RENEWABLE FUNCTIONALIZED PYRIDINES DERIVED FROM MICROBIAL METABOLITES OF THE ALKALOID (*S*)-NICOTINE

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Abstract - (*S*)-Nicotine, which is present in concentrations of 2-8 % in dried leaves of certain tobacco plants, was used as a starting material for the biocatalytic production of renewable functionalized pyridines. Key microbial metabolites of (*S*)-nicotine, such as 6-hydroxy-(*S*)-nicotine and 4-[(6-hydroxypyridine)-3-yl]-4-oxobutyrate were used in subsequent chemical steps for the preparation of a wide variety of 2,5-di- or 2,3,5-tri-substituted pyridines.

INTRODUCTION

Renewable resources are very important starting materials for the chemical synthesis of a wide variety of non-food products. In particular, natural chiral compounds such as amino acids and carbohydrates are essential synthons for the manufacture of many biologically active compounds. Due to generally increased concerns regarding the pollution of the environment, CO_2 emission, limited petrochemical reserves, and agricultural production surplus in some countries there is an interest in using nature's synthetic potential for the development of new products with new properties.

Dried leaves of the tobacco plants *Nicotiana rustica* and *N. tabacum* contain as much as 2-8% of (*S*)-nicotine.[1] Surprisingly, this natural alkaloid has not been seriously considered as a potential renewable resource and text books on this topic generally lack references to (*S*)-nicotine.[2] The only large scale application of nicotine is its use as an insecticide. Before the first synthetic insecticides came to the market approx. 2800 tons of (*S*)-nicotine per year were used as a crop protectant.[3] The lack of any specific applications for nicotine as a renewable chemical may be because nicotine is very toxic and has a limited use as an insecticide, and the selective functionalization of nicotine at the pyridine or the pyrrolidine ring by chemical means is difficult to control.[4,5] This restricts the chemical preparation of semi-synthetic pyridines using nicotine as a starting material. For many years it has been known that micro-organisms de-

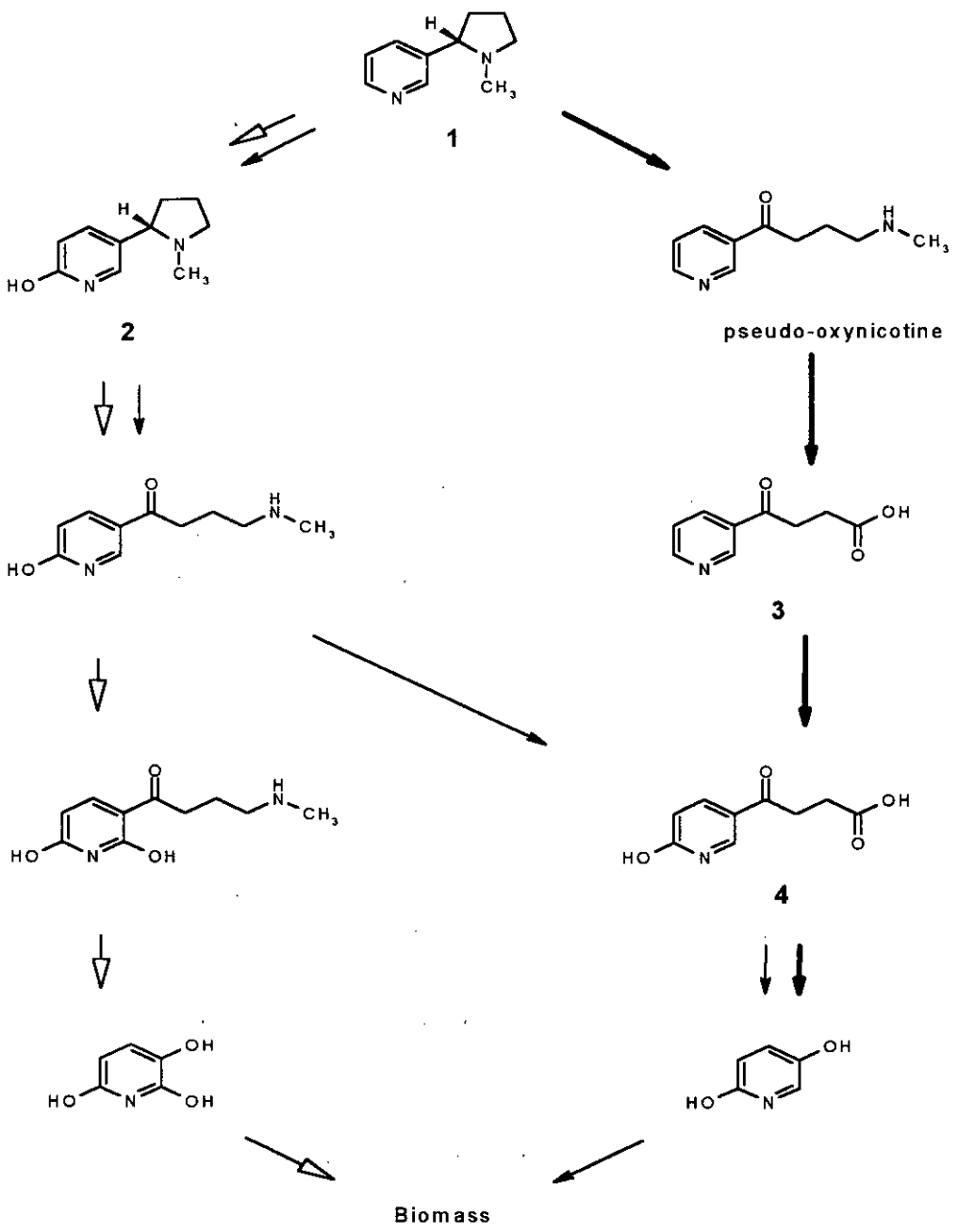


Figure 1. Microbial degradation pathways of (*S*)-nicotine in *Arthrobacter* sp. NRRL-B-3603 (⇨), *Pseudomonas* sp. DSM 8653 (➡) and *Variovorax paradoxus* DSM 8244 (→).

grading (*S*)-nicotine **(1)** form a whole series of substituted pyridines in their metabolic pathways (Figure 1).[6,7] A process for the production of 6-hydroxy-(*S*)-nicotine **(2)** has been developed on this basis,[8] but to our knowledge this metabolite has not been used as a starting material for the synthesis of novel optically

active nicotine analogues until very recently.[9]

In this paper we describe the enzymatic preparation of 4-[6-hydroxypyridin-3-yl]-4-oxobutyrate **(4)** from **1** and the use of **4** and **2** for the synthesis of novel renewable 2,5-di- or 2,3,6-tri-substituted pyridines.[10]

RESULTS AND DISCUSSION

Screening of micro-organisms and production (*S*)-nicotine metabolites

The screening of micro-organisms producing (*S*)-nicotine metabolites was straightforward using TLC-analysis to identify the pathway of the individual isolates. *Arthrobacter oxydans* NRRL-B-3603 was chosen to produce **2**. This strain did not grow well on a mineral salts medium with nicotine as the only C-source and therefore the medium was supplemented with corn steep liquor. Due to the good water solubility of **2** it was necessary to extract the free base from the concentrated aqueous phase. Up to 30 g/L of 6-hydroxy-(*S*)-nicotine were produced with an isolated yield of 51% . *Pseudomonas sp.* DSM 8653 showed a pronounced accumulation of **4** during growth on (*S*)-nicotine. Interestingly, the degradation of **4** only proceeded when no more **1** or **3** was present in the medium. The accumulation of hydroxylated pyridines and pyrazines has already been observed in the degradation of a whole series of substituted pyridines and pyrazines.[11-18] Although the *Pseudomonas* strain grew well in mineral salts medium with (*S*)-nicotine as sole carbon and energy source, the performance of the biotransformation was improved with the addition of citrate and yeast extract. **4** can easily be precipitated as a free acid from the fermentation broth. With this system we were able to produce 15 g/L of **4** with an isolated yield of 91%.

Preparation of semi-synthetic pyridines

The chemical synthesis of 2,5-disubstituted pyridines from nicotinic acid is difficult because the selective functionalization of the pyridine ring in position 2 or 6 is difficult to control and the chain extension in the position of the carboxylate group may require several additional steps. However, starting from the microbial metabolites and using standard chemical methods [19] **2** and **4** were transformed into a whole series of novel substituted pyridines. In particular, it was possible to produce in good yields with total retention of the chirality 6-chloro-(*S*)-nicotine **(5)** and 5,6-dichloro-(*S*)-nicotine **(7)** starting from 6-hydroxy-(*S*)-nicotine (Figure 2).

4-[6-Hydroxypyridin-3-yl]oxobutyrate **(4)** is a versatile synthon for the preparation of di- or tri-substituted pyridines as well as various pyridinyl-substituted heterocyclic compounds. Pyridones such as **4** can undergo a variety of useful reactions, including substitution in the *ortho* position with strong electrophiles, as shown by the formation of compound **(8)** in 67 % yield in the presence of chlorine water

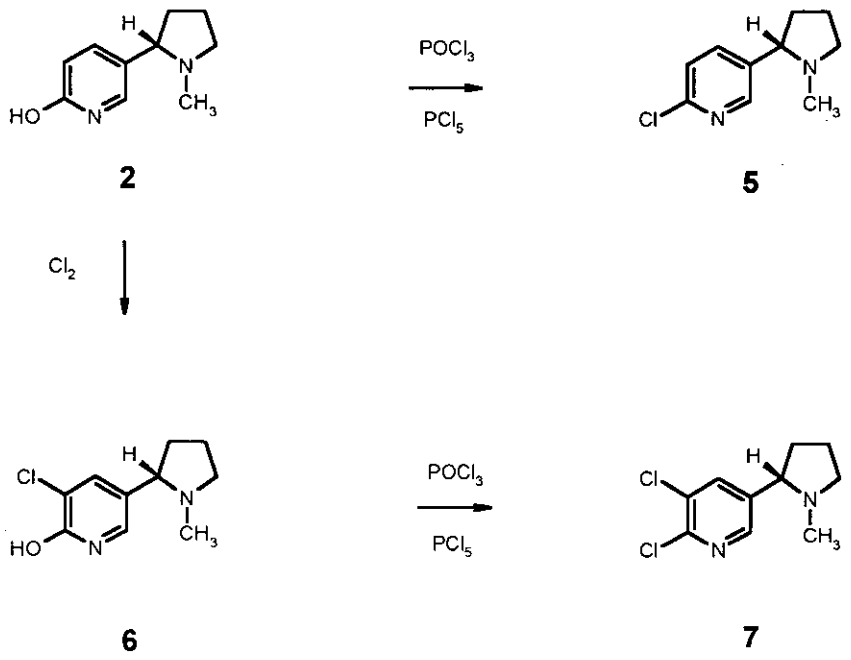


Figure 2. Production of substituted chiral (*S*)-nicotine analogs from 6-hydroxy-(*S*)-nicotine.

(Figure 3). The carboxyl function in **4** and **8** has to be protected in order to allow further manipulation of the pyridine ring. The corresponding esters **(9a,b)** or **(11)** can be efficiently converted into mono- or dichloropyridines **(10)** or **(12)** using phosphorus oxychloride, opening the way to various substituents in position 6.

The 4-oxobutyrate moiety is known to be particularly amenable to a number of synthetic transformations, leading to many different classes of compounds with useful biological activities *via* regio- and/or stereoselective reduction processes, or providing access to various heterocyclic systems *via* cyclocondensations. The reactions described in Figure 4 illustrate some of the compounds readily available from 4-[6-hydroxypyridin-3-yl]oxobutyrate.

The cyclocondensation of compounds **(9a)** or **(10)** results in the formation of the corresponding tetrahydropyrazinones **(13)** and **(14)**, respectively, in good yield and purity. These are structural analogues of a whole class of cardiotonic agents, of which Bemoradan is the prototype.[20] It is remarkable that the strongly nucleophilic hydrazine did not substitute the reactive chlorine in position 6 of the pyridine ring. Unlike hydrazine, hydroxylamine did not form a six-membered ring upon condensation with the oxobutyrate in methanol. This reaction produced an 8/1 quantitative mixture of isomeric (*E*)- and (*Z*)-hydroxyimino esters **(15)**, which were separated by crystallization. Pyridyl-substituted γ-aminobutyric

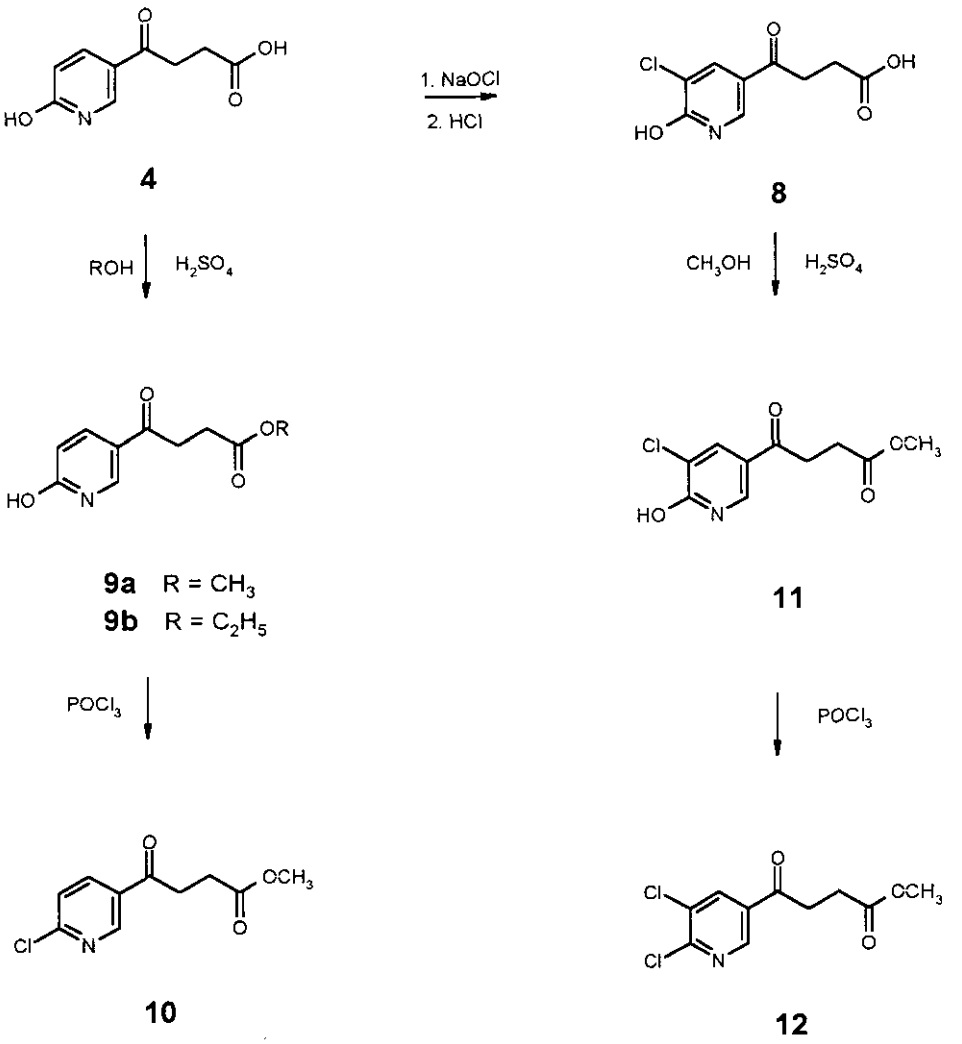


Figure 3. 4-[6-Hydroxypyridin-3-yl]oxobutyrate as a synthon : transformations of the pyridone ring.

acids could be prepared from **15**.

As expected, $NaBH_4$ completely reduced the 4-oxobutyrate **(10)** into the pyridyl-butyrolactone **(16)** under mild conditions. Enantioselective reductions of γ-keto esters are well known, for example the preparation of whisky lactone using baker's yeast,[21] or borohydride in the presence of a chiral auxiliary.[22] Compound **(10)** could be a substrate for an enantioselective reduction, which ultimately would lead to non-racemic γ-hydroxy esters or amides *via* a simple lactone ring-cleavage under basic conditions. The racemic compounds **(17)** and **(18)** were obtained quantitatively in this way.

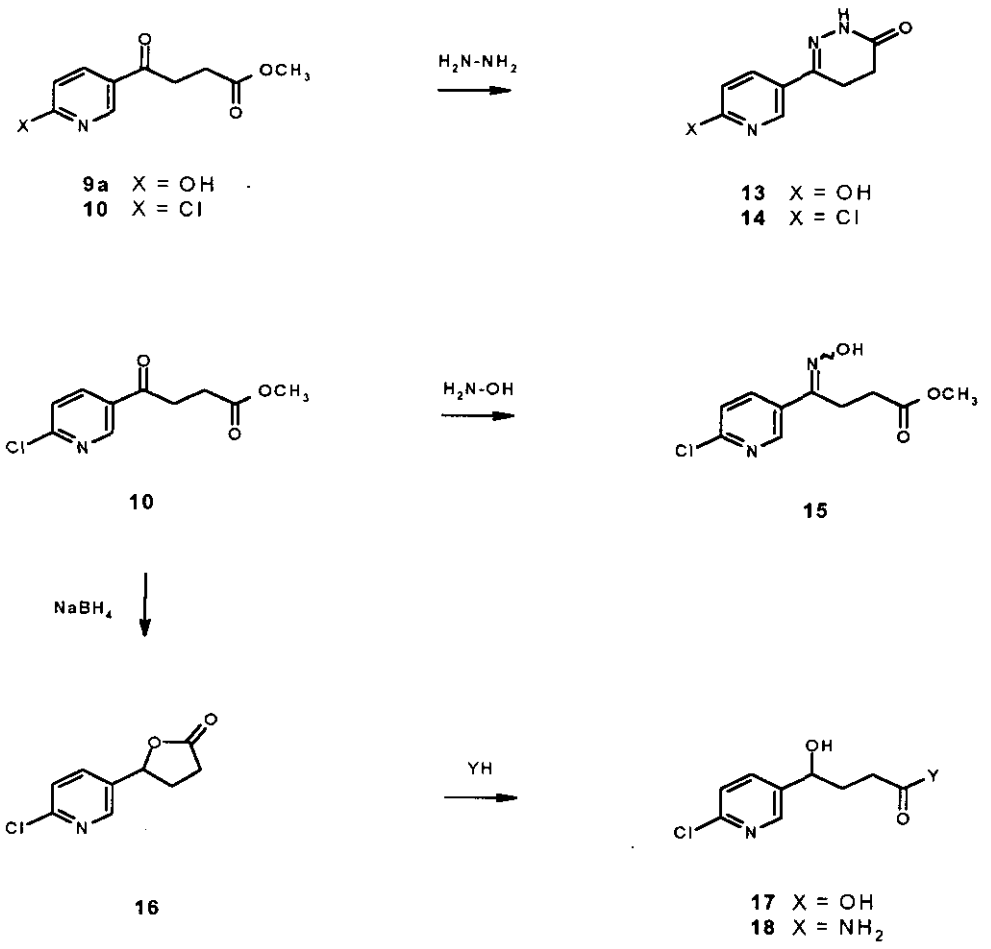
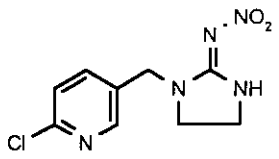


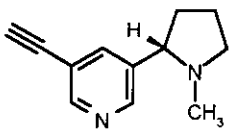
Figure 4. Some transformations of the 4-oxobutyrate sidechain

Our results show that a synergistic biocatalytic/chemical approach opens up new routes for the preparation of semi-synthetic substituted pyridines from (*S*)-nicotine. Although it is possible to prepare a wide range of compounds from these metabolites of (*S*)-nicotine, there is presently no immediate use for these products in the synthesis of commercially available drugs or agrochemicals. However, three biologically active 2,5- or 3,5-substituted pyridines were recently discovered recently. These are the insecticide Imidacloprid,[23] the anti-Parkinson agent SIB-1508Y,[24] and the analgesic Epibatidine, originally isolated from an Ecuadoran frog (Figure 5).[25] (*S*)-Nicotine and its derivatives are also being investigated for therapy of ulcerative colitis, Alzheimer's disease, Parkinson's disease, Tourette's syndrome, sleep apnea and attention deficit disorder.[27-29]

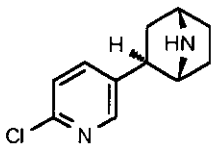
In summary, this paper suggests that the combination of biotechnology and chemistry offers a new approach for the synthesis of renewable functionalized pyridines.



Imidacloprid



SIB-1508Y



Epibatidine

Figure 5. Biologically active 2,5- or 3,5-disubstituted pyridines.

EXPERIMENTAL

Micro-organisms

All micro-organisms used in this study are wild type strains. *Arthrobacter oxydans* NRRL-B-3603 was obtained from the USDA, National Center for Agricultural Utilization Research, 1815 North University Street, Peoria, Illinois 61604, USA. *Pseudomonas* sp. DSM 8653 and *Variovorax paradoxus* DSM 8244 were deposited at the Deutsche Sammlung von Mikroorganismen, Mascheroder Weg 1B, D-38124 Braunschweig, Germany.

Screening

Aerobic (*S*)-nicotine degrading micro-organisms were isolated on a minimal salts medium (MS medium) [30] supplemented with 2 g/L of (*S*)-nicotine (Fluka, Switzerland) at a pH of 7.0 (MS medium +nicotine). Samples used for enrichments were taken from the local waste water treatment plant and a tobacco field in Massongex, Switzerland. Incubation temperature was 30 °C. The resulting bacterial cultures were transferred three times to fresh medium before the suspensions were streaked out to single colonies on the same medium containing 16 g/L of agar. To determine the degradation pathway of the isolated microorganisms, 2 µL of the cell free culture both was applied to a Merck Silicagel 60 F_{254} thin-layer chromatography plate and developed with ethanol / chloroform / 25% aq. NH_4OH / H_2O (55/30/10/5); detection was at 254 nm. The following R_f values were determined: **1** 0.92, **2** 0.70, **3** 0.38, **4** 0.31. Typically, the media of strains degrading **1** *via* 2,3,6-trihydroxypyridine turned very dark during incubation.

Biotransformation of (*S*)-nicotine to 6-hydroxy-(*S*)-nicotine (2)

A 230 mL preculture of *Arthrobacter oxydans* NRRL-B-3603, grown overnight in the MS medium+nicotine with 16 g/L corn steep liquor at a pH of 5.5 and a temperature of 30 °C, was used to inoculate a 1 liter fermenter containing 530 mL of MS+nicotine medium with 12.8 g of corn steep liquor

and 40 g of **1**. The pH during the fermentation was kept constant at 5.5 and the temperature was 30 °C. The conversion of **1** to **2** was followed by TLC analysis. After 14 h an additional 10 g of **1** as a 10% (w/v) solution of the sulfate salt were added to the fermenter. After 24 h the formation of **2** reached a maximum, the biotransformation was stopped and the pH of the cell-free fermentation solution was adjusted to 10.5. The broth was then concentrated under reduced pressure to a volume of 150 mL and **2** was extracted with dichloromethane. The organic phase was dried with Na_2SO_4 and **2** was isolated after removing the solvent. Then 30 g of **2** were isolated (purity according to ^{1}H-NMR >90%) with a yield of 51 %. 6-Hydroxy-(*S*)-nicotine was recrystallized from 2-butanone and had mp 122.3-123.3 °C, $[\alpha]^{20}_D$= - 66.9°, (c = 1, H_2O); $[\alpha]^{20}_D$ = - 112.3° (c = 1, acetonitrile); UV λ_{max} (H_2O) nm (ε): 229 (11000), 299 (5800); MS *m/z* (after silylation) : 249 $(M-1)^+$, 84 (BP); ^{1}H-NMR ($CDCl_3$) : 1.51-1.59 (m, 1H), 1.67-1.82 (m, 2H), 1.96-2.05 (m, 1H), 2.05 (s, 3H), 2.16 (dt, *J* = 9 Hz and 8.6 Hz, 1H), 2.82 (t, *J* = 8.2 Hz, 1H), 3,08 (dt, *J* = 7.3 Hz and 2.2 Hz, 1H), 6.34 (d, *J* = 9.5 Hz, 1H), 7.21 (d, *J* = 2.4 Hz, 1H), 7.42 (dd, *J* = 2.4 and 9.5 Hz, 1H), 11.48 (br s, 1H); ^{13}C-NMR ($CDCl_3$) : 21.87 (CH_2), 33.29 (CH_2), 39.66 (CH_3), 55.94 (CH_2), 66.52 (CH), 119.16 (C), 120.23 (CH), 132.83 (CH), 140.33 (CH), 162.39 (C); Anal. Calcd for $C_{10}H_{14}N_2O$: C; 67.76, H; 7.40, N; 15.81. Found C; 67.57, H; 7.99, N; 15.99.

Biotransformation of (*S*)-nicotine to 4-[6-hydroxypyridin-3-yl]4-oxobutyrate (4) [31]

A 400 mL preculture of *Pseudomonas* sp. DSM 8653 grown overnight in MS medium+nicotine with 0.8 g/L of citric acid and 2 g/L yeast extract at a pH of 7.0 and a temperature of 30 °C was used to inoculate a fermenter containing 4 L of the same medium. The aeration rate was increased from 1 L/min after inoculation to 3 L/min after 5 h. After 7 h the OD_{650} of the cell suspension was 4.0 and 50 g of **1** as a 10% (w/v) solution of the sulfate salt was added to the fermenter. One liter of a neutral aqueous solution containing 20 g of **1**, 8 g of citric acid and 20 g of yeast extract was pumped into the fermenter over 15 h at a constant rate. The conversion of **1** to **4** was followed by TLC analysis. The concentration of **4** was also determined spectrophotometrically. The ε-value at 305 nm in 0.1 M NaOH was 17000 mol^{-1} cm^{-1}. The biotransformation was stopped after 15 h at an OD_{650} of 9.2. For the purification of **4** the cell free broth was acidified with conc. H_2SO_4 to a pH of 2.5. The precipitated acid was removed by filtration and dried. From a total of 70 g of **1** used for the biotransformation 77 g of **4** were isolated, which corresponds to a yield of 91%. No impurities were detected in the ^{1}H-NMR spectrum. Using *Variovorax paradoxus* DSM 8244 as a biocatalyst in a similar experiment, the yield of the biotransformation was 73%. ^{1}H-NMR (DMSO-d_6) : 2.52 (t, *J* = 6 Hz, 2H), 3.04 (t, *J* = 6 Hz, 2H), 6.36 (d, *J* = 9.7 Hz, 1H), 7.86 (dd, *J* = 9.7 and 2.7 Hz, 1H), 8.23 (d, *J* = 2.7 Hz, 1H), 12.06 (br s, 2H), ^{13}C-NMR (DMSO-d_6) : 27.76 (C-2),31.86 (C-3), 116.16 (C-3'), 119.48 (C-5'), 138.08 (C-4'), 140.55 (C-2'), 162.42 (C-6'), 173.66 (C-1), 193.8 (C-4); mp > 250°C (decomposition). MS *m/z* (after silylation) 339(M^+) 324 (BP).

Synthesis of chemically modified (*S*)-nicotine metabolites

6-Chloro-(*S*)-nicotine (5) [32]

6-Hydroxy-(*S*)-nicotine (10 g, 0.0561 mol) was added with efficient stirring to a suspension of PCl_5 (13.1 g, 0.0629 mol) in $POCl_3$ (30 g, 0.196 mol) at 40 °C. Due to the exothermic reaction, the temperature rose to 80 °C and the suspension began to dissolve. The mixture was further heated to 92 °C and kept at that temperature for 1 h. The brown solution was then cooled down to 40 °C and 0.31 g of formic acid was added with caution (evolution of HCl and CO). After 10 min, $POCl_3$ was distilled off under reduced pressure, the residue was taken up with 100 ml of water and the pH adjusted to 10 with aqueous 30 % NaOH. The milky aqueous phase was extracted with 2x 100 mL of toluene and the extracts were washed with 25 mL of water. After evaporating the organic solvent, the oily residue (11.8 g) was purified *via* bulb-to-bulb distillation at 0.05 mbar, 100-140 °C. The yield of 6-chloro-(*S*)-nicotine as a colorless liquid was 9.78 g (88.6 %). n_D = 1.5415, $[\alpha]^{20}_D$ = -154° (c = 1, acetonitrile). ^{1}H-NMR ($CDCl_3$) : 8.30 (d, *J* = 2.4 Hz, 1H), 7.69 (dd, *J* = 2.4 and 8.4 Hz, 1H), 7.29 (d, *J* = 8.4 Hz, 1H), 3.23 (m, 1H), 3.10 (t, *J* = 8.4 Hz, 1H), 2.32 (q, *J* = 8.4 Hz, 1H), 2.20 (m, 1H), 2.16 (s, 3H), 1.92 (m, 1H), 1.83 (m, 1H), 1.68 (m, 1H); ^{13}C-NMR ($CDCl_3$) : 150.15 (C-6), 149.24 (C-2), 138.14 (C-3), 137.87 (C-4), 124.29 (C-5); 68.06 (C-2'), 56.96 (C-5'); 40.33 (CH_3), 35.38 (C-3'), 22.68 (C-4'). MS (EI) : *m/z* : 196 (28), 195 (26), 167 (17), 153 (8), 133 (30), 84 (100); Anal. Calcd for $C_{10}H_{13}N_2Cl$: C; 61.07, H; 6.66, N; 14.24. Found C; 60.14, H; 6.50, N; 14.65.

5-Chloro-6-hydroxy-(*S*)-nicotine (6)

A solution of 6-hydroxy-(*S*)-nicotine (20 g, 0.112 mol) in 330 mL of methanol was placed into a 3-necked flask equipped with a thermometer and a gas inlet tube. 12 g of chlorine were introduced at 19 °C over 5 h The yellow solution was evaporated and the residue was extracted with 130 mL of water and 150 mL of toluene. The desired product remained in the aqueous phase. From the toluene extract, 0.29 g of 3,5-dichloro-2(1*H*)-pyridone was obtained upon evaporation. The aqueous phase was brought to pH 13 with aqueous 30 % NaOH, then washed with 200 mL of toluene in order to remove some minor impurities, and subsequently the pH was adjusted to 9.4 with conc. hydrochloric acid. The product was extracted with 200 mL of toluene and 2x 200 mL of CH_2Cl_2. The organic extracts were washed with 30 mL of water and evaporated to dryness, affording 21.7 g (91 %) of brownish oily product, which crystallized upon standing. mp 114.5 - 115.5 °C; $[\alpha]^{20}_D$ = -139.6° (c = 1, acetonitrile); UV (H_2O) : λ_{max} : 224 nm (ε = 7585), 303 nm (ε = 5835) ^{1}H-NMR ($CDCl_3$) : 13.4 (br s, 1H), 7.75 (d, *J* = 2 Hz, 1H), 7.33 (d, *J* = 2 Hz, 1H), 3.18 (m, 1H), 2.86 (t, *J* = 8.2 Hz, 1H), 2.27 (q, *J* = 8.2 Hz, 1 H), 2.16 (s, 3H), 2.10 (m, 1H), 1.87 (m, 1H), 1,77 (m, 1H), 1.65 (m, 1H); ^{13}C-NMR ($CDCl_3$) : 161.47 (C-6), 139.71 (C-2), 131.19 (C-

4), 125.88 (C-3), 122.80 (C-5), 67.25 (C-2'), 56.63 (C-5'), 40.14 (CH_3), 34.19 (C-3'), 22.54 (C-4'). MS (EI) (trimethylsilyl derivative) : *m/z* : 286 (19), 285 (30), 284 (48), 283 (60), 269 (20), 255 (45), 221 (16), 84 (100); Anal. Calcd for $C_{10}H_{13}N_2OCl$: C; 56.47, H; 6.16, N; 13.17. Found C; 56.83, H; 6.23, N; 13.29.

5,6-Dichloro-(*S*)-nicotine (7)

PCl_5 (23.4 g, 0.112 mol) was added with efficient stirring to a suspension of 5-chloro-6-hydroxy-(*S*)-nicotine (21.7 g, 0.1 mol) in $POCl_3$ (60 g, 0.391 mol) at 50°C . The reaction mixture was warmed up to 95 °C within 20 min and kept for 1 h at that temperature. The resulting solution was cooled to 70 °C and $POCl_3$ was distilled off under reduced pressure. The residue was taken up in water and the pH adjusted to 9.5 with aqueous 30 % NaOH. The solution was extracted with 2x 150 mL of toluene, the extracts were washed with 30 mL of water and evaporated to produce 18.2 g of a brown oil. After bulb-to bulb distillation (0.05 mbar, 130-170 °C, this afforded 16.4 g (70 % of theoretical yield) of 5,6-dichloro-(*S*)-nicotine as a colourless liquid. $[\alpha]^{20}_D$ = - 132° (c = 1, acetonitrile). ^{1}H-NMR ($CDCl_3$) : 8.21 (d, J = 2.2 Hz, 1H); 7.82 (d, J = 2.2 Hz, 1H); 3.23 (m, 1H); 3.13 (t, J = 8.2 Hz, 1H); 2.33 (q, J = 8.2 Hz, 1H); 2.23 (m, 1H); 2.18 (2, 3H); 1.95 (m, 1H); 1.83 (m, 1H); 1.67 (m, 1H). ^{13}C-NMR ($CDCl_3$) : 147.58 (C-6), 146.65 (C-2), 140.25 (C-3), 137.63 (C-4), 130.58 (C-5), 67.43 (C-2'), 56.88 (C-5'), 40.38 (CH_3), 35.49 (C-3'), 22.78 (C-4'). MS (EI) : *m/z* : 232, 231, 230, 229, 201, 187, 167, 132, 97, 84 (100); Anal. Calcd for $C_{10}H_{12}N_2Cl_2$: C; 51.97, H; 5.23, N; 12.12. Found C; 49.98, H; 4.99, N; 12.36.

4-(5-Chloro-1,6-dihydro-6-oxopyridin-3-yl)-4-oxobutyric acid (8)

Twenty-five grams of 4-(1,6-dihydro-6-oxopyridin-3-yl)-4-oxobutyric acid (128 mmol) were suspended in 250 mL of water at rt. A clear solution was obtained upon adjusting the pH to 10.5 with aqueous 30 % NaOH. After cooling to 2 °C, Cl_2 gas was slowly added while keeping the pH between 10.2 and 10.5 and the temperature < 6 °C. A total of 13.2 g of Cl_2 and 72.4 g of aqueous 30 % NaOH were needed. 19.2 g of sodium sulfite (152.3 mmol) were added, and the mixture was heated to 40 °C in order to reduce any excess of chlorine (or *N*-chlorinated pyridone). The solution was acidified to pH 2 with conc. HCl, whereupon the desired product precipitated. The suspension was stirred at rt for 2 h, then cooled to 2° C and filtered, affording 19.66 g of the title acid as a white solid, mp : 275 °C (decomp). Yield : 66.8 %. ^{1}H-NMR (DMSO-d_6) : 2.52 (t, J = 6.8 Hz, 2H), 3.08 (t, J = 6.8 Hz, 2H), 8.07 (d, J = 3.5 Hz, 1H), 8.30 (d, J = 3.5 Hz, 1H), 12.18 (br s, 1H), 12.77 (br s, 1H); ^{13}C-NMR (DMSO-d_6) : 27.71 (C-2), 32.01 (C-3), 116.23 (C-3'), 124.46 (C-5'), 135.83 (C-4'), 139.13 (C-2'), 158.35 (C-6'), 173.59 (C-1), 193.46 (C-4); Anal. Calcd for $C_9H_8NO_4Cl$: C; 47.08, H; 3.51, N; 6.10. Found C; 46.50, H; 3.50, N; 5.97.

Methyl 4-(1,6-dihydro-6-oxopyridin-3-yl)-4-oxobutyrate (9a)

10.4 g of conc. sulfuric acid was added to a suspension of 27 g of 4-(1,6-dihydro-6-oxopyridin-3-yl)-4-oxobutyric acid (138 mmol) in 400 g of methanol. The mixture was then heated to 63 °C for 2 h, during which 100 mL of methanol was distilled off. The resulting brown solution was allowed to cool to rt, then refrigerated, whereupon the product crystallized. The solid was collected by filtration, washed with cold methanol and dried under vacuum. The yield of methyl ester as white crystals was 23.5 g (81 %). mp : 167-168 °C; ^{1}H-NMR (DMSO-d_6) : 2.59 (t, J = 7.5 Hz, 2H), 3.13 (t, J = 7.5 Hz, 2H), 3.58 (s, 3H), 6.37 (d, J = 12 Hz, 1H), 7.86 (dd, J = 12 Hz, 3.5 Hz, 1H), 8.27 (d, J = 3,5 Hz, 1H), 12.14 (br s, 1H); ^{13}C-NMR (DMSO-d_6) : 27.51 (C-2), 31.83 (C-3), 116.00 (C-3'), 119.50 (C-5'), 138.10 (C-4'), 140.85 (C-2'), 162.42 (C-6'), 172.80 (C-1), 193.82 (C-4); MS (EI) : 209 (M^+), 178, 150, 122 (BP); Anal. Calcd for $C_{10}H_{11}NO_4$: C; 57.41, H; 5.30, N; 6.70. Found C; 57.21, H; 5.40, N; 6.83.

Ethyl 4-(1,6-dihydro-6-oxopyridin-3-yl)-4-oxobutyrate (9b)

This was prepared similarly to the methyl ester and crystallized from ethanol. mp : 121.8-122.7 °C; ^{1}H-NMR (DMSO-d_6) : 1.18 (t, J = 8.2 Hz, 3 H), 2.58 (t, J = 7.5 Hz, 2H), 3.11(t, J = 7.5 Hz, 2H), 4.05 (q, J = 8.2 Hz, 2 H), 6.37 (d, J = 12 Hz, 1H), 7.86 (dd, J = 12 Hz, 3.5 Hz, 1H), 8.26 (d, J = 3.5 Hz, 1H), 12.18 (br s, 1H); Anal. Calcd for $C_{11}H_{13}NO_4$: C; 59.19, H; 5.87, N; 6.27. Found C; 58.71, H; 5.90, N; 6.58.

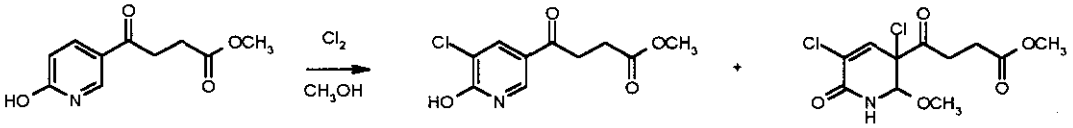
Methyl 4-(6-chloropyridin-3-yl)-4-oxobutyrate (10)

Twenty-five grams of methyl 4-(1,6-dihydro-6-oxopyridin-3-yl)-4-oxobutyrate (0.119 mol) were suspended in 110 mL of $POCl_3$ (1.2 mol) at 20 °C and the mixture was heated with caution to 70 °C, then kept at that temperature for 1 h. $POCl_3$ was distilled off under slightly reduced pressure, leaving a brown oil. This was taken up in 200 mL of toluene and water (1/1), brought to pH 9 with aqueous 30 % NaOH, and extracted twice with toluene. The combined toluene extracts were evaporated, affording 26.15 g of a reddish oil, which was treated with charcoal and crystallized in diisopropyl ether. The product was obtained as a white crystalline substance (24.14 g, 88.7 % yield). mp : 73.4-74 °C. ^{1}H-NMR ($CDCl_3$) : 2.81 (t, J = 7.5 Hz, 2H), 3.32 (t, J = 7.5 Hz, 2H), 3.72 (s, 3H), 7.47 (d, J = 10.2 Hz, 1H), 8.23 (dd, J = 10.2 Hz, 3.6 Hz, 1H), 8.97 (d, J = 3.6 Hz, 1H). ^{13}C-NMR ($CDCl_3$) : 27.71 (C-2), 33.70 (C-3), 51.95 (OCH_3), 124.58 (C-5'), 130.79 (C-3'), 138.02 (C-4'), 149.83 (C-2'), 155.78 (C-6'), 172.89 (C-1), 195.85 (C-4). Anal. Calcd for $C_{10}H_{10}NO_3Cl$: C; 52.76, H; 4.43, N; 6.15. Found C; 53.07, H; 4.47, N; 6.13.

Methyl 4-(5-chloro-1,6-dihydro-6-oxopyridin-3-yl)-4-oxobutyrate (11)

Forty-three grams of crude 4-(5-chloro-1,6-dihydro-6-oxopyridin-3-yl)-4-oxobutyric acid (0.18 mol)

were suspended in 730 mL of methanol at rt, and 16 mL of conc. sulfuric acid were added. The mixture was heated so that *ca.* 100 mL of methanol was distilled off over 4 h. One half of the residual methanol was then removed under reduced pressure, and the resulting suspension was cooled to 2 °C. The product was collected by filtration and washed with 20 mL of cold methanol, yielding 38.06 g of the expected methyl ester as a white crystalline material (≥ 83.4 % yield). mp : 211.5-213.5 °C. ^{1}H-NMR (DMSO-d_6) : 2.60 (t, J = 7.8 Hz, 2H), 3.15 (t, J = 7.8 Hz, 2H), 3.59 (s, 3H), 8.07 (d, J = 3.5 Hz, 1H), 8.32 (d, J = 3.5 Hz, 1H), 12.78 (br s, 1H); Anal. Calcd for $C_{10}H_{10}NO_4Cl$: C; 49.38, H; 4.15, N; 5.76. Found C; 48.30, H; 4.26, N; 6.31. This compound was also prepared *via* the chlorination of methyl 4-(1,6-dihydro-6-oxopyridin-3-yl)-4-oxobutyrate with Cl_2 in methanol :



28 % 70 %

The main product in this reaction is **methyl 4-(3,5-dichloro-2-methoxy-6-oxo-1,2,3,6-tetrahydropyridin-3-yl)-4-oxobutyrate** : ^{1}H-NMR ($CDCl_3$) : 2.69 (t, J = 6.9 Hz, 2H), 2.83 (m, 1H), 3.28 (m, 1H), 3.42 (s, 3H), 3.72 (s, 1H), 5.01 (dd, J = 4.6 Hz, 1.8 Hz, 1H), 7.22 (d, J = 1.8 Hz, 1H), 8.57 (d, J = 4.6 Hz, 1H). ^{13}C-NMR ($CDCl_3$) : 28.02 (C-2), 31.05 (C-3), 52.02 (CH_3OCO), 55.86 (CH_3O), 64.75 (C-3'), 86.59 (C-2'), 130.29 (C-5'), 133.50 (C-4'), 160.00 (C-6'), 172.55 (C-1), 196.27 (C-4). Anal. Calcd for $C_{11}H_{12}NO_5Cl_2$: C; 42.6, H; 4.2, N; 4.5. Found C; 42.9, H; 4.3, N; 4.5.

Methyl 4-(5,6-dichloropyridin-3-yl)-4-oxobutyrate (12)

A suspension of 37 g (151.9 mmol) of methyl 4-(5-chloro-1,6-dihydro-6-oxopyridin-3-yl)-4-oxobutyrate in 230 mL of $POCl_3$ (2.51 mol) was warmed to 75 °C for 2.5 h, then $POCl_3$ was removed under vacuum. The reddish residue was taken up in 200 mL of CH_2Cl_2, 100 mL of water was added, and the pH of the aqueous phase was raised to 8.5 with aqueous 30 % NaOH. After separating the phases, the aqueous phase was re-extracted with 100 mL of CH_2Cl_2. The combined organic extracts were treated with charcoal and evaporated. The residue was crystallized from 300 mL of diisopropyl ether and isolated by filtration at 2 °C, yielding 32.83 g of light rosy crystalline material. A second crop, 1.76 g of product, was obtained from the mother liquor by crystallizing from the same solvent. Overall yield : 87 %. mp : 85.2-86 °C ^{1}H-NMR ($CDCl_3$) :2.83 (t, J = 7.8 Hz, 2H), 3.29 (t, J = 7.8 Hz, 2H), 3.73 (s, 3H), 8.33 (d, J = 2.4 Hz, 1H), 8.87 (d, J = 2.4 Hz, 1H). ^{13}C-NMR ($CDCl_3$) : 27.67 (C-2), 33.87 (C-3), 51.99 (OCH_3), 131.31 and 131.81 (C-3' and C-5'), 137.78 (C-4'), 146.80 (C-2'), 153.27 (C-6'), 172.72 (C-1), 194.99 (C-4); Anal. Calcd for $C_{10}H_9NO_3Cl_2$: C; 45.83, H; 3.46, N; 5.34. Found C; 45.61, H; 3.65, N; 5.60.

6-(1,6-Dihydro-6-oxopyridin-3-yl)-2,3,4,5-tetrahydropyridazin-3-one (13)

Five grams of methyl 4-(1,6-dihydro-6-oxo-pyridin-3-yl)-4-oxobutyrate (23.9 mmol) were suspended in 67 mL of ethanol. 2.4 g of hydrazine hydrate (47.9 mmol) were added, and the mixture was heated to 70 ° C, causing the substrate to dissolve. The colourless solution was refluxed overnight, forming a thick white precipitate. After cooling, the solid was collected by filtration, washed with ethanol and dried under vacuum. The yield of pyridazinone was 4.33 g (94.8 %). mp : > 260 °C; ^{1}H-NMR (DMSO-d_6) : 2.38 (t, J = 9.6 Hz, 2H), 2.80 (t, J = 9.6 Hz, 2H), 6.38 (d, J = 12 Hz, 1H), 7.69 (d, J = 3 Hz, 1H), 7.90 (dd, J =12 Hz, 3 Hz, 1H), 10.80 (s, 1H), 11.84 (br s, 1H); ^{13}C-NMR (DMSO-d_6) : 20.82 (C-5), 25.81 (C-4), 114.38 (C-3'), 120.16 (C-5'), 134.15 (C-4'), 137.33 (C-2'), 146.58 (C-6), 161.97 (C-6'), 166.78 (C-3); Anal. Calcd for $C_9H_9N_3O_2$: C; 56.52, H; 4.75, N; 21.99. Found C; 56.44, H; 4.75, N; 21.61.

6-(6-Chloropyridin-3-yl)-2,3,4,5-tetrahydropyridazin-3-one (14)

1.13 g of hydrazine hydrate (22.6 mmol) was added to a solution of 3 g of methyl 4-(6-chloropyridin-3-yl)-4-oxobutyrate (13.2 mmol) in 55 mL of toluene/methanol (7/4) at rt. The mixture was stirred for 5 days, during which the product slowly precipitated. The suspension was cooled to 2 °C and the product was collected by filtration, then washed with a few mL of cold toluene/methanol mixture. 1.86 g of product was obtained as white needles (67.3 %). Upon evaporation the mother liquor afforded 1 g of residue containing mostly the desired product. mp : 210.8-211.8°C . ^{1}H-NMR (DMSO-d_6) : 2.50 (t, J = 9.6 Hz, 2H), 2.98 (t, J = 9.6 Hz, 2H), 7.58 (d, J = 10.2 Hz, 1H), 8.17 (dd, J = 10.2 Hz, 3.6 Hz, 1H), 8.74 (d, J = 3.6 Hz, 1H), 11.11 (s, 1H); Anal. Calcd for $C_9H_8N_3OCl$: C; 51.67, H; 3.86, N; 20.10. Found C; 50.70, H; 3.98, N; 19.80.

Methyl 4-(6-chloropyridin-3-yl)-4-hydroxyiminobutyrate (15)

Ten grams of methyl 4-(6-chloropyridin-3-yl)-4-oxobutyrate (43.9 mmol) and 3.66 g of hydroxylamine hydrochloride (52.7 mmol) were dissolved in 65 mL of methanol at 45 °C, and 5.33 g of triethylamine were added dropwise over 45 min. The reaction mixture was agitated for 30 min and evaporated. The solid residue was extracted with ethyl acetate/toluene (1/1) and water, the organic phase was dried on sodium sulfate and evaporated. The solid residue (10.1 g) was crystallized in 25 mL of toluene, yielding 7.21 g of methyl 4-(6-chloropyridin-3-yl)-4-hydroxyiminobutyrate as the pure (*E*)-isomer (67.7 %). The mother liquor was evaporated and the solid material crystalized from diisopropylether, affording 0.89 g of the pure (*Z*)-isomer (8.4 %), as determined by NMR spectroscopy.

(*E*)-isomer : mp 126.5-125.5 °C. ^{1}H-NMR ($CDCl_3$) : 2.66 (t, J = 8.5 Hz, 2H); 3.08 (t, J = 8.5 Hz, 2H); 3.65 (s, 3H); 7.34 (d, J = 8.4 Hz, 1H); 7.94 (dd, J = 8.6 Hz and 3 Hz, 1H); 8.67 (d, J = 3 Hz, 1H); 9.20 (br s, 1H). ^{13}C-NMR ($CDCl_3$) : 21.06 (C-3), 30.21 (C-2); 51.97 (OCH_3); 124.22 (C-5'), 130.35 (C-3'),

136.55 (C-4'), 147.57 (C-2'), 151.99 (C-6'), 154.70 (C-4), 172.97 (C-1). Anal. Calcd for $C_{10}H_{11}N_2O_3Cl$: C; 49.58, H; 4.58, N; 11.57. Found C; 48.96, H; 4.73, N; 11.54.

(*Z*)-isomer : mp 80-82.5 °C. ^{1}H-NMR ($CDCl_3$) : 2.62 (t, *J* = 7.5 Hz, 2H); 2.87 (t, *J* = 7.5 Hz, 2H); 3.66 (s, 3H); 7.39 (d, *J* = 8.8 Hz, 1H); 7.86 (dd, *J* = 8.8 Hz and 3.3 Hz, 1H); 8.53 (d, J = 3.3 Hz, 1H); 8.79 (br s, 1H). ^{13}C-NMR ($CDCl_3$) : 30.05 and 30.45 (C-2, C-3), 51.92 (OCH_3), 124.04 (C-3'), 127.93 (C-5'), 138.70 (C-4'), 148.96 (C-6'), 151.76 (C-6'), 152.34 (C-4), 172.97 (C-1). MS (E.I.) : 242/244, 225/227, 265/167, 138/140, 103, 76, 55 (BP); Anal. Calcd for $C_{10}H_{11}N_2O_3Cl$: C; 49.58, H; 4.58, N; 11.57. Found C; 48.71, H; 4.67, N; 11.62.

5-(6-Chloropyridin-3-yl)-2,3,4,5-tetrahydrofuran-2-one (16)

1.97 g of $NaBH_4$ (52.1 mmol) was added portionwise over 20 min to a solution of 15 g of methyl 4-(6-chloropyridin-3-yl)-4-oxobutyrate (65.9 mmol) in 220 mL of methanol at 25 °C, and the mixture was stirred for 1 h. Toluene was added (200 mL) and methanol distilled off, then the mixture was heated further to 100 °C in order to complete the lactone ring closure (1 h). The insoluble material was filtered off and the filtrate evaporated to produce 13.3 g of light brownish oil. This, upon crystallization from 70 mL of diisopropyl ether, afforded 11.95 g of lactone as a white solid (91.8 %). mp : 52.5-53 °C. ^{1}H-NMR ($CDCl_3$) : 2.20 (m, 1H), 2.72 (m, 2H), 2.76 (m, 1H), 5.54 (m, 1H), 7.49 (d, *J* = 10.2 Hz, 1H), 7.68 (dd, *J* = 10.2 Hz, 3 Hz, 1H), 8.38 (d, *J* = 3 Hz, 1H). ^{13}C-NMR ($CDCl_3$) : 28.77 (C-3), 30.63 (C-4), 78.19 (C-5), 124.55 (C-5'), 134.12 (C-3'), 136.10 (C-4'), 147.13 (C-2'), 151.69 (C-6'), 176.02 (C-2); Anal. Calcd for $C_9H_8NO_2Cl$: C; 54.70, H; 4.08, N; 7.09. Found C; 53.98, H; 4.06, N; 7.01.

4-(6-Chloropyridin-3-yl)-4-hydroxybutyric acid (17)

This acid was formed quantitatively upon reacting the lactone with aqueous NaOH (pH 13) at rt. After neutralisation, the solution was evaporated and the residue crystallized from ethanol, yielding the hydroxy-acid as white crystals, 72 % yield, mp : 130.4 - 131.4 °C; ^{1}H-NMR (DMSO-d_6) : 1.83 (m, 2H), 2.16 (m, 2H), 4.66 (dd, *J* = 5.0 and 7.1 Hz, 1H), 7.45 (d, *J* = 8.2 Hz, 1H), 7.6 (br s, 2 H), 7.79 (dd, *J* = 8.2 and 2.1 Hz, 1H), 8.35 (d, *J* = 2.1 Hz, 1H); ^{13}C-NMR (DMSO-d_6) : 34.97, 35.35 (C-2, C-3), 70.38 (C-4), 123.5 (C-5'), 137.23 (C-4'), 141.63 (C-3'), 147.60 (C-2'), 148.14 (C-6'), 178.19 (C-1). MS of disilylated product : 359/361 (M^+), 214/216 (BP); Anal. Calcd for $C_9H_{10}NO_3Cl$: C; 50.13, H; 4.67, N; 6.50. Found C; 50.05, H; 4.59, N; 6.52.

4-(6-Chloropyridin-3-yl)-4-hydroxybutyramide (18)

5-(6-Chloropyridin-3-yl)-2,3,4,5-tetrahydrofuran-2-one (3.6 g, 1.82 mmol) was dissolved in 30 mL of 25 % ammonium hydroxide solution at 45 °C and kept at that temperature for 1 h. The solution was then

evaporated under vacuum to yield 4.65 g of an oily residue. This was dried under vacuum over P_2O_5 to yield 3.9 g of hydroxy amide as a gum (≈ 100 %). ^{1}H-NMR (DMSO-d_6) : 1.85 (m, 2H), 2.12 (t, J = 7.5 Hz, 2H), 4.63 (m, 1H), 5.57 (d, J = 6 Hz, 1H), 6.76 (br s, 1H), 7.30 (br s, 1H), 7.48 (d, J = 9.6 Hz, 1H), 7.80 (dd, J = 9.6 Hz, J = 3.5 Hz, 1H), 8.35 (d, J = 3.5 Hz, 1H); ^{13}C-NMR (DMSO-d_6) : 31.04 (C-2), 34.25 (C-3), 68.94 (C-4), 123.74 (C-5'), 137.27 (C-4'), 140.48 (C-3'), 147.62 (C-2'), 148.56 (C-6'), 174.00 (C-1); Anal. Calcd for $C_9H_{11}N_2O_2Cl$: C; 50.36, H; 5.17, N; 13.05. Found C; 50.04, H; 5.17, N; 12.78.

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