

Synthesis of Heterocyclic Dipeptide Analogues

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Synthesis of constrained mimics of a serine dipeptide is described. Triflated 3-hydroxypicolinic acid methyl ester was carbo-substituted using ethynylstannanes with Pd-catalysis to furnish 3-acetylenic pyridines which were hydrolysed and coupled to Ser(*t*-Bu)OMe before cyclization to 1-alkylidene-3-oxo-1,3-dihydro-pyrrolo[3,4-*b*]pyridine derivatives. Subsequent deprotection provided the dipeptide analogue.

In recent reports we have described stereocontrolled syntheses of cyclic, rigidified serine and threonine analogues which would be expected to exert restrictions on peptide conformations after incorporation into bioactive peptides.¹ This report describes the preparation of dipeptide mimics as a constrained substitute of pyroglutamic acid (pGlu) with serine as the second *C*-terminal α -amino acid. Racemic dipeptides were obtained.

Several bioactive and physiologically important peptides such as thyrotropin-releasing hormone, luteinizing hormone-releasing hormone, neurotensin, bombesin, fibrinopeptides and gastrin have pGlu as the *N*-terminal amino acid.² pGlu is the cyclic derivative of glutamic acid, or alternatively it can be regarded as a 5-oxo derivative of proline which constitutes the substituted heterocyclic system 5-oxopyrrolidine-2-carboxylic acid. It is therefore not surprising that pGlu in a pharmacophoric peptide region in some cases can be replaced by another azaheterocycle carrying an α -carboxylic acid group.³ Our goal has been to find routes for the preparation of conformationally restricted dipeptide mimics from pyridine-2-carboxylic acid. Formally this can be achieved by insertion of a bridge between the amino nitrogen of the amino acid and an *ortho* C-position in the heterocycle. The target molecules were derivatives of the bicyclic pyrrolo[3,4-*b*]pyridin-3-one system, *viz.*, compound **8** in Scheme 2.

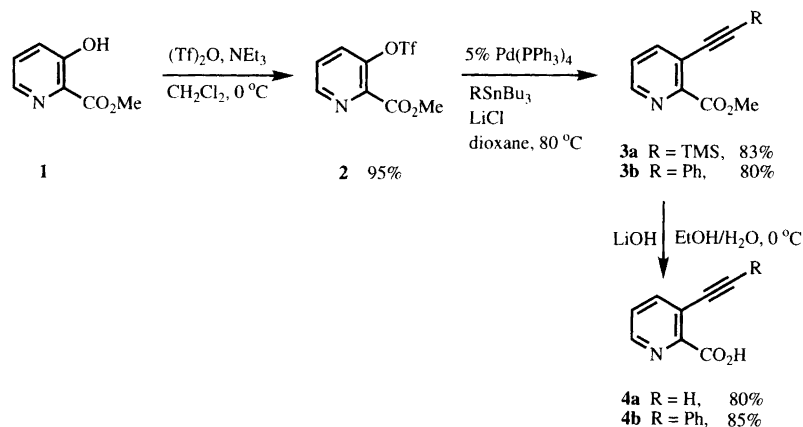
Methyl 3-hydroxypyridine-2-carboxylate (**1**) was used as starting material. The 3-hydroxy position was to be the site for carbo-substitution after conversion into a triflate since triflates are good substrates for carbo-substitution in azines under Pd-catalysis.⁴ The triflate **2** was prepared using triflic anhydride together with triethylamine as the base. The coupling reactions were

effected using either trimethylsilyl (TMS)-protected or phenyl substituted ethynylstannanes and heating with tetrakis(triphenylphosphine)palladium as the catalyst in dioxane in the presence of three mole equivalents of lithium chloride. Yields of the order 80% of the alkynyl coupling products **3** were obtained. The carboxylic acid function was to be used for acylation of the amino acid serine. Therefore the ester group in compounds **3** was hydrolysed with lithium hydroxide in dilute ethanol to the acids **4**. Concomitant cleavage of the terminal silyl substituent on the ethynyl group in the ester **3a** furnished the free acetylene derivative **4a**.

For peptide bond formation, the acid group in the acids **4** was activated by reaction with *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide and subsequently with 1-hydroxybenzotriazole at -10°C in DMF before coupling with *O*-*tert*-butyl serine methyl ester. The coupling products **5a** and **5b** were formed in 65 and 97% yields, respectively. No racemization in the coupling reaction was detected in ^1H NMR experiments using the chiral lanthanide shift reagent $\text{Eu}(\text{tfc})_3$. This was verified in a model experiment by preparing the (*R*)-serine analogue by the same procedure as described for the (*S*)-isomer (96% yield). In neither case was any doubling of ^1H NMR signals observed. When the *S*- and *R*-enantiomers were mixed, however, the expected doubling of ^1H -signals was clearly demonstrated for the *tert*-butyl and the methyl ester singlets. Enantiomeric purity was also supported by optical rotation measurements in chloroform which gave specific rotations $[\alpha]_D^{20} = +31.00^\circ$ for **5a** and $[\alpha]_D^{20} = -31.2^\circ$ for its *R*-isomer.

Attempts to effect cyclization of compounds **5** to a fused cyclic product with palladium catalysis were not successful. Cyclization over the acetylene function, however, resulted under alkaline conditions. The cyclizations failed to proceed satisfactorily under catalytic conditions. For the formation of the terminally unsubstituted acet-

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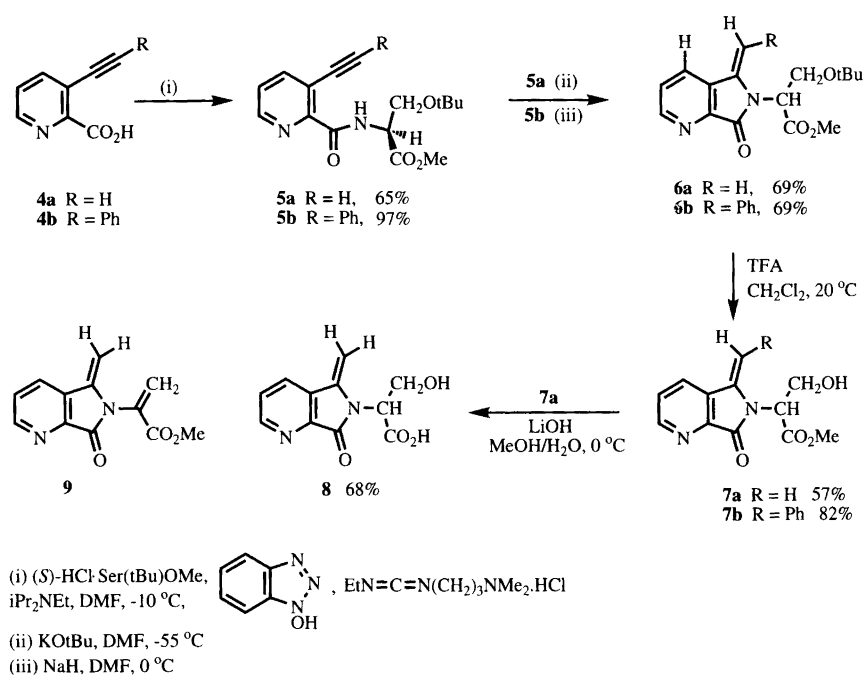


Scheme 1.

ylene derivative **6a** our best condition was the use of 10% potassium *tert*-butoxide in DMF at -55°C for 20 min. For the preparation of the phenyl homologue **6b**, sodium hydride in DMF at 0°C for 30 min was used. Potassium *tert*-butoxide was less satisfactory in the latter case. The initial step in the cyclization reaction was expected to be deprotonation of the amide function and a subsequent Michael-type addition of the amide nitrogen to the triple bond. The regioselectivity was such that only the five-membered ring derivative was seen in the cyclization reaction. The structure assignments of the cyclic products were based on NMR studies. In the ^1H NMR spectrum of the parent structure the two olefinic protons were at 5.20 and 5.31 ppm. The numerical value of the coupling constant J 2.9 Hz is consistent with geminal olefinic coupling constants. The cyclization product was therefore assigned a five-membered ring structure

6a. The assignment of this structure is supported by DEPT NMR which showed the carbon signal for a CH_2 -group at 92.9 ppm. Additional support for the five-membered ring assignment comes from spectra of the hydrolysis products **7a** and **8** (*vide infra*). Thus the olefinic signals in the ^1H NMR spectrum of compound **7a** were at 5.2 and 5.57 ppm, and of target compound **8** at 5.18 and 5.51 ppm, with coupling constants of 3.2 and 2.7 Hz, respectively.

In the phenyl derivative **6b**, obtained from cyclization of the phenylethynyl substrate **5b**, the olefinic proton was seen as a singlet at 6.76 ppm in the chromatographically homogeneous product. The NMR data satisfy a five-membered ring structure with benzylidene *Z*-configuration. Thus NOESY NMR spectra showed interaction between the vinyl-H and H-4 in the pyridine ring. There was also interaction between *ortho*-H in the phenyl ring



Scheme 2.

and the methine α -H in the serine part. No interaction was observed between this proton and the vinyl-H.

Almost total racemization occurred under the cyclization conditions used. This was evident from analyses using the chiral lanthanide shift reagent $\text{Eu}(\text{tfc})_3$, which showed duplication of signals in the NMR spectra. Racemization is also supported by the observation of a by-product in the cyclization reaction, which was identified as the elimination product **9**. Both observations are in accordance with proton abstraction and carbanion formation at the α -carbon in the serine part.

The *tert*-butyl protecting group in substrates **6** was removed by treatment with TFA in dichloromethane at ambient temperature to furnish the methyl esters **7**. If necessary, the methyl ester can be cleaved by using lithium hydroxide in dilute methanol at 0°C as shown for **7a** giving the acid **8**.

In conclusion, we have described a short route for the preparation of racemic conformationally restricted dipeptide mimics.

Experimental

The ^1H NMR spectra were recorded at 200 MHz and the ^{13}C NMR spectra at 50 MHz with a Varian Gemini 200 instrument. NOESY NMR spectra were run at 500 MHz on a Bruker DPX 500 instrument. Mass spectra under electron impact conditions (EI) were recorded at 70 eV ionizing potential, and ammonia was used for chemical ionization (CI); the spectra are presented as m/z (% rel. int.).

Anhydrous dioxane was obtained by distillation from sodium and benzophenone. Dichloromethane was distilled over CaH_2 , and DMF was distilled over BaO .

Methyl 3-hydroxy-2-pyridinecarboxylate (1).⁵ Oxalyl chloride (1.096 g, 8.63 mmol) in dry dichloromethane (10 ml) was added dropwise to a solution of 3-hydroxy-2-pyridinecarboxylic acid (1.0 g, 7.19 mmol) and DMF (2 drops) in dry dichloromethane (10 ml) under argon at 0°C . The reaction mixture was stirred at 0°C for 1 h and at ambient temperature for 2 h. The solution was evaporated, methanol (20 ml) was added and the reaction was mixture stirred at ambient temperature for 72 h before excess methanol was distilled off. Ethyl acetate was then added, and the solution washed with saturated aqueous NaHCO_3 , and then with brine, dried over MgSO_4 and evaporated. Recrystallization of the residue from water gave a white solid; yield 768 mg (70%).

Methyl 3-trifluoromethanesulfonyloxy-2-pyridinecarboxylate (2). Triethylamine (0.295 g, 2.92 mmol) in dry dichloromethane (5 ml) was added dropwise to a solution of methyl 3-hydroxy-2-pyridinecarboxylate (0.446 g, 2.92 mmol) in dry dichloromethane (5 ml) under argon at 0°C . The solution was stirred at 0°C for 2 h before triflic anhydride (0.822 g, 2.92 mmol) was added. The reaction mixture was stirred at 0°C for 60 min, washed

with water, dried over MgSO_4 and evaporated. The crude product was purified by flash chromatography eluting with hexane–EtOAc 1:2. The product was a colourless oil; yield 789 mg (95%). ^1H NMR (CDCl_3): δ 4.01 (s, Me), 7.58–7.74 (m, H-4 and H-5), 8.74 (dd, J 4.4, 1.2 Hz, H-6). ^{13}C NMR (CDCl_3): δ 53.2 (Me), 118.5 (q, $J_{\text{C,F}}$ 319 Hz, CF_3), 128.1 (C-5), 131.3 (C-4), 141.5 (C), 146.2 (C), 148.9 (C-6), 162.8 (CO). MS (CI- CH_4): 286 ($M^+ + 1$, 100), 281 (14), 254 (43), 239 (15), 230 (19), 160 (19), 149 (46), 80 (18). MS (EI): M 284.9943. Calc. for $\text{C}_8\text{H}_6\text{F}_3\text{NO}_5\text{S}$: 284.9919.

Methyl 3-trimethylsilylethynyl-2-pyridinecarboxylate (3a). Methyl 3-trifluoromethanesulfonyloxy-2-pyridinecarboxylate (0.57 g, 2 mmol), [(trimethylsilyl)ethynyl]tributylstannane⁶ (1.16 g, 3 mmol), LiCl (0.25 g, 6 mmol) and $(\text{Ph}_3\text{P})_4\text{Pd}$ (116 mg, 0.1 mmol) were stirred together in dry dioxane (15 ml) under argon at 80°C for 2 h. The reaction mixture was diluted with light petroleum, treated with saturated aqueous KF solution for 30 min and the precipitated tributylstannyl fluoride removed by filtration through a plug of Celite. The reaction mixture was washed with water, dried over MgSO_4 and evaporated. The crude product was purified by flash chromatography eluting with hexane–EtOAc 1:1. The product was a colourless oil; yield 388 mg (83%). Anal. $\text{C}_{12}\text{H}_{15}\text{NO}_2\text{Si}$: C, H. ^1H NMR (CDCl_3): δ 0.23 (s, TMS), 3.95 (s, Me), 7.35 (dd, J 4.7, 8 Hz, H-5), 7.86 (dd, J 1.7, 8 Hz, H-4), 8.56 (br d, J 3.3 Hz, H-6). ^{13}C NMR (CDCl_3): δ -0.4 (TMS), 52.6 (Me), 99.9 (C), 103.4 (C), 120.2 (C), 125.0 (C-5), 141.8 (C-4), 148.0 (C-6), 149.9 (C), 164.9 (CO). MS (EI): 233 (M^+ , 7), 219 (9), 188 (18), 174 (6), 161 (15), 160 (100), 144 (6), 89 (6).

Methyl 3-phenylethynyl-2-pyridinecarboxylate (3b). Methyl 3-trifluoromethanesulfonyloxy-2-pyridinecarboxylate (0.2 g, 0.7 mmol), (phenylethynyl)tributylstannane⁶ (0.357 g, 0.91 mmol), LiCl (89 mg, 2.10 mmol) and $(\text{Ph}_3\text{P})_4\text{Pd}$ (40 mg, 0.035 mmol) were stirred together at 80°C in dry dioxane (10 ml) under argon overnight. The reaction mixture was diluted with light petroleum, treated with saturated aqueous KF solution for 30 min, and the precipitated tributylstannyl fluoride removed by filtration through a plug of Celite. The reaction mixture was washed with water and brine, dried over MgSO_4 and evaporated. The crude product was purified by flash chromatography eluting with hexane–ethyl acetate 1:2. The product was a pale yellow solid; yield 133 mg (80%), m.p. 65°C . Anal. $\text{C}_{15}\text{H}_{11}\text{NO}_2$: C, H. ^1H NMR (CDCl_3): δ 4.03 (s, Me), 7.33–7.40 (4 H, m, Ph and H-5), 7.55–7.60 (2 H, m, Ph), 7.96 (dd, J 1.6, 8 Hz, H-4), 8.62 (br d, J 3.5 Hz, H-6). ^{13}C NMR (CDCl_3): δ 53.1 (Me), 85.0 (C), 97.0 (C), 120.3 (C), 121.9, 127.8, 128.4, 131.1 (Ph), 124.8 (C-5), 140.6 (C-4), 147.1 (C-6), 148.2 (C), 164.0 (CO). MS (EI): 237 (M^+ , 100), 222 (23), 180 (25), 179 (87), 178 (66), 177 (28),

166 (25), 150 (32). MS (EI): M 237.0772. Calc. for $C_{15}H_{11}NO_2$: 237.0790.

3-Ethynyl-2-pyridinecarboxylic acid (4a). LiOH·H₂O (361 mg, 8.6 mmol) in water (5 ml) was added dropwise to a solution of methyl 3-trimethylsilylethynyl-2-pyridinecarboxylate (1.00 g, 4.3 mmol) in ethanol (20 ml) at 0 °C. The solution was stirred at 0 °C for 30 min before the ethanol was distilled off. Water (10 ml) was added, the solution extracted with ethyl acetate and the aqueous solution acidified with 3 M HCl to pH 3. The precipitated product was a pale yellow solid which was collected by centrifugation and dried under high vacuum; yield 505 mg (80%). ¹H NMR (CD₃OD): δ 4.03 (s, CH), 7.59 (dd, J 4.8, 8 Hz, H-5), 8.08 (dd, J 1.6, 8 Hz, H-4), 8.60 (dd, J 1.6, 4.8 Hz, H-6). ¹³C NMR (CD₃OD): δ 79.5 (C), 86.9 (CH), 119.7 (C), 126.1 (C-5), 143.0 (C-4), 148.0 (C-6), 150.9 (C), 166.1 (CO). MS (EI): 147 (M^+ , 51), 103 (100), 102 (25), 91 (21), 76 (56), 75 (30), 64 (17), 50 (34).

3-Phenylethynyl-2-pyridinecarboxylic acid (4b). LiOH·H₂O (177 mg, 4.22 mmol) in water (5 ml) was added dropwise to a solution of methyl 3-phenylethynyl-2-pyridinecarboxylate (500 mg, 2.11 mmol) in ethanol (20 ml) at 0 °C. The solution was stirred at 0 °C for 1 h before the ethanol was distilled off. Water (15 ml) was added and the solution was extracted with ethyl acetate. The aqueous solution was acidified with 3 M HCl to pH 3. The precipitated white solid product was collected by filtration and dried under high vacuum; yield 400 mg (85%), m.p. 123 °C. Anal. $C_{14}H_9NO_2$: C, H. ¹H NMR (DMSO-*d*₆): δ 7.43–7.55 (m, Ph), 7.60 (dd, J 4.8, 8 Hz, H-5), 8.10 (dd, J 1.4, 8 Hz, H-4), 8.62 (dd, J 1.4, 4.8 Hz, H-6). ¹³C NMR (DMSO-*d*₆): δ 85.2 (C), 95.3 (C), 117.3 (C), 121.5, 128.5, 129.1, 131.1 (Ph), 125.0 (C-4), 140.4 (C-5), 147.9 (C-6), 151.5 (C), 166.0 (CO). MS (EI): 223 (M^+ , 54), 180 (14), 179 (100), 178 (31), 152 (12), 151 (13), 125 (10), 75 (8). MS (EI): M 223.0642. Calc. for $C_{14}H_9NO_2$: 223.0633.

(S)-O-tert-Butyl-N-(3-ethynylpyridine-2-carbonyl)serine methyl ester (5a). Diisopropylethylamine (371 mg, 2.87 mmol) was added dropwise to a solution of (S)-HCl·Ser(*tert*-Bu)OMe (304 mg, 1.43 mmol) and 3-ethynyl-2-pyridinecarboxylic acid (211 mg, 1.43 mmol) in dry DMF (5 ml) under argon at –10 °C. 1-Hydroxybenzotriazole (220 mg, 1.43 mmol) and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (275 mg, 1.43 mmol) were added to the resultant solution and the solution stirred at –10 °C for 2 h, then at ambient temperature overnight. The DMF was distilled off under reduced pressure, ethyl acetate added to the residue, and the extracts washed consecutively with saturated aqueous NaHCO₃ and brine, dried over MgSO₄, and evaporated. The product was isolated from the residual material by flash chromatography using hexane–EtOAc 1:2. The product was a white solid; yield

284 mg (65%), m.p. 129 °C. Anal. $C_{16}H_{20}N_2O_4$: C, H. [α]_D²⁰ +31.0° (c =0.58, CHCl₃). ¹H NMR (CDCl₃): δ 1.13 (s, *t*-Bu), 3.58 (s, CH), 3.66 (dd, J 3.3, 9.2 Hz, one H in CH₂), 3.74 (s, OMe), 3.90 (dd, J 3.3, 9.2 Hz, one H in CH₂), 4.85–4.92 (m, CH), 7.39 (dd, J 4.6, 7.5 Hz, H-5), 7.93 (dd, J 1.6, 7.5 Hz, H-4), 8.54 (dd, J 1.6, 4.6 Hz, H-6), 8.65 (br d, J 8.5 Hz, NH). ¹³C NMR (CDCl₃): δ 27.8, 52.5, 53.2, 62.2, 73.4, 80.0, 85.3, 118.0, 124.6, 142.6, 146.5, 148.9, 162.1, 169.6. MS (EI): 304 (M^+ , 3), 231 (34), 218 (68), 160 (65), 130 (54), 103 (76), 102 (100), 57 (49).

(R)-O-tert-Butyl-N-(3-ethynylpyridine-2-carbonyl)serine methyl ester [(R)-5a] was prepared from (*R*)-serine as above in 96% yield; [α]_D²⁰ –31.2° (c =0.58, CHCl₃).

(S)-O-tert-Butyl-N-(3-phenylethynylpyridine-2-carbonyl)serine methyl ester (5b). Diisopropylethylamine (232 mg, 1.80 mmol) was added dropwise to a solution of (S)-HCl·Ser(*t*-Bu)OMe (190 mg, 0.9 mmol) and 3-phenylethynyl-2-pyridinecarboxylic acid (200 mg, 0.9 mmol) in dry DMF (5 ml) under argon at –10 °C. 1-Hydroxybenzotriazole (137 mg, 0.9 mmol) and *N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride (172 mg, 0.9 mmol) were subsequently added, the resultant solution stirred at –10 °C for 2 h, and at ambient temperature overnight before the DMF was distilled off under reduced pressure. The residual material was dissolved in ethyl acetate, and the solution washed consecutively with saturated aqueous NaHCO₃ and brine, dried over MgSO₄ and evaporated. The product was further purified by flash chromatography using hexane–EtOAc 1:1 and was obtained as a white solid; yield 330 mg (97%), m.p. 93 °C. Anal. $C_{22}H_{24}N_2O_4$: C, H. ¹H NMR (CDCl₃): δ 1.11 (s, *t*-Bu), 3.58 (dd, J 3.3, 9 Hz, one H in CH₂), 3.65 (s, OMe), 3.84 (dd, J 3, 9 Hz, one H in CH₂), 4.87 (dt, J 3, 9 Hz, CH), 7.22–7.26 (m, Ph), 7.32 (dd, J 4.6, 8 Hz, H-5), 7.48–7.54 (m, Ph), 7.87 (dd, J 1.6, 8 Hz, H-4), 8.44 (br d, J 3.4, H-6), 8.63 (br d, J 9 Hz, NH). ¹³C NMR (CDCl₃): δ 27.1 (*t*-Bu), 52.1, 52.7, 62.1, 73.2, 86.1 (C), 97.1 (C), 119.5, 122.8, 125.0, 128.0, 128.5, 131.6, 142.3, 146.6, 148.8, 163.2 (CO), 170.7 (CO). MS (EI): 380 (M^+ , 30), 323 (40), 294 (49), 293 (100), 179 (45), 178 (78), 151 (21), 57 (24). MS (EI): M 380.1735. Calc. for $C_{22}H_{24}N_2O_4$: 380.1736.

Methyl 3-tert-butoxy-2-(1-methylene-3-oxo-1,3-dihydropyrrolo[3,4-*b*]pyridin-2-yl)propionate (6a). Potassium *tert*-butoxide (8 mg, 0.07 mmol) was added to a solution of (S)-O-*tert*-butyl-N-(3-ethynylpyridine-2-carbonyl)serine methyl ester (200 mg, 0.65 mmol) in dry DMF (10 ml) at –50 °C. The solution was stirred at –50 °C for 20 min before water was added. The reaction mixture was extracted with ethyl acetate, washed consecutively with saturated aqueous NaHCO₃ and brine, dried over MgSO₄ and evaporated. The crude product was purified by flash chromatography using hexane–EtOAc 1:2. The product thus obtained was a white glassy foam; yield

138 mg (69%). Anal. $C_{16}H_{20}N_2O_4$: C, H. 1H NMR ($CDCl_3$): δ 1.09 (s, *t*-Bu), 3.73 (s, Me), 4.05 (d, *J* 6.4 Hz, CH_2), 5.14–5.23 (m, one H in $CH_2=$ and CH), 5.31 (d, *J* 2.9 Hz, one H in $CH_2=$), 7.50 (dd, *J* 4.8, 7.9 Hz, H-5), 8.02 (dd, *J* 1.4, 7.9 Hz, H-4), 8.81 (dd, *J* 1.4, 4.8 Hz, H-6). ^{13}C NMR ($CDCl_3$): δ 27.2, 52.4, 54.3, 59.7, 73.5, 92.9, 125.9, 128.1, 130.9, 138.1, 146.6, 151.7, 164.7, 168.5. MS (EI): 304 (M^+ , 13), 248 (72), 233 (87), 220 (100), 188 (98), 186 (75), 147 (70), 59 (61). MS (EI): *M* 304.1433. Calc. for $C_{16}H_{20}N_2O_4$: 304.1423.

The second product eluted from the column on chromatography was identified as methyl 2-(1-methylene-3-oxo-1,3-dihydropyrrolo[3,4-*b*]pyridin-2-yl)acrylate (**9**): 1H NMR ($CDCl_3$): δ 3.80 (s, OMe), 4.92 (d, *J* 2.6 Hz, one H in $CH_2=$), 5.30 (d, *J* 2.6 Hz, one H in $CH_2=$), 6.07 (s, one H in $CH_2=$), 6.86 (s, one H in $CH_2=$), 7.56 (dd, *J* 4.7, 7.8 Hz, H-5), 8.08 (dd, *J* 1.3, 7.8 Hz, H-4), 8.87 (dd, *J* 1.3, 4.7 Hz, H-6). MS (EI): 230 (M^+ , 100), 171 (45), 170 (26), 143 (19), 142 (25), 130 (19), 116 (22), 102 (23).

Methyl (Z)-3-tert-butoxy-2-(1-benzylidene-3-oxo-1,3-dihydropyrrolo[3,4-b]pyridin-2-yl)propionate (6b). Sodium hydride (50% in oil) (14 mg, 0.29 mmol) was added to a solution of (*S*)-*O*-*tert*-butyl-*N*-(3-phenylethynylpyridine-2-carbonyl)serine methyl ester (111 mg, 0.29 mmol) in dry DMF (4 ml) under argon at 0 °C. The solution was stirred for 30 min before water was added. The mixture was extracted with ethyl acetate, and the extracts were washed consecutively with saturated aqueous $NaHCO_3$ and brine, dried over Na_2SO_4 and evaporated. The product was isolated after flash chromatography of the residual material using hexane–EtOAc 1:2. The product was a colourless oily material; yield 77 mg (69%). Anal. $C_{22}H_{24}N_2O_4$: C, H. 1H NMR ($CDCl_3$): δ 0.99 (s, *t*-Bu), 3.69 (s, CO_2Me), 3.85 (dd, one H in CH_2 , *J* 4.8, 9.8 Hz), 4.11 (t, one H in CH_2 , *J* 9.5 Hz), 4.79 (dd, CH, *J* 4.8, 9.6 Hz), 6.76 (s, CH=), 7.3–7.54 (m, Ph and H-5), 8.10 (dd, H-4, *J* 1.4, 8 Hz), 8.80 (dd, H-6, *J* 1.4, 4.7 Hz). ^{13}C NMR ($CDCl_3$): δ 27.5 (*t*-Bu), 52.5 (CO_2Me), 57.4 (NCH), 59.7 (CH_2O), 73.2 (C in *t*-Bu), 108.3 (CH=), 125.5 (C-5), 127.2 (C-4), 127.7, 128.1, 129.5, 133.4 (Ph), 132.4 (C=), 132.8 (C-3), 145.7 (C-2), 151.1 (C-6), 166.0 (CON), 168.1 (CO_2Me). MS (EI): 380 (M^+ , 28), 324 (69), 235 (77), 234 (32), 233 (35), 223 (57), 222 (100), 205 (25). MS (EI): *M* 380.1729. Calc. for $C_{22}H_{24}N_2O_4$: 380.1736.

Methyl 3-hydroxy-2-(1-methylene-3-oxo-1,3-dihydropyrrolo[3,4-b]pyridin-2-yl)propionate (7a). A solution of methyl (*S*)-3-*tert*-butoxy-2-(1-methylene-3-oxo-1,3-dihydropyrrolo[3,4-*b*]pyridin-2-yl)propionate (280 mg, 0.92 mmol) and TFA (10 ml) in dry CH_2Cl_2 (10 ml) was stirred at ambient temperature for 30 min. The solution was then evaporated and the white solid product isolated from the residual material by flash chromatography using EtOAc; yield 130 mg (57%), m.p. 119 °C. Anal. $C_{12}H_{12}N_2O_4$: C, H. 1H NMR (CD_3OD): δ 3.73 (s, OMe), 4.24 (d, *J* 7 Hz, CH_2), 5.15–5.25 (m, NCH

and one H in $CH_2=$), 5.57 (d, *J* 3.2 Hz, one H in $CH_2=$), 7.66 (dd, *J* 4.8, 8 Hz, H-5), 8.34 (dd, *J* 1.4, 8 Hz, H-4), 8.75 (dd, *J* 1.4, 4.8 Hz, H-6). ^{13}C NMR (CD_3OD): δ 53.2, 57.4, 60.5, 94.1, 109.3, 127.4, 129.9, 132.2, 139.1, 146.6, 151.7, 168.9. MS (EI): 248 (M^+ , 88), 230 (52), 218 (32), 189 (77), 159 (73), 147 (100), 131 (44), 104 (58). MS (EI): *M* 248.0804. Calc. for $C_{12}H_{12}N_2O_4$: 248.0797.

Methyl (Z)-3-hydroxy-2-(1-benzylidene-3-oxo-1,3-dihydropyrrolo[3,4-b]pyridin-2-yl)propionate (7b). A solution of methyl (*Z*)-3-*tert*-butoxy-2-(1-benzylidene-3-oxo-1,3-dihydropyrrolo[3,4-*b*]pyridin-2-yl)propionate (232 mg, 0.61 mmol) and TFA (10 ml) in dry CH_2Cl_2 (10 ml) was stirred at ambient temperature for 30 min. The solution was then evaporated and the white solid product isolated from the residual material by flash chromatography using ethyl acetate; yield: 162 mg (82%), m.p. 210 °C. Anal. $C_{18}H_{16}N_2O_4$: C, H. 1H NMR ($DMSO-d_6$): δ 3.64 (s, OMe), 3.72–3.84 (m, one H in CH_2), 3.99–4.16 (m, one H in CH_2), 4.62–4.69 (m, OH), 5.04 (t, *J* 6 Hz, NCH), 7.16 (s, CH=), 7.4–7.5 (m, Ph), 7.72 (dd, *J* 4.7, 8 Hz, H-5), 8.55 (dd, *J* 1.2, 8 Hz, H-4), 8.81 (dd, *J* 1.2, 4.7 Hz, H-6). ^{13}C NMR ($DMSO-d_6$): δ 52.4, 58.0, 58.5, 109.3, 126.6, 128.1, 128.6, 128.8, 129.4, 132.2, 133.3, 133.9, 145.2, 151.6, 166.0, 168.4. MS (EI): 324 (M^+ , 100), 306 (84), 247 (96), 246 (54), 235 (54), 233 (50), 223 (50), 219 (64). MS (EI): *M* 324.1111. Calc. for $C_{18}H_{16}N_2O_4$: 324.1110.

3-Hydroxy-2-(1-methylene-3-oxo-1,3-dihydropyrrolo[3,4-b]pyridin-2-yl)propionic acid (8). LiOH \cdot H₂O (21 mg, 0.5 mmol) in water (5 ml) was added to a solution of methyl (*S*)-3-hydroxy-2-(1-methylene-3-oxo-1,3-dihydropyrrolo[3,4-*b*]pyridin-2-yl)propionate (113 mg, 0.45 mmol) in methanol (15 ml) at 0 °C. The solution was stirred at 0 °C for 5 h before water (10 ml) was added. The solution was extracted with ethyl acetate and the aqueous solution was acidified with 3 M HCl to pH 3. Water was thereafter distilled off, the residual material spun in dichloromethane and the product isolated as a white insoluble solid by centrifugation; yield 72 mg (68%), m.p. >230 °C. Anal. $C_{11}H_{10}N_2O_4$: C, H. 1H NMR (CD_3OD): δ 3.34 (s, OH), 4.12 (dd, *J* 11.7, 9.2 Hz, one H in CH_2), 4.25 (dd, *J* 11.7, 5.5 Hz, one H in CH_2), 5.03 (dd, *J* 9.2, 5.5 Hz, CHN), 5.18 (d, *J* 2.7 Hz, one H in $CH_2=$), 5.51 (d, *J* 2.7 Hz, one H in $CH_2=$), 7.62 (dd, *J* 7.8, 4.8 Hz, H-5), 8.29 (dd, *J* 7.8, 1.3 Hz, H-4), 8.71 (d, *J* 4 Hz, H-6). ^{13}C NMR (CD_3OD): δ 59.2, 61.5, 94.7, 127.6, 130.2, 133.0, 139.7, 148.3, 152.0, 156.1, 167.1.

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