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ORIGINAL ARTICLE

Chemoenzymatic routes to enantiomerically pure 2-azatyrosine and 2-, 3- and 4-pyridylalanine derivatives

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Abstract Enantiomerically pure 2-, 3- or 4-pyridylalanine (pya) and 2-azatyrosine (azatyr) are known to present various biological activities. After incorporation into appropriate peptide sequences, these heterocyclic non natural α-amino acids could behave as new substrates or inhibitors of elastase from *Pseudomonas aeruginosa*. This enzyme is known to be involved in nosocomial infections and infections related to the cystic fibrosis disease. New efficient chemoenzymatic preparations of those compounds using α-chymotrypsin (α-CT) are presented.

Keywords Heterocyclic α-amino acids · Pyridyl alanine · Azatyrosine · Chemoenzymatic route · *Pseudomonas aeruginosa elastase*

Introduction

Non-proteinogenic heterocyclic α-amino acids like (L)-2-azatyrosine and pyridylalanines are interesting because of their chemical and medicinal applications.

Pya analogues are well known for their anti-inflammatory activity (Shimeno et al. 1977), or as contraceptive agent for (*D*)-3-pya (US Pat. 1985). *N*-oxide-2-pya and *N*-oxide-3-pya are phenylalanine antagonists (Sullivan et al. 1968). Pyridylalanines are also used as histidine analogues in peptidic sequence (Hoes et al. 1980, Van Batenburg et al. 1977).

(L)-2-azatyr [L-β(5-hydroxy-2-pyridyl)alanine] is an antibiotic agent isolated from *Streptomyces chibanensis* (Inouye et al. 1975). More recently, it has been shown that (L)-azatyr act as an antitumor-antibiotic agent (Lecointe et al. 2000; Adamczyk et al. 2001).

Morever, (*L*)-2-azatyr results in major physiological effects: (*L*)-2-azatyr has been shown to promote permanent reversion of *ras*-dependent transformed cells to the normal phenotype in culture (Shindo-Okada et al. 1989) and to inhibit chemical induction of carcinogenesis in transgenic mice bearing oncogenic human ras (Izawa et al. 1992).

Several methods have been developed for the synthesis of heterocyclic α-amino acids but most of them were not very efficient and produced only enantiomerically enriched or racemic amino acids.

Most of the asymmetric syntheses were based on asymmetric hydrogenation of dehydroamino acid derivatives, with rhodium complex of PROPRAPHOS for example (Doebler et al. 1996; Wang et al. 2002), coupling reactions of suitable functionalized heterocycles with Schöllkopf reagent followed by an acid hydrolysis (Croce et al. 2000) or coupling reactions of optically pure serine derivatives with suitable functionalized halopyridines (Walker et al. 1997; Tabanella et al. 2003).

Dondoni et al. (2003) reported the use of aldehydes or β-ketoester derivatives bearing *N*-Boc-2,2-dimethyl oxazolidine ring as components in Biginelli and Hantzsch cyclocondensations to access two different series of heterocyclic substituted α-amino acids especially pyridylalanines with up to 96% ee.

Asymmetric syntheses with enantiomerically pure 2-hydroxypinan-3-one or their oxazinone derivatives as chiral auxiliaries were commonly used in our laboratory

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(Pappalardo et al. 1999; Bouifraden et al. 1999) to access a large variety of optically pure non natural amino acids. Morever, chemoenzymatic routes were developed for the preparation of the fluorescent probe (*L*)-7-azatryptophane (Lecointe et al. 1998) or (*L*)-7-azaindolylalanine and the antidiabetic amino acid, (*L*)-*β*-pyrazolylalanine (Rolland-Fulcrand et al. 2000) with high enantioselectivity (ee > 99%).

We describe in this paper two chemoenzymatic routes to access to (*L*) and (*D*) 2-, 3- or 4-pya derivatives and one route to obtain (*L*) and (*D*) 2-azatyr. The key step was the kinetic resolution with an appropriate enzyme.

Results and discussion

Pyridylalanines

The first route describes the synthesis of pyridylalanine derivatives via an oxazolone **3a**, **b** obtained from reaction between a pyridylaldehyde **1a**, **b** and acetylglycine **2** (Scheme 1).

Dehydro amino acid derivatives **4a**, **b** were obtained after hydrolysis of **3a**, **b**. **5a**, **b** were obtained by hydrogenation of **4a**, **b**. Hydrolyses of **5a**, **b** led to **6a**, **b** witch were protected on N terminus as N-Fmoc and on C terminus as methyl ester **7a**, **b**. Enantiomerically pure **8a**, **b** and **9a**, **b** were obtained by methyl ester enzymatic hydrolysis using α-CT (ee > 98%) (Tararov et al. 1997).

Enzymatic resolutions with α-CT gave fair yields after purification (Table 1); the separation between Fmoc-(D)-amino ester and Fmoc-(L)-amino acid can be carried out by several extractions with appropriate solvent or by preparative HPLC. The experimental conditions of separation depended on the solubility of N-protected pya in water solutions. Acylase I from *Hog Kidney* was also tested for the enzymatic resolutions of racemic **5a**, **b** but separation between (L)-3-, 4-pya and Ac-(D)-3- and 4-pya was not successful.

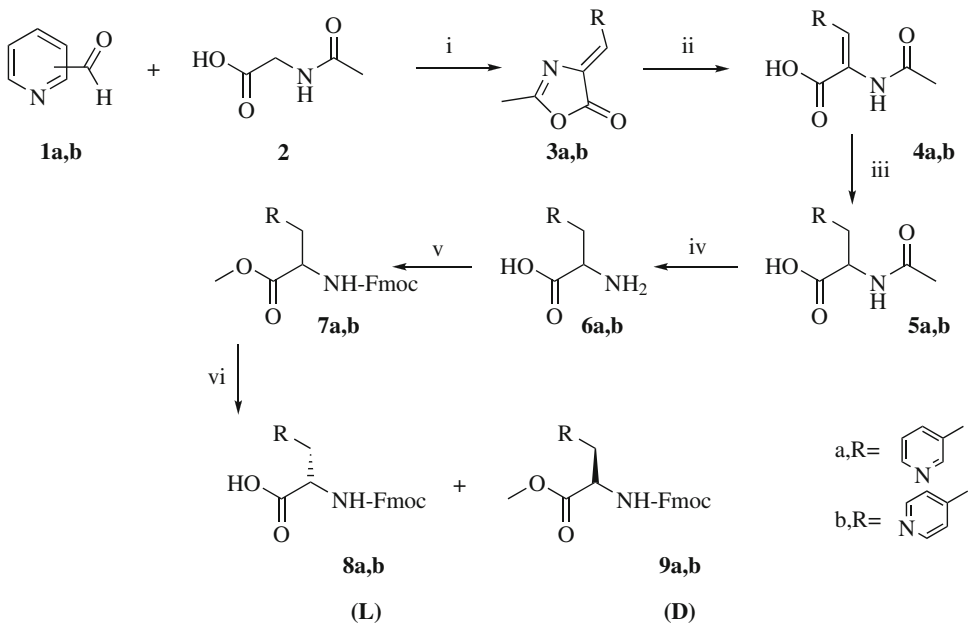
The low overall yield observed in the 4-pya preparation was due to 4-pyridylaldehyde degradation observed in the first step (46% yield in **3b**). Moreover, we observed that the same strategy could not be used for the synthesis of 2-pya. The corresponding oxazolone cannot be obtained because of 2-pyridylaldehyde degradation as already described in the literature (Doebler et al. 1996; Wang et al. 2002).

The second strategy consisted in diethylacetamidomalonate **10** alkylation by commercially available 2-, 3- or 4-bromomethyl pyridines bromohydrates **11a**, **b**, **c**. This strategy was commonly used for amino acid synthesis (Tararov et al. 1997; Cooper et al. 1996, Agafonova et al. 1970) but never described for optically pure pya syntheses (Scheme 2).

We had to optimize the experimental conditions for the alkylation (Table 2) because we observed that previously used Agafonova's conditions (Agafonova et al. 1970) were not successful.

1.5 equiv of alkylating agent proved that it was the optimal quantity: tests were performed with 0.5–3 equiv. Therefore, optimal conditions were: 3 equiv of KHMDS, 1.5 equiv of bromomethyl pyridine in ACN as solvent at −40°C (entry 9).

N-acetyl-monoethyl ester **13a**, **b**, **c** were obtained by one pot saponification and decarboxylation (Cooper et al. 1996) using 2.5 equiv of sodium ethanolate in refluxing dioxan.



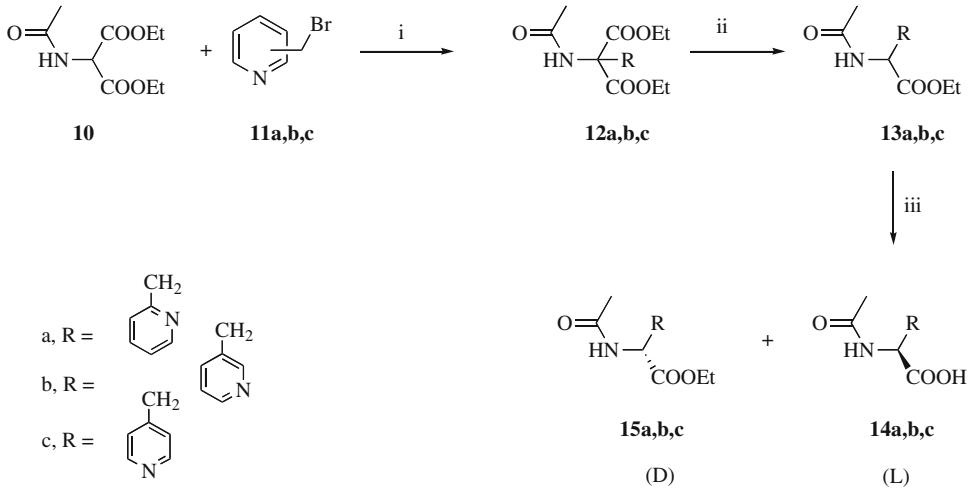
Scheme 1 Reagents and condition: i, anhydrous Ac_2O (0.3 equiv), AcONa (0.1 equiv), 1.5 h, 90–100°C, **3a**:70% yield, **3b**:46% yield; ii, 3% Na_2CO_3, **4a**:70% yield, **4b**:33% yield; iii, H_2/Pd/C (10%), MeOH, overnight r.t., **5a**, **b**:85% yield; iv, refluxing HCl 6 N, 3 h, **6a**, **b**:quantitative yield; v, **1** FmocOSu (0.9 equiv)/10% $NaHCO_3$aq., 22 h, r.t.; 2, MeOH/$ClSiMe_3$ (8 equiv), 36 h, 40°C, **7a**:quantitative yield, **7b**:51% yield; vi, α-CT, Phosphate Buffer, 2 h, separation, **8a**:41% yield, **8b**:40% yield, **9a**:33% yield, **9b**:46% yield

Table 1 Characteristics of optically pure N-protected 3 or 4 pya after separation and purification procedure

Compound	Name of compound	[a]HPLC retention time (min)	$[\alpha]_D$	Overall yield(%)[b]	ee (%)
8a	N-Fmoc-(*L*)-3-pya-OH	8.9	+21 (c = 1, MeOH)	17	>98
9a	N-Fmoc-(*D*)-3-pya-OCH_3	9.4	−36 (c = 1, DMF)	14	>98
8b	N-Fmoc-(*L*)-4-pya-OH	8.8	−31 (c = 1, DMF)	3	>98
9b	N-Fmoc-(*D*)-4-pya-OCH_3	9.2	+16 (c = 1, MeOH)	3	>98

[a] HPLC gradient from 0 to 100% ACN/0.1% TFA (v/v), 15 min

[b] Yield calculated from the racemic mixture



Scheme 2 Reagents and conditions: i, KHMDS (3 equiv), ACN, −40°C, **12a**:70% yield, **12b**:70% yield, **12c**:60% yield; ii, (2.5 equiv) NaOH in EtOH, r.t., 90 min, and refluxing dioxan, **13a**:50% yield, **13b**:60% yield, **13c**:70% yield; iii, α-CT/phosphate buffer pH 6.9, separation, **14a**:35% yield, **14b**:36% yield, **14c**:30% yield, **15a**:42.5% yield, **15b**:30% yield, **15c**:35% yield

Table 2 Optimization of experimental conditions in the alkylation step using 2-bromomethyl pyridine **11a** (1.5 equiv)

Entries	Base (equiv)	Solvent	Temperature (°C)	[a]Yield(%)
1	EtONa (2)	EtOH	−78	9
2	tBuOK (2	THF	−78	11
3	DBU (1.5)	ACN	−20	30
4	DBU (1.5)	ACN	r.t.	34
5	DBU (1.5)	ACN	Reflux	11
6	DBU (3)	ACN	r.t.	38
7	DBU (4)	ACN	r.t.	37
8	KHMDS (3)	ACN	r.t.	53
9	KHMDS (3)	ACN	−40	70

[a] After purification on silicagel chromatography

14a, **b**, **c** and **15a**, **b**, **c** were obtained after α-CT resolution in fair yields and ee (Table 3).

Azatyrosine

The same strategy as described above for pya was used for the synthesis of azatyr derivatives using a new alkylating agent **22** (Scheme 3).

Cooper et al. (1996) have already applied the same acetamidomalonate strategy in 1996. For optically pure 2-azatyrosine synthesis, classical conditions are used for the alkylation step: reflux THF with ethanolate as base and 2-bromomethyl-5-hydroxy pyridine as alkylating agent but the yield was only 59%.

The hydroxyl group present on pyridine ring of the alkylating agent must be protected because of compound instability (Lecointe et al. 2000). Acetoxy protecting group was a good choice because it can be easily removed later during the saponification step. During alkylation, we observed a partial cleavage of acetyl protecting group but it did not affect the synthesis. Using our conditions and this new alkylating agent **22** allowed to increase the yield to 81%. **17** was yielded in 80% yield after one pot saponification and decarboxylation (Cooper et al. 1996) of mixture **16a**, **b**. The α-CT resolution gave fair yields and ee after purification by extractions in appropriate solvents (Table 4).

The alkylating agent **22** was synthesized in two steps in 57% overall yield (Scheme 4). The compound **22** had to be purified immediately in order to avoid its decomposition and polymerisation. The compound **22** has to be stored at low temperature (−20°C), in darkness.

Conclusion

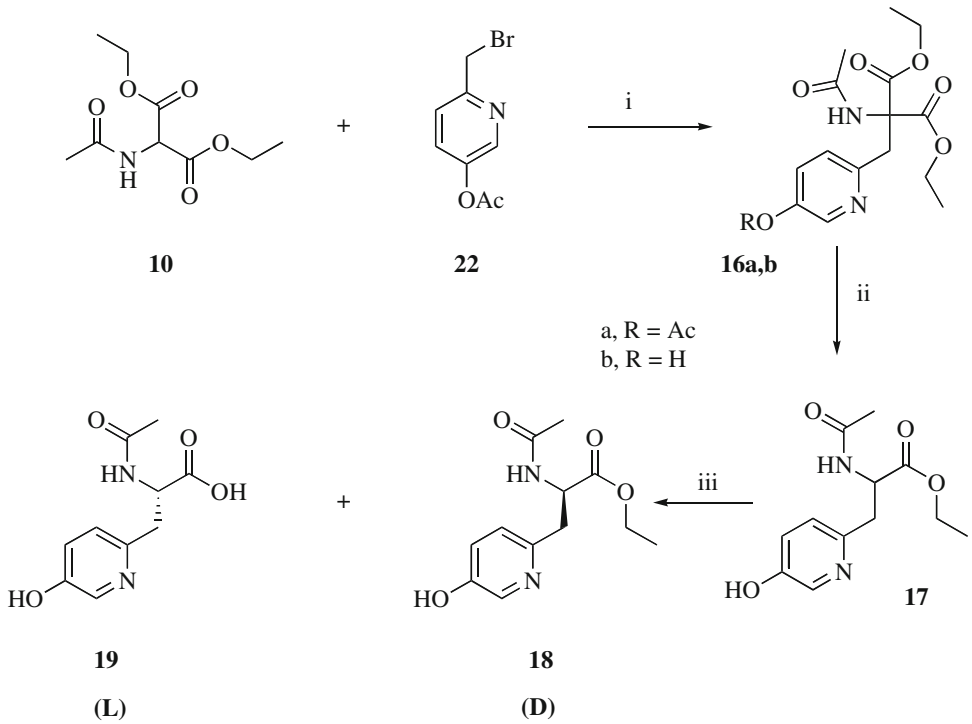
Synthesis of regioisomers of pyridylalanines and 2-azatyrosine derivatives has been described in this paper by two

Table 3 Characteristics of optically pure *N*-acetyl 2 or 3 or 4 pya after separation and purification procedure

Compound	Name of compound	[a]HPLC retention time (min)	$[\alpha]_D$	Overall yield (%)[b]	ee (%)
14a	N–Ac-(L)-2-pya-OH	3.1	+32 (c = 28.7, 1 N HCl)	12	>98
15a	N–Ac-(D)-2-pya-OEt	3.2	−23 (c = 37, AcOEt)	15	>98
14b	N–Ac-(L)-3-pya-OH	2.8	+34 (c = 43, 1 N HCl)	15	>98
15b	N–Ac-(D)-3-pya-OEt	2.9	−25 (c = 36, AcOEt)	13	>98
14c	N–Ac-(L)-4-pya-OH	2.8	+29.5 (c = 1, 1 N HCl)	13	>98
15c	N–Ac-(D)-4-pya-OEt	2.9	−22 (c = 1, AcOEt)	15	>98

[a] HPLC analysis conditions : isocratic system : 90% ACN 0.1% TFA (v/v)

[b] Yield calculated from the racemic mixture



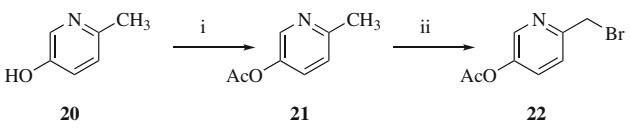
Scheme 3 Reagents and conditions: i, KHMDS, ACN, −40°C, **16a**:44% yield, **16b**:37% yield; ii, NaOH/EtOH, r.t., 90 min and refluxing dioxan, 80%; iii, α-CT/ phosphate buffer pH 6.9, 18:42% yield, 19:40% yield

Table 4 Characteristics of optically pure *N*-acetyl-2-azatyrosine after separation and purification procedure

Compound	Name of compound	[a]HPLC retention time (min)	$[\alpha]_D$	Overall yield (%)[b]	ee (%)
19	N–Ac-(L)-2-azatyr-OH	2.8	+37 (c = 1, 1 N HCl)	26	>99
18	N–Ac-(D)-2-azatyr-OEt	2.9	−24 (c = 1, AcOEt)	27	>99

[a] HPLC analysis conditions: isocratic system: 90% ACN 0.1% TFA (v/v)

[b] Yield calculated from the racemic mixture



Scheme 4 Reagents and conditions: i, Ac_2O, 120°C, 30', 95% yield; ii, NBS, AIBN/CCl_4, 60°C, 48H, 60% yield

different chemoenzymatic strategies leading to enantiomerically pure compounds.

The first one, via oxazolone derivatives, is recommended for 3- and 4-pya stereoselective syntheses but not for 2-pya because of 2-carboxaldehyde instability. The second strategy, via acetamidomalonate derivatives, can be applied for the synthesis of all three regioisomers of pyridylalanine and 2-azatyrosine.

The strategies described include N and C protections in the aim to direct insertion into a correct peptide sequence for kinetic studies of *Pseudomonas aeruginosa* elastase PsE (Rival et al. 1999; Riechmann and Kasche 1986; Miranda and Tominaga 1991, 1986.

Experimental part

General remarks

Melting points were obtained using a Büchi 510 capillary apparatus and were uncorrected. NMR spectra were obtained at room temperature on either a Bruker Avance DPX 200 (200 Hz), a Bruker AC250 (250 Hz) or a Bruker Avance 300 (300 Hz). For ^{1}H NMR or ^{13}C NMR spectra recorded in $CDCl_3$, D_2O, MeOH-d_4 or DMSO-d_6 chemical shifts are quoted in ppm and were referenced to the residual solvent peak. The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Coupling constants were reported in Hertz (Hz). Low resolution mass spectra were recorded on micromass electrospray instrument with only molecular ion and other major peaks being reported. High resolution mass spectra were determined on micromass Q-Tof electrospray machine. Elemental analyses were performed using a Thermo Finnigan Flash EA 1112 Series machine. LC–MS identification was performed by electrospray on a micromass ESI Platform II. Optical rotations were determined on a Perkin-Elmer 241 polarimeter at room temperature in appropriate solvent. Enantiomeric excess was calculated by HPLC using chiral columns: CHIRACEL®. Flash chromatography was carried out using E-Merck Silica Gel (Kieselgel 60, 230–400 mesh) as stationary phase. Thin layer chromatography was carried out on aluminium plates pre-coated with Merck Silicagel 60F254 and were visualized by quenching of UV fluorescence, by iodine vapor or by ninhydrin spray. Analytic HPLC was performed on a Waters apparatus 717 plus autosampler with Millenium 32 program on SymmetryShield™ RP_{18} 3.5 µm 4.6 × 50 mm column and using linear gradient of CH_3CN in H_2O with 0.1% TFA (v/v) in 15 min with 1 mL/min flow or in isocratic conditions on a Beckman LC-508 apparatus with 32 Karat software on Macherey–Nagel CC 250/4.6 nucleosil 100-5 C18 column with 1 mL/min flow. Preparative HPLC was performed on a Waters Delta 4000 apparatus equipped with a Delta-Pak C18 column (15 µm, 40 × 100 mm) and a UV detector, using linear gradient of CH_3CN in H_2O with 0.1% TFA (v/v) with 20 mL/min flow. THF was distilled from sodium/benzophenone ketyl. Reagents and enzymes were supplied from commercial sources (ALDRICH, FLUKA).

*(4Z)-2-Methyl-4((pyridin-3-yl)methylene)oxazol-5(4H)-one or oxazolone derivative of 3-pya (**3a**)*

A mixture of nicotinic aldehyde (0.1 mol, 10.7 g), *N*-acetylglycine (0.1 mol, 11.7 g) anhydrous sodium acetate (0.1 mol, 0.82 g) and acetic anhydride (0.3 mol, 3.06 g) was stirred at 90–100°C for 75 min. After cooling the mixture, ethanol and a mixture of ethanol/water (1/1) were added. **3a** precipitated, was filtered and obtained after recrystallization in carbon tetrachloride in 70% yield as a yellow powder. Mp = 170–172°C. ^{1}H NMR (250 MHz, DMSO-d_6): δ (ppm) 2.4(s, 3H), 7.3(s, 1H), 7.55(m, 1H), 8.7(m, 2H); 9.1(s, 1H). MS (ESI) *m/z* 189 $(M + H)^+$.

A mixture of isonicotinic aldehyde (0.1 mol, 10.7 g), *N*-acetylglycine (0.1 mol, 11.7 g) anhydrous sodium acetate (0.1 mol, 0.82 g) and acetic anhydride (0.3 mol, 3.06 g) was stirred at room temperature and plunged quickly at 90–100°C for exactly 1 min. After cooling the pasty mixture was tritured in ethanol and in a mixture of ethanol/water (1/1). **3b** precipitated, was filtered and purified by recrystallization in benzene in 46% yield as a green powder. Mp = 189–190°C. ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 2.35(s, 3H), 7.0(s, 1H), 7.85(m, 2H), 8.75(m, 2H). MS (ESI) *m/z* 189 $(M + H)^+$.

*(Z)-2-acetamido-3-(pyridin-3-yl) acrylic acid (**4a**)*

3a (0.1 mol, 18.91 g) was suspended in 3% sodium bicarbonate solution (70 mL) and heated in a water bath up to 70°C until complete dissolution. After cooling, **4a** was precipitated with 3 N HCl solution, filtrated and recrystallized in ethanol. **4a** was obtained in 70% yield as a white powder. Mp = 184–185°C. ^{1}H NMR (250 MHz, D_2O) δ (ppm) 2(s, 3H), 7.4(s, 1H), 7.95(dd, 1H, $J_1 = 5.90$ and $J_2 = 5.80$ Hz), 8.5(m, 2H), 8.75(s, 1H). MS (ESI) *m/z* 207 $(M + H)^+$.

*(Z)-2-acetamido-3-(pyridin-4-yl) acrylic acid (**4b**)*

3b (0.1 mol, 18.91 g) was suspended in water (50 mL) and heated up to 100°C until complete dissolution. After cooling, precipitated **4b** was filtrated, recrystallized in ethanol and was obtained in 33% yield as a white powder. Mp = 174°C. ^{1}H NMR (200 MHz, D_2O) δ (ppm) 2(s, 3H), 6.9(s, 1H), 8.45(d, 2H, $J = 6.8$ Hz), 8.75(d, 2H, $J = 6.8$ Hz). MS (ESI) *m/z* 207 $(M + H)^+$.

*N-acetyl-3 Pyridylalanine (**5a**)*

4a (103 mg, 0.5 mmol) was dissolved in methyl alcohol (10 mL). 10% Pd/C (93 mg) was added. The mixtures were stirred under H_2 9 h at room temperature. The solutions were filtered on Celite and methyl alcohol was eliminated

under reduced pressure. *N*-acetyl-3 Pyridylalanine **5a** was yielded after recrystallization as white powder (88.5 mg). *R*f: 0.8 (EtOH/NH_4OH 9/1), HPLC R_t: 2.8 min (isocratic system 90%ACN, 0.1%TFA (v/v)). ^{1}H NMR (250 MHz, MeOH-d_4) δ (ppm) 2(s, 3H), 3.3(m, 2H), 3.9(t, 1H, $J = 6.45$ Hz), 7.95(dd, 1H, $J_1 = 6.1$ and $J_2 = 6.3$ Hz), 8.55(m, 3H). ^{13}C NMR (D_2O, 300 MHz) δ (ppm) 28(CH_3), 33.5(CH_2), 55(CH); 123.8(CH), 131(C), 137.5(CH), 147(CH), 148.5(CH), 173(C=O), 176(C=O). MS (ESI) *m/z* 209 $(M + H)^+$.

*N-acetyl-4-Pyridylalanine (**5b**)*

The same procedure as for **5a** was used to synthesize **5b** with **4b** (103 mg, 0.5 mmol) in place of **4a**. Yield: 85% 88.5 mg. R_f: 0.82 (EtOH/NH_4OH 9/1), HPLC R_t: 2.9 min (isocratic system 90%ACN, 0.1%TFA (v/v)). ^{1}H NMR (250 MHz, MeOH-d_4) δ (ppm) 2 (s, 3H), 3.15(m, 2H), 4.75(dd, 1H, $J_1 = 5.1$ and $J_2 = 5$ Hz), 7.45(d, 2H, $J = 6$ Hz), 8.55(d, 2H, $J = 6.1$ Hz). MS (ESI) *m/z* 209 $(M + H)^+$.

*2-Amino-3-(pyridin-3-yl)propanoic acid (**6a**)*

5a (250 mg, 1.2 mmol) was refluxed 3 h in HCl (10 mL). After filtration the acidic filtrate was neutralized with 3 N sodium hydroxide and extracted with diethyl ether (10 mL). The aqueous layer was treated with active charcoal, filtrated and concentrated under reduced pressure. The 2-amino-3-(pyridin-3-yl)propanoic acid **6a** was obtained after washing with distilled acetone (2 × 10 mL) as white crystals in quantitative yield.

Mp = 250–255°C. $Mp_{[lit]} = 258$–260°C.[28] R_f: 0.6 (EtOH/NH_4OH 9/1). ^{1}H NMR (250 MHz, D_2O) δ (ppm) 3.10(m, 2H), 3.9(t, 1H, $J_1 = 6.4$ Hz), 7.35(m, 1H), 7.70(m, 1H), 8.35(s, 1H), 8.4(d, 1H, $J = 6.4$ Hz). MS (ESI) *m/z* 167 $(M + H)^+$.

*2-Amino-3-(pyridin-4-yl)propanoic acid (**6b**)*

The same procedure as for **6a** was used to synthesize **6b** with **5b** (250 mg, 1.2 mmol) in place of **5a**. Quantitative yield. Mp = 229–232°C. $Mp_{[lit]} = 235$–236°C.[28] R_f: 0.65 (EtOH/NH_4OH 9/1). ^{1}H NMR (250 MHz, D_2O) δ (ppm) 3.10(m, 2H), 3.8(t, 1H, $J_1 = 6.7$ Hz), 7.30(d, 2H, $J = 6.3$ Hz), 8.6(d, 2H, $J = 6.4$ Hz). MS (ESI) *m/z* 167 $(M + H)^+$.

*N-fluorenylmethyloxycarbonyl-3-pyridylalanine-methyl ester (**7a**)*

Fmoc OSu (18.1 mmol, 4.6 g) was dissolved in dioxan (10 mL) and added to a solution of amino acids or amino esters (3.32 g, 20 mmol) dissolved in 10% aqueous sodium hydrogenocarbonate. The mixture was stirred for 22 h at r.t. The pH of the aqueous layer was decreased until pH3 with a concentrated HCl solution and was extracted with diethyl ether or ethyl acetate 2 times. The organic layer was dried on $MgSO_4$, solvent was evaporated under reduced pressure and Fmoc amino acid was obtained.

The Fmoc amino acid (11 mmol, 1 equiv) was dissolved in MeOH (100 mL). $ClSiMe_3$ (88 mmol, 8 equiv) was added dropwise and the solution was stirred for 36 h at 40°C. MeOH and excess of $ClSiMe_3$ were eliminated by evaporation under reduced pressure and **7a** was directly obtained in quantitative yield. Light yellow solid. Mp = 39–41°C. R_f: 0.85 (20%MeOH/CH_2Cl_2). HPLC R_t: 9.33 min (gradient of ACN in water-0.1% TFA (v/v), 15 min).^{1}H NMR (200 MHz, MeOH-d_4) δ (ppm) 3.25(m, 2H), 3.7(s, 3H), 4.15(m, 1H), 4.3(m, 2H), 4.6(m, 1H), 7.3 (t, 2H, $J = 7.55$ Hz), 7.4(t, 2H, $J = 7.25$ Hz), 7.55(t, 2H, $J = 6,8$ Hz), 7.75(d, 2H, $J = 7.34$ Hz), 7.95(m, 1H), 8,54(m, 1H), 8.75(m, 2H). MS (ESI) *m/z* 403 $(M + H)^+$.

*N-fluorenylmethyloxycarbonyl-4-pyridylalanine-methyl ester (**7b**)*

The same procedure as for **7a** was also used to protect **6b.** Yield: 51% as white powder. Mp = 52–55°C. HPLC R_t: 9.2 min (gradient of ACN in water-0.1% TFA (v/v), 15 min).^{1}H NMR (200 MHz, MeOH-d_4) δ (ppm) 3.25(m, 2H), 3.7(s, 3H), 4.15(m, 1H), 4.3(m, 2H), 4.6(m, 1H), 7.3 (t, 2H, $J = 7.45$ Hz), 7,4(t, 2H, $J = 7.25$ Hz), 7.55(t, 2H, $J = 6.8$ Hz), 7.75(d, 2H, $J = 7.34$ Hz), 7.95(m, 2H), 8.75(m, 2H). MS (ESI) *m/z* 403 $(M + H)^+$.

*Enzymatic resolution of 7a by α-CT (**8a, 9a**)*

A solution of **7a** (2.25 g, 5.6 mmol) in DMF (5 mL) was added dropwise to a stirred solution of α-chymotrypsine (15 mg) and ammonium acetate (29 mg, 0.38 mmol) in water (40 mL) at room temperature, the pH being kept constant at 6.9, using a pH Stat, by addition of 1 M ammonium hydroxide. The reaction mixture was stirred for 2 h at room temperature and followed by HPLC comparing peaks area. At the end of the kinetic resolution, the mixture was concentrated by evaporation under reduced pressure. The reaction mixture was diluted with water (10 mL) and neutralized until pH8 with 3 N NaOH solution and the aqueous layer was extracted 3 times with ethyl acetate. The organic layer was dried on Mg SO_4 and concentrated under reduced pressure. The N-protected amino ester (D) was obtained after purification on silica gel chromatography (elution solvent: 20%MeOH/CH_2Cl_2 or 100% AcOEt). The pH of the aqueous layer was decreased until pH4 with pure acetic acid and extracted 3 times with ethyl acetate. The

organic layer was dried on $MgSO_4$ and concentrated under reduced pressure. The N-protected amino acid (L) was obtained after recrystallization in a mixture of diethyl ether/hexane. (**8a**): Yield: 41% (891 mg) as white. R_f: 0.6 (20%MeOH/CH_2Cl_2). HPLC R_t: 8.9 min (gradient of ACN in water-0.1% TFA (v/v), 15 min). ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm) 3.25(m, 2H), 4.05(m, 1H), 4.15 (m, 2H), 4.45 (m, 1H), 7.15(m, 5H), 7.3(m, 2H), 7.55(d, 1H, $J = 7.45$ Hz), 7.75(d, 2H, $J = 7.35$ Hz), 8.35(m, 2H). MS (ESI) *m/z* 389 $(M + H)^+$. $[\alpha]_D = +21$, c = 1 in MeOH; ee > 98%. (**9a**): Yield: 33% (743 mg) as pale yellow solid. Mp = 41–43°C. R_f: 0.85 (20%MeOH/CH_2Cl_2). HPLC R_t: 9.33 min (gradient of ACN in water-0.1% TFA (v/v), 15 min). $[\alpha]_D = -36$, c = 1 in DMF;[7, 6] ee > 98%. See **7a** for NMR analysis.

*Enzymatic resolution of 7b by α-CT (**8b**, **9b**)*

The same procedure as for **8a** and **9a** was also used to synthesize **8b** and **9b** with **7b** (2.25 g, 5.6 mmol) in place of **7a**. **(8b):** Yield: 40% (869 mg) as white powder. R_f: 0.58 (20%MeOH/CH_2Cl_2). HPLC R_t: 8.8 min (gradient of ACN in water-0.1% TFA (v/v), 15 min). ^{1}H NMR (200 MHz, MeOH-d_4) δ (ppm) 3.25(m, 2H), 4.15(m, 1H), 4.3(m, 2H), 4.6(m, 1H), 7.3(t, 2H, $J = 7.45$ Hz), 7.4(t, 2H, $J = 7.25$ Hz), 7.55(t, 2H, $J = 7.4$ Hz), 7.75(d, 2H, $J = 7.35$ Hz), 8 (m, 2H), 8.75(m, 2H). MS (ESI) *m/z* 389 $(M + H)^+$. $[\alpha]_D = -31$ in DMF; ee > 98%. (**9b**): Yield: 46% (1,035 mg) as white powder. Mp = 53–55°C. HPLC R_t: 9.2 min (gradient of ACN in water-0.1% TFA (v/v), 15 min).$[\alpha]_D = +16$ in MeOH; ee > 98%. See **7b** for NMR analysis.

*Diethyl 2-acetamido-2-(methylpyridin-2-yl)malonate (**12a**)*

To the stirred solution in ACN (100 mL) of diethyl acetamidomalonate (2.17 g, 10 mmol, 1 equiv) and KHMDS (5.98 g, 30 mmol, 3 equiv) in cooled ethyl alcohol bath (−40°C), 2-bromomethyl pyridine (2.58 g, 1.5 equiv) diluted in ACN (5 mL) was slowly added (30 min) with a syringe pump. The temperature of the stirred mixture raised until room temperature overnight. The solvent was evaporated under reduced pressure. The residue was diluted in ethyl acetate (100 mL), washed with saturated solution of ammonium chloride (80 mL), dried on $MgSO_4$ and concentrated under reduced pressure. **12a** was purified by chromatography on silicagel column with AcOEt/cyclohexane 1/1 mixture as eluent.

Yield: 70% as transparent crystals. Mp = 82.4–86°C. R_f: 0.45 (AcOEt/cyclohex 1/1). HPLC R_t: 6.5 min (gradient of ACN in water-0.1% TFA (v/v), 15 min). ^{1}H NMR (200 MHz, Acetone-d_6) δ (ppm) 1.3(t, 6H, $J = 7.1$ Hz), 1.95(s, 3H), 3.75(s, 2H), 4.20(q, 4H, $J = 7.1$ Hz), 7.05 (d, 1H, $J = 7.1$ Hz), 7.15(m, 2H), 7.7(m, 1H), 8.45(d, 1H, $J = 7.1$ Hz).^{13}C NMR (300 MHz, Acetone-d_6) δ (ppm) 14.5(2 CH_3), 22.5(CH_3), 41(CH_2), 62.6(2 CH_2), 67(C), 122.6(CH), 125.1(CH), 137(CH), 149.5(CH), 157.6(C), 168.5(2C=O), 169.7(C=O). MS (ESI) *m/z* 309 $(M + H)^+$.

*Diethyl 2-acetamido-2-(methylpyridin-3-yl)malonate (**12b**)*

The same procedure as for **12a** was used to synthesize **12b** with 3-bromomethyl pyridine (2.58 g) in place of 2-bromomethyl pyridine. Yield: 70% as transparent crystals. Mp = 94–95°C. R_f: 0.75 (AcOEt). HPLC R_t: 7 min (gradient of ACN in water-0.1% TFA (v/v), 15 min). ^{1}H NMR (300 MHz, Acetone-d_6) δ (ppm) 1.25(t, 6H, $J = 7,1$ Hz), 2.05(s, 3H), 3.6(s, 2H), 4.25(q, 4H, $J = 7.1$ Hz), 7.3 (m, 1H), 7.4(s, 1H), 7.5(d, 1H, $J = 7.1$ Hz), 8.3(s, 1H), 8.45(d, 1H, $J = 7,1$ Hz).^{13}C NMR (300 MHz, Acetone-d_6) δ (ppm) 14(2CH_3), 22.5(CH_3), 36(CH_2), 63(2CH_2), 68(C), 124(CH), 132.5(C), 138.5(CH), 149(CH), 152(CH), 168(2C=O), 170.05(C=O). MS (ESI) *m/z* 309 $(M + H)^+$.

*Diethyl 2-acetamido-2-(methylpyridin-4-yl) malonate (**12c**)*

The same procedure as for **12a** was used to synthesize **12c** with 4-bromomethyl pyridine (2.58 g) in place of 2-bromomethyl pyridine. Yield: 60% as transparent crystals. Mp = 118–121°C. R_f: 0.5 (AcOEt/cyclohexane 1/1). HPLC R_t: 6.8 min (gradient of ACN in water-0.1% TFA (v/v), 15 min). ^{1}H NMR (300 MHz, Acetone-d_6) δ (ppm) 1.25(t, 6H, $J = 7.1$ Hz), 2.05(s, 3H), 3.6(s, 2H), 4.25 (q, 4H, $J = 7.1$ Hz), 7.05(d, 2H, $J = 7.1$ Hz), 7.4(s broad, 1H, NH), 8.45(d, 2H, $J = 7,1$ Hz).^{13}C NMR (300 MHz, Acetone-d_6) δ (ppm) 14(2CH_3); 23(CH_3), 38(CH_2), 63(2CH_2), 68(C), 126(2CH), 145(C), 150(2CH), 168(2C=O), 170.05(C=O). MS (ESI) m/z 309 $(M + H)^+$.

*Ethyl (D, L)-2-acetamido-3-(pyridin-2-yl)propanoate (**13a**)*

13a was prepared according to the procedure of Cooper et al. 1996. Yield: 50% as white powder. Mp = 72–74°C. HPLC R_t: 3.2 min (isocratic conditions ACN-water (9:1) containing 0.1% TFA (v/v)). ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 1.15(t, 3H, $J = 7.1$ Hz), 2.0 (s, 3H), 3.35(m, 2H), 4.15(q, 2H, $J = 7.1$ Hz), 5(m, 1H), 7.15(m, 3H), 7.65 (m, 1H), 8.55(d, 1H, $J = 7.1$ Hz). MS (ESI) m/z 237 $(M + H)^+$.

*Ethyl (D, L)-2-acetamido-3-(pyridin-3-yl)propanoate (**13b**)*

The same procedure as for **13a** was used to make **13b** with **12b** in place of **12a**. Yield: 60% as white powder. Mp = 112–115°C. HPLC R_t: 2.9 min (isocratic conditions ACN-water (9:1) containing 0.1% TFA (v/v)). ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 1,25(t, 3H, $J = 7.1$ Hz),

1.95(s, 3H), 3-3.15(m, 2H), 4.2(q, 2H, $J = 7.1$ Hz), 4.9(m, 1H), 6.75(s broad, 1H, NH), 7.2(m, 1H), 7.6(m, 1H), 8.35(s, 1H), 8.45(d, 1H, $J = 7.1$ Hz). MS (ESI) *m/z* 237 $(M + H)^+$.

*Ethyl (D,L)-2-acetamido-3-(pyridin-4-yl)propanoate (**13c**)*

The same procedure as for **13a** was used to make **13c** with **12c** in place of **12a**. Yield: 70% as white powder. Mp = 83– 85°C. HPLC R_t: 2.9 min (isocratic conditions ACN-water (9:1) containing 0.1% TFA (v/v)). ^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 1.25(t, 3H, $J = 7,1$ Hz), 1.95(s, 3H), 3–3.15(m, 2H), 4.15(q, 2H, $J = 7.1$ Hz), 4.9(m, 1H), 6.3(s broad, 1H, NH), 7.1(d, 2H, $J = 7.1$ Hz), 8.5(d, 2H, $J = 7.1$ Hz). MS (ESI) *m/z* 237 $(M + H)^+$.

*Enzymatic resolution of 13a (**14a**, **15a**)*

The procedure for the synthesis of **14a** and **15a** was similar to that used for **8a** and **9a** except that 1.18 g (5 mmol) of compound **13a** was used instead of compound **7a** and with different procedure of purification: after concentration of the resolution mixture under reduced pressure, the pasty residue was extracted 3 times with ethyl acetate and the combined ethyl acetate extracts were concentrated under reduced pressure. Purification by flash column chromatography (10%MeOH/CH_2Cl_2) gave N-protected-(D)-amino ester. The solid residue was diluted with water (10 mL) and its pH was acidified by acetic acid (pH4) and the salty aqueous solution was lyophilized. The solid was washed 2 times with ethanol (10 mL) and the N-protected-(L)-amino acid was obtained after recrystallisation in a mixture of diethyl ether/hexane. (**14a**): Yield: 35% (364 mg) as white powder. HPLC R_t: 3.1 min (isocratic conditions ACN-water (9:1) containing 0.1% TFA (v/v)).^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 2,02(s, 3H); 3,35(m, 2H);5(m, 1H); 7,175(m, 3H); 7,65(m, 1H); 8,55(d, 1H, $J = 7,6$ Hz). MS (ESI) *m/z* 209 $(M + H)^+$. $[\alpha]_D = +32.2$ c = 28.7 in 1 N HCl, ee > 98%. (**15a**): Yield: 42.5% (501 mg) as white powder. Mp = 72–74°C. HPLC R_t: 3.2 min (isocratic conditions ACN-water (9:1) containing 0.1% TFA (v/v)). For NMR analysis see **13a**. $[\alpha]_D = -23,1$ c = 37 in AcOEt, ee > 98%.

*Enzymatic resolution of 13b (**14b**, **15b**)*

The same procedure as for **14a** and **15a** was used to prepare **14b** and **15b** with **13b** (1.18 g, 5 mmol) used in place of **13a**. (**14b**): Yield: 36% (374 mg) as white powder. HPLC R_t: 2.8 min (isocratic conditions ACN-water (9:1) containing 0.1% TFA (v/v)).^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 1.95(s, 3H), 3–3.15(m, 2H), 4.9(m, 1H), 6.75(s broad, 1H, NH), 7.2(m, 1H), 7.6(m, 1H), 8.35(s, 1H), 8.45(d, 1H, $J = 6.3$ Hz). MS (ESI) *m/z* 209 $(M + H)^+$. $[\alpha]_D = +34$ c = 28,7 in 1 N HCl, ee > 98%. (**15b**):Yield: 30% (354 mg) as white powder. Mp = 112–115°C. HPLC R_t: 3.2 min (isocratic conditions ACN-water (9:1) containing 0.1% TFA (v/v)). For NMR analysis see **13b**. $[\alpha]_D = -25$ c = 36 in AcOEt; ee > 98%.

*Enzymatic resolution of **13c** (**14c**, **15c**)*

The same procedure as for **14a** and **15a** was used to prepare **14c** and **15c** with **13c** (1.18 g, 5 mmol) used in place of **13a**. (**14c**) Yield: 30% (312 mg) as white powder. HPLC R_t: 2.86 min (isocratic conditions ACN-water (9:1) containing 0.1% TFA (v/v)).^{1}H NMR (200 MHz, $CDCl_3$) δ (ppm) 1.95(s, 3H), 3–3.15(m, 2H), 4.9 (m, 1H), 6.3(s broad, 1H, NH), 7.1(d, 2H, $J = 6$ Hz), 8.5(d, 2H, $J = 5.9$ Hz). MS (ESI) *m/z* 209 $(M + H)^+$.$[\alpha]_D = +29.5$, c = 1 in 1 N HCl, ee > 98%. (**15c**) Yield: 35% (413 mg) as white powder. Mp = 83–85°C. HPLC R_t: 2.9 min (isocratic conditions ACN-water (9:1) containing 0.1% TFA (v/v)). For NMR analysis see **13c**. $[\alpha]_D = -22$°C, c = 1 in AcOEt, ee > 98% .

*Diethyl 2-acetamido-2-((5-acetoxypyridin-2-yl)méthyl) malonate (**16a**)*

The procedure of synthesis of **16a** was similar to that used to synthesize **12a** except 5-Acetoxy-2-bromomethylpyridine was used in place of 2-bromomethyl pyridine. Yield: 44% as white crystals. HPLC *R*t: 8.8 min (gradient of ACN in water-0.1% TFA (v/v), 20 min). RMN ^{1}H (200 MHz, Acetone-d_6) δ (ppm) 1.3(t, 6H, $J = 7.1$ Hz), 1.95(s, 3H), 2.3(s, 3H), 3.75(s, 2H), 4.21(q, 4H, $J = 7.1$ Hz), 7.2(d, 1H, $J = 7.1$ Hz), 7.35(s, 1H, NH), 7.5(m, 1H), 8.29(s, 1H). RMN ^{13}C (300 MHz, Acetone-d_6) δ (ppm) 13.5($2CH_3$), 20(CH_3), 22(CH_3), 40(CH_2), 62($2CH_2$), 67(C), 125(CH), 129.5(CH), 142.5(CH), 146(C), 154(C), 167.5(2C=O), 169(2C=O). MS (ESI) *m/z* 367 $(M + H)^+$.

*Diethyl 2-acetamido-2-((5-hydroxypyridin-2-yl)methyl) malonate (**16b**)*

The same procedure as for **16a** was used to make **16b**. Yield: 37% as white crystals. Mp = 147–149°C. *R*t: 6.7 min (gradient of ACN in water-0.1% TFA (v/v), 20 min). RMN ^{1}H (200 MHz, Acetone-d_6) δ (ppm) 1.3(t, 6H, $J = 7.1$ Hz), 1.95(s, 3H), 3.75(s, 2H), 4.21(q, 4H, $J = 7.1$ Hz), 7.2(d, 1H, $J = 7.1$ Hz), 7.35(se, 1H, NH), 7.5(m, 1H), 8.29(s, 1H). RMN ^{13}C (300 MHz, Acetone-d_6,) δ (ppm) 13.5($2CH_3$), 22(CH_3), 40(CH_2), 62($2CH_2$), 67(C), 125(CH), 129,5(CH), 142,5(CH), 146(C), 154(C), 167.5(2C=O), 169(C=O). MS (ESI) *m/z* 325 $(M + H)^+$.

*Ethyl (D, L)-2-acetamido-3-(5-hydroxypyridin-2-yl) propanoate (**17**)*

17 was prepared according to the procedure using by Cooper et al. 1996. Yield: 80% as white powder. Mp = 175–177°C. HPLC *R*t: 2.9 min (isocratic conditions ACN-water (9:1) containing 0.1% TFA (v/v)). RMN ^{1}H (300 MHz, DMSO-d_6) δ (ppm) 1.3(t, 3H, $J = 7.1$ Hz), 1.9 (s, 3H), 3.1(dd, 1H, $J_1 = 7.13$ and $J_2 = 7.1$ Hz), 4.25–4.35(m, 2H), 4.5–4.6(m, 2H), 7.1(d, 2H, $J = 7.1$ Hz), 8.34(d, 1H, $J = 7.1$ Hz). RMN ^{13}C (300 MHz, DMSO-d_6) δ (ppm) 14(CH_3), 22(CH_3), 41(CH_2), 52.3(CH), 62.6(CH_2), 122.6(CH), 125,1(CH), 137(CH), 149.5(C), 157.6(C), 168.5(2C=O), 169.7(C=O). MS (ESI) *m/z* 253 $(M + H)^+$.

*Enzymatic resolution of **17** (**18**, **19**)*

The same procedure as for **14a** and **15a** was used to prepare **18** and **19** with **17** (1.26 g, 5 mmol) in place of **13a**. (**18**) Yield: 40% (448 mg) as white powder. HPLC *R*t: 2.8 min (isocratic conditions ACN-water (9:1) containing 0.1% TFA (v/v)). RMN ^{1}H (300 MHz, DMSO-d_6) δ (ppm) 1.9(s, 3H), 3.1(dd, 1H, $J_1 = 8$ et $J_2 = 7.9$ Hz), 4.3(m, 2H), 7.1(d, 2H, $J = 8$ Hz), 8.34(d, 1H, $J = 8$ Hz). RMN ^{13}C (300 MHz, DMSO-d_6) δ (ppm) 22(CH_3), 41(CH_2), 52.3(CH), 122.6(CH), 125.1(CH), 137(CH), 149.5(C), 157.6(C), 168.5(C=O), 169.7(C=O). MS (ESI) *m/z* 225 $(M + H)^+$. $[\alpha]_D^{25} = +37$, c = 1 in HCl 1 N ee > 99%. (**19**) Yield: 42% (529 mg) as white powder. For NMR analysis see **17**. $[\alpha]_D^{25} = -24$, c = 1 in AcOEt ee > 99%.

*5-Acetoxy-2-methylpyridine (**21**)*

A solution of 5-hydroxy-2-methylpyridine (1.00 g, 9.1 mmol) in acetic anhydride (4.3 ml, 45.8 mmol) was heated at 120°C for 30 min. After cooling to RT, the mixture was neutralized with 1 N NaOH solution and 5-acetoxy-2-methylpyridine was extracted into dichloromethane (3 × 5 ml). The organic layer was dried over $MgSO_4$ and concentrated under reduced pressure and dried at high vacuum. **23** was obtained as colorless oil in quantitative yield. bp: 120°C (22.5 Torr). ^{1}H NMR ($CDCl_3$, 200 MHz): δ 2.3 (s, 3H), 2.55 (s, 3H), 7.30 (d, 1H, $J = 8.50$ Hz), 7.50 (d, 1H, $J = 8.5$ Hz), 8.45 (d, 1H, $J = 8.5$ Hz). ^{13}C NMR ($CDCl_3$, 300 MHz) δ 21 (CH_3), 23.5 (CH_3), 123.5 (CH), 129.7 (CH), 142 (CH), 145.3 (C), 155.5 (C–O), 169 (C=O). SM-ESI$^+$: 152.05 $[M + H]^+$. HRMS *m/z*: Calcd for $C_8H_{10}NO_2$: 152.0712 $[M + H]^+$. Found 152.0717.

*5-Acetoxy-2-bromomethylpyridine (**22**)*

To a mixture of N-bromosuccinimide (4.048 g, 22.7 mmol) and 2,2′-azobis(2-methylpropionitrile) (400 mg) in carbon tetrachloride (45 ml) under argon and heated to 60°C, was added a solution of **23** (2.45 g, 16.25 mmol) in carbon tetrachloride (30 ml). After 12 h, the mixture was filtered to remove succinimide and the filtrate was evaporated under reduced pressure. The solid obtained was purified immediately on silica gel column (diethyl ether/petroleum ether: 1/1(v/v)) and obtained as white crystals (60% yield), mp. 38.5–40°C. ^{1}H NMR (CD_3CN, 300 MHz) δ 2.3 (s, 3H), 4.65 (s, 2H), 7.55 (d, 2H, $J = 8$ Hz), 8.38 (s, 1H). ^{13}C NMR (CD_3CN, 300 MHz) δ 20 (CH_3), 33 (CH_2), 124 (CH), 130.05 (CH), 143 (CH), 146.5 (C), 154 (C–O), 169 (C=O). HPLC: tR = 8.2 min (0%—20 min— >100%). SM-ESI$^+$: 230.17 and 232.18 $[M + H]^+$. *Anal.* Calcd for $(C_8H_8BrNO_2)_2.H_2O$: C, 40.28; H, 3.59; N, 5.87. Found: C, 40.39; H, 4.12; N, 5.53.

Conflict of interest The authors declare that they have no conflict of interest.

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