be cyclic (Fig. 1). While these observations are not easily incorporated into a model for a chiral catalyst, they do lead to the conclusion that any catalyst for the Strecker synthesis must be compatible with a protic solvent.

Though no catalysts for the Strecker synthesis had been developed, several were known for a closely related reaction, the formation of



Scheme 3. Other chiral auxiliaries employed in the Strecker synthesis

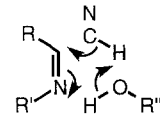
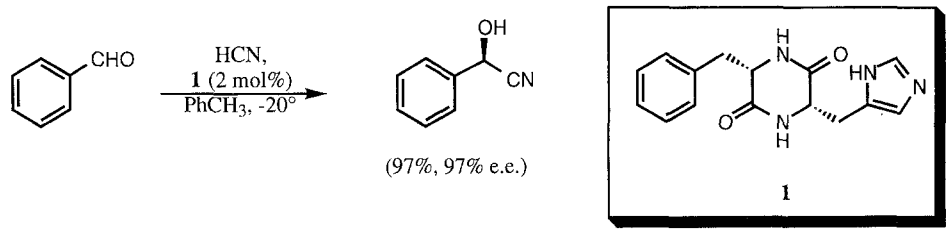


Fig. 1. Proposed termolecular transition state for the Strecker synthesis



Scheme 4. Asymmetric catalysis of cyanohydrin formation by cyclo[(*S*)-histidyl-(*S*)-phenylalanyl]

cyanohydrins. Although aldehydes and aliphatic ketones will react spontaneously with hydrogen cyanide, Lapworth (1903) demonstrated that addition of a base to the reaction led to a rate acceleration. In addition, an enzyme – D-oxynitrilase – catalyzes the enantioselective formation of (*R*)-mandelonitrile from benzaldehyde *in vivo*, as shown by Becker et al. (1965) and Effenberger (1994). In an effort to develop synthetic analogues of oxynitrilase, Inoue and co-workers (Oku and Inoue, 1981) synthesized and evaluated a series of dipeptides, resulting in the remarkable discovery that the diketopiperazine *cyclo*[(*S*)-His-(*S*)-Phe] (**1**, Scheme 4) catalyzed the enantioselective formation of (*R*)-mandelonitrile from benzaldehyde and hydrogen cyanide in high yield and enantiomeric excess (Tanaka et al., 1990; North, 1993).

The presumed mechanistic similarity between cyanohydrin formation and the Strecker synthesis leads to the expectation that **1** may also catalyze the Strecker synthesis, but two problems preclude its use in such a capacity. First, the Strecker synthesis requires the use of a protic solvent, whereas Inoue has shown that **1** affords enantioselectivity in cyanohydrin synthesis only when used in relatively nonpolar solvents such as toluene and diethyl ether (Tanaka et al., 1990).

A second, more subtle problem with the Inoue catalyst involves its mode of catalysis. Although the origin of enantioselectivity is not well understood, one might expect that **1** catalyzes cyanohydrin formation in much the same way as other bases. Prelog and Wilhelm (1954) have studied the mechanism of catalysis of cyanohydrin formation in detail, and concluded that protonated bases function both as general acid catalysts and as the counterion for cyanide anion. Applying this same reasoning to **1** suggests that its complex with hydrogen cyanide serves as a general acid to protonate benzaldehyde in the transition state of cyanohydrin formation. Consideration of acidity functions would imply that, while an imidazolium ion ($pK_a = 6.7$) is fully capable of protonating benzaldehyde ($pK_b = -3.9$), it will be unable to serve as a general

acid catalyst in the presence of the more basic benzaldimine ($pK_b \sim 8$) and thus may not be able to catalyze the Strecker synthesis.

Materials and methods

All solvents were commercial grade and were distilled and dried as follows: tetrahydrofuran and toluene from sodium benzophenone ketyl; dichloromethane and acetonitrile from calcium hydride. Flash chromatography was performed with silica gel grade 60 (230–400 mesh). High pressure liquid chromatography was performed using Vydac C_8 and C_{18} reverse phase columns and a Daicel ChiralPak AD™ chiral stationary phase column.

Results and discussion

In the event, treatment of a variety of imines with hydrogen cyanide in the presence of catalytic **1** in methanol afforded only racemic α-amino nitriles. It was therefore decided to install a more basic sidechain in place of the imidazole of **1**. A guanidine was chosen for the low acidity of its conjugate acid ($pK_a = 13.4$). Because it was unknown whether the proximity of the imidazole to the diketopiperazine ring in **1** was important, it was decided to keep the nearest nitrogen of the guanidine two carbons removed from the diketopiperazine, just as the nearest nitrogen of the imidazole ring is in histidine. Therefore, a new diketopiperazine (**2**, Fig. 2) was synthesized, containing (*S*)-phenylalanine and the non-proteinogenic α-amino acid (*S*)-norarginine, the lower homologue of arginine.

The synthesis of **2** begins with benzyloxycarbonyl-(*S*)-glutamic acid (**3**, Scheme 5), which upon protection as the oxazolidinone **4** and subsequent Curtius rearrangement (Scholtz and Bartlett, 1989) is converted to the carbamate **5** in 81% yield. Hydrolysis of the oxazolidinone using KOH/MeOH and coupling with (*S*)-phenylalanine methyl ester using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide/1-hydroxybenztriazole afforded the dipeptide **7** (79% yield). This coupling step was also used to verify that hydrolysis had occured without racemization. Catalytic hydrogenolysis of the benzyloxycarbonyl group of **7** followed by cyclization in refluxing methanol afforded the diketopiperazine **8** in quantitative yield. Deprotection of the Boc group in **8** using HCl in ethyl acetate was followed by guanidylation with 3,5-dimethylpyrazole-1-carboxamidine nitrate (Scott et al., 1953) to afford the catalyst **2**. Reverse phase HPLC purification of **2** produced the catalyst in 45% yield from **8**.

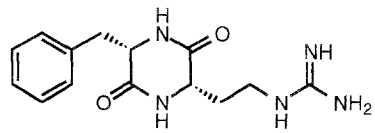


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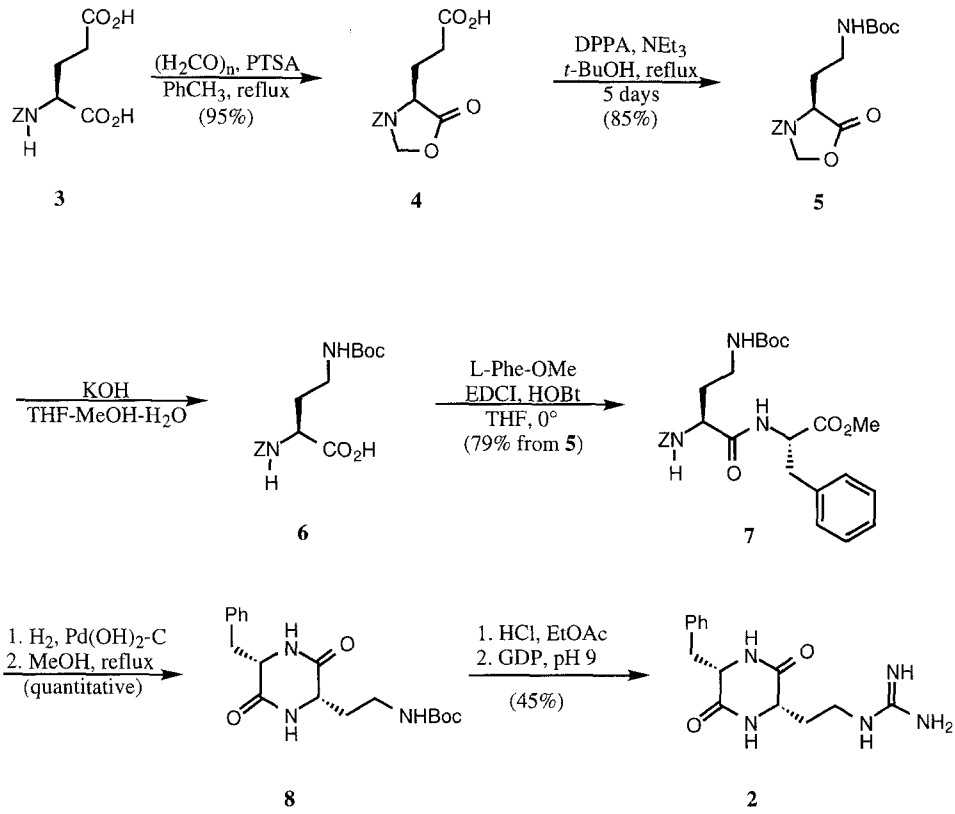
Fig. 2. Cyclo[(*S*)-norarginyl-(*S*)-phenylalanyl], a catalyst for the Strecker synthesis

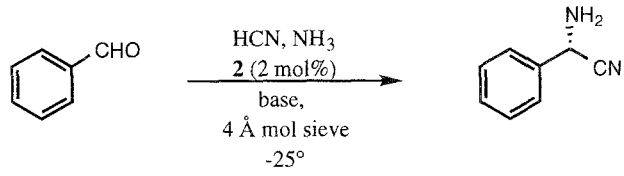


Scheme 5. Synthesis of the diketopiperazine cyclo[(*S*)-norarginyl-(*S*)-phenylalanyl] (**2**)

Initial experiments involved treatment of benzaldehyde with ammonia and hydrogen cyanide in the presence of 2 mol% of catalyst **2** under a variety of reaction conditions (Table 1). The enantiomeric purity of the resultant α-amino nitrile was determined by derivatization with (+)-MTPA chloride (Dale et al., 1969) and comparison of the ^{19}F-NMR spectrum of the crude product with that of an authentic racemate derivatized in identical fashion. The configuration of the products was established by conversion of underivatized α-amino nitriles to phenylglycine and determination of the sign of its optical rotation. Initial experiments showed low to moderate enantioselectivity and moderate to good yield. After reaction conditions were varied with unpredictable results, it was found that the product (2-aminophenylacetonitrile) was configurationally unstable: a purified sample in methanol solution racemized with an approximate half-life of 4 hours.

While the discovery of the configurational instability explained the erratic and disappointing results shown in Table 1, it raised an interesting question: why did the α-amino nitriles obtained from optically active imines not suffer the same problems? Since the only apparent difference was the presence of an alkyl group (e.g., α-methylbenzyl) on nitrogen in those early examples, it was decided to examine the reactions of *N*-substituted imines using catalyst **2** (Table 2). In this version of the reaction, a solution of a pre-formed imine and **2** (2 mol%) in methanol was treated at $-25°$ with hydrogen cyanide (2 equiv.).

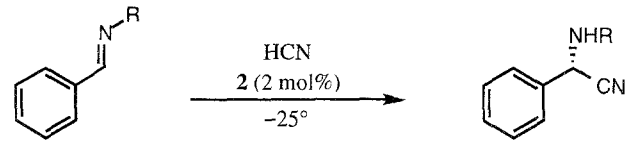
Table 1. Asymmetric catalysis of the Strecker synthesis



solvent	base (eq)	time, h	yield,[a] %	ee,[b] %, S
CH_3OH	PVP	19	20	40
CH_3OH	PVP	32	65	–
CH_3OH	NEt_3	40	68	33
CH_3OH	NEt_3 (2)	96	73	13
CH_3OH[c]	NEt_3	17	83	28
CH_3OH	Py	35	29	23
CH_3OH	Py (2)	40	95	2
CH_3OH	none	52	24	22

[a] Based on ^{1}H-NMR of crude produce. [b] Determined by ^{19}F-NMR of (+)-MTPA derivatives. [c] 147 eq of NH_3 and 11 eq of HCN.

Table 2. Conversion of substituted Imines to α-amino nitriles

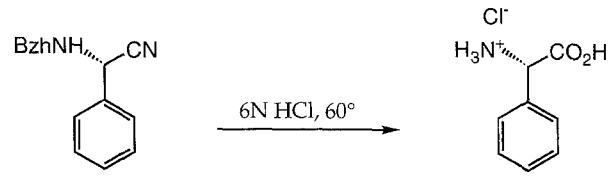


R	solvent	yield,[a] %	ee,[b] %, S
4-OMe-$PhCH_2$	CH_3OH	97	>99
3,4,5-$(OMe)_3PhCH_2$	CH_3OH	98	>98
Ph_2CH	CH_3OH	95	>99
t-BuOCO	i-PrOH	88	75

[a] Based on ^{1}H-NMR of crude product. [b] Determined by ^{19}F-NMR of (+)-MTPA derivatives.

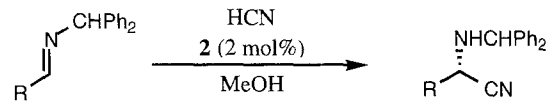
Intriguingly, the presence of alkyl groups on the imine nitrogen resulted in the formation of (S)-α-amino nitriles in high yield and exceptionally high enantiomeric excess. Moreover, all the *N*-substituted α-amino nitriles appeared to be configurationally stable at room temperature, in marked contrast to the parent 2-aminophenylacetonitrile and in accord with literature precedent.

The various *N*-alkyl α-amino nitriles were hydrolyzed according to literature conditions (6*N* HCl, 60°, 6hr) (Marvell, 1920) to attempt simultaneous hydrolysis of the nitrile and deprotection of the amino functionality. Of the derivatives examined, only the benzhydryl-protected α-amino nitrile proved satisfactory in this regard. When the optically active *N*-benzhydryl-α-amino nitrile was subjected to these conditions (Scheme 6), (*S*)-phenylglycine was



Scheme 6. Hydrolysis of (*S*)-*N*-benzhydryl-2-aminophenylacetonitrile to L-phenylglycine

Table 3. Conversion of *N*-benzhydryl imines to α-amino nitriles



R	temp, C	yield,[a] %	ee,[b] %, S
Ph	−25	97	>99
4-Cl-Ph	−25	97	83
4-Cl-P	−75	94	>99
4-OMe-Ph	−25	96	64
4-OMe-Ph	−75	90	96
3-Cl-Ph	−75	80	>99
3-OMe-Ph	−75	82	80
2-furyl	−25	83	19
2-furyl	−75	94	32
i-Pr	−75	81	<10
t-Bu	−75	80	17

[a]Based on ^{1}H-NMR of crude product. [b]Determined by chiral HPLC chromatography using a Daicel ChiralPak AD column.

obtained without loss of optical activity as determined by optical rotation. Benzaldehyde can thus be converted to (*S*)-phenylglycine in 3 steps, in 92% yield and >99% e.e.

The generality of this methodology was explored by subjecting various *N*-benzhydryl imines derived from aromatic and aliphatic aldehydes to the reaction conditions described above (Table 3). Enantiomeric purity of the product α-amino nitriles was determined by chiral HPLC chromatography and absolute configuration was assigned by hydrolysis of the α-amino nitriles and comparison of the optical rotations of the α-amino acids to literature values.

The products derived from aromatic aldehydes were obtained in high enantiomeric excess but those derived from aliphatic aldehydes were obtained only with low enantiomeric excess. In all cases, the enantioselectivity could be improved by lowering the reaction temperature, but preliminary studies indicate that increasing the amount of catalyst did not improve the enantioselectivities of any of the problematic substrates. In all cases examined, the (*S*)-isomer was the major product.

Thus, it would appear that catalyst **2** will be most useful in the synthesis of phenylgycine derivatives. Obtaining this subclass of α-amino acids in optically

pure form represents a formidable synthetic challenge owing to their relative inaccessibility (Williams and Hendrix, 1992). It is therefore envisaged that catalyst **2** will find application in the large-scale production of a wide variety of important arylglycines.

Despite the structural similarities between **1** and **2** and the mechanistic similarities between the reactions they catalyze, several important differences exist which belie the apparent similarity. First, whereas **1** operates as a heterogeneous catalyst, **2** is fully soluble under the conditions of the Strecker synthesis. Second, although both **2** and **1** are composed of (*S*)-amino acids, they catalyze formation of products of *opposite* configuration. Additionally, studies conducted on **1** have also demonstrated a dependence of the enantioselectivity on the method of crystallization of the catalyst (Tanaka, 1990), solvent viscosity (Danada, 1991) and on enantioselective autocatalysis by the product itself (Danda, et al., 1991). Studies are ongoing in our laboratories to investigate the effects of these and other factors on the enantioselectivity of the Strecker synthesis using catalyst **2**.

Acknowledgement

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References

Becker W, Freund H, Pfeil E (1965) Stereospecific synthesis of D-hydroxynitriles and optically active ethanolamines. Angew Chem Int Ed Engl 4: 1079

Chakraborty TK, Reddy GV, Hussain KA (1991) Diastereoselective Strecker synthesis using α-phenylglycinol as chiral auxiliary. Tetrahedron Lett 32: 7597–7600

Chakraborty TK, Hussain, KA, Reddy GV (1995) α-Phenylglycinol as chiral auxiliary in diastereoselective Strecker synthesis of α-amino acids. Tetrahedron 51: 9179–9190

Dale JA, Dull DL, Mosher HS (1969) α-Methoxy-α-trifuoromethylphenylacetic acid, a versatile reagent for the determination of enantiomeric composition of alcohols and amines. J Org Chem 34: 2543–2549

Danda H (1991) Essential factors in asymmetric hydrocyanation catalyzed by cyclo(-(*R*)-Phe-(*R*)-His-). Synlett: 263–264

Danda H, Nishikawa H, Otaka K (1991) Enantioselective autoinduction in the asymmetric hydrocyanation of 3-phenoxybenzaldehyde catalyzed by cyclo[(*R*)-phenylalanyl-(*R*)-histidyl]. J Org Chem 56: 6740–6741

Davis FA, Reddy RE, Portonovo PS (1994) Asymmetric Strecker synthesis using enantiopure sulfinimines: a convenient synthesis of α-amino acids. Tetrahedron Lett 35: 9351–9354

Davis FA, Portonovo PS, Reddy RE, Chiu Y (1996) Asymmetric Strecker synthesis using enantiopure sulfinimines and diethylaluminum cyanide: the alcohol effect. J Org Chem 61: 440–443

Duthaler RO (1994) Recent developments in the stereoselective synthesis of α-aminoacids. Tetrahedron 50: 1539–1650

Effenberger F (1994) Synthesis and reactions of optically active cyanohydrins. Angew Chem Int Ed Engl 33: 1555–1564

Ellman J, Mendel D, Anthony-Cahill S, Noren CJ, Schultz PG (1991) Biosynthetic method for introducing unnatural amino acids site-specifically into proteins. Methods Enzymol 20: 301–306

Harada K (1963) Asymmetric synthesis of α-amino-acids by the Strecker synthesis. Nature 200: 1201

Hruby VJ, Sharma SD (1991) Designing peptide and protein ligands for biological receptors. Curr Opin Biotechnol 2: 599–605

Inaba T, Kzono I, Fujita M, Ogura K (1992) An efficient and practical synthesis of L-α-amino acids using (*R*)-phenylglycinol as a chiral auxiliary. Bull Chem Soc Jpn 65: 2359–2365

Lapworth J (1903) Reactions involving the addition of hydrogen cyanide to carbon compounds. J Chem Soc 83: 995–1009

Marvell CS, Noyes WA (1920) A study of the possible asymmetry of the aliphatic diazo compounds. J Am Chem Soc 42: 2259–2278

North M (1993) Catalytic asymmetric cyanohydrin synthesis. Synlett: 807–820

Ogata Y, Kawasaki A (1970) Mechanistic aspects of the Strecker aminonitrile synthesis. J Chem Soc (B) 1971: 325–329

Oku J, Inoue S (1981) Asymmetric cyanohydrin synthesis catalyzed by a synthetic cyclic dipeptide. J Chem Soc Chem Commun 1981: 229–230

Patel MS, Worsley M (1969) Asymmetric synthesis of optically active α-amino acids by hydrocyanic acid addition to the optically active Schiff base. Can J Chem 48: 1881–1884

Phadtare SK, Kamat SK, Panse GT (1985) Asymmetric synthesis of α-amino acids. Ind J Chem 24B: 811–814

Prelog V, Wilhelm M (1954) Untersuchungen über asymmetrische Synthesen VI). Der Reaktionsmechanismus und der sterische Verlauf der asymmetrischen Cyanhydrin-Synthese. Helv Chim Acta 37: 1634–1660

Scholtz JM, Bartlett PA (1989) A convenient differential protection strategy for functional group manipulation of aspartic and glutamic acids. Synthesis: 542–544

Scott FL, O'Donovan DG, Reilly J (1953) Studies in the pyrazole series. III. Substituted guanidines. J Am Chem Soc 75: 4053–4054

Strecker A (1850) Über die künstlische Bildung der Milchsäure und einen neuen, dem Glycocoll homologen Körper. Liebigs Ann Chem 75: 27–51

Tanaka K, Mori A, Inoue S (1990) The cyclic dipeptide cyclo[(*S*)-phenylalanyl-(*S*)-histidyl)] as a catalyst for asymmetric addition of hydrogen cyanide to aldehydes. J Org Chem 55: 181–185

Walia JS, Bannore SN, Walia AS, Guillot L (1974) Mechanism for the formation of α-aminonitriles: intermediacy of an imine or a cyanohydrin. Chem Lett: 1005–1008

Wallace CJA, Mascagni P, Chait BT, Collawn JF, Paterson Y, Proudfoot AE, Kent SBH (1989) Substitutions engineered by chemical synthesis at three conserved sites in mitochondrial cytochrome c. J Biol Chem 264: 15199–15209

Weinges K, Blackholm H (1980) Herstellung optisch aktiver Aminonitrile. Chem Ber 113: 3098–3102

Williams RM (1989) Synthesis of optically active α-amino acids. In: Baldwin JE, Magnus PD (eds) Organic chemistry series. Pergamon, Oxford

Williams RM, Hendrix JA (1992) Asymmetric synthesis of arylglycines. Chem Rev 92: 889–917

Authors' address: Dr. M. Lipton, Department of Chemistry, Purdue University, West Lafayette, IN 47907, U.S.A.

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