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Nitrilase-Catalyzed Enantioselective Synthesis of Pyrrolidine- and Piperidinecarboxylic Acids

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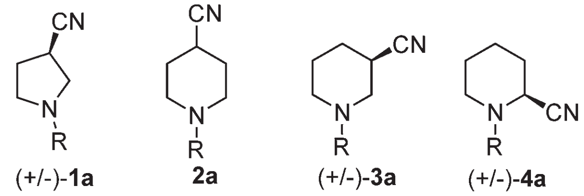
Abstract: The enantioselective synthesis of the non-proteinogenic amino acids β-proline and nipecotic acids from their readily available nitriles is achieved in high enantiomeric excess by commercially available nitrilases. The presented procedure comprises not more than 4 steps, thus considerably reducing the multiple steps generally required. Amide formation is also observed for specific heterocyclic nitriles.

Keywords: biocatalysis; enantioselective synthesis; (iso)nipecotic acid; nitrilases; pipecolic acid; β-proline

Introduction

The heterocyclic non-proteinogenic amino acids β-proline (pyrrolidine-3-carboxylic acid), pipecolic acid (piperidine-2-carboxylic acid), nipecotic acid (piperidine-3-carboxylic acid) as well as isonipecotic acid (piperidine-4-carboxylic acid) are common substructures in natural and synthetic bioactive compounds[1–3] and deserve attention due to numerous other applications. Several successful efforts have been made to synthesize these acids in an enantioselective manner by chemical synthesis, though they generally involve multistep procedures.[4] Some approaches have made use of biocatalysts to introduce asymmetry. Thus, the synthesis of both (+)- and (−)-β-proline by a microbial Baeyer–Villiger oxidation[5] has been reported. The synthesis of (*S*)-pipecolic acid involving (*R*)-hydroxynitrile lyase[2a] and the kinetic resolution of racemic amides by stereospecific amidases from whole cells[2b] as well as by acylase[6] in high enantiomeric excess has also been published.

The availability of ready to use nitrilase preparations[7] with the advantage to avoid the laborious handling of whole cell biotransformation systems has prompted us to search for an efficient and decisively short enantioselective synthesis of these heterocyclic amino acids from their readily available nitriles **1a**–**4a** (Figure 1). In the course of our current research on non-proteinogenic amino acids from nitriles, it became apparent that N-heterocyclic nitriles are highly applicable substrates for nitrilases.



R = toluenesulfonyl (Ts); benzyloxycarbonyl (Cbo);
tert-butyloxycarbonyl (Boc);

Figure 1. Heterocyclic amino nitriles for enzymatic transformations [only one enantiomer is depicted for racemic structures (±)-**1a**, (±)-**3a** and (±)-**4a**].

Results and Discussion

Synthesis of Substrates

The racemic heterocyclic nitrile (±)-**1a-Cbo** as well as **2a-Cbo** and **2a-Boc** were prepared from commercially available (±)-3-pyrrolidinol and 4-hydroxypiperidine, respectively, after protection of the ring nitrogens, following mesylation and S_N2 displacement by cyanide. In case of **1a**–**3a-Ts**, ditosylation of 4- and (±)-**3**-hydroxypiperidine or pyrrolidine and displacement reaction yielded the desired nitriles directly, although accompanied by some elimination products. Pipecolic carbonitriles **4a** were prepared from piperidine according to a literature reported procedure[8] and subsequent *N*-protection.

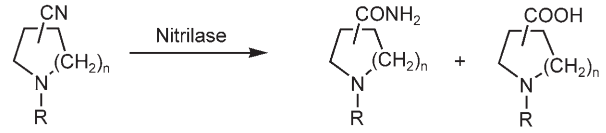
The respective amides **1b**–**4b** were required as HPLC references in those cases were additional product peaks appeared in the biotransformation reactions. They were synthesized according to a standard protocol.[2d]

The spectroscopic and physical data as well as elemental analysis data of the novel compounds are given in the Experimental Section.

Biotransformations

Initially, all the amino nitriles (Figure 1) were subjected to biotransformations on a screening level employing eight nitrilases (see Supporting Information). For better comparability all screening experiments were stopped after 18 h of reaction time. All heterocyclic nitriles (±)-**1a, 2a** and (±)-**3a** are suitable substrates for all nitrilases regarding substrate specificity, except piperidine-2-carbonitrile (±)-**4a**, which is a substrate only for NIT-106. Due to the very fast transformation in the case of (±)-**1a**, screening data were also collected after 0.5 and 2 h to estimate the relative performance of the nitrilases. Not surprisingly, some transformation reactions were accompanied by the formation of the corresponding amides, such as pipecolic amide **4b-Ts** (up to 10%). Surprisingly, 2–4% of isonipecotic amide **2b-Cbo** and pyrrolidine-3-carboxamides **1b-Cbo** and **1b-Ts** were observed throughout in amounts of 1–31% (the latter by NIT-104). Such a nitrilase-catalyzed amide formation has been increasingly noticed in the past and the majority of this formerly unexpected nitrile hydratase activity of nitrilases occurred when substrates activated in the α-position to the nitrile group were reacted.[9–11] Most recently, a study on this subject suggesting a mechanistic rationale of amide formation appeared.[12] In the case of pipecolic carbonitrile (±)-**4a-Ts**, the amide is even the predominantly formed product. All amides were confirmed by comparing their HPLC retention times with the chemically synthesized reference compounds.

On the basis of screening results, reaction protocols were established for the particular nitriles with the appropriate nitrilase (Scheme 1). Nitrilases exhibiting nitrile hydratase activity were omitted, if possible. The reactions were monitored by HPLC and stopped at the time of the maximal expectable enantiopurity/conversion of the acids according to kinetic resolution rationale. Following work-up and isolation/purification, the *ee*s of β-proline and piperidine carboxylic acid derivatives were determined by chiral HPLC (Experimental Section). The attention attracted by the present acids as well as their commercial potential can be appreciated from the number of asymmetric syntheses and patent applications reported.[4] All procedures share some obstacles, such as the requirement of multiple synthetic steps and/or expensive chiral catalysts. It is remarkable that the most atom-economic synthesis for these monocyclic acids, the asymmetric hydrogenation of the respective aromatic compounds, is an unsolved problem until now. Recently, the hydrogenation of nipecotic acid from nicotinic acid was reported not to be achievable in a straightforward manner, instead, partial hydrogenation of nicotinate and subsequent asymmetric hydrogenation had to be carried out to gain a high *ee* of the final product, depending also on a specific N-protecting group.[4e]



rac 1a – 4a **1b – 4b** **1c – 4c**

R = toluenesulfonyl (Ts); benzyloxycarbonyl (Cbo); *tert*-butyloxycarbonyl (Boc); n = 1, 2

Scheme 1. Nitrilase-catalyzed enantioselective transformation of pyrrolidine and piperidine carbonitriles.

A pronounced difference in catalytic activity was noticed depending on ring size. Five-membered pyrrolidine-3-carbonitriles were formed in close to theoretical yields within a maximum reaction time of 24 h. The isolated yields and enantiopurities are given in Table 1. All six-membered piperidine 3- and 4-carbonitriles required transformation times within days. Irrespective of that, the nitrilases performed with nearly unchanged activity over this period of time. Thus, nipecotic acid could be prepared and isolated in 93% *ee*. *N*-Toluenesulfonyl protected acids were formed in enantioselectivities superior to the *N*-Cbo derivatives, a fact we also have observed throughout work on carbocyclic γ-amino acids.[13]

Table 1. Enzymatic preparation of heterocyclic amino acids – isolated yields.

Substrate	Enzyme	Substrate conc.	Enzyme conc.	Time	Conversion [%]	*ee* [%]	Yield of **c** [%]
1a-Cbo	NIT-106	6.80 mM	$1.67\ \mathrm{g\,L^{-1}}$	22.5 h	46	10	38
1a-Cbo	NIT-108	13.4 mM	$2.75\ \mathrm{g\,L^{-1}}$	24 h	34	49	29
1a-Ts	NIT-106	0.247 mM	$0.20\ \mathrm{g\,L^{-1}}$	12.5 h	44	76	42
2a-Cbo	NIT-108	8.24 mM	$2.20\ \mathrm{g\,L^{-1}}$	5 d	32	-	28
2a-Ts	NIT-106	2.24 mM	$1.42\ \mathrm{g\,L^{-1}}$	6 d	86	-	85
3a-Ts	NIT-107	0.382 mM	$0.29\ \mathrm{g\,L^{-1}}$	6 d	50	93	40

Although some product from (±)-**4a** was formed by NIT-106, pipecolic acid can obviously not be synthesized in a preparatively satisfying way with the available nitrilases, in particular because the amide formation is twice as high as the acid formation. This formation is well consistent with the picture of other α-activated nitriles.[8–10] Current enzyme engineering is concerned with modification of particular nitrilases to prepare pipecolic acid as well as further heterocyclic acids of commercial importance.[14]

A comparison of the present heterocyclic β-amino nitriles (±)-**1a** and (±)-**3a**[15] with carbocyclic β-amino nitrile analogues bearing the amino group in an exocyclic position,[16–18] evidences a strictly diverging reactivity with nitrilases: the former heterocycles are excellent substrates, the carbocyclic compounds are non-substrates. Contrary to nitrilases though, such cyclopentane- and cyclohexanecarbonitriles can be transformed enantiosectively to β-amino acids by nitrile hydratases in whole cells.[16–18] This low substrate specificity of nitrilases regarding the carbocyclic structures is likely due to a sterically unfavourable position of the exocyclic amino group in the 2-position. A similar situation is encountered in (±)-**4a**, where the protecting group of the ring nitrogen atom is acting in the manner of an exocyclic substituent in 2-position, thus resulting in substantial hindrance for the enzyme. Consequently, the activity towards (±)-**4a** is also very low (Table S2 in Supporting Information).

Conclusions

The practicability of an enantioselective synthesis to non-proteinogenic N-heterocyclic amino acids of high commercial value was demonstrated by a protocol featuring a maximum of 4 steps. The substrates, saturated N-heterocyclic nitriles have not been subjected to nitrilase transformations so far and contribute to our research interests in understanding nitrilase substrate specificities. Besides, some of the products [**1b-Cbo**, (±)-**3a-Ts**, **4b-Ts**] have not been reported until now. The forthcoming availability of modified nitrilases is also expected to make pipecolic acid and others accessible by this straightforward approach.

Experimental Section

The enzymes NIT-101–NIT-108 were purchased from Biocatalytics, Inc., CA. For preparative reactions, the enzymes used were delivered with the following specifications: NIT-106 (85.0 U/mg solid at 5 mM *p*-tolylacetonitrile), NIT-107 (2.8 U/mg solid at 20 mM benzonitrile) and NIT-108 (2.60 U/mg solid at 5 mM cinnamonitrile). Analytical thin layer chromatography was carried out on Merck Silica gel 60 F_{254} plates. Flash chromatography was performed on Merck Silica gel 60, 230–400 mesh. Analytical HPLC was carried out with an Agilent Series 1100 HPLC using a G1315A diode array detector or MWD detector. For achiral analysis a LiChrospher 100 RP18e column (5 μm) was used. Chiral analysis was carried out with a Daicel Chiralpak AD-H (5 μm) and a Chiralcel OD-H column (5 μm). EI-mass spectra were recorded with a Hewlett–Packard 5972 MSD and HP 6890 Series II GC equipped with an HP5-MS column. ^{1}H (499.82 MHz) and ^{13}C NMR (125.69 MHz) spectra were recorded on a Varian INOVA 500. Melting points were determined on an Electrothermal MEL-TEMP apparatus and are uncorrected. The elemental analyses were performed on a Heraeus vario EL.

Substrate Synthesis

1a-Cbo and **2a-Cbo**; **Step 1:** One equivalent of 3-pyrrolidinol or 4-hydroxypiperidine in a water/diethylether mixture (1:2) was Cbo-protected by addition of 1.6 equivs. of K_2CO_3 and 1.5 equivs. of benzyl chloroformiate at 0 °C. The reaction mixture was stirred at room temperature until completion. The phases were separated and the aqueous phase was extracted with ethyl acetate three times. The products were obtained as colourless oils after drying with Na_2SO_4, evaporation of the solvents and silica gel chromatography.

Step 2: Subsequently, 1.0 equiv. thereof was mesylated in CH_2Cl_2 by addition of 1.5 equivs. of pyridine and 1.2 equivs. of MesCl at 0 °C. The reaction mixture was stirred at room temperature until completion. The mixture was washed with HCl 0.1 N, dried and concentrated to give a yellowish oil which was used for the following S_N2 reaction without further purification.

Step 3: One equivalent of the respective precursor was dissolved in DMSO, 1.0 equiv. of TBACN (***Caution!***) and 2.0 equivs. of powdered KCN (***Caution!***) were added and the mixture was stirred at 65 °C until complete consumption of the starting materials. Subsequently, saturated $NaHCO_3$ was added. The aqueous layer was extracted with ethyl acetate three times. The organic layer was dried with Na_2SO_4, evaporated and separated from by-products by silica gel chromatography.

2a-Boc: One equivalent of 4-hydroxypiperidine in CH_2Cl_2 was Boc protected by addition of 1.1 equivs. of Boc_2O and 1.5 equivs. of triethylamine. The reaction mixture was stirred at room temperature until completion. The mixture was washed with saturated NH_4Cl, dried with Na_2SO_4, concentrated and purified by silica gel chromatography. For further treatment see steps 2 and 3 for **1a-Cbo** and **2a-Cbo**.

1a-Ts–3a-Ts: One equivalent of 3-pyrrolidinol, 3- or 4-hydroxypiperidine in acetonitrile was ditosylated by addition of 2.5 equivs. of TsCl and 3.0 equivs. of triethylamine. The reaction mixture was stirred at room temperature until completion. Acetonitrile was removed and the residue dissolved in CH_2Cl_2. The mixture was washed with HCl 2N, saturated $NaHCO_3$ and saturated NaCl, dried with Na_2SO_4, concentrated and purified by silica gel chromatography. For further treatment see steps 2 and 3 for **1a-Cbo** and **2a-Cbo**.

4a-Ts: See ref.[8]

Screening

For screening experiments, 1.0 mg of enzyme preparation was dissolved in phosphate buffer (497.5 μL, 50 mM, pH 8.00, 1 mM EDTA). The substrate was added as a solution in MeOH or DMSO (2.5 μL, 40 mM) to give a final concentration of 0.2 mM. The reactions proceeded at 30 °C in an Eppendorf Thermomixer at 1100 rpm. After 18 h, acetone (500 μL) was added. The reaction vessels were centrifuged for 5 min at room temperature and 13000 rpm to remove precipitated proteins. The supernatant was analyzed by RP-18 HPLC using a gradient of 0.1% H_3PO_4 and acetonitrile.

Preparative Scale Biotransformations

The commercial enzyme preparation was dissolved in phosphate buffer (50 mM, pH 8.00, 1 mM EDTA) in a round-bottomed flask. Approximately 100 mg of substrate was added as solution in DMSO (max. 5 vol% of cosolvent). Concentrations and absolute amounts are added for the respective compounds listed below. The reaction was stirred with a magnetic bar and the temperature was adjusted to 29–31 °C by the use of an oil bath. The conversion was monitored by HPLC. After completion, the mixture was acidified by addition of HCl and the protein was removed by $(NH_4)_2SO_4$ precipitation and filtration through a plug of celite. The products were isolated by extraction of the aqueous layer with ethyl acetate and purified by silica gel chromatography using ethyl acetate/cyclohexane mixtures with little portions of acetic acid.

(±)-3-Cyanopyrrolidine-1-carboxylic acid benzyl ester (1a-Cbo): Colourless oil; ^{1}H NMR ($CDCl_3$): $\delta=2.17$–2.30 (m, 2H), 3.07–3.14 (m, 1H), 3.48–3.54 (m, 1H), 3.59–3.77 (m, 3H), 5.13 (d, 1H, $J=10.5$ Hz), 5.15 (d, 1H, $J=10.5$ Hz), 7.30–7.37 (m, 5H); ^{13}C NMR ($CDCl_3$): $\delta=27.87$/28.61, 29.55/30.40, 44.70/45.17, 48.95/49.41, 67.46, 119.94/120.03, 128.29, 128.43, 128.79, 136.60/136.63, 154.45/154.61; MS (EI): $m/z=230$ [M$^+$] (2), 139 (1), 123 (3), 107 (2), 91 (100). Chiral separation on Chiralpak AD-H, *n*-heptane/ethanol 1:1, 0.5 mL min^{-1}, 20 °C.

(±)-3-Carbamoylpyrrolidine-1-carboxylic acid benzyl ester (1b-Cbo): White solid, mp 124–125 °C, ^{1}H NMR (DMSO-d_6): $\delta=1.88$–2.00 (m, 2H), 2.87–2.93 (m, 1H), 3.23–3.51 (m, 4H), 5.04 (s, 1H), 6.94 (s, br., 1H, NH_2), 7.30 (s, br., 1H, NH_2), 7.34 (m, 4H), 7.43 (m, 1H); ^{13}C NMR (DMSO-d_6): $\delta=28.95$/29.80, 42.88/43.81, 45.91/46.44, 48.72/49.37, 66.41, 128.16, 128.43, 129.08, 137.84, 154.45, 174.49/174.61; MS (EI): $m/z=171$ (1), 137 (1), 127 (6), 108 (5), 91 (100), 82 (9).

Pyrrolidine-1,4-dicarboxylic acid monobenzyl ester (1c-Cbo): Colourless oil, yield: 38 mg (29% at 34% conversion; *ee*=49%) from 123 mg (13.4 mM) of (±)-**1a-Cbo** with *NIT-108* (2.75 g/L); ^{1}H NMR ($CDCl_3$): $\delta=1.93$–2.11 (m, 2H), 3.00–3.09 (m, 1H), 3.26–3.40 (m, 2H), 3.41–3.53 (m, 2H), 5.05 (s, 2H), 7.29–7.37 (m, 5H), 12.39 (s, br., 1H, COOH); ^{13}C NMR ($CDCl_3$): $\delta=28.33$/29.21, 42.35/43.25, 45.58/46.11, 48.24/48.85, 66.47, 128.16, 128.46, 129.30, 137.78, 154.46, 174.95/175.02; MS (EI): $m/z=158$ (1), 142 (2), 114 (5), 108 (8), 91 (100), 77 (14).

(±)-1-(4-Toluenesulfonyl)-pyrrolindine-3-carbonitrile (1a-Ts): White solid, mp 103–104 °C; ^{1}H NMR ($CDCl_3$): $\delta=$ 2.05–2.11 (m, 1H), 2.19–2.26 (m, 1H), 2.47 (s, 3H), 3.00 (m, 1H), 3.32–3.44 (m, 3H), 3.66 (dd, 1H, $J=7.3$, 10.3 Hz), 7.36 (d, 2H, $J=7.8$ Hz), 7.72 (d, 2H, $J=7.8$ Hz); ^{13}C NMR ($CDCl_3$): $\delta=21.84$, 28.00, 30.01, 46.90, 50.83, 119.40, 127.83, 130.26, 133.11, 144.59; MS (EI): $m/z=251$ [M$^+$] (3), 250 (32), 155 (62), 91 (100). Chiral separation on Chiralpak AD-H, *n*-heptane/ethanol 1:1, 0.8 mL min^{-1}, 20 °C.

1-(4-Toluenesulfonyl)-pyrrolindine-3-carboxylic acid (1c-Ts): White solid, mp 123–125 °C; yield: 28 mg (42% at 44% conversion; *ee*=76%) from 62 mg (0.247 mM) of (±)-**1a-Ts** with *NIT-106* (0.20 g/L); ^{1}H NMR ($CDCl_3$): $\delta=2.06$ (m, 2H), 2.42 (s, 3H), 2.97 (m, 1H), 3.29 (m, 2H), 3.42 (m, 1H), 3.51 (m, 1H), 7.31 (d, 2H, $J=7.1$ Hz), 7.69 (d, 2H, $J=$ 7.1 Hz); ^{13}C NMR ($CDCl_3$): $\delta=21.80$, 28.48, 42.73, 47.49, 49.86, 127.89, 129.98, 133.35, 144.04, 178.34; MS (EI): $m/z=$ 269 [M$^+$] (0.7), 238 (90), 155 (28), 114 (100), 91 (85).

4-Cyanopiperidine-1-carboxylic acid benzyl ester (2a-Cbo): Colourless oil; ^{1}H NMR ($CDCl_3$): $\delta=1.77$–1.92 (m, 4H), 2.80–2.85 (m, 1H), 3.44 (ddd, 2H, $J=13.7$, 7.6, 3.7 Hz), 3.72 (ddd, 2H, $J=13.7$, 7.4, 3.8 Hz), 5.13 (s, 2H), 7.31–7.39 (m, 5H); ^{13}C NMR ($CDCl_3$): $\delta=26.42$ (2), 28.58, 42.12, 67.64, 121.12, 128.23, 128.42, 128.80, 136.66, 155.21; MS (EI): $m/z=244$ [M$^+$] (2), 153 (2), 137 (3), 109 (5), 91 (100).

4-Carbamoylpiperidine-1-carboxylic acid benzyl ester (2b-Cbo): White solid, mp 131–133 °C, ^{1}H NMR (DMSO-d_6): $\delta=1.39$ (dq, 2H, $J=4.3$, 12.3 Hz), 1.68 (d, 2H, $J=12.3$ Hz), 2.26 (tt, 1H, $J=3.7$, 11.6 Hz), 2.70–2.90 (m, 2H), 3.97 (d, 2H, $J=13.2$ Hz), 5.05 (s, 2H), 6.79 (s, br., 1H, NH_2), 7.26 (s, br., 1H, NH_2), 7.28–7.37 (m, 5H); ^{13}C NMR (DMSO-d_6): $\delta=28.70$, 41.94, 43.80, 66.78, 128.19, 128.50, 129.11, 137.71, 155.05, 176.64; MS (EI): $m/z=244$ (1), 171 (2), 127 (18), 109 (3), 91 (100), 82 (19).

Piperidine-1,4-dicarboxylic acid monobenzyl ester (2c-Cbo): White solid, mp 71–73 °C, yield: 30 mg (28% at 32% conversion) from 101 mg (8.42 mM) of **2a-Cbo** with *NIT-108* (2.20 g/L); ^{1}H NMR (DMSO-d_6): $\delta=1.36$–1.44 (m, 2H), 1.80 (d, 2H, $J=13.0$ Hz), 2.40–2.44 (m, 1H), 2.90 (m, br. singlet-like, 2H), 3.89 (d, 2H, $J=13.4$ Hz), 5.05 (s, 2H), 7.28–7.37 (m, 5H), 12.30 (s, br., 1H, COOH); ^{13}C NMR (DMSO-d_6): $\delta=28.36$, 43.55, 49.28, 66.82, 128.21, 128.50, 129.11, 137.69, 155.06, 176.28; MS (EI): $m/z=244$ (1), 171 (2), 127 (19), 109 (2), 91 (100), 82 (26).

4-Cyanopiperidine-1-carboxylic acid *tert*-butyl ester (2a-Boc): Colorless oil; ^{1}H NMR ($CDCl_3$): $\delta=1.45$ (s, 9H), 1.75–1.81 (m, 2H), 1.85–1.89 (m, 2H), 2.79 (m, 1H), 3.33 (ddd, 2H, $J=13.7$, 8.1, 3.7 Hz), 3.65 (ddd, 2H, $J=13.7$, 7.1, 3.7 Hz); ^{13}C NMR ($CDCl_3$): $\delta=26.53$, 28.60, 28.64, 41.60, 80.36, 121.31, 154.65; MS (EI): $m/z=210$ [M$^+$] (0.3), 155 (5), 137 (18), 109 (11), 83 (10), 57 (100).

Piperidine-1,4-dicarboxylic acid mono *tert*-butyl ester (2c-Boc): White solid, mp 146–148 °C under partial decomposition; ^{1}H NMR ($CDCl_3$): $\delta=1.45$ (s, 9H), 1.59–1.67 (dq, 2H, $J=3.9$, 11.2 Hz), 1.88–1.91 (dd, 2H, $J=2.0$, 13.2 Hz), 2.45–2.51 (m, 1H), 2.85 (m, 2H), 4.01 (d, br. 2H, $J=13.2$ Hz), 9.95 (s, br., 1H, COOH); ^{13}C NMR ($CDCl_3$): $\delta=27.94$, 28.64, 41.04, 43.22, 80.03, 154.99, 180.56; MS (EI): $m/z=172$ (3), 156 (9), 128 (10), 122 (5), 105 (8), 84 (11), 57 (100).

1-(4-Toluenesulfonyl)-piperidine-4-carbonitrile (2a-Ts): White solid, mp 136–138 °C; ^{1}H NMR ($CDCl_3$): $\delta=1.90$–2.04 (m, 4H), 2.44 (s, 3H), 2.73 (m, 1H), 3.08–3.16 (m, 4H), 7.33 (d, 2H, $J=8.1$ Hz), 7.62 (d, 2H, $J=8.1$ Hz); ^{13}C NMR ($CDCl_3$): $\delta=1.80$, 25.57, 28.16, 44.07, 120.63, 127.81, 130.14, 132.97, 144.30; MS (EI): $m/z=264$ [M$^+$] (11), 155 (22), 109 (100), 91 (71); anal. calcd. for $C_{13}H_{16}N_2O_2S$: C 59.07, H 6.10, N 10.59; found: C 58.85, H 6.09, N 10.52.

1-(4-Toluenesulfonyl)-piperidine-4-carboxylic acid amide (2b-Ts): White solid, mp 212–215 °C partial decomposition; ^{1}H NMR (DMSO-d_6): δ=1.47–1.55 (m, 2H), 1.71–1.75 (dd, 2H, J=2.7, 13.4 Hz), 1.98–2.03 (tt, 1H, J=3.8, 10.9 Hz), 2.20–2.25 (dt, 2H, J=2.4, 11.7 Hz), 2.39 (s, 3H), 3.51 (m, 2H, dublet-like, J=12.2 Hz), 6.79 (s, br., 1H, NH_2), 7.17 (s, br., 1H, NH_2), 7.42 (d, 2H, J=8.1 Hz), 7.59 (d, 2H, J=8.1 Hz); ^{13}C NMR (DMSO-d_6): δ=21.70 , 28.31, 46.09, 128.17, 130.50, 133.13, 144.17, 176.19; MS (EI): m/z=264 (5), 199 (1), 155 (21), 127 (31), 109 (100), 91 (95), 82 (56), 65 (31).

1-(4-Toluenesulfonyl)-piperidine-4-carboxylic acid (2c-Ts): White solid, mp 169–171 °C (partial decomposition); yield: 54 mg (85% at 86% conversion) from 59 mg (2.24 mM) of **2a-Ts** with *NIT-108* (1.42 g/L); ^{1}H NMR (CDCl$_3$): δ=1.77–1.85 (m, 2H), 1.97–2.00 (m, 2H), 2.09 (m, 1H), 2.25–2.30 (m, 1H), 2.43 (s, 3H), 2.41–2.46 (m, 1H), 3.63–3.66 (m, 2H), 7.31 (d, 2H, J=8.3 Hz), 7.63 (d, 2H, J=8.3 Hz), 9.32 (s, br., 1H, COOH); ^{13}C NMR (CDCl$_3$): δ=21.78, 27.41, 40.02, 45.59, 127.91, 129.95, 133.18, 143.92, 180.11; MS (EI): m/z=266 (1), 238 (1), 155 (7), 142 (96), 91 (43), 82 (100); anal. calcd. for $C_{13}H_{17}NO_4S$: C 55.11, H 6.06, N 4.94; found: C 54.98, H 6.06, N 4.85.

(±)-1-(4-Toluenesulfonyl)-piperidine-3-carbonitrile (3a-Ts): White solid, mp 130–131 °C; ^{1}H NMR (CDCl$_3$): δ=1.59–1.70 (m, 2H), 1.84–1.86 (m, 1H), 1.95–1.99 (m, 1H), 2.45 (s, 3H), 2.64 (t, 1H, J=9.5 Hz), 2.81 (m, 2H), 3.4–3.42 (m, 1H), 3.65 (d, 1H, J=8.8 Hz), 7.35 (d, 2H, J=8.2 Hz), 7.65 (d, 2H, J=8.2 Hz); ^{13}C NMR (CDCl$_3$): δ=21.81, 23.40, 27.50, 27.62, 46.11, 47.97, 119.62, 127.88, 130.15, 133.18, 144.40; MS (EI): m/z=264 [M$^+$] (42), 198 (12), 155 (34), 109 (100), 91 (72); anal. calcd. for $C_{13}H_{16}N_2O_2S$: C 59.07; H 6.10; N 10.60. Found: C 58.34; H 6.04; N 10.54; Chiral separation on Chiralpak ADH, n-heptane/2-propanol 1:1, 0.5 mL min^{-1}, 20 °C.

1-(Toluene-4-sulfonyl)-piperidine-3-carboxylic acid (3c-Ts): White solid, mp 135–138 °C; yield 38 mg (40% at 50% conversion; ee=93%) from 101 mg (0.382 mM) of **(±)-3a-Ts** with *NIT-107* (0.29 g/L); ^{1}H NMR (CDCl$_3$): δ=1.36–1.44 (m, 1H), 1.62–1.69 (m, 1H), 1.79–1.82 (m, 1H), 1.98–2.01 (m, 1H), 2.36 (t, 1H, J=10.2 Hz), 2.43 (s, 3H), 2.52 (t, 1H, J=10.7 Hz), 2.66 (m, 1H), 3.58 (d, 1H, J=11.7 Hz), 3.81 (d, 1H, J=11.2 Hz), 7.33 (d, 2H, J=7.8 Hz), 7.64 (d, 2H, J=7.8 Hz), 9.30 (s, br., 1H, COOH); ^{13}C NMR (CDCl$_3$): δ=21.79, 24.11, 26.47, 40.97, 46.48, 47.60, 127.91, 130.00, 133.15, 143.98, 178.87; MS (EI): m/z=283[M$^+$] (0.2), 238 (100), 155 (20), 128 (5), 91 (32).

2-Cyano-piperidine-1-carboxylic acid benzyl ester (4a-Cbo): Colorless oil; ^{1}H NMR (CDCl$_3$): δ=1.46 (m, 1H), 1.73 (m, 3H), 1.84 (m, 1H), 1.95 (m, 1H), 3.04 (m, 1H), 4.13 (m, 1H), 5.16 (s, 2H), 5.30 (m, 1H), 7.29–7.50 (m, 5H); ^{13}C NMR (CDCl$_3$): δ=20.47, 24.74, 28.68, 41.96, 44.55, 68.33, 117.71, 128.37, 128.64, 128.87, 136.04; MS (EI): m/z=244[M$^+$] (4), 153 (9), 128 (11), 109 (12), 91 (100); anal. calcd for $C_{14}H_{16}N_2O_2$: C 68.83, H 6.60, N 11.47; found: C 68.66, H 6.58, N 10.99.

(±)-1-(4-Toluenesulfonyl)-piperidine-2-carbonitrile (4a-Ts): White solid, mp 121–122 °C; ^{1}H NMR (CDCl$_3$): δ=1.54–1.68 (m, 2H), 1.80 (m, 2H), 1.87–1.98 (m, 2H), 2.44 (s, 3H), 2.67 (m, 1H), 3.84 (d, 1H, J=11.7 Hz), 4.98 (s, 1H), 7.36 (d, 2H, J=8.2 Hz), 7.70 (d, 2H, J=8.2 Hz); ^{13}C NMR (CDCl$_3$): δ=19.95, 21.90, 24.70, 29.65, 43.38, 46.02, 115.46, 128.11, 130.14, 133.50, 144.84; MS (EI): m/z=264 [M$^+$] (2), 212 (100), 155 (70), 91 (69). Chiral separation on Chiralcel OD-H, n-heptane/2-propanol 1:1, 0.38 mL min^{-1}, 20 °C.

(±)-1-(4-Toluenesulfonyl)-piperidine-2-carboxylic acid amide (4b-Ts): White solid, mp 126–136 °C under decomposition; ^{1}H NMR (DMSO-d_6): δ=1.11 (m, 1H), 1.18–1.33 (m, 2H), 1.41 (m, 2H), 1.89 (d, 1H, J=12.7 Hz), 2.36 (s, 3H), 3.30–3.37 (m, 1H), 3.63 (d, 1H, J=12.2 Hz), 4.38 (d, 1H, J=4.4 Hz), 7.08 (s, br., 1H, CONH_2), 7.28 (s, br., 1H, CONH_2), 7.35 (d, 2H, J=8.3 Hz), 7.64 (d, 2H, J=8.3 Hz); ^{13}C NMR (DMSO-d_6): δ=19.88, 21.45, 24.38, 27.60, 43.19, 55.11, 127.51, 130.32, 138.07, 143.52, 172.53; MS (EI): m/z=238 (81), 155 (66), 126 (15), 91 (100); anal. calcd. for $C_{13}H_{18}N_2O_3S$: C 55.30, H 6.43, N 9.92; found: C 54.61, H 6.35, N 9.76.

(±)-1-(4-Toluenesulfonyl)-piperidine-2-carboxylic acid (4c-Ts): White solid, mp 101–102 °C; chemical hydrolysis, yield: 20%; ^{1}H NMR (CDCl$_3$): δ=1.30–1.47 (m, 2H), 1.60–1.75 (m, 3H), 2.15 (m, 1H), 2.41 (s, 3H), 3.20 (dt, 1H, J=2.4, 12.7 Hz), 3.73 (d, 1H, J=12.7 Hz), 4.76 (d, 1H, J=5.3 Hz), 7.26 (d, 2H, J=7.8 Hz), 7.68 (d, 2H, J=7.8 Hz), 8.70 (s, br., 1H, COOH); ^{13}C NMR (CDCl$_3$): δ=21.04, 21.75, 24.69, 27.73, 42.77, 55.08, 127.40, 129.72, 137.17, 143.57, 176.95; MS (EI): m/z=283 [M$^+$] (0.02), 238 (100), 155 (17), 91 (26).

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