Biotransformation of substituted pyridines with dioxygenase-containing microorganisms†

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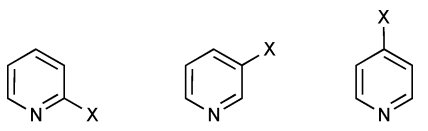
A series of 2-, 3- and 4-substituted pyridines was metabolised using the mutant soil bacterium *Pseudomonas putida* UV4 which contains a toluene dioxygenase (TDO) enzyme. The regioselectivity of the biotransformation in each case was determined by the position of the substituent. 4-Alkylpyridines were hydroxylated exclusively on the ring to give the corresponding 4-substituted 3-hydroxypyridines, while 3-alkylpyridines were hydroxylated stereoselectively on C-1 of the alkyl group with no evidence of ring hydroxylation. 2-Alkylpyridines gave both ring and side-chain hydroxylation products. Choro- and bromo-substituted pyridines, and pyridine itself, while being poor substrates for *P. putida* UV4, were converted to some extent to the corresponding 3-hydroxypyridines. These unoptimised biotransformations are rare examples of the direct enzyme-catalysed oxidation of pyridine rings and provide a novel synthetic method for the preparation of substituted pyridinols. Evidence for the involvement of the same TDO enzyme in both ring and side-chain hydroxylation pathways was obtained using a recombinant strain of *Escherichia coli* (pKST11) containing a cloned gene for TDO. The observed stereoselectivity of the side-chain hydroxylation process in *P. putida* UV4 was complicated by the action of an alcohol dehydrogenase enzyme in the organism which slowly leads to epimerisation of the initial (*R*)-alcohol bioproducts by dehydrogenation to the corresponding ketones followed by stereoselective reduction to the (*S*)-alcohols.

Introduction

As part of our ongoing studies into the oxidative biotransformations of arenes using microorganisms containing dioxygenase enzymes,[1] we have investigated the influence of heteroatoms on the course of the enzyme-catalysed process. It has already been established that electron-rich rings, for example thiophenes and furans,[2] are good substrates for dioxygenase enzymes but it has been generally accepted that electron-deficient rings, especially pyridines, are either poor substrates or completely unreactive. Indeed, when the toluene dioxygenase (TDO) enzyme is presented with competition between a benzene ring and a pyridine ring, for example using quinolines or isoquinolines as substrates,[3,4] then enzymatic attack is almost invariably on the carbocyclic ring. To date there have been very few examples reported of the oxidation of pyridine rings by dioxygenase enzymes, with most involving the dihydroxylation of 2-pyridone or 2-quinolone rings.[4,5] In the few other reported examples of biological oxidations of pyridine rings, the enzymes involved are either unidentified or mono-oxygenases.[6] Another empirical observation of the regioselectivity of dioxygenase enzymes is that when oxidation on the ring is unfavourable, for example when two ring substituents are in a 1,3 relationship (the *meta* effect), then an exocyclic group such as an alkyl chain may be preferentially hydroxylated.[7] *Pseudomonas putida* UV4 has already been demonstrated to catalyse the benzylic hydroxylation of some alkylbenzene substrates, although the transformation is usually accompanied by arene ring *cis*-dihydroxylation to give triols.[8]

With these general observations in mind, we set out to investigate the possibility of using the unreactivity of a pyridine ring to direct dioxygenase activity towards the side-chain of a range of alkyl-substituted pyridines (*e.g.* **1a–3c**, Fig. 1).



a: X = Et **d:** X = Cl
b: X = Pr **e:** X = Br
c: X = Me **f:** X = H

Fig. 1 Substituted pyridine substrates for biotransformation with *P. putida* UV4.

We anticipated that if exocyclic chain oxidation was preferred, then the hydroxylation of prochiral alkylpyridines would be stereoselective, thus providing a novel method of producing enantiopure pyridylalkanols for potential use as chiral synthetic intermediates or ligands, both of which applications have received considerable recent interest.[9] Previously reported stereoselective methods for the generation of pyridylalkanols have relied on either the asymmetric reduction of acylpyridines or the kinetic resolution of racemic esters of pyridylalkanols.[10] The stereoselective oxidation of alkylpyridines would offer a novel, alternative synthetic method for preparing this class of compounds.

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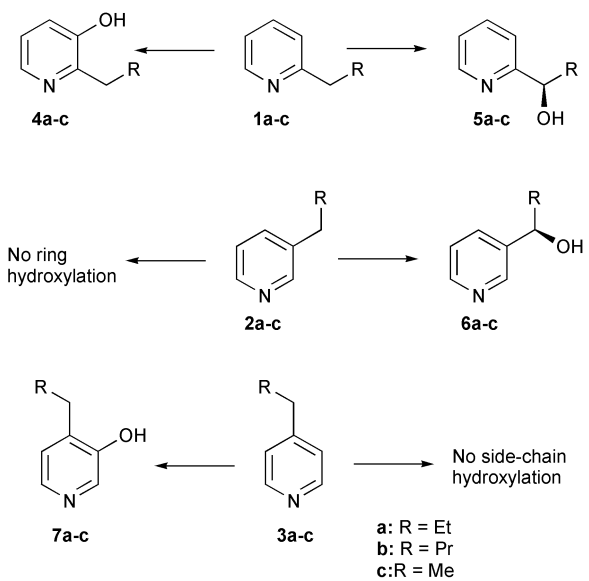
Results and discussion

Initial studies were carried out on 2-, 3- and 4-ethylpyridine (**1a**, **2a** and **3a**) as substrates fed to resting cultures of *P. putida* UV4 at concentrations of 0.5–1.0 g dm^{-3}, and the biotransformations were allowed to proceed for 20–24 hours before work up. It was immediately evident that the three substrates differed considerably in the range of isolated products and therefore the course of the biotransformations (Scheme 1, Table 1). Biotransformation of 2-ethylpyridine **1a** resulted in two products: (*R*)-1-(2-pyridyl)ethanol **5a** and 2-ethyl-3-hydroxypyridine **4a** in a ratio of approximately 3 : 1 and in 40% combined isolated yield. This result was surprising because of the isolation of **4a**, which was apparently a product of direct hydroxylation of the pyridine ring. Even more surprising was the biotransformation of 4-ethylpyridine **3a**, which resulted in a poor yield (15%) of 4-ethyl-3-hydroxypyridine **7a** as the only isolated bioproduct. By contrast, biotransformation of 3-ethylpyridine **2a** gave (*R*)-1-(3-pyridyl)ethanol **6a** as the only product and with 90% ee.



Scheme 1 Biotransformations of alkylpyridines **1a–3c** with *P. putida* UV4.

From these preliminary results it was clear that the position of the substituent on the pyridine ring had a dramatic effect on the regioselectivity of the biotransformation. Extending the range of substrates to propylpyridines (**1b**, **2b**, **3b**) and methylpyridines (**1c**, **2c**, **3c**) gave similar patterns of regioselectivity (Scheme 1, Table 1). The very low isolated yield (2%) of (3-pyridyl)methanol **6c** may be due to its further metabolism to nicotinic acid and subsequent transfer into primary metabolic pathways, but there was no direct evidence to support this supposition. Neither nicotinic acid nor the intermediate aldehyde were observed in the biotransformation medium. The absolute configuration of each pyridylalkanol was established by comparison of the sign of optical rotation with published data, and the enantiopurities of the pyridylalkanols were determined by chiral stationary phase (CSP) HPLC using a Chiralcel OD column. The method was also cross-checked by conversion of the chiral alcohols into diastereoisomeric MTPA esters and NMR analysis, and in each case samples of the racemic alcohols were prepared to validate the methods.

Having demonstrated that TDO regioselectivity could be determined by substituent position on the pyridine ring, attention was turned to substrates where exocyclic oxidation was not an option. Contrary to earlier unpublished reports[11] of its unreactivity with *P. putida* UV4, pyridine **1f** was converted into 3-hydroxypyridine **4f**, albeit in very poor isolated yield (3%). Similarly, a small group of halogenopyridines (**1d**, **1e**, **3d**, **3e** and **2d**), which should have been even less reactive than pyridine, were also hydroxylated, resulting in relatively small but isolable quantities of the corresponding 3-hydroxypyridines (**4d**, **4e**, **7d**, **7e** and **8**, Scheme 2, Table 2). It is interesting that no conversion was observed of the 2-halogenopyridines to 2-pyridone, and subsequent *cis*-dihydroxylation, in contrast to the

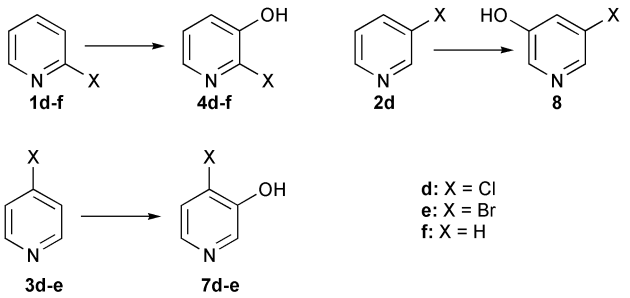
Table 2 Biotransformations of pyridine **1f** and halogenopyridines **1d–e**, **3d–e** and **2d** with *P. putida* UV4

Substrate		Bioproduct	
X			Yield
Cl	**1d**	**4d**	18%
Cl	**2d**	**8**	9%
Cl	**3d**	**7d**	14%
Br	**1e**	**4e**	10%
Br	**3e**	**7e**	10%
H	**1f**	**4f**	3%

Table 1 Biotransformations of alkylpyridines **1a–3c**

Substrate		Hydroxypyridine		Pyridylalkanol			
X			Yield		Yield	Abs. configuration	ee[a]
Et	**1a**	**4a**	11%	**5a**	29%	*R*	83%[b], >99%[c]
Et	**2a**	—	—	**6a**	30%	*R*	90%
Et	**3a**	**7a**	15%	—	—	—	—
Pr	**1b**	**4b**	10%	**5b**	28%	*R*	>99%
Pr	**2b**	—	—	**6b**	70%	*R*	>99%
Pr	**3b**	**7b**	7%	—	—	—	—
Me	**1c**	**4c**	10%	**5c**	20%	—	—
Me	**2c**	—	—	**6c**	2%	—	—
Me	**3c**	**7c**	17%	—	—	—	—

[a] Determined by chiral stationary phase HPLC. [b] Product with *P. putida* UV4 after 24 h biotransformation. [c] Product with *E. coli* pKST11 after 24 h biotransformation.



Scheme 2 Biotransformations of pyridine **1f** and halogenopyridines **1d–e**, **3d–e** and **2d** with *P. putida* UV4.

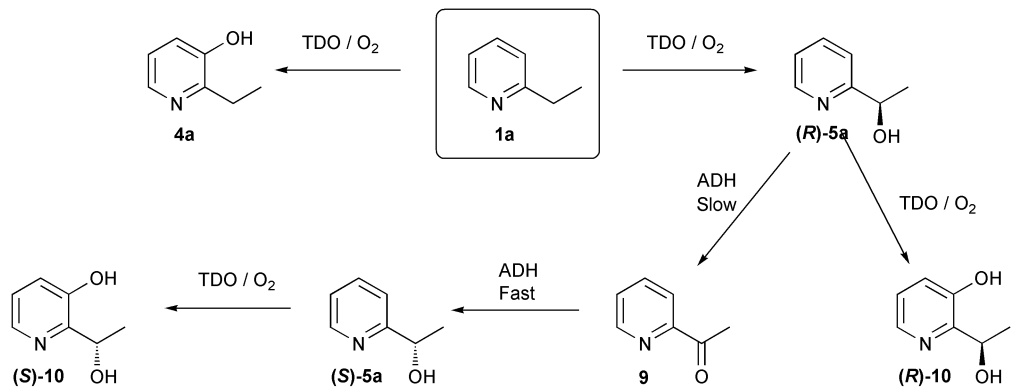
bicyclic 2-chloroquinoline analogue.[3] When the enzyme was given no choice of regioselectivity in the substrate (*i.e.* 3-chloropyridine, **2d**) the ring was hydroxylated in the 5-position to give 5-chloro-3-hydroxypyridine **8**. It thus appears that the TDO enzyme is capable of the catalysing the oxidation of a range of monocyclic pyridine rings, even when the ring is extremely electron-poor.

Further investigation into the stereoselectivity of the side-chain oxidation of 2-ethylpyridine **1a** revealed that a second enzyme was acting on the initial pyridylethanol bioproduct **5a**. This discovery first emerged with the observation that the ee of **5a** decreased with reaction time. A time course study revealed that the ee of the (*R*)-pyridylethanol (*R*)-**5a** decreased from >98% within the first 30 minutes of the biotransformation to 70% after 30 hours. In addition, a trace amount of a minor metabolite, the ketone **9**, was observed in the HPLC analysis of the biotransformation. Ketone **9** was identified by comparison of its retention time and UV spectrum with authentic material and by spiking biotransformation samples for HPLC analysis. This suggested that a secondary enzymatic process, possibly involving an alcohol dehydrogenase (ADH) enzyme, was causing partial racemisation by oxidation of the (*R*)-alcohol (*R*)-**5a** to the ketone **9** and then re-reduction to the enantiomer, (*S*)-**5a** (Scheme 3). There was further support for this hypothesis when another metabolite, the diol **10** resulting from a second oxidation, was observed after prolonged biotransformations, only being detectable by HPLC after the initial substrate **1a** had been completely consumed. To establish the order of events in the overall biotransformation process, the hydroxypyridine **4a** and the pyridyl alcohol **5a** were used separately as substrates for the organism. Hydroxypyridine **4a** remained unchanged after 24 hours but racemic pyridyl alcohol (±)-**5a** was completely consumed during the same time, with the diol **10** as the only observed bioproduct. Stereochemical analysis of **10** produced from the racemic substrate showed it to be predominantly the (*S*)-enantiomer, with an ee of 53%. The two pure enantiomers of **5a**, produced by classical resolution[12] of the racemate, were then fed separately to the organism. (*S*)-**5a** resulted in enantiomerically pure diol, (*S*)-**10**, while (*R*)-**5a** gave (*R*)-**10** but with a considerable degree of epimerisation (ee = 37%). Finally, when the ketone **9** was fed to the organism it was completely and rapidly (2 hours) reduced to enantiopure (*S*)-**5a**. It was subsequently discovered that a range of 2-, 3- and 4-acylpyridines were all biotransformed rapidly (<4 hours), quantitatively and stereoselectively to the corresponding enantiopure (*S*)-alcohols, as reported previously.[13]

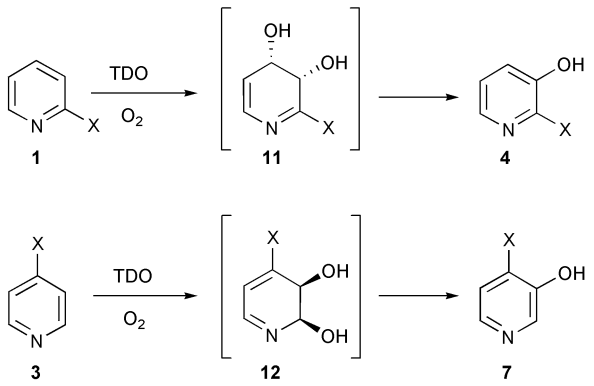
These combined results lead to the overall conclusion that 2-ethylpyridine is initially biotransformed with the TDO enzyme in *P. putida* UV4 to a mixture of the hydroxypyridine **4a**, which undergoes no further reaction, and the (*R*)-pyridylalcohol (*R*)-**5a**. A previously unidentified alcohol dehydrogenase enzyme then epimerises (*R*)-**5a** by slow oxidation to the ketone **9** and more rapid reduction to the (*S*)-enantiomer. Both enantiomers of **5a** are then oxidised further by TDO to the diol **10** (Scheme 3).

It is not possible, by simply isolating and characterising the bioproducts, to be confident about the mechanism of the formation of the ring-hydroxylated pyridinol products. While enzyme-catalysed aromatic ring hydroxylation is well-known for carbocyclic arenes, there is little precedence for the direct hydroxylation of pyridine rings. There is, however, some history of pyridine ring dihydroxylation using this organism. In earlier work[3] on the biotransformations of bicyclic azaarenes it was postulated that minor metabolites monohydroxylated on the heterocyclic ring were the result of rapid dehydration of highly unstable hetero-arene *cis*-dihydrodiol intermediates. It is therefore reasonable to suppose that the hydroxypyridine metabolites **4** and **7** reported here are similarly derived from unstable pyridine *cis*-dihydrodiols **11** and **12** respectively (Scheme 4). The proposed configurations of **11** and **12** shown in Scheme 4 are based on the known stereoselectivity of the TDO enzyme in the carbocyclic arene series. In each case, whether the proposed *cis*-dihydrodiol is 2,3 or 3,4 relative to the pyridine nitrogen, dehydration to the 3-hydroxypyridine product will be favoured.

No direct observation of *cis*-dihydrodiol intermediates was made during any of the biotransformations and, thus far, attempts to trap the *cis*-dihydrodiols by *in situ* transformation methods have failed, suggesting that dehydration of the pyridine *cis*-dihydrodiol intermediates, if formed, is extremely rapid or even



Scheme 3 Summary of biotransformation pathways for 2-ethylpyridine **1a** with *P. putida* UV4.



Scheme 4 Proposed intermediacy of *cis*-dihydrodiols in the ring hydroxylation of substituted pyridines catalysed by the TDO enzyme.

occurs before the release of the bioproducts from the enzyme. To establish that the TDO enzyme was responsible for both ring and side-chain hydroxylation transformations, use was made of the recombinant organism *E. coli* pKST11 containing a cloned gene for the TDO enzyme from *P. putida* UV4. This construct had been used previously[14] to confirm that monosulfoxidations of sulfides were being catalysed by TDO and not by an alternative enzyme-catalysed oxidation. 2-Ethylpyridine **1a** fed to the parent, unmodified *E. coli* JM109 strain was recovered unchanged, but when fed to the pKST11 recombinant strain resulted in the formation of both the pyridinol **4a** and the (*R*)-pyridylethanol (*R*)-**5a**. Furthermore, the alcohol (*R*)-**5a** was enantiomerically pure, thus providing further evidence that the side-chain hydroxylations by *P. putida* UV4 are stereoselective for the (*R*)-pyridylalkanols but that subsequent action by an alcohol dehydrogenase enzyme results in the partial racemisation.

Conclusion

Simple, substituted monocyclic pyridines are substrates for the toluene dioxygenase enzyme and give substituted 3-hydroxypyridines as bioproducts, with the exception of 3-alkylpyridines where exocyclic side-chain oxidation is observed exclusively. That such different outcomes were observed for the biotransformations of 2-, 3- and 4-alkylpyridines suggests that the two pathways are similar in preference hierarchy for the TDO enzyme and that substituent position is sufficient to determine regioselectivity. While the chemical yields of these biotransformations are generally poor to moderate, no attempt has been made to optimise the process and better dioxygenase-containing biocatalysts might be developed using directed evolution or similar techniques. Nevertheless, the chemo- and regioselective hydroxylation of the 3-position of the pyridine ring has few analogues in non-enzymatic chemistry, and this represents a potential new and mild synthetic route to substituted pyridinols.

Experimental

General experimental methods

Substrates. Biotransformation substrates were purchased from commercial suppliers and used without further purification with the exception of 3-propylpyridine **2b**, which was prepared by Wolff–Kishner reduction of 3-propanoylpyridine using a published procedure.[15]

Biotransformations. Shake flask (<0.5 g) biotransformations using *P. putida* UV4 were carried out under reported conditions[16] for 20–24 h (TDO oxidation biotransformations) or 2–4 h (stereoselective reduction of acylpyridine substrates). Biotransformations using the recombinant strain *E. coli* pKST11 and the control unmodified strain *E. coli* JM109 were carried out in the Biological Sciences Department, Warwick University, as described previously.[14] The metabolites obtained after biotransformation were purified and separated by PLC (silica gel, ethyl acetate eluent). The absolute configurations of the chiral pyridylalkanol metabolites (**5a**, **5b**, **6a**, and **6b**) were determined by comparison of the signs of specific optical rotations with literature values. The ee values were determined by CSPHPLC analysis using a Chiralcel OD column (hexane–isopropanol, 95 : 5 or 90 : 10) and corroborated by formation of the corresponding MTPA esters followed by ^{1}H-NMR analysis of the diastereoisomeric composition. Bioproducts with poor volatility were treated with the silylating reagent bis(trimethylsilyl)trifluoroacetamide (BSTFA) prior to mass spectrometric analysis.

Metabolites **4a**, **4c**, **4d**, **4e**, **4f**, **5a**, **5b**, **5c**, **6a**, **6c**, **7a**, **7c**, **7d**, **7e** and **8** have been reported previously. Spectroscopic and other analytical data for these compounds may be found in the Supplementary Information.†

(*R*)-1-(2-Pyridyl)ethanol (5a). From substrate **1a** (500 mg), **5a** was isolated as a colourless oil (167 mg, 29%); $[\alpha]_D$ +50.4 (*c* 0.5, EtOH), (lit.[17a] $[\alpha]_D$ +24.5 (*c* 0.31, $CHCl_3$)). Spectroscopic data were in accordance with those published in the literature for the racemic compound.[17b]

(*R*)-1-(2-Pyridyl)-1-propanol (5b). From substrate **1b** (200 mg), **5b** was isolated as a colourless oil (63 mg, 28%) $[\alpha]_D$ +60.8 (*c* 0.74, EtOH). Spectroscopic data were in accordance with those published in the literature for the racemic compound.[18]

(*R*)-1-(3-Pyridyl)ethanol (6a). From substrate **2a** (200 mg), **6a** was isolated as a colourless oil (69 mg, 30%), $[\alpha]_D$ +33.5 (*c* 0.20, EtOH), (lit.[19a] $[\alpha]_D$ +52.8 (*c* 1.4, $CHCl_3$)). Spectroscopic data were in accordance with those published in the literature for the racemic compound.[19b]

(*R*)-1-(3-Pyridyl)-1-propanol (6b). From substrate **2b** (250 mg), **6b** was isolated as a colourless oil (200 mg, 70%), $[\alpha]_D$ +37.4 (*c* 0.76, MeOH); δ_H (500 MHz, $CDCl_3$) 0.89 (3H, dd, J = 7.4 Hz, J = 3.3 Hz, CH_3), 1.72 (1H, m, CH_2CH$_3$), 1.82 (1H, m, CH_2CH$_3$), 4.45 (1H, br s, OH), 4.60 (1H, m, C*H*OH), 7.22 (1H, m, 5-H), 7.70 (1H, dd, J = 4.1 Hz, J = 1.8 Hz, 4-H), 8.34 (1H, d, J = 4.1 Hz, 6-H), 8.39 (1H, s, 2-H); δ_C (125 MHz, $CDCl_3$) 8.8 (CH_3), 30.8 (CH_2), 71.8 (CH), 122.4 (C), 133.0 (CH), 139.6 (CH), 146.3 (CH), 146.8 (CH); *m*/*z* (EI) 137 (M^+, 60%), 108 (M^+ – CH_2CH_3, 100%), 80 (70%), HRMS (EI) calcd. for $C_8H_{11}NO$ (M^+): 137.084064, found 137.083748.

(2-Pyridyl)methanol (5c). From substrate **1c** (100 mg), **5c** was isolated as a colourless oil (11 mg, 10%). Spectroscopic data were in accordance with those published in the literature.[20]

(3-Pyridyl)methanol (6c). From substrate **2c** (500 mg), **6c** was isolated as a colourless oil (11 mg, 2%). Spectroscopic data were in accordance with those published in the literature.[21]

1-(3-Hydroxy-2-pyridyl)ethanol (10). From (±)-1-(2-pyridyl)-ethanol, (±)-**5a** (150 mg), **10** was isolated as a yellow oil (54 mg, 32%); δ_H (300 MHz, $CDCl_3$) 1.52 (3H, d, $J = 6.6$ Hz, CH_3), 5.13 (1H, q, $J = 6.6$ Hz, C*H*OH), 7.08 (1H, dd, $J = 8.0$ Hz, $J = 4.5$ Hz, 5-H), 7.18 (1H, d, $J = 8.0$ Hz, 4-H), 7.66 (2H, br s, 2 × OH), 7.99 (1H, d, $J = 4.5$ Hz, 6-H); δ_C (75 MHz, $CDCl_3$) 22.6 (CH_3), 70.4 (CH), 123.5 (CH), 124.5 (C), 138.6 (CH), 148.9 (CH), 152.1 (C); *m/z* (EI) 139 (M^+, 72%), 124 ($M^+ - CH_3$, 97%), 120 (95%), 39 (100%); HRMS (EI) calcd. for $C_7H_9NO_2$ (M^+): 139.063329, found 139.063240.

The racemic substrate, (±)-**5a**, yielded (*S*)-**10** with 53% ee, $[\alpha]_D$ −15.7 (*c* 0.8, $CHCl_3$); (*S*)-**5a**, yielded enantiopure (*S*)-**10**, $[\alpha]_D$ −28.8 (*c* 0.8, $CHCl_3$); and (*R*)-**5a** yielded (*R*)-**10** with 37% ee, $[\alpha]_D$ +10.1 (*c* 0.8, $CHCl_3$).

2-Ethyl-3-hydroxypyridine (4a). From substrate **1a** (500 mg), **4a** was isolated as a white solid (62 mg, 11%); mp 135–136 °C, (lit.,[22] 134–136 °C). Spectroscopic data were in accordance with those published in the literature.[23]

3-Hydroxy-2-propylpyridine (4b). From substrate **1b** (200 mg), **4b** was isolated as a white solid (24 mg, 10%); mp 134–135 °C (lit.,[22] 133–135 °C); δ_H (500 MHz, $CDCl_3$) 0.99 (3H, t, $J = 7.4$ Hz, CH_3), 1.71 (2H, m, CH_2CH$_3$), 2.68 (2H, t, $J = 7.3$ Hz, CH_2CH$_2$), 6.15 (1H, br s, OH), 6.94 (1H, dd, $J = 7.8$ Hz, $J = 4.9$ Hz, 5-H), 7.09 (1H, d, $J = 7.8$ Hz, 4-H), 8.02 (1H, d, $J = 4.9$ Hz, 6-H); δ_C (125 MHz, $CDCl_3$) 13.0 (CH_3), 20.8 (CH_2), 30.5 (CH_2), 124.3 (CH), 134.2 (CH), 137.8 (CH), 150.3 (C), 153.0 (C); *m/z* (EI) [silylated with BSTFA] 209 (M^+, 93%), 194 ($M^+ - CH_3$, 100%), 180 ($M^+ - CH_2CH_3$, 65%), 164 (80%), 73 (94%).

3-Hydroxy-2-methylpyridine (4c). From substrate **1c** (100 mg), **4c** was isolated as a white solid (12 mg, 10%); mp 165–168 °C (lit.,[24a] 168.5–169.5 °C). Spectroscopic data were in accordance with those published in the literature.[24b]

4-Ethyl-3-hydroxypyridine (7a). From substrate **3a** (100 mg), **7a** was isolated as a white solid (17 mg, 15%); mp 94–95 °C (lit.,[25a] 93.5–95 °C). Spectroscopic data were in accordance with those published in the literature.[25b]

3-Hydroxy-4-propylpyridine (7b). From substrate **3b** (300 mg), **7b** was isolated as a white solid (24 mg, 7%); mp 94–97 °C; δ_H (500 MHz, $CDCl_3$) 0.99 (3H, dt, $J = 7.4$ Hz, $J = 2.2$ Hz, CH_3), 1.71 (2H, m, CH_2CH$_3$), 2.66 (2H, q, $J = 7.4$ Hz, CH_2CH$_2$), 7.08 (1H, d, $J = 4.9$ Hz, 5-H), 7.93 (1H, d, $J = 4.8$ Hz, 6-H), 8.18 (1H, s, 2-H); δ_C (125 MHz, $CDCl_3$) 11.3 (CH_3), 22.0 (CH_2), 30.6 (CH_2) 124.8 (CH), 134.1 (C), 136.3 (CH), 145.8 (CH), 152.6 (C); *m/z* (EI) [silylated with BSTFA] 209 (M^+, 93%), 194 ($M^+ - CH_3$, 100%), 180 ($M^+ - CH_2CH_3$, 65%), 164 (80%), 73 (94%), HRMS (EI) calcd. for $C_8H_{11}NO$ (M^+): 137.084064, found 137.083839.

3-Hydroxy-4-methylpyridine (7c). From substrate **3c** (100 mg), **7c** was isolated as a white solid (20 mg, 17%); mp 117–120 °C (lit.,[26] 120–121 °C). Spectroscopic data were in accordance with those published in the literature.[25a]

2-Chloro-3-hydroxypyridine (4d). From substrate **1d** (150 mg), **4d** was isolated as a white solid (30 mg, 12%); mp 164–165 °C (lit.,[27a] 164–165 °C). Spectroscopic data were in accordance with those published in the literature.[27b]

2-Bromo-3-hydroxypyridine (4e). From substrate **1e** (100 mg), **4e** was isolated as a pale yellow resin (11 mg, 10%). Spectroscopic data were in accordance with those published in the literature.[28]

3-Hydroxypyridine (4f). From substrate **1f** (100 mg), **4f** was isolated as a yellow oil (3 mg, 3%). Spectroscopic data were in accordance with those published in the literature.[29]

4-Chloro-3-hydroxypyridine (7d). From substrate **3d** (400 mg), **7d** was isolated as a white solid (46 mg, 10%); mp 126–127 °C. Spectroscopic data were in accordance with those published in the literature.[30]

4-Bromo-3-hydroxypyridine (7e). From substrate **1e** (100 mg), **7e** was isolated as a pale yellow resin (11 mg, 10%). Spectroscopic data were in accordance with those published in the literature.[31]

5-Chloro-3-hydroxypyridine (8). From substrate **2d** (300 mg), **8** was isolated as a white solid (10 mg, 9%). Spectroscopic data were in accordance with those published in the literature.[32]

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