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Scaleable catalytic asymmetric Strecker syntheses of unnatural α-amino acids

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Abstract

α-Amino acids are essential building blocks for protein synthesis, and are also widely useful as components of medicinally active molecules and chiral catalysts.1,2,3,4,5 Efficient chemo-enzymatic methods for the synthesis of enantioenriched α-amino acids have been devised, but the scope of these methods for the synthesis of unnatural amino acids is limited.6,7 Alkene hydrogenation is broadly useful for enantioselective catalytic synthesis of many classes of amino acids,8,9 but this approach is not applicable to the synthesis of α-amino acids bearing aryl or quaternary alkyl α-substituents. The Strecker synthesis—the reaction of an imine or imine equivalent with hydrogen cyanide, followed by nitrile hydrolysis—is an especially versatile chemical method for the synthesis of racemic α-amino acids (Fig. 1).10,11 Asymmetric Strecker syntheses using stoichiometric chiral reagents have been applied successfully on gram-to-multi-kilogram scales to the preparation of enantiomerically enriched α-amino acids.12,13,14 In principle, Strecker syntheses employing sub-stoichiometric quantities of a chiral reagent provide a practical alternative to these approaches, but the reported catalytic asymmetric methods have seen only limited use on preparative scales (e.g., > 1 gram).15,16 The limited use of existing catalytic methodologies may be ascribed to several important practical drawbacks, including the relatively complex and precious nature of the catalysts, and the requisite use of hazardous cyanide sources. Herein we report a new catalytic asymmetric method for the syntheses of highly enantiomerically enriched non-proteinogenic amino acids using a simple chiral amido-thiourea catalyst to control the key hydrocyanation step. Because this catalyst is robust and lacks sensitive functional groups, it is compatible with safely handled aqueous cyanide salts, and is thus adaptable to large-scale synthesis. This new methodology can be applied to the efficient syntheses of amino acids that are not readily prepared by enzymatic methods or by chemical hydrogenation.

The urea- and thiourea-catalyzed Strecker synthesis of (*R*)-*tert*-leucine developed several years ago by our group illustrates many of the factors that have limited the application of catalytic asymmetric imine hydrocyanation methods towards routine preparative-scale

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Author Contributions S.J.Z. and M.P.L. synthesized and evaluated the catalysts; M.P.C. evaluated the scope of the TMSCN-mediated reaction; S.J.Z. developed the KCN-mediated syntheses and the largescale procedures; S.J.Z. and E.N.J. wrote the manuscript; E.N.J. guided the research.

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syntheses (Fig. 2).17, 18 Although this synthesis provides (*R*)-*tert*-leucine in high yield and enantiomeric excess (e.e.), the hydrocyanation reaction is run at cryogenic temperatures and uses a hazardous cyanide source: either trimethylsilyl cyanide (TMSCN)/methanol (MeOH) or HCN. In addition, the syntheses of either polystyrene-bound catalyst **1a** or homogeneous analogue **1b** require eight chemical steps, and the conversion of the α-aminonitrile to the α-amino acid requires four chemical steps.

In mechanistically and synthetically guided efforts to identify simpler small-molecule H-bond donors for enantioselective imine hydrocyanation, we discovered that amido-thiourea derivatives lacking the diaminocyclohexane moiety in **1**,19,20 are efficient catalysts for the hydrocyanation of *N*-benzhydryl-protected imines using HCN generated in situ from TMSCN and MeOH (Table 1).21 Whereas simple amido-thiourea **4a** induced low levels of enantioselectivity in hydrocyanation of both aliphatic and aromatic imines (entry 1), the corresponding phenyl-substituted amido-thioureas proved more effective (entries 2–4). Highest enantioselectivities were observed in reactions catalyzed by *N*-benzhydryl-substituted catalyst **4e** (entry 5). This catalyst contains a single stereogenic center and is prepared in three steps from commercially available reagents (74% overall yield on 5-gram scale), and is thus readily accessible compared with chiral Strecker catalysts identified previously.15,16

Amido-thiourea derivative **4e** proved effective and highly enantioselective for the hydrocyanation of imines derived from alkyl (Table 2, entries 1–5), aryl (entries 7–16), heteroaryl (entries 17–19), and alkenyl aldehydes (entries 20–22). Lower enantioselectivities are obtained with less sterically demanding imines (entries 6 and 24). In general, (*R*)-α-aminonitriles are obtained from the catalyst derived from (*S*)-*tert*-leucine. This fortuitous outcome introduces an important practical feature of this methodology, because (*S*)-*tert*-leucine is readily available inexpensively by enzymatic methods,6 but practical catalytic methods do not exist for the synthesis of (*R*)-*tert*-leucine and related (*R*)-amino acids.22

High yields and enantioselectivities are obtained in these imine hydrocyanations catalyzed by **4e**; however, TMSCN is expensive, and the stoichiometric HCN generated upon combination with methanol introduces serious practical and safety liabilities that limit application on preparative scale. Potassium cyanide (KCN) and sodium cyanide (NaCN) represent inexpensive, alternative cyanide sources for Strecker syntheses,13,14,23 but these reagents have found limited application in catalytic asymmetric imine hydrocyanations developed to date. This may be attributed to the poor solubility of cyanide salts in organic solvents, and the incompatibility of known catalysts to aqueous media. In contrast, catalyst **4e** lacks any sensitive functional groups, and therefore might be adaptable to use under aqueous or biphasic conditions. Indeed, treatment of toluene solutions of imine **2a** with KCN, acetic acid, water, and catalyst **4e** led to the formation of α-aminonitriles with similar enantioselectivity as was observed in the homogeneous, TMSCN/MeOH-mediated reaction (Fig. 3). Only small decreases in enantioselectivity were observed at higher temperatures and concentrations, and reactions carried out under these more practical conditions proceeded at substantially higher rates.

The efficiency of this reaction is relatively insensitive to small changes in reagent and catalyst concentration: using an optimized protocol, hydrocyanation experiments using 0.5 mol% catalyst were executed reproducibly and safely on 25–100 mmol scale of aliphatic imines **2a**, **2b**, and **2d** (Fig. 3). These imines were prepared on multi-gram scales in one or two steps from commercially available aldehydes.24 The hydrocyanation reaction mixtures were treated with aqueous K_2CO_3 prior to workup to quench any unreacted HCN generated under the reaction conditions. The enantiomerically enriched α-aminonitriles were isolated in crude form by routine extraction and solvent removal procedures, and converted to the corresponding *tert*-butoxycarbonyl-protected (*R*)-α-amino acids by a two-step sequence involving H_2SO_4/HCl-mediated hydrolysis followed by treatment of the resulting aqueous amino acid solutions with di-*tert*-butyl dicarbonate (Boc_2O).25,26 The highly enantiomerically enriched, sterically demanding protected α-amino acids were then isolated on multi-gram scales by recrystallization. In each case, the synthetic sequence required no chromatographic purification or specialized equipment.

α-Amino acids bearing quaternary alkyl substituents, especially *tert*-leucine, are common components of pharmaceuticals3 or medicinal chemistry targets,27 and their derivatives have been found to be highly effective as components of chiral ligands5 and organocatalysts28 used in small molecule asymmetric catalysis (Fig. 4). Use of these α-amino acids has largely been limited to (*S*)-*tert*-leucine, which may be prepared efficiently by enzymatic methods.6 The method described in this paper allows for efficient access both to the (*R*)-enantiomer of *tert*-leucine and to more sterically demanding analogues, thereby expanding the pool of α-amino acids that can be used in medicinal and other applications.

A detailed experimental and computational29 analysis of the hydrocyanation reaction catalyzed by **4e** points to a mechanism involving initial amido-thiourea-induced imine protonation by HCN to generate a catalyst-bound iminium/cyanide ion pair (Fig. 5). Collapse of this ion pair and C–C bond formation to form the α-aminonitrile then occurs in a post–rate-limiting step. Complete details of this novel mechanistic hypothesis and the basis for enantioselectivity will be reported separately.30

Methods Summary

Reactions were carried out in round-bottomed flasks under nitrogen, unless otherwise noted. Commercially available reagents were purchased and used as received unless otherwise noted. Catalysts, imines, and α-amino acids were characterized by NMR and infrared spectroscopy, and by mass spectrometry. The e.e. of chiral, non-racemic α-amino acids was determined by chiral HPLC analysis of the benzyl ester derivatives. α-Aminonitriles were characterized by NMR and infrared spectroscopy, and the enantiomeric excesses were determined by chiral HPLC analysis. For experimental details and spectroscopic characterization data, chiral HPLC traces of racemic and non-racemic α-aminonitriles and benzyl esters of α-amino acids, ^1H and ^{13}C NMR spectra of catalyst **4e** and α-amino acids, and the geometry of the calculated intermediate, see the Supplementary Information.

Methods

Preparation of α-aminonitrile 3a by KCN-mediated hydrocyanation

Caution! HCN is produced. The experiment should be executed in a well-ventilated fume hood. A 250-mL round-bottomed flask containing a 4-cm long stir bar was charged with KCN (5.21 g, 80 mmol, 2.0 equiv) and toluene (76 mL), capped with a virgin rubber septum, and cooled at 0 °C for 10 min under N_2. Acetic acid (2.75 mL, 48 mmol, 1.2 equiv) and water (2.88 mL, 160 mmol, 4.0 equiv) were added sequentially via syringe, and the N_2 inlet was removed. The resulting white, heterogeneous mixture was stirred vigorously at 0 °C. After 5 min the upper organic layer had become a clear, colorless solution, and the lower aqueous layer contained a chunky, white precipitate. After stirring for 20 min, the N_2 inlet was restored, and a freshly prepared stock solution of **2a** (9.79–9.93 g, prepared from 40 mmol of aminodiphenylmethane as described in the Supplementary Information) and **4e** (116 mg, 0.20 mmol, 0.0050 equiv) in toluene (24 mL) was added via syringe in 10-mL portions over 1 min. The flask containing the stock solution was rinsed with additional toluene (2 × 3 mL), and the rinses were added to the reaction. The N_2 inlet was removed, and the mixture was stirred at 0 °C. The reaction was monitored as follows: a 100 μL aliquot was removed via syringe, filtered through a 1-cm high plug of Na_2SO_4, rinsed with hexanes (2 × 3 mL), and concentrated under reduced pressure. The sample was dissolved in 600 μL $CDCl_3$ and analyzed by ^{1}H-NMR spectroscopy. After 2.5 h, conversion was estimated to be 95% by integration of the benzhydryl resonances of the starting material (5.4 ppm) and product (5.2 ppm). After 4 h, the reaction mixture was allowed to warm to room temperature over 5 min. The septum was removed, and the reaction mixture was treated with 50 mL of a 0.2 g/mL aqueous K_2CO_3 solution. The mixture was transferred to a 250-mL separatory funnel in a fume hood, the reaction flask was rinsed with diethyl ether (3 × 5 mL), and the rinses were added to the separatory funnel. The organic and aqueous layers were thoroughly mixed, and the aqueous layer removed. The organic layer was washed with another 50 mL of K_2CO_3 solution and then with brine (50 mL). The aqueous layers were disposed in a waste container that was maintained at basic pH and stored in a fume hood. The clear, colorless organic layer was dried over Na_2SO_4, decanted into a 500-mL round-bottomed flask, rinsing with diethyl ether (3 × 5 mL), and concentrated to a volume of approximately 100 mL using a rotary evaporator. The flask was then charged with a 2-cm long stir bar, placed in a 25 °C water bath, and concentrated to a volume of approximately 15 mL by vacuum transfer into a dry ice/acetone bath. A sample of the clear, colorless liquid residue was analyzed by chiral HPLC analysis (AS-H, 1 mL/min, 5% *iso*-propanol/hexanes, 220 nm): t_R(minor) = 6.18 min, t_R(major) = 9.09 min, 87–88% ee (range of three experiments). The liquid was transferred to a 100-mL round-bottomed flask, rinsing with CH_2Cl_2 (3 × 4 mL). The solution was concentrated to a mass of 12 g under reduced pressure (30 torr → 1 torr). ^{1}H-NMR analysis of the clear, colorless oil revealed approximately 30 mol % remaining toluene. Crude **3a** was used in the next step without further purification. Experimental procedures for the hydrolysis of **3a** and for the isolation of (*R*)-Boc-*tert*-leucine are provided in the Supplementary Information.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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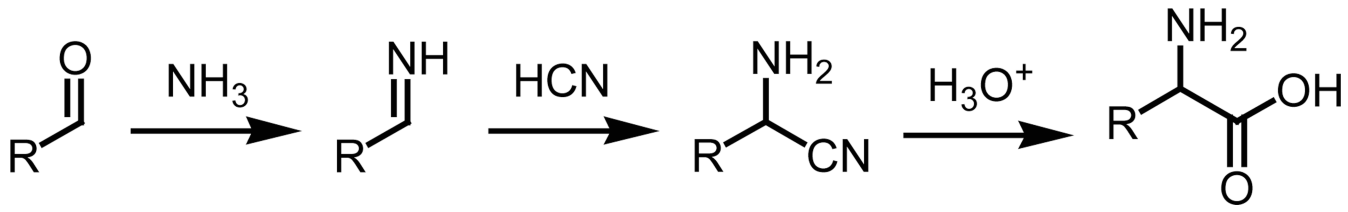


Figure 1. Strecker synthesis of α-amino acids

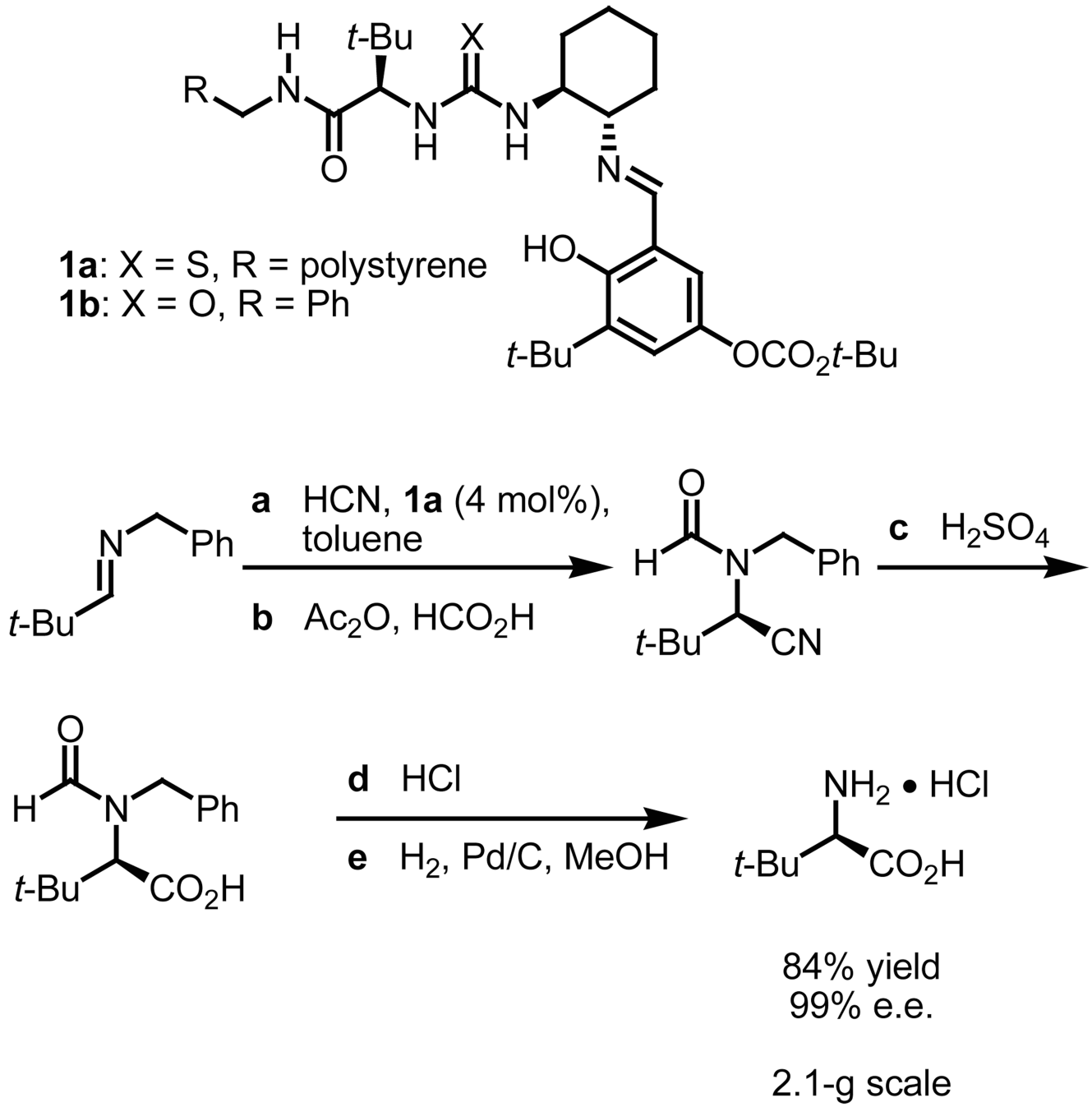


Figure 2. First-generation thiourea-catalyzed asymmetric Strecker synthesis of *tert*-leucine

a, HCN (1.3 equiv.), **1a** (4 mol%), toluene, −75 °C, 15 h. **b**, acetic anhydride (Ac_2O), HCO_2H, 5 min. **c** 65% aqueous H_2SO_4, 45 °C, 20 h. **d**, concentrated HCl, 13 h. **e**, H_2, Pd/C (0.1 equiv.), methanol (MeOH), 8 h. Ph, phenyl; *t*-Bu, *tert*-butyl.

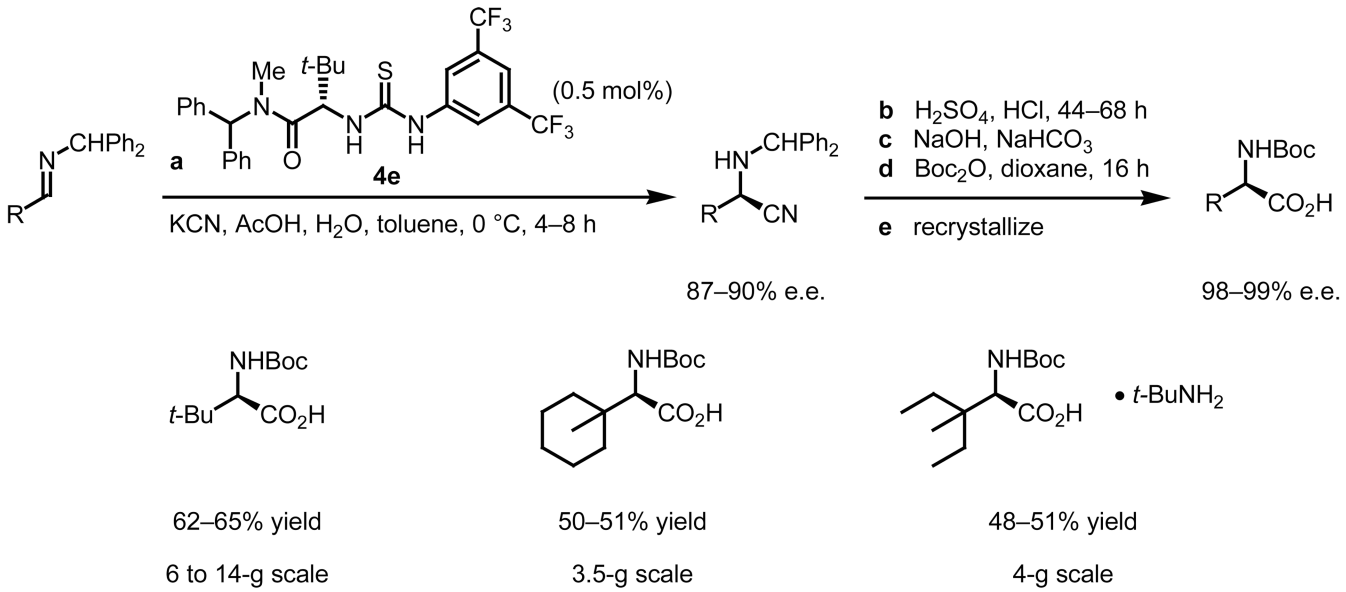
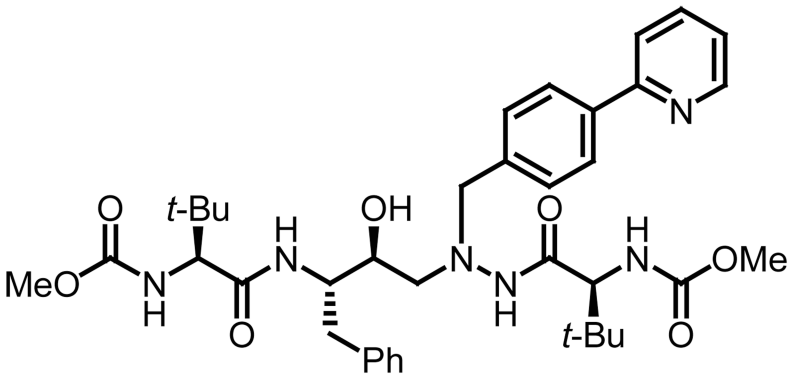
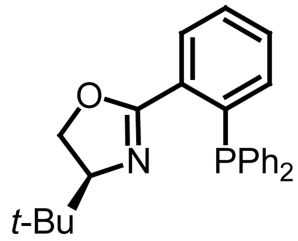


Figure 3. Potassium cyanide-mediated Strecker synthesis

a, Catalyst **4e** (0.5 mol%), KCN (2 equiv.), acetic acid (AcOH, 1.2 equiv.), H_2O (4 equiv.), toluene, 0 °C, 4–8 h. **b**, aqueous H_2SO_4 and HCl, 120 °C, 44–68 h. **c**, NaOH, $NaHCO_3$. **d**, di-*tert*-butyl dicarbonate (Boc_2O, 2.5–3 equiv.), dioxane, 16 h. **e**, recrystallize directly from hexanes/diethyl ether or as the *tert*-butylamine (*t*-$BuNH_2$) salt from tetrahydrofuran/ethanol. Ph, phenyl; *t*-Bu, *tert*-butyl; Boc = *tert*-butoxycarbonyl.



Atazanavir (HIV protease inhibitor)



(*S*)-*t*-Bu-phox
(ligand for asymmetric catalysis)

Figure 4. Examples of a pharmaceutical product and a chiral ligand derived from *tert*-leucine

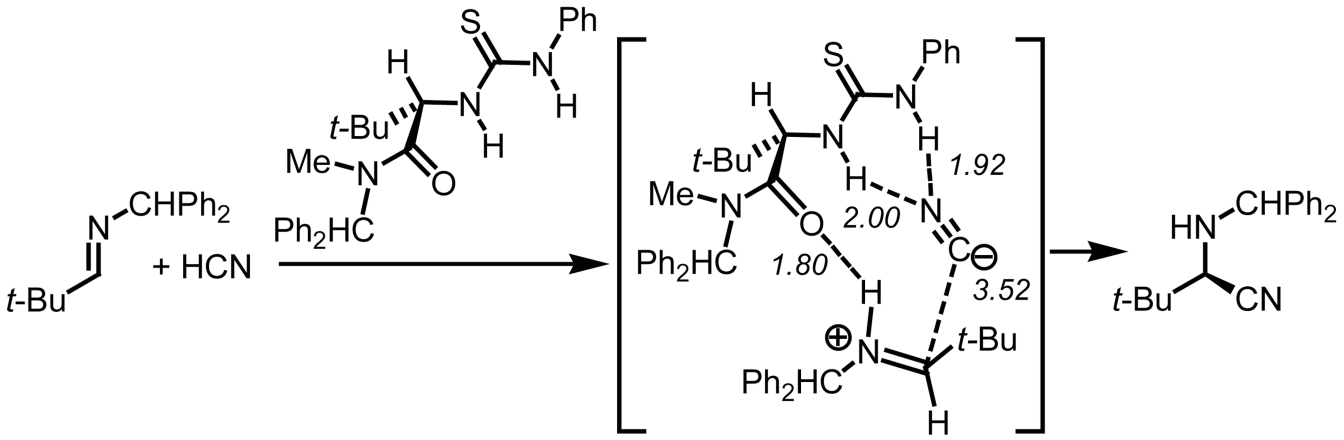
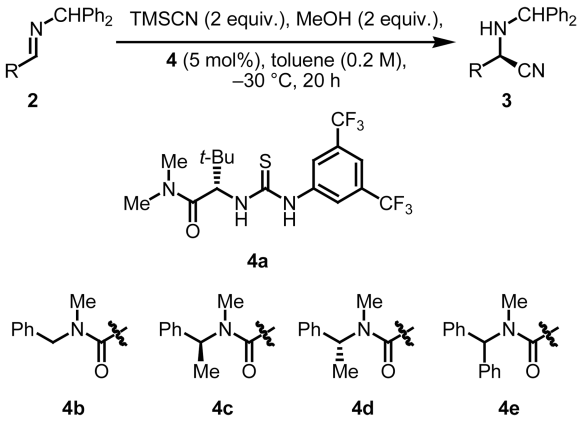


Figure 5. Proposed catalytic mechanism

The structure in brackets is the iminium/cyanide ion pair intermediate that directly precedes C–C bond formation, as calculated using the B3LYP/6-31G(d) level of density functional theory within Gaussian 03. Bond distances are shown in Å. Me, methyl, *t*-Bu, *tert*-butyl, Ph, phenyl. Three-dimensional coordinates are included in the Supplementary Information.

Table 1

Dependence of imine hydrocyanation enantioselectivity on catalyst structure



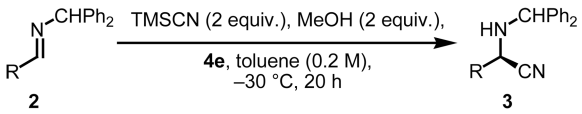
Entry	Catalyst	e.e.[a] (%) R = *t*-Bu	e.e.[a] (%) R = C_6H_5
1	**4a**	−14	41
2	**4b**	30	86
3	**4c**	58	90
4	**4d**	77	97
5	**4e**	93	98

[a] Enantiomeric excess (e.e.) determined by chiral high performance liquid chromatography analysis using commercially available columns.

Me, methyl; Ph, phenyl; *t*-Bu, *tert*-butyl.

Table 2

Scope of asymmetric imine hydrocyanation



Entry	Imine 2 (R =)	4e (mol%)	Yield[a] (%)	e.e.[b] (%)
1	*t*-butyl (**a**)	2	99	93
2	Et_2MeC (**b**)	2	99	96
3	$PhMe_2C$ (**c**)	2	97	85
4	(1-methyl)cyclohexyl (**d**)	2	99	95
5	1-adamantyl(**e**)	2	99	93
6	cyclohexyl (**f**)	2	99	74
7	*p*-$OMeC_6H_4$ (**g**)	2	99	99
8	*p*-MeC_6H_4 (**h**)	2	98	98
9	C_6H_5 (**i**)	2	98	98
10	*p*-ClC_6H_4 (**j**)	2	97	98
11	*p*-$CF_3C_6H_4$ (**k**)	2	98[c]	96
12	*p*-CNC_6H_4 (**l**)	10	96[c]	93
13	*p*-BrC_6H_4 (**m**)	2	98	99
14	*o*-$OMeC_6H_4$ (**n**)	2	97	88
15	*o*-BrC_6H_4 (**o**)	2	96[c]	97
16	*m*-BrC_6H_4 (**p**)	2	97[c]	92
17	2-furyl (**q**)	2	99	93
18	3-furyl (**r**)	2	98	97
19	2-thiophenyl (**s**)	2	97[c]	95
20	1-cyclohexenyl (**t**)	2	99	95
21	(*E*)-1-methyl-pent-1-enyl (**u**)	2	98	91
22	(*E*)-cinnamyl (**v**)	2	99	95
23	(*E*)-hexenyl (**w**)	2	97	73

[a] Isolated yields of **3** after silica gel chromatography of reactions run on 1.0 mmol scale.

[b] Enantiomeric excess (e.e.) determined by chiral high performance liquid chromatography analysis using commercially available columns.

[c] Reaction run at 0 °C.

Et, ethyl; Me, methyl; Ph, phenyl.