SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF A PYRIDO[3',4':5,4]PYRROLO[1,2-c]-[1,4]BENZODIAZEPINE-3,10-DIONE, A NEW BENZODIAZEPINE-β-CARBOLINE TYPE HYBRID MOLECULE

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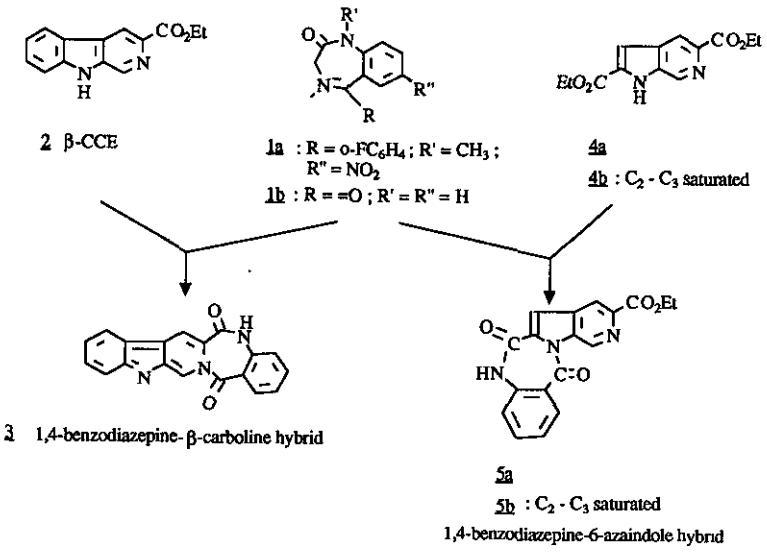
Abstract - Diethyl 2,3-dihydro-6-azaindoline-2,5-dicarboxylate 4b was synthesized and used as starting material in the preparation of a novel benzodiazepine-β-carboline type hybrid molecule (3aR,S)-ethyl 3,10-dioxo-2,3,3a,4-tetrahydro-10H-pyrido[3',4':5,4]pyrrolo[1,2-c][1,4]-benzodiazepine-6-carboxylate 5b. The benzodiazepine receptor binding affinities of this compound and its precursors were found to be very modest *in vitro*. These results confirm our previously proposed model of the configuration of the benzodiazepine and β-carboline binding sites on the receptor as represented by the configuration of these two moieties in the high affinity hybrid 3.

This paper is dedicated to Professor Sir Derek Barton on the occasion of his 70th birthday.

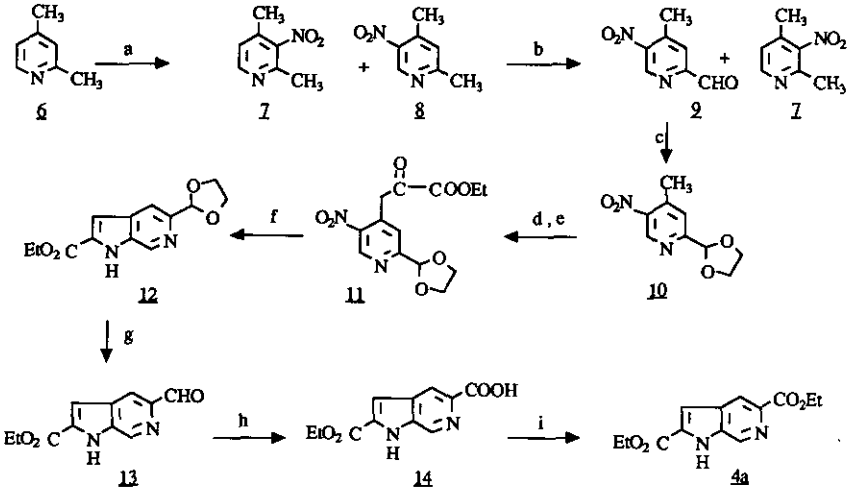
The benzodiazepine receptor of the mammalian central nervous system is known to mediate the anticonvulsant, anxiolytic and sedative properties of 1,4-benzodiazepines (e.g. flunitrazepam, 1a, Scheme 1).[1]



Scheme 1

This receptor also binds compounds of the 3-carboxy-β-carboline class (e.g. ethyl β-carboline-3-carboxylate or β-CCE, 2) with high affinity.[2] Based on the hypothesis that 1,4-benzodiazepines and 3-carboxy-β-carbolines occupy separate but adjacent (or partly overlapping) sites on the receptor protein, we have recently described the synthesis and high binding affinity of a hybrid molecule 3 which combines structural features of both 1 and 2 in a single molecule.[3] This hybrid may thus presumably occupy simultaneously both types of sites on the receptor. In this paper, we present the synthesis and receptor affinity of another type of hybrid molecule 5b in which the 1,4-benzodiazepinedione moiety (1b) is this time fused to the 1,2 positions of 5-ethoxycarbonyl-6-azaindoline (4b). The latter constitutes the reduced form of the 6-azaindole 4a, the essential pharmacophore of active β-carbolines of type 2.

The preparation of dicarboxylic 6-azaindoles of type 4 has not previously been described. Frydman et al.[4] were able to synthesize 2-ethoxycarbonyl-5-methoxy-6-azaindole by condensation of diethyl oxalate with 2-methoxy-4-methyl-5-nitropyridine followed by reductive cyclization of the resulting pyruvate. This methodology was adapted for the synthesis of 4 starting from 2,4-lutidine (6, Scheme 2).



a = KNO_3, H_2SO_4, Δ ; b = $(C_6H_5SeO)_2O$, dioxane, reflux ; c = $HOCH_2CH_2OH$, pTSA, toluene, reflux ;
d = KOEt, EtOH ; e = EtOOCCOOEt ; f = H_2, Pd-C, CH_2Cl_2 ; g = pTSA, CH_3CN, H_2O, reflux ;
h = HCOOH, H_2O_2; i = EtOH, HCl, reflux

Scheme 2

Thus, nitration of 6 with potassium nitrate and fuming sulfuric acid gave a mixture (1:1) of the 3-nitro (7) and 5-nitro (8) isomers, together with unreacted starting material, as described.[5] Compounds 7 and 8 could be separated by careful distillation and the 2-methyl group of 8 was oxidized to the aldehyde 9 with selenium dioxide in refluxing dioxane. However, the poor yields of this combined treatment (< 20%) encouraged us to investigate more efficient methods of transforming 8 into 9. It was found that benzeneseleninic anhydride in dioxane at reflux effected this conversion cleanly.[6] Moreover, when the mixture of 7 and 8 was oxidized with this reagent, only compound 8 reacted, the 2-methyl group of 7 being apparently too hindered to permit easy access by the bulky selenium reagent. Compound 9 could then be easily separated from 7 by chromatography and crystallization. The expensive benzeneseleninic anhydride was regenerated following Barton's procedure.[7] An attempt to use the catalytic benzeneseleninic anhydride procedure[8] was unsuccessful.

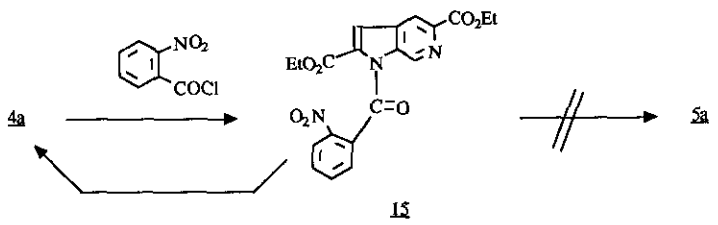
Treatment of the aldehyde 9 with ethylene glycol in refluxing toluene in the presence of catalytic p-toluenesulfonic acid led to the crystalline acetal 10. Following the general

procedure of Frydman and coworkers,[4] the anion of 10, formed by the action of potassium in ethanol, was reacted with diethyl oxalate affording the pyruvate 11 in good yield. It was found that transformation of 11 into the bifunctional 6-azaindole 12 was best achieved by palladium-catalyzed hydrogenolysis of the nitro group in dichloromethane rather than in the usual ethanol. The acetal blocking group of 12 was subsequently removed by acidic hydrolysis, regenerating the aldehyde 13.

The next step in the synthesis of the azaindole synthon 4a required oxidation of the formyl group of 13 to the corresponding carboxylic acid. The commonly used silver oxide procedure was found to be unsatisfactory. However, treatment of 13 in formic acid at 0°C with hydrogen peroxide effected clean, high yield conversion to the acid 14. This oxidation procedure for heterocyclic aldehydes has little literature precedence[9] but its relative mildness should make it a method of choice for such transformations.

Fischer esterification of 14 finally yielded the desired bifunctional 6-azaindole 4a.

Having the correctly substituted 6-azaindole 4a on hand, an attempt was made first to synthesize hybrid 5a (Scheme 3). Thus, condensation of 4a with o-nitrobenzoyl chloride gave 15. However, the amide bond of 15 proved to be extremely sensitive to acid, base and hydrogenolysis such that reduction of the nitro group to the amine under a large variety of conditions invariably led to formation of the precursor 4a.

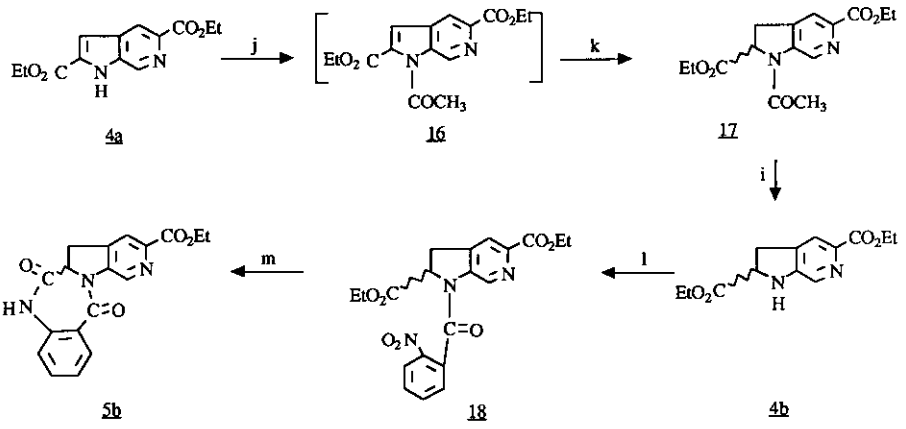


Scheme 3

We reasoned that the amide bond of 15 would be strengthened if the 2,3-bond of the starting azaindole 4a was first reduced to the azaindoline 4b. Moreover, molecular models showed that, in the unsaturated hybrid molecule 5a, the presence of the 2,3-double bond distorts the conformation of the benzodiazepinedione moiety[10] such that recognition by the receptor would be

jeopardized. Saturation of this bond, as in the proposed hybrid 5b, allows the benzodiazepine to assume its pharmacologically important conformation.

Thus, hybrid 5b was synthesized as shown in Scheme 4. Compound 4a was first converted to the



j = DMAP , Et_3N , Ac_2O , CH_2Cl_2 , rt ; k = H_2 , $PdCl_2$, EtOH ; ı = EtOH , HCl , reflux ;
l = o-ClCO-C_6H_4-NO_2 , K_2CO_3 , acetone ; m = H_2 , Pd-C,EtOH

Scheme 4

azaindoline 4b using a technique applied to the reduction of the 2,3 double-bond of 2-substituted indoles.[11] This involved prior N-acetylation of 4a with acetic anhydride in dichloromethane in the presence of DMAP and triethylamine to give the unstable intermediate 16. The latter was hydrogenated with palladium dichloride as catalyst to provide the blocked 2-R,S-azaindoline 17. Removal of the N-acetate group of 17 in refluxing HCl-saturated ethanol yielded the desired 6-azaindoline 4b. Synthesis of hybrid 5b then proceeded uneventfully by way of condensation of 4b with o-nitrobenzoyl chloride followed by reductive cyclization of the resulting adduct 18.

Several attempts were made to form 5a by dehydrogenation of 5b (i.e. DMSO-sulfur, DDQ, palladium on carbon) but all were unsuccessful.

The affinities of the new compounds 4a, 4b, 18 and 5b for the benzodiazepine receptor, as determined in vitro in rat brain preparations as previously described[3] are shown in the Table.

TABLE

Inhibition of specific ^{3}H-flunitrazepam binding in in vitro preparations of rat cerebral cortex membranes at 0°C, expressed as IC_{50} (concentration of drug causing 50% inhibition).

Compound	IC_{50} (nM)
Flunitrazepam 1a	3.0
β-CCE 2	2.5
Hybrid 3	23
4a	84,000
4b	84,000
18	80,000
5b	78,000

Compared to the binding affinities of a typical benzodiazepine, (flunitrazepam, 1a), an active β-carboline (β-CCE, 2) and of the previously synthesized benzodiazepine-β-carboline hybrid molecule 3, the new hybrid molecule 5b binds very poorly to the benzodiazepine receptor. Thus, our original model of the disposition of benzodiazepine and β-carboline binding sites on the receptor, as exemplified by hybrid 3, appears to be more correct.

EXPERIMENTAL

Melting points were determined on a Büchi apparatus and are uncorrected. Ir spectra of samples were obtained in KBr or neat with a Perkin-Elmer 297 instrument. Proton nmr spectra were determined on Varian T-60, Bruker 200- or 400-MHz instruments. Chemical shifts are given as δ

values with reference to Me_4Si as internal standard. Thin-layer chromatography was performed on Merck silica gel 60 plates with fluorescent indicator. The plates were visualized with uv light (254 and 366 nm). Merck silica gel 60 (230-400 mesh) was used for all column chromatography. Mass spectral measurements were done on an AEI MS-9 or an AEI MS-50 spectrometer. Elemental analyses were performed at the ICSN, CNRS, Gif-sur-Yvette, France.

2,4-Dimethyl-5-nitropyridine (8) and 2,4-dimethyl-3-nitropyridine (7).

To 20 ml of fuming sulfuric acid at 0°C was added dropwise 4 ml of 2,4-lutidine (6) followed by slow addition of solid potassium nitrate (7 g ; 69 mM) over 15 min. The mixture was stirred at 0°C for 1 h, then allowed to come to room temperature over 2 h. The mixture was afterwards gradually heated (10°C/15 min) to 100°C, left for 5 h, then heated to 130°C and left for 4 h. The mixture was cooled, poured into ice and water, neutralized with solid $NaHCO_3$, extracted with ether and the organic phase was washed twice with water. The ether extract was dried over $MgSO_4$ and the solvent was evaporated under vacuum to give a brown oil. This oil was distilled under vacuum, affording 25% of 8 (bp_{30} 135-145°C ; lit.[5] bp_{12} 135-140°C), 25% of 7 (bp_{30} 130-135°C ; lit.[5] bp_{12} 118-123°C) and 50% of starting material 6 (bp_{30} 70-75°C). ^1H Nmr of 8 (80 MHz, $CDCl_3$) δ 2.60 (6H, s, $2CH_3$), 7.10 (1H, s), 9.02 (1H, s). ^1H Nmr of 7 (80 MHz, $CDCl_3$) δ 2.16 (6H, s, 2 CH_3), 7.07 (1H, d, J = 5 Hz), 8.42 (1H, d, J = 5 Hz).

4-Methyl-5-nitropyridine-2-carboxaldehyde (9).

To a 4:1 mixture of 8 and 7 (1 g total ; 6.7 mM) in dry dioxane (400 ml) was added benzeneseleninic anhydride (2.6 g ; 7.45 mM). The mixture was refluxed until complete disappearance of starting material was observed by TLC (dichloromethane-ethanol 95:5) and the resulting orange solution was cooled and concentrated under vacuum. Dichloromethane was added to the residue and the solution was washed with saturated aqueous $NaHCO_3$ solution and water, and dried over $MgSO_4$. The solvent was then removed under vacuum and the residue was chromatographed on silica gel (dichloromethane-ethanol 97.5:2.5) to give the aldehyde 9 (533 mg ; 68% from 8), mp 81-82°C (from dichloromethane) ; ir (ν_{max} cm^{-1}, KBr) : 1720 (C=O), 1605 (N=C), 1525, 1305 and 835 ($C-NO_2$) ; eims (m/z) : 166 (M^+), 138, 92, 77 ; ^1H Nmr (80 MHz, $CDCl_3$) δ 2.70 (3H, s, CH_3), 7.87 (1H, s), 9.15 (1H, s), 10.02 (1H, s). Anal. Calcd for $C_7H_6O_3N_2$: C, 50.61 ; H, 3.64 ; N, 16.86. Found : C, 50.82 ; H, 3.55 ; N, 16.90. Compound 7 was recovered unchanged. The diselenide produced in this reaction could be readily recovered and reoxidized to benzeneseleninic anhydride.[7]

2-(1,3-Dioxolan)-4-methyl-5-nitropyridine (10).

A solution of aldehyde 9 (131 mg ; 0.79 mM) in toluene (20 ml) containing ethylene glycol (287 mg ; 4.63 mM) and p-toluenesulfonic acid monohydrate (5 mg ; 0.02 mM) was refluxed until complete disappearance of starting material was observed by TLC (toluene-ethyl acetate 4:1). The solution was cooled, diluted with toluene and washed with saturated aqueous $NaHCO_3$ solution, water and brine. The organic layer was dried over $MgSO_4$, the solvent removed under vacuum and the residue was crystallized in dichloromethane-n-hexane affording 10 (148 mg ; 89%), mp 77°C ; ir (ν_{max} cm^{-1}, KBr) : 1620 (N=C), 1535 and 1365 (C-NO_2), 1190, 1120, 1085 and 1035 (C-O) ; eims (m/z) : 210 (M^+), 209, 167, 164, 137 ; ^1H nmr (60 MHz, $CDCl_3$) δ 2.66 (3H, s, CH_3), 4.13 (4H, s, CH_2CH_2), 5.86 (1H, s), 7.50 (1H, s), 9.10 (1H, s). *Anal. Calcd for* $C_9H_{10}O_4N_2$: C, 51.43 ; H, 4.76 ; N, 13.33. Found : C, 51.72 ; H, 4.80 ; N, 13.55.

Ethyl 2-(1,3-dioxolan)-5-nitro-4-pyridinylpyruvate (11).

To a solution of anhydrous ethanol (500 ml), potassium (3.6 g ; 92.1 meq) and freshly-distilled diethyl oxalate (12.9 ml ; 95 mM) was added over 15 min at room temperature and under nitrogen a solution of compound 10 (17.5 g ; 83 mM) in dry toluene (200 ml). The reaction mixture immediately turned red and after 2 h stirring, the red precipitate which had formed was collected by filtration and washed copiously with dry diethyl ether. The solid was then dissolved in water and acetic acid was added until disappearance of the intense red color was observed. This aqueous solution was extracted with ethyl acetate (3 X), the combined organic extracts were washed once with water, dried over Na_2SO_4 and evaporated to dryness under vacuum. The resulting orange syrup *crystallized on standing, affording* 88% of 11 which could be recrystallized from ethanol, mp 86-87°C ; eims (m/z) : 310 (M^+), 267, 237 (M^+-CO_2Et) ; ^1H Nmr (*200 MHz*, $CDCl_3$) (as 1:1 mixture of enol and keto ester tautomers) δ 1.43 (6H, 2t, J = 8 Hz, $CH_2\underline{CH_3}$), 4.17 (m, 8H, $C\underline{H_2}C\underline{H_2}$), 4.44 (4H, 2 q, J = 8 Hz, $C\underline{H_2}CH_3$), 4.66 (2H, s, $C\underline{H_2}$ of keto form), 4.82 (4H, s, exchangeable with D_2O, OH of enol form + H_2O), 5.92 (1H, s, C<u>H</u>-O), 5.95 (1H, s, C<u>H</u>-O), 7.00 (s, 1H, C<u>H</u>=C), 7.53 (1H, s, H-3), 8.39 (1H, s, H-3), 9.13 (1H, s, H-6), 9.33 (1H, s, H-6). Anal. Calcd for $C_{13}H_{14}O_7N_2$. 3/4 H_2O : C, 48.22 ; H, 4.79 ; N, 8.65. Found : C, 48.14 ; H, 4.71 ; N, 8.54.

Ethyl 5-(1,3-dioxolan)-1H-pyrrolo[2,3-c]pyridine-2-carboxylate (12)

A solution of compound 11 (1 g) in dichloromethane (60 ml) was hydrogenated in a Parr apparatus

at 30 psi for 2 h in the presence of 10% palladium on carbon (1 g) as catalyst. The reaction mixture was filtered on Celite, the catalyst washed copiously with a mixture of dichloromethane-ethanol (1:1) and the combined filtrate and washings were evaporated to dryness under vacuum. The resulting solid was crystallized from dichloromethane-n-hexane, affording 524 mg (62%) of pure 12, mp 190-191°C ; ir (ν_{max} cm^{-1}, KBr) : 3050 (NH), 1705 (C=O), 1615 (C=C) ; eims (m/z) : 262 (M^+), 218 (M^+-OCH_2CH_2) ; ^{1}H nmr (200 MHz, $CDCl_3$) δ 1.43 (3H, t, J = 7 Hz, $CH_2\underline{CH_3}$), 4.16 (4H, m, $O\underline{CH_2}\underline{CH_2}$), 4.46 (2H, q, J = 7 Hz, $\underline{CH_2}CH_3$), 6.03 (1H, s, $\underline{CH}$-O), 7.26 (1H, s, H-3), 7.85 (1H, s, H-4), 9.53 (1H, s, H-7). Anal. Calcd for $C_{13}H_{14}O_4N_2$: C, 59.54 ; H, 5.34 ; N, 10.69. Found : C, 59.72 ; H, 5.34 ; N, 10.69.

2-Ethoxycarbonyl-1H-pyrrolo[2,3-c]pyridine-5-carboxaldehyde (13).

A solution of the acetal 12 (140 mg) in 10% aqueous acetonitrile (10 ml) was refluxed for 5 h in the presence of p-toluenesulfonic acid monohydrate (30 mg). The reaction mixture was cooled and concentrated in vacuo to remove excess acetonitrile. The residue was diluted with chloroform (50 ml) and washed successively with saturated aqueous sodium hydrogen carbonate (2 x 20 ml) and water (20 ml). The organic phase was dried over Na_2SO_4 and the solvent removed under vacuum leaving crude solid 13 which was crystallized from dichloromethane-n-hexane (90 mg, 77%), mp 173-174°C ; ir (ν_{max} cm^{-1}, KBr) : 1740 (C=O), 1700 (C=O) ; eims (m/z) : 218 (M^+), 190 (M^+-CO). ; ^{1}H nmr (60 MHz, $CDCl_3$) δ 1.40 (3H, t, J = 7 Hz, $CH_2\underline{CH_3}$), 4.53 (2H, q, J = 7 Hz, $\underline{CH_2}CH_3$), 7.36 (1H, s, H-3), 8.36 (1H, s, H-4), 9.00 (1H, s, H-7), 10.09 (2H, s + broad s, $\underline{CH}O$, NH). Anal. Calcd for $C_{11}H_{10}N_2O_3$: C, 60.55 ; H, 4.59 ; N, 12.84. Found : C, 60.50 ; H, 4.64 ; N, 12.88.

2-Ethoxycarbonyl-1H-pyrrolo[2,3-c]pyridine-5-carboxylic acid (14).

To a solution of the aldehyde 13 (6.6 g) in formic acid (20 ml) at 0°C was added 33% aqueous hydrogen peroxide (5 ml). The reaction mixture was stored at 4°C overnight. The precipitate which formed was collected by filtration, washed with water and dried, yielding 14 as its formate (6.3 g, 89%), mp 310-311°C ; eims (m/z) : 234 (M^+), 190 (M^+-CO_2) ; ^{1}H nmr (400 MHz, DMSO-d_6) δ 1.37 (3H, t, J = 7 Hz, $CH_2\underline{CH_3}$), 3.36 (bs, D_2O-exchangeable, $COO\underline{H}$ + $HCOO\underline{H}$ + H_2O), 4.41 (2H, q, $\underline{CH_2}CH_3$), 7.37 (1H, s, H-3), 8.14 (1H, s, $\underline{H}COOH$), 8.48 (1H, s, H-4), 8.89 (1H, s, H-7), 9.83 (1H, s, D_2O-exchangeable, NH). Anal. Calcd for $C_{11}H_{10}N_2O_4.HCO_2H$: C, 51.43 ; H, 4.29 ; N, 10.00. Found : C, 51.41 ; H, 4.32 ; N, 9.99.

Diethyl 1H-pyrrolo[2,3-c]pyridine-2,5-dicarboxylate (4a).

A suspension of the acid 14 (6 g) in HCl-saturated ethanol (400 ml) was refluxed for 6 h. The solution was cooled, the solvent was removed under vacuum and the residue was suspended in ethyl acetate (600 ml) before neutralization with saturated aqueous sodium hydrogen carbonate. The aqueous layer was removed and the organic phase was washed twice with water, dried over Na_2SO_4 and the solvent removed under vacuum. The resulting solid was crystallized from dichloromethane-ether, yielding 6.4 g (95%) of white crystals of 4a, mp 191-193°C ; ir (ν_{max} cm^{-1}, KBr) 1700 (C=O) ; eims (m/z) 262 (M^+), 217 (M^+-OEt) ; ^1H nmr (200 MHz, DMSO-d_6) δ 1.53 (6H, 2t, J = 7 Hz, 2 x $CH_2\underline{CH}_3$), 4.53 (4H, 2q, J = 7 Hz, 2 x $\underline{CH}_2CH_3$), 7.60 (1H, s, H-3), 8.70 (1H, s, H-4), 9.10 (1H, s, H-7), 13.00 (1H, s, D_2O-exchangeable, NH). Anal. Calcd for $C_{13}H_{14}N_2O_4$: C, 59.54 ; H, 5.34 ; N, 10.69. Found : C, 59.35 ; H, 5.41 ; N, 10.86.

Diethyl 1-(o-nitrobenzoyl)pyrrolo[2,3-c]pyridine-2,5-dicarboxylate (15).

To a suspension of sodium hydride (60% dispersion ; 110 mg ; 4.6 mM) and potassium iodide (204 mg ; 1.2 mM) in dry tetrahydrofuran (15 ml) at 0°C under nitrogen was added dropwise a solution of the diester 4a (196 mg ; 0.7 mM) in dry tetrahydrofuran (15 ml). The mixture was stirred at room temperature during 2 h, giving a pale yellow solution. To this solution was added dropwise freshly-distilled o-nitrobenzoyl chloride (260 mg ; 1.4 mM) in dry tetrahydrofuran (10 ml). After 30 min stirring, saturated aqueous NH_4Cl and water were added. The solution was extracted with dichloromethane, the organic layer was washed with water and brine, dried over $MgSO_4$ and the solvent was removed under vacuum. The crude product was chromatographed on silica gel (dichloromethane-ethanol 97.5:2.5) affording pure 15 (283 mg ; 92%), mp 152°C (from dichloromethane-n-hexane) ; ir (ν_{max} cm^{-1}, KBr) : 1720 (C=O), 1530 (NO_2) ; eims (m/z) 411 (M^+), 366 (M^+-OEt), 338 (M^+-COOEt) ; ^1H nmr (200 MHz, $CDCl_3$) δ 1.92 (3H, t, J = 8 Hz, $CH_2\underline{CH}_3$), 2.19 (3H, t, J = 8 Hz, $CH_2\underline{CH}_3$), 4.80 (2H, q, J = 8 Hz, $\underline{CH}_2CH_3$), 5.20 (2H, q, J = 8 Hz, $\underline{CH}_2CH_3$), 8.03 (1H, s, H-3), 8.45 (3H, m, H-Ar), 8.90 (1H, m, H-Ar), 9.18 (1H, s, H-4), 9.83 (1H, s, H-7). Anal. Calcd for $C_{20}H_{17}N_3O_7$: C, 58.39 ; H, 4.14 ; N, 10.22. Found : C, 58.46 ; H, 4.02 ; N, 10.11.

(2R,S) Diethyl 1-acetyl-2,3-dihydropyrrolo[2,3-c]pyridine-2,5-dicarboxylate (17).

To the diester 4a (312 mg ; 1.19 mM) in dry dichloromethane (5 ml) was added 4-(N,N-dimethyl)-aminopyridine (16 mg ; 0.13 mM), triethylamine (0.2 ml ;1.43 mM) and acetic anhydride (0.15 ml,

1.59 mM). The mixture was stirred overnight at room temperature. The solution was then washed with brine, dried over $MgSO_4$ and the solvent was evaporated in vacuo, affording a mixture of 4a and the non-isolated intermediate 16 (16 : 4a = 6:1). This mixture, dissolved in ethanol (7 ml) was then hydrogenated at atmospheric pressure in the presence of palladium dichloride (22 mg). After complete consumption of the unstable intermediate 16, as indicated by TLC (dichloromethane-ethanol 95:5), the mixture was filtered and the catalyst was copiously washed with ethanol. The filtrate and washings were evaporated and the residue was chromatographed on silica gel (dichloromethane-ethanol 97.5:2.5) to give the dihydro compound 17 (191 mg ; 52% from the diester 14), mp 103°C (from dichloromethane-n-hexane) ; ir (ν_{max} cm^{-1}, KBr) : 3000 (N-C=O), 1730 (C=O), 1680 (C=O), 1600 (N=C) ; eims (m/z) : 306 (M^+), 264, 261, 233, 191, 160, 117 ; ^1H nmr (200 MHz, $CDCl_3$) δ 1.26 (3H, t, J = 8 Hz, CH_3), 1.43 (3H, t, CH_3), 2.25 (3H, s, CH_3), 3.55 (1H, d, J = 18 Hz), 3.65 (1H, dd, J = 18 Hz and 11 Hz), 4.23 (2H, q, J = 8 Hz, CH_2), 4.46 (2H, q, J = 8 Hz, CH_2), 4.98 (1H, d, J = 11 Hz), 8.00 (1H, s), 9.53 (1H, s). Anal. Calcd for $C_{15}H_{18}O_5N_2$: C, 58.82 ; H, 5.88 ; N, 9.15. Found : C, 58.75 ; H, 5.38 ; N, 9.42.

(2R,S) Diethyl 2,3-dihydro-1H-pyrrolo[2,3-c]pyridine-2,5-dicarboxylate (4b).

A solution of the N-acetyl derivative 17 (126 mg ; 0.41 mM) in HCl-saturated methanol (5 ml) was refluxed until complete disappearance of starting material was observed by TLC (dichloromethane-ethanol 95:5). The solution was then cooled and neutralized with saturated aqueous $NaHCO_3$ solution. The mixture was filtered, the solid was washed with ethanol and the filtrate and washings were evaporated. Dichloromethane was added to the resulting residue, and the solution was washed with water and brine and dried over $MgSO_4$. Evaporation of the solvent left a crude solid which was purified by preparative chromatography (dichloromethane-ethanol 95:5) affording compound 4b (78 mg ; 72%), mp : 132°C (from dichloromethane-n-hexane) ; ir (ν_{max} cm^{-1}, KBr) : 3150 (NH), 1730 (C=O), 1680 (C=O), 1600 (C=N), 1280, 1230 ; eims (m/z) : 264 (M^+), 219, 191, 146, 118 ; ^1H Nmr (400 MHz, $CDCl_3$) δ 1.31 (3H, t, J = 7 Hz, CH_3), 1.41 (3H, t, J = 7 Hz, CH_3), 3.45 (2H, m), 4.25 (2H, q, J = 7 Hz, CH_2), 4.44 (2H, q, J = 7 Hz, CH_2), 4.53 (1H, dd, J = 10 Hz and 10 Hz), 4.86 (1H exchangeable with D_2O, s, NH), 7.92 (1H, s), 8.13 (1H, s). Anal. Calcd for $C_{13}H_{16}O_4N_2$: C, 59.09 ; H, 6.06 ; N, 10.61. Found : C, 59.02 ; H, 6.15 ; 10.90.

(2R,S) Diethyl 2,3-dihydro-1-(o-nitrobenzoyl)pyrrolo[2,3-c]pyridine-2,5-dicarboxylate (18).

To the dihydro derivative 4b (62 mg ; 0.23 mM) in dry acetone (3 ml) was added K_2CO_3 (39 mg ; 0.28 mM) and o-nitrobenzoyl chloride (0.05 ml ; 0.38 mM). The mixture was stirred at room

temperature until complete disappearance of starting material was observed by TLC (dichloromethane-ethanol 95:5), following which it was diluted with dichloromethane (10 ml) and filtered. The solid was washed with dichloromethane and the combined filtrate and washings were evaporated under vacuum. The residue was taken up in dichloromethane, washed with saturated aqueous $NaHCO_3$ solution and brine, dried over $MgSO_4$ and the solvent was removed under vacuum. The crude product was chromatographed on silica gel (dichloromethane-ethanol 95:5) affording pure 18 (88 mg ; 91%), mp 49°C (from dichloromethane-n-hexane) ; ir (ν_{max} cm^{-1}, KBr) : 1710 (C=O), 1690 (C=O), 1640 (N-C=O), 1510 and 1350 (N=O) ; eims (m/z) : 413 (M^+), 368, 340, 263, 190, 150, 145, 117 ; ^1H Nmr (200 MHz, $CDCl_3$) δ 1.38 (6H, m, 2 CH_3), 3.30 (1H, m), 3.70 (1H, m), 4.33 (2H, m, CH_2), 4.43 (2H, m, CH_2), 5.50 (1H, dd, J = 10 Hz and 11 Hz), 7.70 (1H, m), 7.86 (1H, m), 7.98 (1H, s), 8.05 (1H, m), 8.30 (1H, m), 9.60 (1H, s). Anal. Calcd for $C_{20}H_{19}O_7N_3.0.5\ H_2O$: C, 56.87 ; H, 4.73 ; N, 9.95. Found: C, 56.74 ; H, 4.92 ; N, 10.13.

(3aR,8) Ethyl 3,10-dioxo-2,3,3a,4-tetrahydro-10H-pyrido[3',4':5,4]pyrrolo[1,2-c][1,4]benzodiazepine-6-carboxylate (5b).

A solution of the nitro compound 18 (300 mg ; 0.72 mM) in ethanol (20 ml) containing 10% Pd-C (34 mg) was hydrogenated at atmospheric pressure. After complete consumption of the starting material 18, as indicated by TLC (dichloromethane-ethanol 95:5), the mixture was filtered and the catalyst was copiously washed with ethanol. The filtrate and washings were evaporated and the residue was chromatographed on silica gel (dichloromethane-ethanol 20:1) affording the cyclized compound 5b (71 mg ; 29%), mp 281°C (from dichloromethane-n-hexane) ; ir (ν_{max} cm^{-1}, KBr) : 3425 (NH), 1675 (C=O), 1655 (C=O) ; eims (m/z) : 337 (M^+), 292, 264, 191, 145, 117. ^1H Nmr (400 MHz, $CDCl_3$) δ 1.40 (3H, t, CH_3), 3.41 (1H, dd, H-4a, $J_{3a,4a}$ = 11 Hz), 4.24 (1H, dd, H-4b, $J_{3a,4b}$ = 3 Hz), 4.50 (2H, q, J = 7 Hz, CH_2), 4.86 (1H, dd, J = 11 Hz and 3 Hz, H-3a), 7.13 (1H, d, J = 8 Hz, H-Ar), 7.43 (1H, t, J = 8 Hz, H-Ar), 7.65 (1H, m, H-Ar), 8.14 (1H, dd, J = 8 Hz and 1 Hz, H-Ar), 8.18 (1H, s, H-5), 8.28 (1H, s, NH), 8.52 (1H, s, H-8). Anal. Calcd for $C_{18}H_{15}O_4N_3.0.5\ H_2O$: C, 62.42 ; H, 4.62 ; N, 12.13. Found : C, 62.30 ; H, 4.37 ; N, 12.16.

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